

*Postmitotic separation enables selective niche retention of one daughter cell in intestinal crypts and is facilitated by interkinetic nuclear migration and basal tethering*

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# 1 **Postmitotic separation enables selective niche retention of one** 2 **daughter cell in intestinal crypts and is facilitated by interkinetic** 3 **nuclear migration and basal tethering**

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5 ***Thomas D. Carroll<sup>1</sup>, Alistair J. Langlands<sup>2</sup>, James M. Osborne<sup>3</sup>, Ian P. Newton<sup>1</sup>, Paul L. Appleton<sup>4</sup>,***  
6 ***Inke Näthke<sup>1\*</sup>***

7 Affiliations: <sup>1</sup>Cell & Developmental Biology, University of Dundee, Scotland, UK, Dundee, DD1 5EH

8 <sup>2</sup>National Phenotypic Screening Centre, University of Dundee, Scotland, UK, DD1 5EH

9 <sup>3</sup>School of Mathematics and Statistics, University of Melbourne

10 <sup>4</sup>Dundee Imaging Facility, University of Dundee, Scotland, UK, DD1 5EH

11 *\*to whom correspondence should be addressed: i.s.naethke@dundee.ac.uk*

## 13 **Abstract**

14 Homeostasis of renewing tissues requires balanced proliferation, differentiation and  
15 movement. This is particularly important in the intestinal epithelium where lineage tracing  
16 suggests that stochastic differentiation choices are intricately coupled to position. To  
17 determine how position is achieved we followed proliferating cells in intestinal organoids  
18 and discovered that behaviour of mitotic sisters predicted long-term positioning. Normally,  
19 70% of sisters remain neighbours while 30% lose contact separating after cytokinesis. Post-  
20 mitotic placements predict differences in positions of sisters later: adjacent sisters reach  
21 similar positions; one separating sister remains close to its birthplace, the other moves  
22 upward. Computationally modelling crypt dynamics confirmed post-mitotic separation as a  
23 mechanism for placement of sisters into different niches. Separation depends on  
24 interkinetic nuclear migration, cell size, and asymmetric tethering by a basal process. These  
25 processes are altered when *Adenomatous polyposis coli (Apc)* is mutant and separation is  
26 lost. We conclude that post-mitotic placement enables stochastic niche exit and when  
27 defective, supports the clonal expansion of *Apc* mutant cells.

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## 28 INTRODUCTION

29 Fate choices of proliferating cells are critical for intestinal homeostasis. Lgr5(+) stem cells  
30 (SCs) in the intestinal crypt base must be regulated carefully to balance their maintenance  
31 with the production of transit-amplifying (TA) progenitors that can specialise. Similarly, exit  
32 of TA progenitors from their proliferative niche has to be regulated to produce the  
33 appropriate number of post-mitotic, differentiated cells. In the crypt, the position of cells  
34 relative to two niches, the stem cell and transit amplifying compartments, reflects their fate  
35 (Ritsma et al., 2014). Accordingly, stem and transit amplifying compartments differ in  
36 composition. The principal components of the intestinal SC niche are Paneth cells. Together  
37 with the surrounding mesenchyme, they provide Notch ligands, EGF and Wnts, which are  
38 critical for maintaining SCs and this creates a local Wnt gradient along the intestinal crypt  
39 axis (Sato et al., 2011). Displacement of stem cells from Paneth cell contact causes serial  
40 dilution of membrane-bound Wnts, contributing to loss of stemness (Farin et al., 2016).  
41 Neutral competition for niche access by the 12-16 SCs in the crypt base governs net  
42 contraction and expansion of clones, leading to mono-clonal crypts over time (Lopez-Garcia  
43 et al., 2010; Snippert et al., 2010). Stem cells near the border of the stem cell niche are  
44 more likely to enter the transit-amplifying compartment and lose stemness. Stem cells  
45 residing at or near the crypt base are more likely to retain stemness (Ritsma et al., 2014).  
46 Traversing the transit amplifying compartment is similarly accompanied by exposure to  
47 progressively less Wnt and other growth factors. Exit from this niche causes cell cycle exit.  
48 Such direct links between cell positioning and a graded niche signalling also operates in  
49 *Drosophilla* (Reilein et al., 2017). These observations suggest that in intestinal crypts,  
50 position, not the segregation of fate-determinants, regulates cell-fate.

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51 Tissue homeostasis is perturbed in intestinal crypts mutant for key tumour  
52 suppressors such as *Adenomatous polyposis coli (Apc)*, KRAS, p53 and SMAD4. These  
53 mutations provide cells with a selective advantage and increase their ability to colonise  
54 proliferative niches. Measuring the competitive advantage of cells carrying these mutations  
55 using sophisticated lineage tracing experiments demonstrated a competitive advantage over  
56 wild-type cells that allowed their preferential retention in the proliferative niche  
57 (Vermeulen et al., 2013, Song et al., 2014). The expansion of such mutant clones is thought  
58 to underpin field cancerisation, the preconditioning large tissue regions to neoplasia  
59 (Slaughter et al., 1953).

60 Our knowledge about cellular mechanisms that control cell positioning in the  
61 intestinal epithelium is limited, as is our understanding about how changes in such  
62 mechanisms can drive retention of mutant clones. Computational modelling suggests that  
63 the magnitude of the Wnt stimulus received at birth is a deciding factor for proliferative fate  
64 (Dunn et al., 2016). That suggests that decisions about cell position are set at birth. To test  
65 this hypothesis, we investigated daughter cell positioning along the crypt axis in 3D using  
66 intestinal organoids.

67

## 68 **RESULTS**

69 We measured cell positioning during and after mitosis in intestinal organoids, a widely  
70 accepted physiological model of the intestinal epithelium (Sato et al., 2009). They contain  
71 epithelial domains that correspond to crypt-villus architecture *in vivo*, and contain a  
72 comparable cellular composition. Cell division (Figure 1A, S1 Figure) and polarity appear  
73 identical to those *in vivo* (Fatehullah et al., 2013), making organoids an ideal model system

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74 to understand the dynamic behaviour of the intestinal epithelium at temporal and spatial  
75 resolution impossible to achieve in tissue *in vivo*.

76 **Interkinetic nuclear migration (INM) operates in all intestinal epithelial cells and facilitates**  
77 **placement of mitotic sisters cells into different positions**

78 Mitotic cells in the intestinal epithelium are easily distinguished (Figure 1A, S1 Figure).  
79 During interphase, nuclei are positioned basally. Upon entering mitosis, interkinetic nuclear  
80 migration (INM) causes nuclei to migrate apically towards the centrosome, similar to  
81 mitoses in the neuro-epithelium (Spear and Erickson, 2012). During this process, mitotic  
82 cells lose their columnar cell shape, become rounded and assume a position in the top half  
83 of the epithelial layer. Adjacent interphase cells expand into the basal space that is vacated  
84 by the migrating nuclei. Once INM is complete, spindles form and mitosis proceeds. After  
85 cytokinesis, newly formed cells move their nuclei basally and eventually assume columnar  
86 shape. As mitotic cells round up, their apical surface remains aligned with that of the  
87 epithelial layer and they remain attached to the basement membrane by a basal process.  
88 Centrosomes are located apically in interphase cells and align laterally with condensed  
89 chromosomes during metaphase. These mitotic stages are indistinguishable between tissue  
90 and organoids (S1 Figure).

91 **Dynamics of INM during mitosis**

92 The distinct movement of pre- and post-mitotic nuclei in intestinal epithelium is similar to  
93 INM in other tissues, where it has been implicated in cell fate decisions (Spear et al., 2012).  
94 For instance, in the neuro-epithelium, INM facilitates differentiation by moving nuclei along  
95 apical-basal signalling gradients (Del Bene et al., 2008). In the developing foetal intestinal



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96 epithelium, INM has been implicated in the growth of epithelial girth (Grosse et al., 2011).

97 The contribution of INM to intestinal homeostasis has never been examined. We examined  
98 how INM affects placement of mitotic sisters by tracking individual cells and their progeny  
99 during mitosis.

100 We directly monitored the position of mother and daughters during mitosis and  
101 after cytokinesis using live imaging of intestinal organoids expressing Histone2B-GFP (H2B-  
102 GFP). All nuclei in organoids derived from *H2B-GFP* mice robustly express GFP 24 hours  
103 after exposure to doxycycline allowing nuclear position to be used as a surrogate for cell  
104 position. (Figure 1B, C, S1 Movie) (Foudi et al., 2009). Measuring cell position in organoids  
105 required tracking cells in three-dimensional (3D) space. Techniques for accurately tracking  
106 cells in 3D are limited and we were unable to reliably track GFP(+) nuclei using automated  
107 methods. Therefore, daughter cell behaviour was recorded manually by tracking cells using  
108 Imaris (Bitplane) (Figure 1D).

109 Recordings revealed novel dynamic data about cell behaviour during mitosis. Mitosis  
110 lasted approximately 60 minutes. Prophase was characterised by nuclear condensation and  
111 INM, followed by rapid formation of the metaphase plate. After spindle alignment and  
112 cytokinesis, both daughters slowly migrate basally until their nuclei align with adjacent  
113 interphase cells (Figure 1E). During interphase, nuclei moved approximately 25 $\mu$ m/hour in  
114 crypts, which increased to 60 $\mu$ m/hour during INM. Speed during basal cell movement was  
115 comparable to that in interphase suggesting that INM is an active process and that basal  
116 movement is passive (Figure 1F). The unique arrangement of microtubule bundles above  
117 the nucleus in early mitosis suggests that INM involves microtubules (S1 Figure).

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118 **Daughter cells either remain adjacent or are separated from one another after mitosis**

119 Tracking mitotic cells revealed two distinct outcomes for mitotic sisters. They either remain  
120 adjacent (6.0 +/-1.2 $\mu$ m apart) and become neighbours (Figure 2A, S2 Movie), or they  
121 separate (12.9 +/- 2.8 $\mu$ m apart) and exchange neighbours (Figure 2B, S3 Movie). Rendering  
122 mitoses in 4D confirmed their separation by a neighbouring cell (Figure 2C, S4 Movie).  
123 Importantly, we observed similar mitoses *in vivo* with one sister positioned significantly  
124 displaced from the other by neighbouring cells (Figure 2D). This data suggests that post-  
125 mitotic separation occurs in native tissue and in organoids.

126 To determine when mitotic sisters separate, we measured when neighbouring cells  
127 first appeared between them. Specifically, we measured the H2B-intensity across the line  
128 connecting the centre of sister nuclei to visualise nuclear boundaries (Figure 2E). For  
129 adjacent sisters, the line-intensity profile was unchanged over time indicating that the two  
130 nuclei remained in close proximity. In post-mitotic separations, an additional peak  
131 appeared between the peaks representing each sister, indicating the insertion of a  
132 neighbouring cell between them. Insertion of neighbours occurred 72-120 minutes after  
133 cytokinesis, indicating that displacement occurred during basal cell movement (Figure 2E).  
134 Live-imaging of the mitochondrial network using Mitotracker clearly showed distinct cells  
135 between mitotic sisters, further confirming their physical separation (Figure 2F, S5 Movie).

136 We found other situations also favoured separation. Separation could be facilitated  
137 by the movement of daughters of other mitoses in the immediate vicinity (S2 Figure, S6  
138 Movie). Furthermore, separation was favoured when mitoses occurred next to Paneth cells.  
139 Paneth cells are more adherent and stiffer (Langlands et al., 2016) and this could force one  
140 daughter cell out of the way (S2 Figure; S7 Movie)

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## 141 ***Apc* mutation alters placement of daughter cells**

142 APC is required for normal intestinal homeostasis and mutations in *Apc* are common to  
143 most tumours in the colon (Fearnhead et al., 2001). The APC protein functions as a scaffold  
144 in Wnt signalling (McCartney and Näthke, 2008). It contributes to spindle orientation  
145 (Yamashita et al., 2003, Quyn et al., 2010), and cell migration along the crypt-villus axis  
146 (Nelson and Nathke, 2013). Lineage tracing and computational modelling has demonstrated  
147 that *Apc* mutations increase the retention of cells in intestinal crypts (Vermeulen et al.,  
148 2013, Song et al., 2014). To determine if changes in the positioning of mitotic sisters could  
149 explain these observations we isolated organoids derived from *Apc* heterozygous mice  
150 (*Apc*<sup>Min/+</sup>). These organoids are initially indistinguishable from wild-type organoids  
151 (Fatehullah et al., 2013) but transform into spherical, cyst-like structures (Figure 3A)  
152 containing cells that have undergone loss of heterozygosity (LOH) (*Apc*<sup>Min/Min</sup>) (Germann et  
153 al., 2014). Mitoses appeared normal in *Apc*<sup>Min/+</sup> organoids, however, in *Apc*<sup>Min/Min</sup> organoids,  
154 abnormal mitoses with multipolar spindles and mitotic slippage were frequently observed  
155 (S3 Figure), similar to cultured cells which lack APC (Dikovskaya et al., 2007). We compared  
156 the incidence of the two types of cell placements in wild-type and *Apc*<sup>Min/+</sup> mice and in  
157 *Apc*<sup>Min/Min</sup> organoids (S1 Movie).

158 In wild-type epithelium, ca. 30% of daughter cells separated whilst ca. 70% remained  
159 adjacent (Figure 3B). Separation was mainly associated with movement of neighbouring  
160 interphase cells during basal cell movement (75.1% +/- 14.8% of cases). Separation by  
161 surrounding mitotic progeny was less common (29.5% +/-21.6% of cases). The frequency of  
162 the two mitotic types was equal in the stem and transit amplifying compartments,  
163 suggesting that mitotic outcome is independent of cell position and type and can occur in

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164 any cycling cell that undergoes INM (Figure 3C). To further confirm that mitotic separation  
165 is not specific to stem cells, we measured mitotic outcome in organoids treated with the  
166 GSK-3 $\beta$  inhibitor, Chir99021, and the HDAC inhibitor, valproic acid, which increases the  
167 number of Lgr5(+) stem cells in the crypt (Yin et al., 2014). Treatment with Chir99021 and  
168 valproic acid did not significantly change post-mitotic separation of sisters (S4 Figure),  
169 suggesting that the occurrence of post-mitotic separation is similar in all dividing cells along  
170 the crypt axis.

171 In *Apc*<sup>Min/+</sup> organoids there was a significant reduction in the frequency of post-  
172 mitotic separations. Sisters never separated in *Apc*<sup>Min/Min</sup> organoids (Figure 3B). This  
173 suggests that *Apc* mutant sisters are more likely to remain adjacent after division. There  
174 was also a significant overall reduction in cell movement between wild-type and *Apc*<sup>Min/+</sup>  
175 epithelial cells, including nuclear speed during INM (Figure 3D), suggesting that cells remain  
176 adjacent because of reduced cell movement. Loss of post-mitotic separation was also  
177 induced by long-term treatment of organoids with high concentrations of Chir99021. This  
178 treatment caused organoids to grow as cysts, similar to *Apc*<sup>Min/Min</sup> organoids (S5 Figure). This  
179 suggests that hyperactive Wnt signalling induced either by *Apc* mutation or by GSK-3 $\beta$   
180 inhibition can alter the frequency of post-mitotic separation, although it is possible that this  
181 is an indirect consequence of the changes in cells size and shape (see below).

## 182 **Post-mitotic separation of daughter cells directs niche exit**

183 The two types of placements of mitotic sisters we discovered led to the hypothesis  
184 that post-mitotic separation allows differential exit of sisters from proliferative  
185 compartments. For instance, separation of stem cell daughters may increase the probability  
186 for one daughter to remain in the stem cell niche compared to the other. Similarly, in the

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187 transit-amplifying compartment, post-mitotic separation could make it more likely for one  
188 daughter to remain in the proliferative compartment and the other to exit and terminally  
189 differentiate. To test this idea, we measured the distance of mitotic sisters from their  
190 starting positions and from each other after their birth. Shortly after cytokinesis, after both  
191 daughters had assumed their interphase position, regardless of mitosis type, one sister  
192 always remained near its starting position, whereas the other moved upward (Figure 4A, B).  
193 At later times (up to 35 hours after mitosis), differences between sisters were accentuated.  
194 If sisters had separated, one always remained close to its starting position while the other  
195 was displaced significantly upwards. In contrast, adjacently placed sisters were both  
196 displaced upwards (Figure 4A, B). Thus the initial difference in distance between sisters in  
197 the two types of mitoses was amplified over time, consistent with the idea that different  
198 placement of mitotic sisters can produce different outcomes for cell positioning.

199 To provide additional evidence for this idea we used a previously established  
200 computational 3D model of intestinal crypts (Dunn et al., 2016) and asked whether post-  
201 mitotic separation could promote heterogeneous position/fate. To compare modelling  
202 results to our experimental data, simulations were performed with daughters placed  
203 adjacent to each other (as in previous computational models) or separated by a factor larger  
204 than a typical cell diameter. Simulations were performed using parameters derived from  
205 the primary data (materials and methods). These simulations confirmed that post-mitotic  
206 separation often led to one daughter being retained close to its point of birth whilst the  
207 other displaced upward (Figure 4C). There was a significant difference between the  
208 separation velocities between the two mitotic subtypes, indicating that daughters that  
209 initially separated moved apart much faster than those born adjacent (Figure 4D, E).

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210 To test whether post-mitotic separation influences the number of heterogeneous  
211 cell pairs, we imposed a crypt-specific boundary separating a proliferative region from a  
212 non-proliferative region. Heterogeneous pairs are produced when one daughter is retained  
213 in the proliferative boundary and the other exits. Consistent with our experimental results,  
214 simulations showed that separation led to more heterogeneous pairs than adjacent  
215 placements (Figure 4F). The same results were produced for thresholds representing the  
216 TA/Differentiated SC/TA boundaries, similar to other reports (Vermeulen et al., 2013, Song  
217 et al., 2014). A greater separation distance at birth led to a higher number of  
218 heterogeneous pairs (Figure 4G). Together, these data suggest that post-mitotic separation  
219 could enhance divergent daughter fate by promoting the exit of one daughter from a niche  
220 whilst allowing the other to remain.

### 221 **Mechanisms for post-mitotic separation**

222 A number of mechanisms may be involved in post-mitotic separation. For instance,  
223 spindle orientation could direct placement of sisters, supported by the different types of  
224 spindle alignment we previously discovered in intestinal tissue (Quyn et al., 2010). Position  
225 and location of mitotic sisters is likely affected by spindle orientation. To understand how  
226 mitotic placement related to spindle orientation, we measured spindle alignment in  
227 organoids. Consistent with previous data in whole tissue, we observed spindle orientation  
228 bias in the stem cell compartment of wild-type organoids where cells more readily oriented  
229 their spindles perpendicularly to the apical surface. There were no perpendicularly oriented  
230 spindles in the stem cell compartment in *Apc*<sup>Min/+</sup> organoids (S3 Figure). This is consistent  
231 with the idea that separating sisters can result from mitoses with perpendicularly aligned  
232 mitotic spindles. However, perpendicular spindle alignment was less frequent than

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233 separating sisters, indicating that additional processes are involved and that spindle  
234 orientation is not a reliable measure of post-mitotic separation.

235 **Basal tethering of daughter cells contributes to post-mitotic separation and is altered in**  
236 ***Apc* mutant organoids**

237 Another mechanism that may affect placement of daughters involves the basal process that  
238 tethers mitotic cells to the basement membrane (Fleming et al., 2007). This process is  
239 formed during INM and persists throughout metaphase. The basal process is rich in F-Actin  
240 and is tethered at the basement membrane by  $\beta$ 4-Integrin (Figure 5A). Tethering of  
241 daughter cells after cytokinesis and during basal cell movement provides a direct means to  
242 guide daughters. Asymmetric tethering of mitotic cells has been shown to coincide with the  
243 segregation of planar cell polarity markers in the colonic epithelium (Bellis et al., 2012).

244 To determine whether asymmetric tethering of sisters operated in organoids and  
245 contributed to their placement, we measured the position of basal processes relative to  
246 prospective daughters. We distinguished whether the process was positioned  
247 symmetrically or asymmetrically. Processes attached close to the cleavage plane,  
248 equidistant to both centrosomes, were classified as symmetric. Those attached closer to  
249 one centrosome were classified as asymmetric. For asymmetrically placed processes, we  
250 also measured their position relative to the crypt base, i.e. whether the mitotic sister they  
251 were connected to was closer to the bottom or top of the crypt (Figure 5B, C). The basal  
252 process in all separating mitoses was significantly more displaced from the cleavage furrow  
253 than in adjacent mitoses (Figure 5D). Accordingly, symmetrical processes predicted equal

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254 tethering of daughters and adjacent cell placement, whereas asymmetric processes  
255 predicted daughter separation (Figure 5C).

256         The proportion of symmetrically and asymmetrically placed basal processes differed  
257 between the stem cell and transit-amplifying compartments. In the latter, the proportion of  
258 asymmetric basal processes was ca. 30%, similar to the proportion of separating mitotic  
259 daughters. However, in the stem cell compartment, this number increased to 50% (Figure  
260 5D). In both regions, asymmetrically positioned processes tended to localise to the  
261 daughter cell closer to the crypt base, predicting that the untethered daughter was most  
262 likely to be displaced upwards. Asymmetrical basal process placement was a feature of  
263 mitotic cells with perpendicularly aligned spindles, suggesting that spindle orientation and  
264 basal process placement are linked (S3 Figure). Live imaging suggested that the basal  
265 process guides basal cell movement. The tethered daughter migrated basally to assume the  
266 interphase position of the mother, whilst the untethered daughter moved freely and  
267 allowed sister separation. This was particularly obvious when a daughter required multiple  
268 attempts to reintegrate into the epithelium (Figure 5E; S8 Movie). In *Apc<sup>Min/+</sup>* intestinal  
269 organoids fewer processes were placed asymmetrically, consistent with the significantly  
270 reduced frequency of separating sisters in *Apc<sup>Min/+</sup>* organoids (Figure 5D). We propose that  
271 asymmetric processes facilitate the displacement of one daughter cell from the niche by  
272 allowing it to separate from its sister, rather than simply aiding in their retention (Bellis et  
273 al., 2012). To provide evidence for this hypothesis, we performed live imaging of H2B-GFP  
274 organoids treated with SiR-Actin (Lukinavicius, G. et al., 2014). As expected, daughter  
275 separation correlated with asymmetric segregation of the basal process (Figure 5F, S9  
276 Movie).



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277 ***Apc* loss and hyperactive Wnt signalling restrict separation of sisters by inhibiting INM and**  
278 **changing cell size and morphology**

279 We did not detect sister cell separation in *Apc*<sup>Min/Min</sup> organoids. Instead, metaphases  
280 usually lay in the plane of the epithelium in line with interphase nuclei and only had short  
281 compressed basal processes which were difficult to visualise (Figure 6A). In addition, cell  
282 morphology was altered and the distance between apical and basal surfaces was  
283 significantly reduced (ca. 25%) compared to wild-type or *Apc*<sup>Min/+</sup> cells (Figure 6A). To  
284 determine whether this was due to changes in cell shape or overall cell size, we measured  
285 the volume of isolated, single cells from wild-type, *Apc*<sup>Min/+</sup> and *Apc*<sup>Min/Min</sup> organoids using  
286 flow cytometry. There was no significant difference between wild-type and *Apc*<sup>Min/+</sup> but cell  
287 size in *Apc*<sup>Min/Min</sup> organoids was reduced by 25%, indicating that a smaller cell volume was  
288 responsible for the reduced cell height (Figure 6B). This suggests that in cells lacking wild-  
289 type *Apc*, space restriction causes a reduction in apical-basal distance to prevent INM and  
290 restrict basal process formation, preventing post-mitotic separation.

291 To directly determine if and how INM was altered in *Apc*<sup>Min/Min</sup> organoids, we first  
292 measured the distance of mitotic nuclei relative to the basal membrane of the epithelial  
293 layer in wild-type, *Apc*<sup>Min/+</sup> and *Apc*<sup>Min/Min</sup> organoids. The basal reference was established as  
294 the plane formed between neighbouring cells proximal to the mitotic/daughter cells (S5  
295 Figure). In wild-type cells, the distance covered by INM was approximately 4µm (Figure 6C,  
296 D). There was no significant difference in distance covered during INM between wild-type  
297 and *Apc*<sup>Min/+</sup> cells (Figure 6C, D). However, the speed of nuclei during INM was significantly  
298 reduced in *Apc*<sup>Min/+</sup> cells suggesting that they require longer to reach the apical region  
299 and/or spend less time there (Figure 3D). As expected, in *Apc*<sup>Min/Min</sup> organoids there was no

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300 apical displacement and all daughters were placed adjacently. Similar results were achieved  
301 in organoids chronically treated with Chir99021 to hyper-activate Wnt signalling (S5 Figure).  
302 We also observed mitoses in  $Apc^{Min/+}$  organoids that exhibited no INM. One possible  
303 explanation is that some cells in  $Apc^{Min/+}$  organoids had already undergone LOH, which may  
304 dramatically reduce INM. Together, these data show that INM is important for the ability of  
305 sisters to separate (Figure 6C, D).

306 To corroborate these observations *in vivo*, we compared the morphology of  
307 interphase and mitotic cells in normal and transformed tissue isolated from a familial  
308 adenomatous polyposis (FAP) patient (Figure 6E). We detected a striking morphological  
309 change in cells from dysplastic regions. In contrast to  $Apc^{Min/Min}$  organoids, which displayed  
310 greatly reduced apical-basal distance, we detected significant lateral compression of cells in  
311 the human tissue samples (Figure 6F, G) that correlated with the pseudo-stratification  
312 caused by ‘pile-ups’ of cells along the crypt-villus axis (Figure 6E). This lateral compression  
313 likely restricts the ability of nuclei to undergo INM and reach the apical surface and  
314 separate. The resulting decrease in post-mitotic separation may contribute to the observed  
315 pseudo-stratification and also promote cell overcrowding in crypts.

316

## 317 **DISCUSSION**

318 Where a cell is born is linked to its identity. In this study, we show that daughter cells  
319 can separate immediately after cytokinesis and assume increasingly diverging positions over  
320 time (Figure 7). This means that one sister is more likely to exit a compartment where it was  
321 born than the other. For stem cells, this means that one sister is more likely to differentiate  
322 into a progenitor. For transit-amplifying cells, it means that one sister is more likely to exit  
323 the proliferative niche of the transit-amplifying compartment and become post-mitotic. For

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324 simple columnar epithelia, it is possible that post-mitotic separation provides a cellular  
325 mechanism for the neutral drift that governs stem cell population dynamics. All intestinal  
326 cells have a similar probability of undergoing post-mitotic separation, allowing one daughter  
327 to remain in its current niche position and the other to leave. It is unlikely that post-mitotic  
328 separation always produces a heterogeneous cell pair, as this would only readily occur near  
329 a niche boundary. However, this mechanism could influence overall homeostasis and  
330 protect stem cell number by slowing neutral drift i.e. ensuring that one daughter remains  
331 close to its birth place, making it more likely to remain in a proliferative niche.

332         Reduced post-mitotic separation in *Apc* mutant cells provides an explanation for  
333 their increased probability to colonise a niche (Vermeulen et al., 2013, Baker et al., 2014).  
334 Neither mutant sister is likely to be displaced from its birthplace, instead, they remain in  
335 close proximity to each other. Together with their well characterised decreased migration,  
336 which we confirmed in organoids (Figure 4), this could significantly decrease the number of  
337 *Apc* mutant cells exiting proliferative compartments (Nelson et al., 2012). As a result, in *Apc*  
338 mutant epithelia, many sisters would remain in a proliferative niche, resulting in increased  
339 number of proliferating cells. This explains the increased number of cells in the crypt base  
340 of *Apc*<sup>Min/+</sup> tissue (Quyn et al., 2010). A reduction in post-mitotic separation and decreased  
341 migration may confer on *Apc* mutant cells the competitive advantage that causes their  
342 preferred niche retention (Figure 4) (Nelson et al., 2012). Changes in the positioning of wild  
343 type and *Apc* mutant cells could also be responsible for the measurable differences in  
344 histologically normal appearing *Apc*<sup>Min/+</sup> tissue. The decrease in the regularity of crypt shape  
345 and packing that is detectable by high resolution optical imaging and high frequency  
346 ultrasound could reflect altered post-mitotic placement of cells and could be caused by

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347 increased retention of expanding clones of *Apc*<sup>Min/Min</sup> mutant cells in *Apc*<sup>Min/+</sup> tissue  
348 (Fatehullah et al., 2016a)

349 Post-mitotic placement is likely to contribute to crypt fission, the process that  
350 produces two daughter crypts and is responsible for elongation of intestinal tract  
351 (Humphries and Wright, 2008). Initiation of crypt fission involves the formation of a cluster  
352 of stem cells at the crypt base, which marks the point of bifurcation (Langlands et al., 2016).  
353 Dynamic post-mitotic rearrangements of daughters could explain how these clusters form.  
354 We found that in many cases, mitoses next to Paneth cells resulted in separating sisters.  
355 The tight packing at the crypt base and the larger size and stiffness of Paneth cells means  
356 that once mitotic daughters of a dividing stem cell at the crypt base remain adjacent to each  
357 other, it is increasingly difficult for daughters of subsequent divisions to separate due to the  
358 physical constraint generated. This could cause the initial clustering of Lgr5+ cells marking  
359 the initiation of fission.

360 Post-mitotic separation is facilitated by INM and the ability to asymmetrically  
361 segregate basal processes. A role for APC in INM as suggested by our data is consistent with  
362 findings in neuro-epithelia where loss of *Apc* disrupts INM (Ivaniutsin et al., 2009). In the  
363 neuro-epithelium, INM relies on microtubules for nuclear movement and actomyosin  
364 activity for cell rounding (Spear and Erickson, 2012, Xie et al., 2007). In the intestinal  
365 epithelium, INM may also involve microtubules. Specifically, the apical-basal microtubule  
366 scaffold may facilitate the nuclear movement during INM (S1 Figure). APC regulates both  
367 microtubules and actin (Näthke et al., 1996, Zumbunn et al., 2001, Okada et al., 2010) and  
368 cytoskeletal defects resulting from *Apc* mutation could compromise the function of  
369 microtubule bundles reducing the efficiency of INM and sister separation. Indeed, the

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370 number of microtubules in large parallel arrays is significantly reduced in *Apc*<sup>Min/+</sup> cells  
371 (Mogensen et al., 2002). Disruption of the microtubule scaffold may also cause the defects  
372 in cell volume and height observed in *Apc*<sup>Min/Min</sup> cells consistent with recent reports  
373 suggesting that disruptions of the apical-basal orientation of microtubules can reduce cell  
374 height (Toya et al., 2016).

375         The formation and position of the basal process underlies post-mitotic separation.  
376 Unlike previous reports in the colon (Bellis et al., 2012), we demonstrate that asymmetric  
377 process localisation actively promotes neighbour exchange and niche exit. How basal  
378 processes form is unclear, whether as a cause or a consequence of mitosis. In *Apc* mutant  
379 cells, as in the colon (Bellis et al., 2012), processes are usually symmetrically placed and they  
380 form more slowly. The increased time required to complete INM in *Apc* mutant cells (Figure  
381 4) may be responsible, by reducing the time available to establish an asymmetric process.

382         Cell morphology is also important for post-mitotic separation. Cells in highly  
383 abnormal regions of FAP tissue were significantly compressed laterally, suggesting that  
384 mutant cells are smaller and/or softer than wild-type cells. There is growing evidence that  
385 malignant cells are softer than untransformed cells (Plodinec et al., 2012). Reduced cell  
386 volume could cause both lateral and/or apical-basal compression and restrict nuclear  
387 movement and impair INM. This would cause mutant cells *in vivo* to remain close to their  
388 sisters and colonise a niche more successfully than wild-type cells. Altered cell morphology  
389 is evident in human intestinal organoids after *Apc* depletion and also seen with mutations in  
390 KRas, P53 or SMAD4 (Drost et al., 2015), suggesting that post-mitotic separation can be  
391 compromised by other contributing mutations which affect cell morphology.

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392 In summary, we provide evidence that post-mitotic separation is a general  
393 mechanism used by intestinal epithelial cells to control niche access. This cellular  
394 mechanism could further explain the stochasticity of intestinal homeostasis and how it  
395 becomes biased to create a pre-neoplastic state.

396

#### 397 **Contributions of authors:**

398 T.D.C and I.N designed the study; T.D.C collected the data and performed the analysis; A.J.L  
399 assisted with organoid culture and provided images for analysis; J.M.O performed the  
400 computational modelling and associated analysis; I.P.N. assisted with animal handling,  
401 maintenance and assisted with the scoring of mitotic events; P.L.A. assisted with method  
402 development for long-term time-lapse microscopy of organoids; T.D.C and I.N wrote the  
403 manuscript with assistance from J.M.O.

404

#### 405 **Conflicts of interests**

406 The authors report no conflicts of interest.

407

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413 size; Prof. Mark Chaplain (Univ. of St. Andrews) for helpful discussions on measuring spindle  
414 alignment.

415

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## 416 **MATERIALS AND METHODS**

### 417 **Mice**

418 All experiments involving mouse tissue were performed under UK home office guidelines.  
419 CL57BL/6 wild-type, *Lgr5*-EGFP-IRES-creERT2 (*Lgr5*<sup>GFP/+</sup>), *Apc*<sup>Min/+</sup> and R26-rtTA Col1A1-H2B-  
420 GFP (H2B-GFP) mice were sacrificed by cervical dislocation or CO<sub>2</sub> asphyxiation.

421

### 422 **Tissue Preparation: Mouse Small Intestine**

423 Adult mouse small-intestine was washed briefly in PBS and fixed with 4% PFA for 3 hours at  
424 4°C. Intestine was cut into 2x2cm<sup>2</sup> pieces and fixed in 4% PFA overnight at 4°C. The tissue  
425 was embedded in 3% low melting temperature agarose and sectioned at 200µm intervals  
426 using a Vibratome (Leica). Cut sections were washed in PBS and permeabilized for 2 hours  
427 with 2% Triton X-100 and incubated with Blocking Buffer (1% BSA, 3% Normal Goat Serum,  
428 0.2% Triton X-100 in PBS) for 2 hours at 4°C. Tissue was incubated for 48 hours with Hoechst  
429 33342 (Thermo Fisher, 1:500) and phalloidin (Molecular Probes, 1:150) diluted in Working  
430 Buffer (0.1% BSA, 0.3% Normal Goat Serum, 0.2% Triton X-100 in PBS) at 4°C. The tissue was  
431 washed with PBS before mounting in Prolong Gold. Sections were mounted on coverslips  
432 between 2x120µm spacers to preserve tissue structures.

433

### 434 **Organoid culture**

435 Organoids were generated from mouse small intestinal crypts as previously described (Sato  
436 et al., 2009). Briefly, small intestines were removed and washed in PBS and opened  
437 longitudinally. Villi were removed by scraping the luminal surface with a coverslip. Tissue  
438 was washed in PBS, incubated in 30mM EDTA (20 minutes) and crypts dislodged by vigorous

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439 shaking. Crypt suspensions were centrifuged (600rpm, 4°C) and the pellet washed twice in  
440 PBS and dissociated to single cells with TripLE Express (Life Technologies) at 37°C for 5 mins.  
441 Cells were resuspended in Advanced DMEM/F12 (ADF) and filtered through a 40µm cell  
442 strainer (Greiner). Single cells were resuspended in growth factor reduced, phenol red-free  
443 Matrigel (BD Biosciences). Organoids were grown in crypt media (ADF supplemented with  
444 10mM HEPES, 2mM Glutamax, 1mM N-Acetylcysteine, N2 (Gemini), B27 (Life Technologies),  
445 Penicillin-Streptomycin (Sigma-Aldrich), growth factors (EGF, 50ng/ml; Invitrogen, Noggin  
446 (100ng/ml; eBioscience), and R-Spondin conditioned media (1:4). Chiron99021 (3µM;  
447 Invitrogen), valproic acid (1mM; Invitrogen) and Y27632 (10µM; Cambridge Bioscience)  
448 were added to organoids for the first 48 hours. Organoids were passaged by physically  
449 breaking up Matrigel, washing in ADF, dissociation by pipetting and reseeded in Matrigel.

450

#### 451 **Human tissue**

452 Human tissue used in this study was the same as used for a previous study (Fatehullah et al.,  
453 2016b). All tissue collected was approved by the Tayside Tissuebank subcommittee of the  
454 Local Research Ethics Committee and obtained in accordance with approved guidelines. FAP  
455 biopsies from one FAP patient was obtained during routine colonoscopy surveillance.

456

#### 457 **Organoid Immunofluorescence**

458 Organoids were grown in 8-well chamber slides (Ibidi) for 1-2 days at 37°C, 5% CO<sub>2</sub>.  
459 Organoids were fixed with warmed 4% PFA in PBS (pH 7.4) for 30 minutes (37°C),  
460 permeabilized for 1 hour in 1% Triton X-100 (this and subsequent steps were performed at  
461 room temperature), blocked for 1 hour (1% BSA, 3% Normal Goat Serum, 0.2% Triton X-100



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462 in PBS). Organoids were incubated overnight in primary antibodies diluted in Working  
463 Buffer (0.1% BSA, 0.3% Normal Goat Serum, 0.2% Triton X-100):  $\gamma$ -tubulin (Sigma: T6557,  
464 1:500), GFP (Abcam: ab13970, 1:500);  $\beta$ 4-Integrin (Abcam: ab25254, 1:100); YL1/2 (1:200),  
465 washed 5x with Working Buffer before overnight incubation with secondary antibodies  
466 diluted in Working buffer: Alexafluor™ conjugated (1:500, Molecular Probes) along with  
467 5 $\mu$ g/ml Hoechst 33342 and Alexafluor™ conjugated phalloidin (1:150). Organoids were  
468 mounted in Prolong Gold overnight.

469

#### 470 **Microscopy**

471 Images of tissue and organoids were acquired with a Zeiss LSM 710 or LSM 880 with  
472 Airyscan (Carl Zeiss) using 25X or 40X Zeiss objective lenses and immersion oil with  
473 refractive index of 1.514. Serial image stacks were acquired with an optical section size of  
474 0.8 $\mu$ m.

475

#### 476 **Confocal Live Imaging**

477 Organoids were grown in Matrigel and spread thinly onto 35mm<sup>2</sup> glass bottom dishes  
478 (World Precision Instruments). Crypt media was supplemented with 2mg/ml doxycycline to  
479 induce H2B-GFP expression. For live-cell imaging of mitochondrial dynamics, induced H2B-  
480 GFP organoids were incubated with 500nM Mitotracker DeepRed FM (ThermoFisher  
481 Scientific) in crypt media for 1 hour; 37°C 5% CO<sub>2</sub>. Subsequently, staining media was  
482 replaced with fresh crypt media containing growth factors. For live-imaging of actin,  
483 organoids were stained with 100nM SiR-Actin in crypt media overnight. Organoids were  
484 placed in a live-cell imaging chamber attached to a Zeiss 710 confocal microscope

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485 maintained at 37°C, 5% CO<sub>2</sub>. Images were acquired using a 40X Zeiss immersion objective.  
486 Serial image stacks were acquired at optimal interval sizes using minimal laser power every  
487 6 minutes.

488

### 489 **Spindle Angle measurements**

490 Spindle orientation was measured using image stacks and analysed using the Imaris imaging  
491 software (Bitplane). Surfaces of the Hoechst,  $\gamma$ -tubulin and phalloidin signals were rendered  
492 using the isosurface tool. Similar to a report in the mouse colon (**Bellis et al., 2012**), we used  
493 two angles to represent spindle orientation: 1) relative to the crypt axis (Axial angle) and 2)  
494 relative to the apical surface (Apical angle). The apical surface was defined using the F-actin  
495 signal at the luminal surface. To calculate angles, three sets of measurement points were  
496 manually placed in 3D:

497 **(1)** Two points defining the two centrosomes. The connection between them represents the  
498 spindle axis.

499 **(2)** Two points placed at either end of the crypt so that the axis formed between them is  
500 representative of the crypt axis.

501 **(3)** Three points placed on the rendered phalloidin surface to represent the cells apical  
502 surface.

503 Axial angles are the angles between the spindle and crypt-axis. The Apical angle is calculated  
504 using the 3 measurement points to determine the normal surface vector to the apical plane.

505 The Axial angle between this defined apical surface and the spindle axis was calculated as  
506 follows:

507 *Calculating the Axial angle*

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508 The Spindle-axis vector ( $\underline{s}$ ) is calculated using Centrosome point 1,  $[S_{x1}, S_{y1}, S_{z1}]$ , and  
 509 centrosome point 2,  $[S_{x2}, S_{y2}, S_{z2}]$ , (**Equation 1**). The crypt-axis vector ( $\underline{c}$ ) is calculated using  
 510 crypt-axis point 1,  $[C_{x1}, C_{y1}, C_{z1}]$ , and Crypt-axis point 2,  $[C_{x2}, C_{y2}, C_{z2}]$ , (**Equation 2**).

$$511 \quad \underline{s} = (S_{x2} - S_{x1}), (S_{y2} - S_{y1}), (S_{z2} - S_{z1}), \quad [1]$$

$$512 \quad \underline{c} = (C_{x2} - C_{x1}), (C_{y2} - C_{y1}), (C_{z2} - C_{z1}). \quad [2]$$

513 The  $\alpha$ -angle is calculated by projecting the Vector  $\underline{s}$  on Vector  $\underline{c}$  (**Equation 3**)

$$514 \quad \alpha^\circ = \frac{180}{\pi} \left[ \cos^{-1} \left( \frac{\underline{s} \cdot \underline{c}}{|\underline{s}| |\underline{c}|} \right) \right], \quad [3]$$

$$515 \quad \text{where} \quad \frac{\underline{s} \cdot \underline{c}}{|\underline{s}| |\underline{c}|} = \frac{(S_x C_x) + (S_y C_y) + (S_z C_z)}{\left( \sqrt{S_x^2 + S_y^2 + S_z^2} \right) \left( \sqrt{C_x^2 + C_y^2 + C_z^2} \right)}. \quad [4]$$

516 Calculating the Apical angle

517 The Apical angle is calculated using three apical surface points (AP):  $[A_x, A_y, A_z]$ ,  $[B_x, B_y, B_z]$   
 518 and  $[C_x, C_y, C_z]$ , placed on the plane, which approximates the apical surface. The co-  
 519 ordinates of these points are used to determine two vectors,  $\underline{a}$  and  $\underline{b}$  (**Equation 5, 6**).

$$520 \quad \underline{a} = \left( (B_x - A_x), (B_y - A_y), (B_z - A_z) \right) = (a_x, a_y, a_z), \quad [5]$$

$$521 \quad \underline{b} = \left( (C_x - A_x), (C_y - A_y), (C_z - A_z) \right) = (b_x, b_y, b_z). \quad [6]$$

522 These vectors can subsequently be used to determine the normal surface vector ( $\underline{\hat{n}}$ ) to the  
 523 apical plane by finding the cross product between vectors  $\underline{a}$  and  $\underline{b}$ . (**Equation 7**).

$$524 \quad \underline{n} = \underline{a} \times \underline{b} = \left( (a_y b_z - a_z b_y), (a_z b_x - a_x b_z), (a_x b_y - a_y b_x) \right)$$

$$525 \quad \quad \quad = (n_x, n_y, n_z),$$

$$526 \quad \text{and} \quad \underline{\hat{n}} = \frac{1}{\sqrt{n_x^2 + n_y^2 + n_z^2}} (n_x, n_y, n_z). \quad [7]$$

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527 The normal surface vector can then be used to determine the angle between the spindle  
528 vector ( $\underline{s}$ ) and the surface plane (**Equation 7**).

529 
$$\beta^\circ = \frac{180}{\pi} \left[ \sin^{-1} \left( \frac{(\hat{n} \cdot \underline{s})}{|\underline{s}|} \right) \right]. \quad [8]$$

530 Given that  $|\hat{n}| = 1$ .

531

### 532 **INM Measurements**

533 Apical and basal interkinetic nuclear migration was measured by determining the distance  
534 between the nucleus and the plane of the epithelium. The plane of the epithelium is defined  
535 as the plane formed between the neighbours of the query nucleus:

536 Find the absolute distance of the mitotic cell,  $M(x_0, y_0, z_0)$  to epithelial plane,  $P(Ax +$   
537  $By + Cz + D = 0)$ . Where the epithelial plane is defined by the plane formed between 3  
538 neighbouring epithelial cells.

539 Distance to the epithelial plane = 
$$\frac{|Ax_0 + Ay_0 + Az_0 + D|}{\sqrt{A^2 + B^2 + C^2}}$$

540 Distance measurements were calculated for 10 planes encompassing each permutation of  
541 neighbouring cells. The average distance for these 10 planes was taken as the  
542 representative distance of the query cell in reference to the plane of the epithelium in which  
543 it originated. This was to account for variability induced by the curvature within the  
544 organoid branches.

### 545 **Definition of Stem Cell and Transit Amplifying compartments**

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546 Stem cell and transit amplifying compartments were defined based on the average position  
547 of Lgr5+ cells along the crypt-villus axis measured in Lgr5-GFP organoids (S5 Figure). As Lgr5  
548 can also be expressed in the early TA compartment (Quyn et al., 2010), we conservatively  
549 defined the SC compartment based on the average position of Lgr5(+) cells rather than the  
550 average distance of the Lgr5(+) cell furthest from the crypt base.

551

### 552 **Distance measurements**

553 Cell position was determined by placing a measurement point in the centre of each nucleus.  
554 The co-ordinates of individual points were used to measure the distances between nuclei or  
555 from the crypt base. The crypt base was defined by a reference nucleus manually chosen at  
556 the crypt base. The reference nucleus was determined at each time point. Distance between  
557 points was calculated by the standard formula:  $d =$   
558  $\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2}$  [9]

559

### 560 **Time-lapse analysis and scoring**

561 Time-lapse image stacks were analysed manually using Imaris (Bitplane). All mitotic events  
562 were marked using the '3D spots function'. Time-point 0 was denoted as the time-point  
563 immediately before cytokinesis. Each mitotic daughter cell was tracked until its death,  
564 subsequent division, or exit from the imaging window. If daughter cells were separated by a  
565 neighbouring nucleus after basal INM (at 120 minutes) they were scored as separated.  
566 Displacement from the 'point of birth' was calculated as the change in distance between the  
567 start (mitotic mother) and end position of a daughter, divided by the time interval.  
568 Daughter 1 was always classified as the daughter closest to the base of the crypt.

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569

570 **INM measurement**

571 We determined the distance covered by nuclei during INM by measuring the distance of the  
572 mitotic nucleus to a defined basal reference plane. The basal reference was defined as the  
573 plane formed by neighbouring interphase cells. The co-ordinates of 5 neighbouring nuclei  
574 most proximal to the mitotic cell were determined. These interphase cells often did not  
575 form a simple plane due to curvature of the epithelial sheet. To account for the shape, we  
576 calculated the nearest distance from the position of the mitotic cell to the planes formed by  
577 combinations of each of three nuclei for the 5 neighbours. The average distance from these  
578 (10) planes was then used as the estimated apical distance travelled during INM (S5 Figure).  
579 Please refer to supplementary information for further details.

580

581 **Cell height measurement**

582 Apical-basal distance was measured as the distance between the apical and basal surfaces  
583 using Imaris. Distances were calculated in the optical section at the centre of the chosen  
584 cell by recording one 3D measurement point in the middle of the apical and one in the  
585 middle of the basal surface. The distance between these points was the apical-basal  
586 distance.

587

588 **Sample size and Statistical analysis**

589 Analyses were performed using at least three different organoids. Individual cell  
590 comparisons were performed using at least 10 cells. Comparisons had to be made between  
591 organoids imaged in separate imaging sessions because each time lapse took three days. All

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592 statistical tests were performed using Prism 6.0a (GraphPad). Tests were performed as  
593 described in figure legends (significance: ns = not significant, \* $p < 0.05$ , \*\* $p < 0.01$ ,  
594 \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ ).

595

## 596 **Multicellular Computational Model**

597 All simulations were undertaken in the CHASTE framework (Mirams et al., 2013). We  
598 extended the model presented in (Dunn et al., 2016) to permit variable separation of cells  
599 after division. In summary, cells are represented by their centres, which are free to move  
600 on a surface (in 3 dimensional space) that is defined using measured crypt geometry. Cells  
601 move because of forces exerted on them due to compression of, and by, neighbouring cells.  
602 We are using the optimal model identified in Dunn et al., 2016, Model 6. In this model cells  
603 divide after a uniformly distributed time that depends on the level of Wnt (imposed as a  
604 linear gradient) experienced when the parent cell divided. Additionally, if cells are  
605 compressed beyond a given threshold they pause in the G1 phase of the cell cycle. All  
606 parameters used are as described in Dunn et al., 2016. As in previous three-dimensional  
607 models of the crypt (Dunn et al., 2016, Dunn et al., 2013), cell division occurs in a direction  
608 uniformly drawn from the sphere surrounding the centre of the dividing cell. Daughter cells  
609 are placed at a specified distance from one another. Previously, in all existing models, this  
610 distance is chosen so daughter cells are adjacent to each other. We modified this parameter  
611 so that two thirds of all cell divisions resulted in the daughter cells being placed next to each  
612 other (a separation of 1 cell diameter), the remaining third resulted in the daughter cells  
613 being separated by  $S$  cell diameters. We vary this parameter between 0 and 3 to measure  
614 the effects on separation of cells in the virtual crypt.

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## 615 **Supplemental Information**

616 We provide supplemental information describing mitosis in intestinal epithelium and organoids,  
617 showing that they are similar and suggest that INM requires microtubules (S1 Figure). In addition to  
618 asymmetric basal tethering, post-mitotic separation appears to be influenced by the proximity of  
619 other mitotic daughters and by proximity to Paneth cells which are less mobile (S2 Figure). We also  
620 show that asymmetric tethering and spindle orientation are partially linked and that this is altered in  
621 *Apc* mutant tissue (S3 Figure). We show that disruption of INM can also be induced by hyper-  
622 activation of Wnt (S4 Figure). We provide figures demonstrating our definition of the stem and  
623 transit amplifying compartments and an illustration of how INM was measured (S5 Figure).

624

## 625 **Movie Legends**

### 626 **S1 Movie. H2B-GFP Intestinal Organoids**

627 Confocal LSM imaging of induced H2B-GFP organoids derived from wild-type and *Apc*<sup>Min/+</sup> mice (Both  
628 untransformed (*Apc*<sup>Min/+</sup>) and transformed cysts (*Apc*<sup>Min/Min</sup>)).

### 629 **S2 Movie. Adjacent Sister Placement**

630 Confocal LSM imaging of an induced wild-type H2B-GFP organoid showing manual tracking of a  
631 mitotic cell and its progeny. Daughters were tracked manually using Imaris. In this example, both  
632 daughter cells 're-insert' into the epithelium as neighbours.

### 633 **S3 Movie. Post-mitotic Separation**

634 Confocal LSM imaging of an induced wild-type H2B-GFP organoid showing manual tracking of a  
635 mitotic cell and its progeny. Daughters were tracked manually using Imaris. In this example, the  
636 'blue' daughter cell is displaced from its sister.

### 637 **S4 Movie. 4D Visualisation of post-mitotic separation**

638 Confocal LSM imaging of an induced wild-type H2B-GFP organoid showing manual tracking of a  
639 mitotic cell and its progeny undergoing post-mitotic separation. Surface rendering was performed to  
640 highlight the mother (cyan), sisters (blue/red) and neighbour cells (magenta). The respective



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641 timelapse is shown in the top panels and a 3D rotation around the timepoints encompassing  
642 interphase, INM, cytokinesis and after separation are displayed in the bottom panels.

643 **S5 Movie. Mitotracker in Intestinal Organoids**

644 Confocal LSM imaging of an induced H2B-GFP organoid treated with Mitotracker.

645 **S6 Movie – Mitotic neighbours**

646 Confocal LSM imaging of an induced wild-type H2B-GFP organoid showing manual tracking of a  
647 mitotic cell and its progeny undergoing post-mitotic separation. In this example, the daughters of  
648 the original mitosis (red) are displaced by the placement of a daughter cell from an adjacent mitosis  
649 (purple).

650 **S7 Movie – Paneth Cell proximity and displacement**

651 Confocal LSM imaging of an induced wild-type H2B-GFP organoid showing manual tracking of a  
652 mitotic cell and its progeny undergoing post-mitotic separation. In this example, the daughters of  
653 this mitosis are displaced in proximity to a Paneth cell (recognisable by the large space with no  
654 nuclei). This movie was used in stills in S2 figure.

655 **S8 Movie – Delayed Daughter Cell Insertion**

656 Confocal LSM imaging of an induced wild-type H2B-GFP organoid showing manual tracking of a  
657 mitotic cell and its progeny undergoing post-mitotic separation. In this example the left most  
658 daughter takes two attempts to reassume its interphase position, whilst the other is displaced. This  
659 movie was used for stills in Figure 5E.

660 **S9 Movie – Asymmetric process segregation underlies post-mitotic separation**

661 Confocal LSM imaging of an induced wild-type H2B-GFP organoid treated with SiR-Actin. The movie  
662 shows a mitotic cell undergoing post-mitotic separation in which one daughter retains the basal  
663 process. The two daughters (white spheres) are separated by a neighbour after reinsertion.

664

665

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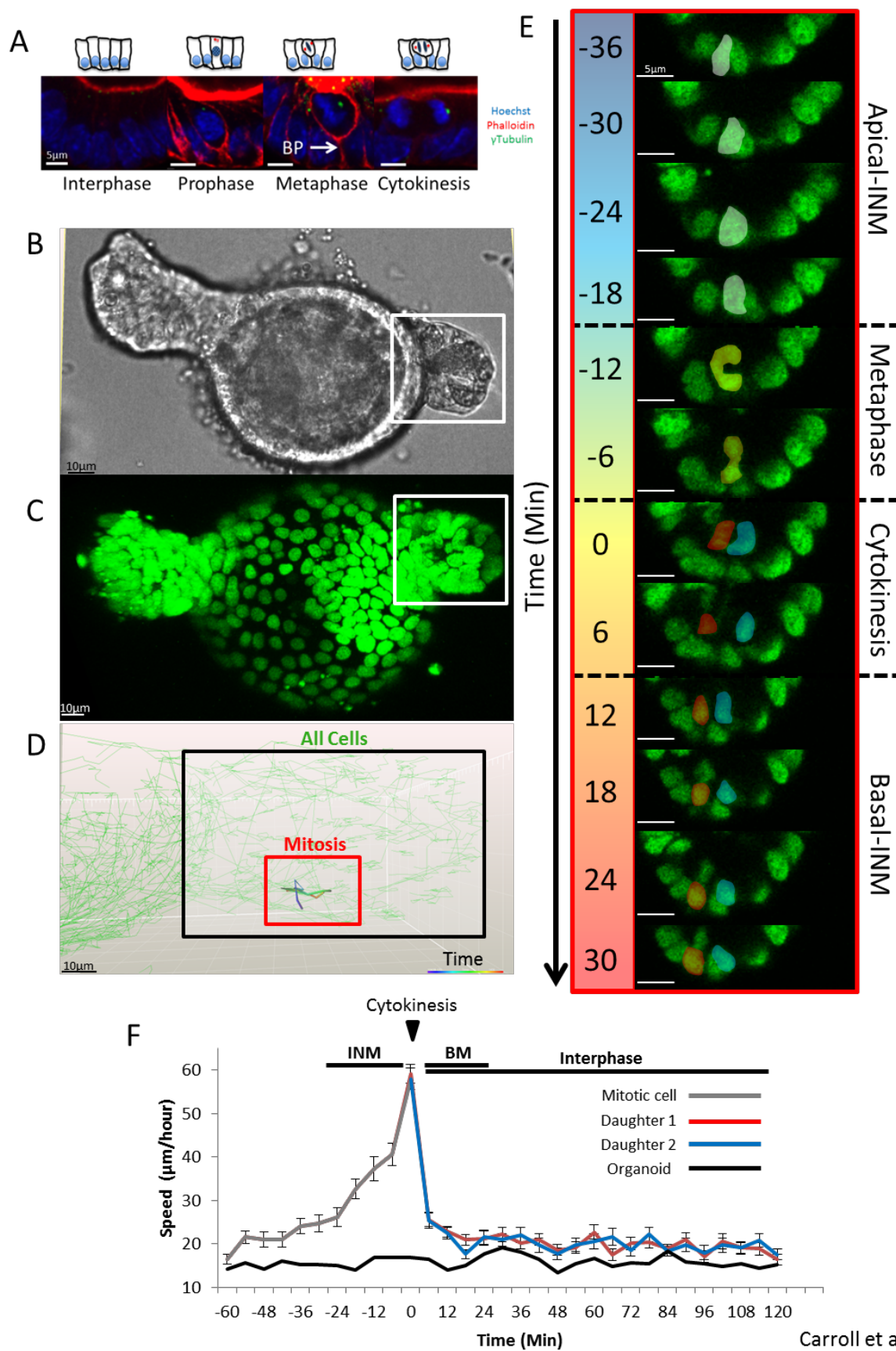
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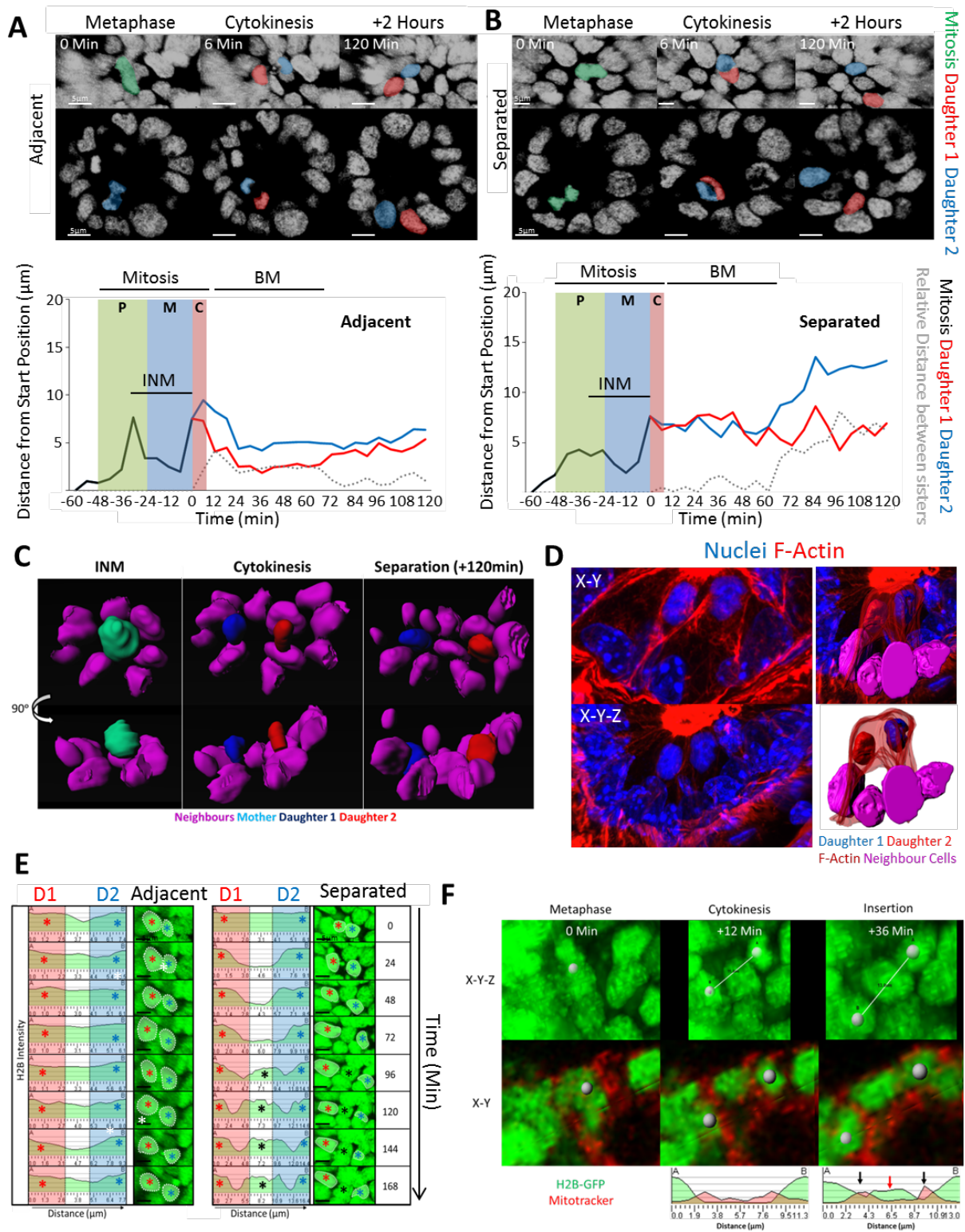
Carroll et al., Figure 1

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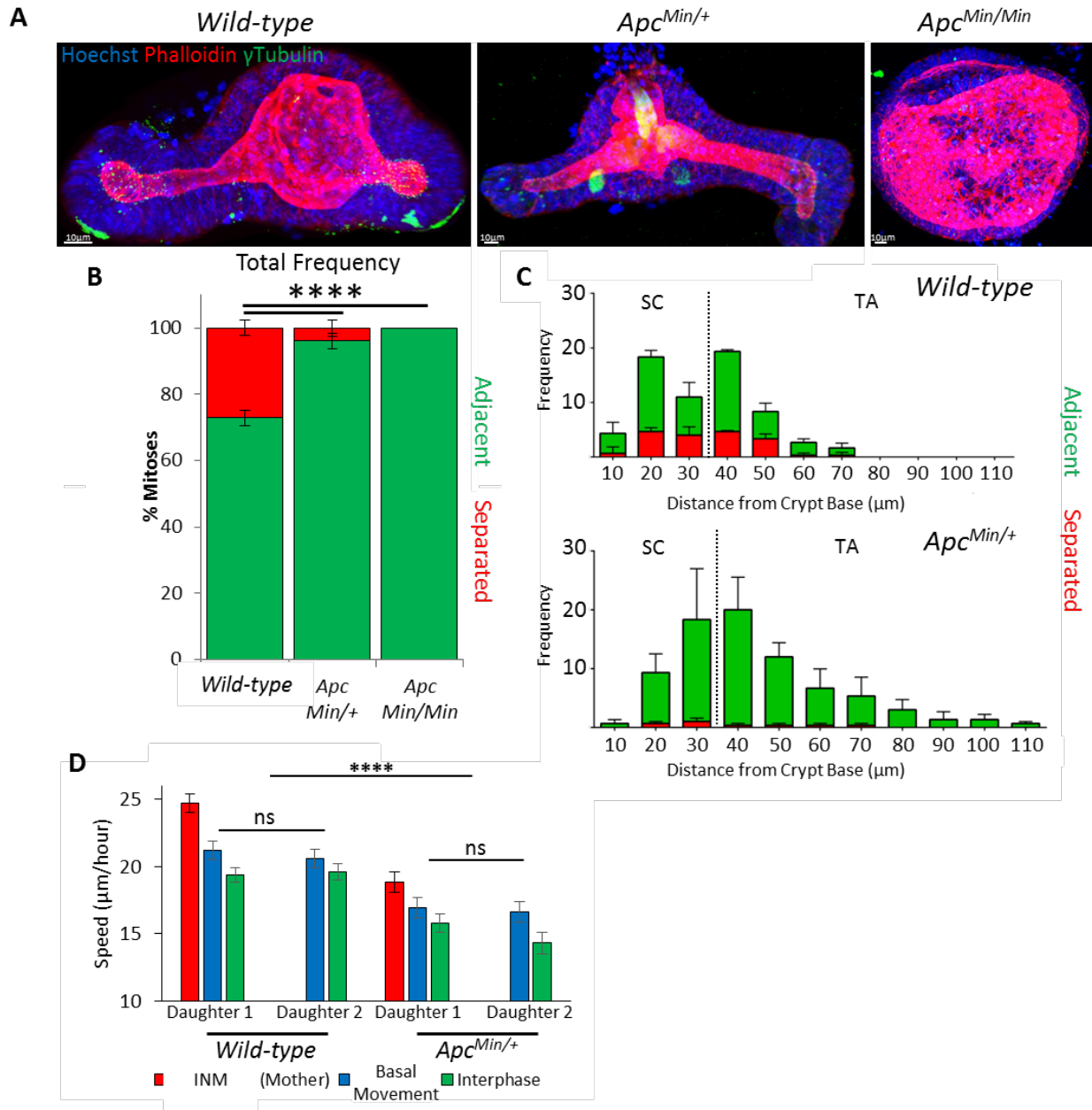


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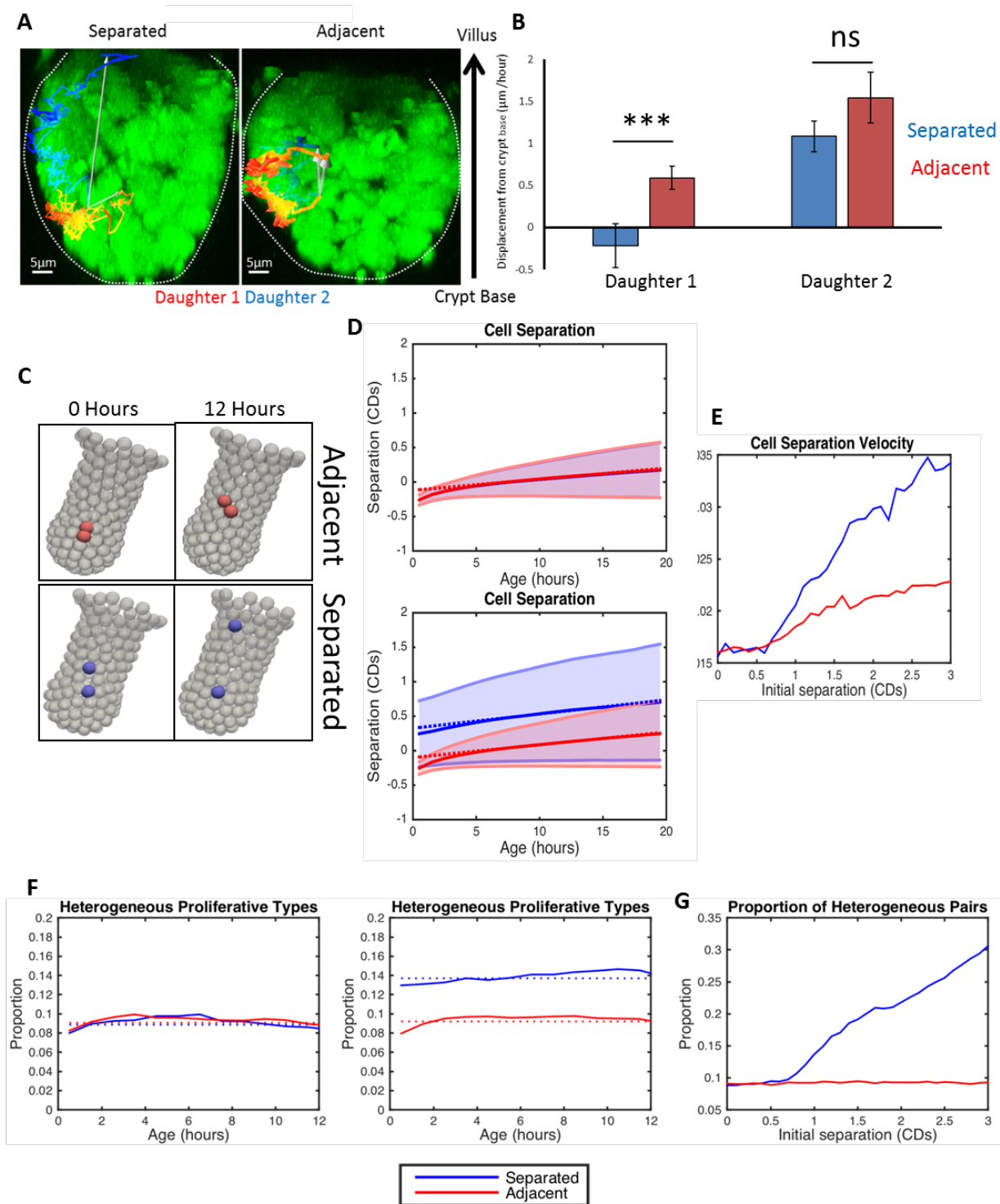


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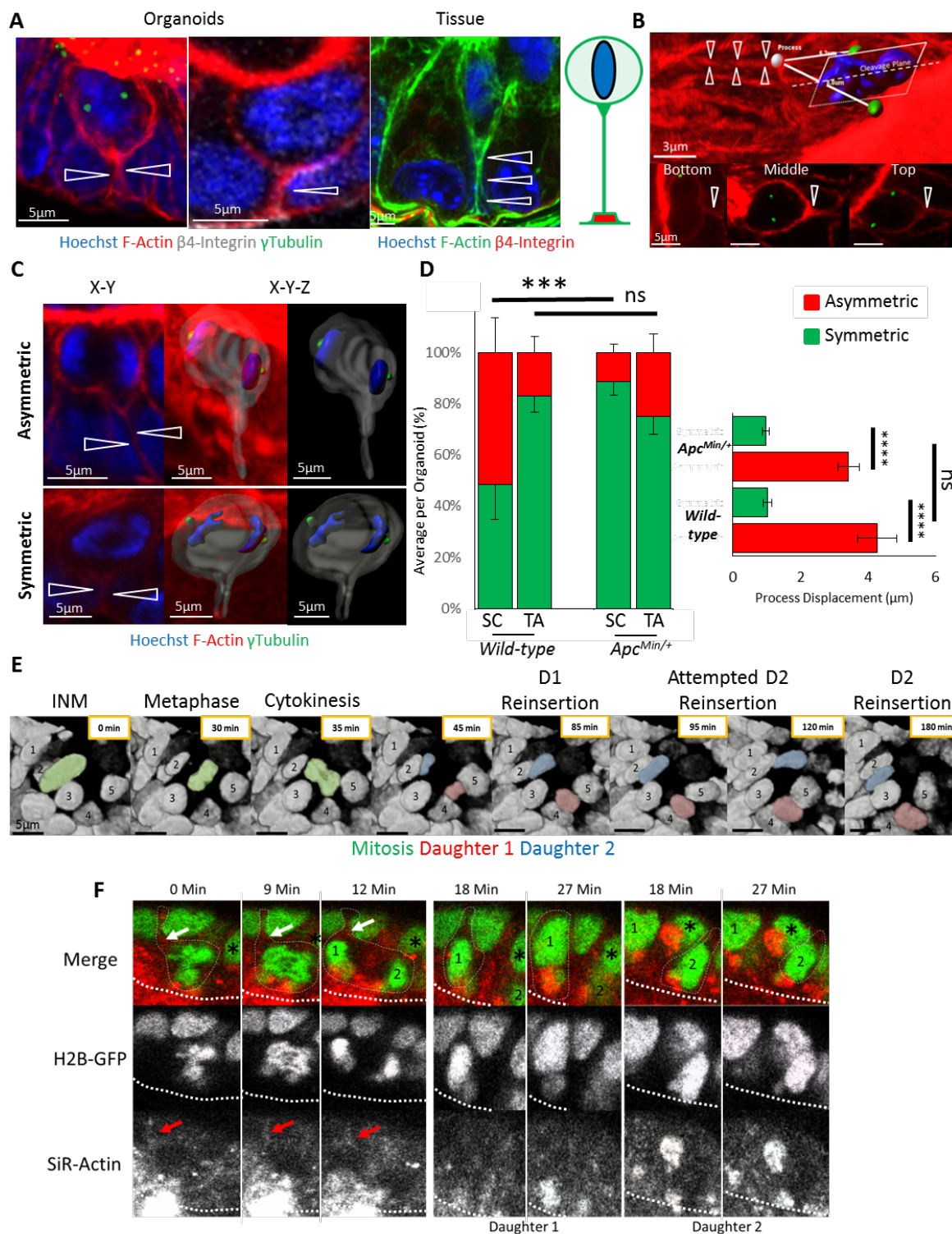
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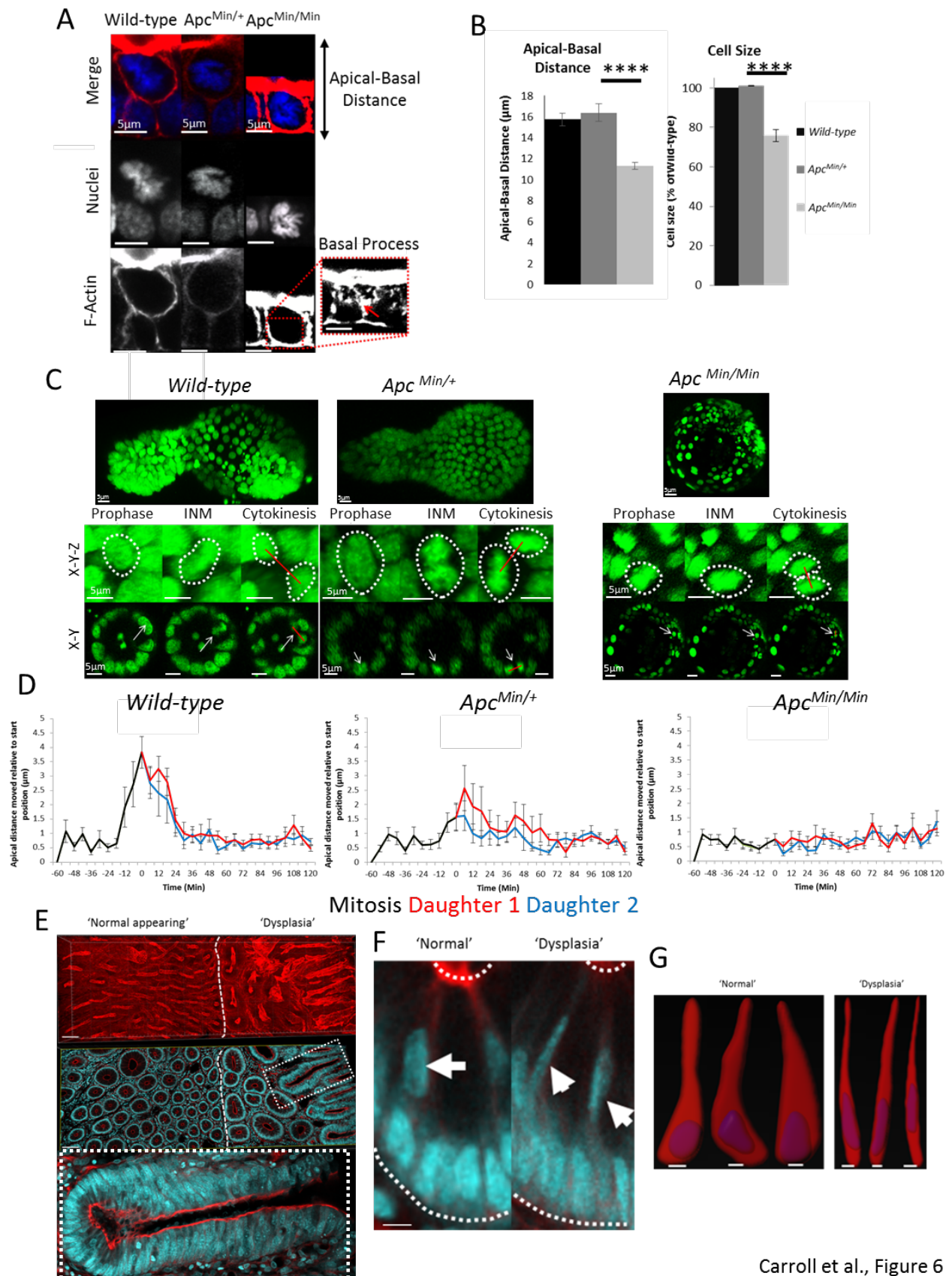


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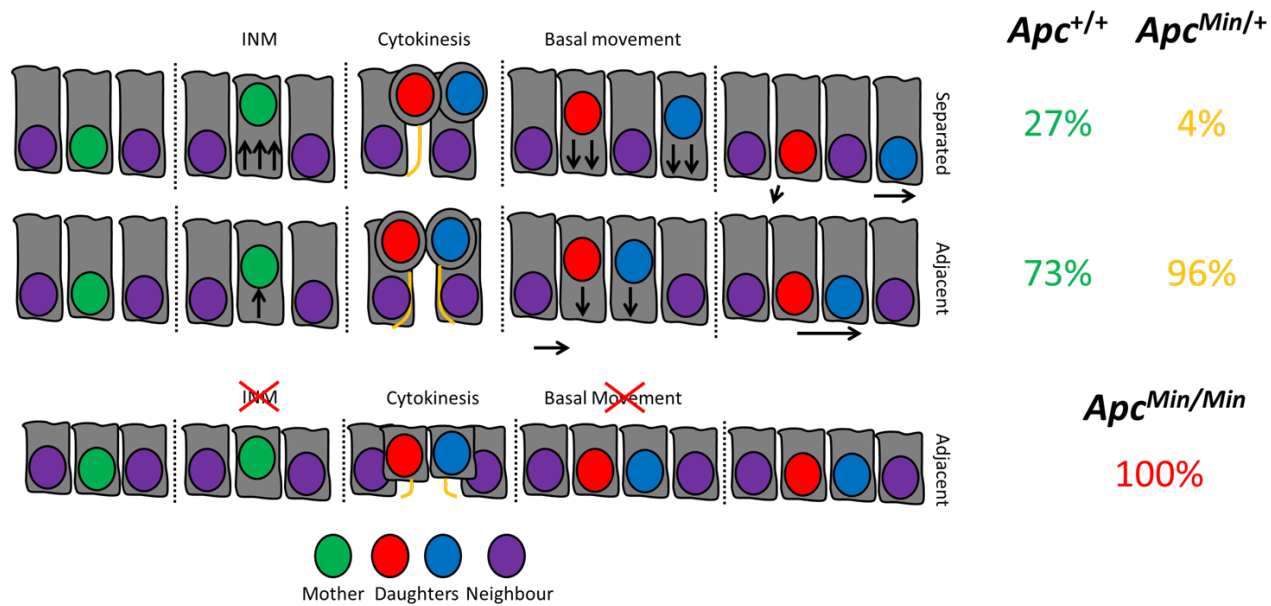
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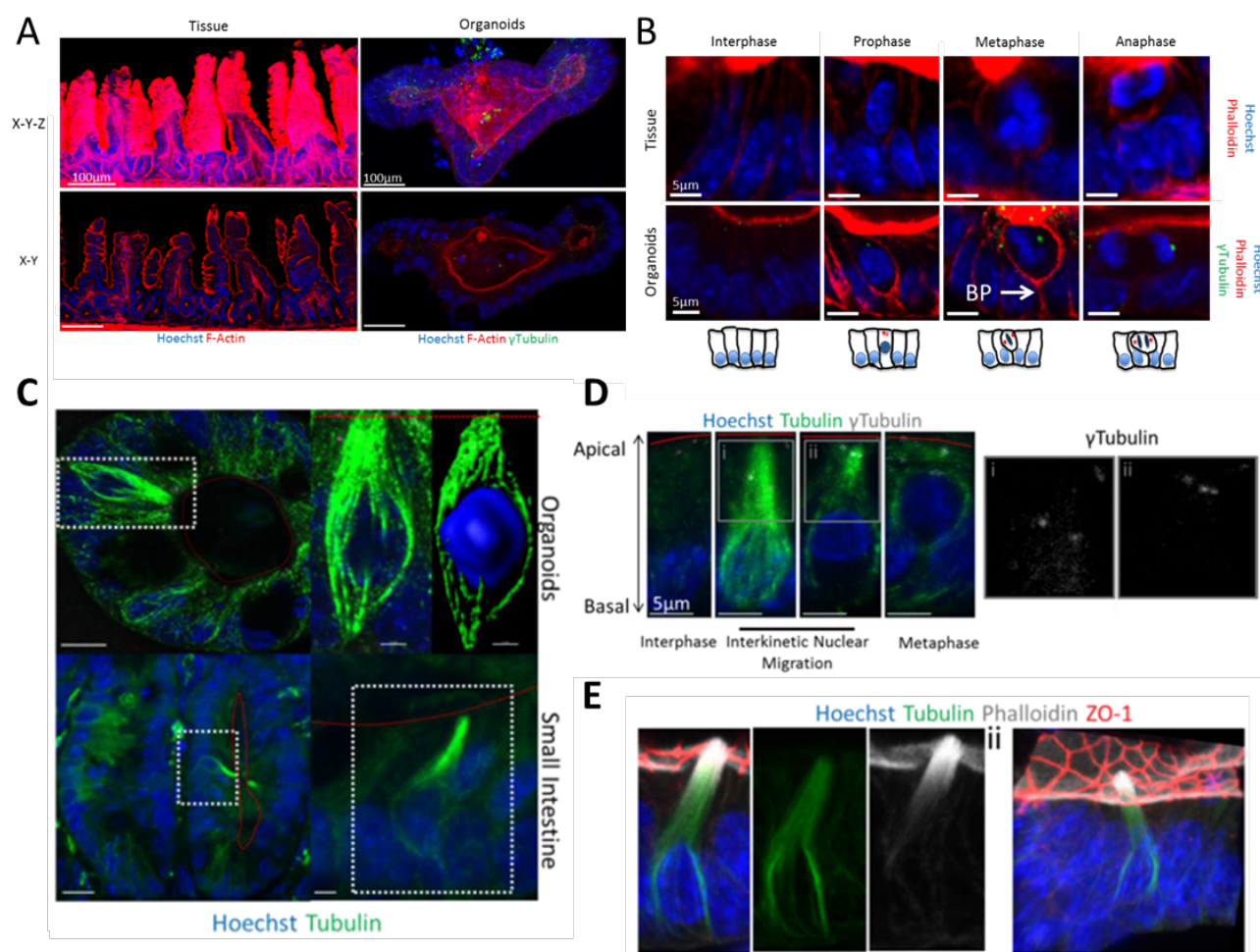
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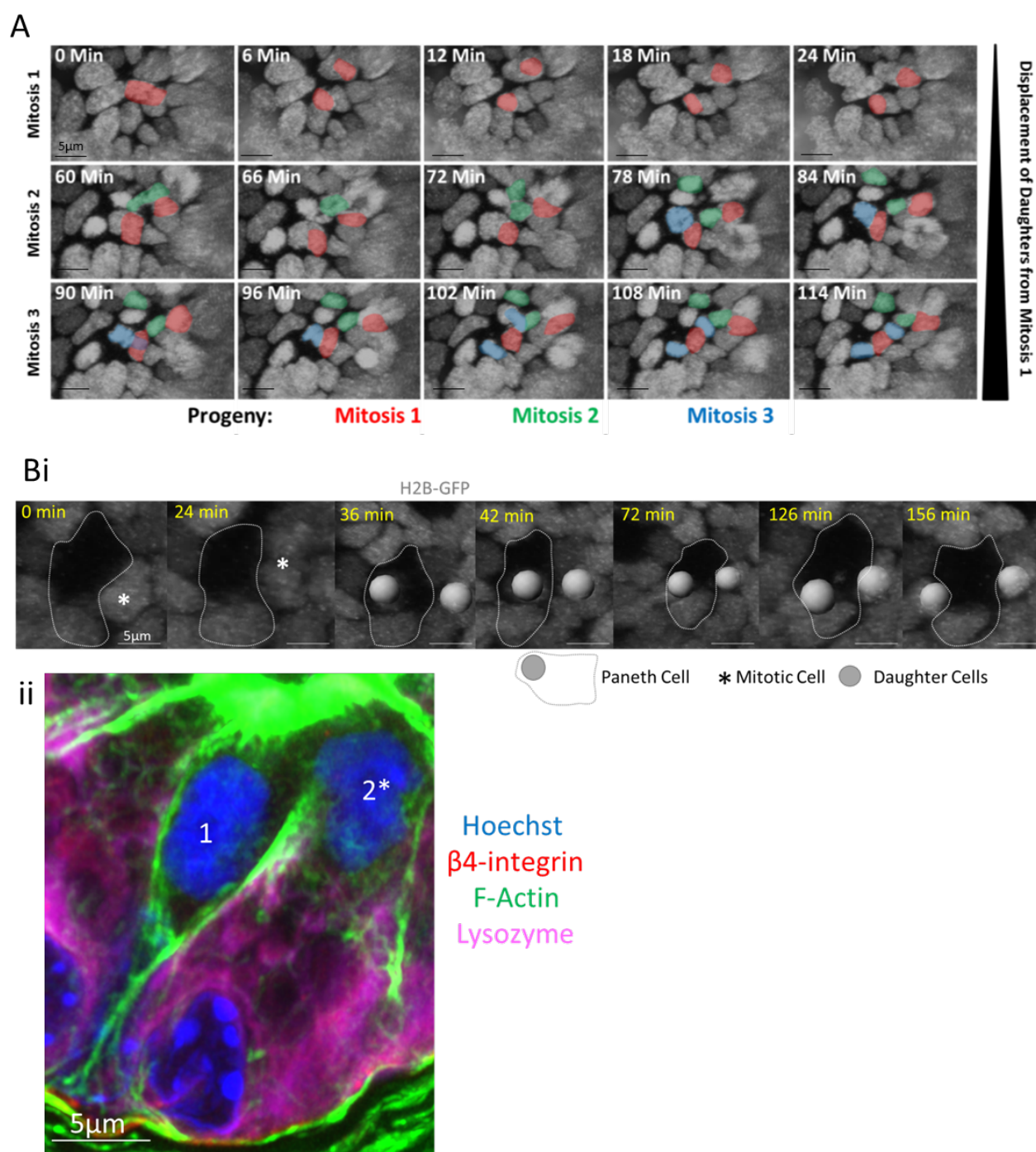


Carroll et al., S1 Figure

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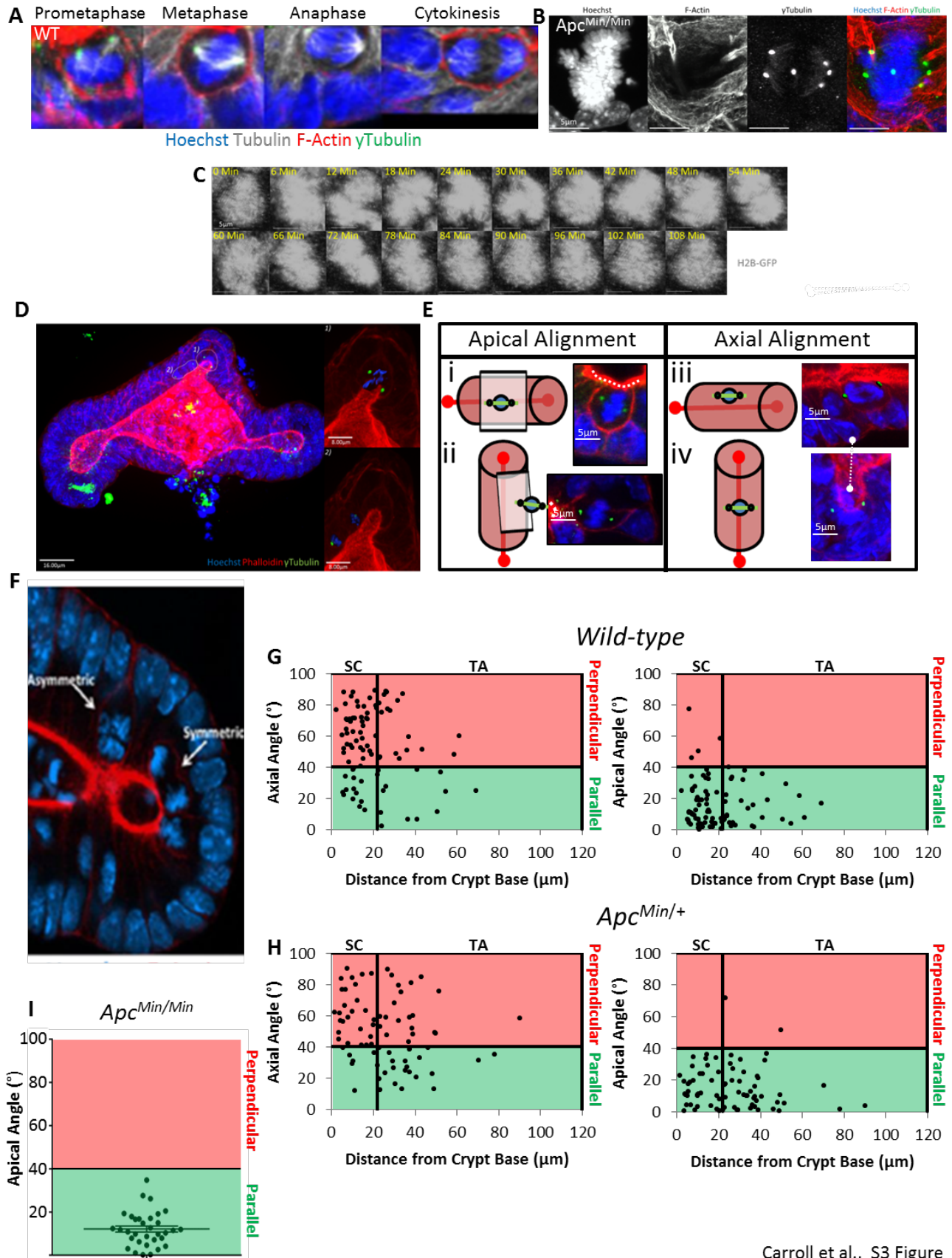


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Carroll et al., S2 Figure

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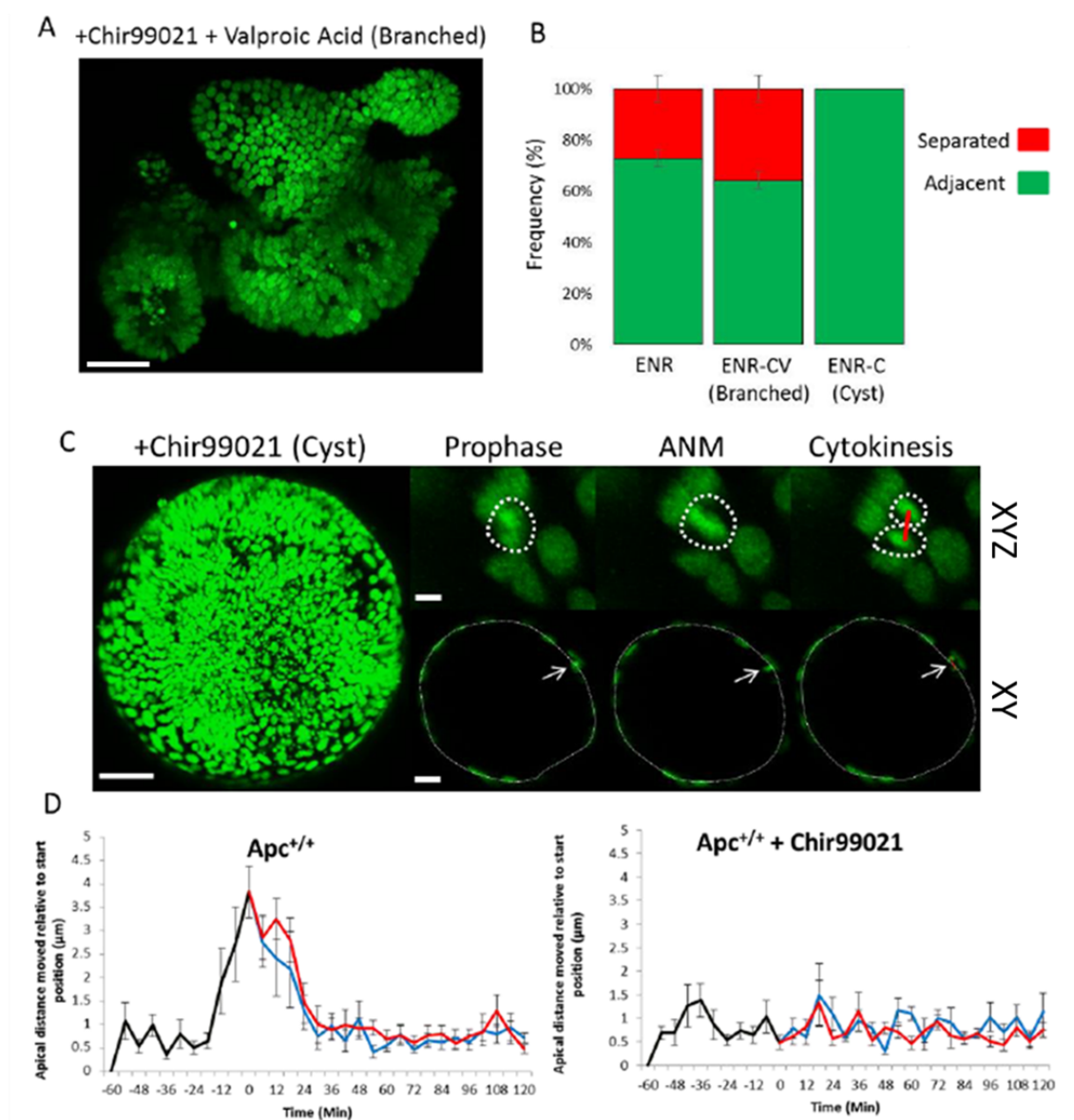
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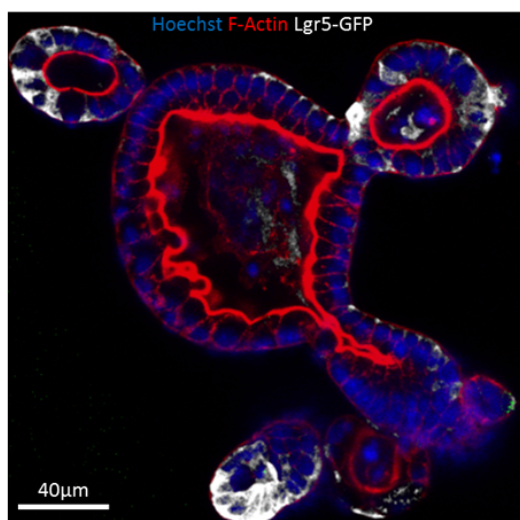
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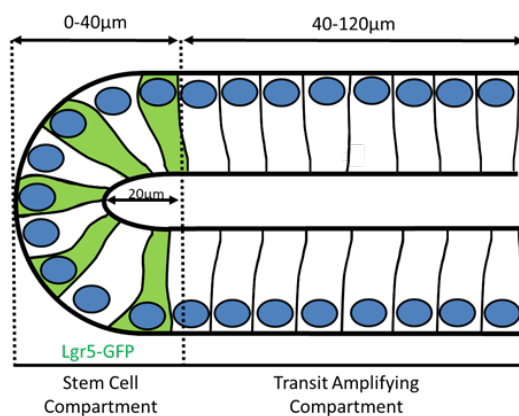
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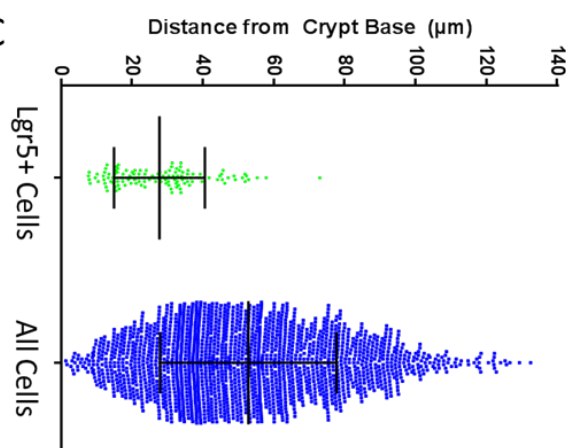
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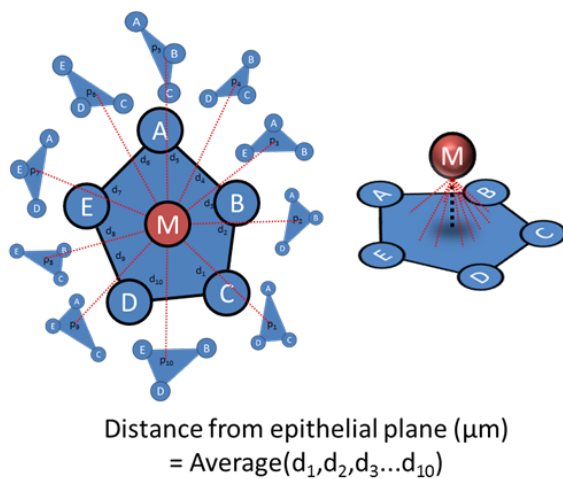
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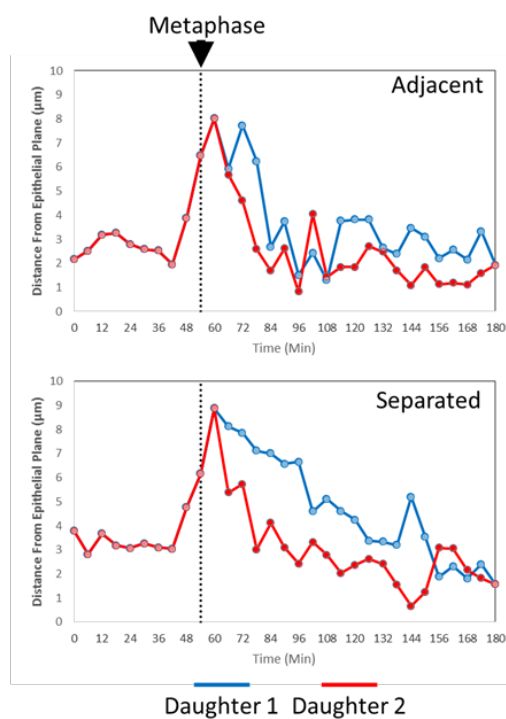
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Carroll et al., S5 Figure



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808 **Figure 1. Dynamics of mitosis in H2B-GFP intestinal organoids**

809 **(A)** Confocal sections of mitotic stages in intestinal organoids. Organoids were stained with  
810 Hoechst, phalloidin and  $\gamma$ -tubulin. (See also S1 Figure.) Representative bright-field **(B)** and  
811 fluorescent **(C)** images of a wild-type H2B-GFP intestinal organoid after 24 hours doxycycline  
812 treatment.

813 **(D)** Manual tracking of a mitotic cell and its daughters. The track is colour-coded based on  
814 time (red box) and is overlaid onto the tracks of neighbouring cells (green), tracked  
815 automatically. In this example, tracks represent a time-lapse covering 66 minutes.

816 **(E)** Dynamics of mitosis in intestinal organoids. Confocal sections (X-Y) of the mitotic cell  
817 highlighted in B. Prophase (white), metaphase (purple), cytokinesis (red) and daughter cell  
818 nuclei (blue and red) are shown.

819 **(F)** Cell speed before, during, and after mitosis, measured for mother (grey line) and  
820 daughters (red/blue lines). Movement of the entire organoid was measured for reference  
821 (black line). The average speed was calculated for 60 mitotic cells from 3 different  
822 organoids. Data is displayed as mean  $\pm$  SEM. Time-points encompassing interkinetic  
823 nuclear migration (INM), cytokinesis, basal cell movement (BM) and interphase are  
824 highlighted.

825

826 **Figure 2. Post-mitotic separation of daughter cells**

827 Mitotic cells were tracked manually for 60 minutes prior to cytokinesis and daughters for a  
828 further 120 minutes. Two types of mitotic types were revealed: **(A)** Daughter cells  
829 positioned adjacent or **(B)** which separated after mitosis. Displayed are 3D projections (top  
830 panels) and 2D sections through an organoid branch. Metaphase (green) and daughters  
831 (red/blue) are highlighted. Representative tracks show the distance of the mitotic mother  
832 (black line) and daughters (red/blue lines) from the original starting position. (P)rophase,  
833 (M)etaphase, (C)ytokinesis, interkinetic nuclear migration (INM), and basal cell movement  
834 (BM) are indicated. Distances between adjacently placed daughters (grey dashed line) are  $\leq$   
835 1 nuclear width ( $6\mu\text{m}$ ) whereas distances between separating daughters is greater.

836 **(C)** 3D rendering of neighbouring nuclei (purple), mother (cyan) and daughters (red/blue) of  
837 a post-mitotic separation event. Displayed are rotated views of cells and their direct  
838 neighbours at time-points encompassing INM, cytokinesis and after separation (120 minutes  
839 after cytokinesis).

840 **(D)** Daughter separation occurs *in vivo*. Representative image of daughters at a crypt base.  
841 Samples were stained with Hoechst (blue) and phalloidin (red). Highlighted are two  
842 prospective daughters (white stars) displayed in X-Y and X-Y-Z views (left panels). Surface  
843 rendering (right panels) highlights cell-cell boundaries and neighbouring cell nuclei.

844 **(E)** H2B-GFP line-intensity profiles were created along a line connecting the centres of sister  
845 nuclei at indicated times after cytokinesis (=Time 0). Reference images (3D projections) are  
846 displayed. Please note that the scaling for the x-axis, indicating distance, changes in the  
847 right-hand panel to accommodate the increased space between the separating daughters.

848 **(F)** Individual frames of an H2B-GFP organoid stained with Mitotracker highlighting a mitotic  
849 cell whose daughters separate shortly after mitosis. Time points reflecting metaphase,  
850 cytokinesis and after return of daughters to their interphase position (reinsertion) are  
851 shown. A line-intensity profile was generated between marked daughters (A and B) during  
852 cytokinesis and after 'insertion'. After reinsertion, a discrete H2B-GFP peak was detected  
853 that correspond to the neighbouring cell that displaces the two daughters (red arrow). The  
854 neighbouring cell has two distinct Mitotracker peaks on either side of its H2B-GFP signal  
855 (black arrows).

856

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857 **Figure 3. *Apc* mutant daughters separate less frequently**

858 **(A)** 3D projections of fixed organoids produced from small-intestinal crypts of wild-type and  
859 *Apc*<sup>Min/+</sup> mice stained with Hoechst (blue), phalloidin (red) and  $\gamma$ -tubulin (green). *Apc*<sup>Min/+</sup>  
860 organoids from cells that have undergone LOH form cysts (*Apc*<sup>Min/Min</sup>). (See also S3 Figure.)

861 **(B)** Types of mitotic daughter placement were scored in organoids (wild-type N=6, 491  
862 mitoses; *Apc*<sup>Min/+</sup>, N=3, 227 mitoses, *Apc*<sup>Min/Min</sup>, N=7, 34 mitoses; T-test). Relative frequency  
863 of each type of mitosis was determined per organoid and averaged for replicate organoids.  
864 There was a significant difference between the number of adjacent and separating  
865 daughters between wild-type, *Apc*<sup>Min/+</sup> and *Apc*<sup>Min/Min</sup> organoids (T-test, p<0.0001).

866 **(C)** Mitotic cell position was determined relative to the crypt base for wild-type and *Apc*<sup>Min/+</sup>  
867 organoids. The frequency of each mitosis type along the crypt-villus axis was measured for 3  
868 organoids. The stem cell (SC) and transit amplifying (TA) compartments are marked as  
869 defined by the average position of Lgr5-GFP(+) cells (see S5 Figure). Data is displayed as  
870 mean +/- SEM.

871 **(D)** Nuclear speed was calculated in wild-type and *Apc*<sup>Min/+</sup> organoids (N=3 organoids, 20  
872 cells) showing average speed for interkinetic nuclear migration (INM), basal cell movement  
873 and interphase. Data is displayed as mean +/-SEM. There was a significant difference  
874 between the speed of cells in wild-type and *Apc*<sup>Min/+</sup> organoids (T-test, p<0.0001).

875

876

877 **Figure 4. Post-mitotic separation promotes both niche retention and exit**

878 **(A)** Representative images show examples of the long-term behaviour of daughter cells  
879 following separate or adjacent placement. An overlay of the track of daughters (Daughter 1,  
880 red; Daughter 2, blue) reveals the total displacement over the time course (white arrow).

881 **(B)** Daughter cell position from the crypt base was measured after reinsertion into the  
882 epithelium (~2 hours) and at the final position able to be recorded (5-35 hours).  
883 Displacement (movement of each daughter from the crypt base over time) was calculated  
884 for each daughter pair. Daughter 1 was defined as the daughter closest to the crypt base.  
885 Values were calculated for separating (Apart, N = 28) or adjacent (Together, N = 84; T-test,  
886  $p < 0.001$ ) sisters.

887 **(C)** Simulation results: representative images showing daughters initially placed adjacent to  
888 each other (red) and placed apart by 1 cell diameter (blue). Snapshots shown represent  
889 these situations immediately after mitosis and 12 hours later (right hand panels).

890 **(D)**. Simulation results show the distribution of cell separation as a function of time since  
891 birth. Results are shown for a homogenous population (left) of cell divisions where  
892 daughter cells are placed adjacent to each other (i.e.  $S = 0$  for all divisions), and for a  
893 heterogeneous population (right) of cell divisions where  $S = 0$  for two thirds of divisions  
894 (red) and  $S = 1$  for the remaining third (blue). The mean separation (solid line) and  
895 standard deviation (shaded region) is displayed. Linear fits to the distribution (from 5-15  
896 hours) are represented by dotted lines.

897 **(E)** Simulation results for the effect of initial cell placement on separation velocity. For each  
898 separation, a heterogeneous population of divisions (two thirds with  $S = 0$  and a third  
899 with  $S \neq 0$ ) is simulated and the corresponding separations (as shown in (D)) are calculated,  
900 and the distribution of values recorded. The separation velocity is calculated by taking the  
901 gradient of the linear fit to the mean of this distribution for both populations of divisions  
902 (adjacent in red and separated in blue). Cells placed initially further apart will separate  
903 more quickly than those placed together.

904 **(F)** Simulation results for the proportion of cell divisions that produce cells in different  
905 niches (i.e one cell remains in the proliferative compartment while the other leaves) for  
906 simulations shown in (D). Results are shown for a homogenous population (left) of cell  
907 divisions where all daughter cells are placed adjacent to each other (i.e.  $S = 0$  for all  
908 divisions) and for a heterogeneous population (right) of cell divisions where  $S = 0$  for two  
909 thirds of divisions (red) and  $S = 1$  for the remaining third (blue). Constant fits to these  
910 distributions (using data for all ages) are denoted by dotted lines.

911 **(G)** Simulation results showing how the average proportion of cell pairs with different  
912 positions (using the constant fit from (F)) depends on the initial separation. The same  
913 simulations were used as in (E). Increasing separation leads to a larger proportion of cell  
914 pairs in different positions.

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915 **Figure 5. Basal tethering of mitotic cells is altered in Apc mutant epithelia**

916 (A) 3D projections of mitotic cells in organoids and whole tissue reveal the basal process  
917 (white arrows). Samples were stained with Hoechst (blue), phalloidin (red),  $\gamma$ -tubulin (green)  
918 and  $\beta$ 4-Integrin (white). A schematic of the basal process is shown.

919 (B) 3D projection of a mitotic cell aligned in metaphase shows the position of its basal  
920 process (white arrow), centrosomes (green), nucleus (blue), and mitotic cleavage plane.  
921 Symmetric or asymmetric process inheritance was scored based on its placement relative to  
922 each centrosome. Accordingly, basal processes could be localised to the cell closer to the  
923 crypt base ('bottom'), equidistant from each centrosome ('middle'), or furthest from the  
924 crypt base ('top'). In the displayed X-Y sections, views were orientated with the crypt base  
925 towards the bottom in each image.

926 (C) Process inheritance was scored by visual inspection of the position of basal processes  
927 relative to the mitotic cell in 3D. Two examples of mitotic cells are shown, one with  
928 asymmetric and one with symmetric process placement (see also S3 Figure). 3D surface  
929 rendering shows the position of the basal process.

930 (D) Process segregation was scored by measuring the distance between the attachment  
931 point of the basal process and the centrosome of each prospective daughter in the stem  
932 cell- and transit-amplifying compartments of wild-type (N=12 organoids, N = 68 mitoses)  
933 and Apc<sup>Min/+</sup> (N=20 organoids, N = 61 mitoses) organoids. Frequencies are displayed as the  
934 average percentage of each outcome per organoid. The frequency is displayed as a  
935 percentage of the mitotic events in each compartment. There was a significant reduction in  
936 the number of asymmetrically localised basal processes in the stem cell compartment in  
937 Apc<sup>Min/+</sup> organoids compared to the stem cell compartment in wild-type organoids (T-test  
938 \*\*\*P<0.001). Processes were scored manually and defined as asymmetric if significantly  
939 displaced from the cleavage furrow. To confirm manual scoring, process displacement was  
940 calculated for all scored asymmetric and symmetric processes. Displacement was defined as  
941 the difference between the distances from the process to each centrosome (right hand  
942 panel). Data is displayed as mean +/- SEM. Process displacement in mitoses scored as  
943 asymmetric was significantly more common than in symmetric mitoses (T-Test  
944 \*\*\*\*p<0.0001).

945 (E) Individual frames of a time-lapse movie reveal the repeated attempt of one daughter  
946 (red) to assume the original position of the mother (green) while the other daughter (blue)  
947 moves on.

948 (F) Individual frames of a time-lapse of H2B-GFP organoids stained with SiR-Actin show a cell  
949 whose daughters undergo post-mitotic separation. Displayed are time points encompassing  
950 metaphase (0 min), cytokinesis (9-12 min) and the two daughters during reinsertion (18-27  
951 min), when they become separated by a neighbour (black stars). An asymmetric process  
952 (white/red arrows) is located closer to daughter 1 on one side of the putative cleavage  
953 furrow. The apical surface is denoted by the thick dashed line.

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954 **Figure 6. *Apc* mutation limits the ability of daughters to separate by preventing**  
955 **interkinetic nuclear migration and reducing cell size**

956 **(A)** Representative images of metaphases in wild-type, *Apc*<sup>Min/+</sup> and *Apc*<sup>Min/Min</sup> intestinal  
957 organoids stained with Hoechst (blue) and phalloidin (red).

958 **(B)** The apical-basal distance of interphase cells was measured in wild-type, *Apc*<sup>Min/+</sup> and  
959 *Apc*<sup>Min/Min</sup> organoids in images (left panel). There was a significant difference in the apical-  
960 basal distance between wild-type and *Apc*<sup>Min/Min</sup> organoids. Cell size was measured in  
961 isolated wild-type, *Apc*<sup>Min/+</sup> and *Apc*<sup>Min/Min</sup> cells using flow cytometry. The median forward  
962 scatter was determined from 3 independent organoid samples for each genotype and  
963 averaged. Data is displayed relative to the size of wild-type cells. There is a significant  
964 difference between the relative cell size of wild-type and *Apc*<sup>Min/Min</sup> organoids (T-test,  
965 \*\*\*\*p<0.0001)

966 **(C)** Individual frames from H2B-GFP organoid movies show interkinetic nuclear migration  
967 (INM). For each genotype, a representative mitosis is shown at prophase, INM and  
968 cytokinesis. 3D (maximum intensity projections) and transverse (X-Y) views through an  
969 organoid branch or cyst are shown.

970 **(D)** Dynamics of INM during mitosis in wild-type, *Apc*<sup>Min/+</sup> and *Apc*<sup>Min/Min</sup> was measured  
971 relative to the starting distance (N = 10 cells per genotype). Data is displayed as mean +/-  
972 SEM. Measurements for mother (black line) and daughters (red and blue lines) are  
973 superimposed (see also S5 Figure).

974 **(E)** A Vibratome section of human FAP colonic tissue was stained with Hoechst (blue) and  
975 phalloidin (red). Displayed are 3D projections (top panel) and section views (bottom panel).  
976 Highlighted are regions of 'normal-appearing' and 'dysplastic' regions. The highlighted area  
977 (dashed line) shows a crypt with cell pile-ups/pseudo-stratification

978 **(F)** Magnified view of a crypt 'normal' and 'dysplastic' regions of FAP colonic tissue in panel  
979 **(E)**. The basal and apical surfaces are highlighted by white dashed lines. Cells undergoing  
980 interkinetic nuclear migration are highlighted by white arrows. Scale bars = 10µm.

981 **(G)** Cell morphology of 3 cells from crypts in 'normal' and 'dysplastic' regions of FAP colonic  
982 tissue. Displayed are surface renders of F-actin and nuclei for individual cells. Scale bars =  
983 5µm.

984

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985 **Figure 7. Model for how prolonged niche retention in *Apc* mutant cells arises**

986 In untransformed intestinal epithelia, normal tissue architecture is maintained. During  
987 mitosis, cells undergo interkinetic nuclear migration, characterised by the apical movement  
988 of nuclei as the cell rounds up. This permits daughter cells to remain proximal or to become  
989 displaced from one another by neighbouring cells. Displacement promotes retention of a  
990 cell at its birthplace and allows the other to exit the niche. This displacement is facilitated by  
991 INM and the asymmetric segregation of the basal process. After mutation of one *Apc* allele  
992 (*Apc*<sup>Min/+</sup>), mitoses become biased towards adjacent placement and migrate slower,  
993 facilitating niche retention. Upon loss of heterozygosity (*Apc*<sup>Min/Min</sup>), all cells lose the  
994 capacity for separation due to reduced cell size and inhibited INM. As a result, symmetrical  
995 cyst growth could be promoted, promoting altered tissue architecture.  
996

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997 **Supplemental Figure Legends**

998 **S1 Figure. Mitosis in intestinal tissue and intestinal organoids.**

999 **(A)** Maximum intensity projection (X-Y-Z) and confocal sections (X-Y) of a vibratome section  
1000 of mouse small-intestine (tissue) and an intestinal organoid stained with Hoechst (blue),  
1001 phalloidin (red), and  $\gamma$ -tubulin (green).

1002 **(B)** Confocal sections (X-Y) of mitotic stages visualised in intestinal crypts of whole tissue and  
1003 organoids. Interphase cells maintain basally positioned nuclei. During mitosis the apical cell  
1004 surface remains aligned with neighbouring cells. Chromatin condensation occurs during  
1005 prophase and the nucleus is displaced apically. During INM, the rounded mitotic cell remains  
1006 attached to the basal membrane by a basal process (BP). After alignment with the apical  
1007 surface the metaphase plate forms apically and is directly followed by anaphase in which  
1008 cells have two clear sets of sister chromatids.

1009 **(C)** An intestinal organoid and small intestinal tissue stained with Hoechst (blue) and an  
1010 antibody against tubulin (green). In the right panel, surface rendering reveals the structure  
1011 of the apical-basal array of microtubules. The apical surface is marked by the red dashed  
1012 line.

1013 **(D)** Representative examples of mitotic cells in organoid epithelium at stages of interkinetic  
1014 nuclear migration. Microtubule polymerisation is most evident when nuclei are basally  
1015 localised. As nuclei move apically, microtubule polymerisation is mostly at the apical-most  
1016 side of the microtubule scaffold. There was no detectable microtubule polymerisation in  
1017 rounded up mitotic cells. Indicated cells are not differentiated due to the detectable pair of  
1018 centrosomes (i and ii). The apical surface is marked by the red dashed line.

1019 **(E)** A tuft cell in an intestinal organoid. Organoids were stained with Hoechst (blue),  
1020 phalloidin (white) and antibodies against tubulin (green) and ZO-1 (red) displayed as a  
1021 section (i) or in 3D (ii). Tuft cells have a 'tuft' of microvilli that protrude apically into the  
1022 lumen. They are fully differentiated, predominantly found within the differentiated zone,  
1023 and lack detectable centrosomes.

1024



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1025 **S2 Figure. Alternative methods of separation**

1026 (A) Separation of daughters is enhanced by movement of neighbouring mitotic cells.  
1027 Displayed are 3D projections of the movements of the progeny of a mitotic cell (Original  
1028 Mitosis, [Mitosis 1; red]) and the progeny of two neighbouring mitotic cells (Mitosis 2,  
1029 green; Mitosis 3; blue). Time 0 marks metaphase of the original mitotic cell.

1030 (B) i) Live imaging of a wild-type H2B-GFP organoid. A Paneth cell can be clearly identified  
1031 based on morphology and distribution of neighbouring nuclei (dashed line). A mitotic cell  
1032 (white stars) proximal to the Paneth cell divides to produce two daughters (white balls) who  
1033 separate and then reenter the epithelial plane adjacent to the Paneth cell. ii) Fixed image of  
1034 small-intestinal tissue, stained with Hoechst (Blue),  $\beta$ 4-Integrin (red), lysozyme (magenta)  
1035 and phalloidin (green). A recent mitosis produced two daughter cells (1, 2\*) which have  
1036 become separated and have reinserted on either side of the Paneth cell. The image is the  
1037 same as that in Figure 5A, showing lysozyme staining.

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1039 **S3 Figure. Spindle orientation in intestinal organoids**

1040 A) Representative mitotic cells in prometaphase, metaphase, anaphase and during  
1041 cytokinesis. Organoids are stained with Hoechst, phalloidin and antibodies against tubulin  
1042 and  $\gamma$ -tubulin to visualise nuclei (blue), F-actin (red), microtubules (white) and centrosomes  
1043 (green). Centrosomes are located equidistantly on either side of the metaphase plate once it  
1044 is fully established.

1045 B) A representative example of an  $Apc^{Min/Min}$  mitotic cell with a multipolar spindle.  $Apc^{Min/Min}$   
1046 organoids were stained with Hoechst (blue), phalloidin (red) and an antibody against  $\gamma$ -  
1047 tubulin (green) to visualise DNA, F-actin and centrosomes.

1048 C) A representative example of an  $Apc^{Min/Min}$  cell undergoing mitotic slippage. Displayed are  
1049 stills from live-imaging of an  $Apc^{Min/Min}$  H2B-GFP organoid. Chromosome condensation is  
1050 clearly observed as the cell enters prophase. Instead of proceeding with mitosis,  
1051 chromosomes de-condense as the cell returns to interphase.

1052 D) A representative example of a wild-type organoid stained with Hoechst (blue), phalloidin  
1053 (red),  $\gamma$ -tubulin (green) to visualise DNA, F-actin and centrosomes. Two mitotic cells are  
1054 highlighted, one in metaphase (top) and one in anaphase (bottom). Surface rendering in  
1055 Imaris can clearly highlight individual cells and their two centrosomes.

1056 E) Potential spindle alignments in intestinal organoids. Spindle orientation was determined  
1057 in reference to: 1) the axis of tissue growth; the crypt-villus axis or 2) the apical surface.  
1058 Spindle orientations are the angle between the spindle and crypt-villus axis (Axial angle) or  
1059 apical surface (Apical angle). Examples of each type of spindle alignment is displayed; i)  
1060 Parallel to the crypt-villus axis (Crypt lengthening), ii) Perpendicular to the crypt-villus axis  
1061 (Crypt widening), iii) Parallel to the apical surface ('symmetric' division) or perpendicular to  
1062 the apical surface ('asymmetric' division). Reference axes are highlighted by the white  
1063 dashed line.

1064 F) Representative example of an asymmetrically and symmetrically oriented division in the  
1065 crypt base of an intestinal organoid. The organoid is stained with Hoechst (blue) and  
1066 phalloidin (red). Note that a pro-daughter cell in the asymmetrically aligned mitoses is  
1067 poised to inherit the basal process.

1068 Spindle orientations were determined for mitoses in G) wild-type, H)  $Apc^{Min/+}$  and I)  
1069  $Apc^{Min/Min}$  organoids. Only apical angles could be calculated for  $Apc^{Min/Min}$  organoids due to  
1070 loss of crypt-villus architecture. Data is displayed in reference to the crypt base. Angles  
1071 greater than  $40^\circ$  were classified as perpendicular. Angles less than  $40^\circ$  are classified as  
1072 parallel. Data is displayed as a function of distance along the crypt-villus axis. The stem cell  
1073 compartment was defined as the curved region at the base of branches, approximately  
1074  $20\mu\text{m}$  from the luminal crypt base.

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1075 **S4 Figure. Disruption of INM can be induced by chronic Chir99021 treatment**

1076 **A)** An H2B-GFP intestinal organoid treated with Chir99021 and valproic acid. Treatment with  
1077 Chir99021 and valproic acid has been shown to increase the frequency and distribution of  
1078 Lgr5(+) cells along the crypt axis (Yin et al., 2014). Treated organoids retain crypt-villus  
1079 architecture. Scale bar = 100 $\mu$ m

1080 **B)** Daughter cell placement after mitosis was scored in H2B-GFP organoids using time-lapse  
1081 movies. Organoids were treated with Chir99021 and valproic acid to increase the stem cell  
1082 content along the crypt-villus axis, or they were chronically treated with 10 $\mu$ M Chir99021  
1083 for 4 days to induce cyst formation in WT organoids. Division subtypes were compared to  
1084 untreated organoids (ENR). Data was compared to the dataset in Figure 4B. (ENR N = 6  
1085 organoids, N = 491 mitoses; ENR-CV (branched) N = 3 organoids, N = 351 mitoses; ENR-C  
1086 (cyst) N = 3 organoids).

1087 **C)** A wild-type H2B-GFP organoid chronically treated with 10 $\mu$ M Chir99021 for 4 days (left  
1088 panel), scale bar = 100 $\mu$ m. Right panels highlight individual frames from live-recordings  
1089 displaying a representative mitosis during prophase, apical interkinetic nuclear migration  
1090 (ANM) and cytokinesis. 3D (maximum intensity projections, X-Y-Z) and transverse (X-Y) are  
1091 shown. Scale bars = 5 $\mu$ m

1092 **D)** Dynamics of interkinetic nuclear migration during mitosis in Chir99021 treated organoids  
1093 were measured relative to the starting distance (N = 10 cells). Data is displayed as mean +/-  
1094 SEM. Measurements of the mother (black line) and daughter cells (red and blue lines) are  
1095 superimposed. The wild-type dataset displayed is the same dataset as displayed in Figure  
1096 7D.

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1099 **S5 Figure. Definition of tissue compartments and interkinetic nuclear migration**

1100 **A)** An Lgr5-GFP expressing intestinal organoid stained with Hoechst (nuclei), phalloidin (F-  
1101 actin) and GFP (Lgr5+ stem cells). Stem cells resided within the base of intestinal organoid  
1102 branches, mostly residing within the curved region of the crypt base.

1103 **B)** The position of each Lgr5-GFP+ cell was recorded and compared to the positions of the  
1104 total cell population with reference to the crypt base. Nuclear position was used as a  
1105 surrogate for cell position and distances were compared to the nucleus closest to the base  
1106 of the crypt. Data was pooled from 6 individual organoids. Data is displayed as mean +/- SD.

1107 **C)** Diagram showing the defined compartments within intestinal crypts. The majority of  
1108 Lgr5+ stem cells were located approximately 0-40µm from the crypt base. This region was  
1109 termed the stem cell compartment. This equated to the curved region at the base of the  
1110 crypt, approximately 20µm from the luminal crypt base. Above this region we defined as  
1111 the transit-amplifying compartment. A small fraction of GFP+ cells resided above the  
1112 defined stem cell compartment, similar to our previous studies in whole intestinal tissue.

1113 **D)** Interkinetic nuclear migration is quantified as the distance of the query cell in reference  
1114 to the epithelial plane in which it originated. The plane of the epithelium is defined as the  
1115 plane in which neighbouring nuclei are located. A plane is defined by the co-ordinates of 3  
1116 points. Therefore the distance was measured between the query cell and the plane formed  
1117 by 3 of its neighbour nuclei. This process was repeated utilizing 5 neighbour cells. The  
1118 average distance for each of these 10 planes was determined as distance from the epithelial  
1119 plane.

1120 **E)** Examples of INM measurement for a cell undergoing adjacent placement (Adjacent) or  
1121 post-mitotic separation (Separated). Distances were determined for each time-point during  
1122 prophase and for each of the daughter cells (red and blue lines).

1123

1124