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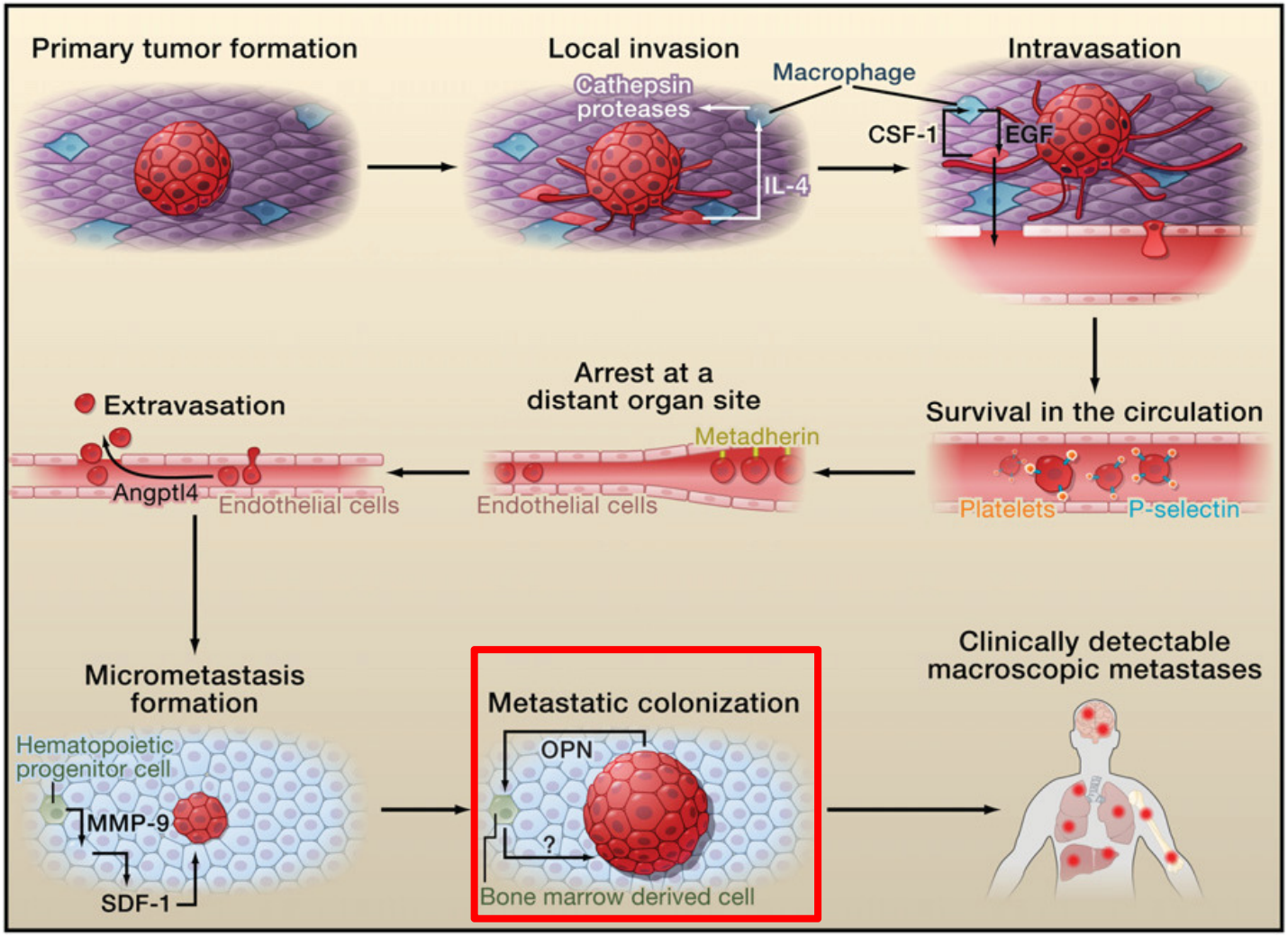
Force Induced Regulation of Epigenetic State in Cancer Cells



Arash Tajik

University of Illinois at Urbana-Champaign

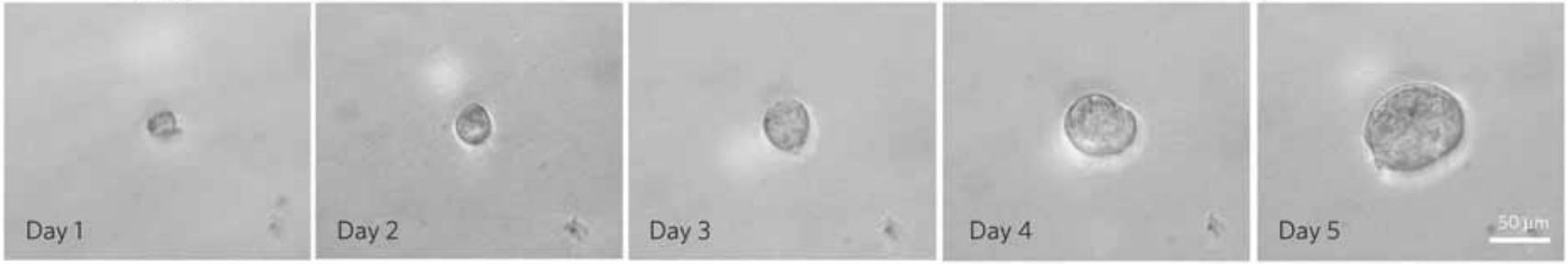
Metastatic colonization is a rare event: ~1 in 10,000 heterogeneous cancer cell population



Valastyan and Weinberg, Cell 2011

Soft Fibrin Matrix Promotes Growth of Tumorigenic Cells

3D Fibrin



Tumor Repopulating Cells (TRC)

3D Fibrin
(10-100 cells)

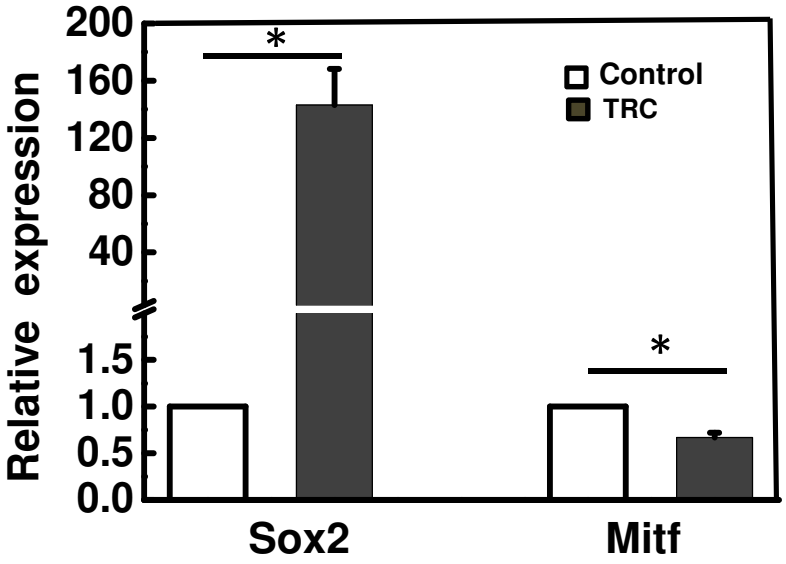


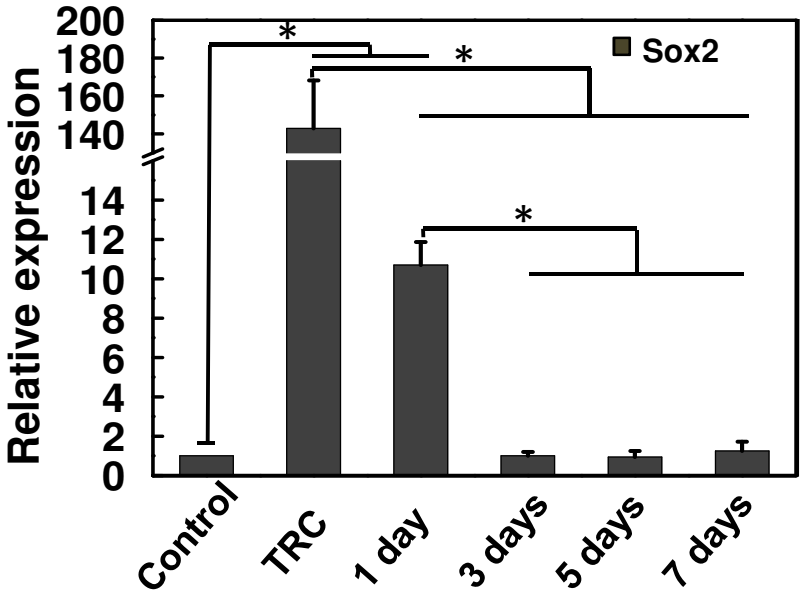
Control
(10,000-100,000 cells)



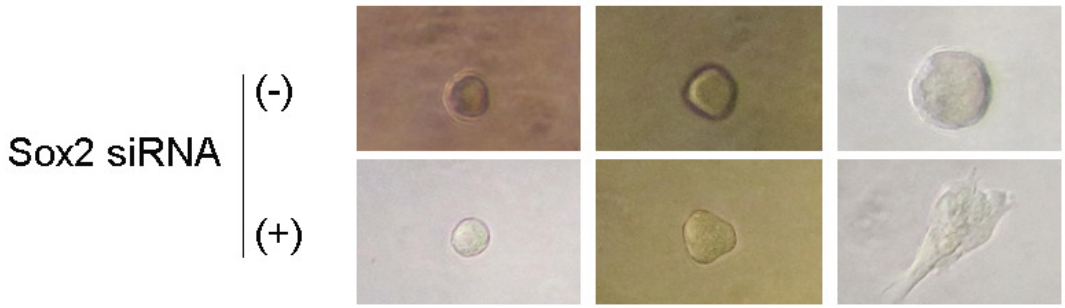
Liu & Tan *et al*, *Nature Mat,* 2012

TRCs are progenitor of control melanoma cells





Mitf: a melanin-related gene
Sox2: a self-renewal marker

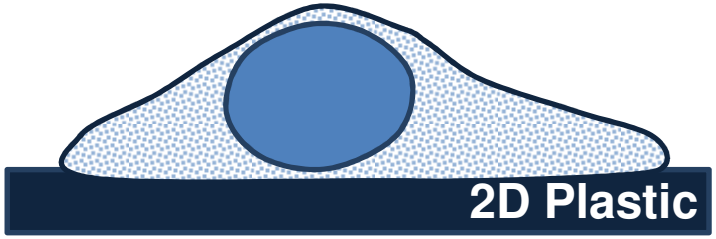


Liu & Tan *et al*, *Nature Mat,* 2012

TRC & control melanoma cells are genetically identical

Control Melanoma Cells

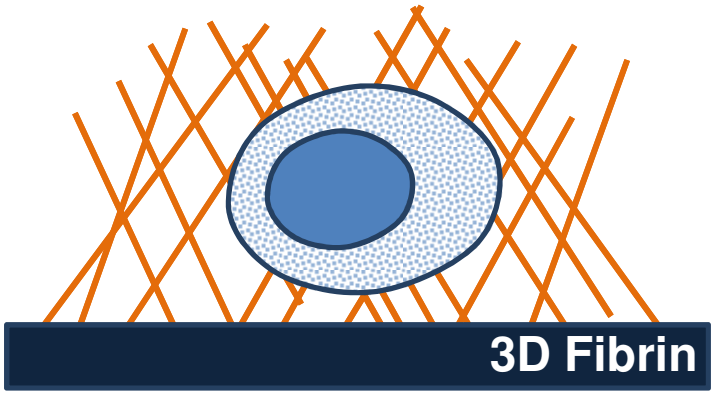
No Sox2
High Mitf

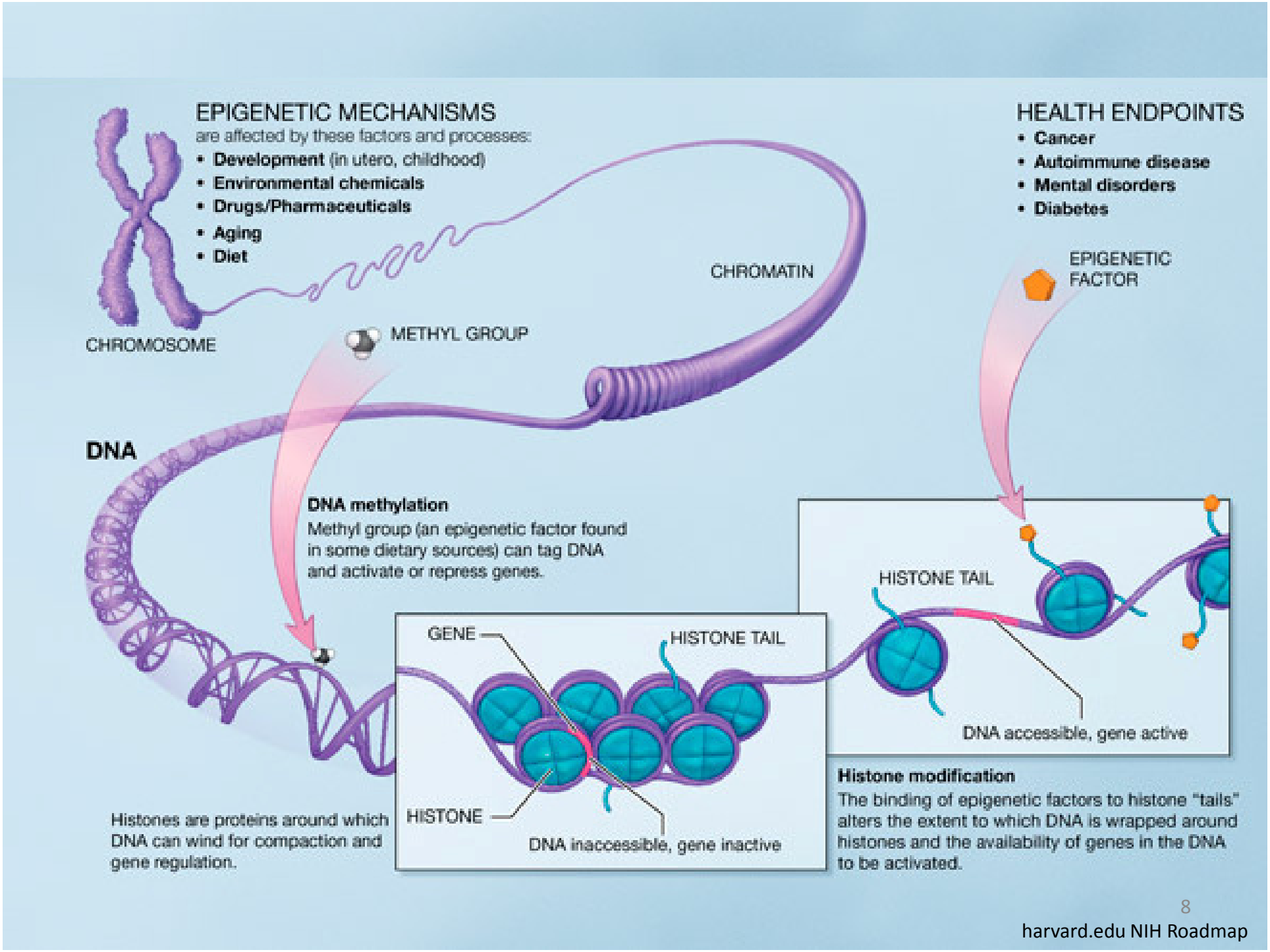


Matrix Stiffening ⇅ **Matrix Softening**

Tumor Repopulating Cells

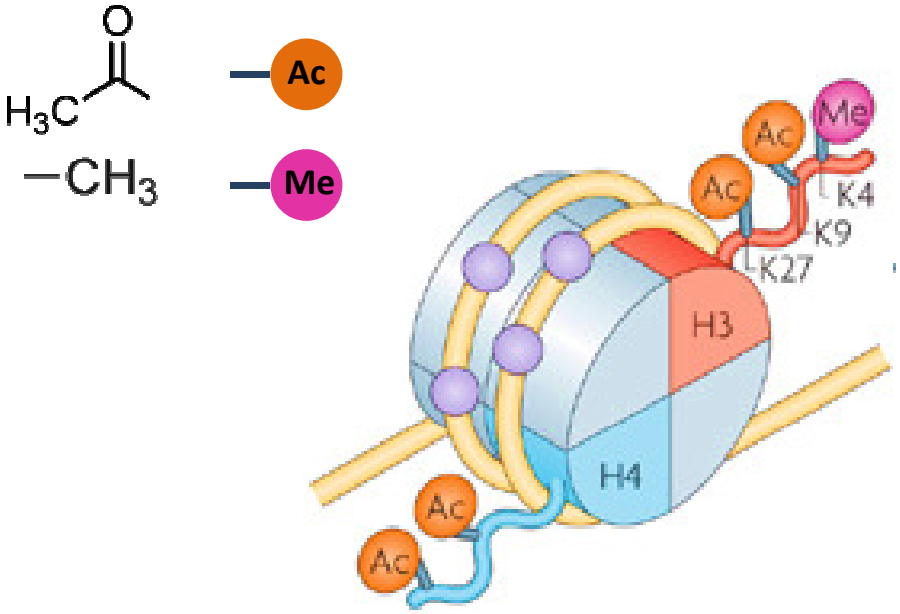
High Sox2
Low Mitf





harvard.edu NIH Roadmap

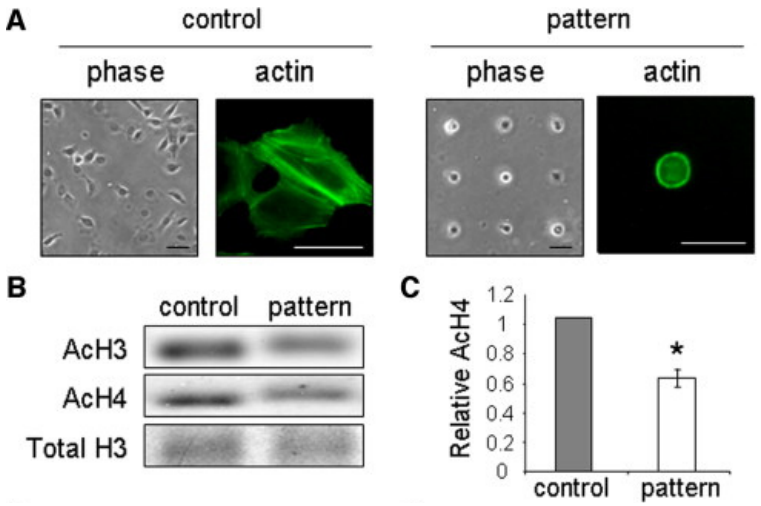
Histone Modification



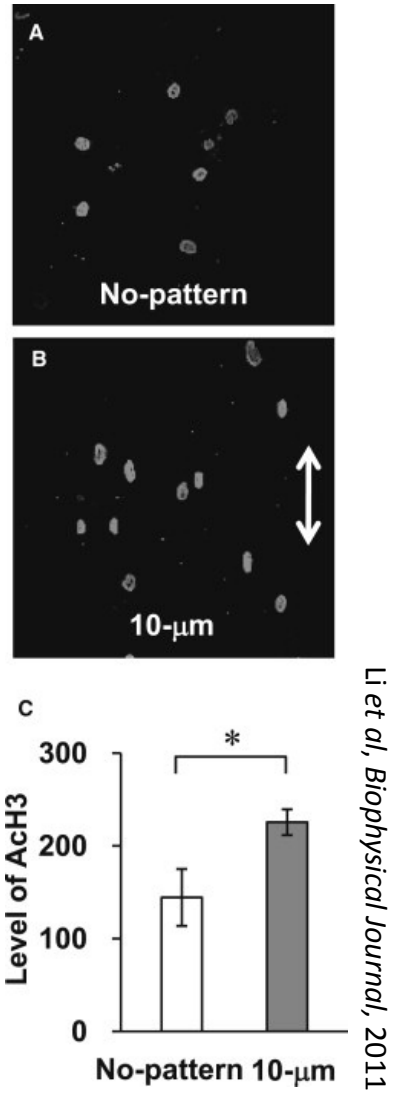
Modification	H3K4	H3K9	H3K27
mono-methylation	Activation	Activation	Activation
di-methylation		Repression	Repression
tri-methylation	Activation	Repression	Repression
acetylation		Activation	

Modified from Cedar & Bergman, *Nature Reviews Genetics,* 2009

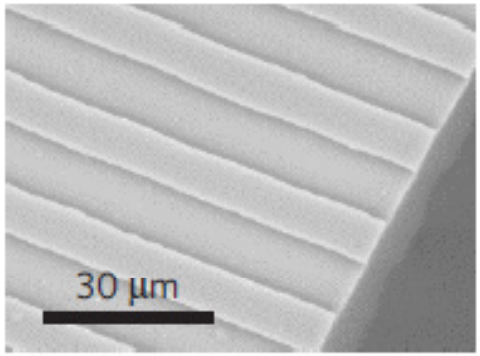
Mechanotransduction & Histone Acetylation

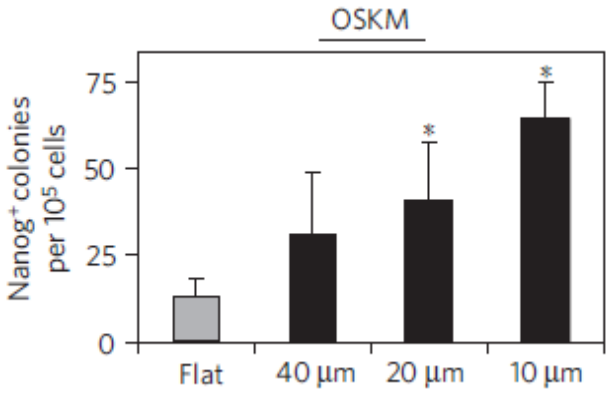


Le Beyec *et al*, *Exp Cell Res*, 2007



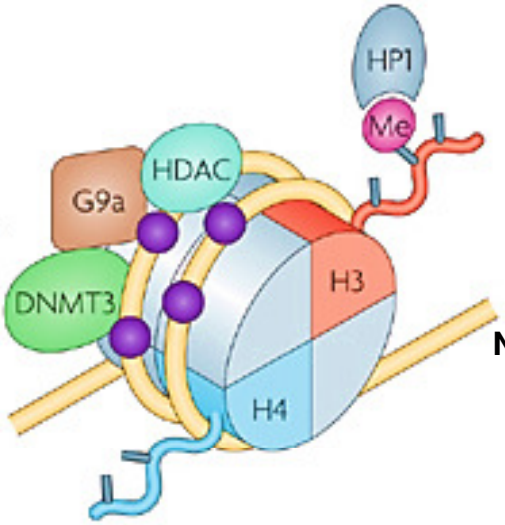
Li *et al*, *Biophysical Journal*, 2011



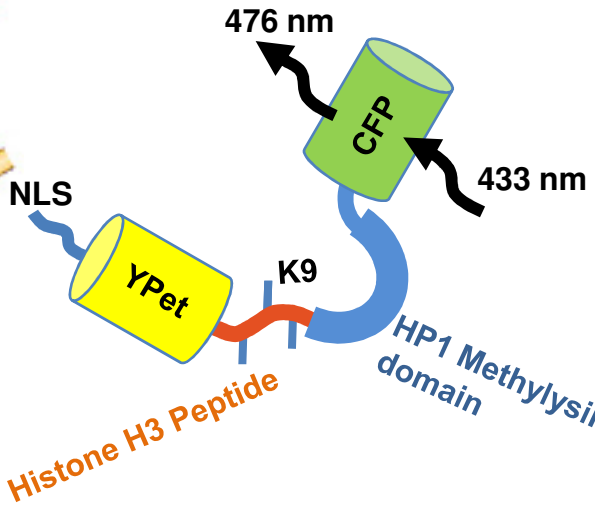


Downing *et al*, *Nat Mat*, 2013

H3K9-me FRET Reporter

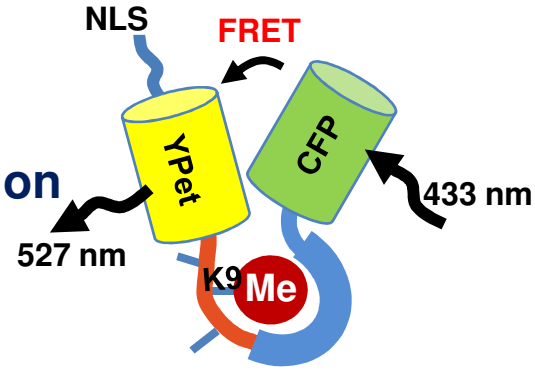


Cedar & Bergman, *Nature Reviews Genetics,* 2009

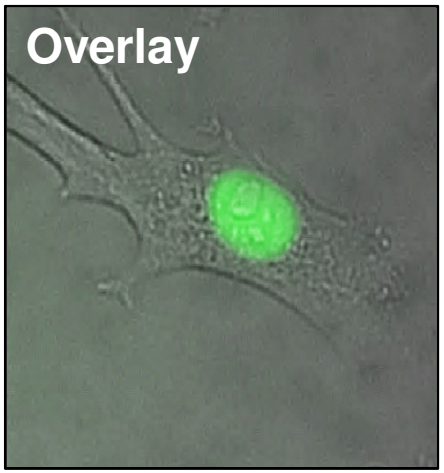


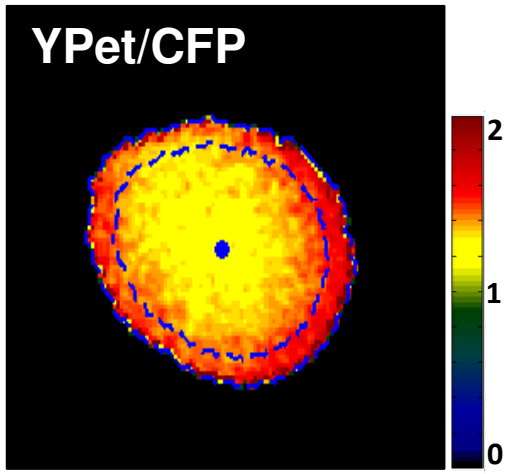
Methylation

Demethylation

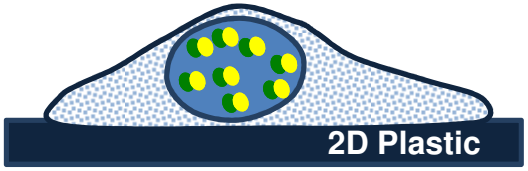


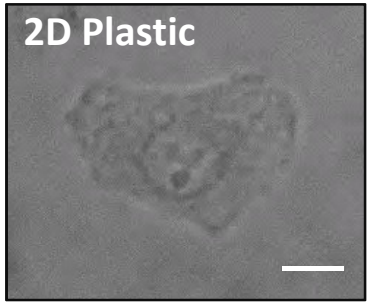
Adapted from Lin *et al*, *JACS,* 2004

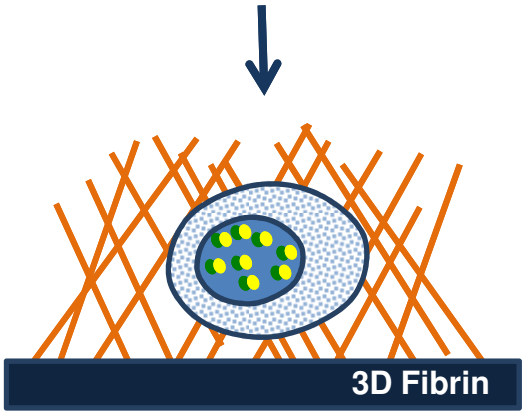


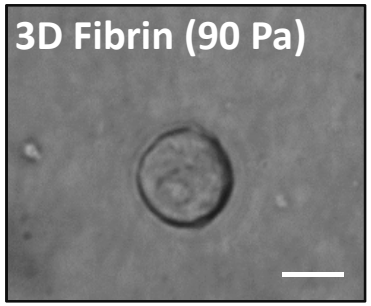


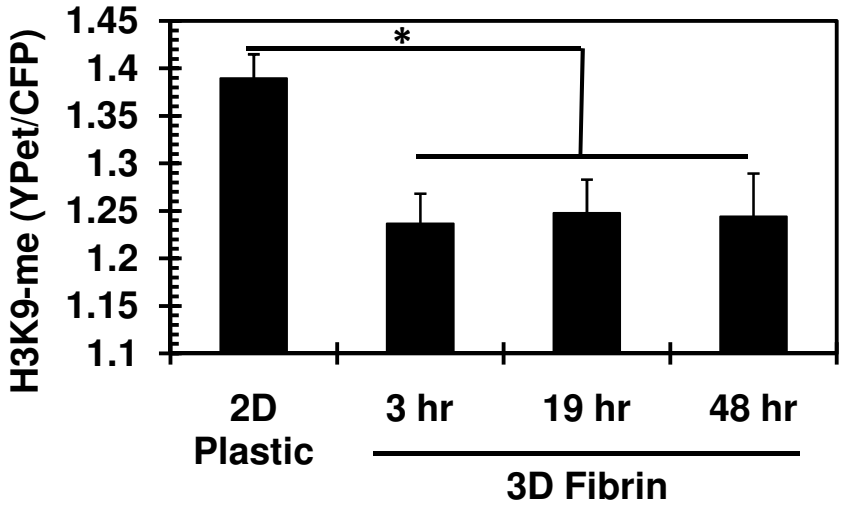
Cells in 3D Fibrin Gel have lower H3K9-me

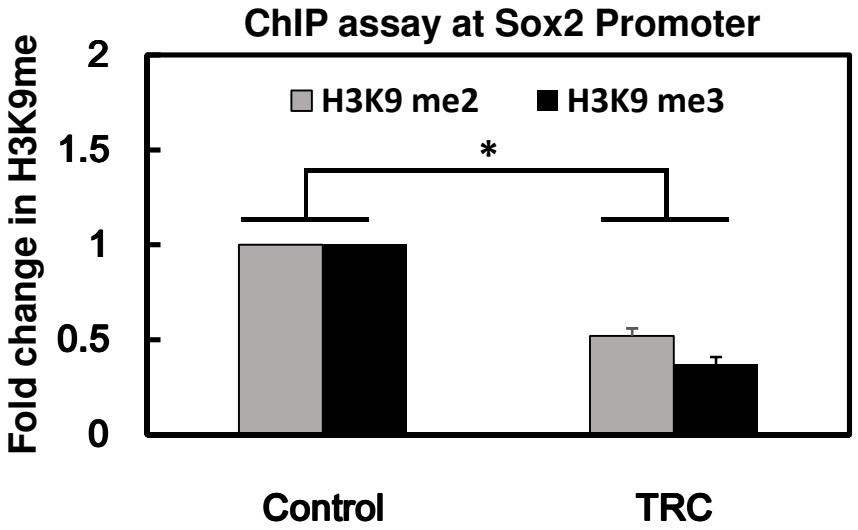




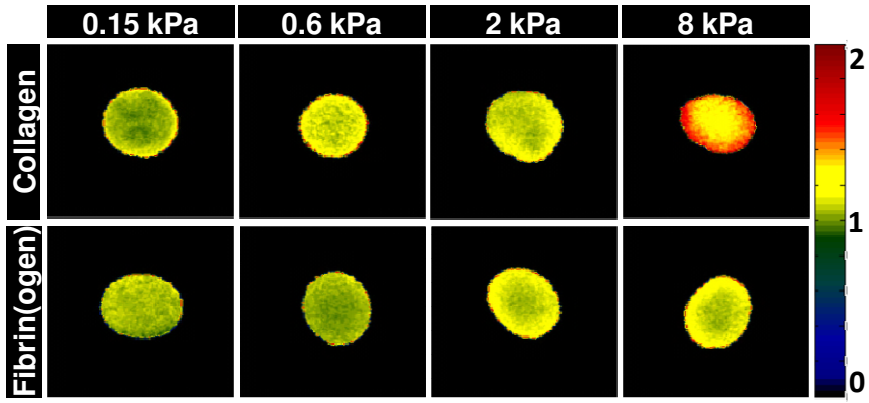


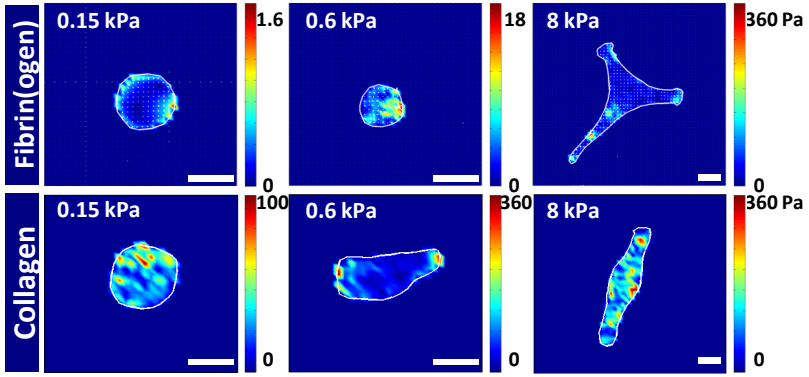


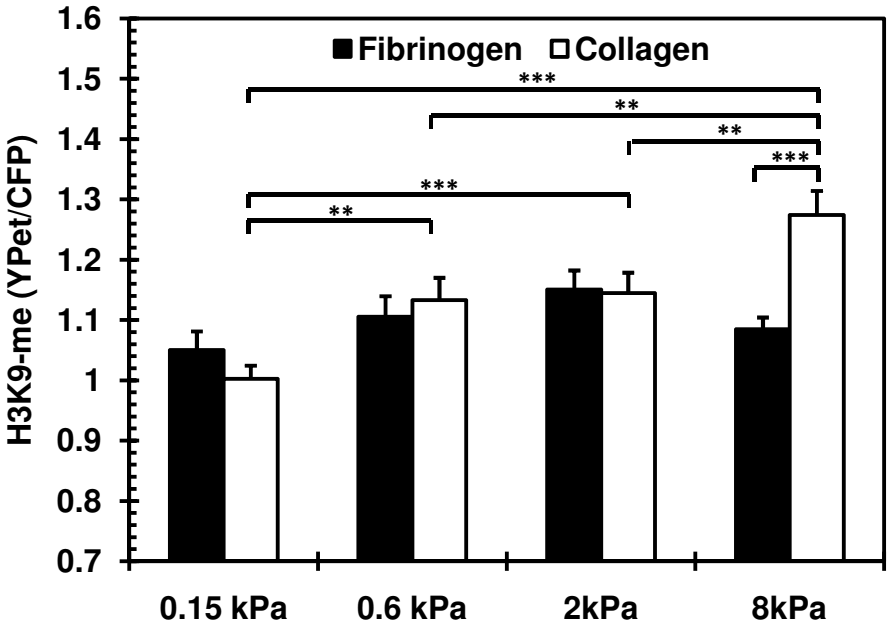


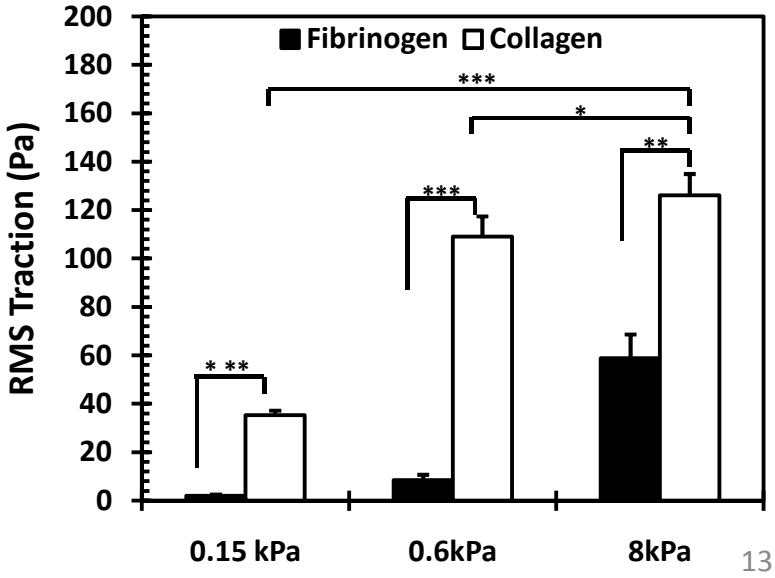


Substrate stiffness regulates H3K9-me in control melanoma cells

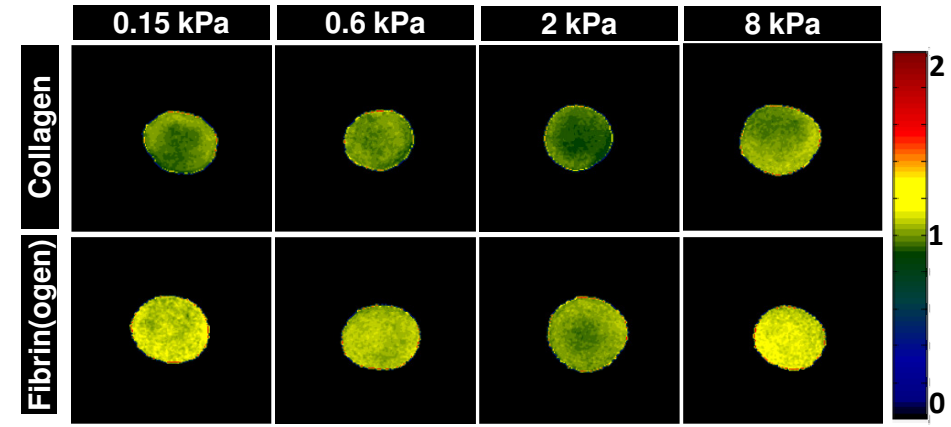


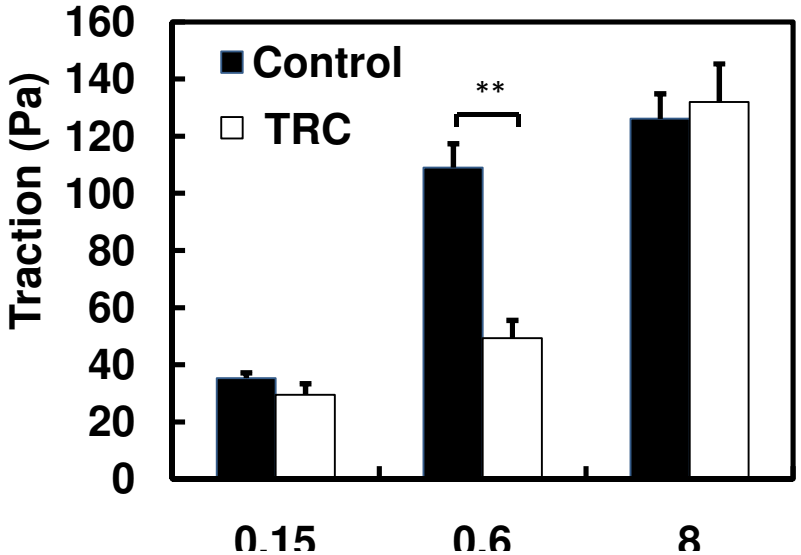


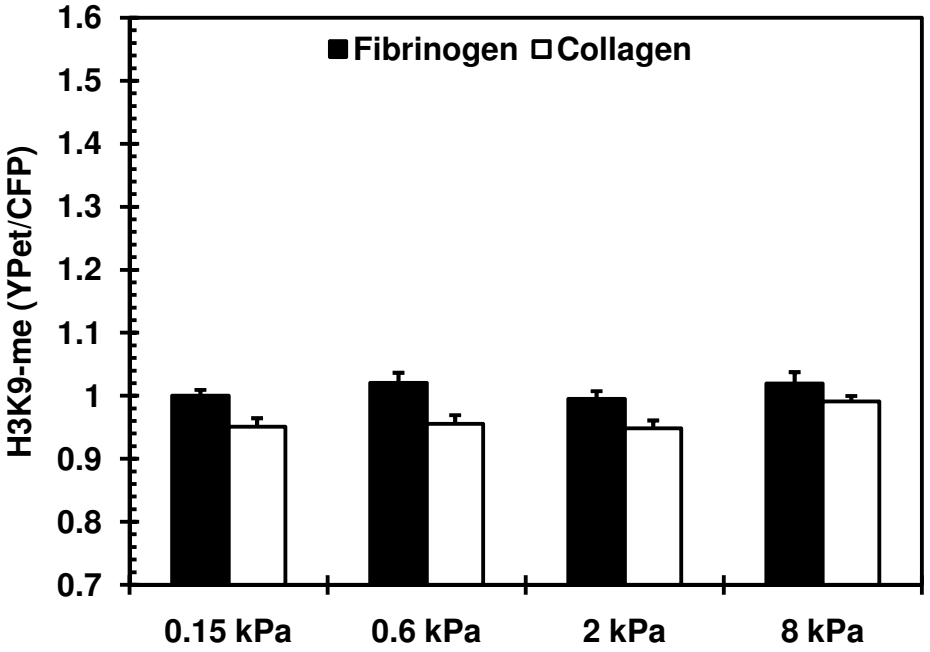


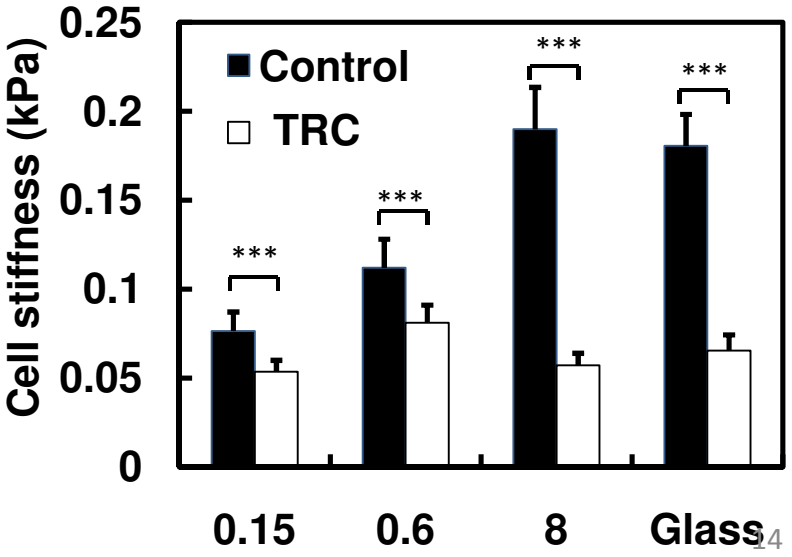


TRCs do not respond to substrate stiffness

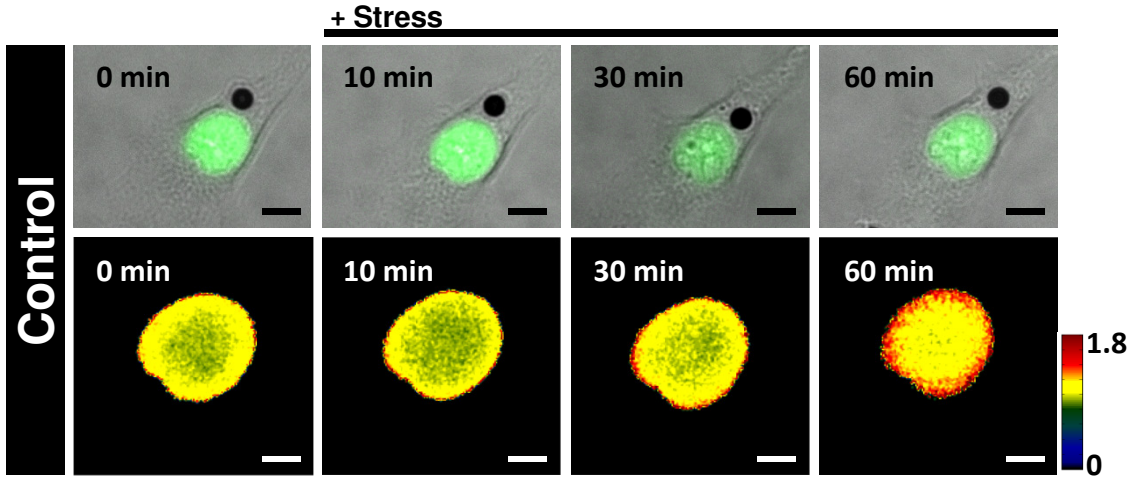


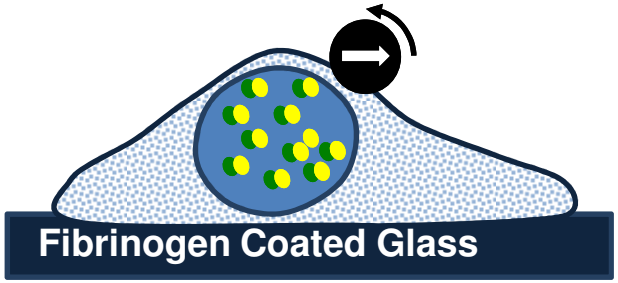


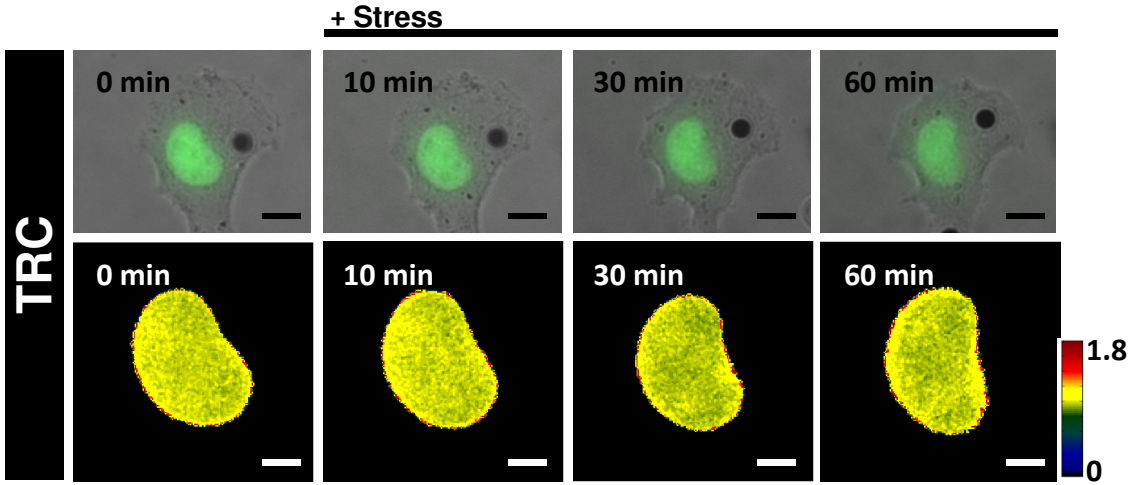


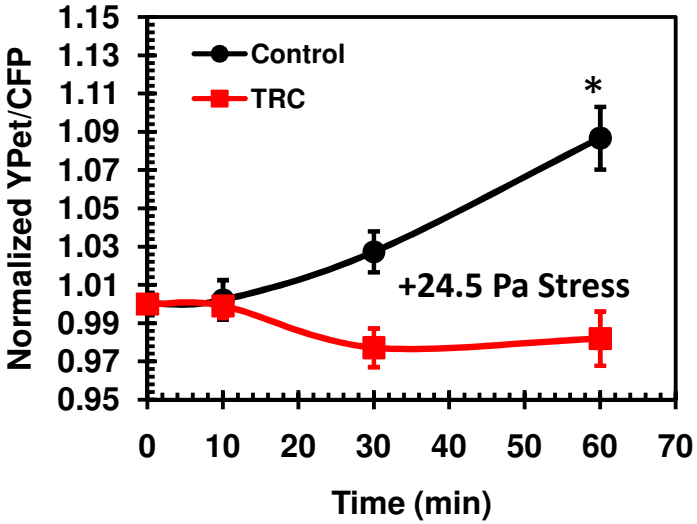


Exogenous forces regulate H3K9-me

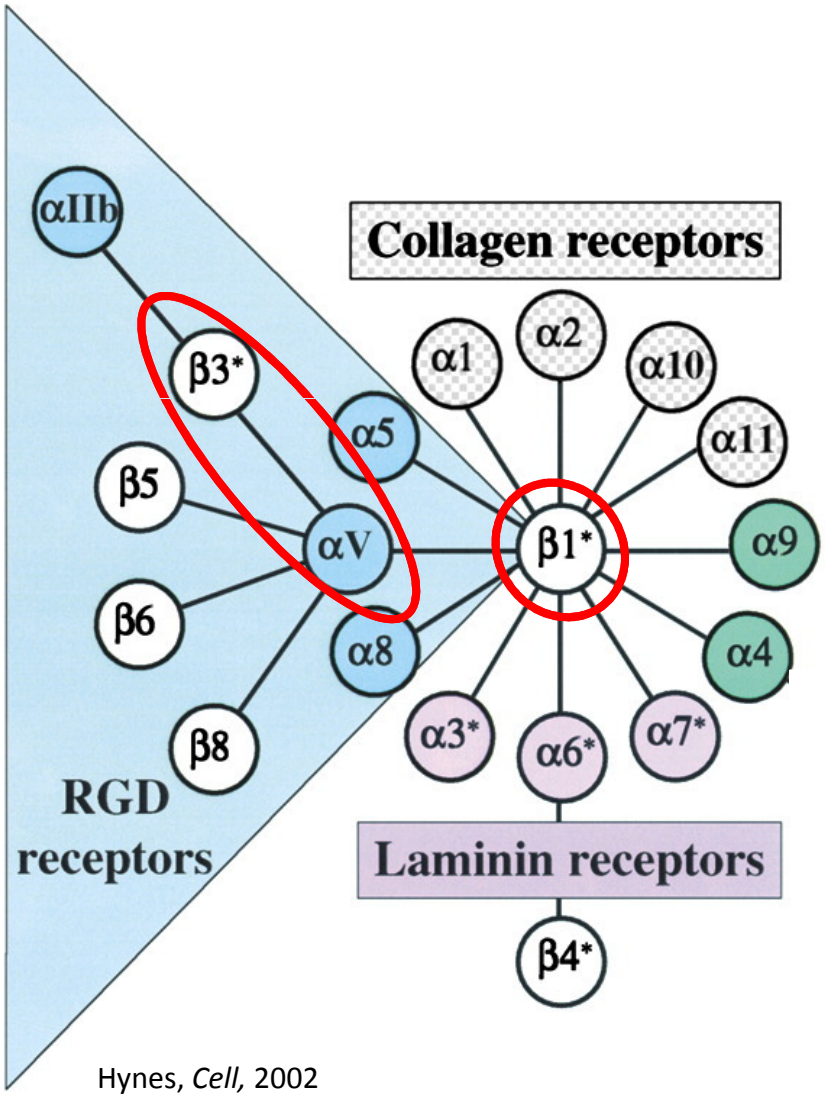




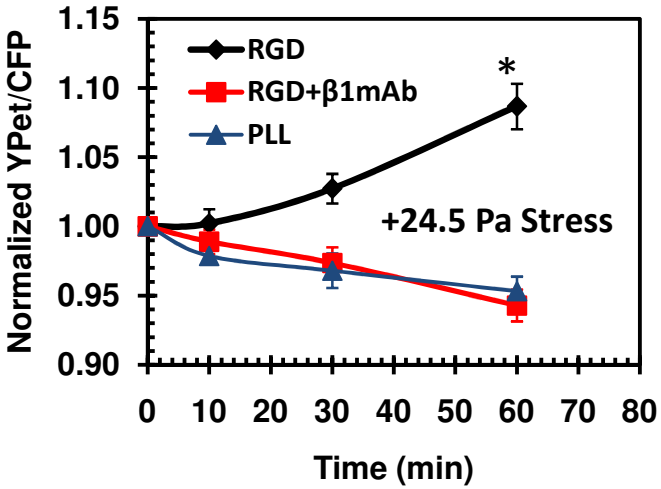


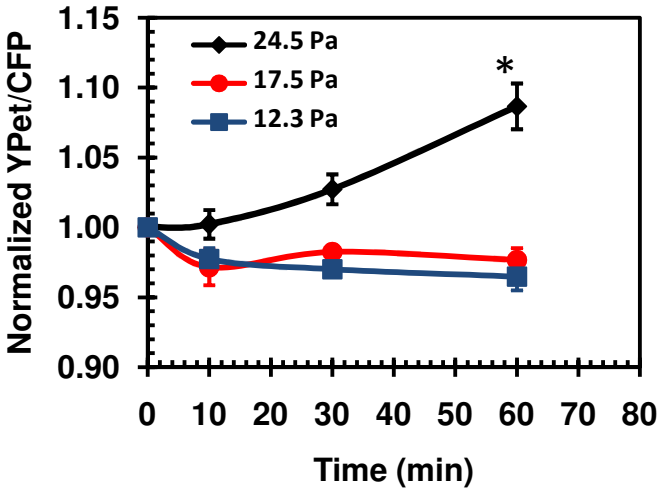


Blocking β1 integrin inhibits force induced H3K9-me in control melanoma cells

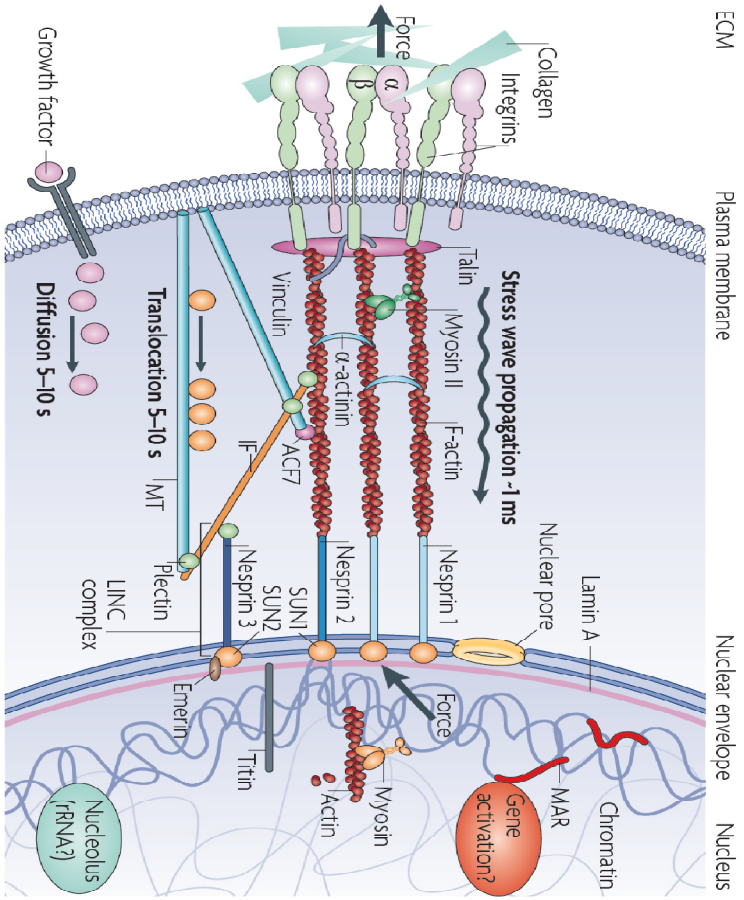


Hynes, *Cell,* 2002

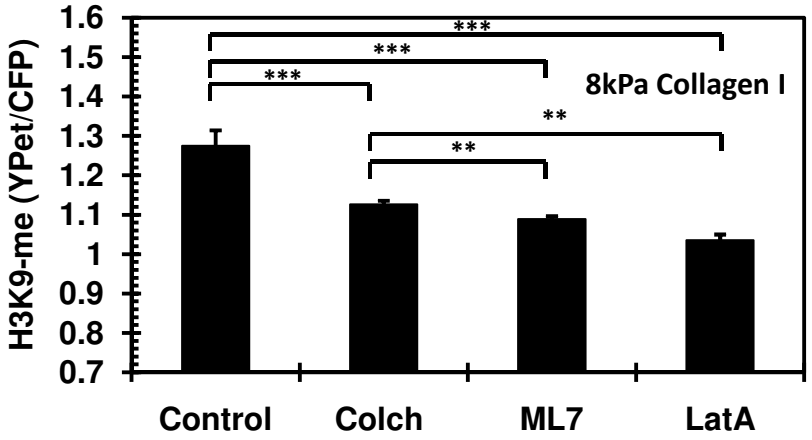




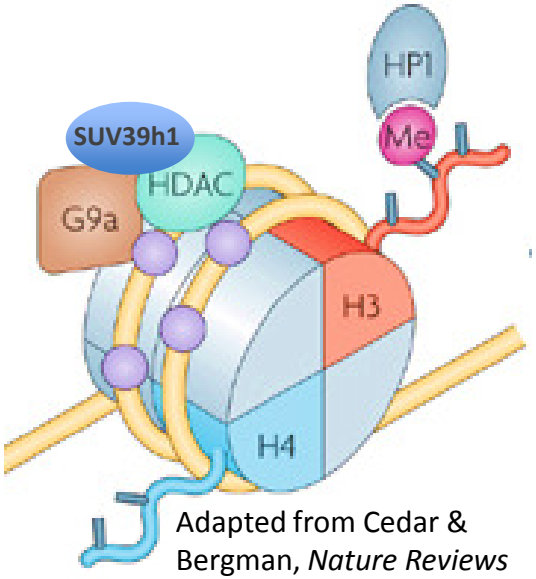
H3K9-me level depends on intact CSK



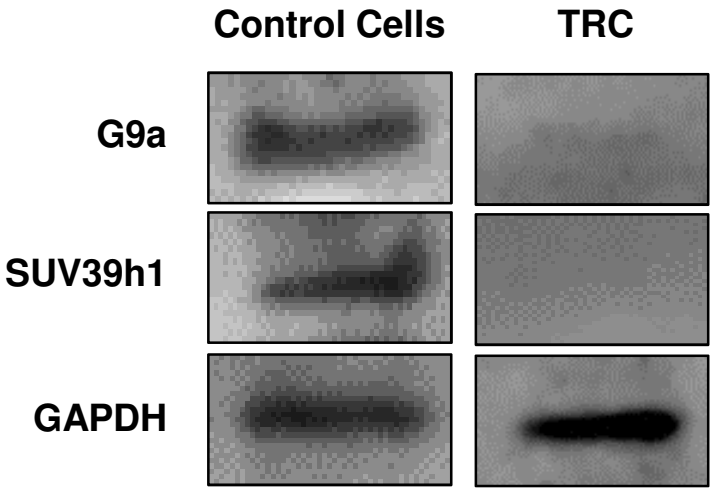
Wang *et al*, *Nature Reviews Molecular Cell Biology,* 2009

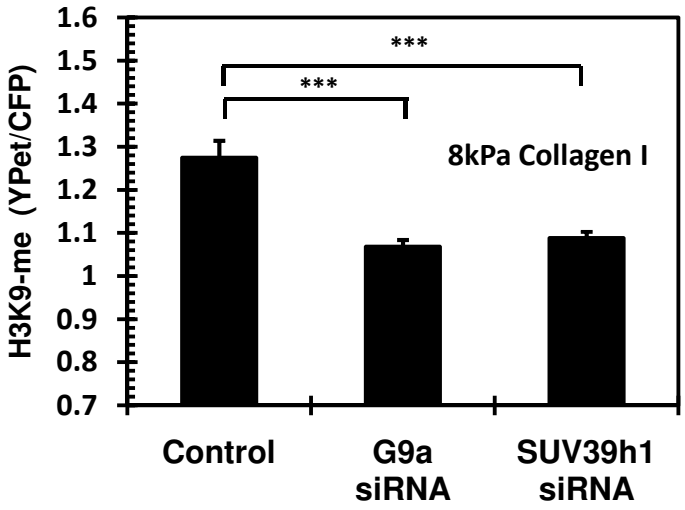


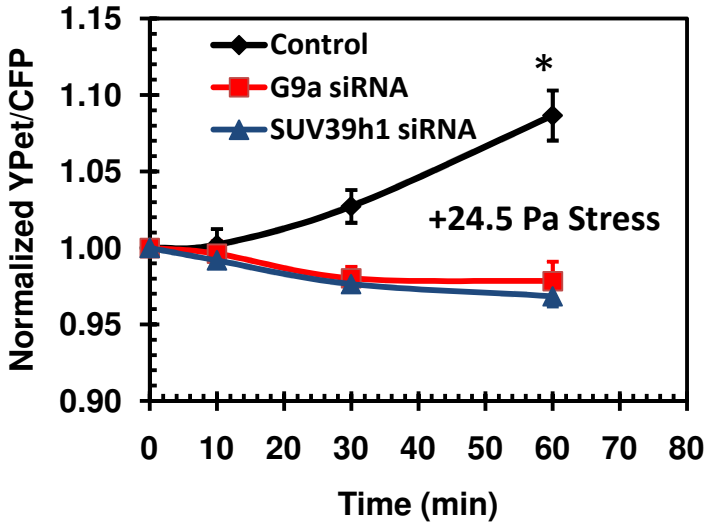
Force-induced H3K9-me depends on methyltransferases



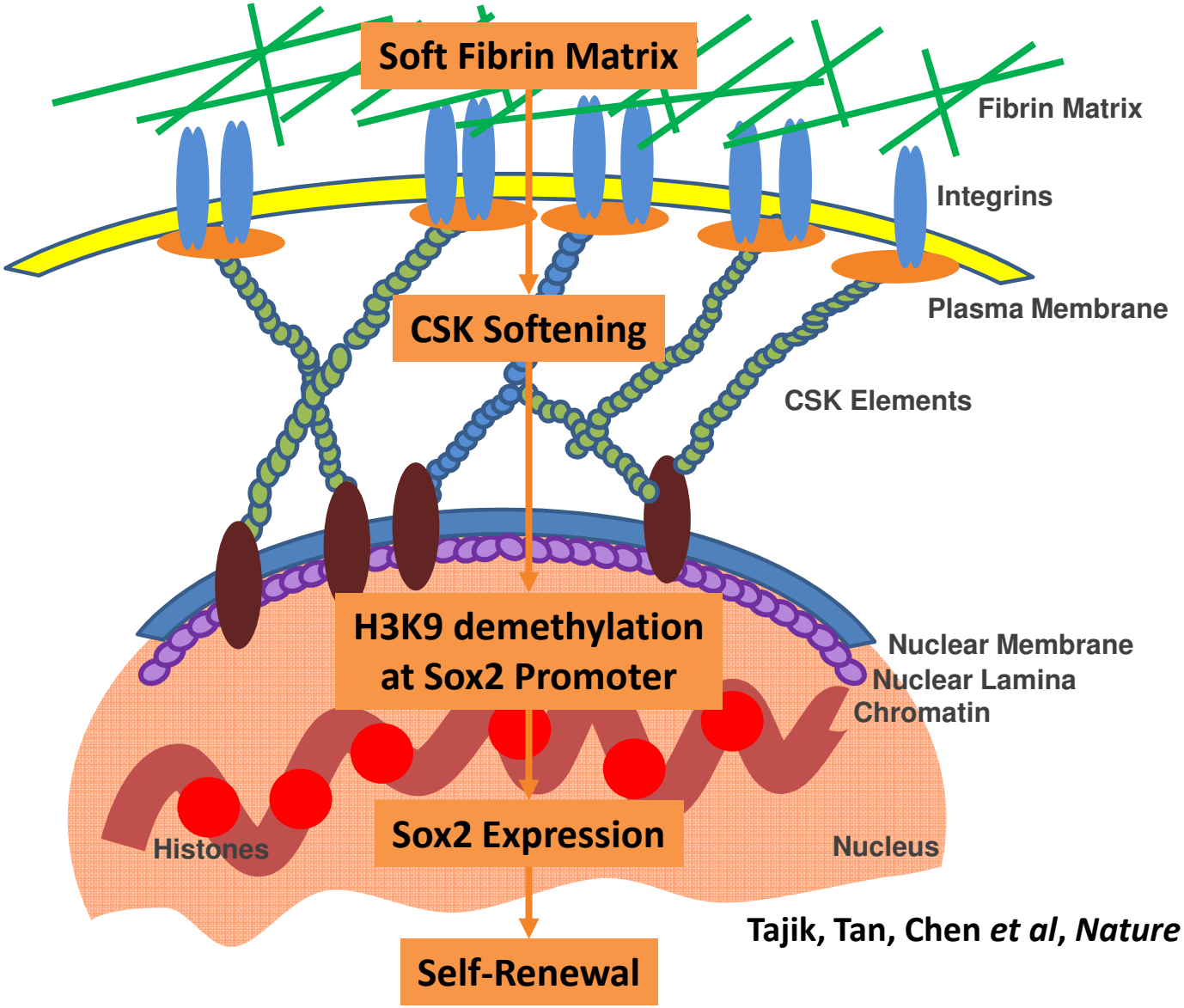
Adapted from Cedar & Bergman, *Nature Reviews Genetics,* 2009







Soft matrix-mediated H3K9 demethylation and Sox2 expression



Tajik, Tan, Chen *et al*, *Nature Comm, 4,* 2014

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  - Dr Jihye Seong
  - Dr Jie Sun

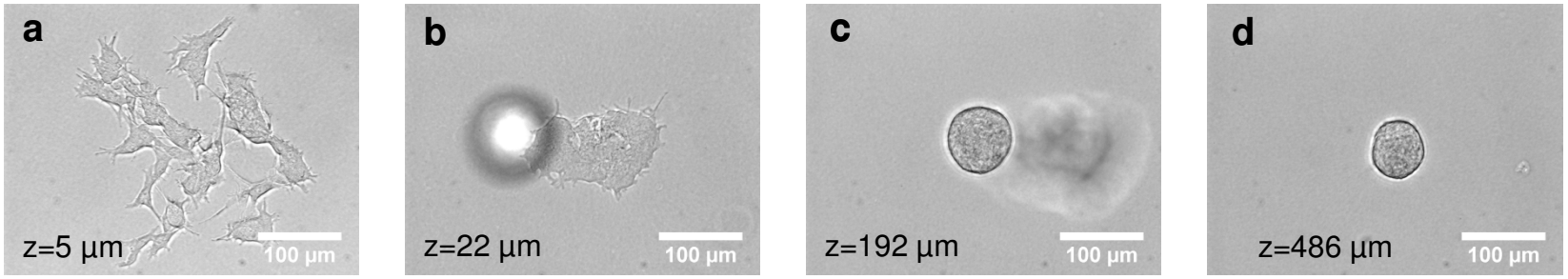
- **Huazhong University of Science and Technology, China**
  - Junwei Chen
  - Qiong Jia



3D matrix stiffness not porosity dictates colony formation

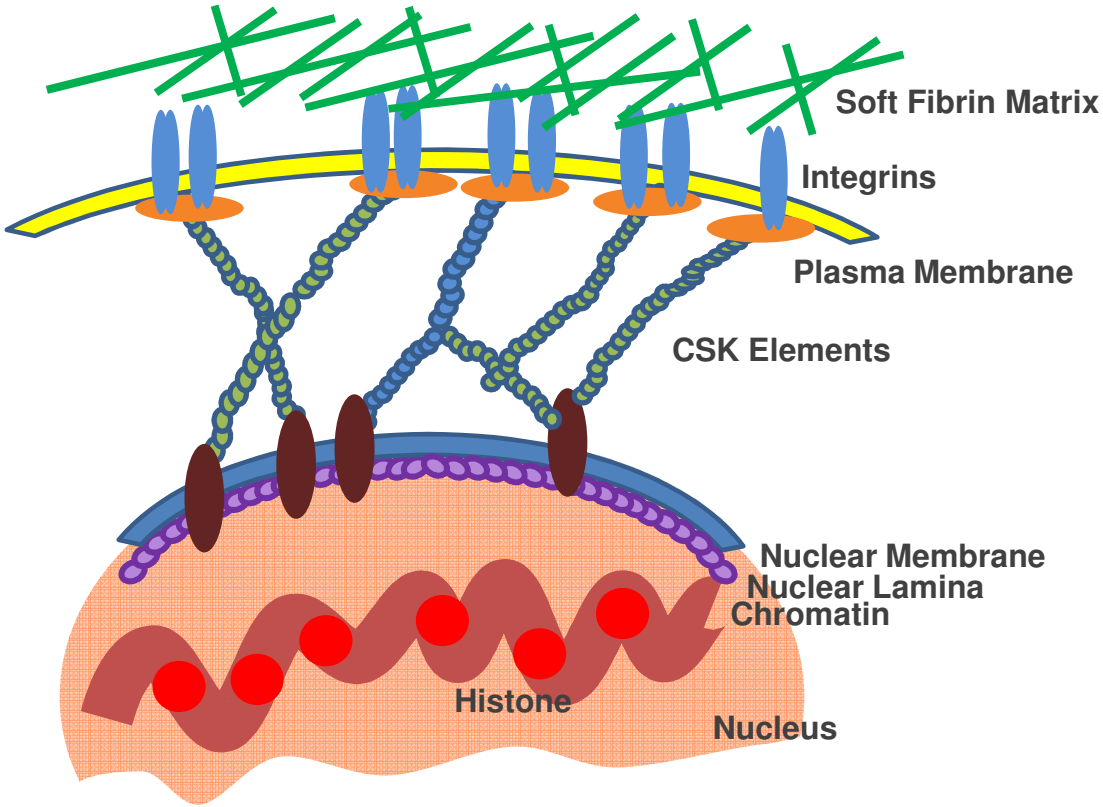
Spread colony near rigid bottom

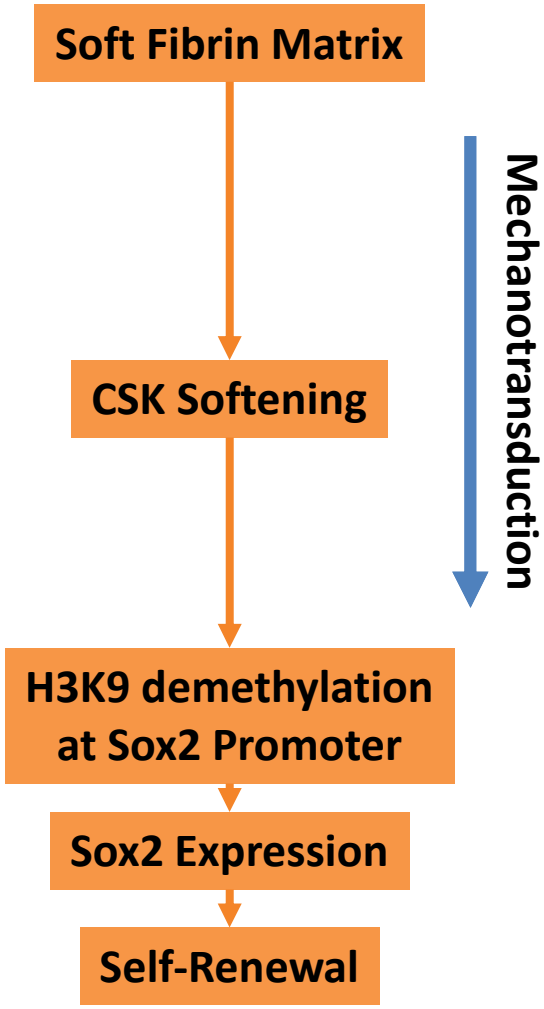
Round colony far away from rigid bottom



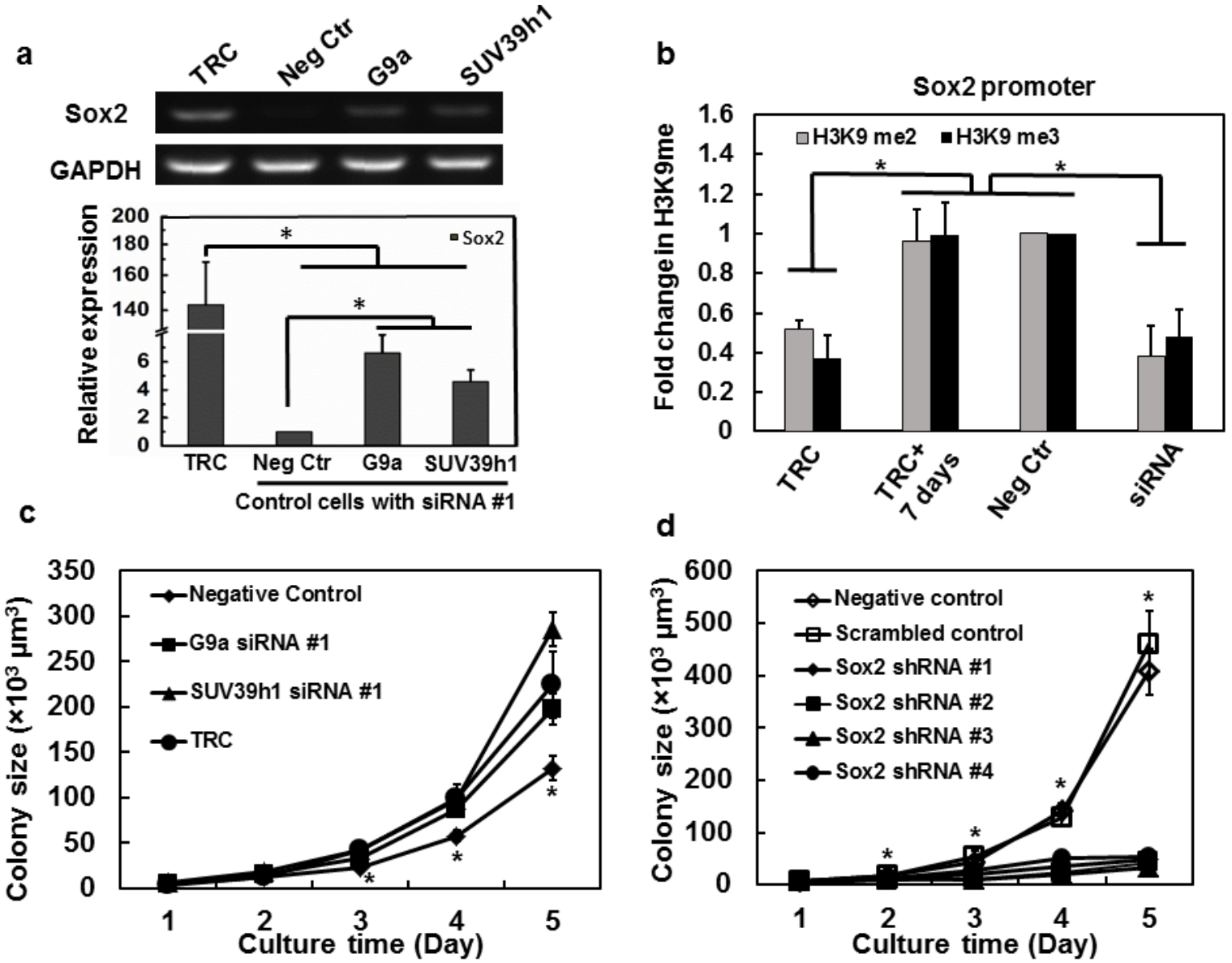
z: distance from the rigid bottom

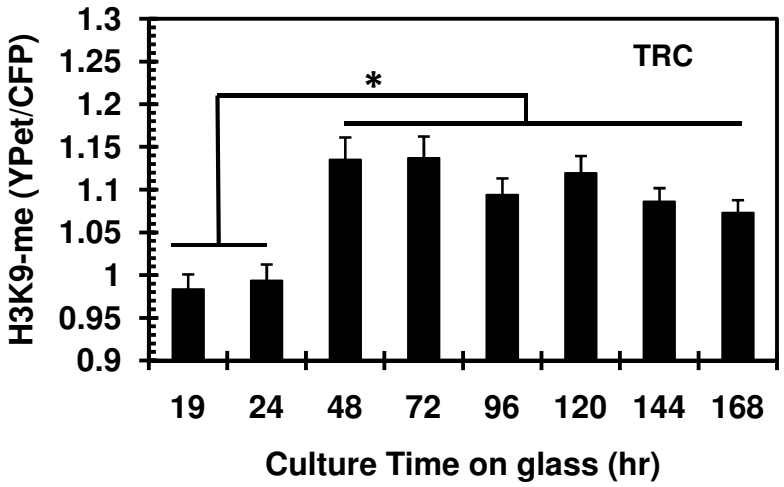
Cytoskeleton-dependent H3K9 demethylation/methylation and Sox2 expression

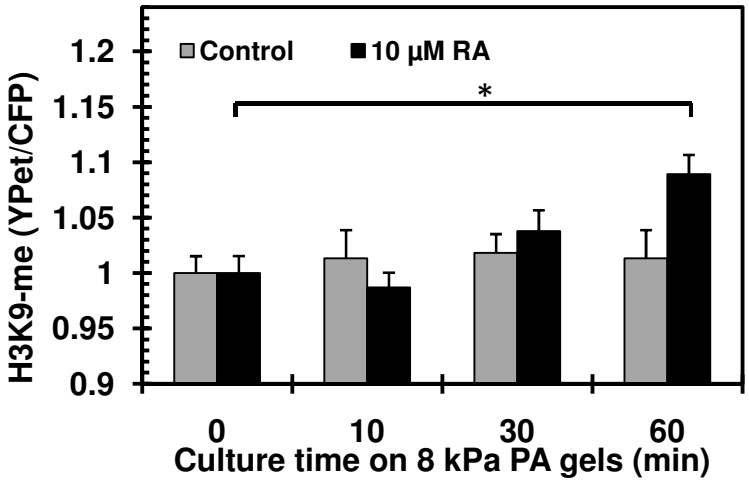




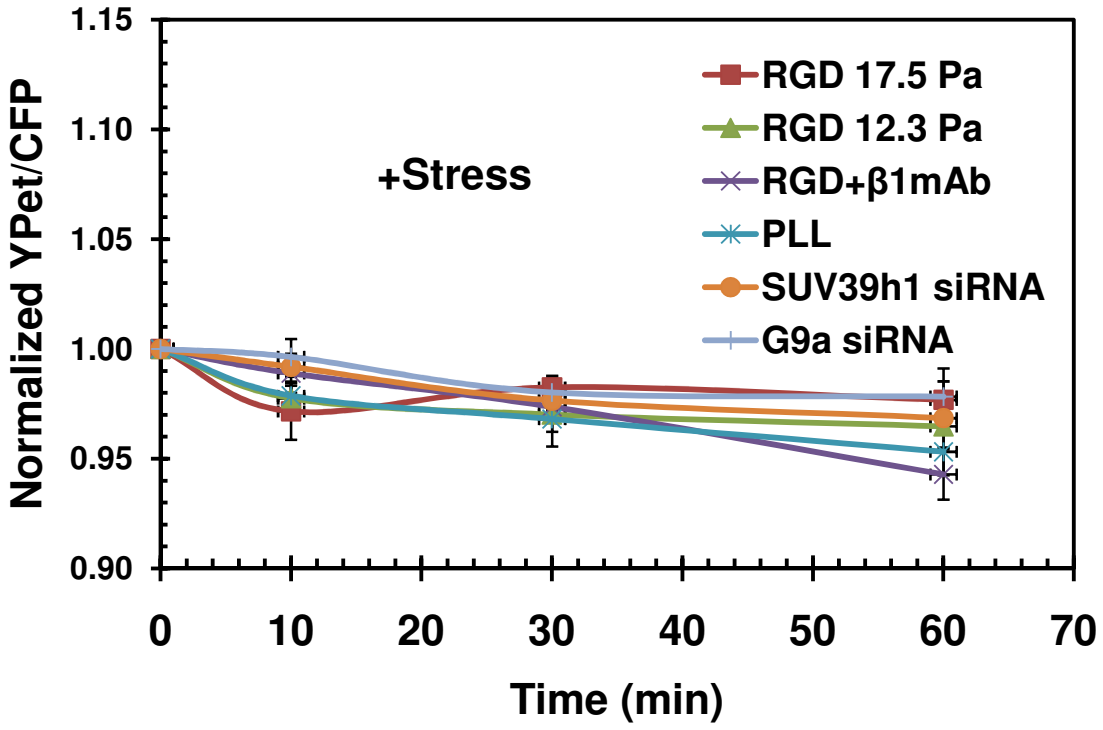
H3K9 demethylation mediates Sox2 dependent self-renewal of TRCs

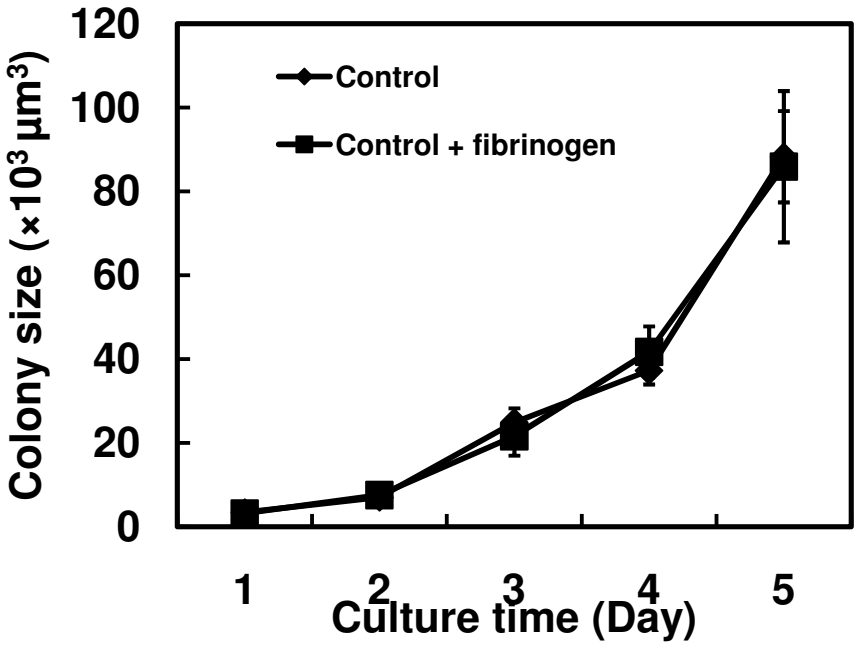


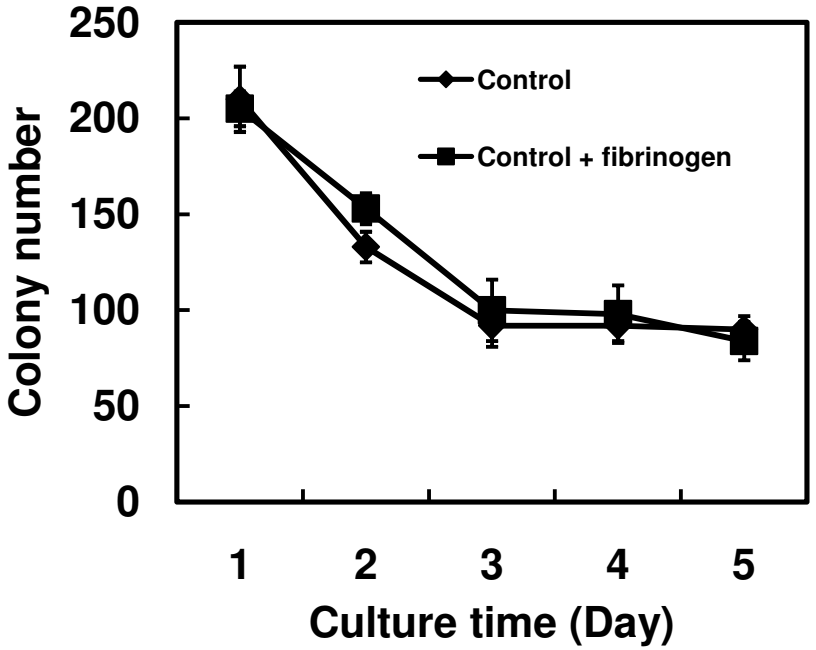

H3K9-me (YPet/CFP)
1.3
1.25
1.2
1.15
1.1
1.05
1
0.95
0.9
TRC
*
19
24
48
72
96
120
144
168
Culture Time on glass (hr)

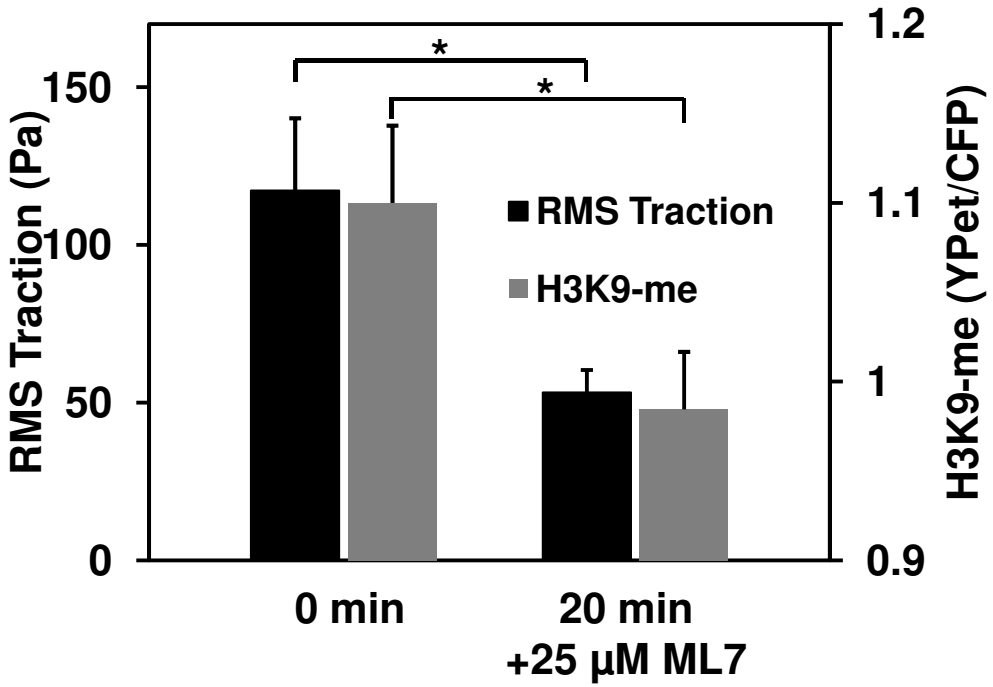

H3K9-me (YPet/CFP)
1.2
1.15
1.1
1.05
1
0.95
0.9
Control
10 μM RA
*
0
10
30
60
Culture time on 8 kPa PA gels (min)

β-1 integrin mediated force-dependent H3K9 methylation




Colony size (×10³ μm³)
120
100
80
60
40
20
0
Control
Control + fibrinogen
1
2
3
4
5
Culture time (Day)


Colony number
250
200
150
100
50
0
Control
Control + fibrinogen
1
2
3
4
5
Culture time (Day)


RMS Traction (Pa)
150
100
50
0
1.2
1.1
1
0.9
H3K9-me (YPet/CFP)
*
*
RMS Traction
H3K9-me
0 min
20 min
+25 µM ML7

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