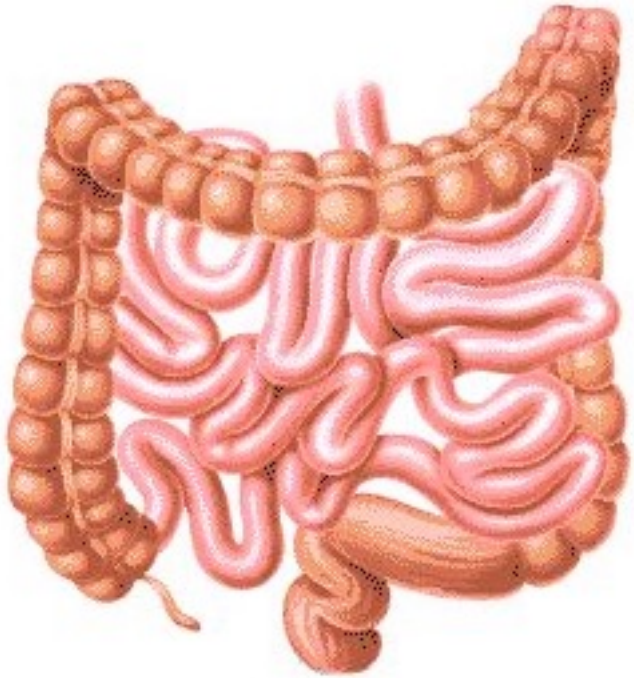
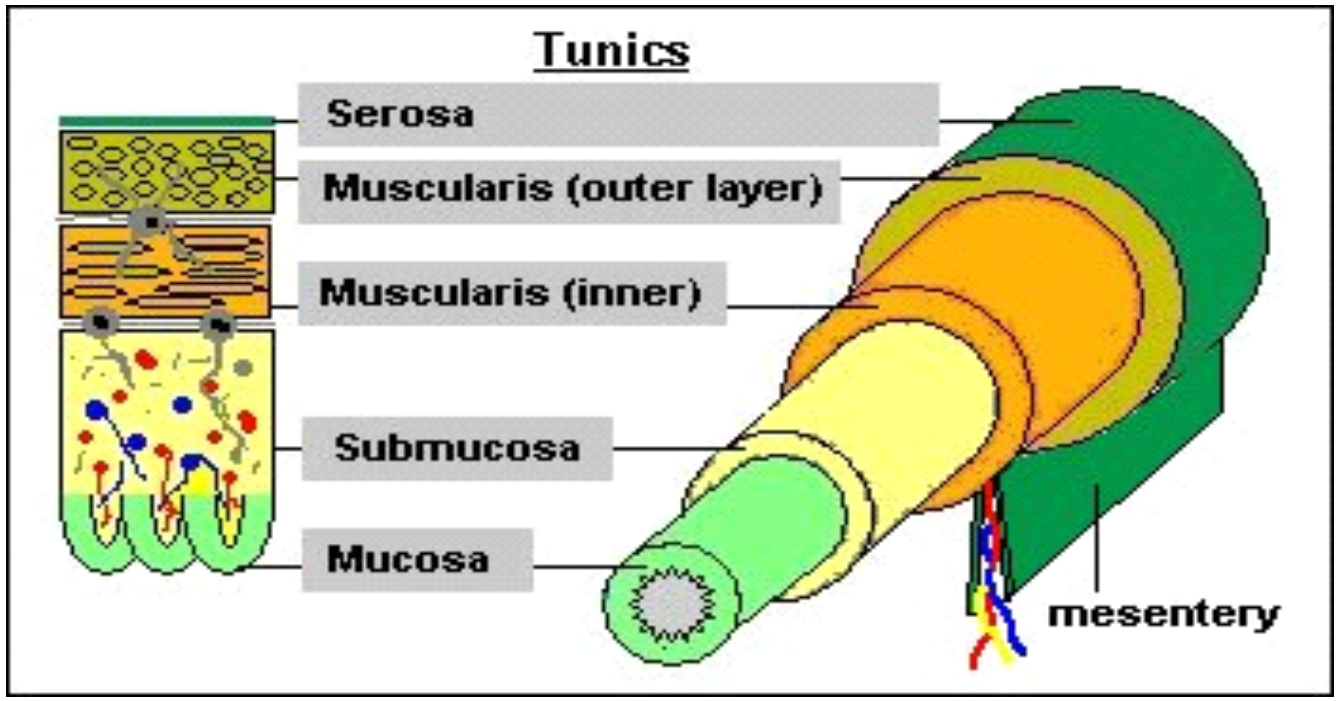
Intestine

Large intestine: The long, tube-like organ that is connected to the small intestine at one end and the anus at the other. The large intestine has four parts: cecum, colon, rectum, and anal canal. Partly digested food moves through the cecum into the colon, where water and some nutrients and electrolytes are removed. The remaining material, solid waste called stool, moves through the colon, is stored in the rectum, and leaves the body through the anal canal and anus.

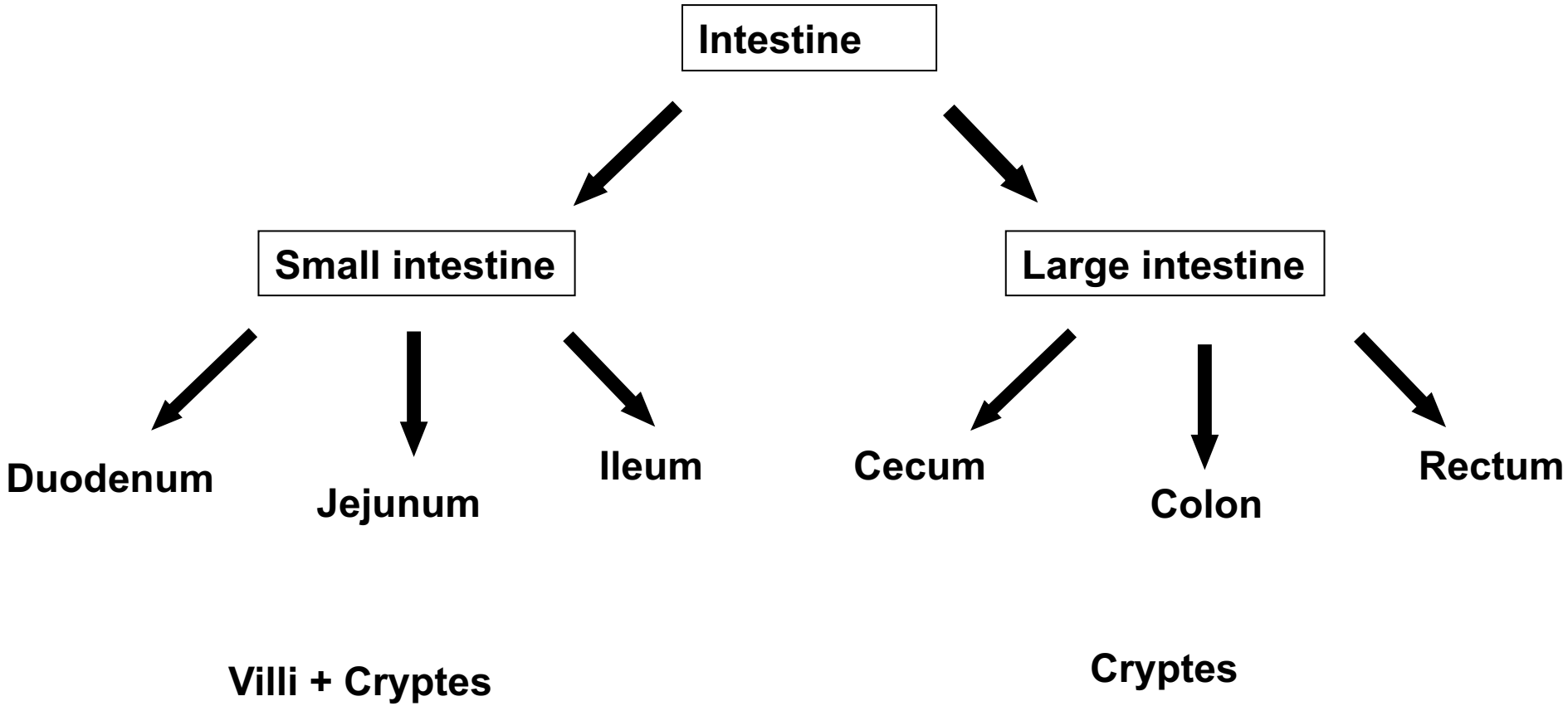


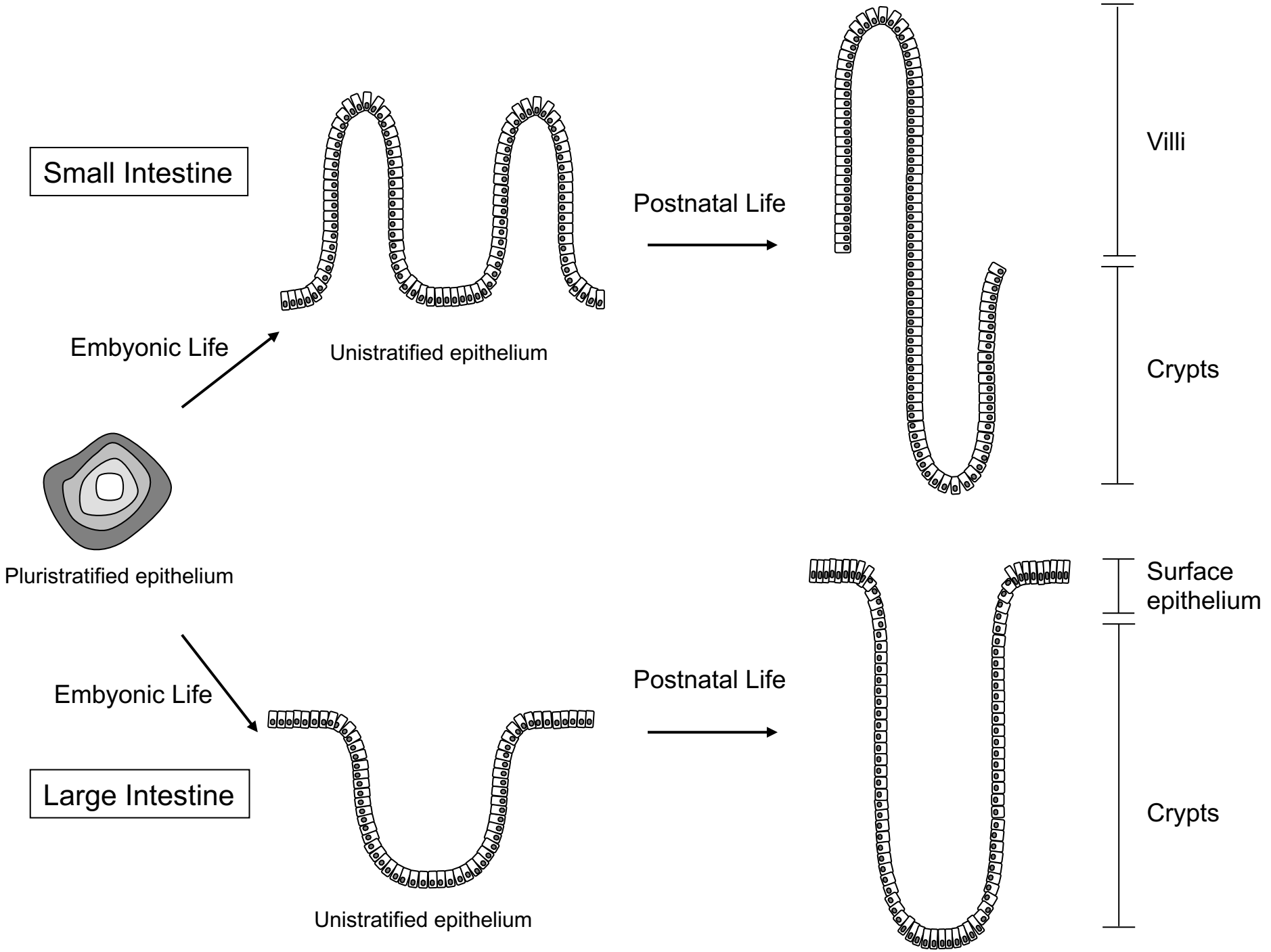
Small intestine: The part of the digestive tract that is located between the stomach and the large intestine.

Structure of the Intestine I

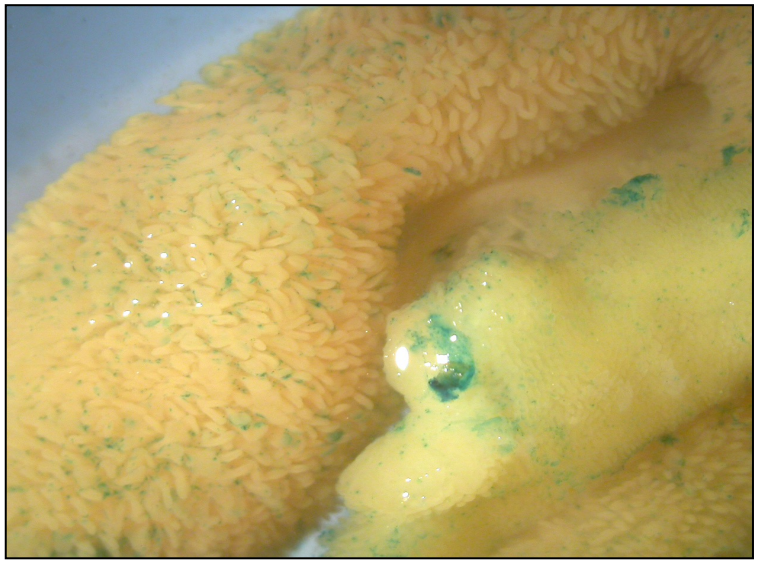


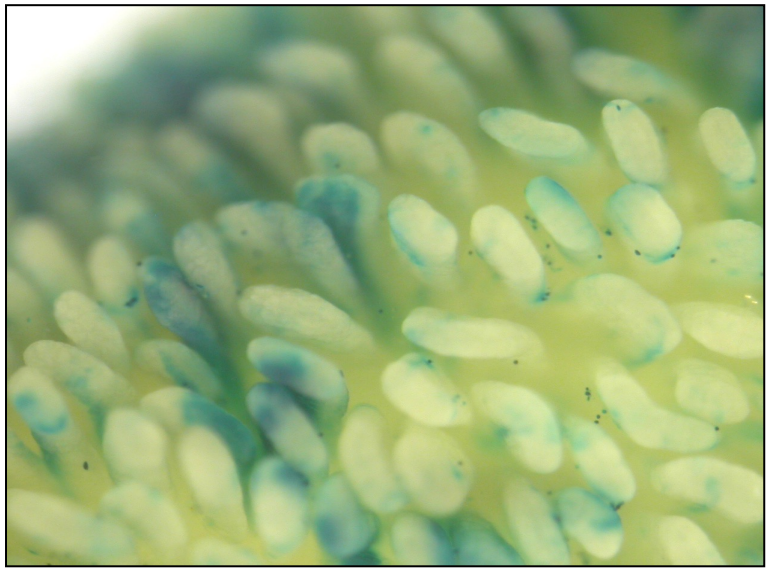
Structure of the Intestine



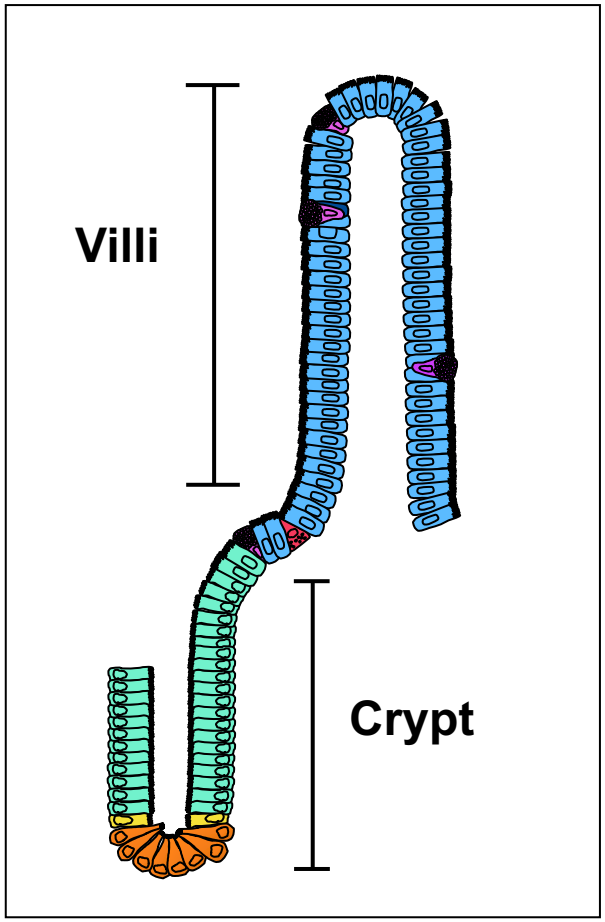

Small Intestine
Embyonic Life
Unistratified epithelium
Postnatal Life
Villi
Crypts
Pluristratified epithelium
Embyonic Life
Large Intestine
Unistratified epithelium
Postnatal Life
Surface epithelium
Crypts

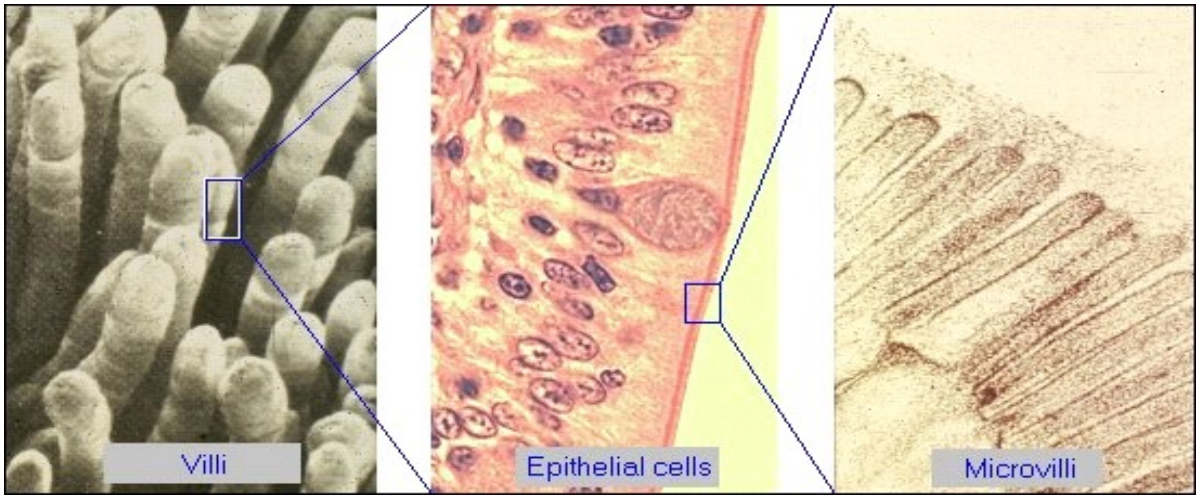
Structure of the Small Intestinal Mucosa



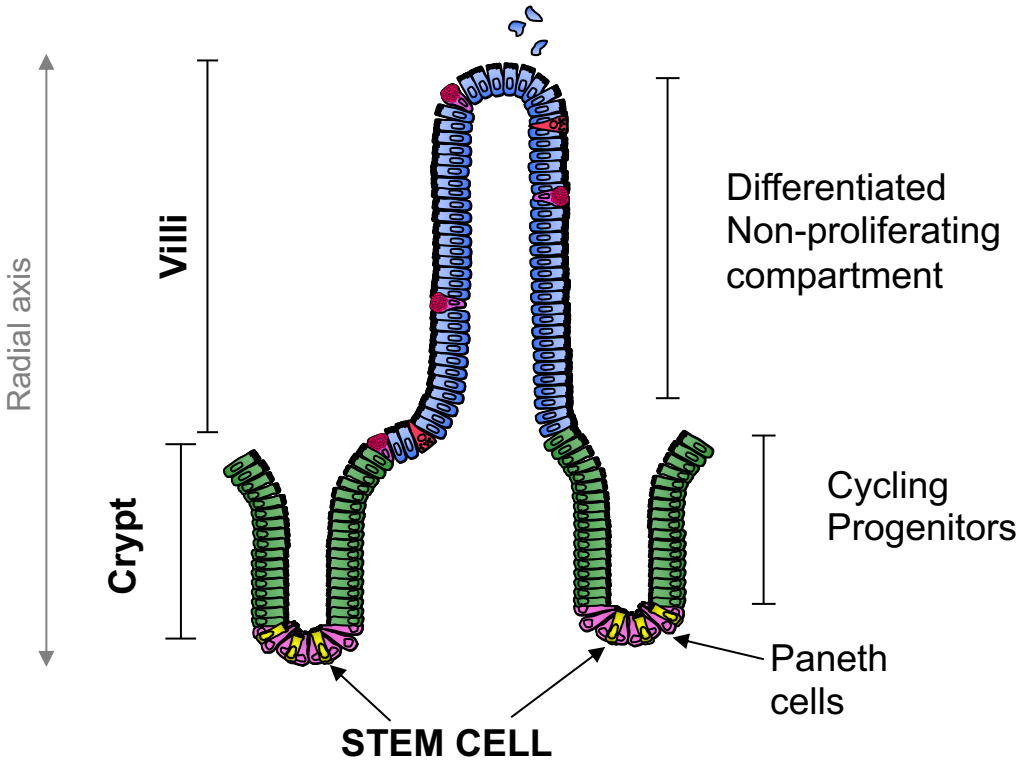


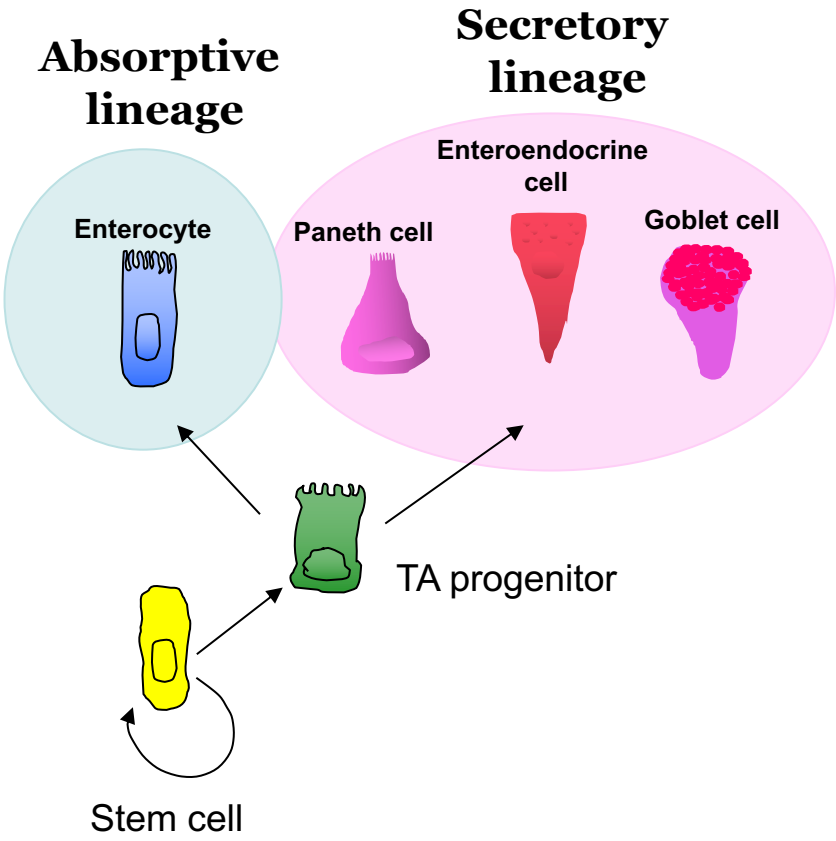
Villi and Crypts





The intestine: model of self-renewing tissue





Cell Stem Cell
Review

Cellular Plasticity in Intestinal Homeostasis and Disease

Felipe de Sousa e Melo[1] and Frederic J. de Sauvage[2,*]
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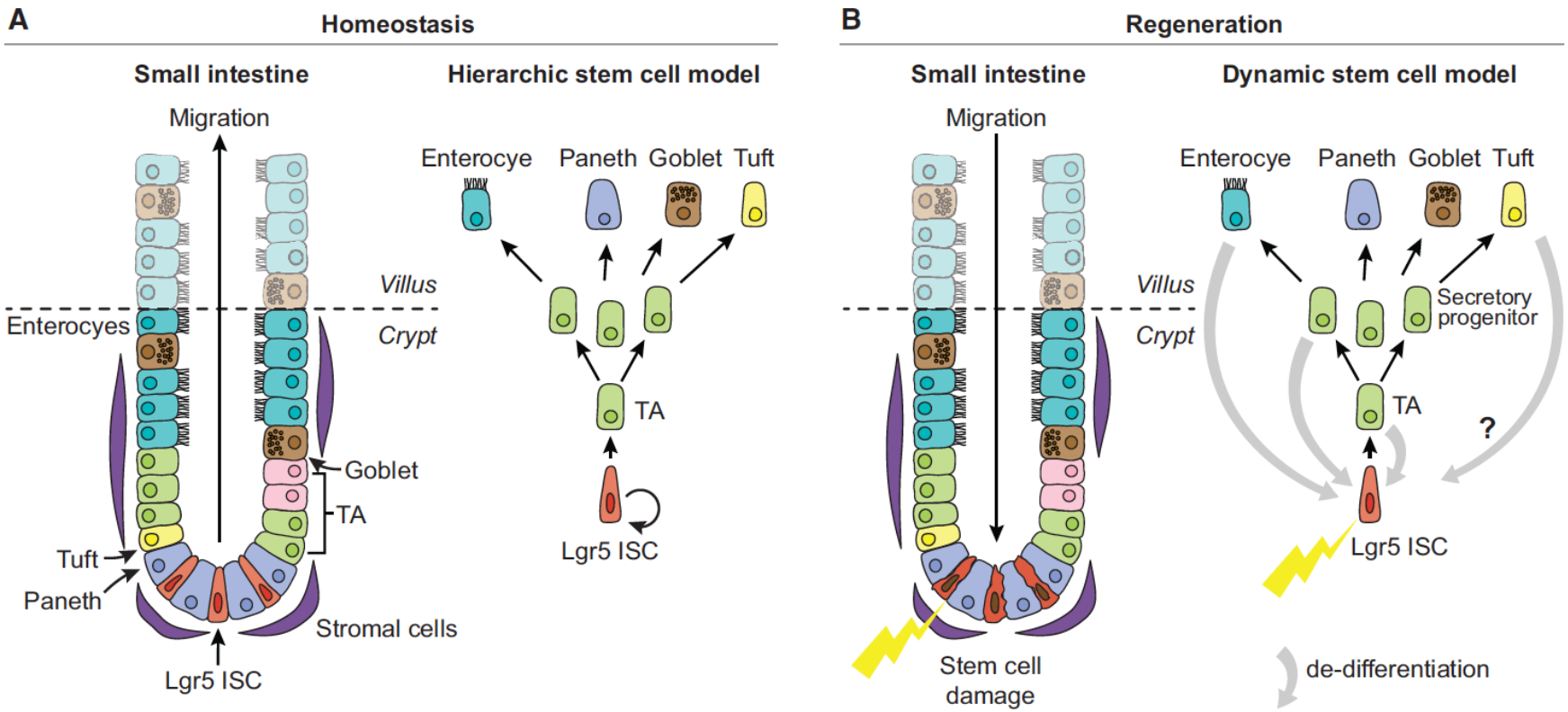
https://doi.org/10.1016/j.stem.2018.11.019

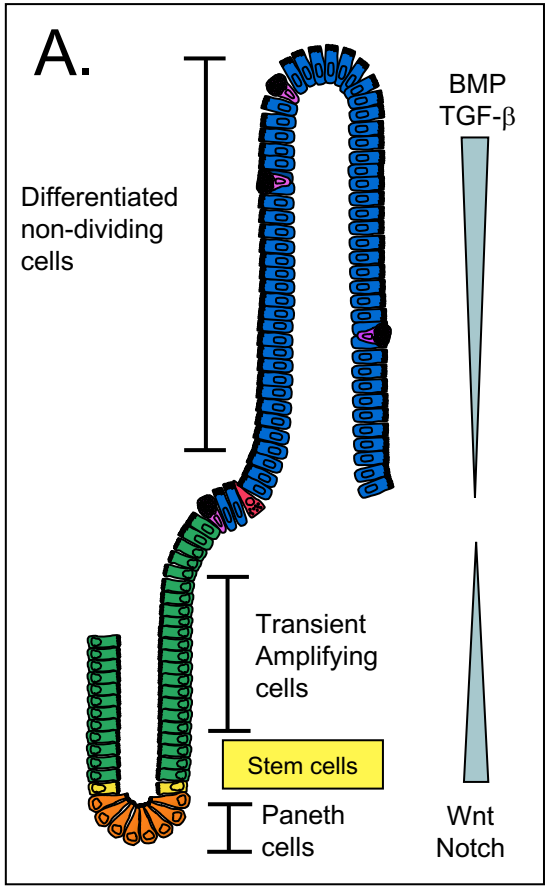
Cell Stem Cell *24*, January 3, 2019



Stem cell research was born in the aftermath of Hiroshima and Nagasaki

Laboratories around the world started to investigate the consequences of radiation on blood, gut, skin and other tissues:

Stem cells of the small intestine have been localized and identified in radiation and label retaining assays



Christopher Potten

A glossary for stem-cell biology

Austin Smith[1]

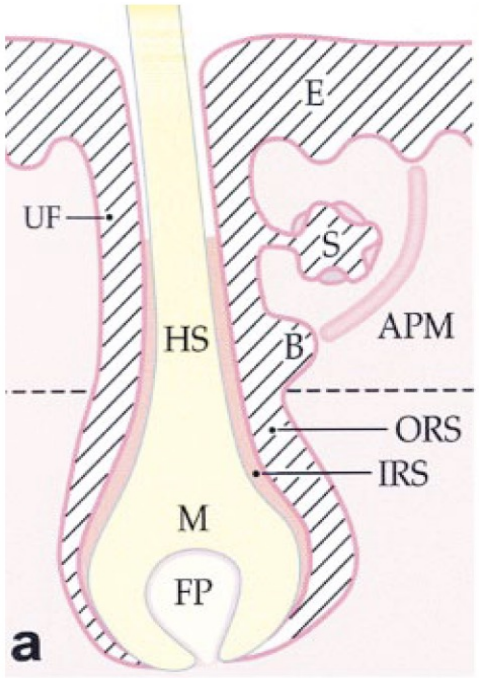
Stem-cell biology is in a phase of dynamic expansion and is forming connections with a broad range of basic and applied disciplines. The field is simultaneously exposed to public and political scrutiny. A common language in the stem-cell community is an important tool for coherent exposition to these diverse audiences, not least because certain terms in the stem-cell vocabulary are used differently in other fields.

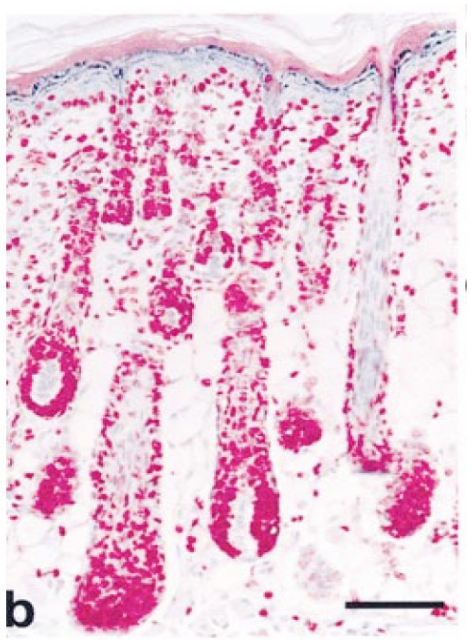
Label-retaining cell Candidate for adult tissue stem cell because of slow division rate and/or immortal strand retention. Interpret with caution.

Label retaining cells assay

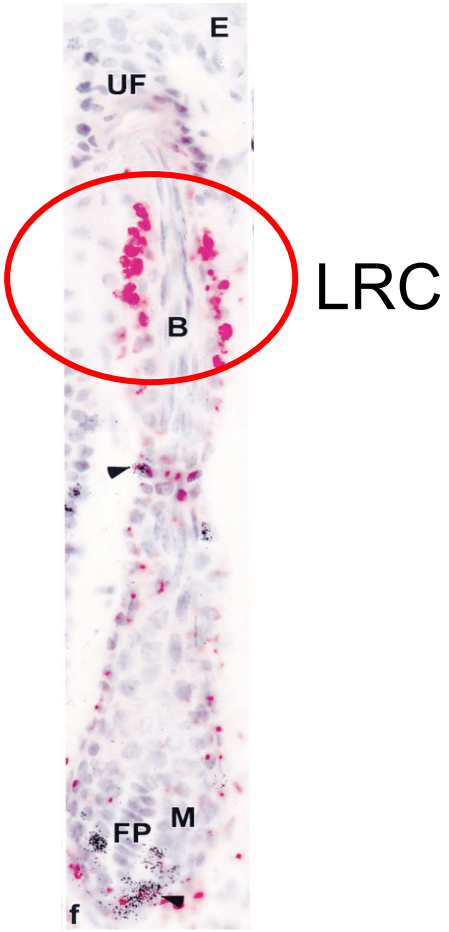
•This method is based on the assumption that normal or cancer stem cells spend long periods not cycling or undergo an “immortal strand” DNA replication.

Label retaining cells are found in the Buldge region





5 day old mice, 2 injections of BrdU s.c. twice per day for 3 days



LRC

^{3H}Thymidin ip injection 1 hr before sacrifice

Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 2302–2306, April 1987
Cell Biology

Three clonal types of keratinocyte with different capacities for multiplication

YANN BARRANDON AND HOWARD GREEN

Department of Physiology and Biophysics, Harvard Medical School, Boston, MA 02115

Primary keratinocytes were cultured to semi-confluency and single cells were picked and cultured

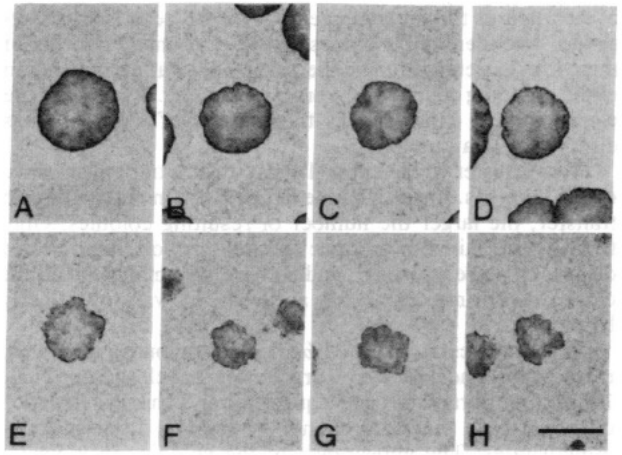


FIG. 1. Macroscopic colony types formed by keratinocytes. Twelve-day colonies formed by strain AY were fixed and stained with rhodamine. Note the smooth perimeter of the colonies in *A–D*, and the wrinkled colonies in *E–H*. The former are typically formed by holoclones and the latter by meroclones. (Bar = 5 mm.)

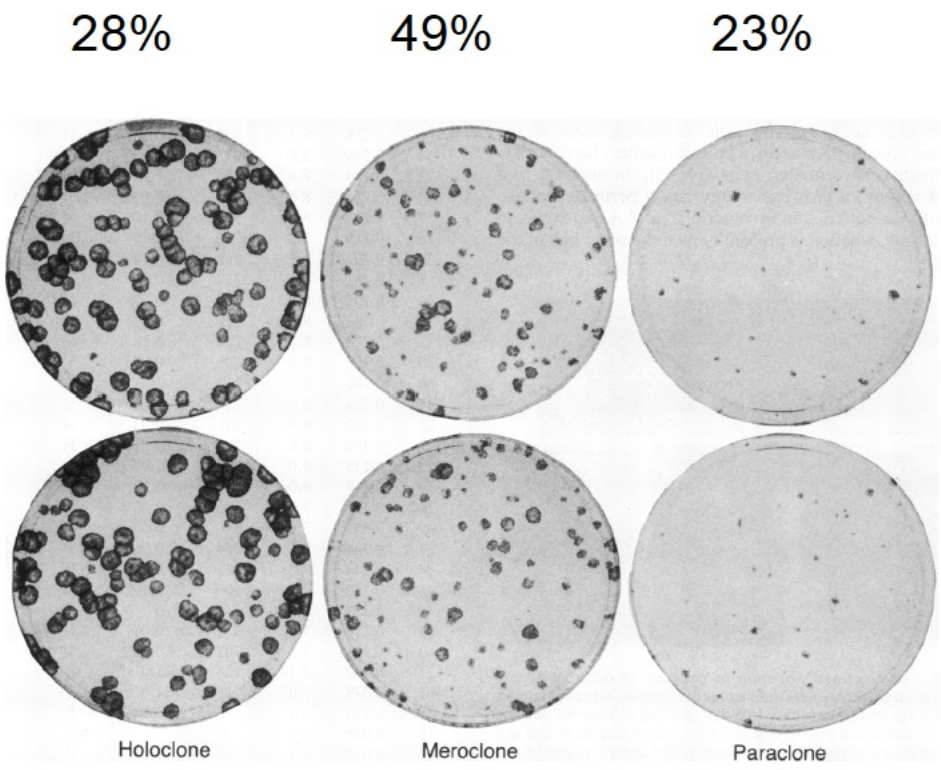
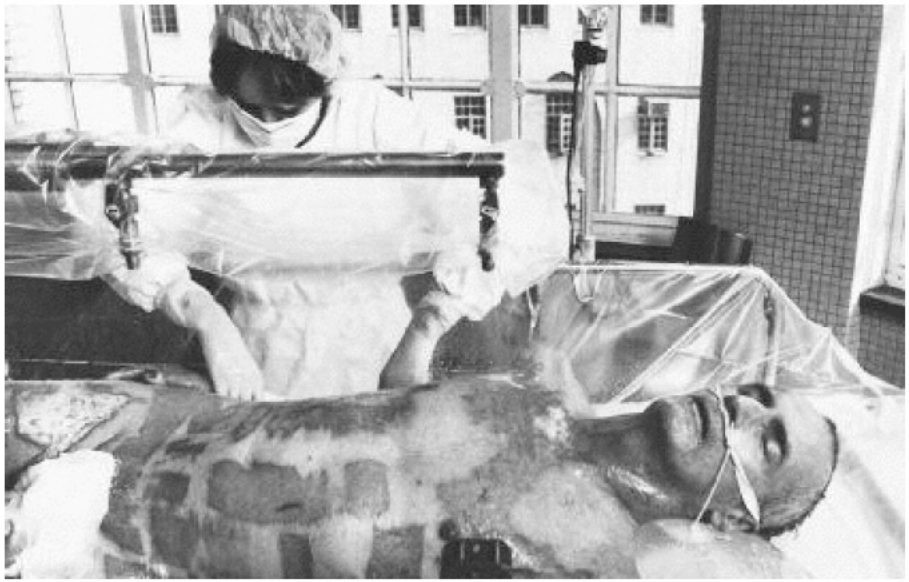
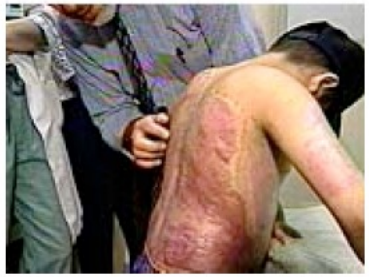


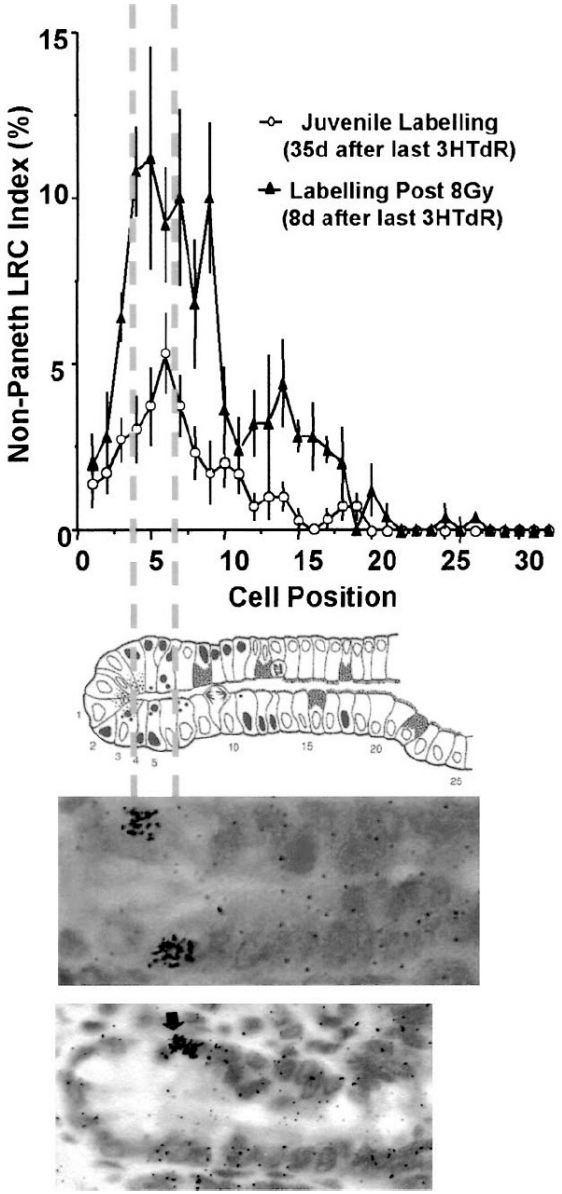
FIG. 3. Colonies produced in indicator dishes by different clonal types of strain AY. Each clone was disaggregated, and one-quarter of the cells was inoculated into each of two indicator dishes containing irradiated 3T3 cells. The cells were allowed to grow for 12 days, when the dishes were fixed and stained with rhodamine. The clones shown are those identified in Table 1 as nos. 44 (holoclone), 33 (meroclone), and 66 (paraclone).

Burned patients can be rescued by autologous skin transplantation (first done in the late 1970s)

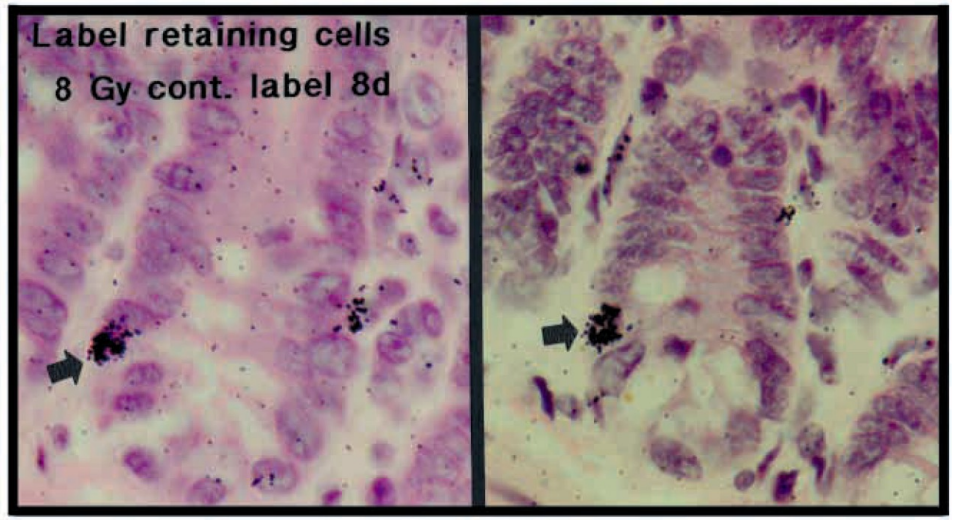




However transplanted skin does not make any sweat glands or hair follicles



Stem cells of the small intestine have been localized and identified in radiation and label retaining assays



Christopher S. Potten Radiation Research 2004

Hypothesis of selective DNA segregation at mitosis

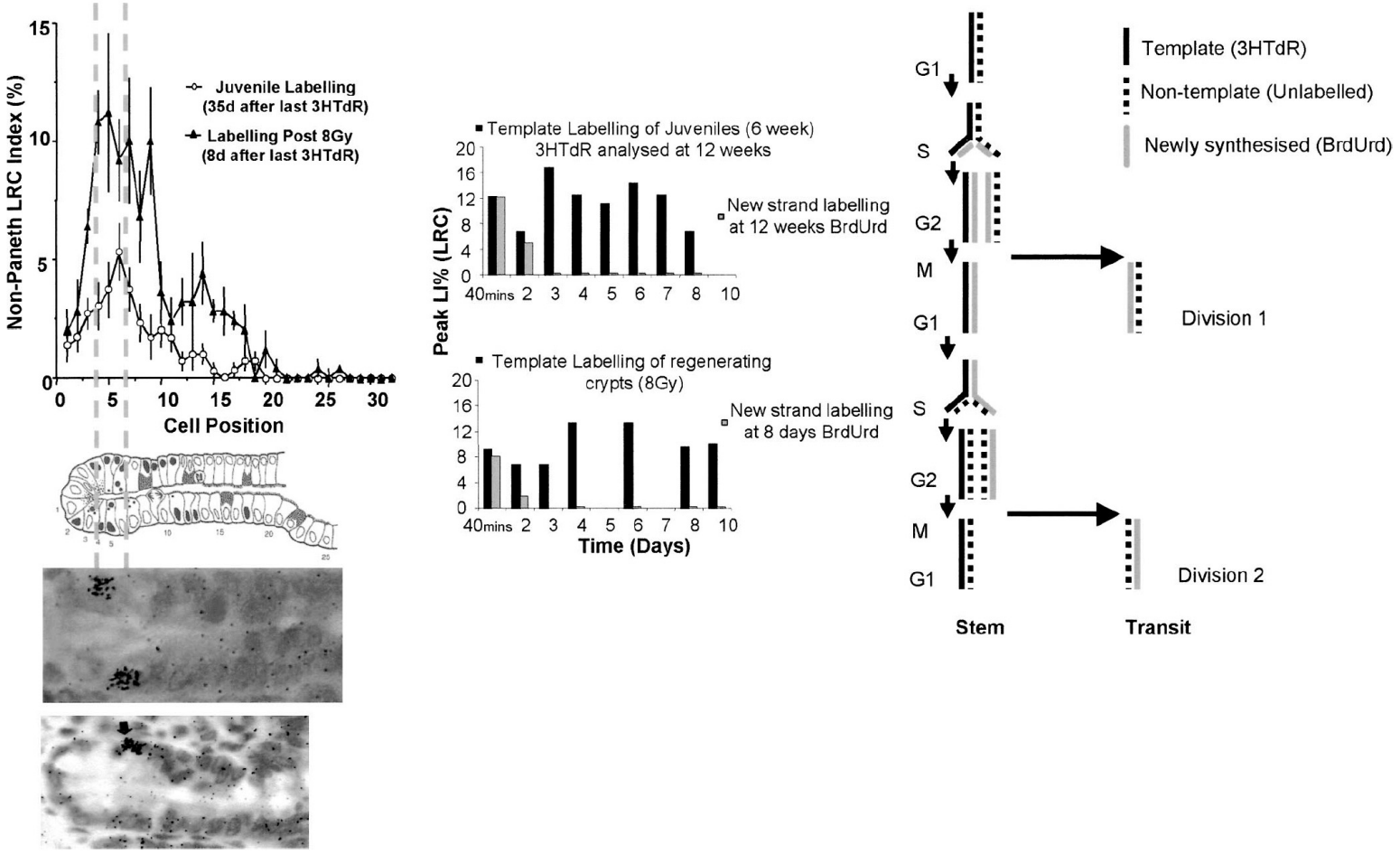
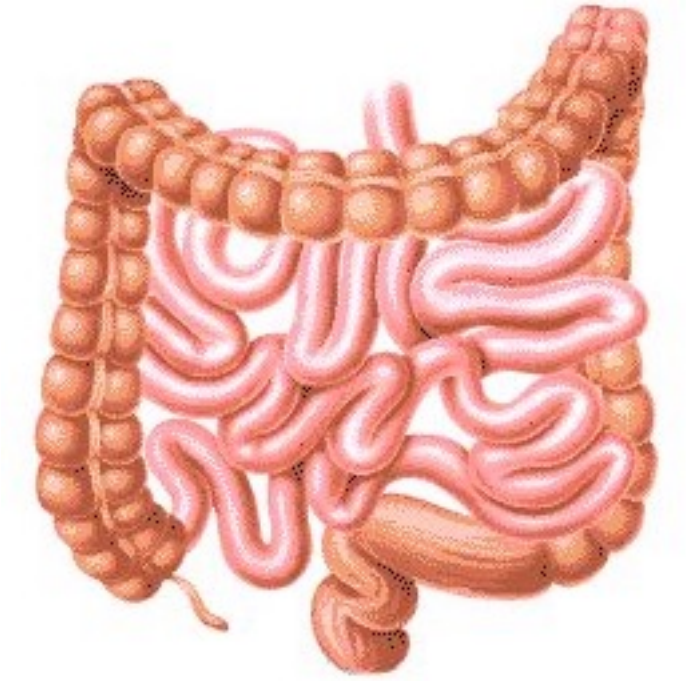
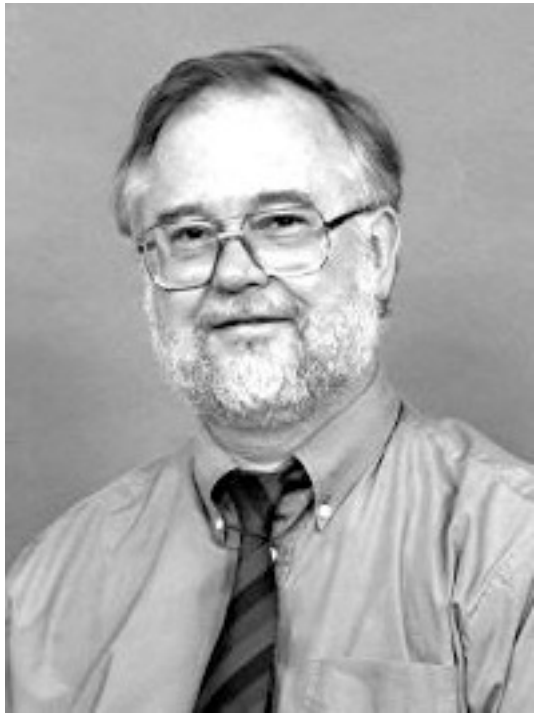


FIG. 8. A figure summarizing the results of a series of experiments designed to generate label-retaining cells (see photomicrographs) as a consequence of selective DNA segregation at mitosis, and the results of an experiment where the DNA strands in label-retaining cells (LRCs) (black bars) were double-labeled with bromodeoxyuridine (gray bars), resulting in both template and newly synthesized strands labeled with different markers. The segregation of these two markers over a series of divisions was studied for the label-retaining cells that were located at the stem cell position. The tritiated thymidine in the template strands persists through many rounds of division, while the bromodeoxyuridine in the newly synthesized strands disappears rapidly over the first two divisions. Reproduced with permission from ref. (*16*).

In humans, cancer of the gut is mostly confined to the colon

Why?





Christopher Potten

Cells of the small intestine are extremely sensitive to radiation

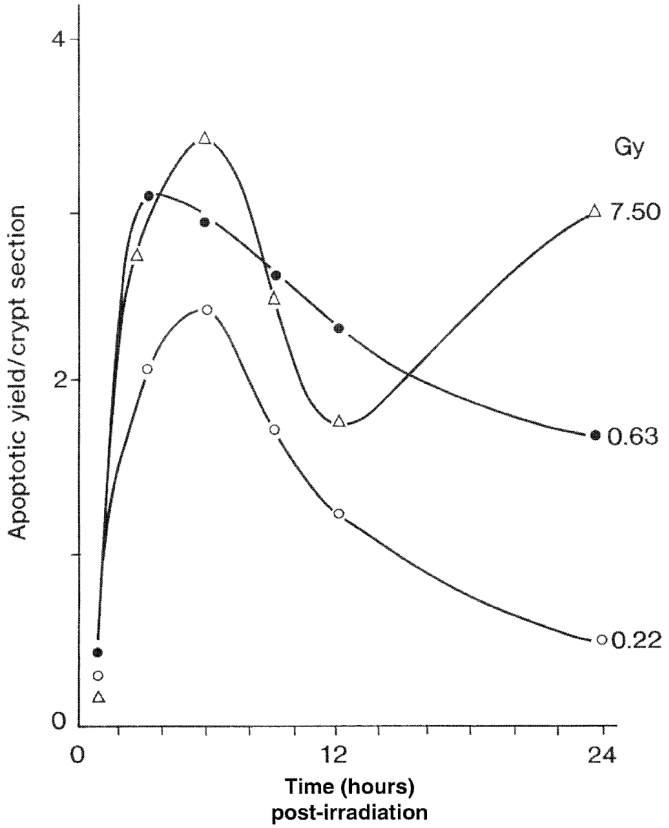


FIG. 5. The yield of apoptotic figures per crypt section as a function of time after exposure to low doses of radiation. The characteristic feature of these graphs is the rapid appearance of apoptosis after exposure and the return to baseline spontaneous levels of apoptosis by about 24 h. Reproduced with permission from Cambridge University Press (*38*).

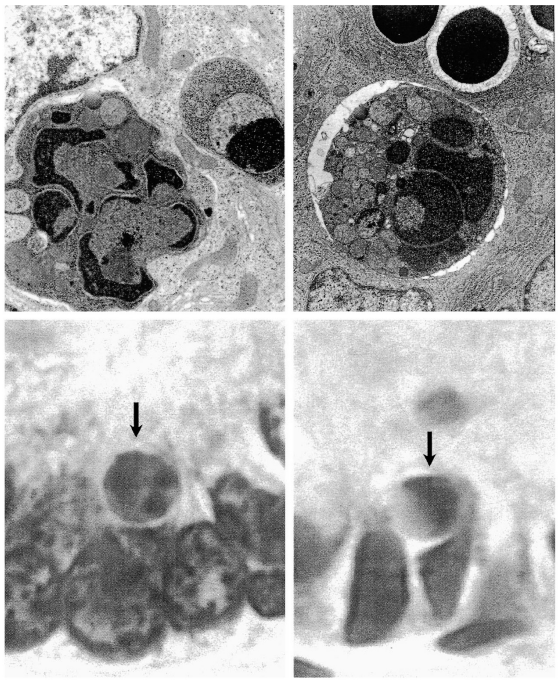


FIG. 4. Small doses of radiation rapidly trigger the morphological changes characteristic of apoptosis in a small number of cells located primarily at the position of the stem cell. These can be identified by the classical morphological changes as seen in transmission electron micrographs, (upper pictures left) an early stage of apoptosis and (right) a later stage showing an apoptotic fragment engulfed in a phagosome. In good-quality hematoxylin and eosin-stained sections, these apoptotic cells can easily be identified (lower panels) using appropriate high-quality light microscopy, and if the sections are correctly oriented in relation to the crypt villus axis, these can be quantified for occurrence against each individual cell position in a crypt section. Lower panels show H&E-stained sections with apoptotic bodies. The lower pictures are reproduced from ref. (*38*) with permission from Cambridge University Press.

Christopher S. Potten Radiation Research 2004

The radiation sensitive cells are found around position +4

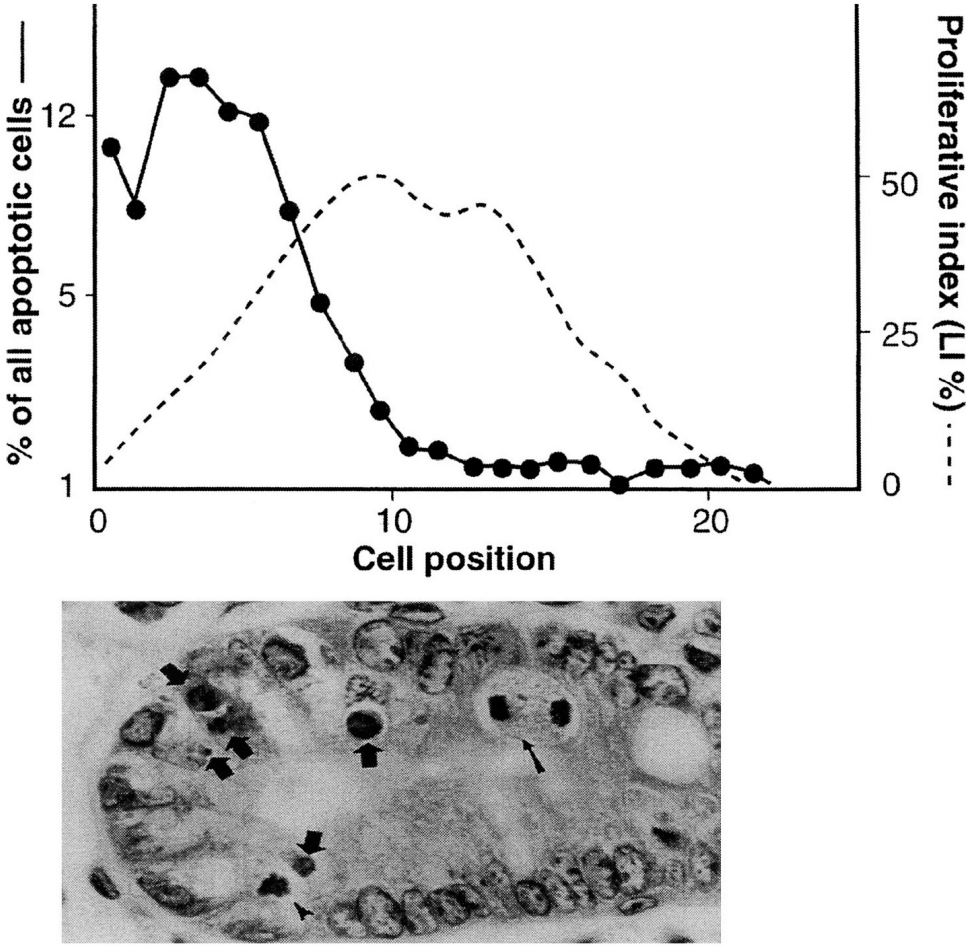


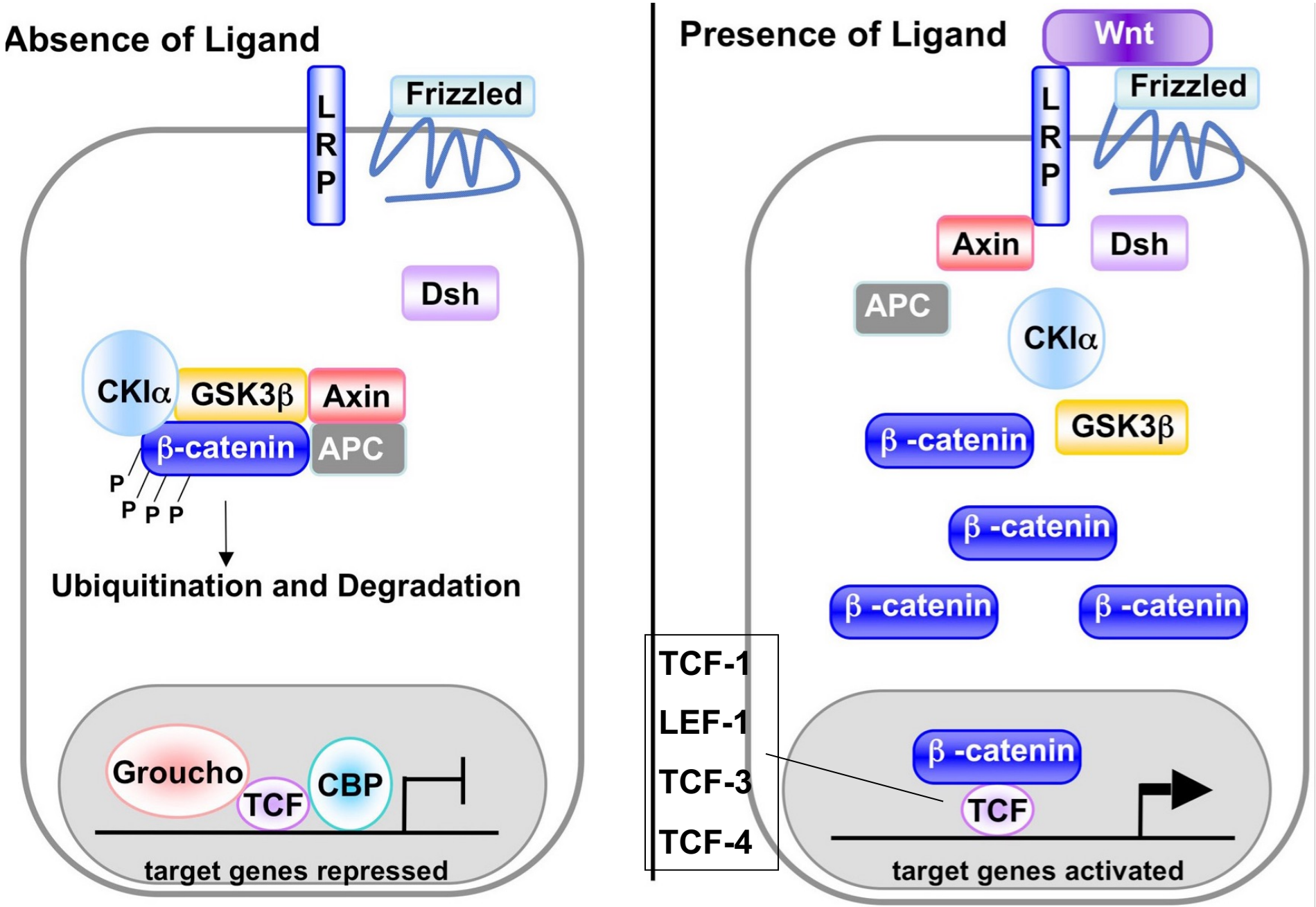
FIG. 6. When the yield of apoptosis at 3–6 h postirradiation is analyzed on the basis of cell position, it can be seen from the closed-circle graph that peak yields occur at cell positions 4–5 from the base of the crypt, i.e. the stem cell positions. Reproduced from ref. (*38*) with permission from Cambridge University Press. The lower photomicrograph shows a crypt section with several apoptotic cells (large arrows) and some mitotic figures (small arrows and arrowhead).

Conclusions

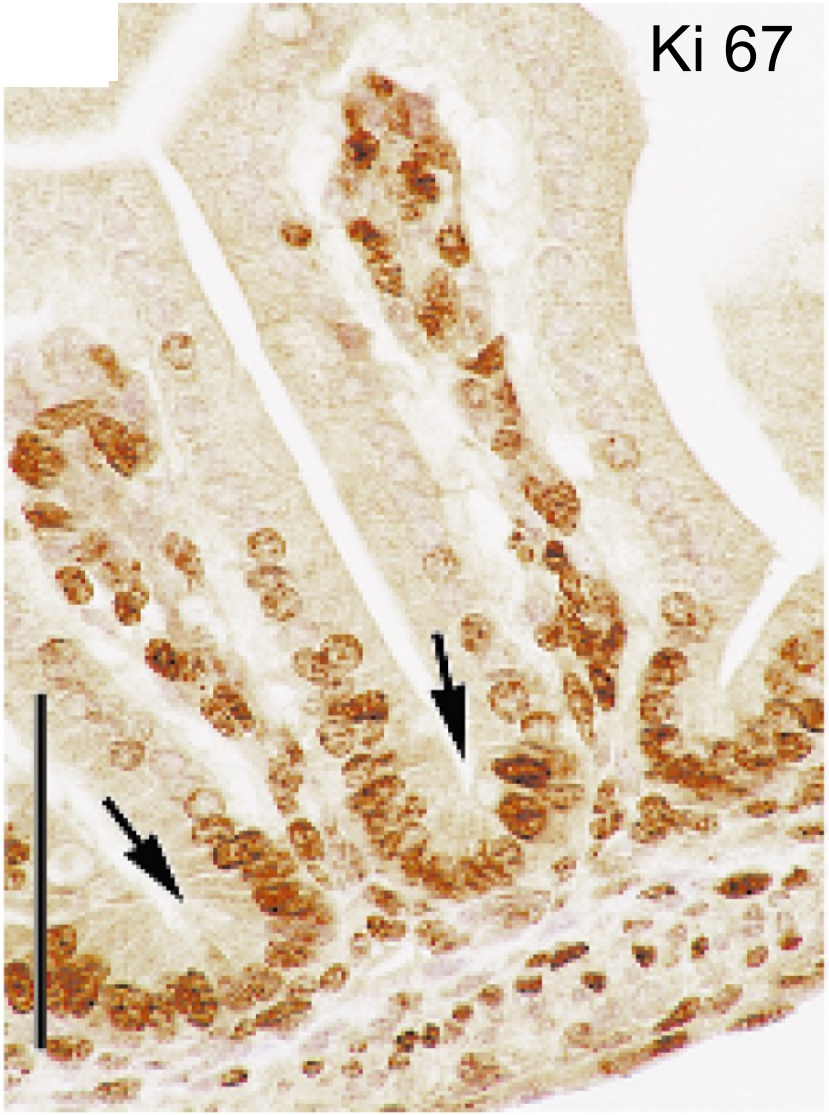
- Stem cells reside at position +4 (label retaining assay)

- The small intestine does not develop tumors because stem cells undergo apoptosis as soon as they acquire mutations.

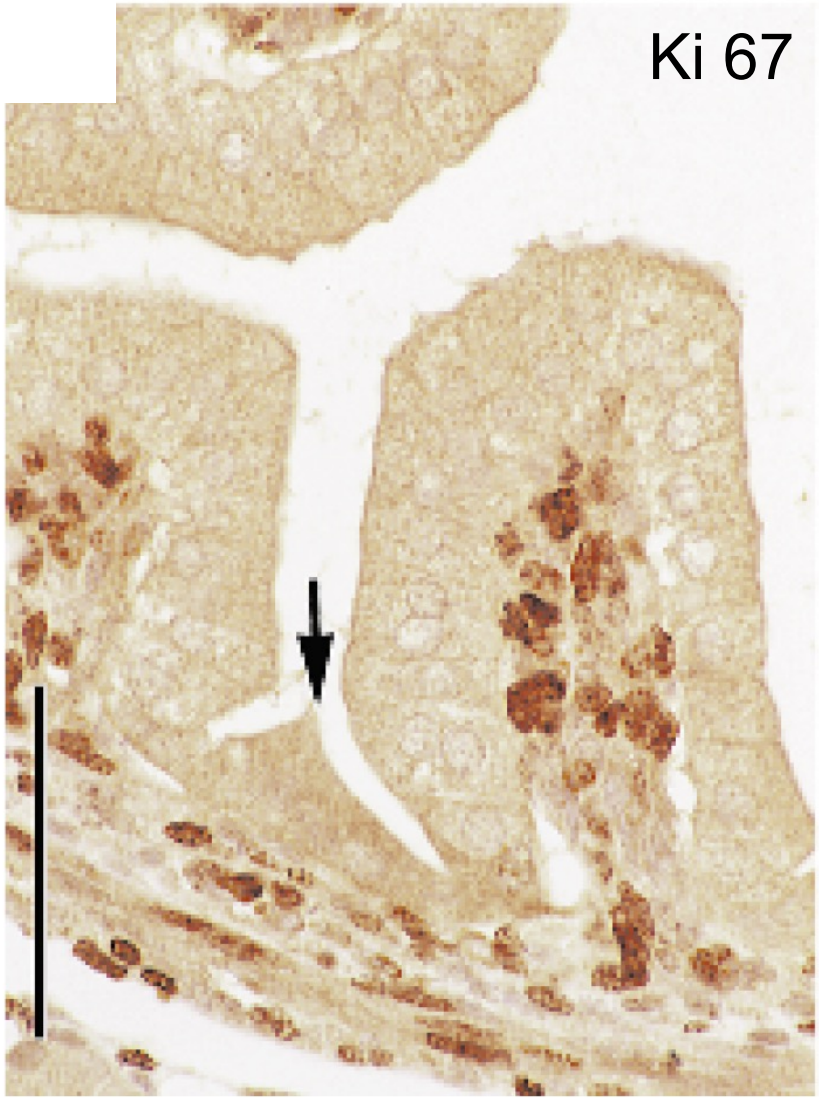
Hans Clevers


Absence of Ligand
LRP
Frizzled
Dsh
CKIα
GSK3β
Axin
β-catenin
APC
P
P P P
Ubiquitination and Degradation
Groucho
TCF
CBP
target genes repressed
Presence of Ligand
Wnt
LRP
Frizzled
Axin
Dsh
APC
CKIα
β -catenin
GSK3β
β -catenin
β -catenin
β -catenin
TCF-1
LEF-1
TCF-3
TCF-4
β -catenin
TCF
target genes activated

TCF4+/-

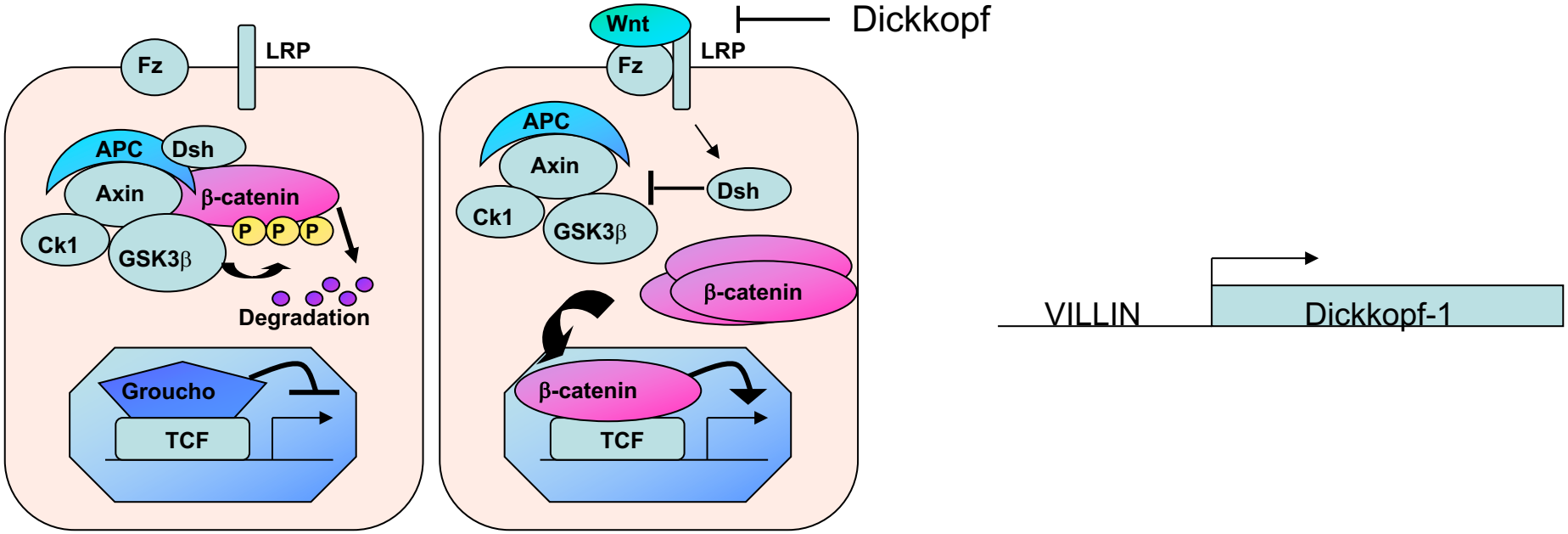


TCF4-/-

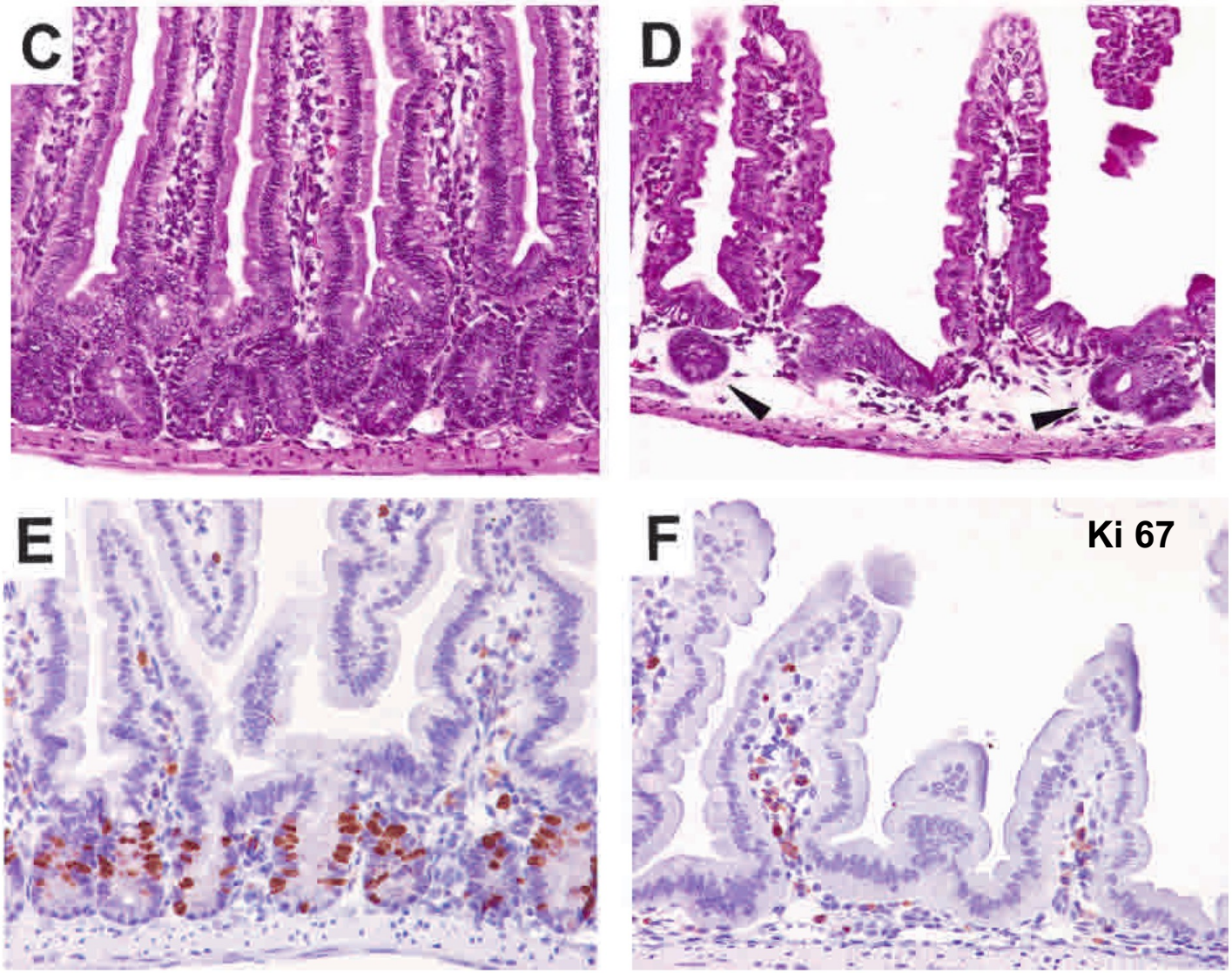


V. Korinek et al. Nat. Gen. 1998

Wnt Signalling is necessary for the maintenance of the Crypt Compartment

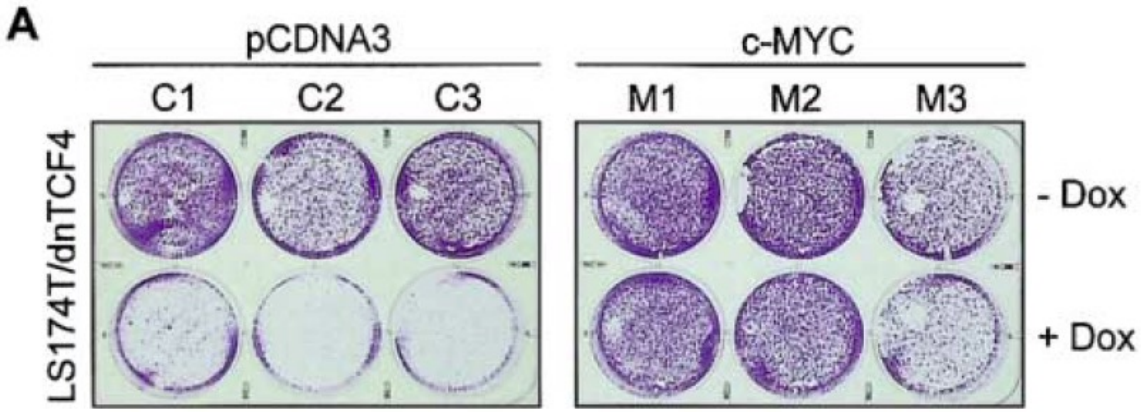


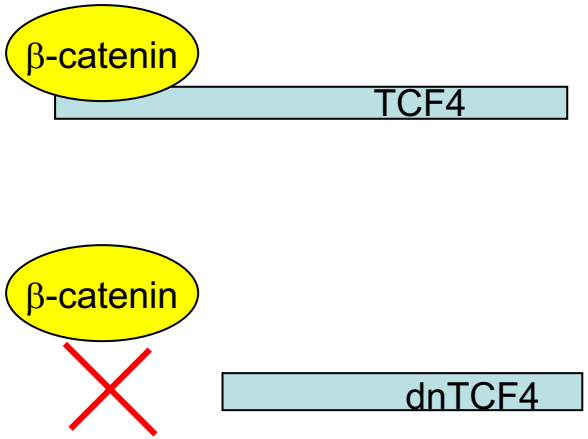
Overexpression of DKK1 results in the loss of the proliferative crypt compartment of the intestine

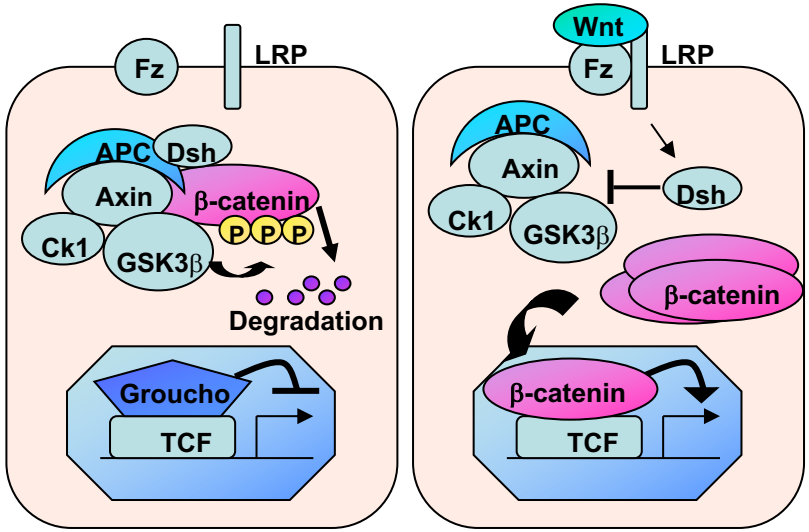


Daniel pinto et al. G&D 2003

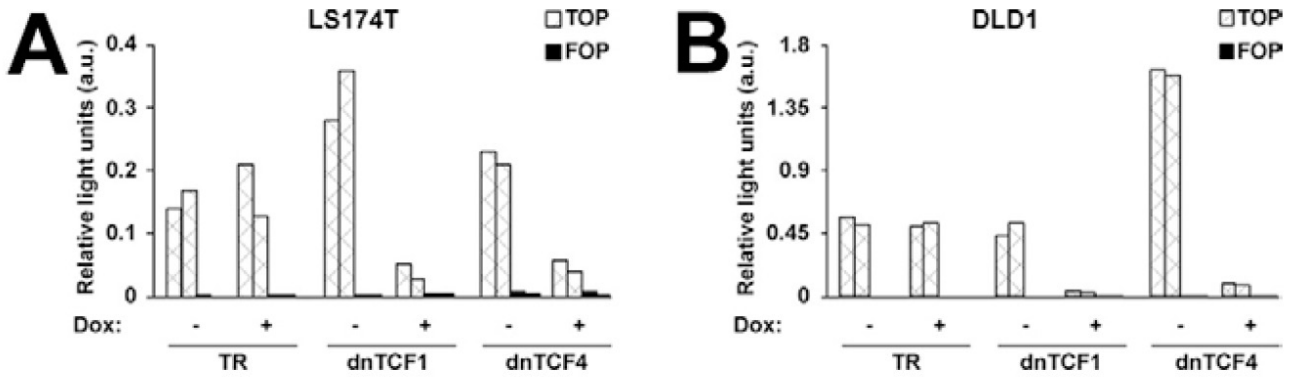
C-Myc represses p21 CIP1/WAF1 expression in LS174T

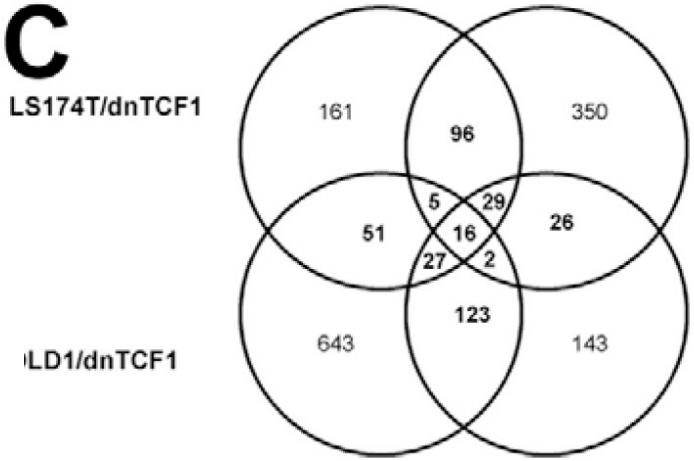


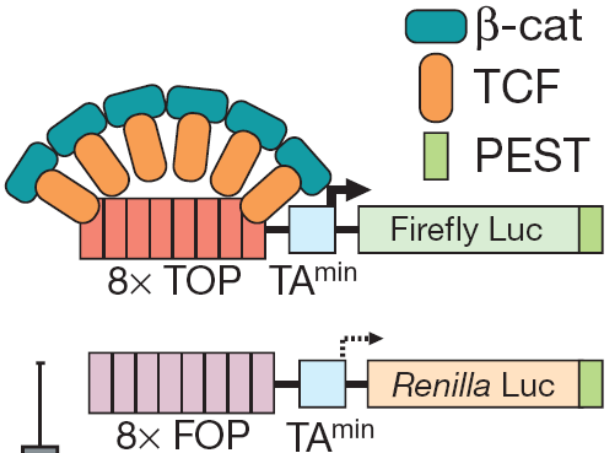


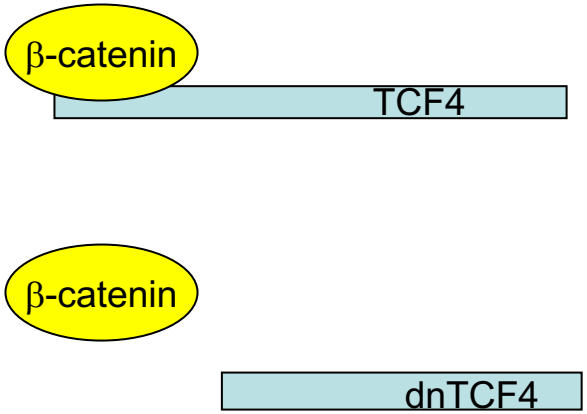


Identification of TCF/Wnt target genes ?



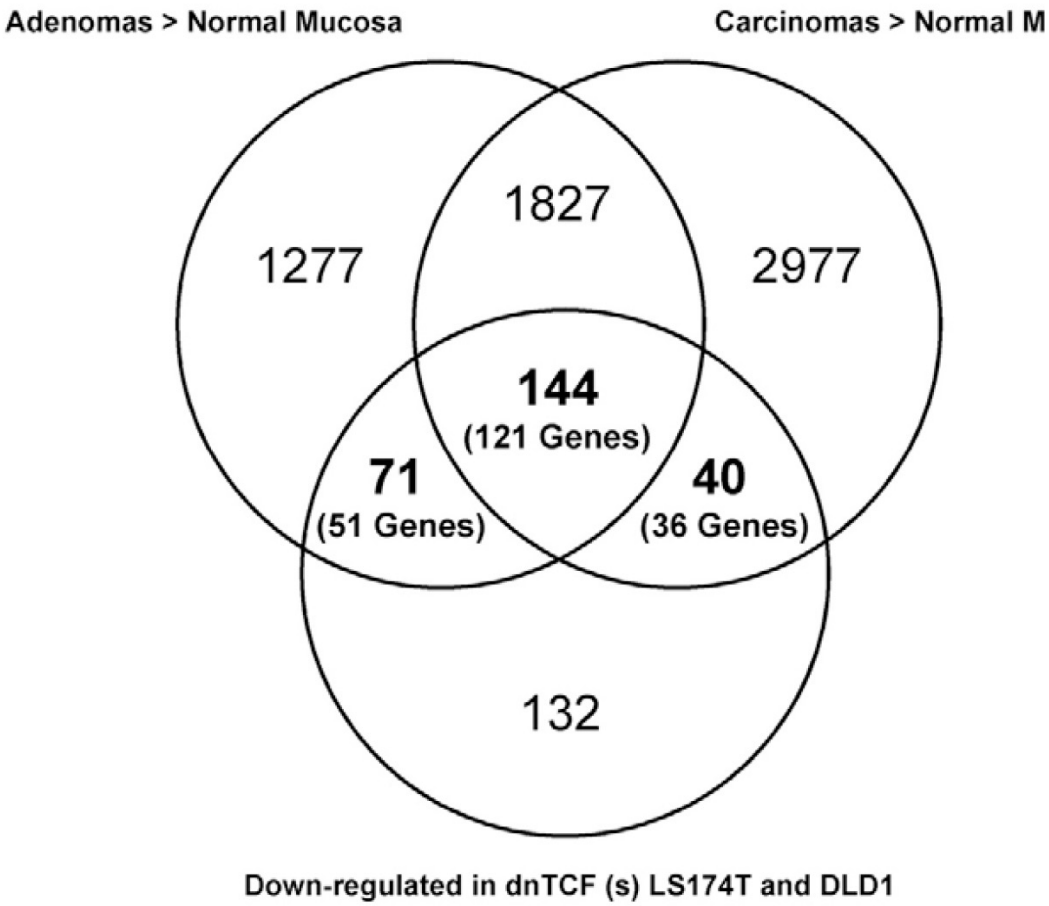






Van der Flier et al. Gasteroenterology2007,132; 628-632

Confirmation by in situ hybridization that TCF target genes are indeed expressed in both the crypt and adenomas of APC min mice



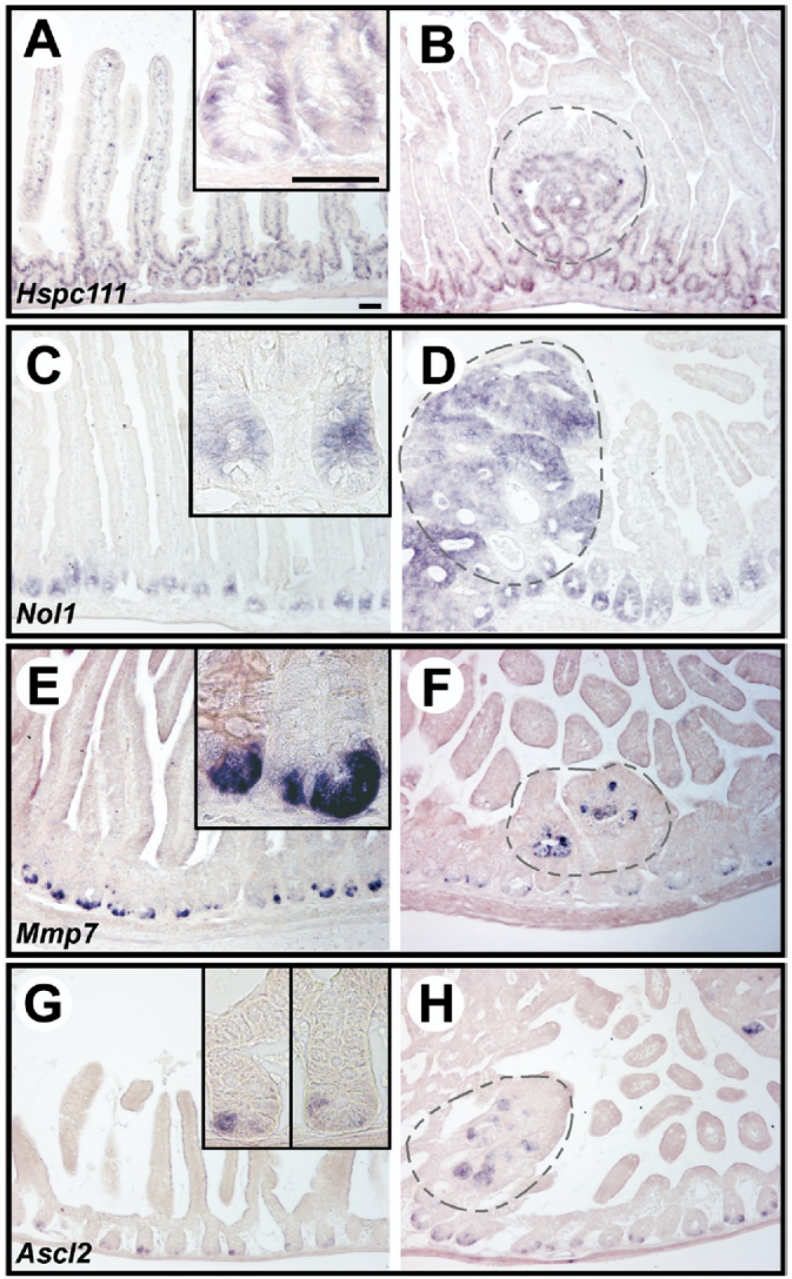
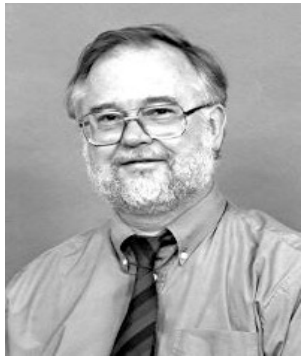


Table 1. Target Genes Down-Regulated in All 4 CRC Cell Lines on Over Expression of dnTCFs

			LS174T dnTCF1		LS174T dnTCF4		DLD1 dnTCF1		DLD1	
Gene symbol	References	Affymetrix ID	10 h	20 h	10 h	20 h	10 h	20 h	10 h	20 h
ASCL2	[18]	229215_at	−4.3	−24	−2.3	−15	−2.8	−7.5	−3.0	−4.0
AXIN2	[14–16]	222696_at	−2.5	−4.9	−2.3	−2.6	−2.8	−3.5	−2.8	−3.7
BMP4[a]	[21]	211518_s_at	−2.0	−2.5	−2.3	−4.9	−3.0	−3.2	−1.6	−2.3
C1orf33[a]		220688_s_at	−1.7	−3.2	−2.3	−2.8	−1.7	−2.0	−1.6	−2.0
HIG2	[20]	1554452_a_at	−1.7	−4.0	−2.1	−3.2	−1.4	−2.6	−1.9	−2.0
HSPC111		203023_at	−1.5	−3.0	−2.3	−2.5	−1.4	−1.6	−1.9	−1.7
HSPC111		214011_s_at	−1.6	−3.0	−2.3	−2.3	−1.5	−1.9	−1.6	−1.6
KITLG[a]		226534_at	−2.6	−3.0	−2.3	−2.3	−4.3	−2.6	−1.6	−2.8
LGR5[a]	[19]	213880_at	−4.3	−9.8	−2.3	−3.5	−7.5	−7.5	−2.1	−3.2
MYC[a]	[17]	202431_s_at	−2.1	−2.3	−2.3	−1.6	−2.8	−3.0	−2.1	−2.3
NOL1		214427_at	−1.7	−2.8	−2.3	−2.1	−1.3	−1.9	−1.6	−1.6
PPIF		201490_s_at	−1.4	−1.6	−2.3	−2.0	−1.5	−1.9	−1.5	−1.5
SOX4	[22]	201416_at	−1.3	−2.1	−2.3	−2.1	−3.0	−3.2	−2.1	−2.0
WDR71		218957_s_at	−2.8	−17	−2.3	−2.8	−1.9	−3.7	−2.0	−3.7
ZIC2		223642_at	−1.7	−2.3	−2.3	−2.3	−1.4	−2.1	−1.7	−1.9
ZNRF3[a]		226360_at	−4.6	−3.2	−2.3	−2.5	−3.5	−3.2	−2.1	−2.8

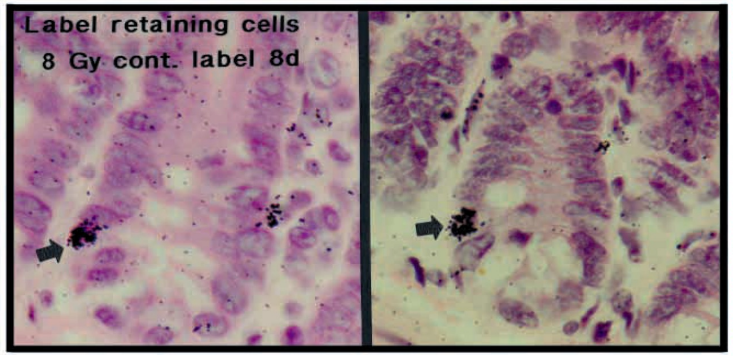
NOTE. Fold changes of genes down-regulated in all 4 CRC cell lines on over expression of dnTCFs are shown.
[a]Genes also have been identified in the Stanford array experiment.[8]



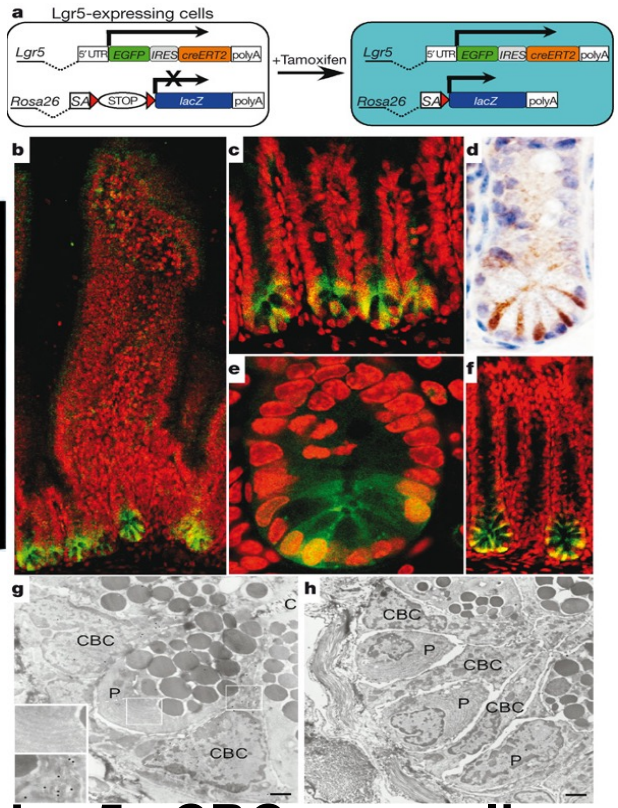
Christopher Potten

Hans Clevers

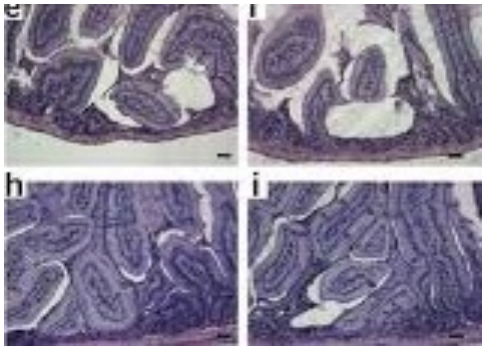
Mario Capecchi



Postulated +4 cells as potential stem cells based on LRC



Lgr5+ CBC are cycling stem cells (based in lineage tracing)

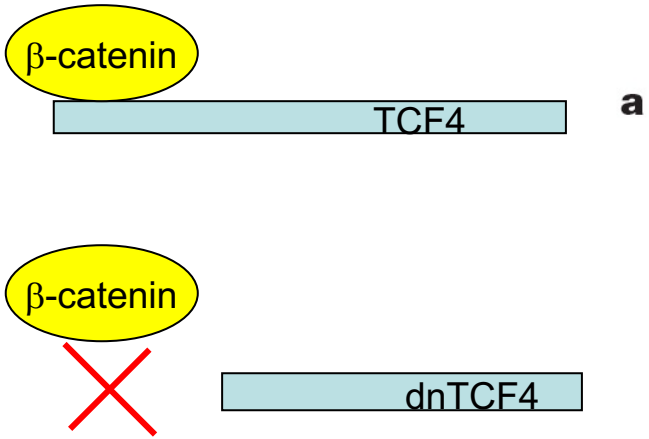


Bmi1+ localize at position +4 are stem cells based on lineage tracing and DT ablation experiments

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



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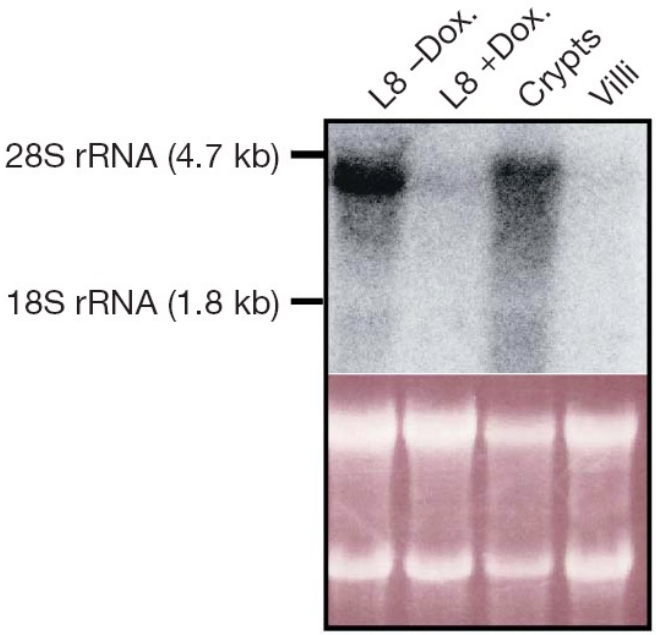
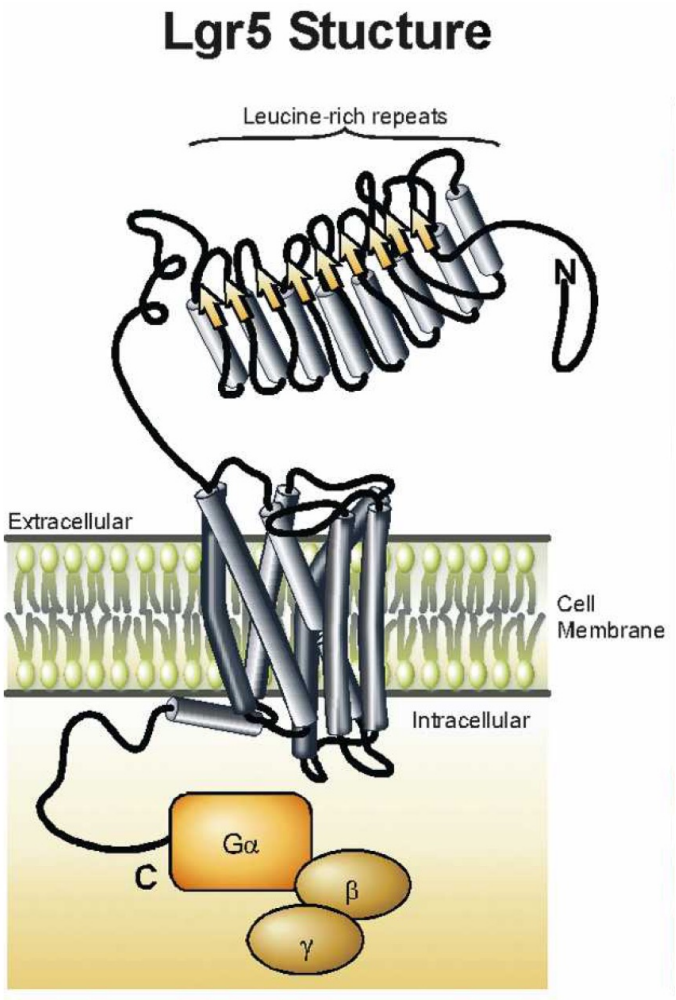
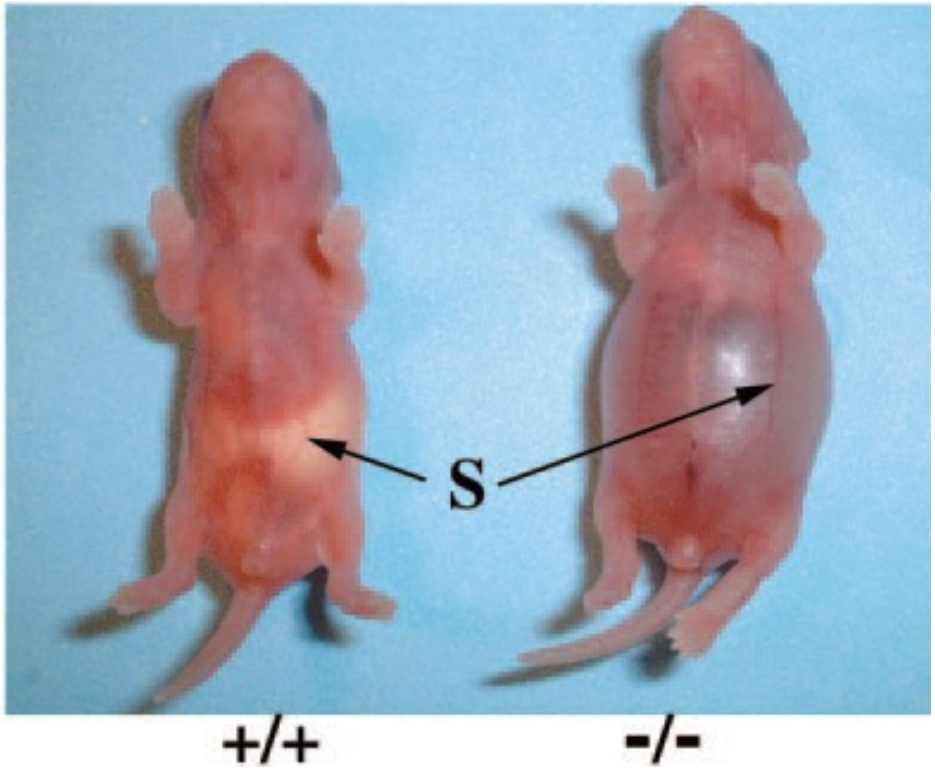


Northern blot analysis showing that Lgr5 is responsive to wnt signaling and is only expressed in the crypt

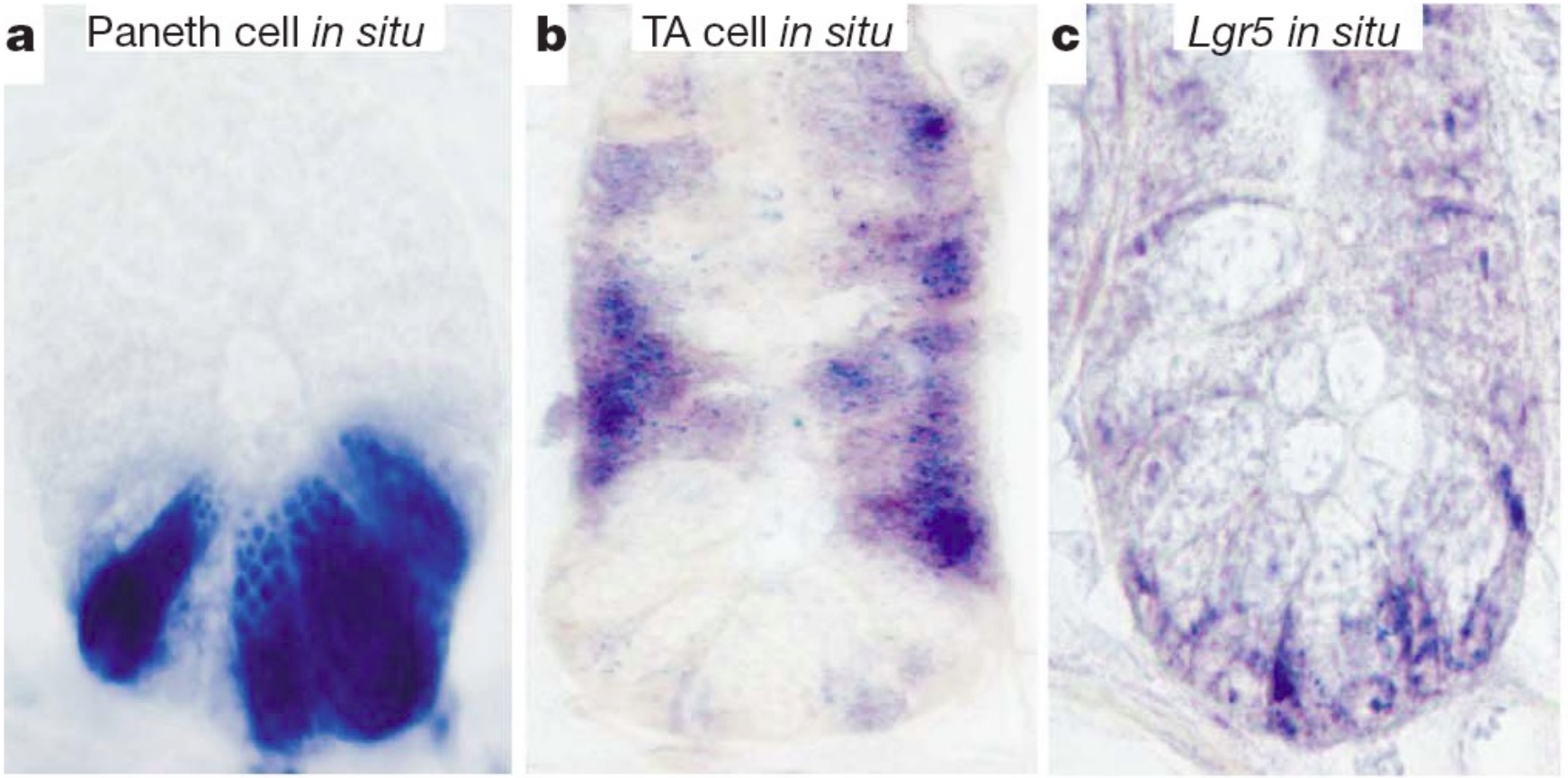
Lgr5/Gpr49 is an orphan G-protein coupled receptor, containing 7 TM-domains and a leucine-rich extracellular ligand binding domain



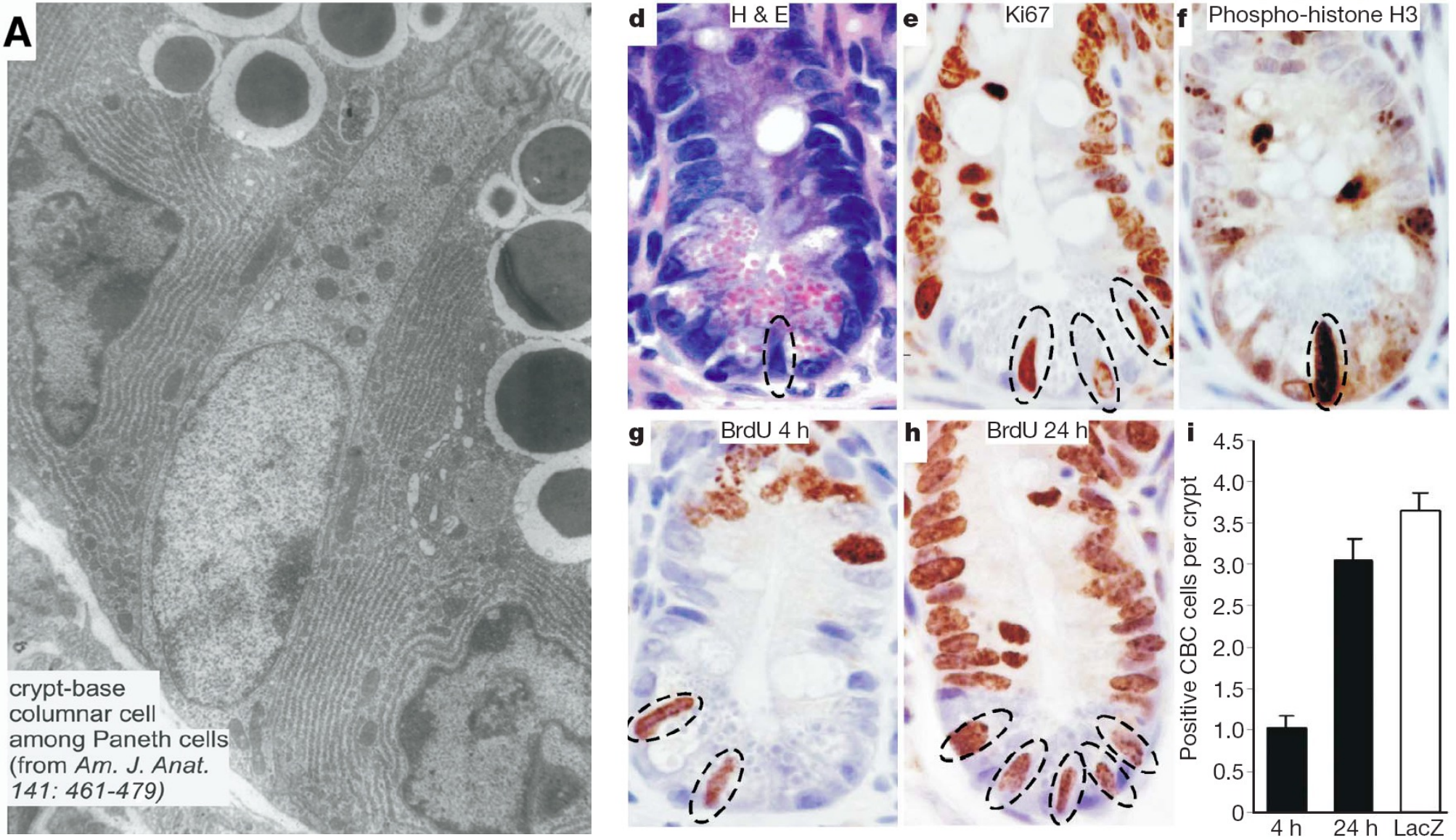
LGr5-/- mice have a malformation of the tongue and lower jaw, which causes newborns to swallow large amounts of air, leading to neonatal death.



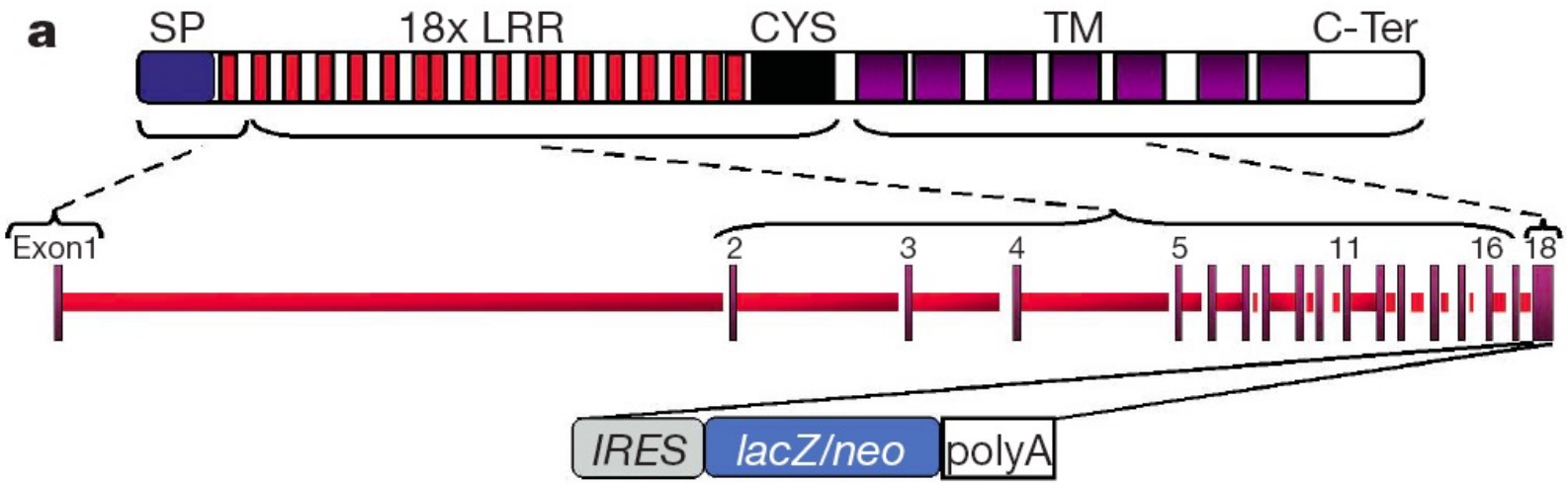
In situ hybridization of Cryptidin1(paneth cells), Wdr43 (TA-cells) and Lgr5 (crypt base columnar cells)



Crypt base columnar cells (CBC) are cycling



LacZ knock-in into the Lgr5 locus in order to follow expression pattern

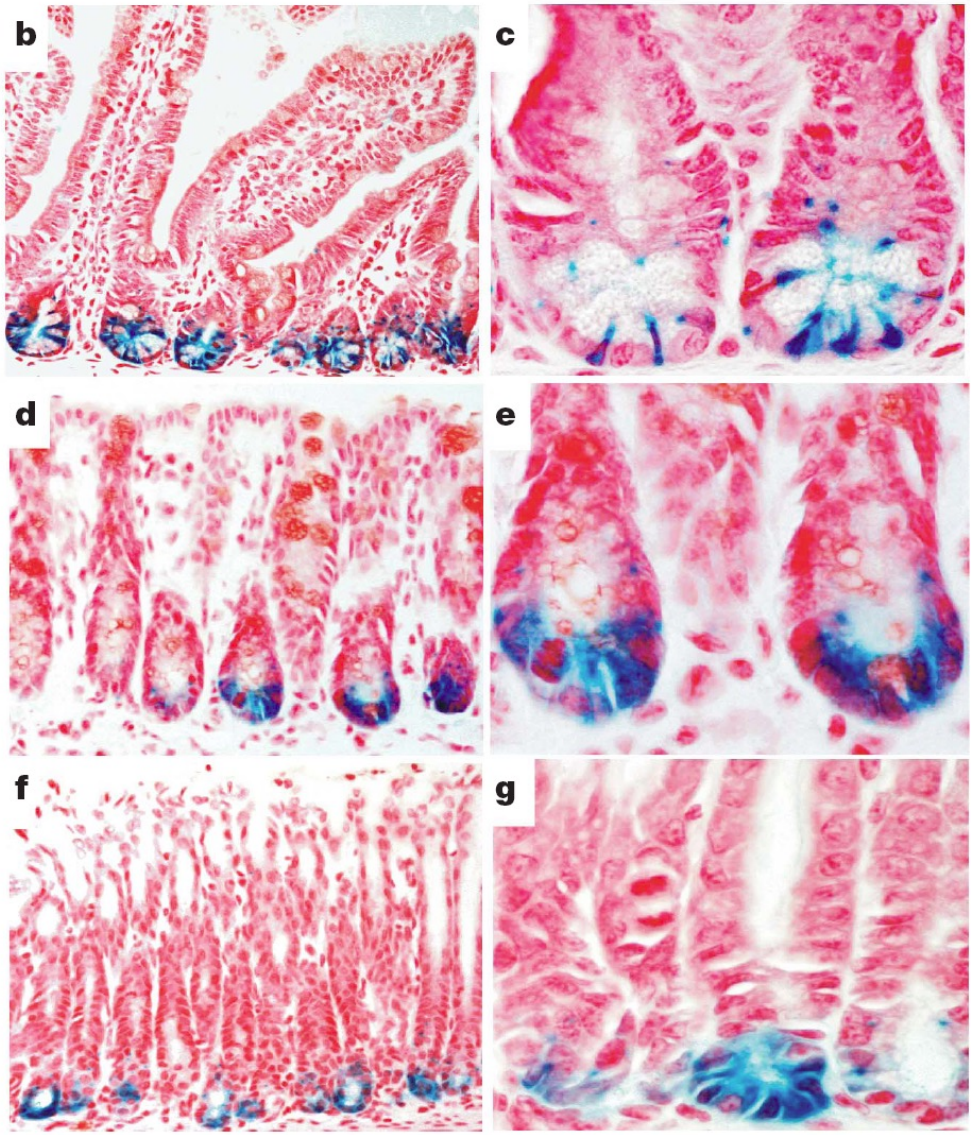


Expression pattern of Lgr-5-lacZ in selected mouse tissues shows restricted expression

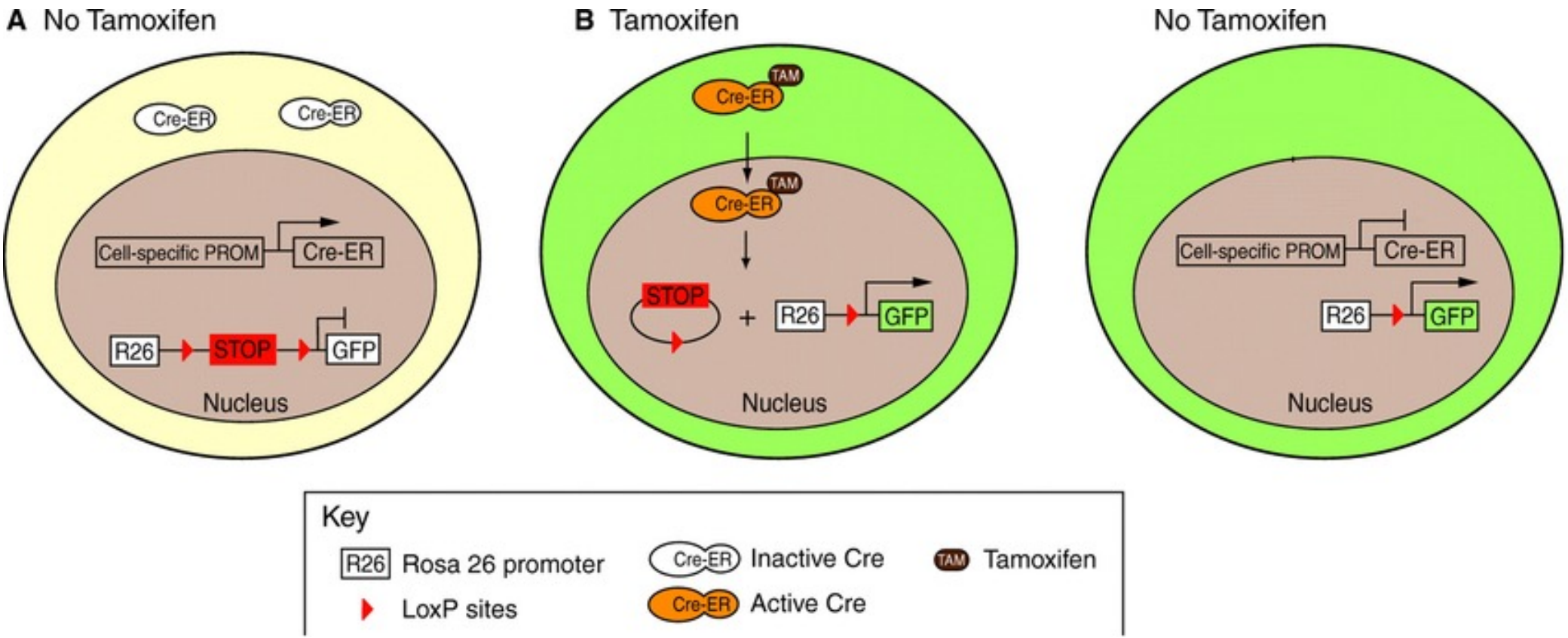
Small intestine

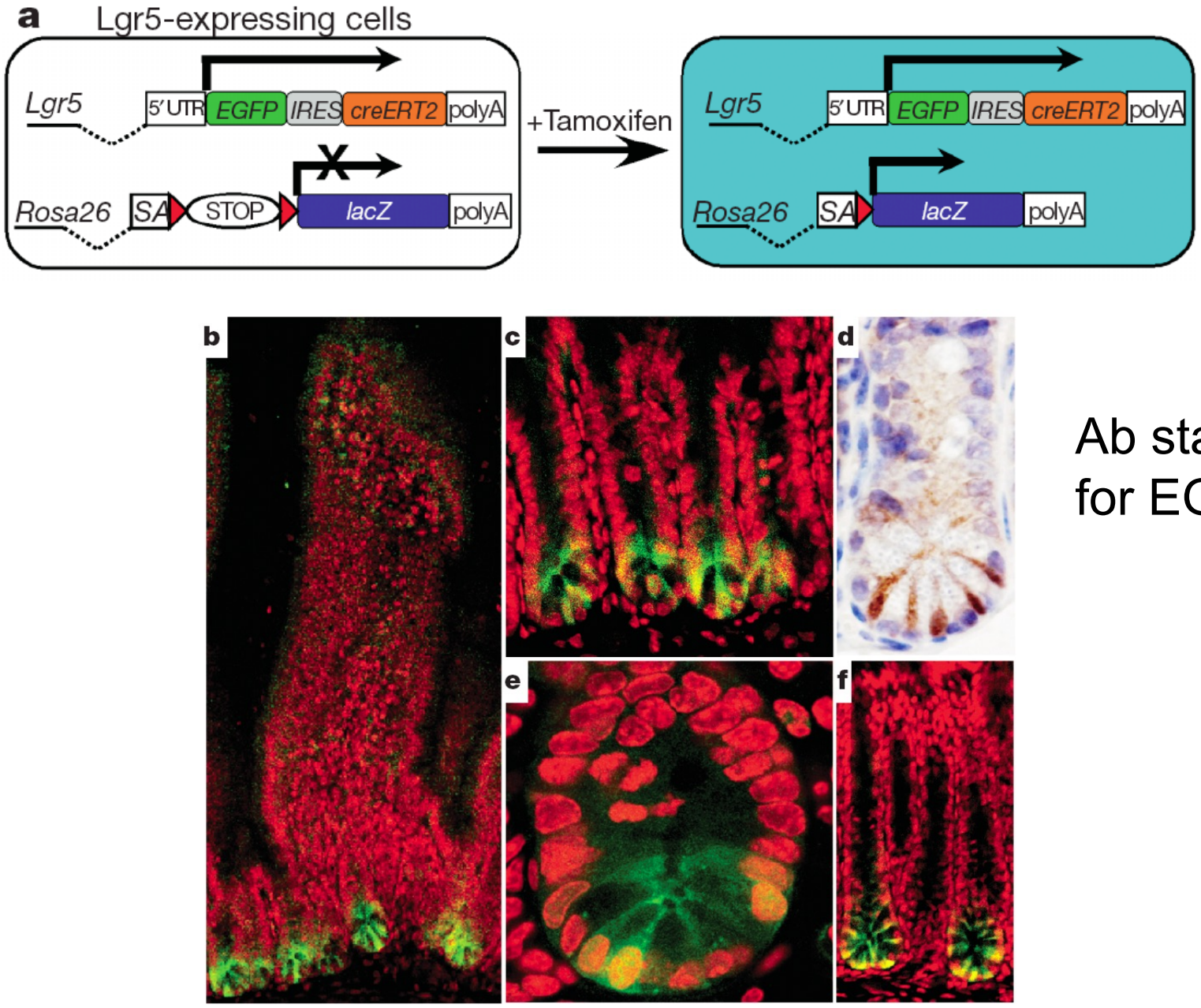
Colon

Stomach



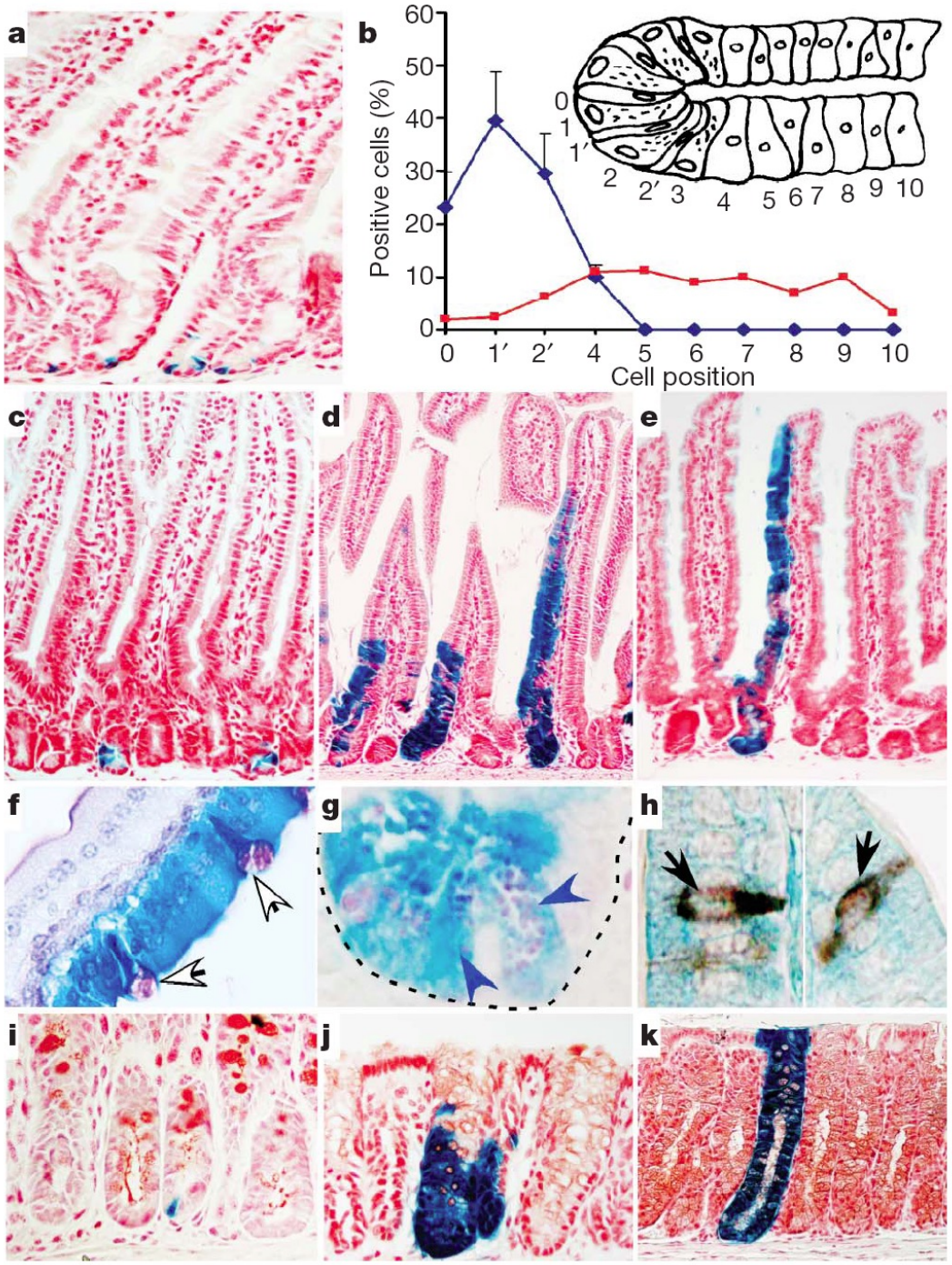
Principle of Cre-ERT-mediate lineage tracing





Ab staining for EGFP

12hrs after tamoxifen injection



Lineage tracing using the Lgr5-EGFP-Cre-ERT2 and RosaR26 mice support hypothesis that Lrg5+ cells are stem cells in the intestine and the colon

Small intestine 1, 5 and 60 days post tamoxifen injection

Double labelling, PAS for goblet and paneth cells and synaptophysin for enteroendocrine cells

Colon 1, 5 and 60 days post tamoxifen injection

- Lgr5 is a marker of Crypt base columnar cells (CBC)
- CBC are cycling (somewhat counterintuitive for stem cells)
- Lineage tracing experiments show that CBC give rise to all major cell lineages within the gut
- Lgr5 is also expressed in scattered cells in the eye, brain, hair follicles, reproductive organs, stomach and intestine.
- Lgr5 is also found in scattered cells of adenomas of APC min mice.

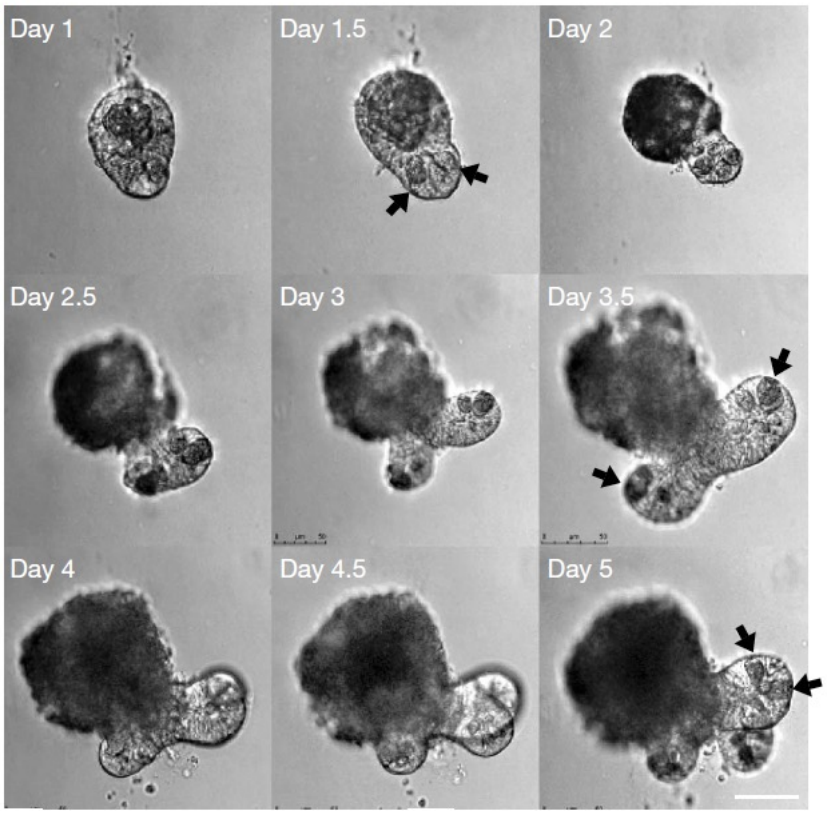
LGR5 seems to be a marker for intestinal stem cells, may be also for other stem cells, and possibly for cancer stem cells of certain tumors.

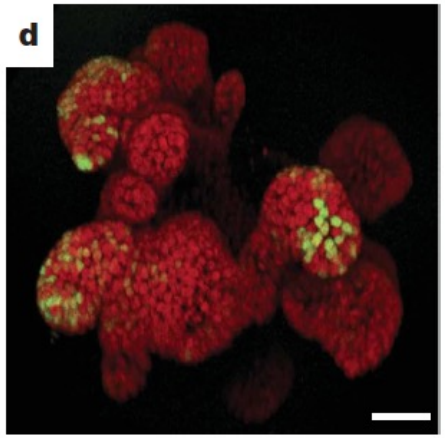

Clevers Lab|Digizyme

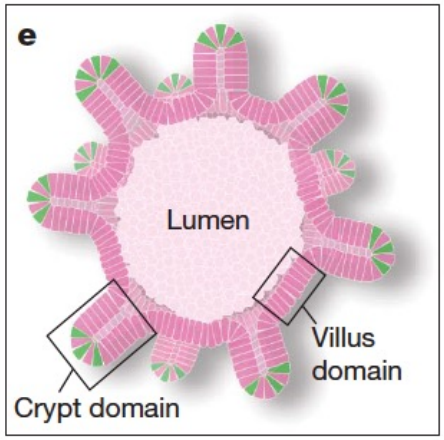
Single Lgr5 stem cells build crypt–villus structures *in vitro* without a mesenchymal niche

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







DMEM/F12+ Matrigel + EGF + R-spondin + Noggin

A single Lgr5+ cells can generate crypt-villus structure in vitro

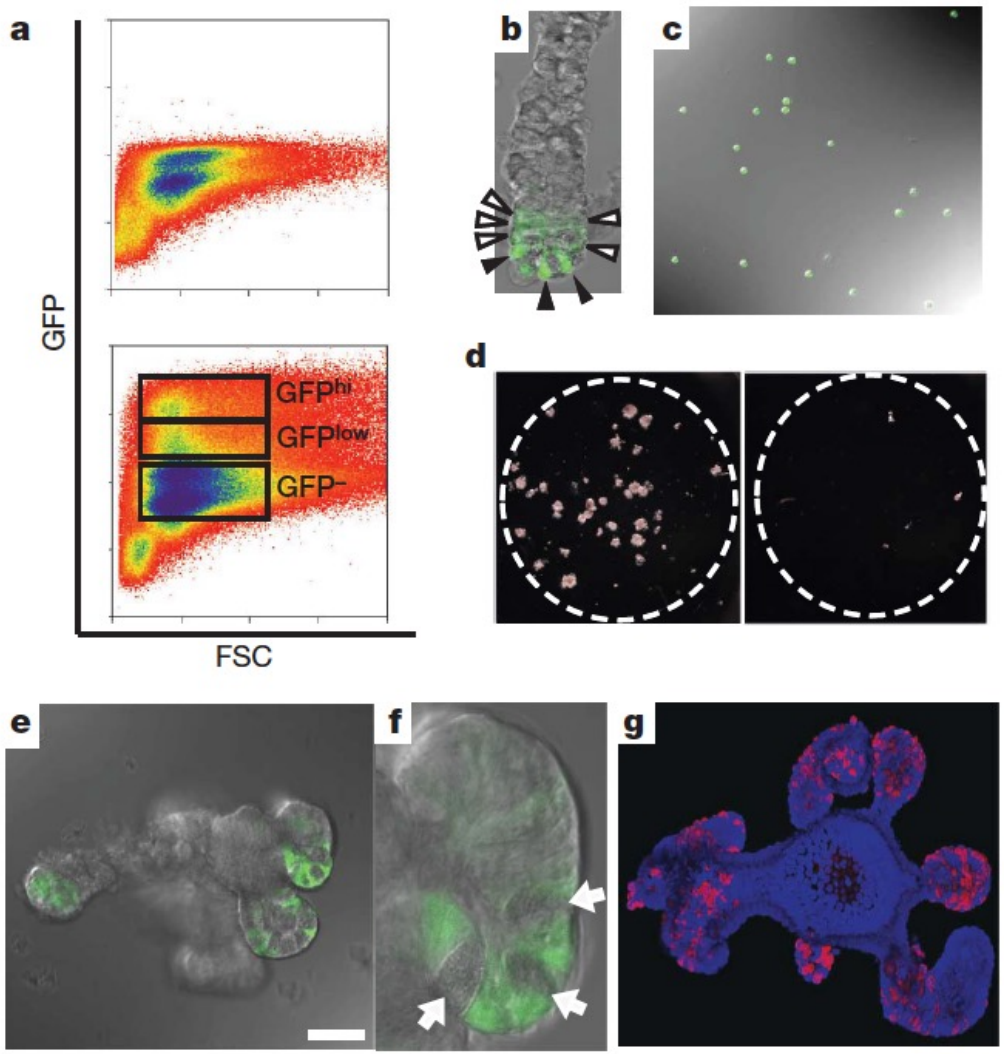
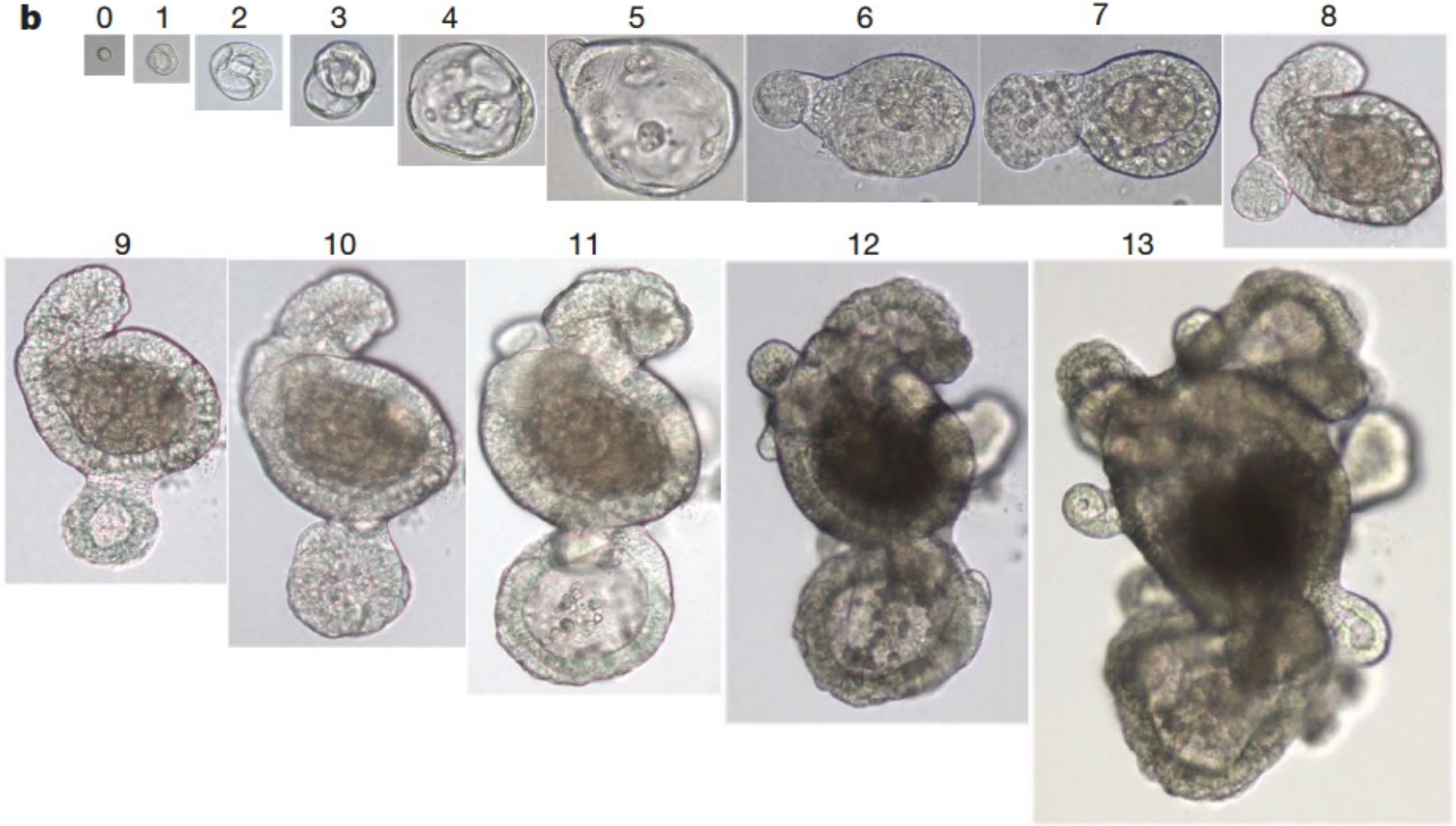


Figure 2 | Single Lgr5^{+} cells generate crypt–villus structures. **a**, Lgr5–GFP^{+} cells from an *Lgr5–EGFP–ires–CreERT2* intestine (bottom); wild-type cells (top). Two positive populations, GFP^{hi} and GFP^{low}, are discriminated. FSC, forward scatter. **b**, Confocal analysis of a freshly isolated crypt. Black arrowheads, GFP^{hi}; white arrowheads, GFP^{low}. **c**, Sorted GFP^{hi} cells. **d**, 1,000 sorted GFP^{hi} cells (left) and GFP^{low} cells (right) after 14 days in culture. **e**, **f**, Fourteen days after sorting, single GFP^{hi} cells form crypt organoids, with Lgr5–GFP^{+} cells and Paneth cells (white arrows) located at crypt bottoms. Scale bar, 50 μm. **f**, Higher magnification of **e**. **g**, Organoids cultured with the thymidine analogue EdU (red) for 1 h. Note that only crypt domains incorporate EdU. Counterstain, 4,6-diamidino-2-phenylindole (DAPI; blue).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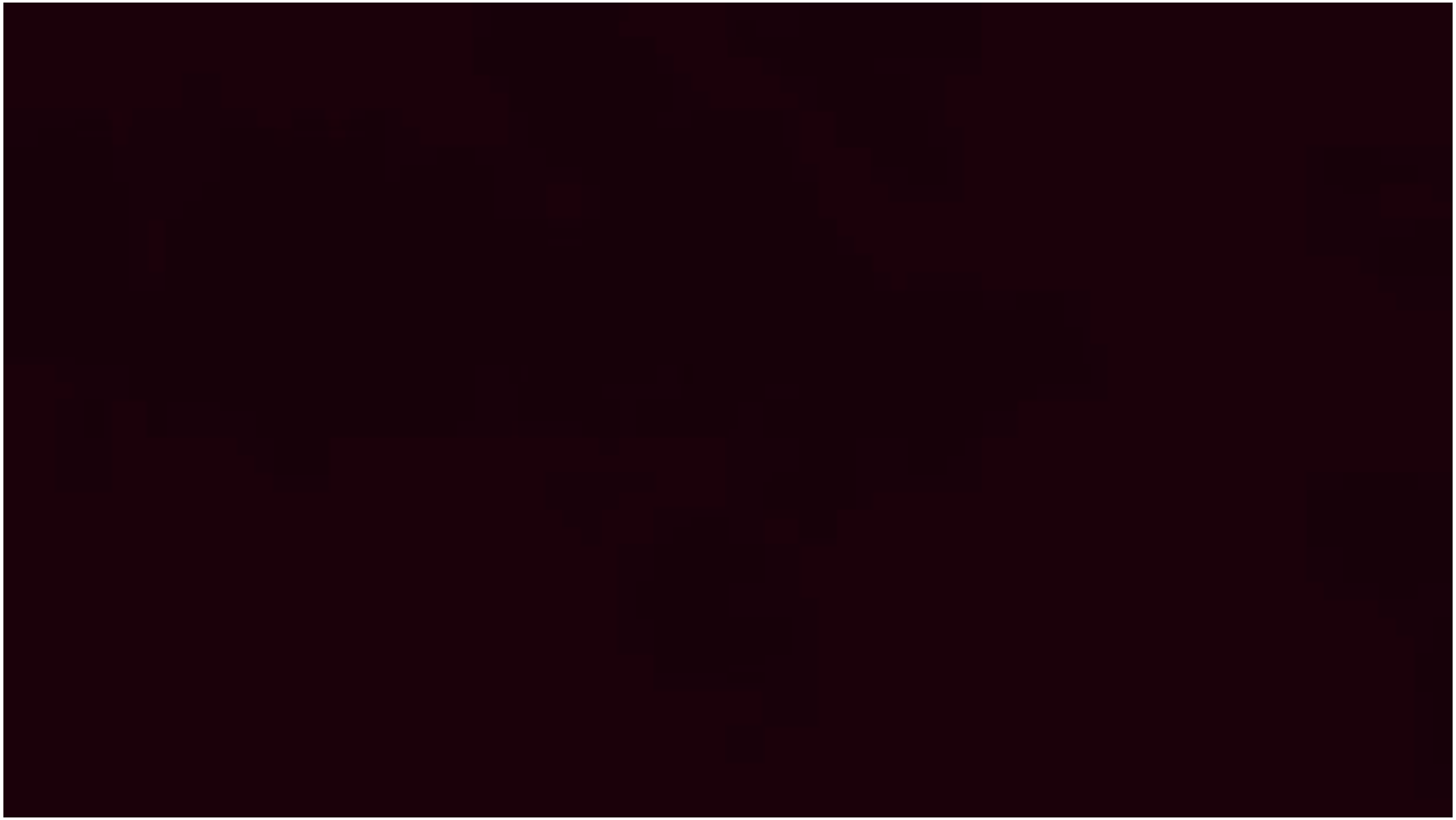


Mario Capecchi

LETTERS

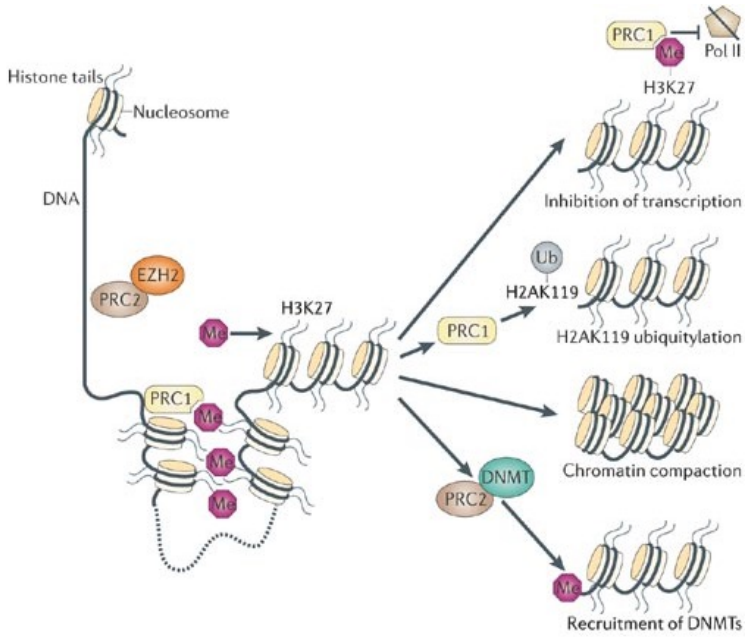
nature genetics

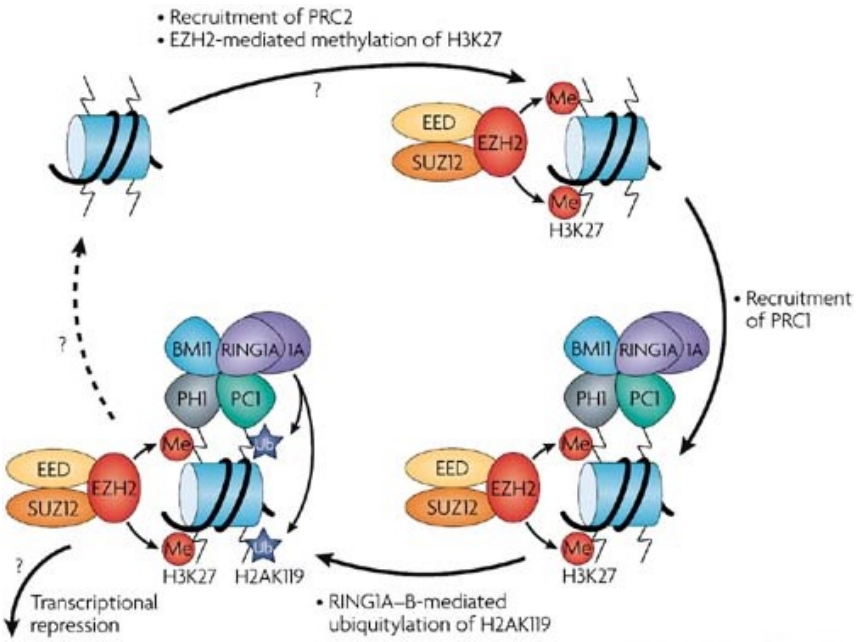
GENETICS VOLUME 40 | NUMBER 7 | JULY 2008

Bmi1 is part of the Polycomb protein epigentic modifier complex

Bmi1 is expressed *in vivo* in intestinal stem cells

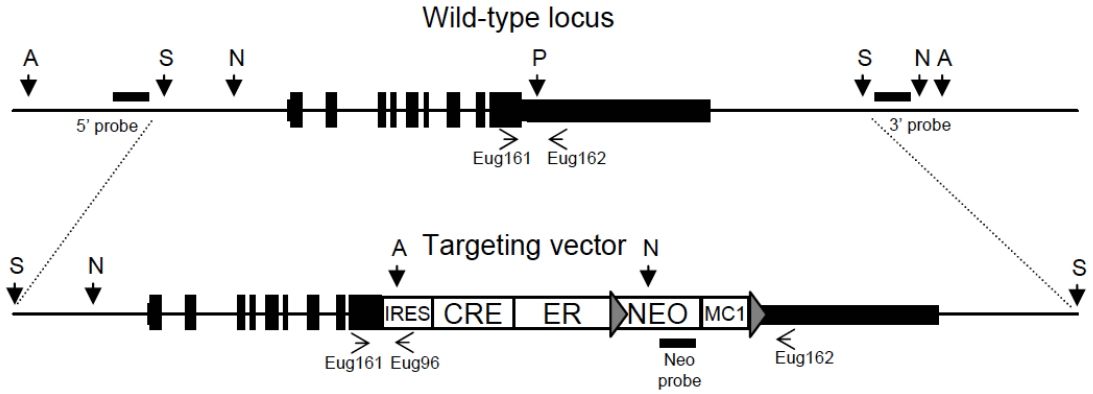
Eugenio Sangiorgi & Mario R Capecchi





In vivo lineage tracing experiment of Bmi1 identies intestinal stem cells

Genetic locus of Bmi1

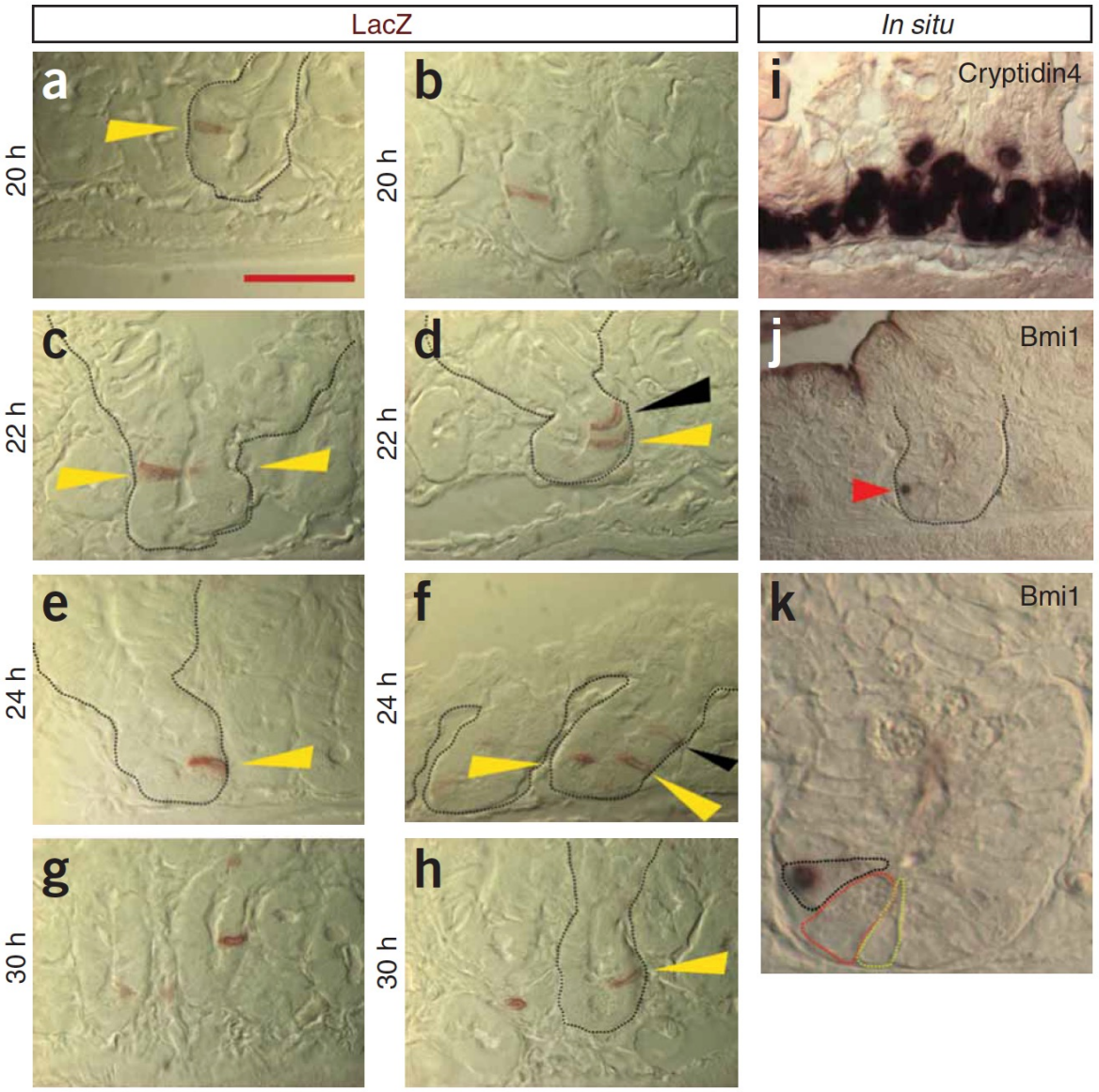


Genetically modified Bmi1 gene locus

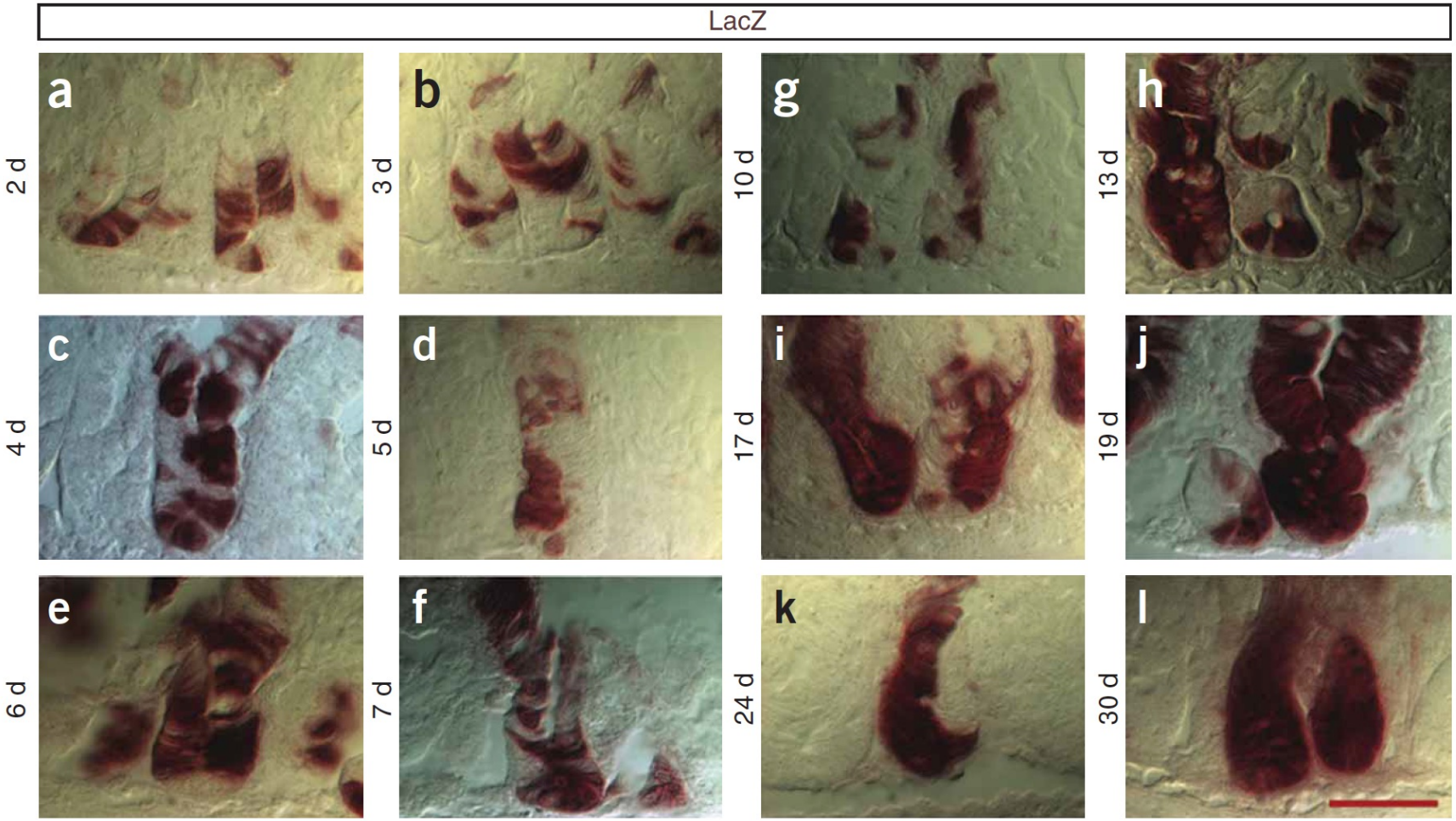
RosaR26 reporter mouse

Genetically modified Bmi1 gene locus RosaR26 reporter mouse

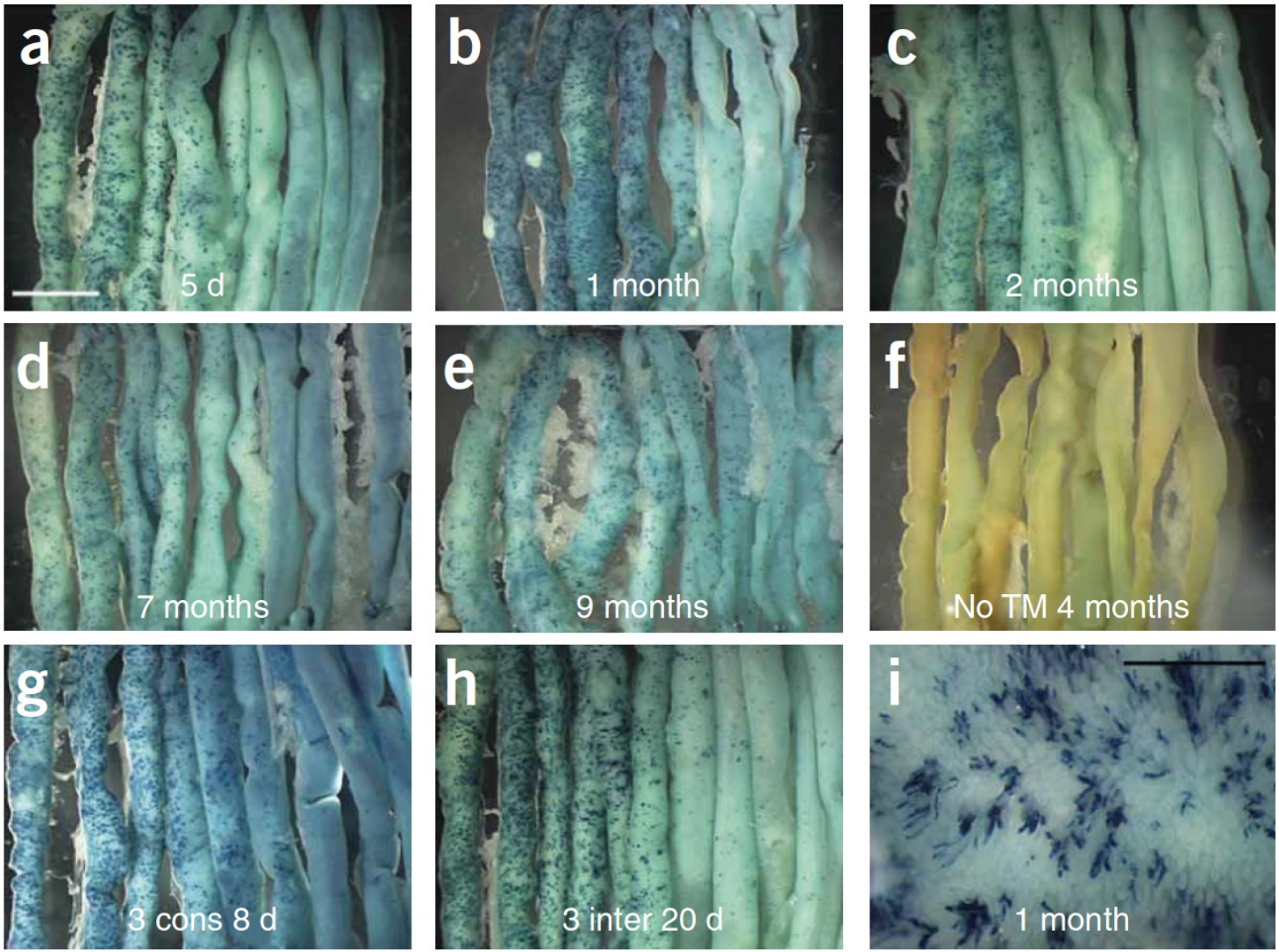
Early Bmi1+ lineage detection mark predominantly cells at position +4 and +5



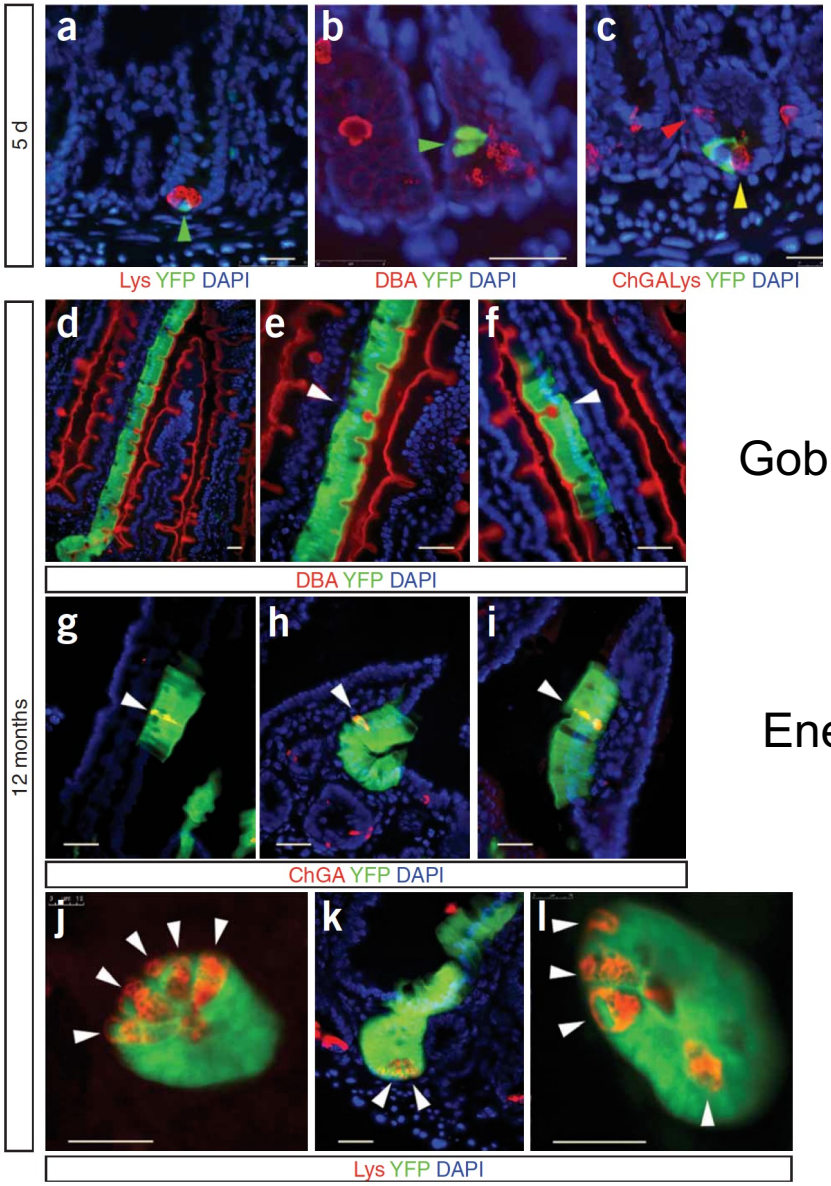
Repopulation kinetics of Bmi1+ lineage



Bmi1+ lineage analysis in the small intestines



Assessment of Bm1+ lineage after 5 days and 12 months to evaluate the colocalization with differentiated cell markers



Goblet cells

Enetroendocrine cells

Paneth cells

Genetically modified Bmi1-Cre-ERT gene locus

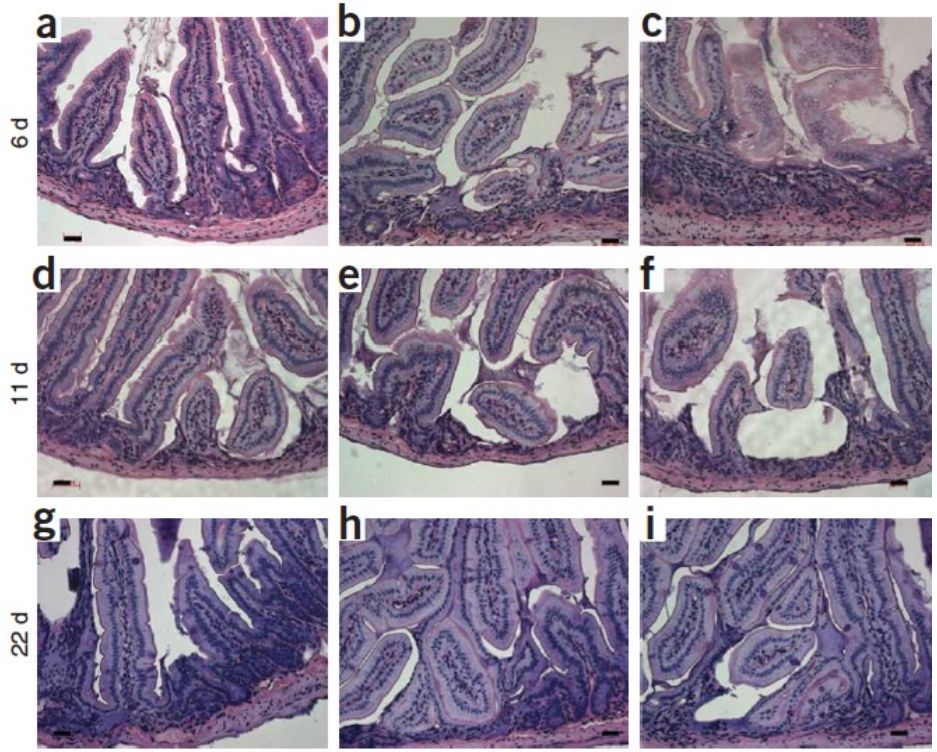


Figure 6 Intestinal stem cell ablation. After one injection of tamoxifen, *Bmi1*$^{CreER/+}$;*Rosa26*$^{DTA/+}$ mice were killed at different time points and their small intestine analyzed. (**a–i**) Representative pictures at 6, 11 and 22 d showing patches of intestinal mucosa disorganized and in disarray, without crypts. The ablation of the *Bmi1*$^{+}$ ISC lineage is responsible for the crypt loss. Scale bar, 50 μm.

What does all of that mean?

We seem to have two pools of intestinal stem cells:

- Slow cycling Bmi1+, position +4
- Fast cycling, Lgr5+, between Paneth cells

Is there a stem cell hierarchy?

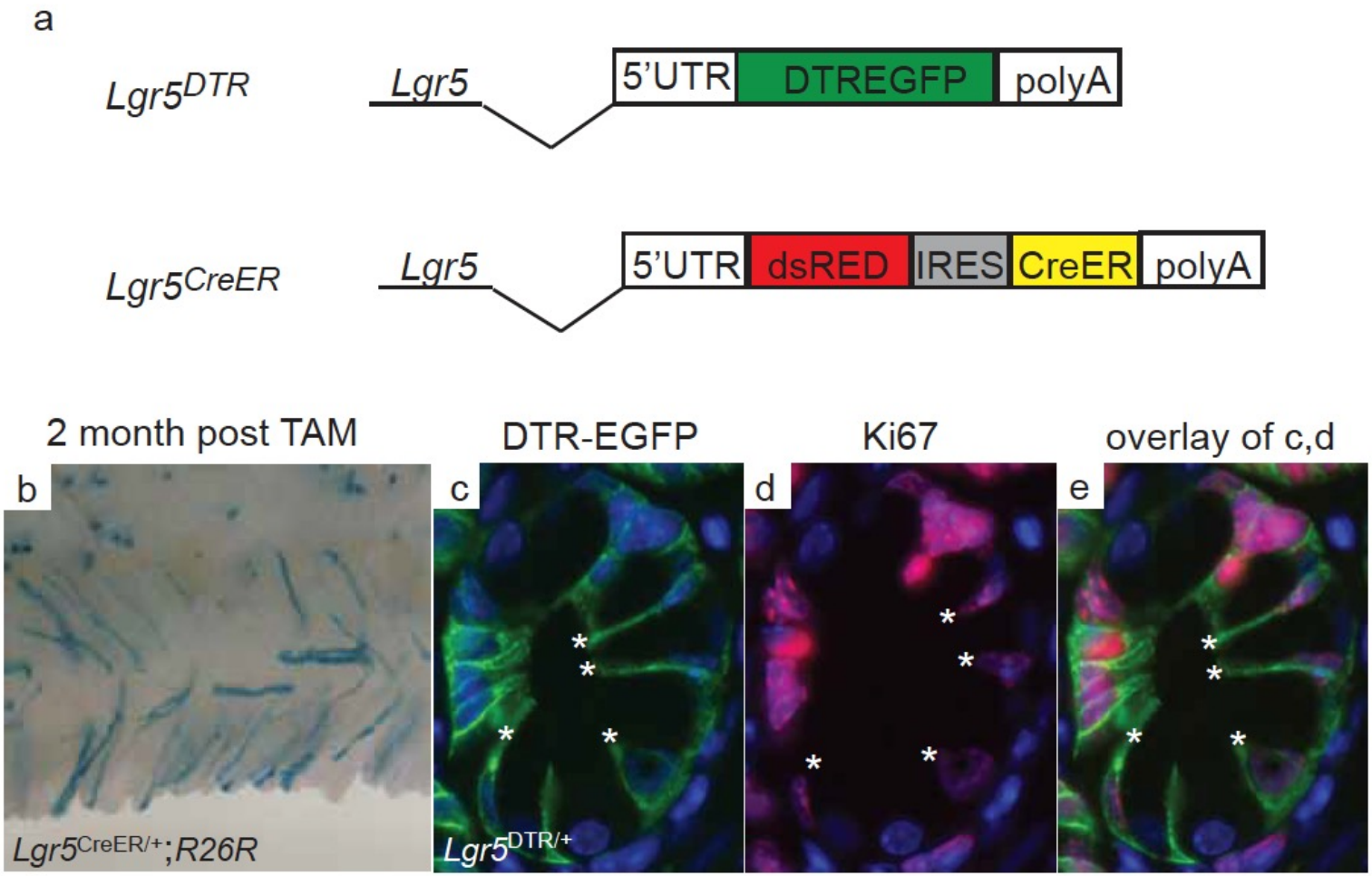
LETTER

doi:10.1038/nature10408

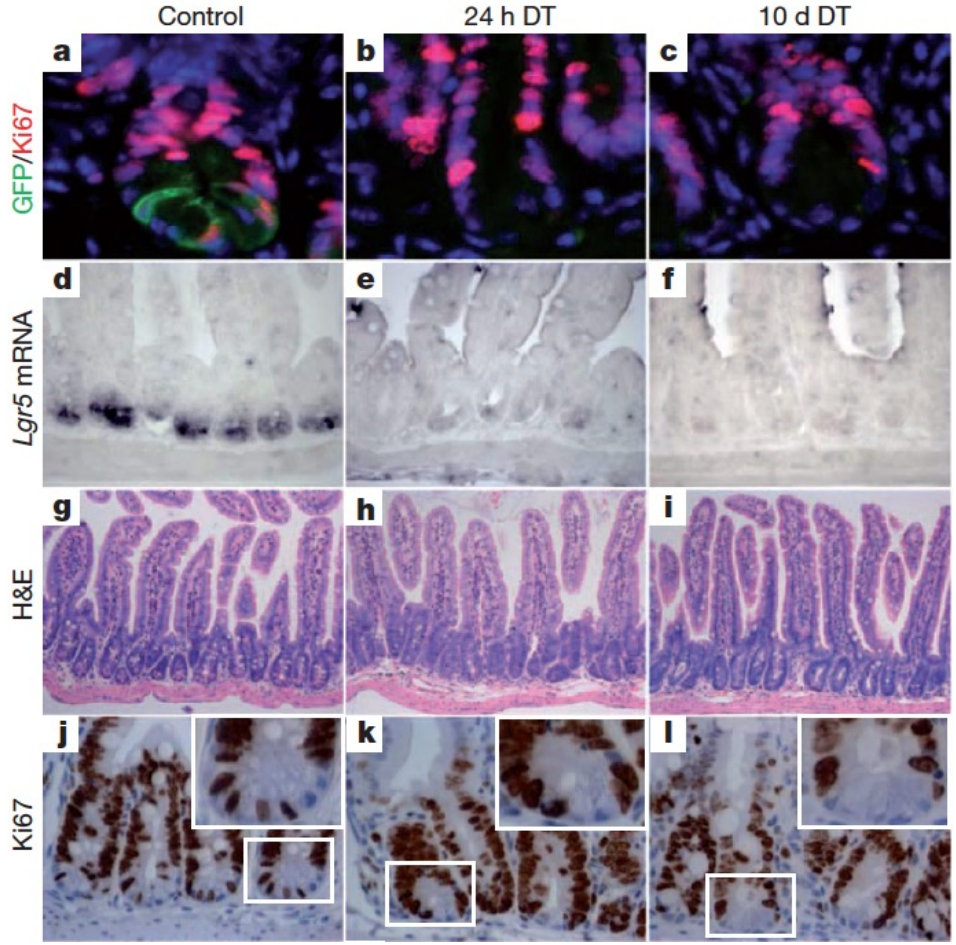
A reserve stem cell population in small intestine renders *Lgr5*-positive cells dispensable

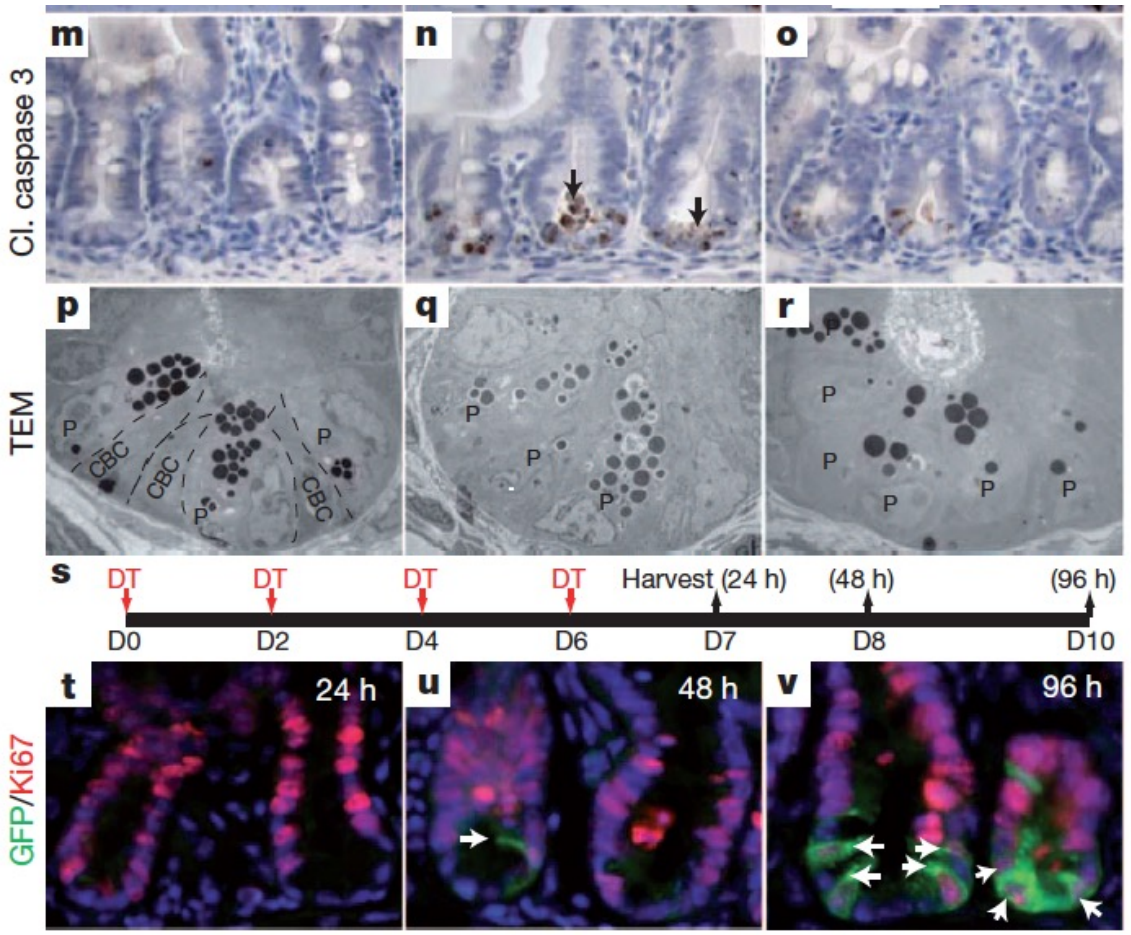
Hua Tian[1], Brian Biehs[2], Søren Warming[1], Kevin G. Leong[3], Linda Rangell[4], Ophir D. Klein[2] & Frederic J. de Sauvage[1]

Experimental strategy

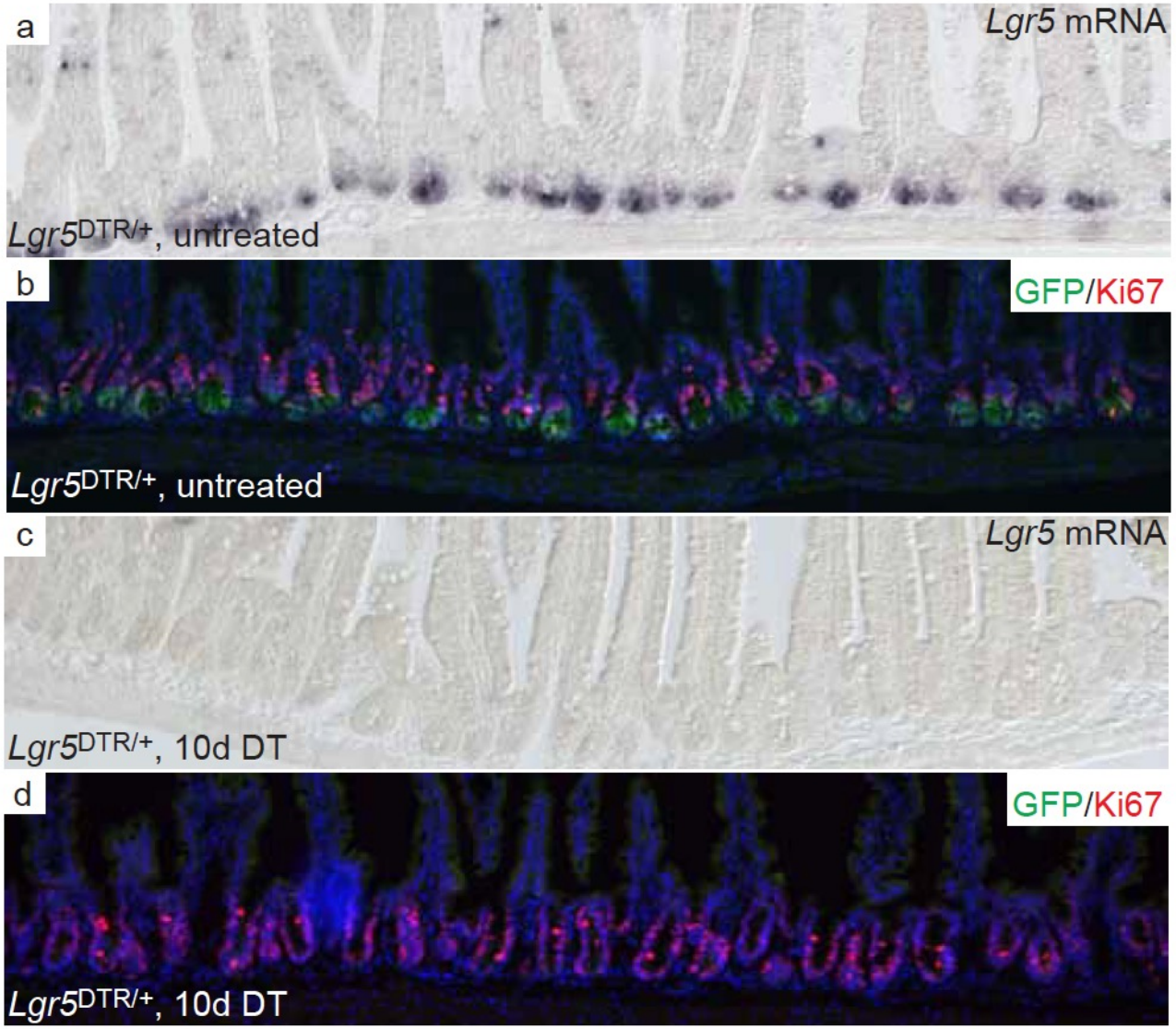


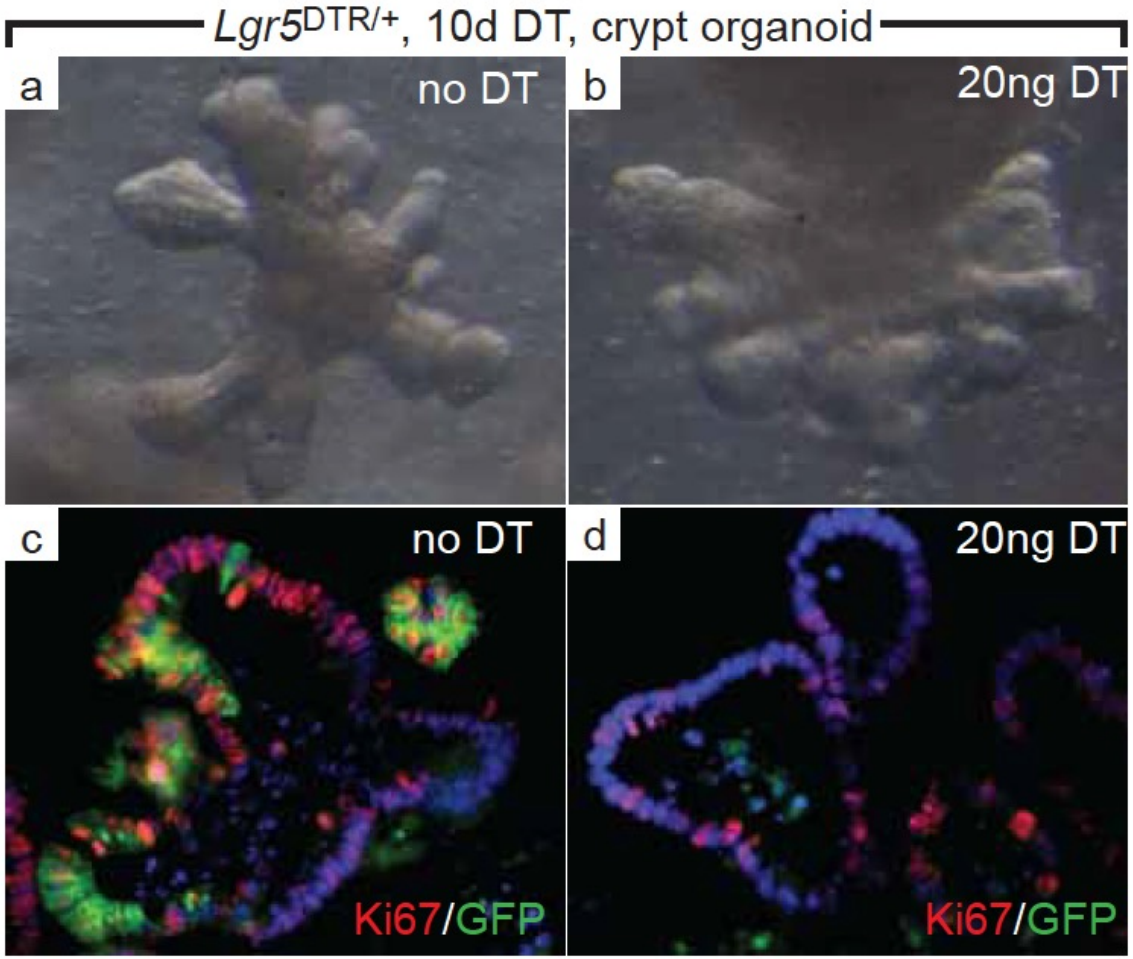
Characterization of DT-mediated CBC ablation





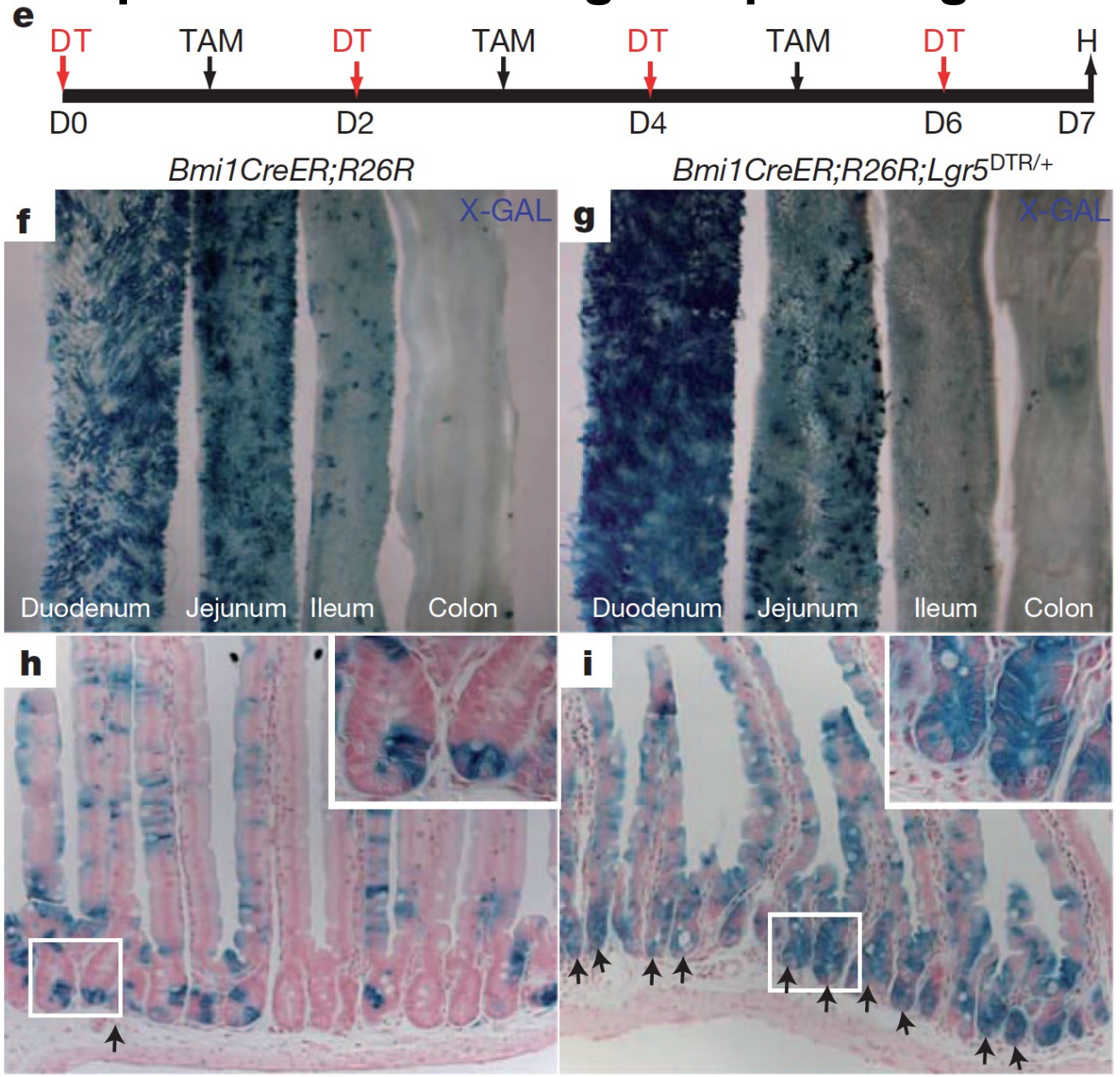
10 day treatment with DT depletes Lgr5+ cells
However the intestinal gut architecture remains normal



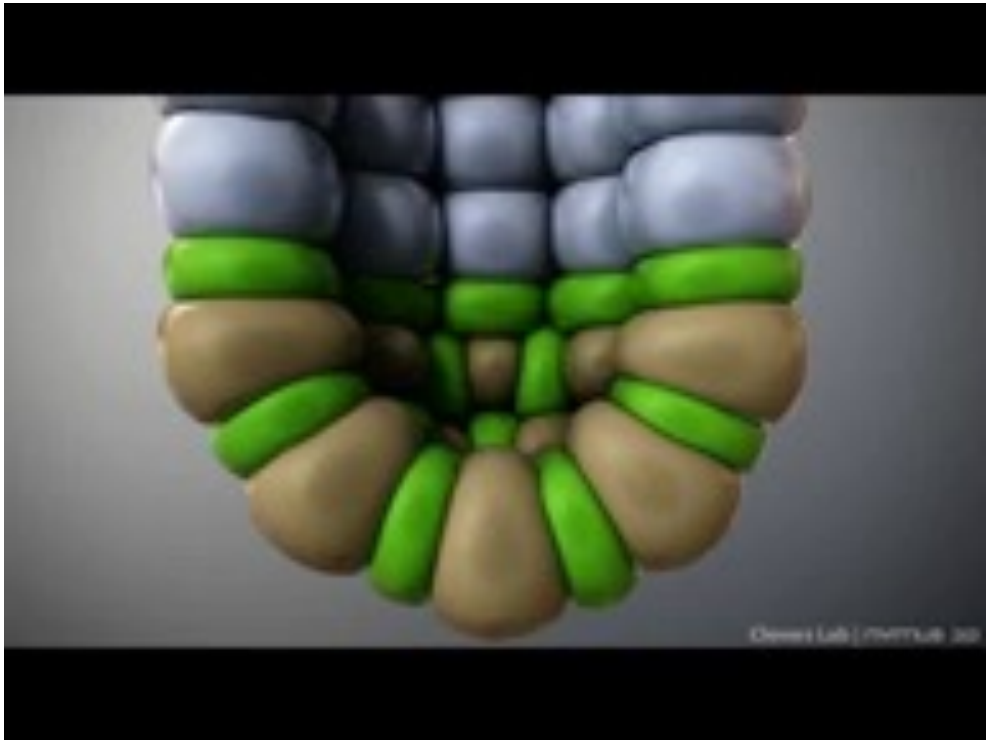


Supplementary Figure 6: *Lgr5*-expressing cells are dispensable for long term crypt organoid culture *in vitro*.

Bmi1-expressing stem cells are mobilized to compensate for loss Lgr5 expressing CBCs



In case of Lgr5+ stem cell loss Bmi1+ stem cells or intestinal progenitors become plastic and can compensate for Lgr5+ stem cells or revert to stem cells



Cell Stem Cell
Article

Lgr5^{+} Stem Cells Are Indispensable for Radiation-Induced Intestinal Regeneration

Ciara Metcalfe,[1] Noelyn M. Kljavin,[1] Ryan Ybarra,[1] and Frederic J. de Sauvage[1,*]
[1]Molecular Oncology Department, Genentech, South San Francisco, CA 94080, USA
*Correspondence: desauvage.fred@gene.com
http://dx.doi.org/10.1016/j.stem.2013.11.008

Cell Stem Cell *14*, 149–159, February 6, 2014

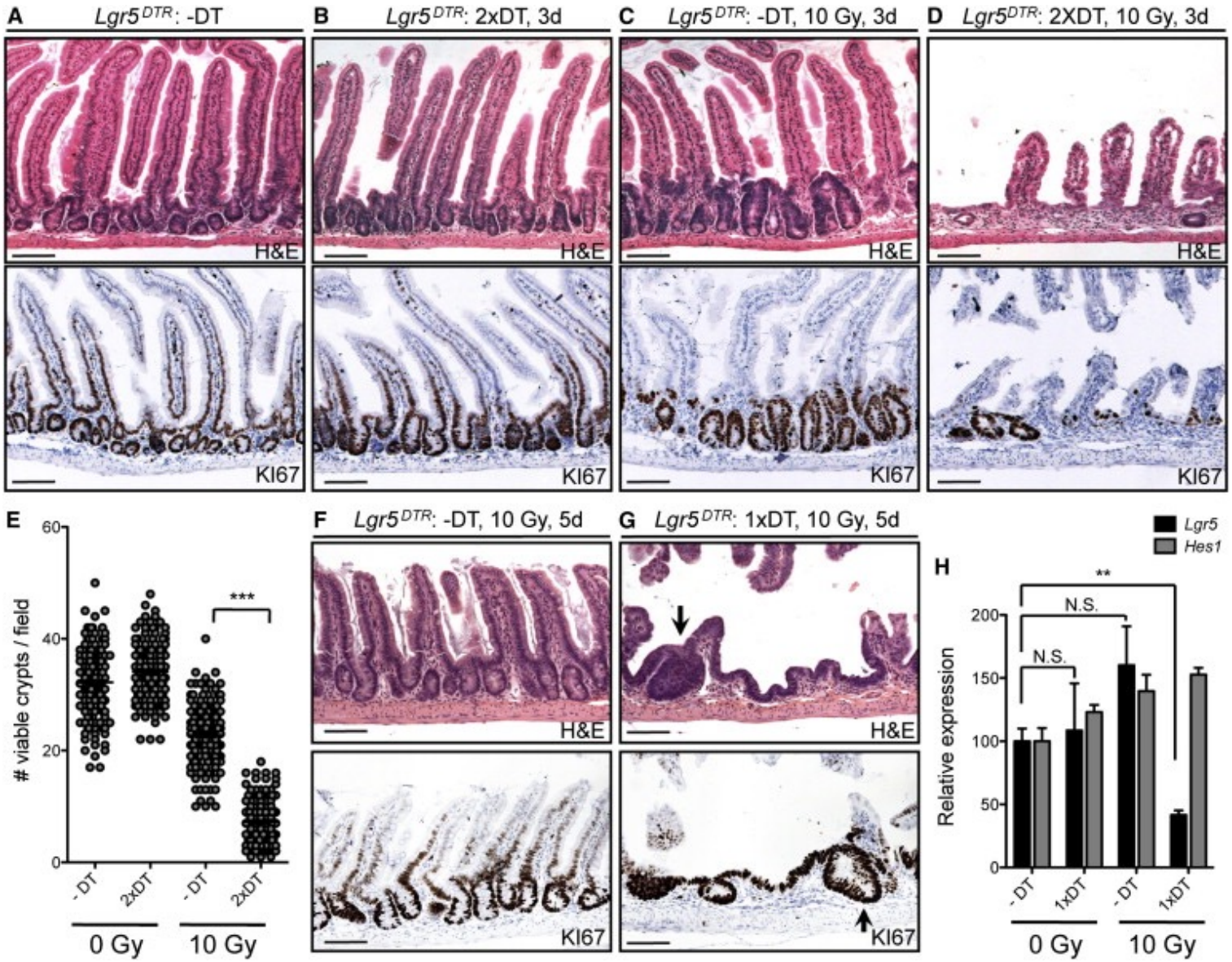
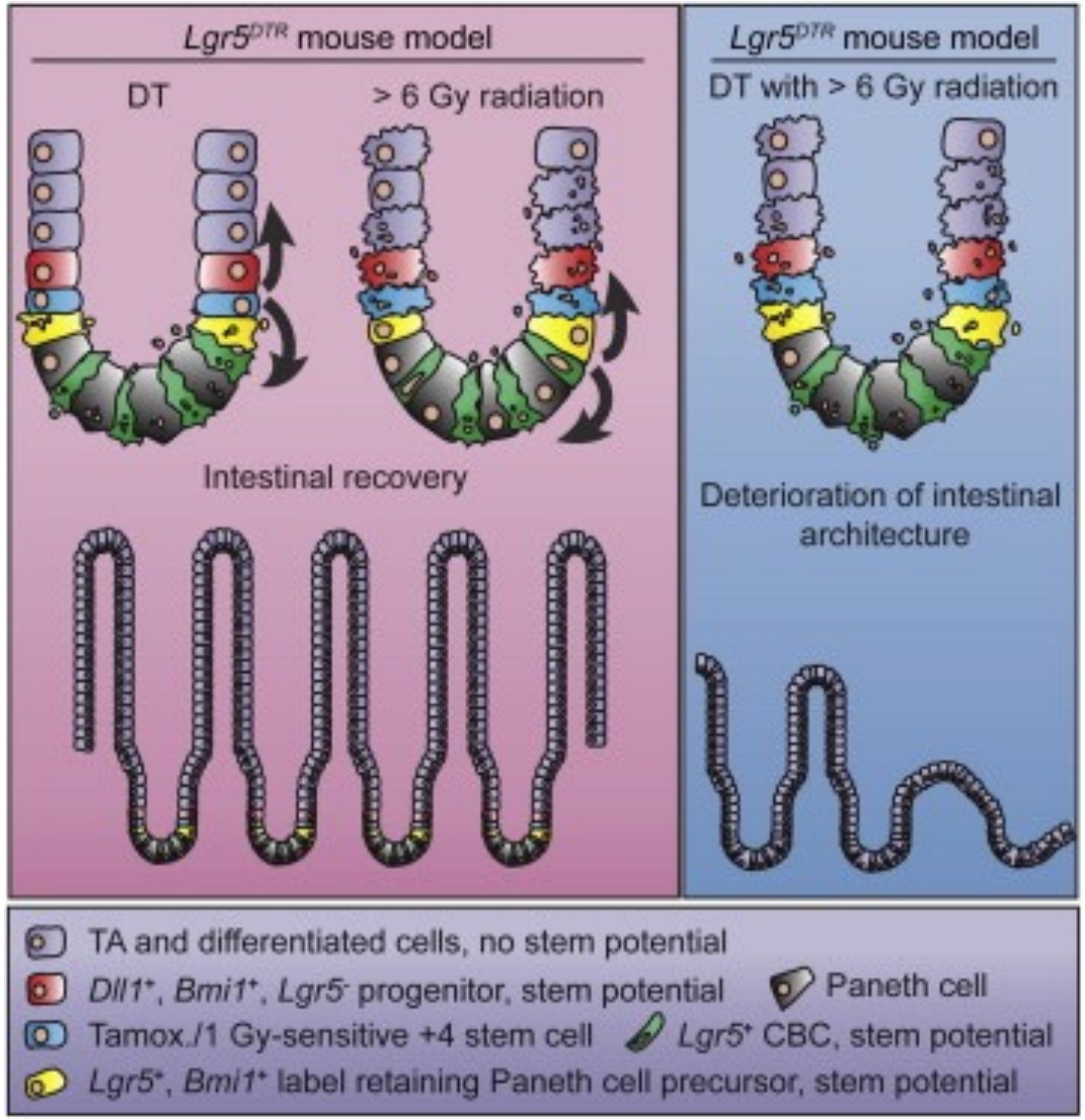
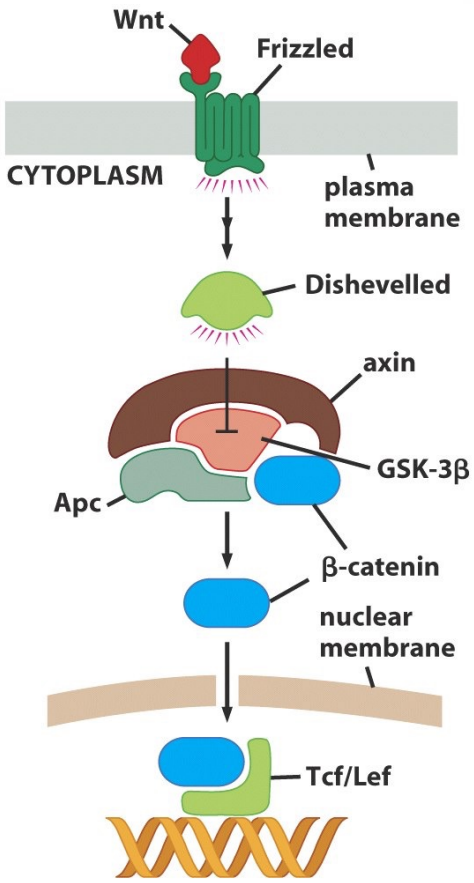
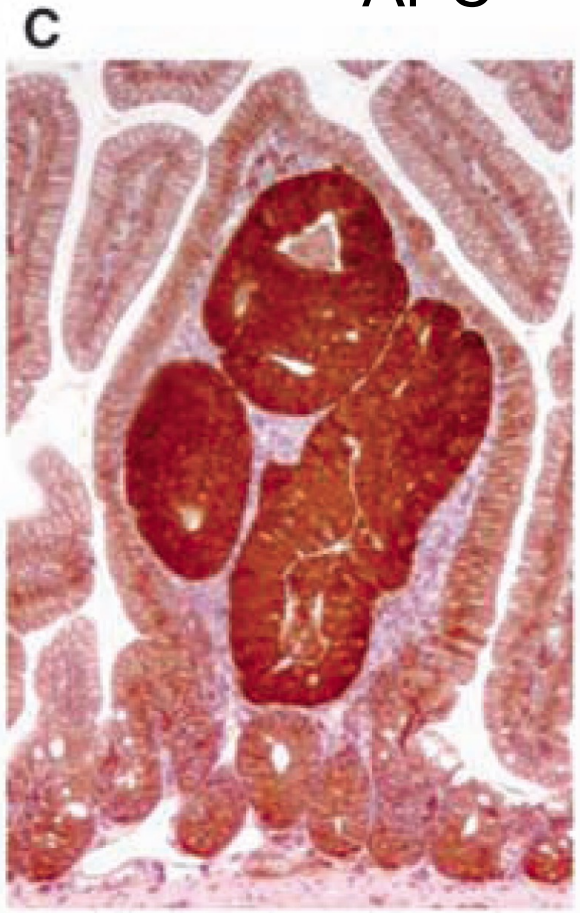


Figure 1 Ablation of Lgr5 + Cells in the Lgr5 DTR Mouse Model Dramatically Alters the Regenerative Response after Exposure to Ionizing Radiation (A–D) Representative H&E- and KI67-stained sections from Lgr5 DTR mouse duodenum treated as s...

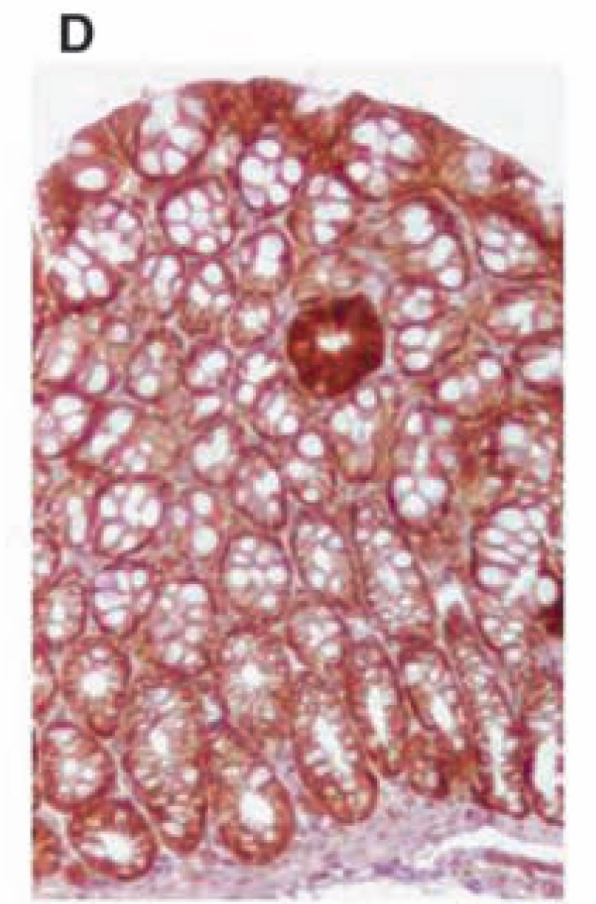

Lgr5DTR mouse model
DT
> 6 Gy radiation
Intestinal recovery
Lgr5DTR mouse model
DT with > 6 Gy radiation
Deterioration of intestinal architecture
TA and differentiated cells, no stem potential
Dll1+, Bmi1+, Lgr5- progenitor, stem potential
Paneth cell
Tamox./1 Gy-sensitive +4 stem cell
Lgr5+ CBC, stem potential
Lgr5+, Bmi1+ label retaining Paneth cell precursor, stem potential

Accumulation of β-catenin throughout the cells in adenomas and aberrant crypt focis in the intestine of APC^{min} mice





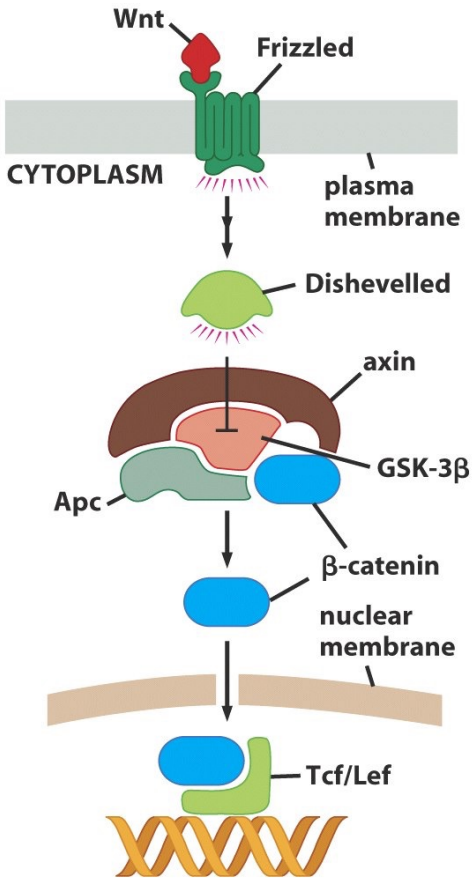
Adenoma in small intestine

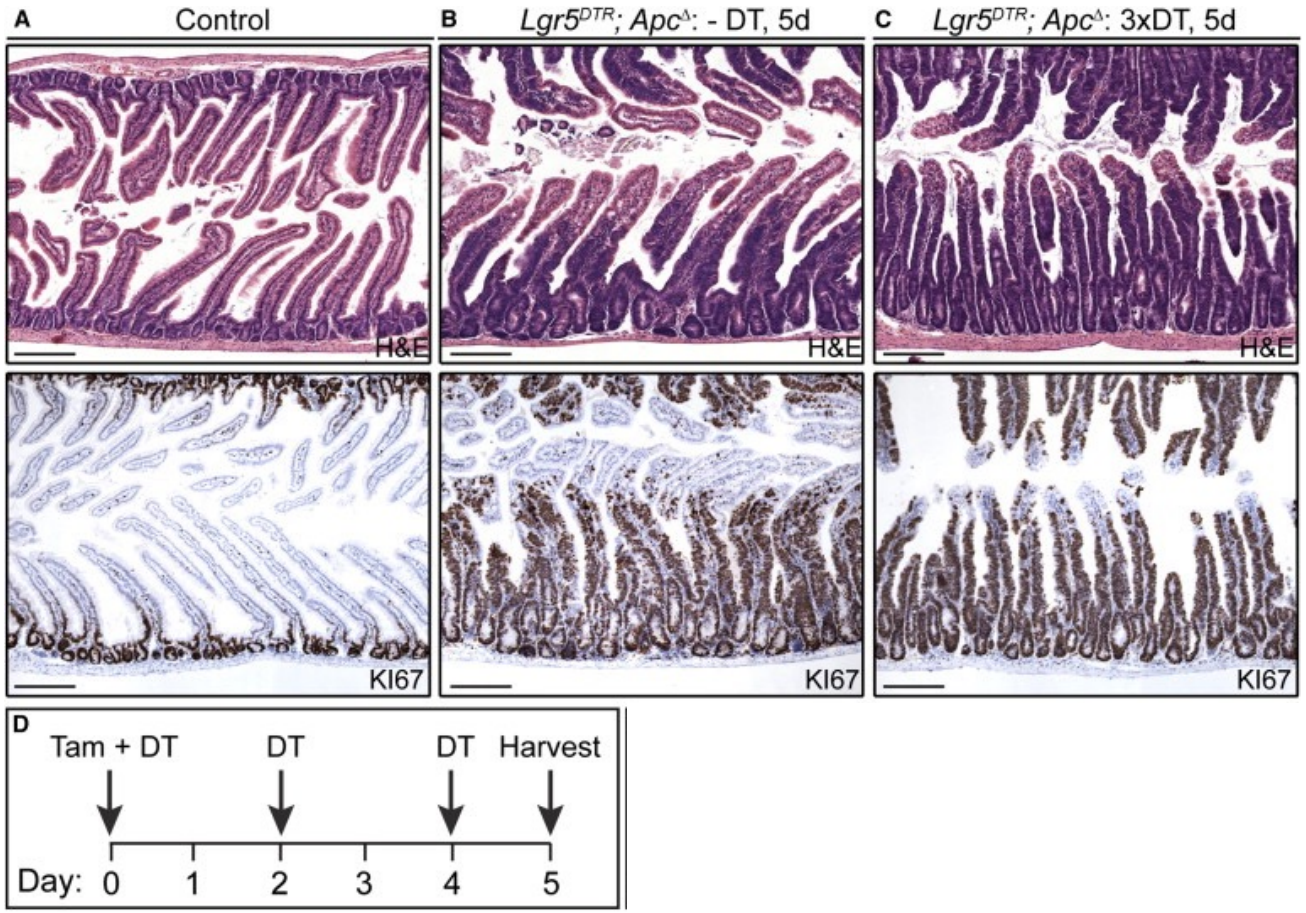


Aberrant crypt focus in colon

Radtke & Clevers, Science 2005

Lgr5 + Cells Are Not Required for Crypt Hyperplasia





What does all of this mean?

1. Lgr5+ cells are true intestinal stem cells
2. Bmi1 + cells are stem cells too
3. Progenitor cells can revert to stem cells in case of stem cell loss.
4. In case of irradiation Lgr5+ cells are indispensable for the regeneration process.