Extracellular matrix mechanical cues regulate lipid metabolism through Lipin-1 and SREBP

Nature Cell Biology. 2019 Mar;21(3):338-347.

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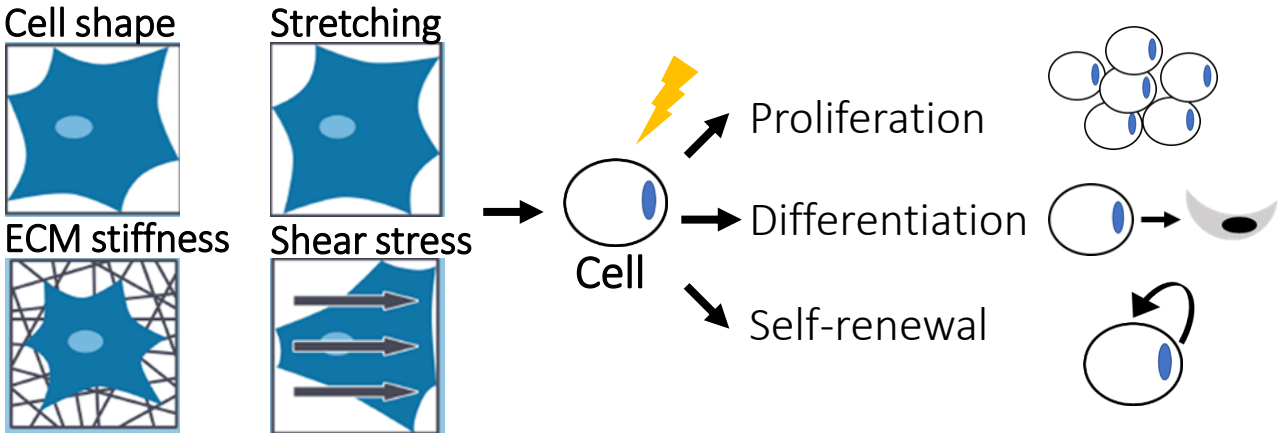
Commentator: Dr. Peng, I-Chen (彭怡禎)

Date: 2019/10/4

Mechanical cues is important to control cell function

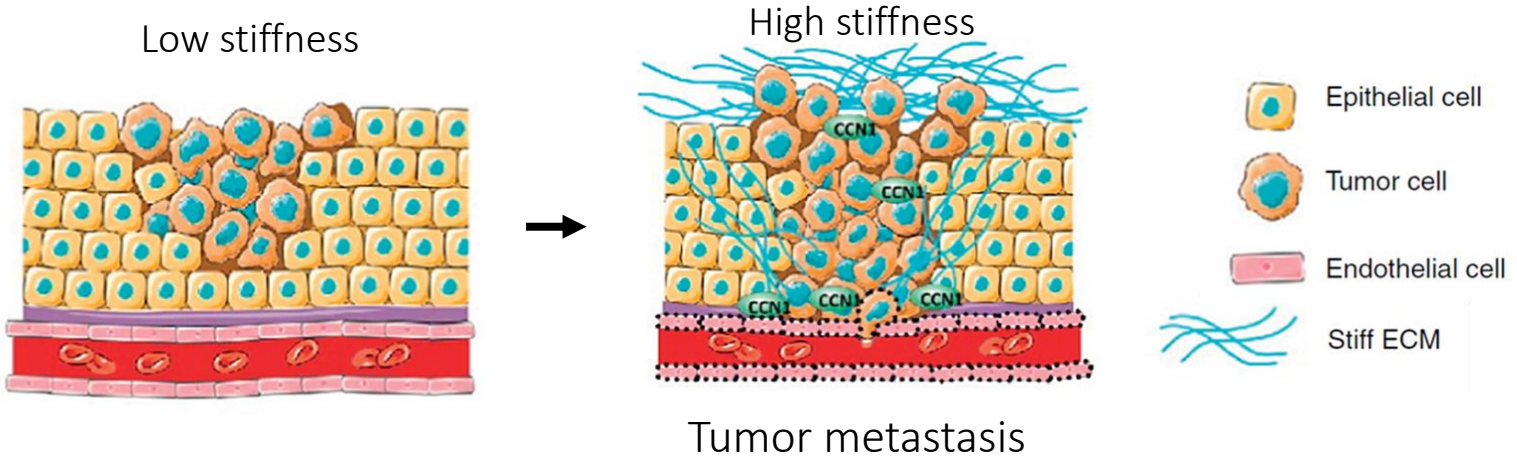
- Mechanical cues regulate cell proliferation, differentiation, and self–renewal.

Nat Rev Mol Cell Biol. 2017 Dec; 18(12): 728–742.

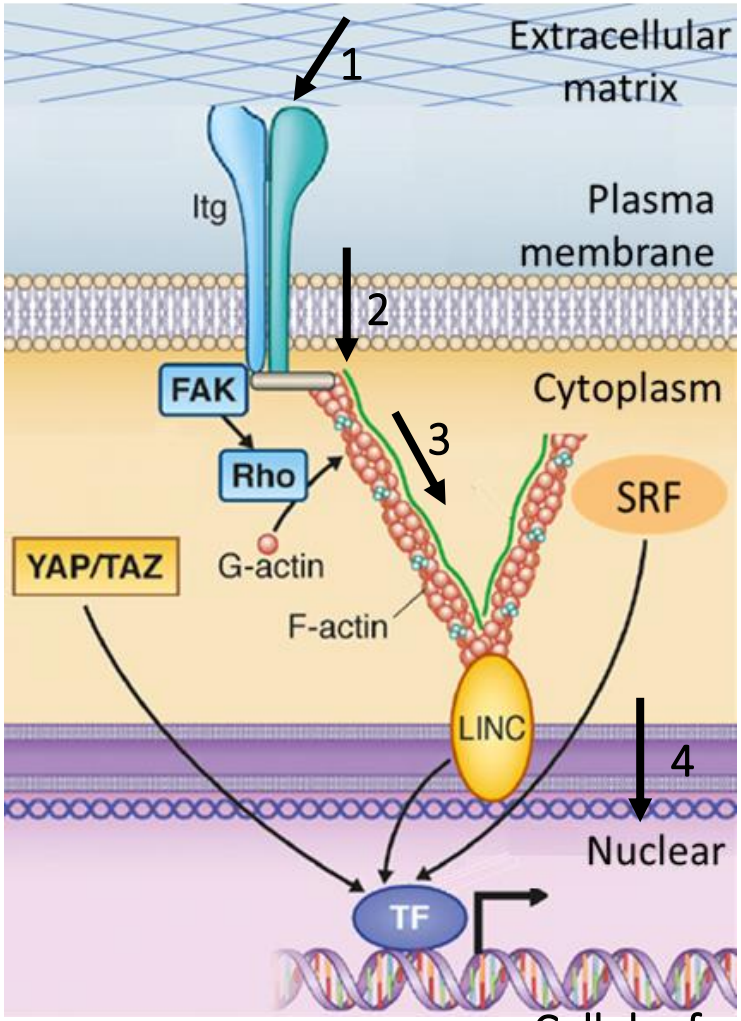


- Extracellular matrix (ECM) mechanical forces promote cancer progression.

J Cell Biol. 2018 May 7;217(5):1571-1587.



Mechanotransduction



1. Mechanical stress

2. Transmembrane receptors transduce the signal into cytoplasm

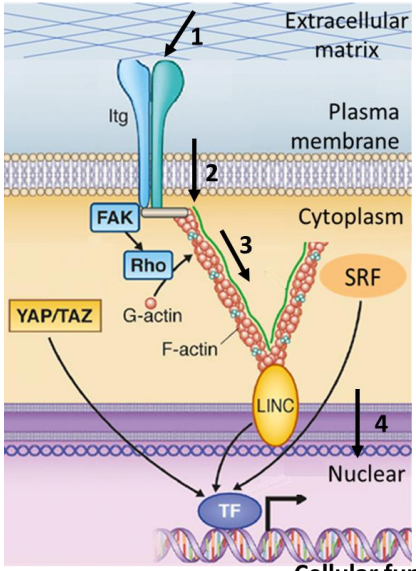
3. Cytoskeleton reorganize

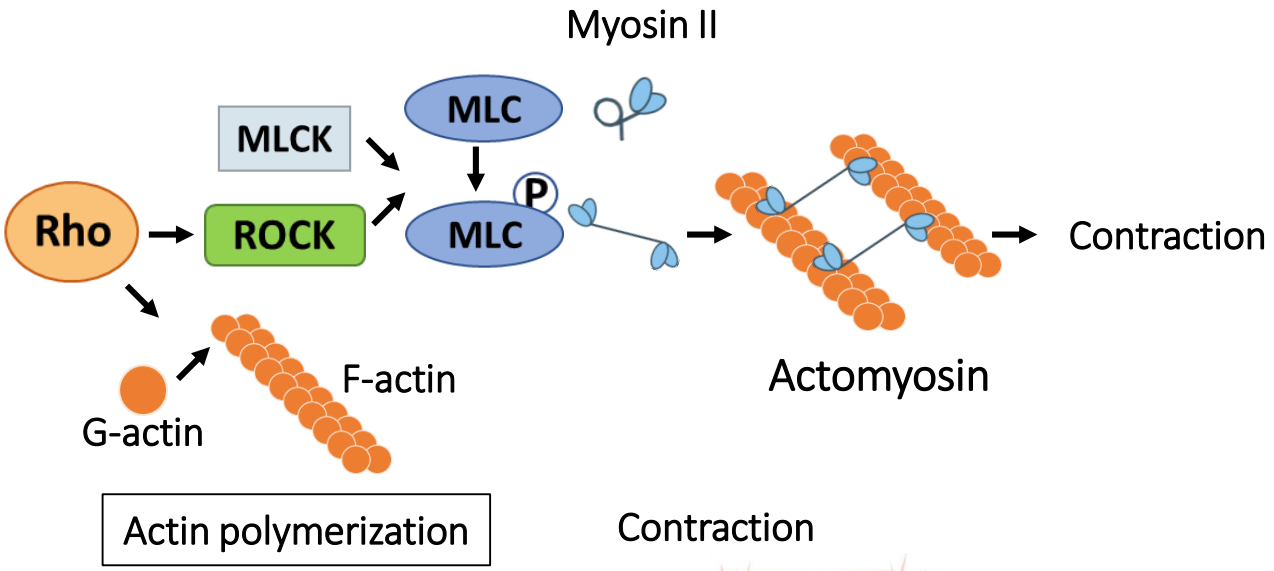
4. Nuclear mechanotransduction

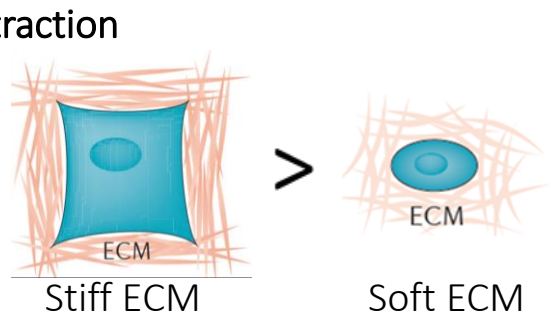
Cellular function

Itg: integrins
LINC: linker of nucleoskeleton and cytoskeleton
Modified from J Clin Med. 2017 May 19;6(5).

Actomyosin contractility is regulated by Rho/ROCK pathway







ROCK: Rho-associated protein kinase
MLC: Myosin light chain
MLCK: Myosin light-chain kinase

Nat Rev Mol Cell Biol. 2014 Dec;15(12):825-33.

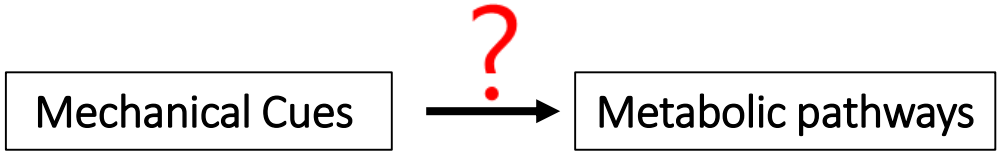
Mechanical forces and cell metabolism regulate cell function

- Linking E-cadherin mechanotransduction to cell metabolism through force mediated activation of AMPK.

  Nat Cell Biol. 2017 Jun; 19(6): 724–731.

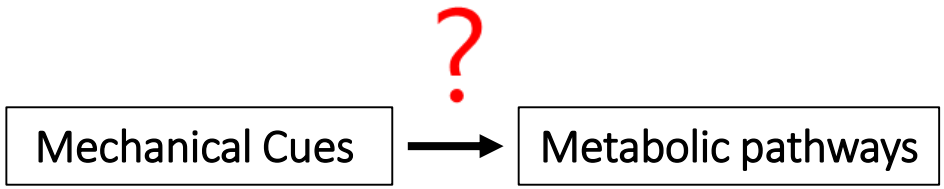
- Metabolic and Mechanical Cues Regulating Pluripotent Stem Cell Fate.

  Trends Cell Biol. 2018 Dec;28(12):1014-1029.



Hypothesis

Mechanical forces regulate metabolic pathway to change cell behaviors

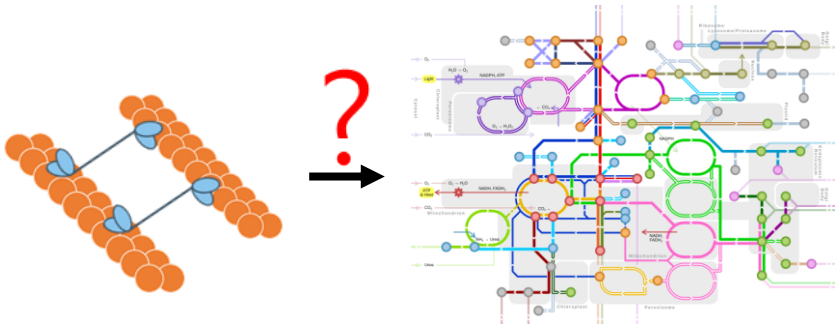


Specific aims

- Aim1: To find metabolic pathways regulated by actomyosin contractility.
- Aim2: To investigate the mechanism of which low contractility induced lipid accumulation.
- Aim3: To find the upstream pathway of which low contractility increased SREBPs activation.
- Aim4: To examine the response of Golgi apparatus on mechanical force.

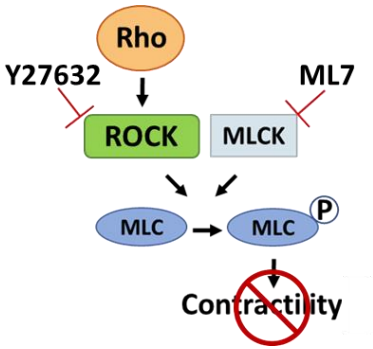
Aim 1

To find metabolic pathways regulated by actomyosin contractility

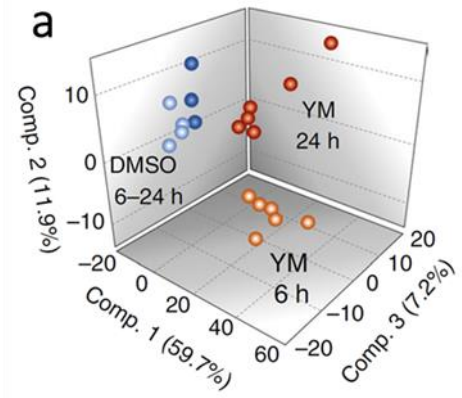


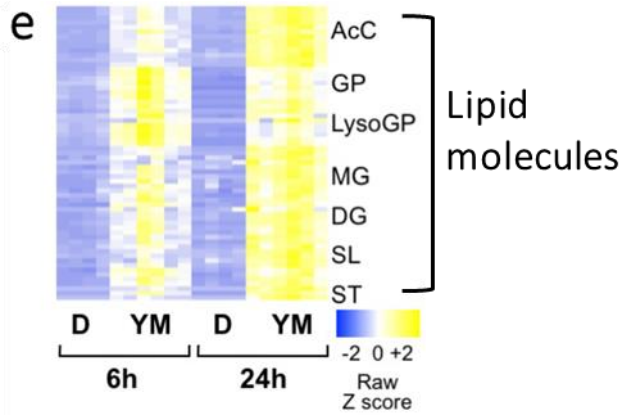
Contractility metabolic pathways

Low contractility increased the expression of lipid molecules

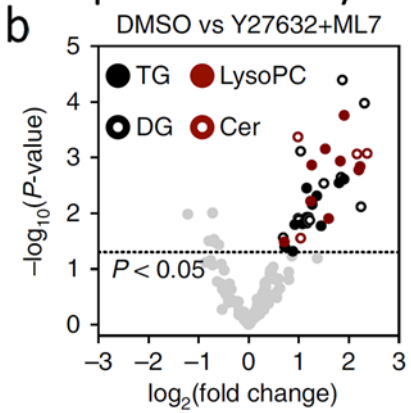


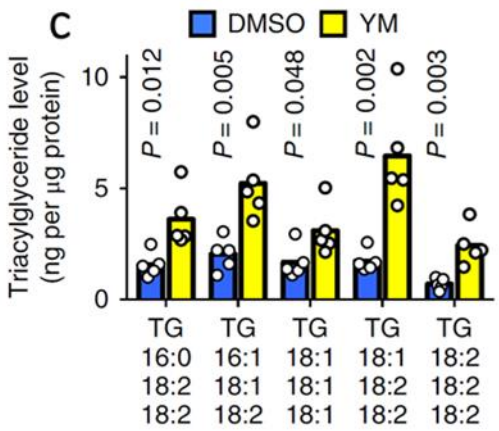
Global metabolomics

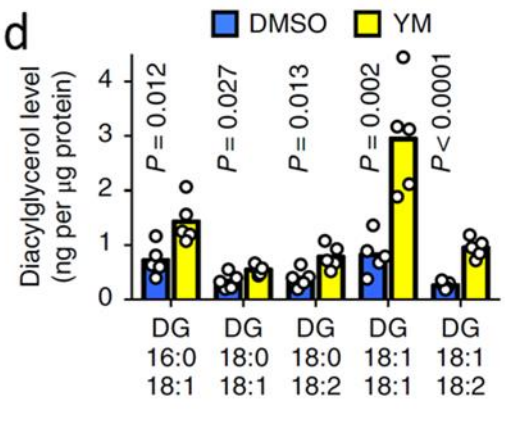




Lipidomic analysis

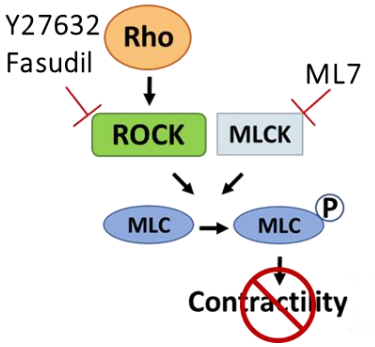


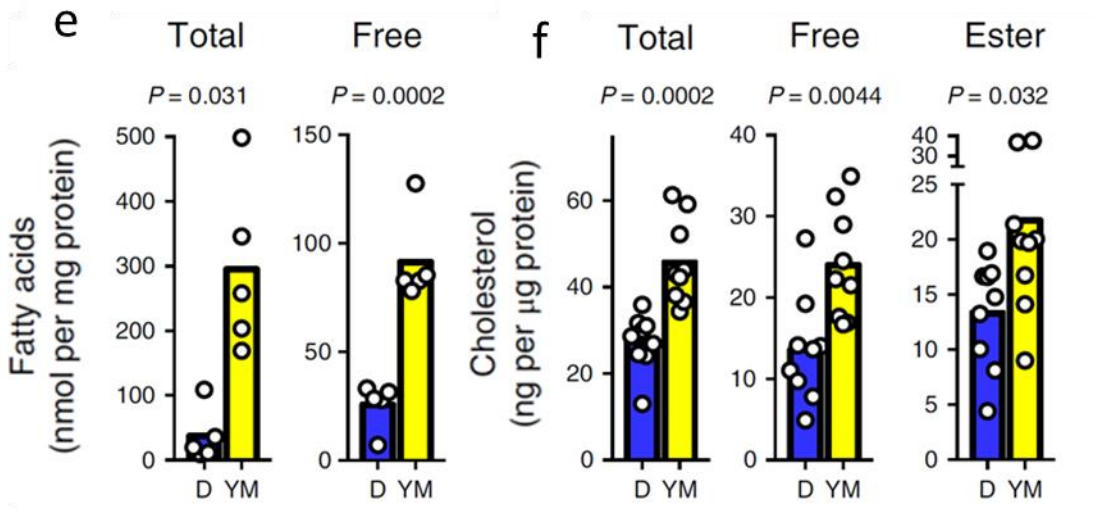


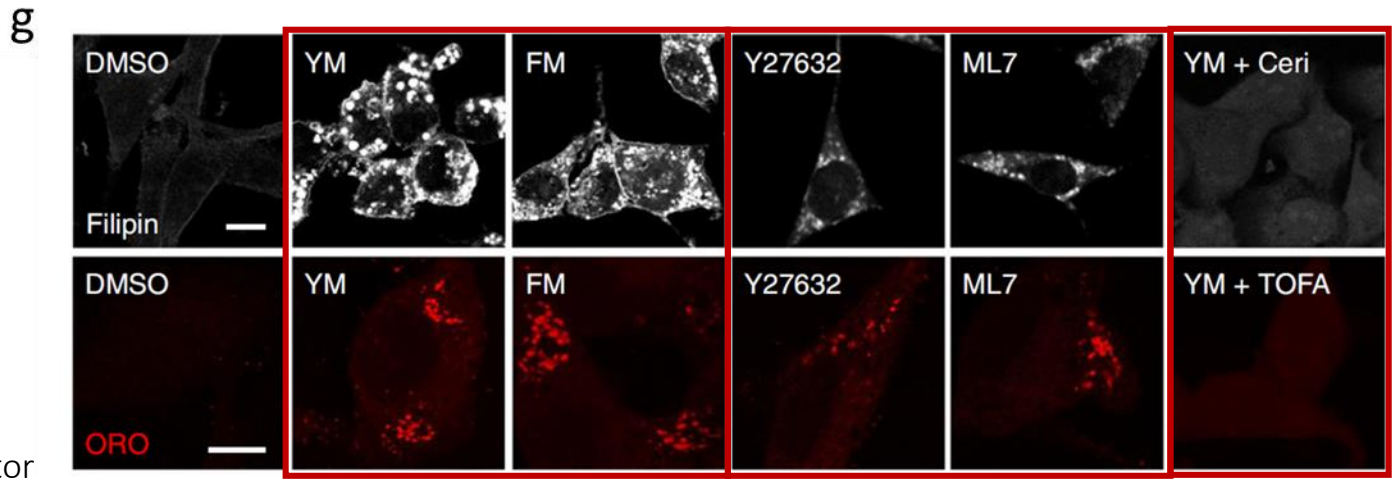


YM: Y27632 and M
TG: triacylglycerols
DG: diacylglycerols
LysoPC: lyso-phosphatidylcholines
Cer: ceramides

Reduction of actomyosin contractility increased cholesterol and fatty acid accumulation

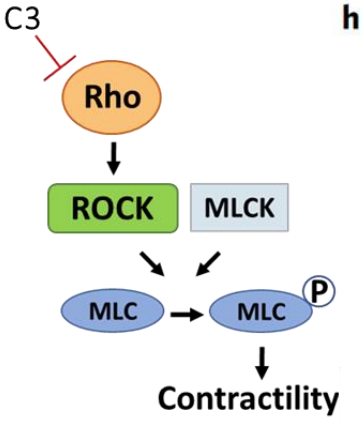


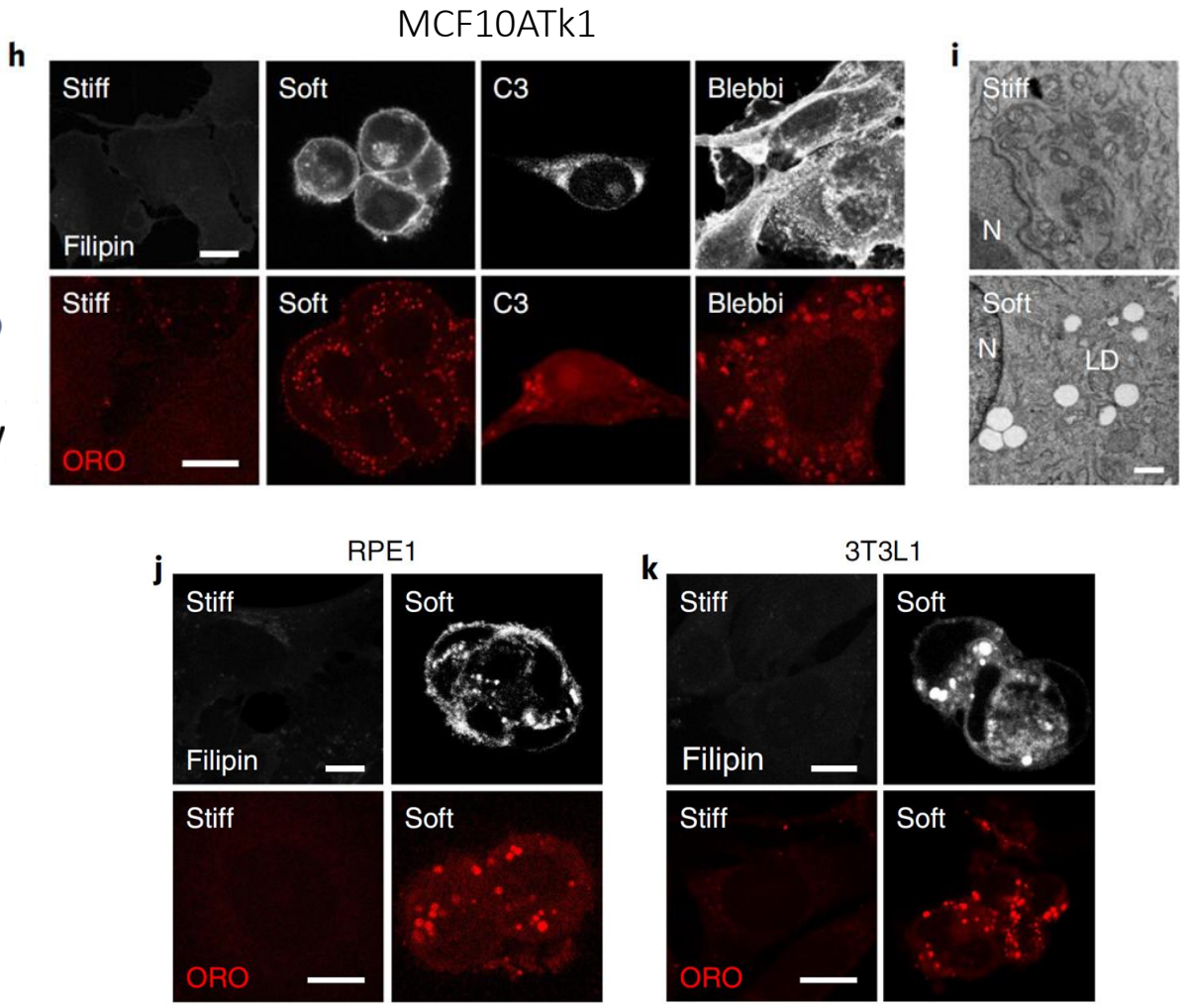




YM: Y27632 +ML7
FM: Fasudil+ ML7
Fasudil: ROCK inhibitor
Cerivastatin (Ceri): cholesterol synthesis inhibitor
TOFA: ACC inhibitor, blocks lipid accumulation
Filipin : fluorescent dye, bind specifically to cholesterol
Oil-Red-O (ORO): stain neutral lipids

Soft ECM increased lipid accumulation

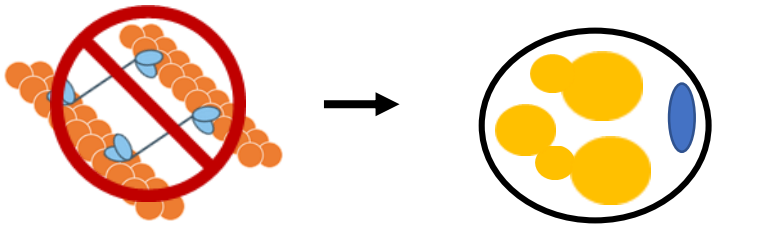




C3: Rho inhibitor
Blebbistatin(Blebbi): myosin II inhibitor

Summary 1

Reduction of actomyosin contractility increased lipid accumulation

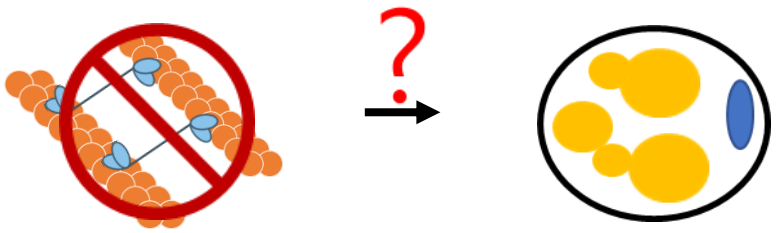


Contractility

Lipid accumulation

Aim 2

To investigate the mechanism of which low contractility induced lipid accumulation

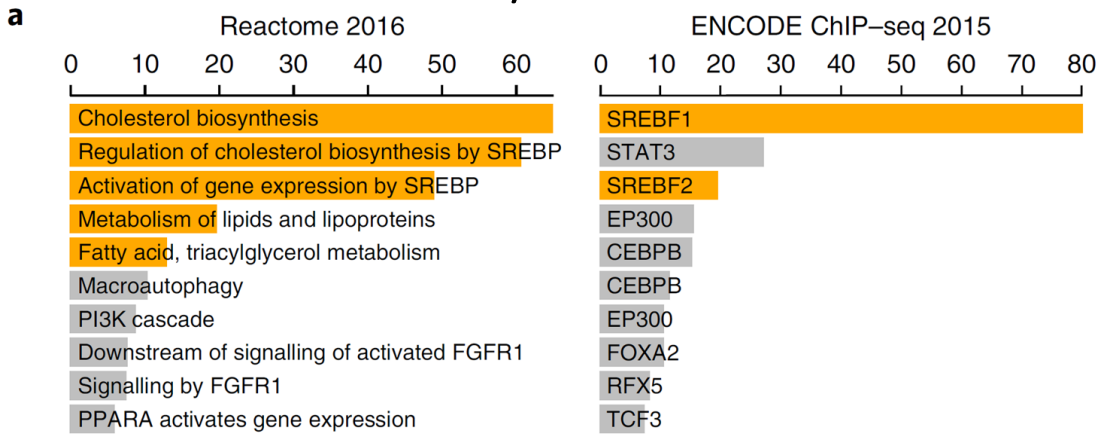


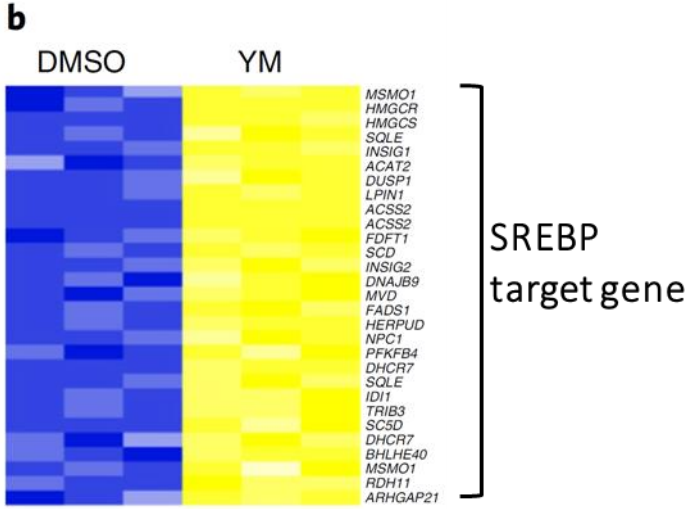
Contractility

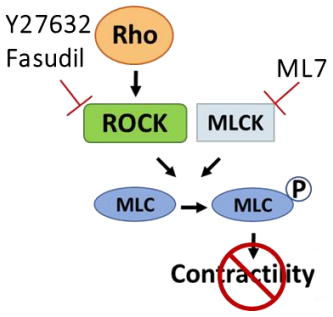
Lipid accumulation

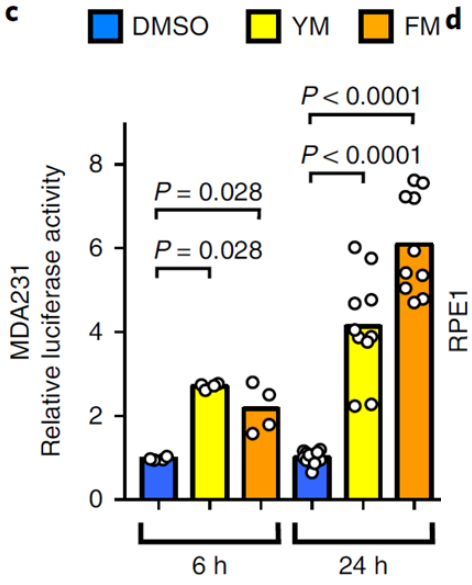
Low contractility induced SREBPs activation

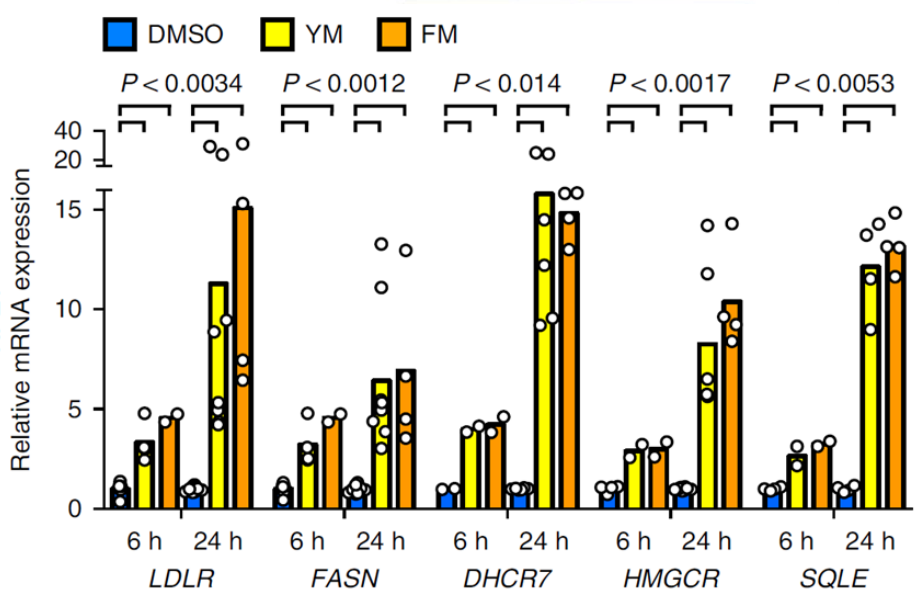
Gene list enrichment analysis





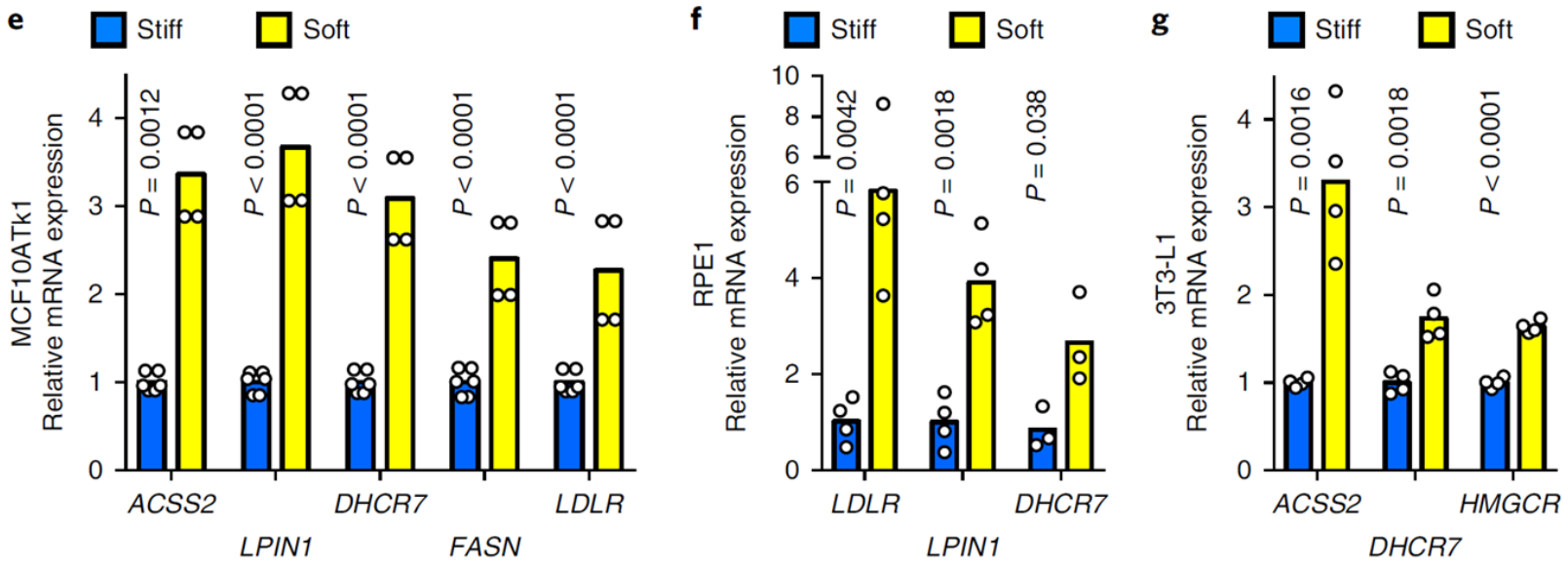




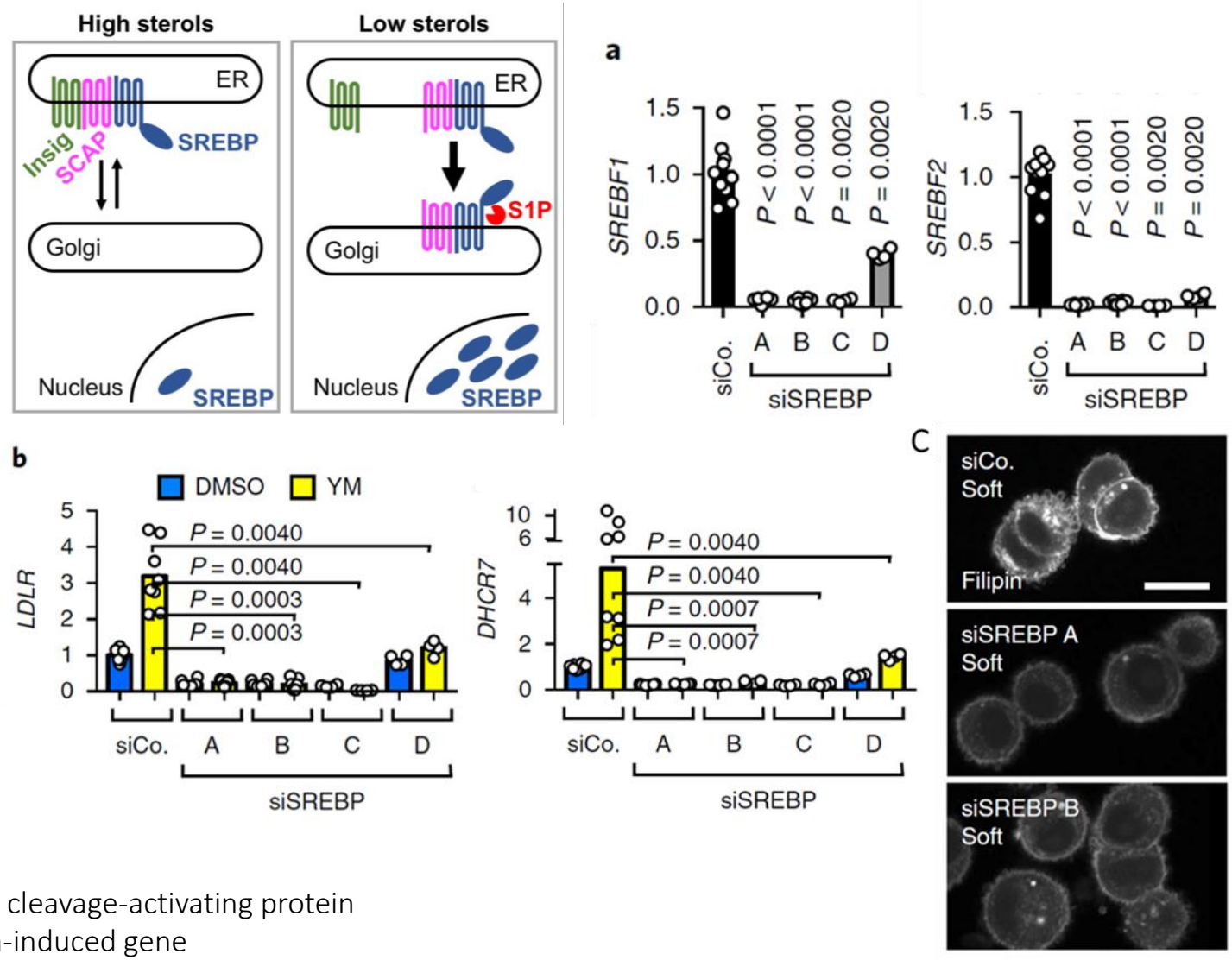


SREBPs: sterol regulatory element binding proteins

Soft ECM increased the expression of SREBP target genes

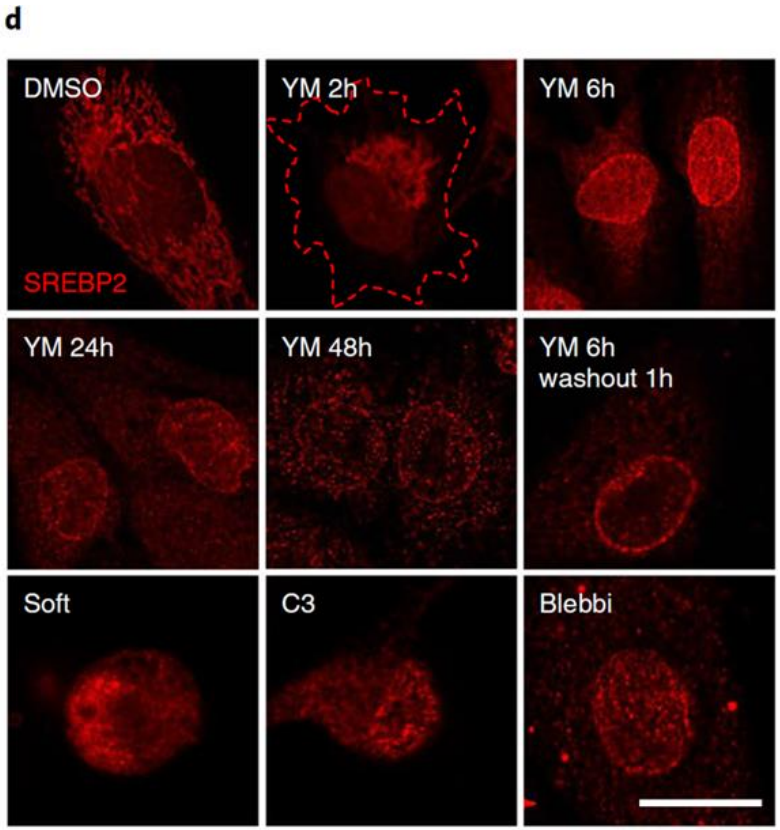


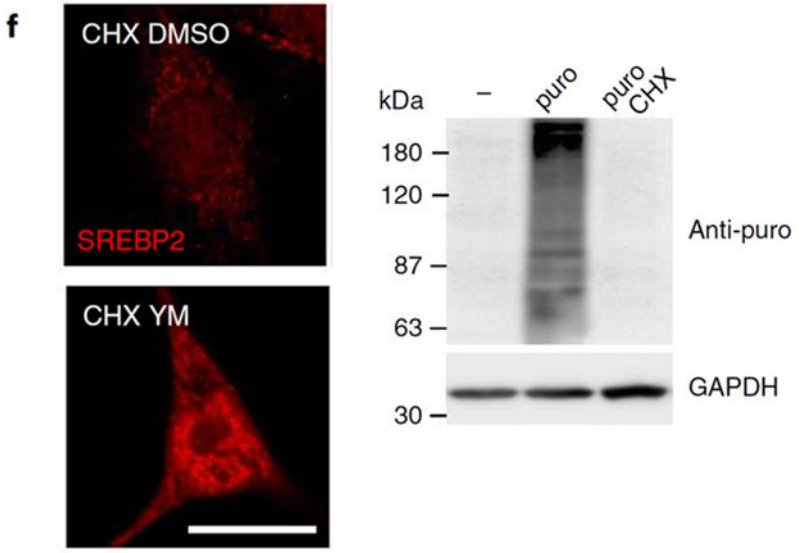
Knockdown of SREBP1/2 decreased lipid accumulation which was induced by YM and soft ECM

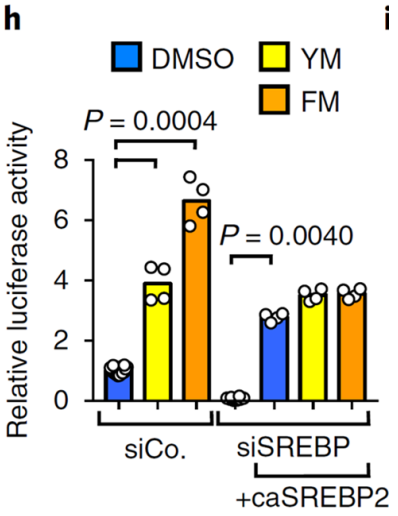


SCAP: SREBP cleavage-activating protein
Insig : insulin-induced gene
S1P: site-1 protease

Low contractility increased the cleavage level of SREBPs



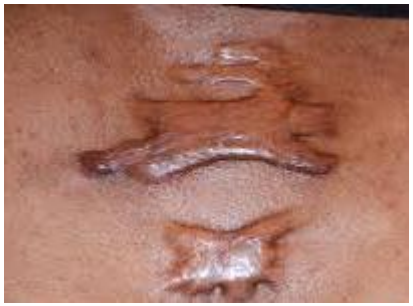




Cycloheximide(CHX): inhibit newly synthesis protein
Puromycin (puro): block translation elongation
caSREBP2: cleaved mature SREBP2 cDNA

Pathological tissue stiffness downregulated SREBP activity

- **Keloid scars**
  fibrous tissue
  Occur on high tension skin



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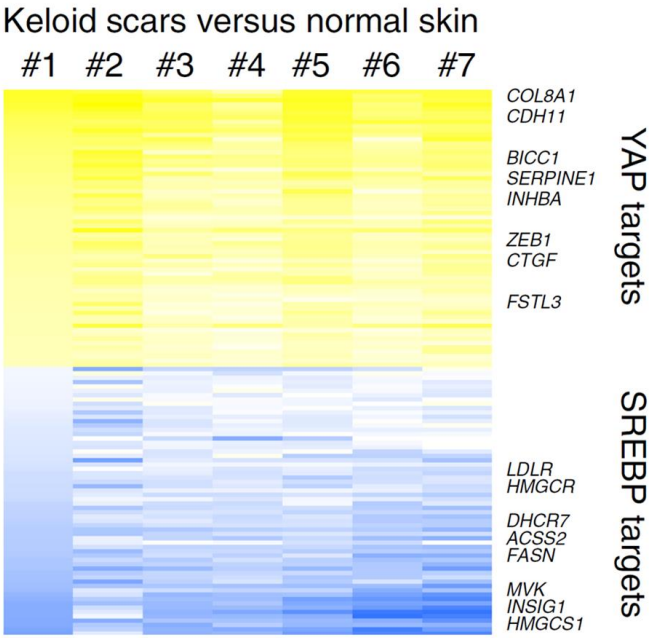


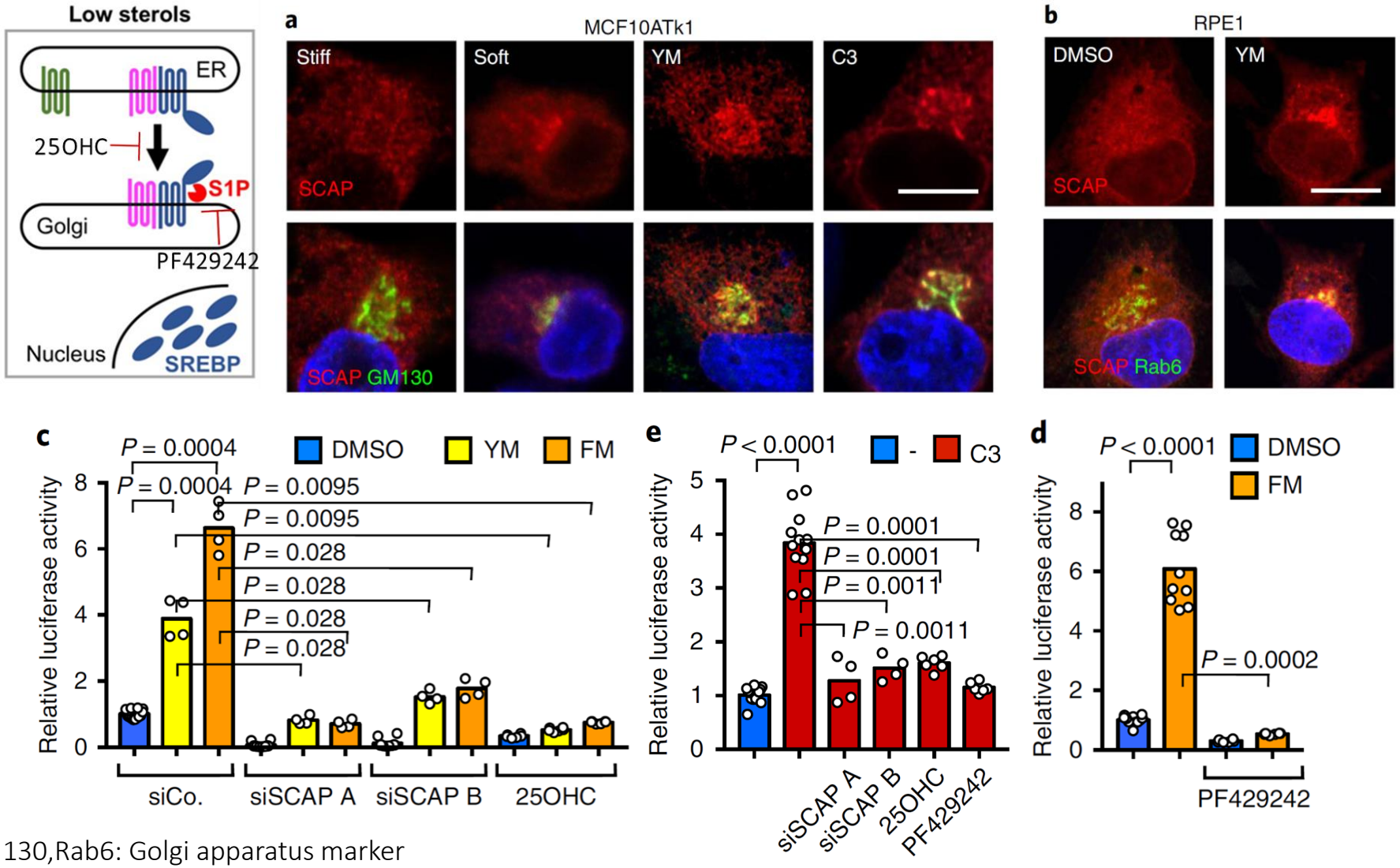
- Keloidal skin have lower level of cholesterol and triglycerides compare with normal skin.

Exp Dermatol. 2008 Apr;17(4):318-23.

↓

May caused by inhibition of SREBP activity

Inhibition of SCAP/SREBP accumulation to Golgi apparatus blocked low contractility-induced SREBPs activation

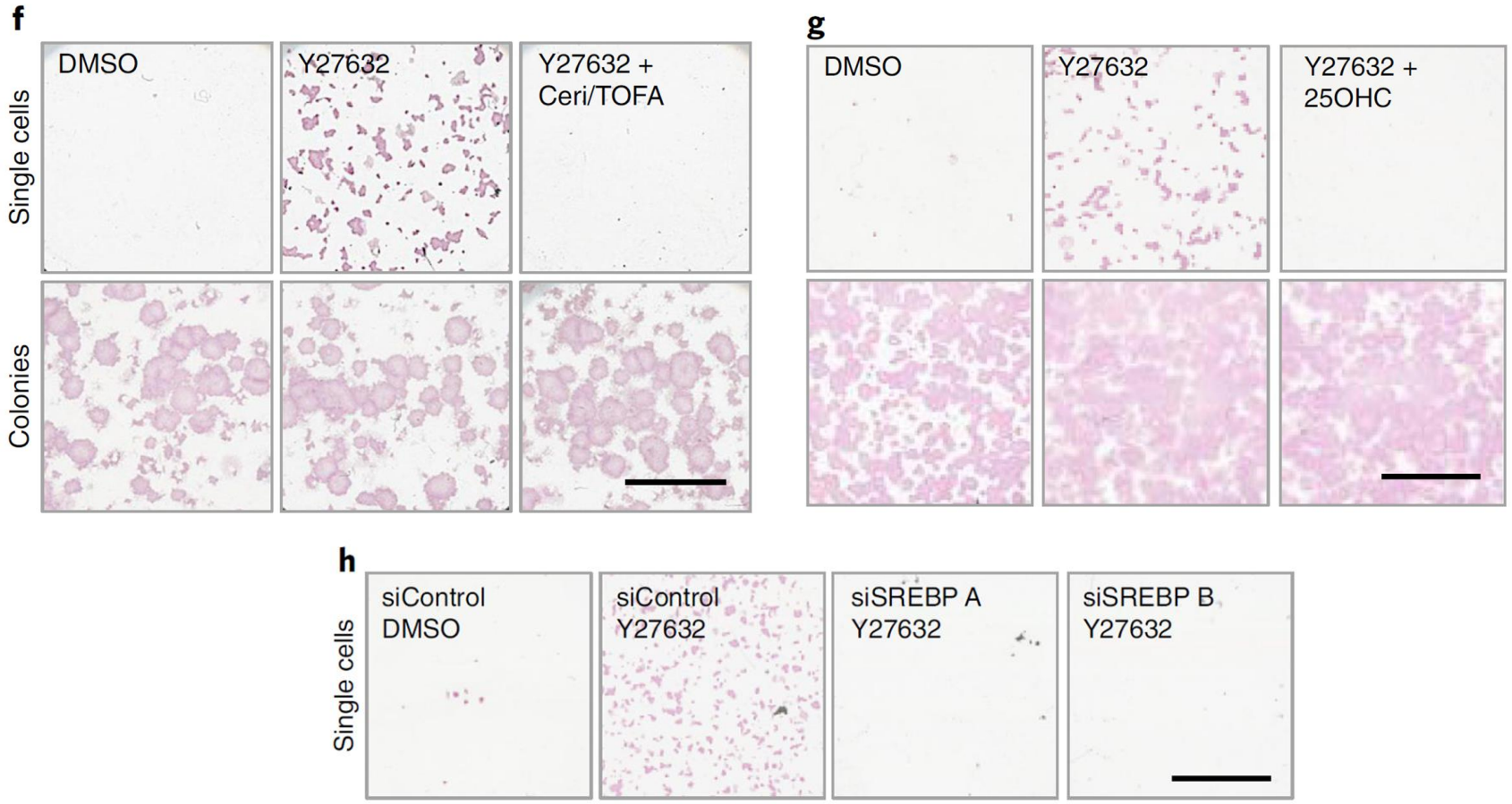


GM130,Rab6: Golgi apparatus marker
25OHC:inhibitor of SCAP transport
PF429242: site-1 protease (S1P) inhibitor

Y27632 increased lipid synthesis to promote human pluripotent stem cells (hPSCs) survival

- hPSCs requires treatment with the ROCK inhibitor to promote single-cell survival.

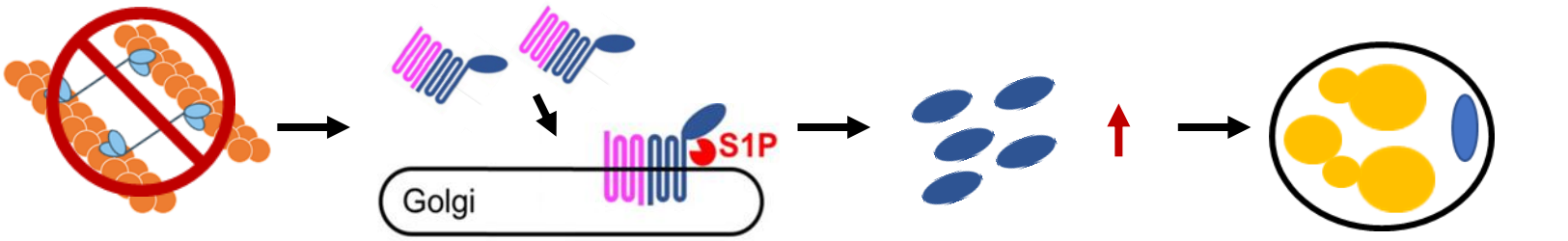
Theriogenology. 2016 Jan 15;85(2):302-14.



Cerivastatin (Ceri): cholesterol synthesis inhibitor
TOFA: ACC inhibitor, blocks lipid accumulation

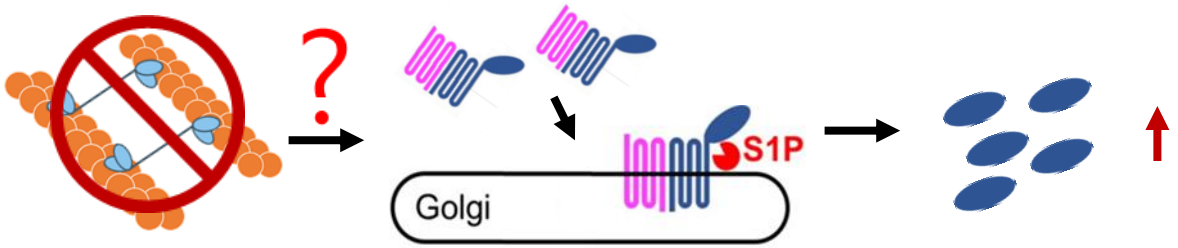
Summary 2

Low contractility increased SCAP/SREBP accumulation to Golgi apparatus to active SREBPs



Aim 3

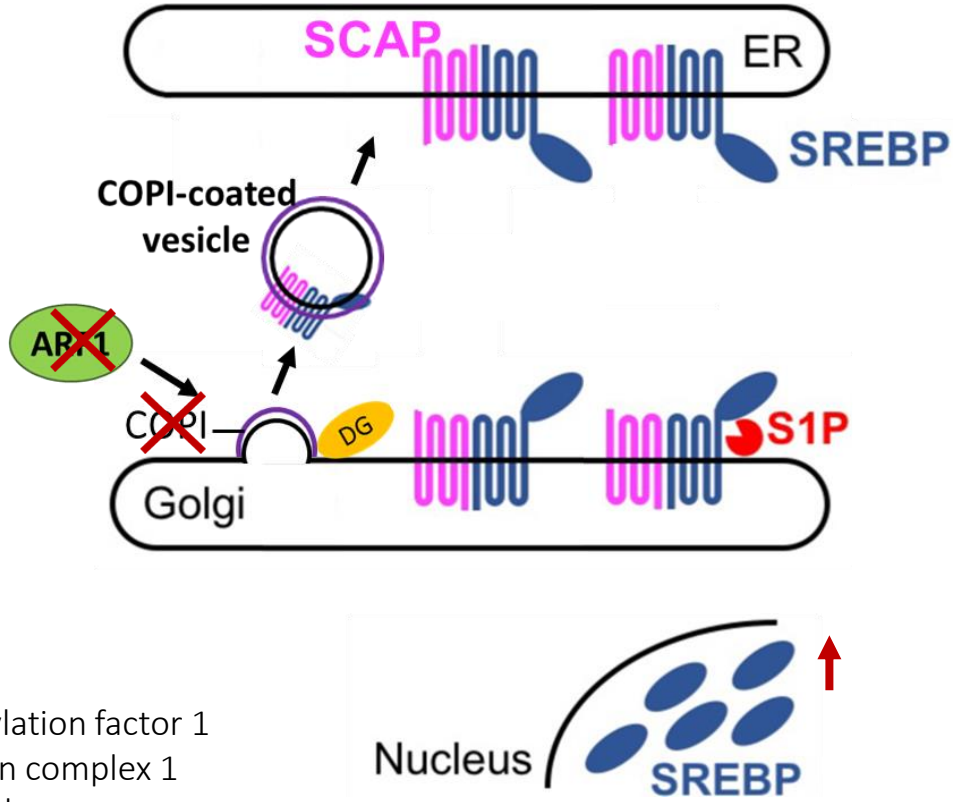
To find the upstream pathway of which low contractility increased SREBPs activation

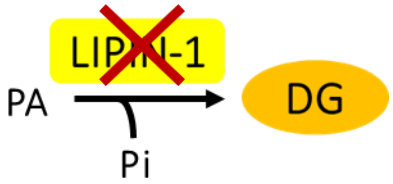


LIPIN-1/ARF1 pathway decreased SREBP activity

- ARF1 and LIPIN-1 are known as inhibitors of SREBPs.

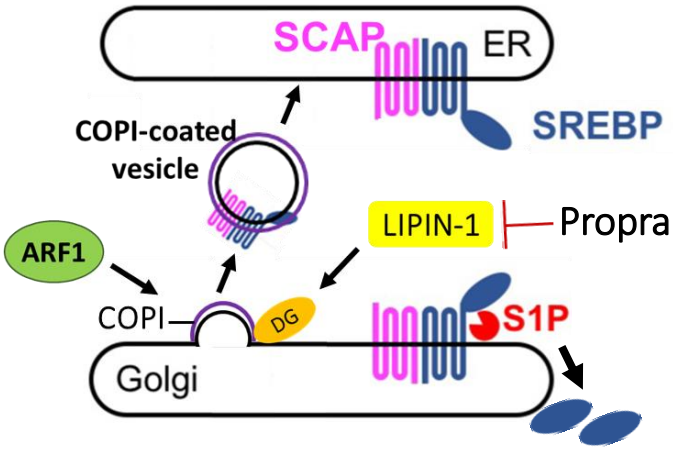
Cell Rep. 16, 9–18 (2016).
Biochim. Biophys. Acta 1859, 1583–1595 (2017).

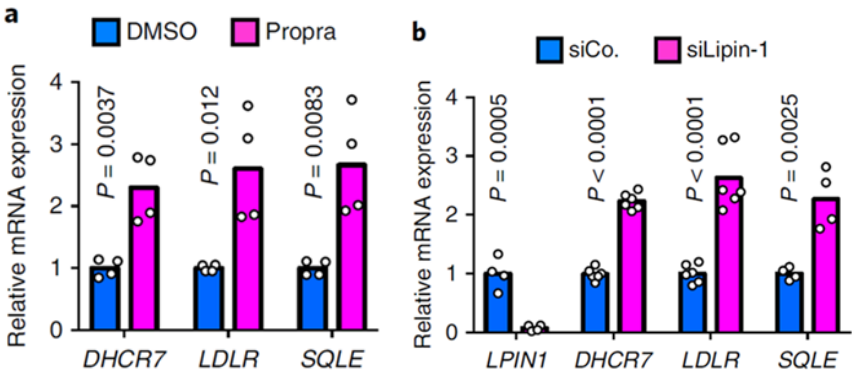


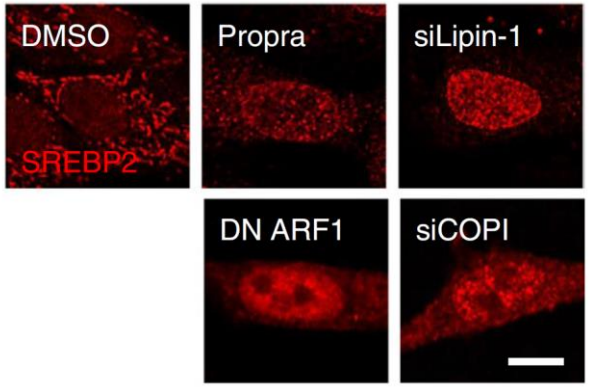


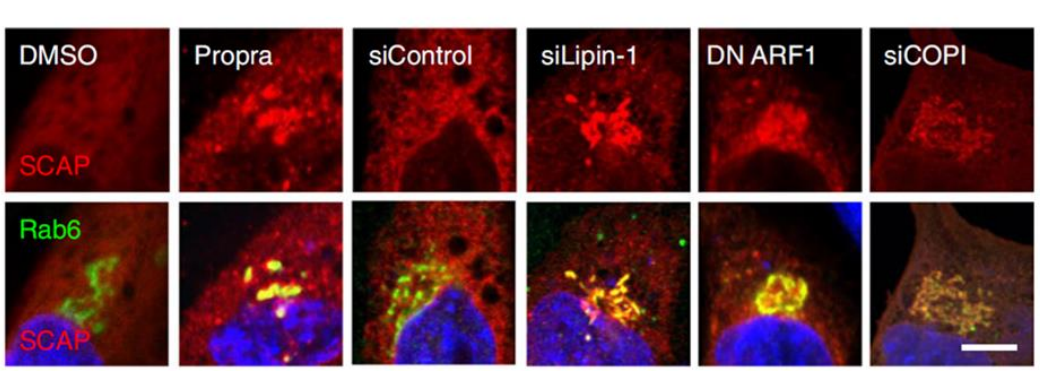
ARF1: ADP ribosylation factor 1
COPI: coat protein complex 1
DG: diacylglycerols
PA: phosphatidic acid

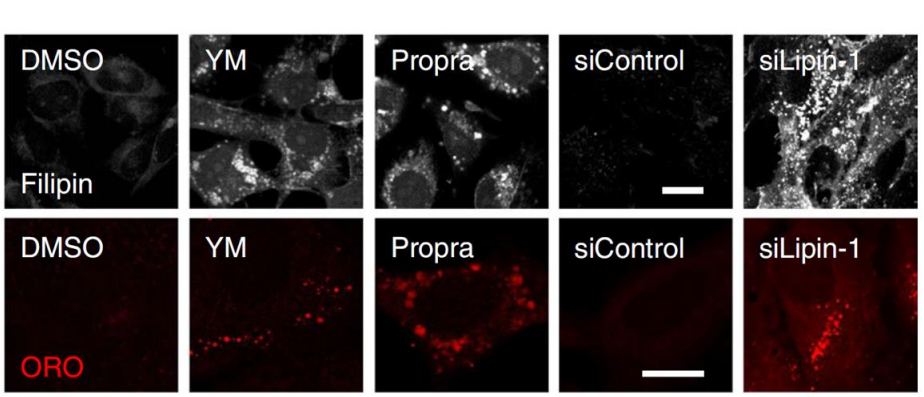
Inhibition of LIPIN-1/ARF1 pathway increased SREBPs activation









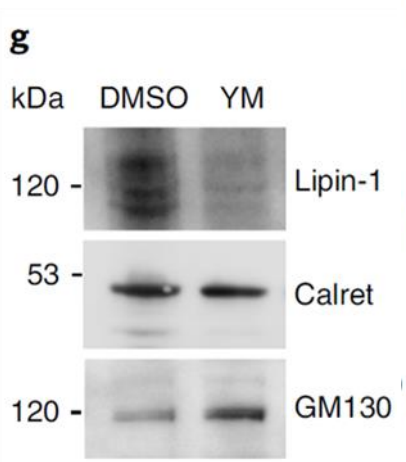


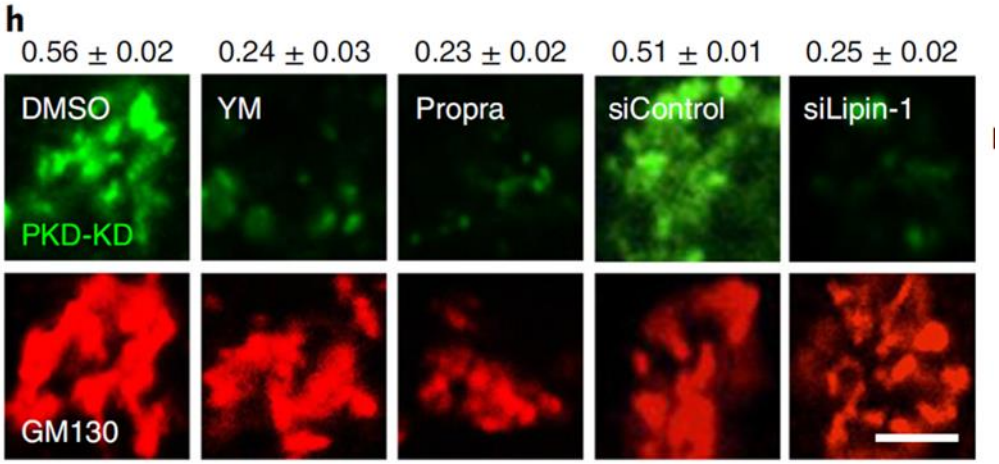
Propra: LIPIN-1 inhibitor
DN ARF1: dominant-negative ARF1-T31N-GFP

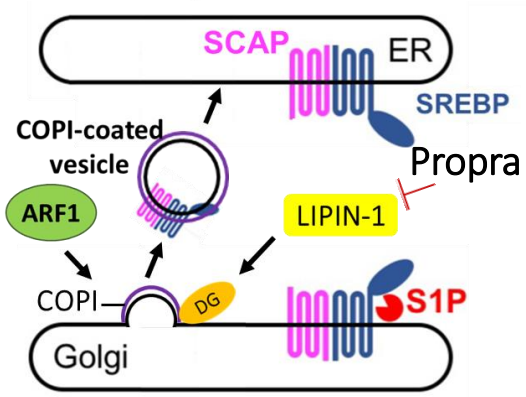
Inhibition of actomyosin contractility reduced ARF1 activation by decreasing LIPIN-1 activity

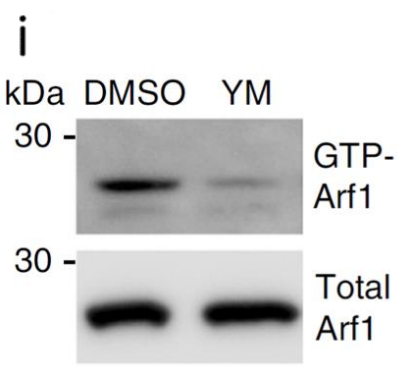
LIPIN-1 activity
- Association with microsomes
- DG content on Golgi membrane (GFP-PKD-KD)

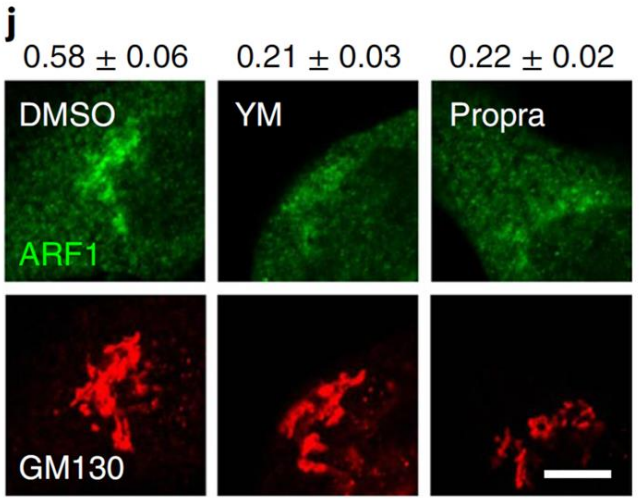
Microsomes fraction





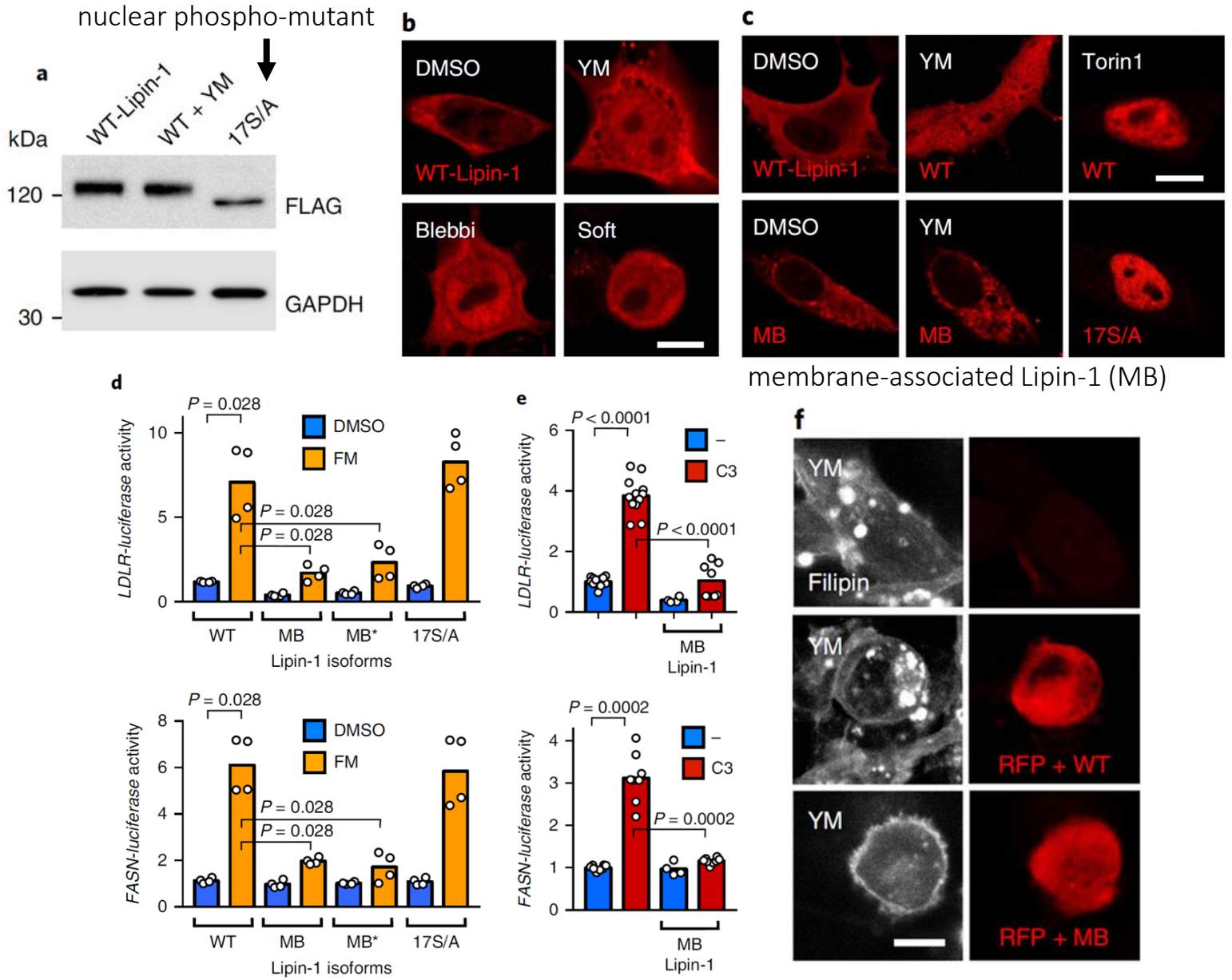






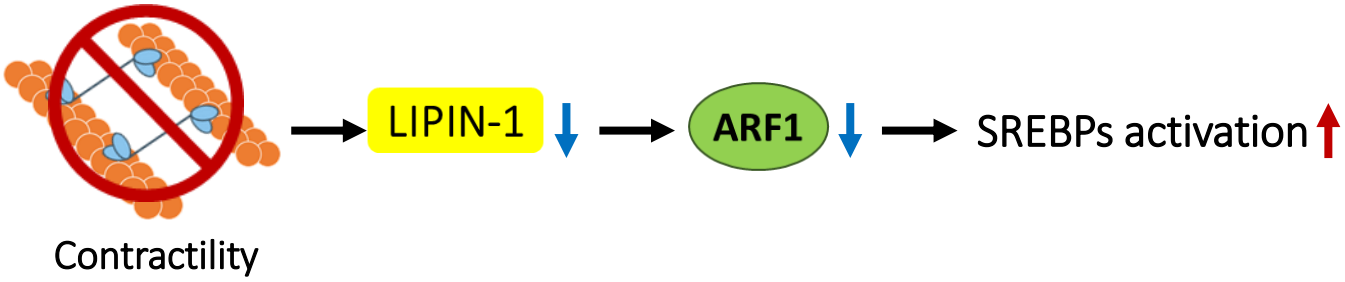
PKD: DG-binding domain
Calret: Microsome marker

Lipin-1 nuclear translocation increased SREBP activation



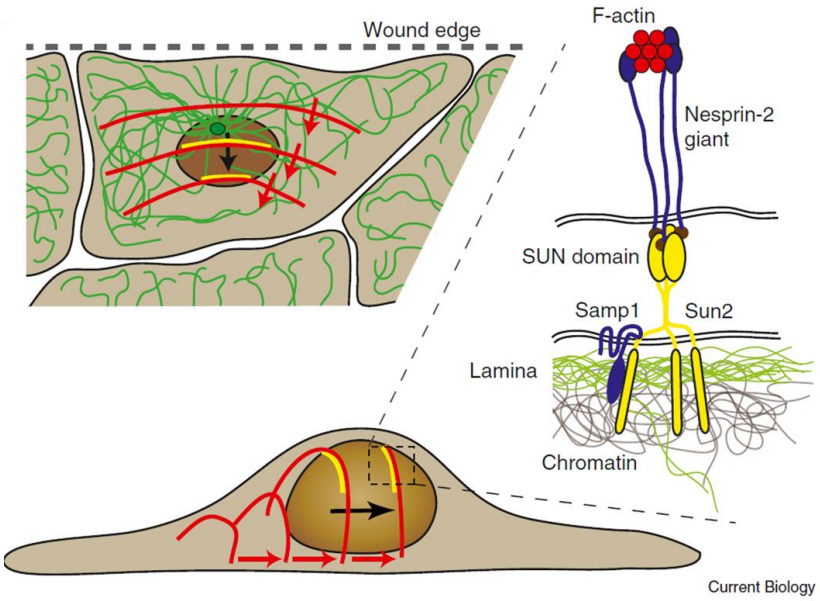
Summary 3

Low contractility blocked LIPIN-1/ARF-1 pathway to increase SREBPs activation

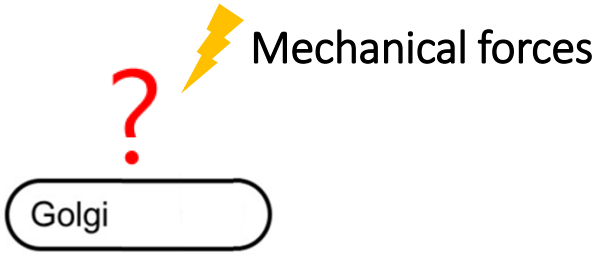


Aim 4

To examine the response of Golgi apparatus on mechanical force.

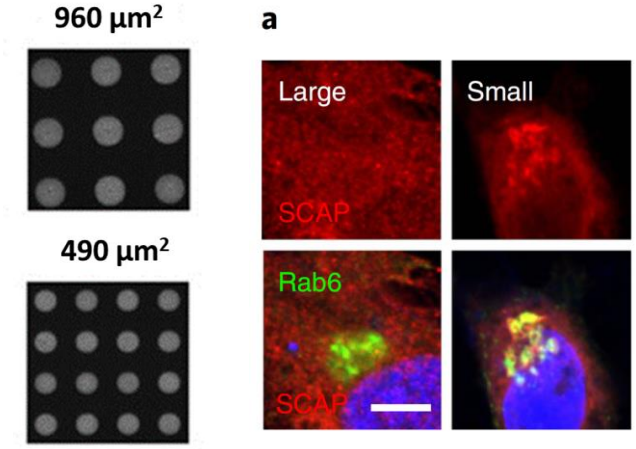


Curr Biol. 2013 Dec 16;23(24):R1113-21

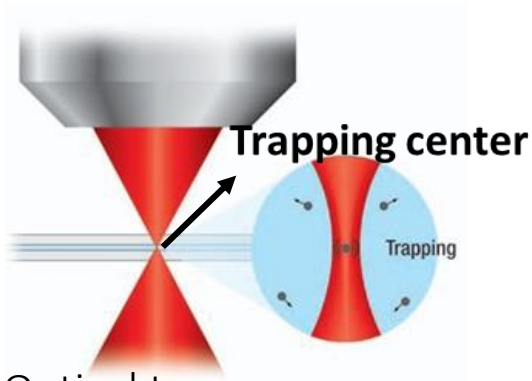


Golgi apparatus responded to extracellular and intracellular mechanical forces

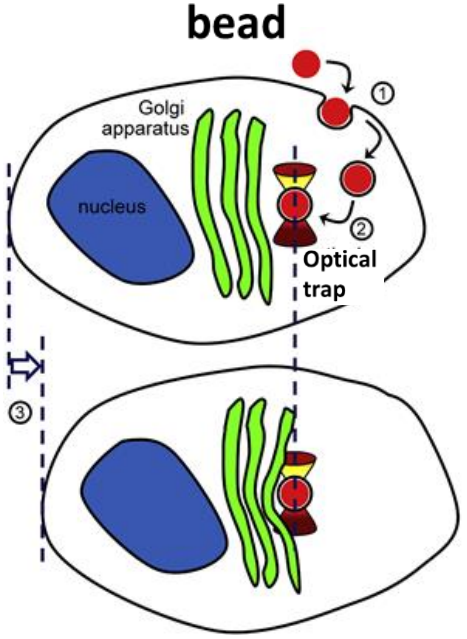
Small micropattern

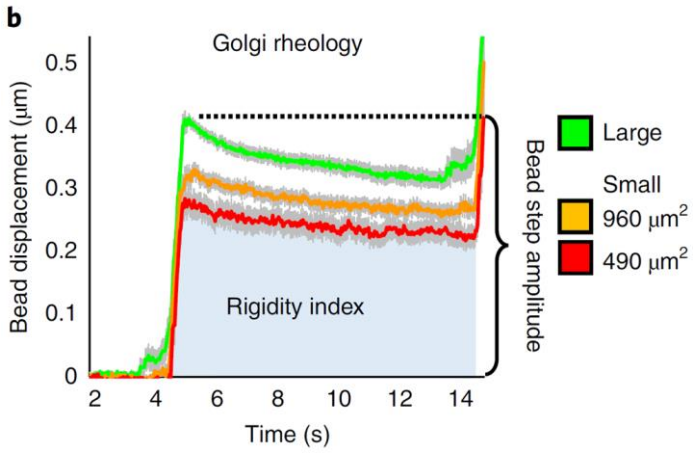


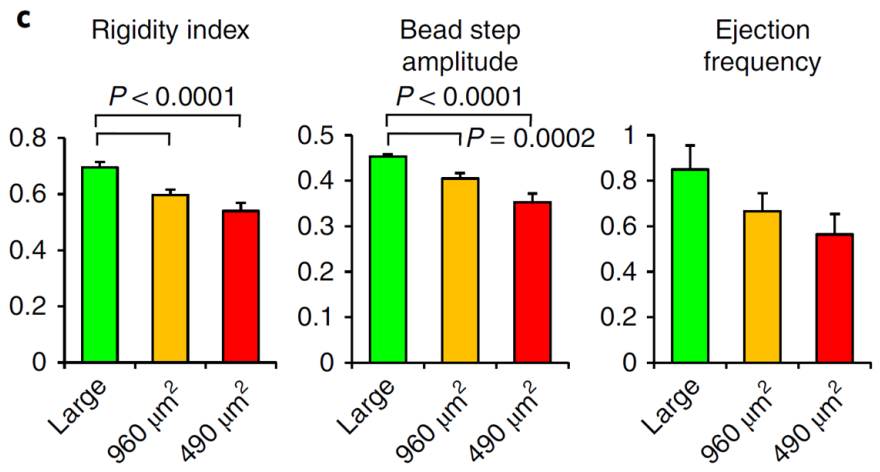
Laser optical trap



Optical tweezers

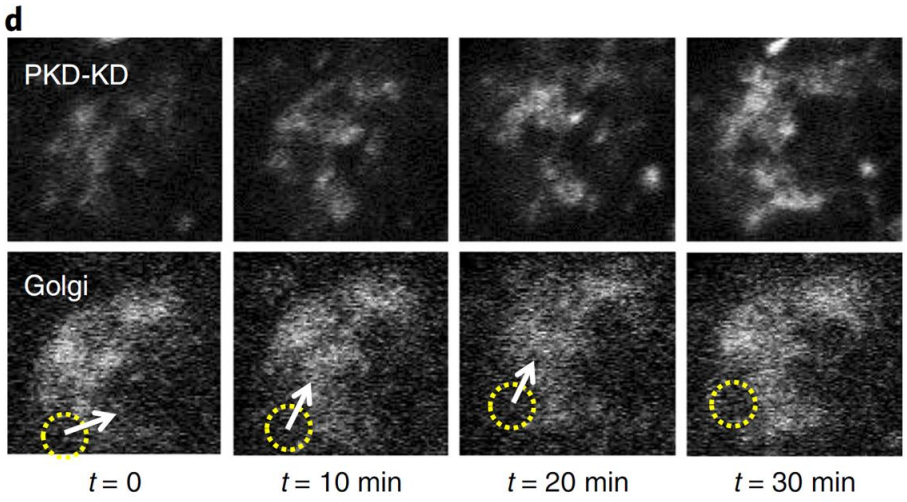


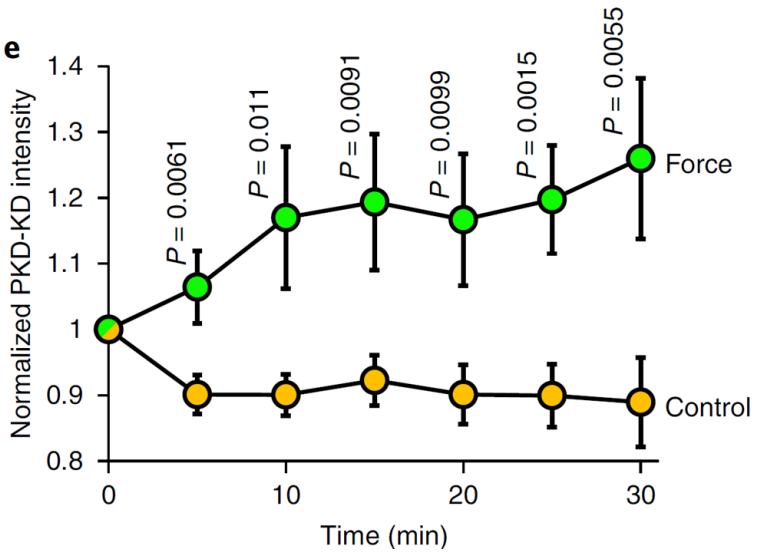




Current Biology. 2014 Aug 4;24(15):1700-11.

Intracellular mechanical forces increased DG content on Golgi membrane





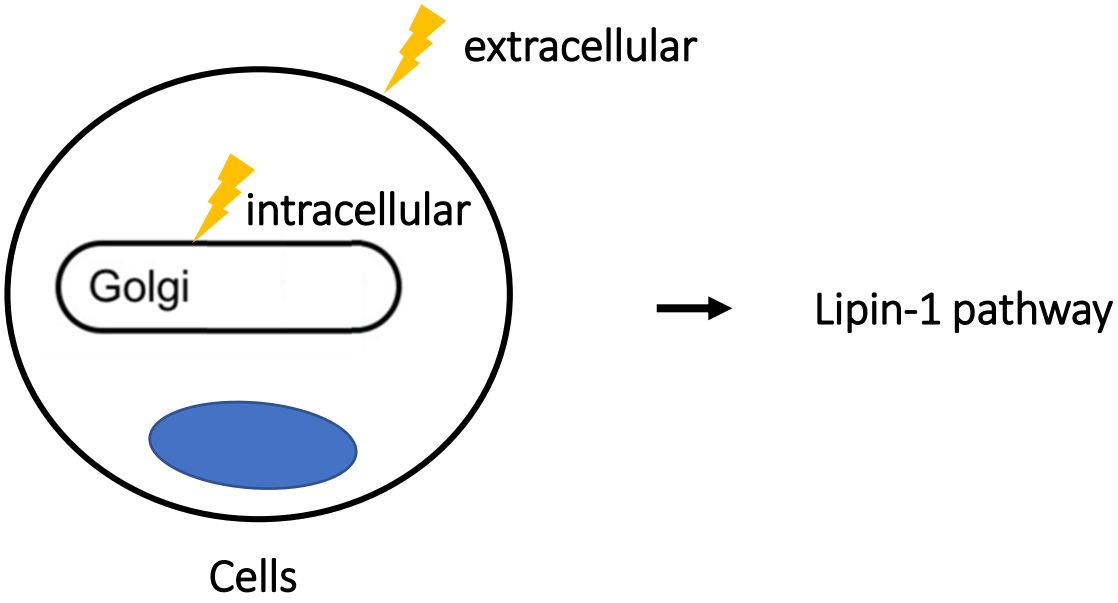
DG content ↑

↓

Indicated Lipin-1 activation ↑

Summary 4

Golgi apparatus responded to extracellular and intracellular mechanical forces



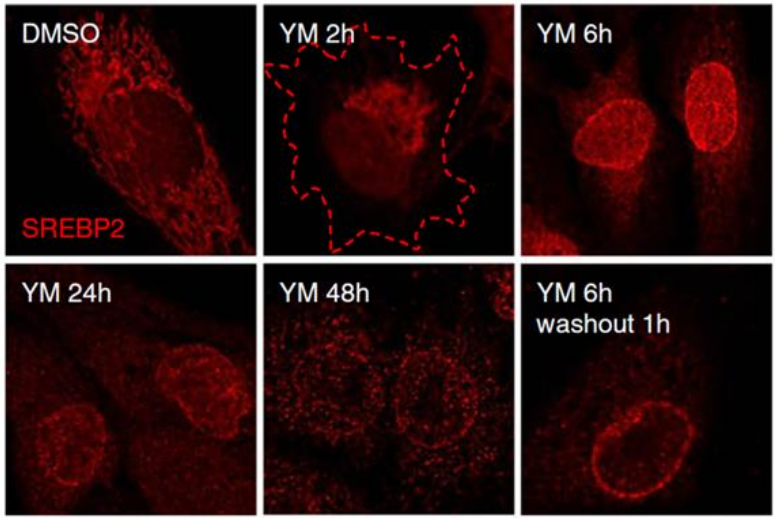
Mechanical forces

Conclusion

Actomyosin contractility regulated lipid metabolism by inhibition of SREBPs activity through LIPIN-1/ARF pathway

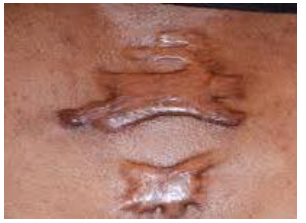
Discussion

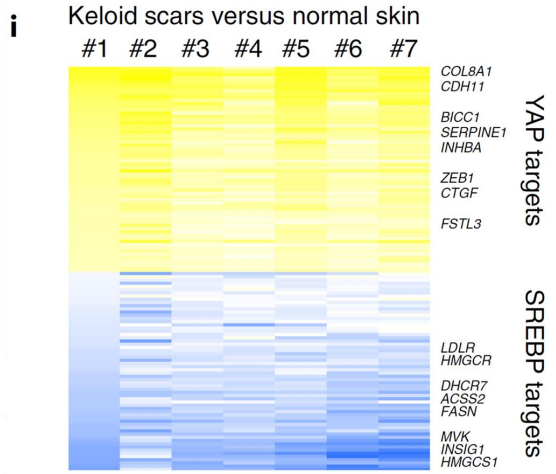
1.



2.

- Keloid scars





May caused by inhibition of SREBP activity

Thank you for your attention!

Ejection frequency