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1 **Reduction of cortical pulling at mitotic entry facilitates aster centration**

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17

18 **Abstract**

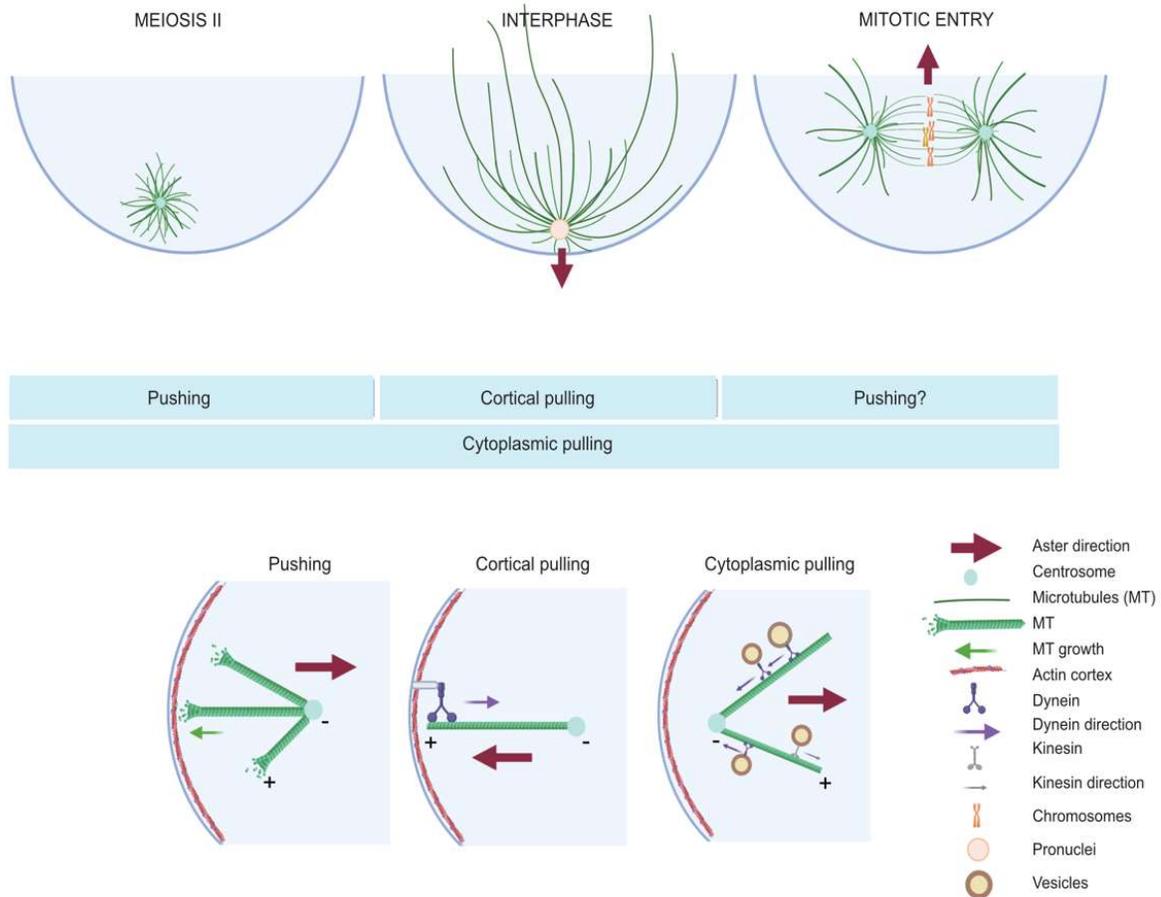
19 Although it has been studied for more than a century, the question of how one cell divides into
20 two equal parts is still not fully resolved. Zygotes have provided much of the mechanistic insight
21 into how the mitotic apparatus finds the center of the cell since the centrally-located mitotic
22 apparatus is created from a large sperm aster that forms at the cortex and thus far from the
23 zygote center. Here we show that in ascidians, the sperm aster extends throughout the cytoplasm
24 during interphase yet remains located near the cortex and does not migrate towards the zygote
25 center. It is only at mitotic entry, when the sperm aster has duplicated and the mitotic apparatus
26 is being assembled, that most of the migration and centration occurs. This temporal pattern of
27 centration behavior is mirrored by primate zygotes (including human). The current mechanisms
28 of aster centration include cytoplasmic pulling that scale with microtubule (MT) length, MT
29 pushing against the proximal cortex or MT-based cortical pulling. However, it is not yet known
30 whether and how these 3 mechanisms are coordinated to prevent aster migration during
31 interphase and trigger migration at mitotic entry. By monitoring quantitatively all three
32 mechanisms (cytoplasmic pulling, pushing and cortical pulling) we have discovered that
33 cortical pulling is switched off as the zygote enters mitosis while both cytoplasmic pulling and
34 proximal cortical pushing remain active. Physical simulations could recapitulate both the static
35 and migratory aspects of sperm aster and mitotic apparatus behavior. We therefore surmise that
36 the reduction in cortical pulling at mitotic entry represents a switch that allows proximal cortical
37 pushing forces and cytoplasmic pulling forces to center the nascent mitotic apparatus.

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40 **Graphical abstract**

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43 **Highlights**

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- 45 • Sperm aster/mitotic apparatus centration occurs at entry into first mitosis
- 46 • MT-based cortical pulling is active during interphase and switched off at mitotic entry
- 47 • Loss of cortical pulling at mitosis entry facilitates centration of the aster
- 48 • MT-based cytoplasmic pulling is active during both interphase and mitosis
- 49 • Agent-based simulations advocate the need for cytoplasmic pulling, a switch in cortical
- 50 pulling and a minor role of pushing for aster centration at mitotic entry.

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55 Introduction

56 Asters are intracellular structures of microtubules (MTs) radially organized around a
57 microtubule organizing center (MTOC), generally a centrosome (Bornens, 2012), or self-
58 organized in presence of molecular motors (Mitchison and Field, 2021; Nédélec et al., 1997).
59 The cell may contain a single aster, as in the case of the sperm aster, or two asters following
60 centrosome duplication, which localize at each pole of the mitotic spindle (Meaders and
61 Burgess, 2020). Asters are involved in many essential functions in the cell including
62 intracellular trafficking and organization (Hamaguchi 1986, Smyth 2015), polarity
63 establishment (Wodarz, 2002), guidance of nuclei (Reinsch and Gönczy, 1998), and
64 determination of the cleavage axis during cell division (Devore et al., 1989; Strome, 1993).
65 Although we have known for more than a century that the protoplasmic mass of the cell
66 becomes equally segregated to the two daughter cells following cell division (Hertwig, 1884),
67 the precise details of the mechanism of migration and centration of the mitotic apparatus is still
68 not fully resolved.

69 Elegant experiments in amphibians, echinoderms, and *C.elegans* zygotes, as well as in yeast
70 and *in vitro* studies, have reported three possible mechanisms of how MTs can generate forces
71 to displace nuclei and whole asters/MTOCs. One mechanism is based on cytoplasmic pulling
72 (Li and Jiang, 2018; Minc and Piel, 2012; Minc et al., 2011; Pelletier et al., 2020; Wühr et al.,
73 2009), one on cortical pulling (Grill et al., 2001; Kotak and Gönczy, 2013; Redemann et al.,
74 2010), and one on MT pushing (Garzon-Coral et al., 2016; Laan et al., 2012; Meaders and
75 Burgess, 2020; Tran et al., 2001). During centration of the sperm aster cytoplasmic pulling
76 exerts forces on the aster by taking advantage of the transport of organelles that travel on the
77 MTs (Kimura and Kimura, 2011; Tanimoto et al., 2016). A cargo moving towards the MTs
78 minus-end at the centrosome experiences an opposing drag force from the cytoplasm (Longoria
79 and Shubeita, 2013; Palenzuela et al., 2020). Cytoplasmic pulling force thus scales with MT
80 length as more cargoes cover longer MTs (De Simone et al., 2018; Kimura and Onami, 2005).
81 Cortical pulling occurs when MTs contact a minus end directed molecular motor localized on
82 the plasma membrane (Laan et al., 2012) often via interaction with cortical LGN/Pins/GPR1-2
83 and NuMA/Mud/Lin-5 complexes (Pietro et al., 2016). Such minus-end directed motor activity,
84 supported by the rigidity of the actin cortex, can pull on MTs bringing the whole aster and
85 mitotic apparatus (notably during asymmetric cell division) towards the cell membrane (Grill
86 and Hyman, 2005; Grill et al., 2001; Kiyomitsu, 2019). Finally, due to the inherent dynamic
87 instability of MTs (Burbank and Mitchison, 2006) and their polymerization against the
88 membrane and its actomyosin rich cortex (Rosenblatt et al., 2004), MTs can exert a pushing
89 force in the opposite direction to MT growth (Sulerud et al., 2020) and thus lead to displacement
90 of asters away from the cell surface (Meaders and Burgess, 2020). Such force generation may
91 be limited however by MT buckling when the MTs exceed about 20 μ m in length (Dogterom et
92 al., 2005), although this would be more difficult to interpret if short branched MTs were also
93 present (Field and Mitchison, 2018; Petry et al., 2013).

94 Striking examples of sperm aster or mitotic apparatus centration occur following fertilization
95 and have been studied in a number of species. Following fertilization a sperm aster forms from
96 the sperm-derived centriole (in many species) at the site of sperm-egg fusion and thus near the
97 plasma membrane and as a consequence far from the center of the fertilized egg (Ishihara et al.,
98 2014). Interestingly however, the cell cycle phase during which aster migration occurs towards

99 the center of the fertilized egg varies depending on species and is independent of the timing of
100 fertilization. For example, sperm aster centration occurs during first interphase in sea urchin
101 immediately after fertilization (Minc et al., 2011) and in mouse oocytes during interphase
102 following exit of meiosis II (Scheffler et al., 2021) whereas it occurs during first prophase in
103 *C.elegans* (Gönczy et al., 1999). In *C. elegans* the sperm aster remains close to the cortex
104 during interphase. In human oocytes (Asch et al., 1995) as well as those of other primates
105 (Hewitson and Schatten, 2002; Simerly et al., 2019) centration also occurs at entry into mitosis
106 (Asch et al., 1995; Simerly et al., 2019) when the sperm aster has duplicated to become the first
107 mitotic apparatus. It is currently unknown how centration of these highly asymmetrically-
108 positioned sperm asters in primates is 1) prevented during interphase and 2) triggered at mitotic
109 entry.

110 To understand the mechanism of sperm aster/mitotic apparatus centration we analyzed the
111 fertilized eggs of ascidians, which are a sister group to the vertebrates (Delsuc et al., 2006). As
112 in mammals, fertilization occurs during meiotic metaphase in the ascidian (Meta I for ascidians,
113 Meta II for primates/humans). We discovered that the sperm aster in ascidians behaves in a
114 similar manner to those in primates/humans: the sperm aster remains cortical during interphase
115 and migrates as a duplicated mitotic apparatus at mitotic entry. By combining experiments and
116 numerical simulations, we investigated how the asymmetrically positioned sperm aster is
117 prevented from migrating towards the center of the zygote during interphase and in addition
118 how centration is triggered at mitotic entry. We found that cortical pulling is switched off at
119 mitotic entry facilitating aster centration at mitotic entry when both cytoplasmic pulling and
120 pushing are active.

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136 **Results**

137 **Aster migration in *Phallusia mammillata* correlates with the cell cycle**

138 To monitor sperm aster and then mitotic apparatus migration while observing cell cycle stages
139 during the first cell cycle, oocytes of *Phallusia* were co-injected with mRNAs encoding MT-
140 binding protein Ensconsin (Ens::3GFP) and Tomato-tagged histone H2B (H2B::Tom). These
141 oocytes were fertilized and imaged from 10 minutes post fertilization (mpf) until cytokinesis.
142 We observed that the aster changes shape, size and position throughout the cell cycle as detailed
143 in the next paragraph (Figure 1A, Movie 1). These changes were associated with cell cycle
144 stages based on recognizable events: polar body (PB) extrusions, pronuclei formation, female
145 pronucleus (PN) migration, nuclear envelope breakdown (NEB), metaphase and anaphase
146 (Figure 1C, 1D).

147 In ascidians, during the completion of meiosis, the sperm aster grows in the vegetal hemisphere,
148 on the opposite side of the fertilized egg from the meiotic spindle which defines the animal pole
149 (Roegiers et al., 1995). During meiosis I, the aster is in the egg cortex (Dumollard and Sardet,
150 2001) whereas at meiosis II (7 to 20 min after fertilization), the aster is spherical and located a
151 few microns (5-10 μm) from the egg cortex (Fig 1A « meiosis II »). Upon entering interphase,
152 when the pronuclei form (Figure 1A “interphase”), the aster remains close to the plasma
153 membrane in the vegetal hemisphere and MTs elongate. Later in interphase, the MTs reach the
154 opposite side of the zygote thus allowing the capture of the female PN formed at the animal
155 pole (Movie 2). This leads to a highly asymmetric aster with long MTs toward the cell center
156 and animal pole, and shorter MTs towards the vegetal pole. The centrosome then duplicates and
157 the two resulting centrosomes position on each side of the male PN which gives the aster an
158 oblong shape extended along the vegetal cell membrane (Figure 1A, “interphase”). Finally, in
159 prophase, which as in subsequent embryonic mitoses occurs 3-4 minutes before NEB
160 (Dumollard et al., 2013), two asters with short MTs are linked by a central mitotic spindle
161 (Figure 1A “early mitosis”). On the time series shown in Fig. 1A the aster does not move
162 between meiosis and interphase whereas between interphase and early mitosis the aster migrates
163 towards the cell center. The separation of the two centrosomes and the formation of the nascent
164 mitotic apparatus both occur during the studied time period; for clarity purposes we refer to the
165 globality of the aster and subsequent spindle migration as “aster migration”.

166 Aster migration was quantified by measuring the distance between the cell center and the male
167 DNA from 28 different embryos at several cell cycle stages (Figure 1B, Figure S1A). In the
168 embryos pooled in the dataset the DNA was labelled either by expressing H2B::Tomato or
169 H2B::Venus or by staining with Hoechst in live zygotes. The first noticeable event in the course
170 of aster migration was the movement of the aster toward the cell cortex upon entry in interphase.
171 Indeed, between the extrusion of the second polar body and the formation of the pronuclei, the
172 aster moved away from the cell center (Fig. 1B). Then, during interphase (9 minutes duration),
173 the distance between the male DNA and the cell center did not change significantly (Fig. 1B)
174 suggesting that the aster did not significantly migrate. The main movement of migration
175 occurred after pronuclei (PN) fusion, at mitosis onset. The distance of the DNA from the cell
176 center decreased between the PN fusion and NEB and further declined between NEB and
177 metaphase (Fig. 1B). Thus, after its initial growth in meiosis which displaces the aster slightly
178 from the cortex, the sperm aster follows three phases of migration: a first movement brings the
179 aster back to the cortex during which the aster changes shape (between 22 min. and 23 min. in

180 Fig. 1B and 1D), then the aster does not move relative to the cell center during interphase
181 (between 23min. and 35 min. in Fig 1B, 1D), and finally the aster starts centering at mitosis
182 entry (after 35 min. in Figure 1C, 1D). This temporal sequence of aster migration was confirmed
183 by the quantification of fixed samples from batches of zygotes sampled every 10 min and
184 immuno-stained to label MTs and DNA (Figure S1B).

185 **Aster migration requires mitosis entry**

186 The main movement of migration starts at mitosis entry (Figure 1). Therefore, we investigated
187 the causal role of mitosis entry on the aster migration. We monitored DNA position as a read
188 out for aster migration and perturbed the cell cycle by injecting oocytes with p21 protein, a
189 cyclin-dependent kinase (CDK) inhibitor (Figure 2). The presence of p21 perturbs CDK activity
190 (Levasseur et al., 2007) and thus prolongs interphase by delaying entry into first mitosis. The
191 injection was considered successful when interphase (the time between PN formation and NEB)
192 lasted more than twice the mean duration in control zygotes or greater than 24 min (Table S1).

193 In zygotes with an unaltered cell cycle (Figure 2A, top panel) the pronuclei formed, the female
194 PN (orange arrow) migrated and reached the male PN (yellow arrowhead), then the mitotic
195 apparatus migrated towards the cell center during mitosis ($t=17$ min post PN formation). In the
196 p21-injected zygotes with prolonged interphase (Figure 2A, bottom panel), the male and female
197 pronuclei formed and the female PN migrated toward the male PN until it reached the
198 centrosome (Figure 2, $t=16$ min) or the male PN. However, the aster and the male PN never
199 migrated, the aster remained asymmetric and uncentered and astral MTs continued to grow
200 (Figure 2, $t=24$ min).

201 Quantification of the aster migration in the two conditions revealed that in control embryos the
202 distance between the cell center and the male PN decreased from $45 \pm$ SEM μm to $30 \pm$ SEM
203 μm from interphase to mitotic entry (Fig 2B). In contrast, during the same timing of interphase
204 in p21-injected zygotes the distance remained at $45 \pm$ SEM μm , showing an absence of aster
205 migration. Thus, prolonging interphase prevents DNA centration and aster migration.

206 **Cytoplasmic pulling is constant during the cell cycle**

207 Given that the aster specifically centers at mitotic entry, we wondered what could cause the
208 absence of migration in interphase. The three mechanisms able to move the aster are
209 cytoplasmic pulling (Li and Jiang, 2018; Minc et al., 2011; Wühr et al., 2009), cortical pulling
210 (Grill et al., 2001; Kotak and Gönczy, 2013; Redemann et al., 2010) and cortical pushing
211 (Garzon-Coral et al., 2016; Laan et al., 2012; Meaders and Burgess, 2020). Since the aster is
212 highly asymmetric and has long MTs directed towards the animal hemisphere throughout the
213 interior of the zygote during interphase (Figure 3A, Fig. 1A), one could expect to observe an
214 aster migration by cytoplasmic pulling (Minc et al., 2011). Thus, we first tested whether the
215 absence of migration during interphase was caused by a lack of cytoplasmic pulling. We
216 examined whether these long interphasic MTs accumulated or transported organelles towards
217 the aster center, which would indicate minus-end directed transport and cytoplasmic pulling
218 (Kimura and Kimura, 2011). The accumulation of organelles was tested by characterizing
219 minus-end directed transport in different contexts. First, during interphase the sperm aster
220 captured the female PN which migrated towards the sperm aster center and male PN (Figure
221 3B). When imaging endoplasmic reticulum during interphase, most of the ER was accumulated

222 around the aster (Fig 3C). Finally, and in order to characterize more precisely the dynamics of
223 organelle movement towards the sperm aster, we imaged the transport of vesicles endocytosed
224 from the plasma membrane (labeled with the membrane dye Cell Mask Orange) and measured
225 their movement towards the sperm aster in the zygote (Movie 3A and 3B, Figure 3D and Figure
226 S2). The presence of minus-end directed transport of the female PN, the ER, and the
227 endocytosed vesicles along the MTs during interphase demonstrated the presence of
228 cytoplasmic pulling forces during interphase. We then wondered if cytoplasmic pulling may be
229 weaker in interphase than in mitosis perhaps explaining the lack of sperm aster movement
230 during interphase versus mitosis. As a proxy for cytoplasmic pulling, we assessed whether the
231 vesicle traffic changed throughout cell cycle. We imaged endocytic vesicles at high-speed (1
232 image/sec) in one confocal plane and made time projections to visualize vesicle trajectories
233 over the course of 3 minutes (Fig 3E). We categorized the vesicles as vesicles moving towards
234 the aster, moving away from the aster or static (Figure 3E, F, Figure S2). The projection of
235 vesicle tracks in an interphasic versus mitotic zygote illustrated that there is no major difference
236 in interphase versus mitosis (Figure 3E). We then compared the speed and total transport of the
237 endocytosed vesicles (Figure 3F). The speed measured is the mean speed of the vesicles on
238 their total trajectories, and the total transport represents the sum of the distance traveled by all
239 vesicles towards the aster center during the 3 min period. No statistically significant difference
240 between interphase or mitosis was found for vesicle speed or the distance travelled (Figure 3F
241 and Figure S2).

242 To conclude, we have demonstrated that there is retrograde transport and hence, active
243 cytoplasmic pulling forces during interphase, and that the organelle transport is not significantly
244 different in interphase and mitosis. This indicates that another mechanism must account for the
245 difference of aster migration behavior between interphase and mitosis, and especially, for the
246 lack of aster migration in interphase.

247 **Cortical pulling is strong in interphase and weak in mitosis**

248 The asymmetric configuration of the sperm aster in interphase together with the accumulation
249 of organelles at the aster center (Figure 1A, “interphase”) should favor an interphasic aster
250 migration by cytoplasmic pulling in the direction of the longer MTs (Kimura and Kimura, 2011;
251 Tanimoto et al., 2016). Since there was no aster migration despite the presence of cytoplasmic
252 pulling, we hypothesized that some mechanism must prevent aster migration. A candidate
253 mechanism to counteract the centering forces of cytoplasmic pulling is cortical pulling, which
254 is able to off-center an aster (Laan et al., 2012). Cortical pulling occurs when minus-end
255 directed motors bound to the cortex interact with MTs (Laan et al., 2012). The transport of the
256 plasma membrane to the centrosome is prevented by the thick actin cortex which provides a
257 support that resists membrane deformation. Hence, with an intact cortex, instead of bringing
258 the plasma membrane towards the MTs minus-end, the molecular motors pull the centrosome
259 to the plasma membrane (Figure 4A). We analyzed whether such cortical pulling forces would
260 thus restrain the aster from migrating to the cell center.

261 We examined cortical pulling over the zygote cell cycle using a membrane invagination assay
262 (Godard et al., 2021; Redemann et al., 2010; Rodriguez-Garcia et al., 2018). We used two
263 pharmaceutical compounds to disrupt the actin cortex: cytochalasin B or latrunculin B. Both
264 perturbed the cortical resistance necessary to prevent membrane deformation. Thus, by labeling

265 the membrane, invaginations of the plasma membrane towards the centrosome could be
266 observed at sites of cortical pulling (Figure 4B, Figure 4C). Depolymerization of MTs with
267 nocodazole completely inhibits such invaginations (Godard et al., 2021). In zygotes treated with
268 latrunculin, the plasma membrane invaginations appear in a cyclic manner (Figure 4B, Movie
269 4). We determined the mean number of invaginations at different phases of the cell cycle
270 (Figure 4B bottom). Invaginations were never observed at meiosis II (6 minutes before PN
271 formation) while an average of 25 +/- 5 (SEM) invaginations was counted during interphase (5
272 minutes before NEB) that decreased to 7 +/- 5 (SEM) invagination at mitosis (1 minute after
273 NEB) (Figure 4A, 4B bottom). These observations demonstrate that cortical pulling is
274 correlated with the cell cycle as it is not active in meiosis and mitosis but very active in
275 interphase.

276 To test the causality between the cell cycle phases and the presence of active cortical pullers,
277 we performed the membrane invagination assay on 2-cell stage embryos in which one cell was
278 either blocked in mitosis or stalled in interphase while the sister cell kept cycling and served as
279 a non-injected control cell. To manipulate the cell cycle we injected either p21 protein, to delay
280 entry into mitosis, or a truncated non-destructible form of cyclin B protein ($\Delta 90$ -cycB) to
281 prevent exit from metaphase (Figure 4C and Figure S3) (Levasseur and McDougall, 2000). As
282 for the zygotes (Figures 4B) the non-injected cell displayed invaginations in interphase while
283 none were visible in mitosis. We found that in p21-injected cells membrane invaginations were
284 detected all throughout the prolonged interphase (Figure 4C top row and Movie 5A). On the
285 contrary, in cyclin B $\Delta 90$ protein injected cells, no invaginations were visible during the
286 prolonged metaphase, while the non-injected sister cell displayed invaginations during
287 interphase (Figure 4C bottom row and Movie 5B). The absence or presence of plasma
288 membrane invaginations as a function of time and cell cycle phase is displayed for each 2-cell
289 stage embryo (Figure S3). In non-injected cells the invaginations disappeared around the time
290 of NEB (stars in Figure S3), and reappeared about 10-15 min after NEB at mitosis exit. We
291 conclude that cortical pulling is cell cycle dependent, it is up-regulated during interphase and
292 down-regulated at mitotic entry, prometaphase and metaphase.

293 **Evaluation of the necessity of both cortical pulling and MT pushing against the cortex** 294 **for aster migration**

295 Cortical pulling is active in interphase therefore it could be responsible for preventing aster
296 migration before mitotic entry. If cortical pulling is inhibited an early aster migration is
297 expected. The inhibition of cortical pulling was performed by adding latrunculin which perturbs
298 the actin cortex. This drug should also inhibit the pushing mechanism since it also requires an
299 intact cortex. By examining aster migration in the presence of latrunculin, we evaluated the
300 necessity of both cortical pulling and MT pushing against the cortex for aster migration. We
301 found that when latrunculin was added during meiosis, the sperm aster was on average 60 μm
302 away from the cell center and remained so for more than 10 minutes. Then the aster migrated
303 toward the cell center during mitosis (Figure 5A, orange). In contrast, the aster in control
304 DMSO-treated zygotes (blue in Figure 5A) was on average 40 μm away from the center and
305 first migrated slightly towards the cortex at interphase entry (to 45 μm away from the center)
306 and remained in position until mitotic entry, when the aster moved toward the cell center. The
307 initial cortex-directed movement (just before PN formation) was not observed in latrunculin-
308 treated zygotes (Figure 5A). Nevertheless, in both latrunculin and DMSO conditions, the aster

309 migrated approximately 15 μ m (total distance) between PN formation and NEB (Figure 5B),
310 suggesting that cytoplasmic pulling is sufficient to move the aster towards the cell center. Since
311 latrunculin inhibited cortical pushing we suggest that the closer proximity of the sperm aster to
312 the plasma membrane is caused by the lack of efficient cortical pushing in meiosis. At two
313 minutes before NEB we noted a slower (though not significant) pace of migration in latrunculin-
314 treated zygotes (Fig 5B) which suggests that an additional mechanism other than cytoplasmic
315 pulling may support aster migration at mitotic entry in control condition.

316 To sum up, the absence of a clear early aster migration when cortical pulling is inhibited (Figure
317 5A, 5B) shows that, in addition to cytoplasmic pulling which can center the aster (Figure 5A),
318 a second mechanism is involved at mitotic entry to permit the fast aster migration. Since the
319 inhibition of cortical pulling is accompanied by the inhibition of pushing, we hypothesized that
320 the pushing mechanism could be an actor of aster centration at mitotic entry, thus we sought to
321 determine when the pushing mechanism is active.

322 **Cortical pushing is active in meiosis and mitosis**

323 Following latrunculin treatment the aster was closer to the membrane than it was in control
324 zygotes. We suggest that the closer proximity of the sperm aster to the plasma membrane caused
325 by latrunculin could be explained by the lack of cortical pushing during meiosis II (Figure 5A).
326 Indeed, we observed during meiosis II that the presence of an aster close to plasma membrane
327 with a perturbed actin cortex creates thin membrane protrusions (i.e., a membrane deformation
328 towards the exterior, Figure 6A). These deformations were not stable as they grew and shrunk
329 (Movie 6) and co-localized with MTs (Figure 6B). This co-localization of MTs and plasma
330 membrane as well as the dynamic nature of the protrusions suggested that the protrusions were
331 caused by MT polymerization. It also supported the notion that an actin cortex was required for
332 the pushing mechanism to move the nascent sperm aster away from the cortex. The protrusions
333 were visible in meiosis, and in cases where the aster did not migrate during the cell cycle, thus
334 permitting the observation of protrusions throughout the complete cell cycle. Protrusions were
335 absent in interphase and present again just after NEB (Figure 6B, Movie 6). These deformations
336 thus appeared to follow the cell cycle as do microtubules themselves, being more dynamic
337 during mitosis but long and stable during interphase (Figure 1). These latter observations must
338 however be taken with caution since the absence of protrusion in interphase could also be due
339 to the presence of the nucleus that may prevent direct contact of MTs on the membrane.

340 **A computer simulation to challenge the contribution of cortical pulling**

341 Based on all the results, we propose the following mechanism for aster migration (see graphical
342 abstract). The sperm aster grows in meiosis and moves off the cortex by pushing and potentially
343 by cytoplasmic pulling. In interphase, cortical pulling brings the aster back to the cortex, despite
344 the constant presence of cytoplasmic pulling. Finally, entry into mitosis triggers the migration
345 of the spindle away from the cortex: cortical pulling stops at mitotic entry, MTs become more
346 dynamic, and the mitotic apparatus moves by combined cytoplasmic pulling and cortical
347 pushing. We designed a 2D agent-based stochastic computer model of aster migration based on
348 the software *Cytosim* (Foethke et al., 2009) (Figure 7A, Movie 7). The parameters of model
349 simulations were set according to past studies on different species (Sup Table S2), and adjusted
350 to our data to match the timing of cell cycle and the cortical pulling activity. We verified the
351 equivalence of the simulated and observed cytoplasmic pulling by comparing a simulation to

352 latrunculin data (Figure S4 A and B, first row). Once an average reference simulation was
353 established, we could remove specific forces to test the contribution of cortical pulling in the
354 global scenario of aster migration (Figure 7), or to explore other situations (Figure S4). With a
355 permanent cortical pulling the aster is quickly brought to the cell cortex in meiosis and did not
356 seem to leave the cortex during the cell cycle (Figure 7A 7B, second row). Thus, in this
357 condition the migration did not fit the control profile of aster migration as the aster was always
358 further from the cell center than in control (Figure 7B, second row).

359 In the complete absence of cortical pulling throughout the cell cycle (Figure 7, third row) the
360 aster position was slightly more centered in interphase, and the overall aster centration hence
361 reached the same distance from the cell center as in the average reference simulation (Figure 7
362 first and third rows). Indeed, in the absence of cortical pulling the aster centered as much as it
363 did in the control dataset, however the centration began earlier, progressed at a constant
364 velocity, and did not show the static phase prior to aster migration (Figure 7B, third row). The
365 comparisons of simulations outputs to the data supported the scenario drawn from our results
366 where aster centration is prevented by cortical pulling activity during interphase, and the
367 inactivation of cortical pulling in mitosis permits aster movement. Only relying on simulations,
368 we pushed the exploration of our model on questions still experimentally unanswered. These
369 theoretical explorations suggested that cytoplasmic pulling is the main contributor to aster
370 centration in mitosis compared to the pushing mechanism (Sup Figure S4A, S4B, bottom row).
371 Finally, we used the simulation to examine a scenario where cortical pulling is nonuniformly
372 inactivated at mitosis entry i.e., if cortical pulling is first turned off near the aster before being
373 completely inactivated (Figure S4C). In this case, the aster centration during mitosis was faster
374 than that obtained by simulating uniform inactivation (Figure 7 top) and the simulation best
375 fitted the experimental data (Figure S4C).

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382 Discussion

383 A central question in the field of cell biology is how a cell divides into two equal sized daughter
384 cells which relies on positioning of the mitotic apparatus to the cell center. The mechanism(s)
385 involved in centering are still not fully resolved although they often depend on MT-based
386 cortical pulling, cytoplasmic pulling or cortical pushing depending on the species, cell type and
387 cell cycle stage. Centration has been studied extensively in *Xenopus*, sea urchin and *C. elegans*
388 zygotes. In the large oocytes of *Xenopus* (circa 1mm) cytoplasmic pulling forces provide the
389 force for aster centration. For example, by microinjecting a dominant negative fragment of the
390 dynactin complex (p150-CC1) sperm aster centration was blocked (Wühr et al., 2010). Also,
391 since MTs of the sperm aster are too short to reach the cortex on the opposite side of the zygote
392 the primary mechanism for centration in *Xenopus* is cytoplasmic pulling in the direction of the
393 longest MTs (Wühr et al., 2010). Sea urchins have smaller zygotes (circa 100µm) and even
394 though MTs of the sperm aster are long enough to reach the opposite cortex, cortical pulling
395 has not been reported to be involved and instead the two mechanisms reported to power sperm
396 aster centration in sea urchin are based on cytoplasmic pulling (Tanimoto et al., 2016) and
397 cortical pushing (Meaders et al., 2020). In *C. elegans* centration occurs during prophase in a
398 dynein-dependent manner (Gönczy et al., 1999). In order to distinguish between cortical and
399 cytoplasmic dynein, factors that recruit dynein to the cortex have been depleted. RNAi
400 knockdown of *goa-1/gpa-16* to deplete cortical dynein led to a higher velocity of sperm aster
401 centration suggesting that cytoplasmic dynein was the primary force generating mechanism for
402 centration (De Simone et al., 2018). In addition, since depletion of cortical dynein increased the
403 velocity of centration cortical pulling forces were suggested to counteract the cytoplasmic
404 forces that power centration of the sperm aster (De Simone et al., 2018).

405 The examples of sperm aster centration in *Xenopus* and sea urchin occur during interphase while
406 in *C. elegans*, primates (Asch et al., 1995; Hewitson and Schatten, 2002; Simerly et al., 2019)
407 and ascidian (here) centration occurs at mitotic entry. Moreover, the position and geometry of
408 the ascidian sperm aster, with long MTs extending into the zygote interior and short MTs
409 directed towards the proximal vegetal cortex, represents a configuration that should favor
410 cytoplasmic pulling to displace the sperm aster towards the zygote center. We were therefore
411 curious about what prevented sperm aster centration until mitotic entry. A cell cycle link that
412 triggers an increase in cortical pulling had been reported previously in *C. elegans* one cell stage
413 embryos (Bouvrais et al., 2021; McCarthy Campbell et al., 2009; Redemann et al., 2010). An
414 increase in posterior cortical pulling forces displaces the mitotic apparatus towards the posterior
415 cortex during anaphase (Grill et al., 2003; McCarthy Campbell et al., 2009; Redemann et al.,
416 2010). Indeed, the number of short-lived MT plus ends engaged in cortical pulling increased at
417 the posterior pole of *C. elegans* one cell stage embryos at anaphase onset (Bouvrais et al., 2021).
418 This increase in cortical pulling has been linked with the fall in cyclin-dependent kinase (CDK)
419 1 activity: either reducing the function of the proteasome, the APC (anaphase-promoting
420 complex), or Cdc20 all delayed spindle displacement while inactivating CDK1 in prometaphase
421 caused premature spindle displacement (McCarthy Campbell et al., 2009). Although these
422 findings indicate that cortical pulling increases at anaphase (Keshri et al., 2020; Kotak et al.,
423 2013) one key additional point may be that cortical pulling is less prominent when CDK1
424 activity is elevated. Here in the ascidian, we have noted a similar phenomenon whereby cortical
425 pulling is elevated during interphase and reduced at mitotic entry when CDK1 activity
426 increases.

427 In the ascidian *P. mammillata*, the sperm aster forms in the vegetal hemisphere of the zygote
428 during meiosis II (Roegiers et al., 1995). Here we demonstrate that during meiosis II the sperm

429 aster grows and remains roughly spherical as it moves slowly away from the proximal vegetal
430 cortex (Figure 1). At this stage the sperm aster remains relatively small (approx. 1/3 zygote
431 diameter) and located in the zygote vegetal hemisphere. Such a vegetal location and relatively
432 small size ensures that sperm astral MTs do not reach the animal pole and thus interfere with
433 the segregation of the meiotic chromosomes, which in smaller *C.elegans* zygotes is
434 accomplished by preventing sperm aster growth during meiosis II (McNally et al., 2012). At
435 entry into interphase astral MTs extend throughout the zygote and capture the female PN
436 located at the animal pole (Figure 1). The female PN then migrates towards the center of the
437 sperm aster during a short interphase (about 10 min) while the cortically-located and highly
438 asymmetric sperm aster remains in position near the vegetal pole (Figure 1). Just prior to NEB,
439 during prophase, the sperm aster begins abruptly to migrate accompanied by NEB and
440 formation of a bipolar mitotic apparatus (Figure 1).

441 What triggers the switch to induce migration at entry into mitosis? First, we delayed entry into
442 mitosis to determine if a causal relationship existed between entry into mitosis and sperm aster
443 migration. Delaying entry into mitosis with the CDK inhibitor p21 prevented sperm aster
444 migration (Figure 2). Next, we teased apart the relative contributions of cortical pushing,
445 cytoplasmic pulling, and cortical pulling to determine which of these three mechanisms
446 displayed a cell cycle-dependent change at mitotic entry that could explain how mitotic entry
447 triggered sperm aster migration. Cortical pushing is present in Meiosis II, and is responsible for
448 the initial aster displacement from the cortex (Figure 6). Even though it seems this mechanism
449 is also active in mitosis (Figure 6B), its contribution to spindle migration at mitosis entry seems
450 minor (Figure S4). We therefore focused on cytoplasmic pulling and cortical pulling.
451 Cytoplasmic pulling can be visualized through the movement of three different endomembrane
452 structures towards the center of the sperm aster: the female PN, endoplasmic reticulum and
453 vesicles (Figure 3). Taking advantage of the opportunity to monitor a large number of vesicles
454 (labelled with Cell Mask Orange) we quantified the movement of the cytoplasmic vesicles to
455 determine whether there was a measurable difference in cytoplasmic pulling between interphase
456 and mitotic entry (Figure 3 and Figure S2). The data demonstrated that vesicles transport was
457 unchanged between interphase and mitotic entry, suggesting a constant cytoplasmic pulling.
458 We then sought to determine whether cortical pulling was more prominent during interphase or
459 mitosis. To do so we exploited the membrane invagination assay following weakening of the
460 cortex (Godard et al., 2021; Redemann et al., 2010; Rodriguez-Garcia et al., 2018).
461 Interestingly, we noted that cortical pulling was greater during interphase than mitotic
462 metaphase (Figure 4). We therefore devised a 2-cell stage assay to determine whether cortical
463 pulling was a feature of interphase and switched off at mitotic entry. By either delaying mitotic
464 entry with p21 or blocking exit from metaphase with $\Delta 90$ cyclin B in one sister cell (Figure 4)
465 we demonstrated that cortical pulling is elevated during interphase and switched off at mitotic
466 entry when CDK1 activity is elevated. This observation could explain the switch-like behavior
467 in migration and suggests that it is the increase in CDK1 activity at mitotic entry that switches
468 off cortical pulling thus liberating the sperm aster from its cortical tethers facilitating centration.
469 Moreover, these data develop further the findings from *C. elegans* where cortical pulling was
470 decreased when CDK activity was elevated (McCarthy Campbell et al., 2009).

471 By using the software *Cytosim* we tested whether cortical pulling could prevent aster migration
472 mediated by cytoplasmic pulling. Simulations showed that cortical pulling was capable of
473 preventing migration caused by long MT-mediated cytoplasmic pulling and re-enforce the data
474 showing that the sperm aster does not migrate during interphase when cortical pulling is
475 elevated (Figure 7). This supports the idea that switching off cortical pulling at mitotic entry is
476 necessary for sperm aster migration. Furthermore, the simulation indicated that a total absence

477 of cytoplasmic pulling prevented aster migration in mitosis (Figure S4). Overall, these findings
478 demonstrate that mitotic apparatus migration in the ascidian occurs at mitotic entry which
479 causes the switching off of cortical pulling while cytoplasmic pulling and pushing remain
480 active. It would be interesting to examine the relationship between mitotic entry and sperm aster
481 migration in primate zygotes to determine whether cortical pulling is also switched off at mitotic
482 entry.

483

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506 **Figure legends**

507 **Figure 1. Migration of the sperm aster correlates with the cell cycle**

508 A. Confocal z-sections from a xyzt series showing microtubules in a live zygotes labeled with
509 Ens::3GFP. Meiosis II, interphase and mitosis (early and late) are displayed. See Movie 1.

510 B. Quantification of the distance (in μm) between the male DNA and cell center measured in
511 3D at each event (see Materials and Methods) during the first cell cycle in 28 live zygotes.
512 Graph showing the distance of the male DNA from the cell center (in μm) at each cell cycle
513 event. The x-axis shows the cell cycle phases corresponding to the time points measured
514 (bottom of the graph), and the timing of the zygote shown in Fig 1D relative to cell cycle event
515 is indicated (top of the graph). The time scale is not linear but adjusted to spread evenly all cell
516 cycle events. The graph is colored according to the cell cycle phases: meiosis II (light grey),
517 interphase (medium grey) and mitosis (darker gray). Error bars represent SEM. Paired t-test
518 adjusted with bonferroni, p-value ≤ 0.05 (*), ≤ 0.01 (**), ≤ 0.001 (***), p-value > 0.05 (ns).

519 C. Schematic representation of the zygotic events that mark the first cell cycle and how they
520 associate with DNA displacement in the cell. X in the first drawing marks the cell center from
521 which distances are measured. Male pronucleus (m).

522 D. Confocal images from a timelapse movie of a zygote with microtubules labelled with
523 ENS::3GFP corresponding to the schematic in C. See also Movie 1.

524 Scale bars in A and D are $50\mu\text{m}$.

525

526 **Figure 2. Entry into mitosis triggers aster migration**

527 (A) Representative time-lapse series of zygotes expressing either H2B::RFP (top panel) or
528 injected with the cyclin-dependent kinase inhibitor p21::GFP protein (bottom panel). The male
529 PN position, reflecting the aster position is shown by the DNA signal in H2B::RFP zygote and
530 by the nuclear localization of p21::GFP in p21 injected zygotes. Male PN is indicated with a
531 yellow arrowhead, female PN with an orange arrow. Time in minutes is indicated on each panel.
532 Scale bars are $50\mu\text{m}$.

533 (B) Quantification of the distance between the male DNA and the cell center (in μm) during
534 interphase (hatched bars, 6 min after PN formation) and during mitosis entry (shaded bar, on
535 average at 12 min after PN formation in controls) in p21-injected zygotes and in zygotes with
536 an unaltered cell cycle. Error bars represent SEM. Paired t test. n=17 p21-injected embryos and
537 n=17 control embryos.

538

539 **Figure 3. Characterization of minus-end directed transport in zygotes**

540 A. Confocal image of a zygote fixed in interphase and immuno-labeled with the anti-tubulin
541 antibody DM1a (white), and stained for DNA with Hoechst (blue). Long microtubules crossing
542 the whole zygote are observed during interphase.

543 B. Time projection of a bright field movie of a zygote showing migration of the female PN
544 towards the male PN during interphase. Yellow dots show the female PN position at several
545 time intervals. The male pronucleus is indicated by a white asterisk (*). An orange arrow
546 indicates initial position of the female PN. See also Movie 2.

547 C. Confocal image showing ER distribution in a zygote expressing Venus::Reticulon (cyan).
548 Note that most of the ER is accumulated around the aster at this stage. The male PN is indicated
549 by a white asterisk (*).

550 D. Confocal image of a zygote stained with Cell Mask Orange showing endocytic vesicles
551 accumulated at the sperm aster (green). The male PN is indicated by a white asterisk (*). Also
552 see Movies 3A and 3B. All scale bars are 50 μ m.

553 E. Left, drawing explaining vesicle trajectories (left panel). Vesicles were classified as moving
554 towards the aster center (retrograde in green), or away from the aster center (anterograde in
555 magenta). Right, time projections over 3 min of a zygote treated with Cell Mask Orange (CMo)
556 in interphase and in mitosis showing the tracks of vesicles endocytosed from plasma membrane.
557 Tracks of anterograde vesicles are in magenta, retrograde vesicles in green. The white circle
558 denotes the area in which vesicle were quantified.

559 F. Quantification of the speed (in μ m/sec) and total transport of CMo vesicles going towards
560 the aster. Vesicles were imaged during 3 minutes of interphase and during 3 minutes of mitosis.
561 Speed is measured over the complete vesicle trajectory, including pauses (left graph) and Total
562 transport is the cumulated distance travelled by all retrograde vesicles in direction of the aster
563 center (right graph). Box plot central mark indicates the median, bottom and top edges of the
564 box indicate the 25th and 75th percentiles. The whiskers extend to the most extreme data points
565 without considering outliers. Paired Wilcoxon test. n=8 embryos.

566

567 **Figure 4. Cortical pulling is stronger in interphase**

568 A. Schematic representation of the invagination assay that tests the presence of cortical pullers
569 attached to the membrane.

570 B. Top panel: Confocal images of the membrane invaginations in meiosis, interphase and
571 mitosis, in a representative zygote treated with latrunculin and labelled with CMo. Images are
572 a z-projection of 10 images from a 25 μ m-thick z-stack of confocal images. Bottom panel:
573 Number of invaginations in meiosis (meiosis II), in interphase (5 min before NEB) and in
574 mitosis (1 min after NEB). Error bars represent +/- SD. n=7 embryos. Also see Movie 4.

575 C. Schematic representation of the injection experiments to perturb cell cycle in one cell of 2
576 cell stage embryo. Plasma membrane was labelled by microinjection of PH::GFP mRNA into
577 eggs prior to fertilization, then at the 2-cell stage 1 cell was injected with the indicated proteins.
578 Images on left show plasma membrane label (white) in both the injected cell and the control
579 sister cell. Images are a projection of 10 images from a 10 μ m-thick z-stacks of confocal images
580 at different time points extracted from xyz movies. The cell cycle stage of each cell is indicated
581 above and below the BF images. Plasma membrane invaginations are observed only in cells in
582 interphase (whether from control side or protein-injected side of the embryos). Black arrow
583 indicates the presence of a nucleus. n=11 embryos for p21 and n=9 embryos for Δ 90. Also see
584 Movies 5A and 5B.

585

586 **Figure 5. In absence of cortical pulling and pushing the aster migration is not advanced** 587 **in time.**

588 A. Average distance (in μ m) from DNA position to cell center over time. The distances are
589 measured in two conditions; in presence of DMSO (control, blue curve, n=16) and in presence
590 of latrunculin (orange, n=20), an actin polymerization inhibitor. To compute the average curve,
591 individual curves were aligned with respect to the time of NEB, here represented by a dotted
592 line. The time from PN formation to NEB is not equivalent in each embryo, thus PN formation

593 is indicated as a span in gray shading. Mitosis is also indicated with a gray shading. The orange
594 and blue shades represent \pm SD, Error bars = \pm SEM.

595 B. Distance travelled by the sperm aster towards the cell center following latrunculin (orange
596 bars) or DMSO (blue bars) treatment. All comparisons are not statistically different except for
597 timepoint 6 to 4 min before NEB which corresponds to the time when the sperm aster is
598 flattened against the cortex in interphase. Error bars = \pm SEM. Wilcoxon test.

599

600 **Figure 6. MTs push on the plasma membrane**

601 A. Representative confocal images of a control zygote (top panel) and of a zygote treated with
602 latrunculin (bottom panel), both in meiosis and expressing the microtubule marker Ens::3GFP
603 and labeled with the plasma membrane dye Cell Mask Orange. Scale bar is 50 μ m.

604 B. Close-up of A on the area containing the sperm aster and the closest piece of plasma
605 membrane in meiosis (first column), in interphase (second column), and mitosis (third column)
606 extracted from time-series of the same zygotes. Black arrows indicate the presence of a nucleus,
607 white arrowheads indicate the presence of thin protrusions. Also see Movie 6. n=3 embryos out
608 of the 9 where protrusions were seen in meiosis. Scale bar is 10 μ m.

609

610 **Figure 7. Cortical pulling dictates the pattern of aster migration**

611 A. Stills from simulations testing the contribution of cortical pulling in the model of aster
612 migration. The selected frames correspond to times of meiosis, interphase and mitosis in the
613 simulation. The mitotic apparatus was simplified as an aster. The aster core (centrosome) is
614 represented by a purple dot at the center of the MTs structure. MTs (in white) are set to become
615 more stable in interphase. The purple cell border illustrates the activity of cortical dyneins, and
616 thus of cortical pulling. The many gray dots represent fixed dyneins in the cytoplasm to reflect
617 the presence of cytoplasmic pulling in the cell. They become green when attached to MTs.
618 Three conditions were tested: a transient cortical pulling in interphase (first row) that serves as
619 a reference simulation, a permanent cortical pulling (second row), and an absence of cortical
620 pulling (third row).

621 B. Comparison of the pattern of aster migration shown as the distance of the aster from the cell
622 center through time, in control experimental data (blue curve), and in the simulations (black
623 curve). The blue and gray shades represent \pm SD. The red margin represents the period of
624 cortical pulling activity in the corresponding simulation. The graphics are aligned with A so
625 that the simulation corresponding to the graphic appear on the same row.

626

627

628 **Supplementary Figures and Table**

629 **Supplementary Figure S1.** Live and fixed data displaying sperm aster position following
630 fertilization.

631 A. Sperm aster position relative to the cell center as a function of time in live zygotes. All
632 individual zygotes exploited in Figure 1 are displayed and aligned with respect to their time of
633 NEB, indicated by a red dotted line.

634 B. Sperm aster position relative to the cell center in fixed zygotes.

635

636 **Supplementary Figure S2.** Vesicle movement parameters.

637 A. Schematic showing vesicles either moving towards (green), away from (pink) the sperm
638 aster center, or stationary (gray arrow). The graph shows the mean percentage of the 2 types of
639 moving vesicles in interphase and in mitosis. The majority of vesicles are stationary (not shown
640 in the graph).

641 B. Statistical comparison of interphase versus mitosis for vesicle movement parameters: mean
642 speed, track persistence, and total transport. Track persistence is the distance traveled towards
643 the aster over the total trajectory of the vesicle. Total transport is the cumulative distance
644 travelled by all vesicles during the 3 minutes time period. No significant difference was found.
645 n=8 embryos, mean number of vesicles tracked per embryo = 1129 in interphase, 1734 in
646 mitosis. Paired Wilcoxon test.

647 C. Statistical comparison of vesicles moving towards or away from the aster center. From the
648 same dataset as presented in B. Paired Wilcoxon test .

649

650 **Supplementary Figure S3.** Effect of cell cycle inhibitors p21 and $\Delta 90$ cyclin B on the presence
651 of membrane invaginations

652 Compiled data showing phases of membrane invaginations as a function of time. 2-cell stage
653 embryos in which membrane was fluorescently labelled (by previous injection of PH::tomato
654 mRNA or incubation in CellMask Orange) were injected with cell cycle inhibitors then treated
655 with cytochalasin and invaginations were imaged by confocal microscopy. Each line represents
656 1 cell. Dark bars represent the presence of invaginations and light bars the absence of
657 invaginations for control cells (n=20), cells injected with $\Delta 90$ Cyclin B protein which blocks in
658 metaphase (n=9) or cells injected with p21 protein which prolongs interphase (n=11). *
659 represents time of NEB. Also see Movies 5A and 5B.

660 **Supplementary Figure S4.** Contribution of pushing and cytoplasmic pulling in aster
661 centration

662 A. Stills from simulations testing the contribution of pushing and cytoplasmic pulling in the
663 aster migration pattern. Like previously (Figure 7), the selected frames correspond to times of
664 meiosis, interphase and mitosis in the simulation. The aster core (centrosome) is represented by
665 a purple dot at the center of the MTs structure. MTs are in white, they are set to become more
666 stable in interphase. The purple cell border illustrates the activity of cortical dyneins, and thus

667 an active cortical pulling. The many gray dots represent fixed dyneins and thus the presence of
668 cytoplasmic pulling in the cell. They become green when attached to MTs. Three conditions
669 were tested. To test the contribution of cytoplasmic pulling alone (first row), as performed
670 experimentally by destroying the cell cortex (Figure 5), the pushing was removed by allowing
671 MTs to ignore boundaries, and the cortical pulling was drastically reduced. Because MTs ignore
672 boundaries, their plus end is often located outside the cell at interphase onset, so cortical dyneins
673 were allowed to bind on all MTs, not only their plus end. The cortical motors were still allowed
674 to bind MT but, as seen with the phenomenon of membrane invagination (Figure 4), they were
675 pulled towards the MT rather than strongly attached to the cell boundary. The second row shows
676 the inhibition of the cytoplasmic pulling, where the fixed cytoplasmic dyneins were removed.
677 Finally, in the third row we tested the inhibition of the pushing mechanism without affecting
678 the cortical pulling, which was experimentally impossible with the tool used in this study. MTs
679 were not allowed to ignore the cell boundary, thus the inhibition of pushing was done by
680 preventing the MT to have a grip on the cell boundary.

681 B. Aster migration, as the distance of the aster from the cell center through time, in simulations
682 (black curve) compared to control experimental data (blue curve) or latrunculin-treatment
683 (orange curves). The blue, orange and grey shades represent \pm SD. The red rectangle shading
684 indicates the period of cortical pulling activity in the corresponding simulation. Each graph in
685 B is aligned with the corresponding simulation in A.

686 C. Simulation exploring a mechanism of aster migration where, at mitosis entry, the cortical
687 pulling is first turned off near the aster before being completely inactivated. The resulting
688 migration (black curve) is compared to control data (blue). The green margin shows the moment
689 of asymmetric cortical pulling.

690

691 **Supplementary Table S1.** Injection of p21 prolongs interphase

692 Analysis of the effect of p21 on the duration of cell cycle phases. Comparison between control
693 and p21-injected zygotes for the duration between pronucleus formation to NEB and between
694 NEB and cytokinesis. p21 significantly prolonged first interphase since the average duration
695 from PN formation to NEB went from 13 min to 28 min. n is displayed together with the SD.

696

697 **Supplementary Table S2.** Parameters used in the simulations related to Figure 7 and Figure
698 S4

699 The *Cytosim* parameters used to create the simulations are listed in the table along with the
700 corresponding references.

701

702

703

704

705

706 **Supplementary Movies**

707 **Movie 1.** Confocal timelapse of aster formation and migration.

708 Confocal z-sections from a xyzt series showing microtubules in a live zygotes injected with
709 Ens::3GFP. Scale bar is 50 μ m. <http://movincell.org/medias/609>

710

711 **Movie 2.** Female PN migration.

712 Confocal z-section from a timelapse series showing microtubule plus ends and associated
713 brightfield showing female pronuclear migration. Microtubules are labelled with EB3::GFP.

714 <http://movincell.org/medias/610>

715

716 **Movie 3.** Cell Mask Orange labelled vesicles accumulating at the sperm aster

717 A. Confocal timeseries with images collected every second in one z plane. The sperm aster is
718 at the bottom and accumulates red fluorescence as vesicles become localized to the sperm aster.
719 <http://movincell.org/medias/611>

720 B. Confocal timeseries with images collected every second in one z plane. Here the zygote was
721 compressed. The sperm aster is at the bottom and accumulates red fluorescence as vesicles
722 become localized to the sperm aster. The female pronucleus in compressed zygotes formed
723 karyomeres which can be seen migrating towards the sperm aster.
724 <http://movincell.org/medias/612>

725

726 **Movie 4.** Cycling invaginations in the zygote

727 Zygote treated with latrunculin and labelled with Cell Mask Orange. No membrane
728 invaginations are present during meiosis II. Membrane invaginations first become visible
729 during interphase. The membrane invaginations are subsequently lost at mitotic entry. Scale bar
730 = 50 μ m. <http://movincell.org/medias/613>

731

732 **Movie 5.** Membrane invagination during interphase in 2 cell stage embryo with one cell injected
733 with either p21 or Δ 90 Cyclin B.

734 A. Confocal timeseries of 2-cell embryo treated with latrunculin and labelled with Cell Mask
735 Orange (rendered cyan). Membrane invaginations are present in the non-injected cell during
736 interphase and are permanently present in the p21::GFP-injected cell (rendered magenta, right
737 cell) during the prolonged interphase. Scale bar is 50 μ m. <http://movincell.org/medias/614>

738

739 B. Confocal timeseries of 2-cell embryo treated with latrunculin and labelled with Cell Mask
740 Orange (rendered cyan). Membrane invaginations are present in the non-injected cell only
741 during interphase, and not in $\Delta 90$ Cyclin B::GFP-injected cell (rendered magenta, cell on the
742 right). Scale bar is 50 μ m. <http://movincell.org/medias/615>

743

744 **Movie 6.** MT pushing on plasma membrane in meiosis/interphase/mitosis.

745 Confocal timeseries of the outward membrane protrusions in the zygote. Microtubules are
746 labelled with Ensconsin::3GFP, the plasma membrane is labelled with Cell Mask Orange and
747 the zygote is treated with latrunculin. <http://movincell.org/medias/616>

748

749 **Movie 7.** Simulation of the aster migration

750 2D simulation of the aster migration from mid-meiosis to mitosis. The aster core (centrosome)
751 is represented by a purple dot at the center of the MTs structure. MTs (in white), are set to
752 become more stable in interphase and when they touch the cortex. The purple cell border
753 illustrates the activity of cortical dyneins, and thus an active cortical pulling, also indicating
754 interphase. The many grey dots represent fixed dyneins and thus the presence of cytoplasmic
755 pulling in the cell. They become green when attached to MTs. <http://movincell.org/medias/617>

756 **Materials and Methods**

757 **Biological material**

758 *Phallusia mammillata* adult animals were collected in Roscoff or Sète and kept at 16°C in the
759 aquaria of the “Centre de Ressources Biologiques” (CRB) of the Institut de la Mer à
760 Villefranche (IMEV) which is an EMBRC-France certified service (see [https://www.embrc-
761 france.fr/fr/nos-services/fourniture-de-ressources-biologiques/organismes-modeles/ascidie-
762 phallusia-mammillata](https://www.embrc-france.fr/fr/nos-services/fourniture-de-ressources-biologiques/organismes-modeles/ascidie-phallusia-mammillata)). The gametes were collected by puncturing separately the oviduct and
763 the sperm duct. The sperm was kept dry at 4°C and could be used for fertilization up to 1 week
764 after collection. Oocytes were used the day of collection after undergoing dechoriation by
765 incubation in 0.1-0.2% trypsin in micro-filtered natural sea water (MSFW) at 19°C for 90
766 minutes, and subsequent washes in MSFW supplemented with 5mM TAPS
767 (tris(hydroxymethyl)methylamino] propanesulfonic acid) pH 8.2. All the subsequent
768 manipulations of live embryos were performed in MSFW 5mM TAPS, using pipette tips,
769 dishes, slides and coverslips coated with 0.1% gelatin and 0.1% formaldehyde (Sardet et al.,
770 2011). For fertilization, a small volume of activated sperm (circa 5µl) was added to the oocytes
771 in a 5ml petri dish. To activate the sperm, 6 µL of dry sperm was incubated for 20 minutes at
772 19°C in 500µL of MSFW pH9.2. Time post fertilization was measured starting when the oocyte
773 first showed a shape change. For fixation of fertilized cultures, the time of fertilization was
774 determined when about 30% of the oocytes showed the first deformation.

775 **mRNA synthesis and injections**

776 Synthetic mRNAs for microinjection were prepared using the mMESSAGE mMACHINE T3
777 kit (Ambion), from plasmids containing the gene of interest (EB3::3GFP, Ensconsin::GFP,
778 PH::GFP, Histone H2b::Rfp1, Venus::Reticulon) flanked by a T3 promoter and a polyA tail.
779 mRNA yield was estimated by spectrophotometry. The mRNAs were stored at high
780 concentration (>10µg/µL) in 1 µl aliquots at -80 °C, then thawed and diluted in distilled water
781 for use. The mRNAs were micro injected into dechorionated oocytes transferred to small glass
782 wedges mounted onto 400 µl Perspex mounting chambers designed for horizontal
783 microinjection (see detailed protocols in McDougall et al., 2014). mRNAs were injected at a
784 pipette concentration of 5-6 µg/µl (injection volume is ~1-2% volume of the egg) using a high
785 pressure system (Narishige IM300). mRNA-injected oocytes were left for 5 h or overnight
786 before fertilization and subsequent confocal imaging.

787 **Protein purification and injections**

788 The p21::mCherry protein (p21 is the cyclin dependent kinase inhibitor 1a) was injected to
789 arrest the cell cycle in interphase. The human p21 protein fused with mCherry was cloned in a
790 pET11a vector with 6 His-tag, and purified with a silica-based resin column (MACHEREY-
791 NAGEL, protino Ni-IDA). Aliquots were flash frozen in liquid nitrogen and stored at -80°C.
792 The protein was injected at a concentration of 30mg/ml.

793 The construction and synthesis of the human Δ90 cyclin B1::GFP plasmid has been described
794 previously (Levasseur and McDougall, 2000). The Δ90cyclin B::GFP fusion protein was stored
795 at -80°C and was injected at a final concentration of approximately 11 mg/ml.

796 The microinjection system described above for mRNAs injection was also used for protein
797 injection. Protein-injected oocytes were left for 45min at 18°C before fertilization and
798 subsequent confocal imaging.

799 **Confocal microscopy imaging**

800 All imaging experiments were performed at 19°C using a Leica TCS SP8 inverted microscope
801 fitted with Hybrid detectors and 40×/1.1NA water objective lens. To image aster migration,
802 each fertilized egg or 2-cell stage embryo was scanned by 4D live imaging of a whole embryo
803 (xyzt) with a frame rate of at least 1 image every 210s. The imaging parameters were adapted
804 to each fertilized egg: z step was between 0.5 and 2µm and time step between 1 min and 3.5
805 min. For fixed samples, z-stacks were acquired at step size of 0.5 µm.

806 **Invagination experiments**

807 Eggs were fertilized in MSFW and transferred after observing the first deformation into a
808 solution of MSFW TAPS containing 5µM of latrunculin B (Sigma Aldrich) diluted from a
809 10mM stock solution (in DMSO). The embryos were then mounted on a slide in the latrunculin
810 SW solution. The zygote plasma membrane was visualized either by microinjection of the
811 PH::Tomato mRNA, or by addition of the membrane dye Cell Mask orange (ThermoFisher,
812 Invitrogen) at 1:1000. For controls, zygotes labeled with PH::Tomato or Cell Mask were treated
813 with DMSO at a dilution of 1:1000.

814 In the case of the embryo injected at the two-cell stage, the fertilized eggs were left to develop
815 at 16°C in MFSW until they started cleaving. Then they were mounted in the injection chamber,
816 and injected with p21 or Δ90cyclinB proteins as soon as the division finished. When two or
817 three embryos were injected they were immediately transferred in MFSW with 5µM of
818 Cytochalasin B (Sigma Aldrich) diluted from a 10mM stock solution in DMSO, and mounted
819 on a slide in this solution for imaging.

820 **Quantification of the number of invaginations**

821 To image membrane invaginations in zygotes or 2-cell stage embryos, 25 µm-thick stacks of
822 confocal images (dz=2.5 µm) were acquired every 10 seconds in Cell Mask Orange stained
823 zygote at around 2-4 minutes after latrunculin incubation. The number membrane invaginations
824 was counted manually by counting invaginations present at 2-5 µm from the plasma membrane.

825 Membrane invaginations were imaged in two-cell embryos 4-5 minutes after incubation in
826 cytochalasin B at a frequency of 1 z-stack every 30 seconds to 1 stack every 2 minutes. The
827 presence or absence of invagination was scored manually on a z-projection image and cell cycle
828 state of the cells were defined as the 15 minutes following NEB.

829 **Quantification of aster migration**

830 The quantification of the distance between the center of the zygote and the DNA throughout
831 the cell cycle was performed in three steps, using Fiji (Schindelin et al., 2012).

832 First, the center of mass of zygotes were determined at each time point. To do so, a Gaussian
833 Blur (sigma =2) was applied to each xyz stack of the timelapse movie. Stacks were then made
834 binary with the “Triangle” method of the “Auto Threshold” function. The Fiji plugin “3D Roi

835 Manager” (Ollion et al., 2015) created objects from the binary stacks, and output their center of
836 mass. This method was verified by comparing the center of mass of the 3D zygote to the
837 centroid of the 2D equatorial section with widest diameter.

838 Secondly, using the Fiji “Point tool” and the “measure” function, the xyz coordinates of the
839 DNA label were obtained at each time point. When DNA was not labeled (in p21-injected
840 embryos), or weak (at NEB), DNA position was approximated to be at equal distance between
841 the two spindle poles, or at the center of the aster when the spindle was not yet formed. The
842 DNA label was chosen over the MT label to measure the aster migration because centrosome
843 duplication occurs before the centration of the spindle, therefore while the spindle as a whole
844 centers, each spindle pole starts centering and then diverges from the cell center to center the
845 DNA.

846 Finally, the distance between the DNA coordinates and the center of mass was computed using
847 the 3D Pythagorean theorem: $d = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2 + (z_2 - z_1)^2}$.

848 **Quantification of the vesicle traffic**

849 To image vesicle trafficking, eggs were fertilized, washed once, and immediately transferred to
850 a GF-coated slide/coverlip in a MFSW containing Cell Mask orange (1/1000). Then, 2D
851 images acquired every second for 3 min. The imaged plane was selected to contain the center
852 of one aster.

853 To measure relative movements of the vesicles, we combined 3 approaches. 1) For vesicles, we
854 used the Fiji tool TrackMate with LoG particle detection and simple LAP tracker (Tinevez et
855 al., 2017). 2) For aster localization we wrote (using Matlab) a manual periodic tracking, with
856 interpolation for intermediate time frames. 3) For cell contour, we developed another Matlab
857 algorithm based on threshold optimization to extract the cell contour. We combined information
858 from those tools to quantify movement.

859 For each vesicle track, we measured the relative path with respect to the aster. In more detail,
860 we defined a radius from the aster center to the centroid of the track, which naturally crosses
861 the cell contour. On this radius, we projected the path to estimate the radial component of the
862 vesicle movement. We also measured the temporal evolution of the contour. To take into
863 account the cell deformation and its impact on vesicle movement, we subtracted from each
864 relative path a yield drift. Considering an elastic behavior on the aster-contour axes, the yield
865 drift of a vesicle at a radius r was defined as follows:

$$866 \quad D_{yield}(r, t) = D_{contour}(t) \times \frac{r}{R(t)}$$

867 with $R(t)$ the distance of the cell contour from the aster center. To segregate vesicles just
868 endocytosed, stagnant below the membrane, from the vesicle moving on the aster, we kept only
869 the tracks 10 μ m away from the cell contour. Based on the path projection on the radius and its
870 orientation (positive if the last position is further from the aster than the first position), we then
871 sorted the tracks as going toward (retrograde) or away from (anterograde) the aster. Vesicles
872 with path projection smaller than 1 μ m were defined as stationary (note that this category
873 includes the static vesicles and vesicles moving orthogonally to the radius).

874 **Modeling**

875 Agent-based simulations were performed in 2D using a custom version of the software *Cytosim*
876 - www.cytosim.org (Foethke et al., 2009) with the parameters provided in Table S2. *Cytosim* is
877 a stochastic simulation engine that includes constituting elements of the cytoskeleton. It has
878 already been used to study spindle and centrosome dynamics and position (Khetan and Athale,
879 2016; Lacroix et al., 2018; Letort et al., 2016).

880 *Aster and microtubules*

881 MTs are modeled as worm-like chains, characterized by a bending stiffness (see Table S2) and
882 inextensibility. MTs can (de)polymerize and their instability is modeled by a stochastic
883 alternation of growing and shrinking phases. The plus-end polymerizes until a catastrophe
884 happens, starting a shrinking period. The aster is modeled as a bead, from which MTs are
885 nucleated. MT minus-ends are anchored to the aster while their plus-end are directed outward
886 from the aster.

887 A pushing force is generated by MTs' plus end polymerizing against the edge of the cell. When
888 MTs push strongly on the cortex, polymerization is slowed down and they have a higher chance
889 to undergo a catastrophe. Previous work (Letort et al., 2016) suggests that pushing cannot center
890 the aster if MTs can glide along the cell membrane. In their work, gliding was prevented by
891 pinning MTs' tips to the point where they first reached the edge of the cell. Pinning and cortical
892 pulling were not activated at the same time though, as pinning would make cortical pulling
893 inefficient. To prevent MT gliding along the cell cortex, we didn't model control cells as a
894 proper circle, but as a crenelated polygon, representing the actomyosin cortex. Once a tip enters
895 an alcove, it cannot slide anymore, as if it were stuck by intertwined actin filaments.

896 *Dynein distributed in the cytoplasm*

897 Dyneins are placed at random positions in the cytoplasm. When a MT comes close, a dynein
898 can bind to the MT and starts moving towards its minus end. As in previous work cited above,
899 a spring-like force pulls the dyneins back to their assigned position when they are displaced.
900 The dynein's velocity depends on the load and the projection of the restoring force along the
901 direction of the MT.

$$902 \quad v = v_{max} \left(1 - \frac{f}{f_s} \right)$$

$$903 \quad f = -k(\vec{x} - \vec{x}_0) \cdot \vec{u}$$

904 v_{max} is the unloaded speed, ie the speed when there is no applied force. f_s is the stall force, the
905 maximal force the dynein can withstand before it stops moving. k is the spring stiffness, \vec{x} the
906 position of the dynein, \vec{x}_0 the rest position it has been assigned and \vec{u} the direction of the MT.

907 *Control of MT dynamics by cortical dynein to generate forces*

908 If cortical pulling is implemented by usual dynein, it often makes the aster spin around the cell.
909 This is due to the fact that MTs tend to align with the cell's edge, as more and more cortical
910 dynein becomes attached to the MTs. The pulling force becomes higher and higher and the aster
911 spins around. *In vitro* experiments suggest that dynein placed in front of a rigid barrier can
912 control MTs' dynamics (Laan et al., 2012). Such dyneins trigger a catastrophe when they bind

913 to the end of a MT, and regulate its shrinking speed thereby generating pulling forces. We
914 modified *Cytosim* to implement such a behavior: cortical dynein works like classical molecular
915 motors except that they can only bind to a MT end, and they chew the MT as they move forward.
916 Like cytoplasmic dyneins, they have a stall force and the load comes from a spring linking the
917 dynein to its original position. However, unlike cytoplasmic dyneins, cortical dyneins cannot
918 move backwards as it would imply the MTs polymerized again.

919 *Code availability and simulation reproducibility*

920 The custom version of *Cytosim* with this interaction implemented is available at
921 <https://gitlab.com/gslndlb/cytosim>, in the branch *dynein_chew*. All configuration files used are
922 in the *cym* folder.

923 **Statistics and diagrams**

924 Statistical tests and graphics were performed using the libraries *rstatix*, *tidyverse*, *dplyr*, *ggpubr*,
925 and *ggplot2* from R software (R Studio, 2020) as well as Microsoft Excel (2013). Tests are
926 provided in the figure legends. Diagrams were created with BioRender.com

927 **Data availability**

928 All main figures are supplied with data used to generate the figures.

929 **References**

- 930 **Asch, R., Simerly, C., Ord, T., Ord, V. A. and Schatten, G.** (1995). The stages at which human
931 fertilization arrests: microtubule and chromosome configurations in inseminated oocytes
932 which failed to complete fertilization and development in humans. *Hum Reprod* **10**, 1897–
933 1906.
- 934 **Bornens, M.** (2012). The centrosome in cells and organisms. *Science* **335**, 422–426.
- 935 **Bouvrais, H., Chesneau, L., Le Cunff, Y., Fairbrass, D., Soler, N., Pastezeur, S., Pécot, T., Kervrann, C.**
936 **and Pécréaux, J.** (2021). The coordination of spindle-positioning forces during the
937 asymmetric division of the *Caenorhabditis elegans* zygote. *EMBO Rep* **22**, e50770.
- 938 **Burbank, K. S. and Mitchison, T. J.** (2006). Microtubule dynamic instability. *Curr Biol* **16**, R516-517.
- 939 **De Simone, A., Spahr, A., Busso, C. and Gönczy, P.** (2018). Uncovering the balance of forces driving
940 microtubule aster migration in *C. elegans* zygotes. *Nat Commun* **9**, 938.
- 941 **Delsuc, F., Brinkmann, H., Chourrout, D. and Philippe, H.** (2006). Tunicates and not
942 cephalochordates are the closest living relatives of vertebrates. *Nature* **439**, 965–968.
- 943 **Devore, J. J., Conrad, G. W. and Rappaport, R.** (1989). A model for astral stimulation of cytokinesis in
944 animal cells. *J Cell Biol* **109**, 2225–2232.
- 945 **Dogterom, M., Kerssemakers, J. W. J., Romet-Lemonne, G. and Janson, M. E.** (2005). Force
946 generation by dynamic microtubules. *Curr Opin Cell Biol* **17**, 67–74.
- 947 **Dumollard, R. and Sardet, C.** (2001). Three different calcium wave pacemakers in ascidian eggs. *J Cell*
948 *Sci* **114**, 2471–2481.
- 949 **Dumollard, R., Hebras, C., Besnardeau, L. and McDougall, A.** (2013). Beta-catenin patterns the cell
950 cycle during maternal-to-zygotic transition in urochordate embryos. *Developmental Biology*
951 **384**, 331–342.
- 952 **Field, C. M. and Mitchison, T. J.** (2018). Assembly of Spindles and Asters in *Xenopus* Egg Extracts.
953 *Cold Spring Harb Protoc* **2018**,.
- 954 **Foethke, D., Makushok, T., Brunner, D. and Nédélec, F.** (2009). Force- and length-dependent
955 catastrophe activities explain interphase microtubule organization in fission yeast. *Mol Syst*
956 *Biol* **5**, 241.
- 957 **Garzon-Coral, C., Fantana, H. A. and Howard, J.** (2016). A force-generating machinery maintains the
958 spindle at the cell center during mitosis. *Science* **352**, 1124–1127.
- 959 **Godard, B. G., Dumollard, R., Heisenberg, C.-P. and McDougall, A.** (2021). Combined effect of cell
960 geometry and polarity domains determines the orientation of unequal division. *Elife* **10**,
961 e75639.
- 962 **Gönczy, P., Pichler, S., Kirkham, M. and Hyman, A. A.** (1999). Cytoplasmic dynein is required for
963 distinct aspects of MTOC positioning, including centrosome separation, in the one cell stage
964 *Caenorhabditis elegans* embryo. *J Cell Biol* **147**, 135–150.

- 965 **Grill, S. W. and Hyman, A. A.** (2005). Spindle positioning by cortical pulling forces. *Dev. Cell* **8**, 461–
966 465.
- 967 **Grill, S. W., Gönczy, P., Stelzer, E. H. and Hyman, A. A.** (2001). Polarity controls forces governing
968 asymmetric spindle positioning in the *Caenorhabditis elegans* embryo. *Nature* **409**, 630–633.
- 969 **Grill, S. W., Howard, J., Schäffer, E., Stelzer, E. H. K. and Hyman, A. A.** (2003). The distribution of
970 active force generators controls mitotic spindle position. *Science* **301**, 518–521.
- 971 **Hertwig, O.** (1884). *Untersuchungen zur Morphologie und Physiologie der Zelle: Das Problem der*
972 *Befruchtung und der Isotropie des Eies, eine Theorie der Vererbung.* Fischer.
- 973 **Hewitson, L. and Schatten, G.** (2002). The use of primates as models for assisted reproduction.
974 *Reproductive BioMedicine Online* **5**, 50–55.
- 975 **Ishihara, K., Nguyen, P. A., Groen, A. C., Field, C. M. and Mitchison, T. J.** (2014). Microtubule
976 nucleation remote from centrosomes may explain how asters span large cells. *Proc. Natl.*
977 *Acad. Sci. U.S.A.* **111**, 17715–17722.
- 978 **Keshri, R., Rajeevan, A. and Kotak, S.** (2020). PP2A--B55y counteracts Cdk1 and regulates proper
979 spindle orientation through the cortical dynein adaptor NuMA. *J Cell Sci* **133**, jcs243857.
- 980 **Khetan, N. and Athale, C. A.** (2016). A Motor-Gradient and Clustering Model of the Centripetal
981 Motility of MTOCs in Meiosis I of Mouse Oocytes. *PLoS Comput Biol* **12**, e1005102.
- 982 **Khetan, N., Pruliere, G., Hebras, C., Chenevert, J. and Athale, C. A.** (2021). Self-organized optimal
983 packing of kinesin-5-driven microtubule asters scales with cell size. *J Cell Sci* **134**, jcs257543.
- 984 **Kimura, K. and Kimura, A.** (2011). A novel mechanism of microtubule length-dependent force to pull
985 centrosomes toward the cell center. *Bioarchitecture* **1**, 74–79.
- 986 **Kimura, A. and Onami, S.** (2005). Computer simulations and image processing reveal length-
987 dependent pulling force as the primary mechanism for *C. elegans* male pronuclear migration.
988 *Dev Cell* **8**, 765–775.
- 989 **Kiyomitsu, T.** (2019). The cortical force-generating machinery: how cortical spindle-pulling forces are
990 generated. *Curr Opin Cell Biol* **60**, 1–8.
- 991 **Kotak, S. and Gönczy, P.** (2013). Mechanisms of spindle positioning: cortical force generators in the
992 limelight. *Curr. Opin. Cell Biol.* **25**, 741–748.
- 993 **Kotak, S., Busso, C. and Gönczy, P.** (2013). NuMA phosphorylation by CDK1 couples mitotic
994 progression with cortical dynein function. *EMBO J* **32**, 2517–2529.
- 995 **Laan, L., Pavin, N., Husson, J., Romet-Lemonne, G., van Duijn, M., López, M. P., Vale, R. D., Jülicher,**
996 **F., Reck-Peterson, S. L. and Dogterom, M.** (2012). Cortical dynein controls microtubule
997 dynamics to generate pulling forces that position microtubule asters. *Cell* **148**, 502–514.
- 998 **Lacroix, B., Letort, G., Pitayu, L., Sallé, J., Stefanutti, M., Maton, G., Ladouceur, A.-M., Canman, J. C.,**
999 **Maddox, P. S., Maddox, A. S., et al.** (2018). Microtubule Dynamics Scale with Cell Size to Set
1000 Spindle Length and Assembly Timing. *Developmental Cell* **45**, 496-511.e6.

- 1001 **Letort, G., Nedelec, F., Blanchoin, L. and Théry, M.** (2016). Centrosome centering and decentering by
1002 microtubule network rearrangement. *Mol Biol Cell* **27**, 2833–2843.
- 1003 **Levasseur, M. and McDougall, A.** (2000). Sperm-induced calcium oscillations at fertilisation in
1004 ascidians are controlled by cyclin B1-dependent kinase activity. *Development* **127**, 631–641.
- 1005 **Levasseur, M., Carroll, M., Jones, K. T. and McDougall, A.** (2007). A novel mechanism controls the
1006 Ca²⁺ oscillations triggered by activation of ascidian eggs and has an absolute requirement for
1007 Cdk1 activity. *Journal of Cell Science* **120**, 1763–1771.
- 1008 **Li, J. and Jiang, H.** (2018). Regulating positioning and orientation of mitotic spindles via cell size and
1009 shape. *Phys Rev E* **97**, 012407.
- 1010 **Longoria, R. A. and Shubeita, G. T.** (2013). Cargo transport by cytoplasmic Dynein can center
1011 embryonic centrosomes. *PLoS One* **8**, e67710.
- 1012 **McCarthy Campbell, E. K., Werts, A. D. and Goldstein, B.** (2009). A cell cycle timer for asymmetric
1013 spindle positioning. *PLoS Biol.* **7**, e1000088.
- 1014 **McNally, K. L. P., Fabritius, A. S., Ellefson, M. L., Flynn, J. R., Milan, J. A. and McNally, F. J.** (2012).
1015 Kinesin-1 prevents capture of the oocyte meiotic spindle by the sperm aster. *Dev Cell* **22**,
1016 788–798.
- 1017 **Meaders, J. L. and Burgess, D. R.** (2020). Microtubule-Based Mechanisms of Pronuclear Positioning.
1018 *Cells* **9**, E505.
- 1019 **Meaders, J. L., de Matos, S. N. and Burgess, D. R.** (2020). A Pushing Mechanism for Microtubule
1020 Aster Positioning in a Large Cell Type. *Cell Rep* **33**, 108213.
- 1021 **Minc, N. and Piel, M.** (2012). Predicting division plane position and orientation. *Trends Cell Biol.* **22**,
1022 193–200.
- 1023 **Minc, N., Burgess, D. and Chang, F.** (2011). Influence of cell geometry on division-plane positioning.
1024 *Cell* **144**, 414–426.
- 1025 **Mitchison, T. J. and Field, C. M.** (2021). Self-Organization of Cellular Units. *Annu Rev Cell Dev Biol* **37**,
1026 23–41.
- 1027 **Nédélec, F. J., Surrey, T., Maggs, A. C. and Leibler, S.** (1997). Self-organization of microtubules and
1028 motors. *Nature* **389**, 305–308.
- 1029 **Ollion, J., Cochenne, J., Loll, F., Escudé, C. and Boudier, T.** (2015). Analysis of nuclear organization
1030 with TANGO, software for high-throughput quantitative analysis of 3D fluorescence
1031 microscopy images. *Methods Mol Biol* **1228**, 203–222.
- 1032 **Palenzuela, H., Lacroix, B., Sallé, J., Minami, K., Shima, T., Jegou, A., Romet-Lemonne, G. and Minc,**
1033 **N.** (2020). In Vitro Reconstitution of Dynein Force Exertion in a Bulk Viscous Medium. *Curr*
1034 *Biol* **30**, 4534-4540.e7.
- 1035 **Pelletier, J. F., Field, C. M., Fürthauer, S., Sonnett, M. and Mitchison, T. J.** (2020). Co-movement of
1036 astral microtubules, organelles and F-actin by dynein and actomyosin forces in frog egg
1037 cytoplasm. *Elife* **9**,.

- 1038 **Petry, S., Groen, A. C., Ishihara, K., Mitchison, T. J. and Vale, R. D.** (2013). Branching Microtubule
1039 Nucleation in *Xenopus* Egg Extracts Mediated by Augmin and TPX2. *Cell* **152**, 768–777.
- 1040 **Pietro, F. di, Echard, A. and Morin, X.** (2016). Regulation of mitotic spindle orientation: an integrated
1041 view. *EMBO reports* **17**, 1106–1130.
- 1042 **Redemann, S., Pecreaux, J., Goehring, N. W., Khairy, K., Stelzer, E. H. K., Hyman, A. A. and Howard,**
1043 **J.** (2010). Membrane invaginations reveal cortical sites that pull on mitotic spindles in one-
1044 cell *C. elegans* embryos. *PLoS ONE* **5**, e12301.
- 1045 **Reinsch, S. and Gönczy, P.** (1998). Mechanisms of nuclear positioning. *J Cell Sci* **111 (Pt 16)**, 2283–
1046 2295.
- 1047 **Rodriguez-Garcia, R., Chesneau, L., Pastezeur, S., Roul, J., Tramier, M. and Pécréaux, J.** (2018). The
1048 polarity-induced force imbalance in *Caenorhabditis elegans* embryos is caused by
1049 asymmetric binding rates of dynein to the cortex. *MBoC* **29**, 3093–3104.
- 1050 **Roegiers, F., McDougall, A. and Sardet, C.** (1995). The sperm entry point defines the orientation of
1051 the calcium-induced contraction wave that directs the first phase of cytoplasmic
1052 reorganization in the ascidian egg. *Development* **121**, 3457–3466.
- 1053 **Rosenblatt, J., Cramer, L. P., Baum, B. and McGee, K. M.** (2004). Myosin II-dependent cortical
1054 movement is required for centrosome separation and positioning during mitotic spindle
1055 assembly. *Cell* **117**, 361–372.
- 1056 **Rusan, N. M., Fagerstrom, C. J., Yvon, A. M. and Wadsworth, P.** (2001). Cell cycle-dependent
1057 changes in microtubule dynamics in living cells expressing green fluorescent protein-alpha
1058 tubulin. *Mol Biol Cell* **12**, 971–980.
- 1059 **Sardet, C., McDougall, A., Yasuo, H., Chenevert, J., Pruliere, G., Dumollard, R., Hudson, C., Hebras,**
1060 **C., Le Nguyen, N. and Paix, A.** (2011). Embryological methods in ascidians: the Villefranche-
1061 sur-Mer protocols. *Methods Mol. Biol.* **770**, 365–400.
- 1062 **Scheffler, K., Uraji, J., Jentoft, I., Cavazza, T., Mönnich, E., Mogessie, B. and Schuh, M.** (2021). Two
1063 mechanisms drive pronuclear migration in mouse zygotes. *Nat Commun* **12**, 841.
- 1064 **Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S.,**
1065 **Rueden, C., Saalfeld, S., Schmid, B., et al.** (2012). Fiji: an open-source platform for biological-
1066 image analysis. *Nat Methods* **9**, 676–682.
- 1067 **Simerly, C. R., Takahashi, D., Jacoby, E., Castro, C., Hartnett, C., Hewitson, L., Navara, C. and**
1068 **Schatten, G.** (2019). Fertilization and Cleavage Axes Differ In Primates Conceived By
1069 Conventional (IVF) Versus Intracytoplasmic Sperm Injection (ICSI). *Sci Rep* **9**, 15282.
- 1070 **Strome, S.** (1993). Determination of cleavage planes. *Cell* **72**, 3–6.
- 1071 **Sulerud, T., Sami, A. B., Li, G., Kloxin, A., Oakey, J. and Gatlin, J.** (2020). Microtubule-dependent
1072 pushing forces contribute to long-distance aster movement and centration in *Xenopus laevis*
1073 egg extracts. *Mol Biol Cell* **31**, 2791–2802.
- 1074 **Tanimoto, H., Kimura, A. and Minc, N.** (2016). Shape-motion relationships of centering microtubule
1075 asters. *J Cell Biol* **212**, 777–787.

1076 **Tinevez, J.-Y., Perry, N., Schindelin, J., Hoopes, G. M., Reynolds, G. D., Laplantine, E., Bednarek, S.**
1077 **Y., Shorte, S. L. and Eliceiri, K. W. (2017).** TrackMate: An open and extensible platform for
1078 single-particle tracking. *Methods* **115**, 80–90.

1079 **Tran, P. T., Marsh, L., Doye, V., Inoué, S. and Chang, F. (2001).** A mechanism for nuclear positioning
1080 in fission yeast based on microtubule pushing. *J Cell Biol* **153**, 397–411.

1081 **Wodarz, A. (2002).** Establishing cell polarity in development. *Nat Cell Biol* **4**, E39-44.

1082 **Wühr, M., Dumont, S., Groen, A. C., Needleman, D. J. and Mitchison, T. J. (2009).** How does a
1083 millimeter-sized cell find its center? *Cell Cycle* **8**, 1115–1121.

1084 **Wühr, M., Tan, E. S., Parker, S. K., Detrich, H. W. and Mitchison, T. J. (2010).** A model for cleavage
1085 plane determination in early amphibian and fish embryos. *Curr. Biol.* **20**, 2040–2045.

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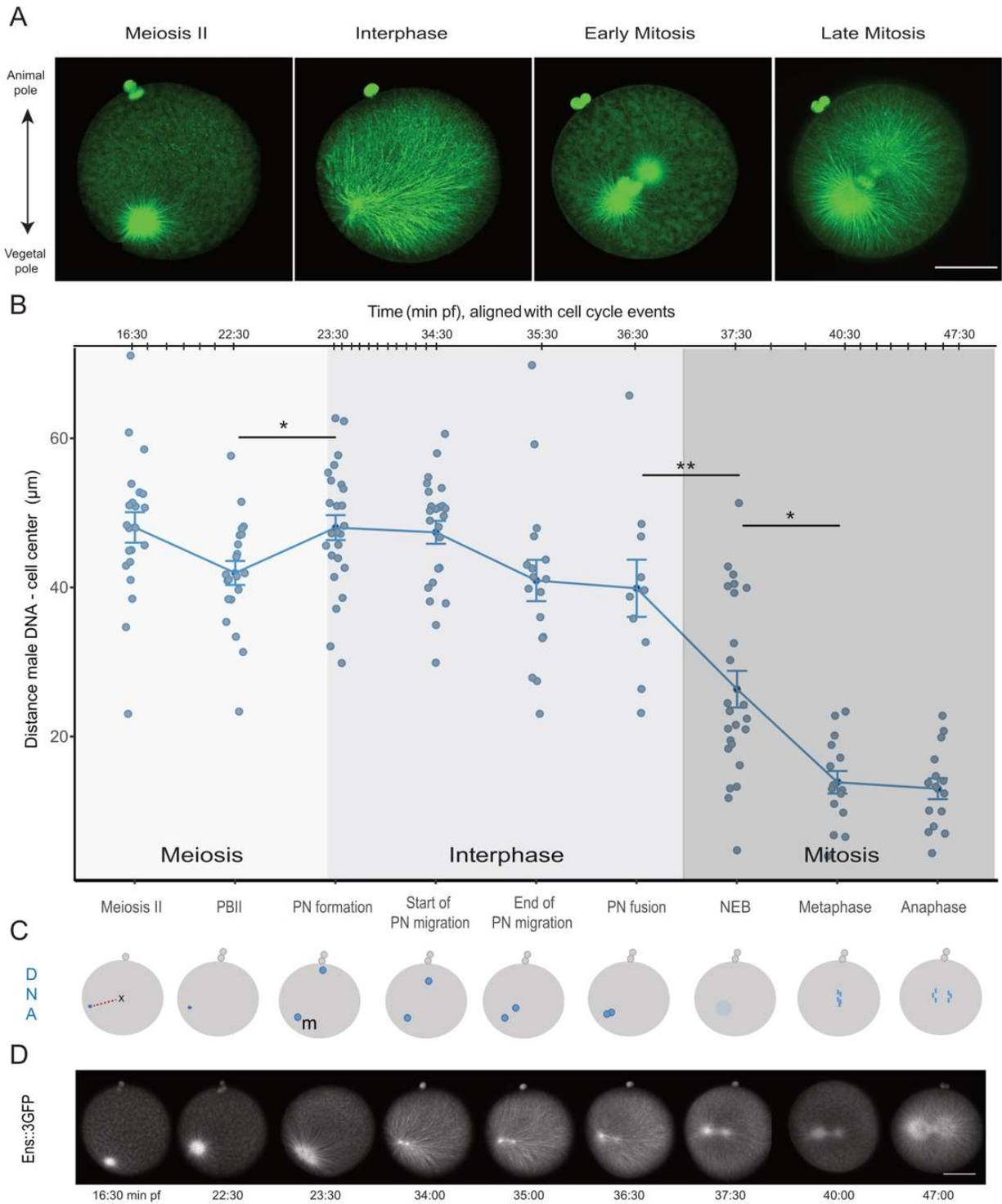
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1105 **Figure 1.**

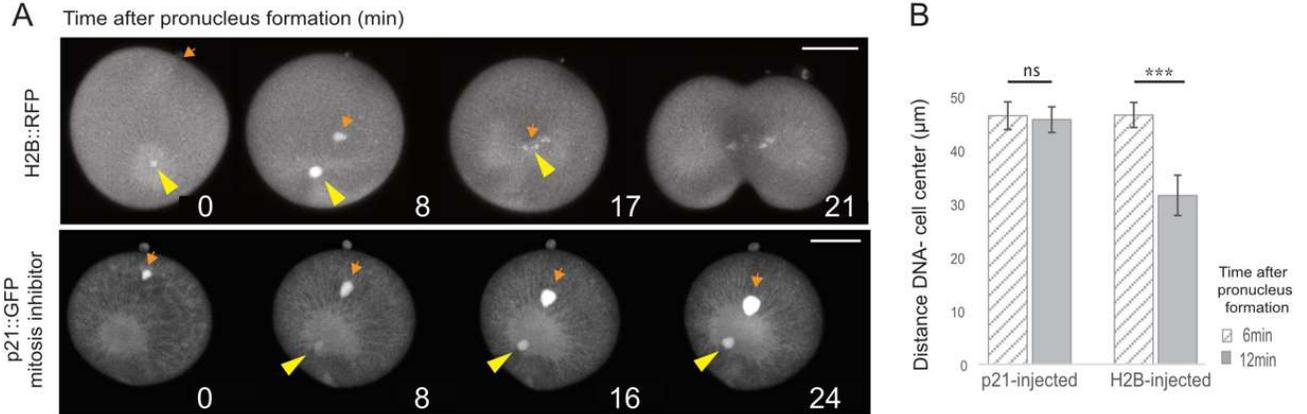


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1109 **Figure 2.**



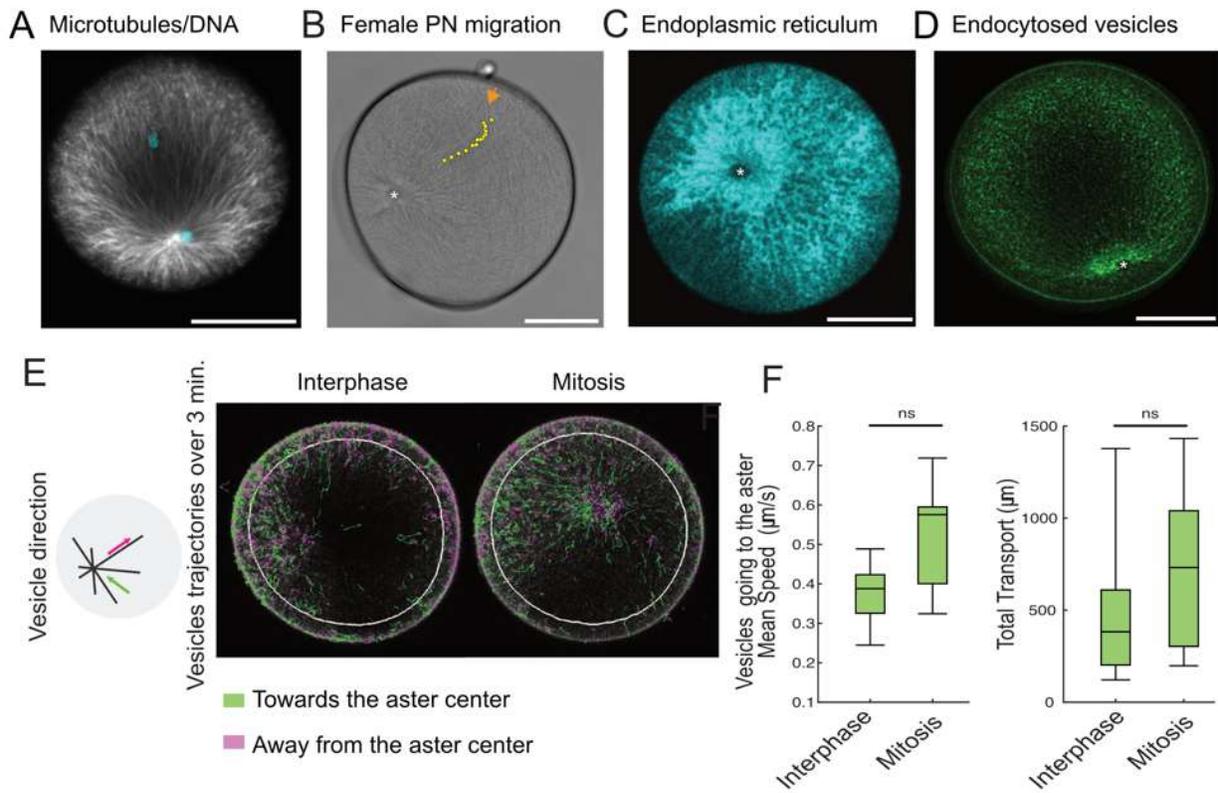
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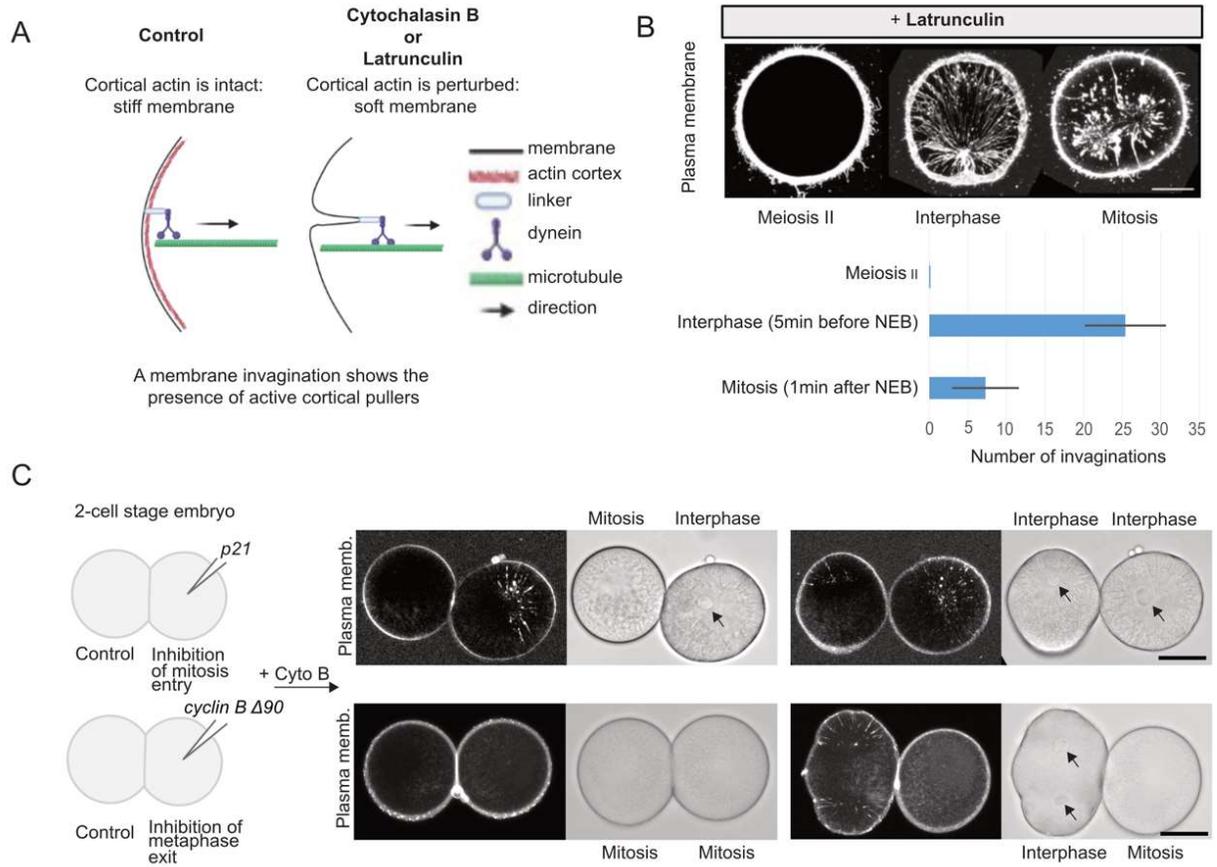
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1114 **Figure 3.**



1115 **Figure 4.**



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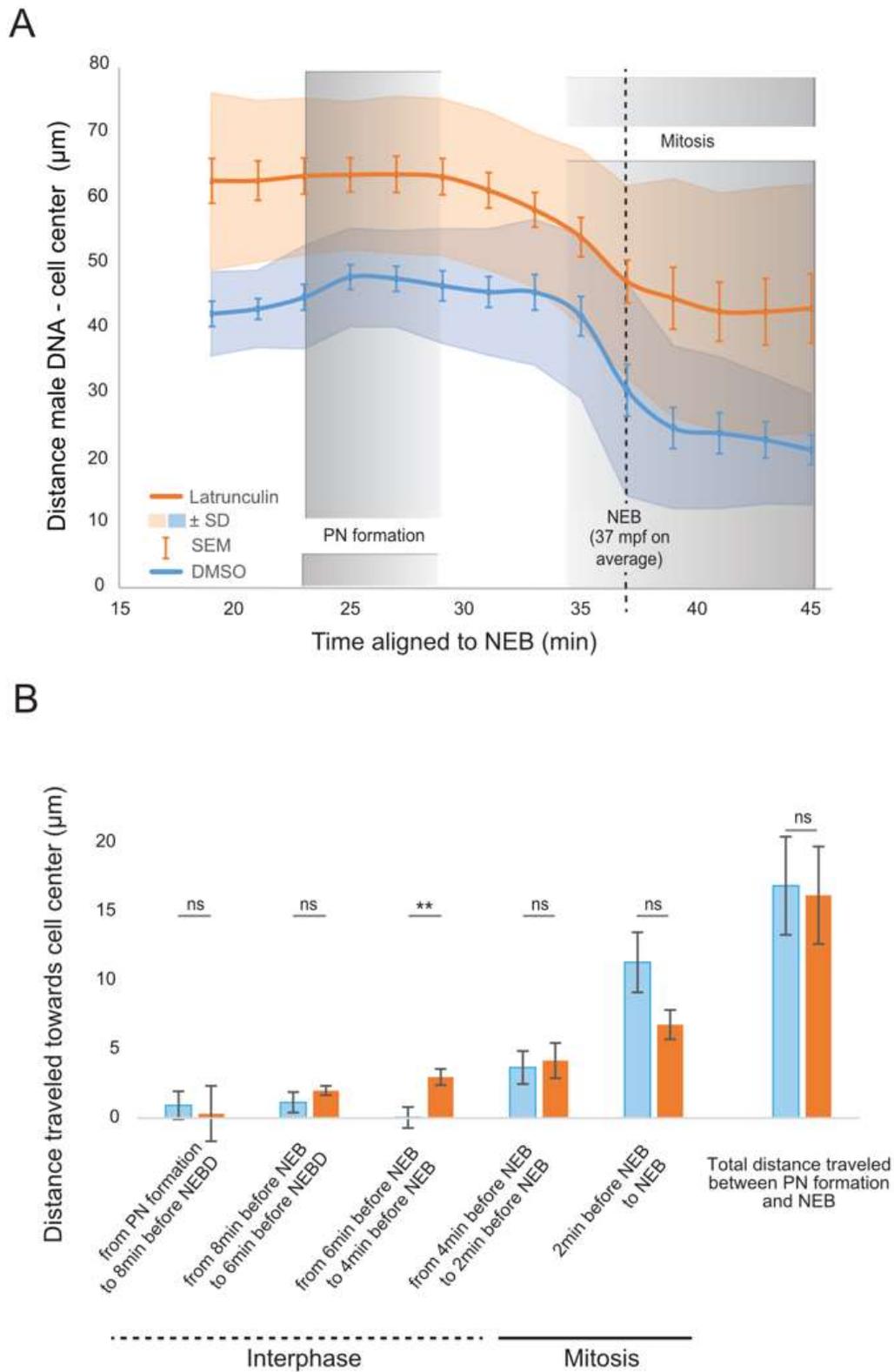
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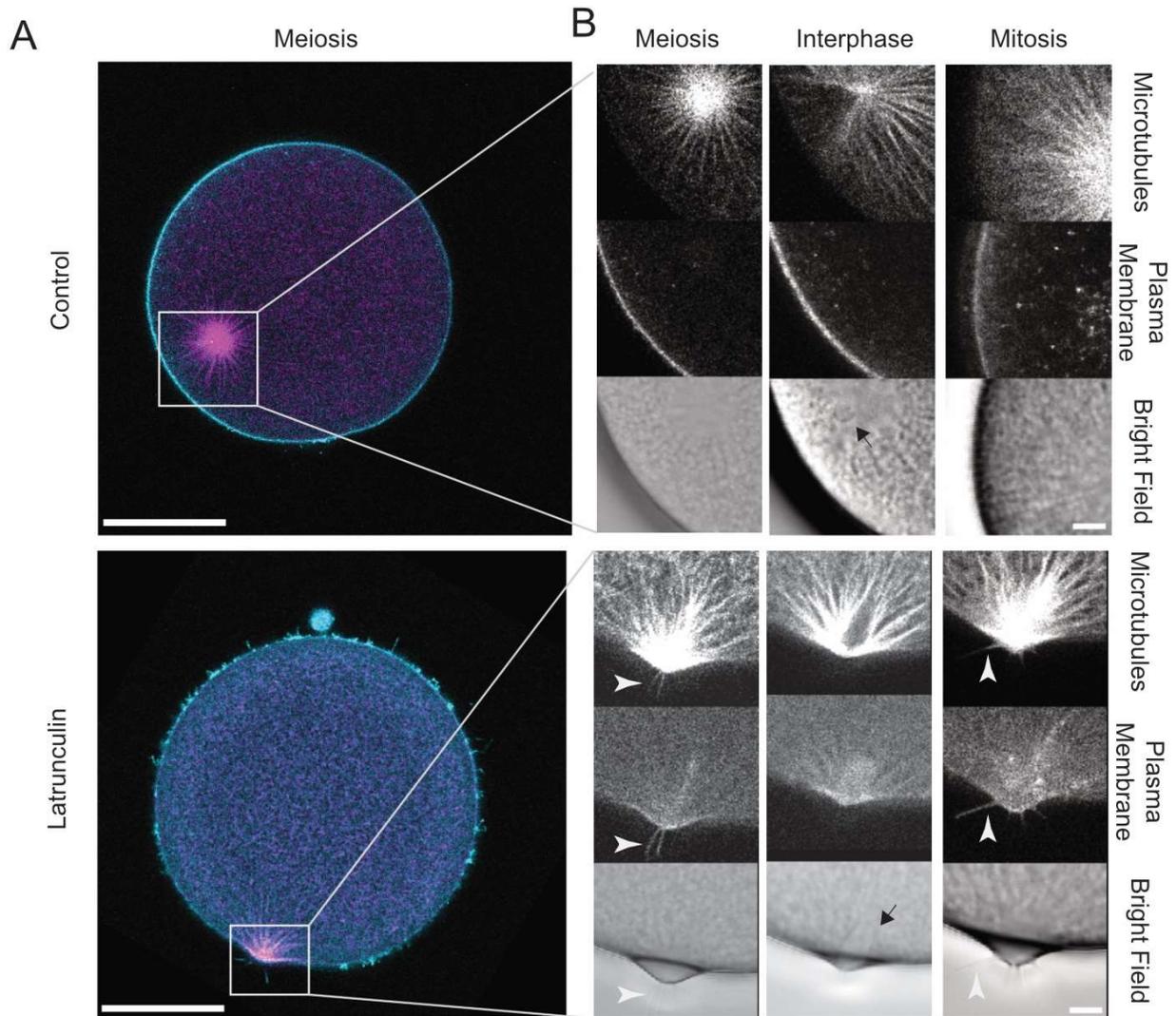
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1130 **Figure 5.**



1136 **Figure 6.**



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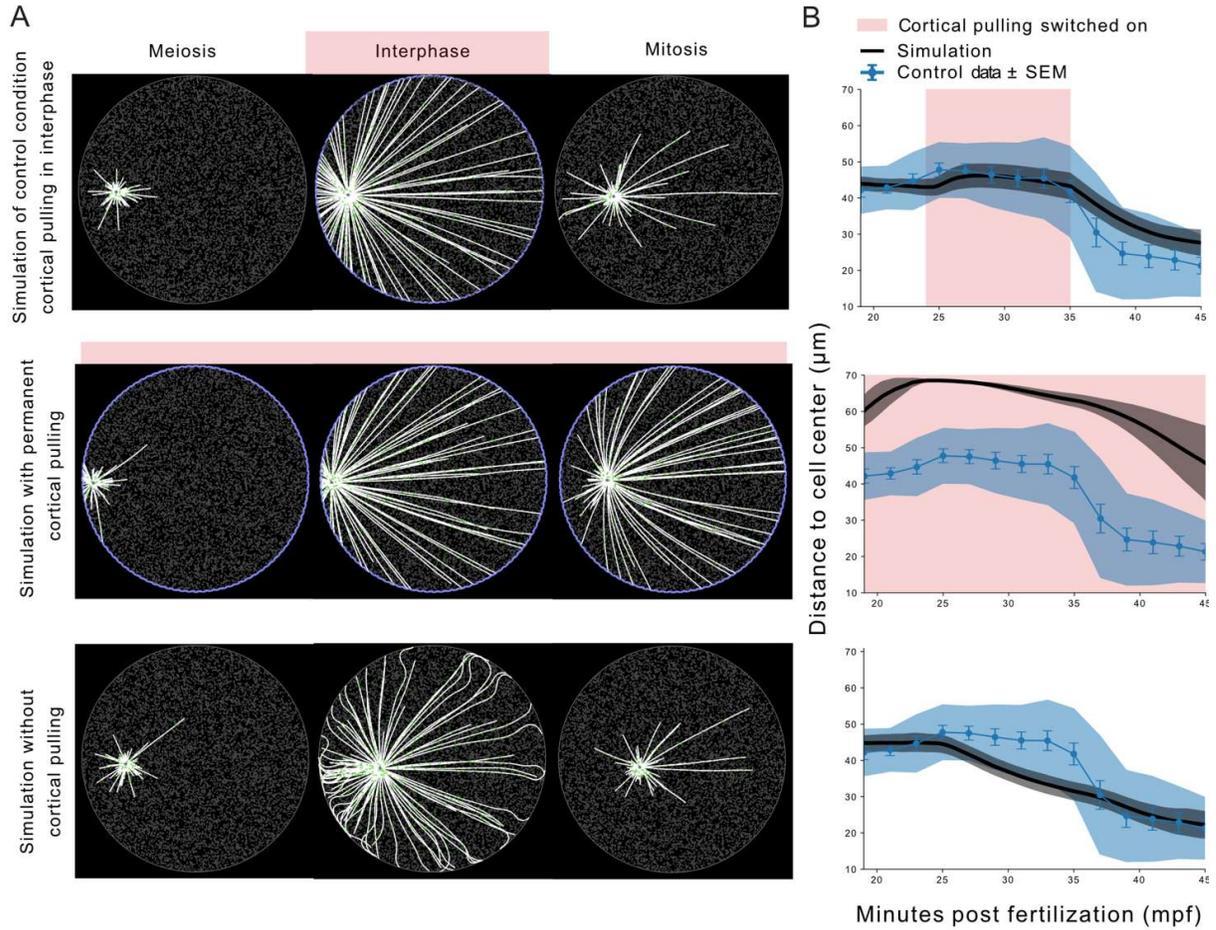
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1147 **Figure 7.**



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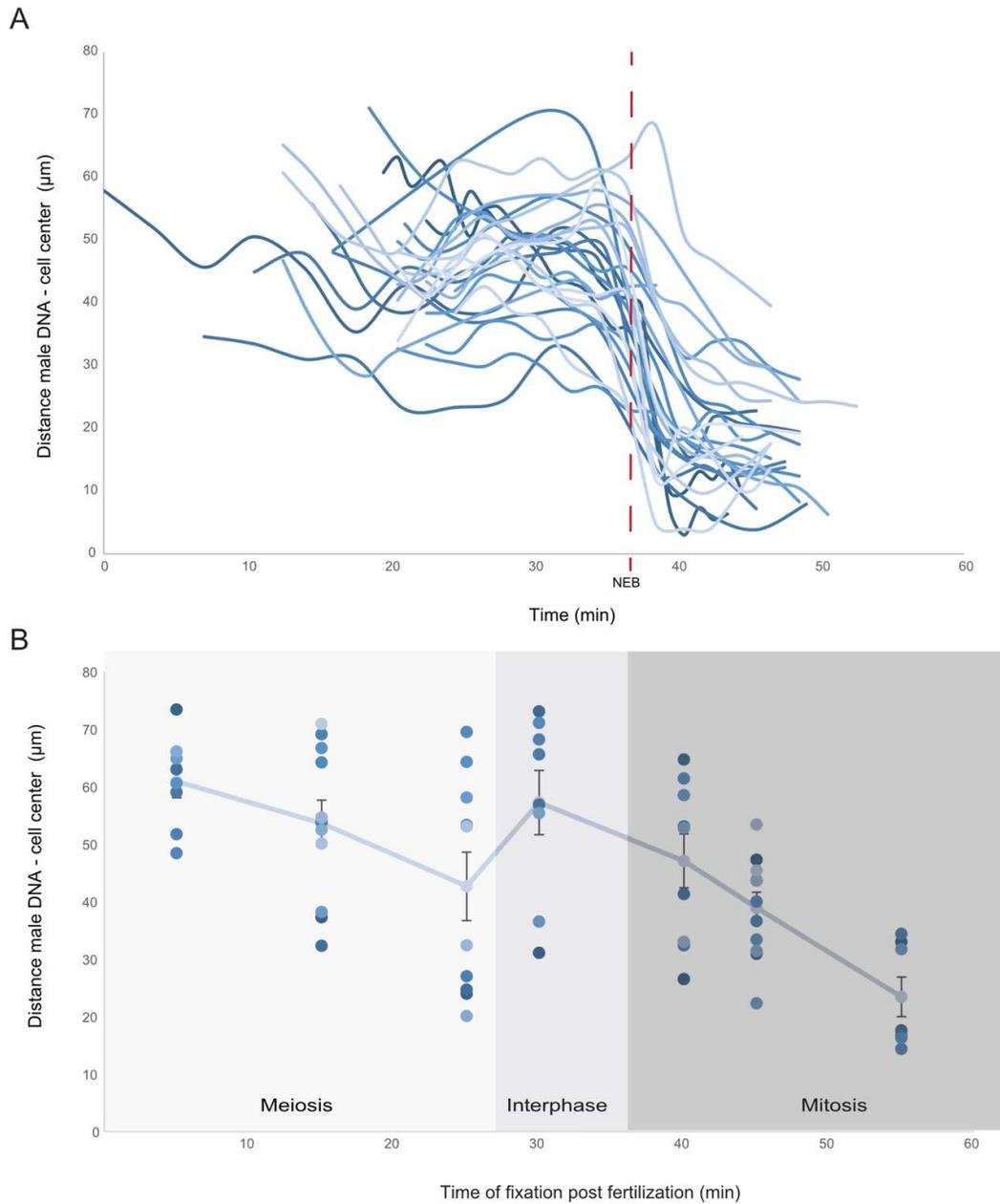
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1161 **Supplementary Figure S1.** Live and fixed data displaying sperm aster position following
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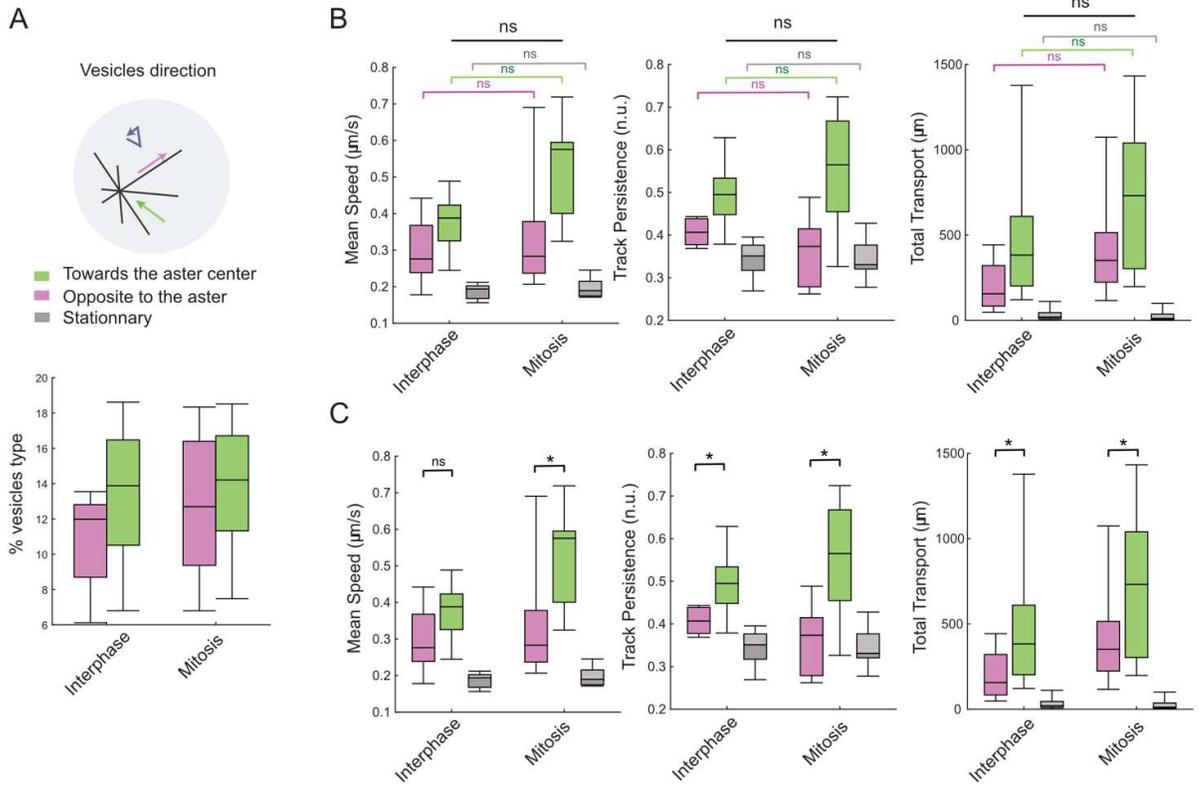
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1170 **Supplementary Figure S2. Vesicle movement parameters**



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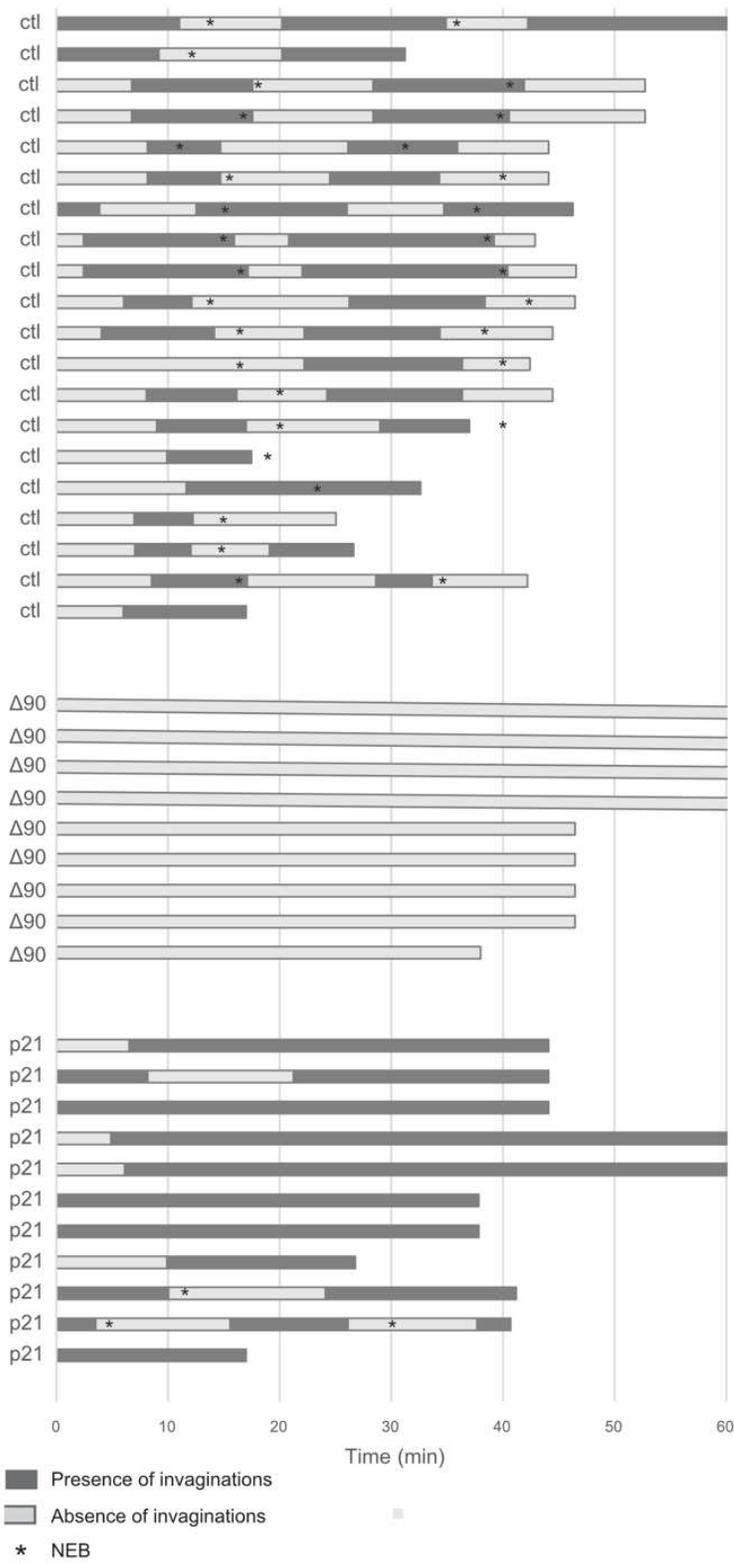
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1187 **Supplementary Figure S3. p21 and $\Delta 90$ cyclin B to delay mitotic entry or block mitotic exit**

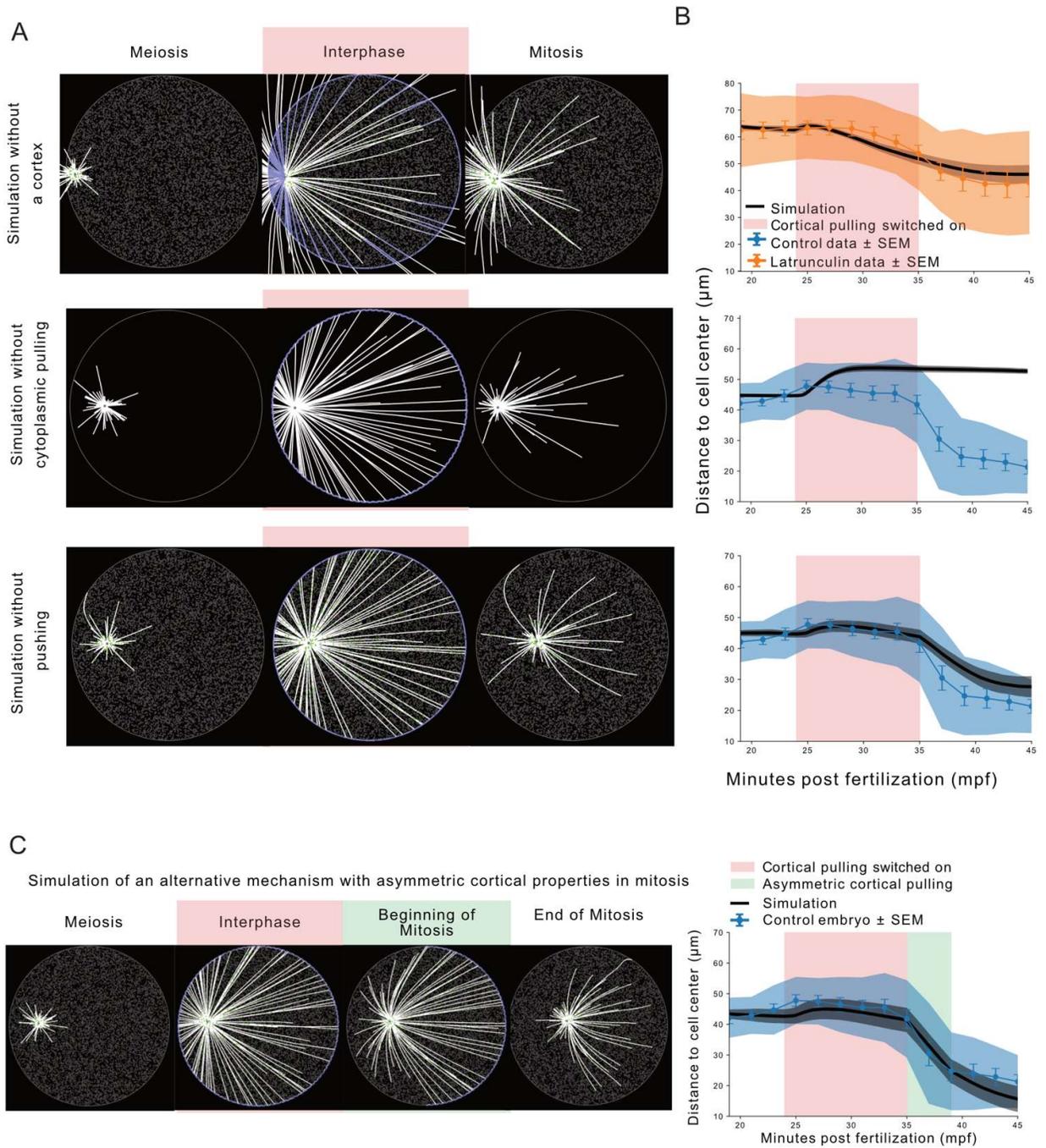
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1190 **Supplementary Figure S4. Contribution of pushing and cytoplasmic pulling in aster**
 1191 **centration**

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1197 **Supplementary Table S1.** Injection of p21 prolongs interphase
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Cell Cycle events	Control			p21-injected		
	min post fertilization	min after PN formation	n	min post fertilization	min after PN formation	n
PN formation	25 ± 2.8	0	22	34.9 ± 11.3	0	17
NEB	37.5 ± 4.2	12	23	65.8 ± 14.2	30	16
Cytokinesis	48 ± 4.5	23	24	65 ± 5.1	29	9
	min			min		
Time between PN formation & NEB	12.7 ± 2.6		20	26.9 ± 2		15
Time between NEB & Cytokinesis	10 ± 2		23	11.3 ± 1.8		9

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1217 **Supplementary Table S2.** Parameters used in the simulations related to Figure 7. General
 1218 units in *Cytosim* are seconds (s), micrometers (μm) and picoNewtons (pN).

Parameter	Value	Reference
General parameters		
Time step	0.01 s	
Cell Radius	70 μm	Average embryo radius
Temperature	18 $^{\circ}\text{C}$	To match experimental temp.
Viscosity	1 pN.s. μm^{-2}	(Lacroix et al., 2018)
Centrosome		
Radius	0.5 μm	(Letort et al., 2016)
Number of MTs	100	(Letort et al., 2016)
MT anchoring stiffnesses	500 pN/ μm	(Letort et al., 2016)
Microtubules		
Bending stiffness	30 pN. μm^2	(Lacroix et al., 2018)
Minimal length	0.5 μm	
Maximal length	140 μm	
Polymerization speed	0.53 $\mu\text{m.s}^{-1}$	Approx. from unpublished data
Depolymerization speed	-0.53 $\mu\text{m.s}^{-1}$	
Growing force	5 pN	(Letort et al., 2016)
Catastrophe rates in meiosis and mitosis	0.12 s^{-1} , 0.48 s^{-1}	(Rusan et al., 2001), ratio between stalled and free (Letort et al., 2016)
Catastrophe rates in interphase	0.018 s^{-1} , 0.075 s^{-1}	
Rescue rate	0.064 s^{-1}	(Letort et al., 2016)
Cytoplasmic dyneins		
Unloaded speed	1.1 $\mu\text{m.s}^{-1}$	Approx. from instantaneous vesicle speed
Stall force	1.75 pN	(Khetan et al., 2021)
Attachment distance	0.02 μm	(Khetan et al., 2021)
Attachment rate	12 μm	(Khetan et al., 2021)
Detachment rate	1 s^{-1}	(Khetan et al., 2021)
Detachment force	1.75 pN	(Khetan et al., 2021)
Motor linker stiffness	100 pN/ μm	(Khetan et al., 2021)
Membrane dyneins		
Unloaded speed	1.1 $\mu\text{m.s}^{-1}$	
Stall force	7 pN	4*cyto. dyneins
Attachment distance	0.02 μm	(Khetan et al., 2021)
Attachment rate	12 s^{-1}	(Khetan et al., 2021)
Detachment rate	0.01 s^{-1}	Approx. To match invagination lifetime (60s)
Detachment force	7 pN	4*cyto. dyneins
Motor linker stiffness (control)	100 pN/ μm	(Khetan et al., 2021)
Motor linker stiffness (latrunculin)	0.222 pN/ μm	To match latrunculin data

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