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# Length-limitation of astral microtubules orients cell divisions in intestinal crypts

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32 **SUMMARY**

33

34 Planar spindle orientation is critical for epithelial tissue organization, and generally instructed  
35 from the long cell shape axis or cortical polarity domains. We introduced mouse intestinal  
36 organoid crypts to study spindle orientation in a monolayered mammalian epithelium. Although  
37 spindles were planar in this tissue, mitotic cells remained elongated along the apico-basal axis  
38 and polarity complexes were segregated to basal poles, so that spindles oriented in an  
39 unconventional manner, orthogonal to both polarity and geometric cues. Using high-resolution  
40 3D imaging, simulations, cell shape and cytoskeleton manipulations, we show that planar  
41 divisions resulted from a length-limitation in mitotic-phase astral microtubules which precludes  
42 them from interacting with basal polarity, and oriented spindles from the local geometry of  
43 apical domains. Accordingly, lengthening microtubules affected spindle planarity, cell  
44 positioning and crypt arrangement. We conclude that microtubule length regulation may serve  
45 as a key mechanism for spindles to sense local cell shapes and tissue forces to preserve  
46 mammalian epithelial architecture.

47

## 48 INTRODUCTION

49

50 Proliferative monolayered epithelia support the morphogenesis, function and renewal of many  
51 stem cell niches and organs during development and adult life (Godard and Heisenberg, 2019;  
52 Macara et al., 2014). They are characterized by an apico-basal polarity (A-B), and an  
53 alignment of mitotic spindles and consequent cell divisions within the tissue planes. These  
54 planar divisions position divided daughter cells side by side in the epithelial layer (Aigouy et  
55 al., 2010; Morin et al., 2007; Peyre et al., 2011) to promote tissue monolayered architecture,  
56 elongation and homeostasis (Campinho et al., 2013; da Silva and Vincent, 2007; Mao et al.,  
57 2013). Accordingly, a mis-regulation of spindle planarity has been shown to impair cell  
58 positioning and tissue integrity, and proposed to drive the emergence of dysplasia,  
59 hyperplasia, or cancer stem cell populations in stem cell niches (Bergstralh and St Johnston,  
60 2014; di Pietro et al., 2016; McCaffrey and Macara, 2011; Morin and Bellaiche, 2011; Quyn et  
61 al., 2010; Xiong et al., 2014). To date, however, spindle orientation in epithelia has been best  
62 studied in model 2D invertebrate and vertebrate tissues, which are amenable to high-  
63 resolution live imaging and genetic manipulations of spindle associated elements (di Pietro et  
64 al., 2016; Morin and Bellaiche, 2011). In contrast, studying cell division orientation in complex  
65 3D mammalian tissues and organs, relevant to human physiology and diseases has been in  
66 general hampered by the difficulty of performing advanced imaging in live animal models, and  
67 by plausible pleiotropic effects of loss of function assays (Dona et al., 2022). Therefore,  
68 addressing detailed mechanisms regulating spindle orientation in proliferating mammalian  
69 epithelial tissues remains an outstanding open endeavor.

70

71 In most animal cells and tissues, mitotic spindles are positioned and oriented from forces and  
72 torques generated by their astral microtubules (MTs) pulled by dynein motors. Dynein may be  
73 evenly distributed over the cytoplasm or cortex, a situation thought to yield length-dependent  
74 MT forces, that function to center spindles and align them with the long geometrical axis of the  
75 cell (Haupt and Minc, 2018; McNally, 2013; Wyatt et al., 2015). Dynein can also be activated  
76 at specific sub-cellular cortical polarity domains enriched in dynein activators including NuMA  
77 (Nuclear and Mitotic Apparatus) and LGN (Leucine-Glycine-Asparagine), such as during  
78 asymmetric divisions (David et al., 2005; Morin and Bellaiche, 2011; Schaefer et al., 2000; Yu  
79 et al., 2000). In tissues, these geometrical and polarity cues often compete to dictate spindle  
80 and division positioning (Chanet et al., 2017; Godard et al., 2021; Niwayama et al., 2019;  
81 Pierre et al., 2016). For instance, the planar orientation of mitotic spindles in columnar  
82 monolayered epithelia is thought to rely on the localization of dynein-regulating polarity  
83 complexes at the level of lateral junctions, as well as on a complete mitotic rounding that  
84 serves to erase the influence of cell geometry and ensures that short mitotic-phase astral MTs

85 bounded by dynamic instabilities reach the cortex to influence spindle orientation (Chanet *et*  
86 *al.*, 2017; di Pietro *et al.*, 2016; Lancaster and Knoblich, 2012; Luxenburg *et al.*, 2011; Morin  
87 and Bellaiche, 2011). In general, however, how cell shape changes, MT dynamics and polarity  
88 effectors intersect to specify spindle planarity in mammalian epithelia remains poorly defined.

89  
90 Given its high renewal rate throughout adult life, the intestinal tissue provides a prime model  
91 to study cell proliferation and division in mammals. The surface of the intestine is lined by a  
92 monolayer of tall columnar epithelial cells, with mitotic stem and transit-amplifying cells  
93 exclusively located in curved tissue invaginations called crypts (Gehart and Clevers, 2019;  
94 Tan and Barker, 2014). Previous studies of cell division in the crypt have reported that mitosis  
95 is associated with an apical migration of cells and DNA, which ends up with the assembly of  
96 a planar spindle in metaphase that specifies cytokinesis orthogonal to the tissue layer (Bellis  
97 *et al.*, 2012; Carroll *et al.*, 2017; Fleming *et al.*, 2007; McKinley *et al.*, 2018; Spear and  
98 Erickson, 2012). However, in part because of the lack of accessibility of the intestine *in vivo*,  
99 detailed mechanisms that control planar spindle orientation and the monolayered architecture  
100 of the crypt are still lacking.

101  
102 Here, we build on intestinal organoids to address mechanisms that control division orientation  
103 in a 3D mammalian epithelium. Organoids are classically generated from isolated crypts  
104 derived from mouse intestinal pieces, and develop regular crypt-like structures with self-  
105 renewing capacity within 4 days of culture in 3D hydrogels, thereby mimicking the organization  
106 and dynamics of the *in vivo* proliferative compartment (Artegiani and Clevers, 2018; Fatehullah  
107 *et al.*, 2016). Using live 3D imaging, physical, chemical and genetic manipulations, we show  
108 that dynein-regulating polarity complexes accumulate at the basal face of mitotic cells and not  
109 at lateral junctions, and that mitotic cells remain largely elongated along the A-B axis,  
110 challenging previous generic models for spindle planarity established in model tissues. We  
111 propose a new quantitative model for planar spindle orientation, based on a local apical cell  
112 shape sensing, mediated by length-limitation of mitotic-phase (M-phase) astral MTs. This  
113 model predicts dose-dependent variations in spindle planarity in normal organoids and in  
114 multiple conditions that affect cell shape and polarity.

115

116

## 117 **RESULTS**

118

### 119 **Mitotic spindles in intestinal crypts orient orthogonal to the cell long axis**

120

121 The spherical geometry of intestinal organoid crypts grown in 3D, provides a unique  
122 opportunity to document cell division with an optimal optical resolution along the A-B axis of

123 the monolayer. We imaged spindle assembly and positioning along this axis, using  $\alpha$ -tubulin-  
124 GFP/H2B-mCherry organoids (Figure 1a). We confirmed that as in many columnar epithelia,  
125 centrosomes were initially located close to the apical pole during interphase, and migrated  
126 towards the DNA at the onset of mitosis to assemble a metaphase spindle that oriented  
127 orthogonal to the A-B axis, and thus in the tissue plane (Figure 1b; Figure S1a; Videos S1 and  
128 S3) (Bellis *et al.*, 2012; Carroll *et al.*, 2017; McKinley *et al.*, 2018). This planar orientation was  
129 maintained through metaphase, anaphase and telophase, yielding cytokinesis that bisected  
130 mother cells along the A-B axis, and placed daughter cells side by side in the monolayer  
131 (Figure 1b; Video S1) (Carroll *et al.*, 2017; McKinley *et al.*, 2018).

132

133 To decipher mechanisms which regulate spindle planarity, we first explored the role of cell  
134 geometry, which generally orients spindles along the long cell shape axis (Lechler and Mapelli,  
135 2021; Salle and Minc, 2021). We imaged cell contours in live tissues using td-Tomato  
136 organoids. This revealed that interphase columnar cells deform at the onset of prophase from  
137 the basal pole, yielding a water drop-like metaphase cell shape, rounder at the apical pole and  
138 elongated towards the basal pole (Figure 1c; Figure S1b). These mitotic shape changes were  
139 concomitant with a marked basal enrichment of myosin-IIA-GFP as well as P-MLC2,  
140 suggesting they are driven by an anisotropic actomyosin-generated cortical tension (Figure  
141 1d-e; Figure S1c-f, Video S2). This singular actomyosin distribution may underpin mitotic  
142 apical migration and neighboring cell rearrangements on the basal side of the epithelium. In  
143 fact, the basal actomyosin clustering mirrored neighboring cell remodeling and multicellular  
144 (3-, 4- and 5-cell) contacts formation in metaphase (Figure S1g). Thus, in contrast to many  
145 epithelial tissue and adherent cells in which the actomyosin cortex homogenously remodels  
146 to ensure a complete mitotic rounding (Chanet *et al.*, 2017; Luxenburg *et al.*, 2011; Nakajima  
147 *et al.*, 2013; They *et al.*, 2005), intestinal organoid crypt cells exhibit an asymmetric and partial  
148 rounding and remain elongated along the A-B axis.

149

150 Full 3D reconstitution of confocal tissue z-stacks allowed to quantify metaphase cell shapes  
151 along with spindle orientations and positions (Figure 1f; Figure S1b). This showed that  
152 metaphase cells exhibit a mean aspect ratio of  $1.33 \pm 0.04$  along the A-B axis, and a sphericity  
153 of  $0.79 \pm 0.02$  (Figure 1g-i), with a mean angle between mitotic spindles and the cell's long axis  
154 of  $66.1 \pm 3.8^\circ$  (Figure 1j). In addition, spindles were not centered at the geometrical cell center.  
155 Rather, they were shifted asymmetrically at an average position of  $40.7 \pm 1.5\%$  of the cell long  
156 axis length towards the apical pole (Figure 1g,i,k). Importantly, similar partial mitotic rounding,  
157 planar and asymmetrically positioned spindles were observed *in vivo* in crypts of mouse  
158 jejunum (Figure S2a-d). Therefore, planar cell division in intestinal crypts may not directly

159 follow long-axis geometrical rules, with a spindle oriented nearly orthogonal to the long cell  
160 shape axis and positioned asymmetrically towards the apical cell domain.

161

162

163 **Dynein-regulating polarity complexes localize to basal poles of mitotic cells and may**  
164 **not contribute to spindle orientation**

165

166 These observations prompted us to assay the localization of dynein-associated polarity  
167 complexes which are potential candidates to override geometric guiding cues (Kotak et al.,  
168 2012; Morin *et al.*, 2007; Niwayama *et al.*, 2019; Peyre *et al.*, 2011; Pierre *et al.*, 2016; Zheng  
169 et al., 2010). We imaged multiple components of evolutionary conserved polarity complexes,  
170 which control spindle orientations in many tissues, including the dynein-regulators NuMA, LGN  
171 as well as LGN-binding partner afadin (Carminati et al., 2016; Wee et al., 2011). During  
172 interphase, NuMA was mostly localized within the nucleus, and re-located to spindle poles  
173 and to the cortex throughout mitosis, as reported in many systems (Compton and Cleveland,  
174 1993; Van Ness and Pettijohn, 1983) (Figure S2e). However, although we expected the  
175 cortical pool of NuMA to localize to lateral poles in face of spindle poles, as described in most  
176 epithelia and adherent cells (di Pietro *et al.*, 2016), it was largely enriched to basal poles of  
177 metaphase cells, away from and orthogonal to spindles, in both intestinal organoids and *in*  
178 *vivo* jejunum (Figure 2a-b; Figure S2e-f). Similar basal cortical enrichments were found for  
179 LGN and afadin (Figure 2c-d; Figure S2g-h). Dynein was in contrast localized throughout the  
180 cytoplasm and cortex, and accumulated at spindle poles (Figure S2i-j). These data show that  
181 canonical polarity complexes are segregated in an unconventional manner, away from lateral  
182 cortices and spindle planes in intestinal crypts. Rather, they appear to follow sites with  
183 increased cortical and junctional tension, as evidenced by the basal accumulation of  
184 actomyosin cytoskeleton (Figure S1c-f) and E-cadherin (Figure S2k-n) as previously reported  
185 in mammalian cell lines (Carminati *et al.*, 2016; Gloerich et al., 2017; Hart et al., 2017).

186

187 To identify potential mechanical designs in astral microtubule (MT) force distributions that  
188 orient planar spindles in this tissue, we developed 2D mathematical models. Starting from a  
189 series of idealized cell shapes elongated along or orthogonal to the tissue plane, we placed  
190 spindles asymmetrically towards the apex, as in experiments, and computed the torque exert  
191 generated by astral MTs as a function of spindle orientation, to identify rotational equilibrium  
192 angles (Bosveld et al., 2016; Minc et al., 2011; They et al., 2007). As expected, when MTs  
193 were grown to fill the whole cell and exert length-dependent forces (shape-sensing system  
194 (Minc *et al.*, 2011)), spindles oriented along the long cell shape axis, in the A-B axis of the  
195 tissue. Similarly, assuming that astral MT forces were scaled to the amount of the dynein-

196 regulator NuMA (Bosveld *et al.*, 2016), also oriented spindles to face NuMA basal domains  
197 along the A-B axis (Figure 2e-g). We conclude that previously established generic models for  
198 spindle orientation may not simply account for the observed spindle planarity, and that  
199 canonical polarity complexes may not influence spindle orientation in this tissue.

200

201

202 **Length-limitations of M-phase astral MTs mediated by the depolymerizing kinesin**  
203 **Kif18B promote spindle planarity.**

204

205 Because M-phase astral MT growth and lengths are bounded by dynamic instabilities, we  
206 tested a model based on a length limitation of astral MTs (Stout *et al.*, 2011; Verde *et al.*,  
207 1992). We reasoned that such limitation could in principle prevent astral MTs from reaching  
208 basal poles of elongated mitotic cells, and create an anisotropy of mitotic aster pair shapes  
209 now longer along the planar axis. Remarkably, in 2D models, this limitation coupled to length-  
210 dependent MT forces robustly predicted planar spindle orientation for a range of cell shapes  
211 and model parameters (Figure 2e-h; Figure S3a-d). Interestingly, however as cells became  
212 too elongated along the A-B axis, spindles eventually turned to a preferred orientation along  
213 the long A-B axis, yielding a bi-stable phase diagram, controlled by the distance from MT (+)-  
214 tips to the basal poles and cell shape aspect ratio (Figure 2f-h). These modelling results  
215 suggest that a cell shape sensing mechanism truncated by a limit in MT length, could in  
216 principle function to orient spindles in the plane of intestinal organoids.

217 To test this hypothesis, we imaged the distribution of astral MTs around spindles. We  
218 implemented expansion microscopy of intestinal organoids to visualize and quantify individual  
219 astral MTs. This revealed the presence of astral MT (+)-tips in close contacts with both apical  
220 and lateral cortices (Figure 3a,c; Figure S3e-g). In sharp contrast, MTs were barely detected  
221 in contact with the basal side, with MT (+)-tips located at a distance  $\sim 4x$  higher on average  
222 from the basal cortex than the apical one (Figure 3a-c; Figure S3e,h). Importantly, these  
223 effects were not caused by a putative anisotropy in MT nucleation, as the number of MTs  
224 growing apically vs basally was similar (Figure 3d). In addition, imaging of the (+)-tip  
225 associated protein EB3-GFP confirmed this exclusion in live tissues, and indicated that the  
226 asymmetric basal exclusion of MTs was inherited from the initial apical migration of  
227 centrosomes and asters (Figure S3i, Video S3). To further test this differential MT-cortex  
228 interaction, we performed laser severing of groups of MTs in  $\alpha$ -tubulin-GFP organoids along  
229 a line-scan orthogonal to the A-B axis, placed either on the apical or basal side of spindles  
230 (Figure 3e-f; Figure S3j-k). Both caused spindle recoils away from the cuts suggesting that  
231 astral MTs predominantly exert pulling forces, presumably by engaging with dynein motors at  
232 the cortex (Farhadifar *et al.*, 2020; Grill and Hyman, 2005). However, in agreement with the



233 lesser extent of MTs reaching the basal cortex, basal cuts caused significantly smaller recoils  
234 than apical ones (Figure 3f; Figure S3k). These data further support an asymmetry in MT  
235 cortex contacts along the A-B axis, and a lack of contribution of basal polarity cues to MT  
236 forces and spindle orientation.

237

238 To directly assay the role of MT length regulation, we next sought to increase astral MTs length  
239 and assess impact on spindle orientation. Kif18B, a (+)-tip depolymerizing kinesin, was shown  
240 to promote MT catastrophe and thus limit the length of M-phase astral MTs in mammalian  
241 cells and tissues (McHugh et al., 2018; Stout *et al.*, 2011). Accordingly, knocking out Kif18B,  
242 using a CRISPR-Cas9 inducible system in organoids, led to significantly longer astral MTs, so  
243 that even the basal-facing MTs now grew long enough to reach the cell cortex (Figure 3g-h;  
244 Figure S3l-m). Strikingly, spindles in Kif18B-KO organoids were not planar anymore, and  
245 rather oriented randomly, with a significant fraction of spindles oriented along the A-B axis  
246 (Figure 3i). Together, these results suggest that a length-limitation of mitotic astral MTs  
247 mediated in part by depolymerizing kinesins, may prevent MTs from interacting with basal  
248 polarity factors and allow spindles to orient in the tissue plane by probing the geometry of the  
249 apical fraction of dividing cells.

250

### 251 **A 3D model for spindle position and orientation in intestinal crypts**

252

253 In order to validate this length-limitation hypothesis, we explored the parameter space in the  
254 model phase diagram (Figure 2f,h) by experimentally altering cell shapes (Figure 4a-c). We  
255 first elongated cells in the tissue plane, to validate a general influence of apical cell shape. We  
256 grew organoids in L-WNR medium, an exogenous global Wnt3a treatment, which results in  
257 the formation of hyper-proliferative and undifferentiated cystic structures (Farin et al., 2012;  
258 Sato et al., 2011b), in which the epithelial monolayer displays a flat squamous morphology  
259 with cells now elongated in the tissue plane (Figure S4a). As predicted by models, and not  
260 surprisingly, spindles oriented parallel to the long cell shape axis in the tissue plane in this  
261 condition (Figure 4a-d; Figure S4a). To increase mitotic cell elongation along the A-B axis,  
262 we next affected contractility by inhibiting myosin-IIA activity. Both blebbistatin treatment or  
263 myosin-IIA-KO drastically impaired mitotic shape changes and rounding, with cells remaining  
264 significantly more elongated along the A-B axis than controls (Figure 4a-c; Figure S4b-e). This  
265 resulted from a loss of cellular rearrangements and the formation of fewer multicellular  
266 contacts in the basal environment of metaphase cells (Figure S4d-h). These results confirmed  
267 the primary function of basal actomyosin contractility in reshaping mitotic cells and its contacts  
268 with neighbors in this tissue. Importantly, as predicted in models (Figure 2f,h), in these more  
269 A-B elongated metaphase cells, spindles now turned to preferentially orient along the long A-

270 B cell shape axis (Figure 4a-b,d). Importantly, in these conditions, polarity complexes  
271 detached from the cell cortex (Figure S4i-j) and spindles were still positioned off-center  
272 towards the apical cell poles (Figure 4b), ruling out putative contributions of basal polarity to  
273 this A-B spindle orientation.

274

275 To test our hypothesis in these multiple conditions affecting cell shapes using realistic 3D cell  
276 geometries, we next turned to simulations. We adapted a gradient descent strategy that can  
277 predict both spindle orientation and position in 3D from the 3D geometry of mitotic cells, the  
278 localization of polarity cues and length limitation in astral MTs (Pierre *et al.*, 2016). Using 3D  
279 segmentations of individual cell contours, spindle poles and basal domains from fixed tissues,  
280 we reconstituted experimental cell shapes, polarity and spindle orientation and position in  
281 mitotic cells (Figure 4e). By inputting the experimental geometry and a limit for MT growth at  
282 the basal pole in the model, we ran simulations starting from random positions and  
283 orientations, and searched for equilibrium. The simulations were robust to initial conditions  
284 and predicted with accuracy in 3D, the planarity of spindles and their apical shifts in control  
285 and L-WNR, as well as their reorientation along the A-B axis in more elongated blebbistatin-  
286 treated cells (Figure 4e-g; Figure S5a-c, and Videos S4-5). In contrast, models in which astral  
287 MTs filled whole cell volumes, or models based on a predominant influence of NuMA domains,  
288 yielded poor agreements with experiments (Figure S5d-g). Importantly, the model predicted  
289 deviations to planarity in a dose-dependent manner, among more or less elongated wt or  
290 blebbistatin treated cells, and also the correct orientation of spindles within the tissue plane,  
291 when shape anisotropies were present in this plane (Figure 4e-f). These direct comparisons  
292 between 3D model predictions and experiments strongly support that length-limitations of  
293 astral MTs can account for spindle position and planar orientation in intestinal crypts.

294

295

### 296 **Lengthening astral MTs affect epithelial tissue layering**

297

298 This truncated shape sensing may have many advantages for the regulation of tissue layering  
299 and cell density. For instance, it may allow cells to adapt division orientation to planar tissue  
300 forces, tilting spindles along the A-B axis if cells become over-compressed by their neighbors,  
301 providing a potential homeostatic mechanism to regulate cell density and monolayered  
302 architecture (Wyatt *et al.*, 2015). Accordingly, in Kif18B-KO organoids, as a consequence of  
303 spindle mis-orientation, planar polarity of cytokinesis and placement of daughter cells were  
304 largely impaired (Figure 5a-b). Hence, the regular basal nuclear arrangement, which is a  
305 hallmark of polarized columnar epithelia, was lost three days after KO induction (Figure 5a,c-  
306 d). Finally, nuclei densities were significantly reduced in the Kif18B-KO (Figure 5e), showing

307 how spindle mis-orientation may impact crypt architecture and cell density. These results  
308 directly demonstrate how a modulation in astral MT length may impact spindle planarity and  
309 architecture of a mammalian tissue.

310  
311

## 312 **DISCUSSION**

313  
314  
315

### **A new generic model for planar spindle orientation in proliferative epithelia**

316 How spindle orientation is regulated in proliferative tissues and stem cell niches is of  
317 fundamental importance for organ morphogenesis and homeostasis, and highly relevant to  
318 human disorders (Caussin and Gonzalez, 2005; McCaffrey and Macara, 2011; Nakajima *et*  
319 *al.*, 2013; Seldin and Macara, 2017). Here, by documenting with high temporal and spatial  
320 resolution spindle orientation together with MT dynamics, cell shape and polarity in a 3D  
321 mammalian proliferative organoid, we propose a new model for the control of planar spindle  
322 orientation and monolayered tissue architecture. This model is based on a partial cell shape  
323 sensing resulting from a length-limitation of M-phase astral MTs. This limitation is coupled to  
324 the A-B polarity of the columnar tissue, and allows initially apically positioned centrosomes to  
325 stop their basal migration at a position shifted towards the apex, and astral MTs to probe the  
326 local apical fraction of mitotic cells to orient spindles in the tissue plane (Figure 5f). This  
327 mechanism has similarities with previous models proposed for asymmetric aster pair  
328 positioning and orientation in some large zygotes featuring anisotropic MT asters (Pierre *et*  
329 *al.*, 2016; Wuhr *et al.*, 2008) (Figure 5f). Importantly, our model contrasts with established  
330 ones in many epithelia, in which the role of polarity effectors including NuMA, LGN or afadin  
331 recruited to lateral cortices, is thought to be predominant to orient spindles in the tissue plane  
332 (di Pietro *et al.*, 2016). In intestinal crypts, their unconventional localization to the basal domain  
333 of mitotic cells suggest they may not contribute to orient spindles, and raise the question of  
334 how they may be segregated there. We found that myosin-IIA inhibition caused their  
335 detachment from the basal cortex (Figure S4i-j). Therefore, we suggest that basal polarity  
336 recruitment could be driven by enhanced local cortical tension associated to basal actomyosin  
337 activity and cellular rearrangements (Figure 1d-e; Figures S1a-g), as proposed in epithelial  
338 cell lines (Bosveld *et al.*, 2016; Carminati *et al.*, 2016; Gloerich *et al.*, 2017; Hart *et al.*, 2017).  
339 Other plausible mechanisms contributing to this basal localization might include a local  
340 clearance of polarity effectors from the apex from chromosome-derived signals  
341 (Dimitracopoulos *et al.*, 2020; Kiyomitsu and Cheeseman, 2012), or a recruitment associated  
342 to the numerous basal multicellular contacts formed in mitosis (Bosveld *et al.*, 2016).

343

344 Interestingly, asymmetric actomyosin activity in other epithelial cells and tissues has been  
345 proposed to directly influence spindle orientation. In *Drosophila*, one study notably reported  
346 that local actomyosin enrichment may override geometrical cues to orient spindles in face of  
347 actomyosin rich zones (Scarpa et al., 2018). In cultured cells, a recent local optogenetic assay  
348 showed an opposite behavior with spindles orienting orthogonal to the most contractile cortical  
349 zone (Kelkar et al., 2020). Our findings in the crypt align more with this latter finding, at least  
350 in term of geometrical outputs. However, our results favor a more indirect role for basal  
351 actomyosin asymmetric enrichment, primarily impacting spindle orientation through its role in  
352 driving a partial and asymmetric mitotic rounding. We currently do not understand which  
353 mechanism may enrich actomyosin at the basal poles of mitotic crypt cells. We suggest it may  
354 reflect the need for apical migrations of dividing cells in columnar tissues, as proposed in the  
355 mouse nervous system or zebrafish retina (Norden et al., 2009; Schenk et al., 2009).  
356 Importantly, in addition to promote anisotropic rounding, we also propose that this pool  
357 functions to maintain epithelial cohesion at the base of mitotic cells (Figure 1d-e; Figures S1a-  
358 g, 4d-f). Further exploration of the regulation and role of this mitotic basal actomyosin  
359 enrichment, may reveal important aspects of intestinal crypt dynamics and architecture.

360

#### 361 **Length-regulation of M-phase MTs, mitotic rounding and spindle orientation**

362 Astral MTs control spindle positioning in organisms ranging from yeast to mammals (McNally,  
363 2013). While most models have thus far largely assumed that astral MTs grow to reach the  
364 cell cortex, to probe cell shapes and polarities, recent studies have suggested that M-phase  
365 astral MTs which are bounded by dynamic instabilities, may be just long enough in mitosis to  
366 reach the cortex of fully rounded cells (Mitchison et al., 2015). Accordingly, impairment of  
367 mitotic rounding has been shown to prevent MTs from properly reaching and interacting with  
368 the cortex, yielding defects in chromosome segregation and spindle orientation (Lancaster et  
369 al., 2013; Luxenburg *et al.*, 2011). In our study, we propose that such cell-cycle regulated  
370 length limitation is directly exploited by the tissue to allow the spindle to only probe apical cell  
371 shapes, position asymmetrically and orient in the tissue plane. Results from Kif18B-KO  
372 organoids favor a global regulation of astral MT catastrophe and length in mitosis, which  
373 translates into an A-B asymmetric MT cortex contact from the initial apical location of  
374 centrosomes. However, we also envisage that more local basal regulation of MT dynamics or  
375 dynein activity could emerge directly from enhanced basal actomyosin activity, or from putative  
376 higher basal organelle crowding, as proposed in other systems (Jimenez et al., 2021; Pierre  
377 *et al.*, 2016; Zhu et al., 2010). While the impact of cell shape on division orientation was first  
378 reported more than 150 years ago (O., 1884), mechanisms by which geometries are being  
379 sensed in multicellular tissues are still at their infancy (Haupt and Minc, 2018). Our study  
380 provides an important new generic mechanism to orient spindles along the short axis in

381 mammalian epithelia, and calls for a better exploration of mechanisms regulating astral MT  
382 length and mitotic shape changes in multicellular tissues.

383

### 384 **Functions of planar divisions for crypt dynamics and architecture**

385 Planar cell divisions are key for maintaining the monolayered architecture of certain epithelial  
386 tissues. Conversely, programmed alterations of planar divisions along the A-B axis of the  
387 tissue are required for epithelial stratification such as during skin development in the mouse  
388 embryo (Biggs et al., 2020; Lechler and Fuchs, 2005). In the curved 3D geometry of intestinal  
389 crypts, we found that cell divisions orient parallel to the local plane of curvature of the tissue.  
390 We propose that such organization of cell divisions may contribute to promote a near-isotropic  
391 expansion of the tissue while maintaining a regular monolayered architecture. Accordingly,  
392 altering spindle planarity resulted in mis-placed daughter cells, often protruding into the apical  
393 lumen (Figure 5a-e). Whether such defects could lead to the long-term formation of tumor  
394 masses, epithelial dysplasia or hyperplasia, or if mechanisms such as apoptosis or daughter  
395 cell reintegration may safeguard the intestinal tissue, remain to be tested (Lough et al., 2019).  
396 In addition, many of the dividing cells in the crypt are Lgr5<sup>+</sup>-stem cells, which may undergo  
397 symmetric fate divisions for self-renewal or asymmetric divisions to generate a daughter cell  
398 that becomes fated. As such, whether alteration of planar spindle positioning affects the auto-  
399 renewal properties of organoids and their homeostasis, as previously described for instance  
400 in Apc-mutated intestinal tissues, is another important open question (Dow et al., 2015; Feng  
401 et al., 2013). More work on the regulation and function of oriented division in mammalian stem  
402 cell niches, tissues and organs may help to better appreciate the emergence of human  
403 epithelial disorders.

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424

425 **Supplemental Information:** Figures S1-S5 and Videos S1-S5 are available in the online  
426 version of the paper.

427

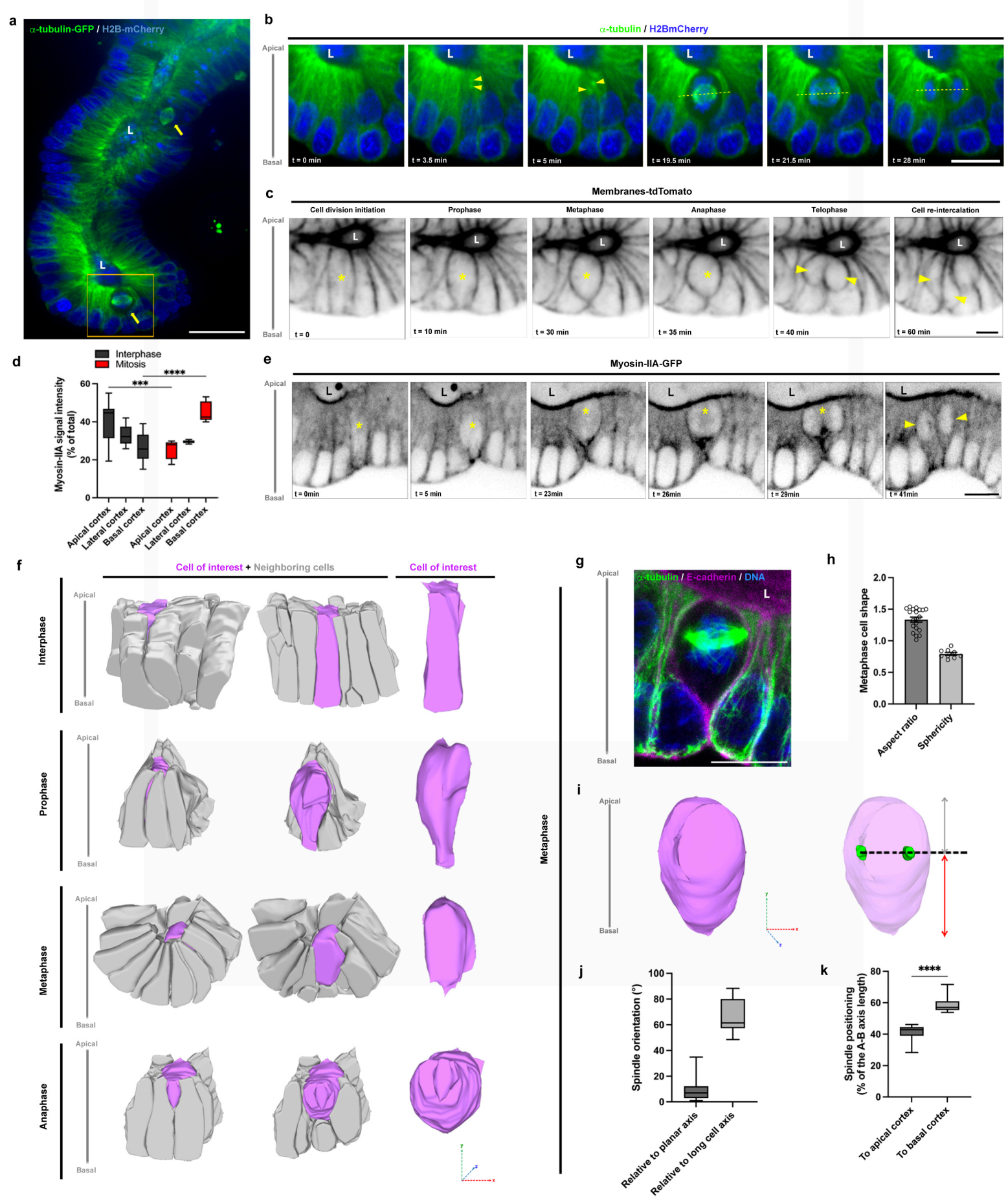
428 **Author contributions:** J.S, O.F, M.A.F, M.S., C.G., T.D., HC, and D.D. performed  
429 experiments. N.M. designed and performed models and simulations. J.S, M.A.F., C.G., N.M.  
430 and D.D. designed the experiments. J.S, M.A.F, C.G., M.S., H.C., N.M. and D.D. performed  
431 analyses. J.S, N.M. and D.D. coordinated the overall research and experiments, and wrote  
432 the manuscript.

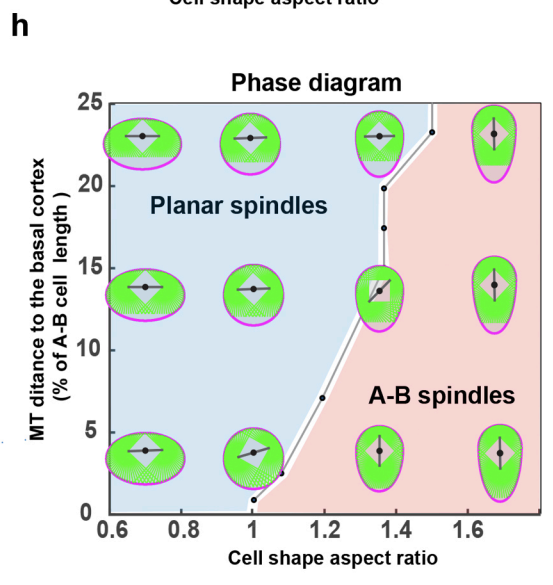
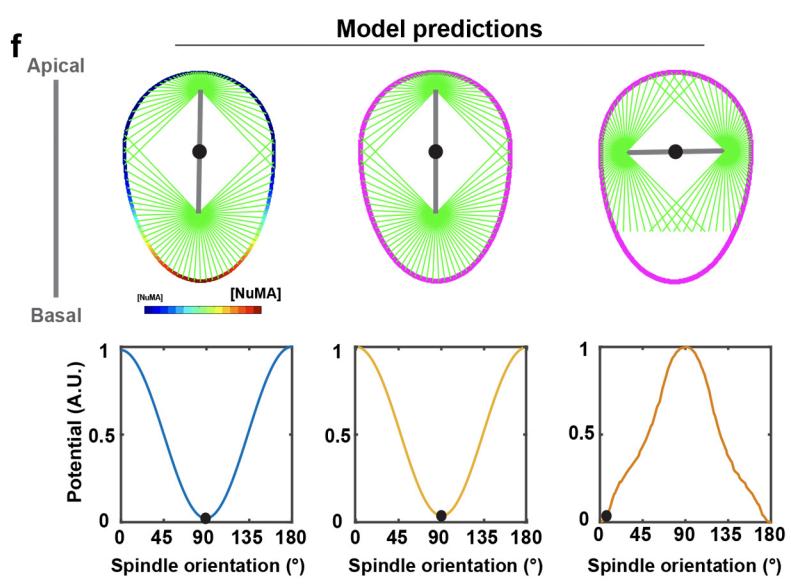
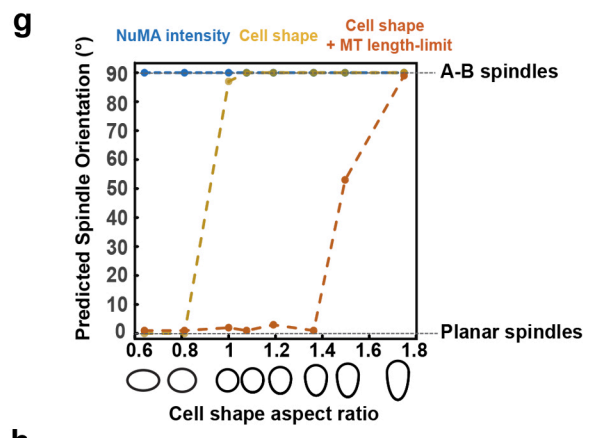
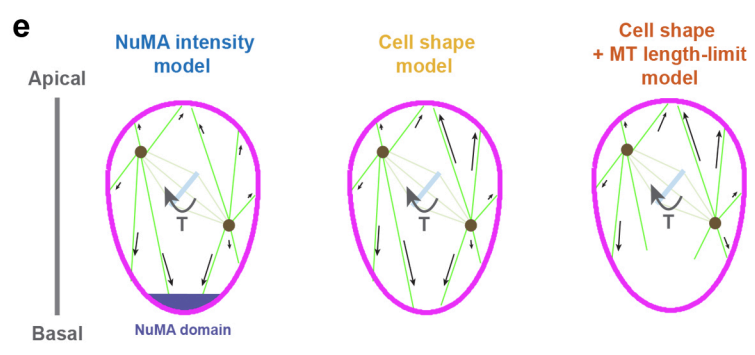
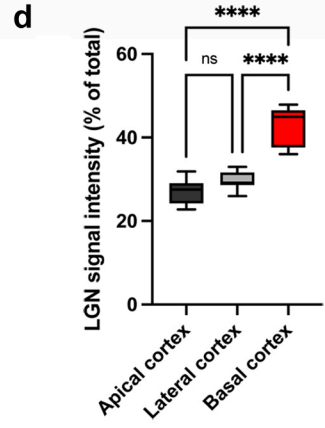
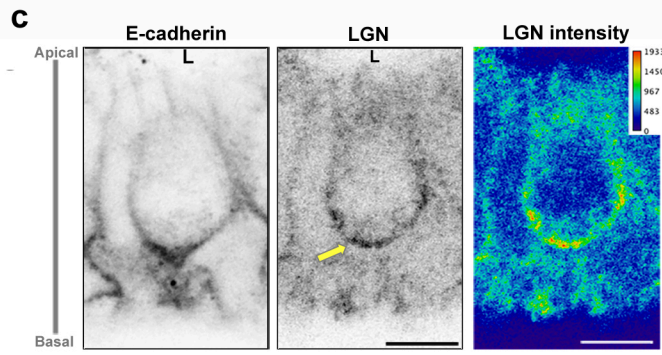
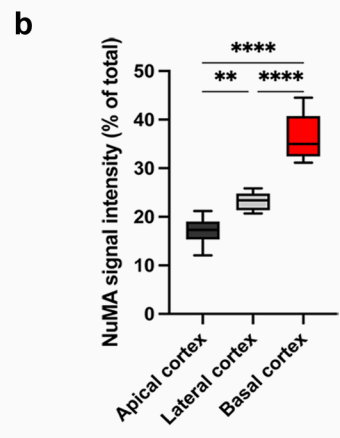
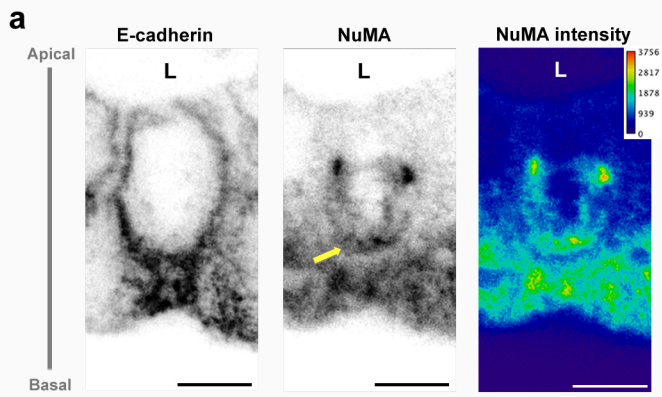
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434 **Conflict of interest:** The authors declare no conflict of interest.

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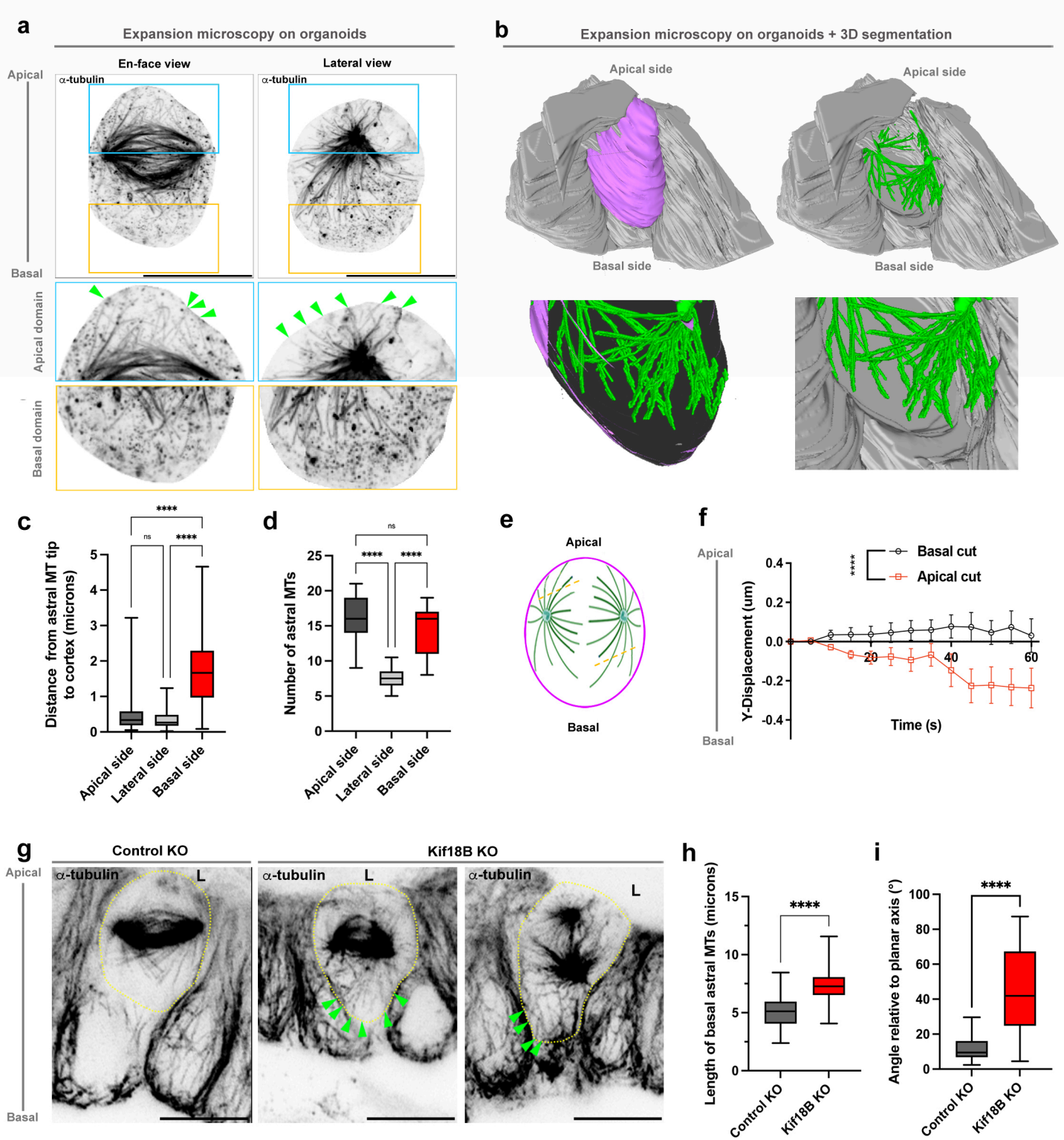
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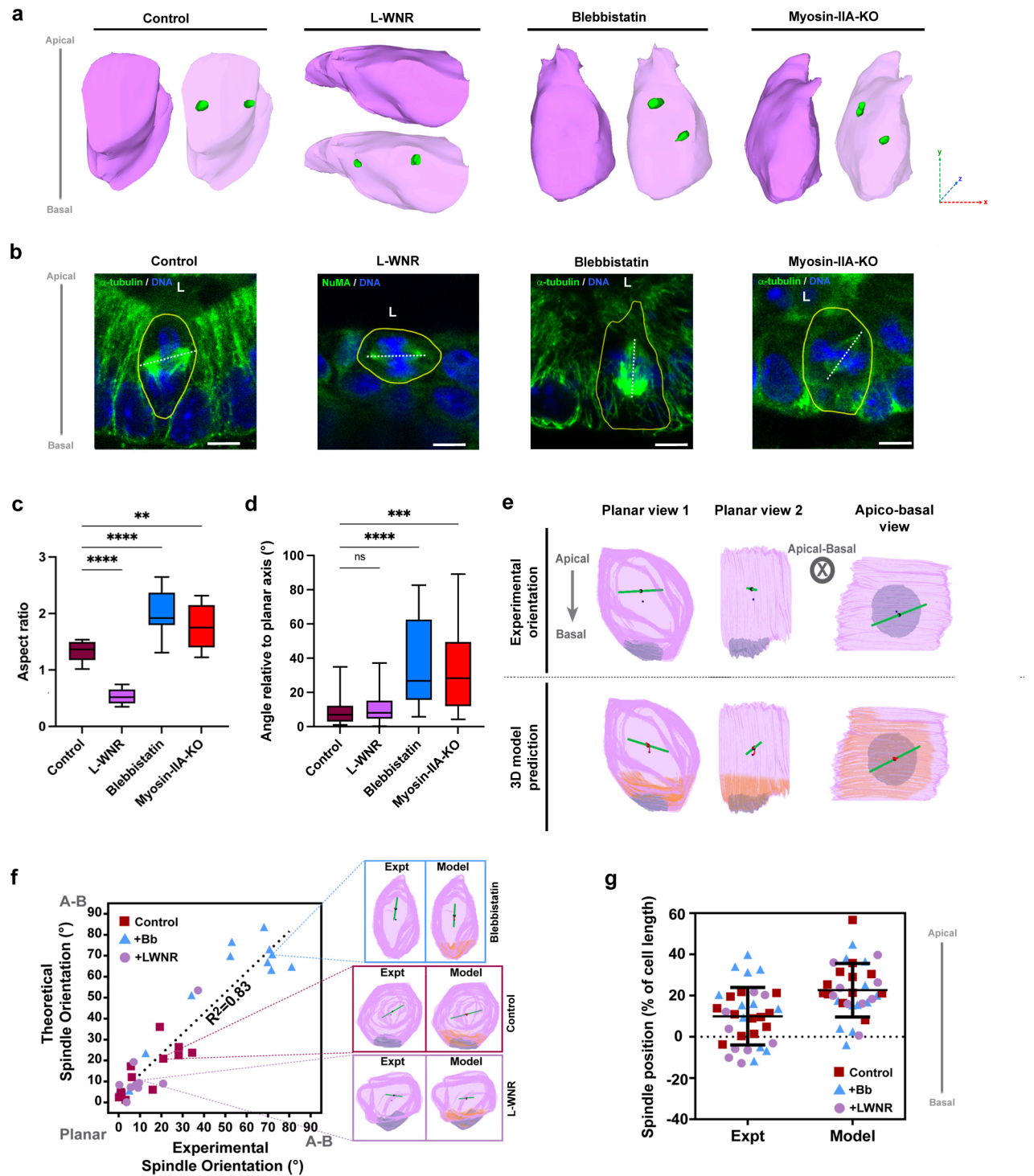


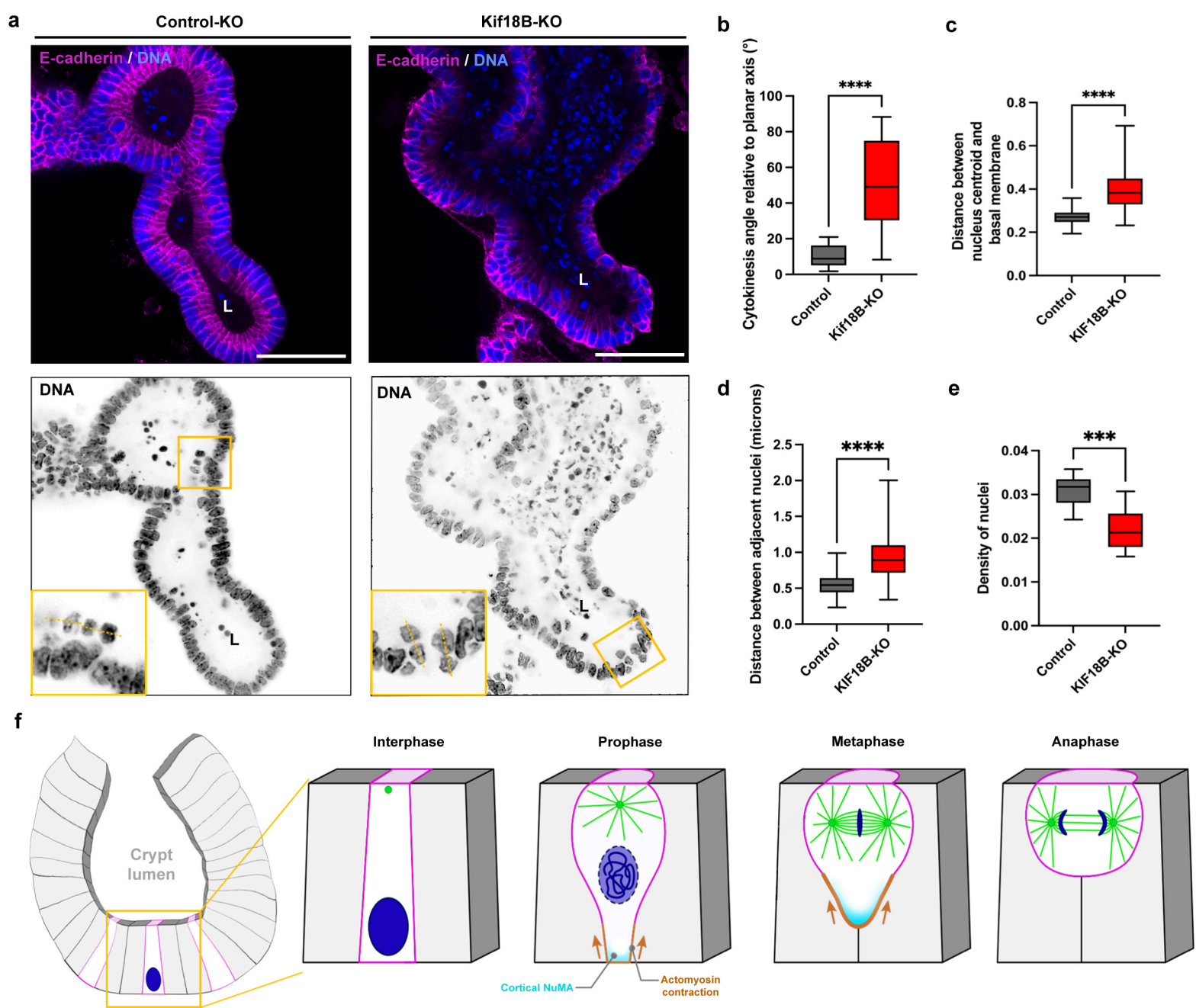


Saleh et al., Figure 2









437 **FIGURE LEGENDS**

438 **Figure 1: An atypical metaphase cell rounding and spindle orientation in intestinal**  
439 **organoids. a** Spinning-disk analysis of  $\alpha$ -tubulin-GFP (green) and H2B-mCherry (blue)  
440 distribution in intestinal crypt organoids. Yellow arrows point on organoid crypt-like structures.  
441 L, lumen. Scale bar, 50 $\mu$ m. **b** Time lapse images of  $\alpha$ -tubulin-GFP (green) and H2B-mCherry  
442 (blue) during mitosis. Yellow arrowheads point on migrating centrosomes. Yellow dotted lines  
443 highlight spindle axis. Scale bar, 10 $\mu$ m. **c** Time lapse images of tdTomato organoids showing  
444 mitosis progression. Yellow star points on a dividing cell. Yellow arrowheads point on daughter  
445 cell separation and re-integration in the epithelial monolayer. Scale bar, 5 $\mu$ m. **d** Statistical  
446 analysis of the percentage of total signal intensity for myosin-IIA-KI-GFP at the apical, lateral  
447 or basal cortex in interphase (grey) or during mitosis (red). n = 10 cells. Two-way ANOVA test  
448 and Tukey's multiple comparison test, \*\*\*p <0.001, \*\*\*\*p <0.0001. **e** Time-lapse of a live-  
449 imaged cell division in myosin-IIA-KI-GFP organoid crypt. Yellow star points on a dividing cell.  
450 Yellow arrowheads point on daughter cell separation and re-integration in the epithelial  
451 monolayer. Scale bar, 10 $\mu$ m. **f** Representative 3D rendering of metaphase cell (magenta) and  
452 neighboring cells (grey) after segmentation of cell membranes based on confocal z-stacks in  
453 crypt organoids. Spatial coordinates are shown. **g** Confocal analysis of  $\alpha$ -tubulin (green) and  
454 E-cadherin (magenta) distribution in organoid metaphase cells. Scale bar, 10 $\mu$ m. **h**  
455 Quantitative analyses of the aspect ratio (distance between the spindle axis and the basal  
456 membrane over the distance between the spindle axis and the apical membrane) (n = 20 cells)  
457 and sphericity (n = 9 cells) of the organoid metaphase cells. Aspect ratio =  $1.33\pm 0.04$   
458 (mean $\pm$ S.E.M), sphericity =  $0.79\pm 0.02$ . **i** Representative 3D rendering after segmentation of  
459 cell membranes and spindle poles from confocal z-stacks of organoid metaphase cells. Cell  
460 shape is depicted in magenta and in transparency, spindle poles in green. Distances between  
461 the spindle axis (black dotted line) and the apical (grey arrow) or basal membrane (red arrow)  
462 are shown. Spatial coordinates are shown. **j** Statistical analysis of the spindle orientation  
463 relative to planar axis (grey) or long cell axis (light grey). Spindle orientation relative to planar  
464 axis =  $8.61\pm 1.52^\circ$  (mean $\pm$ S.E.M), to long cell axis =  $66.13\pm 3.8^\circ$ . N = 3 experiments, n(planar  
465 axis) = 25 cells, n(long cell axis) = 12 cells. **k** Statistical analysis of the percentage of the  
466 distance between the spindle axis and the apical (grey) or basal (light grey) membrane.  
467 Distance from apical membrane =  $40.728\pm 1.485\%$  (mean $\pm$ S.E.M), from basal membrane =  
468  $59.272\pm 1.485\%$ . N = 3 experiments, n = 13 cells. Paired t-test, \*\*\*\*p <0.0001.

469

470 **Figure 2: A 2D model for spindle orientation predicts that length limitation in M-phase**  
471 **astral microtubules may account for spindle planarity. a** Confocal analysis of the

472 distribution of E-cadherin and NuMA in mouse organoid metaphase cells. NuMA signal  
473 intensity is color-coded with Fire LUT table from ImageJ on the right panel. Color scale bar  
474 indicates the gray value intensity. Yellow arrow points to basal accumulation of NuMA. Scale  
475 bar, 5  $\mu\text{m}$ . **b** Statistical analysis of NuMA signal intensity at the apical, lateral or basal cortex.  
476 Signal intensity at the apical cortex =  $17.04 \pm 0.86$  (mean  $\pm$  S.E.M), lateral cortex =  $23.23 \pm 0.58$ ,  
477 basal cortex =  $36.26 \pm 1.5$ . n = 10 cells. One-way ANOVA test and Tukey's multiple comparison  
478 test, \*\*p = 0.008, \*\*\*\*p < 0.0001. For each experiment, three independent experiments were  
479 carried out. **c** Confocal analysis of the distribution of E-cadherin and LGN in human organoid  
480 metaphase cells. LGN signal intensity is color-coded with Fire LUT table from ImageJ on the  
481 right panel. Color scale bar indicates the gray value intensity. Yellow arrow points to basal  
482 accumulation of LGN. Scale bar, 5  $\mu\text{m}$ . **d** Statistical analysis of LGN signal intensity at the  
483 apical, lateral or basal cortex. Signal intensity at the apical cortex =  $26.93 \pm 0.89$  (mean  $\pm$  S.E.M),  
484 lateral cortex =  $29.69 \pm 0.67$ , basal cortex =  $43.14 \pm 1.42$ . n = 10 cells. One-way ANOVA test  
485 and Tukey's multiple comparison test, \*\*\*\*p < 0.0001. For each assay, three independent  
486 experiments were carried out. **e** Schemes representing the core hypothesis of the distribution  
487 of microtubule (MT) length and polarity domains in the 3 tested models for spindle orientation.  
488 **f** Rotational potential energy profiles plotted as a function of spindle orientation angles with  
489 respect to the planar axis predicted by the 3 different models. The black dot marks the  
490 preferred spindle orientation at the minimum of the potential profiles. **g** Predicted preferred  
491 spindle orientation for a range of cell shapes with increasing aspect ratios in the tissue plane  
492 or along the A-B axis. Note how the model based on cell shape coupled to length limitation in  
493 MTs transits from predicting planar spindles to apico-basal oriented spindles when cells  
494 become over-elongated, along the A-B axis. **h** Phase diagram of the predicted preferred  
495 spindle orientations for the model based on cell shape and a limit in MT lengths, drawn as a  
496 function of the 2 control parameter, the distance of MT-(+)tips to the basal side and the  
497 elongation of mitotic cell shapes.

498

499 **Figure 3: Existence of a length-limit for M-phase astral MTs that impact spindle**  
500 **orientation.** **a** Projected confocal z-stacks of  $\alpha$ -tubulin distribution in isolated organoid  
501 metaphase cell. En-face and lateral views are presented. Apical domain areas boxed in blue  
502 and basal domain areas boxed in yellow are presented in the low panels. Green arrowheads  
503 point to astral MTs contacting the apical cortex. Corrected scale bar, 10  $\mu\text{m}$ . **b** Representative  
504 3D rendering of a metaphase cell (magenta), neighboring cells (grey), spindle poles and  
505 microtubules (green) after segmentation of a confocal z-stack of E-cadherin and  $\alpha$ -tubulin  
506 localization. Are depicted A-B views tilted to show basal cortex. **c** Statistical analysis of the  
507 distance between the astral MT tip and the apical, lateral or basal cortex. Distance between

508 astral MT tip and the apical cortex =  $0.466 \pm 0.045$  (mean $\pm$ S.E.M), the lateral cortex =  
509  $0.349 \pm 0.025$ , the basal cortex =  $1.685 \pm 0.086$ . N = 3 experiments, n = 11 cells. N = 104 apical  
510 astral MTS, n = 102 lateral astral MTs, n = 107 basal astral MTS. Two-way ANOVA with  
511 Tukey's multiple comparisons test, \*\*\*\*p < 0.0001; ns, non-significant. **d** Statistical analysis of  
512 the number of astral MTs at the apical, lateral and basal sides. Number of astral MTs at the  
513 apical side =  $16.3 \pm 1.09$  (mean $\pm$ S.E.M), at the lateral side =  $7.6 \pm 0.5$ , at the basal side =  
514  $14.2 \pm 1.06$ . N = 3 experiments, n = 11 cells. Unpaired t-test, \*\*\*\*p < 0.0001; ns, non-significant.  
515 **e** Scheme showing the position of apical or basal laser cut (yellow dotted lines) of astral MTs.  
516 **f** Statistical analyses of the spindle pole displacement in the y-axis after apical or basal laser  
517 cut in organoid metaphase cells. n = 6 cells for each condition. Unpaired t-test, \*\*\*\*p < 0.0001.  
518 **g** Confocal analysis of  $\alpha$ -tubulin distribution in metaphase cells in control-KO or Kif18B-KO  
519 organoids. Yellow dotted line delimits the metaphase cell. Green arrowheads point to astral  
520 MTs contacting the basal cortex. L, lumen. Scale bar, 10 $\mu$ m. **h** Statistical analysis of the length  
521 of basal astral MTs in metaphase cells of control-KO or Kif18-KO organoids. n = 99 astral MTs  
522 in 13 control-KO cells, n = 103 astral MTs in 17 Kif18-KO cells. Unpaired t-test, \*\*\*\*p < 0.0001.  
523 **i** Statistical analysis of the spindle axis orientation relative to the planar axis of the epithelium  
524 in metaphase cells of control-KO or Kif18-KO organoids. n = 32 control-KO cells, n = 50 Kif18-  
525 KO cells. Unpaired t-test, \*\*\*\*p < 0.0001. For each experiment, three independent experiments  
526 were carried out.

527

528 **Figure 4: Metaphase cell shape impacts planar spindle orientation in organoid cells. a**  
529 Representative 3D rendering of cell shape and spindle pole positioning after segmentation of  
530 cell membranes and NuMA spindle-associated signal from confocal z-stacks of control  
531 (DMSO-treated), L-WNR-cultured, blebbistatin-treated or induced myosin-IIA-KO organoid  
532 metaphase cells. Cell shape is depicted in magenta, spindle poles in green. Spatial  
533 coordinates are shown. **b** In control (DMSO-treated), L-WNR-cultured, blebbistatin-treated or  
534 induced myosin-IIA-KO organoid metaphase cells, is shown the confocal image of  $\alpha$ -tubulin  
535 or NuMA (green) and nuclei (blue) Scale bar, 5 $\mu$ m. **c** Statistical analyses of organoid  
536 metaphase cell aspect ratio in control (DMSO-treated), L-WNR-cultured, blebbistatin-treated  
537 or induced myosin-IIA-KO organoid metaphase cells. Aspect ratio of metaphase cells in  
538 control organoids =  $1.33 \pm 0.04$  (mean $\pm$ S.E.M), in L-WNR-cultured organoids =  $0.53 \pm 0.04$ , in  
539 blebbistatin-treated organoids =  $2.01 \pm 0.12$ , in myosin-IIA-KO organoids =  $1.78 \pm 0.13$ . n  
540 (control organoids) = 20 cells, n (L-WNR organoids) = 10 cells, n (blebbistatin-treated  
541 organoids) = 10 cells, n (myosin-IIA-KO organoids) = 10 cells. One-way ANOVA, with Tukey's  
542 multiple comparisons tests, \*\*p = 0.0011, \*\*\*\*p < 0.0001. **d** Statistical analyses of the spindle  
543 axis orientation relative to the plane of the epithelium. Angle deviation in control organoids =

544 8.61±1.52 (mean±S.E.M), in L-WNR-cultured organoids = 11.28±3.74, in blebbistatin-treated  
545 organoids = 37.11±5.04, in myosin-IIA-KO organoids = 34.88±5.22. n (control organoids) = 25  
546 cells, n (L-WNR organoids) = 9 cells, n (blebbistatin-treated organoids) = 25 cells, n (myosin-  
547 IIA-KO organoids) = 25 cells. One way-ANOVA, \*\*\*p = 0.0001, \*\*\*\*p <0.0001. ns, non-  
548 significant. For each experiment, three independent experiments were carried out. **e** (Top) 3D  
549 reconstitution of experimental cells obtained from 3D z-stacks of a representative dividing cell  
550 labeled for E-cadherin and NuMA to extract cell shape (magenta), spindle orientation (green  
551 line) and NuMA accumulation at the basal cortex (grey domain). (Bottom) Corresponding  
552 simulation output, based on MT length-dependent forces (shape sensing) and an exclusion of  
553 MTs length in the basal domain region (orange zone). The red traces represent the history of  
554 spindle axis displacement in the simulation. Both experimental and simulated cells are viewed  
555 in different planes with respect to A-B axis. **f**. Predicted spindle orientation angle with respect  
556 to the A-B axis, plotted as a function of the experimental axis for 10 individual control, L-WNR  
557 treated or blebbistatin-treated crypt cells. Insets provide representative examples of  
558 experimental and simulated spindle orientation in the different treatments. The dotted line is a  
559 linear fit, with a slope of 0.96 (1 being a perfect agreement between models and experiments).  
560 R<sup>2</sup> is the correlation coefficient between the fit and the data. **g**. Experimental and theoretical  
561 prediction of spindle asymmetric position towards the apical cell poles in the indicated  
562 conditions.

563

564 **Figure 5: Impact of spindle mis-orientation in epithelial organization.** **a** Confocal  
565 microscopy analysis of the distribution of the E-cadherin (magenta) and nuclei (blue) in control-  
566 KO and Kif18B-KO organoids. Representative cytokinesis events are boxed in yellow and  
567 presented in bottom left. Yellow dotted lines highlight cell division axis. L, lumen. Scale bar,  
568 50µm. **b** Statistical analysis of cytokinesis angle relative to the planar axis in control-KO or  
569 Kif18B-KO organoids. n = 13 control-KO cells, n = 26 Kif18B-KO cells. Cytokinesis angle  
570 (control-KO) = 10.3±1.68°, cytokinesis angle (Kif18B-KO) = 50.8±4.9°. Unpaired t-test, \*\*\*\*p  
571 < 0.0001. **c** Statistical analysis of interphase nuclear positioning (distance from the centroid of  
572 the interphase nucleus to the basal membrane) (n = 100 cells). Nuclear positioning (control-  
573 KO) = 0.271±0.003 µm (mean±S.E.M), (Kif18B-KO) = 0.399±0.01 µm. Unpaired t-test, \*\*\*\*p <  
574 0.0001. **d** Statistical analysis of distance between adjacent interphase nuclei (n = 100 cells).  
575 Distance between nuclei (control-KO) = 0.559±0.015 µm (mean±S.E.M), (Kif18B-KO) =  
576 0.939±0.032 µm. Unpaired t-test, \*\*\*\*p < 0.0001. **e** Statistical analysis of nucleus density (n =  
577 10 fields). Nucleus density (control-KO) = 0.031±0.001 µm (mean±S.E.M), (Kif18B-KO) =  
578 0.022±0.001 µm. Unpaired t-test, \*\*\*p = 0.0002. **f** Scheme depicting the proposed model of  
579 spindle polarity modulation in organoid metaphase cells.

580 **STAR METHODS**

581

582 **Resource availability**

583

584 **Lead contact**

585 Further information and requests for resources and reagents should be directed and will be  
586 fulfilled by the lead contacts, Delphine Delacour ([delphine.delacour@ijm.fr](mailto:delphine.delacour@ijm.fr)) and Nicolas Minc  
587 ([nicolas.minc@ijm.fr](mailto:nicolas.minc@ijm.fr)).

588

589 **Materials availability**

590 Materials generated in the current study are available from the lead contacts upon request.

591

592 **Data and code availability**

593 New generated codes and data obtained in the current study are available from the lead  
594 contacts on request.

595

596

597 **Experimental model and subject details**

598

599 **Organoid culture and transfection**

600 Wild-type C57/Bl6 mice were provided by the animal house facility of the Institut Jacques  
601 Monod. Mice used for intestinal crypt isolation were between 6 and 12 weeks old. After  
602 euthanization by cervical dislocation, the small intestine was harvested, flushed with PBS to  
603 discard luminal content and cut longitudinally open. The tissue was then cut into small pieces  
604 of 3-5 mm and further washed in PBS. The pieces of intestinal tissue were then incubated on  
605 ice for 10 min in a tube containing 5 mM EDTA. The tube was then vortexed for 2 min to  
606 release villi from the tissue. After EDTA removal, the intestinal pieces were placed in cold PBS  
607 and vortexed vigorously for 3 min to ensure crypt release. This process was repeated 3 times,  
608 with each fraction recovered. The third and fourth fractions are usually concentrated in crypts,  
609 so these are combined and passed through a 70- $\mu$ m cell strainer to remove remaining villi and  
610 centrifuged at 1000 RPM for 5 min. The pellet (crypts) was then washed in advanced  
611 DMEM/F12 (#12634010 ThermoFisher Scientific, Waltham, Massachusetts, USA) and  
612 centrifuged. The final pellet is resuspended in 50  $\mu$ l of 1:1 ratio of advanced DMEM/F12 and  
613 ice-cold Matrigel (#734-1100 VWR, Radnor, PA, USA) and plated as domes. Incubation at  
614 37°C for 20-30 min allowed Matrigel polymerization. Organoid culture was performed in  
615 IntestiCult™ Organoid Growth medium (#06005 STEMCELL Technologies, Vancouver,



616 Canada), from here on termed ENR medium. Organoids were routinely grown in Matrigel with  
617 IntestiCult™ Organoid Growth medium and passaged every 7 to 10 days. Medium was  
618 changed every 2 days. Live-imaging or immunofluorescence experiments were performed on  
619 3-4 days organoids. For cystic growth, intestinal organoids were cultured with L-WNR medium  
620 supplemented with 10 $\mu$ M CHIR99021 for 10 days. The L-WNR cell line was purchased from  
621 ATCC (ATCC CRL-3276™). The L-WNR medium was produced according to the ATCC  
622 recommendations.

623  $\alpha$ -tubulin-GFP and EB3-RFP expression was carried out by lentiviral transduction using the  
624 commercial lentiviral biosensors LentiBrite (#17-10206; #17-10222, Merck, Darmstadt,  
625 Germany) and following an already established protocol for lentiviral transduction in intestinal  
626 organoids (Van Lidth de Jeude et al., 2015). This process involves dissociating intestinal  
627 organoids into single cells using TrypLE Express (#12605010 Thermofisher Scientific,  
628 Waltham, Massachusetts, USA) and seed them on a layer of Matrigel along the lentiviral  
629 vectors overnight, before covering with another layer of Matrigel the next day to allow growth  
630 into 3D organoids. The medium was enriched with 10  $\mu$ M of CHIR99021 and 10  $\mu$ M of Y-  
631 27632 (#72054 and #72304 STEMCELL Technologies, Vancouver, Canada) to prioritize stem  
632 cell proliferation and improve single cell survival respectively. After 48 to 72h, fluorescent  
633 organoids start appearing and can be isolated and expanded.

634 H2B-mCherry organoids were generated from H2B-mCherry-knock-in mice provided by  
635 Renata Basto (Institut Curie, Paris). VillinCreERT2-tdTomato organoids were generated from  
636 mice provided by Danijela Vignjevic (Insitut Curite, Paris) (Krndija et al., 2019). Myosin-IIA-  
637 GFP-knock-in mice (Zhang et al., 2012) were provided by Robert S. Adelstein (NHLBI,  
638 Bethesda) and Ana-Maria Lennon-Dumesnil (Institut Curie, Paris). Myosin-IIA-KO/mTmG  
639 mice (Krndija et al., 2019) were kindly provided by Danijela Vignjevic (Insitut Curite, Paris) and  
640 generated by crossing Myosin-IIA-KO (Jacobelli et al., 2010) and mTmG mice (Muzumdar et  
641 al., 2007). Cre recombinase for Myosin-IIA-KO was induced with 100nM of 4-  
642 hydroxytamoxifen for 24h (#SML1666, Sigma-Aldrich).

643 Kif18B-KO organoids were generated by a CRISPR-Cas9 strategy using the Edit-R All-in-one  
644 lentiviral system from Horizon Discovery (Cambridge, UK). A set of 3 lentiviral sgRNAs were  
645 used to target Kif18B (#GSGM11839-246783580: GGTCAGAACACCCAGTTAAT;  
646 #GSGM11839-246783577: GTGTTTGCCTATGGCGCCAC; #GSGM11839-246783579:  
647 GTGGTGTGAGGTCCCGAGT). Control-KO organoids were generated using a non-  
648 targeting sgRNA (#GSG11811, Horizon Discovery). Organoids were transduced with lentiviral  
649 particles containing an inducible Cas9 along the sgRNAs for the targeted gene. Selection was  
650 then performed using 2 $\mu$ g/ml of puromycin (#A1113803, Thermofisher Scientific) for at least 2  
651 weeks before inducing Cas9 with 600 ng/ml of doxycycline (#D9891 Merck).

652 For human organoid preparation, the project was approved by the Scientific Committee of the  
653 tumor bank of Lille and the Department of Pathology of the Lille University Hospital. The  
654 patient had signed an informed consent. Left colectomy was carried out for a colon  
655 adenocarcinoma in the Department of General and Digestive Surgery of the Lille University  
656 Hospital. The patient had not been treated with neoadjuvant chemotherapy. The normal  
657 mucosa sample (1 cm<sup>2</sup> harvested >10cm distant from the tumor) was cut into small pieces  
658 (<3mm<sup>3</sup>) and washed thoroughly with 1X PBS buffer supplemented with combined antibiotics  
659 (normocin, gentamicin and amphotericin B). Mucosa fragments were then incubated in 25 ml  
660 of 1X PBS with 2.5mM EDTA at 4°C for 30 min under slow rotation. Mechanical release of  
661 colonic crypts was then performed three times in 10 mL 1X PBS. Fractions of colonic crypt  
662 suspension were pooled, centrifuged, resuspended in advanced DMEM/F12 medium and  
663 filtered through a 70- $\mu$ m cell strainer. After counting, cells were resuspended in Matrigel and  
664 seeded in 40  $\mu$ L domes in the wells of a 24-well plate. After Matrigel solidification, domes were  
665 covered with complete colon organoid medium (advanced DMEM/F12 medium supplemented  
666 with 1X Glutamax (Invitrogen), 1 X HEPES (Sigma-Aldrich), B-27<sup>®</sup> Supplement Minus Vitamin  
667 A (Invitrogen), 1 X N2 ( Invitrogen), 1 mM N-acetyl-L-cysteine (Sigma-Aldrich), 50% v/v  
668 Wnt3a/RSPO1/Noggin-conditioned medium, 50 ng/ml EGF (Peprotech), 0.5  $\mu$ M A83-01  
669 (Tocris), 10 mM Nicotinamide (Sigma-Aldrich), 10 nM Gastrin (Sigma-Aldrich) and 10  $\mu$ M Y-  
670 27632 dihydrochloride (Tocris), as recommended in (Sato et al., 2011a). Resulting colonic  
671 organoids were named COL-2920xi. Complete medium without Y-27632 was then renewed  
672 every two days and organoids were passaged through mechanical disruption every week.

673

674

## 675 **Method details**

676

### 677 **Antibodies and reagents**

678 Mouse monoclonal antibody directed against  $\alpha$ -tubulin (DM1A clone, IF dilution, 1:100) was  
679 purchased from Sigma-Aldrich. Rabbit polyclonal antibody directed against EpCAM  
680 (#ab71916, IF dilution, 1:100) and rabbit polyclonal antibody directed against  $\alpha$ -tubulin  
681 (#ab18251, IF 1:100) were from Abcam. Mouse monoclonal antibody directed against E-  
682 cadherin (clone 36, #610181, IF dilution, 1:50) was from BD Biosciences. Rabbit polyclonal  
683 antibody directed against Phospho-Myosin Light Chain 2 (Ser19, #3671, IF dilution, 1:100)  
684 and rabbit monoclonal antibody directed against E-cadherin (clone 24E10, #3195S, IF dilution  
685 1:100) were from Cell Signaling Technology. Rabbit monoclonal antibody directed against  
686 NuMA (EP3976, #ab109262, IF dilution, 1:100) was from Abcam. Rabbit polyclonal antibody  
687 directed against non-muscle myosin heavy chain II-A antibody (poly19098 clone, #909801,

688 WB dilution 1:500) was from Biolegend. Mouse monoclonal antibody directed against GAPDH  
689 (#60004-1-Ig, WB dilution 1:5000). Phalloidin-Alexa488, 568 or 647 were from Life  
690 Technologies. Nuclei were stained with Hoechst 33342 solution incubation (Life Technologies,  
691 Paisley, UK) at a 1:1000 dilution. Blebbistatin and Y-27632 were from Sigma Aldrich (Saint-  
692 Louis, MO, USA).

693

### 694 **Biochemical analysis**

695 For western blots, organoid lysates were prepared 3 days after plating using per condition 6  
696 wells of a 24-well plate. Matrigel was depolymerized by incubating with 1 ml of Gentle Cell  
697 Dissociation Reagent (#07174 STEMCELL Technologies, Vancouver, Canada) for 30 min at  
698 4°C and centrifugation for 5 min at 500 x g at 4°C. The pellet was resuspended in lysis buffer  
699 containing 25mM Tris / 5 mM NaCl / 1mM EDTA / 1mM EGTA / 0.5% NP40 / 1% Triton TX100,  
700 and incubated on ice for 30 min. The solution was then passed 10 times through a syringe  
701 equipped with a 23G needle and centrifuged at 10 000 RPM at 4°C for 10 min. Supernatant  
702 total protein content was measured by Bradford assay (Biorad). For each condition, 50µg of  
703 proteins was loaded per well in Novex Tris-Glycine pre-cast gels (ThermoFischer Scientific).  
704 Proteins were detected with either HRP-linked goat anti-mouse IgG antibody (dilution  
705 1:10,000; Sigma-Aldrich) or HRP-linked donkey anti-rabbit IgG antibody (dilution 1:10,000, GE  
706 Healthcare, Buckinghamshire, UK), and visualized on ImageQuant LAS4000 (GE-  
707 Healthcare). Signal quantification was performed using Fiji software.

708

### 709 **Immunostaining**

710 Routinely, organoids were fixed using 4% paraformaldehyde for 30 min, then permeabilized  
711 using 0.025% saponin solution in PBS for 30 min. Blocking step was performed in 0.025%  
712 saponin/1% BSA solution for 45 min, before proceeding to incubation with primary antibody at  
713 4°C overnight. The next day, the primary antibody was removed and the organoids washed 3  
714 times in PBS for 10 min each, before adding the secondary antibody and left to incubate for  
715 2h at room temperature. Finally, organoids were washed 3 times again for 10 minutes before  
716 incubating in Hoechst 33342 for 15 min to stain nuclei. Immunostained samples were mounted  
717 in home-made Mowiol solution.

718 For the MT immunostaining, we used an established protocol that maintain the MT integrity in  
719 intestinal organoids (Goldspink et al., 2017). Briefly, organoids were isolated from the Matrigel  
720 and fixed in a methanol/formaldehyde solution (92% methanol, 8% formaldehyde). The  
721 blocking step was performed by incubating organoids in 10% goat serum solution in PBS with  
722 0.1% Triton X-100. Immunostained organoids were mounted in home-made Mowiol solution  
723 on a slide.

724 For E-cadherin immunostaining *in vivo*, mouse jejunum was processed as previously  
725 described (Salomon et al., 2017). Briefly, samples were fixed for 2 hours in 4% PFA and  
726 paraffin embedded. 5  $\mu\text{m}$  tissue sections were de-waxed in a xylene bath, rehydrated in  
727 isopropanol and in solutions with decreasing ethanol concentrations. Tissue sections were  
728 then blocked in 10% goat serum (Sigma-Aldrich) for 1 h. Primary antibody incubation was  
729 performed at 4°C overnight and secondary antibody incubation at room temperature for 2 h,  
730 both in 1% goat serum solution. Hoechst33342 staining was used to detect nuclei. Tissue  
731 sections were mounted in home-made Mowiol 488 solution.

732 For NuMA immunostaining *in vivo*, 1-mm pieces of mouse jejunum were fixed in 4% PFA  
733 overnight under shaking. After PBS wash, tissue permeabilization was performed in 1% Triton  
734 X-100 / PBS solution for 1 h, before saturation in 1% BSA / 3% goat serum / 0.2% Triton X-  
735 100 / PBS solution for 1 h. Incubation with primary or secondary antibodies were done in 0.1%  
736 BSA / 0.3 % goat serum / 0.2 % Triton X-100 / PBS overnight at 4°C. Hoechst33342 staining  
737 was used to detect nuclei. Immunostained samples were mounted in Vectashield (Vector  
738 Laboratories, Burlingame, CA).

739

#### 740 **Live imaging**

741 Dynamics experiments on  $\alpha$ -tubulin-GFP/H2B-mCherry, myosin-IIA-KI-GFP or EB3-RFP  
742 organoids were performed using an inverted Zeiss microscope equipped with a CSU-X1  
743 spinning disk head (Yokogawa – Andor), using Zeiss 40X and 63X water objectives.

744

#### 745 **Laser ablation experiments**

746 Laser ablation experiments were performed using a spinning disk microscope equipped with  
747 a CSU-XI spinning disk head, using a 63X oil objective. The ablation was done using a pulsed  
748 355 nm ultraviolet laser at a power of 30% and a thickness of 3, interfaced with an iLas system  
749 (Roper Scientific) piloted in Metamorph.

750

#### 751 **Two-dimensional models for spindle orientation**

752 2D models to predict spindle orientation were adapted from (Bosveld *et al.*, 2016; Minc *et al.*,  
753 2011). These models were developed and executed through Matlab (Mathworks) scripts which  
754 can be made available upon demand. Starting from the shape of a cell, the model positioned  
755 spindle poles and traced MT asters radiating from spindle poles to the contour. For length  
756 limitations, we added a fixed maximal length to MTs, normalized to cell length along the A-B  
757 axis, which was varied in figure 2e. Each MT is associated to a force,  $f_{\text{MT}}$ , which varies  
758 depending on hypothesis. For models based on shape sensing, we posited that  $f_{\text{MT}} = aL_{\text{MT}}^2$   
759 with  $L_{\text{MT}}$  the length of the MT and  $a$ , an arbitrary constant. The scaling to the square was  
760 chosen as it best represents a length-dependent system in which MTs pull at the surface (Hara

761 and Kimura, 2009; Minc *et al.*, 2011). We note however that other models previously proposed  
762 to center and orient spindles with the long axis, including those based on pulling in bulk  
763 cytoplasm or MT pushing at the surface and limited by buckling, yielded to the same outputs.  
764 For NuMA based models, the force per MT was  $f_{MT} = b[NuMA] * L_{MT}^2$ , with  $b$  an arbitrary  
765 constant, and  $[NuMA]$  is the local concentration of NuMA around cells, inputted as a  
766 normalized Gaussian distribution with a peak located at the basal pole of cells. The script then  
767 looped to compute the torque exert generated by all MTs as a function of all possible spindle  
768 angles, and computed a rotational energy potential as a primitive of the torque (They *et al.*,  
769 2007) (Figure 2f). Free parameters in the model were the number of MTs, the size of the  
770 spindle and the spatial extension of asters, which had little influence on model prediction  
771 (Figure S3a-d). For the NuMA model, we added one other parameter which is the width of the  
772 Gaussian, which was estimated from experimental images, and which did not influence  
773 prediction for domain size relatively small as compared to cell contour. Key parameters that  
774 altered spindle orientation outputs were thus cell shape, and the asymmetry of spindle  
775 positioning, as well as the maximal length of MTs related to cell length (Figure 2g-h and Figure  
776 S3a-d)

777

### 778 **Three-dimensional simulations for spindle position and orientation**

779 The simulation package used for 3D models was developed in Matlab (Mathworks) and was  
780 adapted from (Ershov and Minc, 2019; Pierre *et al.*, 2016). This package includes a module  
781 to extract and reconstitute 3D shapes, spindle pole positions and polarity domains from  
782 segmented experimental image, and to add predefined hypothesis similar to those used above  
783 in 2D. In 3D, the exclusion of MTs from the basal pole was introduced as a gradient from the  
784 basal pole of length limitation, which was scaled to cell length along the A-B axis. The size of  
785 spindles and polar basal domains were defined directly from each experimental stack. Once  
786 parameters were defined as inputs, the model placed the spindle in a random position and  
787 orientation, traced MTs from spindle poles to measure their length and associated a force to  
788 each, and computed both global forces and torques exerted by MT asters. To search for  
789 minima, the model used a random walk, which follows the minimization of torques and forces.  
790 This was achieved by randomly modulating one of the 5 spatial parameters (3 for the position  
791 and 2 for the orientation of spindles), and recalculating the force torque at the subsequent  
792 step. The simulations followed the direction of force/torque minimization and stopped at a  
793 position/orientation once the iteration returns to this equilibrium after a given number of  
794 iterations (e.g. 300 runs) (Figure 4e-g, Figure S5a-c, and Videos S4-5). The length of the  
795 simulations, the duration needed to identify a stable equilibrium, the noise added to explore  
796 other parameters, and other intrinsic parameters of the loop, can be modulated, but were fixed  
797 for all the simulations performed in this work. Finally, to ensure that the spindle

798 position/orientation identified did not correspond to a local minimum which could have been  
799 biased by the initial conditions, the simulations were run typically 3-4 times from another  
800 random starting position/orientation. Finally, once a position was found, the model  
801 recalculated the torque landscapes as a function of the 2 possible angles (Figure S5c).

802

### 803 **Segmentation and 3D rendering**

804 Cell shape or spindle pole 3D segmentations are performed based on confocal z-stacks of cell  
805 membranes (i.e. E-cadherin, EpCAM) or MTs (i.e.  $\alpha$ -tubulin, NuMA), respectively.  
806 Segmentations are performed manually to guarantee the best match with the initial data.  
807 Masks corresponding to cell shapes, spindle poles, organoid lumen and basal environment  
808 (“exterior”) were generated on each slice of confocal z-stacks using ImageJ.

809 For generating the 3D rendering of organoid cells, mask outlines were translated into meshes,  
810 which are 3D surfaces connecting the perimeters on each slice, by using a Python program  
811 adapted from an initial program provided by Emmanuel Faure (LIRMM, Montpellier). Briefly,  
812 for a given cell, the mask was first translated into a list of points lying on the perimeters for  
813 every position along z where the cell is present. The list of points was then translated into a  
814 list of polygonal faces linking the points together. Visualization of meshes was done with the  
815 Meshlab software ([www.meshlab.net](http://www.meshlab.net)).

816 Measurements of geometric properties of the segmented cells, in particular their volume V  
817 and surface area A, were provided by Meshlab and used to compute the cell sphericity:  
818  $s = \pi^{1/3}(6V)^{2/3}/A$  (Wadell, 1935). From each pixel of the segmented cells, we computed the  
819 distance to the lumen. For each cell, the mean cell height H in the apico-basal direction was  
820 computed as the difference between the furthest and closest pixels from the lumen, averaged  
821 for every position along z where the cell is present. The mean cell width was computed from  
822 the cell volume and height assuming a cylindrical geometry and using  $W = 2(V/\pi H)^{1/2}$ . The  
823 mean spindle positioning with respect to the apico-basal axis was computed by averaging the  
824 distance from each point of the spindle to the lumen.

825 Multicellular contact distribution was computed from the ImageJ organoid cell shape masks  
826 and by using a custom Python program. The multicellular contacts were identified as any pixel  
827 in the vicinity of the borders of three or more cell masks (within 20 pixels). The “degree” of the  
828 multicellular contact (3-cells, 4-cells etc.) was given by the number of different cell masks in  
829 the vicinity of each multicellular contact. Positioning of computed multicellular contacts was  
830 then visualized together with cell meshes in Meshlab.

831

### 832 **Expansion microscopy of organoids**

833 Expansion microscopy protocol was adapted from Gambarotto et al. (Gambarotto et al., 2019).  
834 Matrigel domes containing mouse organoids were dissociated by using Gentle Cell

835 Dissociation Reagent. After centrifugation, Matrigel debris were eliminated with the  
836 supernatant and organoid pellet kept. Organoids were incubated overnight at 37°C in PBS  
837 containing 2% acrylamide / 1.5% formaldehyde.

838 For gelation, organoids were washed in PBS and pelleted by centrifugation. After  
839 resuspension in PBS, organoids were laid on a parafilm piece in a humid chamber. After PBS  
840 removal with a tip, gelation solution (19.3% sodium acrylate / 10% acrylamide / 0.1% N,N'-  
841 methylenbisacrylamide / 0.5% TEMED / 0.1% Ammonium persulfate, in PBS) was added.  
842 Gelation solution containing organoids was covered with a 5mm coverslip, incubated 5 min on  
843 ice and then 1 hour at 37°C. The coverslip and the gel were placed in a 24-well plate filled  
844 with 500µl of denaturation buffer (200mM SDS / 200mM NaCl / 50mM Tris water, PH9). After  
845 15 min agitation at room temperature, the gel detached from the coverslip, was placed in a  
846 1.5ml microtube filled with denaturation buffer and incubated at 95°C for 1h30min.

847 For immunofluorescence, organoids were transferred in a 24-well plate, blocked and  
848 permeabilized during incubation in 1% BSA / 0.025% saponin, in PBS solution for 1 hour at  
849 room temperature. Primary antibodies were incubated overnight at 4°C in the 1% BSA /  
850 0.025% saponin, in PBS solution, under gentle shaking. The day after, after 3 PBS washes,  
851 secondary antibodies were incubated 4 hours at 4°C and then 1 hour at room temperature  
852 upon gentle shaking. After three PBS washes, nuclei were labelled with Hoechst 33342.

853 For the organoid expansion, the gel was transferred in a 6-well plate filled with ddH<sub>2</sub>O and  
854 wash for 1 hour without agitation. After ddH<sub>2</sub>O change, the gel was incubated overnight at  
855 room temperature. The day after, the expansion index was evaluated based on gel length  
856 before and after expansion. For image acquisition, the expanded gel was placed in a poly L-  
857 lysine-coated glass chamber after exceeding water removal.

858

859

## 860 **Statistical analysis**

861

862 All statistical analyses were performed using Prism (GraphPad Software, San Diego, CA,  
863 USA, version 9.0). All circular graphs and statistics were performed using Oriana version 4  
864 (Kovach Computing). Unless otherwise stated, experiments were replicated 3 times  
865 independently.

866

867 **SUPPLEMENTAL VIDEOS**

868

869 **Video S1:**

870 **Description:** Time lapse imaging of  $\alpha$ -tubulin-GFP (green) dynamics during mitosis in  
871 H2BmCherry (blue) organoids. Images were acquired every 30 sec. Frame rate is 15 fps.

872

873 **Video S2:**

874 **Description:** Time lapse imaging of myosin-IIA-GFP in intestinal organoids. Scale bar, 10 $\mu$ m.  
875 Images were acquired every 15 sec. Frame rate is 15 fps.

876

877 **Video S3:**

878 **Description:** Time lapse imaging of EB3-RFP in prophase (left panel) and metaphase (right  
879 panel) cells in intestinal organoids. Images were acquired every 4 or 1 sec, respectively.  
880 Frame rate is 10 fps.

881

882 **Video S4:**

883 **Description:** Time-lapses of 3D gradient descent simulations to predict planar spindle  
884 orientation, started with spindles close to the apical pole (left) or the basal pole (right). Note  
885 the similar final predicted equilibrium.

886

887 **Video S5:**

888 **Description:** 3D rotating views of spindle orientations in a representative experimental cell,  
889 and as predicted by 3D simulations based on the different indicated hypothesis (NuMA  
890 polarity, Full cell shape, or cell shape coupled to length-limitation in MTs).

891

892

893



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