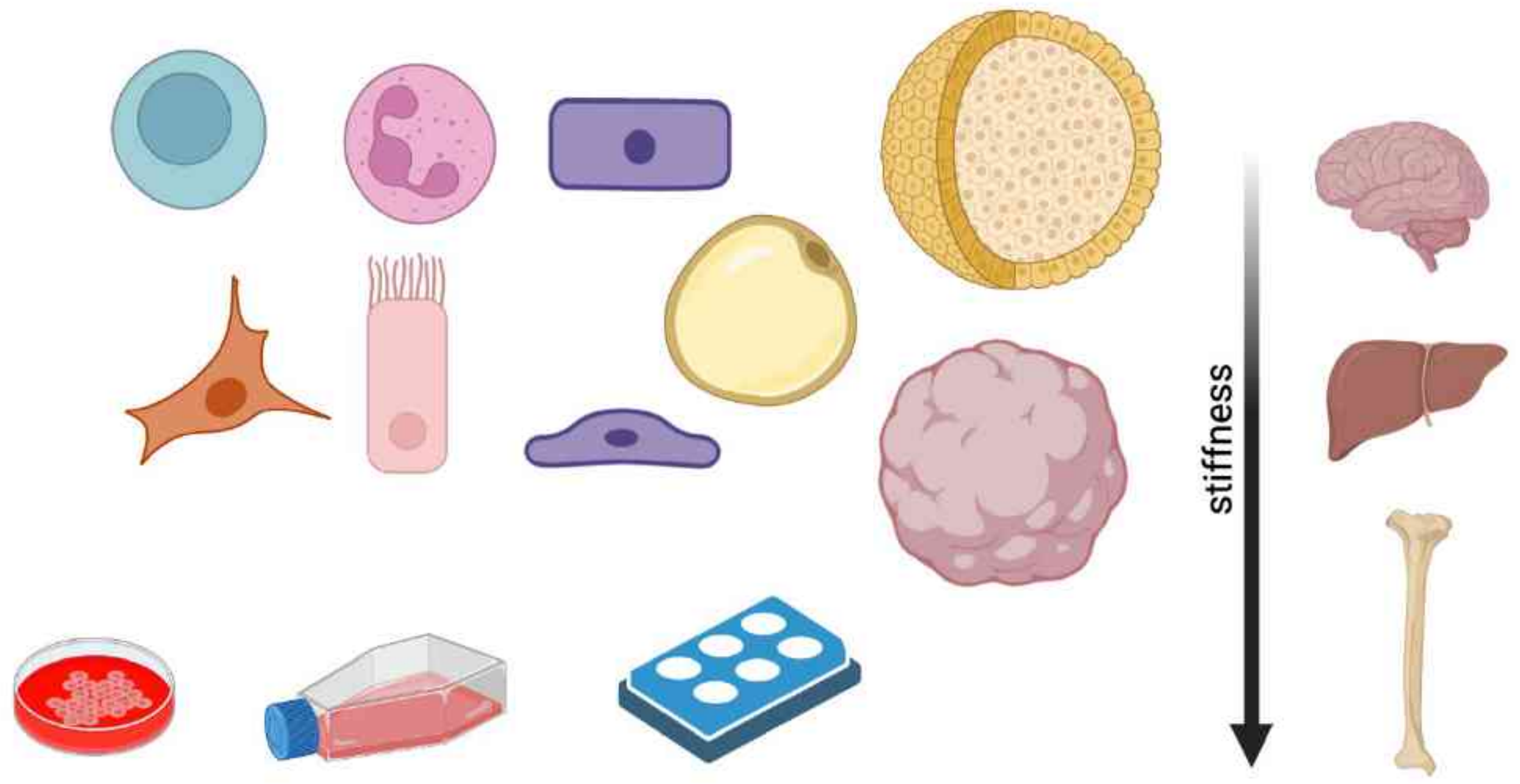
Introduction into Mechanobiology

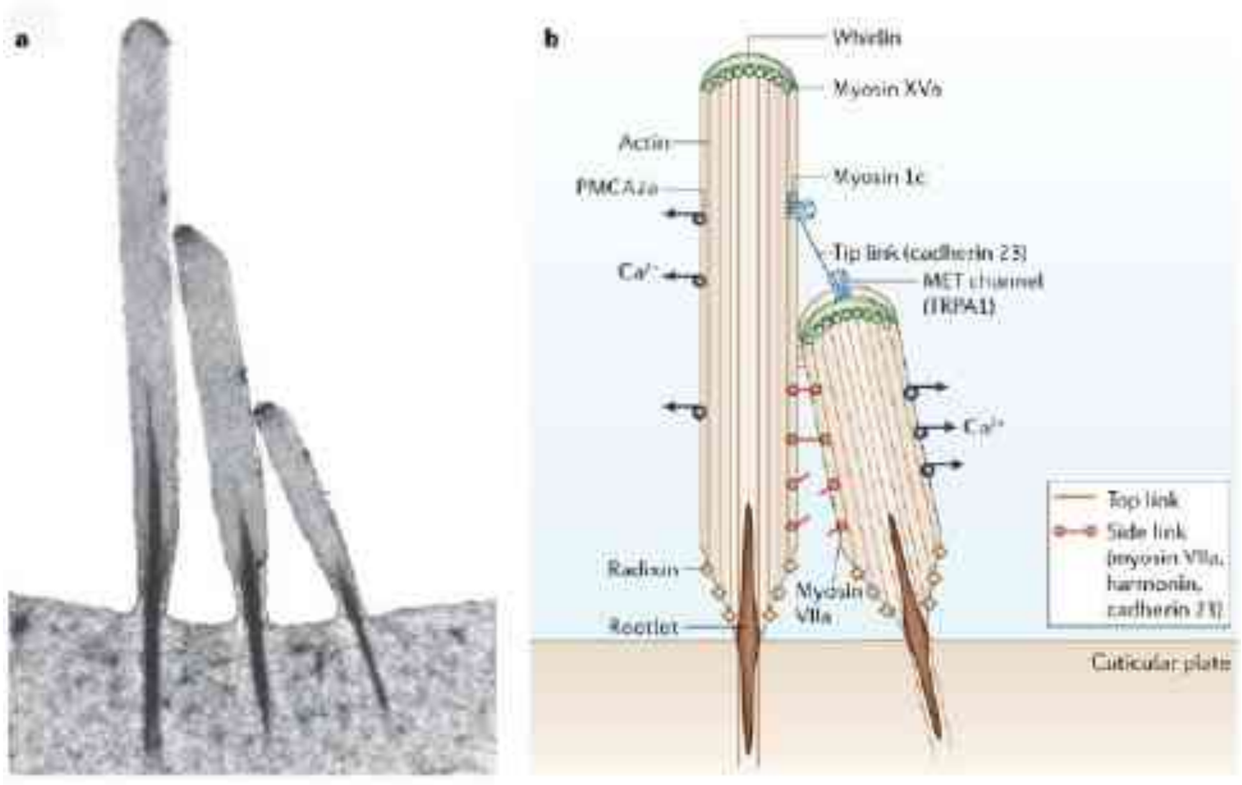
Hesso Farhan

Institute of Pathophysiology


stiffness

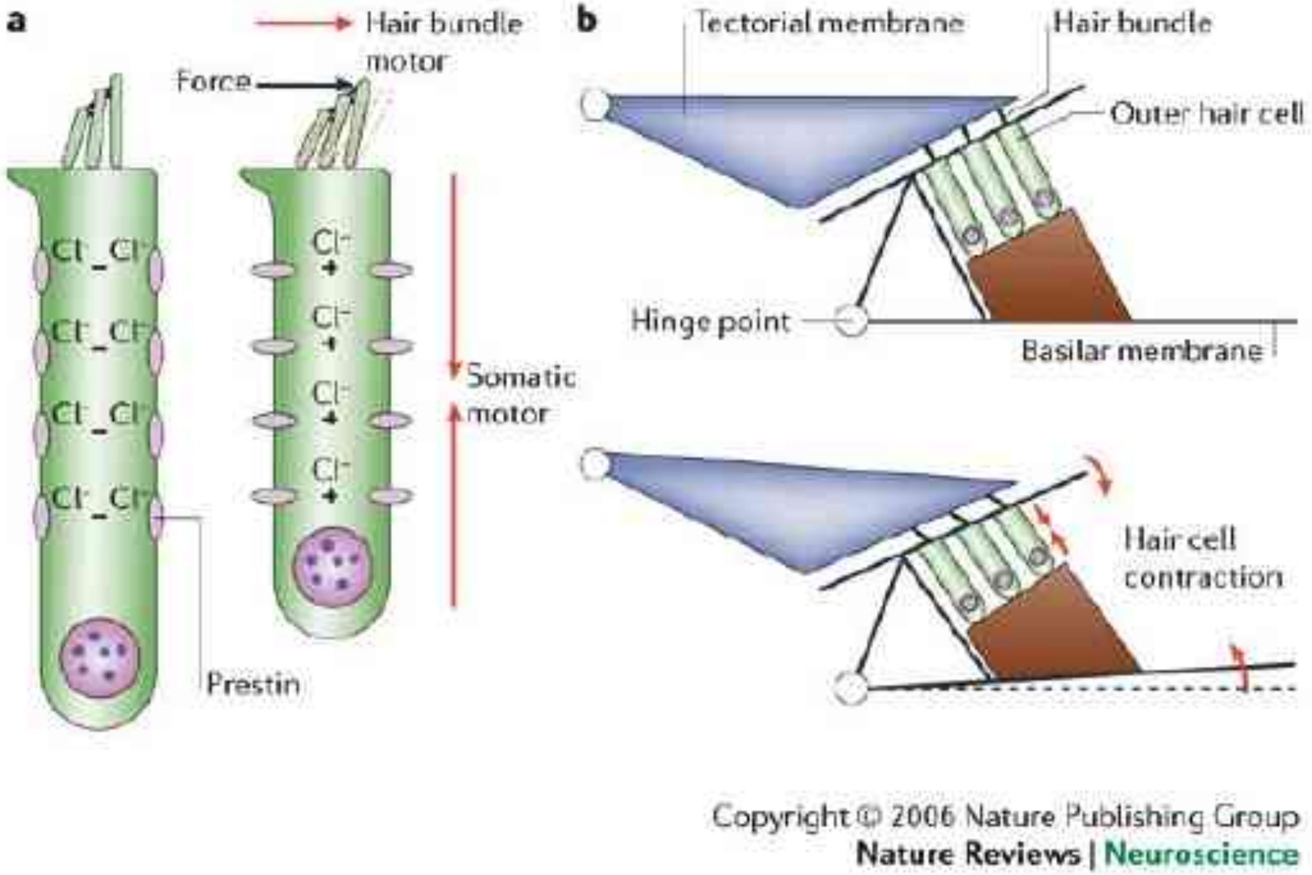
Evidence that cells are exposed to mechanical forces

- 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

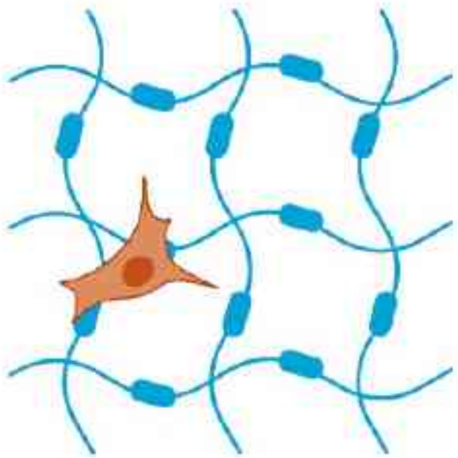
- Prestin is inactive when bound to Cl^-
- Upon depolarization → Prestin changes its conformation → contraction of the cell body

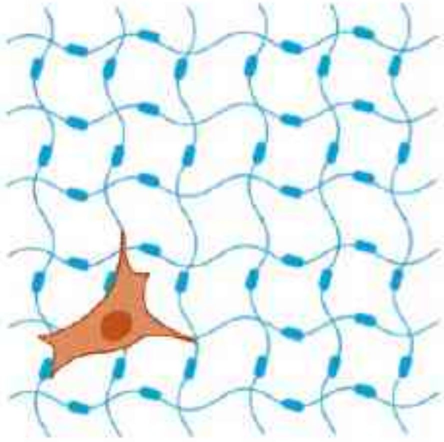


https://www.nature.com/articles/nrn1828

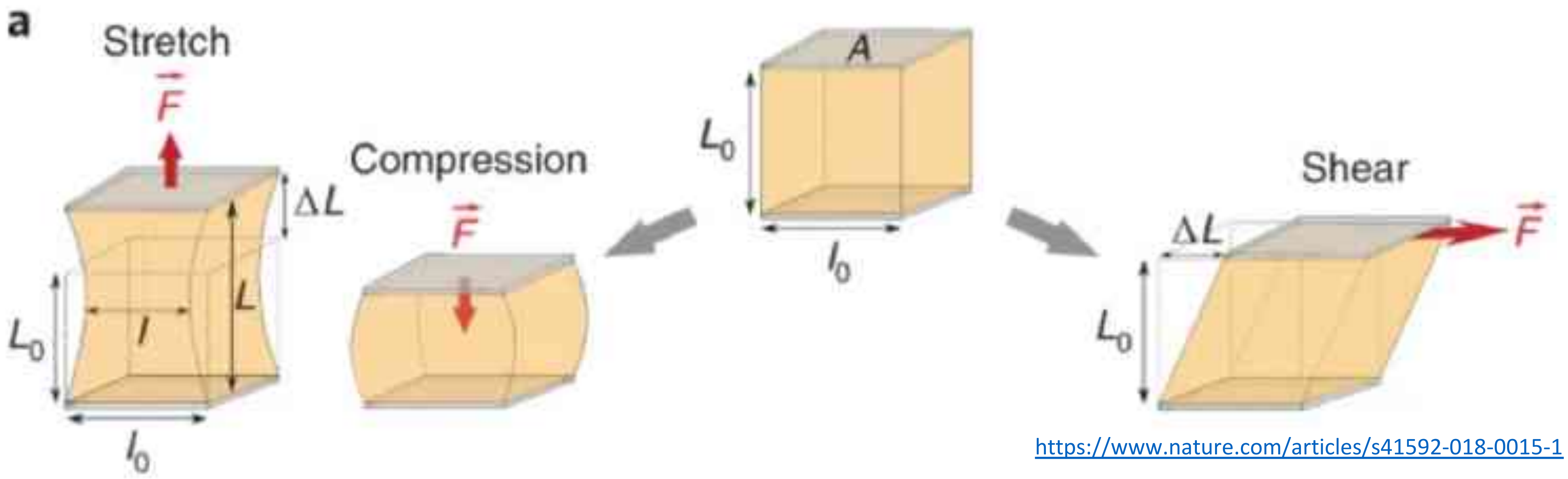
https://auditoryneuroscience.com/ear/dancing_hair_cell

Main types of mechanical forces



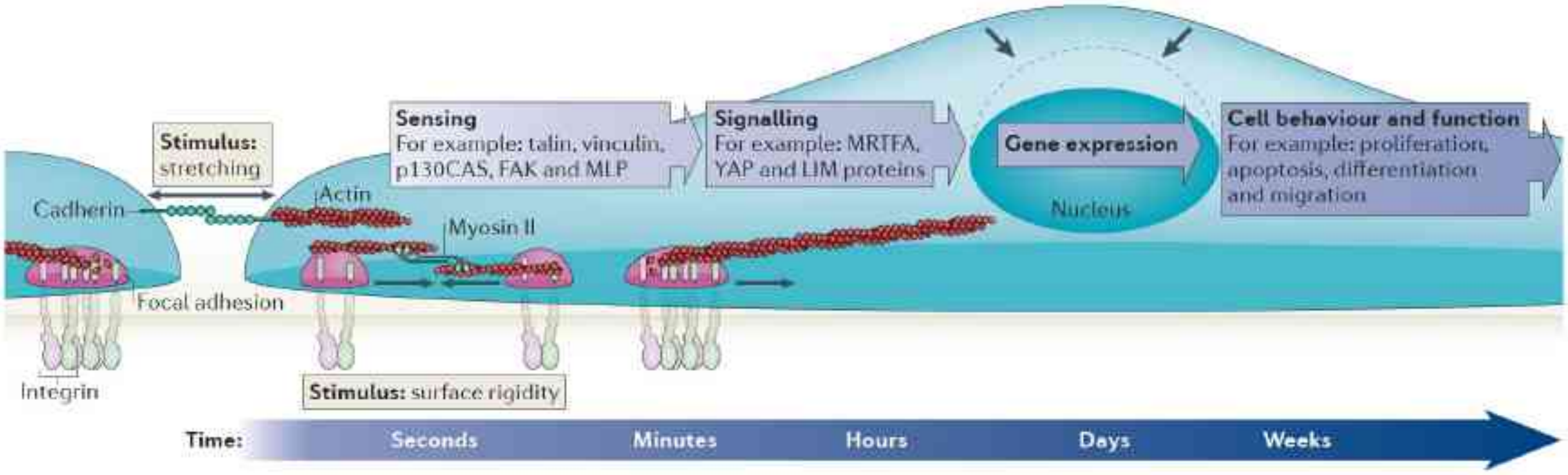


Stiffness and density of substrate

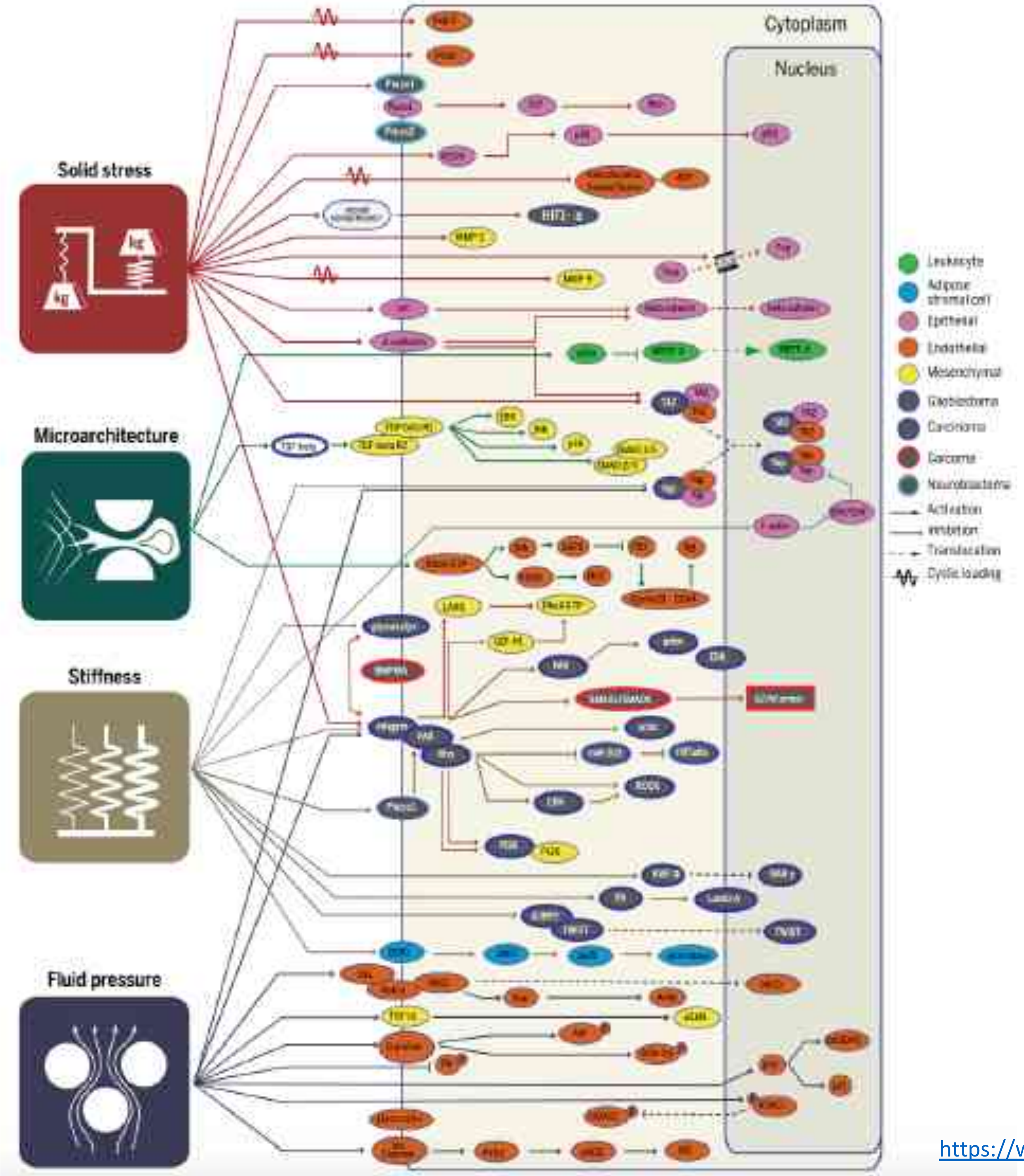


https://www.nature.com/articles/s41592-018-0015-1

Mechanotransduction

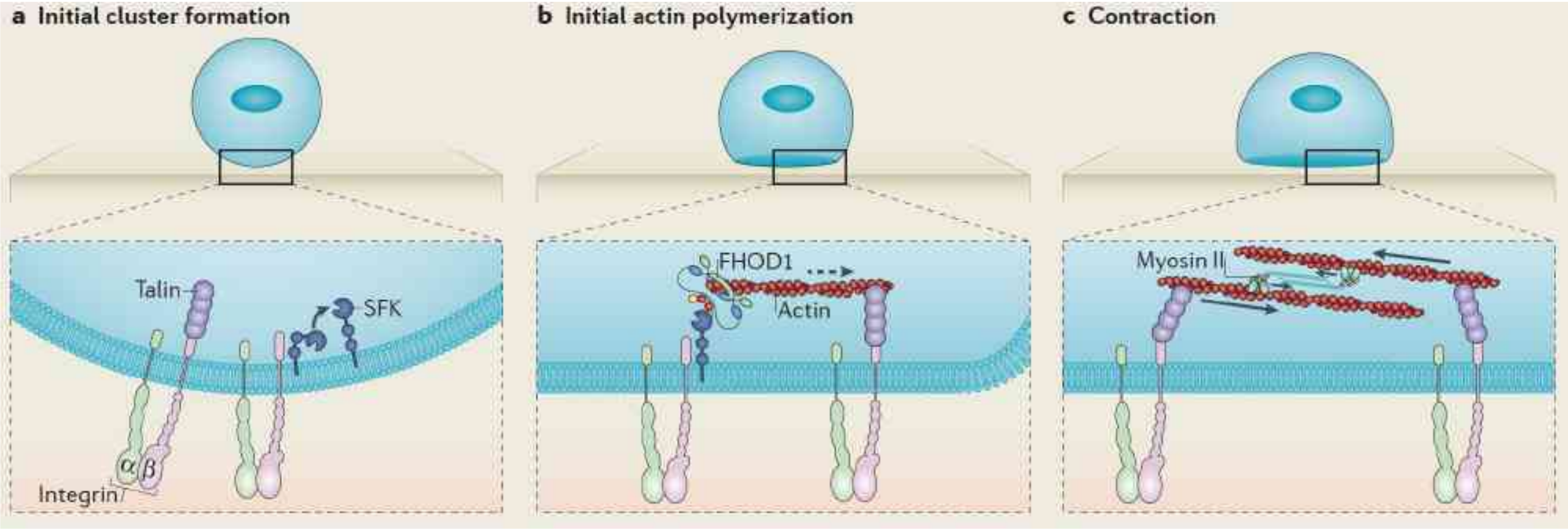


Mechanotransduction is the conversion of mechanical force into a biochemical signal



https://www.science.org/doi/epdf/10.1126/science.aaz0868

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

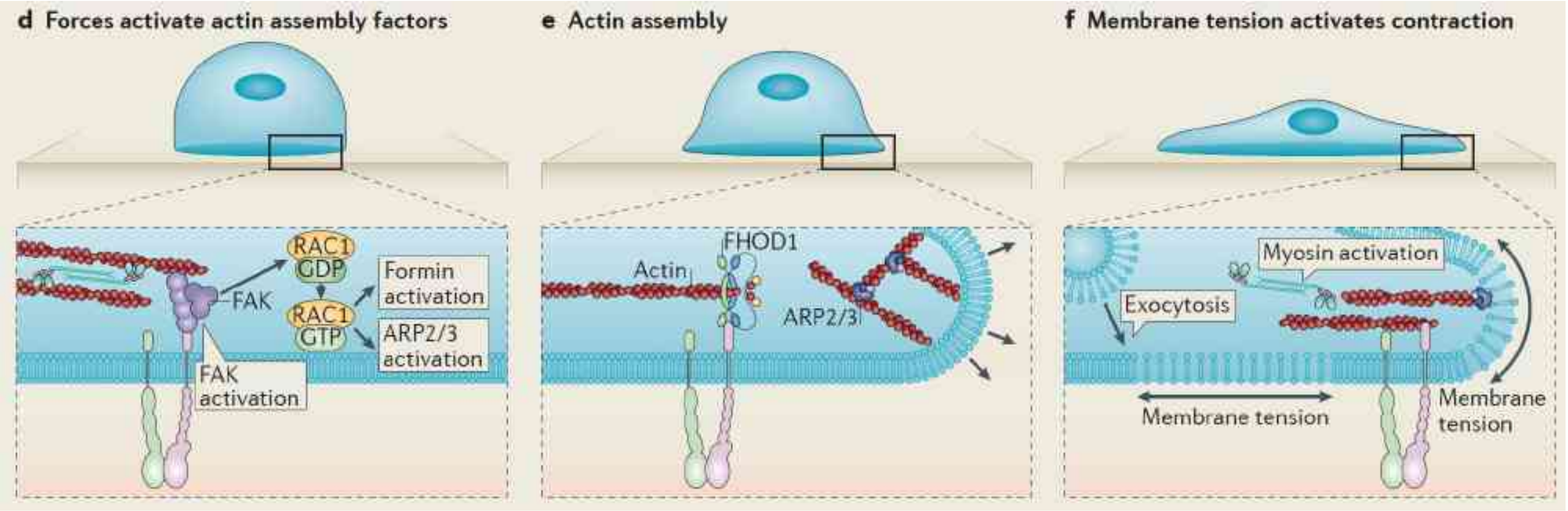


Integrins are activated

Actin polymerization

Myosin recruitment
→ Contraction and flattening of the cell

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

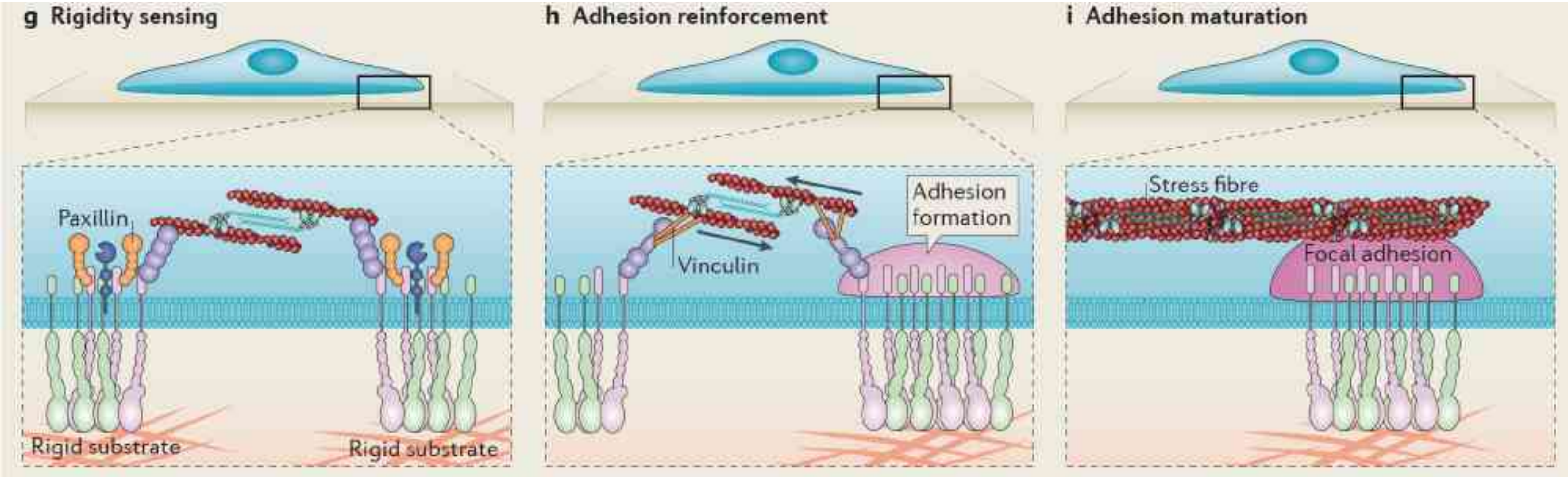


Activation of Rac1
→ More actin polymerization

Increased contractility

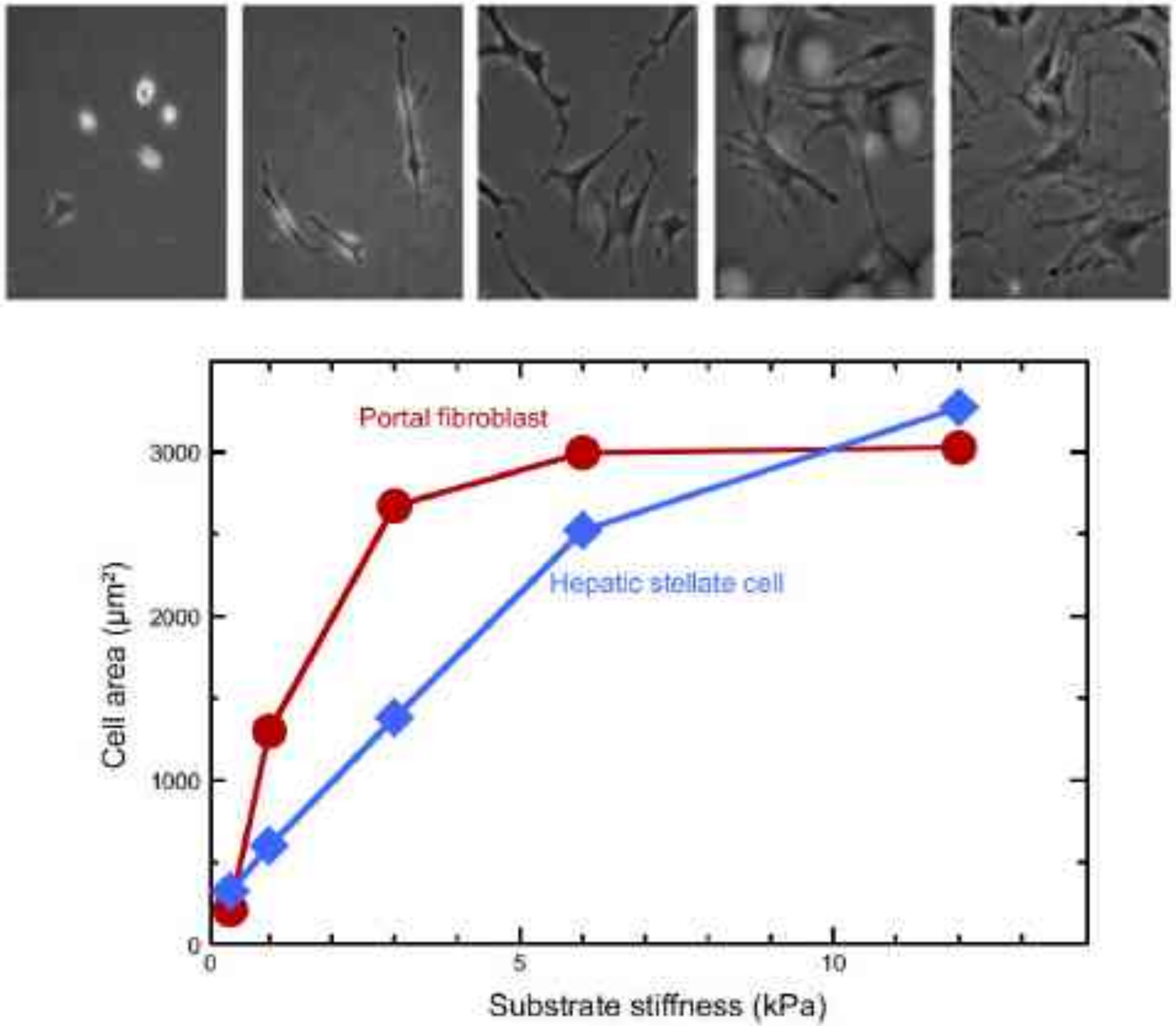
Membrane tension
→ sensor?

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 → biochemistry and physics collaborate

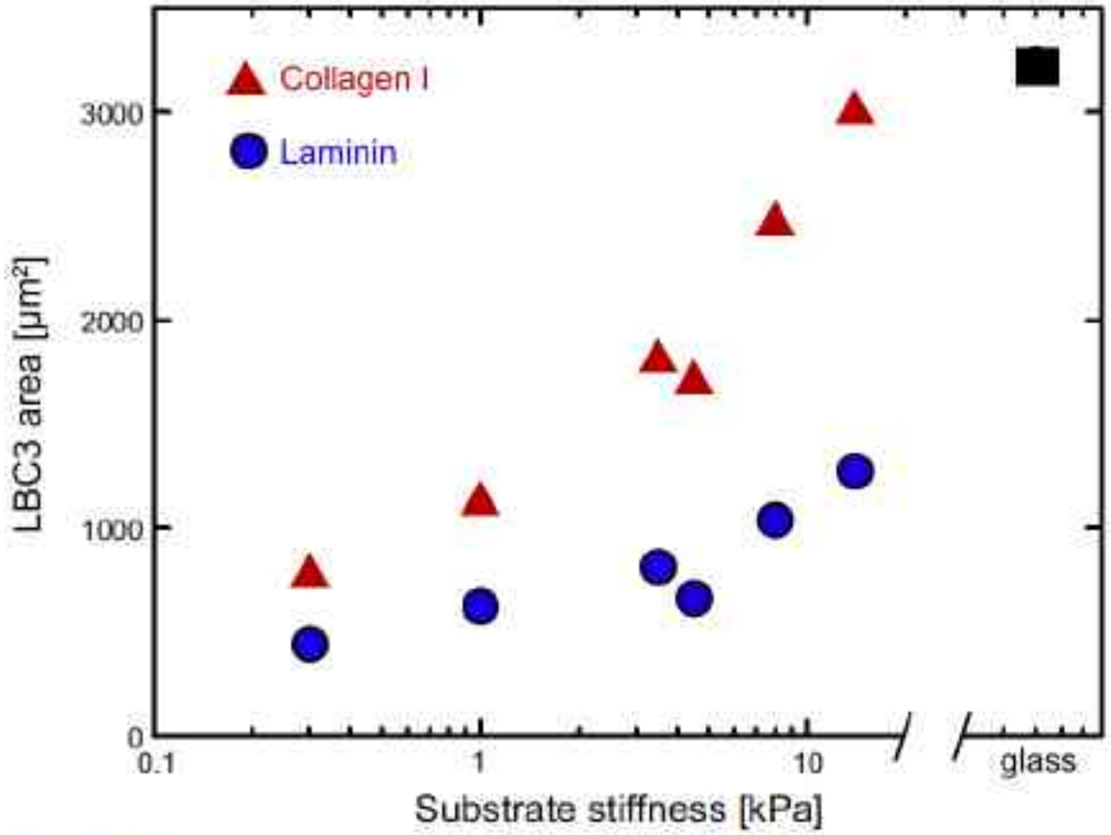
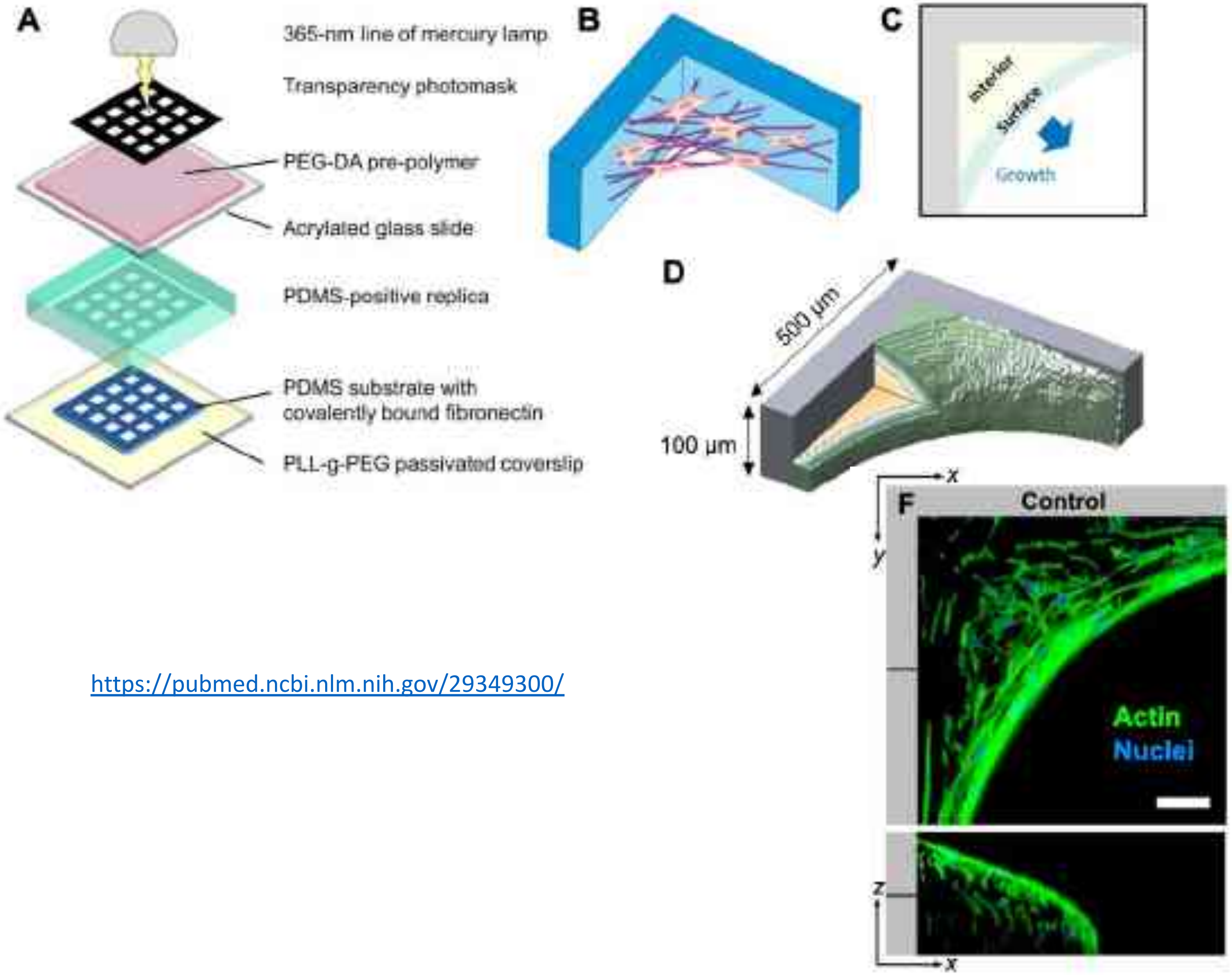


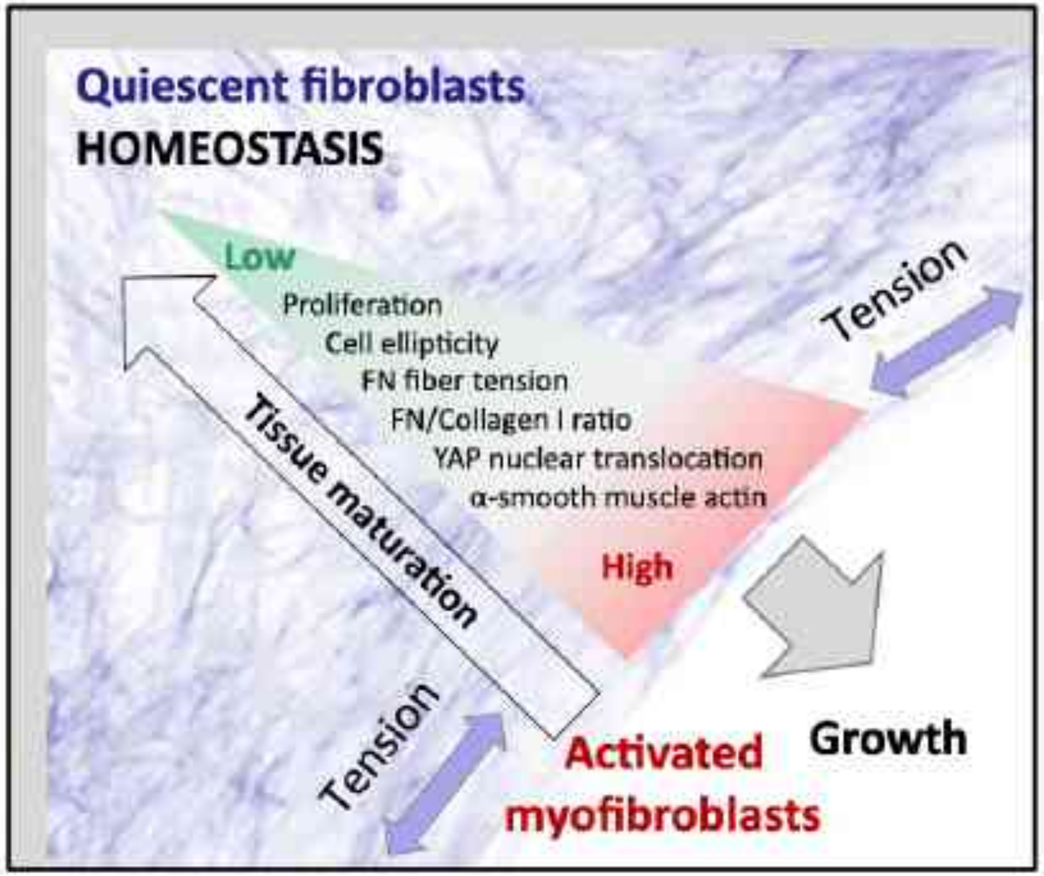
FIGURE 4. Integrin ligand dependence of response to substrate stiffness. Area of LBC3 human glioma cells on polyacrylamide gels coated with collagen I or laminin compared with area on glass after 24 h. [From Pogoda et al. (173).]

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

Dimensionality at the supracellular scale affects cell differentiation

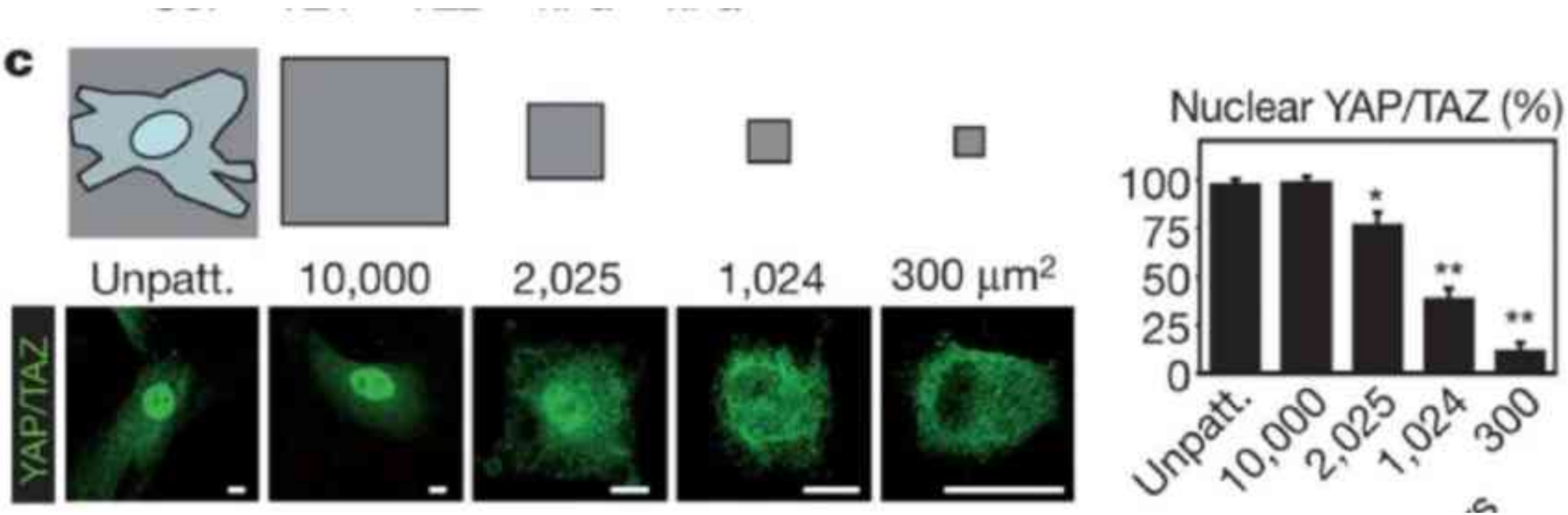


https://pubmed.ncbi.nlm.nih.gov/29349300/



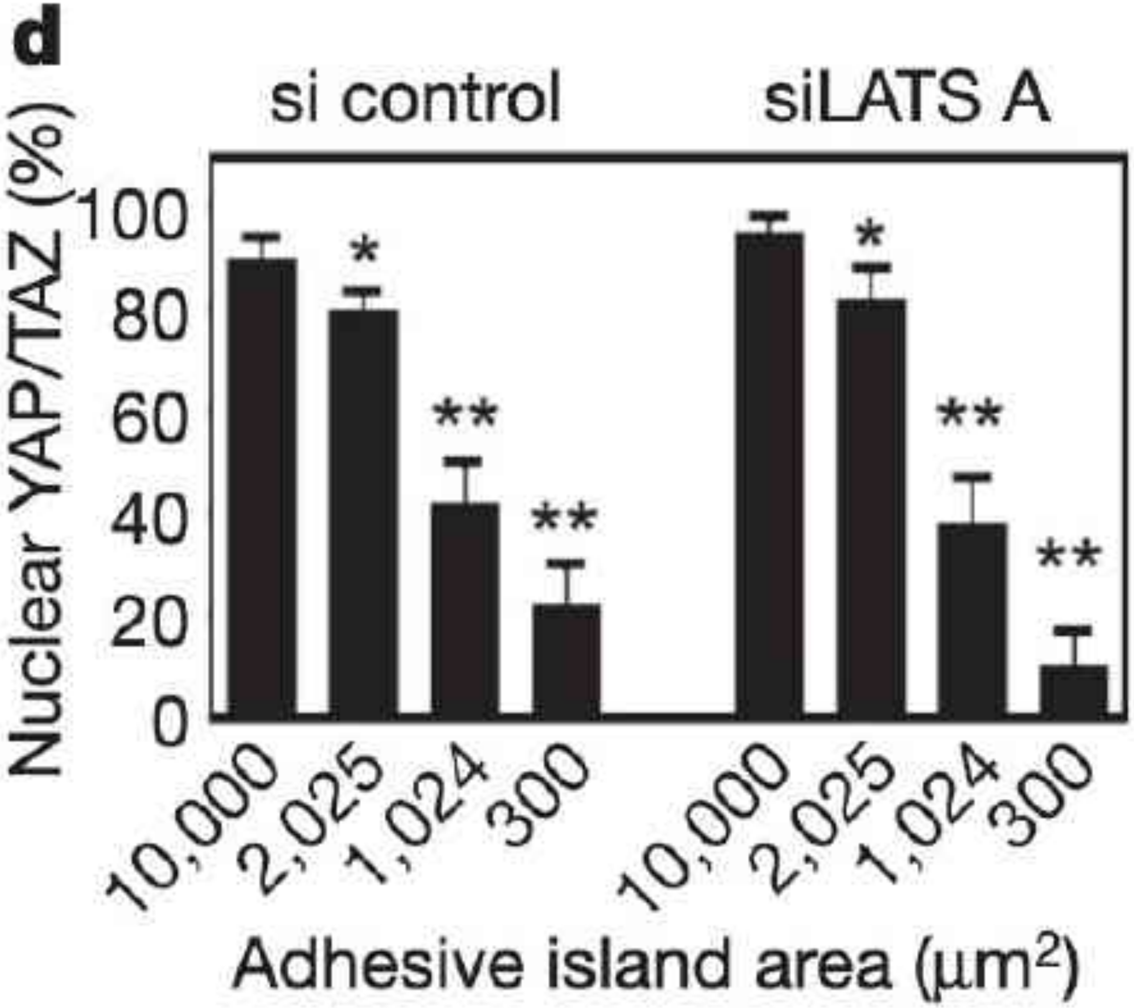
Fibroblasts at the front differentiate into myofibroblasts without the need for external factors

Dimensionality also works at the cellular scale



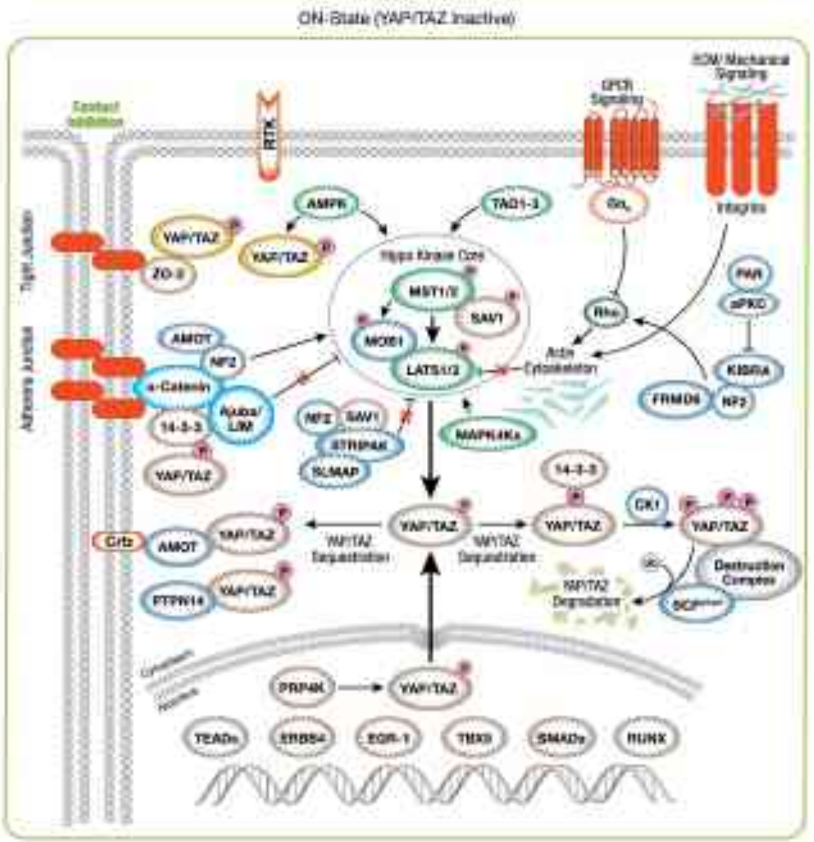
https://www.nature.com/articles/nature10137

YAP/TAZ mechanotransduction is independnet of the Hippo pathway

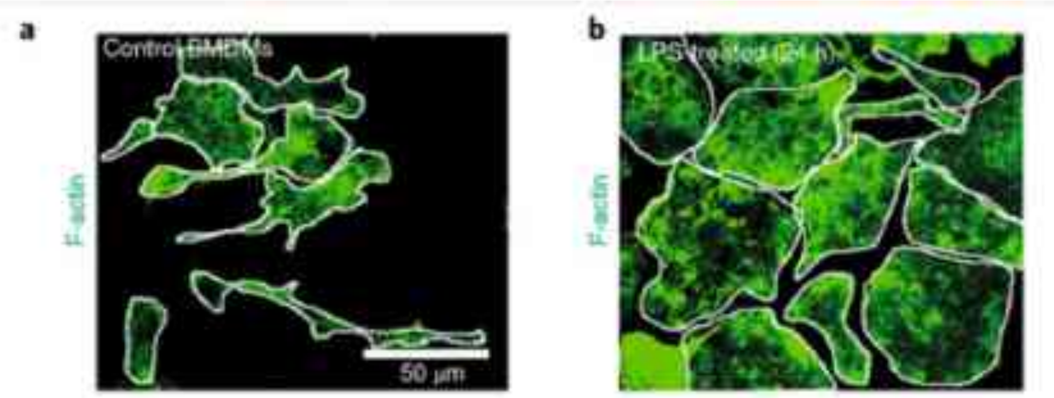


https://pubmed.ncbi.nlm.nih.gov/21654799/

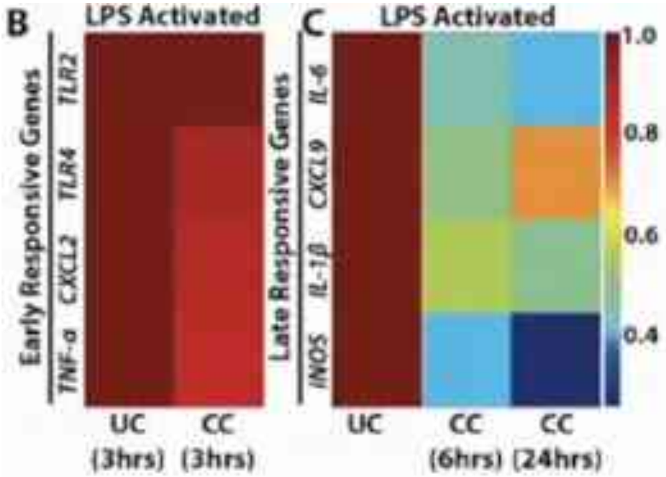
The Hippo Pathway signal via YAP/TAZ to mediate contact inhibition (via LATS)

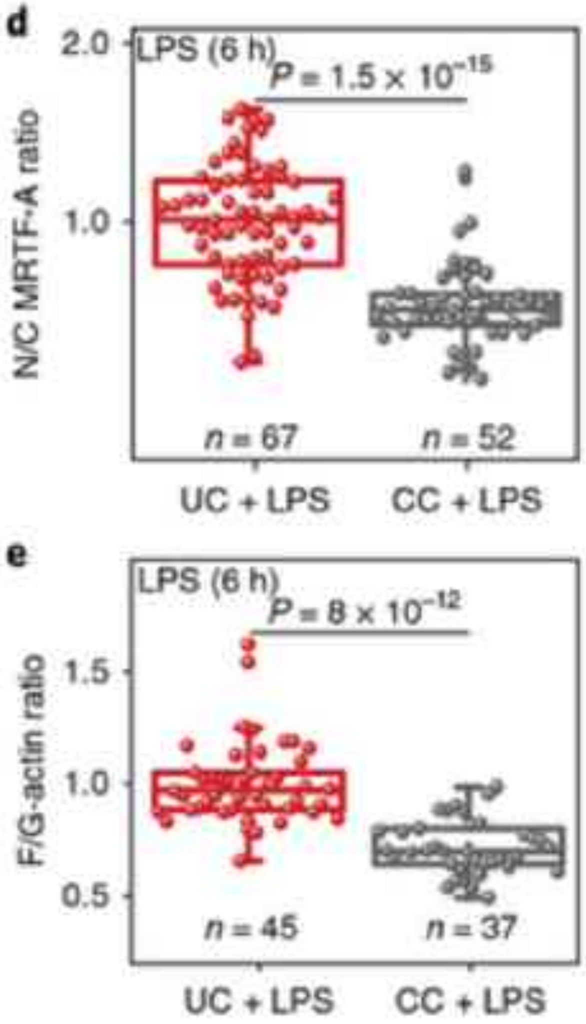


Mechanotransduction regulates cell fate decisions



Macrophages expand when exposed to LPS

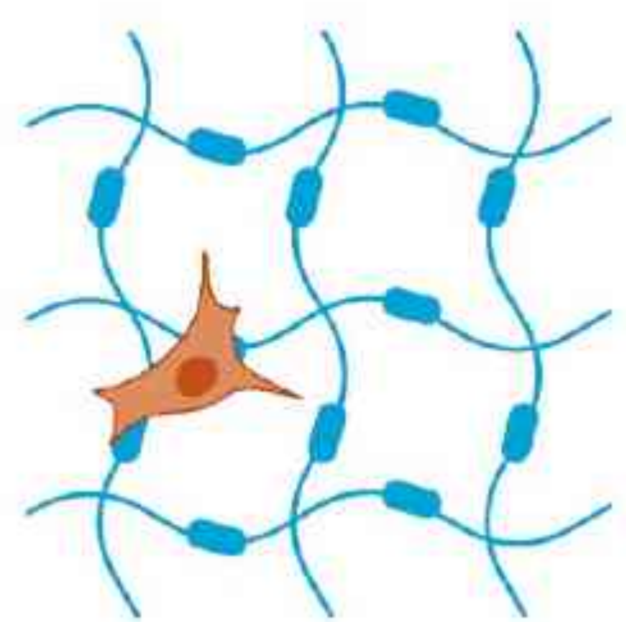


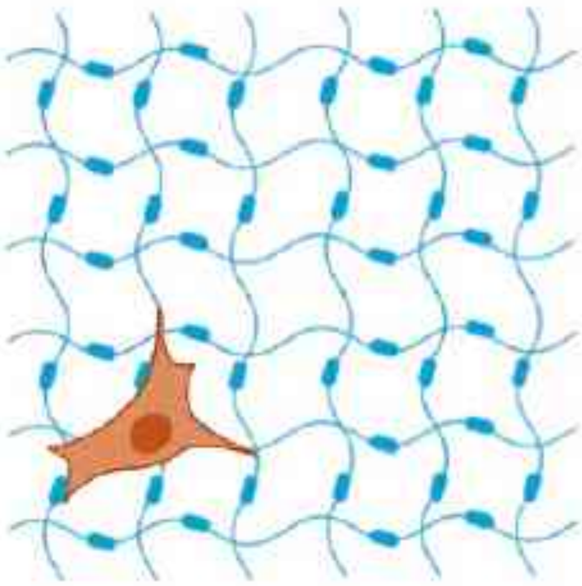


If the cells are confined, they cannot exert their full inflammatory potential

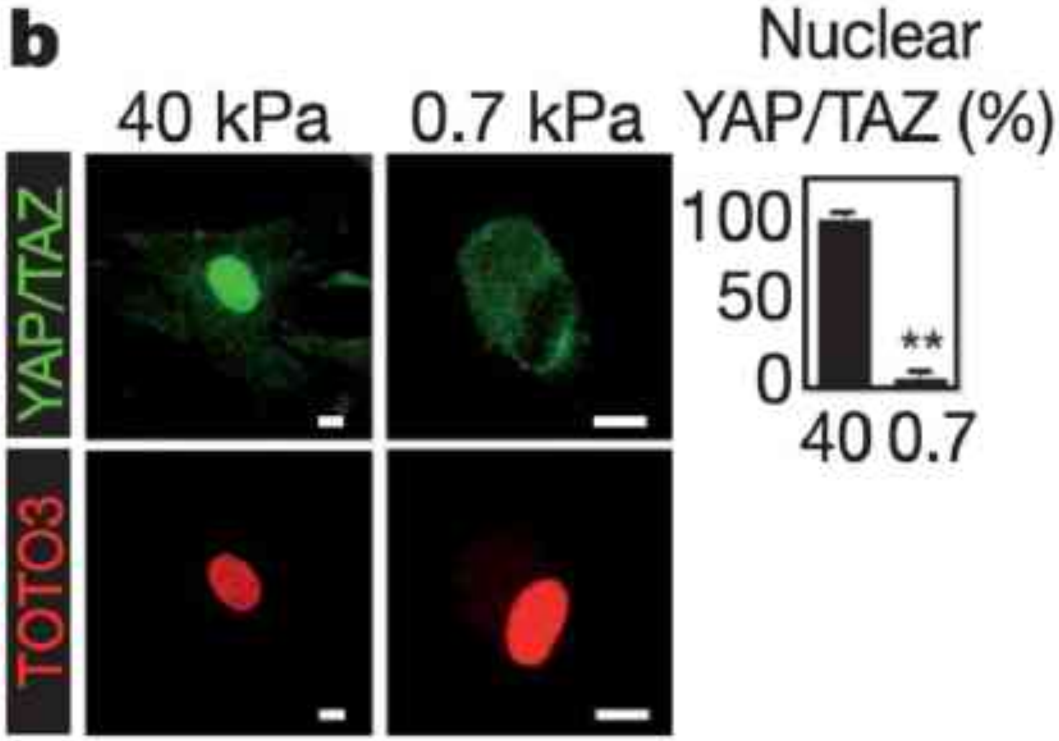
https://pubmed.ncbi.nlm.nih.gov/30349032/

All the exmples above are about tension.
What about Stiffness?



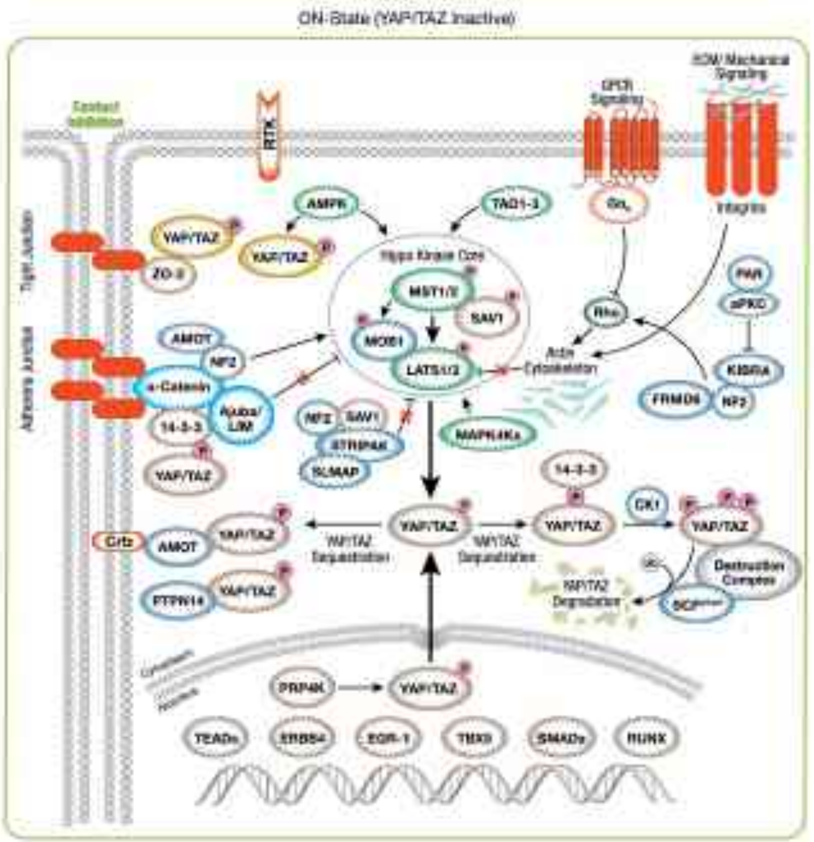


YAP/TAZ mediates response of cells to stiffness

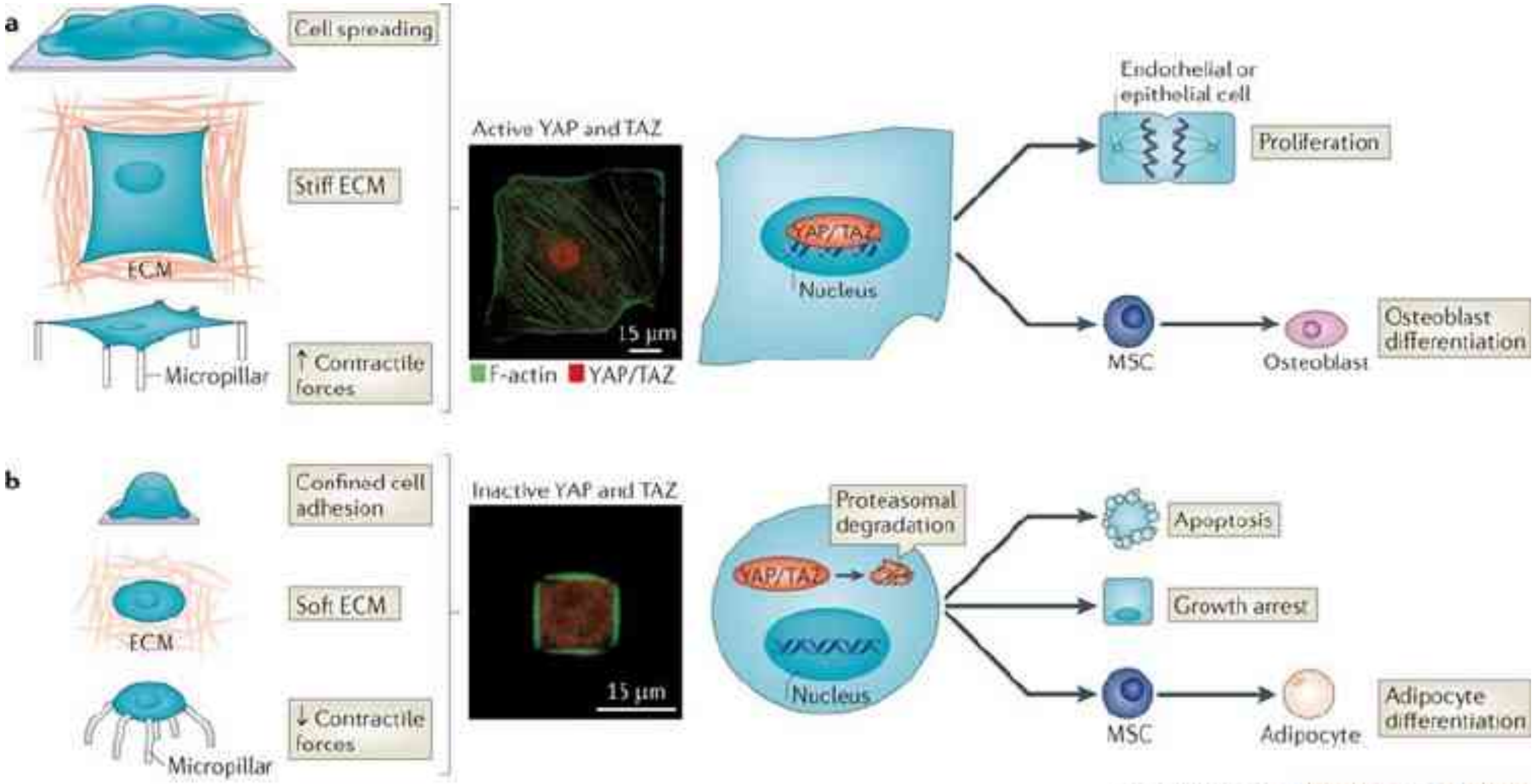


https://pubmed.ncbi.nlm.nih.gov/21654799/

The Hippo Pathway signal via YAP/TAZ to mediate contact inhibition (via LATS)

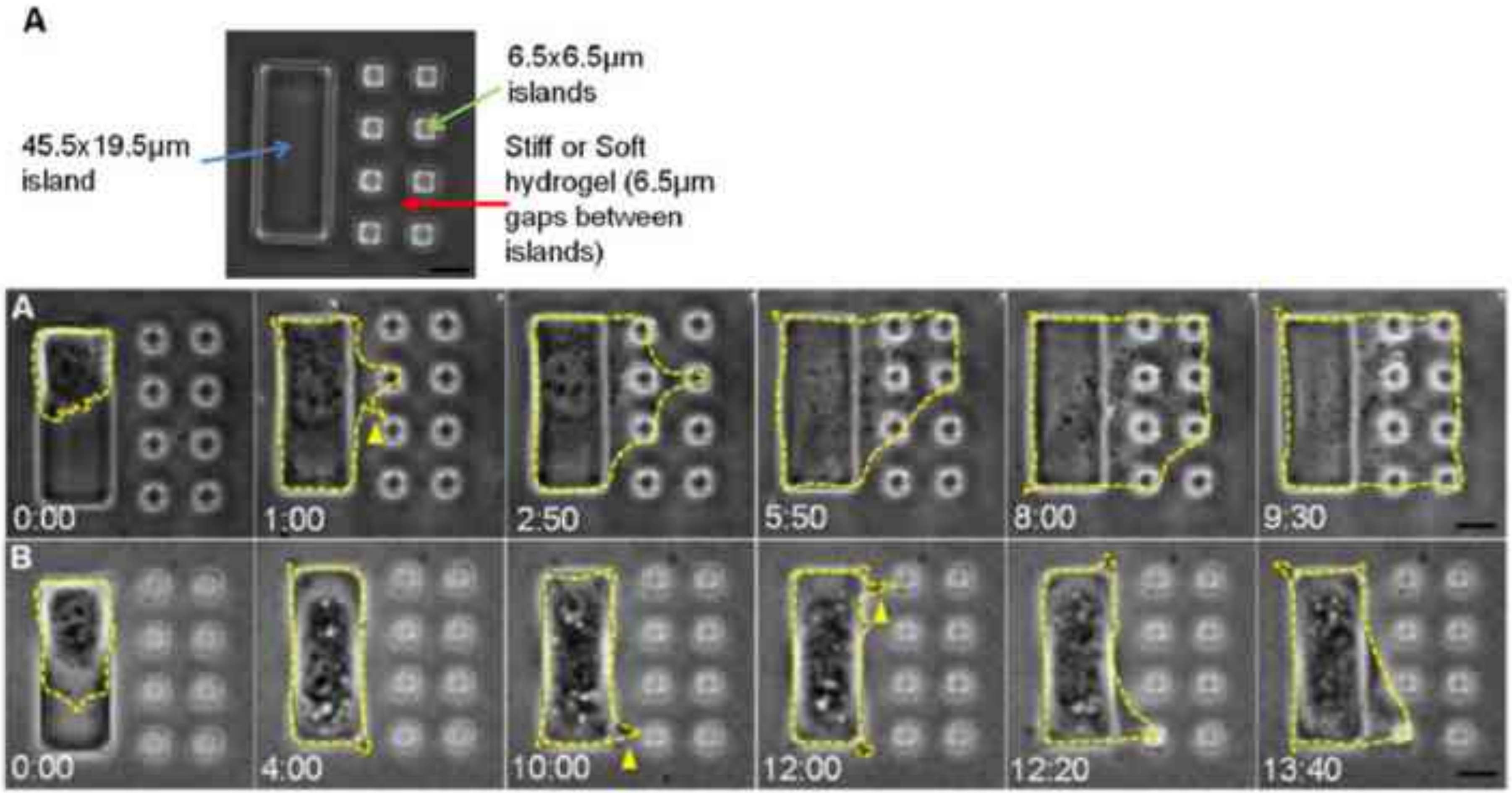


Mechanotransduction regulates cell fate decisions via YAP/TAZ



https://www.nature.com/articles/nrm3416

Cells probe the stiffness of their surrounding with filopodia to “decide” whether to spread



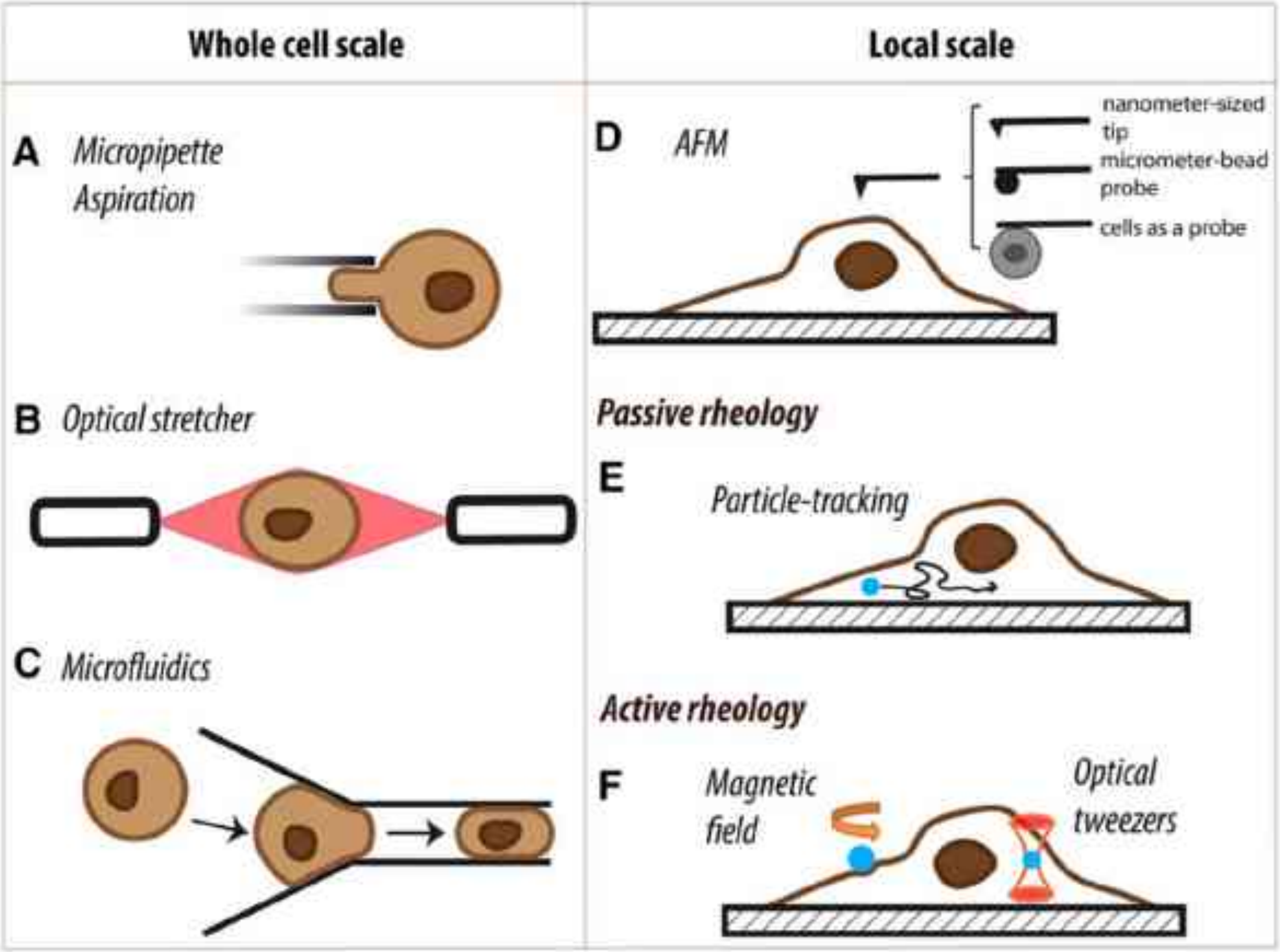
Stiff

Soft

https://www.pnas.org/doi/epdf/10.1073/pnas.1412285111

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

How to measure cell stiffness



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

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

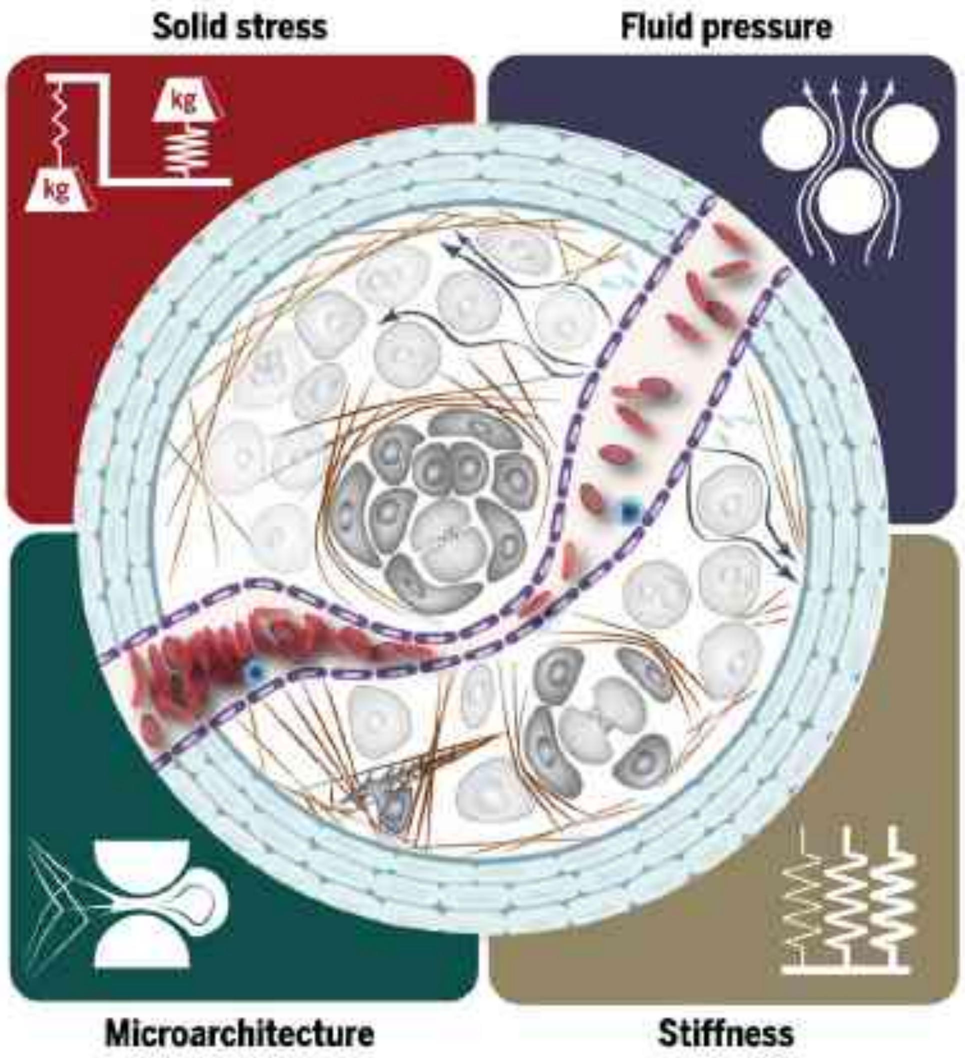
Palpation is often the first and simplest way to detect a tumour, for instance in breast tissues, before a more reliable diagnosis by biopsy. It is well accepted that tumour tissues, at the scale of the whole organ, are stiffer than their normal surrounding environment. Working with entire mammary gland, 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

Mechanobiology is mostly studied in the context of cancer

Force by the solid/elastic elements within a tumor (ECM and cells). Ranges from 0.7 kPa in Glioma to 10 kPa in pancreatic cancer.

Chemotherapy reduces solid stress (less cells = less solid stress)

Mediated by the arrangement of fibers in the ECM and by the loss of normal tissue architecture.



Through leaky vessles, or alterations of lymphatic drainage. The range ist between 1-5 kPa.

Also includes shear stress

Also known as “elasticity”. Mainly through enhanced ECM production in tumors.

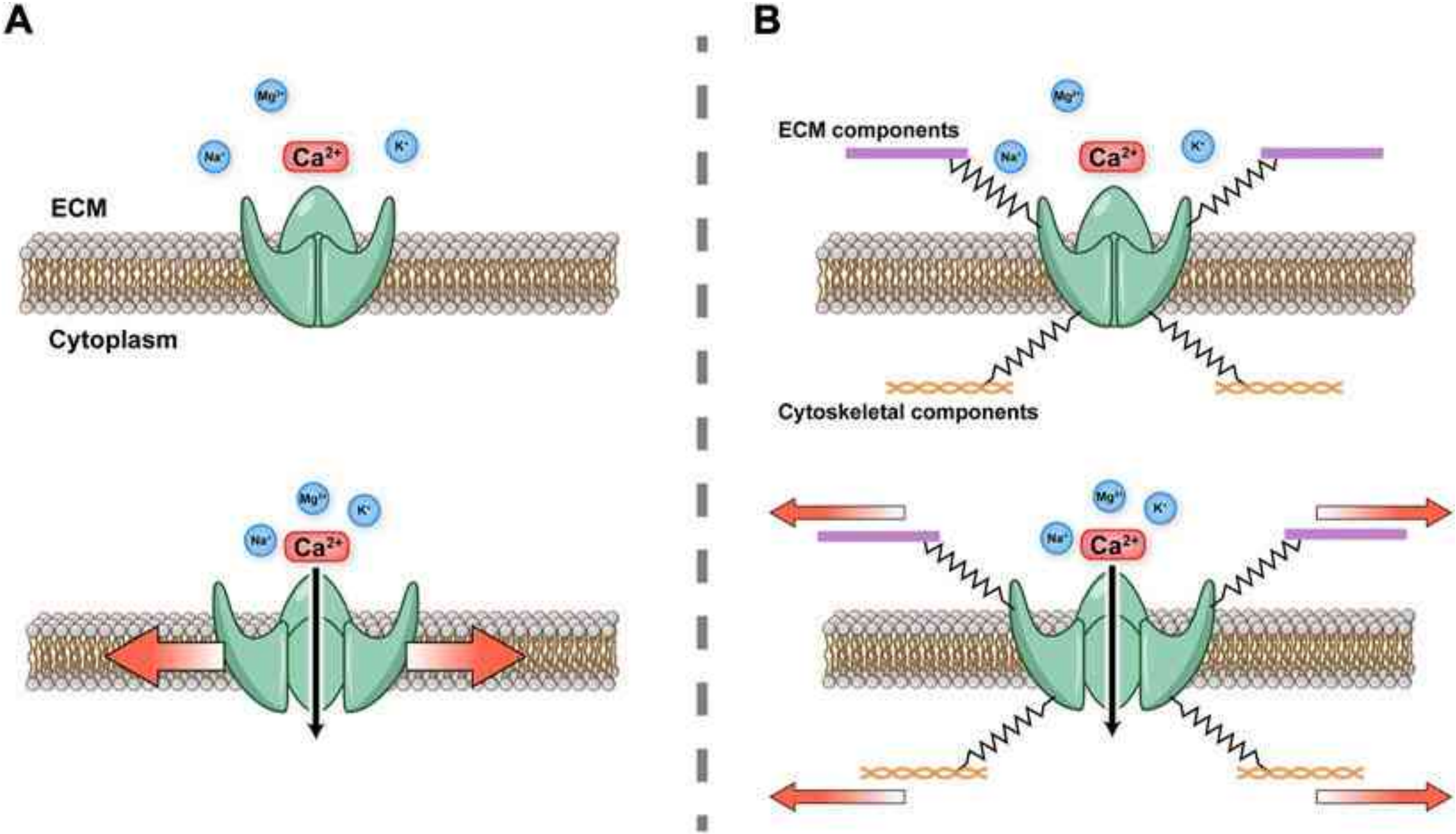
Ranges from 1kPa for brain tumors to 70 kPa in cholangiocarcinoma

https://www.science.org/doi/epdf/10.1126/science.aaz0868

What is a ***mechanosensor***?

→ ***A protein that converts a mechanical force into a biochemical signal***

One of the best understood mechanosensors are ***PIEZO channels***



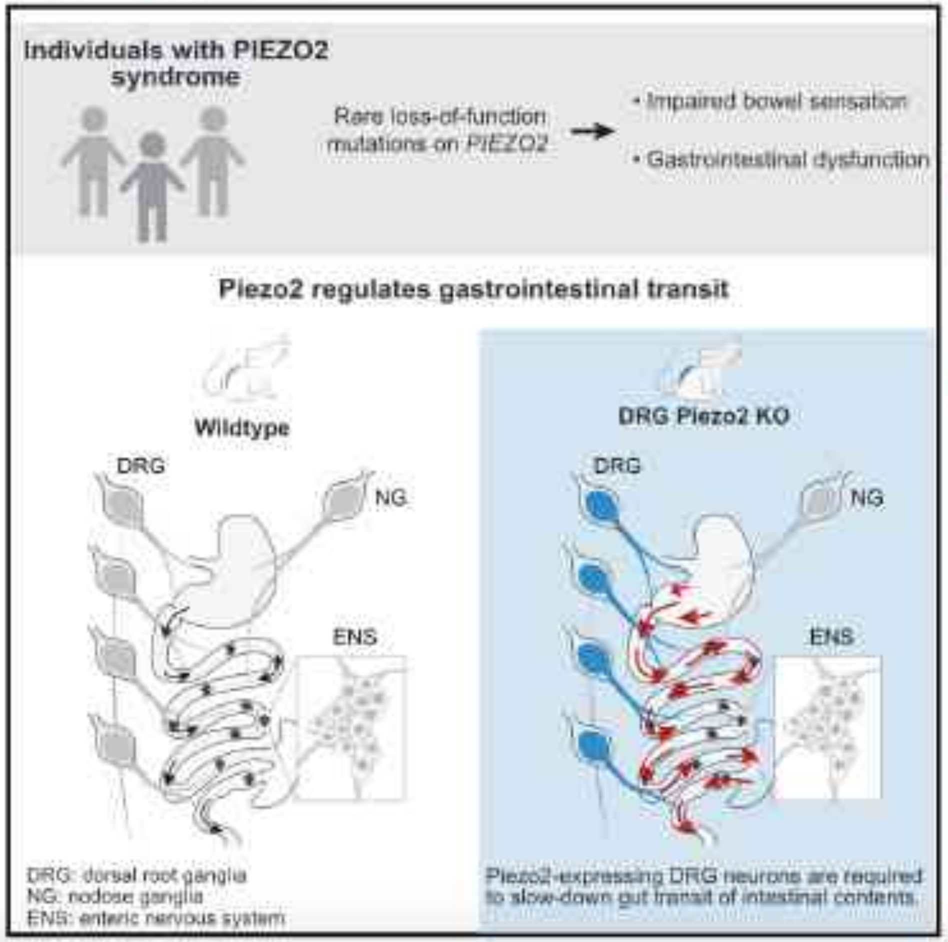
https://www.frontiersin.org/articles/10.3389/fphys.2022.1039714/full

Cell

Article

PIEZO2 in somatosensory neurons controls gastrointestinal transit

Graphical abstract



Authors

M. Rocio Servin-Vences, Ruby M. Lam, Alize Koolen, ..., Carsten G. Bönnemann, Alexander T. Chesler, Ardem Patapoutian

Correspondence

alexander.chesler@nih.gov (A.T.C.), ardem@scripps.edu (A.P.)

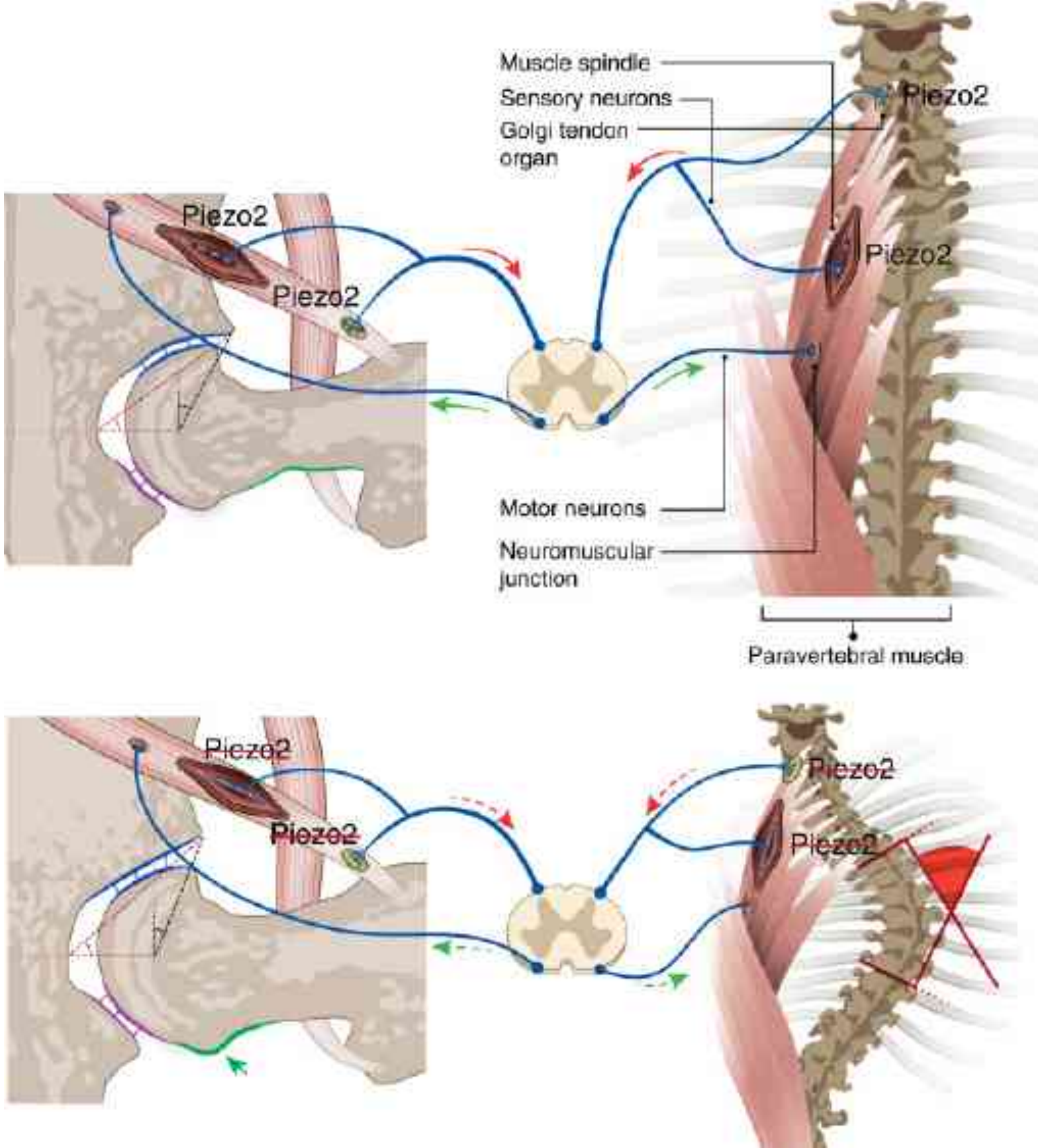
In brief

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:

- watery stool
- diarrhea
- constipation and diarrhea
-

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



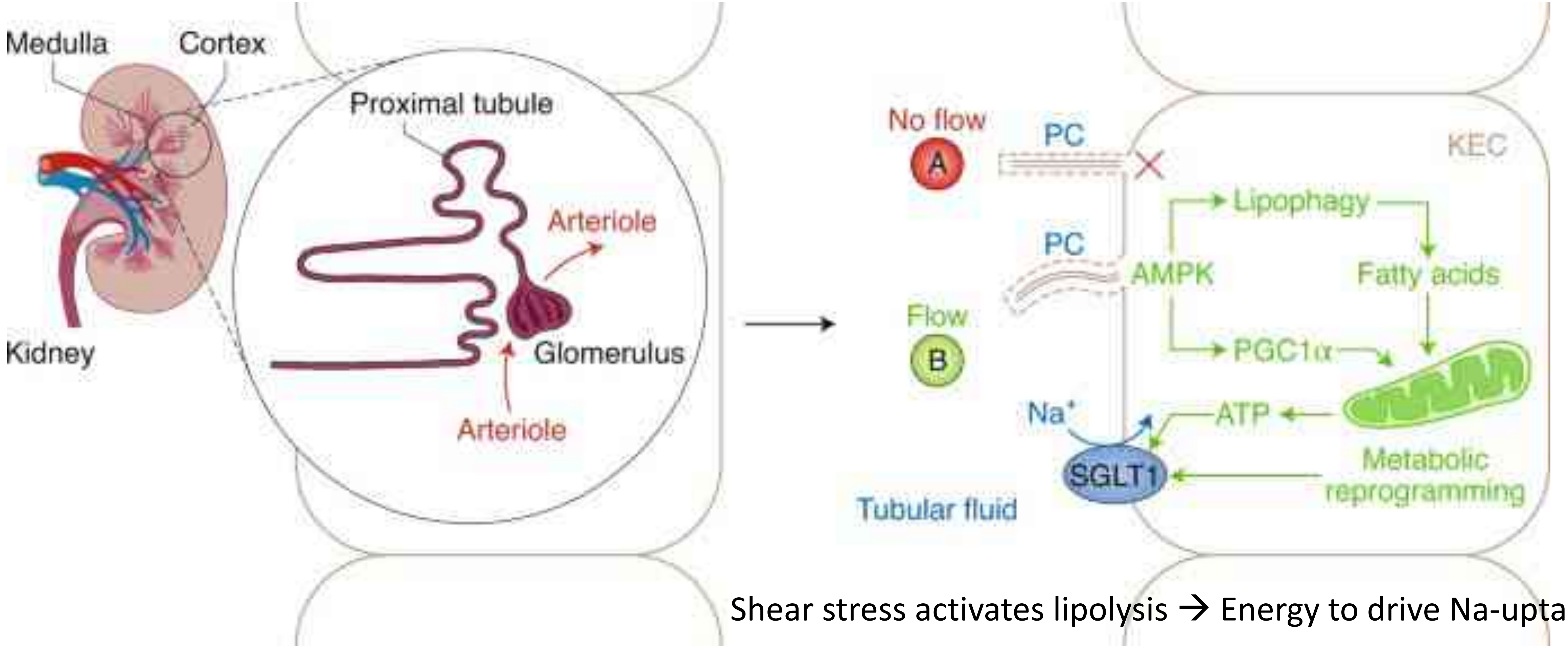
Loss of *Piezo2* in proprioceptive neurons results in spine malalignment and hip dysplasia.

Excessive mechanotransduction in sensory neurons causes joint contractures

SHANG MA, ADRIENNE E. DUBIN, LUIS O. ROMERO, MEAGHAN LOUD, ALEXANDRA SALAZAR, SARAH CHU, NIKOLA KLIER, SAMEER MASRI,
YUNXIAO ZHANG, [...], AND ARDEM PATAPOUTIAN +5 authors Authors Info & Affiliations

SCIENCE • 12 Jan 2023 • Vol 379, Issue 6628 • pp. 201-206 • DOI: 10.1126/science.add3598

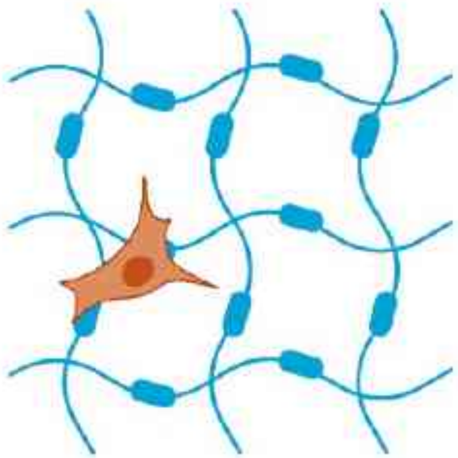
The primary cilium participates in sensing shear stress

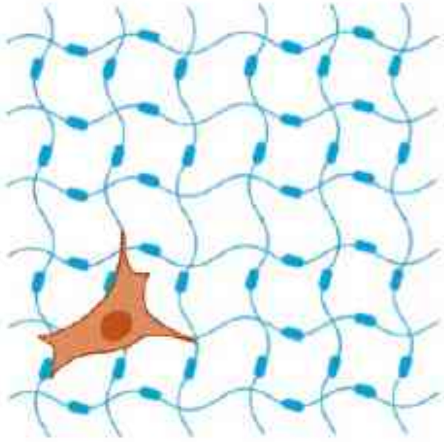


Shear stress activates lipolysis → Energy to drive Na-uptake

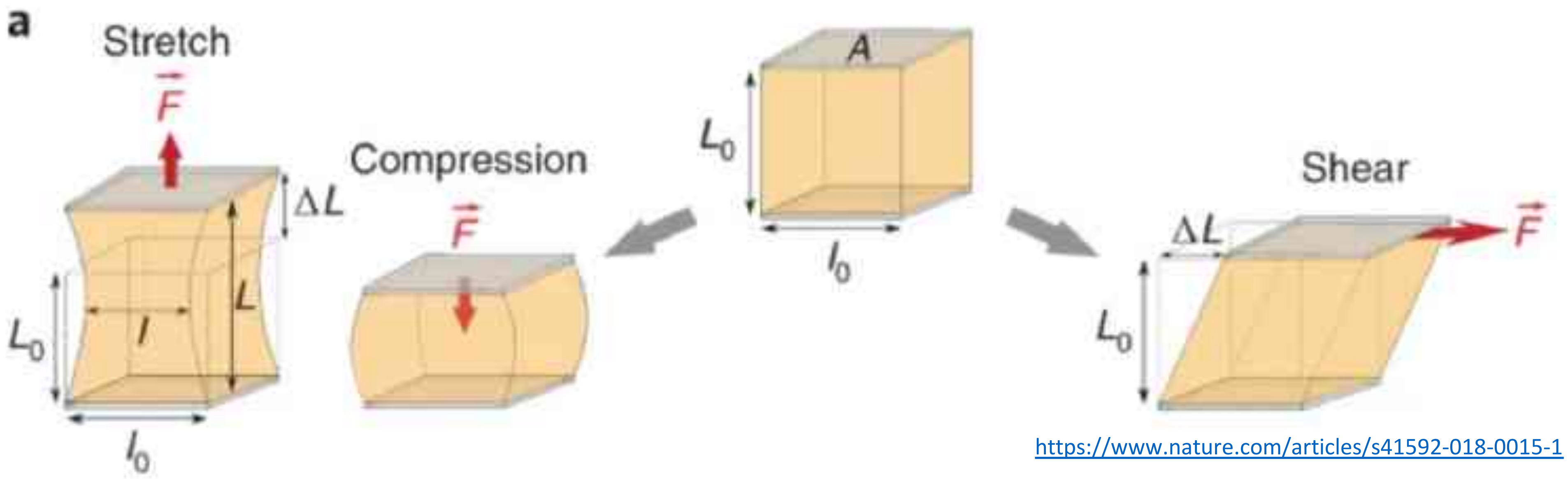
https://www.nature.com/articles/s41556-020-0571-3

Main types of mechanical forces



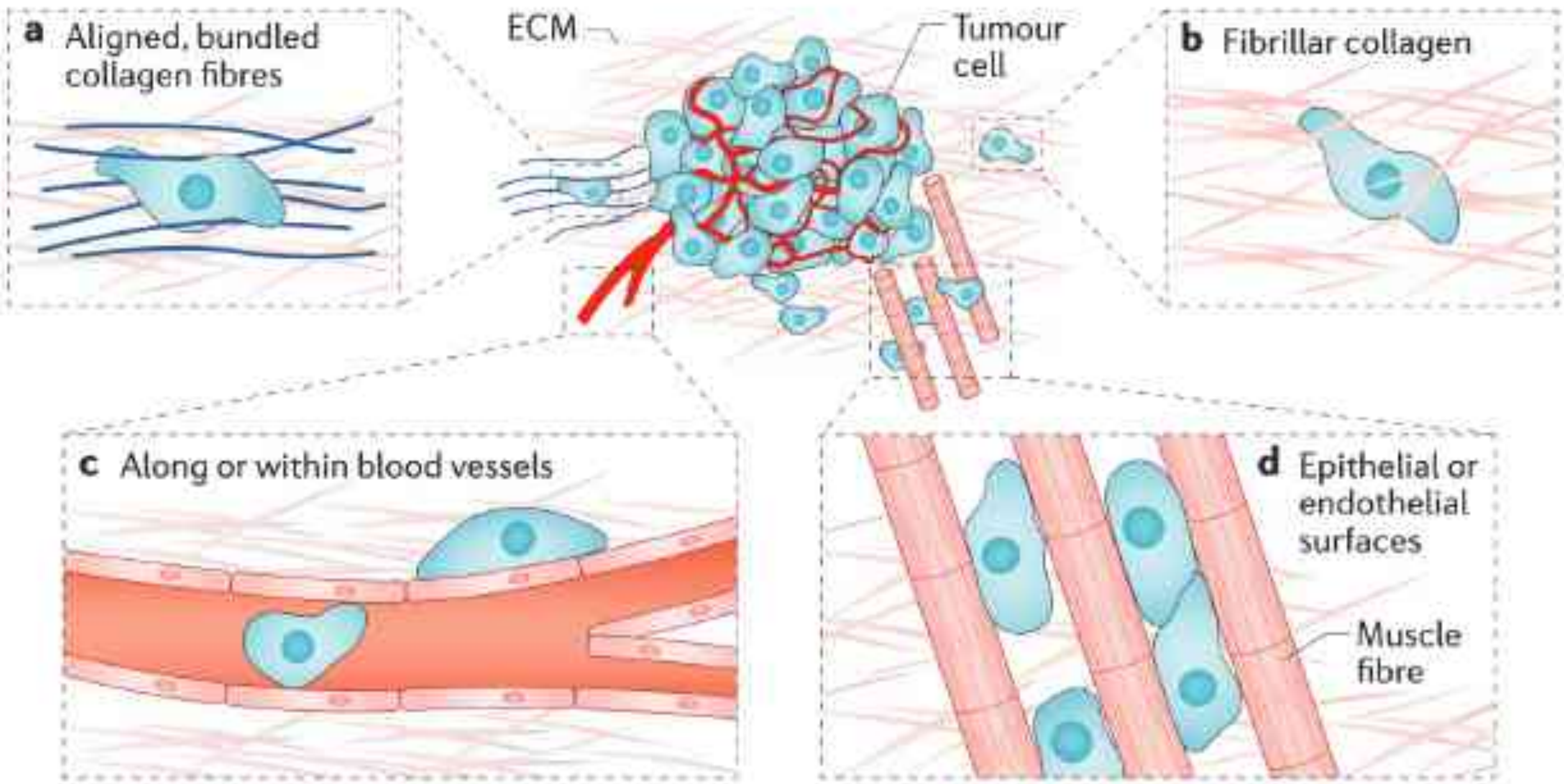


Stiffness and density of substrate



https://www.nature.com/articles/s41592-018-0015-1

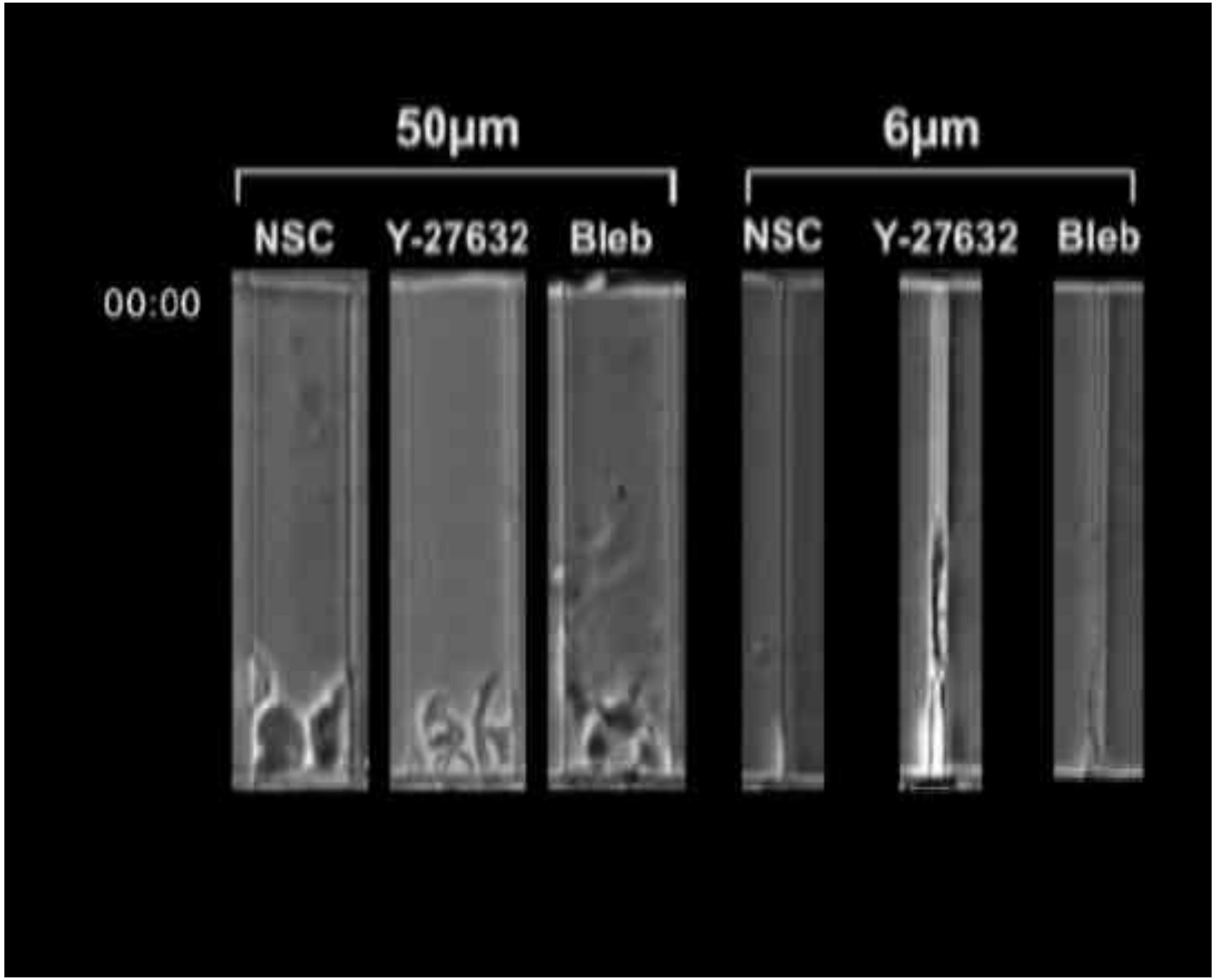
Cells encounter confined environments *in vivo*



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



NSC= inhibitor of Rac1
Y-27632 = inhibitor of ROCK = reduces contractility

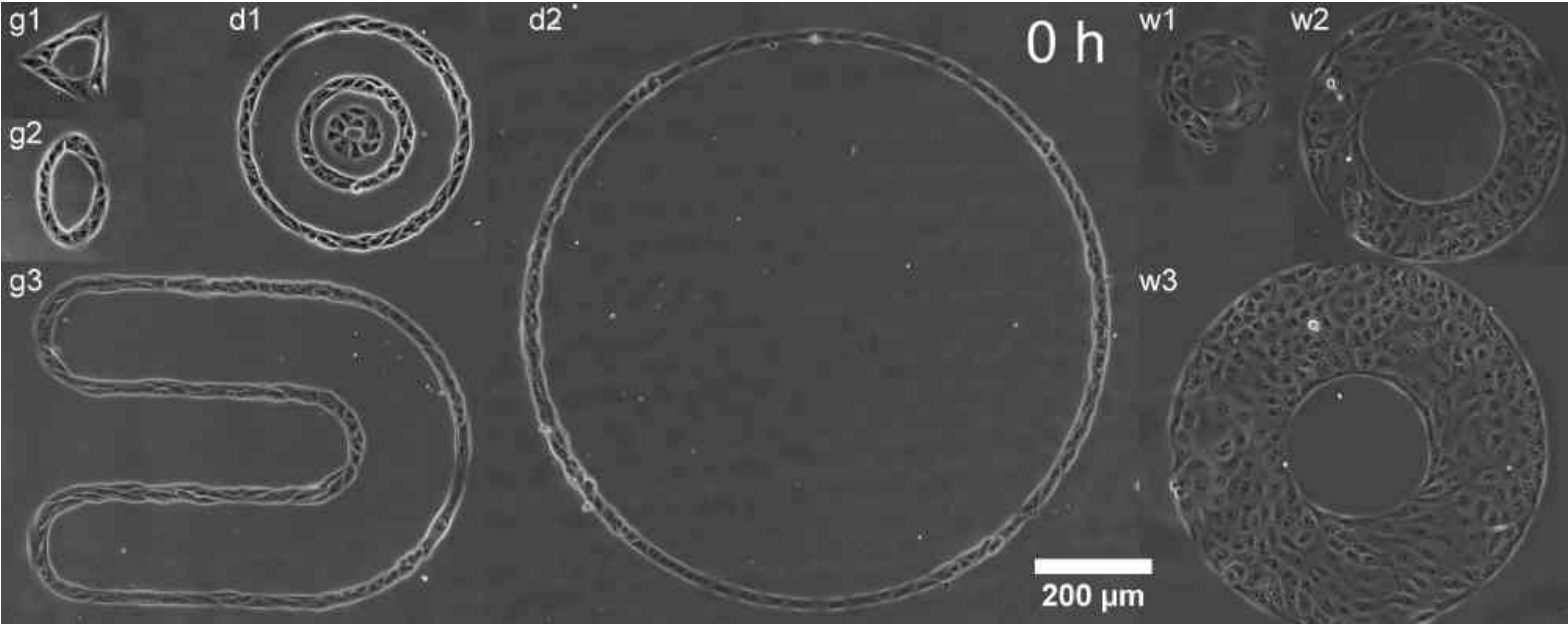
Confined migration does not need Rac1, but rather contractility. Unconfined migration is the opposite.

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

Confined migration does not need Rac1 in channels

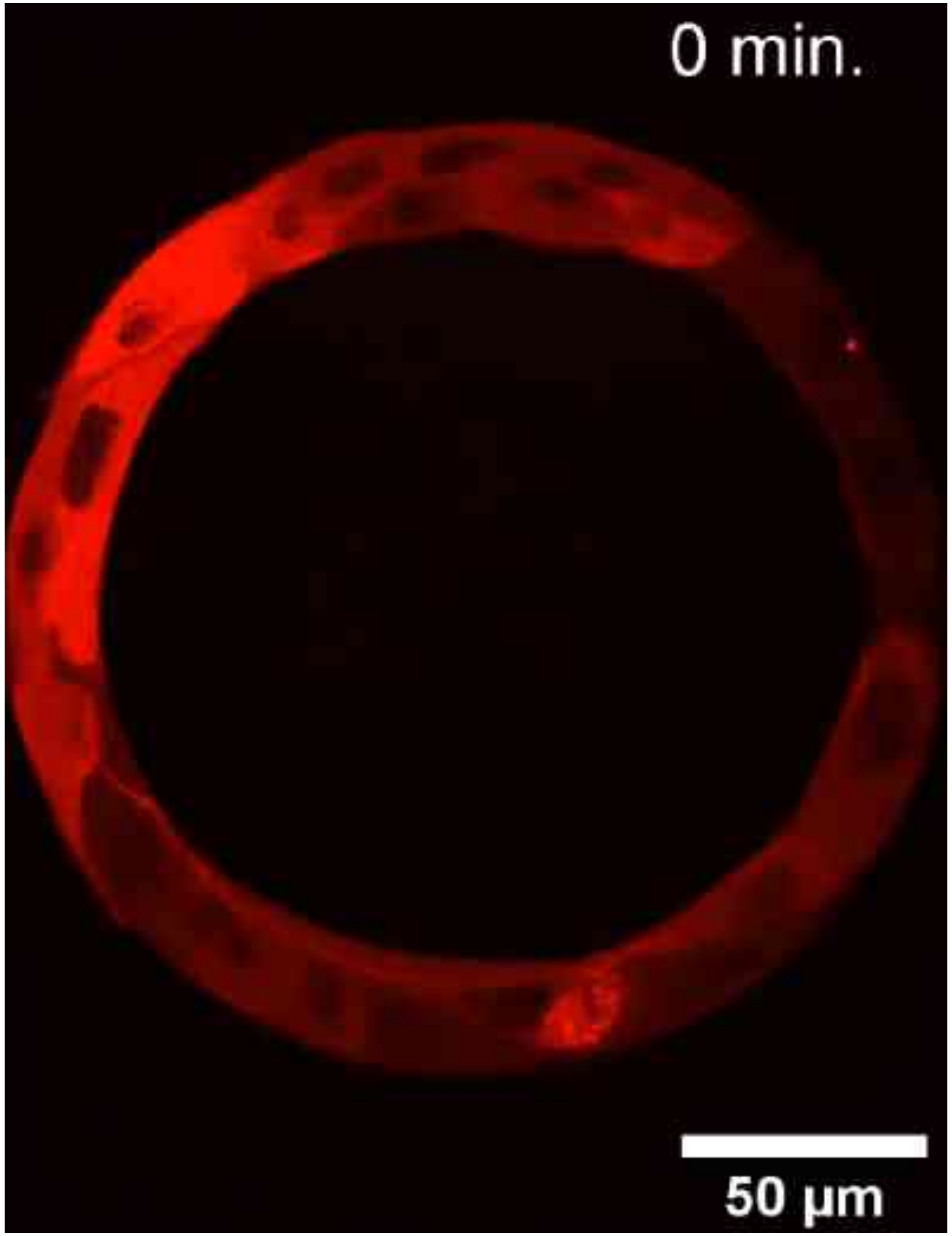
However, it is different in 2D confinemnet

2D confinement



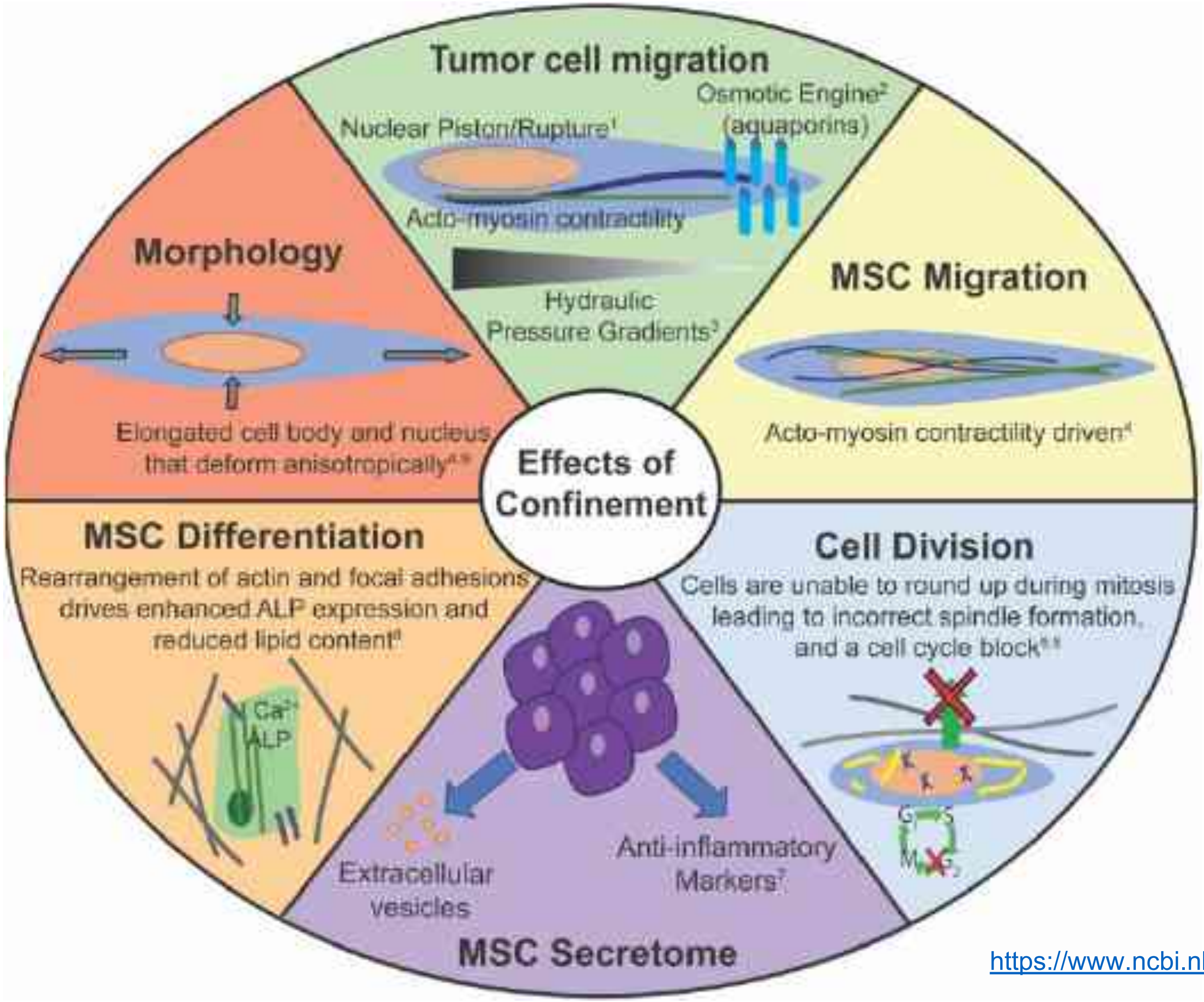
https://pubmed.ncbi.nlm.nih.gov/32641972/

Inhibiting Rac polarization blocks the migration of cells



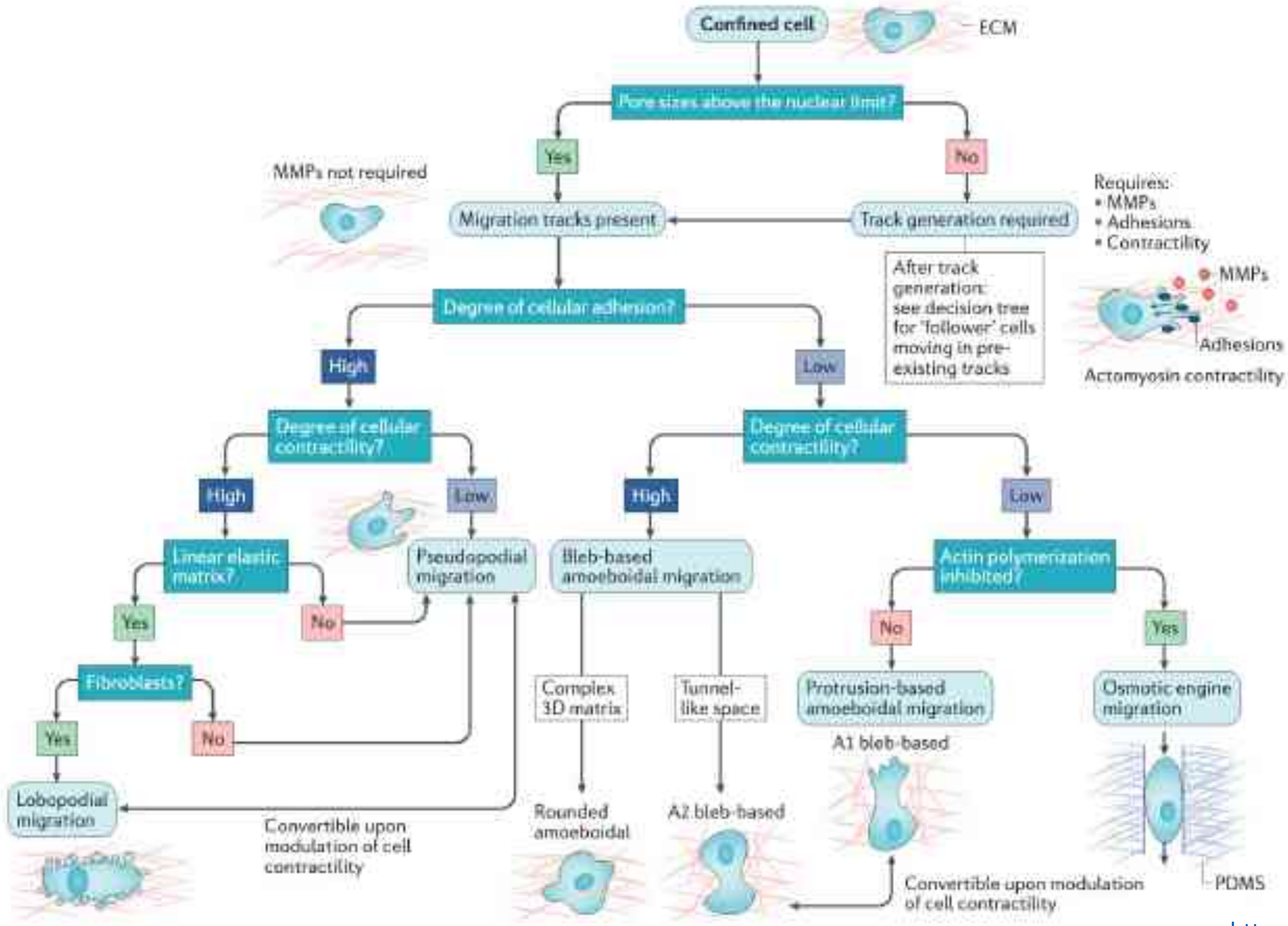
https://pubmed.ncbi.nlm.nih.gov/32641972/

Confinement affects cell behavior



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7193100/

Determinants of cell migration in confinement



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

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

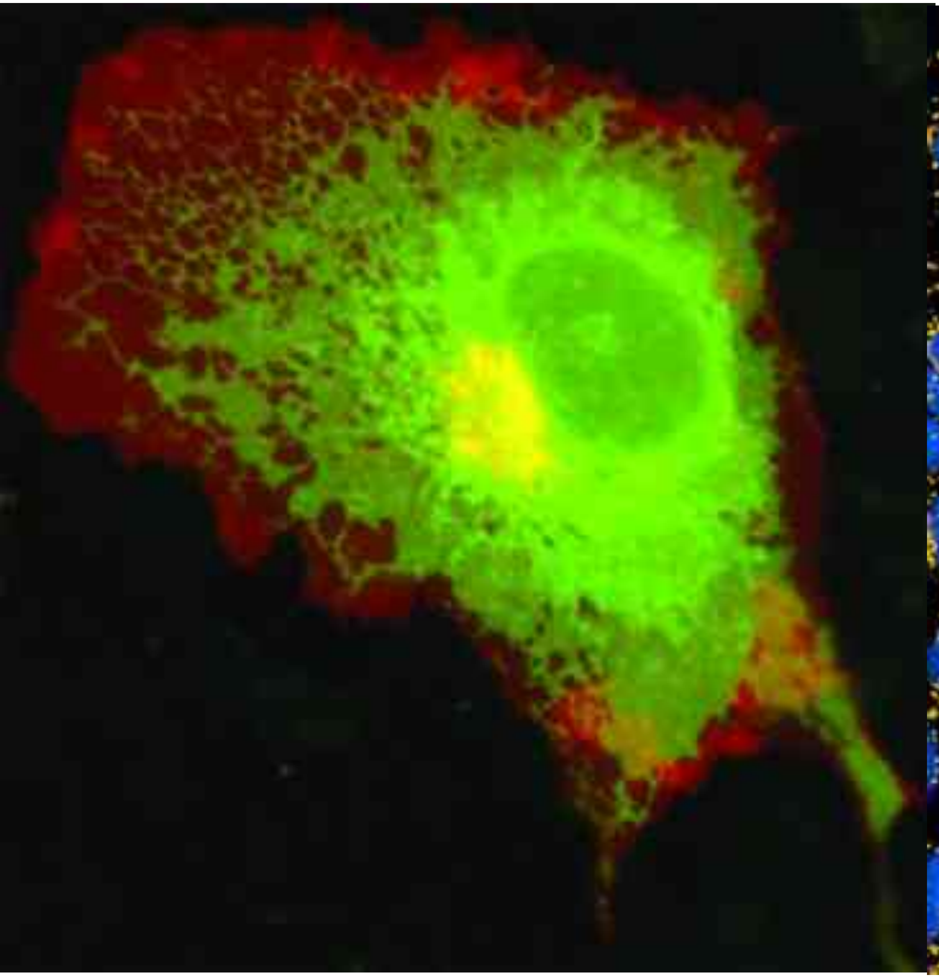
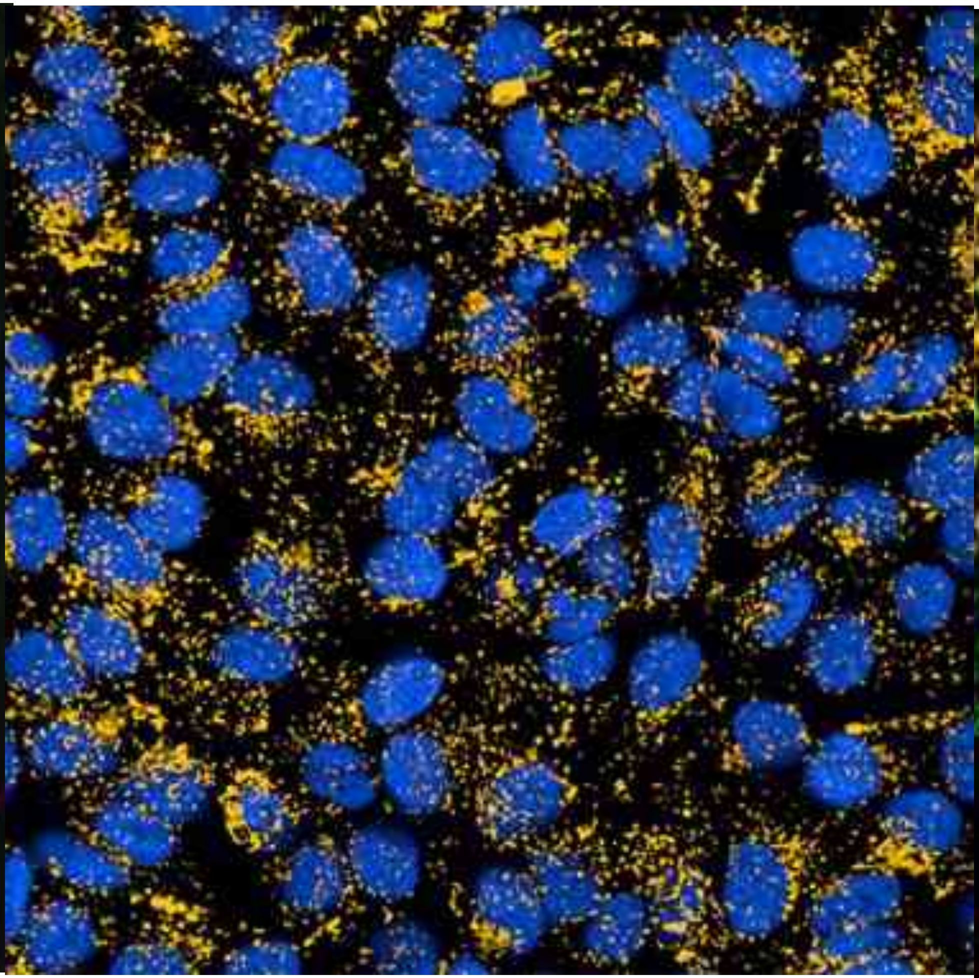


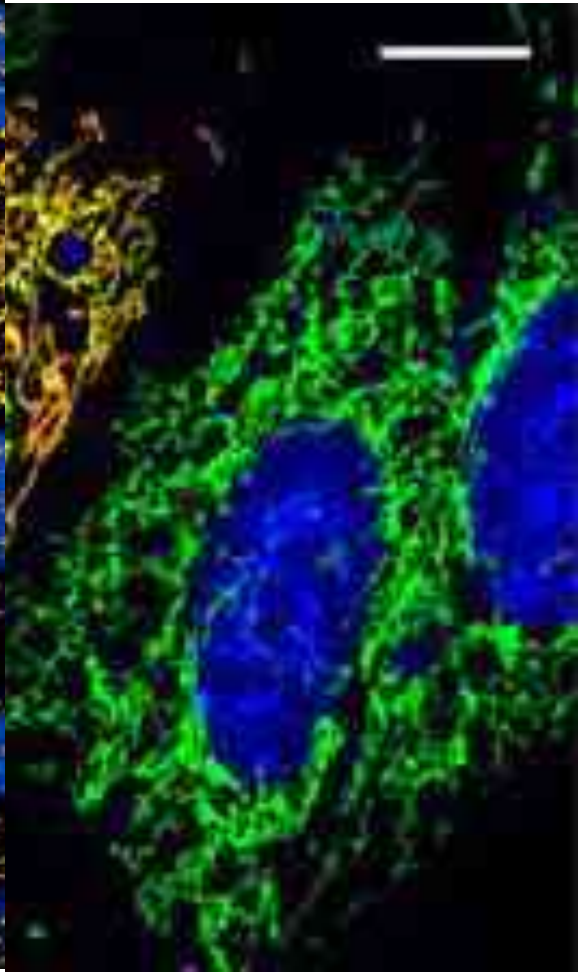
Image from K Hirschberg

Lysosomes



https://www.perkinelmer.com/

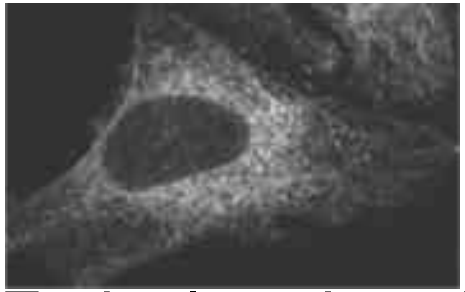
Mitochondria

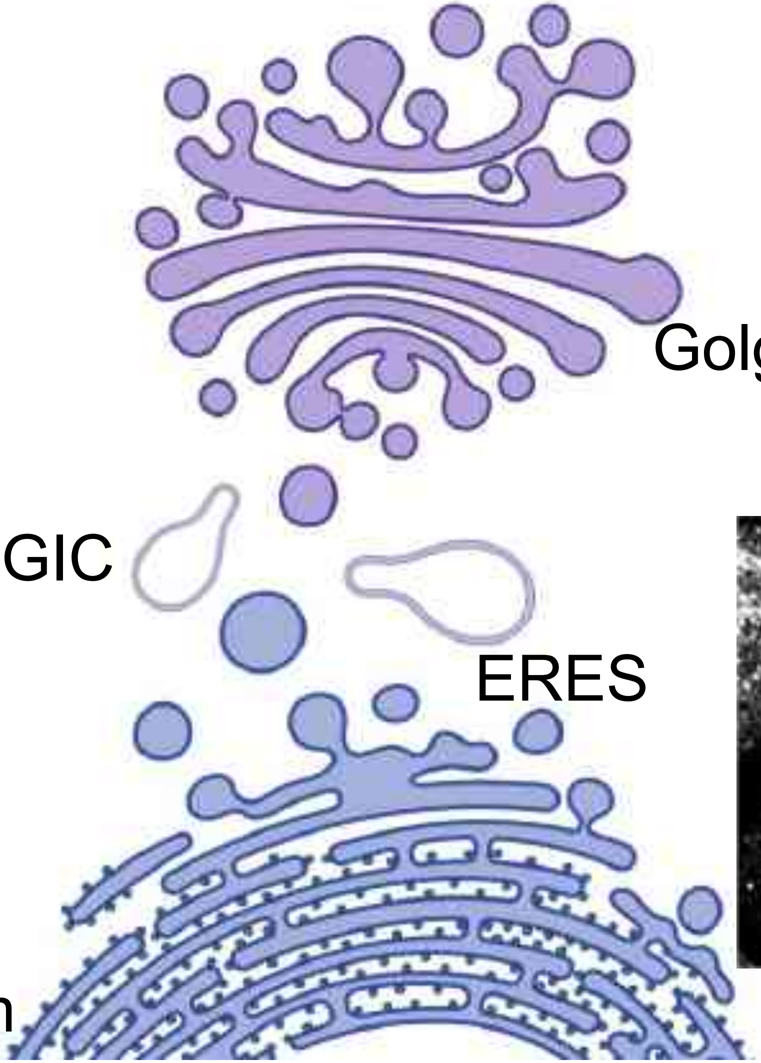


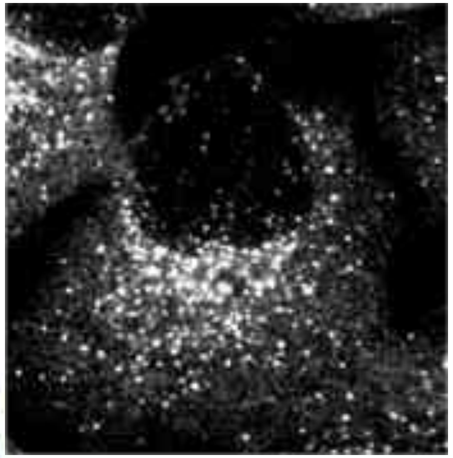
https://www.mpg.de/12309861/gene-therapy-mitochondrial-diseases

Mechanobiology is also linked to secretion

The secretory pathway

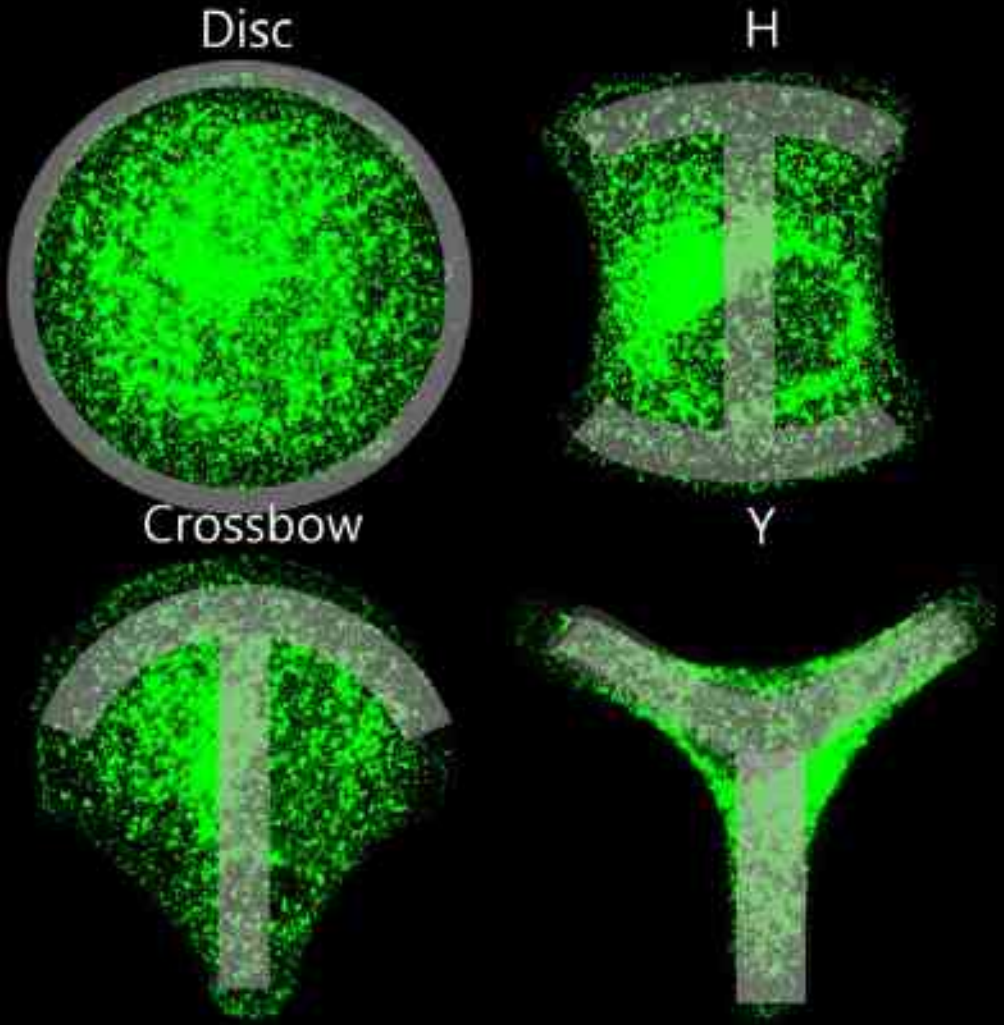




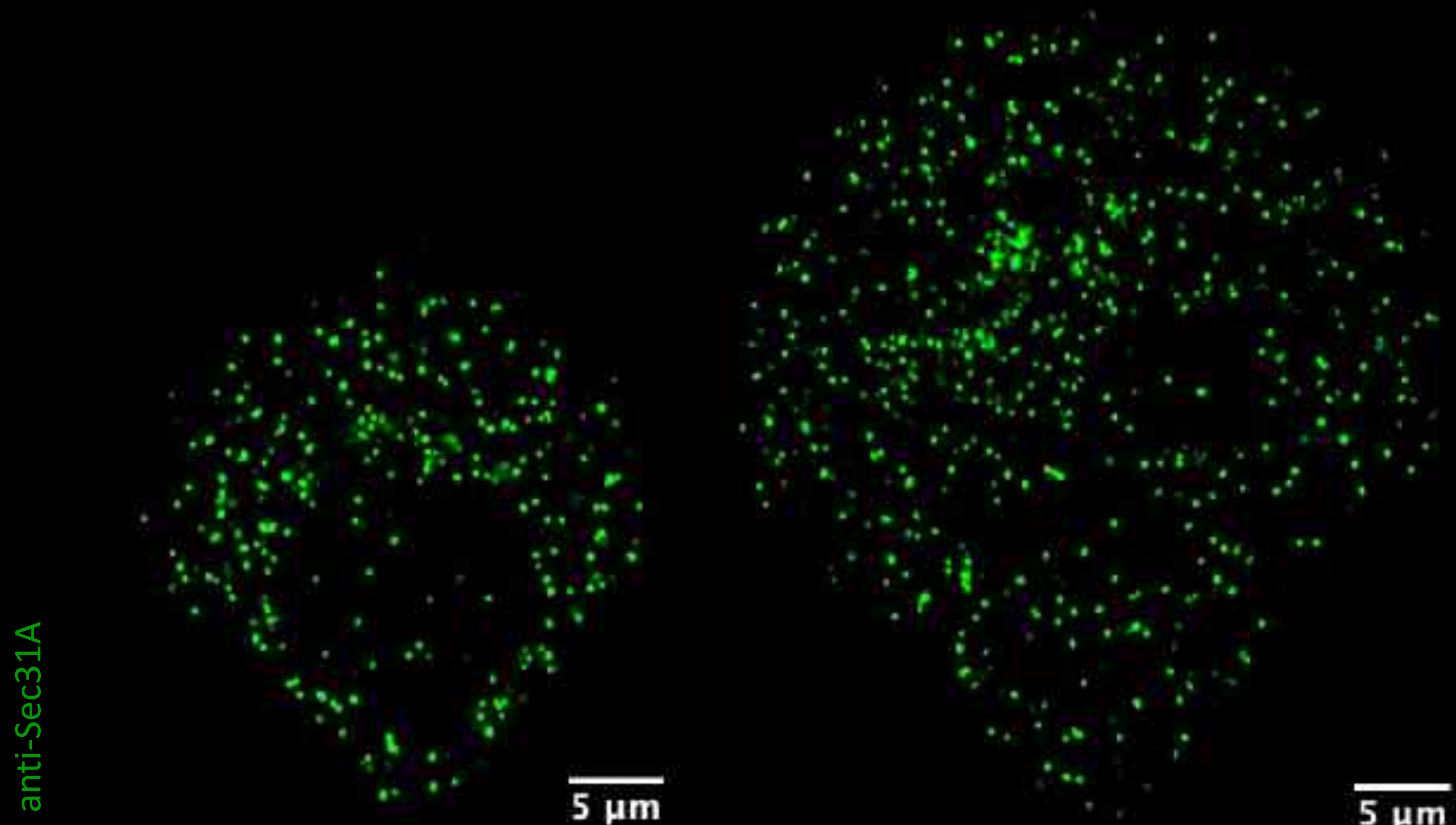


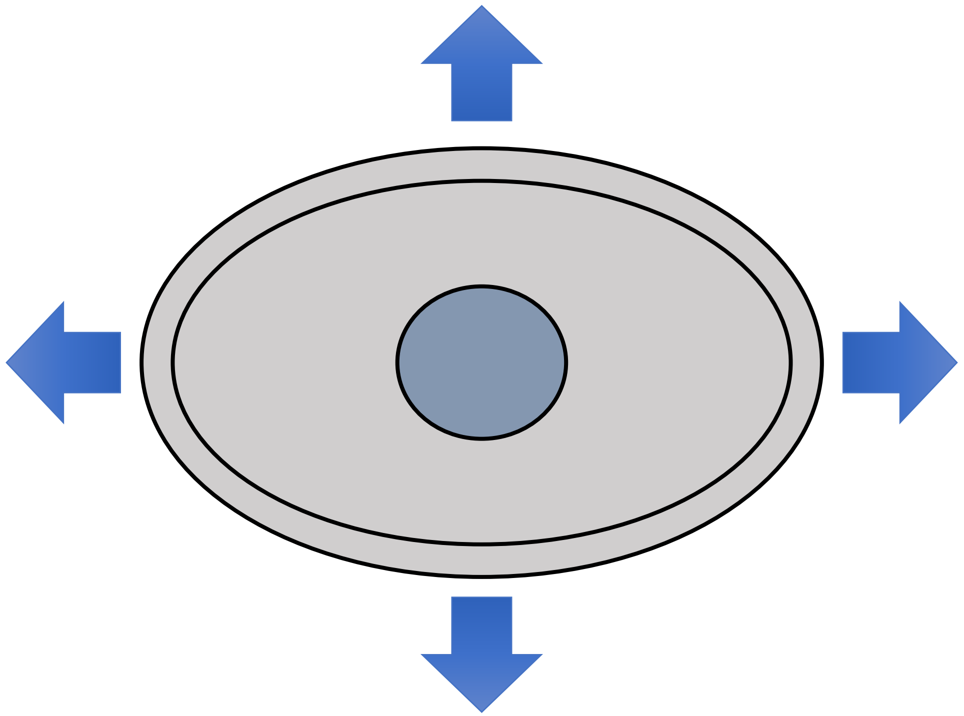
Endoplasmic reticulum

Cells on micropatterns



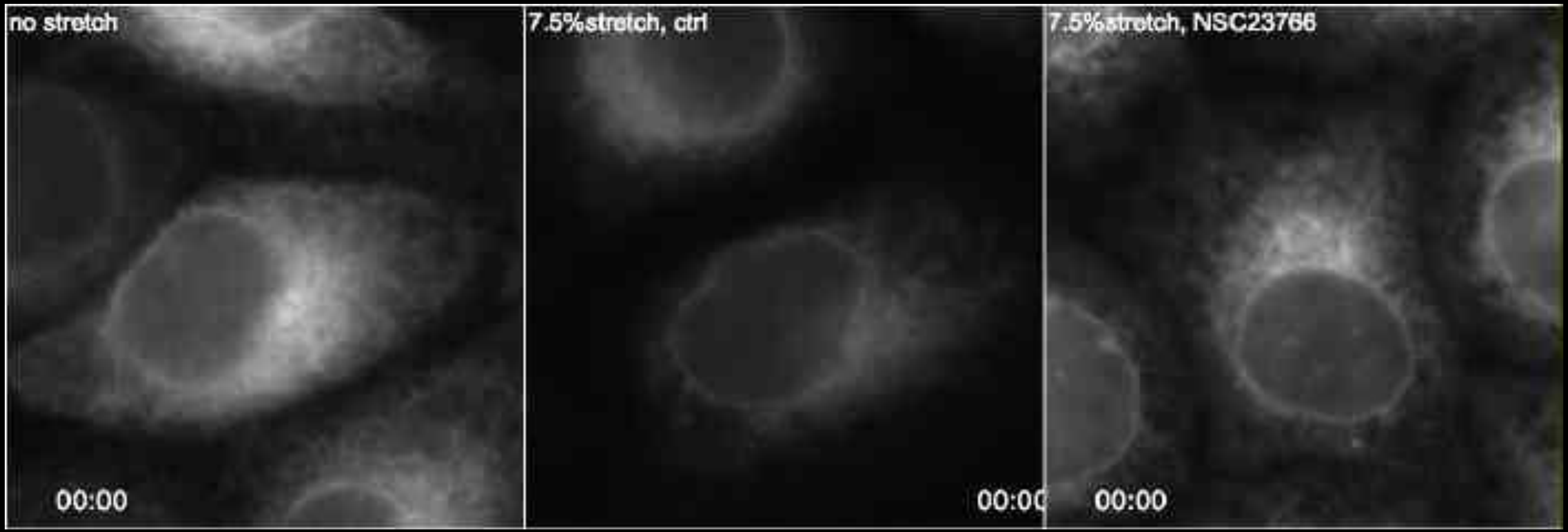
Forcing cells to grow on large pattens increases ERES



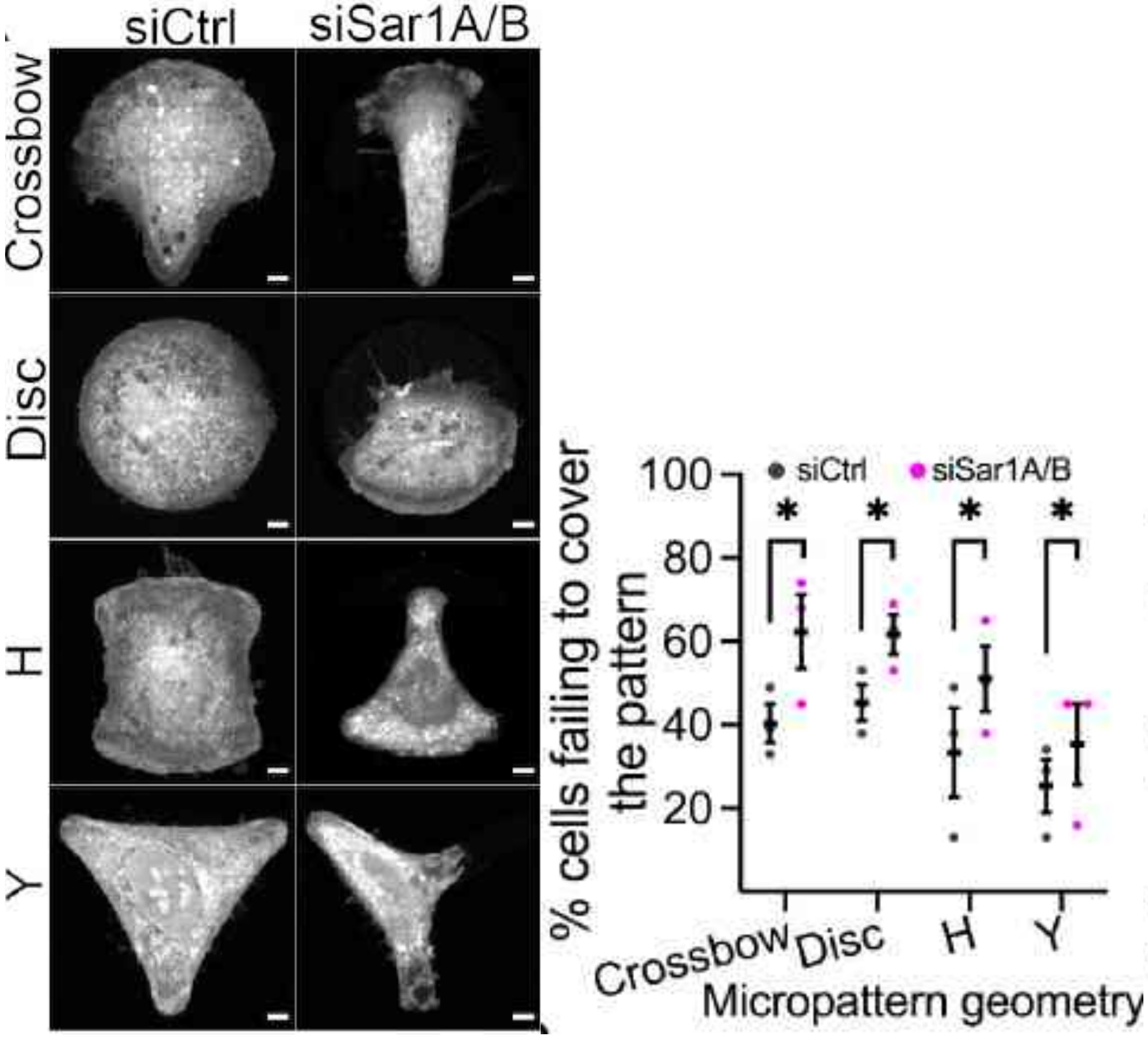


Mechanotension accelerates ER-Golgi transport in a Rac-dependent manner

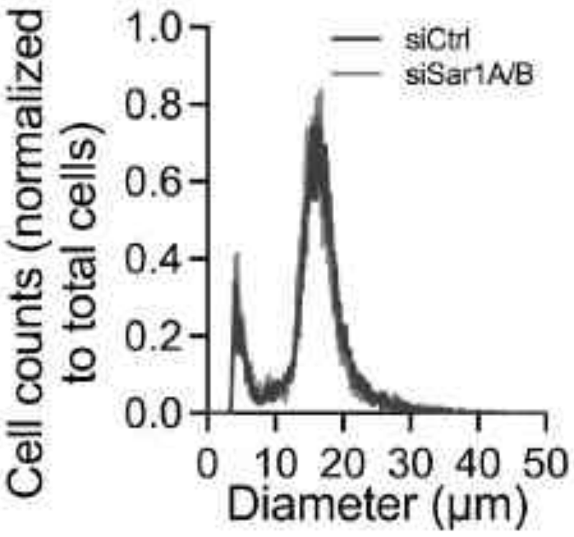
RUSH assay

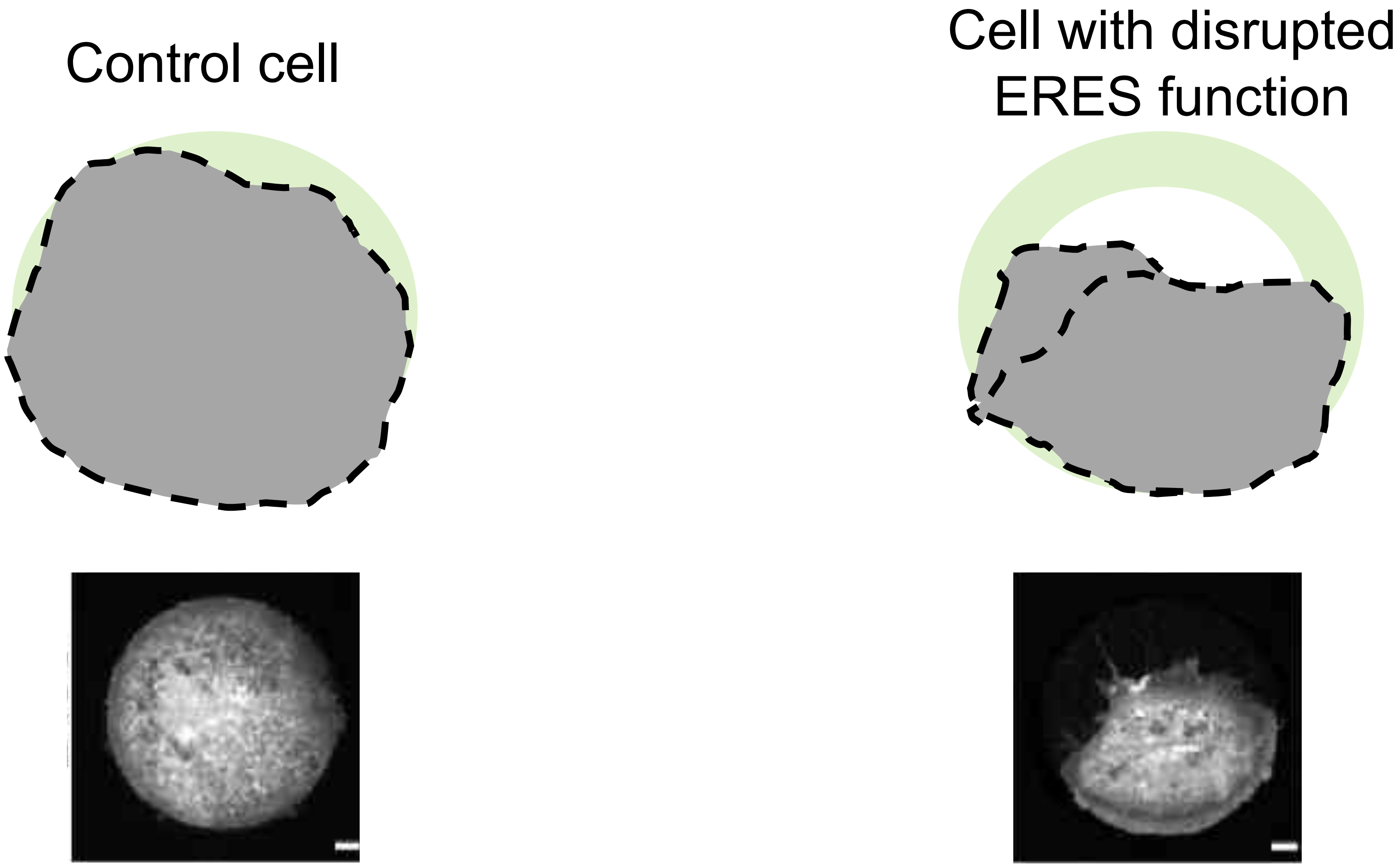


Blocking ERES function prevents cells from spreading on large micropatterns

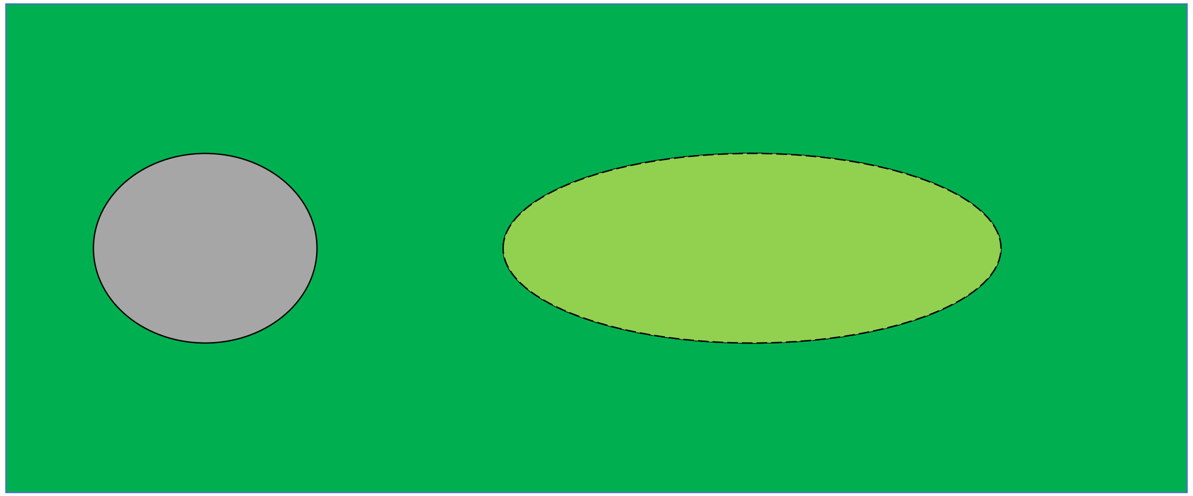


Not due to cell size difference

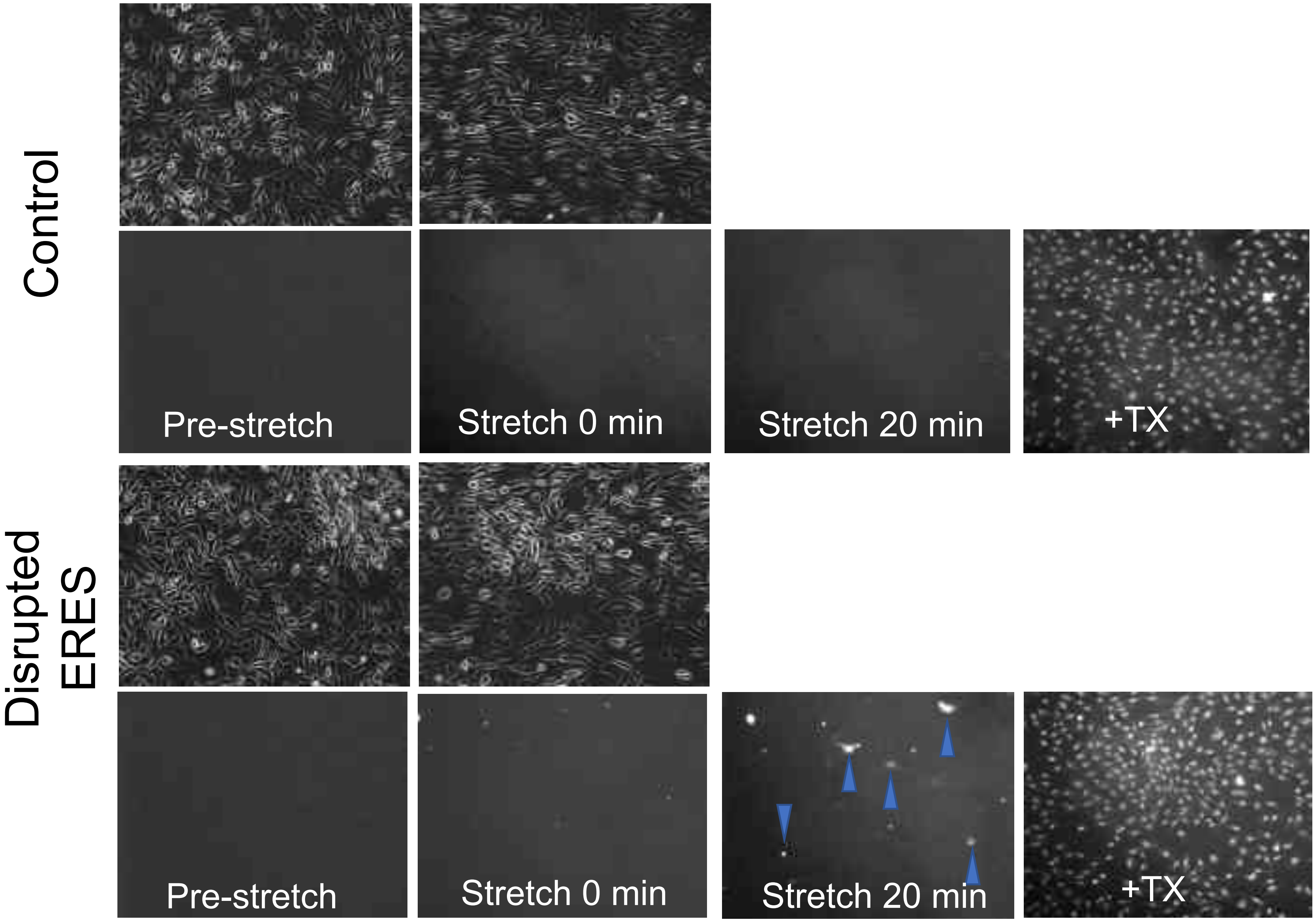



Control cell
Cell with disrupted
ERES function

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
Mechanical control
of ER exit sites
Sar1
Rac1
Rac1
Sar1
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

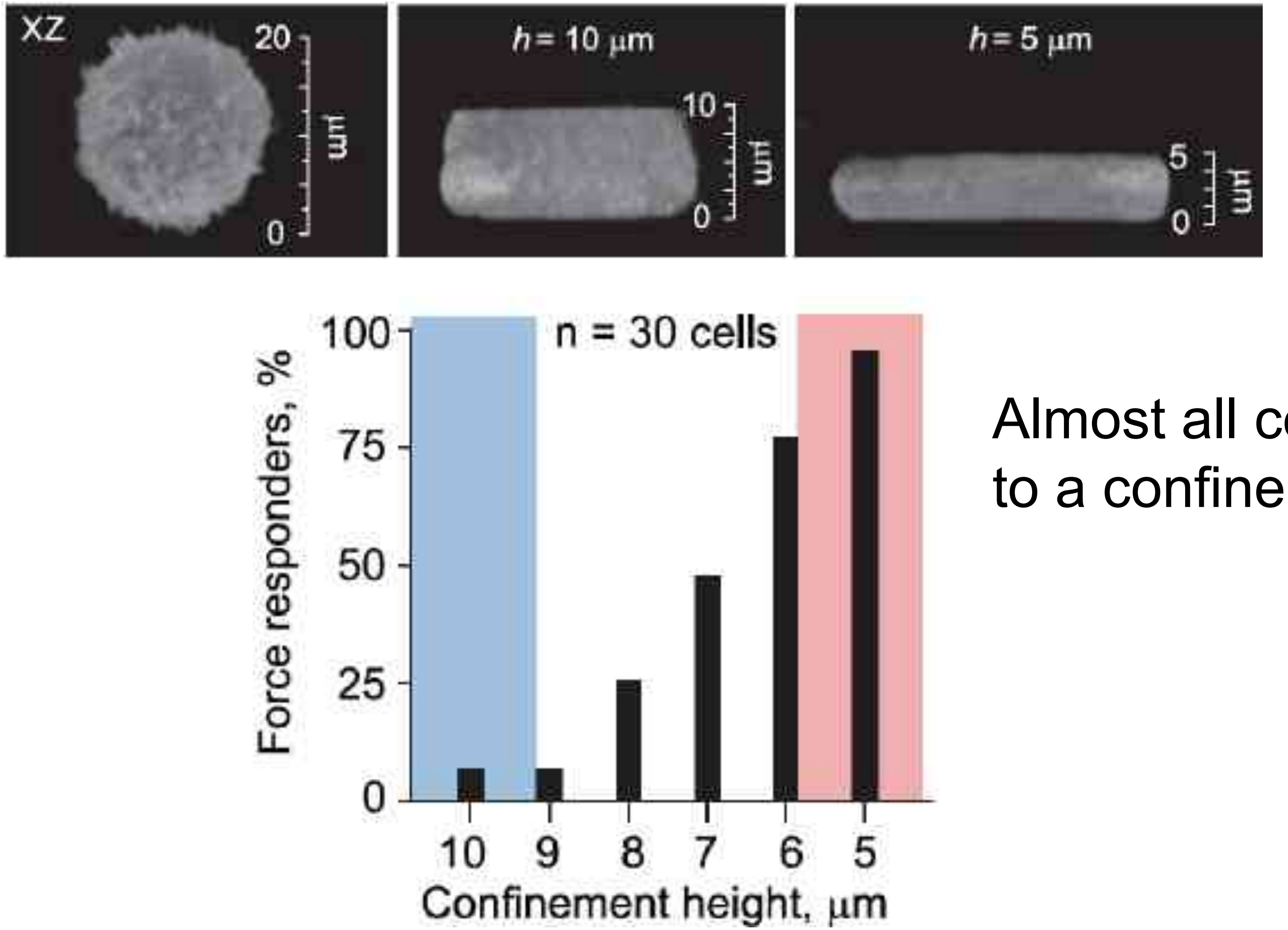
The nucleus measures shape changes for cellular proprioception to control dynamic cell behavior

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/

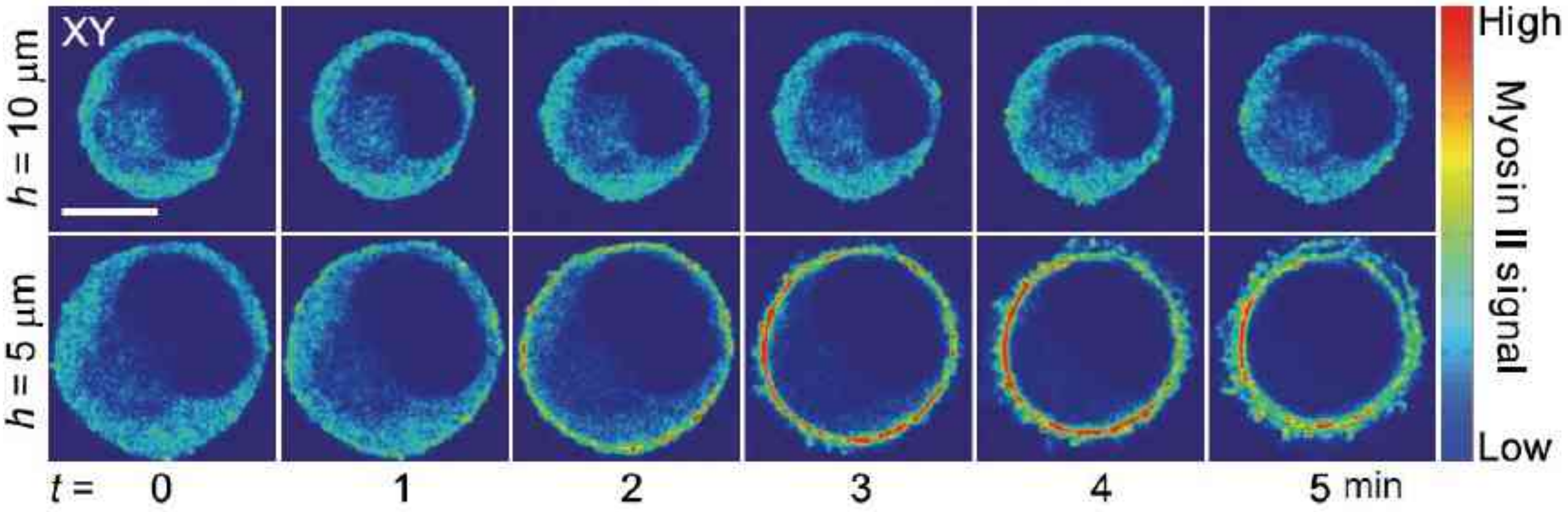
https://pubmed.ncbi.nlm.nih.gov/33060331/

Response of cells to confinement (squeezing)

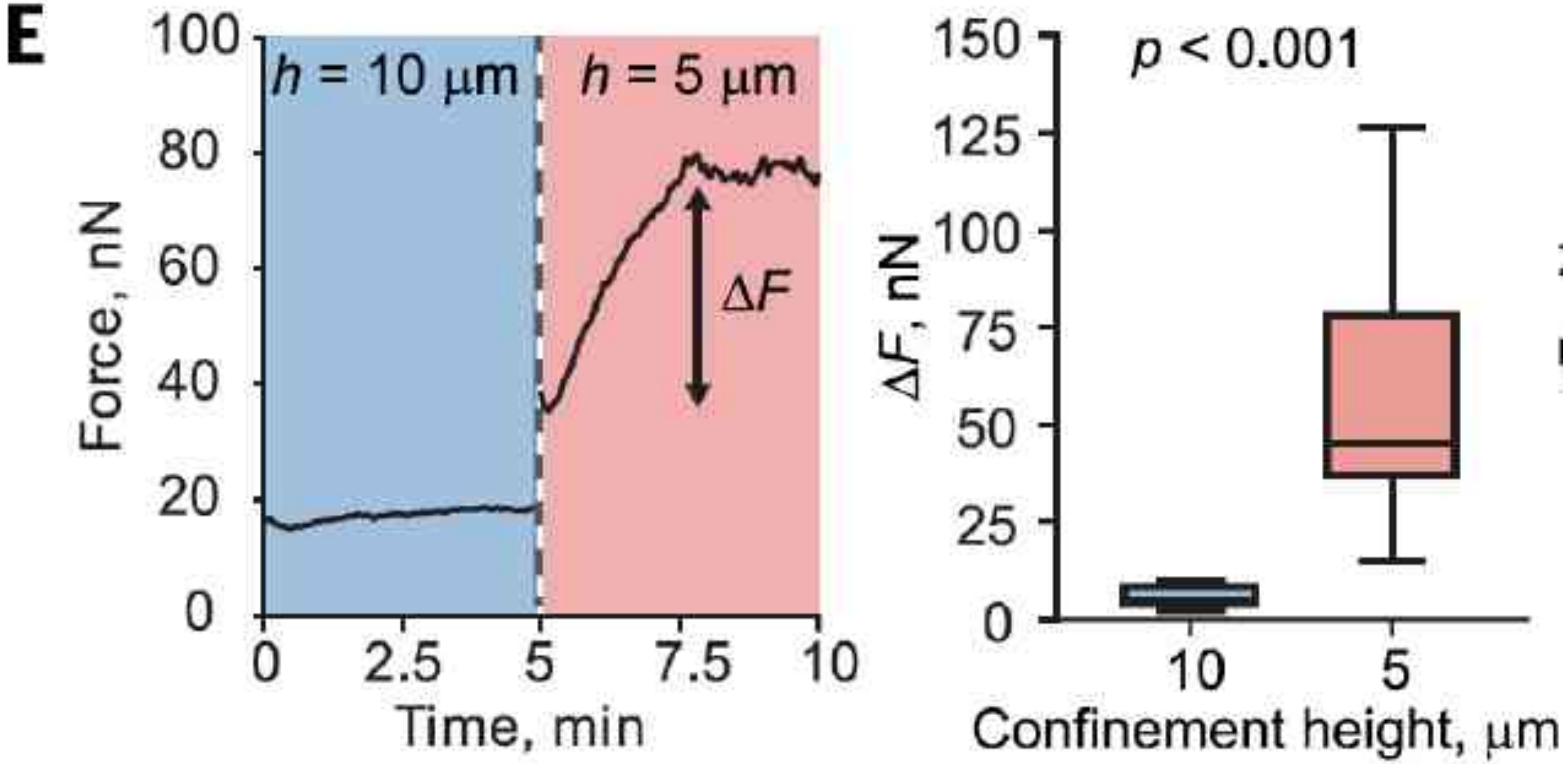


Almost all cells respond to a confinement of 5 µm

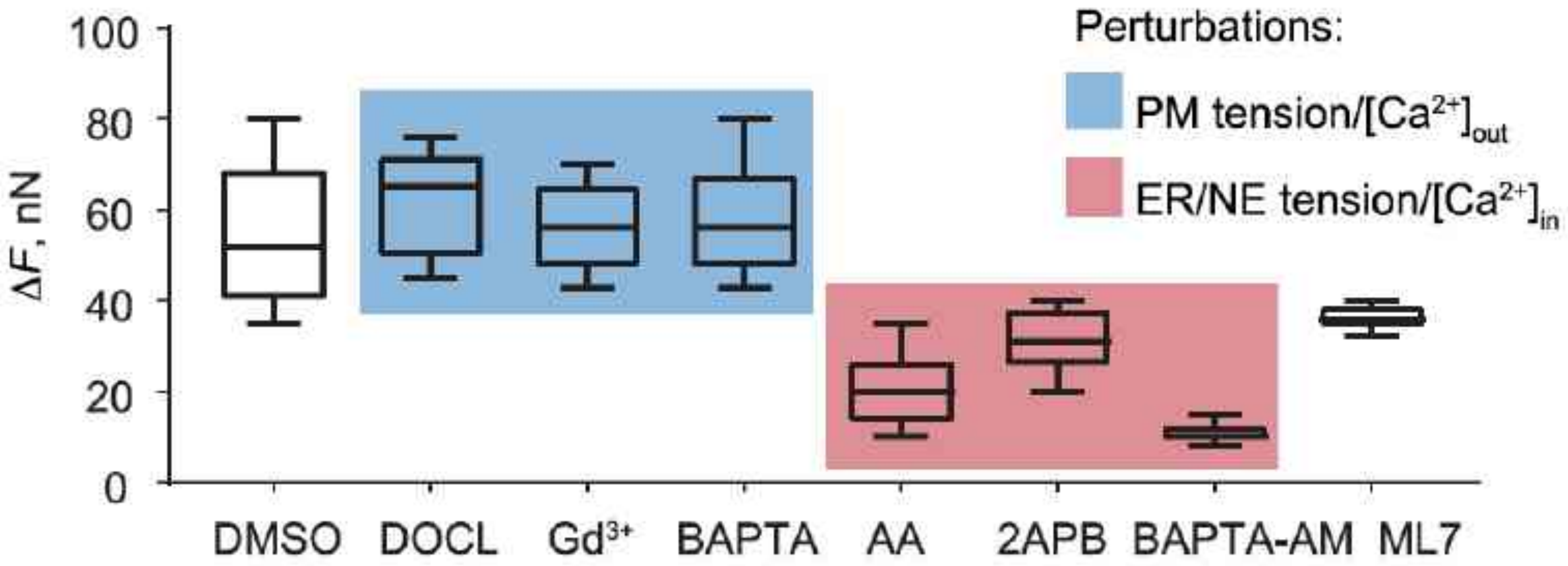
Rapid myosin recruitment to the cell surface upon squeezing



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

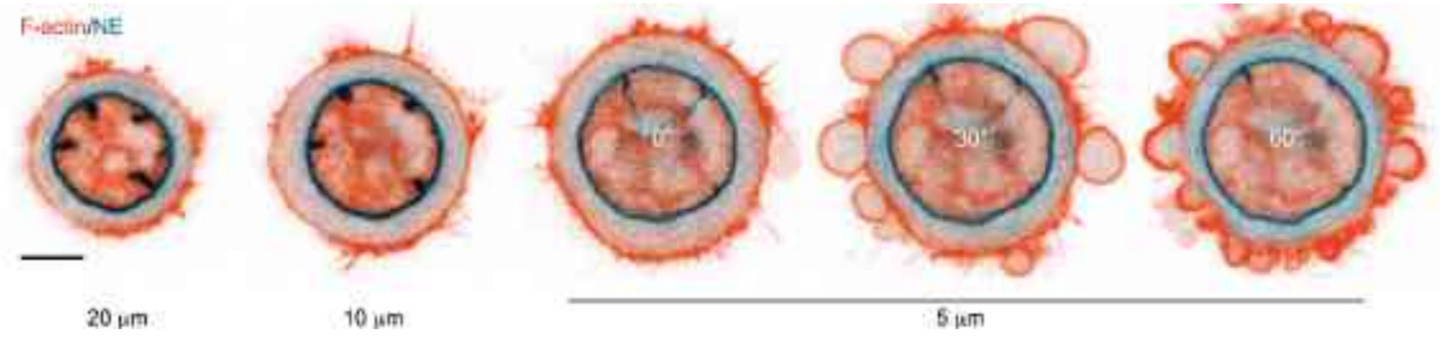
Gd= gadolinium (III) cholride. Inhibits mechanosensitive plasma membrane channels

BAPTA= chaleates calcium extracellularly (-AM is for intracellular calcium)

AA= AACOCF3. Inhibits cPLA2

2APB= Xestospongin. Inhibits stretch-activated IP3 receptors in the ER

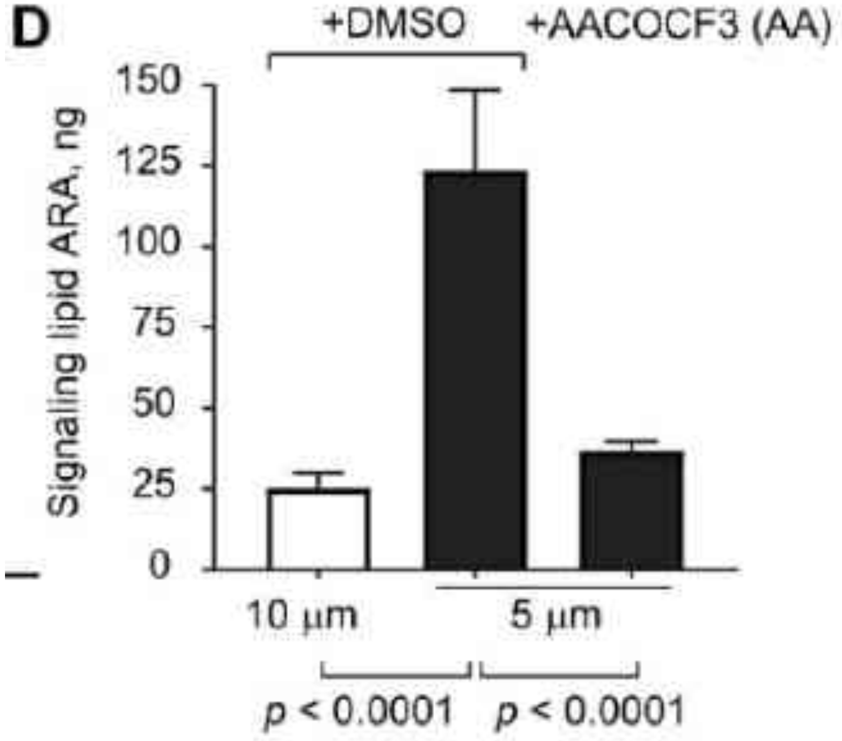
No evidence for nuclear rupture



HeLa
Lap2B-GFP
SiR-actin-stained
confinement
5 s interval
20-10-5 µm confinement

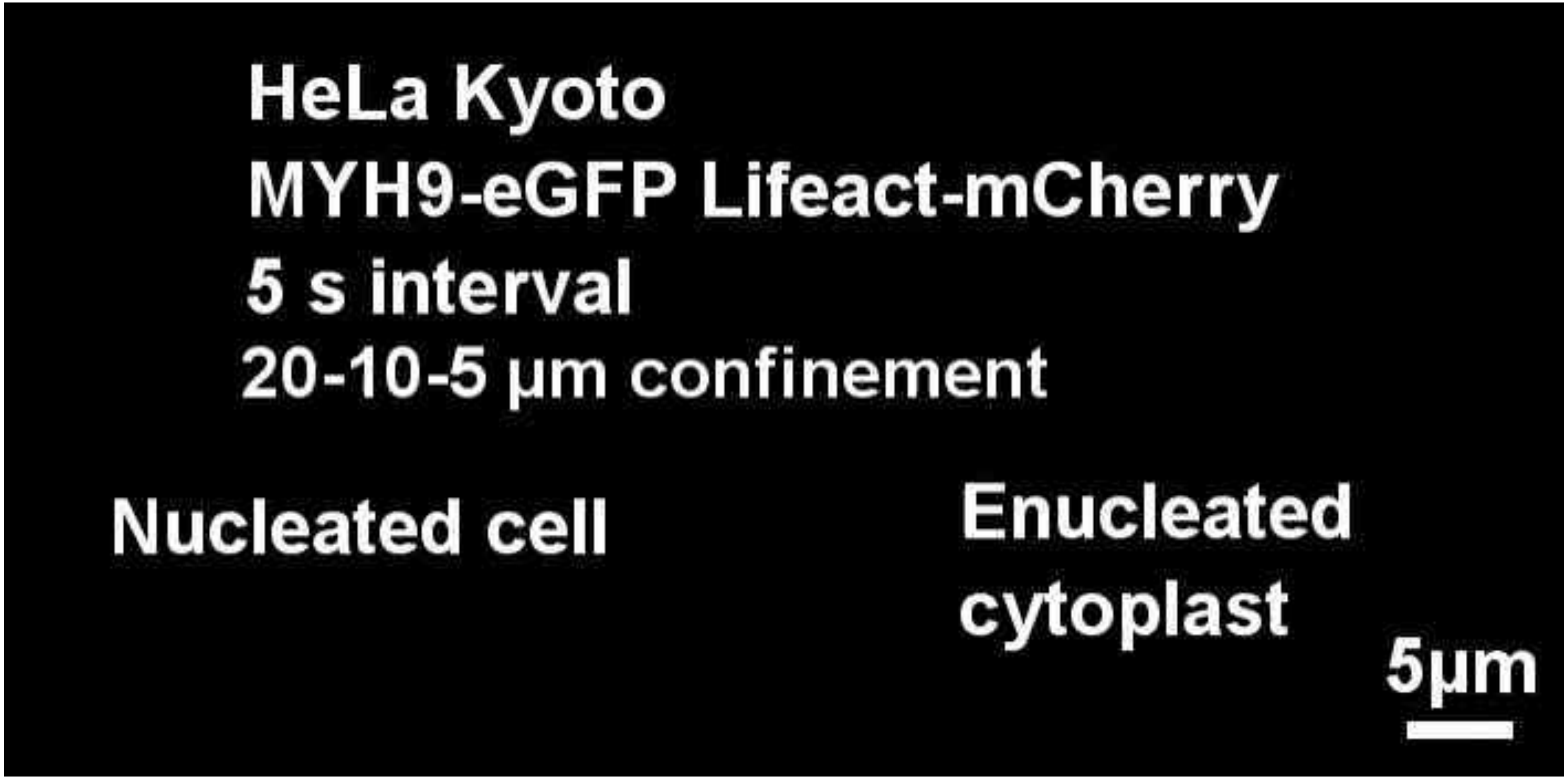
5µm

Confinement induces the production of arachidonic acid → cPLA2 is activated



AACOCF3= inhibitor of cPLA2

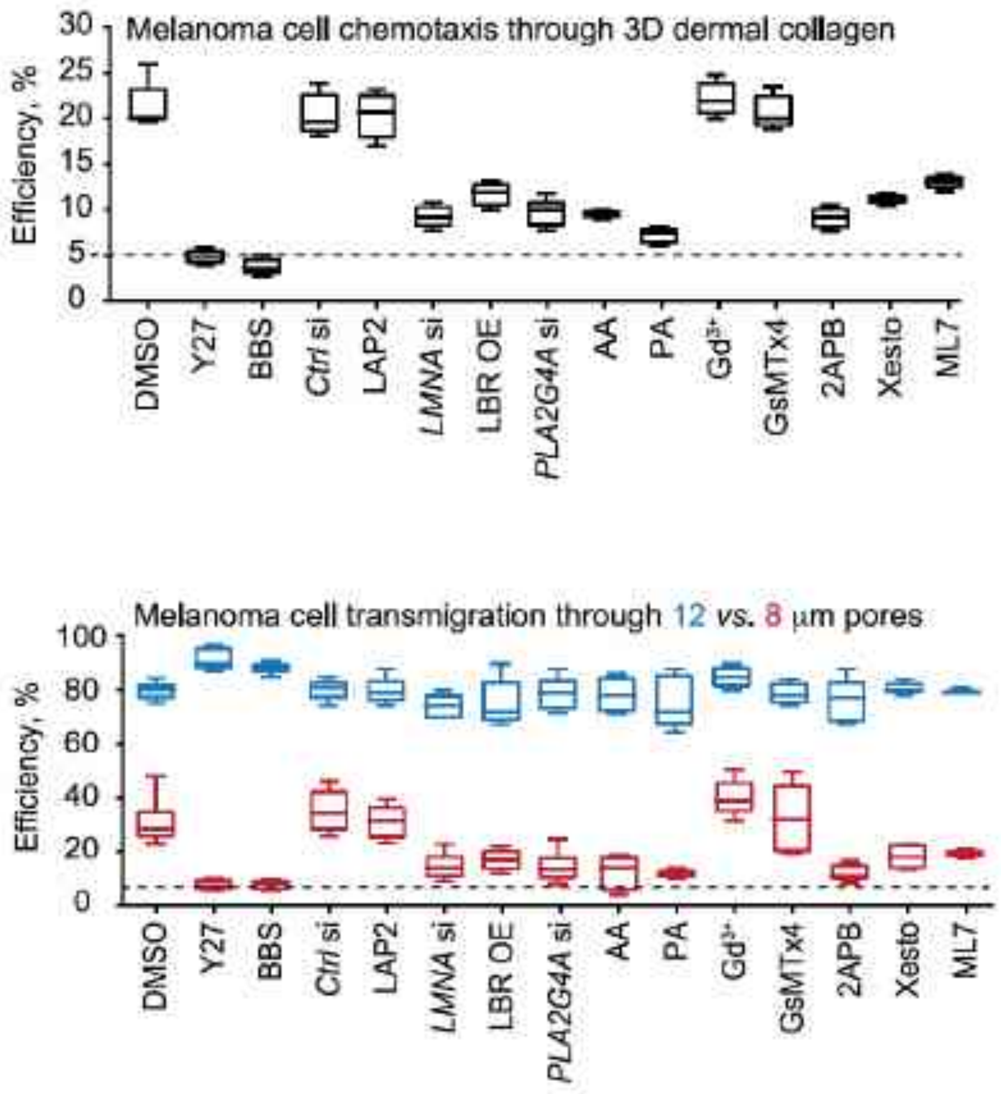
Confinement-induced membrane blebbing requires the presence of the nucleus

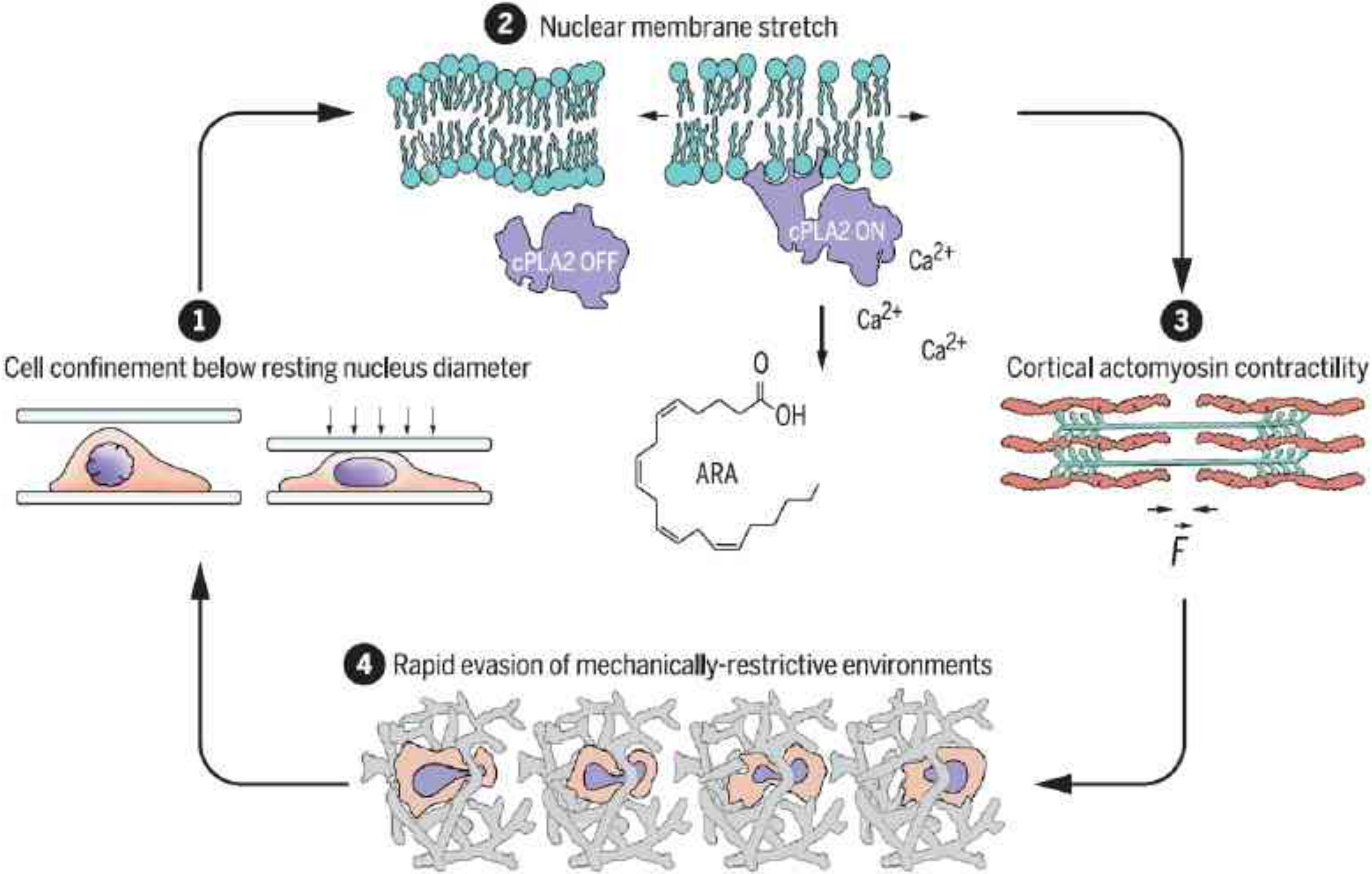


Confinement-induced membrane blebbing requires the presence of the nucleus

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
Ca2+
Ca2+
Ca2+
O
OH
ARA
1
Cell confinement below resting nucleus diameter
3
Cortical actomyosin contractility
F
4 Rapid evasion of mechanically-restrictive environments

Thank you for your attention