ERK-mediated mechanochemical waves direct collective cell polarization

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Summary

During collective migration of epithelial cells, the migration direction is aligned over a tissue-scale expanse. Although the collective cell migration is known to be directed by mechanical forces transmitted via cell-cell junctions, it remains elusive how the intercellular force transmission is coordinated with intracellular biochemical signaling to achieve collective movements. Here we show that intercellular coupling of ERK-mediated mechanochemical feedback yields long-distance transmission of guidance cues. Mechanical stretch activates ERK through EGFR activation, and ERK activation triggers cell contraction. The contraction of the activated cell pulls neighboring cells, evoking another round of ERK activation and contraction in the neighbors. Furthermore, anisotropic contraction based on front-rear polarization guarantees unidirectional propagation of ERK activation, and in turn, the ERK activation waves direct multicellular alignment of the polarity, leading to long-range ordered migration. Our findings reveal that mechanical forces mediate intercellular signaling underlying sustained transmission of guidance cues for collective cell migration.

Keywords

Collective cell migration; ERK/MAPK; EGFR; FRET; front-rear polarity; intercellular signal transfer; mathematical model; mechanochemical feedback; mechanotransduction; wave propagation.

52 **Introduction**

53 Collective cell migration underpins various fundamental biological processes, including
54 embryonic development and tissue repair (Friedl and Gilmour, 2009; Mayor and Etienne-
55 Manneville, 2016). A migrating cell cluster exhibits multicellular coordination of cellular
56 parameters, such as cytoskeleton organization (Farooqui and Fenteany, 2005), organelle
57 positioning (Carvalho et al., 2019; Farooqui and Fenteany, 2005; Reffay et al., 2011), and cell
58 velocity (Petitjean et al., 2010; Tlili et al., 2018). Directionality of these parameters in each cell
59 can be provided by two mechanisms. First, cells sense a direction from global external cues,
60 such as a gradient of chemoattractant or substrate stiffness (Haeger et al., 2015; Shellard and
61 Mayor, 2019). Second, directional cues are transmitted from a leading edge of a cell cluster to
62 the bulk; cells located at the edge, referred to as leader cells, sense the microenvironment to
63 spread out and dictate the direction of follower cells located behind the leader cells
64 (Omelchenko et al., 2003; Reffay et al., 2014; Yamaguchi et al., 2015). In the latter case, all
65 follower cells as well as the leader cells generate mechanical forces to actively migrate, and
66 those forces are orchestrated as cooperative intercellular forces over the cell cluster (Serra-
67 Picamal et al., 2012; Tambe et al., 2011; Trepat et al., 2009). It has also been shown that the
68 mechanical forces transmitted via cell-cell junctions provide local cues to direct an ordered cell
69 migration in a cluster (Das et al., 2015; Tambe et al., 2011); however, our understanding of the
70 signaling molecules responsible for the long-range transmission of the mechanical forces is far
71 from complete.

72 Extracellular signal-regulated kinase (ERK), a serine/threonine kinase, plays critical roles
73 in mechanotransduction that regulates differentiation (Tanabe et al., 2004), epithelial cell
74 division (Gudipaty et al., 2017), and tissue homeostasis (Moreno et al., 2019). Earlier studies
75 have shown that ERK activation propagates as multiple waves from leader cells to the follower
76 cells during collective cell migration using *in vitro* cultured cells (Handly et al., 2015;
77 Matsubayashi et al., 2004; Nikolic et al., 2006) and *in vivo* mouse ear skin (Hiratsuka et al.,
78 2015). Recently, we have demonstrated that the ERK activation waves orient the directed
79 migration of a cell cluster against the wave direction, indicating a critical role of the ERK
80 activation waves in coordinated cell migration (Aoki et al., 2017). However, it remains largely
81 unknown how cells harness the mechanotransduction and the ERK activation waves to
82 coordinate collective cell migration.

83 In this study, by combining Förster resonance energy transfer (FRET)-based biosensors
84 (Harvey et al., 2008; Komatsu et al., 2011), an optogenetic tool (Aoki et al., 2013), traction

force microscopy (Trepat et al., 2009), and mathematical modeling, we show that each follower cell possesses a mechanochemical feedback system, in which stretch-induced ERK activation triggers cell contraction. Intercellular coupling of the ERK-mediated mechanochemical feedback enables sustained propagation of ERK activation and contractile force generation, leading to multicellular alignment of front-rear cell polarity over long distances. Thus, our study clarifies a mechanism of intercellular communication underlying long-range sustained transmission of directional cues for collective cell migration.

Results

Cell deformation waves precede ERK activation waves

To investigate the relationship between ERK activation and cell deformation during collective cell migration, Madin-Darby canine kidney (MDCK) cells confluently seeded within compartments of a silicone confinement were released for collective cell migration. ERK activity and cell deformation were evaluated by FRET imaging with EKAREV-NLS, by which ERK activation is quantified as an increase in the FRET/CFP ratio (Komatsu et al., 2011), and particle image velocimetry (PIV)-based image processing, respectively. As the cells migrated toward free spaces, sustained ERK activation waves were propagated from the leader cells to the follower cells (Figures 1A and 1B; Movie S1), in agreement with previous reports (Aoki et al., 2017; Matsubayashi et al., 2004). The cell deformation, i.e., extension and shrinkage, was quantified by the cell strain rate in the direction of collective cell migration (x-strain rate). Similar to the ERK activity, the x-strain rate exhibited repeated positive and negative values, namely extension and shrinkage, which propagated from the leader cells to the follower cells (Figures 1A and 1C; Movie S1), as described previously (Serra-Picamal et al., 2012; Tlili et al., 2018). Hereinafter, we refer to the propagation of the x-strain rate as cell deformation waves (Rodriguez-Franco et al., 2017).

To analyze the correlation between the ERK activation and the cell deformation, time-series data of the ERK activity and those of the x-strain rate were collected at the single cell level. We then calculated ERK activation rate, i.e., the ERK activity change per min, to compare not ERK activity itself but a time derivative of ERK activity and x-strain rate. We noticed that the ERK activation rate and the x-strain rate oscillated almost synchronously with an approximately 90 min period (Figure 1D). Furthermore, temporal cross-correlation analysis revealed that the ERK activation rate lagged 3 min behind the x-strain rate (Figure 1E),

118 indicating that the cell deformation waves precede the ERK activation waves. In other words,
119 cells are first extended, followed by ERK activation, and then the cells start contracting.
120

121 **Cell extension triggers ERK activation via EGFR signaling**

122 Because the cell extension precedes ERK activation, we reasoned that mechanical stretch
123 activates ERK during collective cell migration. To demonstrate this, we stretched MDCK cells
124 plated on an elastic silicone plate and compared the ERK activity before and after the
125 mechanical stretch. As anticipated, uniaxial 50% stretch of the MDCK cells resulted in ERK
126 activation (Figures 2A and 2B), indicating that passive extension of cells activates ERK.
127 Furthermore, the ERK activation almost linearly responded to different degrees of stretch, and
128 30% stretch was sufficient for triggering significant ERK activation, indicating that ERK
129 activation scales with degrees of stretch.

130 Previously, we reported that EGFR and a disintegrin and metalloprotease 17
131 (ADAM17), which catalyzes the shedding of membrane-tethered EGFR ligands (Maretzky et
132 al., 2011a), affect ERK activation waves (Aoki et al., 2017; Aoki et al., 2013). Consistent with
133 those reports, either treatment with an inhibitor of EGFR, PD153035, or that of matrix
134 metalloproteinases (MMPs) and ADAMs, marimastat, suppressed the ERK activation waves
135 during collective cell migration (Figure S1). These observations strongly suggest that the
136 stretch-induced ERK activation requires EGFR and ADAM17 activity. In fact, both PD153035
137 and marimastat suppressed the stretch-induced ERK activation (Figure 2C). Remarkably,
138 immunoblotting showed that cell stretch transiently increased auto-phosphorylated EGFR,
139 followed by phosphorylation of the downstream kinases including RAF1, MEK1/2, and
140 ERK1/2 (Figures 2D-2H). Together, these results indicate that cell stretch activates EGFR and
141 its downstream signaling molecules, including ERK.
142

143 **ERK activation induces cell contraction via Rho-associated kinase activation**

144 Because the ERK activation precedes cell shrinkage (Figure 1E), we speculated that ERK
145 activation would induce cell contraction. To prove this hypothesis, we used 2paRAF, a light-
146 inducible ERK activation system based on cryptochrome 2 (CRY2)–CIBN dimerization (Aoki
147 et al., 2013; Kennedy et al., 2010; Kinjo et al., 2019). In this system, RAF1 fused with CRY2
148 is recruited to the plasma membrane by heterodimerization of CRY2 and membrane-anchored
149 CIBN upon blue light exposure, culminating in ERK activation. We seeded cells with and
150 without 2paRAF (2paCRY2-RAF1/CIBN-mScarlet-I-CAAX) expression into two separated

151 compartments of a silicone confinement, respectively, and formed an interface of the two cell
152 populations by the removal of the confinement (Figure 3A). Upon ERK activation by blue light
153 exposure, the interface shifted toward the side of the cells expressing 2paRAF (Figures 3B and
154 3C; Movie S2). Moreover, inhibiting the ERK activation with a MAPK/ERK kinase (MEK)
155 inhibitor, trametinib, treatment suppressed the interface shift (Figures 3B and 3C; Movie S2).
156 Furthermore, even with the inhibition of the ERK activity propagation in the cells without
157 2paRAF expression by treating an inhibitor of EGFR PD153035, a degree of the light-induced
158 contraction of the cells expressing 2paRAF was not significantly different from a case without
159 the inhibitor (Figures S2A and S2B), indicating that the cell contraction is due to the optogenetic
160 ERK activation but not to the ERK activation in the neighbors. Thus, these results clearly
161 indicate that the ERK activation induces cell contraction of confluent MDCK cells. To confirm
162 ERK activation also triggers cell contraction during collective cell migration, we tested the
163 effect of ERK inhibition either with an inhibitor of EGFR, PD153035, or that of MAPK/ERK
164 kinase (MEK), trametinib, on deformation of migrating cells. Both PD153035 and trametinib
165 treatment damped the oscillation of the x-strain rate (Figure S2C-S2F). Thus, ERK activation
166 is required for cell deformation during collective cell migration.

167 Cell contraction is driven by actomyosin, which involves Rho-associated kinase
168 (ROCK)-mediated regulation (Amano et al., 1996; Kimura et al., 1996; Totsukawa et al., 2000).
169 Thus, we examined the effects of a ROCK inhibitor, Y-27632, on the ERK-induced cell
170 contraction. We found that Y-27632 suppressed the ERK-induced cell contraction (Figure 3B
171 and 3C; Movie S2). This observation prompted us to examine whether ERK induces ROCK
172 activation. To this end, we used a cytosolic FRET biosensor for ROCK activity (Li et al., 2017).
173 After optogenetic ERK activation, ROCK activity increased, then plateaued within 12 min after
174 the blue light exposure (Figures 3D and 3E). We next examined the ROCK activity dynamics
175 during collective cell migration. Interestingly, ROCK activity exhibited repeated unidirectional
176 wave propagation from leader cells to the follower cells, as did the ERK activity (Figures 3F
177 and 3G; Movie S3). Inhibition of ERK with trametinib abolished the propagation of ROCK
178 activation waves (Figure 3H; Movie S3) as well as temporal oscillatory activation in each cell
179 (Figure 3I). In stark contrast, inhibition of ROCK with Y-27632 abolished the oscillatory
180 activity in each cell and also decreased the basal activity of ROCK drastically (Figures 3J and
181 3K; Movie S3), indicating that ERK activity is responsible only for the oscillatory component
182 of ROCK activity. Thus, we conclude that ROCK is activated downstream of ERK and is
183 integral to ERK-induced cell contraction.

184

185 **ERK activation decreases traction forces and accumulates F-actin at the cell-cell**
186 **interface**

187 How does ERK activation alter mechanical force generation to induce contraction? To answer
188 this question, we combined light-inducible ERK activation with traction force microscopy
189 (Trepat et al., 2009), which allows the measurement of traction forces loaded by cells on the
190 substrate. On a substrate of polyacrylamide gel embedded with fluorescent beads, the interface
191 between the cells with and without 2paRAF expression was formed, as already described
192 (Figure 3A). The traction force exerted by the 2paRAF-expressing cells markedly decreased
193 within 40 min after the start of blue light exposure (Figures 3L and 3M; Movie S4). By contrast,
194 inhibiting ERK with trametinib restored the traction force generation (Figure 3N), confirming
195 that ERK activation suppresses force loading on the substrate. The gradual increase in traction
196 force by the cells without 2paRAF expression in Figure 3M and 3N is independent of the
197 optogenetic ERK activation in the neighboring 2paRAF-expressing cell cluster because the
198 traction force increased over time even without blue light exposure (Figure S2G and S2H). This
199 is likely because increasing cell density by proliferation downregulates ERK activity (Aoki et
200 al., 2013). Moreover, we found that subcellular localization of F-actin in migrating cells is
201 altered depending on the ERK activity level (Figures 3O and 3P). ERK activation by epidermal
202 growth factor (EGF) promoted F-actin localization at the lateral side of the cells. By contrast,
203 ERK inhibition by trametinib treatment produced stress fibers at the basal side of the cells.
204 Therefore, these results suggest that ERK activation triggers cell contraction by accumulating
205 F-actin at the cell-cell interface and triggering predominant force loading on the interface while
206 suppressing traction force generation.
207

208 **Zonal cell contraction initiates sustained unidirectional ERK activation waves at the**
209 **interface**

210 We have shown that each cell possesses an ERK-mediated mechanochemical feedback system
211 coupling cell deformation and ERK activation: ERK is activated by cell extension, and the
212 activated ERK induces cell contraction. Considering the tight physical connection between
213 epithelial cells, contraction of a cell cluster should stretch cells in the adjacent cluster, mainly
214 at its border, thereby generating the ERK activity propagation. To test this hypothesis, we used
215 a rapamycin-activatable (RA) Rho guanine nucleotide exchange factor (GEF) system to induce
216 contraction of a cell cluster (Inoue et al., 2005; van Unen et al., 2015). We first seeded cells

217 carrying the RA Rho GEF expression system into a confinement (Figure 4A), and then seeded
218 EKAREV-NLS cells after the removal of the confinement, resulting in the formation of an
219 interface between cells expressing RA Rho GEF and cells expressing EKAREV-NLS. Before
220 rapamycin treatment, ERK activity was randomly propagated without any preferential direction
221 (Figure 4B; Movie S5). Upon rapamycin treatment, the cells with RA Rho GEF began to
222 contract, resulting in the extension of the adjacent EKAREV-NLS–expressing cell cluster
223 toward the RA Rho GEF-expressing cell clusters (Figures 4C and 4D; Movie S5). With this
224 trigger, ERK activation waves emerged at the interface and were unidirectionally propagated
225 toward the extended EKAREV-NLS–expressing cell cluster (Figures 4C-4E). In addition, the
226 displacement of the EKAREV-NLS–expressing cells was oriented toward the RA Rho GEF-
227 expressing cell clusters and was directed opposite the ERK activation waves (Figure 4F). Thus,
228 these results suggest that cell contraction generates ERK activation waves in the adjacent cell
229 cluster.

230 We then asked whether a cell-cell tight connection is required for intercellular propagation
231 of ERK activation waves. To test this, we knocked-out α-catenin, a major cell-cell junction
232 component (Nagafuchi et al., 1994), in MDCK cells (Figure 4G). As expected, E-cadherin at
233 the cell-cell junction was reduced in the α-catenin KO cells in comparison with wild-type (WT)
234 cells (Figure 4H). Importantly, there was no significant difference in the sensitivity of the WT
235 and α-catenin KO cells to EGFR stimulation by EGF (Figure 4I). Thus, the cells possess equal
236 sensitivity to EGFR stimulation even in α-catenin KO. Nevertheless, coordinated ERK activity
237 propagation was severely disrupted in the α-catenin KO cells (Figures 4J and 4K; Movie S6),
238 indicating that intercellular mechanical linkage is required for ERK activity propagation. Thus,
239 we conclude that intercellular force transmission mediates ERK activation waves.
240

241 **Front-rear cell polarization is required for the unidirectional propagation of ERK**
242 **activation waves**

243 We next investigated how unidirectional propagation of ERK activation waves is achieved
244 during collective cell migration. If cells contract isotropically, the loss of directional
245 information would impede the unidirectional propagation of ERK activation waves. Therefore,
246 there must be a mechanism by which the directional information is retained during the wave
247 propagation. We found that the cell deformed preferentially along the direction of migration
248 (Figures 5A-5C), to an extent sufficient for activating ERK (Fig. 2B), indicating that cells
249 deform anisotropically during collective cell migration. Furthermore, phosphorylated myosin

250 light chain, a marker of contractile myosin, and F-actin accumulated at the basal rear side of
251 migrating cells (Figures 5D, S3A, and S3B), which are indicative of polarized rear contraction.
252 Some cells showing the dense actomyosin fibers elongate almost perpendicular to the direction
253 of cell migration. This suggests that cell contraction toward the migration direction would cause
254 slight extension perpendicular to the direction of migration because the cell volume does not
255 largely change during the cell contraction. The presence of a front-rear cell polarity was also
256 confirmed by the localization of GM130, a Golgi apparatus marker, known to be located in
257 front of the nucleus in some types of migrating cells (Magdalena et al., 2003; Nobes and Hall,
258 1999). When cells were examined immediately after release from confinement (0 h migration),
259 the Golgi orientation relative to the nucleus was not biased toward the leading edge (Figures
260 S3C and S3D). By contrast, 21 h after the initiation of collective cell migration, the Golgi
261 orientation was biased toward the direction of the migration over more than a 1 mm range from
262 the leader cells (Figures 5E and 5F). To quantify the degree of alignment, we defined the
263 directedness of the Golgi orientation (Figure 5G), and found that the Golgi directedness first
264 increased around the leader cells, and then the increase spread through the follower cells over
265 time (Figure 5H). In addition, the directedness of ERK activation waves and that of migration
266 showed similar dynamics to the Golgi directedness (Figures 5I and 5J). Thus, these results
267 clarify that alignment of front-rear cell polarity in follower cells positively correlates with
268 unidirectionality of ERK activation waves.
269 To investigate the requirement of front-rear cell polarization for the unidirectional ERK
270 activation waves, we knocked down Rac1 or Cdc42, which are required for the establishment
271 of front-rear polarization (Nobes and Hall, 1999). Expression of short hairpin RNAs reduced
272 Rac1 and Cdc42 expression to 50% and 30%, respectively (Figures S3E and S3F). In the
273 knocked-down cells, the front-rear polarization of Golgi in the leader cells was still directed in
274 the direction of the migration; however, that of the follower cells was impaired after 21 h
275 migration (Figures 5K and 5L). Moreover, the unidirectionality of the ERK activation waves
276 and the alignment of migration direction were also impaired in the follower cells of the
277 knocked-down cells (Figures 5M and 5N; Movie S7). Taken together, these results demonstrate
278 that the alignment of front-rear polarization toward the direction of the migration is required
279 for the unidirectional ERK activation waves and the ordered cell migration over long distances
280 from the leading edge.
281
282 **ERK activation waves orient front-rear polarity in follower cells**

283 We further addressed the relationship between the ERK activation waves and the front-rear
284 polarization. Previously, it has been proposed that force loading on cell-cell junctions directs
285 front-rear polarization (Das et al., 2015). Consistent with this, disruption of cell-cell junctions
286 by α-catenin KO abolished the alignment of the front-rear polarization toward the direction of
287 migration (Figures S4A-S4C). From this observation, we speculated that ERK-induced
288 contractile force generation would direct the front-rear polarization in neighboring follower
289 cells. As expected, inhibition of ERK activation with trametinib suppressed the alignment of
290 the front-rear polarization and migration directionality in the follower cells at 21 h after
291 migration (Figures 6A-6C and S4D; Movie S8). Interestingly, constitutive ERK activation by
292 12-*O*-tetradecanoylphorbol 13-acetate (TPA) also inhibited the alignment of the front-rear
293 polarization and migration directionality in the follower cells (Figures 6A-6C and S4E; Movie
294 S8), suggesting that periodic ERK activation in the form of waves is important for polarizing
295 follower cells. We then tested whether synthetic ERK activation waves can enhance the
296 directedness of the Golgi polarization in the follower cells. To this end, we used MDCK cells
297 expressing 2paRAF (2paCRY2-RAF1/CIBN-CAAX) and Golgi-7-mCherry as a Golgi
298 apparatus marker. Those cells were confluently seeded in a confinement, and then released for
299 migration with an EGFR inhibitor PD153035 to suppress autonomous ERK activation waves
300 (Figure 6D). Of note, inhibition of EGFR signaling suppresses ERK activation waves in
301 follower cells, but ERK activation in the cells near the leading edge remains (Figure S1B and
302 S1C). Thus, the leader cells still migrate, even with EGFR signaling inhibition, while follower
303 cell migration is abrogated (Aoki et al., 2017). Under this condition, we created optogenetic
304 ERK activation waves by shifting patterned blue light-illumination to mimic spontaneous ERK
305 activation waves; i.e., ~35 μm width and 400 μm intervals at 3 μm min^{-1} velocity (Figure 6D).
306 With the synthetic ERK activation waves, the front-rear cell polarizations were significantly
307 aligned even in follower cells more than 600 μm distant from the leader cells, compared with
308 the samples in the absence of ERK activation waves (Figures 6E and 6F; Movie S9). Thus,
309 these results clearly demonstrate that ERK activation waves orient the front-rear polarity in
310 cells against the direction of the waves. Collectively, we conclude that unidirectional ERK
311 activation waves and multicellular alignment of the front-rear polarization propagate
312 cooperatively, enabling long-distance transmission of directional cues for collective cell
313 migration.
314
315 **Modeling cellular mechanochemical feedback with polarity demonstrates long-range**

316 **unidirectional ERK activation waves**

317 Finally, we developed a mathematical model to understand the role of ERK-mediated
318 mechanochemical waves in collective cell migration. We employed a two-dimensional cellular
319 Potts model (CPM) to represent behavior of the epithelial monolayer at a single cell scale
320 (Glazier and Graner, 1993; Hirashima et al., 2017; Merks and Glazier, 2005). In this model,
321 each cell morphology is represented as a cluster of regular lattices, and a state of lattices
322 determine an energy of the system. The dynamics of a multicellular system proceeds
323 stochastically on the basis of an energy minimization using a Monte Carlo simulation algorithm.
324 We here regard a unit of trials in Monte Carlo simulation (Monte Carlo steps: MCS) as 1 h in
325 experiments. The energy in the model includes essential properties in multicellular mechanics,
326 i.e., intercellular adhesion, cell elasticity, and active cellular contraction (Guillot and Lecuit,
327 2013; Heisenberg and Bellaiche, 2013). In addition, the cells possess an inherent orientation
328 regarding the polarized rear contraction, equivalent to the front-rear polarity defined as the
329 Golgi orientation in our experiments (Figure 7A). In our model, orientations of the cell polarity
330 in individual cells tend to be aligned as a result of the physical interaction with neighboring
331 cells (Supplementary notes) (Hirashima et al., 2013; Peyret et al., 2019; Szabo et al., 2010).
332 The anisotropy along the front-rear polarity and the strength of cell contraction are governed
333 by the cell polarity parameter ω and the ERK activity, respectively. That is, the cell contraction
334 is isotropic when $\omega=0$ and polarized for the rear side of the cell with increasing ω (Figure 7B);
335 we set $\omega=1$ as suggested in experiments (Figures 5A-5J), unless otherwise noted. In simulations,
336 we assumed that the follower cells obey the stretch-induced contraction, while the leader cells
337 keep migrating towards the free space.

338 We performed simulation analysis under a similar condition to the confinement release
339 assay, and confirmed that the unidirectional ERK activation waves from the leader cells to the
340 follower cells were reproduced (Figures 7C and 7D; Movie S10). Moreover, the simulation
341 results well mimicked experimental measurements in terms of spatio-temporal profiles of front-
342 rear polarity directedness, ERK wave directedness, and migration directedness (Figure 7E). We
343 then examined the effect of the cell polarity level ω on the directedness. For any type of
344 directedness, the spatial range with high values becomes more limited around the leading cells,
345 with the decrease of ω (Figure 7F; Movie S10) as observed with disruption of polarity proteins
346 (Figure 5K-5N). This indicates that the cell anisotropy in contraction along the front-rear
347 polarity is essential for long-range multicellular guidance. In addition, we investigated the effect
348 of ERK activation waves on the polarity directedness (Figures 7G and 7H). Even without the

349 ERK activation waves, the front-rear polarity in the follower cells around the leading edges is
350 relatively directed toward the front because the migrating leader cells pull near-follower cells;
351 however, the polarity directedness gradually decreases with distance from the leader cells. By
352 contrast, the polarity directedness is maintained long-range from the leader cells via the ERK
353 activation waves (Figure 7H). Hence, ERK activation waves direct the front-rear polarity of the
354 follower cells, consistent with the experiments with optogenetic ERK activation waves (Figure
355 6F).

356 Further in silico analysis led to the finding that the strength of cell-cell adhesion γ
357 controls synchronization of the ERK activity along a perpendicular axis to its propagation
358 direction (y-axis in Figure 7I). We introduced a spatial correlation length of the ERK activity
359 along the y-axis as a measure of its synchronization, and examined a dependency of γ on the
360 spatial correlation length. Our numerical investigation clearly shows that the spatial correlation
361 length increases with increasing cell-cell adhesion strength γ (Figure 7J), indicating that
362 multicellular integrity via intercellular mechanical linkages plays a key role in the ERK activity
363 synchronization. Taken together, tight connections between cells, each of which experiences
364 mechanochemical feedback with front-rear polarity, effect well-ordered long-range ERK
365 activation waves.
366
367

368 **Discussion**

369 During collective cell migration of epithelial cells, not only leader cells but also follower cells
370 exert traction forces on their substrates to drive cell movement, resulting in long-distance
371 coordinated cell migration (Trepat et al., 2009). Thus, the follower cells can sense the direction
372 of the leader cell migration. Previous studies have revealed that the directional cues are
373 transmitted to the follower cells by intercellular mechanical forces (Serra-Picamal et al., 2012).
374 The mechanical tension varies dynamically and spreads over long distances, leading to
375 establishment of local anisotropic stress, along which the cells tend to migrate (Das et al., 2015;
376 Tambe et al., 2011). However, the molecular mechanism by which the mechanical forces are
377 sustainably transmitted over long distances to direct collective cell migration remains to be
378 revealed. Here we identified that ERK is a key molecule regulating cellular mechanochemical
379 feedback, which governs long-distance sustained propagation of the directional cues. We
380 propose a mechanism of ERK and mechanical force-mediated intercellular communication
381 underlying the collective cell migration (Figure S4G). In the initial process of collective cell

382 migration, advancement of the leader cells toward the free space exerts pulling forces on the
383 adjacent follower cells, directing front-rear cell polarization. The pulling force then stretches
384 the follower cells and activates the EGFR-ERK signaling cascade, which in turn generates
385 contractile forces at the rear side of the follower cells through the ROCK pathway. The
386 contraction of the follower cells exerts pulling forces on the next follower cells, directing front-
387 rear polarization in the next follower cells and likely providing space for cryptic lamellipodia
388 to protrude for active migration (Das et al., 2015; Farooqui and Fenteany, 2005). At the same
389 time, the pulling force evokes another round of cell stretch, ERK activation, and following
390 ERK-mediated rear contraction in the next follower cells. Thus, intercellular coupling of the
391 ERK-mediated mechanochemical feedback via cell-cell junctions enables sustained
392 propagation of pulling forces, front-rear cell polarity, and ERK activation over a large tissue
393 scale, leading to collective cell migration.

394 The combination of optogenetic ERK activation and traction force measurement revealed
395 that ERK plays a critical role in translocating cellular force generators. Our experiments
396 demonstrate that the ERK activation triggers cell contraction through ROCK activation (Figure
397 3B-3E) while it decreases the traction forces (Figures 3L and 3M). The effect of ERK activation
398 on the force generation can be explained by the translocation of dense F-actin fibers from cell
399 bases to cell-cell junctions (Figures 3O and 3P), which should lead to an efficient tug on
400 adjacent cells. It is plausible that the ERK-induced cell contraction through ROCK involves
401 Rho GEF activation localized at cell-cell junctions. Along this line of reasoning, a previous
402 study demonstrated that mechanical stretching of cells caused localization of Rho GEF at cell-
403 cell junctions, giving rise to local activation of RhoA, and thereby the junction-associated
404 actomyosin (Acharya et al., 2018). On the other hand, the decrease in traction forces by ERK
405 activation could be attributed to disruption of focal adhesion-associated actin stress fibers due
406 to promotion of focal adhesion turnover (Colo et al., 2012; Webb et al., 2004). The focal
407 adhesion turnover should increase deformability of cells to efficiently contract upon contractile
408 force generation.

409 Remarkably, our results imply that ERK activation triggers polarized rear contraction in
410 each cell. As the intracellular ERK activation may not be significantly polarized due to rapid
411 ERK diffusion within the cytoplasm (Fujioka et al., 2006), the effect of ERK activation on the
412 polarized rear contraction is explained by an antagonistic activation of Rac1 and RhoA (Guilluy
413 et al., 2011). Force loading on a cell-cell junction activates Rac1 via dissociation of merlin from
414 the junction, which directs the cell front (Das et al., 2015). The preoccupied Rac1 activation at

415 the cell front should restrict the activation of RhoA-regulated contractile machineries to the rear
416 side of the cells, leading to polarized rear contraction upon ERK activation. Therefore, ERK
417 should convey the signal to stimulate the contractile machineries within a cell at the back in
418 response to pulling forces at the front.

419 An important unsolved question is how the mechanical stimuli are converted into
420 signal transduction to activate ERK. We have previously demonstrated that ADAM17 plays a
421 role for the propagation of the ERK activation in MDCK cells (Aoki et al., 2017). It has been
422 proposed that ADAM17 catalyzes the ectodomain shedding of EGFR ligands (Sahin et al.,
423 2004), and the released ligands can bind and activate EGFR in an autocrine or paracrine manner.
424 In this study, we found that stretch of MDCK cells activates EGFR-ERK pathway (Figures 2D
425 and 2E), as reported in other cell types (Kippenberger et al., 2005; Zhang et al., 2007), and the
426 stretch-induced ERK activation requires ADAM proteinase activity (Figure 2C). Therefore, we
427 suppose that the stretch-induced EGFR activation involves the ADAM17-mediated shedding of
428 EGFR ligands. It has been shown that ERK activates ADAM 17 by means of phosphorylation
429 of Thr735 (Diaz-Rodriguez et al., 2002; Li et al., 2018). However, EGFR activation occurs as
430 early as 1 min after stretching and precedes ERK activation (Figures 2D-2H), which is at odds
431 with the involvement of ERK activation in the stretch-induced EGFR activation. It has also
432 been reported that src, a protein tyrosine kinase, is involved in ADAM17 activation (Maretzky
433 et al., 2011b; Niu et al., 2015) and is required for the stretch-induced EGFR activation (Zhang
434 et al., 2007). However, we do not know whether ADAM17 is activated upon stretch to cause
435 the ligand shedding. Another possible mechanism is that EGFR ligands are constitutively shed
436 by ADAM proteinases and the sensitivity of EGFR to the ligands may be increased upon stretch.
437 Thus, future studies will be necessary to explore how the mechanical stimuli activate EGFR in
438 a manner dependent on the ligands in the ERK activation waves. Also, we have recently
439 obtained preliminary data showing that ADAM17 is involved in ERK-induced cell contraction
440 as well as stretch-induced ERK activation. Therefore, further investigations should focus on the
441 regulation of ERK activation waves by ADAM17 through multi-tiered system.

442 In conclusion, we have revealed that mechanical force transmission functions as a
443 mediator of the intercellular ERK signal transduction underlying collective cell migration.
444 Current understanding of the intercellular signal transduction mostly emphasizes the
445 importance of intercellular transfer of biochemical molecules including growth factors,
446 hormones, and neurotransmitters. Thus, our present study raises another consideration, which
447 is the critical role of cellular response to mechanical stimuli in intercellular signal transduction.

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Author contributions

Conceptualization, N.H., M.M., T.H.; Methodology, N.H., K.A., X.T., M.M., T.H.; Software, N.H., X.T., T.H.; Validation, N.H., L.R., A.M.L., T.H.; Formal analysis, N.H., T.H.; Investigation, N.H., L.R., A.M.L.; Resources, N.H., K.A., X.T., T.H.; Data curation, N.H., T.H.; Writing - original draft, N.H., M.M., T.H.; Writing - review & editing, N.H., K.A., M.M., T.H.; Visualization, N.H., T.H.; Supervision, M.M., T.H.; Project administration, M.M., T.H.; Funding acquisition, N.H., X.T., M.M., T.H.

Declaration of interests

The authors declare no competing interests.

471 **Main figure titles and legends**

472 **Figure 1. Cell deformation waves precede ERK activation waves during collective cell**
473 **migration**

474 (A) Phase contrast images (upper), FRET/CFP ratio indicating ERK activity (middle), and
475 strain rate in the direction of collective cell migration (x-strain rate; lower) are represented at
476 1h (left), 8 h (center), and 14 h (right) after start of time-lapse imaging. In the x-strain rate
477 images, extending and shrinking regions are shown in red and blue, respectively. Scale bar, 200
478 µm. The imaging interval is 1 min.

479 (B and C) x-t kymographs of ERK activity (B) and x-strain rate (C), from (A). White arrows
480 indicate the rightward propagation of ERK activation waves (B) and cell deformation waves
481 (C).

482 (D) Temporal change of ERK activation rate and x-strain rate in a representative cell.

483 (E) Temporal cross-correlations between x-strain rate and ERK activation rate. The blue line
484 indicates the average temporal cross-correlation coefficients with standard deviations (SDs). *n*
485 = 212 cells from three independent experiments. See also Movie S1.

486

487 **Figure 2. Passive cell extension triggers ERK activation via EGFR signaling**

488 (A and B) ERK activity change in MDCK cells on an elastic chamber. (A) Representative
489 images before (left) and 20 min after uniaxial 50% stretch (right). The black double-headed
490 arrow indicates the axis of the uniaxial stretch. White and yellow arrowheads each correspond
491 to the same cells in the left and right images. Scale bar, 30 µm. (B) Violin plots of ERK activity
492 20 min after 20-50% stretch or that without stretch (0% stretch). Red and gray lines indicate
493 median and mean values, respectively. *n* = 3612 cells (0% stretch), 4771 cells (20% stretch),
494 3856 cells (30% stretch), 4070 cells (40% stretch), and 3178 cells (50% stretch) from three
495 independent experiments. Dunnett's test; $P = 0.0969$ (0 versus 20% stretch), ****$P < 0.0001$
496 (0 versus 30, 40, or 50% stretch).

497 (C) ERK activity changes in cells treated with DMSO, 1 µM PD153035, and 10 µM marimastat,
498 from immediately after to 20 min after the stretch are shown as means with SDs. *n* = 3634 cells
499 (DMSO), 3237 cells (PD153035), and 4000 cells (marimastat) from three independent
500 experiments. Dunnett's test, ****$P < 0.0001$.

501 (D) MDCK cells were stretched and then lysed at the indicated time points. The cell lysates
502 were analyzed by immunoblotting with the indicated antibodies.

503 (E-H) Normalized phosphorylation levels of EGFR (E), RAF1 (F), MEK1/2 (G), and ERK1/2

504 (H) are represented as means with SDs (*n* = 3).
505 See also Figure S1.
506
507 **Figure 3. ERK activation triggers cell contraction via Rho-associated kinase activation**
508 (A) Schematics of an experiment with the light-inducible system. The boundary between the
509 cell populations with and without 2paRAF expression was imaged.
510 (B) Representative images of CFP (EKAREV-NLS; upper) and CIBN-mScarlet-I-CAAX
511 (2paRAF; middle) treated with DMSO (left), 200 nM trametinib (center), and 10 μM Y-27632
512 (right) were obtained at the boundary between the cells with and without 2paRAF. Lower panels
513 indicate kymographs of the CIBN-mScarlet-I-CAAX (2paRAF) images. Blue lines indicate the
514 blue light illumination. Scale bar, 30 μm. The imaging interval is 5 min.
515 (C) Displacement of the boundary is plotted over time after blue light exposure. The lines
516 represent the average with SDs. *n* = 9 from three independent experiments.
517 (D) ROCK activity images of MDCK cells expressing 2paRAF and a ROCK biosensor are
518 represented at 0 min (left) and 15 min (right) after the start of blue light exposure. Scale bar, 20
519 μm. The imaging interval is 1 min.
520 (E) Quantification of ROCK activity in each cell in (D) after the start of blue light exposure.
521 The line represents the average with SDs. *n* = 15 from three independent experiments. Unpaired
522 *t*-test, *P* = 0.0065.
523 (F) ROCK activity images are represented at 0 h (upper) and 12 h (lower) after removal of
524 confinement. Scale bar, 200 μm. The imaging interval is 2 min.
525 (G) A kymograph of the ROCK activity in (F). White arrows indicate the rightward propagation
526 of ROCK activity.
527 (H and J) A kymograph of ROCK activity is shown before and after 200 nM trametinib (H) and
528 100 μM Y-27632 (J) treatment. The imaging interval is 2 min.
529 (I and K) ROCK activity in 5 representative cells was plotted over time after trametinib (I) and
530 Y-27632 (K) treatment.
531 (L) Traction force microscopy of the cell seeded on polyacrylamide gels with optogenetic ERK
532 activation. Differential interference contrast (DIC) images (upper), CIBN-mScarlet-I-CAAX
533 fluorescence (2paRAF; middle), and traction force (lower) are represented at −1 h (left), 1 h
534 (center), and 4 h after the start of blue light exposure. Scale bar, 50 μm. The imaging interval
535 is 5 min.
536 (M) Mean traction force in cells with and without 2paRAF, as shown in (L), with SDs (n = 3).

537 (N) Mean traction force in cells with 2paRAF with DMSO or 200 nM trametinib treatment at 2
538 h after the start of blue light exposure (n = 3).
539 (O) Fluorescence images of F-actin (phalloidin) in migrating MDCK cells on collagen-coated
540 glass substrates 45 min after treatment with DMSO, 100 ng mL^{-1} EGF, 200 nM trametinib, or
541 both EGF and trametinib. Scale bar, 10 μm.
542 (P) Intensity profile of F-actin along the orange lines in (O). Orange arrowheads indicate edges
543 of cells.
544 See also Figure S2, Movie S3 and S4.
545
546 **Figure 4. ERK activation waves are mediated by intercellular mechanical force**
547 (A) Schematics of an experiment using the rapamycin-inducible system. The boundary between
548 the cell population with RA Rho GEF and that with EKAREV-NLS expression was imaged.
549 (B) DIC images (upper), ERK activity (middle), and ERK wave directionality (lower) are
550 represented at 0 h after DMSO (left) and 50 nM rapamycin (right) treatment. Broken lines
551 indicate the cell population boundary. Arrows represent the direction of ERK activity
552 propagation. For the analysis of ERK wave directionality, the EKAREV-NLS–expressing cells
553 located to the left of the cell population boundary were omitted from the analysis. Scale bar,
554 100 μm. The imaging interval is 5 min.
555 (C) ERK activity (upper) and ERK wave directionality (lower) are represented at 13 h after the
556 drug treatment.
557 (D) Kymographs of ERK activity with DMSO (left) or rapamycin (right) treatment. The
558 EKAREV-NLS–expressing cells located on the left side of the cell population boundary were
559 excluded from the kymographs for visibility.
560 (E and F) Polar histograms showing the distribution of ERK wave (E) and cell displacement
561 (F) direction over 13-13.5 h after treatment with DMSO (left) and rapamycin (right). For ERK
562 wave direction, n = 1078 (DMSO) and 1127 (rapamycin) from three independent experiments.
563 For cell displacement direction, n = 475 (DMSO) and 1167 (rapamycin) from three independent
564 experiments.
565 (G) Analysis of the expression level of α-catenin and β-actin in WT and α-catenin KO MDCK
566 cells by immunoblotting.
567 (H) DIC images (upper) and immunofluorescence images of E-cadherin (lower) in the confluent
568 WT and α-catenin KO MDCK cells.
569 (I) The ERK activity 16 min after the addition of EGF was plotted as log(dose) response. Orange

570 circles (WT cells) and blue squares (α-catenin KO cells) represent the average of ERK activity
571 with SDs. The data were obtained from three independent experiments.
572 (J and K) Kymographs of ERK activity during collective cell migration in WT (J) and α-catenin
573 KO (K) MDCK cells. The imaging interval is 5 min.
574 See also Movie S5 and S6.
575
576 **Figure 5. Multicellular alignment of front-rear polarization underpins unidirectional**
577 **ERK activation waves**
578 (A) A representative cell expressing mCherry in the cytosol is marked by a broken line. The left
579 column shows composite images of phase contrast and mCherry fluorescence (green), and the
580 right column shows ERK activity. Cells showed low ERK activity in the contracted state (upper
581 and lower) and high ERK activity in the extended state (middle).
582 (B) Changes in lengths of cells along the x axis and y axis from contracted (minimum ERK
583 activity) to extended (maximum ERK activity) states are plotted. Each line indicates an
584 individual cell. $n = 30$ cells from three independent experiments. Paired *t*-test, ****$P < 0.0001$.
585 (C) Changes in lengths of cells along the x axis and y axis from extended (maximum ERK
586 activity) to contracted (minimum ERK activity) states are plotted. Each line indicates an
587 individual cell. $n = 30$ cells from three independent experiments. Paired *t*-test, ****$P < 0.0001$.
588 (D) Immunofluorescence images of F-actin (left) and di-phosphorylated MLC (ppMLC; center)
589 at the basal plane in MDCK cells at 17 h after migration. The right column indicates the
590 composite images of F-actin and ppMLC.
591 (E) Immunofluorescence images of the Golgi apparatus (GM130) and the nucleus (EKAREV-
592 NLS) in MDCK cells at 21 h after migration. The upper image shows a wide-view field, and
593 the lower images are magnified images of the regions corresponding to the numbered windows
594 in the upper image.
595 (F) Polar histograms showing the distribution of Golgi orientation relative to the nucleus in the
596 cells at 0-0.5 mm (left), 0.5-1.0 (center), and greater than 1.0 mm (right) distant from the leader
597 cells. $n = 1297$ cells (0-0.5 mm), 2096 cells (0.5-1.0 mm), and 1452 cells (>1.0 mm) from three
598 independent experiments.
599 (G) θ is the angle between the reference direction and sample direction. Directedness was
600 calculated by the indicated equation. The reference direction is set to the left for analysis of
601 Golgi orientation and cell migration direction, and to the right for ERK wave direction, in order
602 to match the signs of their directedness.

603 (H-J) Mean directedness of Golgi orientation (H), ERK wave direction (I), and cell migration
604 (J) binned every 300 μm from the leader cells after 0 h, 6 h, and 21 h migration is plotted over
605 the distance from the leader cells, with SDs. The data were obtained from three independent
606 experiments. The first bin of the ERK wave directedness was excluded from the result because
607 of outliers due to boundary effects.
608 (K) Immunofluorescence images of Golgi (GM130) and nuclei (EKAREV-NLS) in shRac1-
609 and shCdc42-expressing MDCK cells at regions greater than 1 mm from the leader cells after
610 21 h migration.
611 (L-N) Directedness of Golgi orientation (Dunnett's test, ****P* = 0.0001 (0.6-0.9 μm, WT versus
612 shCdc42), *P* = 0.0508 (0.9-1.2 mm, WT versus shCdc42), ****P* = 0.0009 (0.6-0.9 μm, WT
613 versus shRac1), *P* = 0.2023 (0.9-1.2 μm, WT versus shRac1)) (L), ERK wave direction
614 (Dunnett's test, ***P* = 0.0067 (0.9-1.2 mm, WT versus shCdc42), **P* = 0.0290 (1.2-1.5 mm,
615 WT versus shCdc42), ***P* = 0.0019 (0.9-1.2 mm, WT versus shRac1), **P* = 0.0248 (1.2-1.5
616 mm, WT versus shRac1)) (M), and cell migration direction (Dunnett's test, **P* = 0.0238 (1.2-
617 1.5 mm, WT versus shRac1), *P* = 0.0655 (1.2-1.5 mm, WT versus shCdc42)) (N) binned every
618 300 μm from the leader cells after 21 h migration were plotted over the distance from the leader
619 cells with SDs. The data of WT cells is the same as that of 21 h migration in (H-J). The data
620 were obtained from three independent experiments.
621 See also Figure S3 and Movie S7.
622
623 **Figure 6. Synthetic ERK activation waves orient front-rear polarity in follower cells**
624 (A) Immunofluorescence images of Golgi (GM130) and nuclei (EKAREV-NLS) in MDCK
625 cells treated with DMSO (left), 200 nM trametinib (center), and 10 nM TPA (right) at a region
626 more than 1.0 mm distant from the leader cells at 21 h after migration. Scale bar, 100 μm.
627 (B and C) Mean directedness of Golgi orientation (B) and cell migration direction (C) binned
628 every 300 μm from the leader cells after 21 h migration were plotted over distance from the
629 leader cells, with SDs. The data were obtained from three independent experiments.
630 (D) Flow diagram of an experiment on synthetic ERK activation waves.
631 (E) Fluorescence images of Golgi apparatus (Golgi-7-mCherry) and nuclei (EKAREV-NLS) in
632 MDCK cells with or without optogenetic ERK activation waves for 18 h. The upper images are
633 wide-view fields, and the lower images are magnified views of the regions outlined by white
634 windows in the upper images.
635 (F) The mean directedness of Golgi orientation binned every 100 μm from the leader cells in

636 (E) was plotted over distance from the leader cells, with SDs. The data were obtained from
637 three independent experiments. Unpaired *t*-test; ***P* = 0.0011 (0.6-0.7 μm, plus versus minus),
638 **P* = 0.0267 (0.7-0.8 mm, plus versus minus).
639 See also Figure S4, Movie S8 and S9.
640
641 **Figure 7. Modeling cellular mechanochemical feedback with polarity demonstrates long-**
642 **range unidirectional ERK activation waves**
643 (A) Schematics of the mathematical model.
644 (B) Contraction mode depending on the parameter ω, defined as the polarity level.
645 (C) Snapshot of ERK activity in the CPM simulation. Scale bar, 200 μm. (D) Kymograph of
646 ERK activity along the x-axis and simulation step. The color corresponds to values of the color
647 bar in (C).
648 (E) Spatial distribution of mean directedness, with SDs. The color shows different simulation
649 steps. n=5.
650 (F) The effect of polarity level ω on the directedness. The color represents mean values of the
651 directedness. n=5.
652 (G and H) Cell polarity directedness with (+) or without (–) ERK wave. The color in (G)
653 represents the angle of cell polarity. (H) Mean polarity directedness from the leading edge, with
654 SDs. n=5.
655 (I) Snapshot of ERK activity distribution in weak (γ=0.5) and strong (γ=1.5) multicellular
656 integrity.
657 (J) Mean y-axis spatial correlation length of the ERK activity on the intercellular integrity γ,
658 with SDs. n=5. See also Movie S10.
659

660 **STAR Methods**

661

662 **RESOURCE AVAILABILITY**

663 ***Lead Contact***

664 Further information and requests for resources and reagents should be directed to and will be
665 fulfilled by the Lead Contact, Tsuyoshi Hirashima (hirashima.tsuyoshi.2m@kyoto-u.ac.jp).

666

667 ***Materials Availability***

668 Materials developed for this study are available on request to the corresponding authors.

669

670 ***Data and Code Availability***

671 Data collected and computer codes developed for this study are available on request to the
672 corresponding authors.

673

674 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

675 **Cell culture**

676 MDCK cells were from the RIKEN BioResource Center (no. RCB0995). Lenti-X 293T cells
677 were purchased from Clontech (no. 632180, Mountain View, CA). These cells were maintained
678 in D-MEM (no. 044-29765, Wako, Osaka, Japan) supplemented with 10% FBS (no. 172012-
679 500ML, SIGMA, St. Louis, MO), 100 unit mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin (no.
680 26253-84, Nacalai Tesque, Kyoto, Japan) in a 5% CO_2 humidified incubator at 37°C.

681

682 **Establishment of stable cell lines**

683 For the establishment of EKAREV-NLS–expressing MDCK cells, a Tol2 transposon system
684 was used. MDCK cells were co-transfected with pT2A-EKAREV-NLS and pCAGGS-T2TP
685 encoding Tol2 transposase, and sorted by FACS as previously described (Kawabata and
686 Matsuda, 2016; Sakurai et al., 2012). To establish MDCK cells stably expressing 2paRAF,
687 pT2ADWpuro_2paRAF or pT2ADWpuro_2paRAFΔmScarlet-I were used for the Tol2
688 transposon system. For the generation of cells stably expressing Eevee-ROCK-NES and the
689 other ectopic proteins, a lentiviral or retroviral system was employed. To prepare the lentivirus,
690 pCSII-based lentiviral vector (Miyoshi et al., 1998) or lentiCRISPRv2 (Addgene Plasmid: no.
691 52961), psPAX2 (Addgene Plasmid: no. 12260), and pCMV-VSV-G-RSV-Rev were co-
692 transfected into Lenti-X 293T cells using polyethylenimine (no. 24765-1, Polyscience Inc.,

693 Warrington, PA). To prepare the retrovirus, pCX4-based retroviral vector (Akagi et al., 2003)
694 or pSUPER (Oligoengine, Seattle, WA), pGP, and pCMV-VSV-G-RSV-Rev were co-
695 transfected into Lenti-X 293T cells. Stable cell lines of MDCK cells were selected and
696 maintained in media containing the following antibiotics: MDCK/EKAREV-NLS/2paRAF, 4
697 μg mL^{-1} puromycin (no. P-8833, SIGMA); MDCK/EKAREV-NLS/2paRAF/Golgi-7-mCherry,
698 4 μg mL^{-1} puromycin and 10 μg mL^{-1} blasticidin S (no. 029-18701, Wako); MDCK/Eevee-
699 ROCK-NES, 10 μg mL^{-1} blasticidin S; MDCK/Eevee-ROCK-NES/2paRAF, 10 μg mL^{-1}
700 blasticidin S and 4 μg mL^{-1} puromycin; MDCK/EKAREV-NLS/mCherry, 4 μg mL^{-1}
701 puromycin; MDCK/RA Rho GEF, 4 μg mL^{-1} puromycin and 10 μg mL^{-1} blasticidin S.
702

703 **CRISPR/Cas9-mediated KO cell lines**

704 For CRISPR/Cas9-mediated KO of dog *CTNNA1* (α-catenin), single guide RNAs (sgRNA)
705 targeting the exon were designed using the CRISPRdirect (Naito et al., 2015). The following
706 sequence was used for the sgRNA sequence: GTAGAAGATGTTCGAAAACA. Oligo DNAs
707 for the sgRNA were cloned into the lentiCRISPRv2 vector, and the sgRNA and Cas9 were
708 introduced into MDCK cells by lentiviral infection. The infected cells were selected with 4.0
709 μg mL^{-1} puromycin. After the selection, reduction in expression levels of the proteins was
710 confirmed by immunoblotting. Bulk cells were used for the experiments.
711

712 **shRNA-mediated KD cell line**

713 For shRNA-mediated KD of dog *RAC1* and *CDC42*, the DNA oligomers corresponding to the
714 shRNAs targeting the genes were subcloned into pSUPER vector. The following sequences
715 were used for shRNA target sequences: *RAC1*, GCCTTCGCACTCAATGCCAAG; *CDC42*,
716 GAACAAACAGAAGCCTATC. The shRNAs were introduced into MDCK cells by retroviral
717 infection. The infected cells were selected with 4.0 μg mL^{-1} puromycin. After the selection,
718 reduction in expression levels of the target proteins was confirmed by immunoblotting.
719

720 **METHOD DETAILS**

721 **Plasmids**

722 pT2A-EKAREV-NLS and plasmids for RA Rho GEF (pCX4puro-LDR, and pCX4bsr-3HA-
723 FKBP-p63RhoGEF-DH) were described previously (Kawabata and Matsuda, 2016; Komatsu
724 et al., 2011; Li et al., 2017; Urasaki et al., 2006; van Unen et al., 2015). pT2ADWpuro_2paRAF
725 (Addgene plasmid: no. 129653) encoding 2paCRY2-RAF1-P2A-CIBN-mScarlet-I-CAAX was

726 described previously (Kinjo et al., 2019). pT2ADWpuro_2paRAFΔmScarlet-I encoding
727 2paCRY2-RAF1-P2A-CIBN-CAAX was generated by PCR and subcloned into the
728 pT2ADWpuro vector. The FRET biosensor for ROCK activity, Eevee-ROCK-NES, was
729 described previously (Li et al., 2017) and subcloned into a pCSII vector. pCSII and pCMV-
730 VSVG-RSV-Rev were kindly gifted from H. Miyoshi (RIKEN BioResource Center, Ibaraki,
731 Japan). pGP was gifted from T.Akagi. psPAX2 was the kind gift of Didier Trono (Addgene
732 plasmid: no. 12260). mCherry-Golgi-7 was a gift from Michael Davidson (Addgene plasmid:
733 no. 55052) and the cassette was subcloned into a pCSIIbsr vector. pCAGGS-T2TP was a gift
734 from Koichi Kawakami (National Institute of Genetics, Shizuoka, Japan).The cDNA of
735 mCherry was subcloned into a pCSIIpuro vector to generate pCSIIpuro-mCherry.
736
737 **Reagents and antibodies**
738 The following reagents were used: trametinib (no. T-8123, LC Laboratories, Woburn, MA), Y-
739 27632 (no. 253-00513, Wako, Osaka, Japan), PD153035, marimastat (no. SC-202223, Santa
740 Cruz Biotechnology, Dallas, TX), rapamycin (no. R-5000, LC Laboratories), TPA (no. P-1680,
741 LC Laboratories), Rhodamine Phalloidin (no. R415, Invitrogen, Carlsbad, CA, 1:40 dilution
742 for immunofluorescence), EGF (no. E9644, SIGMA, St. Louis, MO).
743 The following primary and secondary antibodies were used for immunoblotting: Anti-
744 EGFR rabbit antibody (no. 4267, Cell Signaling Technology, Danvers, MA, 1:1,000 dilution);
745 anti-phospho-EGFR (Tyr1068) rabbit antibody (no. 3777, Cell Signaling Technology, 1:1,000
746 dilution); anti-RAF1 mouse antibody (no. 610152, BD Biosciences, Franklin Lakes, NJ,
747 1:1,000 dilution); anti-phospho-RAF1 (Ser338) rabbit antibody (no. 9427, Cell Signaling
748 Technology, 1:1,000 dilution); anti-MEK1/2 rabbit antibody (no. 9122, Cell Signaling
749 Technology, 1:1,000 dilution); anti-phospho-MEK1/2 (Ser217/221) rabbit antibody (no. 9121,
750 Cell Signaling Technology, 1:1,000 dilution); anti-ERK1/2 mouse antibody (no. 610123, BD
751 Biosciences, 1:2,000 dilution); anti-phospho-p44/42 MAPK (Erk1/2; Thr202/Tyr204) rabbit
752 antibody (no. 9101, Cell Signaling Technology, 1:2,000 dilution); anti-β-Actin rabbit antibody
753 (no. 4970, Cell Signaling Technology, 1:1,000 dilution); anti-Cdc42 mouse antibody (no.
754 610929, BD Biosciences, 1:1,000 dilution); anti-Rac1 mouse antibody (no. 610650, BD
755 Biosciences, 1:1,000 dilution); anti-α-catenin mouse antibody (no. 610194, BD Biosciences,
756 1:1,000 dilution); IRDye 680-conjugated goat anti-mouse IgG antibody (no. 926-32220, LI-
757 COR Biosciences, Lincoln, NE, 1:10,000 dilution); and IRDye 800CW goat anti-rabbit IgG
758 antibody (no. 926-32211, LI-COR Biosciences, 1:10,000 dilution).

759 The following primary and secondary antibodies were used for immunofluorescence:
760 anti-phospho-Myosin Light Chain 2 (Thr18/Ser19) rabbit antibody (no. 3674, Cell Signaling
761 Technology, 1:50 dilution); anti-E-cadherin rabbit antibody (no. 3195, Cell Signaling
762 Technology, 1:300 dilution); Alexa 647-conjugated goat anti-mouse IgG (H+L) antibody (no.
763 A-21235, Thermo Fisher Scientific, Waltham, MA, 1:1,000 dilution); alexa 647-conjugated
764 goat anti-rabbit IgG (H+L) antibody (no. A-21245, Thermo Fisher Scientific, 1:1,000 dilution);
765 and alexa 568-conjugated goat anti-rabbit IgG (H+L) antibody (no. A-11036, Thermo Fisher
766 Scientific, 1:1,000 dilution).
767
768 **Confinement release assay**
769 To confine the MDCK cell monolayer, a Culture-Insert 2 Well (no. 81176, ibidi, Martinsried,
770 Germany) was placed on a glass-bottom dish coated with 0.3 mg mL^{-1} type I collagen (Nitta
771 Gelatin, Osaka, Japan). MDCK cells (7×10^3 cell) were seeded in the Culture-Insert. 24 h after
772 seeding, the Culture-Insert was removed, and the medium was replaced with Medium 199
773 (11043023; Life Technologies, Carlsbad, CA) supplemented with 10% FBS, 100 unit mL^{-1}
774 penicillin, and 100 µg mL^{-1} streptomycin. 30 min after the removal of the Culture-Insert, the
775 cells were imaged with an epifluorescence microscope every 1 to 10 min.
776
777 **Boundary assay**
778 For the light-induced ERK activation experiment, MDCK/EKAREV-NLS/2paRAF cells were
779 seeded in a well of a Culture-Insert 2 well placed on a 24 well glass bottom plate, and
780 MDCK/EKAREV-NLS cells were seeded in the other well of the insert and outside of the insert.
781 After 15 h incubation, the insert was removed, followed by further incubation for 36h, allowing
782 the cells to fill the gap between the cell populations. The interface between cells with and
783 without 2paRAF expression was imaged, and the cells were exposed to 438 nm blue LED light
784 every 5 min for CRY2 activation.
785 For the drug-induced cell contraction experiment, a well of the insert was removed by
786 cutting, and the remained insert with a well was placed on a 24 well glass-bottom plate. MDCK
787 cells expressing RA Rho GEF were seeded in the well. After 6 h incubation, the insert was
788 removed, and MDCK/EKAREV-NLS cells were plated in the 24 well glass-bottom plate. The
789 interface between cells with and without RA Rho GEF expression was imaged, and dimethyl
790 sulfoxide (DMSO; final 0.1%) or rapamycin (final 50 nM) was added into the medium for the
791 Rho GEF activation. To determine the ERK wave directionality, heat maps of ERK activity

792 were obtained by interpolating the signals in regions between the nuclei of MDCK/EKAREV-
793 NLS cells in the FRET/CFP ratio images. The heat maps of ERK activity were analyzed by
794 particle image velocimetry (PIV) using a free Matlab-toolbox, MatPIV (Sveen, 2004), to
795 calculate the ERK wave directionality. The size and overlap of the interrogation window was
796 set to 349 μm and 75%, respectively. To determine the directionality of cell displacement, the
797 Fiji TrackMate plugin (Jaqaman et al., 2008; Schindelin et al., 2012) was applied to the CFP
798 fluorescence images for tracking each cell over 13-13.5 h after treatment with DMSO or
799 rapamycin.
800
801 **Cell stretch assay**
802 For the cell stretch assay, MDCK cells (2×10^5 cell) were seeded on an elastic silicone chamber
803 (no. STB-CH-04, STREX, Osaka, Japan) coated with 0.3 mg mL^{-1} type I collagen. After 24 h
804 incubation, the MDCK cells on the stretch chamber were uniaxially stretched by 20-50% with
805 a manual cell-stretching system (no. STB-100-04, STREX) on an epifluorescence microscope.
806 The ERK activity 20 min after 20-50% stretch or that without stretch (0% stretch) was shown
807 as violin plots by using a MATLAB code, Violin Plot (Hoffmann, 2020). Red and gray lines
808 indicate median and mean values. For immunoblotting, stretched MDCK cells were lysed with
809 SDS sample buffer containing 62.5 mM Tris-HCl (pH6.8), 12% glycerol, 2% SDS, 40 ng mL^{-1}
810 bromophenol blue, and 5% 2-mercaptoethanol, followed by sonication with a Bioruptor UCD-
811 200 (Cosmo Bio, Tokyo, Japan). After boiling at 95°C for 5 min, the samples were resolved by
812 SDS-PAGE on SuperSep Ace 5-20% precast gels (Wako), and transferred to PVDF membranes
813 (Merck Millipore, Billerica, MA). All antibodies were diluted in Odyssey blocking buffer (LI-
814 COR Biosciences). Proteins were detected by an Odyssey Infrared Imaging System (LI-COR
815 Biosciences).
816
817 **Time-lapse imaging**
818 FRET images were obtained and processed under essentially the same conditions and
819 procedures as previously described (Aoki and Matsuda, 2009). Briefly, cells were imaged with
820 an IX83 inverted microscope (Olympus, Tokyo, Japan) equipped with a UPlanFL-PH 10x/0.3
821 (Olympus), a UPlanSApo 20x/0.75 (Olympus), or a UPlanSApo 40x/0.95 objective lens
822 (Olympus), a DOC CAM-HR CCD camera (Molecular Devices, Sunnyvale, CA), a Spectra-X
823 light engine (Lumencor Inc., Beaverton, OR), an IX3-ZDC laser-based autofocusing system
824 (Olympus), an electric XY stage (Sigma Koki, Tokyo, Japan), and a stage top incubator (Tokai

825 Hit, Fujinomiya, Japan). The filters and dichromatic mirrors used for time-lapse imaging were
826 as follows: for FRET imaging, a 438/24 excitation filter (incorporated in the Spectra-X light
827 engine), a FF458-Di02-25x36 (Semrock, Rochester, NY) dichromatic mirror, and FF01-
828 483/32-25 (Semrock) and FF01-542/27-25 (Semrock) emission filter for CFP and FRET,
829 respectively. For mCherry and mScarlet-I imaging, a 575/25 excitation filter, a glass
830 dichromatic mirror (Olympus), and FF01-624/40-25 (Semrock) emission filters were used.
831

832 **Immunofluorescence and confocal microscopy**

833 MDCK cells were fixed with 4% paraformaldehyde in PBS for 15 min at room temperature,
834 followed by permeabilization with 0.2% Triton X-100 in PBS for 5 min. The samples were then
835 incubated with 1% BSA in PBS for 1 h at room temperature, followed by sequential incubation
836 with primary and secondary antibodies diluted with 1% BSA in PBS overnight at 4°C (primary
837 antibodies) or for 1 h at room temperature (secondary antibodies). Images were collected using
838 a Fluoview FV1000 confocal microscope (Olympus) equipped with a UPlanSApo 60x/1.35 or
839 a UPlanSApo 100x/1.40 objective lens (Olympus).
840

841 **Traction force microscopy**

842 Polyacrylamide gel substrates were prepared as previously described, with slight modifications
843 (Aoki et al., 2017; Rodriguez-Franco et al., 2017). Briefly, glass-bottom dishes (IWAKI,
844 Shizuoka, Japan) were treated with 2% acetic acid (WAKO) and 0.2% 3-
845 (Trimethoxysilyl)propyl methacrylate (SIGMA) in 80% ethanol for 2 min. After the removal of
846 the solution, the dishes were dried for 15 min. For 3 kPa gels, 5.5% acrylamide, 0.09%
847 bisacrylamide, 0.05% ammonium persulfate, 0.05% N,N,N',N'-tetramethyl ethylenediamine,
848 and 0.01% deep red fluorescent carboxylate-modified beads (0.2 μm diameter; F8810, Thermo
849 Fisher Scientific) in PBS were prepared. 18 μL of the solution was put on the glass-bottom
850 dishes and 18 mm glass cover slips (Matsunami, Osaka, Japan) were placed on top of them.
851 After polymerization, the gels were covered with 2 mg mL^{-1} Sulfo-SANPAH (no. ab145610,
852 abcam, Cambridge, UK) and activated by ultraviolet light for 5 min with an Ultraviolet
853 Crosslinker CL-1000 apparatus (UVP, Upland, CA). The procedure was repeated again with 7
854 min UV irradiation. The gels were coated with 100 μm mL^{-1} type I collagen overnight at 4°C.
855 Then, the gels were washed three times with PBS and incubated with culture medium for 1h.
856 For traction force microscopy, the bead fluorescence was imaged with an IX83 inverted
857 microscope equipped with a 632/22 excitation filter (incorporated in the Spectra-X light engine),

a glass dichromatic mirror (Olympus), and FF01-692/40-25 (Semrock) emission filter. A reference image was obtained after the removal of cells by trypsinization. Traction forces were computed by Fourier-transform traction microscopy as described previously (Trepat et al., 2009).

EGF stimulation assay

To evaluate the sensitivity of cells to EGFR stimulation, WT or α-catenin KO MDCK cells (2.5 × 10^3 cell) were seeded on a 96 well glass bottom plate coated with 0.3 mg mL^{-1} type I collagen. After 24 h incubation, the MDCK cells were treated with different dose of EGF (0.01, 0.1, 1, 3, 10, 100 ng mL^{-1}). The ERK activity 16 min after the addition of EGF was plotted as log(dose) response and the plots were fitted to a variable slope model by least squares fit method with GraphPad Prism 7 software.

Optogenetic ERK activation wave assay

To illuminate a defined rectangular region with blue light, transillumination light of a 100-W halogen lamp filtered with BA420-460 (Olympus) was covered by a homemade aperture mask containing a slit. The image of the slit was focused on the sample plane by the condenser lens. The width of the focused image of the illumination light was approximately 35 μm. The illuminated region was moved at 3 μm min^{-1} velocity, and the illumination was patterned with 400 μm spatial intervals at each time point. The procedure was applied to the migrating MDCK/EKAREV-NLS/2paRAF/Golgi-7-mCherry cells treated with 1 μM PD153035, an EGFR inhibitor, to suppress autonomous ERK activation waves.

Kymography

To obtain the kymographs of FRET/CFP ratios and x-strain rate, these values were averaged along the y-axis in a defined region of the images, providing an intensity line along the x-axis. The operation was repeated for the respective time points, and the intensity lines were stacked along the y-axis for all time points.

Mathematical modeling

A cellular Potts model for cell migration

We used a two-dimensional cellular Potts model (CPM), also known as a Glazier-Graner-Hogeweg model, widely used for multicellular dynamics (Balter et al., 2007; Krieg et al., 2008;

891 te Boekhorst et al., 2016; van Helvert et al., 2018). In the CPM, each cell morphology is
892 represented as a cluster of square lattices identified with the identical index σ, denoting a cell;
893 the lattice distribution mainly determines an energy of the system *H*, such that cell behavior
894 depends on a balance of forces defined by the energy. The energy in our model is composed of
895 minimal factors necessary to capture the two-dimensional multicellular dynamics, such as
896 interfacial energy, cell area constraint, and active cell contraction as follows:
897

898 $$H = \sum_{\boldsymbol{r},\boldsymbol{r}'} J_{\tau(\sigma_r)\tau(\sigma_{r'})}\left(1 - \delta_{\sigma_r \sigma_{r'}}\right) + \lambda_A \sum_{\sigma}(A_\sigma - A_0)^2 + H_{contraction}, \quad (1)$$

899

900 where each of $\boldsymbol{r}$ and $\boldsymbol{r}'$ represents a position of lattice site, τ is an attribute of the lattice, i.e.,
901 cell or medium, *J* is the interfacial energy between cell-cell or cell-medium, δ is the Kronecker
902 delta, λ_A is the magnitude of resistance to cell deformation, A_σ is the current cell area, A_0 is
903 the ideal cell area, and $H_{contraction}$ is a term for cell contraction.
904 The first term in Eq. (1) describes a strength of cell-cell adhesion. The energy to
905 maintain cell-cell adhesion is determined by an energy of cell-cell adhesion J_{cc} relative to that
906 of cell-medium adhesion J_{cm} and is expressed as $\gamma = J_{cm} - J_{cc}/2$ (Davies and Rideal, 1963;
907 Glazier and Graner, 1993). We determined $J_{cm} = J_{cc} = 3$ in light of a balance with other
908 parameters for imitating MDCK behaviors, and changed the value in J_{cm} for the change in γ.
909 The second term represents cell elasticity, meaning that the cells attempt to retain the ideal area.
910 We set λ_A as 0.02 and 1 when cells extend beyond and shrink below the ideal area, respectively,
911 because of the unique material property of cells (Latorre et al., 2018; Trepat and Sahai, 2018).
912 The ideal cell area was determined as A_0= 400 μm^2, by the use of actual cell images. The third
913 term relates to the active cell contraction. In this model, individual cells are assigned unit
914 vectors $\boldsymbol{p}_{\boldsymbol{\sigma}}$ corresponding to the front-rear cell polarity, and the cell contraction occurs
915 depending on the polarity level ω with the contraction strength λ_σ, a variable related to the
916 ERK activity as shall be explained later. We define the contraction term as follows:
917

918 $$H_{contraction} = \sum_{\sigma} \sum_{l_\sigma} \lambda_\sigma \Phi, \quad (2)$$

919

920 and

921

922 $$\Phi = \begin{cases} 1 + 2\omega\pi^{-1}(\varphi_{l_\sigma} - \pi) & \text{if} \quad \varphi_{l_\sigma} > \pi(1 - (2\omega)^{-1}) \\ 0 & \text{if} \quad \varphi_{l_\sigma} \leq \pi(1 - (2\omega)^{-1}) \end{cases}, \quad (3)$$

923

924 where l_σ represents the index of lattices composing the cell periphery, and $\varphi_{l_\sigma} \in [0, \pi]$ is an
925 angle between $\boldsymbol{p_\sigma}$ and the vector connecting from the centroid of a cell to a peripheral lattice.
926 Note that $H_{contraction} = \sum_\sigma \lambda_\sigma P_\sigma$, where P_σ is the perimeter of a cell when $\omega = 0$, meaning
927 that cells shrink independent of the direction of polarity. By contrast, cell contraction is biased
928 to the rear of the cell when ω becomes larger.
929 In the CPM, the system transition occurs stochastically by a lattice-based Monte Carlo
930 method; that is, the labeled value of a randomly chosen lattice site $\sigma_{\boldsymbol{r}}$ is attempted to be
931 replaced by a different labeled value of randomly-chosen adjacent lattice site $\sigma_{\boldsymbol{r}'}$. The transition
932 occurs by evaluating a change in energy ΔH associated with its replacement. In the case of
933 energy increase, i.e., ΔH>0, the index replacement occurs stochastically according to a
934 Boltzmann acceptance function $exp(-\Delta H)$, while it deterministically occurs in the case of
935 energy decrease, i.e., ΔH≤0. For the details of CPM, see (Glazier and Graner, 1993; Graner
936 and Glazier, 1992; Hirashima et al., 2017; Marée et al., 2007; Merks and Glazier, 2005; Scianna,
937 2015). We regard the number of trials for lattice replacement as a total number of pixel domains
938 in simulations as a unit simulation step (USS); an update at each lattice site is attempted once
939 per 1 USS on average. We then defined 100 USS as 1 Monte Carlo step, corresponding to 1
940 hour.
941
942 *Dynamics of polarity orientation*
943 Explicit rules that govern the dynamics of front-rear cell polarity have not been clear. Yet, earlier
944 experimental studies have proposed that the front-rear polarity is oriented according to the
945 tensile force on cell-cell junctions (Das et al., 2015; Hayer et al., 2016). In particular, it has been
946 shown that Merlin localized at a cell-cell junction inhibits the activation of Rac1 when the
947 tension is low. In contrast, with high tension by strong contraction of neighbor cells, Merlin is
948 released into the cytoplasm, and Rac1 is locally activated (Das et al., 2015). Thus, orientation
949 of the front-rear polarity in individual cells changes through physical interaction with
950 neighboring cells over time. With this fact, we model the dynamics of cell polarity orientation
951 ϑ_σ as a phenomenological coupling with cell displacement according to earlier theoretical
952 studies (Hirashima et al., 2013; Notbohm et al., 2016; Peyret et al., 2019; Szabó et al., 2010a;
953 Szabó et al., 2010b; Tlili et al., 2018):
954
955 $$\frac{d\vartheta_\sigma}{dt} = \mu \frac{v_\sigma}{\sqrt{A_\sigma}} \Delta\phi_\sigma, \quad (4)$$
956 where μ is the degree of alignment to polarity in neighbor cells, v_σ is the cell speed, and

957 $\Delta\phi_\sigma$ is the angle between cell velocity $\boldsymbol{v}_\sigma$ and the polarity vector $\boldsymbol{p}_\sigma$. $v_\sigma/\sqrt{A_\sigma}$ contributes
958 to weighting of how much the neighboring cells affect reorientation of cell polarity and/or self-
959 reinforcement for a persistent polarization.
960 The behavior of cell polarity according to Eq. (4) can vary. For example, consider a
961 situation in which a cell A pulls an adjacent cell B in a direction opposite to the polarity
962 within cell B, and the center position of cell B is moved toward cell A. When μ is larger, the
963 polarity in cell B will re-orient to the direction in which cell B is pulled. Conversely, when μ
964 is smaller, the polarity orientation in cell B will not change, and it tends to be persistent over
965 time. In addition to values of μ, the magnitude of the cell displacement resulting from
966 interactions between multiple neighboring cells affects multicellular alignment of polarity. We
967 chose $\mu = 1$ for reproducing a proper polarity alignment.
968
969 *ERK-mediated mechanochemical feedback*
970 In the main text, we have shown experimentally that cell extension activates ERK, and that the
971 ERK activation induces cell contraction. Here we incorporated this observation into the
972 framework of CPM.
973 We define the cell areal strain as $\varepsilon_\sigma = A_\sigma/A_0 - 1$, and the dynamics of a normalized
974 ERK activity level $[ERK]_\sigma$, bounded from -1 to 1, is represented as
975
976 $$\frac{d[ERK]_\sigma}{dt} = (\tanh(\alpha\varepsilon_\sigma) - [ERK]_\sigma)/\eta_E, \quad (5)$$
977
978 where α is a sensitivity parameter and η_E denotes a timescale of this dynamics. We set $\alpha =$
979 3 for a proper response to cell areal strains in simulations, and $\eta_E = 3$ min with measured
980 data in Figure 1.
981 The dynamics of cell contraction strength λ_σ is defined as
982
983 $$\frac{d\lambda_\sigma}{dt} = (\lambda U([ERK]_\sigma - [ERK]^*) - \lambda_\sigma)/\eta_c, \quad (6)$$
984
985 where λ is a controlling parameter for conversion of ERK activation to contraction, $[ERK]^*$
986 denotes a threshold of ERK activation-induced contraction, and η_c denotes a timescale of this
987 dynamics. $U(x)$ is a step function: $U(x) = 1$ for $x \geq 0$ and $U(x) = 0$ for $x < 0$. We
988 chose $\lambda = 10$ and $[ERK]^* = 0.5$ since the ERK activation waves are reproduced with them.

989 We set $\eta_c = 30$ min, reflecting the response time delay observed in Figure 3C.
990
991 **Simulations**
992
993 *Matching time*
994 All dynamics on polarity orientation, ERK activity, and contraction (Eq. (4)-(6)) were
995 calculated in their discretized form and were updated every USS. The cell velocity $\boldsymbol{v}_\sigma$ was
996 also calculated by the change in the center of mass of cells per 1 USS. We ran the simulation
997 until steps equivalent to 23 hours after a start of cell migration in the confinement release assay.
998
999 *Confinement release assay, Boundary conditions, and Initial conditions.*
1000 In reference to a size of imaging windows, we set a pixel length of the simulation space as 2.5
1001 μm × 2.5 μm, and the computer simulation was performed in a space with 1200 (horizontal) ×
1002 400 (vertical) pixels, corresponding to 3 mm × 1 mm. We placed a cluster of cells, arranged
1003 with 120 cells in the x-axis direction by 50 cells in the y-axis direction, in a simulation field
1004 with a reflecting boundary condition. There is a free space only along the left side of the cell
1005 cluster, but no free space along the top, bottom, or right side. We assumed that cells facing the
1006 free space were leader cells, so that leaders could be changeable throughout the simulation. At
1007 initiation, each cell area was set to the ideal area value, and the orientation of front-rear cell
1008 polarity in each cell was random except for the leader cells. Cell proliferation was not included
1009 in simulations because it seems to minimally affect the ERK activation wave propagation (Tlili
1010 et al., 2018).
1011
1012 *Leader cells and follower cells*
1013 We defined the leader cells as cells facing a free space, corresponding to medium in the model,
1014 and follower cells as the others. The leader cells sense free spaces to migrate and communicate
1015 to the follower cells located behind the leader cells (Omelchenko et al., 2003; Yamaguchi et al.,
1016 2015). Thereby, we set the following two rules for the leader cells in polarity orientation and
1017 cell contraction. First, the cell polarity in the leader cells is persistently oriented towards the
1018 free space with a white Gaussian noise regardless of Eq. (4). Second, the leader cells keep
1019 generating polarized contractile force ($\omega = 1$) independent of cell extension-induced ERK
1020 activity (λ_σ =100) in Eq. (2) and (3) throughout the simulations.
1021

1022 *Parameters*
1023 The default parameter values are shown in the column "Values" in Table S1. In the rightmost
1024 column, the range of the respective parameters used in Figure 7 is summarized (Table S1). Each
1025 parameter value was determined based on either measured data, observation, or a balance of
1026 other parameter values. Under an energy minimization framework, relative values of a
1027 parameter to values in other parameters should be significant if the system is not far from its
1028 equilibrium.
1029
1030 **Additional comments on modeling and simulations**
1031 Wave propagation of cell velocities and/or mechanical stress during collective cell migration
1032 have been modeled in some earlier studies, and most of them adopt a continuum approach (Alert
1033 and Trepat, 2019; Banerjee et al., 2015; Notbohm et al., 2016; Tlili et al., 2018; Yabunaka and
1034 Marcq, 2017). They are constructed with a relatively simple assumption and include just a few
1035 parameters, which makes the analysis easier. In contrast, we chose a cell-based approach to
1036 express the collective cell behavior and modeled a system on a cellular mechanochemical
1037 feedback at tissue scale. Despite being complex and limited to numerical simulations, our model
1038 can recapitulate multiple unidirectional ERK activation waves in collective cell migration.
1039 Moreover, it simulates conditions under which the waves are not generated, and those are
1040 consistent with our experimental results. We believe that this approach contributes to further
1041 understanding of unique systems within and between cells, as well as refining our theoretical
1042 modeling of such systems. Additional numerical investigations on multicellular dynamics and
1043 ERK activation waves were beyond the scope of this study.
1044
1045 **Repeatability of experiments**
1046 All experiments were performed on at least three independent cell culture preparations.
1047
1048 **QUANTIFICATION AND STATISTICAL ANALYSIS**
1049
1050 **Quantification of ERK activity and cell deformation**
1051 For the analysis of the cell strain rate along an axis of collective cell migration (x-strain rate),
1052 PIV using MatPIV (Sveen, 2004) was applied to phase contrast time-lapse images to calculate
1053 velocity fields of cells. Velocity fields at time T were computed by the displacement between
1054 the images at $T\text{-}\Delta t$ and $T+\Delta t$ by using 'single' option for the method of pattern matching. The

1055 size of the interrogation window was set to 29.1 μm, approximately corresponding to the typical
1056 cell size, and the window overlap was set to 50%. Here, we defined the x-strain rate as spatial
1057 derivative of velocity field along x-axis divided by the distance between the centers of two
1058 adjacent interrogation windows. Thus, the x-strain rate $\dot{\varepsilon}_{i,j}$ was calculated according to

$$\dot{\varepsilon}_{i,j} = \frac{v_{i,j}^{x} - v_{i+1,j}^{x}}{L}, \quad (7)$$

1059 where $v_{i,j}^{x}$ is the *x* component of the velocity at spatial indices of resultant velocity vectors
1060 obtained by PIV analysis (*i, j*), and *L* is the distance between the windows (14.5μm). The value
1061 of x-strain rate is assigned at the center between the x-coordinate of two source velocity vectors.
1062 The obtained discrete data were processed by a cubic interpolation method to fill the gap.
1063 To represent the FRET efficiency, FRET/CFP ratio images were generated after the
1064 background intensity was subtracted from the original fluorescence images in the CFP and
1065 FRET channel, respectively, using Metamorph software (Molecular Devices, Sunnyvale, CA).
1066 Then, the Fiji TrackMate plugin was applied to the CFP fluorescence images for tracking each
1067 cell. The time derivative of ERK activity (ERK activation rate) at time *t* for each cell was
1068 computed by the change in FRET/CFP ratio between t-Δ t and $t+\Delta t$. The obtained time-series
1069 data of the x-strain rate and the ERK activation rate were processed with a Savitzky-Golay filter
1070 to reduce the noise. The cross-correlation coefficient $r(\tau)$ between time-series data of x-strain
1071 rate $f(t)$ and ERK activation rate $g(t)$ was calculated as follows:

$$r(\tau) = \frac{\sum_t f(t+\tau)g(t)}{\sqrt{\sum_t f(t)^2}\sqrt{\sum_t g(t)^2}}, \quad (8)$$

1072 where τ is the lag time.
1073 To quantify the amplitude of x-strain rate oscillation, the time-series data were fitted
1074 to a multi-peak function as described previously (Muta et al., 2018).
1075
1076 **Quantification of directedness**
1077 For quantification of directionality of Golgi positioning, ERK activation waves, and cell
1078 migration, we defined directedness as shown in Figure 5D. The reference direction is set to the
1079 forward direction for analysis of Golgi orientation and cell migration direction, and to the
1080 backward direction for that of ERK wave direction to match the signs of their directedness. To
1081 obtain the sample directions of the Golgi apparatus, the center of mass of the nucleus in each
1082 cell was determined with Fiji. Fluorescence images of Golgi apparatus markers were processed
1083 with a mean filter. The position that showed the highest intensity in the region within 6.8 μm

from the center of mass of the nucleus was defined as the position of the Golgi apparatus. The sample directions were determined by the direction of the Golgi apparatus relative to the center of mass of the nucleus. To determine the sample directions of ERK activation waves, heat maps of ERK activity were obtained by interpolating the signals in regions between the nuclei of MDCK/EKAREV-NLS cells in the FRET/CFP ratio images. The heat maps of ERK activity were analyzed by MatPIV with a 174 μm window size and a 75% window overlap. The directions of the calculated velocity vectors were obtained as the sample directions. For the determination of the sample directions of cell migration, each cell was tracked with a Fiji TrackMate plugin. The direction of cell displacement for 20 min was defined as the sample direction of cell migration for that individual cell.

Statistical analysis

Statistical analyses were performed with GraphPad Prism 7 software (GraphPad Software, San Diego, CA). No statistical analysis was used to predetermine the sample size. The sample sizes, statistical tests, and p-values are indicated in the figures and the figure legends. To compare two sets of data, paired or unpaired *t*-tests were used. To compare multiple sets of data, the samples were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. P-values of less than 0.05 were considered to be statistically significant in two-tailed tests, and were classified as follows: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, and n.s. (not significant, i.e., $P \geq 0.05$).

1106 **Supplemental video titles and legends**

1107 **Movie S1. ERK activation waves and cell deformation waves during collective cell**
1108 **migration, related to Figure 1**

1109 Time-lapse video of collectively migrating MDCK cells expressing EKAREV-NLS. Phase
1110 contrast images are shown at the top. The golden pseudo-color represents the FRET/CFP ratio
1111 indicating ERK activity (middle). Red and blue indicate positive and negative x-strain rate,
1112 respectively (bottom). The color scales correspond to those in Figure 1A. Time in hr:min.

1113

1114 **Movie S2. Cell contraction upon optogenetic ERK activation, related to Figure 3**

1115 Time-lapse video of the boundary between confluent MDCK cells with and without 2paRAF
1116 expression. The upper frames represent the fluorescence of CIBN-mScarlet-I-CAAX, a
1117 component of 2paRAF. The lower images show differential interference contrast (DIC).
1118 The blue light illumination started at 0 min and was repeated every 5 min. The cells were treated
1119 with DMSO (left), 200 nM trametinib (center), 10 μM Y-27632 (right) 30 min before the start
1120 of the imaging. Time in hr:min.

1121

1122 **Movie S3. ROCK activity propagation during collective cell migration, related to Figure**
1123 **3**

1124 First part: Time-lapse video of collectively migrating MDCK cells expressing a FRET biosensor
1125 for ROCK activity. The color represents the FRET/CFP ratio indicating ROCK activity and its
1126 scale corresponds to the one in Figure 3F. Second part shows migrating cells that were treated
1127 with 200 nM trametinib (left) and 100 μM Y-27632 (right) at 0 min. Time in hr:min.

1128

1129 **Movie S4. Traction force microscopy with optogenetic ERK activation, related to Figure**
1130 **3**

1131 Time-lapse traction force microscopy at the boundary between confluent MDCK cells with and
1132 without 2paRAF expression. Upper frames show differential interference contrast (DIC).
1133 Middle frames represent the fluorescence of CIBN-mScarlet-I-CAAX, a component of 2paRAF.
1134 Lower frames show traction forces. The color scale corresponds to the one in Figure 3L. The
1135 blue light illumination started at 0 min and was repeated every 5 min. Time in hr:min.

1136

1137 **Movie S5. Cell contraction triggers ERK activation waves, related to Figure 4**

1138 Time-lapse videos of the boundary between confluent MDCK cells with and without

1139 rapamycin-activatable Rho GEF (RA Rho GEF). The approximate boundary is indicated as
1140 dotted lines at the initial time frame. The color represents the FRET/CFP ratio indicating ERK
1141 activity, and the scale corresponds to the one in Figure 4B. The cells were treated with DMSO
1142 (left) or 50 μM rapamycin (right) at 0 min to induce contraction of the RA Rho GEF-expressing
1143 cells. Time in hr:min.
1144
1145 **Movie S6. Intercellular mechanical linkage is required for ERK activation waves,**
1146 **related to Figure 4**
1147 Time-lapse videos of collectively migrating WT (left) and α-catenin KO (right) MDCK cells.
1148 The color represents the FRET/CFP ratio indicating ERK activity, as shown in Figure 4, and its
1149 upper and lower value is 1.8 and 0.85. Time in hr:min.
1150
1151 **Movie S7. Requirement of Cdc42 and Rac1 for unidirectional ERK activation waves,**
1152 **related to Figure 5**
1153 Time-lapse videos of collectively migrating WT (upper), shCdc42 (middle), and shRac1
1154 (lower)-expressing MDCK cells. The color represents the FRET/CFP ratio indicating ERK
1155 activity, as shown in Figure 1, and its upper and lower value is 1.8 and 0.85 for each of the
1156 videos. Time in hr:min.
1157
1158 **Movie S8. Effect of constitutive ERK activation and inhibition on collective cell**
1159 **migration, related to Figure 6**
1160 Time-lapse videos of collectively migrating MDCK cells. The color represents the FRET/CFP
1161 ratio indicating ERK activity, and the scale corresponds to the one in Figure S4D and S4E. The
1162 cells were treated with DMSO (upper), 200 nM trametinib (middle), and 10 nM TPA (bottom)
1163 at 0 min. Time in hr:min.
1164
1165 **Movie S9. Optogenetic ERK activation waves and front-rear cell polarization, related to**
1166 **Figure 6**
1167 Time-lapse videos of migrating MDCK cells without (upper) and with (lower) Optogenetic
1168 ERK activation waves. Green and red indicate nuclei (EKAREV-NLS) and Golgi apparatus
1169 (Golgi-7-mCherry), respectively. Cyan represents regions illuminated with blue light. The cells
1170 were pre-treated with 1 μM PD153035, an EGFR inhibitor, to suppress autonomous ERK
1171 activation waves. Time in hr:min.

1172

1173 **Movie S10. in silico ERK activation waves, related to Figure 7**

1174 Simulations for ERK activation waves in migrating cells with (ω=1, upper frame) and without
1175 (ω=0, lower frame) cell polarity. The color represents the ERK activity and its scale corresponds
1176 to the one in Figure 7C.

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