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1 **The cell cycle controls spindle architecture in Arabidopsis by**
2 **modulating the augmin pathway**

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30

31

32 **Summary**

33

34 To ensure an even segregation of chromosomes during somatic cell division,
35 eukaryotes rely on specific microtubule structures called mitotic spindles. There are,
36 however, striking differences in overall spindle organization among eukaryotic super
37 groups, and in particular little is known about how spindle architecture is determined
38 in plants. As a foundation for our work, we have measured prime characteristics of
39 Arabidopsis mitotic spindles and built a three-dimensional dynamic model of the
40 Arabidopsis mitotic spindle using Cytosim. Next, we identified the cell-cycle regulator
41 CYCLIN-DEPENDENT KINASE B1 (CDKB1) together with its cyclin partner CYCB3;1
42 as key regulators of spindle shape and organization in Arabidopsis. Loss of CDKB1
43 function resulted in a high number of astral microtubules that are normally absent from
44 plant spindles, as opposed to animal ones. We identified an augmin complex member,
45 ENDOSPERM DEFECTIVE1 (EDE1), as a substrate of the CDKB1;1-CYCB3;1
46 complex. A non-phosphorylatable mutant of EDE1 displayed spindles with extended
47 pole-to-pole distance, resembling the phenotypes of *cycb3;1* and *cdkb1* mutants.
48 Moreover, we found that the mutated EDE1 version associated less efficiently with
49 spindle microtubules. Consistently, reducing the level of augmin in Cytosim
50 simulations largely recapitulated the spindle phenotypes observed in *cycb3;1* and
51 *cdkb1* mutants. Our results emphasize the importance of cell cycle-dependent
52 phospho-control of the mitotic spindle in plant cells. They also support the validity of
53 our computational model as a framework for the exploration of mechanisms controlling
54 the organization of the spindle in plants and in other species.

55

56 **Keywords**

57 Spindle, cell cycle, cyclin, cyclin-dependent kinase, phosphorylation, augmin, cell
58 division, mitosis, developmental biology, plant biology

59

60 **Introduction**

61 Eukaryotes have acquired specific- and robustly-functioning cytoskeletal arrays to
62 accomplish cell divisions. Plants in particular have unique microtubule arrays for cell
63 division, namely the preprophase band (PPB) and the phragmoplast¹. In somatic cells,

64 the preprophase band forms in late-G2 cells committed to division, and marks the
65 future cortical cell-division site. After PPB disassembly and nuclear envelope
66 breakdown, a typical barrel-shaped spindle forms, which is responsible for the
67 segregation of sister chromatids. In telophase, the phragmoplast appears, a
68 cytokinetic array that drives centrifugal cell plate assembly and fusion to the parental
69 cortex. Accurate regulation of the timing and architecture of each of these microtubule
70 structures is essential for plant morphogenesis. While the PPB and the phragmoplast
71 have been addressed in several studies leading to important insights about their
72 organization, relatively little is known about the mechanisms driving assembly and
73 function of the spindle of plant cells.

74 Most land plants form spindles in the absence of a distinct microtubule
75 organizing center (MTOC), responsible for nucleating microtubules in a γ -tubulin
76 dependent manner. In animals, this MTOC is generally the centriole-containing
77 centrosome². γ -tubulin is part of the γ -tubulin ring complex (γ TuRC) that acts as a
78 template for microtubule polymerization³.

79 The augmin complex is a conserved γ TuRC-targeting factor which is composed
80 of eight members^{4,5} and allows microtubule nucleation from existing microtubules, in
81 a parallel or branched fashion⁵. Microtubule-dependent microtubule nucleation
82 mediated by the augmin complex amplifies microtubule number while preserving their
83 polarity⁶. In moss, it has been shown that knocking down augmin subunits leads to a
84 reduction of around 50% in the number of spindle microtubules⁷. Hence, augmin
85 activity is critical for microtubule amplification and organization in the plant spindle. In
86 *Arabidopsis*, ENDOSPERM DEFECTIVE1 (*EDE1*), an *AUG8/HAUS8* homologue,
87 targets the whole complex to spindle microtubules during mitotic cell divisions⁸. A
88 knockdown mutant of *EDE1* displays highly elongated spindles, whereas a null mutant
89 of this gene results in lethality^{8,9}, highlighting the role of the augmin complex in plant
90 spindle architecture.

91 In human cells, Polo-like kinase 1 (*Plk1*) has been shown to promote the
92 association of Augmin-like complex subunit 8 (*HAUS8*, the human homolog of *EDE1*)
93 with spindle microtubules¹⁰. However, plants lack *Plk* homologs, suggesting that
94 cyclin-dependent kinase (CDK) complexes and/or Aurora kinases could take over
95 some of their microtubule-associated functions in plants^{11,12}. Indeed, cell-cycle factors

96 like cyclins and CDKs are prime candidates for the regulation of spindle microtubules
97 because of both their expression pattern as well as their localization^{13,14}. In addition,
98 plant CDK-cyclin complexes are known to be involved in the regulation of microtubule-
99 associated proteins like MAP65-1, whose interaction with microtubules is negatively
100 regulated by CDK phosphorylation at prophase and metaphase¹⁵. Thus, there is
101 strong evidence that CDK-cyclin phosphorylation is essential for the organization and
102 function of mitotic microtubule arrays, including the spindle¹⁶. Accordingly, B1-type
103 cyclin double mutants (namely *cycb1;1 cycb1;2* and *cycb1;2 cycb1;3*) have spindles
104 that show defects in chromosome capture, as well as other defects in the PPB and
105 phragmoplast arrays¹⁷. However, little is known about the regulation of the spindle by
106 CDK-cyclin complexes.

107 Here, we show that the B3-type cyclin of Arabidopsis and its main CDK partner
108 CDKB1;1/CDKB1;2 control spindle morphogenesis. Remarkably, double *cdkb1;1*
109 *cdkb1;2* mutants displayed spindles with prominent astral microtubules reminiscent of
110 centrosome-derived microtubules observed in animal spindles. We identify EDE1, an
111 augmin complex member homologous to AUG8, as a substrate of the CDKB1;1-
112 CYCB3;1 complex. Moreover, we show that a non-phosphorylatable mutant form of
113 EDE1 results in aberrant spindle length, and this phenotype is also seen in *cycb3;1*
114 and *cdkb1;1 cdkb1;2* mutants. Similarly, reducing augmin concentration in a 3D model
115 of the spindle results in elongated spindles, supporting our inference of the role of cell
116 cycle-dependent phosphorylation of augmin in plant cells.

117

118 **Results**

119

120 **Generation of a computational 3D simulation of the spindle**

121 To understand the contribution of different molecular mechanisms to the organization
122 of the spindle, we generated a three-dimensional dynamic model of an Arabidopsis
123 root mitotic spindle using Cytosim that extends significantly over previous simulations
124 of the *Xenopus* spindle (Figure 1A–H and S1)^{18,19}. Microtubules were generated via
125 three different pathways: directly nucleated at the kinetochores, nucleated by augmin
126 on the side of pre-existing microtubules, and nucleated on the spindle-poles, resulting
127 in approximately 100, 500 and 500 microtubules in each pathway respectively. These

128 pathways shared a cellular pool of nucleator, and microtubule assembly was limited
129 by availability of tubulin in the cell. To simulate the spindle poles and anchor the
130 microtubule fibers, we introduced a condensate with particle properties governed by
131 Smoothed Particle Hydrodynamics. In addition to augmin, we included kinesin-5²⁰,
132 kinesin-14²¹, and katanin²² in our simulation. Kinesin-5 and kinesin-14 were added to
133 promote microtubule cross-linking and spindle bipolarity, by sliding microtubules apart
134 and together respectively. Notably, kinesin-5 was important to generate pulling forces
135 on the kinetochores. Katanin was added to the condensate poles to regulate spindle
136 length by severing. Dynein and NuMA were excluded from our simulation due to their
137 presumed absence in plants¹².

138 Several simplifications were made, considering our focus on investigating how
139 the general metaphase steady-state characteristics of the spindle are established.
140 Kinetochores were fixed in position, forming a metaphase plate. When possible,
141 spindle parameters were determined experimentally (Figure S2). First, we estimated
142 the number of spindle microtubules by analyzing Transmission electron microscopy
143 (TEM) images of cross-sections of Arabidopsis roots (Figure S2A and S2B). Second,
144 the number of kinetochore microtubules was estimated by measuring the fluorescence
145 intensity of kinetochore fibers stained against α -tubulin and by counting the number of
146 microtubules in bundles observed by TEM (Figure S2C–G). Third, the growth rate of
147 microtubules was measured by using a reporter fusion for the End-binding protein 1
148 (EB1b; Figure S2H–J)²³. A full list of the parameters used in the simulation is provided
149 in Table S1.

150 Our model produced organized spindles with focused poles and thick
151 microtubule bundles that were attached in a bipolar manner to kinetochores (Figure
152 1A and 1B). At high source rates of augmin, we were able to more closely reproduce
153 the appearance of the barrel-shaped plant spindles with few pole-nucleated
154 microtubules (Figure 7F).

155

156 **CYCLIN B3;1 controls spindle morphogenesis**

157 To complement our simulation approach, we sought for possible cell-cycle regulators
158 of the plant spindle. Since we have previously shown that mitotic B1-type cyclins are
159 key regulators of microtubule organization in Arabidopsis¹⁷, we decided to assess

160 spindle shape in roots of *cycb1;1 cycb1;2* double mutants (Figure 2A). This double
161 mutant combination has the strongest defects in growth and seed development among
162 the B1-type cyclin mutant combinations, while still being viable¹⁷. We measured three
163 spindle shape parameters, namely the lengths of major and minor axes, and the area
164 (Figure 2B–D). Unexpectedly, the *cycb1;1 cycb1;2* mutant did not display any
165 significant changes in spindle shape (Figure 2B–D and Table S2).

166 We therefore hypothesized that other B-type cyclins could be involved in
167 regulating spindle morphogenesis. The single member of the B3-type cyclin class in
168 Arabidopsis was a good candidate as it was previously described to localize to the
169 spindle in both mitosis and meiosis^{13,14}. Indeed, spindles in roots of the *cycb3;1* mutant
170 were more disc-shaped compared to the wild type (WT; Figure 2A) – the major axis
171 was elongated and the minor axis was smaller, whereas the spindle area did not
172 change significantly (Figure 2B–D and Table S2). Thus, we concluded that CYCB3;1
173 is a regulator of spindle morphology in Arabidopsis.

174

175 **CDKB1;1 is the main CDK partner of CYCB3;1 and the *cdkb1* mutant is** 176 **hypersensitive to microtubule-destabilizing stress**

177 To identify the main CDK partner(s) of CYCB3;1, as well as other potential interacting
178 proteins and substrates, we performed affinity purification coupled to mass
179 spectrometry (AP-MS) using CYCB3;1 as a bait in Arabidopsis cell suspension
180 cultures (Figure 3B and Table S3 and S4). Five proteins were identified as potential
181 interactors of CYCB3;1 (Figure 3B). None of them, however, were directly involved in
182 microtubule regulation. Enzyme-substrate interactions are known to be weak and,
183 hence, it is not surprising that we did not detect good substrate candidates in this
184 assay. The presence of CDKB1;1 among the potential interactors, however,
185 suggested that this kinase is the main partner of CYCB3;1. Consistently, CYCB3;1
186 was previously found to copurify with CDKB1;1 in tandem affinity purification
187 experiments²⁴.

188 CDKB1;1 was previously shown to play a role in controlling plant growth²⁵ and
189 stomatal cell divisions²⁶. CDKB1s are key regulators of DNA damage response in
190 Arabidopsis, e.g., in response to cisplatin, by activating homologous recombination
191 repair²⁷. CDKB1s have also been shown to play a minor and partially redundant role

192 with CDKA;1²⁸, and possibly other cell-cycle kinases during cell proliferation and
193 development of Arabidopsis. Because CDKB1;1 and CDKB1;2 have been found to
194 function in a highly redundant manner, and likely act in similar pathways²⁷, we
195 analyzed the double mutant for these two CDKs in the following experiments.

196 To assess a potential role of CDKB1s in spindle regulation and track their
197 localization in mitotic divisions, we first generated a CDKB1;1 reporter by fusing its
198 genomic sequence to GFP. We demonstrated the functionality of the CDKB1;1-GFP
199 reporter through its ability to rescue the root phenotype of *cdkb1* plants growing on a
200 medium with the DNA-damaging drug cisplatin (Figure S3). In the root, the CDKB1;1-
201 GFP reporter was found to be mainly present in the nucleus at the PPB stage, together
202 with a faint cytosolic signal (Figure 3A). Later in mitosis, CDKB1;1-GFP co-localized
203 with the spindle and phragmoplast microtubules (Figure 3A).

204 After confirming the localization of CDKB1;1 on mitotic microtubule arrays, we
205 decided to reassess the phenotype of the *cdkb1;1 cdkb1;2* double mutant (hereafter
206 referred to as *cdkb1*, Figure 3C–J). First, we analyzed root growth on oryzalin (Figure
207 3C). Oryzalin is a microtubule-destabilizing drug²⁹, and many microtubule-related
208 mutants are hypersensitive to this drug in comparison to the WT¹⁷. Under control
209 conditions, the *cdkb1* mutant roots were 20.3% shorter than the WT five days after
210 germination. Upon treatment with 150 nM oryzalin, *cdkb1* had a reduction of 38.5% in
211 root growth, whereas, in the WT, the observed reduction in root growth was only
212 marginally significant (Figure 3C). Thus, we concluded that the root growth phenotype
213 of *cdkb1* is enhanced under mild microtubule destabilization conditions, prompting the
214 hypothesis that CDKB1s could be involved in the control of mitotic microtubule arrays.

215

216 **The *cdkb1* mutant displays PPB and spindle defects**

217 To test the role of CDKB1s in controlling microtubule organization, we first performed
218 wholemount immunolocalization studies using antibodies against KNOLLE and α -
219 tubulin as well as co-staining with DAPI for the DNA and counted the different mitotic
220 stages (Figure 3D and 3F and Table S5). KNOLLE staining allows the identification of
221 G2/M cells where PPBs are normally present in the WT³⁰. First, we found that, in
222 *cdkb1*, 10.67% of KNOLLE-positive mitotic cells had no PPB, in comparison to only
223 1.01% in the WT (Figure 3E and 3F), indicating that *cdkb1* mutants have defects in

224 the establishment of the PPB. Next, we found that the *cdkb1* double mutant had a
225 higher frequency of mitotic cells at the spindle stage in their roots (23.21%) in
226 comparison to the WT (14.70%; Figure 3D).

227 We then wondered if the spindle shape of the *cdkb1* double mutants was also
228 altered. For this analysis, we measured the spindle shape as described above in
229 wholmount immuno-stained roots against α -tubulin and co-stained with DAPI (Figure
230 3G). Indeed, the spindles of *cdkb1* were significantly longer and larger in comparison
231 to the WT (Figure 3G–J and Table S2). Based on these findings, we concluded that
232 CDKB1;1 is a major regulator of mitotic microtubule arrays, particularly at the PPB and
233 spindle stages.

234

235 **The *cycb3;1* and *cdkb1* mutants have an abnormal spindle organization and** 236 **altered γ -tubulin distribution**

237 To further characterize why the spindle shape was altered in *cycb3;1* and *cdkb1*
238 mutants, we used super-resolution imaging with Airyscan (Figure 4). Spindles in *cdkb1*
239 appeared highly disorganized in comparison to the WT, which could explain why they
240 are bigger on average (Figure 3J and 4A). Furthermore, we noticed a striking number
241 of astral microtubules in *cdkb1* spindles, which are essentially absent from the WT
242 (Figure 4A–C). In the *cdkb1* mutant, around half of the spindles (11 out of 23 spindles)
243 had prominent, generally short astral microtubules. This prompted us to check for the
244 presence of astral microtubules in the *cycb3;1* mutant and, indeed, we also observed
245 such microtubule configurations, albeit at a non-statistically significant frequency (2
246 out of 23 spindles; Figure 4A–C). Nevertheless, these structures were never found in
247 the WT (n = 23).

248 Next, given the central function of γ -tubulin in spindle organization and
249 function³¹, we wondered if its distribution was affected in the *cycb3;1* and *cdkb1*
250 mutants. To that end, we performed immunostaining against α - and γ -tubulin in cells
251 of the root apical meristem of the *cycb3;1* and *cdkb1* mutants (Figure 4D–F). The
252 distribution of γ -tubulin, as expressed by the ratio of fluorescence peak distance
253 divided by spindle length, was affected in both *cycb3;1* and *cdkb1* mutants compared
254 to the WT (see material and methods; Figure 4F). Hence, we concluded that the

255 localization of γ -tubulin in both *cycb3;1* and *cdkb1* mutants was strongly biased
256 towards the spindle poles compared to the WT.

257

258 **EDE1 is a substrate of the CDKB1;1-CYCB3;1 complex and its phosphorylation**
259 **is important for its function**

260 The spindle elongation phenotype found in *cycb3;1* and *cdkb1* mutants was
261 reminiscent of the defects previously described in *ede1* mutants⁸. EDE1 is the
262 microtubule-binding component of the augmin complex in mitotic Arabidopsis cells.
263 Additionally, the EDE1 protein contains eight CDK phosphorylation consensus (S-T/P)
264 sites and was previously found to phosphorylated by human Cdk1 in *in vitro* assays³².
265 Hence, we tested if the CDKB1;1-CYCB3;1 complex could phosphorylate EDE1 *in*
266 *vitro*. We found that EDE1 was phosphorylated at several sites, including but not
267 limited to at least six of the eight CDK consensus phosphorylation sites (Figure 5A and
268 Table S6).

269 To address the localization of EDE1 in mitosis and assess the importance of its
270 phosphorylation, we first generated a genomic EDE1 reporter (GFP-EDE1). We also
271 mutated eight CDK phosphosites (seven of them identified *in vitro*) into either an
272 alanine (GFP-EDE1^{8A}), which blocks phosphorylation, or an aspartate (GFP-EDE1^{8D}),
273 which mimics a phosphorylated amino acid (Figure 5A). We introduced the WT and
274 mutated versions in the *ede1-1* mutant background (hereafter referred to as
275 *ede1/GFP-EDE1*, *ede1/GFP-EDE1^{8A}* and *ede1/GFP-EDE1^{8D}*). The *ede1/GFP-*
276 *EDE1^{8A}* plants had a fully rescued seed phenotype (Figure S4A and S4B). However,
277 we found that their root growth was hypersensitive to oryzalin, similarly to the *ede1-1*
278 mutant, whereas *ede1/GFP-EDE1* plants grew similarly to the WT (Figure 5B). When
279 we measured the timing from nuclear envelope breakdown (NEB) to anaphase onset
280 (AO) with or without 150 nM oryzalin in *ede1/GFP-EDE1* plants, we did not find a
281 significant change (Figure 6A and 6B). In contrast, in *ede1/GFP-EDE1^{8A}* plants, the
282 NEB to AO duration was significantly longer in oryzalin-treated plants (Figure 6A and
283 6B). This showed that the functionality of the non-phosphorylatable GFP-EDE1^{8A}
284 protein was affected, especially under stress conditions.

285 To further characterize mitotic defects in *ede1-1* plants rescued by the different
286 GFP-EDE1 versions, we measured the frequency of PPB, spindle and phragmoplast

287 stages in root apical meristems (Figure 5C and Table S5). Similar to *cdkb1* mutants,
288 *ede1/GFP-EDE1^{8A}* had a significant overrepresentation of spindle stages in mitotic
289 cells (20.05% of total mitotic figures versus 14.99% in *ede1/GFP-EDE1*). We found
290 that *ede1/GFP-EDE1^{8A}* plants displayed deformed spindles highly reminiscent of
291 *cycb3;1* (Figure 5D–G). Their major axis was larger and their minor axis was smaller
292 in comparison to *ede1/GFP-EDE1*, whereas the spindle area did not change
293 significantly (Table S2). Conversely, *ede1/GFP-EDE1^{8D}* did not have a significant
294 change in the major axis or spindle area compared to *ede1/GFP-EDE1*, but still had a
295 significantly smaller minor axis, albeit not as reduced as in *ede1/GFP-EDE1^{8A}* (Table
296 S2). We concluded that EDE1 phosphorylation has an impact on spindle architecture
297 under control conditions, and becomes even more critical when microtubules are
298 destabilized.

299 Based on the striking similarity between the phenotypes of *cycb3;1* and
300 *ede1/GFP-EDE1^{8A}*, we hypothesized that EDE1 is a major substrate of CDK-cyclin
301 complexes involving CYCB3;1. To test this hypothesis, we made crosses of *cycb3;1*
302 with *ede1-1* mutants. Indeed, spindle defects in *cycb3;1 ede1-1* double mutants were
303 identical to the single *ede1-1* mutant (Figure S5A–D). We thus concluded that EDE1
304 is the main substrate of CYCB3;1 action, whereas the *cdkb1* mutant phenotype is
305 possibly more pleiotropic and a result of alterations in different CDK-cyclin
306 phosphorylation pathways.

307

308 **EDE1 phosphorylation is important for its localization at the spindle**

309 Since the human homolog of EDE1 has been suggested to stabilize microtubules³³,
310 we wondered if *ede1/GFP-EDE1^{8A}* plants had impaired tubulin turnover³⁴, which
311 results from the combination of many microtubule activities including dynamic
312 instability and could contribute to the above-described phenotypes. To test that, we
313 performed a FRAP assay of microtubules tagged with TagRFP-TUA5 in the
314 *ede1/GFP-EDE1* or *ede1/GFP-EDE1^{8A}* backgrounds and observed their recovery
315 over time (Figure S4C–E). However, we did not find a significant difference in the half
316 maximum values between the two genotypes and fluorescence recovered at similar
317 rates in both cases. Thus, we concluded that tubulin turnover did not change
318 significantly in *ede1/GFP-EDE1^{8A}* plants in comparison to *ede1/GFP-EDE1*.

319 As EDE1 is known to recruit the γ TuRC to spindle microtubules, and given the
320 biased distribution of γ -tubulin in the *cycb3;1* and *cdkb1* mutants, we assessed the
321 localization of the mutated forms of GFP-EDE1 at the spindle in the *ede1-1*
322 background (Figure 5D, 5H and 5I). Indeed, the distribution of GFP-EDE1^{8A} was
323 significantly biased towards the spindle poles in comparison to GFP-EDE1, as
324 expressed by the ratio of peak distance divided by spindle length, whereas the GFP-
325 EDE1^{8D} version did not show a significant difference in localization in comparison to
326 GFP-EDE1 (Figure 5H and 5I). In addition, we found that spindles of *ede1*/GFP-
327 EDE1^{8A} plants also displayed prominent astral microtubules in 2 out of 22 cases
328 (Figure 6C–E), reminiscent of the *cycb3;1* mutant (2 out of 23 spindles). Spindles of
329 *ede1*/GFP-EDE1^{8D} plants also displayed astral microtubules, although at a lower
330 frequency (1 out of 21 spindles). Though the differences were not statistically
331 significant regarding the proportion of spindles displaying astral microtubules in
332 *ede1*/GFP-EDE1^{8A} or *ede1*/GFP-EDE1^{8D} in comparison to *ede1*/GFP-EDE1 (Figure
333 6E), we have shown above that these structures were never found in wild-type
334 spindles (n = 23) and, accordingly, no prominent astral microtubules were found in
335 *ede1*/GFP-EDE1 (n = 12).

336 Since the binding of HAUS8 to microtubules is enhanced upon phosphorylation
337 by Plk1¹⁰, we wondered if the phosphorylation of EDE1 also affected its association
338 with microtubules. We thus performed FRAP assays in spindles of *ede1*/GFP-EDE1,
339 *ede1*/GFP-EDE1^{8A} and *ede1*/GFP-EDE1^{8D} root cells (Figure 6F–H). The half
340 maximum of GFP-EDE1^{8A} was on average 22.96 s \pm 10.42, significantly longer than
341 GFP-EDE1 (12.04 s \pm 6.72). GFP-EDE1^{8D} had an average half maximum of 17.79 s \pm
342 8.78, further confirming that it functions more similarly to GFP-EDE1 than the GFP-
343 EDE1^{8A} version, although this was still a significantly slower recovery compared to
344 GFP-EDE1. Therefore, we concluded that the phosphorylation of EDE1 is important
345 for its association with spindle microtubules and is significantly blocked in the GFP-
346 EDE1^{8A} protein.

347

348 **Altering the amount of augmin in the simulation affects spindle length and**
349 **organization**

350 To validate the role of augmin in overall spindle organization, and considering our
351 experimental observations, we manipulated the amount of augmin in our simulations
352 (Figure 7A–I, n = 132 simulations of 2000 s). As the augmin source rate increased
353 (while all other parameters were constant), spindle length decreased – quickly at first
354 and then slowly (Figure 7A). In the range of augmin source rates we tested, the spindle
355 length decreased by about 50%. With increasing augmin source rates, the average
356 length of each kind of microtubule decreased, with kinetochore microtubules (which
357 are the longest, presumably because their plus ends are stabilized at kinetochores)
358 experiencing the largest percentage decrease (approximately 30%; Figure 7B). The
359 number of augmin-nucleated microtubules increased from zero to more than 1000 and
360 the number of pole-nucleated microtubules decreased from around 500 to 400, while
361 the number of kinetochore-nucleated microtubules remained approximately constant,
362 as expected (Figure 7C). We also tested the effect of varying the augmin binding and
363 nucleation rates as well as the diffusion coefficient on the spindle organization (Figure
364 S6). Increasing binding and nucleation rates (Figure S6A–L) resulted in similar effects
365 to increasing source rate. Increasing the diffusion coefficient (Figure S6M–R),
366 however, had the opposite effect on spindle length. Indeed, the increased mobility,
367 which occurs in random directions, is expected to diminish the chance of augmin to
368 encounter and bind microtubules, effectively decreasing the amplification activity.

369

370 **Discussion**

371 In this work, we have combined computer simulations with experimental approaches
372 to advance our knowledge of spindle formation in plants. We have identified CDKB1
373 in conjunction with CYCB3;1 as a major regulator of the Arabidopsis mitotic spindle.
374 Until now, little was known in plants about how cell cycle regulators control spindle
375 formation. Based on their role in microtubule organization¹⁷, we had initially expected
376 that B1-type cyclins together with their CDK partners, mostly CDKB2s, would be
377 involved in the regulation of spindle shape and organization. However, no obvious
378 spindle defects were found in the most severe mutant combination *cycb1;1 cycb1;2*.
379 Although we cannot rule out that other members of the B1 class participate in spindle
380 architecture, CYCB1s seem mostly involved in other aspects of chromosome
381 segregation, like connection of spindle microtubules to kinetochores¹⁷. Accordingly,

382 the B1-type cyclin from humans binds to and supports the localization of a member of
383 the spindle assembly checkpoint (SAC), MAD1, at the kinetochore³⁵. With respect to
384 plant B1-type cyclins, it will be interesting to explore whether they have a similar role
385 in regulating kinetochore proteins and/or the SAC, especially given that the core SAC
386 machinery appears to be functionally-conserved in *Arabidopsis* albeit in an adapted
387 manner^{36,37}.

388 CDK-cyclin complexes have been previously implicated in the direct control of
389 spindle morphogenesis in other organisms. For instance, the Cdk1-cyclin B1 complex
390 from humans is known to phosphorylate importin- α 1 to inhibit its function, and release
391 spindle assembly factors, such as TPX2, to promote spindle formation³⁸. Furthermore,
392 mutations in the budding yeast CDK1 (*Cdc28*) as well as simultaneous depletion of all
393 budding yeast B-type cyclins also result in abnormal spindle assembly, which mirrors
394 our findings with *CYCB3;1* in *Arabidopsis*. More specifically, budding yeast cells
395 impaired in *Cdc28*/B-cyclin function have duplicated spindle pole bodies (SPBs) that
396 fail to separate³⁹. The *Cdc28*/B-cyclin complex specifically phosphorylates yeast
397 kinesins-5 Kip1 and Cin8, and this phosphorylation plays a role in promoting SPB
398 separation and spindle assembly⁴⁰. Although plants lack a discernable MTOC at the
399 spindle stage like SPBs or centrosomes, here we found that γ -tubulin (a major
400 component of SPBs and centrosomes) distribution is likewise impaired in *cycb3;1* and
401 *cdkb1* mutants. In *Arabidopsis*, no less than 23 kinesins are expressed in mitosis,
402 among which many have potential CDK phosphosites⁴¹. Whether B-type cyclins are
403 involved in the phospho-control of such mitotic kinesins and help establish spindle
404 bipolarity in plant cells remains to be seen.

405

406 **The role of CDKB1 and CYCB3;1 in spindle organization**

407 Here, we found that the *CYCB3;1*-*CDKB1* complex is involved in spindle
408 morphogenesis, at least partly through phospho-regulation of the augmin complex
409 member EDE1. Interestingly, tubulin turnover does not seem to be affected in the non-
410 phosphorylatable version of EDE1 we analyzed; hence, we propose that the elongated
411 spindle phenotype we observed is mostly due to an altered frequency and/or pattern
412 of microtubule-dependent microtubule nucleation within the spindle. If tubulin
413 availability in a cell limits spindle length, spindles can become longer when augmin

414 function is affected because the amount of free tubulin increases, as does the
415 polymerization speed of the remaining spindle microtubules. Indeed, in our
416 simulations, spindles became shorter in response to increasing augmin source rate.
417 As the augmin source rate increased from 0.065 /s to 8.6 /s, the amount of free tubulin
418 (measured as the microtubule length equivalent) decreased from 3300 μm to 2500
419 μm , which means that the microtubule growth speed decreased by about a third
420 relatively to its base speed. Consequently, kinetochore microtubules became shorter,
421 contributing to shortening the spindle (Figure 7G–I). Furthermore, pole-nucleated
422 microtubules were longer and more abundant with lower levels of augmin in our
423 simulations, fitting our observation of a higher and more prominent number of astral
424 microtubules in *cdkb1* mutants. Perhaps parallel nucleation and other augmin-
425 independent nucleation pathways become more common in the mutants we studied,
426 further contributing to the change in spindle shape we observed as previously
427 suggested for the *ede1-1* mutant⁸. Additionally, since the augmin complex nucleates
428 microtubules that generally preserve the polarity of their mother microtubules⁶, the
429 astral microtubules in *cycb3;1* and *cdkb1* represent further evidence of a deficient
430 augmin activity.

431 Why do *cycb3;1* and *cdkb1* mutants display spindles with an altered distribution
432 of γ -tubulin, biased towards the poles? At the prophase stage, the pro-spindle is
433 present normally as two polar caps rich in γ -tubulin at the nuclear envelope⁴², and this
434 structure seems unperturbed in the analyzed mutants. Following NEB, augmin has
435 been shown to critically bind to and amplify the number of microtubules to assist
436 spindle formation¹². Augmin likely translocates γ -tubulin from the spindle poles (which
437 form from remnants of the polar caps following NEB) towards spindle microtubules in
438 the midzone. In the *cycb3;1* and *cdkb1* mutants, however, a faulty augmin-mediated
439 redistribution of γ -tubulin upon NEB likely results in the accumulation of γ -tubulin at
440 the spindle poles.

441 Since the spindle defects seen in *cdkb1* double mutants are stronger than in
442 *cycb3;1* mutants, it seems probable that CDKB1s operate with other cyclins to control
443 spindle morphology. CDKB1s may also be involved in the establishment of the cortical
444 division site, since we often observed cells without a PPB in *cdkb1* mutants (Figure
445 3E and 3F), although we did not examine this further.

446

447 **Robust sister chromatid separation by highly disorganized spindles**

448 It is surprising that the spindles of *cdkb1* mutants, albeit highly disorganized, did not
449 impair chromosome segregation, but rather affected the duration of spindle stages. It
450 is likely that the action of the SAC ensured proper spindle-kinetochore attachments
451 and bipartite sister chromatid segregation by delaying anaphase onset^{36,43}.

452 A recent study in human spindles made use of a co-depletion of both the SAC
453 factor Mad2 and HAUS6 to circumvent extensive mitotic divisions and study the effect
454 of depleting augmin on chromosome segregation without the surveillance mechanism
455 mediated by the SAC⁴⁴. An interesting experiment for the future would be to combine
456 mutations in SAC components with either the *cycb3;1* or *cdkb1* mutants to investigate
457 how severely chromosome segregation is disrupted when both augmin and the SAC
458 are impaired in plants.

459

460 **Basic molecular mechanisms guiding spindle organization**

461 Here, we have modelled a spindle in three dimensions with increased realism in
462 comparison to previous work and including new factors such as augmin and kinesin-
463 14^{19,45}. Whereas a quantitatively accurate model of the *Xenopus* spindle has not yet
464 been achieved due to its size, the smaller size of the Arabidopsis spindle meant it was
465 possible to simulate all of its microtubules within a reasonable computational time.
466 Modelling an Arabidopsis mitotic spindle in particular was interesting because it has
467 an intermediate size that is ideal for simulations when compared to smaller fission
468 yeast or larger *Xenopus laevis* spindles and because plant spindles lack key molecular
469 players seen in animals. For instance, there is only limited evidence of a NuMA
470 homolog in plants^{12,46} and, hence, the pole organization in our simulation differs from
471 the NuMA-organized spindle poles that have previously been employed^{19,45}. Plants
472 also lack the molecular motor dynein, which was also not included in our simulation,
473 but possess an astonishing number of kinesins, including several expressed in
474 mitosis, that likely take over some of dynein's functions⁴⁷.

475 With this work, we shed light on molecular mechanisms governing spindle
476 organization in plants that are likely relevant for other eukaryotic groups as well. Our
477 simulation will serve as a foundation for understanding spindle organization in other

478 species, thus advancing our knowledge of how cells ensure a robustly-functioning
479 spindle structure to separate their chromosomes in cell divisions and thereby
480 proliferate.

481

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483

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493

494 **Author contributions**

495

496 M.R.M. and A.S. conceived the experiments. M.R.M., P.C. and L.B. performed
497 experiments and statistical analyses. F.N., H.S. and C.J. constructed the spindle
498 simulations and analyzed them. E.W. fixed and processed plant samples for TEM and
499 performed TEM imaging. M.P., K.B. and D.B. performed the wholemount
500 immunolocalization of α -tubulin and KNOLLE and corresponding statistical analyses.
501 S.C.S. and H.N. performed the mass spectrometry experiment and data analysis of
502 the *in vitro* kinase assays. E.V.D.S. and G.D.J. performed the AP-MS of CYCB3;1 and
503 corresponding statistical analyses. M.R.M. and A.S. analyzed the data. M.R.M. and
504 A.S wrote the manuscript.

505

506 **Declaration of interests**

507

508 The authors declare no competing interests.

509

510 **Figure legends**

511

512 **Figure 1. Tridimensional simulation of the Arabidopsis root mitotic spindle**

513 (A–B) Snapshots of the simulations performed in Cytosim showing a side (A) and an
514 end-on (B) view of the spindle. Microtubules are here color-coded according to the
515 pathway of nucleation: blue if nucleated by the poles, black if nucleated by
516 kinetochores, and yellow if nucleated by the augmin pathway. Chromosomes were not
517 included in the model for simplicity, but 20 kinetochores were fixed in position such as
518 to form a well-aligned metaphase plate. For more details of the model, see figure S1
519 and supplementary material.

520 (C–H) Distribution of key elements of the simulated spindle. (C) Microtubule plus ends
521 (red and green). (D) Kinesin-5 (yellow). (E) Augmin-activated nucleators (green). (F)
522 Microtubule minus ends. (G) Kinesin-14 (pink). (H) Katanin (red).

523

524 **Figure 2. The *cycb3;1* mutant has an elongated spindle shape**

525 (A) Confocal laser-scanning micrographs of TagRFP-TUA5-tagged microtubules in
526 root cells at the spindle stage of WT, *cycb1;1 cycb1;2* and *cycb3;1* plants. Scale bar 5
527 μm .

528 (B–D) Quantification of the spindle major axis (B), minor axis (C) and area (D) in root
529 cells of WT ($n = 22$), *cycb1;1 cycb1;2* ($n = 21$) and *cycb3;1* ($n = 21$) plants. Median
530 values were plotted as a line for each genotype. The axis or region that was measured
531 is indicated below each graph.

532 The level of significance was determined by an ordinary one-way ANOVA followed by
533 Dunnett's multiple comparisons test (* $P < 0.05$ and ** $P < 0.01$; ns depicts a non-
534 significant difference).

535

536 **Figure 3. The *cdkb1* mutations affect PPB and spindle mitotic microtubule** 537 **arrays**

538 (A) Confocal laser-scanning micrographs of root cells of plants containing the
539 TagRFP-TUA5 and CDKB1;1-GFP reporters at the three main mitotic stages (PPB,
540 spindle and phragmoplast). The two reporters show a co-localization in the spindle
541 and phragmoplast stages. Scale bar 5 μm .

542 (B) Main protein interactors of CYCB3;1 as identified by AP-MS using CYCB3;1 as a
543 bait. CDKB1;1 is highlighted in green, while other interactors that were not explored in
544 this paper are shown in gray.

545 (C) Quantification of root growth assays of WT and *cdkb1* seedlings on the control
546 condition (DMSO) or 150 nM oryzalin. DAG: days after germination. Bars represent
547 the mean value \pm SD of three independent experiments with at least 16 plants per
548 genotype per condition in each experiment. Comparisons on graph: WT control versus
549 WT on oryzalin, $P = 0.0843$; WT control versus *cdkb1* control, $P = 0.0211$; *cdkb1*
550 control versus *cdkb1* on oryzalin, $P = 0.0019$.

551 (D) Quantification of PPB, spindle and phragmoplast stages in the roots of WT,
552 *cycb3;1* and *cdkb1* plants. Different letters indicate significant differences in the
553 proportion of the microtubule array per category in a Chi-squared test followed by the
554 Marascuilo procedure to identify significant pairwise comparisons. Six roots were
555 analyzed per genotype.

556 (E) Confocal laser-scanning micrographs of cells co-stained against α -tubulin
557 (magenta) and KNOLLE (green) in the roots of WT and *cdkb1* plants. Nuclei were
558 counterstained with DAPI for the DNA (cyan). At this stage, the WT shows a clear
559 accumulation of KNOLLE and a PPB, whereas the *cdkb1* mutant shows an
560 accumulation of KNOLLE but no obvious PPB. Scale bar 5 μ m.

561 (F) Quantification of the different PPB types in the roots of WT, *cycb3;1* and *cdkb1*
562 plants. Different letters indicate significant differences in the proportion of the PPB
563 type per category in a Chi-squared test followed by the Marascuilo procedure to
564 identify significant pairwise comparisons. Six roots were analyzed per genotype.

565 (G) Confocal laser-scanning micrographs of roots cells of WT and *cdkb1* plants at the
566 spindle stage stained against α -tubulin (magenta) and counterstained for the DNA with
567 DAPI (cyan). Scale bar 5 μ m.

568 (H–J) Quantification of the spindle major axis (F), minor axis (G) and area (H) in the
569 root cells of WT and *cdkb1* plants ($n = 23$ for both genotypes). Median values were
570 plotted as a line for each genotype.

571 The level of significance was determined by a two-way ANOVA followed by Tukey's
572 multiple comparisons test in (C) and unpaired t tests in (H–J) (* $P < 0.05$, ** $P < 0.01$,
573 **** $P < 0.0001$; ns depicts a non-significant difference).

574

575 **Figure 4. The *cycb3;1* and *cdkb1* mutants have spindles with prominent astral**
576 **microtubules**

577 (A) Maximum intensity projections of confocal laser-scanning micrographs of root cells
578 of WT, *cycb3;1* and *cdkb1* plants at the spindle stage stained against α -tubulin
579 (magenta) and counterstained for the DNA with DAPI (cyan). The astral microtubules
580 are highlighted with dashed white boxes. Scale bar 5 μ m.

581 (B) Close-ups of the images shown in (A) depicting astral microtubules in the spindles
582 of *cycb3;1* and *cdkb1* root cells stained against α -tubulin (magenta) and
583 counterstained for the DNA with DAPI (cyan). Scale bar 0.5 μ m.

584 (C) Quantification of the number of spindles with or without prominent astral
585 microtubules in the root cells of WT, *cycb3;1* and *cdkb1* plants (n = 23 for all
586 genotypes).

587 (D) Confocal laser-scanning micrographs of root cells of WT, *cycb3;1* and *cdkb1* plants
588 at the spindle stage co-stained against α -tubulin (magenta) and γ -tubulin (orange).
589 The white dashed line indicates the axis that was used to measure fluorescence
590 intensity and was further plotted in the graph in (E). Scale bar 5 μ m.

591 (E) Quantification of the fluorescence intensity of γ -tubulin across the spindle axis
592 indicated in (D) in WT (n = 23), *cycb3;1* (n = 23) and *cdkb1* (n = 22) root cells.

593 (F) Quantification of the ratio of the distance between the fluorescence peaks seen in
594 (E) divided by the spindle length value in WT (mean \pm SD; 0.65 ± 0.12 , n = 23), *cycb3;1*
595 (0.83 ± 0.10 , n = 23) and *cdkb1* (0.85 ± 0.04 , n = 22) root cells. The median values
596 were plotted as a line for each genotype. See methods for detail.

597 The level of significance was determined by a two-proportion z-test followed by
598 Bonferroni correction in (C) and an ordinary one-way ANOVA followed by Tukey's
599 multiple comparisons test in (F) (** P < 0.001, **** P < 0.0001; ns depicts a non-
600 significant difference).

601

602 **Figure 5. EDE1 is a substrate of the CDKB1;1-CYCB3;1 complex and its**
603 **phosphorylation is important for its function**

604 (A) Representation of the protein sequence of EDE1. All the eight mutated amino acids
605 in the GFP-EDE1^{8A} and GFP-EDE1^{8D} constructs are represented alongside their

606 amino acid position in the protein. Amino acids represented in black were found to be
607 phosphorylated in the *in vitro* kinase assay with the CDKB1;1-CYCB3;1 complex,
608 whereas the amino acid in gray (S214) was not identified in the *in vitro* kinase assay.

609 (B) Quantification of root growth assays of WT and *ede1-1* seedlings as well as *ede1-*
610 *1* mutants rescued by GFP-EDE1 or GFP-EDE1^{8A} on the control condition (DMSO) or
611 150 nM oryzalin. Growth on the control (mean \pm SD): WT 1.08 cm \pm 0.27; *ede1-1* 0.93
612 cm \pm 0.20; *ede1*/GFP-EDE1 1.00 cm \pm 0.35; and *ede1*/GFP-EDE1^{8A} 0.97 cm \pm 0.30.
613 Growth on oryzalin (mean \pm SD): WT 0.96 cm \pm 0.17; *ede1-1* 0.49 cm \pm 0.23;
614 *ede1*/GFP-EDE1 0.88 cm \pm 0.31; and *ede1*/GFP-EDE1^{8A} 0.52 cm \pm 0.24. DAG: days
615 after germination. Bars represent the mean \pm SD (n = 12–24). Two other rescue lines
616 in the *ede1-1* background were tested for both the GFP-EDE1 and GFP-EDE1^{8A}
617 constructs with similar results.

618 (C) Quantification of PPB, spindle and phragmoplast stages in the roots of *ede-1*
619 mutants rescued by GFP-EDE1, GFP-EDE1^{8A} or GFP-EDE1^{8D}. Different letters
620 indicate significant differences in the proportion of the microtubule array per category
621 in a Chi-squared test followed by the Marascuilo procedure to identify significant
622 pairwise comparisons. Seven roots were analyzed per genotype.

623 (D) Confocal laser-scanning micrographs of GFP-EDE1-tagged microtubules in root
624 cells at the spindle stage of *ede1-1* mutants rescued by GFP-EDE1, GFP-EDE1^{8A} or
625 GFP-EDE1^{8D}. Scale bar 5 μ m.

626 (E–G) Quantification of the spindle major axis (E), minor axis (F) and area (G) in the
627 root cells of *ede1-1* mutants rescued by GFP-EDE1 (n = 20), GFP-EDE1^{8A} (n = 21) or
628 GFP-EDE1^{8D} (n = 20). Median values were plotted as a line for each genotype.

629 (H) Quantification of the fluorescence intensity of GFP-EDE1 across the spindle axis
630 indicated in (D) in root cells of *ede1-1* mutants rescued by GFP-EDE1 (n = 20), GFP-
631 EDE1^{8A} (n = 21) or GFP-EDE1^{8D} (n = 20).

632 (I) Quantification of the ratio of the distance between the fluorescence peaks seen in
633 (H) divided by the spindle length value in root cells of *ede1-1* mutants rescued by GFP-
634 EDE1 (n = 20), GFP-EDE1^{8A} (n = 21) and GFP-EDE1^{8D} (n = 20). The median values
635 were plotted as a line for each genotype. Comparisons on graph: GFP-EDE1 versus
636 GFP-EDE1^{8A}, $P = 0.0048$; GFP-EDE1 versus GFP-EDE1^{8D}, $P = 0.0610$. See methods
637 for detail.

638 The level of significance was determined by a two-way ANOVA followed by Tukey's
639 multiple comparisons test in (B) and one-way ANOVAs followed by Tukey's multiple
640 comparisons tests in (E–G) and (I) (* $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$; ns depicts
641 a non-significant difference).

642

643 **Figure 6. The phosphorylation of EDE1 is important for its localization at spindle**
644 **microtubules**

645 (A) Confocal laser-scanning micrographs of GFP-EDE1- and TagRFP-TUA5-tagged
646 microtubules in root cells of *ede1-1* mutants rescued by GFP-EDE1 and GFP-EDE1^{8A}.
647 Mitotic cells were followed from nuclear envelope breakdown (NEB) through the
648 anaphase onset stage (AO) to cytokinesis. The timepoint is indicated on the top right
649 of the images in seconds. Scale bar 5 μm .

650 (B) Quantification of the length of the NEB to AO stage in root cells of *ede1-1* mutants
651 rescued by GFP-EDE1 and GFP-EDE1^{8A} on the control (mean \pm SD; 617.6 s \pm 104.0
652 for GFP-EDE1 and 588.3 s \pm 98.8 for GFP-EDE1^{8A}, n = 17–18) or 150 nM oryzalin
653 condition (681.2 s \pm 102.7 for GFP-EDE1 and 765.0 s \pm 180.7 for GFP-EDE1^{8A}, n =
654 17–18). The median values were plotted as a line for each genotype and condition.
655 Comparisons on graph: GFP-EDE1 control versus GFP-EDE1 on oryzalin, $P = 0.4673$;
656 GFP-EDE1^{8A} control versus GFP-EDE1^{8A} on oryzalin, $P = 0.0005$.

657 (C) Maximum intensity projections of confocal laser-scanning micrographs of root cells
658 of *ede1-1* mutants rescued by GFP-EDE1, GFP-EDE1^{8A} and GFP-EDE1^{8D} at the
659 spindle stage stained against α -tubulin (magenta) and counterstained for the DNA with
660 DAPI (cyan). The astral microtubules are highlighted with dashed white boxes. Scale
661 bar 5 μm .

662 (D) Close-ups of the images shown in (C) depicting astral microtubules in the spindles
663 of *ede1-1* mutant root cells rescued by GFP-EDE1^{8A} and GFP-EDE1^{8D} and stained
664 against α -tubulin (magenta) and counterstained for the DNA with DAPI (cyan). Scale
665 bar 0.5 μm .

666 (E) Quantification of spindles with or without prominent astral microtubules in the root
667 cells of *ede1-1* mutants rescued by GFP-EDE1 (n = 12), GFP-EDE1^{8A} (n = 22) and
668 GFP-EDE1^{8D} (n = 21).

669 (F) Confocal laser-scanning micrographs of root cells in which the FRAP assay of
670 spindles tagged by GFP-EDE1, GFP-EDE1^{8A} or GFP-EDE1^{8D} in the *ede1-1*
671 background was performed. The white dashed box represents the area that was
672 bleached by the laser. The time is indicated on the top right of the images in seconds.
673 Scale bar 5 μm .

674 (G) Quantification of the fluorescence intensity recovery over time following bleaching
675 of spindles in root cells tagged by GFP-EDE1 ($n = 31$), GFP-EDE1^{8A} ($n = 28$) or GFP-
676 EDE1^{8D} ($n = 24$) in the *ede1-1* background. The fluorescence intensity was normalized
677 in each cell by the maximum and minimum values and plotted as an average (line) \pm
678 SD (shaded area).

679 (H) Quantification of the half maximum values in seconds of fluorescence recovery in
680 *ede1-1* mutants rescued by GFP-EDE1 ($n = 31$), GFP-EDE1^{8A} ($n = 28$) or GFP-
681 EDE1^{8D} ($n = 24$). The median values were plotted as a line for each genotype.
682 Comparisons on graph: GFP-EDE1 versus GFP-EDE1^{8A}, $P < 0.0001$; GFP-EDE1
683 versus GFP-EDE1^{8D}, $P = 0.0452$.

684 The level of significance was determined by ordinary one-way ANOVAs followed by
685 Tukey's multiple comparisons tests in (B) and (H) and a two-proportion z-test followed
686 by Bonferroni correction in (E) (* $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$; ns depicts a
687 non-significant difference).

688

689 **Figure 7. The amount of augmin controls spindle length and organization in the**
690 **simulation**

691 (A–C) Some key spindle properties as a function of the augmin source rate ($/s$). All
692 temporal means are taken over the last half of the simulation, $500s < t < 1000s$.

693 (A) The mean spindle length (μm) decreases with augmin source rate ($/s$). The spindle
694 length is measured as the distance between the center-of-masses of the left and right
695 groups of condensates. The green, yellow, and blue diamonds indicate the three
696 examples shown in (D).

697 (B) Mean lengths (μm) of each group of fibers nucleated at kinetochores (green
698 circles), by augmin (yellow triangles), and at poles (purple squares).

699 (C) Mean number of fibers of each type.

700 (D–F) Visualization of simulated spindles at the final time $t = 1000s$, for augmin source
701 rates as indicated. Kinetochores microtubules are black, augmin-nucleated
702 microtubules are orange, and pole-nucleated microtubules are blue. Kinetochores are
703 variously-colored spheres near the metaphase plate.

704

705 (G–I) Relationships between kinetochore-fiber (k-fiber) properties and the spindle
706 length (μm), with data points colored according to the augmin source rate ($/s$). All
707 quantities are means over the last half of the simulation, $1000s < t < 2000s$, and k-
708 fiber quantities are averaged over all k-fibers. (G) Mean k-fiber length (μm), (H) mean
709 growth (μm) at k-fiber plus ends, and (I) mean severing (μm) at k-fiber minus ends.

710

711 **Figure S1. Essential elements of Cytosim**

712 (A) Microtubules exhibit dynamic instability. They are discretized into points connected
713 by inextensible segments such that microtubules can bend but do not stretch. Points
714 are subjected to forces from bending elasticity, steric interactions, and crosslinking
715 motors (if present).

716 (B) Motors consist of two motor entities. Each motor entity can bind, unbind, and move
717 along a microtubule. Crosslinking motors exert forces on the microtubules they
718 connect via a Hookean spring-like link.

719 (C) Steric interactions are calculated for each model point of a microtubule. For
720 example, a line from P, a point on the blue microtubule, is projected onto the nearby
721 segment of the green microtubule at Q. The line PQ is orthogonal to the green
722 microtubule. An equal and opposite force is applied to the green and blue microtubule
723 along PQ such that the steric forces acting on a pair of microtubules are symmetric
724 and sum to zero.

725

726 **Figure S2. Experimentally-determined spindle parameters**

727 (A) A cross section of a root spindle imaged by TEM. The area which was used to
728 count microtubules is indicated with a box. Scale bar $0.5 \mu m$.

729 (B) Close-up corresponding to $1 \mu m^2$ indicated in (A).

730 (C) A cross section of a root spindle imaged by TEM. Bundles with different
731 microtubule numbers are indicated with colored boxes. Scale bar $0.5 \mu m$.

732 (D–F) Close-ups of microtubule bundles observed in (C). (D) A bundle of two
733 microtubules. (E) A bundle of four microtubules. (F) A bundle of six microtubules.
734 (G) Quantification of the number of microtubules in kinetochore-fibers measured from
735 confocal microscopy images of root spindles stained against α -tubulin ($n = 12$
736 kinetochore-fibers from four different cells). The plotted line indicates the median.
737 (H) A root spindle of a plant expressing *PRO_{EB1b}:EB1b:GFP*. The line indicates the
738 axis from which the kymograph in (I) was plotted. Scale bar 5 μ m.
739 (I) A kymograph generated from the line in (H).
740 (J) Quantification of the microtubule growth speed from three independent spindles (n
741 = 10 microtubules per spindle). The plotted line indicates the median.

742

743 **Figure S3. The CDKB1;1-GFP reporter largely rescues the root growth of *cdkb1***
744 **with and without cisplatin**

745 (A) Pictures of seedlings of WT, *cdkb1* and *cdkb1* rescued by CDKB1;1-GFP grown
746 on ½ MS (control, top) or cisplatin (bottom) for five days. Scale bar 1 cm.

747 (B) Quantification of root growth of WT, *cdkb1* and *cdkb1* rescued by CDKB1;1-GFP
748 grown on ½ MS (control) or cisplatin for five days. Three replicates were performed
749 with 10 plants per genotype per condition. Graph indicates mean \pm SD of the three
750 replicate average values.

751 The level of significance was determined by a two-way ANOVA followed by Tukey's
752 multiple comparisons test (* $P < 0.05$ and **** $P < 0.0001$; ns depicts a non-significant
753 difference).

754

755 **Figure S4. The GFP-EDE1^{8A} construct fully rescues the seed abortion of the**
756 ***ede1-1* mutant and does not significantly affect the microtubule dynamic**
757 **instability at the spindle**

758 (A) Pictures of the seeds from WT and *ede1-1* as well as *ede1-1* mutants rescued by
759 GFP-EDE1 and GFP-EDE1^{8A}. Scale bar 200 μ m.

760 (B) Quantification of the seed abortion of the seeds depicted in (A) in WT ($n = 158$)
761 and *ede1-1* ($n = 190$) as well as *ede1-1* mutants rescued by GFP-EDE1 ($n = 189$) and
762 GFP-EDE1^{8A} ($n = 188$).

763 (C) Confocal laser-scanning micrographs of root cells in which the FRAP assay of
764 spindles tagged by TagRFP-TUA5 in the *ede1-1* mutant rescued by GFP-EDE1 or
765 GFP-EDE1^{8A} was performed. The white dashed box represents the area that was
766 bleached by the laser. The time is indicated on the top right of the images in seconds.
767 Scale bar 1 μ m.

768 (D) Quantification of the fluorescence intensity recovery over time following bleaching
769 of spindles in *ede1-1* mutant root cells tagged by TagRFP-TUA5 and rescued by GFP-
770 EDE1 (n = 21) or GFP-EDE1^{8A} (n = 24).

771 (E) Quantification of the half maximum values in seconds of fluorescence recovery in
772 *ede1-1* mutants rescued by GFP-EDE1 (n = 21) or GFP-EDE1^{8A} (n = 24). The median
773 values were plotted as a line for each genotype. Average half maximum of 42.52 s \pm
774 13.32 for GFP-EDE1 and 40.90 s \pm 13.71 for GFP-EDE1^{8A}. Comparison on graph: *P*
775 = 0.6903.

776 The level of significance was determined by a two-proportion z-test followed by
777 Bonferroni correction in (B) and an unpaired t test in (E) (**** *P* < 0.0001; ns depicts a
778 non-significant difference).

779

780 **Figure S5. A double mutation in both *CYCB3;1* and *EDE1* does not further**
781 **increase the spindle phenotype in comparison to the *ede-1* mutant**

782 (A) Confocal laser-scanning micrographs of TagRFP-TUA5-tagged microtubules in
783 root cells at the spindle stage of WT, *cycb3;1*, *ede1-1* and *cycb3;1 ede1-1* plants.
784 Scale bar 5 μ m.

785 (B–D) Quantification of the spindle major axis (B), minor axis (C) and area (D) in root
786 cells of WT (n = 50), *cycb3;1* (n = 51), *ede1-1* (n = 27) and *cycb3;1 ede1-1* (n = 24)
787 plants. Median values were plotted as a line for each genotype.

788 The level of significance was determined by an ordinary one-way ANOVA followed by
789 Šídák's multiple comparisons test (* *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001, **** *P* <
790 0.0001).

791

792 **Figure S6. Varying other augmin parameters leads, in general, to a similar trend**
793 **in spindle organization to varying augmin source rate**

794 (A–C) Relationship between spindle properties and the augmin binding rate ($/s$). All
795 temporal means are taken over the last half of the simulation, $500s < t < 1000s$.

796 (A) Mean spindle length (μm) against augmin binding rate ($/s$). The spindle length is
797 measured as the distance between the center-of-masses of the left and right groups
798 of condensates.

799 (B) Mean lengths (μm) of each group of fibers nucleated at kinetochores (green
800 circles), by augmin (yellow triangles), and at poles (purple squares).

801 (C) Mean number of fibers of each type.

802 (D–F) Relationships between kinetochore-fiber (k-fiber) properties and the spindle
803 length (μm), with data points colored according to the augmin binding rate ($/s$). All
804 quantities are means over the last half of the simulation, $500s < t < 1000s$, and k-fiber
805 quantities are averaged over all k-fibers. (D) Mean k-fiber length (μm), (E) mean
806 growth (μm) at k-fiber plus ends, and (F) mean severing (μm) at k-fiber minus ends.

807 (G–I) Relationship between spindle properties and the augmin nucleation rate ($/s$). All
808 temporal means are taken over the last half of the simulation, $500s < t < 1000s$.

809 (G) Mean spindle length (μm) against augmin nucleation rate ($/s$). The spindle length
810 is measured as the distance between the center-of-masses of the left and right groups
811 of condensates.

812 (H) Mean lengths (μm) of each group of fibers nucleated at kinetochores (green
813 circles), by augmin (yellow triangles), and at poles (purple squares).

814 (I) Mean number of fibers of each type.

815 (J–L) Relationships between kinetochore-fiber (k-fiber) properties and the spindle
816 length (μm), with data points colored according to the augmin nucleation rate ($/s$). All
817 quantities are means over the last half of the simulation, $500s < t < 1000s$, and k-fiber
818 quantities are averaged over all k-fibers. (J) Mean k-fiber length (μm), (K) mean growth
819 (μm) at k-fiber plus ends, and (L) mean severing (μm) at k-fiber minus ends.

820 (M–O) Relationship between spindle properties and the augmin diffusion coefficient
821 ($\mu m^2/s$). All temporal means are taken over the last half of the simulation, $500s < t <$
822 $1000s$.

823 (M) Mean spindle length (μm) against augmin diffusion coefficient ($\mu m^2/s$). The spindle
824 length is measured as the distance between the center-of-masses of the left and right
825 groups of condensates.

826 (N) Mean lengths (μm) of each group of fibers nucleated at kinetochores (green
827 circles), by augmin (yellow triangles), and at poles (purple squares).

828 (O) Mean number of fibers of each type.

829 (P–R) Relationships between kinetochore-fiber (k-fiber) properties and the spindle
830 length (μm), with data points colored according to the augmin diffusion coefficient
831 ($\mu\text{m}^2/\text{s}$). All quantities are means over the last half of the simulation, $500\text{s} < t < 1000\text{s}$,
832 and k-fiber quantities are averaged over all k-fibers. (P) Mean k-fiber length (μm), (Q)
833 mean growth (μm) at k-fiber plus ends, and (R) mean severing (μm) at k-fiber minus
834 ends.

835

836 **Material and Methods**

837

838 **Arabidopsis root mitotic spindle simulation**

839

840 Mitotic spindles were simulated using Cytosim, an Open-Source project ([gitlab.com/f-](https://gitlab.com/f-nedelec/cytosim)
841 [nedelec/cytosim](https://gitlab.com/f-nedelec/cytosim)). Here, we provide an overview of our methods which are based on
842 Brownian dynamics. The numerical aspects (integration, stability) were described
843 previously¹⁸. Further to this publication, accessibility of the source code should enable
844 the full analysis of our methods, and reproducibility of the results. In brief, microtubules
845 are modeled as incompressible bendable filaments having the persistence length of
846 microtubules, in a medium characterized by a viscosity as measured for cells⁴⁸.
847 Microtubules are represented by vertices distributed regularly along their length.
848 Connections between microtubules, and forces such as steric interactions are
849 represented by Hookean links between the filament's vertices. The forces are linearly
850 interpolated to adjacent vertices on the filament when a link is formed between two
851 vertices. The evolution of the entire network is simulated by solving the equation of
852 motion for successive small-time intervals, updating this equation as the motors move
853 to different positions on the filaments, and motor and crosslinkers bind or unbind, and
854 microtubules grow, shrink, vanish, or are created. In essence, the movement is defined
855 by an over-damped Langevin equation: $\xi \frac{dx}{dt} = f(x, t) + B(t)$, for a large
856 multivariate vector x , where the right-hand terms are elastic and random forces

857 respectively, and ξ is a diagonal matrix of drag coefficients calculated using Stokes'
858 law from the viscosity of the fluid and the dimensions of the objects. Such an equation
859 accurately describes the motion of micrometer-sized objects in a fluid that is
860 dominated by elastic and viscous forces. In addition to Brownian motion at each
861 positional coordinate, the equation includes the bending elasticity of the filaments and
862 the elastic terms associated with the molecules forming bridges between two
863 filaments. The differential equation involving all the coordinates of all vertices is solved
864 using a first-order semi-implicit numerical integration scheme that is numerically very
865 stable. Moreover, at each time step, a variety of biochemical processes are modelled
866 as first-order stochastic processes: activation, binding and unbinding, nucleation,
867 microtubule dynamic instability.

868

869 The cell volume is fixed and cylindrical, with a length $11\mu\text{m}$ and diameter $5\mu\text{m}$,
870 symmetric around the x-axis. The edges of the cell induce microtubule plus ends to
871 stall. With this assumption, no confinement forces were needed. Microtubules thus do
872 not track the cell edges.

873

874 **Microtubule Nucleation.** Microtubules are nucleated by three pathways:

875 N1. Kinetochores

876 N2. Pole-induced

877 N3. Augmin-mediated

878

879 Each pathway is constituted of a fixed number of nucleators entities, with properties
880 adjusted according to the pathways that is represented: kinetochore and pole
881 associated nucleators are anchored to beads, while the augmin-mediated nucleator is
882 part of a diffusible complex. Each nucleator may only nucleate one microtubule at a
883 time, and would remain inactive until this microtubule vanishes, or the nucleator
884 detaches from it.

885

886 For pathway N1 (kinetochores), each kinetochore harbors 5 nucleators, and their
887 nucleation rate is fixed and unregulated. Moreover, the kinetochore-based nucleator

888 will remain attached at the plus end of the microtubules, while for the other pathways
889 the nucleator remains attached to the minus end.

890

891 Nucleation pathway N2 (poles) consists of nucleators attached to the beads that form
892 the condensate at the spindle pole (see below).

893

894 For nucleation pathway N3 (augmin-mediated), individual augmin entities are
895 generated on a random position on the surface of the kinetochores with a fixed source
896 rate. These augmin entities have a finite lifetime characterized by a constant molecular
897 rate. This is implemented using a timer for each augmin entity, initialized with $t =$
898 $-\log(\theta^+)/R$, where $R=5/s$ is the deactivation rate and θ^+ a random number in
899 $]0,1]$. Augmin entities are deleted if their timers reach zero. During its lifetime, an
900 augmin entity diffuses freely, and may bind to existing microtubules within its binding
901 range, with the prescribed binding rate. A bound augmin stays fixed relative to the
902 microtubule on which it is attached, until it unbinds. An augmin entity that is bound to
903 a microtubule (the mother) will nucleate a new microtubule (the daughter) as
904 determined by its nucleation rate. Unbound augmin do not nucleate. A daughter
905 microtubule is orientated parallel to the mother microtubule, in the same direction.
906 During the time that it is bound, the augmin entity is protected from deactivation (the
907 internal timer is frozen). The timer restarts if the augmin detaches from the
908 microtubules to which it is docked. These assumptions are intended to capture the
909 control of the augmin activity by the Ran pathway⁴⁹, where the RanGTP complex is
910 generated at the surface of the chromatin by RCC1 and deactivated elsewhere by
911 RanGAP, forming a sharp gradient of active Ran around the chromosomes⁵⁰. Our
912 assumptions capture the essential condition that daughter microtubules are nucleated
913 parallel to their mother microtubule⁴, in the vicinity of the chromosomes⁵¹, and that
914 augmin can be transported by fluxing microtubules⁵².

915

916 We used a single scalar parameter (noted as γ) to model the fact that pathways 2 and
917 3 share the same molecular nucleator gamma-tubulin. When a nucleator from these
918 two pathways is active, its nucleation rate is multiplied by $(1 - N\gamma)$, where N is the

919 number of microtubules in the system. In this way nucleation is reduced as
920 microtubules become more numerous until it vanishes for $N = 1/\gamma$. We used $\gamma =$
921 0.0001, corresponding to a maximum of 10000 microtubules, which is much above
922 the actual number of microtubules in the simulation (~2000), and this limit is not
923 reached. However, this assumption connects the nucleation activities of pathways 2
924 and 3, with the effect of reducing the number of pole-nucleated microtubules if the
925 augmin activity is increased.

926

927 Microtubules are nucleated with an initial length $L_0 = 32nm$ with their plus ends in
928 the growing state, and undergo dynamic instability at the plus ends. The minus ends
929 are static. Dynamic instability at the plus end is implemented following a stochastic
930 model of the GTP cap that protects microtubules from catastrophes⁵³. The
931 instantaneous microtubule growth speed is set dynamically from the total length of the
932 microtubules at a given time point i.e. $v_g(t) = \alpha \left(1 - \frac{1}{\Omega} \sum L_i(t)\right)$ where α is the
933 maximum growth speed, $\sum L_i(t)$ is the total length of all microtubules at time t and
934 the constant Ω represents the total available tubulin pool, expressed in MT length
935 (4000 μ m). These assumptions intend to represent conditions in which the amount of
936 tubulin from which microtubules polymerize is finite. The growth speed of individual
937 microtubule is further reduced in the presence of an antagonistic force, $f_a < 0$, by an
938 exponential factor, e^{f_a/f_g} , where $f_g > 0$ is a characteristic "growing force"
939 parameter⁵⁴. This factor is always applied, but we believe that it is insignificant for the
940 simulations presented in this work, because cell-edge induced forces were not
941 enabled. We instead assumed that microtubules would stall upon contacting the cell
942 edge, only growing at a fraction of their speed in the cytoplasm; specifically, the growth
943 speed is divided by 10. With this assumption, we recover the conditions in plant cells,
944 where microtubules are not observed to track the edges of the cell, but instead Eb1
945 comets vanish as they reach the cell edge. Given that the stochastic model of dynamic
946 instability is very dependent on the rate of tubulin addition, microtubules contacting
947 the cell edge thus rapidly undergo catastrophes in the simulation, as observed *in vivo*.
948 Microtubules shrink at a constant shrinkage speed v_s and do not undergo rescues.

949 Any microtubule shorter than 24 nm is deleted. After a shrinking microtubule has
950 vanished, its nucleator is free to nucleate again.

951

952 Microtubules experience steric interactions. They repel each other via a soft-core
953 interaction that is repulsive with a diameter $d_0 = 50nm$:

954
$$F(d) = k_{steric}(d - d_0), \text{ if } d \leq d_0$$

955 where d is the distance between the two interacting filaments. This force is applied
956 at every filament vertex that is within the steric diameter of another filament segment.

957 It acts primarily in the direction orthogonal to the filament axis and will not prevent
958 filaments from sliding along each other. Steric forces interfere in this way minimally
959 with the movements induced by crosslinking motors such as Kinesin 5 (Figure S1) but
960 will induce parallel microtubules to separate their center lines $d_0 = 50nm$ apart.

961

962 Moreover, a weak force is added to bring the microtubules closer to the x-axis
963 (parameter 'squeeze'). This force promotes the formation of the spindle poles by
964 focusing the kinetochores fibers on the x-axis. The force magnitude is implemented
965 as $f(u) = F_\epsilon \tanh(u/R_z)$, with $u = \sqrt{y^2 + z^2}$ and $F_\epsilon = 0.05pN$ the maximum
966 magnitude of the force, and $R_z = 3\mu m$ is the range at which it plateaus. The force is
967 applied only at the minus ends, to all microtubules. This force is directed towards the
968 x-axis, with no component parallel to the x-axis: $f(u) \times \{0, \frac{-y}{u}, \frac{-z}{u}\}$.

969

970 Kinetochores are represented by spherical particles with a radius of 100 nm. The 20
971 kinetochores associated with the 10 chromosomes are placed such as to form a
972 regular metaphase plate in the middle of the cell. Ten kinetochores are placed a $x =$
973 $0.25\mu m$, while the other ten are placed at $x = -0.25\mu m$, in a mirror configuration
974 (same y and z coordinates). The two sets of 10 kinetochores are distributed in the YZ
975 plane such as to approximate a disc of uniform density. Specifically, 8 kinetochores
976 are placed at the summit of a regular octagon with $y^2 + z^2 = 2\mu m$, and two
977 kinetochores are placed inside this octagon at $y = 0.65\mu m$ and $z = 0.3\mu m$ and
978 the symmetric position $\{-y, -z\}$. Each kinetochore is immobilized in translation with

979 a Hookean link of stiffness 1000 pN/ μm but is free to rotate. Thus, the metaphase
980 alignment of the chromosomes is assumed in our model. Each kinetochore harbors 5
981 nucleation entities. Microtubules are allowed to grow from the kinetochores in the
982 initialization sequence of the simulation, in the direction of the closest spindle pole
983 (e.g., toward $x > 0$ for microtubules originating from kinetochores placed at $x =$
984 $0.25\mu\text{m}$). This favors the biorientation of all kinetochores in the initial configuration.
985 The alignment of chromosomes in the metaphase plate, and the biorientation of
986 kinetochores are two important aspects of mitotic spindle assembly that were
987 intentionally left aside for future work, to focus on the question of how the length of the
988 spindle is regulated by augmin.

989

990 Each kinetochore has 5 nucleating entities (ndc80) located on a cap directed towards
991 the closest pole. Each entity may nucleate one microtubule and remains attached to
992 its plus end until spontaneous detachment occurs, which is set at a rate of 0.01s^{-1} .
993 The nucleation rate of 1s^{-1} implies that kinetochores have 5 microtubules attached to
994 them most of the time. If the kinetochore unbinds, the associated microtubule plus end
995 is set in a shrinking state and will thus rapidly vanish since there is no rescue.
996 Kinetochores regulate the plus end dynamics of microtubules to which they are
997 attached. The minus ends are not affected. A kinetochore-attached microtubule plus
998 end grows slower than that of a regular microtubule, and its growth speed is regulated
999 by force on the plus end f (the force in the ndc80 entity). Specifically, $v_g(t) =$
1000 $\left(1 - \frac{1}{2} \sum L_i(t)\right) 2\beta / \left(1 + \exp\left(-\sqrt{2}f/f_g\right)\right)$, where $\beta = \alpha/5$ (the amplitude of the
1001 reduction, 5, is set by the parameter 'stabilize') and where $f_g > 0$ is the microtubule's
1002 characteristic "growing force". Compared to other microtubules, the kinetochore
1003 suppresses catastrophes, reduces average growth by a factor 6, and regulates growth
1004 upon force with the factor $2 / \left(1 + \exp\left(-\sqrt{2}f/f_g\right)\right)$, which by construction is in
1005 $]0,2]$. Pulling forces will accelerate microtubule growth up to a factor 2, while pushing
1006 forces will reduce growth by a significant fraction, if the force reaches f_g .

1007

1008 Each spherical particle used to represent a kinetochore contains three vertices on its
1009 surface, constituting, together with the center point, a local reference frame that
1010 provides orientation in space. The ndc80 entities are placed with respect to this local
1011 reference frame, such as to form a small cluster (a 'polar cap') on one side. This cap
1012 is initially orientated towards the closest spindle pole. Kinetochores and associated
1013 microtubules are linked by Hookean links. A first type of link constrains the position of
1014 the plus end to match the position of the “ndc80” entity on the surface of the
1015 kinetochore. This link is of zero resting length and stiffness 222 pN/ μm . A second type
1016 of link (parameter 'anchor_stiffness' 44 pN/ μm) is used to align all the microtubules
1017 from one kinetochore, in the direction of its cap. This link is formed between the vertex
1018 of the microtubule, that is just before the plus end, and a matching virtual point built
1019 on the kinetochore reference frame, away from the kinetochore surface, at a distance
1020 equal to the separation of the microtubule vertex and its plus end. This way a
1021 geometrically simple but realistic configuration of microtubule attachment with the
1022 kinetochore is built.

1023

1024 Molecular motors. Kinesin-5 and Kinesin-14 are modeled as 2 linked units, forming a
1025 complex which can thus be unbound, attached to one microtubule, or attached to two
1026 microtubules. Complexes diffuse in the unbound state, can bind to one or two filaments
1027 and, when bound to two filaments, are modeled as Hookean springs with a resting
1028 length of 50 nm and various stiffness values as specified in the parameter table.
1029 Binding is determined by a rate within a binding range, and these two parameters are
1030 set following typical values for such molecules, initially measured for conventional
1031 kinesin. Subunits bind and unbind independently from each other but cannot bind to
1032 the same position on the same filament when they belong to the same complex.
1033 Diffusion of unbound motors is not modelled explicitly; it is assumed to be sufficiently
1034 fast that a uniform spatial distribution of unbound motors is maintained. The simulation
1035 only keeps track of the number of unbound motors, but not their positions and
1036 evaluates the average number of binding events per time step using the current total
1037 length of microtubules and the cell volume. This estimate is discretized using a
1038 Poisson distribution and the corresponding number of binding events is directly

1039 implemented by picking random positions along microtubules with uniform sampling
1040 (option 'fast_diffusion').

1041

1042 Molecular motor units. Whereas their binding and unbinding are discrete stochastic
1043 events, bound kinesins move deterministically on microtubules at a speed which is
1044 linearly proportional to load, given by $v = v_m(1 + f_{load} \cdot d/f_{stall})$, where d is a
1045 unit vector parallel to the microtubule (in the direction preferred by the motor), f_{load}
1046 the force vector, $f_{stall} > 0$ is a characteristic stall force and v_m is the unloaded
1047 speed of the motor (positive for kinesin5 and negative for kinesin14). Note that with
1048 our conventions, forces that antagonize the motor preferred motion are directed
1049 opposite to d , hence a plus-end-directed motor is slowed down by forces directed
1050 towards the minus end. For a minus-end-directed motor, the unit vector d points
1051 toward the minus end. Motors detach from the microtubule side at a rate k_{off} and
1052 immediately from the microtubule ends. The detachment rates of motors are increased
1053 exponentially by the load on the motor and a characteristic unbinding force f_{unbind} ,
1054 according to Kramer's law; $k = k_{off} \exp(\|f_{load}\|/f_{unbind})$.

1055

1056 Kinesin-5 is modelled as a pair of identical motor units connected by a Hookean
1057 spring-like link with resting length d_m and stiffness K_m . This link can rotate freely at
1058 both attachment points, such that the angle between two crosslinked microtubules is
1059 unconstrained. If one motor of a pair is bound to a microtubule the other can bind to
1060 any microtubule within a range r_b at rate k_{on} . To simulate the observed difference in
1061 Kinesin-5 affinity to parallel vs. antiparallel microtubules configurations⁵⁵, we used two
1062 separate kinesin-5 entities: a 'antikin' that may only bind antiparallel configurations and
1063 a 'parakin' that may bind to all the other configurations. The criteria defining parallel
1064 vs. antiparallel is based on the cosine of the angle formed between the direction
1065 vectors of the relevant microtubule segments (the dot product of the unit direction
1066 vector of the microtubules). The antiparallel motor may bind only if $\cos(\theta) < -0.5$,
1067 and the other motor if $\cos(\theta) > -0.5$. To simulate the observed differences, the

1068 'parakin' as an unbinding rate of 0.1 s^{-1} , whereas the 'antikin' has a lower unbinding
1069 rate of 0.025 s^{-1} . The other characteristics of the two kinesin-5 subtypes are identical.

1070

1071 Kinesin-14 is composed of a minus-end-directed motor domain linked to a diffusible
1072 domain via a Hookean link. The minus-end-directed motors is modelled similarly to
1073 the plus-end-directed motor domains of Kinesin-5, with respect to load and
1074 detachment. The non-motor domain of Kinesin-14 may diffuse passively or be dragged
1075 along the side of a microtubule. It is characterized by a linear mobility coefficient μ . A
1076 domain that is under a force f transmitted through the Hookean link will move along
1077 the microtubule in the direction of the force with an average speed μf . In addition, it
1078 undergoes diffusion with a 1D diffusion constant $D_1 = \mu k_B T$, where T is the absolute
1079 temperature and k_B Boltzman's constant ($k_B T = 4.2 \text{ nm} \cdot \text{pN}$). The movement in a
1080 time interval τ was implemented as $\delta = \mu f \tau + \theta \sqrt{6D_1 \tau}$, where θ is a random
1081 number uniformly distributed over $[-1, 1]$. In contrast to the motor domain, the
1082 diffusible domain does not unbind immediately upon reaching the microtubule minus
1083 end. Instead, it keeps the same unbinding rate at the minus end than when located on
1084 the side of microtubules. Unbinding rates are however still modulated exponentially by
1085 the load according to Kramer's law; $k = k_{off} \exp(-\|f_{load}\|/f_{unbind})$. Given that it
1086 is linked to a slow minus-end-directed motor, a diffusible domain is unlikely to ever
1087 reach a growing plus end, but we have assumed anyhow that it would detach
1088 immediately at the plus end.

1089

1090 Spindle poles. The poles of the spindle in the simulation are made with discrete
1091 particles. Initially, 1000 particles are placed at $x = 2\mu\text{m}$, and another 1000 at $x =$
1092 $-2\mu\text{m}$. Two forces hold particles together and provide them with the ability to form a
1093 fluid phase within the cytoplasm: a specific pressure associated to the density of
1094 particles, and a surface tension. The pressure terms ensure that the beads remain
1095 separated by a distance roughly corresponding to maximal sphere packing density.
1096 The surface tension promotes the fusion of two droplets of beads that would come into
1097 contact, in our case leading to the collapse of the spindle into a monopole. The beads

1098 behave as a fluid phase and form compact droplets at the pole which remain mostly
 1099 spherical and moves very little during the simulation. The density of the condensate
 1100 appears uniform and close to the density value set as parameter (equal to V_{max}).

1101

1102 The bead fluid subsystem is modelled following the 'Smoothed Particle
 1103 Hydrodynamics' (SPH) method⁵⁶. The SPH method, which was originally developed
 1104 for astrophysics, integrates well with Cytosim after adaptation to the microscopic
 1105 physics in which inertia is negligible. All particles are spherical with the same radius
 1106 $R = 64nm$. We assumed a uniform mass density for the particles that is equal to that
 1107 of the cytoplasm, such that we simply used the volume of each particle ($m_a =$
 1108 $4\pi R^3/3$) and not their mass to weigh their contribution in the SPH sums. We note h
 1109 the smoothing length scale ($h = 303nm$) and only used kernels with finite support,
 1110 vanishing for distances d above h . The local density ρ_a is calculated using the
 1111 standard 6th-order polynomial kernel $W_{poly6}(d) = W_6 [h^2 - d^2]^3$, where $W_6 =$
 1112 $315/64\pi h^9$ provides the normalization. With our simplification of density=1, the
 1113 mass density estimated at particles is effectively a dimensionless volume fraction. A
 1114 value of pressure for each bead is then calculated as $P_a = K_{SPH} \cdot \max(0, \rho_a -$
 1115 $V_{max})$, where V_{max} is the desired density, set to the maximum volume fraction
 1116 achieved for packed spheres ($V_{max} = \pi/3\sqrt{2} \approx 0.74$), and K_{SPH} can be seen as a
 1117 compressibility factor, a stiffness associated with the pressure. A pressure force is
 1118 derived from the gradient of density, using Desbrun's spiky kernel $W_{spiky}(d) =$
 1119 $W_S [h - d]^3$, where $W_S = 15/\pi h^6$ ⁵⁷, using Monagan's symmetric formula (Eq. 3.3
 1120 in J. J. Monaghan. Smoothed particle hydrodynamics, 1992):

$$1121 \quad f_{a \leftarrow b} = -m_a m_b \left(\frac{P_a}{\rho_a^2} + \frac{P_b}{\rho_b^2} \right) \nabla W_S(r_a - r_b).$$

1122 We used a cohesion kernel to model the surface tension⁵⁸, with however a modified
 1123 kernel:

$$1124 \quad W_{cohesion}(d) = W_C \begin{cases} C_K [(h-d)d]^3 - (C_K - 1) \left(\frac{h}{2}\right)^6 & \text{if } d \leq h/2 \\ [(h-d)d]^3 & \text{if } d > h/2, \end{cases}$$

1125 where $W_C = 32/\pi h^9$ is for normalization. This kernel is continuous at $d = h/2$, and
1126 $C_K = 275/19$ is adjusted to ensure that the force experienced by a particle located
1127 on the surface of a droplet of constant density would vanish, namely:

$$1128 \quad 0 = \int_{r=0}^h r^2 W_{cohesion}(r) dr$$

1129 The cohesion force is calculated using a symmetric formula:

$$1130 \quad f_{a \leftarrow b} = -\gamma_{SPH} m_a m_b \frac{r_a - r_b}{|r_a - r_b|} \left(\frac{2V_{max}}{\rho_a + \rho_b} \right) W_C(r_a - r_b).$$

1131 Using symmetric SPH formula ensures that the force will always balance, which is
1132 essential. The forces calculated per particle are then scaled by the drag coefficient of
1133 each particle (Stokes' law: $\zeta = [6\pi R]^{-1}$) to obtain their instantaneous speed, from
1134 which a displacement is calculated. We use an explicit integration if the bead is
1135 unconnected with the microtubule system ($\delta x = \zeta f \tau$). Otherwise, for instance if one
1136 of the bead's binder is attached to a microtubule, the SPH force is added to Cytosim's
1137 force engine as an explicit force term, such as to combine the SPH-calculated forces
1138 with the elastic forces associated with the links to microtubules. In any case, a random
1139 force is added to model the unbiased diffusion of beads, calibrated from their size
1140 ($D = \zeta k_B T$), the viscosity and the time-step. The full details of our SPH
1141 implementation will be given in a separate article.

1142

1143 Three activities are associated with the beads forming the condensate at the spindle
1144 pole: microtubule binding, nucleation and severing. The microtubule binding activity is
1145 implemented by attaching discrete binding entities to the center of the beads forming
1146 the condensate. In the spindle simulations, each bead has ~ 4 binders. These binders
1147 may only bind to microtubules near their minus ends, specifically at a location of the
1148 microtubule that is less than 250 nm away from the minus end, provided the distance
1149 to the bead center (where the binder is anchored) is lower than 64 nm. The nucleation
1150 activity is implemented by attaching one nucleator per bead (see above for the detailed
1151 description of the nucleation model). The microtubule severing activity is implemented
1152 similarly to the augmin complex: katanin entities are generated at the surface of the
1153 beads with a 'source' rate and destroyed stochastically with a constant rate of 8 s^{-1} .
1154 Katanin entities are free to diffuse and to bind to microtubules during their lifetime. In

1155 this way a permanent gradient of severing activity is generated within and around the
1156 condensate. A Katanin entity is a complex made of two severing units. Each severing
1157 unit can cut a microtubule to which it is bound with a rate of $0.2s^{-1}$. Upon cutting the
1158 severing unit unbinds. The new plus end is created in the shrinking state, as widely
1159 observed⁵⁹. The new minus end is static.

1160

1161 **Experimental determination of spindle parameters**

1162

1163 For the estimation of the number of spindle microtubules, we analyzed TEM images
1164 of cross-sections of roots. We measured the number of microtubules in a $1 \mu m^2$
1165 square. Next, we extrapolated this value to an area of approximately $16 \mu m^2$ for one
1166 half of the spindle. With this, we reached a value that varied between 576 and 1,408
1167 microtubules for a full root spindle ($n = 5$).

1168

1169 For estimating the number of microtubules in kinetochore fibers, we counted the
1170 number of microtubules in bundles from TEM images and measured the fluorescence
1171 intensity of kinetochore fibers from spindles stained against α -tubulin compared to
1172 single microtubules in the same cell. For the fluorescence measurements, we drew a
1173 line across a single microtubule in Fiji and measured the integrated density divided by
1174 the area analyzed. Next, we measured the integrated density divided by the area
1175 analyzed in kinetochore fibers and divided that by the value obtained for a single
1176 microtubule to obtain an estimate of number of microtubules. This fluorescence
1177 intensity estimate was obtained from four different cells. We obtained similar values in
1178 both experimental approaches.

1179

1180 For determining the growth speed of microtubules, we generated kymographs using
1181 the KymographBuilder Fiji plugin (<https://imagej.net/plugins/kymograph-builder>) from
1182 roots of plants expressing an *EB1b_{PRO}::EB1b-GFP* reporter that were imaged with a
1183 spinning disk microscope with a 0.5 s frame rate. Values were obtained for ten
1184 microtubules per spindle from three spindles, each from an independent plant.

1185

1186 **TEM of Arabidopsis root cross-sections**

1187

1188 Roots were fixed with 2% glutaraldehyde in cacodylate buffer (75 mM, pH 7.0) for 3.5
1189 h, postfixed with 1% osmium tetroxide at 4°C overnight. Samples were dehydrated
1190 through a series of graded acetone concentrations, 30% to 100%, and finally
1191 embedded in plastic according to Spurr⁶⁰. Ultrathin sections were obtained with a
1192 ultramicrotome (Ultracut E, Leica-Reichert-Jung, Nußloch, Germany) and stained with
1193 uranyl acetate followed by lead citrate⁶¹. Sections were viewed with a LEO 906 E TEM
1194 (LEO, Oberkochen, Germany) equipped with the Wide-angle-2K (4Mpx.) Dual Speed
1195 CCD Camera (TRS, Moorenweis, Germany) using the software ImageSP-
1196 Professional to acquire, visualize, analyse, and process image data.

1197

1198 **Plant growth conditions**

1199

1200 *Arabidopsis thaliana* seeds were grown on ½ MS medium (basal salt mixture, Duchefa
1201 Biochemie) containing 0.5% sucrose and 0.8% agar (plant agar, Duchefa Biochemia).
1202 Seeds were initially sterilized with a solution containing 2% bleach and 0.05% Triton
1203 X-100 for 5 min followed by three washes with sterile distilled water and the addition
1204 of 0.05% agarose. Plates with seeds were then stratified at 4°C for 2–3 days in the
1205 dark. Next, plates were placed in an *in vitro* growth chamber at a temperature of 22°C
1206 in a 16-hour light regime. Seedlings were transferred afterwards to soil in a growth
1207 chamber with a 16-hour/21°C light and 8-hour/18°C dark regime with 60% humidity.
1208 Plants were transformed using the floral dipping method⁶².

1209

1210 **AP-MS on CYCB3;1**

1211

1212 Cloning of CYCB3;1 encoding the C-terminal GS^{rhino} tag⁶³ fusion under control of the
1213 constitutive cauliflower tobacco mosaic virus 35S promoter and transformation of
1214 *Arabidopsis* cell suspension cultures (PSB-D) with direct selection in liquid medium
1215 was carried out as previously described⁶⁴.

1216

1217 Pull downs were performed in triplicate, using in-house prepared magnetic IgG beads
1218 and 25 mg of total protein extract per pull down as described⁶⁴. On-bead digested

1219 samples were analyzed on a Q Exactive (ThermoFisher Scientific) and co-purified
1220 proteins were identified with Mascot (Matrix Science) using standard procedures⁶⁴.

1221

1222 After identification, the protein list was filtered versus a large dataset of similar
1223 experiments with non-related baits using calculated average Normalized Spectral
1224 Abundance Factors (NSAFs)⁶⁴. Proteins identified with at least two matched high
1225 confident peptides in at least two experiments, showing high (at least 10-fold) AND
1226 significant [$-\log_{10}(p\text{-value(T-test)}) \geq 10$] enrichment compared to the large dataset were
1227 retained.

1228

1229 **Generation of the CDKB1;1-GFP and GFP-EDE1 reporters**

1230

1231 To create the *PRO_{CDKB1;1}:CDKB1;1:EGFP* construct, the genomic fragment of
1232 *CDKB1;1* was amplified by PCR and cloned into pDONR221. The SmaI site was
1233 inserted in front of the *CDKB1;1* stop codon. *CDKB1;1* constructs were linearized by
1234 SmaI digestion and were ligated to the *EGFP* gene, followed by LR recombination
1235 reactions with the destination vector *pGWB501*. The same approach was employed
1236 to generate the *PRO_{EDE1}:EGFP:EDE1* construct, with the exception that the *EGFP*
1237 gene was inserted at the N-terminus of *EDE1* before the first *ATG* codon. Primers
1238 used in this study are listed in Table S7.

1239

1240 **Spindle morphogenesis image analysis**

1241

1242 First, an ellipse was fitted manually in Fiji to spindles tagged with TagRFP-TUA5 or
1243 immunostained against α -tubulin. Next, the major axis, minor axis and spindle area
1244 measurements were obtained by going to Analyze > Set measurements and checking
1245 the “Area” and “Fit ellipse” boxes. All values are provided in Table S2. To judge the
1246 presence of prominent astral microtubules in individual spindle images, spindle files
1247 were anonymized in Fiji with the Blind Analysis Tools plugin
1248 (<https://imagej.net/plugins/blind-analysis-tools>). To analyse γ -tubulin distribution, the
1249 images (with a 49 nm pixel size) were first equally treated with the Gaussian Blur filter
1250 with a radius of 0.05 scaled units to improve the fluorescence intensity peak definition.

1251 Then, a line was drawn exactly at the middle of the spindle through the pole-to-pole
1252 axis in a perpendicular angle in relation to the spindle midzone and the fluorescence
1253 intensity profile was plotted in Fiji. The fluorescence intensity values were then
1254 normalized by the minimum and maximum values in each cell and combined into a
1255 graph containing the mean and SD values of each replicate. The distance between
1256 the two highest values of fluorescence was calculated individually in every cell and
1257 then corrected by the spindle major axis and plotted as a ratio. In the case of the
1258 analysis of GFP-EDE1 distribution, the images (with a 143 nm pixel size) were treated
1259 with Gaussian Blur with a radius of 0.1 scaled units.

1260

1261 **Root growth assays and timing of mitotic divisions on oryzalin**

1262

1263 For the oryzalin root growth assays, seeds were sown on $\frac{1}{2}$ MS with either 0.05%
1264 DMSO as a control or oryzalin. Root growth was recorded daily up until 5 days after
1265 germination when plates were scanned and subsequently analyzed with Fiji. To follow
1266 mitotic cell divisions on control or oryzalin conditions live, whole five- to seven-day-old
1267 seedlings were placed in a glass-bottom dish and covered in solid $\frac{1}{2}$ MS followed by
1268 the addition of liquid $\frac{1}{2}$ MS containing 0.05% DMSO as a control or 150 nM oryzalin
1269 and incubation for 1 hour. Oryzalin stocks were prepared in DMSO at a concentration
1270 of 100 mM and stored at -20°C.

1271

1272 For the root growth assays to assess the functionality of the CDKB1;1-GFP
1273 reporter, five-day-old seedlings were transferred onto medium with or without 10 μ M
1274 Cisplatin for 5 days. At the end of the experiment, plates were photographed and root
1275 length was measured using ImageJ software.

1276

1277 **Wholemout immunolocalization of α -tubulin and KNOLLE in roots**

1278

1279 Roots of 4-day-old Arabidopsis seedlings were fixed in 4% paraformaldehyde and
1280 0.1% Triton X-100 in MTSB 1/2 buffer (25 mM PIPES, 2.5 mM MgSO₄, 2.5 mM EGTA,
1281 pH 6.9) for 1 hour under vacuum, then rinsed in PBS 1X for 10 minutes. Samples were
1282 then permeabilized in ethanol for 10 minutes and rehydrated in PBS for 10 minutes.

1283 Cell walls were digested using the following buffer for one hour: 2 mM MES pH 5,
1284 0.20% driselase and 0.15% macerozyme. Tissues were hybridized overnight at room
1285 temperature with the B-5-1-2 monoclonal anti- α -tubulin (Sigma) and the anti- KNOLLE
1286 antibody⁶⁵ (kind gift of G. Jürgens, University of Tübingen, Germany). The next day,
1287 tissues were washed for 15 minutes in PBS, 50 mM glycine, incubated with secondary
1288 antibodies (Alexa Fluor 555 goat anti-rabbit for KNOLLE antibody and Alexa Fluor 488
1289 goat anti-mouse for the tubulin antibody) overnight and washed again in PBS, 50 mM
1290 glycine and DAPI 20 ng/ml. Tissues were mounted in VECTASHIED and DAPI and
1291 viewed using an SP8 confocal laser microscope (Leica Microsystems).

1292
1293 Samples were excited sequentially at 405 nm (DAPI), 488 nm (@TUB/Alexa Fluor
1294 488), and 561 nm (@KNOLLE/Alexa Fluor 555), with an emission band of 420-450
1295 nm (DAPI), 495-545 nm (Alexa Fluor 488), and 560-610 nm (Alexa Fluor 555) using a
1296 PMT for DAPI imaging, and hybrid detectors for MT and KNOLLE imaging. All stacks
1297 were imaged using the same zoom (x 1,60) with a voxel size xyz of 200 nm x 200 nm
1298 x 500 nm.

1299
1300 A blind counting was set up to count mitotic MT arrays. Six roots per genotype were
1301 analyzed for WT, *cycb3* and *cdkb1*, and seven roots were analyzed for *ede1-1*
1302 transformed with GFP-EDE1 WT, GFP-EDE1^{8A} and GFP-EDE1^{8D}. All images were
1303 first anonymized, and mitotic MT arrays were counted within each root stack using the
1304 “Cell counter” ImageJ plugin (<https://imagej.nih.gov/ij/plugins/cell-counter.html>).

1305

1306 **Immunolocalization of α - and γ -tubulin in root meristematic cells**

1307

1308 Root cells were immunostained as described in Liu et al. 1993⁶⁶. α -tubulin was stained
1309 using a monoclonal antibody raised in mouse (Sigma, T9026) and γ -tubulin was
1310 stained using a monoclonal antibody also raised in mouse (Agrisera, AS20 4482).
1311 Since the primary antibodies were raised in the same species, a sequential staining
1312 method was employed. First, the slides were incubated with the γ -tubulin antibody
1313 overnight at 4°C followed by incubation with the secondary antibody against mouse
1314 STAR 635P (abberior) at room temperature for 2 hours. Next, the slides were

1315 incubated with the α -tubulin antibody overnight at 4°C followed by incubation with the
1316 secondary antibody against mouse STAR 580 (abberior) at room temperature for 2
1317 hours. Samples were then mounted in VECTASHIELD containing DAPI (Vector
1318 Laboratories). Slides were imaged in a Zeiss LSM 880 microscope equipped with
1319 Airyscan and images were acquired with a voxel size of 49 nm x 49 nm x 160 nm.

1320

1321 **Protein expression and purification and *in vitro* kinase assay**

1322

1323 To generate HisGST-EDE1, the *CDS* of EDE1 was initially amplified by PCR with
1324 primers containing attB1/attB2 flanking sequences followed by a Gateway BP reaction
1325 into pDONR221 and subsequently a Gateway LR reaction into the pHGGWA vector.
1326 The destination vector was then transformed in *E. coli* BL21 (DE3) pLysS cells. For
1327 expression, *E. coli* cultures were grown until an OD of 0.6 followed by addition of IPTG
1328 at a concentration of 0.2 mM and incubation at 16°C overnight. The CDKB1;1-
1329 CYCB3;1 complex was expressed and purified as described in Harashima and
1330 Schnittger⁶⁷. After purification with Ni-NTA agarose or Strep-Tactin in case of the
1331 CDKB1;1 control, all proteins were desalted using PD MiniTrap G-25 columns (GE
1332 Healthcare) and protein quality was checked by CBB staining and immunoblotting.
1333 Kinase assays were incubated at 30°C for 1 hour in a buffer containing 50 mM Tris-
1334 HCl, pH 7.5, 10 mM MgCl₂, 0.5 mM ATP and 5 mM DTT.

1335

1336 **Sample preparation and LC-MS/MS data acquisition for the identification of** 1337 **EDE1 phosphosites**

1338

1339 The protein mixtures were reduced with dithiothreitol, alkylated with chloroacetamide,
1340 and digested with trypsin. These digested samples were desalted using StageTips
1341 with C18 Empore disk membranes (3 M)⁶⁸, dried in a vacuum evaporator, and
1342 dissolved in 2% ACN, 0.1% TFA. Samples were analyzed using an EASY-nLC 1200
1343 (Thermo Fisher) coupled to a Q Exactive Plus mass spectrometer (Thermo Fisher).

1344

1345 For initial assessment of phospho sites, peptides (1:10 dilution) were separated on 16
1346 cm frit-less silica emitters (New Objective, 75 μ m inner diameter), packed in-house

1347 with reversed-phase ReproSil-Pur C18 AQ 1.9 μm resin (Dr. Maisch). Peptides were
1348 loaded on the column and eluted for 50 min using a segmented linear gradient of 5%
1349 to 95% solvent B (0 min : 5%B; 0-5 min -> 5%B; 5-25 min -> 20%B; 25-35 min ->35%B;
1350 35-40 min -> 95%B; 40-50 min ->95%B) (solvent A 0% ACN, 0.1% FA; solvent B 80%
1351 ACN, 0.1%FA) at a flow rate of 300 nL/min. Mass spectra were acquired in data-
1352 dependent acquisition mode with a TOP15 method. MS spectra were acquired in the
1353 Orbitrap analyzer with a mass range of 300–1500 m/z at a resolution of 70,000 FWHM
1354 and a target value of 3×10^6 ions. Precursors were selected with an isolation window
1355 of 1.3 m/z. HCD fragmentation was performed at a normalized collision energy of 25.
1356 MS/MS spectra were acquired with a target value of 5×10^5 ions at a resolution of
1357 17,500 FWHM, a maximum injection time of 120 ms and a fixed first mass of m/z 100.
1358 Peptides with a charge of 1, greater than 6, or with unassigned charge state were
1359 excluded from fragmentation for MS²; dynamic exclusion for 20s prevented repeated
1360 selection of precursors.

1361
1362 For the targeted analysis samples (1:3 dilution) were resolved using the above-
1363 mentioned segmented linear gradient. The acquisition method consisted of a full scan
1364 method combined with a non-scheduled PRM method. The 17 targeted precursor ions
1365 were selected based on the results of DDA peptide search in Skyline. MS spectra were
1366 acquired in the Orbitrap analyzer with a mass range of 300–2000 m/z at a resolution
1367 of 70,000 FWHM and a target value of 3×10^6 ions, followed by MS/MS acquisition for
1368 the 17 targeted precursors. Precursors were selected with an isolation window of 2.0
1369 m/z. HCD fragmentation was performed at a normalized collision energy of 27. MS/MS
1370 spectra were acquired with a target value of 2×10^5 ions at a resolution of 17,500
1371 FWHM, a maximum injection time of 120 ms and a fixed first mass of m/z 100.

1372

1373 **MS data analysis and PRM method development**

1374

1375 Raw data from DDA acquisition were processed using MaxQuant software (version
1376 1.5.7.4, <http://www.maxquant.org/>)⁶⁹. MS/MS spectra were searched by the
1377 Andromeda search engine against a database containing the respective proteins used
1378 for the *in vitro* reaction. Trypsin specificity was required and a maximum of two missed

1379 cleavages allowed. Minimal peptide length was set to seven amino acids.
1380 Carbamidomethylation of cysteine residues was set as fixed, phosphorylation of
1381 serine, threonine and tyrosine, oxidation of methionine and protein N-terminal
1382 acetylation as variable modifications. The match between runs option was disabled.
1383 Peptide-spectrum-matches and proteins were retained if they were below a false
1384 discovery rate of 1% in both cases.

1385

1386 Raw data from the DDA acquisition were analyzed on MS1 level using Skyline
1387 (<https://skyline.ms>)⁷⁰ and a database containing the respective proteins used for the
1388 *in vitro* reaction. Trypsin specificity was required and a maximum of two missed
1389 cleavages allowed. Minimal peptide length was set to seven maximum length to 25
1390 amino acids. Carbamidomethylation of cysteine, phosphorylation of serine, threonine
1391 and tyrosine, oxidation of methionine and protein N-terminal acetylation were set as
1392 modifications. Results were filtered for precursor charges of 2, 3 and 4. For each
1393 phosphorylated precursor ion a respective non-phosphorylated precursor ion was
1394 targeted as a control, furthermore several precursor ions from the backbone of EDE1
1395 were chosen as controls between the samples. In total 17 precursors were chosen to
1396 be targeted with a PRM approach.

1397

1398 After acquisition of PRM data the raw data were again processed using MaxQuant
1399 software, with above-mentioned parameters. Table S6 shows phosphosites and
1400 localization probabilities obtained using the MaxQuant search. The mass spectrometry
1401 proteomics data have been deposited to the ProteomeXchange Consortium via the
1402 PRIDE⁷¹ partner repository with the dataset identifier PXD046697.

1403

1404 **FRAP assay**

1405

1406 For the bleaching of GFP-EDE1, sections of the spindles were bleached with the 405
1407 and 488 lasers both at 100% after 5 frames of imaging and with a scan speed of 7 and
1408 5 iterations. Images were acquired every 0.5 s with a pixel size of 120 nm. For the
1409 analysis of the images, the Stowers Plugins Collection was used
1410 (<https://research.stowers.org/imagejplugins>). The data processing and analysis was

1411 performed as previously described⁷². For the bleaching of TagRFP-TUA5 in the GFP-
1412 EDE1/*ede1-1* and GFP-EDE1^{8A}/*ede1-1* backgrounds, only the 405 laser was used at
1413 100% for fluorescence bleaching, but the other parameters were the same as in the
1414 bleaching of GFP-EDE1. Outliers in the half maximum values were removed using the
1415 ROUT method (Q = 5%).

1416

1417 **Supplemental file legends**

1418

1419 **Table S1. Simulation Parameters**

1420 Whenever possible, we used published, experimentally determined values. The
1421 configuration file of the simulation is also provided as the definitive source of
1422 parameter values.

1423

1424 **Table S2. Quantification and statistical tests of spindle parameters**

1425

1426 **Table S3. Overview of confirmed CYCB3;1 interactors**

1427 A–D: prey annotation. E: number of different bait groups a prey was identified in over
1428 the whole AP-MS dataset. Baits were functionally grouped. The lower the more
1429 specific. F–G: number of replicates in which a prey was identified with at least two
1430 (column F) or with one (column G) unique peptides. H–J: details on the NSAF-based
1431 filtering to identify specifically enriched prey proteins.

1432

1433 **Table S4. Protein Identification details obtained with Q Exactive (Thermo Fisher 1434 Scientific) and Mascot Distiller software (version 2.5.0, Matrix Science) 1435 combined with the Mascot search engine (version 2.6.2, Matrix Science) using 1436 the Mascot Daemon interface and database Araport11plus (contaminants 1437 filtered out)**

1438 prot_score: protein score; prot_mass: protein mass; prot_matches_sig: number of
1439 assigned peptide matches above threshold (high confidence, $p < 0.01$);
1440 prot_sequences_sig: number of significant protein sequences above threshold (high
1441 confidence, $p < 0.01$); prot_cover: percentage of protein sequence covered by
1442 assigned peptide matches; prot_len: protein sequence length (AA); prot_pi: pi of

1443 identified protein; pep_query: peptide query number; pep_rank: rank of the peptide
1444 match, 1 to 10, where 1 is the best match; pep_isbold: peptide is in bold red (Red and
1445 bold typefaces are used to highlight the most logical assignment of peptides to
1446 proteins. The first time a peptide match to a query appears in the report, it is shown in
1447 bold face. Whenever the top-ranking peptide match appears, it is shown in red. Thus,
1448 a bold red match is the highest scoring match to a particular query listed under the
1449 highest scoring protein containing that match. This means that protein hits with many
1450 peptide matches that are both bold and red are the most likely assignments.
1451 Conversely, a protein that does not contain any bold red matches is an intersection of
1452 proteins listed higher in the report.); pep_isunique: peptide is unique to protein;
1453 pep_exp_mz: observed m/z value (precursor); pep_exp_mr: experimental relative
1454 molecular mass; pep_exp_z: observed peptide charge state; pep_calc_mr: calculated
1455 relative molecular mass; pep_delta: difference (error) between the experimental and
1456 calculated masses; pep_start: peptide start position in protein; pep_end: peptide end
1457 position in protein; pep_miss: number of missed enzyme cleavage sites; pep_score:
1458 peptide ions score; pep_ident: peptide score identity threshold; pep_expect:
1459 expectation value for the peptide match (The number of times we would expect to
1460 obtain an equal or higher score, purely by chance. The lower this value, the more
1461 significant the result); pep_res_before: amino acid before peptide sequence; pep_seq:
1462 peptide sequence; pep_res_after: amino acid after peptide sequence; pep_var_mod:
1463 any variable modifications found in the peptide; pep_var_mod_pos: position of
1464 variable modifications in the peptide.

1465

1466 **Table S5. Mitotic division figures in roots of WT, *cycb3;1* and *cdkb1* and GFP-**
1467 **EDE1, GFP-EDE1^{8A} and GFP-EDE1^{8D} in the *ede1-1* background**

1468

1469 **Table S6. Phosphorylated sites in EDE1**

1470

1471 **Table S7. Primers used in this study**

1472

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Figure 1

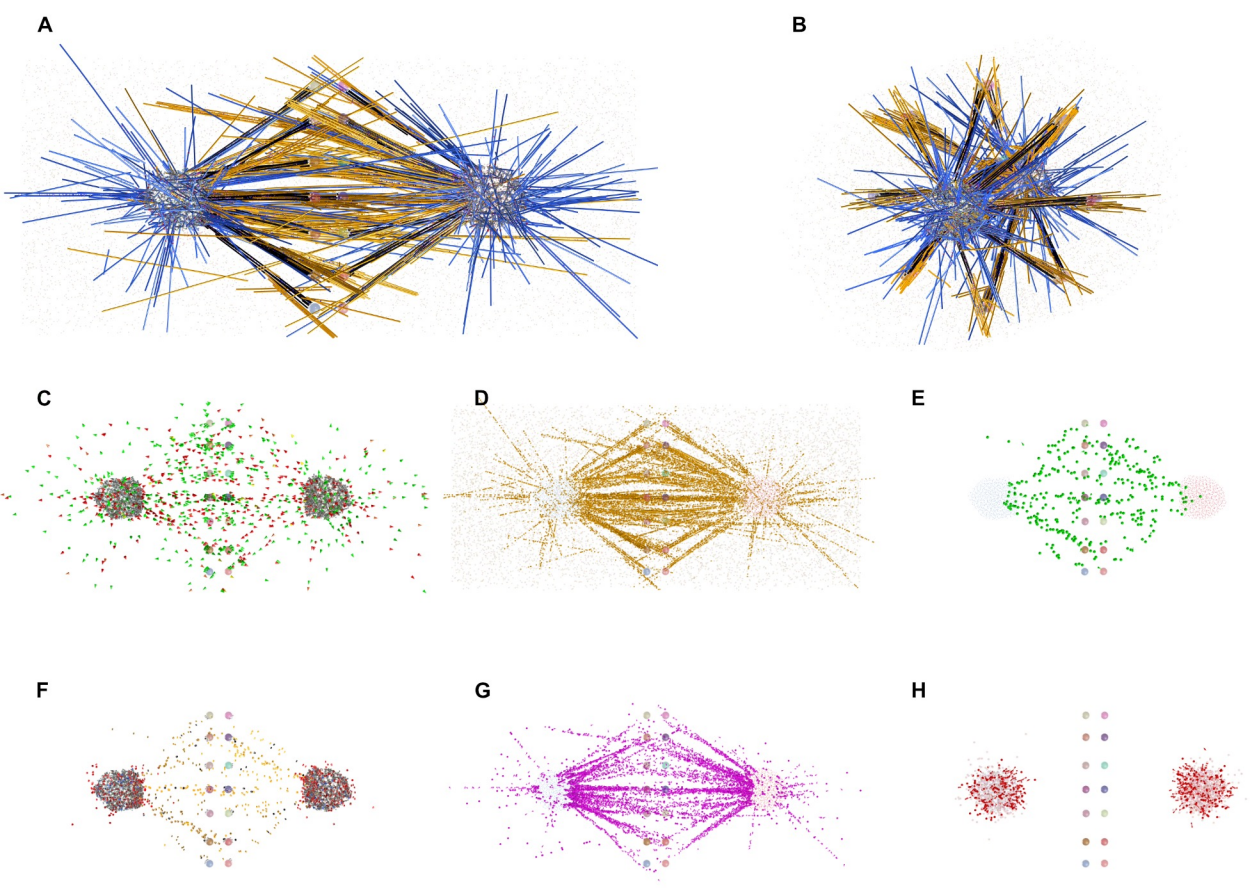


Figure 2

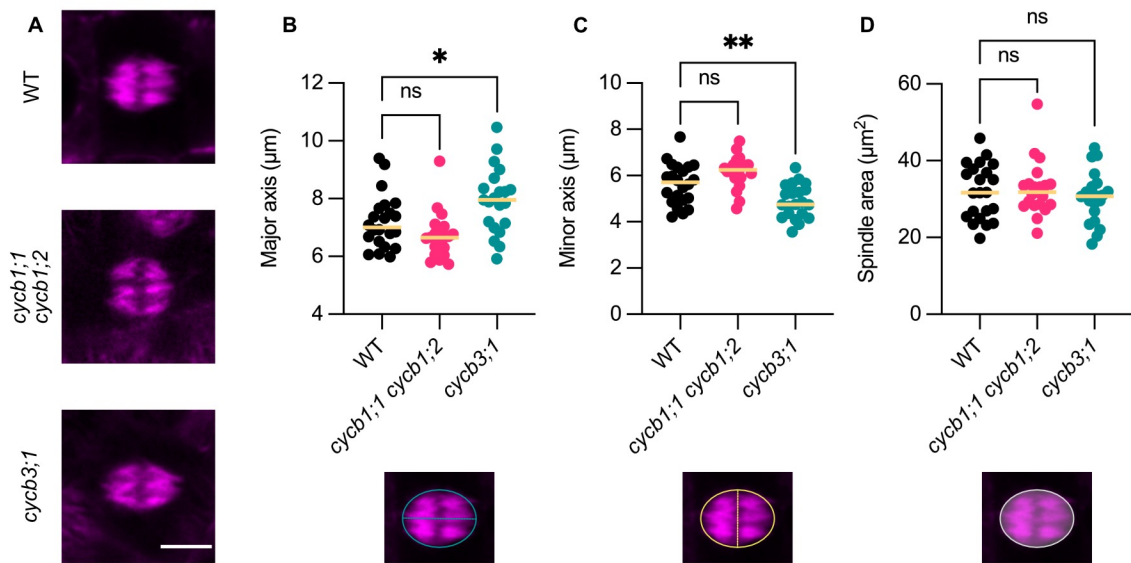


Figure 3

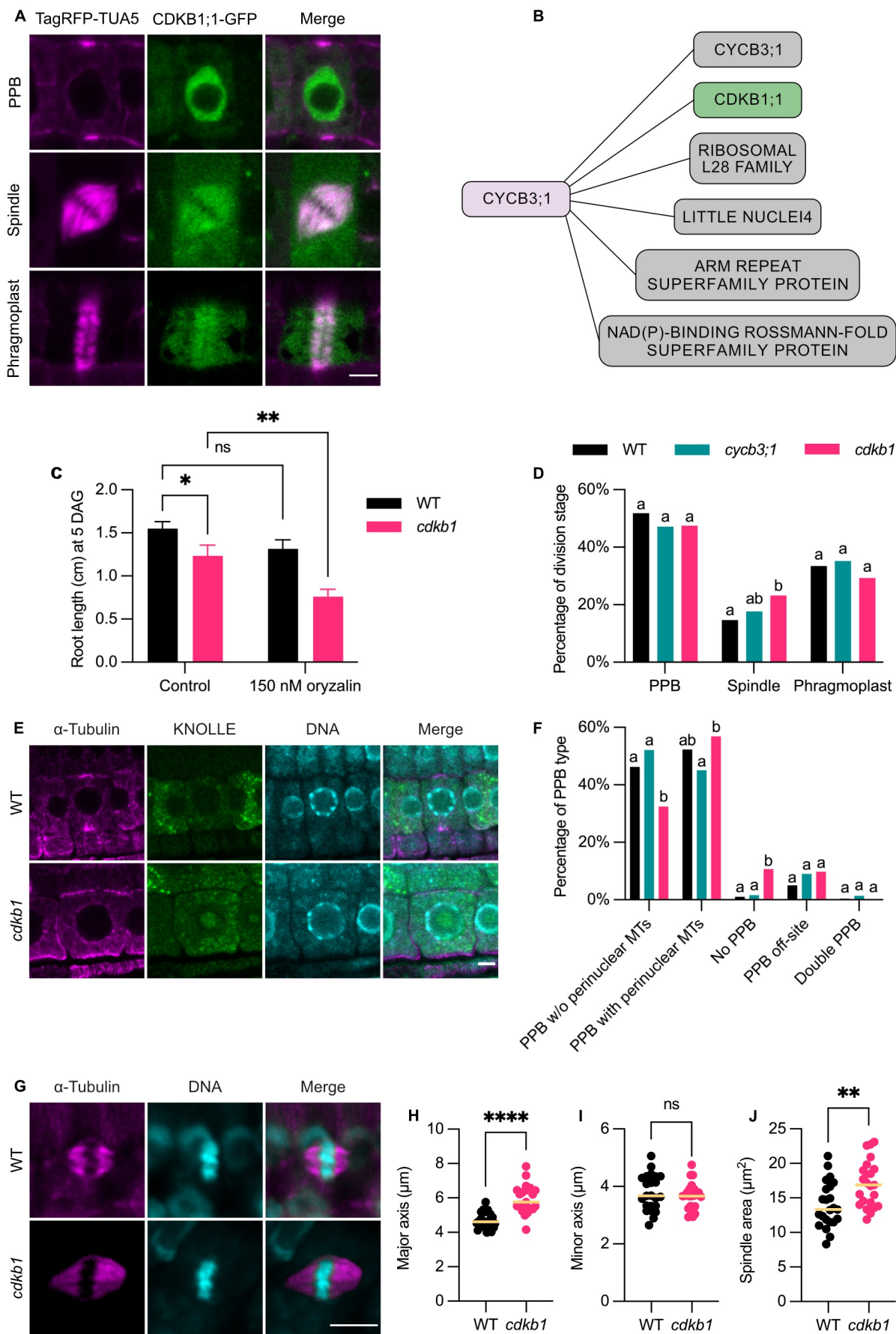


Figure 4

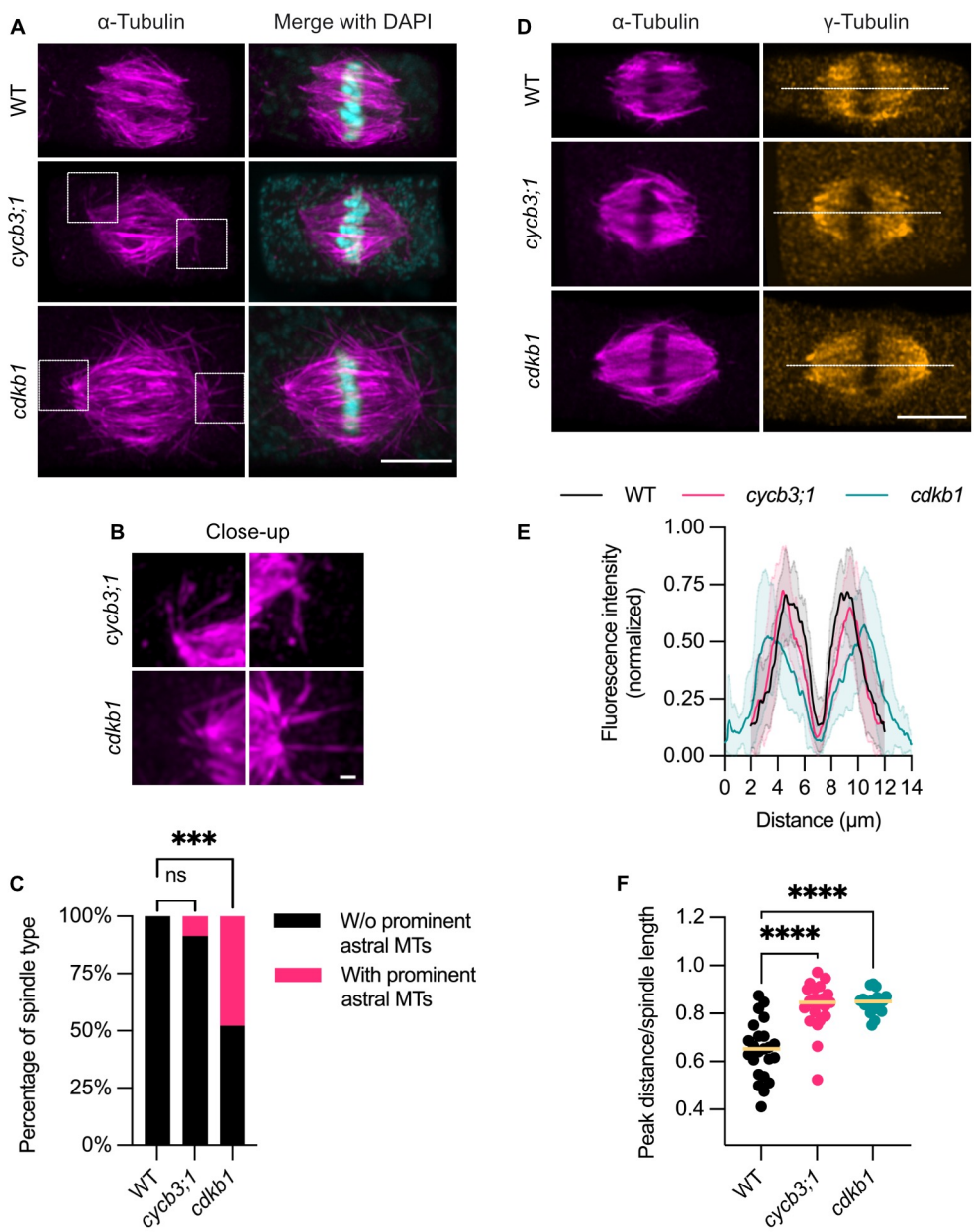


Figure 5

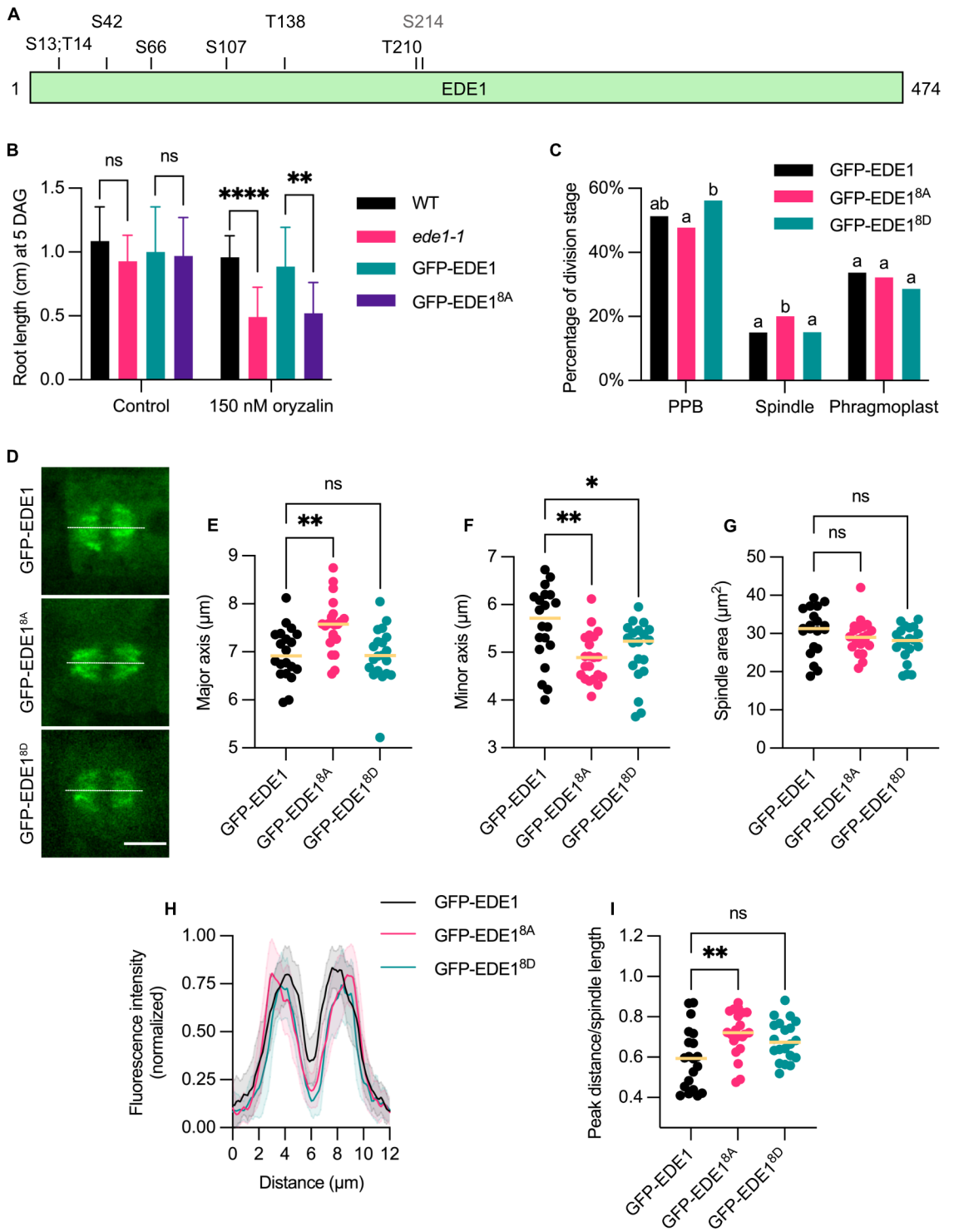


Figure 6

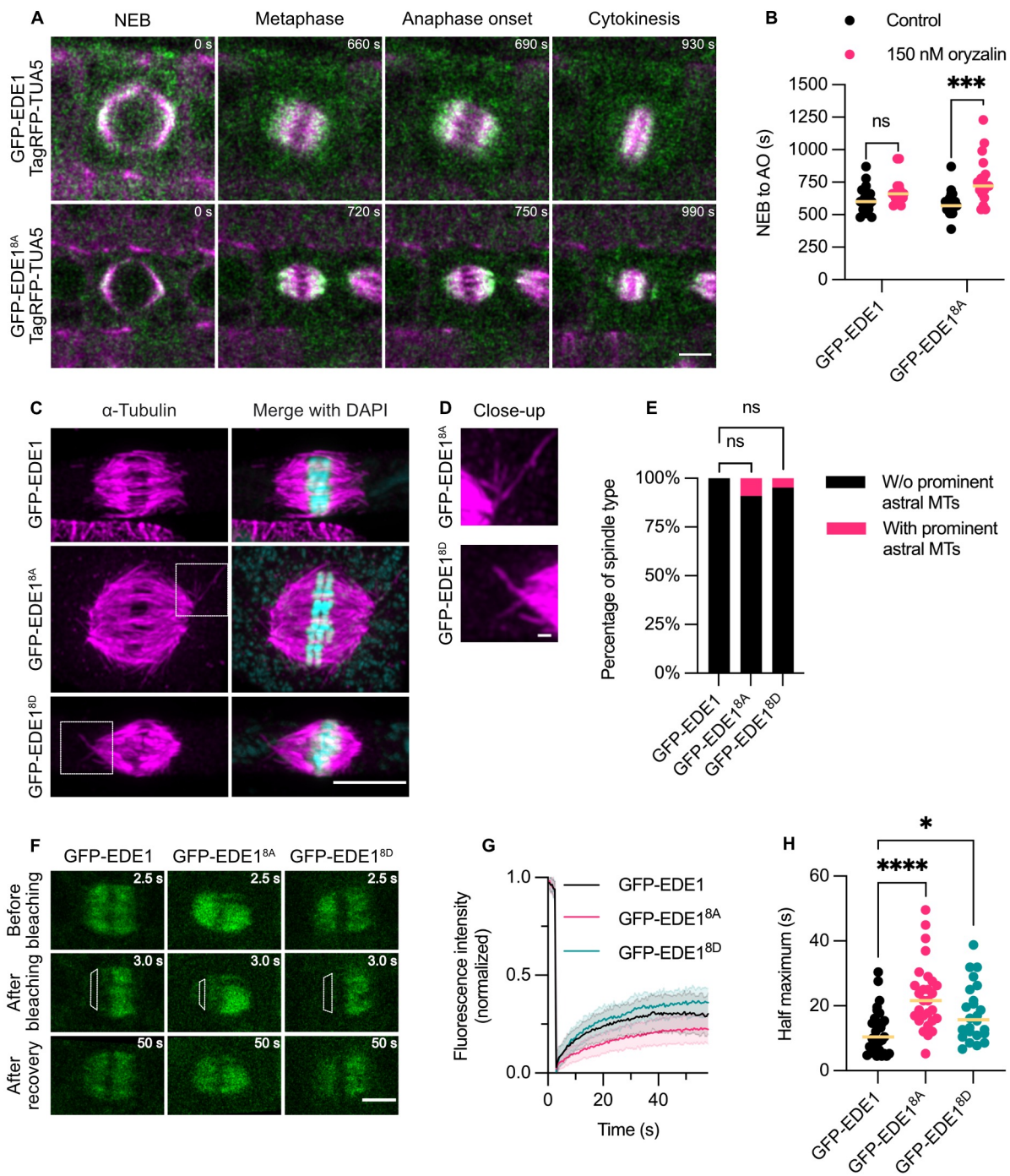


Figure 7

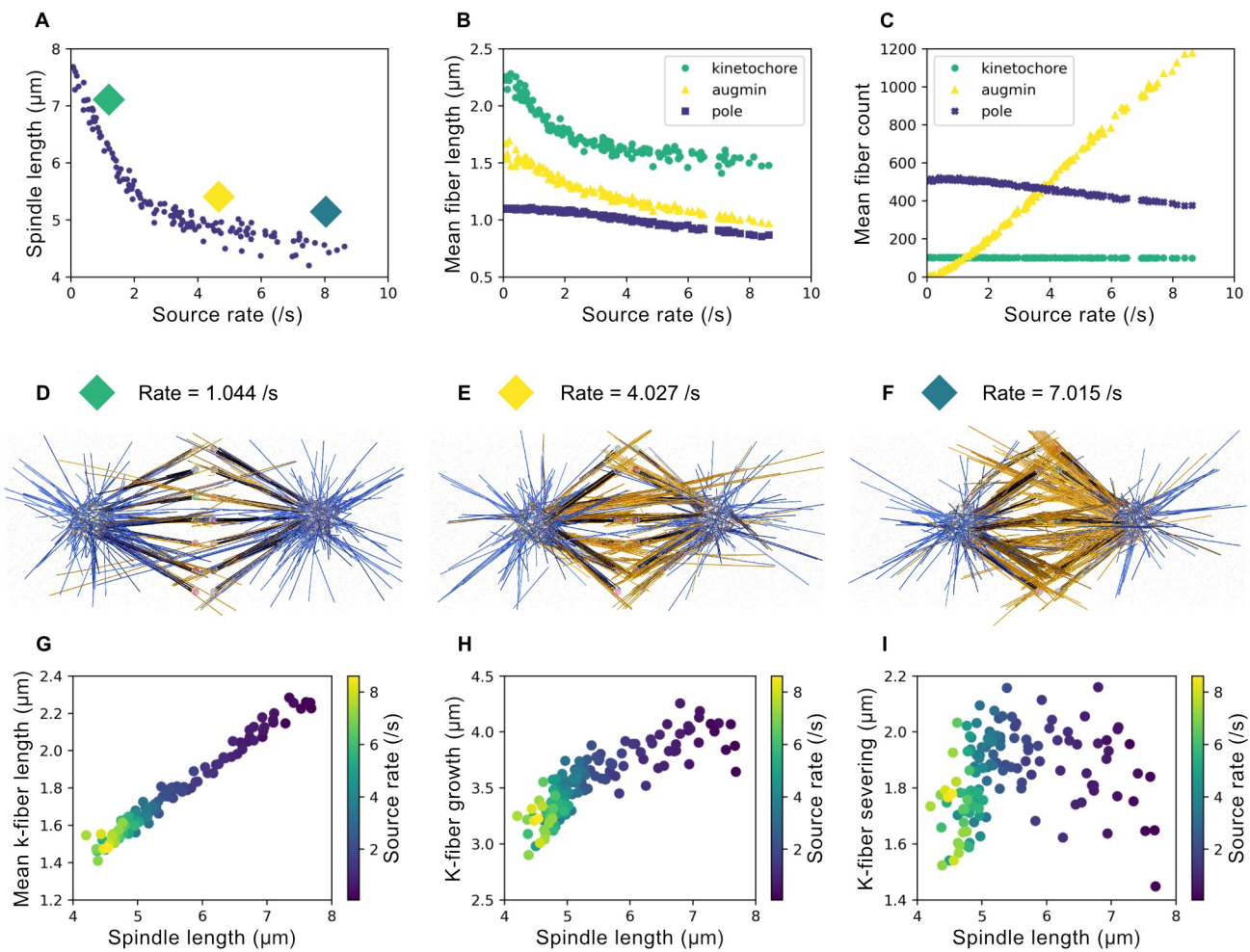


Figure S1

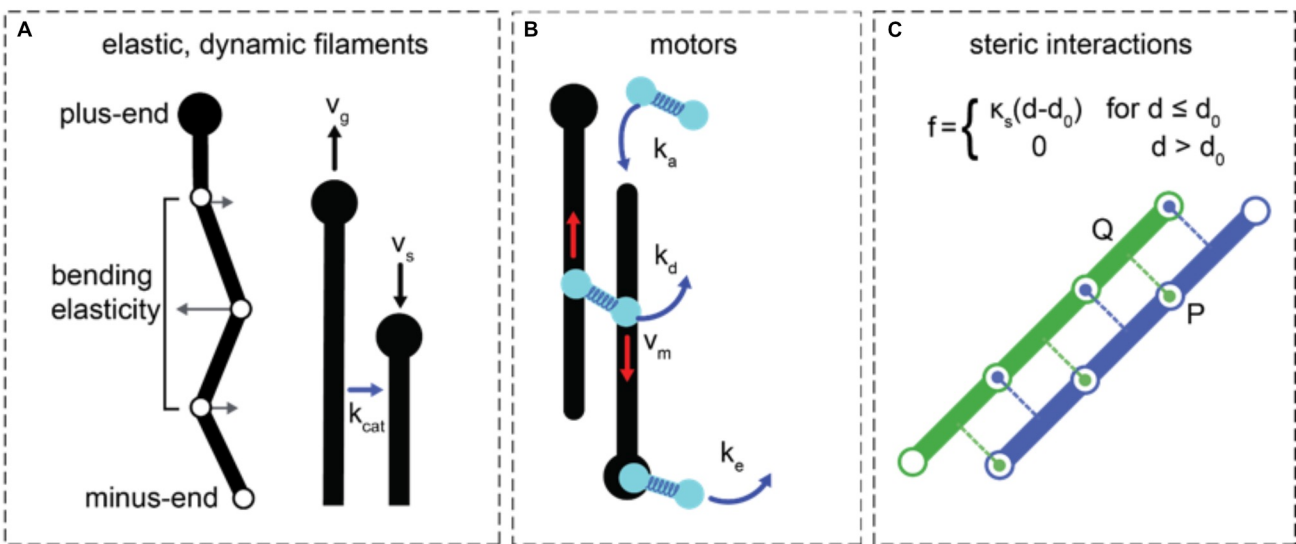


Figure S2

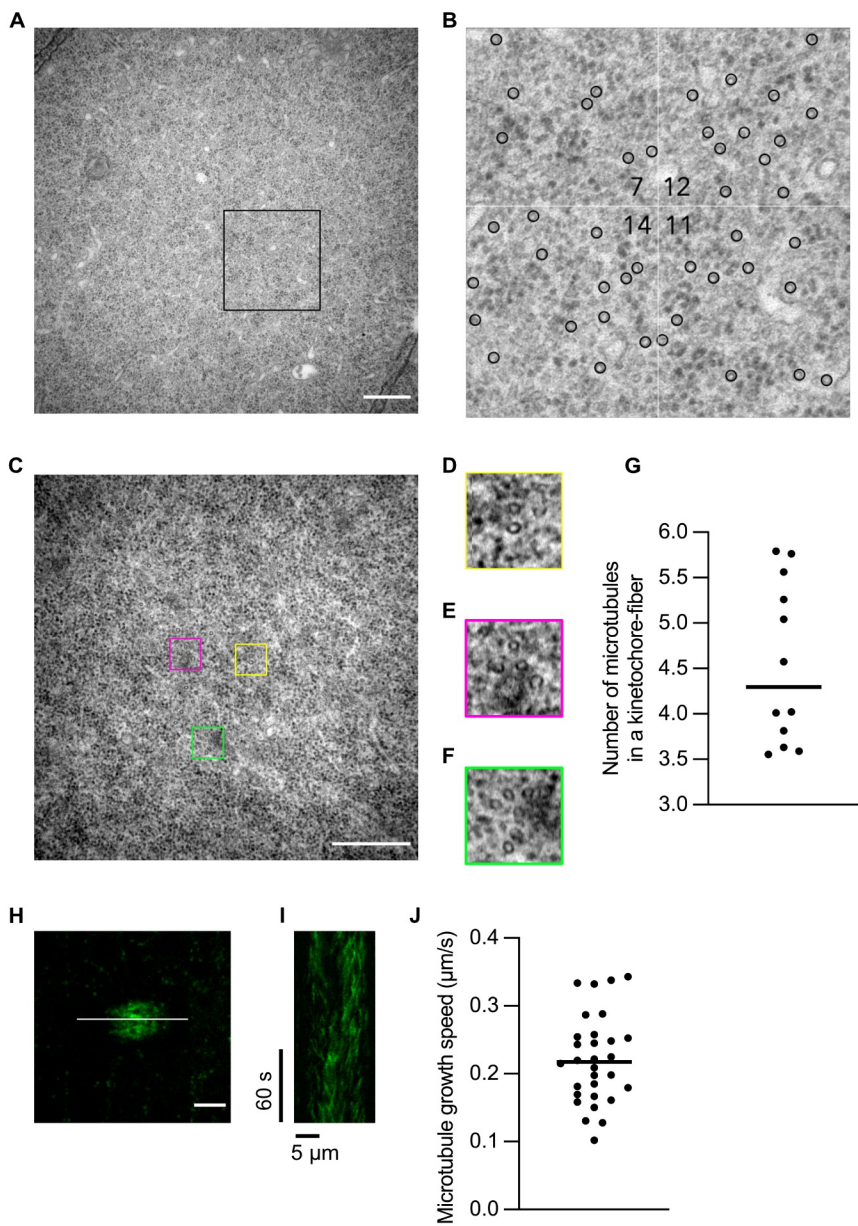


Figure S3

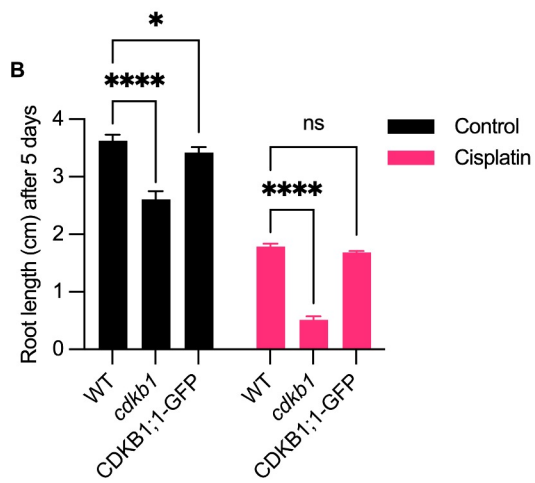
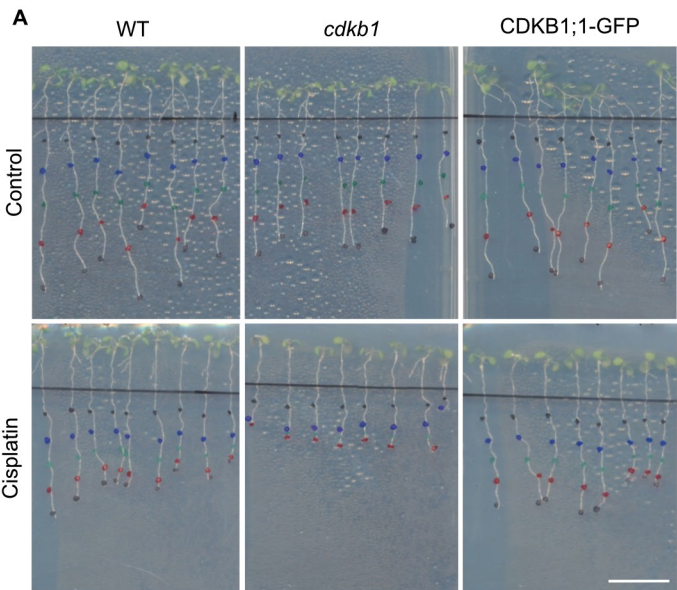


Figure S4

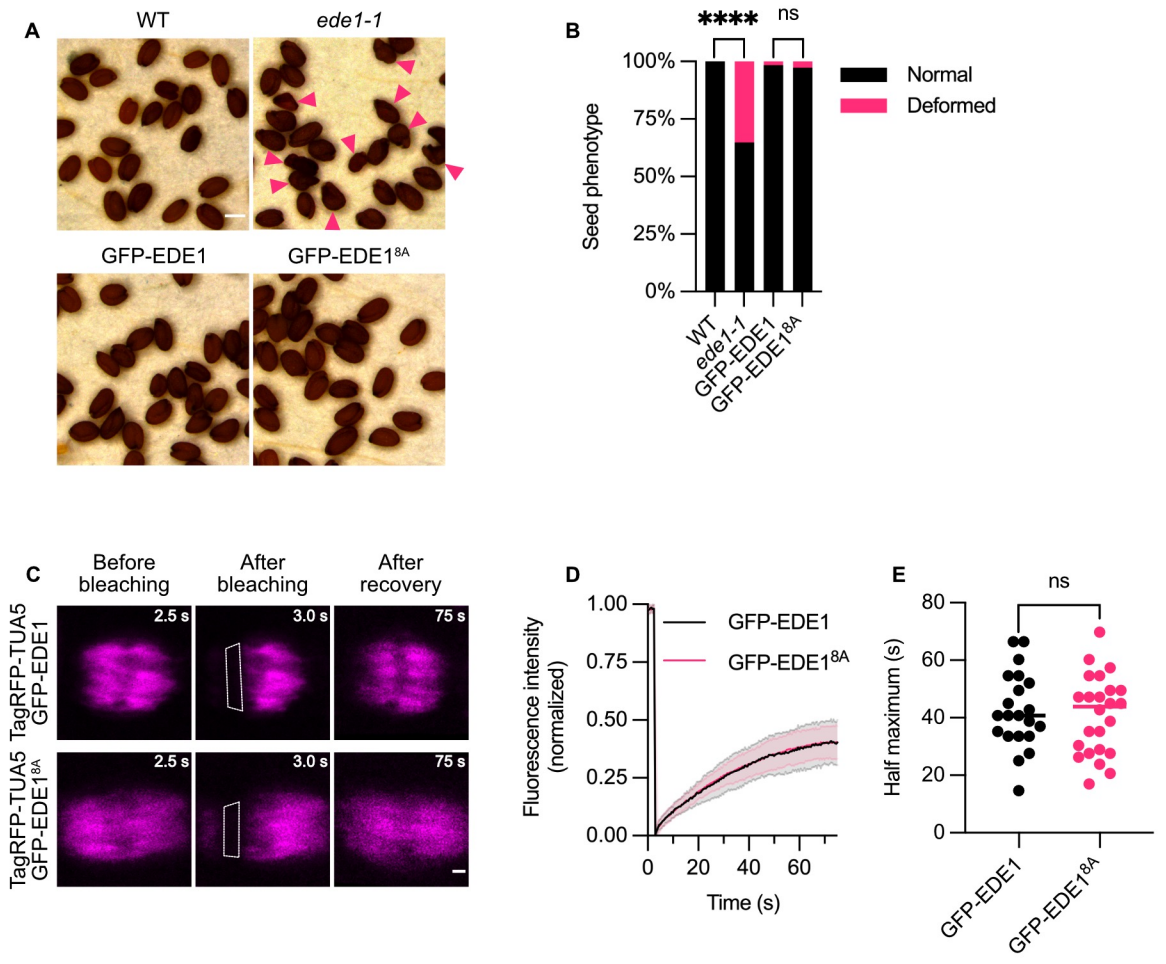


Figure S5

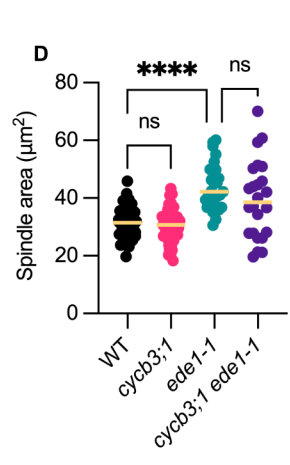
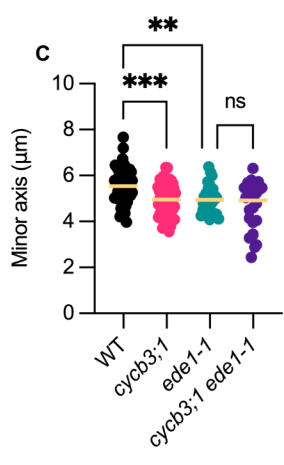
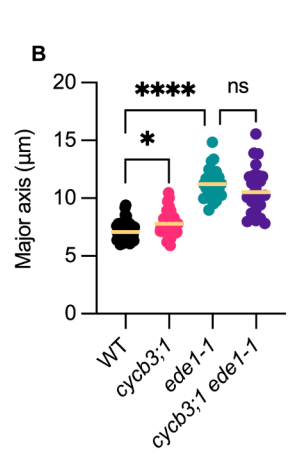
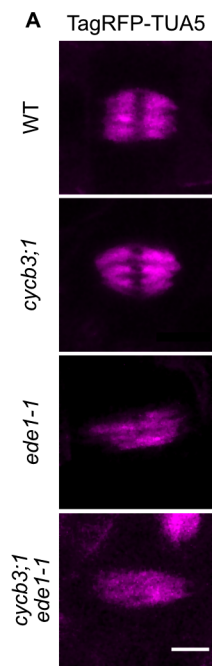


Figure S6

