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E-cadherin-dependent phosphorylation of EGFR governs a homeostatic feedback loop controlling intercellular junction viscosity and collective migration modes.

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1 **Abstract:**

2

3 Actomyosin tension has been shown to be a ubiquitous driver of tissue morphogenesis^{1, 2}.
4 The Rho pathway, a prominent regulatory network influencing cortical tension, plays a central
5 role in both tissue reorganisation and cell migration³⁻⁶. While viscous dissipation in the actin
6 network is commonly regarded as a constant passive parameter in cell migration in both 2D
7 and 3D contexts, there is limited knowledge concerning the regulation of dissipative forces
8 arising from viscous drag between cells during collective rearrangement. Here, we found that
9 the phosphorylation of Epithelial Growth Factor Receptor (EGFR) downstream of *de novo* E-
10 cadherin adhesion^{7, 8} orchestrates a feedback loop, thereby governing intercellular viscosity
11 via the Rac pathway regulating actin dynamics. Our findings highlight how the E-cadherin-
12 dependent EGFR activity controls the migration mode of collective cell movements
13 independently of intercellular tension. Combining molecular cell biology, micropatterning,
14 and *in silico* simulation, our work suggests the existence of a regulatory loop by which cells
15 can tune junctional actin viscosity, with implications for the phenomenology of
16 morphogenetic movements.

17

18 **Main.**

19

20 We postulated the existence of a feedback loop between cell junction elongation and E-
21 cadherin-dependent phosphorylation of EGFR at junctions. This hypothesis was tested on
22 Madin-Darby Canine Kidney (MDCK) cells with 2D migration in a culture dish. All experiments
23 were conducted using both serum-free and serum-rich media, with consistently similar
24 phenotypes observed, albeit more pronounced effects in serum-free conditions. All
25 presented results pertain to serum-free conditions (**Methods**).

26

27 We first compared the migration of MDCK cells under control conditions and following
28 selective inhibition of EGFR phosphorylation using Erlotinib at 1 μ M. Quantification of cell
29 movement on 2D or 1D line patterns (**Extended Data Fig. 1a, c**) demonstrated that the
30 migration of single isolated cells remained insensitive to EGFR inhibition. Consequently, we
31 ruled out the possibility that EGFR inhibition directly altered cell-substrate interactions as well
32 as their single cell migration potential. In contrast, EGFR inhibition significantly impacted
33 collective cell migration on 2D sparse islets and 1D line patterns (**Fig. 1a, Extended Data Fig.**
34 **1b, d, and Supplementary Video 1**). In the former case, EGFR inhibition markedly reduced
35 the cellular swirling motion of cells by inhibiting EGFR phosphorylation. The velocity at which
36 each contact changes length during migration (**Methods**), exhibited a 2-fold decrease upon
37 inhibition (**Fig. 1b**). Thus, EGFR dephosphorylation reduced the dynamics of cell junction
38 deformation.

39

40 Reciprocally, we impeded the physical deformation of contacts during migration by
41 supplementing the medium with 50 μ g/mL of dextran (5, 50 and 270 kDa) to increase the
42 medium viscosity. We carefully ensured that the addition of dextran induced minimal osmotic
43 shock (**Extended Data Fig. 2a**). In media with higher viscosity (270kDa dextran), the migration
44 speed of individual isolated cells decreased by 28 % (**Extended Data Fig. 2b**). Within cohesive
45 patches consisting of 20 to 50 cells, both collective migration and contact deformation ceased
46 within 10 minutes of adding 50 μ g/mL dextran (**Fig. 1c, Extended Data Fig. 2c and**
47 **Supplementary Video 2**). The individual cell velocity within the patch decreased from $13.7 \pm$

48 0.13 $\mu\text{m}/\text{h}$ (N=1735 cells) in the control group to $8.2 \pm 0.06 \mu\text{m}/\text{h}$ (N=1925) for 270kDa
49 dextran. Immunostaining revealed a significant reduction in the phosphorylation of EGFR at
50 the apical junction (**Extended Data Fig. 2d**). Western blots confirmed a 75% reduction in the
51 phosphorylation of the Src-dependent site Y845 pEGFR⁹, while the other phosphorylation
52 sites (Y1068, Y1173) remained inactive under serum-free conditions (**Fig. 1d**). Our data
53 strongly suggest that the physical arrest of cell junction deformation directly or indirectly
54 leads to the dephosphorylation of apical pEGFR at its Src-dependent site.

55
56 We subsequently investigated whether enhancing pEGFR would favour the dynamics of
57 contact deformation. To do so, we transfected MDCK cells with EGFR coupled to the RUSH
58 system (**Methods**). The RUSH-EGFR construct was sequestered on the ER membrane until
59 released by biotin addition in the culture medium¹⁰. We established a high-confluence, non-
60 polarised (95%) MDCK monolayer, resulting in a mosaic expression of RUSH-EGFR, with small
61 patches of positive cells amid non-expressing control MDCK cells (**Fig. 1e**). Prior to the
62 addition of biotin, the cells displayed limited junctional localization and weak recruitment of
63 apical EGFR (**Supplementary Video 3**). Upon the addition of biotin, the RUSH-positive cells
64 showed a substantial recruitment of EGFR at the cell contacts, leading to a 5-fold increase
65 (from 2.6 ± 0.5 to $14.3 \pm 1.2 \mu\text{m}/\text{h}$) in their junction elongation velocity, quantified using
66 Cellpose neural network segmentation of the cell contours (**Fig. 1e, f and Methods**). In RUSH-
67 positive cells, EGFR localized exclusively to cell-cell contacts within minutes (**Extended Data**
68 **Fig. 3a**). To validate these findings, we repeated the experiment in the presence of Erlotinib
69 ($1\mu\text{M}$). Despite the relocalization of EGFR to the junctions (**Extended Data Fig. 3b and**
70 **Supplementary Video3**), the junction elongation velocity remained as low as the control, with
71 very limited cellular rearrangements. These results strongly suggest that the burst increase in
72 pEGFR at cell-cell contacts favours the dynamics of contact deformation.

73
74 Conversely, we induced the physical elongation of cell junctions by embedding obstacles
75 (non-adhesive disks with a diameter of $200\mu\text{m}$, **Methods**) into high confluence monolayers
76 (**Fig. 1g**). Only the limited number of cell layers that spontaneously elongated and encircled
77 the obstacles displayed deforming junctions (**Fig. 1g and Supplementary Video 4**) and
78 elevated levels of apical pEGFR (**Fig. 1g**), in sharp contrast to the immobile bulk cells (**Fig. 1h**).
79 A treatment with Erlotinib inhibited the elongation and circumrotation of the cells around the
80 obstacle (**Supplementary Video 4**). Taken together, our findings support the hypothesis of a
81 positive feedback loop (**Fig. 1i**) between apical EGFR phosphorylation and junction elongation.
82 We subsequently delved into the molecular mechanisms underlying this phenomenon.

83
84 The absence of the soluble ligand EGF and the specific phosphorylation of Y845 suggested an
85 E-cadherin (Ecad)-dependent activation of EGFR^{7, 8, 11}. Confluent patches of WT-MDCK cells
86 displayed a two-fold decrease in apical pEGFR compared to sub-confluent patches ($27.6 \pm$
87 11.1 A.U. vs 51.6 ± 19.0 A.U.) (**Fig. 2a**). In contrast, Ecad-KO tissues showed consistently low
88 levels of apical pEGFR in both confluent and sub-confluent cases (4.0 ± 2.3 A.U. vs 6.1 ± 3.2
89 A.U.), while still forming cohesive patches (likely due to K-cadherins, quantified in (**Extended**
90 **Data Fig. 4c**) with a proper junctional actin structure. In Ecad-KO MDCK cells with rescued
91 expression of Ecad (Ecad-Res), the pEGFR levels in confluent and sub-confluent tissues
92 returned to their control values (22.2 ± 4.0 A.U. vs 30.2 ± 7.9 A.U.). Furthermore, when EGFR
93 was inhibited by adding Erlotinib ($1 \mu\text{M}$), the apical pEGFR for both confluent and sub-

94 confluent patches in WT-MDCK, Ecad-KO MDCK, and Ecad-Res MDCK all dropped to low levels
95 (**Extended Data Fig. 4a, b**).

96
97 We further validated the direct phosphorylation of EGFR at adherens junctions. We used Total
98 Internal Reflection Fluorescence (TIRF) microscopy to image the live recruitment of cytosolic
99 SH2-Grb2 to the membrane as a proxy for EGFR phosphorylation¹². MDCK cells stably
100 expressing tdEOS-labelled SH2-Grb2 were left to spread on E-Cad coated circular patterns
101 (25µm diameter) (**Fig. 2b and Methods**). SH2-Grb2 dynamically accumulated in elongated
102 structures that dynamically followed the progression of cell edges with a time delay Δt . A
103 parallel experiment using E-cad-GFP MDCK cells revealed a similar accumulation of E-cad in
104 structures with similar time delay Δt (1.3 ± 0.2 min for E-cad-GFP, 1.5 ± 0.2 min for SH2-Grb2)
105 (**Fig. 2b**). Our findings imply that the engagement of E-cad during junction elongation results
106 in transient phosphorylation of EGFR. Consequently, the arrest of cell junction elongation
107 leads to the dephosphorylation of pEGFR, whereas its physical induction promotes EGFR
108 phosphorylation.

109
110 We then scrutinized the alteration of recruitment of actin regulators in the same conditions
111 as above. EGFR phosphorylation is a major regulator of Erk, a kinase extensively implicated in
112 collective cell migration mechanisms¹³. Monitoring Erk activity using Fluorescence Resonance
113 Energy Transfer (FRET) did not reveal any changes following the addition of 50µg/mL dextran
114 to migrating cells (**Extended Data Fig. 5a**). This suggests that Erk signaling is not downstream
115 of EGFR in our experimental context. To further dissect the molecular events, we performed
116 pulldown assays to gauge the activity of Rho family GTPases, which are key regulators of actin
117 dynamics¹⁴. The introduction of dextran to migrating cells resulted in a 28.9% reduction in
118 Rac1 activity, with no discernible effects on Cdc42 and RhoA (**Fig. 2c**). However, the broad
119 nature of pulldown assays made it challenging to distinguish whether Rac1 activity was
120 junctional or lamellipodial. Notably, Wave2, a downstream target of Rac1, was present at the
121 apical side of the junction¹⁵ (**Extended Data Fig. 5b**). Surprisingly, the recruitment of Wave2
122 and Arp2/3, two factors promoting branched actin nucleation, was higher in confluent
123 monolayers (58.0 ± 24.0 A.U. and 91.1 ± 18.8 A.U., respectively) than in sub-confluent patches
124 (43.9 ± 22.9 A.U. and 63.9 ± 11.9 A.U., respectively), a difference that is substantially reduced
125 upon treatment with Erlotinib (1µM) (**Fig. 2d, e and Extended Data Fig. 5b, c**). The amount of
126 phosphorylated Myosin light chain (pMLC) remained unaffected by pEGFR both in confluent
127 and sub-confluent culture conditions (**Fig. 2f and Extended Data Fig. 5d**). Our data advocate
128 for a model wherein the trans binding of new E-cadherin in elongating junctions, increasing
129 the junctional level of pEGFR, subsequently activating Rac1, decreasing levels of Wave2 and
130 Arp2/3 with a constant myosin level, thereby establishing a balance between branched and
131 linear junctional actin. **Fig. 2g** illustrates this feedback homeostatic loop.

132
133 Next, we evaluated the impact of EGFR phosphorylation on the turnover rate of junction actin
134 using Fluorescence Recovery After Photobleaching (FRAP). We compared junctions in
135 confluent monolayers, cells surrounding obstacles, and sub-confluent patches with or without
136 EGFR inhibition (**Fig. 2h and Extended Data Fig. 6a-c**). In all cases, we observed a deceleration
137 in actin turnover when pEGFR levels were lower. Additionally, we probed junctional tension
138 by assessing fast actin recoil following laser ablation (**Fig. 2i**). We did not detect any
139 substantial difference, proving that pEGFR does not regulate tension in this specific context.
140 Finally, we probed the viscoelastic properties of junctions using Atomic Force Microscopy

141 (AFM), revealing an increase in the loss modulus upon EGFR inhibition, with no changes in the
142 elastic modulus (**Extended Data Fig. 6d-f**).

143

144 We previously reported that E-cad-dependent phosphorylation of EGFR in suspended cell
145 doublets increases the velocity of *de-novo* junction formation⁸ and the toughness of their
146 adhesion¹⁶. In all cases, the microscopic dynamics of the actin cortex are associated with a
147 change in cell deformability, with minimal impact on cortical tension. This implies that the
148 homeostasis of junction viscosity is regulated by the Ecad-dependent EGFR phosphorylation
149 loop, effectively “self-lubricating” junction elongation.

150

151 By analogy with the transition from laminar to turbulent flows of fluids at various Reynolds
152 numbers, we monitored how the inhibition of EGFR alters patterns of collective MDCK
153 migration along fibronectin strips (width, 400 μm ; length, 3000 μm) (**Methods**). **Fig. 3a** and
154 **Supplementary Video 5** show the leading region (0-500 μm from the front) of migrating cells
155 in ctrl and Erlotinib conditions over 12 hours. Although migration fronts collectively
156 progressed at similar velocities in both conditions, inhibition of pEGFR abolished the vortices
157 observed in the ctrl case, leading to more laminar flows with enhanced cellular elongation in
158 the direction of migration. We subsequently quantified these qualitative observations. The
159 high level of apical pEGFR in Ctrl was significantly reduced in the inhibitory case (**Fig. 3b**). In
160 the bulk regions (3 mm away from the front), the level of apical pEGFR remained constant in
161 both conditions. We computed the cellular flow lines in the monolayer (**Methods**) to
162 established maps of cell velocities and flow vorticity (**Fig. 3c, d**). Additionally, we used CellPose
163 to segment individual cells (**Methods**) and to quantify the individual level of strain on each
164 cell. pEGFR inhibition resulted in around a 3-fold increase in cellular strain (0.06 ± 0.04 in Ctrl,
165 0.14 ± 0.06 in Erlotinib; $n=1060$ cells) (**Fig. 3e**). While the collective velocity of the migration
166 fronts was not affected by pEGFR inhibition (15.0 ± 4.7 $\mu\text{m}/\text{h}$ in Ctrl, 14.8 ± 2.9 $\mu\text{m}/\text{h}$ in
167 Erlotinib; $N=16$ strips), it substantially reduced the individual cell velocity within the
168 monolayer (from 18.7 ± 2.0 $\mu\text{m}/\text{h}$ to 12.6 ± 2.0 $\mu\text{m}/\text{h}$; $n=182$ cells) (**Fig. 3g**). Likewise, the
169 vorticity of the collective flow (**Fig. 3h**) was reduced by 2-fold (0.70 ± 0.10 h^{-1} to 0.33 ± 0.05 h^{-1} ;
170 $n=182$ cells), and the correlation length of the cell velocity increased by 2-folds
171 (**Supplementary Fig. 4**).

172

173 To further substantiate the hypothesis regarding a change in intercellular viscosity, we
174 inferred the average shape relaxation time t_{visc} of cells within the monolayer. This parameter
175 proved to be a reliable indicator of cellular viscoelasticity in migrating monolayers¹⁷. **Fig. 3i, j**
176 illustrates the analysis procedure. Initially, we segmented phase-contrast images of the
177 monolayer at a specific time point. Subsequently, we computed the average initial cell strain
178 on a coarse-grained grid (**Methods**) and used an optic flow method to estimate the flow lines
179 (**Methods**). The evolution of local cellular strain was then evaluated using the approach
180 depicted in **Fig. 3i** and elaborated on in the **Supplementary Information**. The only free
181 parameter in this equation is the intrinsic strain relaxation time t_{visc} , which does not depend
182 on the shear level experienced by the cells. By utilizing the strain map at $t = 0$ and solving the
183 equation along the flow lines, we inferred the final strain map at a later time point (10 h). We
184 varied t_{visc} to maximise the correlation between the observed and measured strain maps. The
185 best fits lead to $t_{\text{visc}} = 75 \pm 15$ min ($R^2=0.4\pm 0.04$; $N=3$) for the control group and $t_{\text{visc}} = 210 \pm 65$
186 min ($R^2=0.34\pm 0.2$; $N=3$) for the pEGFR inhibition group (**Fig. 3k, l**). In the control conditions,
187 cells exhibited shape relaxation when advected in swirling vortices, while under pEGFR

188 inhibitory conditions, they elongated more in directed laminar, plug-like flows
 189 (**Supplementary Video 6**). As pEGFR inhibition did not affect the single-cell migration
 190 (**Extended Data Fig. 1a, c**), the approximately 3-fold increase in cell shape relaxation times
 191 supports the hypothesis that the level of pEGFR controls viscous dissipation in cell-cell
 192 junction elongation.

193

194 Finally, we designed a vertex model with viscosity (**Supplementary Information**) to
 195 demonstrate that a modulation of intercellular viscosity can account quantitatively for our
 196 observations. In our model, the force balance at each tri-cellular junction is expressed as
 197 follows:

198

$$199 \quad \underbrace{\mathbf{F}_i^{(\text{elastic})}}_{\text{elasticity}} + \underbrace{\mathbf{F}_i^{(\text{active})}}_{\text{activity}} + \underbrace{\mathbf{F}_i^{(\text{friction})}}_{\text{friction}} + \underbrace{\mathbf{F}_i^{(\text{viscous})}}_{\text{viscosity}} = \mathbf{0}$$

200 The first 3 terms are standard for vertex models^{18, 19} and correspond to: $\mathbf{F}_i^{(\text{elastic})}$: the elastic
 201 forces accounting for the mechanical regulation of the cell shape²⁰⁻²³, that are assumed to
 202 derive from a mechanical energy E :

203

$$E = \underbrace{\sum_J \frac{1}{2} K_A (A_J - A_0)^2}_{\text{area elasticity}} + \underbrace{\sum_J \frac{1}{2} K_P (P_J - P_0)^2}_{\text{perimeter elasticity}},$$

204 where the two terms account for the cell area elasticity and the cell perimeter elasticity,
 205 respectively. In detail, K_A and K_P are the area stiffness and perimeter stiffness of cells,
 206 respectively; A_J and P_J are the area and the perimeter of the J -th cell, respectively; A_0 and P_0
 207 are the preferred area and preferred perimeter, respectively.

208 $\mathbf{F}_i^{(\text{active})}$ corresponding to the active fluctuations of the cortical tension, assumed to be a
 209 Gaussian white noise of amplitude Λ (**Supplementary Information**), and

210

$$\mathbf{F}_i^{(\text{friction})} = -\xi \mathbf{v}_i$$

211 corresponding to the friction of the cells on the underlying substrates.

212 We complemented this description by adding a viscous dissipation term accounting for
 213 cortical deformation and cytoplasmic flows, which reads:

214

$$\mathbf{F}_i^{(\text{viscous})} = \sum_j \eta_{(i,j)} t_{(i,j)} \cdot (\mathbf{v}_j - \mathbf{v}_i) t_{(i,j)}$$

215 where $t_{(i,j)}$ is a unit vector oriented between the vertex i and j , which is either another vertex,
 216 in which case $\eta_{(i,j)} = \eta^{(s)}$ is the viscous modulus dissipation along the cell surface (cortex), or
 217 the cell barycenter, in which case $\eta_{(i,j)} = \eta^{(b)}$ is a viscous modulus, representing dissipation
 218 within the cell bulk (cytoplasm) (details in the **Supplementary Information**).

219

220 First, we conducted simulations for the RUSH-EGFR experiment (**Fig. 1e, f**), considering a
 221 down-step in intercellular viscosity on selected clusters of cells (N=4 cells) dispersed in a cell
 222 monolayer at equilibrium (**Fig. 4a and Supplementary Information**). We used a set of
 223 parameters summarised in **Supplementary Table I**, which were optimised to quantitatively
 224 reproduce the experimental results, while remaining within the typical range used to describe
 225 MDCK monolayers. In the simulations, a 10-fold decrease (from $\eta=1.4 \text{ nN}\cdot\text{min}\cdot\mu\text{m}^{-1}$ (30 a.u)
 226 to $\eta=0.14 \text{ nN}\cdot\text{min}\cdot\mu\text{m}^{-1}$ (3 a.u)) in intercellular viscosity leads to a 4-fold increase (from $2.6 \pm$
 227 0.1 to $10.6 \pm 3.7 \mu\text{m}/\text{h}$; N=100) in the junction elongation velocity (**Fig. 4b, c and**

228 **Supplementary Video 7**), aligning closely with the experimental values (from 2.6 ± 0.5 to 14.3
229 $\pm 1.2 \mu\text{m/h}$) (**Fig. 1f**).

230

231 Second, we conducted simulations of collective cell migration along the strips (details in
232 **Supplementary Information**). We maintained the same set of parameters while introducing
233 additional conditions: (1) we posited that the inhibition of EGFR by Erlotinib leads to
234 intracellular viscosity equivalent to its downregulation in dense monolayers, given their
235 comparable recruitment levels. We hence set it to $\eta = 1.4 \text{ nN}\cdot\text{min}\cdot\mu\text{m}^{-1}$ (30 a.u) and (2) we
236 imposed the velocity of the migration front to align with experimental values of $15 \mu\text{m/h}$ (**Fig.**
237 **3g**). Subsequently, we tuned the intercellular viscosity of the Ctrl case to match the
238 experimental results, finding that $\eta = 0.28 \text{ nN}\cdot\text{min}\cdot\mu\text{m}^{-1}$ (6 a.u) yielded the best quantitative
239 predictions. The **Supplementary Video 7** shows a typical simulation output (**Fig. 4d-f**). Both
240 experimental and simulated data underwent analysis using the same scheme. Notably, a
241 sizeable increase in cell strain (**Fig. 4g**), a decrease in cell velocity (**Fig. 4h**), a reduction in
242 vorticity (**Fig. 4i**), an expansion in spatial correlation length (**Supplementary Fig. 4**), as well as
243 an elevation in cell shape relaxation times t_{visc} (**Fig. 4j, k**) were observed between $\eta = 0.28$
244 $\text{nN}\cdot\text{min}\cdot\mu\text{m}^{-1}$ (6 a.u) au (Ctrl) and $\eta = 1.4 \text{ nN}\cdot\text{min}\cdot\mu\text{m}^{-1}$ (30 a.u) (Erlotinib). These quantitative
245 findings closely matched the experimental observations (**Fig. 4l**).

246 In conclusion, we propose that the E-cadherin-dependent phosphorylation of EGFR fine-tunes
247 the structure of junctional actin, thereby affecting actin dynamics. On a larger scale, it
248 influences junctional viscosity, governing the collective modes of cell migration (**Fig. 2g**). This
249 insight demonstrates that E-cadherin-dependent EGFR activity could regulate the dynamics
250 of collective cell behavior and sheds light on the role of cellular viscous dissipation in collective
251 cell migration, an important aspect that has been understudied.

252

253 **Methods**

254 **Cell culture and reagents**

255 MDCK strain II cells were cultured at 37°C with 5% CO₂ in high-glucose Dulbecco's Modified
256 Eagle Medium (DMEM, Invitrogen). The medium was supplemented with 10% fetal bovine
257 serum (FBS, Invitrogen), and 100 units/mL of penicillin and 100 µg/mL of streptomycin (Pen-
258 strep, Invitrogen).

259 To investigate collective migration behaviours, we utilised stable cell lines expressing
260 fluorescent markers or knockout variants of MDCK cells. The following cell lines were
261 employed: wild-type MDCK (MDCK-WT), stably transfected GFP–actin MDCK (MDCK-actin-
262 GFP), stably transfected GFP–E-cadherin MDCK (MDCK-E-cad-GFP) (kindly provided by W. J.
263 Nelson), histone-1–stable GFP MDCK (MDCK-H1-GFP), E-cad KO MDCK (MDCK-Ecad KO)
264 (kindly provided by B. Ladoux, Institut Jacques Monod), and E-cad Rescue MDCK (MDCK-Ecad
265 Res) (kindly provided by P. Kanchanawong, Mechanobiology Institute).

266 For serum starvation experiments, cells were subjected to serum starvation by incubating
267 them in a growth medium. This medium consisted of high-glucose DMEM lacking FBS,
268 supplemented with 100 units/mL of penicillin and 100 µg/mL of streptomycin. To inhibit EGFR
269 activity, Erlotinib hydrochloride (1µM, Sigma-Aldrich) was employed.

270

271 **Plasmids and transfection**

272 The Str-KDEL_SBP-EGFP-EGFR plasmid was generously provided by Dr. David Marc Virshup's
273 laboratory at Duke NUS. The "SH2-GRB2-tdEOS" plasmid was kindly gifted by Dr. Jay T Groves'
274 Lab. MDCK-WT cells, with 80% confluence, were transfected with 3 µg of DNA using the Neon
275 electroporation system (Invitrogen), following the manufacturer's instructions. For Erk
276 activity measurement, EKAREV-NLS expressing MDCK cells were a kind gift from Dr. Tsuyoshi
277 Hirashima's Laboratory at Mechanobiology Institute, NUS.

278

279 **Stamp preparation for collective cell migration on line-patterned strips**

280 Master molds featuring the desired pattern were crafted using SU8-3050 resist on silicon
281 wafers through standard lithography techniques. The pattern employed in this study
282 encompasses a sizable rectangular "reservoir" (approximately 5000 x 700 µm),
283 interconnected with 10 rectangular strips (around 3000 x 400 µm each)²⁴. Subsequently,
284 Polydimethylsiloxane (PDMS) stamps were derived from these wafers and utilised for
285 microcontact printing.

286 The PDMS stamps were incubated with Fibronectin (50µg/ml, Merck) for a duration of 45
287 minutes, after which they were transferred onto a 35 mm uncoated imaging dish (Ibidi) via
288 microcontact printing. Prior to Fibronectin stamping, the dish had been pre-coated with a
289 layer of PDMS and exposed to UV light for activation. The PDMS stamps were then air-dried
290 within a laminar hood for 10 minutes and delicately pressed against the dish's bottom for 1

291 minute. Following microcontact printing, the PDMS stamps were carefully lifted without
292 causing any agitation. The dish bearing the Fibronectin-stamped pattern underwent
293 additional passivation by treating it with a 2% Pluronic F127 solution (Sigma) for 1 hour, aimed
294 at preventing cells from attaching and proliferating in the unstamped areas. Subsequent to
295 passivation, the dishes underwent thorough rinsing with PBS on three times. A PDMS block
296 was strategically positioned atop the microcontact-printed pattern, effectively confining cells
297 within the "reservoir" region. MDCK-H1-GFP, MDCK-Ecad KO, or MDCK-Ecad Res cells were
298 pre-treated with Mitomycin C at a concentration of 10 μ g/ml (Roche) for a duration of 1 hour
299 to inhibit cell proliferation. These MDCK cells were trypsinised and strategically seeded along
300 the periphery of the PDMS block to cover the "reservoir" area. Once the cells reached
301 confluence on the PDMS block's sides, the block was gently released, enabling cells to migrate
302 along the strips. Migrating cells were subsequently subjected to specific inhibitors as
303 indicated. The process of live imaging was executed using widefield microscopy (Olympus
304 IX81) with a 10x objective. Throughout imaging, the dishes were maintained within a
305 humidified environment at 37°C with 5% CO₂. Both phase-contrast and fluorescent images
306 were acquired at intervals of 4 minutes over a duration ranging from 12 to 24 hours.

307

308 **Obstacle migration**

309 The design employed for obstacle migration involves a substantial rectangular "reservoir"
310 (approximately 5000 x 700 μ m), which is linked to 10 rectangular strips (around 3000 x 400 μ m
311 each). Each strip features a central circle with a diameter of 200 μ m. The PDMS stamps crafted
312 from these wafers encompass 200 μ m diameter circles within each strip. The subsequent
313 preparation steps remain consistent with those outlined in the preceding section. Following
314 contact printing and passivation, the 200 μ m diameter circles exhibit non-adhesive properties,
315 serving as obstacles during cell migration.

316

317 **Single-cell without confinement, single-cell confined to lines, cell trains and cell patches migration**

319 For the migration of single cell lines, wafers with a pattern of 20 μ m lines were utilised. PDMS
320 stamps were crafted from these wafers and subsequently employed for microcontact
321 printing. The preparation steps mirrored those outlined in the preceding section. Following
322 passivation, the dishes were primed for cell seeding. MDCK-H1-GFP or MDCK-WT cells were
323 trypsinised and their counts were determined prior to seeding. Approximately 4-5 x10⁴ MDCK
324 cells were introduced into the imaging dish and allowed to incubate for 2 hours at 37 °C within
325 a 5% CO₂ incubator, facilitating full cellular spreading. The migrating cells were then subjected
326 to the indicated inhibitors.

327 For the migration of cell trains, the employed pattern comprises a large rectangular
328 "reservoir" (approximately 5000 x 700 μ m), which is linked to 20 rectangular strips
329 (approximately 3000 x 20 μ m). The preparation steps mirror those outlined in the preceding
330 section for the creation of line-patterned strips. The migrating cells were subjected to
331 treatment using the specified inhibitors.

332 In the case of single-cell and cell patch migration, Fibronectin-coated dishes were utilised for
333 direct cell seeding. A quantity of $4-5 \times 10^4$ MDCK cells (for single cells) and $3-4 \times 10^5$ MDCK
334 cells (for cell patches) were seeded into the imaging dish and incubated at 37 °C within a 5%
335 CO₂ incubator for 2 hours to allow for complete cell spreading. The migrating cells were
336 subsequently treated with the indicated inhibitors.

337 Live imaging was conducted using widefield microscopy (Olympus IX81) with either a 10x or
338 20x objective. The dishes were positioned within a humidified chamber at 37 °C with 5% CO₂
339 during the imaging process. Phase-contrast and fluorescent images were captured at 10-
340 minute intervals, spanning a duration ranging from 12 to 24 hours. Migration speeds of
341 individual cells ($n > 30$) were tracked using either the TrackMate plugin for Image J on phase-
342 contrast images or Imaris for fluorescent nucleus images. The speed of junction deformation
343 at cell-cell junctions was quantified by measuring the lengths of these junctions at each time
344 point.

345

346 **Dextran experiments**

347 MDCK-H1-GFP cells (for live imaging) or MDCK-WT cells (for western blotting) were seeded at
348 a low confluence and subjected to overnight serum starvation. Subsequently, 50µg/mL of
349 dextran (00269, 00891, 00894, Sigma-Aldrich) with the specified molecular weight was
350 introduced to the cells before the execution of either western blotting or live imaging. The
351 latter was performed using a widefield microscopy setup (Olympus IX81) equipped with a 20x
352 objective. Cell velocity and confinement ratio were assessed using the Trackmate plugin
353 within Fiji.

354

355 **EGFR release experiment**

356 MDCK-WT cells were transfected with the RUSH plasmid Str-KDEL_SBP-EGFP-EGFR. Following
357 transfection, the cells were plated onto an Ibidi imaging dish pre-coated with Fibronectin and
358 placed in a complete medium at 37°C with 5% CO₂. Once the cells reached confluency,
359 overnight serum starvation was conducted. Subsequently, EGFR-GFP was liberated from the
360 endoplasmic reticulum (ER) through the addition of 40mM biotin. Live imaging was carried
361 out utilising a spinning-disc confocal microscope (Yokogawa CSU-W1) attached to a Nikon
362 Eclipse Ti-E inverted microscope body, equipped with a 60x NA1.3 water lens. Fluorescent
363 images were captured both prior to and subsequent to the biotin introduction, at 5-minute
364 intervals, spanning a duration of 6 hours. The speed of deformation at cell-cell junctions was
365 quantified by measuring the lengths of these junctions at each time point, both 30 minutes
366 before and 30 minutes after the release of EGFR. The measurements were then averaged over
367 this 30-minute period.

368

369 **Western blotting**

370 Cells were incubated on ice with RIPA lysis buffer (Sigma), supplemented with protease and
371 phosphatase inhibitor cocktails (Sigma). Subsequently, lysates underwent SDS-PAGE and
372 were transferred onto nitrocellulose membranes. The membranes were then blocked using
373 5% BSA and 0.05% Tween 20 in TBS, followed by incubation with the specified primary
374 antibodies. Detection of immune complexes was achieved using appropriate HRP-conjugated
375 secondary antibodies (Cell Signaling Technologies) and an enhanced chemiluminescence
376 reagent (Clarity ECL, BioRad). Protein band intensities were quantified using ImageJ Software.
377 The primary antibodies employed were pEGFR^{Y845} (44784G, Thermo Fisher Scientific),
378 pEGFR^{Y1068} (2234, Cell Signaling Technologies), pEGFR^{Y1173} (4407, Cell Signaling
379 Technologies), EGFR (2232, Cell Signaling Technologies), RhoA (sc418, Santa Cruz), Cdc42
380 (ab187643, Abcam), Rac1 (610651, BD Biosciences) and β -actin (MA515739, Thermo Fisher
381 Scientific).

382 To assess Rho family GTPases activity, pull-down assay using GST-PBD and GST-RBD were
383 performed on cell lysates as described previously²⁵.

384

385 **Immunofluorescence**

386 After a collective cell migration period of 12-24 hours, MDCK cells were fixed using pre-
387 warmed 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) at 37 °C for 15
388 minutes. Subsequently, they were permeabilised with 0.2% Triton X-100 in TBS for 30 minutes
389 at room temperature. Samples were then blocked using 1% BSA in TBS for 1 hour. The cells
390 were incubated overnight at 4 °C with primary antibodies: rabbit anti-phospho-EGFR (Y845)
391 polyclonal antibody (44-784G, Thermo Fisher Scientific, diluted 1:200); Purified Mouse anti-
392 E-Cadherin monoclonal antibody (Clone 36) (610181, BD Transduction Laboratories); WAVE2
393 antibody (H-110) (sc-33548, Santa Cruz); Arp3 antibody (A5979, Sigma-Aldrich); Phospho-
394 Myosin Light Chain 2 (Ser19) antibody (3671, Cell Signaling Technologies, diluted 1:50)
395 according to the manufacturer's instructions. Following three washes with PBS at 10-minute
396 intervals, cells were incubated with secondary antibodies (anti-mouse Alexa555-conjugated
397 secondary antibody and anti-Rabbit Alexa647-conjugated secondary antibody, both from
398 Thermo Fisher Scientific) along with Alexa405-coupled phalloidin (Invitrogen) in darkness at
399 room temperature for 1 hour. Subsequently, cells were rinsed with PBS and prepared for
400 imaging acquisition. Confocal images were captured in 3D stacks using a spinning-disc
401 confocal microscope (Yokogawa CSU-W1) mounted on a Nikon Eclipse Ti-E inverted
402 microscope body, equipped with a 100x NA1.5 or 60x NA1.3 lens.

403

404 **Real-time quantitative PCR (qPCR)**

405 The entire RNA was isolated from a single well of a 6-well plate using the RNeasy Plus Micro
406 Kit (QIAGEN), following the guidelines provided by the manufacturer. A quantity of 450ng of
407 total RNA was employed to generate the cDNA utilising the cDNA synthesis kit (SensiFAST™).
408 For qPCR analysis, the FastStart Universal SYBR Green Master (ROX) mix was employed on a
409 CFX96 Touch Real-Time PCR detection system (Bio-Rad). GAPDH was employed as the internal
410 reference gene.

411

412 **Live cell spreading on E-cadherin-coated surface**

413 Disks with a diameter of 25µm were photopatterned onto glass coverslips, following the
414 previously described method²⁶. These patterns were then coated overnight at 4°C with a
415 recombinant E-cadherin Fc Tag protein (10204, Sino Biological) at a concentration of
416 20µg/mL, and subsequently gently washed with PBS. The patterned coverslips were mounted
417 within imaging chambers.

418 MDCK-WT cells were transfected with the SH2-GRB2-TdEos plasmid, and after selection with
419 500 µg/mL Geneticin (10131035, Thermo Fisher Scientific), a stable cell line was established
420 following sorting using an SH800S cell sorter (Sony). MDCK-SH2-GRB2-TdEos or MDCK-Ecad-
421 GFP cells were serum-starved overnight, then seeded onto Ecad-Fc patterns, and allowed to
422 spread for a period of 2 hours before initiating TIRF time-lapse imaging. This imaging was
423 carried out using a Motorised TIRF Module (Nikon) integrated with a Nikon Eclipse Ti-E
424 inverted microscope body.

425

426 **Fluorescence recovery after photobleaching (FRAP)**

427 Bleaching was performed on the actin of apical cell-cell junctions for FRAP measurements.
428 The cortical actin recovery time (t_{half}) was calculated by fitting the following exponential
429 function to the recovery curves:

$$430 \quad I(t) - I(0) = (I_{\infty} - I(0)) \left(1 - \exp\left(-\ln 2 \frac{t}{t_{half}}\right) \right)$$

431

432 **Laser ablation**

433 An initial image was acquired to determine the precise laser spots. In the case of MDCK-actin-
434 GFP cells, the laser spots were positioned at the apical cell-cell junctions. The pre-acquisition
435 process involved capturing five images at 1-second intervals. During the acquisition phase, a
436 laser power of 60% was utilised, with a duration of approximately 1-2 seconds, targeting the
437 predetermined regions. Subsequently, the post-acquisition stage encompassed the capture
438 of 100 images at 1-second intervals.

439 To quantify the recoil velocity, the positions of two nodes within the defined junctions were
440 manually tracked using ImageJ software. Following the laser ablation, the temporal evolution
441 of the distance between these two nodes was fitted using a single exponential function. While
442 the double exponential function is commonly employed in other studies, it proved unsuitable
443 for our analysis. In our investigation, the recoil velocity exhibited a gradual nature, and fitting
444 it with a double exponential function yielded unrealistically high speeds. Consequently, we
445 opted to employ a single exponential function for a more appropriate representation.

446

447 Atomic force microscopy (AFM)

448 AFM experiments were conducted using a Nanowizard IV BioAFM system manufactured by
449 JPK Instruments, Germany. Indentations were performed on randomly chosen cells at the
450 junctional regions. This was achieved using a cantilever (with a nominal k value of 0.03 N/m,
451 provided by Novascan Technologies, Inc., Ames, IA) with an attached polystyrene bead (4.5
452 μm diameter) at its tip. The applied force was set at 3 nN and a loading rate of 5 $\mu\text{m/s}$ was
453 used.

454 For each experimental condition, measurements were taken from over 30 cells across three
455 independent trials and subsequently averaged. Young's modulus values were used to
456 quantitatively describe the cellular stiffness. These values were calculated using the JPK Data
457 Processing Software (JPK Instruments, Germany), which incorporates Hertz's contact model
458 tailored to spherical indenters (with a diameter of 4.5 μm and a Poisson's ratio of 0.5). Energy
459 dissipation, representing the heat-based loss of mechanical energy during each indentation
460 cycle by the AFM tip, was determined by assessing the enclosed area between the approach
461 and retraction curves (hysteresis). This phenomenon is largely attributed to frictional and
462 viscous damping within the cell structure at this low speed²⁷.

463

464 Erk activity measurement

465 30000 EKAREV-NLS expressing cells were seeded into a well of culture-inserts 2 wells (81176,
466 ibidi) and allowed to spread for 8 hours. Simultaneously, the insert was then removed and
467 cells were serum starved overnight. Time-lapse FRET images were obtained using a Nikon AX
468 point scanning confocal microscope mounted on a Ti-2 Nikon inverted body. To represent the
469 FRET efficiency, FRET/CFP ratio images were generated after the background was subtracted
470 from the original images in the CFP and FRET channel using a matlab code kindly provided by
471 Tsuyoshi Hirashima's Laboratory at Mechanobiology Institute, NUS. To quantify FRET Ratio
472 for each cells at each timepoints, the Fiji Trackmate plugin was applied to the CFP channel to
473 track each cell position overtime.

474

475 Segmentation

476 We used Cellpose²⁸ for the cell segmentation. We performed an erosion with a 3x3 square
477 kernel to each mask to limit the occurrence of gaps between cells; we disregard objects with
478 areas lower than 20 pixels. We define the cell inertia tensor, with a constant linear weight
479 density along the segmented cell boundaries, i.e. with xx component $I_{xx} = \iint (x - \bar{x})^2 dx dy$,
480 where (\bar{x}, \bar{y}) is the position of the cell barycenter. We call *average shape tensor* field the
481 spatially and temporally averaged inertia matrix over all cells within 30x30 pixel-large boxes
482 (corresponding to approx. 10 cells within each), regularly spaced on a spatial grid. The strain
483 field is defined as $\epsilon = \log(\lambda_1/\lambda_2)/2$ where λ_1 (resp. λ_2) is the maximum (resp. minimum)
484 eigenvalue of the average shape tensor. For the cell tracking, we used bTrack²⁹.

485

486 Simulation of EGFR release experiments

487 In our EGFR release experiments, cells under investigation are located in the bulk of the tissue,
488 far away from the boundary. In this case, we observe fluctuations in cell edge length but no
489 obvious cell motions, see **Fig. 1e**. To mimic such fluctuations in cell length, we here consider
490 active fluctuations of intercellular tension, $\Lambda_{ij}^{(\text{act})}$, at each cell-cell interface ij . These
491 fluctuations contribute to an active force at each vertex,

$$492 \quad \mathbf{F}_i^{(\text{active})} = \sum_{j \in \text{neighbor}} \Lambda_{ij}^{(\text{act})} \mathbf{t}_{i,j},$$

493 where the summation is over all vertices that connect to the vertex i . We assume such tension
494 fluctuations satisfy an Ornstein-Uhlenbeck stochastic dynamic with time correlation³⁰,

$$495 \quad \frac{d\Lambda_{ij}^{(\text{act})}}{dt} = -\frac{\Lambda_{ij}^{(\text{act})}}{\tau_\sigma} + \zeta_{ij}(t),$$

496 where τ_σ is the relaxation time of the active tension and $\zeta_{ij}(t)$ are independent Gaussian
497 white noises, satisfying $\langle \zeta_{ij}(t) \rangle = 0$ and $\langle \zeta_{ij}(t) \zeta_{kl}(t') \rangle = \Delta_\sigma^2 \delta_{ik} \delta_{jl} \delta(t - t')$ with Δ_σ being
498 the fluctuation intensity.

499 We simulated a cell sheet consisting of $N = 100$ cells in a square box of size $L = \sqrt{NA_0}$, using
500 periodic boundary conditions, see **Supplementary Fig. 1a**. We initialize our simulations from
501 a random Voronoi cell pattern and let the system relax toward a dynamic steady state where
502 the cell elongation parameter and cell motion velocity approach a steady plateau²².

503 To model the effect of the light activation of EGFR and the possibility of a subsequent viscosity
504 modulation, we then randomly selected a small group of four cells in contact and decreased
505 the bulk viscosity $\eta_j^{(b)}$ of those four cells, from the default value (with $\eta_j^{(b)}(t < 0) = \eta^{(\text{CTL})}$)
506 to a lower value (with $\eta_j^{(b)}(t > 0) = \eta^{(\text{EGFR+})}$), see **Supplementary Fig. 1b**. Further, the
507 viscosity $\eta_{ij}^{(s)}$ along the cell-cell interface between the vertices i and j is assumed to be the
508 average viscosity of the two contacting cells (indexed by J and K) as $\eta_{ij}^{(s)} = (\eta_J^{(b)} + \eta_K^{(b)})/2$.

509 We compare the junction remodeling velocity, \dot{l} , before and after the drop in viscosity. We
510 find that the ratio of the junction remodeling velocity $\dot{l}_{\text{after}}/\dot{l}_{\text{before}}$ increases with the ratio of
511 the cell viscosity decrease, $\eta^{(\text{CTL})}/\eta^{(\text{EGFR+})}$ (**Supplementary Fig. 2**). In particular, the data of
512 $\eta^{(\text{CTL})}/\eta^{(\text{EGFR+})} = 10$ agree with our experiments (**Supplementary Fig. 2**).

513 We provide the default parameter values for such simulations in **Supplementary Table I**.

514

515 Simulation of collective cell migration experiments

516 To simulate the collective cell migration experiments, we now turn to collective cells initially
 517 confined in a rectangular geometry of size $L_x \times L_y$ with $L_x = 18\sqrt{A_0}$ and $L_y = 100\sqrt{A_0}$,
 518 which contains around $N_{\text{cell}} \approx 1800$ cells, see **Supplementary Fig. 3**.

519 As in the RUSH-EGFR model simulation, we first initialize the simulations using a Voronoi
 520 tessellation. We then let the system relax, keeping the bottom boundary fixed and simulating
 521 the cell sheet in a confined rectangular geometry to reach a steady state. We next relax the
 522 bottom boundary and run the simulations to reach a dynamic steady state.

523 At the left, top, and right borders, cells are allowed to slip along but adhere to the boundaries;
 524 while at the bottom boundary, we imposed vertices to move at a constant speed

$$525 \quad v_y(\text{bottom boundary vertices}) = -V_{\text{front}}, \quad v_x(\text{bottom boundary vertices}) = 0.$$

526 The value of V_{front} is fixed at a comparable value to the one measured in experiments.
 527 Specifically, we set $V_{\text{front}} = 15 \mu\text{m/h}$ in simulations.

528 With the sole migration at the edge (described above), we were not able to recapitulate the
 529 formation of vortices similar to the one observed in experiments.

530 To recapitulate the formation of vortices similar to the one observed in experiments, we
 531 turned to a Vicsek-like model of cell motility²³. Within such model, we associate each cell with
 532 an active force $\mathbf{F}_J^{(\text{act})} = T_0(\cos\theta_J, \sin\theta_J)$ of magnitude T_0 and direction θ_J ; such model
 533 mimics the cell motility induced by cell protrusions²³, with the polar direction θ_J of each cell
 534 J evolving according to the equation:

$$535 \quad \frac{d\theta_J}{dt} = \frac{1}{n_J} \sum_{K \in \text{neighbor}} \left\{ \mu_{LA} \sin[\theta_K^{(\text{vel})} - \theta_J] + \mu_{CIL} \sin(\alpha_{J,K} - \theta_J) \right\} + \zeta_J(t)$$

536 where μ_{LA} and μ_{CIL} represent the strengths of local alignment interaction and contact
 537 inhibition of locomotion, respectively; $\theta_K^{(\text{vel})} = \arg(\mathbf{v}_K)$ refers to the argument angle of the
 538 velocity \mathbf{v}_K of cell K ; $\alpha_{J,K} = \arg(\mathbf{r}_J - \mathbf{r}_K)$ denotes the argument angle of the vector pointing
 539 from cell K to cell J ; $\zeta_J(t)$ is a white-noise process with zero mean and variance $2D_r$. For the
 540 cells at the free boundary, we constrain their polar active force direction θ_J to orient normally
 541 to the free boundary and toward the free space.

542 To mimic cell flows from the top boundary (bulk region of MDCK cell sheet), we allow cell
 543 divisions on a top region which is within a distance $d < 5$ cell length to the top boundary, see
 544 **Supplementary Fig. 3**. We perform cell divisions once cells within such a region exceed an
 545 area threshold $A_{\text{div}} = 1.5A_0 = 486\mu\text{m}^2$.

546 We provide the default parameter values for such simulations in **Supplementary Table II**.

547 We are interested in and examine the collective cell dynamics in a region near the moving
 548 front (within a distance of ~ 30 cell length to the moving front). Note that to reduce the
 549 artificial effect of the top boundary condition on collective cell migration dynamics in a region

550 near the moving front, we have set a sufficiently large scale of the simulated cell monolayer
551 in the vertical direction, i.e., $L_y \sim 100$ cell length.

552

553 **Data display and statistics**

554 Prism (GraphPad Software) and Matlab (Math Works) were used for data analysis and graph
555 plotting. Graphs were mounted using Adobe Illustrator. ANOVA test and paired or unpaired
556 Student's t-test were carried out to analyse the significant difference levels.

557

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566

567 **Author contributions:**

568 Fu Chaoyu performed the migration experiments, their quantification and wrote the
569 manuscript. F. Dilasser performed the single cell experiments and the dextran experiment,
570 the pull-down assay. Zhao-zhen Lin performed all simulations. Marx Karnat did all the
571 segmentation and tracking, Sound Wai Phow and Tsuyoshi Hirashima helped with the ERK
572 experiments. Hui Ting Ong helped with image analysis. Nai Mui Hoon Brenda performed the
573 AFM experiments. Harini performed the QPCR, Aditya Arora contributed to the
574 understanding of the experimental results. Michael Sheetz initiated the work on EGFR and
575 supervised C.F. Jean-Francois Rupprecht supervised the simulation work and contributed to
576 the manuscript. Sham Tlili performed the relaxation time analysis and contributed to the
577 theoretical part of the work. Virgile Viasnoff designed the experiments, contributed to the
578 manuscript and supervised the work.

579

580 **Conflict of Interest:**

581 The authors declare no conflict of interest.

582

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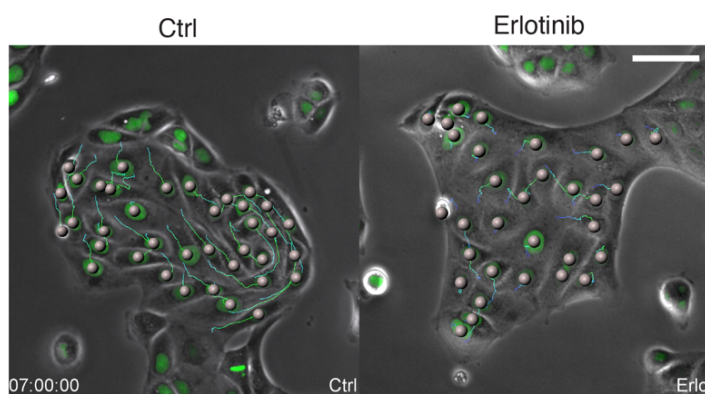
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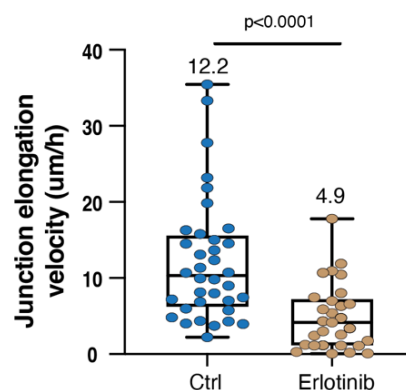
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671 **Figures**

a Reducing EGFR phosphorylation impedes cell junction deformation (I).

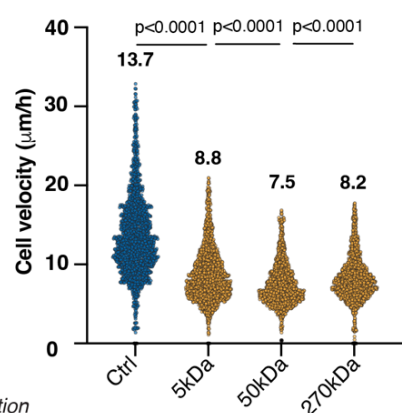
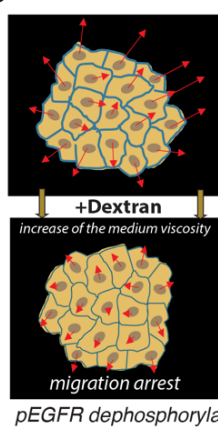


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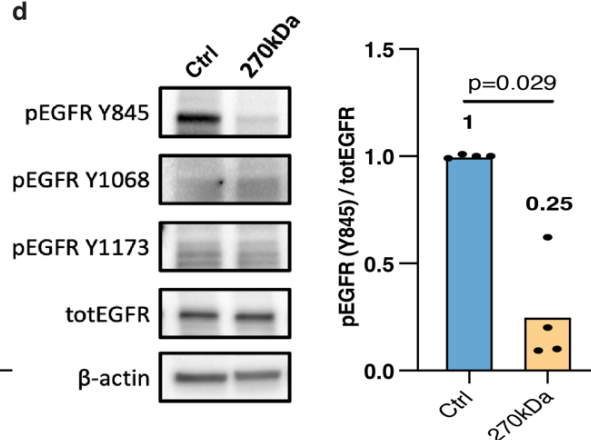


Impeding cell junction deformation reduces EGFR phosphorylation (II).

c



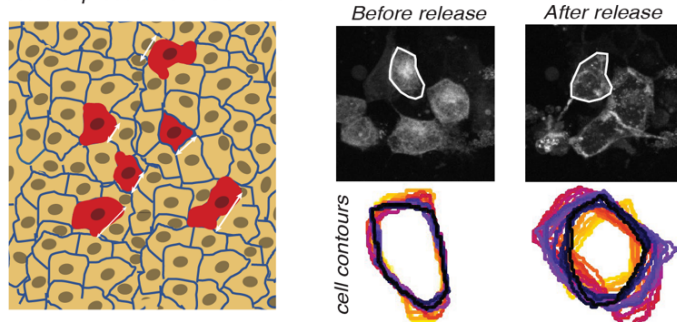
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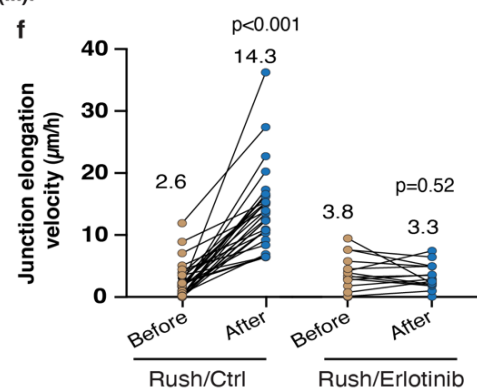
Inducing pEGFR phosphorylation promotes cell junction deformation (III).

e

Mosaic expression of RUSH-EGFR



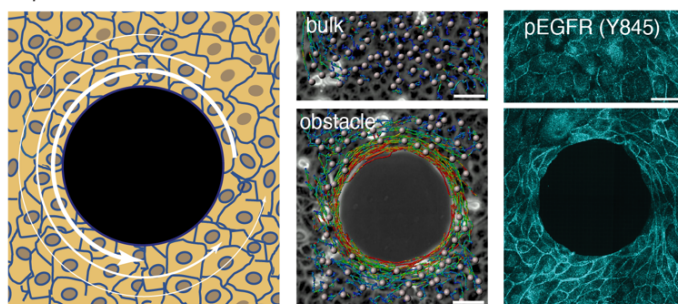
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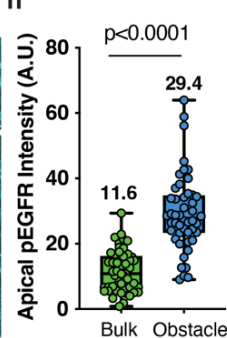
Promoting cell junction deformation induces EGFR phosphorylation (IV).

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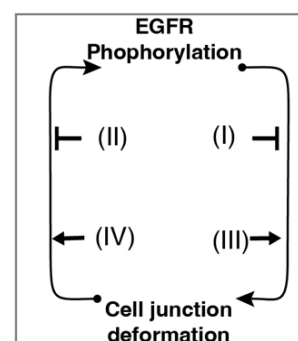
Spontaneous rotation around an obstacle



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673

674 **Fig. 1: A positive feedback loop between apical EGFR phosphorylation and cell junction**
675 **deformation.**

676 **a.** Representative patches of MDCK cells under control and EGFR-inhibited conditions
677 (Erlotinib at 1 μ M) including the tracking or individual cell trajectories. Scale bar: 100 μ m.

678 **b.** Quantification of individual junction elongation velocities in the patches (mean value \pm s.d.)
679 $n_{\text{Ctrl}} = 36$ junctions and $n_{\text{Erlotinib}} = 29$ junctions from 3 independent experiments, two-tailed
680 unpaired t-test, $p < 0.0001$.

681 **c.** Schematics of the experiment for cell arrest by dextran addition. Quantification of
682 individual cell migration velocity 10 minutes after adding dextran with various molecular
683 weights (mean value \pm s.d. $n=1735-1925$ cells from 3 independent experiments.)

684 **d.** Western Blot and its quantification of EGFR phosphorylated states (Y845) before and after
685 cell arrest from 4 independent experiments, two-tailed unpaired t-test, $p = 0.029$.

686 **e.** Experimental setup schematics (left) and segmented contours quantification (right) of cell
687 mosaically expressing RUSH-EGFR before and after its release from the endoplasmic
688 reticulum.

689 **f.** Quantifications of junction elongation velocities upon the release of EGFR, under control
690 and pEGFR-inhibited conditions. $n_{\text{Rush/Ctrl}} = 28$ junctions and $n_{\text{Rush/Erlotinib}} = 15$ junctions from 3
691 independent experiments, two-tailed paired t-test, $p_{\text{Rush/Ctrl}} < 0.001$, $p_{\text{Rush/Erlotinib}} = 0.52$.

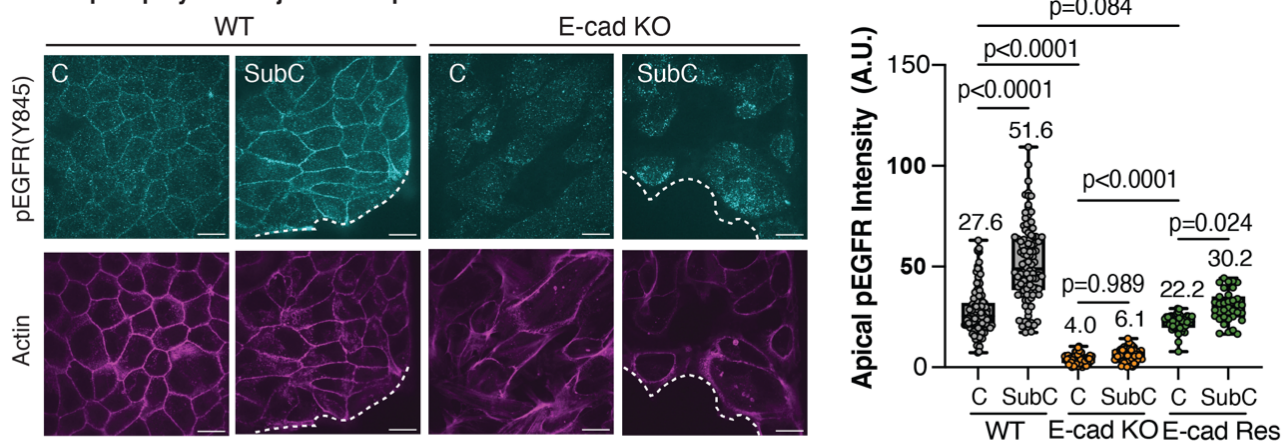
692 **g.** Schematics of the physical induction of cell elongation around obstacles (left). Images of
693 cells encircling obstacles and in bulk regions including single cell tracking and apical
694 localization of pEGFR-Y845 by immunostaining. Scale bar: 50 μ m.

695 **h.** Quantifications of apical pEGFR-Y845 intensity around obstacles (mean value \pm s.d. $n_{\text{Bulk}} =$
696 50 junctions and $n_{\text{Obstacle}} = 48$ junctions from 3 independent experiments, two-tailed unpaired
697 t-test, $p < 0.0001$.)

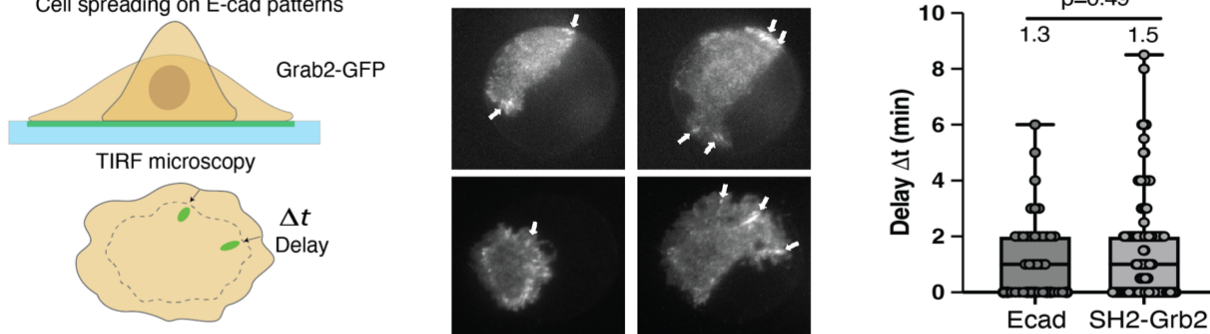
698 **i.** Diagram of a positive feedback loop between apical EGFR phosphorylation and cell junction
699 deformation.

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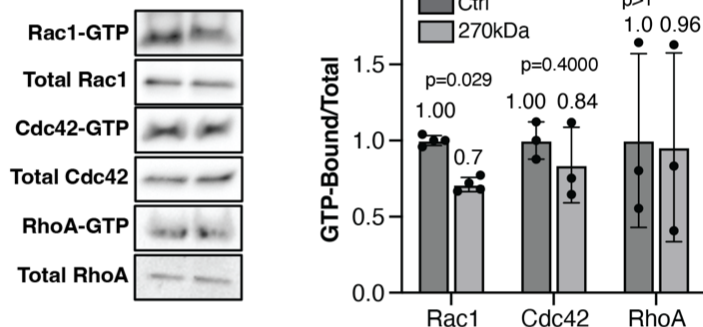
a EGFR phosphorylation at junction depends on E-cadherins



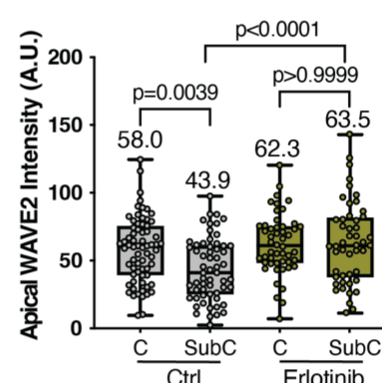
b Cell spreading on E-cad patterns



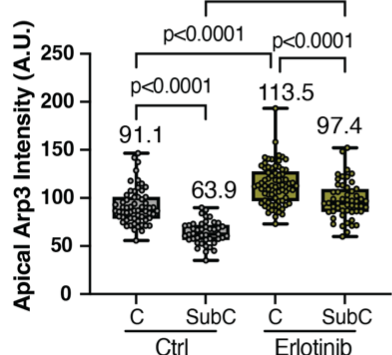
c Ctrl 270kDa



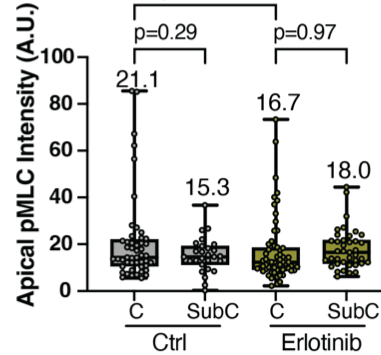
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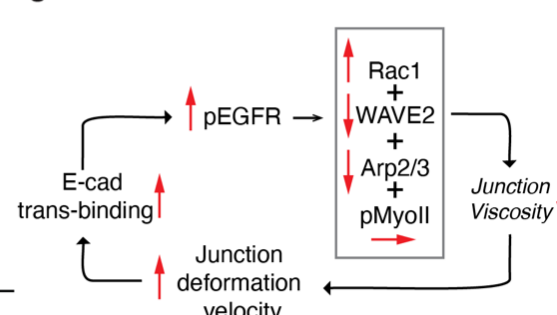
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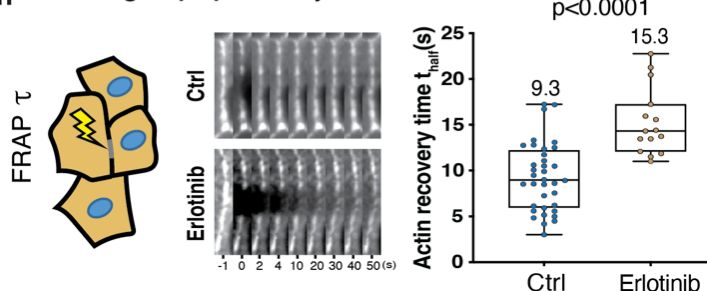
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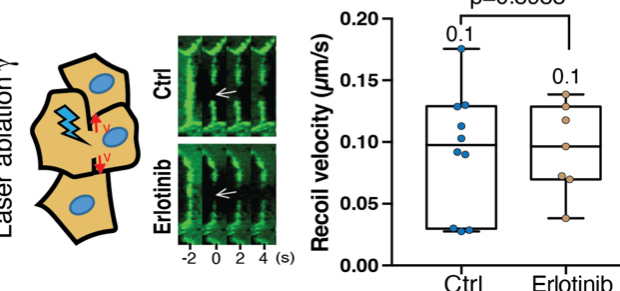
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h Rheological properties of junctional actin



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703 **Fig. 2: E-cadherin-dependent phosphorylation of EGFR fine-tunes actin dynamics with**
704 **minimal impact on cortical tension.**

705 **a.** Immunostaining of apical pEGFR (Y845) and actin in wild-type (WT) and E-cadherin knock-
706 out (Ecad-KO) MDCKs on confluent (C, left) and sub-confluent (SubC, right) regions. Scale Bar:
707 20 μ m. Quantification of apical pEGFR in WT, Ecad-KO and Ecad-KO-rescued (Ecad-Res)
708 tissues. (WT: $n_C = 122$ junctions, $n_{SubC} = 106$ junctions from 4 independent experiments, $p <$
709 0.0001 ; Ecad-KO: $n_C = 67$ junctions, $n_{SubC} = 61$ junctions from 3 independent experiments, $p =$
710 0.9893 ; Ecad-Res: $n_C = 47$ junctions, $n_{SubC} = 34$ cell junctions from 3 independent experiments,
711 $p = 0.0239$. Ordinary one-way ANOVA Tukey's test.

712 **b.** Schematic (left) and time-lapse imaging (middle) of SH2-Grb2 (tdEOS) and E-cadherin (GFP)
713 localization during cell spreading on E-cadherin-coated patterns. Quantification of the
714 recruitment speed of E-cadherin and SH2-Grb2 (right). $n_{Ecad} = 40$ cell adhesions and $n_{SH2-Grb2} =$
715 132 cell adhesions from 3 independent experiments, two-tailed unpaired t-test, $p=0.49$.

716 **c.** Pull-down assays on Rho family GTPases (Rac1, Cdc42 and RhoA) and quantification of GTP-
717 bound GTPases post cell arrest by dextran. $n_{Rac1} = 4$ WB, $p=0.029$, $n_{Cdc42} = 3$ WB, $p=0.4$ and
718 $n_{RhoA} = 3$ WB, $p>1$, two-tailed unpaired t-test.

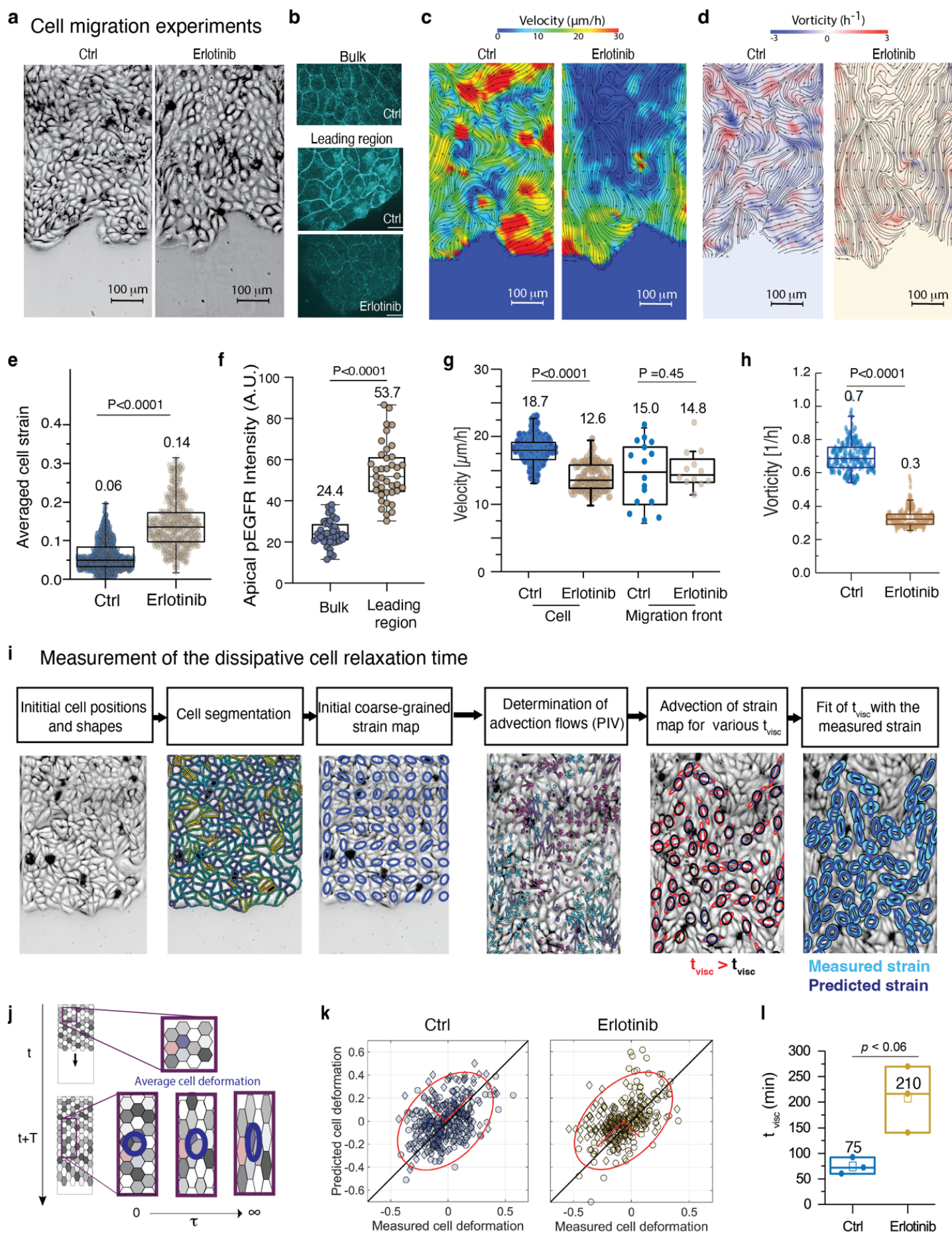
719 **d-f.** Quantification of apical WAVE2 (**d**), Arp3 (**e**) and pMLC (**f**) under control and EGFR
720 inhibited conditions. Data are the mean value \pm s.d. WAVE2: $n_{Ctrl, C} = 68$ cell junctions, $n_{Ctrl, SubC}$
721 $= 59$ cell junctions from 2 independent experiments, $n_{Erlotinib, C} = 50$ cell junctions, $n_{Erlotinib, SubC}$
722 $= 48$ cell junctions from 2 independent experiments; Arp3: $n_{Ctrl, C} = 60$ cell junctions, $n_{Ctrl, SubC} =$
723 43 cell junctions from 2 independent experiments, $n_{Erlotinib, C} = 74$ cell junctions, $n_{Erlotinib, SubC} =$
724 52 cell junctions from 2 independent experiments; pMLC: $n_{Ctrl, C} = 49$ cell junctions, $n_{Ctrl, SubC} =$
725 27 cell junctions from 2 independent experiments, $n_{Erlotinib, C} = 62$ cell junctions, $n_{Erlotinib, SubC} =$
726 39 cell junctions from 2 independent experiments. Ordinary one-way ANOVA Tukey's test.

727 **g.** Proposed model of E-cadherin-dependent phosphorylation of EGFR reducing junction
728 viscosity through the regulation of Rac1, WAVE2, Arp2/3, fine-tuning actin dynamics, with
729 minimal impact on cortical tension.

730 **h-i.** Experimental schematics and characteristic images of fluorescence recovery after
731 photobleaching (FRAP) (left) and laser ablation (right) experiments on intercellular junctions
732 between GFP-Actin-MDCK cells, Scale Bar: 3 μ m. Quantification of fluorescence recovery time
733 (left) and recoil velocities (right) under control and pEGFR-inhibited conditions. FRAP: $n_{Ctrl} =$
734 36 cell junctions, $n_{Erlotinib} = 15$ cell junctions from 3 independent experiments, two-tailed
735 unpaired t-test, $p<0.0001$; Laser ablation: $n_{Ctrl} = 10$ cell junctions, $n_{Erlotinib} = 7$ cell junctions
736 from 2 independent experiments, two-tailed unpaired t-test, $p=0.8983$.

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Fig. 3: Phosphorylated EGFR (pEGFR) modulates cell deformability and influences collective migration.

a. Phase-contrast images of MDCK monolayers migrating on fibronectin-coated line patterns under control and pEGFR-inhibited conditions (Erlotinib at 1 μ M). Scale Bar: 100 μ m. Five independent experiments yielded consistent results.

b. Immunostaining of apical pEGFR(Y845) highlights its localization at cell junctions in bulk and leading regions under control and leading regions under pEGFR-inhibited conditions. Scale Bar: 20 μ m. Four independent experiments corroborate these findings.

c-d. Representative velocity (**c**) and vorticity (**d**) profiles with flow line maps, illustrate MDCK monolayer migration under control and pEGFR-inhibited conditions. Scale bar: 100 μ m. Three independent experiments yielded consistent results.

e. Quantification of cellular strain states in the monolayer under control and pEGFR-inhibited conditions. $n_{\text{Ctrl}} = 1060$ cells and $n_{\text{Erlotinib}} = 1060$ cells from 3 independent experiments, two-tailed unpaired t-test, $p < 0.0001$.

f. Quantification of apical localization of pEGFR (Y845) at the bulk and leading front region of the monolayer. $n_{\text{Bulk}} = 42$ cell junctions and $n_{\text{leading}} = 39$ cell junctions from 3 independent experiments, two-tailed unpaired t-test, $p < 0.0001$.

g. Quantification of cell velocity (left) and migration front velocity (right) under control and pEGFR-inhibited conditions. $n_{\text{ctrl, cell}} = 181$ cells, $n_{\text{Erlotinib, cell}} = 181$ cells from 3 independent experiments, $p < 0.0001$; $n_{\text{ctrl, migration front}} = 16$ strips, $n_{\text{Erlotinib, migration front}} = 12$ strips from 3 independent experiments, $p = 0.45$, two-tailed unpaired t-test.

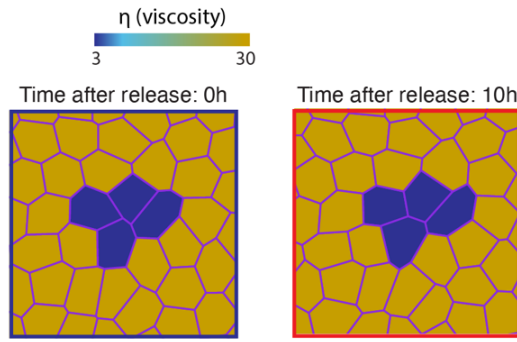
h. Quantification of spatial correlation in the velocity field under control and pEGFR-inhibited conditions. $n_{\text{ctrl}} = 181$ cells, $n_{\text{Erlotinib}} = 181$ cells from 3 independent experiments, two-tailed unpaired t-test, $p < 0.0001$.

i. Schematic representation of the analysis pipeline to measure the average cell shape relaxation time in a migrating monolayer. Average cell strain profiles along the migration axis are depicted, with bold lines indicating mean values and narrow lines representing standard deviations.

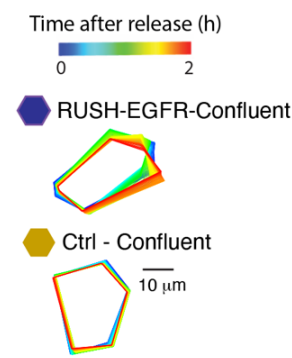
j-k. Correlative plots between measured and advection-based predicted cellular strain for the best fit of the viscoelastic time (t_{visc}) under control and pEGFR-inhibited conditions. Two independent experiments yielded consistent results.

l. Measured viscoelastic time (t_{visc}) under control and pEGFR-inhibited conditions. $n_{\text{ctrl}} = 3$ strips, $n_{\text{Erlotinib}} = 3$ strips from 2 independent experiments, two-tailed unpaired t-test, $p < 0.06$.

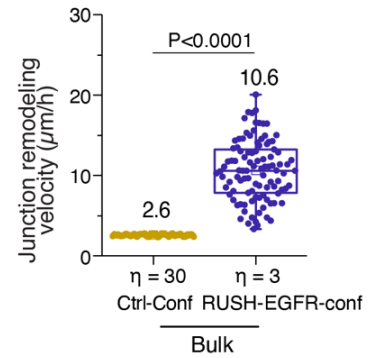
a RUSH-EGFR simulations



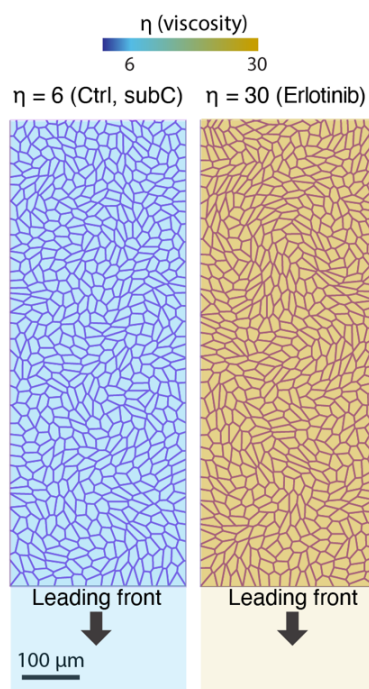
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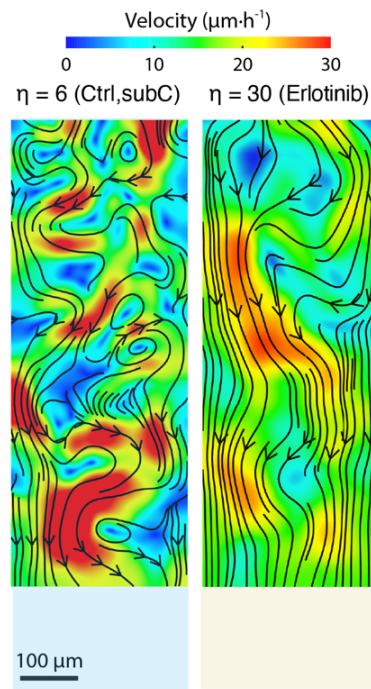
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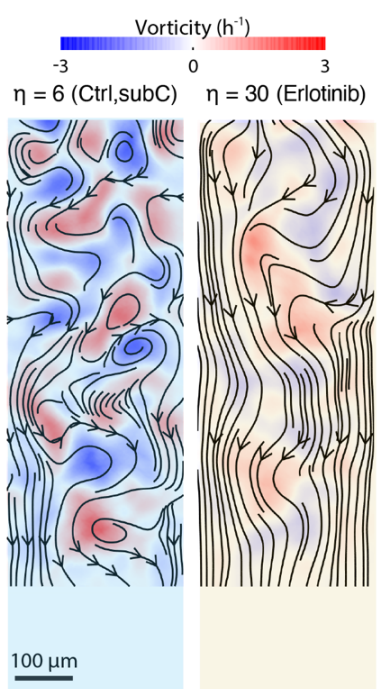
d Cell migration simulations



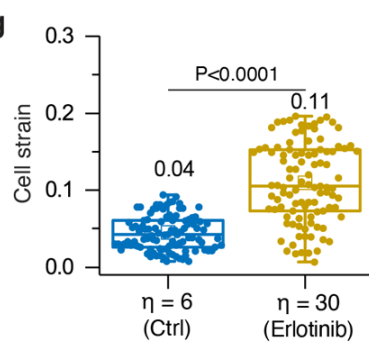
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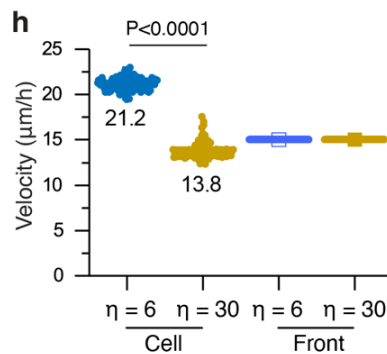
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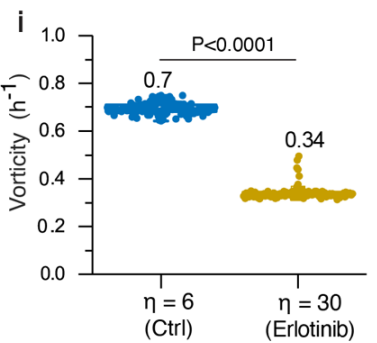
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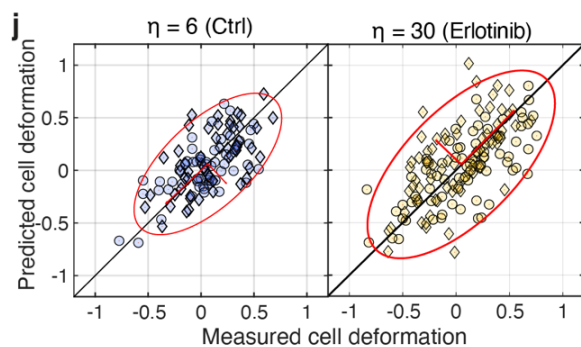
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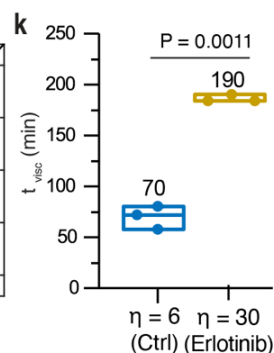
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	Ctrl subC		Erlotinib	
	Exp	Simul η=6	Exp	Simul η=30
Cell Strain (%)	5	4	10	11
Velocity (μm/h)	18	21	13	14
Vorticity (h ⁻¹)	0.9	0.7	0.3	0.3
Corr Length (μm)	110	80	200	150
t _{visc} (min)	75	70	210	190

778

779 **Fig. 4: Modeling the impact of viscosity changes on epithelial migration.**

780 **a.** Simulated cellular arrangement in a vertex model with tension fluctuation, illustrating four
781 low viscosity cells (dark blue, $\eta=3$) within a larger population of normal viscosity cells (orange,
782 $\eta=30$). This models RUSH-EGFR activated cells within a large tissue of inactivated cells.

783 **b.** Temporal variation of two representative cell profiles for low viscosity (RUSH-EGFR) cells
784 (top) and control cells (bottom).

785 **c.** Quantifications of the cell-cell junction remodeling velocity for control cells and low
786 viscosity (RUSH-EGFR) cells ($\eta=30$ or $\eta=3$, respectively). Conf = Confluent. $n_{\text{Ctrl}} = 101$ cell
787 junctions and $n_{\text{RUSH-EGFR}} = 101$ cell junctions from simulations, two-tailed unpaired t-test,
788 $p < 0.0001$.

789 **d.** Simulated cellular arrangement in a vertex model with $\eta=6$ (light blue) and $\eta=30$ (orange),
790 modelling the control and pEGFR-inhibited (Erlotinib) tissues, in the presence of cellular
791 activity and an imposed uniform front migration speed.

792 **e-f.** Velocity (**e**) and, vorticity fields (**f**), both with flow lines (black) for the model of control
793 and pEGFR-inhibited conditions.

794 **g-i.** Distribution in the simulated averaged cellular strain (**g**), velocity (displayed together with
795 the imposed front migration speed) (**h**), and vorticity (**i**), under control and pEGFR-inhibited
796 conditions (see **Methods** for averaging procedure). $n_{\text{Ctrl, strain}} = 101$ cells and $n_{\text{Erlotinib, strain}} = 101$
797 cells from simulations, two-tailed unpaired t-test, $p < 0.0001$. $n_{\text{Ctrl, velocity}} = 100$ cells and $n_{\text{Erlotinib,}}$
798 $\text{velocity} = 100$ cells from simulations, two-tailed unpaired t-test, $p < 0.0001$. $n_{\text{Ctrl, vorticity}} = 100$ cells
799 and $n_{\text{Erlotinib, vorticity}} = 100$ cells from simulations, two-tailed unpaired t-test, $p < 0.0001$.

800 **j.** Correlative plots between the measured and advection-based predicted cellular strain for
801 the best fit of the viscoelastic time (t_{visc}) under control ($\eta=6$) and pEGFR-inhibited ($\eta=30$)
802 conditions.

803 **k.** Viscoelastic time (t_{visc}) for control ($\eta=6$) and pEGFR-inhibited ($\eta=30$). $n_{\text{Ctrl}} = 3$ strips and
804 $n_{\text{Erlotinib}} = 3$ strips from simulations, two-tailed unpaired t-test, $p = 0.0011$.

805 **l.** Table summarizing the experimental and simulation results.

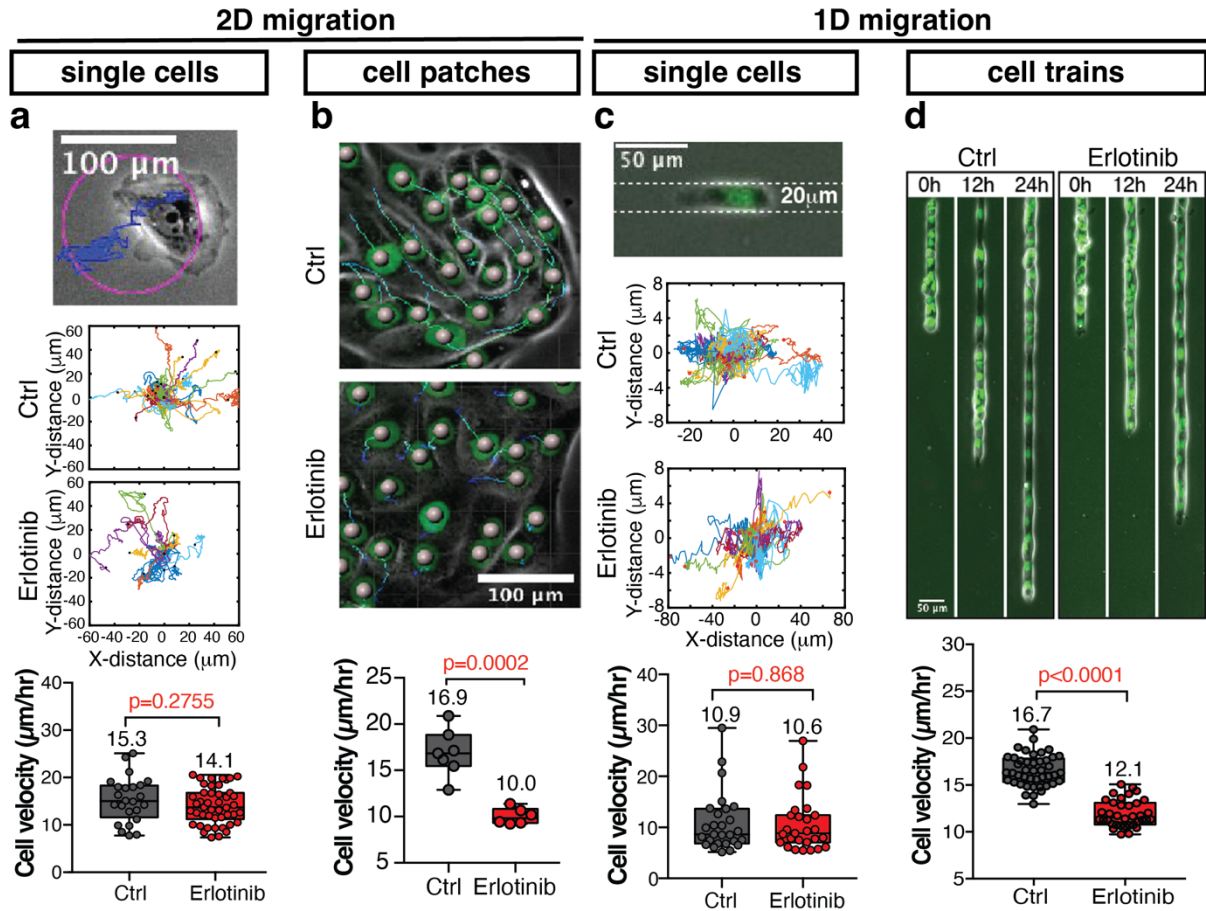
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808 **Extended Data Figures**

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814 **Extended Data Fig. 1: Dephosphorylation of EGFR reduces the dynamics of cell junction**
815 **deformation without directly altering cell-substrate interactions.**

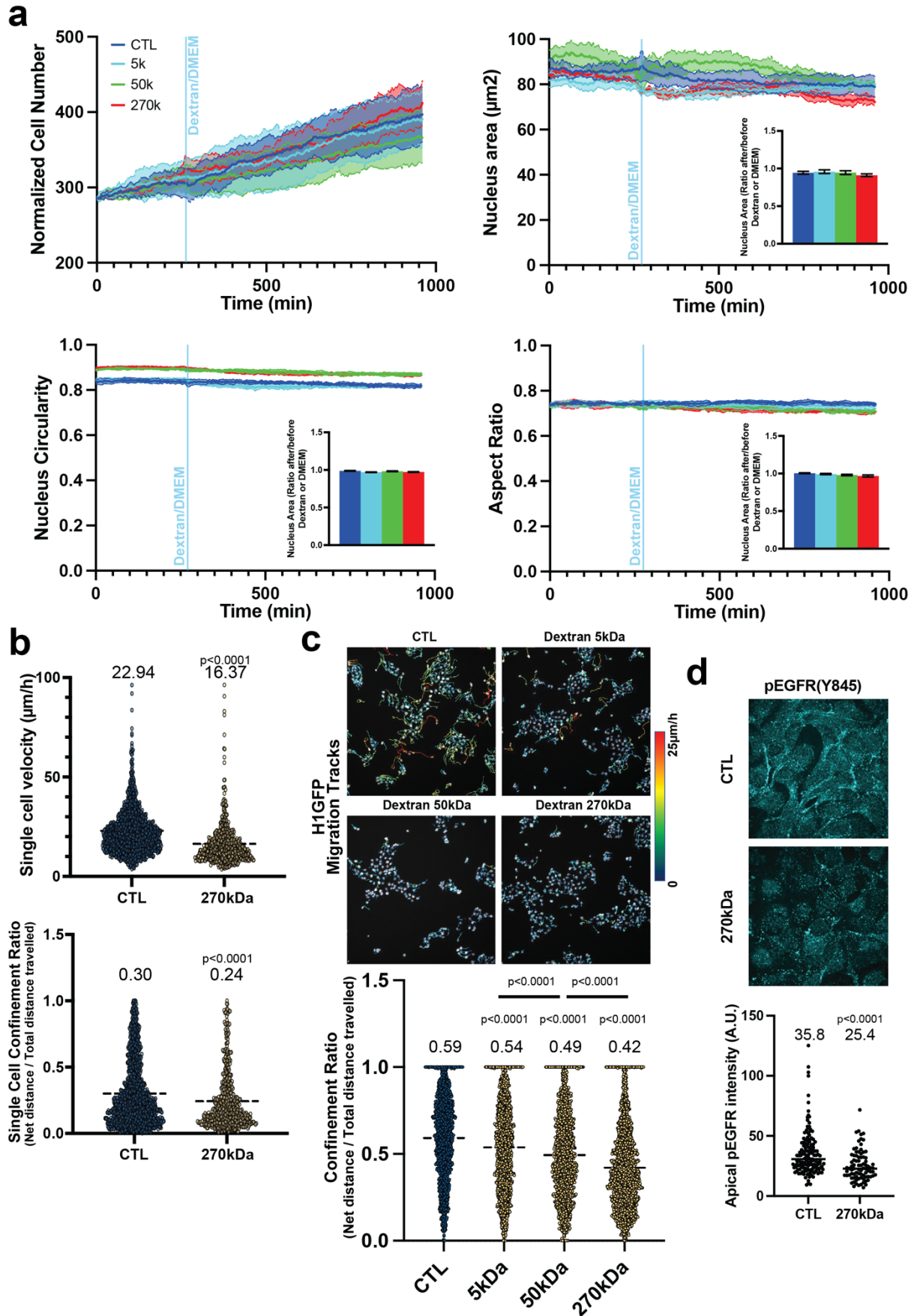
816 **a.** Inhibition of EGFR activity does not disrupt single-cell motility in 2D migration.
817 Representative examples of single-cell 2D migration on fibronectin-coated surfaces (top),
818 Scale Bar: 100 μm . Thirty to forty representative nuclear tracks over 12h in cells randomly
819 migrating under Ctrl and pEGFR inhibition conditions (middle). Average speed of single-cell
820 motility from nuclear movement tracks over 12h under Ctrl and pEGFR inhibition conditions
821 (bottom), $n_{\text{Ctrl}} = 24$ cells and $n_{\text{Erlotinib}} = 46$ cells from 3 independent experiments, two-tailed
822 unpaired t-test, $p=0.2755$.

823 **b.** Representative examples of migration of 2D epithelial patches on fibronectin-coated
824 surfaces under Ctrl and pEGFR inhibition conditions (top), Scale Bar: 100 μm . Average speed
825 of cells in patch migration for 12h from nuclear movement tracks under Ctrl and pEGFR
826 inhibition conditions, $n_{\text{Ctrl}} = 7$ cell patches and $n_{\text{Erlotinib}} = 6$ cell patches from 4 different
827 experiments, two-tailed unpaired t-test, $p=0.0002$.

828 **c.** Inhibition of EGFR activity does not disturb single-cell motility in 1D migration.
829 Representative examples of single-cell migration on 20 μm fibronectin-coated lines (top),
830 Scale Bar: 50 μm . Thirty representative nuclear tracks over 12h in cells migrating on 20 μm
831 line patterns under Ctrl and pEGFR inhibition conditions (middle). Average speed of single-
832 cell motility on 20 μm line patterns from nuclear movement tracks under Ctrl and pEGFR
833 inhibition conditions (bottom), $n_{\text{Ctrl}} = 27$ cells and $n_{\text{Erlotinib}} = 27$ cells from 3 different
834 experiments, two-tailed unpaired t-test, $p=0.868$.

835 **d.** Representative examples of migration of 1D epithelial trains on 20 μm fibronectin-coated
836 lines under Ctrl and pEGFR inhibition conditions (top), Scale Bar: 50 μm . Average speed of
837 cells in train migration for 24h on 20 μm line patterns from nuclear movement tracks under
838 Ctrl and pEGFR inhibition conditions, $n_{\text{Ctrl}} = 44$ cell trains and $n_{\text{Erlotinib}} = 36$ cell trains from 3
839 different experiments, two-tailed unpaired t-test, $p<0.0001$.

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843 **Extended Data Fig. 2: Dextran impact on single and collective cell migration speed and mode**
844 **without evident signs of osmotic shock.**

845 **a.** Quantification of cell proliferation (upper left), nucleus area (upper right), circularity (lower
846 left) and aspect ratio (lower right) on cell patches before and after the addition of dextran of
847 the indicated molecular weight. The light blue line indicates the moment of dextran addition.
848 Four independent experiments yielded consistent results.

849 **b.** Quantification of single-cells velocity and persistence before and after the addition of
850 270kDa dextran, $n_{\text{Ctrl}} = 1217$ cells and $n_{270\text{kDa}} = 543$ cells from 3 different experiments, two-
851 tailed unpaired t-test, $p < 0.0001$.

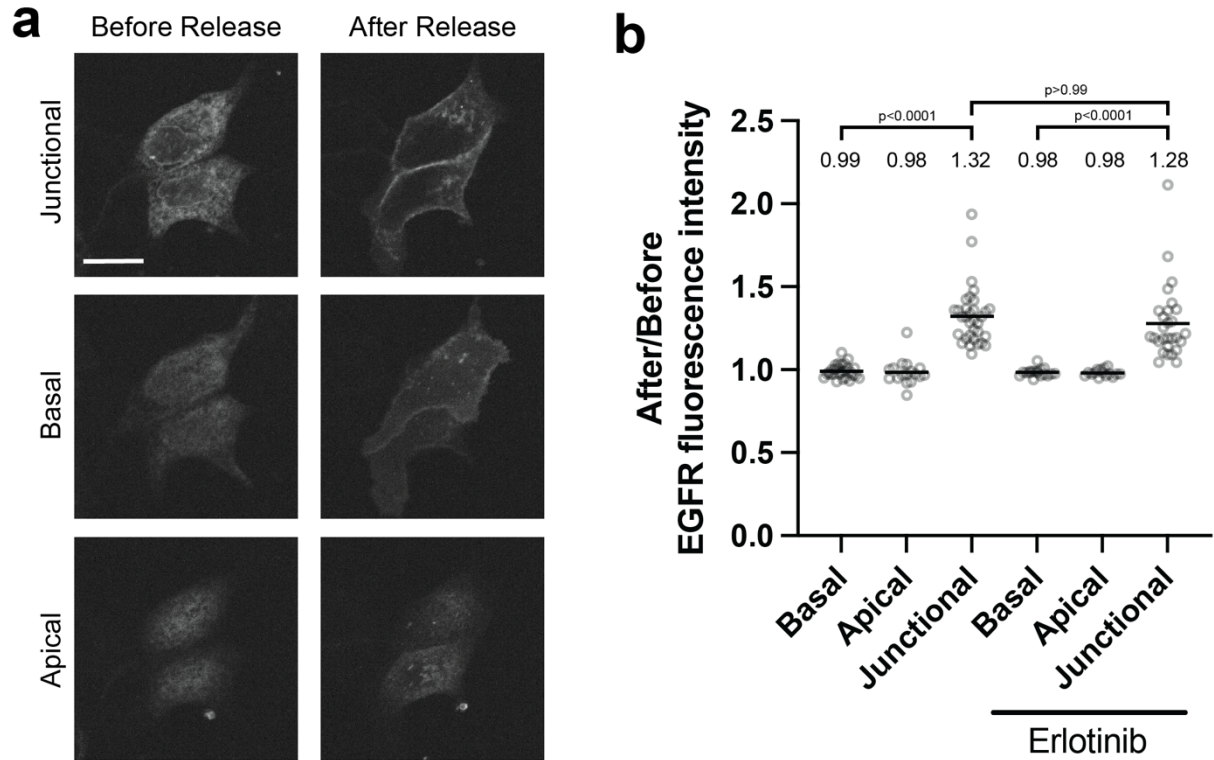
852 **c.** Representative images of cell tracking with or without the presence of dextran of the
853 indicated molecular weight (up). Quantification of individual cell persistence within cell
854 patches under control or indicated molecular weight dextran conditions (down), $n_{\text{Ctrl}} = 1762$
855 cells, $n_{5\text{kDa}} = 1770$ cells, $n_{50\text{kDa}} = 1424$ cells and $n_{270\text{kDa}} = 1968$ cells from 3 different
856 experiments, Ordinary one-way ANOVA Tukey's test, $p < 0.0001$.

857 **d.** Confocal images (up) and quantification (down) of pEGFR(Y845) junctional fluorescence
858 intensities under control and 270kDa dextran conditions, $n_{\text{Ctrl}} = 143$ cell junctions and $n_{270\text{kDa}}$
859 = 84 cell junctions from 3 different experiments, two-tailed unpaired t-test, $p < 0.0001$.

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Extended Data Fig. 3: EGFR preferentially translocates to cell-cell junction after release from the Endoplasmic Reticulum (ER).

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a-b. Confocal images (a) and corresponding quantification (b) of EGFR-GFP fluorescence

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intensities at the junctional, basal and apical side of the cells, both before and after release

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from the endoplasmic reticulum. Scale Bar: 20 μ m. $n_{Ctrl\ Basal} = 27$ cells, $n_{Ctrl\ Apical} = 17$ cells, n_{Ctrl

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$Junctional} = 33$ cell junctions, $n_{Erlotinib\ Basal} = 17$ cells, $n_{Erlotinib\ Apical} = 15$ cells and $n_{Erlotinib\ Junctional} = 28$

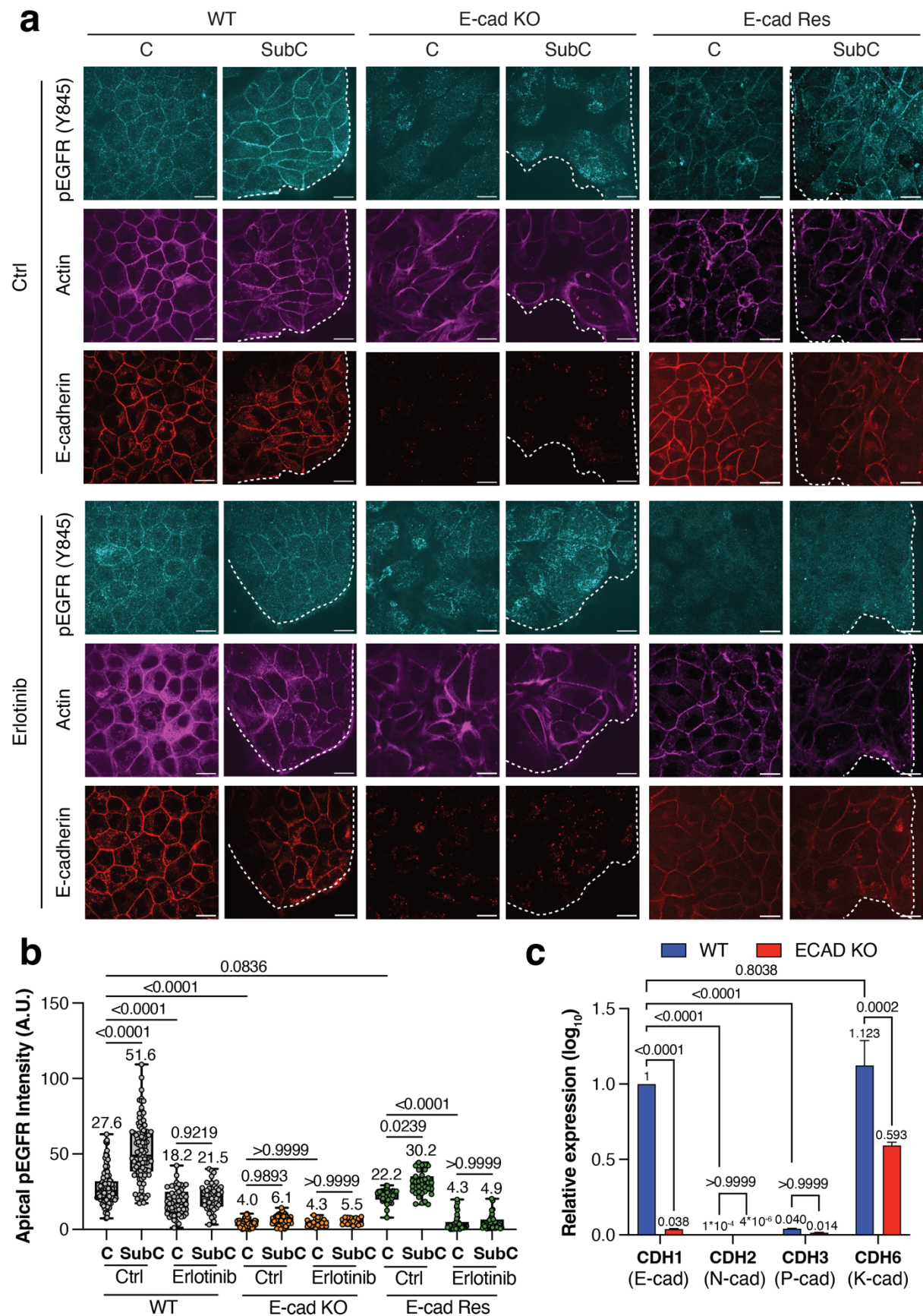
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cell junctions from 3 different experiments, Ordinary one-way ANOVA Tukey's test.

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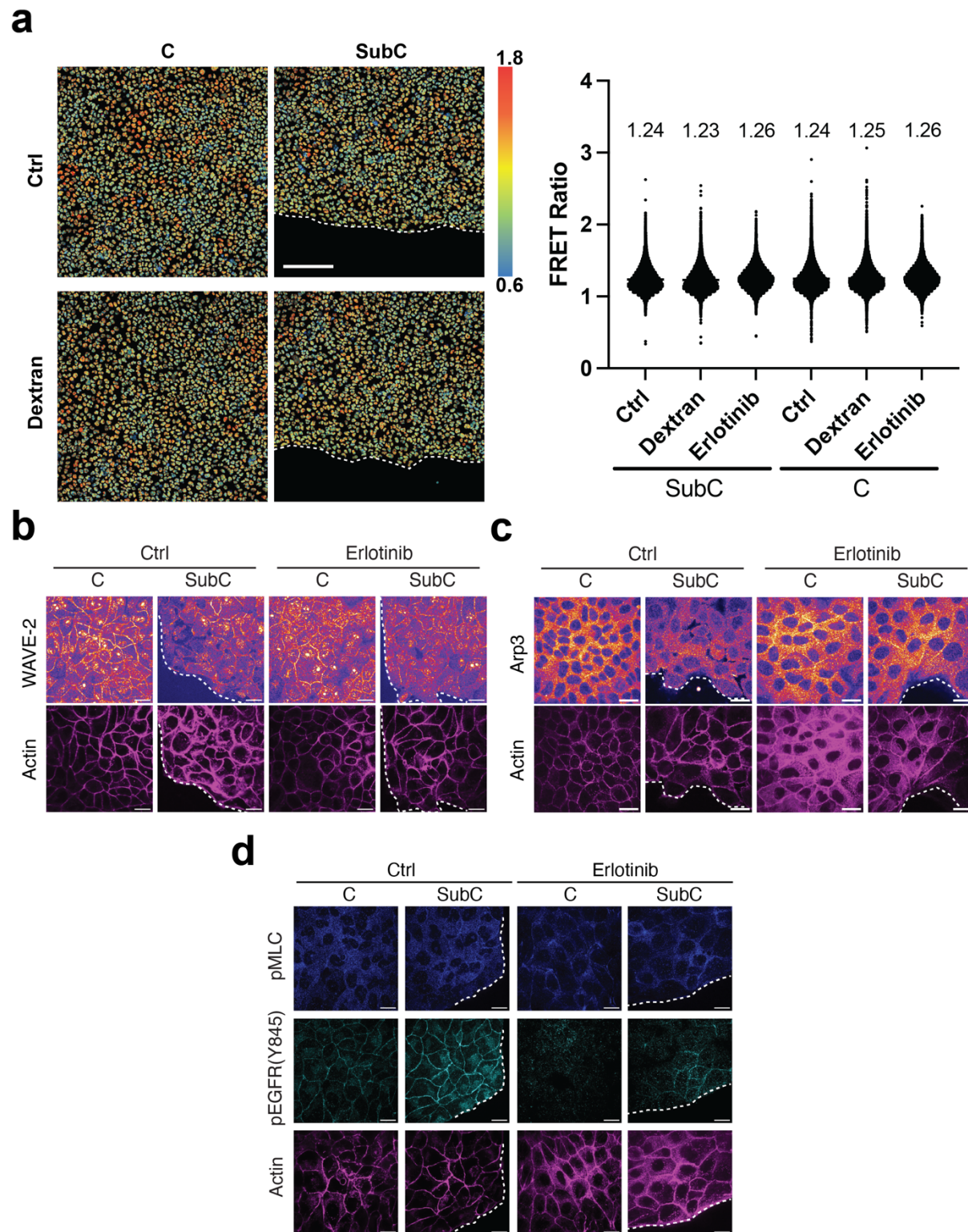
876 **Extended Data Fig. 4: E-cadherin junctions govern phosphorylation of EGFR (Y845) at the**
877 **apical side of MDCK tissues.**

878 **a.** Immunostaining of pEGFR (Y845), actin and E-cadherin in wild-type (WT), E-cadherin knock-
879 out (Ecad-KO) and Ecad-KO-rescued (Ecad-Res) MDCK cells on the apical side of confluent (C,
880 left) and sub-confluent (SubC, right) regions under control and pEGFR inhibition (Erlotinib)
881 conditions. The white dotted line indicates the leading front of the patches. Scalr Bar: 20 μ m.
882 **b.** Quantification of apical pEGFR in WT, Ecad-KO and Ecad-Res MDCK cells. For WT: $n_{Ctrl, C} =$
883 122 cell junctions, $n_{Ctrl, SubC} = 106$ cell junctions from 4 different experiments, $n_{Erlotinib, C} = 51$
884 cell junctions, $n_{Erlotinib, SubC} = 44$ cell junctions from 3 different experiments; For Ecad-KO: $n_{Ctrl,$
885 $C} = 67$ cell junctions, $n_{Ctrl, SubC} = 61$ cell junctions from 3 different experiments, $n_{Erlotinib, C} = 24$
886 cell junctions, $n_{Erlotinib, SubC} = 16$ cell junctions from 3 different experiments; For Ecad-Res: $n_{Ctrl,$
887 $C} = 47$ cell junctions, $n_{Ctrl, SubC} = 34$ cell junctions from 3 different experiments, $n_{Erlotinib, C} = 41$
888 cell junctions, $n_{Erlotinib, SubC} = 32$ cell junctions from 3 different experiments, Ordinary one-way
889 ANOVA Tukey's test.

890 **c.** RT-qPCR results showing the relative expression of CDH1 (E-cad), CDH2 (N-cad), CDH3 (P-
891 cad) and CDH6 (K-cad) in WT and Ecad-KO MDCK cells from 3 different experiments.

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896 **Extended Data Fig. 5: Molecular components underlying EGFR phosphorylation in response**
897 **to cell junction deformation.**

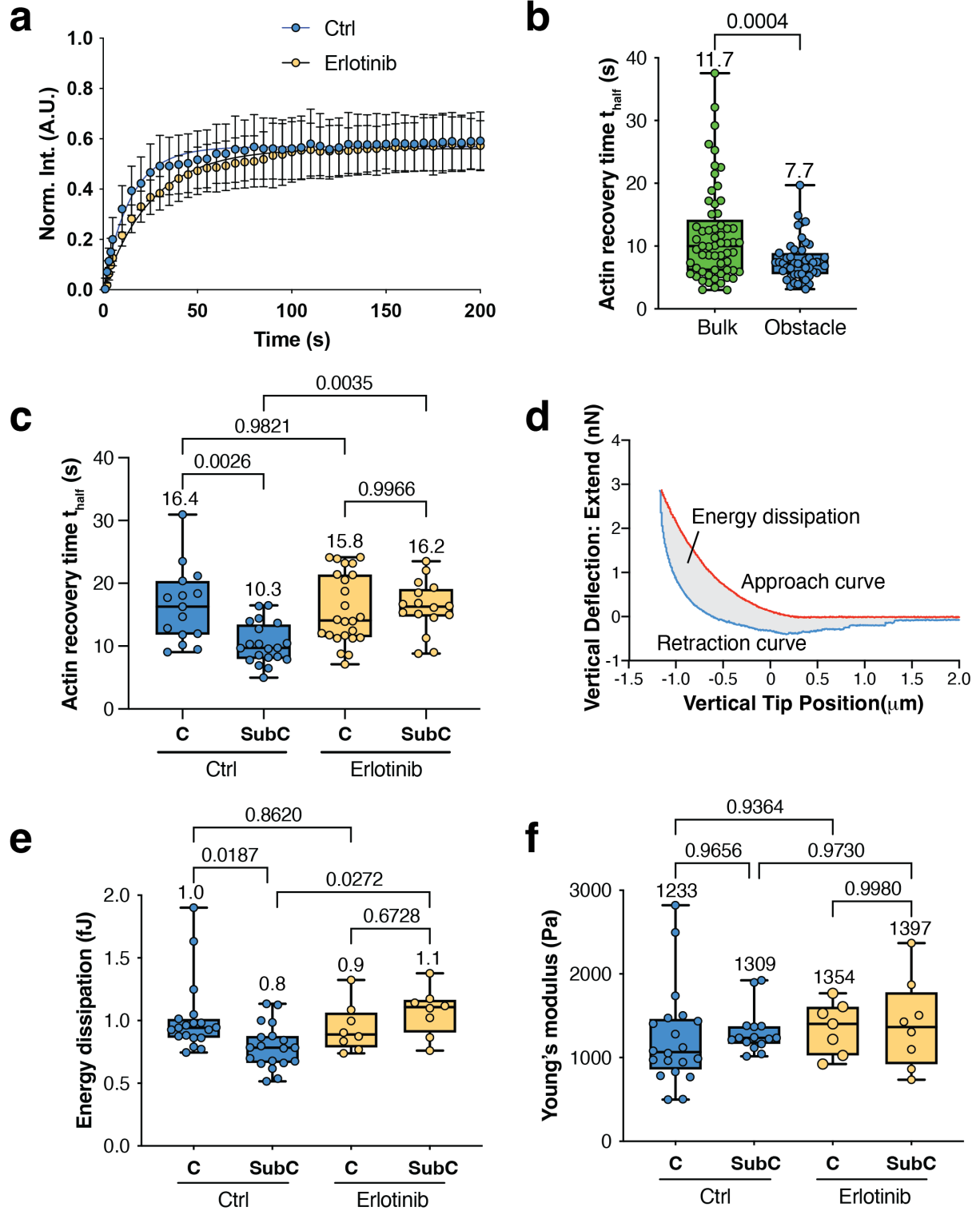
898 **a.** Representative images and corresponding quantification of FRET Ratio in confluent (C, left)
899 and sub-confluent (SubC, right) regions of migrating MDCK monolayers, with or without EGFR
900 inhibition (Erlotinib), $n_{\text{Ctrl, SubC}} = 27014$ cells, $n_{\text{Dextran, SubC}} = 27766$ cells, $n_{\text{Erlotinib, SubC}} = 15316$ cells,
901 $n_{\text{Ctrl, C}} = 43852$ cells, $n_{\text{Dextran, C}} = 43079$ cells and $n_{\text{Erlotinib, C}} = 27305$ cells from 3 independent
902 experiments. Scale Bar: 200 μm .

903 **b-c.** Immunostaining of WAVE-2 and actin (**b**), Arp3 and actin (**c**) on the apical side of confluent
904 (C, left) and sub-confluent (SubC, right) regions under Control and pEGFR inhibition (Erlotinib)
905 conditions. Scale Bar: 20 μm . Two independent experiments yielded consistent results.

906 **d.** Immunostaining of pMLC, pEGFR (Y845) and actin on the apical side of confluent (C, left)
907 and sub-confluent (SubC, right) regions under control and pEGFR inhibition (Erlotinib)
908 conditions. Scale Bar: 20 μm . Two independent experiments yielded consistent results.

909

910



913 **Extended Data Fig. 6: Regulation of actin dynamics and junctional viscoelastic properties by**
914 **EGFR phosphorylation without impact on junctional tension.**

915 **a.** Fluorescence Recovery After Photobleaching (FRAP) experiments of actin were conducted
916 at the apical regions of cell-cell junctions under both control and pEGFR inhibition (Erlotinib)
917 conditions. The data were normalized and fitted with best-fit curves.

918 **b.** Actin dynamics were assessed in cells encircling obstacles and in bulk regions. $n_{\text{Bulk}} = 61$ cell
919 junctions and $n_{\text{Obstacle}} = 47$ cell junctions from 3 independent experiments, two-tailed unpaired
920 t-test, $p=0.0004$.

921 **c.** Measurements of actin dynamics in migrating MDCK monolayers were performed in both
922 confluent (C, left) and sub-confluent (SubC, right) regions under control and pEGFR inhibition
923 (Erlotinib) conditions. $n_{\text{Ctrl, C}} = 15$ cell junctions, $n_{\text{Ctrl, SubC}} = 20$ cell junctions from 2 independent
924 experiments, $n_{\text{Erlotinib, C}} = 24$ cell junctions, $n_{\text{Erlotinib, SubC}} = 16$ cell junctions from 3 independent
925 experiments, Ordinary one-way ANOVA Tukey's test.

926 **d.** Typical force-displacement curves from Atomic Force Microscopy (AFM) force
927 measurements of MDCK monolayers. The area enclosed between the approach and
928 retraction curves, corresponds to energy dissipation.

929 **e-f.** Measurements of energy dissipation (**e**) and Young's modulus (**f**) of migrating MDCK
930 monolayers in confluent (C, left) and sub-confluent (SubC, right) regions under control and
931 pEGFR inhibition (Erlotinib) conditions. For energy dissipation: $n_{\text{Ctrl, C}} = 19$ cell junctions, $n_{\text{Ctrl, SubC}}$
932 $= 19$ cell junctions from 3 independent experiments, $n_{\text{Erlotinib, C}} = 8$ cell junctions, $n_{\text{Erlotinib, SubC}}$
933 $= 8$ cell junctions from 3 independent experiments, Ordinary one-way ANOVA Tukey's
934 test. For Young's modulus: $n_{\text{Ctrl, C}} = 20$ cell junctions, $n_{\text{Ctrl, SubC}} = 15$ cell junctions from 3
935 independent experiments, $n_{\text{Erlotinib, C}} = 7$ cell junctions, $n_{\text{Erlotinib, SubC}} = 8$ cell junctions from 3
936 independent experiments, Ordinary one-way ANOVA Tukey's test.

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940 **Supplementary Discussion:**

941 **E-Cadherin - EGFR - Rac1 - Wave2 - Arp2/3 interplay and relevance at cell-cell junctions.**

942

943 In this study, we elucidated a molecular signaling pathway that plays a crucial role in
944 regulating the viscosity of intercellular junctions. Our findings demonstrate that the trans
945 binding of E-cadherin within highly dynamic cell-cell junctions triggers the activation of EGFR
946 and Rac1, aligning with previous research^{7, 31-33}. Rac1 has been well-established to play a
947 major role in Wave2 complex recruitment and activation³⁴. Specifically, E-cadherins activate
948 Rac1 at the cell-cell junction, contributing to junction stabilization through Wave2^{35, 36}.

949

950 Contrary to existing studies conducted on highly confluent monolayers characterized by
951 mature contacts and non-dynamic cell-cell junctions, our investigation focuses on sub-
952 confluent patches exhibiting elevated apical pEGFR and Rac1-GTP levels. In our unique
953 context of high dynamics, we proposed that the signaling pathways and molecular actors may
954 differ. Notably, Rac1 activation, which has been linked to E-cadherin adhesion disruptions³⁷,
955 could potentially contribute to the rapid turnover of E-cadherin adhesions in highly dynamic
956 cell-cell junctions, thereby hindering Wave2 recruitment for junction stabilization.

957

958 Alternatively, we hypothesize that the impact of pEGFR at the junction is non-local, coupling
959 with recruitment at the basal part of cells and creating competition with the junctional pool.
960 Unfortunately, due to limitations in the available imaging techniques, we were unable to
961 discern the cortex bound to the cytoplasmic fraction. Consequently, while our paper
962 establishes a correlation between the recruitment levels of various proteins, we refrain from
963 asserting a direct causal link.

964

965 **Captions of the Supplementary videos.**

966

967 **Supplementary Video 1:**

968 Cell-cell reorganization in representative patches of MDCK-H1-GFP cells under control and
969 pEGFR-inhibited (Erlotinib at 1 μ M) conditions recorded at 12 frames/hour. Scale Bar: 100 μ m.

970

971 **Supplementary Video 2:**

972 Cell patches migration of MDCK-H1-GFP cells with or without dextran addition recorded at 12
973 frames/hour. Scale Bar: 100 μ m.

974

975 **Supplementary Video 3:**

976 MDCK-WT monolayers with mosaic expression of RUSH-EGFR-GFP. Junction elongation
977 before and after addition of biotin and subsequent release of EGFR from the endoplasmic
978 reticulum under control and pEGFR-inhibited (Erlotinib at 1 μ M) conditions recorded at 12
979 frames/hour. Scale Bar: 20 μ m.

980

981 **Supplementary Video 4:**

982 Cells encircling obstacles and in bulk regions under control and pEGFR-inhibited (Erlotinib at
983 1 μ M) conditions recorded at 15 frames/hour. Scale Bar: 50 μ m.

984

985 **Supplementary Video 5:**

986 Cells migrating on 400 μ m width line strips under control and pEGFR-inhibited (Erlotinib at
987 1 μ M) conditions recorded at 15 frames/hour. Scale Bar: 100 μ m.

988

989 **Supplementary Video 6:**

990 The vorticity of the collective flow for the migrating cells under control and pEGFR-inhibited
991 (Erlotinib at 1 μ M) conditions recorded at 15 frames/hour.

992

993 **Supplementary Video 7:**

994 Vertex-model simulations under control ($\eta=6$) and pEGFR-inhibited (Erlotinib at 1 μ M) ($\eta=30$)
995 conditions. Other parameters see Supplementary Table II.

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998