Leukocyte Rolling

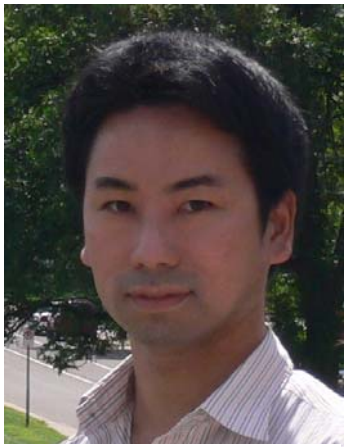
Klaus Ley

Rolling Lab Members

Current and Recent Lab Members

Elisa Ferrante

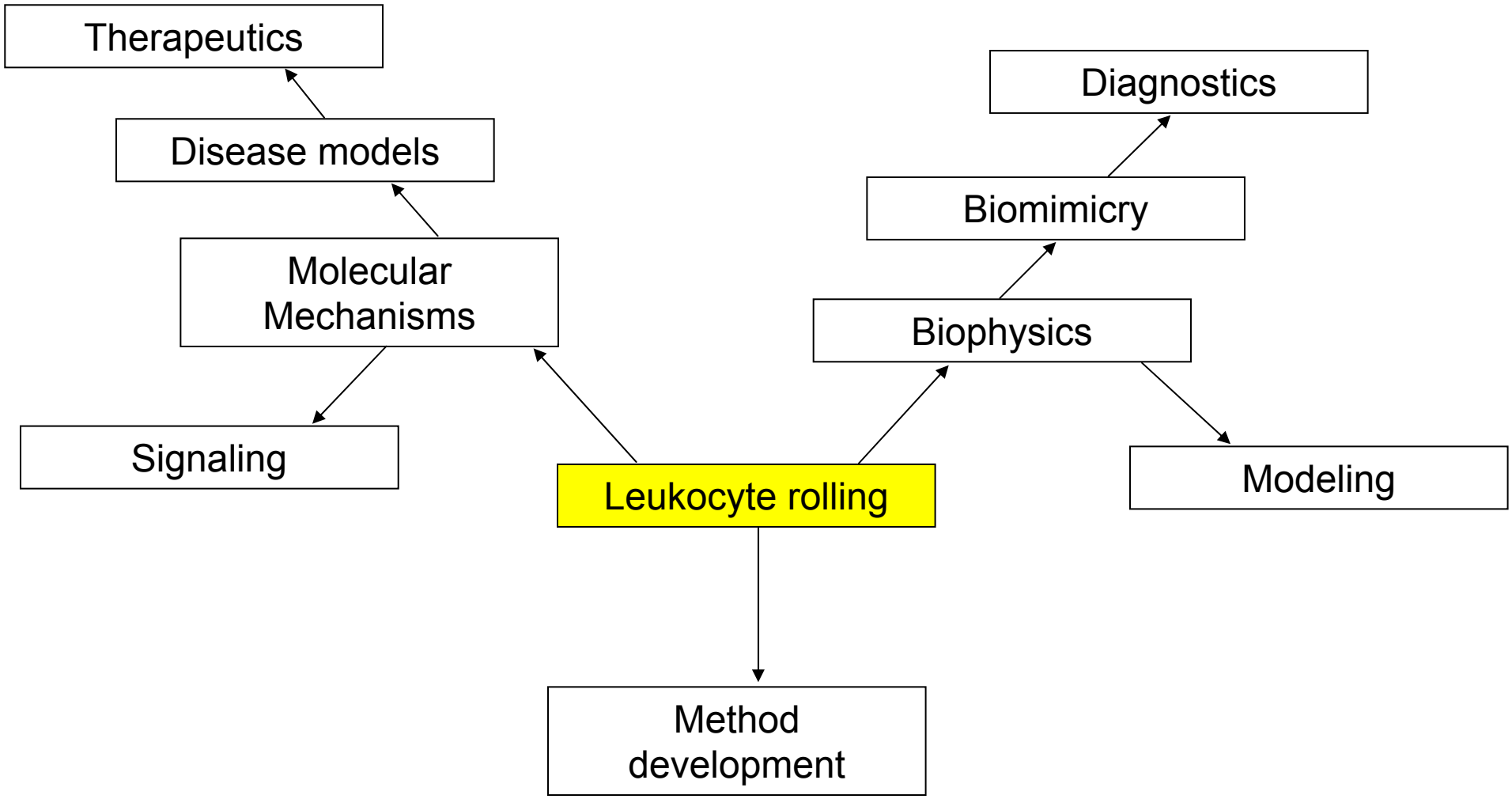
John Pickard



Yoshihiro Kuwano

Maria Pospieszalska

Prithu Sundd


Therapeutics
Disease models
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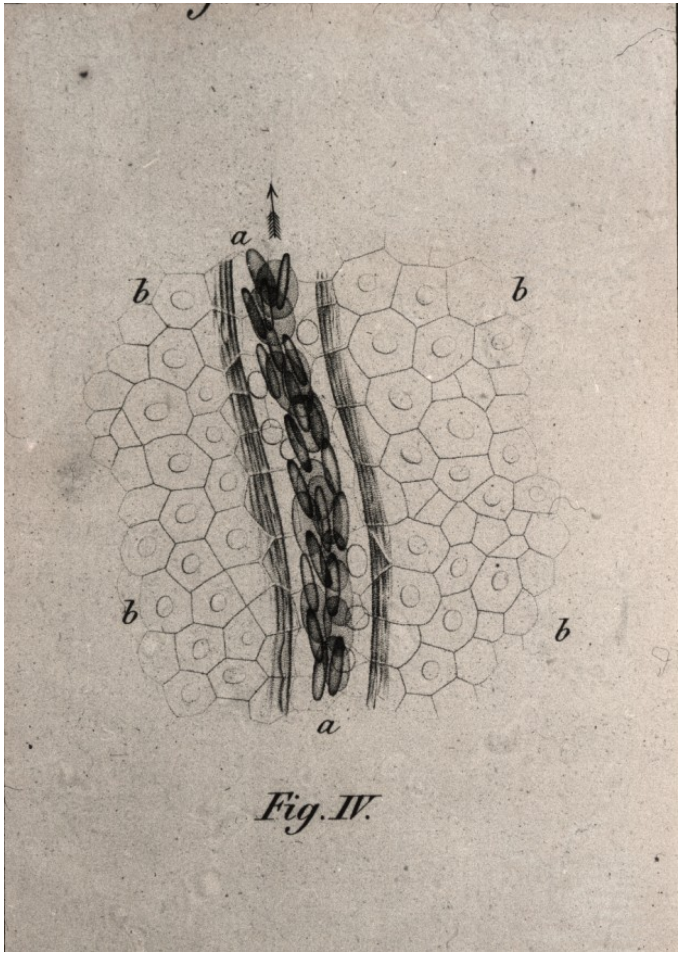
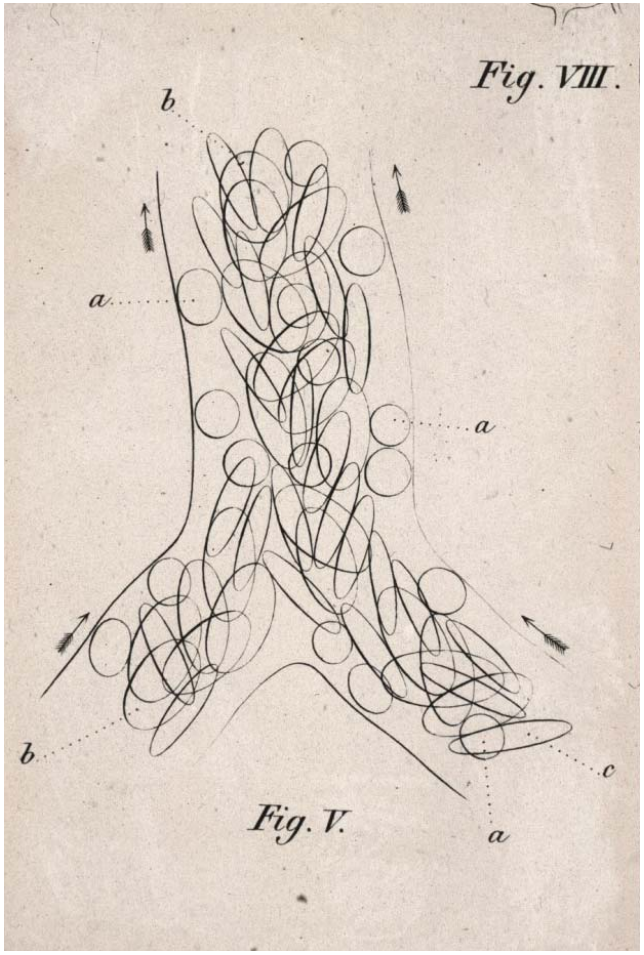
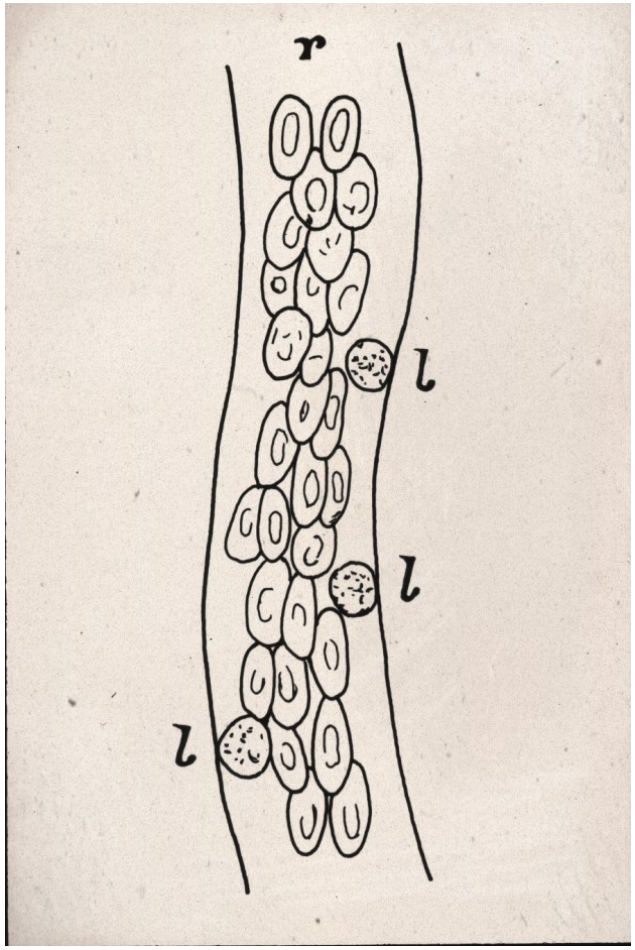
Leukocyte rolling
Method development
Diagnostics
Biomimicry
Biophysics
Modeling

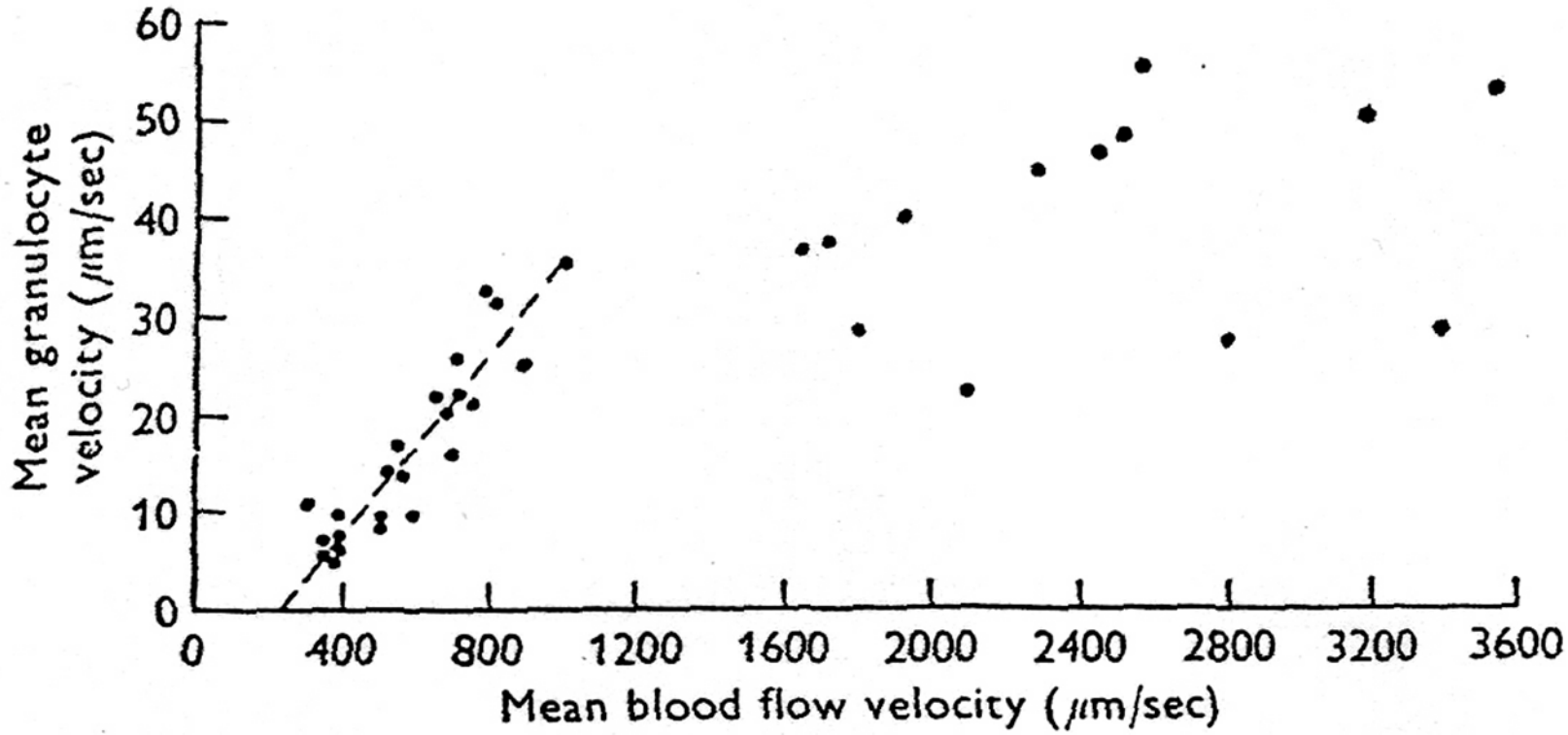






Rudolf Wagner 1835

Rudolf Virchow 1877



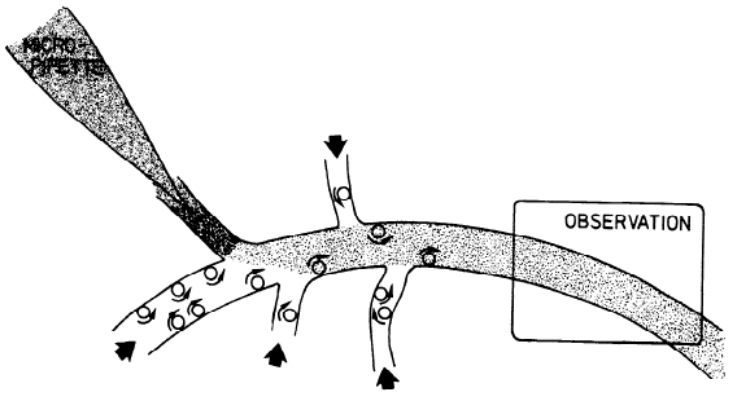
Atherton and Born, 1972

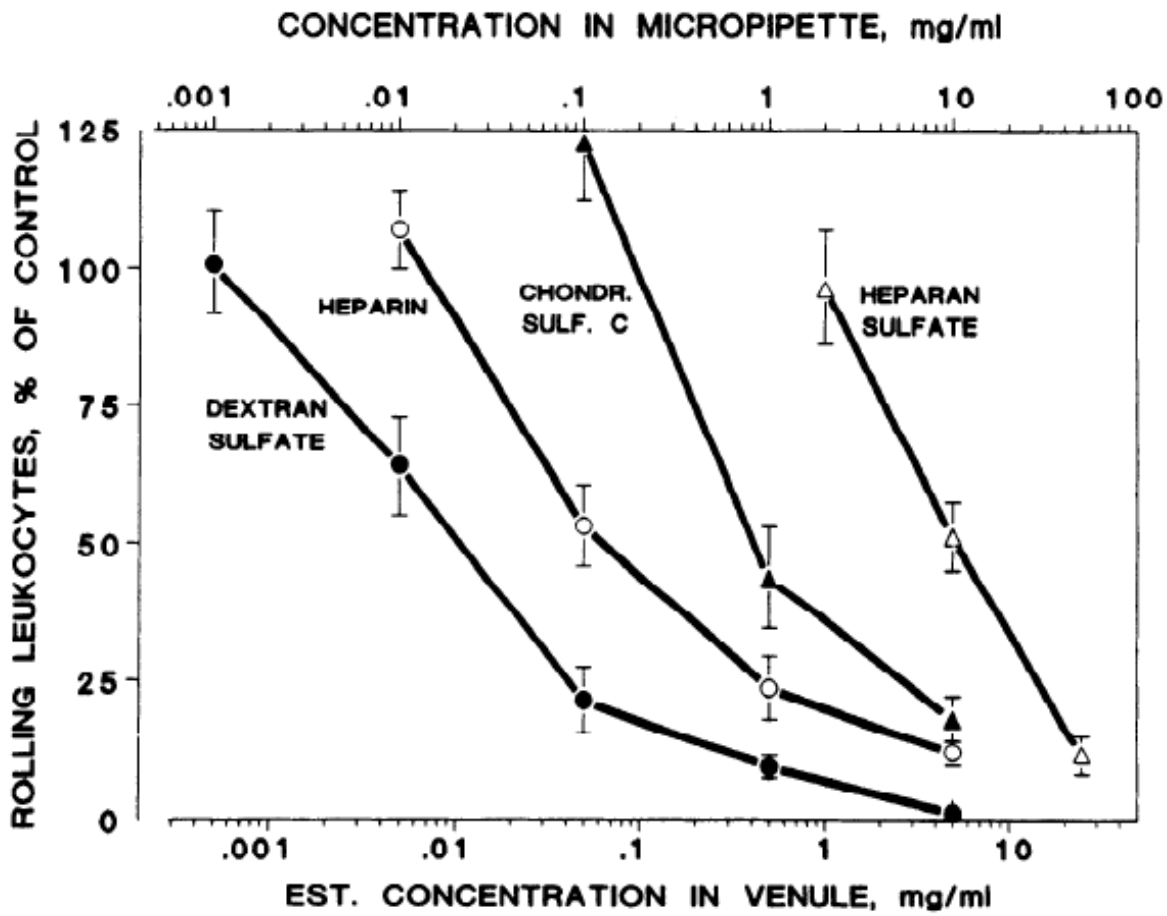
Sulfated polysaccharides inhibit leukocyte rolling in rabbit mesentery venules

KLAUS LEY, MICHELLE CERRITO, AND KARL-E. ARFORS
La Jolla Institute for Experimental Medicine, La Jolla 92037; and University of California San Diego, AMES-Bioengineering, La Jolla, California 92093

In view of this apparent difference, it is speculated that leukocyte integrins may not mediate leukocyte rolling. An interaction between a lectinlike leukocyte surface receptor and carbohydrate moieties on endothelial cells appears to be an attractive alternative working hypothesis.

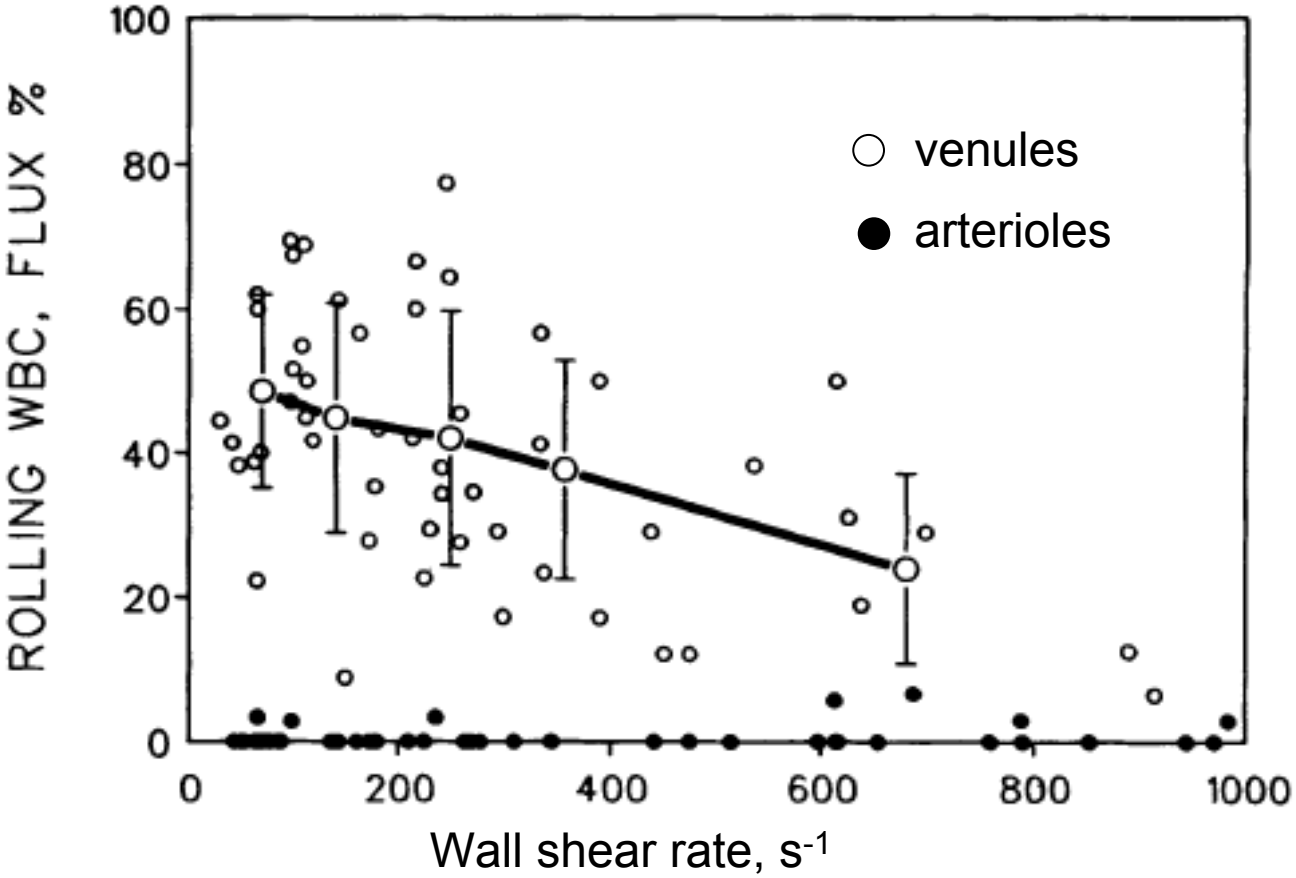
Am. J. Physiol. 260: H1667-H1673, 1991


MICRO-PIPETTE
OBSERVATION

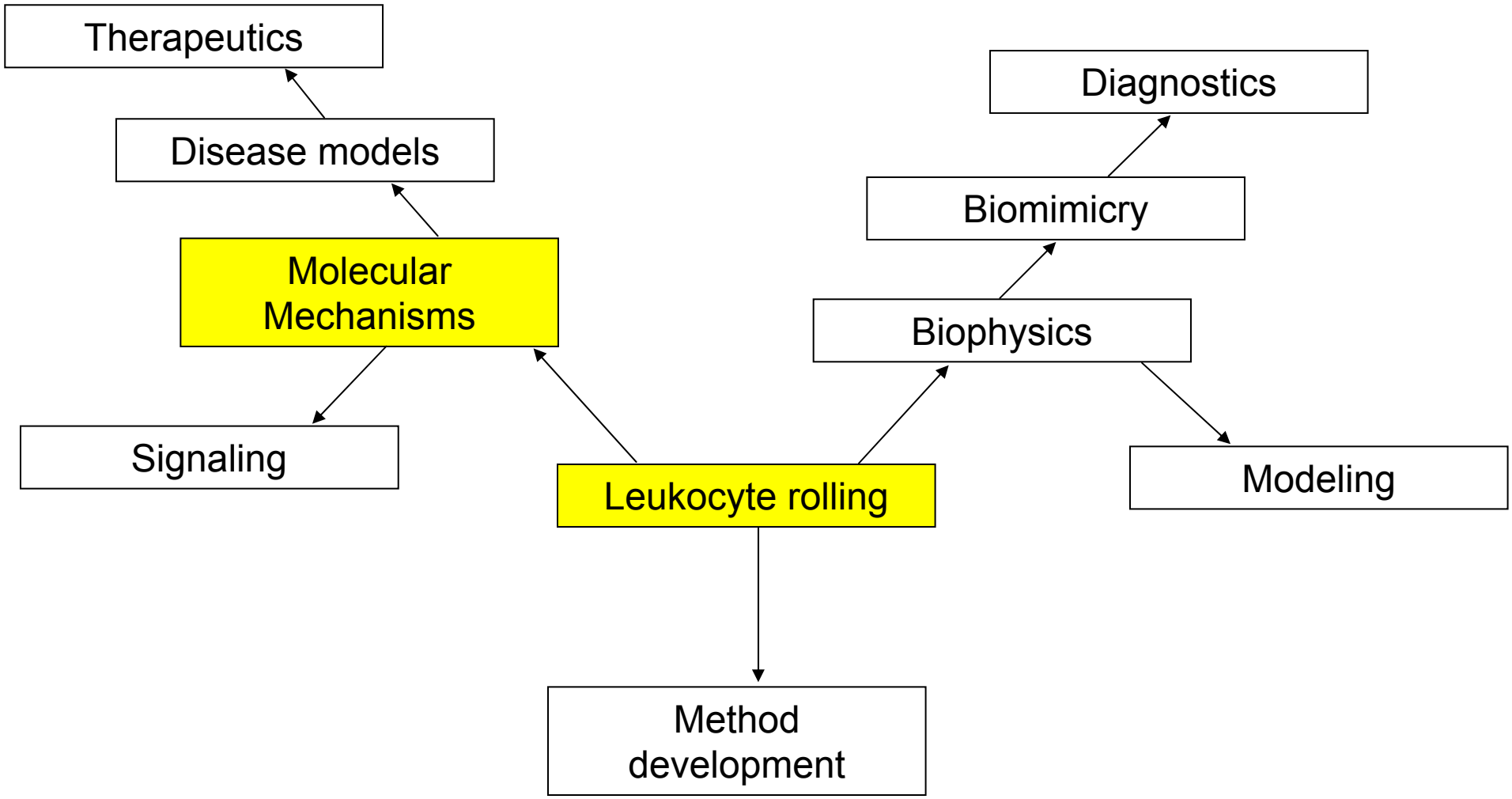

CONCENTRATION IN MICROPIPETTE, mg/ml
.001
.01
.1
1
10
100
ROLLING LEUKOCYTES, % OF CONTROL
125
100
75
50
25
0
HEPARIN
CHONDR. SULF. C
HEPARAN SULFATE
DEXTRAN SULFATE
.001
.01
.1
1
10
EST. CONCENTRATION IN VENULE, mg/ml

Endothelial, Not Hemodynamic, Differences Are Responsible for Preferential Leukocyte Rolling in Rat Mesenteric Venules

Klaus Ley and Peter Gaehtgens



Circ. Res. 69: 1034-1041, 1991


Therapeutics
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Biophysics
Biomimicry
Diagnostics
Modeling

RAPID COMMUNICATION

Lectin-Like Cell Adhesion Molecule 1 Mediates Leukocyte Rolling in Mesenteric Venules In Vivo

By Klaus Ley, Peter Gaehtgens, Christopher Fennie, Mark S. Singer, Laurence A. Lasky, and Steven D. Rosen

These findings indicate that LEC-CAM 1 mediates the adhesive interaction underlying leukocyte rolling and thus may play an important role in inflammation and in pathologic conditions involving leukocytes.

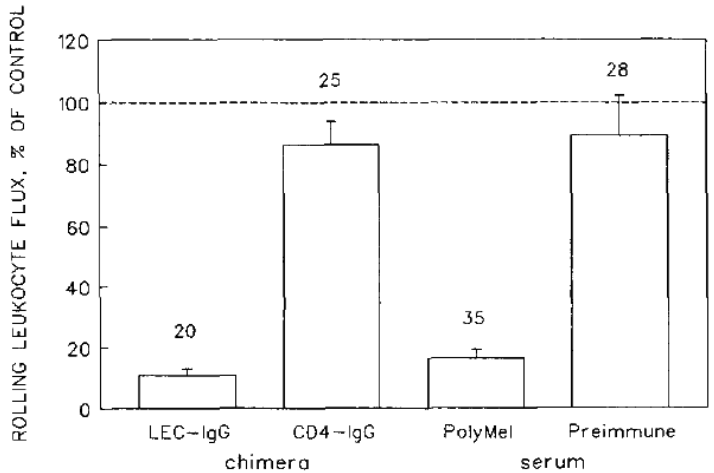
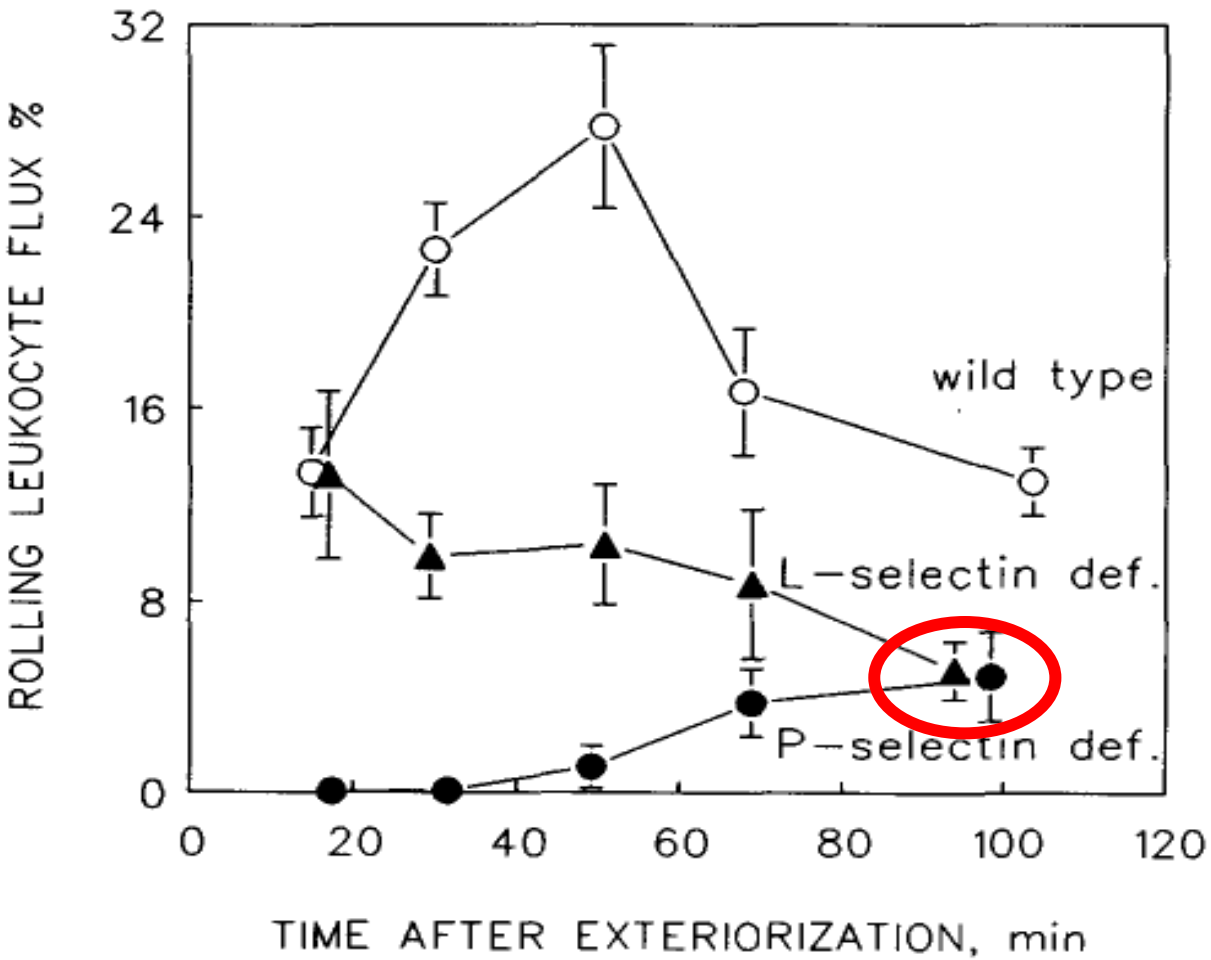


Fig. 1. Flux of rolling leukocytes during micro-infusion. The percent of control flux, mean ± SEM, and number of applications are indicated. LEC-IgG, recombinant chimera of murine LEC-CAM 1[23]; CD4-IgG, similar chimera of CD4[24], both 100 μg/mL; PolyMel, rabbit antimouse LEC-CAM 1 serum (1:10); preimmune, matching preimmune serum.

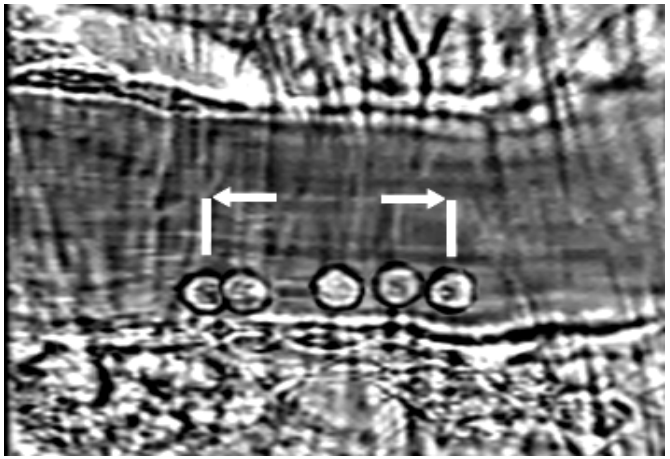
Blood 77: 2553-2555, 1991

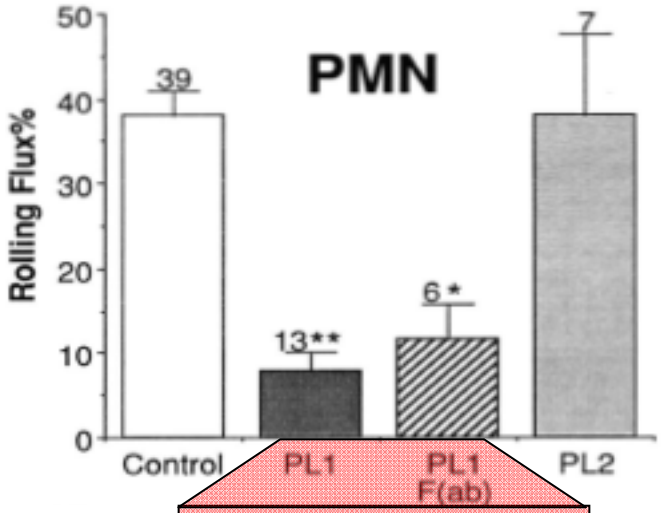
P- and L-selectin in leukocyte rolling



J. Exp. Med. 1995;
181: 669-675.

PSGL-1 in leukocyte rolling





PL1 blocks PSGL-1 binding to P- and L-selectin

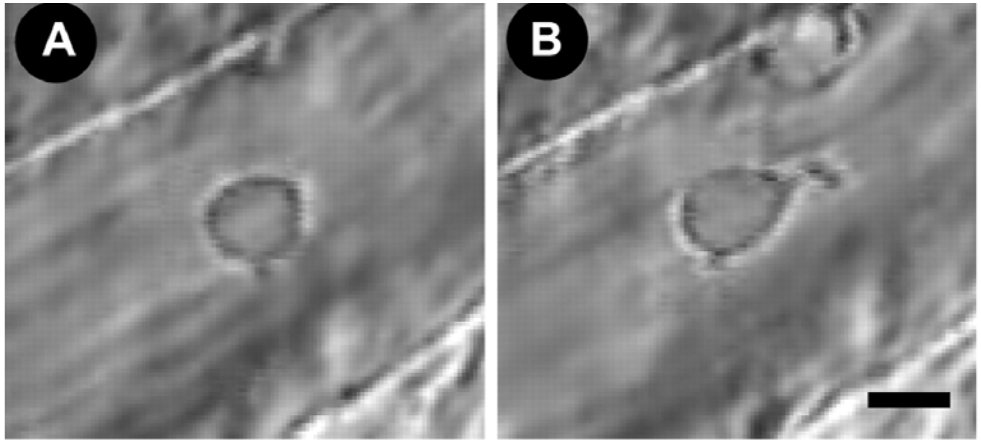
Norman, K.E. et al., Blood 1995; **86**: 4417-4421.

P-selectin Glycoprotein Ligand-1 Mediates L-Selectin–dependent Leukocyte Rolling in Venules

Markus Sperandio,[1,3] Michael L. Smith,[1] S. Bradley Forlow,[1] Timothy S. Olson,[2] Lijun Xia,[3] Rodger P. McEver,[3,4] and Klaus Ley[1,2]

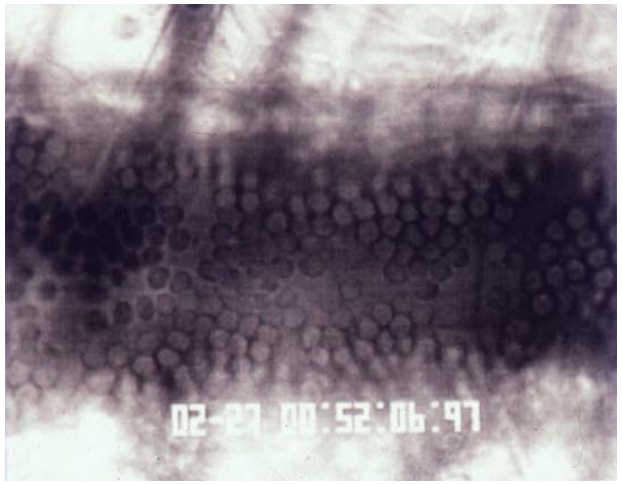
[1]*Department of Biomedical Engineering, and* [2]*Cardiovascular Research Center, University of Virginia, Charlottesville, VA 22908*

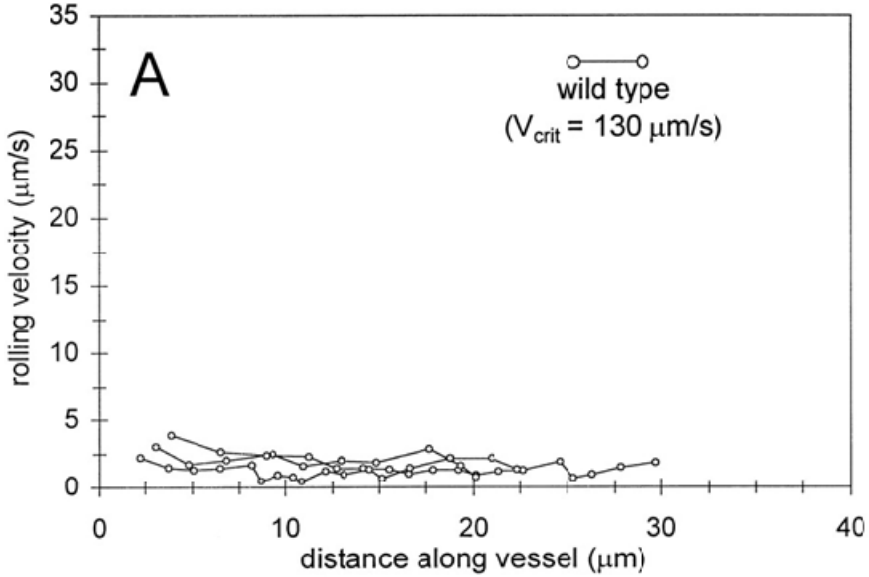
[3]*Cardiovascular Biology Research Program, Oklahoma Medical Research Foundation, and* [4]*Department of Biochemistry and Molecular Biology, and Oklahoma Center for Medical Glycobiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104*

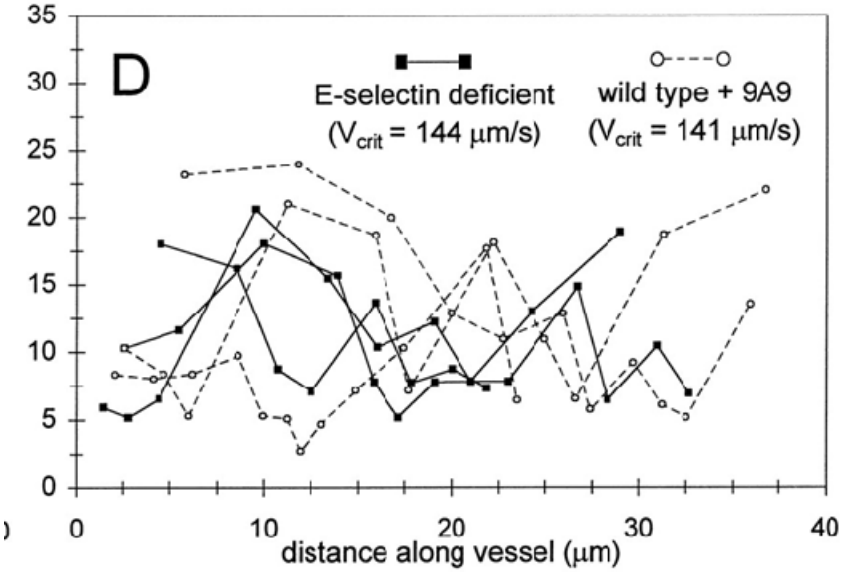


t=0 sec t=13 sec

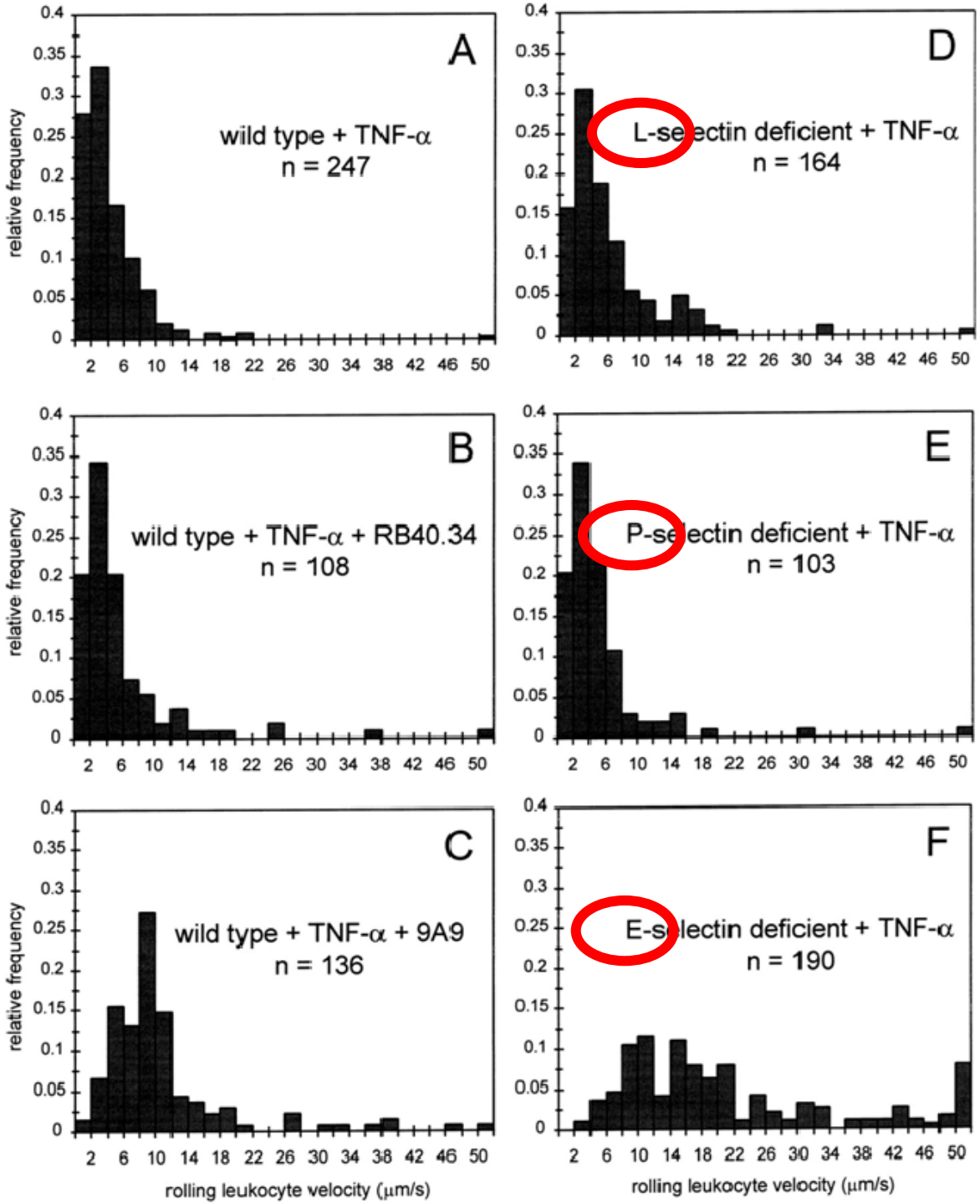
Sperandio, M., et al. 2003. *J. Exp. Med.* 197: 1355-1363.

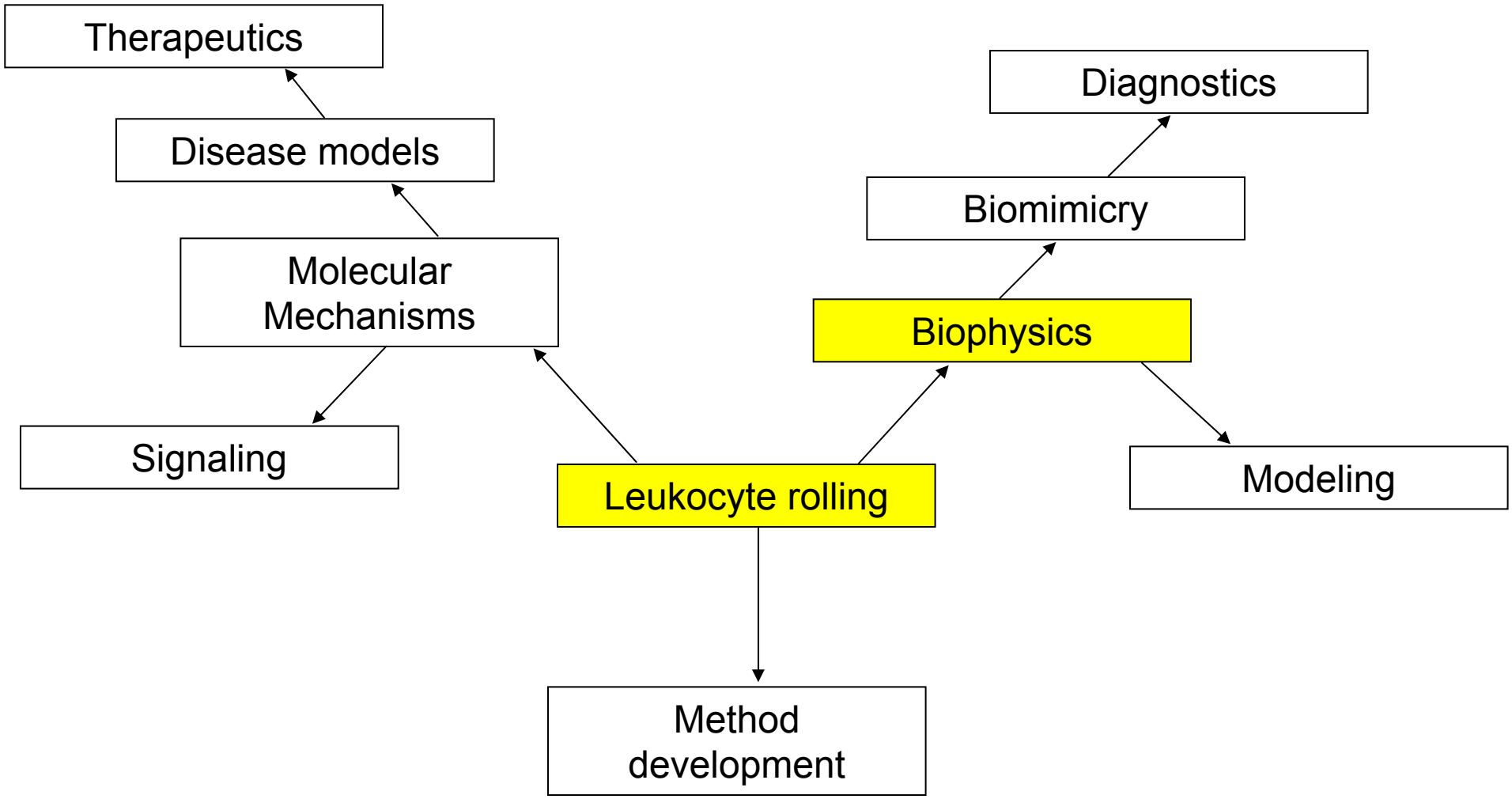






Kunkel, E. J., and K. Ley. 1996. Distinct phenotype of E-selectin deficient mice: E-selectin is required for slow leukocyte rolling in vivo. *Circ. Res.* 79: 1196-1204.


A
wild type + TNF-α
n = 247
B
wild type + TNF-α + RB40.34
n = 108
C
wild type + TNF-α + 9A9
n = 136
D
L-selectin deficient + TNF-α
n = 164
E
P-selectin deficient + TNF-α
n = 103
F
E-selectin deficient + TNF-α
n = 190
relative frequency
rolling leukocyte velocity (μm/s)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
2 6 10 14 18 22 26 30 34 38 42 46 50


Therapeutics
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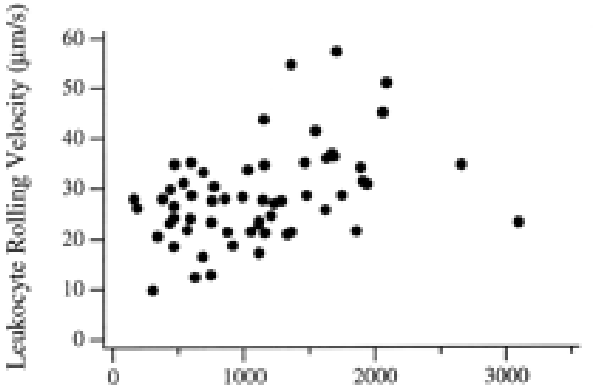
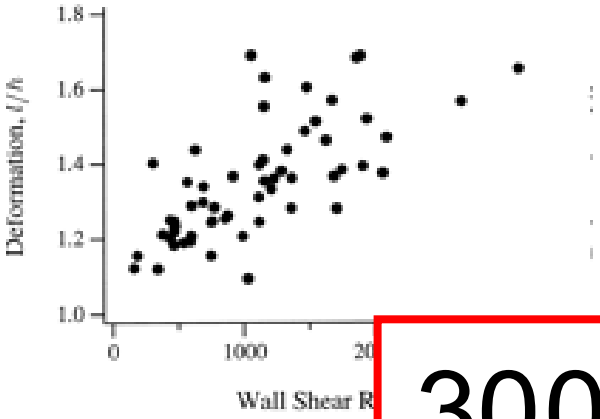
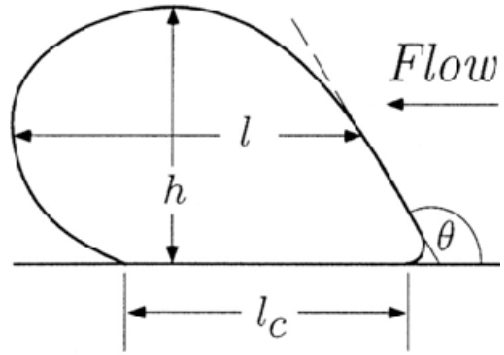
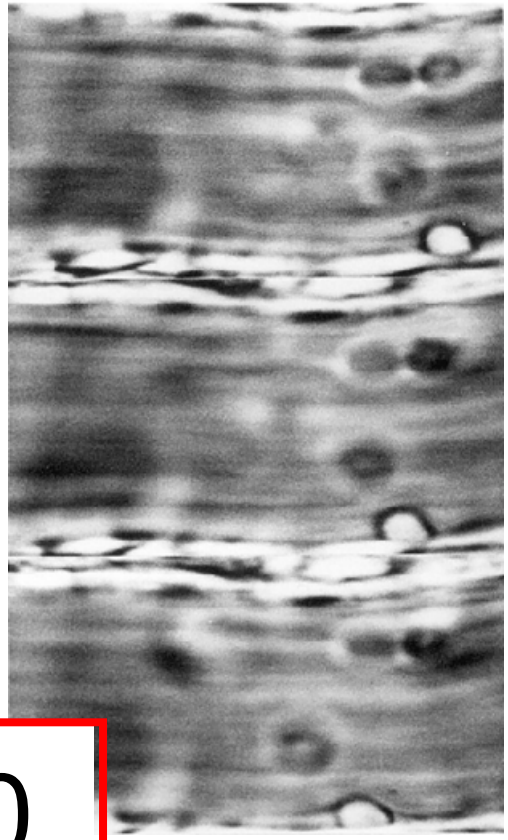
Diagnostics
Modeling


Leukocyte Rolling Velocity (μm/s)
60
50
40
30
20
10
0
0
1000
2000
3000


Deformation, l/h
1.8
1.6
1.4
1.2
1.0
0
1000
Wall Shear R


Flow
l
h
θ
l_c

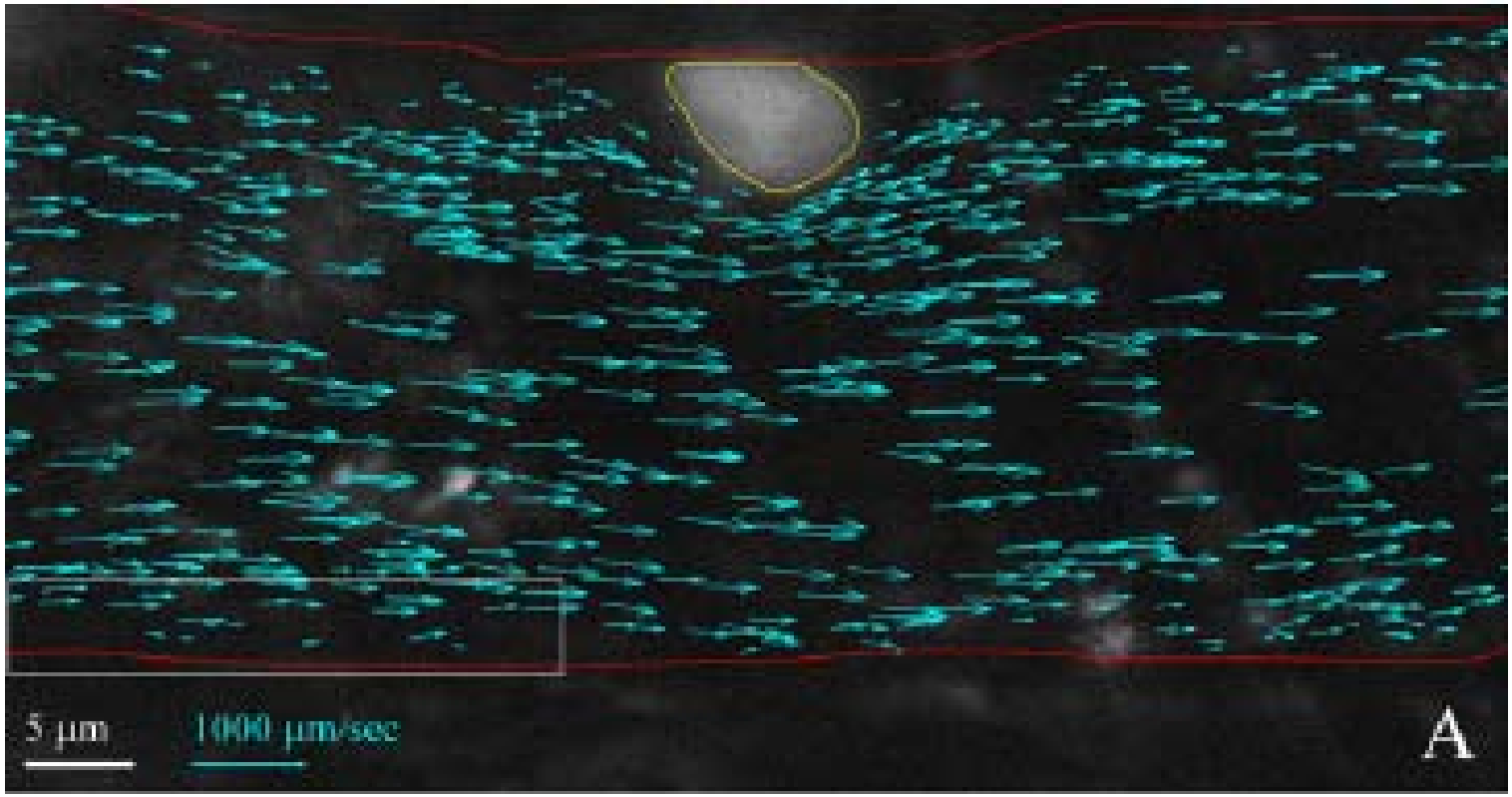


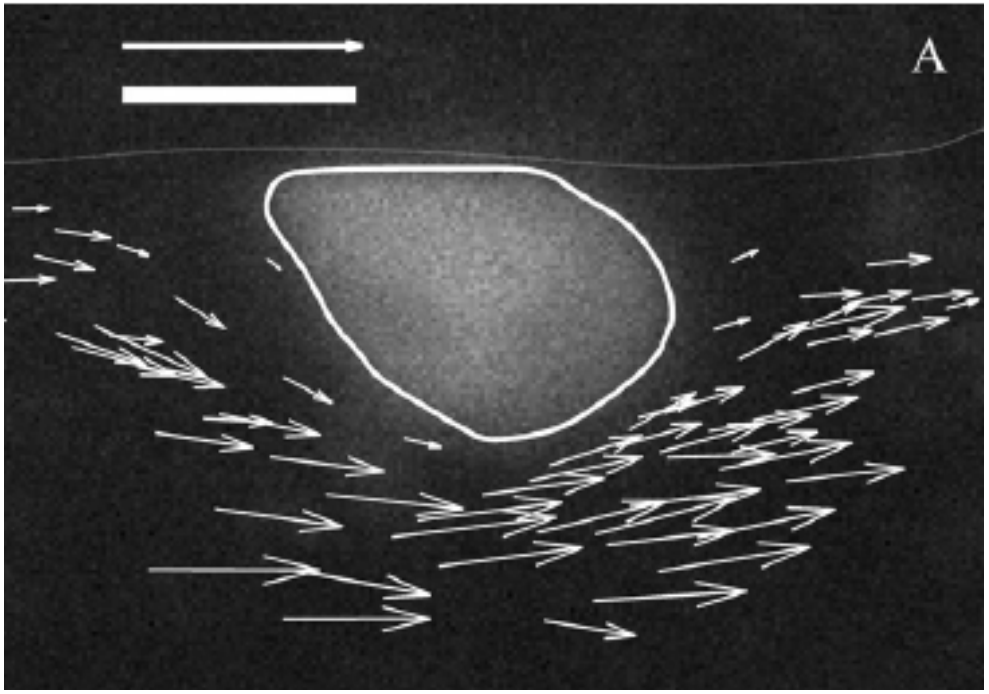
3000

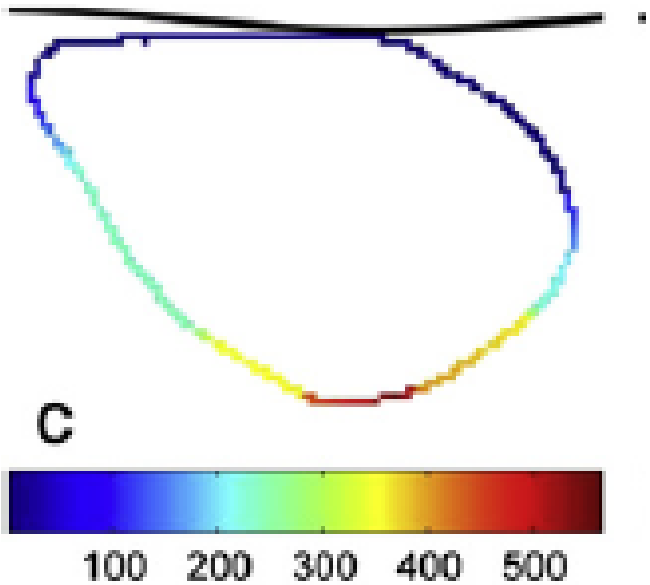
Biophysical Journal Volume 96 May 2009 4249–4259

Micro-PTV Measurement of the Fluid Shear Stress Acting on Adherent Leukocytes In Vivo

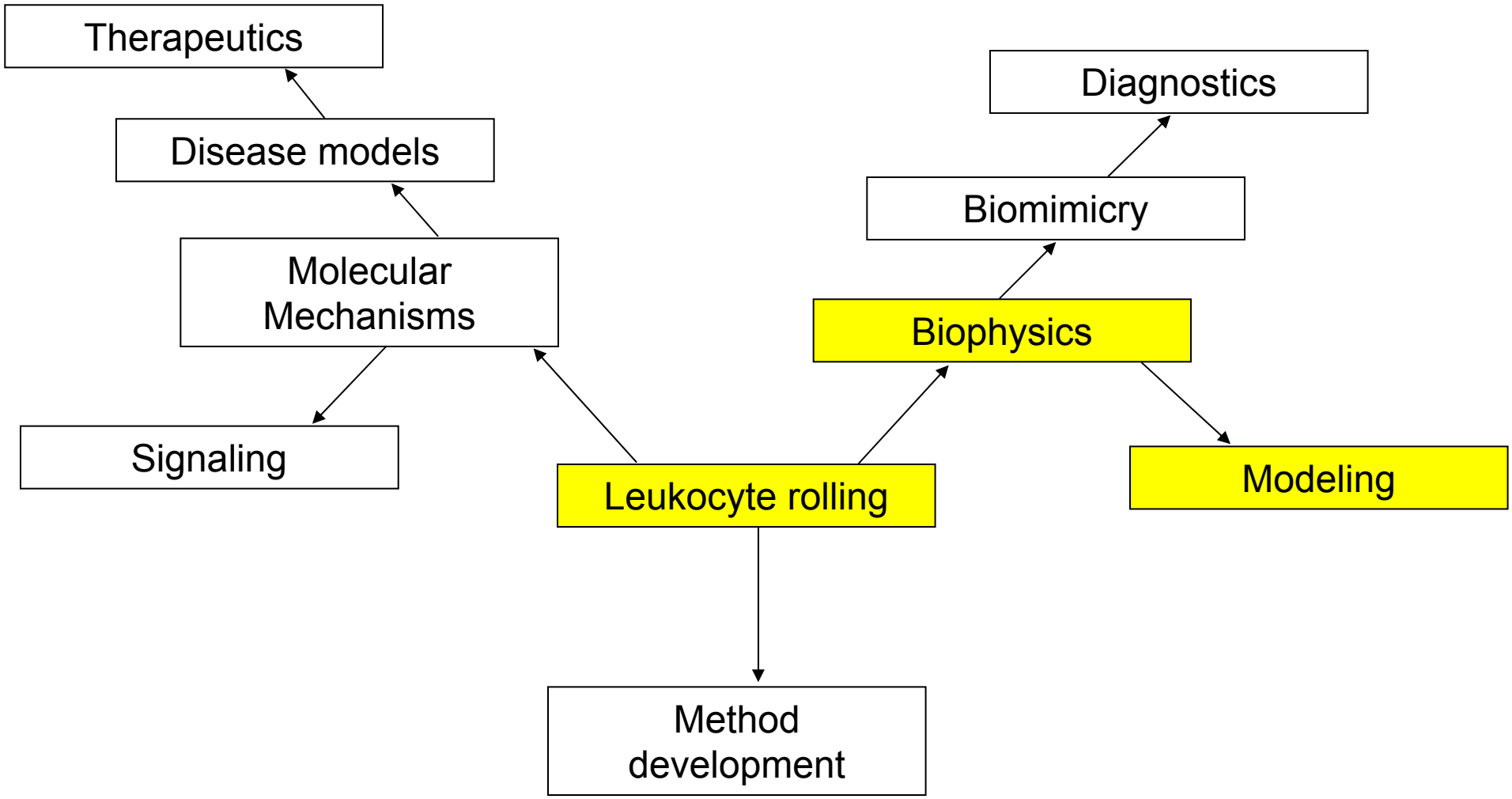
John E. Pickard[†] and Klaus Ley[†‡*]
[†]Department of Biomedical Engineering, University of Virginia, Charlottesville, Virginia; and [‡]La Jolla Institute for Allergy and Immunology, La Jolla, California







Wall shear rate, s^{-1}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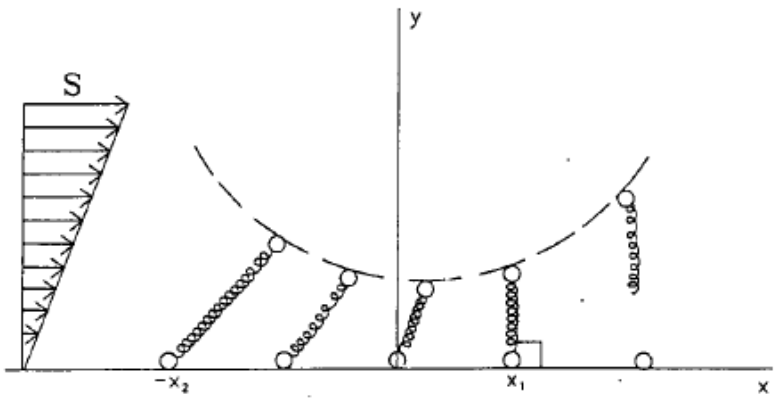
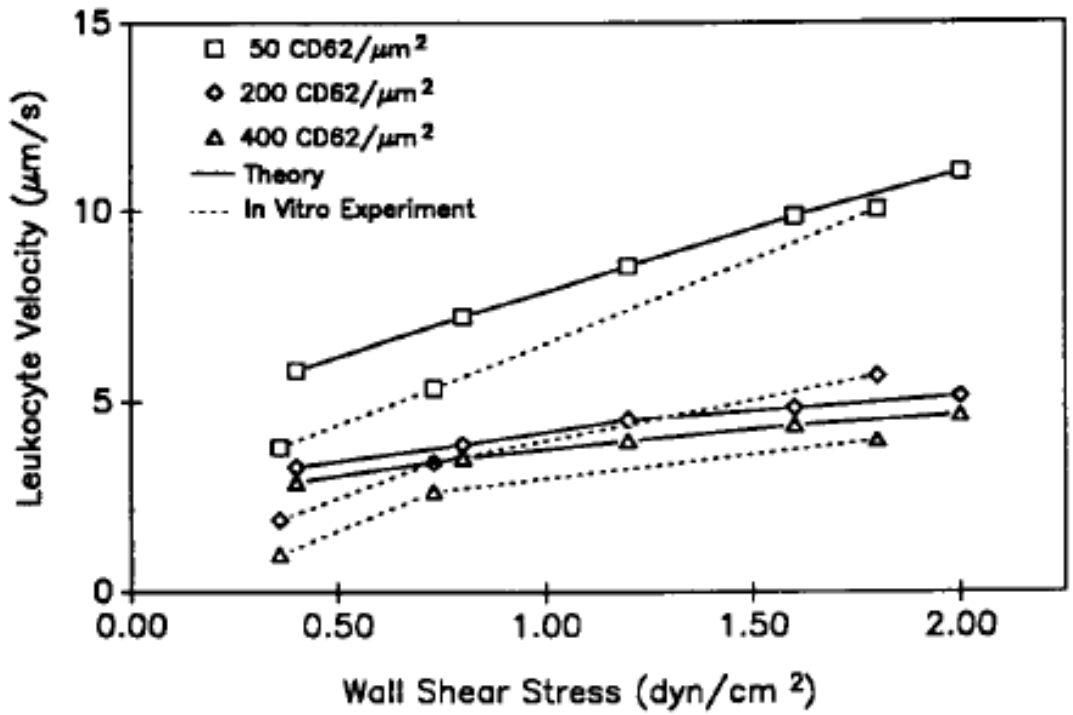
Diagnostics
Modeling

How do selectins mediate leukocyte rolling in venules?

Aydin Tözeren* and Klaus Ley‡
*Department of Mechanical Engineering, The Catholic University of America, Washington, D.C. 20064 USA; and
‡The Institut für Physiologie, Freie Universität Berlin, Berlin 33, Germany





Biophys. J. 63:
700-709, 1992

Microcirculation, 16: 115–130, 2009

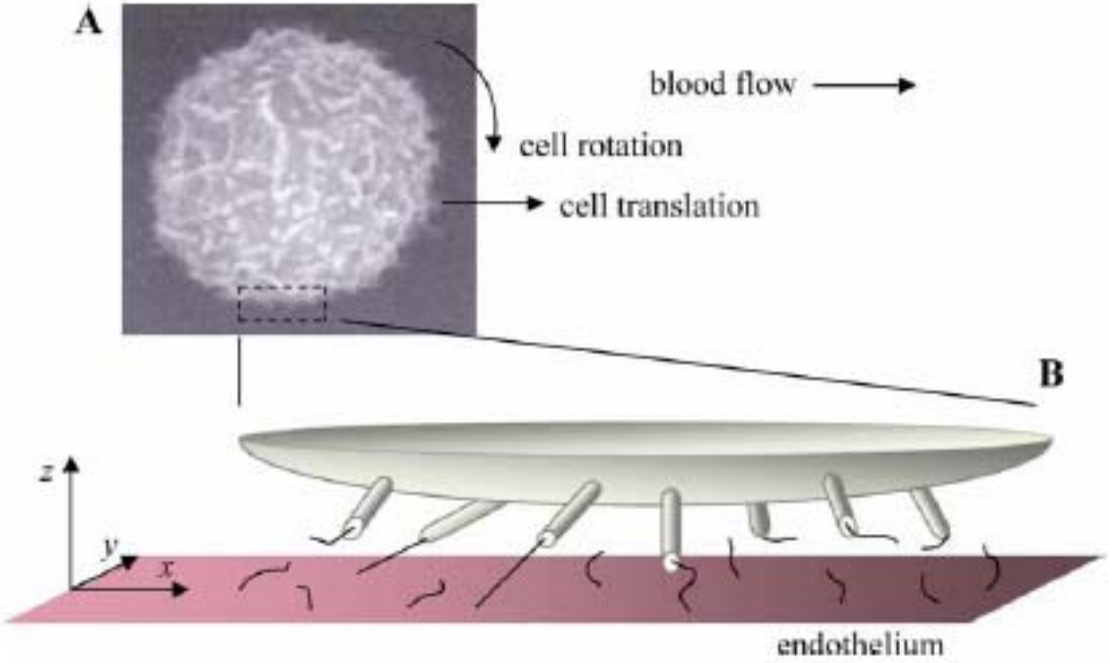
ISSN: 1073-9688 print / 1549-8719 online
DOI: 10.1080/10739680802462792

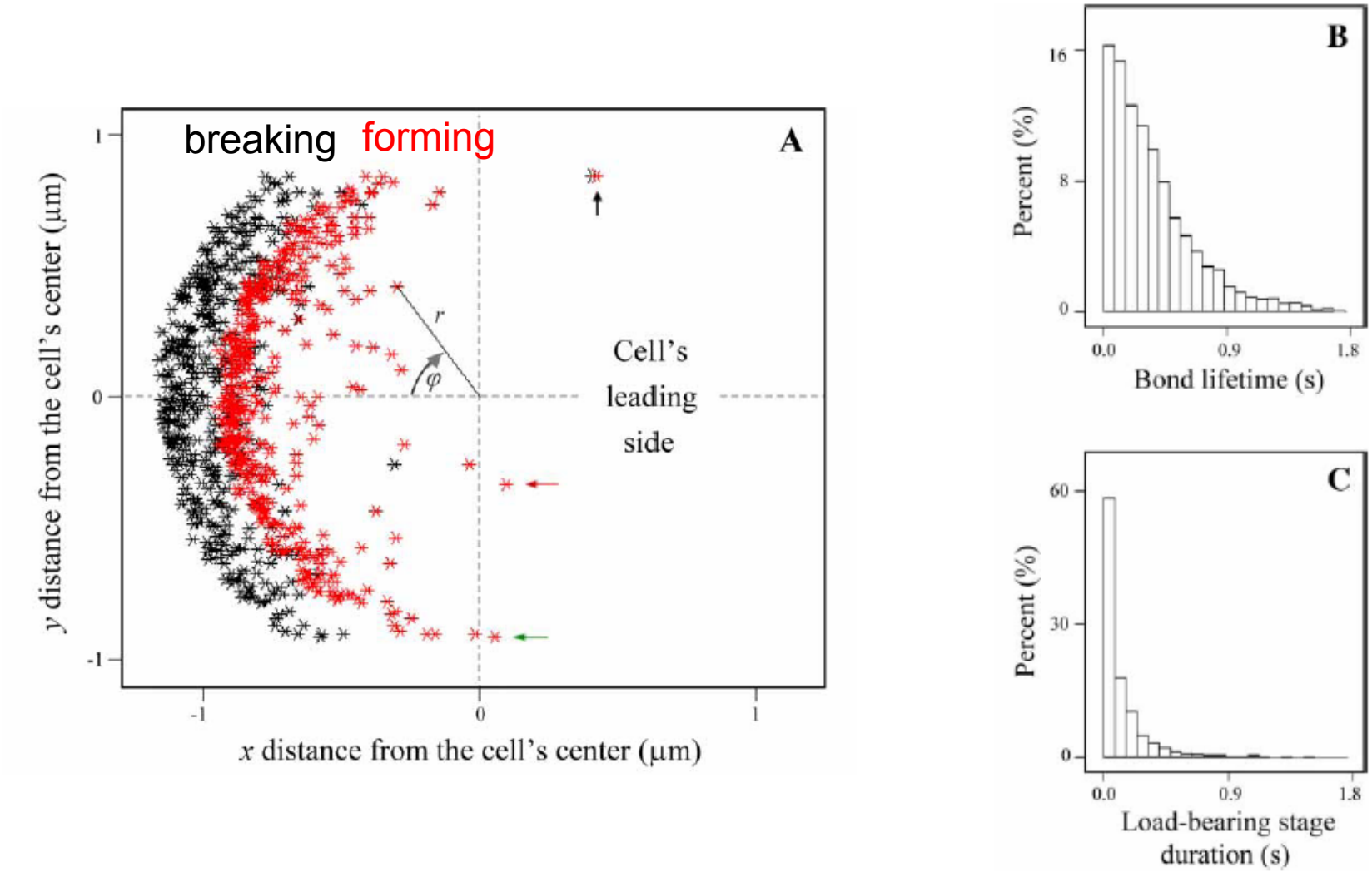
informa
healthcare

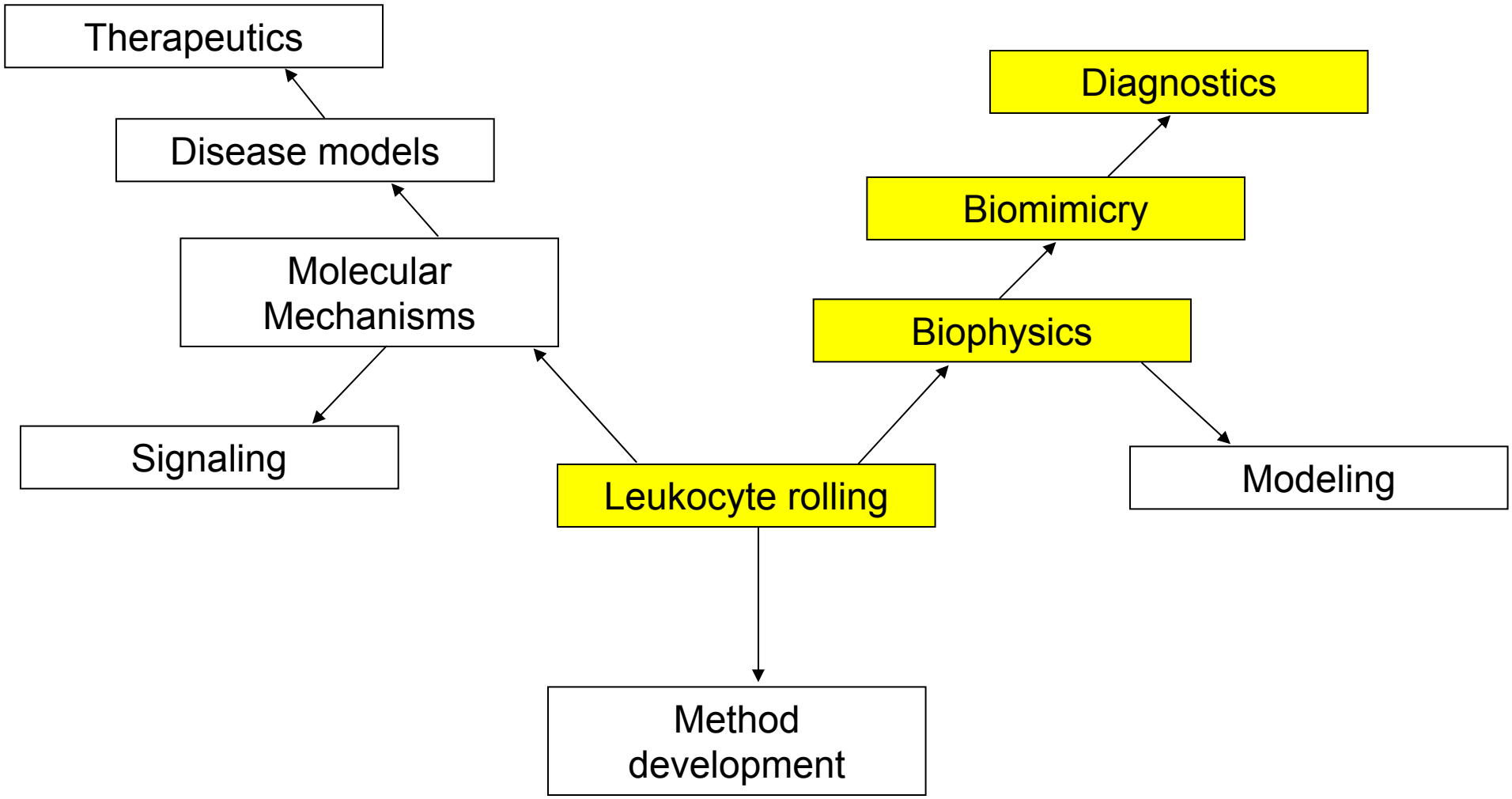
Event-Tracking Model of Adhesion Identifies Load-Bearing Bonds in Rolling Leukocytes

MARIA K. POSPIESZALSKA,* ALEXANDER ZARBOCK,*,† JOHN E. PICKARD,‡ AND KLAUS LEY*

*Division of Inflammation Biology, La Jolla Institute for Allergy and Immunology, La Jolla, California, USA; †Department of Anesthesiology and Intensive Care Medicine, University of Münster, Münster, Germany; ‡Robert M. Berne Cardiovascular Research Center and Department of Biomedical Engineering, University of Virginia, Charlottesville, Virginia, USA




breaking
forming
A
Cell's
leading
side
r
φ
y distance from the cell's center (μm)
x distance from the cell's center (μm)
1
0
-1
-1
0
1
B
Percent (%)
16
8
0
0.0
0.9
1.8
Bond lifetime (s)
C
Percent (%)
60
30
0
0.0
0.9
1.8
Load-bearing stage
duration (s)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Journal of Controlled Release
Volume 114, Issue 3, 12 September 2006, Pages 288-299

doi:10.1016/j.jconrel.2006.06.008 | How to Cite or Link Using DOI

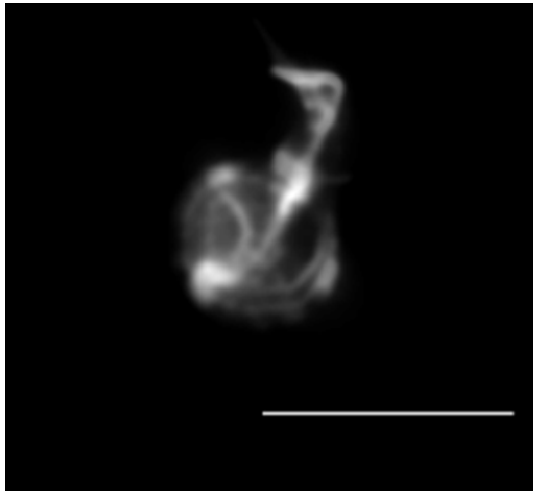
Deformable gas-filled microbubbles targeted to P-selectin

[a]Cardiovascular Division, University of Virginia, Charlottesville, VA 22908-0158, USA

[b]Department of Biomedical Engineering, University of Virginia, Charlottesville, VA 22908-0158, USA

[c]Cardiovascular Research Center, University of Virginia, Charlottesville, VA 22908-0158, USA

Leukocyte



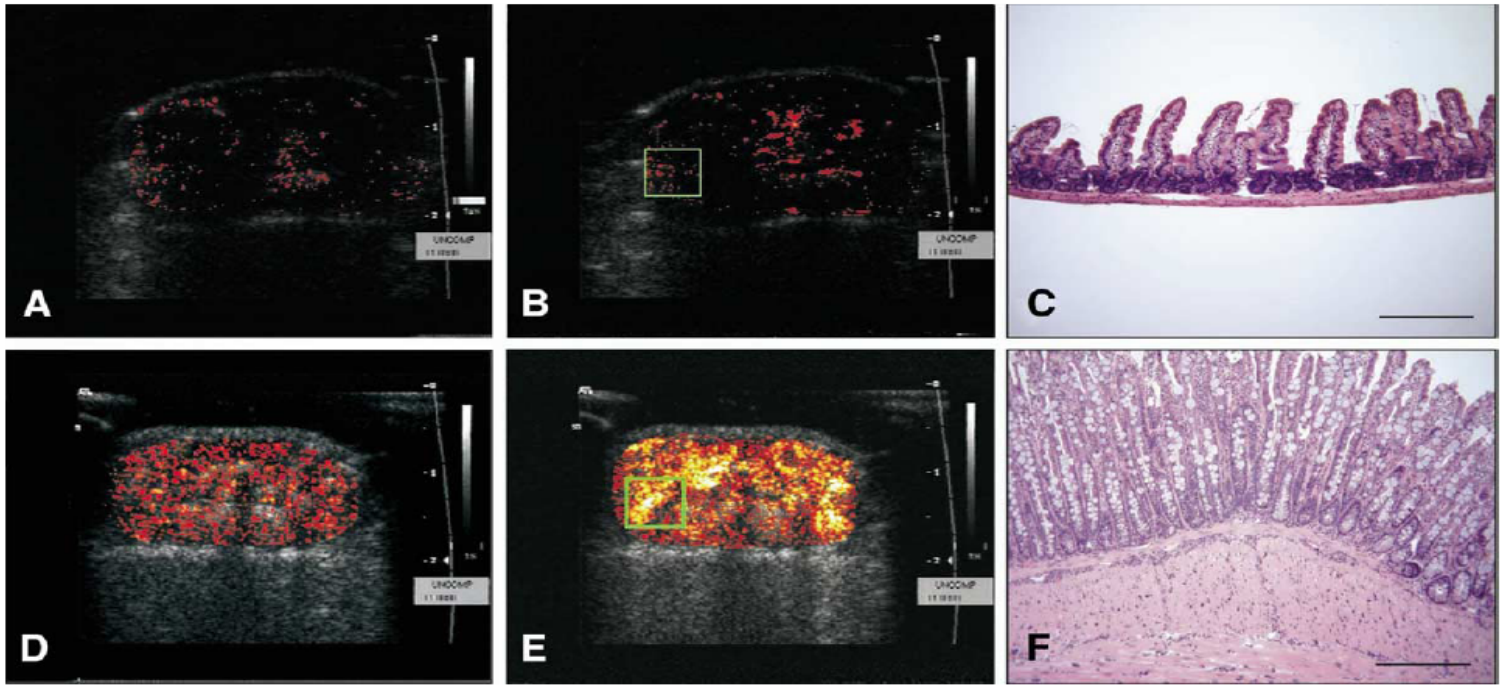
Ultrasound Microbubble

Molecular Ultrasound Imaging of IBD

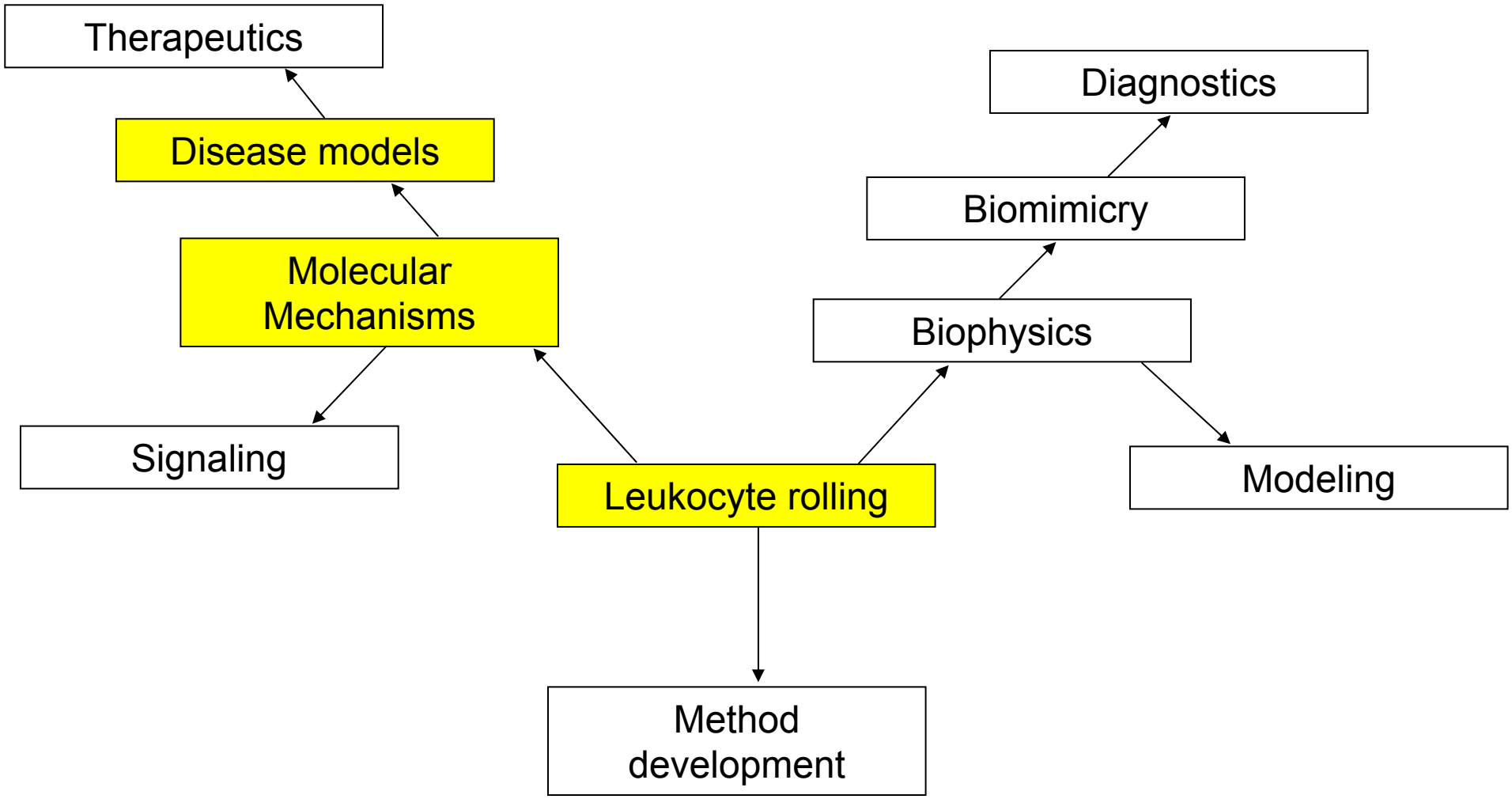
isotype MAdCAM-1 histology

control

ileitis



Bachmann, C. et al.,
Gastroenterology 2006; **130**: 8-16.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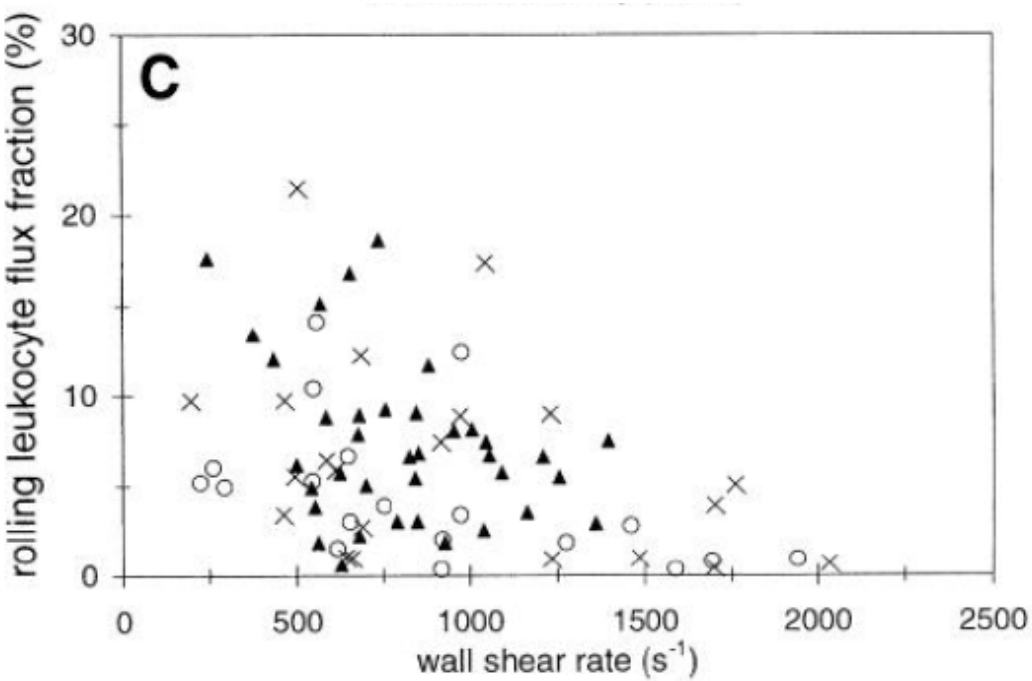
TNF-α induces selectin-mediated leukocyte rolling in mouse cremaster muscle arterioles

ERIC J. KUNKEL, UNSU JUNG, AND KLAUS LEY
Department of Biomedical Engineering, University of Virginia School of Medicine, Charlottesville, Virginia 22908

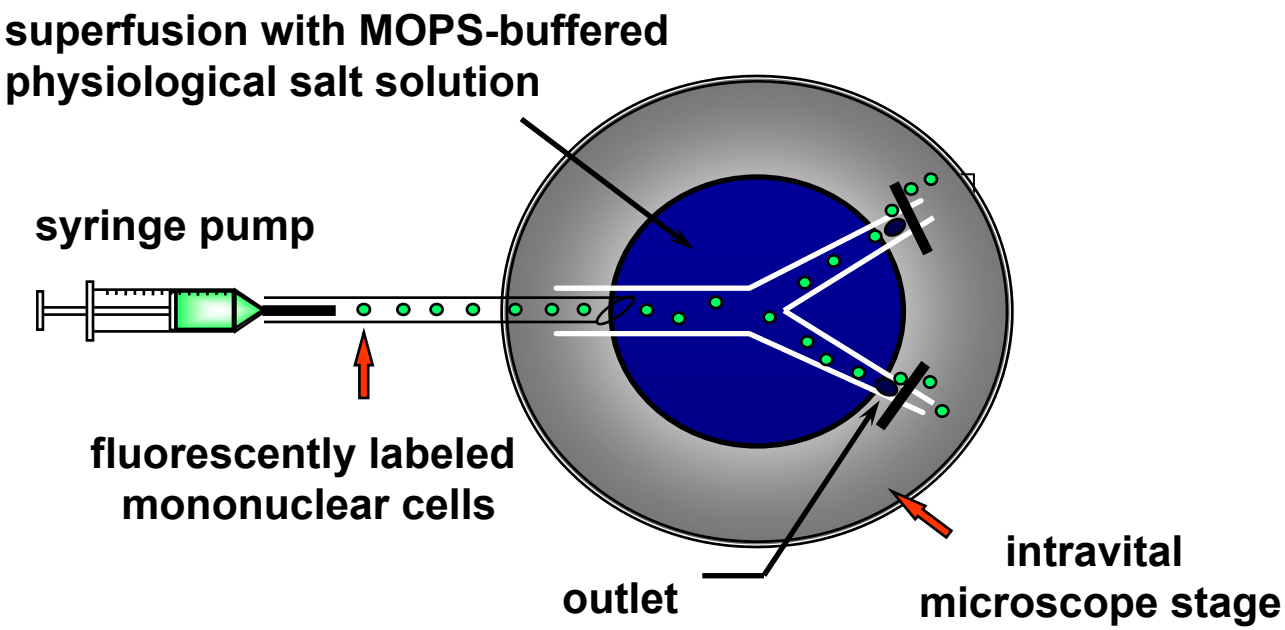


Kunkel, E. J et al. 1997. *Am. J. Physiol.* 272: H1391-H1400.

Original Contributions

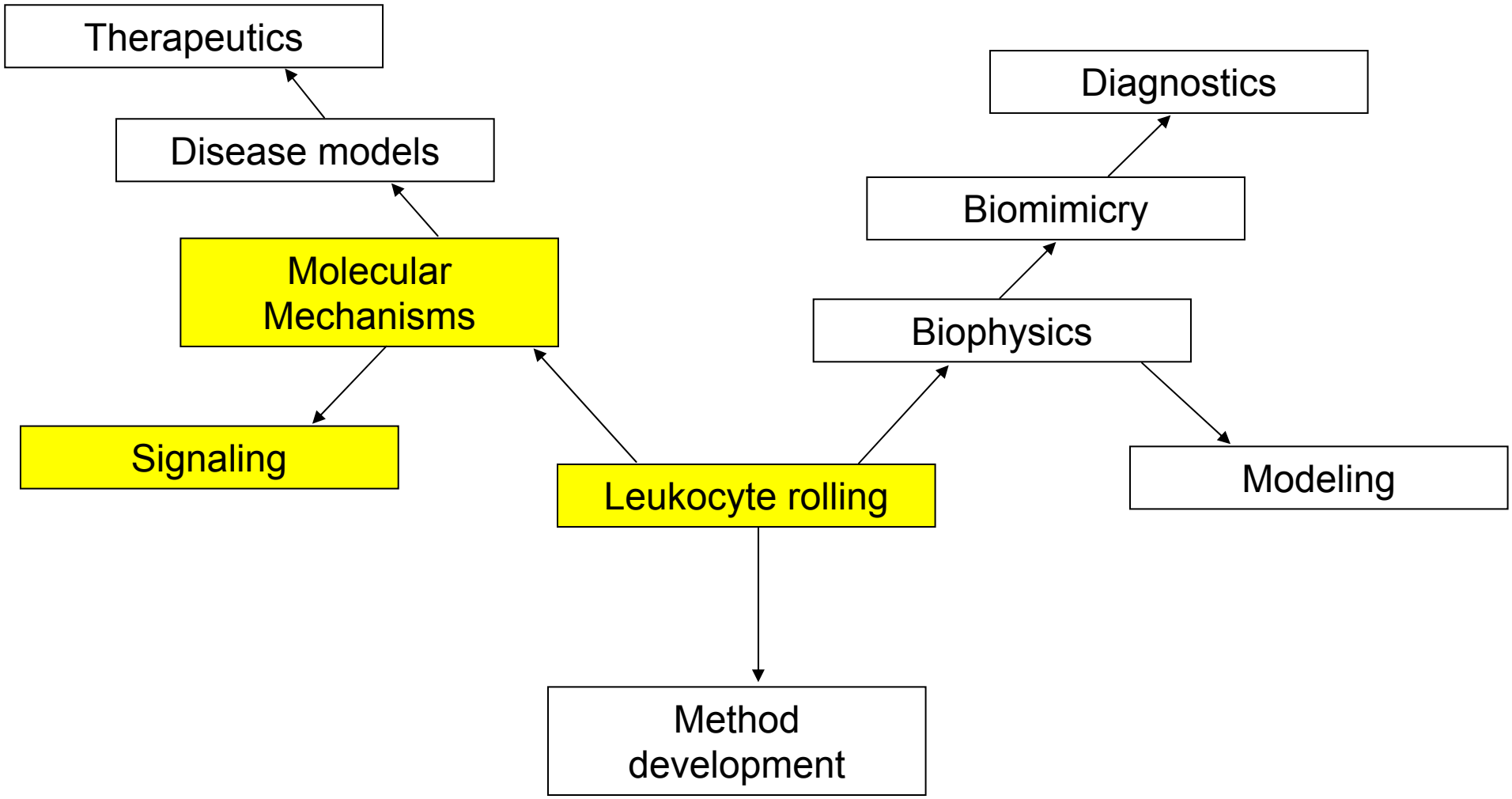
Direct Demonstration of P-Selectin– and VCAM-1–Dependent Mononuclear Cell Rolling in Early Atherosclerotic Lesions of Apolipoprotein E–Deficient Mice

Carroll L. Ramos, Yuqing Huo, Unsu Jung, Shukti Ghosh, David R. Manka, Ian J. Sarembock, Klaus Ley



Circ. Res. 1999; **84**: 1237-1244.

Ex vivo perfused apoE$^{-/-}$ carotid artery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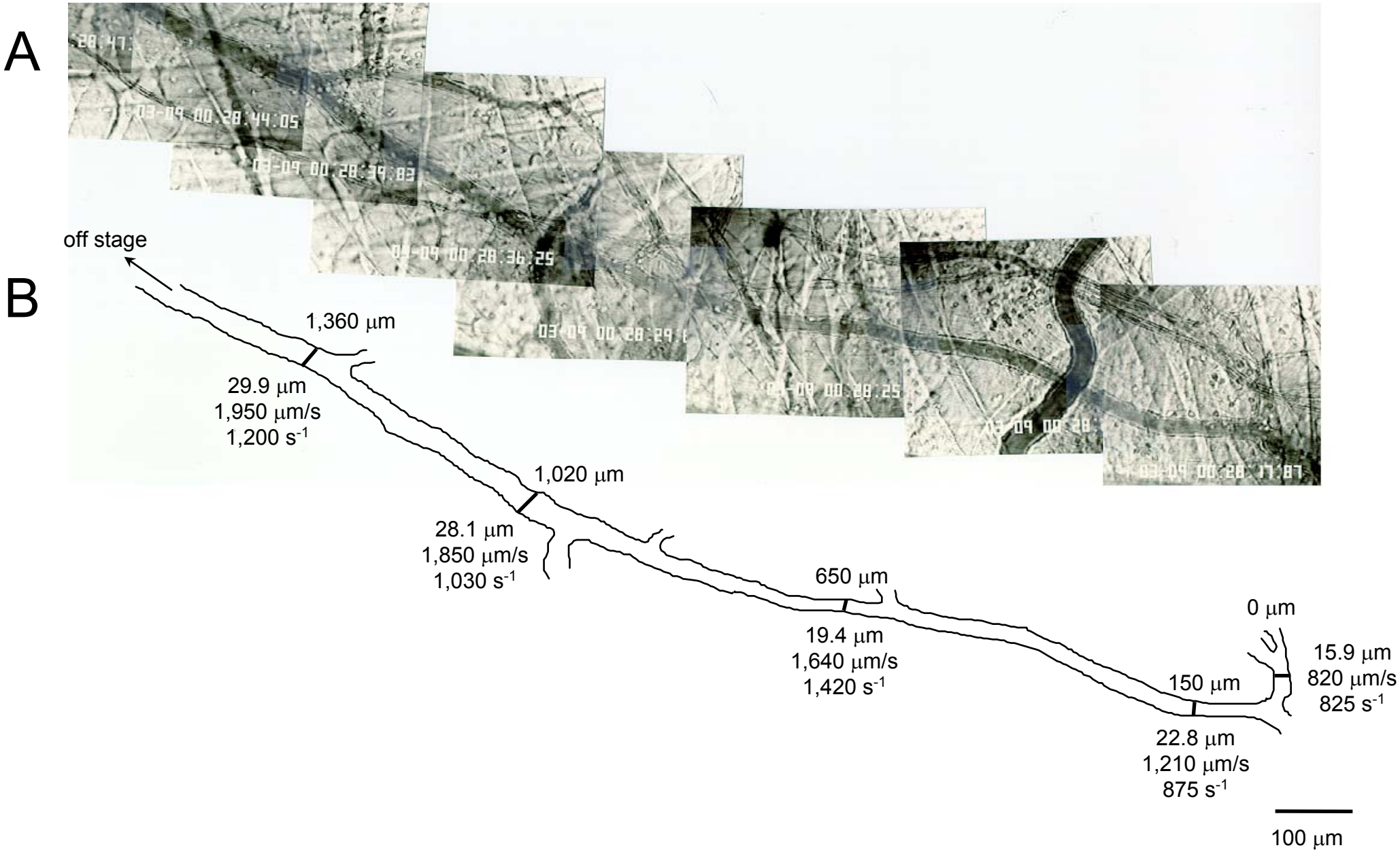
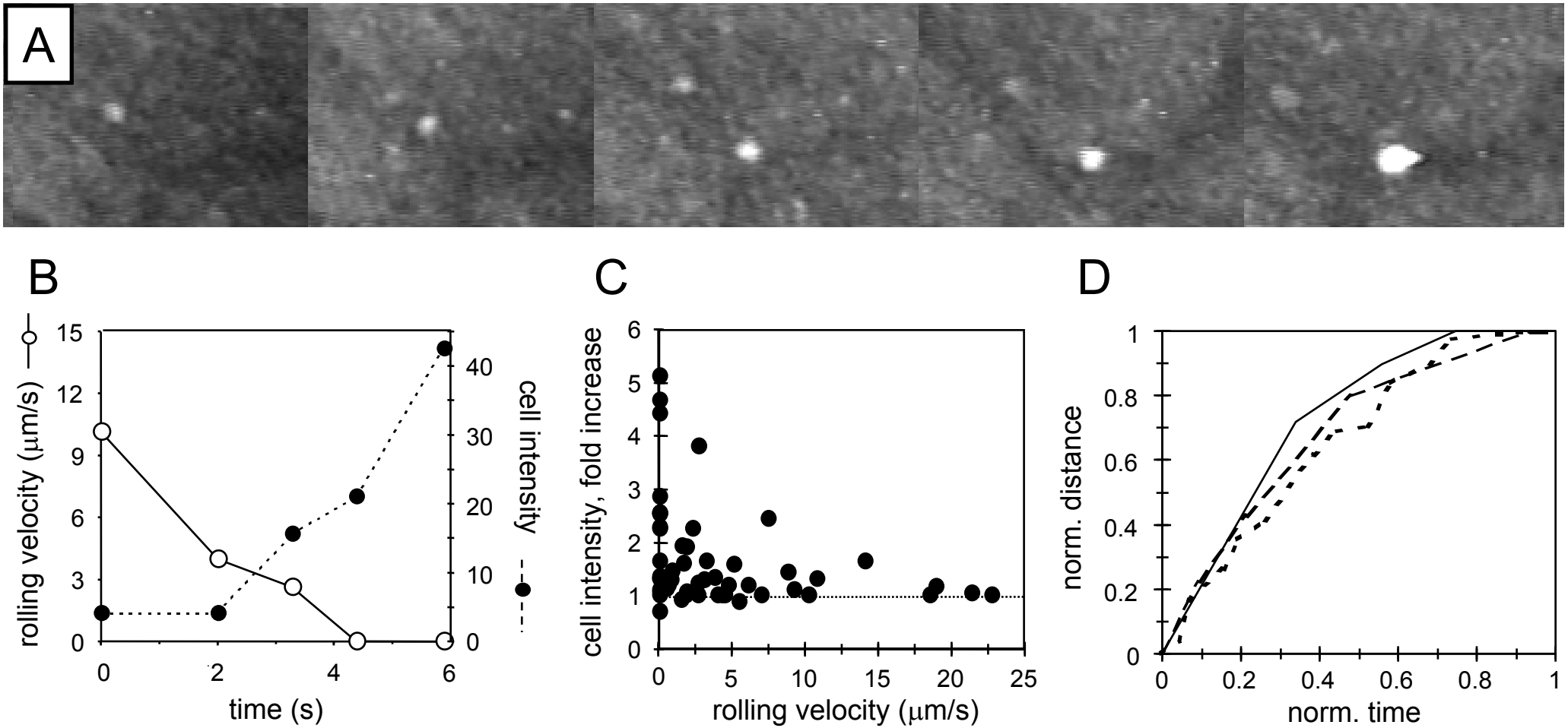
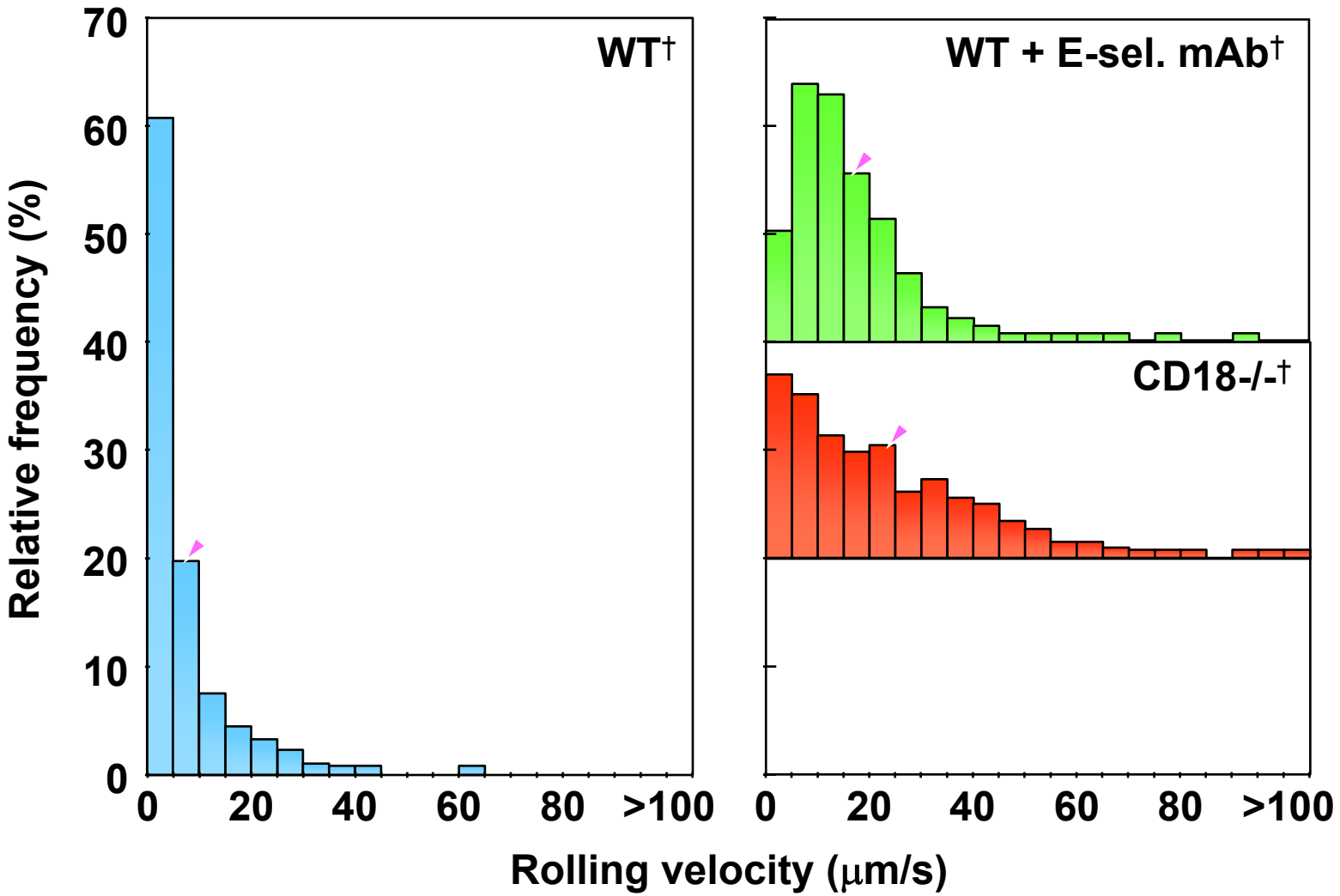

A
B
off stage
1,360 μm
29.9 μm
1,950 μm/s
1,200 s-1
1,020 μm
28.1 μm
1,850 μm/s
1,030 s-1
650 μm
19.4 μm
1,640 μm/s
1,420 s-1
0 μm
15.9 μm
820 μm/s
825 s-1
150 μm
22.8 μm
1,210 μm/s
875 s-1
100 μm

Leukocyte Arrest During Cytokine-Dependent Inflammation In Vivo[1]

Eric J. Kunkel,[2] Jessica L. Dunne, and Klaus Ley[3]

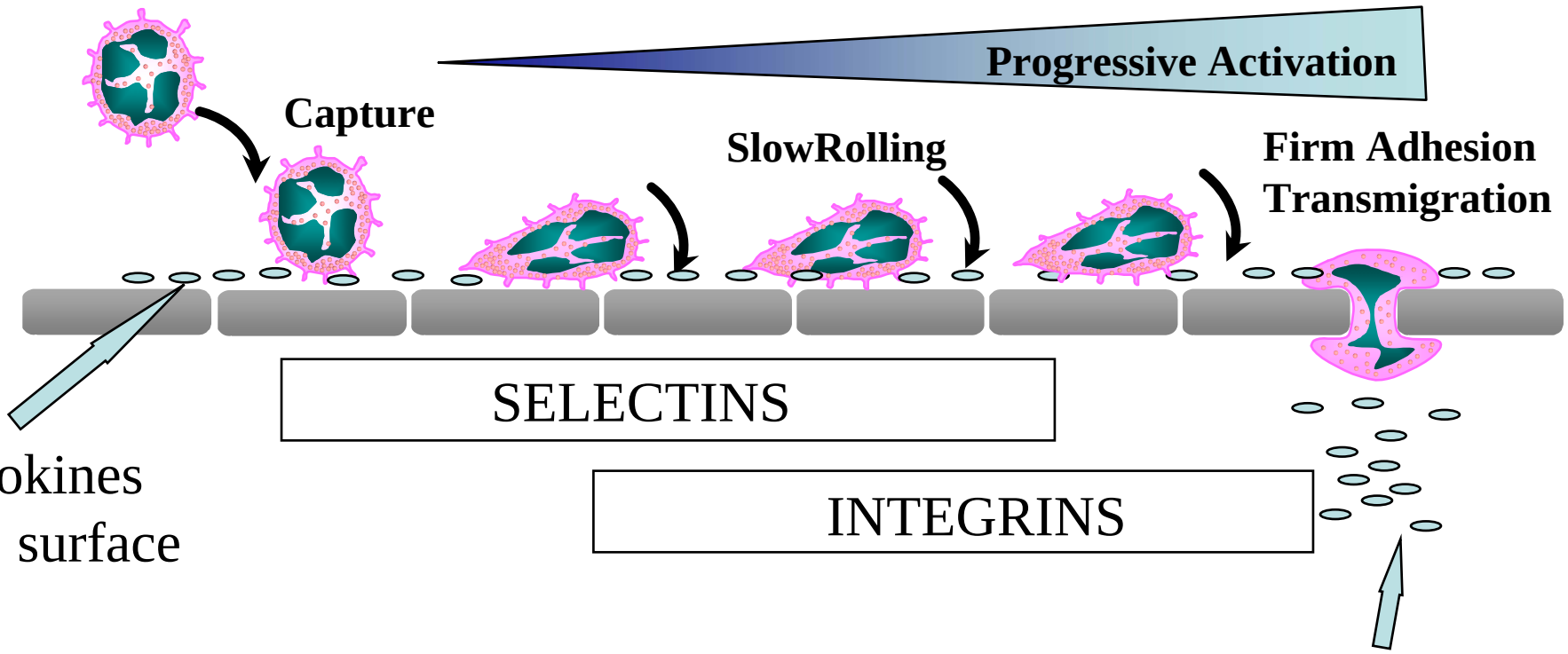


Leukocyte Rolling Velocity Distribution after TNF-α Treatment



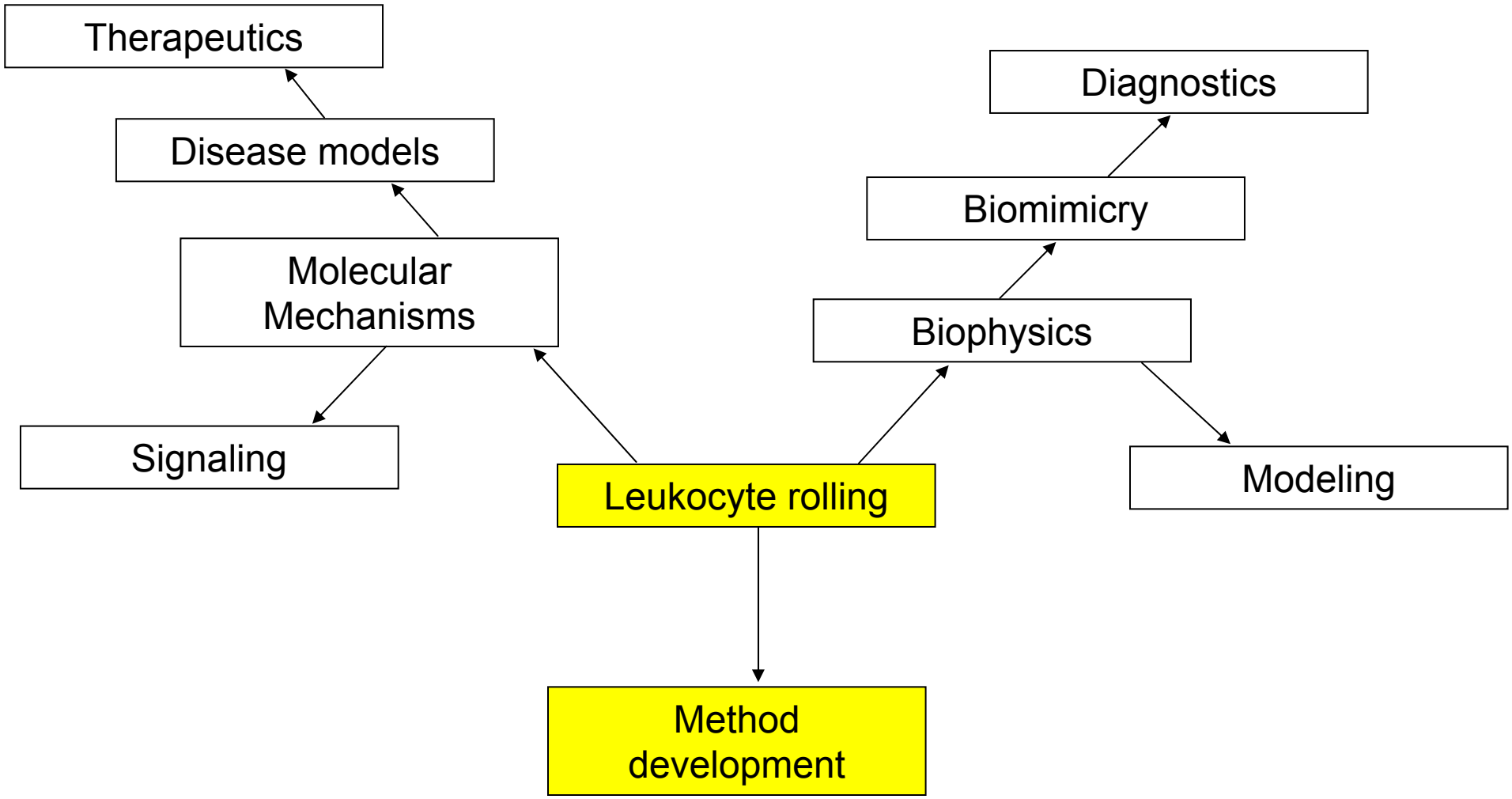
CYTOKINE-INDUCED INFLAMMATION

+ TNF-α



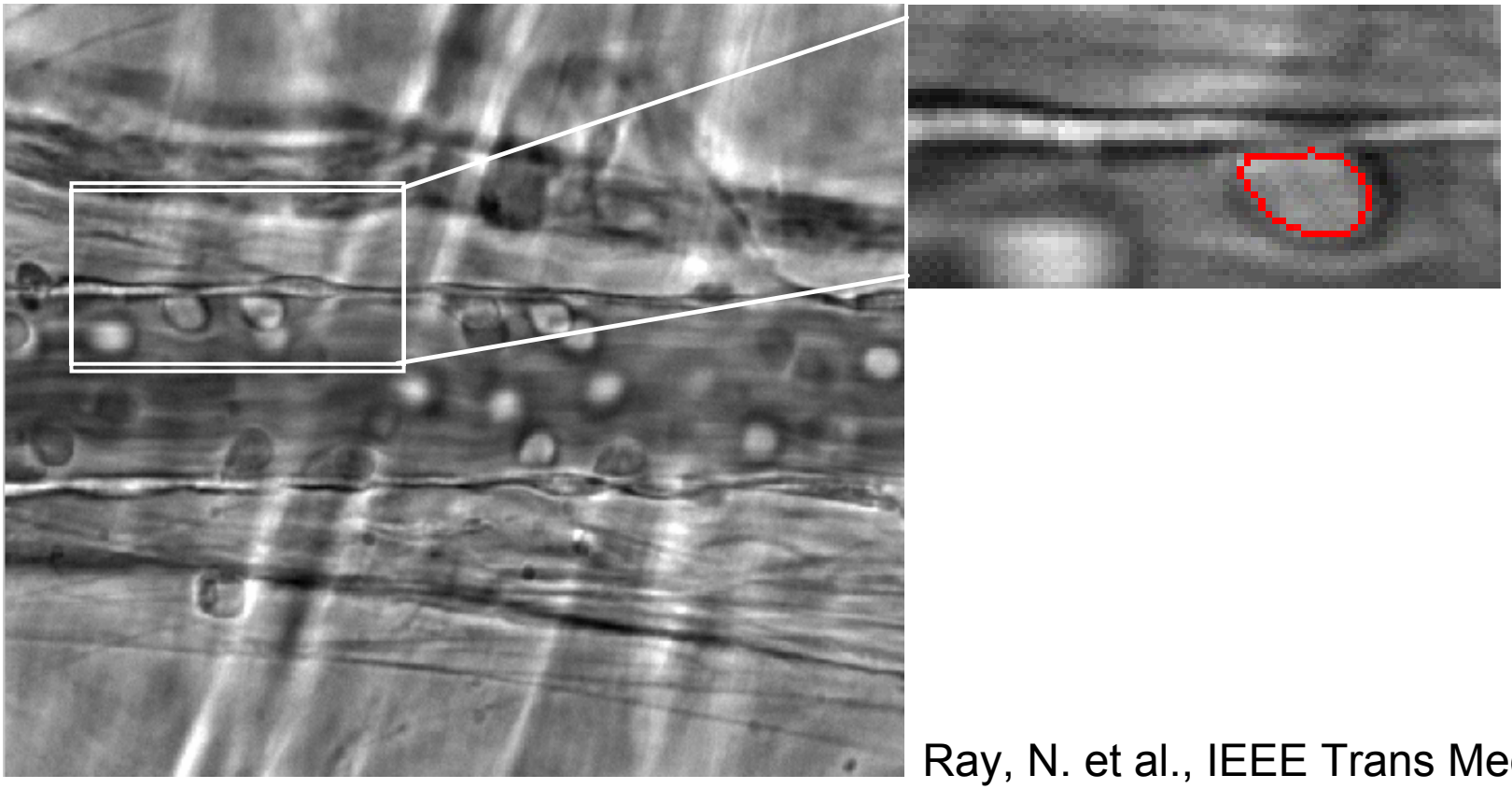
Chemokines
on EC surface

Chemoattractant
in tissue


Therapeutics
Disease models
Molecular Mechanisms
Signaling
Leukocyte rolling
Method development
Diagnostics
Biomimicry
Biophysics
Modeling

Tracking Leukocytes *In Vivo* With Shape and Size Constrained Active Contours

Nilanjan Ray, *Student Member, IEEE*, Scott T. Acton*, *Senior Member, IEEE*, and Klaus Ley

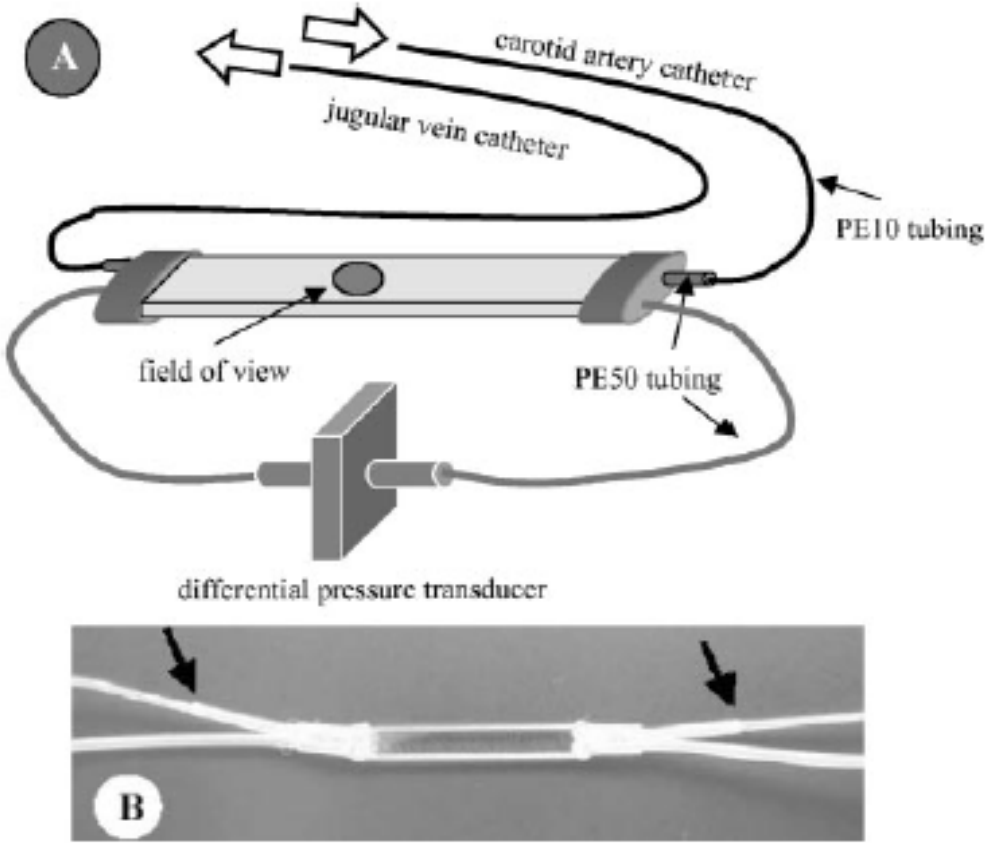


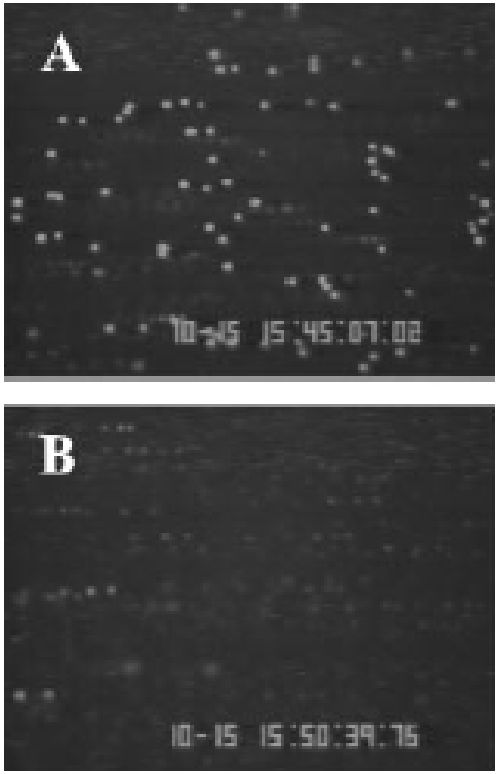
Ray, N. et al., IEEE Trans Med Imaging 2002; **21**: 1222-1235.

Autoperfused mouse flow chamber reveals synergistic neutrophil accumulation through P-selectin and E-selectin

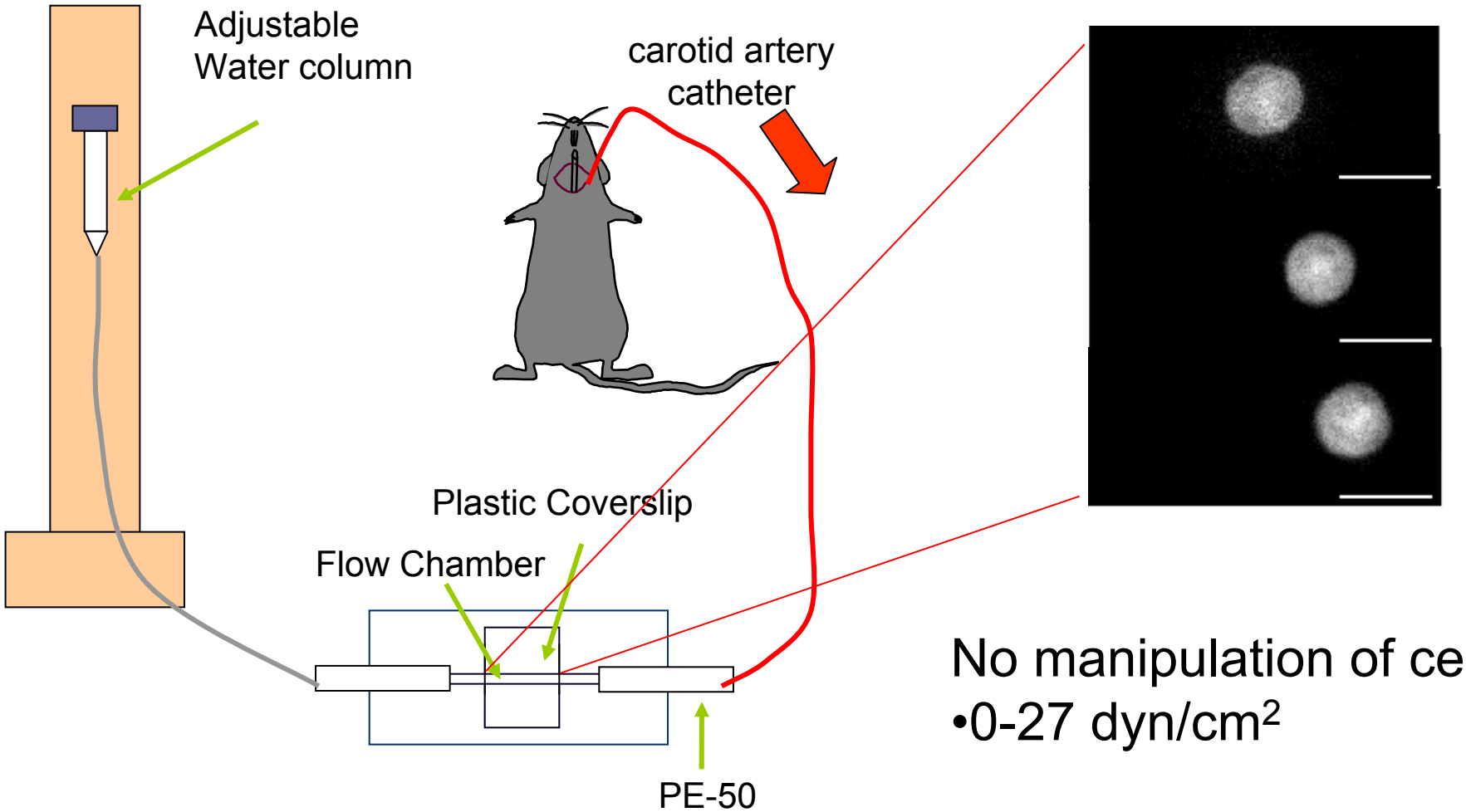
Michael L. Smith,* Markus Sperandio,*,†,1 Elena V. Galkina,‡ and Klaus Ley*,‡

*Department of Biomedical Engineering and ‡Cardiovascular Research Center, University of Virginia, Charlottesville; and †Neonatal Unit, Children's Hospital, University of Heidelberg, Germany





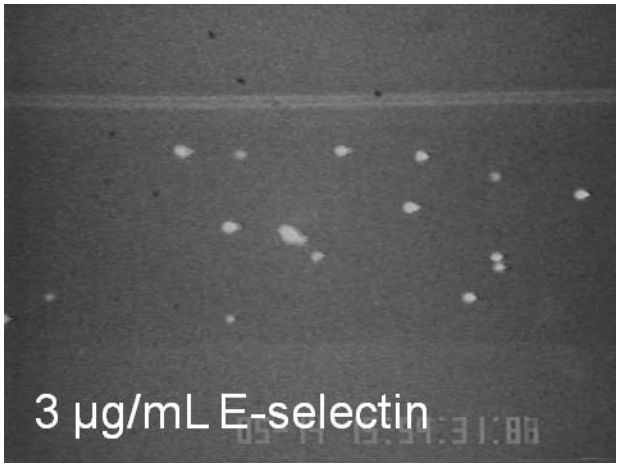
20x200 μm autoperfused flow chamber

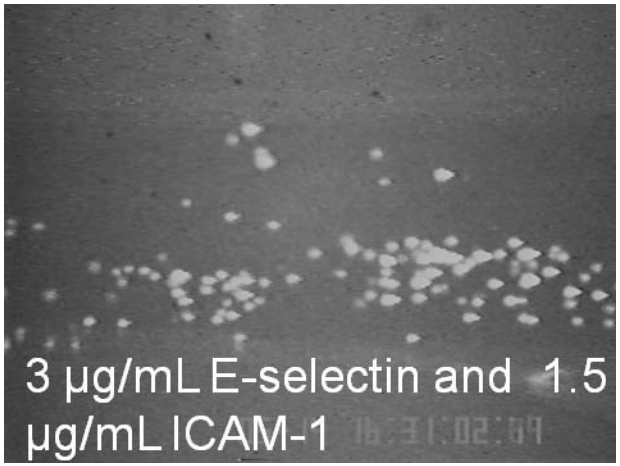


No manipulation of cells

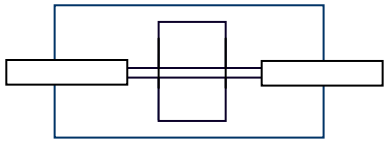
•0-27 dyn/cm^2

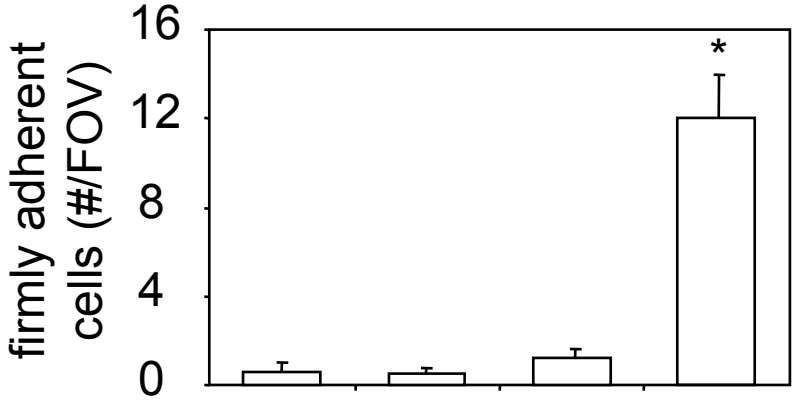
Chesnutt et al.,
Microcirculation 2006


3 µg/mL E-selectin


3 µg/mL E-selectin and 1.5
µg/mL ICAM-1

E-selectin engagement is not sufficient for neutrophil arrest….

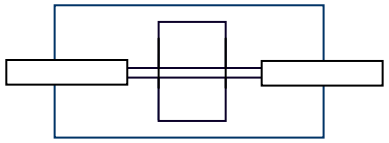


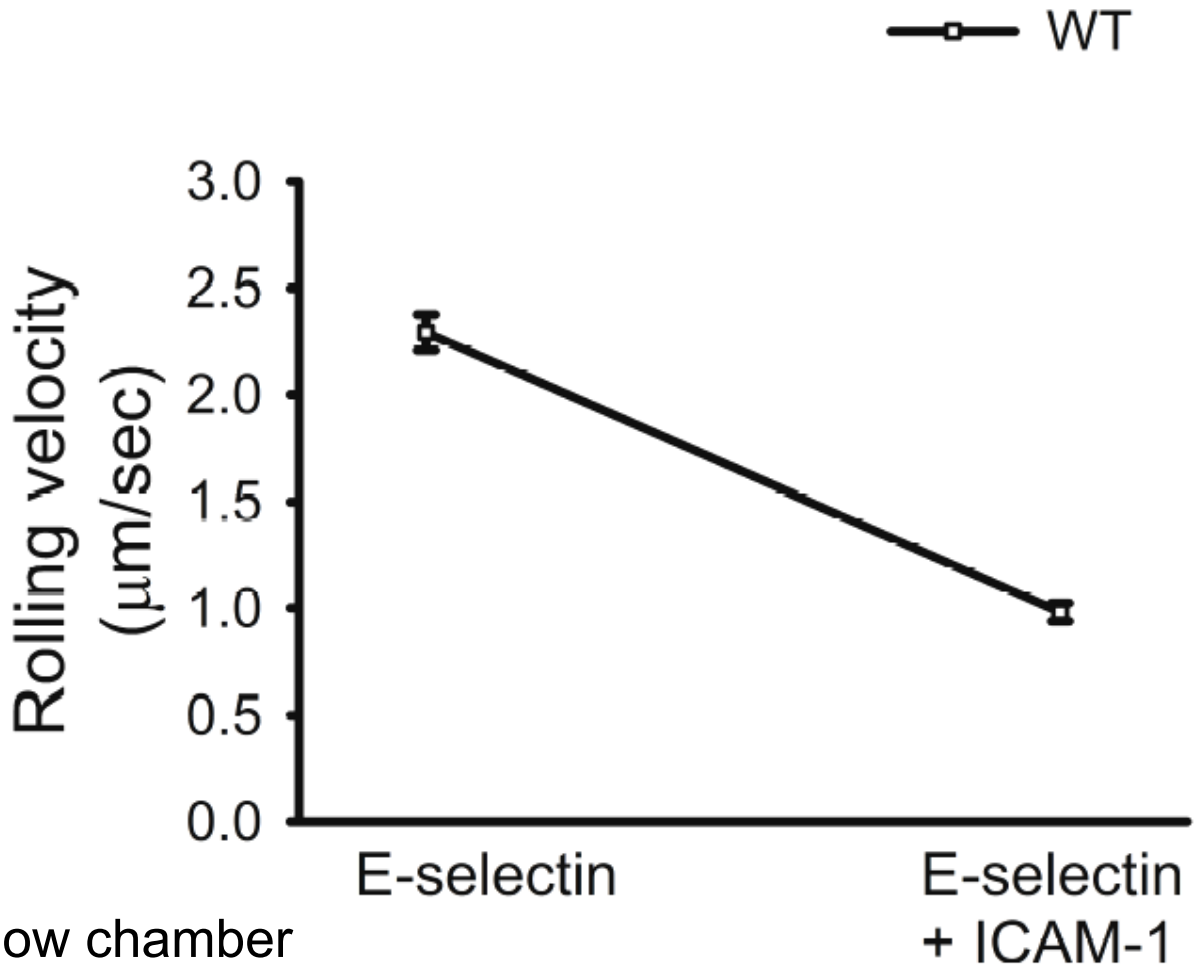


E-selectin	+	+	+	+
ICAM-1	-	+	-	+
CXCL1	+	-	+	+

Chesnutt et al., Microcirculation 2006

….but induces slow rolling when ICAM-1 is available





Autoperfused flow chamber

6 dyn/cm^2

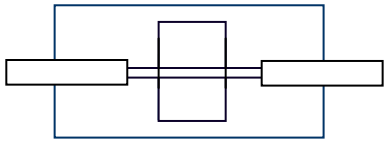
Zarbock et al., Immunity 2007

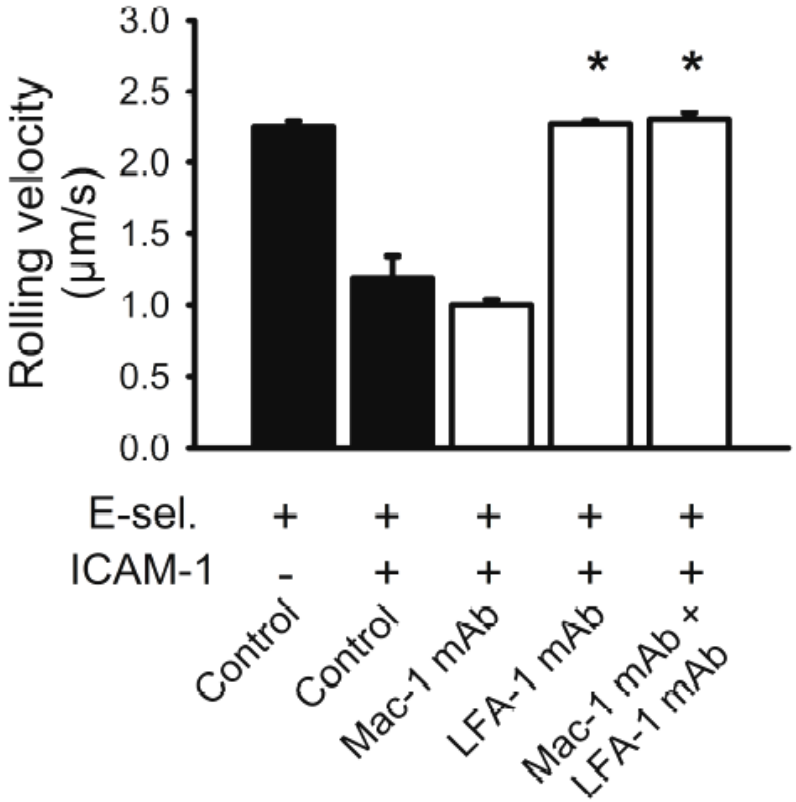
Immunity
Article

Spleen Tyrosine Kinase Syk Is Necessary for E-Selectin-Induced $\alpha_L\beta_2$ Integrin-Mediated Rolling on Intercellular Adhesion Molecule-1

Alexander Zarbock,[1,4] Clifford A. Lowell,[5] and Klaus Ley[1,2,3,*]
[1] Robert M. Berne Cardiovascular Research Center
[2] Department of Physiology and Biological Physics
[3] Department of Biomedical Engineering
University of Virginia, Charlottesville, VA 22908, USA
[4] Department of Anesthesiology and Intensive Care Medicine, University of Münster, 48155 Münster, Germany
[5] Department of Laboratory Medicine, University of California, San Francisco, San Francisco, CA 94143, USA
*Correspondence: klausley@virginia.edu
DOI 10.1016/j.immuni.2007.04.011

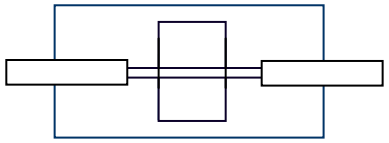
Slow rolling on E-selectin and ICAM-1 is LFA-1-dependent

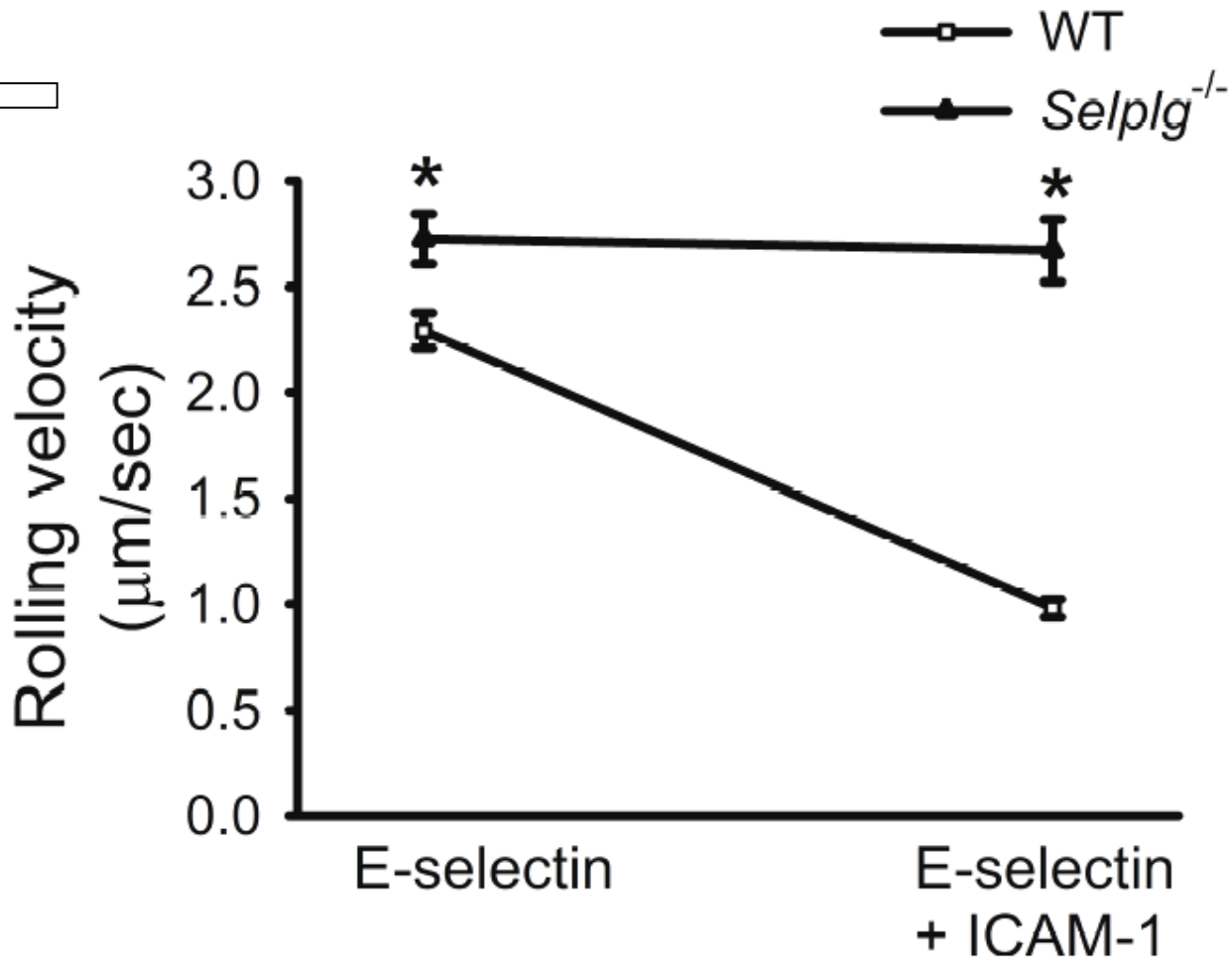




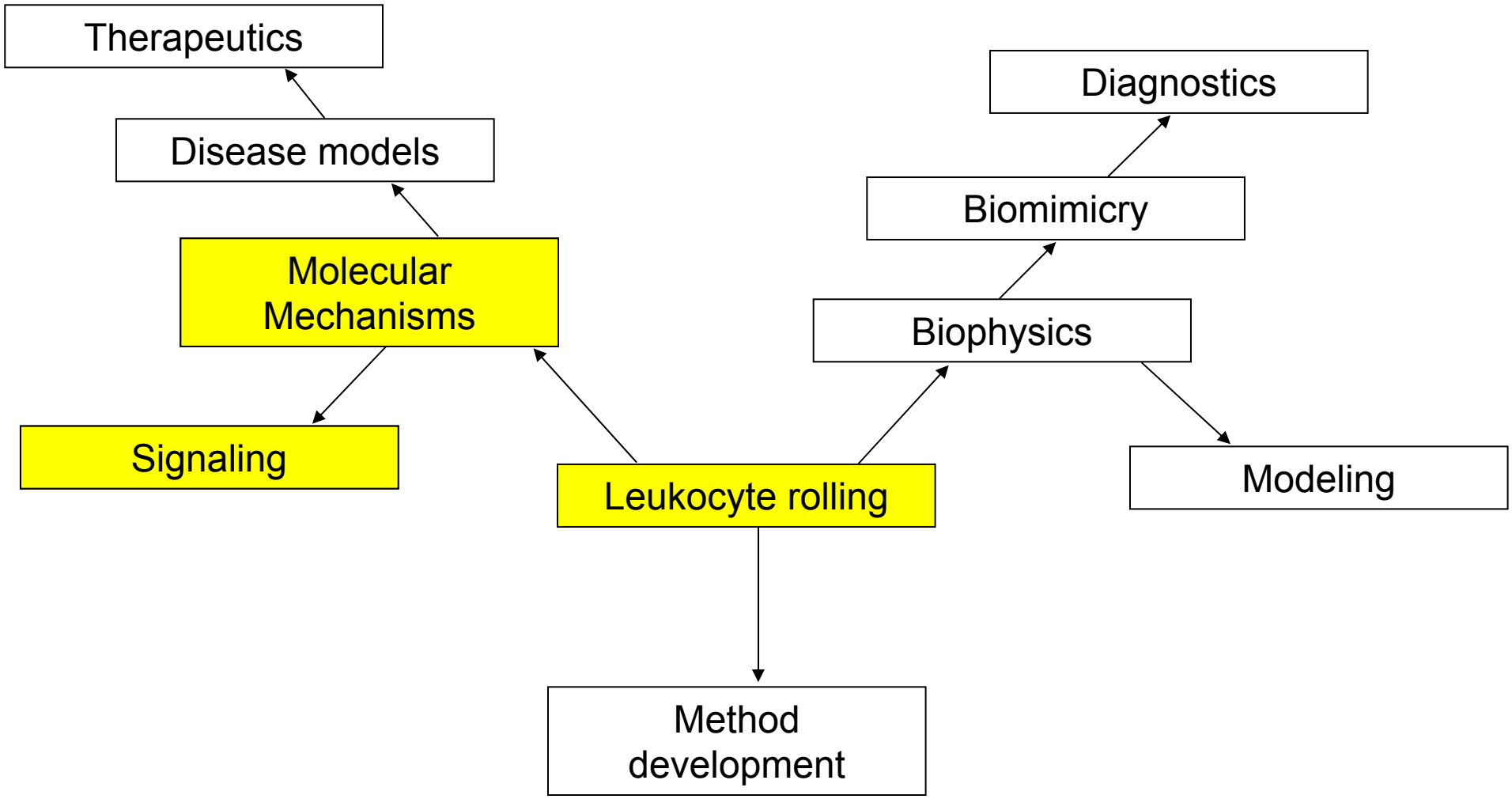
Zarbock et al., Immunity 2007

PSGL-1-deficient neutrophils do not slow down on ICAM-1





Zarbock et al., Immunity 2007


Therapeutics
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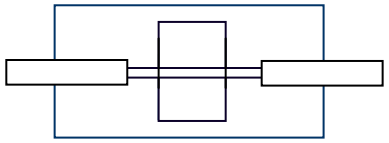
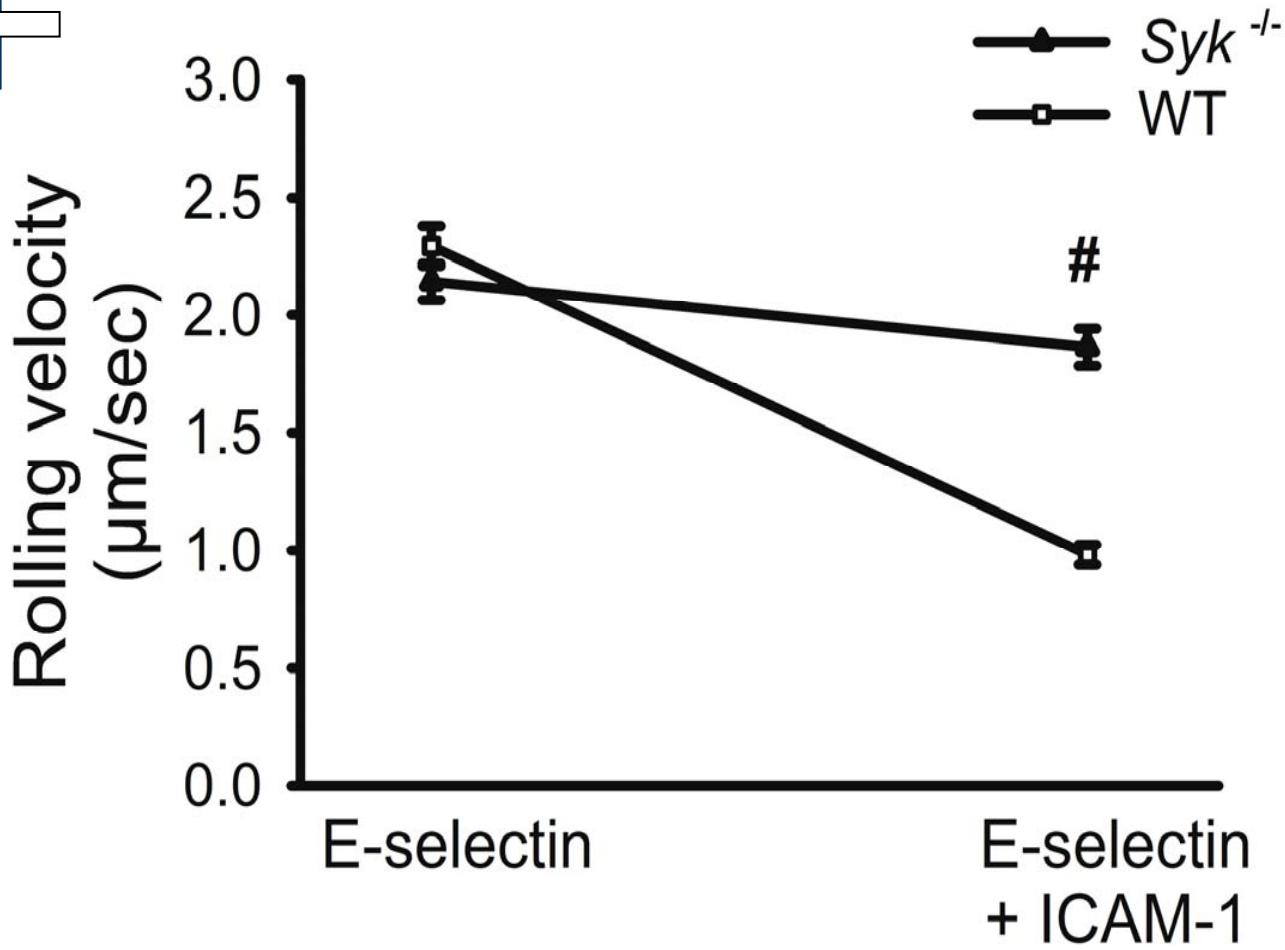
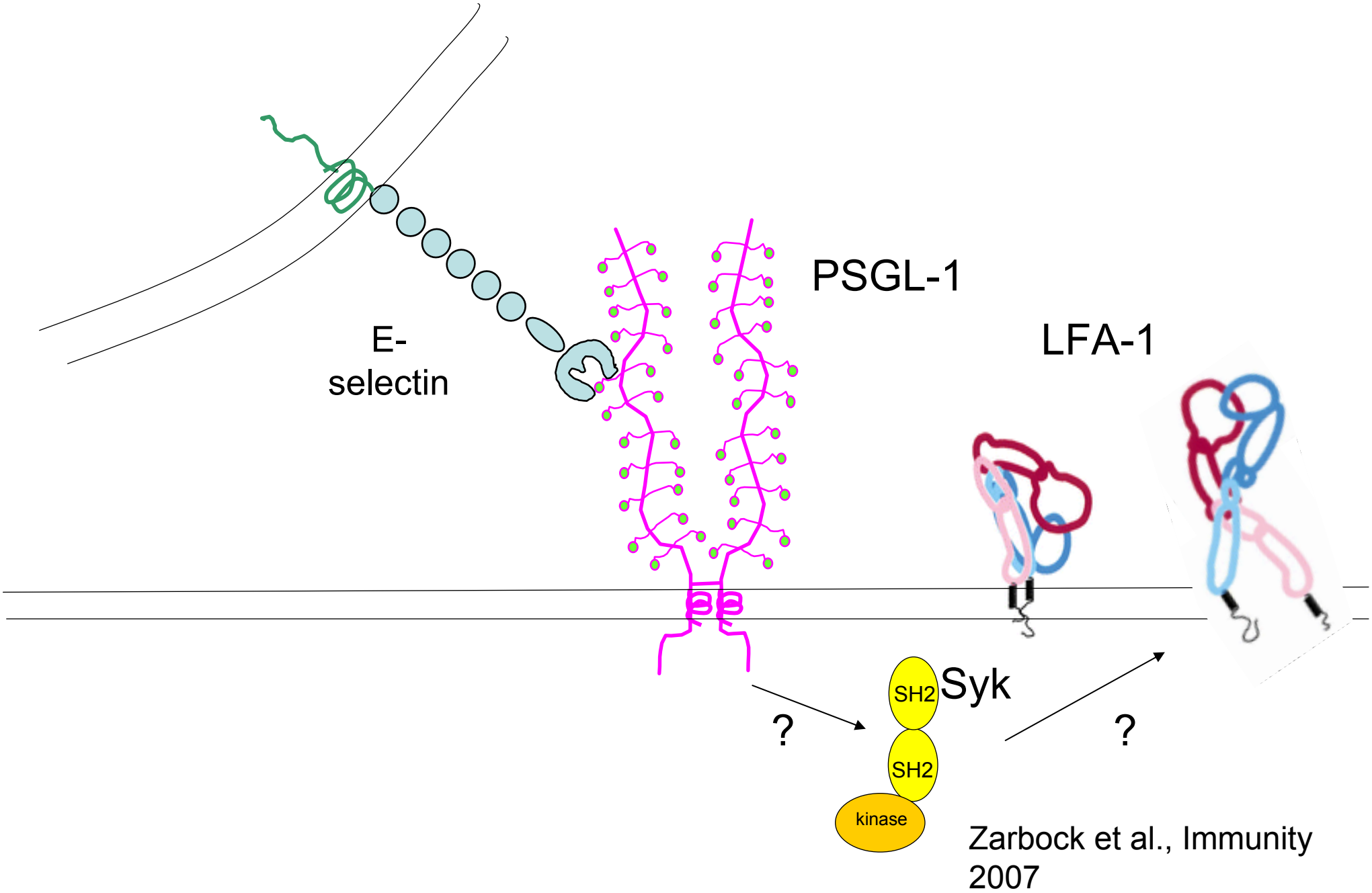
Modeling

Syk$^{-/-}$ neutrophils do not slow down on E-selectin and ICAM-1



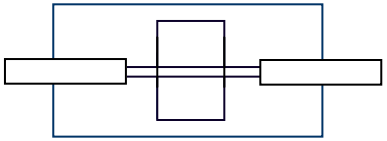


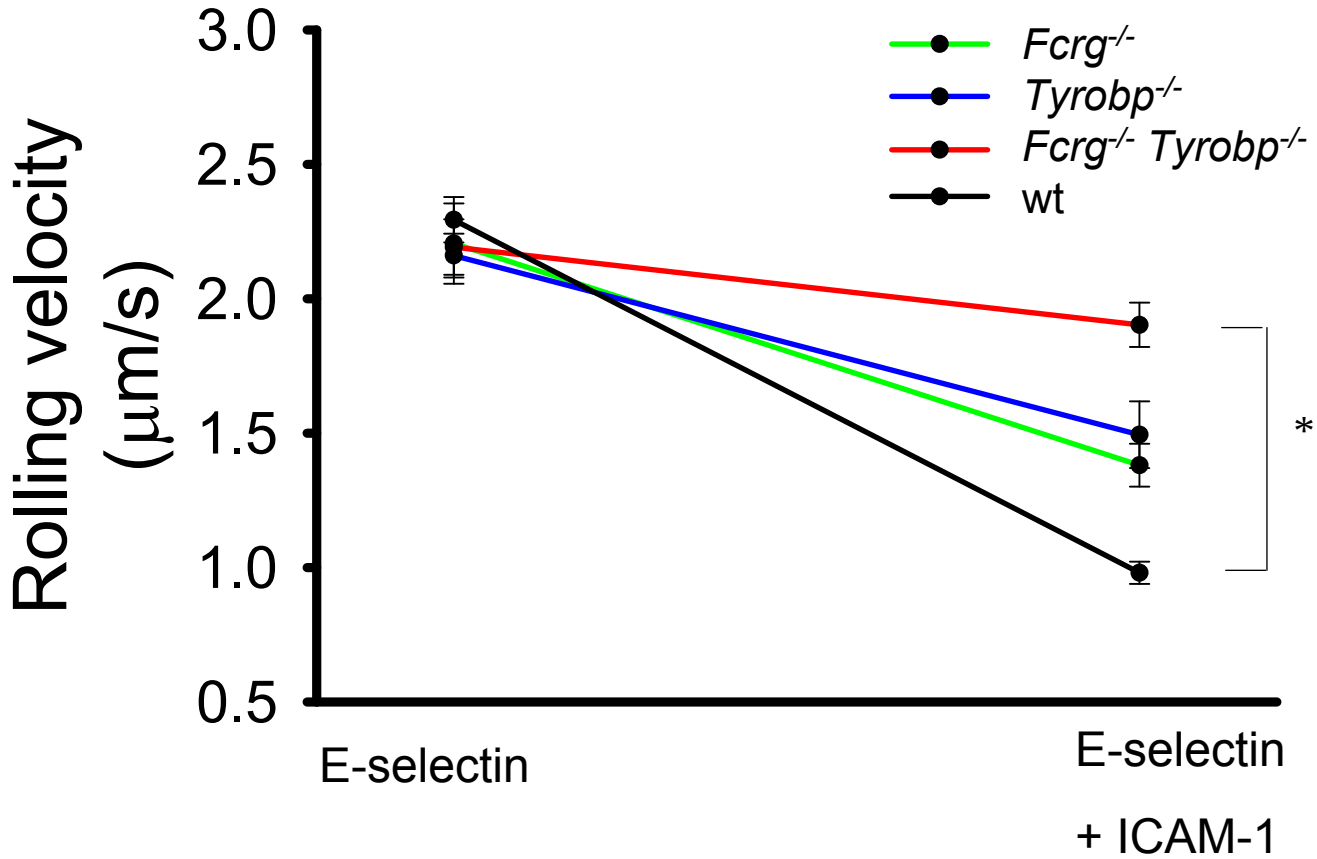
Zarbock et al., Immunity 2007



Zarbock et al., Immunity 2007

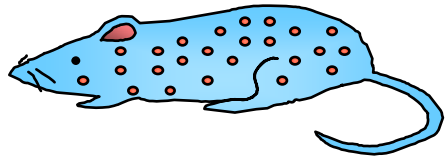
ITAM adaptors

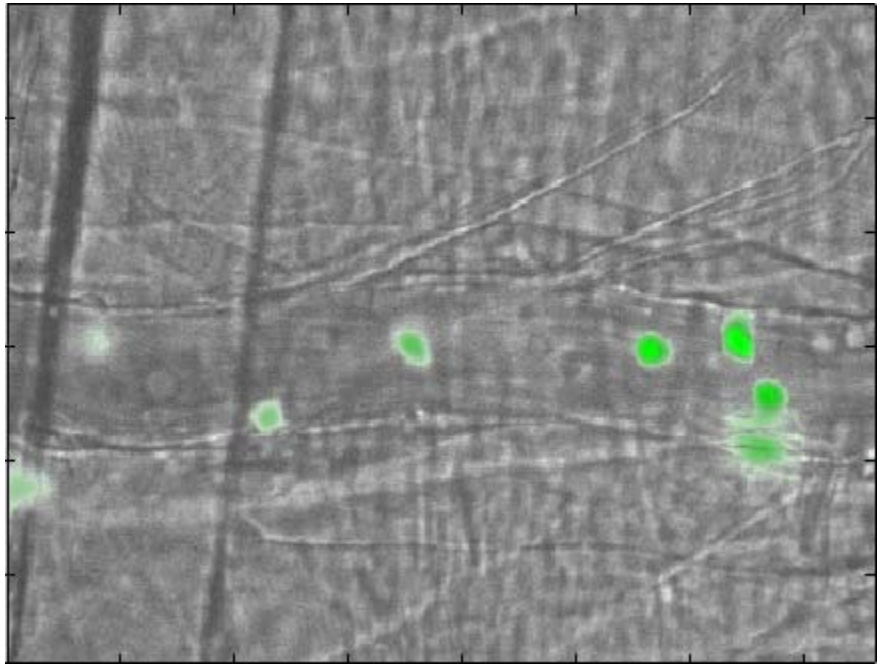


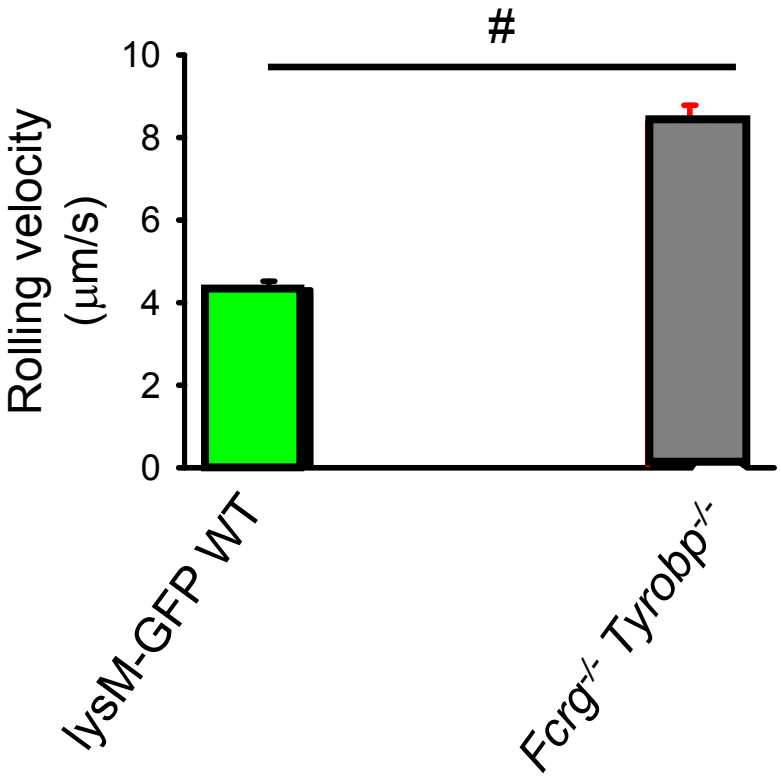


Zarbock et al., JEM 2008

Mixed chimeric mice to study rolling velocities of DAP12/FcRγ double deficient leukocytes

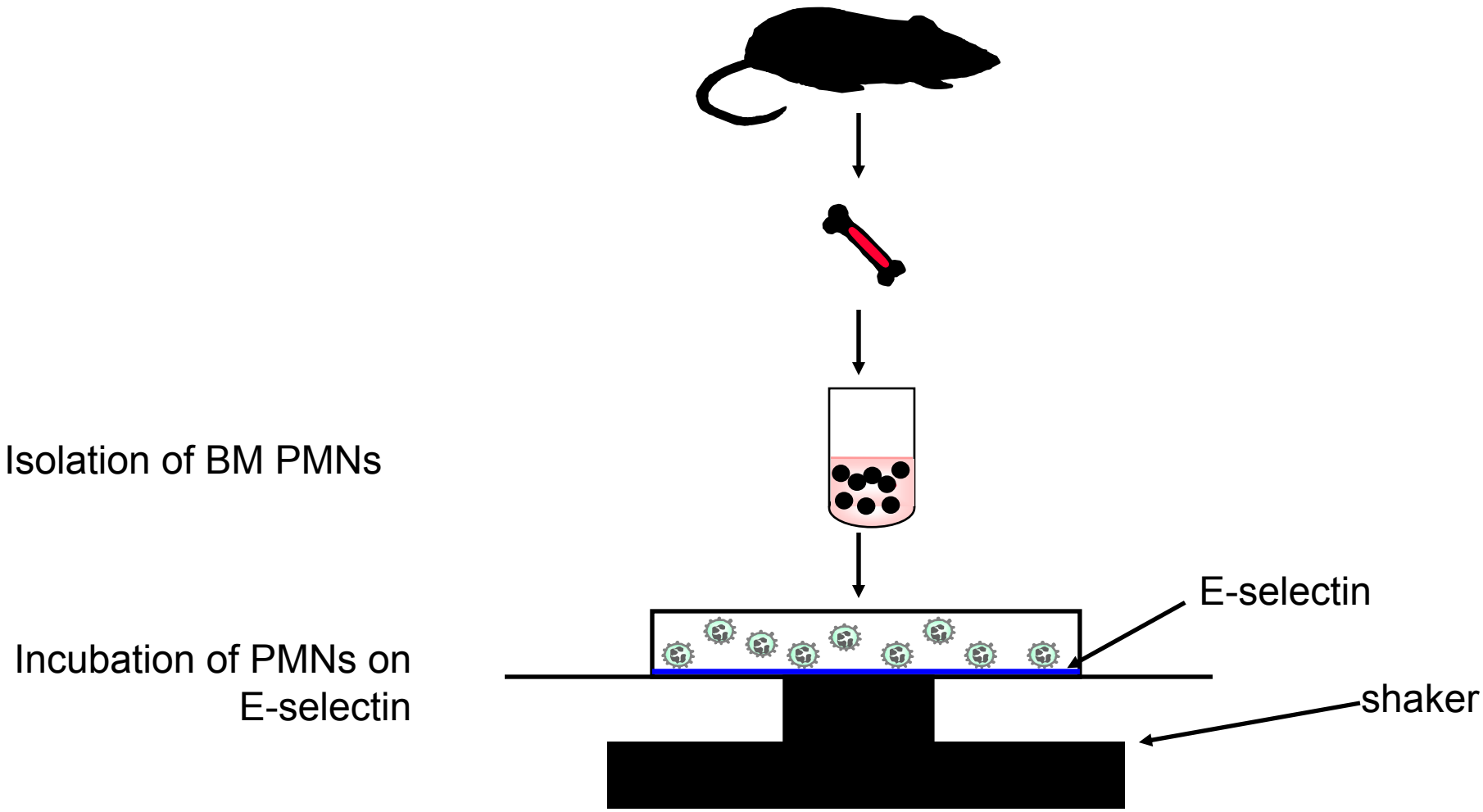




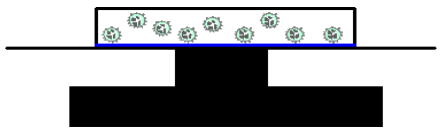


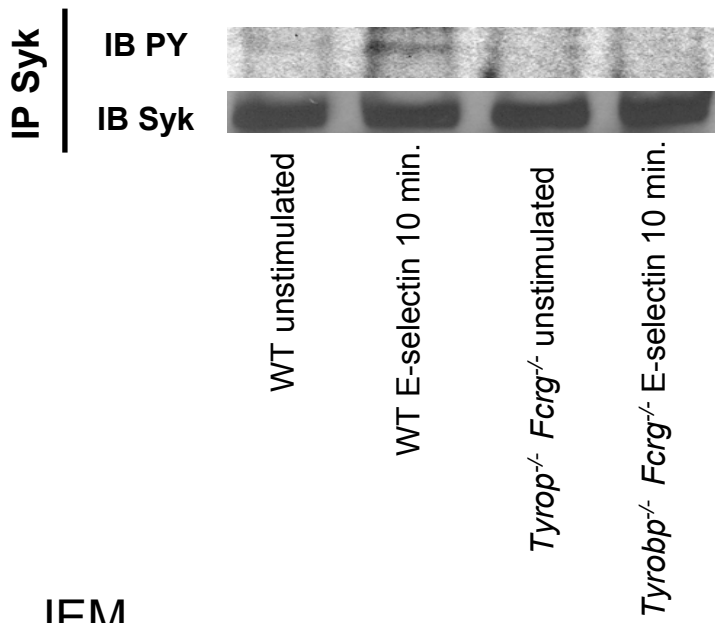
Zarbock et al., JEM 2008

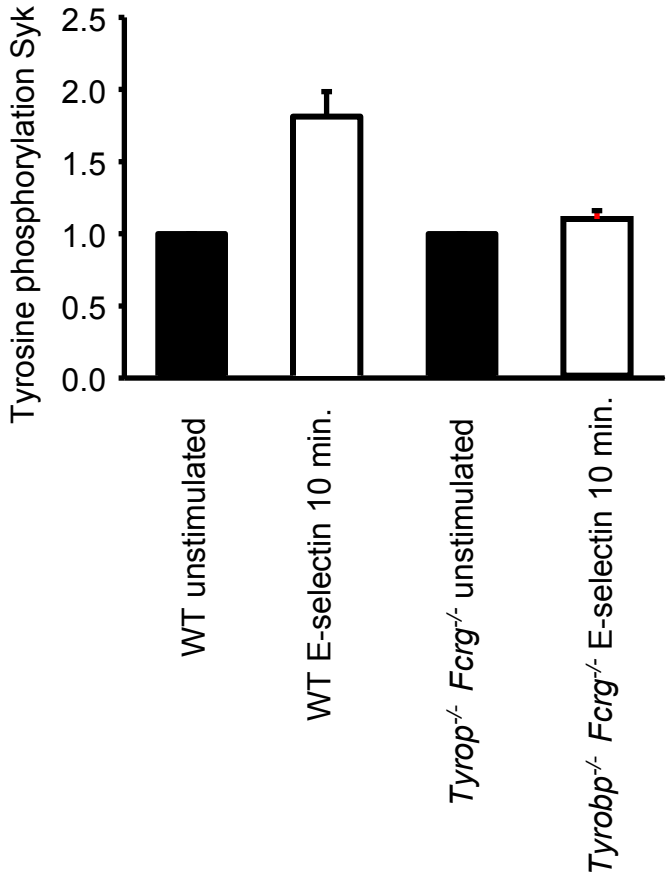
Phosphorylation assay



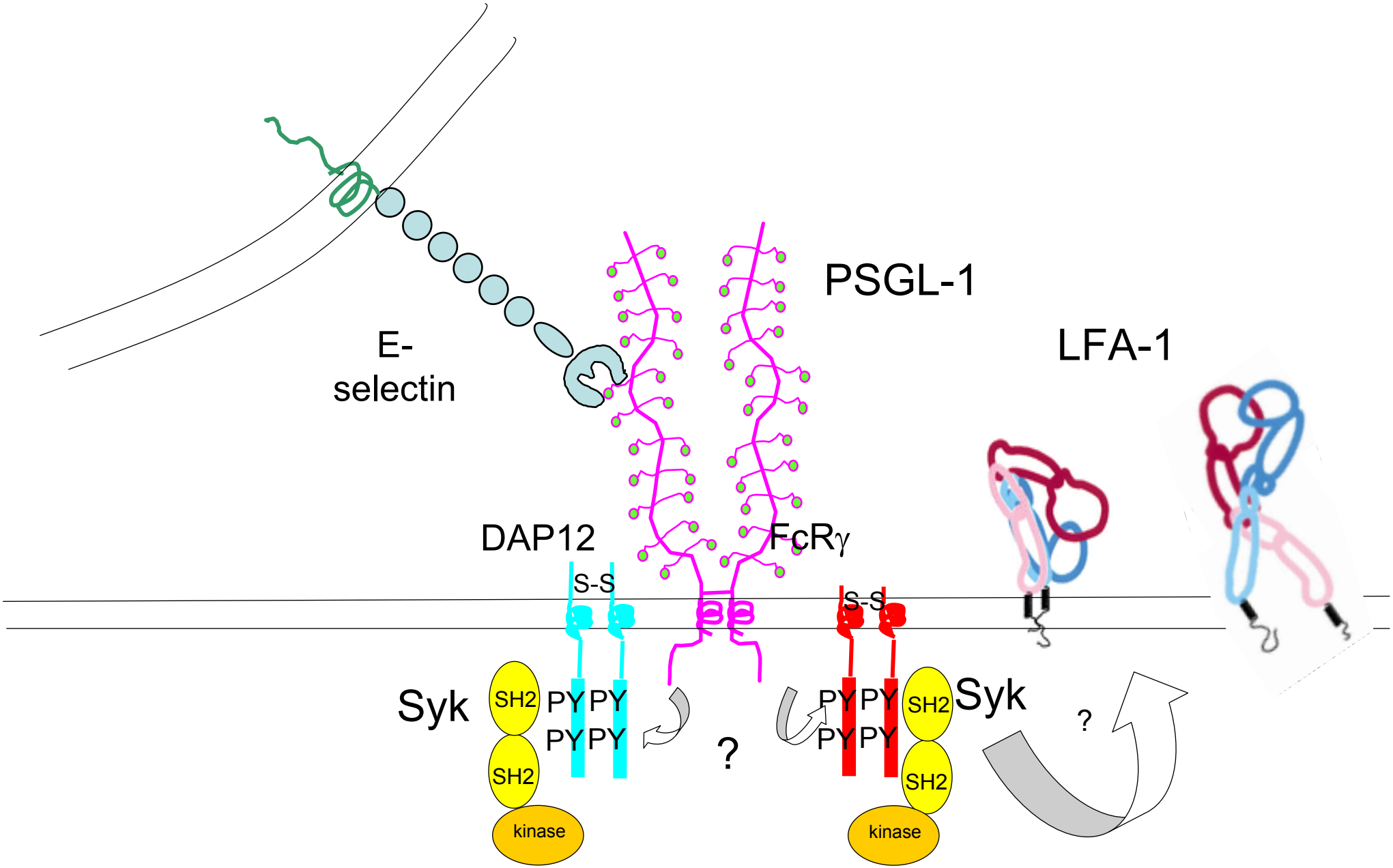
Elimination of DAP12 and FcRγ abolishes Syk phosphorylation following E-selectin engagement



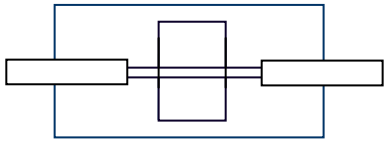


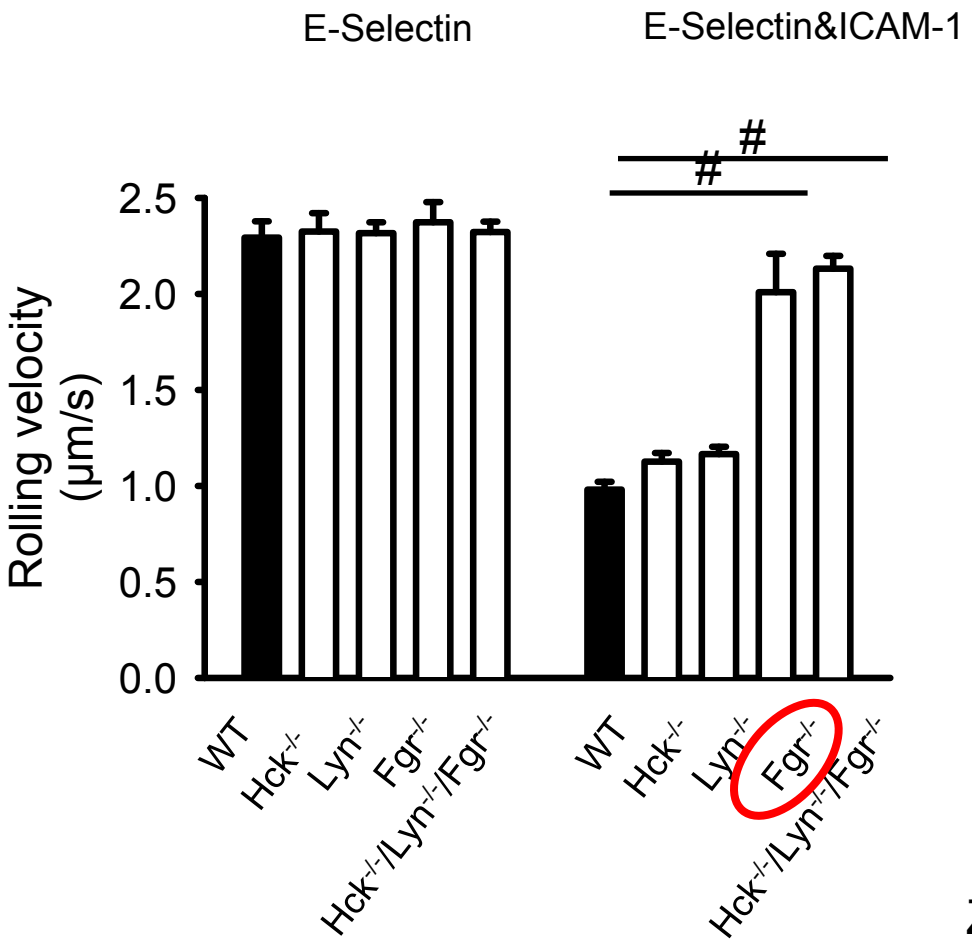


Zarbock et al., JEM 2008


E-
selectin
PSGL-1
LFA-1
DAP12
FcRγ
S-S
S-S
Syk
SH2
SH2
kinase
PY PY
PY PY
?
PY PY
PY PY
SH2
SH2
kinase
Syk
?

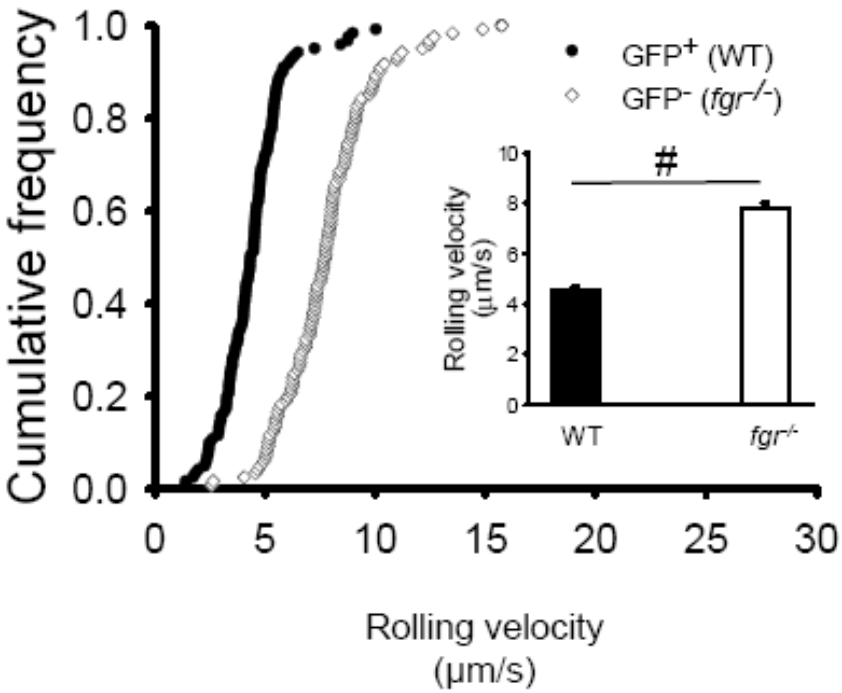
Elimination of Fgr abolishes slow rolling on E-selectin and ICAM-1

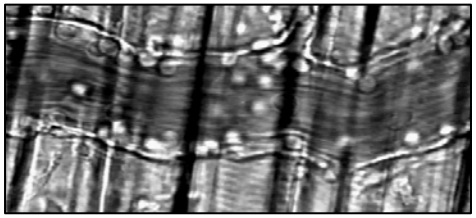


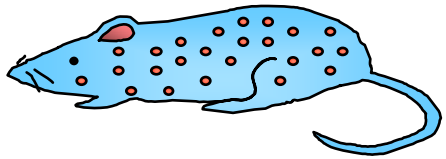


Zarbock et al., JEM 2008

Elimination of *Fgr*$^{-/-}$ elevates rolling velocity in vivo

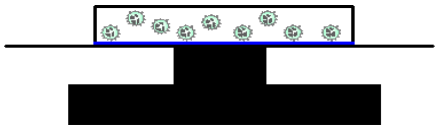


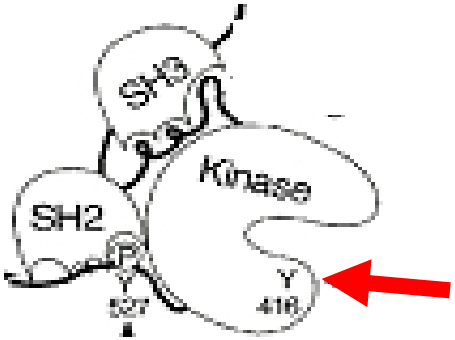


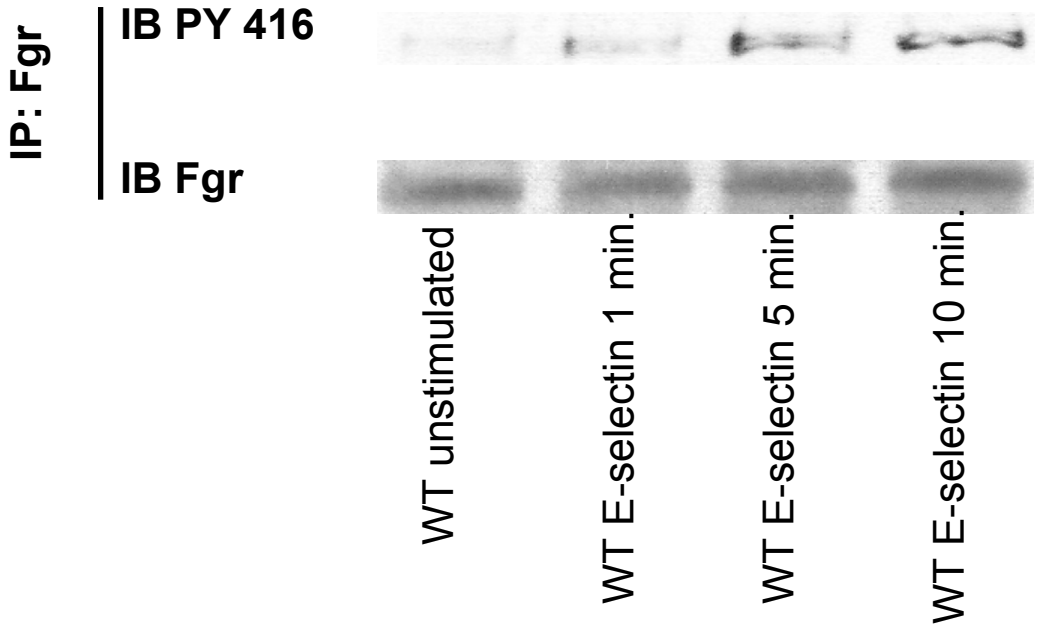


Zarbock et al., JEM 2008

Fgr is phosphorylated at Y416 upon E-selectin engagement

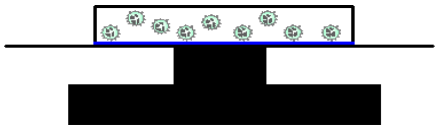


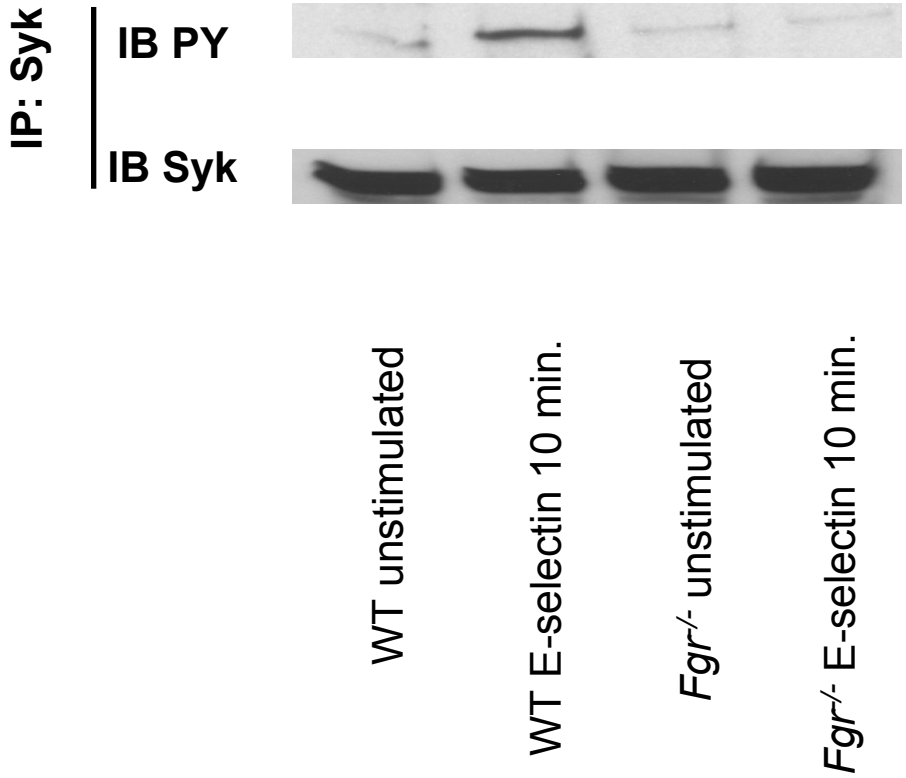




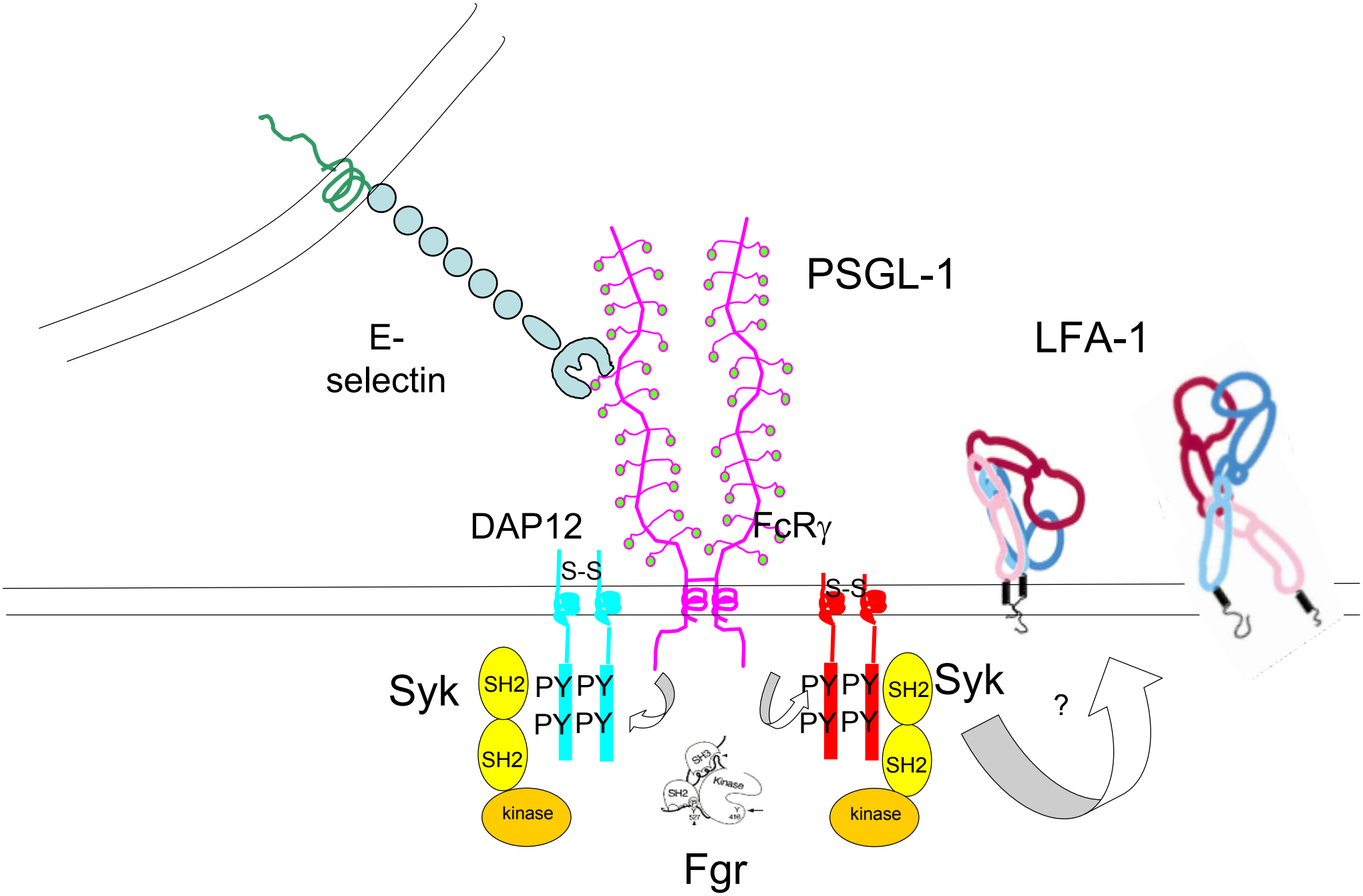
Zarbock et al., JEM 2008

Loss of function:
Fgr is needed for Syk phosphorylation

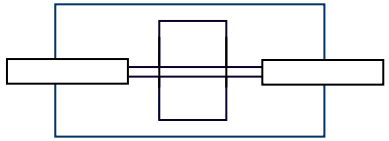


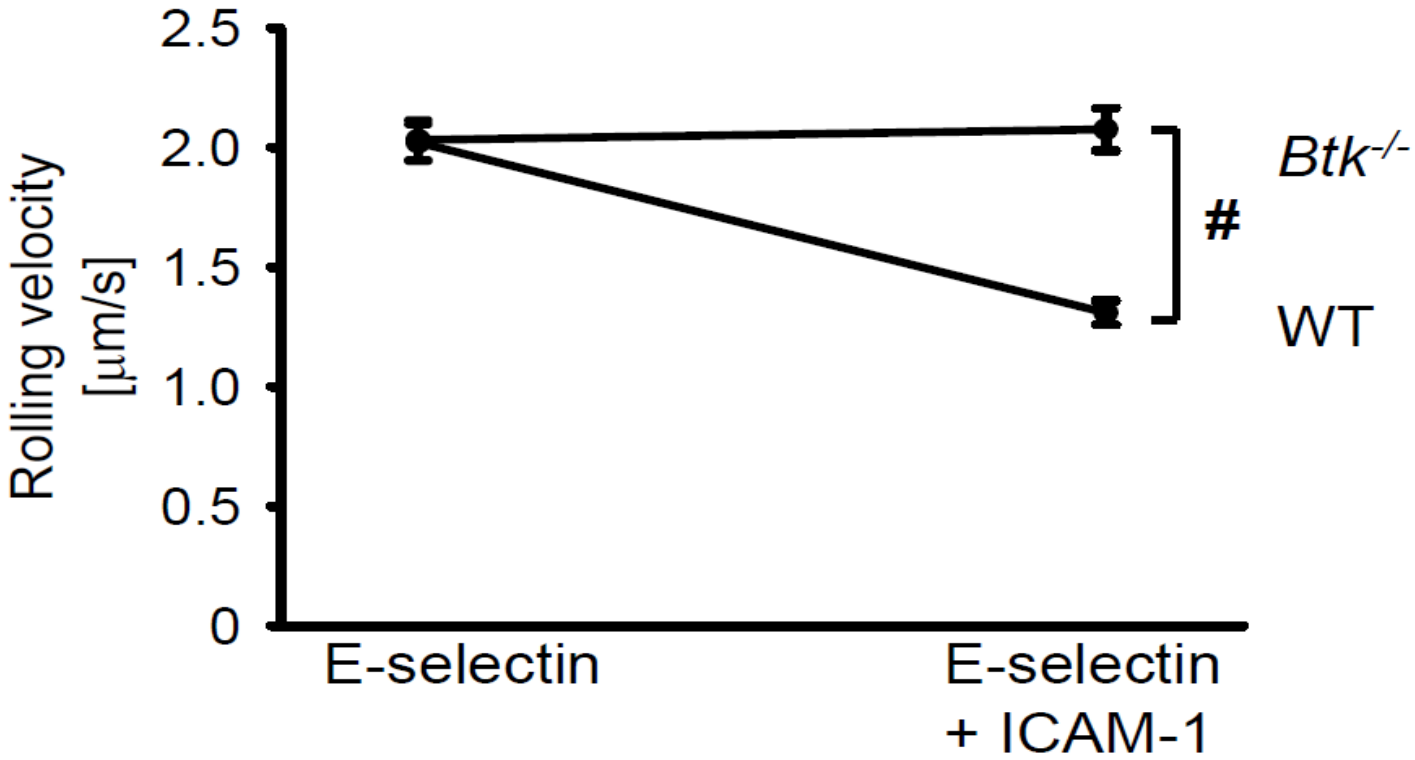


Zarbock et al., JEM 2008


E-
selectin
PSGL-1
LFA-1
DAP12
FcRγ
S-S
S-S
Syk
SH2
PY PY
PY PY
SH2
kinase
PY PY
PY PY
SH2
Syk
SH2
kinase
?
Fgr

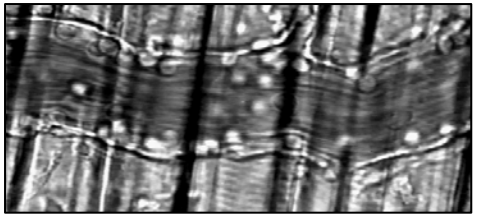
Bruton's Tyrosine Kinase

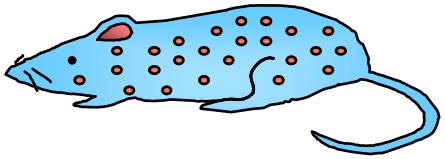


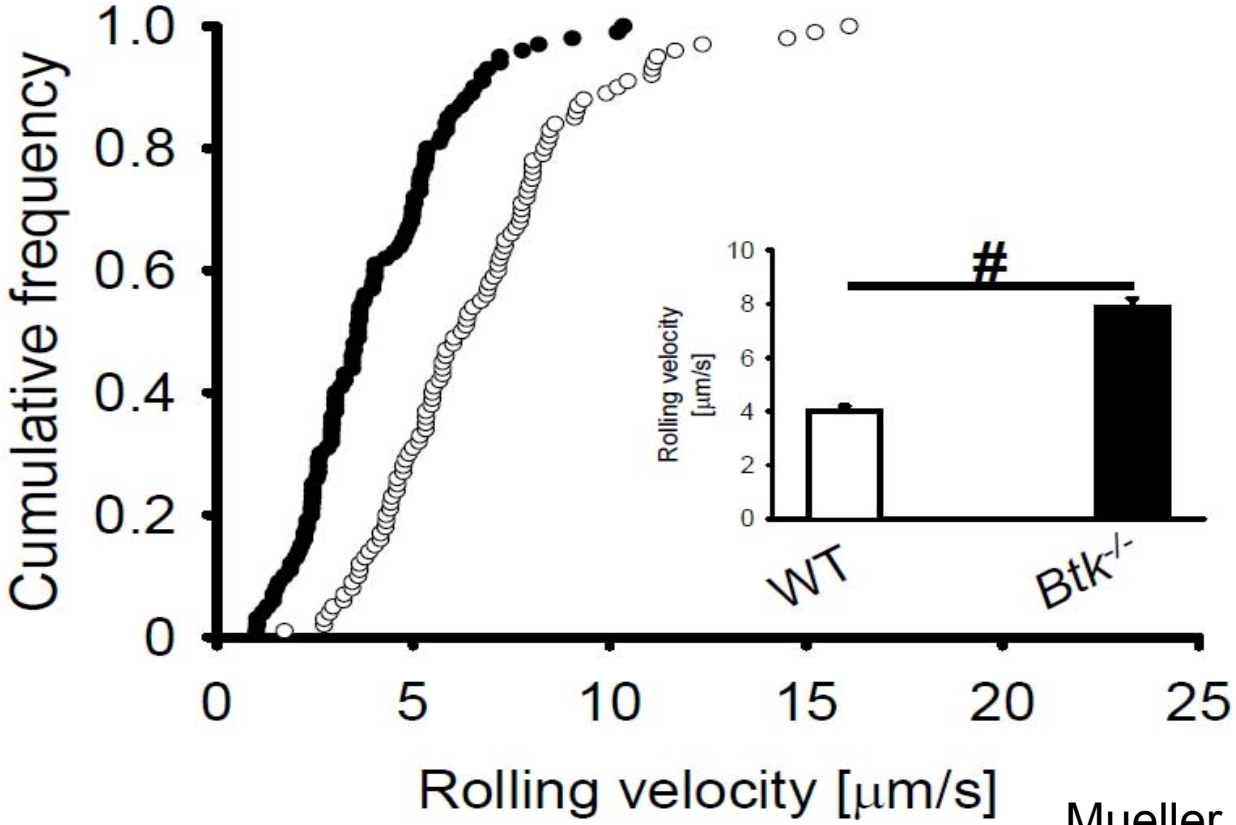


Mueller et al., Blood 2010

Bruton's Tyrosine Kinase

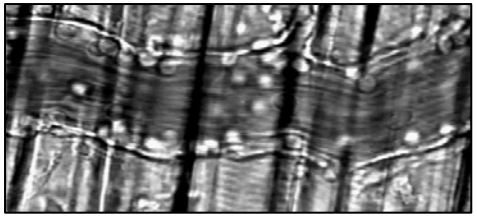


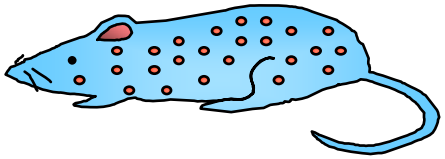


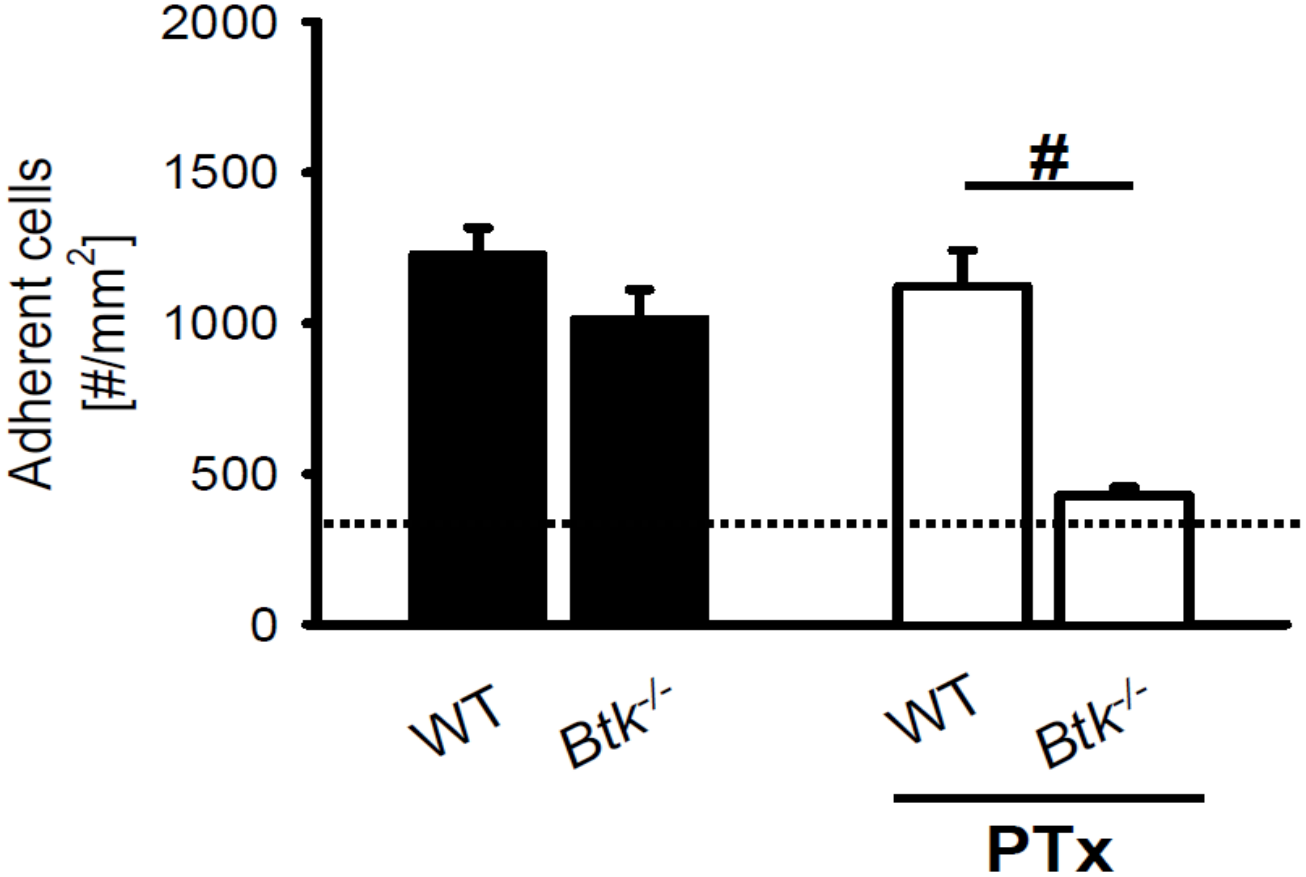


Mueller et al., Blood 2010

Bruton's Tyrosine Kinase

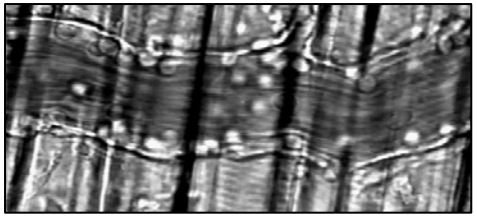


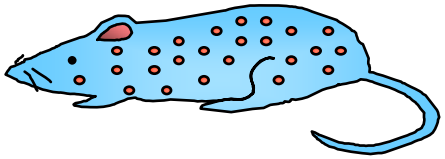


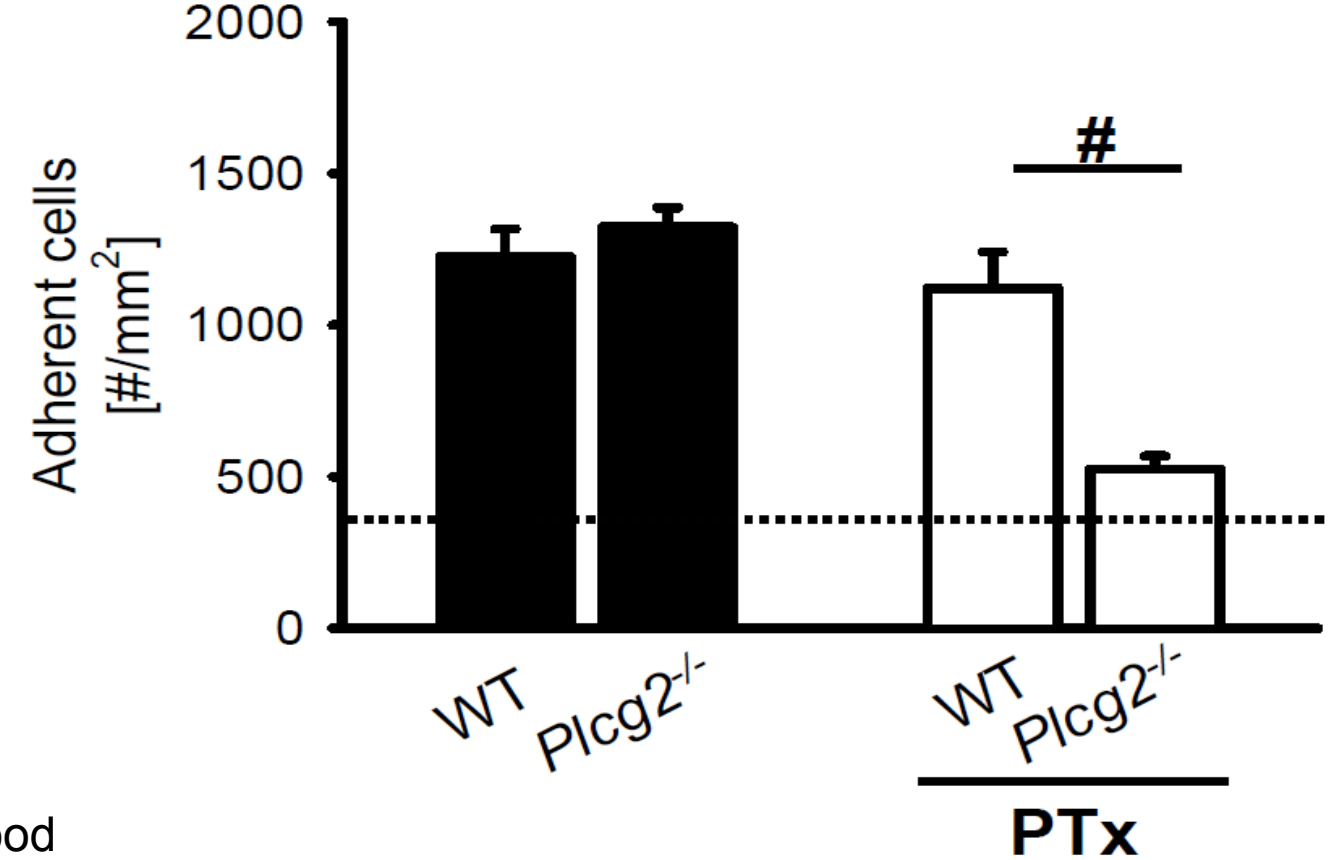


Mueller et al., Blood 2010

Phospholipase γ_2

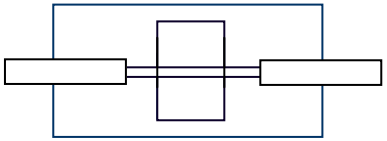


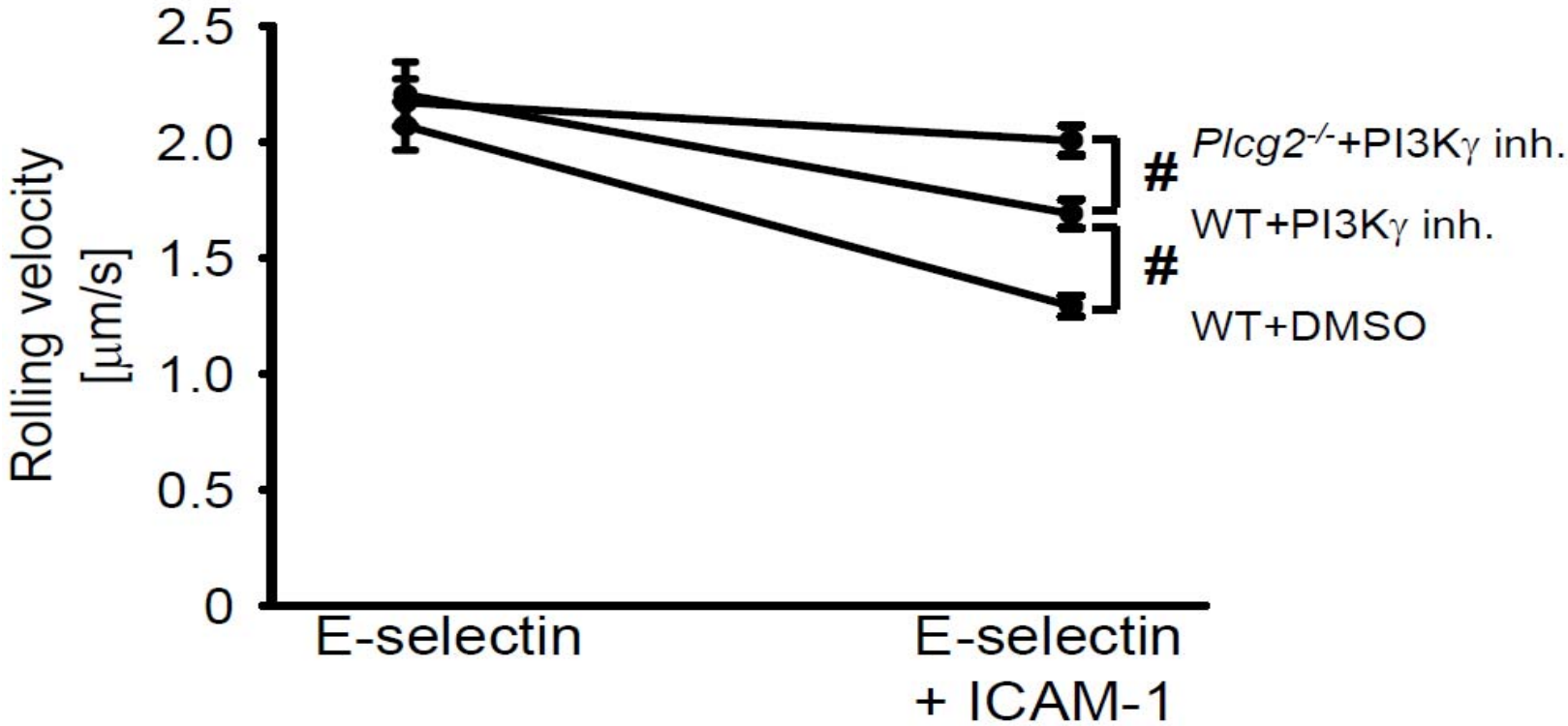




Mueller et al., Blood 2010

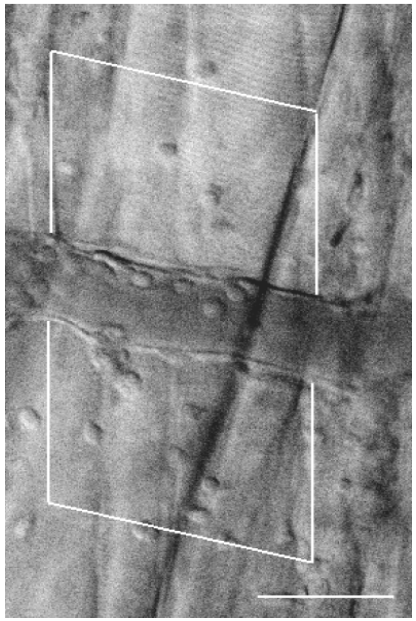
Phospholipase γ_2

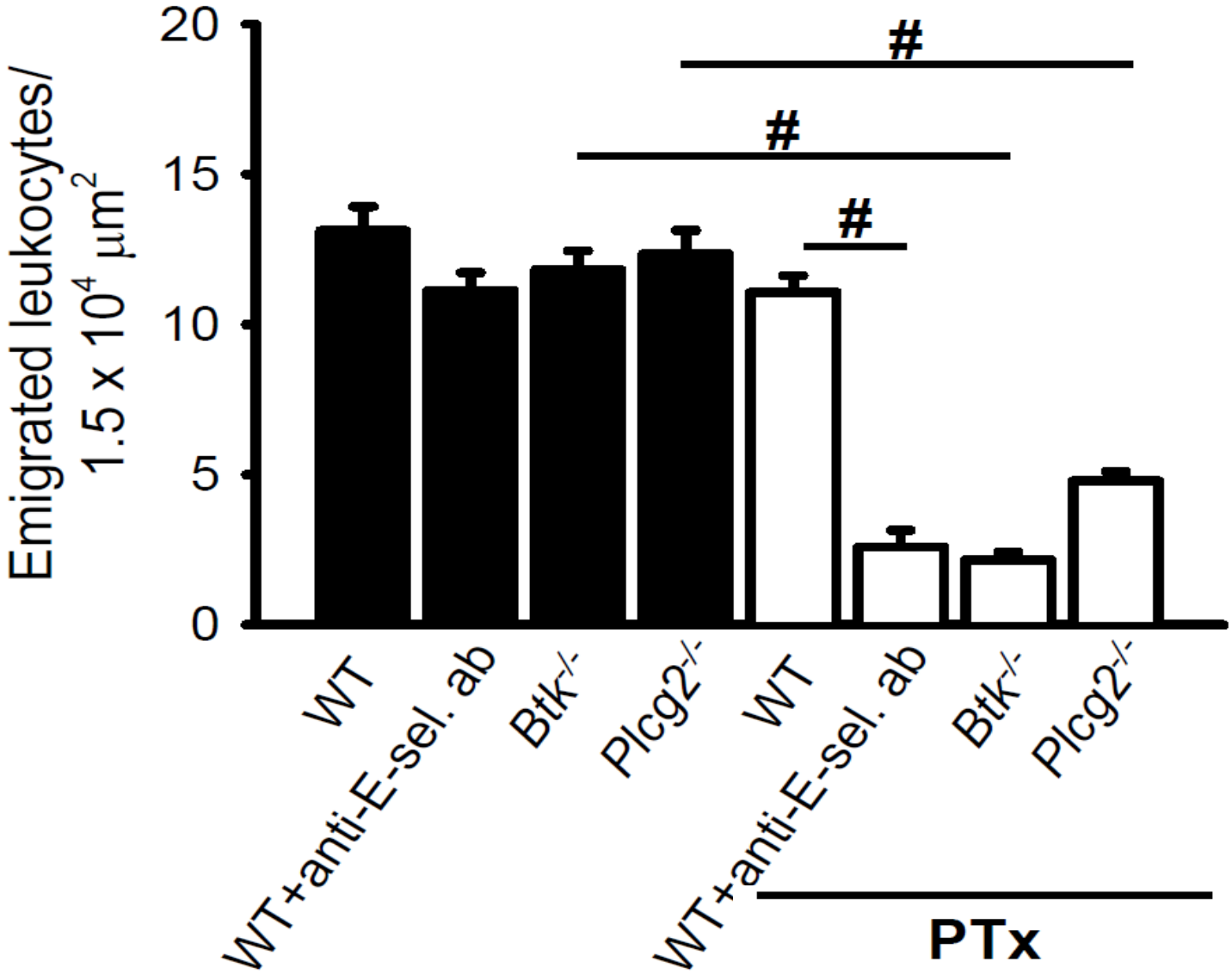




Mueller et al., Blood 2010

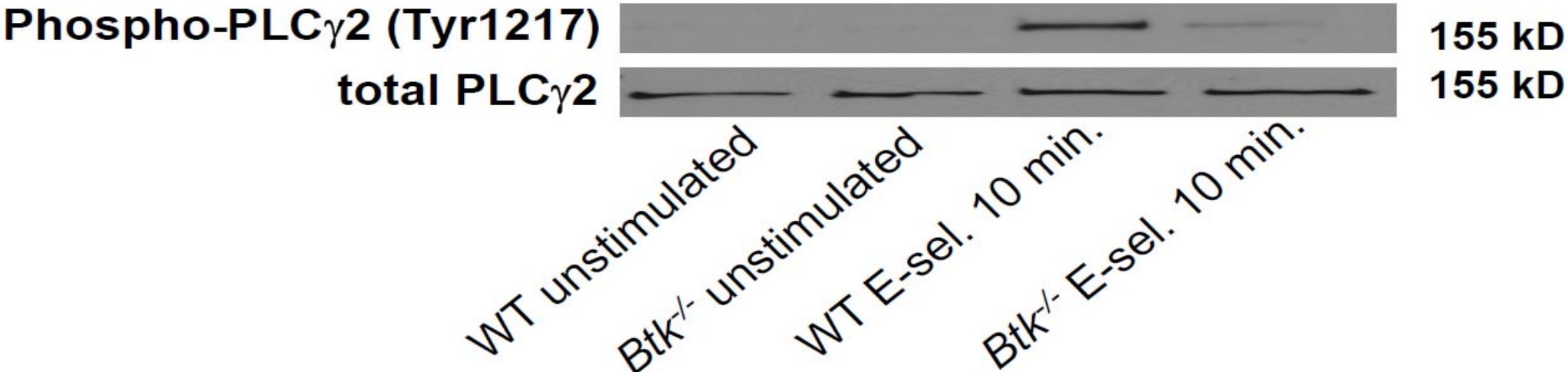
Phospholipase γ_2

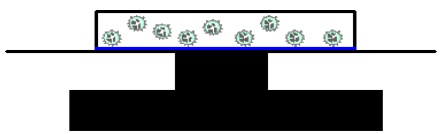


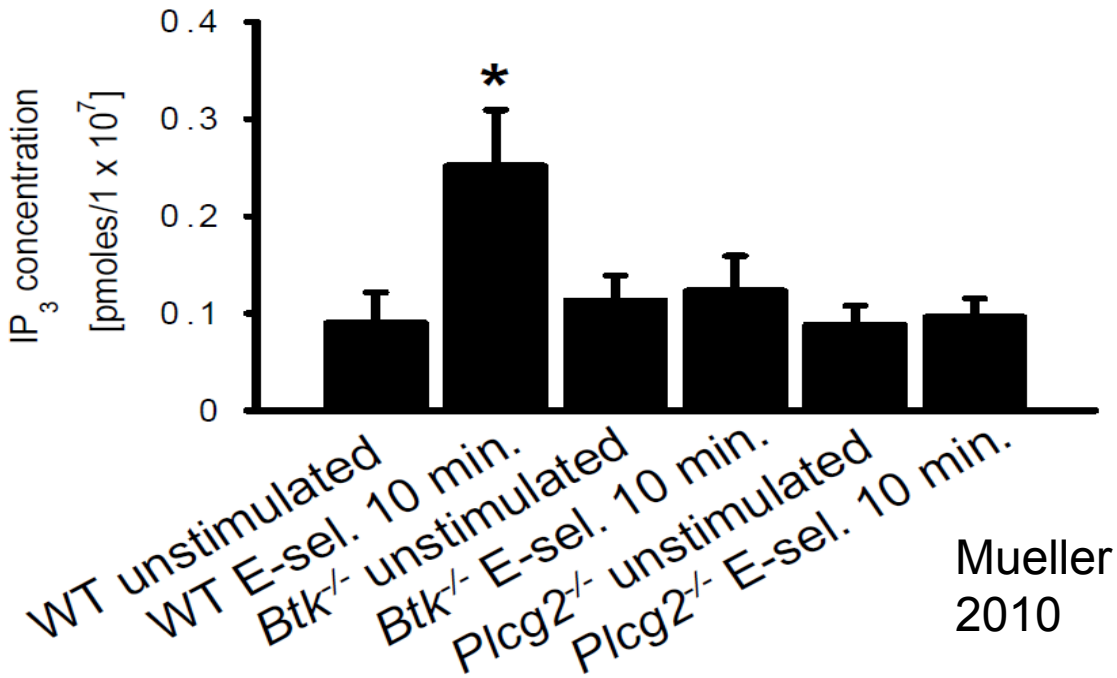


Mueller et al., Blood 2010

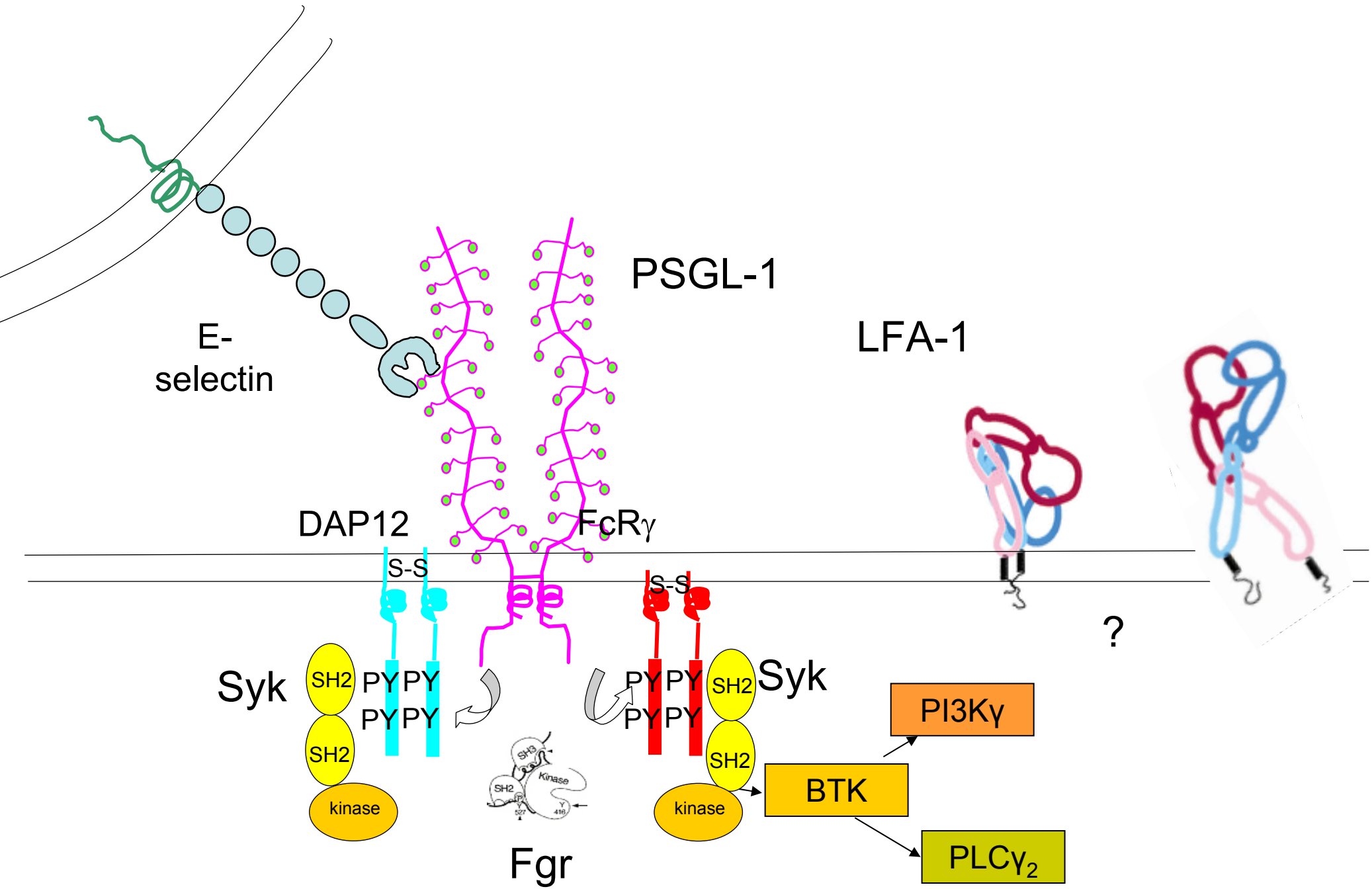
BTK upstream of Phospholipase γ_2



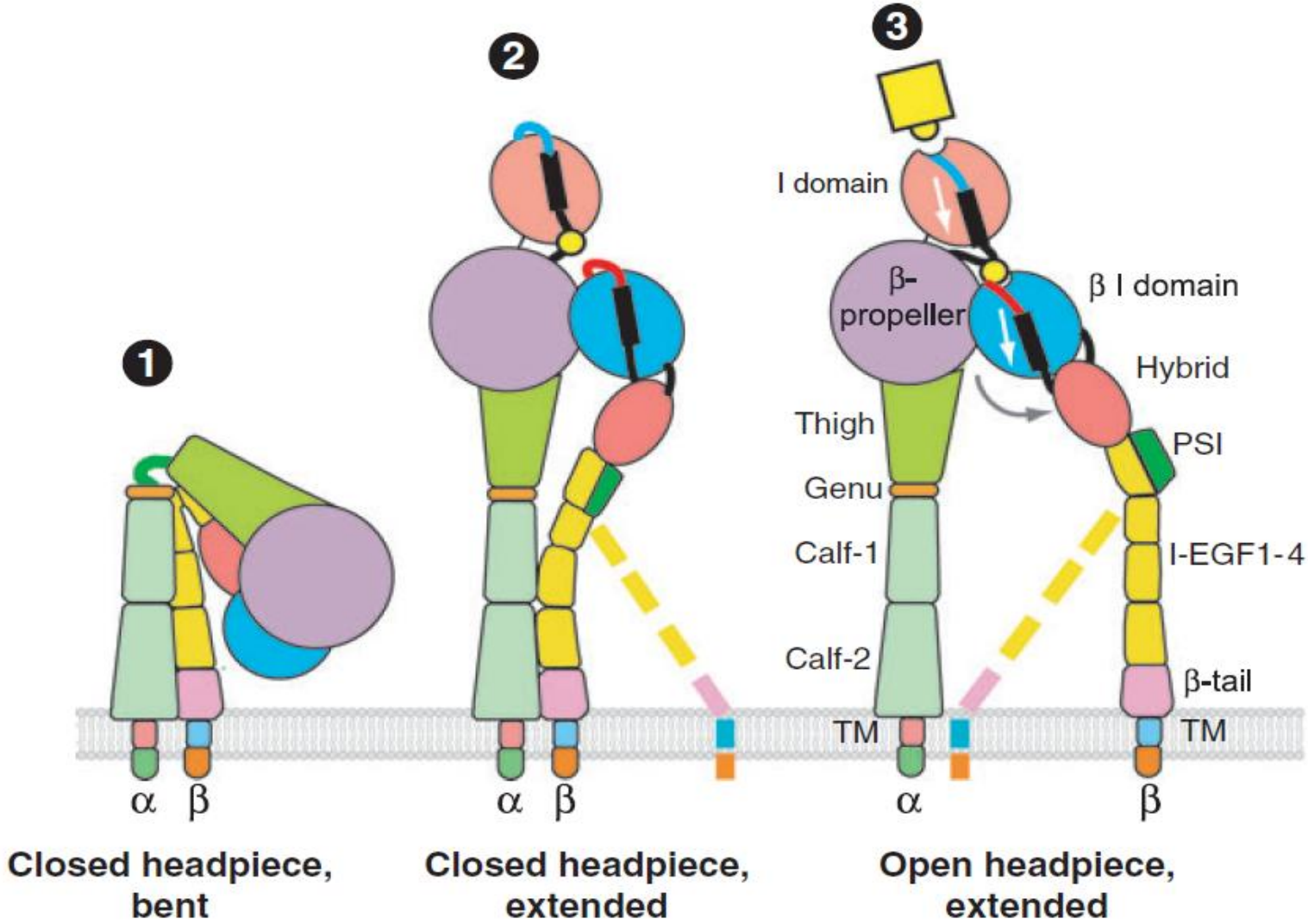




Mueller et al., Blood 2010


PSGL-1
E-
selectin
LFA-1
DAP12
FcRγ
S-S
S-S
?
Syk
SH2
SH2
kinase
PY PY
PY PY
PY PY
PY PY
SH2
SH2
kinase
Syk
Fgr
PI3Kγ
BTK
PLCγ2

Integrin Conformations

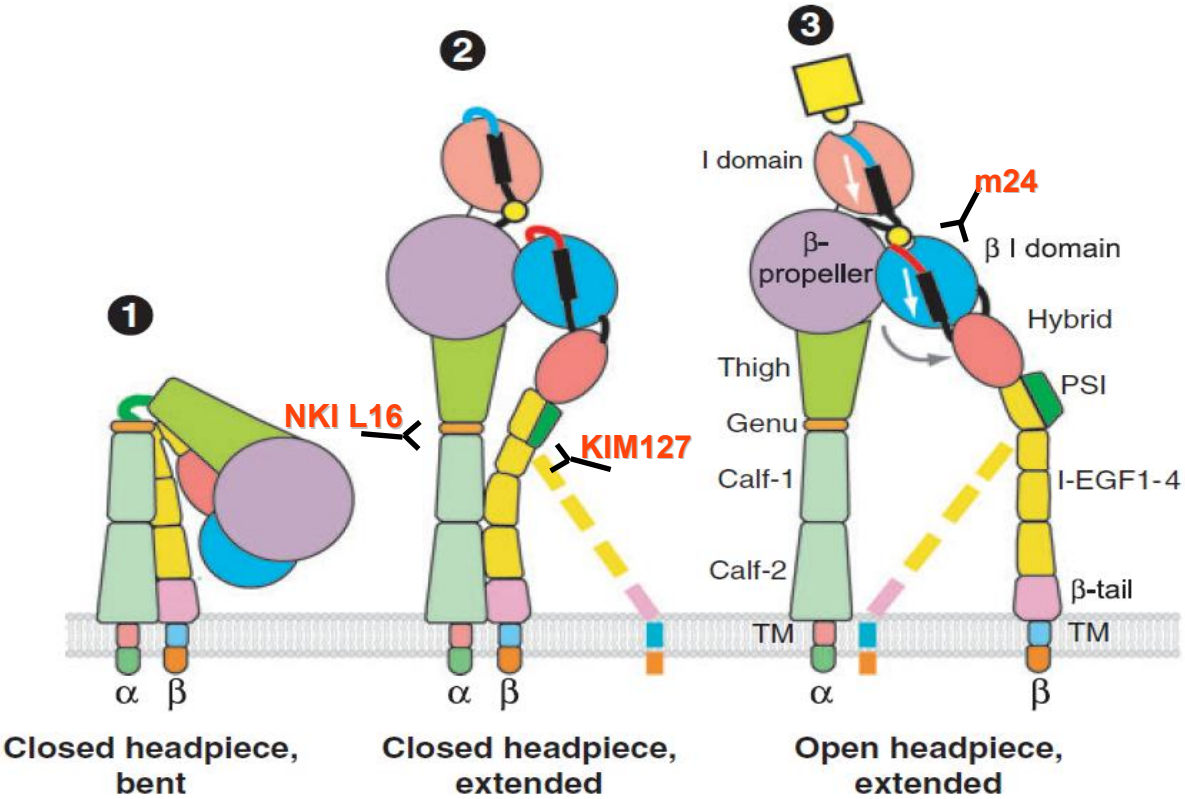


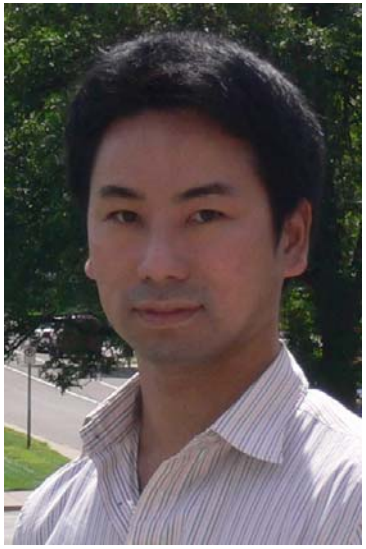
Luo BH et al. Annu Rev Immunol 2007

Rolling on E- or P-selectin induces the extended but not high-affinity conformation of LFA-1 in neutrophils

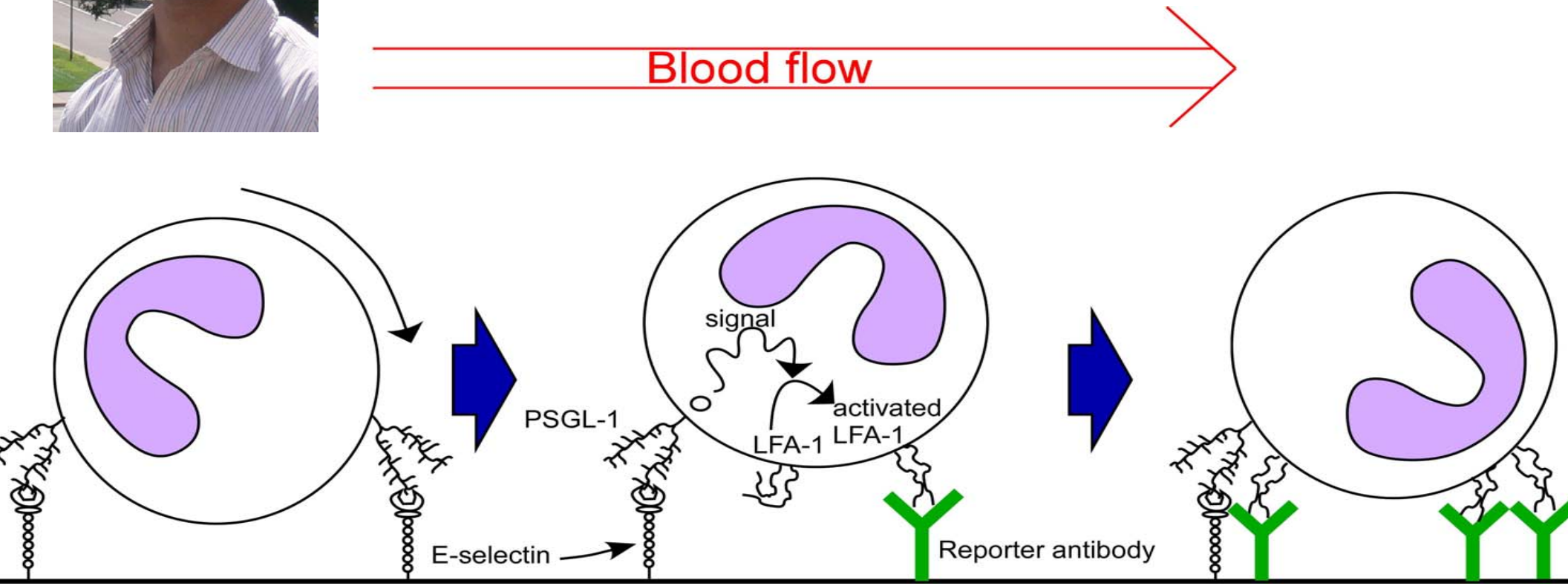
*Yoshihiro Kuwano,[1] *Oliver Spelten,[2,3] Hong Zhang,[1] †Klaus Ley,[1] and †Alexander Zarbock[2,3]

[1]Division of Inflammation Biology, La Jolla Institute for Allergy & Immunology, CA; [2]Department of Anesthesiology and Intensive Care Medicine, University of Münster, Münster, Germany; and [3]Max-Planck Institute for Molecular Biomedicine, Münster, Germany



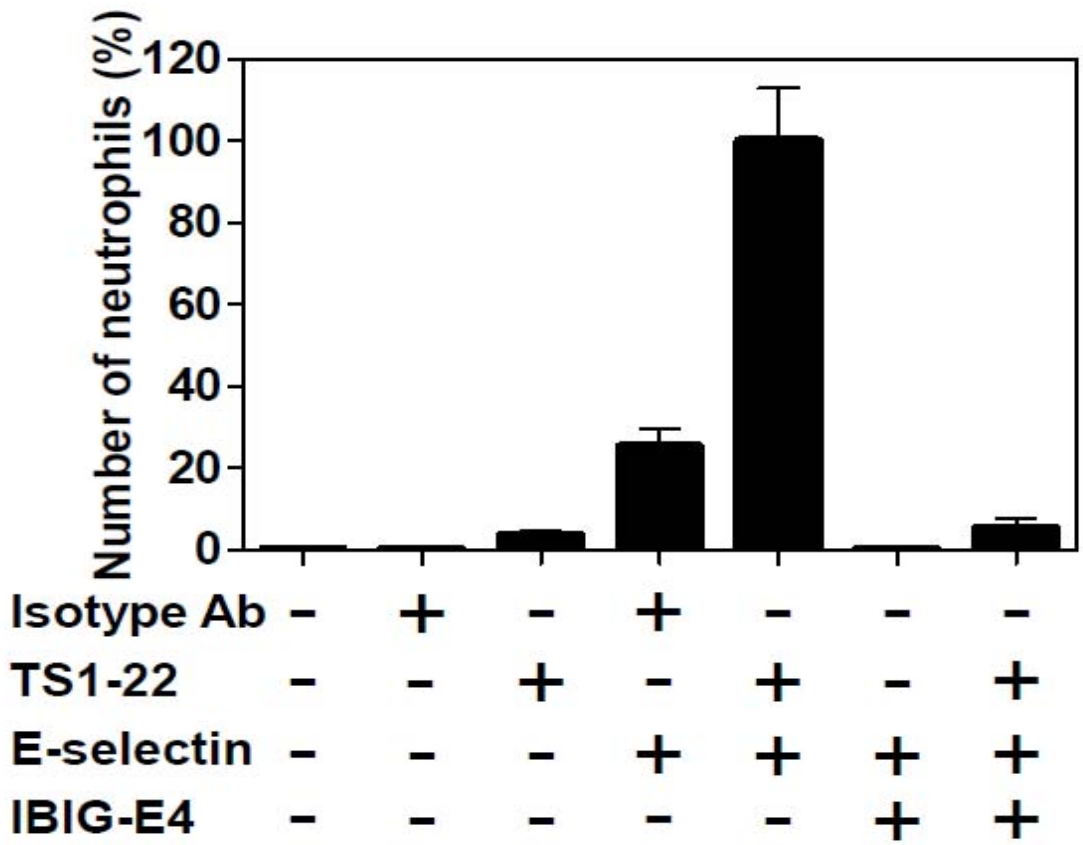


Immobilized reporter assay



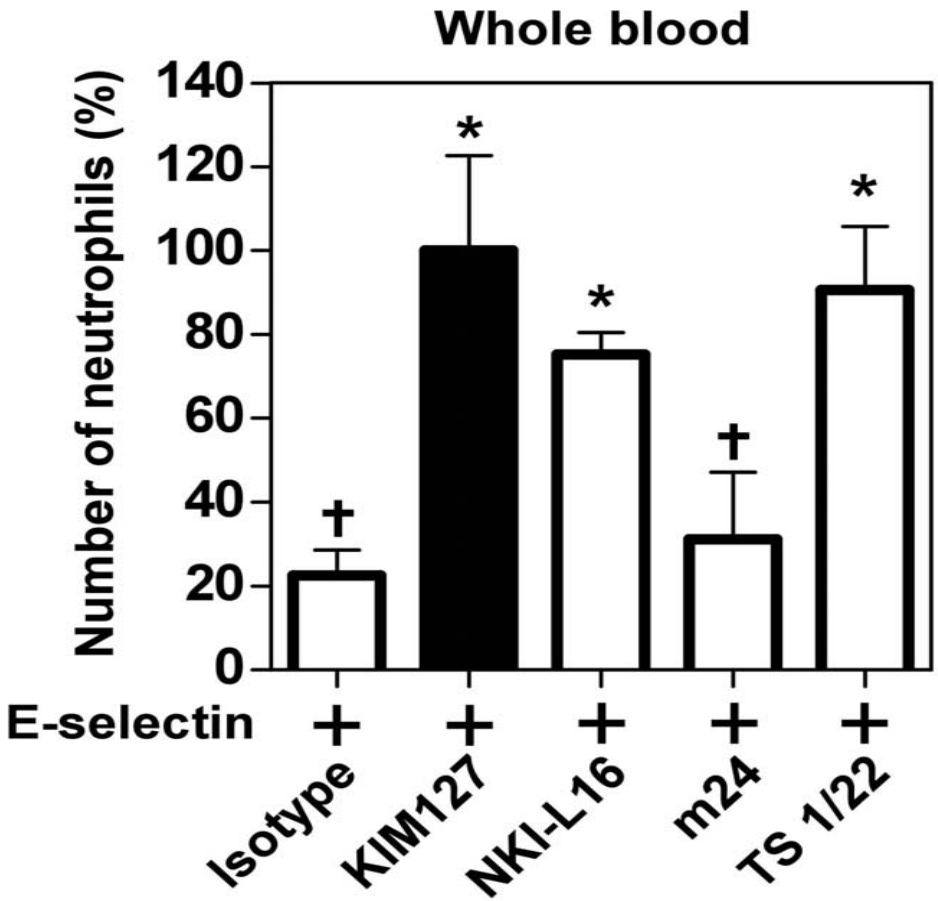
Kuwano et al., Blood 2010

Immobilized reporter assay: Specificity controls



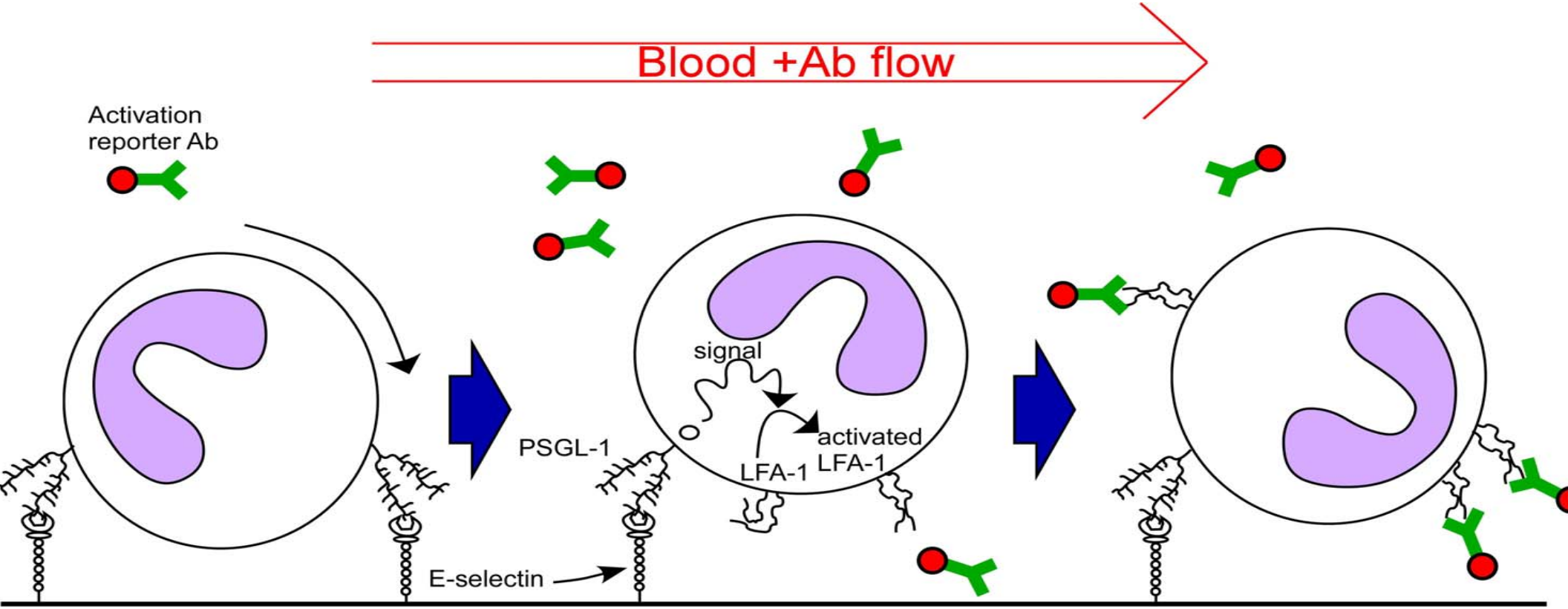
Kuwano et al., Blood 2010

Immobilized reporter assay



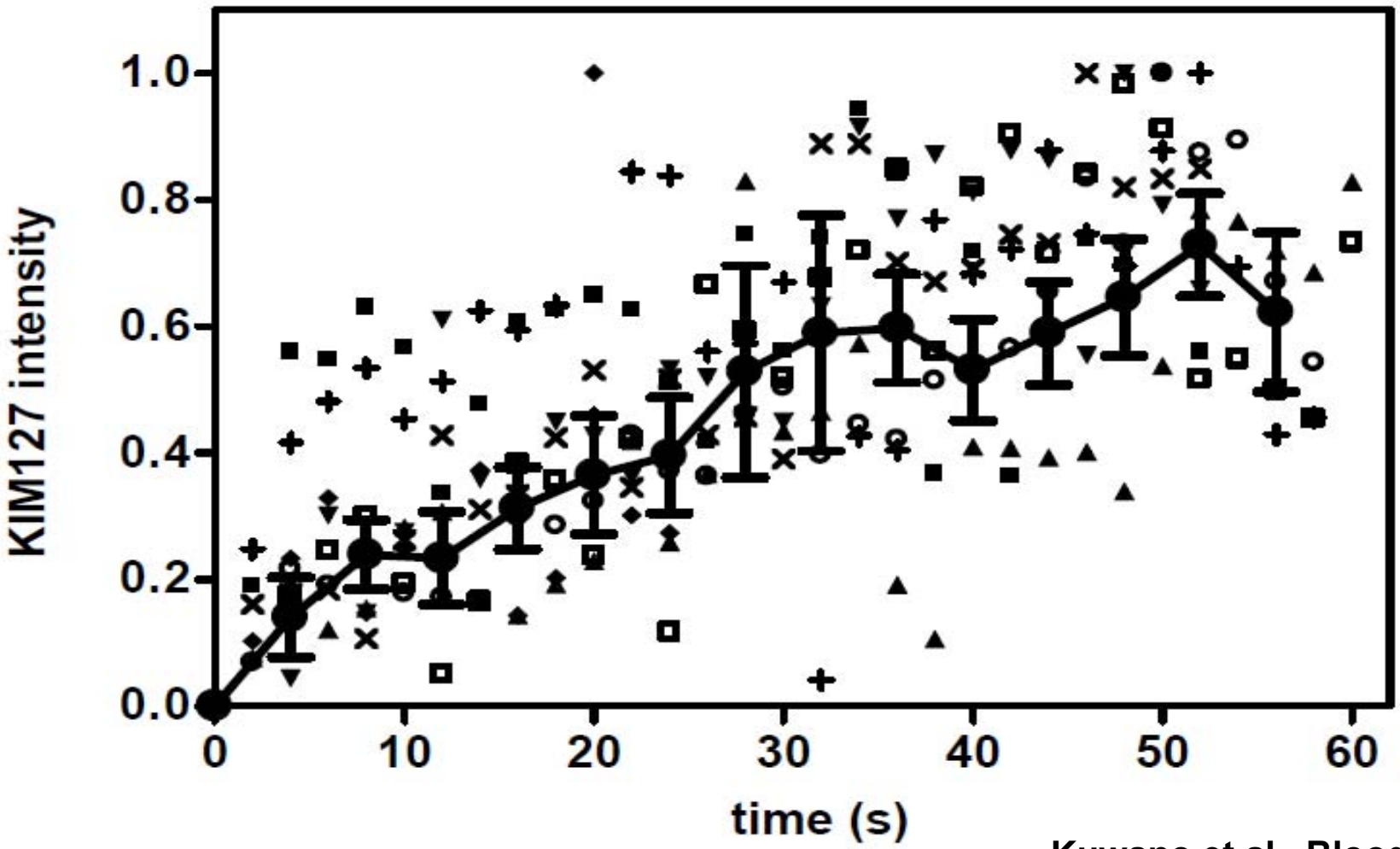
Kuwano et al., Blood 2010

Fluid phase reporter assay



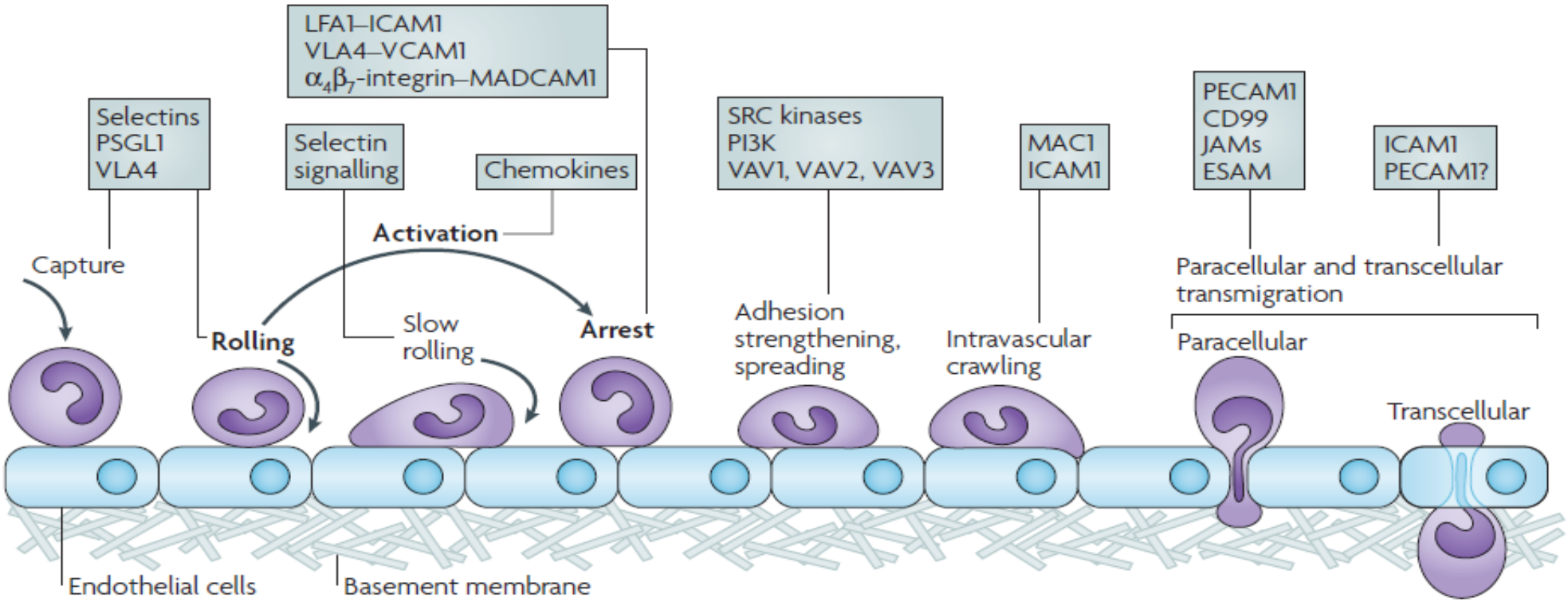
Kuwano et al., Blood 2010

Fluid phase reporter assay

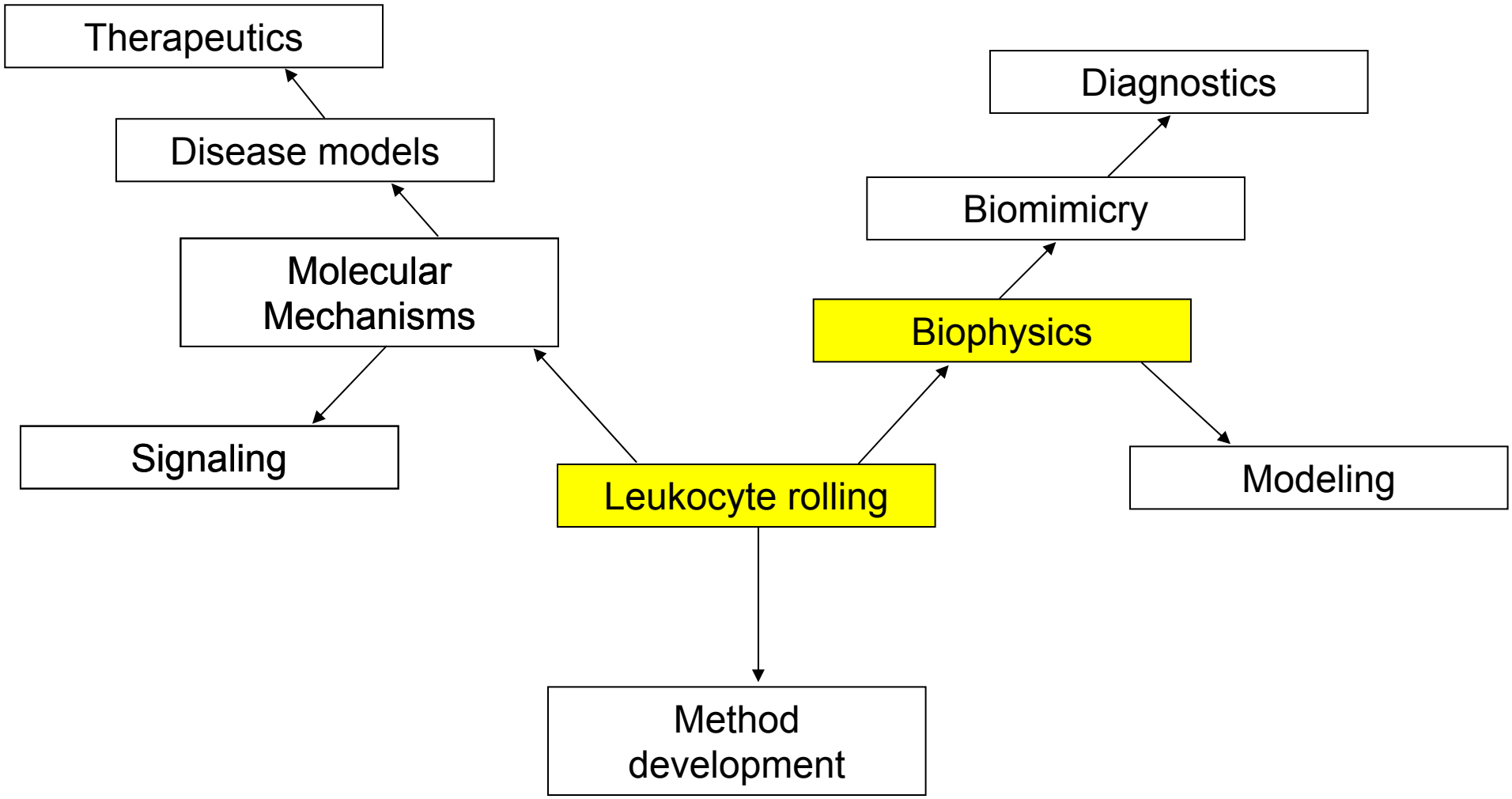


Kuwano et al., Blood 2010

Adhesion Cascade



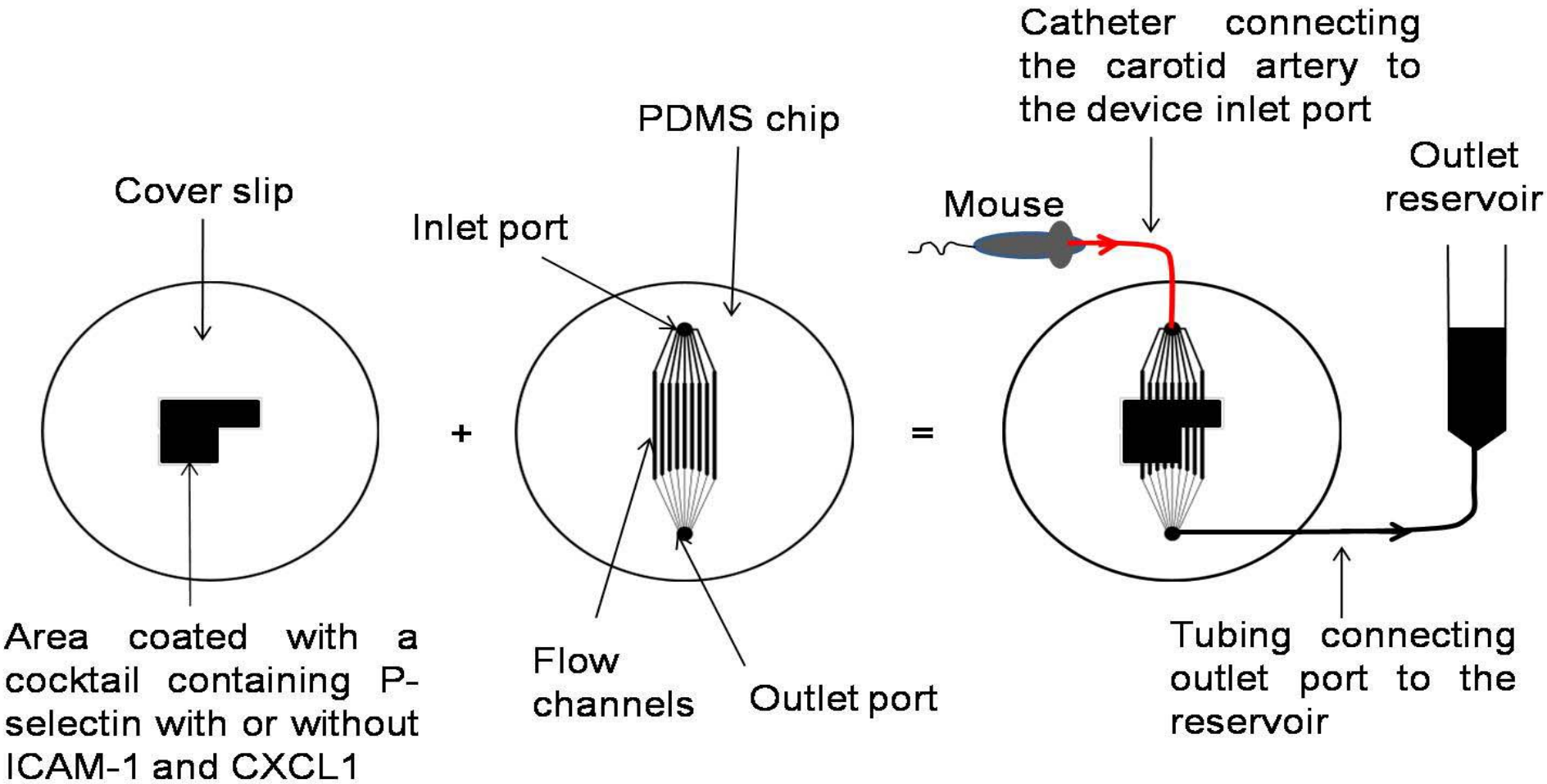
Nat. Rev. Immunol. 7:678-689.

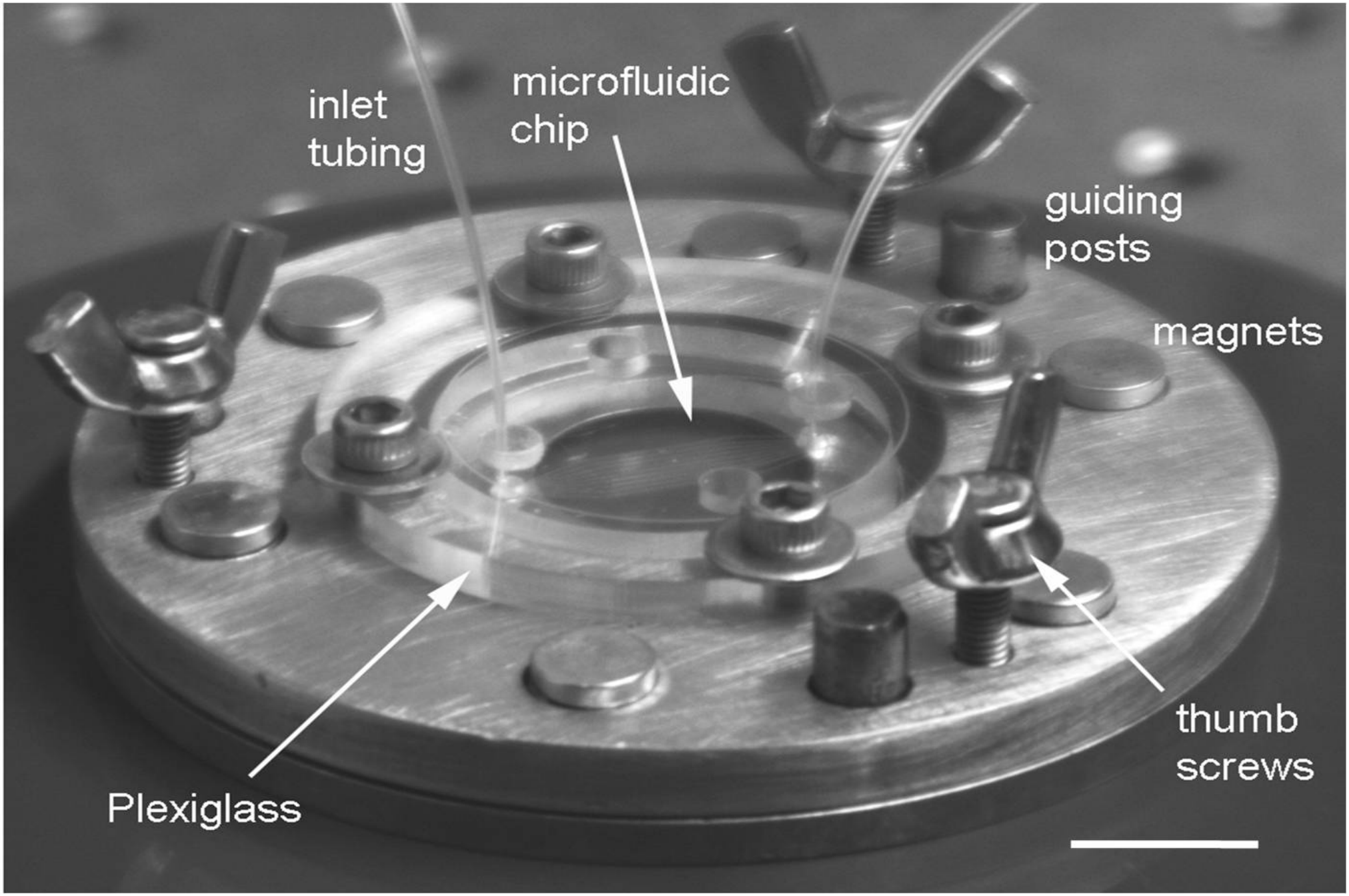

Therapeutics
Disease models
Molecular Mechanisms
Signaling
Leukocyte rolling
Method development
Biophysics
Biomimicry
Diagnostics
Modeling

Quantitative dynamic footprinting microscopy reveals mechanisms of neutrophil rolling

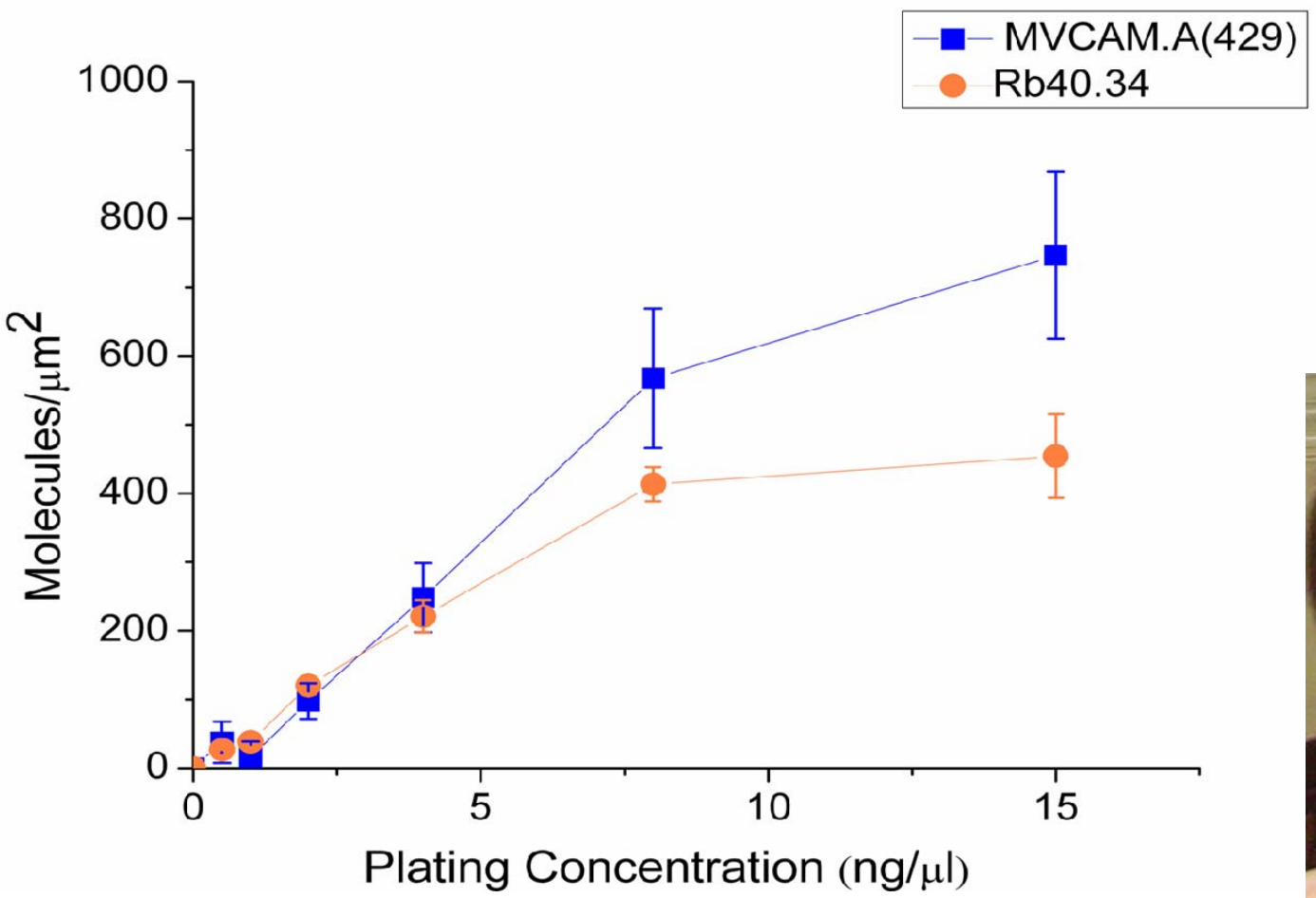
Prithu Sundd[1], Edgar Gutierrez[2], Maria K Pospieszalska[1], Hong Zhang[1], Alexander Groisman[2] & Klaus Ley[1]

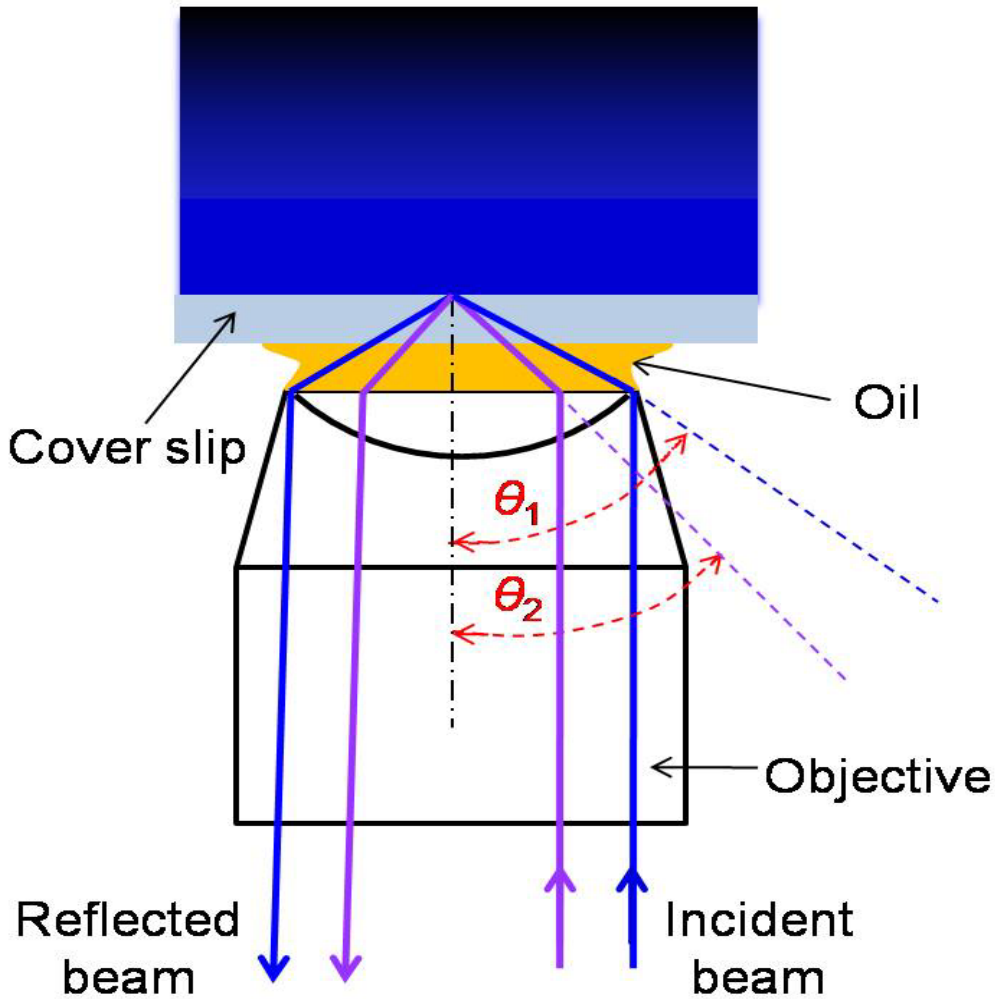
Microfluidics



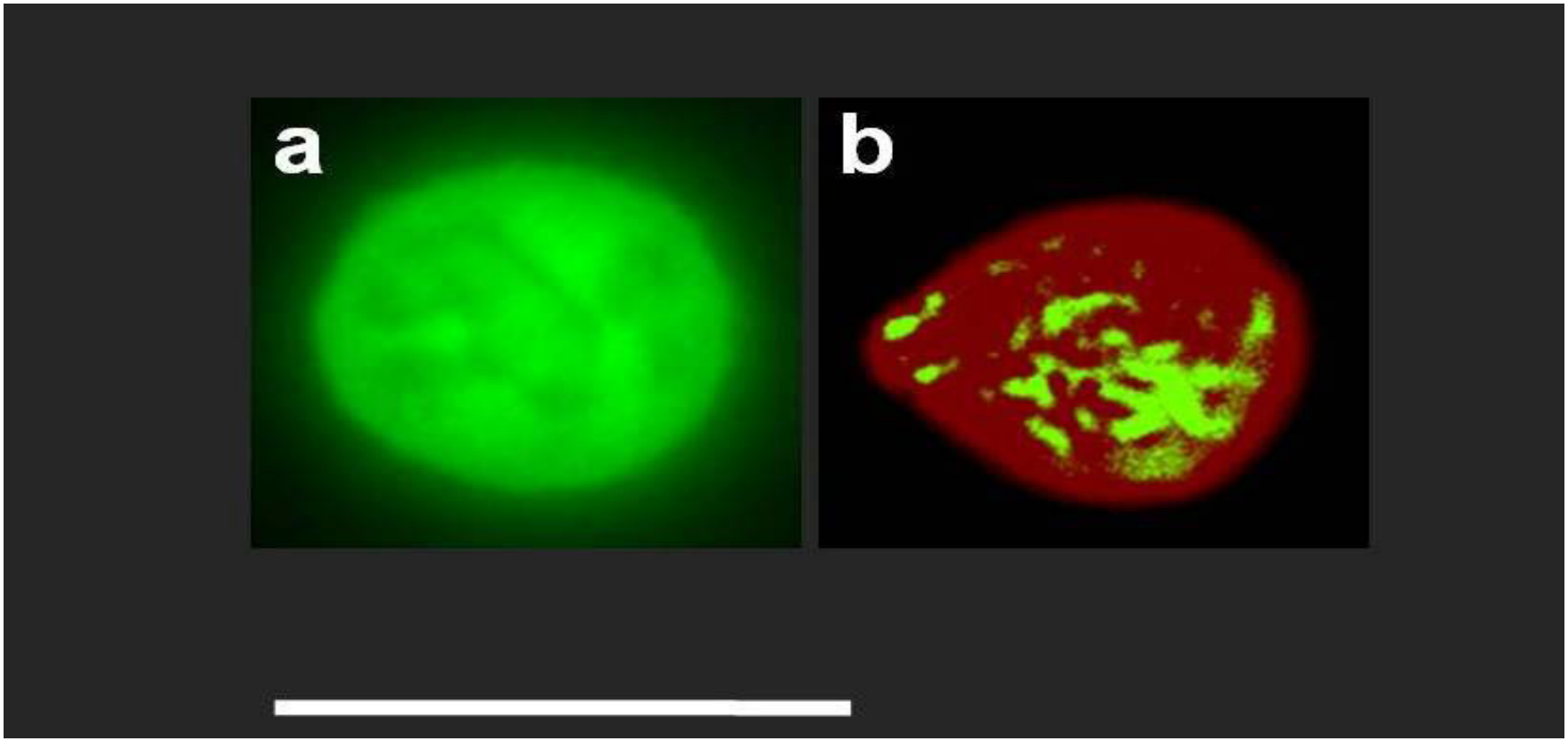

inlet
tubing
microfluidic
chip
guiding
posts
magnets
thumb
screws
Plexiglass

Controlling Site Density

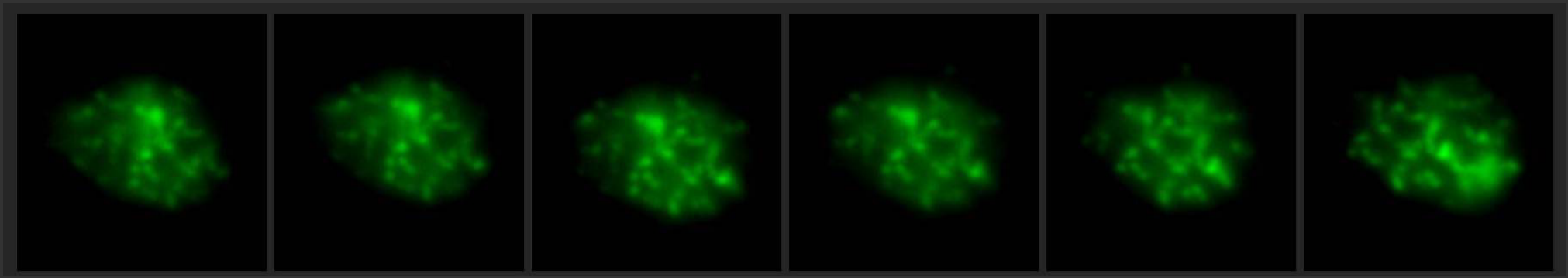


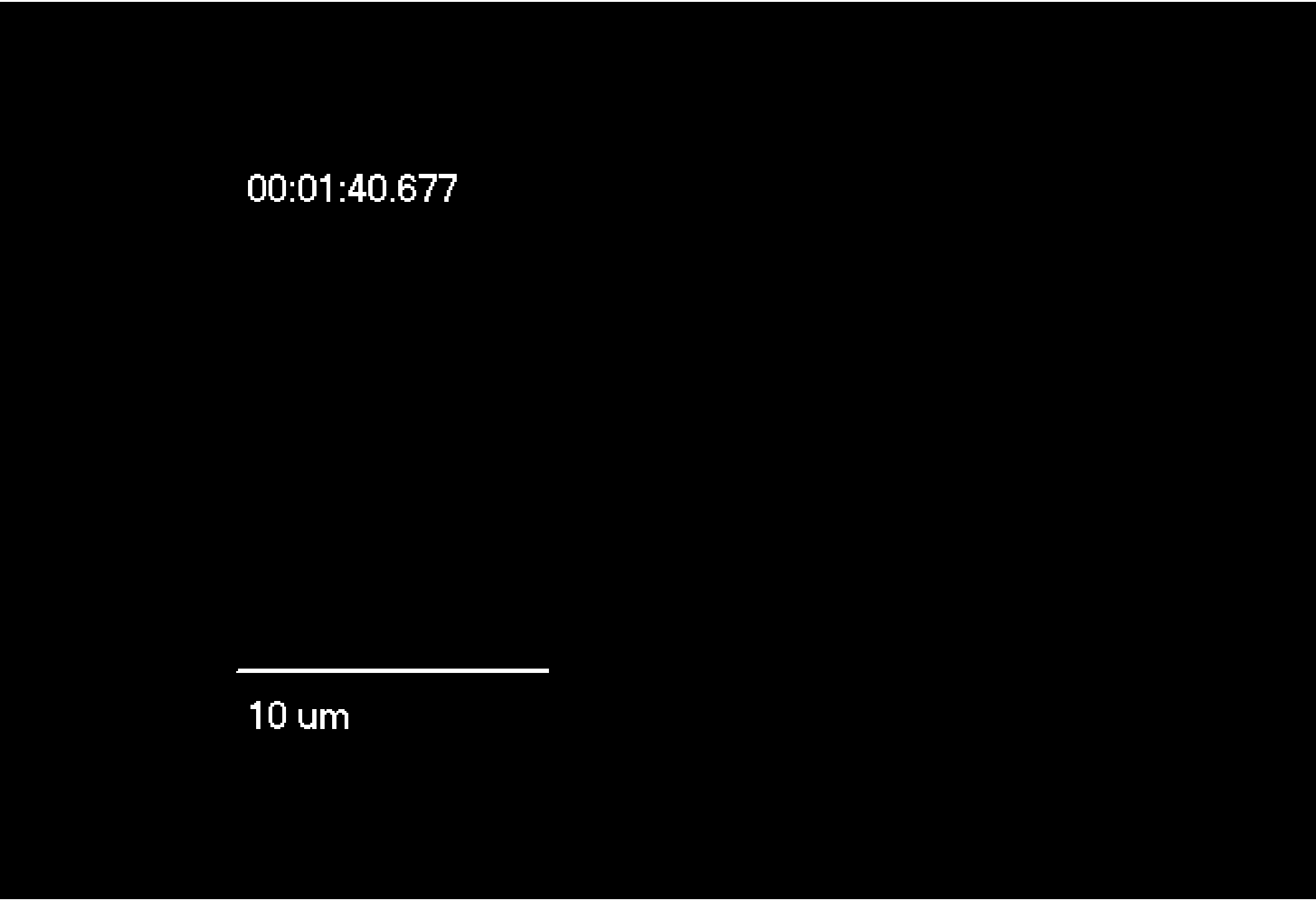

Cover slip
Oil
θ_1
θ_2
Objective
Reflected beam
Incident beam

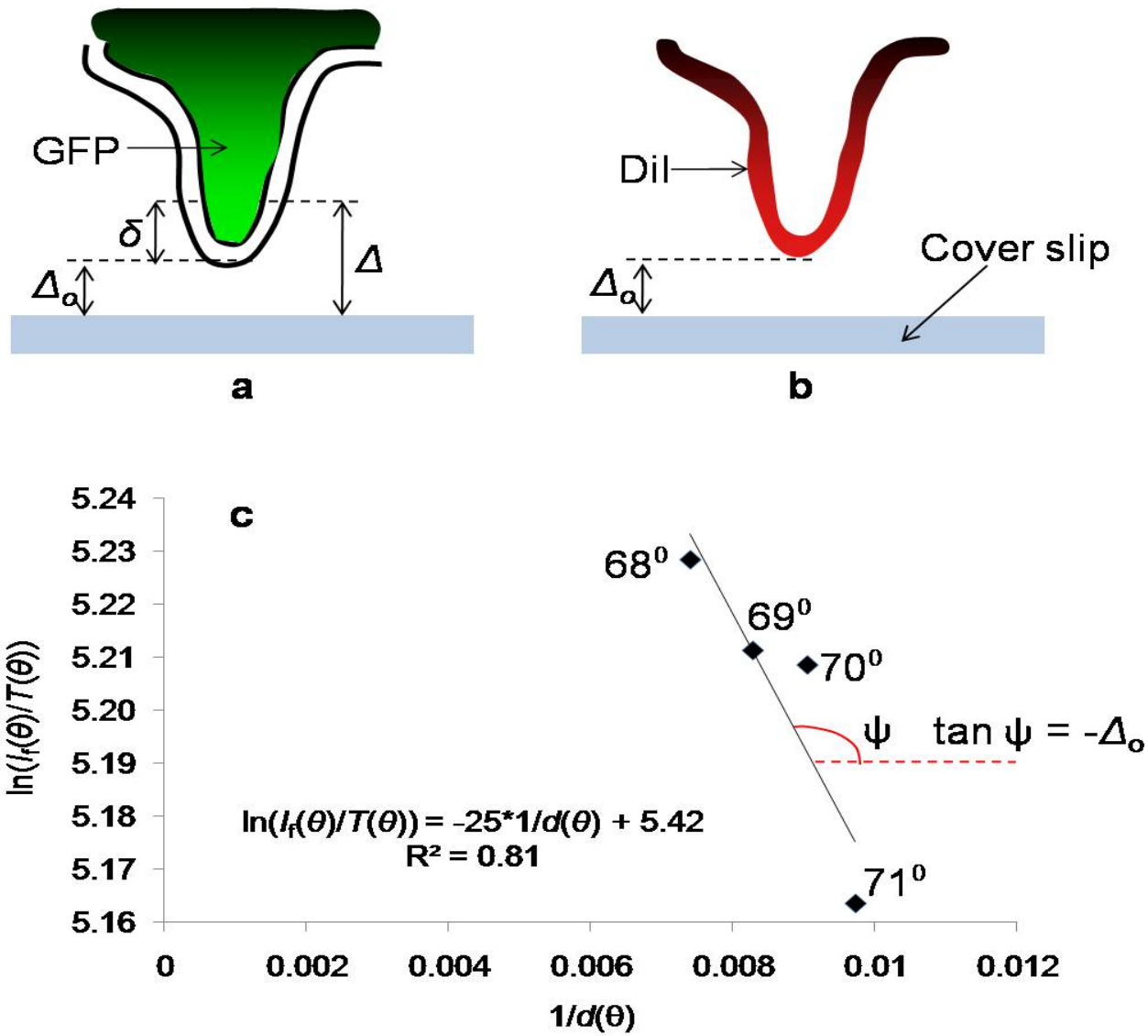
Epifluorescence vs TIRF

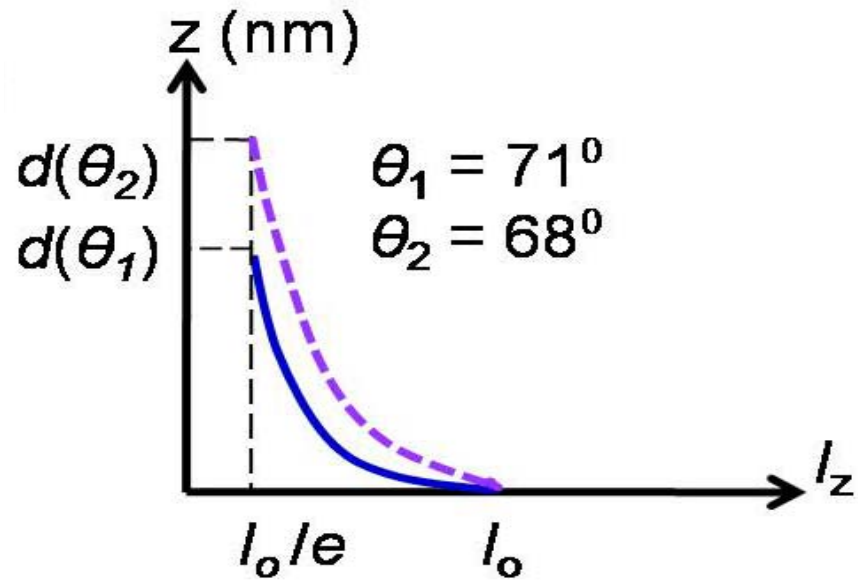


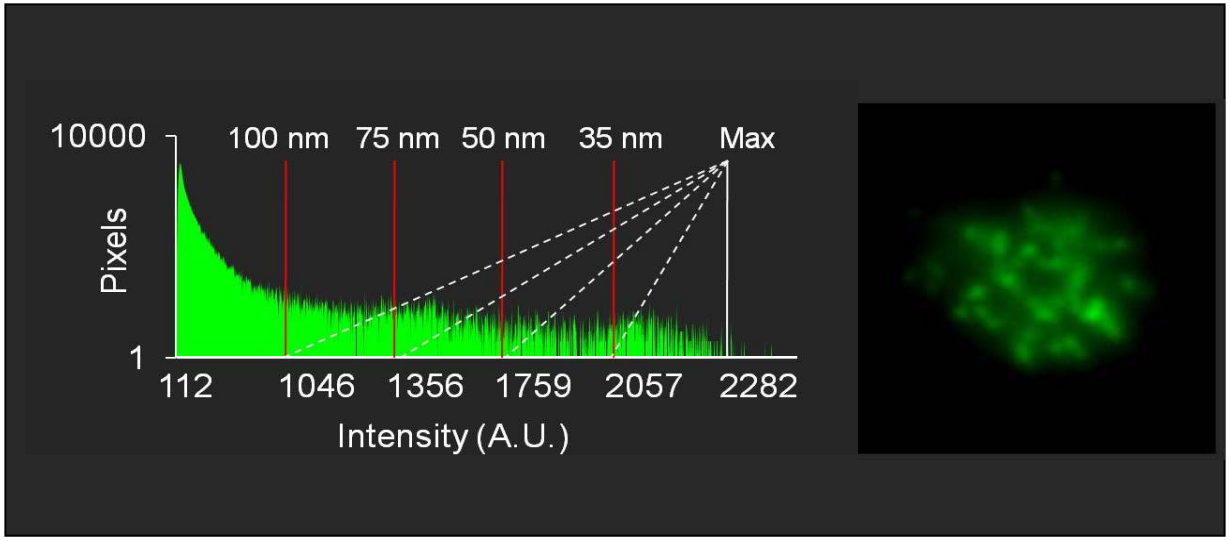
TIRF footprints of rolling *Lysm*^{GFP} neutrophil

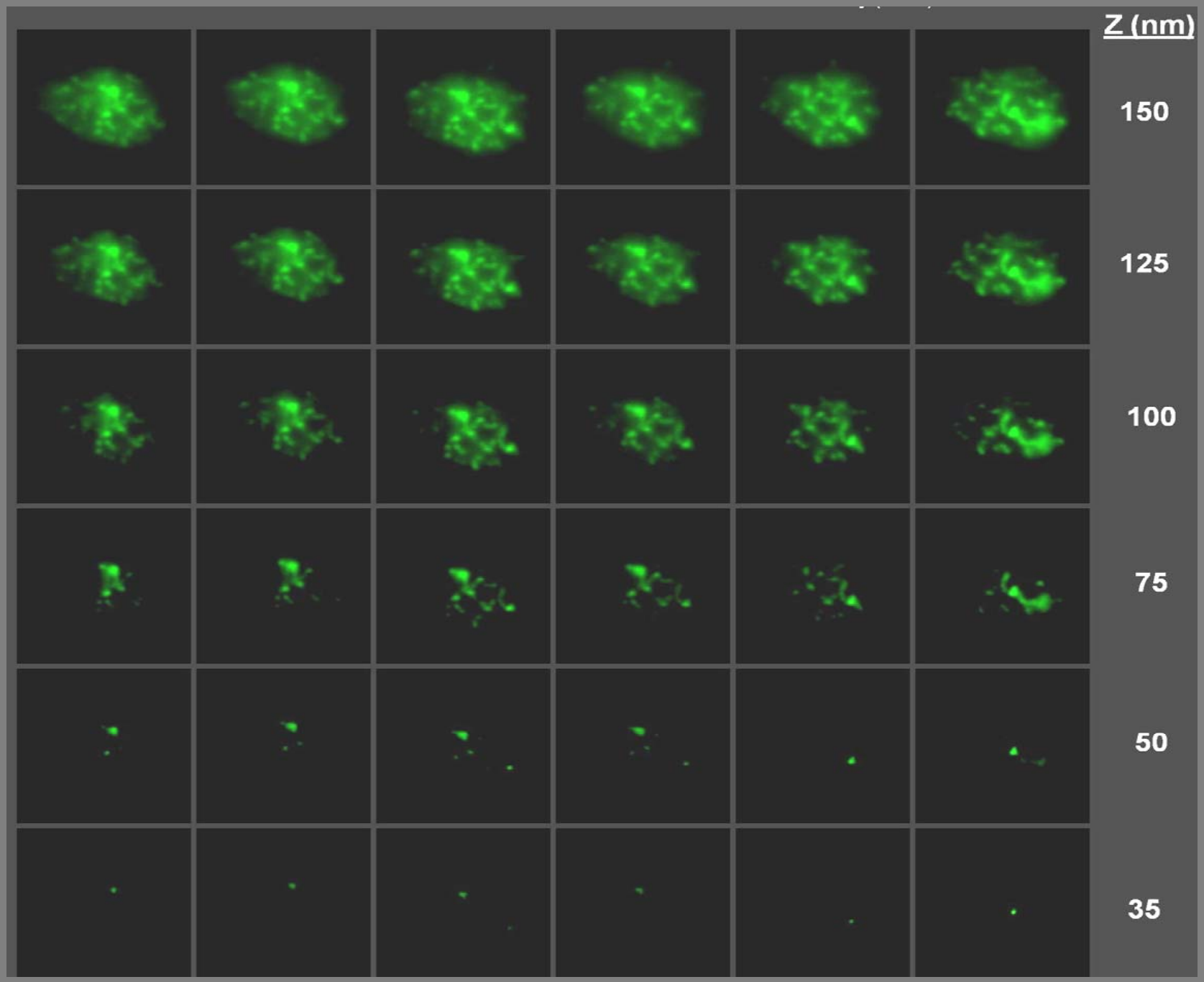



00:01:40.677
10 um

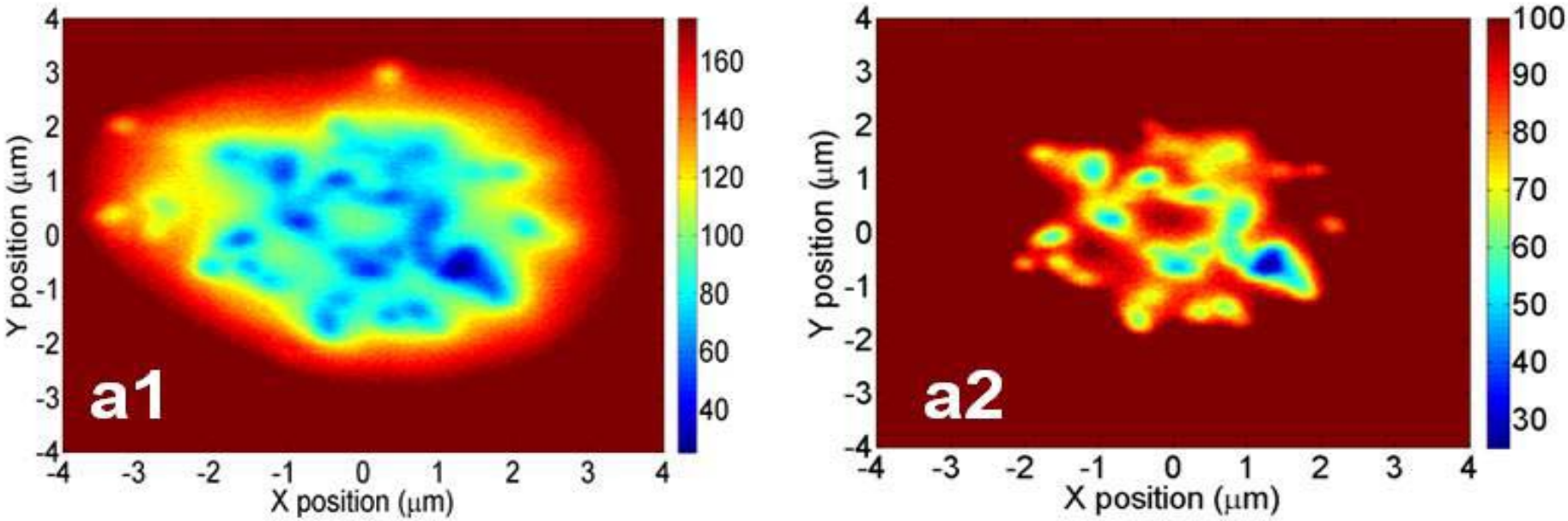

GFP
δ
Δ
Δo
a
DiI
Cover slip
Δo
b
c
5.24
5.23
5.22
5.21
5.20
5.19
5.18
5.17
5.16
ln(If(θ)/T(θ))
68⁰
69⁰
70⁰
71⁰
ψ
tan ψ = -Δo
ln(If(θ)/T(θ)) = -25*1/d(θ) + 5.42
R² = 0.81
0
0.002
0.004
0.006
0.008
0.01
0.012
1/d(θ)


z (nm)
d(θ2)
d(θ1)
θ1 = 71⁰
θ2 = 68⁰
lz
lo/e
lo

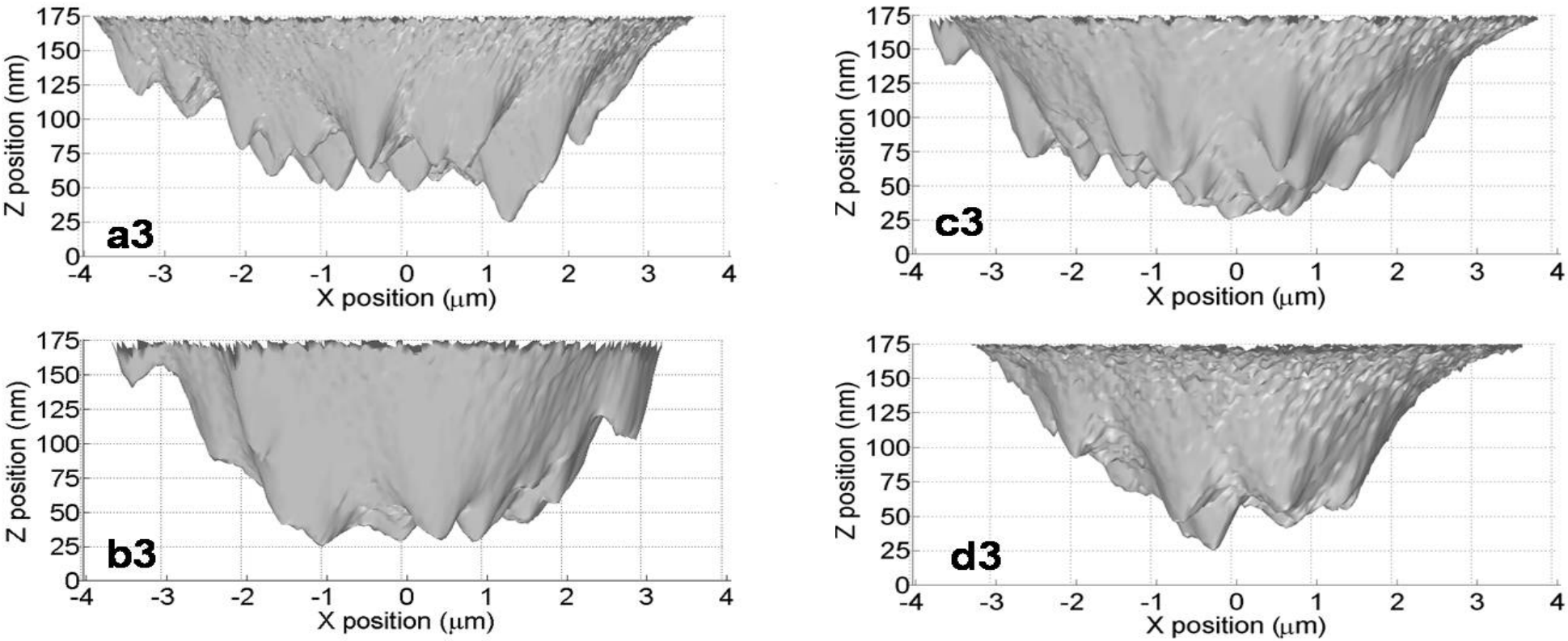

10000
1
Pixels
100 nm
75 nm
50 nm
35 nm
Max
112
1046
1356
1759
2057
2282
Intensity (A.U.)


Z (nm)
150
125
100
75
50
35

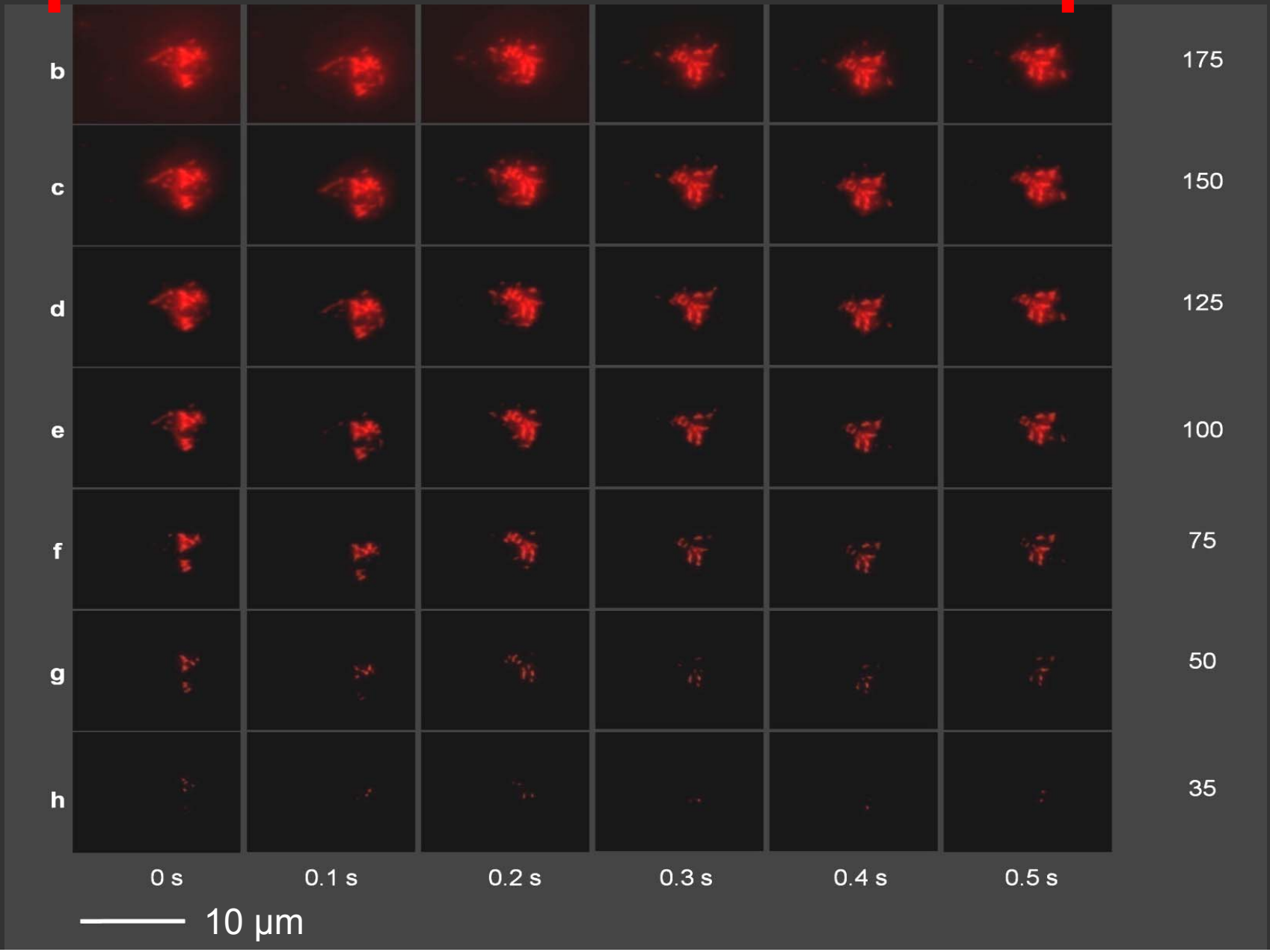
TIRF footprints of rolling *Lysm*^{GFP} neutrophil

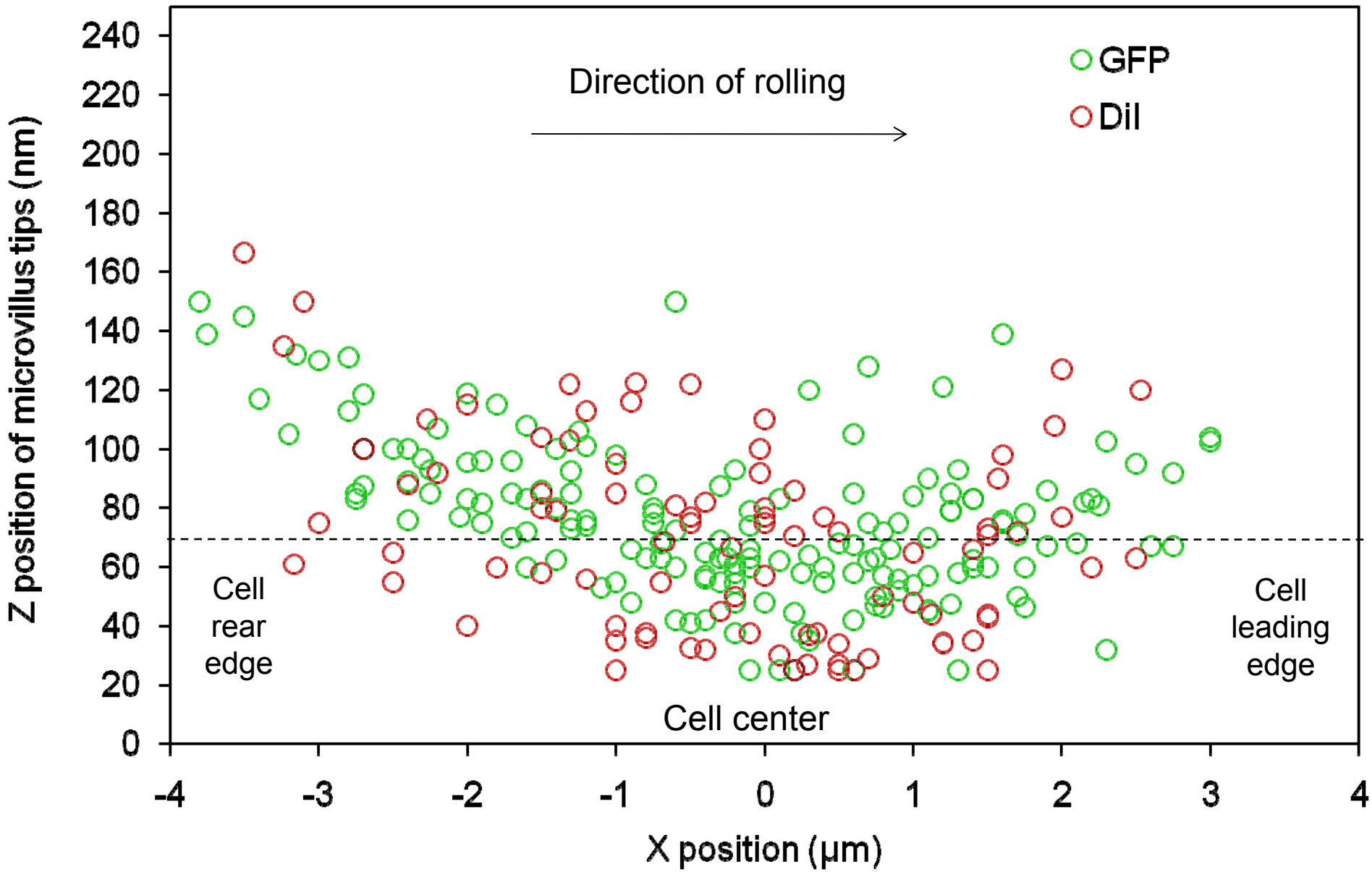


TIRF footprints of rolling *Lysm*^{GFP} neutrophils



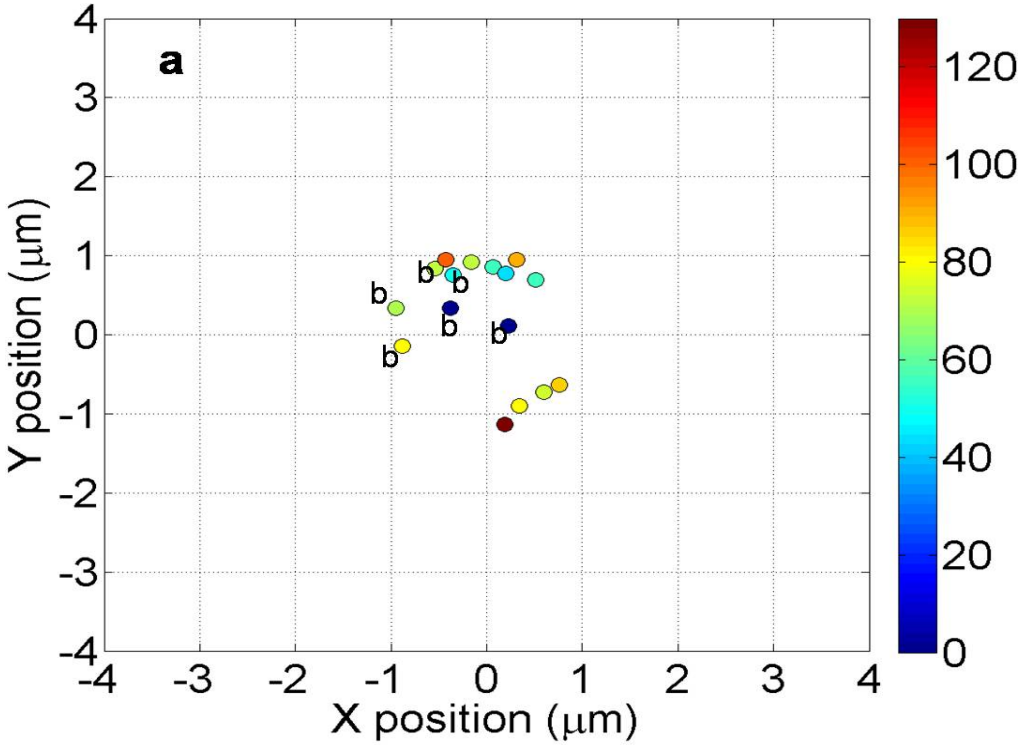
qDF of DiI-labeled neutrophil



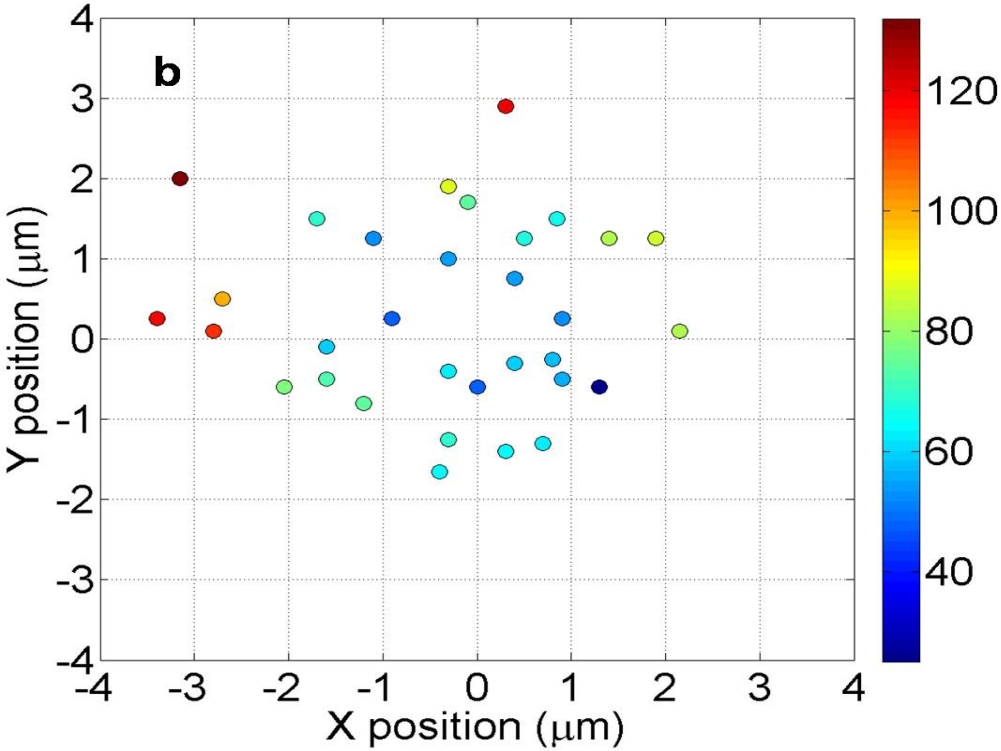

Direction of rolling
GFP
DiI
Cell rear edge
Cell leading edge
Cell center
Z position of microvillus tips (nm)
X position (µm)
240
220
200
180
160
140
120
100
80
60
40
20
0
-4
-3
-2
-1
0
1
2
3
4

Footprints of rolling neutrophil

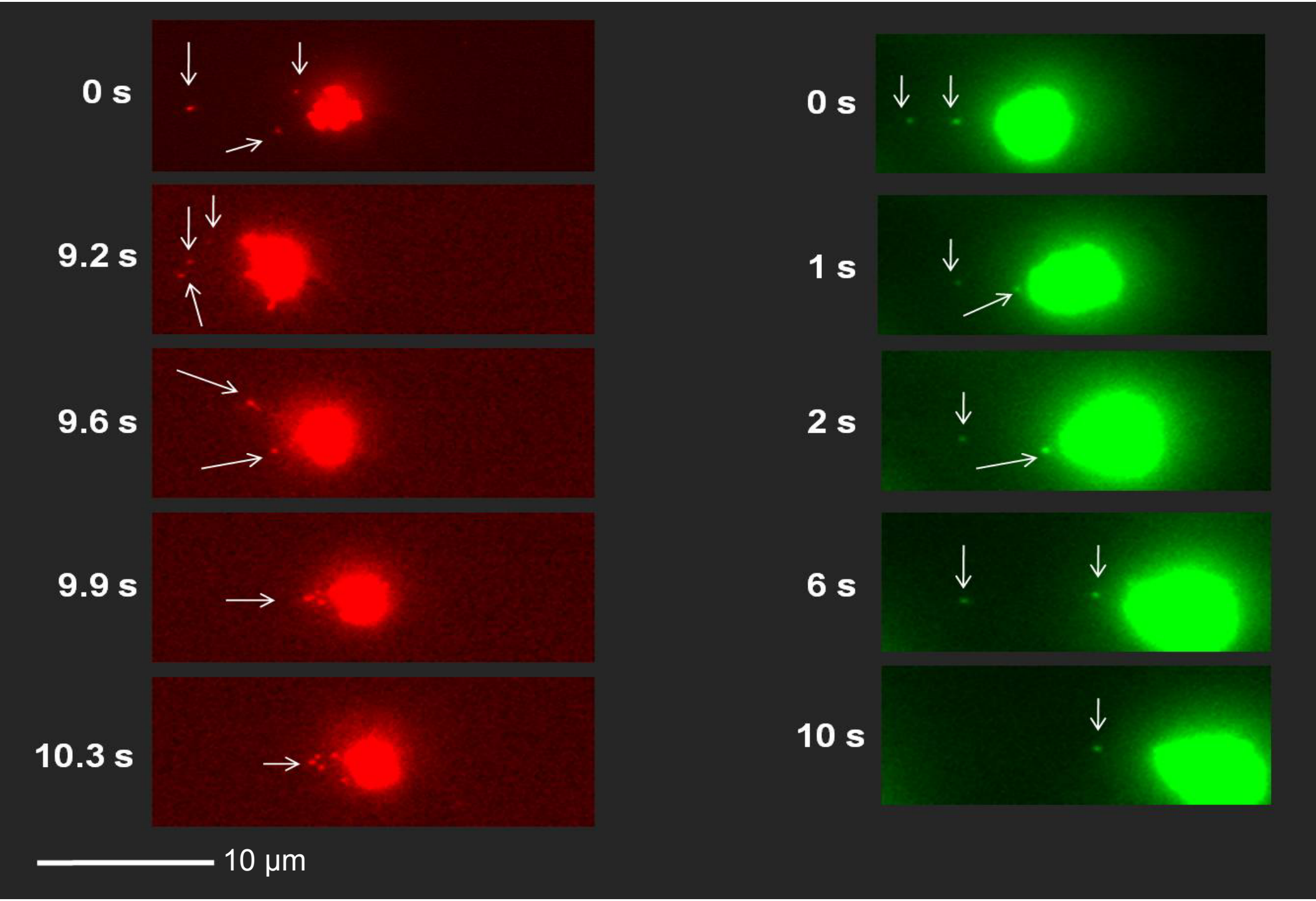
Model (ETMA)

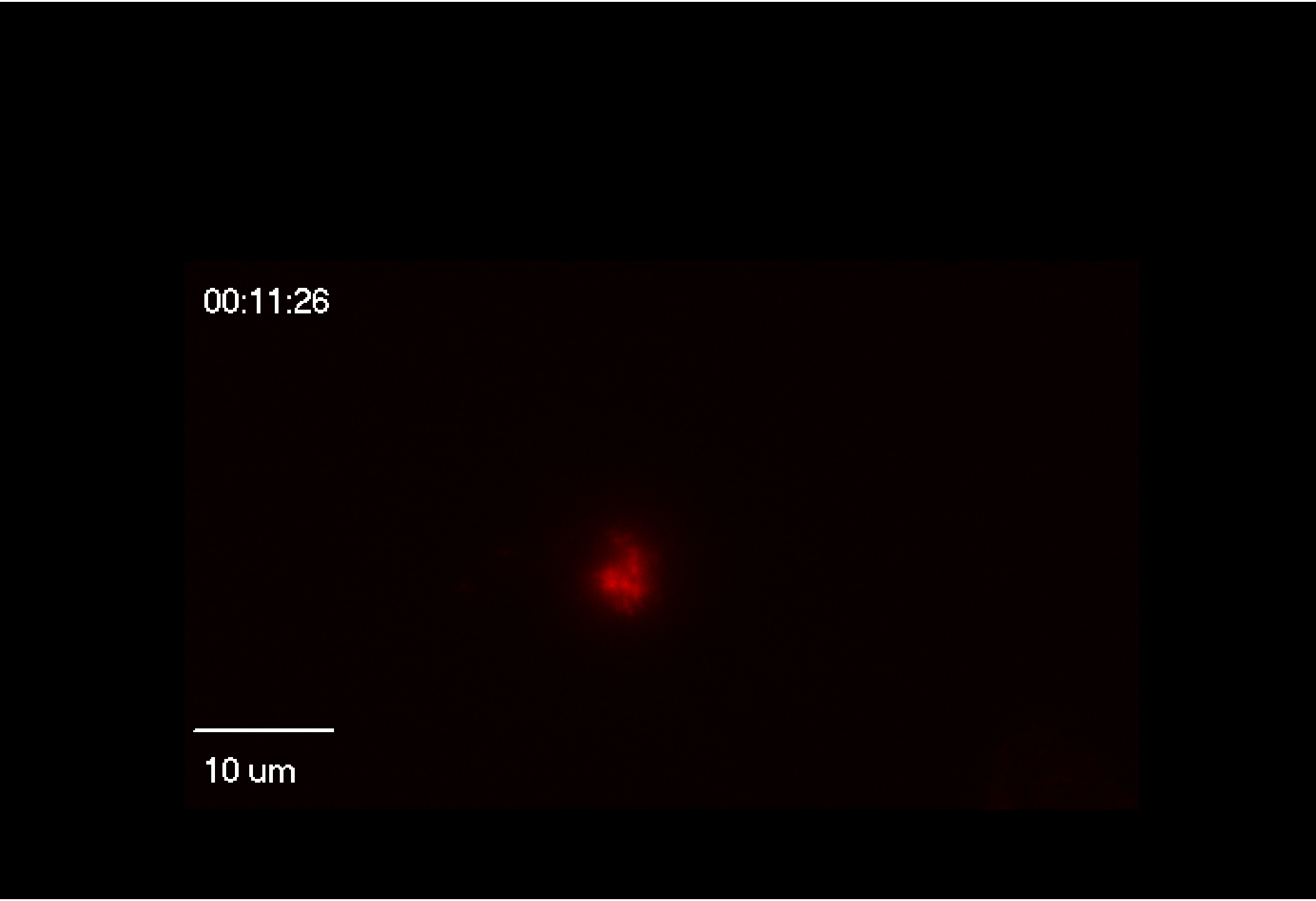


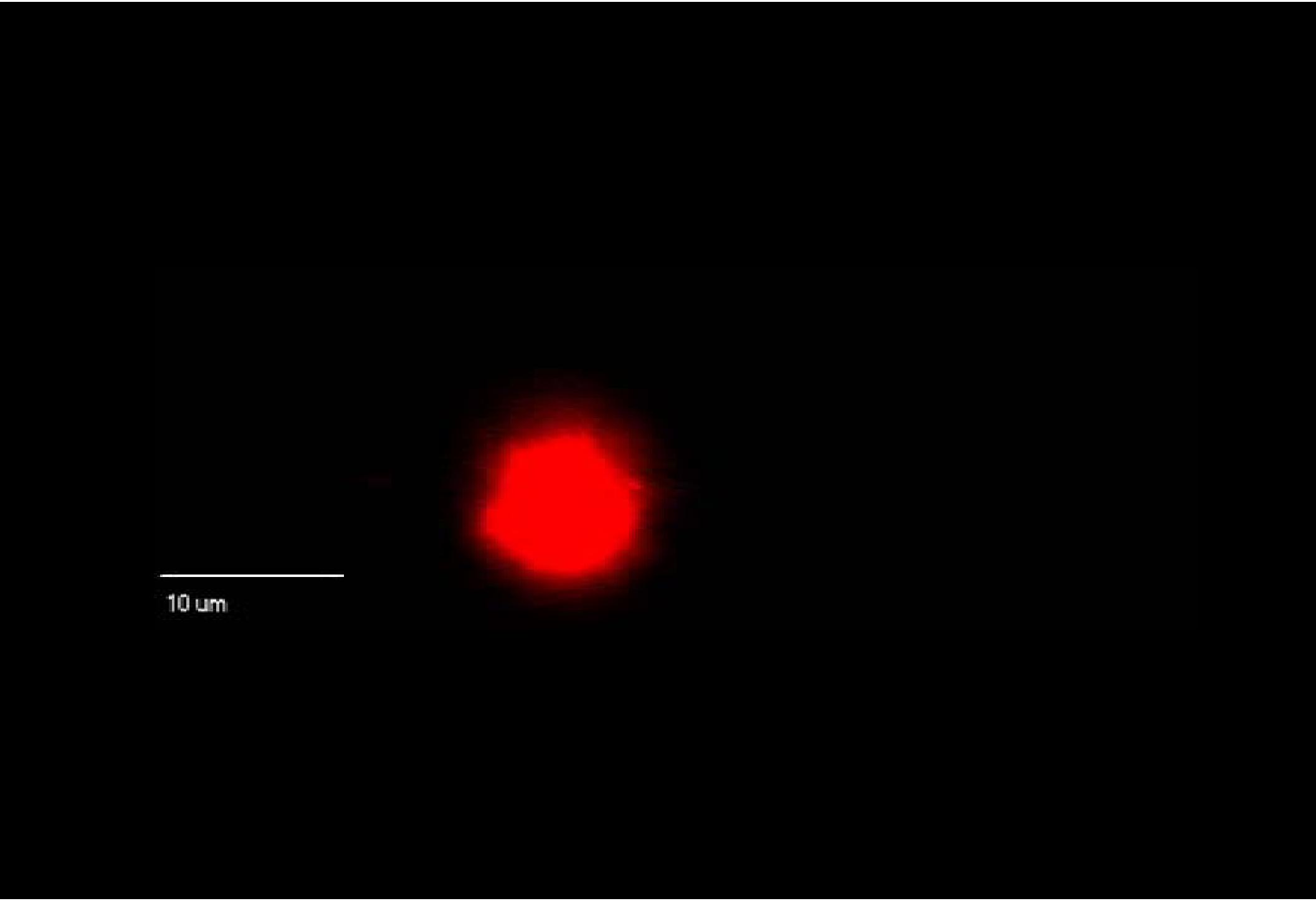
measured (TIRF)



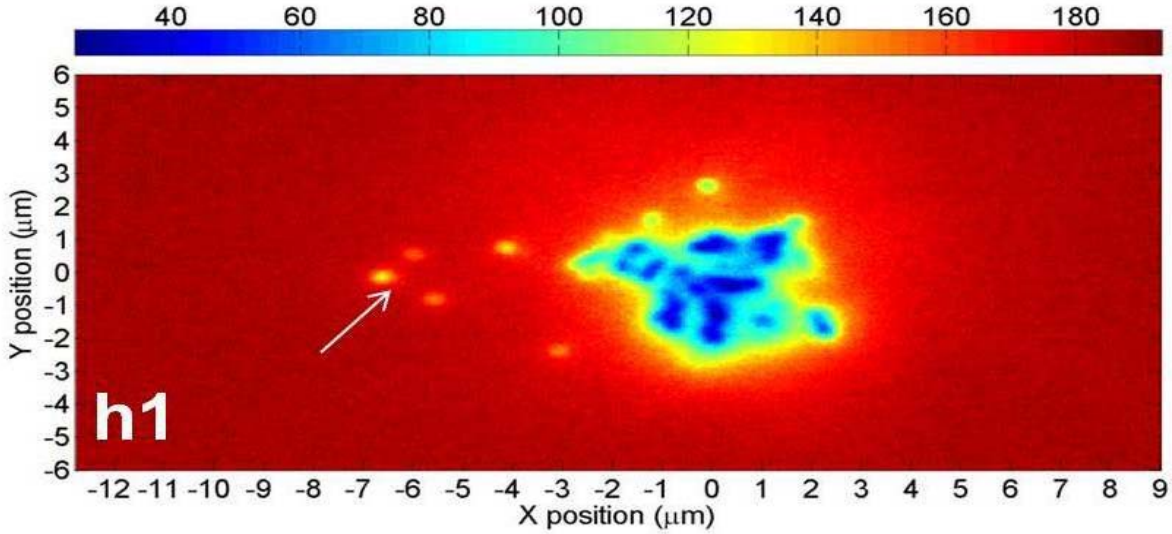
Rolling

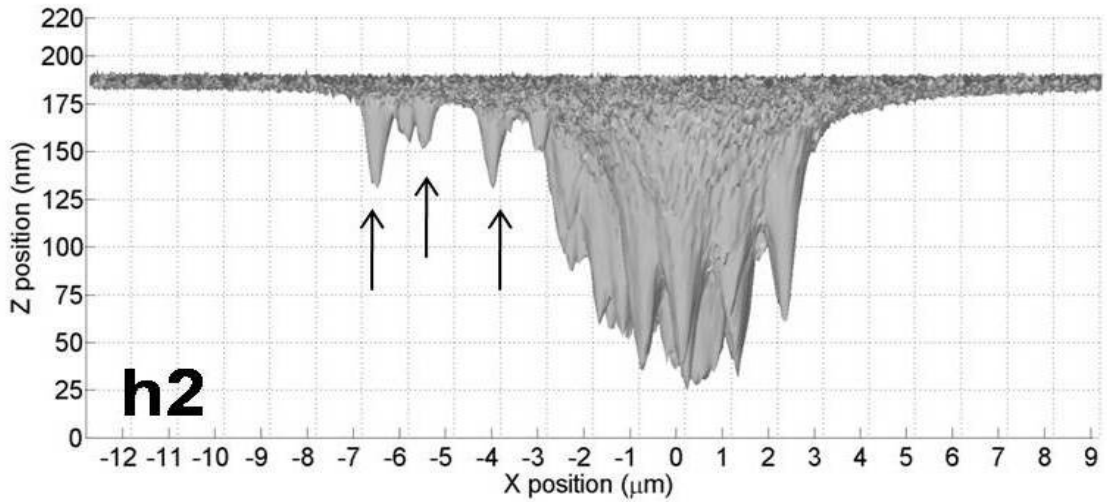

0 s
9.2 s
9.6 s
9.9 s
10.3 s
0 s
1 s
2 s
6 s
10 s
10 µm


00:11:26
10 um

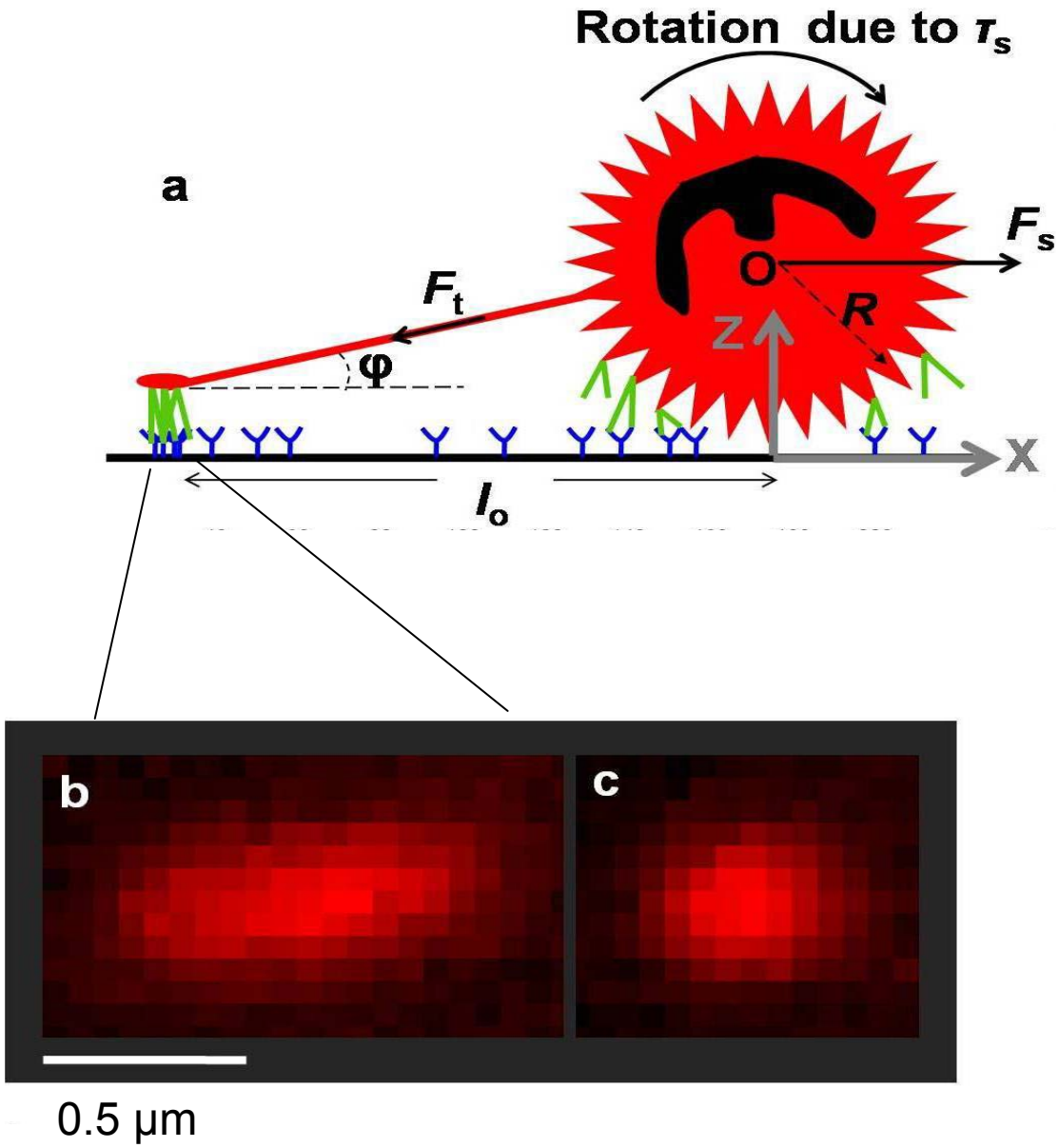

10 um

qDF reveals long tethers





qDF reveals long tethers



National Heart Lung and Blood Institute
National Institute for Diabetes, Digestive and Kidney Diseases
National Institute for Biomedical Imaging and Bioengineering
National Science Foundation
Deutsche Forschungsgemeinschaft
Studienstiftung des Deutschen Volkes
American Heart Association
Crohn's and Colitis Foundation of America
Mizutani Foundation
Whitaker Foundation
Wallace Coulter Foundation
Juvenile Diabetes Research Foundation

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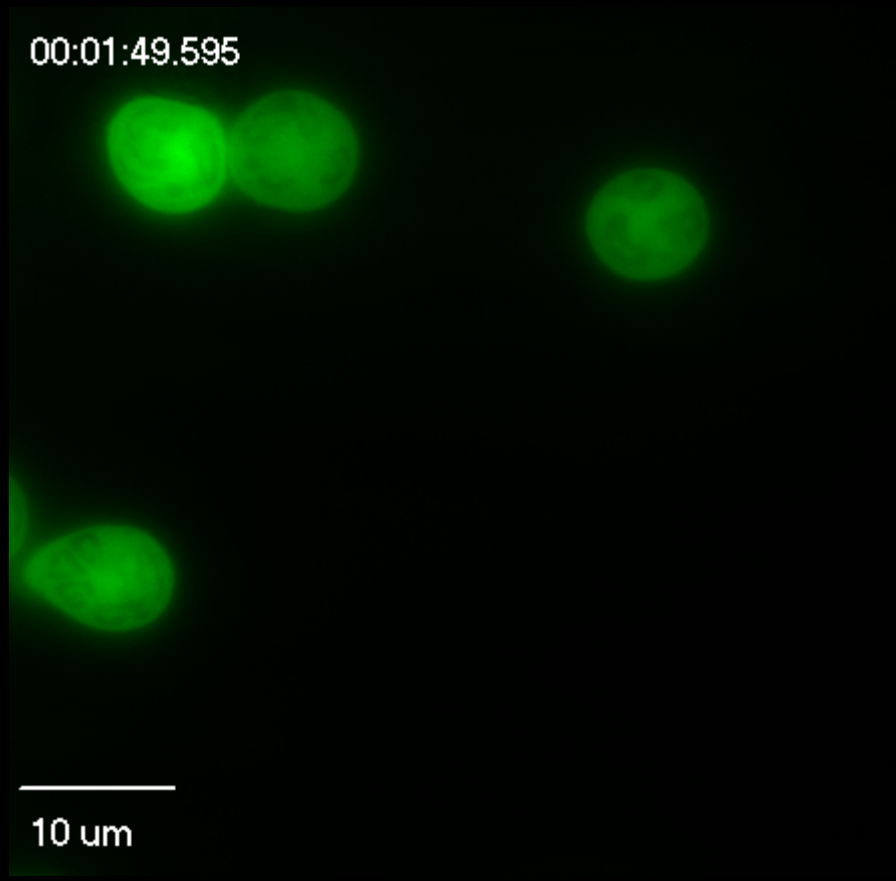
Neutrophil Arrest



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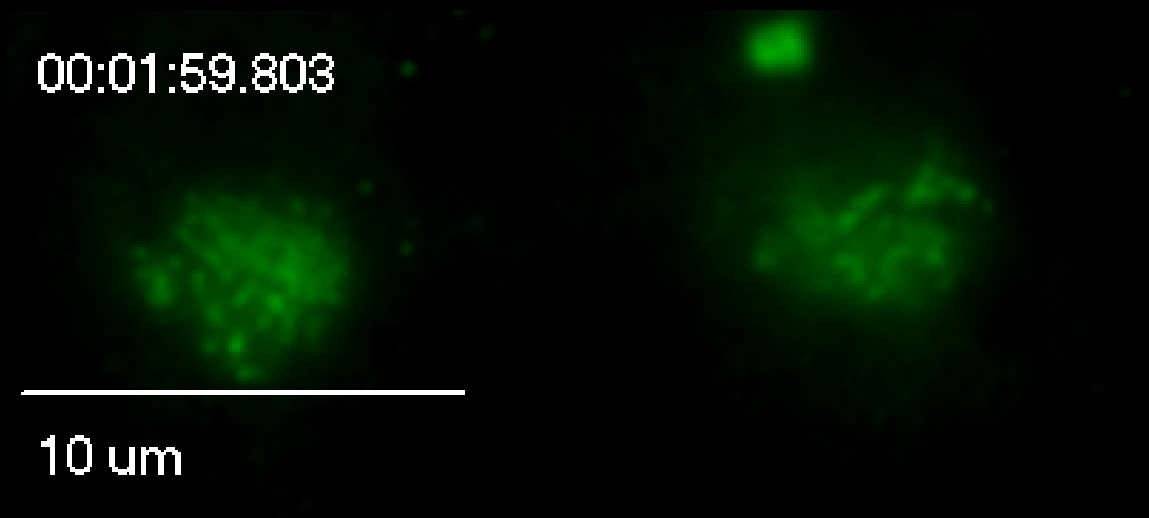

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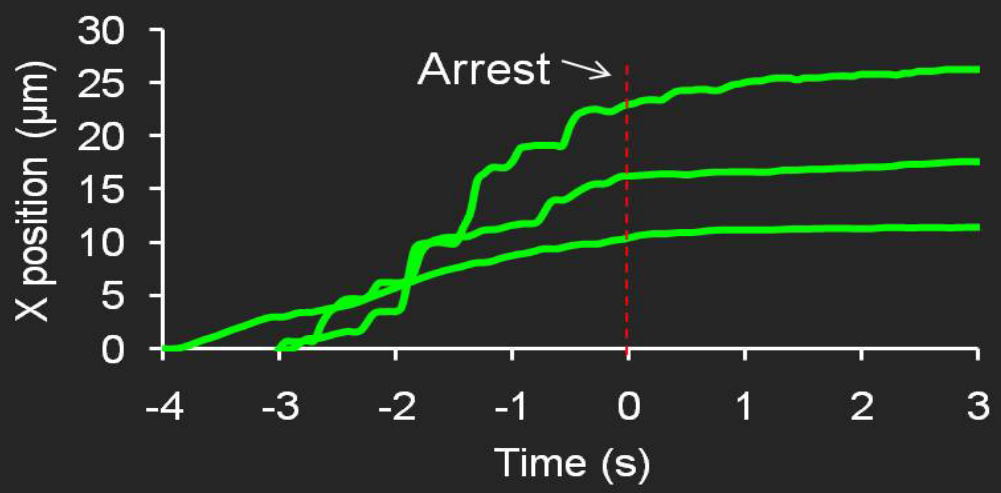

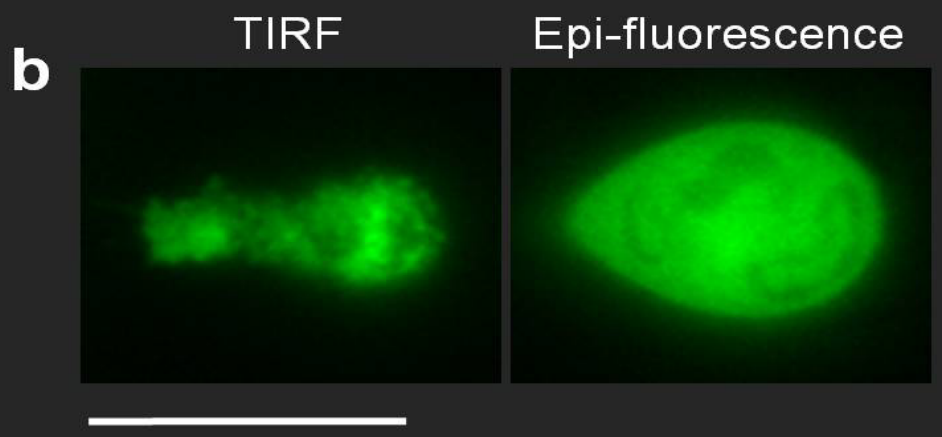

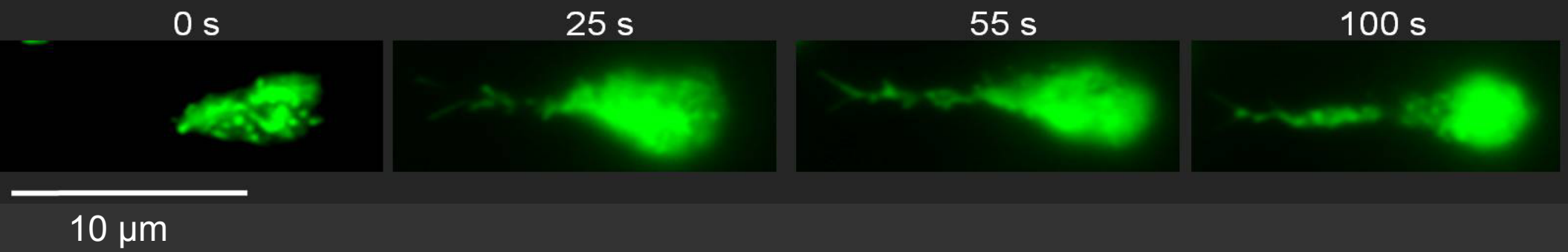

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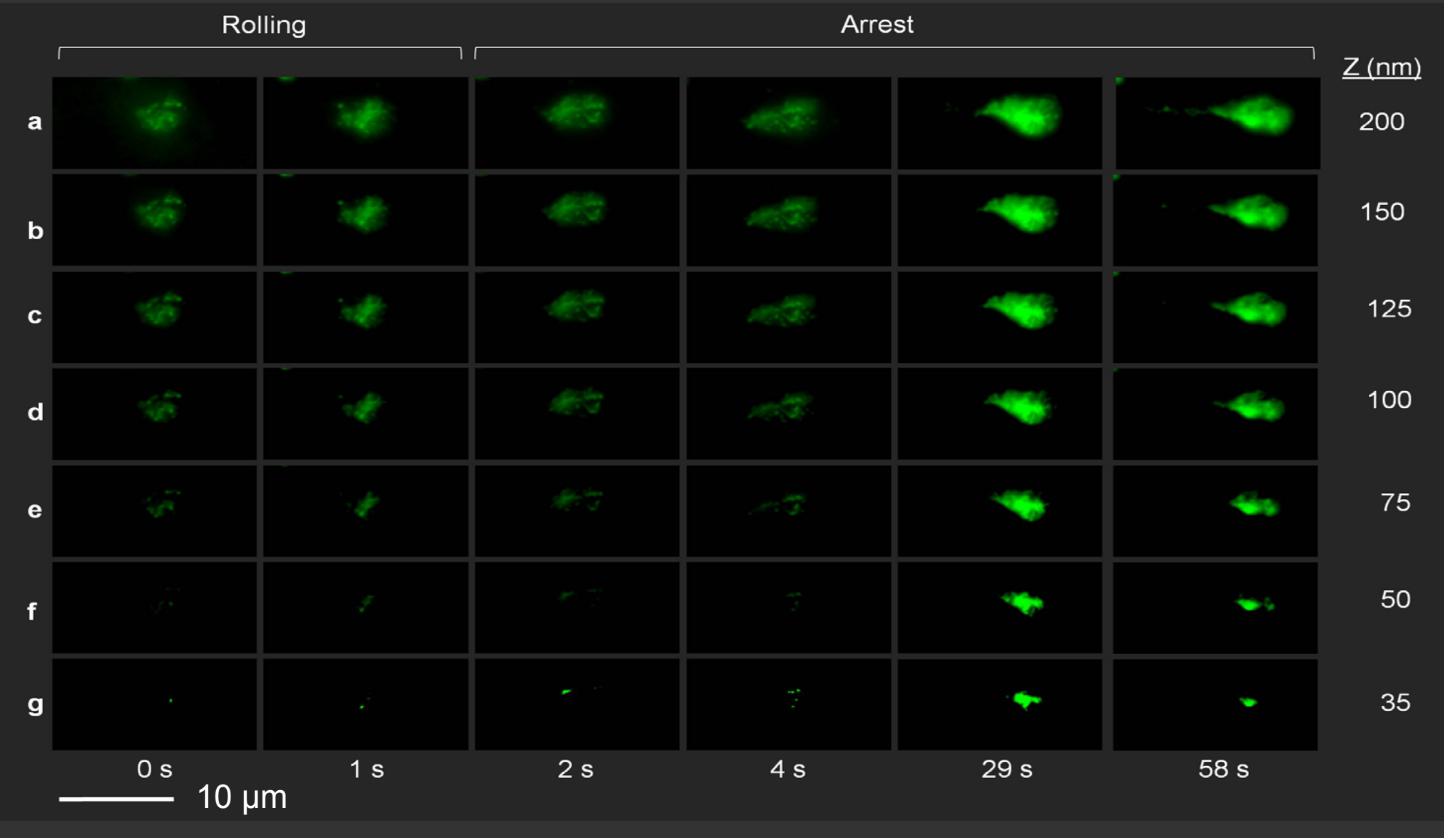
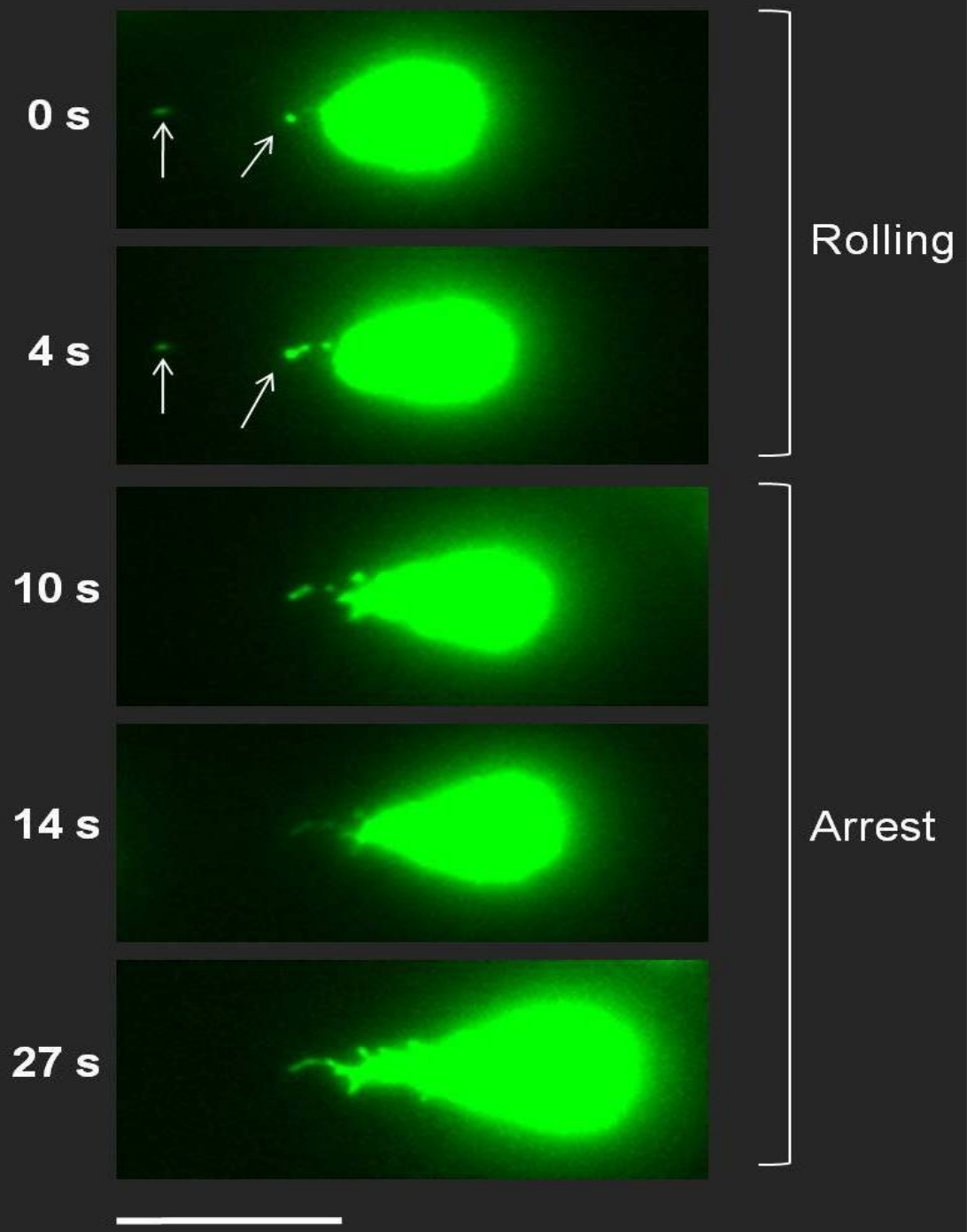
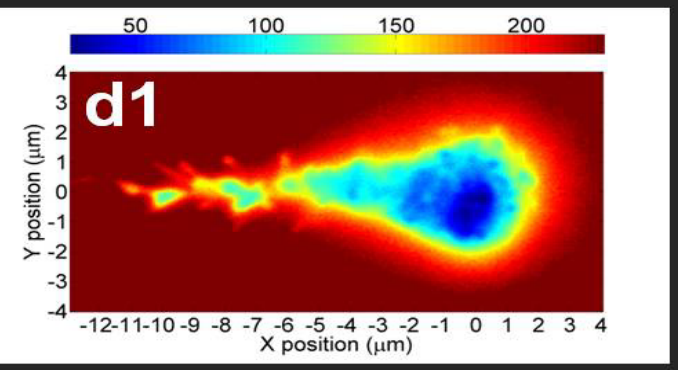
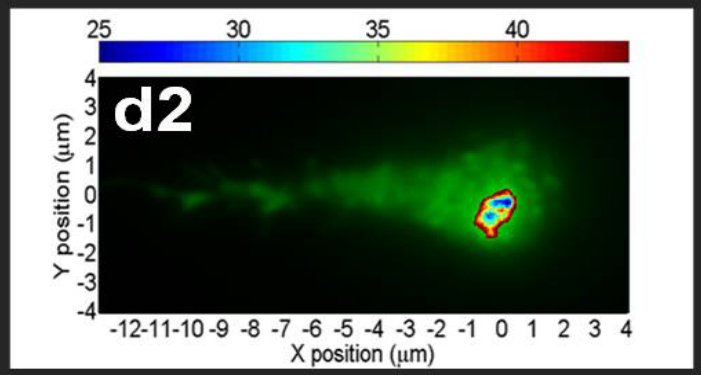
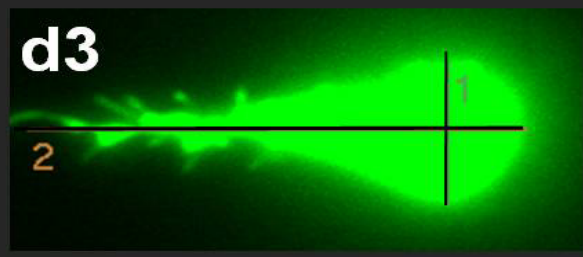


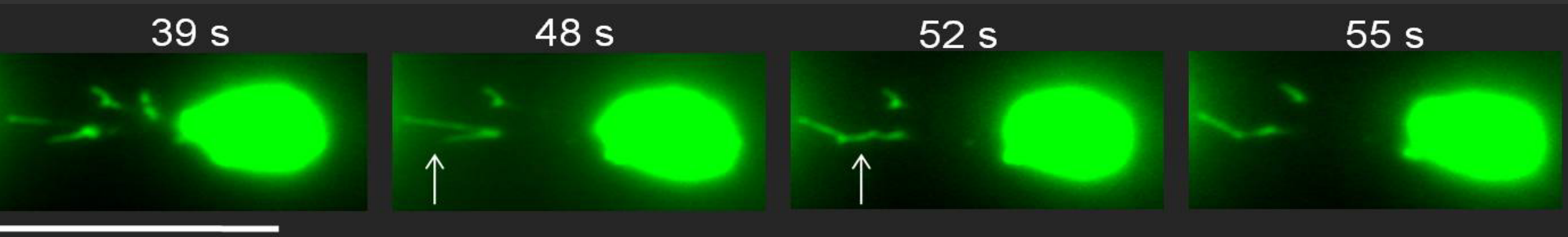

0 s
4 s
10 s
14 s
27 s
Rolling
Arrest

Neutrophil Arrest

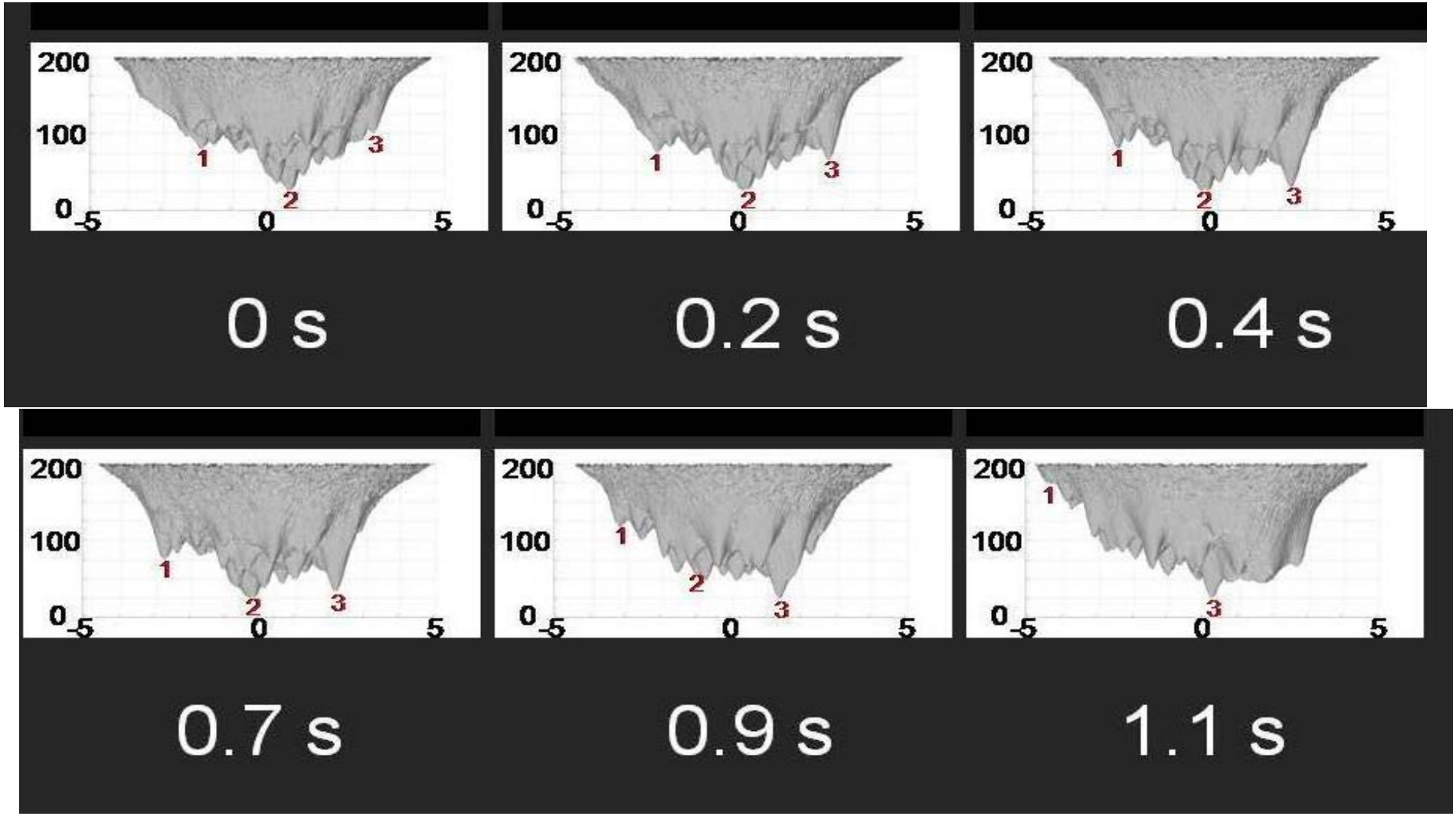




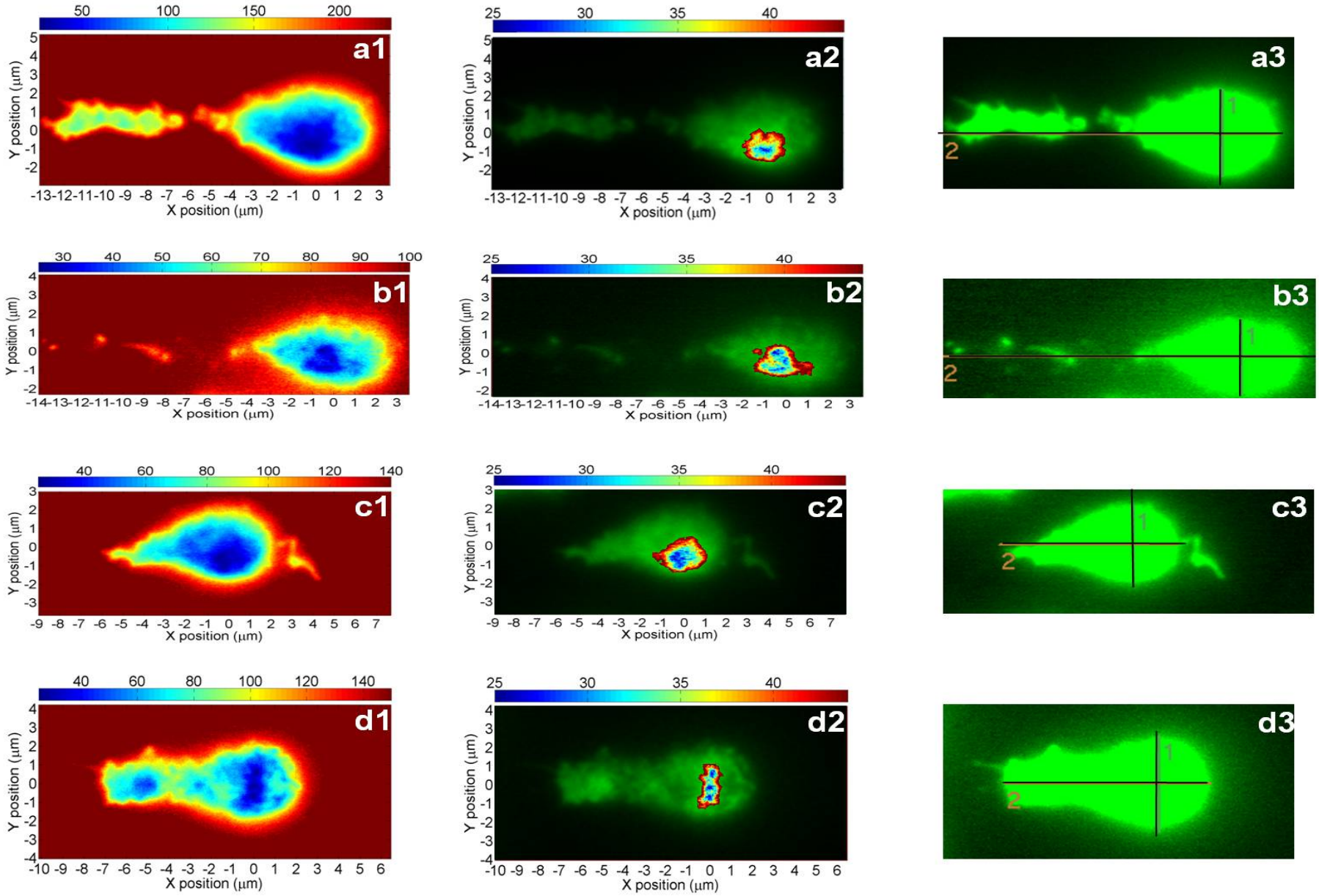




TIRF footprints of rolling *Lysm*^{GFP} neutrophil



Arrested neutrophils



Conclusions

- Contact area of rolling neutrophils about twice as large as previously assumed
- Microvilli preserved while rolling
- Extending long tethers dissipate bond force, detach without leaving material behind
- These two phenomena probably explain rolling at high shear stress
- After arrest, a single thick trunk with tethers forms that leaves material behind when detaching: likely source of microparticles

Ekaterina Koltsova (Multiphoton)
Prithu Sundd (Dynamic Footprinting)
Michel Nussenzweig (*CD11c*^{YFP} mice)
Tom Graf (*LysM*^{GFP} mice)

NIH grants HL 58108 and HL 55798

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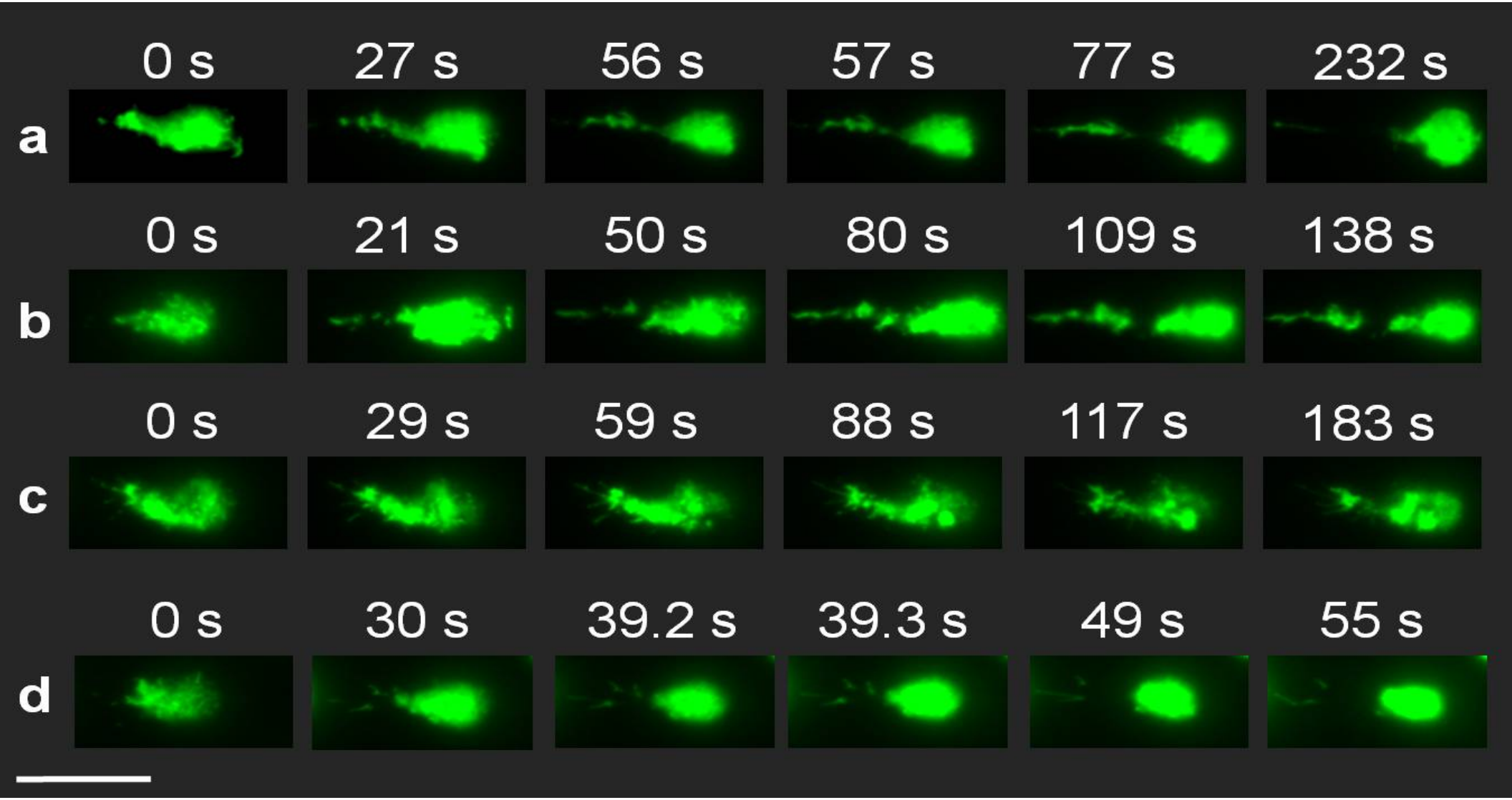
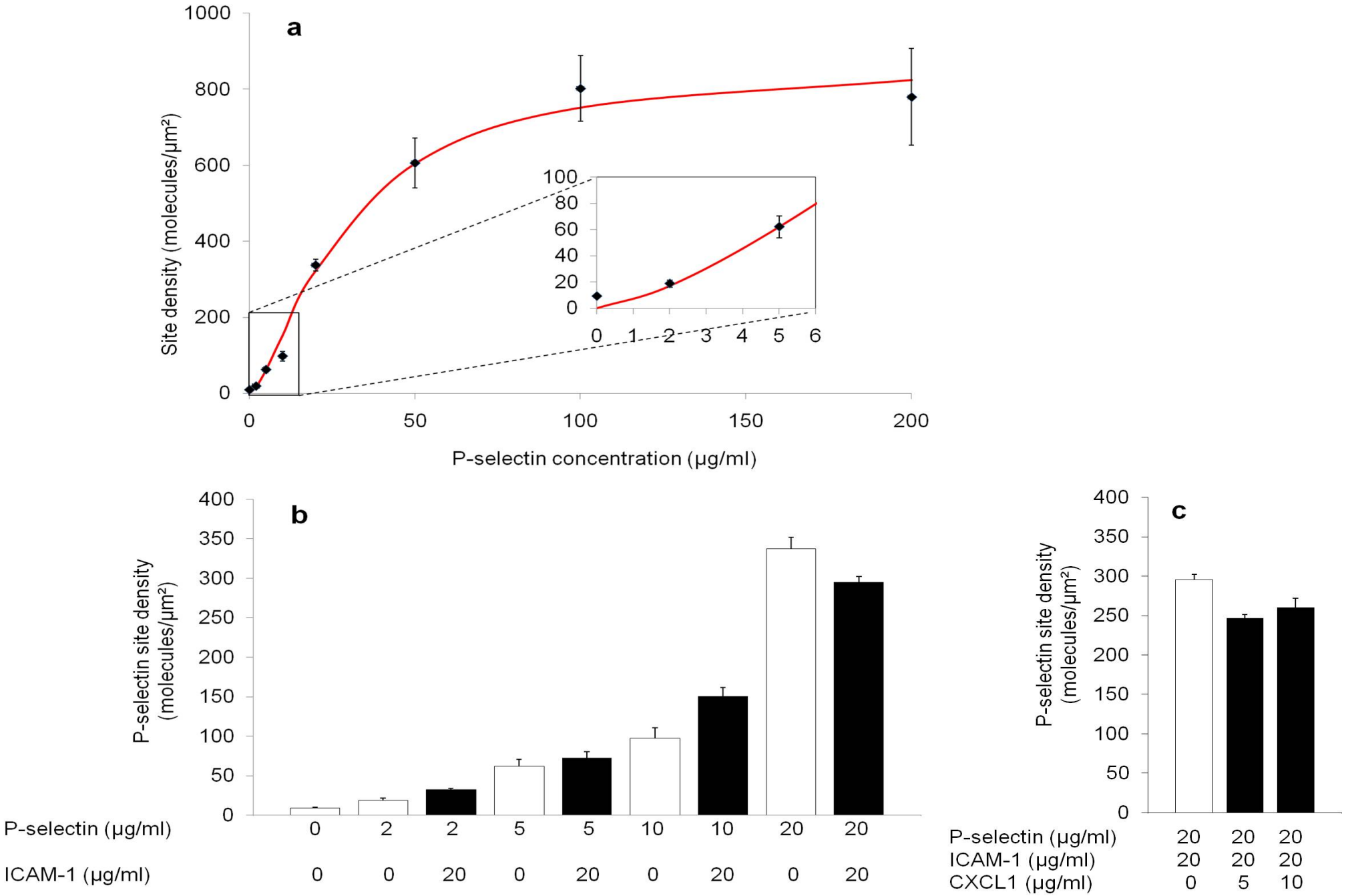
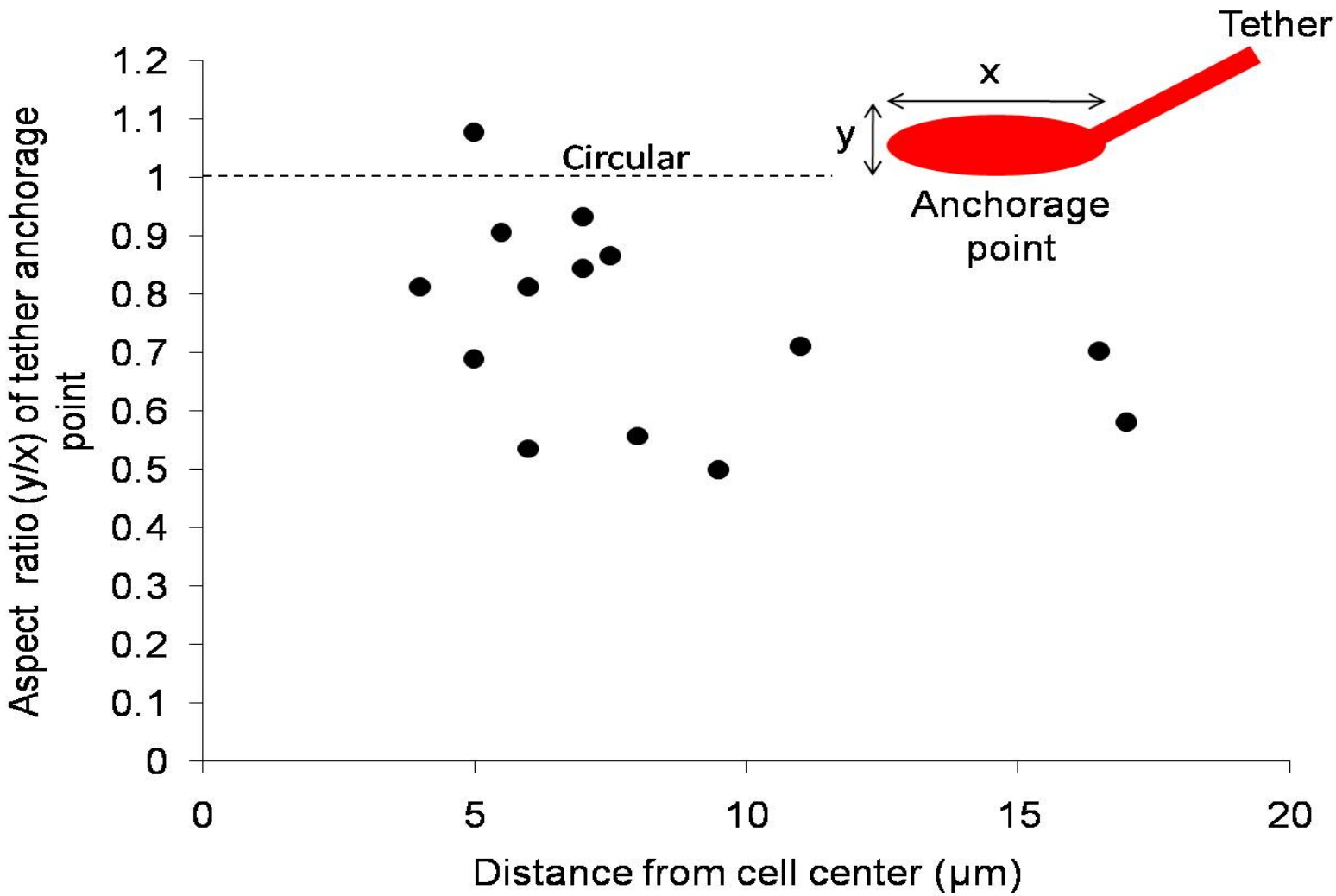
Arrested neutrophils

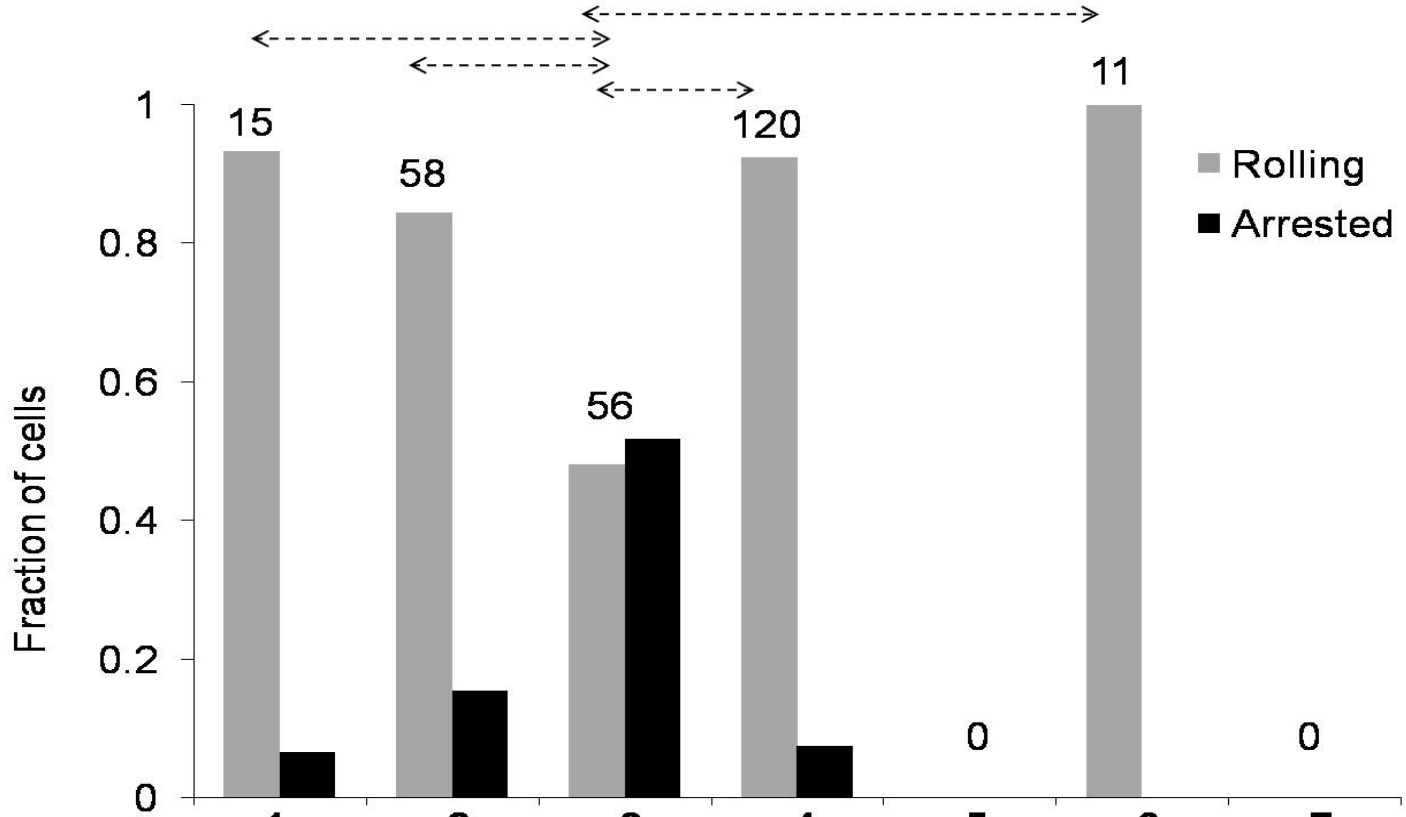



a
Site density (molecules/µm²)
P-selectin concentration (µg/ml)
b
P-selectin site density (molecules/µm²)
P-selectin (µg/ml)
ICAM-1 (µg/ml)
c
P-selectin site density (molecules/µm²)
P-selectin (µg/ml)
ICAM-1 (µg/ml)
CXCL1 (µg/ml)

Tether anchorage points



Specificity controls



Substrate	**1**	**2**	**3**	**4**	**5**	**6**	**7**
P-selectin	+	+	+	+	+	+	+
ICAM-1	-	+	+	+	+	+	+
CXCL1	-	-	+	+	+	+	+
MAb							
Anti LFA-1 (TIB217)	-	-	-	+	+	-	-
Anti PSGL-1 (4RA10)	-	-	-	-	+	-	-
Anti ICAM-1 (YN-1)	-	-	-	-	-	+	+
Anti P-selectin (RB40.34)	-	-	-	-	-	-	+

Total Internal Reflection Fluorescence Microscopy

$$d(\theta) = (\lambda/4\pi)(n_1{}^2 \sin^2\theta - n_2{}^2)^{-1/2}$$

$$T(\theta) = 4\cos^2\theta\,/[1-(n_2/n_1)^2]$$

1. Fluorophore in cytoplasm

$$I_{F(\theta)} = AT(\theta)\int_{\Delta}^{\infty} c(z)e^{-z/d(\theta)}\,dz$$

Assuming *c* and θ as constant

$$I_F = AT(\theta)cd(\theta)e^{-\Delta/d(\theta)}$$

if $\Delta = \Delta_o + \delta$

such that $I_F = I_{Fmax}$ when $\delta = 0$

$$I_F = I_{Fmax}e^{-\delta/d(\theta)}$$

$$\Delta = \Delta_o + d(\theta)ln\frac{I_{Fmax}}{I_F}$$

2. Fluorophore in plasma membrane

$$I_{F(\theta)} = AT(\theta)\int_{\Delta-t/2}^{\Delta+t/2} c(z)e^{-z/d(\theta)}\,dz$$

Assuming *c* and θ as constant

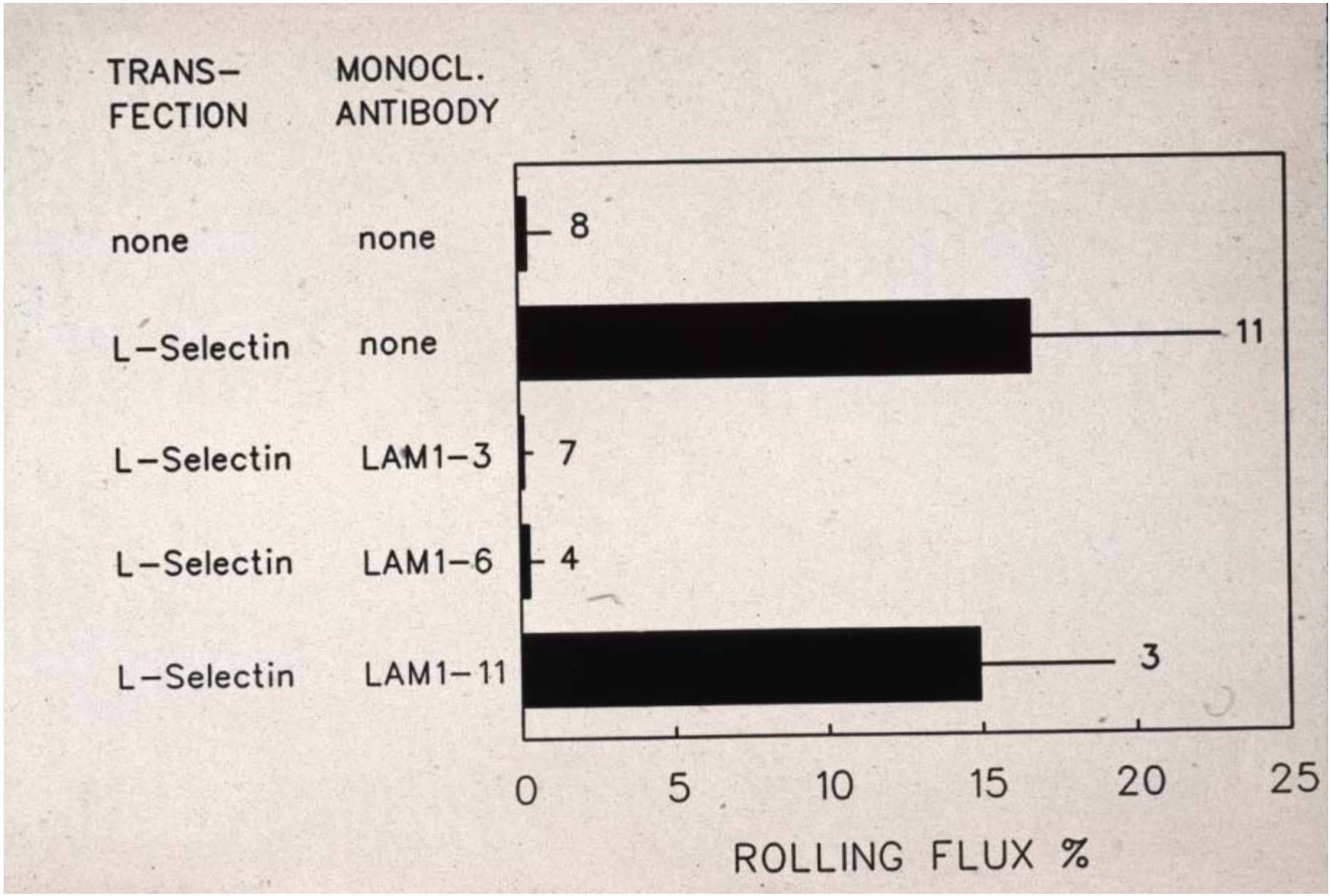
$$I_F = AT(\theta)cte^{-\Delta/d(\theta)}$$

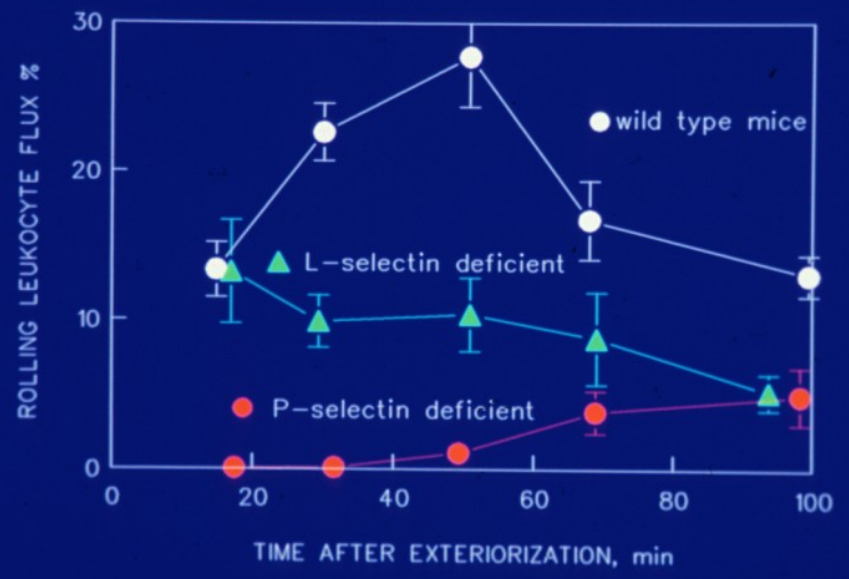
if $\Delta = \Delta_o + \delta$

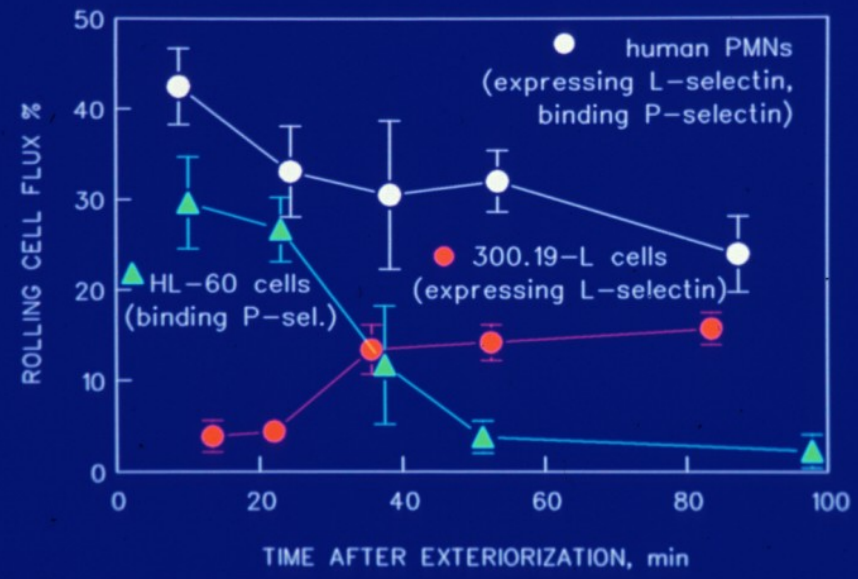
such that $I_F = I_{Fmax}$ when $\delta = 0$

$$I_F = I_{Fmax}e^{-\delta/d(\theta)}$$

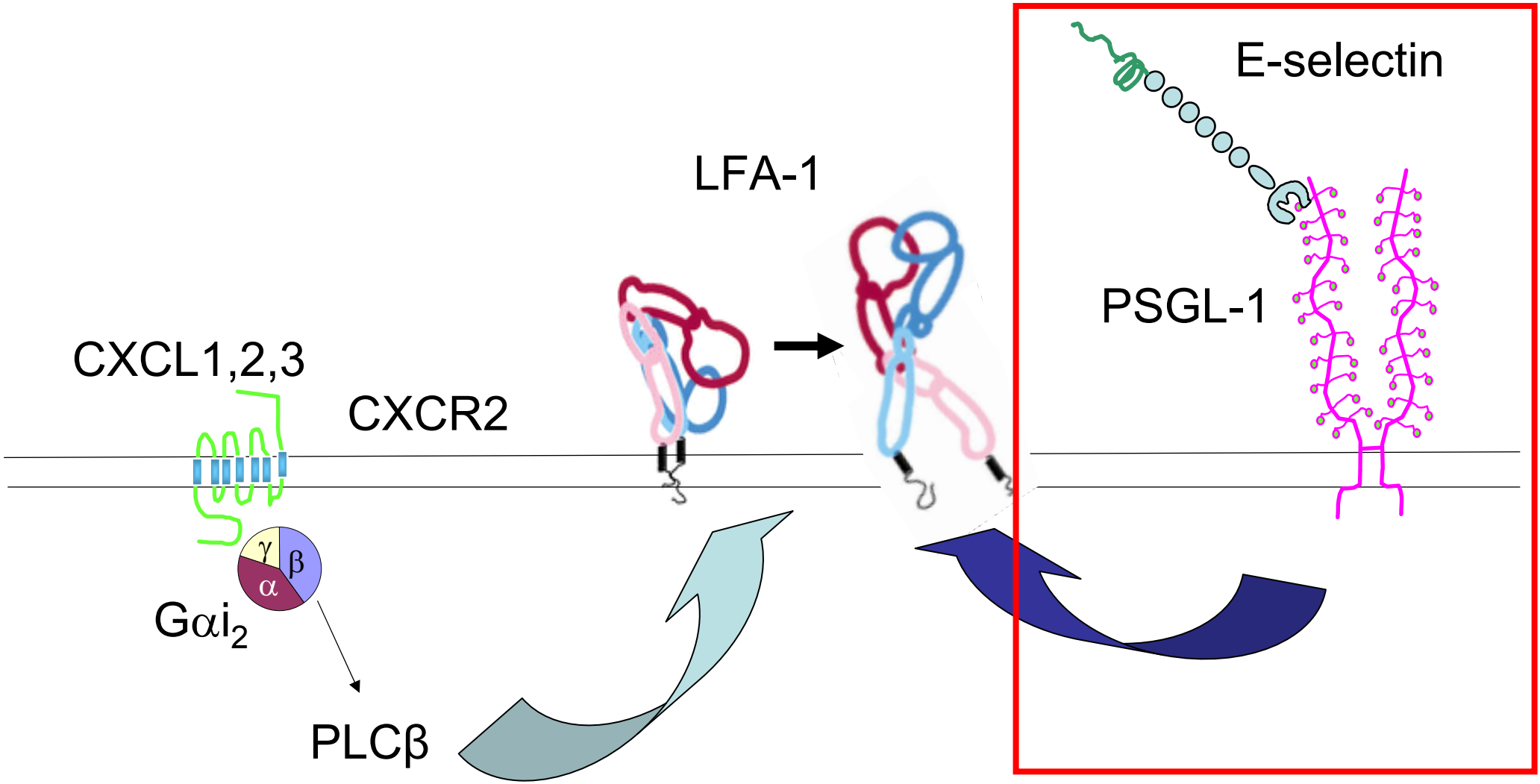
$$\Delta = \Delta_o + d(\theta)ln\frac{I_{Fmax}}{I_F}$$


TRANS-
FECTION
MONOCL.
ANTIBODY
none
none
8
L-Selectin
none
11
L-Selectin
LAM1-3
7
L-Selectin
LAM1-6
4
L-Selectin
LAM1-11
3
0
5
10
15
20
25
ROLLING FLUX %

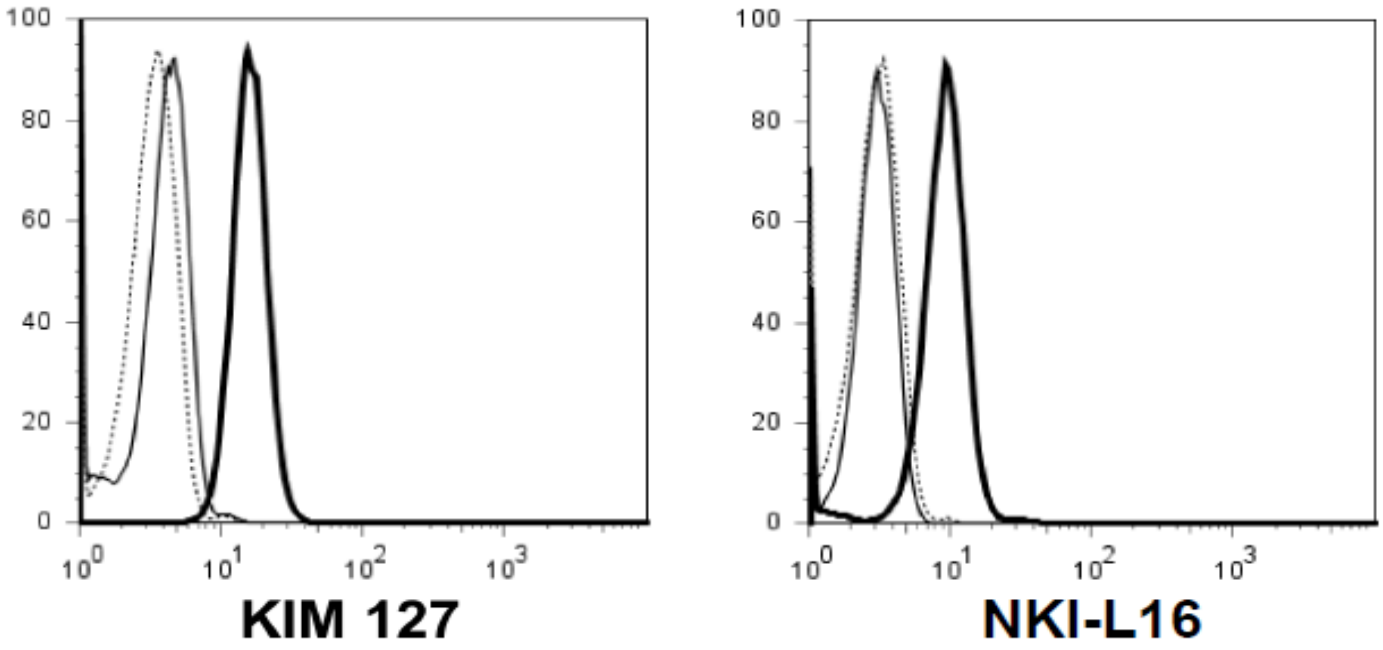

ROLLING LEUKOCYTE FLUX %
30
20
10
0
0
20
40
60
80
100
TIME AFTER EXTERIORIZATION, min
wild type mice
L-selectin deficient
P-selectin deficient


ROLLING CELL FLUX %
50
40
30
20
10
0
0
20
40
60
80
100
TIME AFTER EXTERIORIZATION, min
human PMNs
(expressing L-selectin,
binding P-selectin)
HL-60 cells
(binding P-sel.)
300.19-L cells
(expressing L-selectin)

Integrin Activation



KIM127, NKI-L16 not expressed on whole blood neutrophils



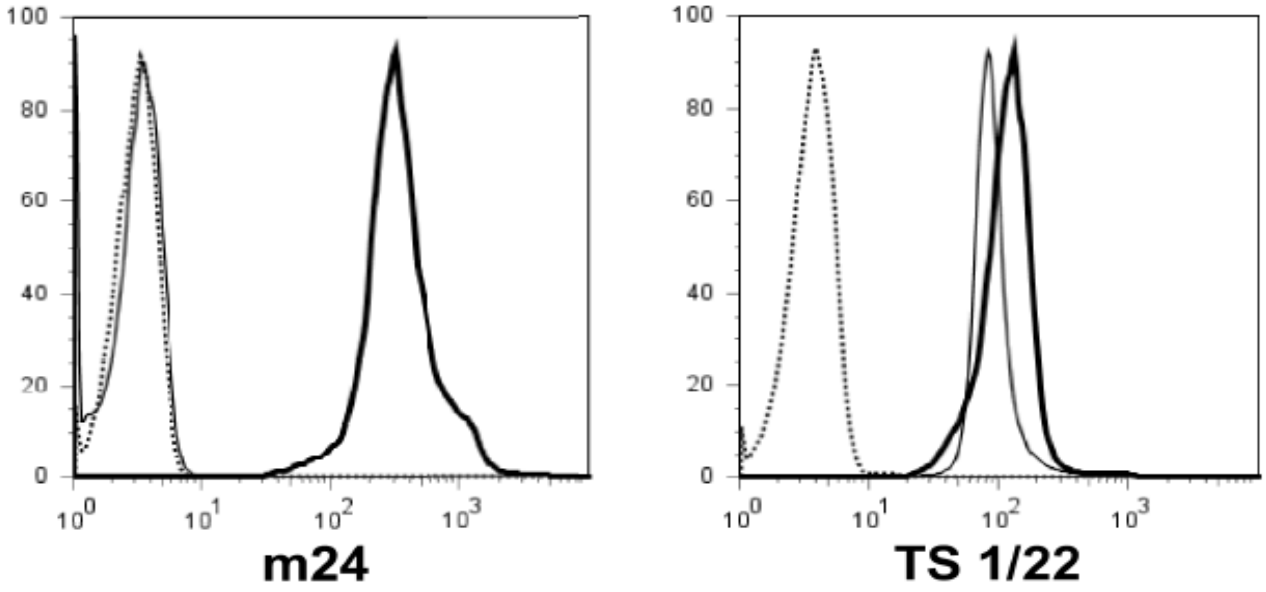
Isotype Ab

No activation

fMLP

Kuwano et al., Blood in press

TS1/22, but not m24 expressed on whole blood neutrophils

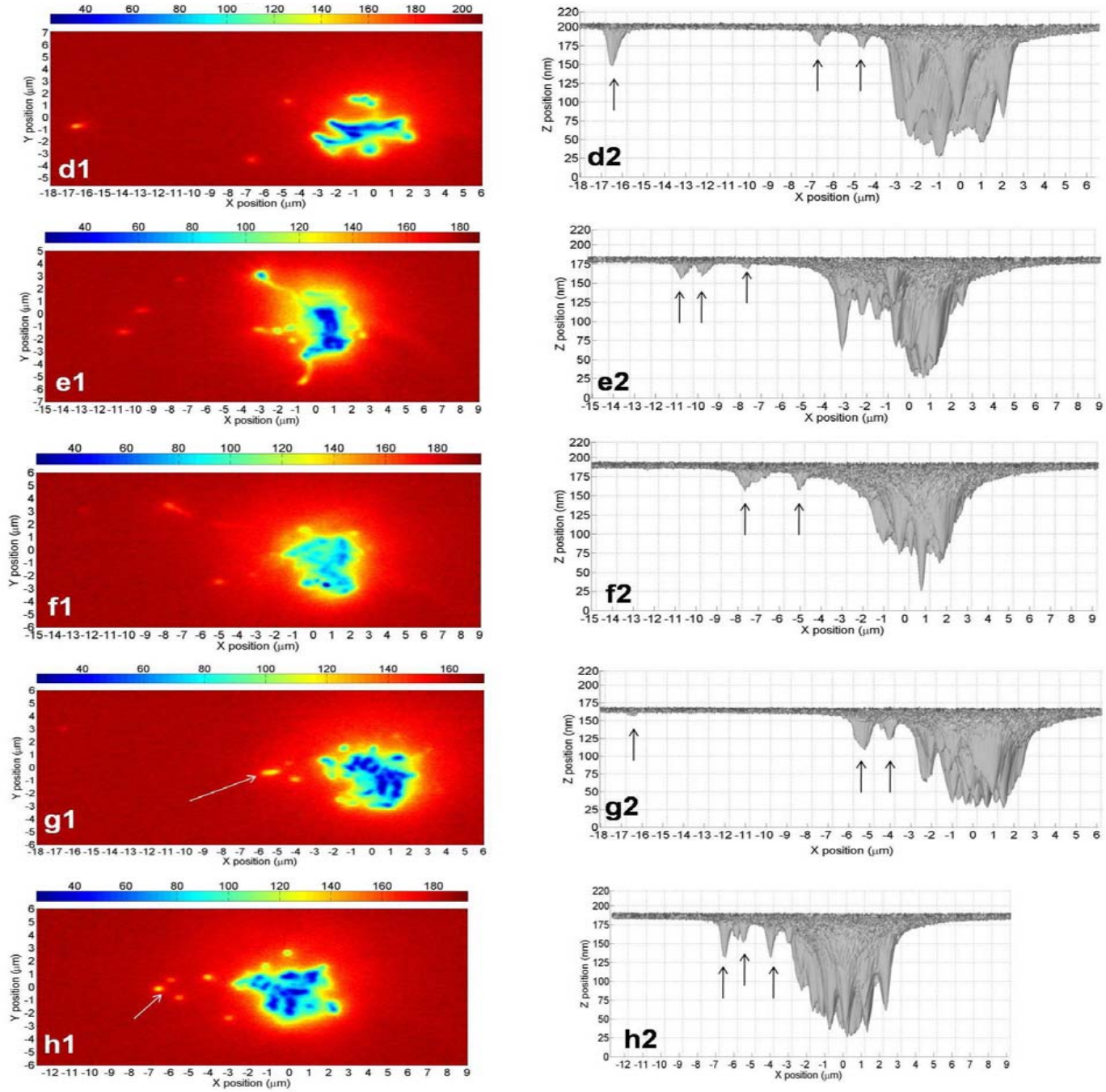


Isotype Ab

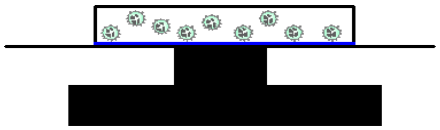
No activation

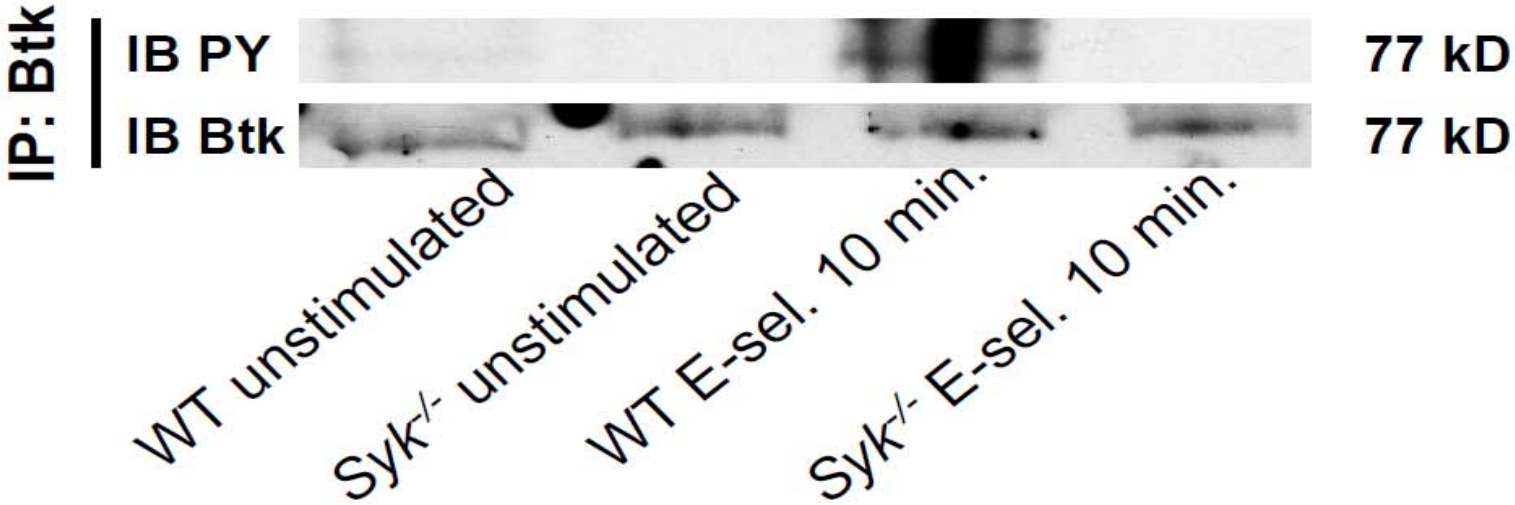
fMLP

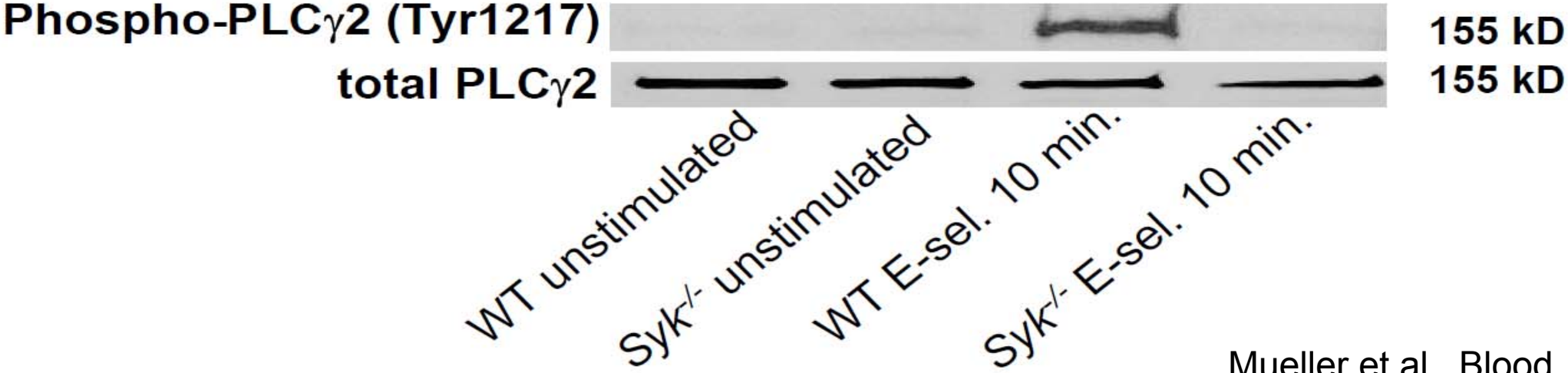
Kuwano et al., Blood in press


d1
e1
f1
g1
h1
d2
e2
f2
g2
h2
X position (μm)
Y position (μm)
Z position (nm)

BTK and Phospholipase γ_2







Mueller et al., Blood 2010