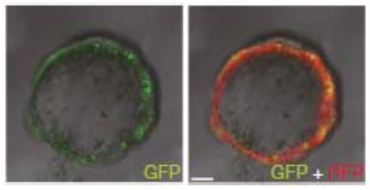
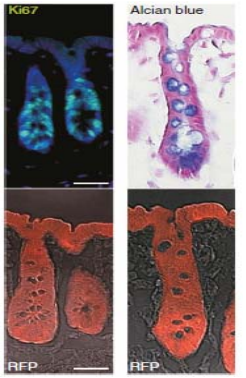
From stem cells to mini-guts:

self-organizing and ever-renewing organoids recapitulate morphogenesis *in vitro*

Journal Club – Timo Böge - 30.07.2013





Overview

I. The intestinal **stem cell niche**

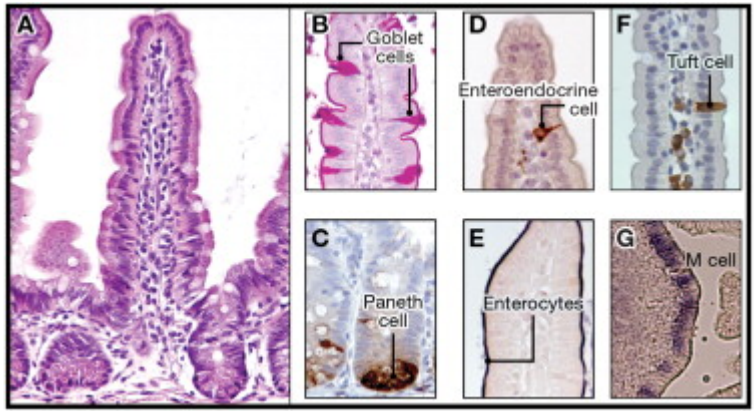
II. The intestinal **mini-gut culture system**

III. **Transplantion** and engraftment of mini-guts

IV. The **liver organoid** culture and transplantation system

V. Generation of mini-guts from i**PSC**

I. The intestinal stem cell niche

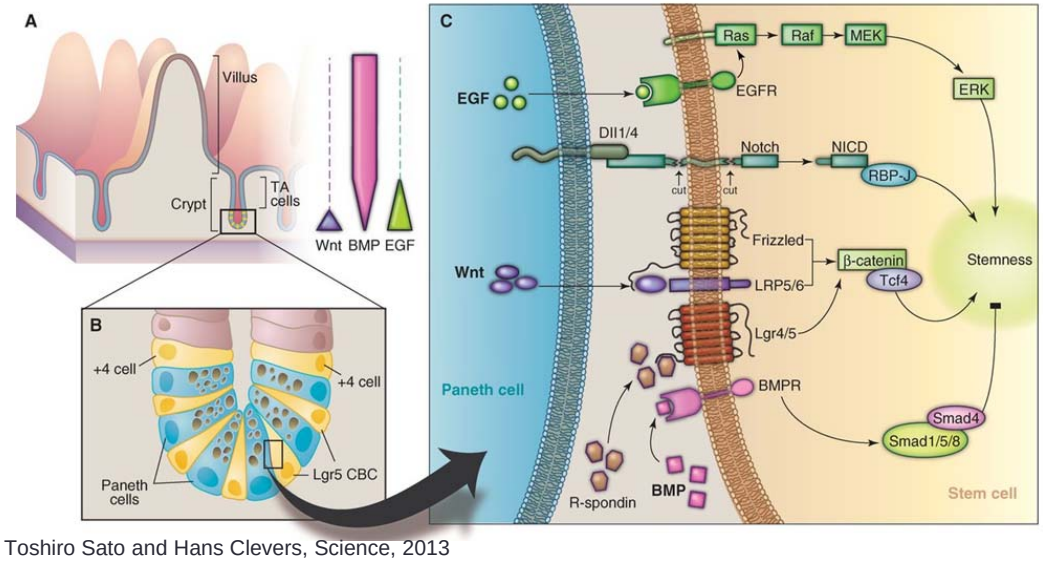


Hans Clevers, Cell, 2013

The intestinal epithelium:

- the highest self renewal rate in the body
- a turn over time of 5 days
- consists of highly differentiated cell types:
  - **Enterocytes** (absobance)
  - **Paneth cells** (secretion of anti-microbial compounds)
  - **Goblet cells** (secretion of mucin)
  - **Enteroendocrine cells** (secretion of various factors)

I. The intestinal stem cell niche

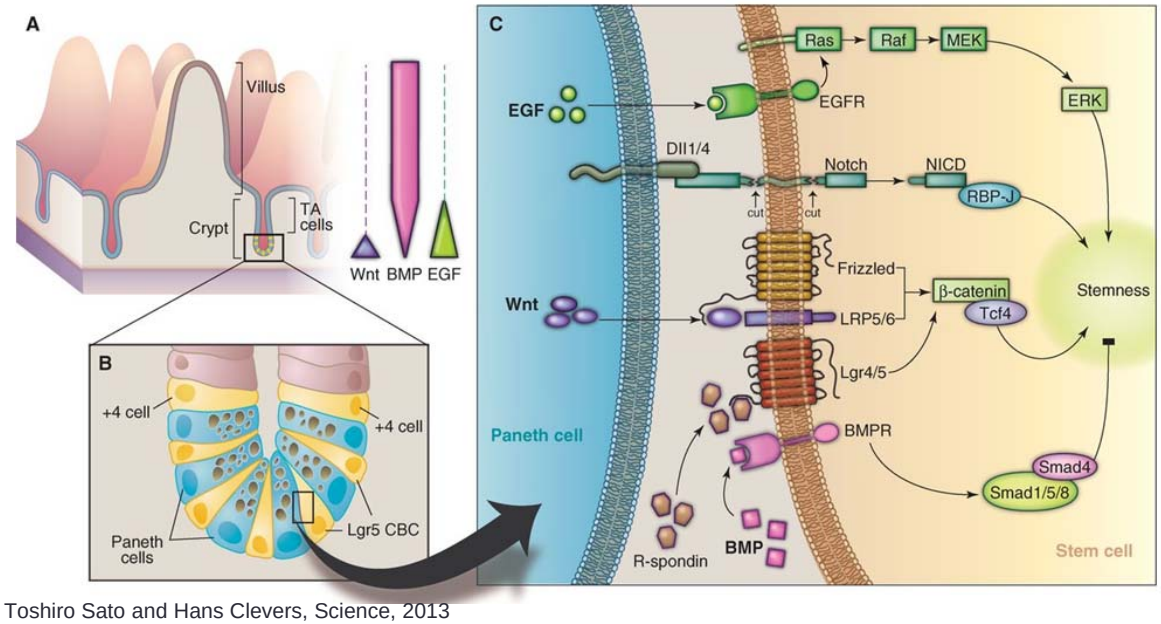


Toshiro Sato and Hans Clevers, Science, 2013

The intestinal stem cell:

- are located at the bottom of the intestinal crypt (**+4 cell, Lgr5-CBC** (crypt base cumnar))
- every crypt contains ~15 Lgr5-CBC stem cells
- cell division rate of Lgr5-CBC : 1 cell division / 24h

I. The intestinal stem cell niche



Toshiro Sato and Hans Clevers, Science, 2013

The intestinal stem cell niche is controlled by four pathways:

- Wnt
- Notch
- EGF
- BMP

Paneth cell contribute to the stem cell microenvironemnt by secreting Wnt3, EGF, R-Spondin and Notch ligands (DII1/4)

Scientific question:

Is it possible to establish long-term cultures:

- from primary adult intestinal tissue ?
- to maintain crypt-villus physiology ?
- without genetic transformation ?
- overcome the Hayflick limit ?

II. The intestinal **mini-gut culture system**

How to establish a long-term culture system with:

- active Wnt signalling for crypt proliferation ?
- EGF signalling for enterocytes proliferation ?
- Noggin expression for expansion of crypts ?
- overcome anoikis ?

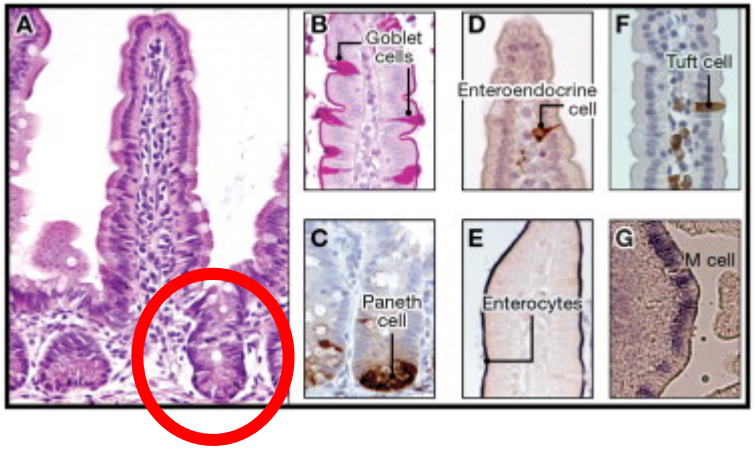

nature Vol 459|14 May 2009|doi:10.1038/nature07935

LETTERS

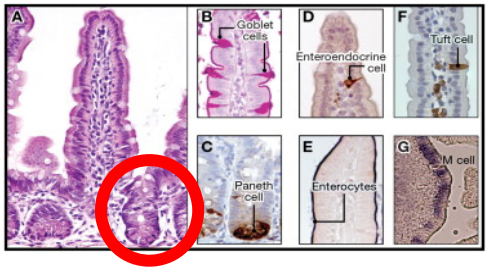
Single Lgr5 stem cells build crypt–villus structures *in vitro* without a mesenchymal niche

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]

→ Culture **intestinal crypts** under optimized conditions



II. The intestinal **mini-gut culture system**

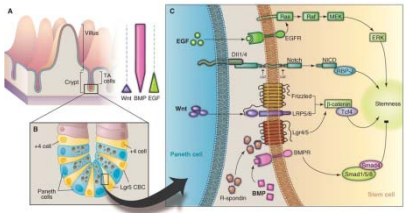


Protocol:

1. **Isolation** of mouse intestinal crypts (Lgr5-GFP+)
- incubation in PBS/EDTA (2mM) for 30min/4°C
- 500 crypts plated in 50ul Matrigel (laminin/collagen mix) in 24 well plates

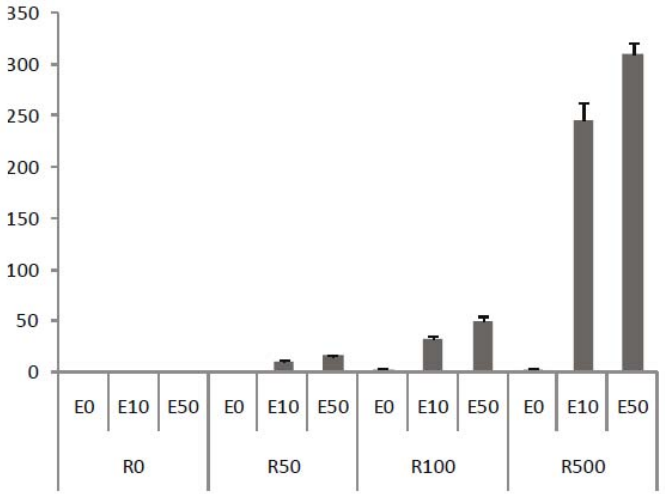
2. Optimization of **culture medium**

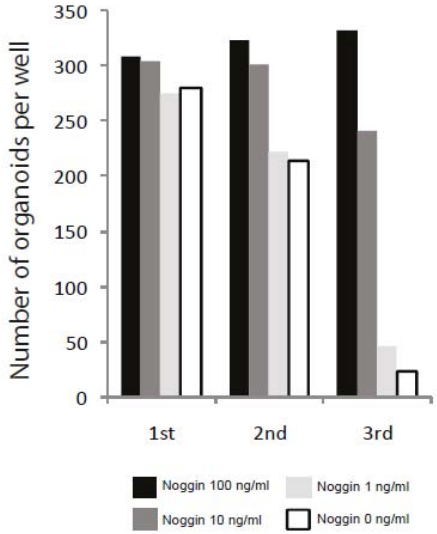
II. The intestinal **mini-gut culture system**



2. Optimization of **culture medium**

- EGF, R-Spondin1 and Noggin are required in high concentrations

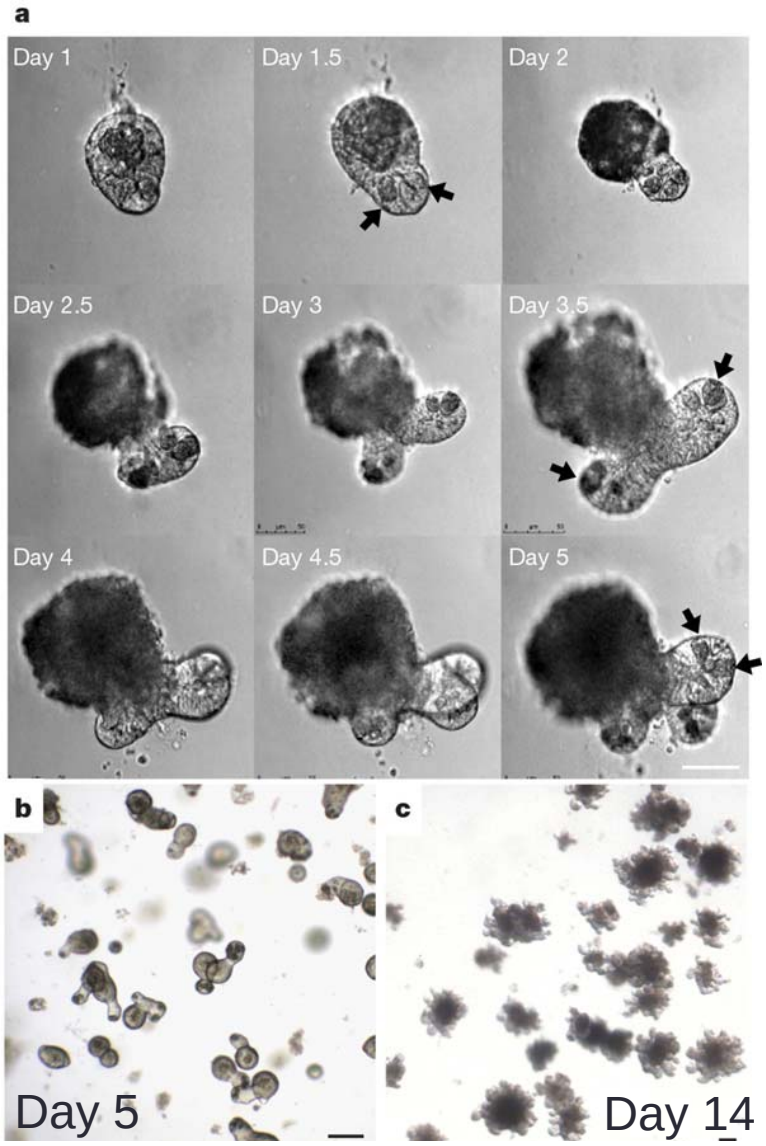




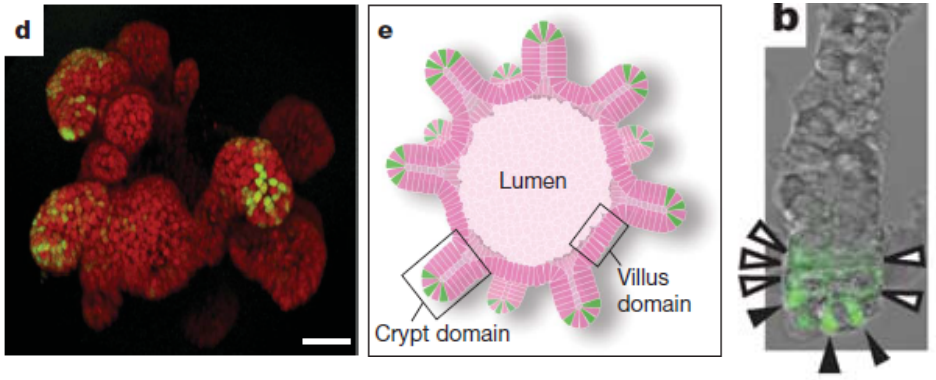
Suppl. Figure 1. Growth factor requirement of crypt culture.
a: 500 crypts were seeded with EGF (E: 0-50 ng/ml) and R-spondin 1 (R: 0-500 ng/ml) in triplicate; crypt organoids were counted 7 days after seeding. **b:** 500 Crypts/crypt organoids were cultured with EGF (50 ng/ml) and R-spondin 1 (500 ng/ml) with the indicated amounts of Noggin and followed for 3 passages. Crypt organoids were counted at each passage. The experiment was repeated three times with comparable results. Error bars, s.e.m.

II. The intestinal **mini-gut culture system**

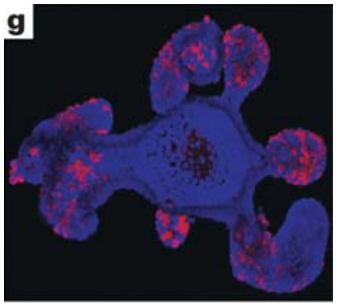
Intestinal crypts grow *in vitro*



Crypts are Lgr5 positive



Only crypts incoorporate BrdU

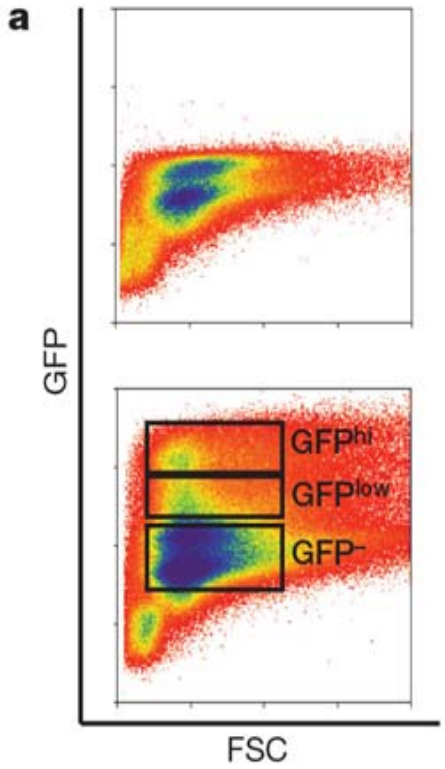


→Video

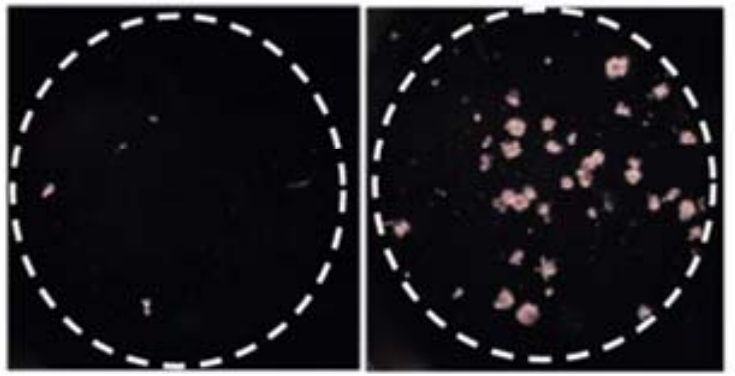
II. The intestinal **mini-gut culture system**

Do organoids also grow from **single stem cells** or only from entire crypts?

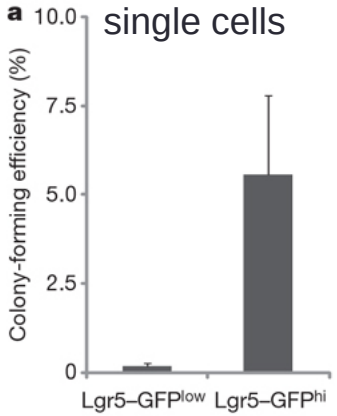
FACS sporting of single cell suspension from Lgr5-GFP+ mice



GFP low GFP high



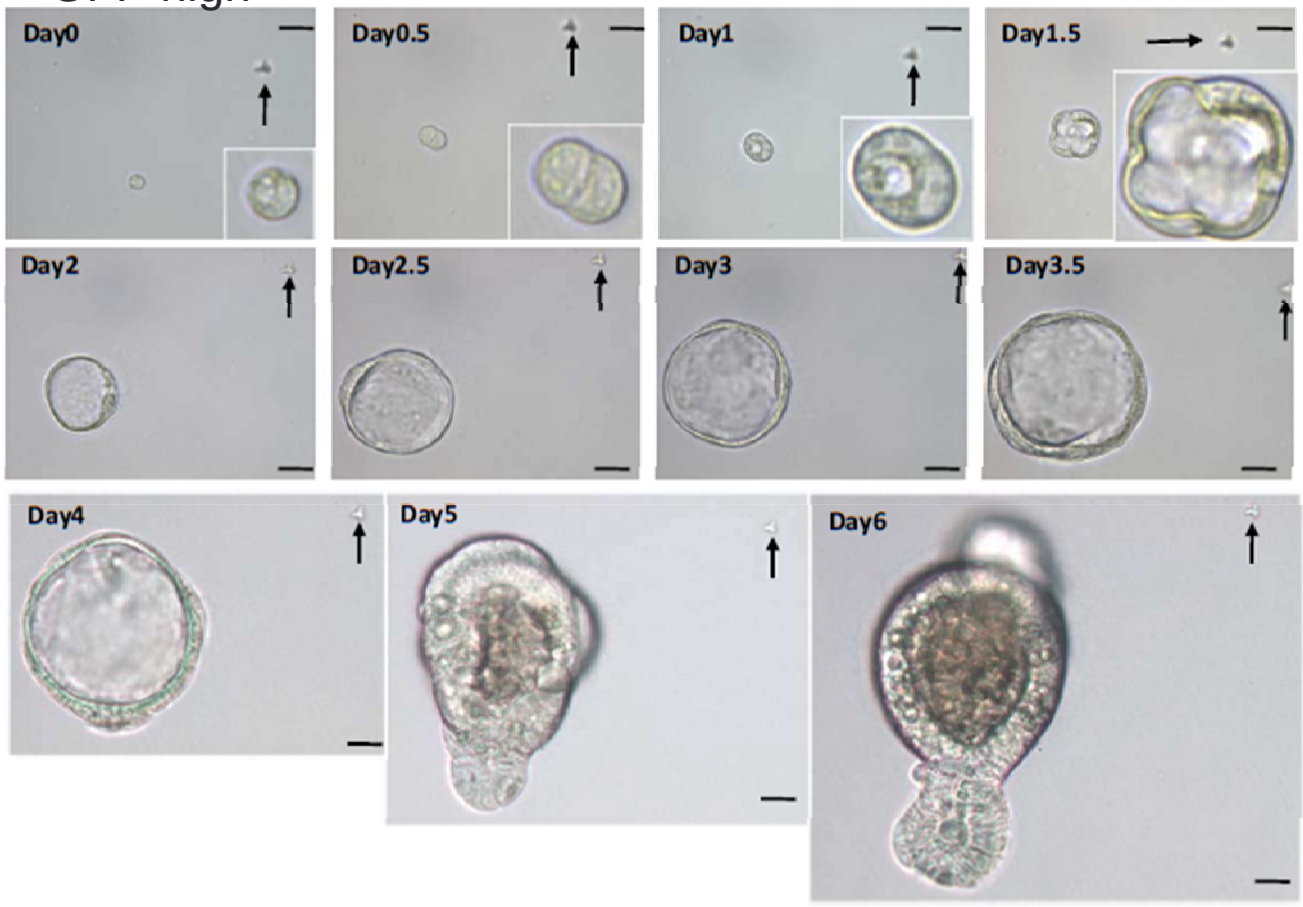
Colony forming efficiency per 100 single cells

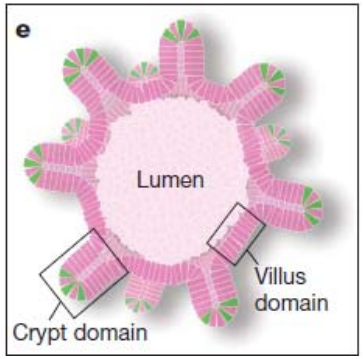


II. The intestinal **mini-gut culture system**

Do organoids also grow from single stem cells or only from entire crypts?

GFP high

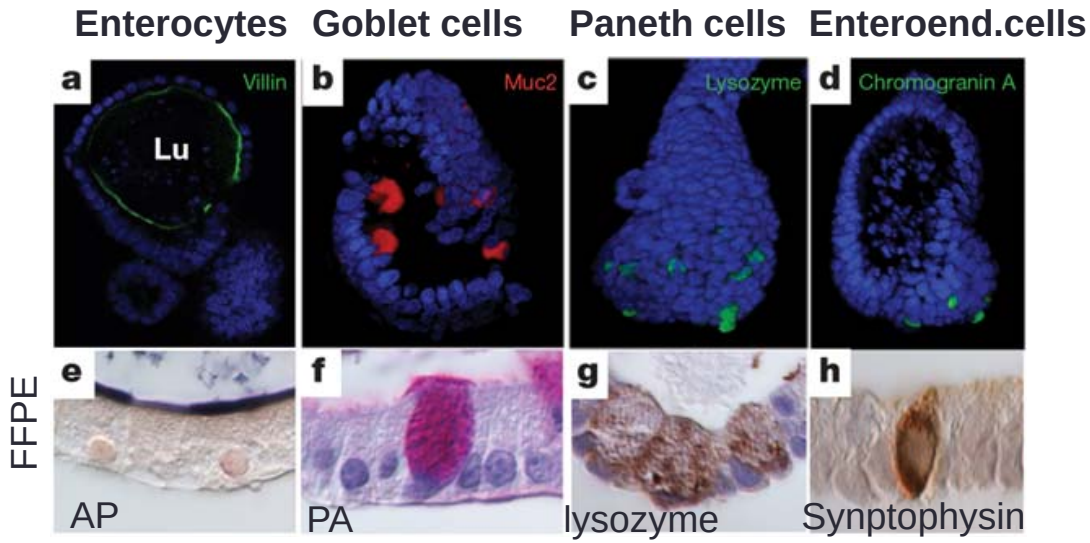


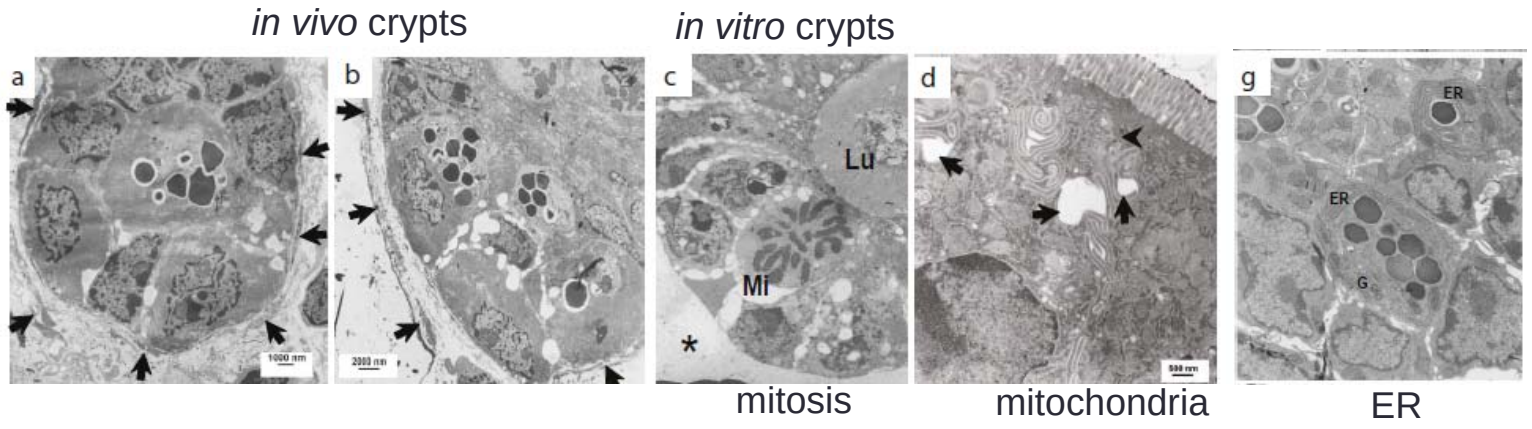


→ single cells build crypt-villus like structures without the stem cells niche

II. The intestinal **mini-gut culture system**

How similar are *in vitro* organoids and *in vivo* crypts?

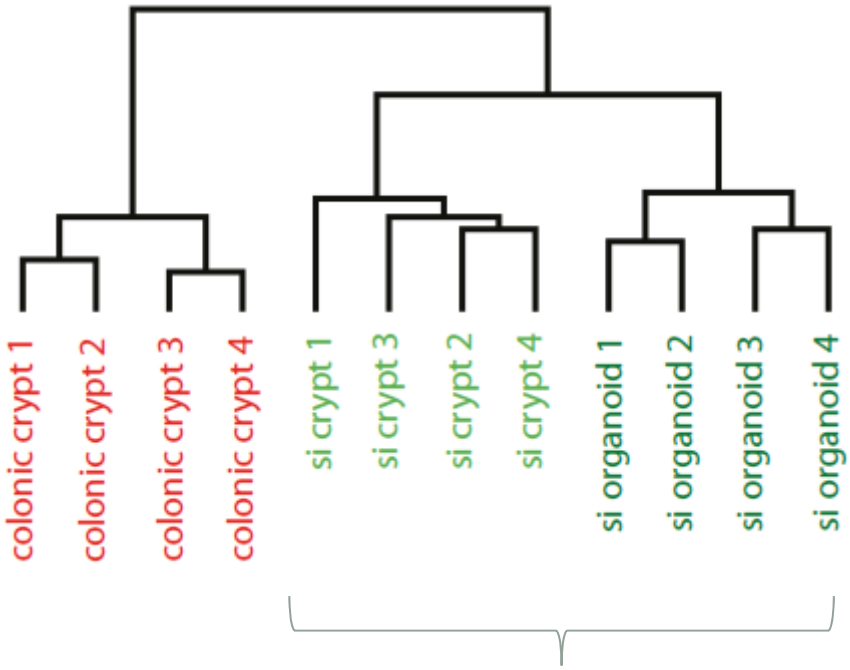




II. The intestinal **mini-gut culture system**

How similar are *in vitro* organoids and *in vivo* crypts?

Gene expression profiling of *in vitro* organoids and corresponding *in vivo* colonic and si crypts

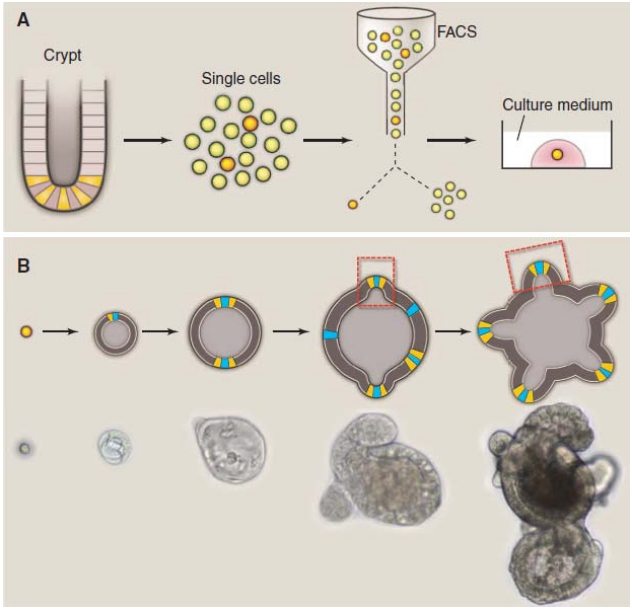


- Only 1.2% of genes were signifcantly enriched in organoids
- 2% of genes were signifcantly enriched in si crypts

→ lymphocyte signature

SUMMARY

- Single Lgr5+ stem cells have the capacity to initiate morphogenesis *in vitro*
- Stem cells grow into ever-expanding epithelial organoids
- Organoids show polarization and differentiation
- Organoids grow stable for at least 1.5 years



→ How functional and useful are mini-guts?
→ Is there a potential use for regenerative medicine?

III. Transplantion and engraftment of mini-guts

TECHNICAL REPORTS

nature medicine

Functional engraftment of colon epithelium expanded *in vitro* from a single adult Lgr5^{+} stem cell

Shiro Yui[1,6], Tetsuya Nakamura[2,6], Toshiro Sato[3,5], Yasuhiro Nemoto[1], Tomohiro Mizutani[1], Xiu Zheng[1], Shizuko Ichinose[4], Takashi Nagaishi[1], Ryuichi Okamoto[2], Kiichiro Tsuchiya[1], Hans Clevers[3] & Mamoru Watanabe[1]

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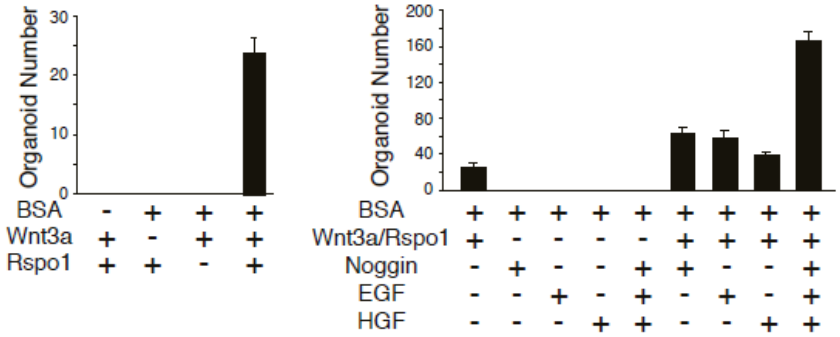
→ Can mini-guts be transplanted and regenerate epithelial tissue *in vivo*?

- Establish a protocol for organoids derived from colonic stem cells
- Develop a transplantation system

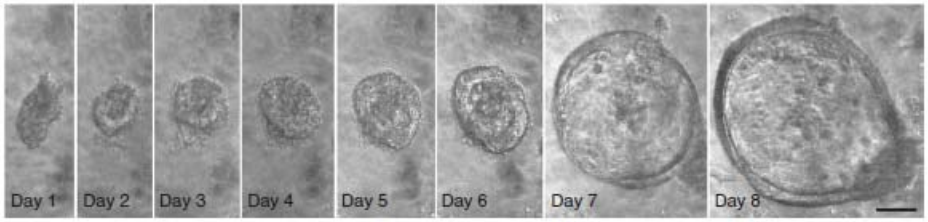
III. Transplantion and engraftment of mini-guts

Protocol for organoids derived from **colonic crypts**

Optimization of culture conditions: + Wnt3a +HGF



a Colonic organoids from **colonic crypts**



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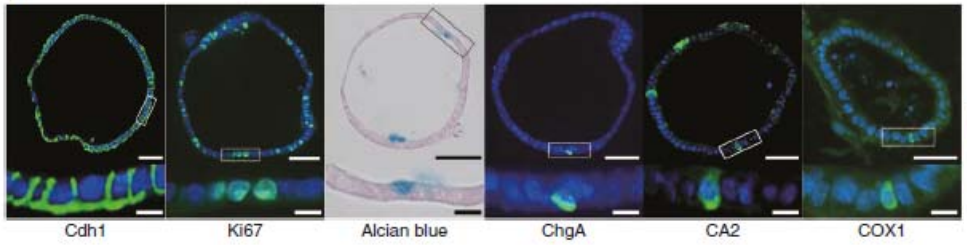
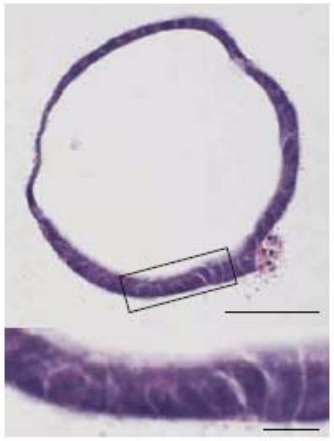


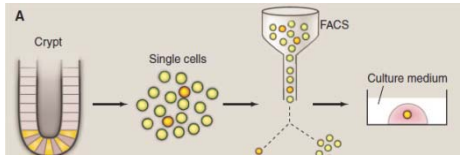


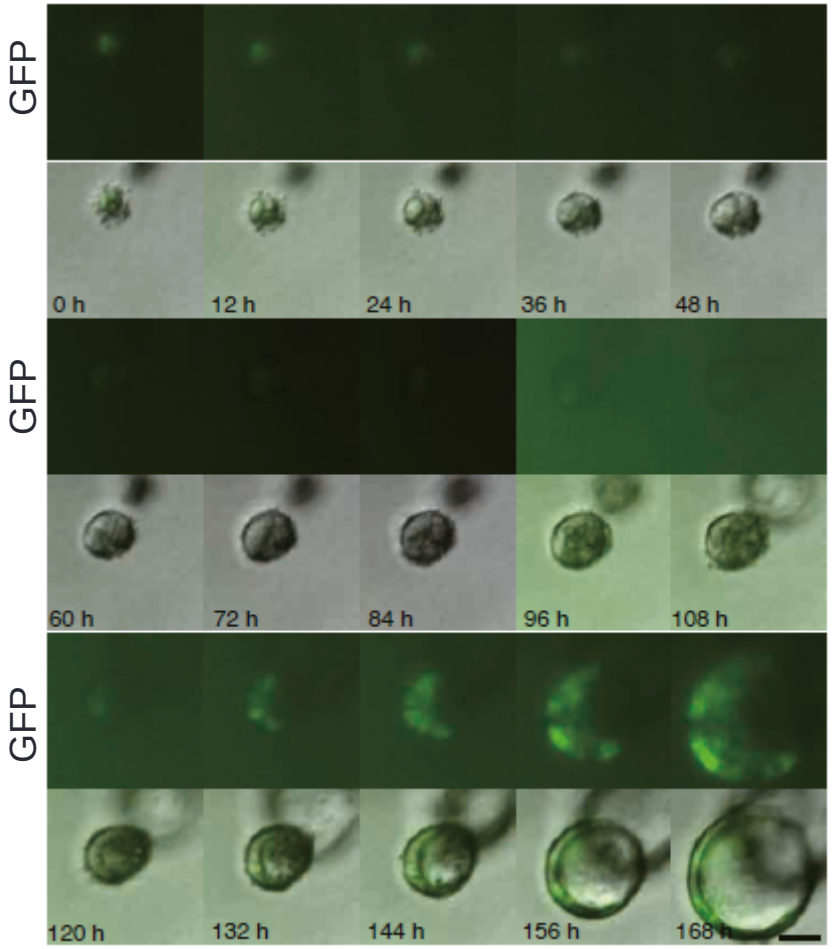
organoids grow as monolayer

organoids rarely have buds

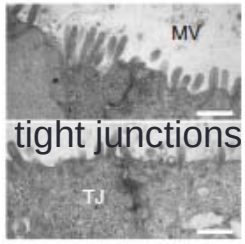
III. Transplantion and engraftment of mini-guts

Colonic organoids from **single cells (Lgr5-GFP+)**

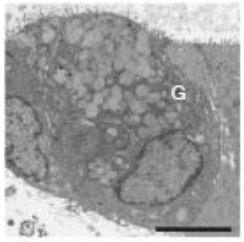


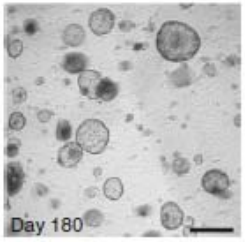


microvilli

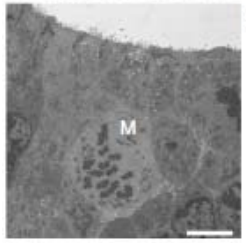


Goblet cells

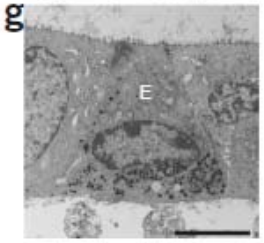


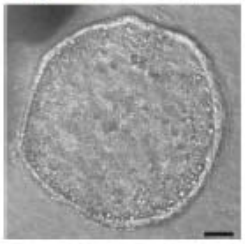


Metaphase spread



mitosis

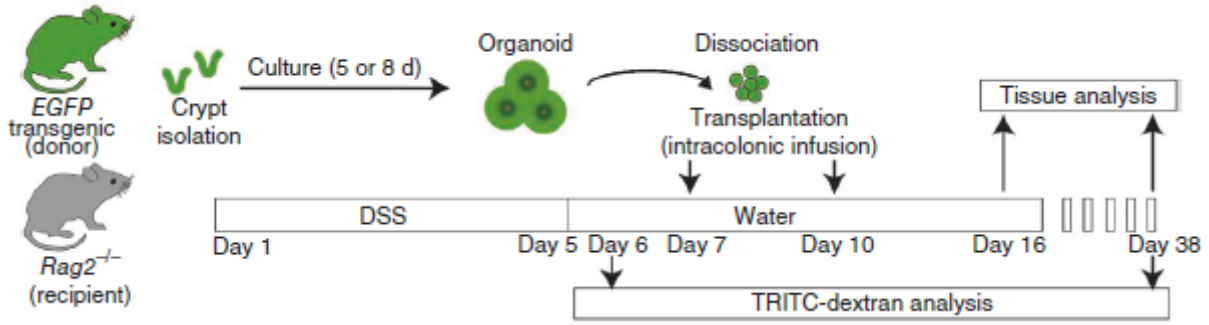




enteroendeocrine cells

III. Transplantion and engraftment of mini-guts

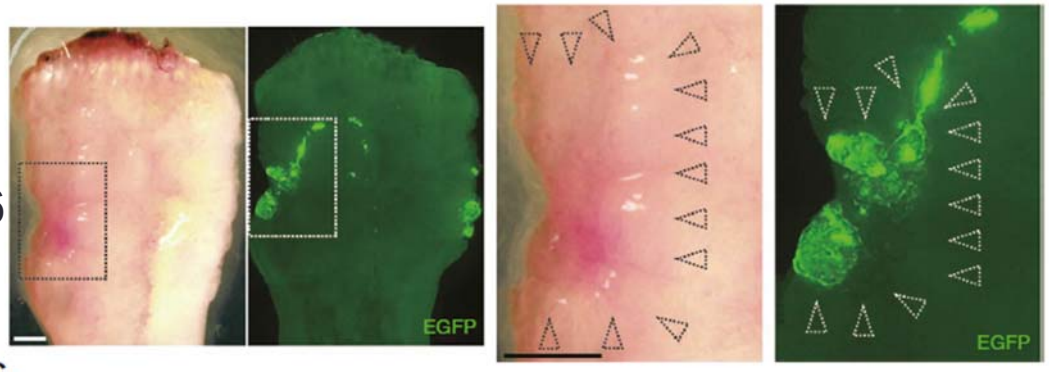
Experimental set-up for transplantation of **crypt derived organoids**



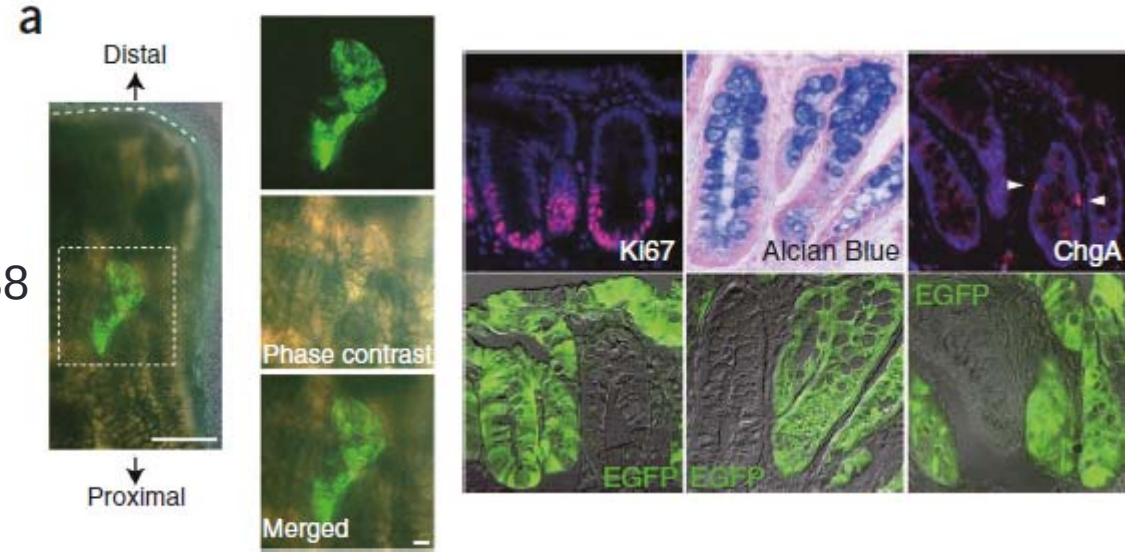
III. Transplantion and engraftment of mini-guts

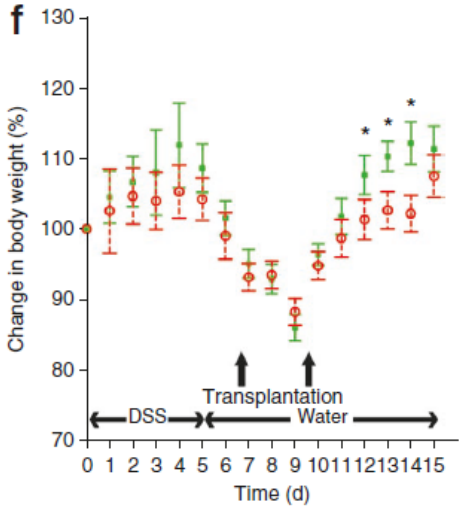
Experimental set-up for transplantation of crypt derived organoids

Day 16



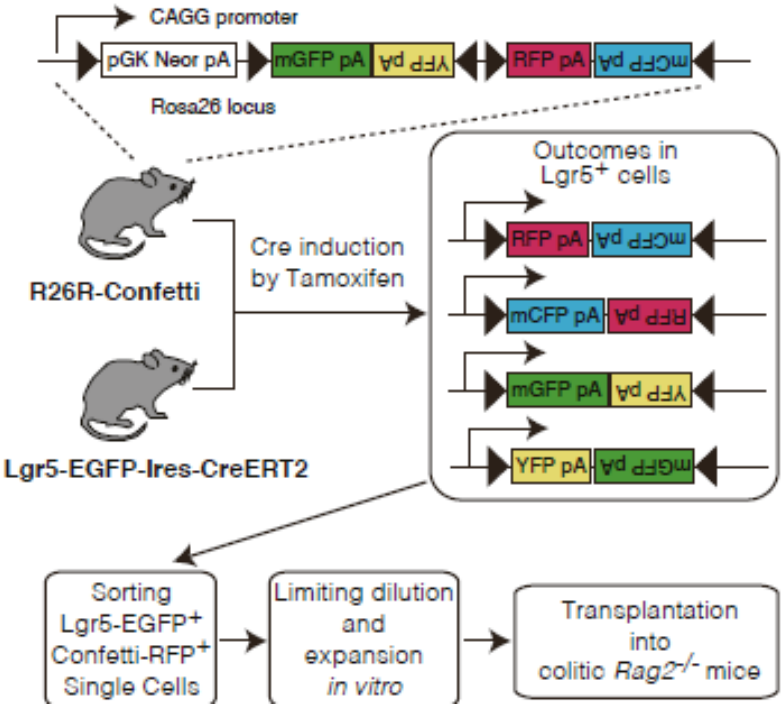
Day 38





III. Transplantion and engraftment of mini-guts

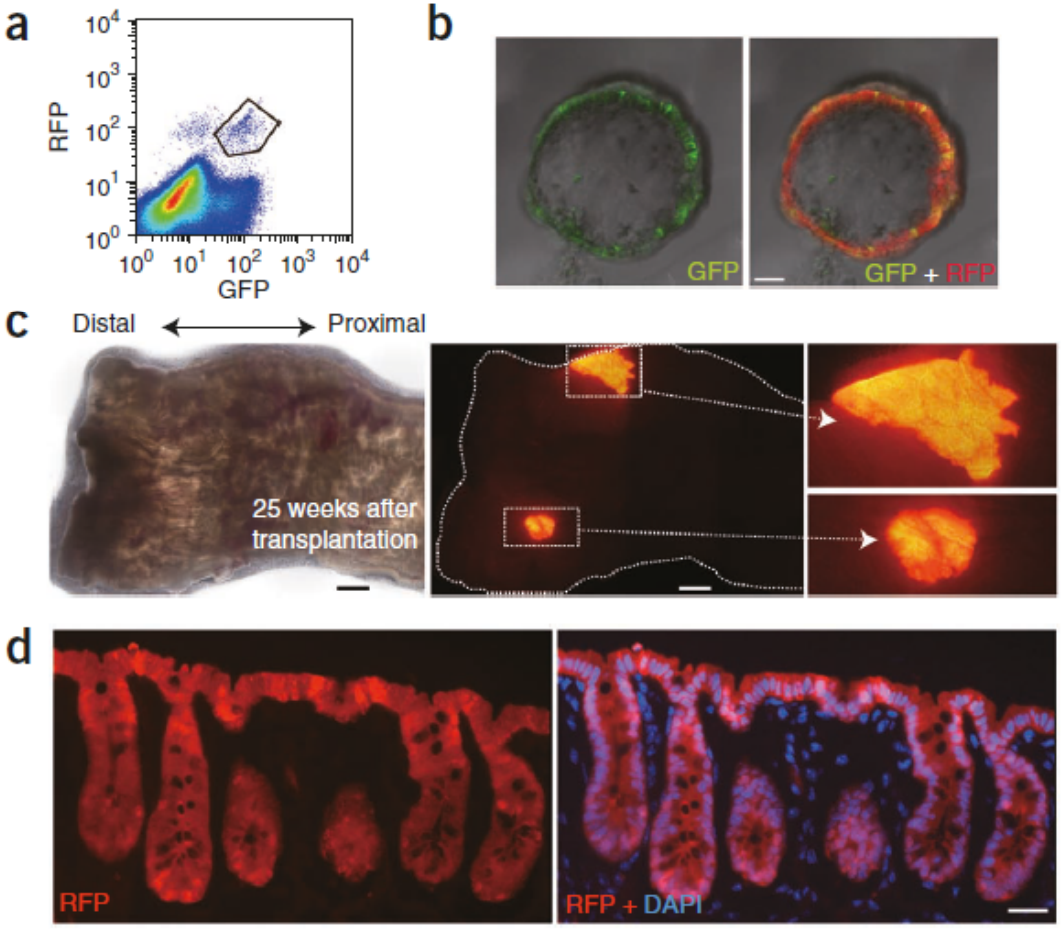
Experimental set-up for transplantation of **single cell derived organoids**

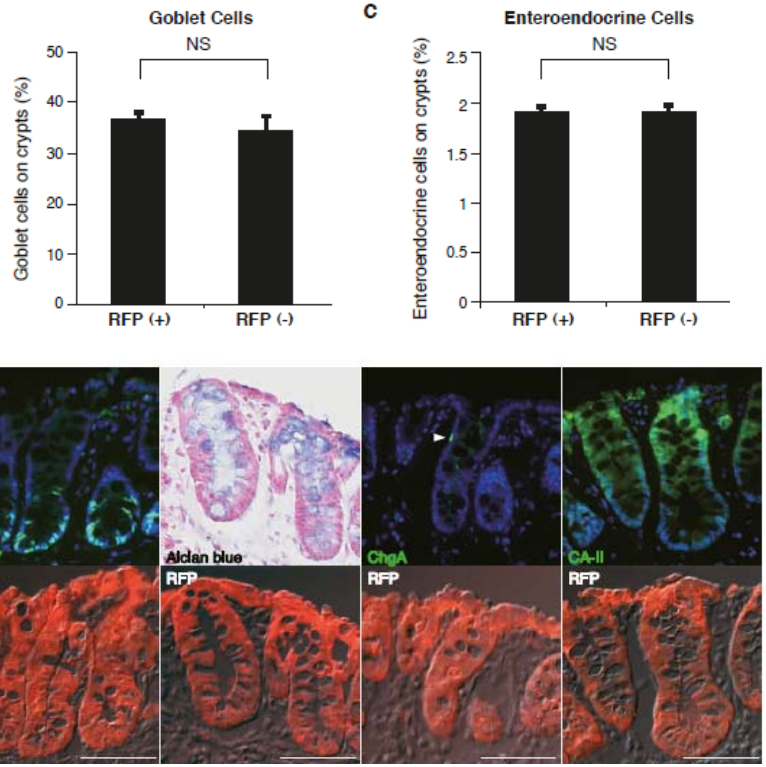


→ organoids derived from single stem cells (Lgr5-GFP+)

→ from the same donor (Confetti-RFP+)

III. Transplantion and engraftment of mini-guts





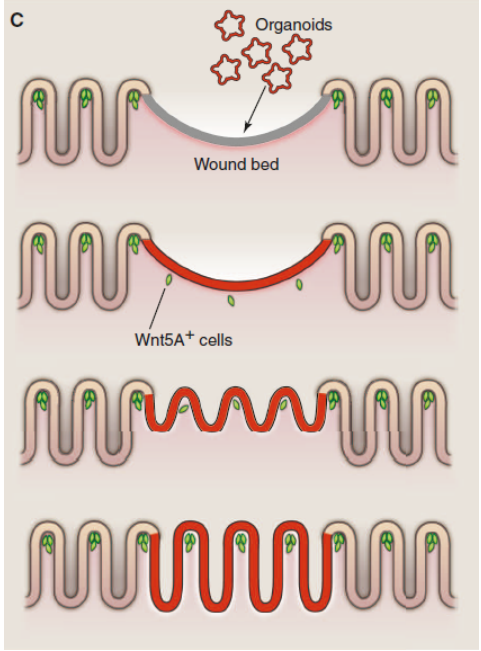
→ Transplantation of 500 organoids/mouse leads to monolayer

→ All differentiated cell types are present in a normal ratio

SUMMARY

- Succesfull isolation and culture of colonic stem cells and organoids
- Crypt and single cells derived organoids show transplantability and engraftment in recipient animals
- Transplanted organoids covered superficially damage colonic tissue
- Improved body weight → beneficial in DSS-induced colitis

→ Is the culture system limited to gastrointestinal stem cells?



III. The liver organoid culture and transplantation system

LETTER

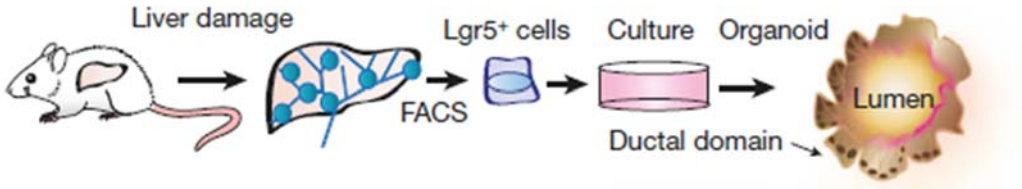
doi:10.1038/nature11826

In vitro expansion of single Lgr5$^+$ liver stem cells induced by Wnt-driven regeneration

Meritxell Huch[1]*, Craig Dorrell[2]*, Sylvia F. Boj[1], Johan H. van Es[1], Vivian S. W. Li[1], Marc van de Wetering[1], Toshiro Sato[1]†, Karien Hamer[1], Nobuo Sasaki[1], Milton J. Finegold[3], Annelise Haft[2], Robert G. Vries[1], Markus Grompe[2] & Hans Clevers[1]

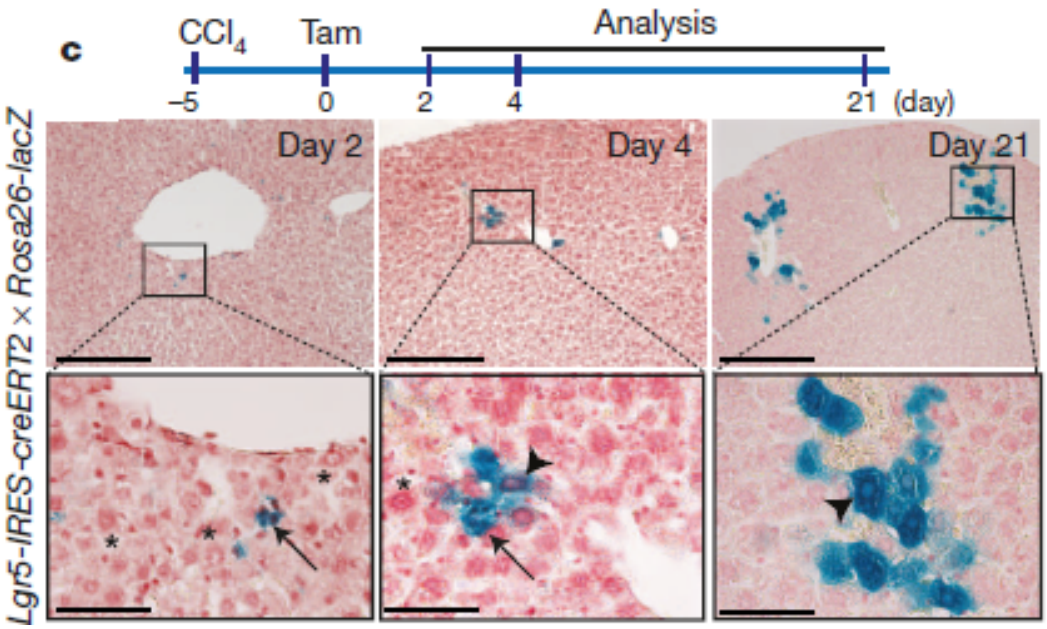
Experimental set-up:

- Isolation of Lgr5+ stem cells from liver tissue:



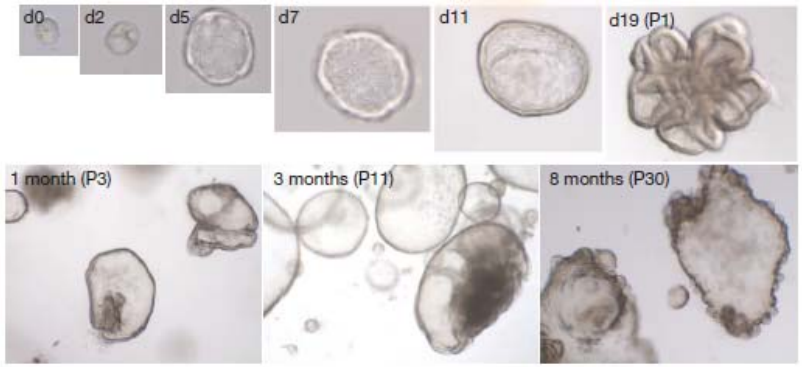
III. The liver organoid culture and transplantation system

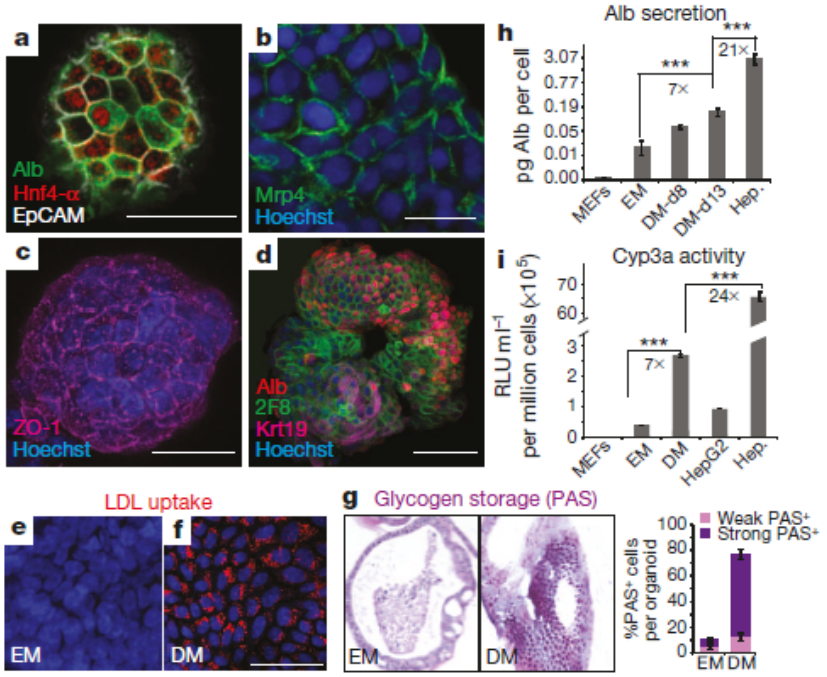
Liver stem cells are quiescent and have to be acitvated by CCL4 treatment



III. The liver organoid culture and transplantation system

Lgr5+ liver stem cells form organoids *in vitro* under optimized culture conditions

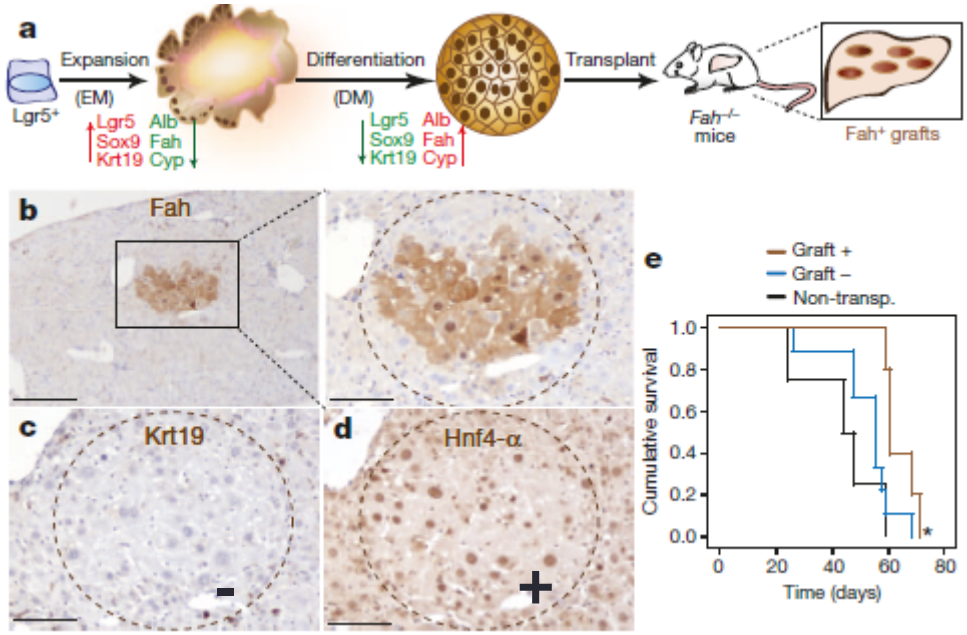




Liver organoids produce hepatocyte specific factors for at least 12 months

III. The liver organoid culture and transplantation system

Transplantation of liver organoids into Fah-/- mice leads to engraftment (1% repopulation) and improved survival of mice



Transplanted organoids engrafted and cells contributed to liver function

BUT: freshly isolated and transplanted hepatocytes repopulated more efficient (30%)

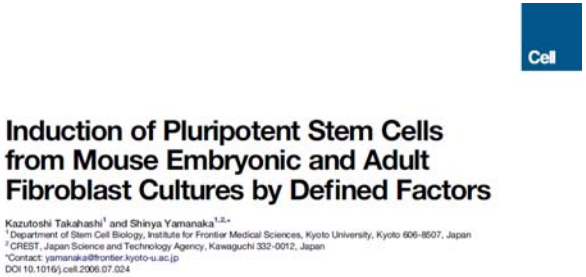
V. Generation of mini-guts from iPSC


LETTER

doi:10.1038/nature09691

Directed differentiation of human pluripotent stem cells into intestinal tissue *in vitro*

Jason R. Spence[1], Christopher N. Mayhew[1], Scott A. Rankin[1], Matthew F. Kuhar[1], Jefferson E. Vallance[2], Kathryn Tolle[1], Elizabeth E. Hoskins[3], Vladimir V. Kalinichenko[1,4], Susanne I. Wells[3], Aaron M. Zorn[1], Noah F. Shroyer[1,2] & James M. Wells[1]


Cell

Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors

Kazutoshi Takahashi[1] and Shinya Yamanaka[1,2,*]

[1] Department of Stem Cell Biology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto 606-8507, Japan

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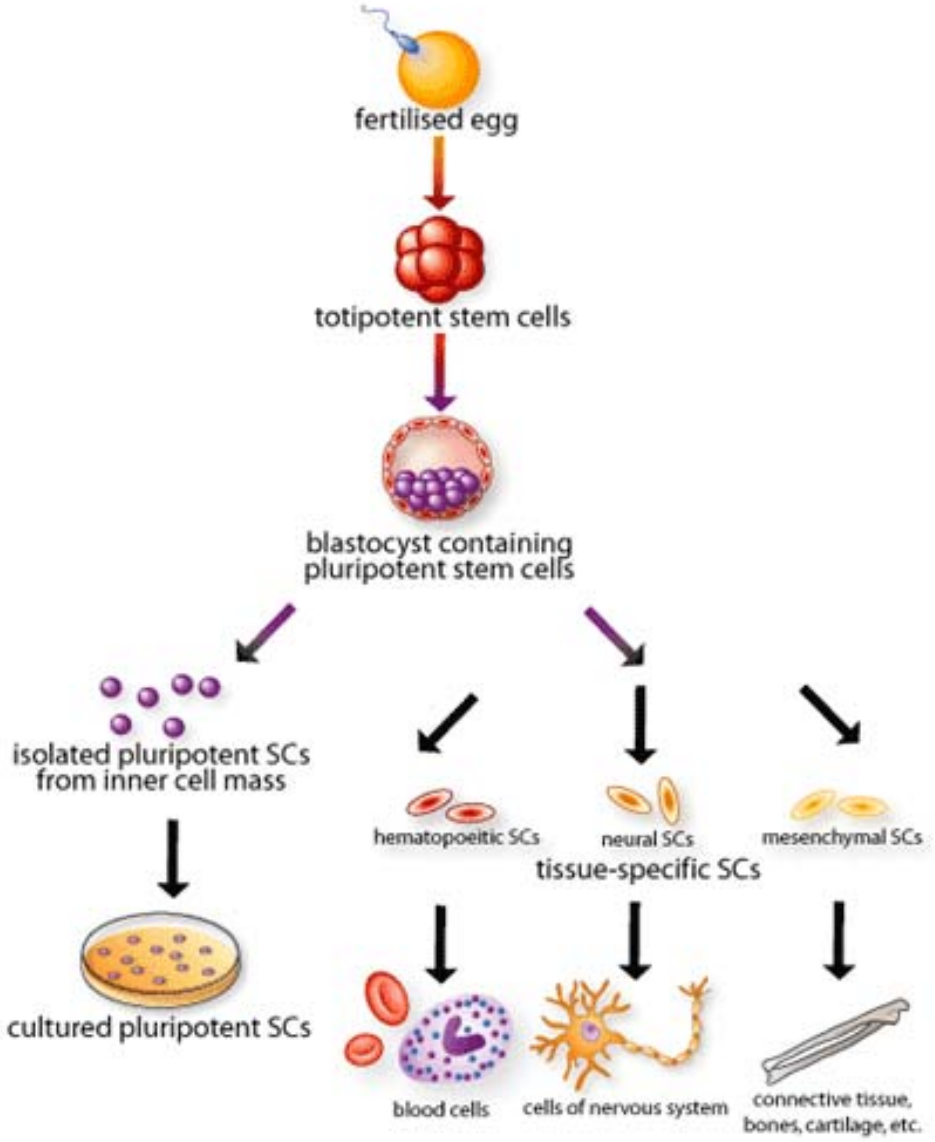
DOI 10.1016/j.cell.2006.07.024

Background:

- Human PSCs and iPSCs are able to differentiate *in vitro* into monolayer cultures (hepatocytes etc.)

- Complex three-dimensional organ structures are not possible?

→ Is it possible to directly differentiate iPSCs into 3D intestinal tissue ?



Totipotent ESC

Pluripotent ESC
-inner blastocyst

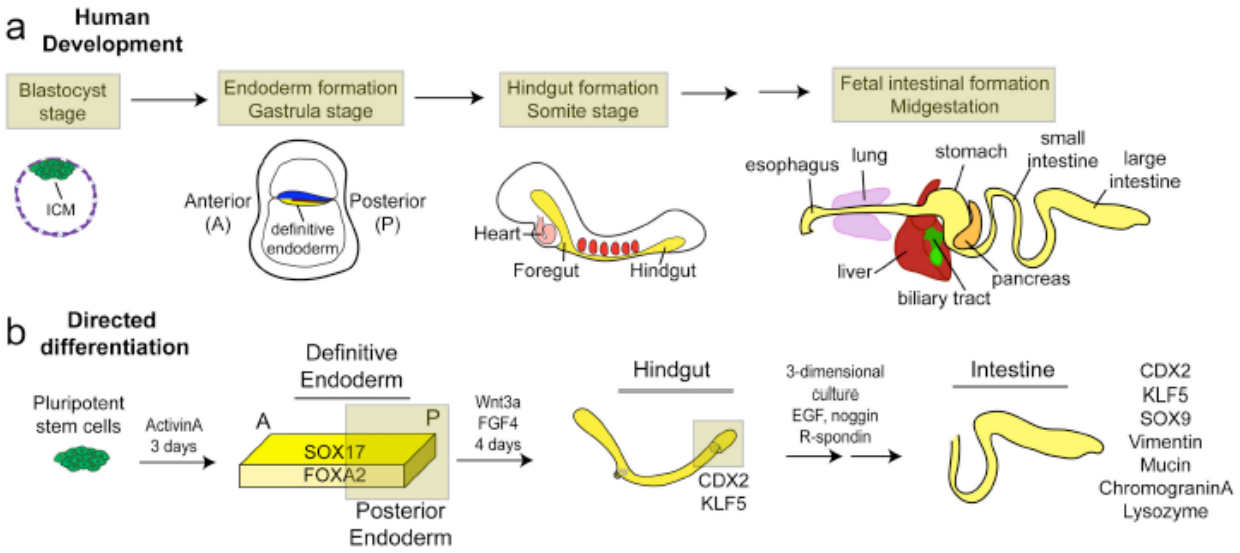
Multipotent SC
-germ layer/ tissue specific progenitor cells

Somatic cell
-highly differentiated

V. Generation of mini-guts from iPSC

Protocol:

1. Differentiation of PSCs into endodermal stem cells
2. Formation of 3D intestine-like organoids

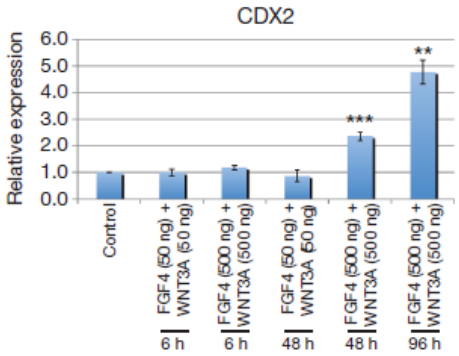


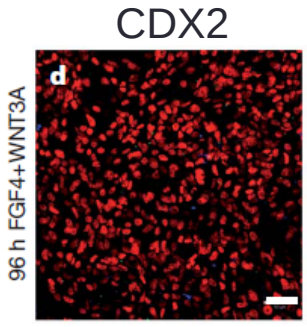
V. Generation of mini-guts from iPSC

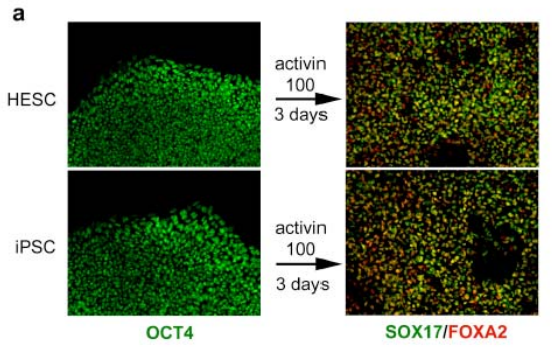
Protocol:

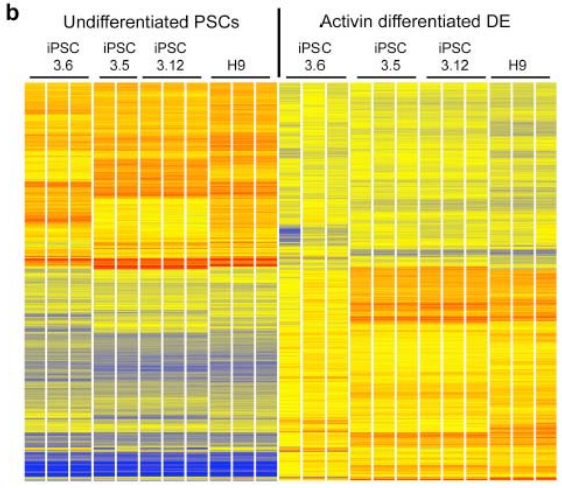
1. Differentiation of PSCs into endodermal stem cells

- Optimization of culture conditions….









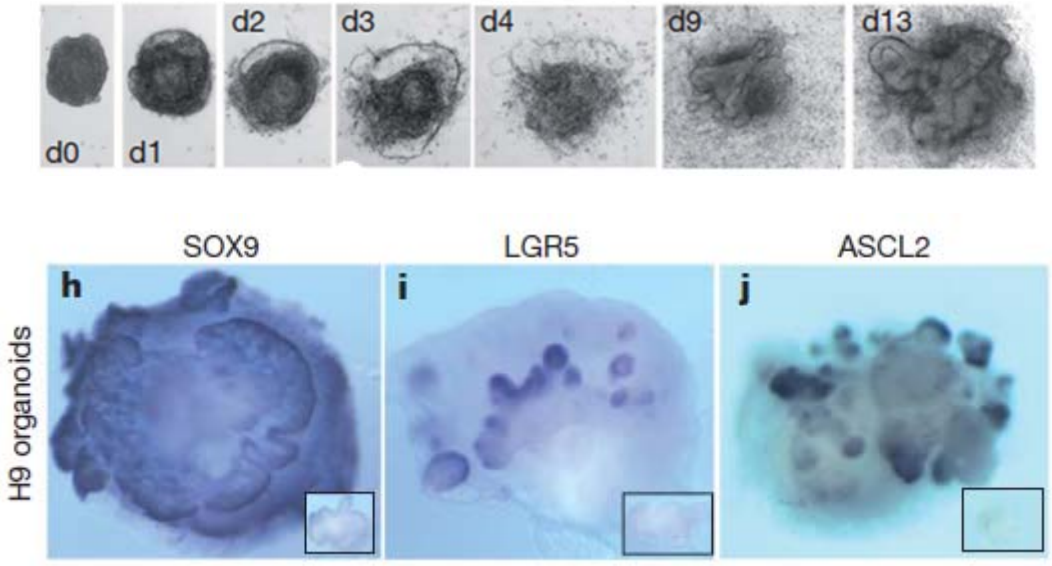
V. Generation of mini-guts from iPSC

Protocol:

2. Formation of 3D intestine-like organoids

- Optimization of culture conditions leads to organoid formation

PSC

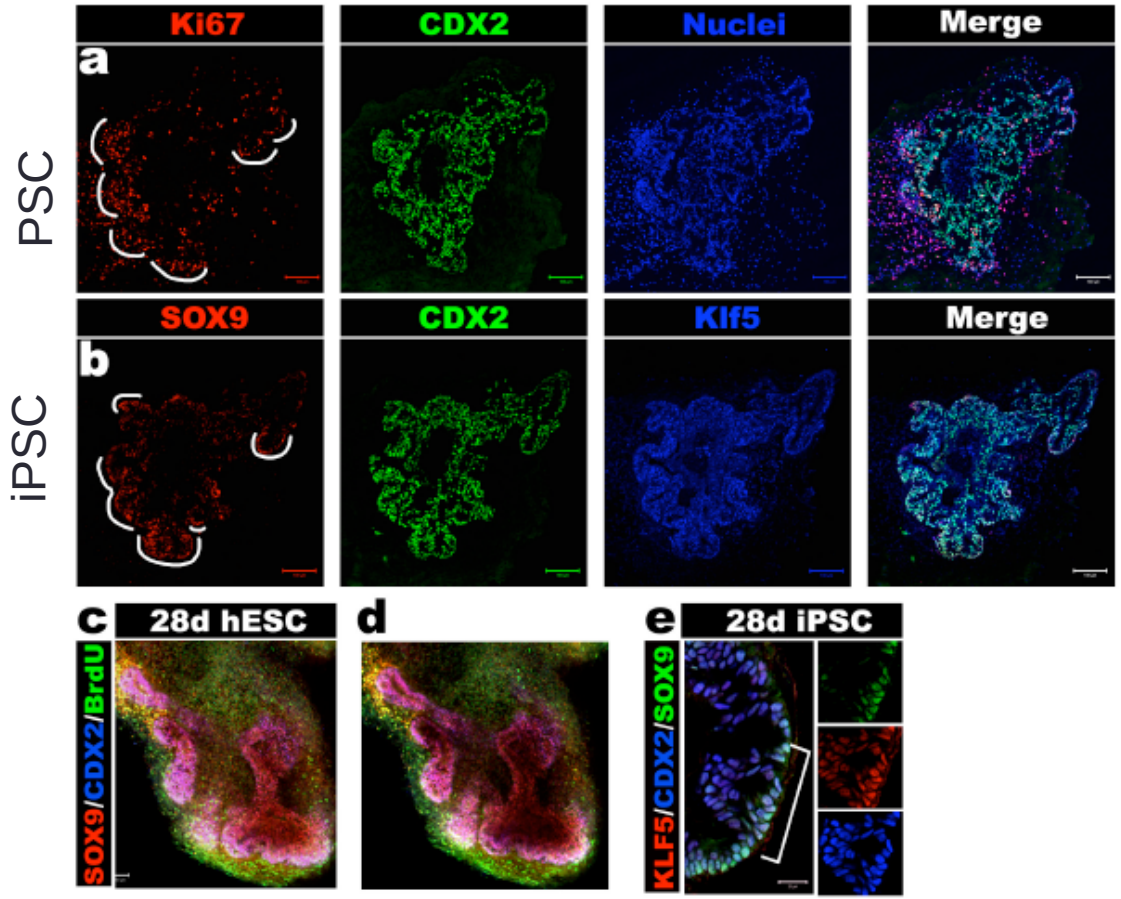


V. Generation of mini-guts from iPSC

Protocol:

2. Formation of 3D intestine-like organoids

- Optimization of culture conditions….

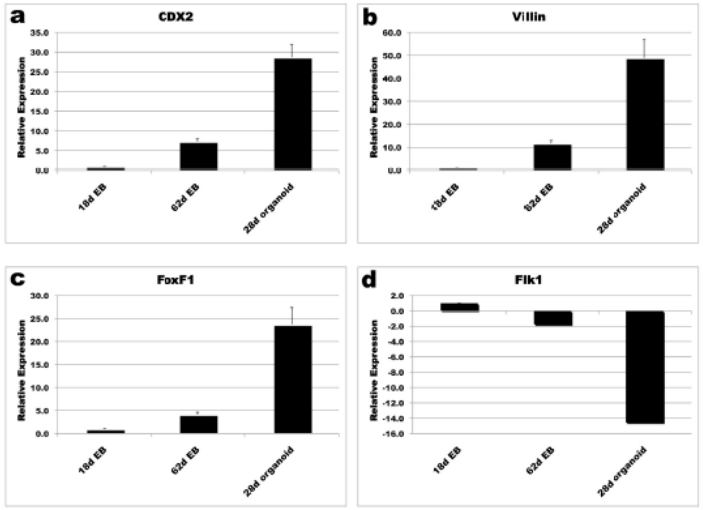


V. Generation of mini-guts from iPSC

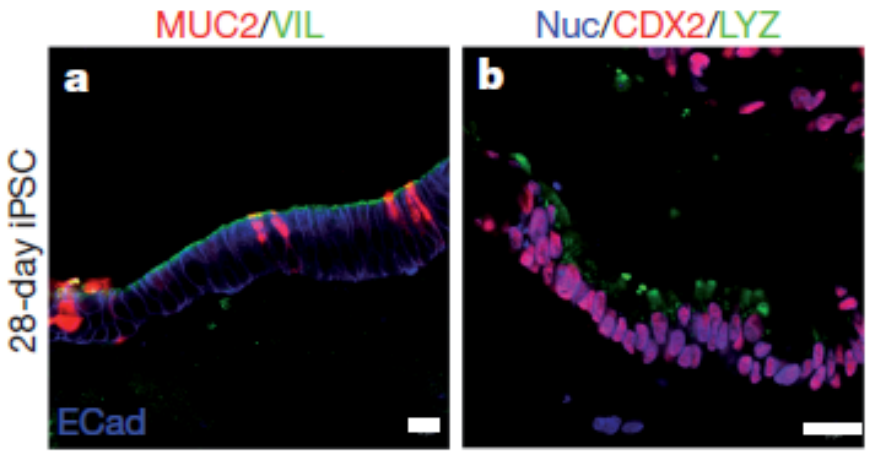
Protocol:

2. Formation of 3D intestine-like organoids

PSC



iPSC

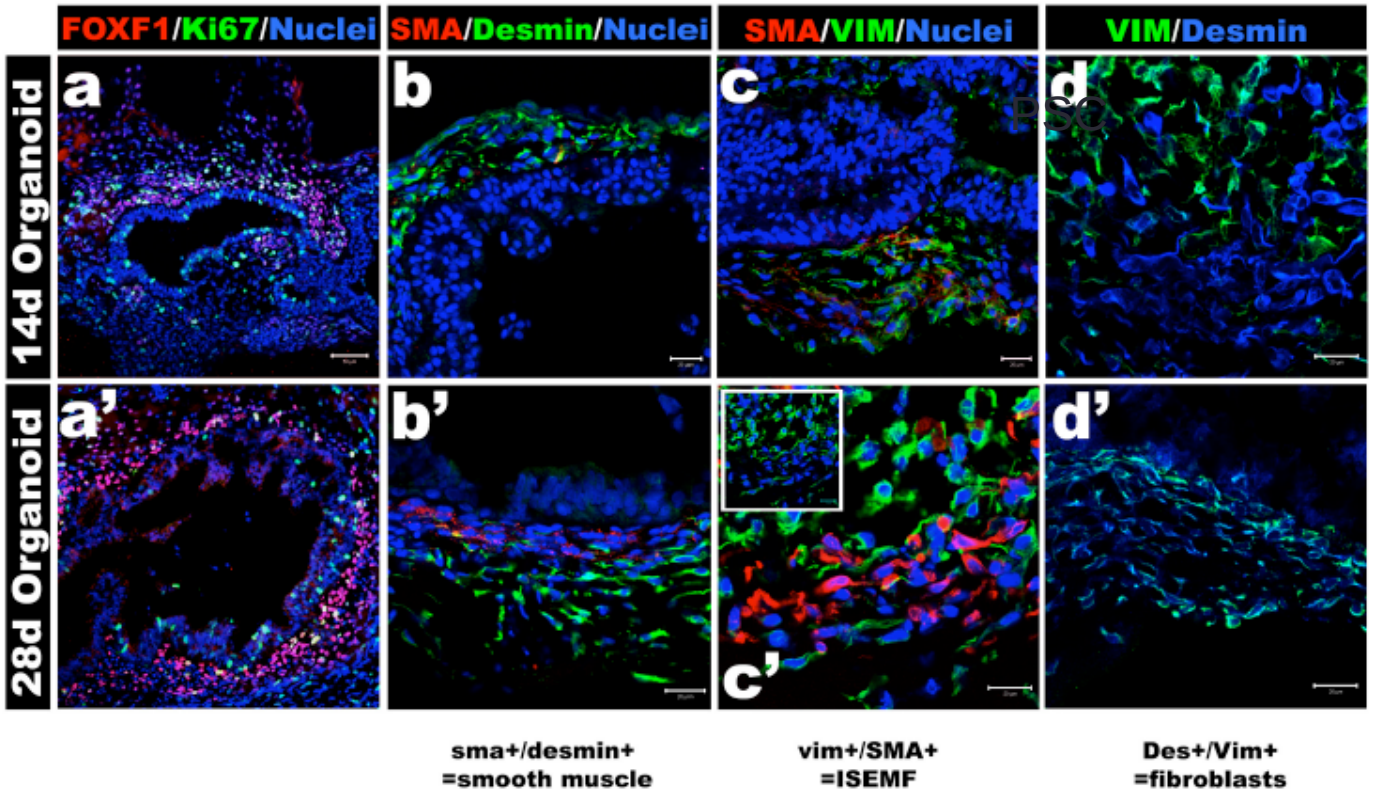


→ Organoids show intestine-like structure and factor expression

V. Generation of mini-guts from iPSC

Protocol:

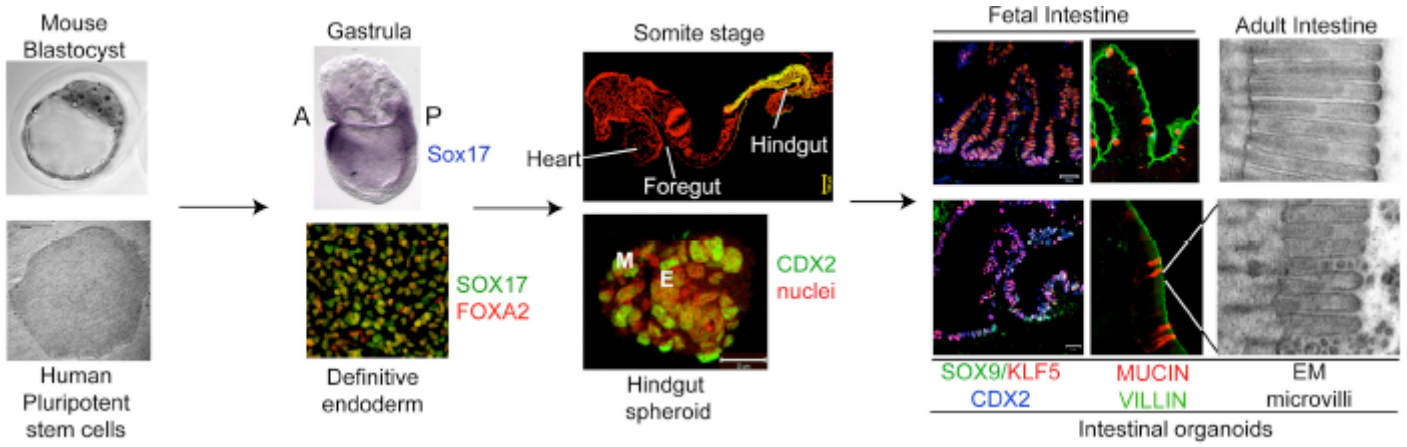
2. Formation of 3D intestine-like organoids



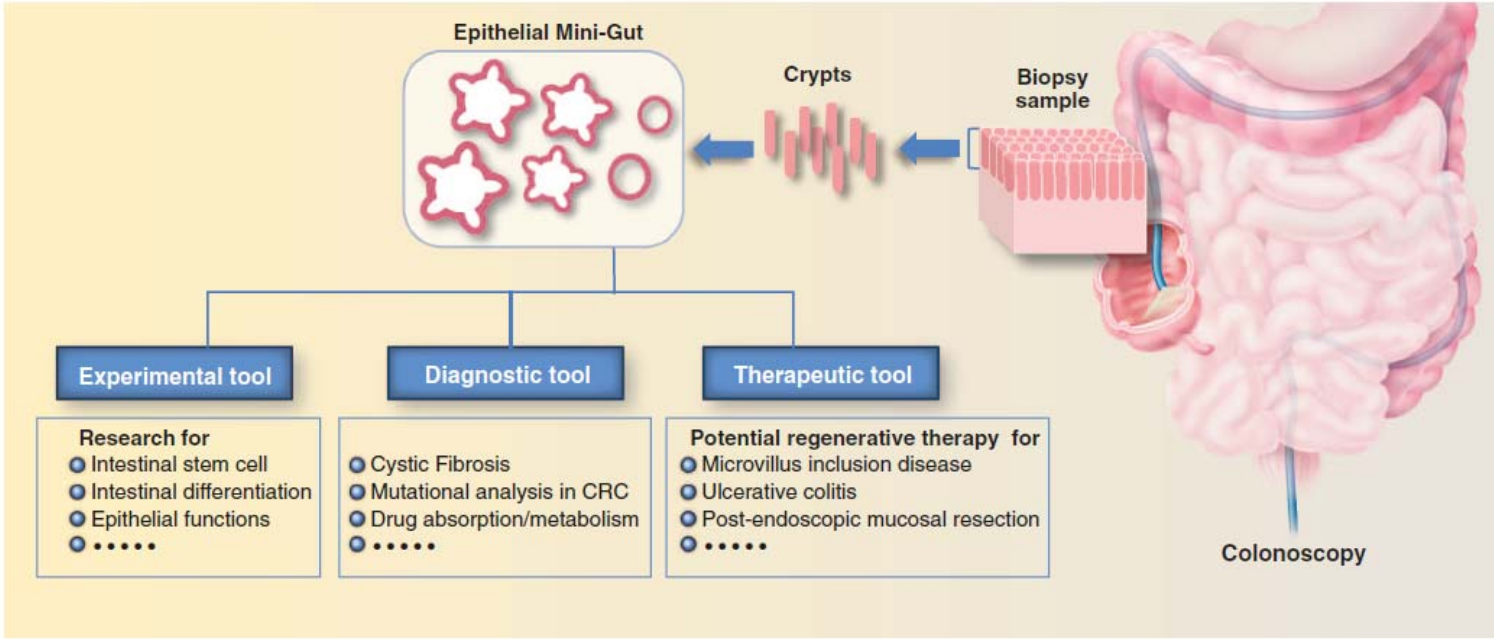
- Organoids show mesenchymal development and maturation

SUMMARY

- hPSC and iPSC are able to differentiate *in vitro* directly in human tissue
- Organoids show 3D architecture and cellular composition similar to intestinal tissue
- Intestinal organoids undergo maturation and develop mesenchymal layer
- → functional studies?



Outlook


Epithelial Mini-Gut
Crypts
Biopsy sample
Colonoscopy
Experimental tool
Diagnostic tool
Therapeutic tool
Research for
Intestinal stem cell
Intestinal differentiation
Epithelial functions
.....
Cystic Fibrosis
Mutational analysis in CRC
Drug absorption/metabolism
.....
Potential regenerative therapy for
Microvillus inclusion disease
Ulcerative colitis
Post-endoscopic mucosal resection
.....