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1 Nature of active forces in tissues: how contractile cells
2 can form extensile monolayers

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4 **Actomyosin machinery endows cells with contractility at a single cell level.**
5 **However, at a tissue scale, cells can show either contractile or extensile be-**
6 **haviour based on the direction of pushing or pulling forces due to neighbour**
7 **interactions or substrate interactions. Previous studies have shown that a**
8 **monolayer of fibroblasts behaves as a contractile system¹ while a monolayer**
9 **of epithelial cells^{2,3} or neural crest cells behaves as an extensile system.⁴ How**
10 **these two contradictory sources of force generation can coexist has remained**
11 **unexplained. Through a combination of experiments using MDCK (Madin**
12 **Darby Canine Kidney) cells, and in-silico modeling, we uncover the mecha-**
13 **nism behind this switch in behaviour of epithelial cell monolayers from ex-**
14 **tensile to contractile as the weakening of intercellular contacts. We find that**
15 **this switch in active behaviour also promotes the buildup of tension at the cell-**
16 **substrate interface through an increase in actin stress fibers and higher trac-**
17 **tion forces. This in turn triggers a mechanotransductive response in vinculin**
18 **translocation to focal adhesion sites and YAP (Yes-associated protein) tran-**
19 **scription factor activation. Our studies also show that differences in extensility**
20 **and contractility act to sort cells, thus determining a general mechanism for**
21 **mechanobiological pattern formation during cell competition, morphogenesis**
22 **and cancer progression.**

23 **Main text**

24 The ability of cell monolayers to self-organize, migrate and evolve depends crucially on the
25 interplay between cell-matrix and cell-cell interactions⁵⁻¹⁰ which controls various phenomena
26 including tissue morphogenesis,^{11,12} epithelial-mesenchymal transition,⁵ wound healing and tu-
27 mor progression.¹³ Cells are active systems, engines that operate away from thermal equilib-

28 rium, transducing chemical energy into motion. Single isolated cells generate contractile force
29 dipoles: the resultant of the forces due to actomyosin contraction, pulling on focal adhesion
30 sites on the substrate, is typically a pair of approximately equal and opposite forces acting
31 inwards along the cellular long axis¹⁴ (Figure 1a). It is reasonable to expect that contractile
32 particles also generate contractile behaviour in the monolayer.¹ However, at the collective cell
33 level, epithelial monolayers display extensile behaviour² i.e. the net force from the neighbours
34 and substrate interaction act to elongate the cell further along its long axis (Figure 1b inset).
35 This immediately poses the question of how such a crossover occurs as the emergence of such
36 differences in active behaviour may be crucial in understanding biological processes such as
37 tissue homeostasis, cell competition and self organization.¹⁵

38 The extensility or contractility within cell populations are based on force balance as shown in
39 Figure 1b and this can be determined by looking at the structure of flow fields around topological
40 defects. Topological defects are singular points in the orientation field of the cell monolayers,
41 where the orientation of cells were defined as the direction of their long axis (see Methods).
42 Having identified the orientation of cells, we use the winding number parameter to identify
43 the location of topological defects, using an automated defect detection method.² In a cellular
44 monolayer two types of topological defects predominate: comet-shaped defects and trefoils
45 (Figure 1c), which correspond to topological defects in nematic liquid crystals with charges
46 $+1/2$ and $-1/2$, respectively.^{1-4,16}

47 Of relevance here in active systems, the active nature of cells results in a directed motion of the
48 comet shaped defects. For extensile systems, the defects move in the direction of the head of
49 the comet, while topological defects in contractile systems move towards the comet tail (Fig-
50 ure 1b). Thus, we measured the average flow field around the comet defects in Madin-Darby
51 Canine Kidney Wild-Type (MDCK WT) monolayers using Particle Image Velocimetry (PIV).

52 The flow and orientation field were obtained from time lapse imaging after they reached con-
53 fluency and before the cells became isotropic as the monolayers grew too dense. The results
54 show clearly that the comet-shaped defects move in the tail-to-head direction (Figure 1d,e, Sup-
55 plementary Figure 1a, c, Video 1), indicating that at a collective level the MDCK monolayer
56 behaves as an extensile active system despite forming contractile dipoles at a single cell level
57 (Figure 1a). The extensile behaviour of comet-shaped defects has been recently reported for
58 Human Bronchial Epithelial Cells (HBEC) as well,³ indicating it to be a property of epithelial
59 monolayers. By contrast, the flow field around comet shaped defects in a monolayer of fibrob-
60 lasts has an opposite flow direction - from head-to-tail of the comet - indicating that fibroblasts
61 behave as a contractile system at the collective level (Supplementary Figure 2a), in agreement
62 with previous studies.¹ This difference in the direction of motion of defects is also reflected
63 in the patterns of strain rates around the defects. While strain rate along the tail-to-head direc-
64 tion (yy -strain rates) show negative values at the head of a comet-shaped defect in MDCK WT
65 monolayers indicating the presence of compression (Figure 1d), this is reversed for a monolayer
66 of fibroblasts, where the yy -strain rate at the defect head is positive, indicating extensional de-
67 formation (Supplementary Figure 2a). But what causes epithelial cells to behave as an extensile
68 system at the collective level, and mesenchymal cells as a contractile system, and what are the
69 consequences during tissue organization are not well understood.

70 One fundamental difference between epithelial and mesenchymal cells is the ability of epithe-
71 lial cells to form strong cell-cell adhesions through E-cadherin based junctional complexes,
72 responsible for active intercellular force transmission.¹⁷ In order to discern the origin of ex-
73 tensile behaviour at a collective level, we performed laser ablation experiments on MDCK WT
74 monolayers (Supplementary Figure 3a,b), where we observed higher recoil at shorter junctions
75 in comparison to long junctions. These results highlight that the smaller cortical tension along
76 long junctions gives rise to a tension distribution that leads to an extensile stress on the cell fur-

77 ther elongating it. We therefore asked if weakening this intercellular adhesion in epithelial cells
78 results in a (mesenchymal-like) contractile behaviour at the collective level. To test this, we in-
79 activated the E-cadherin gene in MDCK cells using CRISPR-Cas9 which was validated through
80 immunostaining and western blot analysis (Supplementary Figure 4a,b). MDCK E-cadherin
81 Knock-Out (E-cad KO) cells can still maintain their contacts through another form of cadherin
82 (cadherin 6),¹⁸ albeit with a significantly weaker adhesion strength as observed through the re-
83 duced level of β -catenin at adherens junctions (Supplementary Figure 4a,b), while still being
84 able to form tight junctions (Supplementary Figure 4a). Strikingly, in these E-cad KO monolay-
85 ers, the average flow field around comet defects switches direction compared to WT monolayers
86 (Figure 1d and e, Supplementary Figure 1a,d, Video 2), indicating a contractile behaviour at the
87 collective level similar to that of fibroblasts where the comet shaped defects move towards the
88 tail direction (Supplementary Figure 2). This change in direction of the flow field around the
89 defect was accompanied by changes in the average strain rate patterns which are positive (ex-
90 tensile deformation) around the head of a comet shaped defect in E-cadherin KO monolayers
91 in comparison to WT monolayers where the strain rate is negative (compressive deformation)
92 around the head of the defect (Figure 1d). Therefore, epithelial monolayers behave as an ex-
93 tensile system due to the presence of strong cell-cell adhesions and loosening this adhesion by
94 removing E-cadherin results in a contractile behaviour.

95 In order to check that this switch from extensile to contractile behaviour is not only specific
96 to MDCK cells, we further validated the results by perturbing cell-cell contacts in the human
97 breast cancer cell line MCF7A, where depleting E-cadherin by RNAi changed the behaviour
98 from an extensile to a contractile system (Supplementary Figure 5). We then validated that
99 this switch was not a clonal effect by re-expressing E-cadherin which restored collective ex-
100 tensile behaviour to MDCK E-cad KO cells (Supplementary Figure 6a). Moreover, the total
101 defect density within the monolayer of MDCK WT and MDCK E-cad KO cells did not reveal

102 changes in the density of defects between WT and E-cad KO monolayers (Supplementary Fig-
103 ure 6b) indicating that the average distance between the defects and defect-defect interactions
104 are not affected by E-cadherin removal. Furthermore, measuring average flows around $-1/2$
105 (trefoil) defects did not show any significant difference between WT and E-cad KO monolayers
106 (Supplementary Figure 2b, and 6c). This is consistent with both simulations (Supplementary
107 Figure 6d) and theories of active nematics,^{19,20} which show that difference in activity affects the
108 self-propulsion of $+1/2$ defects, while not altering the velocity field of $-1/2$ defects. Indeed,
109 comparing the Mean-Square-Displacement (MSD) from defect trajectories in WT and E-cad
110 KO monolayers clearly indicates that while $+1/2$ defects have propulsive behaviour and move
111 faster in WT monolayers, the motion of $-1/2$ defects is diffusive in both conditions (Supple-
112 mentary Figure 6e).

113 We next checked whether the extensile to contractile crossover could be the impact of a change
114 in the behaviour of individual cells. However, based on our traction force data, both single
115 isolated WT and single isolated E-cad KO cells showed contractile behaviour with the forces
116 directed inwards along their elongation axes as cells pulled on the substrate (Figure 1a). This
117 indicates that removing E-cadherin does not change the contractile pattern (intracellular stress)
118 of single cells (Supplementary Figure 7a). Therefore, the change from contractile to extensile
119 behaviour at the collective level can be linked to the presence of E-cadherin which mediates
120 force transmission between neighbouring cells through intercellular interactions.

121 In order to better discern the competition between intracellular contractile stresses (generated
122 by the actomyosin machinery throughout the cell) and the intercellular stresses (due to neigh-
123 bour interactions), we varied these two stresses independently using a cell-based model. The
124 model is based on a phase-field formulation²¹ that captures the deformation of individual cells,
125 and has recently been shown to reproduce the formation of topological defects in MDCK mono-

126 layers, along with their associated flow field and stress patterns.²² In a similar manner as in the
127 experimental analysis, where the orientation of cells were identified through their long axis, in
128 the model a shape tensor, S , characterizes the magnitude and direction of cell elongation (Fig-
129 ure 2a). This parameter continuously evolves with the deformation of cells as they push/pull
130 on their neighbours within the monolayer. Following our recent work,²² intercellular stresses
131 are defined to be proportional to the shape tensor which allows us to model extensile stresses
132 at the cell-cell contacts (Figure 2a). This form of modeling was inspired by previous studies
133 on adherens junctions and actomyosin interaction which showed that force transduction at the
134 junction can modify the actomyosin network and in turn the cell shape²³ and was experimen-
135 tally validated on MDCK WT monolayers through laser ablation experiments where shorter
136 junctions were under higher tension (higher recoil velocity) in comparison to longer junctions
137 which were under lower tension (lower recoil velocity) (Supplementary Figure 3a,b). In ad-
138 dition, an intracellular stress is defined to mimic internal stresses generated by acto-myosin
139 complexes within the individual cells (see Methods for the details of the model). The effect
140 of E-cadherin removal is thus captured in the model by tuning down the intercellular stresses.
141 Just as in the experiments both comet-shaped and trefoil topological defects ($+1/2$ and $-1/2$
142 charges, respectively) are found in the orientation field of the monolayer (Figure 2b) and the
143 average flow fields and strain rate maps around comet shaped defects match those measured
144 for the WT cells (Figure 2c). More importantly, we found that lowering intercellular stresses
145 switched flow direction around comet-shaped topological defects and strain rates in agreement
146 with experimental results of E-cad KO (Figure 2b and c). Quantitative analysis of the simu-
147 lations showed that reducing the intercellular stresses results in slower dynamics characterized
148 by a smaller root mean square (rms)-velocity (Figure 2d) and generates less correlated patterns
149 of motion characterized by a smaller velocity correlation length (Figure 2e). Moreover, due to
150 the dipolar symmetry of intercellular stresses in the model, simulation results predict that the

151 switch from extensile to contractile behaviour does not alter the isotropic stress patterns, i.e.,
152 tension (positive isotropic stress) and compression (negative isotropic stress) around the defects
153 (Figure 3a), when intercellular stresses are reduced.

154 To test the predictions of the model we first experimentally studied the effect of E-cad KO on the
155 stress patterns around topological defects and collective motion of cells. Using traction force
156 microscopy, we obtain traction forces in the monolayer, from which we infer the associated
157 stress patterns using Bayesian Inversion Stress Microscopy (BISM).²⁴ Using a similar approach
158 as strain rate measurements around defects, we are able to compute the average stress fields
159 around comet shaped defects. Our experiments agreed with the simulations in showing no
160 difference in the average isotropic stress patterns around comet shaped defects between the
161 WT (Figure 3b) and E-cad KO monolayers (Figure 3c), while they still show a difference in
162 their flow field (Supplementary Figure 7b),² indicating that the tension and compression around
163 defects are primarily controlled by local cellular organization and elongation, and not the flow
164 field around them. Moreover, measuring the velocity correlation function,²⁵ we found it to be
165 consistent with the numerical predictions whereby removing E-cadherin reduces the correlation
166 length compared to the WT monolayers (Figure 3d). This is also in agreement with previous
167 reports which demonstrate a reduction in velocity correlation length of mesenchymal cells with
168 respect to epithelial cells.²⁵ Interestingly, by performing rescue experiments to put E-cadherin
169 back, we found an increase in velocity correlation length (Figure 3d) which was very close to
170 that of WT monolayers. This indicates that the perturbation of junctional protein E-cadherin can
171 be used as an effective way of tuning the collective contractility and extensibility of the epithelial
172 monolayer.

173 Comparing the average velocities in the monolayers with and without E-cadherin also agreed
174 with the model's prediction that the velocity of the monolayer is reduced upon E-cadherin de-

175 pletion (Figure 3e) at similar density. Interestingly, traction force microscopy measurements re-
176 vealed that this reduction in velocity is accompanied by a significant (about three fold) increase
177 in the average traction forces that E-cad KO monolayers exert on their underlying substrate
178 in comparison to WT monolayers (Figure 3f). Furthermore, we compared average cell areas
179 within the monolayer for both WT and E-cad KO monolayers and did not notice an appreciable
180 difference in spreading area, although for both WT and E-cad KO monolayers the average cell
181 spreading area reduced over time (Figure 3g). In contrast, the aspect ratio of cells within the
182 WT monolayers reduced over time while the aspect ratio of cells within E-cad KO monolayers
183 did not change over time (Figure 3g). These measurements of velocity reduction, traction force
184 increase, and changes in aspect ratio in the monolayers without E-cadherin, combined together,
185 hinted that the cell-substrate interaction increased as the cell-cell interaction was weakened,
186 indicating a possible cross-talk between intracellular and intercellular interactions as reported
187 previously²⁶⁻²⁹.

188 To test this, and based on previous studies that showed changes in cellular response to substrate
189 adhesions^{30,31}, we asked if the increase in the average traction force of E-cad KO monolay-
190 ers was a result of changes in their mechanotransductory response. Using actin staining we
191 first checked for changes in the organization of stress fibers in the cells within a monolayer,
192 as stress fiber formation is an important determinant of force generation by cells on a sub-
193 strate.^{32,33} Indeed, comparing actin staining of WT and E-cad KO monolayers, we found a
194 considerable increase in stress fibers in the absence of E-cadherin (Figure 4a). Concomitantly,
195 phosphomyosin staining of WT and E-cad KO monolayers showed an increase in the number
196 of phosphomyosin light chain (pMLC2) fibers (Figure 4a) generated at the basal surface within
197 E-cad KO cells. Western blot analyses further revealed an increase in the total level of myosin
198 light chains (MLC2) (Supplementary Figure 4c). Considering these results we reasoned that
199 inhibiting cell contractility in E-cad KO cells may alter their active behaviour. Upon treatment

200 with a mild dose of blebbistatin (5 μ M), an inhibitor of Myosin II (Supplementary Figure 8a)
201 E-cad KO monolayers still behave as a contractile system. However, a higher dose (20 μ M)
202 of blebbistatin (Supplementary Figure 8b) or 25 μ M of Y27632, an inhibitor of ROCK 1 and
203 2 (Supplementary Figure 8c), resulted in a switch in behaviour from a contractile to that of
204 an extensile system as summarized in Table 1. As control, we showed that similar treatments
205 did not affect the extensile behaviour of the WT monolayers (Table 1, Supplementary Fig-
206 ure 8d and e). We then measured the traction forces exerted by cells when treated with 20
207 μ M blebbistatin. As reported previously,³⁴ treatment of both WT and E-cad KO monolayers
208 with 20 μ M of blebbistatin results in a drastic reduction of traction forces (Figure 4b). This
209 reinforces the importance of cell substrate interaction in dictating the contractile behaviour of
210 E-cad KO monolayers. Thus, removing E-cadherin not only reduces the extensile intercellular
211 stresses, it also increases the intrinsic contractility (intracellular stress) generated by cells at the
212 cell-substrate interface.

213 Since focal adhesions (FAs) are known to be mechanosensors at cell-matrix interface,³⁵ we then
214 investigated the assembly of FAs in E-cad KO and WT monolayers. By using paxillin staining
215 to determine changes in FAs, we showed a marked increase both in length, and area within
216 the cells (Figure 4c) in the E-cad KO monolayers in comparison to the WT monolayers. More
217 importantly, we found that the E-cad KO modified the subcellular localization of vinculin, a
218 protein which is known to respond and transmit force from both integrin and cadherin based
219 adhesion complexes.^{36,37} While the total level of vinculin remained unchanged in both WT
220 and E-cad KO monolayers (Supplementary Figure 4d), the localization of vinculin was altered,
221 whereby vinculin was mostly present at the cell-cell junctions in WT monolayers, but basally
222 located in E-cad KO monolayers (Figure 4d). We further verified if all paxillin positive focal
223 adhesions were vinculin positive in both WT and E-cad KO monolayers and observed a strong
224 correlation between them (Pearson's coefficient of 0.8842 and 0.8843 for WT and E-cad KO) as

225 shown in Supplementary Figure 9, reiterating our observed increase in cell-substrate interaction
226 in the absence of E-cadherin.

227 Since Yes-associated protein (YAP) transcriptional activity is also known to modify cell me-
228 chanics, force development and FA strength,^{38,39} we investigated the localization of YAP within
229 E-cad KO monolayers. Interestingly, we found that YAP was predominantly localized to the nu-
230 cleus in E-cadherin KO monolayers (Figure 4e), which corresponds to the active state of YAP.
231 This is in agreement with previous studies that reported an activation of YAP through nuclear
232 accumulation in the absence of E-cadherin or in well spread cells.^{30,40,41} Taken together, our
233 results show that removing E-cadherin enhances the formation of stress fibers, promotes YAP
234 activation, alters viculin localization, and leads to a marked increase in the formation of focal
235 adhesions and their linkage to the substrate, in turn triggering a contractile behaviour.

236 Our force measurements together with acto-myosin activity and adhesion patterns establish that
237 the extensile or contractile nature of epithelial cells at a collective level relies on the interplay
238 between active stresses at cell-cell and cell-matrix interfaces. To further explore this crossover
239 we plated cells on a soft (2.3 kPa) polyacrylamide (PA) gels, recalling that cellular responses on
240 soft substrates leads to lower contractility and less stable focal adhesions.⁴² MDCK WT mono-
241 layers remained extensile regardless of substrate stiffness (Supplementary Figure 10a), while
242 E-cad KO cells switched from contractile to extensile behaviour on a soft substrate (around 2.3
243 kPa) (Supplementary Figure 10b). Taken together, these experiments show that tuning cell-cell
244 and cell-substrate adhesion can result in a switch between extensile and contractile behaviour
245 of cell monolayers further validating our observatin that blebbistatin treatment drastically re-
246 duced traction forces (Figure 4d) and switched the behaviour of E-cad KO monolayers from
247 contractile to extensile. It is possible in the simulations to further explore this crossover by con-
248 tinuously varying the strength of intra- and inter-cellular stresses, independently. The results are

249 summarized in a stability-phase diagram that classifies the monolayer behaviour as extensile or
250 contractile based on the direction of the defect motion (Supplementary Figure 11a). The non-
251 symmetric structure of the stability-diagram further highlights the different impacts of intra-
252 and inter-cellular stresses on the direction of defect motion. In our simulations, while intracel-
253 lular stresses act within single cells and are along the direction of cell polarity, the intercellular
254 stresses arise in between neighboring cells and are proportional to the cell deformation. As
255 such, intercellular stresses can reinforce themselves: small cell deformations lead to intercellu-
256 lar stresses that further enhance cell deformation, generating stronger intercellular stresses. We
257 conjecture that this bootstrap mechanism results in intercellular stresses to more strongly affect
258 the collective behavior of the monolayer compared to their intracellular counterparts.

259 Based on this difference in contractile and extensile behaviour we then used the model to sim-
260 ulate the interaction between the extensile and contractile systems. The results showed that
261 cells were able to separate out into two different phases over time when mixed at 50-50 ratio
262 (Figure 5a and Supplementary Figure 11b, Video 3), where extensile cells were surrounded by
263 contractile ones. We were able to replicate this experimentally (Figure 5b and Supplementary
264 Figure 11c, Video 4) whereby WT and E-cad KO cells separate out into two different phases
265 with WT (extensile) cells surrounded by E-cad KO (contractile) cells when plated at a 50-50
266 ratio (Figure 5a). While thermodynamic mechanisms such as differential adhesion and differ-
267 ence in line tension between two cell types have been shown to govern phase separation in 3D
268 cell aggregates,⁴³⁻⁴⁵ active cell sorting in monolayers with strong substrate adhesion, has not
269 been directly observed to the best of our knowledge. We, therefore, sought to further explore
270 the possible distinctions between the cell sorting, as observed here, and the well-established
271 differential adhesion and differential line tension hypotheses. To this end, we first quantified
272 the degree of phase separation by measuring the mixing-index of a mixture of WT and E-cad
273 KO cells defined as the number of homotypic neighbours over the total number of cells.^{46,47}

274 In the segregation mechanism based on differential line tension this mixing-index grows with
275 a power-law exponent with time and approaches one.⁴⁷ However, as evident from both experi-
276 ments and simulations, the mixing-index in our system saturates and complete phase separation
277 is never obtained (Figure 5a and b). We conjecture that this is partly because of strong cell-
278 substrate adhesion that dominates over any possible difference in line tensions and also due to
279 a fundamental difference between activity-driven phase separation and thermodynamic mecha-
280 nisms. In addition, phase separation based on differential line tension posits that – independent
281 of the asymmetry of the binary mixture - the phase with higher line tension always forms ag-
282 gregates that are enveloped by the cells with lower line tension to minimize the free energy of
283 the mixture.^{43,45}

284 To test this, we performed mixing experiments by varying the percentage of WT versus E-cad
285 KO cells, (30/70 and 70/30, respectively; Figure 6a and Supplementary Figure 11d,e). In the
286 latter case, we could even observe E-cad KO colonies surrounded by WT cells which could not
287 be simply explained by the differential adhesion hypothesis and was not observed in previous
288 adhesion based studies governed by cortical/line tension.^{43,45-49} We were able to replicate this
289 in our simulations (Figure 6b). Moreover, to further test the unmixing phase we thought to
290 probe the unmixing of two cell types with and without E-cadherin, but both showing extensile
291 behaviour. Since 20 μ M blebbistatin was shown to reverse the contractile behaviour of E-cad
292 KO monolayers from contractile to extensile (Table 1 and Supplementary Figure 8), we treated
293 a mixture of WT and E-cad KO plated at 50/50 ratio with blebbistatin after unmixing. Upon
294 blebbistatin treatment, we see a drop in the mixing index (Figure 6c, Video 5). In addition, the
295 clear boundaries formed in an untreated sample were lost characterized by the loss of circularity
296 of WT colonies upon blebbistatin treatment (Figure 6c).

297 Taken together, these results reinforce the fundamental distinctions between phase separation

298 in systems with differences in activity in comparison to well-established differential line ten-
299 sion or differential adhesion mechanisms. Even though tissue segregation was first exemplified
300 based on differences in cadherin-mediated surface tension,^{43,48,50} it was later shown that inter-
301 cellular adhesion is not the only mechanism that triggers cell sorting.⁵¹ Theoretical predictions
302 have suggested that cell sorting could be driven by a combination of cell surface tension and
303 contractility.^{45,46} While, we cannot completely rule out the contribution of differential adhesion
304 or differential line tension towards the sorting between WT and E-cad KO cells, our results
305 clearly demonstrate the importance of cell-substrate interaction and intracellular stresses as key
306 regulators of cell sorting in cellular monolayers with strong adhesion to substrate.

307 The results presented in this work show that epithelial cells are able to maintain their collective
308 behaviour through a coordination of intercellular and intracellular stresses. Intercellular stresses
309 are mediated through adherens junctions, while intracellular stresses could be mediated through
310 changes in substrate interaction and actomyosin machinery. Using a combination of in-silico
311 modelling and extensive experimental studies we have shown that perturbation of E-cadherin in
312 MDCK cells, increases their substrate interaction in addition to changing their active nematic
313 behaviour from extensile (WT) to contractile (E-cad KO) similar to a monolayer of fibroblast
314 which behaves as a contractile unit. Our experimental results also show that perturbation of
315 adherens junctions are accompanied by molecular level changes, including reduced levels of
316 vinculin at cell-cell contacts, together with an increase in focal adhesion size and area in the ab-
317 sence of E-cadherin, and increase in the number of actin stress fibers on the basal layer. While,
318 using our numerical model we were able to study how varying inter and intracellular stresses
319 impacts the active behaviour of cells. In addition, mixing the two different systems revealed
320 that these differences in active behaviour were sufficient to drive sorting of these domains into
321 an unmixed phase over time. Comparing our observations of sorting with previously observed
322 studies and hypothesis⁴³⁻⁴⁵ highlights fundamental distinctions that arise due to the difference

323 in the nature of active forces. These observations bring in a new understanding to the existing
324 models of differential adhesion. Having understood the role of extensility and contractility in
325 dictating demixing (sorting) of cells, this approach could be expanded to studying other biolog-
326 ical processes such as tissue growth, development and tissue homeostasis. For instance, recent
327 studies demonstrated the importance of nematic organization of actin cytoskeleton in Hydra
328 during morphogenesis,⁵² while other studies have begun to explore the role of liquid-crystal
329 ordering during morphogenesis⁵³ and *in – vivo* epithelial tissue patterning.⁵⁴ These findings
330 highlight the importance of active nematic behaviours at a collective level to understand tis-
331 sue shape and organization, factors central to morphogenesis.^{52,53,55–58} As such, the adaptation
332 of cellular systems from extensile to contractile behaviours might be a crucial mechanism by
333 which a collective living system undergoes morphological changes (sorting or tissue organiza-
334 tion) based on a transition from a cohesive to a less coordinated organization. Such a transition
335 relying on the cross-talk between cell-cell and cell-matrix interactions may provide a new mech-
336 anism to understand cell migration during development, wound healing, and collective cancer
337 cell invasion.

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358 **Author contributions**

359 L.B, T.B.S, A.D, J.Y, R.M.M and B.L designed the research. G.H.N.S.N and T.D developed
360 the MDCK E-cadherin KO cell line. L.B performed experiments and analysed the results. S.S
361 helped in the PA gel experiments. M.T performed and quantified laser ablation experiments.
362 S.G performed the MCF7A experiments. A.D, R.M, performed the in silico simulations. T.B.S
363 contributed to the analysis tools. A.S.Y, Y.T, R.M.M, A.D, J.Y, and B.L supervised the project.

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367 **Code Availability**

368 Nematic analysis was performed using a custom made code which has been published in Saw
369 et al, Nature 2017. This is available upon request. Numerical analyses were performed using a
370 custom made code "CELADRO: Cells as Active Droplets", which is an open source code that
371 we have deposited on GitHub (<https://github.com/rhomu/celadro>). All the analysis tools are
372 available upon request.

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Figure 1

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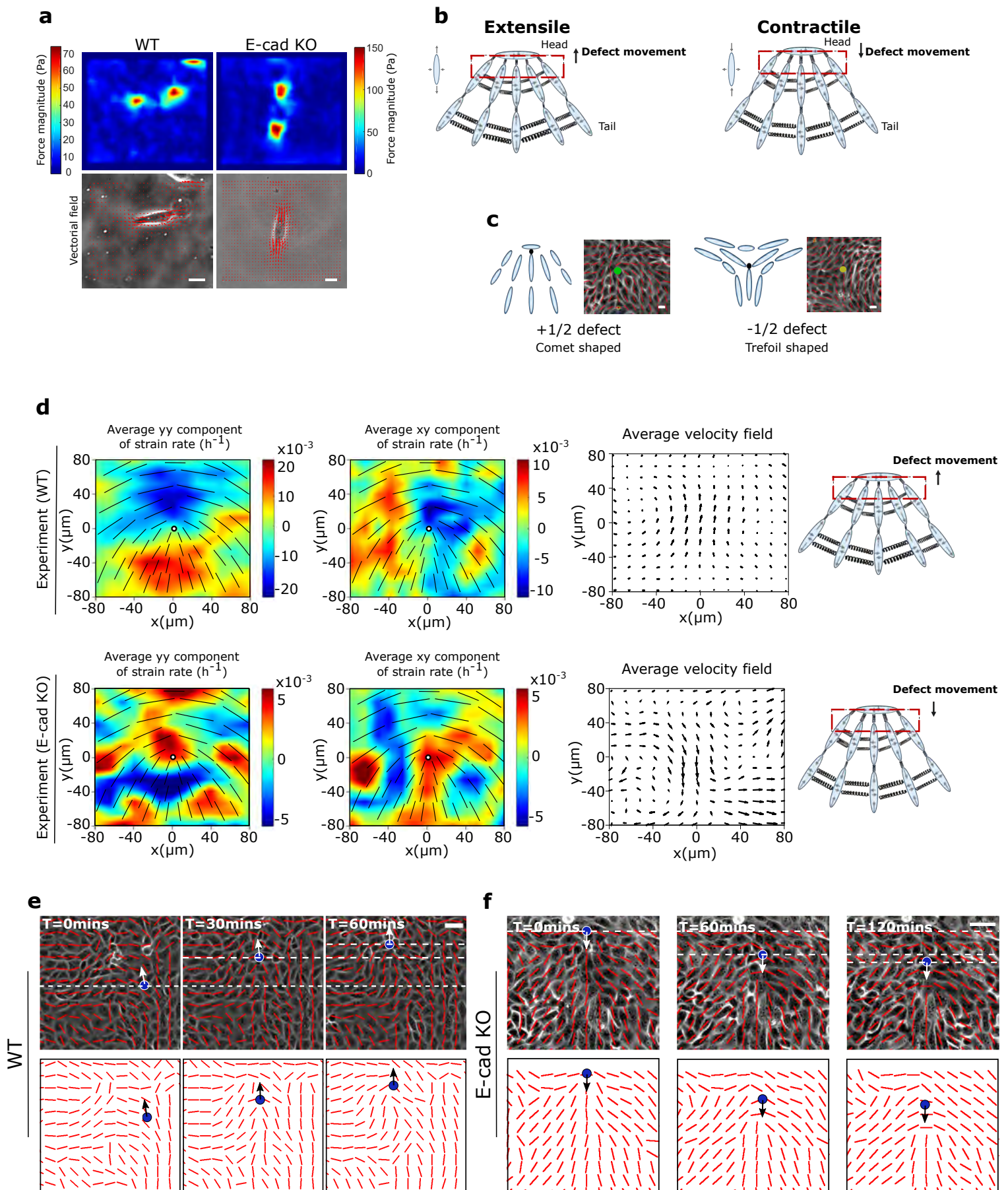


Figure 1| Active nematic behaviour of epithelial cellular systems changes from extensile to contractile in the absence of E-cadherin. a) Top, left and right: typical examples of traction force magnitude maps for a single MDCK WT and E-cadherin KO cell cultured on deformable PDMS surfaces. Bottom, left and right: vectorial maps of traction forces for a single MDCK WT and E-cadherin KO cell on a soft PDMS substrate. Scale bars, $20\mu\text{m}$. b) Schematic showing the defect movement based on force balance for an extensile active nematic system (left) and contractile active nematic system (right) with an inset of forces exerted on neighbours by an extensile (left) and contractile (right) nematic particle. c) Schematic (left) and experimental (right) images of $+1/2$ defect (left, comet configuration) and $-1/2$ defect (right, trefoil configuration). Scale bars, $20\mu\text{m}$. d) Average yy - and xy -components of strain rate map around $+1/2$ defect obtained from experiments (left and middle respectively) and corresponding average flow field (right) for MDCK WT cells (top) ($n = 1934$ defects from 2 independent experiments) and MDCK E-cadherin KO cells (bottom) ($n = 1,884$ defects from 2 independent experiments). Schematic on the extreme right illustrates the movement of defects. Colour code is positive for stretching and negative for shrinkage. e, f) Experimental data for MDCK WT (e) and MDCK E-cadherin KO (f) monolayers. Top panels: phase contrast images of the cells overlaid with the average local orientation of the cells (red lines). Bottom panels: average local orientation of the cells (red lines). The blue circle shows the location of a $+1/2$ defect and the corresponding arrow indicates the direction of motion of this defect over time. Dashed lines have been added for better reading of defect movement. Scale bars, $40\mu\text{m}$.

Figure 2

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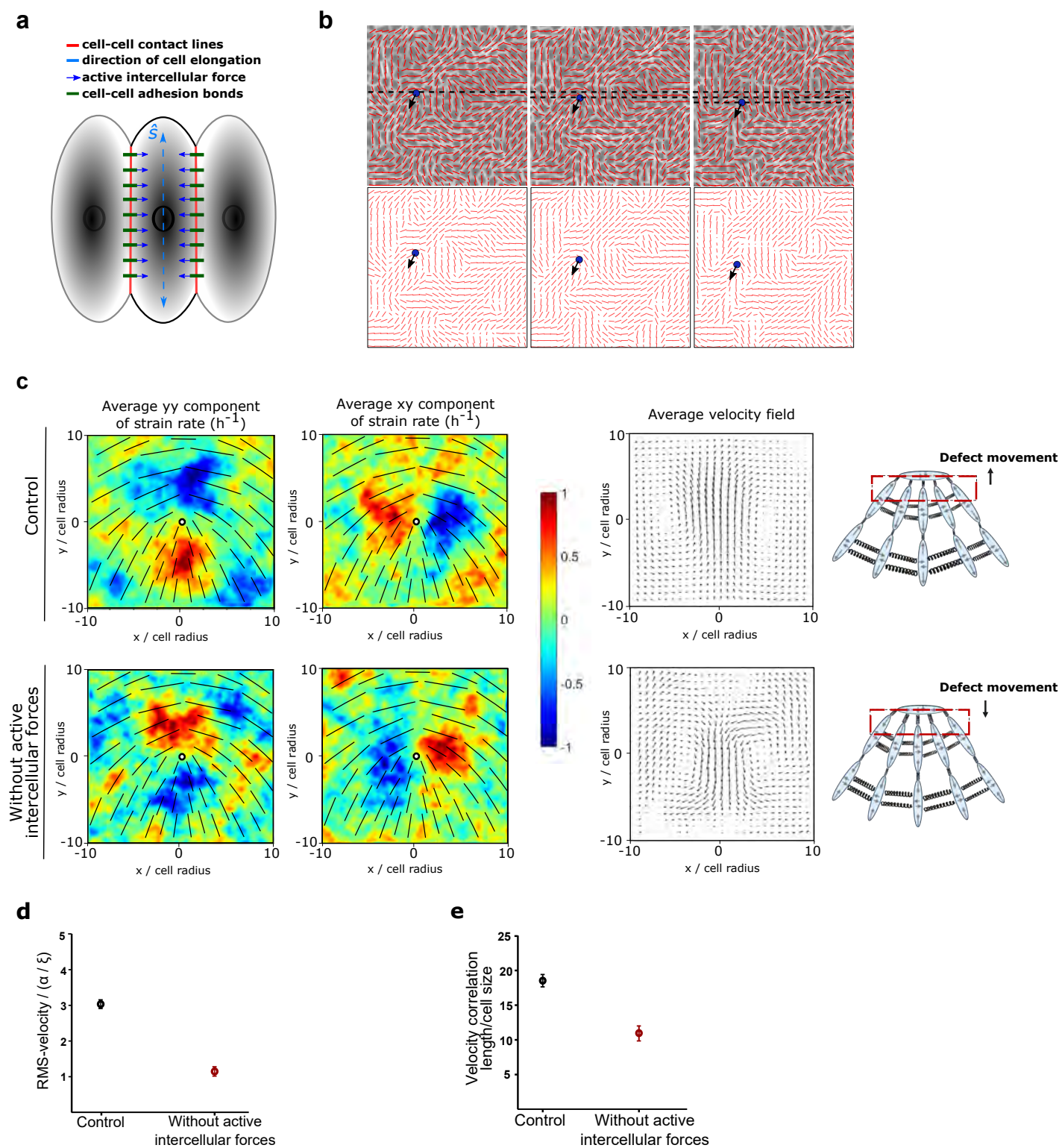


Figure 2| Intercellular stresses change the contractile behaviour of a 2D nematic system a) Schematic illustrating the model used in numerical simulations which incorporates cell-cell interaction through active intercellular forces. The direction of cell elongation is denoted by the headless vector \hat{s} , which is found from the eigenvector corresponding to the largest eigenvalue of the shape tensor \mathbf{S} for each cell. b) Numerical simulations for the case without active intercellular stresses, showing: Top, phase contrast images of the cells overlaid with the average local orientation of the cells (red lines). And bottom, average local orientation of the cells (red lines). The blue circle shows the location of a $+1/2$ defect and the corresponding arrow indicates the direction of motion of this defect over time. c) Average yy - and xy -components of strain rate map around $+1/2$ defect obtained from simulations (left and middle respectively) and corresponding average velocity flow field (right: $n = 2,083$ defects) for the control condition (top) and the condition without active intercellular forces. Colour code is positive for stretching and negative for shrinkage. d) RMS velocity, and e) the velocity correlation length in the monolayer normalized to the individual cell size obtained from $n=30$ different simulations for the control condition and the condition without active intercellular forces.

Figure 3

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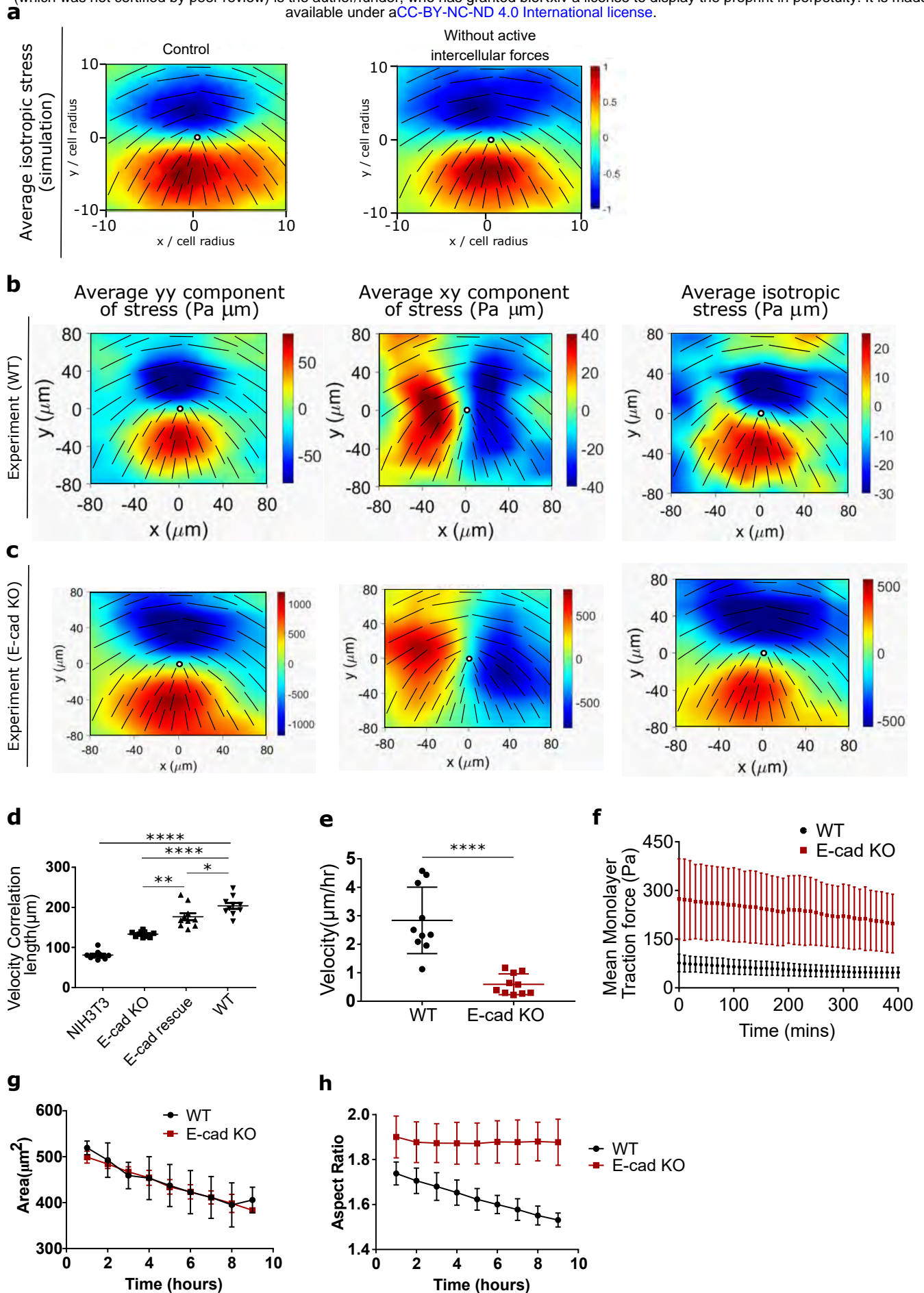


Figure 3| Knocking out E-cadherin increases cell-substrate interactions. a) Average isotropic stress around a $+1/2$ defect obtained from simulations for the control condition (left) and condition without intercellular forces (right) ($n = 2,083$ defects). b,c) Average yy (left)-, xy (middle)- and isotropic (right) components of stress around a $+1/2$ defect obtained from experiments for (b) MDCK WT ($n = 1,899$ defects) and (c) E-cadherin KO ($n = 1,428$ defects) from 2 independent experiments. For a and b colour code represents the strength of the stress with positive for tensile state, negative for compression. d, e, f) velocity correlation length (d) ($n=10$), velocity (e) ($n=10$) and mean traction force (f) ($n=12$) of cells within a monolayer for both MDCK WT and MDCK E-cadherin KO cells. g, h) Cell spreading area (g) and aspect ratio (h) of cells within the monolayer obtained from $n=10$ different images for MDCK WT and E-cadherin KO cells as a function of time from 2 independent experiments. The error bars represent the standard deviation. Unpaired t-test was performed resulting in $*p<0.05$, $**p<0.01$, $***p<0.001$ and $****p<0.0001$.

Figure 4

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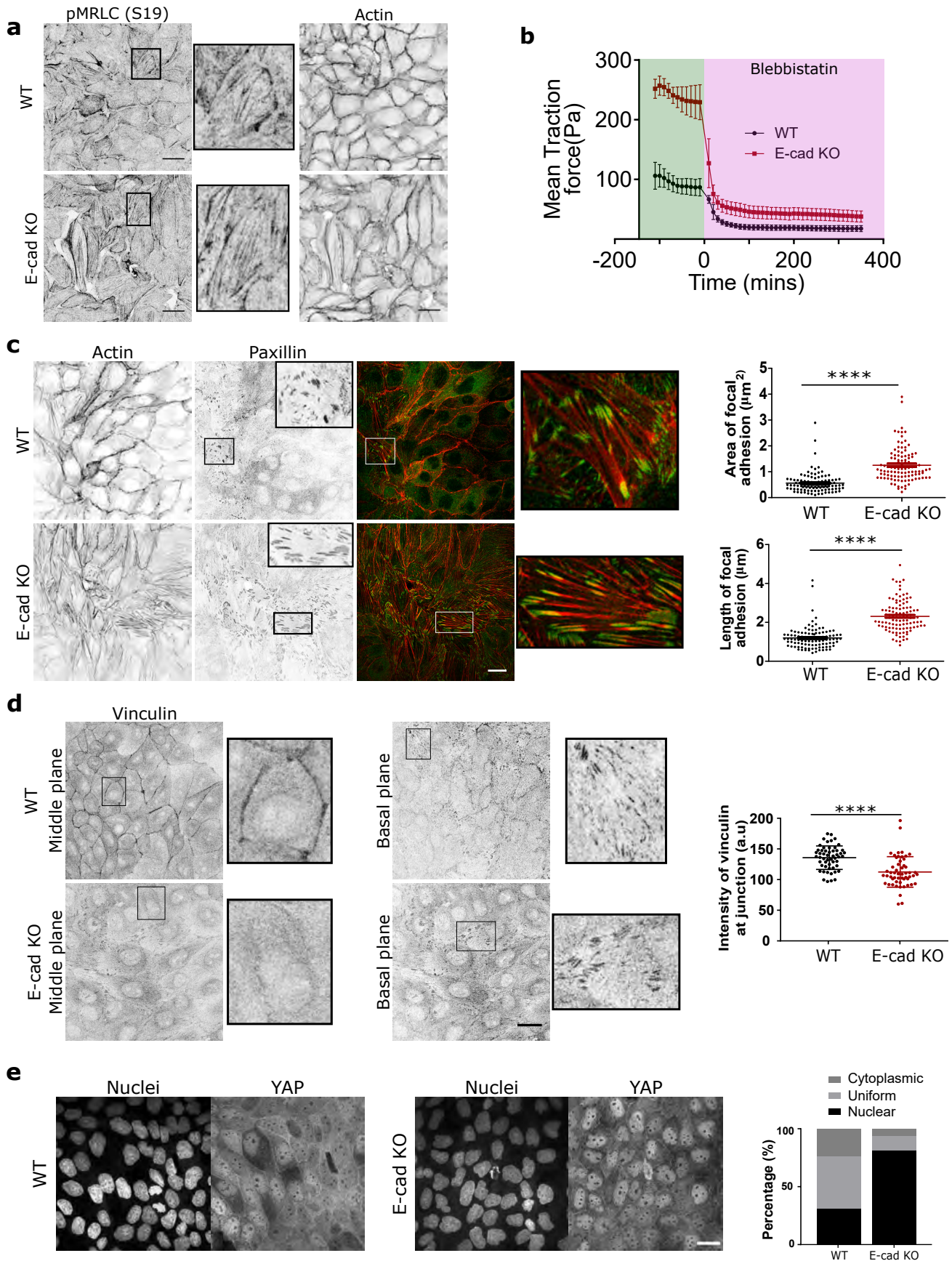


Figure 4| E-cadherin removal triggers mechanotransductive changes within the monolayer. a) pMRLC (left), zoom of pMRLC (middle), actin (right) staining of MDCK WT (top) and E-cadherin KO (bottom) monolayers. b) Evolution of mean traction force of MDCK WT and E-cadherin KO monolayers before and after 20 μ M blebbistatin treatment ($n=10$ from 2 independent experiments). c, d, e) actin and paxillin (c), vinculin (d), YAP (green), and nucleus (blue) (e), staining within a monolayer for both MDCK WT and E-cadherin KO cells. c) Area of focal adhesion (left) and length of focal adhesion within the monolayer for $n=106$ focal adhesions. d) Mean intensity of vinculin at the cell-cell junction in the middle plane ($n=54$). e) Distribution of YAP in nucleus, cytoplasm, or uniform distribution calculated for $n=1162$ cells (MDCK WT) and $n=1008$ cells (MDCK E-cadherin KO). Error bars represent the standard deviation. Unpaired t-test was performed leading to $*p<0.05$, $**p<0.01$, $***p<0.001$ and $****p<0.0001$. Scale bars, 20 μ m.

Figure 5

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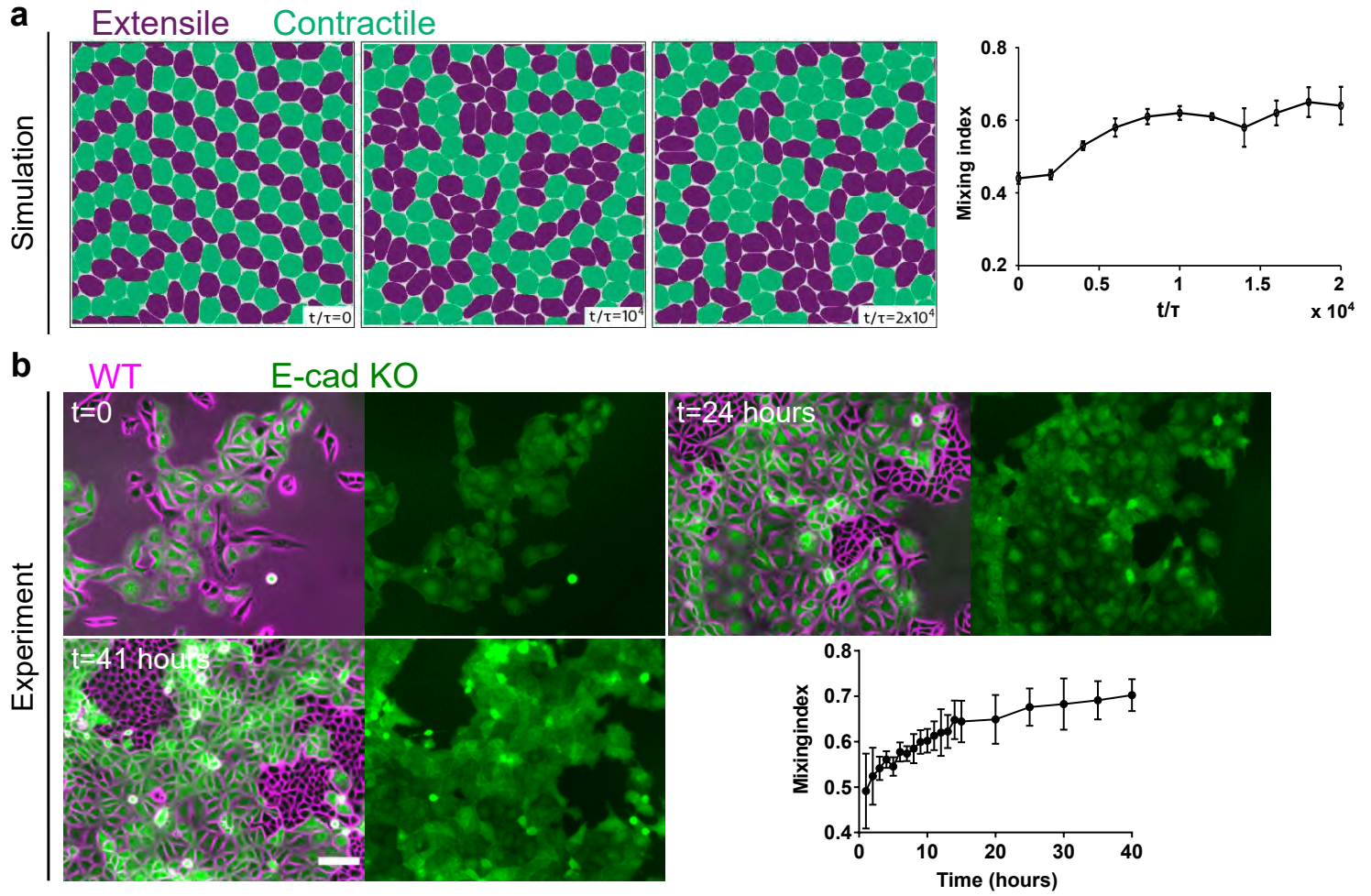


Figure 5| Cell sorting triggered by change in nematic behaviour of monolayers. a,b) Time lapse sorting of extensile and contractile cells observed over time represented by mixing index in simulations (a) and experiments (b) of MDCK WT (magenta) and E-cadherin KO cells tagged with LifeAct GFP (green). In (a) $\zeta_s/R\alpha = 0.042$, $\zeta_0/R\alpha = -0.062$ for the extensile cells and $\zeta_s/R\alpha = 0.0$, $\zeta_0/R\alpha = -0.062$ for the contractile cells. Mixing index was obtained from two independent simulations and the error bars mark the standard deviation. Mixing index in experiments (b) was obtained from n=5 different clusters from 2 independent samples. c, d, e, f, g) Demixing (left) and isotropic stress field (right) for different conditions tested in the simulations, line tension (c), steric repulsion (d), adhesion (e), activity based extensile and contractile (f) and experimentally obtained phase separation (left) and isotropic stress field (right). Error bars represent the standard deviation. Scale bars: $100\mu\text{m}$.

Figure 6

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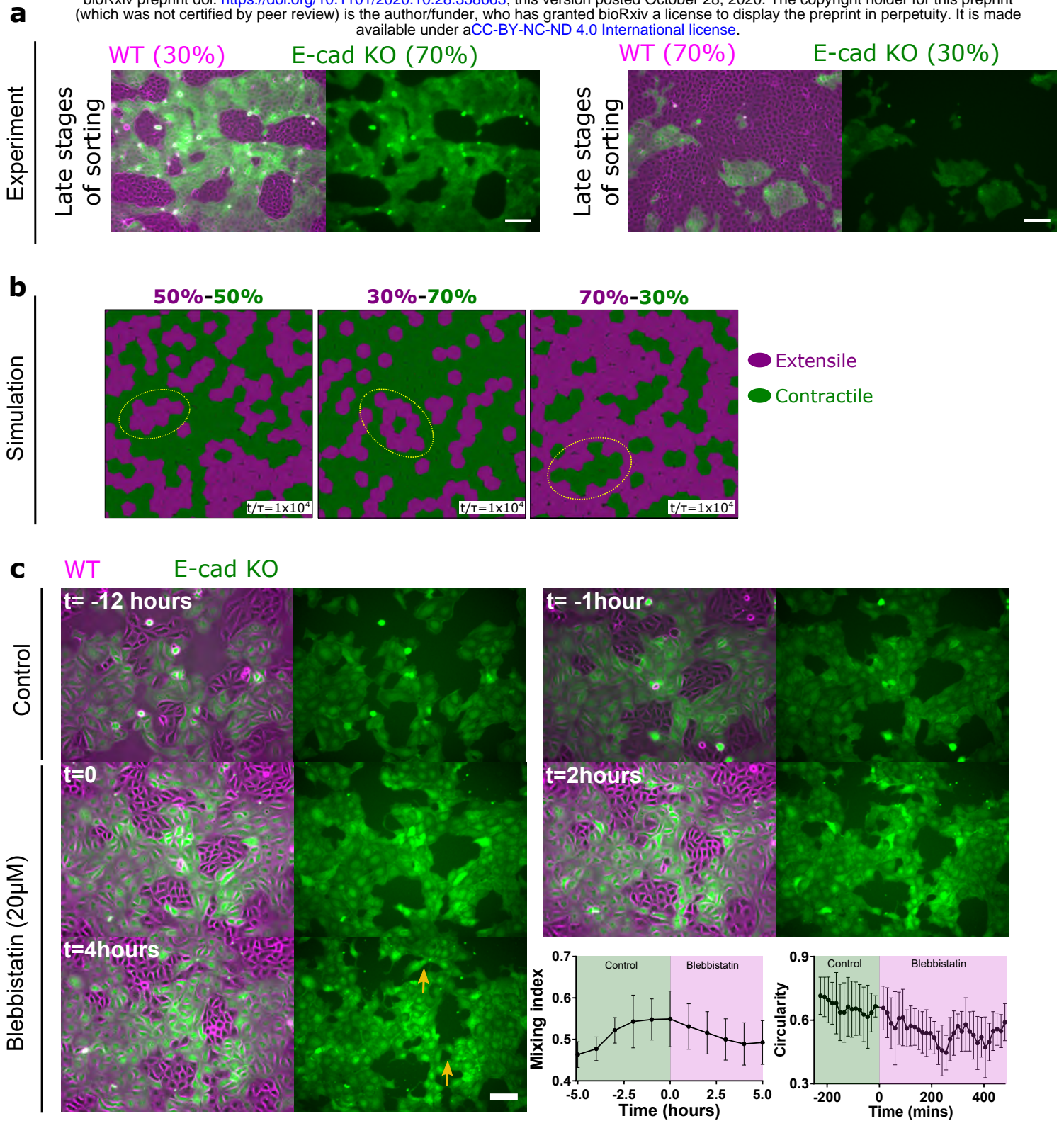


Figure 6| Cell sorting is governed by activity of the system. a) Demixing of MDCK WT and E-cadherin KO at different starting densities, WT (30%) and E-cadherin KO (70%) (left) and WT (70%) and E-cadherin KO (30%) (right). b) Demixing of extensile and contractile particles obtained from simulations at different starting densities. Extensile and contractile particles are mixed at 50-50 (left), 30-70 (middle) and 70-30 (right) respectively. In (b) $\zeta_s/R\alpha = 0.016$, $\zeta_Q/R\alpha = -0.016$ for the extensile cells and $\zeta_s/R\alpha = 0.0$, $\zeta_Q/R\alpha = -0.016$ for the contractile cells. c) Demixing phase observed before and after the addition of 20 μ M blebbistatin characterized by mixing index (left) (n=5) and circularity of several colonies (right) (n=5). Error bars represent the standard deviation. Scale bars: 100 μ m.