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## Adhesion-mediated heterogeneous actin organization governs apoptotic cell extrusion

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### ABSTRACT

Apoptotic extrusion is crucial in maintaining epithelial homeostasis and has implications in diseases of epithelial tissues. Current literature supports that epithelia respond to extrusion to maintain their integrity by the formation of a supracellular actomyosin ring (purse-string) in the neighbors that encompasses the dying cells. However, little is known about whether other types of actin structures could contribute to extrusion as well as how forces generated by mechanosensitive proteins in the cells are integrated. Here, we found that during extrusion, a heterogeneous actin network composed of lamellipodia protrusions and discontinuous actomyosin cables, was reorganized in the neighboring cells and was the main factor driving extrusion forwards. The early presence of basal lamellipodia protrusion participated both in basal sealing of the extrusion site and in orienting the actomyosin purse-string at the later stage of extrusion. These sequential events are essential in ensuring a successful extrusion in apicobasal direction. The co-existence of these two mechanisms is determined by the interplay between the cell-cell and cell-substrate adhesions. A theoretical model integrates the role of these cellular mechanosensitive components to explain why a dual-mode mechanism, which combined lamellipodia protrusion and purse-string contractility, leads to more efficient extrusion than a single-mode mechanism. We anticipate that our approach will be useful to provide mechanistic insight into epithelial homeostasis, morphogenetic events and tumorigenesis.

## 1 INTRODUCTION

2 In adult homeostatic tissues, the epithelia undergo constant turnover with cell division balanced  
3 by cell death [1]. Maintaining integrity requires the removal of dead cells by extrusion to  
4 protect the organ they encase. To achieve this goal, rearrangement of neighboring cells occurs  
5 concurrently with apoptotic cell death to expel the dying cell while maintaining intact  
6 monolayer [2].

7 The purse-string model has been described as the primary extrusion mechanism in  
8 epithelia monolayers. During this process, contractile actomyosin rings are formed by the  
9 extruding cell itself [3, 4] and by the neighboring cells [4-6]. Both intrinsic and extrinsic  
10 apoptotic pathways can elicit extrusion, and caspase activation is essential to activate the Rho  
11 signaling pathway [7], which promotes the actomyosin ring formation in both the extruding  
12 cell and the neighboring cells. In mammalian cells, these rings were suggested to contract  
13 basolaterally and exert an upward force to expel the dying cell and close the gap left behind [4,  
14 5]. There are similarities drawn between the actomyosin contractile ring here and the  
15 supracellular purse-string in the wound healing mechanism [8-10]. Nevertheless, cell extrusion  
16 involves three-dimensional cell-cell junction reorganization at the interface of dying cell-  
17 neighboring interface [11-13], while contractile purse-string in wound healing cases was  
18 described to exert forces in a single plane. Cells adjacent to the extrusion site partially lose  
19 polarity and reorganize their cell-cell junctions and actomyosin network [11, 14]. Thus, the  
20 presence of the extruding cell instead of a void gap, which involves the additional roles of cell-  
21 cell junctions at the interface between dying cells and neighboring cells, distinguishes extrusion  
22 from wound healing. Therefore, how actomyosin purse-string could produce sufficient force to  
23 drive extrusion in three-dimensionally in the polarized epithelia is still ambiguous.

24 Recently, we provided evidence that extrusion of apoptotic cells in epithelia with low  
25 packing density could occur with little purse-string involvement [15]. In this case, neighboring  
26 cells crawl towards extruding site and expel the dying cell via lamellipodia protrusions based  
27 on Rac1-regulated actin polymerization. This result is reminiscent of the wound healing  
28 process when heterogeneous actomyosin networks could concurrently contribute to gap closure  
29 with distinct force signature [16, 17]. Nevertheless, whether the presence of lamellipodia  
30 impedes purse-string formation during extrusion was not clarified in previous studies. Also, as  
31 cells extend lamellipodia, cell-extracellular matrix (ECM) interactions through focal adhesions

32 are remodeled, whether such dynamics of cell-substrate adhesions modulates actin  
33 reorganization during extrusion has not been understood so far.

34 In this study, we sought to determine the relative contributions of cell crawling and cell  
35 contractility to extrusion in relation with cell-cell and cell substrate adhesions. We addressed  
36 these questions using MDCK cell culture as the model and a combination of techniques  
37 including laser-induced cell death, micro-patterning, traction force microscopy, and molecular  
38 biology.

39

## 40 **RESULTS**

### 41 **Extrusion depends on dual-mechanism which combines lamellipodia protrusion and purse-** 42 **string**

43 We monitored the actin organization at extruding cell-neighboring cell interfaces of laser-  
44 induced apoptotic cells from Wild-Type (WT) and fluorescently-labeled actin MDCK cells  
45 cocultured monolayers at high density (40-45 cells per 100 x 100  $\mu\text{m}^2$ ). Thin actin-labeled at  
46 ruffled membrane indicated lamellipodial protrusions and were observed at the early time of  
47 extrusion at the basal plane of neighboring cells in both WT/GFP-actin MDCK cells (Fig. 1a,  
48 b) and WT/Ruby Life-act MDCK cells mosaic cultures (Supplementary Movie 1). Thick  
49 bundles of actin indicating actomyosin cables were also formed in a non-uniform manner in  
50 neighboring cells at the apico-lateral plane (Fig. 1a, b'). These partial apical actomyosin cables  
51 were also observed during naturally-occurring extrusion (Supplementary Fig. 2b). We  
52 validated the presence of lamellipodia by looking at cell extrusions in mosaic cultures of WT  
53 and MDCK expressing YFP-tagged p21 binding domain (PBD), a fluorescent biosensor of  
54 active Rac1 and Cdc42, [18, 19] for both laser-induced cell death extrusions (Supplementary  
55 Fig. 1a, b) and naturally-occurring extrusions (Supplementary Fig. 2a). Enhanced PBD signal  
56 in neighboring cells followed by directional cell edge movement towards the extrusion site  
57 coincided with the rise of caspase-3 signal in the extruding cell (marked by caspase-3 indicator  
58 DEVD-FMK), (Supplementary Fig. 1a & c-d, Supplementary Fig.2 a-a'), indicating that  
59 lamellipodial protrusion emerged in response to apoptosis. This directional movement of  
60 lamellipodia protrusion persists for a longer period than spontaneous lamellipodia fluctuations  
61 unrelated to apoptosis that usually exist in the monolayer (Supplementary Fig. 1e-e'). We  
62 observed similar processes at play for naturally-occurring and laser-induced cell extrusions  
63 based on our established method [15]. In the following, we will present data based on laser-

64 induced extrusions to better control the initial conditions and ensure consistency between  
65 analyses.

66 To further evaluate the influence of cell protrusions *versus* actomyosin cable  
67 contractility, we looked at the evolution of the closing area defined by the edges of neighboring  
68 cells as a function of time in basal and apico-lateral planes. Complete basal sealing beneath the  
69 dying cells occurred in 30 min of extrusion (Fig. 1a), preceding apico-lateral area reduction  
70 (Fig. 1d-d'). MDCK monolayers treated with the Rac1 inhibitor NSC-23766, which inhibits  
71 lamellipodia formation, displayed delayed basal area reduction (Fig. 1e-e', Supplementary Fig.  
72 3a), which was then concurrent with apical area closure. Rac1 inhibition also resulted in an  
73 increased proportion of neighboring cells forming actomyosin cables (Fig. 1c) as well as in a  
74 reduction of the number of successful extrusions (Fig. 1f). On the other hand, perturbing purse-  
75 string formation with myosin II inhibitor - blebbistatin or formin inhibitor - SMIFH2 led to  
76 increased lamellipodia presence and faster closure rate of the basal plane (Supplementary Fig.  
77 3b-d). Nevertheless, all these inhibitors reduced the number of successful extrusions (Fig. 1f  
78 & Supplementary Fig. 3b-d), indicating that extrusion was driven by combined effects of Rac-  
79 induced basal protrusions and apical contractions of the neighboring cells.

80

### 81 **Rac1-mediated lamellipodia protrusions resulted in heterogeneous apico-lateral actin** 82 **organization**

83 In contrast to the expectation that a uniform, multicellular purse-string is prominent during  
84 extrusion as proposed in previous studies [20], actin cables formed by surrounding cells were  
85 instead non-uniform (Fig. 1a, Supplementary Movie 1, Fig. 2a). We then questioned the  
86 contribution of such discontinuous actin cables to extrusion. We first quantified the  
87 inhomogeneity of the actomyosin cable by measuring the intensity of myosin IIA  
88 immunostaining at extrusion sites (Fig. 2a) and compared this parameter with or without the  
89 Rac 1 inhibitor. Caspase-3 was used as the surrogate marker for the initiation of apoptosis and  
90 stage of extrusion since the increase of caspase-3 levels as the function of time correlated with  
91 the reduction in apical closing area (Supplementary Fig. 4a-a'). The inhomogeneity in  
92 actomyosin II staining cable decreased with the intensity of caspase-3 (Fig. 2b; Supplementary  
93 Fig. 4b). Myosin distribution was only uniform at the final stage when the caspase-3 signal was  
94 maximum (Fig. 2b). We also quantified the inhomogeneity of actin distribution as a function  
95 of extrusion area and confirmed that inhomogeneous actomyosin cables persisted at

96 neighboring-extruding cells interface until the end of extrusion (Fig. 2d-f; Supplementary Fig.  
97 5a).

98 We rationalized that the purse-string may not need to span all cells to exert contractility:  
99 the actomyosin cables accumulated at each neighboring cell-extruding cell junctions could still  
100 contract. To test this hypothesis, we measured and cross-correlated the length of each junction  
101 as the function of time with its actin intensity over time (Fig.2f, Supplementary Fig. 5a-c). At  
102 the interfaces which form actin cables, the intensity of actin accumulation at these junctions  
103 correlates with junction's shortening (Supplementary Fig. 5b). Such correlation for junctions  
104 that form cables was higher when compared with junctions that do not form actin cables  
105 (Supplementary Fig.5b'-c, Fig. 2f). This result supported that contractile activity occurred at  
106 the individual junction level over the time-course of extrusion. This persistence of  
107 discontinuous purse-string is opposite from the view that a complete actomyosin ring is  
108 required for junctional reductions in neighboring cells.

109 As perturbing lamellipodia protrusion by NSC-23766 led to simultaneous reduction of  
110 basal and apical extrusion area (Fig. 1e-e'), we questioned the impact of lamellipodia formation  
111 on the assembly of actomyosin cables. We found that inhibiting lamellipodial extensions by  
112 NSC-23766 treatment led to a more uniform distribution of Myosin II (Fig. 2a-e). Also, this  
113 uniform distribution of Myosin-II in NSC-23766-treated cells was independent of the caspase-  
114 3 levels (Fig. 2b). Even when actomyosin distribution became homogeneous and formed  
115 complete purse-strings around the apoptotic cell, these purse-strings were typically tilted in z  
116 over several  $\mu\text{m}$  (Fig. 2g-g'). These actomyosin rings were tilted  $31.6^\circ \pm 10.0^\circ$  with respect to  
117 the horizontal plane, while they were typically more horizontally oriented  $10.5^\circ \pm 2.8^\circ$  in NSC-  
118 23766 treated monolayers (Fig. 2h). Therefore, lamellipodia protrusion might elicit the  
119 heterogeneous actomyosin organization during extrusion.

120 We postulated that the tilting of the full ring at the end stage of extrusion was due to  
121 two factors: non-uniform formation of contractile actin cables at apical levels and lamellipodia  
122 protrusions that delay myosin enrichment to form contractile cables. The representative  
123 example on Fig.1a illustrates further this point. As cell extended lamellipodia (Fig.1a, cell #1,  
124 top view and side view), the interface shared with its neighbors followed cell body extension  
125 and thus localized at the basal plane. This results in cell #2, #3, the side cell #4 connected to  
126 #3 being pulled to the basal plane. However, without lamellipodia protrusion, the rest of cell  
127 #4 remained at apical plane (18 min) when it started to form actomyosin cable. As a

128 consequence, the actomyosin ring observed at late stages was partly connected to the basal part.  
129 To further support this model, we imaged cells co-expressing TdTomato F-Tractin and Myosin  
130 Regulatory Light Chain (MRLC) to distinguish actin at contractile cables and actin-  
131 accumulated at protrusive membranes (Supplementary Fig. 6, Supplementary Movie 2). Actin  
132 protrusion at the basal sides was observed first, followed by myosin enrichment at the same  
133 plane and myosin reduction at 1-2  $\mu\text{m}$  above, suggesting that basal protrusion helped to anchor  
134 actomyosin cables in the apical-to-basal direction.

135 These findings evidenced that lamellipodia protrusions preceded and caused  
136 heterogeneous patterns of actomyosin cables formation. As lamellipodial protrusion is  
137 stochastic, the resulted purse-string ring is heterogeneous, as observed. Early basal sealing was  
138 accomplished by lamellipodial protrusions, and extrusion is completed through apical closure  
139 by purse-string, which can either be partial or uniform. Failure of extrusion in the case of  
140 lamellipodia inhibition implied that such a sequence of events was more important than a  
141 single, uniform purse-string formation.

142

### 143 **Modulation of cell-cell junction strength dictates the dominant mechanism of extrusion**

144 As partial actomyosin cables persisted during extrusion, we next questioned whether the  
145 presence of a full purse-string ring was dispensable for cell extrusion. In oncogenic extrusion,  
146 remodeling of E-cadherin followed by a redistribution of junctional tension was sufficient to  
147 drive extrusion without a prominent purse-string ring [11, 13, 21]. Adherens junctions have  
148 also been reported to mediate the rearrangement of cells surrounding the extrusion site [12]  
149 and the formation of contractile actin cables [14]. Our experiments showed that junctional  
150 complexes between extruding and neighboring cells were remodeled. The reduction of  
151 junctional E-cadherin between extruding and neighboring cells was observed at the middle  
152 stage of extrusion (Supplementary Fig. 7a, 60 min, middle panel, white open arrows,  
153 Supplementary Fig. 7c, 78 min) in a non-uniform manner. Subsequently, inhomogeneous  
154 accumulation of active RhoA (Supplementary Fig. 7a-b) were observed. These sequences of  
155 events where molecular regulators of cellular tension assemble in a non-uniform fashion recall  
156 the partial actomyosin purse-string formation described so far.

157 We thus examined the contribution of cadherin-catenin complexes to cell extrusion by  
158 analyzing extrusion in  $\alpha$ -catenin knocked-down ( $\alpha$ catKD) MDCK cells fully deficient for  
159 adherens junction (AJ) formation. We imaged actin distribution at extrusion sites (Fig. 3).

160 Extrusion of these cells entirely engaged the lamellipodia-based mechanism (Fig. 3a, a'), and  
161 as a result, apical closure was strongly delayed compared to basal closure (Fig. 3b-b').  
162 Occasionally, we observed the accumulation of actin at the apical plane for  $\alpha$ catKD cells facing  
163 the extruded cells (Fig. 3a, 30 min). To exclude the possibility that this accumulation of actin  
164 was part of functional contractile actomyosin cables, we performed laser ablation on these  
165 myosin- enriched interfaces and followed the associated junctional recoil (Fig. 3c-f). Laser  
166 ablation in  $\alpha$ catKD cells induced junctional recoil twice slower than the one observed in WT  
167 cells (Fig.3d-e). Besides, initial recoil velocity of the ablated junction in  $\alpha$ catKD cells during  
168 extrusion was independent of junctional myosin accumulation, in contrast to the linear increase  
169 in WT case (Fig.3f). These results indicated that weakened cell-cell junction (CCJ) in  $\alpha$ catKD  
170 cells caused impaired purse-string contractility.

171 To artificially create a condition with different CCJ strength in between dying cell and  
172 neighboring cells, we co-cultured WT and  $\alpha$ catKD MDCK cells and induced apoptosis in WT  
173 cells, which were surrounded by both WT and  $\alpha$ catKD cells. We found that neighboring  
174  $\alpha$ catKD cells, with weakened CCJ engaged in extrusion could switch to lamellipodia-based  
175 protrusion and participate in basal closure (Fig. 4a-b), while neighboring WT cells formed  
176 contractile actin cable (Fig. 4a, top panel). In these mosaic monolayers, the  $\alpha$ catKD cells  
177 displayed faster basal cell edge movement towards the extrusion center than WT cells (Fig.4b).  
178 This set of experiments confirmed that cells with weaker CCJ strength at the interface with the  
179 extruding cell used lamellipodia protrusion as the dominant mechanism.

180 We further proved that by varying CCJ strength using cells expressing various  $\alpha$ -catenin  
181 mutants that could either reduce or enhance CCJ strength [22-24], we could tune the  
182 mechanism of extrusion. The expression of these different  $\alpha$ -catenin mutants into  $\alpha$ catKD cells  
183 (see Method section) rescued the contribution of actomyosin cables with different degrees  
184 (Fig.4c, Supplementary Movie 3).  $\alpha$ cat-L344P expressing cells, unable to recruit vinculin at  
185 CCJs despite being unable to form actin cables (Supplementary Movie 3), formed pronounced  
186 lamellipodial protrusions around extruded cells. In contrast, cells rescued with  $\alpha$ cat-WT or  
187  $\alpha$ cat- $\Delta$ Mod (constitutively recruiting vinculin) constructs formed more pronounced actin  
188 cables around extruded cells (Supplementary Movie 3).  $\alpha$ catKD MDCK cells, which formed  
189 lamellipodial protrusions closed the basal area faster than the other mutants, while  $\alpha$ cat- $\Delta$ Mod  
190 cells, which preferentially formed purse-string, displayed the most delayed basal area closure  
191 (Fig.4d-e). This further validates that cadherin-mediated adhesions were crucial to modulate  
192 cell protrusion and actin cable activities during extrusion. Although either reducing or



193 enhancing CCJ strength altered basal closure timing, it did not affect the duration of extrusion,  
194 as followed by apical closure timing (Fig.4f). These data indicated that on substrate with  
195 homogeneous adhesion, monolayers with altered CCJ strength could adjust the relative  
196 contribution of lamellipodia protrusion/purse-string to successfully extrude dying cells.

197

### 198 **Basal lamellipodia protrusion controls cell extrusion via cell-substrate adhesion assembly**

199 We further investigated the contribution of lamellipodia protrusion to ensure successful  
200 extrusion. The inhomogeneity of actomyosin cables at the apical plane was suggested to be the  
201 result of lamellipodia protrusion at the basal part, implicating the role of cell-matrix adhesion  
202 in the formation of these cables. Therefore, we aimed to decouple lamellipodia protrusion and  
203 actomyosin cable contractility by manipulating cell-substrate adhesion. We cultured MDCK  
204 monolayers on micro-patterned substrates containing non-adhesive circular patches, and laser-  
205 induced apoptosis on cells sitting on top of these patches. We varied the size of the patches  
206 from 10  $\mu\text{m}$  to 30  $\mu\text{m}$  in diameter to cross-over subcellular and cellular dimensions and, thus,  
207 partially or entirely prevent cellular protrusions of the neighboring cells.

208 Mechanical inhibition of cell protrusions by the non-adhesive patch (diameter = 30  $\mu\text{m}$ )  
209 resulted in isotropic actin cable formation (Fig. 5a, Supplementary Movie 4). Continuous  
210 recruitment of actin cables to the purse-string appeared to pull on cell-cell junction (CCJ) as  
211 revealed by the accumulation of E-cadherin at the tips of surrounding CCJs (Fig. 5a, arrows,  
212 Supplementary Movie 4). The purse-string was also composed of radial cables, which  
213 emanated out of the continuous tangential cables, connecting to focal adhesion complexes at  
214 the edges of the non-adhesive patterns (Fig. 5b, white open arrow & Supplementary Fig.8a &  
215 d) as previously observed during collective cell migration [25]. Some cables connect the focal  
216 adhesion with cell-cell junction (as visualized by enhanced actin at junction, pointed by a pair  
217 of cyan arrowheads in Fig. 5b). These observations revealed that the actin meshwork formed  
218 in response to extrusion was not only supported by multicellular actomyosin cable anchored  
219 across cell-cell junctions but also emerged from cables connected to cell-substrate adhesions.  
220 Laser ablation performed on these radial cables resulted in the recoil of both the purse-string  
221 (Supplementary Fig. 8a, b & b', initial recoil velocity =  $0.13 \pm 0.01 \mu\text{m/s}$ ) and the rear of the  
222 cell away from extrusion site (Supplementary Fig. 8a, c & c', initial recoil velocity =  $0.07 \pm$   
223  $0.01 \mu\text{m/s}$ ) indicating that these cables are under tension.

224 In agreement with our data on pharmacological inhibition of lamellipodia (Fig. 1e-e'),  
225 the uniform purse-string alone was not sufficient to extrude apoptotic cells at 30  $\mu\text{m}$  diameter  
226 non-adhesive patches (Fig. 5a, Supplementary Movie 4). The dying cell detached from its  
227 neighbors without the simultaneous sealing activity from the neighbors (Fig. 5a,  
228 Supplementary Fig. 9a, Supplementary Movie 4). To investigate the extent to which the lack  
229 of substrate adhesion impeded extrusion we varied the non-adhesive patch size (Fig. 5c and  
230 Supplementary Fig. 9). A monotonic trend was observed between the patch size and extrusion  
231 efficiency (Fig. 5c, Supplementary Fig. 9b-b'), supporting that by forming nascent focal  
232 adhesion, protrusions from neighboring cells control the formation of purse-string to extrude  
233 the dying cells.

234

### 235 **Deconstructing the mechanical forces of cell extrusion using micropatterned surfaces**

236 As both lamellipodia protrusion and actomyosin cables can exert mechanical forces [16, 17,  
237 26], we sought to understand how these forces contribute to successful extrusion. Therefore,  
238 we further dissected the mechanical work contribution by lamellipodia versus purse-string.  
239 Traction force cells exerted on the substrate has been used to describe forces generated by both  
240 lamellipodia and purse-string during wound healing processes [16, 25]. These studies shown  
241 that during wound healing, lamellipodia exert traction forces pointing radially and away from  
242 the wound center, while purse-string traction forces are generally tangential or radially inwards.  
243 We thus adapted traction force microscopy to dissect the relative forces contributed to these  
244 two mechanisms during extrusion. We observed an increase of the overall traction forces upon  
245 cell extrusion as compared to forces at random sites (Supplementary Fig. 10 a-b). By  
246 decomposing forces into radial and tangential components, we concluded that both components  
247 played a role in the force increase (Supplementary Fig. 10c), exhibiting an anisotropic  
248 distribution of forces at extrusion sites. Furthermore, the observation of inward- and outward-  
249 pointing forces with respect to the center of the extrusion zone suggested that both mechanisms,  
250 lamellipodia protrusion and purse-string, contributed significantly to cell extrusion, in  
251 agreement with previous studies on wound healing and epithelial gap closure [16, 25]. As  
252 extrusion progressed, the purse-string mechanism appeared more pronounced (Fig. 1a,  
253 Supplementary Fig. 10a) reflected by a more prominent contribution of inward-pointing forces  
254 (Supplementary Fig. 10a).

255 To further investigate the relative mechanical contributions of the two mechanisms at  
256 play during extrusion, we first measured the forces during extrusion over circular (10, 15, and  
257 30  $\mu\text{m}$ -diameter) non-adhesive patches to determine the forces produced by purse-strings. The  
258 fact that contractile purse-string is partially connected to the focal adhesions at the edge of the  
259 patch indicates that the resulting forces generated by purse-string could be measured by traction  
260 force exerted on the edges of non-adhesive patches. Indeed, pronounced traction forces were  
261 observed at the edges of the non-adhesive patches during extrusion (Fig.5d). These forces are  
262 prolonged for 2.5 hours on patches of  $D = 30 \mu\text{m}$  and  $15 \mu\text{m}$  corresponding to incomplete  
263 extrusion on these patches. On  $10 \mu\text{m}$  diameter non-adhesive patches, the forces diminished  
264 after 60 minutes (the duration when extrusion is typically completed). Also, on  $D = 10 \mu\text{m}$  the  
265 orientation of force was more anisotropic (Fig. 5e iii & Supplementary Movie 6) compared to  
266 prominent radial inwards-pointing forces observed on  $15 \mu\text{m}$  and  $30 \mu\text{m}$  diameter non-adhesive  
267 patches (Fig. 5e-i & ii, Radial Traction Force & Orientation of the forces with respect to  
268 extrusion center, Supplementary Movie 7). The dependence of the force pattern on the non-  
269 adhesive patch diameter corroborated with the reduced contribution of lamellipodia to  
270 extrusion as the patch size increased.

271 We estimated the tension contributed by purse-string using force measurement on non-  
272 adhesive patch of  $D = 30 \mu\text{m}$ . We represent the contractile force exerted by purse-string by the  
273 line tension  $\gamma$  (the radial traction force divided by radius of extruding cell). On non-adhesive  
274 patch of  $D = 30 \mu\text{m}$ , we observed that total and radial forces diminished together with the re-  
275 opening of the closing area during extrusion (Fig. 5d-i & e-i, Supplementary Fig.11a). For the  
276 total work done to move the cell edge across the non-adhesive area of radius  $R$ , without  
277 lamellipodia protrusion, the radial traction force  $F_{\text{radial}}$  needs to be large enough to generate the  
278 work to overcome the resistivity of the bulk:  $dW_{\text{radial}} = F_{\text{radial}}.dr \geq \gamma.(2\pi R).dr$ . At non-adhesive  
279 patch size of  $D = 30 \mu\text{m}$ ,  $F_{\text{radial}}$  is only contributed by purse-string. Since extrusion always fails  
280 on this size, we could estimate the force exerted by purse-string by identifying the range at  
281 which the Work  $dW$  changed sign (corresponding to the re-opening of the closing area, range  
282 between the dashed line on Supplementary Figure 11a). As such, we estimated that the tension  
283 by purse-string was  $\gamma = 6.5 \pm 1.4 \text{ nN}/\mu\text{m}$  (a value consistent with the value reported in [25])  
284 (Supplementary Fig. 11a, middle panel).

285 The estimation for purse-string tension was validated again on extrusion on non-  
286 adhesive patch size of  $D = 15 \mu\text{m}$  (Supplementary Fig. 11b). Note that at the range  $\gamma = 6.5 \pm$   
287  $1.4 \text{ nN}/\mu\text{m}$ , the purse-string produces non-zero Work and as such, extruding area is reduced

288 linearly before reaching flat ends. On patch size of  $D = 10 \mu\text{m}$ , we observed that the  $F_{\text{radial}}$  only  
289 started increasing (Supplementary Fig.11c – middle panel) at  $T = 40 \text{ min}$ , corresponding to a  
290 radius around 2-2.5  $\mu\text{m}$ . Since the non-adhesive patch remained smaller than the typical cell  
291 size, we expected that lamellipodia-based forces could contribute to cell extrusion over larger  
292 time scales than in the previous cases, leading to a delayed signal in solely purse-string based  
293 forces (Supplementary Fig. 11c).

294 We further investigated how cell-cell junctions strength modulated the forces  
295 contributed by purse-string during extrusion. We combined non-adhesive patches with the  
296  $\alpha\text{catKD}$ ,  $\alpha\text{cat-L344P}$  and  $\alpha\text{cat-}\Delta\text{Mod}$  cell lines of varying CCJ strengths. We compared their  
297 behaviors on non-adhesive patches with diameters of 10, 15, 20, 25 and 30  $\mu\text{m}$  with those of  
298 WT cells (Fig. 6).  $\alpha\text{catKD}$  cells failed to be extruded successfully from the non-adhesive patches  
299 even at small sizes (Fig. 6a, Supplementary Movie 8). We also estimated the line tension  
300 produced by actin cables formed by  $\alpha\text{catKD}$  cells to be less than 1  $\text{nN}/\mu\text{m}$  (Supplementary Fig.  
301 11d). Although there was actin accumulated around the extruding-neighbor interfaces and  
302 caspase-3 signal was elevated, the extruding area stagnated over 6 hours. In  $\alpha\text{cat-L344P}$  cells,  
303 the actomyosin cables were observed at CCJ as the extruding area reduced (Fig.6b). However,  
304 most of the extrusions failed in this case because the actin cable was not sustained (Fig. 6b).  
305 On the other hands, extrusion of  $\alpha\text{cat-}\Delta\text{Mod}$  cells was generally successful with visibly  
306 actomyosin cable reinforcement (Fig.6c, white arrowheads). Furthermore, there were also  
307 successful extrusions for these cells on larger non-adhesive patches (Supplementary Movie 9).  
308 In summary, enhancing CCJ strength could increase the speed of closure on non-adhesive  
309 patches (Fig. 6d-e).

310 Finally, previous studies have shown that substrate curvature can promote either  
311 lamellipodia-based protrusions or actin cable assembly [17, 25]. Along this line, we developed  
312 anisotropic patterns (Fig. 7) with a positive curvature side which could favor lamellipodia  
313 protrusion and a negative curvature side which could favor actin cable formation [17, 25].  
314 Preferential formation of a cable at the negative-curvature before the formation of actin cable  
315 on the positive-curvature was observed (Fig. 7a). At the positive curvature side, lamellipodia  
316 protrusion was dominant until the protruding front reached the edge of the patch and converted  
317 into actin cable (Fig. 7a,  $t = 12 \text{ min}$  and  $t = 18 \text{ min}$ , white arrowhead). The forces generated by  
318 lamellipodia protrusion and actomyosin cables were also dissected on these anisotropic non-  
319 adhesive shapes (Supplementary Fig. 12). As extrusion progresses, higher radial traction forces  
320 pointing towards the center accumulated at the negative edge than at the positive edges while

321 the overall traction forces increased around the shape's edges (Fig. 7b & c). On positive  
322 curvature, outward-pointing traction forces span the patch edge during early extrusion (Fig. 7d  
323 & f, Supplementary Fig. 12a & b, top panel, 0-30min), that were attributed to the forward cell  
324 crawling movement. The force orientation became tangential to the edge of the cell as it  
325 crawled towards the edge of the non-adhesive patch (Supplementary Fig. 12a & b, top panel,  
326 45min). On the negatively curved region of the patch, forces oriented tangentially to the cell's  
327 edge (Fig. 7d' & f', Supplementary Fig. 12a' & b', 45-72 min). Over time, cells were pulled  
328 inside the non-adhesive area, leading to a change of force patterns with an increase of inward-  
329 pointing forces (Supplementary Fig. 12, 87 min, Fig. 7 e-g). It could be explained by the  
330 formation of cables that linked cellular front over the non-adhesive area to remaining adhesions  
331 at the back (Fig. 7a, 108 min). Altogether, these data helped to clarify the force pattern and the  
332 mechanical signature of the transition associated to lamellipodia and actin cable assembly.

333

### 334 **A theoretical model to reconcile forces produced by purse-string and lamellipodia** 335 **protrusion during extrusion**

336 In order to provide an integrated view of the extrusion mechanism driven by cell protrusions  
337 and contractility, we developed a theoretical model. In two recent works, a theoretical  
338 framework emerged to account for the high variability in the completion time of wound [24,  
339 25]. In spite of similarities, including in terms of time scales, the length scales involved in  
340 extrusion are significantly lower than in the wound closure experiments reported in [24, 25],  
341 indicating discrepancies in the strength of the forces involved in these processes. Our  
342 theoretical model reconciled the relevant forces involved in extrusion and wound closure, as  
343 well as the two experimental features observed here in the context of extrusion over adhesive  
344 and non-adhesive substrates (see Fig. 8a-d).

345 In the adhesive case, we expect basal lamellipodia protrusions to generate forces  
346 oriented towards the extruding cell; such contribution to the tissue spreading dynamic  
347 corresponds to a negative isotropic stress contribution that drives a full extrusion for any initial  
348 size of the ablated cell. As such, the stress exerted at the boundary between neighboring cell-  
349 extruding cell can be written in terms of a boundary stress equation  $\sigma_{r=R(t)} = P_C + \sigma_P + \sigma_\mu +$   
350  $\frac{\gamma}{R(t)} + \xi$  (Supplementary, Theoretical Appendix, Eq. 4) where  $R(t)$  is the radius of the closing  
351 area;  $P_C$  is the overall monolayer prestress;  $\sigma_P$  is the protrusive force the neighboring cells  
352 exerted at the interface ( $\sigma_P > 0$  for lamellipodia protrusion [27, 28]);  $\gamma$  is the tension along

353 the actomyosin cables within the multicellular purse string located on the apical side;  $\xi$   
354 encompasses different sources of short-time scale stress fluctuations, generated either along  
355 the acto-myosin cables or within the bulk of the tissue; finally, we introduce a term  $\sigma_\mu$  to  
356 account for the stress exerted through radial actin cables, providing a resistive contribution to  
357 extrusion ( $\sigma_\mu < 0$ ) in the case of non-adhesive patches.

358 Solving the equations for  $R(t)$  as the function of time (Eq.7-11), we found that the  
359 completion time for the adhesive substrate is largely independent of the value of the  
360 contractility by the actomyosin cable. This is consistent with our experimental results that  
361 modulating actomyosin cables contraction *via* changing CCJ strength does not significantly  
362 alter the duration of extrusion (Fig.4f).

363 The difference in the non-adhesive case lies in the existence of radial stress fibers that  
364 spread from the edges of the non-adhesive patch (basal side) to the edge of the tissue (apical  
365 side, see Fig. 5b, Fig.8c-d, Supplementary Fig. 8d). As they were shown to be tensile  
366 (Supplementary Fig. 8a-d'), these radial cables resist the closure activity of neighbors. These  
367 fibers become increasingly tilted in the apico-basal direction (Fig.8d, Supplementary Fig. 8d'),  
368 and these angles are correlative to the strain exerted when closure occurred on non-adhesive  
369 patches (Supplementary Fig. 8e). Taking into these observations, we consider the simplest  
370 assumption of constant contractility along these fibers. In turn, the projected in-plane tissue  
371 stress is an increasing function of the tilt; hence it increases during the extrusion process (see  
372 Supplementary Note – Fig. 1). We experimentally showed that the cell extrusion failed  
373 whenever the size of the non-adhesive patch is bigger than a critical radius, which can be  
374 estimated by numerical simulation (see Theoretical Appendix- Fig.SI Theory Fig.2).

375 By considering the stress fluctuation  $\xi$  in Eq.5 as the function of  $R$ , which depends on  
376 fluctuation by pressure, purse-string and apical-basal cables, we show that these fluctuations  
377 do contribute to closure of extrusion site (Supplementary Note - Eq.10 and Eq.11). We used  
378 numerical simulation to solve the probability of extrusion as the function of initial size and  
379 probability according to this framework. We compared our simulation with experiments  
380 performed with cells expressing different  $\alpha$ cat mutants (Fig.8e-f) on different non-adhesive  
381 patch sizes. The trend obtained from numerical solutions varying the strength of the purse-  
382 string contractility (see Fig.8e-f) closely follows the trend observed in experiments with  
383 different  $\alpha$ cat mutant background for cells on non-adhesive patches. Our analytical model  
384 demonstrated that the presence of fluctuations may lead to the completion of the extrusion

385 process, even in a phase space of parameters where deterministic forces would prevent it from  
386 occurring. It also suggests that  $\alpha$ -catenin may not just reduce the overall contractility, but also  
387 the level of contractility fluctuations.

388

## 389 **DISCUSSION**

390 Our experimental and theoretical findings establish a new quantitative scenario for the  
391 mechanisms driving cell extrusion in an epithelial sheet. It provides a holistic model of the joint  
392 efforts of two distinct cellular processes, lamellipodia protrusions and contractile actomyosin  
393 cables to eliminate apoptotic cells from tissues while maintaining epithelial integrity. Branched  
394 actin networks of lamellipodia provide fast pushing forces on the membrane to close the  
395 extruding area early at the basal plane. Contractile actomyosin cables first form at apical planes  
396 in a discontinuous manner only become uniform at later stages of extrusion. Lamellipodia  
397 assembly leads to protrusive activity underneath the dying cell but also helps to anchor  
398 actomyosin cables in apical-to-basal direction. Hence, the tilted localization of the complete  
399 purse-string rings shows unanticipated closure processes, which are following not only  
400 horizontal but also apico-basal directions.

401 The dual-mechanism mode involving lamellipodia protrusion and heterogeneous purse-  
402 string could be adjusted by changing cell-substrate adhesion patterns or AJ strength. Such  
403 dual-mechanism ensures that basal sealing is accomplished and further maintains epithelial  
404 integrity during apoptotic extrusion. Our results provide additional models besides the ones  
405 already published [5, 7, 29], which emphasized the dominant role of a complete multicellular  
406 purse-string that fully surrounds the apoptotic cell during extrusion.

407 The combined effect of lamellipodial protrusions and actomyosin contractility during  
408 extrusion is well captured by the observed force patterns and our theoretical approach. We  
409 delineated the efficient forces exerted by the purse-string or lamellipodial protrusions that  
410 should overcome the bulk resistance of the epithelial monolayer by both experimental traction  
411 force analysis and the analytical model. We were able to deconstruct experimentally the force  
412 values for lamellipodia and purse-string during extrusion. The purse-string formation results in  
413 forces pointing radially towards the center of extruding cell on top of non-adhesive patches as  
414 extrusion progresses (Fig. 4e) with a measured line tension of 10-13 nN. These force  
415 magnitudes corroborate with those measured on large wound closure [16, 17, 30]. The  
416 estimated stress exerted by lamellipodial protrusions was between 50-100 Pa, in agreement

417 with previous measurements [15, 31]. By theoretical modeling and comparing the numerical  
418 solution with experimental data, we unveiled the relative contributions of forces at play to expel  
419 dying cells from epithelial monolayers.

420 Heterogeneous mechanisms have been proposed to facilitate wound healing and gap  
421 closure [16, 17, 32, 33]. These studies emphasized that the full, uniform purse-string was  
422 important yet sometimes not sufficient to promote gap closure. Recent *in silico* studies  
423 suggested that a mixed mechanism of contractile purse-string and protrusive crawling were  
424 more efficient than single-mechanism-based closure [34, 35]. Similarly, experiments done in  
425 this work prove that a dual-mechanism is essential for cell extrusion. We provide further  
426 information on how lamellipodia-driven basal protrusions could affect subsequent purse-string  
427 formation three-dimensionally. This is strikingly different from the case of wound closure  
428 since, in extrusion, the closing activity is coupled with partial apico-basal changes when the  
429 extruding cell gradually loses contact with its neighbors. In wound healing, actin reorganization  
430 at the front of the wound only occurs in two dimensions.

431 The heterogeneous changes of actin structures around the extrusion site were found to  
432 be coupled with the non-uniform remodeling of CCJ between the extruding cell and its  
433 neighbors. Previous studies showed that the reduction of E-cadherin at the junctions, together  
434 with a reduction of tension at the lateral membrane, occurs before extrusion [6, 11, 36]. The  
435 reduced tension at the CCJs is necessary for increased contractility that expels the cell [11],  
436 and redistribution of E-cadherin clusters is crucial to recruit factors organizing actomyosin  
437 purse-string like Coronin 1B [14]. Here we provide an additional role of AJ in mediating  
438 extrusion via dual-mechanism mode. The stochastic formation of lamellipodia at early  
439 extrusion stages led to an asymmetrical partial loss of apico-basal polarity and reduction of AJ  
440 at each neighbor-extruding cell interfaces, which corresponds to inhomogeneous actomyosin  
441 purse-string formation. Furthermore, while cadherin-catenin complexes are reduced at  
442 bicellular interfaces, they appear to be enhanced at tricellular contacts together with the  
443 recruitment of actomyosin cables, suggesting that there is likely positive feedback between AJ  
444 reinforcement and actomyosin cable maintenance.

445 Taken together, we established the interplay of the cell-cell junction and cell-substrate in  
446 regulating the extrusion mechanism by tuning lamellipodia protrusion and contractile purse-  
447 string mode. The switching mechanism from lamellipodium protrusion to contractile purse-  
448 string helps epithelia adapt to the environment with heterogeneous substrate adhesion



449 molecules, which typically occurs *in vivo* to maintain homeostasis. The complex and  
450 heterogeneous regulation, even at a small-scale event, epitomizes the robustness of epithelia  
451 and proposes a new framework for understanding epithelial homeostasis as well as extrusion-  
452 related pathological conditions.

453

## 454 MATERIALS AND METHODS

### 455 Cell line and tissue culture

456 Madin-Darby canine kidney (MDCK) strain II was cultured in DMEM medium (Invitrogen)  
457 supplemented with 10% FBS (Invitrogen). To study the function of specific proteins, stable  
458 cell lines or transient expression/knock-down variant of MDCK were used. Stably transfected  
459 cells including GFP-actin MDCK, Lifeact Ruby MDCK,  $\alpha$ -catenin knock-down MDCK,  
460 mCherry E-cadherin MDCK, and GFP E-cadherin MDCK are kind gifts from James W.  
461 Nelson. MDCK PBD-YFP expressing MDCK was kindly provided by Fernando Martin-  
462 Belmonte, Universidad Autónoma de Madrid. These stably-transfected cells are maintained  
463 with media supplemented with 250  $\mu$ g/mL geneticin (Invitrogen) to maintain their gene  
464 expression. Fluorescence-labeled cells are checked regularly for uniform fluorescence intensity  
465 and selected by cell sorting if necessary. Mycoplasma test was done every three months to  
466 control the mycoplasma contamination.

### 467 Plasmids and transfection

468 To study the role of CCJ during extrusion, we manipulated  $\alpha$ -catenin expression. The  $\alpha$ -catenin  
469 mutants were published previously [22]: L344P mutant with the leucine to proline point  
470 mutation at the 344aa of VH2 domain conferring  $\alpha$ -catenin's inability to bind to vinculin, and  
471  $\Delta$ -Mod mutant which has the vinculin-binding modulatory domain deleted (aa 396-631) and  
472 allows  $\alpha$ -catenin to be constitutively bound to vinculin. These mutant constructs were  
473 transiently transfected in  $\alpha$ -catenin knock-down MDCK cells to generate cell populations with  
474 varied CCJ strength. In this study we used:  $\alpha$ -catenin knock-down ( $\alpha$ catKD),  $\alpha$ -catenin L344P  
475 (L344P), wild-type  $\alpha$ -catenin (WTres),  $\alpha$ -catenin  $\Delta$ -Mod ( $\Delta$ Mod). mEmerald-MyosinIIA-N,  
476 mApple-Actin, GFP-Actin, mCherry-ARPP34-N were kindly shared by Pakorn  
477 Kanchanawong. GFP-APHP RhoA sensor was the generous gift from Alpha Yap. Transfection  
478 was performed with Neon electroporation system (Invitrogen).

### 479 Laser Induction System to study apoptotic extrusion

480 We induced apoptosis in monolayer by UV laser to be able to capture early events of extrusion.  
481 The system was designed as in [37], consisting of a Nikon A1R MP laser scanning confocal  
482 microscope, with Nikon Apo 60 X oil-immersion/1.40 objective. To induce apoptosis, UV-  
483 laser (355nm, 300 ps pulse duration, 1kHz repetition rate, PowerChip PNV-0150-100, Teem  
484 Photonics) was focused at the target cell for 7-10s with the power of 10 nW at the back aperture.  
485 With this setting, DNA-strand break is induced without permeabilization of the membrane [15].  
486 This induces cell undergoing apoptosis and being extruded.

### 487 Cell Seeding Setup

488 To distinguish lamellipodial protrusion and actin cable formation at the extruding cell  
489 neighboring cells interface, we used mosaic cocultures of non-fluorescent cells (which would  
490 be laser-induced for apoptosis) and fluorescence-labeled GFP-actin or Lifeact Ruby cells at the  
491 ratio of 1:7. Cells were trypsinized, mixed and seeded at  $2 \times 10^6$  cells/ $\mu$ m<sup>2</sup> on glass bottom  
492 petri dish (Iwaki) coated with fibronectin (Roche, 1hour incubation, 25  $\mu$ g/mL) or on  
493 fibronectin-micropatterned substrates. 16-20 hours before imaging. Cell attachment was  
494 monitored every half an hour and unattached cells were washed by PBS. Cells density was kept  
495 at the range of 40-45 cells per 100 x 100  $\mu$ m<sup>2</sup> unless otherwise specified for density-dependent  
496 measurements.

### 497 Determination of extrusion initiation (t= 0 min)

498 Initiation of apoptotic extrusion was defined by (i) the brightening of caspase-3 indicator, (ii)  
499 a bright spot on phase contrast or bright field image, (iii) the sharp shrinkage of the cell area  
500 and (iv) the initiation of nucleus condensation and fragmentation. Our previous published  
501 results indicate that these four hallmarks occur simultaneously within the time interval of  
502 interest (3-5min) following laser induction pulse [10].

### 503 **Drug Treatment**

504 Drugs were incubated 2 hours prior to imaging unless stated otherwise. The drug  
505 concentrations are as follow: (S)-nitro-blebbistatin (Cayman Chemical) 50  $\mu$ M, NSC-23766  
506 (Sigma-Aldrich) 200  $\mu$ M, SMIFH2 (Sigma) 50  $\mu$ M dissolved in DMSO. Caspase-3 indicator  
507 DEVD-FMK conjugated to Sulfo-rhodamine (Abcam) was used at 1:1000 dilution and  
508 incubated with the cells 30 min before experiment. To visualize F-actin in certain experiments,  
509 siR-actin (Cytoskeleton) was added at 100 nM concentration to the medium 12 hours before  
510 imaging. Hoechst 33342 at 1  $\mu$ g/mL (Sigma) was added into culture media 1 hour and washed  
511 before imaging for nucleus live detection.

### 512 **Micropatterning of the substrate**

513 Wafers with the custom-designed patterns and micropillars were prepared following  
514 photolithographic techniques as described previously [38].

515 For cell monolayer confined on patterns, a stenciling technique was used. PDMS (Sylgard 184,  
516 Dow Corning) was mixed with crosslinking agent to the ratio of 10:1. The mixture was poured  
517 onto the wafer and degassed then cured at 80°C for 2 hours. The stencil with patterns of interest  
518 was deposited onto the glass-bottomed IWAKI dishes and plasma-treated with Oxygen for 30  
519 minutes. By this way, the non-patterned area on glass surface became functionalized and could  
520 attach to 0.1 mg/mL PLL-g-PEG (SuSoS) that was flowed in later by capillary. The dish was  
521 incubated with PLL-g-PEG for 1 hour before removing the stencil and coated with fibronectin.  
522 Fibronectin was washed off and the surface was further treated by 2% pluronics (F127, Sigma)  
523 for further passivation of non-patterned area.

524 For experiments with non-adhesive patches, micro-contact printing was used instead of micro-  
525 stenciling. PDMS substrate was spin-coated at 1000 rpm for 30s then 4000 rpm for 30s on  
526 IWAKI petri dishes and cured for 1.5 hours at 80°C. PDMS stamps were prepared from wafer  
527 molds and coated with mixture of 50  $\mu$ g/mL fibronectin (Roche) and 25  $\mu$ g/mL Atto647-  
528 conjugated fibronectin (Atto dye, Sigma). Stamps were deposited on deep UV-treated surfaces  
529 (15 min) and the unstamped areas were passivated by 2% pluronics (F127, Sigma) for 2 hours  
530 to render non-adhesive surface. The dishes were rinsed with PBS three times before seeding  
531 cells.

### 532 **Immunostaining and antibodies**

533 Cells were fixed with 4% PFA at 37°C for 10 minutes, permeabilized and blocked with 0.1%  
534 Triton X-100 in 1% BSA/PBS overnight. Myosin IIA was stained using rabbit Anti-myosin  
535 IIA antibodies (Sigma M8064) 1:100. Paxillin was stained using rabbit monoclonal anti-  
536 paxillin antibodies [Y113] (Abcam ab32084) 1:100. Actin filaments were stained with Alexa  
537 Fluor® 568 Phalloidin (Life Technologies) 1:100, Alexa Fluor® 488 Phalloidin (Life  
538 Technologies) 1:100 or Alexa Fluor® 647 Phalloidin (Life Technologies) 1:100. Secondary  
539 antibodies Goat Anti-rabbit IgG Alexa Fluor® 568 (Life Technologies) were used at a 1:100  
540 dilution. Nucleus was labeled with Hoechst 33342 at 1  $\mu$ g/mL concentration.

### 541 **Microscopy**

542 Time-lapse confocal imaging was carried out with spinning disk confocal microscopy (Nikon  
543 Eclipse Ti-E inverted microscope body, CSU-W1 Yokogawa head, dichroic filters and 1.27  
544 NA 60X water-immersed objective). GFP fluorescent signal was taken with 488 nm Diode  
545 laser, 50 mW, 5% power, 100 ms exposure. RFP/mCherry/mApple fluorescent signal was  
546 taken with 561 DPSS laser, 25 mW, 5% power, 100 ms exposure. Images were taken with z  
547 step = 0.5  $\mu\text{m}$ , 17-19 stacks. Super-resolution imaging was done using the Live-SR module  
548 (York et al., 2013) integrated with the above-mentioned spinning disk confocal microscope  
549 using a 1.6 NA 100X oil-immersed objective. GFP fluorescent channel was taken with 488 nm  
550 Diode laser, 50mW, 8% power, 100 ms exposure. RFP/mCherry/mApple fluorescent signal  
551 was taken with 561 DPSS laser, 25 mW, 10% power, 100 ms exposure. DAPI fluorescent  
552 channel was acquired with 405 nm Diode laser, 50 mW, 5% power, 100 ms exposure. Images  
553 were taken with z step = 0.1  $\mu\text{m}$ . Microscope settings were kept constant throughout the set of  
554 experiments for keeping consistent quantification.

### 555 **Traction force microscopy**

556 For traction force microscopy, a substrate of 10-20 kPa Young's Modulus was prepared by  
557 mixing CyA and CyB PDMS components (Dow Corning) at the ratio of 1:1 to enable detection  
558 of nanoNewton forces. The mixed PDMS was spin-coated onto glass-bottomed petri dishes  
559 (IWAKI) and cured in 80°C for 30 minutes. The surfaces were then incubated with 5% (3-  
560 Aminopropyl)trimethoxysilane (Sigma) in ethanol for 5 min. carboxylated red fluorescent  
561 beads (100 nm, Invitrogen) were then functionalized on the substrate at a 1:500 dilution in  
562 deionized (DI) water. The beads were passivated with 100 mM Tris solution (Sigma) in DI  
563 water at for 10 min. Finally, fibronectin (50  $\mu\text{g}/\text{ml}$ ) was incubated for 1 hour at 37 °C. This  
564 substrate was patterned on soft gel using indirect micro-contact printing. First, the pattern of  
565 interest was stamped first on thin Polyvinyl Alcohol membrane made up of 5% PVA solution  
566 (Sigma) and transferred the inverted membrane to the substrate. The membrane was dissolved,  
567 and the non-patterned areas were passivated by incubation with 2% Pluronics for 2 hours.  
568 Finally, cells can be seeded onto the substrate as previously described.

569 The bead displacement was calculated using PIVlab (Thielicke & Stamhuis, 2014). The  
570 settings are: 1) Fast-Fourier Transform (FFT), 2) a Gause 2-by-3 point sub-pixel estimator, 3)  
571 linear window interpolator and 4) three-passes (64x64, 32x32, 16x16 pixel size interrogation  
572 window equivalent to 12x12, 6x6, 3x3  $\mu\text{m}^2$  with 50% overlap,). Bead displacements were  
573 converted into traction force (in Pa) by Fourier Transform method using FTTC ImageJ plugin  
574 developed by Martiel et al. (2015). The subsequent analysis was done on customed-written  
575 Matlab scripts.

### 576 **Laser ablation**

577 The laser ablation system composed of a UV-laser (355 nm, 300 ps pulse duration, 1 kHz  
578 repetition rate, PowerChip PNV-0150-100, team photonics) was integrated into a Nikon A1R  
579 MP confocal microscope, with customized optical path and dichroic filter coaligned with the  
580 optical axis of the microscope. Position of the laser can be controlled by a mirror mounted on  
581 two linear actuators (TRA12CC, Newport), and the exposure time of the laser was controlled  
582 by a mechanical shutter (VS25S2ZM0, Uniblitz) using built-in ImageJ plugins from a PC. The  
583 system was mounted on the spinning disk confocal system described above equipped with 60X  
584 oil-immersed/1.4 NA objective. The integrated laser ablation system allows us to perform laser  
585 ablation during imaging.

586 Laser ablation was performed on cells expressing fluorescent-labeled myosin regulatory light  
587 chain (MRLC) to visualize actin cables Apoptosis was induced as previously described and  
588 actin cable formation monitored by imaging at time interval of 10 minutes. Once the cables

589 were visualized, a time-lapse image series was launched on the region of interest (256 x 256  
590 pixels) at time interval of 1-2 seconds. A UV-laser pulse of 5nW, 5s was performed on the  
591 desired myosin-rich region. Images were then taken for 5 minutes. The recoil distance of the  
592 actin cable  $d(t)$  was determined by tracking the displacement of the two ends connecting to  
593 cell-cell junctions. The cable could be modeled as a Kelvin-Voigt fiber (Fernandez-Gonzalez  
594 et al., 2009) by fitting into equation:  $d(t) - d(0) = \frac{F_0}{E} \times (1 - e^{-[\frac{E}{\mu}] \times t})$

595  $d(t)$  as the recoil distance at time  $t$

596  $d(0)$  as the original distance between two CCJ.

597  $F_0$  is the tensile force at the junction before ablation

598  $E$  is the junction's elasticity

599  $\mu$  is the viscosity coefficient related to the viscous drag of the cell cytoplasm

600 The initial recoil, as such, could be derived into equation:  $initial\ recoil = \frac{F_0}{\mu}$

601 By fitting each ablation event into the equation, we could derive the initial recoil value, which  
602 is proportional to the tension exerted at the junction by actin cable.

### 603 **Image Analysis**

604 In order to obtain statistics, image acquisition settings of the same type of experiments were  
605 kept constant. Images were background subtracted and contrast enhanced for visualization.  
606 Intensity measurements were performed on raw images. Fluorescence intensities at the  
607 extruding cell-neighboring cell edges were measured using line scan tool in ImageJ. Bleach  
608 correction for confocal time-lapse imaging was performed using ImageJ EMBL Bleach  
609 Correction plugin. Afterwards, we took the summation image for stack of 6 slices ( $z$ -step = 0.5  
610  $\mu\text{m}$ , from 1  $\mu\text{m}$  to 3.5  $\mu\text{m}$  above the basal plane). Intensity profiles of actin or myosin at cell-  
611 cell junctions were obtained by performing a 10-pixel line scan spanning along the junction  
612 and corrected by deducting the average fluorescence intensity of cell contributing to the  
613 junction of interest. Average intensity and standard deviation values were obtained from this  
614 final line profile. Inhomogeneity of the actin/myosin intensity was defined as Inhomogeneity  
615 = Mean Intensity/90<sup>th</sup> percentile Intensity of the line profile, or as standard deviation  
616 normalized by the mean intensity. To correlate the formation of actin cables with the stage of  
617 extrusion, we used caspase-3 indicator DEVD-FMK conjugated to Sulfo-rhodamine (Abcam)  
618 as the surrogate marker for the extrusion stage.

619 To measure the actomyosin ring localization in 3D, we measured myosin-IIA intensity on fixed  
620 cells imaged by Structured Illumination Microscopy (Nikon Eclipse Ti-E inverted microscope  
621 body, CSU-W1 Yokogawa head, dichroic filters. The 3D reconstruction was performed on  
622 Imaris, and the ring localization can be tracked using manual point selection tool to select the  
623 myosin intensity at cell-cell interface. The coordinations of points corresponding to the  
624 actomyosin rings were fitted into a plane by matlab. By calculating the angle between this plane  
625 and horizontal plane, the angle that the ring forms with horizontal plane can be deduced.

626

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639

640 **Author contributions.**

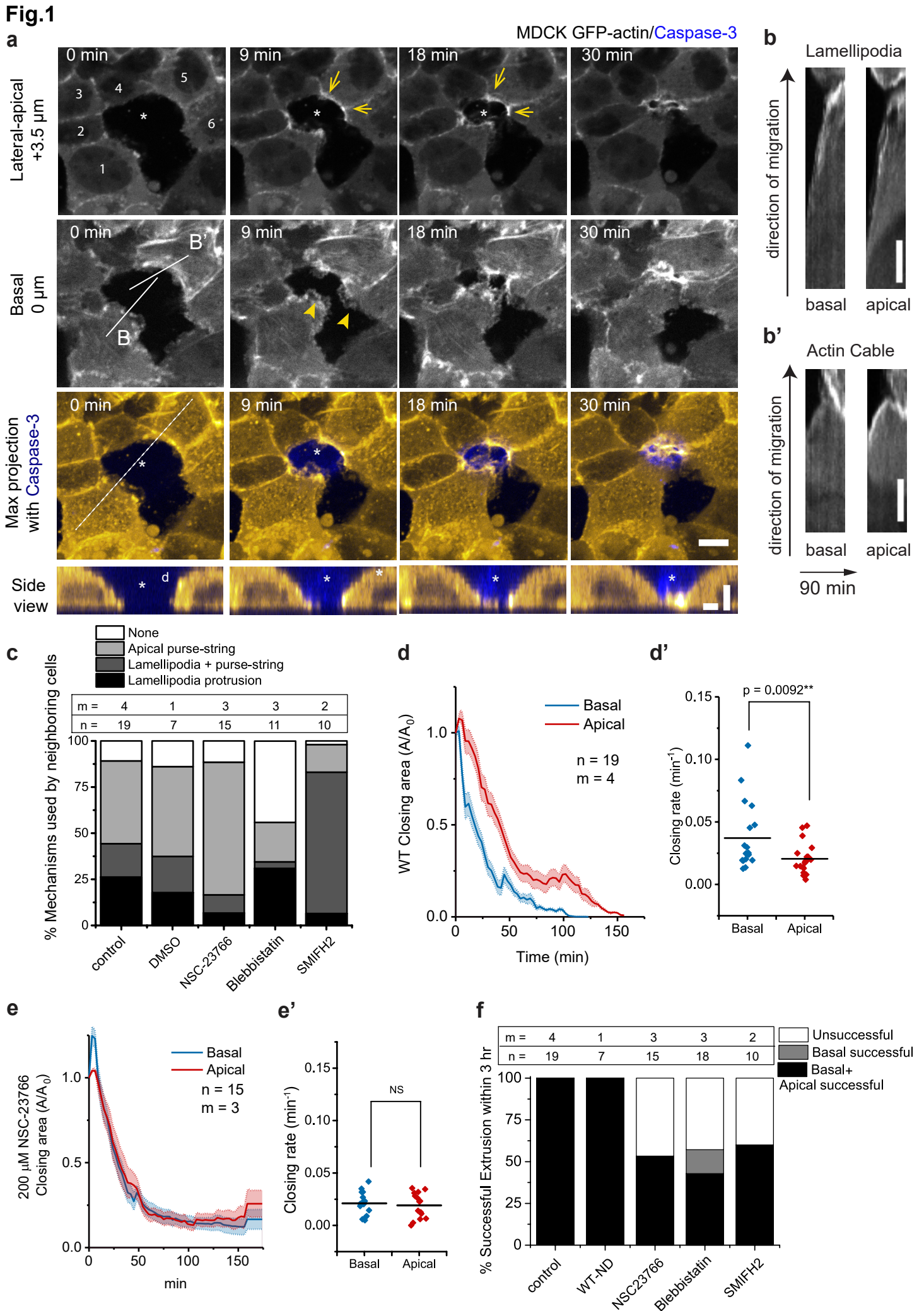
641 BL conceived the project; APL, BL designed research; APL principally conducted the  
642 experiments and analyzed the data with support from BL, RMM, YT and JFR; JFR developed  
643 the theoretical model; RMM, YT, CTL, JFR and BL provided guidance and input; RMM  
644 developed  $\alpha$ -cat mutants; BL and CTL supervised the project; APL, JFR and BL wrote the  
645 manuscript. All authors read the manuscript and commented on it.

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**Fig. 1: Epithelial extrusion depends on dual-mechanism mode: basal lamellipodia protrusion and purse-string formation.**

(a) Confocal time-lapse evolution of a non-fluorescent WT MDCK cell (asterisk) extruding from mosaic monolayer with MDCK GFP-sctin cells as neighbors. Arrows indicate actin cable formation, and arrowheads indicate lamellipodia protrusions. Colored panels represent maximum intensity projections from basal plane to +9  $\mu\text{m}$  apical plane. Scale bar = 10  $\mu\text{m}$ . Bottom panel: side view along dashed line showing wedged-shape when lamellipodia protrusion and non-uniform actomyosin cables take places during extrusion. Vertical scale bar = 5  $\mu\text{m}$ . Horizontal scale bar = 10  $\mu\text{m}$ .

(b – b') Kymographs performed along the connected line for lamellipodia (labeled as B in panel a) and along the connected line for actin cable (labeled as B' in panel a), at basal and apical planes. Vertical scale bar = 5  $\mu\text{m}$ . Time duration for all kymographs was 90 min.

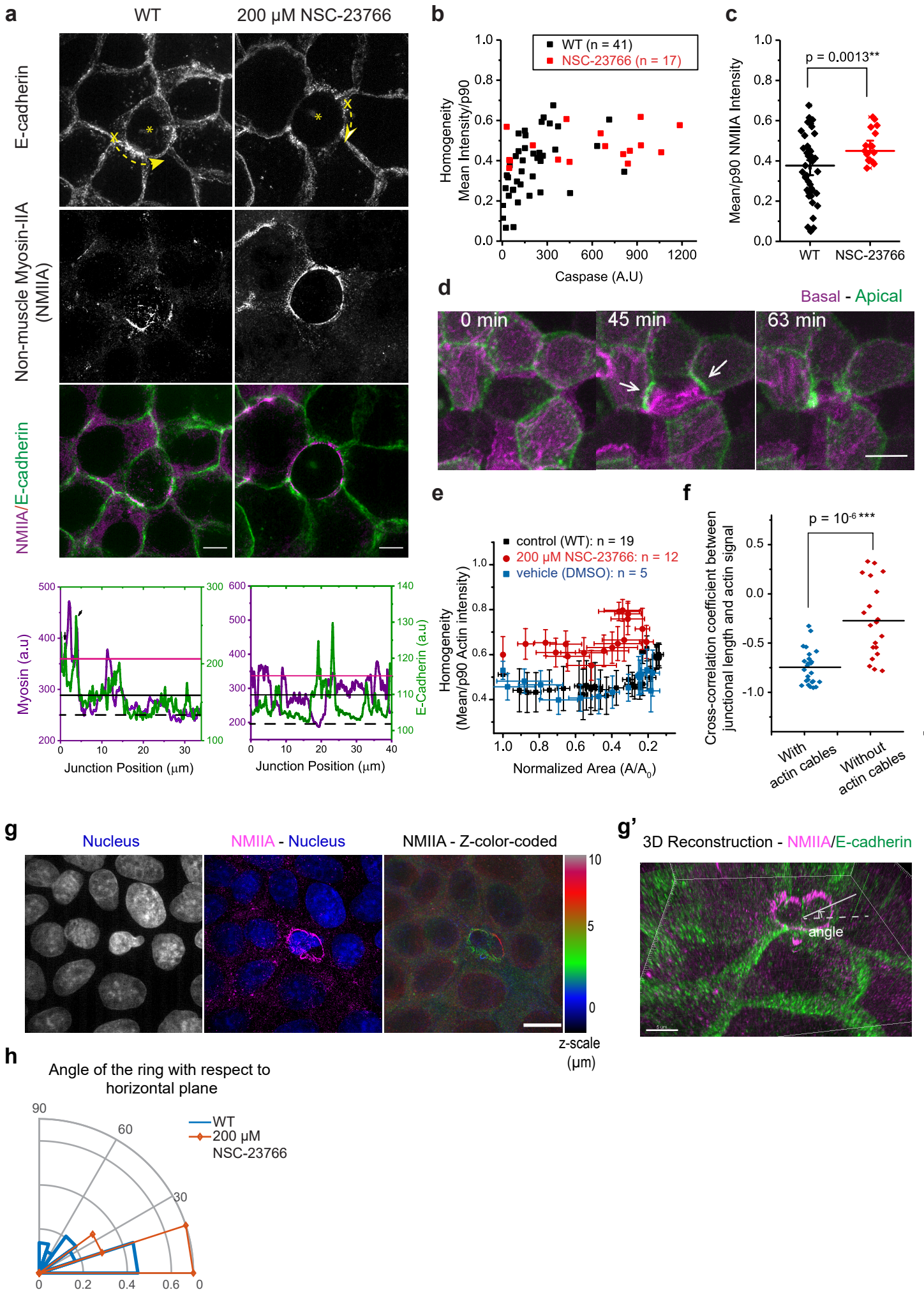
(c) Proportion of the mechanism chosen by neighboring cells in control conditions versus different pharmacological perturbation conditions: 200  $\mu\text{M}$  NSC-23766 (Rac inhibitor) and 50  $\mu\text{M}$  (S)-nitro Blebbistatin (SBB, Myosin activity inhibitor) and 50  $\mu\text{M}$  SMIFH2 (Formin inhibitor). 0.1% DMSO was used as additional control for drug vehicle. n indicates number of extrusion events observed. m indicates number of independent experiments (experiments carried on different days). Source data are provided as a Source Data file.

(d & e) Average relative area closure of basal and apical planes (defined as + 4  $\mu\text{m}$  from the basal plane) as a function of time. The areas were normalized to area at t = 0 min. Shaded area : SEM. Number of extrusion events n = 15, and number of independent experiments m = 3. Source data are provided as a Source Data file.

(d' & e') Closing rate derived by extracting the tangent of initial phase of the area closing curve. Middle lines: mean. Paired t-tests (2-tailed) were performed as comparison for closing speed between apical versus basal plane. Number of extrusion events and number of independent experiments are same as (c). Source data are provided as a Source Data file.

(f) Successful extrusion percentage for all conditions. Basal successful indicates complete basal closure but the cells were not extruded and new junctions between neighboring cells were not formed. Successful extrusion is indicated by basal successful + apical successful. Source data are provided as a Source Data file.

**Fig.2**



**Fig. 2: Inhomogeneous actomyosin cables were observed and persistent during extrusion.**

(a) Immunostaining of non-muscle myosin IIA (NMIIA) on extrusion of MDCK with GFP-E-cadherin stably expressed monolayer in WT *versus* Rac-inhibited monolayer. Images show maximal projection of z-stack from 2-6  $\mu\text{m}$  above basal plane of SIM confocal image of fixed monolayer with extruding cell (asterisk). Scale bar = 10  $\mu\text{m}$ . Bottom panel: line scan of 10 pixels width showing intensity profile along the junction between neighboring cells-extruding cell. X in the first panel indicates the position 0 of the line scan and the dashed arrows indicate the direction the line scan was performed. Inhomogeneous actomyosin cables were observed in WT condition but not in Rac-inhibited condition. Source data are provided as a Source Data file.

(b) Relationship between non-muscle myosin IIA intensity inhomogeneity and caspase indicator fluorescence. (Number of extrusion events:  $n = 41$  for WT and  $n = 17$  for Rac-inhibitor treatment. Number of independent experiments:  $m = 4$  for WT and  $m = 2$  for Rac-inhibitor treatment). Values closer to 1 indicate more homogeneous cables and closer to 0 indicates more non-uniform cables. Source data are provided as a Source Data file.

(c) Comparison for the inhomogeneity of actomyosin cables between WT versus Rac-inhibited monolayers by mean/p90 (normalized against the background myosin intensity). Middle lines: mean. Error bars: SEM. 2-tailed unpaired t-test was performed, and p values shown. Source data are provided as a Source Data file.

(d) Confocal time-lapse evolution of MDCK cell stably expressing Lifeact Ruby for actin undergoing extrusion. Basal plane (magenta) is superimposed with apical plane (green, 3.5  $\mu\text{m}$  above the basal plane). Scale bar = 10  $\mu\text{m}$ .  $T = 0$  is defined as the time point right after laser induction.

(e) Average inhomogeneity of actin cable (indicated by actin signals in live imaging) as a function of area normalized against value at time 0. Values close to 1 indicate more inhomogeneous actin cables and values close to 0 indicate more homogeneous actin cables. Error bars: SEM. Number of independent experiments: WT:  $m = 3$ ; Rac-inhibitor (NSC-23766):  $m = 2$ ; DMSO (drug control):  $m = 1$ . Source data are provided as a Source Data file.

(f) Cross-correlation analysis between junctional length and average junctional intensity on individual junctions. ( $n = 26$  for edges with cables and  $n = 20$  for edges without cables in 7 extrusions, 3 independent experiments). Middle lines: mean. Two-tailed unpaired t-test was performed. See more on Supplementary Fig. 5b. Source data are provided as a Source Data file.

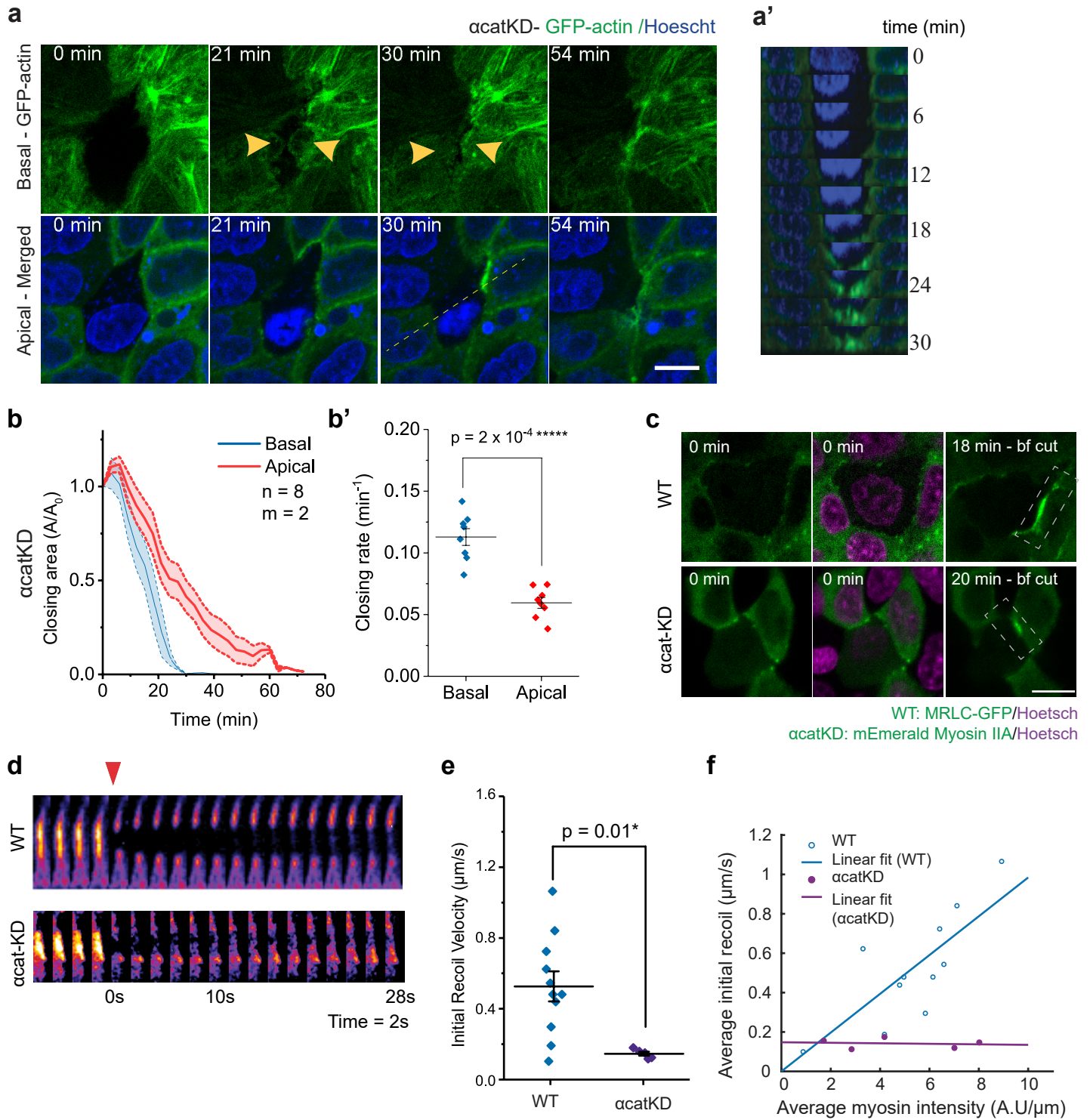
(g & g') Localization of actomyosin cables in 3D shown by z-color coded and 3D reconstruction for immunostaining of myosin IIA in WT cells.

(g) Immunostaining of MDCK cells extruding from monolayer (extruding cell at the center). i) DAPI channel. ii) Merged of DAPI and AlexaFluor-568 channel. iii) Myosin staining color-coded according to the height of the monolayer. The contrasting localization of myosin around nucleus at two separated planes ( $\sim 3 \mu\text{m}$  *versus*  $7 \mu\text{m}$  at each side) suggested the partitioning of the purse-string into two rings which are tilted.

(g') 3D reconstruction for images in (g). A circular fit was performed on each ring on 3D reconstruction images. The angle at which the circle formed with horizontal plane  $xy$  was defined as shown on the image.

(h) Histogram of distribution of angles that the purse-string ring forms w.r.t horizontal plane. (WT:  $n = 32$  rings in  $m = 19$  extrusion events; NSC-23766 (Rac-inhibitor):  $n = 10$  rings in  $m = 11$  extrusion events). Source data are provided as a Source Data file.

### Fig.3



**Fig. 3: Reduction of cell-cell junction (CCJ) strength impairs contractile purse-string formation and renders extrusion depend on lamellipodia protrusion.**

(a-a') Time-lapse imaging of  $\alpha$ cat- knocked-down MDCK cells ( $\alpha$ -catKD-GFP-actin) expressing GFP-actin. The cell in the middle undergoes extrusion. (a) Top panels: basal plane, bottom panels: apical plane (+ 4  $\mu$ m above basal plane). Scale bar = 10  $\mu$ m. (a') Side view along the yellow-dashed line, reconstructed from a Z-stack of 7  $\mu$ m (stacks spaced of 0.5  $\mu$ m).

(b) Average relative area closure of basal versus apical planes (defined as + 4  $\mu$ m from the basal plane) for  $\alpha$ -catKD-GFP-actin MDCK cells. The areas were normalized to area at  $t = 0$ min. Shaded area: SEM.  $n = 8$  indicates number of extrusion from  $m = 2$  independent experiments. Source data are provided as a Source Data file.

(b') Closing rate derived by extracting the tangent of initial phase of the area closing curve. Middle lines: mean. Error bars: SEM. Paired t-tests (2-tailed) were performed. Source data are provided as a Source Data file.

(c-f) Laser ablation at cell-cell junction (area denoted by dashed rectangle) during extrusion on WT MDCK cells expressing GFP-MRLC and  $\alpha$ catKD MDCK cells expressing m-Emerald Myosin IIA.  $N = 11$  for WT and  $N = 5$  for  $\alpha$ catKD indicate number of laser ablation experiments. Source data are provided as a Source Data file.

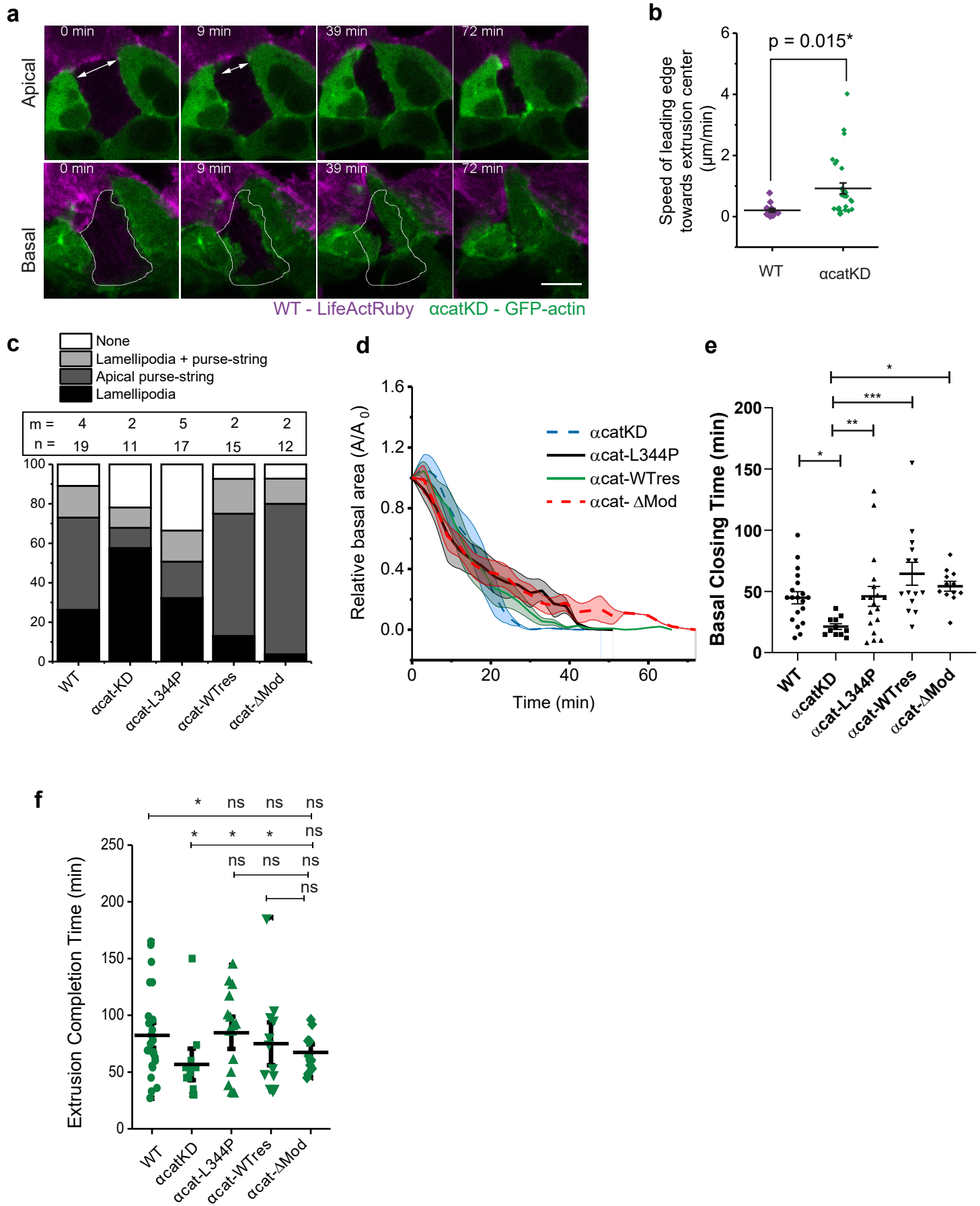
(c) Top view images of myosin accumulation at cell-cell junctions.  $t = 0$  min refers to the initiation of laser-induced extrusion. Images in the last panel (bf cut = before cut) are at the time point the cable becomes visible and at when laser ablation was performed on the cable. Dashed rectangles indicate the junction on which laser ablation was performed.

(d) Evolution of myosin signal from the rectangular area in (c) before and after laser ablation of cell-cell junctions. Enhanced myosin recruitment is shown in FIRE LUT. Arrowhead indicated the point at which laser ablation is performed.

(e) Comparison between the initial recoil velocity – which reflect junction tension - after ablation for WT versus  $\alpha$ catKD. Middle lines: mean. Error bars: SEM. Unpaired t-test was performed. Number of laser ablation experiments WT:  $n = 11$  and  $\alpha$ catKD:  $n = 5$ .

(f) Average initial recoil velocity as a function of average myosin accumulation prior to laser ablation. Middle lines: mean. Error bars: SEM. Linear regression was performed to fit.

**Fig.4**





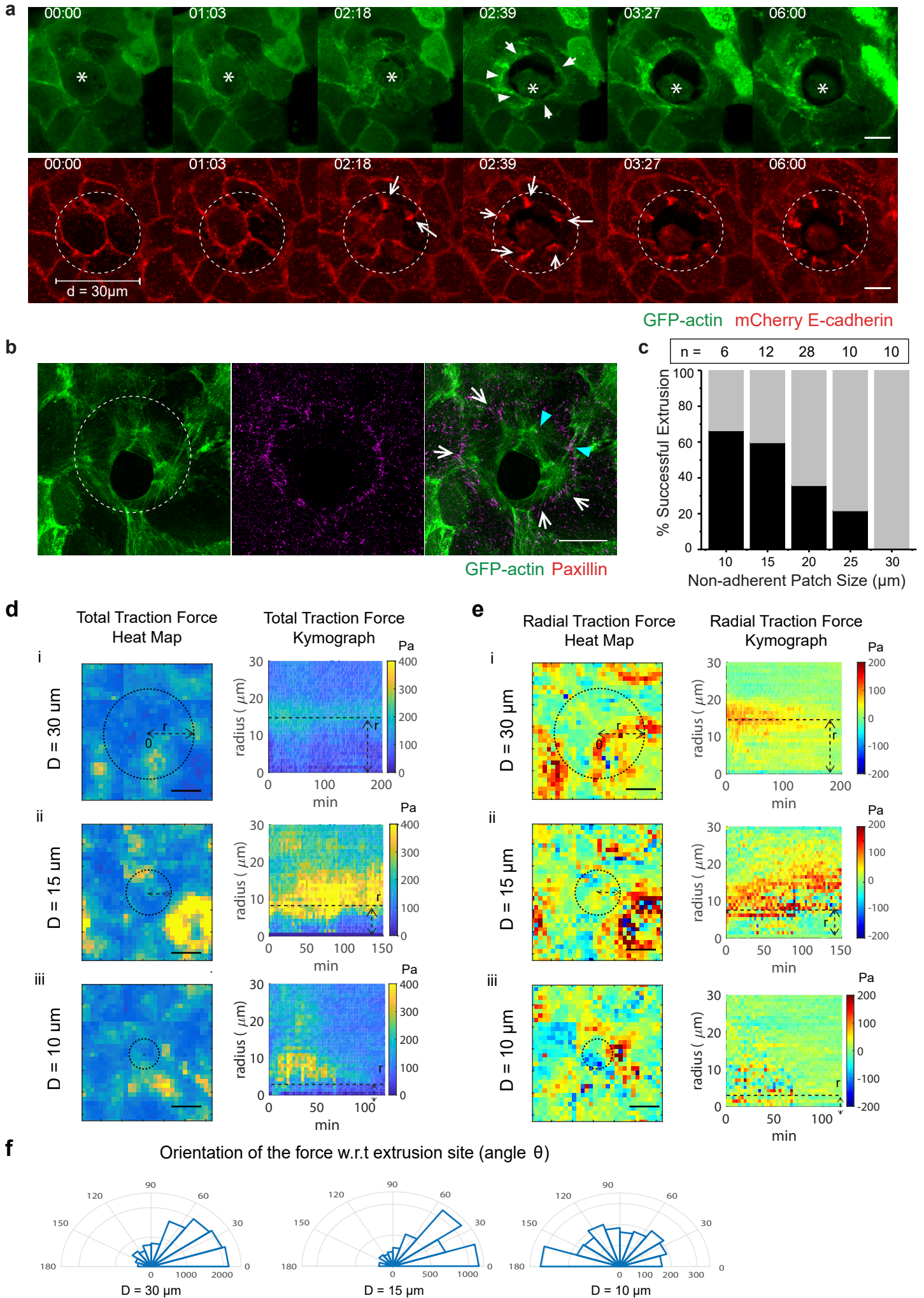
**Fig.4: Tuning cell-cell junction strength by  $\alpha$ -catenin could adjust the preferential mode of extrusion but not extrusion efficiency.**

(a – b) Laser-induced extrusion in mixed WT and  $\alpha$ catKD MDCK co-culture. The extruding cells are WT (a) Top view of an example of extrusion from the mosaic monolayer. Scale bar = 10  $\mu$ m. (b) Velocity towards the center of extrusion at basal side for each type of neighbor. Middle lines: mean. Error bars: SEM. N = 20 extrusion events. 2-tailed, unpaired t-test was performed.

(c) The contribution of mechanism at individual neighbors in single cell type cultures of different  $\alpha$ -catenin mutant cells.  $\alpha$ cat-KD: MDCK cells with stable  $\alpha$ -catenin knock-down.  $\alpha$ cat-L344P: MDCK  $\alpha$ -catenin knock-down cells rescued with  $\alpha$ -catenin-L344P mutant.  $\alpha$ cat-WTres: MDCK  $\alpha$ -catenin knock-down cells rescued with wild-type GFP- $\alpha$ -catenin.  $\alpha$ cat- $\Delta$ Mod: MDCK  $\alpha$ -catenin knock-down cells rescued with  $\alpha$ -catenin- $\Delta$ Mod mutant. n: number of extrusion events. m: number of independent experiments.

(d-f) (d) Evolution with time of relative closure area changes at basal plane for cells expressing the different  $\alpha$ -catenin mutants. Number of extrusion events and number of independent experiments are same in (c). (e): Mean basal closing times. (f) Mean extrusion completion time (both apical and basal area are closed). Middle lines: mean. Error bars: SEM. 1-way ANOVA ( $p < 0.01^{**}$ ) followed by pairwise comparison with Benjamini, Krieger and Yekutieli correction for false discovery rate was performed. Significant level: ns: non-significant;  $p < 0.05^{*}$ ;  $p < 0.01^{**}$ ;  $p < 0.001^{***}$ ,  $p < 0.0001^{****}$ . Source data are provided as a Source Data file.

## Fig.5



**Fig. 5: Substrate adherence influences the uniformity of actomyosin cables, extrusion efficiency and forces contribution by purse-string/lamellipodia.**

(a) Confocal time-lapse imaging of laser-induced extrusion of a mCherry-E-cadherin, GFP-actin expressing MDCK cells sitting on 30  $\mu\text{m}$  nonadherent patch. The dying cell (marked with an asterisk) is not successfully extruded. Whites arrows, dashed circles. Scale bar = 10  $\mu\text{m}$ .

(b) Structural Illuminated Microscopy (SIM) images of a fixed extruding GFP-actin expressing MDCK cell on non-adhesive patch. Cells were fixed and stained with anti-Paxillin antibodies. White arrow shows the radial actin cable connected with focal adhesion. Cyan arrowheads show cables anchoring between cell-cell junctions (indicated by actin converging points) and focal adhesion (indicated by paxillin staining). Scale bar = 10  $\mu\text{m}$ .

(c) Quantification of the percentage of successful extrusion in function on non-adhesive patch size  $i$ .  $n$  indicates the number of extrusion events. Source data are provided as a Source Data file.

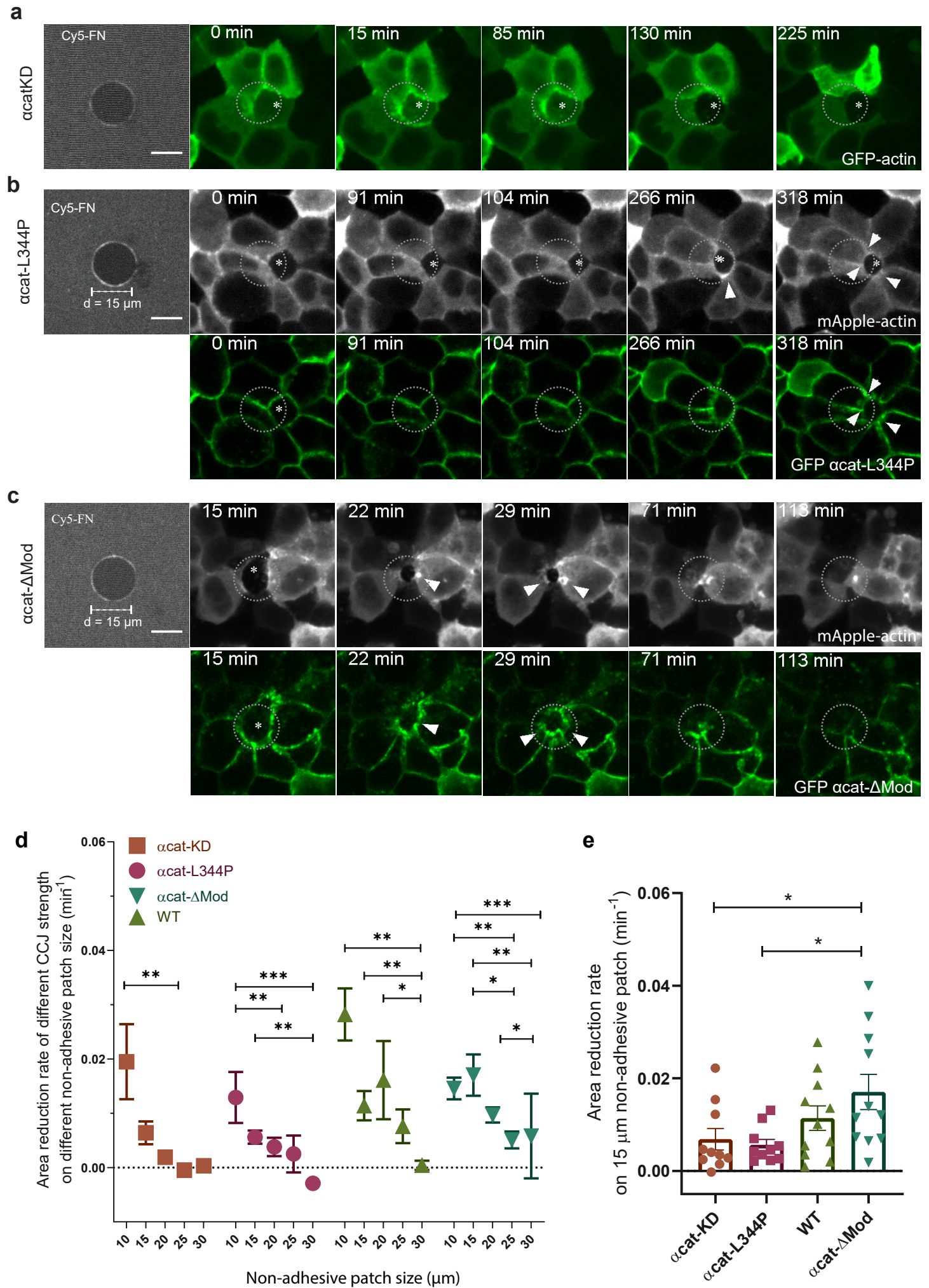
(d & e) Traction force measurement on non-adherent patches. Source data are provided as a Source Data file.

(d) Heat map (left) and kymograph of average total traction magnitude (right) with respected to the center of the non-adhesive patches as a function of time. Color bar indicated the force magnitude (in Pa). Heat map was the average over 3 hours for 5 different extrusions, and kymograph was the average of 5 different extrusions.

(e) Heat map (left) and kymograph of average radial traction force (right) with respected to the center of the non-adhesive patches as a function of time. Color bar indicated the force magnitude (in Pa). +1 indicates forces pointing towards center (inwards) and -1 indicates forces pointing away from center (outwards). Color bar indicated the force magnitude (in Pa). The kymograph was the average of 4-6 different extrusions.

(f) Polar histogram showing the orientation of the force with respected to extrusion site on 10  $\mu\text{m}$ , 15  $\mu\text{m}$  and 30  $\mu\text{m}$  non-adhesive patch. Angle  $\theta$  is the radial angle of the force vector w.r.t the center of non-adhesive patch.  $\theta < 90$  shows inwards pointing forces and  $90 < \theta < 180$  shows outwards pointing forces.

**Fig.6**



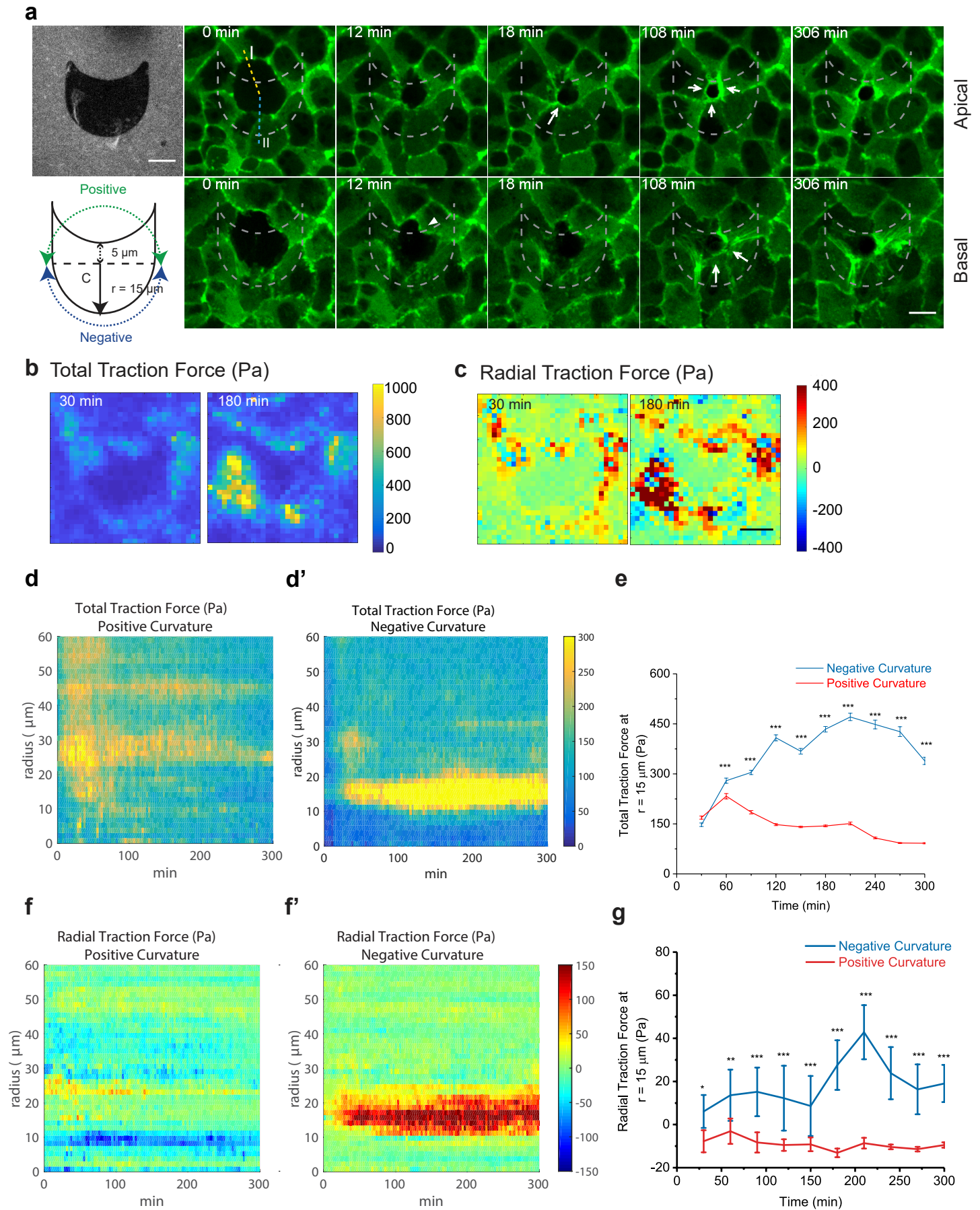
**Fig. 6: Interplay of cell-substrate adhesion and cell-cell junction strength on regulating dual-mechanism modes of extrusion**

(a-c) Representative examples of extrusion of cells with different CCJ strength on 15  $\mu\text{m}$  non-adhesive patches. The first image shows the substrate coated with Cy-5 Fibronectin with darker field represents non-adhesive area. 2<sup>nd</sup> to 5<sup>th</sup> panel: confocal live imaging of (a)  $\alpha\text{catKD}$  cells (b)  $\alpha\text{cat-L344P}$  cells and (c)  $\alpha\text{cat-}\Delta\text{Mod}$  cells. Arrowheads indicated the accumulation of  $\alpha\text{cat}$  at tricellular points corresponding to cable formation. Scale bar = 10  $\mu\text{m}$ .

(d) Quantification of the mean area reduction rate of cell extrusion for the four types of cells on varying non-adhesive patch sizes. Error bars indicated SEM. Non-parametric Kruskal-Wallis test was performed for each group, followed by pair-wise comparisons. Significant level:  $p < 0.05^*$ ;  $p < 0.01^{**}$ ;  $p < 0.001^{***}$ .  $N = 10-28$  for each condition. Source data are provided as a Source Data file.

(e) Quantification of the rate of area closing for the same four types of cells on 15  $\mu\text{m}$  non-adhesive patches. Number of extrusion events: WT:  $N = 10$ ;  $\alpha\text{cat-KD}$ :  $N = 10$ ;  $\alpha\text{cat-L344P}$ : 3:  $N = 10$ ;  $\alpha\text{cat-}\Delta\text{Mod}$ :  $N = 11$ . At least 2 independent experiments were performed for each condition Non-parametric Kruskal-Wallis test ( $p < 0.01^{**}$ ) followed by pair-wise comparison was performed. Significant level:  $p < 0.05^*$ ;  $p < 0.01^{**}$ . Source data are provided as a Source Data file.

**Fig.7**



**Fig. 7: Anisotropic non-adhesive shape dictated preferential mode of extrusion.**

(a) Confocal time-lapse images of cell extrusion on non-isotropic non-adhesive shapes. First panel, first image: substrate coated with Cy5 fibronectin showing non-adherent patches (darker phase). Second panel, first image: schematic of the anisotropic patch design. 2<sup>nd</sup>-5<sup>th</sup> images: time-lapse confocal imaging at apical plane (top panel) and basal plane (bottom panel) of GFP-actin expressing monolayer centered on a cell extruding above a nonadherent patch (non-fluorescent cell). The negatively-curved region induced the earlier formation of contractile actin cables (white filled arrow). Scale bar = 10  $\mu\text{m}$ .

(b-c) Average heat map for total (b) and radial traction forces in Pascal (Pa) (c) averaged over 4 extrusion events (One event each experiment). The force magnitude was color-coded on the color scale bar (in Pa). Scale bar = 10  $\mu\text{m}$ . Source data are provided as a Source Data file.

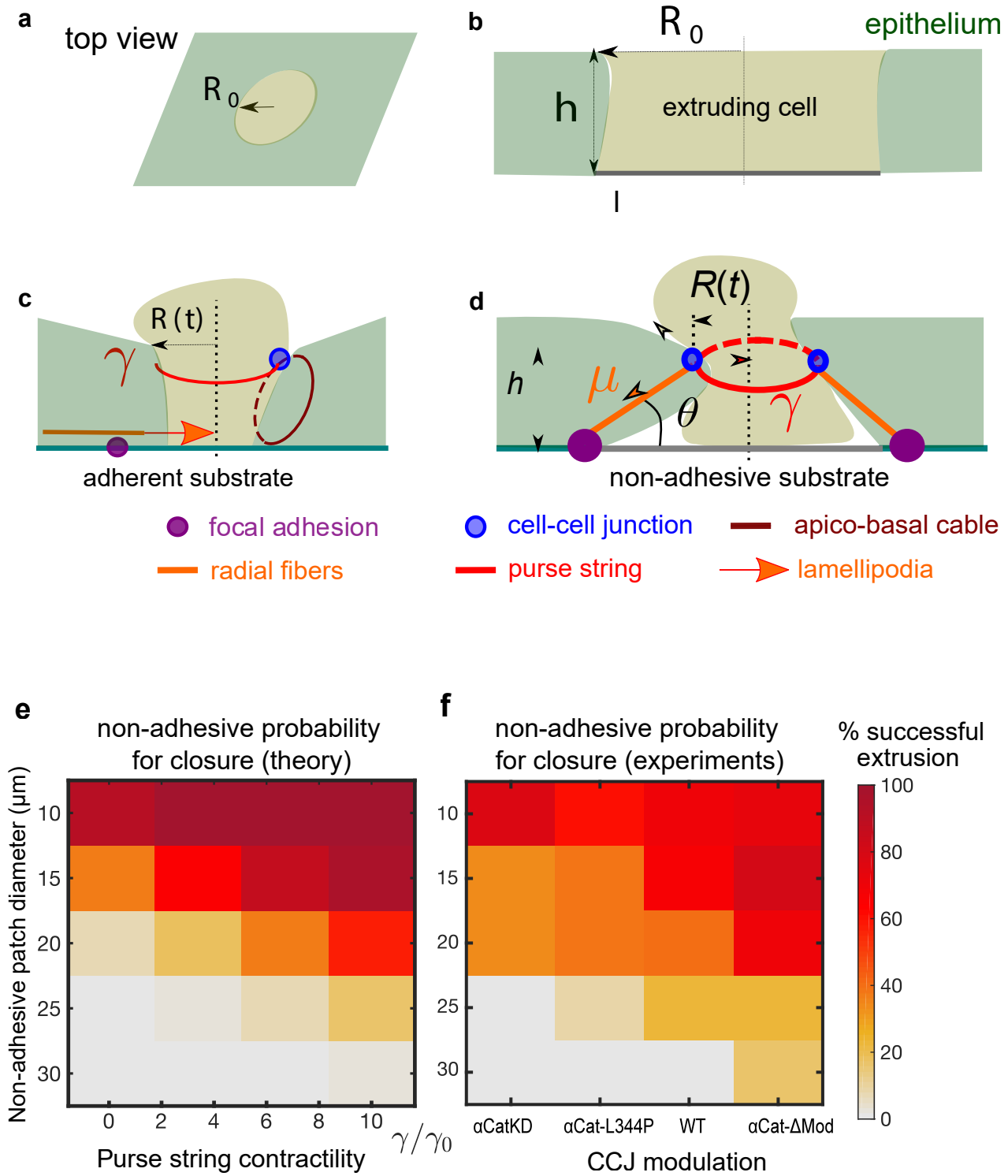
(d-d') Kymographs for total traction forces around extrusion sites for positively-curved (P) regions and negatively-curved (N) regions. The force magnitude was color-coded on the color scale bar (in Pa).  $m = 4$  extrusion events. Source data provided as a Source Data file.

(e) Averaged traction force magnitude at  $r = 15 \mu\text{m}$  (equivalent to the distance between center to negative edges) in function of time.  $n = 20$  averaged force vectors for each time point, averaged from  $m = 4$  extrusion events. Error bars indicate SEM. 2-tailed unpaired t-test was used for comparing between negative curvature and positive curvature.  $p < 0.001^{***}$ . Source data are provided as a Source Data file.

(f-f') Kymographs for radial traction forces around extrusion sites for positively-curved (P) regions and negatively-curved (N) regions. The force magnitude was color-coded on the color scale bar (in Pa).  $n = 20$  averaged force vectors for each time point, averaged from  $m = 4$  experiments. Source data are provided as a Source Data file.

(g) Averaged radial traction force at  $r = 15 \mu\text{m}$  in function of time. Replicates number same as (e). Error bars indicate SEM. 2-tailed unpaired t-test was used for comparison between negative curvature and positive curvature.  $P < 0.001^{***}$ . Source data are provided as a Source Data file.

**Fig.8**





### **Fig.8: Integrative model for extrusion**

(a-d) Sketch of the model.

(a) Side view with the extruding cell (represented as a light green disk) and the surrounding epithelium.

(b) Side view at the initial stage of extrusion with the laser ablated patch (corresponding to one or several cells) of a radius  $R_0$ .

(c) Adherent case: a combination of lamellipodia, apico-basal cable, and non-uniform purse-string contribute to the extrusion process.

(d) Non-adhesive case: a multi-cellular purse-string of tension  $\gamma$  (red circle) contributes to the extrusion dynamics while apico-basal cables (orange line), with a tension denoted  $\mu$ , provide a resistive contribution. Arrows represent the forces acting on a cell-cell junction (represented by a blue circle).

(e) Phase diagram of the predicted percentage of successful extrusions in terms of the non-adhesive patch diameter ( $2 R_0$ ) and of the purse string contractility.

(f) Phase diagram of the experimentally measured percentage of successful extrusions in terms of the non-adhesive patch diameter and of the CCJ perturbation. Number of extrusion events:  $N = 10-28$  for each condition.