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Inhomogeneous mechanotransduction defines the spatial pattern of apoptosis-induced compensatory proliferation

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Summary

The number of cells in tissues is controlled by cell division and cell death, and its misregulation could lead to pathological conditions such as cancer. To maintain the cell numbers, a cell-elimination process called apoptosis also stimulates the proliferation of neighboring cells. This mechanism, apoptosis-induced compensatory proliferation, was originally described more than 40 years ago. While only a limited number of the neighboring cells need to divide to compensate for the apoptotic cell loss, the mechanisms that select cells to divide have remained elusive. Here we found that spatial inhomogeneity in Yes-associated protein (YAP)-mediated mechanotransduction in neighboring tissues determines the inhomogeneity of compensatory proliferation in Madin-Darby Canine Kidney (MDCK) cells. Such inhomogeneity arises from the non-uniform distribution of nuclear size, and the non-uniform pattern of mechanical force applied to neighboring cells. Our findings from a mechanical perspective provide additional insight into how tissues precisely maintain homeostasis.

Introduction

Apoptosis, or programmed cell death, is a mechanism by which unnecessary, aged, or damaged cells are eliminated¹. To maintain the homeostatic cell number in epithelium and organs, an apoptotic event can also induce proliferation of neighboring cells, a process termed apoptosis-induced compensatory proliferation, takes place. This phenomenon of compensatory proliferation was originally described more than 40 years ago². Haynie and Bryant investigated how *Drosophila* imaginal wing disc responds to damage induced by x-ray irradiation. Although irradiation of *Drosophila* larvae killed ~60% of the cells in the wing disc, the remaining cells were able to recover and develop as adult wings with normal structure and size. This suggested that there are cellular mechanisms that promote cell division upon tissue damage to compensate for the cell loss. Since then, the mechanisms of compensatory proliferation, especially the role of mitogenic signals secreted by the apoptotic cell, have been investigated^{3,4}. For instance, in *Drosophila*, it is well characterized that the secretion of Decapentaplegic (Dpp) and Wnt/Wingless (Wg) mitogens from the apoptotic cell results in cell proliferation in neighboring cells⁵⁻⁹. In mice¹⁰ and a mammalian cell line¹¹, the pro-inflammatory metabolite, Prostaglandin E2 (PGE2), is produced and secreted by the apoptotic cell and stimulates cell growth. Notably, it is sufficient for a limited number of the neighboring cells to divide to compensate for the cell loss due to apoptosis. This raises a possibility that biochemical signals secreted by the apoptotic cell, which could influence all the neighboring cells, are crucial, but may not be solely responsible, to explain this spatial inhomogeneity of compensatory proliferation. More recently, the apoptotic process, especially in the context of cell extrusion, has been associated with mechanical force. An apoptotic cell is expelled from a tissue through the contraction of an actomyosin cable formed in the dying cell as well as in the neighboring cells, or via lamellipodium crawling by adjacent cells¹²⁻¹⁶. This cell extrusion process further alters the surrounding tissue tension and morphogenesis¹⁷⁻²⁰. Here we considered the mechanical force associated with an apoptotic process and elucidate how only a small number of cells around an apoptotic cell undergo cell division. We found that the spatial inhomogeneity in mechanotransduction through the growth-promoting transcriptional co-activator Yes-associated protein (YAP) in the neighboring tissue is responsible for the inhomogeneity of compensatory proliferation. Such inhomogeneous mechanotransduction results from the combination of the non-uniform distribution of nuclear size, which is inherent in tissues, and the non-uniform pattern of

mechanical force applied to the neighboring cells upon apoptosis.

Results

Mechanical force propagation and stretching in the neighboring tissue upon apoptosis

To understand how an apoptotic event mechanically influences neighboring cells, we established an *in vitro* platform to quantitatively measure the changes in mechanical force among neighboring tissue upon cell death in Madin-Darby Canine Kidney (MDCK) epithelial cells (Fig. 1A). DNA damage and subsequent apoptotic cell extrusion (Fig. 1B) in the desired cell were induced by using a UV laser^{14,21} among a tissue that was cultured on deformable substrates made from elastic polydimethylsiloxane (PDMS) gel with fluorescent beads²². Upon the induction of apoptosis, a wave-like propagation of bead displacement, which is the proxy for substrate deformation, was observed (Movie 1). Particle image velocimetry (PIV) analysis of the movement of the fluorescent beads (Fig. 1C) showed that initially, the beads moved away from the apoptotic cell (red, $t=5\text{min}$ in Fig. 1C) and this displacement was largest at the nearest neighboring cells (Fig. 1H, Methods). In addition to the outward movement, the inward movement of the substrate emerged at the region close to the extrusion site (blue, Fig. 1C). To quantitatively understand the substrate deformation, we plotted the kymograph of the average radial velocity of the beads as a function of distance from the apoptotic cell (Fig. 1D, Methods). In-between the outwardly and inwardly moving substrate, there was a small region of substrate that showed transiently static behavior (i.e. no or balanced inward and outward movement), and this transiently static region shifted away from the extrusion site over time (white in Fig. 1D). We adopted this static region as one of the hallmarks of substrate deformation and found that the wave propagated a distance of $21.3\pm 2.3\mu\text{m}$ (mean \pm s.e.m.) away from the dead cells in an hour (Fig. 1G). Comparable wave-like propagation was observed upon spontaneous apoptotic cell extrusion without using UV laser induction (Fig. 3A, hereafter referred to as spontaneous extrusion). To understand the cause of the substrate deformation, we treated the tissue with the Rac1 inhibitor NSC23766 to inhibit lamellipodial cell crawling. Lamellipodia crawling is known to occur in neighboring cells and contributes to apoptotic cell extrusion^{14,16} and to deform the substrate to the direction opposite to the cell migration. Although we predicted that NSC23755 treatment would diminish substrate deformation upon apoptosis, we found that the wave propagation increased to a distance of $42.8\pm 2.4\mu\text{m}$ in an hour (Fig. 1E-G, Movie 1). We then

tracked the tissue dynamics by differential interference contrast (DIC) imaging and found that the cells moved away from the dead cell in both control and NSC23766-treated tissue (Fig. 1I-J, Movie 2). We reasoned that the release of epithelial tissue pre-tension, which is an inherent tissue characteristic generated by actomyosin-mediated contractility, leads to the relaxation and the outward movement of the tissue around the apoptotic cell. Indeed, we previously reported a reduction of the adherens junction molecule E-cadherin between apoptotic and neighboring cells after caspase-3 activation in the apoptotic cell in *Drosophila* epithelia¹³ and MDCK cells²³. This reduction of E-cadherin leads to disengagement of the cell-cell junction between apoptotic and neighboring cells, and a release of the tissue pre-tension¹³. To further validate our reasoning, we compared the tissue pre-tension between control and NSC23766-treated monolayer by laser ablation at the cell-cell junction²⁴ without induction of apoptosis. The junctional tension in the NSC23766-treated tissue is higher than that of the control tissue (Fig. S1A-C), which is consistent with the larger wave propagation in NSC23766-treated tissue. We further found that the relative position between the focal adhesions of the neighboring cells and the beads embedded in the substrate did not change drastically during wave propagation, indicating there is minimal sliding between tissue and the substrate during wave propagation (Fig. S1D). Together, our data reveal that the tissue moves away from the dead cell due to the relaxation of the tissue pre-tension upon apoptosis. The wave-like dynamics of the substrate gel caused by the release of the pre-tension of tissue upon apoptosis can be explained by a physical model based on a linear elasticity theory²⁵.

A kymograph of the radial average velocity of the beads showed that there was an inward movement of the substrate toward the apoptotic cell (blue in Fig. 1C-F). We speculate that this is, in part, due to the formation and the contraction of the actomyosin cable in the neighboring cells^{12,16,23}. The appearance of the inward movement emerged around 5~10min after the initial outward movement, consistent with the timing of formation and initiation of actomyosin cable contraction. These observations imply that the neighboring cells are stretched by apoptotic cell extrusion. Indeed, measuring the strain of the neighboring cells showed that the nearest neighbor cells underwent $16.2 \pm 2.3\%$ stretch, which is significantly larger than cells further away from the dead cell (Fig. 1K-L). Taken together, we conclude that the cells in the vicinity of the apoptotic cell experience a tensile stretching, which is due to the combination of tissue relaxation and apoptosis-associated actomyosin cable contraction.

A small number of neighboring cells show nuclear translocation of YAP and cell cycle progression upon apoptosis

To address whether this stretching of the neighboring tissue would further influence the biochemical signaling within the surrounding cells, we examined the dynamics of Yes-associated protein (YAP), a growth-promoting transcription co-activator and an effector of the Hippo pathway²⁶. YAP is also known as a mechanotransducer which transforms the physical stimuli that cells experience through mechanosensing into intracellular biochemical signals²⁷. Upon mechanical stimuli, YAP translocates into the nucleus, which further promotes downstream transcriptional programs including cell proliferation²⁸. We imaged the localization of YAP in the neighboring cells of an apoptotic cell by using MDCK cells that stably express YAP-GFP, which shows similar distribution as endogenous YAP (Methods, Fig. S2A). Only a fraction of neighboring cells exhibited nuclear translocation of YAP (Fig. 2A-B, Movie 3) as cells undergo stretching (Fig. S2B). Furthermore, the cells close to the apoptotic cell had a higher probability of YAP nuclear translocation, compared to cells further away from the dying cell (Fig. S2C). Similar YAP nuclear translocation was observed in response to spontaneous extrusion, and was associated with cell division (Fig. S2D-E). To further understand the consequence of apoptosis-associated YAP nuclear translocation, we imaged the cell cycle progression of the neighboring cells around spontaneous extrusion by using MDCK cells with the cell cycle reporter Fluorescent Ubiquitination-based Cell Cycle Indicator, FUCCI²⁹. The cells were first serum-starved for 24 hours to synchronize the cell cycle at G1 phase. Prior to imaging, serum-free media was replaced with fresh media containing serum. We found that only a few neighboring cells underwent cell cycle progression from G1 to S phase at around 6-7 hours after apoptosis (Fig. 2C-E, Movie 4). The cells close to the apoptotic cell (rows 1 and 2) had a higher probability of mitosis (Fig. 2F-G). Moreover, the probability (Fig. S2F) and the number (Fig. 2H) of mitosis increased at around 16-24 hours after apoptosis. Such characteristics are distinct from the cells irrelevant to and more than 2 cells away from apoptosis (Fig. 2G-H, S2F). Similar cell cycle progression was observed in response to UV-induced apoptosis (Fig. S2G). Together, our data consistently showed that only a few neighboring cells undergo cell division associated with YAP nuclear translocation upon apoptosis (Fig. 2, S2).

Spatial inhomogeneities in force propagation and nuclear size define which of the

neighboring cells undergo cell division following apoptosis

To uncover what defines this spatial inhomogeneity of cell division, we sought out factors that are not homogeneous in space. First, the substrate deformation as measured by bead displacement is spatially inhomogeneous (Fig. 1C, 3A). We noticed that cells that underwent cell cycle progression upon apoptosis spatially correlated to the region with large displacement (Fig. 3A), indicating cells that experienced a larger displacement and consequently underwent more stretching have a higher chance to undergo cell division. Second, the spatial distribution of the size of the nucleus is spatially inhomogeneous. We found that the cells that underwent cell cycle progression upon apoptosis tended to have a larger nucleus before the onset of apoptosis (Fig. 3B). To address whether these two spatially inhomogeneous factors, i.e., substrate deformation and nucleus size, are both required for cell proliferation, we simultaneously monitored bead displacement, nucleus size, and YAP localization. We found a case of 2 neighboring cells that both had a large nucleus (arrowhead and arrow, Fig. 3C), however, only the cell located in the region that underwent large bead displacement showed YAP nuclear translocation (arrowhead, Fig. 3C). To further solidify our observation, we analyzed more than 100 cells that were in the vicinity of UV-induced apoptotic cells (Fig. 3D). Only cells with a large nucleus that experienced large bead displacement showed clear YAP nuclear translocation (red in Fig. 3D, and S3A). In contrast, cells with a large nucleus that experienced small bead displacement, or any cells with a small nucleus, did not show clear YAP nuclear translocation (blue in Fig. 3D). In addition, by analyzing the relationship between strain (Fig. 1K), nucleus size, and cell cycle progression measured by FUCCI, we found that only the cells around spontaneous extrusion with a large nucleus that experienced large strain underwent cell division (red in Fig. 3E, and S3B). To rationalize how strain and nuclear size regulate YAP nuclear translocation and cell proliferation, we measured the number of nuclear pore complexes depending on nuclear size (Fig. 3F-G). The nuclear pore complex is a known structure that facilitates YAP transport from the cytoplasm to the nucleus in a tension-dependent manner³⁰. We found that the number of nuclear pores increases with the size of the nucleus (Fig. 3G, S3C), and speculated that the larger nuclear size helps nuclear translocation of YAP upon mechanical stretching. Together, we found that the spatially limited cell division around the apoptotic process is defined by the spatial inhomogeneity of pre-tension release (measured by bead displacement) which leads to the strain of a cell, and the spatial inhomogeneity of nuclear size.

To understand to what extent the difference in nuclear size before the onset of apoptosis (Fig. 3D-E) represented the intrinsic inhomogeneity of nuclear size among tissue, we measured the changes in nuclear size during cell cycle progression. The nucleus size typically increased $\sim 10\%$ during progression from G1 to G2 phase (Fig. S3D). Moreover, we observed a wide distribution in nuclear size within a tissue with a full width at half maximum (FWHM), which represents the spread of the distribution, of $50.4\mu\text{m}^2$ (Fig. S3E). We thus conclude that the difference in nuclear size between cells that divided or not (Fig. 3E, S3B) and between cells that showed YAP nuclear translocation or not (Fig. 3D, S3A) did not predominantly arise from the changes in the nuclear size during cell division, but from the intrinsic inhomogeneity of nuclear size among tissue.

Compensatory proliferation requires force propagation through the substrate

To understand to what extent the mechanical factors shown earlier play causal roles in compensatory proliferation in addition to mitogenic signaling from the apoptotic cell, which is known to contribute to compensatory proliferation^{3,4}, we aimed to modulate force propagation without altering the biochemical interaction between apoptotic and neighboring cells. To this end, we did not change the mechanical properties of the PDMS substrate, but altered the size of the tissue, or the friction between the gel substrate and the glass-bottom Petri dish. We speculated that tissue pre-tension decreases as tissue size reduces³¹, and first altered the size of the tissue by using microcontact printing technology (Methods). The size of the mini-tissue was reduced until the bead displacement diminished. A mini-tissue of 100 μm diameter (Fig. 4A) showed a reduced bead displacement (Fig. 4B-C, Movie 5), consistent with the reduction of junctional tension in the mini-tissue (Fig. S1A-C). Under this condition, the neighboring cells failed to translocate YAP into the nucleus (Fig. 4D-E, Movie 5) regardless of nucleus size (Fig. 4F) upon induction of apoptosis in a cell in the middle of a mini-tissue. Our theoretical model based on linear elasticity theory²⁵ predicted that the gel substrate with 50 μm thickness is weakly adhered to the surface of the glass-bottom Petri dish (Fig. 1A) and that increasing this adhesion would suppress the wave propagation. To test this prediction, the dish was silanized with 3-aminopropyl trimethoxysilane (APTES) to increase adhesion of the gel to the glass (Methods, Fig. 4G). We found that under this condition the bead displacement upon apoptosis was diminished (Fig. 4H-I) and YAP nuclear translocation in the neighboring cells was abolished regardless of nucleus size (Fig. 4J-L). In addition, cell

cycle progression in neighboring cells surrounding a spontaneous extrusion was also abolished with APTES treatment (Fig. 4M-N). Taken together, our data further support the idea that modulation of the mechanical status of the neighboring cells of the apoptotic cell through the substrate is required for the nuclear translocation of YAP, cell cycle progression, and cytokinesis of the neighboring cells.

Discussion

In this study, we addressed a long-standing question of how only a small number of cells around an apoptotic cell undergo cell division to sustain homeostasis of the cell number in a tissue (Fig. 4O). The spatial inhomogeneity of cell division in response to the apoptotic process is associated with inhomogeneous mechanotransduction, characterized by the nuclear translocation of YAP, and arises from the combination of the large strain and the large nuclear size of the neighboring cells, which is inherent in tissues. Suppression of mechanical stimulus without altering the cell-cell contact between apoptotic and neighboring cells resulted in a lack of mechanotransduction and cell division (Fig. 4A-N). These results highlighted that force propagation through the deformable substrate, which has been overlooked, plays a causal role in compensatory proliferation. Our theoretical model²⁵ suggested that the experimental condition where the gel substrate is weakly adhered to a glass-bottom dish (Fig. 1A) has mechanical features analogous to layers of tissue with viscoelasticity. We speculate that stratified epithelium, such as skin³², shares similar mechanotransduction mechanisms in compensatory proliferation. In contrast, compensatory proliferation may not be apparent with a substrate where apoptosis-associated force cannot be propagated, such as a glass substrate or the gel substrate that strongly adhered to a glass-bottom dish (Fig. 4G), which behaves as an elastic substrate and has been used for traction force microscopy.

One obvious question is whether the number of nuclear pore complexes is the only factor correlated with nuclear size and YAP nuclear translocation. Interestingly, it has been shown that large nuclei are much softer than small nuclei³³. It is possible that large nuclei are more deformable and therefore an increase in nuclear membrane curvature upon cell stretching promotes YAP nuclear translocation through the nuclear pore complex³⁰. Another key question is how biochemical signaling that emerges from the apoptotic process contributes to compensatory proliferation. Our data support the idea that mitogenic signals secreted without specific direction from the apoptotic cell are crucial as demonstrated

before⁵⁻¹¹, but not solely sufficient, to explain the spatially inhomogeneous nature of compensatory proliferation. However, we cannot rule out the possibility that there are additional mechanisms that could suppress cell division of neighboring cells next to the dividing and apoptotic cells. For instance, a paralog of YAP, a transcriptional coactivator with a PDZ-binding motif (TAZ), is shown to be excluded from the nucleus by extrinsic compression and can cause lateral inhibition in cell fate specification³⁴. Indeed, the neighboring cells that experienced a negative strain, i.e., compression, did not undergo cell division (Fig. 3E). Moreover, it will be interesting to investigate how extracellular-signal-regulated kinase (ERK), which is shown to act as a survival factor for neighboring cells^{35,36}, and calcium, which promotes cell extrusion³⁷, contributes to compensatory proliferation. Together, our findings from a mechanical perspective complement the current biochemical understanding of apoptosis-induced compensatory growth and provide additional insights into cellular functions of how tissue precisely maintains homeostasis.

Limitations of the study

Although we showed that the neighbouring cells with YAP nuclear translocation is associated with cell division (Fig. S2D-E), the mechanisms of how YAP nuclear translocation leads to cell cycle progression in the context of apoptosis-induced proliferation needs to be investigated further. Perturbing YAP activity or its nuclear transport would provide insight into this process. However, it is known that inhibiting YAP prevents cell division regardless of apoptosis³⁸, making it difficult to definitively assess its role in compensatory proliferation. Additionally, apoptosis-induced proliferation has been observed in a wide range of cancers, including breast cancer, melanoma, and glioma cells³⁹⁻⁴². It remains to be determined whether the mechanisms identified in this study are general to other context, including tumor pathology.

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Author Contributions

T.K., I.Y., and Y.T. designed the experiments. T.K., I.Y., Y.P., A.P.L., M.L., and X.T. performed the experiments. T.K., I.Y., Y.L., M.L., and M.S. analyzed the data. Y.L., J.P., T.H., and B.L. contributed quantitative analyses and theoretical interpretation. I.Y generated cell lines. T.K. and Y.T. wrote the manuscript. Y.T. oversaw the project. All authors discussed the results and commented on the manuscript.

Declaration of Interests

The authors declare no competing interests.

Main figure titles and legends

Fig 1. Mechanical force propagation and stretching in the neighboring tissue upon apoptosis. (A) Schematic of the experimental setup. A confluent MDCK monolayer (orange) is cultured on a Polydimethylsiloxane (PDMS) gel (green). An apoptotic cell is highlighted in gray. (B) Time-lapse images of an apoptotic cell extrusion within an MDCK monolayer. (C and E) Heat maps with vectors showing the direction and magnitude of bead radial velocity in control and NSC23766-treated tissues. The color bar indicates the magnitude of the outward (red) and inward (blue) velocity from the apoptotic cell in $\mu\text{m}/5 \text{ min}$. (D and F) Kymographs of the average radial velocity in C and E. (G) Average distance from the apoptotic cell to the static region, the white region between red (outward) and blue (inward), in D and F. Time 0 represents the onset of cell extrusion. Data are mean \pm s.e.m. $n = 5$ ROIs for both control and NSC23766. (H) Average bead displacement at different distances from the apoptotic cell (row number) at 1 min after apoptosis. Data are mean \pm s.d.; * $P < 0.05$ and *** $P < 0.001$, one-way ANOVA and Levene test followed by Tukey-Kramer test. $n = 7$ ROIs in 3 independent experiments. (I and J) Velocity vectors of tissue movement upon apoptosis in control and NSC23766-treated conditions. Red dots, apoptotic cells. (K) Diagram illustrating cellular strain (ϵ) for cells near and far (row1 and row4) from an apoptotic cell (blue). L_{t0} and L_{t30} represent the length of the cells at 0 and 30 minutes after apoptosis. (L) Strain of cells in row1 and row4 at 30 min after apoptosis. Data are mean \pm s.d.; *** $P < 0.001$, Mann-Whitney U -test. $n = 53$ cells for both row1 and row4 from 7 ROIs in 3 independent experiments. Scale bars, 20 μm in B; 50 μm in C, E, I, and J.

Fig. 2. A small number of neighboring cells show nuclear translocation of YAP and cell cycle progression upon apoptosis. (A) Time-lapse images showing YAP nuclear translocation (white arrowhead) within an MDCK YAP-GFP monolayer. Time 0 represents the onset of laser-induced apoptosis (asterisk). (B) Evolution of the nuclear/cytoplasmic (Nuc/Cyt) YAP-GFP ratio in cells surrounding an apoptotic cell. Cells were classified as having YAP translocation ($I_{\text{nuc/cyt}} \geq 0.5$, red) or no translocation ($I_{\text{nuc/cyt}} < 0.5$, blue) based on their Nuc/Cyt ratio at 2 hours after apoptosis. $n = 25$ ($I_{\text{nuc/cyt}} \geq 0.5$) and 17 ($I_{\text{nuc/cyt}} < 0.5$) cells in 10 ROIs from 6 independent experiments. Data are mean \pm s.d. (C) Time-lapse images showing cell cycle progression of a cell (white arrowhead) next to a spontaneous apoptosis (black arrowhead) within an MDCK FUCCI monolayer. (D) Representative normalized

fluorescence intensities of mKO2-Cdt1 (red) and mAG-hGem (green) in a cell shown in C (white arrowhead). Dotted lines indicate the time points of cell-cycle phase transition. (E) Distribution of the time of G1-S phase transition in cells surrounding the apoptotic cell. Data are mean \pm s.d. n = 114 cells from 54 ROIs in 6 independent experiments. (F) Illustration of cells in each cell row (rows 1-4) surrounding a live or apoptotic cell. (G) Probability of cell division for each row of cells within 24 hours after extrusion. Cells that underwent the G1-S phase transition prior to extrusion or arbitrary defined time 0 were excluded from the analysis for apoptosis or no cell death case, respectively. Data are mean \pm s.d.; not significant (ns), *P < 0.05 and **P < 0.01, Mann-Whitney *U*-test. (H) Distribution of the time of cell division at each row of cells around control (no cell death) and apoptotic cells. Time 0 represents the time of spontaneous apoptosis. Data shown in G and H are from n = 54 ROIs in 6 independent experiments for both control and apoptosis. Scale bars, 20 μ m in A and C. See also Figure S2.

Fig. 3. Inhomogeneous cell division arises from the combination of spatial inhomogeneities in force propagation and nuclear size. (A and B) Time-lapse images showing an MDCK FUCCI monolayer before (left) and after (right) a spontaneous apoptosis (asterisk), a heat map with vectors of bead displacement at 1 min after extrusion (center in A), and a heat map of the nuclear area at 10 min before extrusion (center in B). A small number of neighboring cells (white arrowhead) show cell-cycle progression. (C) Time-lapse images of MDCK YAP-GFP monolayer before (left) and after (right) laser-induced apoptosis (asterisk), and a heat map of bead displacement and nuclear area (middle). A cell associated with a large nucleus and large bead displacement shows YAP nuclear translocation (white arrowhead), but a cell associated with a large nucleus and small displacement does not (white arrow). (D) A scatter plot showing how cells with (red) or without (blue) YAP nuclear translocation correlate with nuclear area and average bead displacement. Nuclear area and bead displacement were measured at 1 min before and after apoptosis. YAP translocation was measured 2 hours after apoptosis. n = 115 cells in 36 ROIs from 9 independent experiments. (E) A scatter plot showing how dividing (red) and non-dividing (blue) MDCK FUCCI cells correlate with the nuclear area and the strain experienced by the cells. Strain was measured at 30 min after spontaneous apoptosis. n = 103 cells in 16 ROIs from 6 independent experiments. (F) Immunostaining for nuclear pore complex of a WT MDCK cell. (G) Graph

showing the total number of nuclear pore complexes as a function of nuclear volume. $n = 45$ cells in 2 independent experiments. Scale bar, $20\mu\text{m}$ in A-C; $10\mu\text{m}$ in F. See also Figure S3.

Fig. 4. Compensatory proliferation requires force propagation through the substrate.

(A-F) Experiments using mini-tissues to reduce force propagation. (A) MDCK YAP-GFP cells (right) confined to a circular pattern of $100\mu\text{m}$ diameter fibronectin (left). (B) A heat map with vectors showing the bead displacement at 1 min after laser-induced apoptosis. (C) Average bead displacement at different distances from the apoptotic cell (row number) at 1 min after apoptosis. Data are $\text{mean}\pm\text{s.d}$, one-way ANOVA, and Levene test followed by Tukey-Kramer test. $n = 5$ ROIs in 5 independent experiments. (D) Time-lapse images of MDCK YAP-GFP monolayer before and 2 hours after laser-induced apoptosis. (E) Evolution of the nuclear/cytosolic (Nuc/Cyt) YAP-GFP ratio in cells surrounding an apoptotic cell. Data are $\text{mean}\pm\text{s.d}$. $n = 20$ cells in 6 ROIs from 6 independent experiments. (F) A scatter plot showing how cells without YAP nuclear translocation (blue) correlate with nuclear area and average bead displacement. Nuclear area and bead displacement were measured at 1 min before and after apoptosis. $n = 29$ cells in 6 ROIs from 6 independent experiments. Same classifications of YAP translocation are used as Fig. 3D. (G-N) Experiments with the substrate that strongly adhering to a glass-bottom dish to reduce force propagation. (G) Schematic illustration of the experimental setup with an APTES-coated adhesive glass-bottom dish. (H) A heat map with vectors showing the bead displacement at 1 min after laser-induced apoptosis. (I) Average bead displacement at different distances from the apoptotic cell (row number) at 1 min after apoptosis. Data are $\text{mean}\pm\text{s.d}$, one-way ANOVA, and Levene test followed by Tukey-Kramer test. $n = 6$ ROIs in 6 independent experiments. (J) Time-lapse images showing MDCK YAP-GFP monolayer before and after laser-induced apoptosis (asterisk). (K) Evolution of YAP-GFP ratio in cells surrounding an apoptotic cell. Data are $\text{mean}\pm\text{s.d}$. $n = 23$ cells in 4 ROIs from 4 independent experiments. (L) A scatter plot showing how cells without YAP nuclear translocation (blue) correlate with nuclear area and bead displacement. Nuclear area and bead displacement were measured at 1 min before and after apoptosis. $n = 35$ cells in 5 ROIs from 5 independent experiments. (M) Time-lapse images of MDCK FUCCI cells surrounding a spontaneous apoptotic cell (white arrowhead). The cyan dashed line represents the boundary between row2 and row3 from the apoptotic cell. (N) Probability of cell division within 24 hours after spontaneous cell extrusion. Data are

from the neighboring cells associated (gray) and not associated (white) with apoptosis on different substrates, i.e., less-adhesive (Fig. 1A) and APTES-coated adhesive substrates (Fig. 4G). Data are mean \pm s.d., *P < 0.05 and ***P < 0.001, Kruskal-Wallis rank-sum test followed by Steel-Dwass test. n = 54 ROIs in 6 independent experiments (each case with uncoated dish), n = 54 ROIs in 4 independent experiments (each case with APTES-coated dish). (O) A model of apoptosis-induced compensatory proliferation. Scale bars, 20 μ m.

STAR★Methods

Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse Anti-mAb414 antibody	BioLegend	CAT# 902907, RRID:AB_2734672
Mouse Anti-YAP antibody	Novus biologicals	CAT# H00010413-M01, RRID:AB_549187
Chicken Anti-GFP antibody	Aves Labs	CAT# GFP-1020, RRID:AB_10000240
Goat Alexa Fluor 488 anti-mouse IgG (H+L) antibody	Invitrogen	CAT# A-11001, RRID: AB_2534069
Goat Alexa Fluor 568 anti-mouse IgG (H+L) antibody	Invitrogen	CAT# A-11004, RRID: AB_2534072
Goat Alexa Fluor 488 anti-Chicken IgY (H+L) antibody	Invitrogen	CAT# A-11039, RRID: AB_2534096
Chemicals, peptides, and recombinant proteins		
TurboFectin 8.0	OriGene Technologies	CAT# TF81005
Puromycin	Gibco	CAT# A1113803
Penicillin-Streptomycin	Gibco	CAT# 15140
Fibronectin	Gibco	CAT# 33016015
NSC23766 Trihydrochloride	Sigma-Aldrich	CAT# SML0952
CY52-276A	Dow Corning	CAT# 2624028
CY52-276B	Dow Corning	CAT# 2624052
3-aminopropyl trimethoxysilane (APTES)	Sigma-Aldrich	CAT# 440140
Polydimethylsiloxane, PDMS	Dow Corning	Sylgard 184
Hoechst 33342	Invitrogen	CAT# H3570
Carboxylated red fluorescent beads	Molecular Probes	CAT# F8801
Carboxylated dark-red fluorescent beads	Molecular Probes	CAT# F8807
Experimental models: Cell lines		
Dog: MDCK	James W. Nelson lab, Stanford University	RRDI: CVCL_0422
Dog: MDCK E-cadherin-GFP	James W. Nelson lab, Stanford University	N/A
Dog: MDCK FUCCI	Lars Hufnagel lab, European Molecular Biology Laboratory	N/A
Dog: MDCK YAP-GFP	This study	N/A
Dog: MDCK Paxillin-GFP	This study	N/A
Recombinant DNA		
cDNA YAP1	OriGene Technologies	NM_001195044
pLenti-C-mGFP-P2A-Puro	OriGene Technologies	CAT# PS100093
pLenti-C-YAP1-mGFP-P2A-Puro	This study	N/A
cDNA Paxillin	OriGene Technologies	NM_002859
pLenti-C-Paxillin-mGFP-P2A-Puro	This study	N/A
Software and algorithms		
Fiji	Schindelin et al. ⁴³	RRID:SCR_002285

TrackMate, Fiji plug-in	Tinevez et al. ⁴⁶	https://imagej.net/imagej-wiki-static/TrackMate
MTrackJ, Fiji plug-in	Meijering et al. ⁴⁸	https://imagescience.org/meijering/software/mtrackj/
StarDist, Fiji plug-in	Carpenter et al. ⁴⁹	https://imagej.net/plugins/stardist
R	R-project	RRID:SCR_001905
RStudio	RStudio	RRID:SCR_000432
MATLAB	MathWorks	RRID:SCR_001622
PIVlab, MATLAB code	Thielicke & Stamhuis,	https://jp.mathworks.co
Recoil velocity, customized MATLAB code	Hara et al. ²⁴	Yusuke Toyama Lab
Other		
Nikon BioStation IM-Q	Nikon	BioStation IM-Q
NikonA1R MP laser scanning confocal microscope	Nikon	Nikon A1R
Nikon Eclipse Ti-E Inverted microscope	Nikon	Nikon Eclipse Ti-E

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources should be directed to and will be fulfilled by the Lead Contact, Yusuke Toyama (dbsty@nus.edu.sg).

Materials Availability

Cell lines generated for this study are available on request to the corresponding authors. This study did not generate new unique reagents.

Data and Code Availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Cell lines

Madin-Darby canine kidney strain II wild type (MDCK WT, *Canis familiaris*, female) and MDCK E-cadherin-GFP were kindly provided by James W. Nelson (Stanford University). The MDCK FUCCI stable cell line was kindly provided by Lars Hufnagel (European Molecular Biology Laboratory). FUCCI, a cell cycle indicator, effectively labels individual nuclei in G1 phase red (mKO2-hCdt1) and those in S/G2/M phase green (mAG-hGeminin)²⁹. Yes-associated protein (YAP) is known to respond to mechanical signals and act as a growth-promoting cofactor^{26,30}. To monitor YAP localization in cells, we constructed MDCK YAP-GFP cells which stably expressing a YAP1-mGFP fusion protein. cDNA encoding YAP1 (NM_001195044) was cloned into a third-generation lentiviral plasmid, pLenti-C-mGFP-P2A-Puro (OriGene Technologies). To produce lentivirus encoding for YAP1-mGFP, HEK293T cells were transfected with pLenti-C-YAP1-mGFP-P2A-Puro using TurboFectin 8.0 (OriGene Technologies). Viral particles were concentrated, and lentiviral transduction was performed on MDCK WT cells using lentiviral packaging plasmids (OriGene Technologies). MDCK YAP-GFP cell clones were selected with puromycin (Gibco) and then sorted by FACS. To monitor focal adhesion, we constructed MDCK paxillin-GFP cells using paxillin (NM_002859) human-tagged ORF clone lentiviral particles (pLenti-C-Paxillin-mGFP-P2A-Puro) with the same procedure as the construction of MDCK YAP-GFP cells. The distribution of YAP-GFP signal was similar to that of endogenous YAP (Fig. S2A).

METHOD DETAILS

Antibodies

The following primary and secondary antibodies were used for immunocytochemistry: mouse mAb414 antibody (1:1000, BioLegend); mouse anti-YAP antibody (1:250, Novus biologicals, Littleton, CO); chicken anti-GFP antibody (1:1000, Aves Labs); goat Alexa Fluor 488 anti-mouse IgG (H+L) antibody (1:1000, Invitrogen); goat Alexa Fluor 568 anti-mouse IgG (H+L) antibody (1:1000, Invitrogen); and Goat Alexa Fluor 488 anti-chicken IgY (H+L) antibody (1:1000, Invitrogen).

Cell culture and drug treatment

MDCK cell lines were maintained in Dulbecco's modified eagle medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco), and 100 units/ml penicillin-streptomycin (Gibco), and kept in a 37°C humidified incubator containing 5% CO₂. Cells ($1-2 \times 10^5$) were seeded onto a silicone gel substrate coated with pure fibronectin (20 µg/ml, Gibco). To arrest the cell cycle and synchronize cells at G0/G1 phase, cells were serum-starved for 24 hours. Before microscopic imaging, serum-free media was replaced with fresh DMEM containing 10% FBS. To inhibit Rac1, MDCK monolayers were treated with NSC-23766 (200 µM, Sigma-Aldrich) in culture media for 30 min prior to imaging.

Microfabrication of silicone gel substrate

Soft Polydimethylsiloxane (PDMS) gel was used as the substrate in all experiments. To prepare the substrate with a stiffness of 10–20 kPa Young's modulus, CY52-276A and CY52-276B components (Dow Corning) were mixed at 1:1 (wt/wt) ratio. The mixed gel was spin-coated onto the glass-bottom Petri dishes (IWAKI) at 1000 rpm for 1 min and then cured at 80 °C for 2 hours. The flat gel thickness was 50µm (Fig. 1A).

Substrate–glass adhesion

For weaker adhesion between the silicone gel substrate and glass-bottom Petri dish (IWAKI), soft silicone elastomer substrate (CY52-276A/B mixture, 1:1 [wt/wt]) was spin-coated (1000 rpm, 1 min) on a dish without a plasma treatment, which is a procedure for traction force microscopy. For stronger adhesion, 3-aminopropyl trimethoxysilane (APTES) was used as a chemical crosslinking agent was used (Fig. 4G). The dish was silanized with 5% APTES (Sigma-Aldrich) in ethanol for 30 min, rinsed with ethanol, and cured at 80 °C for 60 min. The soft silicone elastomer substrate (CY52-276A/B blended, 1:1 [wt/wt]) was then spin-coated (1000 rpm, 1 min) onto an APTES-coated dish.

Microcontact printing

To confine the cells within a circular pattern (100 µmΦ) on the substrate, we used a micro-contact printing technique¹⁶. Silicon wafers with circular patterns were fabricated using SU-8 photoresist for soft lithography. To form stamps, PDMS (Sylgard 184, Dow Corning) with a crosslinker to silicone elastomer of 1:10 ratio (wt/wt) ratio was poured onto the wafer,

degassed, and then cured at 80°C for 2 hours. The stamps were coated with a mixture of 50 µg/mL pure fibronectin (Roche) and 25 µg/mL Cy5-conjugated fibronectin (Cy5-FN, GE Healthcare) for 1 hour. The pattern of interest was first stamped onto a Polyvinyl Alcohol membrane prepared from 5% PVA solution (Sigma), and the membrane was inverted onto the soft gel substrate (CY52-276A/B mixture, 1:1 [wt/wt]). The membrane was dissolved, and the non-patterned areas were passivated by incubation with 2% Pluronic for 2 hours. Finally, cells were seeded onto the substrate as described above. Circularly patterned mini-tissues were synchronized at G0/G1 phase and imaged using a NikonA1R MP laser scanning confocal microscope.

Laser induction of apoptosis

Laser-based apoptosis induction was performed using NikonA1R MP laser scanning confocal microscope with a Nikon Apo 60x/1.40 oil-immersion objective¹⁴. To induce DNA damage for a single cell, a UV laser (355 nm, 300 ps pulse duration, 1 kHz repetition rate, PowerChip PNV-0150-100, team photonics) with a laser power of 25-50 nW at the back aperture of the objective was focused on the nucleus for 3-5 s²³. The onset of apoptotic extrusion, defined by cell shrinkage and nuclear condensation, was observed within 5-10 min following laser irradiation¹⁶. The nucleus was stained with Hoechst 33342 (1 µg/ml, Invitrogen) in culture medium for 1 hour and washed before the laser induction experiment.

Traction force microscopy (TFM)

Traction force microscopy is a non-invasive technique that measures the local substrate deformations by imaging the displacements of fluorescent beads on the surface of soft elastic gel⁴⁴. The substrate spin-coated Petri dish was silanized with 5% APTES in ethanol for 15 min, rinsed with ethanol, and cured at 80 °C for 60 minutes. Carboxylated red or dark-red fluorescent beads (Molecular Probes) were functionalized on the substrate at a 1:500 dilution in distilled water for 10 min. The beads were then passivated with Tris (100mM, Sigma) for 30 min and washed with distilled water. Pure fibronectin (20 µg/ml) was incubated on the substrate at 37 °C for 1 hour prior to cell seeding. Cells and beads were imaged using a NikonA1R MP laser scanning confocal microscope or Nikon BioStation IM-Q. Images were taken every 1 or 5 minutes with z step = 0.75 µm.

Laser ablation

For junctional ablation, we used MDCK E-cadherin-GFP cells seeded on the silicone gel substrate overlaid on the glass bottom dish. The laser ablation experiment was performed on a NikonA1R MP laser scanning confocal microscope with Nikon Apo 60x/1.40 oil-immersion objective^{16,24}. A UV-laser (355 nm, 300 ps pulse duration, 1 kHz repetition rate, PowerChip PNV-0150-100, team photonics) was focused on the cell-cell junction of the target cell for 0.1 sec at a laser power of 200 nW at the back aperture of the objective (Fig. S1A). Time-lapse image series were started 10 sec before the ablation and acquired every 1 sec.

Immunocytochemistry

MDCK cells were fixed with 4% paraformaldehyde for 10 minutes at room temperature, permeabilized and blocked with 0.1% Triton X-100 in 3% BSA/PBS for 30 minutes. The nuclear pore complex (NPC) was stained with the monoclonal antibody mAb414 (1:1000, BioLegend) which is widely used to detect functionally mature NPCs⁴⁵. MDCK YAP-GFP cells were treated with anti-YAP (H00010413-M01, 1:250, Novus biologicals, Littleton, CO) and anti-GFP antibodies (GFP-1020, 1:1000, Aves Labs). Cells were washed with PBS and incubated with secondary antibodies containing 0.1% Triton X-100 in 3% BSA/PBS for 1 hour at room temperature. The nucleus was labeled with Hoechst 33342 (1 μ g/ml). Confocal images were acquired using a W1 spinning disk confocal microscope.

Microscopy imaging

Prior to imaging, all MDCK monolayer tissues were synchronized at G0/G1 phase, and then fresh media containing serum was added. Cell density was kept at 60-70 cells per 100 x 100 μ m² for imaging. Phase-contrast and fluorescence time-lapse imaging for the MDCK FUCCI monolayer was performed on a Nikon BioStation IM-Q with a 20x objective. For confocal time-lapse imaging and TFM, the nucleus was labeled with Hoechst 33342 prior to imaging, and then images were acquired using a NikonA1R MP laser scanning confocal microscope with a Nikon Apo 60x/1.40 oil-immersion objective under the control of NIS-Elements with a 0.75- μ m interval-z scanning setting. Confocal fluorescence imaging for fixed MDCK monolayer tissues was performed using a Nikon Eclipse Ti-E inverted microscope equipped with a W1 spinning disk and a 100x/1.45 oil-immersion objective under the control of

MetaMorph with a 0.5- μm interval-z scanning setting.

QUANTIFICATION AND STATISTICAL ANALYSIS

Analysis of cell-cycle progression and cell division

To analyze the history of cells around an apoptotic cell within the MDCK-FUCCI monolayer, we recorded apoptotic events and individual cell-cycle progression and cell divisions within the region of interest (ROI, 160 x 160 μm) containing a single extruding cell in the center. For control, ROIs without extruding cells were randomly selected from images that were used for selecting ROIs with apoptosis (Fig. 2F). To quantify the cell-cycle progression of MDCK FUCCI cells, we performed automatic cell tracking and measurement of mKO1 and mAG fluorescence intensities using a Fiji plug-in TrackMate⁴⁶, and obtained individual mean fluorescence intensity profiles. The timings of cell-cycle phase transition (G1-S, S-G2, and G2-M phase) were automatically calculated from the fluorescence intensity profiles using an R package 'segmented'. Briefly, mKO1 and mAG fluorescence intensity were plotted against time and then fitted to the piecewise regression models. Breakpoints where the regression lines intersected in each fluorescence were defined as the transition time points. The first and second breakpoints in mAG were defined as G1-S and G2-M phase transitions, respectively. The second breakpoint in mKO1 was defined as S-G2 phase transition. To calculate the probability of cell division in each row, the number of cell divisions was divided by the total number of cells in each row.

Measurement of bead displacement

Bead displacement in the TFM experiment was calculated by particle image velocimetry (PIV) analysis using an open-source MATLAB code PIVlab⁴⁷. The setting, three passes (64x64, 32x32, and 16x16 pixel size interrogation window with 50% overlap each), was used. The radial displacement of the beads was calculated using a custom MATLAB code (Fig. 1C-F). To create kymographs for the average radial velocity of beads, the radial displacement of beads was averaged at each radius (0 to 73 μm , every 1.66 μm) from the center of the apoptotic cell¹⁴. The extent of mechanical propagation is estimated by the static region, which is the boundary between the radial outward and inward displacements of the beads. The

average distance between the static region and the center of the apoptotic cell was calculated from the data set prepared to calculate the average radial velocity of beads, and then we plotted it every 5 minutes in Fig. 1G. The average bead displacement for each cell was calculated from 25 measurement points (5x5) within a ROI (13.12x13.12 μm) with a nuclear centroid nearly in the center (note that the nuclear diameter was estimated to be $10.73 \pm 1.452 \mu\text{m}$ [mean \pm s.d.], $n = 6719$ cells).

Quantification of cellular deformation (strain)

To evaluate the deformation of cells surrounding an apoptotic cell, we calculated cell strain (ϵ) by dividing the change in cell length ($L_t - L_{t0}$) by the initial length (L_{t0}). To measure the cell length, we manually traced the outline of the apoptotic and the target cells from the phase images, and then the centroid of the cell was determined using the 'Analyze Particles...' function in Fiji. Finally, the cell length of the target cell was measured along a line connecting their centroids.

Recoil velocity

In order to evaluate the tissue pre-tension, we calculated the recoil velocity after laser ablation at the cell-cell junction. The coordinates (i.e., the axes (x, y)) of two junctional nodes were tracked using the Fiji plug-in MTrackJ⁴⁸ (Fig. S1A). The recoil velocity was then calculated as the derivative of the double exponential function using a custom MATLAB code²⁴.

Measurement of YAP-GFP fluorescence intensity

To quantify the distribution of YAP in cells surrounding an apoptotic cell, we used MDCK YAP-GFP cells and measured nuclear and cytoplasmic YAP-GFP fluorescence intensity. Single nuclei labeled with Hoechst 33342 were automatically segmented using the Fiji plug-in StarDist⁴⁹. Nuclear YAP-GFP fluorescence intensity (I_{nuc}) was measured within the segmented area on a single z-frame where the nuclear centroid was present. The same z-frame was used for manual measurement of cytoplasmic YAP-GFP (I_{cyto}) and background fluorescence intensities (I_{back}). The ratio of nuclear/cytoplasmic YAP-GFP fluorescence intensity ($I_{\text{nuc/cyt}}$) in individual cells was calculated as $I_{\text{nuc/cyt}} = (I_{\text{nuc}} - I_{\text{back}}) / (I_{\text{cyt}} - I_{\text{back}})$. Cells surrounding the apoptotic cell 2 hours after laser induction were classified as having “YAP

translocation” ($I_{\text{nuc/cyt}} \geq 0.5$) or “no YAP translocation” ($I_{\text{nuc/cyt}} < 0.5$) status.

Nuclear size and nuclear pore

To quantify the nuclear size, the cross-sectional nuclear area of living cells labeled with FUCCI or a nuclear marker was automatically measured using the Fiji plug-in StarDist. The area measurement was performed on a single z-frame where the nuclear centroid was present. Nuclear volume was estimated on z-series (z step = 0.5 μm) using a 3D object counter function of Fiji. Assuming that the nucleus represents an almost perfectly round sphere, the nuclear radius and surface area (area of nuclear envelope, A_{NE}) were calculated from the nuclear volume according to the equations: $V = 4/3\pi r^3$ and $S = 4\pi r^2$, where V is the nuclear volume, r is the nuclear radius and S is the nuclear surface area (A_{NE}). To count the number of nuclear pores, a fluorescently labeled nucleus was outlined using Fiji, and then the distribution of mAb414-stained nuclear pores was determined using the Find Maxima function in Fiji. Briefly, the randomly selected nucleus was thresholded and outlined individually on each z-series (z step = 0.5 μm), nuclear ROIs were created, and nuclear pores within the ROIs were automatically counted.

Statistical analysis

All experimental data were tested for normality of distribution using the Shapiro-Wilk normality test. Differences were analyzed with the following tests using the R packages: Welch's t-test or Mann-Whitney U -test for non-parametric comparison of two groups; Dunnett's test or Tukey-Kramer test after ANOVA and Levene tests for parametric multiple comparisons: or Steel-Dwass test after Kruskal-Wallis rank-sum test for non-parametric multiple comparisons.

Supplemental movie titles and legends

Movie 1: Substrate deformation upon apoptosis, related to Figure 1. Bead movement (left) and heat map with radial velocity vectors of the beads (right) after laser induction of apoptosis within the MDCK monolayer for control (top) and NSC23766 treated (bottom) conditions. The color bar indicates the magnitude of radial outward (red) and inward (blue) velocity in $\mu\text{m}/5 \text{ min}$. Scale bars, $50 \mu\text{m}$.

Movie 2: Tissue relaxation upon apoptosis, related to Figure 1. MDCK tissue dynamics after laser induction (DIC images, left) and velocity field obtained by PIV analysis of DIC images (right) for control (top) and NSC23766-treated (bottom) conditions. Red dot, apoptotic cell; length of vectors, proportional to their magnitude.

Movie 3: YAP nuclear translocation in the neighboring cell upon apoptosis, related to Figure 2. Time-lapse images of MDCK YAP-GFP monolayer stained with Hoechst 33342 (magenta). Asterisk, apoptotic cell; white arrowhead, YAP-GFP nuclear translocation; scale bars, $20\mu\text{m}$.

Movie 4: Cell cycle progression in the neighboring cell upon apoptosis, related to Figure 2. Time-lapse images of MDCK FUCCI monolayer. Black arrowhead, apoptotic cell extrusion; white arrowhead, cell-cycle progression and subsequent cell division; scale bars, $20\mu\text{m}$.

Movie 5: Responses of neighboring cells in a mini-tissue to apoptosis, related to Figure 4. Time-lapse images of MDCK YAP-GFP mini-tissue confined to $100 \mu\text{m}\Phi$ circular pattern (left) stained with Hoechst 33342 (center left), bead movement (center right), and heat map with vectors of bead displacement (right). Asterisk, apoptotic cell; color bar, the magnitude of bead displacements in μm ; scale bars, $20\mu\text{m}$.

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