

Polarity-driven three-dimensional spontaneous rotation of a cell doublet

Linjie Lu^{*1-4}, Tristan Guyomar^{*1-4}, Quentin Vagne^{*5}, Rémi Berthoz¹⁻⁴, Alejandro Torres-Sánchez⁶, Michèle Lieb¹⁻⁴, Cecilie Martin-Lemaitre^{7,8}, Kobus van Unen⁹, Alf Honigmann^{7,8,10}, Olivier Pertz⁹, Guillaume Salbreux^{**5}, Daniel Riveline^{**1-4}

¹ Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France

² Université de Strasbourg, Illkirch, France

³ Centre National de la Recherche Scientifique, UMR7104, Illkirch, France

⁴ Institut National de la Santé et de la Recherche Médicale, U964, Illkirch, France

⁵ University of Geneva, Quai Ernest Ansermet 30, 1205 Genève, Switzerland

⁶ Tissue Biology and Disease Modelling Unit, European Molecular Biology Laboratory, Doctor Aiguader 88, Barcelona (08003), Spain

⁷ Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

⁸ Technische Universität Dresden, Biotechnologisches Zentrum, Center for Molecular Cellular Bioengineering (CMCB), Dresden, Germany

⁹ Institute of Cell Biology, University of Bern, Bern, Switzerland

¹⁰ Cluster of Excellence Physics of Life, TU Dresden, Dresden, Germany

* Equal contribution

**Co-corresponding authors : Guillaume.Salbreux@unige.ch and riveline@unistra.fr

Abstract:

Cell mechanical interactions play a fundamental role in the self-organisation of organisms. How these interactions drive coordinated cell movement in three-dimensions remains unclear. Here we report that cell doublets embedded in a 3D extracellular matrix undergo spontaneous rotations and we investigate the rotation mechanism using live cell imaging, quantitative measurements, mechanical perturbations, and theory. We find that rotation is driven by a polarized distribution of myosin within cell cortices. The mismatched orientation of this polarized distribution breaks the doublet mirror symmetry. In addition, cells adhere at their interface through adherens junctions and with the extracellular matrix through focal contacts near myosin clusters. Using a physical theory describing the doublet as two interacting active surfaces, we find that rotation is driven by myosin-generated gradients of active tension, whose profiles are dictated by interacting cell polarity axes. We show that interface three-dimensional shapes can be understood from the Curie principle: shapes symmetries are related to broken symmetries of myosin distribution in cortices. To test for the rotation mechanism, we suppress myosin clusters using laser ablation and we generate new myosin clusters by optogenetics. Our work clarifies how polarity-oriented active mechanical forces drive collective cell motion in three dimensions.

Spontaneous rotations *in vivo* and *in vitro*

Spontaneous cell rotational motions have been reported in a variety of contexts *in vivo*. Tissues undergo rotation during development in *Drosophila* in the egg chamber¹, in the ommatidia of the retina², in the testis³, and in zebrafish embryos, where rotation of cell pairs occurs in the zebrafish's lateral line⁴. In early *C. elegans* embryo development, chiral counter-rotating flows break chiral symmetry and play a role in setting the organism's left-right axis^{5,6}.

51 Seminal observations *in vitro* in two-dimensions have shown that endothelial adhering cells
52 migrating on a substrate and confined within a two-dimensional pattern form a stably rotating
53 doublet⁷. The cell-cell interface adopts a curved shape, such that the doublet acquires an overall
54 shape reminiscent of a “yin-yang”. More recently, groups of epithelial cells were reported to
55 undergo rotation within rings^{8,9}. In three-dimensions *in vitro*, during alveologenesis of the
56 human mammary gland, it was shown that organoids also undergo rotation¹⁰. In addition,
57 MDCK cells can assemble into hollow cysts in three-dimensions which undergo spontaneous
58 rotation in an assay within two layers of Matrigel¹¹. There the two layers of Matrigel impose a
59 polarization axis to the cyst which allows to probe for chiral broken symmetry, revealed in a
60 bias in the direction of rotation. Altogether, rotational flow appears to be a common feature of
61 the collective motion of interacting cells.

62
63 However, it is unclear how these rotational movements arise from the distribution of force-
64 generating elements in the cell. Several models have been proposed to explain the rotation of
65 a cell doublet on a two-dimensional substrate confined in a micropattern, using phase-field,
66 particle-based, or cellular Potts models¹²⁻¹⁵. These models exhibit simultaneous doublet
67 rotation and interface deformation, based on a representation of actin polymerization forces
68 and protrusion-forming forces¹²⁻¹⁵ and coupling to a biochemical system exhibiting
69 spontaneous polarization through feedback between an activator and inhibitor¹³, or directly to
70 an internal polarization vector¹⁴. Despite these advances, it is still unclear what biophysical
71 mechanisms underlie collective cell rotation in three dimensions, and notably how force-
72 generating elements in the cell self-organize to drive coherent cell motion.

73
74

75 **Dynamics of MDCK doublet rotation**

76

77 Here, we exploit a novel assay to study the mechanism behind the spontaneous rotation in three
78 dimensions of MDCK cell doublets. We confined cells within a thin layer of Matrigel, close to
79 the coverslip, to optimize imaging resolution (Fig. 1a, Methods). Strikingly, all embedded
80 MDCK cell clusters undergo spontaneous rotation. We focus here on adhering cell doublets
81 which emerge from the division of a single cell. There was no obvious common orientation of
82 the axis of rotation of cell doublets, and in most but not all cases, a lumen at the cell-cell
83 interface rotated with the doublet (Fig. 1b, Supplementary Videos 1,2). Single cells instead did
84 not rotate in Matrigel (Ext. Fig. 1). Cells participating in the rotating doublet however do not
85 have to be sister cells, as two cells with different fluorescent E-cadherin labels could adhere to
86 each other and initiate rotation (Fig. 1c and Supplementary Video 3).

87

88 To investigate quantitatively doublet rotation (Supplementary Information sections 1, 2), we
89 imaged rotating doublets expressing E-cadherin and computationally segmented cell shapes in
90 the doublet (Fig. 1d, Supplementary Video 4 and Methods). We calculated the center of mass
91 and velocity of each doublet cell, which allows defining a doublet rotation vector ω (Fig. 1e).
92 The norm of the rotation vector increased after cell division for ~100 minutes, before reaching
93 a roughly constant rotational velocity of ~ 180 degrees/h for a duration of ~10 hours,
94 corresponding to about 5 continuous full turns along the same direction (Fig. 1f, g). This is
95 consistent with previously reported rotation velocities of MDCK cysts and breast epithelial cell
96 spheres^{11,16}. Plotting the trajectory of the vector ω showed that the axis of rotation is not fixed
97 but appears to drift over time (Fig. 1h and Ext. Fig. 2). This is expected to the extent that no
98 external cue sets a preferred axis of rotation. We note that this also implies that the spinning
99 motion of the doublet does not intrinsically break chiral symmetry. Plotting the correlation

100 function of the normalized rotation vector further indicated a characteristic correlation time of
101 a few hours (Fig. 1i).

102

103 **Dynamics of doublet elongation during rotation**

104

105 We then wondered whether doublet cells were rotating relative to each other or were rotating
106 together as a solid object (Fig. 2a). We reasoned that in the latter case, the doublet elongation
107 axis would rotate together with the rotation vector ω . We extracted from computationally
108 segmented doublet shapes (Fig. 1d) the three orthogonal principal axes of elongation and
109 evaluated the corresponding relative elongation magnitudes (Fig. 2b, [Supplementary](#)
110 [Information section 3](#)). We noted that the elongation of the doublet major axis was maximal at
111 cell division and then relaxed to a nearly constant value, following the same trend as the
112 magnitude of rotation, but in reverse (Fig. 1h, 2b). We then tested whether the rotation axis
113 was correlated with the axis of maximal doublet elongation. This revealed a strong anti-
114 correlation between the major axis of elongation and the direction of rotation (Fig. 2c),
115 indicating that the elongation major axis is within the plane of rotation. The two minor cell
116 elongation axes had instead weak positive correlations to the axis of rotation. The elongation
117 major axis was also aligned with the vector joining the center of mass of the cells, indicating
118 that it rotates together with the doublet (Fig. 2c). Overall, these results suggest that the doublet
119 rotates as a single physical object. Consistent with this idea, patterns of E-cadherin ([Ext. Fig.](#)
120 [3a-b](#) and [Supplementary Information section 6](#), [Supplementary Video 4](#)) and actin
121 ([Supplementary Video 5](#)) at the cell-cell contact remained similar during ~ 1 hour 30 min of
122 observation.

123

124 **Mode decomposition of interface deformation**

125

126 We then quantified the shape of the interface between the two cells of the doublet (Fig. 2d).
127 We found that the average deviation of the interface from a planar shape was increasing with
128 the magnitude of doublet rotation (Fig. 2e, f). Cross-sections of the doublet showed that the
129 interface was curved in a way that evoked a yin-yang shape, as noticed previously for doublets
130 rotating on a substrate⁷ (Fig. 1b-d). However, when looking at the full interface three-
131 dimensional shape, we noticed that the shape was more complex than suggested by this simple
132 picture (Fig. 2e). To make sense of this complex shape, we decomposed it into basic modes of
133 deformations, which we obtained from Zernike polynomials and classified according to their
134 symmetry properties (Fig. 2d, [Supplementary Information section 4](#)). We defined a “bowl”
135 mode corresponding to a rotationally symmetric deviation of the interface; a “saddle-node”
136 mode with the symmetry properties of a nematic, a “three-fold” mode with a three-fold rotation
137 symmetry; and a “yin-yang” shape with a positive and a negative peak of deformation and the
138 symmetry property of a vector ([Ext. Fig. 4](#), [Supplementary Information section 4.2](#)). We then
139 measured the average magnitude of these 4 deformation modes (Fig. 2g). We found that all 4
140 modes contributed to the interface shape and the saddle-node mode was dominating in relative
141 magnitude (Fig. 2g). We then tested whether the orientations of the shape deformation modes
142 were correlated with the axis of rotation (Fig. 2h). We calculated correlation values considering
143 the symmetry properties of each mode of deformation. Only the yin-yang mode, but neither the
144 saddle-node nor the three-fold mode, has an orientation correlated with the direction of rotation
145 (Fig. 2h). Altogether the doublet interface has a complex three-dimensional shape, and one
146 mode of interface deformation correlated with the doublet rotation.

147

148 **Cortical myosin distribution encoded in cell polarity**

149

150 Having characterized doublet rotation and the doublet interface shape, we then asked whether
151 key proteins of the cytoskeleton and adhesion machinery had a distribution correlated with the
152 doublet rotation. We stained cell doublets for phosphorylated Myosin-Regulatory Light Chain
153 (p-MRLC) and F-actin to label the acto-myosin cytoskeleton, and E-cadherin to label cell-cell
154 contacts (Fig. 3a and Methods). Strikingly, we found that while E-cadherin and F-actin were
155 largely concentrated at the cell-cell interface, p-MRLC was almost absent at the interface but
156 concentrated at two bright zones near the boundary of the cell-cell junction. Immuno-
157 fluorescence paxillin staining and live imaging of vasodilator-stimulated phosphoprotein
158 (VASP) further revealed that focal contacts were also concentrated near the interface boundary
159 (Fig. 3b, c). Live imaging combining markers for actin and myosin cytoskeleton, cell-cell
160 adhesion, and focal contacts showed that myosin clusters and focal contacts appear distributed
161 within the cortex on opposite sides near the doublet interface, judging from their position
162 relative to the interface shape (Fig. 3d, e, Supplementary Video 5-7, Ext. Fig. 3, Supplementary
163 Information section 6). Finally, the observed myosin dynamics (Ext. Fig. 3c, d, Supplementary
164 Video 7) shows signs of cluster movements, with typical cluster velocities around $0.1\text{-}1$
165 $\mu\text{m}\cdot\text{min}^{-1}$.

166
167 To quantify the cortical myosin distribution, we defined a polarity vector \mathbf{p} associated with
168 each cell, which was obtained from myosin signal intensity on the cell surface, corrected for a
169 gradient in the z direction away from the microscope objective (Ext. Fig. 4c-e, Supplementary
170 Information section 5). In line with our observation of myosin clusters, the two cell polarity
171 vectors were consistently pointing away from the axis joining the doublet at an angle of nearly
172 90° , in opposite directions in each cell of the doublet (Fig. 3f-i). The cortical myosin polarity
173 axis was correlated with both the doublet rotation axis and with the orientation of the yin-yang
174 interface deformation (Fig. 3j-k). To better visualize the distribution of cortical myosin, we
175 averaged the myosin intensity profile in a reference frame defined by the rotation vector and
176 the cell doublet axis; this again revealed a strong myosin accumulation near the cell-cell
177 interface, towards the direction of cell motion (Fig. 3l, Ext. Fig. 5). Overall, we conclude that
178 the cortical myosin accumulates in clusters whose positions are correlated with rotation and
179 interface deformation (Fig. 3l).

180 181 **An active surface model recapitulates doublet rotation**

182
183 We then wondered if we could understand how doublets were physically rotating and the
184 complex shape of their cell-cell interfaces (Fig. 4a and Ext. Fig. 6). We considered a physical
185 model where each cell is described as an active viscous surface, subjected to active tension
186 (Supplementary Information, section 7). Cell-cell adhesion is described by an interaction
187 potential between cell surface points, with short-range repulsion and intermediate-range
188 attraction. Surface flows are obtained from force balance equations at the cell surface, taking
189 into account an external friction force proportional to the cell surface velocity. Polarity vectors
190 were assigned to each cell, opposite to each other and oriented with a constant angle away from
191 the cell-cell interface. A profile of active tension was imposed around the cell polarity axis
192 (Supplementary Information, section 7.3). This profile was set using measurements of cortical
193 myosin intensity in a reference frame defined by the cell polarity vector and the axis joining
194 the doublet cells (Fig. 4b-c, Ext. Fig. 5, Ext. Fig. 6a-c, Supplementary Information section 5.4).
195 Simulations were performed using the Interacting Active Surfaces (IAS) numerical
196 framework¹⁷ (Fig. 4d, Supplementary Information, sections 7.1 and 7.2). Solving for the
197 doublet dynamics, we found that a cortical flow emerges, the cell doublet rotates, and the cell-
198 cell interface acquires a deformed shape (Fig. 4e, Ext. Fig. 6f and Supplementary Video 8).
199 The simulated interface shape was a pure yin-yang deformation correlated with the direction

200 of rotation (Fig. 4e-f), the doublet was elongated, and the major axis of elongation was rotating
201 with the doublet (Fig. 4g), as experimentally observed (Fig. 2c, h). Other deformation modes
202 were however absent, in contrast to experiments (Fig. 4e). Both the simulated rotational
203 velocity and magnitude of interface deformation increase with the magnitude of the active
204 tension deviation (Fig. 4h, i and Supplementary Information section 7.4). To test this
205 prediction, we compared the magnitudes of the rotation and yin-yang interface deformation
206 mode to the variation in cortical myosin polarity and found that they were indeed correlated
207 (Fig. 4h, i). The simulated rotation magnitude was comparable to experiments for parameters
208 giving rise to cortical flows of $\sim 0.1 \mu\text{m}/\text{min}$, comparable to the observed speed of myosin
209 clusters (Ext. Fig. 6f, Ext. Fig. 3d).

210

211 **Curie principle applied to doublet interface shape**

212

213 We then wondered how we could explain the emergence of modes of interface deformation
214 other than the yin-yang shape. We reasoned that the Curie principle, stating that “the
215 symmetries of the causes are to be found in the effects”^{18,19}, implies that molecular cues guiding
216 interface deformations should satisfy symmetry rules consistent with the observed interface
217 shape (leaving aside the possibility of spontaneous symmetry breaking). We classified a set of
218 configurations of doublets and polarity axis according to their symmetry properties (Fig. 4j).
219 A configuration where cell polarities are in the same plane but shifted in opposite directions
220 away from the doublet axis, belongs to the C_{2h} point group in Schoenflies notation²⁰. As a
221 result, such a doublet should exhibit yin-yang and three-fold interface deformation, as observed
222 in simulations (Fig. 4f, Ext. Fig. 6d). In contrast, tension asymmetry between the two cells of
223 the doublets should give rise to the bowl deformation mode; while a nematic configuration of
224 active tension distribution, with different intensities in each cell, should give rise to the bowl
225 and saddle-node deformation mode (Fig. 4j). Simulating doublets with varying profiles of
226 active tension confirmed these predictions (Fig. 4j). We then verified if this relationship
227 between modes of cortical myosin distribution and modes of interface deformation could be
228 observed in experiments. Indeed, we found that the magnitude of the bowl deformation mode
229 was correlated to the difference in average cortical myosin intensity of the two doublet cells
230 (Fig. 4k). We also noticed that the distribution of cortical myosin had a secondary, less
231 concentrated cluster opposite to the main myosin cluster (Fig. 4b, Ext. Fig. 5). We reasoned
232 that this secondary cluster was giving rise to a nematic distribution of cortical myosin,
233 quantified by a nematic tensor²¹. Indeed, we measured a positive correlation between the
234 nematic tensor of the saddle-node interface deformation mode, and the difference of cortical
235 myosin nematic tensor between the two doublet cells (Fig. 4l). We then verified that simulating
236 a doublet with an active tension profile summing a polar, nematic distribution and a difference
237 in average tension between the two doublets cells, resulted in a complex interface shape with
238 a similar mode decomposition as in experiments (Fig. 4j, last row, Fig. 2g). We conclude that
239 the complex shape of the doublet interface can be understood, based on symmetry principles
240 from the cortical myosin distribution in the doublet.

241

242 **Deletion or ectopic induction of myosin clusters affects doublet interface and motion in** 243 **experiments and in simulations**

244

245 We then reasoned that if the myosin clusters are responsible for cell rotation and interface
246 deformation, perturbing their activity and localization would affect the cell doublet shape and
247 motion. Indeed, treatment with the myosin inhibitor blebbistatin (Fig. 5a) resulted in rotation
248 arrest and simultaneous flattening of the interface (Fig. 5a-c and Ext. Fig. 7). The effect of the
249 blebbistatin-induced arrest of rotation was reversible: when we washed out the inhibitor,

250 doublets retrieved rotational motion and a bent interface (Ext. Fig. 7 and Supplementary Video
251 9). We then aimed at specifically altering the two opposite myosin spots using laser ablation
252 (Fig. 5d). This induced transient arrest of the rotation and interface flattening. Following a lag
253 time of ~ 10 minutes, rotation restarted with a simultaneous increase in rotation velocity and
254 interface deformation (Fig. 5e and Supplementary Video 10). Suppressing the gradient of
255 active tension in simulation also suppressed rotation (Fig. 5d, e and Supplementary Video 11).
256 In order to generate additional ectopic local myosin clusters, we then engineered a stable
257 optogenetic cell line that we used to locally activate Rho (Fig. 5f and Supplementary Video 12,
258 see Methods). Transient Rho activation resulted in ectopic myosin activation at the cell cortex
259 comparable in intensity and size to spontaneous myosin clusters. This new cluster triggered the
260 displacement of the doublet away from the region of activation while the doublet kept rotating
261 (Fig. 5f-h). Introducing an ectopic region of increased active tension in simulations resulted in
262 a similar drift of the doublet (Fig. 5f-h and Supplementary Video 13). Altogether, these results
263 support the role of myosin clusters for driving doublet rotation.

264

265 Discussion

266

267 Our analysis shows that doublet rotation arises from myosin clusters positioned away from the
268 axis joining the two doublet cells. Therefore, the doublet cell rotation requires cell-cell
269 interactions to trigger the shift of the cell polarity axis. Consistent with this picture, in our
270 experimental setup single cells do not rotate. It has been reported that in a bilayered Matrigel,
271 which provides an external polarization axis, single MDCK cells rotate¹¹. In this situation, the
272 environment is providing a preferred direction. It would be interesting to track the cortical
273 myosin distribution in cells rotating in these conditions to test if the polarity-based mechanism
274 we propose also applies in that context.

275

276 Which mechanisms result in myosin cluster formation? Clusters could emerge by spontaneous
277 symmetry breaking from an initially symmetric configuration where the cell polarities are
278 pointing towards each other (Fig. 4j). The dynamics of increase of rotation magnitude after cell
279 division (Fig. 1h), which resembles an exponential increase followed by saturation, is
280 consistent with such a scenario. The position of myosin clusters could be related to protrusions
281 which are sent by each cell beyond the interface at the cortex. Alternatively, they form in
282 response to cell interface deformation, and induce rotation and further cell interface
283 deformation, with the positive feedback loop at the origin of the instability and spontaneous
284 symmetry breaking necessary for rotation. Among the deformation modes we have analyzed,
285 the yin-yang mode has the right symmetry property to be coupled to a shift of the two cell
286 polarities away from the doublet axis, which occurs in opposite directions in each cell of the
287 doublet (Fig. 4j). Interestingly, we also observed that following cell division, a myosin-dense
288 cluster forms at the center of the doublet interface and appears then to relocate towards the
289 periphery of the contact (Ext. Fig. 8 and Supplementary Video 14). We note that myosin
290 clusters have also been reported to generate stress in cytokinesis²². We also observe that focal
291 contacts are polarized within each cell of the doublet, such that a myosin cluster in one cell is
292 in close vicinity to a region dense in focal contacts within the opposite cell. This organization
293 suggests either a common origin for myosin and focal contact distribution or a polarization
294 mechanism relying on negative feedback between cortical myosin and focal contacts. Possibly,
295 forces resulting from the cortical myosin inhomogeneous distribution promote focal contacts
296 through a reinforcement mechanism^{23,24}.

297

298 Our study shows that epithelial cell doublets allow to bridge the gap between microscopic
299 players involved in cell motion and collective tissue dynamics. We propose that the

300 spontaneous rotation we observed here is a manifestation of basic principles of cell interactions,
301 involving cross-talks of cell polarity between neighbouring cells and polarity-oriented
302 mechanical interactions between the cells and their environment. The ubiquity of collective
303 rotations observed in various cell types *in vitro* suggests that they indeed emerge from generic
304 principles. Analysis of the symmetries, here through the Curie principle, helps in making sense
305 of these complex interactions. It would be interesting to see how basic rules of cell polarity
306 interactions combine with mechanical forces to generate tissue self-organisation beyond
307 collective cell rotation.

308
309
310
311

Methods:

312 Cell culture and cell lines.

313 Cells were maintained at 37°C in a 5% CO₂ incubator. All following steps are performed under
314 a sterile hood.

315 The maintenance of MDCK II cell lines was done using high Glutamax Modified Eagle's
316 Medium (Gibco, ref. 41090-028), 5% v/v Fetal Bovine Serum (South America Gibco, ref.
317 10270106), 1% v/v Non-Essential Amino Acid (Gibco, ref. 11140-050), 1% v/v sodium
318 pyruvate (Gibco, ref. 11360-039) and 1% Penicillin-Streptomycin. MDCK cells were replated
319 every 3 to 4 days when they reached 70-95% confluency. Trypsin-EDTA was used to detach
320 the cells from the plate and with a seeding density of $5 \cdot 10^4$ per cm². To generate doublets,
321 single cells were embedded in a Matrigel close to the surface. Coverslips were activated by O₂
322 plasma and 100% Matrigel (Corning BV: 356231) was then used to coat coverslips. Following
323 a 10 min incubation at 37°C, 100% Matrigel was polymerized and formed a basal layer. Single
324 cells were deposited at a density of 15,000 cells/cm². After 10 minutes incubation, unattached
325 cells and excess medium were removed. Next a drop of 10 µl 100% Matrigel was deposited.
326 After polymerization of Matrigel at 37°C in the incubator, culture medium was further added.

327 We prepared the following stable cell lines: MDCK II E-cadherin-GFP/Podocalyxin-
328 mScarlett/Halo-CAAX to visualize the cell-cell junction and the lumen. To generate new
329 myosin clusters by opto-genetics, we prepared a stable MDCK II cell line expressing iLID-
330 LARG::mVenus²⁵, engineered to be membrane-anchored by a slowly diffusing Stargazin
331 membrane anchor²⁶ combined with the DH/PH domain of the Leukemia-associated RhoGEF
332 (LARG)²⁷. This cell line allowed us to activate Rho locally and trigger the local recruitment
333 of myosin. We visualized Rho activity with an active Rho sensor (2xrGBD-dTomato) and
334 myosin localization with MRLC-iRFP703. We also used the following cell lines: MDCK II
335 VASP-GFP for tracking focal contacts²⁸, MDCK II MRLC-KO1/E-cadherin-mNG to follow
336 myosin and cell-cell junctions²⁹, MDCK II MRLC-GFP³⁰, MDCK II E-cadherin-GFP and
337 MDCK II E-cadherin-DsRed³¹. These cell lines allowed us to track specific correlations
338 between rotation and adhesion or cytoskeleton protein localizations. E-cadherin was also used
339 for segmentation purposes. To visualize F-actin live, we also used SiR-Actin (Tebu-Bio,
340 251SC001). Each experimental condition (biological repeat noted N) was reproduced at least
341 3 times on at least 10 doublets (written n in captions).

342

343 Drugs treatments and immuno-fluorescence staining.

344 To investigate the role of myosin, we used blebbistatin (Sigma, B0560) to inhibit myosin with
345 the following steps. Timelapses of samples were acquired under the microscope and the
346 medium was then changed by a medium containing 10 μ M of blebbistatin. Following 1 hour
347 incubation, samples were washed 3 times with fresh medium. Samples were further imaged to
348 visualize the eventual re-initiation of rotation.

349 For immunostaining⁹, samples were washed with PBS and then fixed with 4%
350 paraformaldehyde (PFA) diluted in PBS for 15 minutes. To permeabilize cells, cells were
351 incubated with 0.5% Triton-X-100 for 15 minutes and then a blocking solution made of 1%
352 Normal Goat Serum in PBS 1X was added overnight. The primary antibody was added directly
353 to the blocking solution for two days at 4°C. Following 3 washing steps, samples were stained
354 with the relevant secondary antibodies for 2 hours at room temperature. We used the following
355 primary antibodies: Anti-E-cadherin (Abcam, Ab11512), Anti-Phospho-Myosin Light Chain
356 2 (Cell signaling technology, #3674), Anti-Paxillin (Abcam, Ab32084), and Alexa FluorTM
357 Phalloidin 488 (Thermo Fisher, A12379) to visualize F-actin. Samples were washed three times
358 in PBS and mounted on a home-made sample holder system for imaging and conservation.

359 **Microscopy.**

360 High throughput imaging was done using a spinning disk microscope with an inverted Leica
361 spinning disk DMI8 equipped with an Orca Flash 2.0 camera (2048*2018 pixels with a size of
362 6.5 μ m) using a 63x glycerol objective (NA = 1.3). The microscope was equipped with an
363 incubation chamber to maintain the samples at 37°C, 5% CO₂ and 85% humidity conditions.

364 To record the initiation of rotation, MDCK doublets were imaged 5 hours after cell seeding.
365 3D stacks were acquired with a z-step of 1 μ m and x-y resolution of 0.206 or 0.103 μ m, every
366 10 min up to 12 hours.

367 Confocal imaging of fixed samples was performed using the same setup. Laser power and
368 digital gain settings were unchanged within a given session to ease quantitative comparison of
369 expression levels among doublets.

370 We locally activated Rho with the scanning head of a confocal microscope (Leica SP8-UV)
371 with a 458 nm laser each 10 s for 20 min, taking an image every minute. We could follow
372 RhoA activity with a Rho binding domain sensor for active Rho (2xrGBD::dTomato)³² and
373 myosin with the MRLC::iRFP703 probe.

374 **Image analysis.**

375 3D segmentation of cells was performed using a custom written ImageJ Macro involving
376 LimeSeg plugin³³ (Supplementary Information section 1). The center of mass, surfaces of cells
377 were computed from the segmented 3D meshes using a written Python code. For the velocity
378 measurements, rotational velocity of cells was computed using the cell-to-cell center of mass
379 vector, velocity of cell 1 and velocity of cell 2 (Supplementary Information section 2).

380 Segmentation of the interface was performed using a customized Python code from the 3D
381 meshes of each cell in a doublet. The 3D point cloud of the interface was then fitted to a
382 polynomial function of degree 3. Characterisation of interface deformation and shape was then
383 performed on the fitted surface (Supplementary Information section 4).

384 The fluorescence intensity signal of myosin was extracted from images and attributed to
385 vertices of 3D meshes. The myosin distribution was then characterised by a polarity vector and
386 a nematic tensor. We correlated these descriptors to the interface shape and the doublet rotation
387 (Supplementary Information section 5).

388 Cylindrical and planar projections of the basal (E-cadherin) and interfacial (myosin) signals
389 were created using a custom Python code (Supplementary Information section 6).

390 For the visualization and 3D rendering, we used Paraview³⁴ software.

391 **IAS simulation framework.**

392 Simulations of rotating doublets were performed using the IAS framework. A polarity is
393 introduced for each cell which modulates the active tension profile along its surface. This leads
394 to the emergence of a cortical flow which propels the cells through friction with the external
395 medium. The integrity of the doublet is maintained using an adhesion interaction potential. See
396 Supplementary Information section 7 for the complete description of the simulations and
397 parameters used.

398 **Statistical tests.**

399 We used a bootstrapping approach for all the statistical tests performed. The tests in Figs. 2c,2h,
400 3h, 3j, 3k, 4h, 4i, 4l, were performed by generating 50 000 samples of mean using
401 bootstrapping. For the correlation tests in Figs. 4h,i,k, we generated independent samples of
402 the correlation defined as $\langle (a - \langle a \rangle) \cdot (b - \langle b \rangle) \rangle / (\sigma_a \sigma_b)$ (for two signals a and b
403 with σ_a, σ_b their respective standard deviations). This correlation takes values between -1 and
404 1. For Figs. 4k we used 200 000 samples. All the p-values correspond to one tailed tests.

405

406 **Acknowledgments**

407 We thank Erwan Grandgirard and the Imaging Platform of IGBMC, and the Salbreux and
408 Riveline groups for help and discussions. Q.V. and G.S. are supported by the University of
409 Geneva. L.L. and T.G. are supported by HFSP and by the University of Strasbourg and by la
410 Fondation pour la Recherche Médicale. D.R. acknowledges the Interdisciplinary Thematic
411 Institute IMCBio, part of the ITI 2021-2028 program of the University of Strasbourg, CNRS
412 and Inserm, which was supported by IdEx Unistra (ANR-10-IDEX-0002), and by SFRI-
413 STRAT'US project (ANR 20-SFRI-0012) and EUR IMCBio (ANR-17-EURE-0023) under
414 the framework of the French Investments for the Future Program. O.P. and D.R. thank
415 funding from SNF Sinergia. D.R. and A.H. acknowledge the Research Grant from Human
416 Frontier Science Program.

417

418 **Author contributions**

419 G.S. and D.R. supervised the study. L.L., T.G. and D.R. conceived and analyzed experiments.
420 L.L. performed the experiments with support from T.G., R.B., M.L.; O.P., K.U., and C.M.-L.,
421 A.H. contributed new optogenetic tool and cell lines respectively. T.G., Q.V. and G.S.
422 performed data analysis. Q.V. and G.S. designed the theoretical model. Simulations were
423 performed by Q.V. with support from A.T.-S. The manuscript was written by G.S. and D.R.
424 based on joint discussions with L.L., T.G., Q.V.

425

426 References

427

- 428 1. Cetera, M. *et al.* Epithelial rotation promotes the global alignment of contractile actin
429 bundles during *Drosophila* egg chamber elongation. *Nat Commun* **5**, 5511 (2014).
- 430 2. Founounou, N. *et al.* Tissue fluidity mediated by adherens junction dynamics promotes
431 planar cell polarity-driven ommatidial rotation. *Nat Commun* **12**, 6974 (2021).
- 432 3. Chougule, A. *et al.* The *Drosophila* actin nucleator DAAM is essential for left-right
433 asymmetry. *PLoS Genet* **16**, e1008758 (2020).
- 434 4. Erzberger, A., Jacobo, A., Dasgupta, A. & Hudspeth, A. J. Mechanochemical symmetry
435 breaking during morphogenesis of lateral-line sensory organs. *Nat. Phys.* **16**, 949–957
436 (2020).
- 437 5. Naganathan, S. R., Fürthauer, S., Nishikawa, M., Jülicher, F. & Grill, S. W. Active torque
438 generation by the actomyosin cell cortex drives left–right symmetry breaking. *eLife* **3**,
439 e04165 (2014).
- 440 6. Pimpale, L. G., Middelkoop, T. C., Mietke, A. & Grill, S. W. Cell lineage-dependent chiral
441 actomyosin flows drive cellular rearrangements in early *Caenorhabditis elegans*
442 development. *eLife* **9**, e54930 (2020).
- 443 7. Brangwynne, C., Huang, S., Parker, K. K. & Ingber, D. E. Symmetry breaking in cultured
444 mammalian cells. *In Vitro Cell.Dev.Biol.-Animal* **36**, 563–565 (2000).
- 445 8. Jain, S. *et al.* The role of single-cell mechanical behaviour and polarity in driving
446 collective cell migration. *Nat. Phys.* **16**, 802–809 (2020).

- 447 9. Lo Vecchio, S., Pertz, O., Szopos, M., Navoret, L. & Riveline, D. *Spontaneous rotations*
448 *in epithelia as an interplay between cell polarity and RhoA activity at boundaries.*
449 <http://biorxiv.org/lookup/doi/10.1101/2021.11.11.468187> (2021)
450 doi:10.1101/2021.11.11.468187.
- 451 10. Fernández, P. A. *et al.* Surface-tension-induced budding drives alveologenesis in
452 human mammary gland organoids. *Nat. Phys.* **17**, 1130–1136 (2021).
- 453 11. Chin, A. S. *et al.* Epithelial Cell Chirality Revealed by Three-Dimensional
454 Spontaneous Rotation. *Proc Natl Acad Sci USA* **115**, 12188–12193 (2018).
- 455 12. Leong, F. Y. Physical Explanation of Coupled Cell-Cell Rotational Behavior and
456 Interfacial Morphology: A Particle Dynamics Model. *Biophysical Journal* **105**, 2301–2311
457 (2013).
- 458 13. Camley, B. A. *et al.* Polarity mechanisms such as contact inhibition of locomotion
459 regulate persistent rotational motion of mammalian cells on micropatterns. *Proc. Natl.*
460 *Acad. Sci. U.S.A.* **111**, 14770–14775 (2014).
- 461 14. Segerer, F. J., Thüroff, F., Piera Alberola, A., Frey, E. & Rädler, J. O. Emergence and
462 Persistence of Collective Cell Migration on Small Circular Micropatterns. *Phys. Rev. Lett.*
463 **114**, 228102 (2015).
- 464 15. Thüroff, F., Goychuk, A., Reiter, M. & Frey, E. Bridging the gap between single-cell
465 migration and collective dynamics. *eLife* **8**, e46842 (2019).
- 466 16. Tanner, K., Mori, H., Mroue, R., Bruni-Cardoso, A. & Bissell, M. J. Coherent angular
467 motion in the establishment of multicellular architecture of glandular tissues. *Proc. Natl.*
468 *Acad. Sci. U.S.A.* **109**, 1973–1978 (2012).
- 469 17. Torres-Sánchez, A., Winter, M. K. & Salbreux, G. *Interacting active surfaces: a*
470 *model for three-dimensional cell aggregates.*

- 471 <http://biorxiv.org/lookup/doi/10.1101/2022.03.21.484343> (2022)
- 472 doi:10.1101/2022.03.21.484343.
- 473 18. Curie, P. Sur la symétrie dans les phénomènes physiques, symétrie d'un champ
474 électrique et d'un champ magnétique. *J. Phys. Theor. Appl.* **3**, 393–415 (1894).
- 475 19. Prost, J., Chauwin, J.-F., Peliti, L. & Ajdari, A. Asymmetric pumping of particles.
476 *Phys. Rev. Lett.* **72**, 2652–2655 (1994).
- 477 20. Symmetry Resources at Otterbein University. <https://symotter.org/>.
- 478
- 479 21. de Gennes, P. G., Prost, J. & Pelcovits, R. *The Physics of Liquid Crystals. Physics*
480 *Today* **48**, 70–71 (1995).
- 481 22. Wollrab, V., Thiagarajan, R., Wald, A., Kruse, K. & Riveline, D. Still and rotating
482 myosin clusters determine cytokinetic ring constriction. *Nat Commun* **7**, 11860 (2016).
- 483 23. Riveline, D. *et al.* Focal Contacts as Mechanosensors. *Journal of Cell Biology* **153**,
484 1175–1186 (2001).
- 485 24. Balaban, N. Q. *et al.* Force and focal adhesion assembly: a close relationship studied
486 using elastic micropatterned substrates. *Nat Cell Biol* **3**, 466–472 (2001).
- 487 25. Guntas, G. *et al.* Engineering an improved light-induced dimer (iLID) for controlling
488 the localization and activity of signaling proteins. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 112–
489 117 (2015).
- 490 26. Natwick, D. E. & Collins, S. R. Optimized iLID Membrane Anchors for Local
491 Optogenetic Protein Recruitment. *ACS Synth. Biol.* **10**, 1009–1023 (2021).
- 492 27. Martz, M. K., Grabocka, E., Beeharry, N., Yen, T. J. & Wedegaertner, P. B.
493 Leukemia-associated RhoGEF (LARG) is a novel RhoGEF in cytokinesis and required for
494 the proper completion of abscission. *MBoC* **24**, 2785–2794 (2013).
- 495 28. Lo Vecchio, S. *et al.* Collective Dynamics of Focal Adhesions Regulate Direction of
496 Cell Motion. *Cell Systems* **10**, 535-542.e4 (2020).

- 497 29. Bhat, A. Myosin cluster dynamics and their roles in epithelial wound ring
498 constriction. PhD, University of Strasbourg, <https://theses.hal.science/tel-03510189>
499 (2019)..
- 500 30. Watanabe, T., Hosoya, H. & Yonemura, S. Regulation of Myosin II Dynamics by
501 Phosphorylation and Dephosphorylation of Its Light Chain in Epithelial Cells. *MBoC* **18**,
502 605–616 (2007).
- 503 31. Adams, C. L., Chen, Y.-T., Smith, S. J. & James Nelson, W. Mechanisms of
504 Epithelial Cell–Cell Adhesion and Cell Compaction Revealed by High-resolution Tracking
505 of E-Cadherin– Green Fluorescent Protein. *Journal of Cell Biology* **142**, 1105–1119
506 (1998).
- 507 32. Mahlandt, E. K. *et al.* Visualizing endogenous Rho activity with an improved
508 localization-based, genetically encoded biosensor. *Journal of Cell Science* **134**, jcs258823
509 (2021).
- 510 33. Machado, S., Mercier, V. & Chiaruttini, N. LimeSeg: a coarse-grained lipid
511 membrane simulation for 3D image segmentation. *BMC Bioinformatics* **20**, 2 (2019).
- 512 34. Ahrens, J., Geveci, B. & Law, C. ParaView: An End-User Tool for Large-Data
513 Visualization. in *Visualization Handbook* 717–731 (Elsevier, 2005). doi:10.1016/B978-
514 012387582-2/50038-1.
- 515

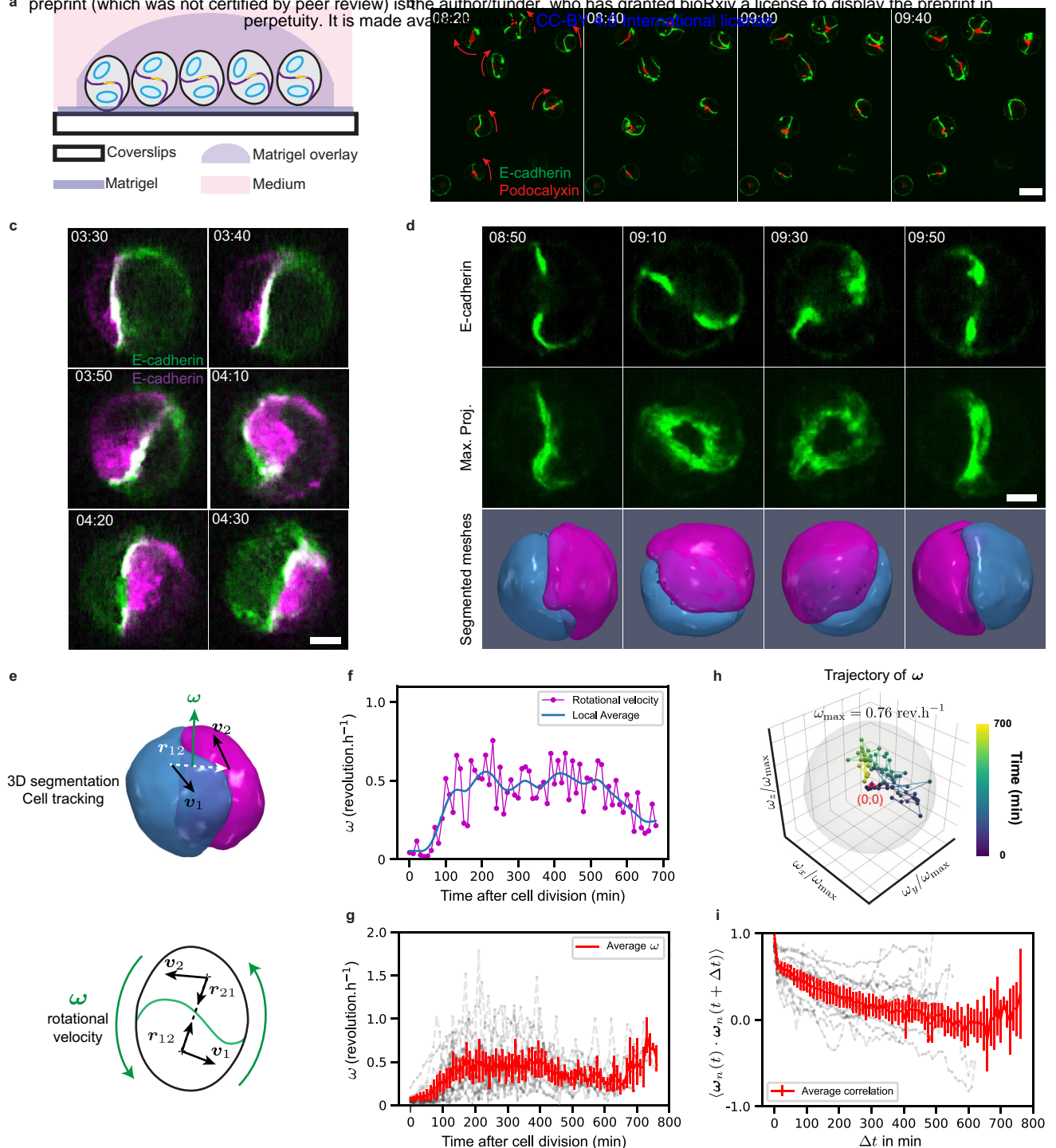


Figure 1. 3D rotation of MDCK cell doublet. **a.** Schematics of the experimental assay. **b.** Snapshots of rotating doublets. Red arrows indicate the direction of rotation. Labels: E-cadherin-mNG (Green), Podocalyxin-mScarlet (Red). Time relative to beginning of Movie 1. **c.** Snapshot of a rotating doublet with two cells expressing E-cadherin of different colours. Labels: E-cadherin-GFP (Green) and E-Cadherin-DsRed (Magenta). Time relative to beginning of Movie 3. **d.** Snapshots of a rotating doublet with labelled E-cadherin-mNG (Green) (from top to bottom, cross-section, maximum projection, and three-dimensional segmentation). $n = 14$ doublets, $N = 3$ biological repeats. Time relative to cell division. **e.** Schematics for the calculation of the rotation vector ω (see Supplementary Information, section 2). **f.** Magnitude of the rotational velocity as a function of time after cell division. **g.** Average and individual trajectories of the magnitude of the rotational velocity of cell doublets after cell division. $n = 14$, $N = 3$. **h.** Trajectory of rotation vector normalized with respect to its maximum amplitude, colormap indicates time from dark blue to yellow. Grey sphere has unit radius. **i.** Autocorrelation of $\omega_n = \omega / \|\omega\|$ as a function of lag time. Scale bars: $5 \mu\text{m}$ except panel b: $20 \mu\text{m}$. Time in panels b,c,d in hh:mm. Error bars: 95% confidence interval of the mean.

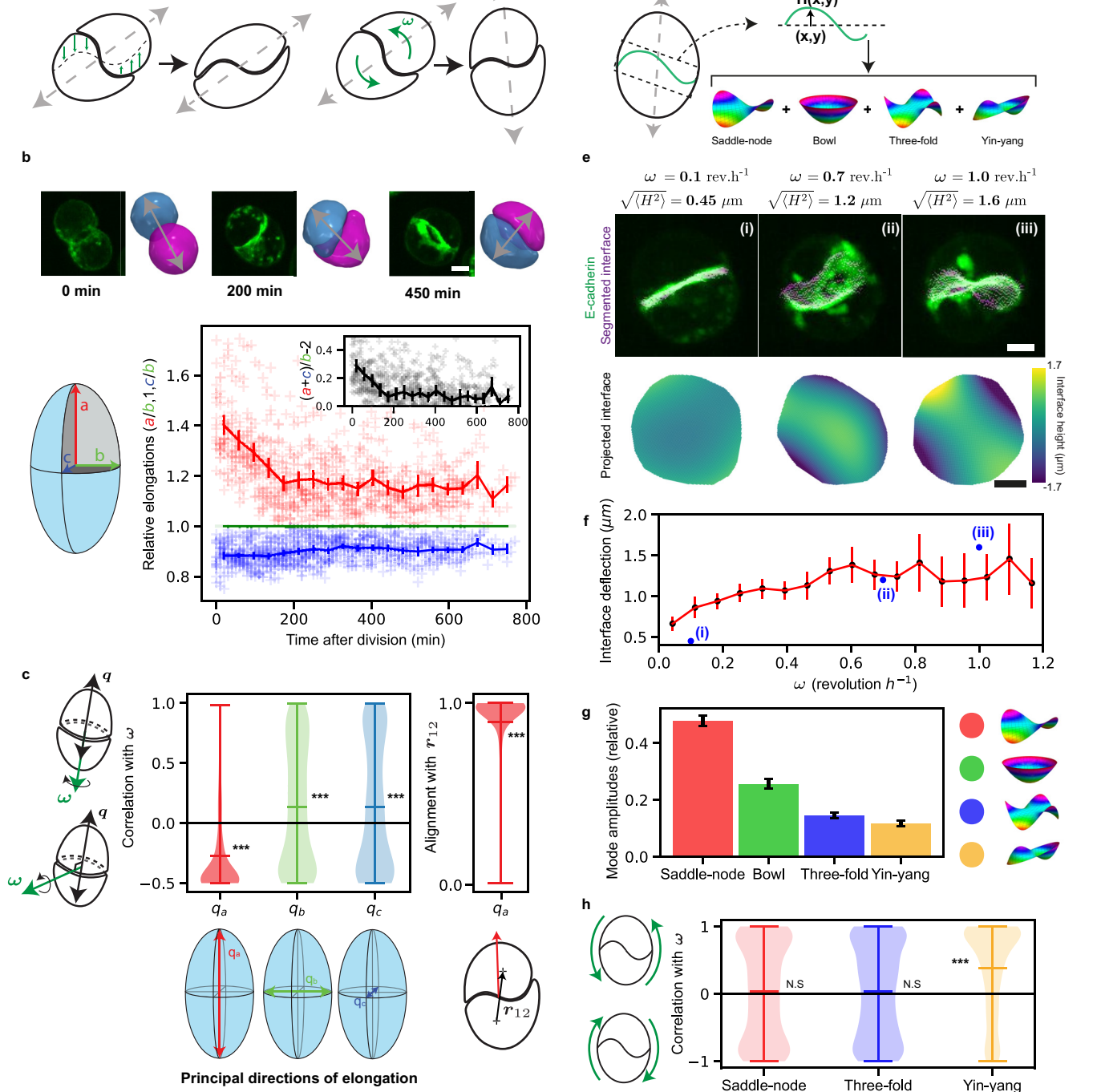


Figure 2. Coordinated rotation of doublet shape and interface. **a.** Schematics of possible scenarios of doublet rotation. Left: The interface is deforming (green arrows), leading to apparent rotation without motion of the doublet outer surface. Right: the doublet is rotating as a solid object and the cell elongation axis rotates with the doublet. **b.** Top: snapshots of rotating doublets with labelled E-cadherin-mNG (Green) and corresponding segmented meshes. Grey double arrows, approximate elongation axis. Bottom left: schematics for definition of three doublet elongation axes. Bottom right: relative doublet elongation magnitudes as a function of time after division. Inset: ratio of elongation magnitudes, positive values indicate a prolate shape. **c.** (Left) Correlation of axis of rotation with axes of elongation, as indicated in the schematics. The direction of maximal elongation, q_a lies in the plane of rotation of the doublet. (Right) Alignment of elongation major axis with the doublet axis. **d.** Schematics for interface shape decomposition into modes with different symmetries. **e.** Representative examples of interface shape for different rotation magnitudes, corresponding to points indicated in **f**. Top row: magnitude of rotational velocity and average interface deflection. Middle row: snapshots of E-cadherin labelled doublets with overlaid interface segmentation. Bottom row: Interface height map. **f.** Average interface deflection as a function of magnitude of rotational velocity. **g.** Average relative magnitude of the interface deformation modes. **h.** Correlation of orientation of deformation mode with direction of rotation (indicated on the left). Statistical tests: ***, $p < 10^{-4}$. Error bars: 95% confidence interval of the mean. $n = 14$ doublets, $N = 3$ biological repeats.

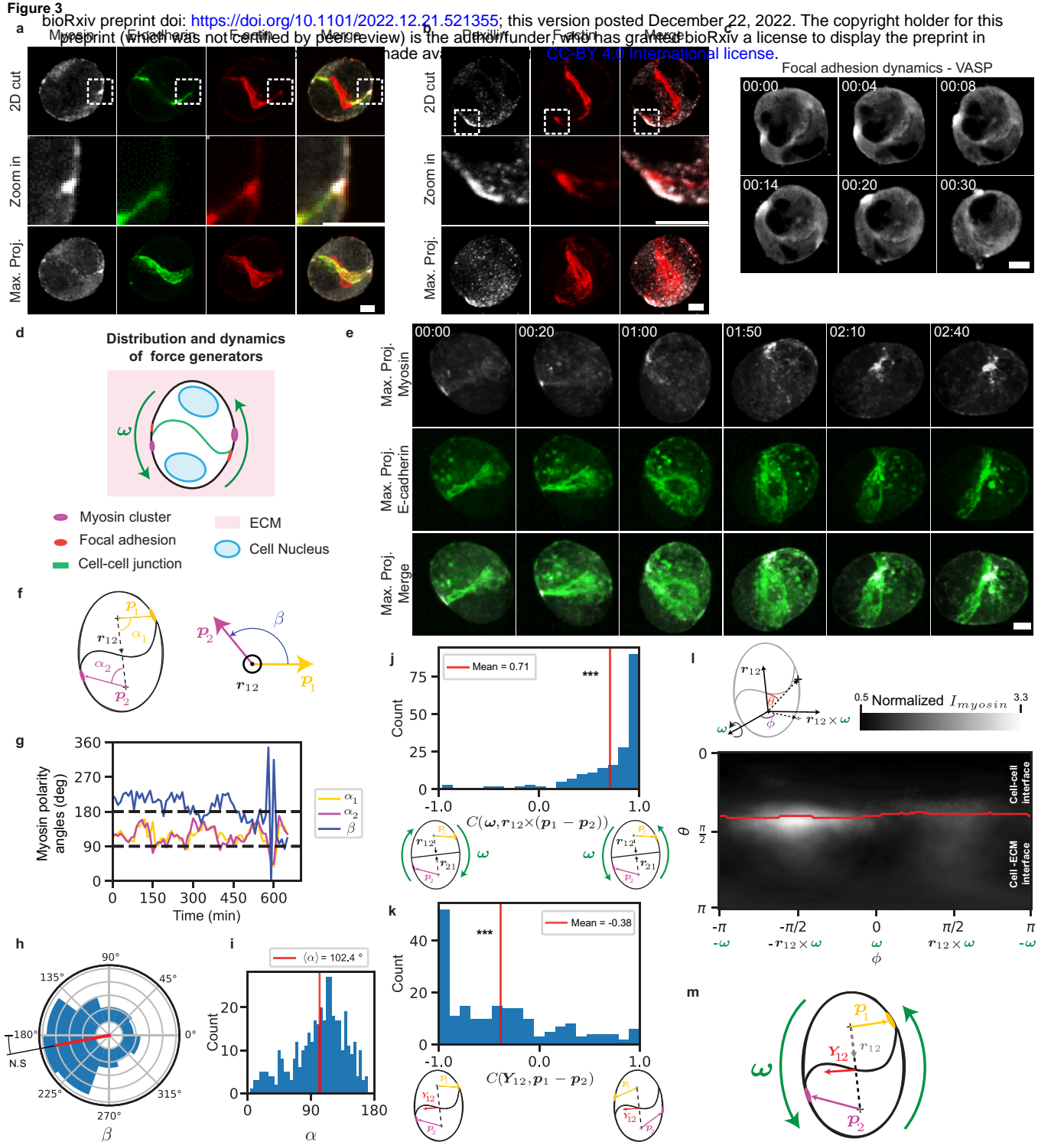


Figure 3. Distribution and dynamics of the force generators and adhesions in cell doublet. Snapshots of representative examples of distribution of (a) myosin, E-cadherin, F-actin and merge, and (b) paxillin, F-actin, and merge. $n > 10$ doublets for a and b. c. Dynamics of focal adhesion in the rotating doublet, labelled with VASP-GFP (grey). Time relative to the beginning of Movie 6. d. Schematics of distribution of force-generating and adhesion proteins in the rotating doublet. e. Snapshots of a rotating doublet with labelled E-cadherin-mNG (green) and myosin-KO1 (grey). f. Scheme of myosin polarity angles α_1 , α_2 and β . g. Myosin polarity angles with respect to the doublet axis as a function of time for a single doublet. h. Histogram of β , the angle between the polarity vectors of cells 1,2, projected on the plane orthogonal to r_{12} . Red line, average orientation of the distribution. i. Histogram of α , the polarity angle relative to the doublet axis. j. Histogram for the correlation between the rotation vector ω and the cross-product between the doublet axis and the difference of cell polarities. Red line: average. k. Histogram for the correlation between the yin-yang orientation vector and polarity difference. Red line: average. l. Map of average myosin intensity in spherical coordinates, in a reference frame defined by the rotation vector ω , the axis of the doublet r_{12} and their cross-product. m. Summary of orientation of myosin polarities and yin-yang interface deformation mode. Scale bars: 5 μm . Time in hh:mm. Statistical tests: ***, $p < 10^{-4}$. $N = 3$ biological repeats. Panel h-l: $n = 12$ doublets.

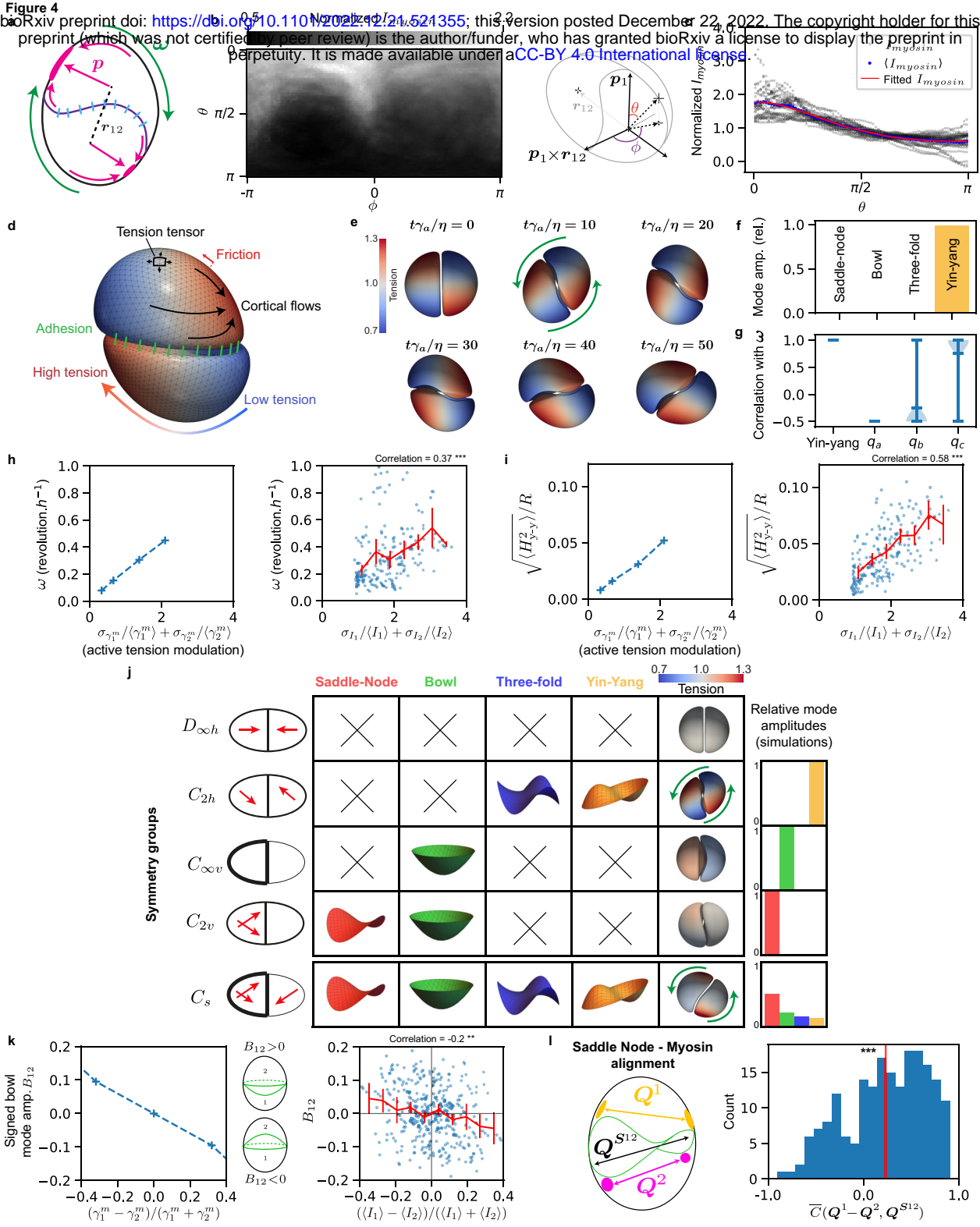


Figure 4. Interacting active surface simulations of a rotating doublet. **a**. Schematics of doublet rotation. **b**. Map of average experimental myosin intensity. **c**. Profiles of myosin intensity as a function of the angle θ defined in panel b. Grey: time average profile of individual cells, Blue: profile averaged over all cells, Red: fitted profile. **d**. Schematics for IAS simulation of a rotating doublet. **e**. IAS simulation results (η cortical viscosity, γ_a reference active tension). Time relative to the beginning of Movie 8. **f**. Amplitude of mode deformation for the simulated cell-cell interface in e. **g**. Correlation of yin-yang mode orientation, and doublet principal elongation, with direction of rotation. **h**. Left: rotation velocity of simulated doublet as a function of active tension modulation ($\eta/\gamma_a = 1\text{min}$). Right: Experimental rotation velocity as a function of myosin variation, Red: binned averages. **i**. Left: Amplitude of yin-yang deformation mode as a function of the amplitude of the active tension modulation. Right: Experimental amplitude of yin-yang deformation mode as a function of myosin variation, Red: binned averages. **j**. IAS doublet simulation for cortical myosin profiles consistent with doublets in different symmetry groups (Schoenflies notation on the left). **k**. Left: Correlation of “bowl” mode amplitude with difference of average rescaled active tension. Right: Experimental correlation of bowl mode amplitude with difference of average myosin intensities. Red: binned average. **l**. Experimental correlation of saddle-node mode with difference of cortical myosin nematic tensor. Error bars: 95% confidence interval of the mean. See Supplementary Information section 7. Statistical tests: **: $p = 3.10^{-4}$, ***: $p < 10^{-4}$.

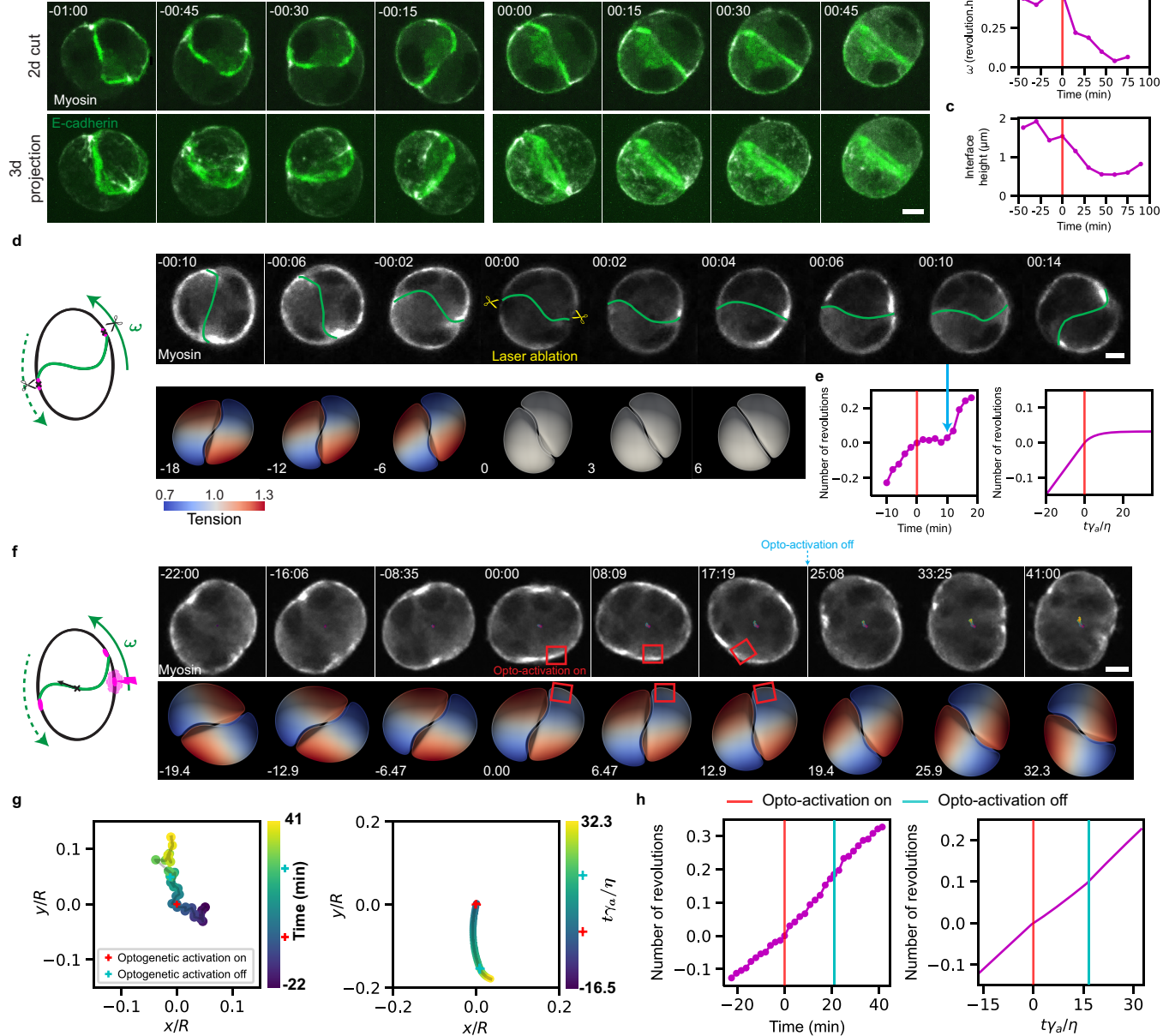
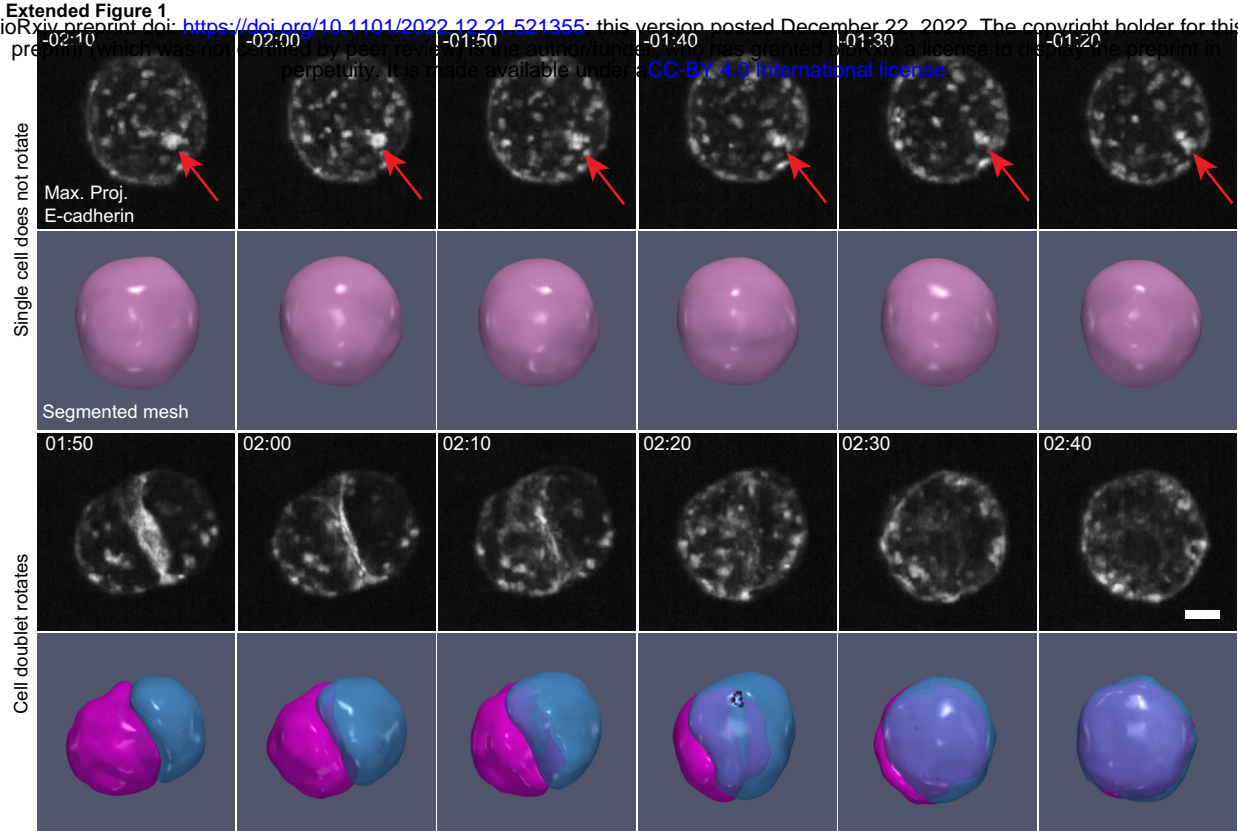
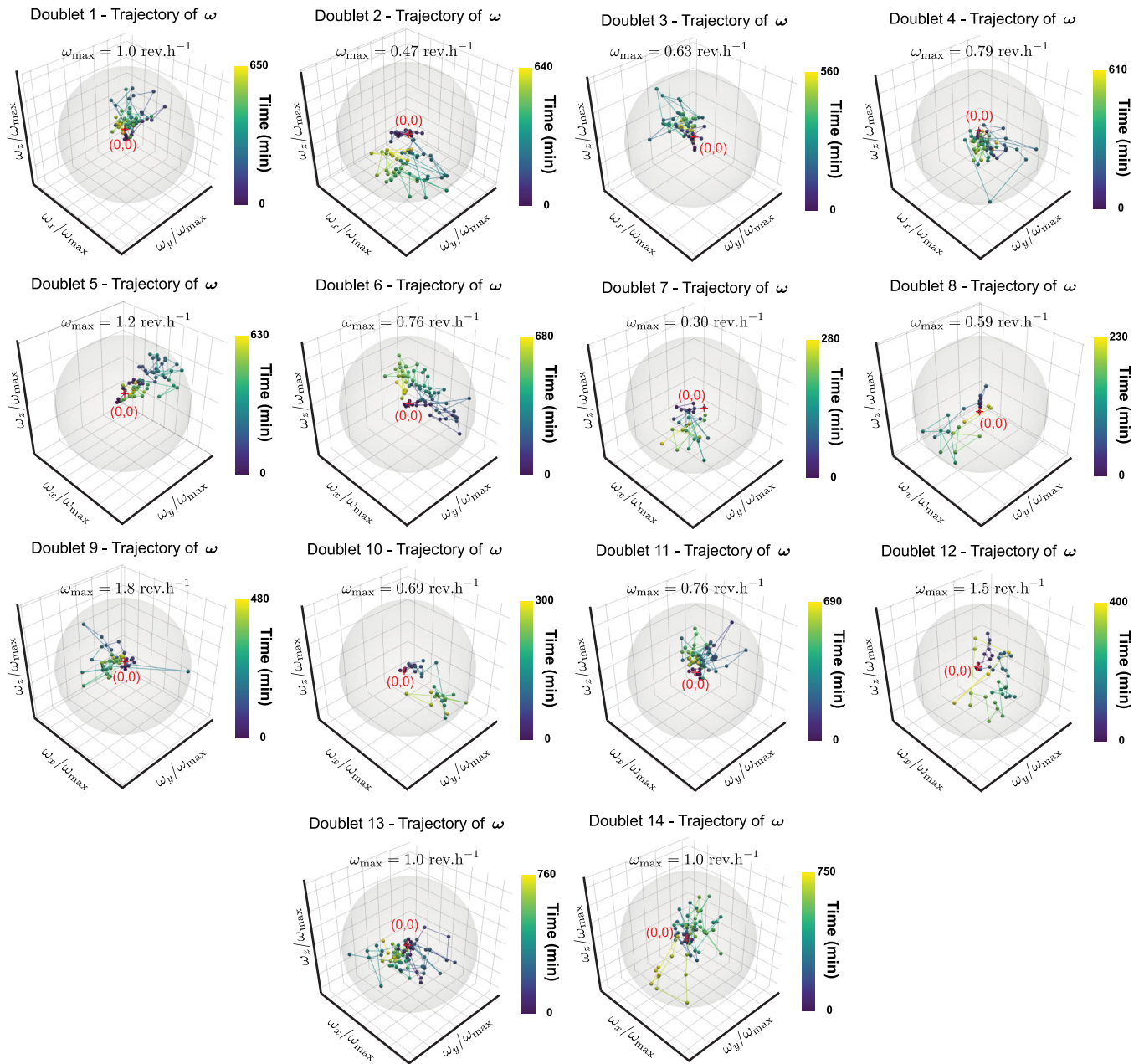


Figure 5. Perturbing the rotation motor. **a.** Snapshot of a rotating doublet, before and after Blebbistatin treatment. Green label: E-cadherin-mNG, grey label: MRLC-KO1 (see also Movie 9). $n = 16$ doublets. **b.** Magnitude of rotation as a function of time, before and after treatment with Blebbistatin. **c.** Magnitude of interface height as a function of time, before and after treatment with Blebbistatin. **d.** Snapshot of a rotating doublet labelled with MRLC-GFP (grey) (top: experiments, bottom: simulation), before and after laser ablation of myosin spots at time 0; ablation spots are indicated by two scissors (see also Movies 10 and 11). **e.** Number of visible turns as a function of time before and after laser ablation of myosin spots; time of ablation indicated by a red line and blue arrow shows the onset of rotation. **f.** Snapshot of a rotating doublet labelled with MRLC-iRFP (grey), before and after optogenetic activation of RhoA (red square) generating a local myosin cluster (top: experiments, bottom: simulation, time 0 activation) (see also Movies 12 and 13). **g.** Number of visible turns as a function of time, before, during and after optogenetic generation of a myosin cluster ($n = 4$ doublets) (left: experiments; right: simulations). **h.** Trajectory of the doublet's center of mass before, during (red line) and after (blue line) optogenetic generation of a myosin cluster (experiment) and induction of a region of increased active tension in one cell (simulation). Scale bars: $5 \mu\text{m}$. Time in hh:mm, panel a,d. Time in mm:ss in panel f.

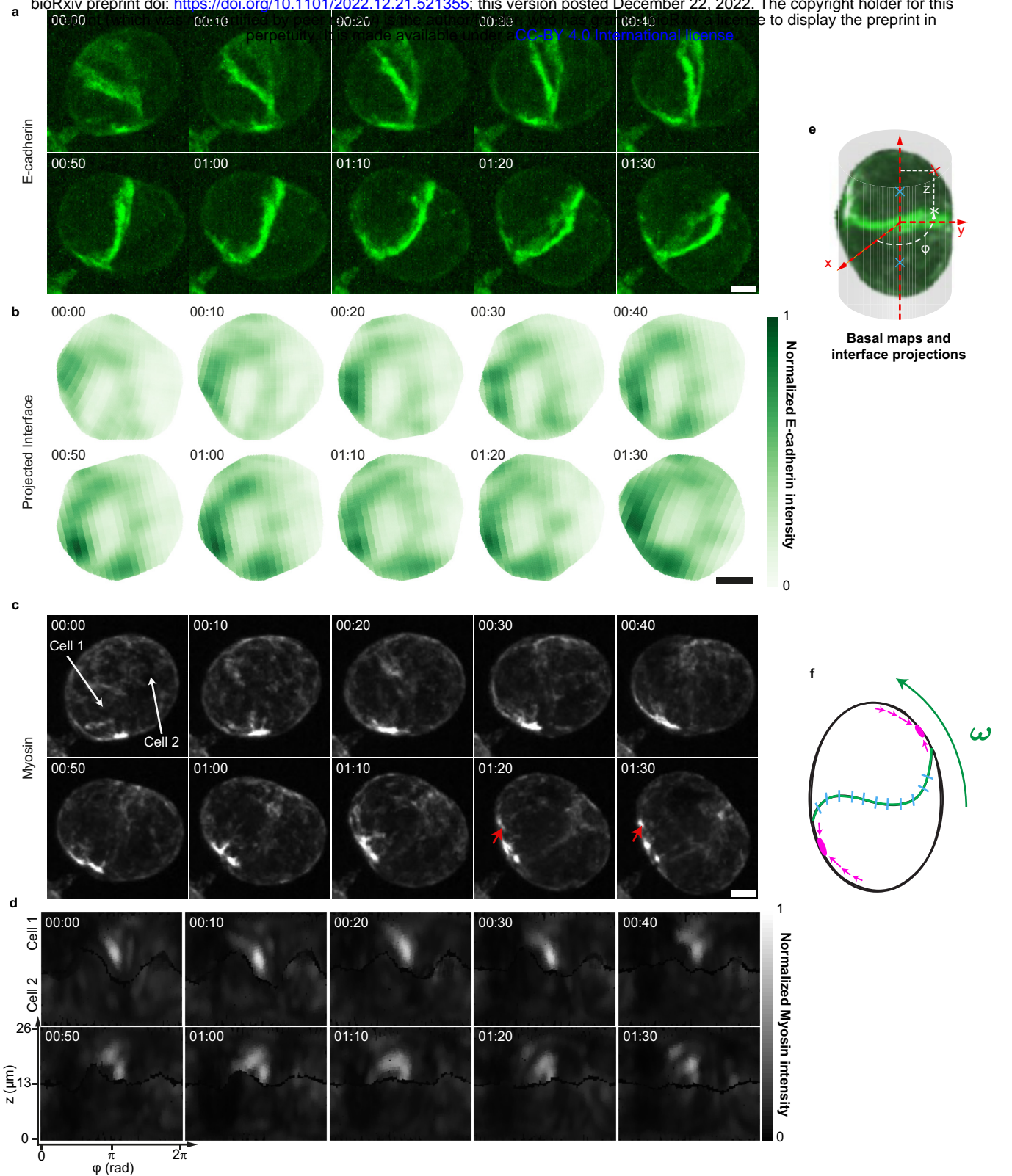


Ext. Fig. 1. Single cells do not rotate. Snapshot of single cell (two top rows) and cell doublet (two bottom rows). Cells labelled with E-cadherin-mNG (grey) - see beginning of Movie 2. For each case, top row: maximum projection of E-cadherin, bottom row: cell segmentation. Time relative to cell division. Scale bars: $5 \mu\text{m}$. Time in hh:mm.

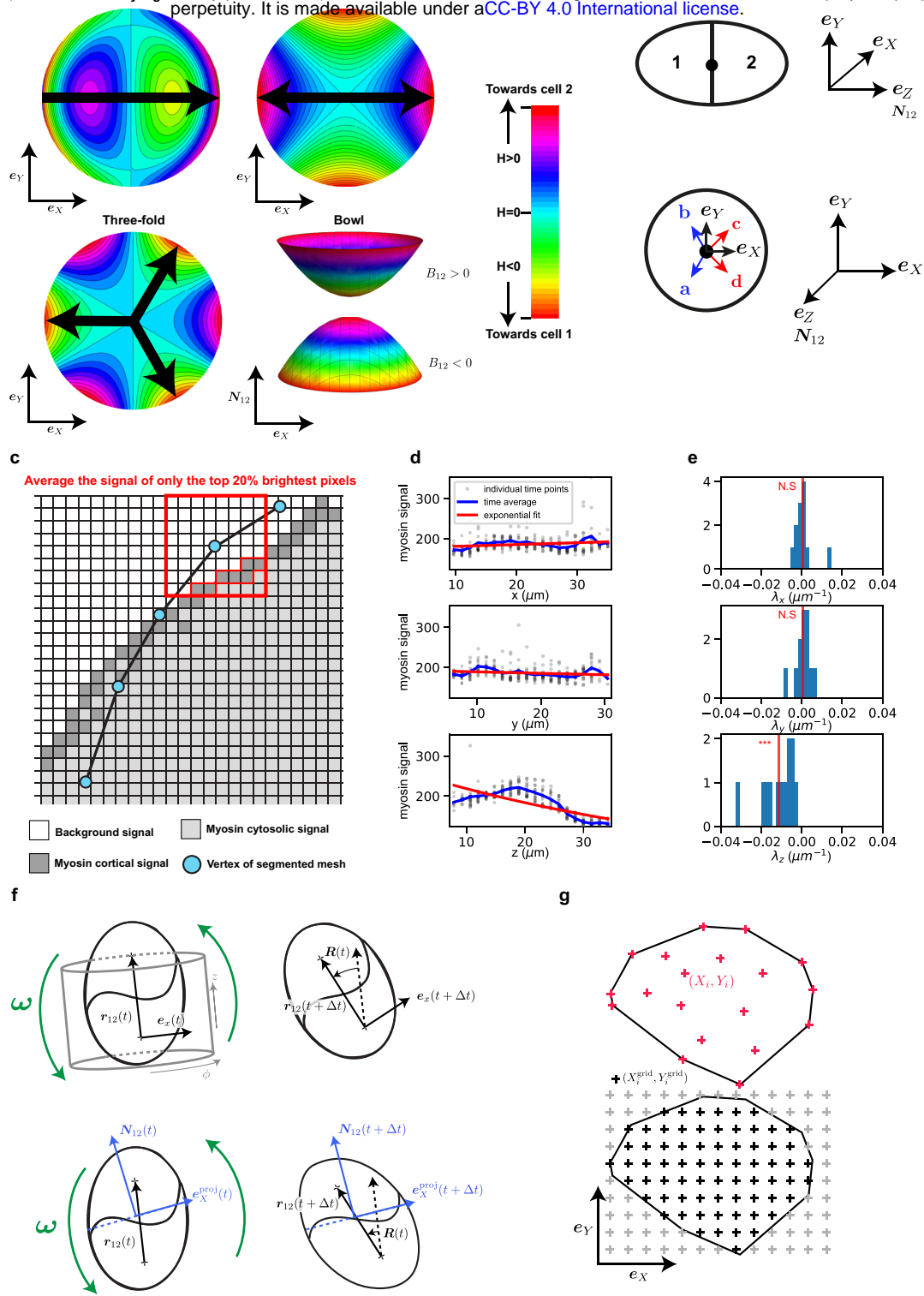
Extended Figure 2



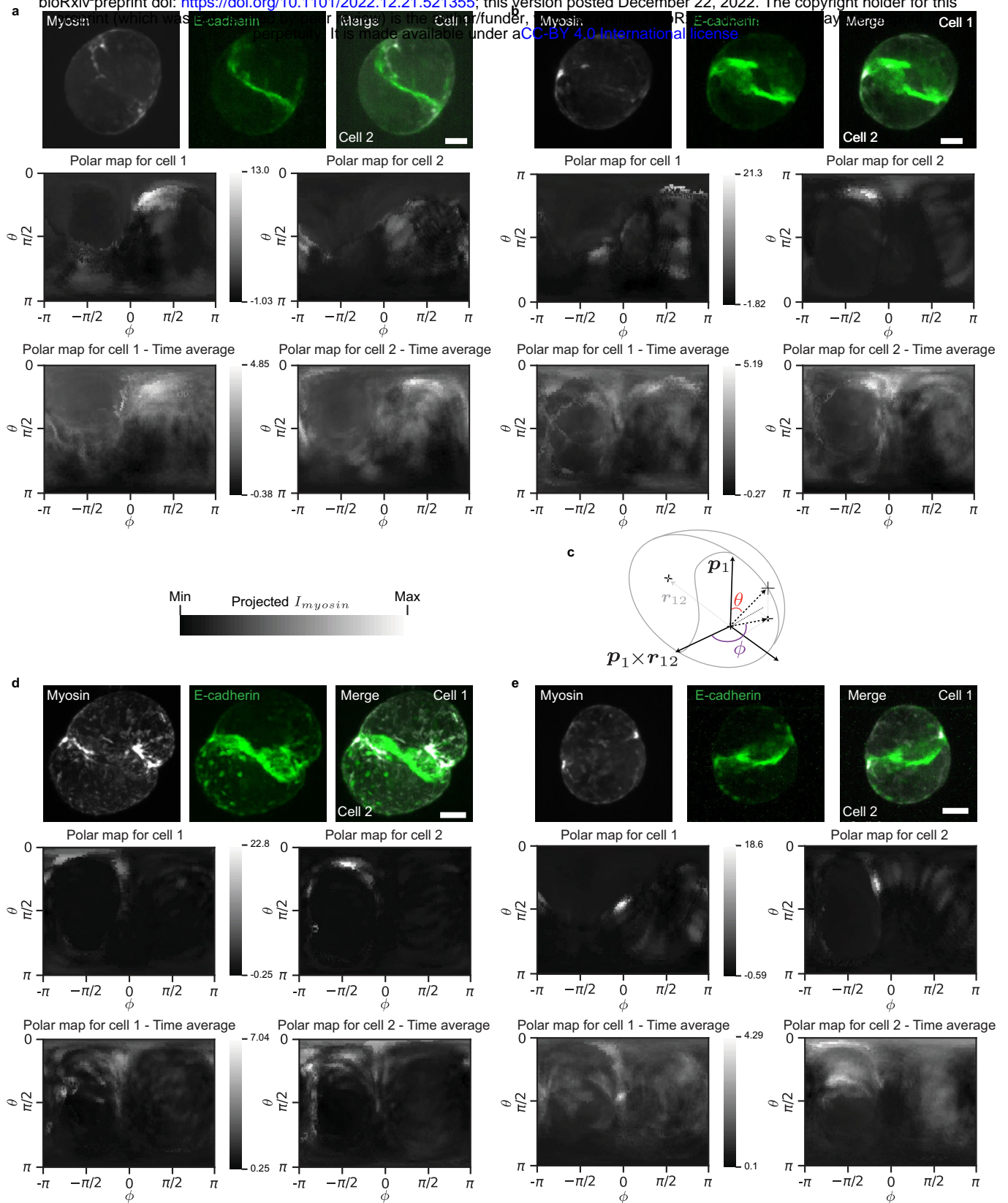
Ext. Fig. 2. Trajectories of rotational velocities. Similar to Fig. 1h: trajectories of the rotation vector of cell doublets after cell division for all 14 doublets, normalized with respect to their respective largest amplitudes (corresponding to Movie 2). Grey sphere has unit radius.



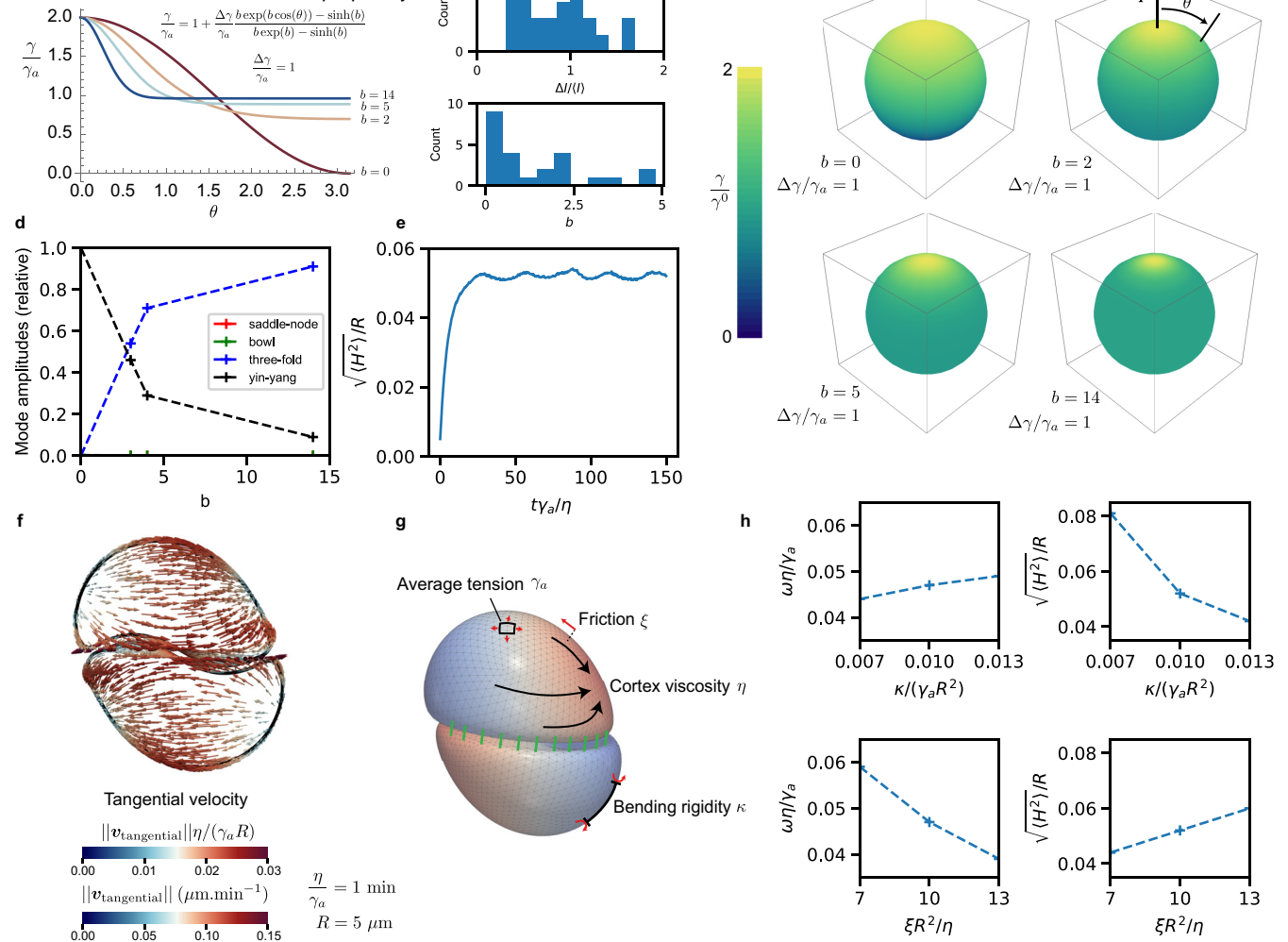
Ext. Fig. 3. Patterns of E-cadherin at the cell-cell interface and myosin dynamics at the cortex.
a. E-cadherin-mNG (grey) labelled rotating doublet. **b.** Patterns of E-cadherin distribution on the doublet cell-cell interface, viewed en-face, for the cell shown in **a.** **c-d.** Mapping of the myosin dynamics at the cortex (normalization described in SI section 6). Myosin clusters highlighted with red arrows exhibit a motion with a velocity of about $0.1\mu\text{m}/\text{min}$. (see Movie 7) **e.** Schematic for projection method. **f.** Schematics for myosin (purple) and cadherin (blue) distribution. Scale bars: 5 μm . Time in hh:mm.



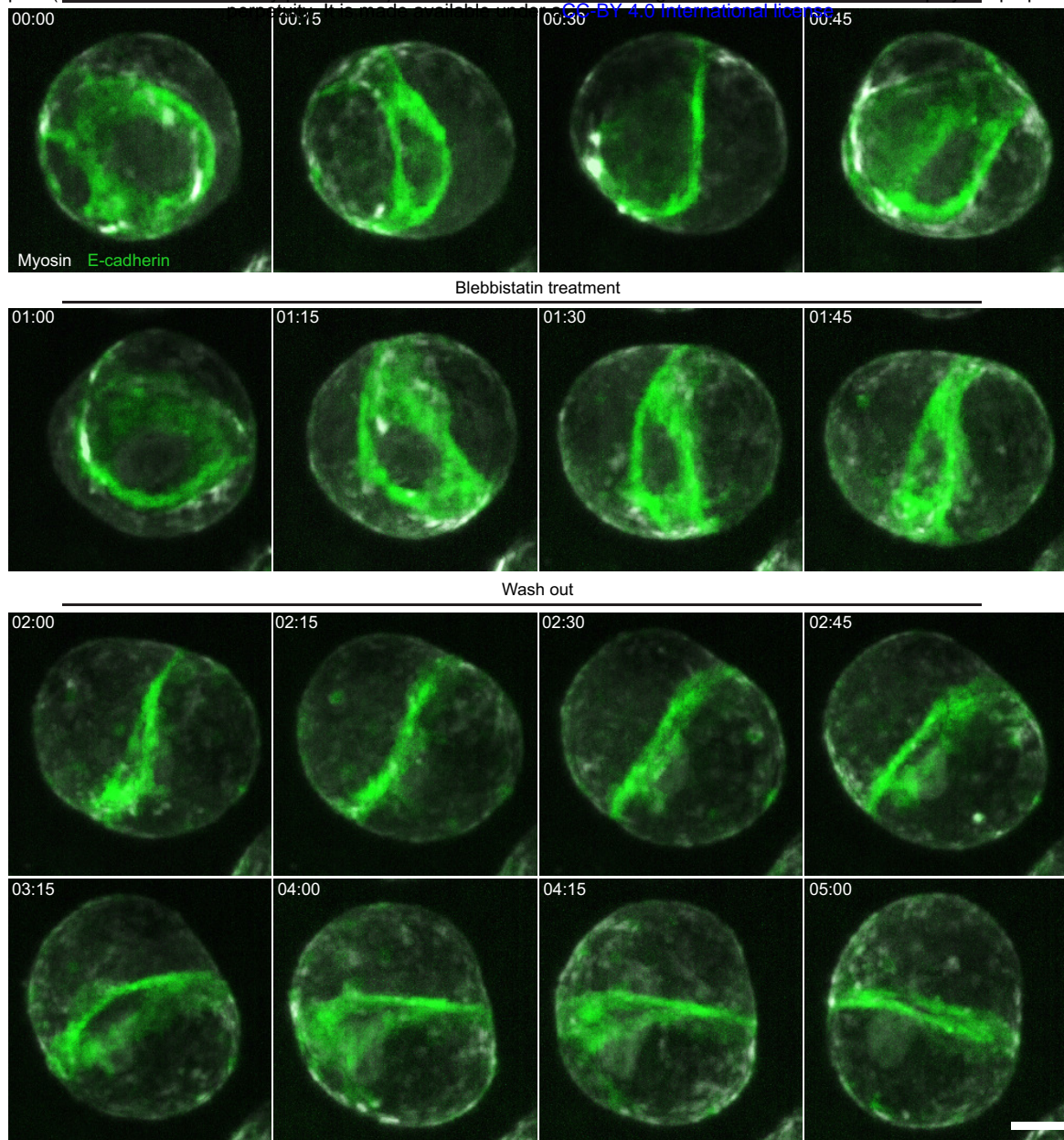
Ext. Fig. 4.: Analysis of interface shape. **a.** Height profile of interface deformation modes. The yin-yang orientation is characterized by a vector, the saddle-node by a nematic, and the three-fold by a three-fold orientational order. **b.** Schematic for the orientation of the vectors $(e_x, e_y, e_z = N_{12})$ associated to the interface of the cell doublet. The vectors a, b, c, d are introduced to define transformations in Supplementary Table 1. **c.** Schematics of method used to obtain cortical intensities from cell segmentation (see Supplementary Information section 5.1) **d.** Average profile of myosin fluorescence intensity in the x, y, z directions, for a representative doublet. **e.** Histogram of fitting parameters characterizing the average myosin profiles, as in d, for all doublets. **f, g.** Procedure to create interfacial and basal maps. **f.** (Top) The vector r_{12} is used as an axis around which to project the myosin signal intensity. The reference frame (e_x, e_y, e_z) defining the cylindrical coordinates (z, ϕ) rotates in a way that is consistent with the doublet rotation. (Bottom) A similar process is used for the interfacial maps of E-cadherin signal. A reference vector is rotated with the doublet to define a consistent viewpoint and is projected at each time t in the plane of the interface defined by N_{12} . **g.** (Top) 2D coordinates (X_i, Y_i) of the interface vertices i , surrounded by their convex hull. (Bottom) A regular grid of new coordinates $(X_i^{\text{grid}}, Y_i^{\text{grid}})$ is created inside the convex hull (black points). *******, $p < 10^{-4}$.



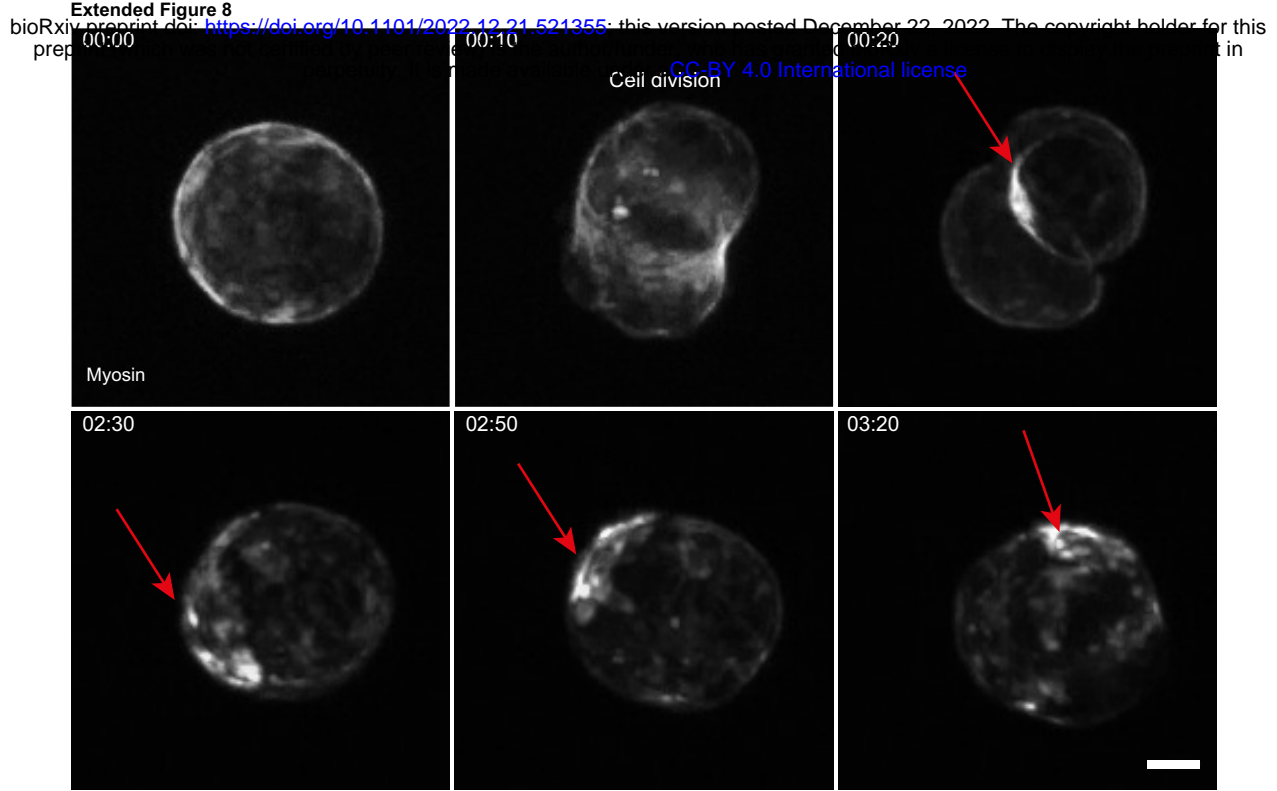
Ext. Fig. 5: Example of polar maps of cortical myosin intensity. **a,b,d,e.** Maps of experimental myosin intensity after calibration in spherical coordinates, in a reference frame defined by the polarity axis \mathbf{p} , the axis of the doublet \mathbf{r}_{12} and their cross-product. For each example, top row: snapshot of doublet, maximum projections of myosin (MRLC-GFP), E-cadherin (Ecadherin-mNG) and merge, middle row: individual cell maps corresponding to the above snapshot, bottom row: time average cell maps corresponding to the time series from which the snapshot in the top row was taken from. **c.** Scheme of the reference frame. Scale bar: 5 μm . Normalization described in Supplementary Information section 6.



Ext. Fig. 6. Additional IAS simulation results. **a.** Examples of tension profiles around the polarity vector, for different values of the parameter b , which controls the spread of the tension around the maximum value (see Supplementary Information section 7.3). **b.** Histogram of fitted values of b and $\Delta I / \langle I \rangle$ to temporally average myosin profiles for individual doublets, showing the distribution of myosin intensity magnitude and spot sizes. The fitting procedure and parameters are described in Supplementary Information section 7.6. **c.** Tension profiles displayed on spheres with values of b identical to panel a. Larger values of b correspond to smaller spots. **d.** Relative amplitudes of the deformation modes as a function of a varying active tension profile whose spread is determined by b . As the active tension spot size becomes smaller (larger values of b), the yin-yang mode is replaced by the three-fold mode. **e.** Interface deflection as a function of dimensionless time for the simulation shown in Fig. 4e. The interface deflection relaxes to a steady-state showing a slightly oscillatory behaviour. **f.** Cortical flow profile at steady-state for the simulation shown in Fig. 4e. For $\eta/\gamma_a = 1 \text{ min}$ and $R = 5 \mu\text{m}$, the typical flow magnitude is $\sim 0.1 \mu\text{m} \cdot \text{min}^{-1}$. **g.** Explanatory scheme of IAS simulation, with key simulation parameters. **h.** Effect of varying the normalized friction coefficient $\xi R^2/\eta$ and the normalized bending rigidity $\kappa/(\gamma_a R^2)$ on the rotation velocity and the interface deflection, around parameter values chosen in simulations of Fig. 4, 5 and other panels of Ext. Fig. 7. See Supplementary Information section 7.5 for additional simulation parameters.



Ext. Fig. 7. Blebbistatin treatment. Snapshots of blebbistatin experiment including before and after blebbistatin treatment followed by washout (see Movie 9). E-cadherin (green). Myosin (grey). Scale bar: 5 μm . Time in hh:mm.



Ext. Fig. 8. Myosin redistribution after cytokinesis. Snapshot of asymmetric myosin distribution before and after cell division (see Movie 14). Scale bar: 5 μ m. Time in hh:mm.

Supplementary Videos legends

- Supplementary Video 1 - All doublets rotate spontaneously. MDCK cells expressing E-cadherin-mNG (in green) and Podocalyxin-mScarlett (in red). Time in hh:mm, scale bar: 20 μm .
- Supplementary Video 2 - All doublets rotate with similar velocity. MDCK cells expressing E-cadherin-mNG (in grey). Time in hh:mm, scale bar: 5 μm .
- Supplementary Video 3 - The doublet also rotates when two cells meet. MDCK cell expressing E-cadherin-GFP (in green) and E-Cadherin-DsRed (in magenta). Time in hh:mm, scale bar: 5 μm .
- Supplementary Video 4 - A typical segmentation of cells. The doublet (left) expressing E-cadherin-mNG is shown next to its segmented version (right). Time in hh:mm, scale bar: 5 μm .
- Supplementary Video 5 - F-actin localises at the cell-cell interface and within protrusions. MDCK cells expressing E-cadherin-mNG (in green) and F-actin labeled with SiR-actin (in grey). Time in hh:mm, scale bar: 5 μm .
- Supplementary Video 6 - Focal adhesions localize near the cell-cell interface. MDCK cells expressing VASP-GFP (in grey). Time in hh:mm, scale bar: 5 μm .
- Supplementary Video 7 - Myosin clusters localize near the cell-cell interface. MDCK cells expressing E-cadherin-mNG (in green) and MRLC-KO1 (in grey). Time in hh:mm, scale bar: 5 μm .
- Supplementary Video 8 - Reference simulation shown in Fig. 4e. Cross-section of a rotating doublet showing a yin-yang interface deformation mode. The colormap indicates the active tension γ/γ_a on the membranes. Dimensionless time $t\gamma_a/\eta$ is indicated. See Supplementary Information section 7.5 for simulation parameters.
- Supplementary Video 9 - Myosin activity is needed for rotation. The doublet rotates and stops its motion when blebbistatin is added (time 01:00); rotation starts again after washout. MDCK cells expressing E-cadherin-mNG in green and MRLC-KO1 in grey. Time in hh:mm, scale bar: 5 μm .
- Supplementary Video 10 - Myosin clusters ablation corresponds to rotation arrests and changes in interface shape. MDCK cells expressing MRLC-GFP (in grey). Time in hh:mm, scale bar: 5 μm .
- Supplementary Video 11 - Laser ablation simulation. Cross-section of a rotating doublet at steady-state, whose tension modulation is switched off at $t=0$. The colormap indicates the active tension γ/γ_a on the membranes. Dimensionless time $t\gamma_a/\eta$ is indicated. See Supplementary Information section 7.5 for simulation parameters.
- Supplementary Video 12 - Local activation of Rho at time 0 (red square) leads to the generation of myosin clusters and this shifts the rotation to translation. The center of mass is tracked throughout the movie and is indicated with changing colours. MDCK cells expressing MRLC-iRFP. Time in hh:mm, scale bar: 5 μm .
- Supplementary Video 13 - Optogenetic simulation. Cross-section of a simulated rotating doublet initially at steady-state. Active tension is increased in a spot in one of the cells, from time $t\gamma_a/\eta = 0$ to $t\gamma_a/\eta=16.57$. The added spot impairs the rotation, makes the doublet asymmetric and creates a drift of its center of mass. See Supplementary Information section 7.5 for simulation parameters.
- Supplementary Video 14 - Myosin clusters appear as a remnant from the cytokinetic ring. MDCK cells expressing MRLC-GFP (in grey). Time in hh:mm, scale bar: 5 μm .