Cell compression: relevance, mechanotransduction mechanisms, and tools

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Summary statement: We review the biological contexts of cell mechanical compression, the associated mechanisms, and the experimental systems engineered to compress cells *in-vitro*.

Abstract:

From border cell migration during Drosophila embryogenesis to solid stresses inside tumors, cells are often compressed during physiological and pathological processes, triggering major cell responses. Cell compression can be observed *in-vivo* but also controlled *in-vitro* through tools such as micro-channels or planar confinement assays. Such tools have recently become commercially available, allowing a broad research community to tackle the role of cell compression in a variety of contexts. This has led to the discovery of conserved compression-triggered migration modes, cell fate determinants and mechanosensitive pathways, among others. In this Review, we will first address the different ways in which cells can be compressed and their biological contexts. Then, we will discuss the distinct mechanosensing and mechanotransducing pathways that cells activate in response to compression. Finally, we will describe the different *in-vitro* systems that have been engineered to compress cells.

KEYWORDS: Mechanobiology, Mechanotransduction, Membrane, Cytoskeleton, *In-vitro* systems, Mechanosensation

Introduction:

Cell responses to mechanical signals have been characterized for decades, starting with the discovery of pressure-triggered opening of ion channels (Chalfie and Sulston, 1981). These responses can be divided into two steps, mechanosensing and mechanotransduction. Mechanosensing refers to the sensing of mechanical forces by cells, normally achieved by mechanosensitive proteins whose conformation or localization changes upon force application. In the example of mechanosensitive ion channels such as Piezo family proteins, mechanical force increases plasma membrane tension, leading to the opening of the channel through a conformational change (Coste et al., 2010; Ge et al., 2015). Mechanotransduction refers to the subsequent transduction of the mechanical inputs into a biochemical signaling cascade. For Piezo proteins, this would be the influx of Ca^{2+} through the channel, and subsequent Ca^{2+}-mediated signaling. Such mechanical responses can be triggered by several types of mechanical signals, including pressure, substrate rigidity, mechanical stretch and compression, among others. In this Review, we focus on cell compression, which happens *in-vivo* both in physiological and pathological contexts as diverse as the creation of signaling centers during embryogenesis (Shroff et al., 2024) and cancer cell invasion (Han et al., 2020; Nishi et al., 2022).

Cells experience different types of compression, which can be classified depending on the dimensions along which compression is applied, and the dimensions that remain unconstrained (Fig. 1). For instance, cells can be compressed along one dimension *in-vitro* by the addition of an agarose pad on top, leaving them free to move within a plane (Aureille et al., 2019). As this compression is generally applied along the apicobasal polarity axis, we will here refer to it as apicobasal compression. Cells can also be subjected to compression along two dimensions, here defined as in-plane compression, such as when they migrate through pores in the extracellular matrix (Boekhorst et al., 2016). Finally, cells can be exposed to mechanical compressions from all directions, as occurs inside tumors. We will designate this as volumetric compression, as it leads to a reduction in cell volume (Stylianopoulos et al., 2012). Of note, the frontier between one or another type of compression can be ambiguous and case-specific; a cell embedded in a matrix with pores small enough to prevent migration will be volumetrically compressed, whereas a cell able to squeeze between pores will only experience in-plane compression.

This diversity of compression forces has driven researchers to engineer a variety of *in-vitro* set-ups, recapitulating each condition to decipher the different mechanisms of signal transduction that occur in each context. In this Review, we first discuss the contexts in which cells experience compression *in-vivo*, specifically focusing on eukaryotic organisms. Then, we discuss the variety of associated mechanotransduction mechanisms involving the plasma membrane, cytoplasm and the nucleus. Finally, we present a range of available *in-vitro* set-ups that allow the study of cellular compression.

Biological contexts and consequences of compression

Cell density regulation

Epithelial tissues act as physical barriers, which must preserve their integrity while being subjected to in-plane tensile or compressive forces. Epithelia maintain homeostatic cell numbers by balancing extrusion (Fig. 2) and division rates *in-vivo* (Campinho et al., 2013; Eisenhoffer et al., 2012; Marinari et al., 2012; Gudipaty et al., 2017). Interestingly, the mechanosensitive ion channel Piezo1 senses both tension and crowding which respectively induce either cell division or delamination (controlled cell extrusion from the epithelial tissue) (Eisenhoffer et al., 2012; Marinari et al., 2012; Gudipaty et al., 2017). High tissue density or confluency also induces contact-inhibition of proliferation, mediated by E-cadherin cell–cell contacts that inhibit the

Hippo pathway (Aragona et al., 2013; McClatchey and Yap, 2012). Live-cell delamination upon inplane cell compression inside a dividing physiological epithelium also initiates stem cell specification. For example, Piezo1-mediated live-cell delamination has been recently shown to trigger neural crest cell migration in mouse embryos (Moore et al., 2024 preprint). In the mammalian epidermis, stem cell proliferation in the basal layer leads to in-plane compression, inducing stem cell delamination and differentiation, which re-establishes homeostatic density in the basal layer (Miroshnikova et al., 2018). In the context of cancer, cell delamination basally after in-plane compression can lead to cell invasion of the surrounding stroma (Fadul et al., 2021). However, preventing epithelial cell extrusion can also lead to the basal accumulation of defective or dead cells and poor epithelial barrier capability, which can eventually result in tumor initiation and progression (Gu et al., 2015). This is an example of the role mechanical forces can play in disease (Box 1).

Cell migration

To promote organ formation or to sustain tissue function, cells need to migrate through diverse environments and can experience compression along one or more axes (Denais et al., 2016; Ratheesh et al., 2018; Szabó et al., 2016; Ventura and Sedzinski, 2022). Moreover, both immune and cancer cells rely on their ability to perform confined migration for immunosurveillance and invasion, respectively (Alexander et al., 2013; Boekhorst et al., 2016; Friedl and Weigelin, 2008; Heuzé et al., 2011; Kameritsch and Renkawitz, 2020; Siekhaus et al., 2010; Wirtz et al., 2011). During confined migration, cells are subject to compression along one or two dimensions as they squeeze within tight spaces (Fig. 2). These spaces can be defined by dense extracellular matrix (ECM), by other cells during intravasation or extravasation, or by tissue structures like that of muscle or adipose tissue (Alexander et al., 2013; Boekhorst et al., 2016; Siekhaus et al., 2010; Szabó et al., 2016; Yamada and Sixt, 2019). To migrate in such distinct three-dimensional (3D) physical environments, cells adopt different migration modes. These are typically categorized as mesenchymal (slow and based on integrin-mediated adhesion) and amoeboid (fast and based on non-specific adhesion) (Boekhorst et al., 2016; Paul et al., 2017; Bergert et al., 2015; Yamada and Sixt, 2019). Interestingly, compressing cells along one dimension (apicobasal compression) is sufficient to induce amoeboid migration or mesenchymal-to-amoeboid transition of various cell types (Liu et al., 2015; Ruprecht et al., 2015).

Cells can also collectively migrate as cohesive clusters. This has been observed in both development and cancer where cells face the challenges of confined migration (Box 1) (Boekhorst et al., 2016; Friedl et al., 2004). In Drosophila, border cells in the ovary delaminate from the epithelium and experience volumetric compression as they squeeze between nurse cells to reach the border of the oocyte (Montell, 2003; Prasad and Montell, 2007). Moreover, in Xenopus and zebrafish embryos, neural crest cells delaminate from the neural tube to migrate collectively through a 3D-confined space during embryogenesis (Szabó et al., 2016).

Cell fate

Compressive signals influence stem cell fate decisions during embryogenesis and tissue homeostasis. During embryogenesis, compression forces contribute to the spatiotemporal coordination of the chemical signals required for organ development. For example, patterns of volumetric compression locate a signaling center necessary for tooth development (Shroff et al., 2024) and determine ectoderm cell competence to Wnt signaling between developmental stages 10 and 12 in Xenopus embryos (Alasaadi et al., 2024). Cell compression can also contribute to maintaining or damaging stem cell niches in adults (Fig. 2) (Xie et al., 2022). Indeed, apicobasal compression promotes the quiescence of muscle stem cells in healthy tissue, whereas its absence during injury leads to cell proliferation and migration, promoting muscle healing (Tao et al., 2023). In contrast, plucking the hair of a rat tail induces in-plane compression of the stem cell

niche and apoptosis of the cells it contains, in a phenomenon resembling aging-associated hair loss (Xie et al., 2022). Of note, researchers have used compression signals to reprogram cells. For instance, Li and collaborators have used volumetric compression via hyperosmotic shock to induce the dedifferentiation of adipocytes into mesenchymal stem cells (MSCs) (Li et al., 2020). Similarly, microchannels inducing in-plane compression have been employed to reprogram fibroblasts into induced pluripotent stem cells (Song et al., 2022).

Box 1: When compression participates in pathologies

Tissues associated with the musculoskeletal system are constantly submitted to compressive forces. A good example of this is cartilage, which is a soft tissue between bones that protects the joints while bearing the weight of the body. It is composed of chondrocytes embedded in a matrix rich in water and proteoglycan (Huselstein et al., 2008; Zhang et al., 2022a). This matrix has a significant role in maintaining tissue homeostasis and reducing the stress gradient applied to cells (Zhao et al., 2020). Although cartilage is constantly subjected to volumetric compression under physiological conditions, aging and excessive compressive forces cause cell apoptosis, leading to osteoarthritis symptoms in more than 300 million people worldwide (Han et al., 2024; Ren et al., 2023). Interestingly, high dynamic compression of chondrocytes leads to their apoptosis (O'Conor et al., 2014; Takeda et al., 2021), whereas lower compression forces trigger mesenchymal stem cell (MSC) differentiation into chondrocytes (Li et al., 2009; Pelaez et al., 2012; Wang et al., 2013). This shows the importance of separating physiological from pathological mechanical signals.

Volumetric compression inside tumors, also called solid stress, is also associated with cancer. Reaching values of 6–16 kPa, it is caused by several factors (Fig. 2) (Helmlinger et al., 1997) including increased cell proliferation, high contractility of the surrounding fibroblasts (Barbazan et al., 2023) and ECM stiffening due to matrix deposition (Stylianopoulos et al., 2012). Compression stresses are not uniform (Stylianopoulos et al., 2013; Zhang et al., 2023) and differentially affect cell proliferation with time (Cheng et al., 2009; Delarue et al., 2014; Fernández-Sánchez et al., 2015; Mary et al., 2022). Compression initially causes inhibition of cell proliferation both through direct effects on cell division and apoptosis (Cheng et al., 2009; Delarue et al., 2014) and by limiting nutrient access (Stylianopoulos et al., 2013; Toi et al., 2017). Subsequently, tumor cells show adaptation to compressive forces, promoting cell proliferation (Fernández-Sánchez et al., 2015; Mary et al., 2022), invasion (Han et al., 2020; Nishi et al., 2022), stemness (Nguyen Ho-Bouldoires et al., 2022; Zhao et al., 2021) and the formation of secondary tumor centers (Gong et al., 2023 preprint).

Mechanosensing and mechanotransduction of compressive forces

Here, we will review current knowledge of mechanosensation mechanisms that can be triggered by compressive forces and the associated mechanotransduction pathways.

At the plasma membrane

Cell compression is first applied on the plasma membrane, leading either to its tension in the case of apicobasal and in-plane compression or to its compression in the case of volumetric compression (Fig. 3). Diverse components of the plasma membrane participate in mechanosensing and mechanotransduction. Some examples are discussed below, but for more detailed information, readers can refer to specific reviews (Le Roux et al., 2019; Li, 2024).

First, the lipid composition and organization of the plasma membrane determine its physical properties (Li, 2024). Moreover, different tension states correlate with different types of membrane organization – high membrane tension is associated with lipid rafts whereas low

membrane tension is associated with reservoirs (passive membrane invaginations or evaginations) (Fig. 3) or caveolae (membrane invaginations actively maintained by cells) (Kosmalska et al., 2015; Le Roux et al., 2019; Sinha et al., 2011; Teo et al., 2020). These structures can be recognized by membrane-binding proteins (Le Roux et al., 2021; Quiroga et al., 2023) or can trigger lipid signaling, therefore transducing changes in membrane tension into biochemical signaling (Teo et al., 2020). For example, in-plane compression of single cells creates membrane reservoirs that are recognized by the BAR protein IRSp53 (also known as BAIAP2). IRSp53 later induces the recruitment of the actin polymerization machinery, promoting the reabsorption of membrane reservoirs (Quiroga et al., 2023) (Fig. 3). Conversely, caveolae disappear when plasma membrane tension increases (Sinha et al., 2011), leading to the depletion of caveolin-1, recruitment of FMNL2 and actin stabilization through phosphatidylinositol-4,5-bisphosphate (Teo et al., 2020). Beyond caveolae, other caveolin-mediated structures also respond to tension (Lolo et al., 2023). Membrane tension also depends on membrane attachment to the underlying actin cortex through force-sensitive membrane-to-cortex attachment proteins, such as ezrin, moesin, and radixin. For instance, membrane tension leads to the loss of the interaction between actin and FilGAP (also known as ARHGAP24), a Rac GTPase-activating protein, mediated by ezrin. This triggers Rac activation and actin polymerization (Ehrlicher et al., 2011). Similarly, myosin 1b interacts more strongly with actin when subjected to tension (Laakso et al., 2008), thus underscoring the importance of applied forces on the cell cortex organization and membrane tension.

Signals can also be transduced at the plasma membrane through the concentration of receptors and the opening of ion channels (Fig. 3). Indeed, in-plane compression of cellular monolayers and volumetric cell compression can induce the reorganization of membrane proteins. For instance, studies have established that in-plane compression causes a reduction in the number of integrin-based adhesions which changes the mechanical homeostasis between cell–ECM and cell–cell adhesions, leading to cell delamination (Miroshnikova et al., 2018). In contrast, volumetric compression can induce the concentration of LRP6 signalosomes, which maintain the activity of the Wnt/β-catenin pathway (Fig. 3) and promote the stemness of compressed cells (Li et al., 2021).

Moreover, ion channels have long been associated with the transduction of mechanical signals, for instance in C. elegans touch neurons (Chalfie and Sulston, 1981; Huang and Chalfie, 1994) and inner ear hair cells (Corey and Hudspeth, 1979), which detect pressure and vibration, respectively. Some channels are permeable to specific ions, whereas others do not discriminate (Peyronnet et al., 2014). Ca^{2+} ions are important second messengers inside cells; thus, channels responsible for their transport are ideal candidates for factors involved in mechanosensing and mechanotransduction. Indeed, both Piezo1 (Coste et al., 2010) and some channels of the transient receptor potential vanilloid (TRPV) family, such as TRPV4 (Fu et al., 2021), promote Ca^{2+} influx into the cell when the membrane is under tension (Fig. 3). These channels can be activated through membrane tension downstream of apicobasal compression (Easson et al., 2023; O'Conor et al., 2014; Shi et al., 2022; Takeda et al., 2021; Wang et al., 2024a) but also enable the detection of in-plane or volumetric compression associated with plasma membrane compression (Gudipaty et al., 2017; Hung et al., 2016; Xie et al., 2022). Interestingly, although in some cases the closing of channels is associated with compression sensing (Delarue et al., 2014; He et al., 2018; Nam et al., 2019), in others their location (at the plasma membrane or inside cells) determines whether tension or compression is detected, highlighting the versatility of mechanisms of action of these channels (Gudipaty et al., 2017).

Following apicobasal or in-plane compression, Ca^{2+} influx into cells has several downstream effects. It promotes cell apoptosis through many pathways, including induction of endoplasmic reticulum stress (Wang et al., 2024a), damage to mitochondria (Shi et al., 2022), caspase-3 activation in combination with tumor necrosis factor (TNF) signaling (Xie et al., 2022),

and the induction of diverse inflammatory pathways (Easson et al., 2023; O'Conor et al., 2014; Takeda et al., 2021). Additionally, Piezo1 activation upon compression can induce ion-dependent cell apoptosis, or ferroptosis, via both Ca^{2+} (Jia et al., 2024) and Fe^{3+} signaling (Xiang et al., 2024) (Fig. 3). Ca^{2+} influxes also promote cell motility through myosin II activation (Lomakin et al., 2020; Venturini et al., 2020) (Fig. 3), as well as Src signaling, which leads to matrix degradation and cancer cell invasion (Luo et al., 2022). Finally, Ca^{2+} influxes can impact cell fate by promoting cellular differentiation, for instance via the inhibition of Notch proteins (Fig. 3) during the differentiation of midgut stem cells into enteroendocrine cells (He et al., 2018), via the activation of the ERK-RANK-OPG pathway during the differentiation of periodontal ligament stem cells into osteoclasts (Jin et al., 2020), or through growth arrest in volumetrically compressed cells (Delarue et al., 2014; Nam et al., 2019). In contrast to apicobasal compression, volumetric compression reduces intracellular Ca^{2+} levels, leading to the downregulation of the phosphoinositide 3-kinase (PI3K)-Akt pathway, in turn promoting the nuclear localization of the cell cycle inhibitor p27Kip1 (also known as CDKN1B) and subsequent cell cycle arrest in G1 phase (Delarue et al., 2014; Nam et al., 2019).

In the cytoplasm and nucleoplasm

By affecting cell volume, volumetric compression alters concentrations and organization of molecules within the cell, which impacts cell behavior and fate. For cells with otherwise identical physical properties, there is an ideal intermediary cell volume at which cells organize their actomyosin cytoskeleton into stress fibers and transmit forces from the substrate to the nucleus, leading to the nuclear translocation of the mechanosensitive transcription factor YAP (also known as YAP1) (Bao et al., 2017). Similarly, our team recently demonstrated that cell volume impacts mechanical behavior, as cells placed in large micro-wells exert contractile forces, whereas cells placed in small wells exert extensile forces (Faure et al., 2024).

Furthermore, cell compression induced by an osmotic shock can induce molecular crowding and phase separation in both the cytoplasm and nucleoplasm (Fig. 3) (Jalihal et al., 2020; Lee et al., 2021a; McCreery et al., 2024 preprint). Cellular compression through hyperosmotic shock induces the phase separation of nuclear 26S proteasome into dense foci, sequestering key nuclear proteins and supporting cell survival under stress (Lee et al., 2021a). Likewise, hyperosmotic shock can cause monomeric proteins to form inactive multimers. This can subsequently affect cellular processes, for example, multimerization of the structural pre-mRNA cleavage and polyadenylation factor CPSF6 following osmotic shock impairs transcription termination (Jalihal et al., 2020). Finally, submitting human induced pluripotent stem cells (hiPSCs) to hyperosmotic shock in basal medium is enough to promote nucleoplasm crowding and chromatin remodeling, leading to lineage transition (McCreery et al., 2024 preprint).

In the nucleus

Because of its intracellular localization and mechanical properties, the nucleus can selectively sense large cell-shape deformations. Large forces on the nucleus have been observed in cell migration (Denais et al., 2016; Renkawitz et al., 2019; Thiam et al., 2016), cell differentiation (Biedzinski et al., 2020) and through transmission of mechanical forces from the microenvironment via protein complexes, such as the linker of nucleoskeleton and cytoskeleton (LINC) complex (Caille et al., 1998; Maniotis et al., 1997). Such forces can deform nuclei to the point of creating nuclear ruptures and DNA damage (Denais et al., 2016; Nader et al., 2021) and can impact processes like cell motility or transcription, as discussed in detail in other reviews (Dupont and Wickström, 2022; Mammoto et al., 2012). Nuclear deformations depend on nuclear composition and mechanics, which are governed by lamin A/C expression levels and chromatin organization (Hobson et al., 2020; Stephens et al., 2017). Nuclear mechanical properties vary

across cell types and during development, differentiation and disease (Hampoelz and Lecuit, 2011; Isermann and Lammerding, 2013) but can also change upon direct force application (Guilluy et al., 2014). For example, as cells migrate through narrow constrictions, the nuclear lamina layer can disassemble, leading to the detachment of lamina-associated chromatin domains and to decreased histone methylation (Song et al., 2022). In epithelia, the Ca^{2+} increase upon uniaxial tissue stretching or cell compression can increase heterochromatin markers and induce nuclear softening (Nava et al., 2020). Forces reaching the nucleus induce the stretching of both the nuclear envelope (NE; which is composed of a double lipid bilayer with an underlying lamin meshwork) and of nuclear pore complexes (NPCs), which control molecular exchange between the cytoplasm and nucleoplasm (Andreu et al., 2022; Elosegui-Artola et al., 2017; Zimmerli et al., 2021). All these elements make the nucleus a key mechanosensor and mechanotransducer of forces, as recently reviewed (Niethammer, 2021).

Inner nuclear membrane (INM) unfolding can be directly sensed by the cytosolic phospholipase A2 ($cPLA_2$; encoded by PLA2G4A) (Fig. 3) (Enyedi et al., 2016). This lipase has a C2 domain that, in the presence of high Ca^{2+}, binds to the INM in a stretch-sensitive way (Enyedi et al., 2016) where it catalyzes the release of arachidonic acid (AA), its first metabolite product (Lomakin et al., 2020; Venturini et al., 2020). Different pathways have been described downstream of $cPLA_2$, supporting an important role for nuclear mechanotransduction of this mechanosensitive protein. In fact, AA can be further processed into molecules known as eicosanoids, which act as chemoattractants, for example attracting leukocytes at wound regions in zebrafish (Enyedi et al., 2016). AA can also act in a cell-autonomous way and activate myosin II activity via the Rho-ROCK pathway in a variety of cell types, including immune cells, cancer cells and zebrafish embryonic stem cells (Lomakin et al., 2020; Venturini et al., 2020) (Fig. 3). This allows single cells to increase their contractility upon apicobasal compression, further inducing cellular blebbing. This equips cells with 'escape mechanisms' that allow them to quickly evade confined environments (Lomakin et al., 2020; Venturini et al., 2020). In dendritic cells, mechanosensing by $cPLA_2$ together with the ARP2/3 actin-nucleating complex controls homeostatic migration to the lymph node (Alraies et al., 2024). Furthermore, confining dendritic cells in between two parallel surfaces at a distance of 3 μm, but not 4 or 2 μm, activated the transcription factor NF-κB (Fig. 3) in a paracrine manner, inducing the expression of CCR7, the chemokine receptor required for homeostatic migration of dendritic cells. This mechanotransduction pathway not only controls migration of dendritic cells but also their immune phenotype and transcription profile (Alraies et al., 2024).

Forces applied to the nucleus are transmitted to NPCs, which expand or shrink because of a respective increase or decrease in force applied to the nucleus via integrin-based focal adhesions and the actin cytoskeleton, or via hypotonic swelling (Elosegui-Artola et al., 2017; Granero-Moya et al., 2024; Schuller et al., 2021; Zimmerli et al., 2021) (Fig. 3). As a result, both passive diffusion and active transport increase when pores dilate (Andreu et al., 2022). However, NPC dilation has a greater effect on active transport than on passive diffusion, leading to the accumulation of mechanosensitive transcription factors, such as YAP, SMAD3, Snail (also known as SNAI1) or Twist proteins, in nuclei subjected to force (Fig. 3) (Andreu et al., 2022). Notably, this feature of NPCs can be harnessed to engineer synthetic molecules to use as probes. Such molecules can assess nuclear deformation and mechanotransduction via nucleocytoplasmic transport in different cell lines, independently of the biochemical signaling that affects mechanosensitive transcription factors such as YAP (Granero-Moya et al., 2024). Interestingly, YAP mechanosensing was first described in the context of ECM rigidity sensing (Dupont et al., 2011; Elosegui-Artola et al., 2016), but this process was later shown to be mediated by actomyosin-induced nuclear compression (Aureille et al., 2019; Elosegui-Artola et al., 2017). Consequently, several studies have observed changes in YAP localization upon compression (Elosegui-Artola et al., 2017; Emon et al., 2024). In Xenopus laevis embryo development, an

increase of hydrostatic pressure promotes the cytoplasmic translocation of YAP (Alasaadi et al., 2024). Furthermore, the authors proposed a model in which YAP transduces volumetric compression by acting as a co-transporter of β-catenin out of the nucleus (Fig. 3), thus impacting cell fate through loss of competence to Wnt signaling.

Engineering systems to compress cells

To decipher how cells sense and transduce compression, researchers have developed a variety of *in-vitro* systems. We will present them based on the dimensionality of compression they apply. All the presented methods, together with the biological model used in each case, are recapitulated in Tables 1. These tools come with the caveat that they are minimalistic systems that aim to mimic and tune the physical properties of the cell micro-environment. Thus, these tools might over-simplify the *in-vivo* scenario or neglect key biophysical or biochemical features. Appropriate controls are often necessary to ensure cells are not being damaged or stressed to induce cellular death programs.

Compression along one dimension

Applying compression along one dimension, generally apicobasal, can be achieved by cell confiners and typically involves squeezing cells towards the surface they are cultured onto (Fig. 4A–D), which can be planar or corrugated (Gaertner et al., 2022). This can be achieved by simply placing an agarose pad on top of adherent cells (Aureille et al., 2019). More sophisticated tools have been designed to culture cells between two parallel surfaces separated at a defined distance. These include glass coverslips with silicone, specifically polydimethylsiloxane (PDMS) micro-pillars (Le Berre et al., 2014) or agarose pads containing pillars (Fig. 4A,C) (Elpers et al., 2023; Prunet et al., 2020). Of note, PDMS pillars can be coated with any desired ECM protein and the confinement can be static (Fig. 4A,C) or dynamic (by coupling the coverslip is with a PDMS suction cup and a pressure controller) (Fig. 4B,C) (Le Berre et al., 2014). These systems are commercially available (4D cell, Bio-Connect). PDMS devices equipped with micro-pistons have also been developed to dynamically and specifically confine the cells underneath the micropiston and not their neighbors by controlling the pressure applied to a control channel that regulates the height of the piston (Onal et al., 2021). Finally, a complex microfluidic design in which observation chambers are placed between air cavities has been designed to apply compression over cell clusters (Jain et al., 2024).

Similar confinements applied to one cell at a time can be achieved by using atomic force microscopy (AFM, available from Nanosurf, JPK, Bruker and others) or cell indenters (Optics11) (Fig. 4D). These systems can be used to confine or locally indent a cell using a probe in the shape of a sphere, an inverted pyramid or a flat surface, among others. Depending on the size and shape of the probe, AFM can be used to confine entire cells (Fig. 4D) (Lomakin et al., 2020; Andolfi et al., 2019; Lulevich et al., 2006) or subcellular regions or organelles, such as the nucleus (Andreu et al., 2022; EloseguiArtola et al., 2017). Probes that have sizes that are comparable to or larger than the nucleus have been used to show that nuclear compression is sufficient to activate nuclear mechanotransduction pathways (Lomakin et al., 2020; Andreu et al., 2022; EloseguiArtola et al., 2017). Smaller and sharper probes offer the possibility of studying subcellular mechanics (Hobson et al., 2020; RocaCusachs et al., 2008). Advantages of AFM and cell indenters over cell confiners are that they can be used not only to confine but also to measure applied forces and deformations (Hobson et al., 2020; Roca-Cusachs et al., 2008) as well as the cellular mechanoresponse to compression (Lomakin et al., 2020). They can also be used on cells cultured on soft substrates (Andreu et al., 2022; EloseguiArtola et al., 2017). However, the sensitivity of these probes comes at the cost of a much lower throughput.

In-plane compression

Various systems that compress cells along two dimensions have been developed, mostly to either study cell migration through pores or cell compression within an epithelia layer (Fig. 4E–K).

To study confined migration, a plethora of *in-vitro* systems are available and used depending on the biological question of interest. Importantly, several tools are often combined in a single study, as in Renkawitz et al. (2019) and Kroll et al. (2023) and many others, emphasizing the generality of the proposed mechanisms as well as the compatibility and complementarity of these tools. Microchannels that constrict cell shape and direct cell migration have been developed in several different ways. Channels are typically fabricated in PDMS (Fig. 4E) (Lautenschläger and Piel, 2013; Renkawitz et al., 2019; Reversat et al., 2020; Thiam et al., 2016), although more recently softer materials like hydrogels have been used (Afthinos et al., 2022; Stöberl et al., 2023). Channels can even be made using liquid–liquid interfaces between aqueous and oil solutions (Mosier et al., 2023). Channel shapes can be freely designed according to the physical parameter or process of interest, and their surfaces coated with any protein of interest (typically ECM proteins). Additionally, chemoattractants can be added to direct cell migration (Fig. 4E). These tools have particularly been used to study immune cell migration in complex and confined environments. For example, channels with constrictions, corrugations or bifurcations of different sizes have been used to study how dendritic cells deform their nucleus while migrating through narrow spaces (Thiam et al., 2016). These channels have also been used to demonstrate that cells can migrate over textured substrates without adhesion molecules by using actin retrograde flow (Reversat et al., 2020). Cells have been found to use their nucleus as a ruler in order to choose wider paths during confined migration in the absence of chemokines (Renkawitz et al., 2019); however, this can be overwritten by chemotactic signals that can force cells to migrate within narrow spaces (Kroll et al., 2023). In comparison, channels made of hydrogels allow measurement of the forces exerted by cells as they migrate. By adding inert markers inside these gels, traction force microscopy can be used to measure the forces exerted by single cells as they pass through a constriction (Stöberl et al., 2023) or by a group of cells migrating in channels with different stiffness (Afthinos et al., 2022). Liquid–liquid interfaces, such as those in between oil and culture media, can also be harnessed to fabricate even softer microchannels (Mosier et al., 2023). These channels have been used to show that migrating neutrophils deform the channel itself. This suggests that the surface tension of the interface induces a confining pressure that is comparable to cell stiffness and confirms that the neutrophil migration modes observed *in-vitro* recapitulate the ones observed *in-vivo* (Mosier et al., 2023).

Chambers containing arrays of PDMS micro-pillars with any desired shape (typically circular or square) and organization can also be used to study cell migration through pores (Fig. 4F). These tools have been especially useful in understanding how nuclei deform and rupture when cells migrate through the spaces in between consecutive pillars spaced at distances smaller than the diameter of their nuclei (Denais et al., 2016). Using these tools, it has also been shown that cells use the actin cytoskeleton to pull their nuclei forward in order to migrate through narrow constrictions (Davidson et al., 2019). PDMS-based channels or pillar arrays can be designed with a very broad variety of geometries and design features using microfabrication technologies, and their imaging is straightforward. However, PDMS is known to be extremely stiff in comparison to biological tissues.

Transwell plates or inlets (Fig. 4G), described in detail in Chung et al. (2018), are used to measure the ability of single cells to migrate across pores with diameters ranging from a few micrometers to submicrometer values. Transwell plates, or Boyden chambers (Chen, 2005), typically consist of a porous membrane (with a defined pore diameter and density) in the middle of a plate that separates two compartments, often filled with ECM components (see Fig. 4G).

Cells are usually added to the upper compartment and transmigrate to the lower one, where chemoattractants can be added and cells can be counted or collected for further experiments (Fanfone et al., 2022; Jung-Garcia et al., 2023). These tools are commercially available (Corning, Thermo Fisher Scientific and others) in the form of single or multi-well plates. For a detailed review on use of porous membranes and Transwell plates as *in-vitro* models for studying cell transmigration see Salminen et al. (2020). These systems have been applied to study the transmigration of immune (Salminen et al., 2019) and cancer cells (Fanfone et al., 2022; Jung-Garcia et al., 2023). For example, human breast cancer cells have been found to develop resistance to anoikis (programmed cell death) and show enhanced invasiveness upon confined migration through pores (Fanfone et al., 2022). Experiments with a series of Transwell plates have been used to test the ability of cancer cells to migrate through pores of decreasing sizes, showing that nuclear adaptability fosters cancer cell migration and invasion (Jung-Garcia et al., 2023). Finally, a cell monolayer can also be added on top of the porous membrane, allowing migrating cells to experience cell–cell contact during their migration (Chakravorty et al., 2006; Lee et al., 2021b).

Confined migration of single immune or cancer cells can also be studied by embedding cells inside a 3D matrix with pores that are sufficiently large to allow cell migration (Fig. 4H) (Kameritsch and Renkawitz, 2020; Krause et al., 2019; Paul et al., 2017; Renkawitz et al., 2019; Wolf et al., 2003). Of note, 3D matrices with very small pore sizes can be used to limit cell growth, thereby confining cells volumetrically. As discussed in more specific reviews (Saraswathibhatla et al., 2023; Vu et al., 2015), these matrices are typically hydrogels made of collagen, alginate, Matrigel, polyethylene glycol (PEG) or a combination of materials, and are commercially available (Ibidi and Thermo Fisher Scientific). Notably, tissue-specific matrices can also be obtained by decellularization of the native ECM (Zhang et al., 2022b). When using hydrogels made of polymers that lack cell binding sites (e.g. alginate), specific peptides can be added to the polymers to ensure homogeneous cell binding (Elosegui-Artola et al., 2017). Depending on their compositions, these hydrogels can be either elastic or viscoelastic (Chaudhuri, 2017; Ma et al., 2021). For instance, by tuning the molecular mass of alginate and the crosslinker concentration, gels can be obtained that have a similar storage modulus (i.e. stiffness when measured at long timescales) but different relaxation times (Chaudhuri et al., 2016; Elosegui-Artola et al., 2023). Interestingly, differences in gel viscoelasticity are sufficient to modulate cancer cell proliferation and invasion (Elosegui-Artola et al., 2023) and stem cell migration (Wu et al., 2023). As migrating cells pull and push onto the fibers of the matrix, this technology allows for highly sensitive measurement of 3D forces (Böhringer et al., 2024). However, cells can also actively remodel their matrix, for example by creating tunnels where more cells can migrate (Driscoll et al., 2024; Yamada and Sixt, 2019). Although interesting, such remodeling renders these systems less controllable and can prevent them from being reused.

Other systems of in-plane compression have been designed to apply cell compression within epithelial monolayers (Fig. 4I), recapitulating conditions of high cellular density. For instance, a pneumatic system allowing the deformation of the bottom of a Petri dish to stretch or compress the cells plated in it was developed in 1985 (Banes et al., 1985). This system was patented and later improved upon and commercialized (e.g. Strex Cell and Cytostretche by Curi Bio). Stretching devices are generally based on a stretchable membrane that can be deformed pneumatically (Shimizu et al., 2011) through an indenter (Huang et al., 2010) or a membrane holder (Gerstmair et al., 2009). This allows cells to be stretched uniaxially, biaxially or equixially (Fig. 3I). Over time, different improvements have enabled cost reductions and the ability to stabilize these systems during microscopy imaging (Dow et al., 2020; Mäntylä and Ihalainen, 2021). To compress cells in these setups, cells are seeded onto pre-stretched elastic membranes, which are released after the cells have spread or monolayers have reached confluency, inducing cellular compression (Fig. 4I). Using this approach, a 28% cell compression was found to induce live-cell extrusion in Madin–Darby canine kidney (MDCK) cell monolayers (Eisenhoffer et al.,

2012) with similar results observed in the epidermis (Miroshnikova et al., 2018). At the single-cell and subcellular levels, this system has also been used to study microtubules under compression (Li et al., 2023) as well as compression-induced plasma membrane reshaping and subsequent mechanotransduction events (Kosmalska et al., 2015; Le Roux et al., 2021; Quiroga et al., 2023). More recently, a tool to stretch suspended monolayers has been developed. In this system, an MDCK monolayer is cultured onto a collagen substrate suspended between two rods; the collagen is then enzymatically removed, and the monolayer can be stretched and compressed between the rods using a micro-manipulator (Harris et al., 2013). With this system, it has been shown that epithelial buckling depends on the speed of compression (Wyatt et al., 2020).

Cell compression inside monolayers can also be achieved by introducing differences in local cell density through substrate design. For example, some cell types self-align and create large-scale patterns inside monolayers. When areas of different alignments coincide in one location, they form a nematic defect (Fig. 4J). Different studies have established that some nematic defects are associated with high cell compression, whereas others correlate with high cell tension (Guillamat et al., 2022; Sonam et al., 2023) (Fig. 4J). Using this knowledge, it is possible to pattern a compression point inside a cell monolayer. In monolayers of C2C12 myoblasts, this induces differentiation into myotubes and controls monolayer organization in 3D (Guillamat et al., 2022). Similarly, seeding cells on a substrate with a frequency of corrugation larger than a few cell lengths has been used to establish a pattern of compression within the monolayer (Fig. 4K). Indeed, cells present in the valleys of these corrugations are compressed in comparison to those placed on the peaks, as characterized by their rounder nuclei and lower nuclear YAP levels (Luciano et al., 2021). These systems allow for easier imaging than stretch setups (Guillamat et al., 2022; Luciano et al., 2021). However, the choice of one versus the other should be predominantly based on the desired compressed area – stretch systems uniformly compress the whole cell monolayer, whereas substrate design can be used to obtain controlled spatial differences in cellular compression within the monolayer.

Volumetric compression

As discussed above, volumetric cell compression is common both during physiological processes, like the maintenance of the intestinal stem cell niche, and under pathological conditions, such as solid stresses generated by cell proliferation in cancer. Here, we present different systems developed to study these processes.

A straightforward way to exert volumetric compression is through osmotic shocks. For instance, adding 1–4% PEG to the cell medium is sufficient to compress cells (Fig. 4L). This quick and simple method has been used to show that volumetric compression induces the dedifferentiation of adipocytes (Li et al., 2020) and the expression of epithelial-to-mesenchymal transition and cancer stemness markers in non-small-cell lung carcinoma cells (Zhao et al., 2021).

As discussed in the context of 3D matrix culture, cells can be embedded in different polymer combinations, offering a simple way to impose volumetric compression progressively as cells proliferate. The amount of stress applied on cells can be controlled through gel stiffness and viscoelasticity (Fig. 4M) (Chaudhuri et al., 2014) as well as by adding pistons above the gel that introduce supplementary compression along one dimension (Fig. 4N) (O'Conor et al., 2014). The latter technique has been extensively used to study the impact of chondrocytes and cells from the intervertebral discs, as it reproduces the *in-vivo* conditions that lead to osteoarthritis and intervertebral disc degeneration (Easson et al., 2023; O'Conor et al., 2014; Shi et al., 2022; Wu et al., 2024). Using commercial piston systems from Flexcell-FESTO, researchers can apply 1.5% to 15% compression at a frequency of ~1 Hz, for a period ranging from a few hours to a few days and study the deleterious effects of compression on cell survival (Jia et al., 2024; Shi et al., 2022; Xiang et al., 2024). Single cells or groups of cells can also be encapsulated in alginate or alginate–

gelatin capsules (Fig. 4O). Details on how to generate these capsules are reviewed in Mohajeri et al. (2022). This technique has enabled researchers to determine that cells stop growing when submitted to a pressure of 1.8–2.2 kPa (Alessandri et al., 2013; Di Meglio et al., 2022). Additionally, this approach has shown that cell compression, but not cell morphology, promotes the cytoskeletal reorganization and cell stemness changes observed during cancer (Fuentes-Chandía et al., 2021). Compression build-up within the capsule also induces fibroblasts to migrate on top of a cancer spheroid instead of forming a segregated cluster (Bertillot et al., 2024). Encapsulation can also be used to cultivate hiPSCs as they organize as cysts in matrigel-coated capsules, which enhances their viability. In bioreactors, encapsulation also eliminates the damage caused by the reactor impeller, drastically enhancing the amplification of hiPSCs to 277- fold over 6.5 days (Cohen et al., 2023). Finally, compression induced by encapsulation of cerebral organoids has been demonstrated to promote their growth and maturation (Tang et al., 2023). Of note, encapsulation requires inert polymers like gelatin to be mixed with matrix proteins, which enable cell attachment and the degradation of the capsule (needed to recover cells at the end of the experiment) (Cohen et al., 2023). Capsules can also be difficult to image due to their shape and floating nature.

Finally, single or groups of cells can be volumetrically compressed in microniches. These consist of wells of different shapes made of hard substrates, like PDMS (Zhang et al., 2020a) (Fig. 4P,Q) and NoA-74 (Li et al., 2016), or hydrogels, such as hyaluronic acid (Bao et al., 2017; Wang et al., 2013) and polyacrylamide (Faure et al., 2024; Wilson et al., 2021). These microniches constrain cells seeded inside, which can lead to compressive forces, while also controlling cell shape and the specificity of cell–substrate adhesions. However, they do not allow for the recovery of the compressed cells after experiments, and their physical features (e.g. rigidity and viscoelasticity) can differ from *in-vivo* conditions (Fig. 4P,Q). Microniches can be used to encapsulate single cells and larger cellular clusters and can be combined with other techniques like AFM (Zhang et al., 2020b) or traction force microscopy (Faure et al., 2024). Combining these techniques enables monitoring of the mechanical status of the cells or tissues during compression and allows the control of cell behavior. Additionally, cells can experience different patterns of tension and compression inside a microniche depending on its shape. Indeed, intestinal organoids seeded in rectangular microniches are subjected to cellular tension in the long end of the rectangle and compression in the short end of the rectangle. This influences cell fate, triggering cells to express villi and crypt phenotypes following tension and compression, respectively (Gjorevski et al., 2022).

Conclusion

As discussed in this Review, cell compression is relevant in a broad variety of pathophysiological contexts and is sensed by cells through a number of pathways depending on the dimensionality of the compression. Some of these pathways have been known for years, such as the opening of Ca^{2+} channels following plasma membrane stretching (Coste et al., 2010), whereas other pathways highlight emerging concepts. For instance, the findings showing that phase separation can interfere with the transcription machinery during volumetric compression could point to a novel type of mechanotransduction mechanism (Jalihal et al., 2020), which might play a role in the cell quiescence displayed by stem cells, as the stemness phenotype is associated with volumetric compression (Li et al., 2020, 2021). These new pathways would not have been identified without the development of a plethora of *in-vitro* systems enabling cell compression in very controlled settings.

As these systems have become broadly available and more user friendly, they have also been adopted outside of the mechanobiology field, broadening their applications. For instance, the

addition of mechanical control systems to standard cell culture protocols might better recapitulate physiological conditions, as in organoids grown on 3D matrices rather than standard plastic plates, or chondroblasts submitted to dynamic compression (Li et al., 2009; Pelaez et al., 2012; Wang et al., 2013). Moreover, compression can improve the efficiency of some cell culture protocols. For instance, encapsulation methods improve yields of hiPSCs cultured in bioreactors (Cohen et al., 2023), and the reprograming of fibroblasts into iPSCs can be boosted through mechanical compression in microfluidic channels (Song et al., 2022). Diagnostic and personalized medicine, particularly for cancer, could also benefit from these techniques. Indeed, cancer studies on human samples have complemented *in-vivo* observations with *in-vitro* tools, such as Transwell assays, to infer the metastatic capacity of cancer cells (Jung-Garcia et al., 2023). Similarly, cancer stem cells present different mechano-phenotypes when apico-basally compressed compared to that seen in their non-stem counterparts, suggesting a differential metastatic potential of these two populations (Conti et al., 2024). This could allow detection of high-metastatic cell types in clinical samples through their mechanical fingerprint, characterized with *in-vitro* tools. Such clinical phenotyping requires the development of systems enabling manipulation and culture of tissues ex vivo, which is already ongoing (Bertillot et al., 2024; He et al., 2018; Jain et al., 2024).

Clearly, the dissemination of tools engineered to impose cell compression, together with the growing evidence of the importance of compressive forces *in-vivo*, will likely popularize their use and boost new discoveries. We expect these discoveries to focus not only on fundamental discoveries in mechanobiology, but also on tools available to the broad biomedical community, with specific applications in diagnostics and therapy.

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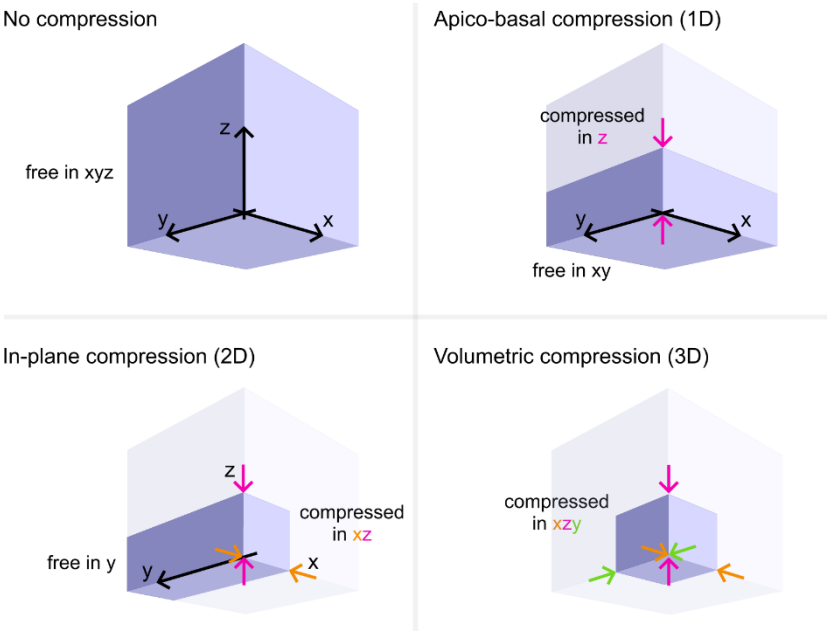

Fig. 1. Definition of type and dimensionality of compression. Top-left, no compression. Cells are free to move in the three dimensions (in xyz) without spatial constrictions. Top-right, apicobasal compression (or compression along one dimension; 1D) refers to cell confinement in one dimension (typically the apicobasal axis) but where cells are free to move in the other two. Bottom-left, in-plane compression (or compression along two dimensions; 2D) occurs when cells are compressed in two dimensions (xz), but free to move in the remaining one (y). Bottom-right, volumetric compression refers to confinement in all dimensions (3D).

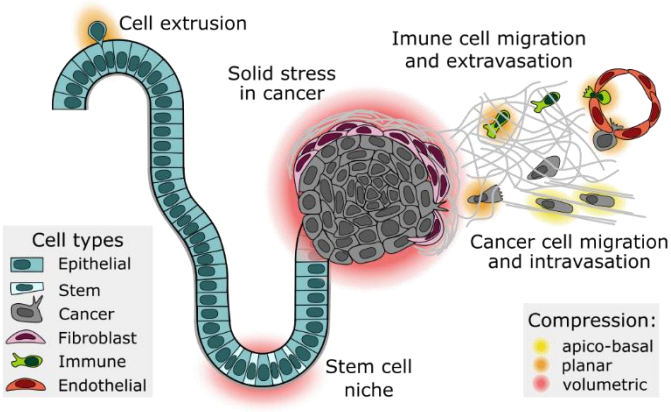

Fig. 2. Scenarios in which cells can experience compression *in vivo*. The yellow, orange and red shaded areas indicate areas where cells are compressed apico-basally, in-plane and volumetrically, respectively. From left to right, in intestinal villi, single cells are compressed by their neighbors (in-plane compression) within the stem cell niche or as they are extruded from the tips of villi. Solid stress (volumetric compression) can be observed in cancer when growth is limited by highly contractile fibroblasts or by surrounding stiffened extracellular matrix (ECM) and stromal tissue as well as in the stem cell niche where cells have been shown to have smaller volumes because of mechanical crowding (Li et al., 2021). When cancer cells escape the main tumor or when immune cells patrol tissues, they migrate through dense ECM or within tissue structures, and perform intravasation or extravasation (migration between endothelial cells into and out of a blood vessel, respectively), thus achieving confined migration. In this scenario, cells experience either apicobasal or in-plane compression depending on whether they migrate between two surfaces or through pores.

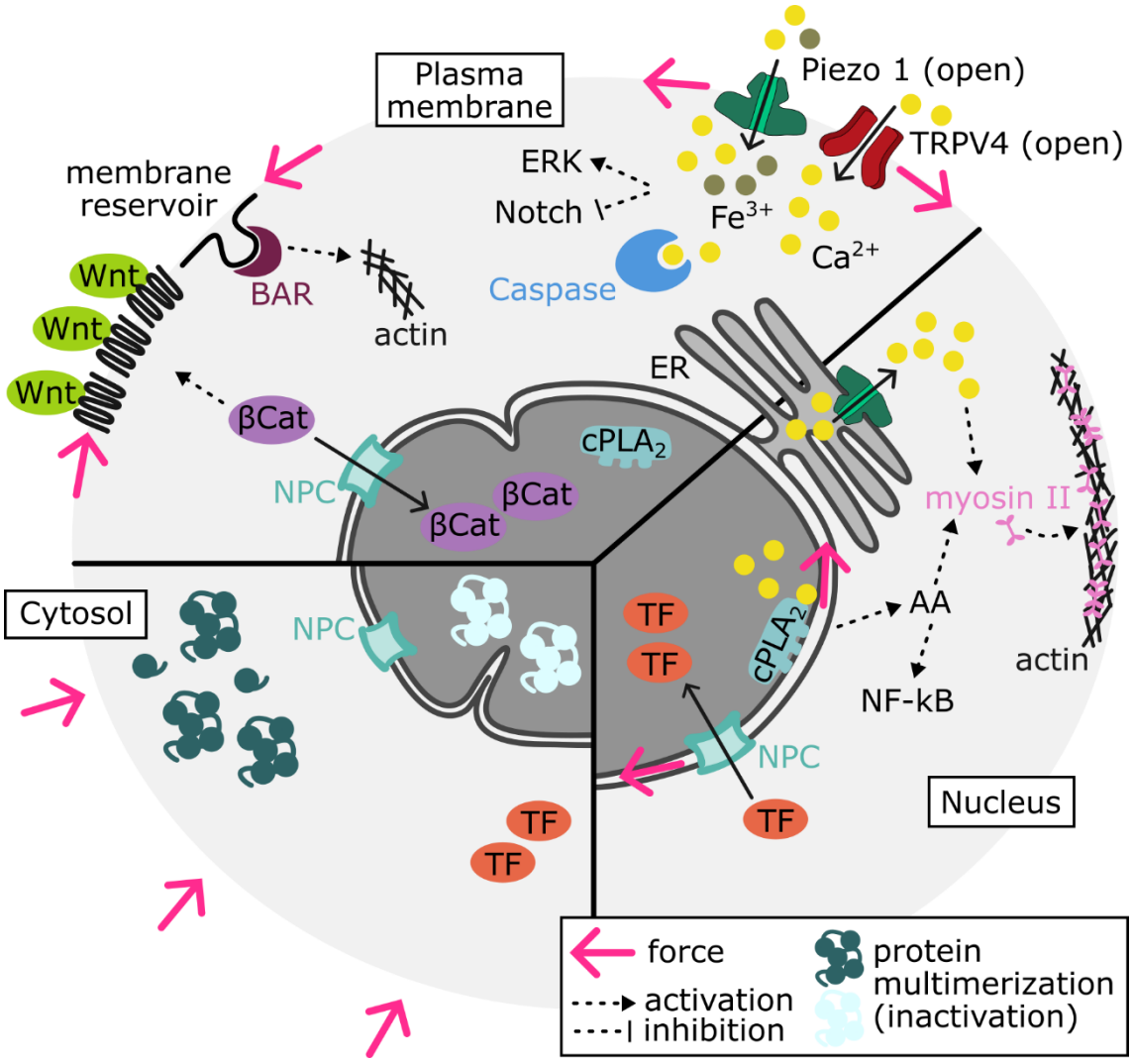

Fig. 3. Mechanosensing and mechanotransduction of cell compression. Pathways are divided according to the cell compartment in which the initial mechanosensing event occurs. At the plasma membrane (top), compression has been shown to maintain the activation of the Wnt/β-catenin pathway through the clustering of the Wnt receptor at the plasma membrane and the subsequent translocation of β-catenin to the nucleus. Plasma membrane compression promotes the formation of reservoirs that can be recognized by BAR proteins, which induces actin polymerization and subsequent reabsorption of the reservoirs. Increase in membrane tension during apicobasal or in-plane compression activates the opening of stretch-sensitive ion channels, such as Piezo1 or TRPV4, leading to the influx of Ca^{2+} and Fe^{3+} inside cells, further tuning the activity of many cellular pathways involving ERK proteins (ERK1/2, also known as MAPK3 and MAPK1, respectively), Notch proteins, caspases and myosin II (among others). In the nucleus (bottom right), nuclear deformation typically results in stretching of the nuclear envelope, inducing NPC dilation and leading to the accumulation of various mechanosensitive transcription factors (TF) like YAP, Smad3, or Twist in the nucleus. Nuclear deformation in the presence of high Ca^{2+}, can also induce the localization of $cPLA_2$ to the nuclear envelope, which can activate myosin II activity (or lead to its accumulation at the actin cortex) and promote NF-κB activation through arachidonic acid (AA) release. In the cytosol (bottom left), volumetric compression directly controls the intracellular concentration of proteins and receptors and can induce their phase separation in both the cytoplasm and nucleoplasm leading to inactivation (protein multimerization).

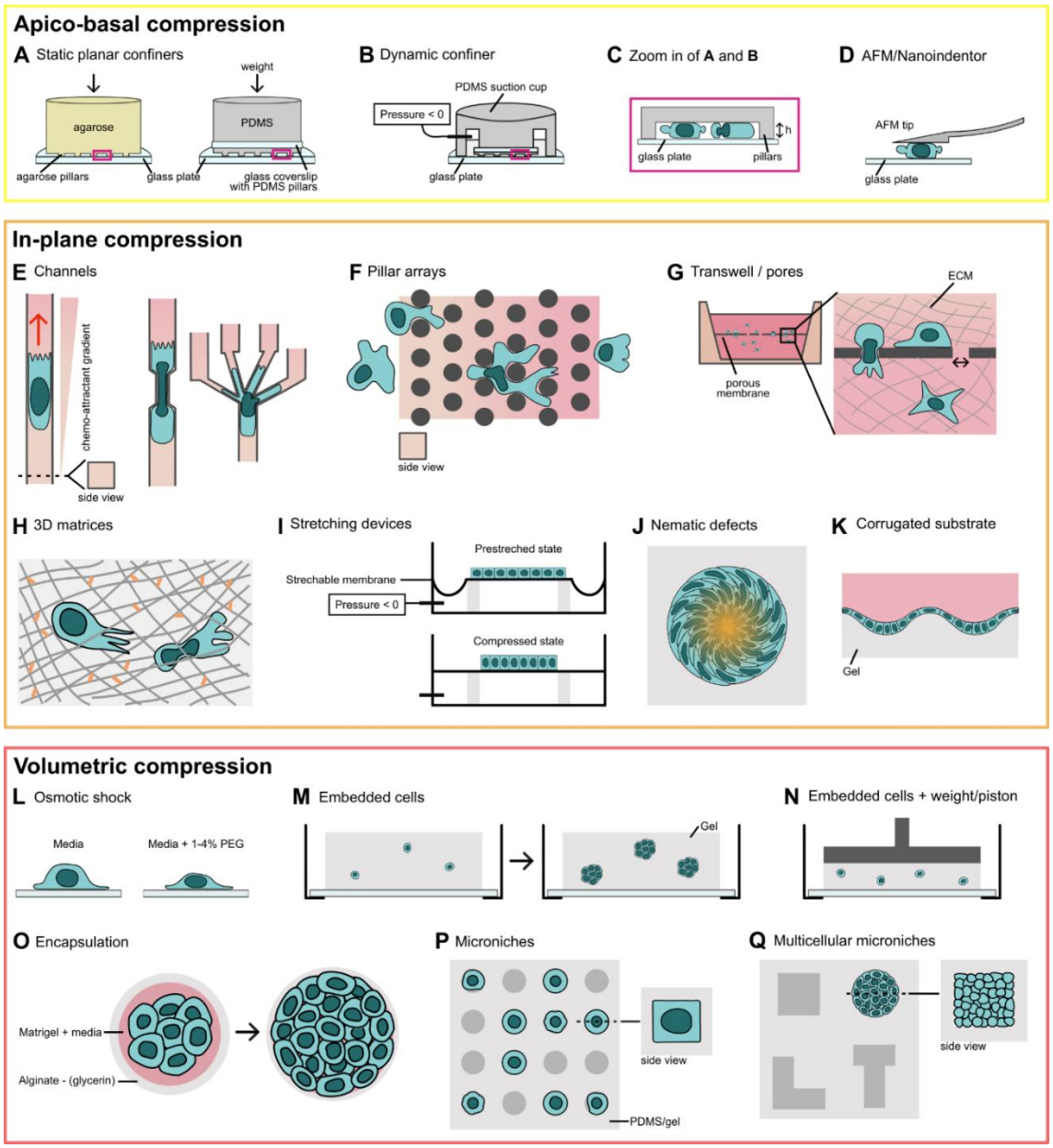

Fig. 4. Techniques to apply one-dimensional, in-plane, or volumetric cell compression *in vitro*. (A–D) Compressing along one dimension. (A) Static planar confiners made of agarose (left) or PDMS (right) allow the confinement of cells on top of a glass surface at a height defined by micro-pillars made of the same material. (B) In dynamic confiners, cells are confined similarly to in A, but the compression can be applied dynamically using a PDMS suction cup connected to a vacuum pump, whose pressure controls the position of the coverslip. (C) Magnified view of cells confined within static or dynamic confiners. (D) AFM cantilever with a flat tip to allow for planar compression over a whole cell. (E–J) In-plane compression. (E) Channels of different geometries, for example, containing constrictions or bifurcations, can be used in the presence of a chemotactic gradient, depicted as a color gradient, to direct cell migration. (F) Pillar arrays contain obstacles of various geometries and constrictions. (G) Transwell plates use a membrane suspended in the middle of a well, with pores of defined size, density, and geometry. (H) 3D matrices made of various ECM components can also be used to study confined cell migration. (I) Stretching devices can be used to impose compression on cells or epithelial tissues by releasing previously applied stretch. (J) Nematic defects, observed when cells align to pre-patterned substrates, induce local cell compression (depicted by the orange-shaded area). (K) Corrugated substrates made of PDMS can induce compression within the valley of a monolayer. (L–Q) Volumetric compression. (L)

Osmotic shock can be induced by adding large polymers or molecules like PEG to the cell medium, resulting in volumetric cell compression. (M,N) Growing cells within gels of different mechanical properties (M) can be used to control volumetric cell compression and (N) a piston can be used to additionally compress cells vertically. (O) Encapsulation methods, such as those using alginate capsules can be used to culture cell aggregates in Matrigel while imposing a maximal volume, thus inducing volumetric cell compression upon tissue growth. (P,Q) Microniches of any desired geometry can be used to compress (P) single cells or (Q) multicellular aggregates in 3D, controlling their shape and size.

Table 1. Compression setups and applications. Publications mostly focusing on methodology are highlighted in gray.

Along one dimension			
	Application	**Biological model**	**Reference**
Agarose Pad	Cell migration – mesenchymal and amoeboid migration	Walker 256 carcinosarcoma cells	(Bergert et al., 2015)
	Cell migration – invasion	Human breast cancer cells (MBA-MB- 231)	(Luo et al., 2022)
	Cell fate – cell division	HeLa, Human embryonic fibroblasts (MRC-5), Normal colon tissue-derived cell (CCD18Co)	(Aureille et al., 2019)
Confiner	Method – agarose		(Elpers et al., 2023)
	Method – PDMS		(Le Berre et al. 2014)
	Compression of nuclei	HeLa	(Enyedi et al., 2016)
	Cell migration – mesenchymal and amoeboid migration	Zebrafish progenitor stem cells	(Ruprecht et al., 2015)
		Screening of various cell lines	(Liu et al., 2015)
	Cell migration – mechanotransduction	Zebrafish progenitor stem cells	(Venturini et al., 2020)
		HeLa, Mouse bone-marrow–derived immature dendritic cells (iDCs)	(Lomakin et al., 2020)
		Human epidermal stem cells	(Nava et al., 2020)
	Cell migration - invasion	Retinal pigment epithelial cells (RPE1), Human breast epithelial cells (MCF10A), Human breast cancer cells (MBA-MB- 231)	(Nader et al., 2021)
	Cell fate – cell division	Hematopoietic cells (TF1), epithelial breast cells (MCF10A), ML2 leukemic cells, mesenchymal stroma cells (HS27A)	(Prunet et al., 2020)
	Cell fate – activate a quiescent stem cell state	Muscle Stem Cells	(Tao et al., 2023)
	Cell fate - differentiation	Dendritic cells	(Alraies et al., 2024)
	Mechanical measurement of spheroids	Coculture of fibroblasts (NIH-3T3) and hepatoma cells (H4-II-EC3) in spheroids	(Jain et al., 2024)
AFM or Cell indenter	Method – AFM		(Lulevich et al. 2006)
	Cell migration – mechanotransduction	HeLa	(Lomakin et al., 2020)
	Nuclear deformation	Fibroblasts	(Elosegui-Artola et al., 2016)
		Breast epithelial cells (MCF10A), fibroblasts	(Andreu et al., 2022; Elosegui-Artola et al., 2017)
		Ovarian Adenocarcinoma cells (SKOV3)	(Hobson et al., 2020)

In-plane compression			
	Application	**Biological model**	**Reference**
Microchannels	Method – PDMS based chips		(Heuzé et al. 2011)
	Cell migration – mechanotransduction	HeLa, Mouse bone-marrow–derived immature dendritic cells (iDCs)	(Lomakin et al., 2020)
	Cell migration – migration through pores	Dendritic cells, T-cells, leucocytes and fibroblasts	(Renkawitz et al., 2019)
		Dendritic cells, Neutrophils (derived from HL60)	(Thiam et al., 2016)
	Cell migration – mesenchymal and amoeboid migration	Walker 256 carcinosarcoma cells	(Bergert et al., 2015)
Forest of pillars	Cell migration – migration through pores	Human breast cancer cells (MBA-MB-231), fibrosarcoma cells (HT1080)	(Denais et al., 2016; Krause et al., 2019)
		Dendritic cells, T-cells, leucocytes and fibroblasts	(Renkawitz et al., 2019)
		Mouse embryonic fibroblast (NIH-3T3)	(Davidson et al., 2019)
Trans-well Plate or inlet	Cell migration – mechanotransduction	Human MCs A375P	(Lomakin et al., 2020)
	Cell migration – migration through pores	Human melanoma cells and primary human melanocytes	(Jung-Garcia et al., 2023)
		Human neutrophils from healthy donors	(Salminen et al., 2019)
	Cell fate – anoïkis resistance	Human breast cancer cells (MDA-MB- 231)	(Fanfone et al., 2022)
3D matrices	Method – 3D hydrogels		(Solbu et al. 2023)
	Cell migration – mechanotransduction	Human MCs A375P, fibroblasts HT1080	(Lomakin et al., 2020)
		Fibrosarcoma cells (HT1080), Human skin fibroblasts,	(Denais et al., 2016; Krause et al., 2019)
	Cell migration – migration through pores	Fibrosarcoma cells (HT1080), Fibrosarcoma (ACC315), Human breast cancer cells (MDA-MB-231), Human CD4+ from healthy donors, Human immune cells from healthy donors	(Wolf et al., 2003; Wolf et al., 2013)
		Dendritic cells, T-cells, leucocytes and fibroblasts	(Alraies et al., 2024; Renkawitz et al., 2019)
		Human melanoma cells and primary human melanocytes	(Jung-Garcia et al., 2023)
	Cell migration - invasion	Human breast epithelial cells (MCF10A), intestinal organoids.	(Elosegui-Artola et al., 2023)
		Mesenchymal stem cells	(Wu et al., 2022)
	Cell migration – mesenchymal and amoeboid migration	Walker 256 carcinosarcoma cells	(Bergert et al., 2015)
		Clonally derived mouse bone marrow stromal mesenchymal stem cells (D1)	(Chaudhuri et al., 2016)
	Cell fate - differentiation	Pre-osteoblasts (MC3T3E1)	(Wu et al., 2023)
Stretching devices	Method – 3D printed stretcher		(Dow et al., 2020)
	Method – LEGO stretcher		(Mäntylä and Ihalainen, 2021)
	Method – suspended monolayer		(Harris et al., 2013)

	Cell organization – membrane and cytoskeleton	Mouse embryonic fibroblasts (MEFs)	(Quiroga et al., 2023)
		Retinal pigment epithelial cells (RPE1)	(Li et al., 2023)
	Cell fate – epithelial extrusion or delamination	Epidermis monolayer	(Miroshnikova et al., 2018)
	Cell fate – epithelial delamination and differentiation	Madin-Darby canine kidney (MDCK)	(Eisenhoffer et al., 2012)
	Cell fate – epithelial folding	Madin-Darby canine kidney (MDCK)	(Wyatt et al., 2020)
	Cell fate – induce stemness	Human colon carcinoma RKO cells	(Li et al., 2021)
Patterning	Cell fate – differentiation	C2C12 - Myoblast	(Guillamat et al., 2022)
Volumetric Compression			
Osmotic shock	Cell organization – aggregation	Human osteosarcoma (U2OS)	(Jalihal et al., 2020)
		Human colorectal carcinoma	(Lee et al., 2021a)
	Cell fate – cell fate changes	Human non-small lung carcinoma	(Zhao et al., 2021)
		Hepatocellular carcinoma cells	(Gong et al., 2023)
	Cell fate – stemness induction	Human colon carcinoma RKO cells	(Li et al., 2021)
	Cell fate – dedifferentiation	Adipocytes	(Li et al., 2020)
Piston (solid or gaseous) associated with encapsulation	Cell fate – stemness induction	Human colon carcinoma RKO cells	(Li et al., 2021)
	Cell fate - differentiation	Bone marrow stem cells, Mesenchymal stem cells	(Li et al., 2009; Pelaez et al., 2012; Wang et al., 2013)
	Cell fate – cell death	Chondrocyte	(O'Conor et al., 2014; Takeda et al., 2021; Wang et al., 2024a)
		Nucleus pulpous cells	(Easson et al., 2023; Jia et al., 2024; Shi et al., 2022; Xiang et al., 2024)
	Cancer – tumor growth	Murine carcinoma cells	(Cheng et al., 2009)
Encapsulation	Method	Mouse colon carcinoma cells	(Alessandri et al., 2013)
	Method – stem cell culture	Human induced pluripotent stem cells	(Cohen et al., 2023)
	Method – cerebral organoid culture	Human embryonic pluripotent stem cells	(Wang et al., 2024b)
	Cell migration	Co-culture of colon cancer cell line (HT29) and fibroblasts (NIH3T3)	(Bertillot et al., 2024)
	Cell fate – stop cell growth	Human colon carcinoma (HT29), mouse colon adenocarcinoma (CT26), human breast cancer cells (BC52), mouse sarcoma cells (AB6) and mouse Schwann cells (FHI)	(Delarue et al., 2014)
		Human breast cancer cells (MDA-MB-231) and normal breast epithelial cells (MCF10A)	(Nam et al., 2019)
		Madin-Darby canine kidney (MDCK)	(Di Meglio et al., 2022)

Encapsulation	Cell fate – stemness induction	Breast cancer cell line (MCF7)	(Fuentes-Chandía et al., 2021)
	Cell fate – epithelial folding	Madin-Darby canine kidney (MDCK)	(Di Meglio et al., 2022)
	Cancer – cell invasion	Breast epithelial cells (MCF10A)	(Chaudhuri et al., 2014; Han et al., 2020)
		Human colorectal cancer cells (DLD-1)	(Nishi et al., 2022)
Microniche	Method		(Comelles et al., 2020)
	Cell organization	Human mesenchymal stem cells	(Bao et al., 2017)
		Mouse Myoblasts C2C12	(Wilson et al., 2021)
		Mouse intestinal organoids	(Gjorevski et al., 2022)
	Cell fate – differentiation	Mouse embryonic stem cells	(Bao and Xie, 2023)

References

Afthinos, A., Bera, K., Chen, J., Ozcelikkale, A., Amitrano, A., Choudhury, M. I., Huang, R., Pachidis, P., Mistriotis, P., Chen, Y., et al. (2022). Migration and 3D Traction Force Measurements inside Compliant Microchannels. *Nano Lett.* 22, 7318–7327.

Alasaadi, D. N., Alvizi, L., Hartmann, J., Stillman, N., Moghe, P., Hiiragi, T. and Mayor, R. (2024). Competence for neural crest induction is controlled by hydrostatic pressure through Yap. *Nat Cell Biol* 26, 530–541.

Alessandri, K., Sarangi, B. R., Gurchenkov, V. V., Sinha, B., Kießling, T. R., Fetler, L., Rico, F., Scheuring, S., Lamaze, C., Simon, A., et al. (2013). Cellular capsules as a tool for multicellular spheroid production and for investigating the mechanics of tumor progression in vitro. *Proc. Natl. Acad. Sci. U.S.A.* 110, 14843–14848.

Alexander, S., Weigelin, B., Winkler, F. and Friedl, P. (2013). Preclinical intravital microscopy of the tumour-stroma interface: invasion, metastasis, and therapy response. *Current Opinion in Cell Biology* 25, 659–671.

Alraies, Z., Rivera, C. A., Delgado, M.-G., Sanséau, D., Maurin, M., Amadio, R., Maria Piperno, G., Dunsmore, G., Yatim, A., Lacerda Mariano, L., et al. (2024). Cell shape sensing licenses dendritic cells for homeostatic migration to lymph nodes. *Nat Immunol* 1–14.

Andolfi, L., Greco, S. L. M., Tierno, D., Chignola, R., Martinelli, M., Giolo, E., Luppi, S., Delfino, I., Zanetti, M., Battistella, A., et al. (2019). Planar AFM macro-probes to study the biomechanical properties of large cells and 3D cell spheroids. *Acta Biomaterialia* 94, 505– 513.

Andreu, I., Granero-Moya, I., Chahare, N. R., Clein, K., Molina-Jordán, M., Beedle, A. E. M., Elosegui-Artola, A., Abenza, J. F., Rossetti, L., Trepat, X., et al. (2022). Mechanical force application to the nucleus regulates nucleocytoplasmic transport. *Nature Cell Biology* 24, 896–905.

Aragona, M., Panciera, T., Manfrin, A., Giulitti, S., Michielin, F., Elvassore, N., Dupont, S. and Piccolo, S. (2013). A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors. *Cell* 154, 1047–1059.

Aureille, J., Buffière-Ribot, V., Harvey, B. E., Boyault, C., Pernet, L., Andersen, T., Bacola, G., Balland, M., Fraboulet, S., Van Landeghem, L., et al. (2019). Nuclear envelope deformation controls cell cycle progression in response to mechanical force. *EMBO reports* 20, 1–11.

Banes, A. J., Gilbert, J., Taylor, D. and Monbureau, O. (1985). A new vacuum-operated stress-providing instrument that applies static or variable duration cyclic tension or compression to cells *in vitro*. *Journal of Cell Science* 75, 35–42.

Bao, M. and Xie, J. (2023). Geometric Confinement-Mediated Mechanical Tension Directs Patterned Differentiation of Mouse ESCs into Organized Germ Layers. *ACS Appl. Mater. Interfaces* 15, 34397–34406.

Bao, M., Xie, J., Piruska, A. and Huck, W. T. S. (2017). 3D microniches reveal the importance of cell size and shape. *Nat Commun* 8, 1962.

Barbazan, J., Pérez-González, C., Gómez-González, M., Dedenon, M., Richon, S., Latorre, E., Serra, M., Mariani, P., Descroix, S., Sens, P., et al. (2023). Cancer-associated fibroblasts actively compress cancer cells and modulate mechanotransduction. *Nat Commun* 14, 6966.

Bergert, T., Erzberger, A., Desai, R. A., Aspalter, I. M., Oates, A. C., Charras, G.,Salbreux, G. and Paluch, E. K. (2015). Force transmission during adhesion-independent migration. Nat. Cell Biol. 17, 524-529.

Bertillot, F., Andrique, L., Ureña Martin, C., Zajac, O., De Plater, L., Norton, M. M., Richard, A., Alessandri, K., Gurchenkov, B. G., Fage, F., et al. (2024). Compressive stress triggers fibroblasts spreading over cancer cells to generate carcinoma in situ organization. *Commun Biol* 7, 184.

Biedzinski, S., Agsu, G., Vianay, B., Delord, M., Blanchoin, L., Larghero, J., Faivre, L., Théry, M. and Brunet, S. (2020). Microtubules control nuclear shape and gene expression during early stages of hematopoietic differentiation. *The EMBO Journal* 39, 1–15.

Boekhorst, V. te, Preziosi, L. and Friedl, P. (2016). Plasticity of Cell Migration In Vivo and In Silico. *Annual Review of Cell and Developmental Biology* 32, 491–526.

Böhringer, D., Cóndor, M., Bischof, L., Czerwinski, T., Gampl, N., Ngo, P. A., Bauer, A., Voskens, C., López-Posadas, R., Franze, K., et al. (2024). Dynamic traction force measurements of migrating immune cells in 3D biopolymer matrices. Nat. Phys. 20, 1816–1823.

Caille, N., Tardy, Y. and Meister, J. J. (1998). Assessment of Strain Field in Endothelial Cells Subjected to Uniaxial Deformation of Their Substrate. *Annals of Biomedical Engineering* 26, 409–416.

Campinho, P., Behrndt, M., Ranft, J., Risler, T., Minc, N. and Heisenberg, C. P. (2013). Tension-oriented cell divisions limit anisotropic tissue tension in epithelial spreading during zebrafish epiboly. *Nature Cell Biology* 15, 1405–1414.

Chakravorty, S. J., McGettrick, H. M., Butler, L. M., Buckley, C. D., Rainger, G. E. and Nash, G. B. (2006). An in vitro model for analysing neutrophil migration into and away from the sub-endothelial space: Roles of flow and CD31. Biorheology 43, 71–82.

Chalfie, M. and Sulston, J. (1981). Developmental genetics of the mechanosensory neurons of Caenorhabditis elegans. *Developmental Biology* 88, 358–370.

Chaudhuri, O. (2017). Viscoelastic hydrogels for 3D cell culture. *Biomater. Sci.* 5, 1480–1490.

Chaudhuri, O., Gu, L., Klumpers, D., Darnell, M., Bencherif, S. A., Weaver, J. C., Huebsch, N., Lee, H., Lippens, E., Duda, G. N., et al. (2016). Hydrogels with tunable stress relaxation regulate stem cell fate and activity. *Nature Mater* 15, 326–334.

Chaudhuri, O., Koshy, S. T., Branco Da Cunha, C., Shin, J.-W., Verbeke, C. S., Allison, K. H. and Mooney, D. J. (2014). Extracellular matrix stiffness and composition jointly regulate the induction of malignant phenotypes in mammary epithelium. Nature Mater 13, 970–978.

Chen, H.-C. (2005). Boyden Chamber Assay. In *Cell Migration: Developmental Methods and Protocols* (ed. Guan, J.-L.), pp. 15–22. Totowa, NJ: Humana Press.

Cheng, G., Tse, J., Jain, R. K. and Munn, L. L. (2009). Micro-Environmental Mechanical Stress Controls Tumor Spheroid Size and Morphology by Suppressing Proliferation and Inducing Apoptosis in Cancer Cells. *PLOS ONE* 4, e4632.

Chung, H. H., Mireles, M., Kwarta, B. J. and Gaborski, T. R. (2018). Use of Porous membranes in tissue barrier and co-culture models. *Lab Chip* 18, 1671–1689.

Cohen, P. J. R., Luquet, E., Pletenka, J., Leonard, A., Warter, E., Gurchenkov, B., Carrere, J., Rieu, C., Hardouin, J., Moncaubeig, F., et al. (2023). Engineering 3D micro-compartments for highly efficient and scale-independent expansion of human pluripotent stem cells in bioreactors. *Biomaterials* 295, 122033.

Comelles, J., Fernández-Majada, V., Berlanga-Navarro, N., Acevedo, V., Paszkowska, K. and Martínez, E. (2020). Microfabrication of poly(acrylamide) hydrogels with independently controlled topography and stiffness. Biofabrication 12, 025023.

Conti, S., Venturini, V., Cañellas-Socias, A., Cortina, C., Abenza, J. F., Stephan-Otto Attolini, C., Middendorp Guerra, E., Xu, C. K., Li, J. H., Rossetti, L., et al. (2024). Membrane to cortex attachment determines different mechanical phenotypes in LGR5+ and LGR5- colorectal cancer cells. Nature Communications 15, 3363–3363.

Corey, D. P. and Hudspeth, A. J. (1979). Ionic basis of the receptor potential in a vertebrate hair cell. *Nature* 281.

Coste, B., Mathur, J., Schmidt, M., Earley, T. J., Ranade, S., Petrus, M. J., Dubin, A. E. and Patapoutian, A. (2010). Piezo1 and Piezo2 Are Essential Components of Distinct Mechanically Activated Cation Channels. Science 330, 55–60.

Davidson, P. M., Battistella, A., Dejardin, T., Betz, T., Plastino, J., Cadot, B., Borghi, N. and Sykes, C. (2019). Actin Accumulates Nesprin-2 at the Front of the Nucleus During Confined Cell Migration. *EMBO Reports* 1–14.

Delarue, M., Montel, F., Vignjevic, D., Prost, J., Joanny, J.-F. and Cappello, G. (2014). Compressive Stress Inhibits Proliferation in Tumor Spheroids through a Volume Limitation. *Biophysical Journal* 107, 1821–1828.

Denais, C. M., Gilbert, R. M., Isermann, P., McGregor, A. L., Te Lindert, M., Weigelin, B., Davidson, P. M., Friedl, P., Wolf, K. and Lammerding, J. (2016). Nuclear envelope rupture and repair during cancer cell migration. *Science* 352, 353–358.

Di Meglio, I., Trushko, A., Guillamat, P., Blanch-Mercader, C., Abuhattum, S. and Roux, A. (2022). Pressure and curvature control of the cell cycle in epithelia growing under spherical confinement. *Cell Reports* 40, 111227.

Dow, L. P., Khankhel, A. H., Abram, J. and Valentine, M. T. (2020). 3D-Printable Cell Crowding Device Enables Imaging of Live Cells in Compression. *BioTechniques* 68, 275–278.

Driscoll, M. K., Welf, E. S., Weems, A., Sapoznik, E., Zhou, F., Murali, V. S., García-Arcos, J. M., Roh-Johnson, M., Piel, M., Dean, K. M., et al. (2024). Proteolysis-free amoeboid migration of melanoma cells through crowded environments via bleb-driven worrying. Developmental Cell 59, 2414-2428.e8.

Dupont, S. and Wickström, S. A. (2022). Mechanical regulation of chromatin and transcription. *Nature Reviews Genetics*.

Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., Zanconato, F., Le Digabel, J., Forcato, M., Bicciato, S., et al. (2011). Role of YAP/TAZ in mechanotransduction. *Nature* 474, 179–183.

Easson, G. W. D., Savadipour, A., Anandarajah, A., Iannucci, L. E., Lake, S. P., Guilak, F. and Tang, S. Y. (2023). Modulation of TRPV4 protects against degeneration induced by sustained loading and promotes matrix synthesis in the intervertebral disc. *The FASEB Journal* 37, e22714.

Ehrlicher, A. J., Nakamura, F., Hartwig, J. H. and Stossel, T. P. (2011). Mechanical strain in actin networks regulates FilGAP and integrin binding to filamin A. *Nature* 478.

Eisenhoffer, G. T., Loftus, P. D., Yoshigi, M., Otsuna, H., Chien, C. B., Morcos, P. A. and Rosenblatt, J. (2012). Crowding induces live cell extrusion to maintain homeostatic cell numbers in epithelia. *Nature* 484, 546–549.

Elosegui-Artola, A., Oria, R., Chen, Y., Kosmalska, A., Pérez-González, C., Castro, N., Zhu, C., Trepat, X. and Roca-Cusachs, P. (2016). Mechanical regulation of a molecular clutch defines force transmission and transduction in response to matrix rigidity. *Nat Cell Biol* 18, 540–548.

Elosegui-Artola, A., Andreu, I., Beedle, A. E. M., Lezamiz, A., Uroz, M., Kosmalska, A. J., Oria, R., Kechagia, J. Z., Rico-Lastres, P., Le Roux, A.-L., et al. (2017). Force Triggers YAP Nuclear Entry by Regulating Transport across Nuclear Pores. *Cell* 171, 1397-1410.e14.

Elosegui-Artola, A., Gupta, A., Najibi, A. J., Seo, B. R., Garry, R., Tringides, C. M., de Lázaro, I., Darnell, M., Gu, W., Zhou, Q., et al. (2023). Matrix viscoelasticity controls spatiotemporal tissue organization. *Nat. Mater.* 22, 117–127.

Elpers, M. A., Varlet, A.-A., Agrawal, R. and Lammerding, J. (2023). Agarose-based 3D Cell Confinement Assay to Study Nuclear Mechanobiology. *Current Protocols* 3, e847.

Emon, B., Joy, M. S. H., Lalonde, L., Ghrayeb, A., Doha, U., Ladehoff, L., Brockstein, R., Saengow, C., Ewoldt, R. H. and Saif, M. T. A. (2024). Nuclear deformation regulates YAP dynamics in cancer associated fibroblasts. Acta Biomater 173, 93–108.

Enyedi, B., Jelcic, M. and Niethammer, P. (2016). The Cell Nucleus Serves as a Mechanotransducer of Tissue Damage-Induced Inflammation. *Cell* 165, 1160–1170.

Fadul, J., Zulueta-Coarasa, T., Slattum, G. M., Redd, N. M., Jin, M. F., Redd, M. J., Daetwyler, S., Hedeen, D., Huisken, J. and Rosenblatt, J. (2021). KRas-transformed epithelia cells invade and partially dedifferentiate by basal cell extrusion. *Nat Commun* 12, 7180.

Fanfone, D., Wu, Z., Mammi, J., Berthenet, K., Neves, D., Weber, K., Halaburkova, A., Virard, F., Bunel, F., Jamard, C., et al. (2022). Confined migration promotes cancer metastasis through resistance to anoikis and increased invasiveness. *eLife* 11, e73150.

Faure, L. M., Gómez-González, M., Baguer, O., Comelles, J., Martínez, E., Arroyo, M., Trepat, X. and Roca-Cusachs, P. (2024). 3D Micropatterned Traction Force Microscopy: A Technique to Control 3D Cell Shape While Measuring Cell-Substrate Force Transmission. *Advanced Science* 2406932.

Fernández-Sánchez, M. E., Barbier, S., Whitehead, J., Béalle, G., Michel, A., Latorre-Ossa, H., Rey, C., Fouassier, L., Claperon, A., Brullé, L., et al. (2015). Mechanical induction of the tumorigenic β-catenin pathway by tumour growth pressure. *Nature* 523, 92–95.

Friedl, P., Hegerfeldt, Y. and Tusch, M. (2004). Collective cell migration in morphogenesis and cancer. *Int. J. Dev. Biol.* 48, 441–449.

Friedl, P. and Weigelin, B. (2008). Interstitial leukocyte migration and immune function. *Nat Immunol* 9, 960–969.

Fu, S., Meng, H., Inamdar, S., Das, B., Gupta, H., Wang, W., Thompson, C. L. and Knight, M. M. (2021). Activation of TRPV4 by mechanical, osmotic or pharmaceutical stimulation is anti- inflammatory blocking IL-1β mediated articular cartilage matrix destruction. *Osteoarthritis and Cartilage* 29, 89–99.

Fuentes-Chandía, M., Vierling, A., Kappelmann-Fenzl, M., Monavari, M., Letort, G., Höne, L., Parma, B., Antara, S. K., Ertekin, Ö., Palmisano, R., et al. (2021). 3D Spheroids Versus 3D Tumor-Like Microcapsules: Confinement and Mechanical Stress May Lead to the Expression of Malignant Responses in Cancer Cells. *Advanced Biology* 5, 2000349.

Gaertner, F., Reis-Rodrigues, P., de Vries, I., Hons, M., Aguilera, J., Riedl, M., Leithner, A., Tasciyan, S., Kopf, A., Merrin, J., et al. (2022). WASp triggers mechanosensitive actin patches to facilitate immune cell migration in dense tissues. Developmental Cell 57, 47-62.e9.

Ge, J., Li, W., Zhao, Q., Li, N., Chen, M., Zhi, P., Li, R., Gao, N., Xiao, B. and Yang, M. (2015). Architecture of the mammalian mechanosensitive Piezo1 channel. Nature 527, 64–69.

Gerstmair, A., Fois, G., Innerbichler, S., Dietl, P. and Felder, E. (2009). A device for simultaneous live cell imaging during uni-axial mechanical strain or compression. *Journal of Applied Physiology* 107, 613–620.

Gjorevski, N., Nikolaev, M., Brown, T. E., Mitrofanova, O., Brandenberg, N., DelRio, F. W., Yavitt, F. M., Liberali, P., Anseth, K. S. and Lutolf, M. P. (2022). Tissue geometry drives deterministic organoid patterning. *Science* 375, eaaw9021.

Gong, X., Ogino, N., Leite, M. F., Chen, Z., Nguyen, R., Liu, R., Kruglov, E., Flores, K., Cabral, A., Mendes, G. M. M., et al. (2023). Adaptation to volumetric compression drives hepatoblastoma cells to an apoptosis-resistant and invasive phenotype.

Granero-Moya, I., Venturini, V., Belthier, G., Groenen, B., Molina-Jordán, M., González-Martín, M., Trepat, X., van Rheenen, J., Andreu, I. and Roca-Cusachs, P. (2024). Nucleocytoplasmic transport senses mechanical forces independently of cell density in cell monolayers. *Journal of Cell Science* 137, jcs262363.

Gu, Y., Shea, J., Slattum, G., Firpo, M. A., Alexander, M., Mulvihill, S. J., Golubovskaya, V. M. and Rosenblatt, J. (2015). Defective apical extrusion signaling contributes to aggressive tumor hallmarks. *eLife* 4, e04069.

Gudipaty, S. A., Lindblom, J., Loftus, P. D., Redd, M. J., Edes, K., Davey, C. F., Krishnegowda, V. and Rosenblatt, J. (2017). Mechanical stretch triggers rapid epithelial cell division through Piezo1. *Nature* 543, 118–121.

Guillamat, P., Blanch-Mercader, C., Pernollet, G., Kruse, K. and Roux, A. (2022). Integer topological defects organize stresses driving tissue morphogenesis. *Nat. Mater.* 21, 588– 597.

Guilluy, C., Osborne, L. D., Van Landeghem, L., Sharek, L., Superfine, R., Garcia-Mata, R. and Burridge, K. (2014). Isolated nuclei adapt to force and reveal a mechanotransduction pathway in the nucleus. *Nature Cell Biology* 16, 376–381.

Hampoelz, B. and Lecuit, T. (2011). Nuclear mechanics in differentiation and development. *Current Opinion in Cell Biology* 23, 668–675.

Han, Y. L., Pegoraro, A. F., Li, H., Li, K., Yuan, Y., Xu, G., Gu, Z., Sun, J., Hao, Y., Gupta, S. K., et al. (2020). Cell swelling, softening and invasion in a three-dimensional breast cancer model. *Nat. Phys.* 16, 101–108.

Han, J., Zhan, L., Huang, Y., Guo, S., Zhou, X., Kapilevich, L., Wang, Z., Ning, K., Sun, M. and Zhang, X. (2024). Moderate mechanical stress suppresses chondrocyte ferroptosis in osteoarthritis by regulating NF-κB p65/GPX4 signaling pathway. *Sci Rep* 14, 5078.

Harris, A. R., Bellis, J., Khalilgharibi, N., Wyatt, T., Baum, B., Kabla, A. J. and Charras, G. T. (2013). Generating suspended cell monolayers for mechanobiological studies. *Nat Protoc* 8, 2516–2530.

He, L., Si, G., Huang, J., Samuel, A. D. T. and Perrimon, N. (2018). Mechanical regulation of stem- cell differentiation by the stretch-activated Piezo channel. *Nature* 555, 103–106.

Helmlinger, G., Netti, P. A., Lichtenbeld, H. C., Melder, R. J. and Jain, R. K. (1997). Solid stress inhibits the growth of multicellular tumor spheroids. 15.

Heuzé, M. L., Collin, O., Terriac, E., Lennon-Duménil, A. M. and Piel, M. (2011). Cell migration in confinement: A micro-channel-based assay. *Methods in Molecular Biology* 769, 415–434.

Hobson, C. M., Kern, M., O'Brien, E. T., Stephens, A. D., Falvo, M. R. and Superfine, R. (2020). Correlating nuclear morphology and external force with combined atomic force microscopy and light sheet imaging separates roles of chromatin and lamin A/C in nuclear mechanics. *Molecular Biology of the Cell* 31, 1788–1801.

Huang, M. and Chalfie, M. (1994). Gene interactions affecting mechanosensory transduction in Caaenorhabditis elegans. *Nature* 367, 467–470.

Huang, L., Mathieu, P. S. and Helmke, B. P. (2010). A Stretching Device for High-Resolution Live- Cell Imaging. *Ann Biomed Eng* 38, 1728–1740.

Hung, W. C., Yang, J. R., Yankaskas, C. L., Wong, B. S., Wu, P. H., Pardo-Pastor, C., Serra, S. A., Chiang, M. J., Gu, Z., Wirtz, D., et al. (2016). Confinement Sensing and Signal Optimization via Piezo1/PKA and Myosin II Pathways. *Cell Reports* 15, 1430–1441.

Huselstein, C., Netter, P., De Isla, N., Wang, Y., Gillet, P., Decot, V., Muller, S., Bensoussan, D. and Stoltz, J. (2008). Mechanobioloy, condrocyte and cartilage. *Biomed Mater Eng.* 213– 220.

Isermann, P. and Lammerding, J. (2013). Nuclear mechanics and mechanotransduction in health and disease. *Current Biology* 23, R1113–R1121.

Jain, S., Belkadi, H., Michaut, A., Sart, S., Gros, J., Genet, M. and Baroud, C. N. (2024). Using a micro-device with a deformable ceiling to probe stiffness heterogeneities within 3D cell aggregates. *Biofabrication* 16, 035010.

Jalihal, A. P., Pitchiaya, S., Xiao, L., Bawa, P., Jiang, X., Bedi, K., Parolia, A., Cieslik, M., Ljungman, M., Chinnaiyan, A. M., et al. (2020). Multivalent Proteins Rapidly and Reversibly Phase- Separate upon Osmotic Cell Volume Change. *Molecular Cell* 79, 978-990.e5.

Jia, C., Xiang, Z., Zhang, P., Liu, L., Zhu, X., Yu, R., Liu, Z., Wang, S., Liu, K., Wang, Z., et al. (2024). Selenium-SelK-GPX4 axis protects nucleus pulposus cells against mechanical overloading-induced ferroptosis and attenuates senescence of intervertebral disc. *Cell. Mol. Life Sci.* 81, 49.

Jin, S., He, D., Wang, Y., Zhang, T., Yu, H., Li, Z., Zhu, L., Zhou, Y. and Liu, Y. (2020). Mechanical force modulates periodontal ligament stem cell characteristics during bone remodelling via TRPV4. *Cell Proliferation* 53, e12912.

Jung-Garcia, Y., Maiques, O., Monger, J., Rodriguez-Hernandez, I., Fanshawe, B., Domart, M. C., Renshaw, M. J., Marti, R. M., Matias-Guiu, X., Collinson, L. M., et al. (2023). LAP1 supports nuclear adaptability during constrained melanoma cell migration and invasion. *Nature Cell Biology* 25, 108–119.

Kameritsch, P. and Renkawitz, J. (2020). Principles of Leukocyte Migration Strategies. *Trends in Cell Biology* 1–15.

Kosmalska, A. J., Casares, L., Elosegui-Artola, A., Thottacherry, J. J., Moreno-Vicente, R., González-Tarragó, V., Del Pozo, M. Á., Mayor, S., Arroyo, M., Navajas, D., et al. (2015). Physical principles of membrane remodelling during cell mechanoadaptation. *Nat Commun* 6, 7292.

Krause, M., Yang, F. W., te Lindert, M., Isermann, P., Schepens, J., Maas, R. J. A., Venkataraman, C., Lammerding, J., Madzvamuse, A., Hendriks, W., et al. (2019). Cell migration through three-dimensional confining pores: speed accelerations by deformation and recoil of the nucleus. *Philos Trans R Soc Lond B Biol Sci* 374, 20180225.

Kroll, J., Hauschild, R., Kuznetcov, A., Stefanowski, K., Hermann, M. D., Merrin, J., Shafeek, L., Müller-Taubenberger, A. and Renkawitz, J. (2023). Adaptive pathfinding by nucleokinesis during amoeboid migration. The EMBO Journal 42, e114557.

Laakso, J. M., Lewis, J. H., Shuman, H. and Ostap, E. M. (2008). Myosin I Can Act As a Molecular Force Sensor. *Science* 321.

Lautenschläger, F. and Piel, M. (2013). Microfabricated devices for cell biology: all for one and one for all. Current Opinion in Cell Biology 25, 116–124.

Le Berre, M., Zlotek-Zlotkiewicz, E., Bonazzi, D., Lautenschlaeger, F. and Piel, M. (2014). Methods for two-dimensional cell confinement. *Methods in Cell Biology*.

Le Roux, A.-L., Quiroga, X., Walani, N., Arroyo, M. and Roca-Cusachs, P. (2019). The plasma membrane as a mechanochemical transducer. *Phil. Trans. R. Soc. B* 374, 20180221.

Le Roux, A.-L., Tozzi, C., Walani, N., Quiroga, X., Zalvidea, D., Trepat, X., Staykova, M., Arroyo, M. and Roca-Cusachs, P. (2021). Dynamic mechanochemical feedback between curved membranes and BAR protein self-organization. *Nat Commun* 12, 6550.

Lee, J., Le, L. T. H. L., Kim, E. and Lee, M. J. (2021a). Formation of Non-Nucleoplasmic Proteasome Foci during the Late Stage of Hyperosmotic Stress. *Cells* 10, 2493.

Lee, J., Kim, S.-E., Moon, D. and Doh, J. (2021b). A multilayered blood vessel/tumor tissue chip to investigate T cell infiltration into solid tumor tissues. Lab Chip 21, 2142–2152.

Li, M. (2024). Research progress on the regulatory role of cell membrane surface tension in cell behavior. Heliyon 10, e29923. doi:10.1016/j.heliyon.2024.e29923.

Li, J., Zhao, Z., Yang, J., Liu, J., Wang, J., Li, X. and Liu, Y. (2009). p38 MAPK mediated in compressive stress-induced chondrogenesis of rat bone marrow MSCs in 3D alginate scaffolds. *Journal Cellular Physiology* 221, 609–617.

Li, Q., Zhang, Y., Pluchon, P., Robens, J., Herr, K., Mercade, M., Thiery, J.-P., Yu, H. and Viasnoff, V. (2016). Extracellular matrix scaffolding guides lumen elongation by inducing anisotropic intercellular mechanical tension. *Nat. Cell Biol.* 18, 311–318.

Li, Y., Mao, A. S., Seo, B. R., Zhao, X., Gupta, S. K., Chen, M., Han, Y. L., Shih, T.-Y., Mooney, D. J. and Guo, M. (2020). Compression-induced dedifferentiation of adipocytes promotes tumor progression. *Sci. Adv.* 6, eaax5611.

Li, Y., Chen, M., Hu, J., Sheng, R., Lin, Q., He, X. and Guo, M. (2021). Volumetric Compression Induces Intracellular Crowding to Control Intestinal Organoid Growth via Wnt/β-Catenin Signaling. *Cell Stem Cell* 28, 63-78.e7.

Li, Y., Kučera, O., Cuvelier, D., Rutkowski, D. M., Deygas, M., Rai, D., Pavlovič, T., Vicente, F. N., Piel, M., Giannone, G., et al. (2023). Compressive forces stabilize microtubules in living cells. *Nat. Mater.* 22, 913–924.

Liu, Y. J., Le Berre, M., Lautenschlaeger, F., Maiuri, P., Callan-Jones, A., Heuzé, M., Takaki, T., Voituriez, R. and Piel, M. (2015). Confinement and low adhesion induce fast amoeboid migration of slow mesenchymal cells. *Cell* 160, 659–672.

Lolo, F.-N., Walani, N., Seemann, E., Zalvidea, D., Pavón, D. M., Cojoc, G., Zamai, M., Viaris De Lesegno, C., Martínez De Benito, F., Sánchez-Álvarez, M., et al. (2023). Caveolin-1 dolines form a distinct and rapid caveolae-independent mechanoadaptation system. *Nat Cell Biol* 25, 120–133.

Lomakin, A. J., Cattin, C. J., Cuvelier, D., Alraies, Z., Molina, M., Nader, G. P. F., Srivastava, N., Garcia-Arcos, J. M., Zhitnyak, I. Y., Bhargava, A., et al. (2020). The nucleus acts as a ruler tailoring cell responses to spatial constraints. *Science* 2894,.

Luciano, M., Xue, S.-L., De Vos, W. H., Redondo-Morata, L., Surin, M., Lafont, F., Hannezo, E. and Gabriele, S. (2021). Cell monolayers sense curvature by exploiting active mechanics and nuclear mechanoadaptation. *Nat. Phys.* 17, 1382–1390.

Lulevich, V., Zink, T., Chen, H.-Y., Liu, F.-T. and Liu, G. (2006). Cell Mechanics Using Atomic Force Microscopy-Based Single-Cell Compression. *Langmuir* 22, 8151–8155.

Luo, M., Cai, G., Ho, K. K. Y., Wen, K., Tong, Z., Deng, L. and Liu, A. P. (2022). Compression enhances invasive phenotype and matrix degradation of breast cancer cells via Piezo1 activation. *BMC Mol and Cell Biol* 23, 1.

Ma, Y., Han, T., Yang, Q., Wang, J., Feng, B., Jia, Y., Wei, Z. and Xu, F. (2021). Viscoelastic Cell Microenvironment: Hydrogel-Based Strategy for Recapitulating Dynamic ECM Mechanics. *Advanced Functional Materials* 31, 2100848.

Mammoto, A., Mammoto, T. and Ingber, D. E. (2012). Mechanosensitive mechanisms in transcriptional regulation. *Journal of Cell Science* 125, 3061–3073.

Maniotis, A. J., Chen, C. S. and Ingber, D. E. (1997). Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proceedings of the National Academy of Sciences* 94, 849–854.

Mäntylä, E. and Ihalainen, T. O. (2021). Brick Strex: a robust device built of LEGO bricks for mechanical manipulation of cells. *Sci Rep* 11, 18520.

Marinari, E., Mehonic, A., Curran, S., Gale, J., Duke, T. and Baum, B. (2012). Live-cell delamination counterbalances epithelial growth to limit tissue overcrowding. Nature 484, 542–545.

Mary, G., Malgras, B., Perez, J. E., Nagle, I., Luciani, N., Pimpie, C., Asnacios, A., Pocard, M., Reffay, M. and Wilhelm, C. (2022). Magnetic Compression of Tumor Spheroids Increases Cell Proliferation In Vitro and Cancer Progression In Vivo. *Cancers* 14, 366.

McClatchey, A. I. and Yap, A. S. (2012). Contact inhibition (of proliferation) redux. *Current Opinion in Cell Biology* 24, 685–694.

McCreery, K. P., Stubb, A., Stephens, R., Fursova, N. A., Cook, A., Kruse, K., Michelbach, A., Biggs, L. C., Keikhosravi, A., Nykanen, S., et al. (2024). Mechano-osmotic signals control chromatin state and fate transitions in pluripotent stem cells.

Miroshnikova, Y. A., Le, H. Q., Schneider, D., Thalheim, T., Rübsam, M., Bremicker, N., Polleux, J., Kamprad, N., Tarantola, M., Wang, I., et al. (2018). Adhesion forces and cortical tension couple cell proliferation and differentiation to drive epidermal stratification. *Nat Cell Biol* 20, 69–80.

Mohajeri, M., Eskandari, M., Ghazali, Z. S. and Ghazali, H. S. (2022). Cell encapsulation in alginate-based microgels using droplet microfluidics; a review on gelation methods and applications. *Biomed. Phys. Eng. Express* 8, 022001.

Montell, D. J. (2003). Border-cell migration: the race is on. *Nat Rev Mol Cell Biol* 4, 13–24.

Moore, E., Zhao, R., McKinney, M. C., Yi, K., Wood, C. and Trainor, P. (2024). Cell extrusion - a novel mechanism driving neural crest cell delamination.

Mosier, J. A., Fabiano, E. D., Ludolph, C. M., White, A. E. and Reinhart-King, C. A. (2023). Confinement primes cells for faster migration by polarizing active mitochondria. *Nanoscale Adv.* 6, 209–220.

Nader, G. P. de F., Williart, A. and Piel, M. (2021). Nuclear deformations, from signaling to perturbation and damage. *Current Opinion in Cell Biology* 72, 137–145.

Nam, S., Gupta, V. K., Lee, H., Lee, J. Y., Wisdom, K. M., Varma, S., Flaum, E. M., Davis, C., West,

R. B. and Chaudhuri, O. (2019). Cell cycle progression in confining microenvironments is regulated by a growth-responsive TRPV4-PI3K/Akt-p27 Kip1 signaling axis. *Sci. Adv.* 5, eaaw6171.

Nava, M. M., Miroshnikova, Y. A., Biggs, L. C., Whitefield, D. B., Metge, F., Boucas, J., Vihinen, H., Jokitalo, E., Li, X., García Arcos, J. M., et al. (2020). Heterochromatin-driven nuclear softening protects the genome against mechanical. *Cell* 181, 1–18.

Nguyen Ho-Bouldoires, T. H., Sollier, K., Zamfirov, L., Broders-Bondon, F., Mitrossilis, D., Bermeo, S., Guerin, C. L., Chipont, A., Champenois, G., Leclère, R., et al. (2022). Ret kinase- mediated mechanical induction of colon stem cells by tumor growth pressure stimulates cancer progression in vivo. *Commun Biol* 5, 137.

Niethammer, P. (2021). Components and Mechanisms of Nuclear Mechanotransduction. *Annual Review of Cell and Developmental Biology* 37, 233–256.

Nishi, R., Oda, Y., Morikura, T. and Miyata, S. (2022). Effect of Compressive Stress in Tumor Microenvironment on Malignant Tumor Spheroid Invasion Process. *International Journal of Molecular Sciences* 23, 7091.

O'Conor, C. J., Leddy, H. A., Benefield, H. C., Liedtke, W. B. and Guilak, F. (2014). TRPV4 - mediated mechanotransduction regulates the metabolic response of chondrocytes to dynamic loading. *Proc. Natl. Acad. Sci. U.S.A.* 111, 1316–1321.

Onal, S., Alkaisi, M. M. and Nock, V. (2021). A Flexible Microdevice for Mechanical Cell Stimulation and Compression in Microfluidic Settings. *Front. Phys.* 9,.

Paul, C. D., Mistriotis, P. and Konstantopoulos, K. (2017). Cancer cell motility: Lessons from migration in confined spaces. *Nature Reviews Cancer* 17, 131–140.

Pelaez, D., Arita, N. and Cheung, H. S. (2012). Extracellular signal-regulated kinase (ERK) dictates osteogenic and/or chondrogenic lineage commitment of mesenchymal stem cells under dynamic compression. *Biochemical and Biophysical Research Communications* 417, 1286–1291.

Peyronnet, R., Tran, D., Girault, T. and Frachisse, J.-M. (2014). Mechanosensitivechannels: feeling tension in a world under pressure. *Front. Plant Sci.* 5,.

Prasad, M. and Montell, D. J. (2007). Cellular and Molecular Mechanisms of Border Cell Migration Analyzed Using Time-Lapse Live-Cell Imaging. *Developmental Cell* 12, 997–1005.

Prunet, A., Lefort, S., Delanoë-Ayari, H., Laperrousaz, B., Simon, G., Barentin, C., Saci, S., Argoul, F., Guyot, B., Rieu, J.-P., et al. (2020). A new agarose-based microsystem to investigate cell response to prolonged confinement. *Lab Chip* 20, 4016–4030.

Quiroga, X., Walani, N., Disanza, A., Chavero, A., Mittens, A., Tebar, F., Trepat, X., Parton, R. G., Geli, M. I., Scita, G., et al. (2023). A mechanosensing mechanism controls plasma membrane shape homeostasis at the nanoscale. *eLife* 12, e72316.

Ratheesh, A., Biebl, J., Vesela, J., Smutny, M., Papusheva, E., Krens, S. F. G., Kaufmann, W., Gyoergy, A., Casano, A. M. and Siekhaus, D. E. (2018). *Drosophila* TNF Modulates Tissue Tension in the Embryo to Facilitate Macrophage Invasive Migration. *Developmental Cell* 45, 331-346.e7.

Ren, X., Zhuang, H., Li, B., Jiang, F., Zhang, Y. and Zhou, P. (2023). Gsmtx4 Alleviated Osteoarthritis through Piezo1/Calcineurin/NFAT1 Signaling Axis under Excessive Mechanical Strain. *IJMS* 24, 4022.

Renkawitz, J., Kopf, A., Stopp, J., de Vries, I., Driscoll, M. K., Merrin, J., Hauschild, R., Welf, E. S., Danuser, G., Fiolka, R., et al. (2019). Nuclear positioning facilitates amoeboid migration along the path of least resistance. *Nature* 568, 546–550.

Reversat, A., Gaertner, F., Merrin, J., Stopp, J., Tasciyan, S., Aguilera, J., de Vries, I., Hauschild, R., Hons, M., Piel, M., et al. (2020). Cellular locomotion using environmental topography.*Nature* 582, 582–585.

Roca-Cusachs, P., Alcaraz, J., Sunyer, R., Samitier, J., Farré, R. and Navajas, D. (2008). Micropatterning of Single Endothelial Cell Shape Reveals a Tight Coupling between Nuclear Volume in G1 and Proliferation. *Biophysical Journal* 94, 4984–4995.

Ruprecht, V., Wieser, S., Callan-Jones, A., Smutny, M., Morita, H., Sako, K., Barone, V., Ritsch- Marte, M., Sixt, M., Voituriez, R., et al. (2015). Cortical contractility triggers a stochastic switch to fast amoeboid cell motility. *Cell* 160, 673–685.

Salminen, A. T., Zhang, J., Madejski, G. R., Khire, T. S., Waugh, R. E., McGrath, J. L. and Gaborski, T. R. (2019). Ultrathin Dual-Scale Nano- and Microporous Membranes for Vascular Transmigration Models. *Small* 15, 1804111.

Salminen, A. T., Allahyari, Z., Gholizadeh, S., McCloskey, M. C., Ajalik, R., Cottle, R. N., Gaborski, T. R. and McGrath, J. L. (2020). In vitro Studies of Transendothelial Migration for Biological and Drug Discovery. *Front. Med. Technol.* 2,.

Saraswathibhatla, A., Indana, D. and Chaudhuri, O. (2023). Cell–extracellular matrix mechanotransduction in 3D. *Nature Reviews Molecular Cell Biology* 24, 495–516.

Schuller, A. P., Wojtynek, M., Mankus, D., Tatli, M., Kronenberg-Tenga, R., Regmi, S. G., Dip, P. V., Lytton-Jean, A. K. R., Brignole, E. J., Dasso, M., et al. (2021). The cellular environment shapes the nuclear pore complex architecture. *Nature* 598, 667–671.

Shi, S., Kang, X.-J., Zhou, Z., He, Z.-M., Zheng, S. and He, S.-S. (2022). Excessive mechanical stress-induced intervertebral disc degeneration is related to Piezo1 overexpression triggering the imbalance of autophagy/apoptosis in human nucleus pulpous. *Arthritis Res Ther* 24, 119.

Shimizu, K., Shunori, A., Morimoto, K., Hashida, M. and Konishi, S. (2011). Development of a biochip with serially connected pneumatic balloons for cell-stretching culture. *Sensors and Actuators B: Chemical* 156, 486–493.

Shroff, N. P., Xu, P., Kim, S., Shelton, E. R., Gross, B. J., Liu, Y., Gomez, C. O., Ye, Q., Drennon, T. Y., Hu, J. K., et al. (2024). Proliferation-driven mechanical compression induces signalling centre formation during mammalian organ development. *Nat Cell Biol* 26, 519–529.

Siekhaus, D., Haesemeyer, M., Moffitt, O. and Lehmann, R. (2010). RhoL controls invasion and Rap1 localization during immune cell transmigration in Drosophila. *Nat Cell Biol* 12, 605– 610.

Sinha, B., Köster, D., Ruez, R., Gonnord, P., Bastiani, M., Abankwa, D., Stan, R. V., Butler-Browne, G., Vedie, B., Johannes, L., et al. (2011). Cells Respond to Mechanical Stress by Rapid Disassembly of Caveolae. *Cell* 144, 402–413.

Solbu A. S. and Strand, B. L. (2023). Assessing cell migration in hydrogels: An overview of relevant materials and methods. *Mater. Today Bio* 18, 100537.

Sonam, S., Balasubramaniam, L., Lin, S.-Z., Ivan, Y. M. Y., Pi-Jaumà, I., Jebane, C., Karnat, M., Toyama, Y., Marcq, P., Prost, J., et al. (2023). Mechanical stress driven by rigidity sensing governs epithelial stability. *Nat. Phys.* 19, 132–141.

Song, Y., Soto, J., Chen, B., Hoffman, T., Zhao, W., Zhu, N., Peng, Q., Liu, L., Ly, C., Wong, P. K., et al. (2022). Transient nuclear deformation primes epigenetic state and promotes cell reprogramming. Nat. Mater. 21, 1191–1199.

Stephens, A. D., Banigan, E. J., Adam, S. A., Goldman, R. D. and Marko, J. F. (2017). Chromatin and lamin a determine two different mechanical response regimes of the cell nucleus. *Molecular Biology of the Cell* 28, 1984–1996.

Stöberl, S., Flommersfeld, J., Kreft, M. M., Benoit, M., Broedersz, C. P. and Rädler, J. O. (2023). Nuclear deformation and dynamics of migrating cells in 3D confinement reveal adaptation of pulling and pushing forces.

Stylianopoulos, T., Martin, J. D., Chauhan, V. P., Jain, S. R., Diop-Frimpong, B., Bardeesy, N., Smith, B. L., Ferrone, C. R., Hornicek, F. J., Boucher, Y., et al. (2012). Causes, consequences, and remedies for growth-induced solid stress in murine and human tumors. *Proc. Natl. Acad. Sci. U.S.A.* 109, 15101–15108.

Stylianopoulos, T., Martin, J. D., Snuderl, M., Mpekris, F., Jain, S. R. and Jain, R. K. (2013). Coevolution of Solid Stress and Interstitial Fluid Pressure in Tumors During Progression: Implications for Vascular Collapse. *Cancer Research* 73, 3833–3841.

Szabó, A., Melchionda, M., Nastasi, G., Woods, M. L., Campo, S., Perris, R. and Mayor, R. (2016). In vivo confinement promotes collective migration of neural crest cells. *J Cell Biol* 213, 543–555.

Takeda, Y., Niki, Y., Fukuhara, Y., Fukuda, Y., Udagawa, K., Shimoda, M., Kikuchi, T., Kobayashi, S., Harato, K., Miyamoto, T., et al. (2021). Compressive mechanical stress enhances susceptibility to interleukin-1 by increasing interleukin-1 receptor expression in 3D- cultured ATDC5 cells. *BMC Musculoskelet Disord* 22, 238.

Tang, X., Wang, Z., Khutsishvili, D., Cheng, Y., Wang, J., Tang, J. and Ma, S. (2023). Volumetric compression by heterogeneous scaffold embedding promotes cerebral organoid maturation and does not impede growth. *Cell Systems* 14, 872–882.

Tao, J., Choudhury, M. I., Maity, D., Kim, T., Sun, S. X. and Fan, C.-M. (2023). Mechanical compression creates a quiescent muscle stem cell niche. *Commun Biol* 6, 1–12.

Teo, J. L., Gomez, G. A., Weeratunga, S., Davies, E. M., Noordstra, I., Budnar, S., Katsuno-Kambe, H., McGrath, M. J., Verma, S., Tomatis, V., et al. (2020). Caveolae Control Contractile Tension for Epithelia to Eliminate Tumor Cells. *Developmental Cell* 54, 75-91.e7.

Thiam, H. R., Vargas, P., Carpi, N., Crespo, C. L., Raab, M., Terriac, E., King, M. C., Jacobelli, J., Alberts, A. S., Stradal, T., et al. (2016). Perinuclear Arp2/3-driven actin polymerization enables nuclear deformation to facilitate cell migration through complex environments. *Nature Communications* 7,.

Toi, M., Asao, Y., Matsumoto, Y., Sekiguchi, H., Yoshikawa, A., Takada, M., Kataoka, M., Endo, T., Kawaguchi-Sakita, N., Kawashima, M., et al. (2017). Visualization of tumor-related blood vessels in human breast by photoacoustic imaging system with a hemispherical detector array. *Sci Rep* 7, 41970.

Ventura, G. and Sedzinski, J. (2022). Emerging concepts on the mechanical interplay between migrating cells and microenvironment in vivo. *Front Cell Dev Biol* 10, 961460.

Venturini, V., Pezzano, F., Català Castro, F., Häkkinen, H.-M., Jiménez-Delgado, S., Colomer- Rosell, M., Marro, M., Tolosa-Ramon, Q., Paz-López, S., Valverde, M. A., et al. (2020). The nucleus measures shape changes for cellular proprioception to control dynamic cell behavior. *Science* 370, eaba2644.

Vu, L. T., Jain, G., Veres, B. D. and Rajagopalan, P. (2015). Cell Migration on Planar and Three-Dimensional Matrices: A Hydrogel-Based Perspective. *Tissue Eng Part B Rev* 21, 67–74.

Wang, Y., Wang, J., Bai, D., Song, J., Ye, R., Zhao, Z., Lei, L., Hao, J., Jiang, C., Fang, S., et al. (2013). Cell proliferation is promoted by compressive stress during early stage of chondrogenic differentiation of rat BMSCs. *Journal of Cellular Physiology* 228, 1935–1942.

Wang, X., Tao, J., Zhou, J., Shu, Y. and Xu, J. (2024a). Excessive load promotes temporomandibular joint chondrocyte apoptosis via Piezo1/endoplasmic reticulum stress pathway. *J Cellular Molecular Medi* 28, e18472.

Wang, Z., Tang, X., Khutsishvili, D., Sang, G., Galan, E. A., Wang, J. and Ma, S. (2024b). Protocol to encapsulate cerebral organoids with alginate hydrogel shell to induce volumetric compression. *STAR Protocols* 5, 102952.

Wilson, R. E., Denisin, A. K., Dunn, A. R. and Pruitt, B. L. (2021). 3D Microwell Platforms for Control of Single Cell 3D Geometry and Intracellular Organization. *Cel. Mol. Bioeng.* 14, 1–14.

Wirtz, D., Konstantopoulos, K. and Searson, P. C. (2011). The physics of cancer: the role of physical interactions and mechanical forces in metastasis. *Nat Rev Cancer* 11, 512–522.

Wolf, K., Mazo, I., Leung, H., Engelke, K., Von Andrian, U. H., Deryugina, E. I., Strongin, A. Y., Bröcker, E. B. and Friedl, P. (2003). Compensation mechanism in tumor cell migration: Mesenchymal-amoeboid transition after blocking of pericellular proteolysis. *Journal of Cell Biology* 160, 267–277.

Wolf, K., te Lindert, M., Krause, M., Alexander, S., te Riet, J., Willis, A. L., Hoffman, R. M., Figdor, C. G., Weiss, S. J. and Friedl, P. (2013). Physical limits of cell migration: Control by ECM space and nuclear deformation and tuning by proteolysis and traction force. *Journal of Cell Biology* 201, 1069–1084.

Wu, D. T., Diba, M., Yang, S., Freedman, B. R., Elosegui-Artola, A. and Mooney, D. J. (2022). Hydrogel viscoelasticity modulates migration and fusion of mesenchymal stem cell spheroids. *Bioeng Transl Med* 8, e10464.

Wu, Y., Jing, Z., Deng, D., Yan, J., Liu, M., Li, L., Zuo, Y., Wu, W., Hu, Q. and Xie, Y. (2023). Dkk-1– TNF-α crosstalk regulates MC3T3E1 pre-osteoblast proliferation and differentiation under mechanical stress through the ERK signaling pathway. *Mol Cell Biochem* 478, 2191–2206.

Wu, S., Zhou, H., Ling, H., Sun, Y., Luo, Z., Ngo, T., Fu, Y., Wang, W. and Kong, Y. (2024). LIPUS regulates the progression of knee osteoarthritis in mice through primary cilia-mediated TRPV4 channels. *Apoptosis* 29, 785–798.

Wyatt, T. P. J., Fouchard, J., Lisica, A., Khalilgharibi, N., Baum, B., Recho, P., Kabla, A. J. and Charras, G. T. (2020). Actomyosin controls planarity and folding of epithelia in response to compression. *Nat. Mater.* 19, 109–117.

Xiang, Z., Zhang, P., Jia, C., Xu, R., Cao, D., Xu, Z., Lu, T., Liu, J., Wang, X., Qiu, C., et al. (2024). Piezo1 channel exaggerates ferroptosis of nucleus pulposus cells by mediating mechanical stress-induced iron influx. *Bone Res* 12, 20.

Xie, Y., Chen, D., Jiang, K., Song, L., Qian, N., Du, Y., Yang, Y., Wang, F. and Chen, T. (2022). Hair shaft miniaturization causes stem cell depletion through mechanosensory signals mediated by a Piezo1-calcium-TNF-α axis. *Cell Stem Cell* 29, 70-85.e6.

Yamada, K. M. and Sixt, M. (2019). Mechanisms of 3D cell migration. *Nature Reviews Molecular Cell Biology* 20, 738–752.

Zhang, Y., De Mets, R., Monzel, C., Acharya, V., Toh, P., Chin, J. F. L., Van Hul, N., Ng, I. C., Yu, H., Ng, S. S., et al. (2020a). Biomimetic niches reveal the minimal cues to trigger apical lumen formation in single hepatocytes. *Nat. Mater.* 19, 1026–1035.

Zhang, Q.-Y., Bai, J.-D., Wu, X.-A., Liu, X.-N., Zhang, M. and Chen, W.-Y. (2020b). Microniche geometry modulates the mechanical properties and calcium signaling of chondrocytes. *Journal of Biomechanics* 104, 109729.

Zhang, S., Liu, W., Chen, S., Wang, B., Wang, P., Hu, B., Lv, X. and Shao, Z. (2022a). Extracellular matrix in intervertebral disc: basic and translational implications. *Cell Tissue Res* 390, 1– 22.

Zhang, X., Chen, X., Hong, H., Hu, R., Liu, J. and Liu, C. (2022b). Decellularized extracellular matrix scaffolds: Recent trends and emerging strategies in tissue engineering. Bioactive Materials 10, 15–31.

Zhang, S., Grifno, G., Passaro, R., Regan, K., Zheng, S., Hadzipasic, M., Banerji, R., O'Connor, L., Chu, V., Kim, S. Y., et al. (2023). Intravital measurements of solid stresses in tumours reveal length-scale and microenvironmentally dependent force transmission. *Nat. Biomed. Eng* 7, 1473–1492.

Zhao, Z., Li, Y., Wang, M., Zhao, S., Zhao, Z. and Fang, J. (2020). Mechanotransduction pathways in the regulation of cartilage chondrocyte homoeostasis. *J Cellular Molecular Medi* 24, 5408– 5419.

Zhao, X., Hu, J., Li, Y. and Guo, M. (2021). Volumetric compression develops noise-driven single- cell heterogeneity. *Proc. Natl. Acad. Sci. U.S.A.* 118, e2110550118.

Zimmerli, C. E., Allegretti, M., Rantos, V., Goetz, S. K., Obarska-, A., Zagoriy, I., Halavatyi, A., Hummer, G., Mahamid, J., Kosinski, J. et al. (2021). Nuclear pores dilate and constrict in cellulo. Science 374, eabd9776.