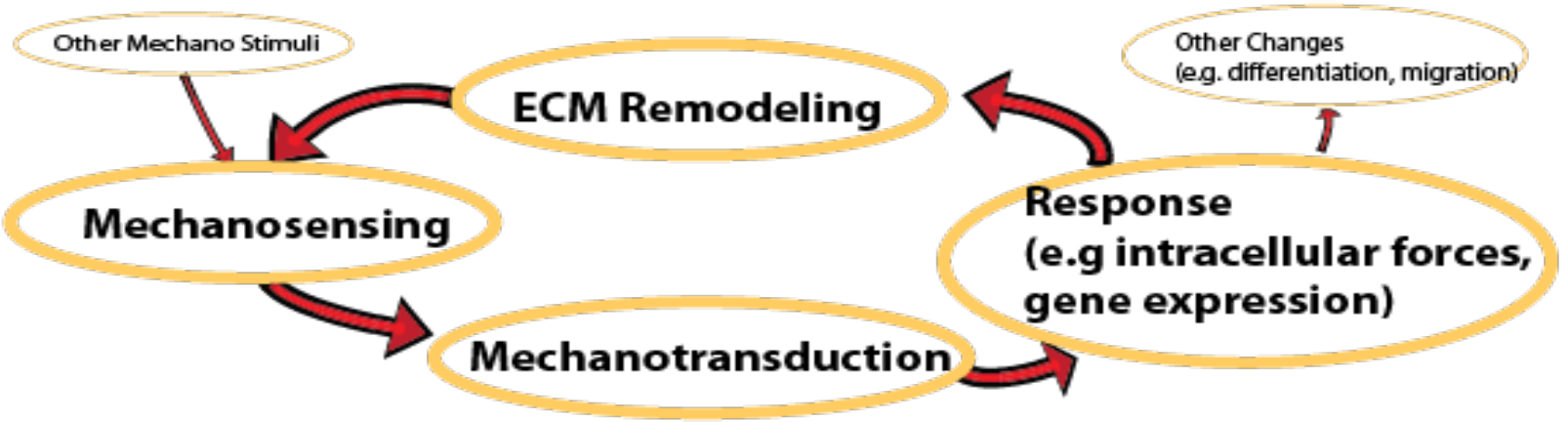
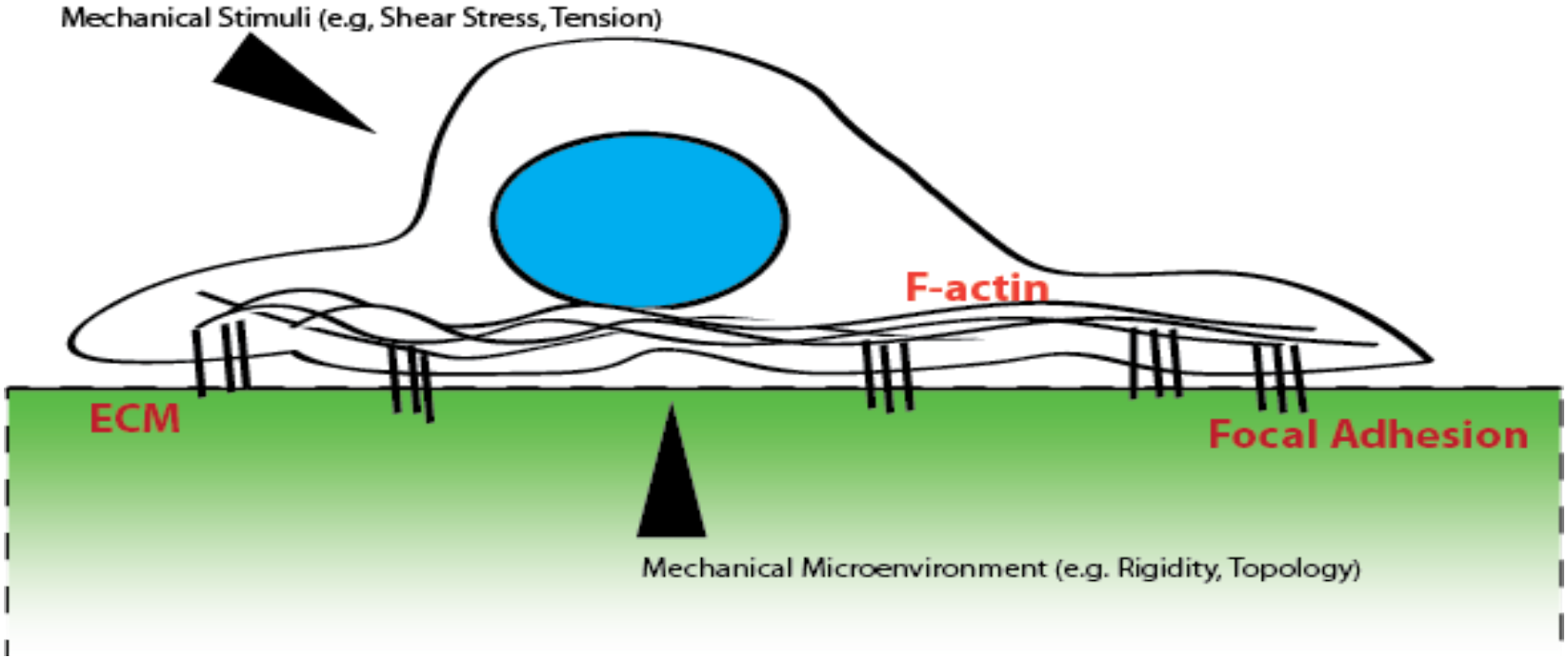
Biological Applications of MechanoBiology

FYS4715 Høst 2021

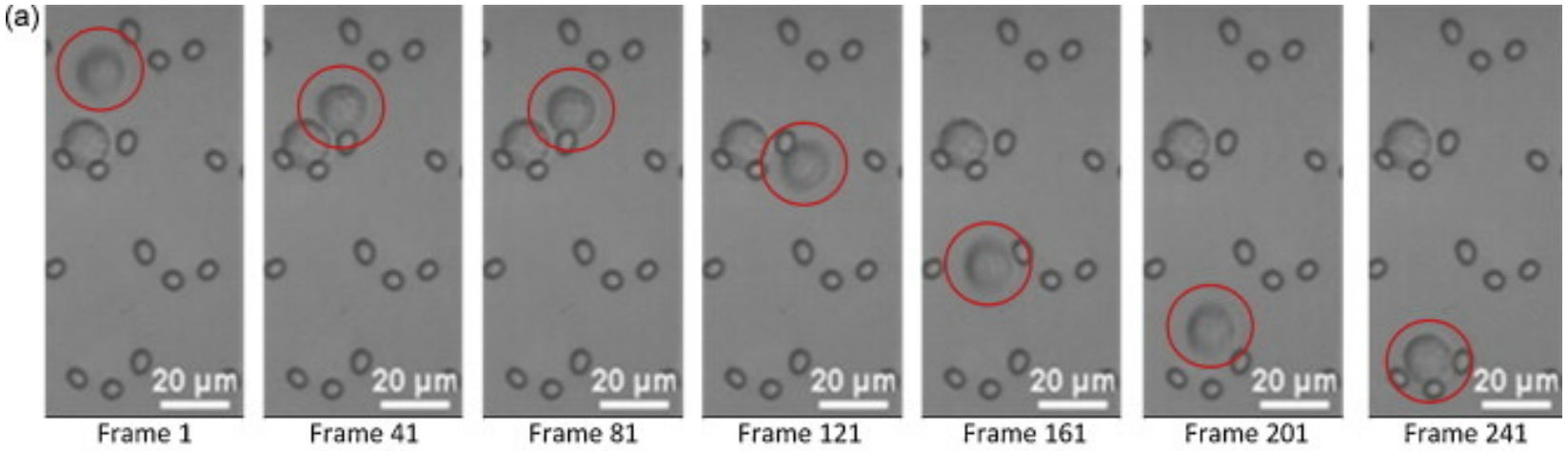
Xian Hu(Edna)


Other Mechano Stimuli
ECM Remodeling
Other Changes
(e.g. differentiation, migration)
Mechanosensing
Response
(e.g intracellular forces,
gene expression)
Mechanotransduction


Mechanical Stimuli (e.g, Shear Stress, Tension)
F-actin
ECM
Focal Adhesion
Mechanical Microenvironment (e.g. Rigidity, Topology)

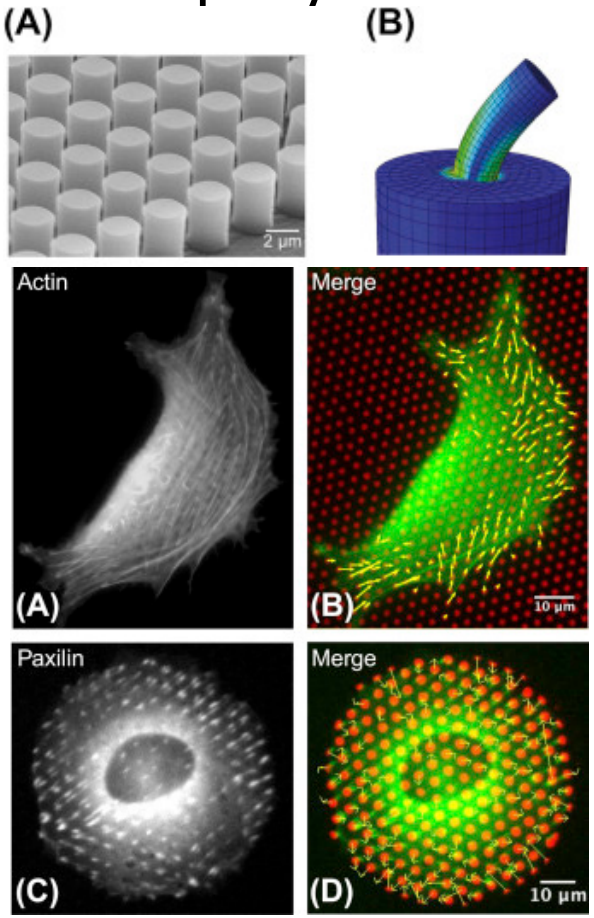
Cellular Rigidity

Microfluidics device that capture CTC by rigidity

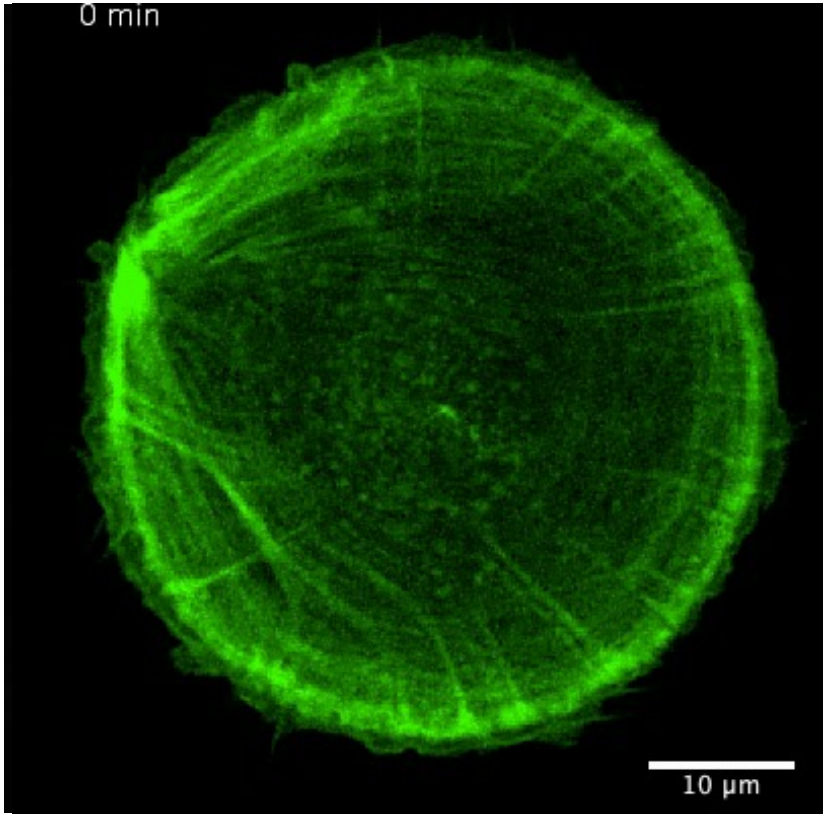


Tan et al, 2010 Biosensors and Bioelectronics

Cell exert physical forces on substrate

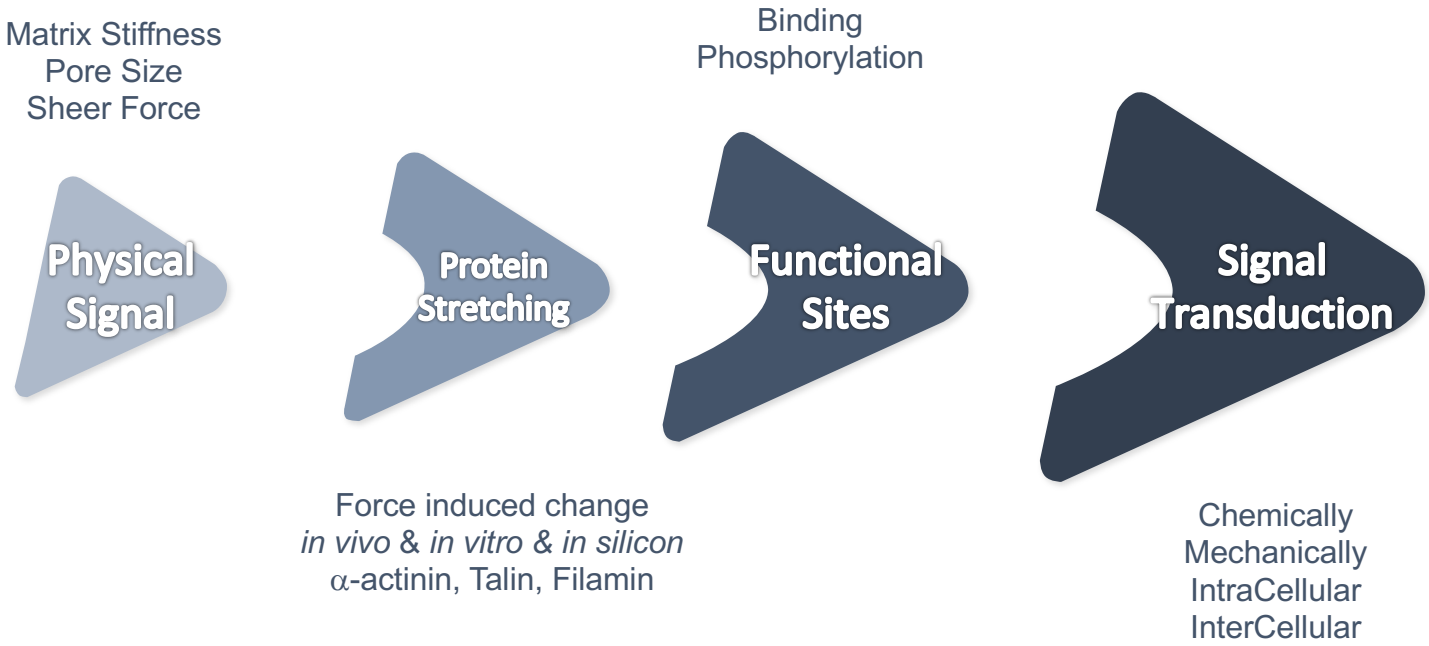


Gupta et al, 2015 Methods in Cell Biology



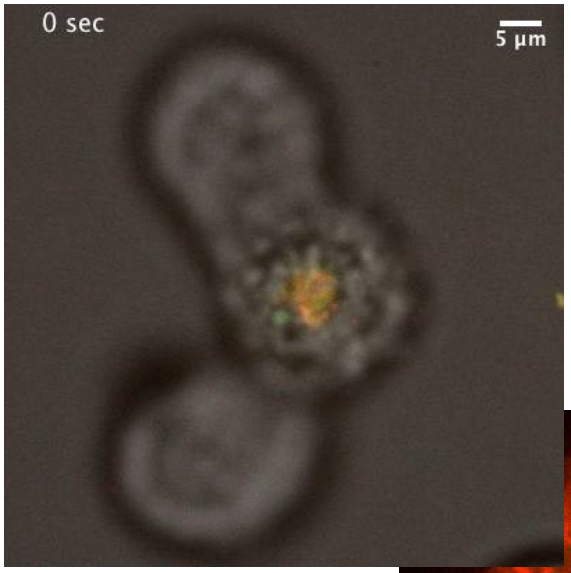
Tee et al, 2015 NCB

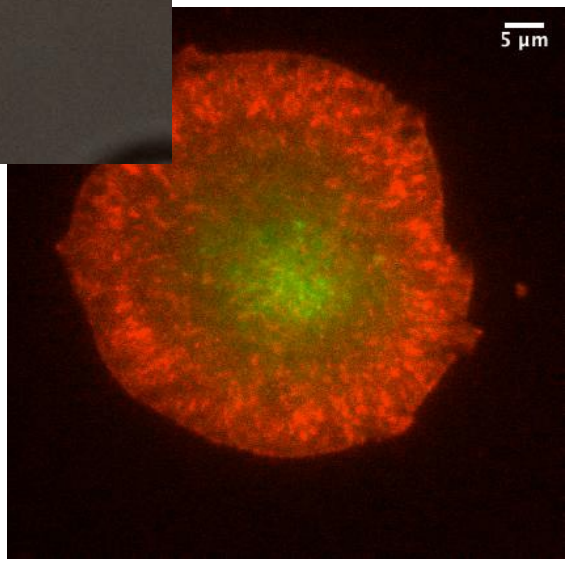
Rigidity Sensing and Molecular Mechanism



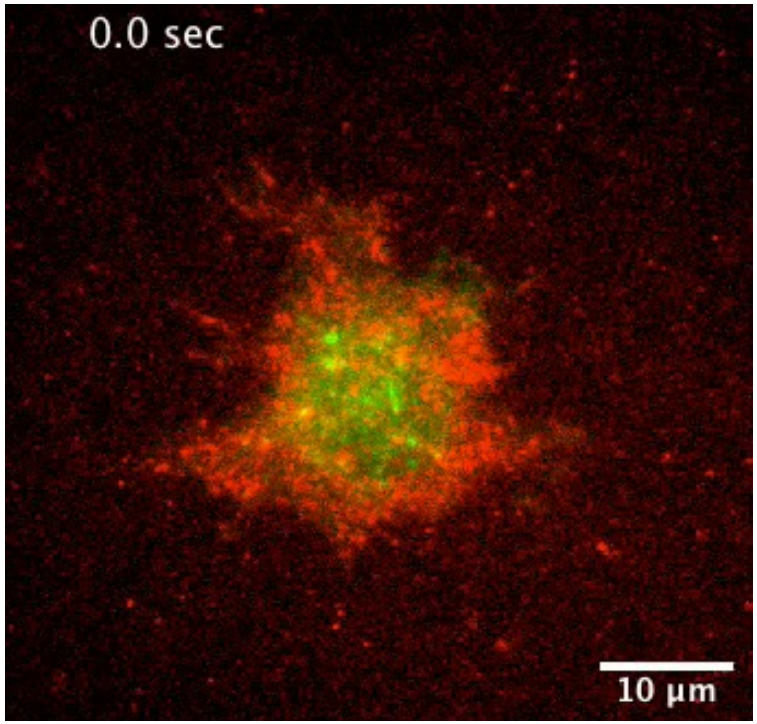
Understand Complex Function in Cells

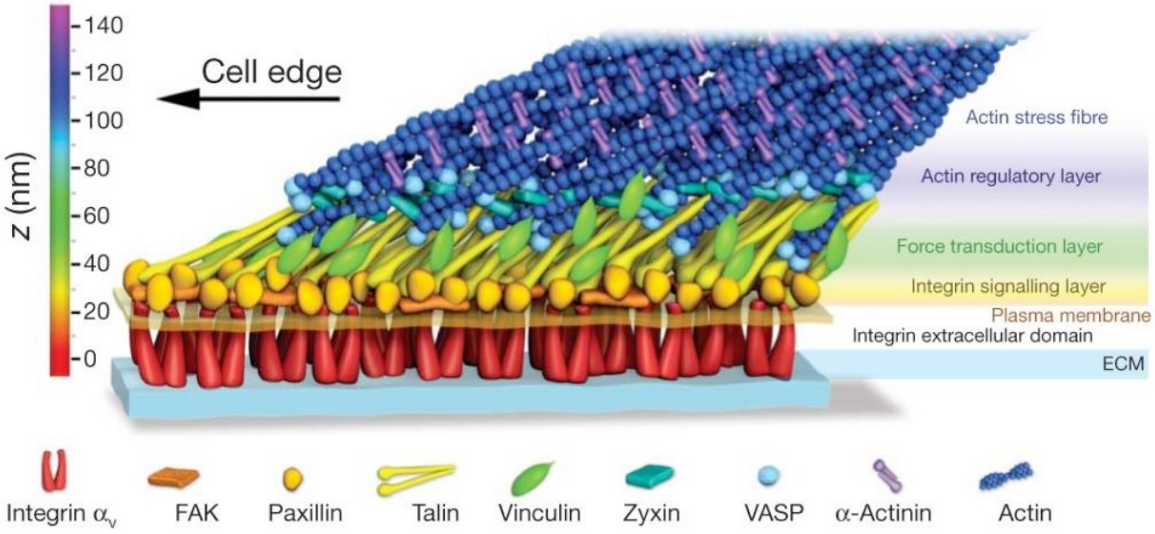
- Cells has many complex functions, integrated by different cellular states—high noise
- Need to standardize experimental procedure, isolate the events and reapeat
- Rigidity Sensing- Cell Sensing Matrix-Cell Binding to Matrix- Cell Spreading Assay
- Have several different experiments to test the same thing



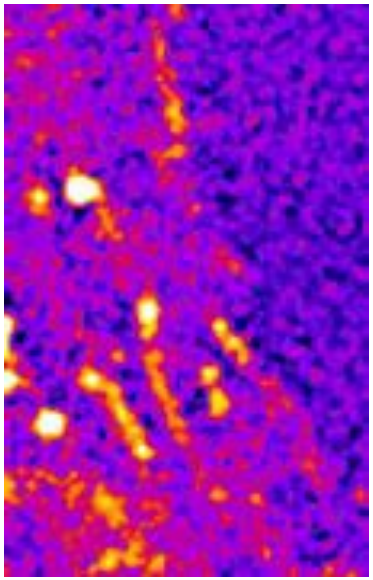


Focal Adhesion

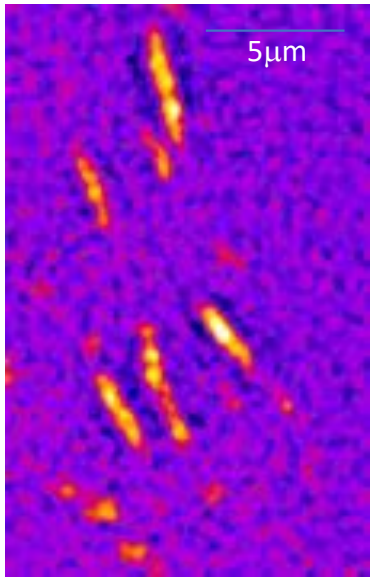




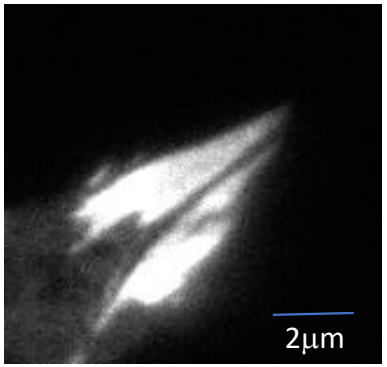
Dynamics of Focal Adhesion Proteins



Talin-GFP



Vinculin-mCherry



Vinculin-GFP

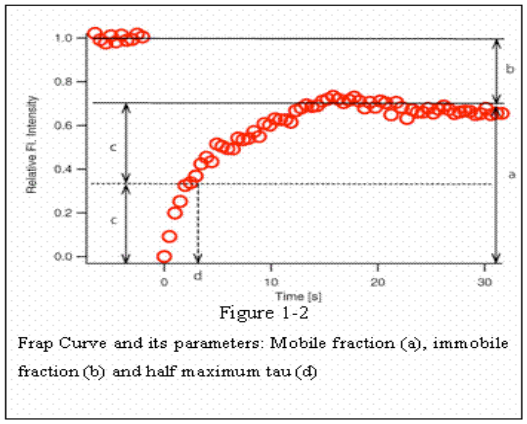


Figure 1-2

Frap Curve and its parameters: Mobile fraction (a), immobile fraction (b) and half maximum tau (d)

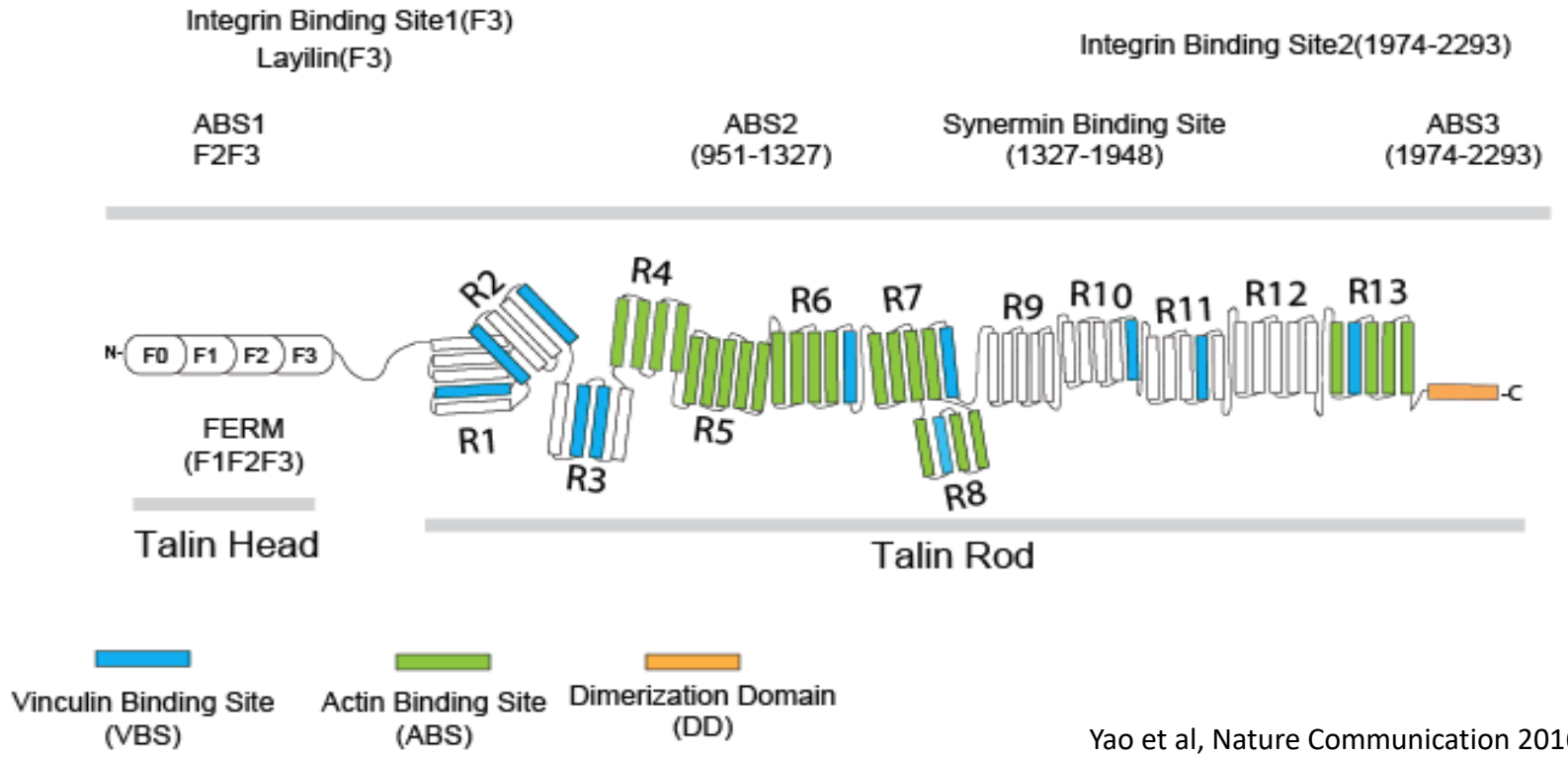
Glass/Fibronectin
100ms/Frame
Stream Mode

MEF(Fibroblast)

Dynamics of FA Proteins

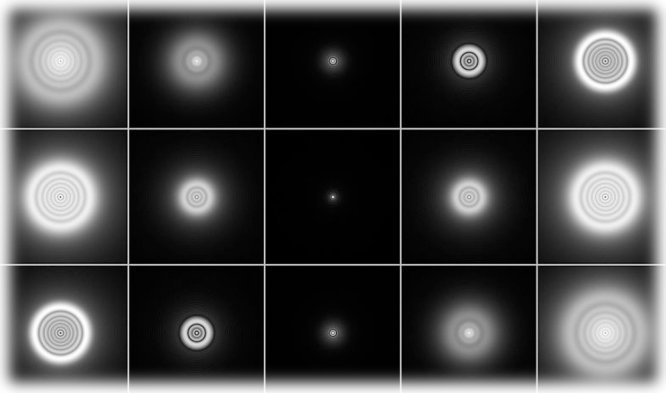
Protein Name	FRAP t1/2(s)	Cell Type
Vinculin	1.9/14/50/11/21	HelaJW/MEF/ REF52
Paxillin	1.5/25/17.7/53.2/20	HelaJW/MEF/ Bovine capillary endothelial cells/MEF, TIRF FRAP/ REF52
FAK	2.1/7/6.7/14	HelaJW/MEF/ Bovine capillary endothelial cells/ REF52
Zyxin	0.5/12/7/10	HelaJW/MEF/ REF52
Talin	2.2/23/77/18	HelaJW/MEF/ Bovine capillary endothelial cells/ REF52
ILK	9.2/12	HelaJW/ REF52
□-actinin	36/14.1/27	MEF/ Bovine capillary endothelial cells/ REF52
beta3-Integrgin	38.5	Bovine capillary endothelial cells
p130Cas	5.8/14	MEF / REF52
Tensin	55	REF52
Src/CSK	9	REF52
Pinch	14	REF52
VASP	0.7/15	HelaJW/ REF52

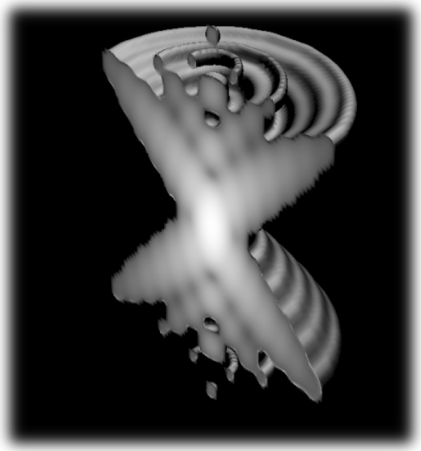
Talin



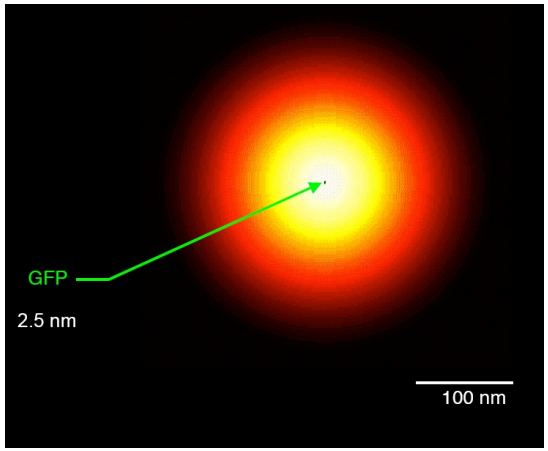
Yao et al, Nature Communication 2016

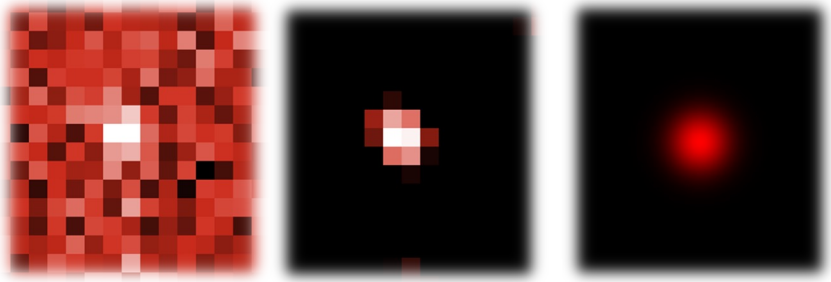
Resolution Limit/PSF

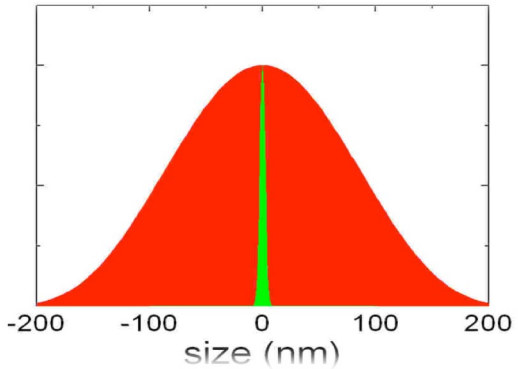




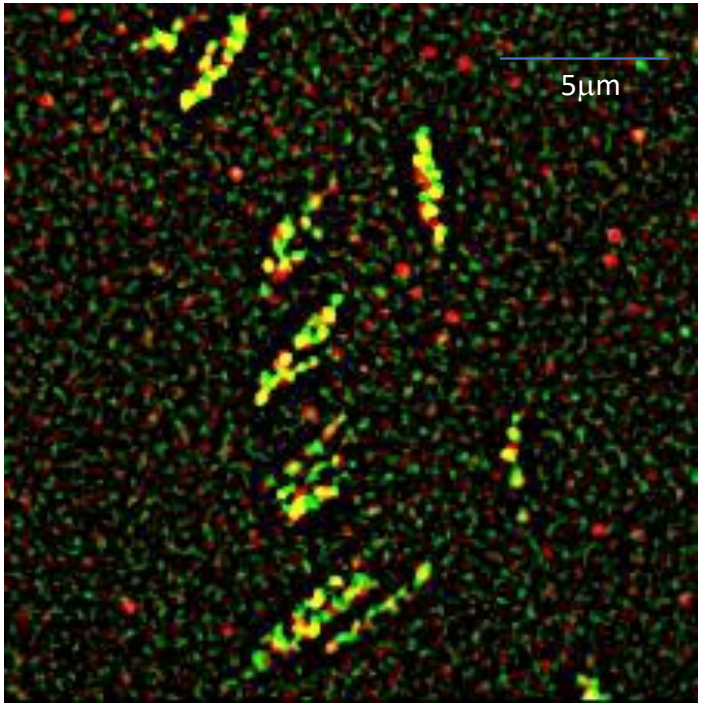
Single Molecule Localization



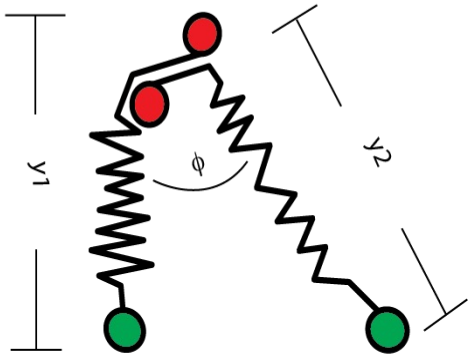




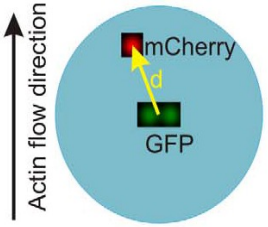
Talin Stretching *in vivo*

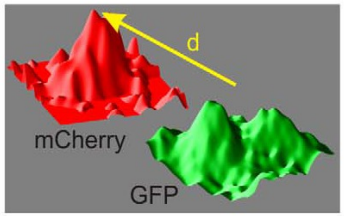


GFP-Talin-mCherry



Labeling of Talin N&C





Dimer Tolerant Detector: Search for Signal Pairs

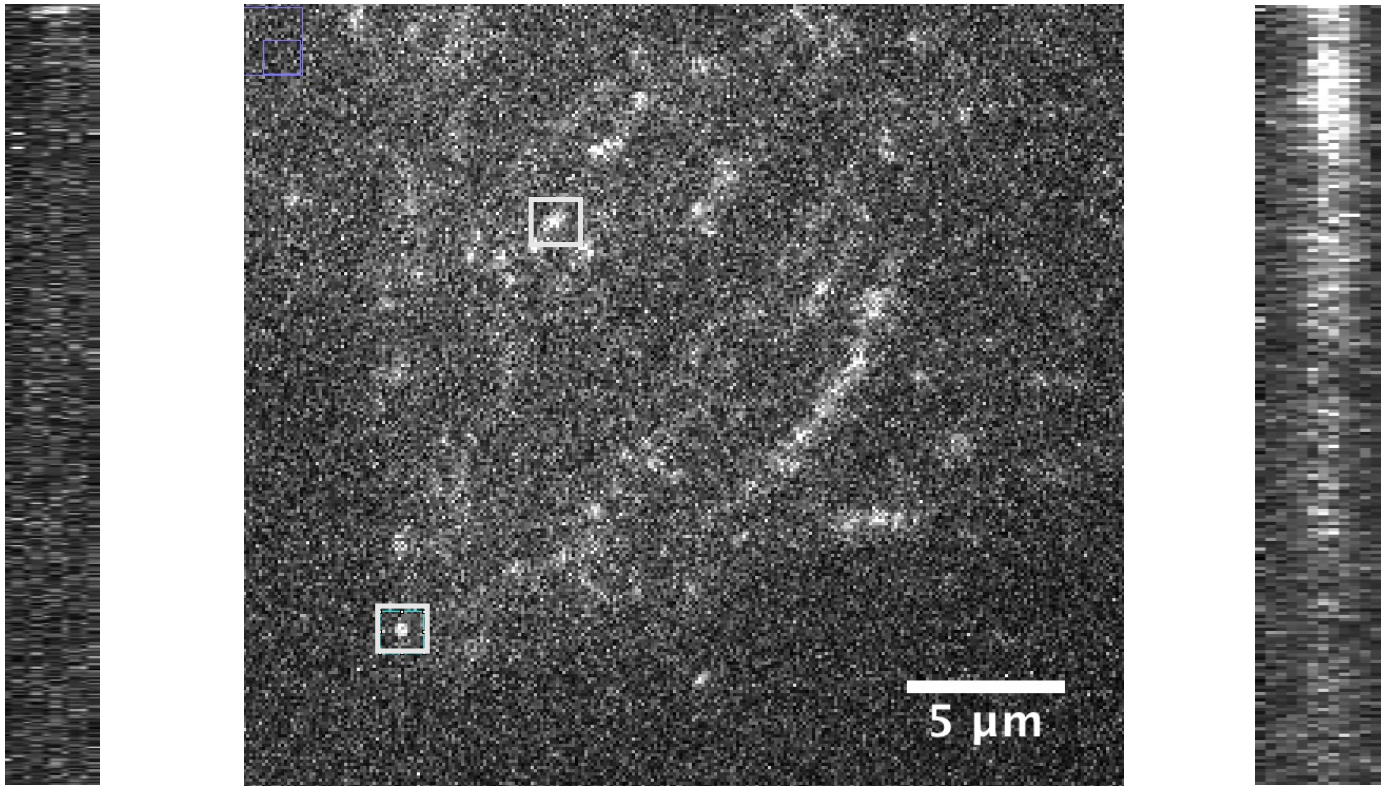
Paired Single Molecule
Live Cell Imaging
site

GFP: integrin binding s
mCherry: actin binding

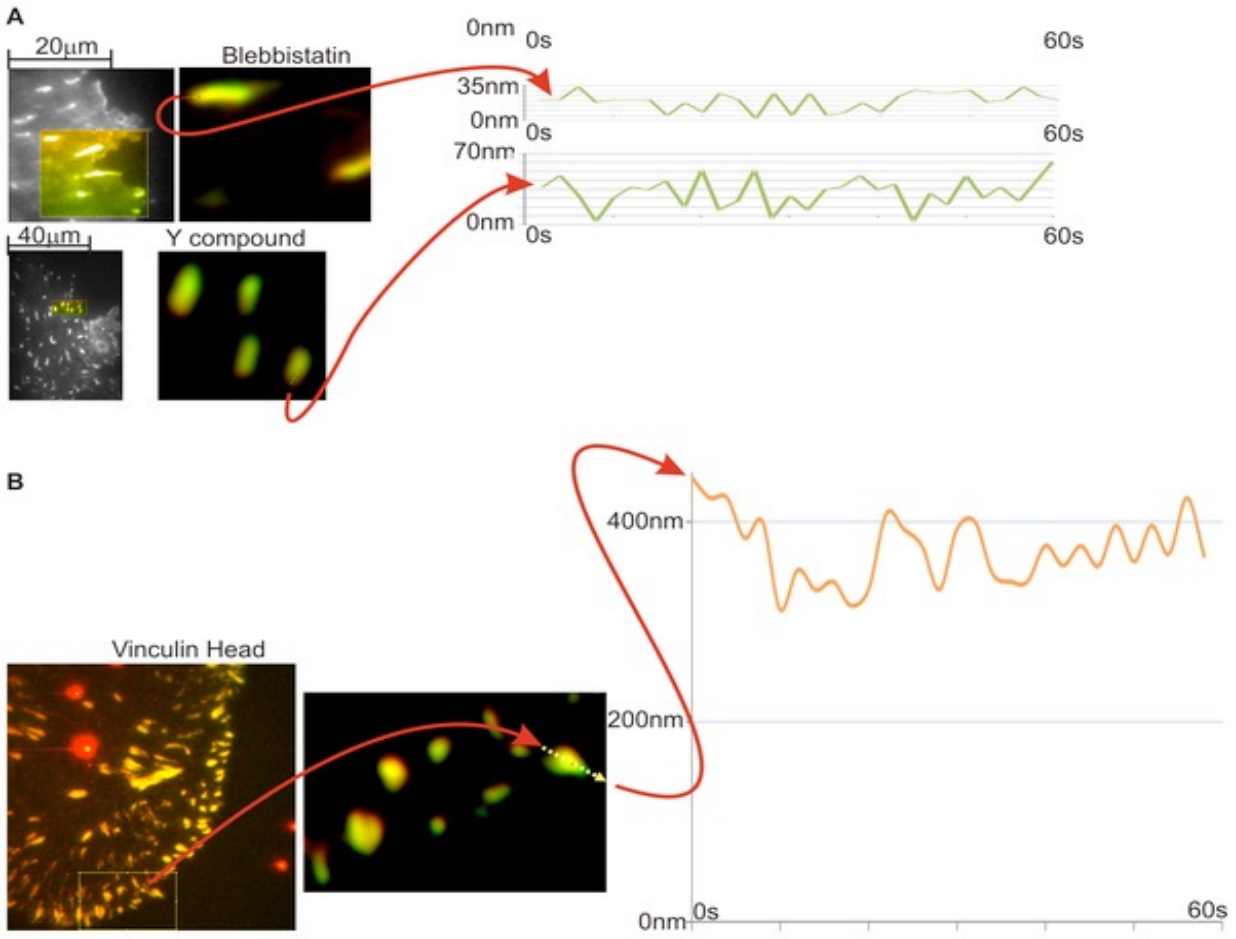
100ms/frame, stream mode, total duration 10s

Margadant.et al, Plos Biology 2011

Single Molecule Imaging

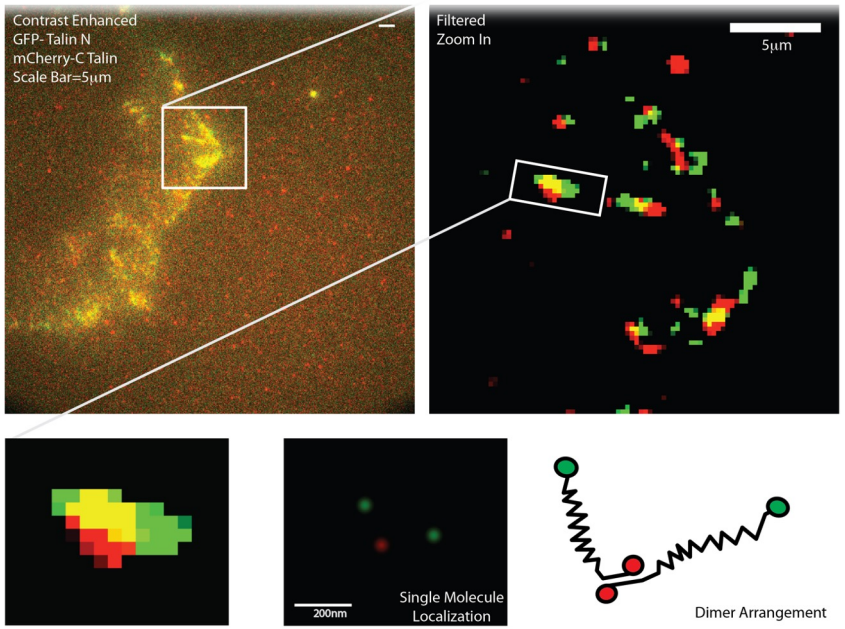


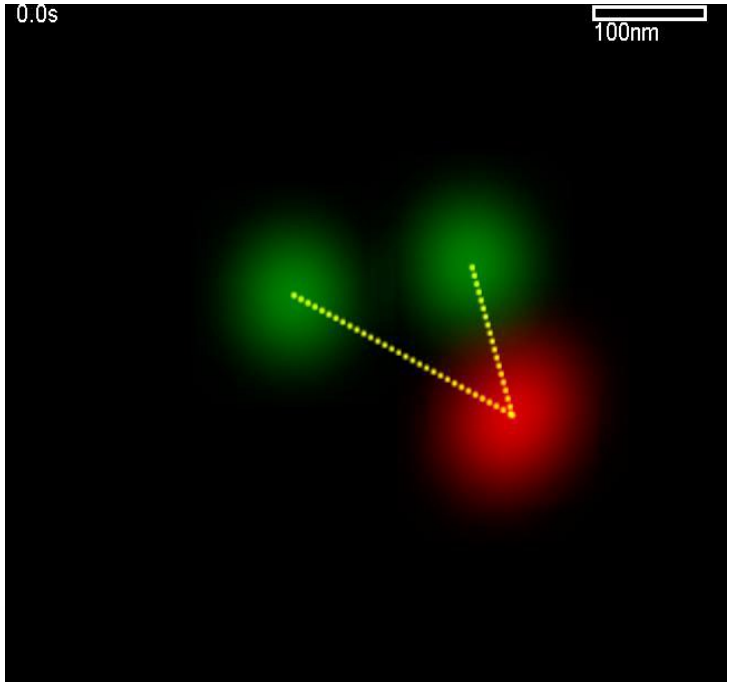
Talin Stretching *in vivo*



Margadant.et al, Plos Biology 2011

GFP-Talin-mCherry
Paired Single Molecule Dynamics



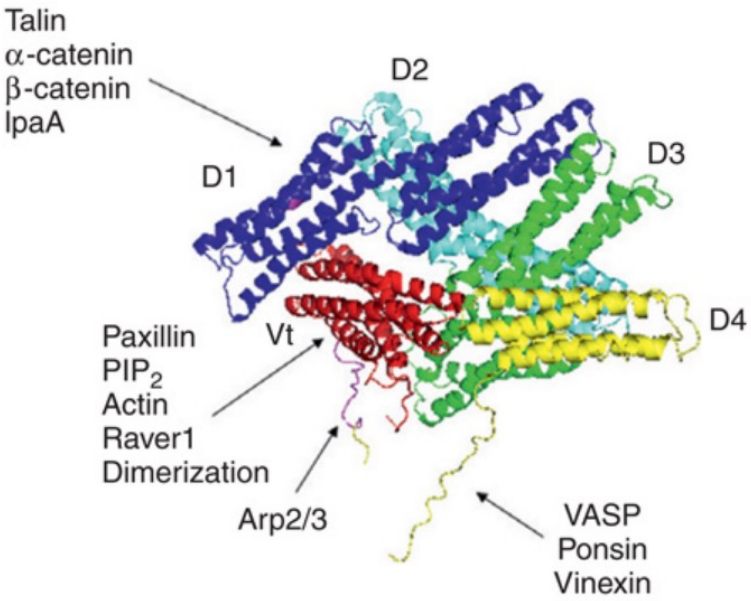


Hu.et al, Nano Letters, 2016

Vinculin

- Function:
  - adaptor
  - non enzymatic activity
  - regulating FA assembly
  - force transmission

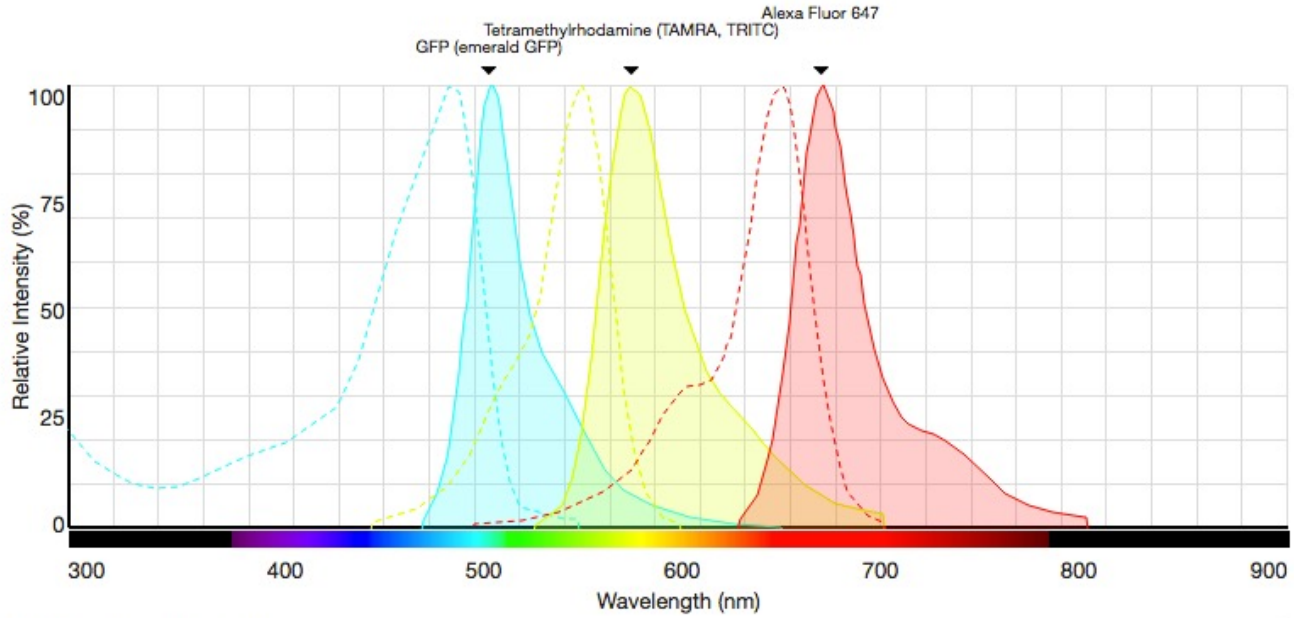
- Work with talin:
  - one talin binding site
  - activated by talin



Peng. et al, Int Rev Cell Mol Biol. 2011

Vinculin and Talin

- **Question: vinculin recruitment event *in situ***
- **Challenge**: Three color live cell imaging with single molecule localization accuracy.
  - Wavelength: ~~Blue~~, ~~Green, Red~~, Far-red
  - Short term imaging
  - Superresolution localization precision
  - Organic dyes: delivering system: eDHFR-TMP



Pointer Location: 635nm, 7%
Fluorophores: Tetramethylrhodamine (TAMRA, TRITC),Alexa Fluor 647

Legend

- GFP (emerald GFP)
- Tetramethylrhodamine (TAMRA, TRITC)
- Alexa Fluor 647

Key
Excitation: - - - Emission: -----

Fluorophores | Light Sources | Excitation Filters | Emission Filters | Reset

	Category (Optional)	Fluorophore	Show plot:	
1.	Click to Select	GFP (emerald GFP)	Excitation Emission	Remove
2.	- All Categories -	Tetramethylrhodamine (TAMRA, TRITC)	Excitation Emission	Remove
3.	Click to Select	Alexa Fluor 647	Excitation Emission	Remove

+ Add

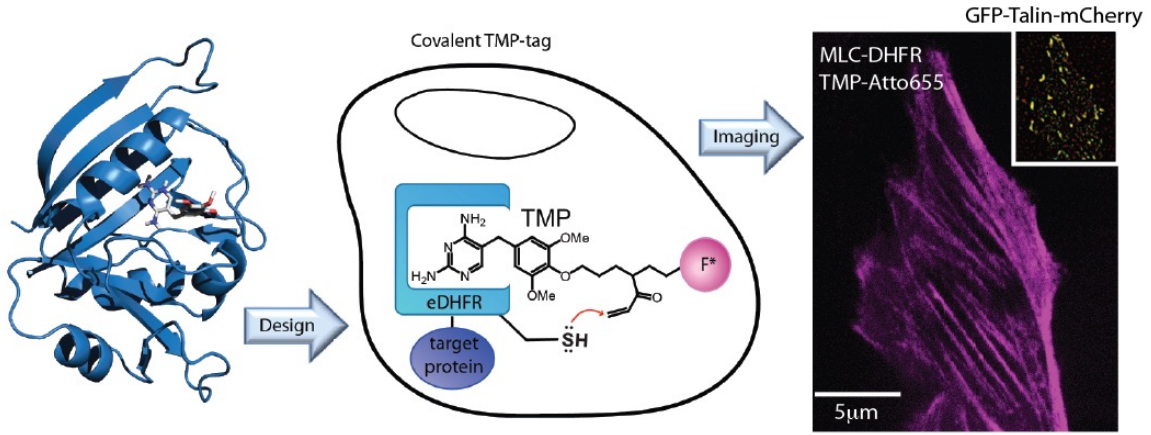
Display Options

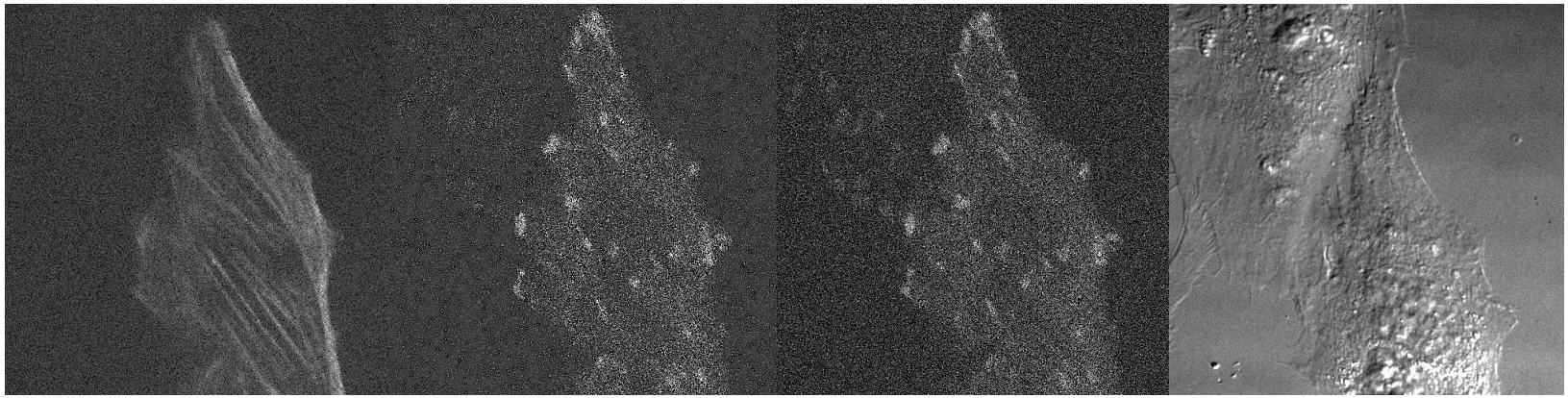
- Grid
- Labels
- Excitation Plots
- Emission Plots
- Light Sources
- Filters
- Show Emission Plot Normalization

ThermoFisher.com

Choosing a Fluorophore	
Excitation and emission	Together with filter set/laser line
Brightness	quantum yield, extinction coefficient
Photo stability	quenching and photo-bleaching rate
Size	GFP 27kDa
Blinking or photometry	Localization or quantitation
Maturation speed	mCherry ~ 15min
Labeling Ratio	monomer/oligomer
Delivering method	SNAP, CLIP, eDHFR-TMP
Biocompatibility	Membrane Permeability, Cytotoxicity, PH stability(pKa)

Labeling Vinculin

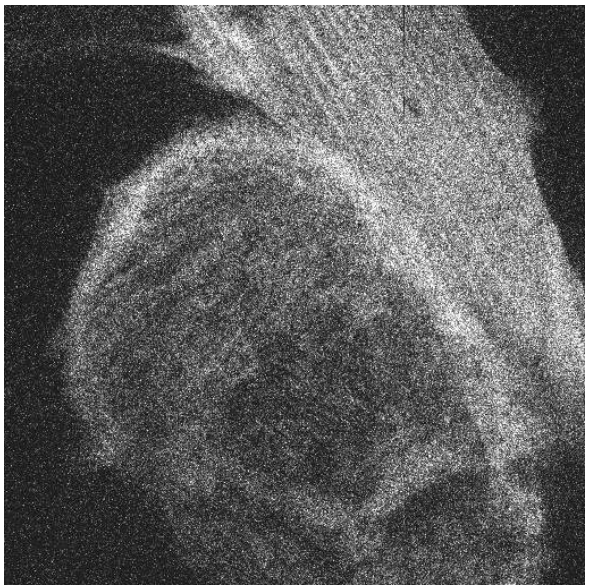




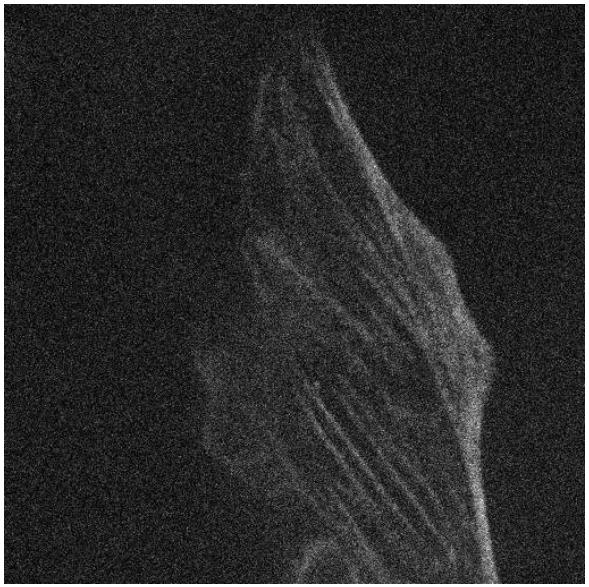
MLC-DHFR, TMP Atto655 GFP-Talin Talin-mCherry DIC

BioCompability

MBI
MECHANOBIOLOGY INSTITUTE
National University of Singapore



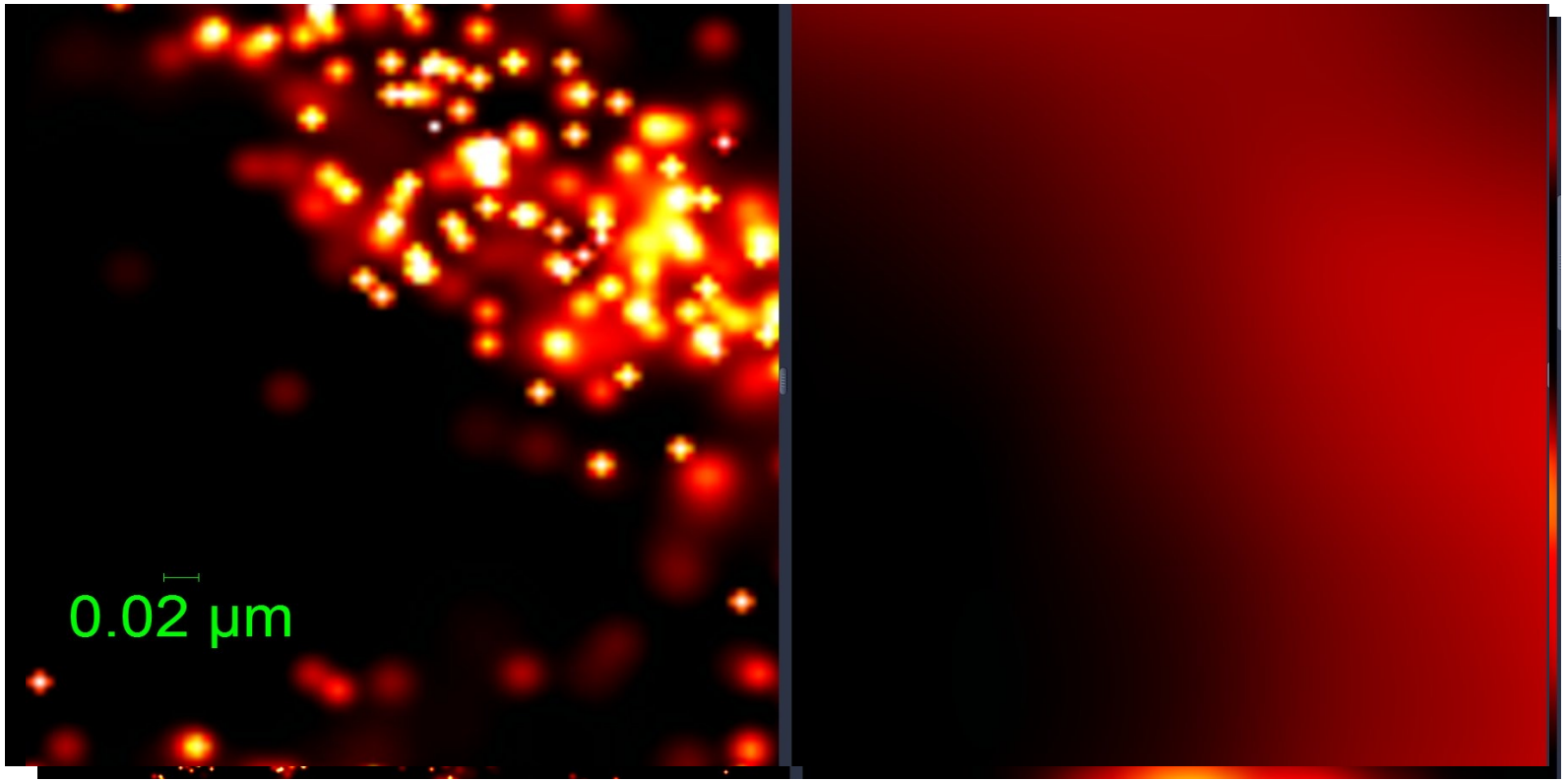
20 uM TMP-Fluorescein, τ=55 sec N=5



20 uM TMP-Atto655, τ=64 sec N=5

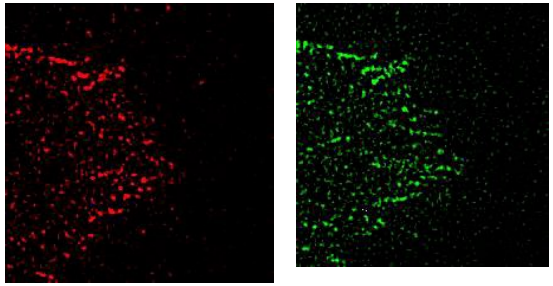
UiO : Universitetet i Oslo

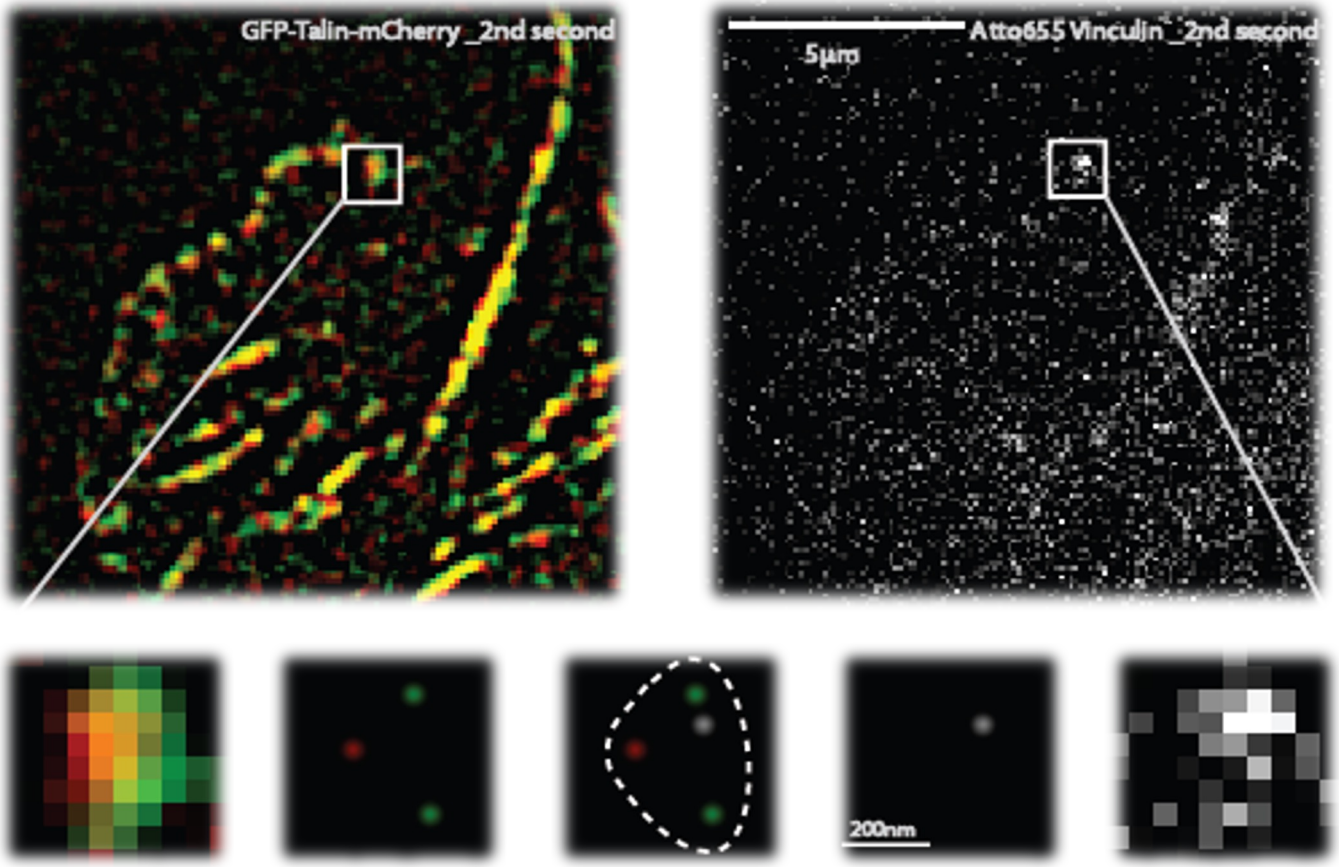
Single Molecule Localization



5µm

GFP-Talin-mCherry
Paired Single Molecule

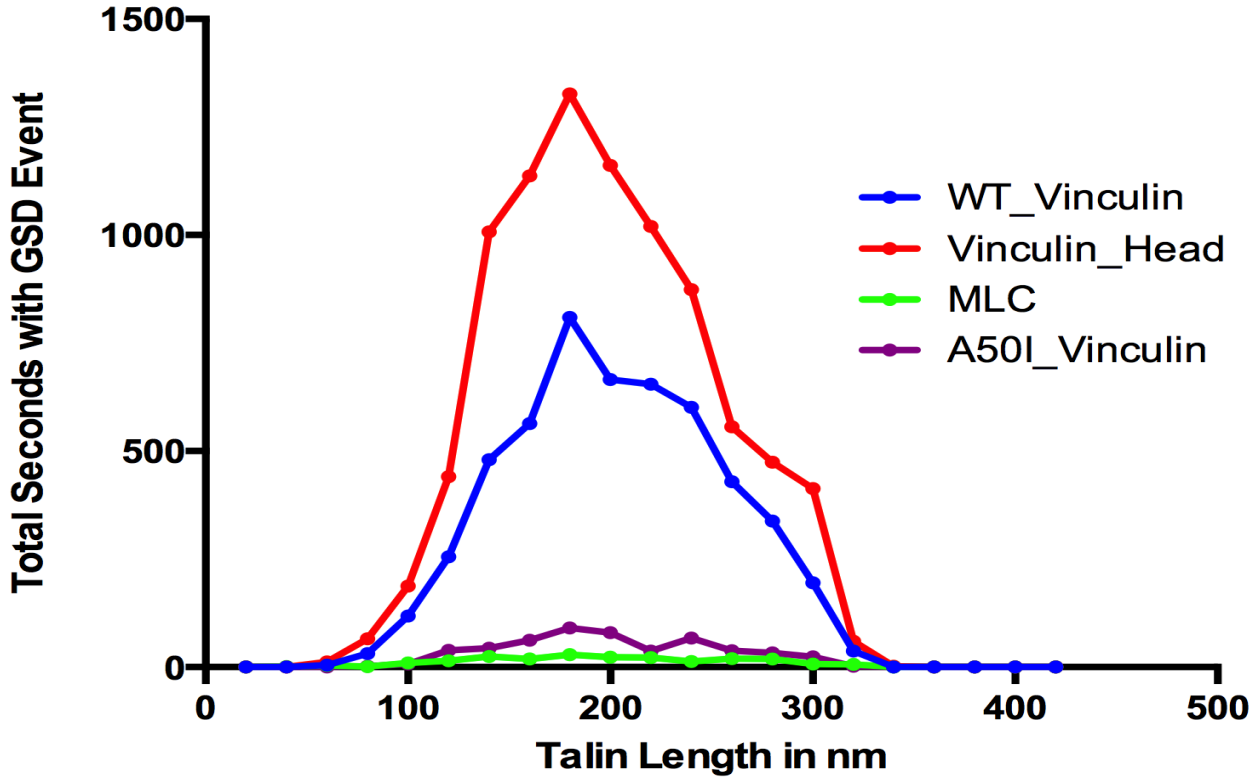




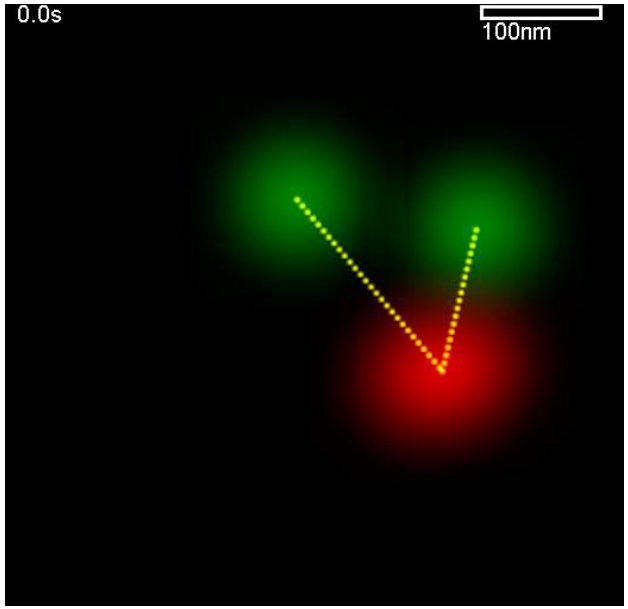
- Simultaneous Imaging of
  - GFP: N terminal, Integrin Binding
  - mCherry:C terminal, Actin Binding, Dimerization
  - Atto655: Vinculin

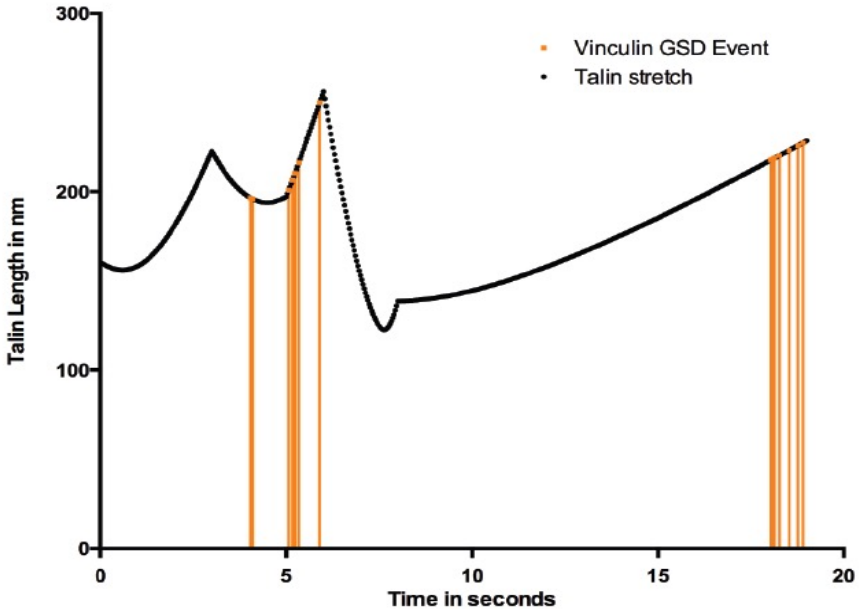
Hu.et al, Nano Letters, 2016

Vinculin binds to talin most when talin is 180nm



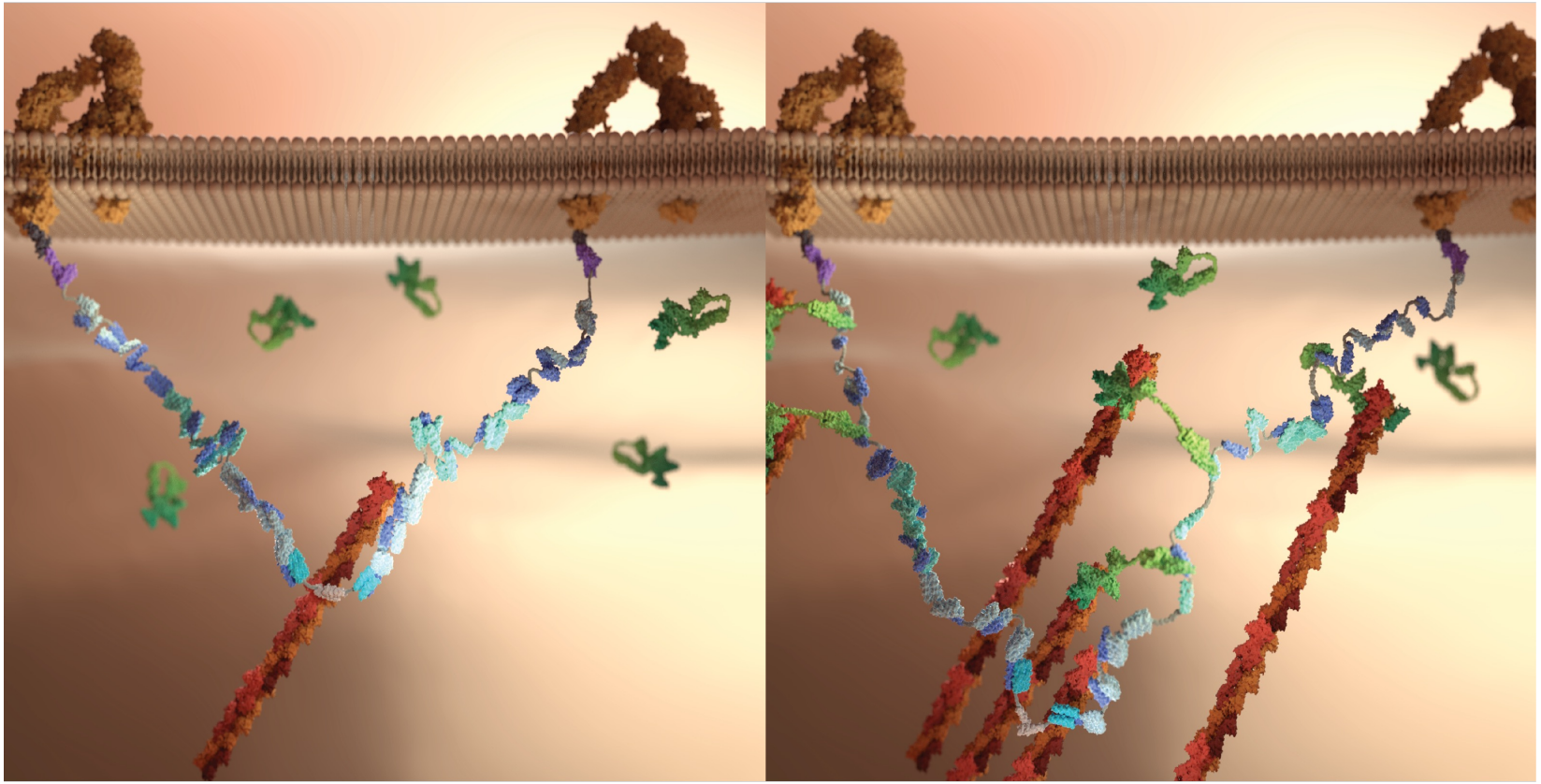
Hu.et al, Nano Letters, 2016





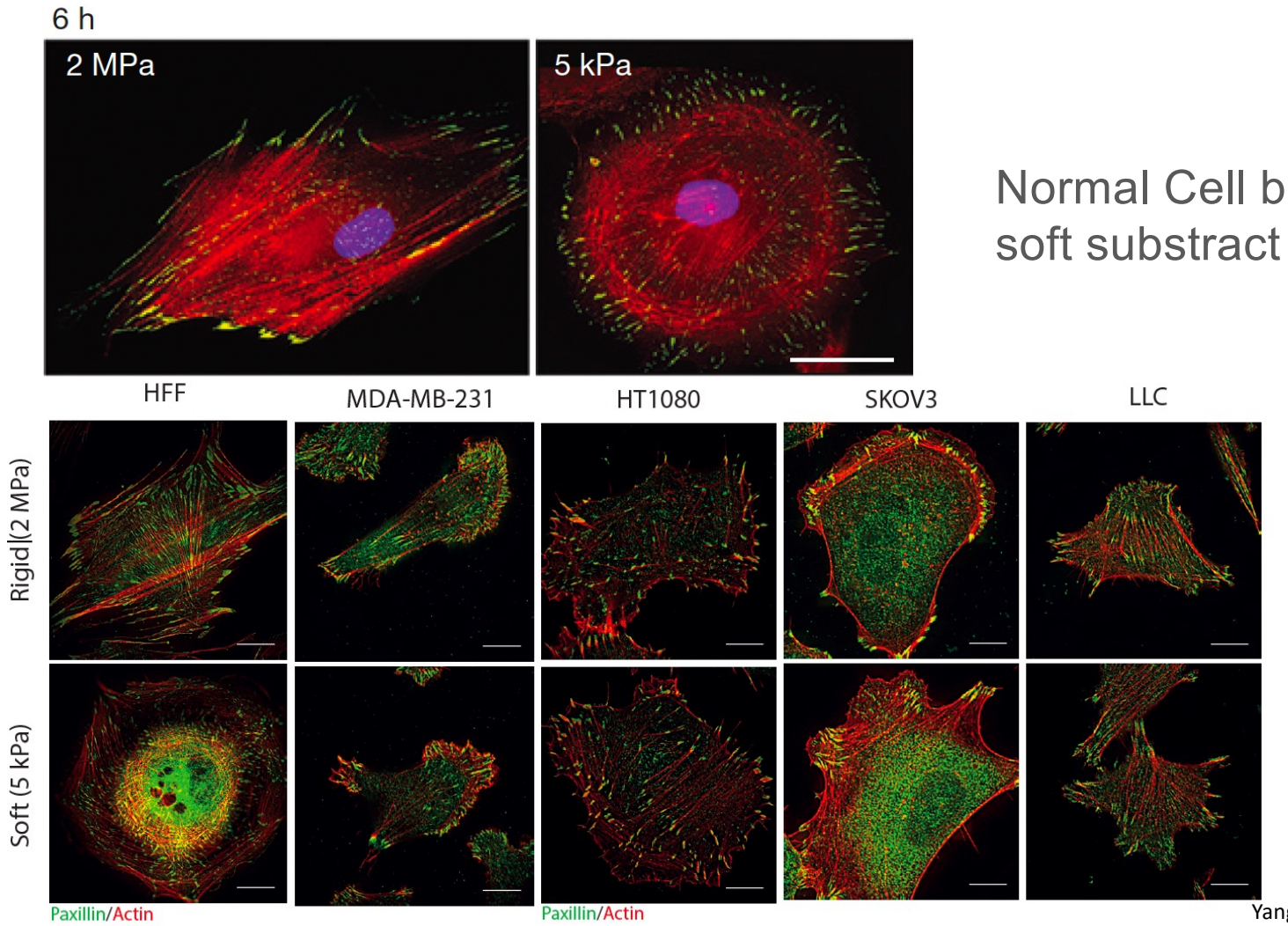
- GFP: N terminal, Integrin Binding
- mCherry: C terminal, Actin Binding
- Atto655: Vinculin

Hu.et al, Nano Letters, 2016



Hu et al, Pro Sci. 2017

Rigidity sensing is impaired in transformed cell

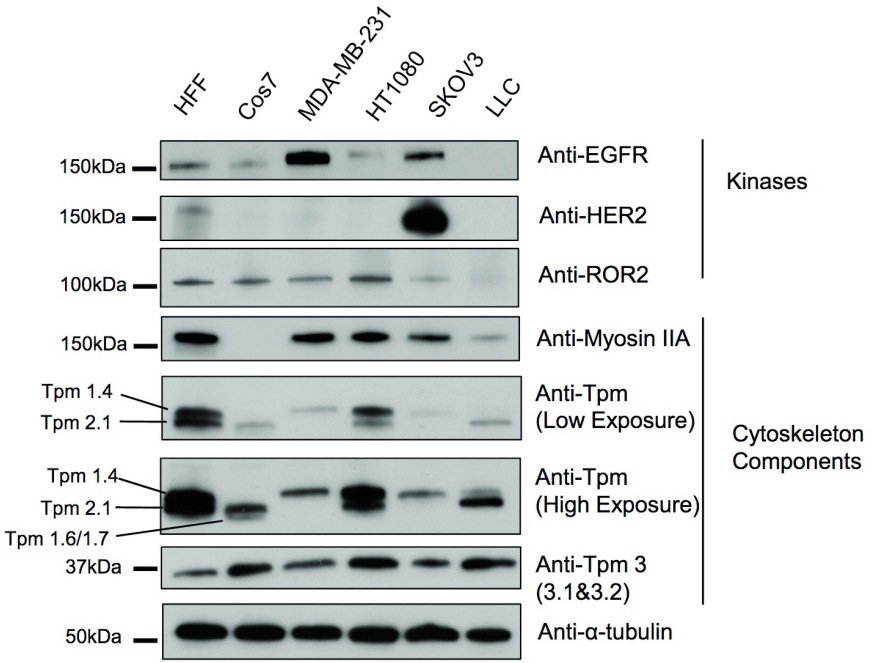


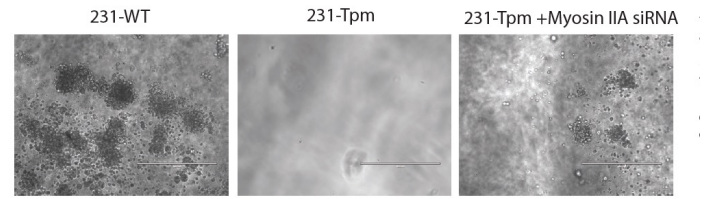
Normal Cell behave differently on soft substract

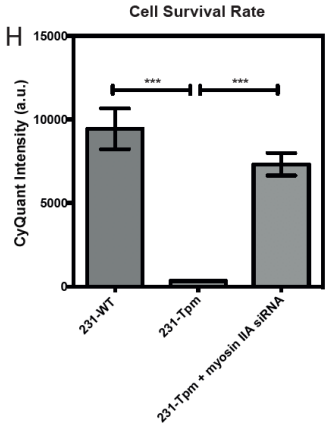
Prager-Khoutorsky, M. *et al;* Nat Cell Bio 2011

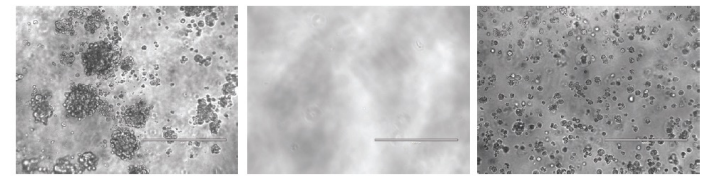
Yang, B. , Wolfenson,H., Nakazawa, N. *et al, Nat Mater* 2019

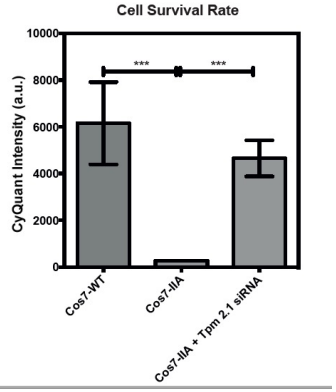
The Missing Mechanosensing Component





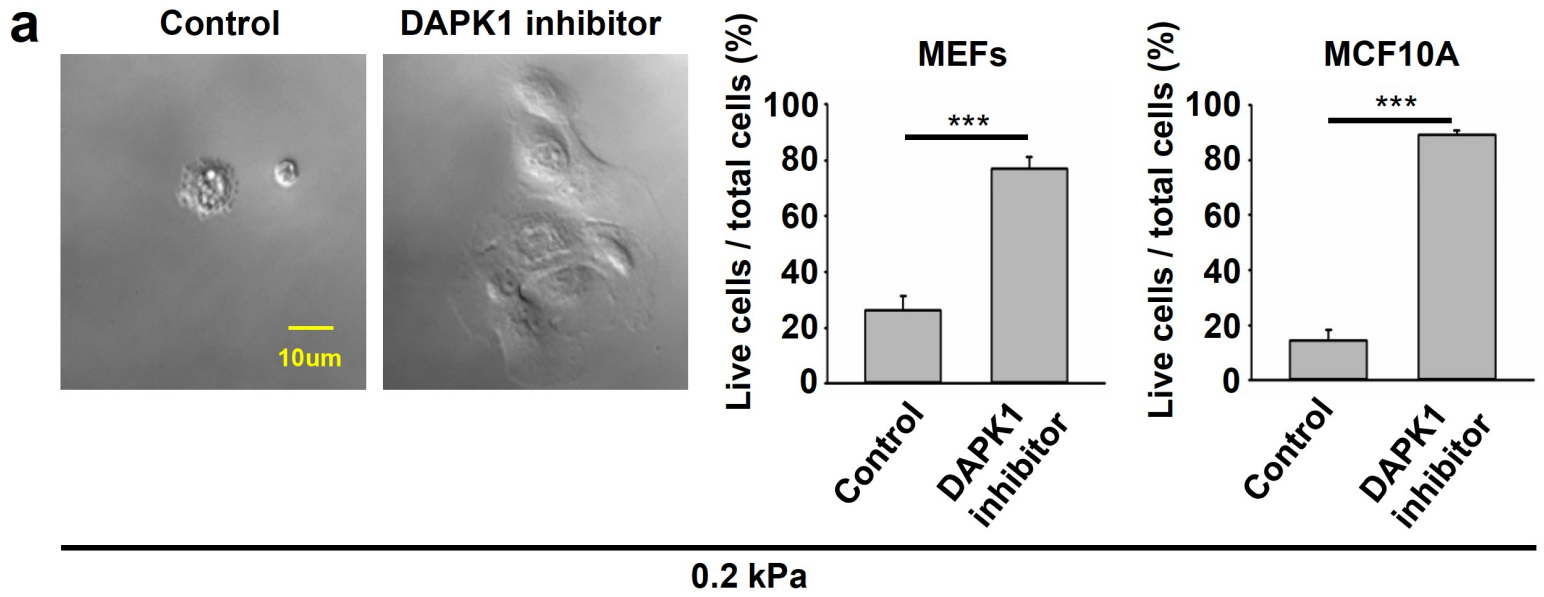




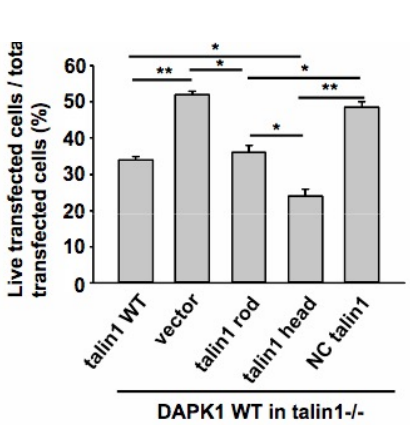


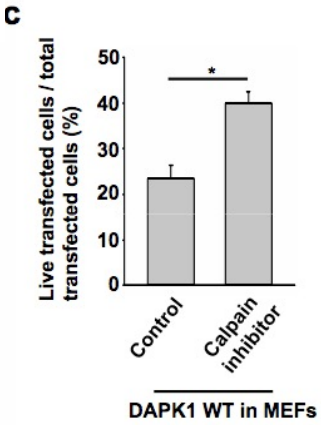
Yang, B. , Wolfenson,H., Nakazawa, N. *et al, Nat Mater* 2019

Missing Link between Sensing and Apoptosis



- DAPKI activity linked with Talin cleavage





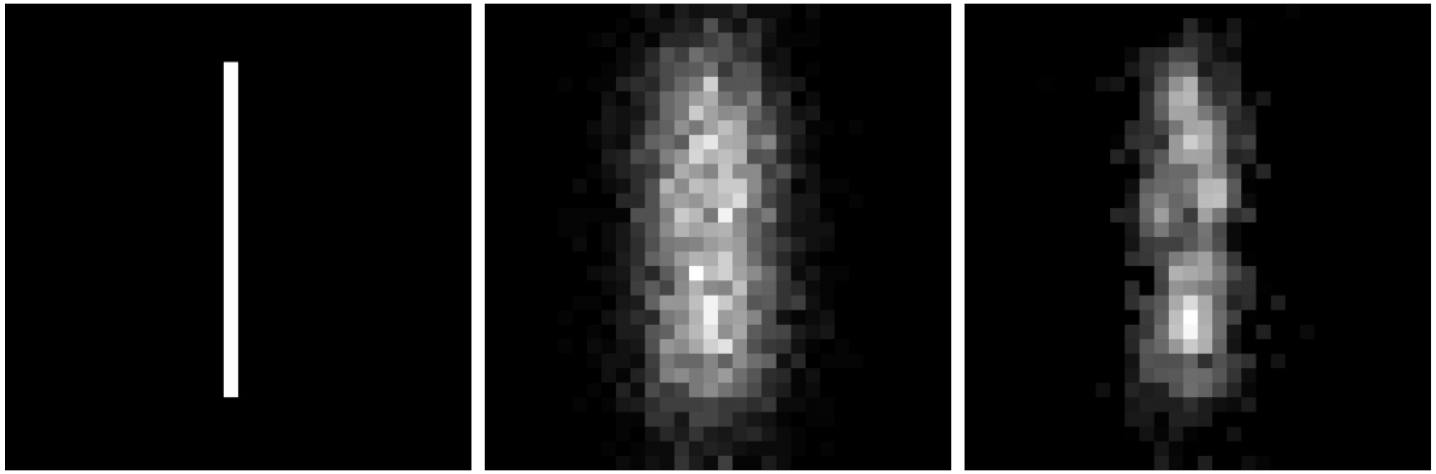
Qin, R., Wolfenson, H.; *et al,* Bioarchiv: 320739

Bio Imaging For Quantitative Measurement

- Qualitative and Quantitative
- What Makes Quantitative Measurement Possible:
  - Scientific grade detectors are linear
  - Fluorescent proteins are linear

- **Live Cell Imaging: Photon budget**

- Artifacts in Quantitative Imaging
  - Protein expression level?
  - Bleaching
  - Laser power
  - Light efficiency of microscope
  - Undersampling



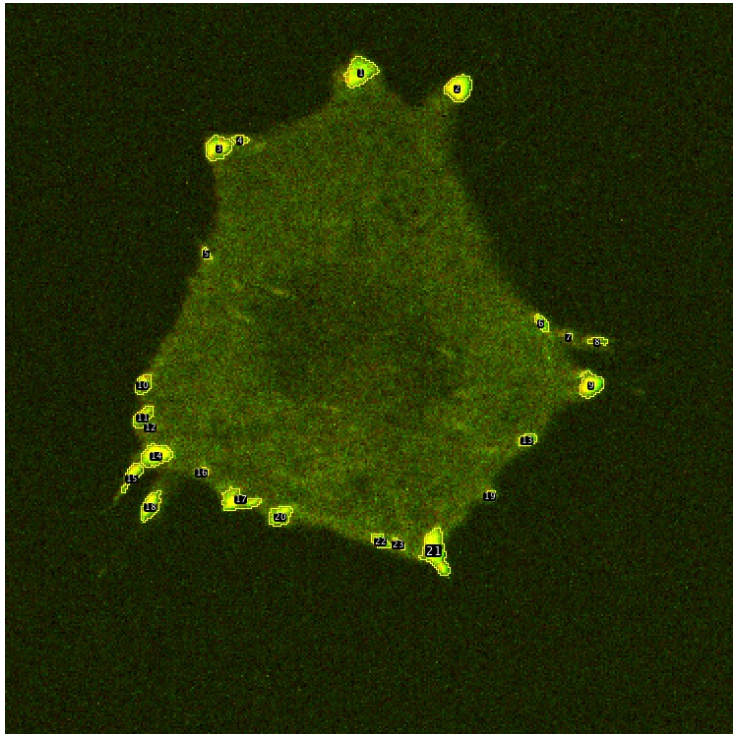
line object

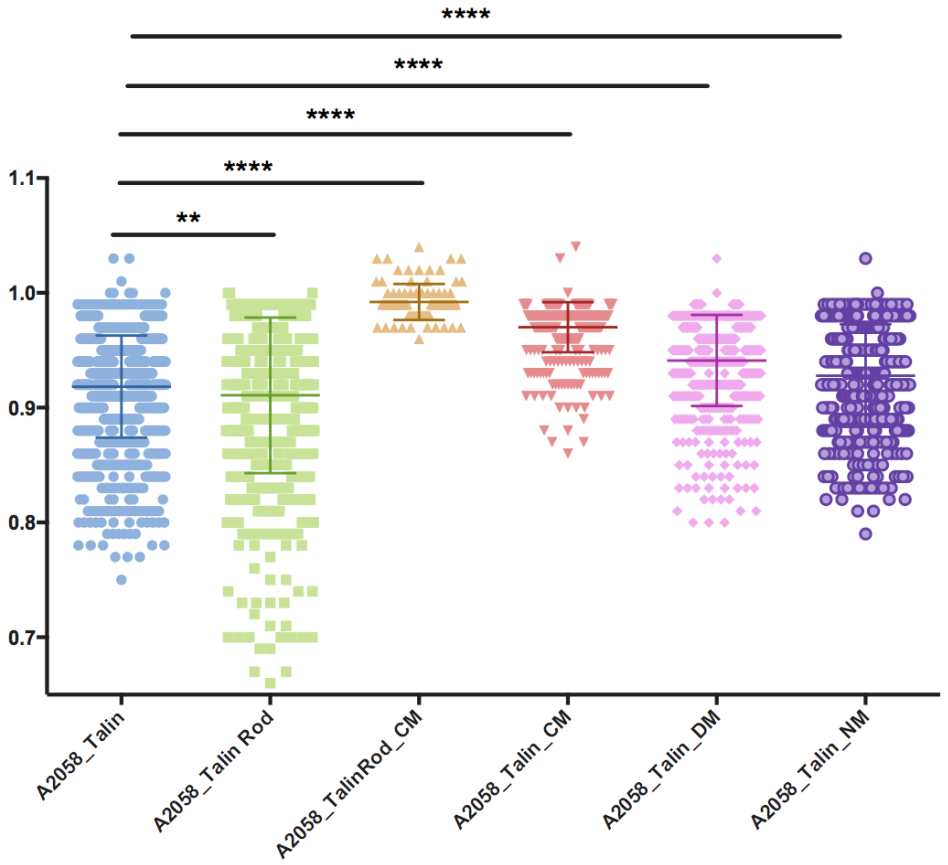
convolved with PSF and Poisson noise

Undersampling simulation

Simulation by Felix Margadant

mCherry/GFP Ratio

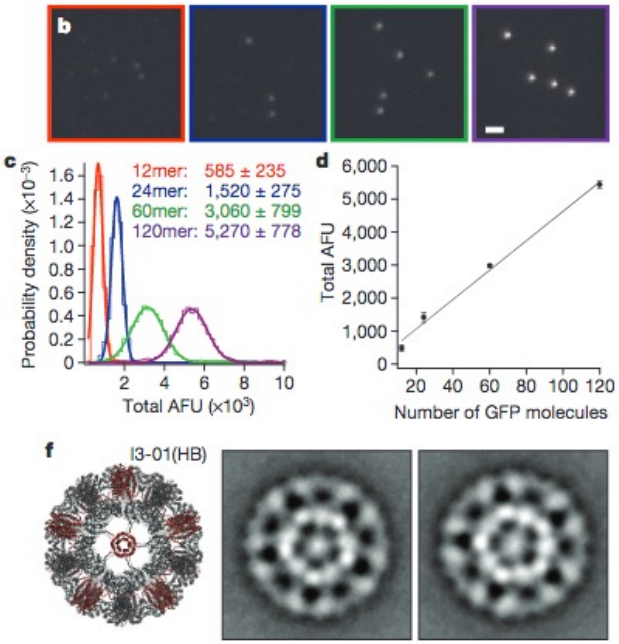




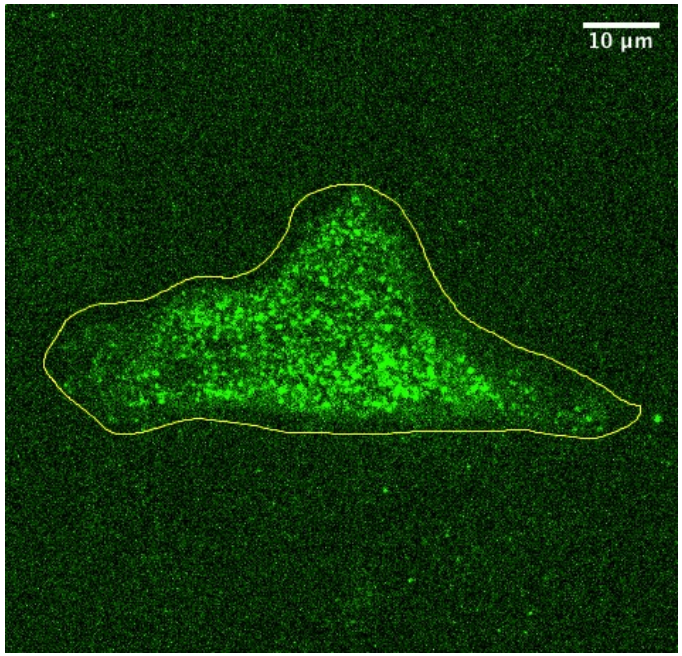
Ratio of mCherry vs GFP in Fas, A2058, SiTalin
Sample Size each group 500+

mCherry/GFP Ratio- Calibration

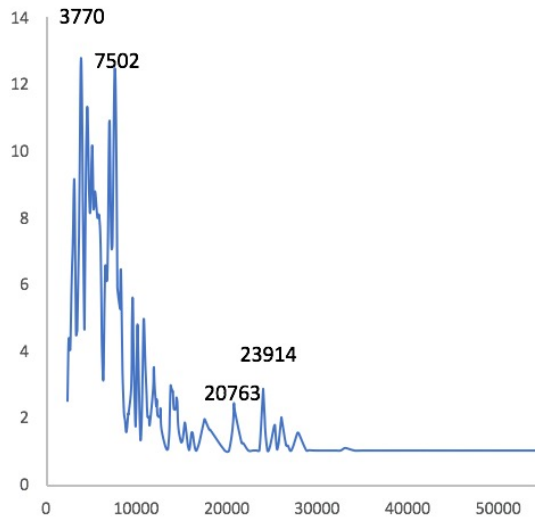
- GFP2-tag, icosahedron, I3-01(14nm)
- Cloned to 12mer mCherry, 12mer GFP, 60mer mCherry, 60mer GFP



Hsia, Y.;.; et al.. *Nature* **2016**, *535* (7610), 136–139



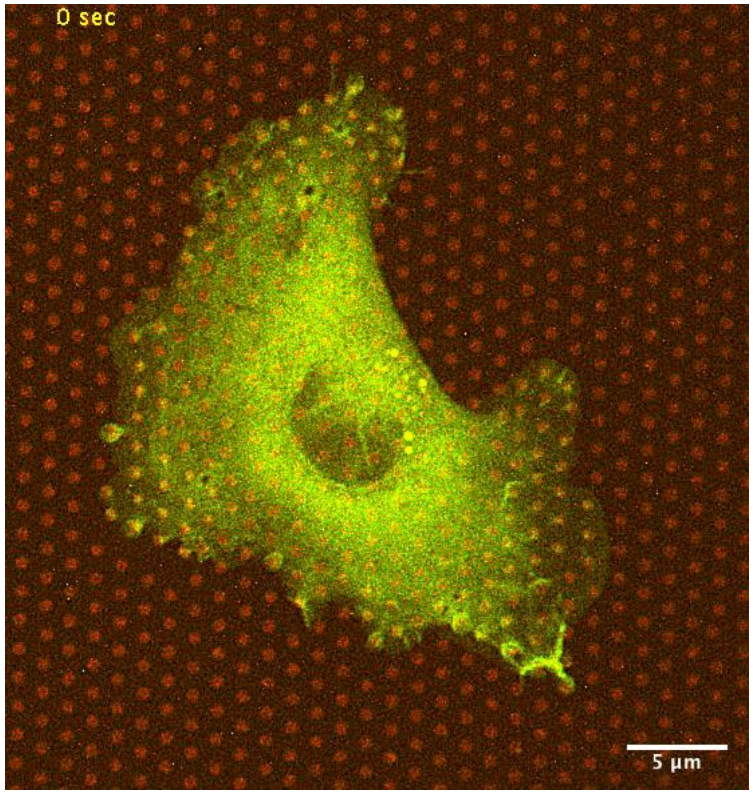
60Mer-GFP



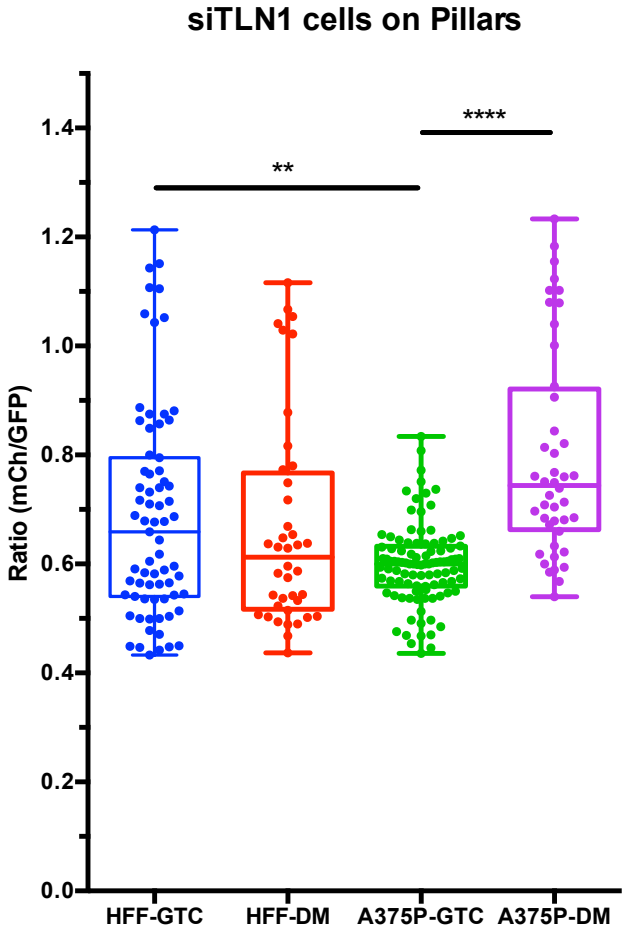
mCherry-60Mer Peaks

1 single GFP intensity ~ 61.2

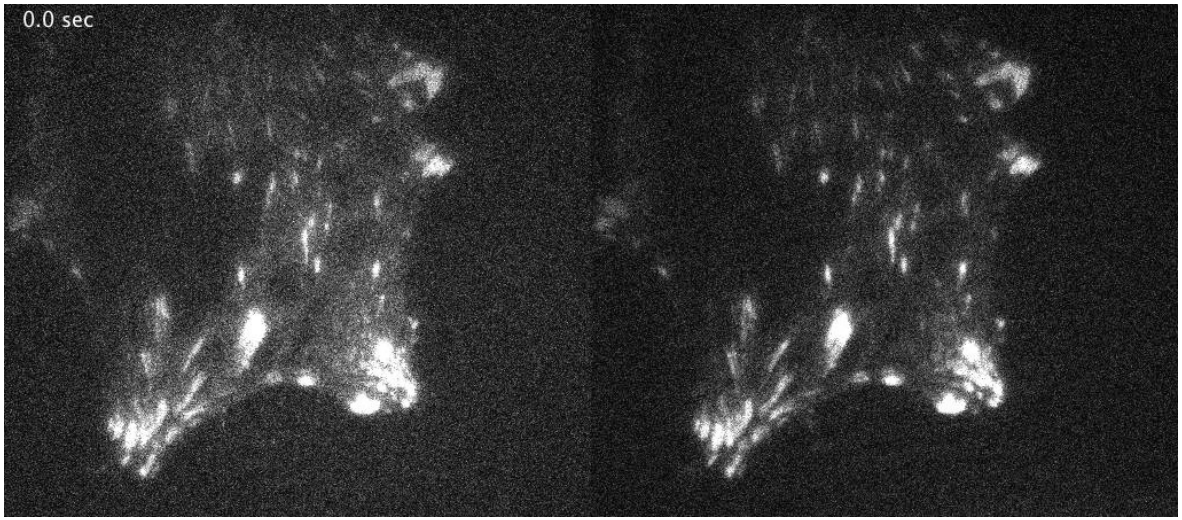
More Cleavage in Transformed Cells on Soft surface



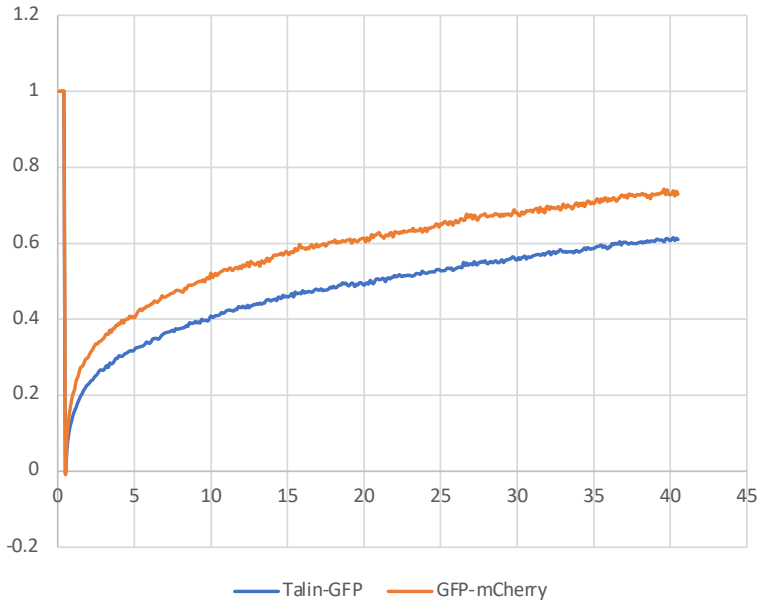
REF, GFP-Talin-mCherry, 2um pillar, 6 hours seeding



FRAP Difference between GFP/mCherry

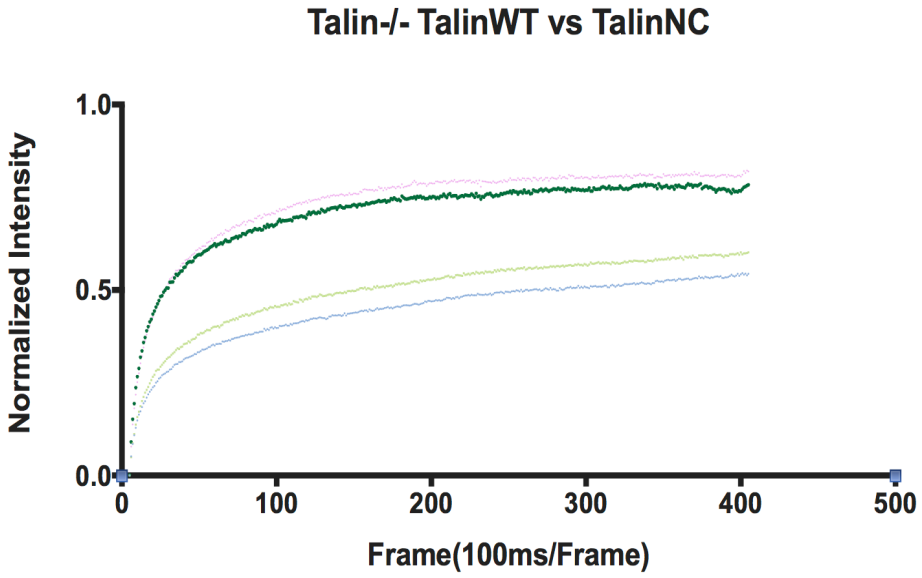


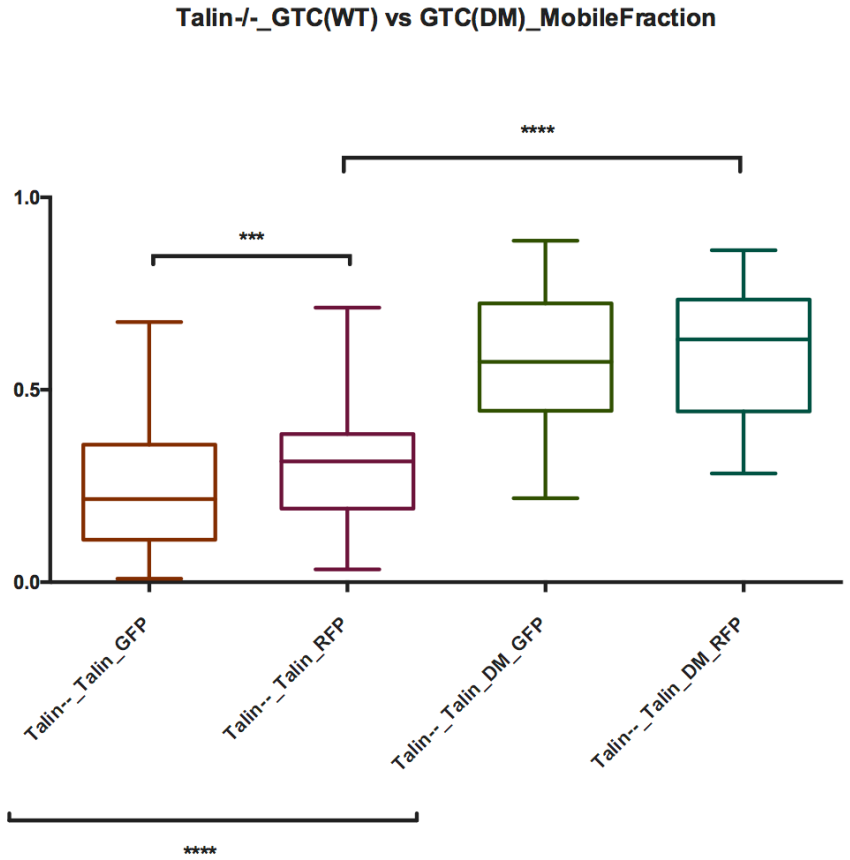
mCherry-C GFP-Talin-mCherry GFP-N



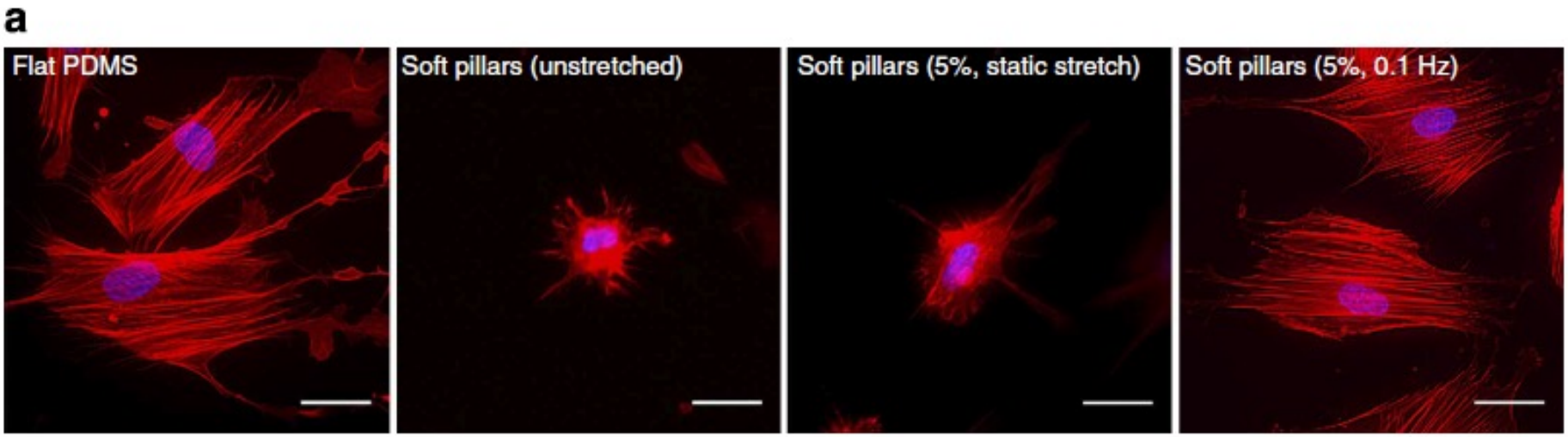
RPTP- GFP-talin-mCherry Dual FRAP, Sample Size: 25 cells

FRAP Difference between GFP/mCherry

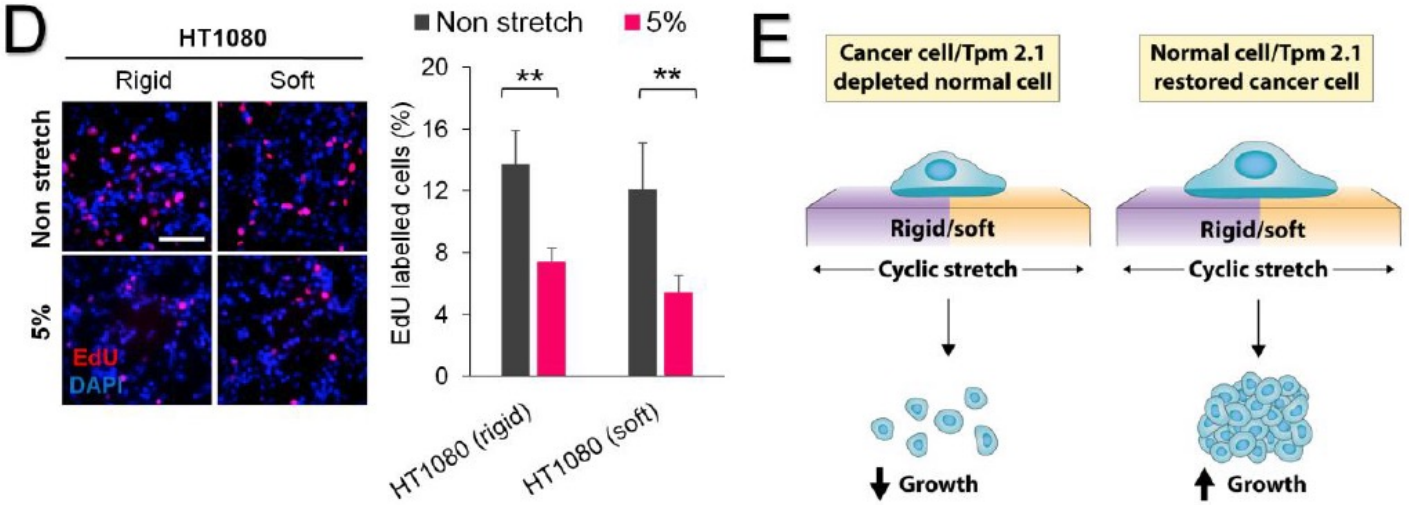




Tau of four groups shows no statistically significant difference, median around 2 sec



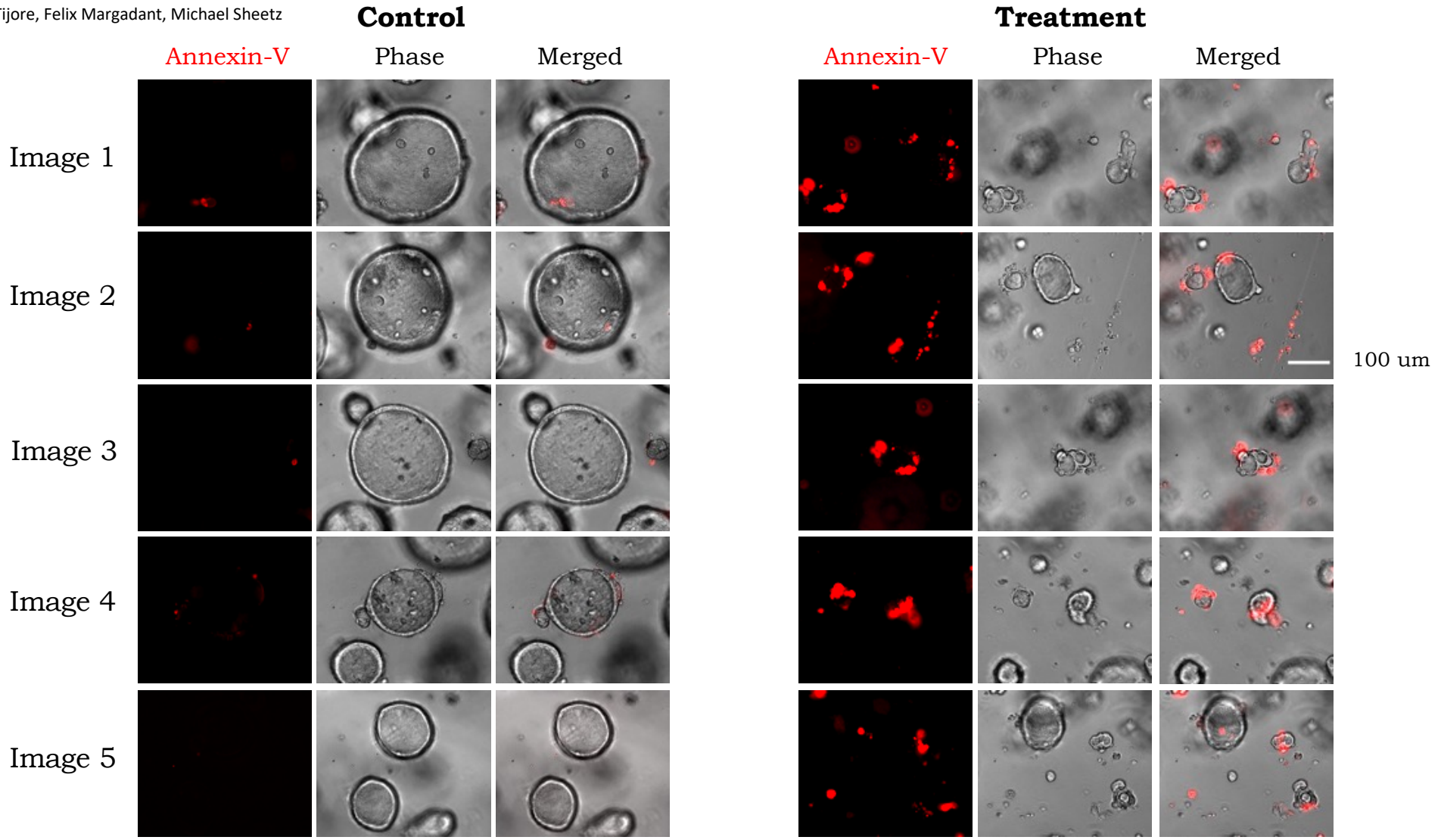
Cui et al, Nat Commun, 2015



Tijore et al, BioArchive, 2018

Ultrasound Promotes Apoptosis in Pancreatic Tumor Organoids

Ajay Tijore, Felix Margadant, Michael Sheetz



Thank you

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National University of Singapore

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Prof. Alexander Bersdhasky
Prof. Hanry Yu
Prof. Oddmund Bakke

Talin Project: Xian Hu, Salma Jalali, MingXi Yao, Felix Margadant, Michael Sheetz

Endosome Project: Xian Hu, Duarte Mateus, Vinodha Manovaseegaran, Felix Margadant & Oddmund Bakke

9th NorMIC Workshop on
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LIVSVITENSKAP
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Image Processing Pipelines
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Localization and Tracking
Deconvolution
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Surfance Rendering

Faculty:

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Felix Margadant, Radium Hospital, Oslo
Ping Ching Cheng, Buffalo, USA
Kay Schink, Radium Hospital, Oslo
Frode Skjeldal, UiO

Harald Stenmark
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Xian Hu (Edna), UiO