Introduction into Mechanobiology

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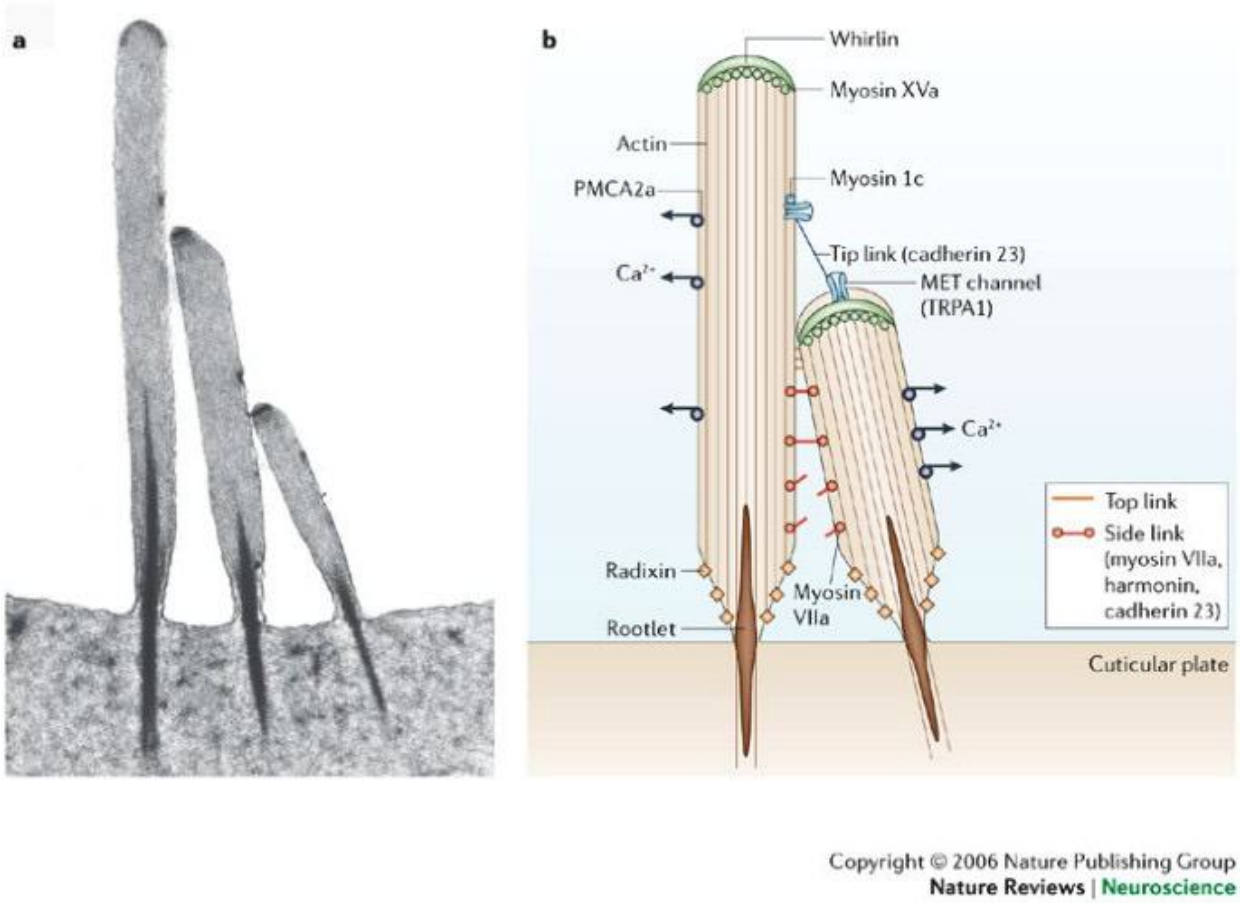
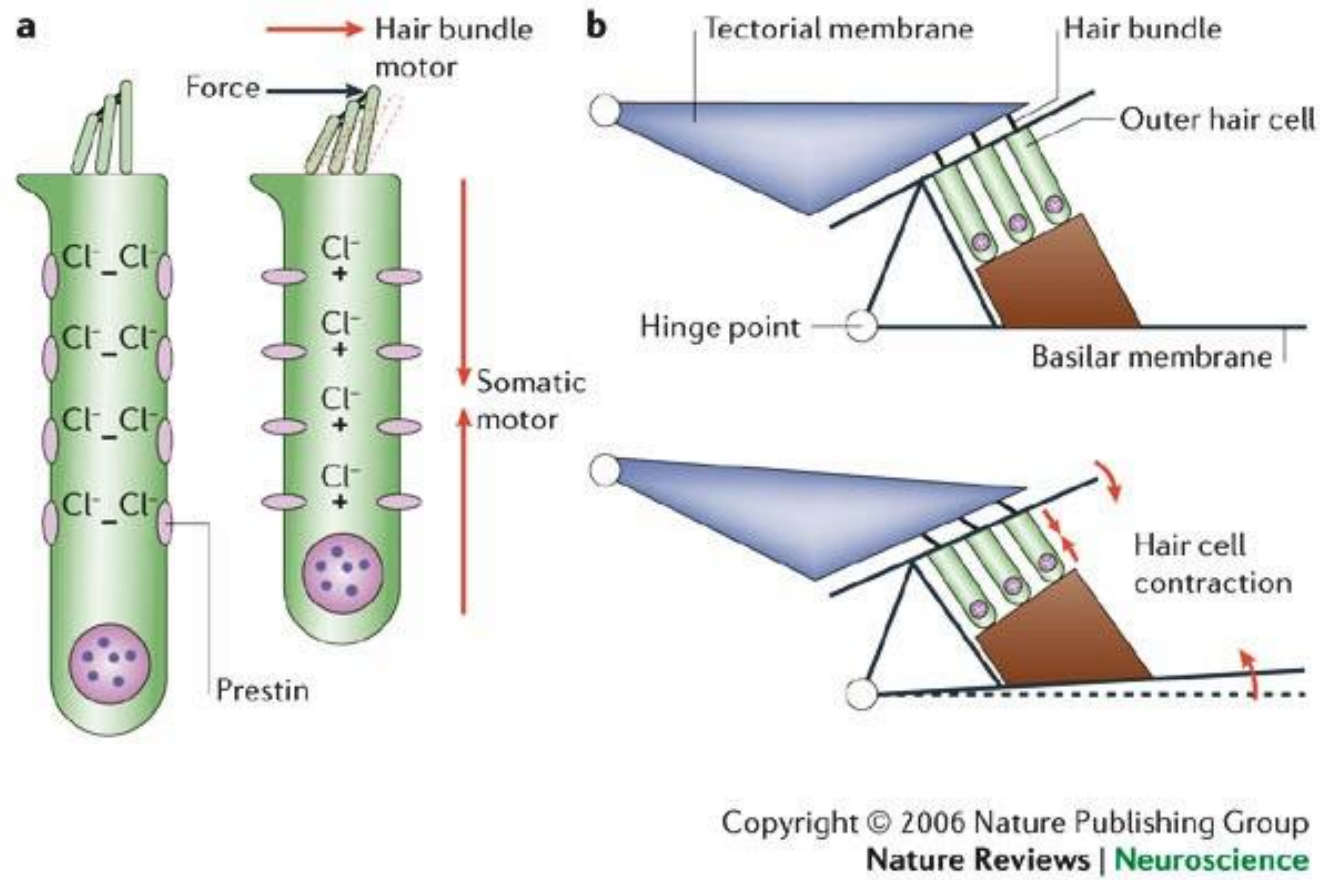
Evidence that cells are exposed to mechanical forces

- Bone and muscle need forces to grow
- Endothelial cells are constantly exposed to shear stress
- Cartilage and bone cells are constantly exposed to compression
- Hair cells (inner ear) sense sound and position via changes in physical forces to their cilia



https://www.nature.com/articles/nrn1828

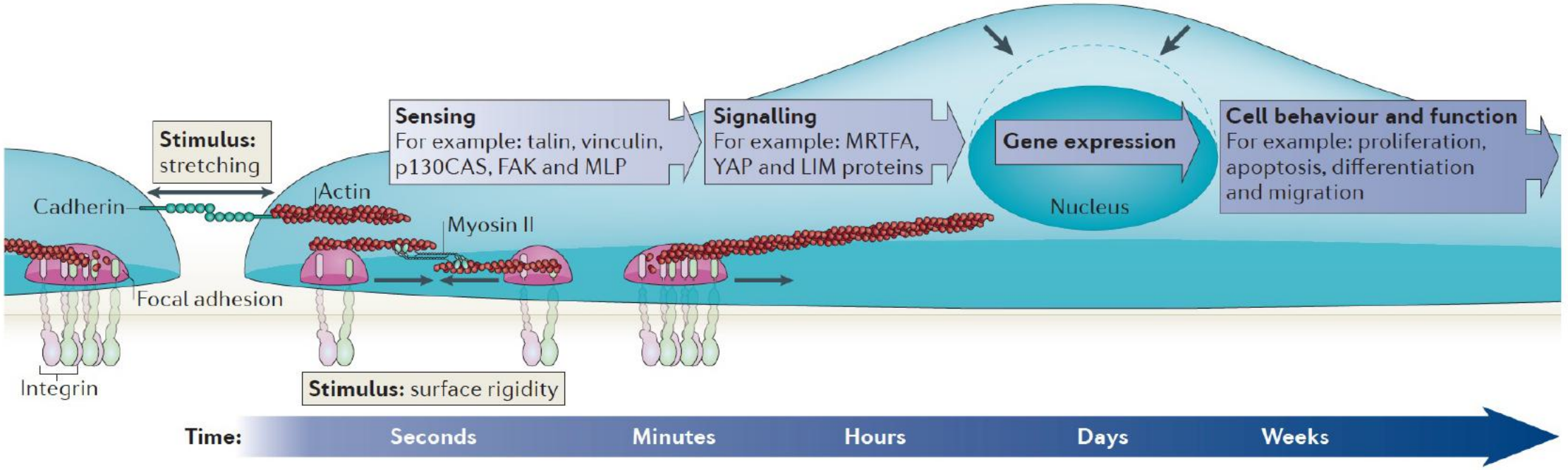
- Prestin is inactive when bound to Cl^-
- Upon depolarization → Prestin changes its conformation → contraction of the cell body



https://www.nature.com/articles/nrn1828

https://auditoryneuroscience.com/ear/dancing_hair_cell

Mechanotransduction



Mechanotransduction is the conversion of mechanical force into a biochemical signal

Mechanosensing → mechanotransduction → biological response

- Mechanosensor: a protein that is affected by the mechanical force and that converts the mechanical signal into a biochemical one.

- Mechanotransducer: the molecules that are downstream of the mechanosensor and that propagate the biochemical signaling cascade

- Some of the problems with this oversimplifications:

➢ All proteins change structure upon exposure to mechanical forces

➢ Some proteins transmit forces, so they do not strictly fit into these two categories

Force vs. Stress vs. Strain

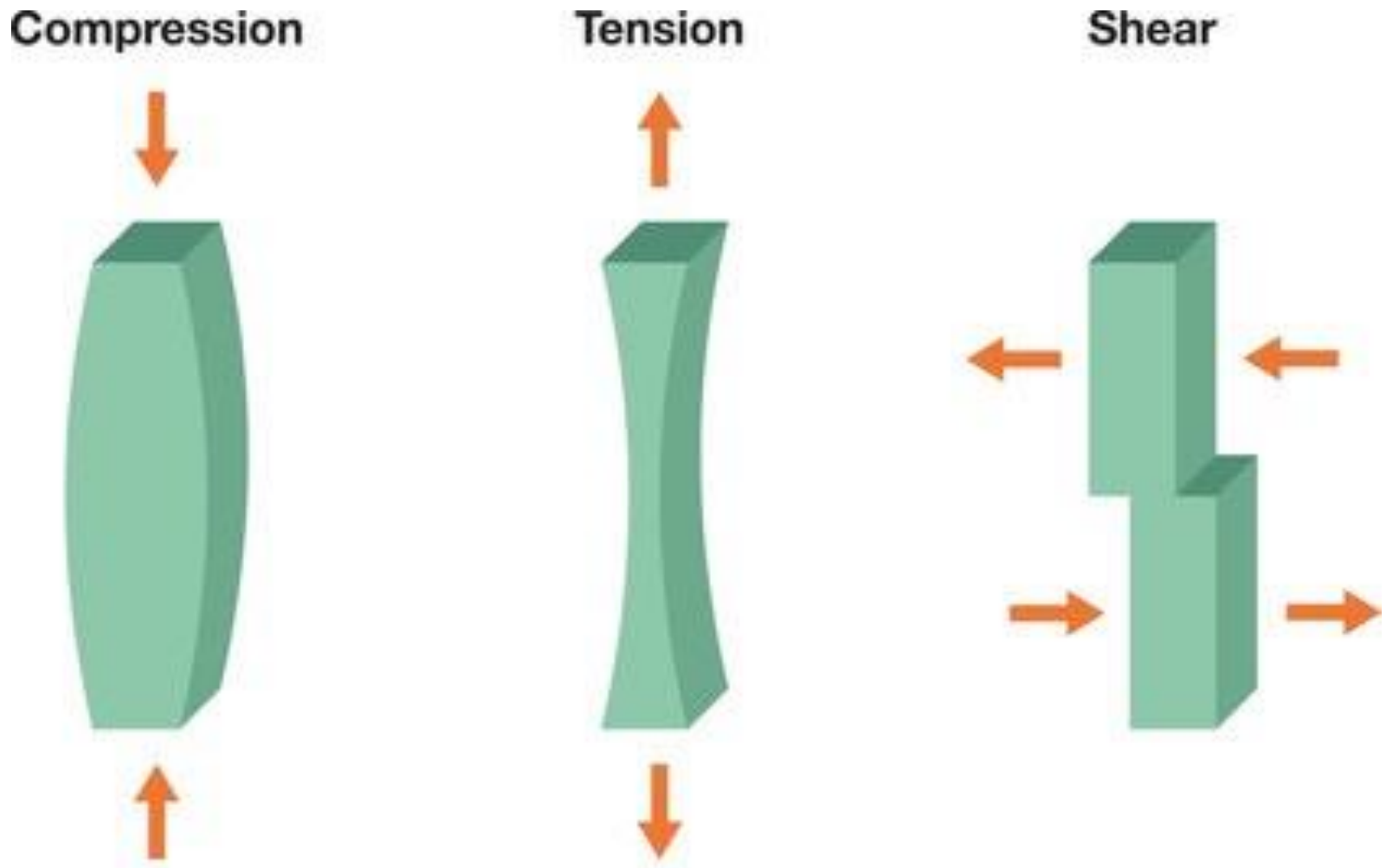
- Mechanical **force**: external physical load acting on a cell or tissue causing it to move or deform. Force is measured in Newtons (N). Example: blood flow, or compression of cartilage during movement

- Mechanical **stress**: the internal responses within tissues or cells to applied forces (i.e., how force is distributed and how cells/tissues resist deformation). $\sigma=A/F$. Stress is measured in Pascal (Pa) or N/m^2.

- Mechanical **strain**: is the deformation of the cell/tissue induced by the stress. While stress can be viewed as the cause, strain is the consequence. Strain has no unit, but is the ratio of deformation.

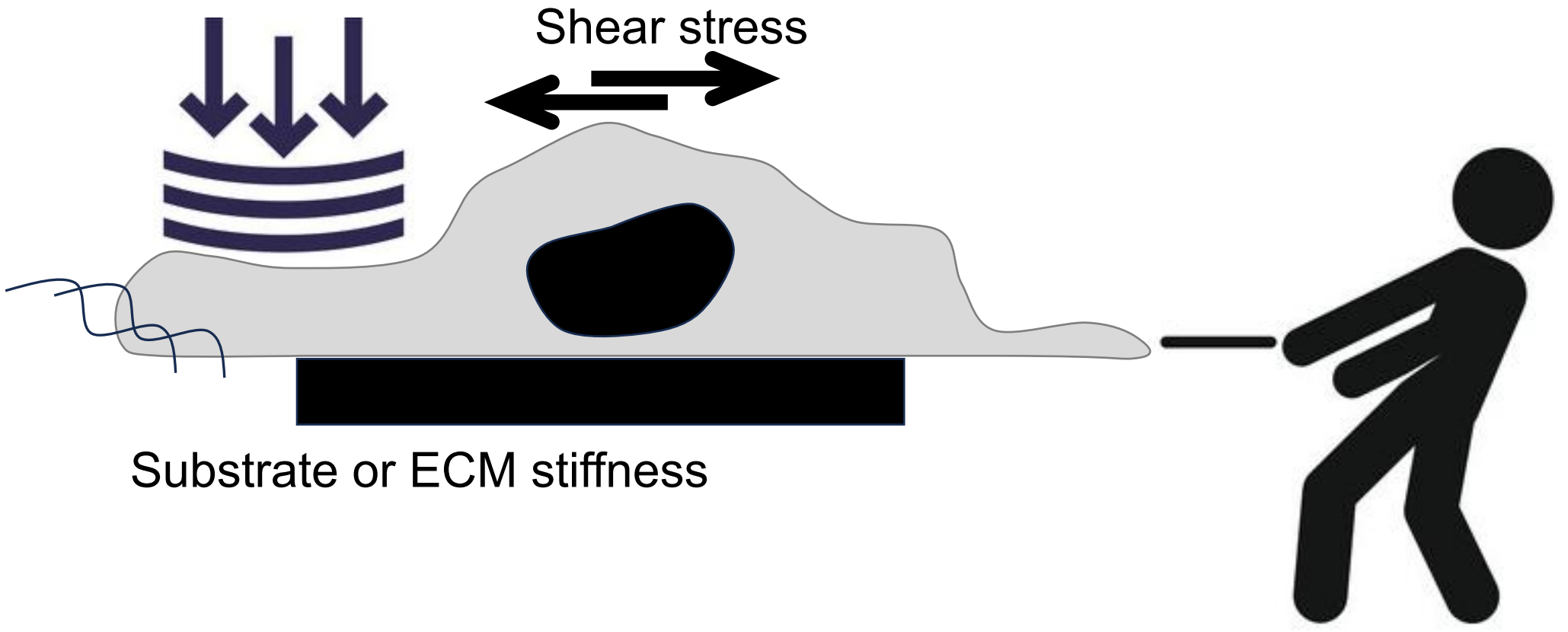
- **Cells sense the stress** (not the force) and respond to it through mechanotransduction.

Force vs. Stress

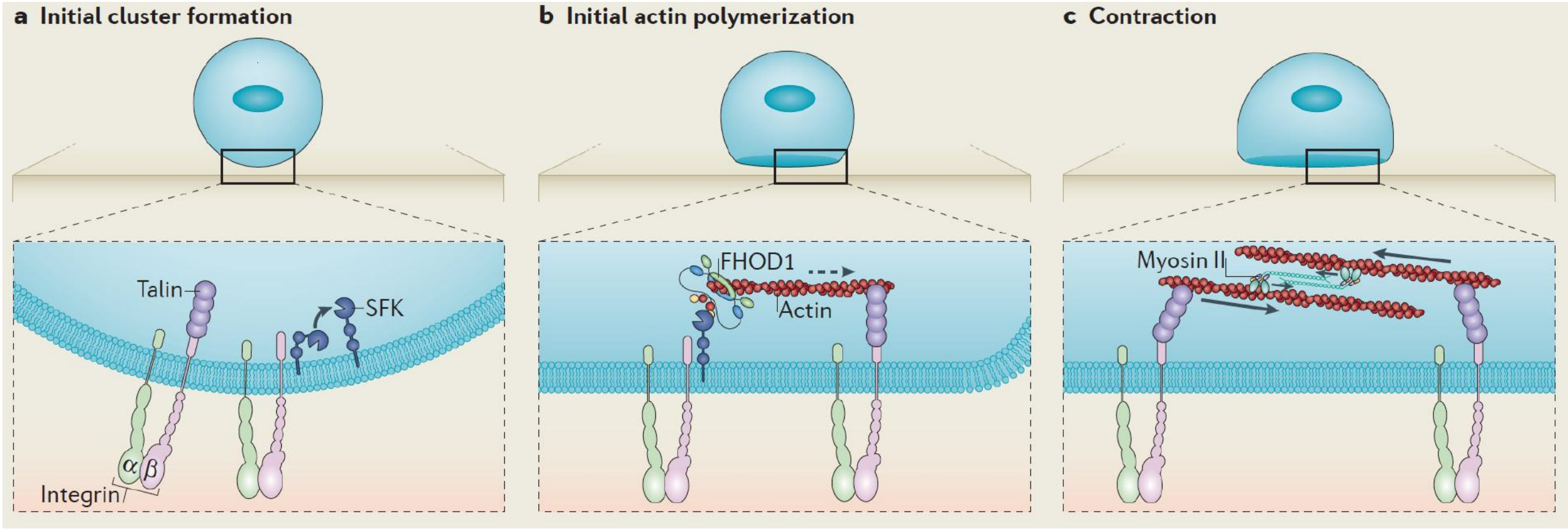


Main types of mechanical stress

Compression (hydrostatic pressure or confinement)



A good example of mechanotransduction can be observed everyday in the lab: cell spreading after seeding.
It is well established how this happens

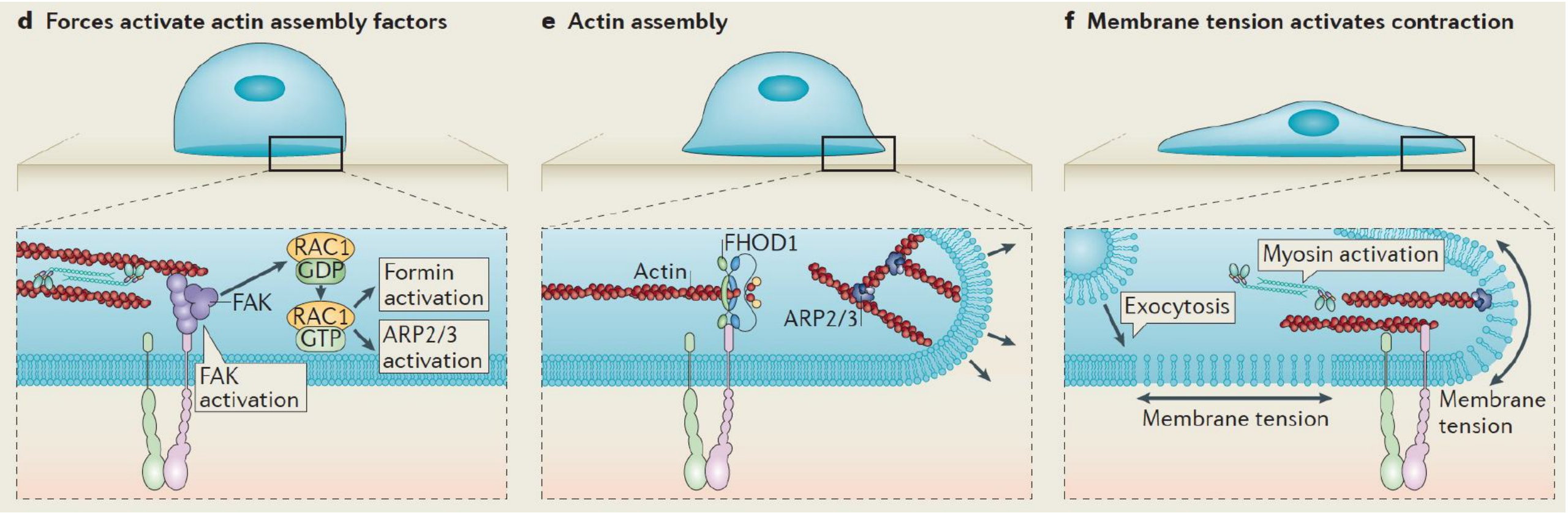


Integrins are activated

Actin polymerization

Myosin recruitment
→ Contraction and flattening of the cell

https://pubmed.ncbi.nlm.nih.gov/25355507/

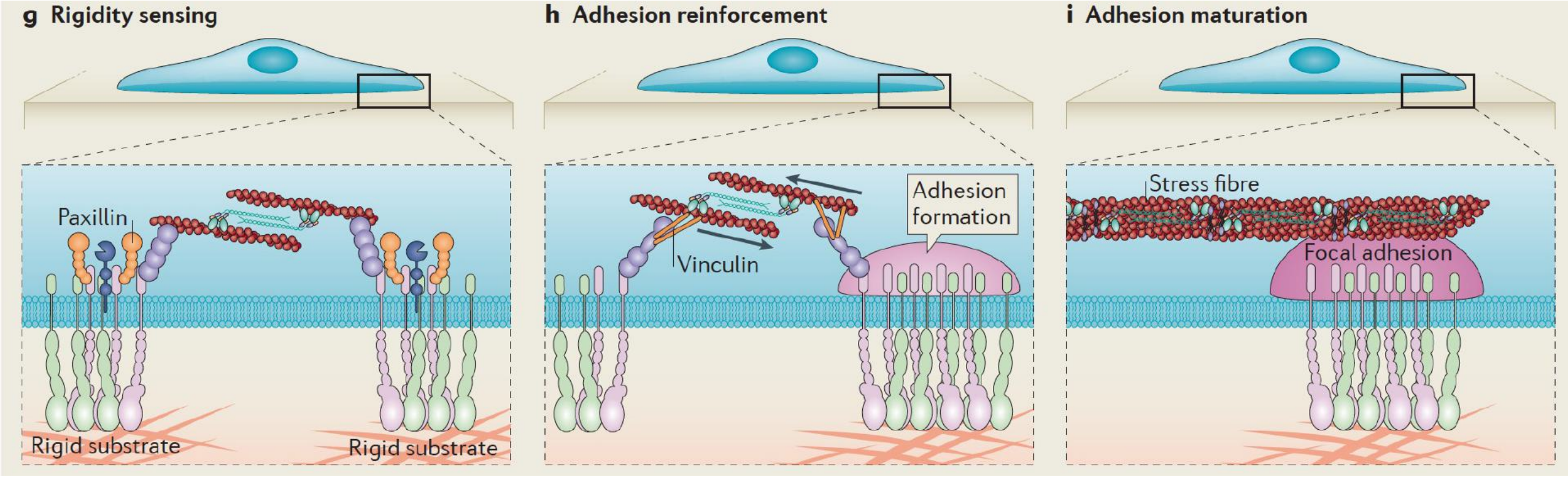


Activation of Rac1
→ More actin polymerization

Increased contractility

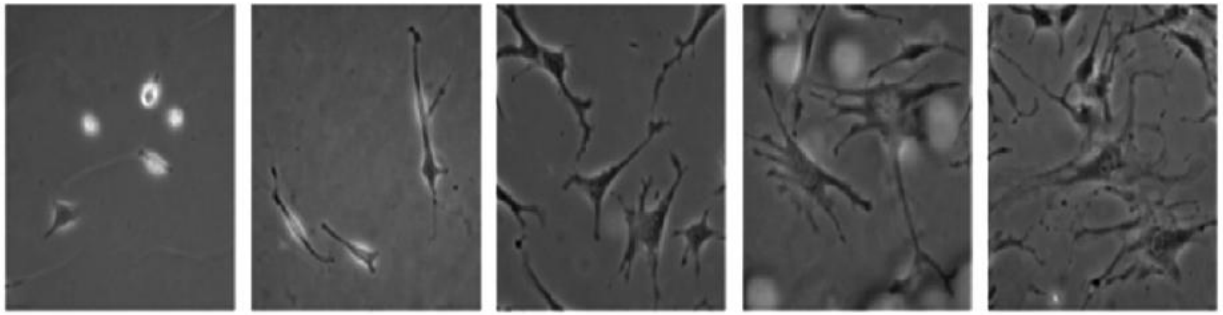
Membrane tension
→ sensor?

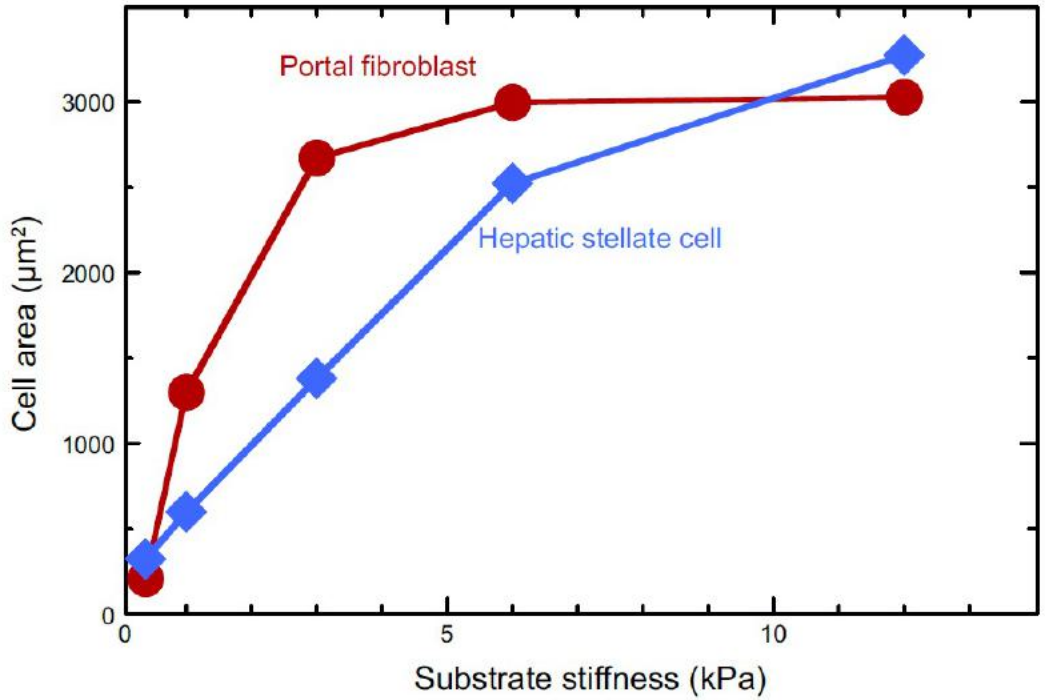
https://pubmed.ncbi.nlm.nih.gov/25355507/



https://pubmed.ncbi.nlm.nih.gov/25355507/

Cells increase their surface area in response to stiffness





https://pubmed.ncbi.nlm.nih.gov/31751165/

The type of matrix affects the response of cells to stiffness → both biochemistry and physics are important

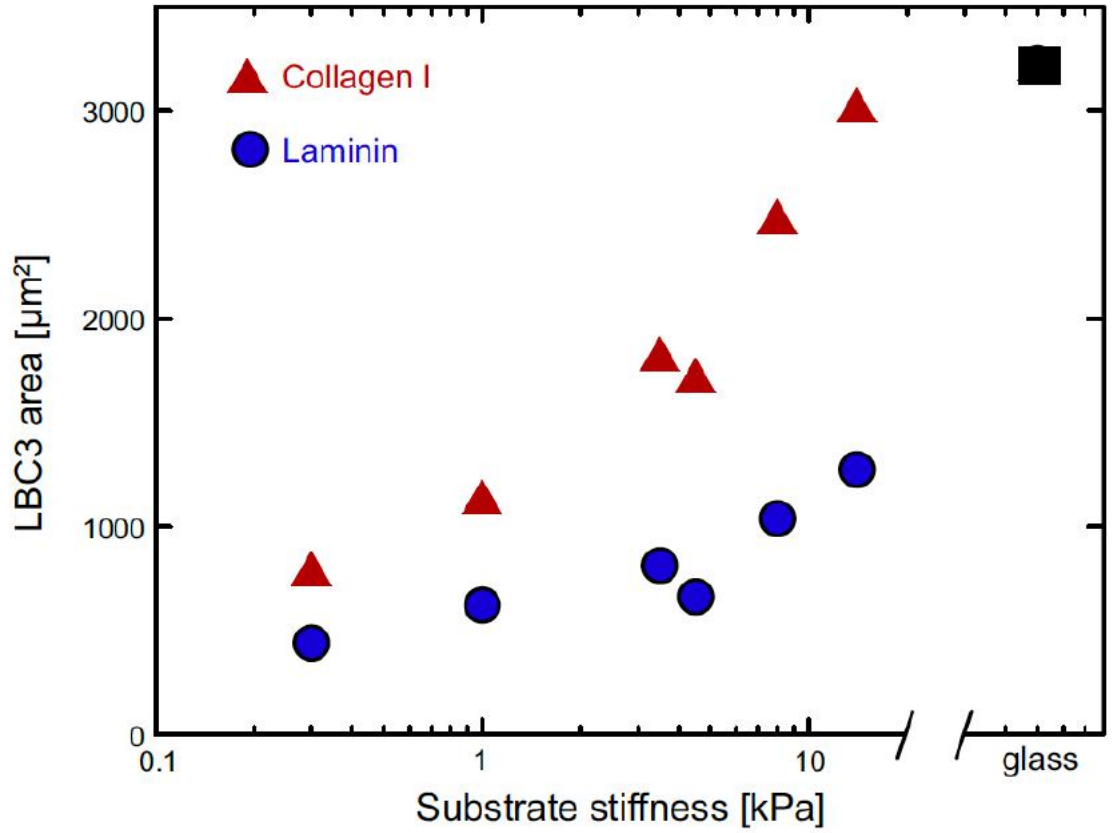


FIGURE 4. Integrin ligand dependence of response to substrate stiffness. Area of LBC3 human glioma cells on polyacrylamide gels coated with collagen I or laminin compared with area on glass after 24 h. [From Pogoda et al. (173).]

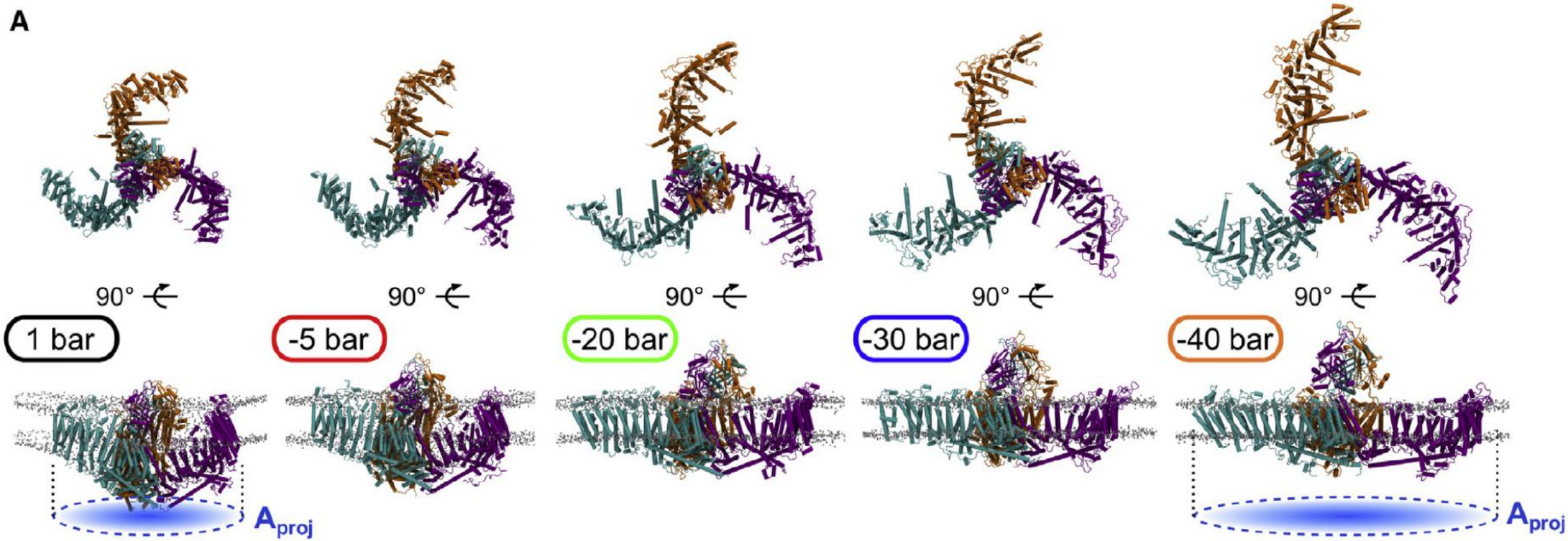
https://pubmed.ncbi.nlm.nih.gov/31751165/

Examples of Mechanosensors:

- Piezo channels

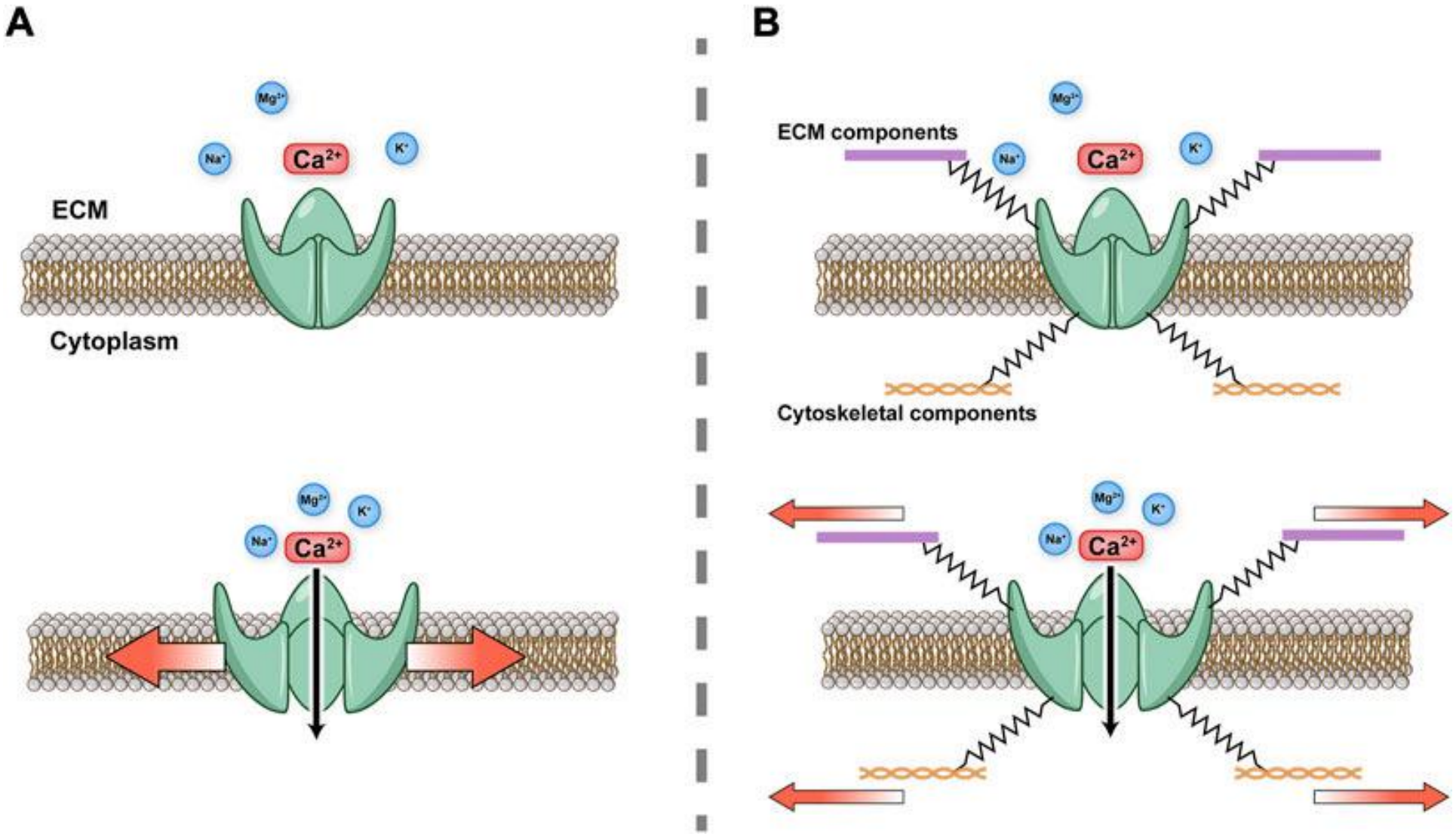
- Integrins

Piezo channels are the best characterized mechanosensors



https://pubmed.ncbi.nlm.nih.gov/33582135/

Piezo channels are the best characterized mechanosensors

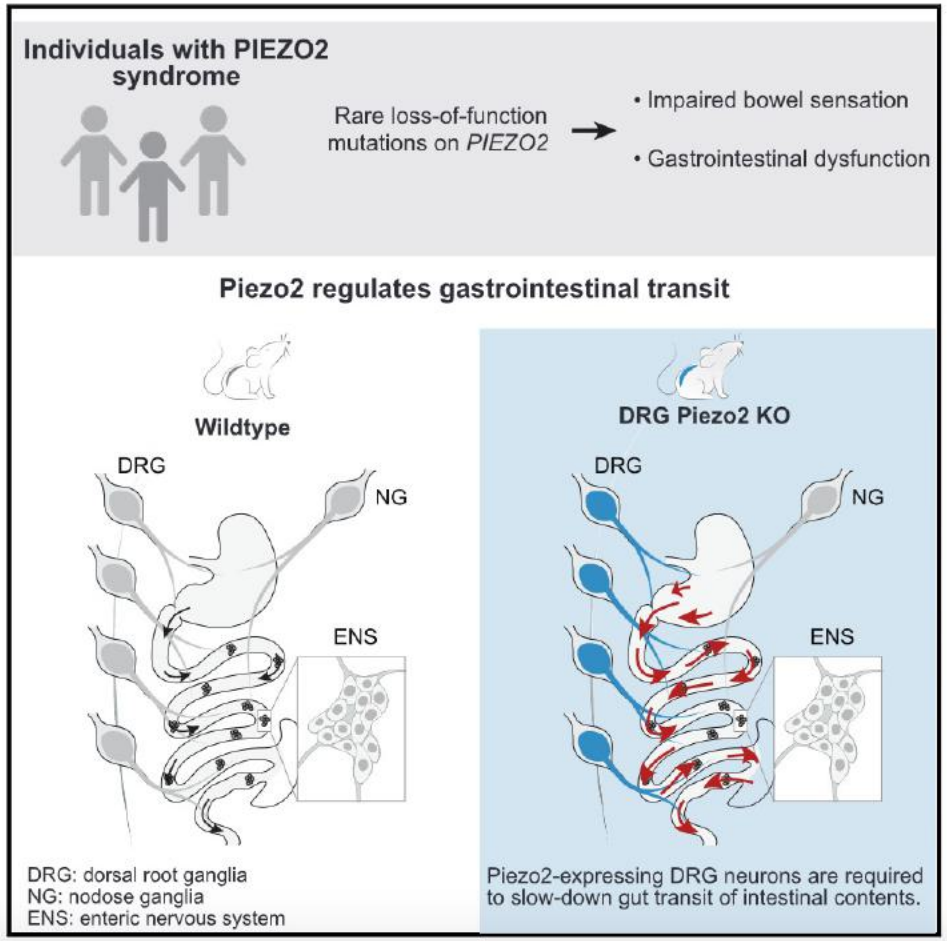


https://www.frontiersin.org/articles/10.3389/fphys.2022.1039714/full

Cell

PIEZO2 in somatosensory neurons controls gastrointestinal transit

Graphical abstract



Authors

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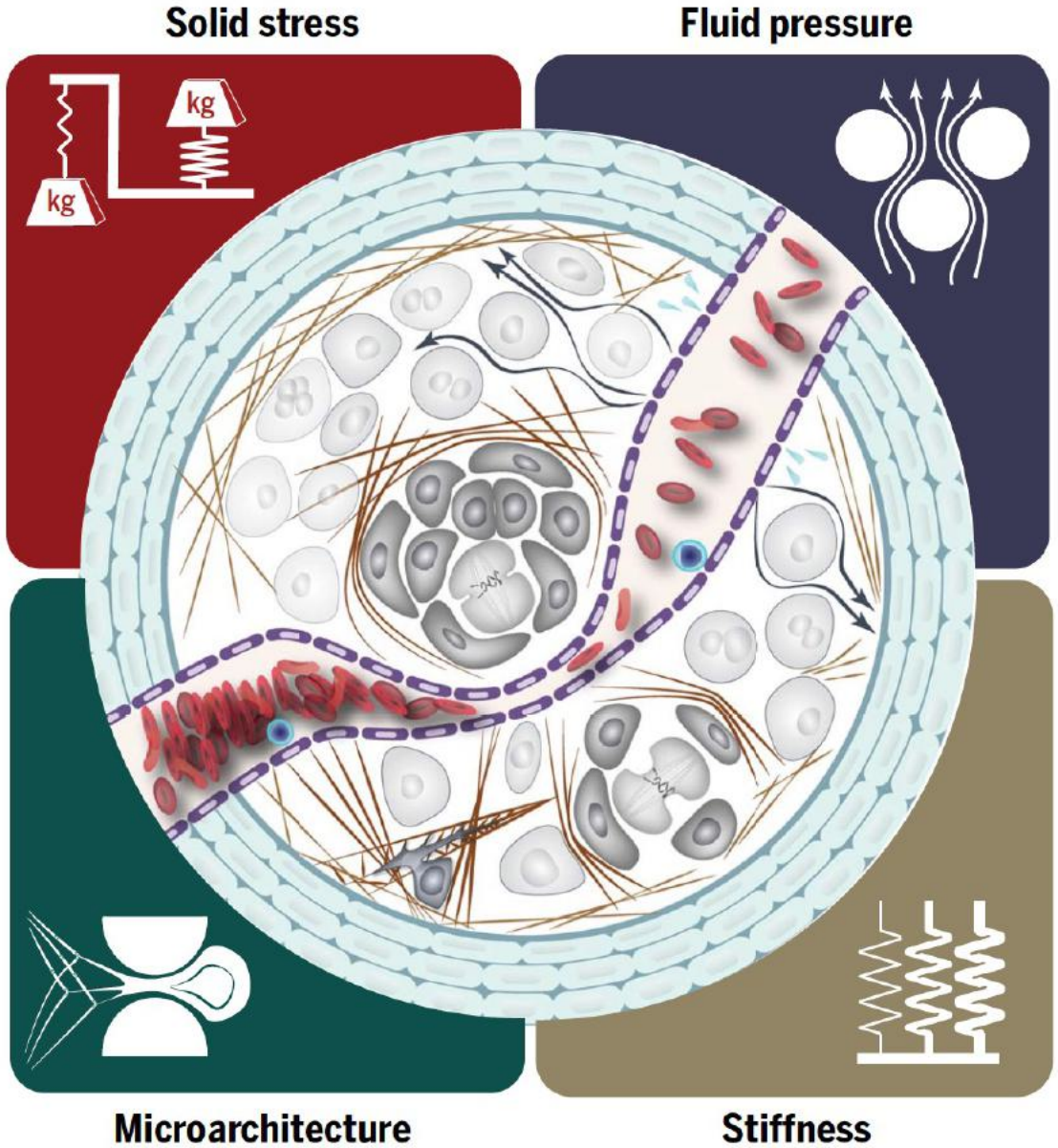
In brief

Piezo2 in dorsal root ganglia neurons is required to sense gut content and slow down food transit rates in the stomach, small intestine, and colon.

GI Symptoms of patients:
- Diarrhea & Constipation

https://pubmed.ncbi.nlm.nih.gov/37541196/

The 4 main types of mechanical stress in solid tumors



The 4 main types of mechanical stress in solid tumors: Solid stress

Solid stress:

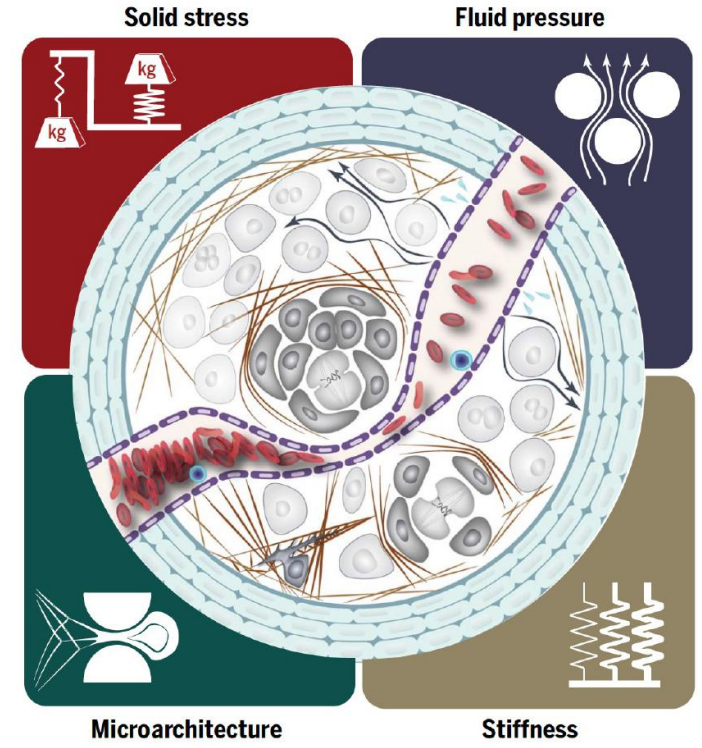
- 3 main types: (i) Compression; (ii) Tensile forces; (iii) Shear stress
- Ranges from 0.5 to 10 kPa
- How is it generated?

➢ Increased tissue volume due to proliferation and immune cell infiltration

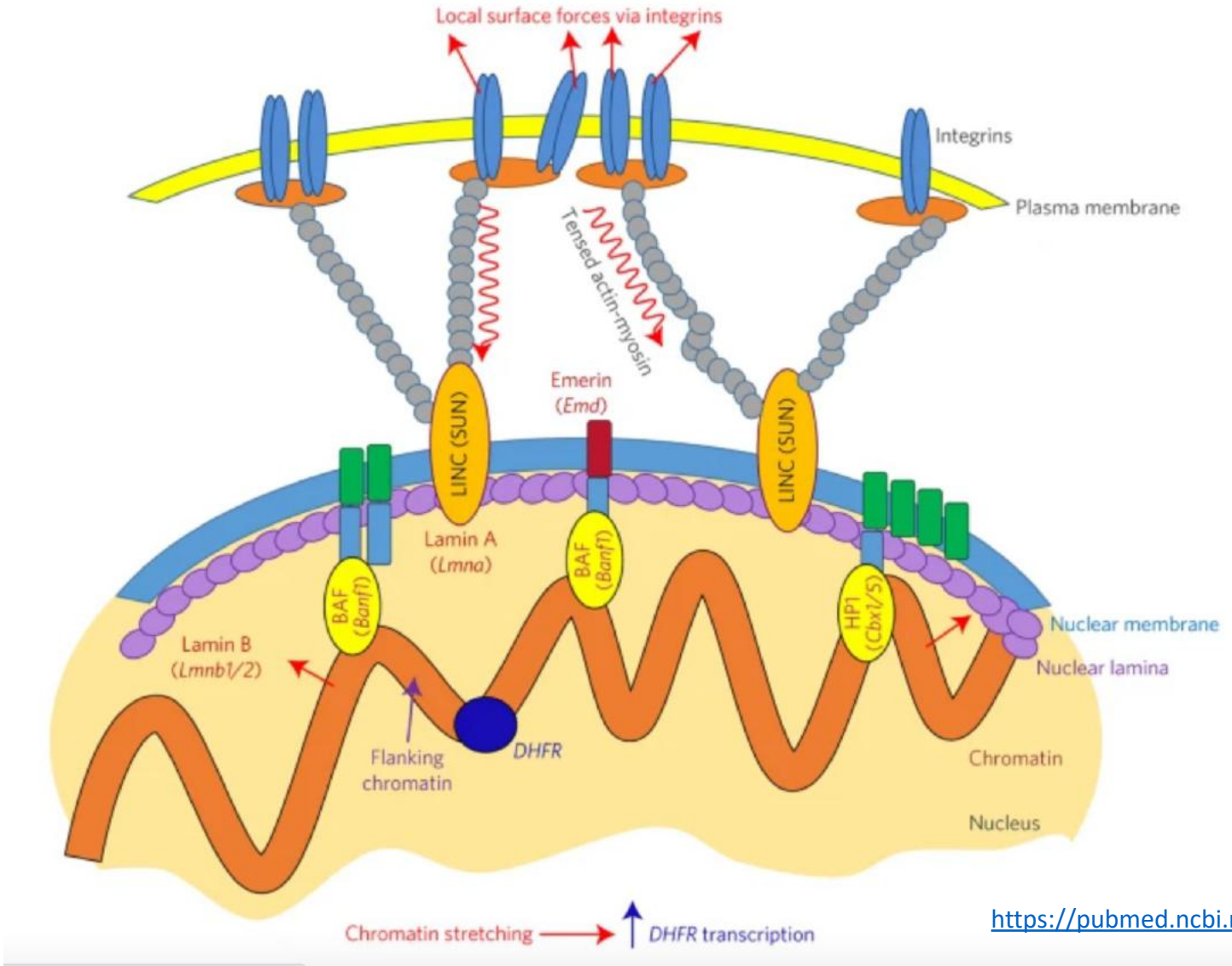
➢ Displacement of normal tissue (many tumors grow as a solid mass)

➢ Swelling of the tumor

➢ Cellular contractility

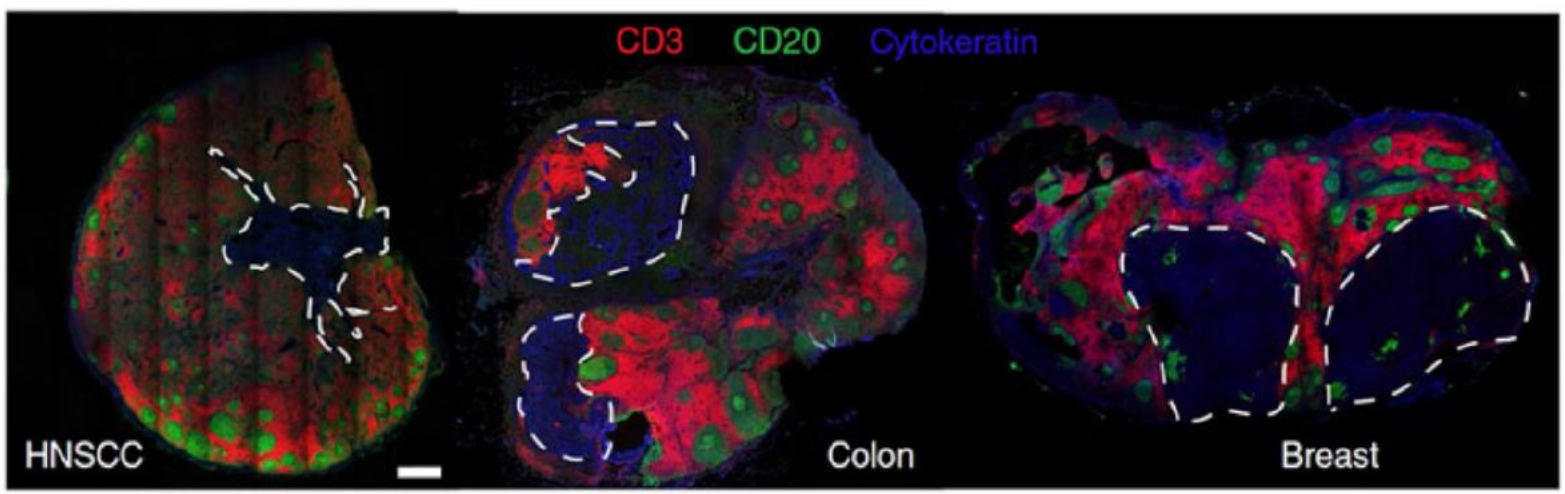


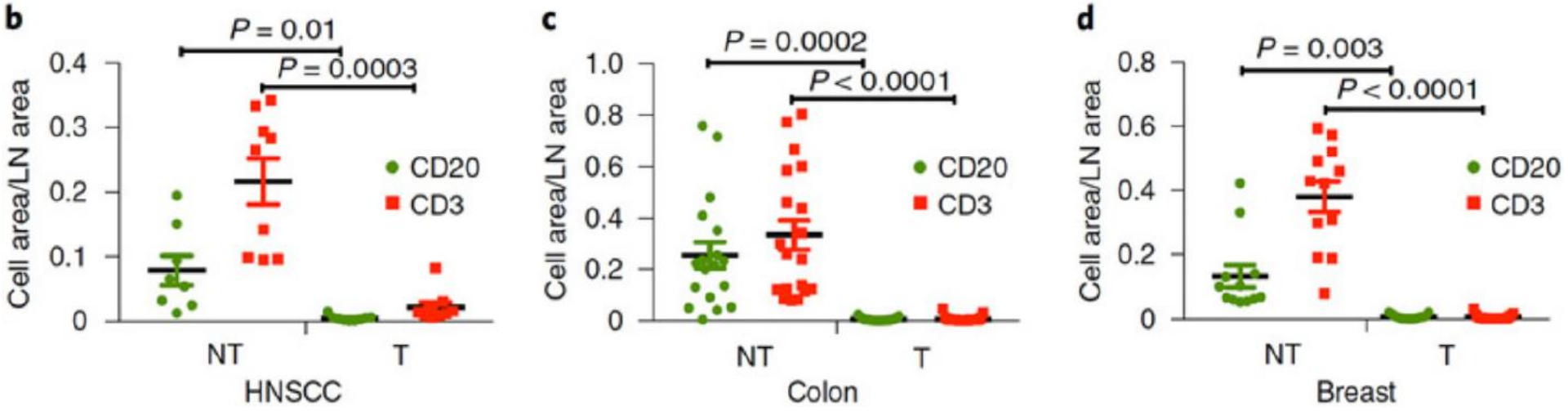
Consequences of solid stress on tumor cells: Changes in chromatin architecture and gene expression



https://pubmed.ncbi.nlm.nih.gov/27548707/

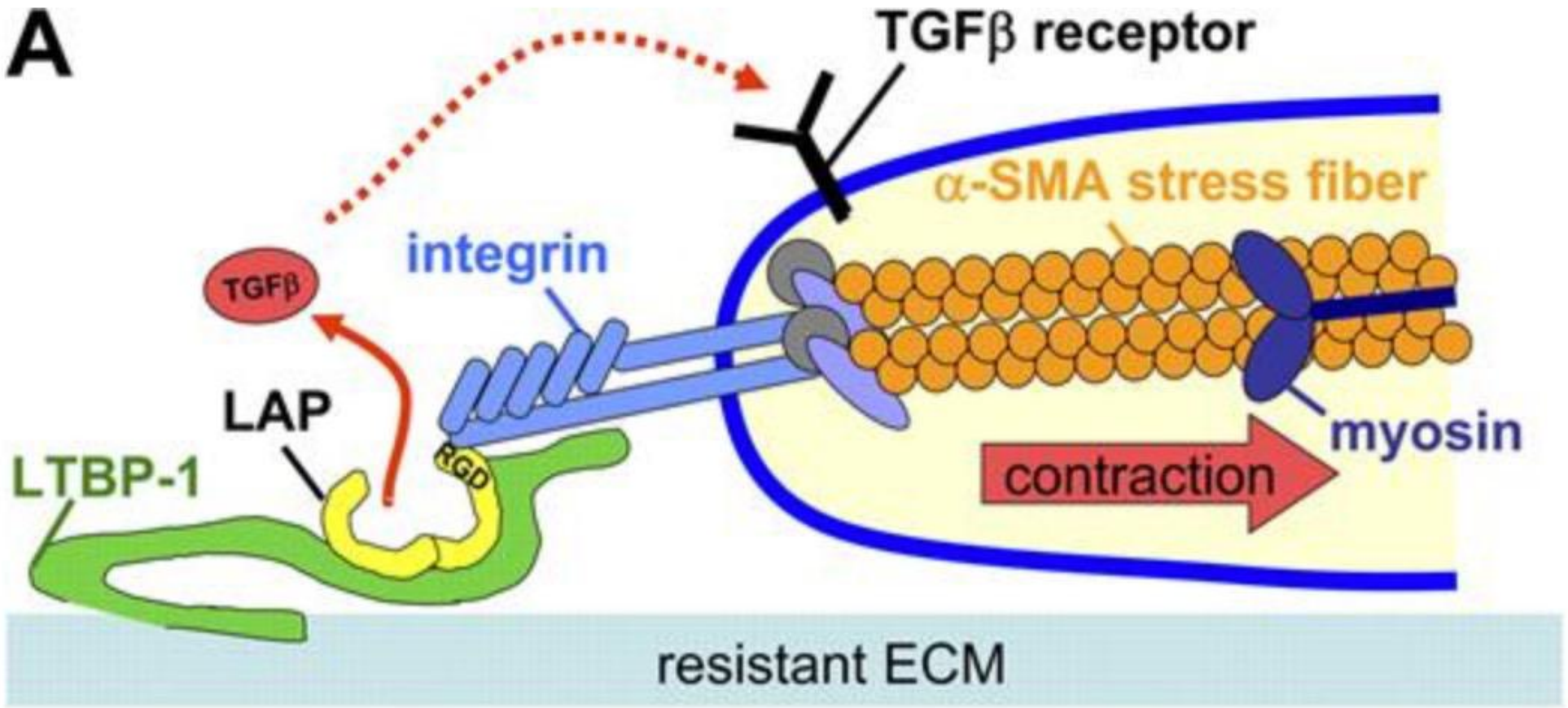
Consequences of solid stress on tumor cells: Impairment of lymphocyte infiltration





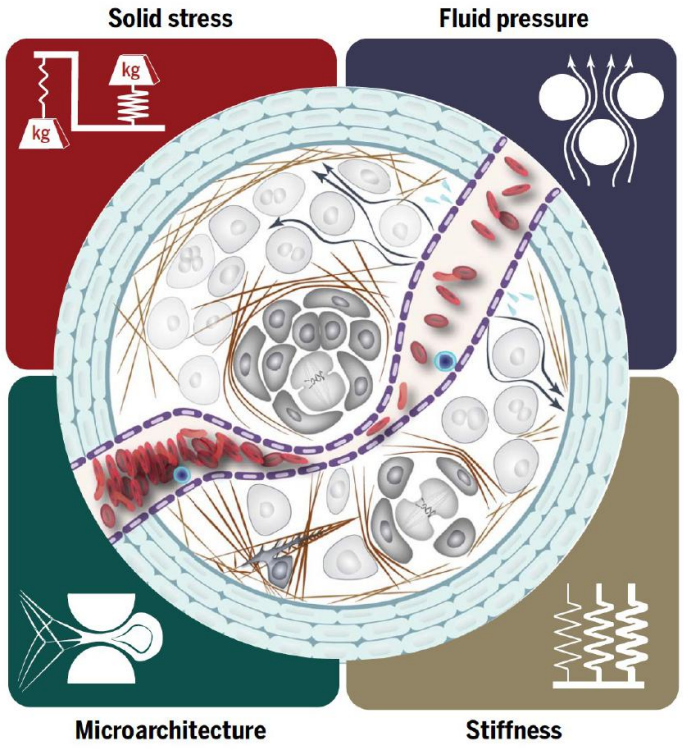
https://pubmed.ncbi.nlm.nih.gov/34282290/

Consequences of solid stress on tumor cells: Liberation of growth factors



https://pubmed.ncbi.nlm.nih.gov/18086923/

The 4 main types of mechanical stress in solid tumors: Interstitial fluid pressure (IFP)



IFP:

- In tumors IFP is elevated and reached values between 1-5 kPa (highest in the tumor center)
- Reasons for elevated IFP: leaky blood vessles, disfunction of lyphatic drainage
- IFP and solid stress are interdependent
- IFP generates shear stress which has the following consequnces:

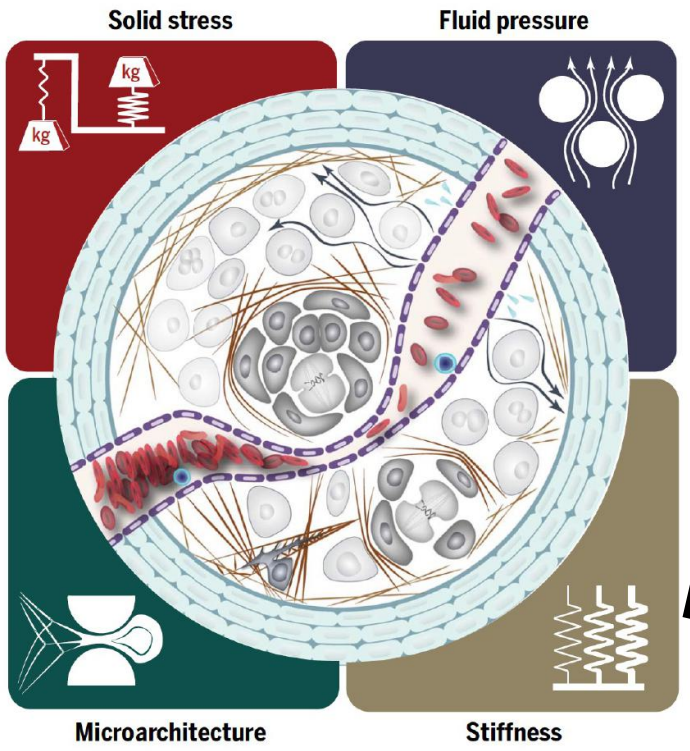
➢ Activation of fibroblasts → matrix secretion → stiffening of tumor

➢ Induction of matrix-metalloproteases (MMPs) → increased tissue invasion of cancer cells

➢ Modulation of angiogenesis

- IFP hinders arrival of drugs to tumors and shortens their retention time

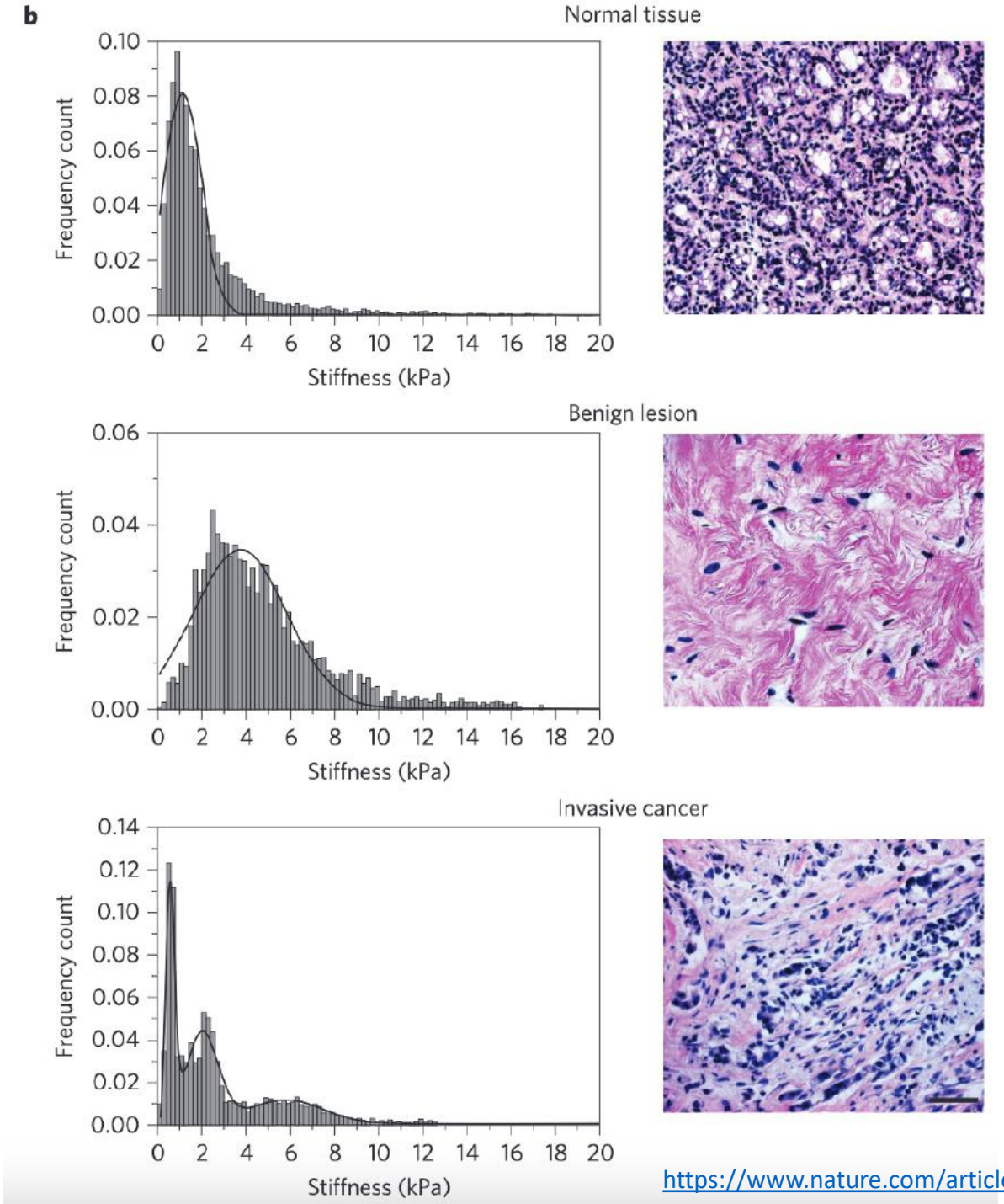
The 4 main types of mechanical stress in solid tumors: Stiffness



Stiffness (rigidity):

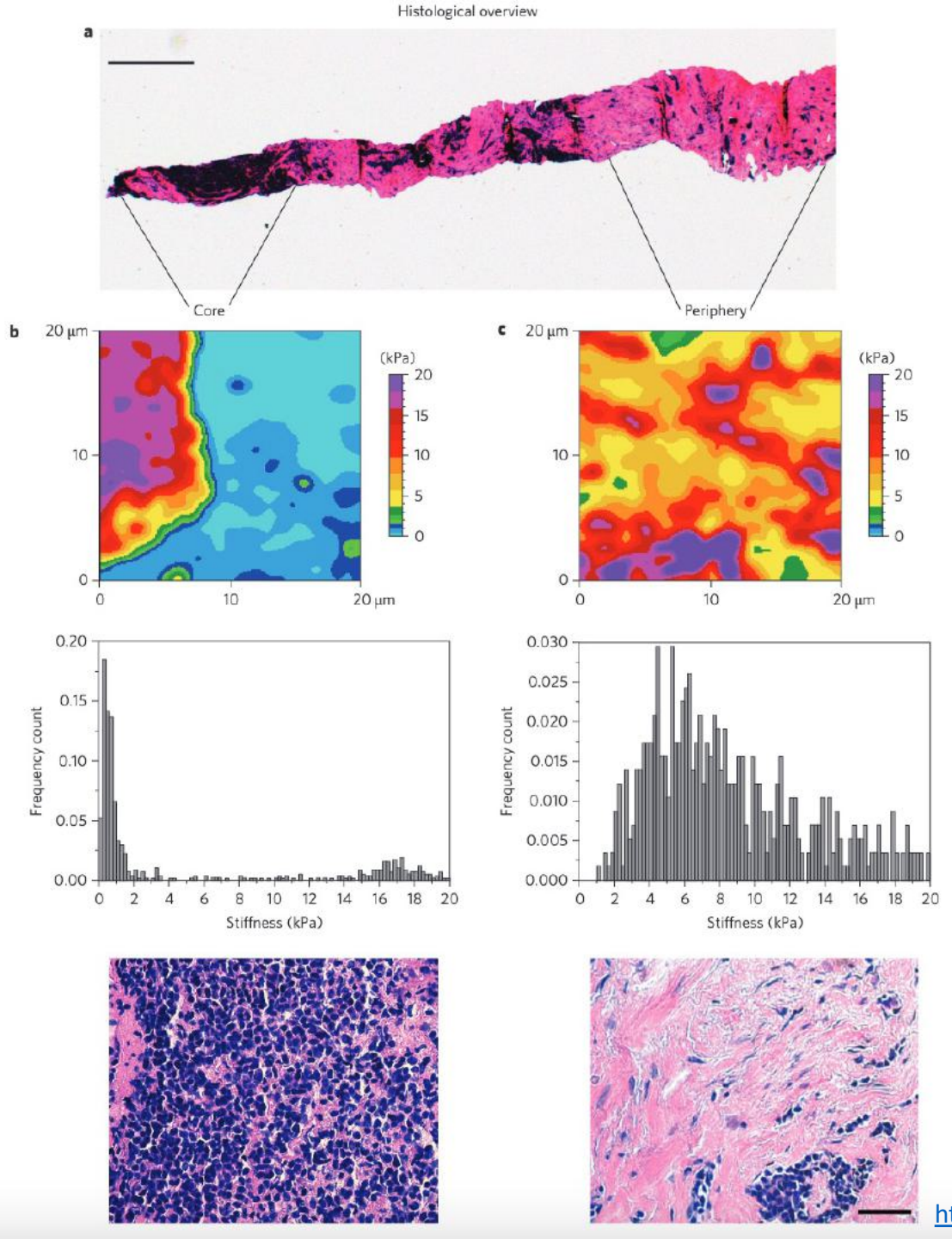
- Cancer cells are softer than normal cells, but the tumor is stiffer than normal tissue. Values range between 1-70 kPa
- Stiffening promotes tumor progression
- Caused by increased secretion and crosslinking of the extracellular matrix (by CAFs)
- Some cells can “remember” stiffness and keep on producing more collagen (=mechanical memory)
- Strain (solid stress) on collagen networks can increase their stiffness
- Sretch of the nucleus → phosphorylation of emerin → stiffening of the nucleus
- Two main signaling pathways are activated: (i) focal adhesion kinase (FAK) and (ii) the YAP/TAZ pathway

Nanomechanical signatures of normal; and cancerous human breast tissue



https://www.nature.com/articles/nnano.2012.167

Stiffness profile varies from core to periphery of a tumor



https://www.nature.com/articles/nnano.2012.167

Cells do not only respond to stiffness of their surrounding. Their own stiffness also matters

Are cancer cells really softer than normal cells?

Charlotte Alibert*†, Bruno Goud*† and Jean-Baptiste Manneville*†[1]

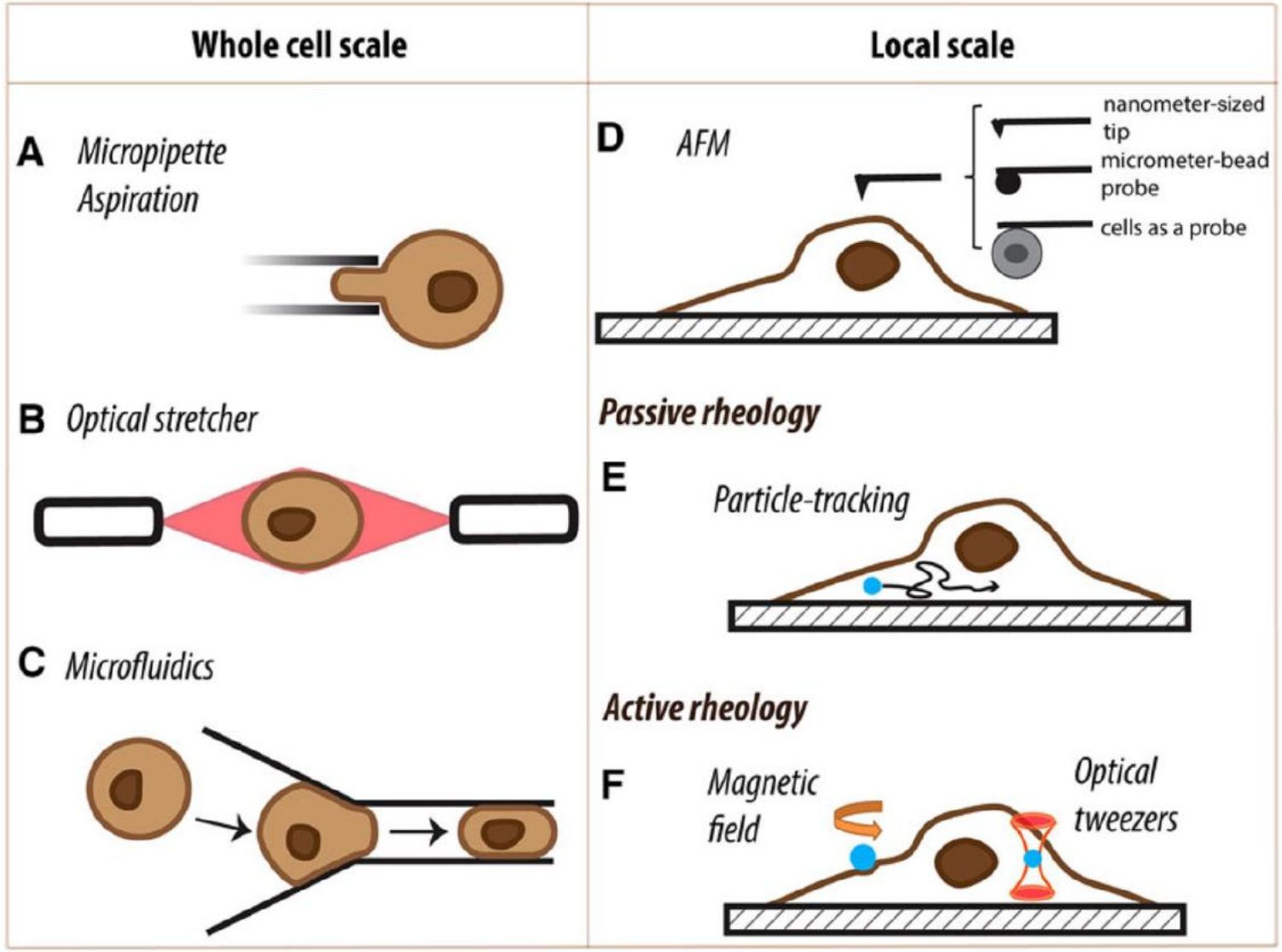
*Institut Curie, PSL Research University, CNRS, UMR 144, Paris, France and †Sorbonne Universités, UPMC University Paris 06, CNRS, UMR 144, Paris, France

https://pubmed.ncbi.nlm.nih.gov/28244605/

Cancer cells really appear to be softer than normal cells

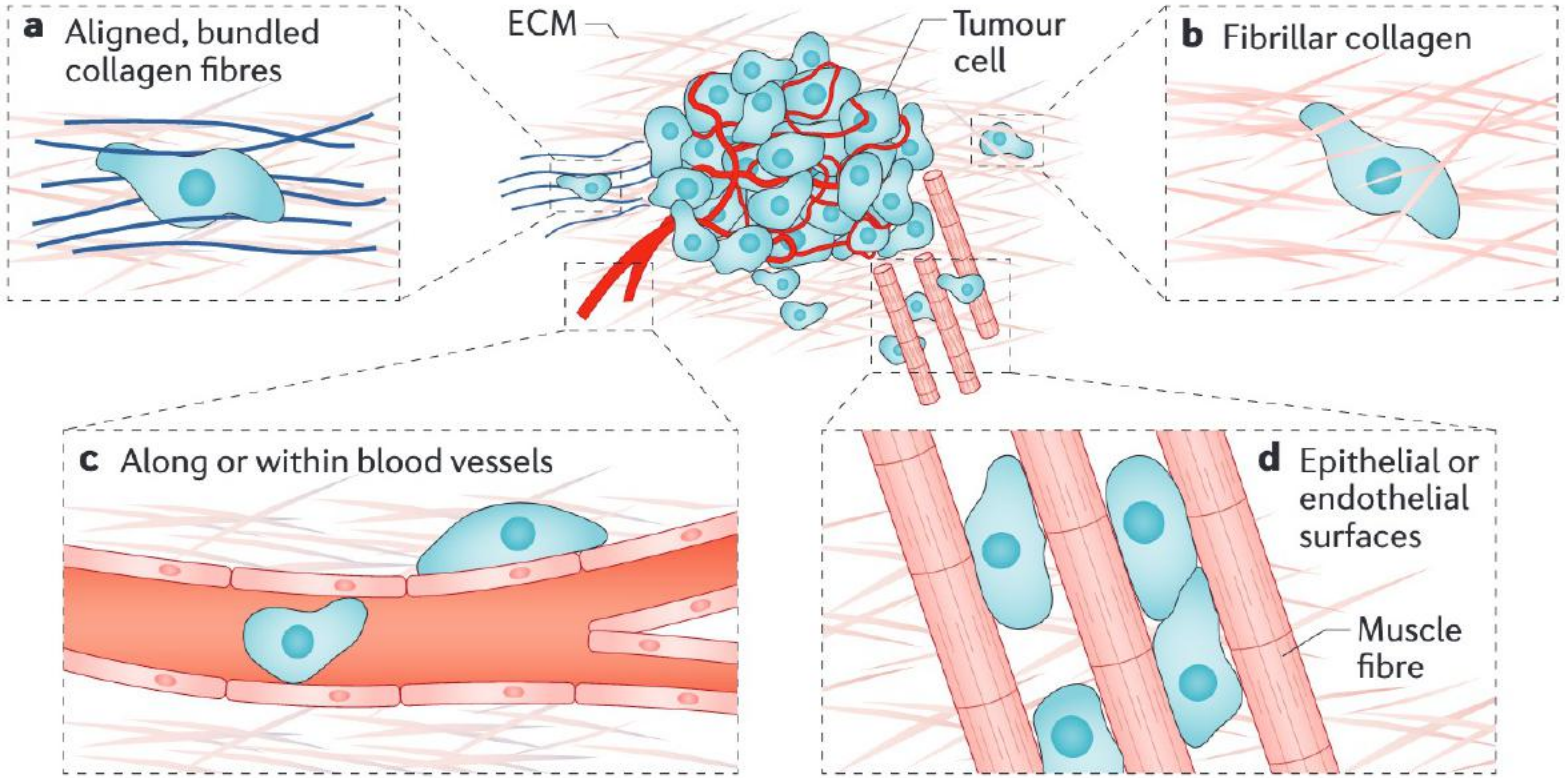
Palpation is often the first and simplest way to detect a tumour, for instance in breast tissues, before a more reliable diagnosis by biopsy. It is well accepted that tumour tissues, at the scale of the whole organ, are stiffer than their normal surrounding environment. Working with entire mammary gland, Levental et al. (2009) showed that the elastic modulus of the tissue indeed increases with tumorigenesis. However, at the level of the single cell, as discussed in section 'Why may cancer cells be softer than normal cells?', several

How to measure cell stiffness



https://pubmed.ncbi.nlm.nih.gov/28244605/

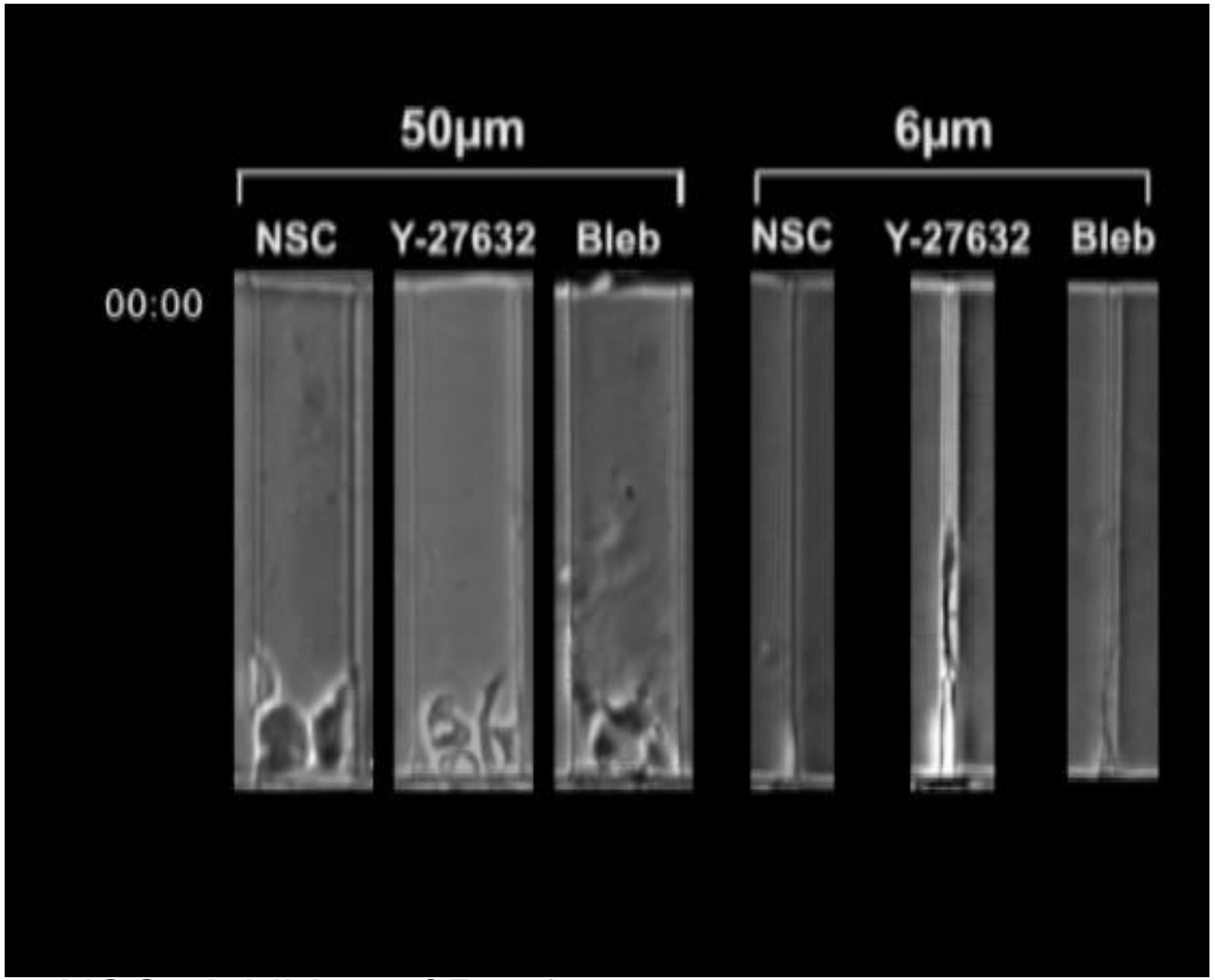
Cells encounter confined environments *in vivo*

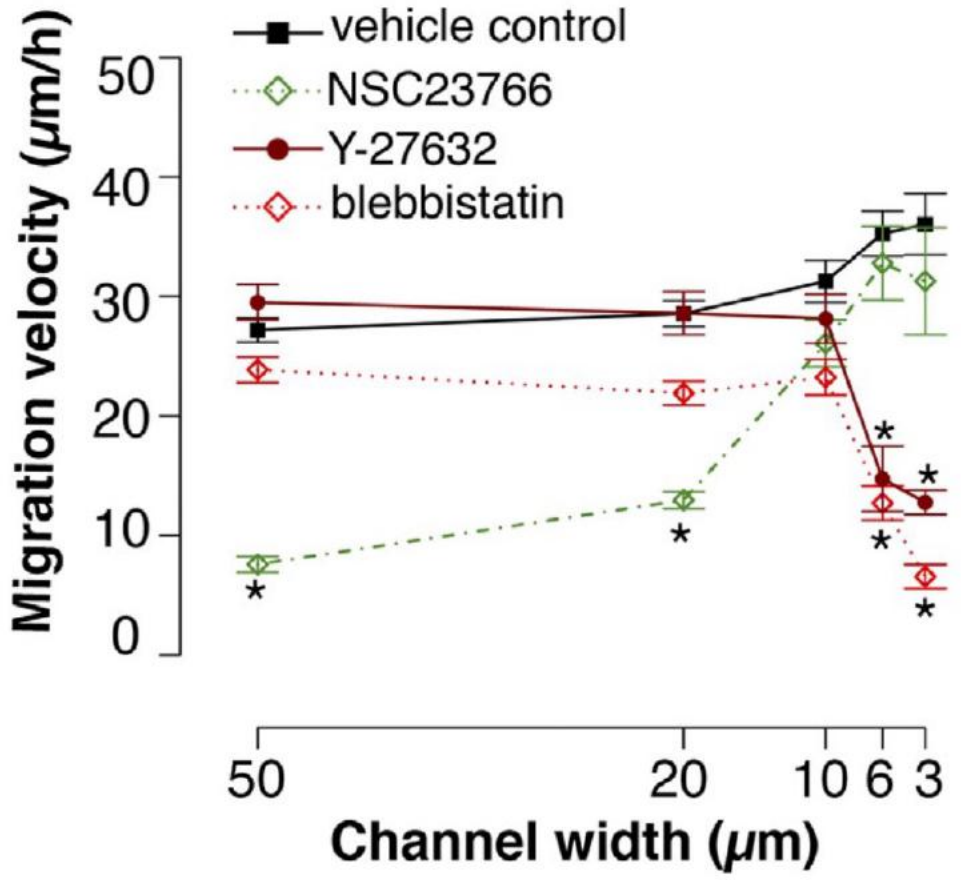


We know from intravital imaging that cells encounter “pores” or “channels” with diameters between 1-30 μm in diameter

https://www.nature.com/articles/nrc.2016.123

Confined vs. unconfined migration





https://pubmed.ncbi.nlm.nih.gov/23979717/

NSC= inhibitor of Rac1

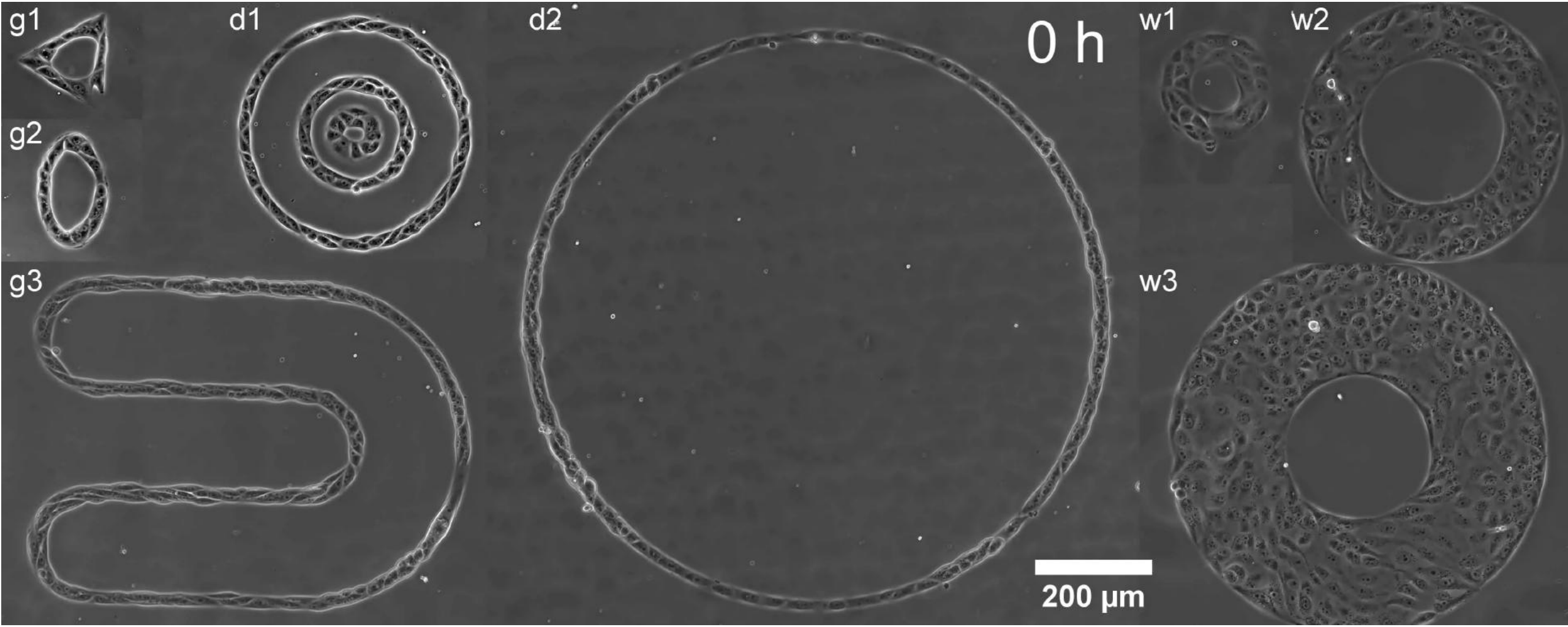
Y-27632 = inhibitor of ROCK = reduces contractility

Confined migration does not need Rac1, but rather contractility. Unconfined migration is the opposite.

Confined migration does not need Rac1 in channels

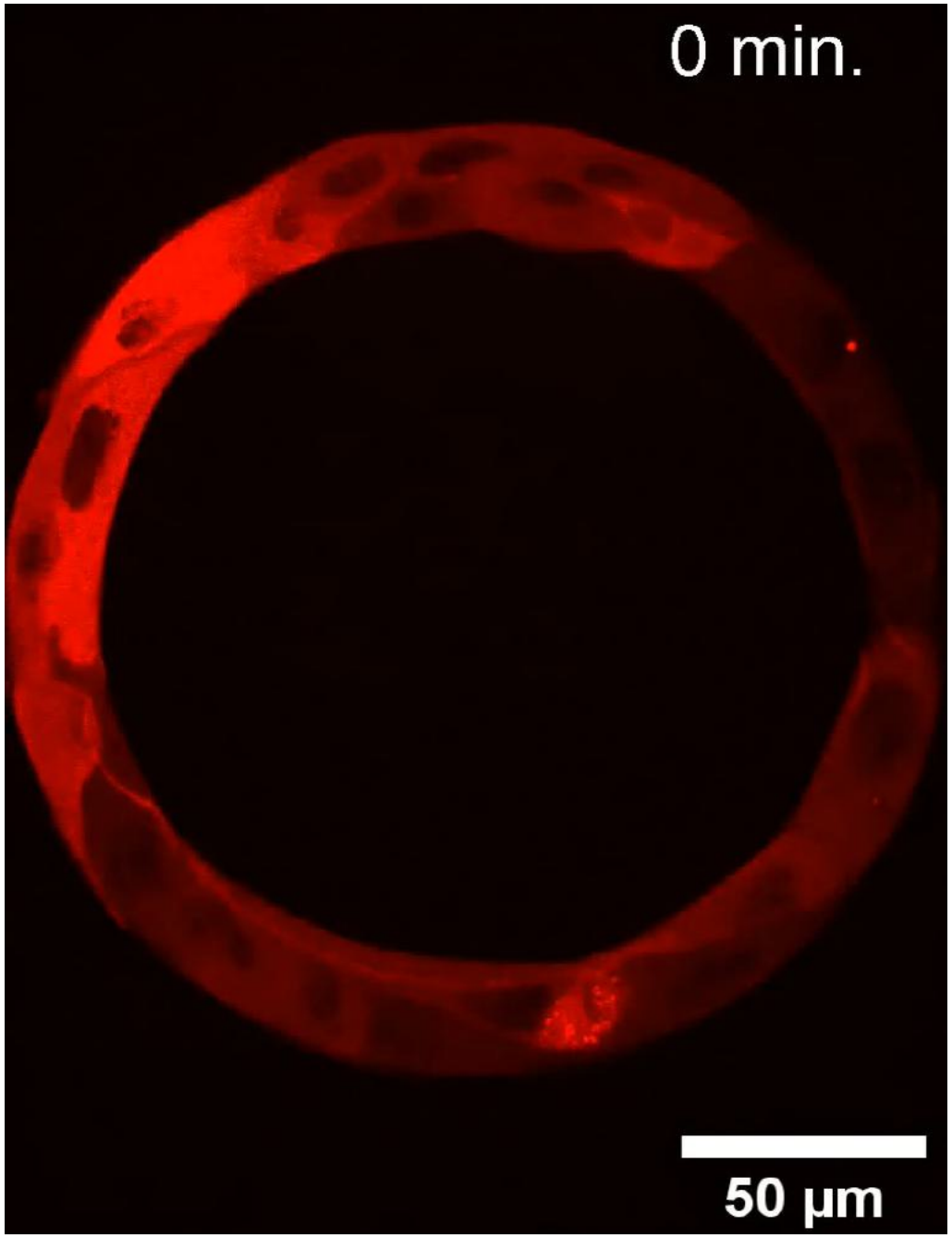
However, it is different in 2D confinemnet

2D confinement



https://pubmed.ncbi.nlm.nih.gov/32641972/

Inhibiting Rac polarization blocks the migration of cells



https://pubmed.ncbi.nlm.nih.gov/32641972/

Cells do not only respond to stiffness of their surrounding. Their own stiffness also matters

So far, we only talked about mechanosensing and mechanotransduction at the cell surface

What about intracellular organelles? Which organelle would you chose for your quest for intracellular mechanobiology?

ER

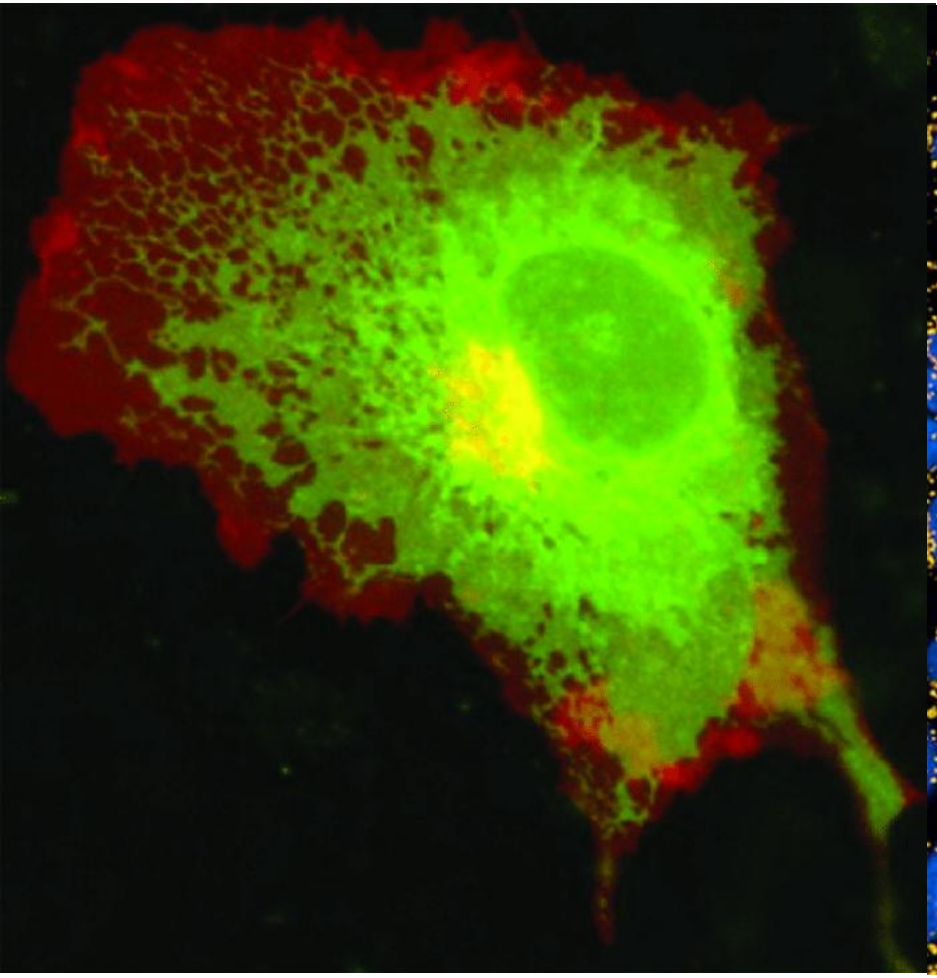
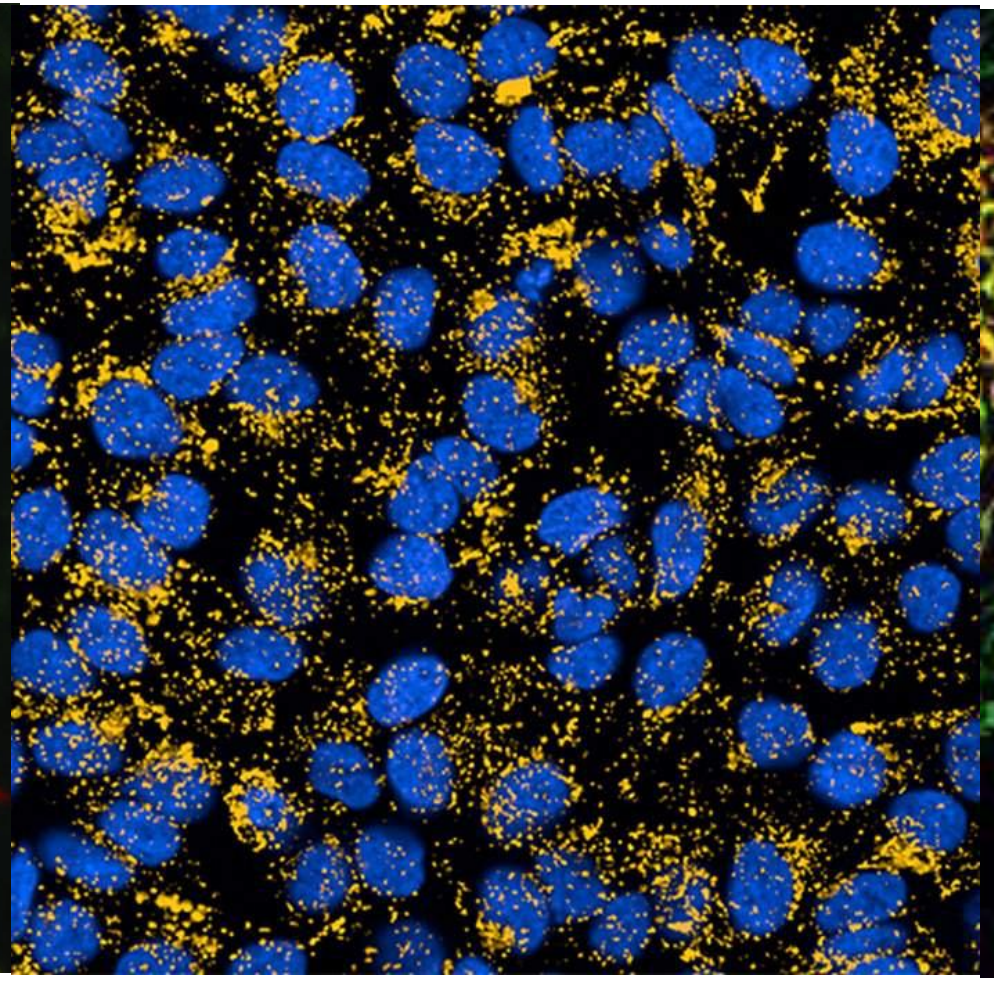


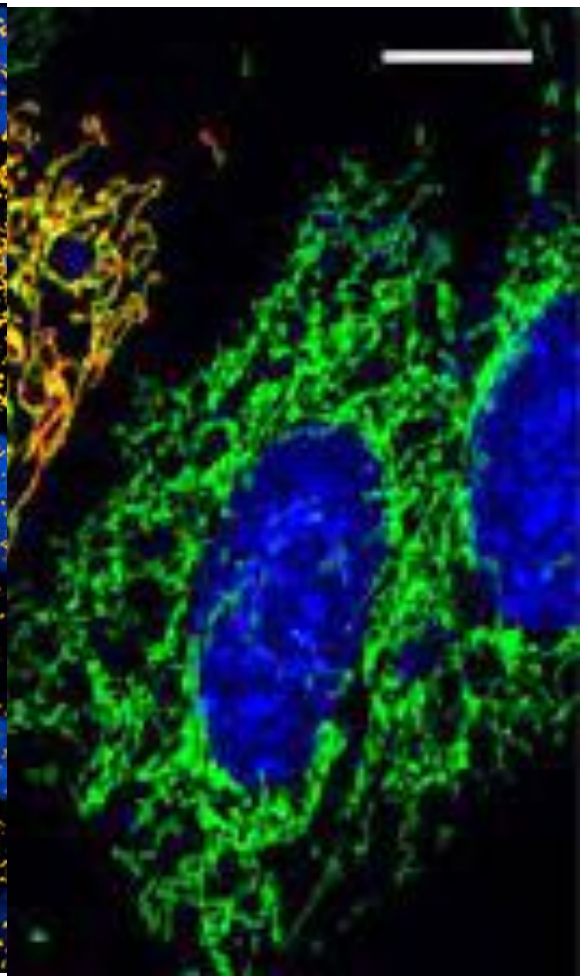
Image from K Hirschberg

Lysosomes



https://www.perkinelmer.com/

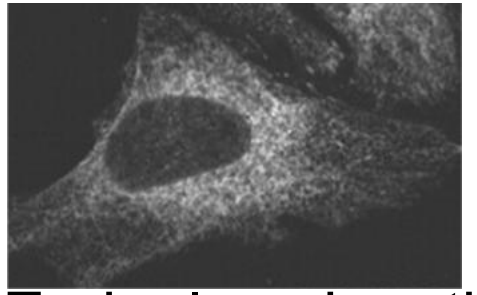
Mitochondria



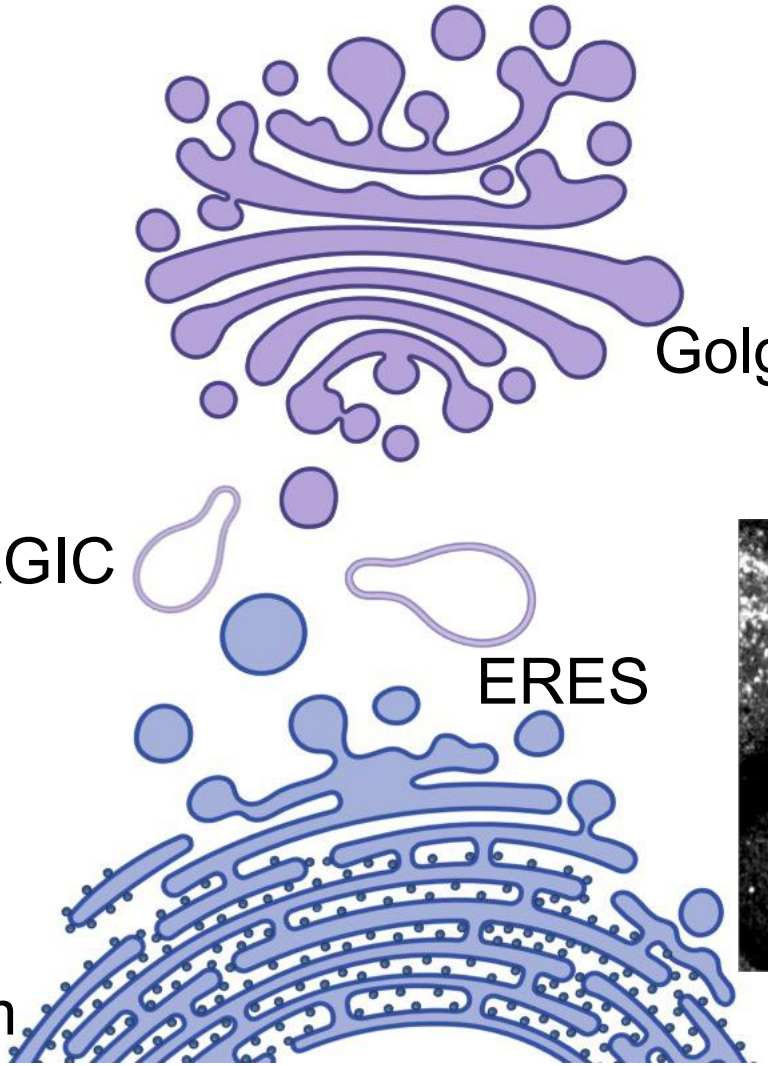
https://www.mpg.de/12309861/gene-therapy-mitochondrial-diseases

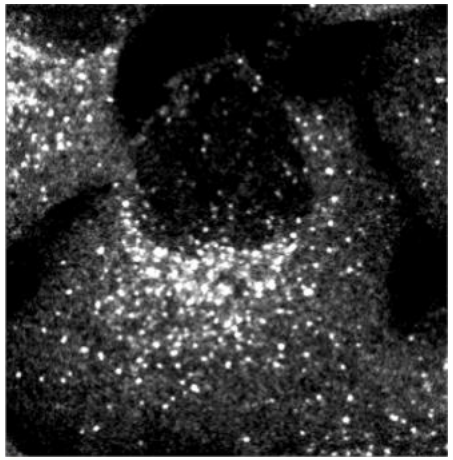
Mechanobiology is also linked to secretion

The secretory pathway

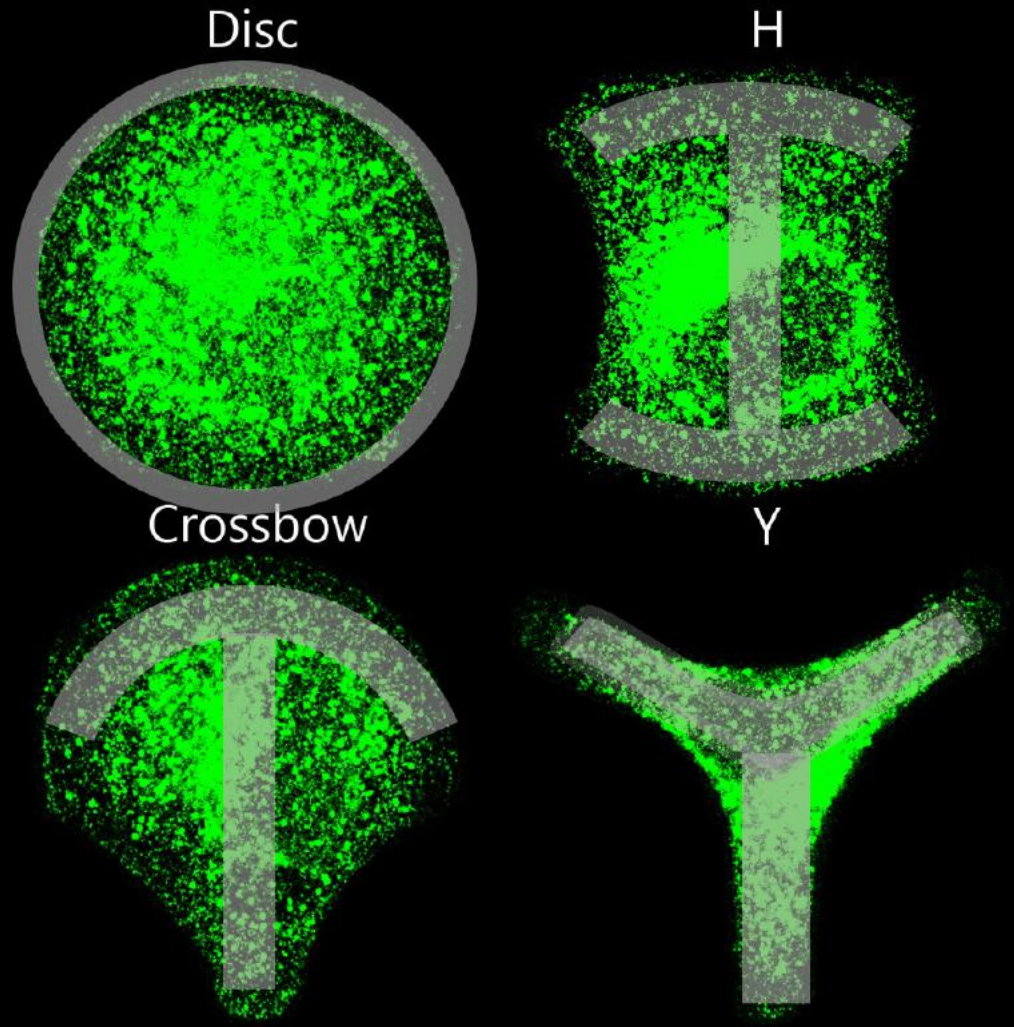


Endoplasmic reticulum

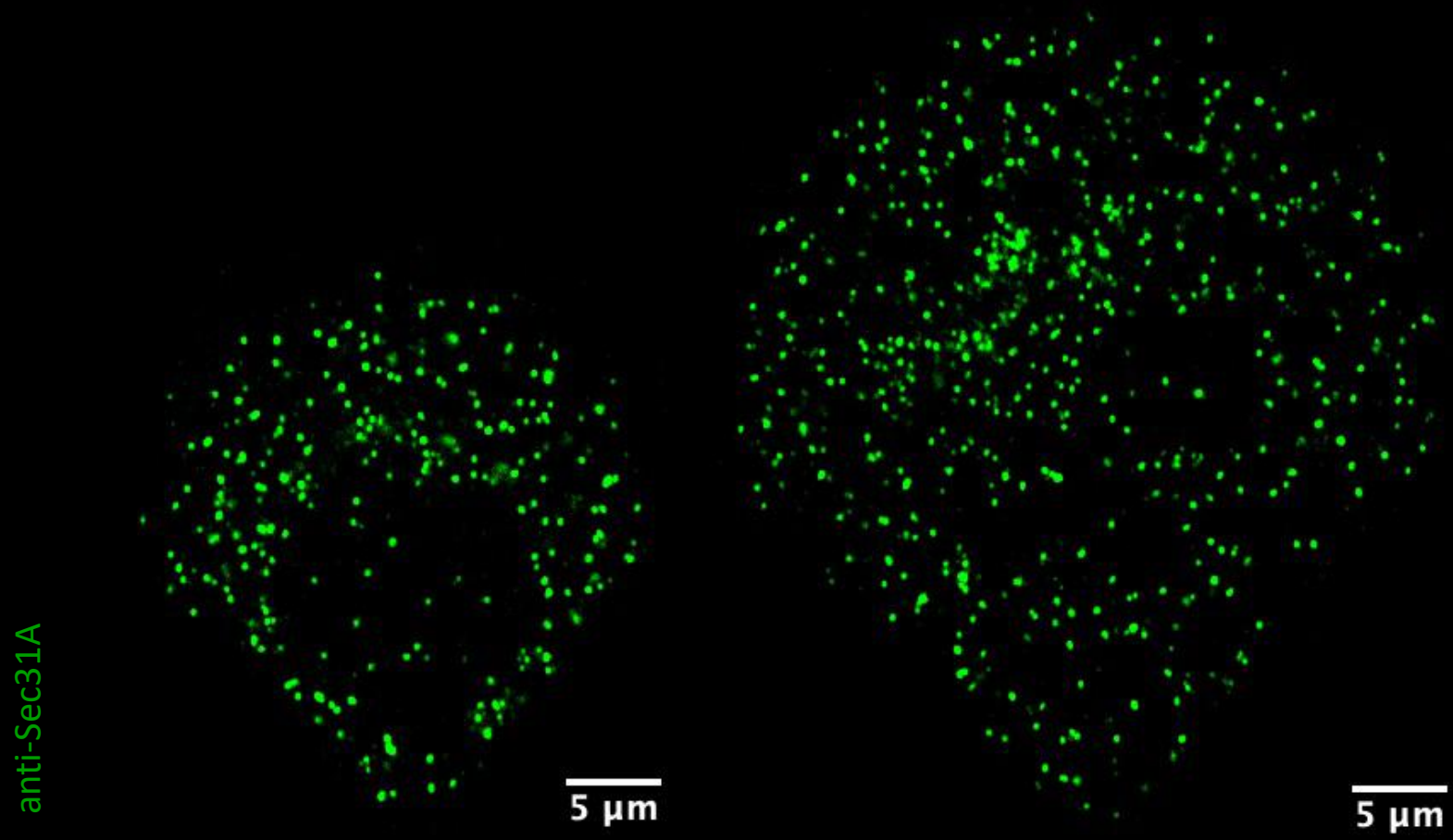


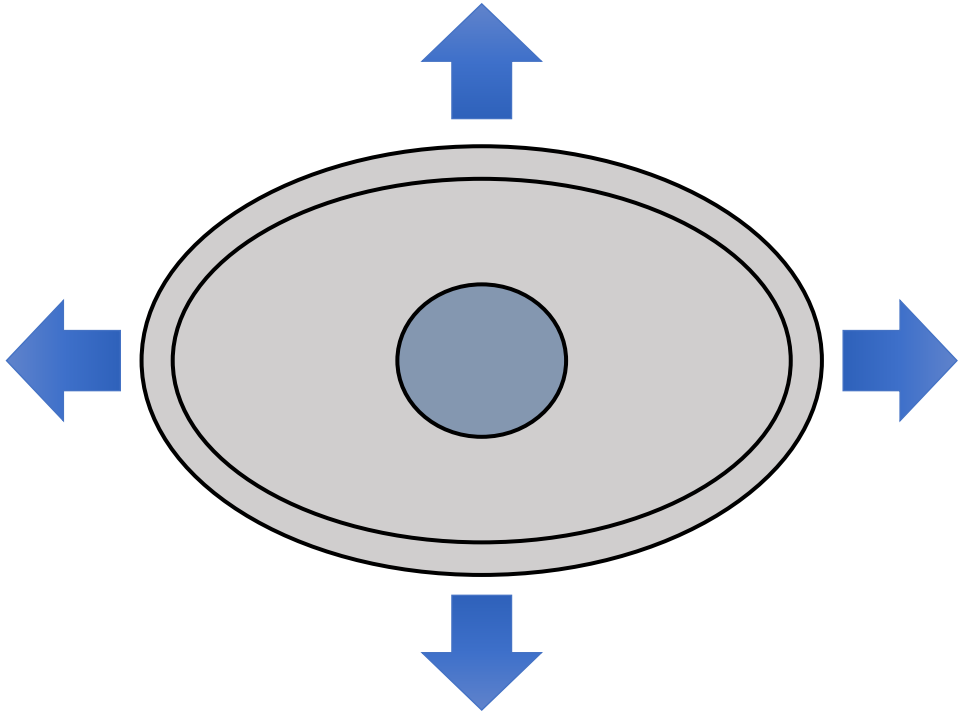


Cells on micropatterns



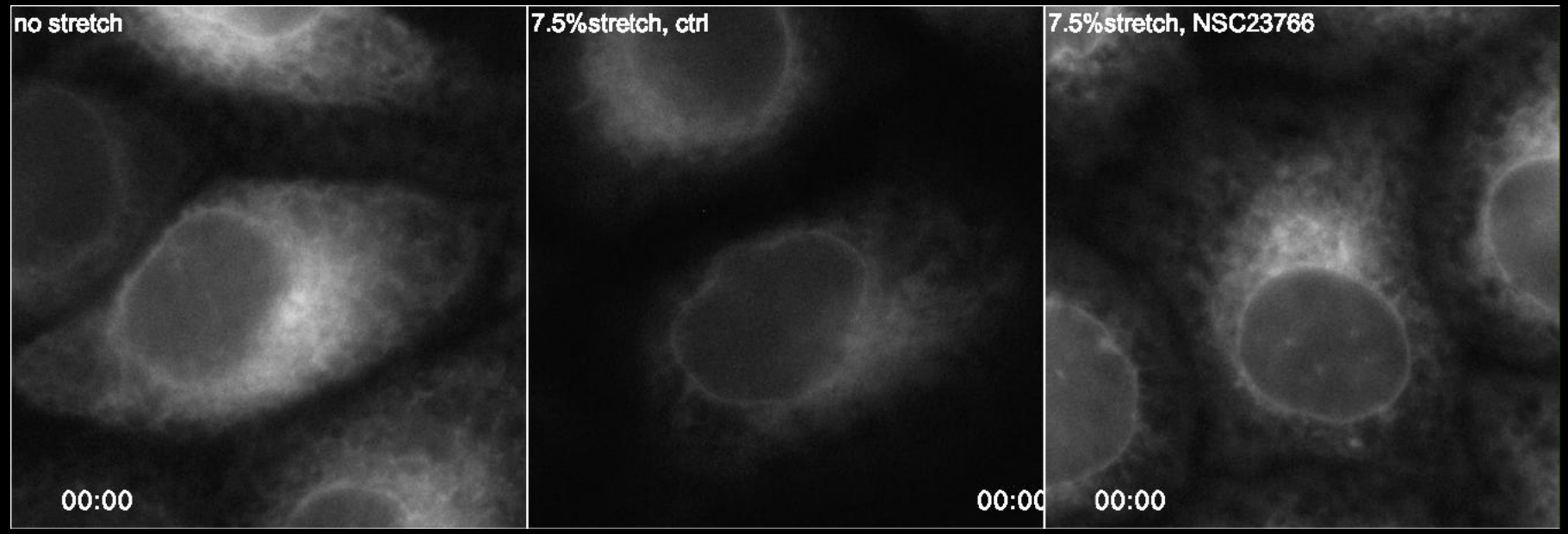
Forcing cells to grow on large pattens increases ERES



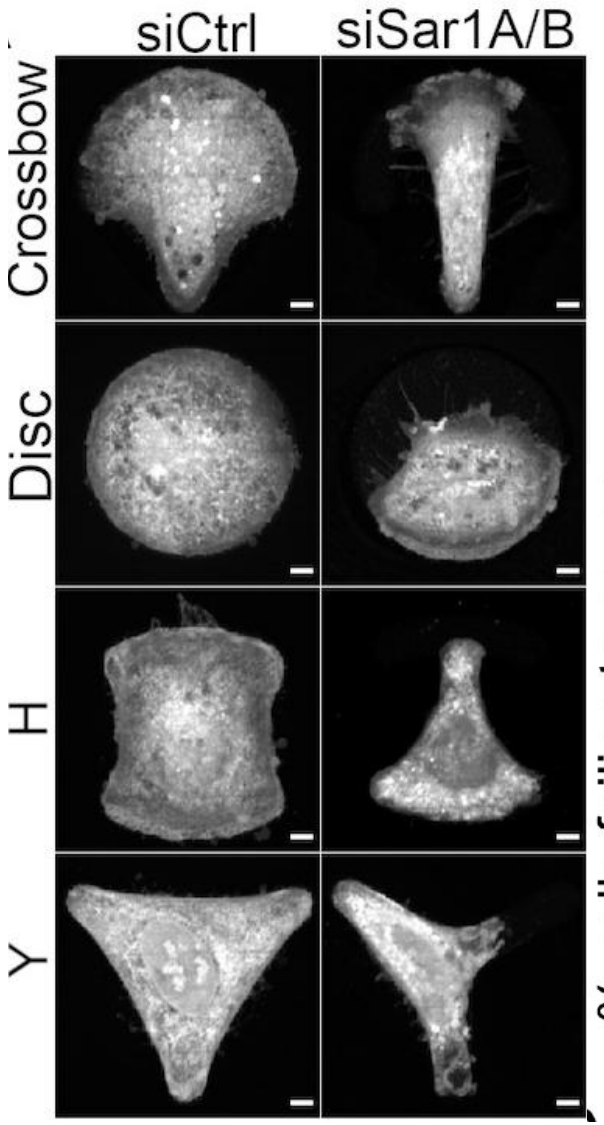


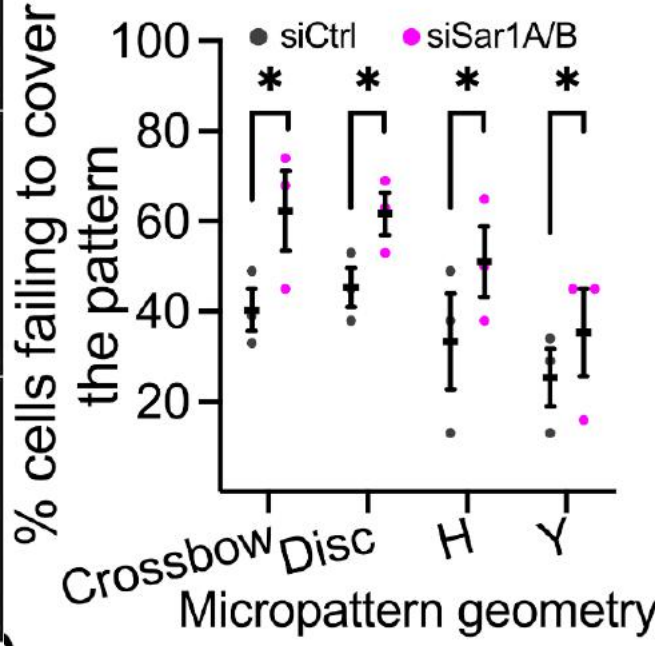
Mechanotension accelerates ER-Golgi transport in a Rac-dependent manner

RUSH assay

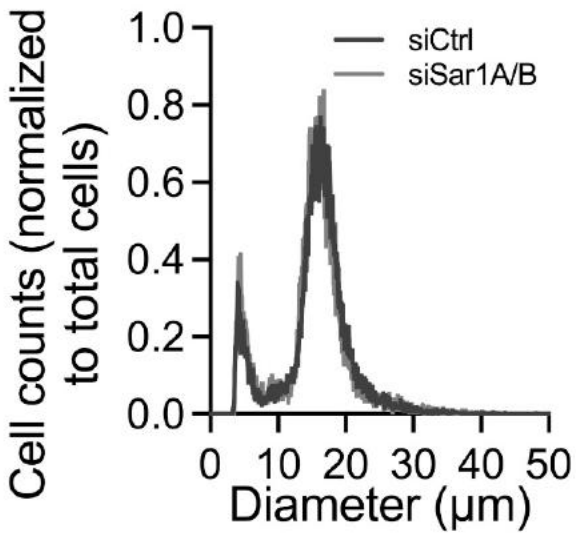


Blocking ERES function prevents cells from spreading on large micropatterns

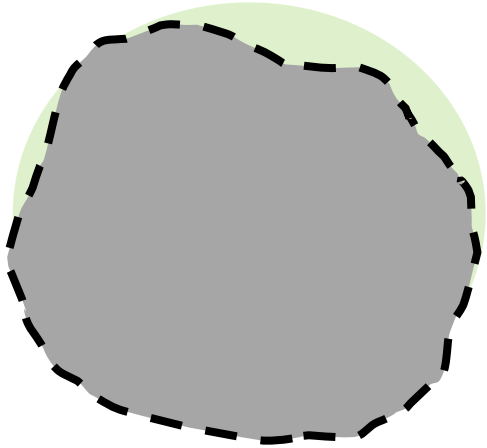


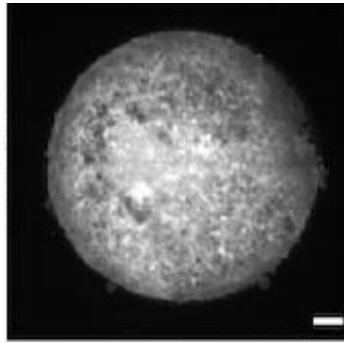


Not due to cell size difference

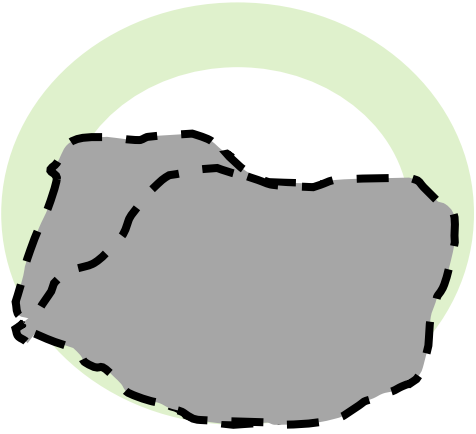


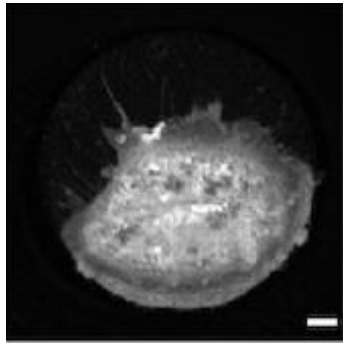
Control cell



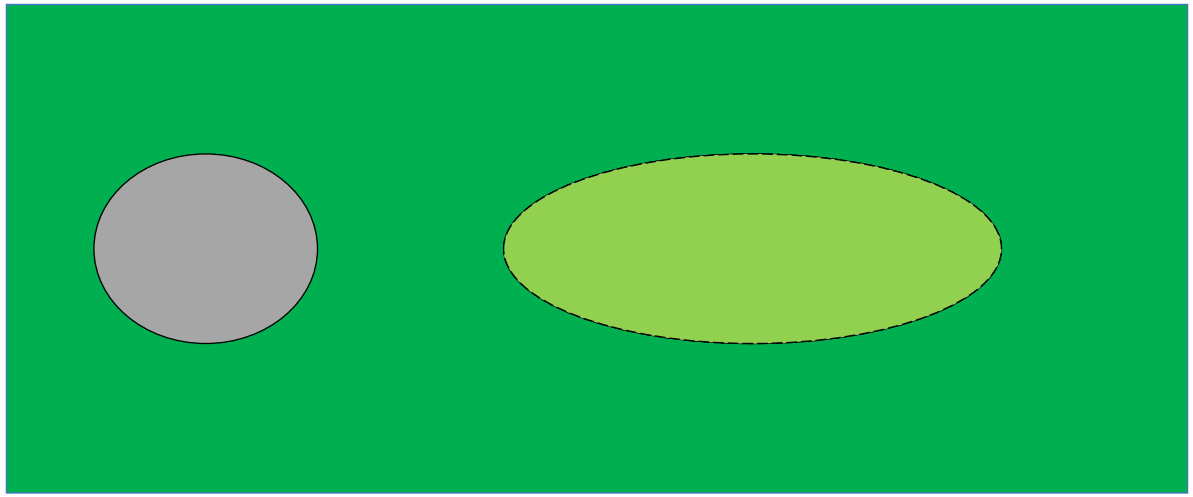


Cell with disrupted ERES function

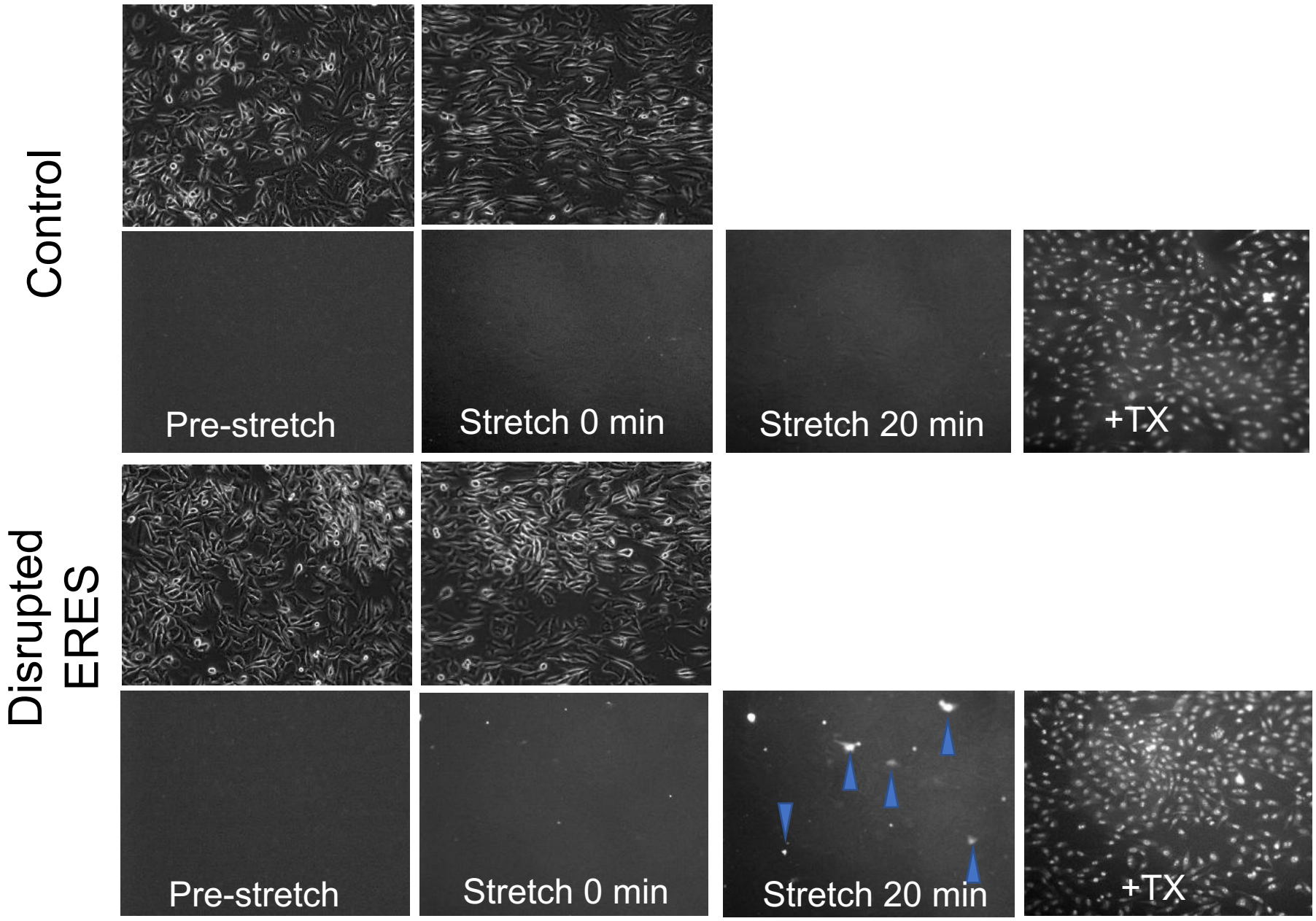




Assay for membrane integrity



ERES function confers resistance to mechanical stress




est. 1982
40 Years
THE EMBO JOURNAL
Volume 41 Issue 18 | 15 September 2022
ERES
ERES
Sar1
Rac1
Mechanical control of ER exit sites
Rac1
Sar1
Illustration by Sandra Krahl
EMBOpress

Is confinement only sensed at the plasma membrane?

RESEARCH ARTICLE SUMMARY

CELL BIOLOGY

The nucleus acts as a ruler tailoring cell responses to spatial constraints

A. J. Lomakin*†‡, C. J. Cattin†, D. Cuvelier§, Z. Alraies§, M. Molina, G. P. F. Nader, N. Srivastava, P. J. Sáez, J. M. Garcia-Arcos, I. Y. Zhitnyak, A. Bhargava, M. K. Driscoll, E. S. Welf, R. Fiolka, R. J. Petrie, N. S. De Silva, J. M. González-Granado, N. Manel, A. M. Lennon-Duménil, D. J. Müller*, M. Piel*‡

RESEARCH ARTICLE SUMMARY

CELL BIOLOGY

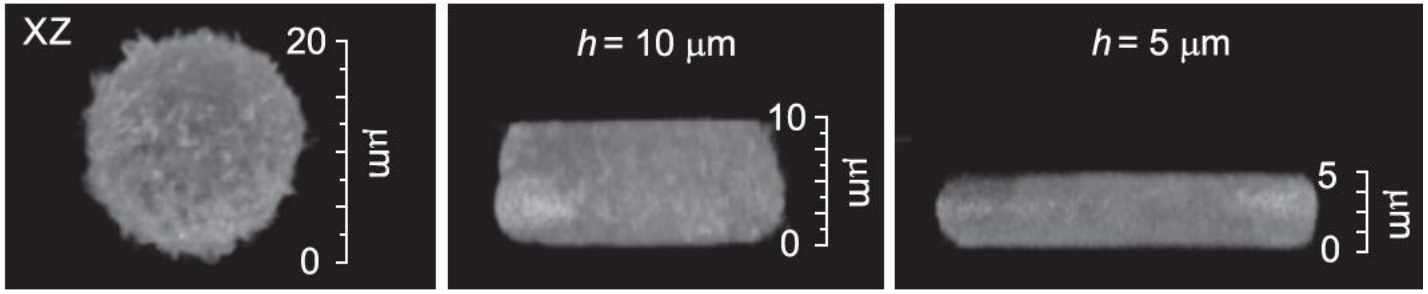
The nucleus measures shape changes for cellular proprioception to control dynamic cell behavior

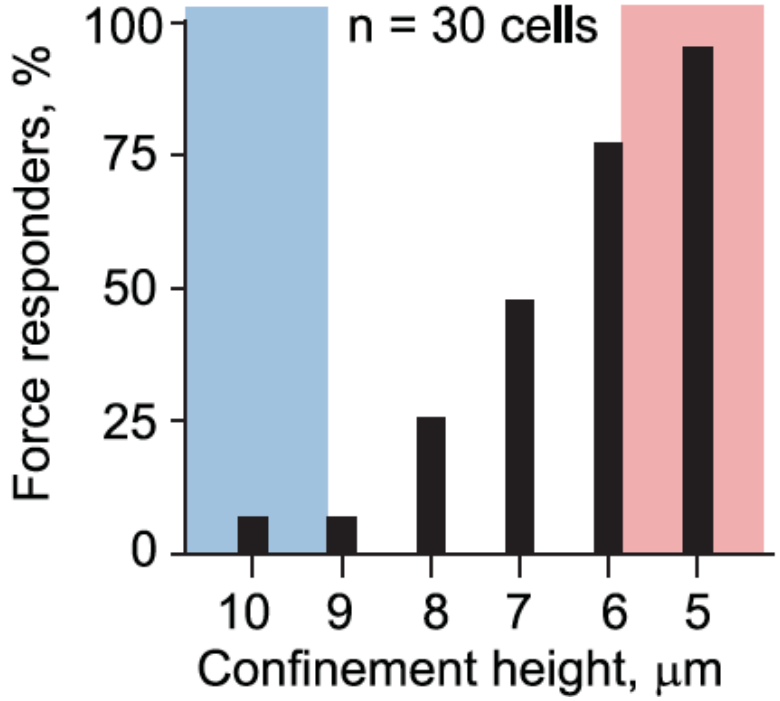
Valeria Venturini, Fabio Pezzano, Frederic Català Castro, Hanna-Maria Häkkinen, Senda Jiménez-Delgado, Mariona Colomer-Rosell, Monica Marro, Queralt Tolosa-Ramon, Sonia Paz-López, Miguel A. Valverde, Julian Weghuber, Pablo Loza-Alvarez, Michael Krieg, Stefan Wieser*, Verena Ruprecht*

https://pubmed.ncbi.nlm.nih.gov/33060332/

https://pubmed.ncbi.nlm.nih.gov/33060331/

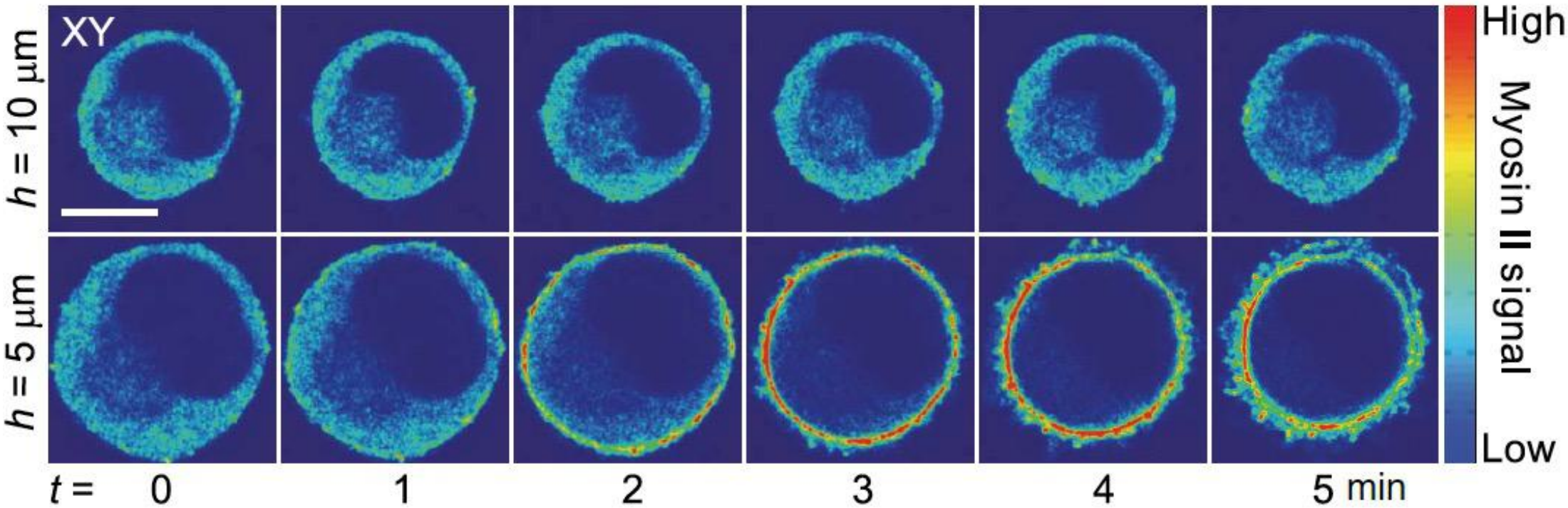
Response of cells to confinement (squeezing)



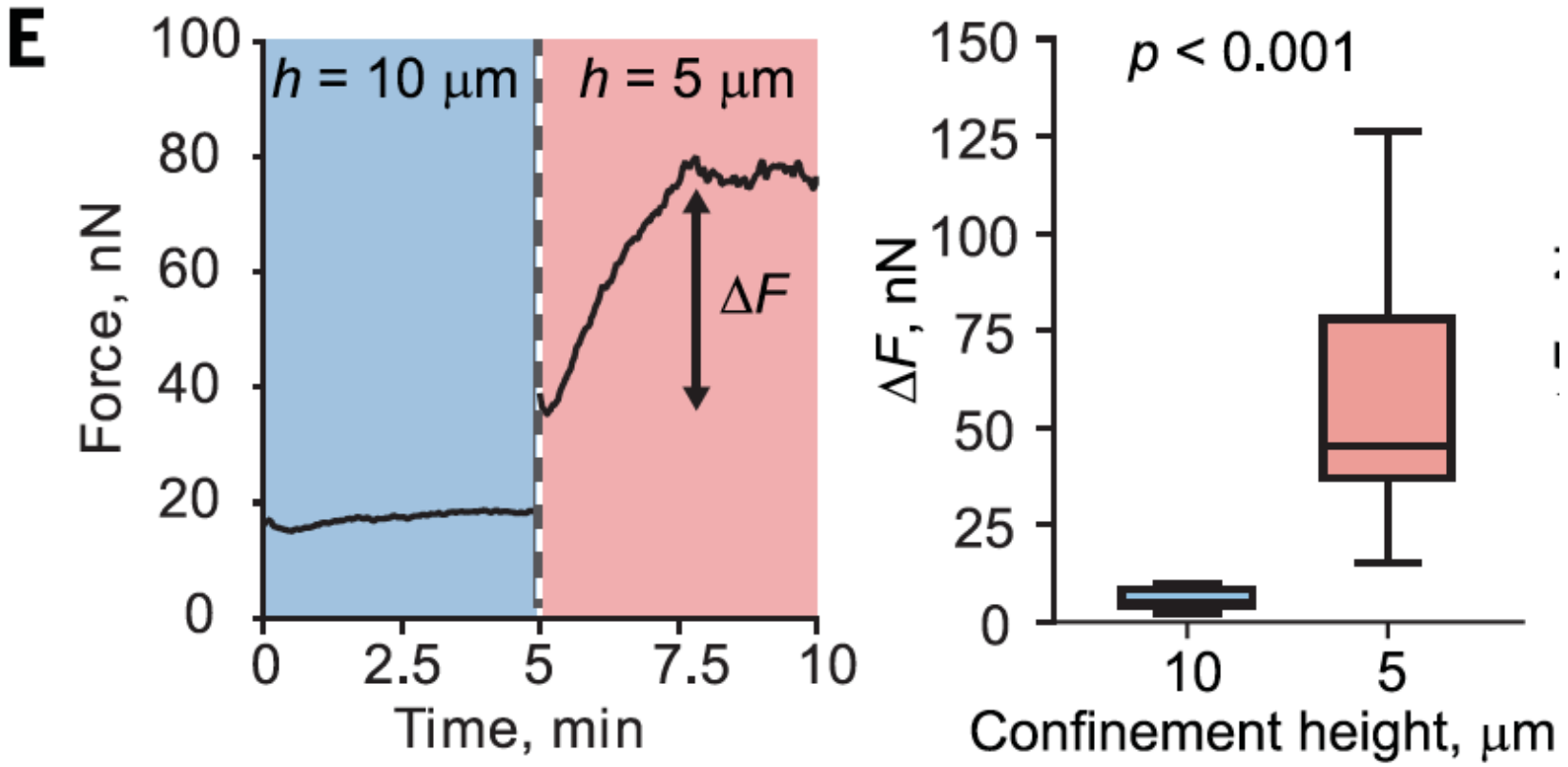


Almost all cells respond to a confinement of 5 μm

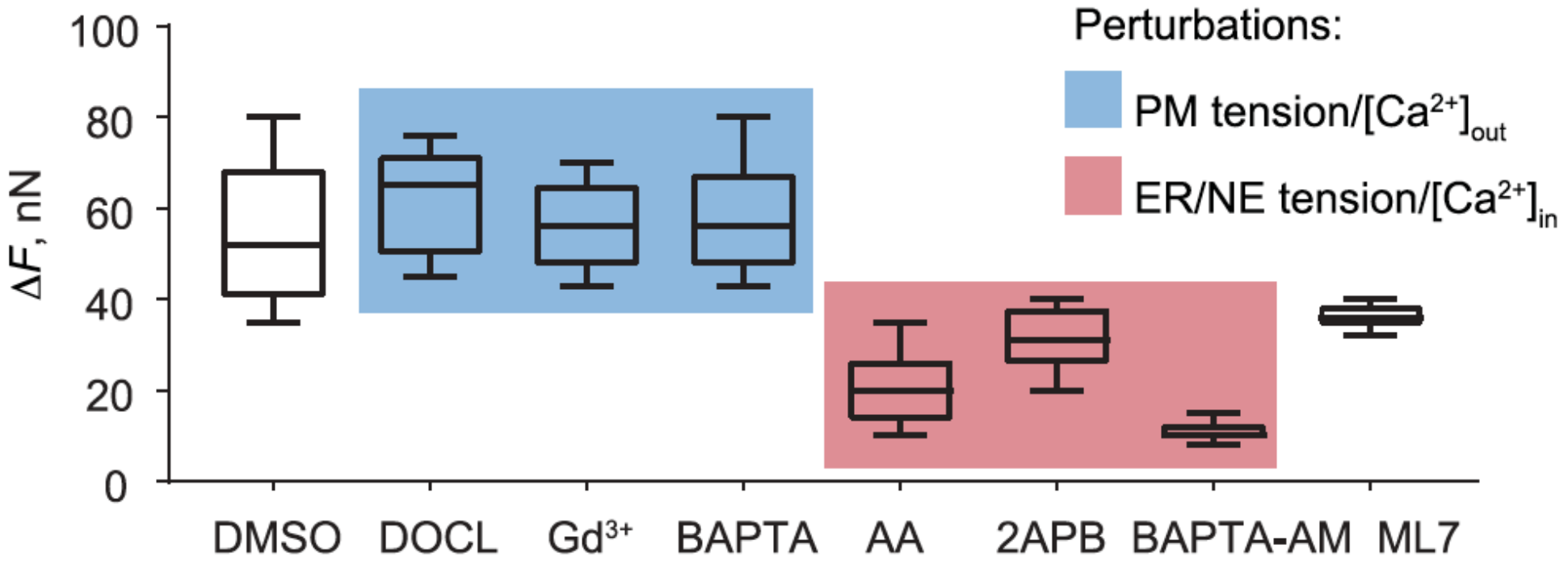
Rapid myosin recruitment to the cell surface upon squeezing



Rapid myosin recruitment to the cell surface upon squeezing → Force-response curve

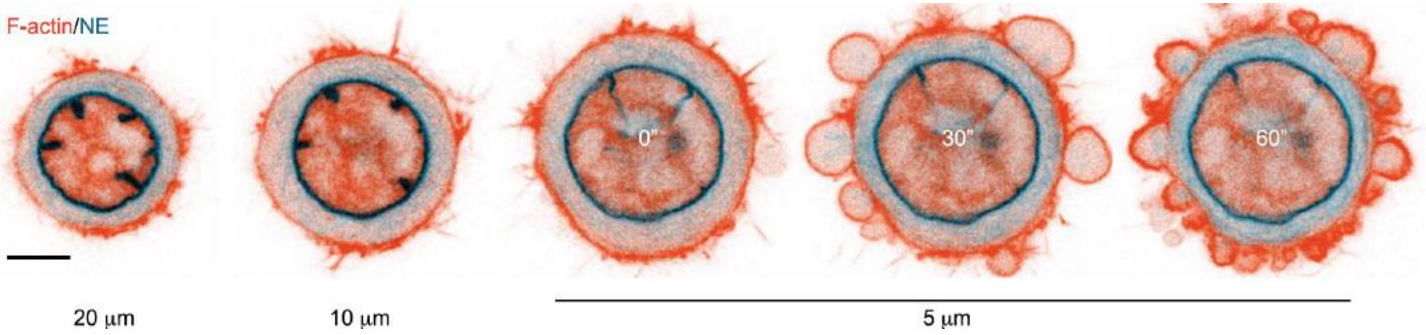


Via a pharmacological screen they identified the ER/NE as relevant cellular compartments

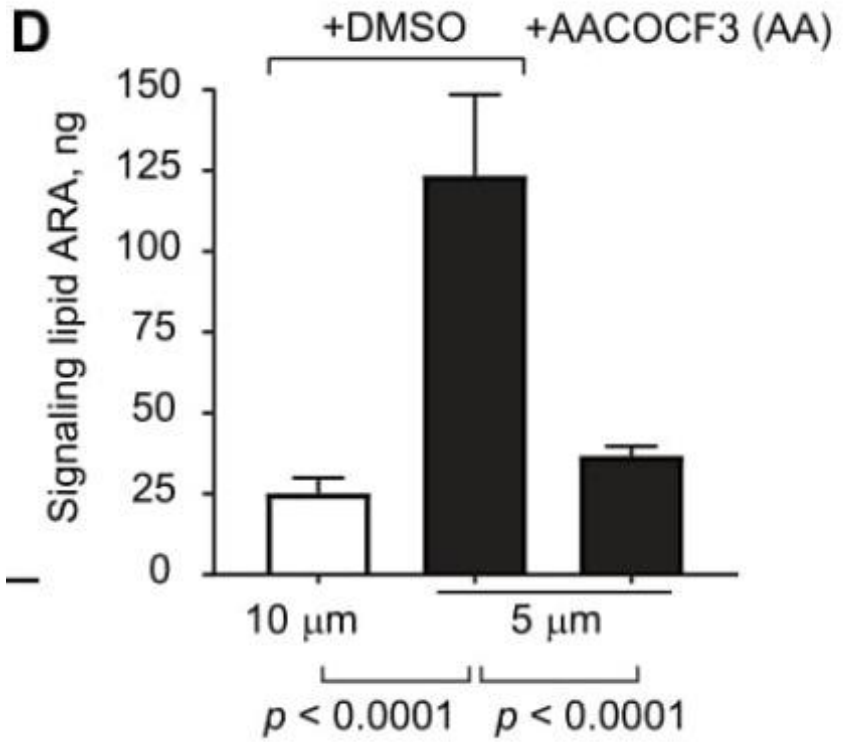


DOCL= deoxycholate. Reduces plasma membrane tension
Gd= gadolinium (III) cholride. Inhibits mechanosensitive plasma membrane channels
BAPTA= chaleates calcium extracellularly (-AM is for intracellular calcium)
AA= AACOCF3. Inhibits cPLA2
2APB= Xestospongin. Inhibits stretch-activated IP3 receptors in the ER

No evidence for nuclear rupture

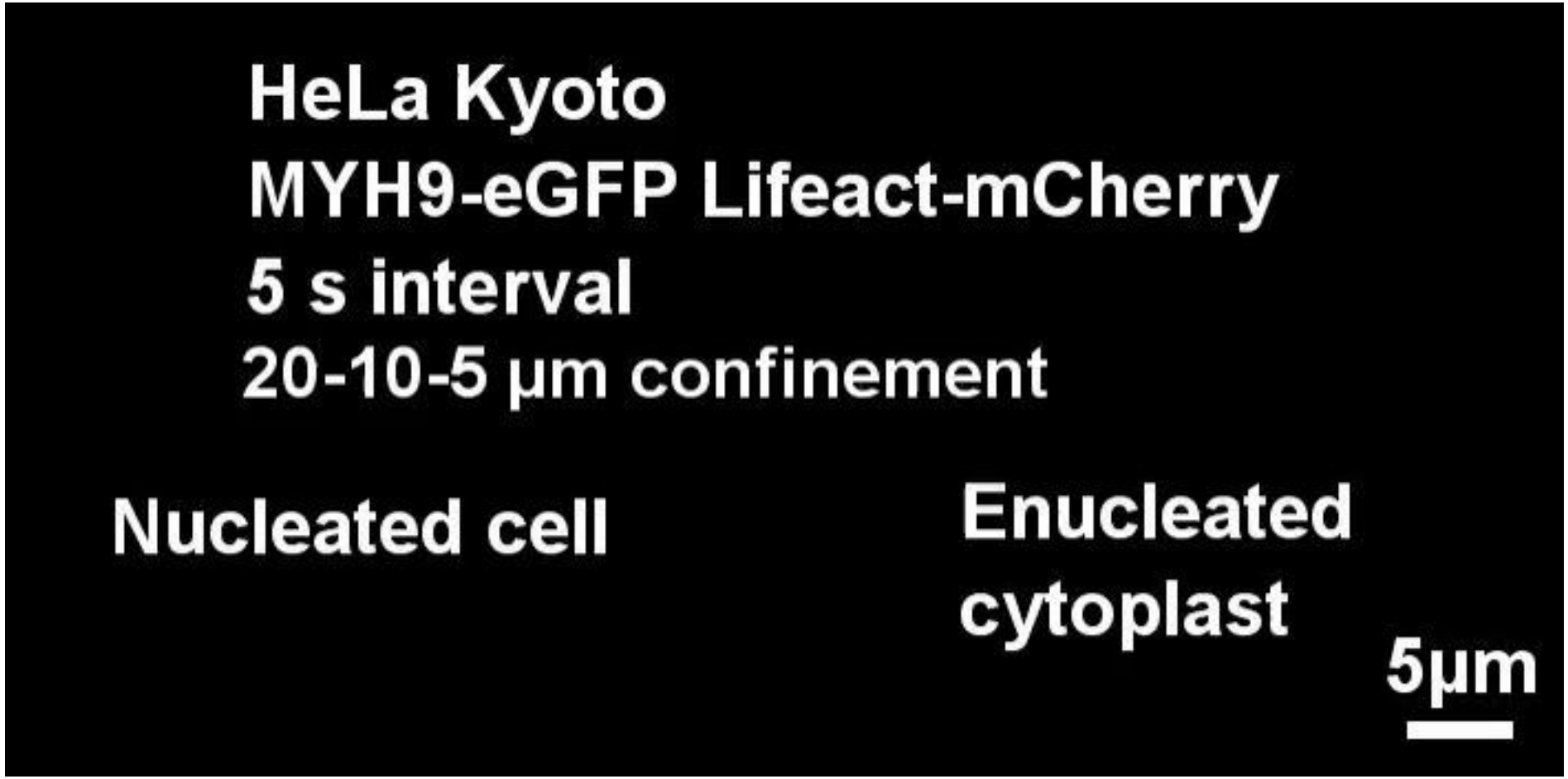


Confinement induces the production of arachidonic acid → cPLA2 is activated



AACOCF3= inhibitor of cPLA2

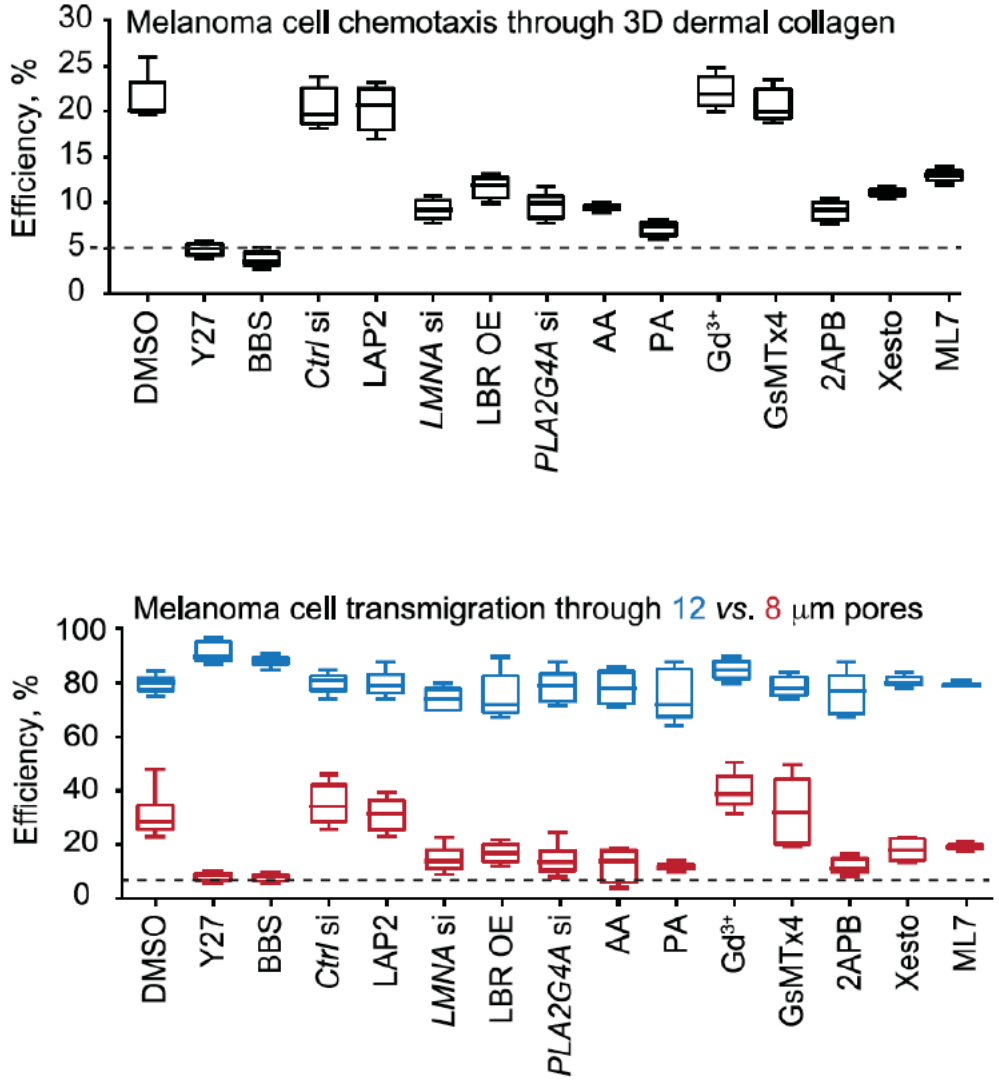
Confinement-induced membrane blebbing requires the presence of the nucleus

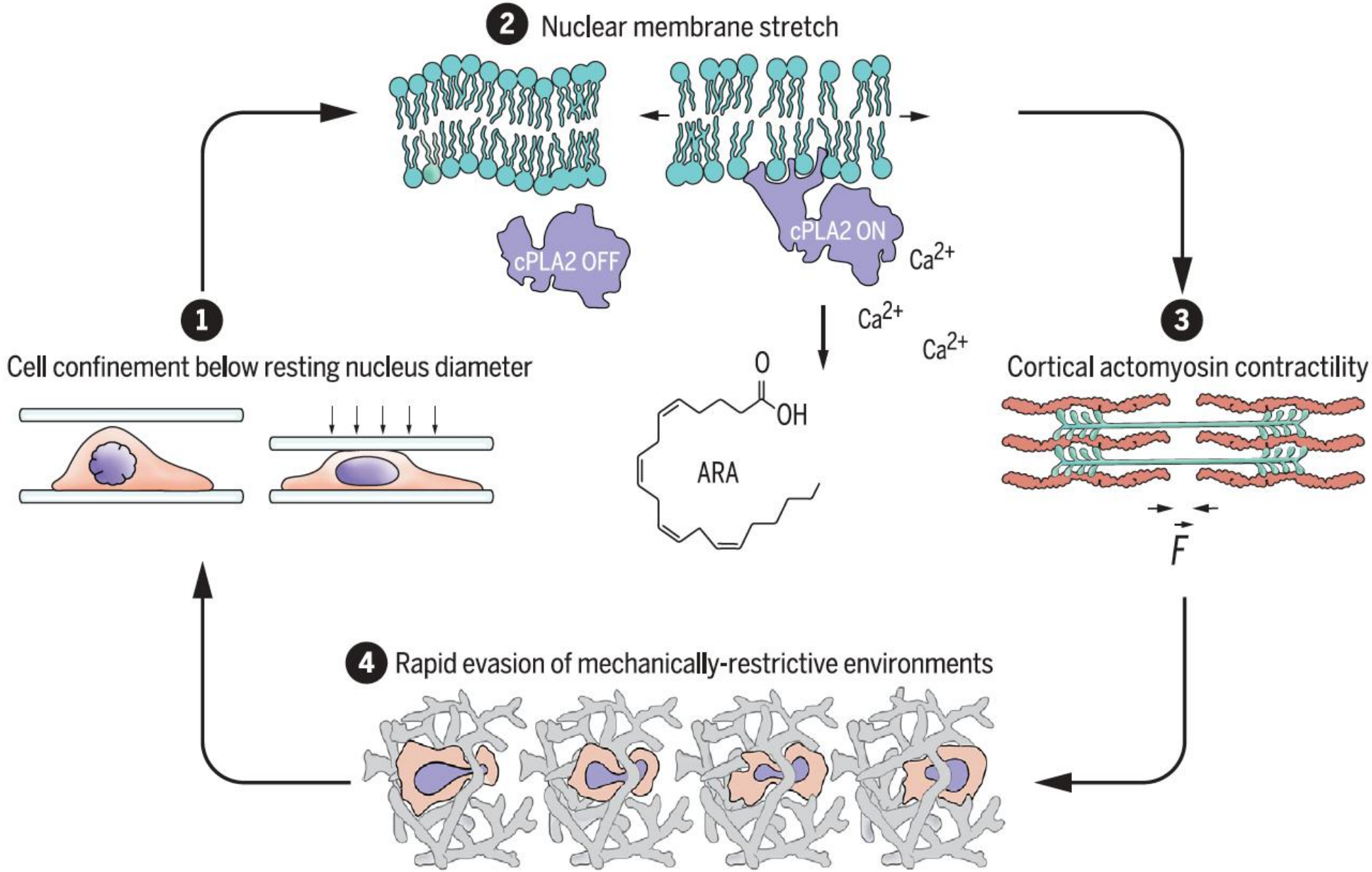


Confinement-induced membrane blebbing requires the presence of the nucleus

Confinement-induced migration of DCs is dependent on cPLA2

Nuclear mechanosensing is relevant for cancer cell migration




2 Nuclear membrane stretch
cPLA2 OFF
cPLA2 ON
Ca^{2+}
Ca^{2+}
Ca^{2+}
1
Cell confinement below resting nucleus diameter
O
OH
ARA
3
Cortical actomyosin contractility
F
4 Rapid evasion of mechanically-restrictive environments

Thank you for your attention

Mechanobiology can be pretty "forceful" when it comes to humor!

Why do mechanobiologists go to Yoga courses?
•They love stretching exercises.

Why don’t cells work well on Mondays?
•They’re still adjusting to the mechanical load from the weekend.

What did the actin filament say to the myosin motor?
•"You complete me—together, we’ve got a lot of force to generate!"

Why do cells refuse to attend the mechanobiology conferences?
•They cannot handle the stress!