Supplementary Note for

Cell fate coordinates mechano-osmotic forces in intestinal crypt formation

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In this Supplementary Note, we provide details on our physical model for the morphogenesis of intestinal organoids. The organoid is treated as a closed epithelial monolayer with two distinct regions, encapsulating an incompressible fluid lumen. We develop a three-dimensional biophysical model to study the mechanics of organoids and use it to derive analytical results of specific morphologies, i.e., bulged and budded shapes, concentrating in particular on the impacts of crypt apical constriction and lumen volume changes on morphogenesis.

1. Two-region vertex model

The macroscopic shape of epithelial tissues and organs can be understood from mechanical interactions at the cellular level, such as cell-cell adhesion and actomyosin-mediated tension along the cell membrane. Vertex models are a class of multiscale mechanical models to understand the interplay between cellular mechanical forces and tissue-scale deformation (*1*-*3*). In vertex models, tissue is described as a set of vertices, where each vertex represents a tri-cellular junction that cell edges meet at, and on which force balance is written (taking into account forces such as surface tensions, line tensions, internal fluid pressure, and external forces from surrounding environment).

An intestinal organoid is initially a spherical epithelial monolayer with a central luminal fluid cavity. After symmetric breaking which creates segregated stem cell and differentiated cell regions, the organoid will evolve towards pear-shaped configurations composed of two regions, crypt and villus. For simplicity, each region in the model is treated as a spherical cap. In the following, we first discuss the free energy of a single cell in the monolayer, then get the total energy of the whole organoid.

1.1. Free energy of a single cell

Consider a single cell with three surface tensions Γ_a, Γ_b, and Γ_l, and three surface areas A_a, A_b, and A_l, where the subscripts a, b, and l respectively represent apical, basal, and lateral surfaces/domains (Fig. 2A). Then, the free energy of a single cell is

$$f = \Gamma_a A_a + \Gamma_b A_b + \frac{1}{2}\Gamma_l A_l, \qquad (1)$$

The apical and basal surfaces are simplified as squares with side lengths d_a and d_b (although more complex shape would give identical results up to pre-factors). With the height of a cell as h, the free energy (1) becomes

$$f = \Gamma_a d_a^2 + \Gamma_a d_b^2 + \Gamma_l h\left(d_a + d_b\right). \tag{2}$$

Each region is treated as a part of a homogeneous sphere shell, which has total cell number N'. In the spherical region, the side lengths are related to the region radii, i.e. $d_a = \sqrt{4\pi/N'}R_a$, $d_b = \sqrt{4\pi/N'}R_b$, where R_a and R_b are the inner (apical) and outer (basal) radii, respectively. Moreover, we have $R_a = R - h/2$, $R_b = R + h/2$, where R is the neutral radius (see Extended Data Fig. 2A for a schematic). Then, the free energy can be rewritten as

$$f = \frac{4\pi}{N'}\left[\left(\Gamma_a + \Gamma_b\right)R^2 + \left(\Gamma_b - \Gamma_a\right)Rh\right] + 2\sqrt{\frac{4\pi}{N'}}\cdot\Gamma_l Rh. \tag{3}$$

For simplicity, a thin-film assumption is employed, which means the thickness of the spherical sheet is much smaller than its radius, i.e. $\left(h/R\right)^2 << 1$ (R/h is typically larger than 2, as we subsequently measure this ratio to fit the morphogenetic evolution and organoid lumen inflation, see Subsection 4.2.1 for further details), which leads to $N'V_{e0} = 4\pi R^2 h\left[1+\frac{1}{12}\left(\frac{h}{R}\right)^2\right] \approx 4\pi R^2 h$, where V_{e0} is the cell volume. This greatly simplifies the analytics, as it yields $h \approx N'V_{e0}/\left(4\pi R^2\right)$. Given that cell volume is under osmotic regulation, involving stresses much larger than the ones produced by actomyosin (*4*), it is reasonable to assume that the volume V_{e0} is independent from tension forces. However, cell volume may change during villus cell differentiation, due to active osmotic regulation, which will be discussed in Subsection 1.4. Under these assumptions, the free energy is only related to radius R:

$$f\left(R\right) \approx \frac{4\pi}{N'}\left(\Gamma_a + \Gamma_b\right)R^2 + \left[\left(\Gamma_b - \Gamma_a\right) + 2\Gamma_l\sqrt{\frac{N'}{4\pi}}\right]\frac{V_{e0}}{R}, \tag{4}$$

and the corresponding neutral radius in free state $\tilde{R}$ should satisfy $\left.\frac{\partial f}{\partial R}\right|_{\tilde{R}} = 0$, which leads to

$$\tilde{R} = \sqrt{\frac{N'}{4\pi}}\left(\frac{V_{e0}\Gamma_l}{\Gamma_a + \Gamma_b}\right)^{\frac{1}{3}}\left(1 + \frac{\Gamma_b - \Gamma_a}{2\Gamma_l}\sqrt{\frac{4\pi}{N'}}\right)^{\frac{1}{3}}. \tag{5}$$

Using Eq. (5), free energy (4) can be recast as

$$f \approx \frac{4\pi}{N'}\left(\Gamma_a + \Gamma_b\right)R^2\left[1 + 2\left(\frac{\tilde{R}}{R}\right)^3\right]. \tag{6}$$

Using Eq. (6) and introducing the deformation ratio $\lambda = R/\tilde{R}$, we can further get the free energy density $f/V_{e0} = \frac{4\pi\left(\Gamma_a+\Gamma_b\right)\tilde{R}^2}{N'V_{e0}}\left(\lambda^2 + 2\lambda^{-1}\right)$, which indicates that $4\pi\left(\Gamma_a + \Gamma_b\right)^2\tilde{R}^2/\left(N'V_{e0}\right)$ acts

as the stiffness of the spherical epithelium. For a large spherical monolayer (N' is a large number), we can neglect the term of apico-basal difference in Eq. (5), and approximate the stiffness as $\left(\Gamma_a+\Gamma_b\right)^{1/3}\Gamma_l^{2/3}V_{\mathrm{e}0}^{-1/3}$, emphasizing the crucial role for the sum of apical and basal tensions in setting in-plane resistance to deformations (which will become crucial to compare the respective responses of villus and crypt regions to lumen inflation, see Fig. 5 of the main text).

1.2. Free energy of a two-region organoid epithelium

The free energy of the whole organoid is the sum of free energies in two regions. For simplicity, every cell in each region is assumed to be the same. Then, the free energy of a two-region epithelium is $F=N_{\mathrm{c}}f_{\mathrm{c}}+N_{\mathrm{v}}f_{\mathrm{v}}$, where N_i and f_i are respectively cell number and cellular free energy in region i, with the index $i=\mathrm{c},\mathrm{v}$ denoting respectively crypt and villus. Using Eq. (6), the free energy of a single cell in region i is $f_i\approx\left(4\pi/N_i'\right)\left(\Gamma_a+\Gamma_b\right)_i R_i^2\left[1+2\left(\tilde{R}_i/R_i\right)^3\right]$, and corresponding free energy of the whole epithelium yields

$$F\approx 4\pi\left(\Gamma_a+\Gamma_b\right)_{\mathrm{c}}\frac{N_{\mathrm{c}}}{N_{\mathrm{c}}'}R_{\mathrm{c}}^2\left[1+2\left(\frac{\tilde{R}_{\mathrm{c}}}{R_{\mathrm{c}}}\right)^3\right]+4\pi\left(\Gamma_a+\Gamma_b\right)_{\mathrm{v}}\frac{N_{\mathrm{v}}}{N_{\mathrm{v}}'}R_{\mathrm{v}}^2\left[1+2\left(\frac{\tilde{R}_{\mathrm{v}}}{R_{\mathrm{v}}}\right)^3\right]. \quad (7)$$

A number of parameters in Eq. (7) can be eliminated as many geometric variables (such as N_i, N_i', and R_i) are related. Firstly, we have organoid volume $V=V_{\mathrm{c}}+V_{\mathrm{v}}$, where $V_i=\pi R_i^3\left(2+3\cos\theta_i-\cos^3\theta_i\right)/3$ is the volume of region i. For simplicity, we introduce an equivalent organoid radius R_{t} satisfying $V=4\pi R_{\mathrm{t}}^3/3$, and considering the geometric relation $R_{\mathrm{c}}\sin\theta_{\mathrm{c}}=R_{\mathrm{v}}\sin\theta_{\mathrm{v}}$, then the region radius R_i is related to radius R_{t} and polar angles θ_i (see Extended Data Fig. 2B for schematic) by

$$R_i=R_{\mathrm{t}}g_i^{-1/3}, \quad (8)$$

with

$$\begin{aligned} g_{\mathrm{c}}&=\frac{1}{2}\left[\left(1+\frac{3}{2}\cos\theta_{\mathrm{c}}-\frac{1}{2}\cos^3\theta_{\mathrm{c}}\right)+\left(\frac{\sin\theta_{\mathrm{c}}}{\sin\theta_{\mathrm{v}}}\right)^3\left(1+\frac{3}{2}\cos\theta_{\mathrm{v}}-\frac{1}{2}\cos^3\theta_{\mathrm{v}}\right)\right] \\ g_{\mathrm{v}}&=\left(\frac{\sin\theta_{\mathrm{c}}}{\sin\theta_{\mathrm{v}}}\right)^{-3}g_{\mathrm{c}} \end{aligned}. \quad (9)$$

Secondly, considering cells in one region have the same geometric shape, the ratio of cell number in the region (which is a spherical cap) to that in the whole spherical shell is proportional to the ratio of surface areas, that is $N_i / N_i' = A_i / A_i'$, where the surface area of region i is $A_i = \pi R_i^2 \left(2 + 2\cos\theta_i\right)$, and the surface are of corresponding spherical shell is $A_i' = 4\pi R_i^2$. Then we can get

$$\frac{N_i}{N_i'} = \frac{1}{4} s_i, \tag{10}$$

where $s_i\left(\theta_i\right) = 2 + 2\cos\theta_i$.

An intestinal organoid evolves from an initial spherical shape toward a two-region configuration. Crypt apical constriction is found to initiate intestinal morphogenesis *in vivo*, and apical surface areas of crypt cells also reduce during the development of intestinal organoids (Fig. 1B). In view of these, we consider that tensions in crypt cells may be distinct from those of villus cells, and evaluate the role of crypt mechanics in organoid morphogenesis. Given that intestinal organoid initially contains identical cell types, prior to the symmetry breaking of fate (*5*), we take all cells to initially have the same surface tensions. For simplicity, we assume that there is no apical-basal tension difference for an initial spherical organoid, and further assume that lateral tensions are unchanged everywhere during development, i.e. $\Gamma_{lc} = \Gamma_{lv} = \Gamma_l$. This assumption was experimentally verified by examining Myosin levels on the lateral surfaces of villus and crypt cells at different time points (Fig. 3B), which makes our choice of non-dimensionalizing tension by lateral tensions natural. We also note that even if lateral tensions did change, e.g. crypt budding driven by increased lateral tension in crypts, this would still be encapsulated in the three classes of mechanisms discussed in the main text (in the case of increased lateral tension in crypts, all things equals otherwise, this is similar to decreasing the in plane contraction in crypts). Then, we can non-dimensionalize Eq. (7) by introducing four dimensionless parameters:

- relative region size of the crypt $\varphi = N_\mathrm{c} / N_\mathrm{t}\left(N_\mathrm{t} = N_\mathrm{c} + N_\mathrm{v}\right)$, which can evolve at different stages (spherical, bulged, budded) given the preferential proliferation of crypt cells.
- in-plane contraction ratio $\alpha = \left(\Gamma_a + \Gamma_b\right)_\mathrm{c} / \left(\Gamma_a + \Gamma_b\right)_0$, which quantifies the relative changes in crypt stiffness due to changes of apical/basal tensions.
- normalized organoid radius $\beta = R_\mathrm{t} / \tilde{R}_0$, where $\tilde{R}_0$ is the radius of the initial spherical

organoid in free state,

- normalized apico-basal tension difference $\gamma_c = \frac{1}{2}\left(\frac{\Gamma_b - \Gamma_a}{\Gamma_l}\right)_c \sqrt{\frac{4\pi}{N_t}}$, which causes the crypt to have a spontaneous curvature.

Submitting Eqs. (8) and (10) into Eq. (7), the dimensionless free energy $\hat{F} = F \Big/ \left[\pi\left(\Gamma_a + \Gamma_b\right)_0 \tilde{R}_0^2\right]$ becomes

$$\hat{F} \approx \alpha\beta^2 \cdot s_c g_c^{-2/3}\left[1 + \frac{2}{\beta^3}\left(\frac{\tilde{R}_c}{\tilde{R}_0}\right)^3 g_c\right] + \beta^2 \cdot s_v g_v^{-2/3}\left[1 + \frac{2}{\beta^3}\left(\frac{\tilde{R}_v}{\tilde{R}_0}\right)^3 g_v\right], \qquad (11)$$

where $\left(\tilde{R}_c / \tilde{R}_0\right)^3 = 8\alpha^{-1}\varphi^{3/2} s_c^{-3/2}\left(1 + \varphi^{-1/2} s_c^{1/2}\gamma_c/2\right)$, $\left(\tilde{R}_v / \tilde{R}_0\right)^3 = 8\left(1-\varphi\right)^{3/2} s_v^{-3/2}$.

To simplify the expression, we redefine geometric parameters $G_c\left(\theta_c, \theta_v\right) = s_c^{-3/2} g_c$, $G_v\left(\theta_c, \theta_v\right) = s_v^{-3/2} g_v$ (which quantify the degree of opening of villus and crypt regions), and introduce the normalized volume $v = \beta^3$. The free energy then reads

$$\hat{F} \approx v^{2/3}\left(\alpha G_c^{-2/3} + G_v^{-2/3}\right) + 16 v^{-1/3}\left[\varphi^{3/2} G_c^{1/3} + \left(1-\varphi\right)^{3/2} G_v^{1/3} + \frac{1}{2}\varphi g_c^{1/3}\gamma_c\right]. \qquad (12)$$

Eq. (12) shows that $\hat{F}$ is a function of only two parameters, i.e. the polar angles θ_c and θ_v, with the minima of $\hat{F}$ (and corresponding θ_c and θ_v) determining the shape of organoids at mechanical equilibrium. In principle, in-plane contraction (α), spontaneous curvature (γ_c), lumen volume (v), and crypt size (φ) can all affect organoid morphogenesis, and we first sequentially explored the influence of each of these parameters separately, to gain intuitive insights into their influence on morphology, which can then be verified in experimental data. Finally, to avoid non-physical minima of this energy, we employed a penalty function to guarantee the inner radii of crypt and villus are always positive, i.e. $R_{ai} = R_i - h_i/2 > 0$. In the calculation, we use $\exp\left\{\eta\left[\left(v/g_i\right)^{1/3} - \left(2\varphi/\tilde{\kappa}_0\right)\left(v/G_i\right)^{-2/3}\right]\right\}$ as a penalty function, where η is chosen as -10^5, $\tilde{\kappa}_0 = 4\pi\tilde{R}_0^3 / \left(N_t V_{e0}\right)$ is a shape factor that characterizes the initial volume ratio between the whole organoid and the epithelial monolayer.

1.2.1. Organoid morphologies

We first study the organoid morphologies with varied volume v and spontaneous curvature γ_c (of crypt region), with $\alpha = 1$ (equal in-plane contraction in villus and crypt

regions). Setting $\alpha = 1$ and crypt size $\varphi = 0.2$, the phase diagram in Extended Data Fig. 2D not only highlights the influence of spontaneous curvature, but also intuitively reveals that the inflation of organoids tends to reopen both the crypt and villus and recover the original spherical shape. In other words, transformation from a budded shape to a bulged one may happen during organoid inflation. This is consistent with classical theoretical result on lipid vesicles with regions of spontaneous curvature, which shows that an increase in vesicle volume will reverse the budding induced by spontaneous curvature (*6*). Examining organoid morphology with $\gamma_c = -0.25$ in the first graph of Extended Data Fig. 2D as an example, its crypt is fully closed under moderate volume expansion, but will open up when the lumen volume increases above a critical threshold. We employed the "degree of crypt opening", defined as $\theta_c / (\pi - \theta_v)$, to quantify the morphogenesis of intestinal organoid. This parameter ranges from 0 to 1, where 0 corresponds to the budded shape with crypt and villus fully closed and 1 to a fully spherical organoid shape.

As shown in Extended Data Fig. 2D, the in-plane contraction in crypt also affects the organoid morphology. Interestingly, examining organoid morphology without spontaneous curvature (i.e. $\gamma_c = 0$), we can find weak in-plane crypt contraction ($\alpha < 1$) can lead to a partially closed crypt. Even without out-of-plane bending, a decrease in in-plane contraction will tend to expand the crypt (by increasing the rest length of crypt cells, or decrease their preferred height). However, the total volume enclosed by the organoid (lumen) is set, so that this mismatch between preferred cell area and lumen volume can engender compressive stresses inside the monolayer and result in a buckling instability (as discussed in Fig. 2B, Extended Data Fig. 3A and main text). Thus, although this cannot occur for swollen organoids, organoids with small lumen volume could conceivably undergo crypt cell-driven buckling from low in-plane contraction in crypts. Importantly however, this then predicts features upon lumen expansion which are very different from the data (Fig. 5). Generally, in the presence of spontaneous curvature, for an organoid with weak in-plane crypt contraction ($\alpha < 1$), the original spherical shape is recovered by lumen volume expansion, while the recovery is harder when the crypt has strong in-plane contraction ($\alpha > 1$). Strikingly, we find that a crypt with a large enough spontaneous curvature may not open up even for arbitrarily large increases in lumen volume. This indicates critical mechanical forces in crypt may exist, beyond which the shape transformation back to spherical shapes never happens.

1.2.2. Morphometric parameters

Upon organoid swelling, the crypt and villus sustain distinct in-plane and out-of-plane deformations, which respectively modulate the thickness and radius of each region. In other words, these geometric quantities can be employed as morphometric parameters to evaluate the mechanical deformations (and corresponding cell tensions) in two regions. For example, profiles of epithelial thickness and radius have been proposed as metrics to infer the nature of forces driving epithelial folds in epithelium-stroma structures (*7*). We thus examine thickness ratio h_c / h_v and radius ratio R_c / R_v to further quantify the morphological evolution during volume expansion. We find in particular that their dependence on two mechanical parameters, i.e., in-plane contraction α and spontaneous curvature γ_c, is qualitatively different (Extended Data Fig. 2E-H). The thickness (or radius) ratio shows two distinct trends during organoid inflation. For an organoid with $\alpha = 1$, $\gamma_c = -0.25$, the thickness ratio increases almost linearly with volume expansion at the early stage, but drops abruptly at $v \approx 2$, while its radius ratio also undergoes both linear and nonlinear variations, but in an opposite way (Extended Data Fig. 2F). These abrupt transitions of thickness and radius ratios are due to shape transformation of organoids (Extended Data Fig. 2D), and clearly indicate that, for organoids with different morphologies, the thickness (or radius) ratio is modulated by lumen volume in distinct ways. Furthermore, we find that crypts with strong in-plane contraction (i.e., $\alpha > 1$) are always thicker than villi (Extended Data Fig. 2E and G), while crypts with $\alpha < 1$ is usually thinner than villi (Extended Data Fig. 2E, H and 3A). This is intuitive as hydrostatic pressure is uniform within the organoid lumen, so that stiffer regions deform less than softer ones (resulting in less thinning). We also find that the inflation of organoids tends to widen the thickness difference between two regions (Extended Data Fig. 2E, G and H), as the softer region tends to accommodate the bulk of the pressure-induced deformation.

Furthermore, as already shown in Fig. 2B, spontaneous curvature γ_c always tends to increase the crypt thickness. This is consistent with results in *Drosophila* gastrulation, where ventral cells are lengthened during furrow formation (*8*). However, Extended Data Fig. 2F-H further indicate that, for a swelling organoid, the influence of γ_c on the thickness ratio h_c / h_v is negligible when the spontaneous curvature is not large enough to close the crypt (as in budded shape). In other words, the thickness ratio of a swelling organoid with a partially opened crypt (e.g., a bulged organoid) is almost independent on γ_c, although increasing crypt apical tension can influence thickness ratio by increasing α.

1.3. Line tension in neck zone

So far, we have only considered changes in the bulk properties of each organoid region, such as in-plane contractions and spontaneous curvatures. However, mechanical forces at the boundary between these two regions may also drive the morphological evolution in biological systems (*9-11*). Here, we assume cells in the neck zone (connection part of crypt and villus) carry distinct surface tensions (and hence the free energy) with cells in two regions. Since the neck zone of organoid is rather narrow, and more like a hollow cylinder rather than a spherical shell, it is reasonable to model the neck zone as a short cylindrical monolayer, and neglect its volume contribution to organoid.

Considering neck cells with longitudinal side length e, height h, and radial side lengths in the apical and basal surfaces d_a and d_b (see Extended Data Fig. 2C for schematic), then the free energy (1) becomes $f = \Gamma_a e d_a + \Gamma_b e d_b + \Gamma_l e h + \frac{1}{2}\Gamma_l h\left(d_a + d_b\right)$. The geometric relationship of a single cell and a cylindrical epithelium can be described by $d_a = 2\pi R_a / N_\mathrm{r}$, $d_b = 2\pi R_b / N_\mathrm{r}$, where N_r is the cell number in the radial direction. Letting R be the neutral radius of the cylindrical epithelium, we obtain $h = N_\mathrm{r} V_{\mathrm{e}0} / \left(2\pi e R\right)$, which recasts the free energy as

$$f = \frac{2\pi}{N_\mathrm{r}}\left(\Gamma_a + \Gamma_b\right) eR + \left[\frac{1}{2}\left(\Gamma_b - \Gamma_a\right) + \Gamma_l \frac{N_\mathrm{r}}{2\pi}\right]\frac{V_{\mathrm{e}0}}{R} + \Gamma_l \frac{V_{\mathrm{e}0}}{e}. \tag{13}$$

Eq. (13) indicates that the free energy depends on two geometric variables R and e, i.e. $f = f\left(R, e\right)$. Considering the free state of cells, which satisfies $\partial f / \partial R = 0$, $\partial f / \partial e = 0$, we can get radius $\tilde{R}$ and length $\tilde{e}$ in the free state

$$\tilde{R} = \frac{N_\mathrm{r}}{2\pi}\left(\frac{V_{\mathrm{e}0}\Gamma_l}{\Gamma_a + \Gamma_b}\right)^{\frac{1}{3}}\left(1 + \frac{\Gamma_b - \Gamma_a}{2\Gamma_l}\frac{2\pi}{N_\mathrm{r}}\right)^{\frac{2}{3}},\quad \tilde{e} = \left(\frac{V_{\mathrm{e}0}\Gamma_l}{\Gamma_a + \Gamma_b}\right)^{\frac{1}{3}}\left(1 + \frac{\Gamma_b - \Gamma_a}{2\Gamma_l}\frac{2\pi}{N_\mathrm{r}}\right)^{-\frac{1}{3}}. \tag{14}$$

Using Eq. (14), the free energy of a cell in the neck can finally be expressed as

$$f = \frac{2\pi}{N_\mathrm{r}}\left(\Gamma_a + \Gamma_b\right)\left[eR + \tilde{e}\tilde{R}\left(\frac{\tilde{R}}{R} + \frac{\tilde{e}}{e}\right)\right]. \tag{15}$$

For an organoid with two regions (crypt and villus) and a neck zone, the total free energy are contributed by three parts, i.e. $F = N_\mathrm{c} f_\mathrm{c} + N_\mathrm{v} f_\mathrm{v} + N_\mathrm{n} f_\mathrm{n}$, where N_n and f_n are respectively the cell number and cellular free energy in the neck zone (f_n follows the expression in Eq. (15).

Since the neck zone is mainly constrained by other regions in its radial direction, we assume a stress-free state in the longitudinal direction, i.e., $\partial f / \partial e = 0$, which leads to $e = \tilde{e}\sqrt{\tilde{R}_{\mathrm{n}} / R_{\mathrm{n}}}$. Then the free energy of neck zone yields

$$F_{\mathrm{n}} = N_{\mathrm{n}} f_{\mathrm{n}} = 2\pi N_{\mathrm{e}} \left(\Gamma_a + \Gamma_b\right)_{\mathrm{n}} \tilde{e}\sqrt{\tilde{R}_{\mathrm{n}} R_{\mathrm{n}}} \left[2 + \left(\frac{\tilde{R}_{\mathrm{n}}}{R_{\mathrm{n}}}\right)^{3/2}\right], \tag{16}$$

where N_{e} is the cell number in the longitudinal direction (therefore we have $N_{\mathrm{n}} = N_{\mathrm{r}} N_{\mathrm{e}}$).

The in-plane contraction ratio $\Lambda = \left(\Gamma_a + \Gamma_b\right)_{\mathrm{n}} / \left(\Gamma_a + \Gamma_b\right)_0$ is introduced to characterize the 'line tension' between two regions. The geometric relationship $R_{\mathrm{n}} = R_{\mathrm{c}} \sin\theta_{\mathrm{c}}$ implies $R_{\mathrm{n}} = R_{\mathrm{t}} g_{\mathrm{n}}^{-1/3}$, with $g_{\mathrm{n}} = g_{\mathrm{c}} / \sin^3\theta_{\mathrm{c}}$. Then, we have $\hat{F}_{\mathrm{n}} = F_{\mathrm{n}} \Big/ \left[\pi \left(\Gamma_a + \Gamma_b\right)_0 \tilde{R}_0^2\right] = 2N_{\mathrm{e}} \Lambda \tilde{e} \tilde{R}_{\mathrm{n}}^{1/2} R_0^{-3/2} \left[2 g_{\mathrm{n}}^{-1/6} \beta^{1/2} + g_{\mathrm{n}}^{1/3} \beta^{-1} \left(\tilde{R}_{\mathrm{n}} / \tilde{R}_0\right)^{3/2}\right]$. To further simplify $\hat{F}_{\mathrm{n}}$, we need to determine N_{r}, which affects both $\tilde{e}$ and $\tilde{R}_{\mathrm{n}}$. The radial cell number of the neck depends on the total cell number of organoid N_{t} and the position of neck (dominated by crypt size φ), that is $N_{\mathrm{r}} = N_{\mathrm{r}}\left(\varphi, N_{\mathrm{t}}\right)$. Specific expression of N_{r} can be estimated as follows: A narrow neck in a spherical organoid in free state satisfies $N_{\mathrm{r}} \tilde{d} = 2\pi \tilde{R}_0 \sin\theta_{\mathrm{n}}$, where θ_{n} is the polar angle of neck, $\tilde{d} = \sqrt{4\pi / N_{\mathrm{t}}}\, \tilde{R}_0$ is the side length of a single cell. Further considering the geometric relation $\varphi = 2\pi \tilde{R}_0^2 \left(1 - \cos\theta_{\mathrm{n}}\right) / \left(4\pi \tilde{R}_0^2\right) = \left(1 - \cos\theta_{\mathrm{n}}\right) / 2$, we can get $N_{\mathrm{r}} = \sqrt{4\pi N_{\mathrm{t}}} \cdot \Delta$, where $\Delta = \sqrt{\varphi - \varphi^2}$. To focus on the in-plane contraction in the neck, the difference of apical and basal tensions (i.e., spontaneous curvature) is neglected, which finally leads to a simplified free energy of the neck

$$\hat{F}_{\mathrm{n}} = 8 N_{\mathrm{e}} \sqrt{\frac{2\pi}{N_{\mathrm{t}}}} \left(\Lambda^{1/2} \Delta^{1/2} \beta^{1/2} g_{\mathrm{n}}^{-1/6} + \sqrt{2} \Delta^2 \beta^{-1} g_{\mathrm{n}}^{1/3}\right). \tag{17}$$

By adding free energy (17) into Eq. (12), we can evaluate the influence of the overall line tension, arising from the in-plane contraction of cells in the neck, on organoid morphogenesis. Fig. 2B and Extended Data Fig. 3A'' shows that, although a contractile neck can promote the bulging and budding of organoids (i.e., decreased radius ratio $R_{\mathrm{c}} / R_{\mathrm{v}}$), it has negligible effects on the thickness ratio $h_{\mathrm{c}} / h_{\mathrm{v}}$. This is in contrast with our experimental findings (Fig. 2C), where bulging of organoids is robustly accompanied by thickness increases on the crypts

compared to villi. This implies that the line tension in neck is not the major driving force for crypt bulging. However, it would be interesting in the future to study its potential effect on longer-term crypt shape maintenance, which would require an extension of the model to consider more complex non-spherical crypt shapes.

1.4. Cell volumes and villus mechanics

The model in Subsection 1.2 considers the influence of crypt mechanics and lumen volume on morphogenesis. However, mechanical contributions from the villus could also impact intestinal organoid development. For example, in the late stage of organoid morphogenesis, the villus shows both cell swelling (Fig. 7B and Extended Data Fig. 8B) and increased intensity of basal myosin (Fig. 3A), which might result in elevated basal tensions (Fig. 2E). To explore this, we extended the previous model, which assumes a constant cell volume in both regions and constant cell tensions in villus during morphogenesis, to incorporate potential variations in cell volumes and villus tensions. We thus introduce normalized cell volumes $v_{\mathrm{ec}} = V_{\mathrm{ec}} / V_{\mathrm{e0}}$, $v_{\mathrm{ev}} = V_{\mathrm{ev}} / V_{\mathrm{e0}}$, where V_{ec} and V_{ev} are respectively the volumes of a crypt cell and a villus cell. In analogy to the definitions in crypt mechanics, in-plane contraction ratio $\alpha_{\mathrm{v}} = \left(\Gamma_a + \Gamma_b\right)_{\mathrm{v}} / \left(\Gamma_a + \Gamma_b\right)_0$, and spontaneous curvature $\gamma_{\mathrm{v}} = \frac{1}{2}\left(\frac{\Gamma_b - \Gamma_a}{\Gamma_l}\right)_{\mathrm{v}} \sqrt{\frac{4\pi}{N_{\mathrm{t}}}}$ are introduced to examine the effects of villus tensions. With these extensions of the model, this rescaled organoid energy $\hat{F}$ now reads:

$$\begin{aligned}\hat{F} = {} & v^{2/3}\left(\alpha_{\mathrm{c}} G_{\mathrm{c}}^{-2/3} + \alpha_{\mathrm{v}} G_{\mathrm{v}}^{-2/3}\right) + 16 v^{-1/3}\left[\varphi^{3/2} v_{\mathrm{ec}} G_{\mathrm{c}}^{1/3} + \left(1-\varphi\right)^{3/2} v_{\mathrm{ev}} G_{\mathrm{v}}^{1/3}\right] \\ & + 8 v^{-1/3}\left[\varphi v_{\mathrm{ec}} g_{\mathrm{c}}^{1/3} \gamma_{\mathrm{c}} + \left(1-\varphi\right) v_{\mathrm{ev}} g_{\mathrm{v}}^{1/3} \gamma_{\mathrm{v}}\right]\end{aligned}. \qquad (18)$$

1.4.1. Influence of cell swelling on morphogenesis

We first evaluate the dependence of organoid morphologies on cell swelling in either crypt or villus. As shown in Extended Data Fig. 2I, both the swelling of crypt cells and villus cells can promote budding. Furthermore, crypt size φ impacts the efficiency of cell swelling on budding. Given the fact that the villus is usually much larger than the crypt, swelling of villus cells is more efficient to promote budding. Furthermore, even when both regions have an equal size, the cell swelling in villus is still more efficient. For a crypt undergoing both cell swelling and tension-modulated deformations, the in-plane contraction will limit the extension of crypt region, while the crypt bending will be hindered by cell swelling. Overall, cell swelling is less efficient on budding when it happens in the tension-enhanced region (i.e., the crypt) than in the

normal region (i.e., the villus). Moreover, Extended Data Fig. 2J shows that the effect of cell swelling on budding can be reversed by lumen expansion (for low crypt apical tension). This is different from the influence of strong mechanical differences in crypts such as high apical actomyosin tension, which leads to maintained closure of the crypt even under infinite lumen expansion.

1.4.2. Influence of villus mechanics on morphogenesis

We then examine the influence of spontaneous curvature of villus γ_v on organoid morphology. Unlike spontaneous curvature γ_c, which is negative due to the enhanced apical tension in crypt, spontaneous curvature γ_v is chosen to be positive in Extended Data Fig. 2K-L, in light of the elevated basal tension and myosin accumulation observed in villus (Fig. 2E and Fig. 3A) as well as basal constriction observed in wild-type cells next to cells with reduced Myosin levels (Extended Data Fig. 4G-G''). Interestingly, the spontaneous curvature γ_v will promote the opening of two regions only when γ_c is quite small ($|\gamma_c| < 0.05$ or estimated value in initial bulging phase), while the out-of-plane bending of villus will facilitate the closure of two regions when the crypt engenders notable spontaneous curvature and strong in-plane contraction (Extended Data Fig. 2K). Importantly, the dependence of thickness (or radius) ratio on γ_v is negligible for an organoid with either equal or stronger in-plane contraction in crypt than in villus (Extended Data Fig. 2L), which we show from Fig. 5 is the relevant case for us. This argues that although basal enrichment of Myosin in the villus region is expected to help and contribute to bulging and budding, it cannot be the dominant/sole driving force (otherwise in-plane contraction of villi would be larger than crypts and lumen inflation would cause crypt dilation), so that we neglect γ_v in first approximation for the fits discussed in Section 4.

1.5. Preferential proliferation of crypt cells

Besides the three mechanical mechanisms hypothesized in Fig. 2B, and additional discussions of cell volumes and villus mechanics in Section 1.4, another possible mechanism of crypt budding is the over-proliferation of crypt cells, which in principle, can also extend the crypt epithelium (like the effect of decreasing in-plane contraction or cell swelling) and thus promote organoid morphogenesis. Indeed, differential cell proliferation is observed in experiments, with cell division occurring predominantly occurs in the crypt region. Although our model is quasi-static (i.e. it predicts an equilibrium shape at time *t* only based on the value of mechanical parameters at that timepoint, independent of their dynamics, which is reasonable

here as morphogenetic timescales of hours/days are very long compared to the timescales of shape relaxation for cells under actomyosin tension – typically minutes), we incorporate this preferential cell division via its effect on the crypt size. For instance, if the cell number in the crypt increases from N_c to N_{cg}, while the cell number in the villus is still N_v, to keep everything consistent with the previous definition, we still keep the relative region size as non-dimensionalized by the original total cell number $N_t = N_c + N_v$, so that the current crypt size is $\varphi_g = N_{cg}/N_t$ $(>\varphi)$, while the villus size is still $1-\varphi$. The free energy (18) of the system then becomes

$$\begin{aligned}\hat{F} = {} & v^{2/3}\left(\alpha_c G_c^{-2/3} + \alpha_v G_v^{-2/3}\right) + 16v^{-1/3}\left[\varphi_g^{3/2} v_{ec} G_c^{1/3} + (1-\varphi)^{3/2} v_{ev} G_v^{1/3}\right] \\ & + 8v^{-1/3}\left[\varphi_g v_{ec} g_c^{1/3}\gamma_c + (1-\varphi) v_{ev} g_v^{1/3}\gamma_v\right]\end{aligned}. \qquad (19)$$

Importantly, in Eq. (19), the crypt size φ_g always multiplies the cell volume in crypt v_{ec}, which indicates that the crypt size (or cell number) may modulate the free energy (and thus the morphology of an organoid) in a similar way as the cell volume. Hence, according to the analysis in Subsection 1.4.1, one can also expect that crypt growth also promotes budding, although its effect will be eliminated by volume expansion. Further theoretical discussion on the influence of crypt growth, combined with specific crypt mechanics, is given in Subsection 3.3.1. In experiments, blocking mitotic cell division shows negligible effects on organoid morphologies (Extended Data Fig. 4F), implying preferential proliferation of crypt cells is not a major promotor of the morphogenesis of intestinal organoids. Thus, to summarize, we take into account differential proliferation of cells in crypt indirectly (as it sets the value of crypt size φ, which we independently measure prior to fitting the data to the model), but can assume that it does not in itself maintain budded shapes (for instance by creating residual stresses in crypts).

1.6. Summary of two-region vertex model

In the subsections above, we have thus proposed a three-dimensional two-region vertex model to describe the morphogenesis of intestinal organoids. The model shows that, altered cell tension, with emphasis on crypt apical constriction, can modulate in-plane contraction and induce out-of-plane bending of the epithelium. As a closed epithelium filled with lumen fluid, the overall volume of an organoid can also modulate its morphology. Other potential mechanisms, including active contraction at the neck zone, cell swelling, altered contractility of villus cells, preferential proliferation of crypt cells, are also evaluated by extending the

model. By combining experimental observations with theoretical results, we find cell swelling also plays an important role in the morphogenesis of intestinal organoids.

2. Analytic approximations

Experimentally, crypt regions are much smaller than villus regions, in particular during the first phases of bulging/budding which we explore here. Based on this, we can simplify the model by considering $V_{\mathrm{c}} \ll V_{\mathrm{v}}$ and $\theta_{\mathrm{v}} \to 0$. The volumetric relation $V = V_{\mathrm{c}} + V_{\mathrm{v}}$ can be expressed as $R_{\mathrm{t}}^3 \approx p_{\mathrm{c}} R_{\mathrm{c}}^3 + R_{\mathrm{v}}^3$, where $p_{\mathrm{c}} = \left(2 + 3\cos\theta_{\mathrm{c}} - \cos^3\theta_{\mathrm{c}}\right)/4$. Considering $V_{\mathrm{c}} \ll V_{\mathrm{v}}$ (or $p_{\mathrm{c}} R_{\mathrm{c}}^3 \ll R_{\mathrm{v}}^3$) leads to $R_{\mathrm{v}} \approx R_{\mathrm{t}}\left[1 - \left(p_{\mathrm{c}}/3\right)\left(R_{\mathrm{c}}/R_{\mathrm{t}}\right)^3\right]$. Combined with Eq. (10), free energy (7) can be rewritten as

$$F \approx \pi\left(\Gamma_a + \Gamma_b\right)_{\mathrm{c}} s_{\mathrm{c}} R_{\mathrm{c}}^2 \left[1 + 2\left(\frac{\tilde{R}_{\mathrm{c}}}{R_{\mathrm{c}}}\right)^3\right] + \pi\left(\Gamma_a + \Gamma_b\right)_{\mathrm{v}} s_{\mathrm{v}} R_{\mathrm{t}}^2 \left\{1 + 2\left(\frac{\tilde{R}_{\mathrm{v}}}{R_{\mathrm{t}}}\right)^3 + \frac{2}{3}\left[\left(\frac{\tilde{R}_{\mathrm{v}}}{R_{\mathrm{t}}}\right)^3 - 1\right] p_{\mathrm{c}} \left(\frac{R_{\mathrm{c}}}{R_{\mathrm{t}}}\right)^3\right\} . \quad (20)$$

Letting $\beta_{\mathrm{c}} = R_{\mathrm{c}} / \tilde{R}_0$ be the normalized crypt radius, one obtains

$$\begin{aligned} \hat{F} \approx\ & \alpha s_{\mathrm{c}} \beta_{\mathrm{c}}^2 + \left(16\varphi^{3/2} s_{\mathrm{c}}^{-1/2} + 8\varphi\gamma_{\mathrm{c}}\right)\beta_{\mathrm{c}}^{-1} + s_{\mathrm{v}}\beta^2 + 16\left(1-\varphi\right)^{3/2} s_{\mathrm{v}}^{-1/2}\beta^{-1} \\ & + \frac{2}{3}\left[8\left(1-\varphi\right)^{3/2} s_{\mathrm{v}}^{-1/2}\beta^{-3} - s_{\mathrm{v}}\right] p_{\mathrm{c}} \beta_{\mathrm{c}}^3 \beta^{-1} \end{aligned} , \quad (21)$$

For a small θ_{v}, using $R_{\mathrm{c}} \sin\theta_{\mathrm{c}} = R_{\mathrm{v}} \sin\theta_{\mathrm{v}}$, we have $\theta_{\mathrm{v}} \approx \left(R_{\mathrm{c}}/R_{\mathrm{v}}\right)\sin\theta_{\mathrm{c}}$, which leads to $s_{\mathrm{v}} \approx 4 - \left(\beta_{\mathrm{c}}/\beta\right)^2 \sin\theta_{\mathrm{c}}^2$. With these approximations, the free energy $\hat{F}$ only depends on θ_{c} and β_{c}:

$$\begin{aligned} \hat{F} \approx\ & 4\beta^2 + 8\left(1-\varphi\right)^{3/2}\beta^{-1} + \alpha s_{\mathrm{c}} \beta_{\mathrm{c}}^2 + \left(16\varphi^{3/2} s_{\mathrm{c}}^{-1/2} + 8\varphi\gamma_{\mathrm{c}}\right)\beta_{\mathrm{c}}^{-1} \\ & + \left[\left(1-\varphi\right)^{3/2}\beta^{-3} - 1\right]\left(\beta_{\mathrm{c}}^2 \sin^2\theta_{\mathrm{c}} + \frac{8}{3} p_{\mathrm{c}} \beta_{\mathrm{c}}^3 \beta^{-1}\right) \end{aligned} . \quad (22)$$

In the following, based on this simplified free energy (22), we will analyze specific organoid morphologies and get corresponding analytical expressions of morphometric parameters. As a limiting case, crypt morphologies under infinite organoid expansion will be discussed. Besides, the influence of cell volumes will be explicitly explored with analytic formulation.

2.1. Scaling laws for thickness and radius modulations

As aforementioned, after the initial symmetric breaking event, an intestinal organoid will evolve towards non-spherical configurations. The organoid first undergoes a bulging phase with the crypt gradually bulges out, then enters into a budded phase. Here, we focus on these two typical morphologies, the bulged shape and the budded one, during the development of intestinal organoids, and make use of their shape features to further simplify the free energy shown in Eq. (22) and get analytical expressions of the radius ratio R_c / R_v and the thickness ratio h_c / h_v.

2.1.1. Bulged organoid

In the bulging stage, the crypt just begins to form and is rather small (i.e., φ is small). These indicate $\theta_* = \pi - \theta_c \sim \varphi^{1/2}$ can be served as a small parameter (i.e., $\theta_* \to 0$), and functions of θ_c in Eq. (22) can be approximated as $s_c \approx \theta_*^2$, $\sin^2\theta_c \approx \theta_*^2$, and $p_c \approx 0$. Then, the free energy (22) is simplified as

$$\hat{F} \approx 4\beta^2 + 8\beta^{-1} + \left(\alpha - 1 + \beta^{-3}\right)\bar{\theta}_*^2 + 16\varphi^{3/2}\bar{\theta}_*^{-1} + 8\varphi\gamma_c\beta_c^{-1}, \tag{23}$$

where $\bar{\theta}_* = \beta_c\theta_*$, and the radius ratio and thickness ratio are respectively approximated as $R_c / R_v \approx \beta_c/\beta$ and $h_c / h_v \approx 4\varphi\beta^2\bar{\theta}_*^{-2}$. One obtains $\bar{\theta}_* = 2\varphi^{1/2}\left(\alpha - 1 + \beta^{-3}\right)^{-1/3}$ from $\partial\hat{F} / \partial\bar{\theta}_* = 0$. To get an estimate of the normalized crypt radius β_c, we need to expand the functions of θ_c (or θ_*) in Eq. (22) to a higher order $O\left(\theta_*^4\right)$, i.e. $s_c \approx \theta_*^2 - \theta_*^4/12$, $\sin^2\theta_c \approx \theta_*^2 - \theta_*^4/3$, and $p_c \approx 3\theta_*^4/16$, which yield an additional sequence of terms in free energy (23) as $(2/3)\varphi^{3/2}\beta_c^{-2}\bar{\theta}_* + \bar{\theta}_*^4\left\{-\alpha\beta_c^{-2}/12 + \left(\beta^{-3} - 1\right)\left(-\beta_c^{-2}/3 + \beta_c^{-1}\beta^{-1}/2\right)\right\}$. Using the extended free energy and considering $\partial\hat{F} / \partial\beta_c = 0$ yield $\beta_c^{-1} = \beta^{-1} - 16\varphi\gamma_c\bar{\theta}_*^{-4}\left/\left(1 - \beta^{-3}\right)\right.$. Thus, the radius ratio and thickness ratio of a bulged organoid can be finally estimated as

$$\frac{R_v}{R_c} \approx 1 - \varphi^{-1}\gamma_c\frac{\left[(\alpha-1)v + 1\right]^{4/3}}{v-1}, \frac{h_c}{h_v} \approx \left[(\alpha-1)v + 1\right]^{2/3}. \tag{24}$$

Eq. (24) thus predicts that the thickness ratio depends only, at first order, on the in-plane contraction ratio α. We found excellent agreement between numerical solutions of the full model, and the analytical criteria of Eq. (24), and confirmed in particular that the thickness ratio depends crucially on α, while it is almost independent on γ_c (Extended Data Fig. 3E).

Furthermore, the radius ratio of a bulged organoid is expected to depend on $\varphi^{-1}\gamma_c$ and α from Eq. (24). In a bulging crypt, the apical actomyosin accumulation is initially small (Fig. 2C), so that it is expected to engender weak in-plane contraction and out-of-plane bending, and corresponding mechanical parameters $\alpha-1$ and γ_c can both be considered small. However, Eq. (24) indicates that the radius ratio is less dependent on $\alpha-1$ than γ_c, and the only leading parameter of R_c/R_v is $\varphi^{-1}\gamma_c$. As verified in Extended Data Fig. 3E, the crypt size φ and spontaneous curvature γ_c are indeed combined to affect the crypt radius, and the resulting parameter $\varphi^{-1}\gamma_c$ can modulate R_c/R_v. Besides, Eq. (24) can fit well with the numerical results of a bulged organoid with varying volumes (Extended Data Fig. 3E).

2.1.2. Budded organoid

For a budded organoid (which is equivalent to a near-closed organoid in our simplified spherical region models), we can take the converse limit of small θ_c (i.e. $\theta_c \to 0$), which results in $s_c \approx 4$, $\sin^2\theta_c \approx 0$, and $p_c \approx 1$. Then the full expression of free energy (22) reduces to

$$\hat{F} \approx 4\beta^2 + 8\left(1-\varphi\right)^{3/2}\beta^{-1} + 4\alpha\beta_c^2 + \left(8\varphi^{3/2} + 8\varphi\gamma_c\right)\beta_c^{-1} + \frac{8}{3}\left[\left(1-\varphi\right)^{3/2}\beta^{-3} - 1\right]\beta_c^3\beta^{-1}, \quad (25)$$

which only depends on the normalized crypt radius β_c. Minimizing this energy with respect to crypt radius (i.e. $\partial\hat{F}/\partial\beta_c = 0$) leads to $\beta_c\beta^{-1} = \left[\alpha - \left(\varphi^{3/2} + \varphi\gamma_c\right)\beta_c^{-3}\right] \Big/ \left[1-\left(1-\varphi\right)^{3/2}\beta^{-3}\right]$, which can be recast as

$$1-\left(\frac{R_c}{\tilde{R}_c}\right)^{-3} = \alpha^{-1}\frac{R_c}{R_t}\left[1-\left(1-\varphi\right)^{3/2}v^{-1}\right]. \quad (26)$$

Experiments indicate that a budding organoid undergoes sustaining apical actomyosin accumulation in the crypt, which will lead to an enhanced in-plane contraction, i.e. $\alpha > 1$. Besides, considering the crypt volume is usually much smaller than the overall volume of the organoid, i.e. $V_c << V$, where $V_c = 4\pi R_c^3/3$ and $V = 4\pi R_t^3/3$ for a budded organoid, we can find that $R_c/R_t < 1$ always holds. Thus, the value of the right side of Eq. (26) is usually close to 0, which indicates that $R_c/\tilde{R}_c \approx 1$ (i.e. $R_c \approx \tilde{R}_c$). Further considering $R_v \approx R_t$, then the radius/thickness ratio of a budded organoid can be approximated as

$$\frac{R_{\mathrm{c}}}{R_{\mathrm{v}}} \approx w_{\mathrm{c}} v^{-1/3},\ \frac{h_{\mathrm{c}}}{h_{\mathrm{v}}} \approx \varphi\left(1-\varphi\right)^{-1} w_{\mathrm{c}}^{-2} v^{2/3}, \tag{27}$$

where $w_{\mathrm{c}} = \tilde{R}_{\mathrm{c}} / \tilde{R}_0 = \varphi^{1/2}\alpha^{-1/3}\left(1+\varphi^{-1/2}\gamma_{\mathrm{c}}\right)^{1/3}$. Eq. (27) indicates that the thickness (or radius) ratio of a budded organoid depends only on the crypt size φ and $u = \alpha\left(1+\varphi^{-1/2}\gamma_{\mathrm{c}}\right)^{-1}$, a parameter coupling the in-plane contraction and spontaneous curvature of crypt. As verified in Extended Data Fig. 3F, the mechanical modulation of the thickness (or radius) ratio can be depicted by a single parameter u. Besides, Eq. (27) indicates a simple scaling law between organoid morphometrics and lumen volume for budded organoids: $R_{\mathrm{c}}/R_{\mathrm{v}} \sim v^{-1/3}$,$h_{\mathrm{c}}/h_{\mathrm{v}} \sim v^{2/3}$, which again shows excellent agreement with numerical solutions to the full model (Extended Data Fig. 3F).

Strikingly, this predicts a key difference between the inflation of bulged vs budded organoids. In the former, the radius ratio is an increasing function of lumen volume (leading to near-spherical shapes upon inflation), while in the latter, the radius ratio always decreases with lumen volume (as the crypt never opens up, and the bulk of the deformation is born by the villus region). As discussed in the main text, we challenged this prediction via two different types of inflation experiments, and found good qualitative and quantitative agreement (Fig. 5B-C, Extended Data Fig. 7A-B), see also Section 4 for details on the fitting strategy used.

Although the above derivations are based on Eq. (22), which can only describe organoids with small crypts, Eq. (27) actually holds for budded organoids with varied crypt sizes. In the following, we will directly use Eq. (7), a generic formulation of free energy, to derive Eq. (27). For a budded organoid, both θ_{c} and θ_{v} are close to 0, which lead to $N_{\mathrm{c}} / N_{\mathrm{c}}' \approx 1$, $N_{\mathrm{v}} / N_{\mathrm{v}}' \approx 1$. Then, Eq. (7) reduces to

$$F \approx 4\pi\left(\Gamma_a + \Gamma_b\right)_{\mathrm{c}} R_{\mathrm{c}}^2\left[1+2\left(\tilde{R}_{\mathrm{c}} / R_{\mathrm{c}}\right)^3\right] + 4\pi\left(\Gamma_a + \Gamma_b\right)_{\mathrm{v}} R_{\mathrm{v}}^2\left[1+2\left(\tilde{R}_{\mathrm{v}} / R_{\mathrm{v}}\right)^3\right], \tag{28}$$

which is a function of two radii R_{c} and R_{v}. These two radii should also satisfy the volumetric constraint, which is simplified as $R_{\mathrm{t}}^3 = R_{\mathrm{c}}^3 + R_{\mathrm{v}}^3$ in the budded case. Hence, the radii can be determined by constructing an auxiliary function that contains both free energy (28) and the volumetric constraint. For the normalized radii $\bar{R}_{\mathrm{c}} = R_{\mathrm{c}} / \tilde{R}_{\mathrm{c}}$ and $\bar{R}_{\mathrm{v}} = R_{\mathrm{v}} / \tilde{R}_{\mathrm{v}}$, the auxiliary function can be written as $y = F \Big/ \left[\pi\left(\Gamma_a + \Gamma_b\right)_0 \tilde{R}_0^2\right] + L\left[w^3\bar{R}_{\mathrm{c}}^3 + \bar{R}_{\mathrm{v}}^3 - R_{\mathrm{t}}^3 / \tilde{R}_{\mathrm{v}}^3\right]$, where L is a Lagrange multiplier, $w = \tilde{R}_{\mathrm{c}} / \tilde{R}_{\mathrm{v}}$. That is

$$y = \alpha w^2 \bar{R}_{\mathrm{c}}^2\left(1+2\bar{R}_{\mathrm{c}}^{-3}\right) + \bar{R}_{\mathrm{v}}^2\left(1+2\bar{R}_{\mathrm{v}}^{-3}\right) + L\left[w^3\bar{R}_{\mathrm{c}}^3 + \bar{R}_{\mathrm{v}}^3 - \bar{R}_{\mathrm{t}}^3 / \tilde{R}_{\mathrm{v}}^3\right]. \tag{29}$$

Calculating $\partial y/\partial \bar{R}_c = 0$ and $\partial y/\partial \bar{R}_v = 0$ lead to $1-\bar{R}_c^{-3} = w\alpha^{-1}\left(\bar{R}_c/\bar{R}_v\right)\left(1-\bar{R}_v^{-3}\right)$, which will further result in Eq. (27) by using $\bar{R}_c \approx 1$ and $\bar{R}_v \approx v^{1/3}\tilde{R}_0/\tilde{R}_v$.

2.2. Infinite volume expansion

As discussed above and in the main text, a key experimental finding is that budded organoids tend to stay closed upon volume expansion, while bulged organoids do not. To further explore the difference between the two morphologies, we examine the limit of infinite organoid inflation (i.e., $\beta \to \infty$), for which the boundary between these two morphologies in phase-space can be derived analytically.

We compare the free energies of organoids in partially open vs fully closed crypts. Since the partially open and fully closed crypt morphologies respectively belong to bulged and budded organoids discussed above, we can approximate their free energies by following the analysis in Subsection 2.1, and considering $\beta \to \infty$. Then, we have $\hat{F}_{po} \approx 4\beta^2 + 12\varphi(\alpha-1)^{1/3}$ for a partially open case, and $\hat{F}_{fc} \approx 4\beta^2 + 12\varphi\left(1+\varphi^{-1/2}\gamma_c\right)^{2/3}\alpha^{1/3}$ for a fully closed shape. The crypt in a budded organoid will stay closed when $\hat{F}_{fc} < \hat{F}_{po}$, which holds for $\left(1+\varphi^{-1/2}\gamma_c\right)^2 < 1-\alpha^{-1}$, which specifies a critical value of crypt apical tension distinguishing the two configurations.

A phase diagram of crypt morphologies under infinite lumen expansion are shown in Extended Data Fig. 3G. The effects of in-plane contraction α and spontaneous curvature γ_c are examined in a representative parameter-regime: an initially large lumen (or thin monolayer) ($\tilde{\kappa}_0 = 10$) and a large crypt region ($\varphi = 0.2$). From the phase diagram, there also exists the third crypt shape: fully closed with vanishing apical surface (i.e. $R_{ac} = 0$). For a fully closed crypt in a budded organoid to get $R_{ac} = 0$, it needs to satisfy $\varphi^{1/2} + \gamma_c = \left(2\tilde{\kappa}_0\right)^{-1}\alpha$.

2.3. Dependence on cell volumes

In aforementioned derivations, cell volumes were set to be constant and identical in crypt and villus regions, i.e., $v_{ec} = 1$ and $v_{ev} = 1$. However, this is typically not the case, as discussed in the main text: Intestinal organoids display increases in cell volume as the lumen volume decreases during morphogenesis (Fig. 7D). To consider effects of cell volumes on specific development stages listed in Subsection 2.1, we incorporate the possibility for varying and different cell volumes to the simplified free energy (22), which is then modified as:

$$\hat{F} \approx 4\beta^2 + 8(1-\varphi)^{3/2} v_{\mathrm{ev}} \beta^{-1} + \alpha s_{\mathrm{c}} \beta_{\mathrm{c}}^2 + \left(16\varphi^{3/2} s_{\mathrm{c}}^{-1/2} + 8\varphi\gamma_{\mathrm{c}}\right) v_{\mathrm{ec}} \beta_{\mathrm{c}}^{-1} + \left[(1-\varphi)^{3/2} v_{\mathrm{ev}} \beta^{-3} - 1\right]\left(\beta_{\mathrm{c}}^2 \sin^2\theta_{\mathrm{c}} + \frac{8}{3} p_{\mathrm{c}} \beta_{\mathrm{c}}^3 \beta^{-1}\right), \quad (30)$$

and follow the similar analysis in Subsections 2.1. In particular, we show that the generalized analytic expressions for the radius (or thickness) ratios, including Eqs. (24) and (27), become:

$$\text{Bulged}: \quad \frac{R_{\mathrm{v}}}{R_{\mathrm{c}}} \approx 1 - \varphi^{-1}\gamma_{\mathrm{c}} v_{\mathrm{ec}}^{-1/3} \frac{\left[(\alpha-1)v + v_{\mathrm{ev}}\right]^{4/3}}{v - v_{\mathrm{ev}}}, \qquad \frac{h_{\mathrm{c}}}{h_{\mathrm{v}}} \approx v_{\mathrm{ec}}^{1/3} v_{\mathrm{ev}}^{-1} \left[(\alpha-1)v + v_{\mathrm{ev}}\right]^{2/3}$$
$$\text{Budded}: \quad \frac{R_{\mathrm{c}}}{R_{\mathrm{v}}} \approx \varphi^{1/2} v_{\mathrm{ec}}^{1/3} u^{-1/3} v^{-1/3}, \qquad \frac{h_{\mathrm{c}}}{h_{\mathrm{v}}} \approx (1-\varphi)^{-1} v_{\mathrm{ec}}^{1/3} v_{\mathrm{ev}}^{-1} u^{2/3} v^{2/3} \quad (31)$$

In view of the fact that cell swelling typically happens during the later development phases (Fig. 1, 2 and Extended Data Fig. 8B), which correspond to the budding stage, we will discuss the dependence of cell volumes v_{ec} and v_{ev} on thickness (or radius) ratio only for budded organoids. For a budded organoid, scaling laws $R_{\mathrm{c}}/R_{\mathrm{v}} \sim v_{\mathrm{ec}}^{1/3}$ and $h_{\mathrm{c}}/h_{\mathrm{v}} \sim v_{\mathrm{ec}}^{1/3} v_{\mathrm{ev}}^{-1}$ are suggested by Eq. (31) and verified by numerical results in Extended Data Fig. 3H. It can be seen from the scaling laws that, cell swelling in crypt always results in an increased radius (or thickness) ratio, while cell swelling in villus decreases the thickness ratio.

2.4. Summary of analytic results

These analytic results provide insights into the physical mechanisms of crypt morphogenesis. As aforementioned, modulated by cell tensions, an epithelial sheet can engender two types of active deformations: in-plane contraction and spontaneous bending, which are respectively described by in-plane contraction ratio α and spontaneous curvature γ_{c}. However, in-plane contraction and bending can both vary at the same time (for instance if only the apical tension in crypt increases, all other parameters being kept constant) which implies the two mechanical variables α and γ_{c} are combined to affect the geometric quantities of organoid epithelium, such as thickness (and radius) ratios. The analytic results in Subsection 2.1 indicates that the initial bulging morphology depends on α for the thickness ratio, $\varphi^{-1}\gamma_{\mathrm{c}}$ for the radius ratio, while the budding configuration is only controlled by $u = \alpha\left(1 + \varphi^{-1/2}\gamma_{\mathrm{c}}\right)^{-1}$.

Here, we restricted ourselves to a two-region morphology (one crypt and one villus), although highly similar results are expected when considering more than one crypt region. Although in principle, budded shapes can arise even in the case of one-region organoids (e.g. absence of mechanical differences between stem and differentiated cells, as explored by

Rozman et al. (*12*), who consider all cells of an organoid have equal properties, and budded shape can occur for remarkable apico-basal tension difference), we note that this unlikely to occur in intestinal organoids, as i) we experimentally observed strong region differences in both actomyosin patterns and apico-basal tensions (assessed both via laser ablation in Fig. 2D and micropipette aspiration in Fig. 2E), and ii) one-region organoids are predicted to become spherical when inflated above a critical size, which is not what we observed in our inflation experiments (Fig. 5B-C, Extended Data Fig. 7A-B).

In view of the fact that shape transformation from budded to open seldom happens even though the lumen volume increases dramatically by ~5 times (Fig. 5C'), the diagrams of crypt morphology with infinite volume (Extended Data Fig. 3G) can be used to determine bounds for the parameters γ_c and α. We thus use these analyses and analytical criteria to guide the fitting of experimental data (both during normal organoid morphogenesis and upon organoid inflation).

3. Morphogenesis with enhanced apical constriction and water uptake

To evaluate the influence of specific parameters on organoid morphologies, parameters in crypt mechanics (e.g. in-plane contraction α and spontaneous curvature γ_c) and volumes (e.g. organoid volume v and volume of a villus cell v_{ev}) are usually analyzed separately in previous sections. Here, we focus on specific biophysical mechanisms uncovered by experiments, showing that these parameters may be coupled together to modulate organoid morphologies.

3.1. Apical tension in crypts modulates both spontaneous curvature and in-plane contraction

Firstly, experiments indicate that enhanced apical constriction of crypt is the leading mechanism in organoid morphogenesis. As assumed in Section 1, the initial spherical organoid has the same tension Γ_0 on both apical and basal surfaces, and the lateral tensions in both regions are Γ_l. With the morphological evolution of organoids, the accumulation of actomyosin on crypt apical surface leads to an increase in crypt apical tension, that is $\Gamma_{ac} = m\Gamma_0$ with m the normalized crypt apical tension satisfying $m \geq 1$, while the other tensions are assumed to be constant, i.e. $\Gamma_{bc} = \Gamma_{av} = \Gamma_{bv} = \Gamma_0$. Considering that the size of the initial spherical organoid are regulated by two tensions Γ_0 and Γ_l, one can easily find the

relation between the shape factor $\tilde{\kappa}_0$ and these two tensions, i.e. $\tilde{\kappa}_0 = \frac{\Gamma_l}{2\Gamma_0}\sqrt{\frac{N_t}{4\pi}}$. Then, one can rewrite in-plane contraction α and spontaneous curvature of crypt γ_c as

$$\alpha = \frac{1+m}{2}, \gamma_c = \frac{1-m}{4\tilde{\kappa}_0}. \tag{32}$$

Eq. (32) shows that crypt apical tension can simultaneously modulate in-plane contraction α and spontaneous curvature γ_c, and that shape factor $\tilde{\kappa}_0$ is also important for the resulting shape. In this study, actomyosin accumulation is considered to be the sole mechanism that modulates cellular tensions, although other regulatory mechanisms, such as stretch-induced cortex dilation (*13*), are reported to be important for epithelia under deformation.

3.2. Lumen/cell volume changes from villus differentiation

Secondly, experiments verify that villus cells up-regulate apical ion pumps that lead to the swelling of villus cells and shrinkage of the lumen (Fig. 7). This water uptake of villus cells will modulate two parameters in our model: the organoid volume v, which is the sum of the lumen volume and half the epithelial volume in villus, and the volume of villus cell v_{ev}. For simplicity, we assume the organoid volume is only modulated by water uptake. Then, during the water uptake of villus cells, the organoid volume is related to volume of a single villus cell v_{ev} as

$$v = 1 - \frac{3(1-\varphi)}{2\tilde{\kappa}_0}(v_{ev} - 1). \tag{33}$$

Obviously, the water uptake from cells will lead to a decrease in organoid volume v. Before specific discussion on the influence of water uptake by villus cells on organoid morphogenesis, we reassess the efficiency of water uptake by different cell types, although the influence of cell swelling has been evaluated in Subsection 1.4.1.

3.2.1. Efficiency for morphogenesis of different scenarios for volume changes

Consider the relative reduction of lumen volume Δv_{lu} is compensated by i) volume increase in all cells, which lead to $v_{ec} = v_{ev} = \tilde{\kappa}_0 \Delta v_{lu}/3 + 1$, ii) volume increase in crypt cells only, which yields $v_{ec} = \tilde{\kappa}_0 \Delta v_{lu}/(3\varphi) + 1$, and iii) volume increase in villus cells only, which results in $v_{ev} = \tilde{\kappa}_0 \Delta v_{lu}/[3(1-\varphi)] + 1$. For the water uptake by cells, including all the three cases above, the overall volume is $v \approx 1 - \Delta v_{lu}/2$. Besides, we also consider the case that the

reduction of luminal fluid is due to the leakage of epithelium, which corresponds to $v_{ec} = v_{ev} = 1$, and $v = 1 - \Delta v_{lu}$. As shown in Fig. 7F, water uptake by villus cells is the most efficient mechanism for organoid budding.

3.2.2. Relevance for *in vivo* morphogenesis

Given that the geometry of the gut *in vivo* is that of a tube, rather than a closed sphere as in organoids, we next wish to discuss the relevance of these findings in the absence of lumen volume changes. From a mechanical perspective, we reason that villus swelling should still promote crypt budding, even in the absence of significant lumen changes, as this can also increase the compressive stresses exerted at the crypt/villus boundary. Indeed, simulating villus cell swelling in the absence of lumen shrinkage still contribute to crypt budding (Fig. 7G). Interestingly, we find a similar situation *in vivo*, with marked increase in villus cell volume in the first days of post-natal development (Extended Data Fig. 8C), which is concomitant to crypt morphogenesis.

3.3. Combined effects of tension and volume changes

Finally, we examine the influence of concomitant crypt apical constriction and water uptake of villus cells on organoid morphology in Extended Data Fig. 6. The water uptake is evaluated by the normalized volume of a villus cell v_{ev}, and causes variations in the lumen volume, as shown in Eq. (33).

3.3.1. Efficiency for morphogenesis

As expected, both the enhanced apical constriction in crypt and water uptake of villus cells can lead to budding (Extended Data Fig. 6A), and the critical apical tension and degree of water uptake are affected by the crypt size φ and shape factor $\tilde{\kappa}_0$ (Extended Data Fig. 6B). It is hard to close a large crypt by apical constriction alone, since the in-plane contraction of the epithelium will lead to the elevation of luminal fluid pressure, which further hinders the bending of the crypt, and a larger contractile region (i.e., the crypt) will lead to a higher fluid pressure. As shown in Eq. (32), spontaneous curvature is inversely related to the shape factor $\tilde{\kappa}_0$, thus strong apical constriction is needed for the budding of an organoid with a thin epithelium or a big lumen (i.e. large shape factor in Extended Data Fig. 6B). Besides, as observed in experiments, an organoid usually undergoes enhanced apical constriction in the bulging stage, which is followed by the water uptake of villus cells. Setting the normalized

crypt apical tension to $m = 2$, we also examine the degree of water uptake that resulting in the closure of two regions in Extended Data Fig. 6B.

Although the phase diagrams in Extended Data Fig. 6B clearly show that, a large crypt size is not favorable for apical constriction-driven budding, the results are obtained under the assumption that region sizes keep constant during development. If the organoid displays preferential proliferation of crypt cells, then one can expect that enlarging crypt size would promote budding, since an increase in cell number should have similar influence on morphogenesis with swelling of crypt cells, as discussed in Subsection 1.5. Indeed, with enhanced apical tension, crypt growth can promote budding (Extended Data Fig. 6C), although the cell number in the crypt needs to be doubled. An increase in cell number engenders an equivalent volume effect of lumen shrinkage, which compresses the crypt/villus boundary and thus promotes budding. After removing the volume effect by rescaling the organoid volume v in Extended Data Fig. 6D, we can find that the morphologies are all quite close to those in the scenario considering constant crypt size (i.e. $\varphi' = \varphi_g / (1 + \Delta\varphi)$ with $\Delta\varphi = \varphi_g - \varphi$), and the negative effect of crypt size on organoid morphologies uncovered in Extended Data Fig. 6B is restored. For a swollen organoid (e.g., the one with $v = 1.2$ in Extended Data Fig. 6E), the volume effect is weakened, and an enlarging crypt no longer benefits organoid morphogenesis.

3.3.2. Influence on morphometric parameters

The morphometric parameters, i.e., thickness ratio h_c / h_v and radius ratio R_c / R_v, also evolve with apical constriction and water uptake. An organoid with enhancing crypt apical constriction may undergo three phases: Bulging, budding, and budding with vanishing crypt apical surface (i.e., $R_{ac} = 0$). In the first two phases, enhanced apical constriction in crypt leads to an increase in the thickness ratio and a decrease in the radius ratio (Extended Data Fig. 6F-G), and the transformation from the bulged to the budded shape results in negligible variations in the trends of thickness ratios but notable changes in those of radius ratios. With continued enhancement of apical constriction, the apical surface of a closed crypt will contract towards a point, then the crypt will stop thickening and the thickness (or radius) ratio goes into a plateau, as shown in Extended Data Fig. 6F.

Morphometric parameters in these three phases are also affected by shape factor $\tilde{\kappa}_0$ and region size φ. As aforementioned, spontaneous curvature γ_c results in an increased thickness and decreased radius of the crypt. Considering γ_c is inversely proportional to shape factor $\tilde{\kappa}_0$

(Eq. (32)) , one can find that a larger $\tilde{\kappa}_0$ will leads to a smaller thickness ratio h_c / h_v and larger radius ratio R_c / R_v in the bulging and budding phases, as verified in Extended Data Fig. 6F. While crypt size φ has negligible influence on the thickness (or radius) ratio in these two phases (Extended Data Fig. 6G). However, both shape factor $\tilde{\kappa}_0$ and crypt size φ are crucial on the third phase. Since the apical surface of an organoid with a thin epithelium/large lumen is hard to contract into a point, a large shape factor will delay the transition towards the third phase (Extended Data Fig. 6F). And a large crypt in the budding phase will become a closed sphere with large radius R_c, which makes it hard to get $R_{ac} = R_c - h_c / 2 = 0$ and enter into the third phase (Extended Data Fig. 6G). After enhanced apical constriction in crypt, water uptake of villus cells will keep promoting the morphogenesis. As expected, water uptake of villus cells will decrease the thickness ratio and promote the closure of two region (Extended Data Fig. 6H). Morphometric parameters show distinct trends in bulging and budding phases. With the water uptake, the thickness ratio decreases more sharply in a budded organoid than in a bulged one, while the radius ratio only shows notable changes in the bulging phase.

We further explore the influence of crypt apical constriction on the evolution of thickness ratio h_c / h_v and radius ratio R_c / R_v during organoid expansion. As expected and shown in Extended Data Fig. 6I, the thickness ratio always increases with volume expansion for an organoid with enhanced crypt apical constriction, which prevents the crypt from inflating with organoid expansion. As already found in Extended Data Fig. 2 and analyzed in Subsection 2.1, for a bulged organoid with weak crypt apical constriction, the radius ratio increases with volume expansion, while the radius ratio of a budded organoid decreases with volume expansion (Extended Data Fig. 6I). Besides, the transformation from a budded shape to a bulged one can also happen, and will also affect the thickness (or radius) ratio. We also discuss morphologies of crypts with enhanced apical constriction under infinite volume expansion in Extended Data Fig. 6J. Setting crypt size $\varphi = 0.2$ (which is the average value that we measured experimentally, see Section 4 for details), the crypt morphologies are modulated by two parameters: normalized crypt apical tension m and shape factor $\tilde{\kappa}_0$. The phase diagram indicates that the three morphologies discussed in Extended Data Fig. 3G (partially open, fully closed, and fully closed with vanishing apical surface) still exist for crypts with enhanced apical constriction. Inserting Eq. (32) into the critical condition in Subsection 2.2, one obtains that the crypt will never open up if the normalized crypt apical tension m satisfies $\left[1+(1-m)/\left(4\varphi^{1/2}\tilde{\kappa}_0\right)\right]^2 + 2/(1+m) < 1$ (whose lower bound is named as m_{crit} afterwards), no

matter the lumen volume. However, when m is larger than $2\varphi^{1/2}\tilde{\kappa}_0$, the apical surface will contract into a point, resulting in $R_{ac}=0$. The influence of cell swelling on thickness (or radius) ratio have been discussed in Subsection 2.3, and both numerical and analytic results indicate that the crypt morphologies under infinite organoid expansion are irrelevant to cell volume v_{ev}.

4. Organoid morphometric measurements and fitting strategy

To validate the theory and extract mechanical parameters, we measured the thickness (and radius) ratios of crypt and villus during normal organoid morphogenesis (Fig. 2C) and inflation experiments, when the lumen volume is increased by PGE treatment (Fig. 5B-C) or micropipette injection (Extended Data Fig. 7A-B). In the measurements, a dimensionless volume $\bar{v}$, which is the current volume of a sample normalized by its originating volume, is used to characterize the organoid inflation. Let v_0 be the initial volume, $\tilde{v}$ be the volume in free mechanical state, which can be estimated as $\tilde{v}\approx\left(1-\varphi\right)^{3/2}v_{ev}$ by using $\partial\hat{F}/\partial\beta=0$ in Eq. (30), then $\bar{v}$ is related to the volume v employed in the model as $\bar{v}=v/\left(v_0\tilde{v}\right)$. Considering the crypt apical constriction as the main mechanical cue of organoid morphogenesis in bulging phase, then the crypt mechanical parameters can be described by Eq. (32). Moreover, luminal volume decreases and swelling of villus cells occurs in the budding phase (Fig. 7). In view of these, one can find that the evolutions of thickness (and radius) ratios depend on four parameters: m, $\tilde{\kappa}_0$, φ, and v_0, for bulged samples, and one more parameter v_{ev} for budded ones. We can directly measure some of them, and determine the remaining parameters by fitting experimental data with analytic formulation or numerical results.

4.1. Independently-measured geometric parameters

Firstly, to estimate the shape factor of organoids, we can measure the shape factor of villus $\kappa_v=4\pi R_v^3/\left(N_vV_{ev}\right)\approx R_v/h_v$, which is linearly dependent on $\bar{v}$ as $\kappa_v\approx\kappa_{v0}\bar{v}$, with the initial shape factor of villus κ_{v0} related to $\tilde{\kappa}_0$ as $\kappa_{v0}\approx\tilde{\kappa}_0v_0$ for bulged organoids and $\kappa_{v0}\approx\left(1-\varphi\right)^{1/2}\tilde{\kappa}_0v_0$ for budded ones. Secondly, we also directly measured the crypt size φ of bulged organoids as $\varphi\approx h_cl_c^2/\left(4h_vR_v^2\right)$, where the arclength of crypt section is denoted l_c, to further constrain the system.

4.2. Parameters extracted via direct fitting

Analytic results in Section 2 provide guidance on the fitting of morphometric data. Replacing volume v by the new normalized volume $\bar{v}$ in Eq. (31) leads to

$$\begin{aligned} &\text{Bulged}: \quad \frac{R_\mathrm{v}}{R_\mathrm{c}} \approx 1-\varphi^{-1}\gamma_\mathrm{c}\bar{v}_\mathrm{e}^{-1/3}\frac{\left[(\alpha-1)v_0\bar{v}+1\right]^{4/3}}{v_0\bar{v}-1}, \quad \frac{h_\mathrm{c}}{h_\mathrm{v}} \approx \bar{v}_\mathrm{e}^{1/3}\left[(\alpha-1)v_0\bar{v}+1\right]^{2/3} \\ &\text{Budded}: \quad \frac{R_\mathrm{c}}{R_\mathrm{v}} \approx \varphi^{1/2}\left(1-\varphi\right)^{-1/2}\bar{v}_\mathrm{e}^{1/3}\left(uv_0\right)^{-1/3}\bar{v}^{-1/3}, \quad \frac{h_\mathrm{c}}{h_\mathrm{v}} \approx \bar{v}_\mathrm{e}^{1/3}\left(uv_0\right)^{2/3}\bar{v}^{2/3} \end{aligned}, \quad (34)$$

where $\bar{v}_\mathrm{e} = v_\mathrm{ec}/v_\mathrm{ev}$ is the volume ratio of a crypt cell to a villus cell. In this new formulation, thickness (or radius) ratio is only related to cell volumes by $\bar{v}_\mathrm{e}^{1/3}$. As aforementioned, cell swelling is insignificant in the bulging phase, but the swelling of villus cells becomes important in the budding phase. Therefore, we have $\bar{v}_\mathrm{e} \approx 1$ for a bulged organoid, and $\bar{v}_\mathrm{e} = v_\mathrm{ev}^{-1} < 1$ for a budded one. During the bulging of organoids, the crypt mechanical parameters vary with time, while the lumen volume stays almost constant. In contrast, the inflation experiments provide a setting where lumen volumes change drastically while crypt mechanics can be considered constant. In view of these, based on the analytical expressions of Eq. (34), we further discuss specific relations between morphometric parameters, i.e. thickness (and radius) ratios, and bulging time or lumen volume in the following, and also derive the relation between thickness ratio and radius ratio. Using these analytical relations, we can fit and rescale the experimental data.

4.2.1. Dynamics of organoid bulging

First, we consider the bulging dynamics of organoids. Experiments show that the volume stays constant in this process, that is $\bar{v} \approx 1$. According to Eq. (34), the morphometric parameters $R_\mathrm{c}/R_\mathrm{v}$ and $h_\mathrm{c}/h_\mathrm{v}$ are then linked to each other via a simple relation:

$$\frac{R_\mathrm{v}}{R_\mathrm{c}} \approx 1 + \mathrm{pm1}\cdot\left(h_\mathrm{c}/h_\mathrm{v}\right)^2\left[\left(h_\mathrm{c}/h_\mathrm{v}\right)^{3/2}-1\right], \quad (35)$$

where $\mathrm{pm1}=\left[2\varphi\kappa_{\mathrm{v}0}\left(v_0-1\right)\right]^{-1}$ is a single fitting parameter. Importantly, this expression is independent on the dynamics of how crypt apical tension varies in time, providing a simple and robust model prediction. The six samples in Fig. 2C have different characteristic sizes, we can get shape factor $\kappa_{\mathrm{v}0}$: 2.7±1.8 (mean ± SD) and crypt size φ: 0.2±0.06 (mean ± SD). The measurement result of $\kappa_{\mathrm{v}0}$, which is obtained by using $\kappa_{\mathrm{v}0} \approx R_\mathrm{v}/\left(h_\mathrm{v}\bar{v}\right)$ as given in Subsection 4.1, indicates that the radius to thickness ratio $R_\mathrm{v}/h_\mathrm{v}$ is typically larger than 2 (volume $\bar{v} \approx 1$

for bulging organoids), providing a safety check on the " thin-film assumption" employed in Subsection 1.1. Extracting pm1 from these samples allows us to rescale the morphometric parameters of every sample to verify that, even for organoids with distinct initial shape factors and crypt sizes, their morphometric parameters can be well-fitted by Eq. (35), as shown in Fig. 2C''. In the fitting and rescaling, the initial volume v_0 is estimated as 2.5±0.5 (mean ± SD, from the analytical theory), which gave consistent values across different organoids. We note for instance that Sample 3 appears as an outlier in terms of thickness ratio (with a much larger value than others), but this is explained by the fact that it is larger in size (κ_{v0} = 6.2), and larger organoids need larger crypt apical tension (which results in a thicker crypt monolayer) to bud, as discussed in Section 3. Despite its specific morphometric character, this sample can also be fitted by choosing a reasonable volume value ($v_0 = 2.5$), consistent with other organoids.

On the other hand, to reproduce the evolution of each morphological ratio in time, one must assume a specific dynamic relation for tension changes in time. For simplicity, we consider a linear increase of the normalized crypt apical tension m with time t, that is $m = m_0 + m't$, where m_0 and m' are respectively the initial value and the slope. Then, the evolution of thickness ratio h_c / h_v and radius ratio R_c / R_v can be estimated as

$$\frac{h_c}{h_v} \approx \left(\mathrm{pm2} + \mathrm{pm3}\cdot t + 1\right)^{2/3},\ \frac{R_v}{R_c} \approx 1 + \mathrm{pm1}\cdot\left(\mathrm{pm2} + \mathrm{pm3}\cdot t\right)\left(\mathrm{pm2} + \mathrm{pm3}\cdot t + 1\right)^{4/3}, \quad (36)$$

where pm2=$\left(m_0 - 1\right)v_0 / 2$, pm3=$m'v_0 / 2$. We can get pm2 and pm3 simultaneously by fitting the experimental data of thickness ratios, and obtain pm1 by fitting the data of radius ratios (both of the evolutions were well-fitted by these analytic forms, see Extended Data Fig. 3C-D). For the six samples we measured, by using analytic fitting, we can get their initial crypt apical tension m_0:1.2 ± 0.2 (mean ± SD). We can also get the enhanced crypt apical tension m at the end of the bulging phase (prior to water uptake by villus cells): 1.6 ± 0.4 (mean ± SD). Besides the data fitting using analytic equation (36), we also use full numerical results (e.g. those in Section 1 and 3) to fit the experimental data, as shown in Fig. 2C' and Extended Data Fig. 3B. In this way, we can get the initial apical tension m_0:1.3 ± 0.3 (mean ± SD), and the apical tension m at the end of the bulging phase: 1.7 ± 0.6 (mean ± SD). We can find that both fittings get quite close estimations of apical tension m, providing a safety check on the fitting procedure. The estimation of m at the end of the bulging is interesting, as it remains significantly smaller (60%) of the critical value of m that leads to crypt budding (this proportion is calculated for three samples with representative crypt sizes and shape factors), and also argues that changes

in lumen volume will play a key role on crypt morphogenesis. Furthermore, *m* is also much smaller than the critical value m_{crit} that allows to remain budded upon infinite volume expansion (40%).

4.2.2. Inflation of bulged organoids

Our analysis of the dynamics of bulging organoid suggests that their apical tension *m* is below the critical point of Fig. 5A, so that these organoids would be expected to open up upon inflation, a key prediction we now test. For the inflation of bulged samples, we can assume that tensions remain constant, and eliminate volume from the equation to derive again a relation between $R_{\mathrm{c}} / R_{\mathrm{v}}$ and $h_{\mathrm{c}} / h_{\mathrm{v}}$:

$$\frac{R_{\mathrm{v}}}{R_{\mathrm{c}}} \approx 1 + \frac{\left(h_{\mathrm{c}}/h_{\mathrm{v}}\right)^2}{\mathrm{pg1}\cdot\left[\left(h_{\mathrm{c}}/h_{\mathrm{v}}\right)^{3/2} - \alpha\right]}, \tag{37}$$

where $\mathrm{pg1} = -\varphi\gamma_{\mathrm{c}}^{-1}\left(\alpha - 1\right)^{-1}$. In contrast to the relation of morphometric parameters in Eq. (35) for bulging evolution, the thickness ratio $h_{\mathrm{c}} / h_{\mathrm{v}}$ and the radius ratio $R_{\mathrm{c}} / R_{\mathrm{v}}$ show similar trends during organoid inflation. The dependence of morphometric parameters on volume $\bar{v}$ yields

$$\frac{h_{\mathrm{c}}}{h_{\mathrm{v}}} \approx \left(\mathrm{pg2}\cdot\bar{v} + 1\right)^{2/3},\ \frac{R_{\mathrm{v}}}{R_{\mathrm{c}}} \approx 1 + \frac{\left(\mathrm{pg2}\cdot\bar{v} + 1\right)^{4/3}}{\mathrm{pg3}\cdot\left(v_0\bar{v} - 1\right)}, \tag{38}$$

Where $\mathrm{pg2} = \left(m - 1\right)v_0/2$, $\mathrm{pg3} = 2\varphi\kappa_{\mathrm{v0}}/\mathrm{pg2}$. The experimental data again was in agreement with these analytic forms (Extended Data Fig. 7C), so that by fitting the experimental data of thickness ratios, we can get pg2, which can be further used to estimate pg3. Then, the initial volume v_0 is employed as the only fitting parameter to fit the data of radius ratios. Further using the relations between parameters in Eq. (37) and those in (38): $\mathrm{pg1} = v_0 \cdot \mathrm{pg3}/\mathrm{pg2}$, $\alpha = 1 + \mathrm{pg2}/v_0$, we can find that the functional form of Eq. (37) predicts the evolution of all six bulged inflation samples (from PGE or pipette), as shown in Fig. 5D. For the six samples we measured, we can get estimates of the initial volume v_0: 1.5 ± 0.3 (mean ± SD) (i.e. always larger than 1, consistent with initially swollen organoids as found in the fits of the bulging evolution, see Subsection 4.2.1), and the normalized crypt apical tension *m*: 1.3 ± 0.05 (mean ± SD), which is close to the initial tension m_0 estimated for the six bulging samples in Subsection 4.2.1, providing another consistency check of the fitting approach and model (and

showing in particular that the parameters used in the fits/collapse of Fig. 2 can be validated by independent datasets).

4.2.3. Inflation of budded organoids

Finally, as aforementioned, the morphometric parameters of budded samples obey a simple scaling law, and we can easily get the relation between R_c / R_v and h_c / h_v:

$$\frac{R_c}{R_v} = \left(\mathrm{pd1}\cdot h_c / h_v\right)^{-1/2}, \tag{39}$$

where $\mathrm{pd1} = \varphi^{-1}\left(1-\varphi\right)v_{ev}$, and their relation with $\bar{v}$ can be recast as

$$\frac{h_c}{h_v} \approx \left(\mathrm{pd2}\cdot\bar{v}\right)^{2/3},\ \frac{R_c}{R_v} = \left(\mathrm{pd3}\cdot\bar{v}\right)^{-1/3}, \tag{40}$$

where $\mathrm{pd2} = v_{ev}^{-1/2}\cdot uv_0$, $\mathrm{pd3} = \mathrm{pd1}^{2/3}\cdot\mathrm{pd2}$. These scaling relationships can in fact be derived from purely geometric considerations, under the assumption that near-spherical villi bear the deformation alone. As shown in Fig. 5E and Extended Data Fig. 7C', they can fit the evolution of budded inflation samples (from PGE or pipette) very well. To estimate the normalized crypt apical tension m, we can further introduce the shape factor of crypt $\kappa_c = 4\pi R_c^3 / \left(N_c V_{ec}\right)$, which can be estimated either by directly using $\kappa_c \approx R_c / h_c$ or by using its relation with other fitting parameters, that is $\kappa_c = \kappa_{v0}\left(\mathrm{pd2}^2\cdot\mathrm{pd3}\right)^{-1/3}$, then the normalized crypt apical tension is

$$m = \frac{4\varphi^{1/2}\tilde{\kappa}_0 + 2}{2\kappa_c + 1} - 1. \tag{41}$$

Although $\varphi^{1/2}\tilde{\kappa}_0$ is hard to get by direct measurement, we can use the analytic critical conditions of crypt morphologies under infinite volume expansion (discussed in Subsection 2.2 and Section 3) to estimate the lower bound of m (i.e., m_{crit}). Now we have $\left(\frac{m+1}{m-1}\right)^{3/2} - \frac{m+1}{m-1} < \frac{1}{2\kappa_c}$ to forbid the crypt to open up with volume expansion and $\kappa_c > 0.5$ to guarantee that $R_{ac} > 0$ always holds. For the six samples we measured, we can get an estimation of m_{crit}: 3.6 ± 0.8 (mean ± SD).

4.3. Validation of tension estimation

Finally, we can use our measurements of Myosin levels, as well as our laser ablation experiments on apical junctions (both measured for different times and regions) to semi-quantitatively constrain the crypt apical tension. As aforementioned, lateral myosin intensity

does not show strong spatio-temporal changes (Fig. 3B), so that we consider constant lateral tension Γ_l in the fitting. Furthermore, apical Myosin intensity in crypts increases by around 50% from spherical to bulged shapes, and by around two-fold from bulged to budded shapes, leading us to hypothesize that tension increases is a key driver of the bulged-budded transformation. This is also consistent with laser ablation experiments on bulged *vs.* budded crypts, showing a roughly two-fold increase (Fig. 2D). We note that because these ablations are done in a highly local manner, they only probe the local tensions of the cell-cell junctions, whereas more global tissue-wide ablation, such as used in *Drosophila* notum, is used instead to estimate global tissue tensions (*14*), which would also depend on parameters like lumen swelling in our systems.

Importantly, these magnitudes of apical crypt tensions for an organoid changes from spherical to bulged to budded shapes are consistent with the values extracted from aforementioned morphogenesis/inflation fittings (we had estimated m_{crit} : 3.6 ± 0.8 from budded sample inflation, and 1.7 ± 0.6 at the end of bulged state from Fig. 2, arguing that a doubling of tension estimated from laser-cutting/Myosin intensity would be sufficient to bud), providing an independent validation for the parameter set we propose here, and the range of the theoretical phase diagram proposed in Fig. 5A.

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