

Control of Lumen Morphology by Lateral and Basal Cell Surfaces

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Across development, the formation of fluid-filled lumina enclosed by epithelial tissues is inextricably linked to the establishment of apicobasal cell polarity. Here, we reveal how the mechanical differences between cell surfaces resulting from this polarity enter the interplay of lumen pressure, mechanics of the cell cortex, and cell-cell adhesion that determines lumen morphology: Madin–Darby canine kidney cysts under tight junction perturbations display a shape instability of apical surfaces that is associated with changes of cyst pressure and apical belt tension [Mukenhörn *et al.*, *Dev. Cell* **59**, 2886 (2024)]. Here, we discover that the cysts also respond to these perturbations by significant modulations of lateral and basal surface tensions. We develop a mean-field three-dimensional vertex model of these cysts that reproduces the experimental shape space and instability quantitatively. This model explains the observed increase of lateral contractility to be a cellular response that counters the instability. Our work thus shows how regulation of the mechanics of all cell surfaces conspires to control lumen morphology.

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I. INTRODUCTION

The formation of fluid-filled lumina enclosed by epithelial cells [Fig. 1(a)] is a fundamental process in organ development, enabling fluid transport, nutrient absorption, and secretion in tissues such as the kidney, lung, and intestine. The diversity of the shapes of these lumina is paralleled by the diversity of the mechanical forces, molecular mechanisms, and morphogenetic processes involved in their formation [2–10]. Physically, the shape of the lumen is controlled by a balance between hydrostatic pressure and mechanical forces associated with tensions in the apical, lateral, and basal cell cortices, cell-cell adhesion at the lateral cell surfaces, and adhesion of the cells to the surrounding extracellular matrix (ECM) at their basal surfaces [Figs. 1(a) and 1(b)].

These mechanical forces are intertwined with the cell biology of the epithelial cell surrounding the lumen. Indeed, these cells are polarized along their apicobasal axis [5,11], which

defines three distinct yet interconnected surface domains [11] that, in turn, define the mechanical properties of the cells: the apical, lateral, and basal interfaces [Figs. 1(a) and 1(b)].

The apical surface faces the lumen and contains an actin-rich cortex including actin-based microvilli that is anchored by the apical junctional complex, composed of tight junctions (TJs) and adherens junctions [12–14]. This complex defines the boundary of the apical domain, regulates paracellular permeability, and can generate contractile tension via the activation of myosin. The lateral surface mediates intercellular adhesion via cadherin-based adherens junctions [11,14], while the basal surface anchors the epithelium to the ECM through focal adhesions [11,15,16]. A continuous cortical actin network [Fig. 1(b)] supports these domains and underlies the apical membrane [17,18]. Forces generated at one interface (e.g., contractile forces at the apical belt) can redistribute across the tissue, and basal ECM anchorage can influence overall cell shape [7,19,20].

The epithelial layer surrounding the lumen must be sealed to sustain osmotic and hence hydraulic pressure differences [9,10]. TJs are therefore key regulators of this system [1,12,21]: Claudins form the transmembrane strands that limit ion permeability [12], while ZO-1 and ZO-2 act as cytoplasmic scaffolds linking junctions to the actin cytoskeleton and shaping cortical architecture [12].

Physical models thus describe lumina as pressurized cavities enclosed by a contractile cell layer [1,9,10,22–28]. Many of these models focus on the interplay of hydraulic and osmotic pressure that drives lumen growth dynamics [9,23,27] and associated hydroelectric effects [24,26]. The mechanics of the cells surrounding the lumen often enter these models via

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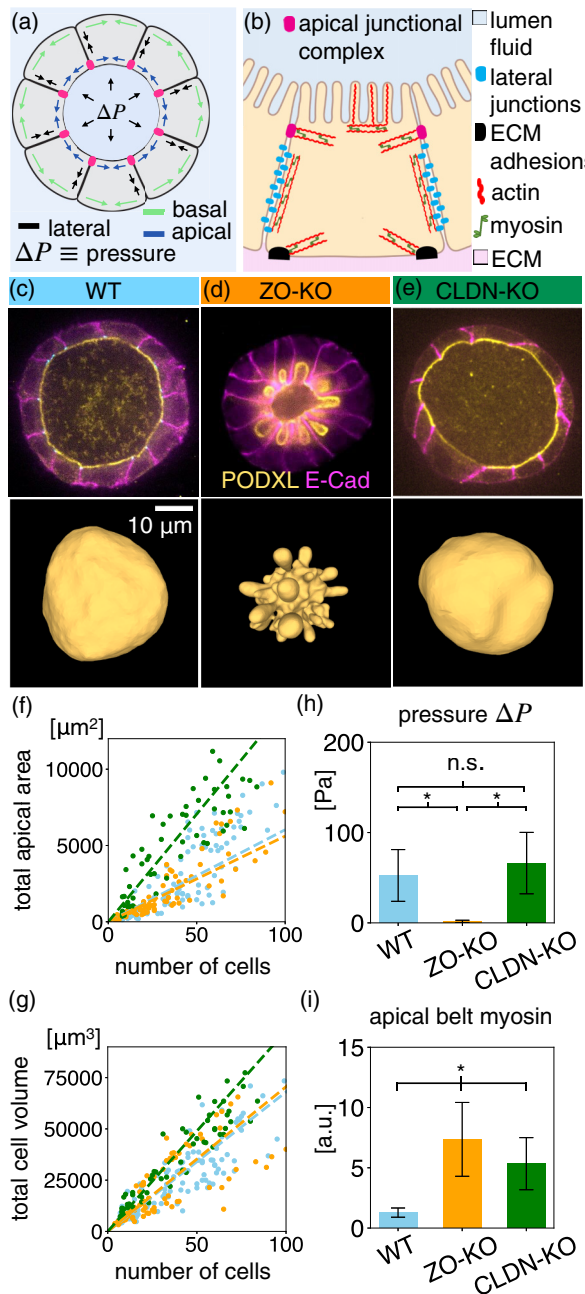


FIG. 1. Mechanics and cell biology of epithelial cysts and behavior of cysts of Madin–Darby canine kidney (MDCK) cells under tight-junction (TJ) perturbations [1]. (a) Schematic of an epithelial cyst. The lumen is a pressurized, fluid-filled spherical cavity surrounded by a monolayer of polarized epithelial cells. Arrows indicate the hydrostatic pressure difference ΔP and the cortical tensions acting along the apical, lateral, and basal surfaces, which together determine cyst shape and stability. (b) Schematic depiction of epithelial cell interfaces and cytoskeletal organization. The apical junctional complex, composed of TJs and adherens junctions, encircles the apex of the cell and anchors the apical actomyosin belt, which generates contractile tension. The lateral membrane forms adhesive contacts between neighboring cells via cadherin-based adherens junctions. At the basal side, focal adhesions mediate interactions between the cells and the extracellular matrix (ECM) surrounding the cyst. A cortical actin cytoskeleton supports these lateral and basal interfaces and underlies the apical membrane, including actin-rich microvilli.

a Young–Laplace equation [29] linking the hydraulic pressure to a constant or elastic tension. Other models focus on the mechanics of the apical surface [1]. These models do not resolve the mechanical differences between the apical, lateral, and basal cell surfaces that result from the apicobasal polarization of epithelial cells. The role of the lateral and basal cell surfaces for lumen morphology thus remains open.

A widely used model that can resolve these differences between the cell surfaces is the vertex model [30–34]. Motivated both by biological applications and fundamental physical questions, recent work has begun to focus on fully three-dimensional instantiations of this vertex model [19,20,35–38]. In particular, mean-field limits [10] could yield insights into the role of the different cell surfaces for lumen morphology.

The role of the different cell surfaces in lumenogenesis also remains poorly understood experimentally, partly because quantifying their mechanics is challenging. Indeed, perturbing cortical tensions or cell–cell adhesion without altering cell mechanics globally is difficult in general [39].

In vitro models of lumen formation are a means of meeting this challenge: Madin–Darby canine kidney (MDCK) cells are widely used to model this process. In three-dimensional culture, MDCK cells polarize and form spherical cysts with a central lumen [1,25,40–42], as shown in Fig. 1(c). A recent study [1] introduced tight-junction (TJ) perturbations into this system: Loss of TJ scaffolds [ZO-KO; Fig. 1(d)] led to lumen volume collapse and apical buckling, while cysts lacking TJ strands [CLDN-KO; Fig. 1(e)] formed rounded lumina despite increased leakiness. Strikingly, the single-cell apical area was observed to be constant in each of these conditions [Fig. 1(f)], in agreement with earlier observations [25]. Less surprisingly, perhaps, the single-cell volume is also conserved in each condition [Fig. 1(g)]. The apical buckling in ZO-KO cysts is associated with strongly

(c) Midplane confocal sections of a wild-type (WT) MDCK cyst and three-dimensional (3D) segmentation of its lumen. Lateral membranes are labeled with E-cadherin (E-Cad, magenta) and apical membranes with podocalyxin (PODXL, yellow). (d) Corresponding images for a TJ mutant cyst (ZO-KO), showing an instability of the apical cell surfaces that buckle into the cells. (e) Corresponding images for a different TJ mutant cyst (CLDN-KO); there is no instability of the apical surfaces. (f) Quantification of apical surface area from 3D segmentations of lumen and cyst surfaces, plotted against total cell number per cyst in WT ($n = 118$ cysts), ZO-KO ($n = 87$ cysts), and CLDN-KO ($n = 64$ cysts). The dashed straight-line fits indicate that the apical area per cell is constant in each condition. (g) Quantification of the combined volume of all cells in the cyst from 3D cyst segmentations, plotted against total cell number per cyst in WT ($n = 118$ cysts), ZO-KO ($n = 87$ cysts), and CLDN-KO ($n = 64$ cysts). The dashed straight-line fits show that the single-cell volume is constant in each condition. (h) Hydrostatic lumen pressure measurements in MDCK cysts in WT ($n = 22$ cysts), ZO-KO ($n = 10$ cysts), and CLDN-KO ($n = 14$ cysts). (i) Quantification of myosin enrichment at the apical junctions in two-dimensional monolayers of WT ($n = 125$ junctions), ZO-KO ($n = 107$ junctions), and CLDN-KO ($n = 92$ junctions) cells. *, $p < 0.01$; n.s., not significant. Images in panels (c)–(e) reproduced and data in panels (f)–(i) taken from Ref. [1].

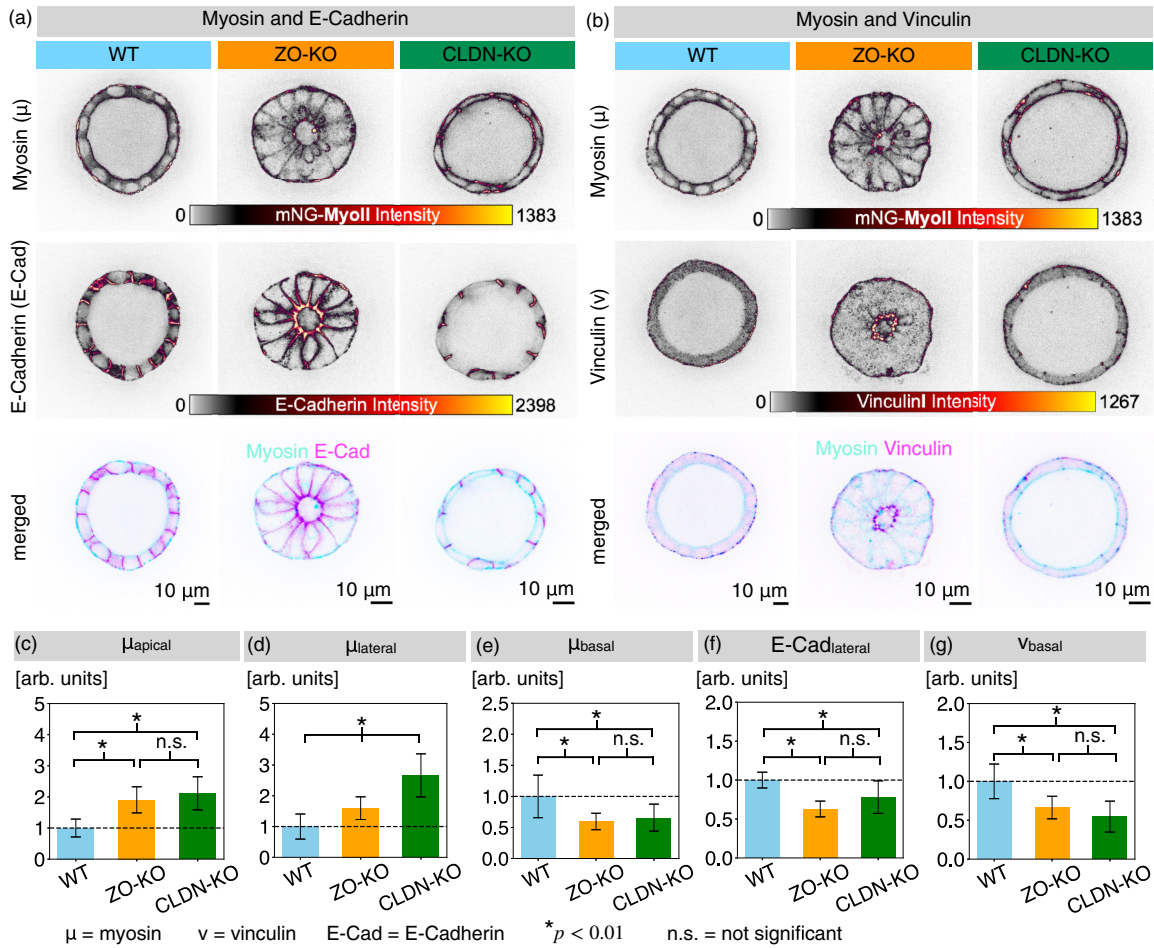


FIG. 2. Experimental quantification of contractility and adhesion across all cell surfaces for wild-type (WT), ZO-KO, and CLDN-KO MDCK-II cysts. (a) Distribution of myosin IIA-mNeonGreen (top row) and of E-cadherin (middle row) in WT, ZO-KO, and CLDN-KO cysts. (b) Distribution of myosin IIA-mNeonGreen (top row) and of vinculin (middle row) in WT, ZO-KO, and CLDN-KO cysts. Fluorescence intensity is shown in arbitrary units (arb. units), visualized using MQ-div-Magma LUT in FIJI [43]. The bottom rows in panels (a) and (b) show the merged signal. Scale bars: 10 μm. (c) Quantification of myosin IIA-mNeonGreen intensity (in arb. units, chosen so that WT = 1) at apical regions of WT ($n = 13$ cysts), ZO-KO ($n = 13$ cysts), and CLDN-KO ($n = 11$ cysts). (d) Analogous quantification for lateral regions. (e) Analogous quantification for basal regions. (f) Quantification of lateral E-cadherin intensity (arb. units) in WT ($n = 13$ cysts), ZO-KO ($n = 13$ cysts), and CLDN-KO ($n = 11$ cysts). (g) Quantification of basal vinculin intensity (arb. units) in WT ($n = 14$ cysts), ZO-KO ($n = 15$ cysts), and CLDN-KO ($n = 14$ cysts). Data represent fluorescence signal intensities measured from fixed cysts in 3D culture; data are reported for culture-matched cysts that are matched by cell number (Appendices A and B).

reduced lumen pressure, while CLDN-KO cysts retained pressure [Fig. 1(h)]. TJ loss was found to increase apical belt tension via myosin accumulation [Fig. 1(i)]. These results pointed to control of apical tension as a key factor in cyst stability [1], with TJs maintaining lumen shape by supporting hydrostatic pressure and suppressing apical contractility.

Here, we use these MDCK cysts with TJ perturbations to study the role of the lateral and basal cell surfaces for their morphological instability and hence for lumen mechanics more generally. We combine new quantitative imaging of MDCK cysts and a mean-field vertex model of the cyst that reproduces the shape instability quantitatively. We reveal that TJ perturbations are accompanied by strong modulations of the mechanics of the lateral and basal cell sides. We show that the increase of the ratio of lateral to basal contractility that we observe stabilizes cyst architecture. This suggests that

epithelial cells coordinate mechanics across the apical, lateral, and basal surfaces to maintain lumen shape under stress.

II. EXPERIMENTAL RESULTS

To investigate the role of the lateral and basal cell surfaces for lumen morphology, we analyze how TJs affect all cell surfaces of wild-type (WT), CLDN-KO, and ZO-KO MDCK-II cysts [Figs. 1(a)–1(c) and 2]. As described above, ZO-KO cysts frequently display collapsed or buckled lumina, but CLDN-KO cysts maintain a round morphology similar to WT cysts [Figs. 1(a)–1(c)]. Two mechanical properties of these cysts were quantified in previous work [1]: Lumen pressure measurements revealed that CLDN-KO cysts retained normal hydrostatic pressure, while ZO-KO cysts were depressurized [Fig. 1(h)]. Quantification of myosin enrichment at the apical junctional belt [1], based on two-dimensional (2D)

monolayers in which apical junctions are easier to resolve, showed the lowest levels in WT, higher levels in CLDN-KO, and the highest levels in ZO-KO cysts [Fig. 1(i)].

To examine how tight-junction perturbations affect the mechanical and structural properties of all cell surfaces, we need to quantify myosin contractility and adhesion at the apical, lateral, and basal surfaces of 3D cysts (Fig. 2). For this, we acquire and analyze new imaging data (Appendices A and B). In more detail, we quantify the localization of key cytoskeletal and adhesion markers: myosin IIa (actomyosin contractility), E-cadherin (lateral cell-cell adhesion), and vinculin, which is a mechanosensitive protein that is recruited to basal focal adhesions via talin [44]. We measure fluorescence intensities across apical, lateral, and basal compartments [Figs. 2(a) and 2(b)]. Our quantification of these signals distinguishes between apical and lateral surfaces and the apical belt region (Appendix B).

Using these markers as functional proxies for contractility, cell-cell adhesion, and focal adhesion, respectively, is consistent with established mechanobiological relationships: Reference [1] showed that junctional myosin IIa levels correlate with recoil velocities on ablation and hence junctional belt tension in MDCK cysts. Similarly, Ref. [45] established a quantitative link between E-cadherin intensity and cell-cell adhesion by dual-pipette aspiration measurements of cell doublets. Finally, accumulation of vinculin at adhesions increases with applied tension and with stiffer substrates, reinforcing the integrin-talin-actin linkage [46,47], so higher intensities of vinculin at focal adhesions report stronger, load-bearing focal adhesions.

Apical and lateral cortical myosin intensities are higher in CLDN-KO and ZO-KO cysts than in WT [Figs. 2(c) and 2(d)], indicating increased contractility at these interfaces in the mutants. This is similar to the previously observed apical belt tension in the mutants [Fig. 1(i)]. By contrast, basal myosin levels [Fig. 2(e)] are highest in WT and reduced in both mutant lines. Lateral E-cadherin intensity [Fig. 2(f)] is strongest in WT, consistent with intact cell-cell adhesion, and decreases in ZO-KO and CLDN-KO cysts. Similarly, basal vinculin intensity [Fig. 2(g)], marking integrin-based adhesions to the extracellular matrix, is higher in WT than in ZO-KO or CLDN-KO cysts. Vinculin is also recruited to adherens junctions via α -catenin, where it reflects mechanical force transmission [44]. Its strong apical localization in ZO-KO cysts [Fig. 2(g)], consistent with previous observations [48], thus indicates elevated apical belt tension. By contrast, WT cysts show little to no apical vinculin, all of which agrees with the previously reported apical belt tension of WT, ZO-KO, and CLDN-KO cysts [Fig. 1(i)].

These data indicate that tight-junction scaffolds not only regulate pressure and tension in the apical surface and the apical belt, but also orchestrate contractility and adhesion across lateral and basal surfaces, which are expected to contribute to the mechanical integrity of epithelial cysts.

III. MEAN-FIELD MODEL OF LUMEN MORPHOLOGY

In order to understand the effect of basal and lateral tension on cyst morphology and the observed lumen instability, we therefore build a mean-field mechanical model of a spherical

cyst that resolves the mechanical properties of all cell surfaces.

The cyst has radius R and consists of a monolayer of N cells surrounding a lumen of radius r [Fig. 3(a)]. We approximate the shapes of individual cells as truncated circular cones [Fig. 3(b)]. Their lateral sides thus have length $R - r$, and the radii of the apical and basal surfaces are x and $X > x$, respectively. The cells are incompressible, of fixed volume V . The apical surface areas of the N cells add up to the surface area of the lumen, while their basal surface areas add up to the surface area of the cyst. This means that

$$4\pi r^2 = N\pi x^2, \quad 4\pi R^2 = N\pi X^2. \quad (1)$$

Similarly, the volumes of the cells and the volume of the lumen add up to the volume of the cyst, whence

$$\frac{4\pi}{3}R^3 = \frac{4\pi}{3}r^3 + NV. \quad (2)$$

We assume that the mechanics of the cells are dominated by cortical tensions, cell-cell adhesion, and cell-matrix adhesion. We adopt a minimal description [10,33,34,36] in which these properties define surface tensions Γ_a , Γ_b , and Γ_ℓ of the apical, basal, and lateral cell surfaces, respectively, and a line tension Λ in the apical belt of the cell [Fig. 3(b)]. The apical and basal surfaces have areas πx^2 and πX^2 , respectively; the area of the lateral surface is $\pi(RX - rx)$. The apical belt has length $2\pi x$, and so the energy of a single cell is

$$e_{\text{cell}} = \Gamma_a(\pi x^2) + \Gamma_b(\pi X^2) + \Gamma_\ell[\pi(RX - rx)] + \Lambda(2\pi x). \quad (3)$$

The pressure in the lumen is P_{lumen} , that in the cell monolayer is P_{cells} , and the external pressure is P_0 . An infinitesimal deformation causes infinitesimal volume changes δV_{lumen} and δV_{cyst} of the lumen and of the cyst, respectively. Since the cell monolayer is incompressible, $\delta V_{\text{cyst}} = \delta V_{\text{lumen}}$. This deformation is therefore resisted by the infinitesimal work

$$\begin{aligned} \delta W &= (P_{\text{lumen}} - P_{\text{cells}})\delta V_{\text{lumen}} + (P_{\text{cells}} - P_0)\delta V_{\text{cyst}} \\ &= \Delta P \delta V_{\text{lumen}}, \end{aligned} \quad (4)$$

where $\Delta P = P_{\text{lumen}} - P_0$ is the hydraulic pressure difference. The lumen is pressurized by osmotic imbalances controlled, e.g., by ion pumps [9]. These define an osmotic pressure difference $\Delta \Pi > 0$ to which ΔP relaxes, over a timescale of mechanical relaxation that we expect to be much faster than the biological timescales of ion regulation and cell division that set $\Delta \Pi$ and N .

We therefore work in the thermodynamic ensemble in which $\Delta P = \Delta \Pi$ and N are fixed. In this ensemble, δW balances the mechanical work $N \delta e_{\text{cell}}$, so the cyst minimizes its enthalpy,

$$E = N e_{\text{cell}} - \Delta P V_{\text{lumen}}. \quad (5a)$$

Explicitly, on substituting Eqs. (1) and (2) into Eq. (3),

$$\begin{aligned} \frac{E}{4\pi} &= -\frac{\Delta P}{3}r^3 + \Gamma_a r^2 + \Gamma_b \left(r^3 + \frac{3NV}{4\pi} \right)^{2/3} \\ &\quad + \frac{\Gamma_\ell}{2} \sqrt{N} \left[\left(r^3 + \frac{3NV}{4\pi} \right)^{2/3} - r^2 \right] + \Lambda \sqrt{N} r. \end{aligned} \quad (5b)$$

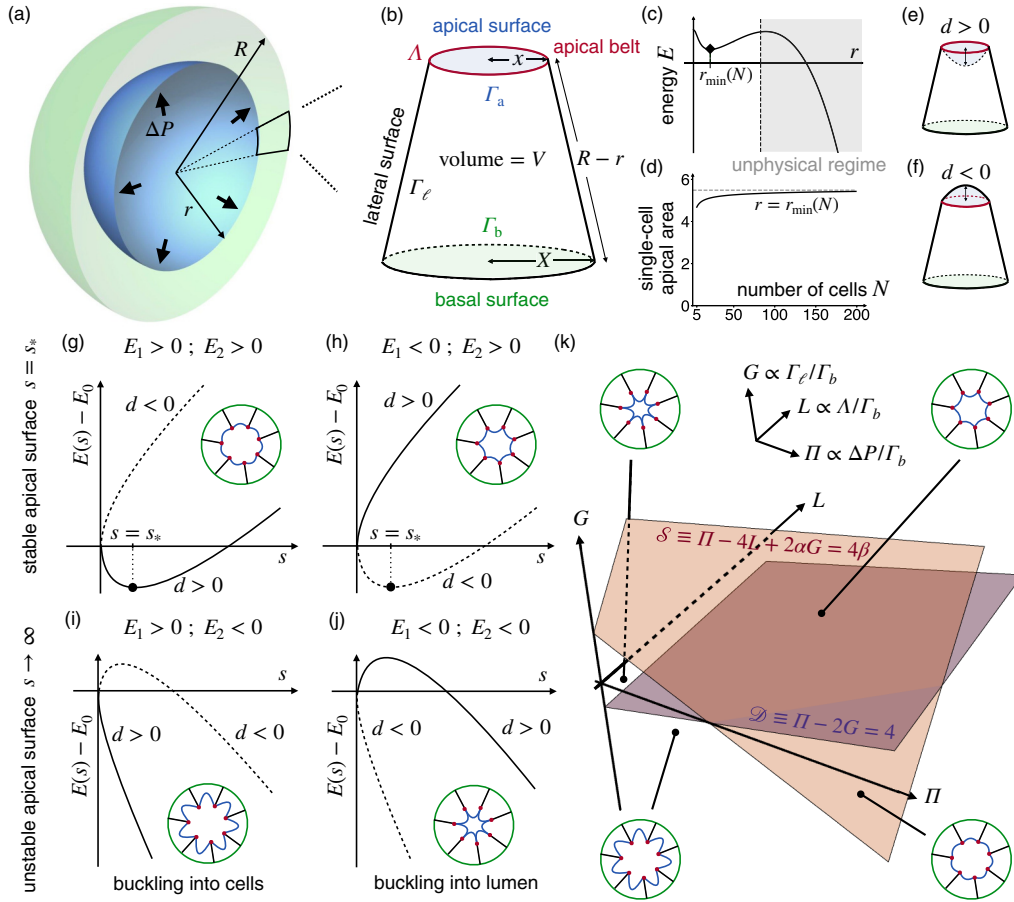


FIG. 3. Mean-field model of the instability of lumen morphology. (a) Geometry of a spherical lumen of radius r within a spherical cyst of radius R . The pressure difference between lumen and the outside of the cyst is ΔP . (b) The shape of each single cell is approximated as a truncated cone of apical radius x , basal radius X , and lateral length $R - r$. The apical, basal, and lateral sides have respective surface tensions Γ_a , Γ_b , and Γ_ℓ , and the apical belt tension is Λ . The cells are incompressible, and have volume V . (c) Generic plot of the cyst energy E against lumen radius r , displaying a local minimum at $r = r_{\min}(N)$, where N is the number of cells. The unphysical global minimum $r \rightarrow \infty$ is regularized by including nonlinear effects (shaded area). Parameter values: $\Gamma_a = \Gamma_b = 1$, $\Gamma_\ell = 20$, $\Delta P = 0.1$, $V = 1$, and $N = 200$. (d) Plot of the apical area πx^2 of a single cell at the energy minimum $r = r_{\min}(N)$ against N . For low pressure differences, ΔP , the single-cell apical area is approximately constant, and converges as $N \rightarrow \infty$ (dotted line). Parameter values as in panel (c), except $\Delta P = 0.01$. (e) Spherical cap of height $d > 0$ buckling into the cell. (f) Spherical cap of height $d < 0$ buckling into the lumen. (g) Plot of nondimensionalized energy $E(s)$ against the perturbation coordinate $s = r_* - r$, in the case $E_1 > 0$, $E_2 < 0$, where r_* and the coefficients E_1 and E_2 are defined in the text. The plot shows the branches for $d > 0$ (solid line) and $d < 0$ (dashed line). There is a single minimum at $s = s_*$, with $d > 0$: The apical surface is stable and bends slightly into the cells. (h) Analogous plot for $E_1 < 0$, $E_2 > 0$. There is again a single minimum, now with $d < 0$. The apical surface is again stable, but now bends slightly into the lumen. (i) Analogous plot for $E_1 > 0$, $E_2 < 0$. There is no local minimum ($s \rightarrow \infty$). The branch with $d > 0$ has the lowest energy, so the apical surfaces are unstable and buckle into the cells. (j) Analogous plot for $E_1 < 0$, $E_2 < 0$. Again, there is no local minimum but now the energy is lowest on the branch with $d < 0$: The apical surfaces are unstable and buckle into the lumen. (k) Phase diagram of the buckling instability. The axes are the dimensionless parameters $G \propto \Gamma_\ell/\Gamma_b$, $L \propto \Lambda/\Gamma_b$, and $\Pi \propto \Delta P/\Gamma_b$ defined in the main text. The planes $\mathcal{S} \equiv \Pi - 4L + 2\alpha G = 4\beta$ and $\mathcal{D} \equiv \Pi - 2G = 4$, where α and β are defined in the text, decide the stability and direction of bending of the apical surfaces and divide parameter space into two stable regions and two unstable regions in which the apical surfaces buckle into the lumen and into the cells, respectively (insets). See text for further explanation.

This energy has a global minimum as $r \rightarrow \infty$ [Fig. 3(c)]. This minimum is unphysical and would be regularized by nonlinear effects such as the mechanics of the cell nucleus and the confinement energy of the cytoplasm [36] that are not included in our model. Generically, the energy has a local minimum at $r = r_{\min}(N)$ that determines the lumen radius.

A. Conservation of single-cell apical area

The apical area of a single cell is conserved in spherical cysts as the number N of cells increases [Fig. 1(f)], but it is unclear *a priori* whether this is a consequence of the mechanics or whether this conservation is regulated actively.

Here, we note that this model *can* provide a mechanical explanation of this experimental observation. Indeed, the

single-cell apical area is $A = 4\pi r_{\min}(N)^2/N$ from the first of Eqs. (1), and Fig. 3(d) illustrates that there are parameter values for which A changes very little as N varies within its experimentally relevant range.

To explain this result, we note that $A = \text{const}$ requires $r_{\min}(N) \sim \sqrt{N}$. We change variables by defining $\eta = r/\sqrt{N}$. To prove that $A = \text{const}$ is now to prove that η is independent of N at the energy minimum. Equation (5b) becomes

$$\begin{aligned} \frac{E}{4\pi N} = & -\frac{\Delta P}{3}\sqrt{N}\eta^3 + \Gamma_a\eta^2 + \Gamma_b\eta^2\left(1 + \frac{3V}{4\pi\sqrt{N}\eta^3}\right)^{2/3} \\ & + \frac{\Gamma_\ell}{2}\sqrt{N}\eta^2\left[\left(1 + \frac{3V}{4\pi\sqrt{N}\eta^3}\right)^{2/3} - 1\right] + \Lambda\eta, \end{aligned} \quad (6a)$$

whence

$$\frac{E}{4\pi N} \sim -\frac{\Delta P}{3}\sqrt{N}\eta^3 + \left[(\Gamma_a + \Gamma_b)\eta^2 + \frac{\Gamma_\ell V}{4\pi\eta} + \Lambda\eta\right], \quad (6b)$$

for $N \gg 1$. If ΔP is sufficiently small, the energy minimum is determined by the terms in square brackets only. Now the single-cell volume is independent of N [Fig. 1(g)]. Hence so is η , assuming that the (relative) tension magnitudes do not change with N . Thus, single-cell apical area is indeed conserved in the limit in which ΔP is sufficiently small.

However, this limit is not appropriate for our experimental system, because the lumen deflates on laser ablation [1], which shows that ΔP matters in setting the energy minimum.

Single-cell apical area conservation can also emerge in the converse limit in which ΔP is very large. In this limit, the energy has a single minimum with $r \rightarrow \infty$. This would imply that $R - r \rightarrow 0$, which is resisted by the stiffness of the cell nuclei. Hence, in this limit, $R - r = \varrho$, where ϱ is the size of the cell nuclei. Thus,

$$\varrho = \left(r^3 + \frac{3NV}{4\pi}\right)^{1/3} - r \sim \frac{NV}{4\pi r^2} \implies r \sim \left(\frac{NV}{4\pi\varrho}\right)^{1/2}, \quad (7)$$

for $N \gg 1$. Since V is independent of N [Fig. 1(g)], this exhibits the scaling $r \sim \sqrt{N}$, so the single-cell apical area is conserved in this limit, too.

However, this collapsed limit does not describe our experimental system either: In Appendix C, we show that the cell volumes in the cysts are significantly larger than the volumes of their nuclei. This agrees with observations in flat monolayers [49].

This argument does not of course prove that single-cell apical area must be regulated actively, because it cannot exclude another mechanical origin of its conservation. We also note that neither of these mechanisms can explain the observed conservation of single-cell apical area in the nonspherical ZO-KO lumina. In what follows, we simply take single-cell apical area conservation as an experimental fact [Fig. 1(f)], similarly to Ref. [25].

B. Instability of lumen morphology

To describe the instability of the apical surfaces in the ZO-KO cysts, we therefore extend our model by replacing the flat apical surfaces of the truncated-cone shapes in

Fig. 3(b) with spherical caps of height d [Figs. 3(d) and 3(e)]: If $d > 0$, the spherical cap points into the cells, while it points into the lumen if $d < 0$. The spherical cap has volume $\pi|d|(3x^2 + d^2)/6$. Using the first of Eqs. (1), Eq. (2) is therefore replaced with

$$\frac{4\pi}{3}R^3 = \left[\frac{4\pi}{3}r^3 + \frac{N\pi}{6}d\left(\frac{12r^2}{N} + d^2\right)\right] + NV, \quad (8a)$$

in which the term in square brackets is the volume of the lumen. This rearranges to

$$R = \left(r^3 + \frac{3dr^2}{2} + \frac{Nd^3}{8} + \frac{3NV}{4\pi}\right)^{1/3}. \quad (8b)$$

The single-cell apical area is now $A = \pi(x^2 + d^2)$. As announced above, in line with experimental observations [Fig. 1(f)], we now assume that A is conserved, so that

$$d = \pm\left(a - \frac{4r^2}{N}\right)^{1/2}, \quad (9)$$

in which $a = A/\pi$ is therefore constant, and where we have used, again, the first of Eqs. (1).

1. Energy of the cyst

With these geometric results and the assumption of single-cell apical area conservation, Eq. (3) is replaced with

$$\begin{aligned} e_{\text{cell}} = & \Gamma_a A + \Gamma_b(\pi X^2) + \Gamma_\ell[\pi(RX - rx)] + \Lambda(2\pi x) \\ = & \text{const} + \frac{4\pi\Gamma_b}{N}R^2 + \frac{2\pi\Gamma_\ell}{\sqrt{N}}(R^2 - r^2) + \frac{4\pi\Lambda}{\sqrt{N}}r, \end{aligned} \quad (10)$$

using Eqs. (1). Equation (10) does not include the bending energy of the apical surfaces, because this can be ignored at the onset of the instability. Indeed, if the apical surfaces have curvature κ , then $A = 2\pi\kappa^{-1}d$, so $\kappa \sim d/A$. The bending energy of the apical surfaces now scales as $NBA\kappa^2 \sim NBd^2/A$, where $B \sim Et^3$ is their bending modulus [50], with E the elastic modulus and t the cortex thickness. Meanwhile, the energy of the apical surfaces scales as $N\Gamma_a A$, with $\Gamma_a \sim Et$ by comparing Eqs. (4) and (8) of Ref. [10]. The ratio of these energies thus scales as $t^2 d^2/A^2 \lesssim 10^{-4} \ll 1$, estimating $t \lesssim 0.5 \mu\text{m}$ [51], $d \lesssim 1 \mu\text{m}$ for stable apical surfaces or at the onset of the instability, and $A \sim 60 \mu\text{m}$ (Appendix B). This confirms that we can neglect the bending energy of the apical surfaces for our purposes, although it may not be negligible in the fully buckled shapes [1].

On discarding the constant single-cell apical area and substituting into the expression for the enthalpy of the cyst in Eq. (5a), and on nondimensionalizing lengths with $\sqrt{a}$ and energies with $4\pi\Gamma_b a$, we find

$$\begin{aligned} E = & -p\left(\frac{r^3}{3} + \frac{dr^2}{2} + \frac{Nd^3}{24}\right) + R^2 + \frac{\gamma}{2}\sqrt{N}(R^2 - r^2) \\ & + \lambda r\sqrt{N}, \end{aligned} \quad (11)$$

where we define

$$p = \frac{\sqrt{a}\Delta P}{\Gamma_b}, \quad \gamma = \frac{\Gamma_\ell}{\Gamma_b}, \quad \lambda = \frac{\Lambda}{\Gamma_b\sqrt{a}}, \quad (12)$$

and where, from Eqs. (8b) and (9),

$$d = \pm \left(1 - \frac{4r^2}{N}\right)^{1/2}, \quad (13a)$$

$$R = \left[r^3 \pm \left(1 - \frac{4r^2}{N}\right)^{1/2} \left(r^2 + \frac{N}{8}\right) + Nv \right]^{1/3}, \quad (13b)$$

in which we define $v = 3V/4\pi a^{3/2}$. Again, this model does not include cell compressibility (which the experimental data suggest to be negligible) and nonlinear cell mechanical effects, associated, for example, with the cell nuclei. It does not include the bending energy of the apical surface either. The latter may not be negligible for highly buckled shapes, but can be neglected at the onset of the instability which we seek to describe here.

2. Stability analysis

If $d = 0$, then, from Eq. (13a), $r = r_* \equiv \sqrt{N}/2$. For $d \neq 0$, Eq. (13a) implies that $r < r_*$. We analyze the stability of the apical surfaces by expanding the energy close to $d = 0$, i.e., for $s \equiv r_* - r \ll 1$. This leads to

$$E = E_0 \mp E_1 s^{1/2} + E_2 s + O(s^{3/2}), \quad (14)$$

in which

$$E_1 = \frac{N^{5/12}}{4(\sqrt{N} + 8v)^{1/3}} \{p[N(\sqrt{N} + 8v)]^{1/3} - 2\sqrt{N}\gamma - 4\}, \quad (15a)$$

$$E_2 = \frac{Np}{4} - \sqrt{N}\lambda - \frac{N^{1/6}}{(\sqrt{N} + 8v)^{1/3}} \left(\sqrt{N} + \frac{1}{\sqrt{N} + 8v} \right) + \frac{N\gamma}{2} \left\{ 1 - \frac{1}{[N(\sqrt{N} + 8v)]^{1/3}} \left(\sqrt{N} + \frac{1}{\sqrt{N} + 8v} \right) \right\}. \quad (15b)$$

An expression for E_0 can be obtained similarly, but is of no further interest, as it only sets a reference state of the energy. There are now four possible behaviors [Figs. 3(g)–3(j)], depending on the signs of E_1 and E_2 : If $E_2 > 0$, the energy has a minimum, either on the branch with $d > 0$ [$E_1 > 0$, Fig. 3(g)] or on the branch with $d < 0$ [$E_1 < 0$, Fig. 3(h)]. In both cases, the apical surface is stable; it bends slightly into the cells in the first case [Fig. 3(g)] and into the lumen in the second case [Fig. 3(h)]. If $E_2 < 0$, there is no energy minimum on either branch, and the apical surfaces are unstable. If, additionally, $E_1 > 0$, then the branch with $d > 0$ has the lowest energy [Fig. 3(i)], and the apical surfaces buckle into the cells [Fig. 3(i)], while, if $E_1 < 0$, then the lowest-energy branch is the one with $d < 0$ [Fig. 3(j)], and so the apical surfaces buckle into the lumen [Fig. 3(j)].

The minimum for $E_2 > 0$ discussed above is attained at $s = s_* \equiv (E_1/2E_2)^2$. Extending the expansion in Eq. (14) to $E = E_0 \mp E_1 s^{1/2} + E_2 s \mp E_3 s^{3/2} + O(s^2)$, asymptoticity demands $E_2 s_* \gg E_3 s_*^{3/2}$, i.e., $E_2^2 \gg E_1 E_3$. This condition holds and hence the asymptotic expansion is valid in the limit $N \gg 1$: using *Mathematica* (Wolfram, Inc.), we find

$$\frac{E_2^2}{E_1 E_3} \sim -\frac{6p^2}{(p - 2\gamma)(19\gamma - 14p)} N + O(N^{1/2}) \gg 1. \quad (16)$$

3. Phase diagram of lumen morphology

The phase diagram of the instability thus depends on the signs of E_1 and E_2 . Now

$$E_1 \geq 0 \iff \Pi - 2G - 4 \geq 0, \quad (17a)$$

$$E_2 \geq 0 \iff \Pi - 4L + 2\alpha G - 4\beta \geq 0, \quad (17b)$$

where, from Eqs. (15),

$$\begin{aligned} \Pi &= p[N(\sqrt{N} + 8v)]^{1/3}, \quad G = \sqrt{N}\gamma, \\ L &= \frac{(\sqrt{N} + 8v)^{1/3}}{N^{1/6}} \lambda, \end{aligned} \quad (18)$$

and

$$\alpha = \frac{(\sqrt{N} + 8v)^{1/3}}{N^{1/6}} \left\{ 1 - \frac{1}{[N(\sqrt{N} + 8v)]^{1/3}} \left(\sqrt{N} + \frac{1}{\sqrt{N} + 8v} \right) \right\}, \quad (19a)$$

$$\beta = 1 + \frac{1}{\sqrt{N}(\sqrt{N} + 8v)}. \quad (19b)$$

In particular, $\alpha \sim 8v/3\sqrt{N}$ and $\beta \sim 1$ for $N \gg 1$. The planes $\Pi - 2G = 4$ and $\Pi - 4L + 2\alpha G = 4\beta$ divide (Π, L, G) parameter space into four regions [Fig. 3(k)]. This calculation shows that these regions corresponding to the four possibilities discussed above [Fig. 3(k), insets]. In particular, the plane $\mathcal{D} \equiv \Pi - 2G = 4$ determines the direction of bending of the apical surfaces (into the lumen or into the cells), while the plane $\mathcal{S} \equiv \Pi - 4L + 2\alpha G = 4\beta$ determines the stability of the apical surfaces.

4. Mechanisms determining lumen morphology

Equation (17b) and Fig. 3(k) show that higher values of $\Pi - 4L + 2\alpha G$ favor stability. Thus, high Π or G are stabilizing and high L is destabilizing. Indeed, at high L (i.e., high belt tension), the apical belt contracts, so the apical surface must buckle to maintain constant area. This contraction reduces the radius of the lumen, which is resisted by high Π (i.e., high pressure), which is therefore stabilizing. Similarly, high G (i.e., high lateral tension) shrinks the lateral cell sides, so the lumen radius must increase by cell volume conservation, which stabilizes the apical surfaces.

Similarly, Eq. (17a) and Fig. 3(k) show that higher values of $\Pi - 2G$ drive bending or buckling of the apical surface into the cells. Thus, unsurprisingly, the apical surfaces bend or buckle into the cells at high Π (i.e., at high pressure). Since the lateral cell sides shrink at high G (i.e., high lateral tension), volume conservation favors bending or buckling of the apical surfaces into the lumen. More surprisingly, perhaps, the direction of bending or buckling is independent of L , i.e., of apical belt tension. This independence emerges from the full calculation, but it is tempting to explain it by noting that the direction of bending or buckling as the apical belt contracts does not affect apical area conservation at leading order.

IV. DISCUSSION

To test our theoretical model and to understand the mechanical role of the different cell surfaces for lumen

morphology, we combine our model with the experimental measurements.

A. MDCK cysts in model parameter space

We begin by quantifying the experiments in the parameter space of the stability diagram. To leading, linear order, any tension T in our model can be expressed as $T = k\mu - k'a$, where μ and a are the fluorescence intensities of contractile proteins (myosin) and adhesive proteins (E-cadherin or vinculin), respectively, and k and k' are proportionality constants that are, in general, different for different cell surfaces (Appendix B).

Since we have nondimensionalized our model parameters with the basal tension, these nondimensional parameters can be expressed in terms of the experimental measurements in Figs. 2(c)–2(g) rescaled with basal myosin intensities.

The pressure, apical belt myosin intensity, and lateral myosin intensity still change significantly between WT and the perturbations if they are rescaled with the basal myosin intensity in this way [Fig. 4(a)]. By contrast, the changes of the lateral E-cadherin and basal vinculin intensities are compensated by changes of the basal myosin intensity, so the corresponding rescaled intensities do not change significantly [Fig. 4(b)].

We also note that the ratio of apical to basal myosin intensities, which does not enter our model founded on the experimentally observed conservation of apical areas [Fig. 1(f)], does change significantly between WT and the two perturbations [Fig. 4(c)]. These changes may be related to active regulation of the single-cell apical area.

As explained in Appendix B, these rescaled measurements allow us to estimate ratios or differences of model parameters. In particular, we obtain the changes, between WT, ZO-KO, and CLDN-KO cysts, of the parameters Π , L , and G , the effect of which on lumen morphology we discuss above. As shown in Fig. 4(d), (i) the pressure parameter Π increases, i.e., stabilizes, from ZO-KO to WT to CLDN-KO; (ii) the belt tension parameter L increases, i.e., destabilizes, from WT to CLDN-KO to ZO-KO; and (iii) the lateral tension parameter G increases, i.e., stabilizes, from WT to ZO-KO to CLDN-KO.

The destabilizing response of the apical belt to both TJ perturbations was already highlighted in Ref. [1]. Strikingly, however, our analysis also reveals that the increase of the lateral tension parameter G counteracts the instability in both TJ perturbations. This may indicate that the lateral cell sides are regulated to stabilize apical surfaces and lumen morphology.

The destabilizing decrease of the pressure parameter Π in the ZO-KO is hardly surprising. Unexpectedly, however, Π increases in the CLDN-KO in spite of increased leakiness. Importantly, the increased pressure difference in the CLDN-KO compared to WT [Fig. 1(h)] is not statistically significant. The significant increase in Π [Fig. 4(a)] rather results from the statistically significant decrease in basal myosin levels [Fig. 2(e)]. This hints at regulation of the basal cell surfaces to stabilize the apical surfaces and lumen morphology.

Since the proportionality constants between tensions and intensities remain undetermined, we can only estimate ratios of the parameters Π , L , and G , but not their absolute values (Appendix B). Still, we can make our comparison between

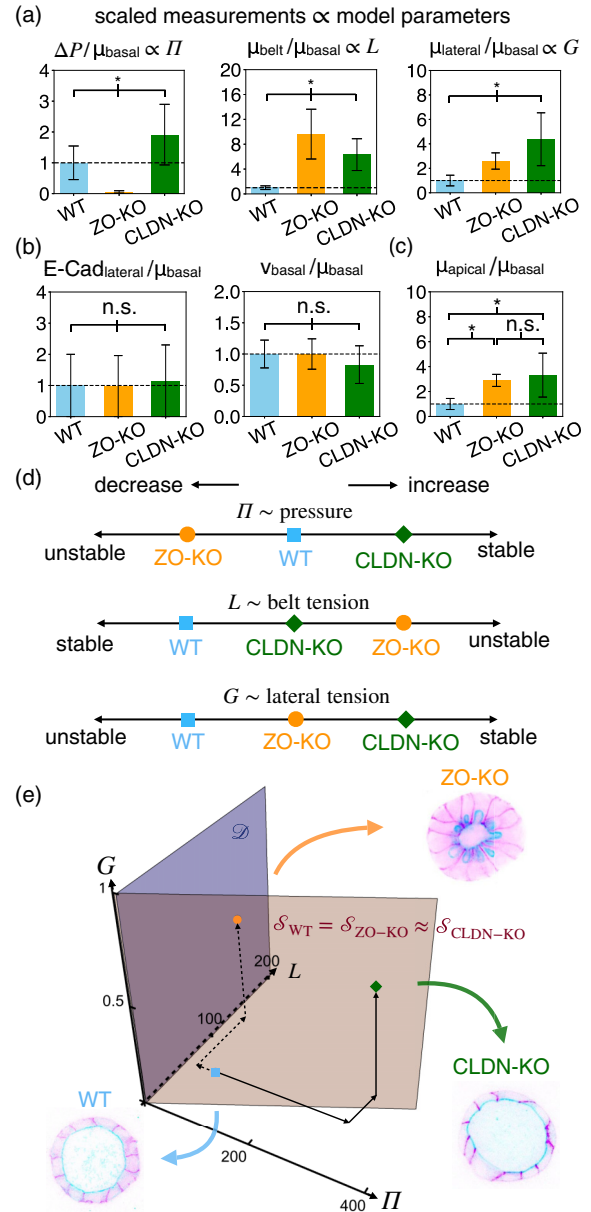


FIG. 4. Experimental quantification of the instability of lumen morphology. (a) Plots of the ratios $\Delta P/\mu_{\text{basal}}$, $\mu_{\text{belt}}/\mu_{\text{basal}}$, and $\mu_{\text{lateral}}/\mu_{\text{basal}}$ that determine the parameters Π , L , and G of the phase diagram of the instability [Fig. 3(k)], for WT, ZO-KO, and CLDN-KO cysts. All three ratios change significantly (*, $p < 0.01$). (b) Similar plots of the ratios $E\text{cad}_{\text{lateral}}/\mu_{\text{basal}}$ and $v_{\text{basal}}/\mu_{\text{basal}}$, showing that they do not change significantly (n.s.) between WT, ZO-KO, and CLDN-KO conditions. (c) Plot of the ratio $\mu_{\text{apical}}/\mu_{\text{basal}}$, which does change significantly between WT and ZO-KO or CLDN-KO, but does not enter the model because of the experimental observation that single-cell apical area is conserved [Fig. 1(f)]. Plots in panels (a)–(c) are based on the data in Figs. 1(h), 1(i), and 2(c)–2(g), and values are scaled with the WT values (so that WT = 1). (d) Effect of the parameters Π , L , and G on stability, illustrating the stabilizing or destabilizing effects of the changes of each of these parameters, between WT, ZO-KO, and CLDN-KO conditions, observed in panel (a). (e) Phase diagram, analogous to Fig. 3(k), showing a set of values of Π , L , and G consistent with the observed cyst morphologies and the experimental measurements in panel (a); parameter values are in Table IV. See text for further explanation. Arrows indicate the

model and experiment more quantitative: There exist values of the parameters Π , L , and G that are consistent with these measured ratios and also with the observed stability behavior. An instance of this is given by the set of parameter values plotted in the phase diagram in Fig. 4(e).

B. Additional model predictions

Our mean-field model makes two more testable predictions about the direction of bending or buckling of apical surfaces.

First, cysts with $\Pi = 0$ (corresponding to zero pressure difference) and $G > 0$ (corresponding to contractile rather than extensile lateral sides) lie above the plane $\mathcal{D} \equiv \Pi - 2G = 4$ in model parameter space. Hence, their apical surfaces will bend or buckle into the lumen [Fig. 5(a)]. This prediction can be tested by laser cuts opening the cyst [1] that equalize hydrostatic pressures instantaneously, $\Delta P = 0$. In the WT [Fig. 5(b)] and consistently with the predictions of the model, this ablation causes the apical surfaces that were initially bent into the cells to bend or buckle into the lumen. This inversion is observed, too, in the CLDN-KO, but not in the ZO-KO, as illustrated in Video S2 of Ref. [1]. It is likely that nonlinear effects prevent inversion of the buckled apical surfaces in the ZO-KO.

Second, for fixed values of the dimensionless pressure p , apical belt tension λ , and lateral tension γ defined in Eq. (12), $(\Pi(N), L(N), G(N))$ trace out a curve in (Π, L, G) parameter space as the number N of cells increases, given explicitly by Eqs. (18). It can start out above the plane $\mathcal{D}$ at small N , then cross below $\mathcal{D}$ at larger N [Fig. 5(c)]. This predicts that the apical surfaces of “small” cysts with few cells bend into the lumen, while those of “large” cysts with more cells bend into the cells. This agrees with our observations [Fig. 5(d)] and those of Ref. [25]. Whether changes of p , λ , and γ , assumed constant to arrive at this prediction, also contribute to this observed change of the bending direction of the apical surfaces remains an open question.

Unstable apical surfaces can, according to our model [Fig. 3(k)], buckle into the lumen or into the cells, but we only observe the latter in our ZO-KO cysts. Interestingly, the apical surfaces of flat monolayers of ZO-KO cells do curve outward [52–54]. Outward buckling would happen at even lower pressures (lower Π) or higher lateral tensions (higher G) according to Fig. 3(k). In particular, because $G > 0$, this implies that, consistently with direct measurements [Fig. 1(h)], the ZO-KO cysts are able to maintain a small, but nonzero, lumen pressure [Fig. 5(a)].

Still assuming that p , λ , and γ are constant, Eqs. (18) and (19) imply the scalings $\Pi \sim N^{1/2}$ and $\alpha G, L, \beta \sim N^0$ for $N \gg 1$. Hence, $\Pi - 4L + 2\alpha G - 4\beta > 0$ for $N \gg 1$. This inequality predicts that, even in ZO-KO cysts, the apical surfaces curve into the lumen for large enough N . We do not observe this in our experiments. One reason might be that as N increases, so does the number of leaky cell junctions in

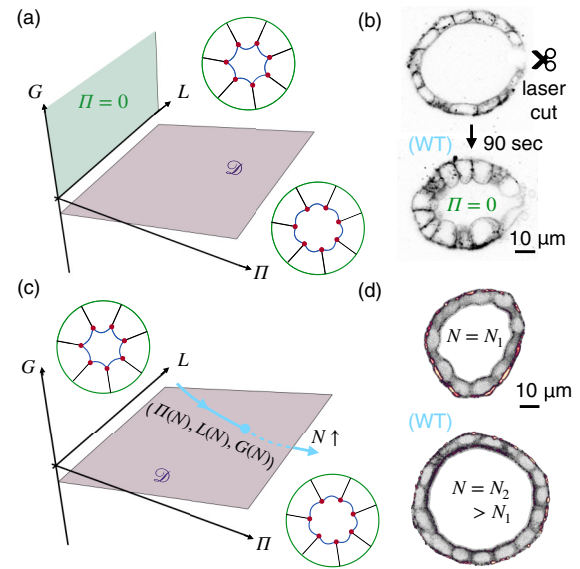


FIG. 5. Additional model predictions. (a) At zero pressure ($\Pi = 0$) and for contractile lateral sides ($G > 0$), the model predicts that the apical cell surfaces bend or buckle into the lumen (shape insets on either side of the plane $\mathcal{D}$ determining the direction of bending). (b) Laser cuts opening cysts set $\Delta P = 0$ and lead to bending of apical cell surfaces into the lumen in WT cysts. Images taken from Ref. [1]. (c) As the number N of cells increases, (Π, L, G) trace out a path in parameter space that can cross $\mathcal{D}$, so the model allows the apical surfaces of “small” cysts to bend into the lumen, while those of “large” cysts bend into the cells. (d) Instances of a WT cyst with $N = N_1$ cells and apical cell surfaces bent into the lumen and of a WT cyst with $N = N_2 > N_1$ cells and apical cell surfaces bent into the cells. Scale bars: 10 μm .

the ZO-KO cysts, which might imply decreasing p and which could in turn break the prediction [55].

V. CONCLUSIONS

In this paper, we study the cell mechanical basis for lumen morphology using MDCK cysts, relying on tight-junction mutations to perturb cyst mechanics and morphology. Our mean-field mechanical model can reproduce the buckling instability of ZO-KO cysts quantitatively. This model predicts the onset of the instability, but not the buckled shapes, which are determined by unknown nonlinear mechanisms. Our combined experimental and theoretical results reveal that lumen shape is also set by lateral and basal tensions, and not only by apical belt tension and pressure. Moreover, our results indicate the tensions in the lateral and basal cell surfaces are regulated to stabilize lumen shape. Thus, the mechanics of all cell surfaces combine to control lumen morphology. Elucidating the cell biological basis for these observations remains, however, an open problem.

Quantifying the fluorescence intensities of contractile and adhesive proteins allows us to estimate ratios of cortical tensions across WT cysts and the perturbations (Appendix B), but does not yield absolute values of these tensions because of the unknown prefactors relating intensities to tensions. Indeed, we rely on the laser ablation experiments of Ref. [56] to decide

← changes of Π , L , and G for WT $\rightarrow$ ZO-KO and WT $\rightarrow$ CLDN-KO transitions [based on panels (a) and (d)]; insets show WT, ZO-KO, and CLDN-KO cysts for illustration.

that contractility dominates adhesion in the lateral and basal cell sides. These prefactors could in principle be determined by simultaneous measurement of intensities and quantitative tension measurements using ablation or atomic force microscopy experiments [56,57]. However, these prefactors could be different in different cell surfaces [56], and the experimental challenge is compounded by the fact that existing experimental approaches often rely on protein overexpression. It is for this reason that absolute measurements that resolve contractile and adhesive contributions to the tensions in the different cell surfaces remain a formidable challenge for the future.

The origin of the observed conservation of single-cell apical area [1,25] during lumen growth [Fig. 1(f)] remains another open question. While we show that it can emerge passively in different parameter regimes of our model, our experimental system is outside these regimes. Whether the system relies on a different passive mechanism or uses active mechanisms such as balancing apical endo- and exocytosis [58–61] remains unanswered. Mechanical nonlinearities, such as the bending energy of the apical surface, could also contribute to a passive mechanism for conservation of the area of the strongly buckled apical surfaces of ZO-KO cysts.

More generally, our work focuses on the mechanical side of the interplay between mechanics and hydraulics [10] that determines lumen dynamics and shape. The dynamics of ion pumping [9] and, possibly, their active regulation and hence active regulation of ΔP as the number N of cells increases through cell division add to this interplay. The other mechanical parameters of the model could also be regulated actively with increasing N . How such effects control lumen dynamics together with these mechanical aspects remains a fascinating question for future work that promises biological and physical insights alike.

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DATA AVAILABILITY

The data that support the findings of this article are openly available [62].

APPENDIX A: EXPERIMENTAL METHODS

1. MDCK cell culture and maintenance

Madin–Darby canine kidney-II (MDCK-II) cell lines with tight-junction perturbations used for this study are as follows: the ZO1/2 knockout MDCK-II (ZO-KO) cell line was generated by Beutel *et al.* [21] using CRISPR/Cas9, and the claudin quin-KO (CLDN-KO) MDCK-II cell line was ob-

tained from the Furuse laboratory [48]. In more detail, ZO-KO denotes a double knockout of the TJ scaffold proteins ZO1 and ZO2 [1,21]; CLDN-KO were created by sequentially knocking out the five major claudins expressed in MDCK-II cells, viz., claudin-2, claudin-4, claudin-3, claudin-7, and claudin-1 [21,48]. Myosin II was endogenously labeled in these cell lines as described in Ref. [1]. Briefly, N-terminal endogenous labeling of myosin II-A with mNeon in WT, ZO-KO, and CLDN-KO cells was achieved by targeting the MYH9 exon using CRISPR/Cas. All cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 5% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were maintained at 37 °C in a humidified atmosphere with 5% CO₂. Media were refreshed every 2–3 days, and cells were passaged at 80–90% confluence.

2. 3D culture of MDCK cysts

MDCK cysts were cultured following established protocols. Briefly, MDCK monolayers were dissociated and embedded in 50% Matrigel (Corning, USA). Cells were maintained for 5 days before immunofluorescence staining.

3. Immunofluorescence staining

Cells were seeded onto glass coverslips and cultured until they reached the desired confluence. They were then fixed with 4% paraformaldehyde for 15 min at room temperature, permeabilized with 0.1% Triton X-100 for 15 min, and blocked with 5% bovine serum albumin (BSA) for 1 hour. Primary antibodies against vinculin (Sigma-Aldrich, No. V4505) and E-cadherin (Cell Signaling, No. 3195) were applied overnight at 4 °C. After washing, fluorescently labeled secondary antibodies were applied for 1 hour at room temperature. Images were acquired using a spinning disk confocal microscope. Cysts for the same condition (WT, ZO-KO, CLDN-KO) were culture matched, i.e., grown and imaged in parallel.

APPENDIX B: DATA ANALYSIS

1. Analysis of experimental images

Reference [1] quantified the intensity of junctional myosin in the apical belt in two-dimensional (2D) monolayers. In this dataset, endogenous myosin IIa tagged with mNeonGreen was imaged in MDCK-II WT and mutant monolayers. To quantify apical belt myosin, the average fluorescence intensity at apical cell-cell junctions was measured and compared between genotypes. Regions of interest (ROIs) were manually drawn along the apical junctions in z-projected confocal images, and mean intensities were extracted using FIJI [43]. We reuse these quantifications [Fig. 1(i)] in our study.

Three-dimensional quantification of myosin intensity in cysts is performed on confocal z-stacks of MDCK cysts stained for endogenous myosin IIa, E-cadherin (lateral marker), and vinculin (basal marker). To quantify average myosin intensity at specific membrane domains, hand-drawn ROIs are placed at the apical surface, lateral membrane, and basal side in individual confocal z-sections. Care is taken to exclude the apical junctional complex from both the apical

and the lateral ROIs by restricting each ROI to its corresponding subcellular domain. For example, both apical and lateral ROIs are drawn to avoid the apical belt region, ensuring separation of apical and lateral myosin signals. Average fluorescence intensities within each region are measured, and background signal is subtracted using a nearby extracellular region. In what follows, “intensity” refers to these averaged data corrected for background for signal. All image analysis is performed in FIJI [43].

Across conditions, cysts are matched by cell number. Figure 2 reports data for cysts with $N = 73 \pm 33$.

2. Estimation of model parameters

These measurements allow us to estimate or constrain the three model parameters Π , G , and L , and the two parameters α and β that define the slopes of the planes in the phase diagram in Fig. 4(e), for the WT and the two perturbations, ZO-KO and CLDN-KO.

a. Estimation of tensions

We begin by estimating the surface and line tensions from the measured intensities of myosin, E-cadherin, and vinculin. As discussed in the main text, the tensions are, at linear order, directly proportional to the intensity of contractile proteins (mainly myosin μ) and negatively proportional to the intensity a of adhesive proteins [E-cadherin (e) and vinculin (v)]. In general, a tension T can therefore be written as

$$T = k\mu - k'a, \quad (\text{B1})$$

in which the proportionality constants $k, k' > 0$ relate arbitrary intensity units to tensions, and depend both on the mechanical properties of the protein as well as the cortex. Hence, these constants are in general different at different cell surfaces [56]. Below, we find that this difference does not affect the ratios of physical parameters that we compare across mutant perturbations.

The surface tensions of the apical, basal, and lateral surfaces, and the line tension in the apical belt, are thus given by

$$\Gamma_a = k_a\mu_a, \quad \Gamma_b = k_b\mu_b - k'_b v_b, \quad (\text{B2a})$$

$$\Lambda = k_{\text{belt}}\mu_{\text{belt}}, \quad \Gamma_\ell = k_\ell\mu_\ell - k'_\ell e_\ell, \quad (\text{B2b})$$

where μ_a , μ_b , and μ_ℓ are the respective myosin intensities of the apical, basal, and lateral surfaces, μ_{belt} is the myosin intensity of the apical belt, v_b is the intensity of vinculin on the basal surface, and e_ℓ is the intensity of E-cadherin on the lateral surface.

The expression for Λ in Eqs. (B2b) does not include any adhesive term. The reason is that apical belt tension is proportional to apical belt myosin intensity [1], so that myosin contractility swamps adhesive effects despite the substantial E-cadherin accumulation in the apical belt, especially in the ZO-KO [Fig. 2(a)].

Since our final model parameters Π , L , and G are nondimensionalized with respect to the basal surface tension, we do the same with all of our measurements. Rearranging

TABLE I. Measured values of the single-cell apical area A and single-cell volume V for WT, ZO-KO, and CLDN-KO cysts. Values are obtained from the slopes of the dashed lines in Figs. 1(f) and 1(g). Data taken from Ref. [1].

Parameter	WT	ZO-KO	CLDN-KO
A (μm^2)	60 ± 33	56 ± 22	141 ± 61
V (μm^3)	679 ± 247	705 ± 273	978 ± 261

Eqs. (B2),

$$\Gamma_b = k_b\mu_b \left(1 - \frac{k'_b v_b}{k_b\mu_b}\right), \quad \Gamma_\ell = k_b\mu_b \left(\frac{k_\ell\mu_\ell}{k_b\mu_b} - \frac{k'_\ell e_\ell}{k_b\mu_b}\right). \quad (\text{B3})$$

Now we leverage the experimental observation that the normalized intensities of E-cadherin and vinculin [Fig. 4(b)] do not change significantly across the WT and the perturbations. Thus,

$$c_1 = 1 - \frac{k'_b v_b}{k_b\mu_b}, \quad c_2 = \frac{k'_\ell e_\ell}{k_b\mu_b}, \quad (\text{B4})$$

are constants, across the WT and the perturbations. Therefore, Eqs. (B3) become

$$\Gamma_b = k_b\mu_b c_1, \quad \Gamma_\ell = k_b\mu_b \left(\frac{k_\ell\mu_\ell}{k_b\mu_b} - c_2\right). \quad (\text{B5})$$

The recoil of the lateral and basal sides on laser ablation observed in Ref. [56] shows that $\Gamma_b, \Gamma_\ell > 0$ in the WT cysts. In particular, $c_1 > 0$.

b. Estimation of parameters of the phase diagram

We can now estimate (relations between) the model parameters Π , G , and L , which are defined in Eqs. (12) and (18). From Eqs. (B5), we find

$$\Pi = \frac{\sqrt{a}\Delta P}{k_b\mu_b c_1} [N(\sqrt{N} + 8v)]^{1/3}, \quad (\text{B6})$$

in which ΔP is given in Fig. 1(h), $N = 73 \pm 33$ is the number of cells in the cysts analyzed, and $a = A/\pi$, $v = 3V/(4\pi a^{3/2})$, defined above, depend additionally on the constant single-cell apical area A and volume V , given in Table I, based on the data in Figs. 1(f) and 1(g). These measurements allow estimation of Π in WT and CLDN-KO and ZO-KO cysts up to the unknown constant $k_b c_1$. Since $c_1 > 0$, we have $\Pi^{\text{WT}}, \Pi^{\text{ZO}}, \Pi^{\text{CLDN}} > 0$. The independent ratios $\Pi^{\text{WT}}/\Pi^{\text{ZO}}$ and $\Pi^{\text{ZO}}/\Pi^{\text{CLDN}}$ do not depend on this constant, however, and their values are reported in Table II. All of this implies that $0 < \Pi^{\text{ZO}} < \Pi^{\text{WT}} < \Pi^{\text{CLDN}}$ [Fig. 4(d)].

Using Eqs. (12), (18), and (B5) again, the second model parameter, G , can be written as

$$G = \frac{\sqrt{N}}{c_1} \left(\frac{k_\ell\mu_\ell}{k_b\mu_b} - c_2\right). \quad (\text{B7})$$

Here, computing the ratios across the WT or the perturbations would still leave us with an unknown offset c_2 . This offset results from the contribution of adhesion, due to E-cadherin, to the lateral tension. In Table II, we still report a quantity

TABLE II. Estimates of ratios of parameters determining cyst stability and buckling direction for the phase diagram in Fig. 4(e).

Parameter ratio	Estimated value
$\frac{\Pi^{\text{WT}}}{\Pi^{\text{ZO}}}$	19
$\frac{\Pi^{\text{ZO}}}{\Pi^{\text{CLDN}}}$	0.019
$\frac{G^{\text{WT}} - G^{\text{ZO}}}{G^{\text{ZO}} - G^{\text{CLDN}}}$	0.89
$\frac{L^{\text{WT}}}{L^{\text{ZO}}}$	0.097
$\frac{L^{\text{ZO}}}{L^{\text{CLDN}}}$	2.93

independent of both c_1 and c_2 , viz.,

$$\frac{G^{\text{WT}} - G^{\text{ZO}}}{G^{\text{ZO}} - G^{\text{CLDN}}}. \quad (\text{B8})$$

The fact that $\Gamma_b, \Gamma_\ell > 0$ in the WT cysts shows that $G^{\text{WT}} > 0$. We can further compute $c_1(G^{\text{WT}} - G^{\text{ZO}})$, $c_1(G^{\text{ZO}} - G^{\text{CLDN}}) < 0$. As noted above, $c_1 > 0$, whence $0 < G^{\text{WT}} < G^{\text{ZO}} < G^{\text{CLDN}}$ [Fig. 4(d)].

The third model parameter is L , which, from Eqs. (12), (18), and (B5), can be written as

$$L = \frac{(\sqrt{N} + 8v)^{1/3} k_{\text{belt}} \mu_{\text{belt}}}{N^{1/6} \sqrt{a k_b} \mu_b c_1}. \quad (\text{B9})$$

Since $c_1 > 0$, $L^{\text{WT}}, L^{\text{ZO}}, L^{\text{CLDN}} > 0$. We compute the ratios $L^{\text{WT}}/L^{\text{ZO}}$ and $L^{\text{ZO}}/L^{\text{CLDN}}$ between perturbations once again, which are independent of the unknown constant $k_b c_1/k_{\text{belt}}$ and which we report in Table II. These results imply that $0 < L^{\text{WT}} < L^{\text{CLDN}} < L^{\text{ZO}}$ [Fig. 4(d)].

The stability diagram also depends on the slope parameters α and β . We compute their values, reported in Table III, using the definitions in Eqs. (19). The corresponding stability planes $\mathcal{S}_{\text{WT}}$, $\mathcal{S}_{\text{ZO-KO}}$, and $\mathcal{S}_{\text{CLDN-KO}}$, plotted in Fig. 4(e), are indistinguishable.

c. Identification of points in the phase diagram

The stability diagram is determined by the values of the three model parameters for each of WT, ZO-KO, and CLDN-KO, i.e., nine model parameters in total. The experimental quantification yields five equality constraints involving these parameters, given in Table II. In addition, the observed stability and buckling direction of the apical surfaces and Eqs. (17) yield six inequality constraints between these parameters. They are

$$\Pi^{\text{WT}} - 2G^{\text{WT}} - 4 > 0, \quad (\text{B10a})$$

TABLE III. Estimates of the parameters α and β determining the slopes of the phase diagram in Fig. 4(e).

Parameter	WT	ZO-KO	CLDN-KO
α	0.41 ± 0.28	0.45 ± 0.23	0.19 ± 0.12
β	1.0049 ± 0.0028	1.0044 ± 0.0021	1.0079 ± 0.0023

TABLE IV. Parameter values satisfying the equality constraints in Table II and inequalities (B10) and (B11) used for plotting the phase diagram in Fig. 4(e).

Parameter	WT	ZO-KO	CLDN-KO
Π	127.8	6.7	359.2
G	0.25	0.48	0.75
L	14.2	146.9	49.6

$$\Pi^{\text{ZO}} - 2G^{\text{ZO}} - 4 > 0, \quad (\text{B10b})$$

$$\Pi^{\text{CLDN}} - 2G^{\text{CLDN}} - 4 > 0, \quad (\text{B10c})$$

$$\Pi^{\text{WT}} - 4L^{\text{WT}} + 2\alpha^{\text{WT}}G^{\text{WT}} - 4\beta^{\text{WT}} > 0, \quad (\text{B10d})$$

$$\Pi^{\text{ZO}} - 4L^{\text{ZO}} + 2\alpha^{\text{ZO}}G^{\text{ZO}} - 4\beta^{\text{ZO}} < 0, \quad (\text{B10e})$$

$$\Pi^{\text{CLDN}} - 4L^{\text{CLDN}} + 2\alpha^{\text{CLDN}}G^{\text{CLDN}} - 4\beta^{\text{CLDN}} > 0, \quad (\text{B10f})$$

in which the values of the slope parameters are given in Table III. Additionally, we have the inequality constraints

$$0 < \Pi^{\text{ZO}} < \Pi^{\text{WT}} < \Pi^{\text{CLDN}}, \quad (\text{B11a})$$

$$0 < G^{\text{WT}} < G^{\text{ZO}} < G^{\text{CLDN}}, \quad (\text{B11b})$$

$$0 < L^{\text{WT}} < L^{\text{CLDN}} < L^{\text{ZO}}, \quad (\text{B11c})$$

obtained above. We find parameter values that satisfy all these equality and inequality constraints using the FindInstance function of *Mathematica* and plot these values, reported in Table IV, in the phase diagram in Fig. 4(e).

APPENDIX C: 3D SEGMENTATION AND VOLUMETRIC ANALYSIS OF CELLS AND NUCLEI

1. Image segmentation of MDCK cysts

Three-dimensional segmentation of live MDCK cysts stained with Hoechst (nuclei) and CellMask Orange (membrane) is performed with Cellpose 4 (Cellpose SAM) [63] in three dimensions, using the two-channel (i.e., membrane and nuclei) image stack for whole cell masks and the nuclei stack for nuclear masks. All datasets are processed using identical parameters across cysts.

2. Quantification of nuclear and cell volumes

Nuclear and cell volumes are computed from the resulting 3D label masks using voxel dimensions provided by microscope metadata. For each cyst, voxel counts are converted into physical volumes, and distributions are pooled across four independent WT cysts. These volumetric measurements form the basis of the data presented in Fig. 6. All computations are performed on the full 3D datasets, and visualizations shown in the main text correspond to central optical sections for consistency across samples.

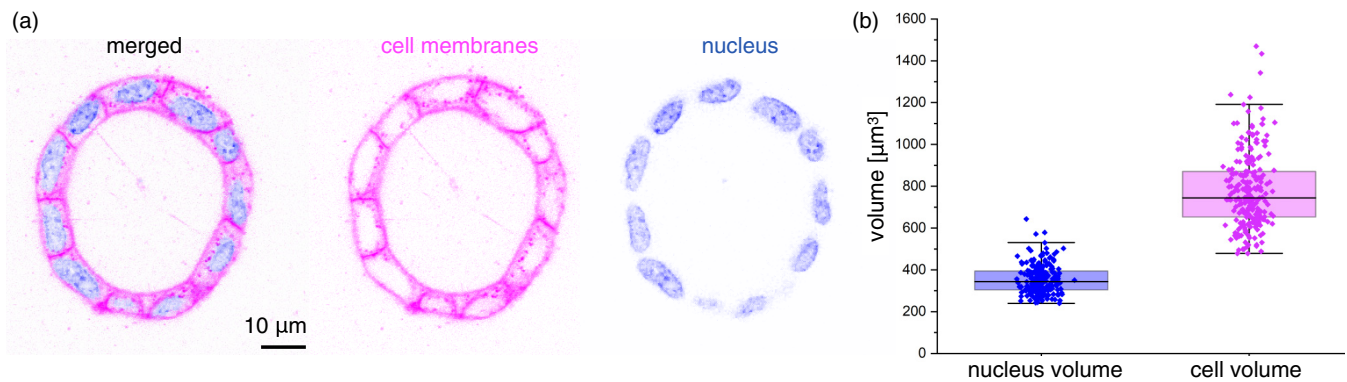


FIG. 6. 3D segmentation-based quantification of nuclear and cell volumes in WT MDCK cysts. (a) Representative optical sections of WT cysts stained with Hoechst (nuclei, blue) and CellMask Orange (cell membrane, magenta). Scale bar: 10 μm . (b) Quantification of nuclear and cellular volumes obtained from full 3D segmentations. Each point represents a single cell or nucleus pooled across four cysts. Bars show means $\pm$ standard deviations (SDs), and horizontal lines indicate medians. Nuclear volume: mean = 355 μm^3 , median = 344 μm^3 , SD = 68 μm^3 . Cell volume: mean = 780 μm^3 , median = 744 μm^3 , SD = 178 μm^3 . Volume values are derived from voxel-resolved 3D masks computed with Cellpose-SAM [63] using identical parameters for all datasets.

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