Tractions and stress fibers control cell shape and rearrangements in collective cell migration

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Much of this work is published in Saraswathibhatla & Notbohm, Phys Rev X, 10:011016, 2020.

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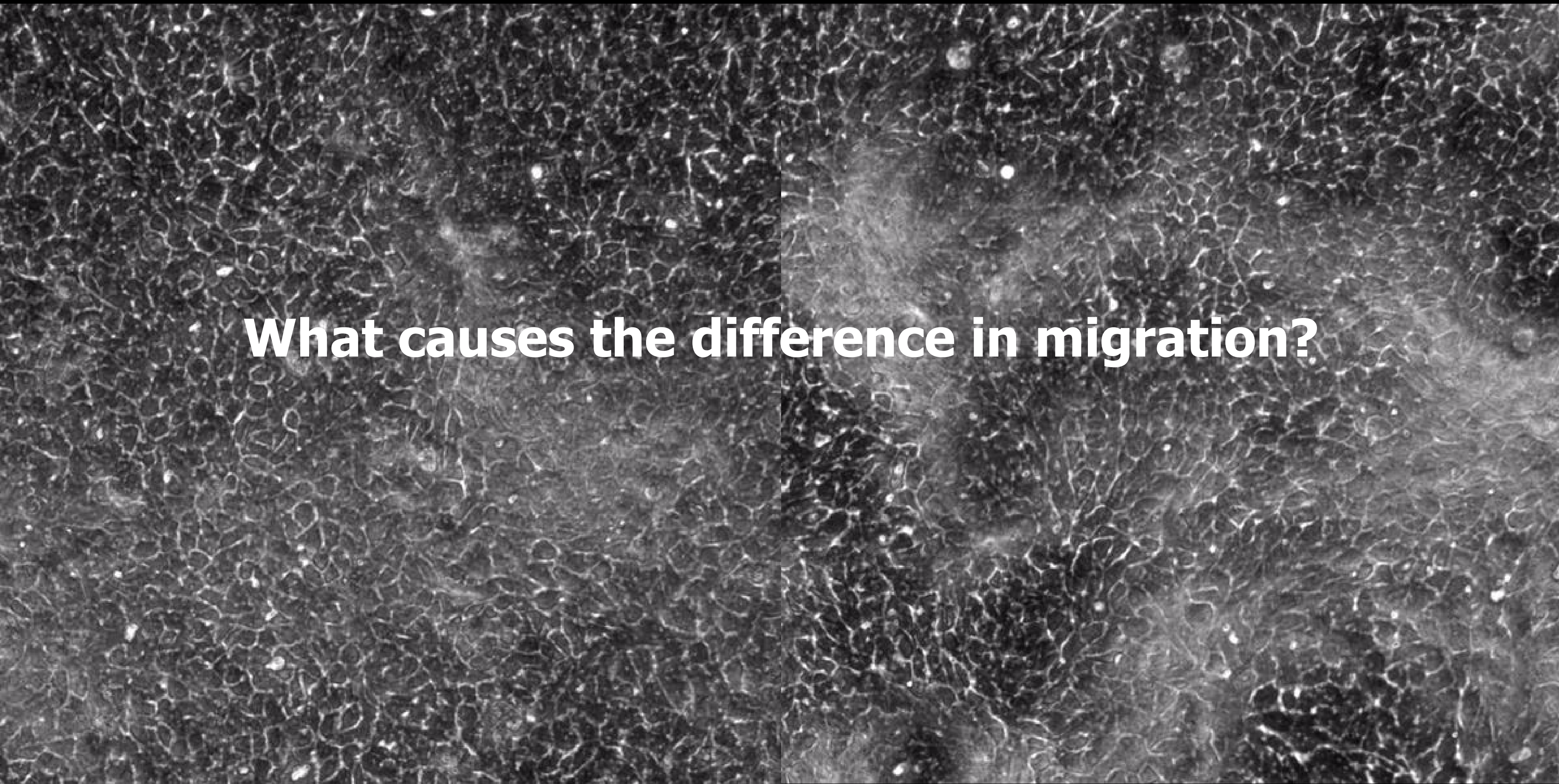
Healthy **Asthmatic**



100 µm

Human bronchial epithelial cells cultured in air-liquid interface

Movies from Jen Mitchel & Jin-Ah Park, Harvard School of Public Health

Park et al, Nat Mater 2015, 14, 1040

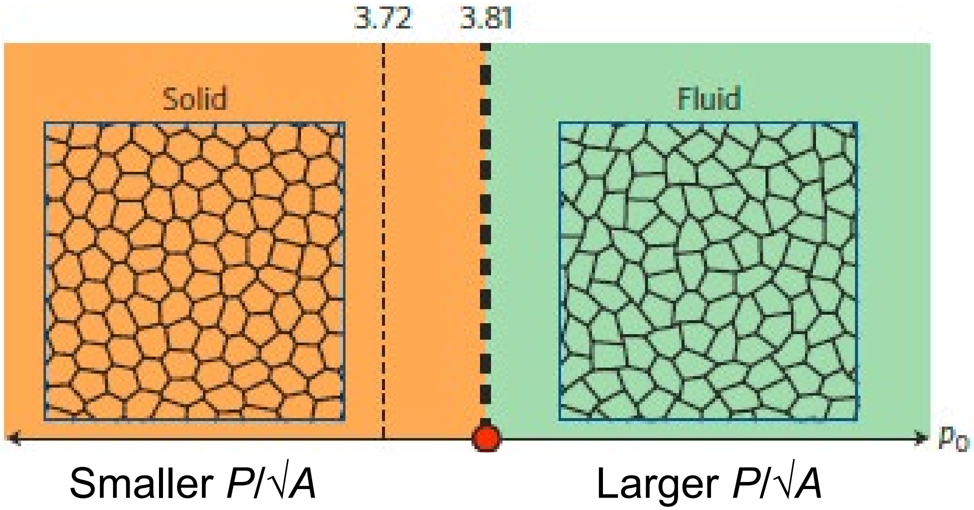
Prediction of vertex model

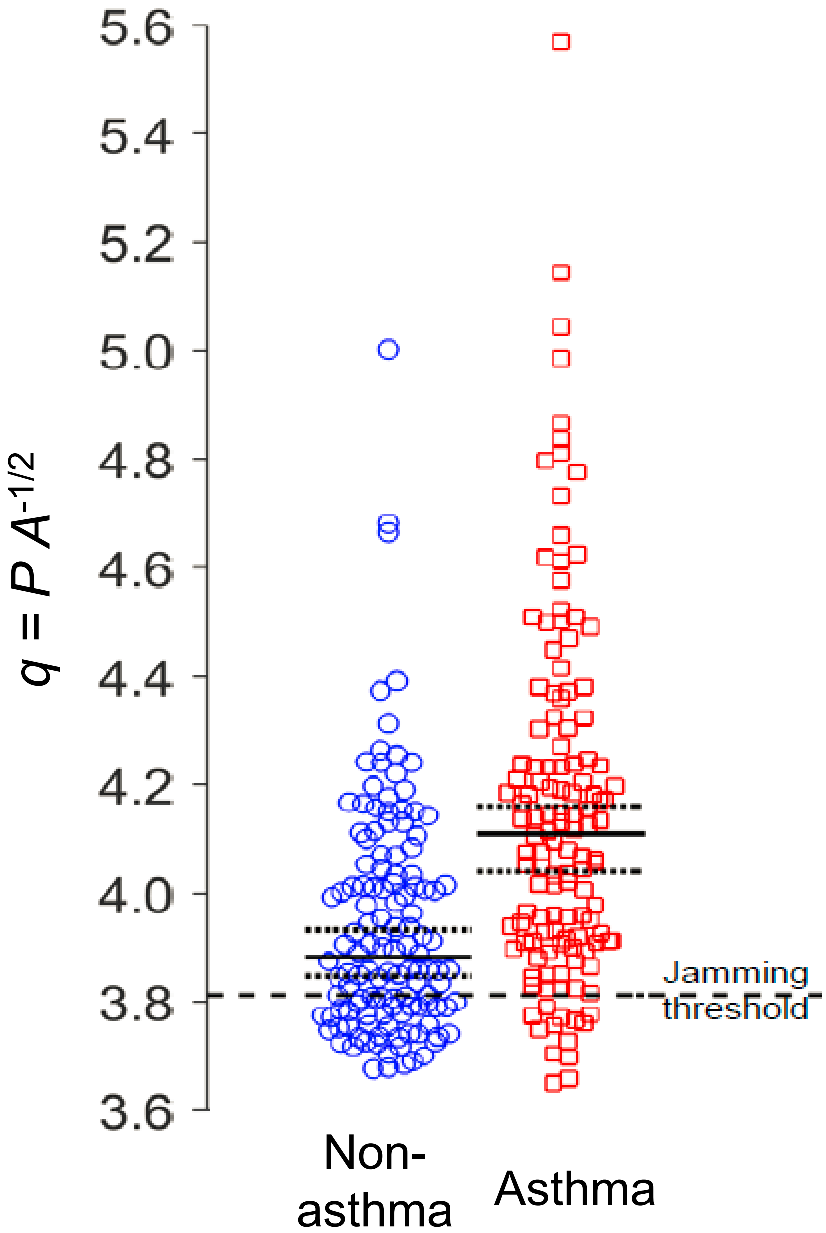
Tendency to maintain constant area

Tendency to maintain constant perimeter

$$E_i = K_A (A_i - A_0)^2 + K_P (P_i - P_0)^2$$

Normalized perimeter $P_0/\sqrt{A_0}$ predicts the transition between static and moving cell layers.





Bi, Lopez, Schwarz, Manning, Nat Phys, 2015, 11, 1074
Park et al, Nat Mater 2015, 14, 1040

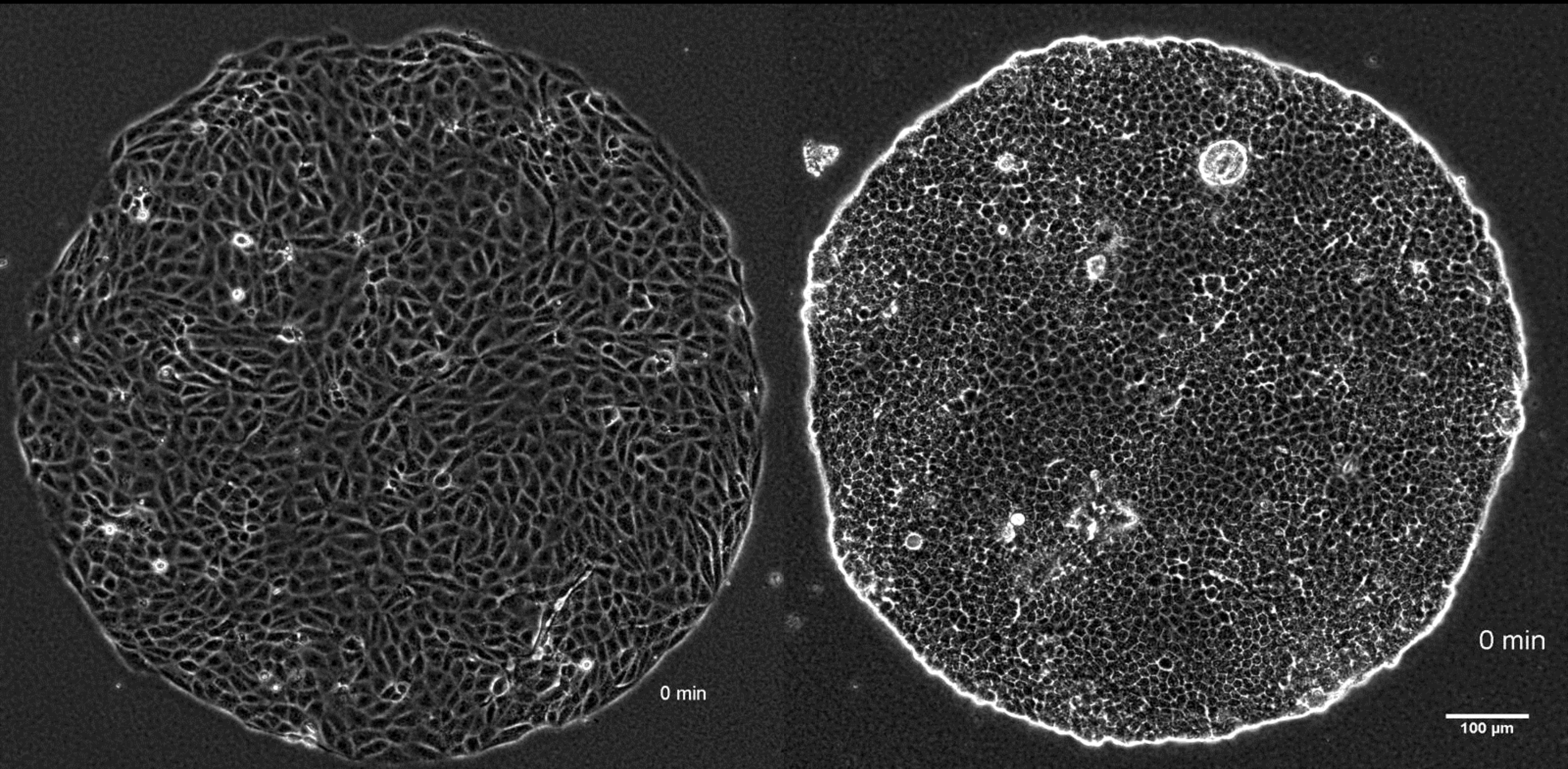
Well-established effect of density on collective migration

Angelini et al, P Natl Acad Sci USA, 2011, 108, 4714
Tambe et al, Nat Mater, 2011, 10, 469
Puliafito et al, P Natl Acad Sci USA, 2012, 109, 739
Nnetu et al, Soft Matter, 2013, 9, 9335

and others

Low density

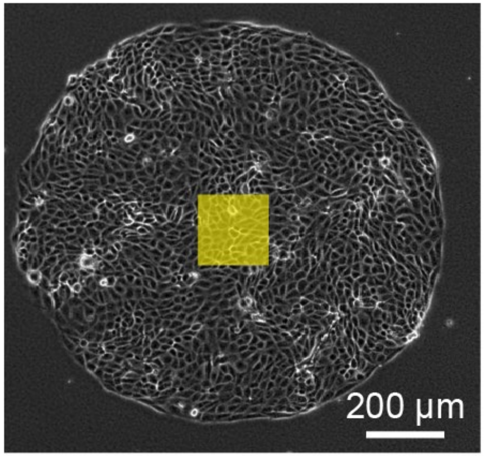
High density

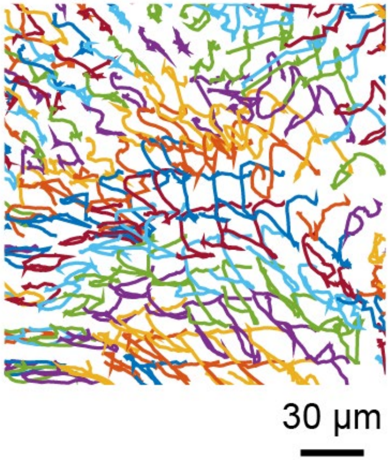


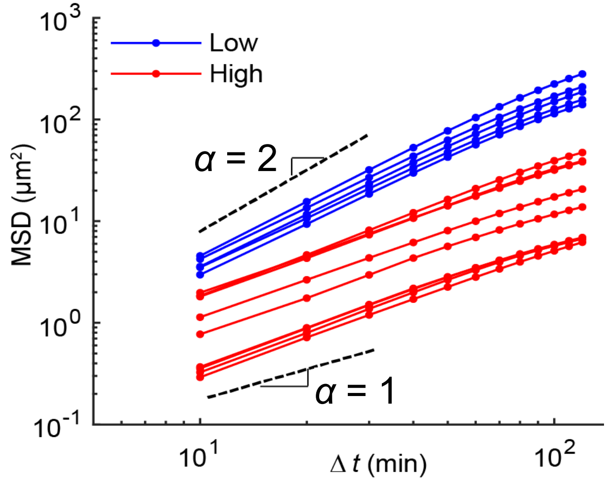
Quantify migration by rearrangements

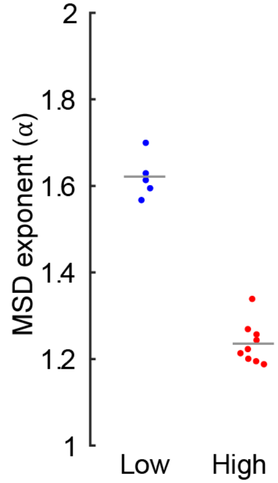
$$MSD\ (\Delta t) = <|r_i(t+\Delta t) - r_i(t)|^2>$$

Low density







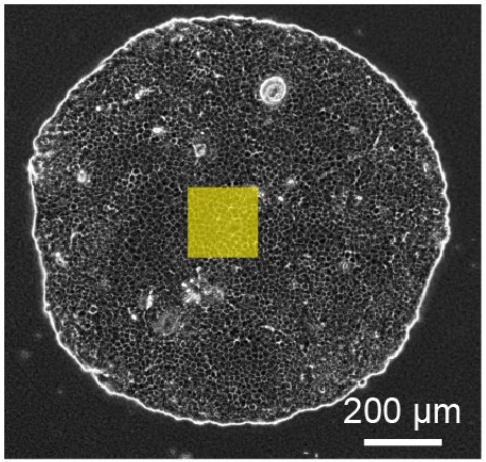


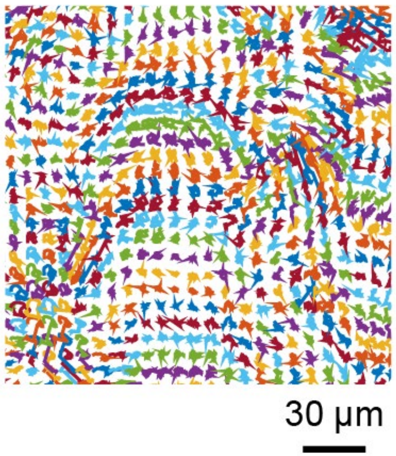
α quantifies rearrangements

α = 1 → random walk

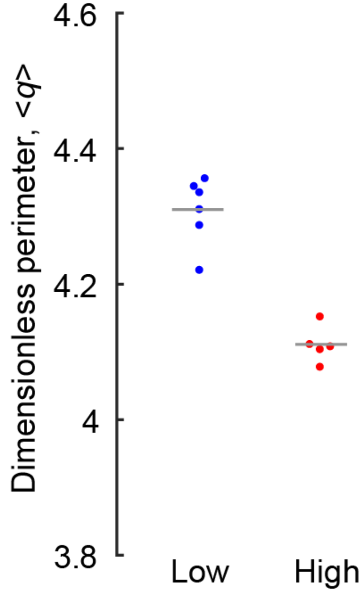
α = 2 → straight trajectory

High density





Dimensionless perimeter



How does density affect migration?

Cells aren't passive particles. The notion of packing free space doesn't necessarily apply.

Hypothesis

1. **Effects of density can be explained by preferred perimeter $P_0 / \sqrt{A_0}$.**
2. **Preferred perimeter $P_0 / \sqrt{A_0}$ affects actual perimeter $q = P/\sqrt{A}$ and cell rearrangements.**

Preferred perimeter $P_0 / \sqrt{A_0}$ controlled by cell periphery

→ actomyosin in cortex reduces it

→ adhesion increases it

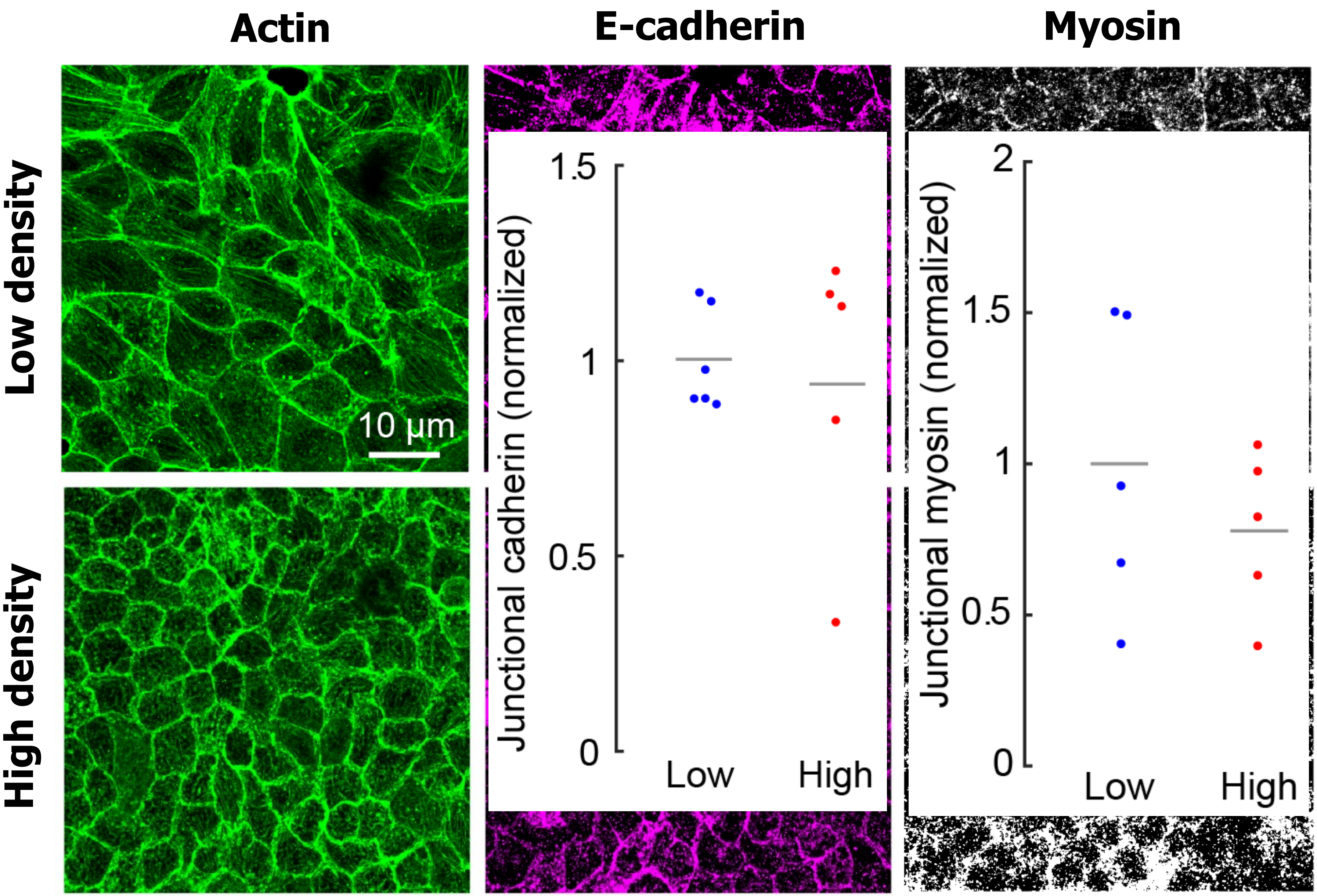
↓ cortical actomyosin, ↑ adhesion → ↑ perimeter, rearrangements

The hypothesized relationship between preferred perimeter and actual perimeter is common in the literature.

Brodland, J Biomech Eng, 2002, 124, 188.
Farhadifar et al, Curr Biol, 2007, 17, 2095.
Staple et al, Eur Phys J, 2010, 33, 117
Bi, Lopez, Schwarz, Manning, Nat Phys, 2015.
Park et al, Nat Mater 2015, 14, 1040.
Chiang & Marenduzzo, Europhys Lett, 2016, 116, 28009.
Bi et al, Phys Rev X, 2016, 6, 021011.
Moshe, Bowick, Marchetti, 2018, Phys Rev Lett, 120, 268105.
Czajkowski et al, Soft Matter, 2018, 14, 5628.

Density | Cell peripheries/cortical tension/adhesion

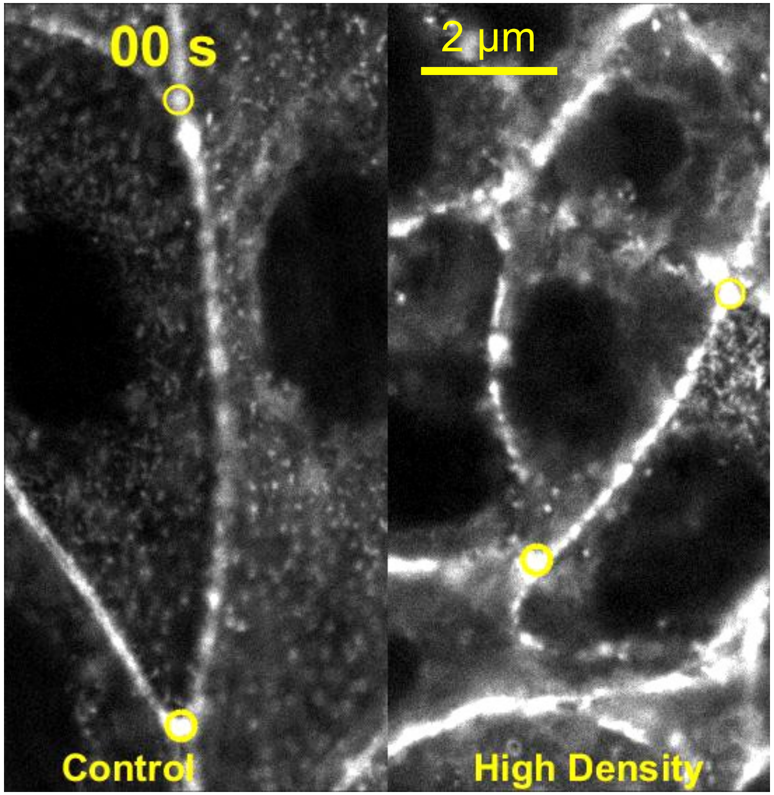
Hypothesis: ↓ cortical actomyosin, ↑ adhesion → ↑ perimeter, rearrangements

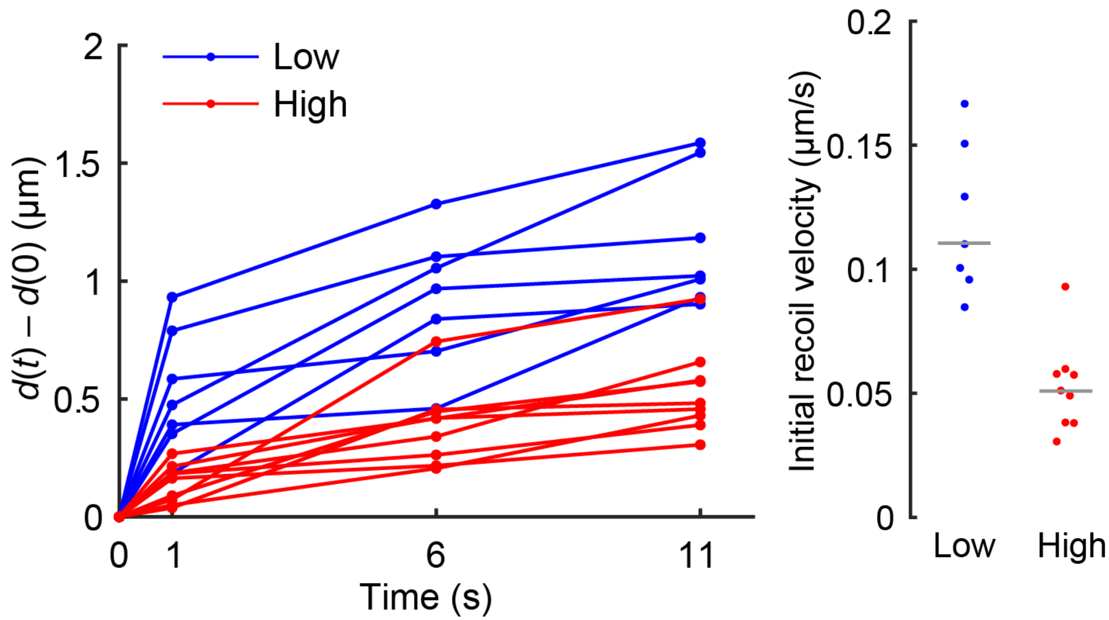


Density | Cell peripheries/cortical tension/adhesion

Hypothesis: ↓ cortical actomyosin, ↑ adhesion → ↑ perimeter, rearrangements

Laser ablation at *t* = 0



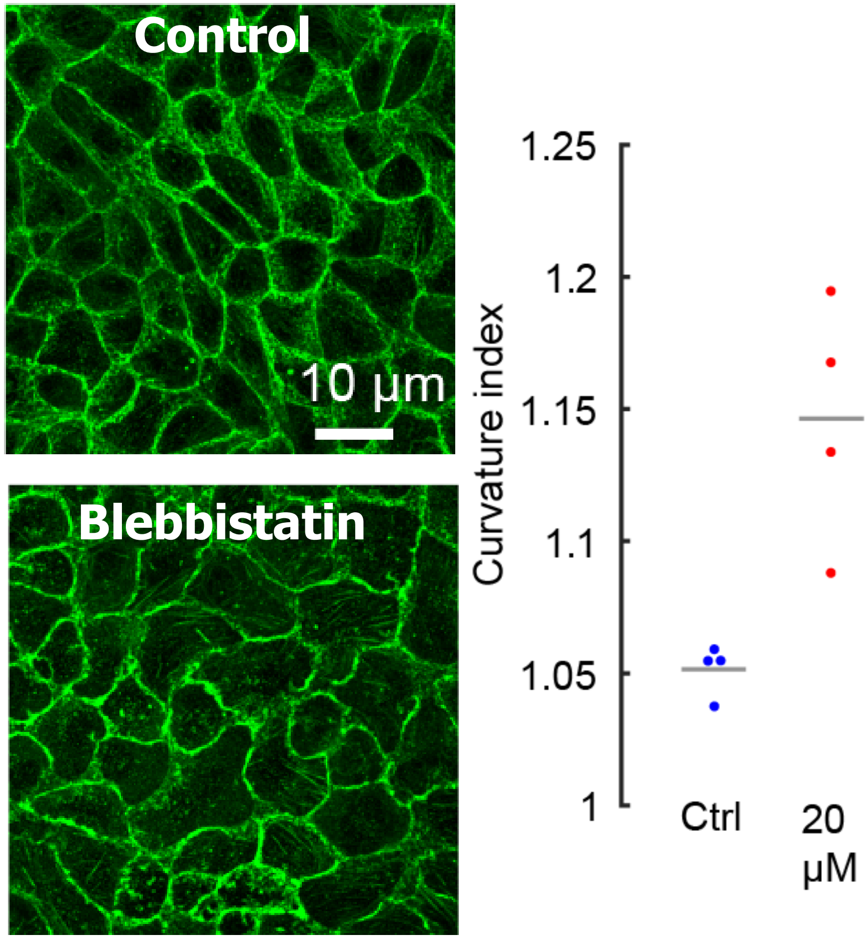


Observation: ↓ cortical tension ↔ ↓ perimeter, rearrangements

Directly reduce cortical tension using actomyosin inhibitors

Blebbistatin inhibits myosin contraction

Actin in cortex

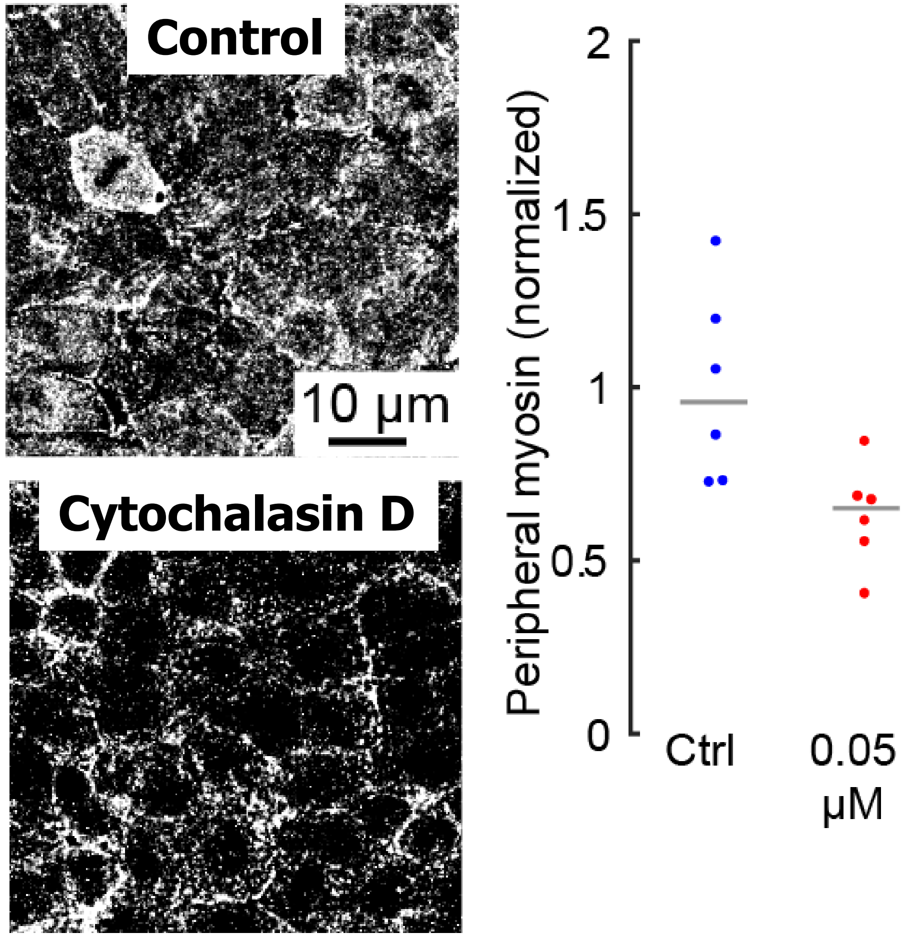


Greater curvature implies reduced cortical tension

Malinverno et al, Nat Mater, 16, 587–596, 2017.
Verma et al, Mol Biol Cell, 23, 4601–4610, 2012.

Cytochalasin D disrupts actin polymerization

Phosphorylated myosin in cortex

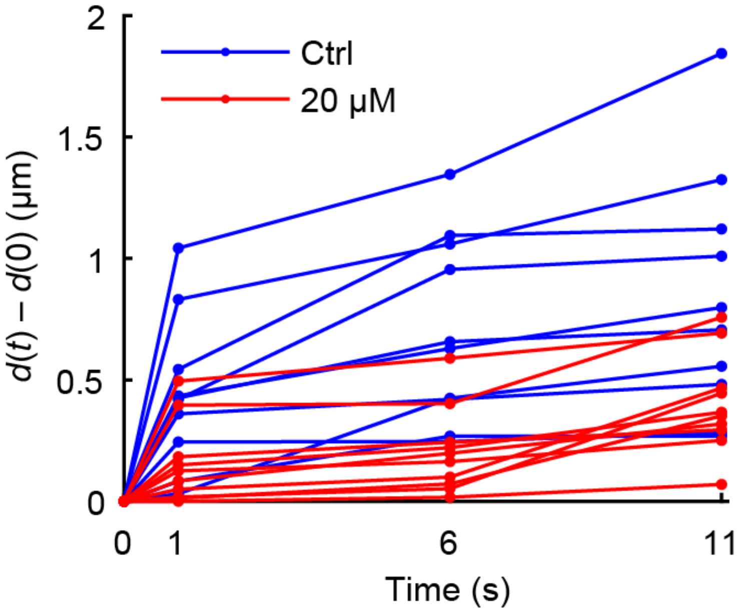


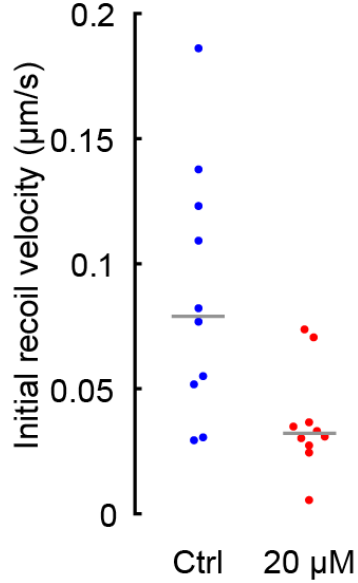
Reduced peripheral myosin implies reduced cortical tension

Verma et al, Mol Biol Cell, 23, 4601–4610, 2012.
Curran et al, Dev Cell, 43, 480-492, 2017.
Kale et al, Nat Comm, 9, 5021, 2019.

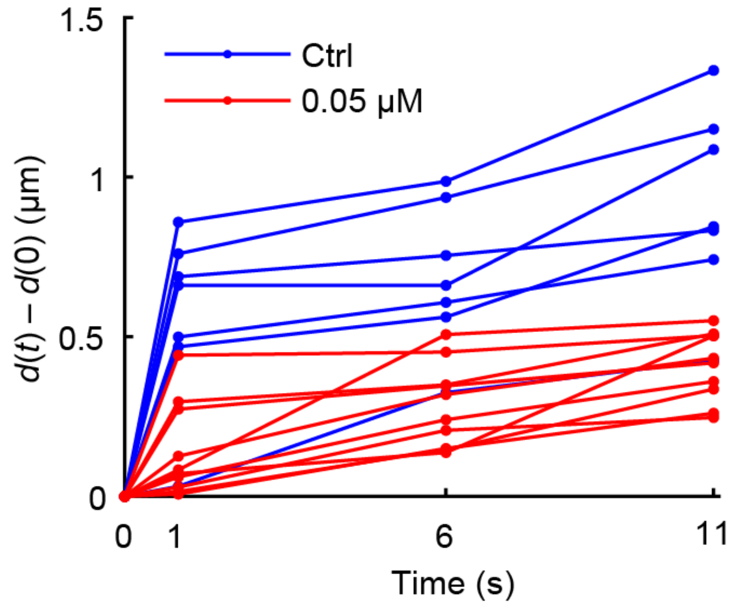
Directly reduce cortical tension using actomyosin inhibitors

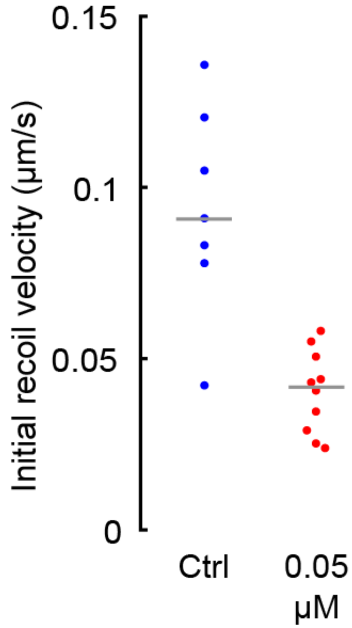
Blebbistatin





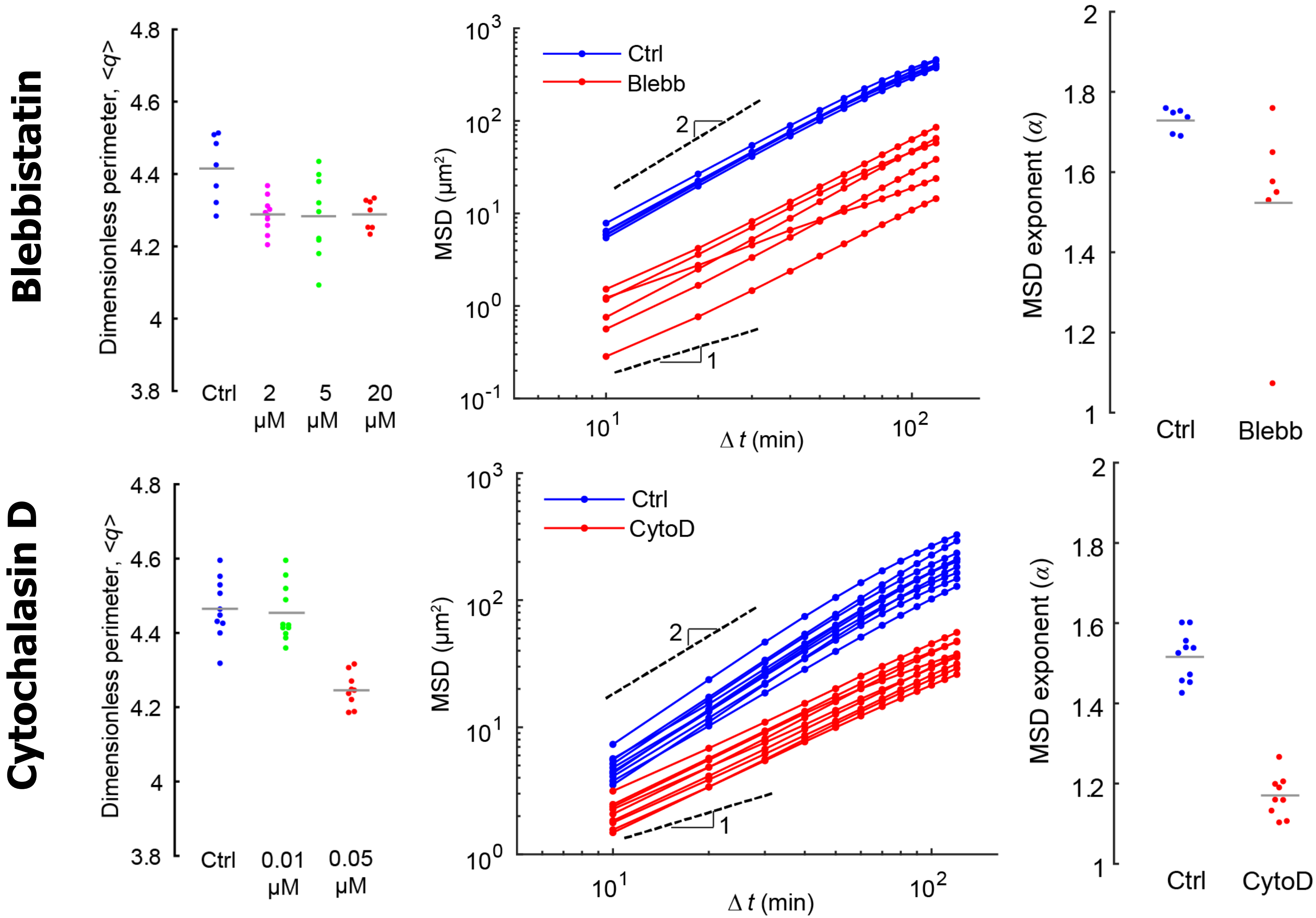
Cytochalasin D





Directly reduce cortical tension using actomyosin inhibitors

Hypothesis: ↓ cortical actomyosin, ↑ adhesion → ↑ perimeter, rearrangements



Observation: ↓ cortical tension → ↓ perimeter, rearrangements

Initial hypothesis

↓ cortical actomyosin, ↑ adhesion → ↑ perimeter, rearrangements

Observation

↓ cortical tension → ↓ perimeter, rearrangements

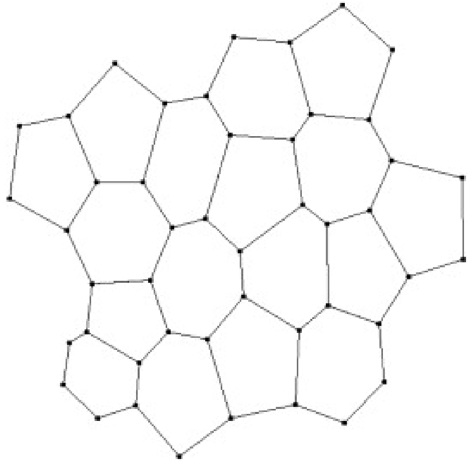
Conclusion

We're missing something.

Force balance for collective shape and rearrangements

In vertex model, combine energy equation with equation of motion

Bi et al, Phys Rev X, 6, 021011, 2016.



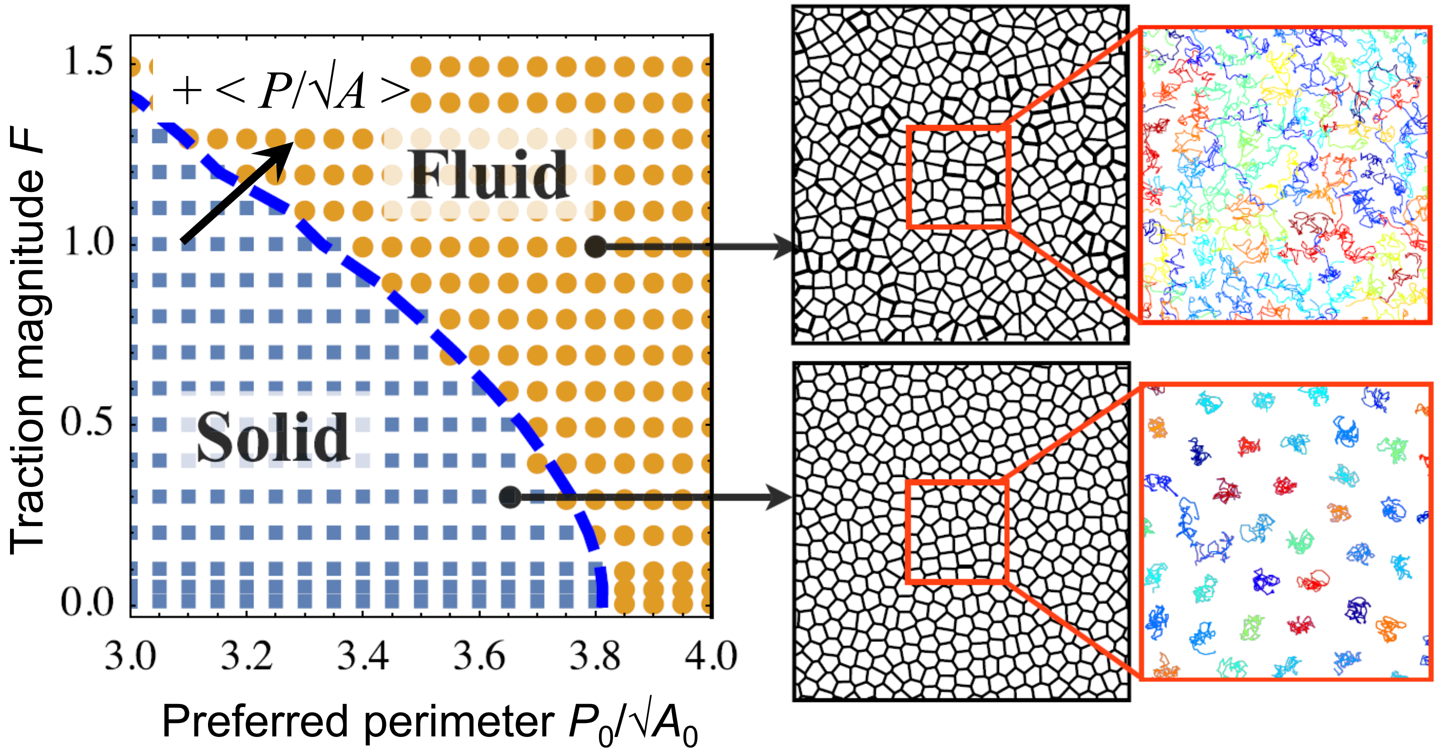
$$E_i = K_A (A_i - A_0)^2 + K_P (P_i - P_0)^2$$

Self-propulsion

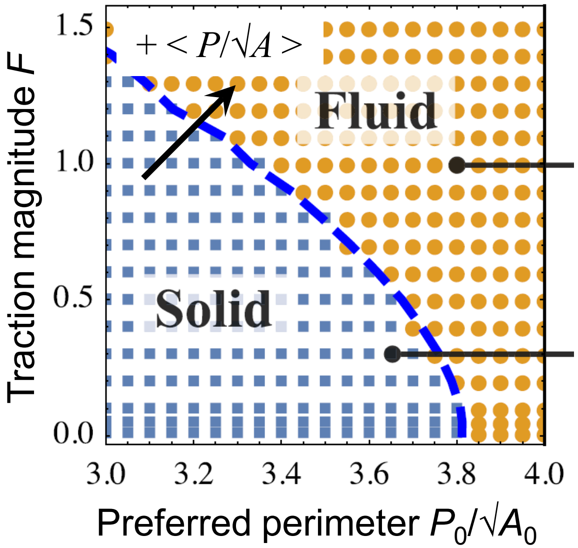
$$\frac{d\boldsymbol{x}}{dt} = \frac{-\nabla E}{\mu} + \boldsymbol{v}$$

Active traction

$$\eta \frac{d\boldsymbol{x}}{dt} = -\nabla E + \boldsymbol{F}$$



The theory predicts 2 factors can affect cell shape and motion



$$E_i = K_A (A_i - A_0)^2 + K_P (P_i - P_0)^2$$

$$\eta \frac{d\boldsymbol{x}}{dt} = -\nabla E + \boldsymbol{F}$$

Preferred perimeter

$$\frac{P_0}{\sqrt{A_0}}$$

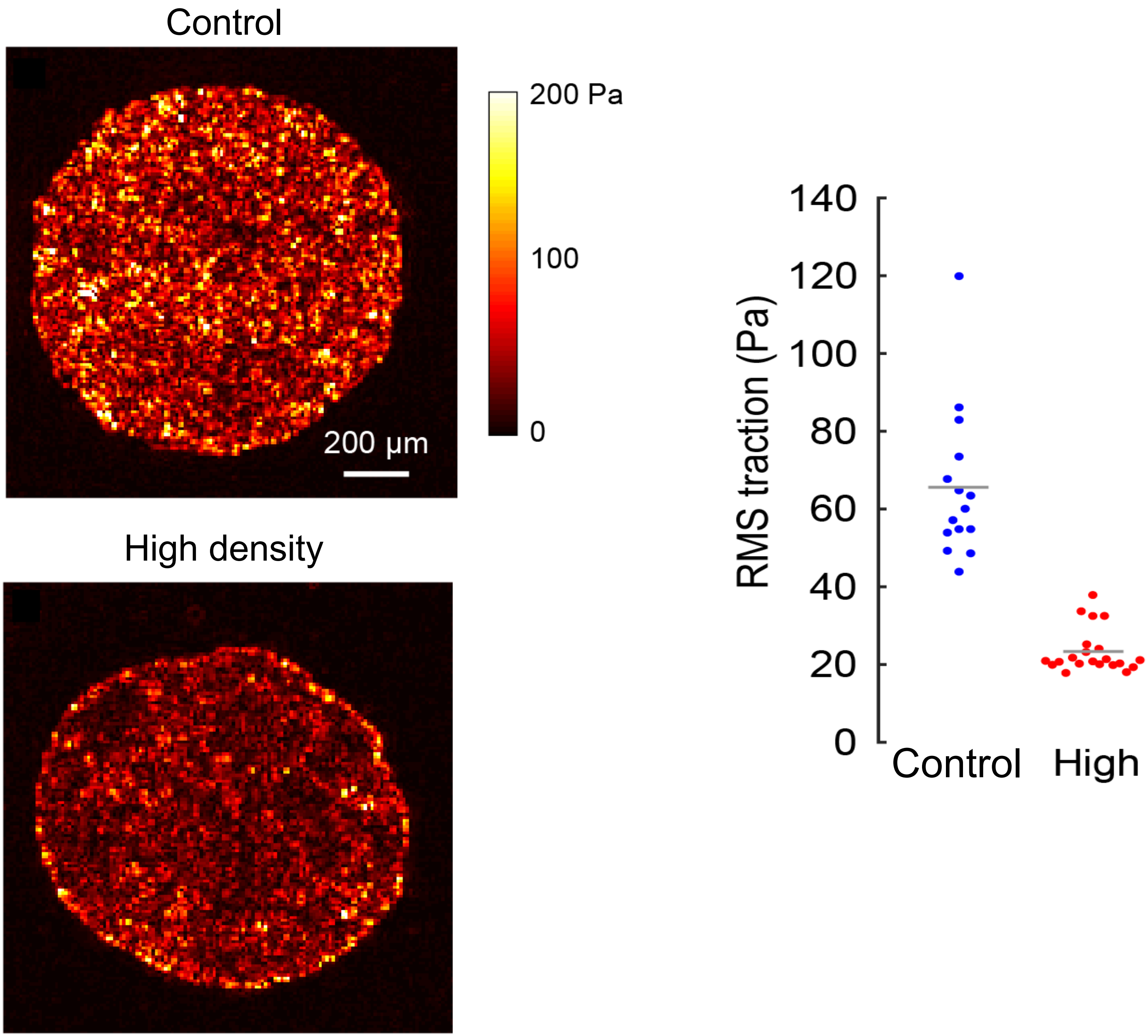
Propulsive force (cell-substrate traction)

$$\boldsymbol{F}$$

New hypothesis

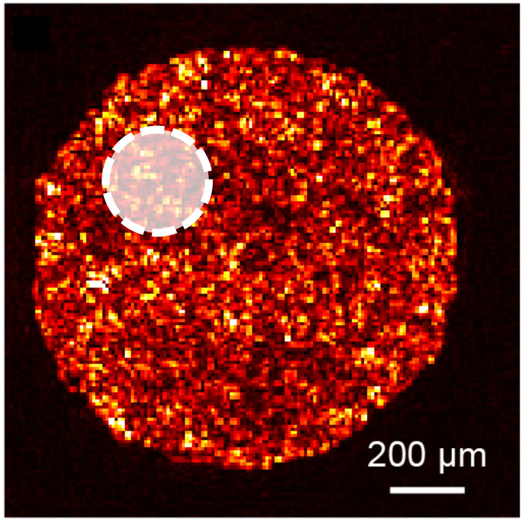
↑ traction → ↑ perimeter, rearrangements

Cell-substrate tractions



Relate experimental traction to motility force

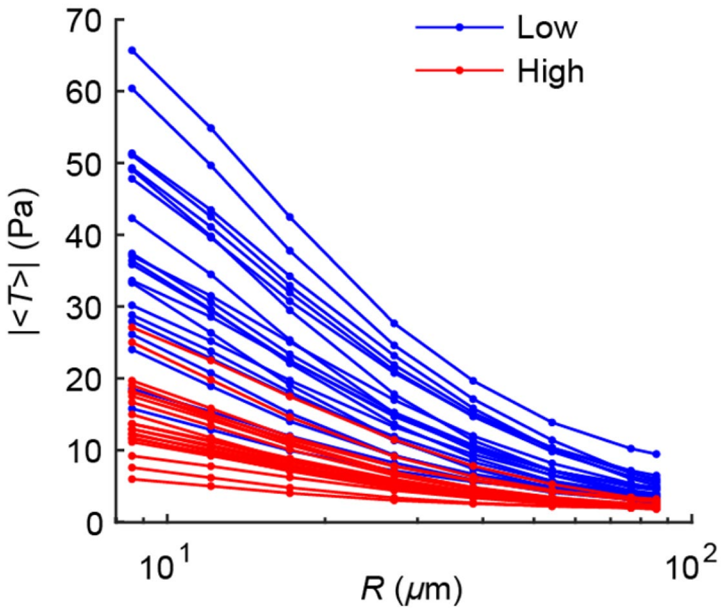
$$\eta \frac{d\boldsymbol{x}}{dt} = -\nabla E + \boldsymbol{F}$$

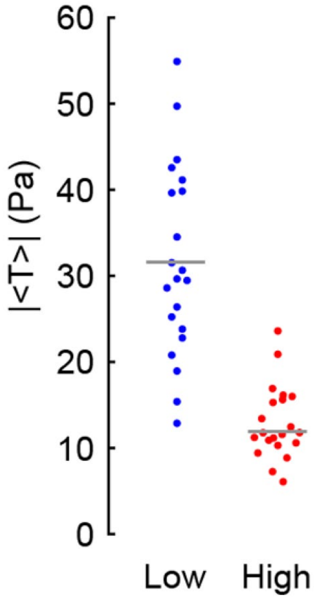


Traction imbalance

- Pick a position in cell island.
- Compute vector sum of data in circle of radius *R*.
- Divide by number of data points.
- Repeat for different *R*.

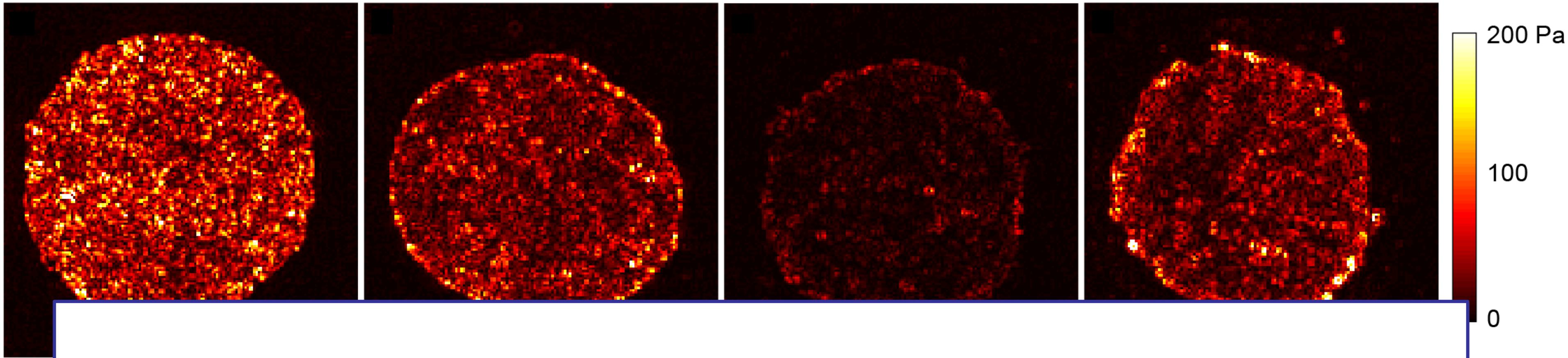
- Repeat for different positions and take mean over all positions.



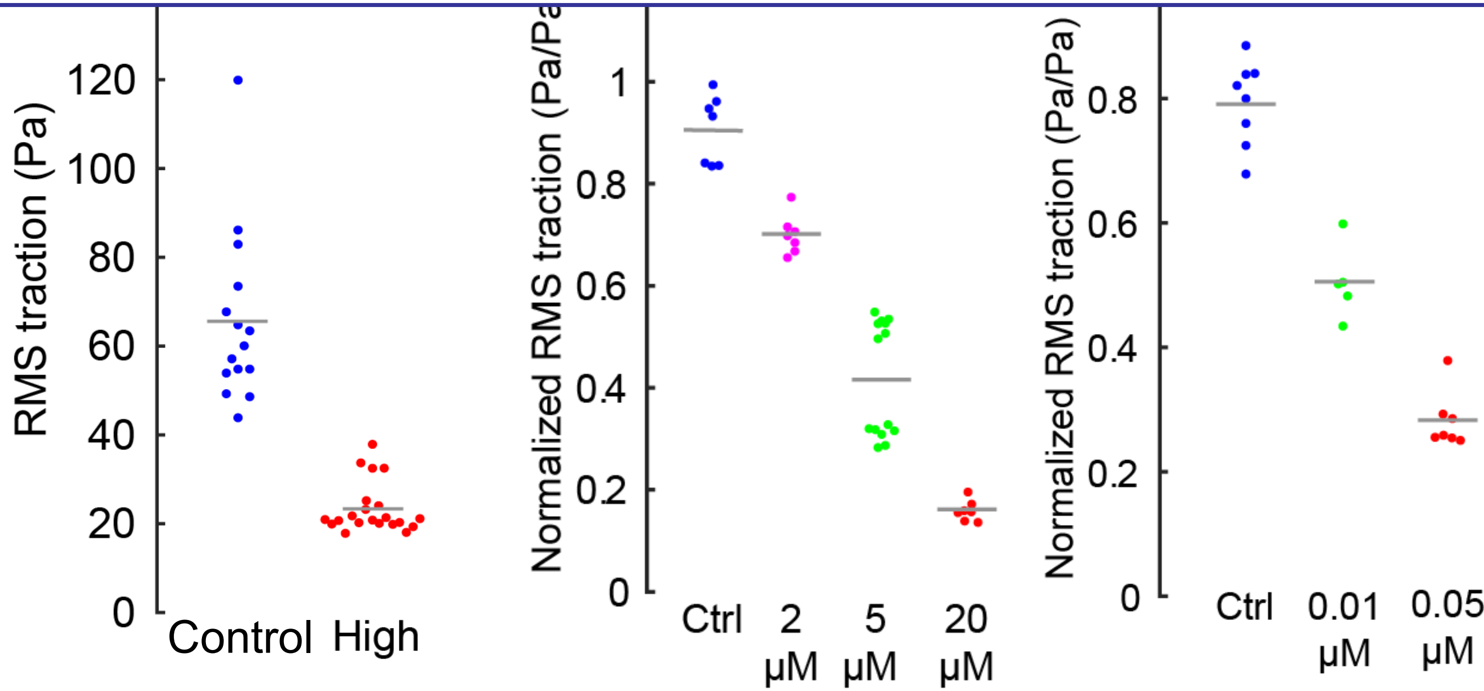


Traction imbalance and rms traction have same trends.

Cell-substrate tractions



New hypothesis: ↑ traction → ↑ perimeter, rearrangements

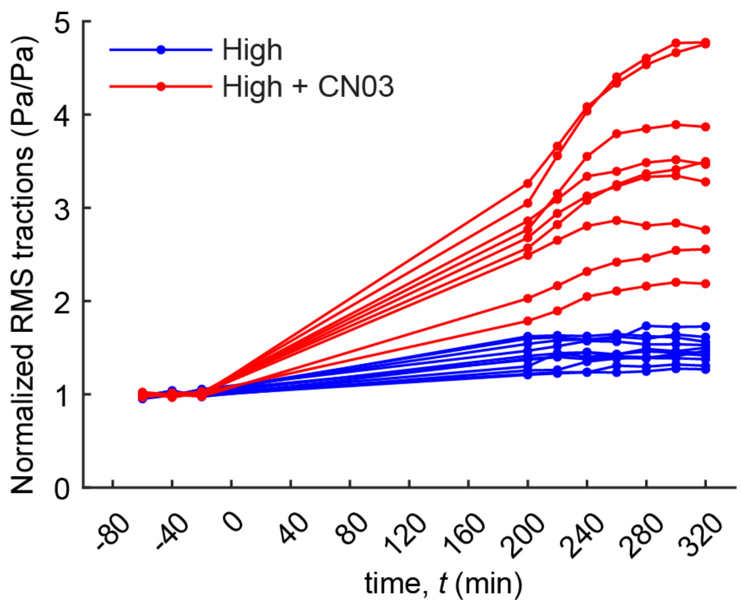


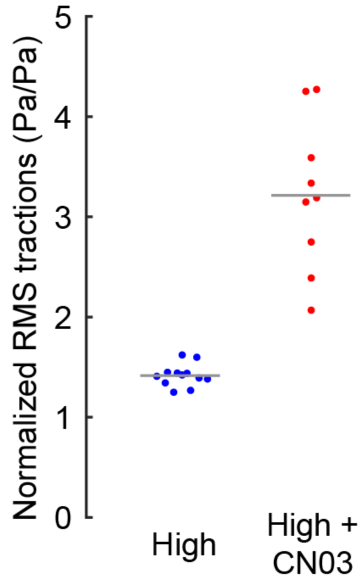
Effect of increasing traction

CN03 activates Rho

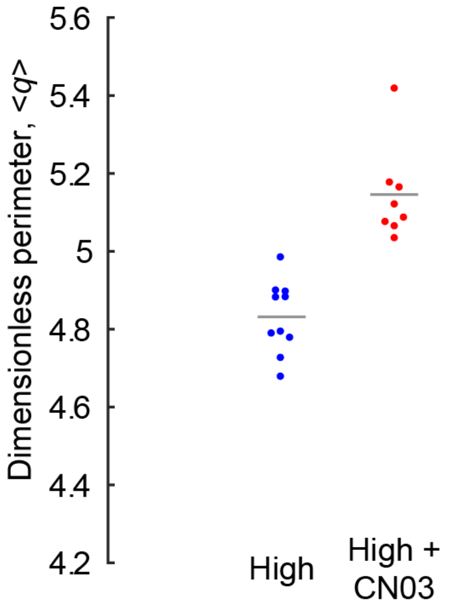
New hypothesis:
↑ traction → ↑ perimeter, rearrangements

Increased tractions

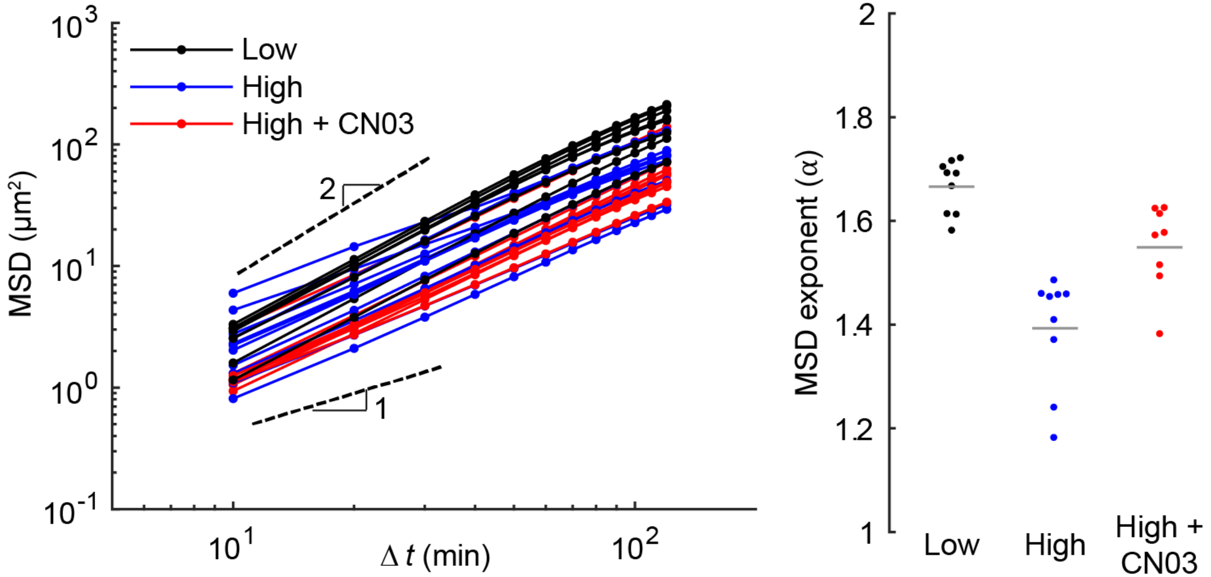




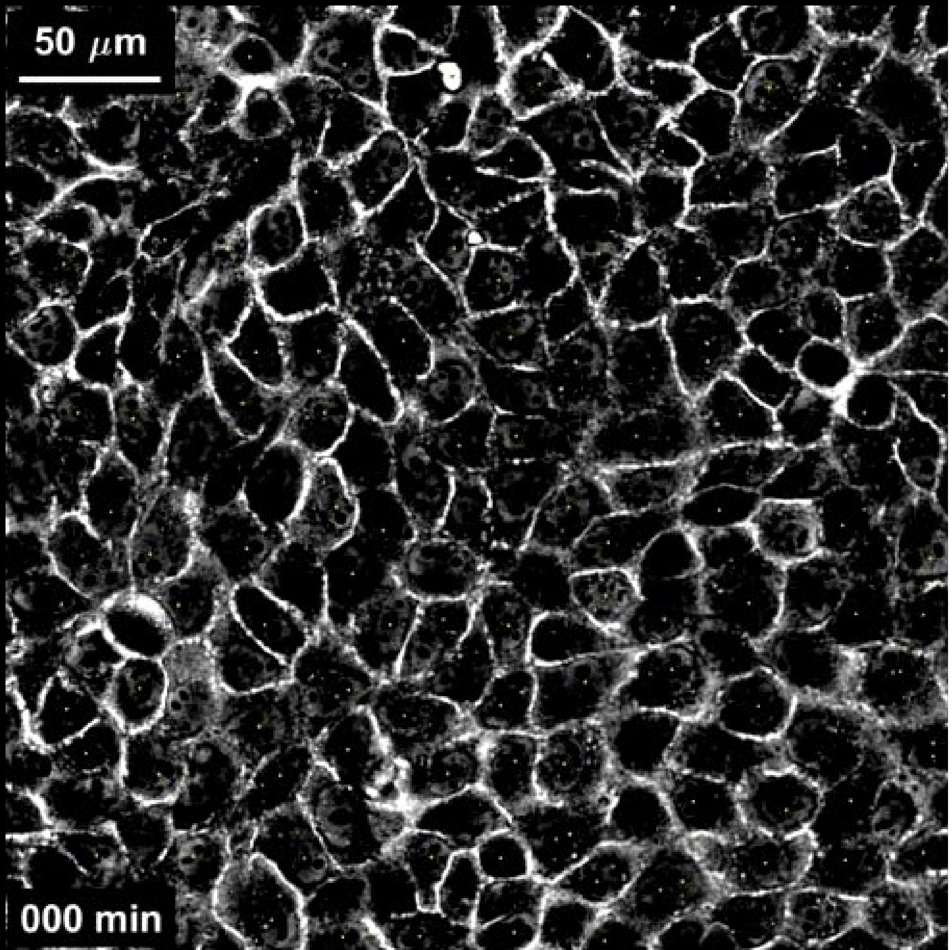
Increased perimeter



Increased rearrangements

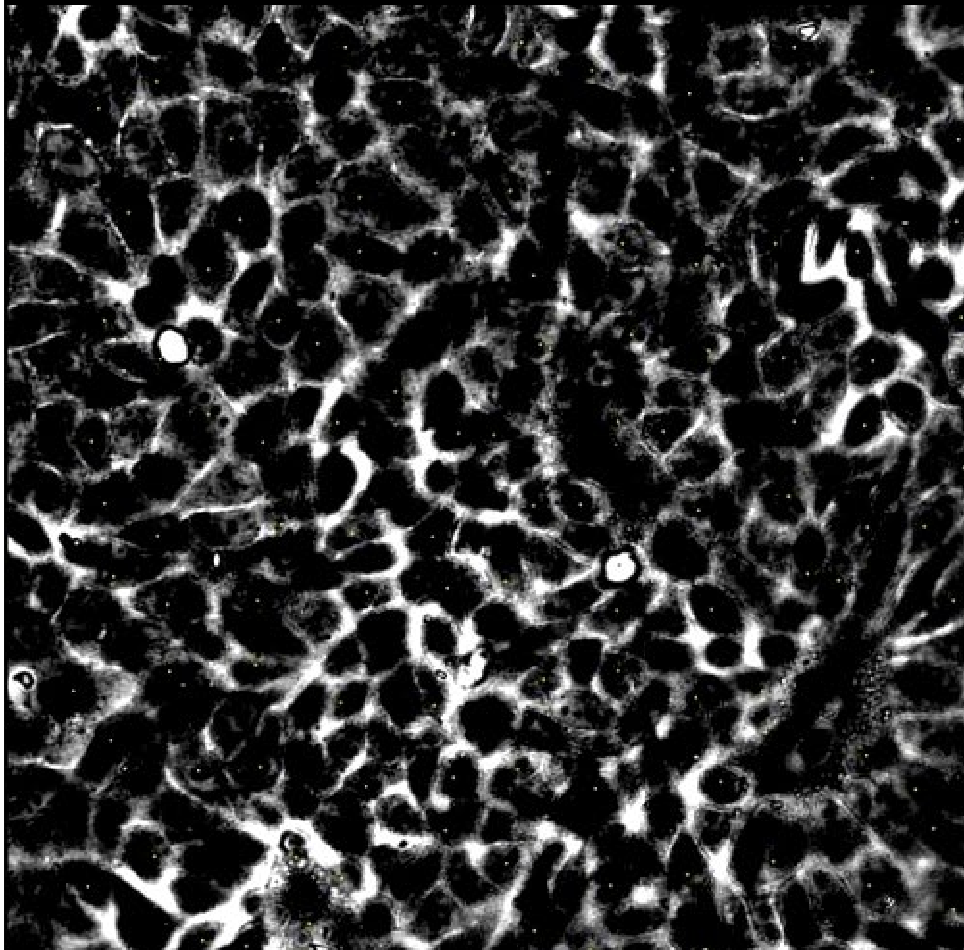


Control

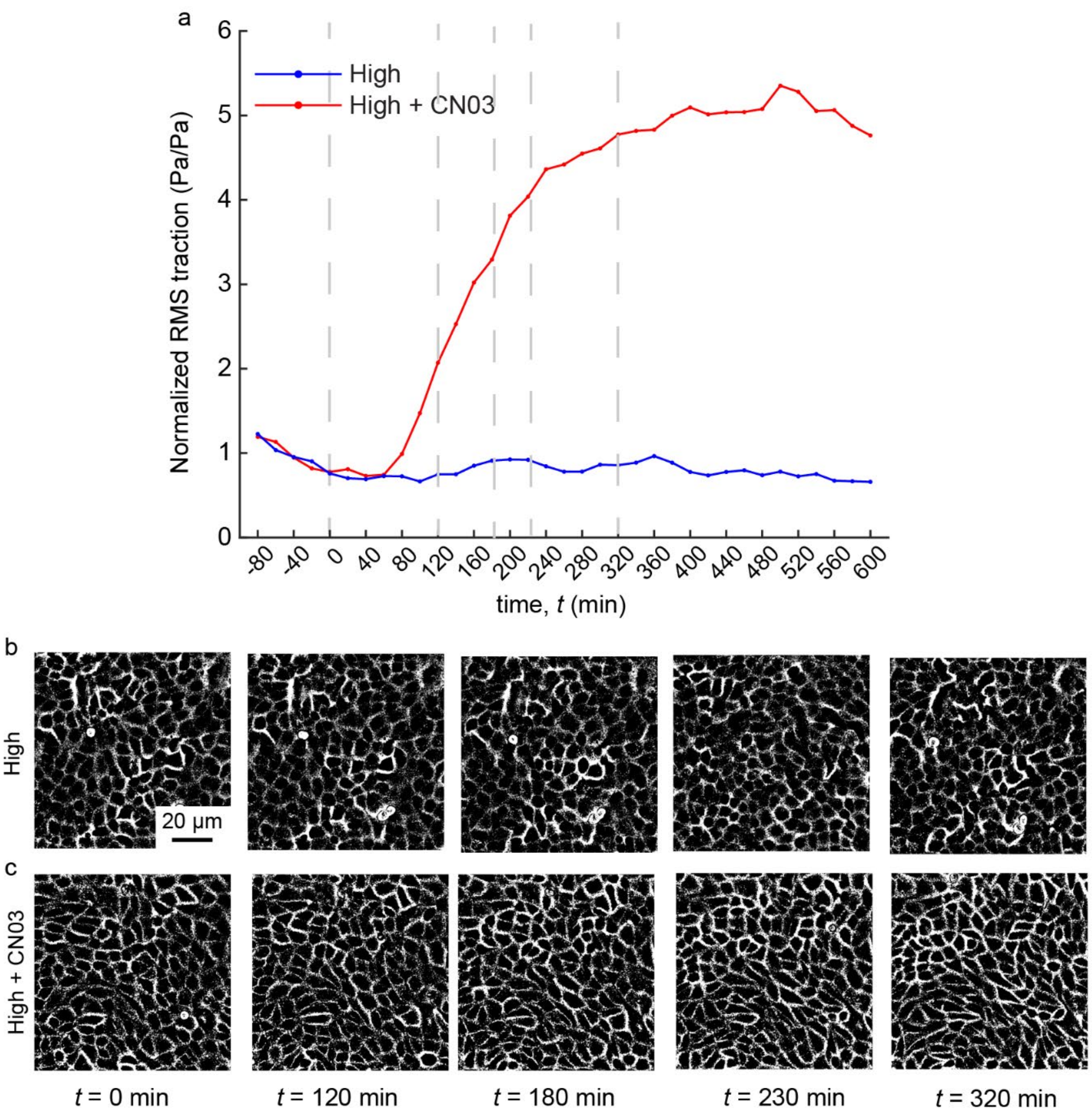


Rho activator CN03

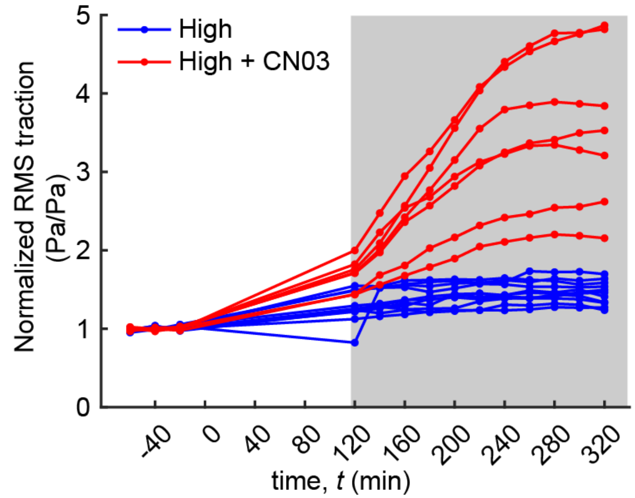
increases rearrangements
elongates cells

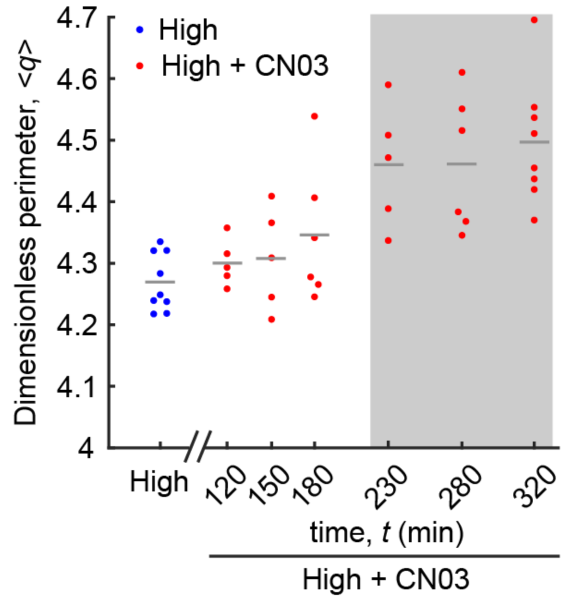


Tractions increase before perimeter does



Gray shading shows when data are statistically different from baseline/control.





Tractions increase before perimeter does

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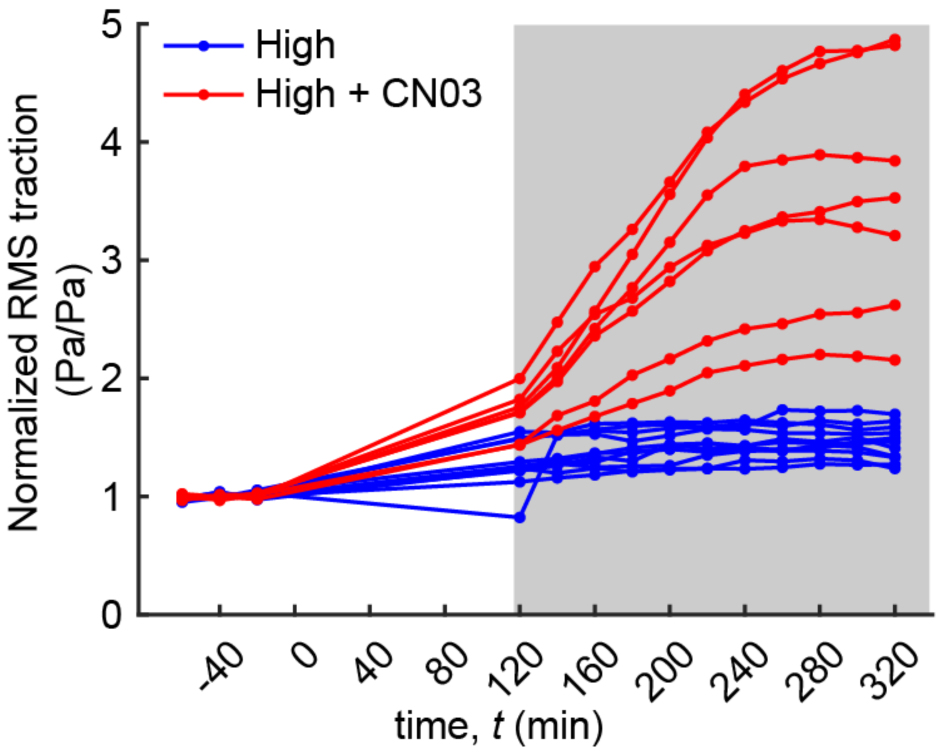
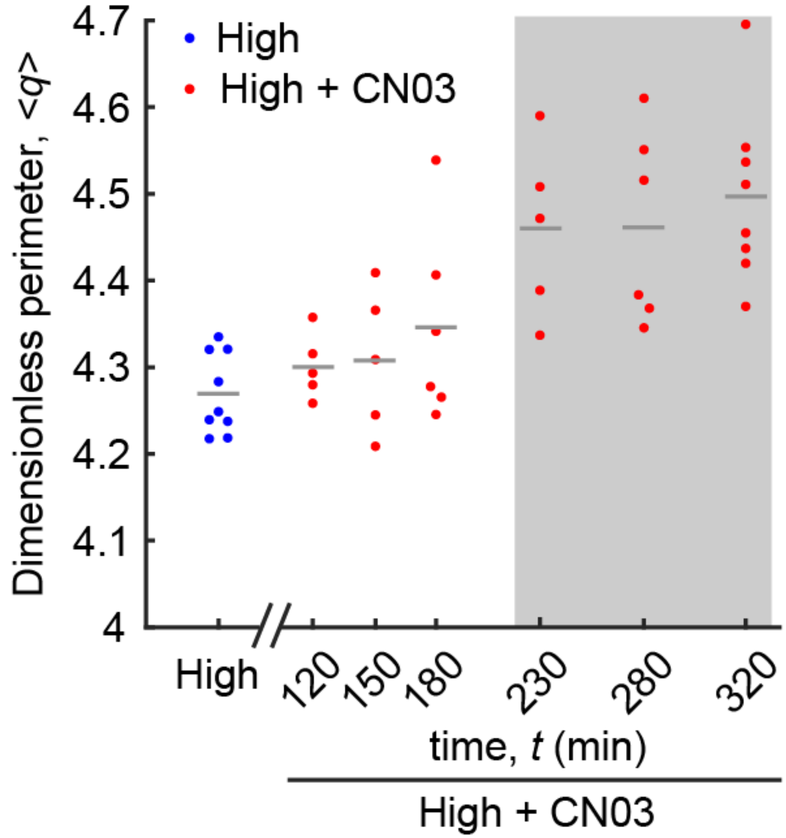
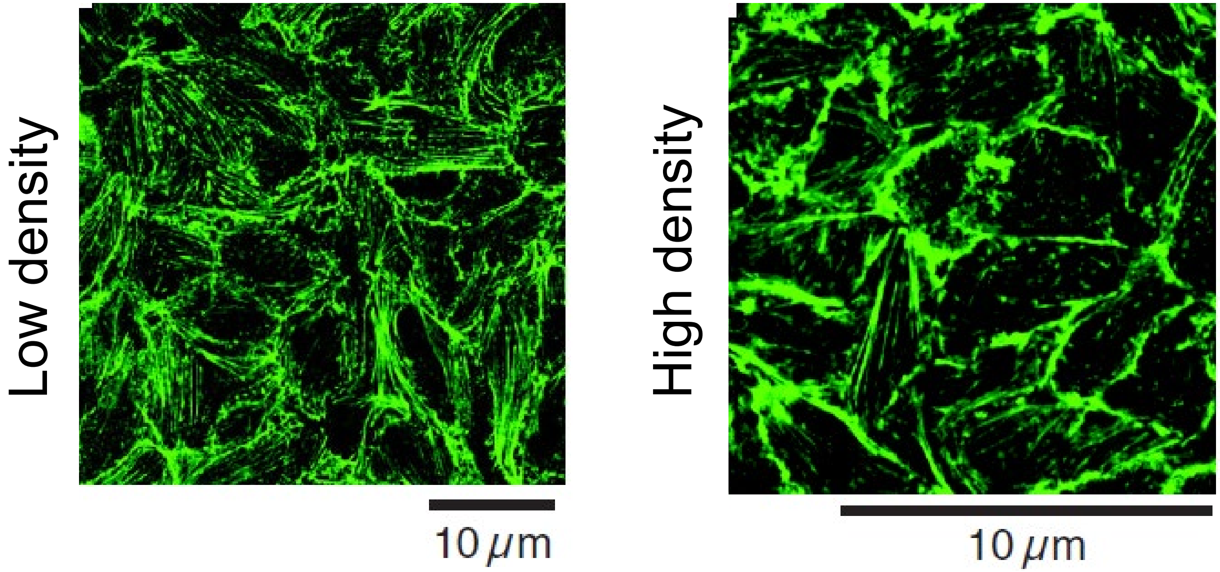


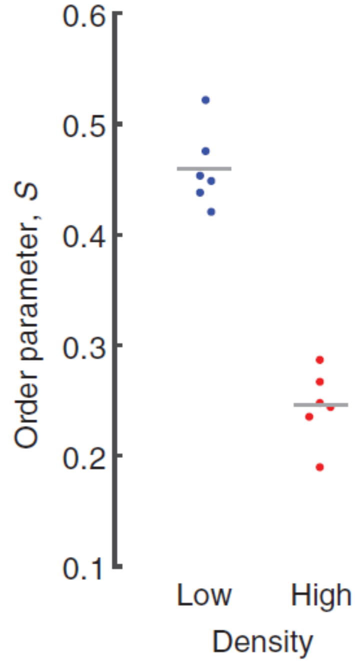


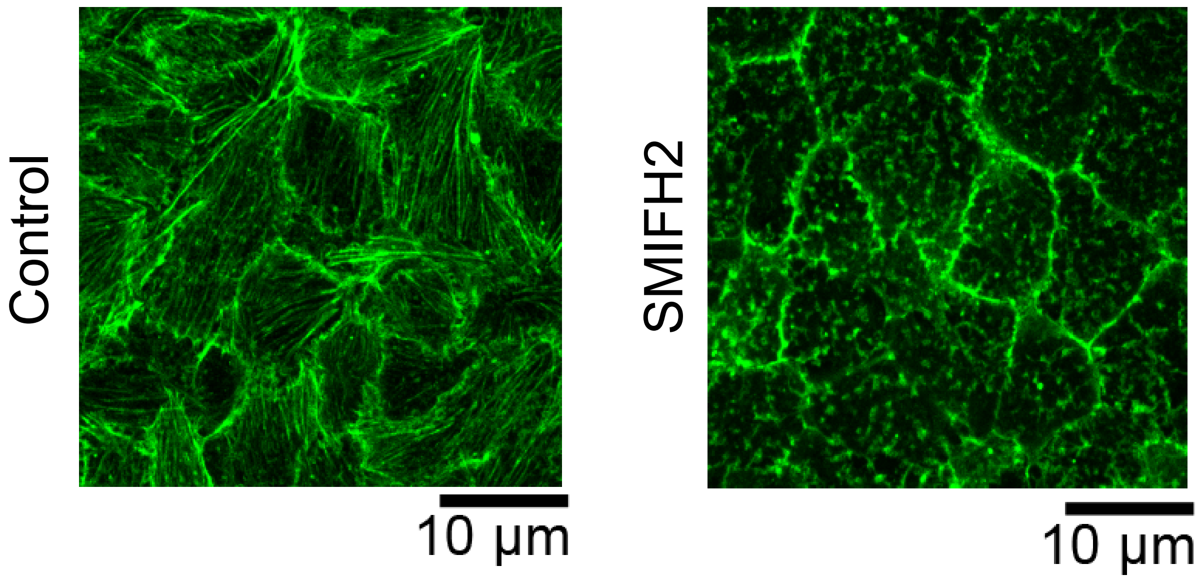
Implication: Tractions have a causal effect on perimeter (and motion).

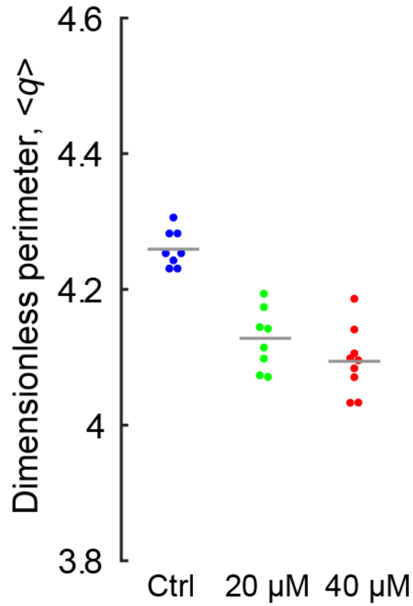
What causes tractions?

Stress fibers









Anisotropy of stress fibers are not built into the model

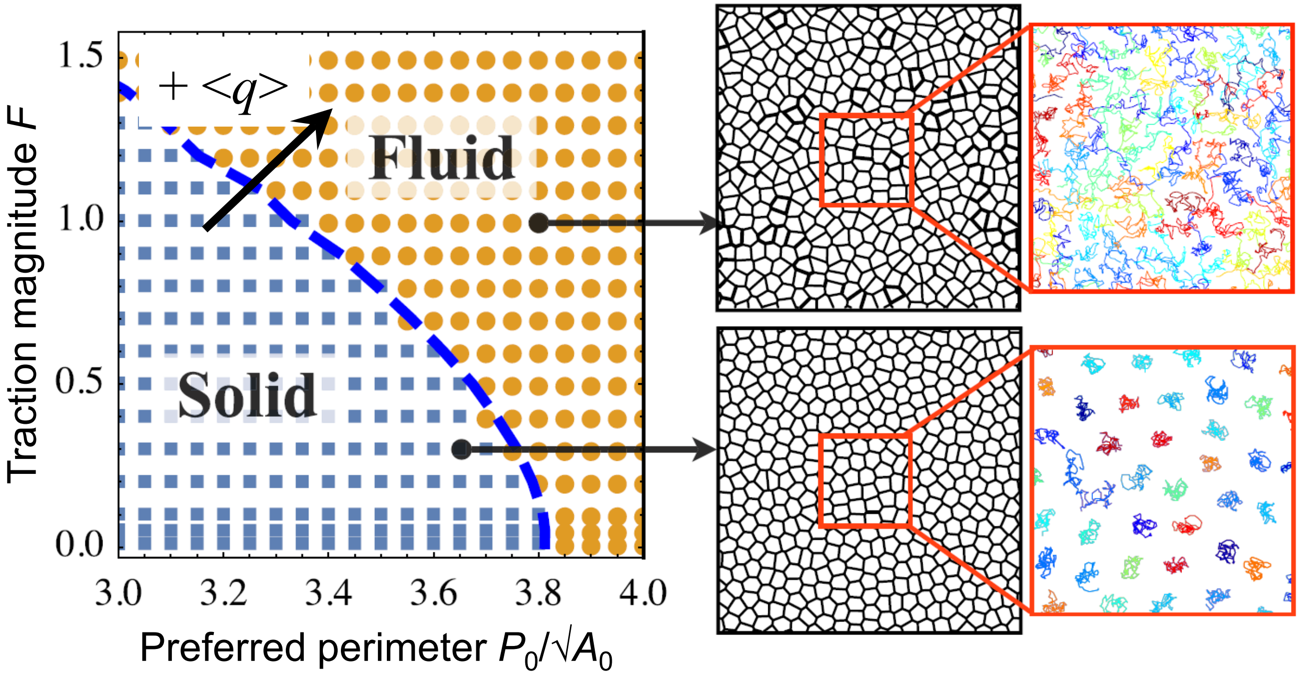
$$E_i = K_A (A_i - A_0)^2 + K_P (P_i - P_0)^2$$

$$\eta \frac{d\boldsymbol{x}}{dt} = -\nabla E + \boldsymbol{F}$$

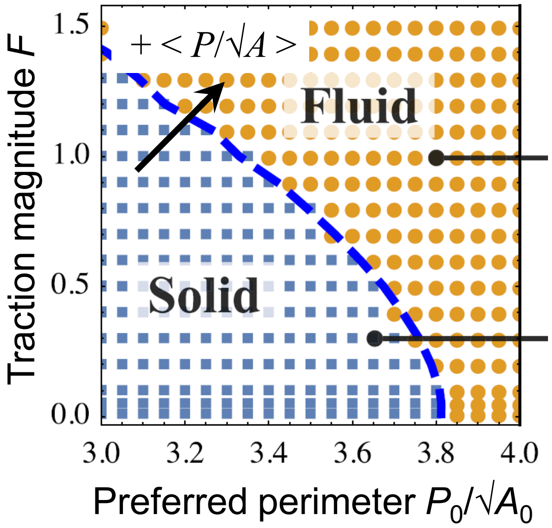
It's possible that greater cell perimeter q is caused by stress fibers.

It's also possible that greater cell perimeter q is caused primarily by traction.

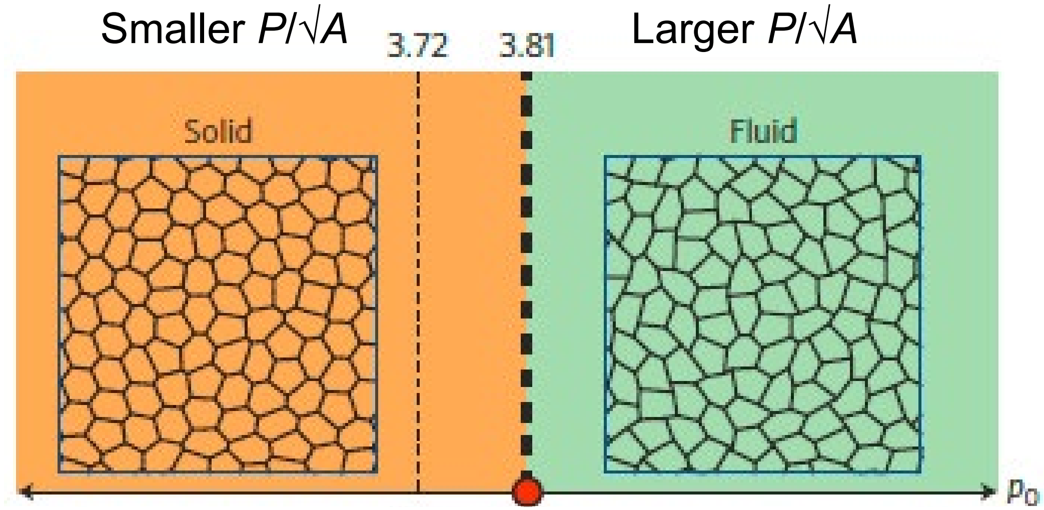
Bi et al, Phys Rev X, 6, 021011, 2016.



Original hypothesis



Bi et al, Phys Rev X, 6, 021011, 2016.



Bi, Lopez, Schwarz, Manning, Nat Phys, 2015, 11, 1074

↓ cortical actomyosin, ↑ adhesion → ↑ perimeter, rearrangements

Conclusions

1. ↑ ***cell-substrate tractions and stress fibers*** → ↑ perimeter $q = P/\sqrt{A}$ → ↑ rearrangements

2. Tractions can reverse the well-established effect of density on rearrangements in collective migration.

Acknowledgments

Notbohm Research Group

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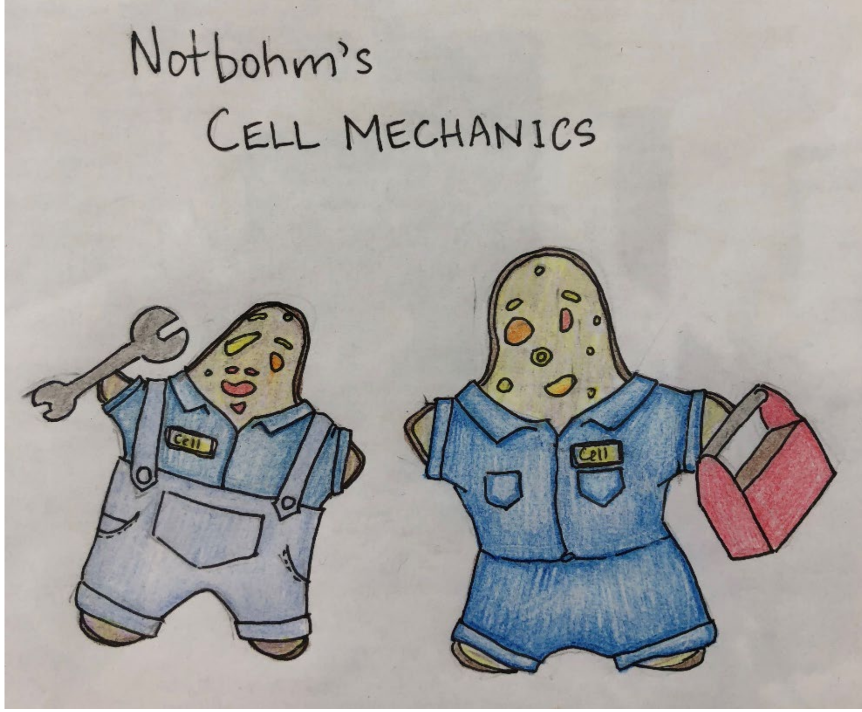

Drawing by Shauna Bennett

Funding

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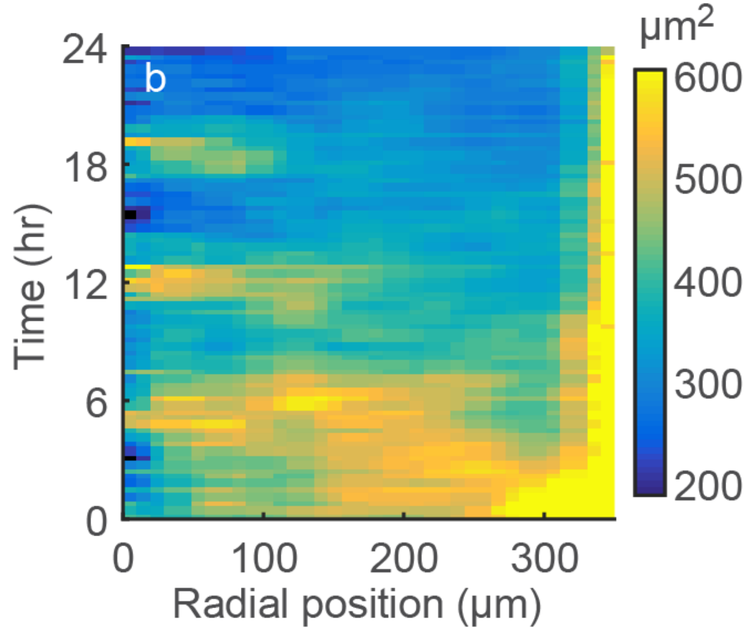
Former Advisors

Jeff Fredberg and Jim Butler at the Harvard Chan School of Public Health

EXTRA SLIDES

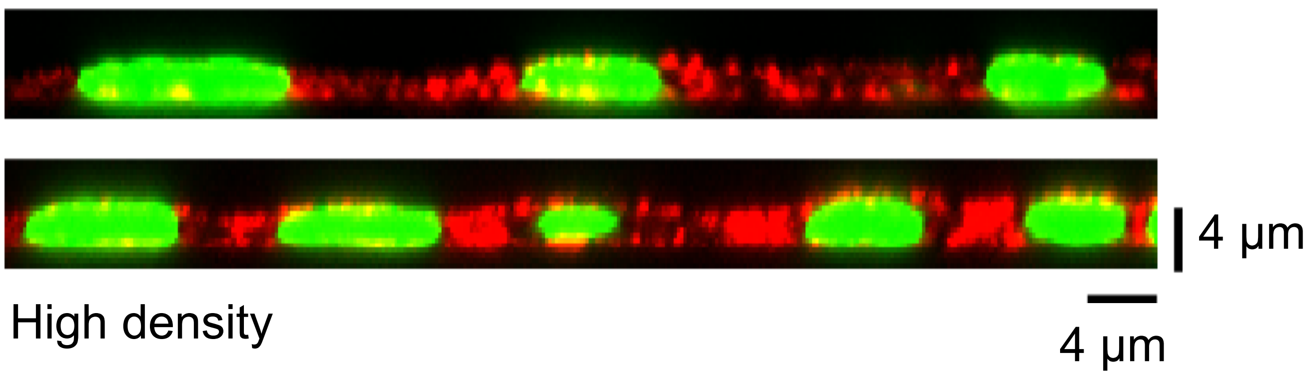
How much do cell volumes change?

Areas change by a factor of 3



Heights change by a factor of 1.2

Low density



High density

Green: nuclei
Red: actin