Topological defects in epithelia govern cell death and extrusion

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Epithelial tissues (epithelia) remove excess cells through extrusion, preventing the accumulation of unnecessary or pathological cells. The extrusion process can be triggered by apoptotic signalling1, oncogenic transformation2,3 and overcrowding of cells4–6. Despite the important linkage of cell extrusion to developmental7, homeostatic2 and pathological processes2,8 such as cancer metastasis, its underlying mechanism and connections to the intrinsic mechanics of the epithelium are largely unexplored. We approach this problem by modelling the epithelium as an active nematic liquid crystal (that has a long range directional order), and comparing numerical simulations to strain rate and stress measurements within monolayers of MDCK (Madin Darby canine kidney) cells. Here we show that apoptotic cell extrusion is provoked by singularities in cell alignments3,10 in the form of comet-shaped topological defects. We find a universal correlation between extrusion sites and positions of nematic defects in the cell orientation field in different epithelium types. The results confirm the active nematic nature of epithelia, and demonstrate that defect-induced isotropic stresses are the primary precursors of mechanotransductive responses in cells, including YAP (Yes-associated protein) transcription factor activity11, caspase-3-mediated cell death, and extrusions. Importantly, the defect-driven extrusion mechanism depends on intercellular junctions, because the weakening of cell–cell interactions in an α-catenin knockdown monolayer reduces the defect size and increases both the number of defects and extrusion rates, as is also predicted by our model. We further demonstrate the ability to control extrusion hotspots by geometrically inducing defects through microcontact printing of patterned monolayers. On the basis of these results, we propose a mechanism for apoptotic cell extrusion: spontaneously formed topological defects in epithelia govern cell fate. This will be important in predicting extrusion hotspots and dynamics in vivo, with potential applications to tissue regeneration and the suppression of metastasis. Moreover, we anticipate that the analogy between the epithelium and active nematic liquid crystals will trigger further investigations of the link between cellular processes and the material properties of epithelia.

To understand the mechanisms that underlie apoptotic cell extrusion (Fig. 1a), we investigated the relationship between extrusion and epithelial monolayer remodelling. By culturing Madin Darby canine kidney (MDCK) epithelial cells at confluence on micropatterned substrates coated with extracellular matrix proteins (Methods), we observed that extrusion events were preceded by a coordinated, long-range flow of cells towards the eventual location of the extrusion (Fig. 1b, −160 min; Supplementary Movie 1). The group of cells that constituted these flows consistently formed a comet-like shape, with the head portion of the comet pointing towards the cell destined for extrusion (Fig. 1c, d). Since the cells in the monolayer were anisotropic in shape over long periods of time (Extended Data Fig. 1a, b) and demonstrated supracellular orientational order in their alignment (Extended Data Fig. 1c, d), the comet shape can be identified as a singularity in the cellular alignment8,10. We note that the singularity takes the form of a topological defect with +1/2 topological charge, where the orientational ordering is destroyed in a nematic liquid crystal. There are predominantly two types of defects in nematic liquid crystals, that is, +1/2 and −1/2, and both were identified in the monolayer (Fig. 1e, Methods)12. Such nematic topological defects have been identified in a wide variety of biological systems including lipid vesicles13, fibroblast cell colonies14,15, suspensions of microtubule bundles16, motility assays of driven filaments17, and growing Escherichia coli colonies16.

We found that extrusion events were strongly correlated with the positions of a subset of +1/2 defects (and less so with −1/2 defects) (Fig. 1f, Extended Data Fig. 1e–h, Methods). We further found similar extrusion–defect links in different types of epithelium (Fig. 1f and Extended Data Fig. 1e–h), including a cell-division-inhibited (treated with mytomycin C) MDCK monolayer, a breast cell line (MCF10A) and human epithelial skin (HaCaT). In the last case, we found a correlation between extrusions and defects, but with a stronger correlation with −1/2 defects, which may be attributed to the multi-stratified organization of HaCaT cells as well as the HaCaT cell layers being more elastic than the MDCK monolayer17. We then analysed the temporal correlation between nematic defects and cell extrusions within MDCK epithelial monolayers. Defects occurred well before cell extrusion and caspase activation (at about 100 min) (Extended Data Fig. 1i), consistent with spatio-temporal cellular flows observed in these regions. It suggests that singularities in cellular alignment are spontaneously generated in the epithelial monolayer in the form of nematic topological defects, and the defects in turn trigger cell apoptosis and extrusion.

To probe the first part of the hypothesis, we studied the properties of singular points of cellular alignment in wild-type (WT) MDCK to confirm their identification with topological defects in active nematic liquid crystals. We used particle image velocimetry18 (PIV; see Methods) to measure experimentally the velocity and strain rate fields around the singular points in cell alignment (Fig. 2a top row, Experiment), and compared them to numerical simulations (see Methods) of active nematic liquid crystals (Fig. 2a bottom row, Simulation)19. The close match between strain rate patterns and velocity fields around +1/2 topological defects in experiments and simulations (Fig. 2a) revealed that the epithelium monolayer of cells indeed behaves as an extensile, active nematic. The extensile nature is manifest as flow along the elongated axis of the cells that moves towards the head region of the defect (Fig. 2a rightmost column, Average velocity field), in contrast to contractile flow, which is directed towards the tail region (Extended Data Fig. 2a).
originates to a large extent from the actomyosin activity, we tested this by introducing blebbistatin treatment. When blebbistatin was added to reduce activity at various concentrations (10 μM and 50 μM), there was a steady and considerable drop in the defect density with respect to the concentration we used (Extended Data Fig. 2d), and this trend was reversed during washout (Fig. 2b, Supplementary Movie 3). The rates of defect density decrease and increase were similar, as the defect density dropped to about 33% of its original value after approximately 10 h of blebbistatin treatment, and increased back to about 66% of the original density in approximately 6 h of washout. The blebbistatin-treated monolayer maintained an extensile flow field (Extended Data Fig. 2b, Blebbistatin 10 μM). In addition, the extrusion–defect spatial correlation was slightly reduced under blebbistatin treatment, suggesting a stress regulation around the defects through actomyosin activity (Extended Data Fig. 2e). Altogether, these results confirm that the epithelium is behaving as an active nematic liquid crystal and that topological defects are spontaneously formed by active stresses in the monolayer. This is in contrast to a recent finding that fibroblast cells in a packed environment behaved as a non-active, nematic liquid crystal, where the number of topological defects relaxed to the equilibrium state.

To understand why extrusions were related to defects, we first speculated that defects might generate regions of high local cell density that induced extrusions, as tissue crowding is known to play a role in cell extrusion. However, we did not find a clear spatial correlation between extrusions and the regions of highest local cell density (Extended Data Fig. 3a, Methods). The next clue came from liquid crystal theory, in which spontaneously formed topological defects are expected to generate mechanical stress in their vicinity owing to large distortions in the cell orientation. We thus hypothesized that +1/2 defects generated spatially localized, elevated compressive stress that is sufficient to provoke cell response, apoptosis and extrusion. This hypothesis is supported by simulations showing that the highest compressive isotropic stresses are strongly correlated with the locations of +1/2 defects and less so with –1/2 defects (Extended Data Fig. 3b), which is reminiscent of the stronger extrusion correlation with +1/2 defects than with –1/2 defects in experiments (Fig. 1f, Extended Data Fig. 1f). To investigate the impact of mechanical stress on cell extrusions, we measured the mechanical traction exerted by cells on the underlying substrate using traction force microscopy (TFM, see Methods), and converted the traction to two-dimensional stress in the monolayer (Fig. 2c) using Bayesian inversion stress microscopy, (BISM, see Methods). Consistent with the velocity field results, the stress pattern around +1/2 defects in the experimental measurements is again highly similar to that in extensile nematic simulations (Fig. 2d).

To prove directly that cells are more compressed at +1/2 defects, we calculated the isotropic contribution to the monolayer stress measured experimentally around topological defects. The isotropic stress provides a clear distinction between +1/2 and –1/2 defects as it is only highly compressive (negative) at the head portion of a +1/2 defect, while being more tensile at a –1/2 defect (Fig. 3a, Experiment). These distinguishing isotropic stress distributions around the defects are again reflected in the simulations (Fig. 3a, Simulation). We then measured the time evolution of the average isotropic stress for cells that were going to extrude, and found that such cells were being increasingly compressed (increasingly negative isotropic stress with time, see Fig. 3b) in the time leading up to their extrusion. Notably, the time at which stress started to become more compressive (about ~110 min) was consistent with the start of the long-range flow and with the rise in the defect–extrusion correlation (Fig. 1b, Extended Data Fig. 1i). These results matched the observation that a +1/2 defect is first created and propagated in the monolayer, and that a cell is then extruded at the head region of the defect where the compressive stress is concentrated (Fig. 3c). There was also a higher probability for a defect with higher compressive stress to induce an extrusion (Extended Data Fig. 3c, Methods). We observed that the +1/2 defects tended to maintain their overall orientation as
the neighbour cells elongated to compensate for the extruding cell (Extended Data Fig. 3d), leading to a slow relaxation (about 250 min) of the average compressive stress (Extended Data Fig. 3e).

We then investigated potential mechanotransductive effects of the stress localization observed at defects on biochemical signals within the monolayer. To this end, we first checked the YAP transcription factor distribution in cells (see Methods), as YAP is known to respond to compressive mechanical signals and act as a potent inhibitor of apoptosis by translocating between the nucleus and cytoplasm—*it is also implicated in extrusion*. We observed that there were substantially more cells at the head region of +1/2 defects than in −1/2 defect cores that had YAP sequestered in their cytoplasm (Fig. 3d, e). As YAP partially more cells at the head region of apoptosis by translocating between the nucleus and cytoplasm—it is

Figure 2 | MDCK WT epithelia behave as 2D, extensile, active nematic liquid crystals. a, Top, average (Avg.) yy- and xy-components of strain rate map around +1/2 defect in experiments (left and middle respectively) and corresponding average velocity flow field (right; n = 2,142 defects from 4 independent movies in 3 independent experiments), compared with simulations of extensile, active nematic liquid crystal (bottom). Colour code is positive for stretching and negative for shrinkage. b, Time evolution of (total) defect areal density under 50 μM blebbistatin treatment and washout (arrow). Data for each time point are binned over duration of 120 min (n = 6 different time frames), in n = 4 independent movies. A t-test is performed for each time point against time = 600 min. Data are represented as n = 24 scatter points for each time, and bars represent mean ± s.e.m of the scatter points. **P < 0.01, ***P < 0.001. c, Diagram of TFM set-up to measure traction (tx and ty) and washout (arrow). Data for each time point are binned over duration of 400 min (spatially averaged over 65 μm × 65 μm; n = 32 extrusions in 2 independent experiments). A t-test is performed for each time point against a normal distribution centred at zero. *P < 0.0001. d, Isotropic stress around a +1/2 defect moving to top left corner of image, and extrusion. Top panel, nematic directors (red lines) overlaid on monolayer. Bottom panel, corresponding isotropic stress heat map. Colour code, positive for tensile state, negative for compression. e, Average isotropic stress evolution for cells experiencing negative (compressive) stress values during extrusion initiation at t = 0 min (spatially averaged over 65 μm × 65 μm; n = 32 extrusions in 2 independent experiments). e, Average isotropic stress map around a +1/2 defect, with composite image at right (red, nucleus; green, YAP). f, Percentage of cells with YAP positive for tensile state, negative for compressive stresses. d, Distribution of YAP and nuclei of cells at a +1/2 defect, with composite image at right (red, nucleus; green, YAP). Green arrowheads, cells with YAP in cytoplasm. e, Percentage of cells with YAP in nucleus, in cytoplasm or uniformly distributed, at head of +1/2 defects, at core of −1/2 defects, or at random points. n = 78 (+1/2), n = 77 (−1/2), n = 78 (random) from 17 independent movies in 2 independent experiments. See Methods. Data represented as minimum, first and third quartiles, median, maximum (lines) and mean (circle). ks-test, ***P < 0.001. f, Average isotropic stress around +1/2 defect (α-catKD experiments). n = 1,940 defects from 3 independent movies. Similar colour code as a, g, h, Average defect areal density (g) and extrusion rate (h) in WT and α-catKD MDCK. g, n = 685 frames from 3 independent movies in 2 independent experiments (WT), n = 360 frames from 3 independent movies (α-catKD). Two sample t-test, **P < 0.001. h, n = 6 independent movies in 4 independent experiments (WT), n = 6 independent movies in 2 independent experiments (α-catKD). ks-test, **P < 0.01. All data, except in e, represented as mean ± s.e.m. Black lines show representative nematic directors, grey circle denotes defect core. Red lines show local cell orientation. Scale bars, 10 μm.

there was an extrusion (Extended Data Fig. 3f). Along these lines, we found that caspase-3 inhibition largely eliminated the occurrence of extrusions (Extended Data Fig. 3g), even though the defect density was maintained. These results showed that compressive stress localization at defects induced cell death signals which in turn triggered extrusion.
the mechanical connection between cell–cell adhesion proteins and the contractile actomyosin cytoskeleton facilitates the relative movements of adjacent cells\textsuperscript{17,18}. More importantly, nematic theory predicts that the number of topological defects is inversely related to the orientational elasticity (Extended Data Fig. 4d), therefore a reduction in the orientational elasticity is expected to result in a larger number of topological defects. Indeed, experiments showed that there was an increase of about 40% in the number of defects on going from WT cells to $\alpha$-catKD cells (Fig. 3g), which can explain a marked increase in the extrusion rate in $\alpha$-catKD cells (Fig. 3h). These results suggest that the weakening of cell–cell junctions in $\alpha$-catKD cells facilitated collective in-plane bending of multiple cells that decreased orientational elasticity, $K$, thus increasing defect formation and extrusion.

To further prove the causal role of defects in extrusions, we sought to control defect locations in the monolayer\textsuperscript{29}, thus allowing the control of extrusion hotspots. Since MDCK cells preferentially align tangential to the boundary between monolayer-adherent and non-adherent substrates\textsuperscript{18}, we microcontact printed (Methods) a star-shaped cell monolayer (Fig. 4a) to geometrically force comet-like defects to the four tips of the star. The length scale of the tip of the star (about 100–200 $\mu$m) was chosen to match the size of the defects. We indeed found that the defect density increased at the corners of the star, and that extrusions predominantly happened close to the four tips (Fig. 4b, c, Extended Data Fig. 5a, Supplementary Movie 5). In contrast, the $-1/2$ defect density became larger near the centre of the star, but there was no increase in extrusion events in this region. This biased distribution of extrusions was not found in a circle-shaped monolayer (Fig. 4d–f, Extended Data Fig. 5a). The extrusions were also more correlated with $+1/2$ defects (more decorrelated with $-1/2$ defects) in the star-shaped than in the circle-shaped monolayer (Extended Data Fig. 1e–h, Extended Data Fig. 5b, Fig. 1f). Thus, we demonstrated that extrusions could be controlled by artificially controlling the positions of $+1/2$ defects in the monolayer.

These findings reinforce the idea that comet-like defects in epithelia can mechanically induce cell apoptosis and extrusions. However, the reverse question may be asked: can apoptotic cells destined for extrusion produce certain biochemical signals that can increase cell activity\textsuperscript{1,3} and hence generate new local defects to expedite their own extrusion? To investigate this possibility, we first checked defect-related properties in a caspase-3 inhibited monolayer, and found that the defect density was similar to that of a non-treated monolayer, and the flow field patterns at $+1/2$ defects still showed an extensile flow field albeit having a reduced pattern size (Extended Data Fig. 5c). This suggested that cell death signals did not contribute to the extensile nature and activity of the epithelium. In another more direct experiment, we used an ultraviolet laser to induce a single cell apoptosis\textsuperscript{31} (Methods), and followed the time evolution of the number of $+1/2$ defects immediately afterward (within a radius of 80 $\mu$m, Fig. 4g) and up to several hours, until the first extrusion occurred. We did not observe any increase in the average number of defects after the laser induction of cell apoptosis (Fig. 4h), which confirmed that there was minimal influence of apoptotic signalling on the triggering of more defects.

To further test the contribution of intercellular junctions to this mechanism, we perturbed collective cell movements and intercellular forces by knocking down $\alpha$-catenin\textsuperscript{17,18}, which is known to be a core mechanosensor of force transmission at cell–cell junctions\textsuperscript{29}. We observed that $\alpha$-catenin knock down ($\alpha$-catKD) MDCK cells maintained similar levels of orientational order and similar extrusion–defect correlation patterns as WT MDCK at a similar cell density range (Extended Data Fig. 4a, b, Fig. 1f, Extended Data Fig. 1e–h). However, the defects were smaller in size and had more spatially focused stress patterns compared to WT MDCK (Fig. 3a, f, Supplementary Movie 4). From the nematic model, the size of the defect core is known to scale as $R \propto \sqrt{K}$, where $K$ is the orientational elasticity constant characterizing the resistance of nematic directors to a change in the orientation\textsuperscript{12}, and thus the smaller defect size seen in $\alpha$-catKD experiments can be interpreted as a reduction in $K$ (Extended Data Fig. 4c). Collective cell bending may be favoured in epithelial $\alpha$-catKD cells, as the alteration of...
measured stress values in two- and three-dimensional epithelium or cell aggregates. This suggests that extreme physical environments or niches (for example, extremely curved substrate surfaces, overcrowding) are not the only places where mechanical activation of apoptosis, extrusions and other mecanosensitive cell activities could be triggered.

We conclude that an epithelial cell monolayer behaves as an active extensile nematic material, and that spontaneous formation of singularities in cellular alignment in the form of nematic topological defects is a previously unidentified cause of cell apoptosis, suggesting that such defects govern cell fate. Hence it is anticipated that this defect-induced extrusion mechanism could be a common strategy for preserving homeostasis of a normal epithelium in vivo and for suppressing tumour invasion. It would be interesting to explore how differences in the intrinsic mechanical properties of different epithelia can influence the stress distributions around topological defects and thus alter the influence of defects on apoptosis and extrusion. Another interesting investigation would be to probe the role of topological defects in pathological conditions, including oncogenic cell extrusion.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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METHODS

Experimental techniques. MDCK strain II WT (MDCK WT, gift from J. W. Nelson, Stanford Univ) and HaCaT (Cell Lines Service) cells were maintained in a culture medium composed of high glucose DMEM 1X medium (Invitrogen), 100 μg/ml penicillin, 100 μg/ml streptomycin (Invitrogen), and 10% fetal bovine serum (Invitrogen). Culture medium for MDCK with stable α-catenin knockdown MDCK (α-catKD), gifted by James W. Nelson, Stanford University, was additionally supplemented with 250 μg/ml gentamicin (Invitrogen). MCF10A cells (ATCC) were maintained in MFGM (Lonza) medium supplemented with cholera toxin (100 ng/ml). All cells were grown at 37 °C and 5% CO2. For microscopy imaging, DMEM was replaced with low glucose Leibovitz (Sigma-Aldrich). MCF10A imaging was done in its culture medium.

To reduce MDCK WT actomyosin activity, blebbistatin (Selleckchem) was added at 10 μM for 1 h before imaging. To observe the defect temporal dimension as a function of activity, 50 μM blebbistatin was added and washout was performed after a certain amount of time. To inhibit MDCK proliferation, mytomycin-C (Sigma) was added at 10 μg/ml for 1 h, and rinsed before imaging. For live imaging of fluorescent cell nuclei, the confluent monolayer was incubated with Hoechst 33342 (ThermoFisher) at 1 μg/ml for 5 min and rinsed. To monitor cell viability in experiments, caspase-3 indicator NucView (Abcam) was added to the medium at 1:10,000 dilution for ~30 min before imaging. Cell apoptosis was inhibited by adding Z-VAD-FMK (Promega) to the medium at 50 μM. Cell lines tested for mycoplasma but not authenticated are MDCK WT, MCF10A, and HaCaT.

Microscopy imaging. A mature and confluent monolayer was allowed to develop overnight before experiments started. Confluency is reached when all space present to the epithelium is filled. Typical experiments were run for 1–2 days. Phase contrast and fluorescence time-lapse imaging for monolayer on a glass-bottom Petri dish, PDMS spin-coated dish, and TFM soft gel were performed using a Biostation (Nikon) or Olympus 1X2-UCB inverted microscope. Confocal time-lapse images were obtained on a Nikon A1R MP laser scanning microscope or a Zeiss upright Z2 microscope.

Microcontact printing. Silicon wafers with desired patterns were made using SU-8 photoresist for soft lithography. To form stamps, PDMS (Sylgard 184, Dow Corning) was mixed at a 1:10 mixture ratio (curing agent: silicone elastomer) and moulded onto silicon wafers to achieve a flat substrate of height ≈0.1 μm. To form stamps, PDMS (Sylgard 184, Dow Corning) at 1:10 mixture ratio (curing agent: silicone elastomer) was moulded onto a silicon wafer with desired patterns. PDMS (Sylgard 184, Dow Corning) at 1:10 mixture ratio (curing agent: silicone elastomer) was moulded onto a silicon wafer with desired patterns. PDMS (Sylgard 184, Dow Corning) at 1:10 mixture ratio (curing agent: silicone elastomer) was moulded onto a silicon wafer with desired patterns.

Preparation of substrate. To prepare the gel with a stiffness of 10–20 kPa, CyA microcontact printing and PDMS spin-coated dish were performed using a Biostation (Nikon) or Olympus 1X2-UCB inverted microscope. Confoocal time-lapse images were obtained on a Nikon A1R MP laser scanning microscope or a Zeiss upright Z2 microscope.

Traction Force Microscopy (TFM). Soft silicone gel with attached fluorescent beads was used as the substrate for TFM so that in-plane cell traction forces on the substrate could be measured. To prepare the gel with a stiffness of 10–20 kPa, CyA and CyB components (Dow Corning) were mixed at a 1:1 ratio and spin-coated on a Petri dish to achieve a flat substrate of height ~60–100 μm. After curing at 80 °C for 2 h, the substrate was silanized with 5% 3-aminopropyl trimethoxysilane (Sigma) in ethanol for 5 min. Carboxylated fluorescent beads (100 μm, Invitrogen) were functionalized on the substrate at 1:500 dilution in deionized water. The beads were passivated with 1X Tris (Sigma) for 10 min and pure fibronectin (50 μg/ml) was incubated on the substrate for 1 h before cell seeding. Between each step, the samples were rinsed 3 times with 1X PBS. After the experiment, the cells were completely removed by adding SDS, so that the resting state of the gel could be measured. Bead displacements (with respect to its resting state) acquired during experiments were measured and converted to cell traction forces with an ImageJ plugin.

Cell apoptosis induction. Laser induction of cell apoptosis was done on a Nikon A1R MP laser scanning microscope with the help of wobbling beads (100 μm, Invitrogen) at the head of a linear stage. Using an in-house MATLAB script, the nucleus-to-cyttoplasm YAP intensity ratio (NCYap-ratio) of cells in the monolayer was measured, where cells were classified as having YAP in the nucleus (NCYap-ratio > Max_threshold), in the cytoplasm (NCYap-ratio < Max_threshold) or uniformly distributed. In fluorescence microscopy, YAP antibody staining was used to label the nuclei of the cells. YAP antibody staining was used to label the nuclei of the cells. YAP antibody staining was used to label the nuclei of the cells.
its closest defect in the preceding frame was determined. The number of closest defects (+1/2 or −1/2 defined separately) within a given distance range from their corresponding extrusions was converted into the areal probability of the defects at the specific distance, and the areal probabilities were normalized such that they sum to 1 (to facilitate comparison between different experimental conditions). A similar closest defect areal probability as a function of distance was also obtained for random points for comparison. For each extrusion, a random point was generated in the same frame as the extrusion, but at a random location (uniformly spaced in the monolayer). For the closest defect probability curve with random points, n = 30 (different sets of random points). A defect probability curve which shows a higher spatial correlation between extrusion and defect has larger positive values at r_c close to 0 μm. The ratio of the defect probability at r_c = 10 μm (closest region to extrusions, between r_c = 0–20 μm) to r_c = 120 μm (furthest point, between r_c = 110–130 μm) is calculated as a measure of the strength of the extrusion−defect correlation. For example, since this ratio for the +1/2 MDCK WT case is ~7 (Extended Data Fig. 1h), this tells us that there is an approximately sevenfold higher probability to find the closest defect for an extrusion situated at ~10 μm compared to ~120 μm from it. To quantify when the corresponding defects start to spatially approach the extrusions, this defect probability ratio is calculated as a function of time (t = 0 min) leading to extrusion at t = 0 min, comparing at each time frame between the defects in that frame and the eventual extrusion spots at t = 0 min.

Strain rate and stress measurements. Strain rate was calculated using the formula η_i = (∂u_i/∂t + u_j ∂u_j/2), with i ∈ (x, y) and velocity field, u, obtained from PIV measurements. Stress was estimated from traction force data by inverting the force balance equation [equation (1)]. This underdetermined problem was solved by Bayesian inversion from the BISS method, independently of the epithelial rheology (details below). The stress estimate was defined as the mode of the posterior stress distribution function (maximal a posteriori estimate, see below for details). Isotropic stress was taken as half the trace of the stress tensor, (σ_{xx} + σ_{yy})/2 in the tissue. Average strain rate and stress maps for the defects were calculated by rotating and aligning the defects to the y axis at the origin, and averaging the values of the rotated vectors and matrices (in their new basis) for the corresponding pixel locations in all defects. Smoothing was done by linear interpolation of maps.

+1/2 defect stress magnitude as indicator of the ability of defect to induce extrusion. For each +1/2 defect, the distance to the closest extrusion within the next 40 min after the time of the defect was determined. These distances were pooled into groups based on the values of the compressive isotropic stress at the head regions (area of 60 μm × 60 μm) of their corresponding defect. The number of extrusions within a given distance range was converted into the areal density of the defects at the specific distance, and the areal densities were normalized such that they sum to 1. The peak in each areal density curve increases near r = 0 μm, as the compressive stress categorizing the group increases. This shows that as the defect compressive stress magnitude increases, the probability of the defect inducing an extrusion increases.

Simulations, models and robustness studies of techniques. Robustness of the nematic director and defect detection method. To verify the effectiveness of the winding angle approach in detecting half-integer topological defects, a different algorithm based on diffusive topological charge [43] to detect the defects was used, and compared to the original approach. The results were found to be identical in both cases. Extended Data Fig. 6b shows an example of topological defects detected using the winding angle approach and the diffusive charge approach.

In addition, errors could occur in the identification of the nematic director field from the pattern of cells in the images, rather than in identifying the defects from the director field. As a check, we have performed a systematic variation of the relevant parameters in the orientation analysis and confirmed the robustness of our approach against small variations in the parameter values. Extended Data Fig. 6c shows the effect of varying the coarse-grained window size (for each nematic director field) and therefore the alignment parameter, on the number of topological defects detected. To reduce the number of false defects detected: (1) each nematic director represents the average orientation of 3–5 cells within the window (window size of ~50 μm). This reduces the possible errors due to fluctuations in the orientation of each cell. (2) Only the stably detected defects were kept (defects that appear in at least 2 consecutive frames).

(3) The final window size was chosen as ~51 μm (80 pixels) (after checking a range of 60–110 pixels). The minimum window size checked was ~38 μm (60 pixels), corresponding to 2–4 cells, which clearly showed detection of noisy defects. The maximum window size checked was ~71 μm (110 pixels), which was too big so that the orientation field was too smooth and certain clear defects were clearly missed. 51 μm was chosen as a compromise between these values (Extended Data Fig. 6c).

Active nematic model for epithelial monolayer. Individual cells in a confluent epithelial monolayer have an in-plane anisotropic shape, which can be approximated by an ellipse, and are constantly moving in the epithelium, corresponding to a non-zero velocity field. Cell alignments show local orientational order of the cells.

The orientational order is destroyed at singular points called topological defects. The strength of a defect is determined by the change in the orientations of cells in a closed curve around the defect core. Integer (±1) and half-integer (±1/2) topological defects correspond to ±2π and ±π rotation of the cell alignment along a closed curve around a singular point [1,2]. The strength of topological defects in cell alignment is an important determinant of the type of orientational order of cells, with ±1 specific to polar materials and ±1/2 specific to nematic materials. Therefore, the emergence of ±1/2 defects in the experiments indicates that despite their individual polarity, MDCK cells behave as nematic materials (the orientation vector is ‘head-tail’ symmetric) at the level of the epithelium.

In order to capture the dynamics of cell movement and orientation, we use a continuum model of nematohydrodynamics for the monolayer [15,44] to account for the combined effects of cell velocity and orientation. This nematohydrodynamic approach has proven successful in describing active systems [94–98]. The orientational order of cells is characterized by the nematic tensor Q = 3S(μn − 1)/2, valid for a 3D or quasi-2D simulation used here, where μ is the cell orientation, S is the magnitude of the order and I is the identity matrix. The nematic tensor is evolved according to the Beris–Edwards equation [12],

\[ \frac{∂Q}{∂t} + u_0(∂Q/∂x) = S - \frac{μ}{κ} H_p \]

(1)

where \( \frac{∂Q}{∂t} + u_0(∂Q/∂x) \) is the total derivative with \( u_0 \) denoting the velocity field and

\[ S = (λE_u + Ω)ΔQ + ΔQ/3 + (Q_0 + ΔQ/3)λE_u - Ω - 2λQ_1/2 \]

(2)

is the co-rotation term accounting for the response of cell orientation to the velocity gradients, where \( δ \) is the identity matrix or the Kronecker delta. Here, velocity gradients are characterized by the strain rate tensor \( E_u = (μ_0 + μ_1)/2 \) and the vorticity tensor, \( Ω = (μ_2 - μ_1)/2 \), corresponding to extensional and rotational flows, respectively. The relative strength of extensional and rotational flows is determined by the alignment parameter \( λ \). Therefore, the alignment parameter accounts for the different responses of particles of different shapes to the symmetric and asymmetric parts of the velocity gradient tensor [99]. Mapping the alignment parameter to the Leslie–Ericksen equation for liquid crystal dynamics gives \( λ = \frac{1}{2β} - \frac{1}{2μ_0} \) (refs 12, 50), where \( β = ab/b \) is the ratio of the length of the cell along its axis of symmetry, \( a \), to its length perpendicular to this axis, \( b \). Therefore, for prolate ellipsoids \( β > 1 \), while for oblate ellipsoids \( β < 1 \) and for spherical particles \( β = 1 \), which correspond to \( λ > 0 \), \( λ < 0 \), and \( λ = 0 \), respectively. The experiments show that MDCK cells exhibit an in-plane anisotropic shape in the form of prolate ellipses and therefore are characterized by \( λ > 0 \). The molecular field in equation (1),

\[ H_p = -\frac{δF}{δQ_1} + \frac{δF}{3δQ} \]

(3)

describes the relaxation of the orientational order to the minimum of the free energy, \( F = F_u + F_0 \). The bulk free energy \( F_b \) is calculated from the Landau–De Gennes expansion,

\[ F_b = \frac{A_0(Q_0Q_1)}{2} + \frac{B_0(Q_0Q_1Q_3)}{3} + \frac{C_0(Q_1Q_3)^2}{4} \]

(4)

where \( A_0, B_0 \) and \( C_0 \) are material constants. In addition, the free energy corresponding to spatial inhomogeneities in the orientation field is described by the Oseen–Frank expansion using a single elastic constant approximation [12],

\[ F_{01} = \frac{K(δQ_1)^2}{2} \]

(5)

where \( K \) is the elastic constant. There are in general two elastic constants, corresponding to bend, \( K_b \) and splay, \( K_s \), in 2D systems. However, setting different values of \( K_b \) and \( K_s \) gives only small qualitative changes in the flow fields around topological defects in the simulations, as these are predominantly controlled by active stresses (introduced below).

The velocity field in the monolayer is evolved according to the incompressible Navier–Stokes equation:

\[ ∂u_1/∂t + u_0(∂u_1/∂x) = 0 \]

(6)

which reduces to the force balance equation \( ∂u_1/∂x = 0 \) in the low Reynolds number limit relevant to monolayer mechanics. The assumption of an incompressible monolayer in equation (6) is supported by experimental measurements of the
divergence of the flow field in the epithelium. Although divergence hotspots (larger values) do arise at singular points in the epithelium, the temporal average of this divergence field shows only small deviations from zero (1 < 4% every 10 min, Extended Data Fig. 7a, b).

Equation (7) describes how the rate of change of linear momentum is driven by stress gradients in the monolayer. The total stress, \( \sigma \), consists of four contributions: isotropic pressure, \(-\phi_{0}\) viscous stress, \( \sigma_{n}^{\text{nonas}} = 2nE_{n} \) nematic elastic stress, \( \sigma_{g}^{\text{ext}} \), and active stress, \( \sigma_{a}^{\text{active}} \). The elastic stress
\[
\sigma_{g}^{\text{el}} = 2\lambda(Q_{g} + \delta_{3}/3)(Q_{g}H_{S}) - \lambda H_{S}(Q_{g} + \delta_{3}/3) - \lambda(Q_{g} + \delta_{3}/3)\delta_{3}H_{S} - \partial Q_{g}\delta F/\partial H_{S} + Q_{g}H_{S} - H_{S}Q_{g}
\]

(8)
corresponds to distortions in cell alignments. Finally, the active stress
\[
\sigma_{a}^{\text{active}} = -\zeta Q_{g}
\]

takes into account the local stresses generated by active processes in the cells, including actomyosin polymerization and cell contractility\(2^{2},2^{31}-2^{33}\). The activity coefficient, \( \zeta \), determines the strength of the activity, with positive and negative values for extensile and contractile stresses. The extensile stress corresponds to the flow generated by the cell activity outward along the elongated axis of the cell, while contractile stress characterizes the flow outward along the shorter axis. The measurements of strain rates around defects in the experiments and comparison with the simulations show that the cells produce extensile stresses in the epithelium and that the epithelium behaves as an extensile active nematic.

Within this framework, the isotropic contribution to the total stress reduces to just the pressure and an isotropic contribution from the nematic elastic stress, as the trace of \( \sigma_{g}^{\text{ext}} \) is zero because \( Q_{g} \) is traceless, and the trace of \( \sigma_{a}^{\text{active}} \) is also zero due to the incompressibility condition, equation (6). Thus, the specific spatial patterns of the isotropic part of the total stress in the simulations are determined only by the elastic stress.

The hybrid Lattice Boltzmann algorithm is used to solve the equations of motion (equations (1), (6) and (7)). Details of the algorithm can be found elsewhere\(2^{34},2^{35}\). The simulation parameters used are \( \Gamma = 0.34, A_{0} = 0, B_{0} = -0.3, C_{0} = 0.3 \), \( K = 0.08, \zeta = 0.006, \Lambda = 0.7 \) and \( \eta = 2/3 \), in lattice units, unless otherwise is stated. Bayesian Inversion Stress Microscopy (BISM). According to Newton’s laws, the stress in a cell monolayer is balanced by the traction forces the monolayer exerts on its substrate everywhere in space and time. In 2D and neglecting inertia, the force balance reads
\[
\nabla \cdot \sigma = t
\]

(10)
where \( \sigma \) is the stress tensor field (units Pa \( \mu \)m in 2D) and \( t \) is the 2D in-plane cell–substrate traction force field. In Cartesian component form, the equations
\[
\frac{\partial \sigma_{x x}}{\partial x} + \frac{\partial \sigma_{x y}}{\partial y} = t_{x}
\]

(11a)
\[
\frac{\partial \sigma_{y x}}{\partial x} + \frac{\partial \sigma_{y y}}{\partial y} = t_{y}
\]

(11b)
can be discretized on a square grid of spatial resolution \( L \) and rewritten in a matrix format,
\[
A \sigma = T
\]

(12)
where the vectors \( \sigma \) and \( T \) respectively consist of all stress and traction components over the whole of space (see ref. 24 for the exact forms of \( A, \sigma \) and \( T \)). Since \( T \) is readily measured by Traction Force Microscopy (TFM), the stress components could be determined by inverting the matrix \( A \). However, the equation is underdetermined as \( \sigma \) has three components \( \sigma_{x x}, \sigma_{y y}, \sigma_{x y} \) at each space position, while \( t \) only has two components \( (t_{x}, t_{y}) \), and thus cannot be solved for \( \sigma \) even if \( T \) is fully known. Bayesian inference provides a statistical framework for the integration of current data, prior knowledge and reasonable assumptions about the system to extract information (in the form of a probabilistic distribution) from such ill-conditioned situations\(2^{36},2^{37}\). Two distributions, that is, the likelihood function, \( L(T|\sigma) \), and prior density, \( \pi(\sigma) \), need to be constructed to calculate the desired output distribution that describes \( L(T|\sigma) \) accounts for the physical relation between measured data, \( T \) and \( \sigma \) through equation (10) up to an additive noise. Assuming the noise has a Gaussian profile with zero mean, the likelihood can be written as
\[
L(T|\sigma) \propto \exp \left[ -\frac{(T - A\sigma)^{T}(T - A\sigma)}{2\sigma_{0}^{2}} \right]
\]

(13)
where \( \sigma_{0}^{2} \) the variance of the noise, allows the definition of a diagonal covariance matrix \( S = \sigma_{0}^{2}I \) with \( I \) the identity matrix. \( \pi(\sigma) \) accounts for any additional constraints and assumptions on the stress components. Specifically, the off-diagonal components of the stress are enforced to be equal, that is, \( \sigma_{x y} = \sigma_{y x} \) (with an extra hyperparameter, \( \alpha \) and stress is assumed to be Gaussian with zero mean and covariance \( \sigma_{0}^{2}I \). The prior is written as
\[
\pi(\sigma) \propto \exp \left[ -\frac{|\sigma_{x y}|^{2} + |\sigma_{x y} - \sigma_{y x}|^{2}}{2\sigma_{0}^{2}} \right]
\]

(14)
with \( \Pi = \sigma_{0}^{2}B \) the covariance matrix of the prior, embedding the two quadratic terms in \( B \).

Bayes’ theorem links the posterior distribution \( \Pi(\sigma|T) \) on \( \sigma \) (conditional probability of \( \sigma \) given data and assumptions), with \( L(T|\sigma) \) and \( \pi(\sigma) \) by a product rule
\[
\Pi(\sigma|T) \propto L(T|\sigma) \times \pi(\sigma)
\]

(15)
Since the likelihood and prior are both Gaussian, \( \Pi(\sigma|T) \) is also Gaussian with covariance matrix \( S_{T} \) and mean \( \sigma_{T} \) written as,
\[
S_{T} = (S_{0}^{2} + A^{T}S^{-1}A)^{-1}
\]

(16)
and
\[
\sigma_{T} = S_{T}A^{T}S^{-1}L
\]

(17)
where \( A^{T} \) is the transpose of \( A, \sigma_{T} \) is the inference of monolayer stress that we need. Recall that whereas actual stress, \( \sigma \), cannot be obtained (because the matrix \( A \) itself cannot be inverted), \( \sigma_{T} \) can be readily calculated since the inversion of \( (S_{0}^{2} + A^{T}S^{-1}A) \) is possible. The Maximum A-Posteriori (MAP) solution to the inference problem is the posterior mode, identical to the mean for a Gaussian: \( \hat{\sigma} = \sigma_{T} \) (see Extended Data Fig. 8a for a schematic of the BISM algorithm). The solution depends on the dimensionless parameter \( A = \frac{\sigma_{0}^{2}}{\sigma_{s}^{2}} \), which is found using the L-curve method\(5\). Note that error bars on the inferred stress are provided by the posterior covariance if needed.

In this Letter, the hyperparameter \( \alpha \) is fixed to a value \( (10^{5}) \) large enough to enforce the equality of off-diagonal components of the stress tensor. With a spatial resolution \( L = 5.12 \mu \)m, the L-curve method yields a value of \( \Lambda = 10^{-5} \) that we use for all stress inferences.

Robustness study. Validation of BISM with unknown boundary conditions. BISM has been validated primarily for confined systems where the boundary conditions are known (free boundary conditions \( \sigma \cdot n = 0 \), where \( n \) is the vector normal to the boundary)\(24\), but not yet in the case of unknown boundary conditions (applicable for our current study, as explained below). For the former case, the boundary conditions are incorporated in the prior, with a supplementary term (compare with equation (14)):
\[
\pi(\sigma) \propto \exp \left[ -\frac{|\sigma_{x y}|^{2} + \alpha^{2}|\sigma_{x y} - \sigma_{y x}|^{2} + \beta^{2}|\sigma_{BC}|^{2}}{2\sigma_{0}^{2}} \right]
\]

(18)
with a modified covariance matrix \( S_{BC} = \sigma_{0}^{2}B_{BC} \), where the three quadratic terms are embedded in \( B_{BC} \). The inferred stress is then obtained from equations (16) and (17) using \( S_{BC} \) instead of \( S_{0}^{2} \). BISM has been validated using numerical simulations that yield stress and traction force data sets, as well as using experimental data in a quasi-1D geometry\(24\). In particular, we showed that the absolute value of the stress could be inferred in confined systems. Further, the robustness of the method for different likelihood and prior distribution functions has also been tested. In particular, we checked that a non-Gaussian prior, a non-Gaussian likelihood, or a smoothness prior all lead to the same level of accuracy as the Gaussian model presented here.

Since the focus in this Letter is on extrusions and defects occurring in the bulk of the monolayer, we needed to perform stress inference away from physical boundaries, without knowledge of the correct boundary conditions applying to the epithelium patch that surrounds the extrusion. We now present an additional numerical validation for this case. Briefly, we used BISM on a larger and confined area using free boundary conditions in the prior (equation (18)), and compared the stress thus inferred in a smaller, central region to that obtained without imposing the boundary conditions in the prior (equation (14)), see Extended Data Fig. 8b–j. Good agreement between the two inferences showed that, except in the vicinity of the boundaries, BISM also infers the absolute value of the stress in the case where the boundary conditions are unknown.
Following previous work, we used a simplified, viscous rheology, neglecting orientational degrees of freedom, with the constitutive equation

$$\sigma = y(\nabla \cdot u) + (\nabla \cdot u')^2 + \gamma(\nabla \cdot u)I$$

(19)

and drove the fluid layer with active moving force dipoles. Including a dissipative interaction with the substrate through an effective fluid friction force, the traction force reads:

$$t = \xi u - f_{act}$$

(20)

The active force $f_{act}$ derives from the sum of $n_d$ dipole forces, $f_{act}(x) = \sum_{n=1}^{\infty} p_n^a(x)$, with a density of $10^{-2}$ dipoles per $\mu m^2$, such that for each dipole $n$, $p_n^a(x)$ is defined as:

$$p_n^a(x) = \frac{10^6 kPa \mu m}{\theta_n^2} D_n \delta(x-x_n)$$

(21)

with deviatoric angular matrices $D_n = \begin{pmatrix} \cos \theta_n & \sin \theta_n \\ -\sin \theta_n & \cos \theta_n \end{pmatrix}$, and amplitudes $p_n^a = 10^6 kPa \mu m$ and $p_n^\theta = 10^6 kPa \mu m$ for the trace and deviator respectively (numerical values taken from experiments). The dipole positions $x_n$ and orientations $\theta_n$ are random variables uniformly distributed over the spatial domain and $[0, \pi]$ respectively. The delta functions are implemented as finite-size Gaussian distributions, with a spatial extension $2d = 10 \mu m$ of the order of a typical cell size. We chose material parameter values typical for cell monolayers: shear viscosity $\eta = 10^{-3} kPa s$, shear viscosity $\gamma = 10^{-3} kPa s$ and compression viscosity $\sigma = 10^6 kPa \mu m^2$.

Using the finite element software FreeFem++, we solved the equations (equations (10), (19), (20)) for $u$ on a $300 \times 300 \mu m^2$ square with boundary conditions $\sigma = n = 0$. The stress $\sigma_{num}$ (Extended Data Fig. 8e, h) and traction force fields are derived from the velocity field and sampled over a regular Cartesian grid of spatial resolution $I = 2 \mu m$, giving $N = 50$ points for $100 \mu m$. To account for the measurement error, we add a white noise to the traction force field with an amplitude $\eta$ equal to 5% of the maximum traction error amplitude. We apply our algorithm to these numerical traction forces in two cases. (1) Whole monolayer of area $300 \times 300 \mu m^2$ with zero stress boundary conditions and prior (equation (18)). We obtain the stress $\sigma_{shad}$ with $3N \times 3N$ values for each component (Extended Data Fig. 8f, i). (2) Central part of the monolayer of area $100 \times 100 \mu m^2$; without boundary conditions and prior (equation (14)). We obtain the stress $\sigma_{central}$ with $N \times N$ values for each component (Extended Data Fig. 8g, j).

We compare the outcomes of two inferences with the exact stress field $\sigma_{num}$, and find excellent agreement between $\sigma_{num}$ and both $\sigma_{shad}$ (blue dots) and $\sigma_{central}$ (red dots, Extended Data Fig. 8b–d). Note that the inference is less accurate close to the border of the central part (black circles), yet allows us to obtain the correct absolute values of the stress in the bulk of the central domain with an accuracy equivalent to that of the whole system inversion.

Statistical analysis. No statistical methods were used to predetermine sample size. The sample size was chosen to see a statistical difference between data sets. In the case where no differences were observed (Fig. 4h), the sample size chosen was at least as big as those where differences were observed. Blinding was achieved when comparing results between experimental analysis, active nematic numerical simulations and Bayesian stress inference as these are done independently in three different institutes. All experimental data were tested with the Anderson–Darling test to check for normality of the distribution. In the case when we were comparing data which were normally distributed and have similar variance, the two-sided $t$-test was used (Figs. 2b, 3b, g, and Extended Data Figs 2d, 3e, 4a). Otherwise, the non-parametric Kolmogorov–Smirnov test ($\kappa$-test) was used. Throughout, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, apart from data in Fig. 3b and Extended Data Fig. 3e, where $*P < 0.0001$. All relevant statistics are reported in the corresponding legends.

Data and code availability. Source data and codes for cell orientation detection, active nematic simulations and Bayesian inference of tissue stress are available upon request.

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Extended Data Figure 1 | Further characterization of cell, monolayer and extrusion–defect correlation properties. a, b, Time evolution of cell aspect ratio (a) and cell area (b) in a confluent MDCK epithelium. Data for each time point are binned over a duration of 120 min. From lowest to highest time points, \( n = 5,101, 5,537, 5,772, 6,549, 6,572, 6,876, 6,593 \) and 6,831 cells. c, Time evolution of nematic measure (averaged local order parameter, \( S \)) of corresponding epithelium (Methods), \( n = 294 \) data points for each bar. d, MDCK monolayer in circular confinement (left). Red lines (represented again as black lines in middle) show local cell orientation, colour coded at right. Scale bar, 100 \( \mu m \). e, Number of closest +1/2 defects per unit area as function of \( r_e \) normalized such that area underneath the density curve sums to 1. \( n = 30 \) different random point sets. See Methods. f, Left, diagram of determination of correlation between −1/2 defects and extrusions: distance, \( r_e \), of each extrusion to its closest −1/2 defect in preceding frame is measured, and the number of these defects per unit area as function of \( r_e \) is normalized (right). See Methods. \( n = 50 \) (MDCK WT) extrusions from 4 independent movies in 3 independent experiments; \( n = 61 \) (MDCK, mytomycin-c) extrusions from 3 independent movies in 2 independent experiments; \( n = 85 \) (MCF10A) extrusions in 2 independent movies; \( n = 79 \) (HaCaT) extrusions in 2 independent movies. g, Similar to e, but is the normalized density curve between random points and −1/2 defects. h, A measure of correlation strength between extrusions and defects: ratio of density curve values at \( r_e = 10 \mu m \) to that at \( r_e = 120 \mu m \) (first and last points in respective curves, Fig. 1f and Extended Data Fig. 1f). i, Measure of correlation strength for eventual extrusion points and +1/2 defects as function of +1/2 defect distributions at each time point (WT-MDCK). All data represented as mean ± s.e.m.
Extended Data Figure 2 | Further examination of active nematic properties and the correlation of extrusion with defects in the epithelium under different conditions. a, Velocity field around +1/2 defect in contractile, active nematic liquid crystal simulation. Director configuration around the defect is same as Fig. 2a.b. Average velocity field around +1/2 defect for mytomycin C and blebbistatin treated MDCK. n = 2,003 (mytomycin C) defects from 3 independent movies in 2 independent experiments; n = 3,061 (blebbistatin) defects from 3 independent movies. +1/2 defect has same orientation and position as in Fig. 2a. c, Total defect areal density evolution in simulation, activity parameter decreased at simulation time t = 0, then increased at t = 5. d, Average total defect density for WT MDCK (‘Non-treated’) and blebbistatin (10μM and 50μM) treated MDCK. n = 314 frames from 4 independent movies in 3 independent experiments (WT); n = 155 frames from 3 independent movies (blebbistatin 10μM); n = 26 frames from 4 independent movies (blebbistatin, 50μM). t-test, ***P < 0.001. e, Left, number of closest +1/2 and −1/2 defects per unit area as function of distance, r_e, measured from respective extrusions (Ext, +1/2) and (Ext, −1/2), in 10μM blebbistatin treated monolayer. Similar curves also plotted for +1/2 and −1/2 defects measured from random points (Random, +1/2) and (Random, −1/2). Each density curve is normalized such that area under each curve sums to 1. n = 78 extrusions in 3 independent movies; n = 30 different random point sets. Right, ratio of density curve values at r_e = 10μm to r_e = 120μm for the curves (Ext, +1/2) and (Ext, −1/2) on the left. All data represented as mean ± s.e.m.
Extended Data Figure 3 | Further investigation of the relation between local cell density, compressive stress, defects and extrusions, and the role of caspase-3 activation. 

**a.** Number of closest high cell density spots per unit area as function of radius, $r$, measured from extrusion points in monolayer (Ext, High cell density). Similar curve plotted for high cell density spots measured from random points (Random, High cell density). $n = 50$ (MDCK WT) extrusions from 4 independent movies in 3 independent experiments; $n = 30$ different random point sets. 

**b.** Normalized number of closest defects per unit area as function of distance, $r_e$, against spots with top 5% of all compressive stresses in simulation domain. See Methods for calculation of simulation stress. 

**c.** Normalized number of closest extrusions per unit area as a function of distance against $\pm 1/2$ defects, grouped by magnitude of compressive stress at head regions of defects. Extrusions are within 40 min after the frame of defect. From lowest (least negative) to highest compressive isotropic stress (most negative), $n = 331$, 215, 180 and 72 defects in 2 independent experiments. All density curves in **a**, **b** and **c** are normalized such that area under the curve sums to 1. 

**d.** Typical example of extrusion event, showing configuration of $\pm 1/2$ defect before and after extrusion. The same neighbour cell before ($t < 0$ min) and after ($t > 0$ min) extrusion is outlined with same colour. 

**e.** Normalised closest extrusion area probability as function of radius (Lattice-Boltzmann units). 

**f.** Typical example of extrusion event with caspase-3 activation (yellow arrowhead) ($t = 0$ min). Top panel, nematic directors (red lines) overlaid on monolayer. Bottom panel, corresponding caspase-3 signalling. 

**g.** Average extrusion rate in non-drug-treated (ND) and caspase-3 inhibited MDCK. $n = 8$ (ND) independent movies in 3 independent experiments; $n = 7$ (caspase-3 inhibited) independent movies in 2 independent experiments. $**P < 0.001$. All data represented as mean ± s.e.m.
Extended Data Figure 4 | Order parameter and extrusion–defect correlation for α-catKD MDCK, and simulations comparing small and large nematic bending elasticity. a, Average local order parameter, $S$, for WT and α-catKD MDCK epithelium. $n = 3$ (WT) independent movies in 2 independent experiments; $n = 3$ (α-catKD) independent movies. $t$-test, ***$P < 0.001$. b, Left, number of closest $+1/2$ and $−1/2$ defects per unit area as a function of distance, $r_e$, measured from respective extrusions (Ext, $+1/2$) and (Ext, $−1/2$) in α-catKD MDCK. Similar curves plotted for $+1/2$ and $−1/2$ defects measured from random points (Random, $+1/2$) and (Random, $−1/2$). Each density curve is normalized such that area under each curve sums to 1. $n = 56$ extrusions in 3 independent movies; $n = 30$ different random point sets. Right, ratio of density curve values at $r_e = 10\, \mu m$ to $r_e = 120\, \mu m$, for the curves (Ext, $+1/2$) and (Ext, $−1/2$) on the left. c, d, Simulations comparing size of defects (c) and total defect areal density (d) for small and large nematic bending elasticity, $K$. $K$ is 0.02 and 0.08 for c, and 0.04 and 0.08 for d. All data represented as mean $\pm$ s.e.m.
Extended Data Figure 5 | Further analysis of topologically induced defects and extrusions. a, Maps of normalized number of $+\frac{1}{2}$ (left column) and $-\frac{1}{2}$ (right column) defects per unit area in star (top row) and circle (bottom row) epithelium confinements (Methods). $n = 6,738$ ($+\frac{1}{2}$) defects and $n = 5,083$ ($-\frac{1}{2}$) defects from 12 independent movies in 2 independent experiments (star); $n = 5,389$ ($+\frac{1}{2}$) defects and $n = 4,858$ ($-\frac{1}{2}$) defects from 8 independent movies in 3 independent experiments (circle). b, Top, number of closest $+\frac{1}{2}$ and $-\frac{1}{2}$ defects per unit area as function of distance, $r_e$, measured from respective extrusions (Ext, $+\frac{1}{2}$) and (Ext, $-\frac{1}{2}$) in star-shaped monolayer. Similar curves plotted for $+\frac{1}{2}$ and $-\frac{1}{2}$ defects measured from random points (Random, $+\frac{1}{2}$) and (Random, $-\frac{1}{2}$). Each density curve is normalized such that area under each curve sums to 1. $n = 145$ extrusions from 12 independent movies in 2 independent experiments; $n = 30$ different random point sets. Bottom, ratio of density curve values at $r_e = 10\mu m$ to $r_e = 120\mu m$, for the curves (Ext, $+\frac{1}{2}$) and (Ext, $-\frac{1}{2}$) on the top. c, Average velocity field around $+\frac{1}{2}$ defect in caspase-3 inhibited monolayer. $n = 2,993$ defects from 7 independent movies in 2 independent experiments. Defect has same orientation and position as in Fig. 2a. d, Isotropic stress measured around cells just before extrusion ($t = 0$ min is time of extrusion). >70\% (>30\%) of cells experienced negative (positive) stress, denoted as Group 1 (Group 2 respectively). $n = 44$ total number of extrusions in 2 independent movies. $\chi^2$-test, **$P < 0.001$. All data represented as mean ± s.e.m.
Extended Data Figure 6 | Automated nematic director detection and robustness study. a, 5 step process of automated nematic director detection. Step 1, phase contrast image of monolayer is obtained. Step 2, details of image are smoothed. Step 3, local orientation of cells are obtained using OrientationJ. Step 4, local contrast is applied to identify cell body regions. Step 5, nematic directors are obtained. b, Example of defect detection in a given nematic director field using the winding number approach (left, red for +1/2 defect, blue for −1/2 defect) and the diffusive charge approach (right, yellow for +1/2 defect, blue for −1/2 defect). c, Number of stable defects detected as a function of window size. A bigger window size allows the orientation of more cells to be averaged for the direction of a nematic. Averaging is done over \( n = 50 \) frames of a monolayer movie, for each window size analysis. \( ks \)-test, **\( P < 0.01 \), ***\( P < 0.001 \). Data are represented as mean ± s.e.m.
Extended Data Figure 7 | Epithelium can be modelled as a 2D incompressible material. a, Instantaneous (for every 10 min frame interval) velocity divergence field (colour coded) in circularly confined epithelium for one time point. b, Temporal average of velocity divergence field in circularly confined epithelium (averaged over about 20 h or 128 images consecutively).
Extended Data Figure 8 | Bayesian inference stress method (BISM) and robustness study. a, Diagram of inference algorithm. b–d, Plots of inferred stress versus simulated stress for each component, in kPa μm. Red line, bisector $y = x$; blue dots, $3N \times 3N$ stress, $\sigma_{\text{whole}}$ for the whole system; red dots, $N \times N$ stress, $\sigma_{\text{central}}$ for the central region; black circles, stresses obtained less than 2 μm from the boundary of the central region, $N = 50$ points. e–g, Pressure, and h–j, shear stress fields in kPa μm for the whole system: from left to right are shown exact values, $\sigma_{\text{num}}$; inferred values obtained for the whole monolayer, $\sigma_{\text{whole}}$; and inferred values obtained for the central region, $\sigma_{\text{central}}$. Black dashed box represents the central region.