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1 **A transient radial cortical microtubule array primes cell division in Arabidopsis**

2

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6

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18

19

20 **ABSTRACT**

21

22 Because all cells experience mechanical stress, they have to develop resistance mechanisms to
23 survive. In plants, this notably involves guidance of cellulose deposition by cortical
24 microtubules in the direction of maximal tensile stress. Although the formation of new walls
25 during cell division tends to follow maximal tensile stress direction too, analyses of individual
26 cells over time reveal a much more variable behavior. The origin of such variability, as well as
27 the exact role of interphasic microtubule behavior before cell division have remained
28 mysterious so far. To approach this question, we took advantage of the Arabidopsis stem, where
29 the tensile stress pattern is both highly anisotropic and stable. Although cortical microtubules
30 generally align with maximal tensile stress, we detected a specific time window, ca. 3 hours
31 before cell division, where cells form a radial pattern of cortical microtubules. This pattern was

32 observed in different growth conditions, and was not related to cell geometry or polar auxin
33 transport. Interestingly, this cortical radial pattern correlated with the well-documented increase
34 of cytoplasmic microtubule accumulation before cell division. This radial organization was
35 prolonged in cells of the *trm678* mutant, where cortical microtubules are partially disorganized.
36 Whereas division plane orientation in *trm678* is noisier, we found that cell division symmetry
37 was in contrast more precise. We propose that an increased cytoplasmic microtubule
38 accumulation in late G2 disrupts cortical microtubules alignment with tissue stress, allowing
39 the cell to transiently explore its own geometry in order to select a future division plane with
40 correct orientation and symmetry.

41

42 **SIGNIFICANCE STATEMENT**

43

44 In all kingdoms, cells divide according to their own geometry as well as external cues. We
45 discovered a transient stage in plant cells, where part of the division machinery becomes blind
46 to mechanical forces originating from the tissue. Using quantitative imaging and mutant
47 analysis, we propose that this new pre-mitotic stage allows cells to take their own geometry
48 into account and increase the precision of the following division.

49

50 **INTRODUCTION**

51

52 Cell division is first and foremost a geometric question: cells generally divide along a given
53 division plane orientation, and more or less symmetrically, as defined by the relative volume
54 of the two daughter cells. How mitosis operates is now well described in all kingdoms(1).
55 However, we lack a clear understanding of how the cell division machinery achieves precision
56 (i.e. reducing the variance in symmetric division) despite broad variation in cell shapes and
57 division rates.

58

59 In *Drosophila* epithelia, cell division symmetry does not occur by default, but instead involves
60 an active process. A “reset” stage indeed occurs, where cells become round. This pre-mitotic
61 cell rounding has been proposed to allow cells to identify their centroid, thus triggering more
62 symmetric divisions, independently of extracellular cues(2). Conversely, disruption of cell
63 rounding in myosin motor mutants puts more weight on pre-mitotic cell geometry, through the
64 maintenance of cell packing, thus biasing division plane orientation(3). Whether this also
65 affects the precision of cell division symmetry remains unknown.

66

67 In plants, the presence of stiff cell walls mechanically hinders any cell deformation before
68 mitosis. However, in late G2, the cortex of plant cells becomes largely depopulated of cortical
69 microtubules (CMTs), and a dense ring forms, named the preprophase band (PPB). This
70 transient pre-mitotic structure precisely predicts the position of the future division plane,
71 established at cytokinesis. Since its discovery, the PPB was thought to be an essential attribute
72 of cell division in most plants tissues(4), until specific PPB-less mutants were obtained and
73 shown to have only mild developmental defects, at least under controlled conditions(5)(6).
74 Furthermore, the PPB contributes to the robustness of division plane orientation, independently
75 of division symmetry, since both symmetrically and asymmetrically dividing cells produce a
76 PPB. Strongly asymmetric divisions, as in meristemoid cells, involve polarity cues that are
77 inherited from interphase(7). However, this does not apply to most plant tissues, where cells
78 divide symmetrically. How plant cells control the symmetry of division and its robustness is
79 mostly unknown.

80

81 During interphase, the parallel alignment of CMTs has been shown to regulate the deposition
82 of cellulose microfibrils in the wall(8). With few exceptions, the PPB, formed in G2 prior to
83 mitosis, adopts the same orientation as the former interphasic cortical array(9). In addition,
84 CMTs have also been found to align with maximal tensile stress in different organs or
85 conditions(10). Because of the co-alignment between interphasic CMTs, PPB and division
86 plane over time, division planes are also often aligned with maximal tensile stress(11). This
87 provides a scenario in which plant cells and tissues would resist tensile stress, through cellulose-
88 dependent wall reinforcement during interphase and new cell wall formation at mitosis, all
89 parallel to maximal tensile stress orientation.

90

91 However, this scenario suffers from a number of experimental and conceptual issues. In
92 particular, CMTs are not always co-aligned during interphase. For instance, basket/star
93 configurations have been reported in hypocotyl cells(12). Besides, CMT array orientation is not
94 necessarily homogeneous within a cell, and can change over time, for instance displaying global
95 rotations(13), consistent with cellulose deposition dynamics too(14). Whether such behaviors
96 could be reconciled with a response to tensile stress and the control of cell division remains to
97 be investigated.

98

99 Here, using the *Arabidopsis* stem as a model system, and taking advantage of its stereotypical
100 tissue stress pattern, we identified a transient radial CMT organization that appears prior to PPB
101 formation, and that diverges from alignment with tissue stress. We propose that this reflects a
102 stage where, in preparation to mitosis, cells sense their own geometry rather than tissue-related
103 cues.

104

105

106

107 RESULTS

108

109 A stereotypical radial CMT organization before cell division

110

111 To observe CMTs in dividing cells over time, we used plant lines expressing the MAP4
112 microtubule binding domain (MBD) fused to mCitrine and focused our analysis on dissected
113 shoot apices, as previously described(15), but here with higher spatial and temporal resolution
114 (see Material and Methods).

115

116 In the Shoot Apical Meristem (SAM), as previously reported, we typically observed CMT
117 alignment at the tissue scale, with a bias towards circumferential direction in the organogenetic
118 (peripheral) zone, whereas and no particular bias was observed in the central zone (Figure
119 1A)(16). However, when considering individual cells over time, CMT behavior appeared much
120 more variable. In particular, before cell division, we often observed cells with radial CMT
121 orientations, as viewed from the top (Figure 1B, Figure S1A and S1B).

122

123 To explore this further, we needed higher spatial resolution. This led us to analyze this behavior
124 in the stem from *in vitro*-grown plants. We focused on the stem region immediately below the
125 shoot apical meristem, a region of active cell division, where cells are 2 to 3-fold larger (Figure
126 1C). Using cell-cell adhesion mutants, we previously demonstrated that the *Arabidopsis* stem
127 exhibits a stereotypical and stable tensile stress pattern, with a maximum in the transverse
128 orientation(17). In this tissue, we confirmed the presence of a strong and global bias of CMTs,
129 towards the circumferential orientation (see Figure S2), parallel to the predicted maximal tensile
130 stress (Figure 1C)(17). Again, when analyzing individual cells over time, we consistently
131 observed a switch from the interphasic alignment of the MT array to a radial array before the
132 formation of the PPB (Figure 1D). In order to increase the number of observable dividing cells,

133 we analyzed cell division in stems from plants grown *in-vitro* on cytokinin-supplemented
134 medium (ACM medium). This readily increased the number of dividing cells imaged in time-
135 lapses: we observed 24 dividing cells on 2 independent stems on ACM medium, vs. 13 dividing
136 cells on 4 independent stems on the growth Arabidopsis Medium (AM)). A transient radial
137 CMT behavior could be observed in all cases (Figure 1E). Note that this result was also
138 confirmed when using a *pUBQ10::RFP-TUA6* marker line, albeit with lower spatial resolution
139 (Figure S3A). From 5 time-lapse experiments with a time resolution of 1 hour, we estimated
140 that this behavior preferentially occurred around 3 hours before cell division, lasts for about 1
141 hour, and is followed by the formation of the PPB (Figure 1D).

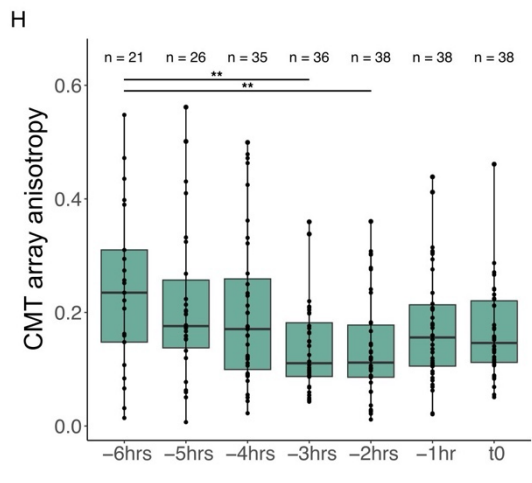
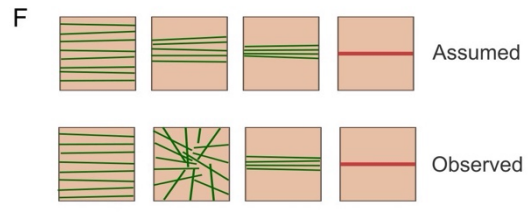
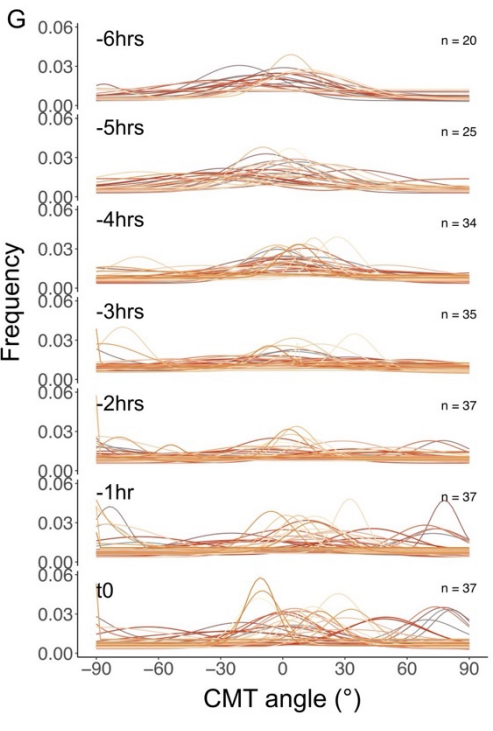
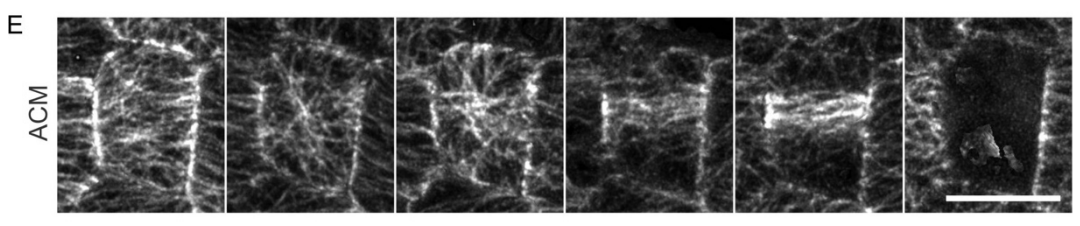
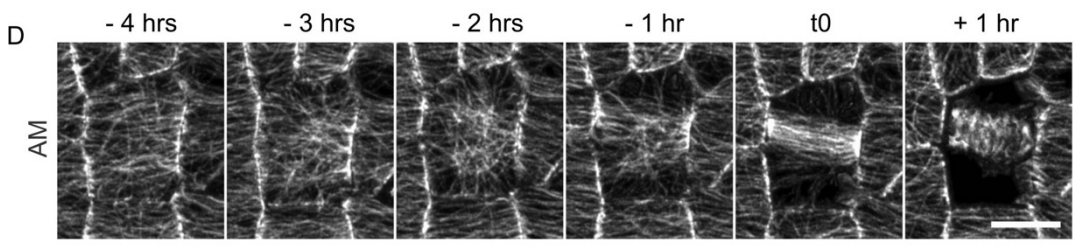
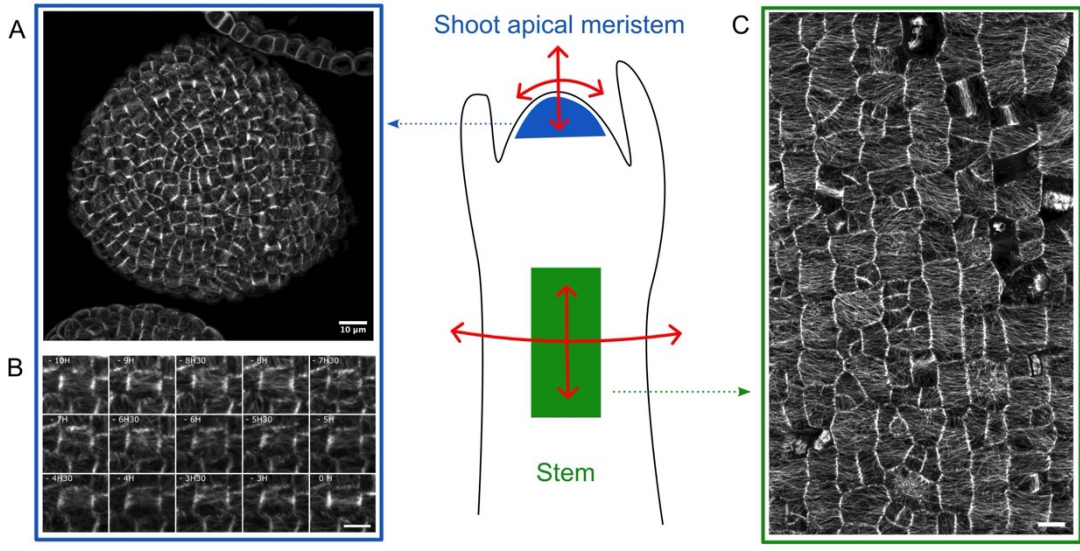
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143 To go beyond these qualitative observations, we measured the distribution of orientations of
144 the CMT arrays using the Directionality plugin in ImageJ for each time-lapse experiment. In
145 all quantifications, time 0 corresponds to the closest timepoint preceding nuclear envelope
146 breakdown, as inferred from the disruption of the PPB, and the initiation of the mitotic spindle.
147 As expected, at $t = -4$ hours, we found a bias towards the transverse orientation (-2.8° at -4
148 hours; 1st quartile = -10.6° , 3rd quartile = 12.1°), which corresponds to the transverse axis of the
149 stem (also the predicted maximal tensile stress direction) (Figure 1G, $n=34$ cells). From 1 hour
150 before $t=0$ up to PPB formation, CMT arrays also exhibited an orientation bias, reflecting the
151 PPB orientation (Figure 1G, $n=37$ cells). However, 3 to 2 hours before $t=0$, the bias was much
152 weaker (-11.9° at -3 hours; 1st quartile = -65.7° , 3rd quartile = 20.2° ; -23.6° at -2 hours; 1st
153 quartile = -90° , 3rd quartile = 5.1°) (Figure 1G, $n=35$ cells). To assess the statistical differences
154 between CMT orientation distribution at each timepoints, we performed Kolmogorov-Smirnoff
155 tests. We found differences to be statistically significant between -2 or -3 hours and earlier
156 timepoints, meaning that the radial array of CMTs appear significantly different from previous
157 interphasic CMT co-alignments (Figure S4). As a negative control, we also found no statistical
158 differences between the distributions at -6 hours and -5 hours, or between the distributions at $-$
159 3 hours and -2 hours, at a threshold of 0.05 (Figure S4).

160

161 To confirm this observation, we used the FibrilTool plugin in ImageJ(18) to quantify the
162 anisotropy of the CMT arrays in each cell and analyzed the anisotropy distribution per time
163 point (Figure S3B). This analysis revealed a drop in the anisotropy value two to three hours
164 before $t=0$ (Figure 1H, $n=36$ cells). Anisotropy then increased again from 1 hour before $t=0$ up
165 to PPB maturation (Figure 1H, $n=38$ cells). Based on these quantifications, we established that,
166 in the stem, the radial array starts ca. 3 hours before the PPB formation and lasts for ca. 2 hours

167 until the start of the PPB maturation. This finding thus challenges the often assumed continuity
168 between interphasic CMTs and PPB (Figure 1F). We propose to call this stage where CMTs
169 form a radial pattern 3 hours before cell division, the “radial step”.



171 **Figure 1.** A transient pre-PPB radial pattern of CMTs in the meristem and the stem.

172 (A) Top view of the shoot apical meristem expressing the *pPDF1::mCitrine-MBD* marker. Red
173 arrows indicate a predicted isotropic tensile stress pattern in the central zone. (B) Time-lapse
174 of a pre-mitotic cell of the SAM, showing the CMT dynamics before the formation of the PPB,
175 using the *pPDF1::mCitrine-MBD* marker line. CMT organization changes from co-aligned
176 arrays (-10h to -7h30) to radial arrays (-7h to -6h) before the start of PPB maturation. (C) Side
177 view of the stem expressing the *pPDF1::mCitrine-MBD* marker. Red arrows indicate the
178 transverse direction of maximal tensile stress, as demonstrated in(17). (D, E) Time lapse of pre-
179 mitotic cells in the stem, showing CMT dynamics before PPB formation, using the
180 *pPDF1::mCitrine-MBD* marker line on the growth medium (AM, D) and cytokinin-
181 supplemented medium (ACM, E). Note the presence of radial CMTs ca. 3 hours before cell
182 division. (F) Schematic summary of the transition between the aligned, interphasic CMT array
183 and the PPB array. The top panel illustrates the simplest scenario, and the bottom panel
184 recapitulates the observations made with the time-lapse experiments. (G) Distribution of CMT
185 orientations overtime in cells from the stem. The angle scale varies between -90° and $+90^{\circ}$, 0°
186 being the transverse orientation to the stem axis. Each row represents the time before $t=0$ (last
187 timepoint before the nuclear envelope breakdown). Each color-coded curve represents the
188 results obtained for one cell. (H) Graphical representation of the evolution of the anisotropy
189 values obtained with FibrilTool overtime (** p -value < 0.01 , Tuckey test). For all images in
190 this figure, SurfCut projections were used, projecting the mCitrine signal between 0 and 4 μm
191 from the surface. The Brightness & Contrast parameters have been modified for a better
192 visualization of CMTs. Scale bars: 10 μm (A, C); 5 μm (B, D, E).

193

194 **The radial step can be uncoupled from cell shape and final division plane orientation**

195

196 To assess whether the radial step relates to cell division plane orientation, we analyzed its
197 behavior in cells of different shapes. In stem tissues, isodiametric cells tend to divide
198 perpendicular to the stem axis (Figure 2B), whereas elongated cells tend to divide along the
199 shortest plane (Figure 2C). Such difference could be related to cellular stress patterns, as
200 inferred from the curvature of the outer wall: the shape of elongated cells prescribes transverse
201 derived maximal tensile stress direction whereas the shape of isodiametric cells prescribes
202 isotropic tensile stress direction(11) (Figure 2A). Note that in contrast to the tensile stress

203 pattern in the stem, which was experimentally validated(17), this cell-shape-derived stress
204 pattern is only predicted from a pressure vessel model of an epidermal cell(11)(19). We thus
205 explored whether the radial step may depend on differences in cell shape and/or related stress
206 pattern.

207

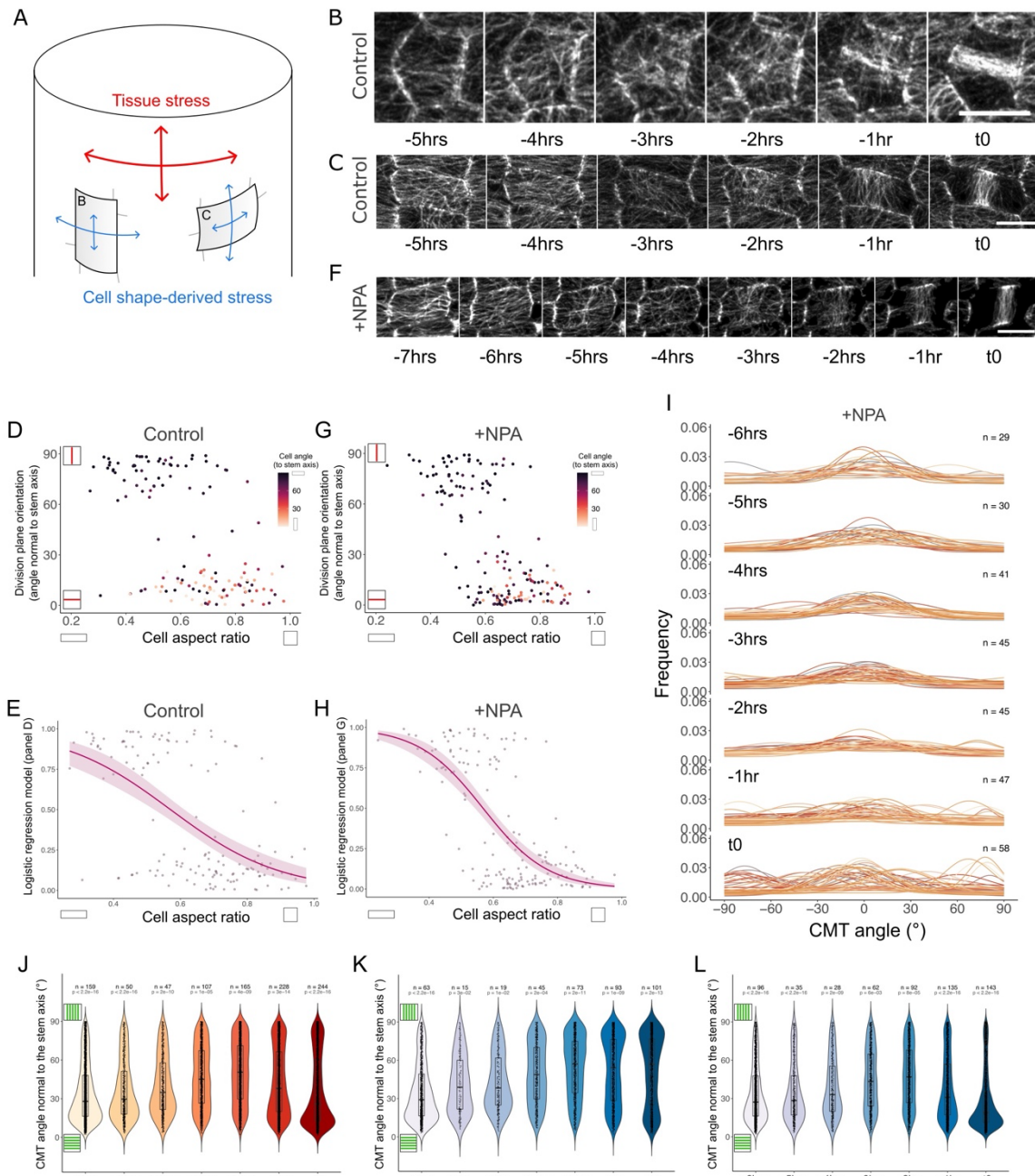
208 To monitor this quantitatively, we calculated the cell aspect-ratio and plotted cell division
209 orientation with respect to the stem axis, using either the PPB as a predictor of the future
210 division plane, or the plasma membrane resulting from a division (Figure 2D, n=141 cells).
211 This confirmed a dual behavior according to cell aspect ratio, at least for cells with an extreme
212 aspect ratio. In particular, more isodiametric cells tend to display division planes perpendicular
213 to the stem axis (i.e. parallel to predicted maximal tissue stress); whereas more elongated cells
214 displayed division planes rather parallel to the stem axis (i.e. perpendicular to maximal tissue
215 stress) (Figure 2D). In order to find the threshold value at which cells switch their behavior, we
216 fitted a logistic regression using the binomial method of the generalized linear models in R
217 (with a p -value of $3.40e^{-11}$). This method only partially fit the data ($R^2 = 0.312$), due to the noise
218 in division orientation in either group, but allowed us to successfully retrieve an inflection point
219 at 0.6 (Figure 2E, see Material and methods). To monitor the orientation of division relative to
220 the cell axis, we measured the orientation of the long axis of the cell relative to the axis of the
221 stem. Measuring the orientation of the long axis of the cell relative to the axis of the stem
222 revealed that most anisotropically shaped cells were elongated transversely to the stem (“C cell”
223 in Figure 2A, see Cell angle color code in Figure 2D), implying that their shortest axis, and thus
224 the maximal tension derived from the cell shape, was orthogonal to the tissue-derived maximal
225 tensile stress. Taking advantage of this dual behavior according to cell shape, we could confirm
226 that in the stem, cells with an aspect ratio below 0.6 tend to divide following cell-shape-derived
227 tension, respecting the local minima rule(20) (Besson and Dumais, 2011). The ones with an
228 aspect ratio above 0.6 (rounder cells) would tend to divide following the tissue-derived maximal
229 tensile stress, that is perpendicular to the stem axis.

230

231 Even though the conflict between maximal tension orientations derived from the cell shape or
232 the tissue may affect the positioning of the division plane, the radial step was comparable in
233 both types of cells, whether elongated (Figure 2B) or not (Figure 2C). We also confirmed this
234 quantitatively with the Directionality measurements, by creating two data subsets: one for cells
235 with a PPB oriented along the stem axis, and the other for cells with a PPB perpendicular to the
236 stem axis. In both cases, the CMT array underwent the radial step (Figures S5A and S5B). To

237 further confirm that the radial step appears independently from final cell division plane
 238 orientation, we observed asymmetrically dividing cells in stems, and we could find a radial step
 239 again around 3 hours before cell division (Figure S3C and S3D).

240
 241



242

243 **Figure 2.** The radial step occurs independent of cell shape or polar auxin transport

244 (A) Diagram representing the predicted tensile stress patterns for epidermal cells in stems. The
245 cylindrical shape of the stem prescribes maximal tensile stress transverse to the stem axis. At
246 cell scale, the minor axis of the cell is also the predicted tensile stress maximal direction.
247 Depending on cell orientation, tissue stress and cell shape-derived stress are synergistic or
248 antagonistic (B) Representative example of a cell expressing the *pPDF1::mCitrine-MBD*
249 marker, with its major axis parallel to the stem axis. The cell forms a radial CMT organization
250 before dividing perpendicular to the stem axis. (C) Representative example of a cell expressing
251 the *pPDF1::mCitrine-MBD* marker, with its major axis perpendicular to the stem axis. The cell
252 forms a radial CMT organization before dividing parallel to the stem axis. (D) Dotplot
253 representing the orientation relative to the stem of the PPB or division planes of cells, in the
254 stem in function of their aspect ratio (the ratio between the longest and the shortest axis of an
255 ellipse fitted around the cell contours). The color code reflects the angle between the major axis
256 of the cell and the stem axis. Light colors highlight cells with their longest axis along the stem
257 axis, dark colors highlight cells with their longest axis transverse to the stem axis. (E) Same as
258 (D), with normalized the data to fit a logistic regression (scale 0 to 1), using the glm function
259 in R studio ($R^2 = 0.312$). (F) Time lapse showing pre-mirotic cells in the stem expressing the
260 *pPDF1::mCitrine-MBD* marker, with NPA from germination. (G) Same as (D), with NPA from
261 germination. (H) Same as (E), with NPA from germination ($R^2 = 0.457$). (I) Same as Figure
262 1D, with NPA from germination. (J) Distribution of CMT angles normal to the stem axis
263 overtime from NPA-treated plants using the OrientationJ - vector field function in Fiji. The *p*-
264 values correspond to the result of a Kolmogorov-Smirnoff statistical test comparing the
265 distribution of the data to a uniform distribution. (K) same as (J) but for cells with an aspect
266 ratio below 0.6 (most elongated cells). (L) same as (J) but for cells with an aspect ratio above
267 0.6 (most isodiametric cells). All images are SurfCut projections between 0 to 4 μm from the
268 surface of the mCitrine signal. Scale bars: 5 μm .

269

270

271 **The radial step can be uncoupled from polar auxin transport**

272

273 Our analysis so far shows that the radial step is independent of cell shape variability. Next, we
274 investigated whether the radial step depends on larger polarizing cues. To do so, we impaired
275 polar auxin transport using Naphtylphtalamic acid (NPA), as auxin has previously been shown
276 to affect cell division rate in roots(21) and plane orientation in embryos(22). We grew plants

277 on NPA-containing medium and, using the same *pPDF1::mCitrine-MBD* marker line, we
278 observed the effect of NPA on CMT organization in stems. It is worth noting that the inhibition
279 of flower formation on stems vastly facilitated the imaging and thus significantly increased
280 sample size.

281

282 We observed the presence of the radial step before PPB formation in the presence of NPA as
283 well (Figure 2F, n=58 cells). Note that the radial step could also be observed in the
284 *pRPS5a::RFP-TUA5* marker line treated with NPA (Figure S3E). This demonstrates that the
285 occurrence of the radial step is not dependent upon polar auxin transport. However, the duration
286 of the radial step was longer with NPA (ca. 3 hours) than in its absence (ca. 2 hours) (Figure
287 2F and 2I, Figure S3E). While cells were usually more isodiametric on NPA than in absence of
288 NPA, the division plane orientation followed a bimodal distribution (transverse or longitudinal)
289 similar to untreated cells (Figure 2G and 2H, n=194 cells). We also fitted a logistic regression
290 with the glm function in R to the data and found an inflection point at 0.6 as well (p -value <
291 $2.2e^{-16}$). The function fit the data better than in control conditions ($R^2 = 0.457$), probably due to
292 the larger sample size. This suggests that the observation that cells tend to divide following the
293 maximal tension derived either from their shape, or from the tissue is dependent on their aspect
294 ratio, remains true in NPA conditions. When dividing the data in two subsets (one for cells with
295 PPBs following the stem axis, and one for cells with PPBs normal to the stem axis), we could
296 also conclude that occurrence of the radial step is not dependent on the final position of the PPB
297 (Figure S5C and S5D).

298

299 Taking advantage of the larger sample size, we next analyzed CMT orientation with respect to
300 the stem axis in NPA-treated plants, using the OrientationJ plugin in Fiji. CMTs were highly
301 perpendicular to the stem axis (i.e. parallel to predicted maximal tensile stress direction) before
302 -3 hours (Figure 2J), and this trend was transiently lost between -4 hours and -3 hours, with a
303 more uniform distribution of CMT orientations reflecting the radial step (Figure 2J). We then
304 did the same analysis for cells with an aspect-ratio below 0.6 (i.e. the most elongated cells,
305 Figure 2K) and above 0.6 (i.e. the most isodiametric cells, Figure 2L). The conclusions were
306 conserved, further confirming that the radial step is independent of cell shape and auxin
307 transport. In the end, most of the elongated cells divided parallel to the stem axis (i.e.
308 perpendicular to maximal tensile stress direction, Figure 2K), in contrast to most isodiametric
309 cells, which divided perpendicular to the stem axis (i.e. parallel to maximal tensile stress

310 direction, [Figure 2L](#)). This analysis further confirms that the presence radial step is not
311 dependent on the final cell division plane orientation.

312

313

314 **The radial step coincides with the pre-mitotic increase in cytoplasmic microtubule content**
315 **and is prolonged when this process is promoted**

316

317 Because the radial step occurs independently of microtubule markers used, growth media or
318 polar auxin transport, we hypothesized that its presence and timing of appearance is mainly
319 controlled by intrinsic factor(s). In plants, and contrary to many eucaryotes, there is no
320 microtubule organizing center (MTOC) and the formation of new microtubules is mostly
321 distributed, and strongly relies on microtubule-dependent γ -tubulin recruitment and branched
322 nucleation(23)(24)(25). Consistently, γ -tubulin mutants display mitotic and interphasic
323 microtubule defects(26). However, there are multiple evidence of microtubule nucleation
324 specifically at the nuclear envelope(27)(28) at the end of the G2 phase, before and during PPB
325 formation(29)(9)(23). This well-established behavior is generally thought to ensure a
326 connection between the PPB and the nucleus(9)(23), and to provide a population of peri-nuclear
327 microtubules to complete mitotic tasks, such as spindle formation(30)(31). Here we
328 investigated whether this behavior also occurs in the stem and whether it temporally correlates
329 with the radial step.

330

331 To assess the cytoplasmic microtubule content, we analyzed the microtubule signal in XZ
332 maximal projections, i.e. through anticlinal cell sections, and calculated the ratio between the
333 endoplasmic signal and the cortical signal. As expected, we observed a gradual increase of the
334 internal microtubule content as the cell reached the PPB stage, with an average increase
335 between the first and the last timepoints of 162% ([Figure 3A and 3B](#), see [Figure S7A](#)).
336 Cytoplasmic microtubules started to accumulate around 3 hours before cell division, i.e.
337 coincident with the radial step.

338

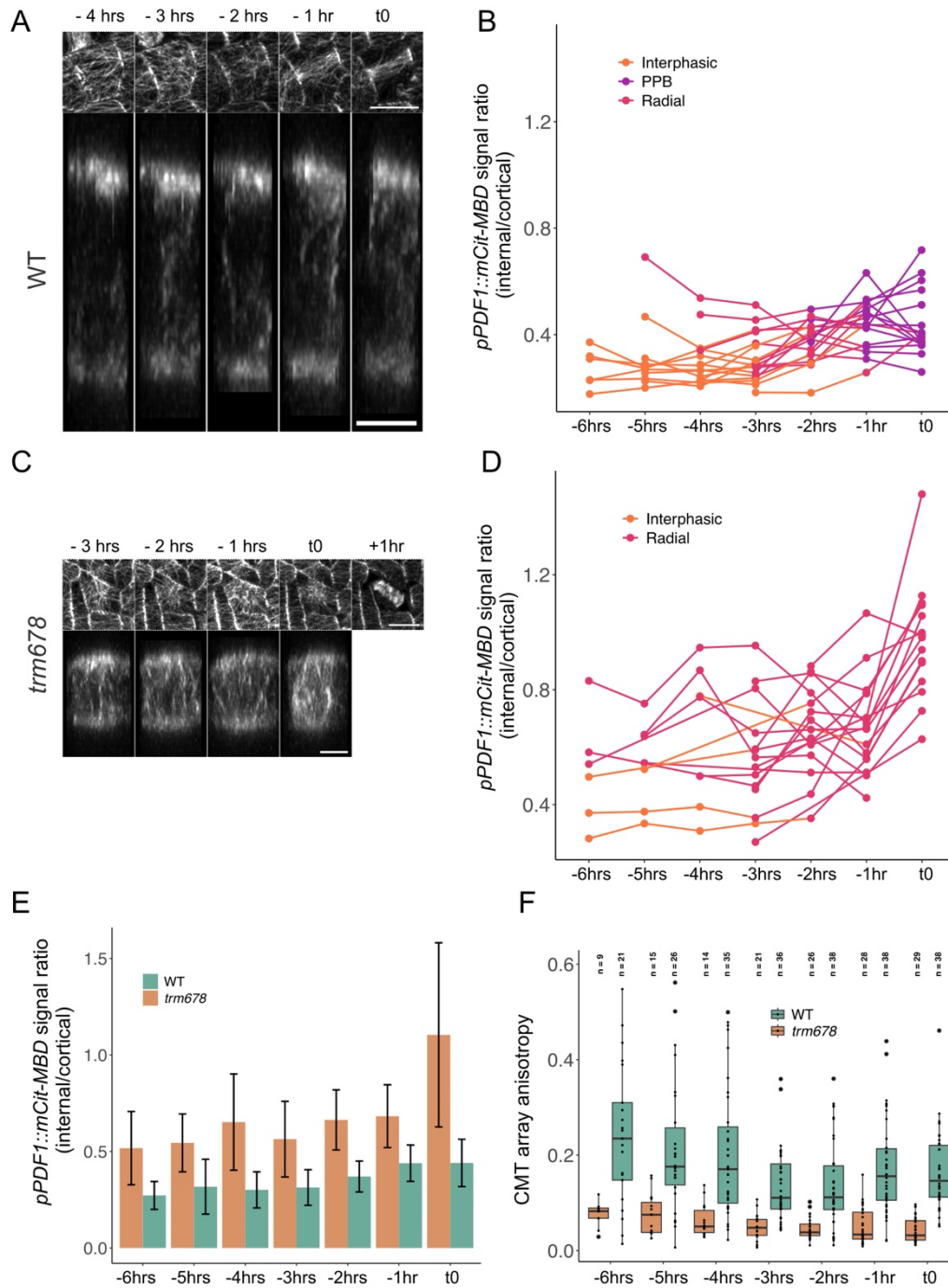
339 To test the connection between the radial step and cytoplasmic microtubule accumulation, we
340 next investigated the radial step in a *trm678* mutant background, grown and imaged in control
341 conditions, as described for the WT. This mutant, as other PPB-deficient mutants, displays a
342 strong accumulation of perinuclear MTs before mitosis(5)(6). The *fass* mutant exhibits an
343 increased microtubule nucleation(32), and this could be a feature of most TTP complex

344 mutants, such as *trm678*, with some evidence pointing towards this hypothesis⁴. Because *ton1*
345 and *fass* mutants exhibit very disrupted interphasic microtubules(5), this would hinder the
346 analysis of more subtle changes in CMT behavior, and notably the radial step. Among these
347 PPB-deficient mutants, *trm678* exhibit WT-like interphasic microtubules(6) (Figure S6A),
348 allowing us to investigate the radial step behavior in that background.

349

350 The mitotic index was comparable in WT and *trm678* stems (1.56% +/- 1.04% in WT stems,
351 and 2.00% +/- 1.21% in *trm678* stems). In the stem, the average duration of mitosis was roughly
352 50% longer in *trm678* (ca. 3 hours) than in the WT (ca. 2 hours, Figure S6B and S6C), even
353 though the exact difference is difficult to assess due to our time resolution. As previously seen
354 in the wild-type, we observed a radial step in *trm678* stems (Figure 3C, n= 29 cells). As
355 expected for a PPB-deficient mutant, the division orientations in the *trm678* stems were noisier
356 than in of the WT (Figure 3C)(33), further confirming that the radial step appears independent
357 of final cell division plane orientation. However, the radial step exhibited two unique features
358 in *trm678*. First, it seemed visually clearer than in the WT (Figure 3C). To ascertain this, we
359 measured the anisotropy of CMT arrays, and found it to be twice lower 3 hours before cell
360 division in the *trm678* mutant compared to the WT, consistent with the observation of a better-
361 defined radial step (p -value = $6,05e^{-6}$ at -2 hours, TuckeyHSD test; Figure 3F). Second, the
362 radial step appeared to last much longer than in the WT. The exact average duration of the radial
363 step in *trm678* is difficult to estimate, because in most of our kinetics, the radial step lasted at
364 least 6 hours, and we missed the full sequence (from interphasic CMTs to the radial step to
365 mitosis) within a multi-hour kinetics (Figure 3C and Figure S6A). We could observe that switch
366 only in exceptional cases, i.e. when the radial step was shorter (ca. 4 hours) before cell division
367 (Figure S7B). In the *trm678* mutant, the internal microtubule signal was significantly higher
368 than in the WT, as shown when following individual kinetics (Figure 3D, Figure S7) and when
369 pooling measurements (a 213% increase between $t = -6$ hours and $t = 0$, p -value = $2.38e^{-4}$ for
370 *trm678*, and a 162 % increase between $t = -6$ hours and $t = 0$, p -value = 0.062 for the WT,
371 Wilcoxon test, Figure 3E). We also measured the anisotropy of CMT array, using ImageJ
372 FibrilTool, and observed reduced anisotropy in *trm678* on average, when compared to WT,
373 consistent with the prolonged radial step in the mutant (Figure 3F). This analysis further
374 suggests that the transient radial organization of CMTs reflects the start of cytoplasmic
375 microtubule accumulation before cell division.

376



377

378 **Figure 3.** The radial array of CMTs correlates with a pre-mitotic increase of internal
 379 microtubule content.

380 (A-D) Evolution of cytoplasmic microtubules overtime in WT background (A) or in the *trm678*
 381 mutant background (C). (A) Time-lapse images of pre-mitotic cells, the top panel being the
 382 cortical projection with SurfCut and the bottom panel an orthogonal projection through the cell.

383 Cortex images are 8-bit, with the B&C range varying between 0 and 160, and orthogonal
384 projections are 16-bit, with the B&C range varying between 0 and 1400. (B) Ratio between the
385 average internal signal and the average of the cortical mCitrine-MBD signal over time. The
386 colors correspond to qualitative classifications of cortical arrays (interphasic, radial step and
387 PPB). Each line represents the kinetics for a given cell. (C) same as (A) but in the *trm678*
388 mutant. (D) same as (B) but in the *trm678* mutant. (E) Evolution of the ratio of mCitrine-MBD
389 signal (internal/cortical) overtime in WT vs. *trm678*. (F) Evolution of CMT array anisotropy
390 overtime, using the FibrilTool plugin in Fiji. Results from the WT are reproduced here from
391 Figure 1H for ease of reading, to compare with *trm678*. Scale bars: 10 μm (surface projections),
392 5 μm (orthogonal projections).

393

394 **Disruption in the balance between cortical and cytoplasmic microtubule accumulation in** 395 ***trm678* increases the precision of cell division**

396

397 Altogether, the radial organization of CMTs coincides with increased cytoplasmic microtubule
398 accumulation around the nucleus (Figure S8), which is even more pronounced in the absence
399 of TRM6/7/8. This provides a scenario in which the radial step emerges from a destabilization
400 of CMTs, either from increased connections between CMTs and the nucleus, or from
401 competition between different sites of microtubule nucleation. This also transiently disrupts
402 CMT alignment with tensile stress at the outer periclinal wall. What could be the function of
403 this reset sequence?

404

405 The reset stage where cells become blind to their mechanical environment echoes data in
406 *Drosophila epithelia* where cell rounding occurs to allow the cell to perceive its centroid,
407 independently from adjacent cells influence, in order to define the degree of cell division
408 symmetry(2)(3). The radial step may have a comparable function in plants, in terms of
409 shielding. Indeed, the inner periclinal cortex also displays a radial reorganization of the CMT
410 array before the formation of the PPB (Figure S9).

411

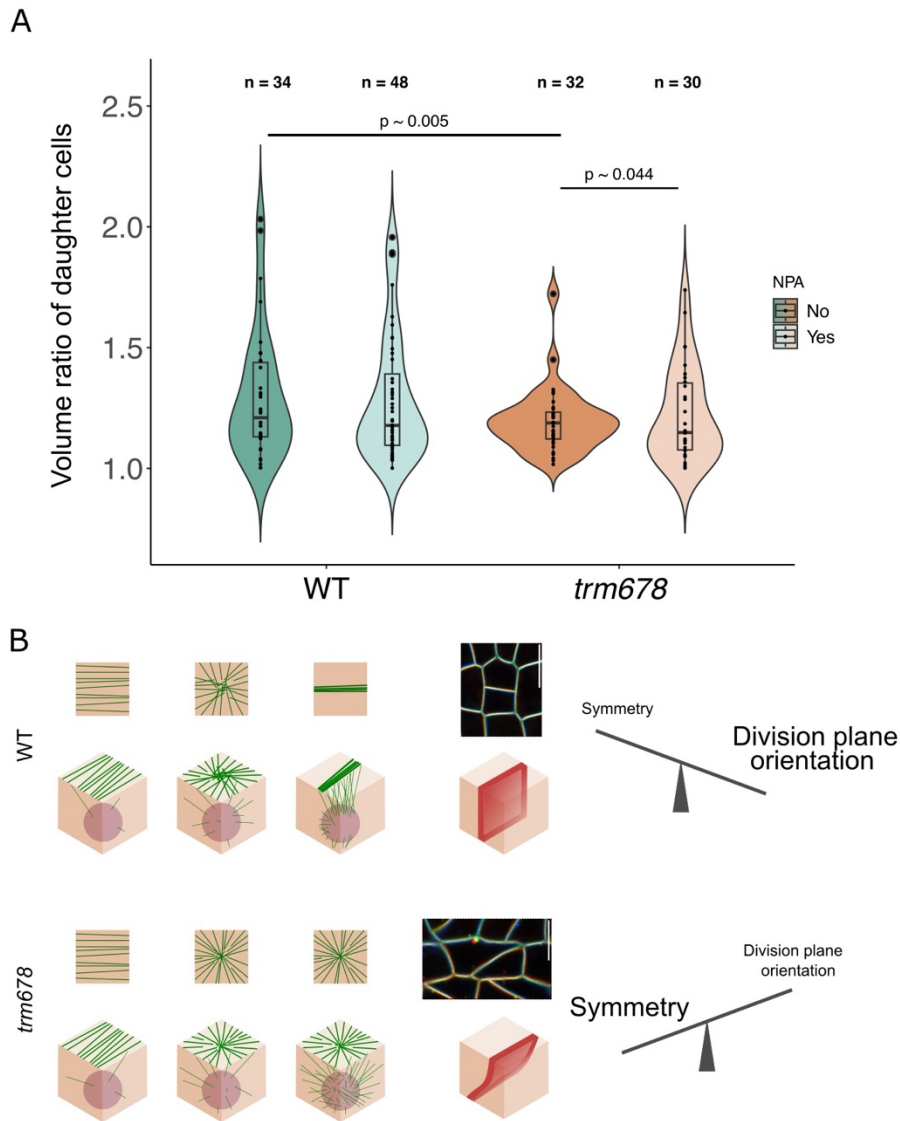
412 To address this question, we analyzed daughter cell volumes in WT and *trm678* stems, and
413 plotted the volumes ratio for both genotypes. The mean ratios were comparable (1.30 +/- 0.26
414 in WT vs. 1.20 +/- 0.14 in *trm678* (Figure 4A)). Note that the means are higher than 1 because
415 we measure the ratio between the larger daughter cell over the smaller one. Interestingly, while

416 the averages were not significantly different (Wilcoxon test p -value = 0.253), the variances
417 were significantly different (0.07 in WT and 0.02 in *trm678*, Fligner test p -value = 0.005),
418 *trm678*'s symmetry between daughter cells being 3.79 times less variable than in wild-type.
419 This suggests that a longer and better-defined radial step correlates with more precise divisions,
420 reducing the variability in daughter cell volume ratios.

421

422 Because the *trm678* has a prolonged radial step and a prolonged M phase (Figure S6B), we next
423 checked whether increased precision is simply the result of a slower pre-mitotic stage. To do
424 so, we performed the same analysis in NPA-treated WT plants, which exhibit slightly longer
425 radial step and slightly longer mitosis than non-NPA treated plants. This did not affect the
426 variance of daughter cell volumes (Figure 4A). When performing the same analysis on NPA-
427 treated *trm678* stems, we instead observed a partial rescue: the variance in NPA-treated *trm678*
428 stems was lower than NPA-treated WT stems, but higher than control *trm678* stems (Figure
429 4A). Thus, the precision in cell division does not correlate with a slower pre-mitosis and mitosis
430 dynamics, but instead, with a longer and qualitatively more clear-cut radial step, in *trm678*.
431 Altogether, we propose that the accumulation of cytoplasmic microtubules near the nuclear
432 envelope 3 hours before cell division in stems transiently disrupts the co-alignment of CMTs
433 with tensile stress, forming a radial organization of CMTs, thus allowing the cell to control the
434 precision of its division, by becoming less sensitive to external cues.

435



436

437 **Figure 4.** The radial step could be a marker for cell shape sensing and symmetry precision in
 438 assessing the division plane positioning.

439 (A) Violin plot showing the distribution of the ratio between the volume of the two daughter
 440 cells in WT and *trm678* backgrounds, in both control and NPA conditions. The measurements
 441 were made using images from cells expressing *pUBQ10::Lti6b-TdTomato* membrane marker.
 442 The ratio is always between the bigger daughter cell and the smaller daughter cell, for
 443 comparison purposes. The *p*-values displayed are the ones obtained from the Fligner's statistical
 444 test using R studio. (B) Model: In the WT (top panel), a suboptimal radial step allows the cell
 445 to sense its own geometry to define cell division symmetry loosely, while the formation of the
 446 PPB defines robust division plane orientation; in the *trm678* mutant (bottom panel), a better-

447 defined radial step improves the division precision (daughter cell volumes), to the detriment of
448 division plane orientation robustness. Radial step and PPB reflect a trade-off in robustness
449 (division precision vs. division plane orientation, respectively). Images: RGB projection of
450 recently divided cells at different focal planes (red: top of the cell, green: middle of the cell,
451 blue: bottom of the cell) in the WT (top cell) and *trm678* (bottom cell). Scale bars: 10 μm .

452

453 **DISCUSSION**

454

455 Altogether, our results are consistent with a sequential model in which the progressive
456 accumulation of cytoplasmic microtubules during the G2 phase would disrupt CMT alignment
457 with tensile stress to allow the cell to sense its own geometry for a short time window and to
458 ensure the precision of cell division. We do not exclude that this stage could also help the cell
459 to acquire information on other key cell division parameters, such as centroid position(34).
460 After this transitory stage, CMTs would resume their alignment either with the short axis of the
461 cell when cells are elongated (Errera's rule, also matching cell-shape derived maximum of
462 tensile stress) or with tissue stress, when cells are isodiametric, to control the final division
463 plane orientation.

464

465 **The transient CMT organization before mitosis as an exception to the CMT alignment** 466 **with tensile stress**

467

468 So far, the alignment of CMTs with maximal tensile stress has been verified in most plant
469 tissues(35)(10), and suffers only a few exceptions. Although this needs to be formally
470 investigated, most of these exceptions could be explained by invoking differences in stress
471 levels. For instance, one could propose that a drop of turgor pressure in hypocotyls switching
472 from dark to light could explain the rapid growth arrest and associated switch in CMT
473 orientation(36). Similarly, the progressive reinforcement of cell walls during differentiation,
474 and thus reduction in stress level, could explain the loss of CMT co-alignment in leaf
475 epidermis(37). This notably builds on recent investigations in protoplasts that are confined in
476 microwells: in this case, CMTs are transverse, aligned with the shortest axis of an elongated
477 cells, which is also a local maximum of stress, following Laplace-Young law(38). However,
478 when they are placed in a hyperosmotic medium, i.e. when tension decreases, CMTs switch to

479 the long axis of the cell: they follow the flattest part of the protoplast contour, which is also the
480 minimum of bending energy(38)(39).

481

482 To our knowledge, the only undisputable exception of CMTs alignment with tensile stress is
483 thus provided in this work, in the sense that during the timeframe of the radial step, the global
484 pattern of tension should not change: 3 hours before cell division, cells in stems exhibit a radial
485 organization of their CMT arrays. This cannot be explained by a change in tissue stress pattern,
486 as adjacent cells usually exhibit CMTs that are aligned with predicted tissue stress direction. A
487 change in cell wall properties would be too slow to affect the CMTs so transiently. Although
488 we cannot exclude the possibility that turgor level would transiently change 3 hours before cell
489 division, this would be unlikely to cause a radial CMT organization. As shown in the protoplast
490 experiments, following a drop of turgor pressure, CMTs would align with the long axis of the
491 cell, and would not make a radial pattern.

492

493 **The radial CMT step as a reset mechanism**

494

495 Our observation goes against a common assumption that PPB orientation usually follows that
496 of CMT during interphase. We instead identified a reset stage where CMTs become
497 disorganized for a duration of ca. 2 hour and at a well-defined time window (3 hours before cell
498 division). The radial step reflects a stage during which CMTs switch from their role in guiding
499 the deposition of cellulose microfibrils, to their role in controlling cell division. Other cortical
500 microtubule arrays have been shown to have links with cell division (typically, the PPB, and
501 more recently cortical-telophase microtubules(40)), further suggesting that close to mitosis,
502 there is a change in CMTs function. Based on the temporal correlation between cytoplasmic
503 microtubule enrichment during the G2 phase and on the prolongation and better-definition of
504 the radial step when cytoplasmic microtubule accumulation is promoted (in *trm678*), we
505 propose that the radial step is a period during which increased cytoplasmic microtubule
506 accumulation disrupts the CMT self-organization, thereby making the CMT network transiently
507 blind to external mechanical cues.

508

509 Our time-lapse analyses shed a new light on past reports on guard mother cells (GMCs). In
510 particular, to quote Yoshinobu Mineyuki in 1999(41), notably building on studies by Mullinax
511 and Palevitz in 1989(42), "graminean GMCs have "interphase microtubule bands" that orient
512 perpendicular to PPBs. This transverse interphase microtubule band precedes a radial

513 microtubule array, which is then replaced by a PPB prior to the longitudinal cell division".
514 These past reports together with our analysis suggest that the radial step is not a guard mother
515 cell exception, but more likely the rule.

516

517 To go one step further, the proposed function of the radial step may be similar to the proposed
518 role of the cell rounding step in *Drosophila* epithelia. Indeed, upon disruption of cell rounding
519 through myosin motor inactivation, epithelial cells remain packed as they divide, and their
520 division plane follows cell geometry instead of tissue stress(3). Even though the mechanical
521 properties of a plant tissue are different than that of an animal due to the presence of cell walls,
522 our analysis of cell division plane orientation according to cell aspect ratio also fits the idea that
523 a threshold of stress, either prescribed by cell shape or tissue shape, determines the final
524 orientation of division plane. Interestingly, in our study, we could explore the consequence of
525 a prolonged, well-defined, radial step, and we found an increase in cell division volume
526 precision. This opens the question as to whether this would also be true in *Drosophila* epithelia.

527

528 **A trade-off in precision between division plane orientation and division precision**

529

530 The idea of a competition between internal and cortical microtubules is not totally new. Indeed,
531 cytoplasmic microtubules have major impacts on cortical array in non-dividing cells(43).
532 Cytoplasmic microtubules can generate an isotropic array overriding the pre-existing
533 microtubule array through the tethering function of CLASP(44). Because pre-mitotic
534 cytoplasmic microtubules are prominent in late G2 phase, it could cause great impact on cortical
535 microtubule array. This is what we find here, also revealing that the accumulation of
536 cytoplasmic microtubules before cell division does not only serve as a reservoir of microtubules
537 to prepare mitosis, but it also contributes to the precision of cell division, possibly by making
538 the cell more self-centered on its nucleus.

539

540 We showed that the radial step is robust to different cell shapes, cell identity (shoot apical
541 meristem, stem, meristemoids) and growth conditions (AM and ACM medium, with or without
542 NPA), where cortical and cytoplasmic microtubule behavior is likely to vary. Such robustness
543 may emerge from a more fundamental parameter: the conflict between cytoplasmic and cortical
544 microtubules. Whereas long-term tracking of dividing cells with microtubule markers and at
545 high enough resolution are still rare in the literature, we could find examples of *Datura*
546 *stramonium* cells with a transient pattern reminiscent of what we observed in *Arabidopsis* (45).

547 We thus propose that the radial step reflects the sequential control of cell division, where
548 division precision precedes division plane robustness. We can speculate that the radial step is
549 an integral part of the preparation for mitosis and therefore could be extended to other tissues
550 and cell types. This sequence also suggests that division precision is more variable than division
551 plane orientation (as its control is temporally more distant from mitosis), possibly because a
552 small error in division precision has less implications than a small error in division plane
553 orientation, at least from a mechanical point of view.

554

555

556 MATERIAL AND METHODS

557

558 Plant material and growth conditions

559 All *Arabidopsis thaliana* lines were in the Col-0 ecotype. Wild-type Col-0 and the *trm678* triple
560 mutant(6) were transformed using the dipping floral method(46). Transgenic lines were
561 expressing a microtubule marker (*pPDF1::mCitrine-MBD*(47)) and a plasma membrane
562 marker (*pUBQ10::Lti6-TdTomato*(48)). As controls, we also used plants expressing the
563 *pRPS5a::RFP-TUA5* tubulin marker, and plants expressing the *pUBQ10::RFP-TUA6* tubulin
564 marker.

565 For SAM imaging, *Arabidopsis* Wild-type and *trm678* mutant lines were sown on soil and
566 grown in climatic chambers under short-day conditions (8-h light/16-h dark) for 6 weeks and
567 then grown under long-day conditions (16-h light /8-hdark regime) for 4 weeks. Dissected
568 apices were embedded in ACM culture medium (2.2 g/L Murashige and Skoog medium without
569 vitamins (Duscheffa), 10 g/L sucrose, pH 5.8 KOH (10 M), 8 g/L agarose) supplemented with
570 1x Morel and Wetmore vitamins and 570 nmol/L trans-zeatin (12.5ul of 1mg/mL of stock
571 solution in 100ml of ACM medium), and maintained under long-day conditions (16-h light 8-
572 h/dark regime, 21°C) as described in (49).

573 For stem imaging, seeds were sterilized using a sterilization solution (88 mg/mL sodium
574 dichloroisocyanurate and 90% ethanol). 50 to 300 µL of seeds were first incubated in 1 mL of
575 the sterilization solution for 6 minutes, and then washed twice with 96% ethanol and left to dry.
576 Seeds were sown on solid *Arabidopsis* medium (11.82 g/L *Arabidopsis* medium (Duscheffa, a
577 custom-made medium for the lab, recipe in the Supplementary Material), 2 mM Ca(NO₃)₂
578 4H₂O, pH 5.8 KOH (10 M), 10 g/L granulated agar (Merck)). In NPA conditions, this medium

579 was supplemented with 10 μm of naphthylphthalamic acid (NPA). The seeds were left for
580 stratification for 2 days at 4°C in the dark, and plants were grown for around 3 weeks in long
581 day conditions (16h light/8h dark) at 20°C, until the first flower buds developed.

582 It is worth noting that, while the cell division is very active at the tip of the stem, imaging
583 conditions can inhibit division and affect microtubule dynamics. This happened in both
584 Arabidopsis and ACM media. We noticed that the winter season is usually more prone to induce
585 slow division rates.

586

587 **SAM dissection and imaging**

588 Dissected shoot apices were imaged with a Leica SP8 upright confocal microscope ([Figures](#)
589 [1A](#), [1B](#), and [S1A](#)). Confocal z-stacks were acquired (every 30 min for 10 hours) at a resolution
590 of $0.09 \times 0.09 \times 0.2 \mu\text{m}$ per voxel using a HC FLUOTAR L 25x/0.95 N.A. water immersion
591 objective. The confocal scan speed was no more than 7, and line averaging was set to 2.

592 **Stem preparation and imaging**

593 Arabidopsis stems from *in vitro* plants with a few unopened flower buds were selected. For
594 easier dissection, we selected stems that were ca.1.5 cm long. Then, the organs impairing a
595 correct imaging were dissected (cauline leaves and flower buds). The imaging medium was
596 either the same as the growth medium (Arabidopsis Medium, AM), or Apex Culture Medium
597 (ACM). In the NPA conditions, the ACM medium was supplemented with 10 μm of
598 naphthylphthalamic acid (NPA). The plants with prepared stems were transferred to an imaging
599 medium and fixed in the medium by placing the roots and leaves inside the agar, exposing the
600 area of interest. The plate containing the samples was placed in the growing chamber for one
601 day before the experiment. [Figure S10A](#) illustrates the different steps of stem preparation.

602 The images for all figures, except [Figures 1A](#), [1B](#), [S1A](#) and [S3A](#), were acquired using the
603 LSM980 – Airyscan 2 upright confocal microscope (Zeiss). All the samples were imaged under
604 the W Plan-Apochromat x20 water immersion lens (NA: 1.0), and were kept under immersion
605 during the whole experiment. All images were made using the airyscan mode of the microscope.
606 For this, the size of the image needed to be optimized for the Airyscan module, which resulted
607 in a pixel size of around $0.065 \mu\text{m} \times 0.065 \mu\text{m}$. The Z resolution was always $0.500 \mu\text{m}$. The

608 pixel time could vary between experiments, but the scanning was always bidirectional and
609 averaged twice by frame. Airyscan parameters are detailed in [Figure S10B](#).

610 Some of the images used for [Figures 2D-2G, 2H, 2I](#) acquired using the LSM980 – Airyscan 2
611 inverted confocal microscope (Zeiss). All the samples were imaged under the C-Apochromat
612 x40 water immersion lens (NA: 1.2), and were kept under immersion during the whole
613 experiment by placing a droplet of water directly on the objective. The rest of the parameters
614 are the same as described above.

615 The images for [Figure S3A](#), as well as for the production of the measurements of [Figures 2D-](#)
616 [E, 2G-H, 2J-2L](#) were acquired using the SP8 confocal microscope (Leica). The samples were
617 imaged using a x25 water-immersion lens (NA = 0.95), with the resonant scanner switched off,
618 to obtain better resolution. 8-bit images were taken in 1024x512 $\mu\text{m}/\text{pixels}$ format, with the
619 XY-resolution of 0.152 $\mu\text{m}/\text{pix}$ and Z-stack slices of 0.500 μm . Images were acquired with
620 a scanning speed of 1000 and averaged 4 times. The mCitrine signal was imaged using the
621 514 nm laser (0.5% intensity) and a HyD 1 detector between 518 nm and 549 nm. The results
622 for [Figures 2D, 2G, 2E, 2H](#) were made using images from the three microscopes.

623 **Image processing**

624 All the processing of the images was done on the Fiji (ImageJ) software.

625 The LSM980 images were processed using the Airyscan Processing program in the Zeiss
626 software, with the “All Images (2D)” program and the Auto Filter. They were then converted
627 from the Carl Zeiss Image format (.czi) to Tagged Image Format File (.tiff) format, using a
628 slightly modified version of the lifRxiv program (<https://github.com/sverger/lifRxiv>).

629 The SP8 images were converted from Leica Image File Format (.liff) to Tagged Image Format
630 File (.tiff) format, using the lifRxiv program.

631 The image stacks were then processed using the SurfCut program(50) projecting the upper
632 signal from the top slices (between 0 and 4 μm of the epidermis). The SurfCut projections
633 coming from the same sample at different time points were then assembled into stacks. We
634 selected the cells that divided or exhibited a PPB by the end of the time frame of the experiment.

635 **Division plane orientation analysis**

636 Cells of interest, showing either a PPB or a cell plate, were identified. The longest and shortest
637 axis were measured by manually drawing a region of interest to the cell's contours, and
638 extracting the fitted ellipse using Fiji. The aspect ratio was then obtained by dividing the
639 shortest plane by the longest plane of this fitted ellipse.

640 The orientation of the division plane, or the PPB, was also measured manually using the Fiji
641 software. The orientations were normalized to keep the results between 0° and 90°, with the
642 0° orientation being horizontal.

643 The division angles were all divided by 90, to get a dataset between 0 and 1, in order to perform
644 the logistic regression. The regression was made with the “glm” fit in R, with the “quasi-
645 binomial” family to account for the dispersion of the data, and the “logit” link. To assess the
646 goodness of the fit, we calculated the R-squared value using the null deviance, and the residual
647 deviance given by the glm function, as followed:

648
$$R^2 = 1 - \left(\frac{\text{Residual deviance}}{\text{Null deviance}} \right)$$

649 To find an approximation of the inflection point of the fitted curve, we acknowledged that the
650 “logit” link uses the following regression:

651
$$\log\left(\frac{p}{1-p}\right) = ax + b$$

652

653 With p being the probability of the response being 1, a being the slope, and b the intercept.

654 We therefore calculated x with p set at 0.5, and a and b being the result of the “glm” fit.

655 **Cortical microtubule orientation analysis**

656 We manually drew rectangles in the center of the cortex, avoiding the edges (to prevent biases
657 in the measurements) on SurfCut projections, for the cells of interest. To measure the local
658 orientations of microtubules overtime, we used the Directionality function in Fiji, using the

659 “Local gradient orientation” method. The output of this program is a distribution of the pixel
660 orientations between -90° and 90° (with 0° being the vertical orientation), as well as a gaussian
661 fit to the data. We plotted the gaussian fit, for each cell at each timepoint.

662 We used the OrientationJ package’s “vector field” function in Fiji
663 (<http://bigwww.epfl.ch/demo/orientation/>) as another way of measuring CMT orientation
664 (Figure 2J, 2K, 2L). For this, we wrote a homemade macro allowing to extract the vectors inside
665 the region of interest. Briefly, we defined the ROI manually and blacken it. We then extract the
666 coordinates of the vectors inside this blackened area by setting a threshold on the energy value
667 given by the “vector field” output. Once these coordinates are saved, we run the “vector field”
668 on the unchanged image and retain the orientation of the vectors of interest. To test the
669 differences in the distribution of these vectors’ orientations overtime, we ran the Kolmogorov-
670 Smirnov test in RStudio. With this test, we compared the distribution of the data at each
671 timepoint with a uniform sequence, generated with RStudio.

672 To measure the anisotropy of the microtubule arrays, we used the SurfCut images, and
673 delimited the region of interest by hand, following the cell contour, but excluding the anticlinal
674 cortex to avoid any bias. We ran the FibrilTool plugin(18) in Fiji to extract the anisotropy. To
675 test the differences between the different genetic background and the different timepoints, we
676 ran the Tukey-Kramer test in RStudio.

677 **Cytoplasmic microtubule content analysis**

678 To analyze the cytoplasmic microtubule, we used Z-stacks of images from lines expressing
679 *pPDF1::mCitrine-MBD*. To project the cytoplasmic microtubule signal, we used the reslice
680 function in Fiji, with “Output spacing” set at $0.5\ \mu\text{m}$, starting at the top. We then made a
681 maximal projection of the reslice output to obtain the cytoplasmic microtubule signal. The
682 region of interest for the reslice projection was determined by hand, by being careful of
683 excluding the anticlinal cortex of the cell to only project the internal signal.

684 The outer periclinal cortex, as well as the inside of the cell were marked by hand, and the
685 average signal was measured using Fiji. For each cell, at each timepoint, the ratio between the
686 internal signal and the cortical signal was plotted, to be able to compare all the measurements,
687 and balance the photobleaching that the samples experienced overtime. We tested the

688 differences in these values between the different genetic backgrounds and between each
689 timepoint using the Tukey-Kramer test in RStudio.

690 **Volume measurements**

691 Membrane marker images were resized to obtain a voxel size with a X of around 250 μm . The
692 images were then processed using the MorphoGraphX software(51). First, images were blurred
693 using the Gaussian blur stack function, and then the segmentation was made using the ITK
694 Watershed Auto Seeded function, and the parameters were fine-tuned to obtain a suitable 3D
695 segmentation. A mesh was then applied using the Marching Cube 3D function, and the volumes
696 were extracted with the Cell Analysis 3D function.

697 Recent divisions were spotted by eye, as newer membranes separating two daughter cells are
698 straight, anchored at 90° from the edges and are thinner than older membranes. The ratio
699 between the two volumes was then always measured by dividing the bigger daughter cell's
700 volume by the smaller ones. For statistical tests, we first performed a Wilcoxon test in RStudio,
701 comparing the averages of the ratios with respect to the NPA conditions (with or without NPA),
702 for each genetic background (WT and *trm678*). As there was not a statistically significant
703 difference between the averages, with a threshold of 0.05, and due to the aspect of the data, we
704 performed a Fligner's test in RStudio that focuses on the variances. The *p*-values are displayed
705 on [Figure 4A](#).

706

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708

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715

716

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842 **Supplementary Information for:**

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845 **A transient radial cortical microtubule array is involved in premitotic control of cell**
846 **division in Arabidopsis**

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848 Isaty Melogno¹⁺, Shogo Takatani^{1,2+}, Paula Llanos³, Chie Kodera⁴, Marjolaine Martin¹, Claire
849 Lionnet¹, Magalie Uyttewaal⁴, Martine Pastuglia⁴, Christophe Trehin¹, David Bouchez⁴,
850 Jacques Dumais³, Olivier Hamant^{1,*}

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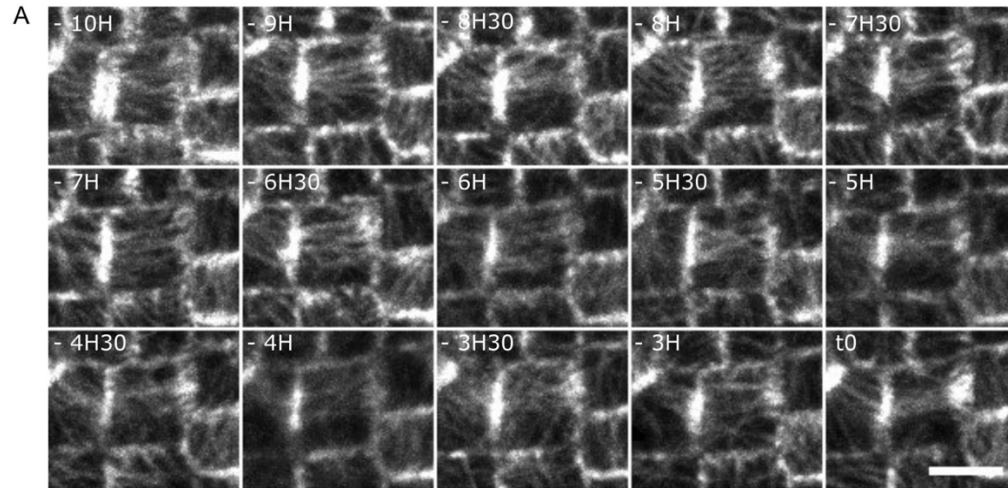
855 **Table 1.** Detail of the composition of the Arabidopsis medium.

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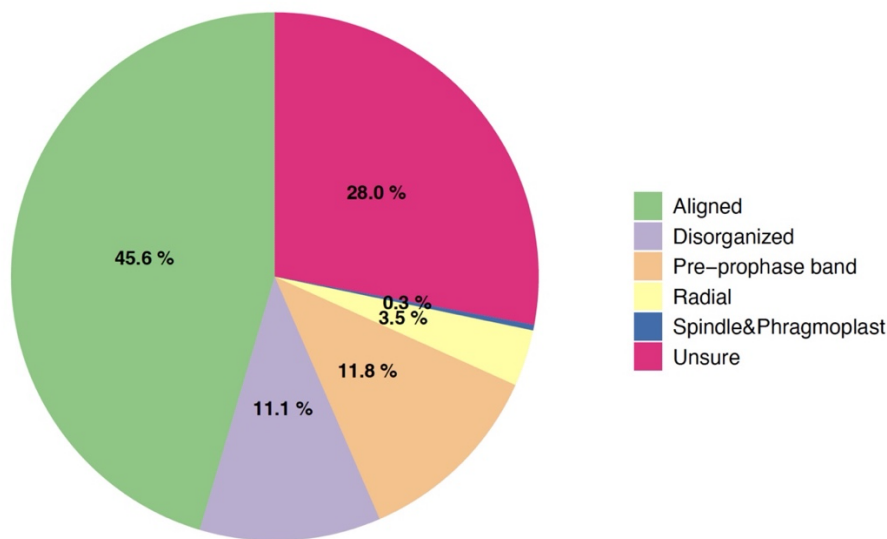
Arabidopsis medium	
Formula	mg/mL
CoCl ₂ .6H ₂ O	0.0025
CuSO ₄ .5H ₂ O	0.13
Ferric Ammonium Citrate	50
NaCl	0.58
H ₃ BO ₃	4.33
MnCl ₂ .4H ₂ O	2.77
Na ₂ MoO ₄ .2H ₂ O	0.05
ZnSO ₄ .7H ₂ O	0.29
KH ₂ PO ₄	340.25
KNO ₃	500.5
MgSO ₄ .7H ₂ O	246.5
Myo-Inositol	100
Nicotinic Acid	1
Pyridoxine HCl	1
Thiamine HCl	1
Ca-Panhotenate	1
Biotine	0.01
Sucrose	10000
MES	700

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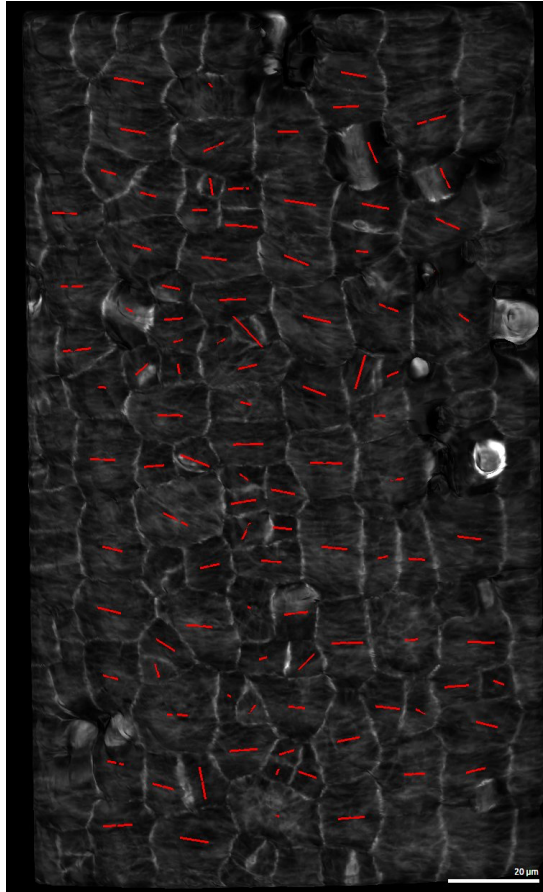
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861 **Figure S1.** Radial array of CMTs in pre-mitotic cells at the shoot apical meristem

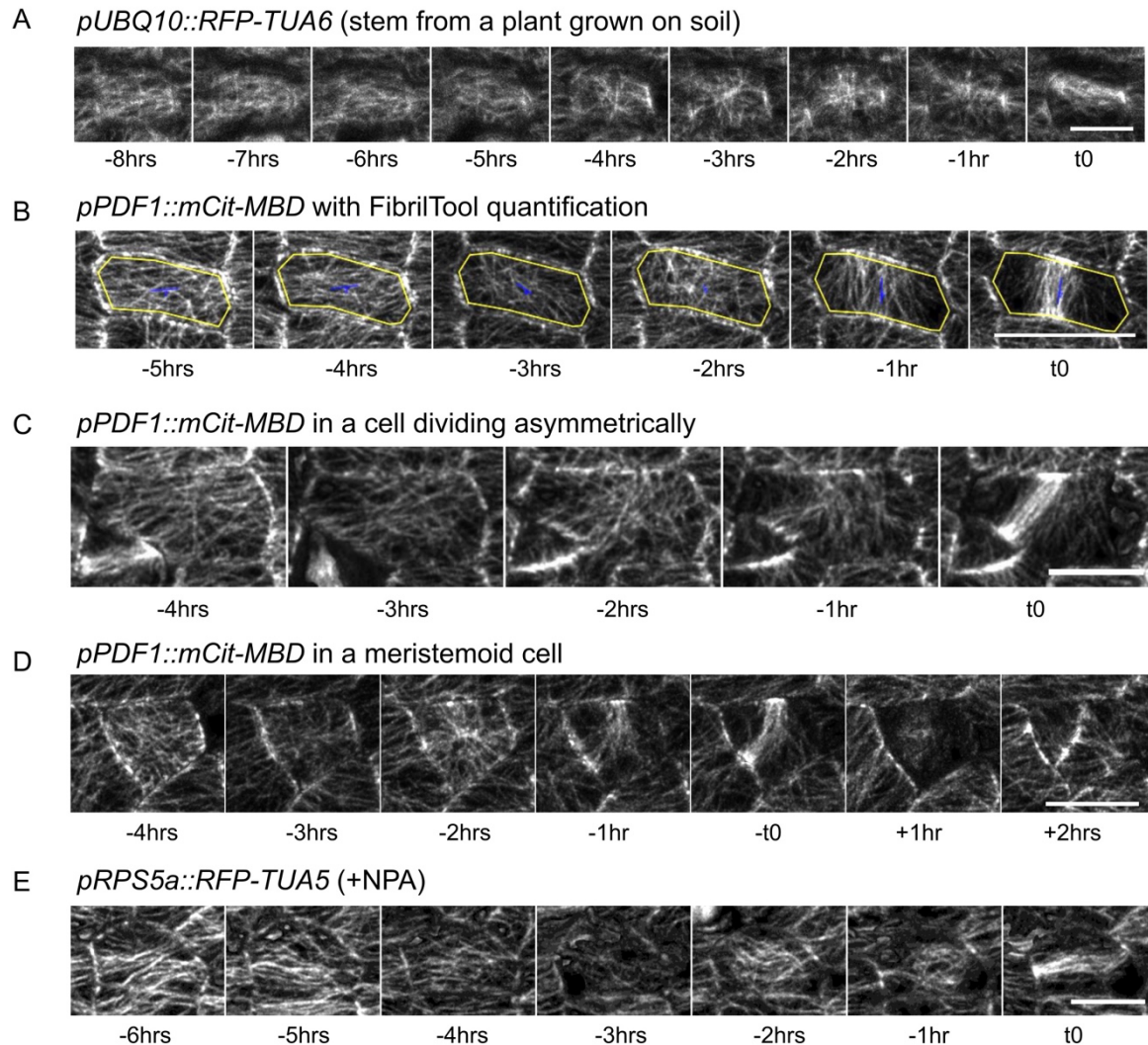
862 (A) Other example of a time lapse showing cortical microtubules dynamics before the
863 establishment of the PPB. From -10 hours to -6 hours, CMTs maintain a stable orientation.
864 From -5h30 onwards, CMTs exhibit less consistent orientations, up to PPB formation. Images
865 are SurfCut projections of the area between 0 and 4 μm of the surface of the *pPDF1::mCit-*
866 *MBD* signal. The scale bar corresponds to 5 μm . The Brightness and Contrast parameters have
867 been modified for a better visualization of the microtubules. (B) Pie chart representing the
868 average percentages of the different organizations of CMT arrays in shoot apical meristems.
869 Aligned reflect the directional organization, typically at interphase. PPB corresponds to the
870 cells where a clear band of microtubules is observed. Radial shows the radial array we described
871 above. Spindle/Phragmoplast corresponds to cells that are undergoing mitosis. Disorganized is
872 when the array is visible, but all of the above categories are not suited to describe it, whereas
873 unsure reflects cells where no clear classification could be made (either the resolution was too
874 low or the image was blurry). These classifications were made qualitatively on a total of 4 shoot
875 apical meristems, where only the central zone was observed.



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Figure S2. Average CMT orientations in an *Arabidopsis* stem.

This image represents the projection of the *pPDF1::mCitrine-MBD* signal between 0 and 4 μm , using the MorphoGraphX software. Cells were segmented using this software, and the average orientation of CMTs was extracted and displayed. The red lines represent the average orientation of CMTs. The length of the line corresponds to the anisotropy of the CMT arrays: the longer the line, the higher the anisotropy value. Most of the cells display transverse cortical microtubules, following the predicted maximal tensile stress pattern.



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890 **Figure S3. Additional evidence of radial CMTs before cell division**

891 (A) CMT dynamics before cell division in stems, using the *pUBQ10::RFP-TUA6* marker line

892 grown on soil. (B) Example of FibrilTool measurement: the yellow contour defines the region

893 of interest from which the measurements are made. The blue line represents the anisotropy of

894 the CMT arrays with its length, and the predominant orientation of the CMTs with its

895 orientation. (C) Example of CMT dynamics in an asymmetrically dividing cell. (D) Example

896 of CMT dynamics in a meristemoid cell (dividing asymmetrically). (E) CMT dynamics before

897 cell division in stems, using the *pRPS5a::RFP-TUA5*, with NPA from germination on plants

898 grown in vitro. All images reveal the fluorescent signal, and are SurfCut projections from 0 to

899 4 μm from the surface of the stem. Scale bars: 10 μm

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901 A)

WT (Control)	-6 hrs	-5 hrs	-4 hrs	-3 hrs	-2 hrs	-1 hr	t0
-6 hrs	XXXXXX	0.1646	5.02e ⁻⁰⁶	< 2.2e ⁻¹⁶	< 2.2e ⁻¹⁶	7.10e ⁻⁰⁴	1.84e ⁻¹¹
-5 hrs	XXXXXX	XXXXXX	8.00e ⁻⁰⁷	< 2.2e ⁻¹⁶	< 2.2e ⁻¹⁶	0.00591	4.76e ⁻¹⁰
-4 hrs	XXXXXX	XXXXXX	XXXXXX	2.18e ⁻¹³	4.90e ⁻¹⁰	2.15e ⁻¹⁴	< 2.2e ⁻¹⁶
-3 hrs	XXXXXX	XXXXXX	XXXXXX	XXXXXX	0.3048	< 2.2e ⁻¹⁶	< 2.2e ⁻¹⁶
-2 hrs	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	< 2.2e ⁻¹⁶	< 2.2e ⁻¹⁶
-1 hr	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	1.54e ⁻⁰⁵
0	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX

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903 B)

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WT (+ NPA)	-6 hrs	-5 hrs	-4 hrs	-3 hrs	-2 hrs	-1 hr	t0
-6 hrs	XXXXXX	0.1548	0.1552	7.18e ⁻⁰⁵	< 2.2e ⁻¹⁶	3.62e ⁻⁰⁴	< 2.2e ⁻¹⁶
-5 hrs	XXXXXX	XXXXXX	0.6853	8.33e ⁻⁰⁴	< 2.2e ⁻¹⁶	0.005563	< 2.2e ⁻¹⁶
-4 hrs	XXXXXX	XXXXXX	XXXXXX	9.04e ⁻⁰⁵	< 2.2e ⁻¹⁶	2.21e ⁻⁰⁴	< 2.2e ⁻¹⁶
-3 hrs	XXXXXX	XXXXXX	XXXXXX	XXXXXX	< 2.2e ⁻¹⁶	0.763	< 2.2e ⁻¹⁶
-2 hrs	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	< 2.2e ⁻¹⁶	< 2.2e ⁻¹⁶
-1 hr	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	< 2.2e ⁻¹⁶
0	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX	XXXXXX

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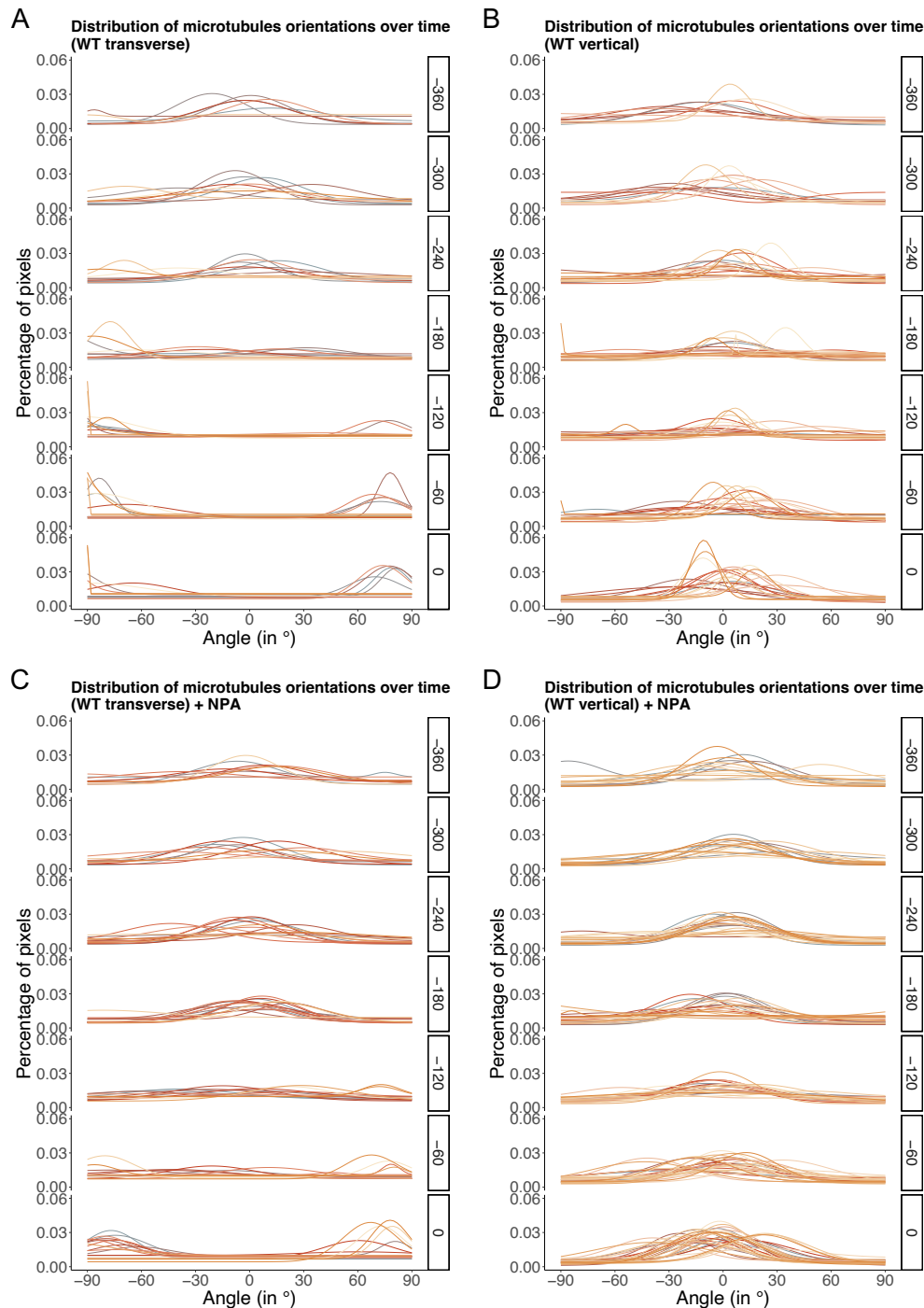
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Figure S4. *p*-value results for Kolmogorov-Smirnoff tests, comparing the distributions of CMT orientations under control conditions (A), or NPA conditions (B), between the different timepoints. The dataset used for this test was the results obtained with the Directionality plugin in Fiji. The null hypothesis for the Kolmogorov-Smirnoff test is “There are no differences between the two distributions”.

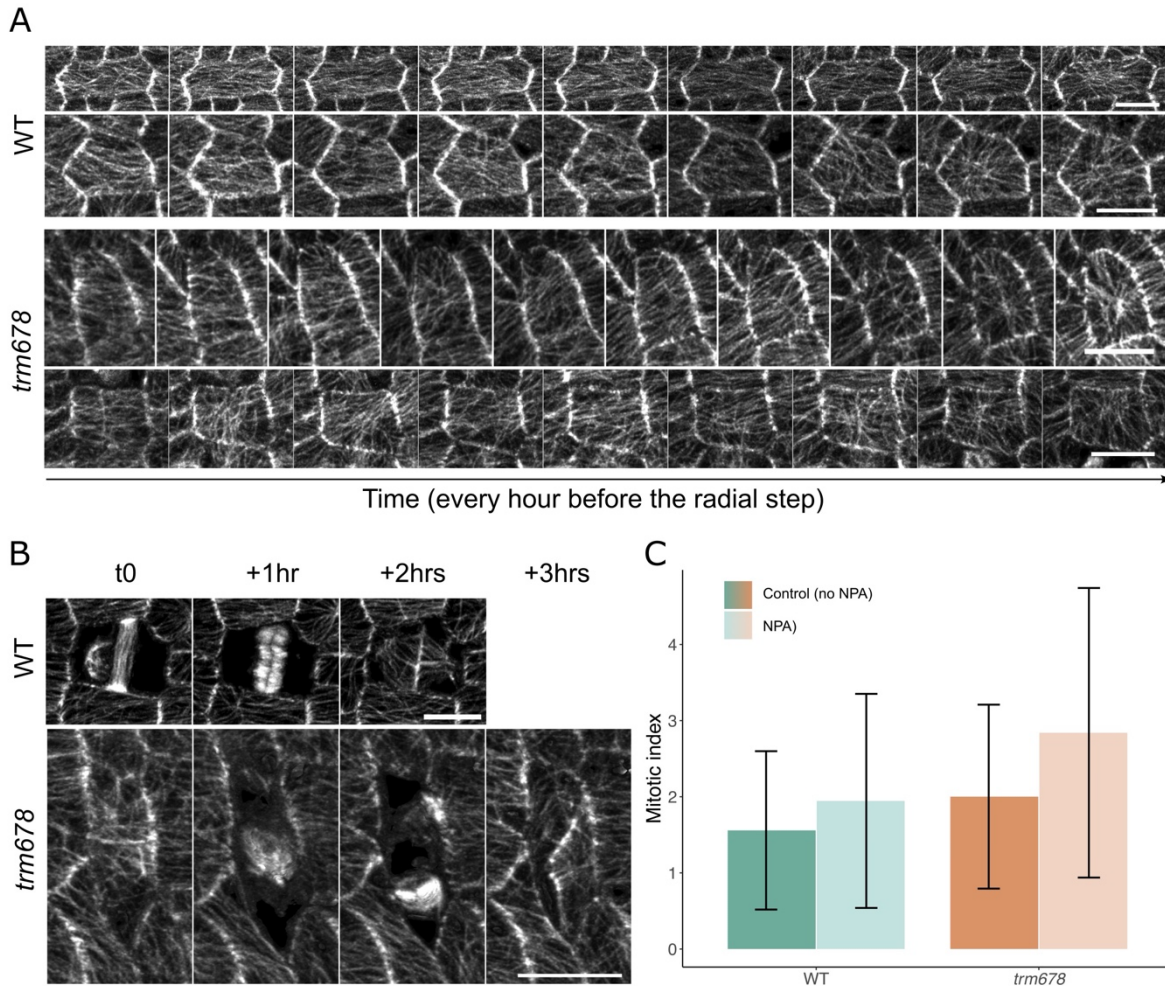


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914 **Figure S5.** Evolution of CMT orientation over time in WT pre-mitotic cells from the stem,
 915 divided in four data subsets.

916 (A-D) The angle scale varies between -90° and $+90^\circ$, 0° being the transverse orientation to the
 917 stem axis. Each row represents the time before $t=0$ (last timepoint before the nuclear envelope
 918 breakdown). Each color-coded curve represents the results obtained for one cell. The dataset
 919 used is the same as the one used for Figures 1G and 2I. (A) Results obtained for cells that
 920 produce a preprophase band oriented along the stem axis (i.e. between -30° and 30°), grown on
 921 media without NPA. (B) Results obtained for cells that produce a preprophase band oriented
 922 transverse to the stem axis (i.e. between -60° and -90° , and between 60° and 90°), grown on
 923 media without NPA. (C) Similar to (A), for cells grown on NPA conditions. (D) Similar to (B),
 924 for cells grown on NPA conditions.

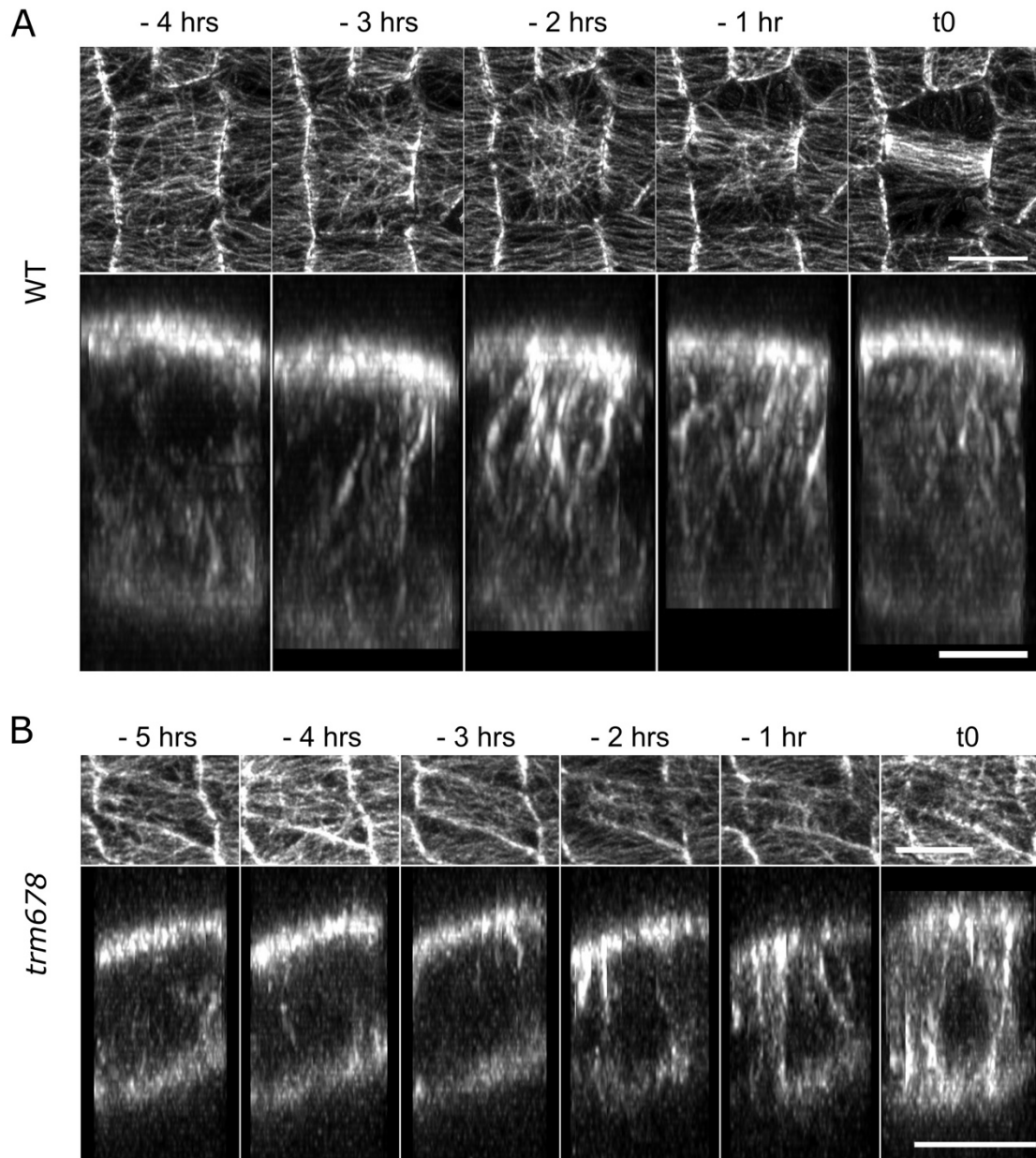
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Figure S6. CMT dynamics and mitosis in WT and *trm678*

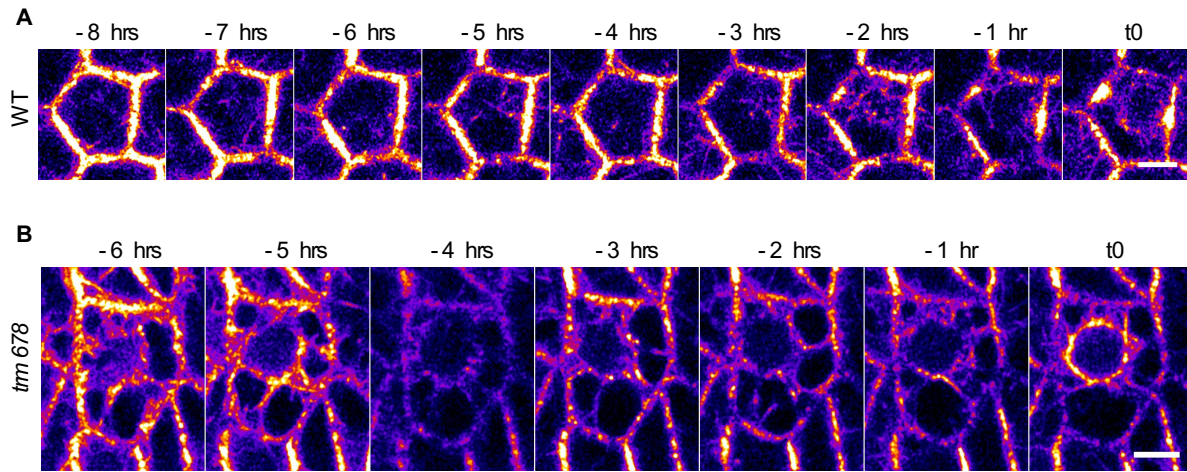
(A) CMT dynamics before the radial step in the WT and *trm678*. Images were taken every hour. (B) CMT behavior during division in a representative WT cell (top panel) and a representative *trm678* cell (bottom panel). The images are SurfCut projections between 0 and 4 μm from the surface of the stem, and the scale bars correspond to 10 μm . (C) Mitotic indexes for cells in both genetic background and in control, or NPA conditions. The mitotic index corresponds to the ratio between mitotic cells (where we can observe spindle or phragmoplast structures) and the total number of cells in the image. *trm678* cells have a slightly higher mitotic index than the WT, and the addition of NPA increases the mitotic index in both backgrounds. $n_{\text{WT, control}} = 12$; $n_{\text{WT, NPA}} = 5$; $n_{\text{trm678, control}} = 16$; $n_{\text{trm678, NPA}} = 9$.



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943 **Figure S7.** Other examples of the evolution of cytoplasmic microtubule content in pre-mitotic
944 cells.

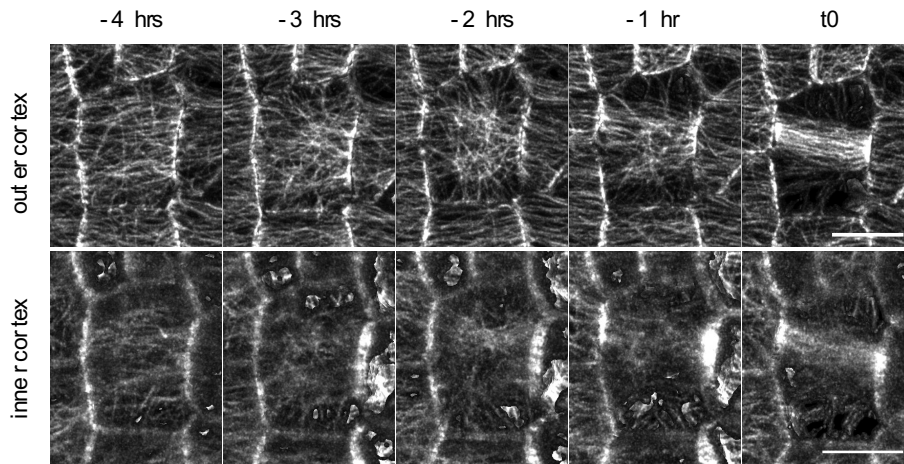
945 (A) Time-lapse images of pre-mitotic cells, the top panel being the cortical projection with
946 SurfCut and the bottom panel an orthogonal projection through the cell. Cortex images are 8-
947 bit, with the B&C range varying between 0 and 160, and orthogonal projections are 16-bit, with
948 the B&C range varying between 0 and 2600. (B) Same as (A) but in *trm678* mutant cell. Note
949 that this is an exceptional case where the radial step is short in *trm678*, which allows the
950 visualization of the full sequence: interphase - radial step - mitosis, further showing the
951 correlation between radial step and perinuclear microtubule accumulation. Scale bars: 10 μm
952 (surface projections), 5 μm (XZ orthogonal projections).



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955 **Figure S8.** *pPDF1::mCitrine-MBD* signal at the equator of a cell from the stem overtime.
956 (A,B) These images were made showing a single slice of the Z-stack in the middle of the cell.
957 (A) WT cell: these 16-bit images are obtained using the fire Look-up table in Fiji, with the
958 Brightness&Contrast values set between 0 and 1000. (B) *trm678* mutant cell: these 16-bit
959 images are obtained using the fire Look-up table in Fiji, with the Brightness&Contrast values
960 set between 0 and 3000. The scale bars correspond to 5 μm .
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Figure S9. Cortical microtubule dynamics of a pre-mitotic cell, displaying the signal from the periclinal outer (top) and inner (bottom) cortex. The images are SurfCut projections of the *pPDF1::mCitrine-MBD* signal between 0 and 4 μm , either starting from the top (top images) or bottom (bottom images) of the cell. The signal from the inner cortex is noisier due to the weaker signal, and the more difficult extraction of the tissue surface. The scale bars are 10 μm .



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	<u>mCitrine</u>	<u>TdTomato</u>
Laser (λ , power)	514 nm, 0.10 %	561 nm, 0.10 %
Main Beam Splitter	MBS 445/514/561/639	MBS 488/561
Secondary Beam Splitter	SBS SP 615	SBS SP 615
Emission Filters for the <u>Airyscan</u> detector	BP 420-480 + BP 495-550	Plate (transmission 100%)

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Figure S10. Protocols: additional data

(A) Step-by-step representation of the stem sample preparation (left to right). First, select a seedling with a stem of around 1.5 cm in length and with a few unopened flower buds. The area of interest (the stem portion under the meristem) is often covered by a few organs. These organs are dissected out in order to expose the area of interest (red arrow). Finally, insert the roots and a few leaves inside the agar to fix the sample, while leaving the area of interest on top of the agar for it to be accessible to the microscope. (B) Airyscan detection parameters.