

Biomedical Engineering Society
2013 Annual Meeting
Seattle, Washington

09/26/2013

Cell Motility I

8:45 AM, Room 611

** Denotes movies replaced by representative snapshots.

Fibronectin on PDMS Elicits a Well-Spread Morphology in Migrating hNeutrophils via β_2 Integrin

Steven J. Henry, John C. Crocker, PhD, and
Daniel A. Hammer, PhD



Funding:

NIH HL18208

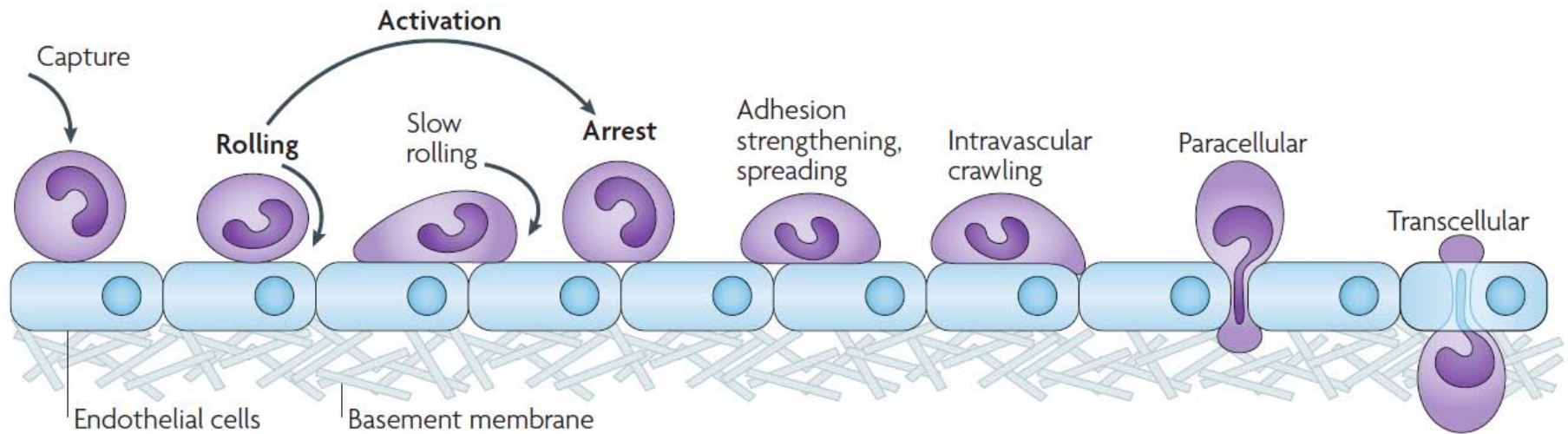
NSF GRFP to SJH

Gratitude to:

Eric Johnston

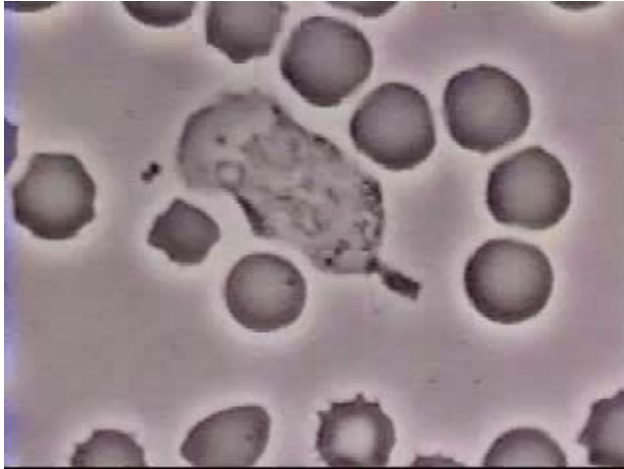
Christopher Chen, PhD

Motility is central to neutrophil function

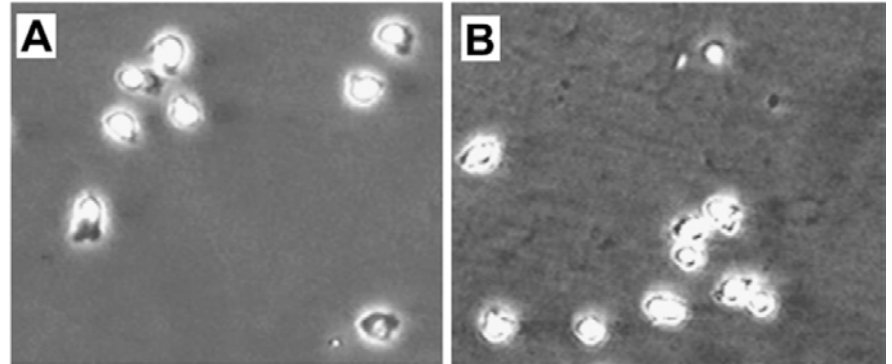


Canonical amoeboid phenotype of neutrophils

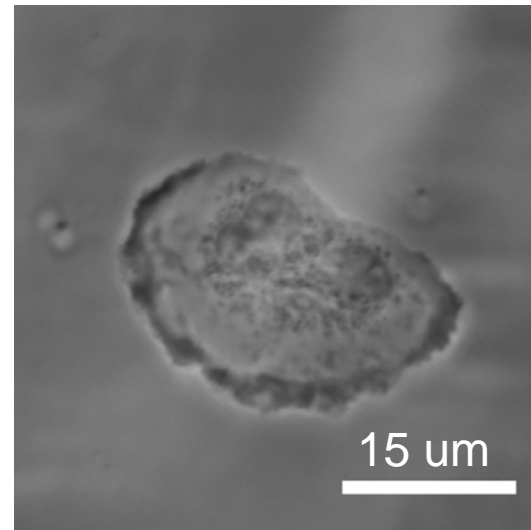
**Rogers, 1950s



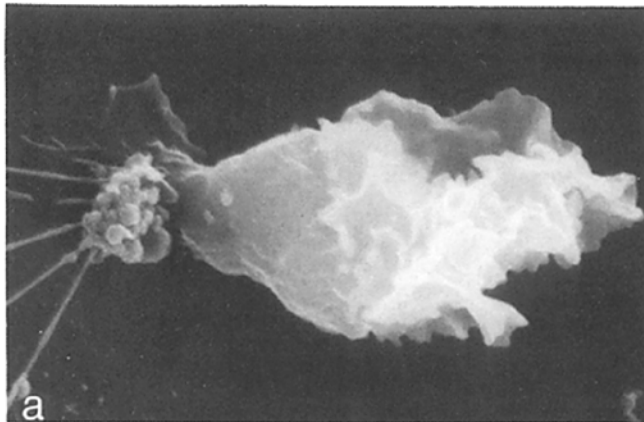
Butler et al. 2008



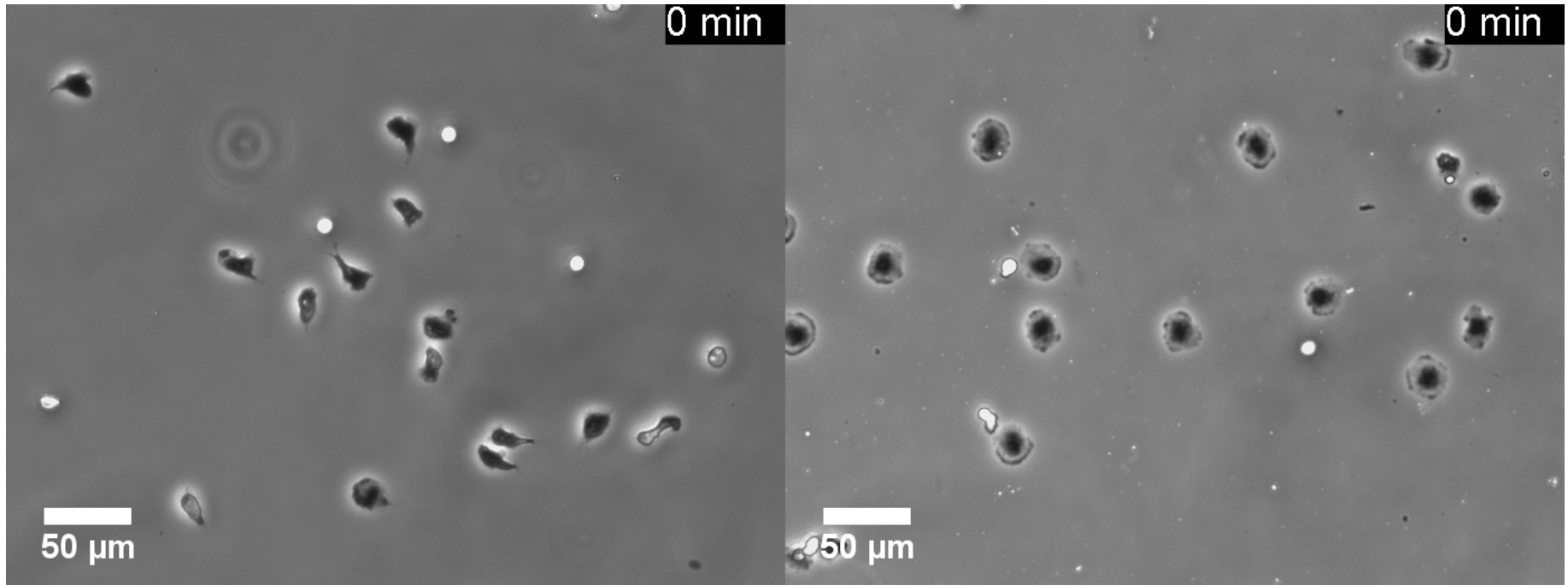
Henry, Crocker, Hammer 2013



Cassimeris et al. 1990

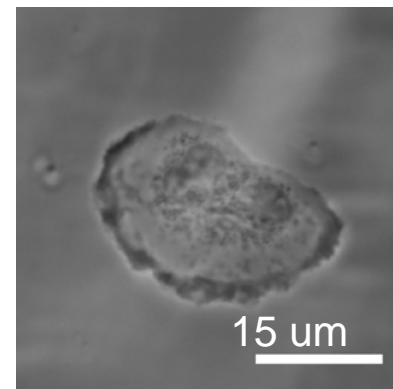
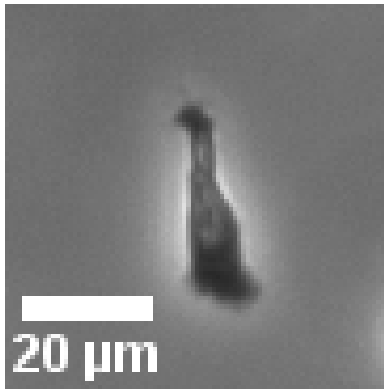


One cell type, two phenotypes

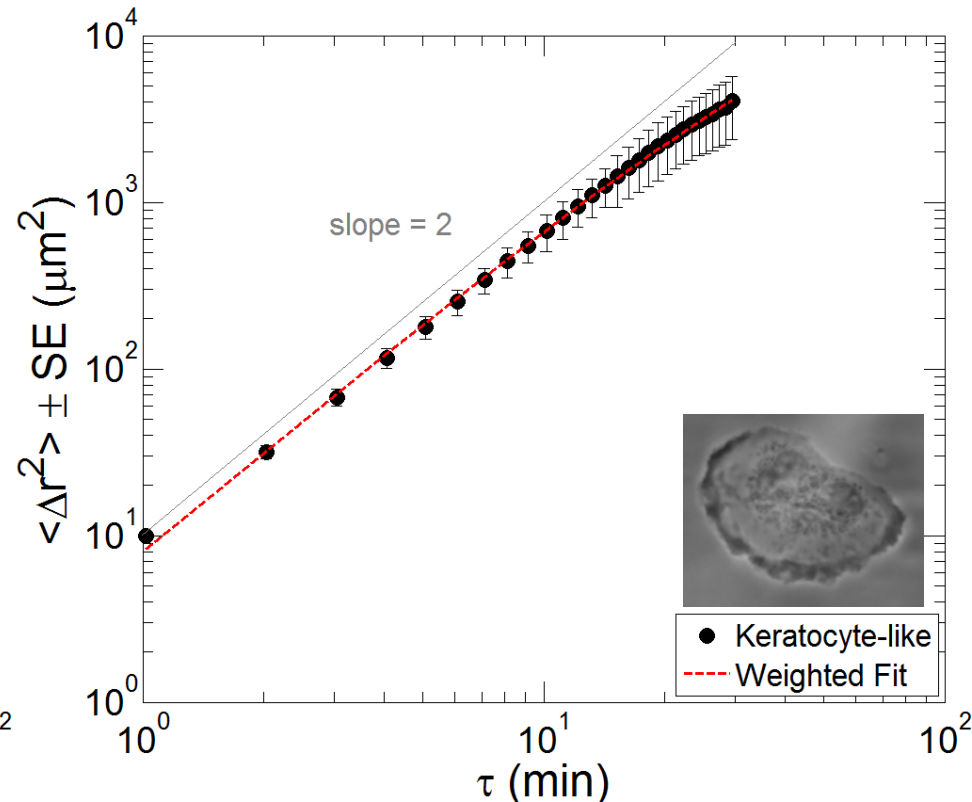
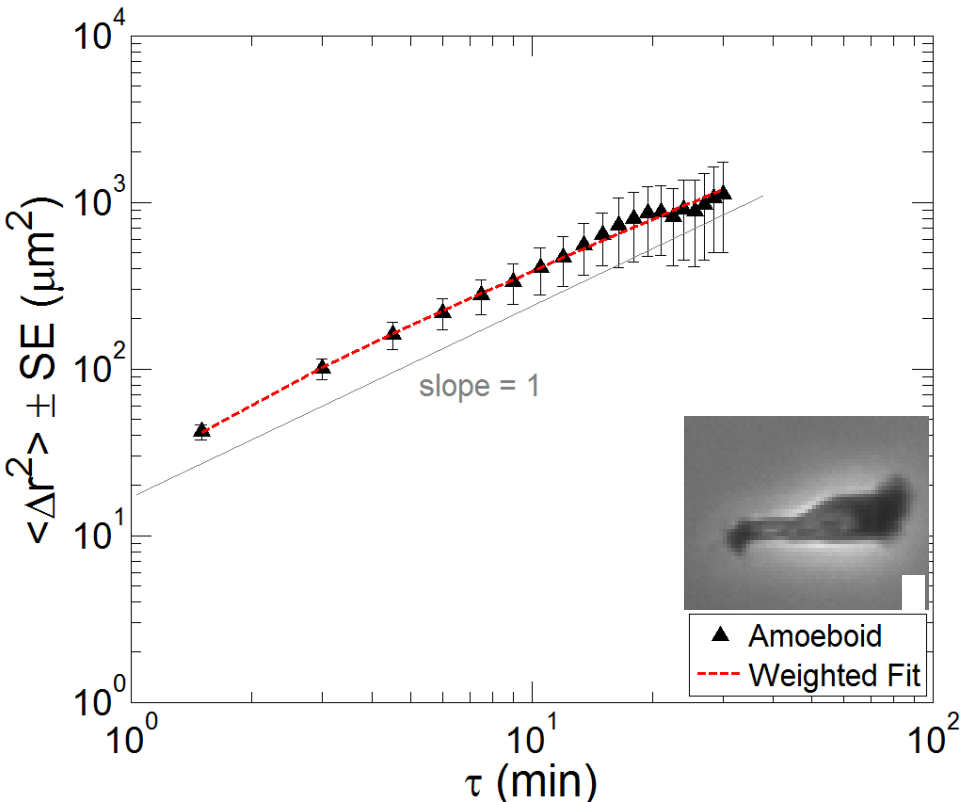


**Amoeboid

**Keratocyte-Like



Quantifying motility: persistent random walk



$$\langle \Delta r^2(\tau) \rangle = 2S^2P[\tau - P(1 - \exp(-\tau/P))]$$

$$S = 6 \mu\text{m}/\text{min}$$

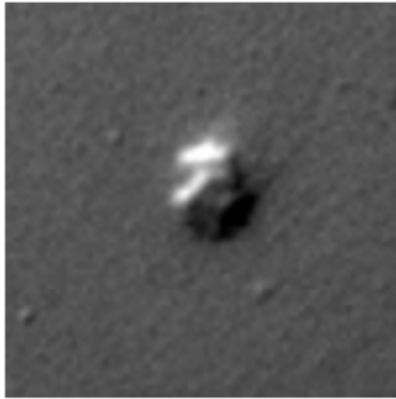
$$P = 0.5 \text{ min}$$

$$S = 3 \mu\text{m}/\text{min}$$

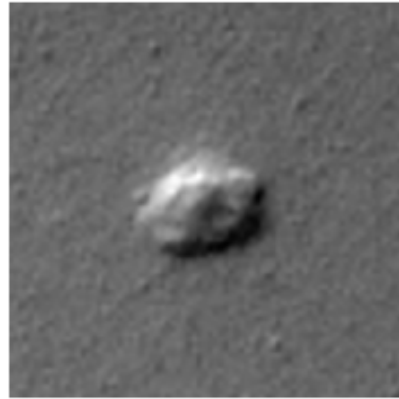
$$P = 15 \text{ min}$$

Well-spread phenotype seen elsewhere

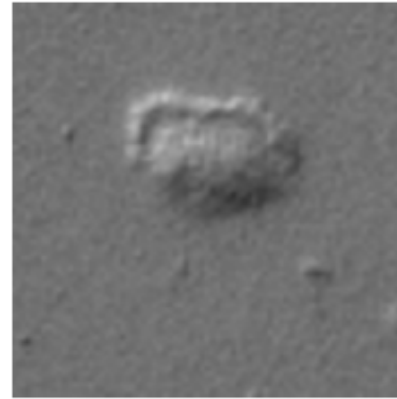
5 kPa



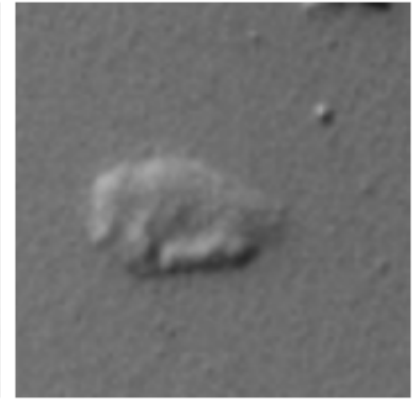
10 kPa



20 kPa



50 kPa

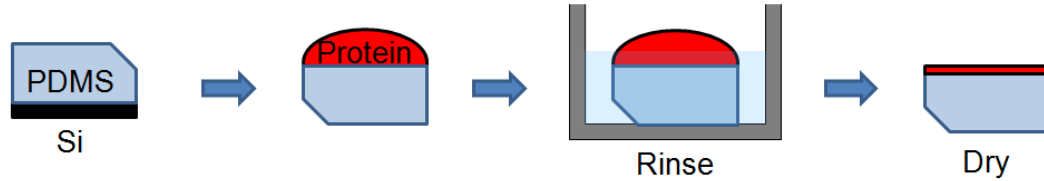


Oakes et al. 2009
FN PA gels

Our Hypothesis: surface ligand density can direct neutrophil phenotype (i.e. shape/motility).

Printing sub-saturating densities of ligand

Ink

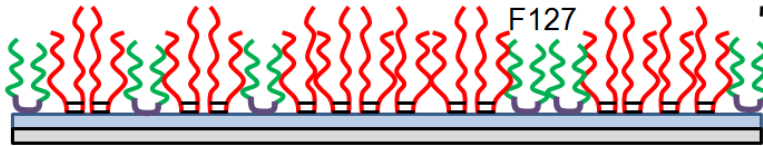


UV Ozone

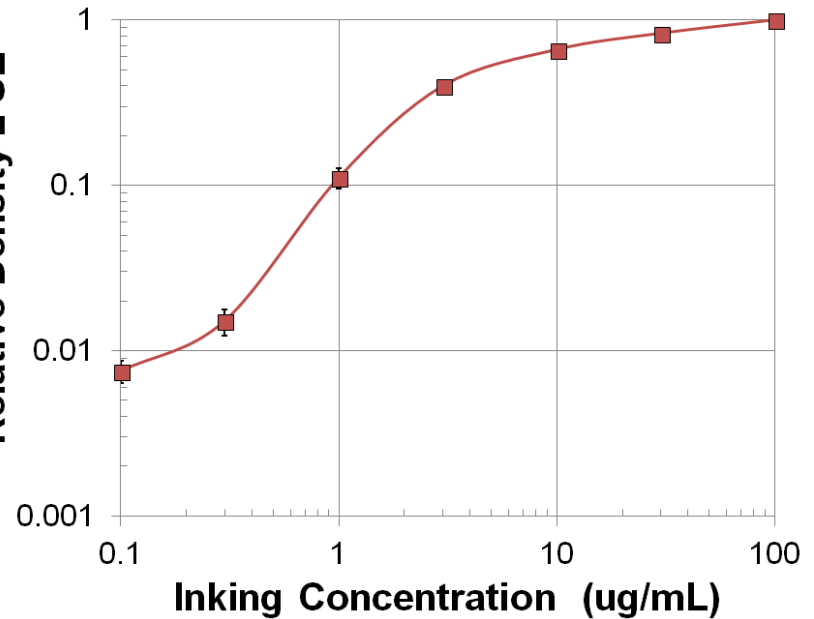
PDMS
Glass

Print

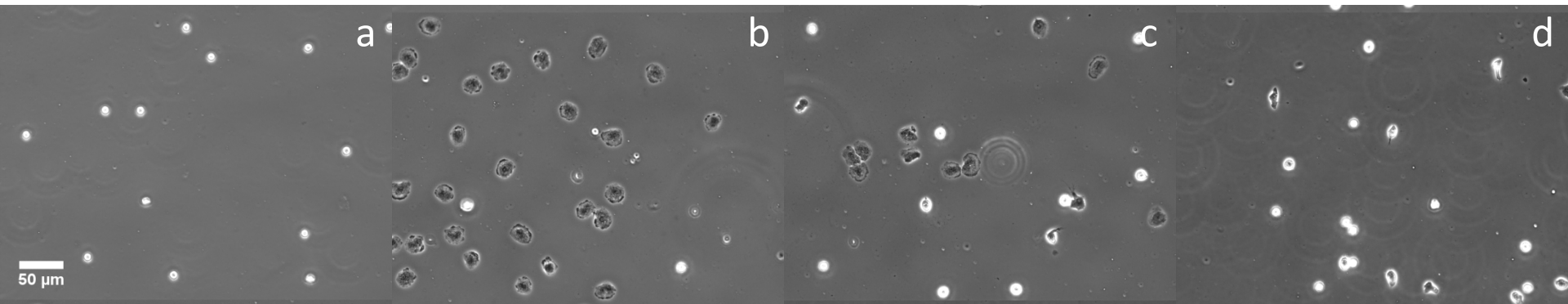
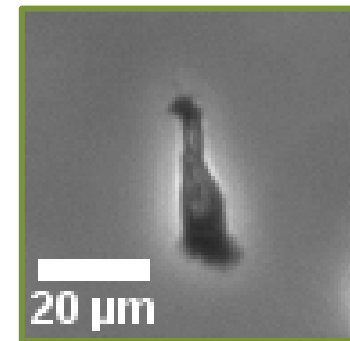
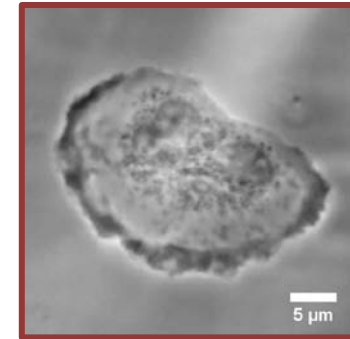
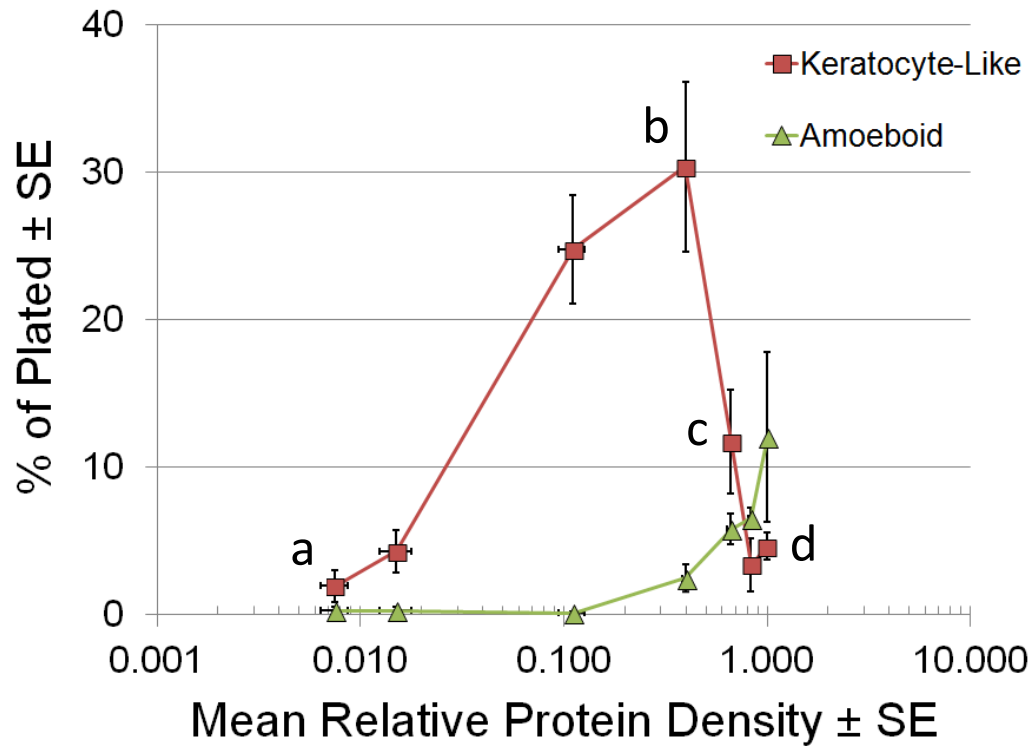
Passivate



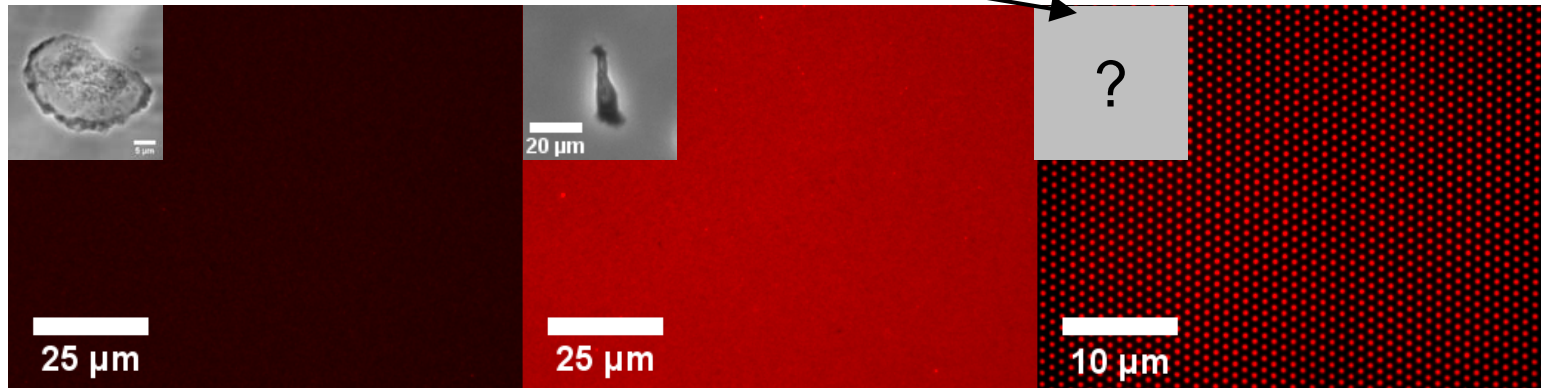
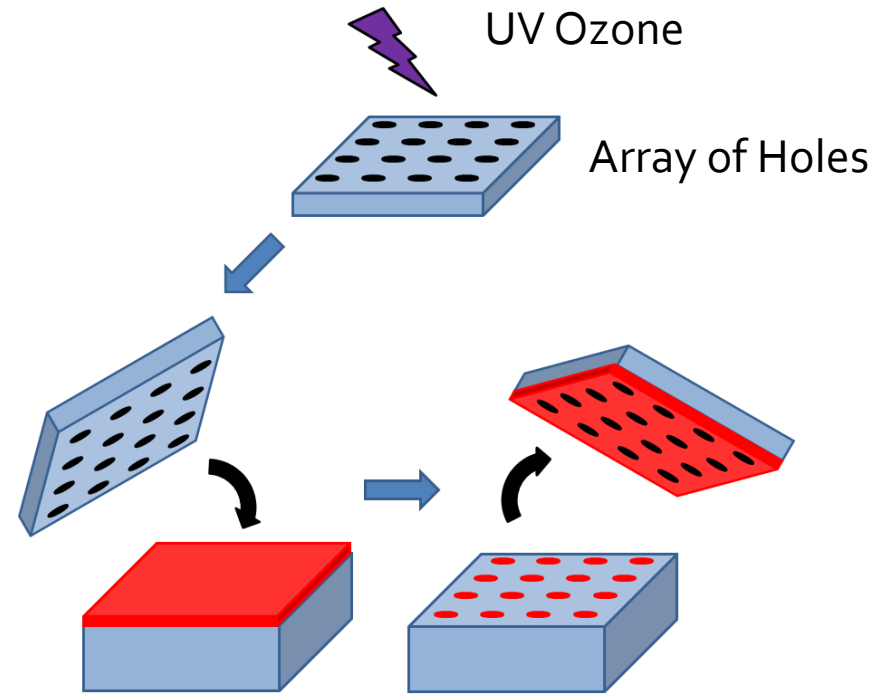
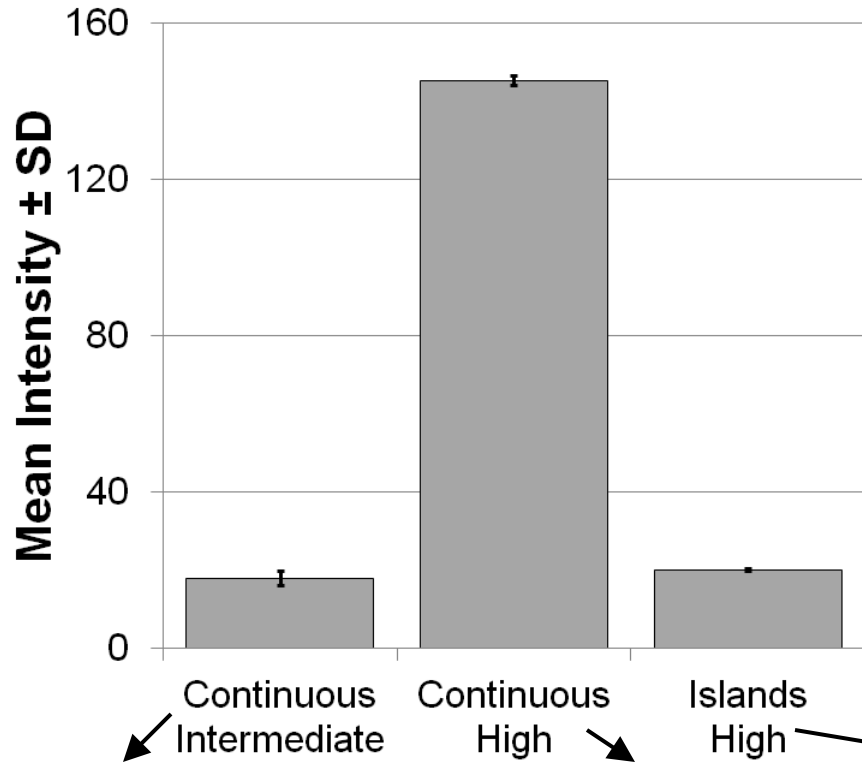
Relative Density \pm SE



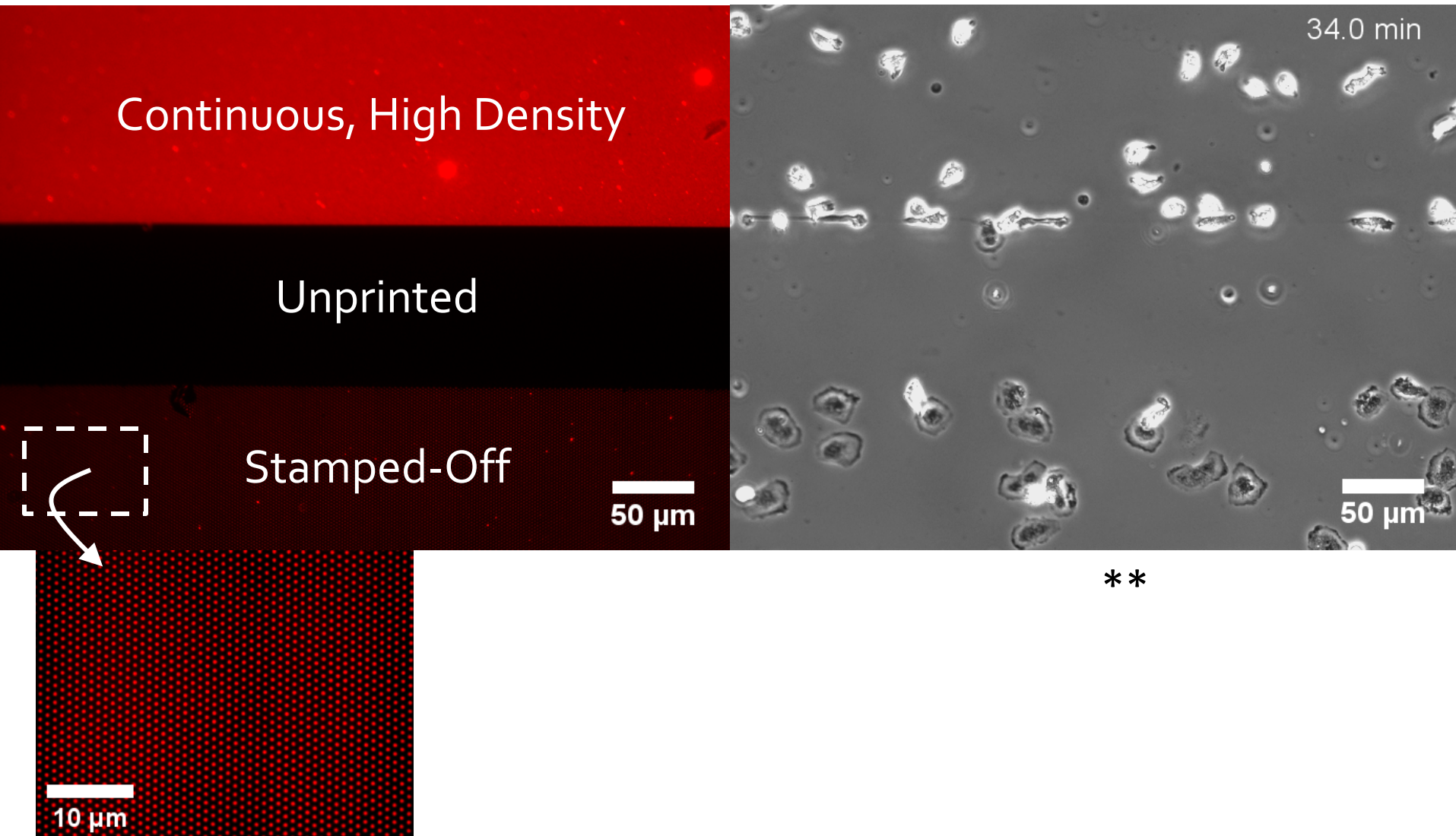
Neutrophil Phenotype Correlates to Density



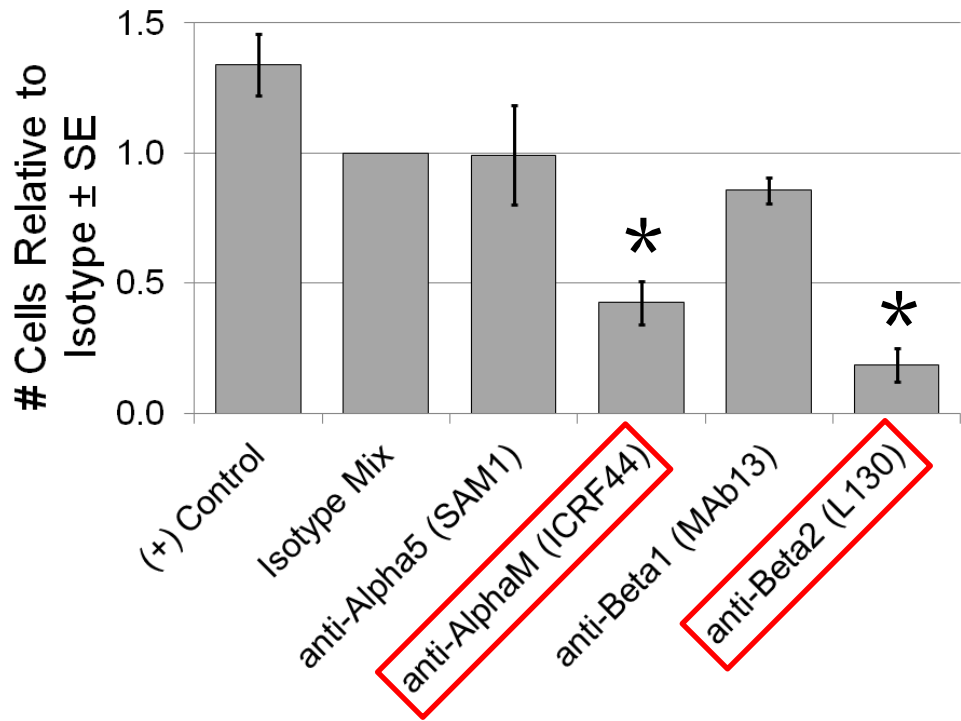
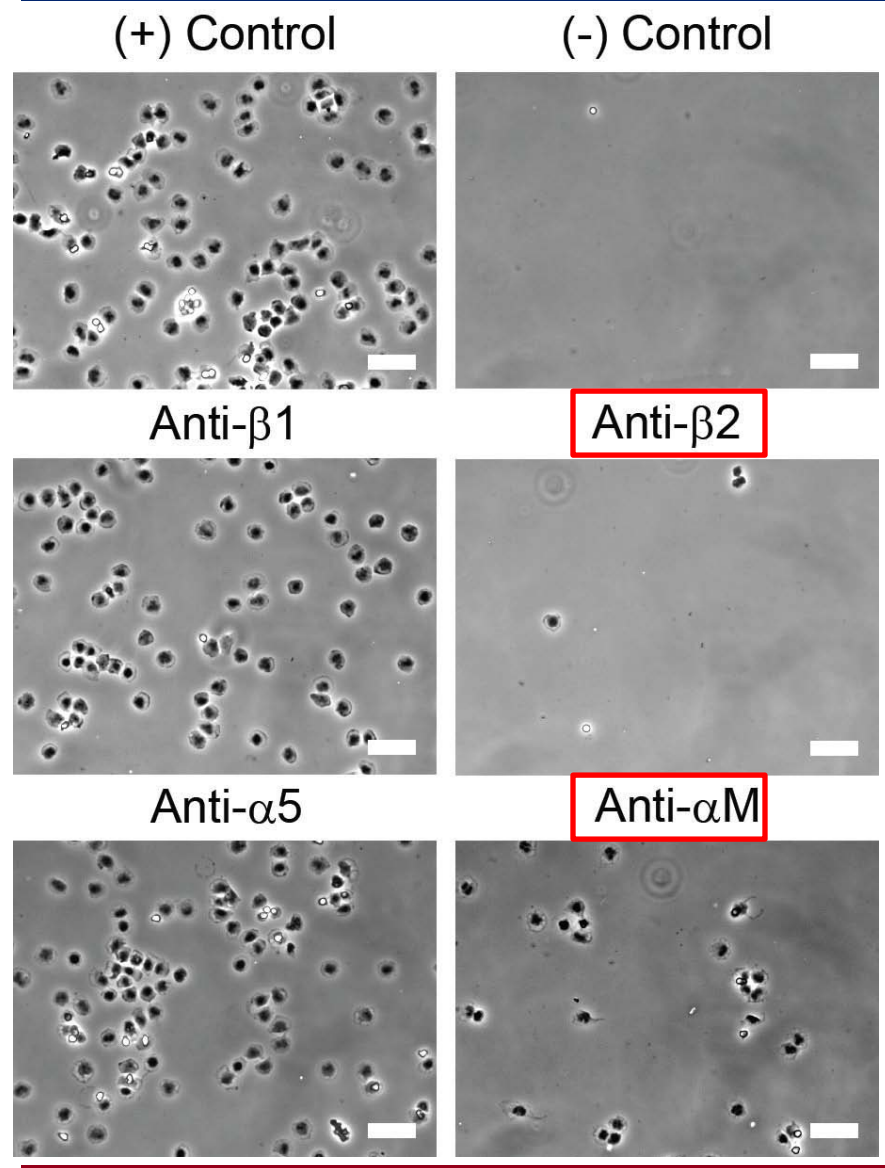
Sensing density at receptor or cell length-scale?



Neutrophils sense density at cell length-scale



$\alpha_M\beta_2$ (Mac-1) Mediates Adhesion to FN

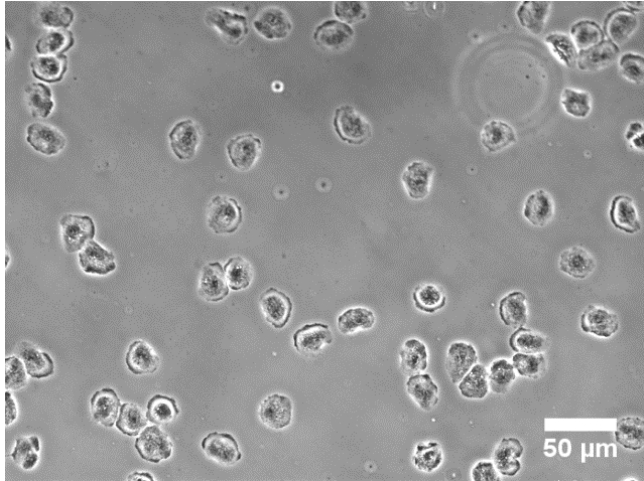


Scalebars = 50 μ m

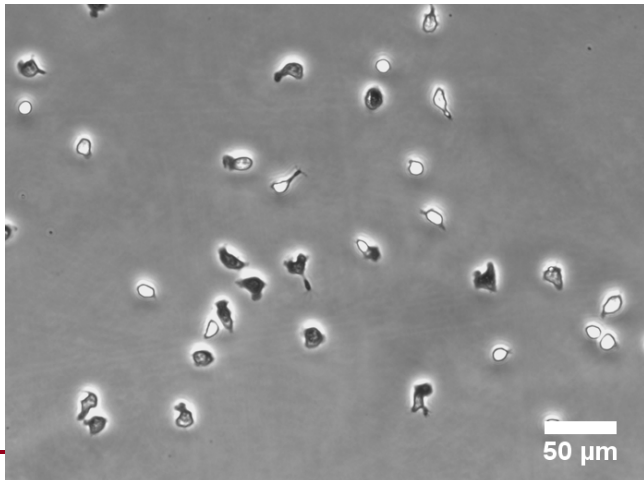
* $P < 0.05$, Dunnet's One Way ANOVA

$\alpha_M\beta_2$ (Mac-1) Promiscuity

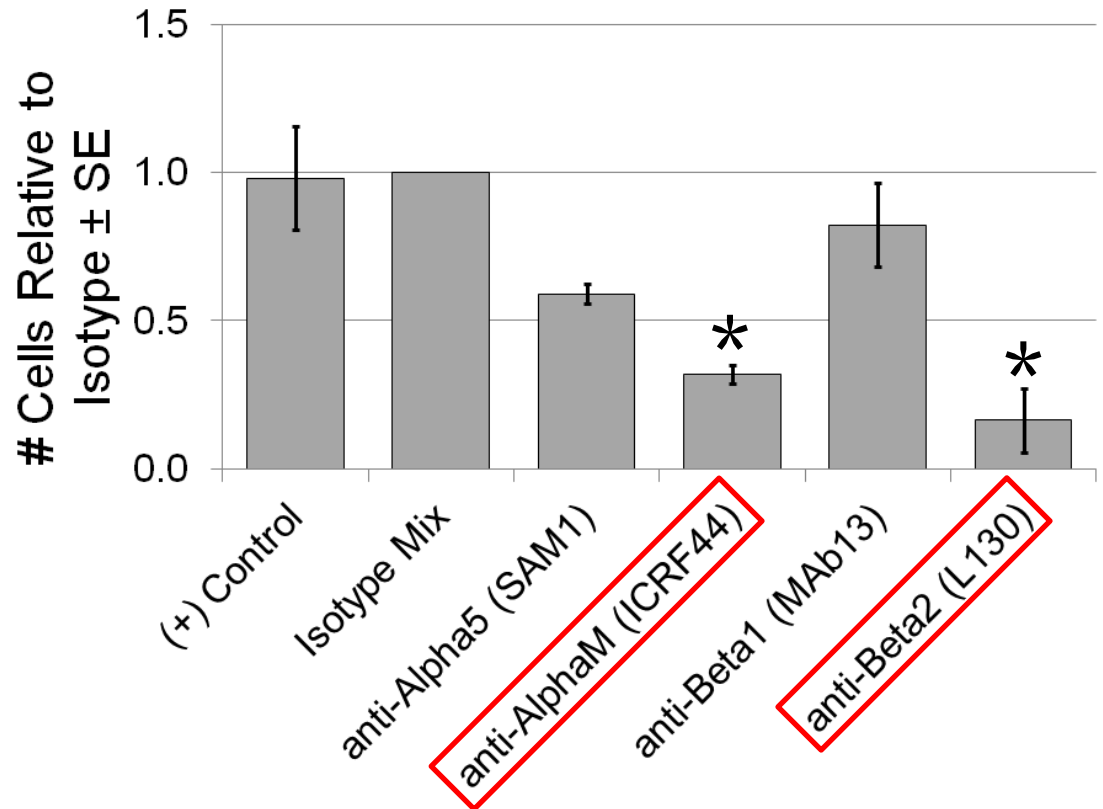
Keratocyte-like on **BSA**
(intermediate density)



Amoeboid on **BSA**
(high density)



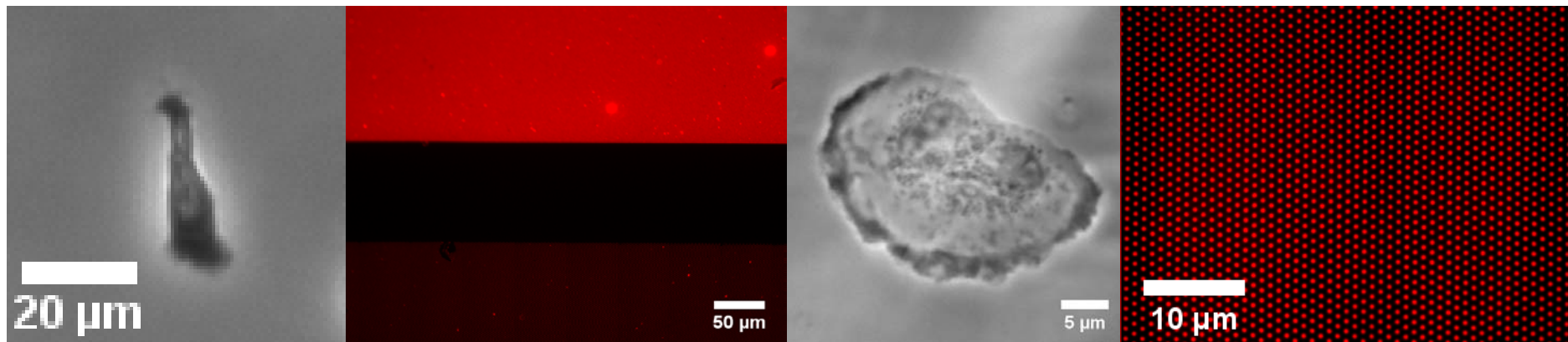
BSA adhesion also via Mac-1



* $P < 0.05$, Dunnet's One Way ANOVA

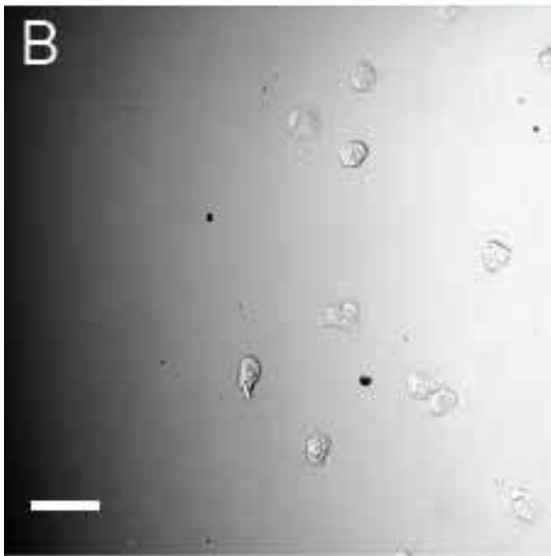
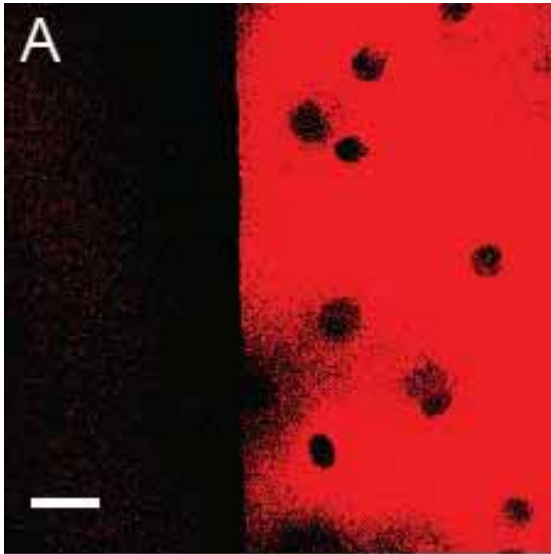
Conclusions

- One cell, two phenotypes
 - Amoeboid: Low Persistence, High Speed
 - Keratocyte-like: High Persistence, Low Speed
- Phenotype can be tuned by matrix density
 - Intermediate density \rightarrow Keratocyte-like
- Density sensing at cell length scale
- Adhesion is Mac-1 mediated

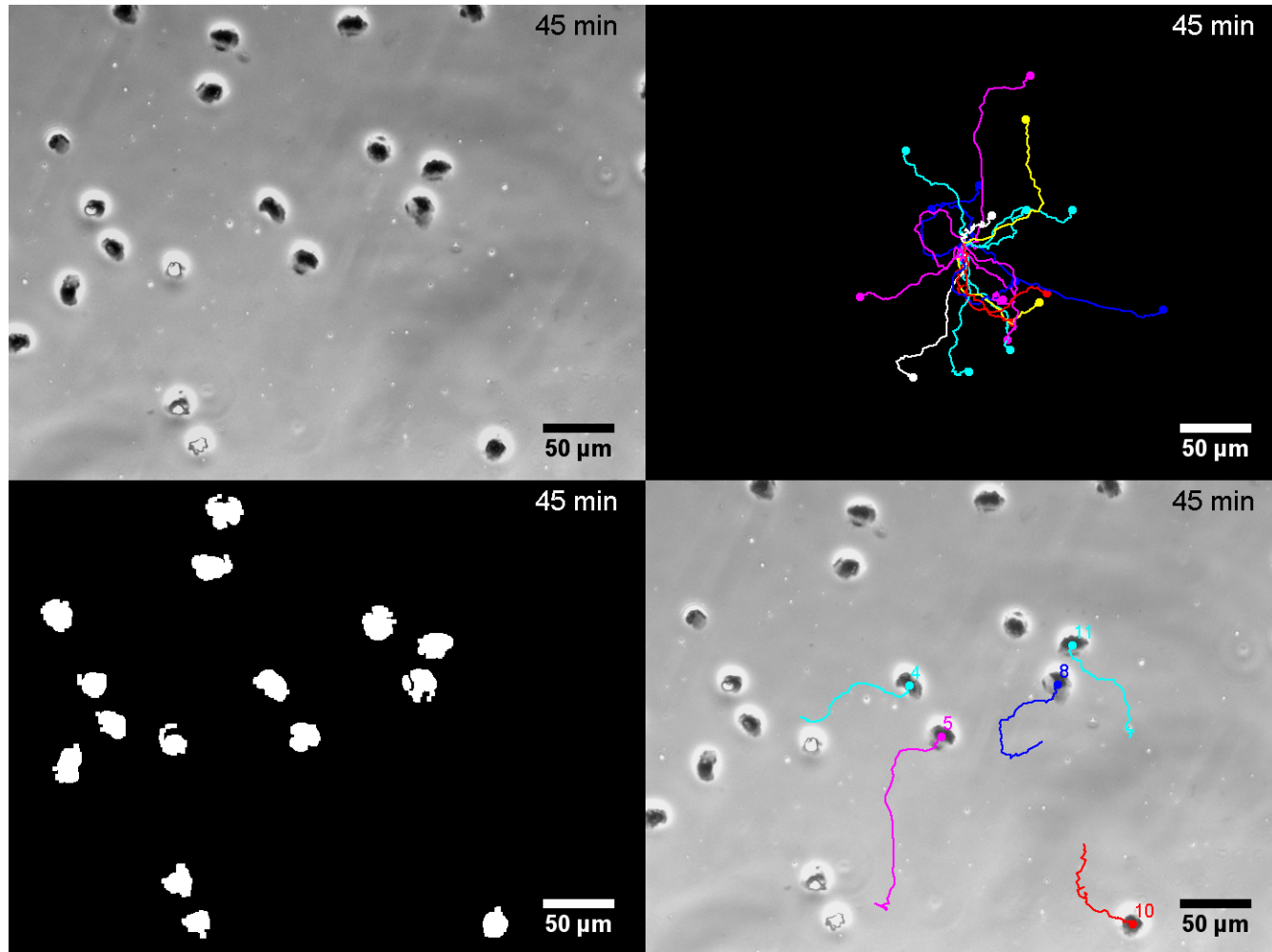


Prepared Supplemental Evidence
(Not Delivered in Talk)

Exquisite cell-ligand specificity



**Quantifying Motility



Segment



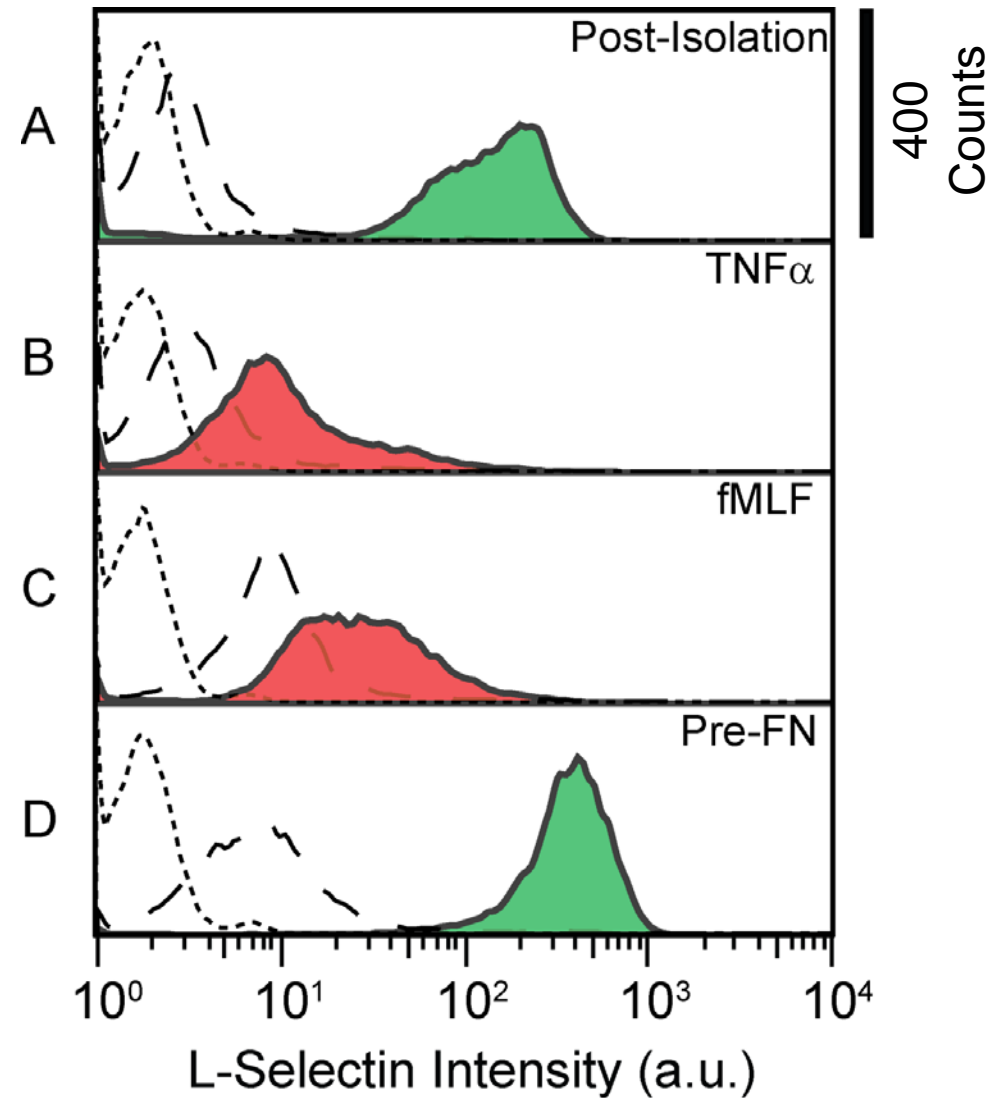
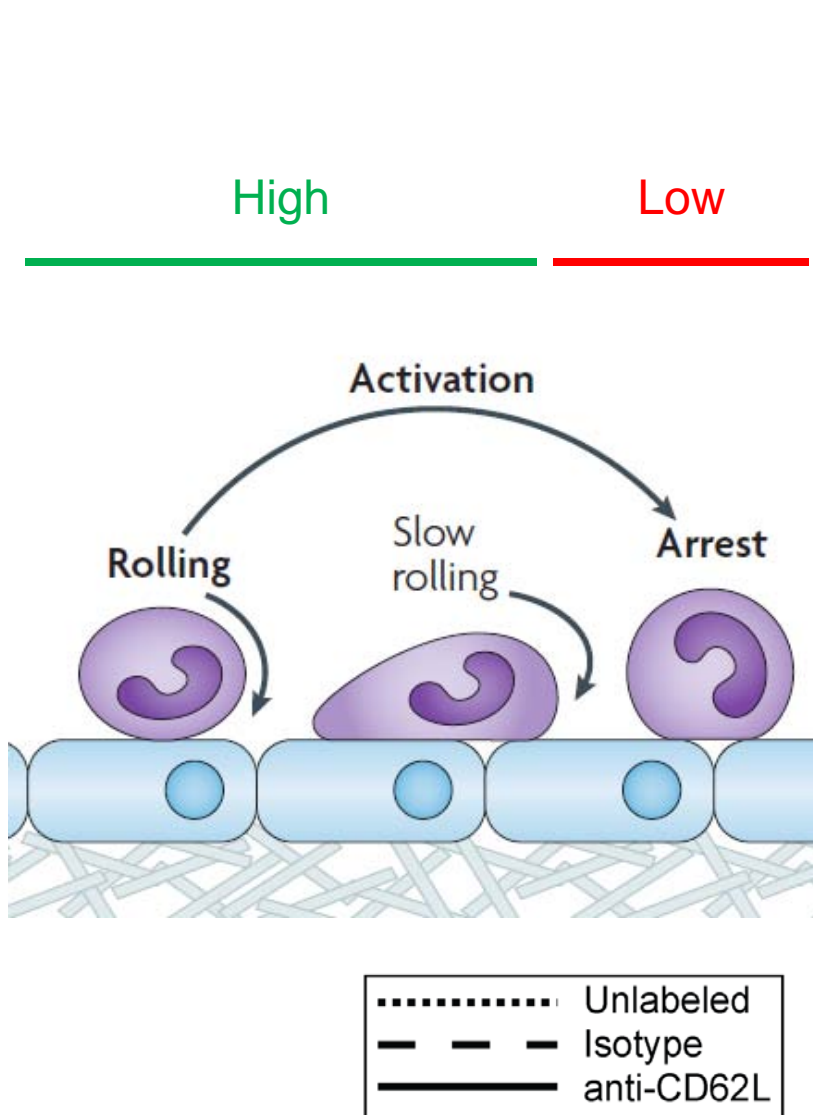
Merge



Link Centroids

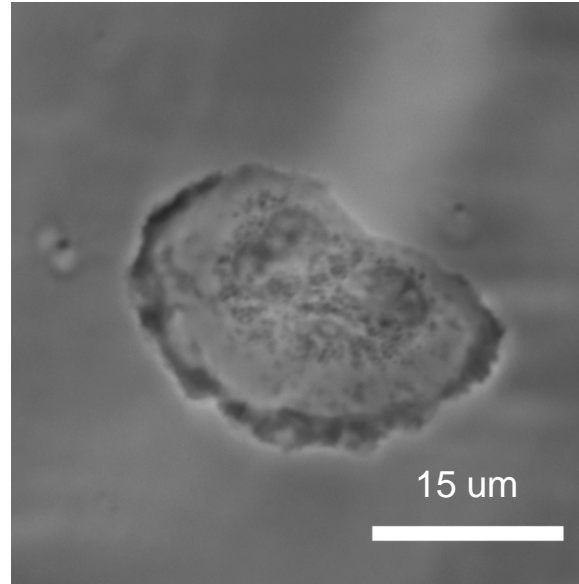


Selectin as "activation" marker



“Keratocyte-like” morphology

Neutrophil



Keratocyte

