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Supplementary information

Theory of mechanochemical patterning and optimal migration in cell monolayers

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Supplementary Information: Theory of mechano-chemical patterning and optimal migration in cell monolayers

I. MODEL DERIVATION

A. Equilibrium of cellular shape

We start by considering the equilibrium of a single cell, under differential tensions on its apical, lateral and basal areas (resp denoted γ_a, γ_l and γ_b). The energy of a single cell of height h and characteristic length l then reads [1–6]:

$$U = \gamma_l h l + (\gamma_a + \gamma_b) l^2 \tag{S1}$$

Because we model 3D cells restricted to a flat substrate, we need only consider stable shapes with zero average spontaneous curvature such that apical and basal areas are equal. Moreover, epithelial cells may be considered incompressible, with constant volume $V_0 = hl^2$, given the small forces generated by actomyosin structures (compared to osmotic forces) [7], and since cell volume does not change appreciably over the time scales considered (minutes to hour). Minimizing the energy with respect to l provides the equilibrium shape of a given cell $l_0 = (\frac{V_0}{2}\frac{\gamma_l}{\gamma_a+\gamma_b})^{1/3}$, and expanding up to 2nd order around this minimum energy yields:

$$\begin{aligned} U(l) =& U(l_0) + (l-l_0)U'(l_0) + \frac{(l-l_0)^2}{2!}U''(l_0) + ... && \text{(S2)} \\ =& U(l_0) + \frac{(l-l_0)^2}{2!}U''(l_0) + ... && \text{(S3)} \\ \approx& U(l_0) + k(l-l_0)^2, && \text{(S4)} \end{aligned}$$

which is the potential of a harmonic oscillator, valid for small perturbations around the equilibrium cell diameter l_0. Computing the value of the second derivative at l_0 provides a value for the spring constant $k = 3(\gamma_a + \gamma_b)$. Such a linearization is valid as we find that the amplitude of oscillations in cell area are typically small compared to the average area (around $5-10\%$, see Extended Data Fig. 3a). Note that we discarded potential line tensions in this analysis, although this would not qualitatively affect the results as we are considering only small deviations from mechanical equilibrium. We also neglect the role of proliferation, as it occurs on much longer time scales than the period of the wave and is not required for wave formation in MDCK layers [8]. For now we also neglect visco-elasticity of the epithelial monolayer, which can arise from re-arrangements or

division/apoptosis events, but will incorporate it in Section II E as a sensitivity analysis to show that it does not qualitatively affect results.

Given the reported role in ERK/MAPK in regulating the actomyosin cytoskeleton [9, 10], one can in principle write a dependency of any tension γ_i on ERK activity. This means that the equilibrium length of a given cell will generically depend on ERK activity such that $l_0 = l_0(ERK)$. Recent experiments have indeed shown that varying ERK activity causes relative changes in F-Actin intensity between lateral and basal surfaces of MDCK cells [10]. We will next consider mechanical couplings between cells in monolayers and the interaction of ERK with mechanics. This description will account for differences in biochemical activities and mechanical properties in different biological settings which lead to differences in spatio-temporal dynamics of ERK activity.

B. Overdamped chain of mechano-chemical springs

A major function of epithelial tissues is to form tight barriers, which is achieved through strong mechanical couplings between neighbouring cells mediated by adhesion molecules such as E-Cadherin [11]. We write down a force balance equation for a cohesive 1D chain of epithelial cells, with cell i characterized by ERK activity $E_i(t)$ and delimited by vertices $r_i(t)$ and $r_{i+1}(t)$. For vertex r_i, which is under frictional contact with a substrate (modelled as fluid friction with coefficient ζ), this reads

$$\zeta\left(r_i(t+dt) - r_i(t)\right) = -k_i\left(r_i(t) - r_{i-1}(t) - l_i^0(t)\right) + k_{i+1}\left(r_{i+1}(t) - r_i(t) - l_{i+1}^0(t)\right) \tag{S5}$$

where l_i^0 and k_i are the rest lengths and spring constants of a single cell and depend on 3D tensions as derived above. In the following, we will look only up to linear stability of the model so we discard the spatial variation in k_i which manifests as non-linear terms. In the continuum limit the vertex model becomes

$$\tau_r \partial_t r = \partial_{xx} r - \partial_x l^0. \tag{S6}$$

Here, lengths have been scaled by a characteristic cell length $< l >$ and we have defined a characteristic mechanical timescale $\tau_r = \zeta/k$. This timescale defines how quickly a mechanical stress diffuses in the monolayer - infinitely fast in the limiting case of vanishing friction, $\zeta \to 0$.

We now incorporate mechanosensation and response involving the chemical species ERK:

$$\begin{cases} \tau_r \partial_t r = \partial_{xx} r - \partial_x l_0 \\ \tau_l \partial_t l_0 = -(l_0 - l_0^{eq}) - \alpha(E - E_{eq}) \\ \tau_E \partial_t E = -(E - E_{eq}) + \beta \partial_x r \end{cases} \tag{S7}$$

The second equation represents the tendency of the rest length towards an intrinsic equilibrium cell length and the action of ERK on decreasing rest length. The third equation represents decay of ERK towards an equilibrium activity, E_{eq}, and the action of increasing cell length ($l = \partial_x r \sim r_{i+1} - r_i$) on increasing ERK activation. Since ERK and rest lengths are carried within cells we could also write advection terms on l_0 and E with cell velocity $\partial_t r$. However, these terms would not affect the pattern forming behaviour that we wish to study since the linear stability around the homogeneous equilibrium state is unchanged. Alternatively, dropping advection terms is equivalent to assuming that waves of ERK and strain propagate fast compared to cell velocity which is valid for this system [9, 10]. Before proceeding it will help us to rewrite the model using the change of variables $l_0 \leftarrow l_0 - l_0^{eq}$ and $E \leftarrow E - E_{eq}$:

$$\begin{cases} \tau_r \partial_t r = \partial_{xx} r - \partial_x l_0 \\ \tau_l \partial_t l_0 = -l_0 - \alpha E \\ \tau_E \partial_t E = -E + \beta \partial_x r \end{cases} \tag{S8}$$

Linear stability around the homogeneous fixed state yields the following dispersion relation:

$$(\tau_l \omega + 1)(\tau_E \omega + 1)(\tau_r \omega + q^2) = -\alpha\beta q^2 \tag{S9}$$

From this we predict a patterning instability at the critical point defined by $Re[\omega] = 0$ and $\frac{dRe[\omega]}{dq} = 0$, which translates to:

$$\alpha\beta > (\alpha\beta)_{\text{crit}} = \frac{(\tau_E + \tau_l)(\sqrt{\tau_E} + \sqrt{\tau_l})^2}{\tau_E \tau_l} \tag{S10}$$

The real and imaginary parts of ω at the critical point then define the wavenumber and angular frequency of the predicted instability, which read:

$${q_c}^2 = \frac{\tau_r}{\sqrt{\tau_E \tau_l}}, \qquad {\omega_c}^2 = \frac{1}{{\tau_l}^{1/2}{\tau_E}^{3/2}} + \frac{1}{{\tau_l}^{3/2}{\tau_E}^{1/2}} + \frac{1}{\tau_l \tau_E}. \tag{S11}$$

Interestingly, both the wavelength and period of the instability are independent of the strength of the couplings α and β. Instead, they involve only the three time scales of the problem, which can

all be measured from macroscopic observation. Furthermore the period depends only on τ_l and τ_E and not on τ_r. For $\alpha\beta > 0$ this is expected since the system is just a classical activator-inhibitor with delay which generically produces temporal oscillations even in the absence of spatial couplings. The mechanical time scale τ_r affects only in the wavelength of the instability (see Extended Data Fig. 2a). Its role is clearest when considering the limiting case (valid experimentally as described below) of $\tau_E \ll \tau_l$. In this limit, the period of the instability scales as $T \propto \tau_E^{1/4}\tau_l^{3/4}$ so that the wavelength scales as

$$\lambda \propto \frac{T}{\sqrt{\tau_l \tau_r}}. \tag{S12}$$

Here $\sqrt{\tau_l\tau_r}$ is the characteristic timescale of mechanical signal propagation upon biochemical change, so that the length scale of the instability is determined by how far a signal can propagate during a single temporal period of the mechano-chemical oscillation between ERK and cellular shape.

Although the linear instability threshold depends only on the product of mechano-chemical couplings $\alpha\beta$, the amplitude of mechanical vs. chemical fluctuations does depend on the relative values of couplings. We simulate the dynamics of the 1D system above the critical point ($\alpha\beta = 1.2 \times (\alpha\beta)_{\text{crit}}$) for different ratios $\alpha/\beta = 0.2, 1, 5$. As anticipated, cell shape is nearly un-affected for very small α while ERK levels vary drastically. In the reverse case of large α, small ERK oscillations are accompanied by major lattice deformations and cellular stretching (Extended Data Fig. 2b). Note that for numerical simulations of the model, we have to specify a non-linearity in the problem to prevent infinite activation of ERK. For this we took the simplest leading-order expansion on ERK activity:

$$\tau_E \partial_t E = -E - E^3 + \beta \partial_x r. \tag{S13}$$

although other non-linearities (for instance on the rest length equation l_0) have similar consequences.

C. Active migration and direction sensing

Although the above theory reproduces ERK/density waves at an appropriate wavelength and period (see Main Text), the appearance of persistent directed waves in migrating monolayers remains to be understood theoretically, together with the biophysical mechanisms through which they might guide long-range active migration [10].

1. Incorporation of active migration in the model

To address this, we define a cell polarity p, a continuous variable taking both positive and negative values, which controls the direction and magnitude of active migration forces generated between cell and substrate (and thus the direction of active migration), and explore the influence of a coupling between local cell polarity p and gradients of stress $\partial_x \sigma_{xx} = \partial_x(\partial_x r - l_0)$, which is based on experimental evidence in multiple systems [12–16] (see Section II D for a discussion of alternative couplings). This gives us the following model:

$$\begin{cases} \tau_r \partial_t r = \partial_{xx} r - \partial_x l_0 + p \\ \tau_l \partial_t l_0 = -l_0 - \alpha E \\ \tau_E \partial_t E = -E + \beta \partial_x r \\ \tau_p \partial_t p = -p + D_p \partial_{xx} p - \gamma \partial_x \sigma_{xx} \end{cases} \tag{S14}$$

where polarity feeds back on the first (force-balance) equation as an external traction force. The last equation is the simplest one can write on polarity, developing at linear order around a $p = 0$ state (which cells relax to in the absence of external stimuli/force). τ_p characterizes the time scale of polarity changes, the diffusion coefficient D_p represents possible short-range polarity alignment between neighboring cells, and γ controls the strength of coupling between gradients of stress and polarity [16].

Such a stress-polarity coupling has been shown to be mediated by Merlin [13], a mechanosensitive protein that re-localizes from cortical cell-cell junctions to the cytoplasm in the presence of external forces during cell migration. This relocalization coordinates polarized Rac1 activity and lamellipodium formation, thus acting exactly as the γ parameter in linking gradients of stress from cell-cell contacts to traction forces. In Fig. 4, we thus use Merlin knock-outs to compare to model predictions for reduced γ. A further indication that the role of Merlin is mainly to modulate the γ parameter is that wavelengths and periods of the oscillations (which depend on τ_R, τ_l, τ_E) are unaffected in Merlin KO (Extended Data Fig. 9d).

Importantly however, this model still requires a non-linearity to break symmetry of wave propagation/cell migration directionality: without it polarity oscillates around mean zero, such that there is no long-range order and no persistent directional migration. In the main text we discuss experimental evidence that suggests traction forces are a decreasing function of ERK activity and use this to motivate a non-linearity on the coupling between polarity and gradients of stress $\gamma = \gamma(E)$ [10].

This addition allows phase differences between ERK and stress (which naturally exist and are fully generic to our mechano-chemical patterns) to control the degree of symmetry breaking (see Fig. 3c for a schematic). As mentioned in Section I A, ERK activity is observed to modulate the distribution of F-actin between the lateral and basal surfaces [10]. This indicates that ERK modulates (possibly in a related manner) both the relative surface tensions (and thus the stable cell shape/aspect ratio) as well as the polar traction forces from directional migration described here. The main difference between the two is that basal surface tension (either from cortical actomyosin or stress fibers) is a scalar term, which modulates the active stresses inside the monolayer, whereas directed migration forces are polar terms, which impact on the external forces exerted on/from the substrate. Strictly speaking traction forces have two active contributions: the first originating from gradients in scalar basal surface tensions and associated with changes in cell/tissue size; the second originating from directional actomyosin flows at basal surfaces. In what follows this latter contribution, proportional to the variable p, is what we refer to as traction force and we will be interested in how this term responds to mechano-chemical waves.

We analyse this in more detail below by assuming a simple saturating non-linearity of the form $\gamma(E) = \gamma/(1+e^E)$, although all of our conclusions only require a variation of γ with E. It is useful to non-dimensionalize by $T = \tau_l$ and $L = a\sqrt{\tau_l/\tau_r}$ with a the average cell size (set to one), make the change of variables $E \leftarrow \alpha E$, $l_0 \leftarrow L l_0$ and absorb parameters $\beta \leftarrow \alpha\beta$, $D_p \leftarrow D_p/L^2$, $\tau_E \leftarrow \tau_E/T$ and $\tau_p \leftarrow \tau_p/T$ so that the model reads

$$\begin{cases} \partial_t \sigma = \partial_{xx}\sigma + \partial_x p + l_0 + E \\ \partial_t l_0 = -l_0 - E \\ \tau_E \partial_t E = -E + \beta \partial_x r \\ \tau_p \partial_t p = -p + D_p \partial_{xx} p - \frac{\gamma}{1+e^{\frac{E}{\alpha}}} \partial_x \sigma. \end{cases} \tag{S15}$$

To gain insight into this system we first derive analytical solutions for the response of an initially un-polarized tissue to an applied ERK wave (as performed for instance in Ref. [9] - although note the differences between our theory and the theory in Ref. [9], which neglected polarity, set $\tau_l \to 0$ and had a different sign $\alpha < 0$ for the length-ERK coupling to the one revealed by our optogenetic experiments, see Fig. 2c-f). We impose a right-left traveling ERK wave $E(x,t) = \cos(qx + \omega t)$ and assume that the traction term $\partial_x p$ can be neglected (an approximation which renders analytical expressions more tractable, and which we verify/relax in numerical simulations of the phase diagram of Fig. 3d). We also neglect diffusion of polarity ($D_p = 0$), which would otherwise act to soften

oscillations without qualitatively changing the results, leaving us with a relatively simple model which can be solved analytically:

$$\begin{cases} \partial_t \sigma = \partial_{xx}\sigma + l_0 + E \\ \partial_t l_0 = -l_0 - E \\ \tau_p \partial_t p = -p - \frac{\gamma}{1+e^{\frac{E}{\alpha}}}\partial_x \sigma. \end{cases} \tag{S16}$$

For $l_0(x,t)$ and $\sigma(x,t)$ we find sinusoidal solutions:

$$l_0\left(x,t\right) = -\frac{1}{\sqrt{1+\omega^2}}\cos\left(qx+\omega t-\operatorname{atan}\left(\omega\right)\right) \tag{S17}$$

$$\sigma\left(x,t\right) = A_\sigma \cos\left(qx+\omega t+\phi_\sigma\right) \tag{S18}$$

with A_σ and ϕ_σ given by

$$A_\sigma = \frac{1}{\sqrt{\left(1+\omega^2\right)\left(1+\frac{q^4}{\omega^2}\right)}} \qquad \phi_\sigma = \operatorname{atan}\left(\frac{q^2-\omega^2}{\omega\left(1+q^2\right)}\right) \tag{S19}$$

If we assume that $\gamma(E)$ is far from saturation we can use a linear approximation $\gamma(E) = \gamma(1/2 - E/(4\alpha))$ to obtain a solution also for the polarity:

$$\begin{aligned} p(x,t) = -\frac{qA_\sigma}{8\alpha}\Big(\sin(\phi_\sigma) &+ \frac{4\alpha}{\sqrt{1+\tau_p^2 w^2}}\cos\left[\omega t + qx + \phi_\sigma + \operatorname{atan}\left(\frac{1}{\tau_p\omega}\right)\right] \\ &- \frac{1}{\sqrt{1+4\tau_p^2 w^2}}\cos\left[2\omega t + 2qx + \phi_\sigma + \operatorname{atan}\left(\frac{1}{2\tau_p\omega}\right)\right]\Big) \end{aligned} \tag{S20}$$

Here, we see that symmetry is broken by the appearance of a constant term and that the shape of the oscillation is altered by the appearance of a phase-shifted second harmonic. The constant term is also the average polarity $\overline{p}$ which is approached in the limit $\tau_p \to \infty$:

$$\overline{p}(\omega,q) = \lim_{\tau_p\to\infty} p(x,t|\omega,q) = -\frac{qA_\sigma(\omega,q)}{8\alpha}\sin\left(\phi_\sigma(\omega,q)\right) = \frac{1}{8\alpha}\frac{\omega q\left(\omega^2-q^2\right)}{\left(1+\omega^2\right)\left(q^4+\omega^2\right)} \tag{S21}$$

2. Optimal polarization of the monolayer

Thus, the average polarity is determined by the angular frequency ω and wavenumber q of the applied wave (plotted in Extended Data Fig. 8a). From Eq. S21 we see that there is a boundary between net-positive and net-negative polarization at $\omega = q$.

Importantly, we find that polarity is maximized in the direction opposite to the ERK/density waves for a unique value of the wavelength and period of the wave. This allows us to define, as

discussed in the main text, an optimality criterion, for which the global average polarization of the monolayer is maximal (and given the fact that large-scale monolayer expansion arises from polarized migration forces in the tissue bulk [17], this also corresponds to waves inducing maximal displacement).

This optimality criteria corresponds to a phase difference $\phi_\sigma = \pi/4$ with ERK preceding stress (with the coefficient α changing only the value of the optimum and not its position). Since stress, $\sigma = \partial_x r - l_0$, involves the hidden variable l_0 it is more informative to compare the phase difference between cell length $\partial_x r$ and ERK (see Fig. 1d) for which we find that $\partial_x r$ precedes ERK by $\pi/4$ at optimum.

Analysis gives expressions for the optimal wavelength and period:

$$q = 1 \text{ and } \omega = 1 + \sqrt{2} \tag{S22}$$

or adding back dimensions,

$$\lambda = 2\pi\sqrt{\frac{\tau_l}{\tau_r}} \text{ (in units of cell length)} \quad \text{and} \quad T = \frac{2\pi}{1+\sqrt{2}}\tau_l \tag{S23}$$

With experimentally estimated values of τ_r and τ_l (see Fig. 2 and note that τ_E does not appear because we are enforcing the dynamics on ERK) we predict optimal waves at wavelengths of $10-40$ cells and periods of $2-10$ hours (Extended Data Fig. 8a). However, this analytical criteria does not take into account ERK de-sensitisation, occurring on time scales of roughly an hour, which manifests in slow, long-term, decays of ERK activity following both mechanical stretch (Fig. 2b) and optogenetic ERK activation experiments [10]. We anticipate that this effect will decrease sensitivity to long-period oscillations and when we include it in numerical simulation of the full model ($\tau_d = 1$h, see Section II A for further theoretical details), we see that the optimum moves towards shorter periods and wavelengths (albeit relatively little, displaying robustness of the results, see Fig. 3d and Extended Data Fig. 8). The predicted optimum then occurs at wavelengths of $\lambda \approx 15-30$ cells and periods of $T \approx 2-7$ hours.

3. Survey of wavelengths and periods observed in the literature

Strikingly, this predicted range is close to the values observed in multiple systems, including the MDCK cells which we study:

- In our conditions, (Fig. 3d and Extended Data Fig. 8) we find ranges, for confluent tissue $\lambda = 20 \pm 1$ cells $T = 108 \pm 3$ min and migrating tissue $\lambda = 21 \pm 5$ cells $T = 96 \pm 4$ min. Using

the range of estimates for τ_l, τ_r and τ_E we also plot the point $\lambda = 10^{+6}_{-3}$ cells $T = 70 \pm 20$ min sitting on the theoretical line from the dispersion relation (Eq. 5) from the main text (asymmetrical error bars appear on λ because upper and lower bounds depend non-linearly on the ranges of three different parameters - see Eq. 6 on the main text). Ref. [9, 10] also found ranges of $\lambda \approx 10 - 20$ cells, $T \approx 1 - 2$ h.

- In Ref. [8]: $\lambda \approx 50$ cells (our estimation from their report of a wavelength around 1mm and cell diameter of 20μm), $T \approx 2$ h

- In Ref. [18]: $\lambda \approx 18$ cells (our estimation from their report of a wavelength of $370\mu m$ and cell diameter of 20μm), $T \approx 4.7$ h. In Fig. 3d and Extended Data Fig. 8, we plot extracted ranges $\lambda = 18.5 \pm 1.5$ cells $T = 282 \pm 42$ min.

- In Ref. [19]: $T \approx 6$h, and a wavelength of at least 700μm (as cells oscillate coherently on a 700μm diameter disk), i.e. approx 35 cells

- From figures in Ref. [20]: we estimate a period $T \approx 5$h and a wavelength of $\lambda \approx 350\mu$m (approx. 15-20 cells)

- From Ref. [21]: $T \approx 2.6$h and a wavelength of $\lambda \approx 15$ cells (estimated combining their measurement of period with their estimation of propagation speed of the wave, leading to around 300μm).

- In Ref. [22], which examines *in vivo* mouse skin upon wound healing, we went back to the data and estimated $\lambda \approx 8$ cells, $T \approx 1.3$ h. Point plotted in Fig. 3d and Extended Data Fig. 8.

We note that some discrepancies in the wavelength and period observed for MDCK cells are likely to be due to differences in experimental set-ups. Different substrates for instance are expected to modify the friction coefficient ζ of monolayers ($\tau_r = \zeta/k$), thus directly impacting the wavelength of the instability, as well as potentially affecting the time scales of ERK signalling (τ_E and τ_l) via mechano-sensation. However, it remains striking that our measurements, as well as published values of wavelength and period, indicate that the system is close to optimality in terms of polarization strength in response to a mechano-chemical wave.

Furthermore, although we have so far considered the limiting case of driven ERK dynamics (in which any wave can be applied on the system), the biophysical origin of the instability places

additional constraints on the system. In particular, it restricts the period and wavelength of the instability to a curve in $\omega - q$ space (i.e. the dispersion relation of our instability, see Fig. 3d or Extended Data Fig. 8). Interestingly, we find that the emergent waves possible from our biophysical model have dominant modes governed by $\omega^4 = q^4+q^3+q^2$ such that they can only induce polarization in the direction opposite to ERK propagation (i.e. sitting in the side of the diagram where $\omega > q$), for any values of model parameters! This makes it an extremely robust mechanism for coordinating cell migration in a unidirectional manner.

II. SENSITIVITY TO MODEL ASSUMPTIONS

In this section, we discuss different extensions to our model, as well as alternative couplings, to provide insights as to its robustness.

A. Adaptation of ERK to mechanical stresses

Our model for ERK dynamics (Eq. S8) can be extended to account for slow adaptation of ERK in response to constant stretch or long-term optogenetic activation ([10] and Fig. 2). In its simplest form, a model of long-term ERK adapation reads:

$$\tau_E \partial_t^2 E + \partial_t E = -E/\tau_d + \beta \partial_t \partial_x r. \tag{S24}$$

As can be seen from the limiting case of this equation: on time-scales longer than τ_d, ERK relaxes towards zero (irrespective of cell area), whereas on time-scales shorter than τ_d, we recover the previous behavior, with ERK linearly following the deformation $\partial_x r$ with a delay τ_E. One can then recalculate the dispersion relation for this extended/adaptive model:

$$(\tau_E \omega^2 + \omega + 1/\tau_d)(\tau_l \omega + 1)(\tau_r \omega + q^2) = -\alpha\beta\omega q^2 \tag{S25}$$

As expected this reduces to the previous dispersion relation for $\tau_d \to \infty$.

We estimated a lower bound on τ_d by fitting mechanical stretch experiments (Fig. 2a,b) with numerical solutions of Eq. S24 for an instantaneously applied constant stretch (temporal step function in $\partial_x r$ - see Extended Data Fig. 4c) and estimate $\tau_d \approx 1$h. Importantly, the dispersion relation for corresponding values of τ_d shows only slight differences (even for short de-sensitization time scales of $\tau_d = 30$min, Extended Data Fig. 4d), arguing that adaptation has a negligible effect on pattern formation in this system. One should note however that adaptation should be important for

longer-term expansion experiments on time scale of days, although in this case other effects such as cell division [8, 23] also become important. We also discussed above (Section I C) how adaptation slightly reduces the effectiveness of very-long period (and wavelength) waves for inducing strong polarization (see Extended Data Fig. 8).

B. Direct cell-cell biochemical communication

In our model, ERK is restricted to the subcellular level, and does not diffuse directly from one cell to the next, instead propagating via mechanical couplings and the mechano-sensitivity of ERK (which is validated by the analysis of the experiments of Fig. 2 and [10]). An extension could consider that biochemical relays act as an effective diffusion coefficient D_E appearing directly in the equation on ERK dynamics:

$$\tau_E \partial_t E = \tau_E D_E \partial_{xx} E - E + \beta \partial_x r. \tag{S26}$$

This allows for diffusive biochemical communication over the lengthscale $\sqrt{D_E \tau_E}$ and translates into a modified dispersion relation:

$$(\tau_E \omega + 1 + D_E q^2)(\tau_l \omega + 1)(\tau_r \omega + q^2) = -\alpha \beta q^2 \tag{S27}$$

Plotting this dispersion relation for $D_E = 1$, a typical diffusion rate for biological proteins given the Stokes-Einstein equation ($D_E \approx 10 \mu m^2 s^{-1}$ in real units for cell length $< l > \approx 20 \mu m$), shows that if present this would have only very weak effects on the instability and pattern formation (Extended Data Fig. 4b).

C. Alternative couplings of ERK to cell strain

In principle, ERK could also respond to cell strain or strain rate, rather than absolute cell length (as is assumed and main text, and used to fit the mechanical stretching experiment). For a coupling to cell strain the model becomes

$$\begin{cases} \tau_r \partial_t r = \partial_{xx} r - \partial_x l_0 \\ \tau_l \partial_t l_0 = l_0^{eq} - l_0 - \alpha E \\ \tau_E \partial_t E = -E + \beta(\partial_x r - l_0) \end{cases} \tag{S28}$$

with the following dispersion relation resulting from linear stability

$$(\tau_l\omega + 1)(\tau_E\omega + 1)(\tau_r\omega + q^2) = \alpha\beta\tau_r\omega \tag{S29}$$

With increasing $\alpha\beta$ the system first becomes unstable at infinite wavelength with no oscillatory component (an exponentially growing homogeneous instability). Therefore, this alternative coupling cannot explain the patterns seen in data. Similarly, we consider the following couplings to strain rate, defined either on absolute cell size or with reference to the cells rest length:

$$\begin{cases} \tau_r\partial_t l = \partial_{xx}(l - l_0) \\ \tau_l\partial_t l_0 = l_0^{eq} - l_0 - \alpha E \\ \tau_E\partial_t E = -E + \beta\partial_t l \end{cases} \tag{S30}$$

and

$$\begin{cases} \tau_r\partial_t l = \partial_{xx}(l - l_0) \\ \tau_l\partial_t l_0 = l_0^{eq} - l_0 - \alpha E \\ \tau_E\partial_t E = -E + \beta\partial_t(l - l_0) \end{cases} \tag{S31}$$

which give the dispersion relations

$$(\tau_l\omega + 1)(\tau_E\omega + 1)(\tau_r\omega + q^2) = -\alpha\beta\omega q^2 \tag{S32}$$

and

$$(\tau_l\omega + 1)(\tau_E\omega + 1)(\tau_r\omega + q^2) = \alpha\beta\tau_r\omega^2 \tag{S33}$$

The first case is always stable for $\alpha\beta > 0$ and the second shows similar behaviour to the previous coupling to strain (Eq. S29). This rules out couplings to strain and strain rate as an explanation for the patterns seen data, consistent also with mechanical perturbation experiments, where an almost instantaneous strain is applied (Fig. 2b). A coupling between ERK and absolute cell size is therefore the simplest coupling that can describe the patterns seen in data.

D. Alternative coupling of polarity to local velocity

In the main text, we explore the influence of a coupling between local cell polarity p and gradients of stress, which is based on experimental evidence in multiple systems [12–16]. There are, however,

other proposals for how tissue mechanics impacts cellular polarity, and in particular the possibility that cellular polarity $\vec{p}$ aligns with cellular velocity $\vec{v}$ (which are not necessarily identical in confluent monolayers due to mechanical couplings between cells [16, 24]). This would modify the equation for polarity in Eq. S14 as follows:

$$\tau_p \partial_t p = -p + D_p \partial_{xx} p + \gamma v \tag{S34}$$

Notice however that gradients of stress and cell velocity $v = \partial_t r$ are closely related by force balance:

$$\tau_r \partial_t r = \partial_x \sigma_{xx} + p \tag{S35}$$

such that re-injecting velocity into Eq. S34 returns a term on gradients of stress:

$$\tau_p \partial_t p = -(1 - \frac{\gamma}{\tau_r}) p + D_p \partial_{xx} p + \frac{\gamma}{\tau_r} \partial_x \sigma_{xx}. \tag{S36}$$

This term is similar, up to a rescaling factor, to the coupling assumed in main text but with opposite sign. This sign difference is important because it reverses the direction in which cells polarize in response to ERK waves, i.e. polarity-velocity coupling produces migration in the opposite direction compared to the model in the main text (stable only as long as $\gamma < 1$.) All of the conclusions regarding optimality are therefore reversed and emergent waves robustly produce polarity in the "wrong" direction, leading us to favour our original coupling. Finally, considering a direct polarity alignment between cells (modelled as a diffusion coefficient D_p in the polarity equation), does not change qualitatively any of the results discussed in the main text so we omit it in simulations.

E. Visco-elasticity

On long time scales the viscous properties of cells and tissues become important and we can modify our system of equations to determine whether this has any effect on our predictions for pattern formation. In the elastic limit it is equivalent to consider either an ERK-dependent active stress $\sigma_a(ERK)$ or an ERK-dependent reference length $l_0(ERK)$. However, these two approaches are not equivalent for a viscoelastic material (c.f. creep vs stress relaxation). We therefore begin by reformulating our model in terms of an active stress before incorporating viscosity for each of the two cases.

1. ERK-dependent active stress in an elastic medium

For an active system we can decompose total stress into passive and active parts

$$\sigma = \sigma_p + \sigma_a \tag{S37}$$

For an elastic medium the passive stress is related to the strain by Hooke's law

$$\sigma_p = \sigma - \sigma_a = k\epsilon \tag{S38}$$

where k is the stiffness and ϵ is strain. Combining force balance between substrate friction and gradients of stress $\partial_x \sigma = \zeta v$ with the equation for strain rate $\dot{\epsilon} = \partial_x v$ gives

$$\partial_{xx}\sigma = \zeta \partial_x v = \zeta \dot{\epsilon} \tag{S39}$$

Using the constitutive equation to eliminate σ gives us an equation of motion relating strain and active stress

$$\zeta \dot{\epsilon} = k\partial_{xx}\epsilon + \partial_{xx}\sigma_a \tag{S40}$$

Once again, we incorporate mechano-sensitive ERK, which we take to depend on absolute cell length $l \sim (1+\epsilon)$, to give a second dynamic equation

$$\tau_E \dot{E} = (E_{eq} - E) + \beta(1+\epsilon) \tag{S41}$$

Comparing with our previous model using the connection $l = (1 + \partial_x r) = (1 + \epsilon)$ shows that $\sigma_a = -kl_0$ and allows us to write a final dynamic equation for active stress

$$\tau_\sigma \dot{\sigma_a} = \sigma_a^{eq} - \sigma_a + \alpha(E - E_{eq}) \tag{S42}$$

As expected, this gives an identical result to the one coarse-grained from the 3D vertex and spring model and presented in the Main Text. While this shows that there is no difference between active stress and an active rest length in the elastic limit, we discuss below that these cases need to be considered separately in visco-elastic models, or in models with a non-linear ERK-dependent stiffness (see Section II F).

2. ERK-dependent active stress in a visco-elastic medium

On timescales longer than cell re-arrangements (or division/apoptosis) biological tissues can be described as visco-elastic Maxwell fluids, and we thus use a classical Maxwell rheology as a starting

point given by the following constitutive equation

$$\frac{\dot{\sigma_p}}{k} + \frac{\sigma_p}{\eta} = \dot{\epsilon} \tag{S43}$$

with η the viscosity of the tissue and other variables defined as in the section above. Using force balance ($\partial_{xx}\sigma_p = \zeta\dot{\epsilon} - \partial_{xx}\sigma_a$) to eliminate σ_p gives an equation of motion relating strain to active stress

$$\zeta\ddot{\epsilon} + (\frac{\zeta}{\tau_m} - k\partial_{xx})\dot{\epsilon} = \partial_{xx}\dot{\sigma_a} + \frac{1}{\tau_m}\partial_{xx}\sigma_a \tag{S44}$$

with $\tau_m = \eta/k$ a visco-elastic timescale. This modifies our system of equations as follows:

$$\begin{cases} \tau_r\ddot{\epsilon} + (\frac{\tau_r}{\tau_m} - \partial_{xx})\dot{\epsilon} = \partial_{xx}\dot{\sigma_a} + \frac{1}{\tau_m}\partial_{xx}\sigma_a \\ \tau_l\dot{\sigma_a} = -\sigma_a + \alpha E \\ \tau_E\dot{E} = -E + \beta\epsilon \end{cases} \tag{S45}$$

which reduces, as expected, to the elastic model described above for $\tau_m \to \infty$. Linear stability around the stationary state now gives the following dispersion relation

$$(\tau_l\omega + 1)(\tau_E\omega + 1)(\tau_r\omega + q^2 + \frac{\tau_r}{\tau_m}) = -\alpha\beta q^2(1 + \frac{1}{\tau_m\omega}) \tag{S46}$$

Comparing to Eq. S9, and using the measured values of the period and wavelength $T = 90$ min and $\lambda = 20$ cells and the fitted value of $\tau_r = 10$ min, we can estimate that visco-elasticity will qualitatively modify the dispersion relation only when $\tau_m < \tau_r/(q^2 + \tau_r\omega) = 13$ min or $\tau_m < 1/\omega = 14$ min. These are much small than typical values of $\tau_m = 60$ min [8], and plotting (Extended Data Fig. 4e) for such values confirms that viscosity has little effect on the dispersion relation or initial patterning, justifying the approximations made in the main text.

3. ERK dependent rest length in a visco-elastic medium

For completeness we also study the case of an ERK dependent rest length in a visco-elastic medium. The previous constitutive equation for the elastic limit, $\sigma/k = \epsilon - l_0$, is replaced by

$$\frac{\dot{\sigma}}{k} + \frac{\sigma}{\eta} = \dot{\epsilon} - \dot{l_0} \tag{S47}$$

where we now consider a non-constant reference state l_0 instead of active stresses. Combining this with force balance ($\zeta\dot{\epsilon} = \partial_{xx}\sigma$) provides the following system of equations:

$$\begin{cases} \tau_r\ddot{\epsilon} + \frac{\tau_r}{\tau_m}\dot{\epsilon} = \partial_{xx}(\dot{\epsilon} - \dot{l_0}) \\ \tau_l\dot{l_0} = -l_0 - \alpha E \\ \tau_E\dot{E} = -E + \beta\epsilon \end{cases} \tag{S48}$$

The dispersion relation from a linear stability analysis is

$$(\tau_l\omega + 1)(\tau_E\omega + 1)(\tau_r\omega + q^2 + \frac{\tau_r}{\tau_m}) = -\alpha\beta q^2 \tag{S49}$$

which again reduces, as expected, to the results of the main text for $\tau_m \to \infty$. As for the case of an ERK dependent active stress (Eq. S46) viscosity has little effect on patterning for realistic values of τ_m.

F. Alternative non-linear couplings for symmetry breaking

In order to break symmetry and produce a net traction force, we introduced a non-linear ERK dependency to the coefficient $\gamma(ERK)$ for the coupling between polarity and gradients of stress (see Section I C 1). This was supported by observations of lower traction forces in cells upon optogenetic ERK activation [10].

However, we also wish to explore the impact of alternative non-linearities on symmetry-breaking. The first case that we consider is a non-linearity on the traction force $f_+(E)$ (which we had previously set to one without loss of generality as it could be absorbed upon non-dimensionalization within the polarity). Indeed, because the observations of Ref. [10] were on traction forces, this feedback could conceivably occur in force generation, rather than in polarization, $\gamma_+(ERK)$, so we again consider a decreasing function $f_+(E)$ for which we keep the same form, $f_+(E) = 1/(1+\exp[E])$. The system of equations, non-dimensionalized as in Eq. S15, becomes

$$\begin{cases} \partial_t r = \partial_{xx} r + \partial_x \sigma_a + f_+[E]p \\ \partial_t \sigma_a = -\sigma_a + E \\ \tau_E \partial_t E = -E + \beta\partial_x r \\ \tau_p \partial_t p = -p - \gamma\left(\partial_{xx} r + \partial_x \sigma_a\right) \end{cases} \tag{S50}$$

In the limit $\tau_p \to 0$ traction forces are identical to the case of a non-linearity on $\gamma(E)$, however symmetry in polarity remains nearly unbroken. We demonstrate this in Extended Data Fig. 7b

where we simulate the system with an applied ERK wave and measure how the net traction force depends on the period and wavelength of this wave. We also plot the spatial profile of the traction force $f_+(E)p$ (red) and cell polarity p (grey dashed) at the point indicated on the diagram. In these simulations we used a lower estimate of τ_p so that we compare the cases of $\gamma(E)$ (Extended Data Fig. 7a) and $f_+(E)$ when they are most similar. The traction forces are nearly identical in both the diagrams and spatial profiles. However, whereas for $\gamma(E)$ the profiles of polarity and traction force are identical (bar a rescaling) and show the same degree of symmetry breaking, for $f_+(E)$ the polarity profile differs greatly from its respective traction force profile and shows close to zero symmetry breaking. Since symmetry-breaking observed in data occurs both on polarity and traction forces (Fig. 3a and [10])), we rule out such a coupling on $f_+(E)$ in favour of $\gamma(E)$.

The second case assumes a non-linearity in the stiffness $k(E)$, which has differing effects depending on whether an ERK dependent active stress or ERK dependent rest length is considered (see also Section II E for a comparison of these two cases in the context of viscoelasticity). From the derivation of l_0 and k in terms of active surface tensions in Section I A, such a non-linearity can occur as k depends on cellular tensions which can be modified by ERK, and we accordingly considered it as an increasing function of ERK, using again a sigmoidal dependency $k(E) = kf_-(E) = k/(1+\exp[-E])$. For the first sub-case of an ERK dependent stiffness with an active reference length the system of equations becomes

$$\begin{cases} \partial_t r = f_-[E]\left(\partial_{xx} r - \partial_x l_0\right) + p \\ \partial_t l_0 = -l_0 - E \\ \tau_E \partial_t E = -E + \beta \partial_x r \\ \tau_p \partial_t p = -p - \gamma\left(\partial_{xx} r - \partial_x l_0\right) \end{cases} \tag{S51}$$

Here, we find an inverse diagram (Extended Data Fig. 7c) with net polarization and traction force in the opposite direction compared to $\gamma(E)$ (Extended Data Fig. 7a). This coupling is therefore inconsistent with experimental observations. An even more dramatic difference occurs for the second sub-case of an ERK dependent stiffness with an active stress (note that $\sigma_a \sim -l_0$ for the an elastic

medium with a constant stiffness):

$$\begin{cases} \partial_t r = f_-[E]\partial_{xx} r + \partial_x \sigma_a + p \\ \partial_t \sigma_a = -\sigma_a + E \\ \tau_E \partial_t E = -E + \beta \partial_x r \\ \tau_p \partial_t p = -p - \gamma \left(\partial_{xx} r + \partial_x \sigma_a\right) \end{cases} \tag{S52}$$

Net-polarisation and traction force is zero or positive (i.e. counter to ERK) for all wavelengths and periods but with an optimum located at very short wavelength and long period (Extended Data Fig. 7d). The degree of symmetry breaking is small in regions corresponding to observed waves which seems to rule this out as a mechanism for inducing net active migration in our biological setting.

We note that the different non-linearities in the cases presented here do not affect the linear stability determining pattern formation or modify the timescales estimated in the main text. The simulations in Extended Data Fig. 7 make use of these timescales (τ_l and τ_r were used to non-dimensionalize) and therefore offer a fair comparison between models. Other parameters were set throughout as $\gamma = 0.1$ and $\tau_p = 15$ min (β does not feature for an applied ERK wave). Finally, a proposed alternative [9] was to have a non-linear cell viscosity (effectively non-linear τ_r in our framework) which decreased with ERK. Although this produced symmetry-breaking in the correct direction, it relied on an instantaneous relationship between cell rest length and ERK, which corresponds to a simplification of $\tau_l \to 0$ in our model, as well as negative α - rather than positive when assessed by optogenetic experiments.

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