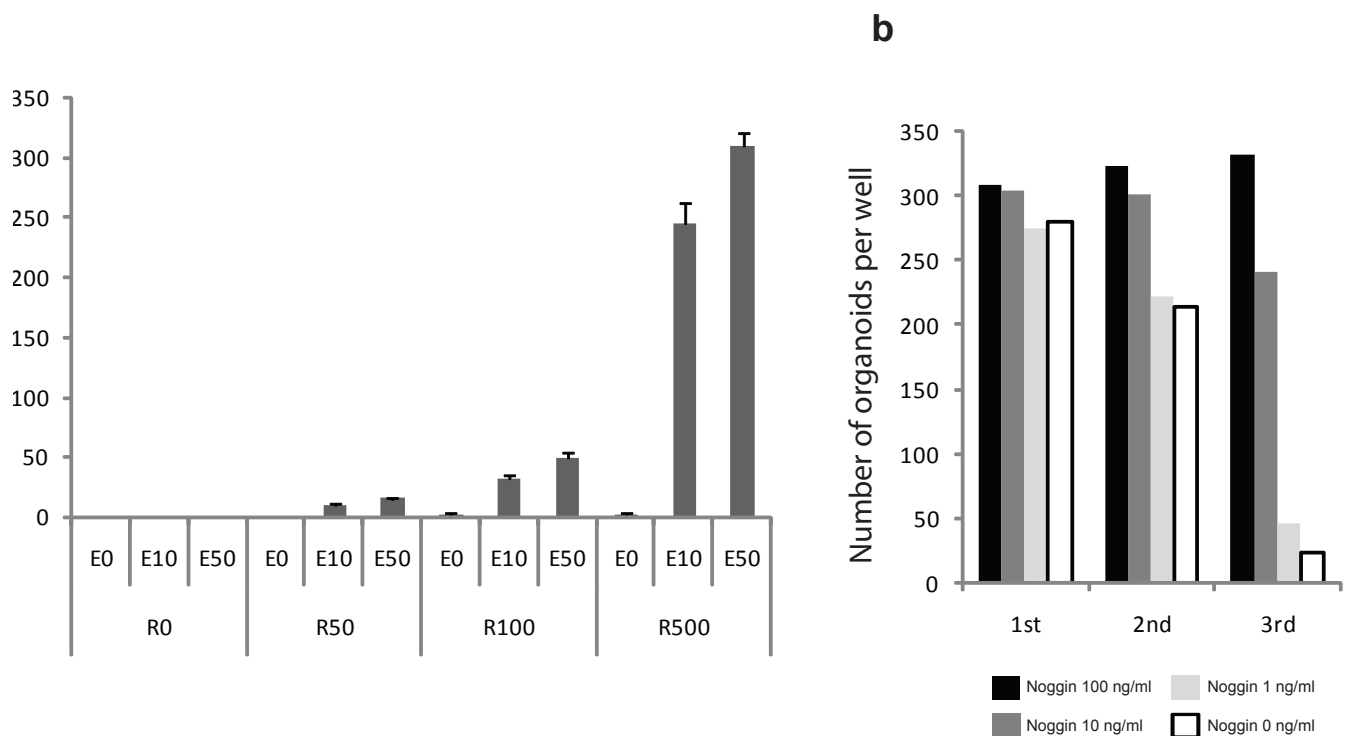
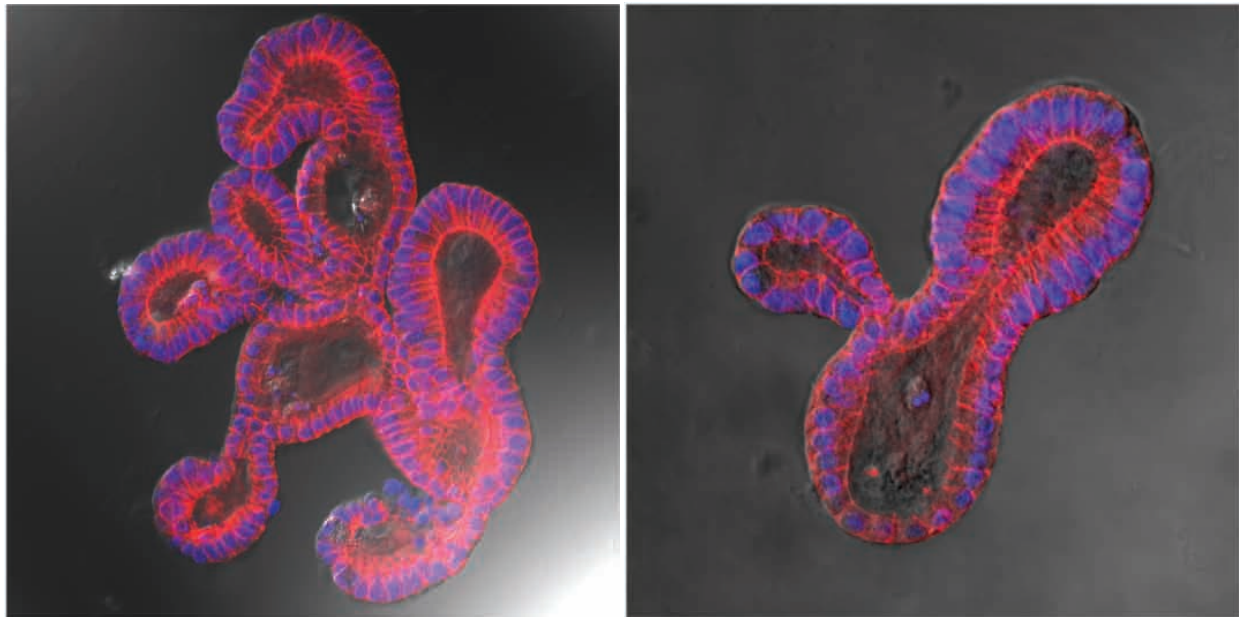


SUPPLEMENTARY INFORMATION

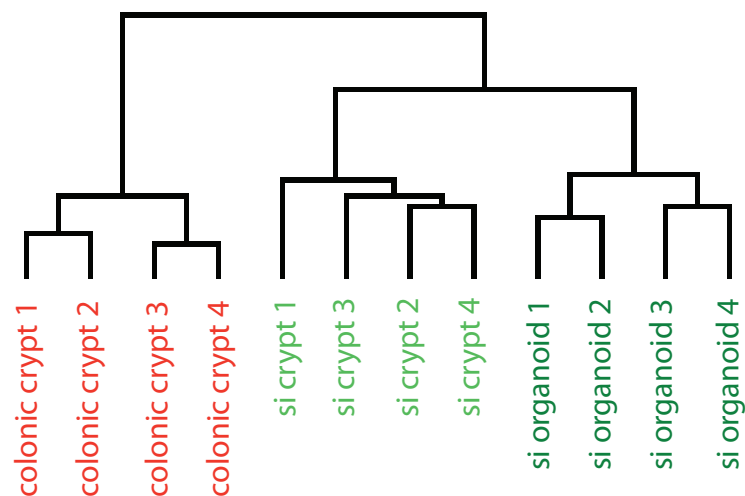
**Suppl. Figure 1. Growth factor requirement of crypt culture.**

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.**



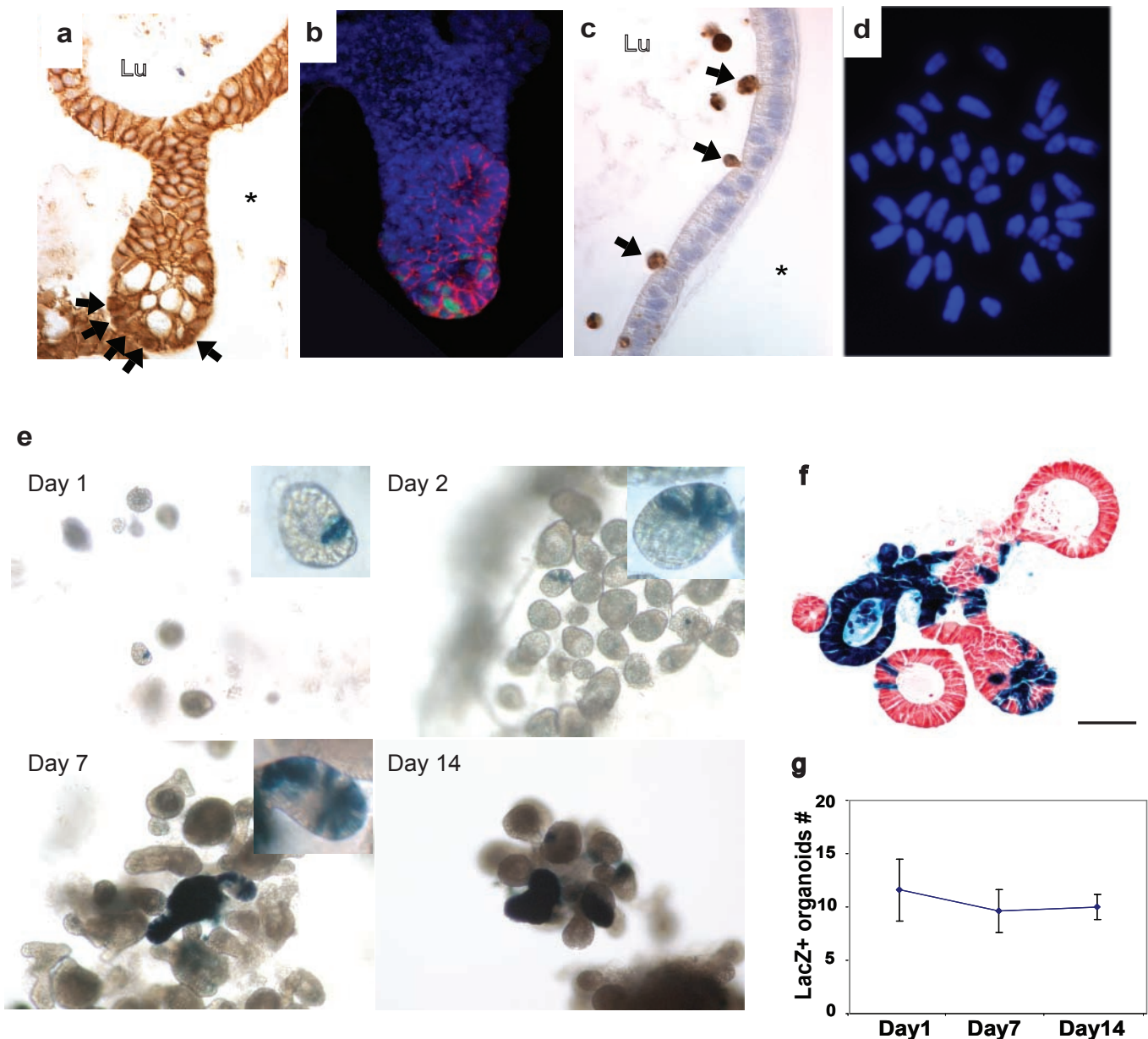
Suppl. Figure 2. E-cadherin staining shows a single cell layer of crypt organoid.

Organoid stained for E-cadherin in red and counter stained with nuclei in blue, reveals the single-layered nature of the organoid epithelium.



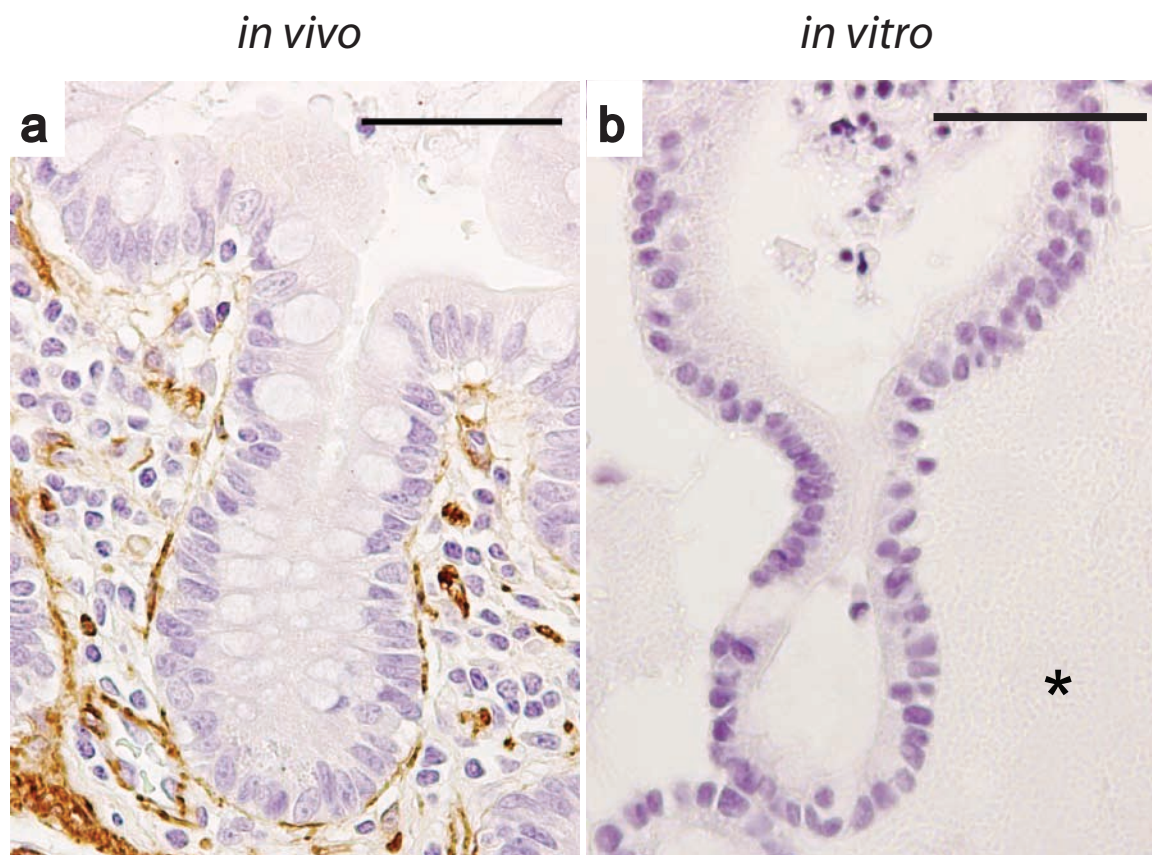
Suppl. Figure 3. Cluster analysis of gene expression profiling.

Cluster analysis of expression levels using freshly isolated colonic and small intestinal crypts as well as small intestinal organoids showed high degree of similarity between small intestinal organoids and the tissue they were derived from, small intestinal crypts. Colonic crypts clustered on a separate branch, indicating a different gene expression pattern of this closely related tissue. Of note, only 1.2% of all genes expressed were significantly enriched in organoids relative to small intestinal crypts, while *vice versa*-2% were enriched in small intestinal crypts. Ingenuity Pathway analysis on these differential genes revealed the specific presence of a lymphocyte signature in freshly isolated crypts, while no significant pathway could be identified in the small number of genes enriched in the organoids (not shown). We conclude that the latter group represents biological noise, while the lymphocyte signature derives from contaminating intraepithelial immune cells, lost upon culture.



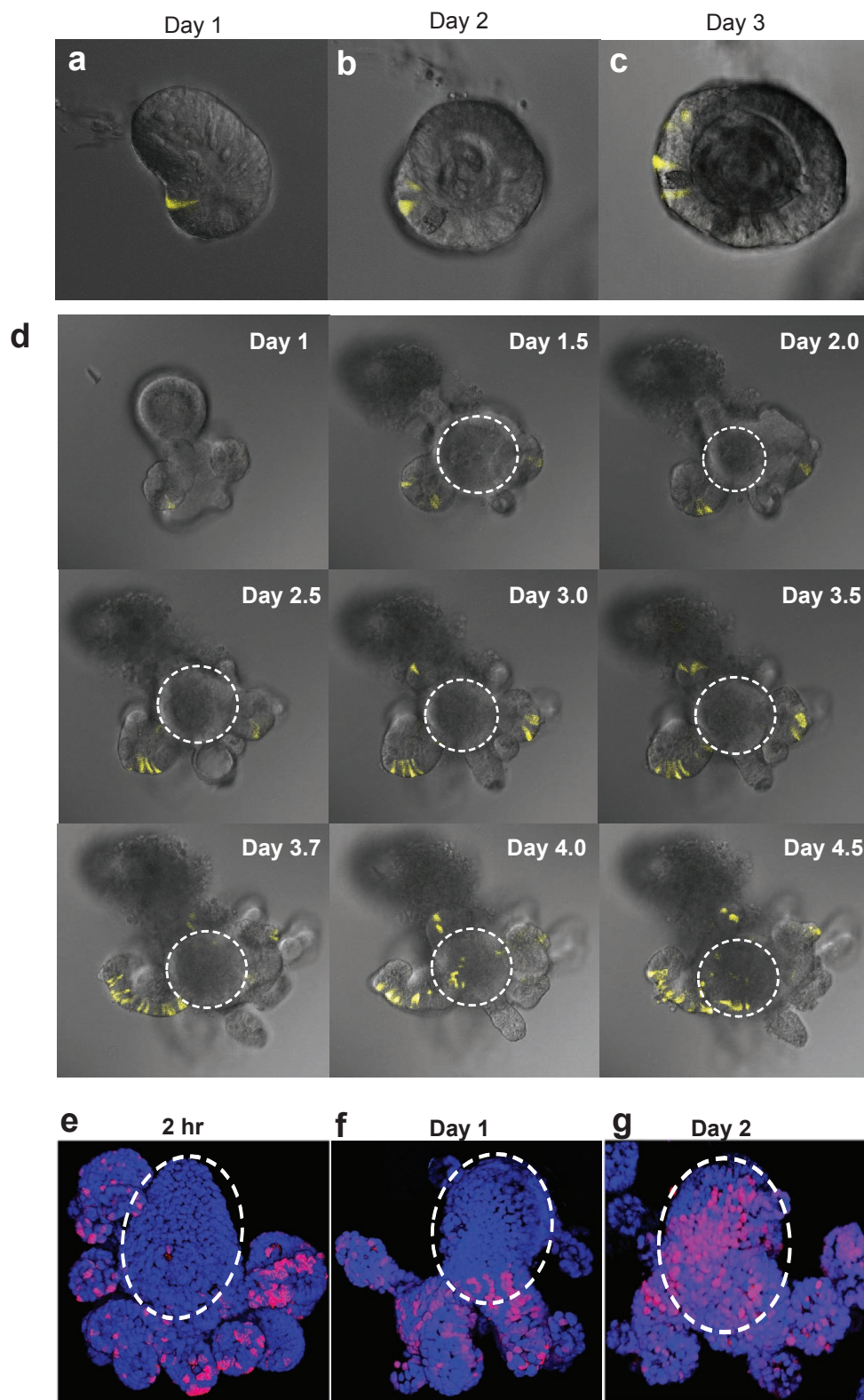
Suppl Figure 4. Crypt organoids preserve basic crypt-villus characteristics.

a-e The Wnt activation code is preserved in crypt domains. **a**: Nuclear β -catenin (brown, arrows) was only seen in crypt domains. Higher resolution image in Suppl Fig 9. Asterisk, matrigel; Lu, lumen **b**: EphB2 (red) is expressed in a gradient on CBC cells and TA cells. Note Lgr5-GFP⁺ stem cells **c**: Caspase-3⁺ apoptotic cells (brown, arrows) shedding into the central lumen lined by enterocytes. **d**: 40 chromosomes in a spread of cells from a > 3 months old crypt culture **e-g**: Lineage tracing of Lgr5⁺ stem cells *in vitro*. **e**: Crypts from *Lgr5-EGFP-ires-CreERT2/Rosa26-lacZ* reporter mice were stimulated by tamoxifen *in vitro* for 12 hr, and cultured for the indicated days. LacZ staining (blue) shows that scattered single blue cells (day 1) generated entirely blue crypts *in vitro* (Day2-14). Insets show higher magnification of stained crypt organoids. **f**: Histological analysis shows an entirely blue crypt-domain feeds into the villus domain. **g**: The percentage of crypt organoids with LacZ⁺ cells remained steady over time, indicating that Lgr5⁺ cells possess long-term stem cell activity. 500 crypts were seeded in triplicate, and LacZ⁺ crypt organoids were counted. Error bars are standard deviation of triplicates. The experiment was repeated three times with similar results.

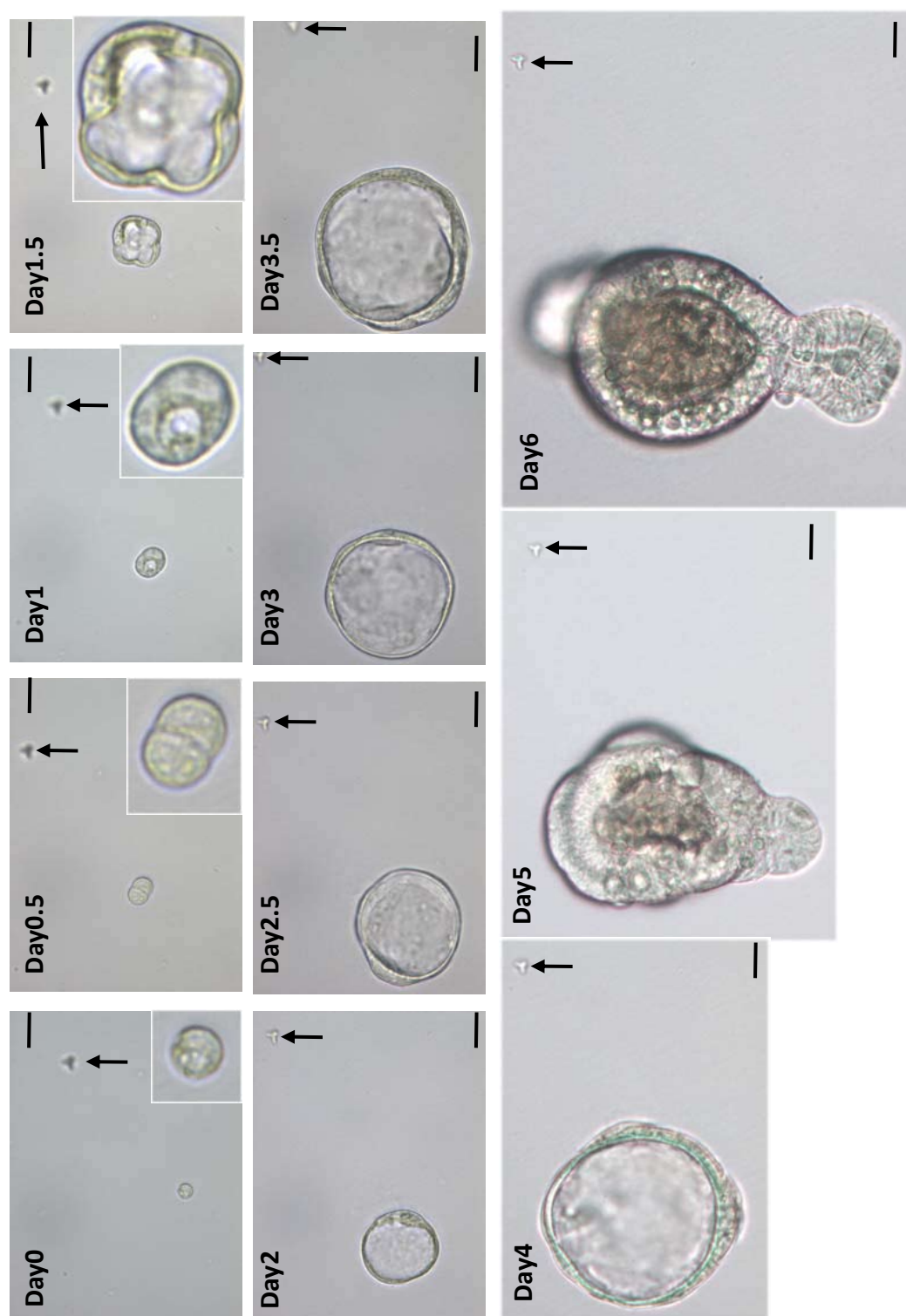


Suppl. Figure 5. No evidence of subepithelial fibroblasts in crypt organoids.

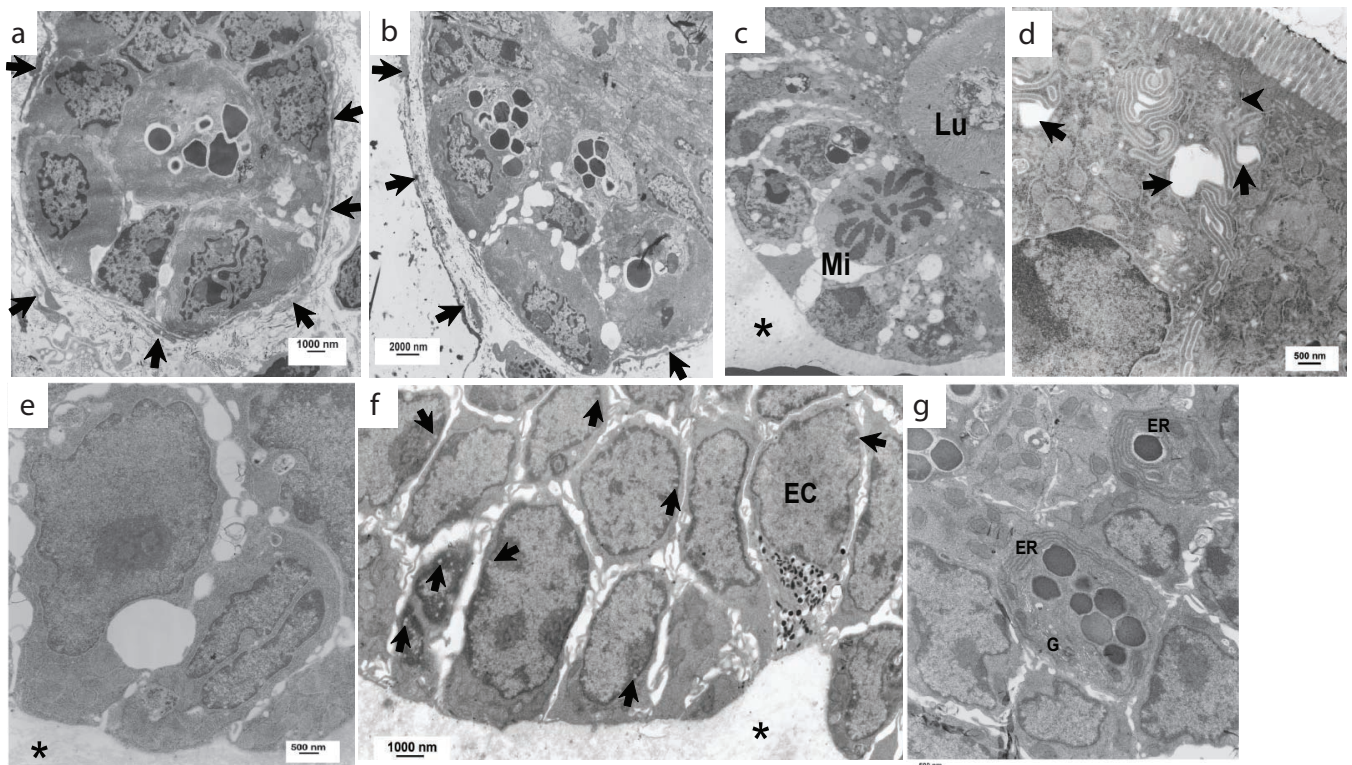
a: Immunostaining for smooth muscle actin (SMA; brown) demonstrates the presence of subepithelial fibroblasts beneath the epithelial layer. **b:** Absence of SMA+ cells in matrigel (asterisk) indicates the absence of subepithelial fibroblasts in the culture system. Scale bar; 50 μ m.



Suppl. Figure 6. **a-c:** A crypt from an *Lgr5-EGFP-ires-CreERT2/Rosa26-YFP* reporter mouse was stimulated by tamoxifen *in vitro* for 12 hr, and imaged for the indicated days. **d:** Seven-day-old organoids derived from an *Lgr5-EGFP-ires-CreERT2/Rosa26-YFP* crypts were stimulated by tamoxifen *in vitro* for 12 hr, and cultured and imaged for the indicated days. YFP fluorescence (yellow) shows that scattered single yellow cells (day 1) generated multiple offspring *in vitro* over the next five days. The villus domain burst during Day1-1.5, following by new villus domain formation (white circle). Note that YFP+ cells are migrating toward villus domain. **e-g:** After a 2 hour-EdU pulse to label S-phase of crypt cells, the labels were chased for 0-2 days. EdU was visualized as red. **e:** immediately after pulse. The label moves to the central luminal domain on day 1 (**f**) and day 2 (**g**). Nucleus was counterstained with DAPI.



Suppl. Figure 7. Colony-forming potency of a single cell sorted in an individual well. An example of a successfully growing single GFP^{hi} cell. The arrows point to a dust particle as a landmark. Scale bar: 50 μ m.



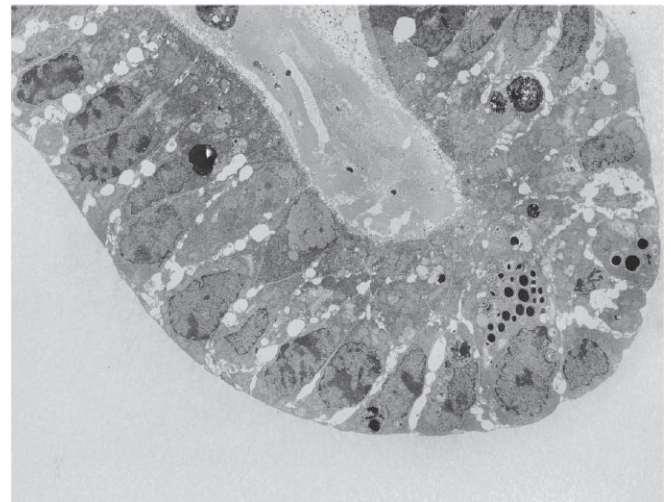
Suppl. Figure 8. Comparison of electron microscopic images between *in vivo* crypt and *in vitro* cultured crypt. **a, b:** Normal intestine at the base of the crypt with the connective tissue underneath (arrows). For comparison see **c-g** of the organoids also taken at the base of a crypt.

d: High magnification image from the apical membrane; There are intercellular clefts (arrows) between the membranes of two adjacent cells. Note the desmosome (arrow head) followed by an intercellular cleft. **e:** High magnification from the basal site where the membrane of two adjacent cells can be followed by intracellular clefts. These images are comparable to **a** and **b** from the intestine of a normal mouse. The origin of these intercellular clefts may be osmotic shock during aldehyde fixation. **f,g:** All cells that make up the organoid are in a healthy state and lack large vacuoles or other signs of stress. One can observe mitosis figures (**c**) and in each cell many nuclear pores (**f**, arrows) and intact mitochondria. ER and Golgi (**g**) can be seen without any evidence of swelling. There is no sign of karyorexis, karyolysis or karyopyknosis. Therefore, no sign of cell lysis or apoptosis is observed. Cells in the lumen of the organoid show the expected apoptotic features as one can observe in the gut of a normal mouse. **f** shows another example of an enteroendocrine cell.

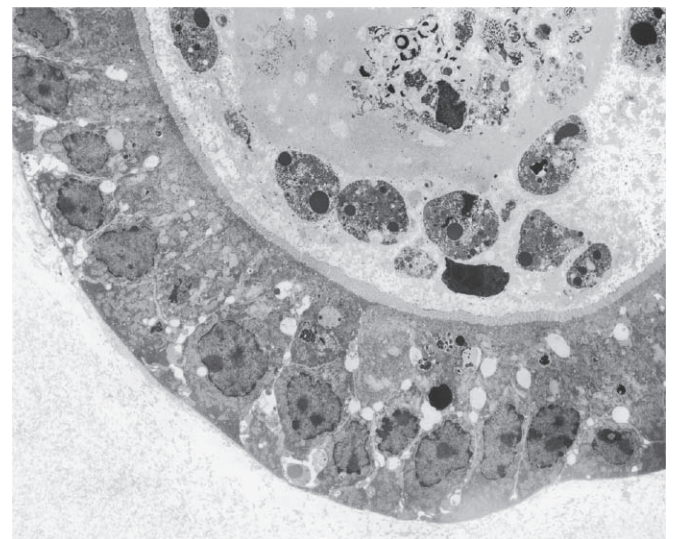
Mi: mitotic cells, Lu: lumen, EC: enteroendocrine cells, G: Golgi.



High resolution image of Suppl Fig4a



High resolution image of Fig 4m



High resolution image of Fig 4p

Suppl. Figure 9. Higher resolution image of Suppl. Fig 4a, Fig 4m and 4p.