Cell fate coordinates mechano-osmotic forces in intestinal crypt formation

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Intestinal organoids derived from single cells undergo complex crypt-villus patterning and morphogenesis. However, the nature and coordination of the underlying forces remains poorly characterized. Here, using light-sheet microscopy and large-scale imaging quantification, we demonstrate that crypt formation coincides with a stark reduction in lumen volume. We develop a 3D biophysical model to computationally screen different mechanical scenarios of crypt morphogenesis. Combining this with live-imaging data and multiple mechanical perturbations, we show that actomyosin-driven crypt apical contraction and villus basal tension work synergistically with lumen volume reduction to drive crypt morphogenesis, and demonstrate the existence of a critical point in differential tensions above which crypt morphology becomes robust to volume changes. Finally, we identified a sodium/glucose cotransporter that is specific to differentiated enterocytes that modulates lumen volume reduction through cell swelling in the villus region. Together, our study uncovers the cellular basis of how cell fate modulates osmotic and actomyosin forces to coordinate robust morphogenesis.

Intestinal crypts, which are composed of stem cells (SCs) and Paneth cells, are an essential unit for epithelial homeostasis. In both mice and humans, crypts form after the emergence of villi, which include other differentiated cells. Mouse villus formation is initiated by the mechanical condensation of the mesenchymal cells underneath the epithelium, which leads to tissue folding and the establishment of Shh and BMP gradients along the villus–crypt axis. The morphogen gradients induce cell differentiation that further promotes villus morphogenesis. Although villus morphogenesis has been well studied, less is known about the mechanism of crypt morphogenesis. Apical constriction of the intravillus tissue has been shown to initiate the invagination of the crypts; however, the mechanisms of crypt formation and especially the coordination between cell type emergence and tissue morphogenesis remains to be investigated.

Major challenges in studying intestinal morphogenesis lie in the difficulty of live imaging and force manipulation owing to the limited accessibility of internal organs. In vitro, mouse intestinal organoids contain functional crypts and villi, providing an opportunity to overcome these difficulties. Mouse intestinal organoids develop from single cells through two sequential symmetry breaking events. The first one is at the level of cell fate, leading to the emergence of the first Paneth cell between the 8-cell and 32-cell cyst stages under the regulation of the notch and hippo pathways. Paneth cells then induce Lgr5+ SCs, after which a small bulge forms around Paneth cells and SCs around day 3 (refs. 15–17). Around day 4, a crypt buds from the bulge with a narrow neck, and this shape transformation determines the second symmetry-breaking event (Fig. 1a). Mouse intestinal organoids share strong similarities with the in vivo tissue, including cell type composition, tissue organization and functionalities. Interestingly, mouse intestinal organoids are one of the few organoid systems that can recapitulate the shape of the tissue and crypt morphogenesis without in vivo mesenchymal cells.

In this Article, we combine four-dimensional multiscale imaging, single-cell RNA sequencing (scRNA-seq), and pharmacological and mechanical perturbations with biophysical modelling to show that cell-fate emergence orchestrates the coordination of mechanical forces through switches in myosin patterns and osmosis. The resulting tissue-scale mechanical responses, which are the differential spontaneous curvature in two regions and the net fluid relocation from lumen to villus cells, determine crypt formation.

Crypt budding follows tissue tensions and lumen shrinkage. Crypt apical constriction initiates crypt morphogenesis in vivo. To investigate the global morphological changes that occur during crypt formation in organoids, we examined their formation using image-based time-course experiments at a high spatiotemporal resolution at days 3, 3.5 and 4 (Fig. 1a,b). We quantified the sizes of cell apical and basal membrane domains through segmentation of the tight junction protein ZO-1 (Fig. 1b and Extended Data Fig. 1a). Mouse intestinal organoids develop from single cells through two sequential symmetry breaking events. The first one is at the level of cell fate, leading to the emergence of the first Paneth cell between the 8-cell and 32-cell cyst stages under the regulation of the notch and hippo pathways. Paneth cells then induce Lgr5+ SCs, after which a small bulge forms around Paneth cells and SCs around day 3 (refs. 15–17). Around day 4, a crypt buds from the bulge with a narrow neck, and this shape transformation determines the second symmetry-breaking event (Fig. 1a). Mouse intestinal organoids share strong similarities with the in vivo tissue, including cell type composition, tissue organization and functionalities. Interestingly, mouse intestinal organoids are one of the few organoid systems that can recapitulate the shape of the tissue and crypt morphogenesis without in vivo mesenchymal cells.

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Spontaneous curvature drives crypt morphogenesis

To understand the potential contributions of crypt apical contraction, villus basal constriction or lumen shrinkage to crypt morphogenesis, we turned to a three-dimensional (3D) vertex model. We modelled organoids as closed monolayers with cells under apical, lateral and basal tensions, enclosing a lumen of fixed volume (Extended Data Fig. 2 and Supplementary Note). On timescales of hours/days, we can assume that the evolution is quasi-static, and examine only the equilibrium configuration of organoids of different sizes. Given the above experimental findings, we allowed cell basal, lateral and apical tensions to be different in two spatially distinct regions (crypt and villus; Fig. 2a). We then computationally screened parameter space to understand how each parameter influences the equilibrium shape, and derived a simplified model that enabled us to predict scaling laws for organoid morphometrics (Fig. 2a). Importantly, we found three classes of mechanisms that could lead to a budded shape (Fig. 2b, Extended Data Fig. 3a–a* and Supplementary Note): (1) a buckling-type instability, whereby crypts have smaller in-plane contraction α than villi and therefore tend to expand, which is constrained by the lumen volume; (2) differential spontaneous curvature γ in crypts versus villi, leading to crypt bending; (3) a contractile boundary of the crypt–villus region, leading to bulging and budding through boundary contraction.

We note that the term spontaneous curvature is used in analogy to the physics of lipid membranes, as synonymous to intrinsic curvature. We found that each class of mechanisms gave rise to qualitatively different predictions on crypt morphometrics, such as the ratio of epithelial thickness or of curvature in crypts versus villi (Supplementary Note). For example, in scenario 1, morphogenesis occurs through differential expansion of the budded region, leading to lower epithelial thickness in the crypt compared with the villus, which is similar to observations during lung buds/alveoli formation; by contrast, in scenario 2, bulging and budding occurs through tissue bending, which increases the epithelial thickness in the crypts (Fig. 2b and Extended Data Fig. 3a,a*). The main difference in the three scenarios is therefore the evolution of epithelial thickness ratios (Fig. 2b and Extended Data Fig. 3a–a*).

We next analysed the dynamics of bulging and found that only scenario 2, namely the differential γ-driven crypt bulging, agreed with the experimental data, as we detected the increased crypt:villus thickness ratio (h/c/h) and the decreased crypt:villus radius ratio (R/Rc). This confirms that crypt apical constriction and spontaneous curvature are a major driving force for crypt morphogenesis (Fig. 2c, Extended Data Fig. 3b and Supplementary Note). Importantly, plotting Rc/R, as a function of h/c/h, was predicted to follow a simple decreasing curve, independent of the dynamics of tension evolution (Fig. 2c (right) and Extended Data Fig. 3c). The data revealed a good agreement with the predicted curve (with a single fitting parameter being the initial lumen volume v0; Supplementary Note), enabling us to validate the spontaneous curvature mechanism. Furthermore, independently measuring organoid and crypt sizes, and assuming a linear increase in crypt apical tension over time enabled a good fit of the individual temporal evolutions of the Rc/R and h/c/h ratios (Fig. 2c, Extended Data Fig. 3b–d and Supplementary Note). We next further tested the model using mechanical measurements, as well as mechanical and pharmacological perturbations.

Myosin II patterns induce tissue spontaneous curvatures

To quantify apical tensions, we performed laser nanosurgery on the apical domains of bulged and budded organoids. The analysis showed higher recoil velocities in crypt apical domains and, therefore, apical contraction in crypts was higher than in villi (Fig. 2d and Extended Data Fig. 4a). Interestingly, the crypt apical tension significantly increases from bulged to budded stages. Through micropipette aspiration experiments, the basal tension of the villi was higher than in crypts (Fig. 2e). The combination of high apical contraction and low basal tension in crypts is therefore fully consistent with the prediction of the model in which spontaneous curvature drives crypt morphogenesis (Supplementary Note).

On the basis of the actomyosin role in generating active tension, we investigated whether patterns of F-actin and myosin correlate with tension and spontaneous curvature that the model predicts. LifeAct–GFP and the phosphorylated myosin light chain (pMLC), which binds to the myosin-II heavy chain and generates contractile force, were enriched in the villus basolateral domain and the apical domain of both crypts and villi (Extended Data Fig. 4b,c). However, in villus apical domains, F-actin and pMLC do not necessarily contribute to apical contraction, but are probably required for microvilli formation. To follow the dynamics of myosin, we generated an organoid line from a Myh9–GFP mouse. We found that Myh9–GFP gradually increases at the crypt apical side as organoids evolve from spherical to bulged and budded (Extended Data Fig. 4d and Supplementary Video 2). In contrast to Myh9–GFP patterns, the membrane reporter (mG) gradually increases at the villus apical side, indicating that the increase in Myh9–GFP is not an artefact due to tissue compaction (Extended Data Fig. 4e). Moreover, in budded organoids, the expression pattern of Myh9–GFP is high in crypt apical and villus basolateral domains, and low in crypt basolateral and villus apical domains (Fig. 3a,b, Extended Data Fig. 4d and Supplementary Video 2). By contrast, the lateral Myh9–GFP
did not change in a region-specific manner (Fig. 3a, b). These Myh9–GFP patterns are in agreement with region-specific tension changes predicted by the spontaneous curvature mechanism (Supplementary Note).

We next tested whether actomyosin is functionally required for crypt morphogenesis. In mouse intestinal organoids, although inhibiting myosin increases organoid survival, blocking actomyosin contractility at day 3 before bulging prevents crypt morphogenesis (Extended Data Fig. 4c). Moreover, inhibiting myosin activity after budding disrupted the crypt morphology (Extended Data Fig. 4f and Supplementary Videos 3 and 4), whereas blocking cell division (through aphidicolin) did not (Extended Data Fig. 4f and Supplementary Videos 3 and 5). This is consistent with actomyosin-driven γ-driven crypt morphogenesis, rather than residual stresses from crypt cell proliferation (Supplementary Note). Nonetheless, as myosin IIA exhibits a differential pattern in crypts and villi, we validated the involvement of myosin IIA in the generation of spontaneous curvature. We constructed mosaic
organoids composed of Myh9<sup>+/−</sup> heterozygous mutant cells and Myh9-GFP cells<sup>32</sup> (Extended Data Fig. 4g–g<sup>″</sup>). In villi, the Myh9<sup>+/−</sup> cells adjacent to Myh9-GFP cells have an expanded basal domain, while the Myh9-GFP cells contract basally, indicating that myosin IIA is required for the generation of basal tension, which propagates to neighbouring cells (Extended Data Fig. 4g–g<sup>″</sup>) and contributes to crypt morphogenesis by creating a reverse spontaneous curvature (Supplementary Note). Thus, domain-specific patterns of myosin IIA modulate cell apical and basal tensions in both crypts and villi, which in turn determine tissue spontaneous curvatures.

In villi, enterocytes (which constitute the majority of villus cells) correlate with basal-enriched Myh9–GFP and basal tension. In crypts, whether Paneth or SCs provide the dominant contribution to spontaneous curvature is unclear. Thus, we compared apical Myh9–GFP levels in enterocytes, in organoids enriched in Paneth cells (PC-enriched organoids, treated with CHIR99021 (CHIR) and DAPT) or in organoids enriched in SCs (SC-enriched organoids, treated with CHIR and valproic acid (VPA)) (Fig. 3c–f). Compared with enterocytes (which had more basal Myh9–GFP, matching villus cells in organoids), the PC-enriched organoids and SC-enriched

![Diagram](image-url)
Fig. 3 | Myosin patterns in SCs and enterocytes determine region-specific spontaneous curvature. a, From left to right, Myh9–GFP in organoids before bulging, bulged organoids and budded organoids, maximum z-projection of a budded organoid, and magnifications of the regions indicated by boxes. Villi (blue arrows) and crypts (red arrows) are indicated. b, Plots of the ratios of Myh9–GFP intensity in cells. ca, crypt apical; cb, crypt basal; va, villus apical; vb, villus basal. c–f, Stem cell fate is responsible for apically enriched Myh9 in the crypt. c, d, Representative images of organoids treated by CHIR and VPA and enriched with SCs (SC-enriched), treated with CHIR and DAPT and enriched with Paneth cells (PC-enriched) or enriched with enterocytes (enterocyst). c, Maximum z-projection of Lgr5-DTR-GFP organoids with Lyz and 4,6-diamidino-2-phenylindole (DAPI) staining (top) and wild-type organoids with CD44, AdlB and DAPI staining (bottom). c, Maximum z-projection (top) and single-layer (bottom) images of Myh9–GFP organoids with Lyz and DAPI staining. e, Quantification of eccentricity from the maximum z-projection images in d. f, Quantification of Myh9–GFP intensity from single-layer images in d. For the box plots in b, e and f, the n values are stated in parentheses and represent organoids selected for multiple single-cell (b) or whole-organoid (d) measurements. For b, e and f, P values were determined using two-tailed t-tests. The box-plot elements show the 25% (Q1, upper bounds), 50% (median, black lines within the boxes) and 75% (Q3, lower bounds) quartiles, and the whiskers denote 1.5 × the interquartile range (maximum = Q3 + 1.5 × (Q3 – Q1); minimum = Q1 – 1.5 × (Q3 – Q1)) with outliers (rhombuses). The experiments in a and c–e were repeated more than three times independently with similar results. Scale bars, for a, 50 µm (organoid) and 5 µm (magnification); and for c and d, 50 µm.
**Fig. 4 | Region-specific localization of cell junctions.** a, b. scRNA-seq analysis of budded organoids. $t$-distributed stochastic neighbour embedding (tSNE)-based visualization of the single-cell degree of expression of marker genes for cell types (SCs/crypt columnar cells, Paneth cells and enterocytes) (a) and cell junctions (Cldn1, Cldn2 and Occln) (b). c. ZO-1 staining in a budded organoid in a maximum z-projection image (left) and a heat-map visualization of the basal signal (right). d. ZO-1 staining in the intestine of a 6-month-old mouse in a section along the crypt–villus axis, and perpendicular sections of the villus and crypt regions along the positions are indicated by black dashed rectangles. e, Middle single-layer section and maximum z-projection of Claudin-2, Occlusin and N-cadherin staining, with DAPI staining for cell nuclei (blue). f, Co-localization of ZO-1 and Myh9–GFP in cell basolateral domains in the villus region. From left to right, z-projection (top) and magnified region (bottom) of ZO-1, Myh9–GFP, and merged ZO-1 and Myh9–GFP. g. Single-layer section and maximum z-projection of ZO-1 (merged with Lyz) and Claudin-2 (merged with DAPI) co-staining in organoids before bulging, bulged organoids and budded organoids. The crypt regions (red arrows) and the villus regions (blue arrows) are indicated. For c–g, the experiments were repeated more than three times independently with similar results. Scale bars, 50 µm.
organoids both had higher apical Myh9–GFP (Fig. 3d,f). Interestingly, SC-enriched organoids had an even higher apical versus basal Myh9–GFP ratio than PC-enriched organoids, suggesting that SCs provide the dominant contribution to spontaneous curvature. In the PC-enriched organoids, few Lgr5+ SCs remained, and generated higher regional spontaneous curvature, leading to local bulges (Fig. 3c,e). Together, these results suggest that SCs in crypt region are the dominant factor in creating the actomyosin-based apical constriction necessary for morphogenesis.

To examine the molecular mechanism that contributes to the emergence of the region-specific actomyosin pattern, we analysed the expression patterns of actomyosin regulators, using an scRNA-seq dataset that distinguishes between crypt and enterocyte-rich villus regions (Fig. 4a,b), and using immunostaining (Fig. 4c–g). An interesting candidate is the tight junction protein ZO-1 that orchestrates the apical organization of actomyosin in mouse intestines43. We detected the region-specific expression patterns from both tight junction proteins (ZO-1, claudin-2, occludin) and adherens junction proteins (N-cadherin) (Fig. 4e). In crypt morphogenesis, claudin-2 is gradually enriched in the maturing crypt (Fig. 4g), whereas ZO-1 is expressed at the whole epithelial apical side and overlaps with the basal pool of Myh9–GFP in the villus region (Fig. 4c–g). In vivo crypt morphogenesis, the restriction of claudin-2 to the crypt region has been detected from postnatal stage 11 (P11), at which stage ZO-1 also starts to exhibit villus basolateral localization (Extended Data Fig. 5). The in vitro and in vivo data suggest that region-specific junction proteins in crypts and villi coordinately contribute to the differential tissue properties and the patterning of actomyosin that is necessary for crypt budding.

Lumen volume reduction accelerates crypt budding

Next, we investigated whether the striking decrease in lumen volume at the onset of crypt budding (Fig. 1d–f) had a functional role in crypt formation. We hypothesized that, in analogy to lipid vesicles with region-specific spontaneous curvature43, lumen volume reduction could contribute to budding by changing the area/volume ratio of the organoid, therefore helping out-of-plane deformations. We computed a phase diagram of organoid morphology as a function of normalized lumen volume and relative crypt apical tension (Fig. 5a, Extended Data Fig. 6 and Supplementary Note). For constant lumen volume, crypts undergo bulging and then budding for increasing values of crypt apical tension (Extended Data Fig. 2d). For low apical tension, increasing the lumen volume above a critical threshold (Fig. 5a) unfolds the budded crypt into spherical shapes, as expected in lipid vesicles44. Unexpectedly, for high values of apical tension, the model predicted that budded shapes cannot be reversed by increasing the lumen volume (Fig. 5a). Theoretically, this is because high apical tension increases not only spontaneous curvature in crypts, but also in-plane rigidity (which resists deformation of crypts and results in preferential expansion of villi to bear the lumen-induced deformation). This prediction of a phase transition provides a critical test of the model. Moreover, the fits of bulging evolution (Fig. 2c) predict that bulged organoids are below the transition point (around 40% of the critical apical tension $m_{\text{crit}}$ at which crypts remain closed after large inflation; Supplementary Note) in the phase diagram of Fig. 5a, whereas budded crypts should be able to sustain large luminal inflation given their twofold increase in crypt apical myosin (Fig. 3a,b) and tension (Fig. 2d and Extended Data Fig. 4a). Thus, we tested this with experiments manipulating the lumen volume (Fig. 5a) to probe the relative material properties of the crypt and villus regions.

We first inflated the lumen in less than 30 min by treating bulged and budded organoids with prostaglandin E2 (PGE), which induces lumen swelling without altering cell fate during the first 6 h (refs. 36,38), and measured several morphometric parameters as a function of lumen volume (Fig. 5b,c) (right) and Supplementary Video 6). Crucially, we found that budded crypts could not be opened by PGE treatment (even increasing the volume up to 498%, which nearly exclusively stretches the villus region, leading to a decreased radius ratio $R_v/R_c$; Fig. 5b), in excellent agreement with the prediction that bulged and budded crypts react distinctly to lumen inflation. To further confirm this, we performed fluid microinjection to rapidly and controllably increase the lumen volume, and also confirmed the opposite response of bulged versus budded organoids (Extended Data Fig. 7a–b” and Supplementary Video 7). This provides a key qualitative test of the spontaneous curvature mechanism that we propose. We note that other mechanisms, such as budding through crypt cell proliferation or crypt cell expansion (that is, buckling, which has been examined theoretically previously15,46), would predict a qualitatively different behaviour, with crypt opening upon inflation (Supplementary Note).

To test this more quantitatively, we analytically derived scaling laws to predict how the thickness and radius ratios ($h_v/h_c$ and $R_v/R_c$) should vary as a function of lumen volume for bulged and budded organoids. To constrain the predictions, we experimentally measured the geometric parameters of the model in preinflation organoids (the fraction occupied by crypt cells and the organoid aspect ratio; Supplementary Note). Strikingly, after fitting of two parameters (crypt apical tension $m$ and initial lumen volume $V_o$ which gave values consistent with the ones fitted from Fig. 2c and Supplementary Note), all inflation data could be collapsed onto the predicted scaling laws (Extended Data Fig. 7c,c”), providing good agreement between the theory and experiments, including opposite trends of the radius ratio $R_v/R_c$, as a function of volume in bulged versus budded crypts (Extended Data Fig. 7c,c”). With the same parameter set, the model also predicted simple scaling relationships between $R_v/R_c$ and $h_v/h_c$, independent of lumen volume (Supplementary Note), which again were in excellent agreement with the data (Fig. 5d,e).

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**Fig. 5 | Lumen volume reduction promotes crypt budding.** a, Theoretical prediction ($κ_0 = 6$, $φ = 0.2$) for the relative contribution of the crypt apical tension ($m$) versus lumen volume ($v$) for organoid shape (left), and schematic of the experimental designs for phase diagram validation (right). The horizontal dashed line at $m = 4.2$ indicates the critical value, above which the budded shapes cannot be reversed by increasing the lumen volume. b,c, Fitting of the lumen inflation experiments with the theoretical model. The images show the organoid morphology before and after lumen inflation by PGE treatment of bulged (b) and budded (c) organoids; tissue and lumen were visualized by LifeAct–GFP (white). Scale bars, 20 μm. Experimental measurements (black dots, data) from the representative samples (the epithelial thickness ratio $h_v/h_c$ and radius ratio $R_v/R_c$; Fig. 5c). Measurements of epithelial thickness ratio and radius ratio in bulged (b, middle), whereas budded crypts opened up (increased radius ratio $R_v/R_c$) (c, middle). Measurements of epithelial thickness ratio and radius ratio in bulged (b, right; $n = 9$ biologically independent samples) and budded (c, right; $n = 10$ biologically independent samples) organoids are shown. P values were calculated using two-tailed paired Student’s t-tests. d,e, All of the samples from PGE and pipette inflation (bulged (d) and budded (e)) can be collapsed by fitting two parameters (Supplementary Note) to the same theoretical master curve ($R_v/R_c$ versus a function of $h_v/h_c$) independent of volume as predicted by the model (black line), showing opposite trends in bulged (d) versus budded (e). The images in b and c are representative of three independent experiments.
Furthermore, our model predicts that lowering contractility in budded organoids should allow lumen pressure to open up the crypt again as in bulged organoids. We confirmed this by repeating the PGE inflation experiment with blebbistatin, which led to budded-crypt opening without perturbing SC fate (Fig. 6a,b and Supplementary Video 8). Finally, we decreased the lumen volume experimentally to achieve the opposite effect to lumen inflation (Fig. 5a–c and Extended Data Fig. 7a,b). Four types of organoids were treated with osmotic shock: (1) day-2.5 organoids without crypt/villus regional difference; (2) day-3 spherical organoids with established regional difference in crypts and villi; (3) day-3.5 bulged organoids; and (4) day-3.5 spherical organoids treated with CHIR that consist only of crypt region15 (Fig. 6c). Seconds after the osmotic deflation, the day-3 spherical organoids formed bulges and day-3.5 bulged organoids formed buds in the crypt regions enriched in Lgr5+ SCs, while the day-2.5 and day-3.5 CHIR-treated organoids remained spherical and did not display significant bulging (Fig. 6c). This provides direct evidence that the reduction in the lumen...
volume can efficiently accelerate crypt morphogenesis in organoids in which domains of cellular fate (and therefore of spontaneous curvature) already exist.

Osmotic changes in enterocytes drives lumen shrinkage

Next, we addressed the mechanisms of volume reduction. Overall organoid volume varies little (Fig. 1d,e); we therefore hypothesized

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**Fig. 6 | Coordination of lumen volume reduction and region-specific spontaneous curvature promotes crypt morphogenesis.**

**a,** Actomyosin maintains crypt morphology in inflated budded organoids. Left, time-lapse recording of a budded *LifeAct-GFP* organoid treated with PGE to inflate the lumen (top) or PGE (the whole time) and blebbistatin (Blebb.) at 3:20 h (bottom). Right, corresponding plot for the radius ratio $R_c/R_v$ of the organoids. The lines in the middle represent the average values and the shadow regions represent the s.d. Times are shown as h:min.

**b,** Osmotic deflation in organoids with different spontaneous curvatures. Cartoon and images of a day-2.5 DMSO-treated organoid, day-3 organoid before bulging, day-3.5 bulged organoid and day-3.5 CHIR-treated organoid without bulging. Left, Paneth cells (red), SCs (yellow) and cells in villus tissue (blue) are indicated in the cartoons. Middle, fluorescence images of organoids expressing Lgr5–DTR–GFP and H2B–iRFP before and after osmotic shock. Right, quantification of the eccentricity of organoids before and after osmotic shock. The red arrows in **d** and **f** indicate the crypt regions. For the plots in **a**–**c**, *n* values are stated in parentheses and represent the number of independent recordings (**a**) or independent organoids (**b** and **c**) selected for measurements. *P* values were calculated using two-tailed *t*-tests (**b**) or two-tailed paired Student’s *t*-tests (**c**). The box-plot elements show the 25% (Q1, upper bounds), 50% (median, black lines within the boxes) and 75% (Q3, lower bounds) quartiles, and the whiskers denote 1.5× the interquartile range (maximum = Q3 + 1.5×(Q3 – Q1); minimum = Q1 – 1.5×(Q3 – Q1)) with outliers (rhombuses). For **a**–**c**, the experiments were repeated at least three times independently with similar results. Scale bars, 50 µm.
that the lumen volume is redistributed to epithelial cells. To distinguish whether a specific cell type was responsible for the reduction in lumen volume, we analysed organoids enriched in different cell types (Fig. 7a). Differentiated enterocytes display a marked reduction in lumen volume (Fig. 7b, Methods, Extended Data Fig. 8a and Supplementary Video 9), whereas CHIR-treated organoids, which are composed mainly of SCs and Paneth cells15, do not (with lumen volume increase; Fig. 7c,e, Extended Data Fig. 8a and Supplementary Video 10). These results suggest that lumen volume reduction is dependent on enterocyte swelling. Indeed, single-cell volumes in the villus region increased during crypt budding, whereas single-cell volumes in the crypt region did not (Extended Data Fig. 8b). We next refined our biophysical model to include that lumen volume reduction could be compensated by (1) a volume increase of all cells, (2) a volume increase of crypt cells or (3) a volume increase of villus cells. Strikingly, hypothesis 3 with enterocytes increasing their cell volume is the most efficient at producing budded crypts, as it decreases villus monolayer tension, while increasing crypt cell volume would be detrimental by increasing the bending rigidity of crypts (Fig. 7f and Supplementary Note). This

Fig. 7 | Enterocytes control lumen volume reduction through SGLT1. a, Schematic of the experimental design used. b, Light-sheet time-lapse recording of an enterocyst, an organoid that is composed of only enterocytes, from day 3 with tissue and lumen visualized by LifeAct–GFP (white). Times are shown as h:min. c, Light-sheet time-lapse recording of a day-3 organoid that is highly enriched with SCs and Paneth cells and that was treated with 3 μM CHIR. The tissue and lumen were visualized by LifeAct–GFP (white). Times are shown as h:min. d, The eccentricity, lumen volume, tissue volume and total volume (lumen + tissue) of the organoid in b. n = 5 independent recordings. e, The eccentricity, lumen volume, tissue volume and total volume of the organoid in c. n = 3 independent recordings. For d and e, the solid lines represent average values and the shaded regions represent the s.d. f, Model prediction of how an increased volume in different regions promotes budding, showing that fluid uptake specifically by villus cells is best for budding. g, Model prediction of increased cell volume in the villus region (v), with and without corresponding lumen volume changes, showing that cell volume changes alone can still promote budding (solid line). h, ISNE-based visualizations of scRNA-seq data indicate the expression of Slc5a1 (which encodes SGLT1). i, Immunostaining of SGLT1 (white) in a budded organoid with crypt regions visualized by Lgr5–DTR–GFP (green) indicates the enrichment of SGLT1 in the villus apical domain. j, SGLT1 is required for lumen volume reduction. Left, representative images of day-4 organoids from DMSO-treated control and sotagliflozin-treated samples (with lumen volume increase; Fig. 7c,e, Extended Data Fig. 8a and Supplementary Video 10), whereas CHIR-treated organoids, which are composed mainly of SCs and Paneth cells15, do not (with lumen volume increase; Fig. 7c,e, Extended Data Fig. 8a and Supplementary Video 10). These results suggest that lumen volume reduction is dependent on enterocyte swelling. Indeed, single-cell volumes in the villus region increased during crypt budding, whereas single-cell volumes in the crypt region did not (Extended Data Fig. 8b). We next refined our biophysical model to include that lumen volume reduction could be compensated by (1) a volume increase of all cells, (2) a volume increase of crypt cells or (3) a volume increase of villus cells. Strikingly, hypothesis 3 with enterocytes increasing their cell volume is the most efficient at producing budded crypts, as it decreases villus monolayer tension, while increasing crypt cell volume would be detrimental by increasing the bending rigidity of crypts (Fig. 7f and Supplementary Note). This
strongly suggests that luminal fluid uptake specifically by villus cells is an efficient and robust mechanism to promote crypt budding. We next returned to the in vivo situation—although the open geometry of the gut tube could make lumen volume changes less substantial, modelling only villus volume increase at a constant lumen volume still provided substantial forces mediating crypt budding (Fig. 7g). Interestingly, segmenting mouse villus cells in vivo during crypt formation (P1 and P11) revealed a volume increase in villus enterocytes (Extended Data Fig. 8c). This shows that, in vivo, the coordination of contractility patterns (Extended Data Fig. 1c,c') and enterocyte volume increase is associated with crypt morphogenesis.

Finally, to test mechanistically how lumen volume reduction is regulated in enterocytes, we analysed membrane transporters that regulate osmotic gradient and transcellular water transfer and selected three groups of candidates on the basis of their expression in scRNA-seq data (Fig. 7h and Extended Data Fig. 8d). Although classical mechanosensitive ion channels such as Piezo1 and Piezo2 are not specifically expressed or have specific function in this system (Extended Data Fig. 9), we found that the family members of the sodium/glucose cotransporter (SGLT1), the water channel aquaporins and the Na+/K+ ATPases (Atp1a1 and Atp1b1) have enterocyte-specific mRNA expression (Fig. 7h and Extended Data Fig. 8d). Interestingly, at the protein level, only SGLT1 is enriched at the villus apical domain (Fig. 7i), while aquaporins and Na+/K+ ATPase are expressed homogeneously throughout the epithelium42–45. Inhibition of each of the membrane transporters led to the failure of lumen volume reduction and organoid budding (Fig. 7j and Extended Data Fig. 8e). Remarkably, the inhibition of the SGLT1 (by sitagliptin) leads to the strongest defects (Fig. 7j and Extended Data Fig. 8e), supporting the role of the SGLT1 transporter in enterocytes in actively driving lumen volume reduction and villus cell swelling46.

Taken together, our findings suggest that membrane transporters, especially SGLT1, in enterocytes promote lumen volume reduction through the regulation of osmotic gradient and transcellular water transport.

**Discussion**

Here we demonstrated that apical contraction in crypts and basal tension in villi generate the region-specific spontaneous curvatures that lead to crypt bulging and budding, and that enterocytes further promote this process by swelling. Apical contraction drives tissue folding of many systems, such as the Drosophila mesoderm invagination, the vertebrate neural tube closure, the morphogenesis of mouse lens placode and gastrulation in various organisms47–49. Basal tension has recently been revealed to be the driving force for tissue morphogenesis50,51. In our model, the two essential requirements are the presence of differential cellular spontaneous curvatures (crypt versus villus), and a larger in-plane contraction of crypts compared to villi (Supplementary Note). Together, these two features result in a critical value of crypt apical tension, above which crypt shape becomes robust to lumen inflation, which could be relevant to in vivo pathological situations such as diarrhoea caused by infections52,53.

Interestingly, it has been reported that basal tension in Madin–Darby canine kidney cells leads to increased spontaneous curvature, which is able to bend 2D-cultured cell layers54. The basal tension in the villus region emerges during enterocyte differentiation and is potentially regulated by specific relocation of ZO-1 to the basolateral domain of the villus. The actomyosin regulator Rac-1 is required for the basal constriction of hinge cells that guides crypt spacing in vivo55, and could have a role in the longer-term maintenance of crypt shape, which could be probed theoretically by further considering boundary forces at the crypt-villus interface (Supplementary Note). As differential patterns of actomyosin56 and ZO-1 are also observed in crypts in vivo (Fig. 4d and Extended Data Fig. 5b), our findings can be extended to in vivo gut development. In mice, the space between villi is strongly reduced during crypt morphogenesis, and villus cells show extensive volume increase during crypt morphogenesis (Extended Data Fig. 8c), suggesting that osmotic effects also have a possible role during in vivo morphogenesis. Indeed, from the perspective of our model, lumen reduction in organoids and villus cell swelling both help curvature-driven bending by increasing compressive stresses within the epithelium. Finally, it is interesting that, although crypt morphogenesis is conserved from in vivo to in vitro, the shape of the villus domain remains flat in vitro. This is probably due to the fact that in vivo villus morphogenesis occurs before crypt morphogenesis, and involves a buckling instability from smooth muscle differentiation which restricts mesenchymal growth57, features that are absent in organoids. Strikingly, however, we found that perturbing ECM remodelling in organoids did not cause defects in crypt morphogenesis (Extended Data Fig. 10), which explains that crypt morphogenesis occurs in organoids without co-culture of mesenchymal cells.

Interestingly, in an accompanying paper on 2D intestinal organoids, Perez-Gonzalez et al.58 also showed that apical actomyosin tension is a key driver of crypt folding morphogenesis, and that this apical tension in SCs increases as a function of time throughout crypt formation. Using 3D traction force measurements and vertex models, the authors therefore reach similar conclusions on the role of bending rather than buckling in crypts. Together, these approaches show the power of organoid assays in uncovering the mechanochemochemical principles of self-organization and morphogenesis59.

To conclude, our findings implicate cell differentiation in orchestrating differential mechanical and epithelial osmotic properties, coordinating organoid morphogenesis, with implications for multiple developmental and cellular processes. Fluid can exert substantial morphogenetic forces, as shown in early mouse embryo or lungs60–65, which are rapidly transmitted spatiotemporally. The manipulation of lumen volume in combination with biophysical modelling is therefore a powerful tool and could be broadly applicable to other model systems. The coupling between cell fate, mechanics and osmotic changes that we uncovered enable stable and robust shape transformation. Interestingly, although formed budded crypts are able to sustain extensive fluid inflation, preventing lumen volume reduction before morphogenesis results in partially bulged, immature crypts, indicating additional potential feedbacks between luminal volume and crypt apical tension. Overall, a comprehensive understanding of the spatiotemporal coordinated behaviours during development and regeneration requires a link between large-scale tissue mechanics and molecular and cellular functions.

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41556-021-00700-2.

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Methods

Animal work. All of the animal experiments were approved by the Basel Cantonal Veterinary Authorities and conducted in accordance with the Guide for Care and Use of Laboratory Animals. Fifteen C57BL/6 outbred mice from three litters and a mother were used to obtain small intestines at the age of P1 (two mice), P2 (two mice), P3 (one mouse), P7 (one mouse), P11 (two mice), P12 (one mouse), P13 (one mouse), P14 (one mouse), P15 (one mouse), P16 (one mouse), P17 (one mouse) and 6 months (one female mouse) for time-course immunohistochemistry (animals from P1 to P17 were too young and therefore not distinguished on genders from external genitalia morphology). Male and female outbred mice from 7 weeks old and onwards were used for generating wild type and Lgr5-DTR-eGFP organoid lines as reported previously16. One male mouse at 10 weeks was used to generate the LifeAct-GFP organoid line. One male mouse at 10 weeks was used to generate the Myh9-GFP organoid line.

The following reagents and antibodies were used: C57BL/6 wild type (Charles River Laboratories), Lgr5-DTR-eGFP (Genentech, de Sauvage laboratory), LifeAct-GFP and Myh9-GFP (Lennon-Dumenil laboratory, Institut Curie).

Mice were kept in housing conditions under a 12 h–12 h light–dark cycle, 18–23°C ambient temperature and 40–60% humidity.

Organoid culture. Organoids were generated from isolated crypts of the murine small intestines as previously described12. Organoids were kept in IntestiCult Organoid Growth Medium (StemCell Technologies) with 100 μg/ml penicillin-streptomycin for amplification and maintenance.

Time-course experiments of fixed organoid samples. The method was adapted from a previous study16. Organoids were collected 5–7 days after passage and digested with Tryple (Thermo Fisher Scientific) for 20 min at 37°C. Disassociated cells were passed through a cell strainer with a pore size of 30 μm (Syrmex). Collecting cells were mixed with Matrigel (Corning) in a 1:1 ratio of ENR medium to Matrigel17. In each well of a 96-well plate, 5 μl droplets with 2,500 cells were seeded. After 15 min of solidification at 37°C, 100 μl of medium was overlaid. From days 0 to 1, ENR was supplemented with 25% Wnt3a-conditioned medium (Wnt3a-CM), 10 μM Y-27632 (ROCK inhibitor, StemCell Technologies) and 3 μM of CHIR99021 (GSK3B inhibitor, StemCell Technologies). From days 1 to 3, ENR was supplemented with 25% Wnt3a-CM and 10 μM Y-27632. From days 3 to 5, only ENR was added to the cells. Wnt3a-CM was produced in-house by Wnt3a mouse at 10 weeks was used to generate the LifeAct-GFP organoid line. From days 0 to 1, ENR was supplemented with 25% Wnt3a-conditioned medium (Wnt3a-CM), 10 μM Y-27632 (ROCK inhibitor, StemCell Technologies) and 3 μM of CHIR99021 (GSK3B inhibitor, StemCell Technologies). From days 1 to 3, ENR was supplemented with 25% Wnt3a-CM and 10 μM Y-27632. From days 3 to 5, only ENR was added to the cells. Wnt3a-CM was produced in-house by Wnt3a L-cells (gift from Novartis).

5, only ENR was added to the cells. Wnt3a-CM was produced in-house by Wnt3a L-cells (gift from Novartis).

Organoid immunostaining and imaging. The method was adapted from a previous report17. Primary and secondary antibodies were diluted in blocking buffer and applied as indicated in Supplementary Table 1. Cell nuclei were stained with 20 μg/ml DAPI (Invitrogen) in PBS for 5 min at room temperature. Cells were stained with 1 μg/ml of Alexa Fluor 647 carboxylic acid succinimidyl ester (CellTrac, Invitrogen) in carbonate buffer (1.95 ml of 0.5 M NaHCO3, 50 μl of 0.5 M Na2CO3, both from Sigma-Aldrich, in 8 ml of water for 10 ml of buffer).

High-throughput imaging was performed using an automated spinning-disk microscope from Yokogawa (0.63X/Voyager 4000S), with enhanced CSU-W1 spinning disk (micosc伦s-enhanced dual Nipkow disk confocal scanner), a x40/0.95 NA Olympus objective and a Neo sCMOS camera (Andor, 2,560 x 2,160 px). For imaging, an intelligent imaging approach was used in the Yokogawa CV7000 (Search First module of Wako software 1.0) as described previously18, z planes spanning a range of up to 90 μm and 2 μm or 3 μm z steps were acquired.

Confocal imaging of fixed samples was performed using the Nikon Ti-E Eclipse inverted motorized stage with the Yokogawa CSU W1 Dual camera (CAM1, X:11424; CAM2, 11736) T2 spinning-disk confocal scanning unit, CF1 P-Fluor x40/1.4NA oil-immersion objective and Vivoview v.4.4.0.8 software. The laser power was adjusted included Tiptop Smart 405/488/639 nm system (Coherent). Laser lines used included Toptica iBeam Smart 405/488/639 nm, a 516 nm Laser power and digital gain settings were unchanged within a given session to permit direct comparison of expression levels among organoids stained in the same experiment. Image stacks were acquired with a slice thickness of 2 μm or less.

Time-course image analysis. Organoid segmentation in maximum intensity projections (MIPs) was adapted from a previous study19. For each acquired confocal z-stack field, MIPs were generated. All MIP fields of a well were stitched together to obtain MIP well overviews for each channel. The high-resolution well overviews were used for organoid segmentation and feature extraction. To this end, we applied the segmentation framework of Serra et al.20, which segmented organoid instances observed until 72 h after fixation with a watershed algorithm and with a fully convolutional neural network (FCN)-augmented watershed for organoids observed at or after 72 h post-fixation. For the organoid segmentation based on DAPI staining, we substituted the segmentation of Serra et al.20 with a U-Net trained as FCN that was trained to generate instance masks for marker channels and used a fixed bandwidth and soft Jaccard loss18. Artefacts of segmentations were identified by setting the size threshold and visual inspection, annotated in Fiji (v.2.1.0 for all of the measurement involved in this study) and removed using a customized Python script. From the segmented MIPs, we calculated the 2D eccentricity of each individual organoid using scikit-image’s regionprops method. Using the individual organoid masks from the MIP, we cropped z stacks from the full 3D images (Yokogawa CV7000) and segmented them individually with the same FCN as used previously18. The 3D volume was approximated by the sum of voxel volume segmented as foreground. The 3D reconstruction of organoids in the osmotic shock experiment was performed using MorphoGraphX22 (the only version) (Fig. 6c), and rotated to demonstrate the change of crypt before and after osmotic shock. Selected 3D views were recorded for segmentation of the organoid shape and calculation of the 2D eccentricity as performed previously.

Single-cell apical and basal domains and single-cell volume were segmented and quantified in 3D using the MorphographX22. For apical and basal domains in day-3.5 and day-4 organoids, cells in the crypt and villus regions were manually segmented, starting from the top of the crypt–villus axis and multiplied by 10 (cell number per 10 μm).

Compound treatments. Compound treatments were tested in a dilution series of various concentrations from 1 mM to 5 nM, with at least three concentrations that induced changes in phenotype instead of organoid death; each medium concentration was repeated for quantification.

Single cells derived from LifeAct-GFP organoids were plated in a 96-well plate chamber or light-sheet chamber and exposed to 7.5 μM (+)-blebbistatin (myosin II inhibitor, Abcam, AB120925), 1 μM cytochalasin D (inhibitor of actin polymerization, Abcam, AB143844), 0.6 μM aphidicolin (DNA polymerase inhibitor, Sigma-Aldrich, A4487), 0.5 μM PGE (gift from Novartis), or 5 μM or 10 μM DMSO (Sigma-Aldrich, D8418) diluted in ENR medium, from 72 h until fixation at 96 h (96-well plate), or from 96 h for 10 h (light-sheet chamber, Extended Data Fig. 6f). From 96 h to 100 h, 90 μM Y-27632 (ROCK inhibitor, StemCell Technologies) and 3 μM of CHIR99021 (GSK3B inhibitor, StemCell Technologies) were added (Extended Data Fig. 6f). After measuring the mean grey value of 1 μm, at least five nuclei were recognized as continued next to each other and counted.

Myh9–GFP intensity ratios in single cells were measured using Fiji. A square of 1 x 1 μm was drawn onto the middle of cell apical, lateral or basal membrane in the image of the middle section of a stack. After measuring the mean grey value for the intensity of the signal on the membranes, the square was shifted to another part of the image of the middle section of a stack. After measuring the mean grey value for membrane signal was then normalized by comparing the ratio of the signal within same cell or same organoid (the term of ratios are shown in Fig. 3b). Myh9–GFP intensity ratios in apical versus basal in organoids were measured in an automated line selection (line width: 5 μm, 0.8125 μm) in Fiji. The entire apical and basal sides from the middle section of organoids were selected for measurement. Samples of each organoid type were selected from three independent experiments with inspection of Lyz co-staining, which indicates the successful induction of the organoid type (Fig. 3d, f). GFP mean intensity in Lgr5-DTR–GFP-positive cells was determined using the wand tool section in Fiji on the sum intensity projection of randomly selected organoid z stacks from each group (Fig. 3b).

Light-sheet microscopy. Light-sheet microscopy was conducted using the LS1 Live light sheet microscope system (Vivenist) or a similar customized microscope system described previously23. Sample mounting was performed as described previously23. For organoid imaging, LifeAct-GFP and Myh9-GFP organoids were collected and digested with Tryple (Thermo Fisher Scientific) for 20 min at 37°C. GFP-positive cells were sorted by fluorescence-activated cell sorting and collected in medium containing advanced DMEM/F-12 with 15 mM HEPES (StemCell
Technologies) supplemented with 100 μg/ml penicillin–streptomycin, 1X GlutaMAX (Thermo Fisher Scientific), 1X B27 (Thermo Fisher Scientific), 1X N2 (Thermo Fisher Scientific), 1mM N-acetylcyesteine (Sigma-Aldrich), 500 ng/ml R-spondin (StemCell Technologies); on the final day, 100 ng/ml murine EGF (R&D Systems). Cells (2,000) were then embedded in a 5 μl drop of Matrigel/medium at a 50:50 ratio. Drops were placed into the imaging chamber and incubated for 20 min before being covered with 1 ml of medium. For the first 3 d, medium was supplemented with 20% Wt3a-CM and 10 μg/ml Y-27632 (ROCK inhibitor; StemCell Technologies); on the final day, 3 μM of CHER90021 (StemCell Technologies) was also supplemented. After more than 2 d culture in a cell culture incubator, the imaging chamber was transferred to the microscope and kept at 37 °C under 5% CO₂. Different organoids were selected as starting positions and imaged every 10 min for up to 4 d, or in PGE treatment, every 3 min for up to 30 min. Budded organoids and enterocysts (the organoids that consist of only enterocytes) were separated from the segmented regions using scikit-image’s regionprops method. In enterocyst-projection of each time frame, the 2D eccentricity was extracted along the centre of an apical/basal circle (basically by the lateral texture of epithelium), and the average value of the apical and basal radii. For each region, we first tried to find the organoid lumen was then performed using the Olympus IX71 microscope equipped with micromanipulators (Eppendorf, NK2) and FemtoJet (Eppendorf).

For the 2 μm droplet of ENR medium, which was covered by mineral oil and placed onto the stage of a microscope (Olympus X71). Microinjection of ENR medium into the organoid lumen was then performed using the Olympus IX71 microscope equipped with micromanipulators (Eppendorf, NK2) and FemtoJet (Eppendorf). The inner walls of the micropipettes were made non-adhesive with Sigmacote (Sigma-Aldrich) to prevent tissue adherence during aspiration. Organoids were collected by Matrigel removal, with three washes in cold PBS. Organoid morphology did not significantly change for up 6 h in ENR medium after Matrigel removal. Random organoids were selected for experiments. In every experiment, the same pipette was used to measure the villi and crypts of the same organoids, and data were pooled from three independent experiments.

Organoid microinjection. Organoids were collected by Matrigel removal, with three washes in cold PBS. Organoid morphology did not significantly change for up 6 h in ENR medium after Matrigel removal. Random organoids were collected for experiments. In every experiment, the same pipette was used to measure the villi and crypts of the same organoids, and data were pooled from three independent experiments.

### Immunohistochemistry and data analysis

Jejunums were dissected for fixation in 10% PFA (Electron Microscopy Sciences) in PBS for 24 h at 4 °C, washed with 70% ethanol, embedded in paraffin, and 3 μm sections were prepared and processed for haematoxylin staining and immunohistochemistry. Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded tissue sections using a Ventana Benchmark fully automated staining system with anti-ZO-1 (Thermo Fisher Scientific, 33-9100, 1:50), anti-beta-catenin (BD Transduction Laboratories, 610154, 1:000) and anti-cytokeratin 14 (Bio-Rad, Molecular Probes, 1:100) antibodies. The immunohistochemical density was measured using the same method as described for organoids (see the “Time-course image analysis” section). Single-cell areas were measured manually by Fiji using the Python selection tool and visualized using ROI manager and a customized ImageJ macro script. Area size varies from apical to basal domains in single cells and does not vary much in parallel to lateral domains. Cell areas in the middle sections that were parallel to lateral domains from the recorded z-stack images were therefore selected for measurements. The distance between villi was measured using the following method: the curves c and c₂ of two closed edges of neighbouring villi were annotated using Fiji’s ROI Manager and exported as ROI files. In a second step, the files were imported into Python and a bivariate polynomial p was fitted, which imported correctness. A correlation between the polynomial and the data was checked manually. The polynomial method was used to define sample size. In compound time-course experiments, we assumed that a minimum of around 50 organoids at day 4 would be sufficient to recognize differences between control and perturbations on the basis of experimental samples. Sample size was determined on the basis of previous related studies in the field.

### Statistics and reproducibility

All statistical analysis was performed using the Python library SciPy. Statistical significance for data displaying normal distributions was calculated using two-tailed Student’s t-tests; for the paired samples in Figs. 1c–f, 4g (right) and 5c, and Extended Data Fig. 7a″, b″, statistical significance was calculated using two-tailed paired Student’s t-tests. P values were performed in Fig. 1c: (1) for apical versus basal domain size in the violin plot P_apical < 10⁻¹⁰ (two-tailed t-test); in the dot plot P_apical < 0.05 (two-tailed paired Student’s t-test). (2) Apical size (two-tailed t-test): day-3 (n = 331) versus day-4 (n = 365), P_apical < 10⁻¹⁰; day-3 (n = 307) versus day-4 (n = 319), P_apical < 0.001; day-3 versus day-4 (n = 550), P_apical < 0.008; day-3.5 crypt versus day-4 crypt, P_apical < 10⁻⁶; day-3.5 crypt versus day-4 crypt, P_apical < 0.0035. (3) Basal size (two-tailed t-test): day-3 (n = 531) versus day-4 (n = 507), P_basal < 0.05; day-3 (n = 507) versus day-3.5 villus (n = 1,249), P_basal < 10⁻¹³; day-3 (n = 507) versus day-4 villus (n = 1,249), P_basal < 10⁻¹³. For box-plot analysis, see the “Time-course image analysis” section.
**Author contributions**
P.L. and Q.Y. conceived the project and designed the experiments. Q.Y. performed and analysed the experiments. E.H. proposed the physical theory. S.-L.X. designed the physical model, performed the simulations. C.J.C. supported the micropipette aspiration. M.R. and D.V. assisted with the image processing and quantifications. F.M.G. generated organoid lines. C.J.C. and T.H. helped to design the micropipette aspiration and mosaic experiments. Q.Y. wrote the first version of the manuscript, S.-L.X. wrote the first version of the Supplementary Information, and P.L., E.H. and Q.Y. supervised the study. P.L. and E.H. helped to design the project and finalize the manuscript.

**Competing interests**
The authors declare no competing interests.

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Extended Data Fig. 1 | Cell and tissue quantification. **a**, Example of selecting crypt and villus region for quantification of single-cell apical and basal size in bulged organoid. Upper panel, from left to right: Z-projection of ZO-1 and Lyz staining in bulged organoid, selected apical segmentation with Lyz signal in 3D and ZO-1 signal (white) projected in 3D apical side in villus from xy view, in villus from yz view, in crypt from xy view and in crypt from yz view. Lower panel, from left to right: selected basal segmentation with Lyz signal in 3D and ZO-1 signal (white) projected in 3D basal side in villus from xy view, outline of villus basal segmentation from xy view, crypt basal segmentation from xy view, outline of crypt basal segmentation from xy view and yz view. Colour of heatmap indicates the size of single-cell apical or basal membranes. **b-c′**, Tissue compaction along crypt-villus axis in the development of intestinal organoid and in vivo tissue. **b**, Representative images of bulged and budded organoids with CD44 and DAPI staining. **b′**, Plot of cell density in crypt (red dashed double arrowhead line) and villus (blue dashed double arrowhead line) tissue as indicated in B. Two-tailed t-test for bulged crypt (n = 22) and bulged villus (n = 22), p < 10^{-11}; for budded crypt (n = 19) and budded villus (n = 19), P < 10^{-11}. **c**, Representative images of immunostaining on the section of mouse intestine at the age of P1 and P11. **c′**, Plot of cell density in crypt (red dashed double arrowhead line) and villus (blue dashed double arrowhead line) regions as indicated in C. Two-tailed t-test for P1 crypt (n = 32) and P1 villus (n = 31), p < 10^{-10}; for P11 crypt (n = 40) and P11 villus (n = 44), P < 10^{-23}. Experiments and imaging analysis in A, and experiments in B-C were repeated at least three times independently with similar results. Scale bars, 20 μm. Violin plot lines in B′ and C′ denote quartile for each group.
Extended Data Fig. 2 | See next page for caption.
Extended Data Fig. 2 | Sensitivity analysis for how model parameters affect crypt morphology. a–c, Schematic of the model and morphometric parameters used (see Supplementary Note). d, Phase diagrams of crypt morphologies with varying volumes \( v \) and spontaneous curvature of crypt \( \gamma_c \), for different values of in-plane contraction \( \alpha \) (left to right: 1, 0.6, and 1.5). e–h, Evolution of thickness ratio \( h_c/h_v \) and radius ratio \( R_c/R_v \) during the inflation of an organoid (crypt size \( \phi = 0.2 \), shape factor \( \tilde{\kappa}_0 = 10 \))): increasing \( \alpha (\gamma_c = 0.1) \) (panel E) and \( \gamma_c (\alpha = 1, 1.5, \text{and} 0.6) \) (panels F–H). i, Influence of cell swelling on crypt morphology (degree of crypt opening) with varied crypt size \( \phi \) (\( \alpha = 1.5, \gamma_c = -0.02 \)), \( \alpha (\gamma_c = -0.1, \phi = 0.5) \), and \( \gamma_c (\alpha = 1, \phi = 0.5) \). j, Morphological evolution during the inflation of an organoid (\( \alpha = 1.5, \gamma_c = -0.02 \)) with swollen villus cells (\( v_{ev} = 5 \)). k, Influence of spontaneous curvature of villus \( \gamma_v \) on crypt morphology (\( \phi = 0.2 \)) with varied \( \gamma_c (\alpha = 1) \) and \( \alpha (\gamma_c = -0.08) \). l, Influence of \( \gamma_v \) on the evolution of \( h_c/h_v \) and \( R_c/R_v \) during the inflation of an organoid with \( \alpha \) is respectively 1 and 1.5 (\( \gamma_c = -0.01, \phi = 0.2 \)).
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 | Comparison between numerical solutions of the full model and analytical scaling laws. a-a″. Three possible mechanical scenarios that could drive crypt morphogenesis. Stable organoid configuration is calculated by minimizing the energy $F(\alpha, \gamma_c, \gamma_v, \phi, v, \Lambda)$, which depends on a few key parameters (see Supplementary Note): $\alpha$, ratio of in-plane contractions in crypt and villus regions; $\gamma_c$, spontaneous curvature crypt region; $\gamma_v$, spontaneous curvature of villus region; $\phi$, relative size of the crypt region; $v$, normalized lumen volume; $\Lambda$, potential line tension along the crypt/villus boundary.

a, crypt budding driven by smaller crypt in-plane contraction $\alpha$, leading to thinner crypts (decreased epithelial thickness ratio $h_c/h_v$ and decreased radius of curvature $R_c/R_v$). a′, crypt budding driven by spontaneous curvature $\gamma_c$, leading to thicker crypts (increased $h_c/h_v$ and decreased $R_c/R_v$). a″, organoid budding driven by line boundary tension, leading to constant thickness (constant $h_c/h_v$ and decreased $R_c/R_v$). The width and location of the green lines indicate the strength and distribution of the driving forces in each model. b, Radius ratio ($R_c/R_v$) from six experimental samples with the corresponding model fit (see Supplementary Note). c-d, All six samples of bulged organoids can be collapsed via $h_c/h_v$ vs. time (C) or $R_c/R_v$ vs. time (D). e-f, Comparison of numerical (symbol) and analytic (line) results to verify that, thickness ratio $h_c/h_v$ and radius ratio $R_c/R_v$ respectively depend on in-plane contraction $\alpha$ (with crypt size $\phi = 0.05$) and coupled parameter $\phi^{-1}\gamma_c$ (with $\alpha = 1.15$) for a bulged organoid (normalized volume $v = 5$) (panel E), $h_c/h_v$ and $R_c/R_v$ depend on parameter $u$ for a budded organoid ($\phi = 0.2$) (panel F), and analytic results also fit well with numerical results for the inflation of a bulged organoid ($\alpha = 1.15, \gamma_c = -0.025, \phi = 0.05$) or a budded organoid ($u = 6, \phi = 0.2$). g, Phase diagram of crypt morphologies of an organoid ($\phi = 0.2, \tilde{\kappa}_0 = 10$) under infinite volume expansion ($v = 10^8$) (see Supplementary Note), with varying spontaneous curvature $\gamma_c$ and in-plane contraction $\alpha$. h, Influence of normalized volume of a crypt cell $v_c$ (top) and that of a villus cell $v_v$ (bottom) on $h_c/h_v$ and $R_c/R_v$ of a budded organoid ($u = 6, \phi = 0.2$).
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Actomyosin drives and maintains crypt budding. a-a’. Montage images across the regions of laser cutting and opening with surrounded LifeAct-GFP signal. Dashed lines outline the size of opening. a’, Plot for distance of openings after cutting. b, Staining of phosphorylated Myosin light chain (pMLC) in budded organoid with maximum z-projection (upper panel) and single section (lower panel). c, Day4 LifeAct-GFP organoids in Control (n = 96), Blebbistatin (n = 188) or Cytocalasin D (n = 45) cultures. Left images, maximum z-projection of LifeAct-GFP; right images, merged maximum z-projection of LifeAct-GFP with Lyz and DAPI staining. Plots, quantification of organoid eccentricity and volume ratio (lumen vs total). d, Representative time-lapse recording of organoid expressing Myh-9-GFP during crypt morphogenesis. Upper images, middle sections. Lower images, maximum z-projections. e, Membrane-targeted GFP (mG) in organoid before bulging (n = 12), bulged (n = 20) and budded (n = 22) organoids in the middle section (images); quantification for ratios of mG intensity in cells (box plots). f, Representative time-lapse recording of budded LifeAct-GFP (white) organoid treated with DMSO control, Blebbistatin or Aphidicolin (left images), and corresponding plot for aspect ratio of the organoids (right plot, numbers of recordings in bracket). Solid lines represent average values, shadow regions standard deviations. g-g’, Merged images for Myh-9-GFP and ZO-1 staining in wild-type/Myh-9-GFP (G, left image) or in myh-9+/Myh-9-GFP (G’, left image) mosaic organoid with zoom-in areas (G and G’, right image) from yellow dashed rectangles. White dashed line outlines the morphology of Myh-9-GFP cells. g”, quantification on percentage of basal constriction in Myh-9-GFP cells next to wild-type (n = 62) or myh-9+ (n = 79) cells Arrows: red (crypt), blue (villus), white (G and G’, basal domain of Myh-9-GFP cells). P-values are calculated from two-tailed t-test. Box-plot elements show 25% (Q1, upper bounds), 50% (median, black lines within the boxes) and 75% (Q3, lower bounds) quartiles, and whiskers denote 1.5x the interquartile range (maxima: Q3 + 1.5x (Q3-Q1); minima: Q1-1.5x (Q3-Q1)) with outliers (rhombuses). Scale bars: B (20µm), C-D, F, G-G’ left (50µm), G-G’ right zoom-in (10µm). Images in A-G are representative of three independent experiments. Data in G” are pulled from two independent experiments.
Extended Data Fig. 5 | In vivo tissue-specific localization of Claudin2 and ZO-1. In vivo staining of Claudin2 (A) and ZO-1 (B) in mouse small intestine at P1, P2, P5, P7, P11, P12, P13, P14, P15, P16, P17 and 6-month adult stages. Stainings were repeated at least three times independently with similar results. Yellow dashed rectangles in B indicate zoom-in regions of villi randomly selected from the same field of views in the presented images. Scale bars: A (100 µm), B (100 µm, zoom-in, 20 µm).
Extended Data Fig. 6 | Sensitivity analysis of model results as a function of crypt apical tension and osmotic changes. a, Phase diagram of crypt morphologies ($\tilde{\kappa}_0 = 6, \varphi = 0.2$) as a function of normalized crypt apical tension $m$ and villus cell volume $v_{ev}$, showing the villus cell swelling favors crypt budding synergistically with crypt apical tension. b, Influence of crypt size $\varphi$ and shape factor $\tilde{\kappa}_0$ on critical values of $m$ (with $v_{ev} = 1$, that is no cell swelling) and $v_{ev}$ (with $m = 2$, that is constant crypt apical tension) for organoid budding. c–e, Influence of preferential proliferation of crypt cells, characterized as crypt size $\varphi_w$ on crypt morphology, with normalized organoid volume $v = 1$ (panel C) or 1.2 (panel E). After rescaling volume $v$, crypt morphologies with preferential crypt growth (solid line) are close to those without preferential growth (dash line) (panel D, see Supplementary Note). f–h, Dependence of thickness ratio $h_c/h_v$ and radius ratio $R_c/R_v$ on normalized crypt apical tension $m$ ($v_{ev} = 1$), with varied $\tilde{\kappa}_0$ ($\varphi = 0.2$) (panel F) and $\varphi$ ($\tilde{\kappa}_0 = 6$) (panel G), and on villus cell volume $v_{ev}$ (panel H), with varied $m$ ($\tilde{\kappa}_0 = 6, \varphi = 0.2$). i, Evolution of $h_c/h_v$ and $R_c/R_v$ during organoid inflation with varied $m$ ($\tilde{\kappa}_0 = 6, \varphi = 0.2, v_{ev} = 1$). j, Phase diagram of crypt morphologies upon infinite volume expansion ($v = 10^8$) (showing three possible phases: fully closed, partially opening, or fully closed with vanishing apical surface), with varying $\tilde{\kappa}_0$ and $m$ ($\varphi = 0.2$).
Extended Data Fig. 7 | The impact of lumen volume on organoid morphology. a-b′, Fitting for lumen inflation experiments with theoretical model. Images, microinjection of bulged (A) and budded (B) organoids for lumen inflation. Fittings (A′ and B′), fitting experimental measurements with its predictive model based on epithelial thickness ratio (h_c/h_v), organoid radius ratio (R_c/R_v) and lumen volume (v) change. Plots (A″ and B″), measurements of h_c/h_v and R_c/R_v in bulged (A″, n=7 biologically independent organoids) and budded (B″, n=12 biologically independent organoids) organoids. P-values are calculated from two-tailed Paired Student’s t-test. c-c′, Evolution of morphometric parameters in bulged (C) or budded (C′) organoids during volume inflation, induced by PGE treatment (n=3 biologically independent organoids) and microinjection (n=3 biologically independent organoids), can be collapsed via R_c/R_v vs. v and h_c/h_v vs. v. Scale bars, 50 µm.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Regulation of lumen volume by enterocytes and the membrane transporters. a, Representative images of DMSO-treated control (n = 96), CHIR- (n = 145) and IWP2- (n = 78) treated organoids (left panels), and corresponding quantification on eccentricity and lumen ratio (right panels). b, Segmentation (Seg.) of single-cell volume based on β-Catenin signal in bulged and budded organoids (left images), violin plot for the corresponding quantification of single-cell volume (left plot), swarm plot for the lumen ratio of each organoids (right plot). Two-tailed t-test for cells in crypt (n = 385) vs. villus (n = 218) in bulged organoid (p = 0.311), crypt (n = 551) vs. villus (n = 769) in budded organoids (p < 10^{-7}), and bulged villus vs. budded villus (p < 10^{-15}); for lumen ratio bulged (n = 8) vs. budded (n = 6) (p < 10^{-14}). c, Immunostaining of β-Catenin in the section of mouse intestinal tissue at P1 and P11 stages, segmentation (seg.) of single-cell area, zoom-in crypt (red dashed rectangles) and villus (blue dashed rectangles) areas in β-Catenin staining image overlapping with single-cell segmentations (left images), violin plot for the quantification of single-cell areas (left plot), swarm plot for the quantification of distance between villi (right plot). Two-tailed t-test for cells in P1 crypts (n = 54) vs. villi (n = 156) (p < 10^{-14}), in P11 crypts (n = 82) vs. villi (n = 159) (p < 10^{-14}), and cells in P1 villi vs. P11 villi (p < 10^{-14}); for distance between villi, P1 (n = 32) vs. P11 (n = 30) (p < 10^{-7}). d, TSNE-based visualizations of single-cell RNA sequencing data indicate mRNA expressions of aquaporins, atp1a1 and atp1b1. e, Day4 organoid treated with DMSO control, Ouabain and CuSO4 (left images), quantification on the eccentricity and lumen ratio of control (n = 155), Ouabain (n = 146), CuSO4 (n = 192) and Sotagliflozin (n = 119) (right plots). P-values in box plots (A, E) are calculated from two-tailed t-test. Box plot elements show 25% (Q1, upper bounds), 50% (median, black lines within the boxes) and 75% (Q3, lower bounds) quartiles, and whiskers denote 1.5× the interquartile range (maxima: Q3 + 1.5×(Q3-Q1); minima: Q1-1.5×(Q3-Q1)) with outliers (rhombuses). Violin plot lines (B, C) denote quartile for each group. Scale bars: A-B (50 μm), C (left, 50 μm, zoom-in, 10 μm), E (20 μm). Images in A, B, C and E are representative of > 3 independent experiments.
Extended Data Fig. 9 | Piezo channels do not strongly regulate crypt budding. a, TSNE-based visualizations of single-cell RNA sequencing data indicate the marker gene expression for crypt (left panel), villus (middle panel) and piezo1 (right panel). b, Piezo1 and DAPI staining in budded organoid. Piezo1 is detected in few single cells. Staining was repeated at least three times independently with similar results. c–e, Inhibition of Piezo1 (GdCl3 and GsMtx4) did not cause any defect in crypt morphogenesis, while activation of Piezo1 (Yoda-1) leads to slight increased lumen volume and reduced eccentricity. Reduced enterocytes (indicated by AdlB signal) are detected in Yoda-1-treated organoids. c, Representative images of Day 4 organoids treated in DMSO-control, GdCl3, GsMtx4 or Yoda-1 condition with AdlB, CD44 and DAPI staining in maximum z-projection. d–e, Corresponding box-plot quantifications of eccentricity (D) and lumen ratio (E) for organoids from C. Experiment (C–E) was repeated three times independently with similar results. Sample numbers: DMSO-control (n = 94 organoids), GdCl3 (n = 143 organoids), GsMtx4 (n = 68 organoids) and Yoda-1 (n = 71 organoids). P-values are calculated from two-tailed t-test. Box-plot elements show 25% (Q1, upper bounds), 50% (median, black lines within the boxes) and 75% (Q3, lower bounds) quartiles, and whiskers denote 1.5× the interquartile range (maxima: Q3 + 1.5× (Q3-Q1); minima: Q1-1.5× (Q3-Q1)) with outliers (rhombuses). Scale bars, 25 μm.
Extended Data Fig. 10 | ECM remodelling is nonessential for crypt budding. 

**a**, Representative images of Day 4 organoids treated by DMSO as control, or broad-spectrum inhibitors of matrix metalloproteinases (GM6001 and Marimastat) with Laminin staining for Matrigel and DAPI staining for cell nuclei in maximum z-projection. Experiment was repeated >3 times independently with similar results. 

**b**, Corresponding box-plot quantifications of eccentricity for organoids from A. Experiments were repeated three times independently with similar results. Sample numbers: DMSO-control (n = 265 organoids), GM6001 (n = 102 organoids), and Marimastat (n = 94 organoids). P-values are calculated from two-tailed t-test. Box-plot elements show 25% (Q1, upper bounds), 50% (median, black lines within the boxes) and 75% (Q3, lower bounds) quartiles, and whiskers denote 1.5x the interquartile range (maxima: Q3 + 1.5x (Q3-Q1); minima: Q1-1.5x (Q3-Q1)). Scale bars, 50 µm.
Reporting Summary

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Statistics

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

To collect time-course imaging data, Wako Software Suite (version 1 and 2) and custom written segmentation code was used. The code was written in the Liberali lab and implemented with Python 3.6 (relying on multiple open source Python libraries for scientific computing and image analysis).

ZEN Black software was used for collecting data of laser nanosurgery. Visview (version 4.4.0.9) was used for collecting confocal imaging data.

Data analysis

To analyze imaging data, Fiji (version 2.1.0), MorphoGraphX as well as custom written code in Python 3.6 (relying on multiple open source Python libraries for scientific computing and image analysis) was used. To analyze RNA-seq data, Rtsne R package (version 3.6.0) was used for tSNE projections.

Code availability

Codes generated and analysed during the current study are available on https://github.com/fmi-basel/glib-nature_cell_biology2021-materials.git.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
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Sequencing data that support the findings of this study have been deposited in the Gene Expression Omnibus (GEO) under accession code GSE115956. Source data are submitted for all the plots. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No particular statistical method was used to define sample size. In compound time-course experiments, we assumed a minimum of around 50 organoids at Day4 would be sufficient to recognize differences between control and perturbations based upon historical experiments. Sample size was determined based on previous related studies in the field (15). |
| Data exclusions | Parameters used to exclude datapoints were defined based on data distributions. Exclusion criteria for segmentation were pre-defined as reported previously (15). (In all time-course experiments for organoids, objects with size smaller than the number of 200 pixels were excluded as they are recognized as smaller than single cells.) |
| Replication | All experiments were replicated at least twice with similar findings. Compound time-course experiments were repeated on different days with different culture of organoids. |
| Randomization | Samples were randomly assigned. |
| Blinding | Same investigator performed experiments and data analyses therefore it is not blinding. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a  ☒ Antibodies  ☐ Eukaryotic cell lines  ☒ Palaeontology and archaeology  ☒ Animals and other organisms  ☒ Human research participants  ☐ Clinical data  ☒ Dual use research of concern | n/a  ☐ ChIP-seq  ☐ Flow cytometry  ☒ MRI-based neuroimaging |

Antibodies

Antibodies used
- anti-ZO-1 (Thermo fisher scientific 33910, clone ZO1-1A12 (monoclonal), dilution 1:200),
- anti-SGLT-1 (Abcam Ab14685, clone NA (Polyclonal), dilution 1:200),
- anti-CD44v6 (BioRad MCA1967, clone 9A4 (monoclonal), dilution 1:200),
- anti-MLC-S1 (Abcam Ab157747, clone NA (Polyclonal), dilution 1:200),
- anti-Occludin (Thermo fisher scientific 711500, clone NA (Polyclonal), dilution 1:200),
- anti-N-Cadherin (BD transduction laboratories 610920, clone NA, dilution 1:200),
- anti-Claudin2 (Thermo fisher scientific 325600, clone 12H12 (monoclonal), dilution 1:200),
- anti-Lysozyme (Abcam Ab36362, clone BGN/06/96 (monoclonal), dilution 1:500),
anti-Beta-Catenin [BD transduction laboratories 610154, clone 14, dilution 1:200],
anti-AldolaseB (Abcam ab75751, clone ERP3138Y, various lots for example GR121452-7, dilution 1:400),
anti-Laminin (Sigma-Aldrich L9393, clone NA (Polyclonal), dilution 1:200),
anti-Piezo1 (Sigma-Aldrich HPA047185, clone NA(Polyclonal), dilution 1:200),
Alexa Fluor 488 goat anti rabbit IgG (Thermo fisher scientific B40943, dilution 1:2000),
Alexa Fluor 568 goat anti rat IgG (Invitrogen A11017, dilution 1:2000),
Alexa Fluor 488 donkey anti mouse IgG (Thermo fisher scientific A-21202, dilution 1:2000).

Validation statements available from manufacturers:
- anti-ZO-1(https://www.thermofisher.com/antibody/product/ZO-1-Antibody-clone-ZO1-1A12-Monoclonal/33-9100)
- anti-SGLT-1(https://www.abcam.com/sgt1-antibody-ab14685.html)
- anti-CD44v6 (https://www.bio-rad-antibodies.com/monoclonal/Mouse-cd44v6-antibody-9a4-mca1967.html?pf=purified),
- anti-MLC-51 (https://www.abcam.com/myosin-light-chain-phospho-s1-antibody-ab157747.html),
- anti-Occludin (https://www.thermofisher.com/order/genome-database/details/antibody/71-1500),
- anti-N-Cadherin (https://www.bdbiosciences.com/us/applications/research/stem-cell-research/cancer-research/human/purified-mouse-anti-n-cadherin-32n-cadherin/p/610920),
- anti-Claudin2 (https://www.thermofisher.com/antibody/product/Claudin-2-Antibody-clone-12H12-Monoclonal/32-5600),
- anti-Lysozyme (https://www.abcam.com/lysozyme-antibody-bgn06961-as36362.html),
- anti-Beta-Catenin (https://www.bdbiosciences.com/us/applications/research/stem-cell-research/cancer-research/human/purified-mouse-anti-beta-catenin-14beta-catenin/p/610154),
- anti-AldolaseB(https://www.abcam.com/aldolase-b--aldolase-c-antibody-epi3138y-ab75751.html),
- anti-Laminin (https://www.sigmaaldrich.com/catalog/product/sigma/l9393?lang=de&region=CH&gclid=EAIaIQobChMI3aXt_fPe7gVzRoGAB2OQuQEAAYASAAEgl69fd_BwE),
- anti-Piezo1 (https://www.sigmaaldrich.com/catalog/product/sigma/hpa047185?lang=de&region=CH&gclid=EAIaIQobChMI3aXt_fPe7gVzRoGAB2OQuQEAAYASAAEgl69fd_BwE).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s) Wnt3a L-cells , gift from Novartis
Authentication The cell line used was not authenticated.
Mycoplasma contamination The cell line is tested negative with mycoplasma contamination.
Commonly misidentified lines (See ICLAC register) No commonly misidentified lines in this lab.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals All animal experiments were approved by the Basel Cantonal Veterinary Authorities and conducted in accordance with the Guide for Care and Use of Laboratory Animals. 35 C57BL/6 outbred mice from three litters and a mother were used to obtain small intestines at the age of P1 (two mice), P2 (two mice), P5 (one mouse), P7 (one mouse), P11(two mice), P12 (one mouse), P14 (one mouse), P15 (one mouse), P16 (one mouse), P17 (one mouse) and 6-month (one female) for time-course immunohistochemistry (animals from P1 to P17 were too young and therefore were not distinguished on genders from external genitalia morphology). Male and female outbred mice from 7 weeks old onwards were used for generating organoid lines of wild type and Lgr5-DTR-EGFP as reported previously (15). One male mouse at 10 weeks was used to generate organoid line of LifeAct-GFP. One male mouse at 10 weeks and one male mouse at 11 weeks were used to generate organoid line of Myh-9+/-. One male mouse at 10 weeks was used to generate organoid line of Myh-9-GFP.

Mouse lines used: C57BL/6 wild type (Charles River Laboratories), Lgr5-DTR-EGFP (Genentech, de Sauvage laboratory), LifeAct-GFP and Myh-9-/- (T. Hiiragi laboratory, EMBL), Myh-9-GFP (Lennon-Duménil laboratory, Institut Curie).

Mice were kept in housing conditions with 12-hour light/12-hour dark cycle, 18-23 degree ambient temperature and 40-60% humidity.

Wild animals The study did not involve wild animals.
Field-collected samples The study did not involve samples collected from field.
Ethics oversight All animal experiments were approved by the Basel Cantonal Veterinary Authorities and conducted in accordance with the Guide for Care and Use of Laboratory Animals.

Note that full information on the approval of the study protocol must also be provided in the manuscript.