Despite acting as a barrier for the organs they encase, epithelial cells turn over at some of the fastest rates in the body. However, epithelial cell division must be tightly linked to cell death to preserve barrier function and prevent tumour formation. How does the number of dying cells match those dividing to maintain constant numbers? When epithelial cells become too crowded, they activate the stretch-activated channel Piezo1 to trigger extrusion of cells that later die. However, it is unclear how epithelial cell division is controlled to balance cell death at the steady state. Here we show that mammalian epithelial cell division occurs in regions of low cell density where cells are stretched. By experimentally stretching epithelia, we find that mechanical stretch itself rapidly stimulates cell division through activation of the Piezo1 channel. To stimulate cell division, stretch triggers cells that are paused in early G2 phase to activate calcium-dependent phosphorylation of ERK1/2, thereby activating the cyclin B transcription that is necessary to drive cells into mitosis. Although both epithelial cell division and cell extrusion require Piezo1 at the steady state, the type of mechanical force controls the outcome: stretch induces cell division, whereas crowding induces extrusion. How Piezo1-dependent calcium transients activate two opposing processes may depend on where and how Piezo1 is activated, as it accumulates in different subcellular sites with increasing cell density. In sparse epithelial regions in which cells divide, Piezo1 localizes to the plasma membrane and cytoplasm, whereas in dense regions in which cells extrude, it forms large cytoplasmic aggregates. Because Piezo1 senses both mechanical crowding and stretch, it may act as a homeostatic sensor to control epithelial cell numbers, triggering extrusion and apoptosis in crowded regions and cell division in sparse regions.

To investigate how epithelial cell division is controlled at steady state, we seeded Madin–Darby canine kidney (MDCK) epithelial cells and measured the percentage of mitotic cells daily by immunostaining cells for phospho-histone H3 (H3P) (Extended Data Fig. 1a, b). Although epithelial cells never stop dividing, the rates of cell division reach a slow steady state by around day 5, at an average density of 11 cells per 1,000 μm², 3 days after reaching confluence (Extended Data Fig. 1a, asterisk). The approximately 7% mitotic rate at seeding slows to 0.7% at steady state, when most cells are in the G0/G1 stage of the cell cycle (Extended Data Fig. 1a–c and Supplementary Videos 1, 2).

How epithelial cells regulate cell division once they reach an optimal density is unclear. Although the overall cell division rates decrease as the monolayer reaches steady state, videos reveal that cells divide in sparse sub-regions of the epithelium (Supplementary Video 2). Furthermore, cell division occurs in zones that are consistently around 1.6-fold less dense than zones in which no division occurs, as quantified by cell lengths in dividing versus non-dividing regions in human colon crypts (1.6-fold), zebrafish epidermis (1.5-fold), and MDCK monolayers (1.7-fold) (Supplementary Videos 2, 3 and Extended Data Fig. 2).

These observations led us to wonder whether cell stretch due to low cell density could activate epithelial cell division. To test this hypothesis, we experimentally stretched MDCK cells at the steady state by either wounding or directly stretching cells in plates designed for uniaxial tension, and analysed mitotic rates at different times after stretch. Stretching cells 1.4-fold using a previously published device or a newly designed stretch device (Extended Data Fig. 3a) was sufficient to induce an approximately 5-fold increase in cell division within only 1 h (Extended Data Fig. 3b and Fig. 1a). Although the increased proliferation rate was low (1.3%), it returned cells to homeostatic densities, as measured by averaged cell lengths, within 4 h (Fig. 1b). Furthermore, scratching an MDCK monolayer caused cells to stretch 2.5-fold times their original length once cell migration ceased and triggered a wave of cell division (Fig. 1c, d and Supplementary Video 4, n = 6), similar to that seen previously. Cell division typically occurred within 1–2 h of wound closure, similar to the kinetics occurring after stretch.

To determine what regulates stretch-induced mitosis, we inhibited a variety of candidate proteins implicated in cellular stress or stretch responses. Gadolinium, a generic stretch-activated channel inhibitor, consistently blocked stretch-induced proliferation, compared to a generic inhibitor of mitosis, the CDK1 inhibitor roscovitine (Fig. 2a, Fig. 2a). Because Piezo1 is a stretch-activated channel required for crowding-induced, extrusion-dependent epithelial cell death, we tested whether it might also control stretch-dependent proliferation. Knockdown of Piezo1 by short interfering RNA (siRNA; or a short hairpin RNA (shRNA) Piezo1 construct, not shown) reduced Piezo1 to 7% of wild-type levels and prevented stretch-induced mitosis (Fig. 2a, b). Notably, long-term knockdown of Piezo1 also reduced steady-state rates of proliferation (Fig. 2a, unstretched). Transfection of a green fluorescent protein (GFP)-tagged Piezo1 construct not targeted by the siRNA rescued stretch-induced proliferation, indicating that proliferation after stretch requires Piezo1 (Fig. 2a, b). Piezo1 knockdown also blocked cell division after wound closure, in comparison to control monolayers (Fig. 2c). To test whether Piezo1 controlled epithelial cell division in vivo, we examined its role in zebrafish epidermis, a model for the simple epithelia that coat our organs. We assayed for H3P-positive cells in epidermis from 4-day-old zebrafish larvae, a stage at which cell division reaches a steady-state rate (Extended Data Fig. 4a and ref. 1). Using a CRISPR-based technique to knockdown Piezo1 mosaically in the F₀ generation, we found that reducing Piezo1 levels to approximately 50% decreased the number of H3P-positive epidermal cells by 5-fold (Fig. 2d–f). Furthermore, a photo-activated morpholino targeting Piezo1 also reduced epidermal cell proliferation by around 5-fold (Extended Data Fig. 4b–d). Together, our data show that epithelial cells at the steady state become stretched before they divide, that stretch is sufficient to activate cell division rapidly, and that cell division either at the steady state or after experimental stretch requires the stretch-activated channel Piezo1.

We next tested where in the cell cycle stretch activates Piezo1 to induce mitosis. Because the mitotic response to stretch is so rapid, it seemed unlikely that stretch-activation of Piezo1 triggers cells to transit through an entire cell cycle from the G0 phase (~12–24 h). Indeed, 2 h of stretch did not increase the number of cells entering S phase, as
measured by EdU incorporation (Extended Data Fig. 5a). Moreover, other reports find that stretch induces epithelial cells to enter the cell cycle but reach G1 after only 6 h\textsuperscript{[7–9]}. To determine whether stretch activates mitosis at G2 or M, we tested whether stretch stimulates cyclin B, which accumulates in the cytoplasm at G2. To prevent cyclin B degradation as cells shift into mitosis, we measured cytoplasmic cyclin B accumulation in the presence of the CDK1 inhibitor, roscovitine. We found that stretch induces cytoplasmic cyclin B accumulation over time (Fig. 3a and Extended Data Fig. 5b). Inhibiting Piezo1 with gadolinium or transcription with α-amanitin blocks stretch-induced cyclin B synthesis (Fig. 3a), suggesting that stretch-induced cyclin B induction requires both Piezo1 and transcription. However, because Piezo1 is a transmembrane protein that relays calcium in response to mechanical stress\textsuperscript{[3–11]}, it probably activates transcription indirectly. Inhibiting calcium with either ruthenium red or BAPTA-AM blocked stretch-induced mitosis and cyclin B production, respectively, confirming that Piezo1 acts through calcium to activate mitosis (Fig. 3b, c). Videos of MDCK monolayers expressing CMV-R-GECO1 (ref. 12) reveal that a single spark of calcium precedes mitosis entry (noted by physiological cell rounding) by 65 min (Extended Data Fig. 5b, c). Because both cyclin B and mitosis require Piezo1 and calcium influx (Fig. 3b, c), we next investigated what induces proliferation in response to calcium influx. A top candidate is the extracellular signal–regulated kinase (ERK1), a calcium-activated target of MEK1/2 (refs 13–15) with roles in controlling G2/M\textsuperscript{[16–18]}. We found that stretch-induced calcium activates ERK1/2 (measured by phospho-ERK1/2 immunoblot) within only 5 min, but is blocked by gadolinium addition (Fig. 3d). Using the MEK1/2 inhibitor AZD6244 (and U0126, not shown), we found that both stretch-induced mitosis and synthesis of cyclin B require the ERK1/2–MEK1/2 pathway (Fig. 3b, c). Thus, we propose that stretch causes Piezo1 to trigger calcium currents that activate ERK1/2 via MEK1/2, which triggers cyclin B transcription and initiate mitosis (Fig. 3e).

It is curious that one protein, Piezo1, can control two opposing processes—cell division and cell death—to regulate cell numbers. This

![Figure 1](https://example.com/figure1.png)

**Figure 1 | Mechanical stretch induces epithelial monolayers to rapidly divide.** a, b, Proliferation rates (a) and cell lengths (b) at various times after stretch show that stretch-induced cell divisions return cell densities to control (ctrl) levels. Values are means of 6 areas each from 3 experiments, error bars denote s.e.m. *P < 0.01, **P < 0.001, ***P < 0.0005, unpaired t-tests compared to control. c, Phase-contrast images showing cumulative where and when cells divide (red dots) during wound healing of an MDCK monolayer, with time in hours. Wound edge is highlighted by the white line. d, Cell divisions after monolayer wounding. Arrow denotes wound closure. Graph in d is representative of 14 experiments.

![Figure 2](https://example.com/figure2.png)

**Figure 2 | Stretch-induced mitosis requires the stretch-activated channel Piezo1.** a, Inhibition or siRNA-mediated knockdown (KD) of Piezo1 blocks stretch-induced mitosis, and is rescued by GFP–Piezo1. Values are means of 6 measurements each from 5 experiments. Gd\textsuperscript{3+}, gadolinium; rosco., roscovitine; scr. siRNA ctrl, scrambled siRNA control. b, Immunoblot confirming knockdown and rescue (Supplementary Fig. 1 for full blots). c, Piezo1 knockdown blocks cell division after wound closure. Graphs are representative of 20 similar experiments. d–f, CRISPR-mediated Piezo1 knockdown reduces Piezo1 mRNA by 50% (n = 3) (d) and markedly decreases mitosis rates (e, f), measured in 26 wild-type and 53 knockout 4-day-old zebrafish larvae. Scale bar, 100 μm. Images are representative of a total of 12 images for each condition. Error bars in a, d, and e denote s.e.m. **P < 0.005, ***P < 0.0005, ****P < 0.0001, unpaired t-test.
seems especially surprising, given that Piezo1 triggers opposing outcomes through the same second messenger: calcium. However, the type of mechanical tension is crucial for the type of response: we find that stretch induces cell division but not cell extrusion, whereas crowding induces cell extrusion but not cell division (Fig. 4a, b). Notably, the magnitude of mechanical strain for each response is similar: 1.5–1.7-fold stretch induces cell division but not cell extrusion, whereas crowding induces cell extrusion but not cell division (Fig. 4a, b). Notably, the magnitude of mechanical strain for each response is similar: 1.5–1.7-fold stretch induces cell division but not cell extrusion, whereas crowding induces cell extrusion but not cell division (Fig. 4a, b). Notably, the magnitude of mechanical strain for each response is similar: 1.5–1.7-fold stretch induces cell division but not cell extrusion, whereas crowding induces cell extrusion but not cell division (Fig. 4a, b).

controls opposing outcomes in other systems, but typically through secreted molecules. For instance, IL-2 maintains homeostatic T cell numbers by controlling both cell division and cell death depending on the levels secreted. Thus, Piezo1 signalling through calcium may represent a new mechanism for regulating a paradoxical feedback loop. While the benefit of having a single regulator control two opposing processes is that its loss should downregulate both processes, Piezo1 mutations and misexpression frequently found in colorectal, gastric and...
thyroid cancers suggest that Piezo1 may act as a tumour suppressor. Future work is needed to determine whether Piezo1 misregulation is sufficient to drive tumorigenesis, and if the relevant mutations affect both epithelial cell death and division.

We next sought to determine how Piezo1 differentially interprets crowding versus stretching. Cellular responses could depend on how the cell is primed, the levels and localization of Piezo1, as well as the type of tension. Our findings show that stretch-activation of Piezo1 target cells primed in early G2 to divide rapidly. It is not clear whether Piezo1 also collaborates with the transcriptional regulators Yap, Taz and β-catenin upon stretch to promote cell cycle entry at the G1 phase, although it is a tempting prospect, since tension-dependent nuclear translocation of Yap requires Piezo1 in neuronal stem cells. Older, crowded cells prone to extrusion may be post-mitotic, as typically seen in villus tips, and less likely to divide in response to calcium. The type of mechanical tension may also have a role in how Piezo1 signals. We find that while chemical activation of calcium currents is sufficient to activate cell division, it is not sufficient to promote cell extrusion and death (Fig. 4c, d). Thus, either thapsigargin or the calcium ionophore A23187 activates calcium at sites required for mitosis, or crowding-induced extrusion requires mechanical crowding in addition to calcium currents. Finally, the ability of Piezo1 to sense different types of tension may depend on its subcellular localization and levels. At subconfluence, Piezo1 levels are low, mainly localizing to the nuclear envelope, but accumulate over time. In confluent but sparser epithelial regions prone to divide, Piezo1 localizes to the plasma membrane and endoplasmic reticulum, whereas in crowded regions prone to extrude, Piezo1 develops into large cytoplasmic aggregates (Fig. 4e). Notably, although Piezo1 accumulates over time, stretching dense MDCK monolayers either mechanically or by wounding causes Piezo1 levels to drop markedly and eventually relocalize to the nucleus (Fig. 4f). At lower densities, when Piezo1 is predominantly at the nucleus, stretch does not appear to have a significant role in regulating mitosis (Extended Data Fig. 7b, c), potentially because the division rate is already very high. In this way, Piezo1 levels and localization could adjust to regulate cell numbers once cells reach a steady state: in sites where cells are sparse, Piezo1 localizes to sites where it may sense stretch, whereas in sites where cells are older and crowded, Piezo1 accumulates into structures where it may sense crowding (Extended Data Fig. 6b). Excessive cell death or wounding may reset the cell clock by degrading Piezo1 so that it would start measuring cell numbers only once cells reach the steady state again. Future studies will need to test whether Piezo1 abundance and localization control its response to different mechanical forces.

Our work has defined a role for mechanical tensions regulating constant epithelial cell numbers, essential for maintaining a tight barrier and preventing cancers from forming. We have found that Piezo1 acts to link the number of cells dying with those dividing by measuring tensions. If epithelial cells become too dense, crowding induces some cells to extrude and die, but if they become too sparse, stretch triggers some cells to divide rapidly to equilibrate epithelia to optimal densities.

**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

Cell culture and stretching assays. MDCK II cells (tested for mycoplasma but not authenticated) (gift from K. Matlin) were cultured in DMEM high glucose with 5% FBS (Atlas, Biologicals) and 100 μg/ml -penicillin/streptomycin (Invitrogen) at 5% CO2, 37°C. Cell stretching was done using a custom-designed Teflon chamber fabricated to culture cells on a flexible silicone membrane (2 x 2.4 cm, 0.5 mm thickness). Before cell seeding, 2 x 2.4 cm silicone membranes were stretched and coated with 5 μg/ml −fibronectin (Sigma) at 37°C for 1h. To stretch membranes with our device, one edge of the silicone membrane was clamped in place, while the other side was clamped to a movable shaft, which moves through a Teflon chamber with a sealing gasket. The movable shaft was pulled out between 2 and 6 mm, resulting in stretch of 10–33%. Approximately 2 x 106 cells were plated onto the 2 x 2.4 cm silicone matrices in a neutral taut state in the device and grown to confluence, then stretched to desired lengths with control chambers being left in a neutral state. Epithelial monolayers were fixed in stretched or neutral state and stained. As an alternative stretching method, 2 x 106 cells were grown to confluence in pronectin-coated flexcell plates designed for uniaxial stretch (Flexcell International Corporation), and stretched by clamping onto a custom-designed base plate (publicly available at https://www.shapeways.com/ as ‘Narrow Bump Base in white strong flexible nylon plastic’) using two ‘Clamp Bars in Stainless Steel’ (Shapeways). The clamp bars and base must be tapped with a 0.25 inch (6.35 mm)-20 tap to match steel 0.25 inch-20 fully threaded studs and rods and capped with knurled style thumbscrews (both Essenta Components).

Wounding assays and live cell imaging. Control MDCK II cells or those expressing a pTRIPZ piezo1 shRNA construct were grown to confluence in 24-well glass bottom dishes (MatTek) or 8-chamber slides (Labtec, Fisher Scientific). Cells were treated with 1.5 μg/ml −doxycycline (Sigma) for 48h to knockdown Piezo1. For wounding experiments, monolayers were scratched with a 10-μl pipet tip. Cells were imaged in complete DMEM supplemented with 10 mM HEPES (Invitrogen) on a Nikon TE2000 inverted microscope at 37°C or an EVOS FL Auto with a Stable Z controller heated stage (Bioptechs) at 37°C. Time-lapse videos were analysed using ImageJ.

For calcium imaging, MDCK cells were transfected with CMV-R-GECO1, a gift from R. Campbell (Addgene plasmid 32444)1 and imaged continuously (3=s intervals) for 4h. For cell cycle imaging, MDCK cells were transfected with pEGFP-PCNA-IRES-puro2b, a gift from D. Gerlich (Addgene plasmid 26461)1.

Drug treatments. The following drugs were used in experiments: 10 μM gadolinium (tri) chloride (for both zebrafish and cell culture) to block Piezo1, and 30 μM ruthenium red or 10 μM BAPTA-AM was used to inhibit calcium (all from Sigma); 10 μM roscovitine was used to block CDK1; 5 μM α-amanitin (Sigma) was used to inhibit RNA polymerase II and III; 1 μM AZD6244 (Cayman Chemical) or 1 μM U0126 (Sigma) was used to inhibit MEK1 and MEK2; 1 μM A23187 or 5 μM thapsigargin (both from Sigma) was used to induce calcium influx.

Cell immunostaining. Cells were fixed with 4% formaldehyde in PBS at room temperature for 20 min. Fixed cells were rinsed three times in PBS, permeabilized for 10 min with PBS containing 0.5% Triton X-100, and blocked in AB Dil PB5 (PBS with 0.1% Triton X-100 and 2% BSA), then incubated in primary antibody (in AB Dil) for 1 h, washed three times with PBS and 0.1% Triton X-100, and incubated in secondary antibodies. Antibody concentrations used for immunostaining were: 1:1,000 mouse phospho-histone H3 (S10) (Cell Signaling Technology); 1:1,000 rabbit histone H3 (phospho S10) (Abcam); 1:200 mouse -3-catenin (Cell Signaling Technology); 1:200 rabbit cyclin B1 XP (Cell Signaling Technology); 1:200 rabbit Piezo1 (Novus, NBPI-78466). Only this Piezo1 antibody reliably worked for Piezo1 immunostaining, as it vanishes in cells mosaically expressing a Piezo1-shRNA-mCherry construct (Extended Data Fig. 5a). Actin was detected using 0.1 μg/ml −Alexa Fluor 647−phalloidin (Invitrogen). Replicating DNA was detected using a Click-it Edu labelling kit, according to the manufacturer's protocol (Life Technologies). Alexa Fluor 488, 568 and 647 goat anti-mouse and anti-rabbit IgG were used as secondary antibodies (Invitrogen). DNA was detected with 1 μg/ml −DAPI in all fixed cell experiments.

Fluorescent microscopy. Images were captured on a Nikon Eclipse 90i using a x40 plan fluor 0.75 lens with a Retiga 2000R cooled mono 12-bit camera (Q Imaging) driven by NIS Elements (Nikon) or a Nikon Eclipse TE300 inverted microscope converted for spinning disc confocal microscopy (Andor Technologies) using a 20 x or 60 x plan fluor 0.95 oil lens with an electron-multiplied cooled CCD camera, 1,000 x 1,000, 8 x 8 mm2 driven by the IQ software (Andor Technologies). NIS Elements was used to quantify cell densities and measure cell length. Cell staining with cytoplasmic cyc1 and H3P was quantified per 10,000 cells using Nikon Elements Software.

FACScan. One million MDCK II cells were collected by trypsin treatment and rinsing twice with 2 ml PBS. The cell pellet was resuspended in 250 μl cold PBS and fixed for 2 h with 800 μl ice-cold 100% ethanol at −20°C. The tubes were warmed to 37°C for 10 min and cells were washed twice with 1 and 2 ml PBS. The cell pellet was resuspended in 500 μl propidium iodide (PI) staining solution (50 μg/ml −PI, 0.1% Triton-X, 0.2 mg/ml RNase A in PBS), vortexed and incubated for 20 min at 37°C. The samples were filtered and then analysed for propidium iodide fluorescence with a BD FACScan Analyzer.

Piezo1 knockdown and western blot analysis. Zebrafish (Danio rerio): For CRISPR-mediated knockdown of Piezo1, exon 2 of the zebrafish piezo1 gene was targeted for CRISPR knockdown and the target sequence was GTAGCCAAATATGCAGCAGtccagg (exononic sequence is uppercase, intronic sequence is lowercase). The 20 nucleotides minus the NGG motif were cloned into a plasmid containing the RNA loop structure required for recognition by the Cas9 enzyme and a T7-binding site, which allowed for the synthesis of single-guide RNA (sgRNA). To induce knockdown of Piezo1, 75 pg of sgRNA and 1,200 pg of Cas9-NLS encoding RNA were injected into the 1-cell-stage into wild-type (AB) embryos and mitosis rates were measured in mosaic F1 larvae at day 4 after fertilization. For Cas9-NLS mRNA synthesis, ptT3s-nCas9n was obtained from the Chen laboratory via Addgene (46757).

Piezo1 morphants were made by mixing 4 ng translation blocking morpholino 1:1 with a 25 bp antisense containing a photolinker and a 4 bp mismatch, and injecting into 1-cell-stage wild-type (AB) embryos. At 28–32 hours after fertilization, embryos were exposed to 350 nm light for 20s to activate the morpholino, then fixed and immunostained at 4 days post-fertilization. All experiments on zebrafish were done with accordance to our zebrafish IACUC protocol, approved by the University of Utah IACUC board.

MDCKs: siRNA to canine Piezo1 mRNA (from Sigma to the sense sequence GUUCUAGGUUCUGCUCCGAUDdTdT) was transfected with RNAiMAX (Thermoﬁsher) into MDCK cells 1 day after plating. For rescue, 1 μg GFP-Piezo1 construct (gift from A. Patapoutian) was transfected after 1 day after addition of siRNA. Alternatively, a pTRIPZ human PIEZO1 lentiviral plasmid (from Fisher Scientific) to the sense sequence GGUCUCCACACACAUCUA was transfected with psPAX and p60 (gift from M. VanBrocklin) into MDCK cells and selected for 14 days with 2 μg/ml −puromycin, and expression was induced by treating with 1.5 μg/ml −doxycycline (Sigma) for 3 days. Cell lysis was obtained by trypsinising cells, resuspending the pellet in NP40 cell lysis buffer containing 1× PMSF and protease inhibitors (Life Technologies), and centrifuging for 10 min at 16,873 g at 4°C. Then 30 μg of protein lystate (determined by Bradford assay) was run on a 3–8% Tris-acetate gel at 150 V for 1h, transferred to PVDF, and probed for Piezo1 (Proteintech) or mouse-anti-tubulin (Sigma) antibody by standard enhanced chemiluminescence.

Statistical analysis. No statistical methods were used to predetermine sample size. For statistical analysis, all experiments, including those in zebrafish, were repeated on three separate days to ensure enough variation and numbers to measure significance by unbiased t-tests. To ensure randomization of measurements, we took random pictures within the middle (region of consistent stretch or crowding) of silicone membrane for quantification. For zebrafish experiments, all fish were analysed, so not randomized or blinded. After noticing a trend in low-density epithelia not responding to stretch, we excluded them in our main data, but included them in Supplementary Fig. 4. Variance between compared samples was similar and expressed as s.e.m. in graphs.

Data availability. The data that support the findings of this study are available from the corresponding author on reasonable request; see author contributions for specific datasets. The source data for the western blots in the figures are available in Supplementary Fig. 1. 27. Held, M. et al. CellCognition: time-resolved phenotype annotation in high-throughput live cell imaging. Nat. Methods 7, 747–754 (2010).
Extended Data Figure 1 | Profile of epithelial cell proliferation and cell density over time. a, Daily proliferation rates of MDCK cells, as measured by H3P immunostaining, in which the asterisk indicates when confluence is reached (n = 3). Cell division slows but does not stop at day 5. b, Cell density plateaus by 5 days of growth when cells reach around 11 per 1,000 μm² (n = 3). All values are means of 10 fields of view from 3 independent experiments; error bars denote s.e.m. c, Cell cycle profiles by FACS at days 1 and 5 after plating at high density.
Extended Data Figure 2 | Epithelial cells divide in sparsest regions of epithelia. a–c, Density of cells in dividing versus non-dividing regions of epithelia, as measured by the cell length along the longest axis of the cell in four videos (except fixed colon sections) from human colon tissue (n = 8; a), developing zebrafish epidermis (n = 50; b), and MDCK cells in culture (n = 50; c). ****P < 0.0001, unpaired t-test. Error bars denote s.e.m.
Extended Data Figure 3 | Stretching steady-state monolayers. a, Left, new printable device used for stretching cells uniaxially on flexcell plates, unassembled (top) and assembled in the stretched state (bottom). Right, schematic of uniaxial stretch on an epithelium in the unassembled (top) and assembled (bottom) states (photo credit: J.R.). b, Immunostained MDCK monolayers before (top) and after (bottom) stretching. Images are representative of more than 200 captured images each. Scale bar, 10 μm.
Extended Data Figure 4 | Piezo1 morphants have reduced cell division in zebrafish epidermis at the steady state. a, Cell division in zebrafish reaches a low steady state at days 4–5 after fertilization. \( n = 50 \) fish each day; error bars denote s.e.m. b, Photo-activation of zebrafish injected with a Piezo1 translation-blocking morpholino (MO) results in knockdown of Piezo1 protein, as shown by an immunoblot. See Supplementary Fig. 1 for full blots. c, d, Zebrafish Piezo1 morphants have notably reduced epidermal mitoses at 5 days post-fertilization when cells homeostatic growth rate. Values in c are means from 3 separate experiments, error bars denote s.e.m. \(* * * * p < 0.005\), unpaired t-test. Each micrograph in d is representative of approximately 75 samples. Scale bars, 100 μm.
Extended Data Figure 5 | A single calcium spark occurs around 1 h before cells divide. a, Blocking stretch-induced proliferation with gadolinium (Gd³⁺) at 2 h after stretch does not affect the percentages of cells in the S phase, as measured by EdU incorporation. Data are mean and s.e.m. from 6 independent experiments. P > 0.05, t-test (compared to non-stretched control). b, Knockdown (KD) of Piezo1 with Gd³⁺ or inhibition of transcription with α-amanitin blocks stretch-induced cytoplasmic cyclin B accumulation and mitosis (H3P). Micrographs are representative of more than 100 samples, except for α-amanitin (40 samples). Scale bar, 10 μm. c, Quantification of time from calcium spark to cell rounding, measured from 10 mitotic events in 8 videos. Error bars denote s.e.m. d, Sample graph measuring the time (min) from calcium spark (arrow) to cell rounding (arrowhead) in MDCK cells expressing the calcium indicator CMV-R-GECO1 using Nikon Elements Software.
Extended Data Figure 6 | Models for how Piezo1 controls cell division in response to stretch, and cell extrusion in response to crowding.

a, Theoretical graph of density-dependent Piezo1 function for cell division and cell death. Epithelia trend to a steady-state density, $X$. If density is reduced, stretching causes Piezo1 to activate cell division; if density increases, crowding causes Piezo1 to activate cells to extrude and die.

b, Schematic showing how Piezo1 (green) localizes to the plasma membrane in sub-regions of the epithelia in which cells are sparser, and divides and accumulates into cytoplasmic aggregates in sub-regions in which cells are older, crowded, and prone to extrude.
Extended Data Figure 7 | Stretch causes cells at the steady state to enter mitosis. **A**, Characterization of the Piezo1 antibody using a Piezo1 shRNA construct tagged with mCherry indicates that cells expressing the mCherry shRNA construct (red) lack Piezo1 (green). Micrographs are representative of approximately 50 samples. Scale bar, 10 μm. **B**, High-density MDCK monolayers (more than 500 cells per 40× field) in which the intrinsic proliferation rate is low (less than 50 H3P-positive cells per 10,000 cells) proliferate in response to mechanical stretch (left) or wounding (right). Error bars denote s.e.m. ***P = 0.0007, unpaired t-test. **C**, Low-density MDCK monolayers just reaching confluence with a higher intrinsic rate of proliferation (more than 50 H3P-positive cells per 10,000 cells) do not significantly proliferate in response to stretch or wounding. *P > 0.05, t-test. n = 12 experiments for each case.