

1    **Probing tissue-scale deformation by *in vivo* force application reveals a fast**  
2    **tissue softening during early embryogenesis**

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5    **Arturo D'Angelo<sup>1,2,\*</sup>, Kai Dierkes<sup>1,2,\*</sup>, Carlo Carolis<sup>1,2</sup>, Guillaume Salbreux<sup>3,#</sup>,**  
6    **Jérôme Solon<sup>1,2,#</sup>**

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8    1. Cell and Developmental Biology Programme, Centre for Genomic Regulation (CRG), The Barcelona Institute of  
9    Science and Technology, Dr. Aiguader 88, Barcelona 08003, Spain.

10    2. Universitat Pompeu Fabra (UPF), Barcelona 08003, Spain.

11    3. The Francis Crick Institute, 1 Midland Road, London NW1 1AT, United Kingdom.

12    \*These authors contributed equally to this work

13    #Co-corresponding authors

14

15    **Abstract**

16    **During development, cell-generated forces induce tissue-scale deformations to**  
17    **shape the organism. Here, we present a method that allows to quantitatively**  
18    **relate such tissue-scale deformations to spatially localized forces and measure**  
19    **mechanical properties of epithelia *in vivo*. Our approach is based on the**  
20    **application of controlled forces on microparticles embedded in individual cells of**  
21    **an embryo. Combining measurements of the bead displacement with the analysis**  
22    **of induced deformation fields in a continuum mechanics framework, we can**  
23    **quantify tissue material properties and follow their change over time. In**  
24    **particular, we uncover a rapid change in tissue response occurring during**  
25    ***Drosophila* cellularization, resulting from a softening of the blastoderm and an**  
26    **increase of external friction. Pharmacological treatments reveal that in addition**  
27    **to actomyosin, the microtubule cytoskeleton is a major contributor to epithelial**  
28    **mechanics at that stage. Overall, our method allows for measuring essential**  
29    **mechanical parameters governing tissue-scale deformations and flows occurring**  
30    **during morphogenesis.**

31

32

33 **Introduction**

34 During animal development, cell-generated forces, which are modulated in space  
35 and time, are translated into tissue-scale deformations that shape the living  
36 organism (Collinet et al., 2015; Lye et al., 2015; Petridou et al., 2017; Rauzi et al.,  
37 2015). The extent and pattern of these deformations depends not solely on the  
38 temporal and spatial profile of the generated force fields but also on the  
39 mechanical properties of tissues that the force acts on. For instance, when a thin  
40 sheet of elastic material moves with friction relative to an external substrate, the  
41 range of deformation triggered by an external force is larger in a stiffer elastic  
42 material. It is thus conceivable that, similar to cell-generated forces, the  
43 mechanical properties of tissues are modulated during development in order to  
44 drive morphogenesis towards specific developmental endpoints. However, the  
45 direct assessment of the material properties governing tissue-scale deformation  
46 in living organism is challenging, as it requires the application of controlled  
47 ectopic forces and the concurrent measurement of epithelial deformation within  
48 developing embryos. Recent approaches have started to develop methodologies  
49 to apply controlled forces employing tissue-embedded (ferromagnetic) fluid  
50 droplets combined with force application by means of a magnetic field or by  
51 trapping cell contacts with optical tweezers (Bambardekar et al., 2015; Campas  
52 et al., 2014; Serwane et al., 2016). Due to the applied force amplitudes, however,  
53 droplet movements as well as tissue deformations reached with these  
54 experimental techniques remain highly localized, thus hindering the accessibility  
55 of mechanical properties emerging on the tissue scale. Here, we present a  
56 method overcoming this limitation. In the spirit of previous *in vitro* methods, we

57 make use of a micron-sized functionalized magnetic particle that is pulled on by  
58 means of a custom made electromagnet (Bausch et al., 1998; Kollmannsberger  
59 and Fabry, 2007). Due to the high force amplitudes available within our assay,  
60 large bead displacements and, in particular, large-scale tissue deformations can  
61 be induced. We show that quantifying the tissue response to force application on  
62 the bead allows to characterize key tissue mechanical properties. Importantly,  
63 we do not only monitor bead displacement, but also quantify tissue deformation  
64 based on image-segmentation data. By analyzing the deformation field within a  
65 continuum mechanics framework, quantitative insights into global mechanical  
66 tissue properties can be obtained. In particular, we show that a rapid change in  
67 tissue mechanics occurs during cellularization on time-scales of as little as  
68 several minutes. Using drug treatments, we find that in addition to the  
69 actomyosin cytoskeleton, microtubules also contribute to the material properties  
70 of the syncytial blastoderm. Finally, we detect changes in the friction coefficient  
71 that correlate with changes in the distance between the blastoderm and the  
72 vitellin envelope, indicating that friction is modulated on short time scales  
73 during development.

74

## 75 **Results**

### 76 **Magnetic particle injection and positioning**

77 We have developed a protocol for injecting an individual magnetic microparticle  
78 into a living *Drosophila* embryo and for a consecutive embedding of the particle  
79 into a tissue of interest: (i) a magnetic bead with a diameter of  $4.5 \mu\text{m}$  is injected  
80 into the embryo at preblastoderm stage, (ii) the bead is then directed with a  
81 permanent magnet towards the surface of the embryo (Figure 1A). The bead is

82 afterwards encapsulated within an individual cell while cellularization occurs  
83 (Figure 1A and see Material and methods). The cell accommodates to the  
84 embedded bead and the organism develops normally until larval stage (Video 1).  
85 By choosing the orientation of the embryo during the steering phase, we can  
86 ensure the bead to be encapsulated in predefined groups of cells that will  
87 develop into specific tissues at a later stage. In particular, we were able to  
88 incorporate magnetic beads in the blastoderm at early stages (Figure 1B) and in  
89 the epidermis and amnioserosa at later stages (Figure 1- figure supplement 1C).  
90 To specifically target intracellular compartments, the microparticle was coated  
91 with a GFP nanobody (Kubala et al., 2010), which specifically binds GFP-tagged  
92 proteins. In particular, in order to attach the bead to the cell surface, we made  
93 use of fly lines expressing GFP-tagged versions of Resille, a membrane protein  
94 (Morin et al., 2001).

95

96 **Bead displacement and deformation field measurements upon force  
97 application**

98 After calibration (see Material and methods), we applied a controlled force step  
99 of 65s duration and amplitudes taking values around 115pN to the magnetic  
100 bead by means of an electromagnet (Figure 1C, Figure1-figure supplement 1A-B,  
101 Suppl. Info. and Material and methods). We obtained two complementary  
102 readouts in order to characterize the mechanical response of the tissue: (i) the  
103 bead displacement over time and (ii) the deformation field of the apical surface  
104 area of the epithelium (Figure 1B-C, see Material and methods).

105 Applying forces at consecutive times in the blastoderm, spanning a period of 50  
106 min before gastrulation of Resille-GFP expressing embryos, we can observe

107 significant changes in both the displacement of the bead and the induced  
108 deformation field (Figure 1B-C, Figure 1-figure supplement 1D, video 2 and video  
109 3). Defining the origin of time as the onset of gastrulation, we find that the  
110 amplitude of bead displacement, i.e. the maximal displacement of the bead at the  
111 end of the force step relative to its position before the force step, changes  
112 abruptly from approximately  $2 \mu\text{m}$  to  $8 \mu\text{m}$  around  $t=-20$  min (Figure 1C). This  
113 change in maximal bead displacement is associated with a concurrent change in  
114 the spatial profile and range of the deformation field (Figure 1B and Figure 1-  
115 figure supplement 1-D). Our data therefore show that the same localized force  
116 can lead to a significantly different deformation pattern when applied at  
117 developmental time points differing only by a few minutes.

118

119 **Spring dashpot analysis shows a fast mechanical switch during  
120 cellularization**

121 To obtain a first mechanical description of the response of the tissue upon force  
122 indentation, we analyzed bead responses by means of an effective rheological  
123 model (Figure 2A). We found that the response to a force step could be well  
124 captured by fitting a viscoelastic Maxwell-Kelvin-Voigt model to the individual  
125 bead displacement curves. In this mechanical circuit, a viscous element, with  
126 viscosity coefficient  $\mu_1$ , acts in parallel with an elastic element, with stiffness  
127 coefficient  $K$ , both operating in series with a viscous element described by a  
128 second viscosity coefficient  $\mu_2$  (Figure 2A). In such a rheological scheme, the long  
129 time behavior is a fluid relaxation occurring on a timescale dictated by the ratio  
130 of the viscosity parameter  $\mu_2$  to the stiffness coefficient  $K$ . Introducing a long-  
131 time viscous response was necessary to account for the incomplete relaxation of

132 the bead after the application of the force (Figure 2A), which indicates that the  
133 tissue does not behave like a purely elastic material on long time scales.  
134 We determined the effective parameters introduced above for 20 force  
135 application experiments, performed at successive time points during  
136 cellularization, the process during which cellular membranes extend basally  
137 towards the interior part of the embryo(Figard et al., 2013; Lecuit and  
138 Wieschaus, 2000; Royou et al., 2004) (Figure 2B). Plotting them as a function of  
139 developmental time reveals a rapid step-like change of all three mechanical  
140 parameters, occurring at -17 min before the onset of gastrulation. Indeed, we  
141 found that the observed time-courses could be well fitted by sigmoids (Figure  
142 2B). The effective viscoelastic timescales,  $\mu_1/K$  and  $\mu_2/K$ , in contrast, did not  
143 change significantly (Figure 2-figure supplement 1C). This observation indicates  
144 that both elasticity and viscosities change in the same extent, suggesting that  
145 these two parameters are not independent from each other. The observed switch  
146 in mechanical parameters coincides with a change in the velocity of progression  
147 of cellularization(Figard et al., 2013) (Figure 1-figure supplement 1E-F). We  
148 therefore refer to the phases before and after the change in mechanical  
149 parameters as early and late cellularization (Figure 1B, Figure 1-figure  
150 supplement 1D and Figure 2-figure supplement 1A).

151

152 **Identification of changes in intrinsic tissue mechanical parameters and**  
153 **external friction with a continuous 2D viscoelastic description**

154 The analysis of observed bead responses with an effective rheological scheme  
155 allows for the detection of rapid changes in the mechanical environment the  
156 bead is embedded in. However, effective response parameters are not direct

157 read-outs of actual material properties of the tissue, and cannot relate the spatial  
158 deformation of the tissue to the applied force. To overcome this limitation, we  
159 analyzed two complementary measurements of the tissue response in pulling  
160 experiments (bead displacement and tissue deformation) in the framework of a  
161 continuum description of tissue mechanics (Figure 3A). In our description, we  
162 represent the tissue as a two-dimensional flat sheet with tension tensor  $t_{ij}$ ,  
163 moving with friction relative to a substrate and subjected to a 2D effective  
164 external force density from the bead  $f_i$ , such that force balance on the tissue  
165 reads

166 
$$\partial_i t_{ij} + f_j = \alpha v_j, \quad (1)$$

167 where  $\alpha$  is a friction coefficient and  $v$  the velocity vector. In addition, we used  
168 the following constitutive equation for the tension tensor:

169 
$$\left(1 + \tau \frac{D}{Dt}\right) t_{ij} = \frac{E\tau}{1+v} v_{ij} + \frac{E\tau v}{1-v^2} v_{kk} \delta_{ij} + \tau \frac{D}{Dt} (2\eta v_{ij} + (\bar{\eta} - \eta) v_{kk} \delta_{ij}), \quad (2)$$

170 where  $v_{ij} = \frac{\partial_i v_j + \partial_j v_i}{2}$  is the velocity gradient tensor,  $E$  and  $v$  are a two-  
171 dimensional Young's modulus and Poisson ratio,  $\eta$  and  $\bar{\eta}$  are a shear and bulk  
172 viscosity, and  $\tau$  is a viscoelastic relaxation time. The tissue is described here as a  
173 linear Maxwell-Kelvin-Voigt material: the tissue is assumed to have a viscous  
174 response characterized by  $\eta$  and  $\bar{\eta}$  on short time scales  $t < \eta/E$ , an elastic  
175 response on intermediate time scales  $\frac{\eta}{E} < t < \tau$ , and that elastic stresses are  
176 relaxed above the Maxwell viscoelastic time scale  $\tau$ . In line with our choice of a  
177 continuous linear description, we note that for forces ranging from 50pN to  
178 115pN, bead displacement and epithelial deformations scale approximately  
179 linearly with the force amplitude (Figure 2-figure supplement 1B, Figure 3-figure  
180 supplement 1D). Note that Eq. (2) is, however, a description of the tissue

181 rheology which is not equivalent to the effective description of the bead  
182 behaviour introduced above, as it distinguishes explicitly between dissipative  
183 processes internal to the tissue (characterized by viscosities) and external to the  
184 tissue (characterized by a friction coefficient). In addition, Eq. (2) discriminates  
185 between isotropic and anisotropic elastic moduli and viscosities.

186

187 We then solved Eqs. 1 and 2 and used a fitting procedure to adjust the  
188 theoretically predicted bead displacement as a function of time and the  
189 displacement field at the end of force application to experimental results (Figure  
190 3B, Suppl. Info. and Material and methods). We found that both the bead  
191 displacement and tissue deformation field could be reproduced with the  
192 continuum description in the early and late phases of cellularization (Figure 3B,  
193 Video 4 and Video 5).

194

195 We then compared the values of mechanical parameters extracted at different  
196 stages. We found changes in elasticity from  $E \simeq 29 \text{ pN}/\mu\text{m}$  to  $7 \text{ pN}/\mu\text{m}$ , shear  
197 viscosity from  $\eta \simeq 540 \text{ pN.s}/\mu\text{m}$  to  $105 \text{ pN.s}/\mu\text{m}$ , and Maxwell viscoelastic  
198 timescale from  $\tau \simeq 530\text{s}$  to  $300\text{s}$  (Figure 3C). These changes correspond to an  
199 overall softening of the epithelium, while the characteristic timescale of viscous  
200 to elastic transition ( $\sim 10\text{s}$ ) and long-time plasticity ( $\sim 100\text{s}$ ) does not vary  
201 significantly. Interestingly, these values match relaxation timescales measured  
202 during the Zebra fish tail bud (Serwane et al., 2016).

203

204 In addition, we found that the friction coefficient was increasing from  $\sim 0.8$   
205  $\text{pN.s}/\mu\text{m}^3$  to  $2.2 \text{ pN.s}/\mu\text{m}^3$ . We wondered why the friction coefficient was

206 changing over the course of cellularization, given that it reflects resistance to  
207 motion between the tissue and surrounding structures. We therefore analyzed  
208 the width of the perivitelline space as a function of time, and found that the  
209 apical surface of the cells is getting in closer contact with the vitellin envelope at  
210 late cellularization (Figure 3-figure supplement 2A-B). This observation suggests  
211 that as the embryo approaches the vitellin envelope, increased mechanical  
212 interactions lead to an increase in the external friction coefficient.

213 Finally, we note that the long time-scale hydrodynamic length  $\sqrt{E\tau/\alpha}$   
214 characterizes the spatial range of mechanical interactions in the tissue (Bonnet  
215 et al., 2012; Mayer et al., 2010). We find that this length decreases from  $\sim 140 \mu\text{m}$   
216 to  $\sim 30 \mu\text{m}$  over the course of cellularization.

217 Altogether, our method allowed us to identify a significant tissue softening and  
218 an increase in external friction occurring during cellularization. Both are  
219 contributing to the strong reduction in the spatial propagation of deformation  
220 generated by otherwise similar force fields.

221

222 **Actomyosin and microtubule cytoskeletons impact the mechanical  
223 properties of the blastoderm**

224 To relate the observed changes in mechanical properties of the epithelium  
225 during this softening to intracellular components, we performed our mechanical  
226 probing experiments in embryos injected with pharmacological drugs affecting  
227 cytoskeletal components. We used a Rho kinase inhibitor (Rho-K), Y27632  
228 (Uehata et al., 1997), and a microtubule depolymerizing drug, Colcemid, to affect  
229 actomyosin contractility and the microtubule cytoskeleton, respectively. It has  
230 been reported that both components of the cytoskeleton contribute to the

231 developmental remodeling occurring during cellularization (Foe and Alberts,  
232 1983; Royou et al., 2004; Schejter and Wieschaus, 1993; Xue and Sokac, 2016).  
233 Pharmacological drugs were injected after magnetic particle injection and before  
234 performing pulling experiments. The output of the experiments was then  
235 analyzed with both the spring-dashpot model and our 2D continuous viscoelastic  
236 model.

237

238 Rho-K inhibited embryos did not show major changes in the effective  
239 parameters  $K$ ,  $\mu_1$ , and  $\mu_2$  compared to WT in both the early and late phases of  
240 cellularization (Figure 2- figure supplement 1B, 1D, Video 6 and Video 7). A fit to  
241 our 2D description indicated that epithelial elasticity is not affected by Rho-K  
242 treatment in both phases (Figure 3C and Figure 3- figure supplement 1B-C).  
243 However, we observed a major change in bulk viscosity  $\bar{\eta}$  of  $\sim 180\%$  at early  
244 cellularization, in the friction coefficient  $\alpha$  compare to WT (a 310% increase at  
245 early cellularization and a 34% decrease in late cellularization), and a decrease  
246 in the viscoelastic relaxation timescale  $\tau$  (a 66% decrease at early cellularization  
247 and a 43% decrease at late cellularization). This is reflected in changes in the  
248 spatial propagation of the deformation and of the hydrodynamic length scale by  
249 a factor  $\sim 3$  compared to WT early cellularization (Supp. Info. Table II, Figure 3C,  
250 Figure 3- figure supplement 1-B). To identify whether the changes in friction  
251 observed in Rho-K injected embryos arise from the interaction with the vitellin  
252 envelope, we estimated the width of perivitellin space. We found that the  
253 distance between the apical cell surface and the vitelline membrane is smaller in  
254 Rho-K treated embryos at early cellularization, i.e. when we measure a higher

255 external friction and, conversely, increased in late cellularization when we  
256 measure a decrease in external friction (Figure 3- figure supplement 2A-B).

257

258 In the absence of microtubules, apico-basal progression of membranes is  
259 impaired and embryos fail to cellularize (Foe and Alberts, 1983; Lecuit and  
260 Wieschaus, 2000; Royou et al., 2004). We therefore used Colcemid treatment to  
261 measure the effect of microtubule depolymerization on epithelial mechanics  
262 during cellularization. We observed that the transition in mechanical parameters  
263 observed in WT was absent in Colcemid-treated embryos, indicating that the  
264 shift in mechanical parameters is associated with the process of cellularization  
265 (Figure 2- figure supplement 1D). Interestingly, Colcemid treatment also affected  
266 the response of the epithelium to mechanical forces in the early phases of  
267 cellularization. With our spring-dashpot analysis, we identified that the major  
268 effect of Colcemid treatment was an increase in the fluidity  $1/\mu_2$  by a factor  $\sim 2$   
269 compared to WT in the early phase (Figure 2- figure supplement 1B and D).  
270 Interestingly, these modulations are also associated with changes in the  
271 deformation field. The spatial range of experimentally observed deformation is  
272 smaller than in WT at early cellularization (Figure 3B, Figure 3- figure  
273 supplement 1B, Video 2 and Video 8). As for WT, we could recapitulate the  
274 deformation field and bead displacements measured in Colcemid injected  
275 embryos with our 2D theoretical description of the epithelium (Figure 3- figure  
276 supplement 1B). Compared to WT in early cellularization, our fitting procedure  
277 indicated a 5-fold increase in the friction  $\alpha$ , a 2-fold decrease of the shear  
278 viscosity  $\eta$  and a 5-fold decrease in the long-time viscoelastic timescale  $\tau$  (Figure  
279 3C and Figure 3- figure supplement 1B). This decrease in the Maxwell

280 viscoelastic timescale in the continuum model indicates that long time-scale  
281 relaxation of elastic forces is considerably accelerated in Colcemid-treated  
282 embryos. This observation suggests that relaxation of stresses stored in the  
283 microtubule network is responsible for the slow dynamics of tissue deformation  
284 in the WT embryo. Using these values, we find that the hydrodynamic length is  
285 about 25  $\mu\text{m}$ , i.e.  $\sim$ 5 times shorter than WT in early cellularization, thus  
286 accounting for the decrease in the spatial extent of deformation observed in  
287 Colcemid-treated embryos. The observed changes in frictional forces are, here as  
288 well, consistent with a reduction in the space between the vitellin envelope and  
289 the blastoderm cells (Figure 3- figure supplement 2A-B). Altogether, we observe  
290 an inverse correlation between friction coefficients and perivitellin space widths  
291 (Figure 3- figure supplement 2A-B), suggesting that cytoskeletal components  
292 may affect the distance between the tissue and the vitellin membrane, and  
293 consequently external friction.

294

## 295 **Discussion**

296 Here, we presented a methodology to apply controlled forces, extract tissue-  
297 scale deformations and measure mechanical properties of epithelia within a  
298 generic continuum mechanics framework. Our method allows for the *in vivo*  
299 measurement of essential physical parameters governing tissue-scale  
300 deformations and flows and therefore to relate force patterns to spatial  
301 deformation profiles. We can monitor mechanical changes during development,  
302 and could uncover a rapid step-like softening of the blastoderm preceding the  
303 onset of gastrulation. Our work therefore indicates that, in addition of the  
304 modulation of cellular forces, mechanical properties of tissues can change

305 significantly on time-scales of the order of minutes during morphogenesis. Such  
306 changes must impact morphogenetic processes, as the pattern and amplitude of  
307 deformations in response to the same force field is set by the mechanical  
308 properties of the involved tissues. In particular, we can speculate about an  
309 essential role for the observed softening during cellularization in the consequent  
310 rearrangements associated with gastrulation. Indeed, low tissue stiffness and  
311 high external friction reduce the range of propagation of deformations, and  
312 therefore allows for local deformation without impacting cells at larger distances.  
313 This feature could be important when several morphogenetic rearrangements  
314 occur concurrently, such as during gastrulation. In addition, our results obtained  
315 in drug-injected embryos identified the microtubule network as one of the main  
316 factors in determining tissue mechanics at this stage. Our work therefore  
317 suggests that the observed modulation of tissue mechanics is due to the  
318 rearrangement of the microtubule network occurring during cellularization (Foe,  
319 1993; Mazumdar and Mazumdar, 2002). Microtubules emanating from the asters  
320 associated to different nuclei display transient connections that disappear during  
321 cellularization. A highly connected microtubule meshwork before celularization  
322 thus rearranges into a network of only weakly interacting microtubules asters.  
323 One hypothesis could be that this change in connectivity is at the origin of the  
324 observed changes in mechanics.

325  
326 Finally, our method for mechanical measurements, due to its versatility, low cost  
327 and adaptability to different microscopy techniques can easily be employed in  
328 other systems. It thus paves the way for further studies mapping out epithelial  
329 mechanics. Therefore, it will serve as an important tool for understanding the

330 emergence of mechanical properties at the tissue-scale in developmental  
331 contexts and in cases of disease.

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333

334

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345  
346 **Material and methods**

347 **Preparation of *Drosophila* Embryos**

348 To inject the bead at pre-blastoderm stage, the embryos are collected during  
349 15min, dechorionated in 100% bleach and mounted on an heptane/glue  
350 coverslip(Fish et al., 2007). The tissue in which the bead will be located has to  
351 face the coverslip. Once mounted, the embryos are dehydrated during 10 min at  
352 25°C and then covered with voltalef 10S oil.

353

354 **Particle microinjection**

355 Micro-needles are generated by pulling 1mm glass capillaries (Narishige G1)  
356 using a micro-puller (Sutter instruments P30). The needle tip is then opened and  
357 beveled in a controlled manner to facilitate the injection using a micro grinder  
358 (Narishige EG-44). The internal diameter is set to be slightly smaller than the  
359 particle, i.e., 3.5-4  $\mu\text{m}$  (Fig 1-A). This allows, by tuning the pressure inside the  
360 micro-needle with a microinjector (WPI PV 820), to hold and inject an individual  
361 particle within the *Drosophila* embryo. Once inside the embryo, the bead can be  
362 oriented on the A-P and dorso-lateral axes using an electromagnet. After  
363 injection, the embryos are positioned above a permanent magnet and are let  
364 aged (2h to apply force during cellularization) in a wet chamber at 25°C.

365

### 366 **Calibration of the electro-magnet**

367 To induce controlled forces we designed an electromagnet similarly to previous  
368 *in vitro* studies(Kollmannsberger and Fabry, 2007). A core of soft metal with a tip  
369 shape (Mumetal, Sekels GmbH) is surrounded by 100 coils of copper cable  
370 alimented by a power supply in order to generate a solenoid. An additional  
371 radiator is placed in between the soft metal core and the copper coils to evacuate  
372 the thermal dissipation. The electric current circulating within the copper coils  
373 will generate a magnetic field that will be focused at the tip of the soft metal core.  
374 The magnetic force exerted on the paramagnetic micro-particle is directly  
375 proportional to the gradient of magnetic field(Kollmannsberger and Fabry,  
376 2007). Therefore, the force would be the highest close to the tip and decay as a  
377 power law with the distance to the tip of the electro-magnet (Figure 1- figure  
378 supplement 1-B). In order to calibrate the magnetic force exerted on the micro-  
379 particle, we have established a calibration assay with micro-particle embedded

380 in PDMS (Sigma Aldrich). Because its viscosity is well established, by measuring  
381 the velocity of the micro-particles within the magnetic field generated by the  
382 electro-magnet, one can determine precisely the force applied to the particle as a  
383 function of its distance to the tip for a specific current applied in the solenoid.  
384 For a current of 0.3A, the typical force-distance curve range from ~1nN at 60  $\mu$ m  
385 from the tip to ~100 pN at 200  $\mu$ m (Figure 1- figure supplement 1A-B). Due to  
386 geometrical constrains arising from the embryo shape, the typically used bead-  
387 magnet distances in our experiments were about 190 $\mu$ m (inset Figure 1- figure  
388 supplement 1B). This corresponds to forces of ~115pN (high force condition, for  
389 0.3A) and ~50pN (low force condition, for 0.15A). Note that for currents above  
390 0.5A the magnetic force saturates.

391

## 392 **Imaging and force application**

393 Embryos were imaged at room temperature (22°C) using an Andor spinning disk  
394 confocal microscope. Z stacks of 4-5 sections spaced by 1 micron interval were  
395 collected every 5 seconds with 100X magnification.

396 The magnet was precisely positioned with a three-axis micromanipulator  
397 (Narishige UMM-3FC) mounted on the microscope stage. In the experiments, the  
398 electromagnet has been positioned approximately at 190  $\mu$ m from the bead, and  
399 the force has been applied systematically for 65s. Data considered valid for  
400 analysis are cases when the bead is attached to the apical cell membrane.  
401 Occasionally, the bead detaches from the membrane, displaces in the basal  
402 direction or is attached to a wrong site in the cell for pulling experiment. In those  
403 cases, we did not include the data in the analysis.

404

405 **Analysis of the bead displacement**

406 Analysis of the bead displacement was performed using Fiji. Tracking of the bead  
407 was performed on images acquired on the red channel where the auto-  
408 fluorescence of the particle is emitted. The following analysis step were  
409 implemented: (1) A median filter (radius 2) is applied on maximum intensity  
410 projections of each z stacks, (2) the projection is then manually thresholded, (3)  
411 MTrack2 plugin was used to automatically extract the x-y coordinate of the bead.  
412 In some occasions, samples showed drift. In order to detrend extracted bead  
413 displacements, we fit a linear function,  $f(x) = mx + c$ , to the bead displacement  
414 in the 100s preceding the force application. Assuming this drift pertained during  
415 the force application, we subtracted this linear trend from the bead displacement.

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429 **Statistics**

430 The table below reports a Welch test, checking the Null hypothesis that two  
431 sample have equal mean, without assuming they have the same standard  
432 deviation. All the conditions are compared with the respective WT at early and  
433 late cellularization for the data generated using the spring-dashpot model as  
434 reported in Figure2-figure supplement 1B.

435

	<b>Condition</b>	<b>Stage</b>	<b>Delta</b>	<b>Parameter</b>	<b>cmax</b>	<b>cmin</b>	<b>p</b>	<b>p_values</b>
<b>0</b>	DMSO	Early Cell.	43%	$K$	0.107667	-0.061682	-	5,46E-01
<b>1</b>	DMSO	Early Cell.	-12%	$\mu_1$	0.246043	-0.611750	-	3,75E-01
<b>2</b>	DMSO	Early Cell.	-36%	$l/\mu_2$	0.413671	-0.505623	-	8,20E-01
<b>3</b>	Colcemid	Early Cell.	68%	$K$	0.071115	0.001740	**	4,08E-02
<b>4</b>	Colcemid	Early Cell.	13%	$\mu_1$	0.564621	-0.191551	-	3,16E-01
<b>5</b>	Colcemid	Early Cell.	134%	$l/\mu_2$	0.232951	0.101975	**	3,37E-05
<b>6</b>	RhoK	Early Cell.	26%	$K$	0.038090	-0.010224	-	2,43E-01
<b>7</b>	RhoK	Early Cell.	26%	$\mu_1$	0.734543	0.015826	**	4,16E-02
<b>8</b>	RhoK	Early Cell.	6%	$l/\mu_2$	0.102569	-0.086092	-	8,55E-01
<b>9</b>	RhoK	Late Cell.	14%	$K$	0.013819	-0.007168	-	4,96E-01
<b>10</b>	RhoK	Late Cell.	13%	$\mu_1$	0.302732	-0.139462	-	4,43E-01
<b>11</b>	RhoK	Late Cell.	0,09	$l/\mu_2$	0.156488	-0.258295	-	6,08E-01
<b>12</b>	Low force	Early Cell.	26%	$K$	0.062561	-0.034499	-	5,21E-01
<b>13</b>	Low force	Early Cell.	-4%	$\mu_1$	0.488387	-0.621007	-	7,94E-01
<b>14</b>	Low force	Early Cell.	98%	$l/\mu_2$	0.380733	-0.135324	-	2,93E-01
<b>15</b>	Low force	Late Cell.	-26%	$K$	0.002841	-0.014751	-	1,63E-01
<b>16</b>	Low force	Late Cell.	-35%	$\mu_1$	-0.017881	-0.422326	**	3,50E-02
<b>17</b>	Low force	Late Cell.	-7%	$l/\mu_2$	0.404143	-0.487061	-	8,33E-01

436

437

438 **Analysis of the experimental deformation field**

439 Experimental images were aligned using the position of the bead at t=0s. Cell  
440 shapes were determined from the outlines of cell membranes at the onset and at  
441 the end of the period of force application, i.e. at t=0s and t=65s, respectively,  
442 using the software Packing Analyzer v.2.0(Aigouy et al., 2010). Cells were  
443 tracked using the same software. We determined the displacement vector,  $d(x)$ ,  
444 corresponding to the centroid movement between t=0s and t=65s of all cells  
445 within the field of view. Here,  $x=(x,y)$  is a vector denoting the position of the  
446 centroid of the cell at t=0s relative to the bead at t=0s. We considered the total  
447 set of obtained deformation vectors,  $\{d(x_k) \text{ for } k = 1, \dots, M\}$ , as the readout of  
448 the experimental deformation field. Note that cells have a typical diameter of  
449 about  $\sim 5\mu\text{m}$ , which then defines the spatial resolution of the deformation field  
450 measurement.

451

452 **Vitelline envelope-apical cell surface distance estimation analysis**

453 The estimation of the distance between the vitellin envelope and apical cell  
454 surface was performed using z-reslice along the AP axis (using FIJI) of z-stacks  
455 spaced by 0.5  $\mu\text{m}$  of embryo expressing Resille-GFP injected with Dextran Texas-  
456 Red 70000MW(Molecular Probes) in the perivitelline space. We use the  
457 fluorescence intensity of Dextran Texas Red as a proxy for the position of the  
458 perivitelline space. With a homemade Matlab script, an average z-fluorescence  
459 profile is obtained by: 1) performing a sliding average of 5 pixels along the z-  
460 reslice and 2) by realigning in z each local average to the maximal dextran-RFP  
461 fluorescence intensity. A spline interpolation has been performed on the Resille-  
462 GFP intensity curves to determine the position of the maximum of intensity.

463

464 **Fly stocks**

465 Stock used for live imaging: Resille-GFP(Morin et al., 2001) and Sqh<sup>AX3</sup>, Sqh-

466 GFP(Royou et al., 2004).

467

468 **Drug Microinjection**

469 Developing embryos have been injected in early phase of cellularization using a

470 Femtojet Injector (Eppendorf). Rho-K inhibitor (Y-27632 Sigma Aldrich) has

471 been injected at 30mM; Colcemid (Santa Cruz Biotechnology) has been injected

472 at 1mM and Dextran Texas-Red 70000MW(Molecular Probes) has been injected

473 at concentrations between 7 and 25mg/ml. We estimate ~20 fold dilution in the

474 embryo.

475

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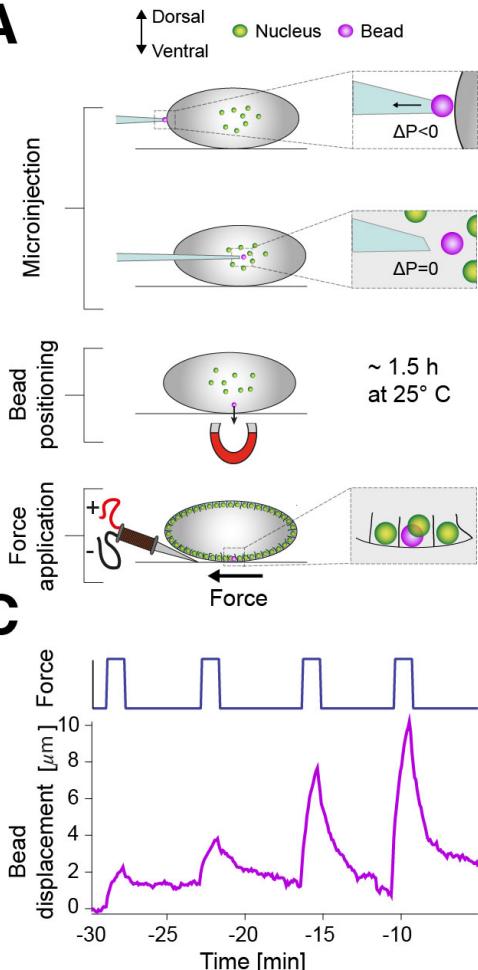
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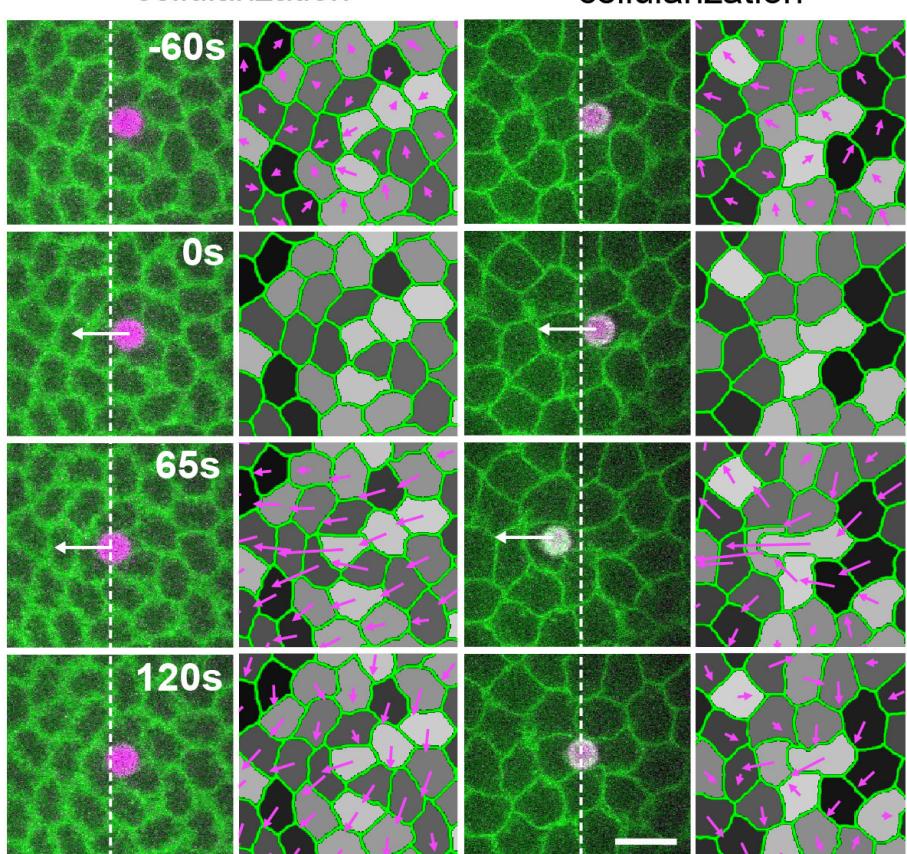
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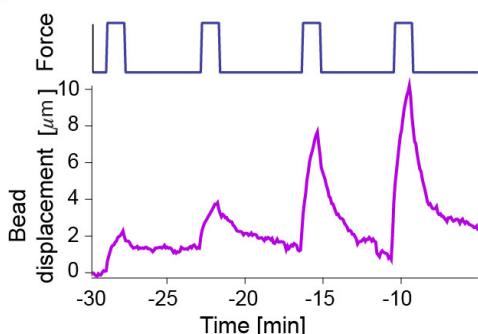
**B**

Early cellularization

Late cellularization



**C**



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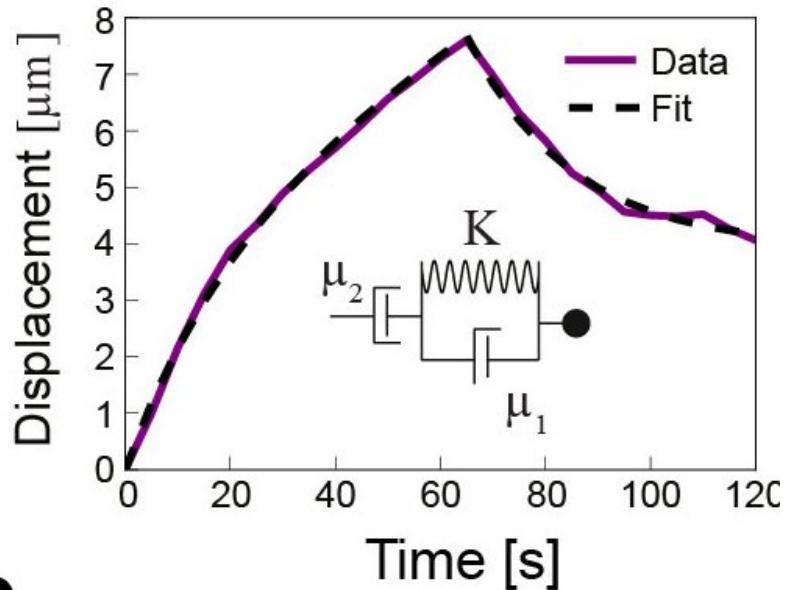
578 **Figure 1. Bead injection and force application.**

579 (A) Injection procedure. An individual magnetic bead (purple) of  $4.5 \mu\text{m}$   
580 diameter is injected into the yolk of an embryo at developmental stage 2. In  
581 order to position the bead apically, the embryo is staged on top of a permanent  
582 magnet post injection ( $\sim 1.5\text{h}$  at  $25^\circ\text{C}$ ). When cellularization begins, force steps of  
583  $65\text{s}$  duration are applied to the bead with an electromagnet.

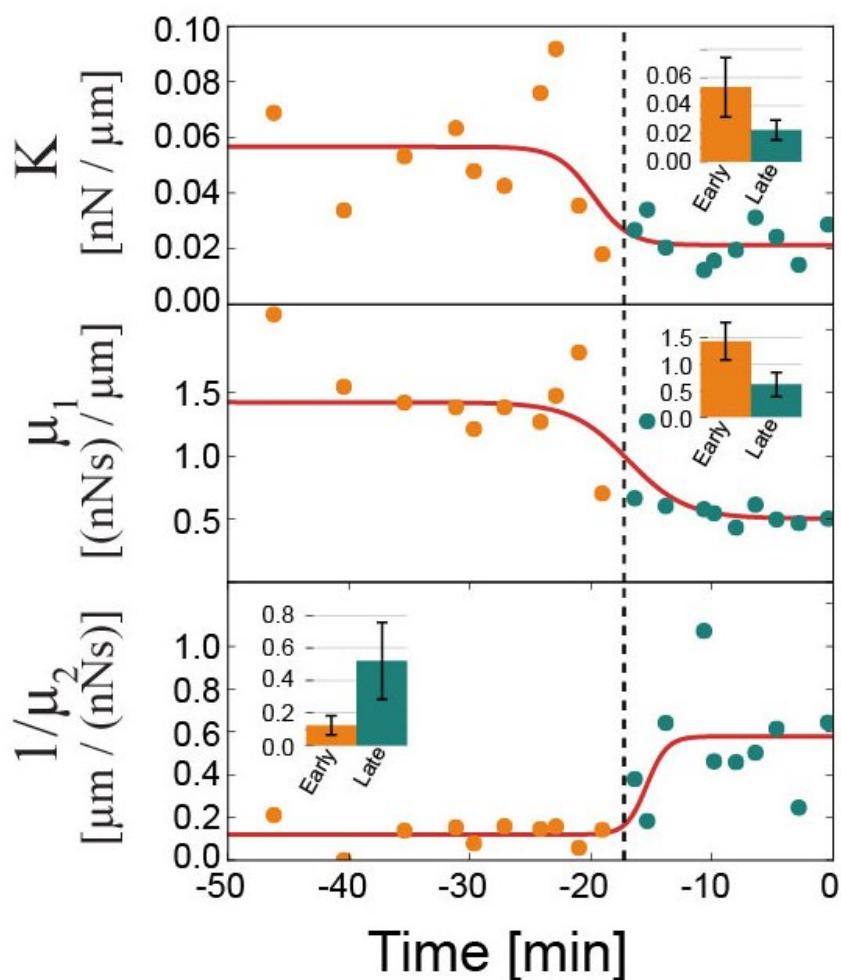
584 (B) Time lapse images showing bead displacement and tissue deformation  
585 (purple arrows) in response to a force step (high force condition, onset at 0s).  
586 Here, the bead was embedded into an individual cell of a Resille-GFP embryo.  
587 Force applications are shown for early cellularization (left,  $>20$  min before  
588 gastrulation) and late cellularization (right,  $<20\text{min}$  before gastrulation). White  
589 arrows indicate force application. White dashed lines mark the left side of the  
590 bead at time 0s. Deformations are calculated relative to the onset of force  
591 applications ( $t=0\text{s}$ ). Scale bar is  $10 \mu\text{m}$ .

592 (C) Bead displacement for four consecutive force applications at high force  
593 condition performed over the time-course of cellularization (bottom) and  
594 corresponding forcing curve over time (top). Time is relative to the onset of  
595 gastrulation at  $t=0\text{min}$ .

**A**



**B**



597 **Figure 2. Fitting bead displacement to an effective spring-dashpot model.**

598 (A) Example of a bead displacement curve after the application of force (high  
599 force condition, purple line) fitted with a Maxwell-Kelvin-Voigt model described  
600 by the three effective parameters  $\mu_1$ (0.61 (nN.s)/ $\mu\text{m}$ ),  $\mu_2$ (1.99 (nN.s)/ $\mu\text{m}$ ) and  $K$   
601 (0.03 nN/ $\mu\text{m}$ ) (see inset for a schematic of the corresponding rheological model).  
602 (B) Effective parameters as a function of developmental time relative to the  
603 onset of gastrulation. Each dot represents a single force application at high force  
604 condition. We observe a step-like change of the three parameters at the onset of  
605 the fast phase of cellularization, i.e. around -20 min. Red lines are fits of  
606 experimental data to a sigmoid function. Black dashed lines represent the mean  
607 of the middle of sigmoid curves and is used to separate between force  
608 applications in the early (orange) and late (blue) phase of cellularization. Insets  
609 are average parameters over the early and late phases, with error bar indicating  
610 standard deviations.

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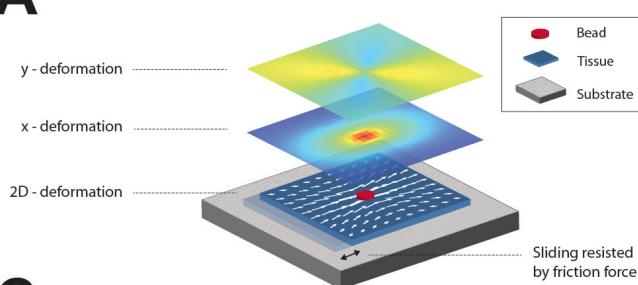
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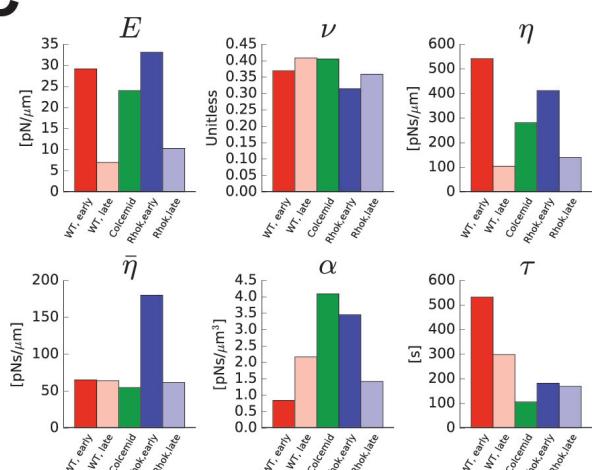
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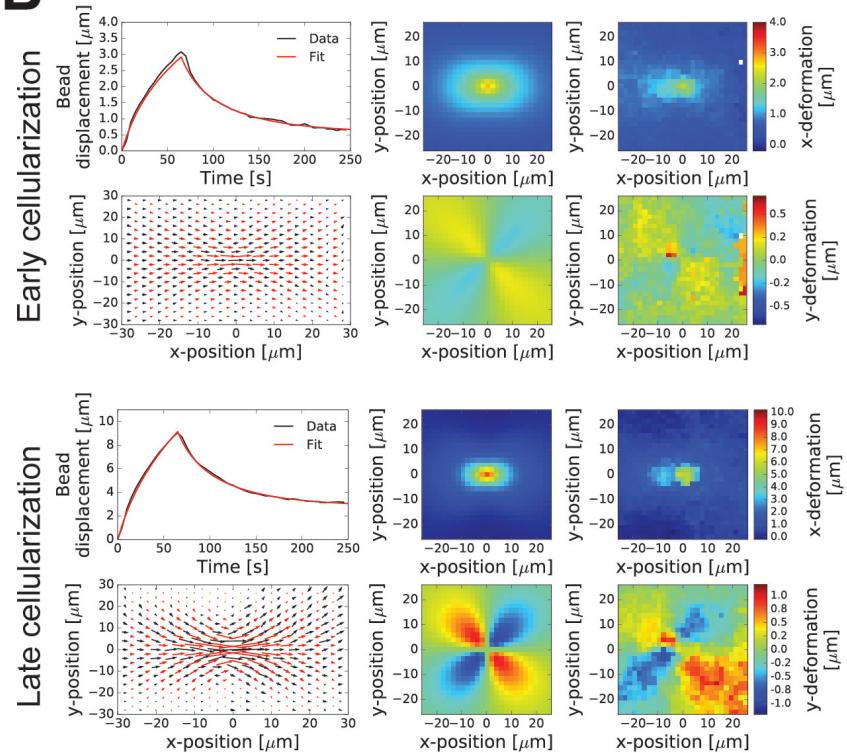
**A**



**C**



**B**



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633 **Figure 3. Determining mechanical tissue parameters within a 2D  
634 continuum mechanics framework.**

635 (A) Schematic of the continuum description used to calculate deformation fields.

636 The epithelial tissue is considered to be a 2D sheet moving relative to a substrate.

637 Sliding of the tissue is resisted by friction. Bead-induced tissue deformation

638 (represented by arrows) is decomposed in x- and y-deformation (shown as

639 heatmaps). Mechanical tissue parameters are extracted from experimental data

640 by fitting the continuum description to both the averaged time-evolution of bead

641 displacement and deformation field at the end of the forcing step.

642 (B) Comparison of experimental and continuum description of bead

643 displacement at early (n=10) and late cellularization (n=10) and deformation

644 field at early (n=10) and late cellularization (n=8).

645 For both conditions, average experimental and fitted bead displacement (top-left

646 panel), fitted (red arrows) and experimental (black arrows) deformation field

647 (bottom-left panel), and fitted and experimental x- and y-deformations (middle

648 and right panels, respectively) are shown.

649 (C) Mechanical parameters as extracted from the fit of experimental data to our

650 continuum description. Values of the elasticity  $E$ , the poisson ratio  $\nu$ , the shear

651 viscosity  $\eta$ , the bulk viscosity  $\bar{\eta}$ , the friction coefficient  $\alpha$ , and the Maxwell

652 viscoelastic timescale  $\tau$  are shown for both WT and embryos injected with

653 Colcemid and Rho-K inhibitor.

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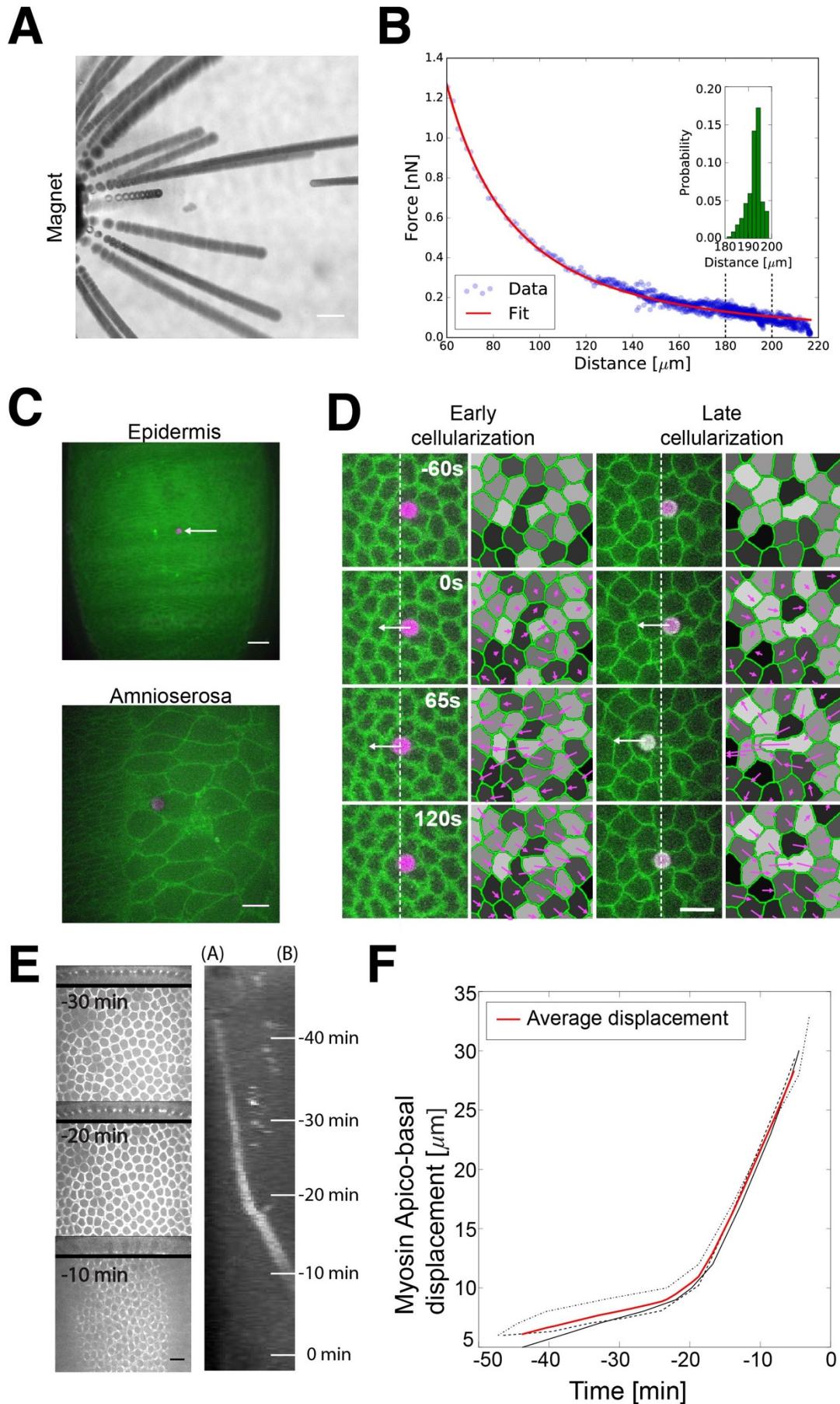
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662 **Figure 1- figure supplement 1**

663 **Force calibration, Bead positioning in tissues and cellularization process.**

664 (A) Time projection movie of  $4.5 \mu\text{m}$  magnetic beads embedded in PDMS  
665 moving toward the magnet during application of the magnetic field generated  
666 with a current of 0.3A. The dark lines indicate bead trajectories. Scale bar is  
667  $20 \mu\text{m}$

668 (B) Example of force-distance calibration curves of  $4.5 \mu\text{m}$  beads (dots) and  
669 power law fit (in red). Inset shows the distribution probability of bead-magnet  
670 distances for the used force application experiments.

671 (C) Snapshot of a single  $4.5 \mu\text{m}$  bead (in magenta) embedded in the ventral  
672 epidermis (top, scale bar is  $20 \mu\text{m}$ ) and in the Amnioserosa (bottom, scale bar  
673 is  $10 \mu\text{m}$ ) at late stages of embryonic development.

674 (D) Time lapse images showing the force application (high force condition) on  
675 a bead embedded into a single cell of a Resille-GFP embryo at early  
676 cellularization (on the left,  $>17$  min before gastrulation) and late cellularization  
677 (on the right,  $<17$  min before gastrulation) and the corresponding velocity  
678 fields. White arrows indicate when force is applied. White dashed lines  
679 indicate the left side of the bead at time -60s. Velocities are calculated  
680 between individual cell positions in the displayed time frames. Scale bar is  $10$   
681  $\mu\text{m}$ .

682 (E) Snapshot of Sqh-GFP embryo at -30, -20 and -10 min before the onset of  
683 gastrulation. Right: kymograph of myosin intensity along the z apico-basal  
684 direction of the tissue, integrated along the midline in the A-P axis,  
685 corresponding to the Sqh-GFP embryo shown on the left. A myosin intensity  
686 peak is displaced basally during the slow and fast phases of cellularization.  
687 Apical (A) is on the left and Basal (B) is on the right. The origin of time is set  
688 at the onset of gastrulation. Scale bar is  $10 \mu\text{m}$ .

689 (F) Traces of the apico-basal displacement of the apicobasal intensity peak of  
690 myosin during cellularization in three different embryos (black lines), and their  
691 average (red line). The origin of time is set at the onset of gastrulation.

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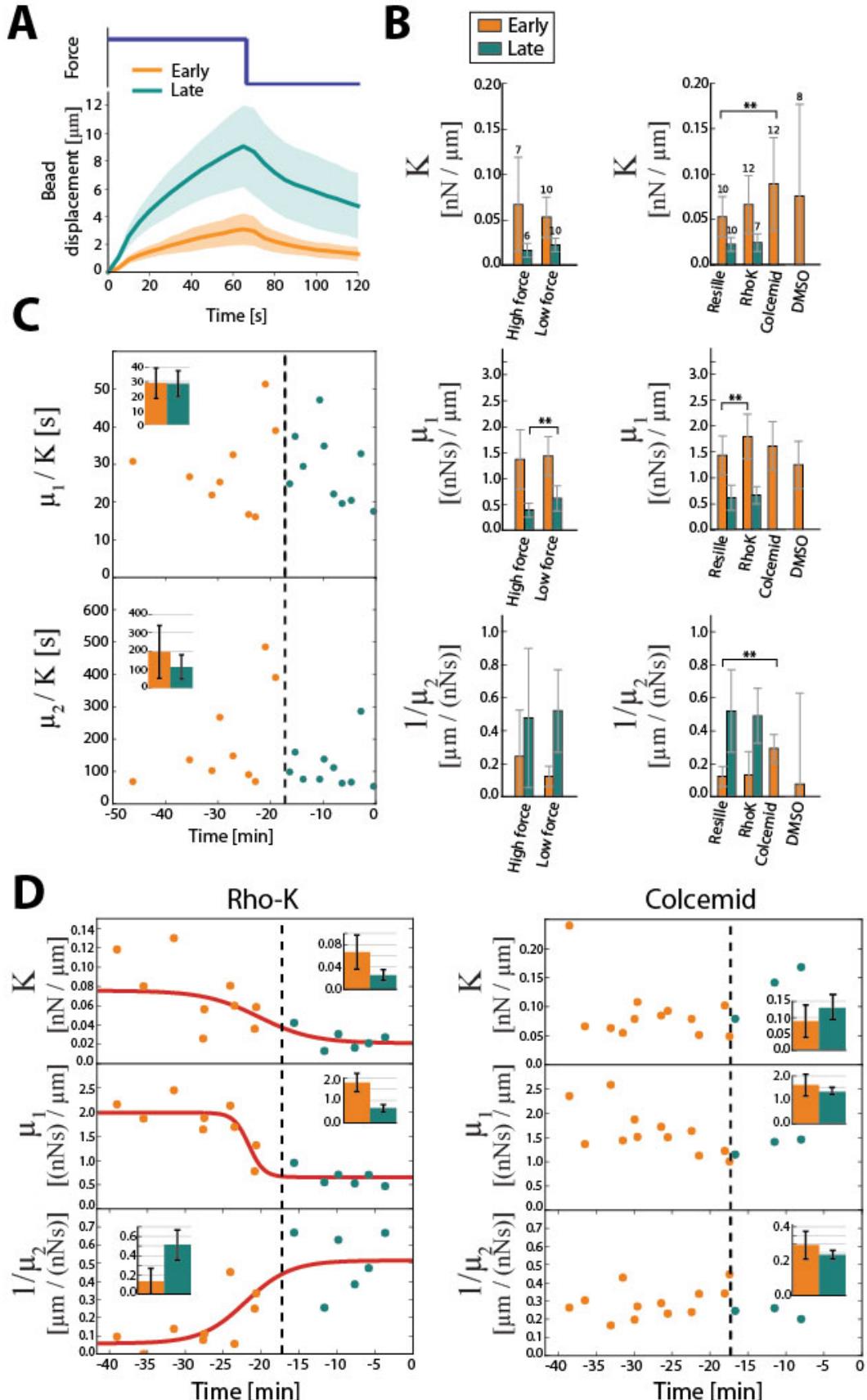
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717 **Figure 2- figure supplement 1**

718 **Analysis of bead displacements with a Maxwell Kelvin-Voigt model.**

719 (A) Average bead displacement for force steps of about 115 pN at early (n=10  
720 applications) and late (n=10 applications) cellularization in Resille-GFP  
721 embryos. Shaded region represents standard deviations.

722 (B) Estimation of stiffness  $K$ , viscosity coefficient  $\mu_1$  and fluidity  $1/\mu_2$  using the  
723 Maxwell-Kelvin-Voigt spring-dashpot model. On the left, the parameters are  
724 estimated for WT embryo (Resille-GFP) at low force steps of ~50pN and high  
725 force steps of ~115pN. The estimated three parameters are approximately  
726 the same for 50pN and 115pN forces indicating that the tissue behaves  
727 linearly in this force regime. On the right, parameters are estimated for the  
728 same force amplitude (~115pN) for Resille-GFP expressing embryos and  
729 Resille-GFP embryos injected with Rho-K, Colcemid and DMSO. Numbers on  
730 the error bars are the number of force application. \*\* indicate p values smaller  
731 then 0.05 calculated using two sided t-test. Error bars represent standard  
732 deviations.

733 (C) Viscoelastic timescales  $\mu_1/K$  and  $\mu_2/K$  as a function of time. The origin of  
734 time is defined as the onset of gastrulation. The dashed lines indicate the  
735 separation between early and late cellularization.

736 (D) Estimation of the three parameters stiffness  $K$ , viscosity coefficient  $\mu_1$  and  
737 fluidity  $1/\mu_2$  over time for Resille-GFP embryos injected with Rho-K (left)  
738 inhibitor and Colcemid (right). For Rho-K injected embryos, we still observe a  
739 step-like behavior in the evolution of the three parameters. The red line is a  
740 sigmoidal fit recapitulating the time evolution of the parameters. For embryos  
741 treated with Colcemid there are no significant changes between early and late  
742 cellularization. In both cases the black dashed line represents the separation  
743 between early (in orange) and late (in blue) force applications. The insets  
744 show the mean value for each parameter for early and late cellularization.  
745 Error bars represent standard deviations.

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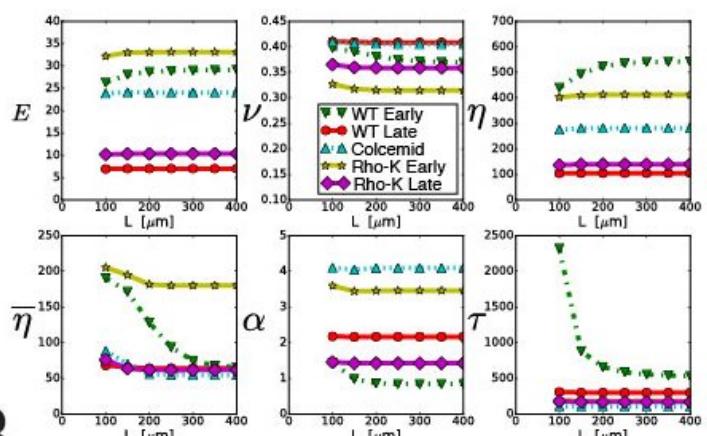
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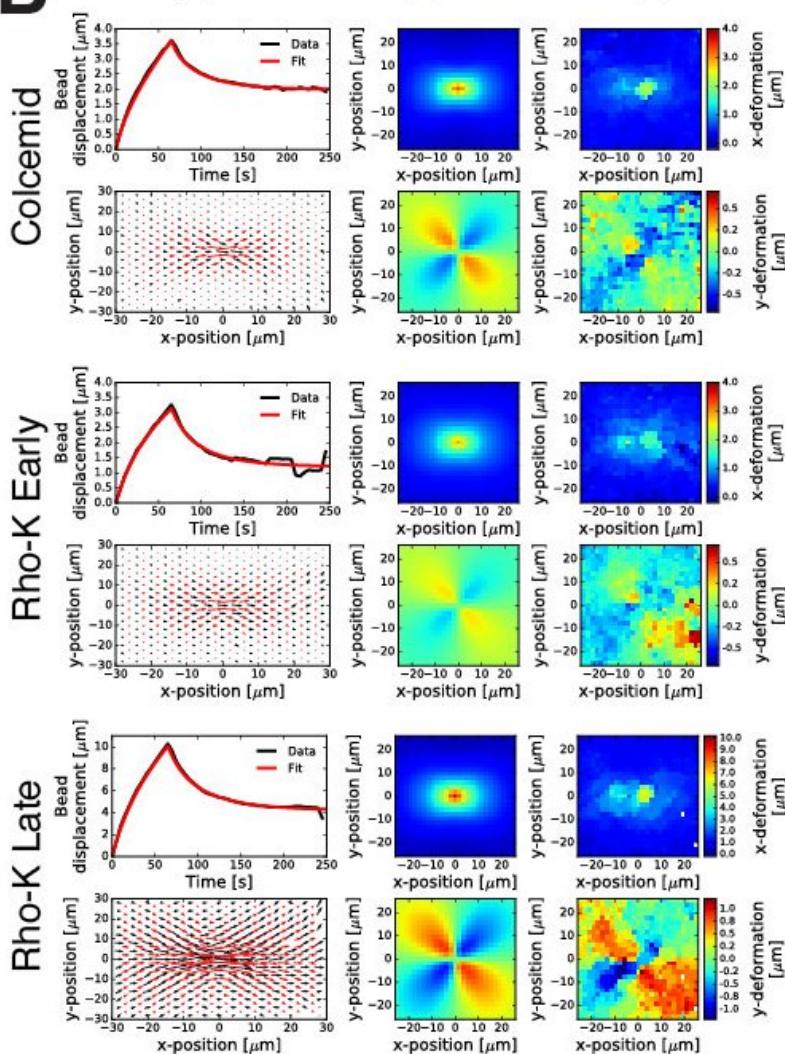
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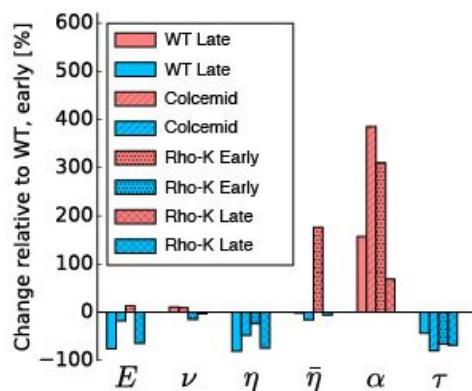
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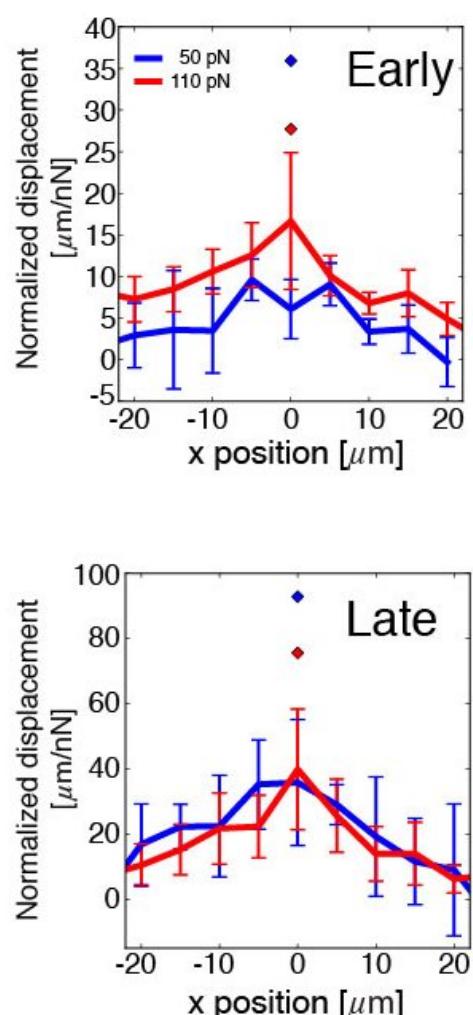
**B**



**C**



**D**



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771 **Figure 3- figure supplement 1**

772 **Analysis of bead displacement and deformation field with a 2D continuum**  
773 **mechanics model.**

774 (A) Mechanical parameters extracted from the fit of WT late cellularization bead  
775 displacement and deformation field as a function of the size L of the theoretical  
776 2D viscoelastic tissue. Beyond 200 $\mu$ m, parameters are independent on the size of  
777 the 2D tissue.

778 (B) Comparison of experimental and continuum description of average bead  
779 displacement in Colcemid (n=12), Rho-K Early (n=12) and Rho-K Late (n=7) and  
780 deformation field in Colcemid (n=8), Rho-K Early(n=8) and Rho-K Late (n=4).  
781 For all conditions, (top-left panel) average experimental and fitted bead  
782 displacement, (bottom-left panel) experimental (black arrows) and fitted  
783 deformation field (red arrows) and fitted and experimental x- and y-  
784 deformations (middle and right panels respectively) are shown.

785 (C) Relative changes in the different mechanical parameters compared to WT  
786 early cellularization upon the different perturbations for early and late  
787 cellularization.

788 (D) x-displacement of the tissues along the axis of force application, normalized  
789 by the pulling force magnitude taken at t=65s, for two force amplitudes 50 pN  
790 and 115 pN at early (Top) and late cellularization (Bottom). The Rhombuses  
791 indicates maximal bead displacements normalized by the force magnitude.  
792 Normalized displacements at 50pN and 115pN are similar, indicating a linear  
793 response of the tissue in this regime.

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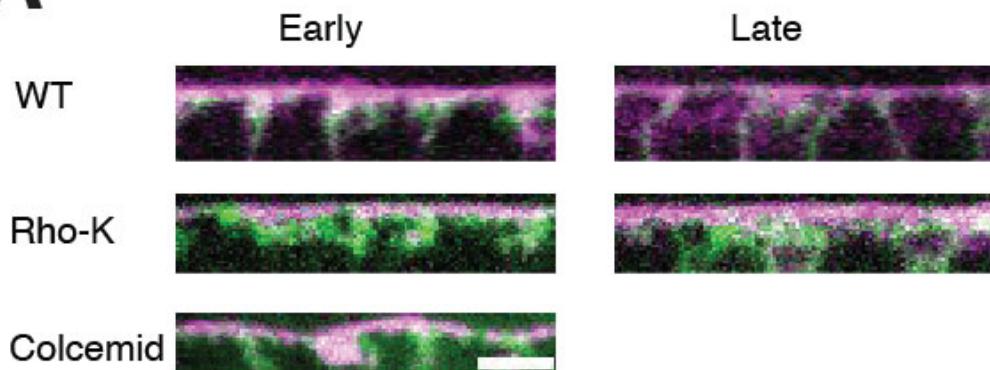
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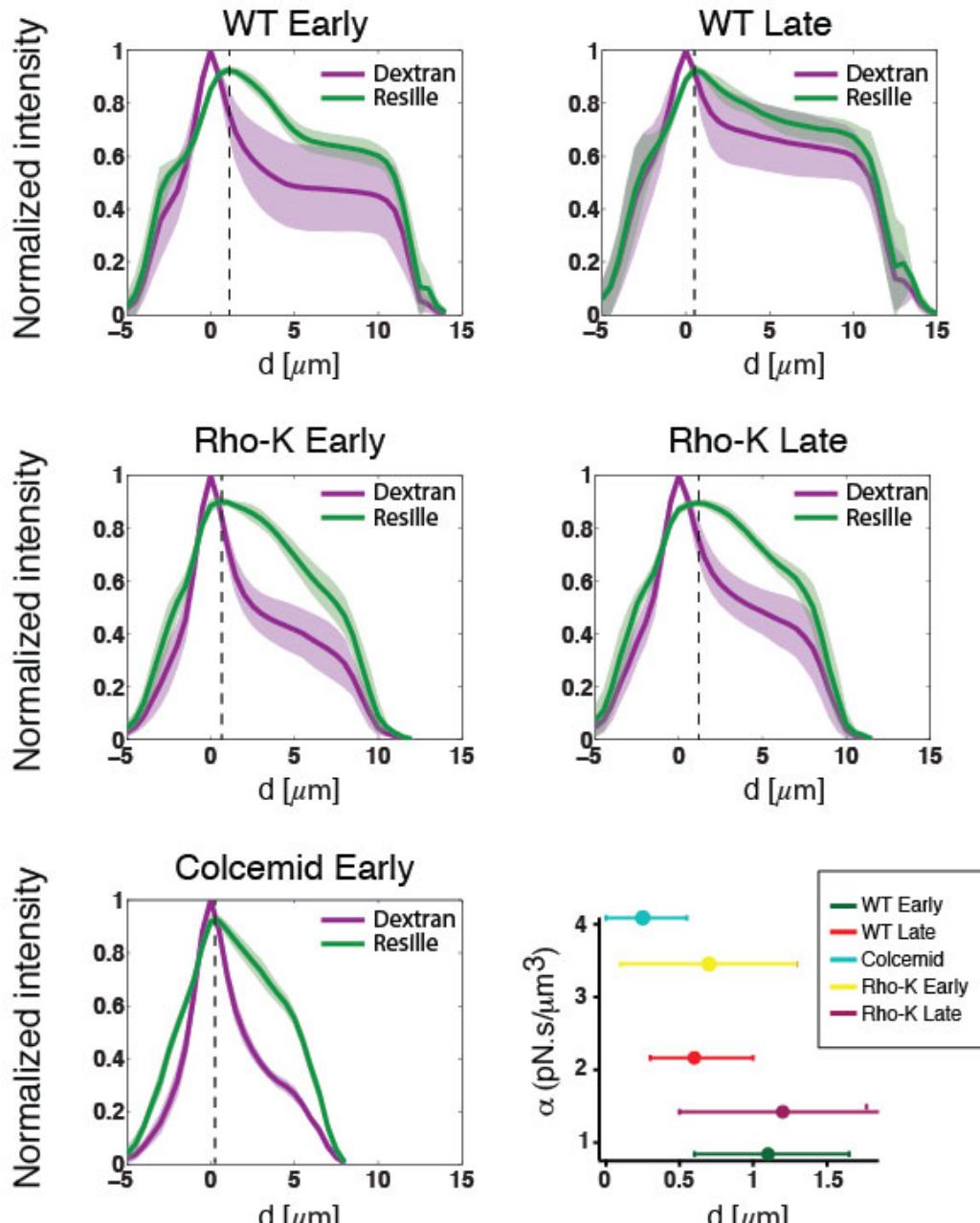
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**A**



**B**



813 **Figure 3- figure supplement 2**  
814 **Estimation of perivitelline width for WT, Colcemid and Rho-K treated**  
815 **embryos**

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817 (A) Z-reslices along the A-P axis of the embryo showing dextran texas red (in  
818 magenta) injected in the perivitellin space and Resille GFP signal (in green) for  
819 WT embryos, Rho-K injected embryos at early and late cellularization and  
820 Colcemid injected embryos at early cellularization. Scale bar: 5 $\mu$ m.  
821 (B) Average fluorescence intensity profiles of the dextran and membrane marker  
822 Resille-GFP labeling cellular membranes for WT and Rho-K treated embryos at  
823 early and late cellularization stages and in colcemid treated embryo at early  
824 stages ( $N_{WT\text{early}}=22$  profiles on 3 embryos,  $N_{WT\text{late}}=21$  profiles on 3 embryos,  $N_{Rho-}$   
825  $K\text{early}=26$  profiles on 3 embryos,  $N_{Rho-K\text{late}}=30$  profiles on 3 embryos,  $N_{Col}=10$   
826 profiles on 2 embryos). The dashed line defines the position of the maximum of  
827 the fluorescence peak for Resille (see methods). The bottom right graph shows  
828 the friction coefficient  $\alpha$  (see fig 3-C) as a function of the dextran-Resille-GFP  
829 maximum peak intensity distance (as a proxy of perivitellin space width) for WT  
830 early and late and Rho-K inhibitor and Colcemid injected embryos. We can  
831 observe a linear relationship between friction coefficient and the inter-peak  
832 distance. The error bars represent the width of the Resille peak at 99% of the  
833 maximal height.

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**863 Video1 *Drosophila* larvae with a 4.5  $\mu$ m bead.**

864 Transmission movie of a drosophila larvae pre-injected at pre-blastoderm stage.  
865 The white arrow indicates the bead position.

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**867 Video 2 Force application experiment at early cellularization.**

868 Time-lapse movie showing a force application experiment on a Resille-GFP  
869 embryo at early cellularization. The purple arrow indicates when a force of  
870  $\sim$ 115pN is applied on the bead.

871

**872 Video 3 Force application experiment at late cellularization.**

873 Time-lapse movie showing a force application experiment on a Resille-GFP  
874 embryo at late cellularization. The purple arrow indicates when a force of  
875  $\sim$ 115pN is applied to the bead.

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**877 Video 4 Predicted tissue deformation upon force application at early  
878 cellularization.**

879 Kinetics of the deformation upon force application, resulting from the fit of  
880 pulling experiments at early cellularization with our 2D theoretical description.  
881 The left panel indicates the deformation field as a quiver plot, the middle panel  
882 the x-component and the right panel the y-component of the deformation field.  
883 The origin of time is the onset of the pulling force.

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**885 Video 5 Predicted tissue deformation upon force application at late  
886 cellularization.**

887 Kinetics of the deformation upon force application, resulting from the fit of  
888 pulling experiments at late cellularization with our 2D theoretical description.  
889 The left panel indicates the deformation field as a quiver plot, the middle panel  
890 the x-component and the right panel the y-component of the deformation field.  
891 The origin of time is the onset of the pulling force.

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**893 Video 6 Force application experiment at early cellularization in an embryo  
894 treated with Rho-K inhibitor.**

895 Time-lapse movie showing a force application experiment on a Resille-GFP  
896 embryo treated with Y27632 at early cellularization. The purple arrow indicates  
897 when a force of  $\sim$ 115pN is applied.

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**899 Video 7 Force application experiment at late cellularization in an embryo  
900 treated with Rho-K inhibitor.**

901 Time-lapse movie showing a force application experiment on a Resille-GFP  
902 embryo treated with Y27632 at late cellularization. The purple arrow indicates  
903 when a force of  $\sim$ 115pN is applied.

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**905 Video 8 Force application experiment at early cellularization in a Colcemid  
906 treated embryo.**

907 Time-lapse movie showing a force application experiment on a Resille-GFP  
908 embryo treated with Colcemid at early cellularization. The purple arrow  
909 indicates when a force of  $\sim$ 115pN is applied.