Metabolismo idro-elettrolitico

- L’acqua totale corporea (ATC) è circa il 60% del peso
- Minore nelle donne (55%) e negli obesi
- 2/3 intracellulare, 1/3 extracellulare
- 2/3 dei liquidi extracellulari (LEC) si trovano negli spazi interstiziali e nel connettivo, soltanto 1/4 è intravascolare.

Water Absorption

- 95% of water is absorbed in the small intestines by osmosis
- Water moves in both directions across intestinal mucosa
- Net osmosis occurs whenever a concentration gradient is established by active transport of solutes into the mucosal cells
- Water uptake is coupled with solute uptake, and as water moves into mucosal cells, substances follow along their concentration gradients

Fabbisogno quotidiano

- Un adulto richiede 400, 500ml di acqua al giorno per riuscire ad espellere il carico di soluti delle urine
- 200-300ml si formano per il catabolismo. La quantità minima per evitare il blocco renale è circa 200-300 ml
- La quantità minima per compensare tutte le perdite è di 700-800 ml.
- Una ingestione di più di 25L può portare ad ridotta osmolalità e alterazione della omeostasi dei liquidi corporei

Perdite di acqua

- Evaporazione (aria e pelle); 0,5ml/h/Kg
- Febbre: 50-70 ml/h/Kg per ciascun grado aumentato di temperatura
- Perdite gastrointestinali importanti nel caso di diarrea e vomito.

Osmolalità

- Differenze nella composizione ionica dei liquidi intra ed extracellulare
- All'interno della cellula c'è più K ed all'esterno più Na. Queste differenze vengono mantenute da pompe Na/K.
- L'osmolalità dei liquidi intra ed extra cellulare è simile

$$\text{Plasma osmolality (mOsm/kg)} = 2\,[\text{serum (Na)}] + \frac{[\text{Glucose}]}{18} + \frac{[\text{BUN}]}{2.8}$$

Il sodio

- Quando il Na diminuisce, i fluidi extracellulari si riducono
- La riduzione di volume viene sentita da recettori cardiaci e venosi, ed il rene viene stimolato a conservare più Na.
- Al contrario se c'è un accumulo c'è ritenzione idrica, i sensori carotidei e renali l'avvertono e c'è una maggiore escrezione di Na

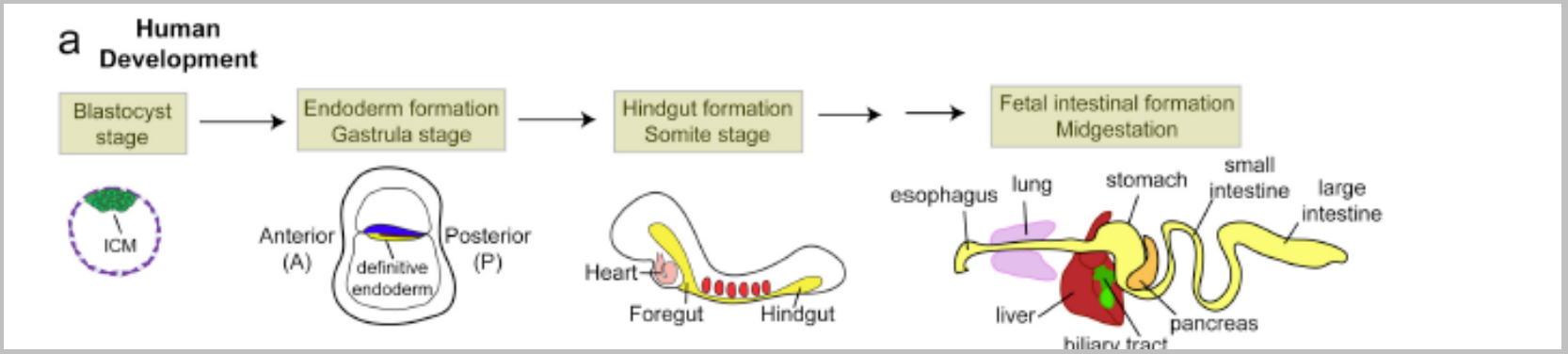
Il contenuto di sodio nell'organismo:

- Assunzione/escrezione renale
- Asse renina- angiotensina. L'angiotensina 2 (clivata dall'angiotensin convertin enzieme ACE) aumenta il riassorbimento di sodio.
- Fattori natriuretici. Uabaina (inibisce le pompe Na-K), i peptidi natriuretici atriali (ANP)

Embryonic Development of the Digestive System

- 3rd week – endoderm has folded and foregut and hindgut have formed
- The midgut is open and continuous with the yolk sac
- Mouth and anal openings are nearly formed
- 8th week – accessory organs are budding from endoderm

Sviluppo intestinale embrionale



Embryonic Development of the Digestive System

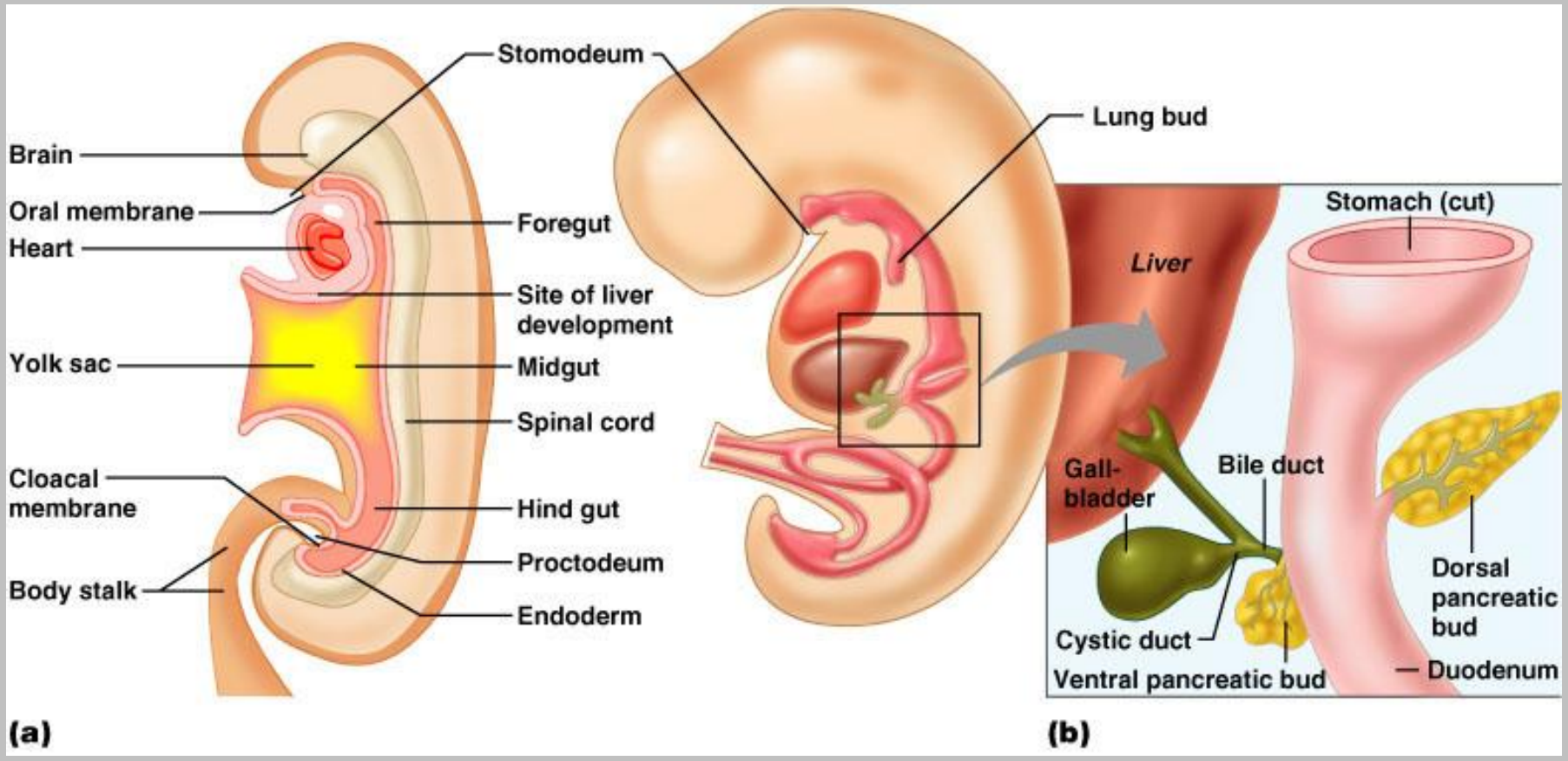


Figure 23.37

Developmental Aspects

- During fetal life, nutrition is via the placenta, but the GI tract is stimulated toward maturity by amniotic fluid swallowed in utero
- At birth, feeding is an infant's most important function and is enhanced by
  - Rooting reflex (helps infant find the nipple) and sucking reflex (aids in swallowing)
- Digestive system has few problems until the onset of old age
- During old age the GI tract activity declines, absorption is less efficient, and peristalsis is slowed

Organoidi:

Strutture che assomigliano ad un organo

Organoidi intestinali

- **Cultura di cripte intestinali**. *“Crypts organoids”*. Possono essere propagate per mesi, non possono essere congelate. Topo, uomo (varie malattie)
- **Organoidi da staminali.** Topo intestino (Lgr5), uomo colon (EPHB)
- **Organoidi da iPS.** (Fibroblasti)

Organoidi da staminale intestinale

- TOPO. 2009. Identificazione del marker della staminale intestinale (Lgr5, **Leucine-rich repeat-containing G-protein coupled receptor 5**)
- TOPO2009-2012. Organoidi da topi transgenici overexpressing Lgr-5-GFP. Impianto in topi con infiammazione. Ricrescita di intestino
- UOMO. 2011. Identificazione di marker di staminale di colon EPHB Ephirin type b receptor 2. E Lgr5

Stem cells organoids

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]

Le cripte intestinali si possono coltivare e propagare in coltura

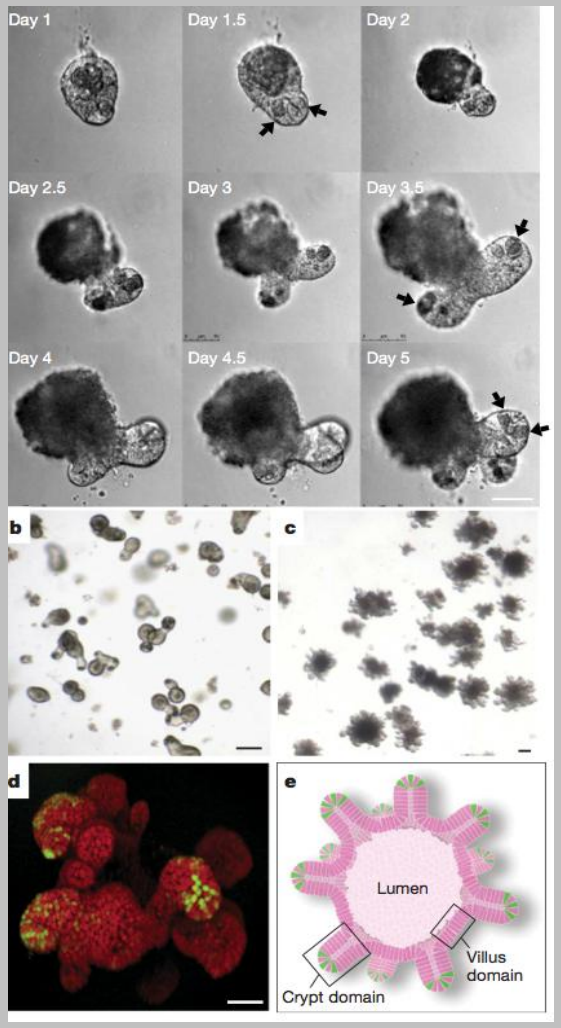


Figure 1 | Establishment of intestinal crypt culture system. **a**, Time course of an isolated single crypt growth. Differential interference contrast image reveals granule-containing Paneth cells at crypt bottoms (arrows). **b**, **c**, Single isolated crypts efficiently form large crypt organoids within 14 days; **b**, on day 5; **c**, on day 14. **d**, Three-dimensional reconstructed confocal image after 3 weeks in culture. Lgr5–GFP^{+} stem cells (green) are localized at the tip of crypt-like domains. Counterstain, ToPro-3 (red). **e**, Schematic representation of a crypt organoid, consisting of a central lumen lined by villus-like epithelium and several surrounding crypt-like domains. Scale bar, 50 μm.

Una singla cellula Lgr5 positiva genera strutture cripte-villi

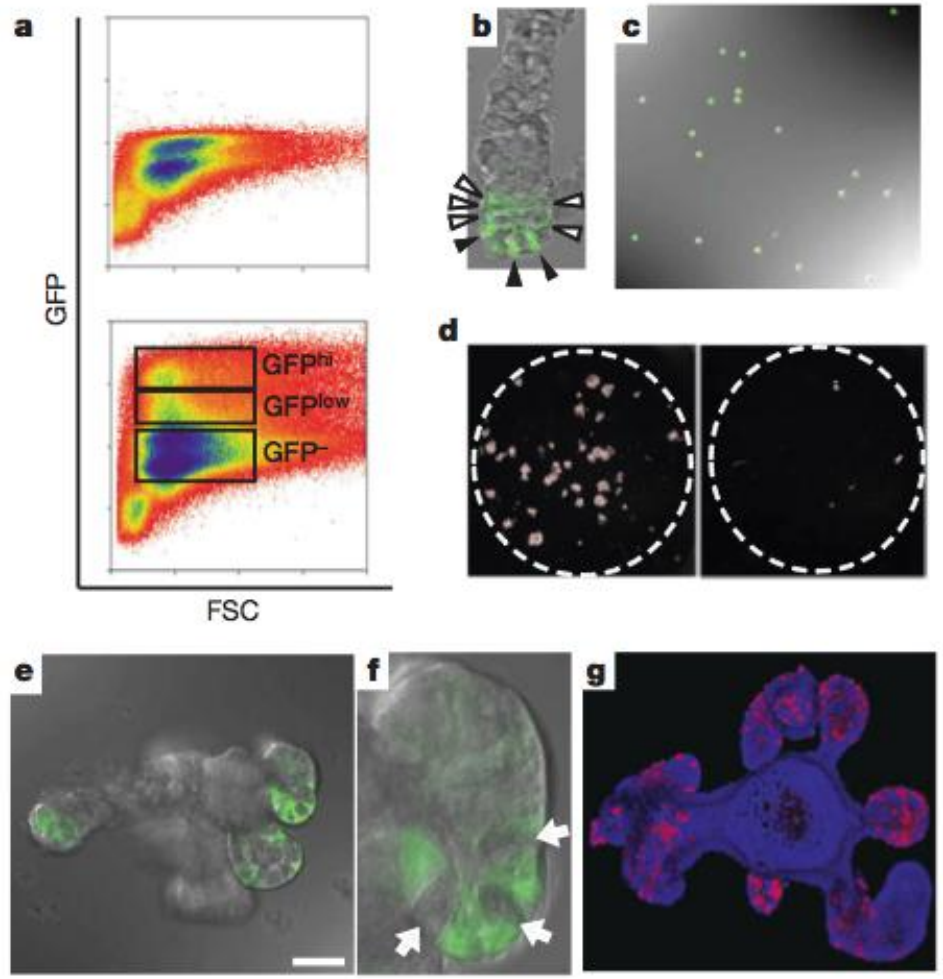


Figure 2 | Single Lgr5$^+$ cells generate crypt–villus structures. **a**, Lgr5–GFP$^+$ cells from an *Lgr5–EGFP–ires–CreERT2* intestine (bottom); wild-type cells (top). Two positive populations, GFP^{hi} and GFP^{low}, are discriminated. FSC, forward scatter. **b**, Confocal analysis of a freshly isolated 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).

Trattamenti:

- R spondin, agonista di WNT (induce crypts iperplasia in vitro)
- Nogging, inibisce TGF beta (aumenta il numero delle cripte)
- -EGF, proliferazione enterociti

Necessario attivare il pathway di WNT che agisce su EPHB2 e Lgr5

Efficienza

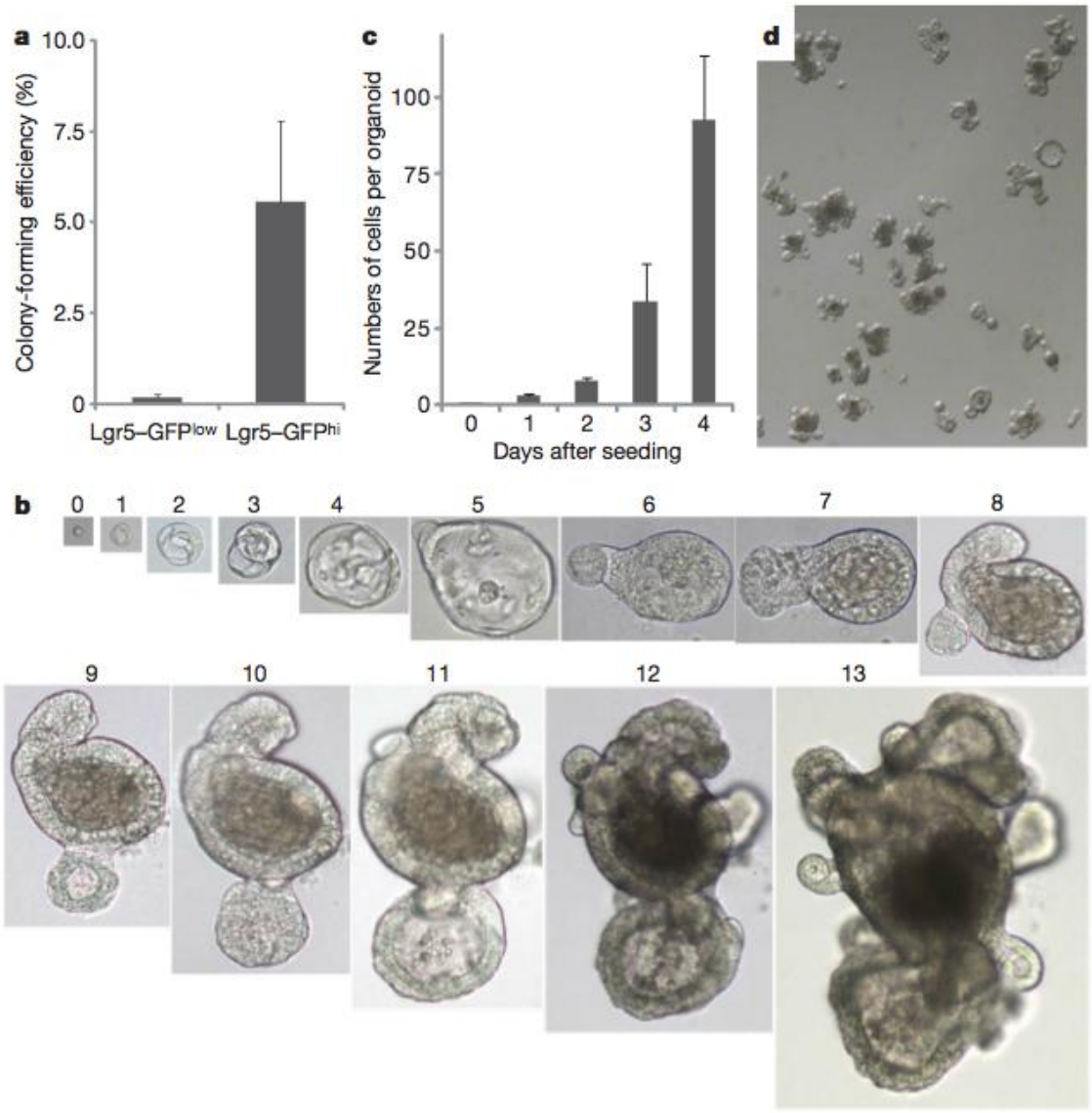


Figure 3 | Colony-forming efficiency of single cells sorted in individual wells. **a**, Colony-forming efficiency was calculated from 100 single sorted GFP^{hi} cells. **b**, An example of a successfully growing single GFP^{hi} cell. Numbers above the images are the days of growth. **c**, Numbers of cells per single organoid averaged for five growing organoids. **d**, A single-cell suspension derived from a single-cell-derived-organoid was replated and grown for 2 weeks. Error bars in **c** and **d** indicate s.e.m. Original magnifications in **b**: days 0–4, ×40; days 5–7, ×20; days 8–11, ×10; days 12 and 13, ×4.

Organizzazione di organoidi derivati da singola cellula Lgr5 positiva

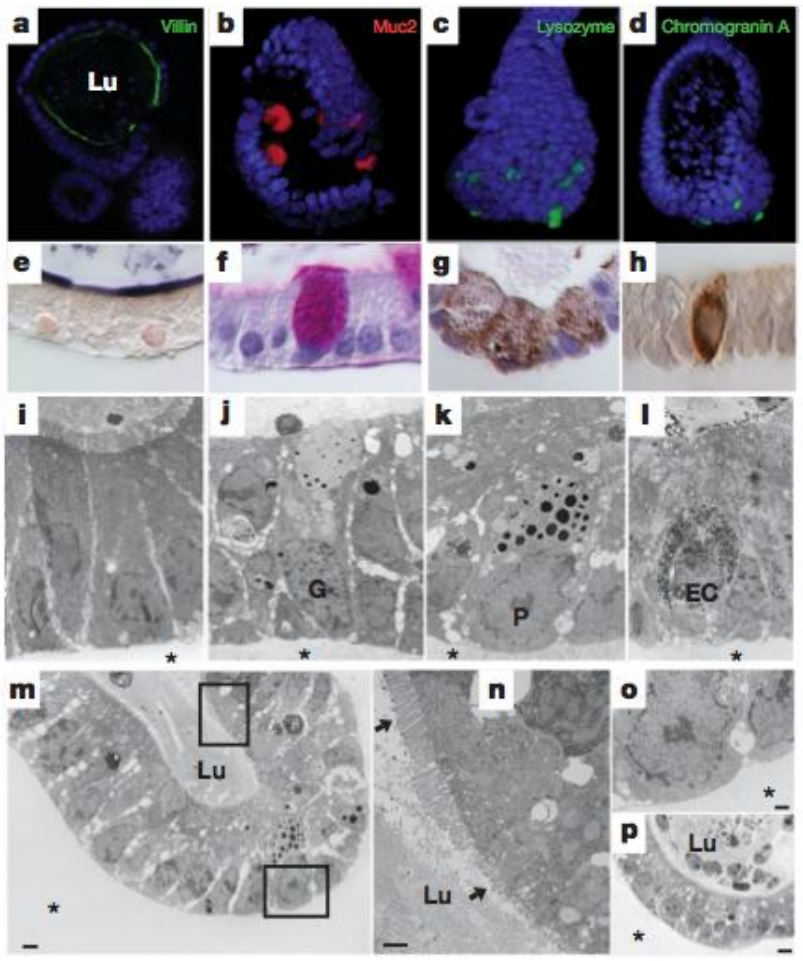


Figure 4 | Composition of single stem cell-derived organoids. **a–d**, Confocal image for villin (**a**, green, enterocytes), Muc2 (**b**, red, goblet cells), lysozyme (**c**, green, Paneth cells) and chromogranin A (**d**, green, enteroendocrine cells). Counterstain, DAPI (blue). **e–h**, Paraffin sections stained for alkaline phosphatase (**e**, green, enterocytes), periodic acid-Schiff (**f**, red, goblet cells), lysozyme (**g**, brown, Paneth cells) and synaptophysin (**h**, brown, enteroendocrine cells). **i–p**, Electron microscopy demonstrates enterocytes (**i**), goblet cells (**j**), Paneth cells (**k**) and enteroendocrine cells (**l**). **m–o**, Low-power crypt images. **n**, **o**, Higher magnifications of **m**. **n**, Maturation of brush border (black arrows). **p**, Low-power villus domain image. Lu, lumen with apoptotic bodies, lined by polarized enterocytes. G, goblet cells; EC, enteroendocrine cells; P, Paneth cells; *, Matrigel. Scale bars, 5 μm (m, p) and 1 μm (n, o).

L'attivazione del pathway di WNT e di EGF è necessaria per la formazione di organoidi

METHODS SUMMARY

Mice. Outbred mice 6–12 weeks old were used. Generation and genotyping of the *Lgr5–EGFP–Ires–CreERT2* allele[1] has been described previously[3]. Rosa26–lacZ or YFP–Cre reporter mice were obtained from Jackson Labs.

Crypt isolation, cell dissociation and cell culture. Crypts were released from murine small intestine by incubation for 30 min at 4 °C in PBS containing 2 mM EDTA (Supplementary Methods). Isolated crypts were counted and pelleted. A total of 500 crypts were mixed with 50 µl of Matrigel (BD Bioscience) and plated in 24-well plates. After polymerization of Matrigel, 500 µl of crypt culture medium (Advanced DMEM/F12 (Invitrogen)) containing growth factors (10–50 ng ml^{-1} EGF (Peprotech), 500 ng ml^{-1} R-spondin 1 (ref. 11) and 100 ng ml^{-1} Noggin (Peprotech)) was added. For sorting experiments, isolated crypts were incubated in culture medium for 45 min at 37 °C, followed by trituration with a glass pipette. Dissociated cells were passed through cell strainer with a pore size of 20 µm. GFP^{hi}, GFP^{low} and GFP^{-} cells were sorted by flow cytometry (MoFlo; Dako). Single viable epithelial cells were gated by forward scatter, side scatter and pulse-width parameter, and by negative staining for propidium iodide. Sorted cells were collected in crypt culture medium and embedded in Matrigel containing Jagged-1 peptide (1 µM; AnaSpec) at 1 cell per well (in 96-well plates, 5 µl Matrigel). Crypt culture medium (250 µl for 48-well plates, 100 µl for 96-well plates) containing Y-27632 (10 µM) was overlaid. Growth factors were added every other day and the entire medium was changed every 4 days. For passage, organoids were removed from Matrigel and mechanically dissociated into single-crypt domains, and then transferred to fresh Matrigel. Passage was performed every 1–2 weeks with a 1:5 split ratio.

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

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

Distribuzione delle cellule paneth e delle cellule Lgr5 positive

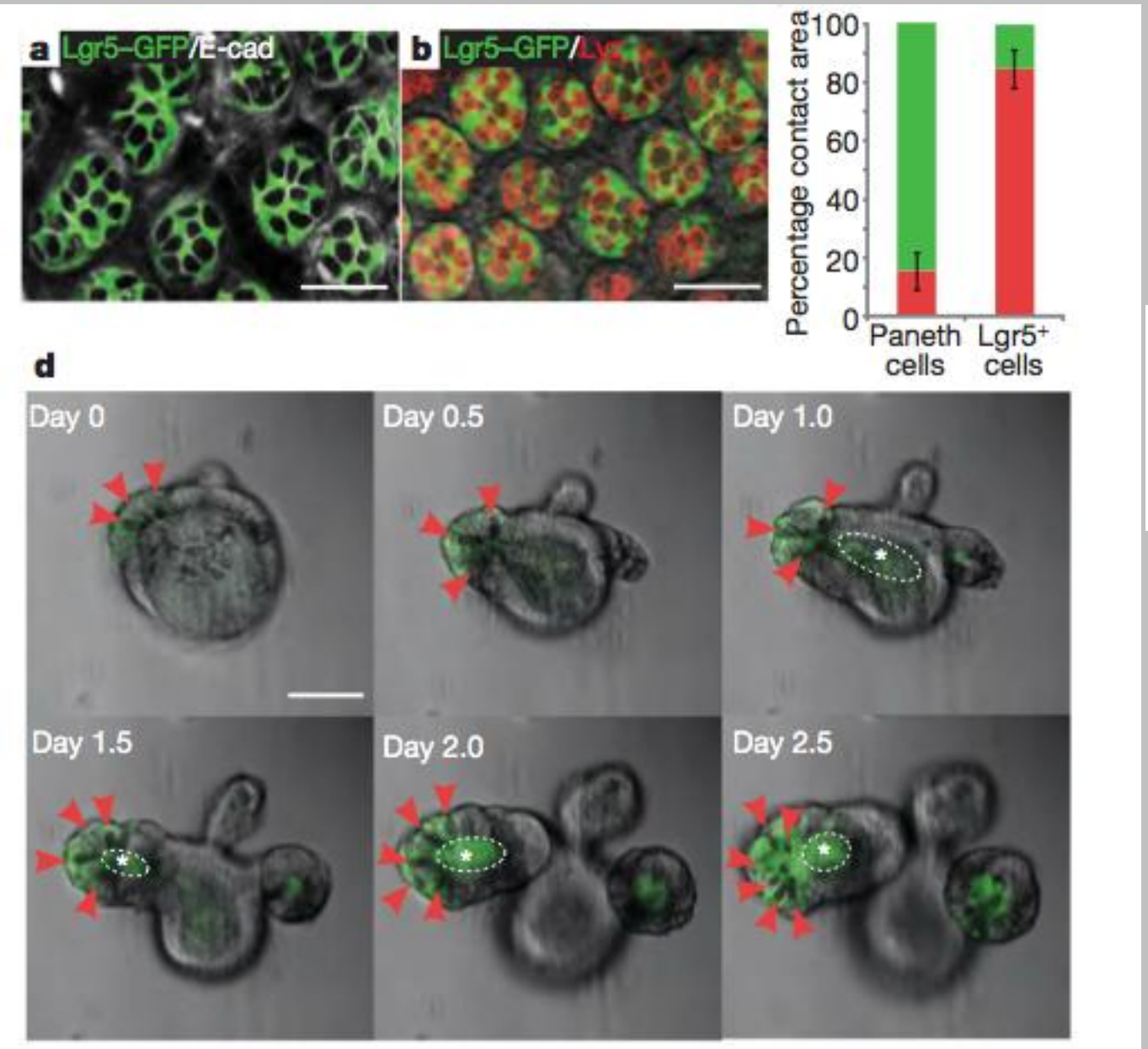


Figure 1 | Geometric distribution pattern of Paneth cells and Lgr5 stem cells. **a**, **b**, Confocal cross-section of *Lgr5-EGFP-ires-creERT2* intestine. E-cadherin (**a**; white) demarcates cell borders. Lgr5 stem cells (green) and Paneth cells (**a**, black; **b**, lysozyme, red) are shown. **c**, Contact area of either Paneth cells or Lgr5 stem cells was quantified with Image J. The values are depicted as mean ± s.d. from three independent mice. Red columns and green columns indicate contact area with Paneth cells and Lgr5 stem cells, respectively. **d**, Stills from Supplementary Movie 1. Time course of crypt organoid growth. Differential interference contrast image reveals granule-containing Paneth cells (red arrowheads) at the site of budding where a new crypt forms. Lgr5–GFP (green) stem cells expand at the crypt base in close proximity to Paneth cells. Asterisk and dotted oval indicate autofluorescence. Scale bar: 50 µm.

Le cellule paneth supportano la crescita delle Lgr5 stem cells

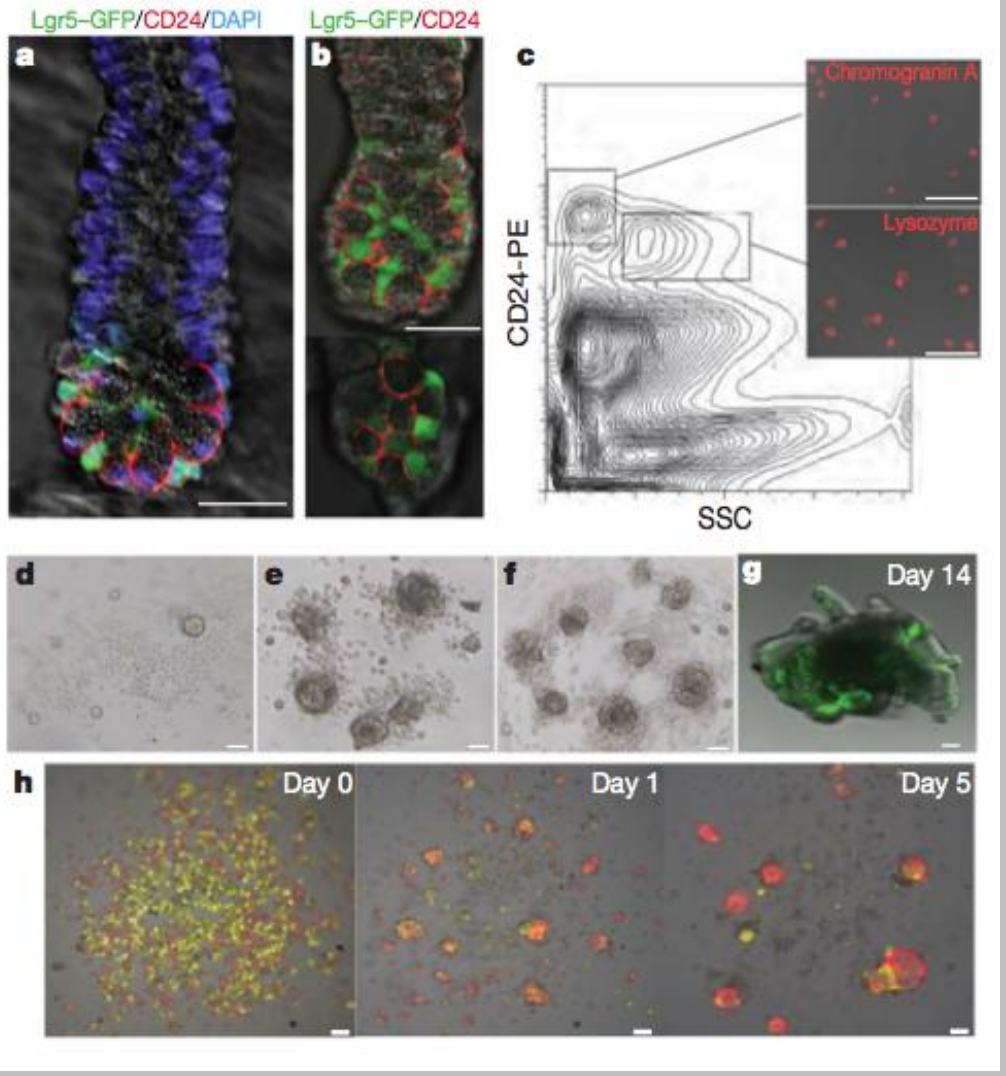


Figure 2 | Paneth cells express CD24 and support growth of Lgr5 stem cells. **a**, Isolated small intestinal crypt. CD24 (red) is expressed by Paneth cells in which granules are visualized by differential interference contrast. Lgr5–GFP^{+} stem cells are green. Counter stain: DAPI (blue). **b**, Isolated colonic crypt. CD24^{+} cells (red) are in intimate contact with Lgr5 stem cells (green). Top, longitudinal crypt section; bottom, section through crypt bottom. **c**, FACS plot of dissociated single cells from small intestinal crypts. Two CD24 bright populations differ by side-scatter (SSC) pattern. Sorted CD24^{hi}SSC^{low} and CD24^{hi}SSC^{hi} cells are subsequently stained. CD24^{hi}SSC^{low} cells are positive for the enteroendocrine marker chromogranin A (top right), whereas CD24^{hi}/SSC^{hi} cells are positive for the Paneth marker lysozyme (bottom right). **d–g**, Single sorted Lgr5 stem cells from *Lgr5-EGFP-ires-creERT2* small intestine (**d**), sorted single Paneth cells (**e**) from wild-type small intestine, and a combination of the two cell types (**f**) were seeded in round-bottomed wells and cultured for 2 days. **g**, Lgr5 stem cells form expanding Lgr5–GFP^{+} (green) organoids only when reassociated with Paneth cells. **h**, Stills from Supplementary Movie 2. Time course of the reassociation culture with RFP^{+} Lgr5–GFP stem cells (red) and YFP^{+} Paneth cells (yellow). Scale bar, 50 μm.

Paneth cells producono WNT3 ed altri segnali necessari

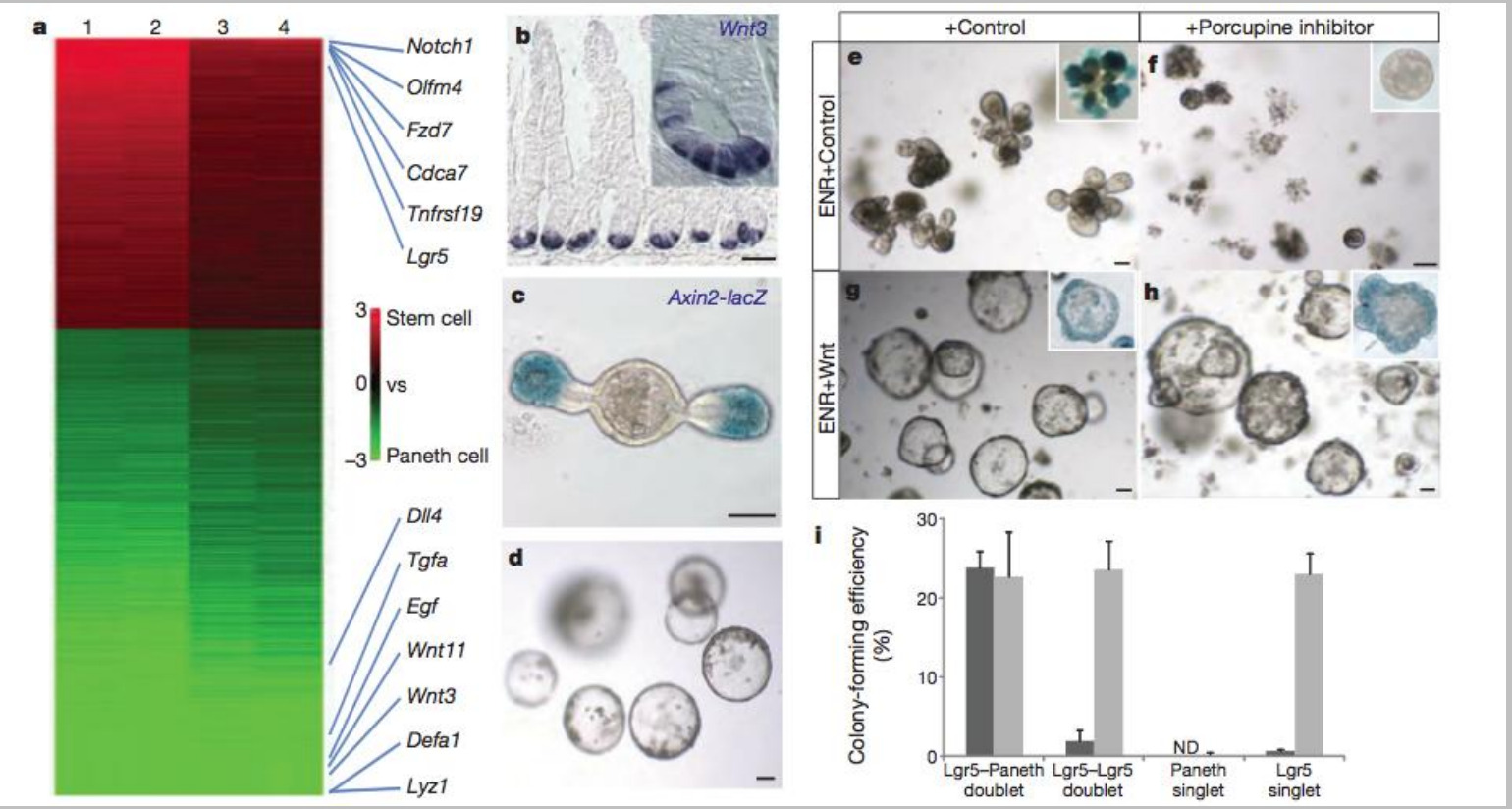


Figure 3 | Paneth cells produce Wnt3 and other essential niche signals for Lgr5 stem cells. **a**, Heat-map of two independent microarray expression experiments (1 and 2; 3 and 4) performed with dye-swap (1 versus 2 and 3 versus 4) from sorted Paneth cells versus Lgr5 stem cells. **b**, *Wnt3* is expressed by Paneth cells at crypt bottoms as analysed by *in situ* hybridization. **c–h**, Localized Wnt production regulates crypt–villus morphogenesis in culture. **c**, Freshly isolated crypts from an *Axin2-lacZ* mouse were cultured in standard EGF/noggin/R-spondin 1 medium (ENR) for 4 days. LacZ response is only seen near the bottoms of the two crypts. **d**, Intestinal adenoma samples from APC^{min} mice were cultured in ENR medium in the absence of R-spondin for 7 days. **e**, Axin2-LacZ crypts grown in ENR medium. **f**, As in **e**, with the addition of porcupine inhibitor IWP1 at 1 μM. **g**, Crypts from an *Axin2-lacZ* mouse cultured in ENR medium plus Wnt3A. **h**, Same as **g**, with the addition of IWP1. Insets in **e–h** depict Axin2-LacZ expression (blue). **e**, **g**, **h**, Six days culture; **f**, 3 days culture after which the organoids disintegrate. See also Supplementary Fig. 2. **i**, Plating efficiency of Lgr5 stem cell–Paneth doublets, Lgr5 stem cell doublets, single Paneth cells and single Lgr5 stem cells with (grey) or without (black) Wnt3A at 100 ng ml^{-1}. Assays were read out as budding organoids at 14 days after sorting. The values are depicted as mean ± standard error of the mean (s.e.m.) from three independent experiments. ND, not detected. See also Supplementary Fig. 3 and Methods for details of doublet isolation and culture. All scale bars, 50 μm.

Le cellule di Paneth regolano il numero di cellule staminali in vivo

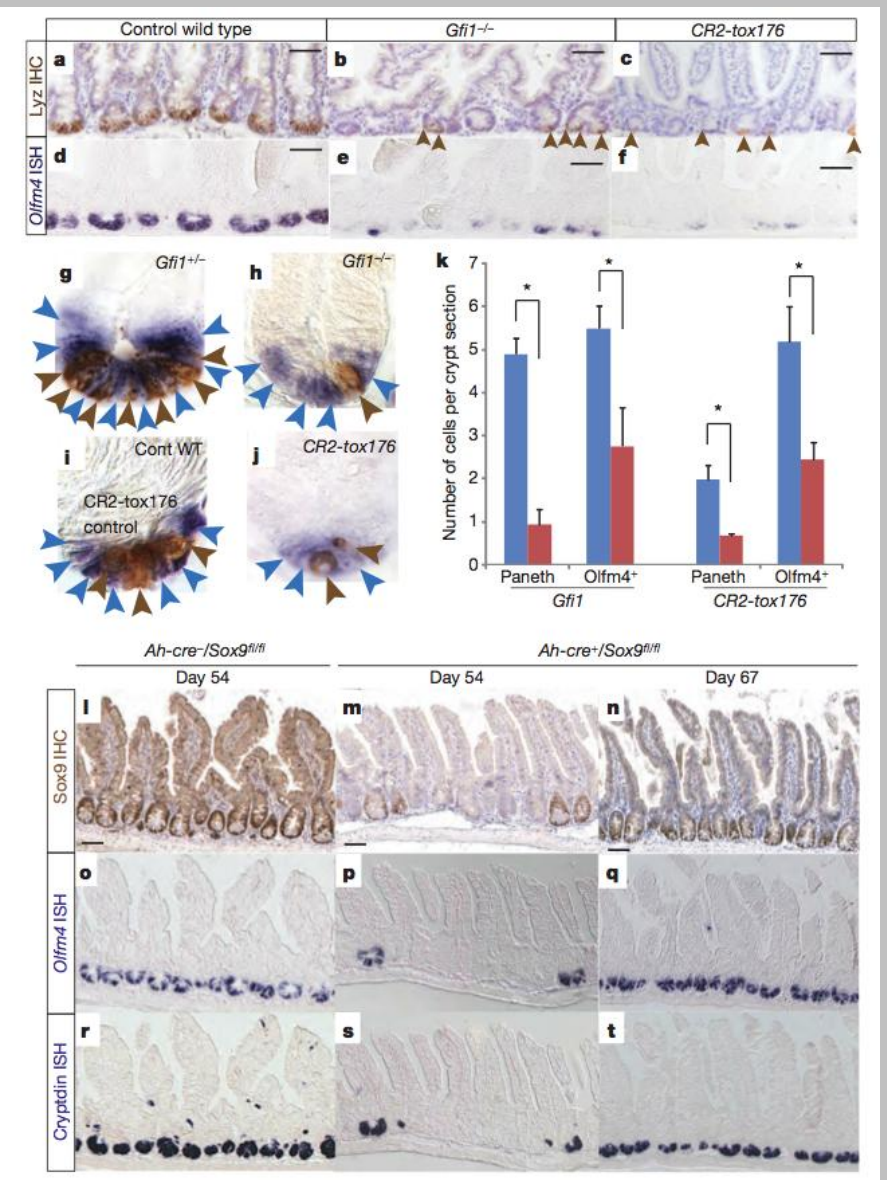


Figure 4 | Paneth cells regulate numbers of intestinal stem cells *in vivo*. **a–k**, Paneth cells and stem cells in constitutive models of Paneth cell decrease. **a–f**, Lysozyme stain (**a–c**; brown arrowheads indicate positive cells) and *Olfm4* staining (**d–f**) of crypts of adult (6–7-week-old) mice of the indicated genotype. **g–j**, Double stain (lysozyme, brown; *Olfm4*, blue) of a representative crypt of the indicated genotype. Brown and blue arrowheads indicate Paneth cells and stem cells, respectively. **k**, Quantification of stem and Paneth cell numbers in both models. For each bar, 100 crypts were scored for each of three mice. Mutant mice are indicated by red bars and control mice by blue bars. $*P < 0.01$. **l–t**, Paneth cells and stem cells in inducible Paneth cell depletion model. Mice of the indicated genotype were injected with β-napthoflavone to induce Cre and were analysed 54 or 67 days after Cre induction by staining for Sox9 protein (brown), *Olfm4* or cryptdin mRNA (blue). Serial sections: **i**, **o**, **r**; **m**, **p**, **s**; and **n**, **q**, **t**. See text for experimental detail. Note the absence of Paneth cells (**s**) and stem cells (**p**) in $Sox9^{-/-}$ crypts (**m**). All scale bars, 50 μm.

Long-term Expansion of Epithelial Organoids From Human Colon, Adenoma, Adenocarcinoma, and Barrett's Epithelium

Toshiro Sato[*], Daniel E. Stange[*], Marc Ferrante[*, ‡, §], Robert G.J. Vries[*], Johan H. van Es[*], Stieneke van den Brink[*], Winan J. van Houdt[‖, ¶], Apollo Pronk[‖], Joost van Gorp[#], Peter D. Siersema[‡], Hans Clevers[*]

Table 1. Optimized Culture Conditions

		Stem cell culture condition										Differentiation condition
Species	Organ	Basal culture medium	EGF	Noggin	R-spondin	Wnt3A	Nicotinamide	Gastrin	A-83-01	SB202190	Fibroblast growth factor 10	Withdraw (−) or add (+) indicating factors
Mouse	Small intestine	○	○	○	○	–	–	–	–	–	–	
	Colon	○	○	○	○	○	–	–	–	–	–	−Wnt3A
	Adenoma	○	○	–	–	–	–	–	–	–	–	
Human	Small intestine	○ + gastrin	○	○	○	○	○	○	○	○	–	−Wnt3A−SB202190−nicotinamide
	Colon	○ + gastrin	○	○	○	○	○	○	○	○	–	−Wnt3A−SB202190−nicotinamide
	Colon cancer	○ + gastrin	Varied	–	–	–	–	–	Varied	Varied	–	
	Barrett's epithelium	○ + gastrin	○	○	○	○	○	○	○	○	○	−Wnt3A−SB202190−nicotinamide+DBZ

NOTE. Basal culture medium: Advanced Dulbecco's modified Eagle medium/F12 supplemented with penicillin/streptomycin, HEPES, Glutamax, 1× N2, 1× B27, and *N*-acetylcysteine. Concentration is indicated in Supplen

Colture di colon umane

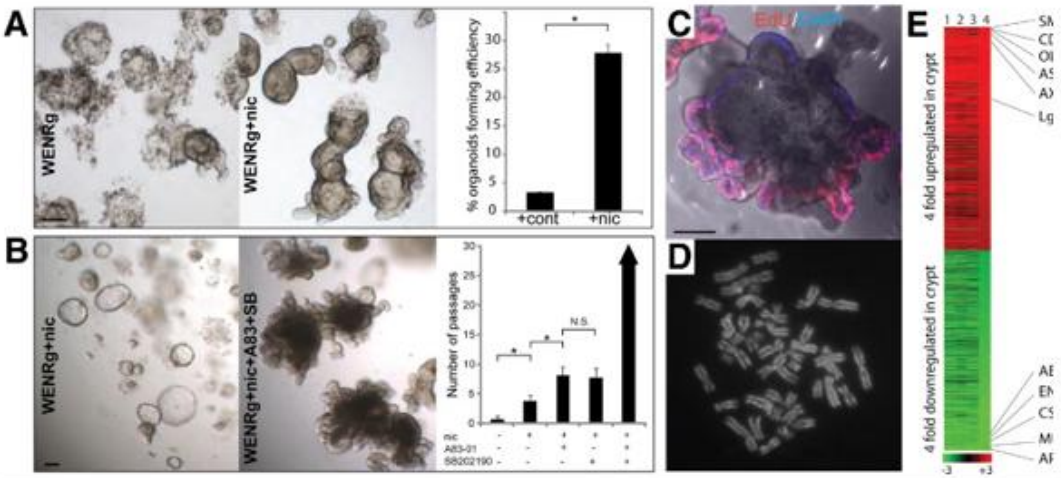


Figure 2. Human colon culture. (*A*) The effect of nicotinamide on human colon crypt organoids. The majority of human colon crypt organoids die within a few days in WENR + gastrin (WENRg) condition (*left panel*). Addition of nicotinamide (*middle panel*: WENRg + nicotinamide [nic]) improves culture efficiency and life span of human colon organoids. **P* < .001. (*B*) The effect of small molecule inhibitor for Alk4/5/7 (A83-01) and for the mitogen-activated protein kinase p38 (SB202190) on human colon crypt organoids. (*Left panel*) Human colon organoid cultures in WENRg + nicotinamide–containing medium form cystic structures 3 to 4 weeks after culture. (*Middle panel*) Human colon organoids retain their characteristic budding structure under the HISC condition (WENRg + nicotinamide + A83-01 + SB202190). (*Right panel*) A83-01 and SB202190 synergistically increase the number of passages of the human colon organoids. **P* < .001. N.S., not statistically significant. *Error bars* indicate SEM. n = 5. (*C*) Proliferating cells visualized by the incorporation of 5-ethynyl-2′-deoxyuridine (EdU) (*red*) are confined to the budding structures. Counterstain is DAPI (*blue*). (*D*) Representative picture of a karyotype from a 3-month-old human colon crypt organoid. *Scale* = 100 μm. (*E*) Heat map of the expression profile of cultured human intestinal organoids. Organoids cultured in vitro clearly exhibit a similar expression profile to freshly isolated small intestinal crypts and express known stem cell markers. *Lane 1*, human small intestinal organoids #1; *lane 2*, human small intestinal organoids #2; *lane 3*, human colon organoids; *lane 4*, freshly isolated human small intestinal crypts. The 4 samples are compared with human small intestinal villus.

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Composizione degli organoidi intestinali

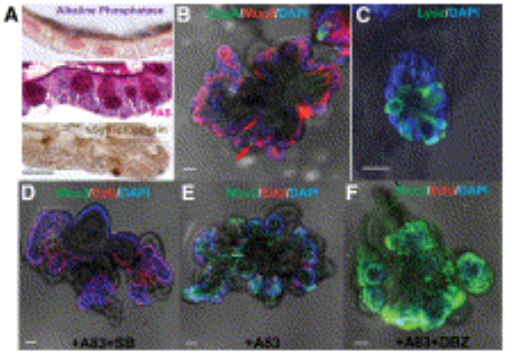

Figure 3. Human intestinal organoid cell type composition. (*A–C*) Human organoids differentiate into the different cell types of the intestine after withdrawal of nicotinamide and SB202190. Markers of the different cell types were used to show differentiation. (*A*) (*top panel*) Alkaline phosphatase (*purple*) for mature enterocytes. (*Middle panel*) Periodic acid–Schiff (PAS) staining (*purple*) for goblet cells. (*Bottom panel*) Synaptophysin (*brown*) for enteroendocrine cells. (*B*) Mucin2 (Muc2; *red*) for goblet cells and Chromogranin A (ChgA; *green*) for enteroendocrine cells (*red arrow and inset*). (*C*) Lysozyme (Lysz; *green*) for Paneth cells. (*D–F*) Goblet cell differentiation (Muc2, *green*) is blocked by SB202190 treatment of organoids (*D*), while the Notch inhibitor DBZ increases goblet cell number in the human organoids (*F*). Proliferating cells (5-ethynyl-2′-deoxyuridine [EdU] incorporation: *red*) are increased in SB202190-treated organoids (*D*) or decreased in DBZ-treated organoids (*F*). Organoids are cultured under the following conditions for 5 days: (*A*, *top*) ENRg + A83-01 + SB202190 + nicotinamide, (*A*, *middle and bottom*, *B*, *and C*) WENRg + A83-01, (*D*) WENRg + A83-01 + SB202190, (*E*) WENRg + A83-01, and (*F*) WENRg + A83-01 + DBZ. *Scale bar*: *A*, 20 μm; *B–F*, 50 μm. *A*, *B*, *and D–F*, human colon crypt organoids; *C*, human small intestinal organoids.

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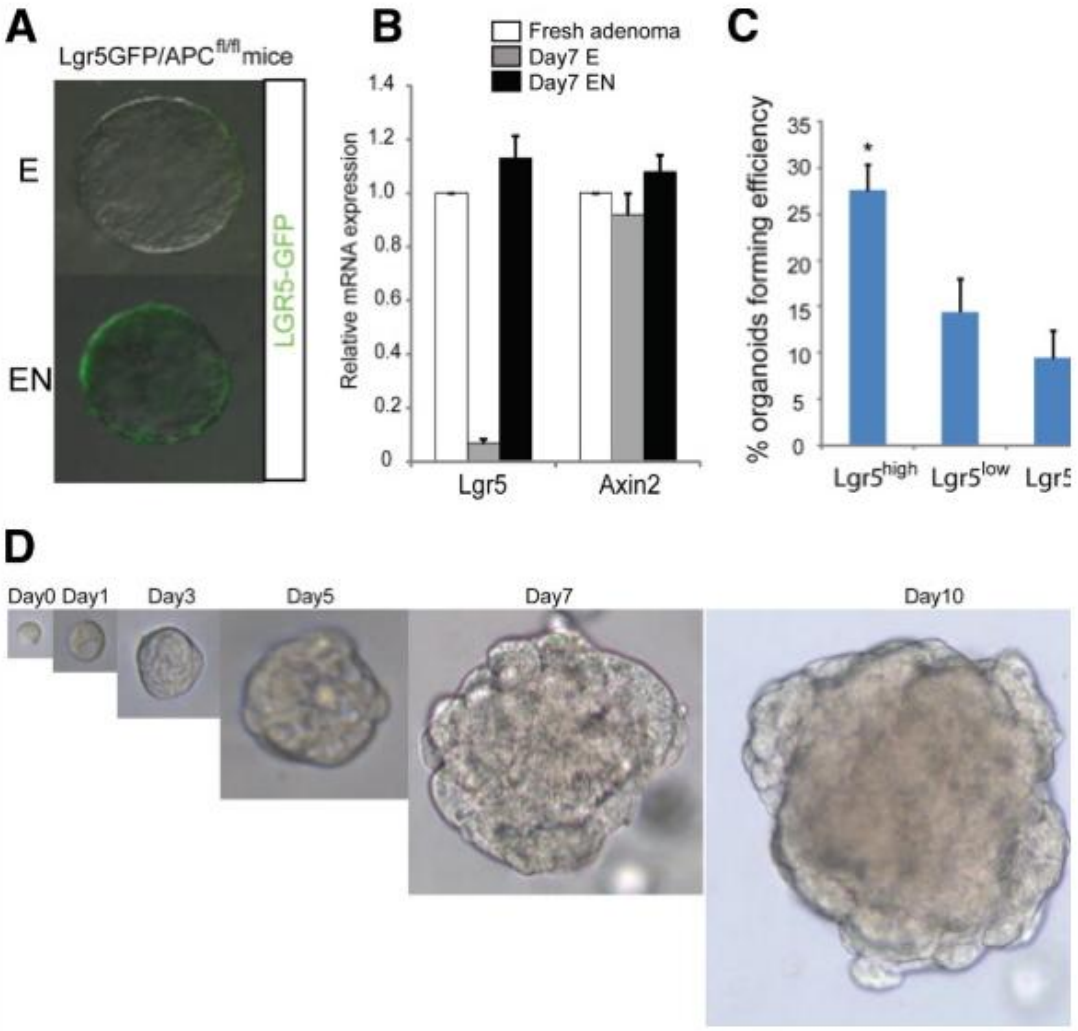

Figure 4. Adeno(carcino)ma cultures. (*A*) *Lgr5-GFP-ires-CreER/APC*$^{fl/fl}$ crypts cultured with EGF (E) (*top*) or EGF + Noggin (EN) (*bottom*) for 10 days. (*B*) Relative messenger RNA expression of Lgr5 and Axin2. Freshly isolated adenoma cells (*white*) were cultured with EGF (*gray*) or EGF + Noggin (*black*). (*C*) Culture efficiency of organoids from sorted Lgr5-GFP^{hi}, Lgr5-GFP^{lo}, and Lgr5-GFP^{-ve} cells. **P* < .01. One-way analysis of variance. *Error bars* indicate SEM. n = 3. (*D*) Time course culture of human colon adenocarcinoma cells.

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Adeno carcinoma

Esofago di Barret

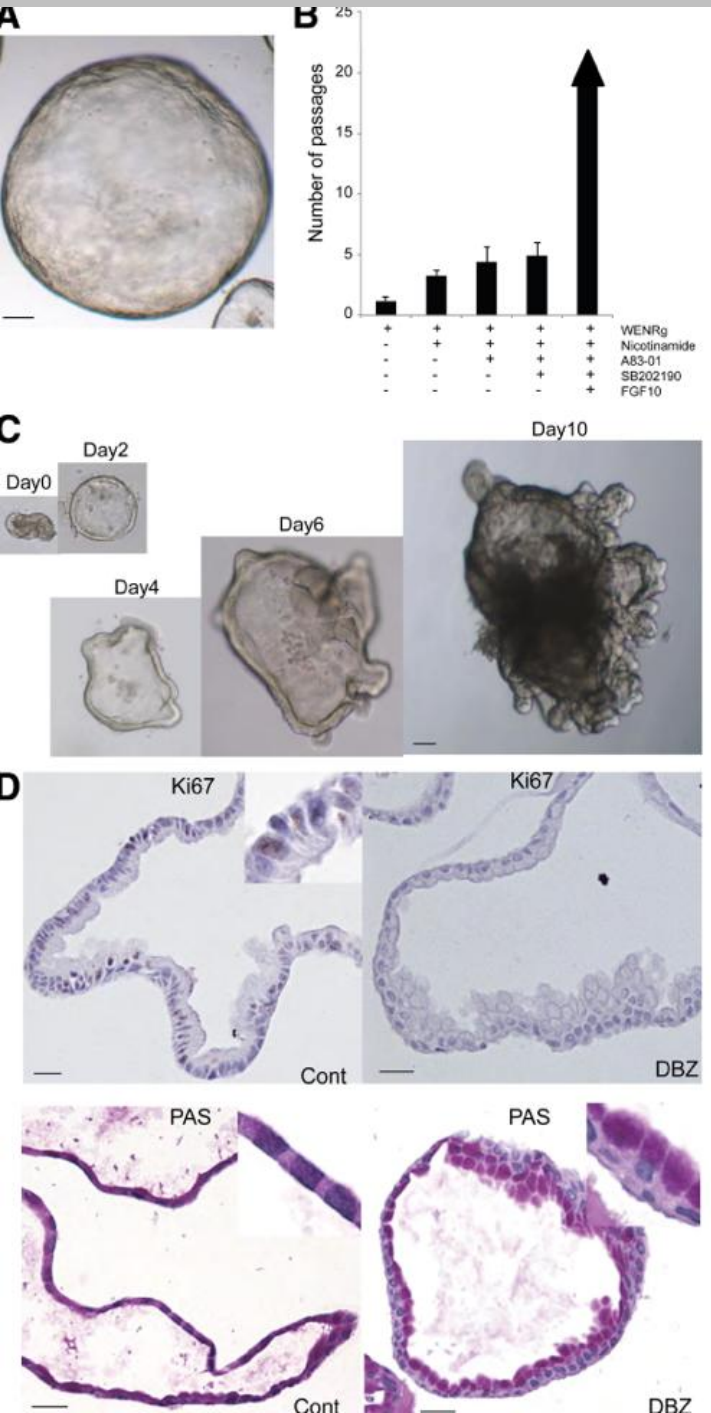


Figure 5. Culture of Barrett's esophagus and treatment with Notch inhibitor. (*A*) Isolated epithelium from Barrett's esophagus cultured with HISC condition for 7 days forms cystic structures. (*B*) Addition of fibroblast growth factor 10 (FGF10) significantly increases the number of passages for Barrett's esophagus organoids. Error bars indicate SEM. n = 3. (*C*) Representative time course of a Barrett's esophagus organoid. (*D*) Paraffin sections from Barrett's esophagus organoids. Nicotinamide and SB202190 are withdrawn for 4 days with (*right*) or without (*left*) the Notch inhibitor DBZ added to the medium. Proliferating cells (Ki67: *brown*) disappear and periodic acid–Schiff (PAS)-positive goblet cells increase with DBZ treatment.

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]

Tranplantation of cultured cells improves acute colitis

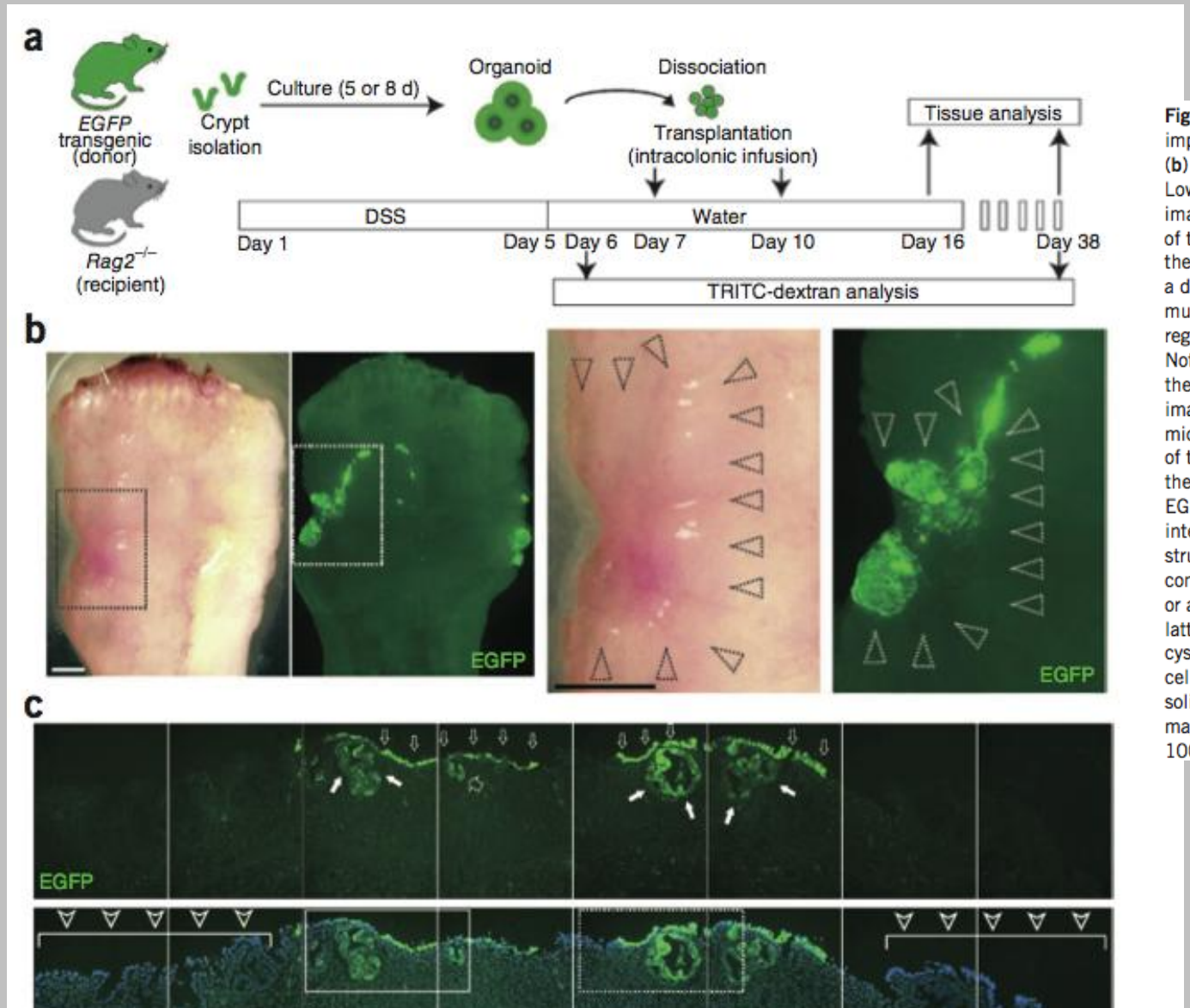
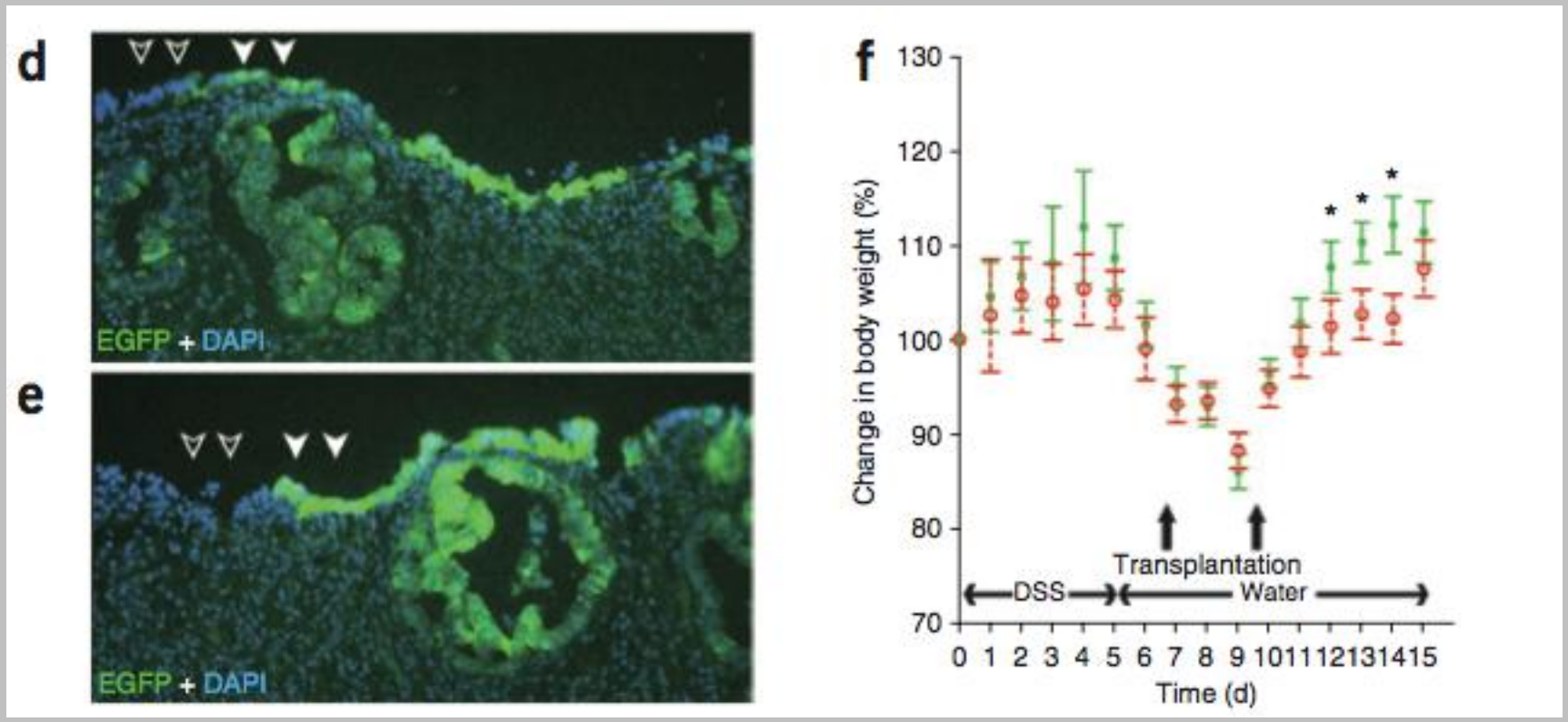


Figure 3 Transplantation of cultured cells improves acute colitis. (**a**) Experimental protocols. (**b**) Recipient colon at 6 d after transplantation. Low-power views (stereoscopic and fluorescent images) are shown on the left. High-power views of the areas in the dotted squares are shown in the right. The black dotted arrowheads show a depressed area surrounded by edematous mucosa. EGFP$^+$ areas overlapping the damaged region (white dotted arrowheads) are also shown. Note that the outline of the tissue is not precisely the same in the stereoscopic and fluorescent images, as they were acquired on different microscopes. Scale bars, 1 mm. (**c**) Histology of the EGFP$^+$ area shown in **b**. EGFP (top) and the merged image with DAPI staining (bottom). EGFP$^+$ cells cover the damaged mucosa that intervene separate areas preserving crypt structures (bottom, arrowheads). EGFP$^+$ cells constitute flat linings (top, narrow open arrow) or an invagination (top, wide open arrows), the latter of which is reminiscent of crypts. EGFP$^+$ cystic structures were also observed in the EGFP$^+$ cells (top, filled white arrows). The regions in the solid- and dotted-line boxes are shown at higher magnification in **d** and **e**, respectively. Scale bar, 100 μm. (**d**) High-power view of the solid box in **c**.


d
EGFP + DAPI
e
EGFP + DAPI
f
Change in body weight (%)
130
120
110
100
90
80
70
Transplantation
DSS
Water
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
Time (d)
*
*
*

Donor- derived cells generano epitelio di colon funzionale

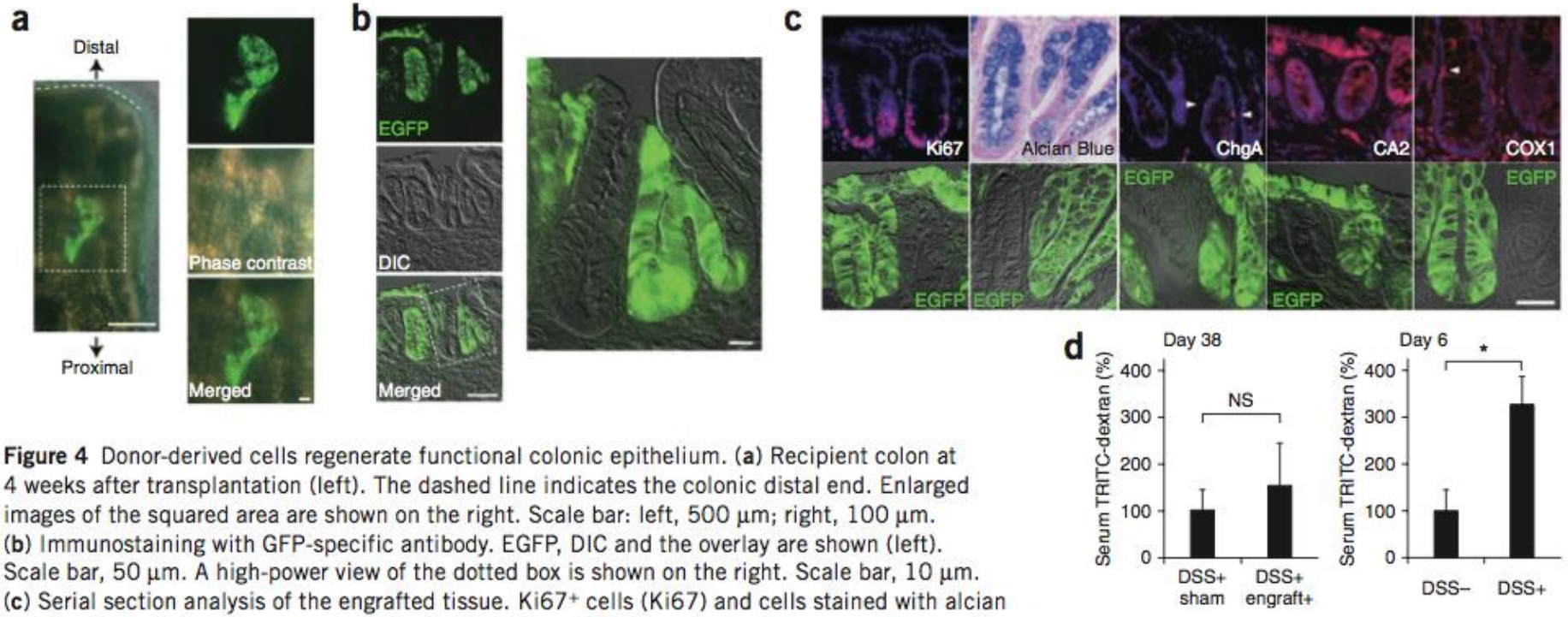


Figure 4 Donor-derived cells regenerate functional colonic epithelium. (**a**) Recipient colon at 4 weeks after transplantation (left). The dashed line indicates the colonic distal end. Enlarged images of the squared area are shown on the right. Scale bar: left, 500 µm; right, 100 µm. (**b**) Immunostaining with GFP-specific antibody. EGFP, DIC and the overlay are shown (left). Scale bar, 50 µm. A high-power view of the dotted box is shown on the right. Scale bar, 10 µm. (**c**) Serial section analysis of the engrafted tissue. Ki67$^+$ cells (Ki67) and cells stained with alcian blue (goblet cells) or immunostained for ChgA, CA2 and COX1 are shown. Images are shown with or without DAPI staining. The bottom row shows neighboring sections stained for GFP. Arrowheads point to ChgA$^+$ or COX1$^+$ cells. Scale bar, 50 µm. (**d**) After DSS colitis induction, transplantation ($n = 6$) or sham transplantation ($n = 6$) was performed. Mice were administered TRITC-dextran by gavage before killing on day 38. Four out of six colons in the transplanted group had EGFP$^+$ engraftment, and the serum TRITC concentration in these mice is shown (DSS+ engraft+; $n = 4$) as a percentage of that in the sham-transplanted group (DSS+ sham; $n = 6$). As a control, DSS colitis was induced (DSS+; $n = 6$) or uninduced (DSS–; $n = 6$) in *Rag2*$^{-/-}$ mice, and these mice were subjected for the same assay on day 6 without transplantation. Data are shown as a percentage of the concentrations in uninduced mice. Error bars, s.e.m. $^*P < 0.05$, NS, not significant (Student's *t* test).

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

JR Spence *et al. Nature* **000**, 1-5 (2010) doi:10.1038/nature09691

nature

Human ES cells and iPSCs form three-dimensional intestine-like organoids.

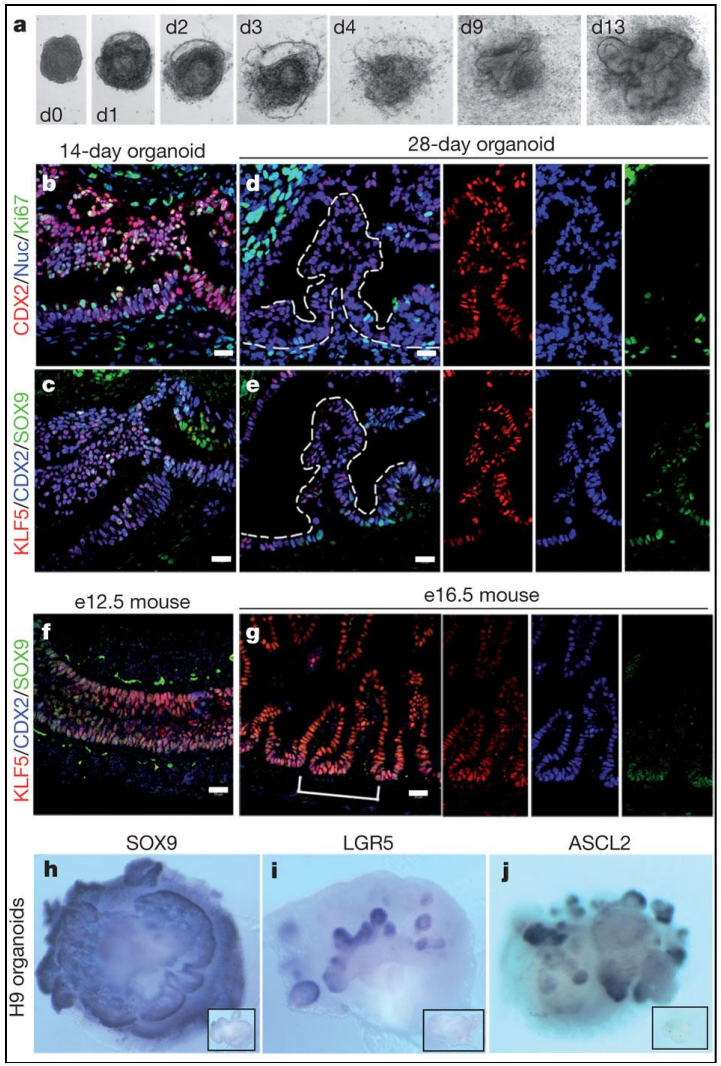
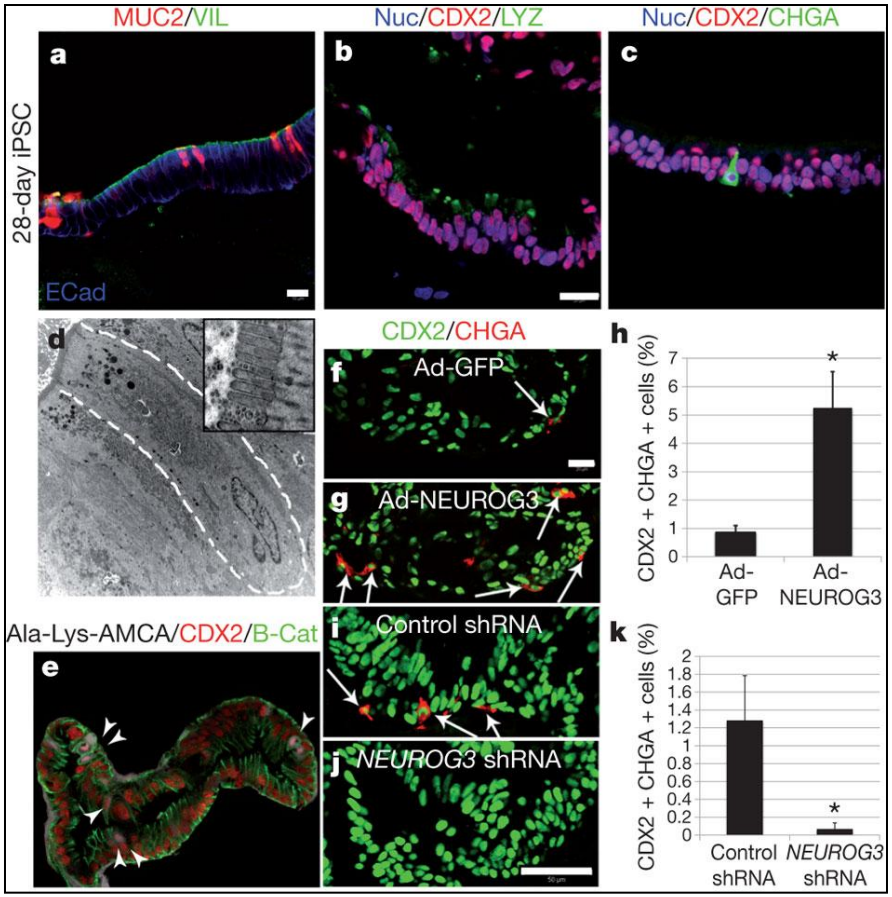


Figure 3

hESCs and hiPSCs form 3-dimensional intestine-like organoids

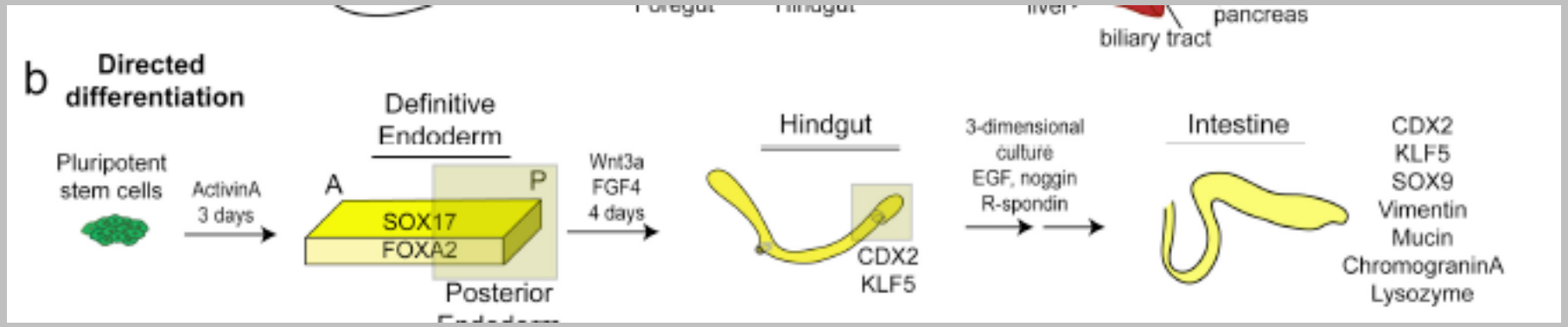
a, A time course shows that intestinal organoids formed highly convoluted epithelial structures surrounded by mesenchyme after 13 days. **b-e,** Intestinal transcription factor expression (KLF5, CDX2, SOX9) and cell proliferation on serial sections of organoids after 14 and 28 days (serial sections are b and c, d and e). **f and g,** Expression of KLF5, CDX2, and SOX9 in mouse fetal intestine at e14.5 (**f**) and e16.5 (**g**) is similar to developing intestinal organoids. The right panels show separate color channels for d, e and g. **h, i and j,** whole mount *in situ* hybridization of 56 day old organoids showing epithelial expression of Sox9 (h) and restricted "crypt-like" expression of the stem cell markers Lgr5 (i) and Ascl2 (j). Insets show sense controls for each probe.

Formation and function of intestinal cell types and regulation of enteroendocrine differentiation by NEUROG3.



Formation and function of intestinal cell types and regulation of enteroendocrine differentiation by NEUROG3

28 day iPSC-derived organoids were analyzed for **a,** villin (VIL) and the goblet cell marker mucin (MUC2), **b,** the paneth cell marker lysozyme (LYSO) or **c,** the endocrine cell marker chromogranin A (CGA). **d,** Electron micrograph showing an enterocyte cell with a characteristic brush border with microvilli (inset). **e,** Epithelial uptake of the fluorescently labeled dipeptide d-Ala-Lys-AMCA (arrowheads) indicating a functional peptide transport system. **f-h,** Adenoviral expression of Neurog3 (pAd-NEUROG3) causes a 5-fold increase in CGA+ cells compared to a GFP control (pAd-GFP); (n=4 biological samples);*(p=0.005). **i-k,** Organoids were generated from hESCs that were stably transduced with shRNA-expressing lentiviral vectors. Compared to control shRNA organoids, NEUROG3 shRNA organoids had a 95% reduction in the number of CgA+ cells; (n=3 for shRNA controls and n=5 for Neurog3-shRNA); *(p=0.018). Error bars for **h,k** are S.E.M.

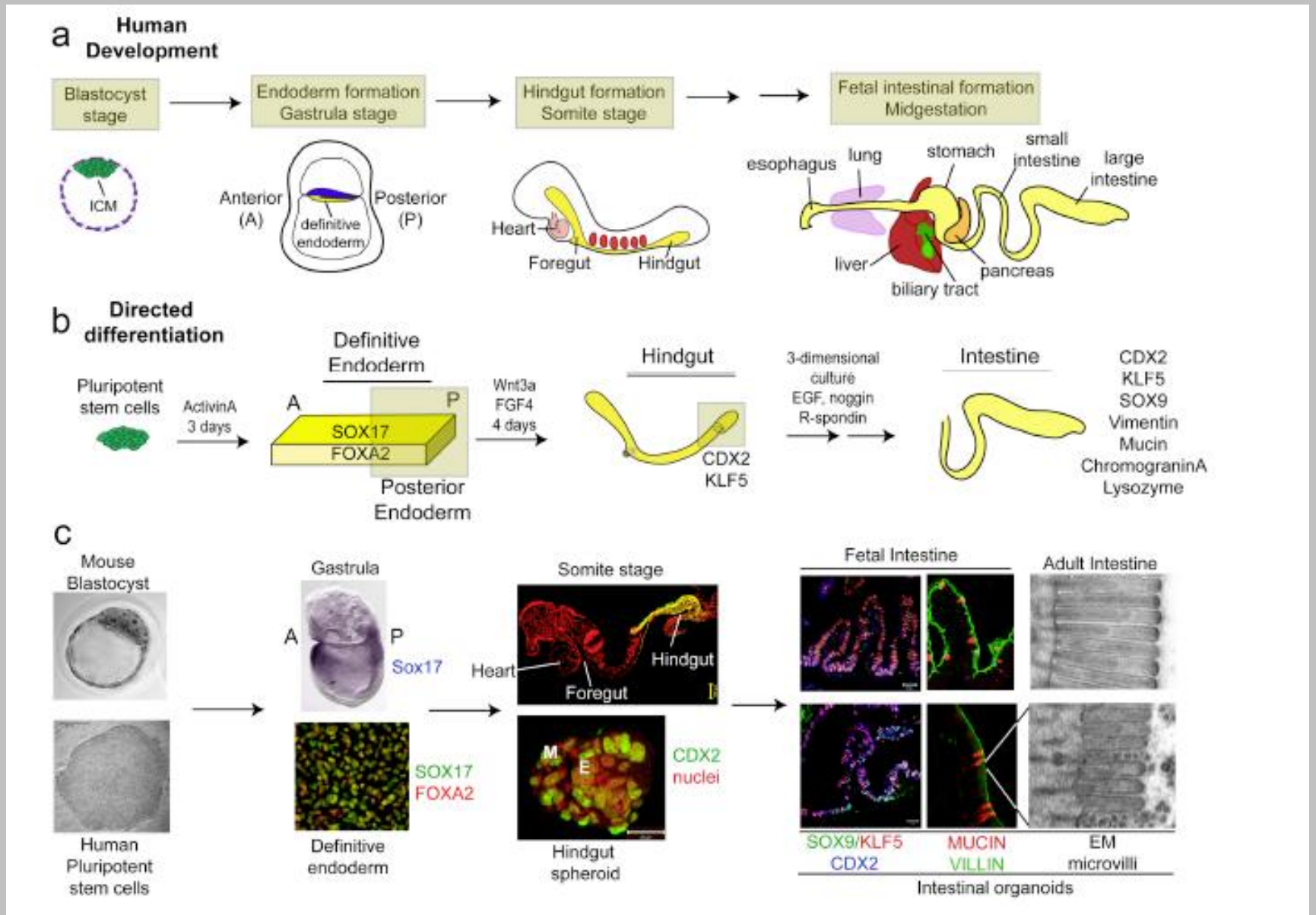


Generation and characterization of induced pluripotent stem cell lines

Normal human skin keratinocytes (NHSK) were obtained from donors with informed consent (CCHMC IRB protocol CR1_2008-0899). NHSKs were isolated from punch biopsies following trypsinization and subsequent culture on irradiated NIH3T3 feeder cells in F media [34]. For iPSC generation, NHSKs were transduced on two consecutive days with a 1:1:1:1 mix of recombinant RD114-pseudotyped retroviruses expressing Oct4, Sox2, Klf4 and cMyc [35,36] in the presence of 8μg/mL polybrene. Twenty-four hours after the second transduction the virus mix was replaced with fresh F media and cells were incubated for an additional three days. Cells were then trypsinized and seeded into 6 well dishes containing $1.875x10^5$ irradiated mouse fibroblasts per well and Epilife medium. On the following day, media was replaced with DMEM/F12 50:50 media supplemented with 20% knockout serum replacement, 1mM L-glutamine, 0.1mM β-mercaptoethanol, 1x non-essential amino acids, 4ng/mL basic fibroblast growth factor, and 0.5mM valproic acid. Morphologically identifiable iPSC colonies arose after 2-3 weeks and were picked manually, expanded and analyzed for expression of human pluripotent stem cell markers Nanog, DNMT3b, Tra1-60 and Tra1-81 [37,38]. Early passage iPSC lines were adapted to feeder-free culture conditions consisting of maintenance in mTeSR1 (Stem Cell Technologies) in culture dishes coated with matrigel (BD Biosciences) and lines were karyotyped.

Protocol early Steps

Differenze tra lo sviluppo intestinale embrionale e la differenziazione diretta da cellule pluripotenti



Materials

Generation of iPS cells: STEP 1

REAGENTS	Brand	**CATALOG NUMBER**	Qt
Knockout Serum Replacement	INVITROGEN	10828-028	1
bFGF human recombinant	PEPROTECH	100-18B	size B
mTeSR1 medium	Stem Cell Technologies	5870	1
MATRIGEL	Becton Dickinson	354277	1
EpiLife Medium	INVITROGEN	MEPICF500	2

Generation of human intestinal organoids: STEP 2

Activin A- Human	R&D systems	1100 9500 10	2
Hyclone defined fetal bovine serum	dFBS; Thermo Scientific		2
FGF-4 - Human	R&D systems	1372 9500 25	1
WNT-3a	R&D systems	5036-WN-010	1
R-Spondin1	R&D systems	4645-RS-025	1
Noggin- Human	R&D systems	1750 9550 20	2
EGF - Human	R&D systems	1325 9505 00	2
N2 Supplement	R&D systems	AR003	1
B27 supplement	INVITROGEN	0080085SA	2
Dispase	INVITROGEN	17105-041	1
Human BMP4	R&D systems	314-BP-010	1

Organoidi che stiamo attivando in laboratorio e perchè

- Crypts organoids: consentono di ottenere cellule epiteliali “pure” del compartimento delle cripte. Potremmo impiegarle allo stesso modo delle cellule da pazienti CD di altri compartimenti. Valutazione della risposta immune innata dopo gliadina o altri fattori (virus), proliferazione, valutazione del trafficking vescicolare etc

- Organoidi da staminali totipotenti (fibroblasti).

-Possono essere ingegnerizzati.

-Si può seguire il processo di differenziamento.

- Incollaborazione con Biogem e Tigem.