# Mechanical regulation of transcription

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# Abstract

Cells and tissues generate and are exposed to a variety of mechanical forces that act across scales of tissues, cells and organelles and provide critical signals to influence cell behavior during development and adult homeostasis. Consequently, a large number of common diseases involve alterations in these mechanical forces, triggering feedback loops that have the potential to influence disease progression. Recent technical advances that have facilitated measuring and manipulating biologically relevant forces have led to identification of mechanosensitive pathways and TFs with key roles in regulating cell states and behaviors. This review highlights the mechanisms by which forces regulate transcription and the emerging roles of these mechanisms in mammalian physiology and disease.

# Introduction

A hallmark of tissue morphogenesis is the patterning of groups of cells into communities with defined common properties, including expression of specific genes that determine cell state, unique morphological features, and specialized behavioral patterns. These functional units of patterned tissue are maintained in adult tissues, some of which undergo constant homeostatic renewal or can self-repair after injury. The generation, maintenance, and repair of functional tissues thus requires intricate coordination of cell fate and position, and the ability of cells to respond and adapt to dynamic changes in their microenvironment. The close, highly reproducible correlation between cell type- specific morphology and cell identity points to constant, bidirectional feedback between microenvironmental signals, the contractile cytoskeleton that responds to these signals and determines cell shape, and the transcriptional circuitry that establishes and maintains cell identity [1–3].

One central microenvironmental signal modality is mechanical force [G] that acts on scales of tissues, cells, organelles and molecules, and that has fundamental impacts on cell behavior through regulating gene transcription. This review will discuss how mechanical signals are propagated and relayed into the nucleus to regulate transcription, and critically evaluate the current evidence for the physiological relevance of this regulation.

# Mechanisms of force sensing

Tissue structures and cell morphologies are adapted to their specialized functions. While executing these specialized functions, tissues and single cells are exposed to and generate tissue-specific mechanical forces such as compression, shear [G], tensile stress [G], or hydrostatic pressure [G] (Figure 1). To generate and sustain their distinct force environments, tissues display specific mechanical properties, dictated by cells and their surrounding extracellular matrix (ECM), including elasticity, viscosity, and friction [G]. Importantly, the manner in which cells interact and respond to these dynamic forces is determined by the physical properties of the cells and ECM (for recent reviews see for example [4–7]. As many diseases lead to alterations in tissue function and architecture, they almost invariantly also change the mechanical properties and forces within tissues, for example through fibrotic reactions [8]. Further, a large number of common diseases such as atherosclerosis [G], arthritis, osteoporosis, and cardiomyopathies, as well several developmental disorders, including Hutchinson-Gilford progeria [G] and Duchenne's muscular dystrophy [G], entail abnormal physiological responses

to mechanical forces [9,10]. This highlights the importance of understanding biological forces and their effects on cell state and behavior.

As in any three-dimensional structure, forces within cells are transmitted across structures that are physically interconnected - the ECM, adhesions, cytoskeleton, and the nucleus (Figure 1). Thus, all cells contain structures that respond to these local forces, resulting in changes in cell behavior. In this review we refer to coupling of extrinsic forces to the intracellular force sensing machineries as mechanotransmission. A central location for mechanotransmission is the plasma membrane, where transmembrane receptor complexes, such as integrin-based cell-matrix adhesions and cadherin-based cell-cell adhesions connect with the contractile actomyosin cytoskeleton, to both exert forces on their surroundings, and sense mechanical properties or dynamic deformation of neighboring cells or the ECM substrate [11–13] (Figure 1). Mechanical forces applied on these multiprotein adhesion complexes can be converted into biochemical signals, for example through mechanical unfolding of individual proteins/complexes (referred to as mechanosensing) and subsequent activation of signaling molecules (mechanotransduction) [14,15] (Figure 2a, b). Another central mechanism of force sensing at the plasma membrane is the stretch-induced activation of ion channels such as Piezo1/2 or TRPV (Figure 2c). They are activated in response to stretch, compression, shear and possibly even hydrostatic pressure to trigger ion-dependent intracellular mechanosignaling [16–21]. Also other plasma membrane structures such as caveolae, as well as membrane tension itself, are involved in mechanotransmission [22–24] (Figure 2d).

In addition to the plasma membrane, recent evidence points to the nucleus as a mechanosensor that senses its own deformation to trigger mechanosignaling. Nuclear deformation can occur downstream of extrinsic forces as a result of force transmission from adhesions, via the cytoskeleton and the LINC complexes, to the nuclear envelope, nuclear lamina and chromatin, resulting in mechanosignaling in a manner similar to what occurs at cell adhesions [10,25,26] (Figure 2d). The nuclear lamina, composed of the intermediate filament proteins Lamin A/C and Lamin B, participates in the regulation of mechanosensitive transcription factors (TFs) [27–31]. In addition, Lamin A protein levels scale with increasing ECM stiffness, providing a mechanism that couples tissue and nuclear stiffness and amplifies mechanosensitive transcription [27,31–33]. Due to its high stiffness compared to the rest of the cell, the nucleus also undergoes direct deformation in response to cell compression or stretch. This nuclear deformation triggers nuclear membrane stretching and subsequent Ca2+ and phospholipase signaling, which activates migratory escape responses and mechanoprotective changes in nuclear and chromatin stiffness in conditions of genotoxic compression [20,34–37]

(Figure 2d). In addition to membrane stretching, nuclear deformation may regulate nuclear pore distribution and permeability to impact intranuclear levels of TFs as a means of nuclear mechanosensing [38–40]. This possibility is particularly interesting in light of the recent observation that the diameter of the pore opening is gated by membrane tension [41] (Figure 2d).

Finally, emerging evidence further suggests that other organelles such as the Golgi and mitochondria display mechanosensitive properties and respond to mechanical forces and actomyosin contraction by changing their structure and function, resulting in propagation of downstream biochemical signals ultimately impacting the activity of TFs (see below, Emerging concepts) [42–46]. Collectively, current evidence indicates that mechanical stress results in strain (deformation) of a broad range of mechanosensitive structures and potentially organelles, triggering downstream signaling. What determines which mechanosensory organelle/structure is activated is unclear but most likely depends on the type and magnitude of the biological force as well as the properties of the cell itself, including contractile state of the cytoskeleton and tension of the plasma and nuclear membranes. It could also be envisioned that multiple mechanosensors can be activated simultaneously, after which their signals will be integrated further downstream. In the following sections we will discuss how mechanical signals are relayed into the nucleus, what is the nature of these signals, and how they regulate transcription to produce physiologically meaningful responses.

# Chromatin responses to nuclear force transmission

Chromatin is a disordered, variably compacted polymer chain that is assembled into a complex, hierarchical 3D configuration through interactions with itself on multiple scales (compartments, topologically associating domains, loops) as well as with the nuclear periphery [47–49]. The organization of chromatin determines its accessibility to TFs and thus represents an important layer of transcriptional regulation. Consequently, cell fate transitions involve changes in chromatin organization, where histone- and DNA-modifying enzymes and chromatin remodelers collaborate with TFs to generate cell-type specific gene expression patterns, and to and maintain these patterns across cell divisions [50–52]. The association of chromatin with the nuclear lamina provides a direct mechanical link all the way from the extracellular environment through adhesion complexes, the cytoskeleton, the LINC complex to chromatin (Figure 3a). The potential functional role of this direct mechanical link in regulating chromatin organization and the various post-translational modification states of histones in response to mechanical force has been under intense investigation in recent years. Although our understanding of this

process remains rudimentary, emerging data indicates that the impact of force on chromatin is time-, magnitude- and cell type-dependent.

Mechanical force transmitted from cell adhesions to the nucleus has indeed been shown to result in immediate displacement of chromatin, which correlates with activation of mechanosensitive gene expression [53,54]. How this specificity of force-mediated activation of only certain genes is achieved remains a key open question. One possibility is that it depends on the positioning of the genes. For example, the *epidermal differentiation complex (EDC)* gene cluster is inactive in epidermal stem cells, where the locus resides in close proximity to the lamina. Application of tension on the lamina through the LINC complex is critical for maintaining the compaction of chromatin at this locus and gene silencing, preventing precocious expression of differentiation genes [55].

On longer time scales of minutes, both cell-extrinsic (dynamic tensile loading) and cell-intrinsic (elevation of cellular contractility) mechanical stimulation triggers ATP-dependent condensation of euchromatin into heterochromatin in mesenchymal stem cells, leading to a suppression of gene expression in the condensed regions [56,57]. In contrast, cyclic uniaxial mechanical stretch in epidermal stem cells leads to a decrease in H3K9me3-marked condensed heterochromatin within 30 minutes of force application, but this decrease has no substantial impact on expression of protein-coding genes as the changes occur mainly at non-coding regions. Instead, the decrease in stiff, lamina proximal heterochromatin contributes to nuclear softening that is required to dissipate mechanical energy to prevent DNA damage [20]. If this mechanical stretch stimulus persists for several hours, cells align perpendicular to the direction of stretch, thereby minimizing strain of the nucleus, allowing the cells to restore steady state chromatin architecture [20]. Interestingly, if the stretch is biaxial and thus cells are not able to align to avoid strain, the regions that have lost H3K9me3 will, in the scale of days, gain H3K27me3, which is also a silencing mark, most likely as a compensatory mechanism to ensure proper silencing of these regions [58]. The application of long-term biaxial stretch will further deplete free nuclear G-actin to trigger transcriptional repression accumulation of H3K27me also at promoters of genes that are expressed at low levels, preventing epidermal stem cell differentiation [58]. The potential of nuclear strain to trigger heterochromatin changes thus appears cell type- and cell state-specific, determined by the steady state nuclear stiffness [20,56,59]. For example cancer cell lines with low Lamin A and thus low nuclear stiffness and membrane tension are refractory to force-induced changes in H3K9me3 and in some cases even increased H3K9me3 in response to force, but could be rendered mechanosensitive by overexpressing Lamin A [20] (Figure 3a).

The notion that histone modifications respond to changes in cell geometry [G] are supported by studies using extrinsic constraints on cell morphology. Forcing mammary epithelial cells into rounded shapes using adhesive micropatterns resulted in global histone deacetylation, chromatin condensation, and overall reduction in gene expression [60]. Similarly, forcing mesenchymal stem cells into elongated shapes led to increased histone deacetylase activity and subsequent decreased histone acetylation [61], whereas increasing cell spread area of fibroblasts triggered increases histone acetylation and changes in gene expression of cytoskeletal genes through effects on actomyosin contractility [62]. Studies in melanoma cells further showed that tissue curvature, most likely through increasing mechanical stress, promotes increased deposition of H3K4me2 and H3K9ac to enhance expression of pro-oncogenic genes [63]. Intriguingly, chromatin architecture can feed back to regulate the nuclear mechanical state as it has been shown that haploinsufficiency of the chromatin modifier MLL4/COMPASS complex in Kabuki syndrome leads to increased H3K27me3 and polycomb complex clustering, leading to increased nuclear stiffness [64].

The mechanisms by which stretch, compression and changes in cell and nuclear shape drive epigenetic and transcriptional changes are still unclear but some mechanistic insights are beginning to unravel. As changes in cell and nuclear shape trigger stretch-induced ion channels, intracellular Ca2+ signaling has been implicated in heterochromatin regulation downstream of mechanical deformation [20,59,65] (Figure 3a). Interestingly, formation of the perinuclear actin ring that mediates nuclear actin levels is also driven by elevation of intracellular Ca2+, linking nuclear strain with actin-driven effects on transcription [20,58,66,67]. In addition, actomyosin contractility and subsequent local tension on the nuclear envelope is likely to play a role, as illustrated by the effects of manipulating LINC complex or Lamin A on chromatin and gene expression in response to force [31,55,68]. Whether these effects of local tension are driven by nuclear strain, thus converging with the above described mechanisms involving nuclear deformation, remains an intriguing open question. Another alternative or parallel layer of regulation could impart from more local nuclear deformations, driven by the perinuclear cytoskeleton and leading to highly localized nuclear invaginations and intranuclear polarization [69]. The amount of perinuclear actin-rich invaginations correlates with the degree of dedifferentiation in a variety of cell types [70]. Compressive forces from the microtubules have also been recently shown to trigger lobulated nuclear shapes and local loss of H3K9me3 heterochromatin from within these NE invaginations, resulting in gene expression changes in human hematopoietic stem cells during their early differentiation [71]. Similarly, the cytomegalovirus has been shown to utilize positioning of the microtubule organizing center to

regulate the nuclear lamina and intranuclear polarization to spatially segregate viral DNA from compact chromatin of the host DNA, thus maximizing virus replication [72].

Collectively these studies emphasize the intimate, functionally relevant connection between cell and nuclear shape changes and 3D chromatin organization and epigenetic state to regulate gene expression. The key question is how these changes interface with biochemical signals to provide specificity while preventing uncontrolled changes in activity that could result from the constant exposure of cells to mechanical force. This crosstalk between mechanical and biochemical signals and their physiological relevance will be the focus of the rest of this review.

# Actomyosin dynamics and nuclear actin in transcriptional regulation

A large majority of the above-described mechanisms of sensing and responding to mechanical forces converge on actomyosin dynamics either through direct regulation of the cytoskeleton or by impacting signaling pathways that influence actin dynamics. There are three central mechanisms by which actin can regulate transcription downstream of mechanical signals: i) regulation of the core transcriptional machinery and chromatin modifiers by nuclear actin, ii) regulation of the SRF/MRTF signaling pathway, and iii) regulation of YAP/TAZ mechanosensitive transcriptional coactivators that respond to changes in actomyosin.

## Nuclear actin in mechanical regulation of core transcription.

After initial controversy, it has become well accepted that actin is also found in the nucleus, both as monomeric G-actin and as filamentous F-actin [73]. Actin is transported through the nuclear pore as a monomer, and the availability of monomers is rate-limiting for the transport in both directions [74]. Thus, any mechanochemical signaling process that impacts actin dynamics and thus the ratio of free G-actin to bound filamentous F-actin in the cytoplasm, including processes such as cell spreading, is likely to influence nuclear actin levels [75] (Figure 3a).

Nuclear actin plays multiple roles in regulation of transcription regulation and initiation, chromatin reorganization, and DNA repair (for a recent comprehensive review see for example Ref.[73]). Nuclear G-actin levels positively correlate with global transcription rates [58,76–80]. Although the precise molecular mechanism(s) still need to be worked out, interactome studies have revealed association of actin with several proteins involved in transcription such as components of the preinitiation complex, pre-mRNA splicing and processing factors, and transcription

elongation factors [77,79,81] (Figure 3a). In Drosophila oocytes actin associates with RNA Polymerase II (RNAPII) on gene bodies of actively transcribed genes [80], whereas specific actin association with chromatin in mammalian cells awaits demonstration. Nuclear actin levels are dynamically regulated by force, and decreased nuclear actin observed is in response to substrate stretching. Here, formation of a tight perinuclear actin ring leads to a decrease in RNAPII transcriptional elongation, subsequently allowing Histone 3 tri-methylation at lysine 27 (H3K27me3) at promoters to silence epidermal differentiation genes [58]. Signals from the ECM substrate can also reduce nuclear actin levels, suppressing transcription and promoting quiescence in mammary epithelial cells [82]. This signaling axis is disrupted in human breast cancer cells resulting in continuous proliferation [76]. Whether this laminin 111-dependent effect is of mechanical or biochemical nature remains to be investigated.

G-actin has been also identified as a structural component and allosteric regulator of several chromatin remodeling complexes including Ino80, SWI/SNF and Tip60/NuA4 [83,84] (Figure 3a). Chromatin remodeling complexes control chromatin accessibility for replication, transcription, and DNA damage repair. Functionally, the G-actin-containing structural modules participate in allosteric control of the motor subunit of the complexes. Indeed β-actin-null mouse embryonic fibroblasts display reduced chromatin association and activity of the ATPase subunit of the BAF complex and show defects in gene expression [85–87]. Curiously, these complexes also contain an additional actin-like subunit, ACTL6A or BAF53, with no known cytoskeletal function, suggesting evolution of a dedicated factor that might retain only the transcription-associated functions of actin in this context. Whether these functions of actin and/or of ACTL6A are impacted by dynamic regulation of nuclear actin, and thus could potentially be mechanosensitive, remains an important open question.

Also the Wave Regulatory Complex (WRC) and Arp2/3 complexes, as well as certain myosin motor proteins, have been linked to transcription or transcription-related processes, indicating a role for nuclear F-actin in transcriptional regulation [88–90]. Such F-actin-mediated transcriptional processes include reactivation of the otherwise silenced *Oct4* pluripotency gene during oocyte-mediated nuclear reprogramming of somatic nuclei [91–93].

Taken together these findings indicate that the dynamic communication between the cytoplasmic and nuclear actin pools can transmit information from the extracellular environment into the nucleus (Figure 3a). Further studies are needed to establish the biochemical basis of actin-mediated transcriptional regulation, and the role of actin dynamics in functional regulation of chromatin remodeling complexes. Advances in this field requires development of tools to better dissect the causative relationships between co-occurring regulation of the transcriptional

machinery, TF activity, and chromatin remodelling in response to changes in actomyosin dynamics.

## The SRF/MRTF system and transcriptional reinforcement of cell-ECM adhesion and actomyosin.

The second main mechanism by which actin regulates gene expression is by controlling the assembly of SRF/MRTF [G] TF complexes (Figure 3b). SRF can associate with different transcriptional co-activators such as TCFs [G] and MRTFs [G] in response to ERK signaling and RHO GTPase [G] activity, respectively [94–98]. SRF/MRTF transcriptional complexes mediate gene activation by either facilitating RNAPII recruitment, or promoter escape of pre-loaded RNAPII, depending on the genomic context [99]. G-actin directly binds to and inhibits MRTFs, so that upon RHO activation and cytoplasmic F-actin polymerization (and subsequent a drop in free G-actin level) MRTFs are set free to bind SRF [100]. Evidence also indicates a specific role for the nuclear F-actin pool in the regulation of SRF/MRTF [75,101,102]. Upon higher demand of F-actin (e.g. due to increased motility or in response to mechanical stress), the SRF/MRTF system is activated and promotes expression ECM, cell-ECM adhesion and cytoskeletal genes to facilitate a feedback mechanoadaptation [G] [103–109]. This central role of SRF in regulating transcription of cytoskeletal and ECM components and mechanoadaptation is also important for epidermal homeostasis and skin barrier function [110–114], and in endothelial cells to promote vessel growth and maturation [115–117]. Finally, SRF can additionally link mechanical cues to the regulation of cell proliferation and differentiation, which depends on a balance between TCFs and MRTFs [118–121]. The SRF/MRTF system thus relays changes in extracellular forces and cytoskeletal actin dynamics into the nucleus (Figure 3b).

## The YAP/TAZ coactivators as a “mechanics to biology” transduction module.

Changes in the actomyosin cytoskeleton also modulate the activity of the ubiquitously expressed paralogous factors YAP and TAZ that play a central role in regulation of transcription downstream of mechanical force, including cell geometry, ECM stiffness, stretching and shear stress [122,123] (Figure 3c). YAP/TAZ dynamically shuttle between the cytoplasm and the nucleus in response to multiple inputs including the Hippo pathway, a kinase cascade that controls tissue and organ size across animals, and their subcellular localization approximates their transcriptional activity. In the nucleus, YAP and TAZ act as transcriptional coactivators and bind

the TEAD family of TFs (TEAD1-4). Moreover, the YAP/TEAD complexes can interact with additional TF complexes that bind DNA in the vicinity of TEADs including AP1, SRF/MRTF, E2F and Myc that cooperate with YAP/TEAD, or TRPS1 that dampens YAP/TEAD-regulated chromatin remodeling and transcription. YAP/TEAD complexes are found preferentially at distal super-enhancer elements, and regulate transcription by recruiting the Mediator and BRD4 epigenetic coactivators to drive RNAPII activity at proximal promoters [124].

The "mechanosensitive" control of YAP/TAZ is an integral part of the mechanisms that coordinate local cell growth with global tissue size such as contact inhibition of growth [G] [125–127], cell competition [G] within epithelia [128–135], the recently proposed "lateral inhibition" or "leader selection" process [136,137], and compensatory tissue growth in response to mechanical expansion [110]. YAP/TAZ respond to F-actin, rather than to G-actin, in a manner which is largely independent of the type of adhesive ECM ligands and integrins involved, but dependent on the mechanosensitive Talin1/2 proteins [122,123,138,139]. Various mechanisms to control YAP mechanoactivation have been proposed, and they likely act in parallel. These include the regulation of LATS1/2 kinases downstream of the RAP2 small GTPase, of the nuclear actin-binding ARID1A protein, and of nuclear pore permeability [38–40,140–142] (Figure 3c).

Interestingly, cells do not only passively respond to extracellular forces, but can actively tune the cytoskeletal response to extracellular forces and the ensuing YAP/TAZ activation. Key factors involved in this feedback tuning process are the F-actin capping and severing proteins CAPZ, Cofilin1/2 and Gelsolin, the focal adhesion component CCM3 (Cerebral Cavernous Malformation 3, also known as PDCD10), and the F-actin bundling protein Fascin1 [125,143–146]. Regulated expression of these cytoskeletal factors by oncogenes provides transformed cells with a cell-autonomous [G] mechanism to overcome a soft, tumor-suppressive mechanical microenvironment [144,147].

Similar to SRF/MRTF, also YAP/TAZ signaling feeds back into the expression of cytoskeletal genes, facilitating mechanoadaptation [148–151]. However, YAP/TAZ differ from the MRTF/SRF system as they translate mechanical inputs into broader biological responses. Indeed YAP/TAZ communicate the degree of extracellular forces, and the corresponding degree of intracellular tension, to the nucleus to regulate proliferation, apoptosis, differentiation and their associated metabolic pathways [122,152]. Thus, the relevance of the mechanically regulated YAP/TAZ transcriptional response appears widespread and spans a large number of organ systems in physiological and pathological scenarios.

In addition to MRTF/SRF and YAP/TAZ a number of other TFs have been shown in certain circumstances to display mechanosensitivity (summarized in Table 1). However, the

physiological relevance of these pathways is more specific or remains to be demonstrated. Below we will discuss the most prominent examples of physiologically-relevant mechanosensitive transcriptional pathways for which in vivo genetic evidence has been provided, or which occur in tissues/organs that have been shown to display physiologically-relevant mechanoresponses.

# Mechanosensitive transcription during development and in stem cells

During development, cell fate needs to be tightly coordinated with cell morphology and position. In addition, morphogenetic movements generate dynamic stretching and compression forces as well as changes in tissue curvature [1–3]. Thus, it has been long speculated that changes in forces and cell morphologies could impact TF activity to provide a feedback control of development. Although the direct link between mechanical forces and the transcriptional response downstream of the TFs still needs to be demonstrated in most cases, gene knockout studies of mechanosensitive TFs have revealed essential roles for these pathways in development.

## Tissue deformation, cell-cell tension and the regulation of β-Catenin.

A number of *in vivo* studies demonstrate the role of mechanical regulation of the WNT/β-Catenin pathway in coupling morphogenetic movements and transcriptional regulation. In Drosophila embryos, tissue compression rescues expression of the Twist mesoderm TF in mutants with defective morphogenetic movements [153] (Figure 4a). Build-up of tissue tension results in Src activation, leading to phosphorylation of β-Catenin to facilitate its release from cell-cell adhesions for nuclear entry [154,155]. Nuclear β-Catenin acts as a transcriptional coactivator for TCF/LEF TF, and is mainly regulated by the WNT pathway [156]. The coactivator function of β-Catenin depends on recruitment of multiple transcriptional and chromatin regulatory factors [157,158]. Tension-mediated regulation of β-Catenin also operates in vertebrate embryos during mesoderm induction as well as in the mouse intestinal epithelium [159,160], and has been observed in the skin of mice overexpressing active Rho kinase (ROCK) [161]. In human pluripotent stem cells, a soft ECM promotes the formation of cell-cell junctions that function as a “reservoir” for WNT-induced β-Catenin activation, so that upon localized high cell-cell tension β-Catenin can be locally activated to drive mesoderm specification [162,163]. The precise mechanical input driving such activation remains elusive, also because of conflicting studies where β-Catenin is shown

not to be released but recruited to stabilize the junctions in response to tension [164]. On the other hand, mechanical stretch in endothelial cells can unmask a phosphorylation site on VE-cadherin that is normally masked by β-Catenin binding, enabling increased turnover of cell-cell adhesions [165]. Thus, a possible reconciliation of these different findings is that the ability of cell-cell tension to release and activate β-Catenin depends on cell-type specific cell-cell adhesion dynamics [166], and in cells with more dynamic, unstable adhesions such as endothelial cells, tension leads to adhesion disassembly and β-Catenin release [167], whereas in cells with stable adhesions such as epithelial cells, tension retains β-Catenin at junctions [164], resulting in opposing transcriptional outcomes.

## YAP/TAZ mechanotransduction in stem cell regulation.

YAP/TAZ promote cell proliferation in multiple cell types, including terminally differentiated cells when overexpressed. The cell cycle re-entry of terminally differentiated cells is often linked to cell de-differentiation, which has suggested a role for YAP/TAZ in regulating stem cells. Studies in adult mice have indicated that YAP/TAZ are important in the context of "emergency" stem cell activation for tissue regeneration after damage, rather than for homeostatic stem cell renewal [124,168–170]. In human stem cells YAP activation can favor reprogramming to the naive pluripotent state and their differentiation, but its requirement for hPSC self-renewal is rather limited [150,171,172]. This may reflect the physiological function of YAP/TAZ in the context of the early mouse embryo, where YAP/TAZ promote differentiation of the trophectoderm [G] or trophoblast stem cell fate, where they are active in response to ROCK and tissue tension, but do not control the pluripotent inner cell mass [G] fate, where they are actively inhibited by the Hippo pathway [173–178]. Indeed, while *Yap1*-deficient mouse embryos arrest around E8.5 due to severe embryonic and extraembryonic defects [179], and *Taz (or WWTR1)*-deficient embryos are viable but develop multicystic kidney disease during development and rarely survive to adulthood [180–182], double knockout embryos die at the morula stage, indicating that YAP and TAZ are redundant but collectively essential for very early embryogenesis [176].

## Exploiting mechano-responsive transcription for regenerative medicine.

In addition to critical roles in morphogenesis, mechanics of the cell microenvironment have profound effects on *ex vivo* stem cell amplification and *in vivo* engraftment [183–186]. In the case of intestinal organoids [G] or intestinal injury in mice, a stiff ECM promotes stem cell expansion and regenerative stem cell reprogramming through YAP/TAZ [187,188]. Another example is the

mechano-chemical regulation of mesenchymal stem cell differentiation on stiff substrata, which occurs by regulating YAP/TAZ and RARG [31,33,123,146,189–191]. This signaling axis is likely of physiological relevance, as modulation of ECM rigidity or actomyosin contractility alter mesenchymal stem cell differentiation trajectories also *in vivo* [192,193]. These findings have triggered interest in exploring the druggability of the ECM-YAP/TAZ-transcription axis, and some studies have used actomyosin inhibitory drugs to shut down unwanted YAP/TAZ activity to enable the *in vitro* differentiation of pancreatic β-cells [194–197] (Figure 4b). Conversely, the availability of small-molecule compounds to activate YAP/TAZ [198–200] may enable a more efficient expansion of stem cells from patients affected by genetic deficiencies in cell-ECM adhesion receptors [201–203]. Thus, the regulation of ECM mechanotransduction and the associated transcriptional responses bears great potential in the context of regenerative medicine [G], although the ability to target such a pleiotropic mechanism to drug specific mechanotransduction processes while avoiding unwanted side effects remains a key challenge in the field.

# The cardiovascular system as paradigm for mechanical force-mediated transcriptional regulation

The physiological relevance of force-mediated transcriptional control is probably most well understood in the cardiovascular system, thus serving as an excellent paradigm for highlighting the in vivo evidence for mechanosignaling. Tangential shear forces associated with blood flow and sensed by endothelial cells (ECs) are major determinants of vascular morphogenesis during development, and of vascular remodelling during adult life. Moreover, variations in the magnitude and pattern of blood flow can contribute to inflammatory responses and to disease. As with other mechanical inputs, these responses entail both morphological and cytoskeletal rearrangements, and the modulation of TF activity [9,204] (Figure 4c).

## Protective effects of high laminar flow.

High laminar flow guides angiogenesis during development, stabilizes vessels, promotes the alignment of ECs in the direction of flow, decreases EC turnover, suppresses inflammation and activates antioxidant pathways, preventing the formation of atherosclerotic plaques (Figure 4c). On the level of intracellular signaling, high laminar flow promotes the activation of the NRF2 TF. NRF2 regulates transcription by forming heterodimers with members of the sMaf protein family,

and is activated by oxidative stress. Consequently, the main transcriptional targets of NRF2 are antioxidant protective genes [205,206]. Activation of NRF2 by blood flow not only empowers endothelial cell antioxidant metabolism, but also suppresses inflammation. This anti-inflammatory response depends on the transmission of shearing forces by the glycocalyx, entails the activation of PI3K/AKT and the production of mitochondrial ROS, and is modulated by COX2 activity and prostaglandins [207–213].

High laminar flow also promotes the activation of ERK5, which induces the activity of the MEF2 TF and chromatin acetylation, leading to increased expression of *KLF2* [214–218]. This induction is further reinforced by flow-regulated expression of *miR-92a* [219]. KLF2 is a TF whose elevated expression in response to flow coordinates the expression of vasoactive compounds that signal to smooth muscle cells to maintain the vascular tone, and also primes the expression of *NRF2* [220–222].

Fluid shear stresses can also regulate gene expression through Notch. Tension across the Delta/Notch signaling complex at cell-cell contacts facilitates its proteolytic cleavage and the release of the Notch intracellular domain (NICD), which acts as nuclear coactivator for the RBPJ DNA-binding factor [223–228]. This mechanism is relevant for the regional specification of the endothelium during development, for the cross-talk with vascular smooth muscle cells, and for the formation of the heart structures [229–235]. Interestingly, activation of Notch depends on the extent and tugging force of cell-cell contacts in epithelial cells [236–239], suggesting the interesting possibility that forces between adjacent cells in a monolayer can also regulate Notch.

Also the regulation of YAP/TAZ by mechanical cues is important for adjusting EC proliferation [123,240,241], during the maintenance of tissue stiffness homeostasis [242], and during developmental sprouting angiogenesis [G] where YAP/TAZ are required for the maturation of the vascular barrier and for direct angiogenesis along a tissue stiffness gradient [243–247]. In this context, YAP/TAZ co-operate with the two antagonistic TFs TFII-I (also known as GTF2I) and GATA2 to regulate the expression of the key angiogenic *Vascular Endothelial Factor Receptor-2* gene [248].

## Homeostatic remodeling in response to low laminar flow.

When arteries are subjected to low laminar flow, a homeostatic mechanism reduces lumen diameter by inward remodeling to restore the optimal shear stress (Figure 4c). This mechanism is based on the cross talk between two pathways: at low shear stress intensity, only SMAD2/3/4 are activated and induce inward remodeling; when higher levels of shear stress are reinstalled, also the ERK5/KLF2 system is activated, leading to inhibition of SMAD2/3 [249] . A similar

cross-talk also occurs for the SMAD1/5/4 transcription complex. SMAD1/5/4 is activated in response to shear stresses, downstream of the BMP type I receptor Alk1 and of its coreceptor Endoglin [250–252]. This is relevant for the stabilization of vessels by flow shear stress, and contributes to arteriovenous malformations observed in HHT patients [250]. At the same time, activation of KLF2 prevents excessive BMP signalling, which accounts for the protective effects of high laminar shear stress against vascular calcifications [253]. Taken together, a number of transcriptional regulators are activated by laminar flow to regulate vascular development and homeostasis. A critical next step for the field is to understand the specific versus overlapping roles of these pathways as well as the mechanisms of crosstalk and co-operativity by which transcriptional specificity in response to specific flow magnitudes is achieved.

# Pathological roles of mechanosensitive transcription

## Pathological transcriptional responses to disturbed blood flow.

Not only the magnitude of shear stress, but also the type (i.e. disturbed vs. laminar flow) is a fundamental regulator of endothelial homeostasis [254,255]. Disturbances in fluid flow dynamics associated with branching or turning points of the arterial tree facilitate the emergence of pathological phenotypes including ECs misalignment, proliferation, and a low level of chronic inflammation. This, over long periods and in cooperation with other risk factors, predisposes to formation of atherosclerotic plaques at such regions (Figure 4c). Disturbed flow acting on the apical surface of ECs is transmitted through the membrane and cortical cytoskeleton to cell-cell junctions, where tension is sensed by a complex between VE-cadherin, PECAM-1 and VEGFR2, and to the basal surface, where tension is sensed at integrin attachment sites [256,257]. In response to tension, several signalling mediators are activated including PI3K, SRC, PLC/PKC, the small GTPase RAC1 and NOX. These in turn promote the activation of the NFkappaB, AP1 and YAP/TAZ TF complexes, resulting in the promotion of proinflammatory gene expression and enhancing proliferation [258–263]. This may indicate YAP/TAZ inhibition as a potential therapy against atherosclerosis, or to normalize vascular malformations in Hereditary Hemorrhagic Telangiectasia [G] [264,265].

The interplay between shear stress and cell-ECM adhesions in regulating the response to flow is complex, because specific ECM ligands that engage their specific integrin receptors can have opposite effects on NFkappaB [266]. This may also account for the opposite regulation of NFkappaB in response to ECM stiffness observed in different cell types [267,268]. Moreover,

transmission of tension from cell-ECM attachment sites to the nucleus can regulate NFkappaB in response to cell stretching [29,269,270].

## A loop of mechanosensitive transcription in fibrosis.

A key paradigm of pathological mechanotransduction is the fibrotic response [G]. During fibrosis, a self-sustaining loop between fibroblast activation/proliferation and ECM secretion/contraction is key for pathological tissue remodeling and stiffening [8]. This loop was key to discovering how cells "read" ECM stiffness by developing active tension to resist extracellular rigidity [12,271]. Multiple mechano-sensitive TFs participate in this loop and establish an integrated and multi-tiered feed-forward system (Figure 4d).

Several data indicate a role for YAP/TAZ in fibrotic reactions in mice and humans [45,148,272–275]. This YAP/TAZ-driven mechanical feedback loop also maintains the activated myofibroblast phenotype during the contraction model of alveolar formation and regeneration and mediate the ECM-mediated crosstalk between cancer cells and cancer associated fibroblasts [G] [146,148,276]. Also the mechanoadaptive transcriptional function of SRF in promoting transcription of cytoskeletal and ECM genes plays a key role during fibrosis, and is amplified in fibrotic disease. Thus, small-molecule modulators of SRF/MRTF can be used to prevent tissue fibrosis or, conversely, to promote wound healing [277–290]. During fibrosis, SRF/MRTF cooperates with YAP/TEAD transcription complexes at adjacent promoter elements [104,105,291]. This crosstalk is also hijacked in cancer cells to sustain malignant growth [G] [292–296].

Another example of the role mechanosensitive TFs in the fibrotic response is the activation of Smad2/3/4 [G] TF complexes [297] upon liberation of TGF-β signalling molecules from "ECM traps" by active pulling of cells on the ECM via integrin complexes [298–305], which cooperates with mechanically-regulated SRF/MRTF [106,107]. Finally, also the SNAIL1 and ZNF1416 TFs can be activated in the context of fibrosis and contribute to this self-amplification loop [306,307] (Figure 4d).

## YAP/TAZ as mediators of mechanically-induced breast cancer progression.

ECM stiffness plays an important role for breast cancer development, often dominating over the cell's genetic and oncogenetic makeup to promote the loss of epithelial polarity and epithelial-to-mesenchymal transition [G] (EMT), the acquisition of migratory and invasive behavior, and proliferation [308–310]. YAP/TAZ can promote several of these phenotypes in cancer cells [311–319] and are relevant in the crosstalk between ECM stiffness and oncogenes in promoting

pro-tumorigenic phenotypes [147,320] (Figure 4e). Moreover, YAP/TAZ lay at the center of a multi-layered feed-forward loop between high mammographic density / ECM stiffness, EMT and microRNAs [312,321–324]. In line with these data, YAP/TAZ promote breast cancer progression in mice, even if genetic evidence for a role of ECM mechanotransduction components in mammary tumorigenesis, and on their effects of YAP/TAZ, awaits stronger experimental evidence [314,325–329].

### Mechanical control of liver homeostasis and cancer through YAP/TAZ.

Hepatocytes were among the first cell types used to study ECM stiffness mechanoresponses [330], and the liver tissue is a model system for organ-size regulation by the Hippo pathway [331]. Recent data indicate that control of YAP/TAZ by the soft tissue mechanical properties are key to maintain hepatocyte proliferative, metabolic and cell fate homeostasis, and to control liver organ size [143]. This could relate with the recent finding that mechanical pressure homeostasis of liver sinusoids is important during tissue regeneration [332]. Liver tissue softness also represents a tumor-suppressive mechanism that can be bypassed by cell-autonomous and oncogene-driven regulation of the Fascin1 actin bundling protein, which supports YAP/TAZ mechanotransduction, cholangiocellular transdifferentiation and the formation of cholangiocarcinomas [144].

Overall, experimental evidence obtained by the cross-contamination of several fields of research has led to the definition of multiple molecular mechanisms by which tissue mechanics can influence genome organization, chromatin epigenetics and gene expression, and in particular genetic ablation experiments in model organisms point to the importance of these mechanisms in the context of normal and diseased cells. As most of the factors highlighted in this section can also be activated by purely biochemical signals, the challenge in the field is now to more precisely dissect the precise, direct role of mechanical forces in modulating these pathways in physiological and pathological scenarios *in vivo*.

## Emerging concepts

The direct transmission of forces through the actin cytoskeleton, and its structural remodelling in response to forces, is a powerful mechanism by which mechanical information can be communicated inside the cell. This is interesting in light of the notion that not only the nucleus, but several other organelles are surrounded by actin filaments, which are important for their subcellular localization, transport and dynamics.

We already discussed evidence implying force transmission to the nucleus, which results in calcium release from the nuclear envelope and/or in response to deformation of the ER proximal to the nucleus [20,34–37]. This could perhaps affect the activity of TFs that originate as ER-resident transmembrane proteins [333]. Another example of organelle mechanosensitivity is the Golgi apparatus, whose rheology mirrors cell tension in response to extracellular mechanical cues [42,45]. This tensional adaptation links extracellular mechanical cues with activation of the SREBP1/2 TFs, by regulating trafficking of the SREBP1/2 transmembrane precursors between the Golgi and the ER [45,334] (Figure 4f). This mechanism accounts for lipid accumulation in cells on a soft ECM, and couples the promotion of mesenchymal stem cell differentiation into adipocytes with switching on the corresponding lipogenic metabolism program.

In other recent studies, mitochondrial morphology has been shown to be regulated by the interplay between peri-mitochondrial actin and mechanical cues from the ECM [44,46,335–341] (Figure 4f). Mechanical forces can even bypass the requirement for actin for mitochondrial fission, by directly inducing mitochondrial deformation [43]. Cells on a soft ECM display enhanced DRP1-dependent mitochondrial fission, which likely depends on cooperation between multiple mechanisms, and which mediates activation of the NRF2 TF to empower antioxidant metabolism, ultimately making cells on a soft ECM able to better resist oxidative stress [44,46,336,342–344]. The finding that a similar pathway is relevant for neural stem cell commitment in the mouse brain, the softest among tissues, suggests this link may have multiple physiological roles beyond redox homeostasis [345,346].

These studies collectively suggest the notion that forces may regulate peri-organelle actin pools, an emerging concept in the field of mechanotransduction with potentially significant relevance in mediating mechano-responsive signalling and downstream transcriptional responses.

# Concluding remarks

Taken together, recent advances in the field of mechanical regulation of transcription indicate that multiple organelles including the plasma membrane, nucleus, mitochondria and ER display mechanosensitivity and are capable of activating downstream signaling to regulate transcription. Going forward it will be critical to determine under which scenarios/in response to what kind of forces these various organelles are triggered to respond and how they cooperate to determine coherent and integrated transcriptional outputs. In addition, the molecular mechanosensors that are responsible for direct sensing of forces within the specific mechanosensitive organelles and thus the mechanisms of initial conversion of mechanical force into a biochemical signal remain

elusive, in particular *in vivo*. One key obstacle to progress is the absence of tools that allow direct visualization and quantification of tension and stress in intact tissues *in vivo*. Such tools, in combination with single molecule imaging of transcriptional output will allow establishment of quantitative relationships between specific mechanical forces and their transcriptional outputs. This would also allow distinguishing the force-mediated effects of mechanosensitive TF systems from their non-mechanosensitive functions. In addition, how TFs cooperate with the pleiotropic effects of force on chromatin and transcription remains an important open question. Unraveling the molecular details of these processes will facilitate understanding if the specific mechanical properties of tissue could function as a layer of “epigenetic” regulation to maintain stable gene transcription and cell identity, as a mechanism to “template” appropriate transcriptional responses during regeneration and repair.


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# Author contributions

S.D. and S.W. conceptualized and wrote the manuscript. All authors approved the final content.

# Competing Interests

The authors declare no competing interests.

# Glossary

mechanical forces: cells in tissues are subjected to multiple forces including applied, frictional, tension and spring (i.e. stretch or compression) forces

shear: shear occurs when a fluid applies a tangential force that pushes one part of the cell in one direction, and the rest of the cell is dragged in the opposite direction by adhesion to the ECM or to other cells

tensile stress: the action-reaction forces acting at the cell-ECM or cell-cell contact sites, and the opposite of compression

elasticity: the ability of a material to return to its original shape after the deformation-inducing force is removed

viscosity: The ability of a material to resist deformation. Most biological materials are considered visco-elastic, i.e they display properties of both elastic and viscous materials. Viscoelastic materials have a strain (deformation) rate that is dependent on time and they dissipate energy when a load is applied and removed whereas a purely elastic material does not.

friction: a resistance a surface encounters when its moving over another surface, eg. in joints, tendons, eye or skin.

Hutchinson-Gilford progeria: a rare autosomal-dominant genetic disorder characterized by premature aging symptoms and caused by mutations in the gene encoding for the Lamin-A/C nucleoskeletal protein.

Duchenne's muscular dystrophy: a genetic disorder characterized by progressive muscle degeneration and weakness and caused by mutations in the gene encoding for the Dystrophin cytoskeletal protein.

cell geometry: intended here as the degree of cell spreading on an adhesive substrate, which regulates actomyosin tension accordingly.

SRF/MRTF: a transcriptional protein complex composed of the DNA-binding Serum Response Factor (SRF) protein and of one of the MRTFs

TCF: Ternary Complex Factor family members Elk1, Net and SAP1 ETS-domain TFs

MRTF: Myocardin-Related TF family members Myocardin, MRTFA and MRTFB

RHO GTPase: small monomeric GTPases that function as molecular switches in response to chemical or mechanical stimuli to regulate actin polymerization and its organization in contractile bundles.

mechanoadaptation: the process by which a cell subjected to mechanical forces reinforces its force-bearing structures

fibrotic response: tissue remodelling characterized by the deposition of collagenous ECM. This can have a physiological function during wound healing (scarring) or a pathological function which can interfere with or totally inhibit the normal architecture and function of the underlying organ or tissue.

malignant growth: the ability of cancer cells to grow without control and to invade neighboring tissues

Smad2/3/4: a heterotrimeric DNA-binding transcriptional protein complex composed of one Smad4 and two Smad2 and/or Smad3 subunits, whose association is regulated by phosphorylation of Smad2 and Smad3 in response to extracellular TGF-β ligands.

contact inhibition of growth: the process by which cell crowding, through the establishment of cell-cell contacts and the reduction of cell geometry, inhibits cell proliferation.

cell competition: the active elimination of a viable but undesirable cell population by competitive interactions within a tissue

Endomitosis: the division of chromosomes not followed by nuclear and cell division that results in a bigger cell with an increased number of chromosomes

cell-autonomous: a property conferred by gene mutation or activation to a cell in a multicellular structure, which is not observed in neighboring cells

cancer-associated fibroblasts: a population of cells found in tumors that are negative for epithelial, endothelial and leukocyte markers, with an elongated morphology, lacking the mutations found in cancer cells, and likely deriving from the fibroblast lineages(s).

keloid fibroproliferative disorders: benign growth of dermal cells, associated to continuous deposition of collagen, that spreads beyond the margin of the original wound, commonly recurs following excision, and rarely regresses spontaneously.

Hereditary Hemorrhagic Telangiectasia: a genetic disorder that results in the development of multiple abnormalities in the blood vessels, often leading to hemorrhages.

sprouting angiogenesis: the growth of new capillary vessels out of preexisting vessels.

epithelial-to-mesenchymal transition : the differentiation process by which cells lose epithelial identity and the ability to form stable cell-cell adhesions, and gain expression of mesenchymal markers associated with increased migratory ability.

trophectoderm: the tissue of the preimplantation mammalian embryo that will contribute to formation of the placenta

inner cell mass: the tissue of the preimplantation mammalian embryo that will contribute to formation of the tissues of the fetus

regenerative therapy: the clinical use of stem cells to stimulate repair mechanisms and restore function in damaged body tissues or organs

organoids: three-dimensional structures obtained by culturing stem cells in vitro, which recapitulate key morphological and differentiation aspects of the real tissue/organ

glossary term

**Table 1. The main transcription factors (TF) regulated in response to mechanical stimuli.**

| TF | DNA binding motif | Mechanical input |
|---|---|---|
| SRF/MRTF | 5'-CC$(A/T)_6$GG-3 | ECM stiffness; Cell geometry; Cell stretching |
| SMAD2/3/4 | 5'-GTCTAGAC-3' (Smad Binding Element motif), 5'-CCAGACA-3' (CAGA motif) | Traction force-mediated unfastening of TGFβ from "ECM traps"; Shear stresses (laminar flow) |
| TEAD/YAP and TEAD/TAZ | 5'-(G/A)CATTCC(A/T)-3' | ECM stiffness; Cell geometry; Cell crowding; Cell stretching; Cell compression; Shear stresses (disturbed flow) |
| β-Catenin/LEF/TCF | 5'-AGATCAAAGG-3' | Tissue compression |
| NRF2 | 5′-TGACtcaGCa-3′ Antioxidant Response Elements | Shear stress (laminar flow); ECM stiffness |
| NICD/RBPJ | 5'-GTGGGGAA-3' | Cell-cell tugging forces, Shear stress (laminar flow) |
| MEF2C (driving expression of *KLF2*) | 5'-(C/T)TA(A/T)4TA(G/A)-3' | Shear stress (laminar flow) |
| SMAD1/5/4 | 5'GGC/AGCC-3' (GC-rich SBE) in the vicinity of a 5'-AGAC-3' (SBE) | Shear stress (laminar flow) |

DNA binding motifs are based on a consensus from the literature, and do not take into account alternative binding sites identified through chromatin-immunoprecipitation experiments.

# Figure legends

**Figure 1.** Diagram of an epithelial cell subjected to extracellular or extrinsic forces, and developing opposing intrinsic tension in response to these by contraction of the actomyosin cytoskeleton. Multiple adhesion complexes can mediate the transmission of forces between the cell and the surrounding ECM or other cells, including integrin-based focal adhesions, cadherin-based adherens junctions and tight junctions (based on Tetraspanins, i.e. occludins and claudins). These receptors are directly connected to F-actin by junctional proteins such as Talin, α- and β-Catenin, and Zonula Occludens proteins. Shear stress results from blood flowing on the cell surface, and is transmitted through the glycocalyx and actin cortex, and on opposing resistance provided by cell attachment.

**Figure 2.** Mechanotransduction of forces into biochemical information. **a.** Cells attach to the ECM through integrin receptor complexes, which are connected to the F-actin cytoskeleton via a number of adaptor proteins, here depicted for simplicity by the Talin protein. On soft ECM, absence of resisting forces and of opposing cytoskeletal tension leave Talin in a closed conformation, limiting the maturation of Focal adhesions. On a stiff ECM, higher forces lead to Talin unfolding, and recruitment of additional proteins to focal adhesions, depicted here by Vinculin. Recruitment of these proteins in response to force initiates signalling within the cell. **b.** Cells attach to neighboring cells' cadherin receptors, connected in the cytoplasm with F-actin via Catenin adaptor proteins. Analogous to Talin, higher forces flowing across cell-cell adhesions lead to unfolding of α-Catenin and recruitment of Vinculin and possibly of other signalling molecules (purple). **c.** Membrane tension induced by direct deformation of the lipid bilayer, or indirectly by application of forces on the ECM and/or cytoskeleton, causes a structural rearrangement of the Piezo channel protein and allows inward ion currents. **d.** Forces can be transmitted to the nucleus either directly (deformation) or indirectly through the actin cytoskeleton, which is tethered to the nuclear envelope and to the nuclear lamins (lamina) by LINC complexes. The inward permeability of Nuclear Pore Complexes (NPC), associated to transmembrane nuclear-associated actin (TAN) lines, is promoted upon nuclear compression. Nuclear membrane tension can trigger the release of calcium ions from intracellular reservoirs, which synergistically induce the recruitment of Phospholipase (cPLA2) at the Inner Nuclear Membrane and its activation. The resulting increase in Arachidonic Acid stimulates cytoplasmic actin dynamics. Formation of endocytic caveolae also responds to membrane tension, acting as

a membrane reservoir to accommodate stretching, and regulating signaling pathways. Adhesion receptors indicate both cell-ECM and cell-cell adhesions.

**Figure 3.** Mechanisms by which mechanical forces can regulate gene expression. **a.** Forces can influence chromatin structural organization, epigenetic modifications and gene transcription. Peripheral chromatin is physically connected, via the nuclear lamins (Lamina) and LINC complexes, to the cytoplasmic actomyosin cytoskeleton. Several chromatin and DNA-associated factors interact with, and are regulated by, nuclear globular and filamentous actin. Nuclear and cytoplasmic actin dynamics are connected by the rate-limiting transport of G-actin across the Nuclear Pore Complexes (NPC). Tension-dependent release of calcium within the cell can also influence such processes. P, Ac and Me exemplify chromatin modifications. **b.** Binding of monomeric globular actin to the MRTF coactivator protein precludes the interaction of MRTF with the DNA-binding SRF TF. When cells are subjected to forces that induce cytoskeletal tension and F-actin polymerization, the decrease of G-actin results in activation of SRF/MRTF-dependent gene expression. **c.** Actomyosin tension regulates the nuclear localization of the YAP/TAZ (Y/T) coactivators and their association with the TEAD family of TFs via multiple parallel mechanisms. Force applied through focal adhesions leads to activation of the RAP2 small GTPase, which relieves the inhibitory action of the LATS1/2 kinases on cytoplasmic YAP/TAZ. Deformation of the nucleus in response to adhesion forces facilitates the nuclear translocation of YAP/TAZ through Nuclear Pore Complexes (NPC). Binding of the ARID1A protein to nuclear F-actin titrates away ARID1A from YAP/TAZ, facilitating the interaction with TEAD TFs. Y/T indicates either YAP or TAZ proteins.

**Figure 4.** Selected functional implications of mechanically-regulated transcription for physiology, regenerative medicine, and disease. **a.** Deformation of Drosophila embryonic tissues, which occurs as a result of morphogenetic movements, reinforces expression of the Twist TF in mesoderm cells, coordinating cell fate specification with morphogenesis. Lower, left: a field of cells undergoing localized compression activate Twist expression (red nuclei). Lower, right: a section of a Drosophila embryo stained for Twist in mesoderm cells undergoing folding and invagination movements. **b.** The *in vitro* production of functional pancreatic endocrine β-cells is a key objective for cell-based replacement therapies. Terminal differentiation of human induced pluripotent stem cells (iPS) into insulin-secreting β-cells is hampered by culture on stiff Tissue Culture plastics (gray line). Disabling YAP/TAZ mechanotransduction in pancreatic progenitors with the F-actin inhibitor latrunculinB enables expression of Neurogenin3 and efficient terminal

differentiation (red arrow). Y/T indicates YAP/TEAD or TAZ/TEAD complexes. **c.** The TF network that controls endothelial responses to shear stresses. High intensity laminar shear stress (LSS) maintains endothelial homeostasis by flow-dependent regulation of multiple pathways and TFs. Low intensity LSS leads to activation of Smad2/3/4 (SM2/3) transcription complexes and inward vessel remodeling. Disturbed shear stress (DSS) activates a distinct set of pro-inflammatory and pro-atherosclerotic TFs. LSS can modulate activation of the indicated membrane receptors/coreceptors for the TGFβ (Alk5, Neuropilin1), BMP (Alk1, Endoglin) and Notch pathways. **d.** TF and signalling networks controlling tissue fibrosis. Deposition and remodeling of a collagenous ECM activates multiple mechano-sensitive TFs that cooperate to maintain fibroblast activation and to further amplify ECM remodeling. Increased ECM stiffness and cell contractility also facilitates the liberation of extracellular TGFβ1-2-3 ligands from ECM-bound «traps» composed of the Latency Associated Peptide (LAP) and Latent TGFβ Binding Protein (LTBP), by pulling via αVβ integrins, leading to activation of pro-fibrotic Smad2/3/4 TFs. MRTF indicates MRTF/SRF complexes. **e.** During breast cancer development, cancer cells and cancer associated fibroblasts (CAF) cooperate to remodel and stiffen the ECM. This maintains the activated state of CAFs, enables metabolic cross-talk between CAFs and cancer cells, and promotes malignant cancer cell behavior by regulating mechano-sensitive TFs. **f.** Metastatic breast cancer cells often disseminate to organs with a soft ECM microenvironment. Reduced actomyosin tension reverberates on peri-organelle F-actin pools, leading to decreased rigidity of the Golgi apparatus, and to increased peri-mitochondrial F-actin. The resulting alterations of organelle dynamics activate the SREBP1/2 and NRF2 TFs, which mediate metabolic reprogramming and influence chemotherapy resistance.

# References


1. Collinet, C. & Lecuit, T. Programmed and self-organized flow of information during morphogenesis. *Nat Rev Mol Cell Biol* **22**, 245–265 (2021).
2. Mishra, N. & Heisenberg, C.-P. Dissecting Organismal Morphogenesis by Bridging Genetics and Biophysics. *Annu Rev Genet* (2021) doi:10.1146/annurev-genet-071819-103748.
3. Stooke-Vaughan, G. A. & Campàs, O. Physical control of tissue morphogenesis across scales. *Curr Opin Genet Dev* **51**, 111–119 (2018).
4. Janmey, P. A., Fletcher, D. A. & Reinhart-King, C. A. Stiffness Sensing by Cells. *Physiological Reviews* **100**, 695–724 (2020).
5. Murrell, M., Oakes, P. W., Lenz, M. & Gardel, M. L. Forcing cells into shape: the mechanics of actomyosin contractility. *Nature Reviews Molecular Cell Biology* **16**, 486–498 (2015).
6. Vining, K. H. & Mooney, D. J. Mechanical forces direct stem cell behaviour in development and regeneration. *Nature Reviews Molecular Cell Biology* **18**, 728–742 (2017).
7. Vogel, V. Unraveling the Mechanobiology of Extracellular Matrix. *Annu. Rev. Physiol.* **80**, 353–387 (2018).
8. Tschumperlin, D. J., Ligresti, G., Hilscher, M. B. & Shah, V. H. Mechanosensing and fibrosis. *J. Clin. Invest.* **128**, 74–84 (2018).
9. Humphrey, J. D. & Schwartz, M. A. Vascular Mechanobiology: Homeostasis, Adaptation, and Disease. *Annu Rev Biomed Eng* **23**, 1–27 (2021).
10. Maurer, M. & Lammerding, J. The Driving Force: Nuclear Mechanotransduction in Cellular Function, Fate, and Disease. *Annu Rev Biomed Eng* **21**, 443–468 (2019).
11. Charras, G. & Yap, A. S. Tensile Forces and Mechanotransduction at Cell-Cell Junctions. *Curr. Biol.* **28**, R445–R457 (2018).
12. Iskratsch, T., Wolfenson, H. & Sheetz, M. P. Appreciating force and shape—the rise of

mechanotransduction in cell biology. *Nat. Rev. Mol. Cell Biol.* **15**, 825–833 (2014).

13. Kechagia, J. Z., Ivaska, J. & Roca-Cusachs, P. Integrins as biomechanical sensors of the microenvironment. *Nat. Rev. Mol. Cell Biol.* **20**, 457–473 (2019).
14. Hu, X., Margadant, F. M., Yao, M. & Sheetz, M. P. Molecular stretching modulates mechanosensing pathways. *Protein Sci* **26**, 1337–1351 (2017).
15. Saini, K. & Discher, D. E. Forced Unfolding of Proteins Directs Biochemical Cascades. *Biochemistry* (2019) doi:10.1021/acs.biochem.9b00839.
16. Cox, C. D., Bavi, N. & Martinac, B. Origin of the Force: The Force-From-Lipids Principle Applied to Piezo Channels. *Curr Top Membr* **79**, 59–96 (2017).
17. Douguet, D. & Honoré, E. Mammalian Mechanoelectrical Transduction: Structure and Function of Force-Gated Ion Channels. *Cell* **179**, 340–354 (2019).
18. Martinac, B. The ion channels to cytoskeleton connection as potential mechanism of mechanosensitivity. *Biochim Biophys Acta* **1838**, 682–691 (2014).
19. Murthy, S. E., Dubin, A. E. & Patapoutian, A. Piezos thrive under pressure: mechanically activated ion channels in health and disease. *Nature Reviews Molecular Cell Biology* **18**, 771–783 (2017).
20. Nava, M. M. *et al.* Heterochromatin-Driven Nuclear Softening Protects the Genome against Mechanical Stress-Induced Damage. *Cell* **181**, 800-817.e22 (2020).
21. Prystopiuk, V. *et al.* A two-phase response of endothelial cells to hydrostatic pressure. *J Cell Sci* **131**, jcs206920 (2018).
22. De Belly, H. *et al.* Membrane Tension Gates ERK-Mediated Regulation of Pluripotent Cell Fate. *Cell Stem Cell* **28**, 273-284.e6 (2021).
23. Sinha, B. *et al.* Cells respond to mechanical stress by rapid disassembly of caveolae. *Cell* **144**, 402–413 (2011).
24. Torrino, S. *et al.* EHD2 is a mechanotransducer connecting caveolae dynamics with gene transcription. *J Cell Biol* **217**, 4092–4105 (2018).

25. Discher, D. E. *et al.* Matrix Mechanosensing: From Scaling Concepts in 'Omics Data to Mechanisms in the Nucleus, Regeneration, and Cancer. *Annu Rev Biophys* **46**, 295–315 (2017).
26. Miroshnikova, Y. A. & Wickström, S. A. Mechanical Forces in Nuclear Organization. *Cold Spring Harb Perspect Biol* a039685 (2021) doi:10.1101/cshperspect.a039685.
27. Buxboim, A. *et al.* Matrix elasticity regulates lamin-A,C phosphorylation and turnover with feedback to actomyosin. *Curr. Biol.* **24**, 1909–1917 (2014).
28. Cho, S. *et al.* Mechanosensing by the Lamina Protects against Nuclear Rupture, DNA Damage, and Cell-Cycle Arrest. *Dev. Cell* **49**, 920-935.e5 (2019).
29. Lammerding, J. *et al.* Lamin A/C deficiency causes defective nuclear mechanics and mechanotransduction. *J Clin Invest* **113**, 370–378 (2004).
30. Lammerding, J. *et al.* Abnormal nuclear shape and impaired mechanotransduction in emerin-deficient cells. *Journal of Cell Biology* **170**, 781–791 (2005).
31. Swift, J. *et al.* Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation. *Science* **341**, 1240104 (2013).
32. Buxboim, A. *et al.* Coordinated increase of nuclear tension and lamin-A with matrix stiffness outcompetes lamin-B receptor that favors soft tissue phenotypes. *MBoC* **28**, 3333–3348 (2017).
33. Ivanovska, I. L. *et al.* Cross-linked matrix rigidity and soluble retinoids synergize in nuclear lamina regulation of stem cell differentiation. *Mol Biol Cell* **28**, 2010–2022 (2017).
34. Enyedi, B., Jelcic, M. & Niethammer, P. The Cell Nucleus Serves as a Mechanotransducer of Tissue Damage-Induced Inflammation. *Cell* **165**, 1160–1170 (2016).
35. Itano, N., Okamoto, S., Zhang, D., Lipton, S. A. & Ruoslahti, E. Cell spreading controls endoplasmic and nuclear calcium: A physical gene regulation pathway from the cell surface to the nucleus. *PNAS* **100**, 5181–5186 (2003).
36. Lomakin, A. J. *et al.* The nucleus acts as a ruler tailoring cell responses to spatial

constraints. *Science* **370**, eaba2894 (2020).

37. Venturini, V. *et al.* The nucleus measures shape changes for cellular proprioception to control dynamic cell behavior. *Science* **370**, eaba2644 (2020).
38. Aureille, J. *et al.* Nuclear envelope deformation controls cell cycle progression in response to mechanical force. *EMBO Rep* **20**, (2019).
39. Elosegui-Artola, A. *et al.* Force Triggers YAP Nuclear Entry by Regulating Transport across Nuclear Pores. *Cell* **171**, 1397-1410.e14 (2017).
40. Hoffman, L. M. *et al.* Mechanical stress triggers nuclear remodeling and the formation of transmembrane actin nuclear lines with associated nuclear pore complexes. *MBoC* **31**, 1774–1787 (2020).
41. Zimmerli, C. E. *et al.* Nuclear pores dilate and constrict in cellulo. *Science* eabd9776 (2021) doi:10.1126/science.abd9776.
42. Guet, D. *et al.* Mechanical role of actin dynamics in the rheology of the Golgi complex and in Golgi-associated trafficking events. *Curr. Biol.* **24**, 1700–1711 (2014).
43. Helle, S. C. J. *et al.* Mechanical force induces mitochondrial fission. *eLife* **6**, e30292 (2017).
44. Romani, P. & Dupont, S. Mitochondrial fission links ECM mechanotransduction to metabolic redox homeostasis and metastatic chemotherapy resistance. *Nature Cell Biology* **In press**,.
45. Romani, P. *et al.* Extracellular matrix mechanical cues regulate lipid metabolism through Lipin-1 and SREBP. *Nat. Cell Biol.* **21**, 338–347 (2019).
46. Tharp, K. M. *et al.* Actomyosin-Mediated Tension Orchestrates Uncoupled Respiration in Adipose Tissues. *Cell Metabolism* **27**, 602-615.e4 (2018).
47. Pombo, A. & Dillon, N. Three-dimensional genome architecture: players and mechanisms. *Nat Rev Mol Cell Biol* **16**, 245–257 (2015).
48. Szabo, Q., Bantignies, F. & Cavalli, G. Principles of genome folding into topologically

associating domains. *Sci Adv* **5**, eaaw1668 (2019).

49. Yu, M. & Ren, B. The Three-Dimensional Organization of Mammalian Genomes. *Annu Rev Cell Dev Biol* **33**, 265–289 (2017).
50. Bickmore, W. A. & van Steensel, B. Genome architecture: domain organization of interphase chromosomes. *Cell* **152**, 1270–1284 (2013).
51. Morgan, M. A. J. & Shilatifard, A. Reevaluating the roles of histone-modifying enzymes and their associated chromatin modifications in transcriptional regulation. *Nat Genet* **52**, 1271–1281 (2020).
52. Tessarz, P. & Kouzarides, T. Histone core modifications regulating nucleosome structure and dynamics. *Nat Rev Mol Cell Biol* **15**, 703–708 (2014).
53. Sun, J., Chen, J., Mohagheghian, E. & Wang, N. Force-induced gene up-regulation does not follow the weak power law but depends on H3K9 demethylation. *Sci Adv* **6**, eaay9095 (2020).
54. Tajik, A. *et al.* Transcription upregulation via force-induced direct stretching of chromatin. *Nat Mater* **15**, 1287–1296 (2016).
55. Carley, E. *et al.* The LINC complex transmits integrin-dependent tension to the nuclear lamina and represses epidermal differentiation. *Elife* **10**, e58541 (2021).
56. Heo, S.-J. *et al.* Differentiation alters stem cell nuclear architecture, mechanics, and mechano-sensitivity. *Elife* **5**, e18207 (2016).
57. Heo, S.-J. *et al.* Mechanically Induced Chromatin Condensation Requires Cellular Contractility in Mesenchymal Stem Cells. *Biophys J* **111**, 864–874 (2016).
58. Le, H. Q. *et al.* Mechanical regulation of transcription controls Polycomb-mediated gene silencing during lineage commitment. *Nat Cell Biol* **18**, 864–875 (2016).
59. Stephens, A. D. *et al.* Physicochemical mechanotransduction alters nuclear shape and mechanics via heterochromatin formation. *Mol Biol Cell* **30**, 2320–2330 (2019).
60. Le Beyec, J. *et al.* Cell shape regulates global histone acetylation in human mammary

epithelial cells. *Exp Cell Res* **313**, 3066–3075 (2007).

61. Li, Y. *et al.* Biophysical regulation of histone acetylation in mesenchymal stem cells. *Biophys J* **100**, 1902–1909 (2011).
62. Jain, N., Iyer, K. V., Kumar, A. & Shivashankar, G. V. Cell geometric constraints induce modular gene-expression patterns via redistribution of HDAC3 regulated by actomyosin contractility. *Proc Natl Acad Sci U S A* **110**, 11349–11354 (2013).
63. Lee, J. *et al.* Geometric regulation of histone state directs melanoma reprogramming. *Commun Biol* **3**, 1–9 (2020).
64. Fasciani, A. *et al.* MLL4-associated condensates counterbalance Polycomb-mediated nuclear mechanical stress in Kabuki Syndrome. *Nat Genet* **52**, 1397–1411 (2020).
65. Stephens, A. D., Banigan, E. J., Adam, S. A., Goldman, R. D. & Marko, J. F. Chromatin and lamin A determine two different mechanical response regimes of the cell nucleus. *Mol Biol Cell* **28**, 1984–1996 (2017).
66. Shao, X., Li, Q., Mogilner, A., Bershadsky, A. D. & Shivashankar, G. V. Mechanical stimulation induces formin-dependent assembly of a perinuclear actin rim. *Proc Natl Acad Sci U S A* **112**, E2595-2601 (2015).
67. Wales, P. *et al.* Calcium-mediated actin reset (CaAR) mediates acute cell adaptations. *Elife* **5**, e19850 (2016).
68. Jung, H.-J. *et al.* An absence of nuclear lamins in keratinocytes leads to ichthyosis, defective epidermal barrier function, and intrusion of nuclear membranes and endoplasmic reticulum into the nuclear chromatin. *Mol Cell Biol* **34**, 4534–4544 (2014).
69. Nastały, P. *et al.* Role of the nuclear membrane protein Emerin in front-rear polarity of the nucleus. *Nat Commun* **11**, 2122 (2020).
70. Johnson, N. *et al.* Actin-filled nuclear invaginations indicate degree of cell de-differentiation. *Differentiation* **71**, 414–424 (2003).
71. Biedzinski, S. *et al.* Microtubules control nuclear shape and gene expression during early

stages of hematopoietic differentiation. *EMBO J* **39**, e103957 (2020).

72. Procter, D. J., Furey, C., Garza-Gongora, A. G., Kosak, S. T. & Walsh, D. Cytoplasmic control of intranuclear polarity by human cytomegalovirus. *Nature* **587**, 109–114 (2020).
73. Ulferts, S., Prajapati, B., Grosse, R. & Vartiainen, M. K. Emerging Properties and Functions of Actin and Actin Filaments Inside the Nucleus. *Cold Spring Harb Perspect Biol* **13**, a040121 (2021).
74. Dopie, J., Skarp, K.-P., Rajakylä, E. K., Tanhuanpää, K. & Vartiainen, M. K. Active maintenance of nuclear actin by importin 9 supports transcription. *Proc. Natl. Acad. Sci. U.S.A.* **109**, E544-552 (2012).
75. Plessner, M., Melak, M., Chinchilla, P., Baarlink, C. & Grosse, R. Nuclear F-actin formation and reorganization upon cell spreading. *J. Biol. Chem.* **290**, 11209–11216 (2015).
76. Fiore, A. P. Z. P. *et al.* Laminin-111 and the Level of Nuclear Actin Regulate Epithelial Quiescence via Exportin-6. *Cell Reports* **19**, 2102–2115 (2017).
77. Hofmann, W. A. *et al.* Actin is part of pre-initiation complexes and is necessary for transcription by RNA polymerase II. *Nat Cell Biol* **6**, 1094–1101 (2004).
78. Philimonenko, V. V. *et al.* Nuclear actin and myosin I are required for RNA polymerase I transcription. *Nat Cell Biol* **6**, 1165–1172 (2004).
79. Qi, T. *et al.* G-actin participates in RNA polymerase II-dependent transcription elongation by recruiting positive transcription elongation factor b (P-TEFb). *J Biol Chem* **286**, 15171–15181 (2011).
80. Sokolova, M. *et al.* Nuclear Actin Is Required for Transcription during Drosophila Oogenesis. *iScience* **9**, 63–70 (2018).
81. Viita, T. *et al.* Nuclear actin interactome analysis links actin to KAT14 histone acetyl transferase and mRNA splicing. *J Cell Sci* **132**, jcs226852 (2019).
82. Spencer, V. A. *et al.* Depletion of nuclear actin is a key mediator of quiescence in epithelial cells. *Journal of Cell Science* **124**, 123–132 (2011).

83. Hurst, V., Shimada, K. & Gasser, S. M. Nuclear Actin and Actin-Binding Proteins in DNA Repair. *Trends in Cell Biology* **29**, 462–476 (2019).

84. Jungblut, A., Hopfner, K.-P. & Eustermann, S. Megadalton chromatin remodelers: common principles for versatile functions. *Current Opinion in Structural Biology* **64**, 134–144 (2020).

85. Xie, X., Jankauskas, R., Mazari, A. M. A., Drou, N. & Percipalle, P. β-actin regulates a heterochromatin landscape essential for optimal induction of neuronal programs during direct reprograming. *PLoS Genet* **14**, e1007846 (2018).

86. Xie, X. *et al.* β-Actin-dependent global chromatin organization and gene expression programs control cellular identity. *FASEB J* **32**, 1296–1314 (2018).

87. Zhao, K. *et al.* Rapid and Phosphoinositol-Dependent Binding of the SWI/SNF-like BAF Complex to Chromatin after T Lymphocyte Receptor Signaling. *Cell* **95**, 625–636 (1998).

88. Wu, X. *et al.* Regulation of RNA-polymerase-II-dependent transcription by N-WASP and its nuclear-binding partners. *Nat Cell Biol* **8**, 756–763 (2006).

89. Xia, P. *et al.* WASH is required for the differentiation commitment of hematopoietic stem cells in a c-Myc-dependent manner. *J Exp Med* **211**, 2119–2134 (2014).

90. Yoo, Y., Wu, X. & Guan, J.-L. A novel role of the actin-nucleating Arp2/3 complex in the regulation of RNA polymerase II-dependent transcription. *J Biol Chem* **282**, 7616–7623 (2007).

91. Miyamoto, K., Pasque, V., Jullien, J. & Gurdon, J. B. Nuclear actin polymerization is required for transcriptional reprogramming of Oct4 by oocytes. *Genes Dev* **25**, 946–958 (2011).

92. Miyamoto, K. *et al.* Nuclear Wave1 is required for reprogramming transcription in oocytes and for normal development. *Science* **341**, 1002–1005 (2013).

93. Yamazaki, S. *et al.* The Actin-Family Protein Arp4 Is a Novel Suppressor for the Formation and Functions of Nuclear F-Actin. *Cells* **9**, 758 (2020).

94. Dalton, S. & Treisman, R. Characterization of SAP-1, a protein recruited by serum

response factor to the c-fos serum response element. *Cell* **68**, 597–612 (1992).

95. Hill, C. S., Wynne, J. & Treisman, R. The Rho family GTPases RhoA, Racl , and CDC42Hsregulate transcriptional activation by SRF. *Cell* **81**, 1159–1170 (1995).
96. Norman, C., Runswick, M., Pollock, R. & Treisman, R. Isolation and properties of cDNA clones encoding SRF, a transcription factor that binds to the c-fos serum response element. *Cell* **55**, 989–1003 (1988).
97. Wang, D.-Z. *et al.* Potentiation of serum response factor activity by a family of myocardin-related transcription factors. *PNAS* **99**, 14855–14860 (2002).
98. Wang, Z. *et al.* Myocardin and ternary complex factors compete for SRF to control smooth muscle gene expression. *Nature* **428**, 185–189 (2004).
99. Esnault, C. *et al.* Rho-actin signaling to the MRTF coactivators dominates the immediate transcriptional response to serum in fibroblasts. *Genes Dev.* (2014) doi:10.1101/gad.239327.114.
100. Miralles, F., Posern, G., Zaromytidou, A.-I. & Treisman, R. Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell* **113**, 329–342 (2003).
101. Baarlink, C., Wang, H. & Grosse, R. Nuclear Actin Network Assembly by Formins Regulates the SRF Coactivator MAL. *Science* **340**, 864–867 (2013).
102. Vartiainen, M. K., Guettler, S., Larijani, B. & Treisman, R. Nuclear Actin Regulates Dynamic Subcellular Localization and Activity of the SRF Cofactor MAL. *Science* **316**, 1749–1752 (2007).
103. Cui, Y. *et al.* Cyclic stretching of soft substrates induces spreading and growth. *Nat Commun* **6**, 6333 (2015).
104. Fearing, B. V. *et al.* Mechanosensitive transcriptional coactivators MRTF-A and YAP/TAZ regulate nucleus pulposus cell phenotype through cell shape. *The FASEB Journal* **33**, 14022–14035 (2019).
105. Foster, C. T., Gualdrini, F. & Treisman, R. Mutual dependence of the MRTF–SRF and

YAP–TEAD pathways in cancer-associated fibroblasts is indirect and mediated by cytoskeletal dynamics. *Genes & Development* **31**, 2361–2375 (2017).

106. O'Connor, J. W. & Gomez, E. W. Cell adhesion and shape regulate TGF-beta1-induced epithelial-myofibroblast transition via MRTF-A signaling. *PLoS One* **8**, e83188 (2013).
107. O'Connor, J. W., Riley, P. N., Nalluri, S. M., Ashar, P. K. & Gomez, E. W. Matrix Rigidity Mediates TGFβ1-Induced Epithelial-Myofibroblast Transition by Controlling Cytoskeletal Organization and MRTF-A Localization. *J Cell Physiol* **230**, 1829–1839 (2015).
108. Olson, E. N. & Nordheim, A. Linking actin dynamics and gene transcription to drive cellular motile functions. *Nat. Rev. Mol. Cell Biol.* **11**, 353–365 (2010).
109. Taskinen, M. E. *et al.* MASTL promotes cell contractility and motility through kinase-independent signaling. *J Cell Biol* **219**, e201906204 (2020).
110. Aragona, M. *et al.* Mechanisms of stretch-mediated skin expansion at single-cell resolution. *Nature* **584**, 268–273 (2020).
111. Koegel, H. *et al.* Loss of serum response factor in keratinocytes results in hyperproliferative skin disease in mice. *J Clin Invest* **119**, 899–910 (2009).
112. Luxenburg, C., Amalia Pasolli, H., Williams, S. E. & Fuchs, E. Developmental roles for Srf, cortical cytoskeleton and cell shape in epidermal spindle orientation. *Nat Cell Biol* **13**, 203–214 (2011).
113. Verdoni, A. M. *et al.* A pathogenic relationship between a regulator of the actin cytoskeleton and serum response factor. *Genetics* **186**, 147–157 (2010).
114. Verdoni, A. M., Ikeda, S. & Ikeda, A. Serum response factor is essential for the proper development of skin epithelium. *Mamm Genome* **21**, 64–76 (2010).
115. Hinkel, R. *et al.* MRTF-A controls vessel growth and maturation by increasing the expression of CCN1 and CCN2. *Nat Commun* **5**, 3970 (2014).
116. Weinl, C. *et al.* Endothelial SRF/MRTF ablation causes vascular disease phenotypes in murine retinae. *J Clin Invest* **123**, 2193–2206 (2013).

117. Weinl, C. *et al.* Endothelial depletion of murine SRF/MRTF provokes intracerebral hemorrhagic stroke. *PNAS* **112**, 9914–9919 (2015).
118. Connelly, J. T. *et al.* Actin and serum response factor transduce physical cues from the microenvironment to regulate epidermal stem cell fate decisions. *Nat Cell Biol* **12**, 711–718 (2010).
119. Gualdrini, F. *et al.* SRF Co-factors Control the Balance between Cell Proliferation and Contractility. *Molecular Cell* **64**, 1048–1061 (2016).
120. Watt, F. M., Jordan, P. W. & O'Neill, C. H. Cell shape controls terminal differentiation of human epidermal keratinocytes. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 5576–5580 (1988).
121. Wozniak, M. A. *et al.* Adhesion Regulates MAP Kinase-Ternary Complex Factor Exchange to Control a Proliferative Transcriptional Switch. *Curr Biol* **22**, 2017–2026 (2012).
122. Dupont, S. Role of YAP/TAZ in cell-matrix adhesion-mediated signalling and mechanotransduction. *Exp. Cell Res.* **343**, 42–53 (2016).
123. Dupont, S. *et al.* Role of YAP/TAZ in mechanotransduction. *Nature* **474**, 179–183 (2011).
124. Pocaterra, A., Romani, P. & Dupont, S. YAP/TAZ functions and their regulation at a glance. *J Cell Sci* **133**, (2020).
125. Aragona, M. *et al.* A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors. *Cell* **154**, 1047–1059 (2013).
126. Benham-Pyle, B. W., Pruitt, B. L. & Nelson, W. J. Cell adhesion. Mechanical strain induces E-cadherin-dependent Yap1 and β-catenin activation to drive cell cycle entry. *Science* **348**, 1024–1027 (2015).
127. Zhao, B. *et al.* Inactivation of YAP oncoprotein by the Hippo pathway is involved in cell contact inhibition and tissue growth control. *Genes & Development* **21**, 2747–2761 (2007).
128. Baker, N. E. Emerging mechanisms of cell competition. *Nat Rev Genet* **21**, 683–697 (2020).
129. Chen, C.-L., Schroeder, M. C., Kango-Singh, M., Tao, C. & Halder, G. Tumor suppression

by cell competition through regulation of the Hippo pathway. *Proc Natl Acad Sci U S A* **109**, 484–489 (2012).

130. Ishihara, E. *et al.* Prostaglandin E2 and its receptor EP2 trigger signaling that contributes to YAP-mediated cell competition. *Genes Cells* **25**, 197–214 (2020).
131. Levayer, R. Solid stress, competition for space and cancer: The opposing roles of mechanical cell competition in tumour initiation and growth. *Semin. Cancer Biol.* (2019) doi:10.1016/j.semcancer.2019.05.004.
132. Liu, Z. *et al.* Differential YAP expression in glioma cells induces cell competition and promotes tumorigenesis. *J Cell Sci* **132**, jcs225714 (2019).
133. Moya, I. M. *et al.* Peritumoral activation of the Hippo pathway effectors YAP and TAZ suppresses liver cancer in mice. *Science* **366**, 1029–1034 (2019).
134. Nishio, M. *et al.* Hippo pathway controls cell adhesion and context-dependent cell competition to influence skin engraftment efficiency. *FASEB J* **33**, 5548–5560 (2019).
135. Price, C. J. *et al.* Genetically variant human pluripotent stem cells selectively eliminate wild-type counterparts through YAP-mediated cell competition. *Developmental Cell* **56**, 2455-2470.e10 (2021).
136. Dupont, S. & Morsut, L. Tissue Patterning: The Winner Takes It All, the Losers Standing Small. *Curr. Biol.* **29**, R334–R337 (2019).
137. Xia, P., Gütl, D., Zheden, V. & Heisenberg, C.-P. Lateral Inhibition in Cell Specification Mediated by Mechanical Signals Modulating TAZ Activity. *Cell* **176**, 1379-1392.e14 (2019).
138. Elosegui-Artola, A. *et al.* Mechanical regulation of a molecular clutch defines force transmission and transduction in response to matrix rigidity. *Nature Cell Biology* **18**, 540–548 (2016).
139. Wada, K.-I., Itoga, K., Okano, T., Yonemura, S. & Sasaki, H. Hippo pathway regulation by cell morphology and stress fibers. *Development* **138**, 3907–3914 (2011).
140. Chang, L. *et al.* The SWI/SNF complex is a mechanoregulated inhibitor of YAP and TAZ.

*Nature* **563**, 265–269 (2018).

141. Meng, Z. *et al.* RAP2 mediates mechanoresponses of the Hippo pathway. *Nature* **560**, 655–660 (2018).
142. Shiu, J.-Y., Aires, L., Lin, Z. & Vogel, V. Nanopillar force measurements reveal actin-cap-mediated YAP mechanotransduction. *Nat Cell Biol* **20**, 262–271 (2018).
143. Pocaterra, A. *et al.* F-actin dynamics regulates mammalian organ growth and cell fate maintenance. *J. Hepatol.* **71**, 130–142 (2019).
144. Pocaterra, A. *et al.* Fascin1 empowers YAP mechanotransduction and promotes cholangiocarcinoma development. *Commun Biol* **4**, 1–13 (2021).
145. Sansores-Garcia, L. *et al.* Modulating F-actin organization induces organ growth by affecting the Hippo pathway: Modulating F-actin organization induces organ growth. *The EMBO Journal* **30**, 2325–2335 (2011).
146. Wang, S. *et al.* CCM3 is a gatekeeper in focal adhesions regulating mechanotransduction and YAP/TAZ signalling. *Nat Cell Biol* **23**, 758–770 (2021).
147. Panciera, T. *et al.* Reprogramming normal cells into tumour precursors requires ECM stiffness and oncogene-mediated changes of cell mechanical properties. *Nat Mater* (2020) doi:10.1038/s41563-020-0615-x.
148. Calvo, F. *et al.* Mechanotransduction and YAP-dependent matrix remodelling is required for the generation and maintenance of cancer-associated fibroblasts. *Nat. Cell Biol.* **15**, 637–646 (2013).
149. Link, P. A. *et al.* Combined control of the fibroblast contractile program by YAP and TAZ. *Am J Physiol Lung Cell Mol Physiol* (2021) doi:10.1152/ajplung.00210.2021.
150. Pagliari, S. *et al.* YAP-TEAD1 control of cytoskeleton dynamics and intracellular tension guides human pluripotent stem cell mesoderm specification. *Cell Death Differ* **28**, 1193–1207 (2021).
151. Porazinski, S. *et al.* YAP is essential for tissue tension to ensure vertebrate 3D body

shape. *Nature* **521**, 217–221 (2015).

152. Romani, P., Valcarcel-Jimenez, L., Frezza, C. & Dupont, S. Crosstalk between mechanotransduction and metabolism. *Nature Reviews Molecular Cell Biology* 1–17 (2020) doi:10.1038/s41580-020-00306-w.
153. Farge, E. Mechanical induction of Twist in the Drosophila foregut/stomodeal primordium. *Curr Biol* **13**, 1365–1377 (2003).
154. Desprat, N., Supatto, W., Pouille, P.-A., Beaurepaire, E. & Farge, E. Tissue deformation modulates twist expression to determine anterior midgut differentiation in Drosophila embryos. *Dev Cell* **15**, 470–477 (2008).
155. Röper, J.-C. *et al.* The major β-catenin/E-cadherin junctional binding site is a primary molecular mechano-transductor of differentiation in vivo. *eLife* https://elifesciences.org/articles/33381 (2018) doi:10.7554/eLife.33381.
156. Nusse, R. & Clevers, H. Wnt/β-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. *Cell* **169**, 985–999 (2017).
157. Doumpas, N. *et al.* TCF/LEF dependent and independent transcriptional regulation of Wnt/β-catenin target genes. *EMBO J* **38**, e98873 (2019).
158. Städeli, R., Hoffmans, R. & Basler, K. Transcription under the Control of Nuclear Arm/β-Catenin. *Current Biology* **16**, R378–R385 (2006).
159. Brunet, T. *et al.* Evolutionary conservation of early mesoderm specification by mechanotransduction in Bilateria. *Nat Commun* **4**, 2821 (2013).
160. Fernández-Sánchez, M. E. *et al.* Mechanical induction of the tumorigenic β-catenin pathway by tumour growth pressure. *Nature* **523**, 92–95 (2015).
161. Samuel, M. S. *et al.* Actomyosin-mediated cellular tension drives increased tissue stiffness and β-catenin activation to induce epidermal hyperplasia and tumor growth. *Cancer Cell* **19**, 776–791 (2011).
162. Muncie, J. M. *et al.* Mechanical Tension Promotes Formation of Gastrulation-like Nodes

and Patterns Mesoderm Specification in Human Embryonic Stem Cells. *Developmental Cell* **55**, 679-694.e11 (2020).

163. Przybyla, L., Lakins, J. N. & Weaver, V. M. Tissue Mechanics Orchestrate Wnt-Dependent Human Embryonic Stem Cell Differentiation. *Cell Stem Cell* **19**, 462–475 (2016).
164. Ray, S., Foote, H. P. & Lechler, T. β-Catenin protects the epidermis from mechanical stresses. *Journal of Cell Biology* **202**, 45–52 (2013).
165. Arif, N. *et al.* PECAM-1 supports leukocyte diapedesis by tension-dependent dephosphorylation of VE-cadherin. *EMBO J* **40**, e106113 (2021).
166. Gayrard, C., Bernaudin, C., Déjardin, T., Seiler, C. & Borghi, N. Src- and confinement-dependent FAK activation causes E-cadherin relaxation and β-catenin activity. *J Cell Biol* **217**, 1063–1077 (2018).
167. Miroshnikova, Y. A. *et al.* Calcium signaling mediates a biphasic mechanoadaptive response of endothelial cells to cyclic mechanical stretch. *Mol Biol Cell* **32**, 1724–1736 (2021).
168. Chung, H. *et al.* Yap1 is dispensable for self-renewal but required for proper differentiation of mouse embryonic stem (ES) cells. *EMBO Rep* **17**, 519–529 (2016).
169. Lian, I. *et al.* The role of YAP transcription coactivator in regulating stem cell self-renewal and differentiation. *Genes Dev* **24**, 1106–1118 (2010).
170. Tamm, C., Böwer, N. & Annerén, C. Regulation of mouse embryonic stem cell self-renewal by a Yes–YAP–TEAD2 signaling pathway downstream of LIF. *Journal of Cell Science* **124**, 1136–1144 (2011).
171. Qin, H. *et al.* Transcriptional analysis of pluripotency reveals the Hippo pathway as a barrier to reprogramming. *Human Molecular Genetics* **21**, 2054–2067 (2012).
172. Qin, H. *et al.* YAP Induces Human Naive Pluripotency. *Cell Reports* **14**, 2301–2312 (2016).
173. Kono, K., Tamashiro, D. A. A. & Alarcon, V. B. Inhibition of RHO–ROCK signaling enhances ICM and suppresses TE characteristics through activation of Hippo signaling in

the mouse blastocyst. *Developmental Biology* **394**, 142–155 (2014).

174. Maître, J.-L. *et al.* Asymmetric division of contractile domains couples cell positioning and fate specification. *Nature* **536**, 344–348 (2016).
175. Mihajlović, A. I. & Bruce, A. W. Rho-associated protein kinase regulates subcellular localisation of Angiomotin and Hippo-signalling during preimplantation mouse embryo development. *Reproductive BioMedicine Online* **33**, 381–390 (2016).
176. Nishioka, N. *et al.* The Hippo signaling pathway components Lats and Yap pattern Tead4 activity to distinguish mouse trophectoderm from inner cell mass. *Dev Cell* **16**, 398–410 (2009).
177. Royer, C. *et al.* Establishment of a relationship between blastomere geometry and YAP localisation during compaction. *Development* **147**, dev189449 (2020).
178. Wang, X. *et al.* Characterizing Inner Pressure and Stiffness of Trophoblast and Inner Cell Mass of Blastocysts. *Biophysical Journal* **115**, 2443–2450 (2018).
179. Morin-Kensicki, E. M. *et al.* Defects in yolk sac vasculogenesis, chorioallantoic fusion, and embryonic axis elongation in mice with targeted disruption of Yap65. *Mol Cell Biol* **26**, 77–87 (2006).
180. Hossain, Z. *et al.* Glomerulocystic kidney disease in mice with a targeted inactivation of Wwtr1. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 1631–1636 (2007).
181. Makita, R. *et al.* Multiple renal cysts, urinary concentration defects, and pulmonary emphysematous changes in mice lacking TAZ. *Am. J. Physiol. Renal Physiol.* **294**, F542-553 (2008).
182. Tian, Y. *et al.* TAZ Promotes PC2 Degradation through a SCFβ-Trcp E3 Ligase Complex. *Mol Cell Biol* **27**, 6383–6395 (2007).
183. Gilbert, P. M. *et al.* Substrate Elasticity Regulates Skeletal Muscle Stem Cell Self-Renewal in Culture. *Science* **329**, 1078–1081 (2010).
184. Han, W. M. *et al.* Synthetic matrix enhances transplanted satellite cell engraftment in

dystrophic and aged skeletal muscle with comorbid trauma. *Sci Adv* **4**, eaar4008 (2018).

185. Moyle, L. A. *et al.* Three-dimensional niche stiffness synergizes with Wnt7a to modulate the extent of satellite cell symmetric self-renewal divisions. *Mol Biol Cell* **31**, 1703–1713 (2020).
186. Urciuolo, A. *et al.* Collagen VI regulates satellite cell self-renewal and muscle regeneration. *Nat Commun* **4**, 1964 (2013).
187. Gjorevski, N. *et al.* Designer matrices for intestinal stem cell and organoid culture. *Nature* **539**, 560–564 (2016).
188. Yui, S. *et al.* YAP/TAZ-Dependent Reprogramming of Colonic Epithelium Links ECM Remodeling to Tissue Regeneration. *Cell Stem Cell* **22**, 35-49.e7 (2018).
189. Engler, A. J., Sen, S., Sweeney, H. L. & Discher, D. E. Matrix elasticity directs stem cell lineage specification. *Cell* **126**, 677–689 (2006).
190. Hong, J.-H. *et al.* TAZ, a transcriptional modulator of mesenchymal stem cell differentiation. *Science* **309**, 1074–1078 (2005).
191. McBeath, R., Pirone, D. M., Nelson, C. M., Bhadriraju, K. & Chen, C. S. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* **6**, 483–495 (2004).
192. Sordella, R., Jiang, W., Chen, G.-C., Curto, M. & Settleman, J. Modulation of Rho GTPase signaling regulates a switch between adipogenesis and myogenesis. *Cell* **113**, 147–158 (2003).
193. Tang, Y. *et al.* MT1-MMP-Dependent Control of Skeletal Stem Cell Commitment via a β1-Integrin/YAP/TAZ Signaling Axis. *Developmental Cell* **25**, 402–416 (2013).
194. Cebola, I. *et al.* TEAD and YAP regulate the enhancer network of human embryonic pancreatic progenitors. *Nat Cell Biol* **17**, 615–626 (2015).
195. Hogrebe, N. J., Augsornworawat, P., Maxwell, K. G., Velazco-Cruz, L. & Millman, J. R. Targeting the cytoskeleton to direct pancreatic differentiation of human pluripotent stem

cells. *Nature Biotechnology* **38**, 460–470 (2020).

196. Mamidi, A. *et al.* Mechanosignalling via integrins directs fate decisions of pancreatic progenitors. *Nature* **564**, 114–118 (2018).
197. Rosado-Olivieri, E. A., Anderson, K., Kenty, J. H. & Melton, D. A. YAP inhibition enhances the differentiation of functional stem cell-derived insulin-producing β cells. *Nat Commun* **10**, 1464 (2019).
198. Fan, F. *et al.* Pharmacological targeting of kinases MST1 and MST2 augments tissue repair and regeneration. *Science Translational Medicine* **8**, 352ra108-352ra108 (2016).
199. Kastan, N. *et al.* Small-molecule inhibition of Lats kinases may promote Yap-dependent proliferation in postmitotic mammalian tissues. *Nat Commun* **12**, 3100 (2021).
200. Pobbati, A. V. *et al.* Identification of Quinolinols as Activators of TEAD-Dependent Transcription. *ACS Chem. Biol.* **14**, 2909–2921 (2019).
201. De Rosa, L. *et al.* Laminin 332-Dependent YAP Dysregulation Depletes Epidermal Stem Cells in Junctional Epidermolysis Bullosa. *Cell Reports* **27**, 2036-2049.e6 (2019).
202. Morikawa, Y. *et al.* Actin cytoskeletal remodeling with protrusion formation is essential for heart regeneration in Hippo-deficient mice. *Science Signaling* **8**, ra41–ra41 (2015).
203. Morikawa, Y., Heallen, T., Leach, J., Xiao, Y. & Martin, J. F. Dystrophin–glycoprotein complex sequesters Yap to inhibit cardiomyocyte proliferation. *Nature* **547**, 227–231 (2017).
204. Min, E. & Schwartz, M. A. Translocating transcription factors in fluid shear stress-mediated vascular remodeling and disease. *Exp. Cell Res.* **376**, 92–97 (2019).
205. Rojo de la Vega, M., Chapman, E. & Zhang, D. D. NRF2 and the Hallmarks of Cancer. *Cancer Cell* **34**, 21–43 (2018).
206. Tonelli, C., Chio, I. I. C. & Tuveson, D. A. Transcriptional Regulation by Nrf2. *Antioxid Redox Signal* **29**, 1727–1745 (2018).
207. Chen, X.-L. *et al.* Laminar flow induction of antioxidant response element-mediated genes

in endothelial cells. A novel anti-inflammatory mechanism. *J. Biol. Chem.* **278**, 703–711 (2003).

208. Dai, G. *et al.* Biomechanical Forces in Atherosclerosis-Resistant Vascular Regions Regulate Endothelial Redox Balance via Phosphoinositol 3-Kinase/Akt-Dependent Activation of Nrf2. *Circulation Research* **101**, 723–733 (2007).
209. Hosoya, T. *et al.* Differential responses of the Nrf2-Keap1 system to laminar and oscillatory shear stresses in endothelial cells. *J. Biol. Chem.* **280**, 27244–27250 (2005).
210. Hsieh, C.-Y. *et al.* Regulation of shear-induced nuclear translocation of the Nrf2 transcription factor in endothelial cells. *Journal of Biomedical Science* **16**, 12 (2009).
211. Jones, C. I. *et al.* Regulation of Antioxidants and Phase 2 Enzymes by Shear-Induced Reactive Oxygen Species in Endothelial Cells. *Ann Biomed Eng* **35**, 683–693 (2007).
212. Psefteli, P.-M. *et al.* Glycocalyx sialic acids regulate Nrf2-mediated signaling by fluid shear stress in human endothelial cells. *Redox Biology* **38**, 101816 (2021).
213. Warabi, E. *et al.* Shear stress stabilizes NF-E2-related factor 2 and induces antioxidant genes in endothelial cells: Role of reactive oxygen/nitrogen species. *Free Radical Biology and Medicine* **42**, 260–269 (2007).
214. Dekker, R. J. *et al.* Prolonged fluid shear stress induces a distinct set of endothelial cell genes, most specifically lung Krüppel-like factor (KLF2). *Blood* **100**, 1689–1698 (2002).
215. Huddleson, J. P., Ahmad, N., Srinivasan, S. & Lingrel, J. B. Induction of KLF2 by Fluid Shear Stress Requires a Novel Promoter Element Activated by a Phosphatidylinositol 3-Kinase-dependent Chromatin-remodeling Pathway*. *Journal of Biological Chemistry* **280**, 23371–23379 (2005).
216. Lee, D.-Y. *et al.* Role of histone deacetylases in transcription factor regulation and cell cycle modulation in endothelial cells in response to disturbed flow. *PNAS* **109**, 1967–1972 (2012).
217. Parmar, K. M. *et al.* Integration of flow-dependent endothelial phenotypes by Kruppel-like

factor 2. *J Clin Invest* **116**, 49–58 (2006).

218. Sohn, S. J., Li, D., Lee, L. K. & Winoto, A. Transcriptional Regulation of Tissue-Specific Genes by the ERK5 Mitogen-Activated Protein Kinase. *Molecular and Cellular Biology* **25**, 8553–8566 (2005).
219. Wu, W. *et al.* Flow-Dependent Regulation of Krüppel-Like Factor 2 Is Mediated by MicroRNA-92a. *Circulation* **124**, 633–641 (2011).
220. Dekker, R. J. *et al.* Endothelial KLF2 Links Local Arterial Shear Stress Levels to the Expression of Vascular Tone-Regulating Genes. *The American Journal of Pathology* **167**, 609–618 (2005).
221. Fledderus, J. O. *et al.* KLF2 Primes the Antioxidant Transcription Factor Nrf2 for Activation in Endothelial Cells. *Arteriosclerosis, Thrombosis, and Vascular Biology* **28**, 1339–1346 (2008).
222. Lee, J. S. *et al.* Klf2 Is an Essential Regulator of Vascular Hemodynamic Forces In Vivo. *Developmental Cell* **11**, 845–857 (2006).
223. Ahimou, F., Mok, L.-P., Bardot, B. & Wesley, C. The adhesion force of Notch with Delta and the rate of Notch signaling. *Journal of Cell Biology* **167**, 1217–1229 (2004).
224. Gordon, W. R. *et al.* Mechanical Allostery: Evidence for a Force Requirement in the Proteolytic Activation of Notch. *Developmental Cell* **33**, 729–736 (2015).
225. Langridge, P. D. & Struhl, G. Epsin-Dependent Ligand Endocytosis Activates Notch by Force. *Cell* **171**, 1383-1396.e12 (2017).
226. Meloty-Kapella, L., Shergill, B., Kuon, J., Botvinick, E. & Weinmaster, G. Notch Ligand Endocytosis Generates Mechanical Pulling Force Dependent on Dynamin, Epsins, and Actin. *Developmental Cell* **22**, 1299–1312 (2012).
227. Sprinzak, D. & Blacklow, S. C. Biophysics of Notch Signaling. *Annu Rev Biophys* **50**, 157–189 (2021).
228. Wang, X. & Ha, T. Defining Single Molecular Forces Required to Activate Integrin and

Notch Signaling. *Science* **340**, 991–994 (2013).

229. Caolo, V. *et al.* Shear stress activates ADAM10 sheddase to regulate Notch1 via the Piezo1 force sensor in endothelial cells. *eLife* **9**, e50684 (2020).
230. van Engeland, N. C. A. *et al.* Vimentin regulates Notch signaling strength and arterial remodeling in response to hemodynamic stress. *Sci Rep* **9**, 12415 (2019).
231. Fang, J. S. *et al.* Shear-induced Notch-Cx37-p27 axis arrests endothelial cell cycle to enable arterial specification. *Nat Commun* **8**, 2149 (2017).
232. Lee, J. *et al.* 4-Dimensional light-sheet microscopy to elucidate shear stress modulation of cardiac trabeculation. *J Clin Invest* **126**, 1679–1690 (2016).
233. Mack, J. J. *et al.* NOTCH1 is a mechanosensor in adult arteries. *Nat Commun* **8**, 1620 (2017).
234. Masumura, T., Yamamoto, K., Shimizu, N., Obi, S. & Ando, J. Shear Stress Increases Expression of the Arterial Endothelial Marker EphrinB2 in Murine ES Cells via the VEGF-Notch Signaling Pathways. *Arteriosclerosis, Thrombosis, and Vascular Biology* **29**, 2125–2131 (2009).
235. Polacheck, W. J. *et al.* A non-canonical Notch complex regulates adherens junctions and vascular barrier function. *Nature* **552**, 258–262 (2017).
236. Chu, Y.-S. *et al.* Force measurements in E-cadherin–mediated cell doublets reveal rapid adhesion strengthened by actin cytoskeleton remodeling through Rac and Cdc42. *Journal of Cell Biology* **167**, 1183–1194 (2004).
237. Hunter, G. L. *et al.* A role for actomyosin contractility in Notch signaling. *BMC Biology* **17**, 12 (2019).
238. Liu, Z. *et al.* Mechanical tugging force regulates the size of cell-cell junctions. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 9944–9949 (2010).
239. Shaya, O. *et al.* Cell-Cell Contact Area Affects Notch Signaling and Notch-Dependent Patterning. *Developmental Cell* **40**, 505-511.e6 (2017).

240. Chen, C. S., Mrksich, M., Huang, S., Whitesides, G. M. & Ingber, D. E. Geometric control of cell life and death. *Science* **276**, 1425–1428 (1997).
241. Folkman, J. & Moscona, A. Role of cell shape in growth control. *Nature* **273**, 345–349 (1978).
242. Moro, A. *et al.* microRNA-dependent regulation of biomechanical genes establishes tissue stiffness homeostasis. *Nat Cell Biol* **21**, 348–358 (2019).
243. Astone, M. *et al.* Zebrafish mutants and TEAD reporters reveal essential functions for Yap and Taz in posterior cardinal vein development. *Sci Rep* **8**, 10189 (2018).
244. Kim, J. *et al.* YAP/TAZ regulates sprouting angiogenesis and vascular barrier maturation. *J. Clin. Invest.* **127**, 3441–3461 (2017).
245. Neto, F. *et al.* YAP and TAZ regulate adherens junction dynamics and endothelial cell distribution during vascular development. *Elife* **7**, (2018).
246. van der Stoel, M. *et al.* DLC1 is a direct target of activated YAP/TAZ that drives collective migration and sprouting angiogenesis. *Journal of Cell Science* **133**, (2020).
247. Wang, X. *et al.* YAP/TAZ Orchestrate VEGF Signaling during Developmental Angiogenesis. *Dev. Cell* **42**, 462-478.e7 (2017).
248. Mammoto, A. *et al.* A mechanosensitive transcriptional mechanism that controls angiogenesis. *Nature* **457**, 1103–1108 (2009).
249. Deng, H. *et al.* Activation of Smad2/3 signaling by low fluid shear stress mediates artery inward remodeling. *PNAS* **118**, (2021).
250. Baeyens, N. *et al.* Defective fluid shear stress mechanotransduction mediates hereditary hemorrhagic telangiectasia. *Journal of Cell Biology* **214**, 807–816 (2016).
251. Poduri, A. *et al.* Endothelial cells respond to the direction of mechanical stimuli through SMAD signaling to regulate coronary artery size. *Development* **144**, 3241–3252 (2017).
252. Zhou, J. *et al.* Force-specific activation of Smad1/5 regulates vascular endothelial cell cycle progression in response to disturbed flow. *PNAS* **109**, 7770–7775 (2012).

253. Huang, J. *et al.* KLF2 Mediates the Suppressive Effect of Laminar Flow on Vascular Calcification by Inhibiting Endothelial BMP/SMAD1/5 Signaling. *Circulation Research* **129**, e87–e100 (2021).

254. Hahn, C. & Schwartz, M. A. Mechanotransduction in vascular physiology and atherogenesis. *Nat Rev Mol Cell Biol* **10**, 53–62 (2009).

255. Nakajima, H. & Mochizuki, N. Flow pattern-dependent endothelial cell responses through transcriptional regulation. *Cell Cycle* **16**, 1893–1901 (2017).

256. Shay-Salit, A. *et al.* VEGF receptor 2 and the adherens junction as a mechanical transducer in vascular endothelial cells. *PNAS* **99**, 9462–9467 (2002).

257. Tzima, E. *et al.* A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. *Nature* **437**, 426–431 (2005).

258. Ishida, T., Takahashi, M., Corson, M. A. & Berk, B. C. Fluid Shear Stress-Mediated Signal Transduction: How Do Endothelial Cells Transduce Mechanical Force into Biological Responses? *Annals of the New York Academy of Sciences* **811**, 12–24 (1997).

259. Khachigian, L. M., Resnick, N., Gimbrone, M. A. & Collins, T. Nuclear factor-kappa B interacts functionally with the platelet-derived growth factor B-chain shear-stress response element in vascular endothelial cells exposed to fluid shear stress. *J Clin Invest* **96**, 1169–1175 (1995).

260. Lan, Q. X., Mercurius, K. O. & Davies, P. F. Stimulation of Transcription Factors NFκB and AP1 in Endothelial Cells Subjected to Shear Stress. *Biochemical and Biophysical Research Communications* **201**, 950–956 (1994).

261. Mohan, S. *et al.* Low shear stress preferentially enhances IKK activity through selective sources of ROS for persistent activation of NF-κB in endothelial cells. *American Journal of Physiology-Cell Physiology* **292**, C362–C371 (2007).

262. Nagel, T., Resnick, N., Dewey, C. F. & Gimbrone, M. A. Vascular Endothelial Cells Respond to Spatial Gradients in Fluid Shear Stress by Enhanced Activation of

Transcription Factors. *Arteriosclerosis, Thrombosis, and Vascular Biology* **19**, 1825–1834 (1999).

263. Tzima, E. *et al.* Activation of Rac1 by shear stress in endothelial cells mediates both cytoskeletal reorganization and effects on gene expression. *EMBO J* **21**, 6791–6800 (2002).
264. Liu, D. *et al.* Atheroprotective effects of methotrexate via the inhibition of YAP/TAZ under disturbed flow. *Journal of Translational Medicine* **17**, 378 (2019).
265. Park, H. *et al.* Defective Flow-Migration Coupling Causes Arteriovenous Malformations in Hereditary Hemorrhagic Telangiectasia. *Circulation* **144**, 805–822 (2021).
266. Orr, A. W. *et al.* The subendothelial extracellular matrix modulates NF-κB activation by flow : a potential role in atherosclerosis. *Journal of Cell Biology* **169**, 191–202 (2005).
267. Drain, A. P. *et al.* Matrix compliance permits NF-κB activation to drive therapy resistance in breast cancer. *Journal of Experimental Medicine* **218**, (2021).
268. Ishihara, S. *et al.* Substrate stiffness regulates temporary NF-κB activation via actomyosin contractions. *Experimental Cell Research* **319**, 2916–2927 (2013).
269. Liang, F. & Gardner, D. G. Mechanical strain activates *BNP* gene transcription through a p38/NF-κB–dependent mechanism. *J Clin Invest* **104**, 1603–1612 (1999).
270. Yamamoto, K. *et al.* Induction of Tenascin-C in Cardiac Myocytes by Mechanical Deformation: ROLE OF REACTIVE OXYGEN SPECIES*. *Journal of Biological Chemistry* **274**, 21840–21846 (1999).
271. Mochitate, K., Pawelek, P. & Grinnell, F. Stress relaxation of contracted collagen gels: Disruption of actin filament bundles, release of cell surface fibronectin, and down-regulation of DNA and protein synthesis. *Experimental Cell Research* **193**, 198–207 (1991).
272. Aramaki-Hattori, N., Okabe, K., Hamada, M., Takato, T. & Kishi, K. Relationship between Keloid Formation and YAP/TAZ Signaling. *Plastic and Reconstructive Surgery – Global*

*Open* **5**, e1357 (2017).

273. Huang, C. *et al.* Keloid progression: a stiffness gap hypothesis. *International Wound Journal* **14**, 764–771 (2017).
274. Liu, F. *et al.* Mechanosignaling through YAP and TAZ drives fibroblast activation and fibrosis. *Am. J. Physiol. Lung Cell Mol. Physiol.* **308**, L344-357 (2015).
275. Mannaerts, I. *et al.* The Hippo pathway effector YAP controls mouse hepatic stellate cell activation. *J. Hepatol.* **63**, 679–688 (2015).
276. Bertero, T. *et al.* Tumor-Stroma Mechanics Coordinate Amino Acid Availability to Sustain Tumor Growth and Malignancy. *Cell Metab* **29**, 124-140.e10 (2019).
277. Bell, J. L. *et al.* Optimization of novel nipecotic bis(amide) inhibitors of the Rho/MKL1/SRF transcriptional pathway as potential anti-metastasis agents. *Bioorganic & Medicinal Chemistry Letters* **23**, 3826–3832 (2013).
278. Fan, L. *et al.* Cell contact-dependent regulation of epithelial-myofibroblast transition via the rho-rho kinase-phospho-myosin pathway. *Mol Biol Cell* **18**, 1083–1097 (2007).
279. Haak, A. J. *et al.* Targeting the Myofibroblast Genetic Switch: Inhibitors of Myocardin-Related Transcription Factor/Serum Response Factor–Regulated Gene Transcription Prevent Fibrosis in a Murine Model of Skin Injury. *J Pharmacol Exp Ther* **349**, 480–486 (2014).
280. Hutchings, K. M. *et al.* Pharmacokinetic optimitzation of CCG-203971: Novel inhibitors of the Rho/MRTF/SRF transcriptional pathway as potential antifibrotic therapeutics for systemic scleroderma. *Bioorganic & Medicinal Chemistry Letters* **27**, 1744–1749 (2017).
281. Johnson, L. A. *et al.* Novel Rho/MRTF/SRF Inhibitors Block Matrix-stiffness and TGF-β–Induced Fibrogenesis in Human Colonic Myofibroblasts. *Inflammatory Bowel Diseases* **20**, 154–165 (2014).
282. Luchsinger, L. L., Patenaude, C. A., Smith, B. D. & Layne, M. D. Myocardin-related transcription factor-A complexes activate type I collagen expression in lung fibroblasts. *J*

*Biol Chem* **286**, 44116–44125 (2011).

283. Sandbo, N., Kregel, S., Taurin, S., Bhorade, S. & Dulin, N. O. Critical role of serum response factor in pulmonary myofibroblast differentiation induced by TGF-beta. *Am J Respir Cell Mol Biol* **41**, 332–338 (2009).
284. Shi, Z., Ren, M. & Rockey, D. C. Myocardin and myocardin-related transcription factor-A synergistically mediate actin cytoskeletal-dependent inhibition of liver fibrogenesis. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **318**, G504–G517 (2020).
285. Shiwen, X. *et al.* A Role of Myocardin Related Transcription Factor-A (MRTF-A) in Scleroderma Related Fibrosis. *PLoS One* **10**, e0126015 (2015).
286. Sisson, T. H. *et al.* Inhibition of Myocardin-Related Transcription Factor/Serum Response Factor Signaling Decreases Lung Fibrosis and Promotes Mesenchymal Cell Apoptosis. *The American Journal of Pathology* **185**, 969–986 (2015).
287. Small, E. M. *et al.* Myocardin-related transcription factor-a controls myofibroblast activation and fibrosis in response to myocardial infarction. *Circ Res* **107**, 294–304 (2010).
288. Tian, W. *et al.* Myocardin related transcription factor A programs epigenetic activation of hepatic stellate cells. *J Hepatol* **62**, 165–174 (2015).
289. Velasquez, L. S. *et al.* Activation of MRTF-A-dependent gene expression with a small molecule promotes myofibroblast differentiation and wound healing. *Proc Natl Acad Sci U S A* **110**, 16850–16855 (2013).
290. Xu, H. *et al.* Myocardin-Related Transcription Factor A Epigenetically Regulates Renal Fibrosis in Diabetic Nephropathy. *J Am Soc Nephrol* **26**, 1648–1660 (2015).
291. Francisco, J. *et al.* Blockade of Fibroblast YAP Attenuates Cardiac Fibrosis and Dysfunction Through MRTF-A Inhibition. *JACC: Basic to Translational Science* **5**, 931–945 (2020).
292. Er, E. E. *et al.* Pericyte-like spreading by disseminated cancer cells activates YAP and

MRTF for metastatic colonization. *Nature Cell Biology* **20**, 966–978 (2018).

293. Girard, C. A. *et al.* A Feed-Forward Mechanosignaling Loop Confers Resistance to Therapies Targeting the MAPK Pathway in BRAF-Mutant Melanoma. *Cancer Res.* **80**, 1927–1941 (2020).
294. Kim, T. *et al.* A basal-like breast cancer-specific role for SRF–IL6 in YAP-induced cancer stemness. *Nature Communications* **6**, (2015).
295. Kim, T. *et al.* MRTF potentiates TEAD-YAP transcriptional activity causing metastasis. *EMBO J* **36**, 520–535 (2017).
296. Yu, O. M. *et al.* YAP and MRTF-A, transcriptional co-activators of RhoA-mediated gene expression, are critical for glioblastoma tumorigenicity. *Oncogene* **37**, 5492–5507 (2018).
297. Budi, E. H., Schaub, J. R., Decaris, M., Turner, S. & Derynck, R. TGF-β as a driver of fibrosis: physiological roles and therapeutic opportunities. *J Pathol* **254**, 358–373 (2021).
298. Ahamed, J., Janczak, C. A., Wittkowski, K. M. & Coller, B. S. In Vitro and In Vivo Evidence that Thrombospondin-1 (TSP-1) Contributes to Stirring- and Shear-Dependent Activation of Platelet-Derived TGF-β1. *PLOS ONE* **4**, e6608 (2009).
299. Buscemi, L. *et al.* The Single-Molecule Mechanics of the Latent TGF-β1 Complex. *Current Biology* **21**, 2046–2054 (2011).
300. Froese, A. R. *et al.* Stretch-induced Activation of Transforming Growth Factor-β1 in Pulmonary Fibrosis. *Am J Respir Crit Care Med* **194**, 84–96 (2016).
301. Klingberg, F. *et al.* Prestress in the extracellular matrix sensitizes latent TGF-β1 for activation. *Journal of Cell Biology* **207**, 283–297 (2014).
302. Munger, J. S., Harpel, J. G., Giancotti, F. G. & Rifkin, D. B. Interactions between Growth Factors and Integrins: Latent Forms of Transforming Growth Factor-β Are Ligands for the Integrin αvβ1. *MBoC* **9**, 2627–2638 (1998).
303. Shi, M. *et al.* Latent TGF-β structure and activation. *Nature* **474**, 343–349 (2011).
304. Wipff, P.-J., Rifkin, D. B., Meister, J.-J. & Hinz, B. Myofibroblast contraction activates latent

TGF-beta1 from the extracellular matrix. *J. Cell Biol.* **179**, 1311–1323 (2007).

305. Zhou, Y., Hagood, J. S., Lu, B., Merryman, W. D. & Murphy-Ullrich, J. E. Thy-1-Integrin αvβ5 Interactions Inhibit Lung Fibroblast Contraction-induced Latent Transforming Growth Factor-β1 Activation and Myofibroblast Differentiation*. *Journal of Biological Chemistry* **285**, 22382–22393 (2010).
306. Jones, D. L. *et al.* ZNF416 is a pivotal transcriptional regulator of fibroblast mechanoactivation. *J Cell Biol* **220**, (2021).
307. Zhang, K. *et al.* Mechanical signals regulate and activate SNAIL1 protein to control the fibrogenic response of cancer-associated fibroblasts. *Journal of Cell Science* **129**, 1989–2002 (2016).
308. Butcher, D. T., Alliston, T. & Weaver, V. M. A tense situation: forcing tumour progression. *Nat. Rev. Cancer* **9**, 108–122 (2009).
309. Gehler, S., Ponik, S. M., Riching, K. M. & Keely, P. J. Bi-Directional Signaling: Extracellular Matrix and Integrin Regulation of Breast Tumor Progression. *CRE* **23**, (2013).
310. Mohammadi, H. & Sahai, E. Mechanisms and impact of altered tumour mechanics. *Nat. Cell Biol.* **20**, 766–774 (2018).
311. Chen, D. *et al.* LIFR is a breast cancer metastasis suppressor upstream of the Hippo-YAP pathway and a prognostic marker. *Nature Medicine* **18**, 1511–1517 (2012).
312. Cordenonsi, M. *et al.* The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. *Cell* **147**, 759–772 (2011).
313. Hiemer, S. E., Szymaniak, A. D. & Varelas, X. The transcriptional regulators TAZ and YAP direct transforming growth factor β-induced tumorigenic phenotypes in breast cancer cells. *J Biol Chem* **289**, 13461–13474 (2014).
314. Lamar, J. M. *et al.* SRC tyrosine kinase activates the YAP/TAZ axis and thereby drives tumor growth and metastasis. *J Biol Chem* **294**, 2302–2317 (2019).
315. Overholtzer, M. *et al.* Transforming properties of YAP, a candidate oncogene on the

chromosome 11q22 amplicon. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 12405–12410 (2006).

316. Rashidian, J. *et al.* Ski regulates Hippo and TAZ signaling to suppress breast cancer progression. *Sci Signal* **8**, ra14 (2015).
317. Yang, C.-S. *et al.* Glutamine-utilizing transaminases are a metabolic vulnerability of TAZ/YAP-activated cancer cells. *EMBO Rep* **19**, e43577 (2018).
318. Zanconato, F. *et al.* Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth. *Nature Cell Biology* **17**, 1218–1227 (2015).
319. Zanconato, F. *et al.* Transcriptional addiction in cancer cells is mediated by YAP/TAZ through BRD4. *Nature Medicine* **24**, 1599–1610 (2018).
320. Lin, C.-H. *et al.* Microenvironment rigidity modulates responses to the HER2 receptor tyrosine kinase inhibitor lapatinib via YAP and TAZ transcription factors. *MBoC* **26**, 3946–3953 (2015).
321. Ding, X., Park, S. I., McCauley, L. K. & Wang, C.-Y. Signaling between Transforming Growth Factor β (TGF-β) and Transcription Factor SNAI2 Represses Expression of MicroRNA miR-203 to Promote Epithelial-Mesenchymal Transition and Tumor Metastasis*. *Journal of Biological Chemistry* **288**, 10241–10253 (2013).
322. Moes, M. *et al.* A Novel Network Integrating a miRNA-203/SNAI1 Feedback Loop which Regulates Epithelial to Mesenchymal Transition. *PLOS ONE* **7**, e35440 (2012).
323. Northey, J. J. *et al.* Stiff stroma increases breast cancer risk by inducing the oncogene ZNF217. *J Clin Invest* **130**, 5721–5737 (2020).
324. Wei, S. C. *et al.* Matrix stiffness drives epithelial–mesenchymal transition and tumour metastasis through a TWIST1–G3BP2 mechanotransduction pathway. *Nature Cell Biology* **17**, 678–688 (2015).
325. Barry-Hamilton, V. *et al.* Allosteric inhibition of lysyl oxidase–like-2 impedes the development of a pathologic microenvironment. *Nat Med* **16**, 1009–1017 (2010).
326. Bui, T. *et al.* Functional Redundancy between β1 and β3 Integrin in Activating the

IR/Akt/mTORC1 Signaling Axis to Promote ErbB2-Driven Breast Cancer. *Cell Rep* **29**, 589-602.e6 (2019).

327. Chan, S. W. *et al.* A Role for TAZ in Migration, Invasion, and Tumorigenesis of Breast Cancer Cells. *Cancer Res* **68**, 2592–2598 (2008).
328. Chen, Q. *et al.* A temporal requirement for Hippo signaling in mammary gland differentiation, growth, and tumorigenesis. *Genes Dev.* **28**, 432–437 (2014).
329. Levental, K. R. *et al.* Matrix Crosslinking Forces Tumor Progression by Enhancing Integrin Signaling. *Cell* **139**, 891–906 (2009).
330. Mooney, D. *et al.* Switching from differentiation to growth in hepatocytes: control by extracellular matrix. *J. Cell. Physiol.* **151**, 497–505 (1992).
331. Driskill, J. H. & Pan, D. The Hippo Pathway in Liver Homeostasis and Pathophysiology. *Annual Review of Pathology: Mechanisms of Disease* **16**, 299–322 (2021).
332. Ishikawa, J. *et al.* Mechanical homeostasis of liver sinusoid is involved in the initiation and termination of liver regeneration. *Commun Biol* **4**, 1–13 (2021).
333. Ye, J. Transcription factors activated through RIP (regulated intramembrane proteolysis) and RAT (regulated alternative translocation). *J Biol Chem* **295**, 10271–10280 (2020).
334. Bertolio, R. *et al.* Sterol regulatory element binding protein 1 couples mechanical cues and lipid metabolism. *Nat Commun* **10**, 1326 (2019).
335. Basu, H. *et al.* FHL2 anchors mitochondria to actin and adapts mitochondrial dynamics to glucose supply. *Journal of Cell Biology* **220**, (2021).
336. Chen, K., Wang, Y., Deng, X., Guo, L. & Wu, C. Extracellular matrix stiffness regulates mitochondrial dynamics through PINCH-1- and kindlin-2-mediated signalling. *Current Research in Cell Biology* **2**, 100008 (2021).
337. Korobova, F., Ramabhadran, V. & Higgs, H. N. An actin-dependent step in mitochondrial fission mediated by the ER-associated formin INF2. *Science* **339**, 464–467 (2013).
338. Majstrowicz, K. *et al.* Coordination of mitochondrial and cellular dynamics by the

actin-based motor Myo19. *Journal of Cell Science* **134**, (2021).

339. Manor, U. *et al.* A mitochondria-anchored isoform of the actin-nucleating spire protein regulates mitochondrial division. *Elife* **4**, (2015).
340. Moore, A. S., Wong, Y. C., Simpson, C. L. & Holzbaur, E. L. F. Dynamic actin cycling through mitochondrial subpopulations locally regulates the fission-fusion balance within mitochondrial networks. *Nat Commun* **7**, 12886 (2016).
341. Moore, A. S. *et al.* Actin cables and comet tails organize mitochondrial networks in mitosis. *Nature* **591**, 659–664 (2021).
342. Schwartz, M. A., Both, G. & Lechene, C. Effect of cell spreading on cytoplasmic pH in normal and transformed fibroblasts. *Proc. Natl. Acad. Sci. U.S.A.* **86**, 4525–4529 (1989).
343. Tominaga, T. & Barber, D. L. Na–H Exchange Acts Downstream of RhoA to Regulate Integrin-induced Cell Adhesion and Spreading. *Mol Biol Cell* **9**, 2287–2303 (1998).
344. Yang, H. *et al.* Materials Stiffness-Dependent Redox Metabolic Reprogramming of Mesenchymal Stem Cells for Secretome-Based Therapeutic Angiogenesis. *Advanced Healthcare Materials* **8**, 1900929 (2019).
345. Khacho, M. *et al.* Mitochondrial Dynamics Impacts Stem Cell Identity and Fate Decisions by Regulating a Nuclear Transcriptional Program. *Cell Stem Cell* **19**, 232–247 (2016).
346. Khacho, M. *et al.* Mitochondrial dysfunction underlies cognitive defects as a result of neural stem cell depletion and impaired neurogenesis. *Hum Mol Genet* **26**, 3327–3341 (2017).

Figure 1

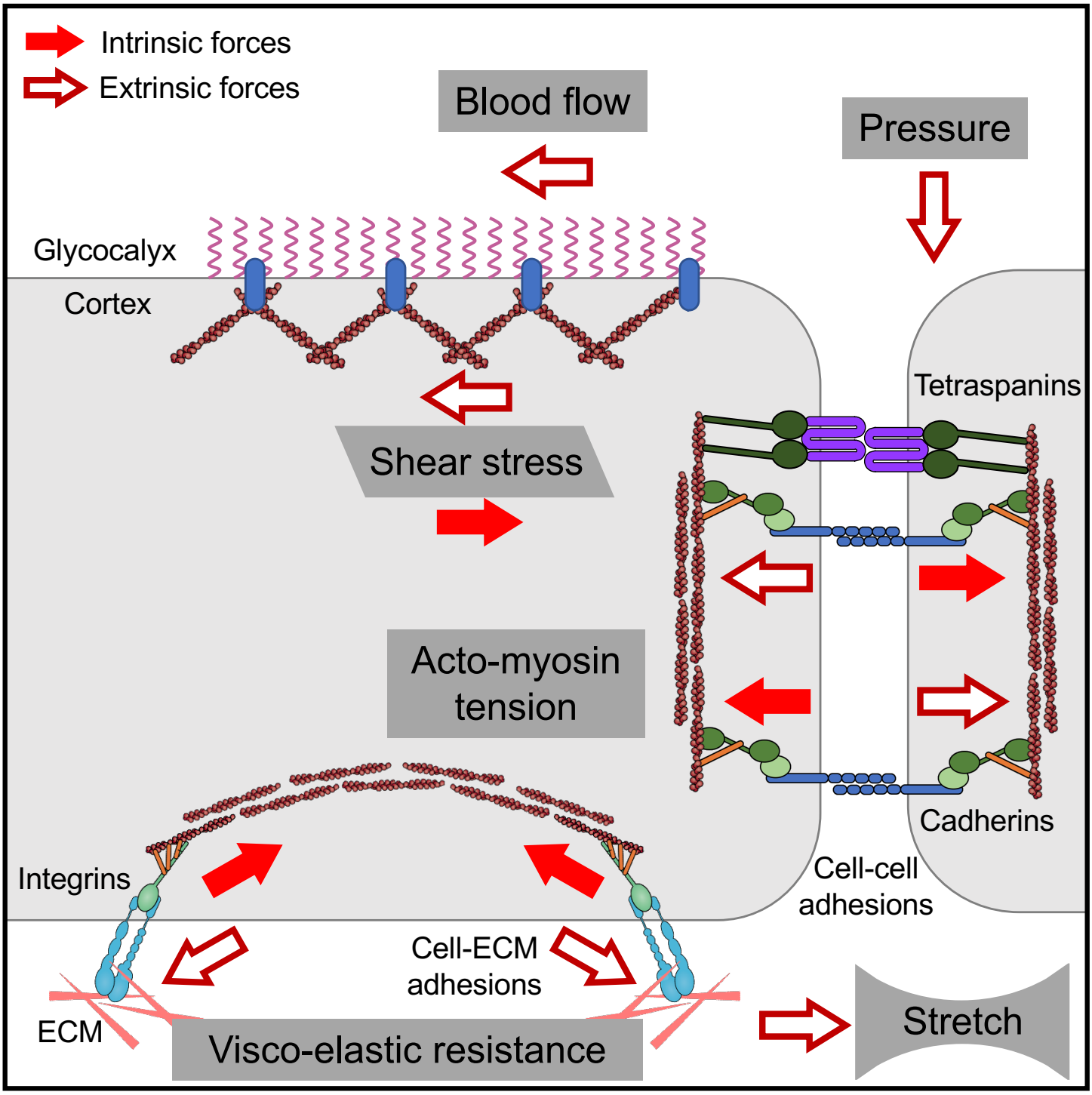
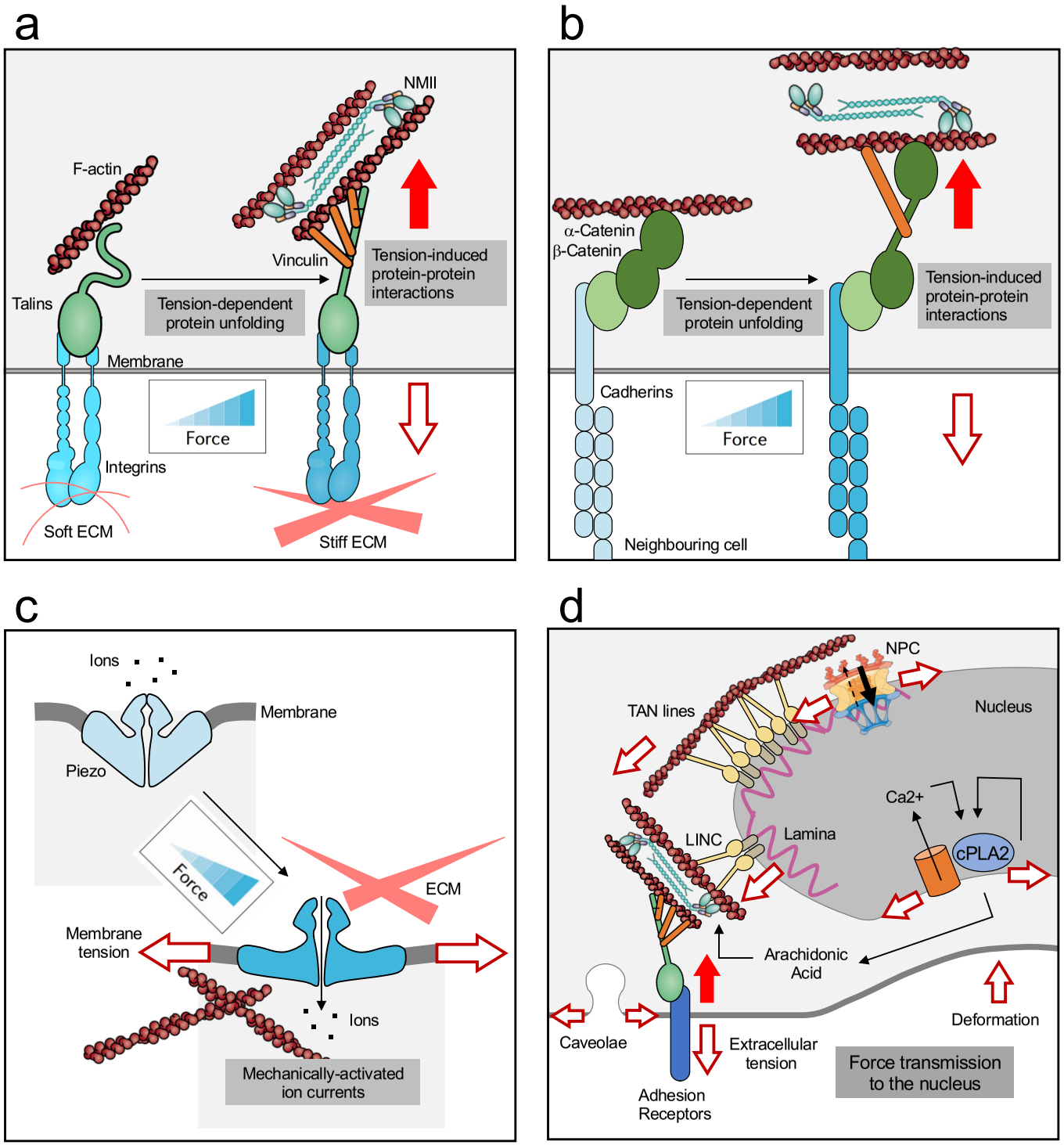

Intrinsic forces
Extrinsic forces
Blood flow
Pressure
Glycocalyx
Cortex
Tetraspanins
Shear stress
Acto-myosin tension
Integrins
Cadherins
Cell-cell adhesions
Cell-ECM adhesions
ECM
Visco-elastic resistance
Stretch

Figure 2


a
NMII
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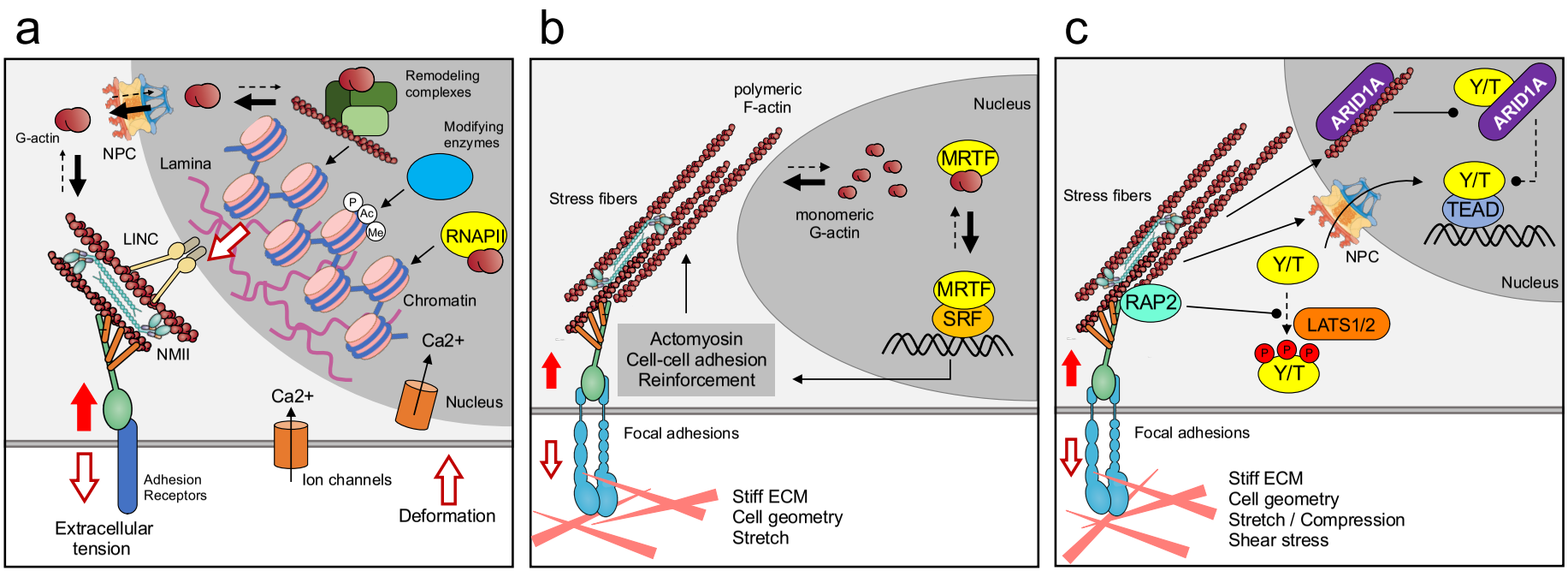
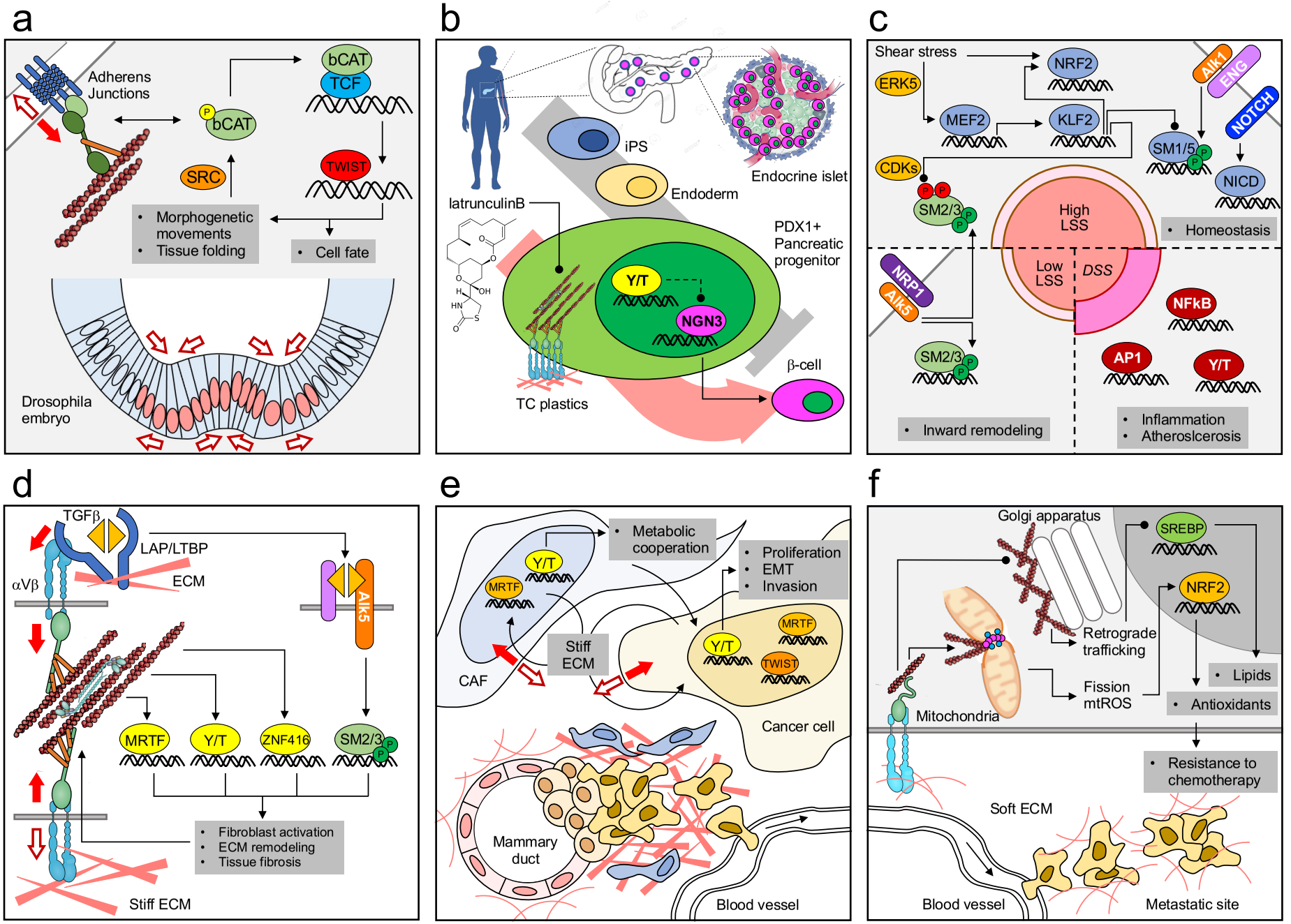
Vinculin
Tension-induced protein-protein interactions
Talins
Tension-dependent protein unfolding
Membrane
Force
Integrins
Soft ECM
Stiff ECM
b
α-Catenin
β-Catenin
Tension-dependent protein unfolding
Tension-induced protein-protein interactions
Cadherins
Force
Neighbouring cell
c
Ions
Membrane
Piezo
Force
ECM
Membrane tension
Ions
Mechanically-activated ion currents
d
NPC
TAN lines
Nucleus
Ca2+
LINC
Lamina
cPLA2
Arachidonic Acid
Deformation
Caveolae
Extracellular tension
Force transmission to the nucleus
Adhesion Receptors

Figure 3


a
Remodeling complexes
G-actin
NPC
Lamina
Modifying enzymes
P
Ac
Me
RNAPII
LINC
Chromatin
Ca2+
NMII
Nucleus
Ca2+
Adhesion Receptors
Ion channels
Deformation
Extracellular tension
b
polymeric F-actin
Nucleus
MRTF
Stress fibers
monomeric G-actin
MRTF
SRF
Actomyosin
Cell-cell adhesion
Reinforcement
Focal adhesions
Stiff ECM
Cell geometry
Stretch
c
ARID1A
Y/T
ARID1A
Stress fibers
Y/T
TEAD
NPC
Y/T
Nucleus
RAP2
LATS1/2
P
P
P
Y/T
Focal adhesions
Stiff ECM
Cell geometry
Stretch / Compression
Shear stress

# Figure 4


a
Adherens Junctions
bCAT
TCF
P
bCAT
SRC
TWIST
• Morphogenetic movements
• Tissue folding
• Cell fate
Drosophila embryo
b
iPS
Endoderm
Endocrine islet
latrunculinB
PDX1+ Pancreatic progenitor
Y/T
NGN3
β-cell
TC plastics
c
Shear stress
NRF2
ERK5
MEF2
KLF2
Alk1
ENG
NOTCH
SM1/5
NICD
CDKs
SM2/3
High LSS
• Homeostasis
NRP1
Alk5
Low LSS
DSS
NFkB
AP1
Y/T
SM2/3
• Inward remodeling
• Inflammation
• Atherosclerosis
d
TGFβ
LAP/LTBP
αVβ
ECM
Alk5
MRTF
Y/T
ZNF416
SM2/3
• Fibroblast activation
• ECM remodeling
• Tissue fibrosis
Stiff ECM
e
• Metabolic cooperation
Y/T
MRTF
• Proliferation
• EMT
• Invasion
Stiff ECM
CAF
Y/T
MRTF
TWIST
Cancer cell
Mammary duct
Blood vessel
f
Golgi apparatus
SREBP
NRF2
Retrograde trafficking
Fission mtROS
Mitochondria
• Lipids
• Antioxidants
• Resistance to chemotherapy
Soft ECM
Blood vessel
Metastatic site