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1 **Tight junctions regulate lumen morphology via hydrostatic**
2 **pressure and junctional tension**

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21

22

23 **Summary**

24 Formation of fluid filled lumen by epithelial tissues is a fundamental process for organ
25 development. How epithelial cells regulate the hydraulic and cortical forces to control lumen
26 morphology is not completely understood. Here, we quantified the mechanical role of tight
27 junctions in lumen formation using genetically modified MDCKII cysts. We found that the
28 paracellular ion barrier formed by claudin receptors is not required for hydraulic inflation of
29 lumen. However, depletion of the zonula occludens scaffold resulted in lumen collapse and
30 folding of apical membranes. Combining quantitative measurements and perturbations of
31 hydrostatic lumen pressure and junctional tension with modelling, we were able to predict
32 lumen morphologies from the pressure-tension force balance. We found that in MDCK tissue
33 the tight junction promotes formation of spherical lumen by decreasing cortical tension via
34 inhibition of myosin. In addition, we found that the apical surface area of cells is largely
35 uncoupled from lumen volume changes, suggesting that excess apical area contributes to
36 lumen opening in the low-pressure regime. Overall, our findings provide a mechanical
37 understanding of how epithelial cells use tight junctions to modulate tissue and lumen shape.

38 **Introduction**

39 Luminal cavities are key structural components of organs, functioning as compartments for
40 fluid transport, nutrient absorption or waste secretion. Establishment of lumen during
41 development occurs via different morphogenetic mechanisms¹. Lumen that form between
42 existing cell contacts such as acini, blastocytes or canaliculi require mechanisms that enable
43 expansion of a fluid filled cavity against the resistance of the surrounding cells.

44 On the molecular level lumen formation is often coupled to the establishment of apical-basal
45 polarity. In particular, formation of an apical membrane with a distinct composition that
46 excludes cell-cell adhesion receptors and enriches ion transporters is important for lumen
47 formation². Molecular activities such as ion pumping, and acto-myosin constriction have been
48 related to the mechanical forces that are required to open a luminal cavity between cells^{3,4}.
49 Current physical models explain lumen shapes by considering osmotic, hydraulic and cortical
50 forces with a special emphasis on luminal pressure^{3,4}. Lumen inflation results from water
51 influx that arises from ion gradients and hydrostatic pressure. However luminal shape is a
52 result of the competition between hydrostatic pressure and mechanical properties of the

53 surrounding cells. In addition to this hydraulic based mechanism, it has been suggested that
54 lumen can also grow in a pressure independent way via forces that arise from formation and
55 maintenance of a constant apical membrane area per cell⁵. Despite this progress, it remains
56 challenging to link the control of mechanical forces that govern lumen growth and shape to
57 the molecular regulation that happens on the cellular and tissue level.

58 Here, we investigated the role of epithelial tight junctions in lumen growth and shape using
59 MDCKII cysts, an organotypic cell-culture model for lumenogenesis. Tight junctions control
60 the paracellular flow of ions and water across the tissue by forming a trans-epithelial
61 diffusion barrier via claudin strands⁶. Knock-out of claudins in MDCKII cells (CLDN-KO)
62 results in complete loss of TJ-strands and CLDN-KO tissue is permeable for small molecules
63 such as ions but remains impermeable to large macro-molecules^{7,8}. Interestingly, the
64 cytoplasmic TJ-scaffold of ZO proteins remains largely unaffected by claudin depletion. The
65 ZO scaffold of tight junctions has been shown to regulate the structure and activity of the
66 actomyosin cortex⁹⁻¹¹. Depletion of ZO1/ZO2 in MDCKII cells causes complete loss of all tight
67 junction proteins from the sub-apical belt including claudins^{7,12,13}. In addition, the actin
68 cytoskeleton is drastically remodeled⁹⁻¹¹. Accordingly, ZO-KO tissue is highly permeable for
69 small and large molecules^{7,14}. Since the control of both transepithelial permeability and
70 cytoskeletal tension are key mechanical parameters for lumen formation, tight junctions are
71 expected to be an important molecular regulator of lumen morphology^{7,9-11,14}. Here, we
72 studied lumen formation of genome edited MDCKII cysts mutated for key tight junction
73 proteins (WT, CLDN-KO, ZO-KO) using quantitative microscopy and mechanical
74 measurements of lumen pressure and junctional tension.

75 Surprisingly, we found that the opening of the transepithelial barrier to ions, using MDCKII
76 cells depleted of all expressed claudins (CLDN-KO,^{7,8}), does not result in a decrease of
77 hydrostatic lumen pressure. Despite high ion leakage, lumen pressure is maintained close to
78 wild-type levels and lumen expand to a round shape similar to wild-type tissue. This suggests
79 that cells can compensate para-cellular leakage by increased ion pumping. In contrast,
80 depletion of the cytoplasmic tight scaffold (ZO-KO)¹³ resulted in lumen volume collapse
81 accompanied by a loss of hydrostatic pressure and a strong increase in junctional tension.
82 Interestingly, the surface area of the collapsed lumen remained similar to WT-tissue causing
83 strong deformations of the apical membrane, suggesting that the apical membrane area is

84 largely independent of hydrostatic lumen pressure and cortical tension. Using a mechanical
85 force balance model with a constrained apical area, we predicted lumen morphologies as a
86 function of lumen pressure and junction cortical tension and found strong agreements with
87 experimental phenotypes. Finally, we used perturbation of lumen pressure and junctional
88 tension to verify the mechanical model using specific inhibitors and a pipette-mediated
89 inflation assay. Taken together, our results revealed that an important function of tight
90 junctions is to reduce the mechanical tension of cells, which enables lumen expansion at low
91 hydrostatic pressure. We envision that the regulation of the pressure-tension force balance
92 by tight junctions is an important control node that allows tuning tissue and lumen shapes
93 during morphogenesis.

94

95 **Results**

96 ***Tight junction (TJ) mutants used and their properties***

97 To investigate the function of TJs in lumen formation (Fig. 1A), we used genome edited
98 MDCKII cells lacking either the set of both cytoplasmic tight junction scaffold proteins ZO1
99 and ZO2 (ZO-KO,¹³) or the set of five major claudins expressed in MDCKs (CLDN-KO,⁷ Fig. 1B).
100 ZO1 and ZO2 as well as claudins are essential for the assembly of tight junction strands and
101 formation of a tissue permeability barrier. In addition, ZO proteins are involved in regulation
102 of cell mechanics via interactions with the acto-myosin cortex^{6,7,13,15}.

103 Previous studies of these TJ-mutant cell lines have been performed using mostly 2D tissue
104 culture. To study epithelial barrier formation in 3D, we used MDCKII cysts embedded in
105 Matrigel and determined the permeability of a small molecular tracer (Alexa488) and a lipid
106 dye to the luminal side of the tissue. In WT cysts, the lipid dye and the soluble small molecule
107 tracer were excluded from the apical membrane (Fig. 1C). In contrast, lumen of cysts grown
108 from CLDN-KO and ZO-KO cells were permeable to the tracer as well as the lipid dye (Fig. 1C).
109 We noted that CLDN-KO cysts were able to form a large, inflated lumen despite being
110 permissive to small solutes, whereas ZO-KO cysts had significantly smaller and more
111 convoluted lumen shapes (Fig. 1C). In line with previous reports^{7,13}, these results
112 demonstrate that loss of tight junctions causes increased epithelial barrier permeability and
113 that loss of ZO1/ZO2 altered lumen morphology.

114 ***Quantification of cyst and lumen morphology of TJ mutants***

115 To quantify the impact of TJs on lumen formation, we compared changes in lumen and tissue
116 morphology of WT, ZO-KO and CLDN-KO during cystogenesis in Matrigel (Fig. 2A).
117 Immunofluorescence staining (IF) was performed on cysts at different stages of cyst
118 development and a 3D image segmentation and analysis pipeline was established to acquire
119 morphometric parameters of lumen and cysts.

120 Overall, we observed that CLDN-KO and ZO-KO cells formed cysts with a single lumen and
121 polarity markers such as podocalyxin (apical) or E-Cadherin (lateral) that were correctly
122 polarized as in WT tissue (Fig. 2A). Furthermore, lumen morphology at initial cyst stages (2-
123 6 cells) was comparable between WT, CLDN-KO and ZO-KO cysts (Fig. 2A, B). Interestingly,
124 at later stages of cyst development (>20 cells), clear differences in lumen morphology
125 emerged: While both WT and CLDN-KO cysts developed large spherical lumen, ZO-KO cysts

126 failed to inflate their lumen, which resulted in intricately folded apical membranes often
127 bending into the cell bodies (see Video S1).

128 Quantification of lumen volume and apical surface area as a function of cell number revealed
129 that on average lumen volume of WT and CLDN-KO cysts increased monotonically with cell
130 number to a value of $8 \times 10^4 \mu\text{m}^3$ at 100 cells, with CLDN-KO lumen showing slightly faster
131 growth than WT (Fig. 2B). In contrast, the lumen volume of ZO-KO cysts inflated significantly
132 slower and remained below $1 \times 10^4 \mu\text{m}^3$ even at 100 cells. Interestingly, despite ZO-KO cysts
133 having a strongly reduced lumen volume, the apical surface area of lumen increased linearly
134 with cell number with the same slope as WT cysts. Thus, the apical surface area per cell was
135 constant over time and comparable between WT and ZO-KO cells. This indicates that lumen
136 volume and surface are not coupled, e.g., cells do not adapt their apical area according to
137 changes in lumen volume.

138 To quantify the deviation from a spherical shape we calculated the sphericity of the lumen
139 over time. After an initially irregular shape, sphericity of WT and CLDN-KO quickly reached
140 values close to a perfect sphere around the 10 to 20 cell stage. In contrast, sphericity of ZO-
141 KO lumen dropped as a function of cell number, showing that the lumen surface to volume
142 ratio increased as more and more cells compete for the relatively small lumen volume over
143 time. To determine how the lumen volume grows in relation to the whole cyst volume we
144 calculated the lumen occupancy, i.e., the ratio of lumen to cyst volume. Lumen occupancy
145 saturated around 50% for WT and CLDN-KO cysts. However, the latter reached saturation
146 already at the 20-cell stage while WT cyst took 60-80 cells to reach a similar lumen
147 occupancy, which indicates that lumen expansion in CLDN-KO cysts happens faster than WT
148 despite higher trans-epithelial leakage. ZO-KO lumen occupancy was significantly lower than
149 WT and saturated around 20%. The low lumen occupancy of ZO-KO cysts went along with a
150 significant thickening of the epithelial cell layer with cells having columnar shapes compared
151 to the cuboidal shapes of WT cysts (see Fig. 2A).

152 Taken together, the morphometric analysis revealed that perturbing TJs via claudin or ZO
153 depletions has differential effects on lumen morphogenesis. First, lumen inflation is strongly
154 reduced by ZO-KO, while CLDN-KO cysts inflate spherical lumen like WT cysts. Second, the
155 apical area of cells is largely constant and uncoupled from the collapse of lumen volume in
156 ZO-KO tissue. Since both mutations produce a strong increase in trans-epithelial ion

157 permeability compared to WT tissue, it is interesting that CLDN-KO cysts are able to inflate
158 large spherical lumen whereas ZO-KO depletion caused a deflated lumen. To address this
159 difference, we turned to quantification of mechanical parameters starting by designing a new
160 method to measure hydrostatic pressure of lumen.

161 ***Quantification of hydrostatic lumen pressure by lumen drainage***

162 In many epithelial tissues lumen inflation has been linked to establishment of hydraulic
163 forces¹⁶. Apical secretion of chloride ions has been suggested to drive osmotic water influx
164 into the lumen of MDCK cysts, producing a hydrostatic pressure difference that inflates the
165 lumen¹⁷. To test whether the morphological difference in lumen morphology between WT,
166 CLDN-KO and ZO-KO cysts are related to changes in hydrostatic pressure, we designed a
167 lumen drainage experiment.

168

169 To measure the hydrostatic pressure difference between the lumen and the extracellular
170 fluid, we used a two-photon laser to cut the cyst open via a short line scan through a single
171 cell (Fig. 3A). Cyst opening resulted in subsequent collapse of the lumen volume, which we
172 quantified by segmenting the whole cysts and lumen from 3D confocal stacks over time (Fig.
173 3A, Video S2). We found that the lumen volume of WT and CLDN-KO cysts decreased with an
174 exponential-like kinetic to $\approx 20\%$ after 300 s, while ZO-KO showed significantly less decrease
175 to $\approx 70\%$ of its initial volume (Fig. 3D).

176

177 We quantified the hydrostatic lumen pressure ΔP using the Hagen-Poiseuille law, which
178 describes a flow Q of a fluid with viscosity η through a channel with radius R and length L due
179 to a hydrostatic pressure difference ΔP (Fig 3B and Material and Methods). To measure the
180 fluid flow together with the exit channel size, we expressed a fluorescently tagged, apically
181 secreted sialomucin (Dendra2-CD164) in WT cysts, which provided a strong staining of the
182 luminal fluid. This allowed us to visualize the fluid outflow from the lumen after laser-cutting
183 the cyst and also to determine the average length and radius of the exit channel assumed to
184 be cylindrical (Fig. 3C, Video S3, and Supplementary Materials). The outflow was quantified
185 via segmentation of luminal volume change from 3D confocal stacks over time (Fig. 3A). The
186 flow rate Q of luminal fluid was obtained from the initial change of lumen volume after cyst
187 cutting (Fig. 3D).

188 Finally, to determine the viscosity of the luminal fluid, we measured the diffusion coefficient
189 of Dendra2-CD164 in the lumen and in PBS buffer using fluorescence correlation
190 spectroscopy. Lumen fluid viscosity was ≈ 5 times higher than water (Suppl. Fig. 1). Based on
191 these four measured quantities (flow, radius, length and viscosity), we calculated the average
192 hydrostatic lumen pressure to be $\Delta P_{WT} = 65 \pm 15$ Pa, $\Delta P_{CLDN-KO} = 84 \pm 21$ Pa and $\Delta P_{ZO-KO} = 0.5 \pm$
193 0.5 Pa (Fig. 3E).

194 Taken together, the hydrostatic pressure measurements revealed that WT cysts build up
195 hydrostatic pressure of ≈ 65 Pa to inflate a spherical lumen, which is close to previously
196 reported values¹⁸. In addition, despite CLDN-KO cyst being permeable for small molecules,
197 they build up even higher lumen hydrostatic pressure than WT cysts ≈ 80 Pa. In contrast, ZO-
198 KO cyst do not build up a significant hydrostatic pressure difference, which may explain why
199 their lumen are collapsed. To understand how the changes in hydrostatic pressure are related
200 to the morphological changes of the lumen, we next determined the corresponding changes
201 of junctional tension of the TJ mutants.

202 ***Tight junction mutations increase apical-junctional tension via myosin activation***

203 To determine the influence of TJ-mutations on mechanical tissue tension, we quantified
204 endogenous myosin-IIa distributions and performed laser ablation experiments. To visualize
205 myosin distribution, we tagged endogenous Myosin-IIa with mNeonGreen in WT, CLDN-KO
206 and ZO-KO cells using CRISPR/Cas genome editing. We observed that in WT monolayers Myo-
207 IIa was mostly cytoplasmic, while in CLDN-KO and ZO-KO Myo-IIa was clearly enriched at the
208 apical junctional complex (Fig. 4A). Quantification of the enrichment of Myo-IIa at the
209 junction with respect to the cytoplasm, i.e., the junction to cytoplasm ratio (JCR), showed that
210 myosin in WT tissue was only slightly enriched at the junction $JCR \approx 1$, while in CLDN-KO
211 monolayers myosin was strongly enriched at the TJ with $JCR \approx 5$. ZO-KO showed an even
212 stronger enrichment with $JCR \approx 7$ (Fig. 4B).

213 Increased junctional myosin levels suggest that cortical tension of the tight junction mutants
214 is increased. In order to estimate junctional tension, we laser dissected apical junctions and
215 analyzed initial recoil velocities (Fig. 4C, D, E). WT tissue showed little recoil with a velocity
216 of $v = 1.1 \mu\text{m/s}$, indicating that the junctions are not under significant tension and the tissue
217 is in a relaxed state. In contrast, we found that CLDN-KO recoil was 3x faster ($v = 3.6 \mu\text{m/s}$)
218 and ZO-KO even 10x faster ($v = 11 \mu\text{m/s}$) compared to WT. Relating the junctional myosin

219 levels to the junctional recoil velocities showed a strong positive correlation (Fig. 4F), which
220 suggests that depletion of CLDN and even more so of ZO proteins increases junctional tension
221 via local myosin accumulation.

222 Taken together, myosin localization and junctional recoil quantification confirmed that tight
223 junctions play an important role in regulating junctional tension in MDCK tissue. ZO proteins
224 and to a lesser extent claudins affect junctional myosin levels and as a consequence lower
225 mechanical tension at the apical junctional complex, effectively making the tissue softer.
226 These results are in line with previous observations that loss of ZO1/ZO2 and loss of claudins
227 are linked to myosin accumulation at the apical junction and increased tissue tension⁹⁻¹¹.
228 Increased junctional tension has also been linked to apical constriction of cells, which can
229 induce folding of tissues¹⁹. In the context of lumen formation, apical constriction of cells is
230 expected to counteract the hydrostatic pressure of the lumen (Fig. 1A). Therefore, the
231 combined loss of hydrostatic pressure together with the increased apical-junctional tension
232 in ZO-KO cyst may explain the collapsed lumen volume and the folding of apical membranes.

233 The combined pressure and tension results suggest that tight junctions promote lumen
234 inflation by two independent mechanisms. First, the junctional permeability barrier
235 promotes the formation of hydrostatic pressure via maintaining ion gradients which drive
236 osmotic water influx. Second, tight junctions inhibit myosin contractility, which reduces
237 apical tension and allows lumen inflation at lower hydrostatic pressure.

238 ***Minimal force balance model recapitulates and predicts lumen phenotypes***

239 To better understand how the changes in hydrostatic pressure and junction tension relate to
240 3D shape changes of lumen, we used an analytical model to describe the force balance of the
241 luminal cavity (Fig. 5A, Material and Methods and Suppl. Material). We assumed that the
242 volume and shape of the lumen are determined by the minimisation of free energy of the
243 lumen surface that includes contributions from hydrostatic pressure and the junctional
244 tension. In addition, we included the finding that the apical surface per cell does not adapt to
245 changes in lumen volume in our experiments (Fig. 2). Therefore, we introduced a constant
246 apical area per cell (experimental value). This surface also contributes to the force balance
247 via a bending modulus (Suppl. Material). Thus, deforming the apical surface is energetically
248 costly.

249

250 Varying lumen pressure and junctional tensions in the regime of experimentally determined
251 values resulted in two lumen states (Fig. 5B). First, when junctional tension dominates the
252 lumen volume is collapsed. Second, when hydrostatic pressure dominates the lumen volume
253 is inflated to a spherical shape. Introduction of constant apical surface area with a bending
254 modulus prevented full collapse of the lumen volume in the tension dominated regime (Suppl.
255 Fig. 2A, B). Thus, the bending energy of the constant apical surface area promotes lumen
256 opening especially in the regime when hydrostatic pressure is not sufficient to inflate the
257 lumen (Suppl. Fig. 2C).

258

259 To compare the morphologies of the lumen predicted by our model with the experimental
260 observed shapes, we calculated the energy minimized shape of a lumen surrounded by 42
261 cells (truncated icosahedron) for 12 positions in the pressure-tension range of our
262 experiments with a vertex model using Surface-Evolver²⁰ (Fig. 5B). The calculated shapes
263 revealed a striking qualitative agreement with the experimental observations (Fig. 5C). In the
264 tension dominated collapsed state the excess apical membrane bends away from the lumen
265 forming the characteristic lobes that are observed in the ZO-KO cysts (Fig. 5C states:1,2,4,7).
266 In the fully inflated state, the apical surface is a smooth sphere resembling WT cysts (states:
267 8,9,6). Close to the transition line apical membranes slightly bend outwards (states: 5+3),
268 which resembles CLDN-KO cyst morphologies.

269

270 In summary, force balance model is sufficient to recapitulate lumen morphologies of WT and
271 TJ-mutants of MDCK cysts in the experimentally determined parameter regime. Thus, the
272 model supports the notion that TJs control lumen morphology by tuning the mechanical force
273 balance of tension vs pressure and bending of the apical surface. Mapping the pressure-
274 tension parameter space, showed how TJ in WT tissue promote efficient inflation of spherical
275 lumen at relatively low hydrostatic pressure by decreasing apical-junctional tension. The
276 model also highlights the mechanical role of the apical membrane, which due to its conserved
277 area and resistance to bending prevents full collapse of the lumen at high tensions and
278 effectively facilitates lumen opening in the low tension vs pressure regime, which is in line
279 with findings from the Dunn lab⁵.

280

281 To further test the mechanical model, we performed perturbations experiments focusing on
282 the rescue of the collapsed lumen phenotype of ZO-KO cysts. To facilitate lumen opening we

283 reduced junctional tension via myosin inhibition and we increased luminal pressure via
284 micropipette inflation.

285

286 ***Increasing hydrostatic lumen pressure partially reverses lumen collapse in ZO-KO cysts***

287 To increase luminal pressure of MDCK cysts, we developed a micropipette inflation approach.
288 For this purpose we grew cysts in shallow PDMS micro-cavities^{21 22}, which allowed us to apply
289 a fluid jet directly against the surface of cysts without detaching the cyst from the surface and
290 the Matrigel. This setup enabled us to efficiently quantify morphological changes upon
291 pipette inflation using confocal microscopy with high throughput (Fig. 6A). We found that the
292 fluid jet applied to the cyst surface was able to open tight junctions and press additional fluid
293 into the lumen increasing the hydrostatic lumen pressure. This was confirmed by seeing
294 fluorescent dextran entering the lumen from the pipette solution (Fig. 6B).

295

296 Fluid addition to the lumen resulted in significant expansion of WT and ZO-KO cysts. In both
297 cases the lumen volume on average increased 7-fold (Fig. 6C). However, since ZO-KO cysts
298 started with much smaller lumen, the absolute volume after inflation remained smaller
299 compared to inflated WT cysts. To quantify how the cell monolayer responded to the lumen
300 volume increase we quantified the change in cell height relative to the initial values (Fig. 6D).
301 We found that the average cell height decreased 0.7 ± 0.1 -fold in WT cyst and significantly
302 less in ZO-KO with 0.9 ± 0.2 -fold. Overall, ZO-KO cells started significantly more elongated
303 compared to WT and even after lumen inflation did not reach the cell height of pre-inflation
304 WT cysts. Relating the cell height change to the change of lumen occupancy after lumen
305 inflation (Fig. 6D), showed that WT and ZO-KO cysts follow the same trend, e.g., increase in
306 lumen occupancy and decrease in cell height. However, ZO-KO cysts could not be inflated to
307 the same degree as WT cysts. While lumen occupancy in WT cysts increased from 0.2 ± 0.1 to
308 0.5 ± 0.1 , the cell height decreased from $10 \pm 2 \mu\text{m}$ to $8 \pm 2 \mu\text{m}$. In contrast, lumen occupancy
309 of ZO-KO cysts only increased from 0.08 ± 0.04 to 0.22 ± 0.1 , while the cell height remained
310 relatively high with a change from $22 \pm 2 \mu\text{m}$ to $19 \pm 3 \mu\text{m}$.

311

312 Taken together, increasing hydrostatic lumen pressure in WT and ZO-KO cysts via pipette
313 inflation caused lumen expansion in both cases. However, cells in ZO-KO cysts remained
314 significantly more elongated and lumen volume remained smaller compared to WT cysts.
315 Thus, ZO-KO cells resisted lumen inflation and cell thinning in response to increased

316 hydrostatic pressure significantly more than WT tissue, which is in line with the increased
317 junction tension of ZO-KO tissue.

318

319 ***Reduction of junctional tension increases lumen volume in ZO-KO and CLDN-KO cysts***

320 To reduce junctional tension we inhibited Rho-kinase (ROCK), an upstream myosin IIa
321 activator, via the compound Y-27632^{23,24}. Inhibition of ROCK via 200 μ M Y-27632 resulted in
322 efficient reduction of myosin accumulation at apical junctions after 120 min to almost WT-
323 levels in ZO-KO and CLDN-KO monolayers (Suppl. Fig 3A,B). In line with the 2D monolayer
324 experiments, Myosin-IIa was mostly enriched at the basal membrane in WT cysts, basal and
325 apical-junctional in CLDN-KO cysts and basal, lateral and apical-junctional in ZO-KO cysts. Y-
326 27632 treatment on 3D cysts resulted in qualitatively similar behavior as in 2D monolayers,
327 e.g., myosin accumulation at the cell membrane including the apical junction complex was
328 decreased (Fig. 7A, B). Since junctional myosin levels strongly correlate with junctional
329 tension (Fig. 4F), Y-27632 treatment is expected to decrease junctional tension.

330

331 In WT-cysts, addition of Y-27632 had no significant influence on cyst and lumen morphology
332 (Fig. 7C). In contrast, in ZO-KO cysts we observed a significant increase of lumen volume
333 starting 20 min after Y-27632 addition and reaching a plateau after 40 min. Overall, the lumen
334 volume of ZO-KO cysts increased 1.2-fold after inhibition of myosin (Fig. 7C). In CLDN-KO
335 cysts, inhibition of myosin also resulted in an increase of lumen volume, however with a
336 slower kinetic and to a lesser extent compared to ZO-KO cysts.

337 In summary, ROCK inhibition resulted in release of junctional myosin-IIa accumulation in TJ
338 mutant tissues to almost WT levels. The release of myosin caused strong lumen expansion in
339 ZO-KO and medium expansion in CLDN-KO cysts, while WT cysts were not affected. The
340 perturbation results support the force balance model and show that releasing apical-
341 junctional tension is an important function of TJs to promote lumen inflation by hydrostatic
342 pressure.

343

344

345

346 Discussion

347 In this work, we addressed the question how tight junctions contribute to formation of lumen
348 in an organotypic 3D tissue culture model system (MDCKII cysts). By measuring and
349 modeling the force balance of hydrostatic lumen pressure and junctional tension of TJ
350 mutants, we found that TJs have a strong impact on the amount of apical-junctional tension
351 and to a lesser degree on the amount of hydrostatic pressure established by solute pumping.
352 In addition, our measurements revealed that cells control the area of the apical membrane
353 independently from hydrostatic pressure, junctional tension and lumen volume.

354 *Role of TJs in regulating tissue permeability and hydrostatic lumen pressure*

355 Comparing hydrostatic lumen pressure of wild-type and TJ-mutant cysts revealed that the
356 paracellular barrier for ions formed by Claudins strands is not required to build up
357 hydrostatic lumen pressure and inflate spherical lumen (CLDN-KO, Fig. 2, 3). Previous trans-
358 epithelial permeability measurements of small molecule tracers showed that permeability
359 for molecules with a size of ≈ 1 nm is ≈ 9 -fold increased for CLDN-KO tissue and ≈ 12 -fold
360 increased in ZO-KO tissue compared to WT⁷. In addition, ZO-KO tissue is also permeable for
361 larger macro-molecules of up to ≈ 15 nm⁷. The significantly increased trans-epithelial leak
362 for solutes of CLDN-KO and the ZO-KO tissue should, in principle, reduce the osmotic pressure
363 difference between the lumen because the para-cellular leak channels equilibrate
364 concentration gradients established by active solute pumping into the lumen¹⁶. Indeed,
365 previous work focusing on pressure independent mechanisms of lumen formation assumed
366 that, due to the increased solute permeability, CLDN-KO cysts have reduced hydrostatic
367 pressure⁵. However, we found that, despite the strongly increased permeability, CLDN-KO
368 cysts establish a hydrostatic lumen pressure comparable to WT tissue and are able to inflate
369 spherical lumen (Fig. 2, 3). This suggests that cells can compensate increased para-cellular
370 solute permeability. One mechanism could be that cells increase active ion pumping into the
371 lumen to compensate the increased para-cellular leak. On the other hand, our results suggest
372 that ZO-KO cysts are not able to establish significant hydrostatic pressure (Fig. 3). This could
373 imply that cells are not able to compensate the further increased solute permeability in the
374 ZO-KO which would reveal an upper limit for the pumping capacity. However, since the ZO-
375 KO cysts also have strongly increased junctional tension and very convoluted lumen shapes
376 it is possible that our lumen volume analysis was not able to resolve small changes in volume
377 after drainage in particular of the folded lobes of the lumen. Thus, it remains possible that

378 also ZO-KO cysts can maintain an osmotic pressure in the lumen. Along these lines, it has been
379 suggested that MDCKII cysts grow in a regime in which the osmotic pressure $\Delta\Pi$ is
380 significantly larger than the hydrostatic pressure ΔP ($\Delta\Pi \gg \Delta P$)¹⁶. Thus, a significant
381 reduction of osmotic pressure due to increased paracellular leaks may still lead to conditions
382 in which lumen can expand by osmotic water influx ($\Delta\Pi > \Delta P$) albeit at slower speeds.
383 Further development of methods that can directly measure local osmotic pressure will be
384 required to understand the feedback between paracellular permeability, solute pumping and
385 hydrostatic pressure.

386 Overall, our results suggest that epithelial cells can maintain sufficient osmotic lumen
387 pressure to drive water influx and promote lumen expansion even when tight junctions open
388 the para-cellular barriers. This points to a feedback mechanism that regulates solute pumping
389 activity to maintain a certain luminal pressure. Recent work suggests that MDCK cells can
390 sense the apical-basal pressure difference and modulate active fluid pumping via localization
391 of the Na/K ATPase¹⁸.

392 ***Role of TJs in regulating apical-junctional tension during lumen formation***

393 The TJ scaffold proteins ZO1 and ZO2 have been shown to be important regulators of the
394 apical actin cortex⁹⁻¹¹. In addition, ZO1 is known to inhibit myosin activity via inhibition of
395 the RhoA exchange factor GEF-H1²⁵, and depletion of ZO1 leads to increased junctional
396 tension^{26,27}. Our quantification of endogenous junctional myosin-IIa levels in combination
397 with junction tension measurements confirm the key role of ZO proteins in regulating apical-
398 junctional contractility via myosin inhibition (Fig. 4,7, Suppl. Fig. 3). In addition, we found
399 that claudins also play a critical role in tension regulation. Depletion of claudins causes an
400 increase of myosin and junctional tension, albeit to lesser extent than ZO1 depletion. Since
401 junctional ZO1 levels are not affected by claudin depletion, myosin regulation via claudins
402 likely involves a different pathway. Along these lines, recent work on transient tight junction
403 breaching via mechanical stretching has revealed that breaks in the tight junction network
404 cause local calcium bursts, which activate RhoA and lead to a contractile response via
405 myosin^{28,29}. This response leads to efficient healing of TJ breaks and ensures active
406 maintenance of the TJ barrier in a mechanically challenging tissue environment. While the
407 mechano-sensor that responds to TJ stretching has not been identified, we speculate that the

408 same mechanical feedback may also be activated in ZO-KO and CLDN-KO tissue, which could
409 explain the increase in junctional tension of CLDN-KO cells despite normal ZO1 levels.

410 Taken together, our results are in line with previous reports that TJ regulate junctional
411 tension via control of acto-myosin activity⁹⁻¹¹. Importantly, we found that a key role of TJ for
412 lumen formation is to decrease apical-junctional tension which promotes spherical lumen
413 inflation at lower hydrostatic pressures.

414 ***Tuning of lumen shapes by TJs and the role of the apical membrane cortex***

415 In line with previous observations⁵, we found that the apical surface area per cell is mostly
416 constant during lumen growth (Figure 2). Even when the lumen volume is collapsed in ZO-
417 KO cyst the apical area remains close to that of WT cells. This suggests that cells control the
418 size of the apical membrane independently of hydrostatic pressure and tension probably by
419 tuning the balance of apical endo- and exocytosis^{30,31} and/or by independently regulating the
420 apical actin cortex. This may also suggest that the size of the apical area is genetically encoded
421 in a cell type specific manner rather than being a parameter that can freely adapt to
422 morphological and mechanical changes of cells and tissues. Therefore, we included an apical
423 area constraint in our mechanical force-balance model of lumen morphology (Fig. 5). Our
424 model is able to capture the key morphological changes of CLDN-KO and ZO-KO compared to
425 WT cysts and therefore provides a useful tool to understand morphological transitions during
426 lumen growth and remodeling. In future work, it will be important to (i) further consolidate
427 the role of the mechanical properties of the apical membrane and its area regulation, (ii)
428 include the contribution of lateral and basal surface mechanics, and (iii) include the role of
429 active pumping and osmotic pressure into the modelling framework to explore the dynamics
430 of lumen formation^{3,16,32}.

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442

443 **Author Contributions**

444 A.H. conceived the project. D.R. and T.G. conceived cyst drainage assay for the estimate of
445 luminal pressure, A.H. developed the experimental implementation and M.M., R.M. performed
446 measurements. D.R. conceived the pipette assay with microfabricated substrate and the
447 lumen inflation with T.G. and L.L. who performed the experiments and measurements. C.H.W.
448 performed morphological characterization of TJ-mutants. M.M. performed junctional tension
449 measurements and myosin perturbation experiments. T.H., M.S., D.R. and T.G. conceived the
450 mechanical model, T.G. performed the associated numerical simulations. M.J.B. and M.F.S.
451 implemented the model in Surface Evolver. C.M-L. did the cell-culture work and created
452 stable cell lines. A.H. and M.M. wrote the manuscript with input from all authors.

453 **Star Methods**

454 ***Cell culture maintenance and cell lines***

455 MDCK-II cells were cultured in MEM (Sigma, USA), 5% FBS (Sigma, USA) with 1 mM of sodium
456 pyruvate (Thermo Fisher Scientific, USA) and 1% of 100x MEM Non- Essential Amino Acids
457 (Thermo Fisher Scientific, USA) at 37°C with 5% CO₂. ZO1/2 knockdown MDCKII as well as
458 MDCKII WT-ZO1-mNeonGreen were created by Beutel et al. using Crispr/Cas¹³. Claudin quin-
459 KO MDCKII cells were obtained from the Furuse lab⁷. For N-terminal endogenous labelling of
460 myosin with mNeon in WT, ZO-KO, CLDN-KO cells, the myosin-2-A exon was targeted using
461 Crispr/Cas.

462

463 ***Three-dimensional culture of MDCK cysts and drug treatment***

464 MDCK cysts were cultured as previously described³³, in brief: MDCK monolayers were
465 dissociated and either embedded in Matrigel (Corning, USA) for the pseudo-time-laps assay
466 or seeded on 0.5 mg/ml laminin (Roche Diagnostics, Switzerland) coated 35-mm glass-
467 bottom dish (MatTek Corp., USA) containing 5% of Matrigel for the other assays referred to
468 in this manuscript. Cells were then cultured for 4-5 days and subsequently imaged. For

469 perturbation of actomyosin contractility by ROCK inhibition, Y-27632 dissolved in DMSO was
470 applied to the culture media with 200 μ M working concentration.

471

472 ***Trans-epithelial permeability assay***

473 3D adherent cysts were cultured as already described for 4 to 5 days in 37 °C 5% CO₂ until
474 reaching 30 to 40 μ m in diameter. To measure permeability of small solute (Dextran-
475 Alexa488) and a lipid tracer (DPPE-TMR), cyst medium was supplemented with 1 μ M DPPE-
476 TMR, which was complexed with fat-free BSA in 1:1 molar ratio, for 8min at 37°C. After
477 washing, the medium was supplemented with 10 μ M Dextran-Alexa488. After 15min
478 incubation, cysts were imaged on a confocal microscope to evaluate distribution of the lipid
479 tracer and Dextran-Alexa488.

480

481 ***Lumen sphericity measurement***

482 To determine how close the lumen shape resembles a sphere, the sphericity (Ψ) was
483 calculated by comparing the surface area (A) and to the surface area of a perfect sphere with
484 the measure lumen volume (V): $\psi = \frac{\pi^{1/3}(6V)^{2/3}}{A}$. A sphericity of 1 refers to a perfect
485 sphere while highly irregular lumen approaches a sphericity of 0.

486

487 ***Drainage of lumen and quantification of hydrostatic lumen pressure***

488 WT, CLDN-KO and ZO-KO cells stably expressing HaloTag-CAAX were cultured with the
489 laminin-based method for 5 days to form cysts. Prior to the experiment, cysts were incubated
490 in culture media with 200 nM of Janelia Fluor® 646 HaloTag® ligand overnight to visualize
491 the membranes.

492 The cysts were cut open by ablating one cell using a Zeiss LSM 780 NLO system (Zen Black
493 software version 11.00) with a 2-photon laser (Titanium/Sapphire) at 800 nm (Coherent,
494 Santa Clara, USA) with a power of 3.2 W at the laser head and 100% laser output set in Zen
495 Black. A line scan with a width of 3 μ m and 5-line repetitions across the epithelium at the
496 middle plane of a cyst was typically sufficient to open the lumen to the outside. Lumen
497 collapse was imaged by taking 3D stacks of the cysts with a time resolution of 15 seconds.

498 To characterize the experiments quantitatively, we segmented the lumen in 3D over time and
499 plotted the change of lumen volume as a function of time. These measurements allow us to

500 calculate the initial flow as a key readout to evaluate hydrostatic luminal pressure. We also
501 measured the channels dimensions using the passage of the fluorescent mucin (see Fig. 3C.).
502 To obtain the hydrostatic lumen pressure before the cut, we modelled the flow going out of
503 the cyst as a Hagen-Poiseuille flow of a liquid of viscosity η at a flow rate Q in a channel of
504 radius R and length L :

$$505 \quad \Delta P = P_{in} - P_{out} = \frac{8\eta QL}{\pi R^4}$$

506 The viscosity of the lumen fluid η , was determined by measuring the diffusion of Dendra-
507 mucin in the lumen using Fluorescence Correlation Spectroscopy experiments

508

509 ***Measurement of lumen fluid viscosity***

510 Viscosity of the luminal fluid was measured using fluorescence correlation spectroscopy
511 (FCS) of dendra2-tagged soluble sialomucin (Dendra2-CD164) on an Abberior STED
512 microscope (Abberior Instruments, Göttingen, Germany). The concentration of dendra2-
513 mucin in the luminal fluid was typically too high for FCS. Therefore, we photo-converted a
514 small fraction of dendra2 into the red-emitting form using a short scan of 405 nm light. FCS
515 measurements were then performed using 561 nm excitation (20 μ W back focal plane) using
516 a 60x water objective to measure the diffusion of the photo-converted dendra2-mucin in the
517 lumen. FCS curves were fitted using a 3D diffusion model including triplet blinking as
518 described previously³⁴. To determine the viscosity in the lumen the diffusion times of
519 dendra2-mucin in the lumen were compared to diffusion times of the same molecule in
520 standard 2D cell culture medium with μ (DMEM @ 37°C) = 0.9 mPa*s (MDCK monolayer on
521 glass) (Figure S1).

522

523 ***Three-dimensional segmentation of MDCK cysts***

524 Fluorescent Z-stacks of MDCK cysts were acquired by confocal microscopy. The images were
525 processed with median filter to better preserve the structures. All reconstructions of three-
526 dimensional images were performed with LimeSeg³⁵ plugin from Fiji185. The segmentation
527 on the 4-D recordings of lumen formation was performed using a groovy script that
528 automatically operated LimeSeg to multiple time points where segmented dots of present
529 time point were applied to next time point as ROI for segmentation. The geometrical
530 information measured with segmented mesh was automatically saved. The number of cells
531 of a cyst was quantified with TrackMate of Fiji on the nuclei signals.

532

533 ***Tension measurement***

534 Laser ablation was performed on Zeiss LSM 780 NLO system driven by Zen Black software
535 version 11.00. Images pixel size was 0.268 μm x 0.2268 μm . Objective used was Zeiss C-
536 Apochromat 40x/1.2 W. Ablation was performed with 800 nm Titanium/Sapphire
537 femtosecond pulsed laser Chameleon from Coherent (Santa Clara, USA) with a power of 3.2
538 W at the laser head, 60% laser output set in Zen Black, reflected by MBS 760+, with pixel
539 dwell time for photomanipulation of 7.2 μs , single iteration, ablation area was line scan, 10
540 pixels. For measuring the recoil velocity, the lateral junction of MDCK WT or CLDN-KO and
541 ZO-KO cells was highlighted by ZO1-mNeonGreen or MyosinIIa-mNeon green respectively
542 with the following settings: mNeonGreen was excited with 488 nm (Argon Laser) with MBS
543 488/561/633, emission filter used was 490-570 nm, pixel dwell time 2.83 μs , approximately
544 7.7 fps with GaAsP detector. Recoil velocities were determined by dividing the distance
545 covered by the vertices in a considered span of time, by the same time span.

546

547 ***Immunostainings of MDCK tissue***

548 Samples were fixed with 4% w/v Paraformaldehyde (PFA) pH 7.25 in PBS for 10 minutes at
549 room temperature. For pseudo time-lapse imaging samples were fixed with 4% PFA +
550 0.025% v/v Glutaraldehyde in PBS for 10 minutes at room temperature to prevent cells being
551 washed away due to PFA incubation. Cells were then permeabilized with 0.5% v/v Triton X-
552 100 in PBS for 10 minutes and blocked with the blocking buffer (2% w/v BSA, 0.02% v/v
553 NaN₃) in PBS for 1 hour at room temperature. Primary antibodies were diluted by the
554 blocking buffer and incubated with cells for 1 hour at room temperature or overnight at 4°C
555 followed by diluted secondary antibodies and labelled phalloidin incubation. Primary
556 antibodies used were: Rabbit anti-gp135/podocalyxin (1:200; from Ünal Coskun's group);
557 Mouse anti-E-Cadherin (1:100; from MPI-CBG antibody facility).

558

559 ***Confocal microscopy***

560 Live cell imaging: the images were acquired with a Zeiss Axio Observer.Z1 inverted
561 microscope (ZEISS, Germany) equipped with a CSU-X1 spinning disc confocal scanning unit
562 (Yokogawa, Japan), driving a Zeiss AxioCam MRm, Monochrome CCD camera. The images
563 were acquired with a x63 1.2 NA water objective lens (ZEISS, Japan) and a x100 1.46NA alpha

564 Plan-Apochromat oil objective lens (ZEISS, Japan) and ZEN blue software (ZEISS, Germany).

565 Z-stacks were acquired with a distance of 300 nm.

566

567 ***Cyst inflation in microfabricated cavities***

568 To increase the volume of the lumen, we applied a local liquid flow using a micro-pipette to
569 cysts growing in microfabricated cavities, which prevented detachment and movements of
570 cysts (see below). Application of liquid flow led to the rapid inflation of the cyst within
571 seconds, which was reversible (see Fig. 6). In addition, we found that fluorescent Dextran,
572 present in the pipette solution, entered the lumen, which indicates that the liquid stream
573 opened cell junctions and presses liquid into the lumen (see Fig. 6A-B).

574 The inflation experiments were quantified by segmenting lumen and cyst shape before and
575 after inflation to extract tissue and cell shape changes in response to the hydrostatic pressure
576 perturbations.

577 The micro-cavities were prepared by soft lithography²¹. Briefly, a mask with 18 μm disks with
578 100 μm inter-distance was designed with Autocad, which was then printed on photomask.
579 The pattern was transferred to SU8 silicon wafer by photolithography. Then a PMDS mold
580 was generated by soft lithography. The PDMS pillar replica were transferred on a cover-glass
581 using plasma activation and chlorotrimethylsilane for passivation and subsequently filled
582 with liquid PDMS. With overnight polymerization at 65°C, the PMDS formed a solid
583 membrane on the cover-glass, and cavities could be obtained by gentle removal of the PDMS
584 stamp. After obtaining through-hole cavities on the cover-glass, the surface was activated by
585 plasma and then incubated with 5 $\mu\text{g}/\text{ml}$ laminin at room temperature. Cells were seeded in
586 cavities by centrifugation³⁶. Then 15 μl Matrigel was added on the top of the cavities, which
587 allowed MDCK to form cysts. Cysts were used 5 days after cell seeding for inflation
588 experiments. To facilitate the experiments, Matrigel on top of micro-wells was removed using
589 a needle.

590 The lumen was inflated by using a pipette pulled with a Sutter pipette puller (P 2000), the tip
591 of the pipette was then broken using a scalpel under a binocular to the expected diameter.

592 The flow was controlled with the Eppendorf Cell Tram Vario. We injected medium (MEM with
593 5% serum) containing fluorescent Dextran to track the localisation of the liquid. Finally,
594 changes in cell and cyst shapes were monitored live by imaging by using Leica spinning disk
595 (Spinning disk LEICA CSU W1).

596

597 **Lumen Model**

598 The lumen shape was calculated based on the minimization of the free energy given by the
599 sum of the contributions from the hydrostatic pressure in the lumen, the line tension at apical
600 junctions of all neighbouring cell pairs and apical bending energy from the lumen surface.

$$601 \quad F = F_{hydr.press} + F_{line\ tension} + F_{bending}$$

$$602 \quad F = -PV + TL + K \int H^2 \cdot dA$$

603 where P is the pressure of the lumen, V its volume, T the apical line tension, L the total apical
604 line length, K the effective bending energy of the apical membrane, H its mean curvature. To
605 obtain an analytical solution to this problem, several geometrical assumptions must be made
606 which are detailed in the supplementary note 1.

607 To estimate the junctional line tension $T = \zeta v$ from the recoil velocities v determined by
608 laser ablation experiments (Figure 4) we used a damping coefficient of $\zeta = \eta_{cortex} \ell_{cortex} =$
609 $50 pN \cdot s / \mu m$. For this we take the typical value of $\eta_{cortex} = 200 \text{ Pa} \cdot s$ ³⁷ and a cortical length
610 of $\ell_{cortex} = 500 \text{ nm}$. Using these values with $v = 10 \mu m/s$ gives line tensions in the range of T
611 $= 1000 \text{ pN}$ (see parameter table below), which corresponds to about 10^4 myosin motors
612 applying a 0.1 pN mean force³⁸. We assume the same ζ damping coefficient for the different
613 MDCKII-mutants because the change in myosin expression levels is related to the changes in
614 tension and not in viscosity of the cortex.

615 Values for the cortex modulus have been previously measured to be $2 \cdot 10^2 k_B T$ ³⁹. To take the
616 rigidity of the cell layer into account, we estimated an effective bending modulus of $2 \cdot 10^5 k_B T$
617 $= k = 2 \cdot 10^{-16} \text{ J}$ (see Table 1). We integrate in this value the effective resistance of the cell layer
618 by assuming a typical height of about $10 \mu m$ and a rough estimate for the associated change
619 in bending modulus following similar scaling arguments proposed in⁴⁰.

620

621 3D shapes of lumen were calculated using Surface Evolver²⁰ by minimizing the energy:

$$622 \quad F = -PV + TL + K \int dA \cdot H^2 + \sum_{i=1}^N M(A_i - A_0).$$

623 By term, the contributions are: the contribution from the fluid inside the lumen, where P is
624 the pressure and V is the volume, contribution from the junctions, where T is the tension and
625 L is the total junction length, bending of the apical surface, where K is the bending modulus
626 and H is the mean curvature which we integrate over the surface, and finally area

627 conservation, where M is the area modulus, A_i is the area of cell i and A_0 is the preferred cell
628 area (equal to S/N , where S is the total surface area and N is the total number of cells).
629 Energy minimization was accomplished using the Surface Evolver programming language ²⁰.
630 Briefly, a surface consisting of 42 polygonal faces was imported into Surface Evolver. These
631 42 faces consist of a mixture of pentagons and hexagons, as dictated by Euler's formula. The
632 edges between the polygonal faces were marked to represent junctions. The surface was then
633 triangulated and transformed into a sphere of surface area S . The energy function above was
634 then applied and the surface evolved toward minimum energy with Surface Evolver's
635 gradient descent tools. A high area modulus was used to ensure cell areas remain
636 approximately constant.

637 Parameters taken for computation of the phase diagram of Fig.5:

Common to all parameter sets	Value
Total surface area S	2500 μm^2
Number of cells N	42
Effective Bending energy K	$2 \cdot 10^{-16} \text{ J} = 200 \text{ pN } \mu\text{m}$
Parameter with ranges	Range
Hydrostatic Pressure	From 0 to 100 Pa
Apical Line Tension	From 0 to 3000 pN

638

639 Parameters taken for computation of the lumen shapes (surface evolver) of Fig.5:

Common to all parameter sets	Values
Total surface area S	2500 μm^2
Number of cells N	42
Bending energy K	$2 \cdot 10^{-16} \text{ J} = 200 \text{ pN } \mu\text{m}$

Area modulus M	$1 \cdot 10^{-14} \text{ J}/\mu\text{m}^2 = 10000 \text{ pN}/\mu\text{m}$
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640

Parameter set label	Tension T (pN)	Pressure P (Pa = pN/micron ²)
1	3000	10
2	3000	50
3	3000	90
4	2000	10
5	2000	50
6	2000	90
7	1000	10
8	1000	50
9	1000	90
10	0	10
11	0	50
12	0	90

641

642

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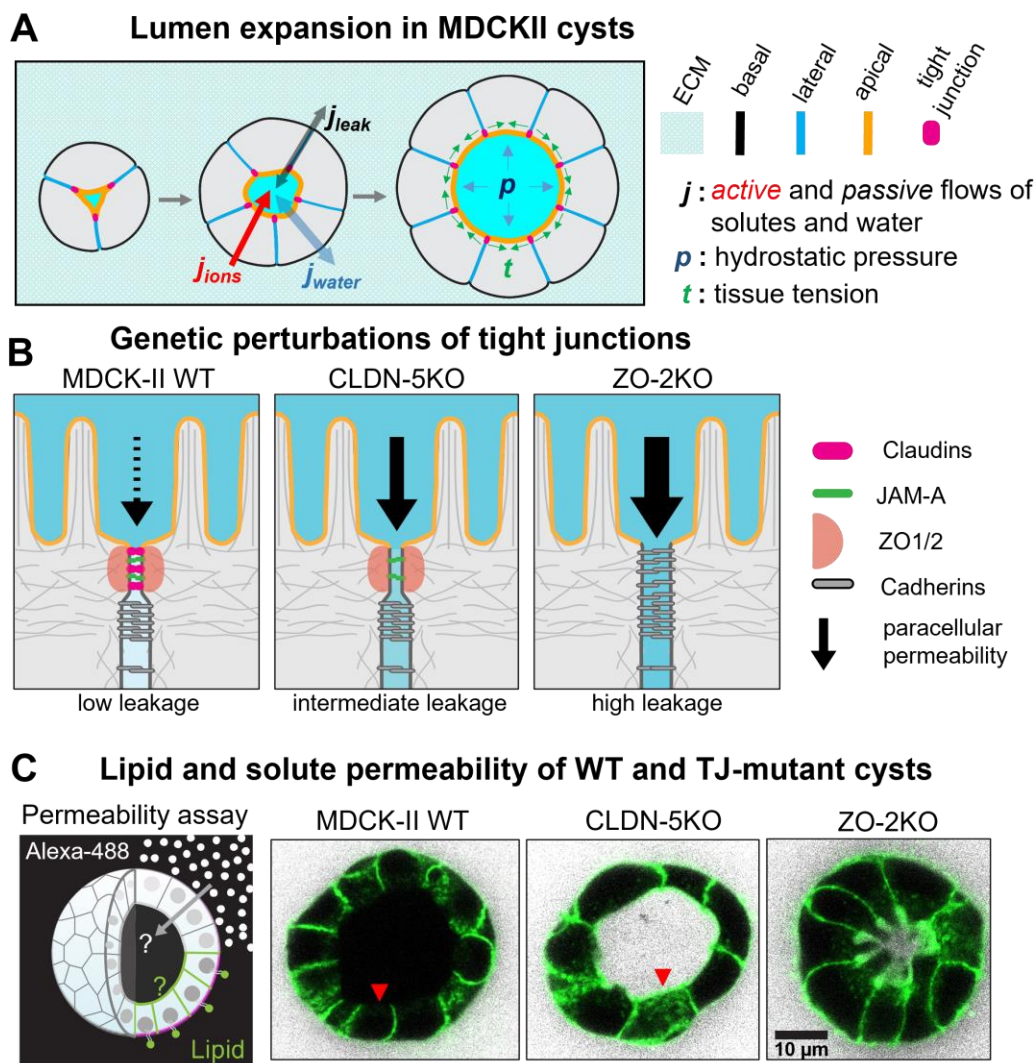
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734 **Figures**

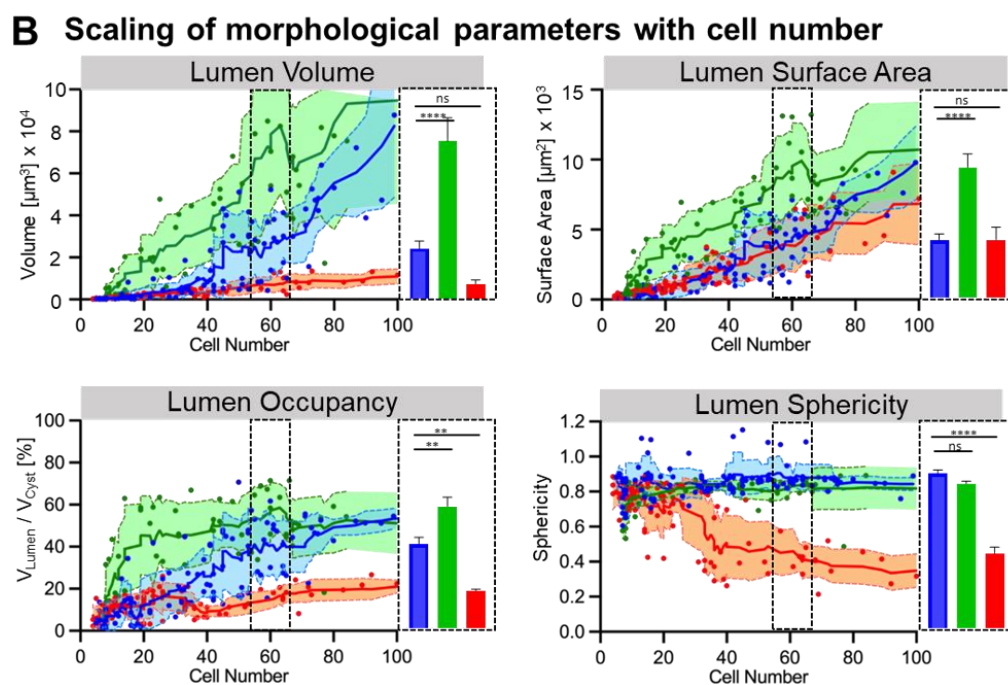
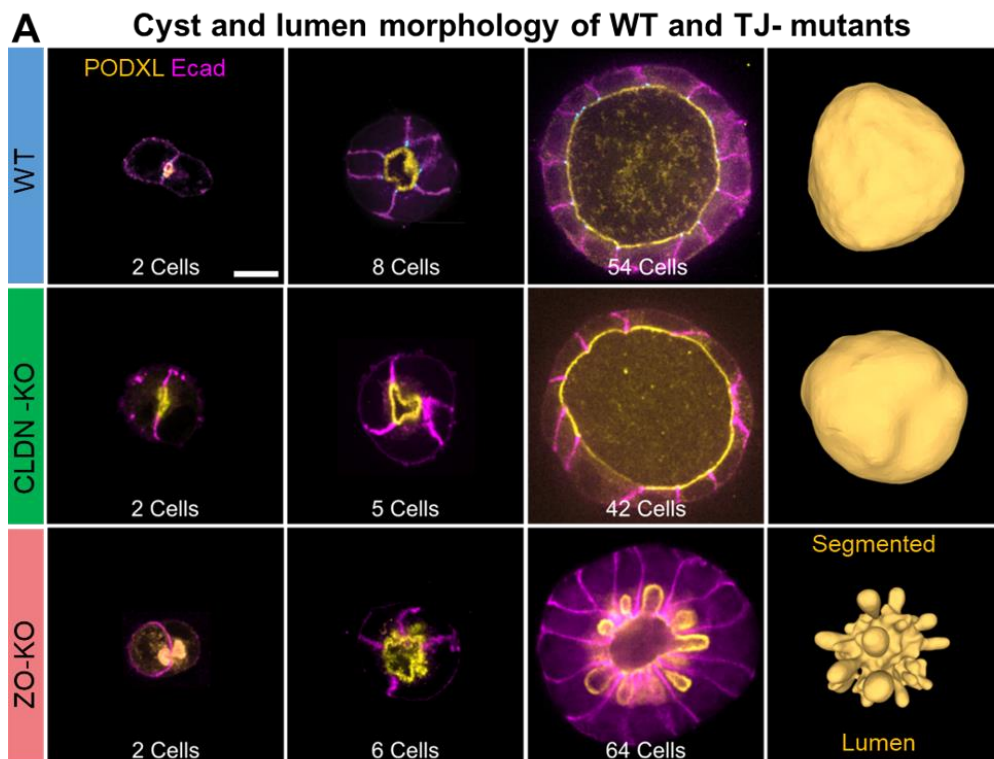


735

736 **Figure 1: Lumen formation in MDCKII cysts and tight junction mutants used in this study**

737 (A) Scheme of lumen expansion in MDCKII cysts. Polarized membrane domains and tight junctions are
 738 highlighted (right panel). Cysts are growing over time due to cell divisions. Osmotic gradients due to
 739 the balance of active ion transport into the lumen and passive para-cellular leaks result in water influx
 740 and establishment of hydrostatic lumen pressure. Hydrostatic lumen pressure is balanced by cortex
 741 tension of the apical-junctional surface. (B) Tight junction mutants used in this study. MDCKII-WT
 742 tissue produces tight junctions that have very low permeability for ions and water. CLDN-5KO tissue
 743 is permeable for small molecules and ZO-2KO tissue is permeable for small- and macromolecules. (C)
 744 Permeability assay in MDCKII cysts for small molecules (Alexa-488) and a lipid tracer (Cell-Mask).
 745 Shown are midplane cross-section of WT and TJ-mutant cysts, white is the small molecule tracer and

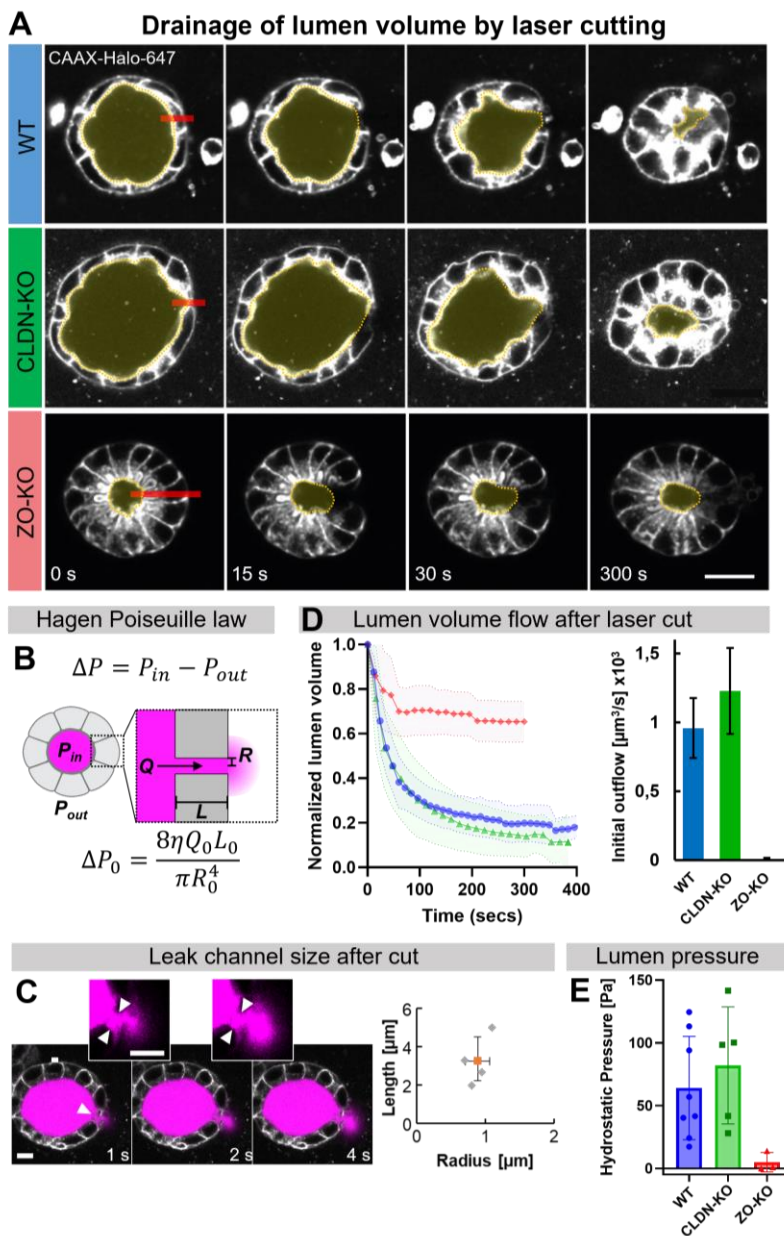
746 green the lipid tracer. WT cysts are tight for both molecules. CLDN-KO and ZO-KO cysts are permeable
747 for both molecules. However, CLDN-KO cysts produce a large lumen while ZO-KO cysts produce small,
748 convoluted lumen. Arrows indicate apical membranes, which are not accessible for the lipid tracer in
749 WT cysts but in CLDN-5KO and ZO-2KO the lipid tracer can cross the junctional barrier.
750



751 **Figure 2: Lumen morphological differences between WT and TJ-Mutants cell lines**

752 (A) Midplane cross-section of WT and TJ-mutant cysts as a function of cell number (indicated at the
 753 bottom of the panel). Lateral membranes are stained by E-cadherin (magenta) and apical membranes
 754 by Podocalyxin (yellow). The right panels show 3D segmentation of the lumen at the later stage. WT
 755 and CLDN-KO cyst form large spherical lumen, ZO-KO cysts form smaller, convoluted lumen with apical

756 membranes curving into the cell bodies. Scalebar is 10 μ m. (B) Quantification of lumen volume, surface
757 area, sphericity and occupancy based on 3D segmentation of lumen and cyst surfaces of WT (n =114),
758 CLDN-KO (n=64) and ZO-KO cysts (n=88) as a function of the total cell number per cyst. Bar graphs
759 indicate quantification of lumen volume, surface area, sphericity and occupancy for 55 - 65 cells
760 (dashed box) and WT (n =15), CLDN-KO (n=6) and ZO-KO cysts (n=9), Oneway-Anova was used for
761 statistical analysis.
762



763

764

Figure 3: Influence of TJ-mutations on hydrostatic lumen pressure in MDCKII cysts.

765

(A) Lumen volume drainage MDCKII TJ mutant cysts by laser cutting. Midplane cross-section of cysts

766

before and after laser cutting. Position of cuts are indicated by red lines. The lumen of WT and CLDN-

767

KO cysts quickly collapsed after the cut, while the lumen of ZO-KO cyst remained relatively constant.

768

Scalebar is 20 μ m. See supplemental movie-1 for an example of lumen drainage. (B) Hagen-Poiseuille

769

law used to determine initial hydrostatic pressure in the lumen: ΔP hydrostatic pressure difference, Q

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fluid flow, L and R channel length and radius, η fluid viscosity. Lumen fluid viscosity was measured

771

using FCS (Suppl. Fig. 1). (C) Geometry of the leak channel after laser cutting was determined by

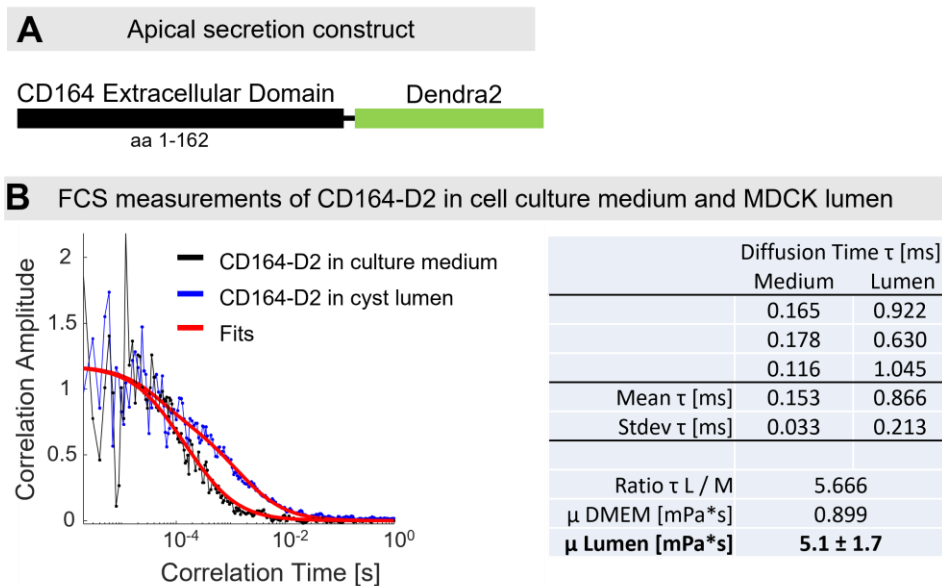
772

imaging the exit of the lumen fluid, which was stained by expression of Dendra2-CD164 (magenta).

773

Scalebar 10 μ m. (D) Lumen volume change after laser cutting determined from 3D segmentation of

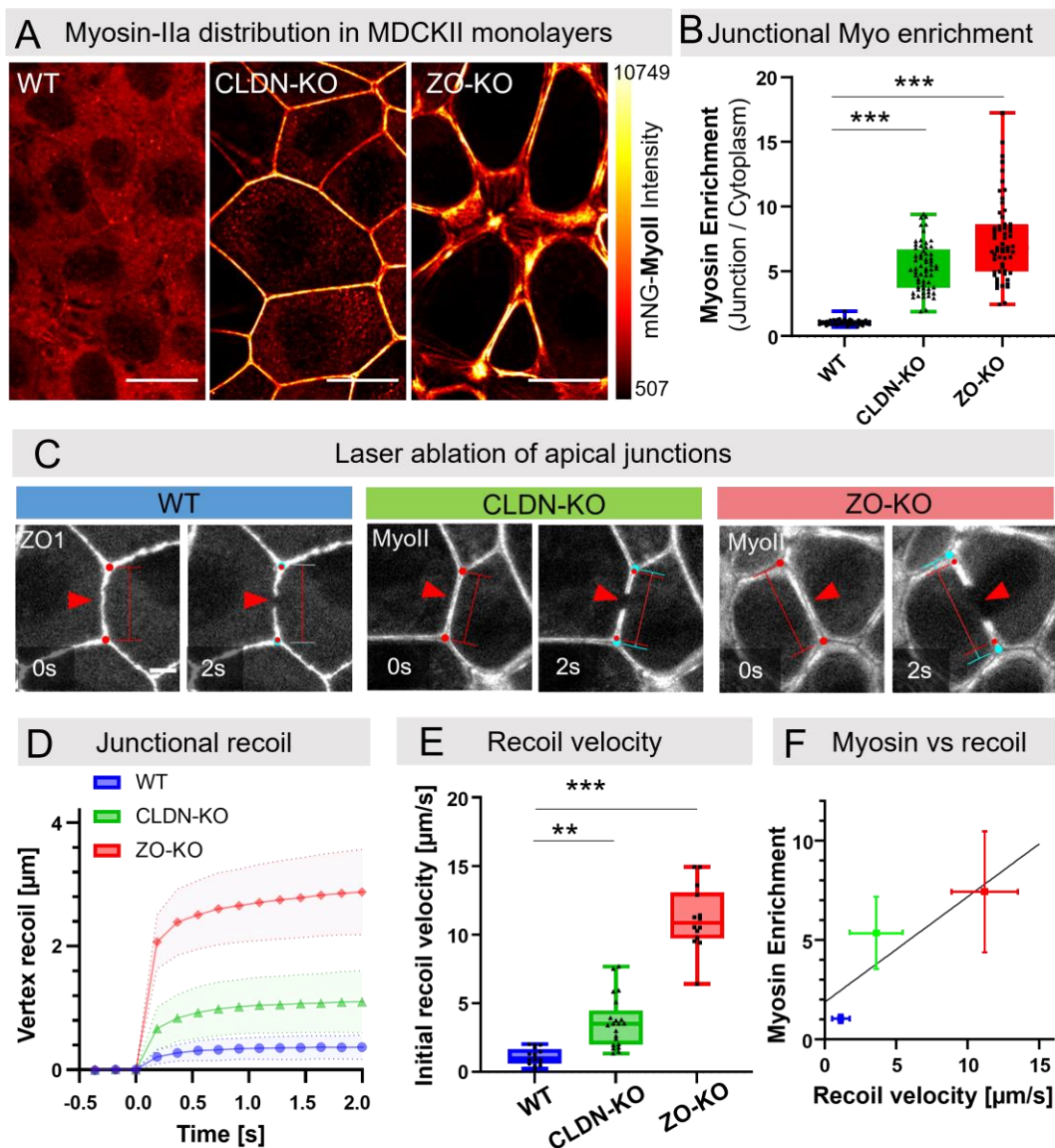
774 lumen over time. Values are normalized to the initial lumen volume before the cut. Right plot shows
775 mean initial flow rate with standard error, calculated from the initial slope. WT (n=12), CLDN-KO (n=6)
776 and ZO-KO cysts (n=5). (E) Hydrostatic lumen pressure of MDKC-II mutants. WT and CLDN-KO have a
777 lumen pressure of ≈ 65 Pa and ≈ 85 Pa while ZO-KO cysts are not able to build up significant pressure.
778



779

780 **Suppl. Figure 1: Measurement of lumen fluid viscosity**

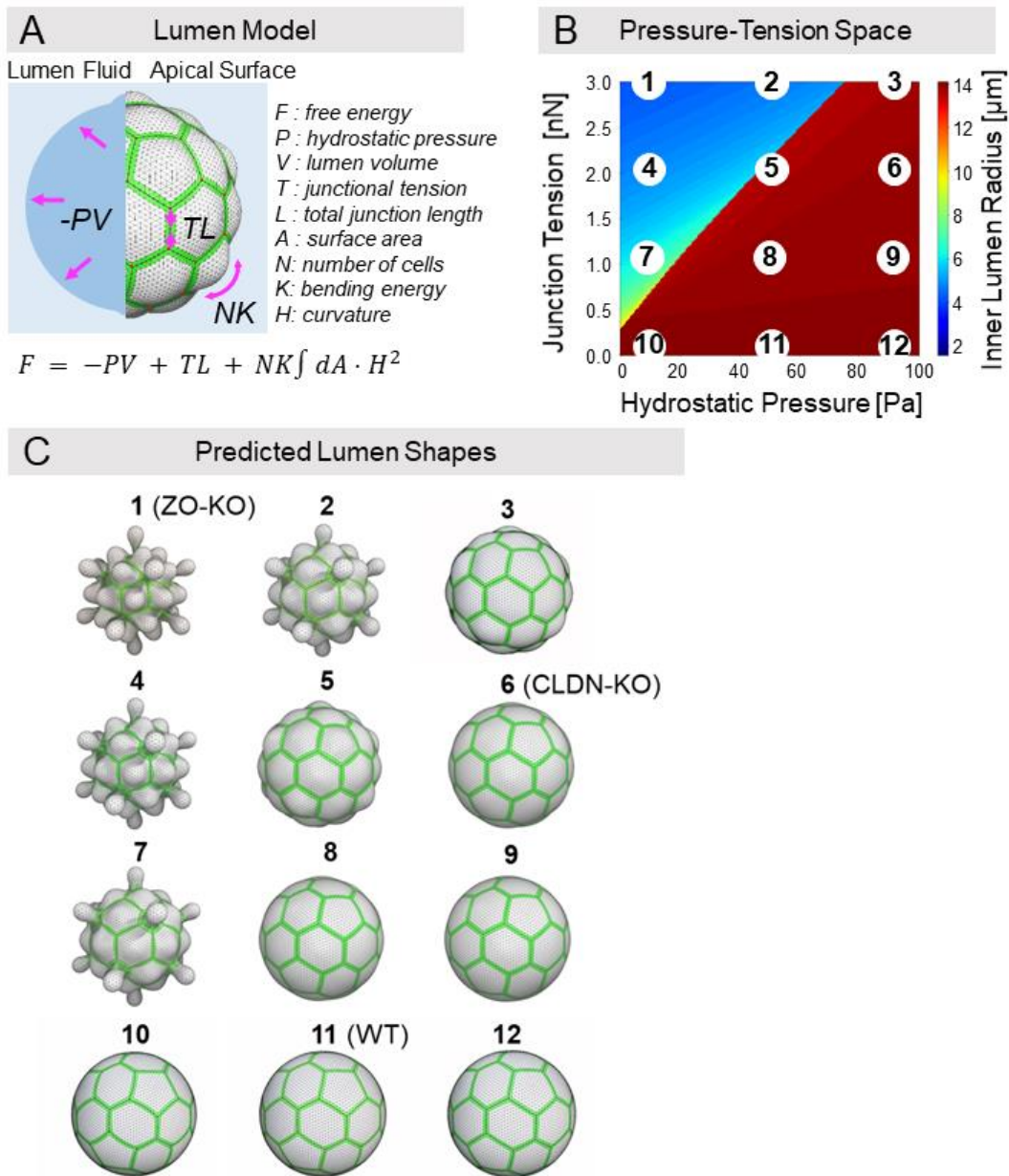
781 (A) Schematic of apically secreted CD164 construct. (B) Fluorescence correlation spectroscopy
782 measurement was performed at 37°C to compare diffusion times of Dendra2-CD164 in the lumen and
783 in cell culture medium. Viscosity in the lumen was determined to be $\eta = 5.1 \pm 1.7$ mPa*s.



784 **Figure 4: Quantification of junctional tension of MDCKII mutants**

785 (A) Distribution of endogenous myosin-IIa tagged with mNeon in MDCK-II monolayers. In WT tissue
 786 myosin is predominantly in the cytoplasm with only minor punctae at cell junctions. CLDN-KO tissue
 787 showed significantly increased levels of myosin at the junctions and ZO-KO monolayer had strong
 788 accumulation of thick myosin bundles along cell-cell junctions. Scalebar is 5 μ m. (B) Quantification of
 789 junctional myosin enrichment at cell-cell junctions versus cytoplasm. WT (n =50), CLDN-KO (n=61)
 790 and ZO-KO cysts (n=60). (C) Laser ablation of cell-cell junctions of MDCK-II mutants. Position of cut is
 791 indicated by red arrow. Junctional recoil distance was measured by tracking the position of tricellular
 792 vertices over time. (D) Dynamics of junctional recoil after laser ablation of MDCK-II WT and mutants.
 793 WT (n =16), CLDN-KO (n=21) and ZO-KO cysts (n=14). Laser cut was done at 0s. Initial distance before
 794 cut was subtracted to highlight the change due to recoil. (E) Mean initial recoil velocities of MDCK-II

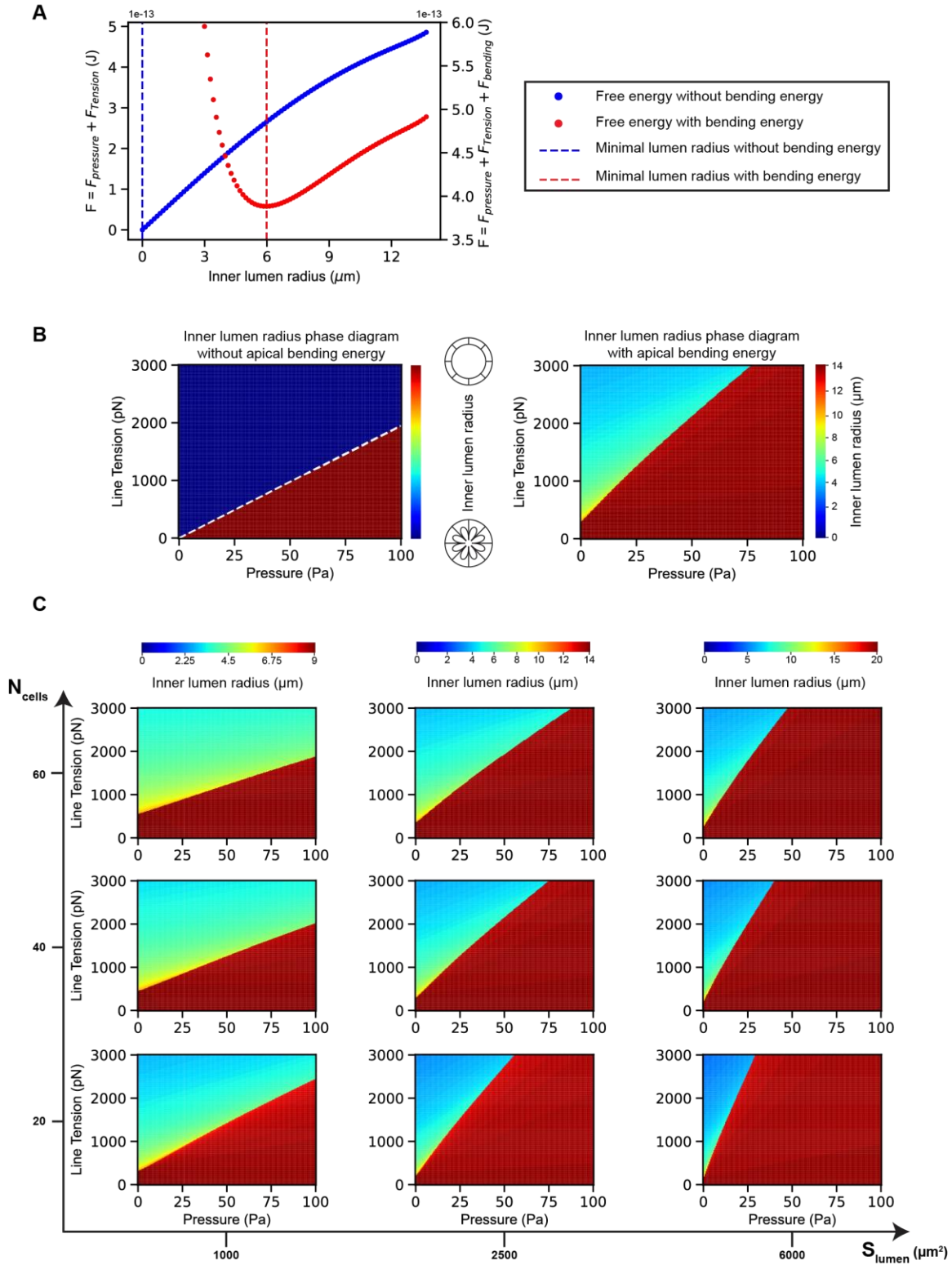
795 mutants show that WT tissue is under low mechanical tension. CLDN-KO tissue has increased tension
796 and ZO-KO tissue builds up strong junction tension. Initial recoil velocities were determined from the
797 slope of first data points after laser cutting. WT (n =16), CLDN-KO (n=21) and ZO-KO cysts (n=14). (F)
798 Correlation between myosin junctional enrichment and initial recoil velocity with a linear fit of WT,
799 CLDN-KO and ZO-KO data from B and E ($R^2= 0.46$).
800



801

802 **Figure 5: Apical surface model recapitulates lumen phenotypes of TJ-mutants**

803 (A) Schematics of the free-energy model of lumen surface. The shape of the lumen is obtained by
 804 minimizing the free energy F of its area A computed with contributions from the hydrostatic pressure
 805 P of the lumen, the apical junctional tension T and the bending rigidity K of apical surface. (B) Phase
 806 diagram of the inner lumen radius in the apical line tension - hydrostatic pressure parameter space.
 807 For this phase diagram, the bending energy is $K = 2 \cdot 10^{-16}$ J and the number of cells is $N_{\text{cells}} = 42$ cells.
 808 (C) Minimum energy 3D shapes of the lumen surface for the points labelled 1 to 12 in (B), calculated
 809 using Surface Evolver.



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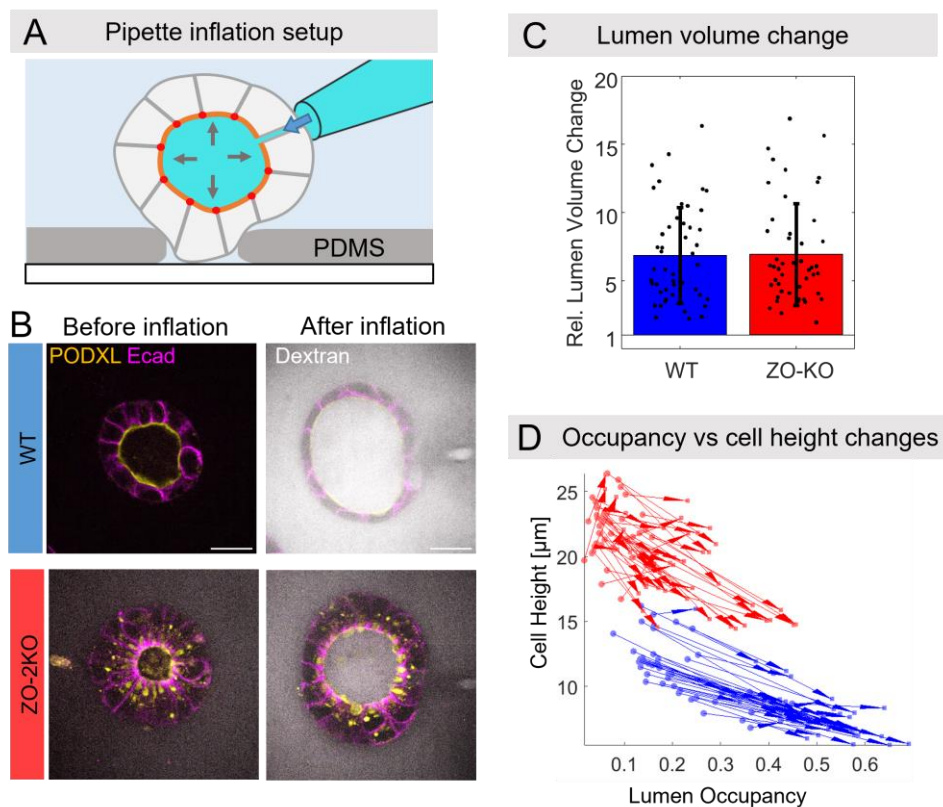
812 **Suppl. Fig. 2 : Contribution of bending energy, cell number and surface area on phase**
813 **diagrams of lumen morphology.**

814 (A) Free energy of the lumen surface as a function of inner lumen radius computed for a total
815 number of cells - $N_{\text{cells}} = 42$ cells, a total lumen surface - $S_{\text{lumen}} = 2500 \mu\text{m}^2$, hydrostatic
816 pressure of lumen - $P = 20$ Pa, apical junctional tension - $T = 1500$ pN. The minimum of free
817 energy determines the value of the inner lumen radius at equilibrium. Blue curve represents
818 the case without apical membrane bending energy, the minimal inner radius in this case is R
819 $= 0 \mu\text{m}$. Red curve represents the case accounting for an apical bending energy ($K = 2.10^{-16}$ J),
820 the minimal inner radius in this case is non-zero. (B) Comparison of the phase diagrams of
821 the inner radius of lumen determined by minimizing the free energy of the lumen surface
822 without (left panel) and with (right panel) bending energy of the apical surface. Blue zones
823 correspond to flower-like lumen shape and red zones to spherical inflated lumen. Total
824 number of cells - $N_{\text{cells}} = 42$ cells, total surface of lumen - $S_{\text{lumen}} = 2500 \mu\text{m}^2$, bending energy K
825 $= 2.10^{-16}$ J. (C) Phase diagrams generated for number of cells N_{cells} - total surface of lumen
826 S_{lumen} pair values for a bending energy - $K = 2.10^{-16}$ J. Note that the inflated region (red zone)
827 increases with larger S_{lumen} values and decreases with larger N_{cells} .

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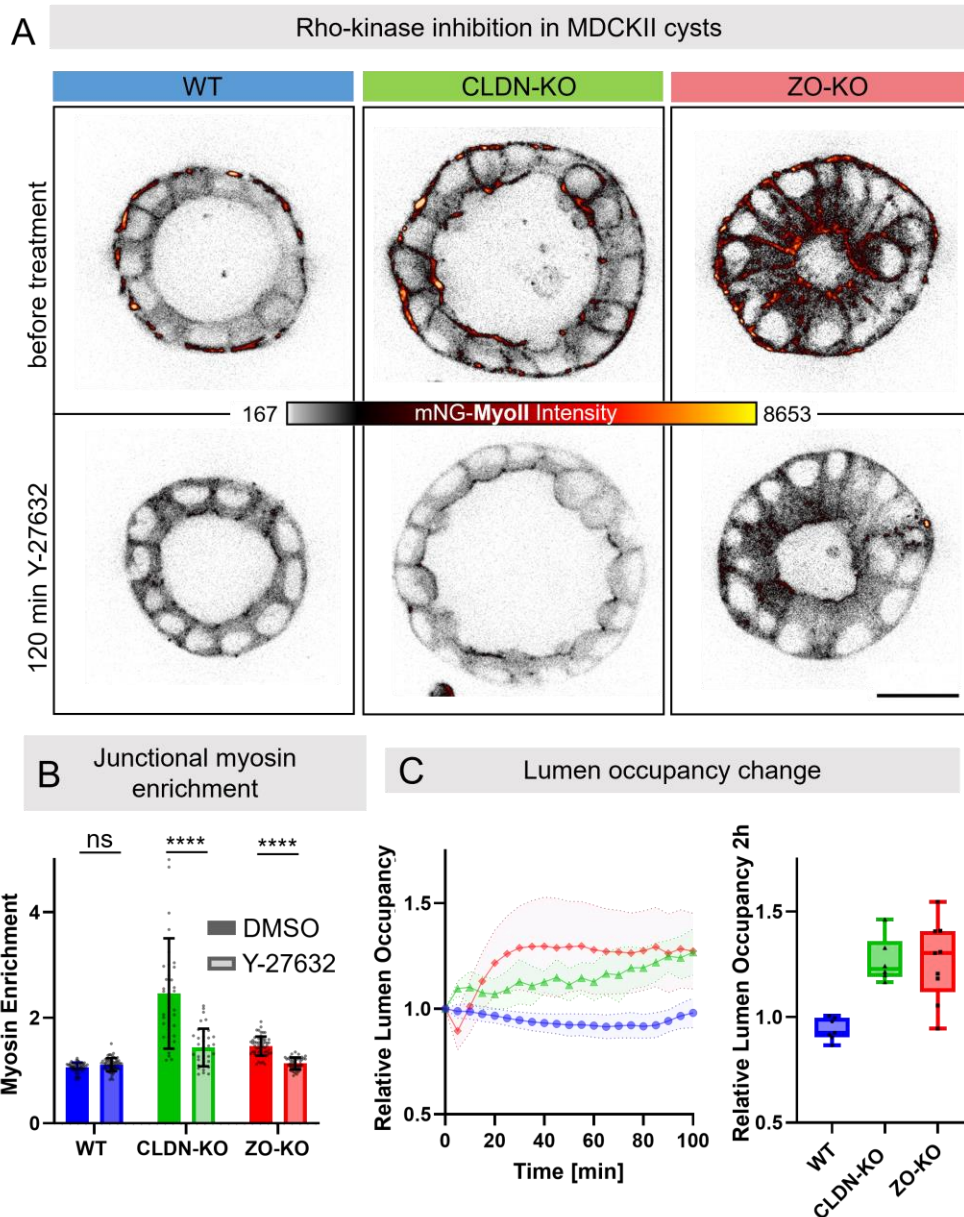
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832 **Figure 6: Increasing hydrostatic lumen pressure promotes lumen opening**

833 (A) Schematic of pipette inflation assay. (B) WT and ZO-KO cysts before and after lumen inflation.
834 Fluorescently labelled Dextran in the pipette solution was used to demonstrate entry of fluid into the
835 lumen. (C) Quantification of lumen volume change normalized to the volume values before inflation.
836 (D) Trajectories of lumen occupancy vs cell height before and after inflation represented by arrows
837 show that ZO-KO cysts cannot be inflated to the same level as WT cysts and cells remain more
838 elongated. WT in blue and ZO-KO in red. WT (n =96), ZO-KO (n=96) for C,D.

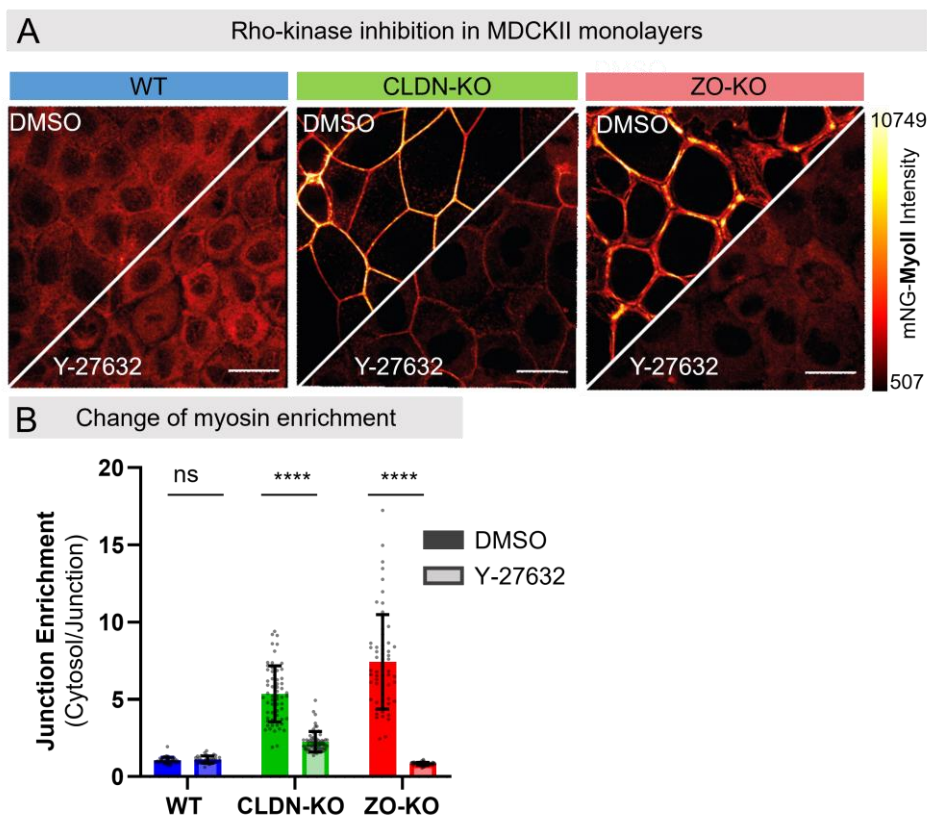


839

840 **Figure 7 Release of junctional tension promotes lumen opening in CLDN-KO and ZO-KO cysts**

841 (A) Distribution of endogenous myosin-IIa tagged with mNeon in MDCK-II cysts before and after
 842 addition of Rho kinase inhibitor Y-27632 (200 μ M) in WT and CLDN-KO and ZO-KO cysts. Scalebar
 843 20 μ m. A clear increase in lumen size is visible in ZO-KO cysts. (B) Quantification of junctional myosin
 844 enrichment at cell-cell junctions versus cytoplasm and comparison between incubation with Y-27632
 845 and the solvent DMSO 120 minutes after treatment, WT (n =57), WT Y-27632 (n=64), CLDN-KO (n=41)
 846 CLDN-KO Y-27632 (n=37), ZO-KO (n=57) and ZO-KO Y-27632 (n=) cysts (n=48).. (C) (Left) Normalized
 847 lumen occupancy after Y-27632 addition. WT cysts showed no significant change of lumen volume. ZO-
 848 KO cysts significantly increased lumen occupancy 30min after myosin inhibition. WT (n =7), CLDN-KO

849 (n=6) and ZO-KO cysts (n=9). (Right) Mean change of lumen occupancy after 120min of Y-27632
850 addition. WT cysts show no significant change of lumen volume after tension release while CLDN-KO
851 and ZO-KO cyst show a 40% increase of their lumen volume. WT (n =7), CLDN-KO (n=6) and ZO-KO
852 cysts (n=9).



853

854 **Suppl. Fig. 3: Release of junctional tension by ROCK inhibition**

855 (A) Distribution of endogenous myosin-IIa tagged with mNeon in MDCK-II monolayers before and 2h
856 post Y-27632 treatment (200 μ M). Scalebar 5 μ m. (B) Quantification of release of junctional myosin
857 enrichment after Y-27632 with DMSO incubation as control. WT (n =50), WT Y-27632 (n=35), CLDN-
858 KO (n=61) CLDN-KO Y-27632 (n=66), ZO-KO (n=60) and ZO-KO Y-27632 (n=) cysts (n=64).

859