**Supplementary Figure 1.**
a) Changes in junction length for orthogonal and distant junctions before and after extrusion in MCF-7 cells. Per extrusion event, a total of 8 junctions per junction type were analyzed. Values correspond mean ± SEM. (*, p<0.05; Two tailed paired t-test, n=5 extrusion events).
b) Scheme of laser microirradiation in the zebrafish periderm.
c) Time sequence showing cell and junctional remodeling of neighbor cells as extrusion proceeds.
d) Frequency distribution of $\Delta \text{ number of junctions} = \text{number of junctions after extrusion} - \text{number of junctions before extrusion}$ for cells that surrounds apoptotic cells. Data are the means for 5 extrusion events (8 cells per extrusion event).
e) $\eta = \frac{a}{p^2}$ measured before and after extrusion for cells experiencing or not a reduction (-1) in their number of sides. Data are the mean of 5 extrusion events (**** p<0.001, Two Way ANOVA Sidak’s multiple comparisons test).
f) Changes in junction length for orthogonal and distant junctions before and after extrusion of death cells after laser microirradiation. Values correspond to means ± SEM (*, p<0.05; Two tailed paired t-test, n=10 extrusion events). Scale bars, 20 µm.

**Supplementary Figure 2.**
a) Junctional recoil measurements in i) untreated monolayers and ii) etoposide treated monolayers, where tension was measured on distant and orthogonal junctions. Data are mean ± SEM, n=3 independent experiments, at least 15 junctions per experiment per condition.
b-d) Etoposide-treated monolayers and staining for of Vinculin and ZO1 (B), α-18 and α-catenin (C) and ZO1 and pMRLC (D). Image show an apoptotic cell surrounded by neighbors before complete extrusion/apoptotic rosette. Scale bars, 20 µm.

**Supplementary Figure 3.**
a) Analysis of junctional Src-Bio-tK FRET index in a 1 dpf Zebrafish periderm in response to laser mediated cell injury. At time = 0 min, cells in the center were irradiated with a multiphoton laser as described in material and methods. Then, images were taken every 2 min. Still images of time sequence with rainbow pseudocolor are shown.
b) Representative western blot analysis of Src and Yes expression in WT, Src KD and Yes KD cells.

**Supplementary Figure 4.**
a) Representative images of pY419 SFK and E-cadherin immunostaining in control, blebbistatin (Blebb) or Y-27632 (Y-27632) treated monolayers.
b) Representative images of YFP fluorescence and FRET index ratio in Src-Bio-tK MCF-7 cells treated (Y-27632) or not (Control) with Y-27632. Scale bars, 20 µm.

Supplementary Figure 5.
Related to Supplementary Figure 3. Uncropped western blot images and scanned western blot membranes.

Supplementary File 1.
MATLAB code for monolayer energy calculation during extrusion.
Supplemental Figure 2_R1

(a) Graph showing the recoil (µm) over time (sec) for Control, Etoposide-Close, and Etoposide-Away conditions. The graph includes error bars indicating variability.

(b) Image showing ZO-1 localization.

(c) Image showing α-Cat localization.

(d) Image showing pMRLC localization.
Supplemental Figure 4

(a) pY419 SFK and E-cad images under different treatments:
- DMSO
- Blebb
- Y-27632

(b) YFP and YFP/FRET images under different treatments:
- DMSO
- Y-27632

Src activity is indicated by the color scale.
Supplementary Figure 5_R1
Src kinases relax adherens junctions between the neighbors of apoptotic cells to permit apical extrusion.

Jessica L. Teo1*, Vanesa M. Tomatis1*, Luke Coburn2, Anne K. Lagendijk1, Irin-May Schouwenaar1, Srikanth Budnar1, Thomas E. Hall1, Suzie Verma1, Robert W. McLachlan1, Benjamin M. Hogan3,8, Robert G. Parton1,4, Alpha S. Yap1** and Guillermo A. Gomez1,5**

1Division of Cell and Developmental Biology, 2Division of Genomics of Development and Disease, Institute for Molecular Bioscience and 4Centre for Microscopy and Microanalysis, The University of Queensland; 2Institute of Complex Systems and Mathematical Biology, University of Aberdeen, United Kingdom; and 5Centre for Cancer Biology, SA Pathology and the University of South Australia, Adelaide, SA, Australia.

*Current address: Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia 3000
*Equal contributions.

**To whom correspondence should be addressed:
Dr. Guillermo A. Gomez: guillermo.gomez@unisa.edu.au
Prof. Alpha S. Yap: a.yap@uq.edu.au
CONTENTS.

1. Mechanics of confluent epithelial cell monolayers.
2. Average junctional tension within epithelial monolayers and its dependence on cell density and topological organization.
3. Energetics and mechanics of apoptotic cell extrusion.
4. Mechanics of topological transitions within a confluent epithelial monolayer.
5. Experimental validation of the model: Part I. Non-linear regression analysis of initial recoil measurements for calculation of modelling parameter (ratio $\frac{K}{J}$).
6. Experimental validation of the model: Part II. Calculation of the amount of mechanical relaxation that is needed in tensile monolayers for topological transitions to occur.
7. References
8. Appendix I
1. Mechanics of confluent epithelial cell monolayers.

To analyze the mechanics of cell-cell interactions in confluent epithelia, we model the apical surface of epithelial cells by the use of vertex models (Farhadifar et al., 2007; Bi et al., 2015; Coburn et al., 2016). In this approximation, the apical adherens junctions of an epithelial cell monolayer are represented by connected polygons in a two-dimensional lattice. Within this lattice, the energy for the $i$th cell ($\hat{E}_i$) and the energy of a monolayer ($\hat{E}_T$) formed by $N$ cells are calculated as:

\[
\hat{E}_i = -\tilde{J} p_i + \tilde{R} p_i^2 + \tilde{\lambda}[a_i - a_o]^2 \quad \text{(Eq. 1)}
\]

\[
\hat{E}_T = \sum_i^N \hat{E}_i = -\tilde{J} \sum_i^N p_i + \tilde{R} \sum_i^N p_i^2 + \tilde{\lambda} \sum_i^N [a_i - a_o]^2 \quad \text{(Eq. 2)}
\]

where $a_i$ and $p_i$ are the cell’s apical area and perimeter, respectively. $a_o$ is the preferred apical area for all cells and the parameters $\tilde{J}$, $\tilde{R}$ and $\tilde{\lambda}$ weight the contribution of adhesion, junctional contractility and volume elasticity (at constant cell height), respectively (Coburn et al., 2016; Coburn et al., 2018). Note that in Eq.1 and Eq.2 we neglected the contribution of adhesion to the substrate since it did not affect significantly the mechanics of a non-migrating/stationary confluent cell monolayer in an earlier analysis (Coburn et al., 2018).

To characterize the presence of junctional tension in the model and its dependence on cell density, topological organization and cell’s adhesive and contractile properties, we reduced the parameter space of Eq. 1 and 2 by

i) normalizing the energy per cell $\hat{E}_i$ and the parameters $\tilde{J}$ and $\tilde{R}$ by the cell elasticity parameter $\tilde{\lambda}$, thus defining

\[
E_i = \frac{\hat{E}_i}{\tilde{\lambda}}; \quad J = \frac{\tilde{J}}{\tilde{\lambda}}; \quad R = \frac{\tilde{R}}{\tilde{\lambda}} \quad \text{and} \quad E_i = \sum_i^N E_i \quad \text{(Eq.3)}
\]

ii) expressing $a_i$, the area of a cell within the monolayer, as the sum of the average area of cells in the monolayer $\langle a \rangle$ plus its deviation $(\Delta a_i)$ from this average value

\[
a_i = \langle a \rangle + \Delta a_i \quad \text{(Eq. 4)}
\]

Eq. 4 has the advantage of introducing explicitly in the model the average packing of cells $\langle a \rangle$, which is related to monolayer cell density ($\delta_{\text{monolayer}}$) by:

\[
\langle a \rangle = \frac{1}{\delta_{\text{monolayer}}} \quad \text{(Eq. 5)}
\]

Then we use Eq. 4 to replace $a_i$ in the last term of Eq. 2, for which we obtain

\[
\sum_i (a_i - a_o)^2 = \sum_i (\langle a \rangle - a_o)^2 + \sum_i 2(\langle a \rangle - a_o)\Delta a_i + \sum_i \Delta a_i^2 \quad \text{(Eq. 6)}
\]
The term $\sum_i 2(\langle a \rangle - a_o)\Delta a_i$ does not contribute to the overall sum across all the cells in the monolayer since the variation of individual cell areas $\Delta a_i$ cancel each other. With this consideration and by replacing Eq.3, Eq.4 and Eq.6 into Eq.2, the normalized energy of the entire monolayer $E_T$ (Eq. 7) and its corresponding single cell contribution $E_i$ (Eq. 8) are given by:

$$E_T = -J \sum_i p_i + K \sum_i p_i^2 + \sum_i^N (\langle a \rangle - a_o)^2 + \sum_i^N (a_i - \langle a \rangle)^2 \quad (Eq. 7)$$

$$E_i = -J p_i + K p_i^2 + (\langle a \rangle - a_o)^2 + (a_i - \langle a \rangle)^2 \quad (Eq. 8)$$

We then considered the geometrical organization of cells within the monolayer and include this in the model. For this it was useful to define $\eta_i$ (and $\eta^*$), the ratio between the cell area $a_i$ (and average area $\langle a \rangle$) and perimeter square $p_i^2$ (and average perimeter $\langle p \rangle^2$).

$$a_i = \eta_i p_i^2 \quad (Eq. 9)$$

$$\langle a \rangle = \eta^* \langle p \rangle^2 \quad (Eq. 10)$$

$$\eta^* = \langle \eta \rangle \quad (Eq. 11)$$

Whereas, $\eta^*$ describes the average topological organization of cells within the monolayer, $\eta_i$ may or may not be the same for all the cells depending whether cells uniformly or non-uniformly tile a surface. For example, when cells form concave polygons that can uniformly tile a surface without leaving gaps (i.e. covering a surface with identical polygons, like squares for example), they present a characteristic $\eta_i =$
\(\eta_{\text{polygon type}}\) value that is specific for the type of polygon (e.g. square, triangle, irregular pentagon) and is the same for all polygons that form the monolayer (Box1).

For these types of cells and monolayer organization, the following rules apply:

\[
\eta_i = \eta_j = \eta_k = \cdots = \eta_{\text{polygon type}} = \langle \eta \rangle = \eta^* \quad (\text{Eq. 12a})
\]

\[
a_i = a_j = a_k = \cdots = \langle a \rangle \quad (\text{Eq. 12b})
\]

\[
p_i = p_j = p_k = \cdots = \langle p \rangle \quad (\text{Eq. 12c})
\]

\[
\langle a \rangle = \eta_{\text{polygon type}} \langle p \rangle^2 \quad (\text{Eq. 12d})
\]

Conversely, when cells tiles a surface non-uniformly, Eq.9-11 still apply, but for this case the cell area \(a_i\), cell perimeter \(p_i\), and \(\eta_i\) parameters are not necessarily the same for all the cells within the monolayer, which is represented by:

\[
\eta_i \neq \eta_j \neq \eta_k \neq \cdots \neq \langle \eta \rangle = \eta^* \quad (\text{Eq. 13a})
\]

\[
a_i \neq a_j \neq a_k \neq \cdots \neq \langle a \rangle \quad (\text{Eq. 13b})
\]

\[
p_i \neq p_j \neq p_k \neq \cdots \neq \langle p \rangle \quad (\text{Eq. 13c})
\]

In the next sections we will use these concepts and consider the role of cell density and cell organization on both the general (non-uniform) and the particular (uniform) cases of cell organization in monolayers.

### 2. Average junctional tension within epithelial monolayers and its dependence on cell density and topological organization.

As described in (Magno et al., 2015; Coburn et al., 2016; Coburn et al., 2018), it is possible to find an expression for junctional tension \(T_i\), and the parameter conditions for liquid-to-solid transitions to occur. This is obtained from the first derivative (at \(\eta_i\) constant) of the energy function equation (Eq. 11, which is the result of substituting Eq. 9 into Eq. 8) with respect to cell’s perimeter.

\[
E_i = -J p_i + K p_i^2 + (\langle a \rangle - a_o)^2 + (\eta_i p_i^2 - \langle a \rangle)^2 \quad (\text{Eq. 14})
\]

\[
T_i = \frac{dE_i}{dp_i} = -J + 2 K p_i + 4\eta_i p_i (\eta_i p_i^2 - \langle a \rangle) \quad (\text{Eq. 15})
\]

After re-arranging Eq. 15, we obtain

\[
T_i = -J + (2K - 4\eta_i \langle a \rangle) p_i + 4\eta_i^2 p_i^3 \quad (\text{Eq. 16})
\]

We then use a 3\textsuperscript{rd} order Taylor series to expand this expression as a function of \(p_i\) and \(\eta_i\) around \(\langle p \rangle\) and \(\eta^*\), for which we obtain:
\[ T_i = -J + (2K - 4\eta^* \langle a \rangle) \langle p \rangle + 4\eta^* \langle p \rangle^3 - (\eta^* - \eta_i)(8\eta^* \langle p \rangle^3 - 4\langle a \rangle \langle p \rangle) \]
\[- \langle \langle p \rangle - p_i \rangle \rangle (12\eta^* \langle p \rangle^2 - 4\langle a \rangle \eta^* + 2K) + 4\langle p \rangle^3 (\eta^* - \eta_i)^2 \]
\[ + 12\eta^* \langle p \rangle (\langle p \rangle - p_i)^2 - (24\eta^* \langle p \rangle^2 + 4\langle a \rangle) (\eta^* - \eta_i) (\langle p \rangle - p_i) \]
(Eq. 17)

Using Eq. 17, the average junctional tension \( T \) within the monolayer is given by:
\[ T = \frac{\sum_i^N T_i}{N} = -J + (2K - 4\eta^* \langle a \rangle) \langle p \rangle + 4\eta^* \langle p \rangle^3 - (8\eta^* \langle p \rangle^3 - 4\langle a \rangle \langle p \rangle) \sum_{i=1}^N \frac{(\eta^* - \eta_i)}{N} \]
\[- (12\eta^* \langle p \rangle^2 - 4\langle a \rangle \eta^* + 2K) \sum_{i=1}^N (\langle p \rangle - p_i) + 4\langle p \rangle^3 \sum_{i=1}^N \frac{(\eta^* - \eta_i)^2}{N} \]
\[ + 12\eta^* \langle p \rangle \sum_{i=1}^N \frac{(\langle p \rangle - p_i)^2}{N} - (24\eta^* \langle p \rangle^2 + 4\langle a \rangle) \sum_{i=1}^N (\eta^* - \eta_i) (\langle p \rangle - p_i) \]
(Eq. 18)

For a monolayer at steady state, the terms \((8\eta^* \langle p \rangle^3 - 4\langle a \rangle \langle p \rangle) \sum_{i=1}^N \frac{(\eta^* - \eta_i)}{N}\) and \((12\eta^* \langle p \rangle^2 - 4\langle a \rangle \eta^* + 2K) \sum_{i=1}^N (\langle p \rangle - p_i)\) in Eq. 18 become null when extending the sum to all the cells of the monolayer. This is because steady state cell-to-cell variations in topology and perimeter cancel each other across different cells. Also, Eq. 9 states an inverse relationship between the perimeter and topological organization (measured by \( \eta \)) suggesting that the term \( \sum_{i=1}^N (\eta^* - \eta_i) (\langle p \rangle - p_i) \) in Eq. 18 does not contribute significantly to the average junctional tension. Finally, the terms \( \sum_{i=1}^N \frac{(\langle p \rangle - p_i)^2}{N} \) and \( \sum_{i=1}^N \frac{(\eta^* - \eta_i)^2}{N} \) are related to the variance \( (\sigma^2) \) of the perimeter \( (\sigma_p^2 = \sum_{i=1}^N \frac{(\langle p \rangle - p_i)^2}{N}) \) and topological organization \( (\sigma_{\eta}^2 = \sum_{i=1}^N \frac{(\eta^* - \eta_i)^2}{N}) \) of cells within the monolayer. With these considerations, Eq. 18 becomes
\[ T = -J + (2K - 4\eta^* \langle a \rangle) \langle p \rangle + 4\eta^* \langle p \rangle^3 + 4\langle p \rangle^3 \sigma_{\eta}^2 + 12\eta^* \langle p \rangle \sigma_p^2 \] (Eq. 19)

and by replacing Eq. 10 into Eq. 19 we obtain:
\[ T = -J + 2K \langle p \rangle - 4\eta^* \langle p \rangle^3 + 4\eta^* \langle p \rangle^3 + 4\langle p \rangle^3 \sigma_{\eta}^2 + 12\eta^* \langle p \rangle \sigma_p^2 \] (Eq. 20a)
\[ T = -J + 2K \left( \frac{\langle a \rangle}{\eta} \right) + 4 \left( \frac{\langle a \rangle}{\eta} \right)^{3/2} \sigma_{\eta}^2 + 12\eta^* \left( \frac{\langle a \rangle}{\eta} \right) \sigma_p^2 \] (Eq. 20b)
\[ T = -J + (2K + 12\eta^* \sigma_p^2) \left( \frac{\langle a \rangle}{\eta} \right) + 4\sigma_{\eta}^2 \left( \frac{\langle a \rangle}{\eta} \right)^{3/2} \] (Eq. 20c)

This analysis reveals that, in our model, the average junctional tension of a cell in a non-uniformly tiled monolayer is given by:
\[ T = -J + \left( \frac{2K}{\sqrt{\eta}} + 12\eta^{3/2}\sigma_p^2 \right) \sqrt{\langle a \rangle} + \frac{4\sigma_p^2}{\eta^{1/2}} \left( \sqrt{\langle a \rangle} \right)^3 \] (Eq. 21)

and which for uniform tiling (i.e. \( \sigma_p^2 = \sigma_p^2 = 0 \)) simplifies to:

\[ T = -J + \left( \frac{2K}{\sqrt{\eta}} \right) \sqrt{\langle a \rangle} \] (Eq. 22a)
\[ T = -J + 2K\langle p \rangle \] (Eq. 22b)

Eq. 22 is a key result of our analysis even for the more general case of non-uniform tiling as those found experimentally since cells in monolayers have small variations in perimeter (i.e. \( \sigma_p^2 \approx 0 \)) and organization (\( \sigma_p^2 \approx 0 \)) suggesting that this can be used for analysis of experimental data.

To further analyze the dependence of junctional tension on monolayer cell density (\( \delta_{\text{monolayer}} \)), we first define
\[ \langle a \rangle = a_o \zeta \] (Eq. 23)

The parameter \( \zeta \) relates to how far the cell average cell area in the monolayer deviates from the preferred area \( a_o \) (described in Eq. 1). Replacing Eq. 23 into Eq. 22a, we obtain:

\[ T = -J + \left( \frac{2K}{\sqrt{\eta}} \right) \sqrt{\zeta a_o} \] (Eq. 24)

As we mentioned before, \( \langle a \rangle = \frac{1}{\delta_{\text{monolayer}}} \), which results in
\[ \delta_{\text{monolayer}} = \frac{1}{\zeta a_o} \] and \( \zeta a_o = \frac{1}{\delta_{\text{monolayer}}} \) (Eq. 25)

\[ T = -J + \left( \frac{2K}{\sqrt{\eta}} \right) \sqrt{\frac{1}{\delta_{\text{monolayer}}}} \] (Eq. 26)

To verify our theoretical analysis, we then use numerical simulations of cell monolayers using cell vertex model (Coburn et al., 2016). In our simulations, we measured the dependence of junctional tension upon monolayer density and compared this result to the results obtained from Eq. 26 (Box 2). The results of the simulations confirmed the overall dependency of junctional tension on monolayer density predicted analytically by Eq. 26. With this, we then used Eq. 26 to experimentally validate and extract parameters of our model using laser ablation as is explained in detail later.
Box 2. Result from numerical simulations of cell monolayers using cell vertex models (as described in (Coburn et al., 2016)). Left, K vs J phase diagram showing the solid and liquid state of monolayers (hard and soft regimes). Right, Comparison of the dependency of junctional tension with monolayer density between simulations (monolayer sitting in the solid state close to the boundary with the liquid state) and the analytical solution (Eq. 26) for different type of topological organization ($\eta$).

Box 3. Summary of key expressions for the calculation of cell energy, and junctional tension in our model as well as the definitions of monolayer density and polygonal type descriptor ($\eta$).

Energy per cell

$$E_i = -Jp_i + Kp_i^2 + \left((a) - a_o\right)^2 + \left(\eta_i p_i^2 - \left\langle a \right\rangle\right)^2 \quad (Eq. 14)$$

Junctional tension per cell

$$T_i = \frac{dE_i}{dp_i} = -J + 2Kp_i + 4\eta_i p_i \left(\eta_i p_i^2 - \left\langle a \right\rangle\right) \quad (Eq. 15)$$

Average junctional tension

$$T = -J + \left(\frac{2K}{\eta^2}\right)\sqrt{(a)} = -J + \left(\frac{2K}{\eta^2}\right)\sqrt{\frac{1}{\delta_{monolayer}}} \quad (Eq. 21/26)$$

Cell density

$$\delta_{monolayer} = \frac{1}{\left\langle a \right\rangle} \quad (Eq. 5)$$

Average topological organization

$$\eta^* = \frac{\left\langle a \right\rangle}{\left\langle p \right\rangle^2} \quad (Eq. 10)$$
3. Energetics and mechanics of apoptotic cell extrusion.
Then we assessed the energetics and mechanics associated with topological transitions within the monolayer as the process of extrusion occurs. Based on our experimental observations (Figure 1), we developed our analysis considering the elongation of the orthogonal junctions on neighboring cells and the reduction in cell area of the apoptotic cell. In particular, we focused on those cell rearrangements that lead to a change in \( \eta \) on neighboring cells (Figure 1) by analyzing the time sequence and topological changes that occurs during and after the extrusion of one apoptotic cell; this accompanies the formation of an epithelial rosette that resolves after extrusion is completed (Box 4). We use different geometrical considerations to calculate the area and perimeter of neighboring cells and apoptotic cells, to allow us to calculate the Energy of the system and average junctional tension throughout the process using Eq. 8 and 21 (Box 3 and 4).

**BOX 4.** Cell shape changes in neighbor and apoptotic cells as extrusion proceeds.

At the beginning of the calculation (i.e. \( t=0 \)), we consider all cells in the system being perfectly hexagonal, having the same initial area \( (a_o) \), perimeter \( (p_o) \) and junctional length (i.e. cell side \( s = \frac{1}{6}p_o \)). We then analyzed how these parameters changed for both the neighbor and apoptotic cells as extrusion occurs. In particular we found that from the sequence of images (Box 4), it is possible to break-down the process into two stages: one stage that relates to those re-arrangements that occur **before** extrusion and the second stage that relates to those rearrangements that occur **after**
the cell is extruded from the monolayer. In the next two sections we consider these two stages in detail.

- *Calculation of monolayer energy and junctional tension before extrusion.*

First of all, we calculated the area $a$ and perimeter $p$ for each cell in the system as extrusion starts to occur. In this first stage we consider the change in the area of the neighbor cell as extrusion proceeds and express this in terms of geometrical variable of the system (Box 3).

\[
a_{\text{neighbor}} = a_o + \Delta a_{\text{neighbor}} \quad \text{(Eq. 27)}
\]
\[
p_{\text{neighbor}} = a_o + \Delta p_{\text{neighbor}} \quad \text{(Eq. 28)}
\]

Then we expressed $a_{\text{neighbor}}$ and $p_{\text{neighbor}}$ as functions of the fraction $x$ of the apoptotic cell’s apothem $r$ \((r = \frac{\sqrt{3}}{2}s \text{, Box 4})\), for which we obtain:

\[
a_{\text{neighbor}} = a_o \left(1 + \frac{(2-x)x}{6}\right) \quad \text{(Eq. 29)}
\]
\[
p_{\text{neighbor}} = p_o \left(1 + \frac{x}{6}\right) \quad \text{(Eq. 30)}
\]

Note that we expressed geometrical quantities of cells as function of the cell’s area \((a_{\text{neighbor}})\) and perimeter \((p_{\text{neighbor}})\), so then it is possible to calculate changes in monolayer energy and tension using the equations listed in Box 3. Note also that this system is integrated by 6 “neighbors” and 1 “apoptotic” cell.

We then consider the changes in area and perimeter for the apoptotic cell. This is a simple case as in our approximation the apoptotic cell preserves its hexagonal shape and its apothem is equal to \(x \frac{\sqrt{3}}{2}s\) and the total area of the system is maintained throughout the process. Thus, using Eq. 27 and the relationship between area and perimeter for a regular hexagon it is possible to write:

\[
a_{\text{apoptotic}} = a_o - 6 \Delta a_{\text{neighbor}} \quad \text{(Eq. 31)}
\]
\[
a_{\text{apoptotic}} = a_o \left[1 - (2 - x)x\right] \quad \text{(Eq. 32)}
\]
\[
p_{\text{apoptotic}} = p_o \sqrt{1 - (2 - x)x} \quad \text{(Eq. 33)}
\]
Since the variable $x$ is an index for how far the process of cell extrusion has progressed, we defined it as the time variable $t$ in our calculations.

\[ x = time = t \text{ (Eq. 34)} \]

Thus, at $t = 0$, both neighbor and apoptotic cell perimeters and areas are equal to their initial conditions (i.e. $p_o$ and $a_o$, respectively), whereas at $t = 1$, the cell neighbors have stretched to their maximum and the area and perimeter of the apoptotic cell is null.

- **Calculation of monolayer energy and junctional tension after extrusion.**

For the analysis of the mechanics and energetics after cell extrusion, we considered the case where multicellular rosettes that form during extrusion resolve forming four tri-cellular junctions (Box 4), which then could reach a configuration that is as much as possible, close to the initial configuration (i.e. tri-cellular junctions forming 120 degrees angles, $t=2$).

From the figure (Box 4), it is noticed that neighbor cells fall within either of two groups, one where cells acquire *heptagonal polygonal shape* and another one, where cells acquire *pentagonal polygonal shape*. The solutions for the area $a$ and perimeter $p$ for each cell type (heptagonal and pentagonal) as a function of the length variable $y$, defined as the length of the bi-cellular junction between heptagonal cells (Box 4), is given by:

\[
\begin{align*}
\text{a}_{\text{pentagonal}} &= a_o \left(\frac{7}{6} - \frac{2}{3} y\right) \text{ (Eq. 35)} \\
\text{a}_{\text{heptagonal}} &= a_o \left(\frac{7}{6} + \frac{2}{3} y\right) \text{ (Eq. 36)} \\
\text{p}_{\text{pentagonal}} &= p_o \left(\frac{1}{2} + \frac{1}{3} \sqrt{1 + 3(1-y)^2}\right) \text{ (Eq. 37)} \\
\text{p}_{\text{heptagonal}} &= p_o \left(\frac{1}{2} + \frac{1}{3} \sqrt{1 + 3(1-y)^2} + \frac{\sqrt{3}}{3} y\right) \text{ (Eq. 38)}
\end{align*}
\]

For the calculations of energy and tension after extrusion is completed and during the period where the rosette is resolving, we use the variable $y$ as an index of time as it indicates how much the rosette has resolved, and the monolayer restored, after the extrusion of the apoptotic cell has completed. Based on this, we defined $t = time$ as.

\[ y = t - 1 \text{ (Eq. 39)} \]

Note that we only used this definition for values of time that are greater than one (i.e. $t > 1$).
Box 5 show the corresponding analysis of the area covered by the cells in the analysis as well as the measurements of the $\eta$ parameter for non-extruding cells in the calculations. As predicted, neighboring cells reduce their $\eta$ as extrusion progress, as we also observed experimentally (Figure 1e).

Box 5. Measurements of cell area changes in the apoptotic cell and its neighbors as extrusion proceeds (Eq. 29, 32, 35 and 36) as well as changes in the cell parameter $\eta$ on neighboring cells throughout this process. Note the reduction of 15% in this parameter as the multicellular rosette forms, which is in agreement to what was observed experimentally (Figure 1e).

Box 6 summarizes the changes in cell shape and energy and tension calculations for our analysis.

Box 6. A. Summary of equations for calculations of cell area and perimeter for all cells (apoptotic and neighbors) during the process of extrusion (Box3). B. Summary of equations that were used for calculation of the changes in Energy and junctional tension in the system during the extrusion process.
4. Mechanics of topological transitions within confluent epithelial monolayer.

Cells that rearrange within apoptotic rosettes experience a significant change in the \( \eta \) parameter, a reflection of a change in their topological organization (Figure 1e, Box 5). Therefore, we analyzed what mechanical requirements were necessary for these topological transitions to occur. Although we analyzed a detailed case above, this section refers to a more general case that also applies to those cases where cells do not extrude.

For this, we first considered a topological transition that involves only a small group of cells that experience a specific topological rearrangement like for example apoptotic cell neighbors considered in this study or the cells experiencing T1 transitions, where a junction shrinks to cause local changes in cell topology (for example from a hexagonal to a pentagonal cell (Martin et al., 2009; Fernandez-Gonzalez and Zallen, 2013). Of note, the topological organization of these cells might or might not differ from the rest of their neighboring cells within the monolayer, but since the rest of the cells do not experience a topological rearrangement, these neither contribute to the transition itself nor to the changes in monolayer energy during the transition.

Then, for this group of cells experiencing a topological transition (i.e. that change their \( \eta \) value), there is a corresponding average change (per cell) in energy of the monolayer (\( \Delta E_{TT} \)) that is given by:

\[
\Delta E_{TT} = \Delta E^{\text{Topological Transition}} = E^a - E^b \quad \text{(Eq. 40)}
\]

Where \( a \) and \( b \) correspond to “after” and “before” the topological change, respectively. Note also that only cells that change their topology (i.e. change their \( \eta \) from a \( \eta^b \) to a \( \eta^a \) value) contribute to this change in energy. Thus, by replacing \( p_i = \sqrt{\frac{\eta_i}{\eta}} \) (Eq.9) into Eq. 8, we obtain for a cell \( i \) that experience a topological change:

\[
E_i^b = -J \sqrt{\frac{\eta_i}{\eta}} a_i^b + K \frac{a_i^b}{\eta_i} + (\langle a \rangle - a_o)^2 + (a^b - \langle a \rangle)^2 \quad \text{(Eq. 40a)}
\]

\[
E_i^a = -J \sqrt{\frac{\eta_i}{\eta}} a_i^a + K \frac{a_i^a}{\eta_i} + (\langle a \rangle - a_o)^2 + (a^a - \langle a \rangle)^2 \quad \text{(Eq. 40b)}
\]

and for
\[ \Delta E_{TT}^{i} = -J \left( \frac{a_i^a}{\sqrt{\eta_i^a}} - \frac{a_i^b}{\sqrt{\eta_i^b}} \right) + K \left( \frac{a_i^a}{\eta_i^a} - \frac{a_i^b}{\eta_i^b} \right) + \left[ (a_i^a - \langle a \rangle)^2 - (a_i^b - \langle a \rangle)^2 \right] \] (Eq. 40c)

Which reads simpler by removing the \( i \)'s cell sub-indexes.

\[ \Delta E_{TT} = -J \left( \frac{a^a}{\sqrt{\eta^a}} - \frac{a^b}{\sqrt{\eta^b}} \right) + K \left( \frac{a^a}{\eta^a} - \frac{a^b}{\eta^b} \right) + \left[ (a^a - \langle a \rangle)^2 - (a^b - \langle a \rangle)^2 \right] \] (Eq. 41)

Since the same number of cells remains in the same area before and after the transition, meaning that the local density does not change significantly, this allows us to simplify Eq. 41, which is a function of \( a^a \), using a Taylor approximation (order 1) around \( a^b \), by which we obtain

\[ \Delta E_{TT} = -J \sqrt{a^b} \left( \frac{1}{\sqrt{\eta^a}} - \frac{1}{\sqrt{\eta^b}} \right) + K a^b \left( \frac{1}{\eta^a} - \frac{1}{\eta^b} \right) + 2 (a^a - a^b)(a^b - \langle a \rangle) \] (Eq. 42)

Note that if the area of cells before \( (a^b) \) the transition is close to the average area of the monolayer, then we should expect the factor \( (a^b - \langle a \rangle) \) be close to zero or negligible when compared to the other terms of Eq. 42. With this assumption and using \( a^b = \langle a \rangle = \zeta a_o \), then the energy change per cell \( \Delta E_{TT} \) during the transition can be expressed as

\[ \Delta E_{TT} = -J \sqrt{\zeta a_o} \left[ \frac{1}{\sqrt{\eta^a}} - \frac{1}{\sqrt{\eta^b}} \right] + K \zeta a_o \left[ \frac{1}{\eta^a} - \frac{1}{\eta^b} \right] \] (Eq. 43)

We then use Eq. 43 to analyze the dependence of topological transitions upon junctional tension. Here the new topological state is defined by \( \eta^a \) ("after") and \( \eta^b = \eta^* \), and \( \zeta a_o = \langle a \rangle \) since we considered the system to start from a stable average condition. Using Eq. 43 and defining \( K_{TT} \) as the junctional contractility parameter at which there is no change in monolayer energy due to a topological transition (i.e. \( \Delta E_{TT} = 0 \)), we obtain

\[ K_{TT}^{\eta_{TT}} = \frac{\sqrt{\eta^a}}{\langle a \rangle} \left[ \frac{\sqrt{\eta^a} - \eta^a}{\eta^* - \eta^a} \right] \] (Eq. 44)

Eq. 43 and 44 are key results as these relationships allow us to analyze the regimes in junctional contractility for which topological transitions are allowed (i.e. \( \Delta E_{TT} < 0 \)). In order to do this, we first solve the following inequality:
\[ \Delta E^{TT} = -J \sqrt{\zeta a_o \left[ \frac{1}{\sqrt{\eta^a}} - \frac{1}{\sqrt{\eta^b}} \right]} + K \zeta a_o \left[ \frac{1}{\eta^a} - \frac{1}{\eta^b} \right] < 0 \quad \text{(Eq. 45)} \]

\[ K \left[ \frac{1}{\eta^a} - \frac{1}{\eta^b} \right] < \frac{J}{\sqrt{\zeta a_o}} \left[ \frac{1}{\sqrt{\eta^a}} - \frac{1}{\sqrt{\eta^b}} \right] \quad \text{(Eq. 46)} \]

Then if \( \eta^a < \eta^b \rightarrow \left[ \frac{1}{\eta^a} - \frac{1}{\eta^b} \right] > 0 \) (Table I), and by introducing \( \eta^b = \eta^* \) and \( \zeta a_o = \langle a \rangle \)

\[ \left[ \frac{K}{J} \right] < \left[ \frac{K}{J} \right]^{TT} = \frac{\sqrt{\eta^*}}{\sqrt{\langle a \rangle}} \left[ \frac{\eta^* - \eta^a}{\eta^* - \eta^b} \right] \quad \text{(Eq. 48)} \]

This result shows that topological transitions that decrease \( \eta \) are allowed if \( \left[ \frac{K}{J} \right] < \left[ \frac{K}{J} \right]^{TT} \). Moreover, this also indicates that \( \left[ \frac{K}{J} \right]^{TT} \) is a limit for contractility \( \left[ \frac{K}{J} \right] \) to favor \( \left[ \frac{K}{J} \right] < \left[ \frac{K}{J} \right]^{TT} \) or prevent \( \left[ \frac{K}{J} \right] > \left[ \frac{K}{J} \right]^{TT} \) cell rearrangements.

Finally, we analyzed if there were any relationships between \( \left[ \frac{K}{J} \right]^{TT} \) and \( \left[ \frac{K}{J} \right]^{s-l} \) (the boundary between solid and fluid monolayer states (Coburn et al., 2016; Coburn et al., 2018)). For this, we previously found \( \left[ \frac{K}{J} \right]^{s-l} = \frac{\sqrt{\eta^*}}{2\sqrt{\langle a \rangle}} \) (Coburn et al., 2016), and replacing this in Eq. 48 results in

\[ \left[ \frac{K}{J} \right] < \left[ \frac{K}{J} \right]^{TT} = \left[ \frac{\sqrt{\eta^*} - \eta^a}{\eta^* - \eta^b} \right] 2 \left[ \frac{K}{J} \right]^{s-l} \quad \text{(Eq. 49)} \]

For \( \eta^a < \eta^* \) the term \( \left[ \frac{\sqrt{\eta^*} - \eta^a}{\eta^* - \eta^b} \right] < \frac{1}{2} \), therefore and under these circumstances

\[ \left[ \frac{K}{J} \right] < \left[ \frac{K}{J} \right]^{s-l} \quad \text{(Eq. 50)} \]

This implies that this type of topological transition is more likely to occur when cells sit in the liquid state and monolayers are fluid. We found also this relationship still holds even for those cases of topological transitions where the value of \( \eta \) does not change as a result of the topological change (See Box 1 for different topological arrangements with the same \( \eta \) value). Overall, this is a general result and is further evidenced by the analysis of \( \eta \) in a \( K \) vs \( J \) phase diagram of a confluent monolayer,
where cells with lower $\eta$ values (triangle, pentagon, square) appears in the liquid region in simulations (Box 7).

**Box 7.** Topological organization $\eta$ of cells and its dependence with the parameters of the model $K$ and $J$. The lines delimit the $\frac{K}{J}$ values for different type of cell rearrangement $\eta$. Left, phase diagram of tension (red, high tension), Right, same phase diagram but showing average values of $\eta$ for simulated monolayers.

5. Experimental validation of the model: Part I. Non-linear regression analysis of initial recoil measurements for calculation of modelling parameter (ratio $\frac{K}{J}$).

According to our model, Eq. 26 is a key expression that permits calculation of the ratio of modeling parameters $K$ and $J$, that can be experimentally informed by measuring junctional tension (by recoil after laser ablation) in monolayers plated at different densities (Figure 1e). Using this data, we then use Eq. 26 to perform non-linear regression analysis.

$$T = -J + \left(\frac{2K}{\sqrt{\eta}}\right) \frac{1}{\sqrt{\delta_{\text{monolayer}}}}$$ (Eq. 26)
In particular, a useful expression for non-linear regression analysis results from defining:

\[ x = \sqrt{\Delta_{\text{monolayer}}} \]  
\[ T = -J + \frac{2K}{\sqrt{\langle \eta \rangle}} \frac{1}{x} \]  

(Eq. 51)  
(Eq. 52)

Note that \( \frac{2}{\sqrt{\langle \eta \rangle}} \) can be measured independently based on measurement of cell area and cell perimeter (Box 8). From our data we obtain an average value of \( \langle \eta \rangle = 0.0562 \), from which we obtain \( \frac{2}{\sqrt{\langle \eta \rangle}} = 8.4329 \).

Since we measure \( T \) based on initial recoil measurements (Liang et al., 2016) is important to note that:

\[ \text{initial recoil} = \frac{d\varepsilon(0)}{dt} = \frac{T}{\mu} \]  

(Eq. 53)

In Eq. 53, \( \mu \) is the viscosity coefficient related to the viscous drag experienced during recoil by cell-cell junctions after ablation and which encompasses the cortex, plasma membrane and cell cytoplasm and \( \frac{d\varepsilon(0)}{dt} \) is the derivative, at time=0, of the amount of recoil or strain \( \varepsilon(t) \) after ablation (Liang et al., 2016). Using Eq. 53, then it is possible to define

\[ T = \text{initial recoil} \times \mu = -J + \frac{2K}{\sqrt{\langle \eta \rangle}} \frac{1}{x} \]  

(Eq. 54)

\[ \text{initial recoil} = -\frac{J}{\mu} + \frac{2K}{\mu \sqrt{\langle \eta \rangle}} \frac{1}{x} \]  

(Eq. 55)

We then define the non-linear regression variables \( \beta \) and \( \alpha \) as

\[ \beta = \frac{J}{\mu} ; \alpha = \frac{K}{\mu} \]  

(Eq. 56)

Replacing Eq. 56 in Eq. 55, results in the following expression that we used for our non-linear regression analysis with fitting parameters \( \beta \) and \( \alpha \).
initial recoil = −β + α \frac{2}{\sqrt{\eta}} \sqrt{s} (Eq. 57)

And from the non-linear regression results, then it is possible to measure the ratio $[\frac{K}{J}]$ using:

\[ [\frac{K}{J}] = \frac{\alpha}{\beta} \] (Eq. 58)

We use Eq. 58 for the calculations of the parameters shown in Figure 1f. Note also Eq.55 implies that, if initial recoil is expressed in $\mu m \ s^{-1}$ and the $\delta_{monolayer}$ in $\mu m^{-2}$ (i.e. cells per unit area), the units for $\alpha = \frac{K}{\mu}$ are $s^{-1}$, for $\beta = \frac{J}{\mu}$ are $\mu m \ s^{-1}$, for $\alpha = \frac{K}{\mu}$ are $s^{-1}$ and for $[\frac{K}{J}]$ are $\mu m^{-1}$. The table below summarizes the data we obtained from our experimental data from control and Y-27632 treated MCF-7 cells that is shown in Figure 1f.

| Best-fit values ± SD | Control            | Y-27632          |
|----------------------|--------------------|------------------|
| $\alpha$            | 0.00117 ± 0.00010 $s^{-1}$ | 0.00070 ± 0.00009 $s^{-1}$ |
| $\beta$             | 0.08335 ± 0.01731 $\mu m \ s^{-1}$ | 0.08335 ± 0.01731 $\mu m \ s^{-1}$ |
| $[\frac{K}{J}] = \frac{\alpha}{\beta}$ | 0.01401 ± 0.00314 $\mu m^{-1}$ | 0.00836 + 0.00205 $\mu m^{-1}$ |

For this non-linear regression analysis $\beta$ was constrained as a shared parameter between Control and Y-27632 treated conditions. We based this on the assumption that this treatment would affect only the contractile properties of cells as these still adhere to one another and we observed changes in junctional tension (Acharya et al., 2017).

6. Experimental validation of the model: Part II. Calculation of the amount of mechanical relaxation that is needed in tensile monolayers for topological transitions to occur.
In this section we include the details regarding the results shown in Figure 1j. For this we start by considering Eq. 48 that expresses a relationship between $\frac{K}{J}$ and $\frac{\sqrt{\eta^*}}{\sqrt{(\langle a \rangle)} \sqrt{\eta^*}}$ (Eq. 48)

$$\frac{K}{J} < \frac{\sqrt{\eta^*}}{\sqrt{(\langle a \rangle)} \sqrt{\eta^*}}$$

We then consider the situation where for a monolayer the $\frac{K}{J}$ ratio is such that topological transitions are forbidden. In this regime $\frac{K}{J}$ should be reduced by a fraction $f$ of its current value (Eq. 59) such that Eq. 48 is satisfied. Introducing this into Eq. 48 we obtain:

$$\frac{K}{J} - f \frac{K}{J} = \frac{\sqrt{\eta^*}}{\sqrt{(\langle a \rangle)} \sqrt{\eta^*}}$$

And rearranging:

$$f = 1 - \frac{\sqrt{\eta^*}}{\sqrt{(\langle a \rangle)} \sqrt{\eta^*}}$$

We use Eq. 60 to calculate how much softening is needed for cells to change their topological organization. For the data in Figure 1j, we measured the values of $\eta$ (shape parameter) for neighbor cells before ($\eta^*$) and after extrusion ($\eta^a$), as well as average area of these cells $\langle a \rangle$ and the $\frac{K}{J}$ that we obtained from our non-linear regression analysis (as explained in Section 5.1). Below is a table with a summary of these results.
Table 2. Summary of results for calculation of conditions for topological transitions to occur in the neighborhood of apoptotic cells.

| Parameter          | Value ± SEM (n=4) |
|--------------------|-------------------|
| \( \eta^\ast \)    | 0.0613 ± 0.0016   |
| \( \eta^a \)       | 0.0521 ± 0.0030   |
| \( \frac{\eta^\ast}{\eta^a} \) | 1.1766 ± 0.075 |
| \( \langle a \rangle \) | 618.97 ± 87.21 \( \mu m^2 \) |
| \[ \frac{[K^f]}{[K^T]} \] (in control monolayers) | 0.01401 ± 0.00314 \( \mu m^{-1} \) |
| \[ \frac{[K^TT]}{[K^T]} \] (for topological transitions) | 0.0049 ± 0.0003 \( \mu m^{-1} \) |
| \( f \)            | 0.653 ± 0.023     |

Data is derived from the analysis of 4 independent process of cell extrusion and measurements made on cell neighbors (7 neighbors per extrusion event, see also Figures 1a-e). \( \langle a \rangle \) is the average apical area of cell neighbors that experience a change in topology (change in number of sides = -1, Figure 1d and e).

Note: from our experimental results we obtained \( \left[ \frac{\sqrt{\eta^\ast}}{\sqrt{\eta^a}-1} \right] \approx 0.479 ± 0.004 \). This value obtained using Eq. 48 is not significantly different from the exact value of 1/2 (Eq. A3, Appendix) predicted for transitions to shapes that has the same value of \( \eta \).
7. References

Acharya, B.R., Wu, S.K., Lieu, Z.Z., Parton, R.G., Grill, S.W., Bershadsky, A.D., Gomez, G.A., and Yap, A.S. (2017). Mammalian Diaphanous 1 Mediates a Pathway for E-cadherin to Stabilize Epithelial Barriers through Junctional Contractility. Cell Rep 18, 2854-2867.

Bi, D., Lopez, J.H., Schwarz, J.M., and Manning, M.L. (2015). A density-independent rigidity transition in biological tissues. Nature Physics 11, 1074-1079.

Coburn, L., Lopez, H., Caldwell, B.J., Moussa, E., Yap, C., Priya, R., Noppe, A., Roberts, A.P., Lobaskin, V., Yap, A.S., Neufeld, Z., and Gomez, G.A. (2016). Contact inhibition of locomotion and mechanical cross-talk between cell-cell and cell-substrate adhesion determine the pattern of junctional tension in epithelial cell aggregates. Mol Biol Cell 27, 3436-3448.

Coburn, L., Lopez, H., Schouwenaar, I.M., Yap, A.S., Lobaskin, V., and Gomez, G.A. (2018). Role of contact inhibition of locomotion and junctional mechanics in epithelial collective responses to injury. Phys Biol 15, 024001.

Farhadifar, R., Roper, J.C., Aigouy, B., Eaton, S., and Julicher, F. (2007). The influence of cell mechanics, cell-cell interactions, and proliferation on epithelial packing. Curr Biol 17, 2095-2104.

Fernandez-Gonzalez, R., and Zallen, J.A. (2013). Wounded cells drive rapid epidermal repair in the early Drosophila embryo. Mol Biol Cell 24, 3227-3237.

Liang, X., Michael, M., and Gomez, G.A. (2016). Measurement of Mechanical Tension at Cell-cell Junctions Using Two-photon Laser Ablation. Bio Protoc 6.

Magno, R., Grieneisen, V.A., and Maree, A.F. (2015). The biophysical nature of cells: potential cell behaviours revealed by analytical and computational studies of cell surface mechanics. BMC Biophys 8, 8.

Martin, A.C., Kaschube, M., and Wieschaus, E.F. (2009). Pulsed contractions of an actin-myosin network drive apical constriction. Nature 457, 495-499.
8. Appendix I. Special cases of topological transitions

Here we considered the following special case of topological transitions where the value of $\eta$ does not change as a result of the topological change. This correspond, for example, to transitions involving different types of irregular pentagons which have exactly the same value of $\eta$ (Box 1).

To calculate $[K]^{TT}$ for this special case, we applied the L'Hôpital's rule to the term $\left[ \frac{\sqrt{\eta^a \eta^a - \eta^a}}{\eta^a - \eta^a} \right]$ in Eq. 47 and evaluate the limit when $\eta^a \rightarrow \eta^*$. After this, we obtained

$$[K]^{TT} = \frac{\sqrt{\eta^*}}{2\sqrt{\langle a \rangle}} \quad (Eq. A1)$$

$$[K]^{TT} = \frac{\sqrt{\eta^*}}{2\sqrt{\langle a \rangle}} \quad (Eq. A2)$$

Note, however, that a topological transition between two regular hexagonal lattices is not possible as it involves an intermediate state corresponding to irregular pentagons or rosettes. This means that the changes in energy we are discussing could be interpreted as energy barriers between (intermediate) states that are involved for junctional rearrangements and topological transitions to occur within cellular monolayers.

We then considered the relationship between $[K]^{TT}$ and $[K]^{s \rightarrow l}$, to analyze the regimes in which topological $\eta^* \rightarrow \eta^*$ transitions are allowed in the system. For topological transitions to occur, $K < K^{TT}$ and therefore:

$$[K] < [K]^{TT} = \frac{1}{2\sqrt{\langle a \rangle}} \quad (Eq. A3)$$

Eq. A3 shows that this type of topological changes is more likely in cell monolayers that sit in the soft regime.