

1 **Combined effect of cell geometry and polarity domains determines the orientation of**
2 **unequal division**

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13

14 **Abstract**

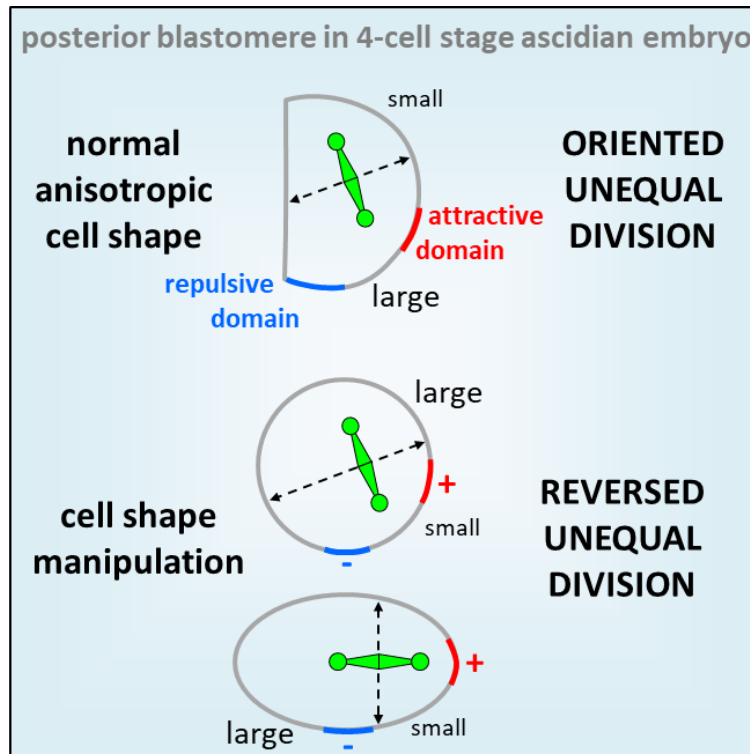
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16 Cell division orientation is thought to result from a competition between cell geometry and
17 polarity domains controlling the position of the mitotic spindle during mitosis. Depending on the
18 level of cell shape anisotropy or the strength of the polarity domain, one dominates the other
19 and determines the orientation of the spindle. Whether and how such competition is also at
20 work to determine unequal cell division (UCD), producing daughter cells of different size,
21 remains unclear. Here, we show that cell geometry and polarity domains cooperate, rather
22 than compete, in positioning the cleavage plane during UCDs in early ascidian embryos. We
23 found that the UCDs and their orientation at the ascidian third cleavage rely on the spindle
24 tilting in an anisotropic cell shape, and cortical polarity domains exerting different effects on
25 spindle astral microtubules. By systematically varying mitotic cell shape, we could modulate
26 the effect of attractive and repulsive polarity domains and consequently generate predicted
27 daughter cell size asymmetries and position. We therefore propose that the spindle position
28 during UCD is set by the combined activities of cell geometry and polarity domains, where cell
29 geometry modulates the effect of cortical polarity domain(s).

30

31 **Graphical abstract**

32



33

34 **Highlight**

35

- 36 • Spindle tilting in anisotropic cell shape induces unequal cell division
- 37 • Cortical polarity domain can exert attractive or repulsive effect on spindle
- 38 • Cell geometry and polarity domain cooperate to position the spindle
- 39 • Cell geometry modulates the effect of polarity domain

40

41 **Introduction**

42

43 The century old observation of cells dividing orthogonal to their long axis (Minc and Piel, 2012)
44 is the result of spindle alignment with the longest axis of the cell mediated by cytoplasmic
45 dynein-dependent pulling forces that scale with astral microtubules length (Li and Jiang, 2018;
46 Minc and Piel, 2012; Minc et al., 2011) and possibly implemented by a combination of
47 microtubules pushing and pulling (Howard and Garzon-Coral, 2017; Pavin et al., 2012).
48 Deviation from this default spindle positioning mechanism arises through local alteration of
49 microtubule-associated forces by polarity domains. Such polarity domains can be cortical
50 enrichment of molecular motors such as Dynein (Grill et al., 2001; Kotak and Gönczy, 2013),
51 polarity proteins such as Ezrin (Hebert et al., 2012; Korotkevich et al., 2017), or organelles like
52 yolk granules (Pierre et al., 2016) as well as local actomyosin driven tension (Scarpa et al.,
53 2018). This has led to the notion that cell geometry and polarity domains are in direct
54 competition to orient the spindle (Niwayama et al., 2019; Pierre et al., 2016). Such competition
55 is thought to occur during early embryonic cleavages to shape whole embryos (Pierre et al.,
56 2016) and to ensure robust cellular patterning in the mouse blastocyst (Niwayama et al., 2019).
57 Yet, whether and how cell geometry and polarity domains compete with each other not only to
58 determine the orientation but also the centering of the mitotic spindle leading to equal or
59 unequal cell divisions (UCDs) remains unclear.

60

61 Unequal Cell Division (UCD) divides the mother cell into two daughter cells of different sizes.
62 This process shapes early embryogenesis in several animal phyla (Hasley et al., 2017; Martín-
63 Durán et al., 2016; McDougall et al., 2019) and sculpts entire organs (Winkley et al., 2019).
64 On top of these morphological outcomes, UCDs are often associated with asymmetric
65 segregation of determinants leading to asymmetric cell division creating sibling cells with
66 different cell fates (Gönczy, 2008; Sardet et al., 2005). While UCDs are a widespread
67 phenomenon in metazoan embryogenesis, the molecular and cellular mechanisms underlying
68 UCD are still being debated.

69

70 One major mechanism to generate UCD relies on spindle off-centering, which can be achieved
71 by local cortical pulling on microtubules or microtubules pushing against the cortex. Cortical
72 anchoring of the microtubule minus-end directed motor Dynein, by interaction with
73 LGN/Pins/GPR1-2 and/or NuMA/Mud/Lin-5 (Pietro et al., 2016), exerts pulling forces able to
74 effectively displace centrosomes and mitotic spindles (Grill and Hyman, 2005). Such local
75 microtubule pulling forces from the posterior pole of *C.elegans* zygotes leads to UCD during
76 the first cleavage (Grill et al., 2001; Kotak and Gönczy, 2013; Redemann et al., 2010). A similar
77 mechanism is thought to operate also during micromere formation in echinoderm embryos

78 (Poon et al., 2019). Cortical pushing can arise when the tip of growing astral microtubule
79 touches the cell cortex creating pushing forces on the spindle (Garzon-Coral et al., 2016; Pavin
80 et al., 2012). Asymmetric microtubule cortical pushing caused by asters size asymmetry is
81 thought to be implicated in the UCD of the first cleavage in some spiralian embryos (Ren and
82 Weisblat, 2006; Shimizu et al., 1998) and in ascidian germ line precursors (Costache et al.,
83 2017). In addition to unequal microtubule pulling and pushing forces, UCD can also emerge
84 from mother cells displaying an anisotropic shape. For instance, during ascidian notochord
85 development, mother cells with conical shape divide along their centre of mass, giving rise to
86 daughter cells with unequal size (Winkley et al., 2019). Finally, a rare but extensively studied
87 mechanism of UCD is found in neuroblasts of *Drosophila* and *C.elegans* where instead of the
88 spindle the cleavage furrow is off-centered by polarized cortical contractility (Cabernard et al.,
89 2010; Kaltschmidt et al., 2000; Ou et al., 2010).

90

91 The early embryo of the ascidian *Phallusia mammillata* has been an emergent model to study
92 mechanisms of UCD due to its invariant cleavage pattern with UCDs in the germline precursors
93 (Costache et al., 2017; Prodon et al., 2010). Whereas spindle positioning and cleavage pattern
94 in somatic lineages is predominantly determined by cell shape, a macromolecular cortical
95 structure, called the Centrosome-Attracting-Body (CAB), overrides the influence of cell shape
96 to off-center the spindle, thereby inducing three successive UCDs from the 8-cell stage
97 (Dumollard et al., 2017). The CAB is inherited by the smaller daughter cells at the posterior
98 pole of the embryo from 16-cell stage onwards, consistent with a decisive role of the CAB in
99 off-centering of the mitotic spindle. However, during the division from the 4 to the 8-cell stage,
100 when the CAB is already functional in orienting the mitotic spindle (Negishi et al., 2007), the
101 posterior vegetal blastomeres inheriting the CAB are larger than their posterior animal
102 daughters (Tassy et al., 2006). Why the CAB at this stage is unable to off-center the mitotic
103 spindle, as found in later divisions, remains unclear.

104

105 Here, we show that the UCDs at the third cleavage of ascidian embryo are determined by the
106 combined activities of cell shape and cortical polarity domains. We found that UCDs at the 4-
107 cell stage rely on mitotic spindle tilting in an anisotropic cell shape, which modulates the
108 influence of polarity domains within the dividing cells. We also identified a yet unknown polarity
109 domain localized in the vegetal cortex, devoid of cortical microtubule pulling forces, which is
110 necessary for UCD of anterior cells.

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112

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114

115 **Results**

116

117 **The third cleavage in ascidian embryo creates large vegetal blastomeres by spindle**
118 **tilting away from the vegetal pole at anaphase onset**

119

120 The invariant cleavage pattern of ascidian embryos is characterized by three rounds of UCDs
121 in two vegetal-posterior blastomeres from the 8-cell stage onwards, leading to the formation of
122 two small germline precursor cells at the 64-cell stage (Figure 1A) (Hibino et al., 1998). These
123 UCDs are due to spindle attraction by the CAB (Nishikata et al., 1999; Prodon et al., 2010),
124 which is consistently inherited in the smallest blastomeres that will give rise to the germline
125 precursors. Before these three canonical UCDs, the orientation of the third cleavage is thought
126 to be determined by the CAB attracting one mitotic spindle pole thereby conferring the slanted
127 shape of the 8-cell stage embryo with the CAB located in the pair of vegetal-posterior
128 blastomeres B4.1 (Figure 1B) (Negishi et al., 2007). However, the vegetal blastomeres at the
129 8-cell stage, containing the CAB, are larger than their animal siblings (Nishida, 2005; Sardet
130 et al., 2007), raising the question why the CAB is inherited by the larger, rather than the smaller
131 daughter cell as found in subsequent UCDs. To address this question, we first confirmed the
132 size asymmetry between the animal and the vegetal blastomeres in *Phallusia mammillata*
133 embryos by measuring cell volumes from 3D reconstructed live 8-cell stage embryos and found
134 that vegetal blastomeres are indeed larger than their animal siblings (Figure 1C). Our measurement
135 also showed that the size asymmetry is slightly but significantly more pronounced in the
136 anterior compared to the posterior pairs of blastomeres (Figure 1C).

137

138 To determine how the UCDs are set during the 4-cell stage, we used 2D confocal imaging to
139 capture spindle dynamics. Sagittal views (Figure 2A) showed that, at metaphase, the spindle
140 in the posterior blastomere B3 is not parallel to the AV axis of the embryo but is slightly tilted
141 by 9.6° as observed previously (Negishi et al., 2007). This tilting became more pronounced at
142 anaphase onset with an angle of 21.3° due to the rapid displacement of the vegetal spindle
143 pole towards the CAB (Figure 2A and 2B), suggesting that the CAB attracts the spindle.
144 Consistent with such a role of the CAB at anaphase onset, the spindle was centered along its
145 axis at metaphase and then became vegetally off-centered at anaphase onset (Figure 2C).
146 Interestingly, however, cleavage plane prediction on 2D confocal sections showed that the
147 larger size of the vegetal daughter blastomeres (inheriting the CAB) is set at anaphase onset
148 (Figure 2D), even though the spindles were attracted toward the CAB (Figure 2B and 2C). This
149 suggests that a mechanism other than spindle off-centering generates the UCD in the posterior
150 blastomere B3 pair. Contrary to the posterior blastomere B3 pair, no clear spindle displacement
151 was observed during the division of the anterior blastomere A3 pair in sagittal views (Figure

152 S1A-S1C). Imaging anterior A3 blastomeres in transversal views, however, revealed the
153 presence of a spindle tilting by 5.67° occurring at anaphase onset apparent by a lateral
154 displacement of the vegetal spindle pole away from the vegetal pole (Figure 2E and 2F). In
155 addition, the spindle was slightly off-centered toward the animal pole in anterior blastomeres
156 at anaphase onset (Figure 3G). Prediction of the cleavage plane showed that the larger size
157 of the vegetal daughter blastomere is set at anaphase onset (Figure 2H), coinciding with the
158 timing of both spindle off-centering and tilting. Collectively, these findings reveal that spindle
159 tilting at anaphase onset is a common feature in both anterior A3 and posterior B3 blastomeres,
160 preceding UCD.

161
162 Since spindle tilting correlates with UCDs at the third cleavage, we performed a virtual analysis
163 to estimate the contribution of spindle tilting to the generation of differently sized daughter cells.
164 To do so, we used transversal confocal sections of anterior A3 blastomeres at anaphase onset
165 from which we extracted the cell contours and the spindle tilting angles measured in Figure
166 3E. We then predicted the relative daughter cell size asymmetry for two virtual cases (Figure
167 3A): in the first case, we first repositioned the spindle parallel to the AV axis and centered it on
168 this axis. In the second case, we applied the measured tilted angles while keeping the virtual
169 spindle centered along its axis (Figure 3A). For both cases, we measured the predicted
170 cleavage plane and found that UCD occurred only when tilting is applied to the spindle (Figure
171 3A). This supports the notion that spindle tilting can contribute to UCD independently of spindle
172 off-centering.

173
174 To understand better how cell shape affects UCD caused by spindle tilting, we turned to virtual
175 simulations of cleavage plane positioning using two different cell geometries (Figure 3B): a
176 circle representing an isotropic cell shape and half-circle representing an anisotropic cell
177 shape. The virtual spindle was then aligned and centered at the centre of mass, which led to
178 equal divisions for both shapes. However, when we gradually applied a tilting of the virtual
179 spindle, UCD occurred in the anisotropic geometry as the cleavage plane was gradually shifted
180 from the centre of mass, whereas no UCD occurred in the isotropic geometry as the cleavage
181 plane always crossed the center of mass (Figure 3B). This suggests that anisotropy of cell
182 shape can lead to off-centering of the cleavage plane, an effect associated to the activity of
183 spindle tilting in UCD.

184
185 **The vegetal pole contains a polarity domain which lacks cortical microtubule pulling**
186 **forces and is necessary to generate large vegetal blastomeres**

187

188 Next, we sought to understand the mechanisms underlying spindle tilting, apparent by the
189 displacement of the vegetal spindle pole away from the vegetal pole in both anterior A3 and
190 posterior B3 blastomeres. To this end, we monitored membrane invaginations after cell cortex
191 weakening as an indicator of microtubule cortical pulling sites, as previously shown in
192 *C.elegans* zygotes (Redemann et al., 2010). Membrane-labelled embryos at 4-cell stage
193 treated from Nuclear Envelop Breakdown with low concentration of Cytochalasin B to weaken
194 the actomyosin cell cortex, displayed largely normal embryo shape, but also showed distinct
195 and localized membrane invaginations at the animal pole of both anterior A3 and posterior B3
196 blastomeres at anaphase onset (Figure 4A). Furthermore, while no membrane invaginations
197 were detected at the vegetal pole, some invaginations were observed at the CAB site (visible
198 by a dense membrane staining) in posterior B3 blastomeres and at the anterior/lateral side in
199 anterior A3 blastomeres (Figure 4A). These findings are consistent with the previously reported
200 ability of the CAB to effectively pull on the spindle at 4-cell stage (Negishi et al., 2007), but also
201 indicate that the vegetal pole of both anterior A3 and posterior B3 blastomeres is devoid of
202 microtubule cortical pulling forces even though the spindle was pointing toward this domain.
203 Importantly, the observed cortical pulling forces are sufficient to explain the spindle tilting
204 motion observed in those blastomeres, suggesting that no other mechanism may be involved.
205

206 The absence of cortical pulling at the vegetal pole could be due to the absence of microtubules
207 contacting the cortex at this site. An animal-vegetal (AV) yolk gradient with higher yolk granule
208 density at the vegetal pole of blastomeres has previously been proposed to account for the
209 UCDs at the third cleavage of ascidian embryos by limiting microtubule growth toward the
210 vegetal pole (Pierre et al., 2016). However, staining of yolk granules showed a homogeneous
211 distribution in oocytes and no such AV gradient at the 4-cell stage, with yolk evenly
212 accumulating at the cell periphery (Figure S2A and S2B). Consistent with this, we found that
213 astral microtubules in live embryos, which become detectable in mitosis from anaphase onset,
214 were able to reach the vegetal cortex of these cells (Figure 4B). We thus conclude that the
215 lack of microtubule pulling forces at the vegetal pole, as evidenced by the lack of membrane
216 invagination in Cytochalasin B treated embryos, is not due to microtubules being unable to
217 reach the cortex, but rather that this cortex is devoid of microtubule pulling force generators.
218

219 Our data further point at the possibility that the vegetal pole contains a polarity domain
220 characterized by a lack of microtubule pulling force generators, leading to spindle tilting and
221 UCD. To test whether the vegetal pole indeed contains a polarity domain, we conducted
222 microsurgeries at the late zygote stage, when embryonic polarities are established after two
223 phases of ooplasmic segregations (Roegiers et al., 1999), followed by 3D reconstruction at the
224 8-cell stage for cell volume measurements (Figure 4C). To validate the ablation of the vegetal

225 pole, we verified that the determinant for gastrulation (located in the vegetal cortex of the
226 zygote) was indeed removed by confirming the absence of endoderm invagination at the 128-
227 cell stage, and we also noticed the presence of a blastocoel (Figure S2C) (Nishida, 1996).
228 Such vegetal pole ablated embryos had an equal division in the anterior lineages and an
229 inverted UCD in the posterior lineages at the third cleavage (Figure 4C). In contrast, proper
230 UCDs leading to larger vegetal blastomeres were maintained after control ablation of the
231 animal pole or the posterior pole (containing the CAB) (Figure 4C; note that gastrulation
232 occurred normally in those control ablation, Figure S2C). These microsurgery experiments
233 suggest that the vegetal pole contains a polarity domain necessary for proper spindle tilting
234 and, consequently, the generation of larger vegetal blastomeres.

235

236 **Anisotropic cell shape modulates the effect of polarity domains on UCD**

237

238 Our results so far suggest that cell shape anisotropy during mitosis at the 4-cell stage - in
239 conjunction with spindle tilting - is critical for UCD and determines the interaction between the
240 spindle and the surrounding cortical polarity domains. The anisotropic shape of mitotic
241 blastomeres at the 4-cell stage is characterized by large cell-cell contacts and a pronounced
242 long axis of the cell oriented along the AV axis of the embryo (Figure 2A and 2E). This
243 elongated cell shape is due to these cells not undergoing mitotic rounding when transiting from
244 interphase to mitosis, apparent by cell sphericity decreasing rather than increasing, as
245 expected for cells undergoing mitotic rounding (Figure S3A). Given such pronounced shape
246 anisotropy of the mitotic blastomeres, we hypothesized that decreasing their shape anisotropy
247 should perturb UCD (Figure 3B), leading to a loss of daughter cell size asymmetry in the
248 anterior blastomeres A3, and an inversion of cell size asymmetry in the posterior blastomeres
249 B3, where the spindle is attracted by the CAB. To test this hypothesis, we conducted manual
250 isolation of pairs or single blastomeres at the 4-cell stage to increase the sphericity of those
251 blastomeres when entering mitosis outside of the spatial confinement of the embryo (Figure
252 5A and 5B). Mitochondrial staining allowed us to identify the corresponding animal and vegetal
253 blastomeres after the cell division of isolated blastomeres as vegetal blastomeres inherit more
254 mitochondria (Figure S3B) (Chenevert et al., 2013). Consistent with our expectations, 3D cell
255 reconstructions before and after the division showed that an increase in mother cell sphericity
256 was associated with a decrease of the UCDs in the anterior lineages and an inversion of the
257 size asymmetry in the posterior lineages (Figure 5A and 5B and S3C-E). Live observation of
258 the mitotic spindle in isolated anterior A3 and posterior B3 blastomeres further showed that
259 spindle tilting and spindle off-centering still occurred between metaphase and anaphase,
260 suggesting that the activity of the polarity domains is maintained in such isotropic cell shape
261 (Figure 5C-5H). Yet, contrary to the situation in anisotropic blastomeres, the spindle tilting is

262 incapable of inducing UCD in these isotropic blastomeres, and, consequently, spindle off-
263 centering led to a strongly reduced UCD in anterior A3 blastomeres and an inversion of UCD
264 in posterior B3 blastomeres (Figure 5B). Collectively, these findings underline the importance
265 of cell shape anisotropy for UCD at the 4-cell stage.

266

267 Given the importance of cell shape anisotropy for blastomere UCD, we asked how the
268 orientation of cell's longest axis influences UCD. Our results so far have revealed the existence
269 of different polarity domains within the blastomeres at the 4-cell stage, with the CAB in posterior
270 cells exerting microtubule pulling forces, and a newly identified domain at the vegetal cortex of
271 both anterior and posterior blastomeres devoid of those pulling forces. We thus hypothesized
272 that the cell geometry, influencing spindle orientation, represents a critical parameter
273 modulating the interaction of the spindle with the surrounding cortex and its polarity domains.
274 In particular, we hypothesize that blastomere geometry determines the general orientation of
275 the spindle along the AV axis of the embryo, and that the presence of a vegetal polarity domain
276 lacking microtubule pulling forces causes vegetal spindle pole displacement from the vegetal
277 pole, spindle tilting and, consequently, off-centering of the cleavage plane and an UCD
278 generating a larger vegetal cell (Figure 6A). Furthermore, in the posterior blastomeres, spindle
279 tilting would be even more pronounced due to the presence of the CAB pulling on the spindle
280 (Figure 6A). In order to test this hypothesis, we sought to physically manipulate the cell
281 geometry to induce a different long axis that would in turn modify the contribution of the two
282 cortical polarity domains (vegetal cortex and CAB). Our model predicted that an AV
283 compression would reduce the influence of the vegetal polarity domain and increase the
284 influence of the CAB due to reduced blastomere AV elongation and thus spindle alignment
285 along this axis (Figure 6B). As expected, AV compression led to spindle alignment in the plane
286 of the compression (perpendicular to the AV axis) and its displacement in direction to the
287 posteriorly located CAB in posterior B3 blastomeres and, consequently, an inversion of the
288 directionality of the UCD in the posterior B3 blastomeres with the CAB now being inherited in
289 the smaller daughter cells (Figure 6C). Taken together, these findings suggest that the
290 combined activities of cell geometry and polarity domains determine spindle off-centering and
291 UCD.

292

293

294 **Discussion**

295

296 Different mechanisms have been proposed to generate UCDs among metazoans such as
297 spindle off-centering (i.e. *C.elegans*, some spiralian) (Grill and Hyman, 2005; Kotak and
298 Gönczy, 2013; Pavin et al., 2012; Ren and Weisblat, 2006; Shimizu et al., 1998), polarized

299 cortical contractility (*D.melanogaster* and *C.elegans* neuroblast) (Cabernard et al., 2010;
300 Kaltschmidt et al., 2000; Ou et al., 2010) or cell shape anisotropy (ie, some spiralian, ascidian
301 notochord) (Toledo-Jacobo et al., 2019; Winkley et al., 2019). Our findings reveal spindle tilting
302 in an anisotropic cell shape as a yet unknown mechanism determining UCD within the early
303 ascidian embryo. Importantly, this mechanism can in principle work independently of spindle
304 off-centering, since it can even override the impact of spindle pulling by the CAB in the 4-cell
305 stage ascidian embryo, but relies on cells not undergoing pronounced mitotic rounding. It is
306 thus conceivable that spindle tilting also determines UCD in other cell types that do not undergo
307 complete mitotic rounding as in early *Xenopus* embryos (Chalmers et al., 2003), in the
308 enveloping cell layer of zebrafish embryos (Campinho et al., 2013) or in *Drosophila* follicle cell
309 epithelium (Aguilar-Aragon et al., 2020).

310

311 The reasons why cells undergo different degrees of mitotic rounding and, thus, might use
312 spindle tilting as a mechanism for UCD, could be manifold. In ascidian 4-cell stage embryos,
313 cells upon entry into mitosis slightly increase cortical tension at their cell-medium interface
314 (Godard et al., 2020), but also retain low cortical tension at their cell-cell interfaces evident by
315 the presence of large cell-cell contacts (Turlier and Maître, 2015), leading to a highly
316 anisotropic cell shape during mitosis. This effect is restricted to early cleavage stages, as from
317 the 16-cell stage onwards cells undergo more pronounced mitotic rounding by decreasing their
318 apical cortical tension, which also allows mitotic cells to be deformable by extrinsic forces
319 implementing tension-oriented cell divisions (McDougall et al., 2019). Thus, in ascidians
320 spindle tilting as a mechanism determining UCD dependent of cell shape anisotropies might
321 be largely restricted to early cleavage stages, while at later stages other mechanisms, such as
322 spindle off-centering by polarity domains, might become more important, as found in the
323 germline precursors with spindle off-centering by the CAB (Costache et al., 2017; Prodon et
324 al., 2010).

325

326 A key finding of our study is that anisotropic cell shape functions in concert with different
327 polarity domains modulating microtubule pulling activities during UCD. In ascidian embryos,
328 the CAB is thought to attract one spindle pole toward the posterior cortex of the embryo,
329 thereby generating three successive rounds of UCD from the 8-cell stage onwards where the
330 CAB is consistently inherited by the smaller daughter cell, eventually giving rise to two germ
331 line precursors at the 64-cell (Negishi et al., 2007; Prodon et al., 2010). However, a major
332 paradox has been that, although the CAB is already active at the 4-cell stage (Negishi et al.,
333 2007), it is not inherited in the smaller, but rather the larger daughter cell after UCD at the 8-
334 cell stage. Our data resolve this paradox by showing that (i) in addition to the CAB, there is a
335 vegetal polarity domain with no microtubule pulling forces and (ii) cell shape anisotropy at

336 mitosis directs spindle alignment to the vegetal cortex away from the CAB, thereby mitigating
337 the activity of the CAB in pulling the spindle towards the posterior of the cell. Importantly, this
338 not only explains how the CAB can be inherited by the larger cell during the 4 to 8-cell stage
339 cleavage, but also more generally provides insight into the way by which UCD is determined
340 by the combined activities of cell shape and polarity domains.

341

342 The forces exerted on microtubules to position the spindle in early ascidian embryo were
343 proposed to originate from cytoplasmic dynein (Pelletier et al., 2020) which has also been
344 proposed to constitute the main mechanism operating in very large cells (Kotak et al., 2013).
345 However, in smaller cells like in ascidians, the astral microtubules can easily reach the cell
346 cortex, which can then also apply pulling forces on those microtubules. Thus, a combination
347 of cytoplasmic and cortical microtubule forces is likely involved in precise spindle positioning
348 in early ascidian embryos. The nature of the cortical force generator remains to be elucidated.
349 However, the cortical pulling occurring at anaphase onset suggests a possible link to a CDK-
350 1-dependent loss of NuMA phosphorylation known to drive its cortical localization (Pierre et
351 al., 2016). In addition to cytoplasmic and cortical pulling provided by microtubules, we also
352 provide evidence for the existence of a yet unknown polarity domain at the vegetal pole of the
353 embryo, which is characterized by the lack of pulling activity. Whereas polarity domains are
354 usually considered as cortical sites that provide a local elevated source of microtubule cortical
355 pulling forces, as shown for the CAB, the vegetal polarity domain clearly displays reduced
356 microtubule pulling activity. The molecular basis for the reduced pulling activity of the vegetal
357 cortical polarity domain remains to be determined; previous studies and our own preliminary
358 data suggest that this polarity domain is established after the 2nd phase of ooplasmic
359 segregation in the zygote and is probably neither a sub-cortical accumulation of yolk (Pierre et
360 al., 2016) nor a cortical gradient of the PI3-kinase shown in the ascidian *H.Roretzi* (Takatori et
361 al., 2015).

362

363 The role of cell geometry in cell division orientation is well-established (Howard and Garzon-
364 Coral, 2017; Li and Jiang, 2018; Minc and Piel, 2012; Minc et al., 2011; Pavin et al., 2012) and
365 is now understood as the result of microtubule length-dependent force generation which
366 positions the spindle along the longest axis of the cell (Pierre et al., 2016). Likewise, there is
367 now ample evidence for the presence of cortical polarity domains, able to locally influence the
368 forces exerted on microtubules and thus bias the position of the spindle (Niwayama et al.,
369 2019; Pierre et al., 2016). However, cell geometry and cortical polarity domains have generally
370 been proposed to be in competition, with one of them eventually dominating in positioning of
371 the spindle (McDougall et al., 2014). Our study, in contrast, suggests that cell geometry and
372 polarity domains act in concert to determine spindle positioning, with cell geometry modulating

373 the effect of cortical polarity domains by influencing the position of the spindle relative to those
374 polarity domains. It will be interesting to determine whether the activity of cortical polarity
375 domains can be affected by cell shape also in other systems where cells remain non-spherical
376 during mitosis and contain cortical polarity domains. In *C. elegans* embryogenesis, for
377 instance, where different polarity domains were shown to affect UCD, embryo deformation
378 alters the precise placement of the cleavage planes (Yamamoto and Kimura, 2017), pointing
379 at the intriguing possibility that the co-action of cell shape and polarity domains represents an
380 evolutionary conserved principle determining UCD.

381

382

383

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393

394 **Figure legends**

395

396 Figure 1: Unequal cell division at the third cleavage of ascidian embryos creates larger vegetal
397 blastomeres which inherit the CAB.

398 (A) Bright field images of vegetal view of an embryo from 8-cell stage to 64-cell stage. The
399 double black arrows on the left side of the embryo mark sister cells and the dashed lines on
400 the right side of the embryo delineate the sister cells of different sizes. Asterisks mark the CAB.
401 (B) Bright field images of left side view of an embryo positioned in a microwell with animal side
402 on top and posterior to the right at 4-cell stage (left), during cytokinesis (middle) and at 8-cell
403 stage (right). The double black arrows show the sister cells. Asterisks mark the CAB. (C)
404 Images (top panel) of left side view of an 8-cell stage embryo imaged on confocal microscope
405 with CellMask staining and the corresponding 3D reconstruction. Plot (bottom panel) of the
406 ratio of the animal daughter cell volume relative to total daughter cells volume for anterior (in
407 blue) and posterior (in red) lineages at 8-cell stage (center line, median; box limits, upper and
408 lower quartiles; whiskers, min and max; cross, mean). The *P* values correspond to two-tailed
409 paired Wilcoxon tests for comparisons of anterior or posterior lineages to 0.5 (equal division)
410 and for anterior/posterior lineages comparison. Asterisks mark the CAB. All scale bars, 20 μ m.
411

412 Figure 2: The spindles tilt at anaphase onset during UCDs.

413 (A) Sagittal views showing spindle position at metaphase and anaphase onset at the 4-cell
414 stage. Schematic illustration (left panel) of a 4-cell stage embryo with the imaging plane in a
415 sagittal view (shown by a dotted double arrow) with anterior blastomeres A3 in blue and
416 posterior blastomeres B3 in red. Fluorescence images of a 4-cell stage embryo in sagittal view
417 (anterior to the left and animal on top) in metaphase (top) and anaphase onset (bottom)
418 previously injected with mRNAs coding for Ensconsin::3xGFP (2 μ g/ μ l, green) and
419 H2B::mCherry (2 μ g/ μ l, red) while plasma membrane is stained by CellMask deep red (1 μ g/ μ l,
420 cyan). The asterisks indicate the position of the CAB in B3 blastomeres. (B) Plot of the spindle
421 angle with the AV axis in posterior blastomere B3 measured on sagittal views in metaphase
422 and anaphase. Positive angle means that the mitotic spindles point toward the posterior
423 pole/side of the embryo. (C) Plot of the spindle centering along its axis (see materials and
424 methods for details) measured in posterior blastomere B3 from sagittal views in metaphase
425 and anaphase. 0.5 means centered spindle and above 0.5 means spindle off-centered toward
426 the vegetal pole. (D) Plot of the relative animal area after bisection according to the spindle
427 position in posterior blastomere B3 in sagittal view. 0.5 means animal and vegetal area are
428 equal and below means vegetal area is larger. (E) Transversal views showing spindle position
429 in anterior blastomeres A3 in metaphase and anaphase onset at the 4-cell stage. Schematic
430 illustration (left panel) of 4-cell stage embryo showing the transverse optical plane across the

431 anterior blastomeres with anterior blastomeres A3 in blue and posterior blastomeres B3 in red.
432 Fluorescence images of 4-cell stage embryo in transversal view (animal on top) in metaphase
433 (top) and anaphase onset (bottom) previously injected with Ensconsin::3xGFP (2 μ g/ μ l, green)
434 and H2B::mCherry (2 μ g/ μ l, red) mRNAs while plasma membrane is stained with CellMask
435 deep red (1 μ g/ μ l, cyan). (F) Plot of the spindle angle with the AV axis in anterior blastomere
436 A3 in transversal view in metaphase and anaphase. Positive angle means that the mitotic
437 spindles point away from the midline. (G) Plot of the spindle centering along its axis in anterior
438 blastomere A3 in transversal view in metaphase and anaphase. 0.5 means a centered spindle
439 and below 0.5 indicates a spindle off-centered toward the animal pole. (H) Plot of the relative
440 animal area after bisection according to the spindle position in anterior blastomere A3 in
441 transversal view. 0.5 means animal and vegetal area are equal and below means vegetal area
442 is larger. All the *P* values correspond to two-tailed paired Wilcoxon tests for comparisons to
443 0.5 (equal division) or for metaphase/anaphase comparisons. The box plots are built with
444 center line, median; box limits, upper and lower quartiles; whiskers, min and max; cross, mean.
445

446 Figure 3: Spindle tilting in an anisotropic cell geometry induces UCD by displacing the cleavage
447 plane from the cell center of mass.

448 (A) Simulation of the effect of a tilted but centered spindle on the bisection of the cell.
449 Schematic illustration of the simulations realized using real cell contour of anterior blastomeres
450 from transversal view. First scenario, the spindle is positioned parallel to the AV axis (not tilted)
451 and centered along this axis (top panel). Second scenario, the spindle tilting is applied (tilted)
452 while kept centered along its axis (bottom panel). Plot of the relative animal area to total cell
453 area after bisection according to the spindle angle using the tilting angles measured at
454 anaphase onset (n=22) (right panel) (center line, median; box limits, upper and lower quartiles;
455 whiskers, min and max; cross, mean). 0.5 means animal and vegetal area are equal and below
456 means vegetal area is larger. Grey lines connect simulation for the same real cell contour and
457 tilting angle. The *P* values correspond to two-tailed paired Wilcoxon tests for comparisons to
458 0.5 (equal division) and for comparison between the two conditions. (B) Theoretical evaluation
459 of spindle tilting in different cell geometries. Schematic illustration of the division plane from a
460 gradually tilted but centered spindle (the top spindle pole remains fixed) in an isotropic shape
461 (circle) (top panel) and in a anisotropic shape (half-circular shape) (bottom panel). Plot of the
462 relative top area to total area after bisection according to the spindle tilting (left panel). 0.5
463 means top and bottom area are equal and below means bottom area is larger.

464

465 Figure 4: The vegetal cortex is a polarity domain lacking microtubule cortical pulling forces and
466 is necessary for making larger vegetal blastomeres.

467 (A) Cortical pulling sites on astral microtubules at mitosis during the 4-cell stage observed as
468 plasma membrane invaginations in embryos with a weakened cortex. Maximum projection
469 (26 μ m thick) of confocal images of 4-cell stage embryo in sagittal view (animal on top) during
470 mitosis (left panel). Embryos were treated with cytochalasin (15 μ M) from Nuclear Envelop
471 Break Down to soften the cell cortex during mitosis and the plasma membrane is stained with
472 CellMask Green (at 1 μ g/ μ l). Orthogonal section (bottom right) at the level indicated by the
473 dashed lines showing the location of first invaginations at the site of CAB in B3 and in lateral
474 position in A3. Schematic illustration summarizing all the membrane invaginations observed in
475 6 embryos (top right). The blue and red arrow heads indicate sites of membrane invaginations
476 for the anterior blastomere A3 and the posterior blastomere B3 respectively. Asterisks show
477 the CAB localization. Scale bar, 20 μ m. (B) Astral microtubules reach the cortex at anaphase
478 onset. Fluorescent confocal images of 4-cell stage embryo in sagittal view (anterior to the left
479 and animal on top) in metaphase (top) and anaphase onset (middle) injected with
480 Ensconsin::3xGFP (2 μ g/ μ l, green) and Utrophin::mCherry (2 μ g/ μ l, red) mRNAs. Enlarged view
481 (bottom) corresponding to the area delineated by the dashed box where white arrow heads
482 indicate astral microtubules contacting the cortex at the vegetal pole at anaphase onset. Scale
483 bar, 20 μ m. (C) Ablations of cell cortex at zygote stage and resulting blastomeres size at 8-cell
484 stage. Schematic illustration (left panel) of the ablation performed at zygote stage after the
485 second phase of ooplasmic segregation (animal on top) and the corresponding 8-cell stage
486 visualized in 3D reconstruction in sagittal view (anterior to the left and animal on top). Plots of
487 the ratio of animal daughter cell volume relative to total daughter cell volume for anterior
488 lineage (in blue, middle) and posterior lineages (in red, right) at 8-cell stage measured from 3D
489 reconstructions in control embryos and embryos with the vegetal pole (VP), or the animal pole
490 (AP) ablated, or CAB ablated (center line, median; box limits, upper and lower quartiles;
491 whiskers, min and max; cross, mean). The *P* values correspond to two-tailed paired Wilcoxon
492 tests for comparisons of anterior or posterior lineages to 0.5 (equal division). Asterisks show
493 the CAB localization.

494

495 Figure 5: Decreasing cell shape anisotropy of the mitotic blastomeres changes the UCDs.

496 (A) Left panel: Bright field images and corresponding 3D reconstruction (anterior blastomeres
497 (A3) in blue, posterior blastomeres (B3) in red) of 4-cell stage embryos in mitosis either whole
498 or after isolations of blastomeres (1/4) or pair of blastomeres (1/2). Right panel: Bright field
499 images and corresponding 3D reconstruction at 8-cell stage (anterior blastomeres (a4.2, A4.1)
500 in blue and cyan, posterior blastomeres (b4.2, B4.1) in red and yellow) either whole or after
501 isolation of blastomeres (1/4) or pair of blastomeres (1/2). Isolations were performed at the 4-
502 cell stage. Note that the whole embryo at 4-cell stage is oriented with an animal view whereas
503 all other embryos are shown in side views. Scale bar, 20 μ m. (B) Plot showing the ratio of

504 animal daughter cell volume relative to total daughter cells volume at 8-cell stage as a function
505 of their mother cell sphericity in mitosis for control embryos and isolated blastomeres (1/4) or
506 pair of blastomeres (1/2) for anterior (blue) and posterior lineages (red). Error bars are standard
507 deviation. (C) Fluorescence images of isolated anterior A3 blastomere previously injected with
508 mRNAs coding for *Ensconsin::3xGFP* (2 μ g/ μ l, green). The spindle is labelled in red and green
509 for the time corresponding to metaphase and anaphase onset, respectively. (D) Plot of the
510 angle between the spindle at metaphase and anaphase onset in isolated anterior A3
511 blastomeres. (C) Plot of the spindle centering along its axis (see materials and methods for
512 details) measured in isolated anterior A3 blastomere in metaphase and anaphase. 0.5 means
513 centered spindle and above 0.5 means spindle off-centered toward the vegetal pole. (E)
514 Fluorescence images of isolated posterior B3 blastomere previously injected with mRNAs
515 coding for *Ensconsin::3xGFP* (2 μ g/ μ l, green). The spindle is labelled in red and green for the
516 time corresponding to metaphase and anaphase onset, respectively. (F) Plot of the angle
517 between the spindle at metaphase and anaphase onset in isolated posterior B3 blastomeres.
518 (C) Plot of the spindle centering along its axis (see materials and methods for details)
519 measured in isolated posterior B3 blastomere in metaphase and anaphase. 0.5 means
520 centered spindle and above 0.5 means spindle off-centered toward the vegetal pole/CAB. All
521 the *P* values correspond to two-tailed paired Wilcoxon tests for comparisons to 0.5 (equal
522 division) or for metaphase/anaphase comparisons. The box plots are built with center line,
523 median; box limits, upper and lower quartiles; whiskers, min and max; cross, mean. Scale bar,
524 20 μ m.

525

526 Figure 6: Anisotropic cell shape influences the spindle interaction with polarity domains and
527 together determines the orientation of UCD.

528 (A) Schematic illustration (left panel) of a 4-cell stage embryo in sagittal view (anterior to the
529 left, animal on top) showing the spindle interaction with the different cortical polarity domains.
530 Illustration of the cleavage plane positioning mechanism (right panel) where the cleavage
531 plane position is a result of the interplay between cell geometry, the posterior CAB polarity
532 domain and the vegetal pole polarity domain (VP). (B) AV compression of 4-cell stage embryo.
533 Schematic illustration a 4-cell stage embryo in sagittal view with an AV compression (top left
534 panel) to bring the long axis of the cell towards the CAB and away from the vegetal pole domain
535 (bottom left panel). Optical confocal section of antero-posterior compressed embryo (middle
536 panel) at 4-cell stage anaphase and at 8-cell stage with membrane stained with CellMask Deep
537 orange (at 1 μ g/ μ l). Plot of the relative animal daughter cell area (right) for posterior lineages at
538 8-cell stage in AV compressed embryos (center line, median; box limits, upper and lower
539 quartiles; whiskers, min and max; cross, mean). The *P* values correspond to two-tailed paired

540 Wilcoxon tests for comparisons to 0.5 (equal division). Asterisks show the CAB localization.
541 VP, Vegetal Pole. Scale bar, 20 μ m.

542

543 Figure S1 – related to Figure 2: Spindle dynamics in anterior blastomeres in sagittal view.

544 (A) Plot of the spindle angle with the AV axis in anterior blastomere A3 measured on sagittal
545 views (see Figure 3A) in metaphase and anaphase. Positive angle means that the mitotic
546 spindles point toward the posterior pole/side of the embryo. (B) Plot of the spindle centering
547 along its axis (see materials and methods for details) measured in anterior blastomere A3 from
548 sagittal views (see Figure 3A) in metaphase and anaphase. 0.5 means centered spindle and
549 above 0.5 means spindle off-centered toward the vegetal pole. (C) Plot of the relative animal
550 area after bisection according to the spindle position in anterior blastomere A3 in sagittal view
551 (see Figure 3A). 0.5 means animal and vegetal area are equal and below means vegetal area
552 is larger. All the *P* values correspond to two-tailed paired Wilcoxon tests for comparisons to
553 0.5 (equal division) and for metaphase/anaphase comparisons. The box plots are built with
554 center line, median; box limits, upper and lower quartiles; whiskers, min and max; cross, mean.

555

556 Figure S2 – related to Figure 4: Yolk distribution in oocyte and 4-cell stage; gastrulation
557 phenotypes after zygote microsurgeries.

558 (A) Confocal fluorescence image showing section across an oocyte (animal on top) with the
559 yolk granules stained with Bodipy 493/503 (1 μ g/ μ l, green). Scale bars, 20 μ m. (B) Confocal
560 fluorescence image showing sagittal section at 4-cell stage (animal on top) with the yolk
561 granules stained with Bodipy 493/503 (1 μ g/ μ l, green). Scale bars, 20 μ m. (C) Schematic
562 illustration (left panel) of the ablation performed at zygote stage after the second phase of
563 ooplasmic segregation (animal on top) and the corresponding confocal fluorescence image of
564 a sagittal section at the gastrula stage (right panel) with the plasma membrane stained with
565 CellMask orange (at 1 μ g/ μ l, white). The white arrow indicates invagination of the endoderm at
566 the vegetal pole. Scale bars, 20 μ m.

567

568 Figure S3 – related to Figure 5: Cell sphericity during 4-cell stage ; Blastomere identification
569 and morphometric analysis of isolated blastomeres.

570 (A) Bright field images of vegetal view of an embryo during the 4-cell stage with anterior to the
571 left (top left panel) and the corresponding 3D reconstructions with anterior blastomeres (A3) in
572 blue and posterior blastomeres (B3) in red (bottom left panel). Plot (right panel) of the cell
573 sphericity for the anterior blastomeres (blue) and posterior blastomeres (red) during the 4-cell
574 stage. Error bars are standard deviation. NEBD, Nuclear Envelop Break Down. Asterisks mark
575 the CAB. (B) Confocal optical section in isolated blastomeres or pair of blastomeres with the
576 mitochondria stained with Mitotracker deep red (at 1 μ g/ μ l, magenta) and the plasma

577 membrane with Cell Mask orange (at 1 $\mu\text{g}/\mu\text{l}$, white) at 4-cell stage and after the cell division at
578 8-cell stage. The blue and red arrowheads at 8-cell stage show the highest mitochondria
579 concentration in the vegetal blastomere in anterior and posterior lineages respectively. (C) Plot
580 of the blastomere sphericity in control (whole) embryo and after blastomere isolations in the
581 anterior lineage. The P values correspond to two-tailed unpaired Mann-Whitney test. (D) Plot
582 of the blastomere sphericity in control (whole) embryo and after blastomere isolations in the
583 posterior lineage. The P values correspond to two-tailed unpaired Mann-Whitney test. (E) Plot
584 of the relative animal daughter cell volume to total daughter cell volume in control (whole)
585 embryo and after blastomere isolations in the anterior lineage. The P values correspond to
586 two-tailed paired Wilcoxon tests for comparisons to 0.5 (equal division) and two-tailed unpaired
587 Mann-Whitney test to compare the different conditions between each other. (F) Plot of the
588 relative animal daughter cell volume to total daughter cell volume in control (whole) embryo
589 and after blastomere isolations in the posterior lineage. The P values correspond to two-tailed
590 paired Wilcoxon tests for comparisons to 0.5 (equal division) and two-tailed unpaired Mann-
591 Whitney test to compare the different conditions between each other. Scale bars, 20 μm .
592
593
594

595 **Materials and Methods**

596

597 Animal collection/maintenance and embryo handling

598 Adults individuals of the European ascidian *Phallusia mammillata* were either purchased from
599 the Roscoff marine station (France) and kept in artificial sea water (ASW) or collected from
600 Sète (France) and kept in filtered natural sea water (FSW) at 16°C. Eggs and sperm were
601 collected by dissection. Eggs were dechorionated in sea water implemented with Trypsin
602 (T8003, Sigma-Aldrich) at 0.1% for 1-2h, and sperm was activated in pH 9.0 sea water prior
603 fertilizations (Schindelin et al., 2012).

604

605 Embryo orientation, imaging and 3D reconstruction

606 Transparent circular microwells were used to orient embryos and image them with a confocal
607 microscope. For this, a home-made array of little pillars made in PDMS was used to cast
608 circular microwell in a polymer of the same refractive index as water (MY-134, MY Polymers)
609 in a glass bottom petri dish (P35G-1.5-14-C, MatTek). For 3D reconstruction, images of
610 $1.48\mu\text{m}^3$ voxel size were acquired every 2min on an inverted Leica TCS SP5, equipped with a
611 HC PL APO CS2 40x/1.10 water immersion objective (Leica), a Hybrid detector and a home-
612 made cooling stage set at 19°C. The images were processed with Ilastik software to segment
613 the membrane signal followed by an intensity threshold applied with ImageJ (Yasuo and
614 McDougall, 2018). 3D reconstruction was performed on the segmented images with Imaris 9.0
615 (Bitplane), which provided measurement of the cell volume and cell sphericity. The relative
616 animal daughter cell volume corresponds to the volume of the animal cell divided by the total
617 volume of the daughter cells (animal and vegetal).

618

619 Blastomere isolation

620 Blastomere or pair of blastomeres were isolated at 4-cell stage using a thin glass filament
621 gently inserted in between blastomeres observed on an Olympus SZX16 stereomicroscope.
622 After isolation, the blastomeres were transferred in ASW supplemented with CellMask Orange
623 ($1\mu\text{g}/\text{ml}$; C10045, Invitrogen) and MitoTracker deep red ($1\mu\text{g}/\mu\text{l}$; M22426, Invitrogen) and
624 imaged on a confocal microscope as for 3D reconstruction detailed above.

625

626 Microtubules live imaging and spindle dynamics quantification

627 Dechorionated oocytes were injected with *Ensconsin::3xGFP* and *H2B::mCherry* or
628 *Utrophin::mCherry* polyA tailed mRNA at $2\mu\text{g}/\mu\text{l}$, synthesized using mMessage mMachine SP6
629 Transcription Kit (Invitrogen, AM1340) on a microinjection setup mounted on an Olympus
630 SZX16 stereomicroscope as in (Schindelin et al., 2012) except that single embryos were
631 oriented in an agarose circular well made from a homemade PDMS pillar microarray. After a

632 minimum 4 hours of incubation, injected oocytes were fertilized and early 4-cell stage embryos
633 were placed in a circular microwell (see above) containing ASW and CellMask deep red at
634 $1\mu\text{g}/\mu\text{l}$ ($1\mu\text{g}/\text{ml}$; C10046, Invitrogen). Confocal time-lapse acquisition of embryos left/right side
635 or anterior facing the objective were performed on an inverted Leica TCS SP5, equipped with
636 a HC PL APO CS2 40x/1.10 water immersion objective (Leica), a Hybrid detector and a home-
637 made cooling stage set at 19°C . All the quantification of spindle position were measured using
638 ImageJ software (Schindelin et al., 2012) on 2D confocal section allowing to precisely capture
639 the anaphase onset. The spindle angle is relative with the AV axis, the spindle centering is the
640 ratio of the distance between chromosomal plate and animal cell edge divided by the distance
641 between the chromosomal plate and the vegetal cell edge, measured on the spindle axis. The
642 division prediction is calculated as the ratio of animal and vegetal areas with a cleavage plane
643 positioned perpendicularly to the spindle at its midzone.

644

645 Simulations of the impact of spindle tilting on cleavage plane positioning

646 To simulate spindle tilting without spindle off-centering, a virtual repositioning of the spindle
647 was performed using ImageJ (Schindelin et al., 2012) on real confocal images of anterior views
648 of 4-cell stage embryos (Figure 2E). Using the time point of anaphase onset, the spindle was
649 first aligned with and centered along the AV axis. Then, a tilting was applied by using the
650 measured angles for the corresponding embryo, while still keeping the spindle centered along
651 its axis. For these two scenarios, the cell area was divided perpendicularly from the centre of
652 the spindle and the relative animal area was measured. For the full virtual simulation of the
653 effect of spindle tilting in a circular (non-constrained) and a half-circular (constrained)
654 geometry, all the scenarios were performed using the different drawing tools of ImageJ
655 (Schindelin et al., 2012).

656

657 Imaging of microtubules cortical pulling sites

658 Embryo were placed in ASW with $15\mu\text{M}$ of CytochalasinB (Sigma-Aldrich, C6762) and
659 CellMask Green at $1\mu\text{l}/\text{ml}$ (Invitrogen, C37608) at the time of Nuclear Envelop Break Down
660 (NEBD) in circular microwell (see above). Fast timelapse confocal acquisition of the embryo
661 left or right side were performed on an inverted Leica TCS SP5 equipped with a HC PL APO
662 CS2 40x/1.10 water immersion objective (Leica). Around 40-60 sec after the first membrane
663 invagination occurred, corresponding to the time of anaphase, a maximum projection of
664 selected planes was done to generate the image on which counting of the sites of membrane
665 invaginations was performed.

666

667 Zygote microsurgeries

668 Ablations of the cell cortex were performed on zygotes after the second phase of ooplasmic
669 segregation (45 to 50 min after fertilization) on an Olympus SZX16 stereomicroscope
670 microinjection setup where the injection needle was replaced by a blunt-end glass pipette with
671 an internal diameter of 40µm and a 30° bent angle (Biomedical Instruments). The vegetal pole
672 or the animal pole were aspirated within the pipette and sectioned from the embryo with a thin
673 glass needle. The volume of the ablated CP cytoplasm was around 12% of the oocyte volume.
674 At 8-cell stage, ablated embryos were placed in ASW with CellMask Orange (1µg/ml ; C10045,
675 Invitrogen) in a circular microwell for 3D reconstruction (see above). After 8-cell stage, the
676 embryo were kept on the microscope to monitor the phenotypes associated with each
677 microsurgery at gastrula stage.

678

679 Embryo compression

680 For Antero-Posterior (AP) compression, early 4-cell stage embryo were placed in a dish
681 containing ASW with CellMask orange (1µg/ml ; C10045, Invitrogen) with a piece of PDMS
682 sculpted with a cavity of rectangular geometry. The compression was realized by placing a
683 coverslip on top of the PDMS. The embryos placed in a cavity providing the proper AP
684 compression were then imaged on an upright Leica TCS SP5 or SP8 equipped with a HC PL
685 APO 20x/0.8 (Leica). For AV compression, early 4-cells stage embryos were placed in ASW
686 with CellMask orange (1µg/ml ; C10045, Invitrogen) on a glass slide. Then a coverslip was
687 gently added on the top and pressed under a stereoscope until the compression was observed.
688 Embryos were then imaged on an inverted Leica TCS SP5 or SP8 equipped with a HC PL
689 APO 20x/0.8 (Leica).

690

691

692 Quantification and statistical analysis

693 Graphical analysis of data and statistics were performed with Excel (Microsoft). All the p-value
694 were calculated with either two-tailed unpaired Mann-Whitney or two-tailed paired Wilcoxon
695 tests. No statistical method was used to predetermine sample size and the experiments were
696 not randomized.

697

698 **References**

699

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Figure 1: Unequal cell division at the third cleavage of ascidian embryos creates larger vegetal blastomeres which inherit the CAB.

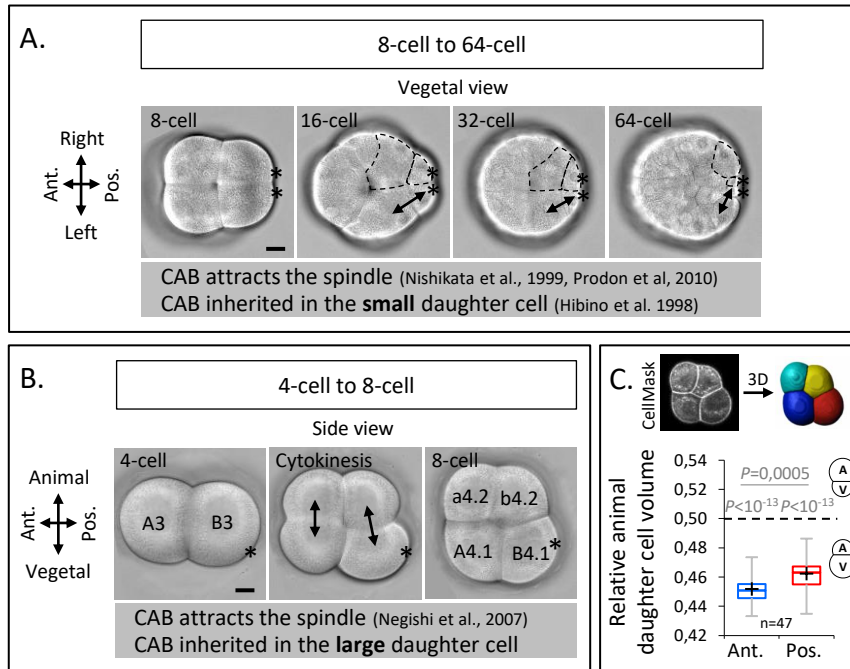


Figure 2: The spindles tilt at anaphase onset during UCDs.

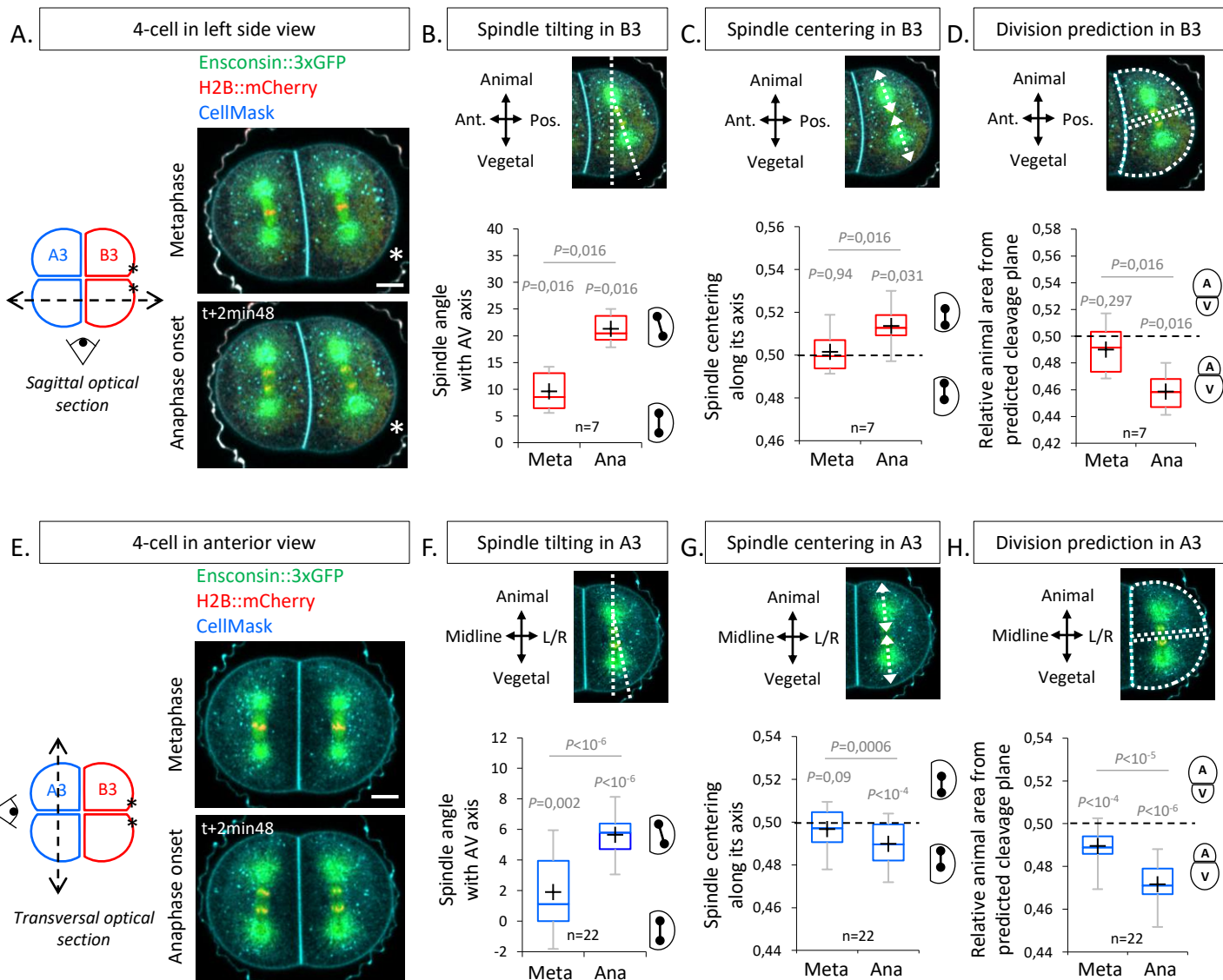


Figure 3: Spindle tilting in an anisotropic cell geometry induces UCD by displacing the cleavage plane from the cell center of mass.

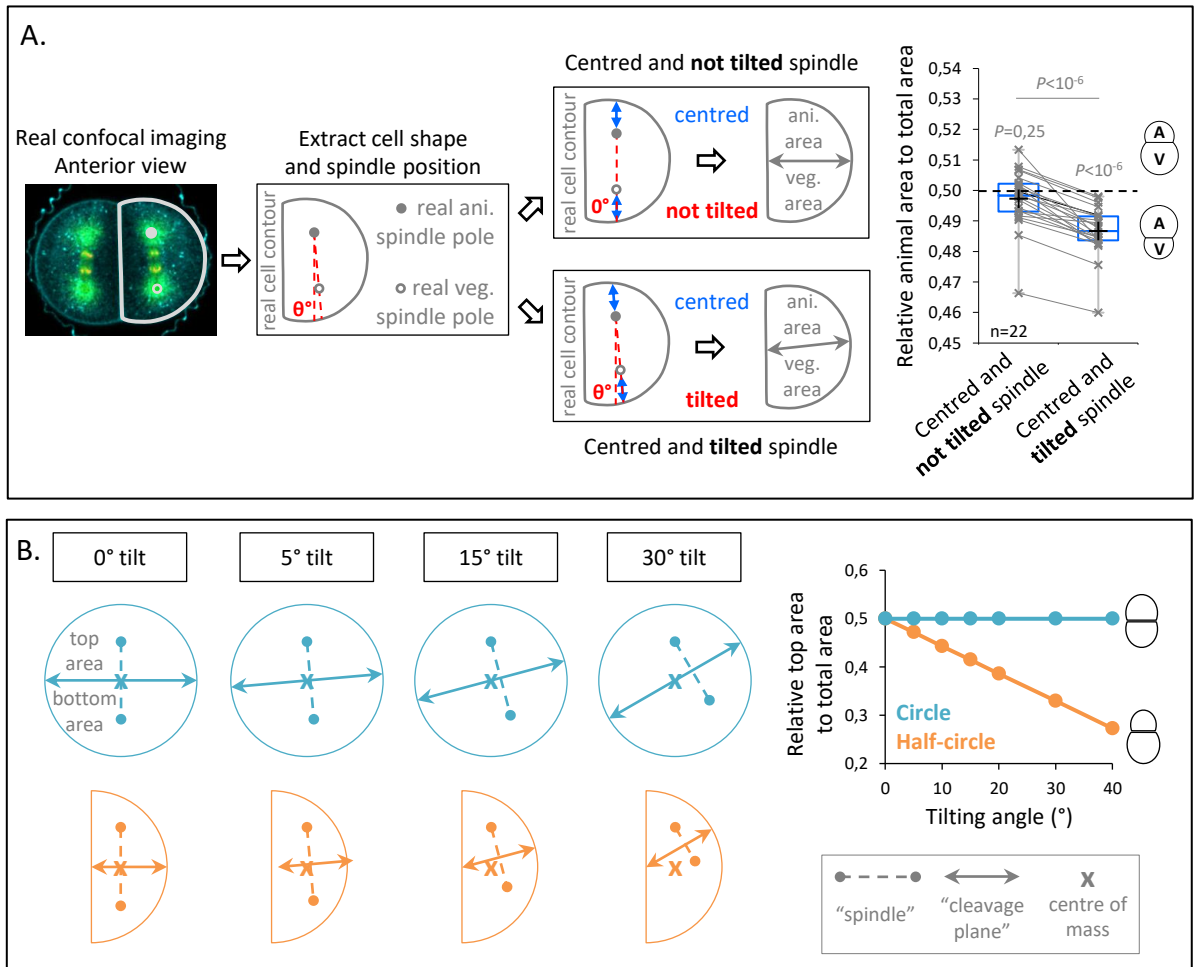


Figure 4: The vegetal cortex is a polarity domain lacking microtubules cortical pulling forces and is necessary for making larger vegetal blastomeres.

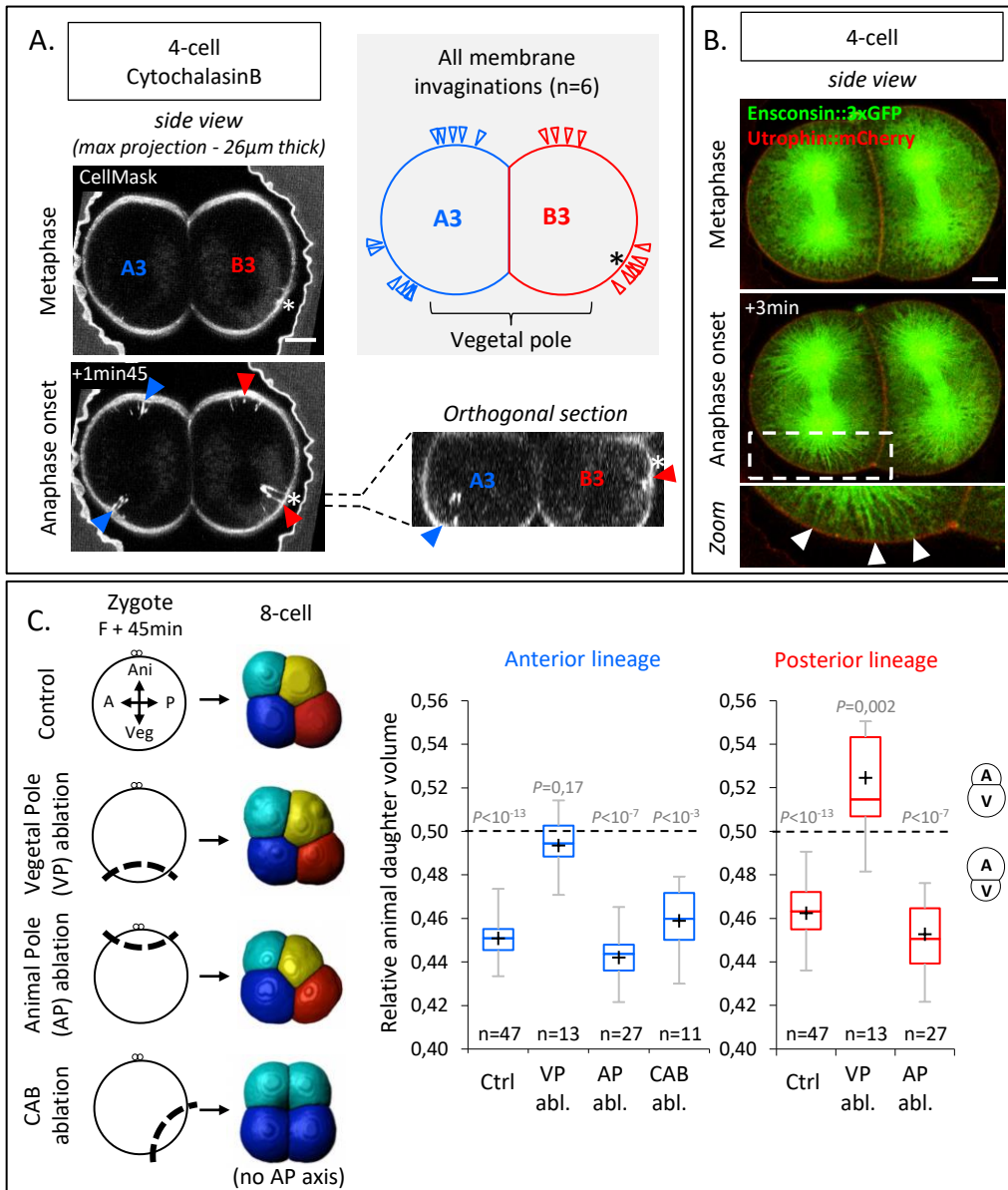


Figure 5: Decreasing cell shape anisotropy of the mitotic blastomeres changes the UCD

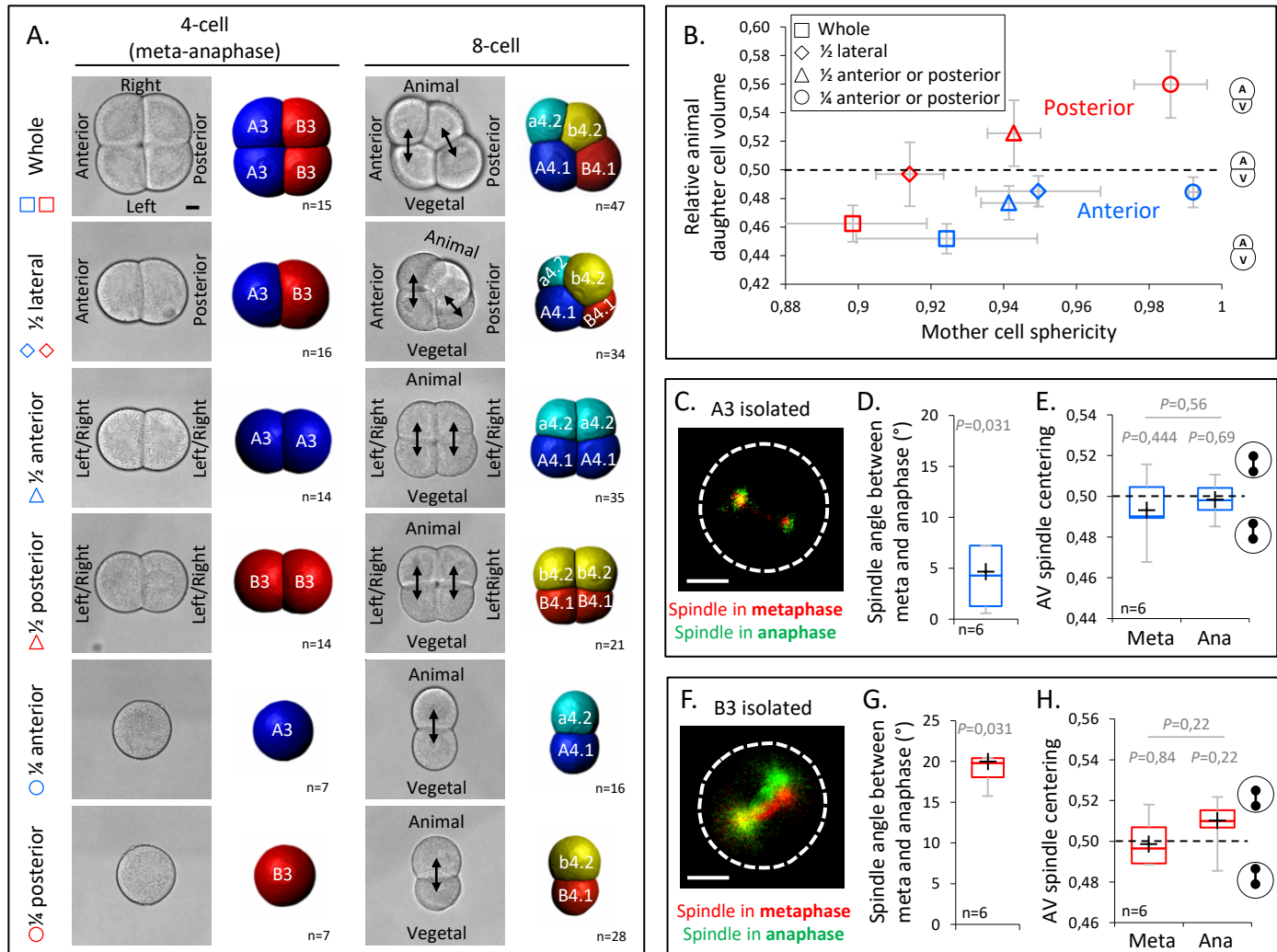


Figure 6: Anisotropic cell shape influences the spindle interaction with polarity domains and together determines the orientation of UCD.

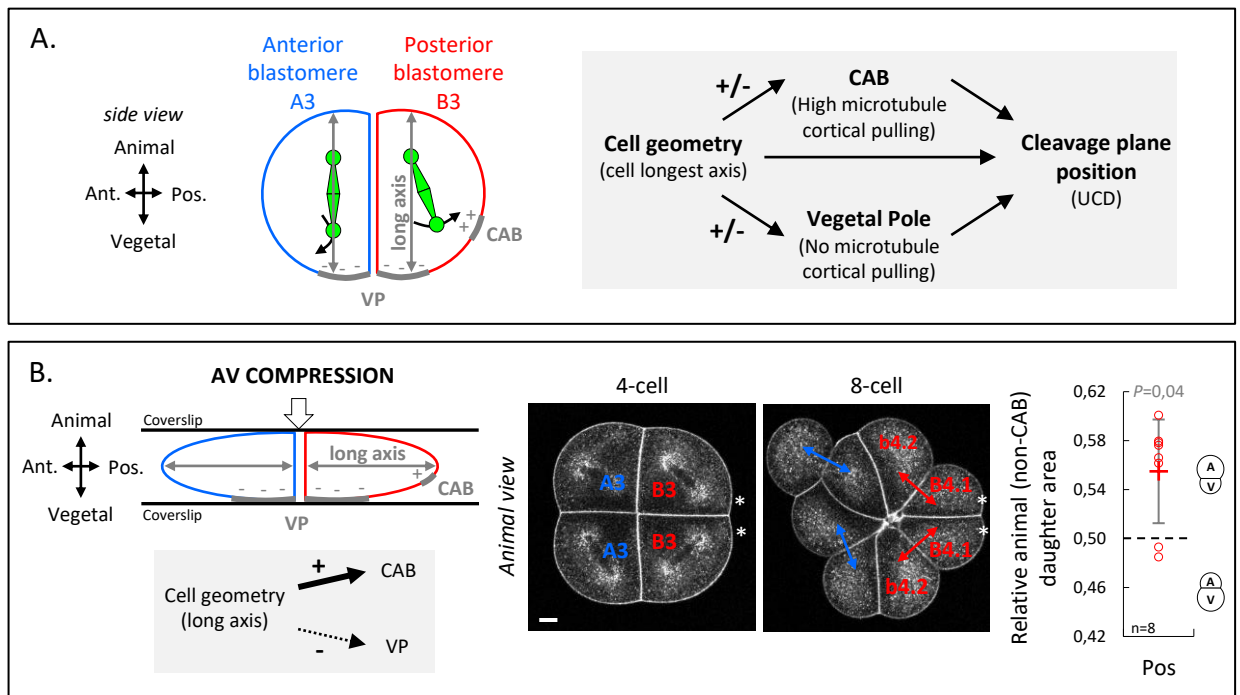


Figure S1 – related to Figure 2: Spindle dynamic in anterior blastomere in sagittal view.

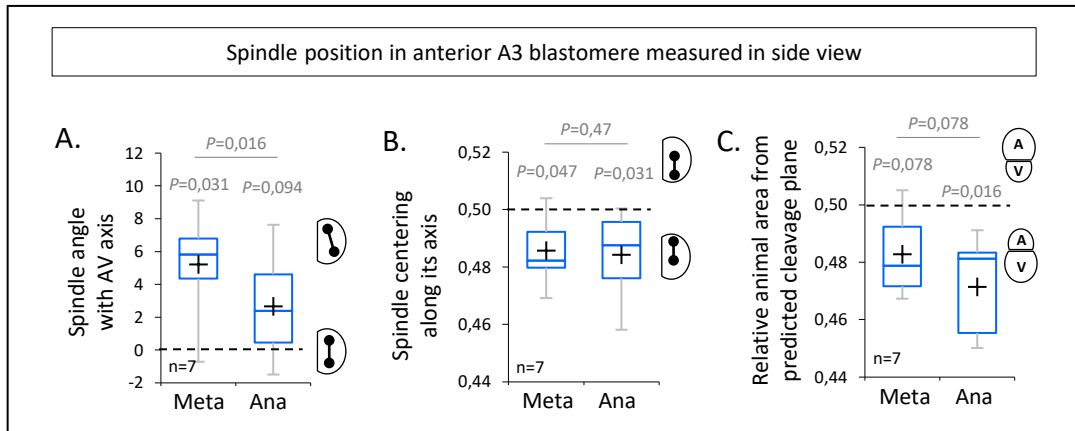


Figure S2 – related to Figure 4: Yolk distribution in oocyte and 4-cell stage ; gastrulation phenotypes after zygote microsurgeries.

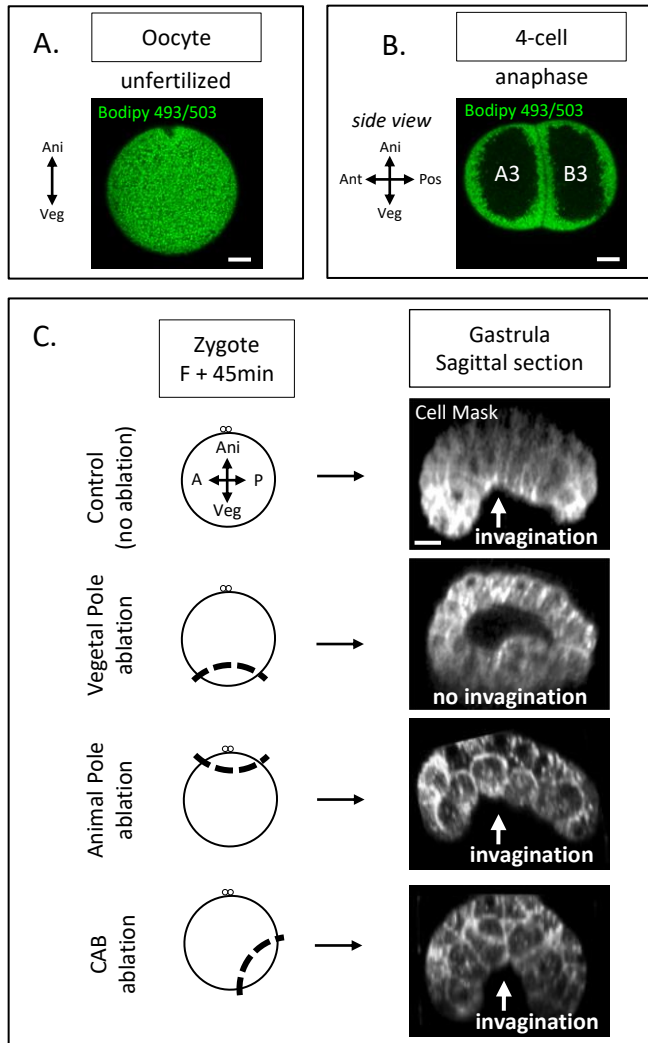


Figure S3 – related to Figure 5: Cell sphericity during 4-cell stage ; Blastomere identification and morphometric analysis of isolated blastomeres.

