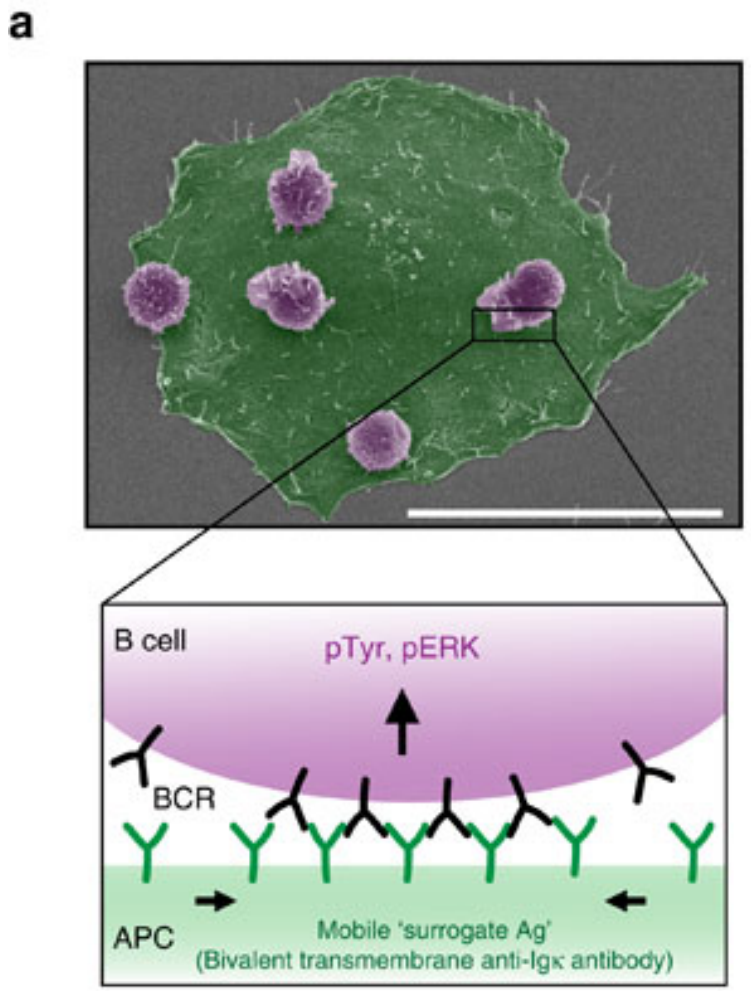
Nat Commun. 2015 Feb 3;6:6168.

Toll-like receptor ligands sensitize B-cell receptor signalling by reducing actin-dependent spatial confinement of the receptor.

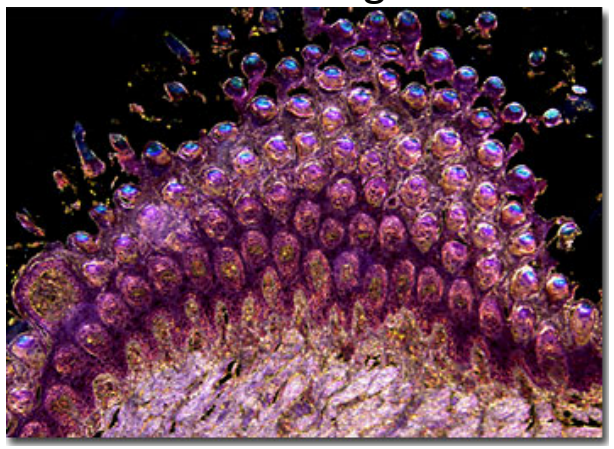
Freeman SA[1], Jaumouillé V[2], Choi K[3], Hsu BE[3], Wong HS[2], Abraham L[4], Graves ML[5], Coombs D[6], Roskelley CD[7], Das R[8], Grinstein S[2], Gold MR[3].



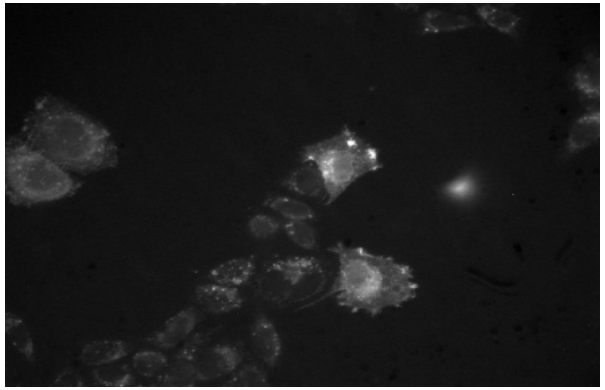
Diffraction limit of light

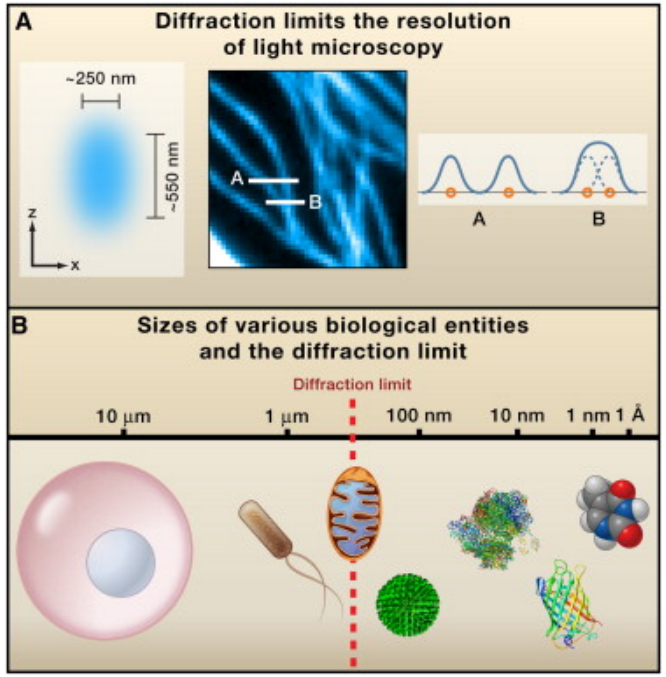
Light microscopy has a resolution limited to ~250 nm

Mouse Tongue

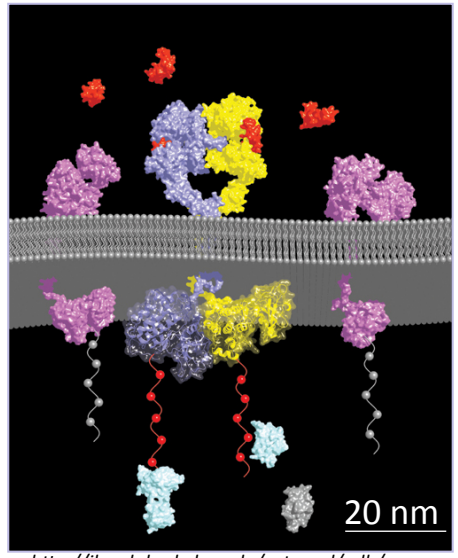


Cells expressing ErbB3

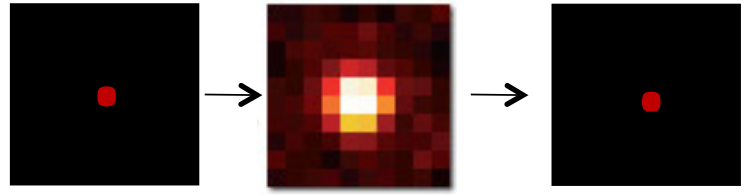




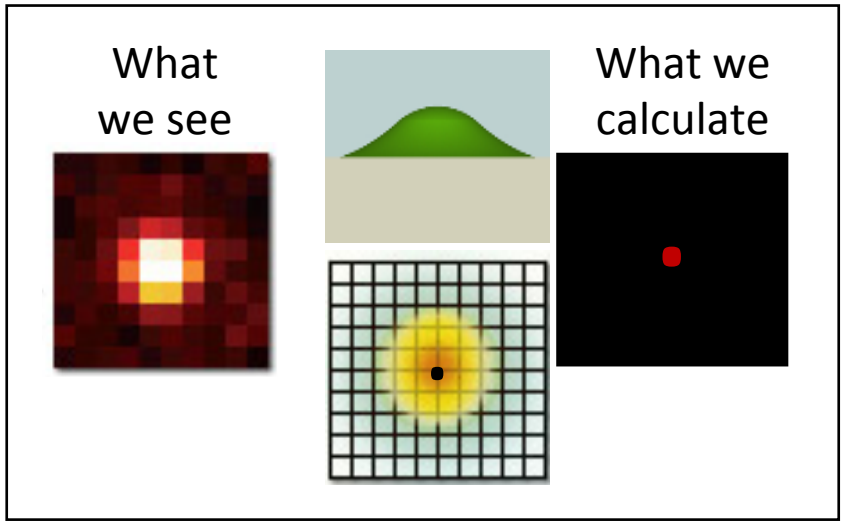
Huang et al. 2010 Cell 143:7, 1047–1058



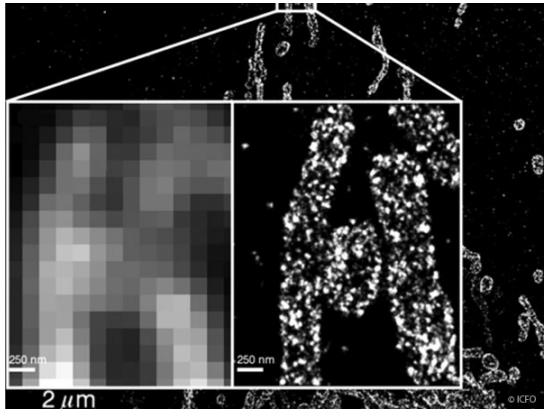
http://jkweb.berkeley.edu/external/pdb/2006/zhang-egfr/index.html

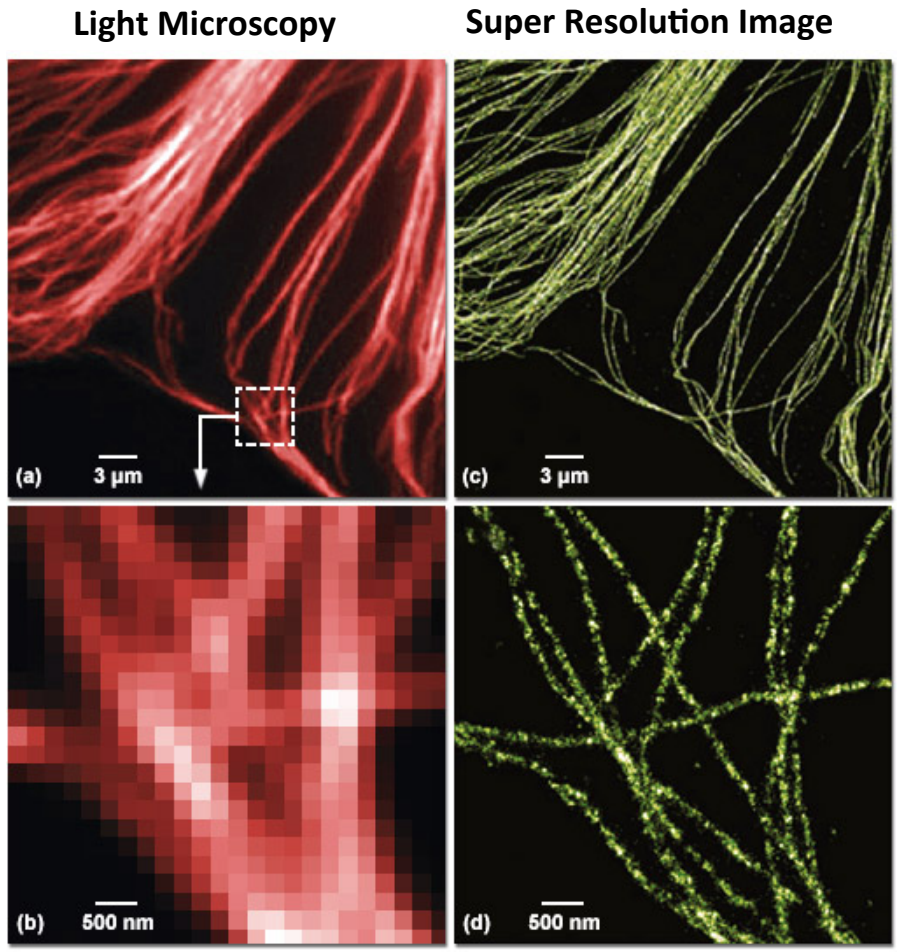


Super resolution microscopy uses single point emitters to go beyond the diffraction limit



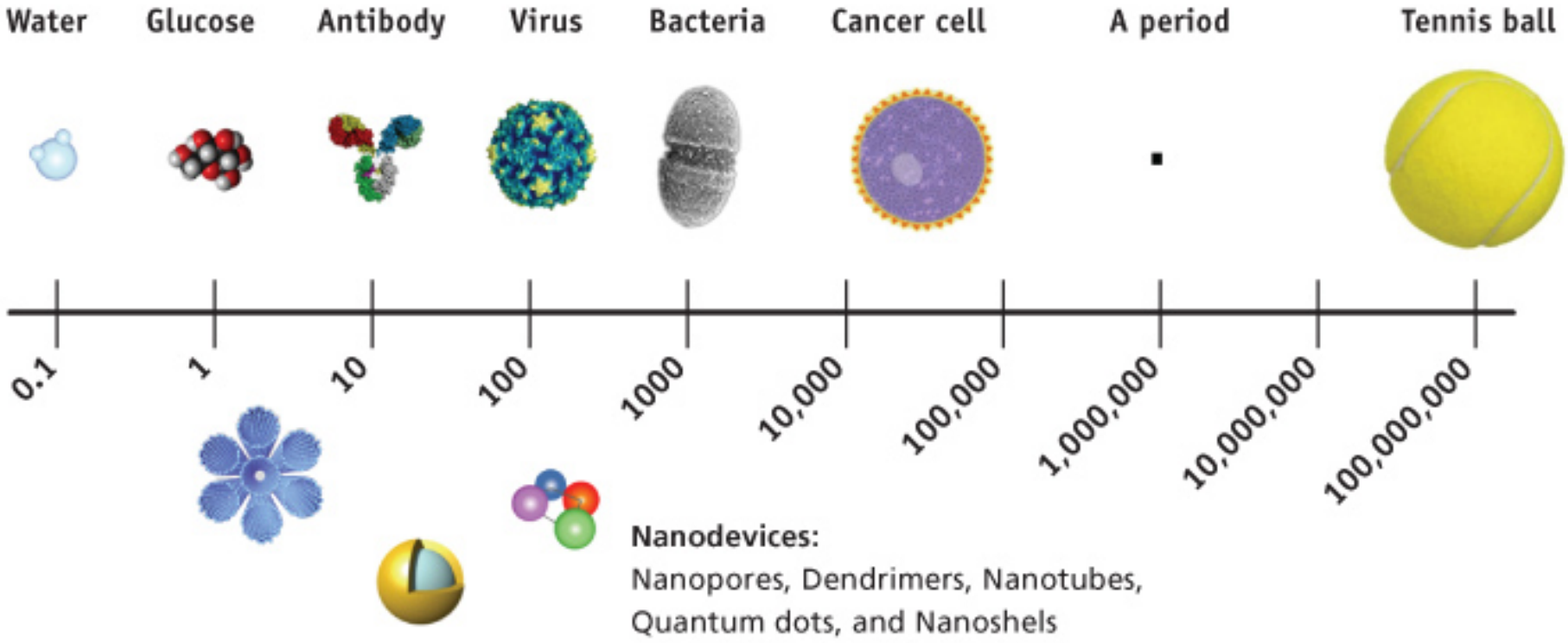
Mitochondria





Images from Nikon Microscopy U

Labeling Proteins on the Membrane with Quantum Dots (QD's)



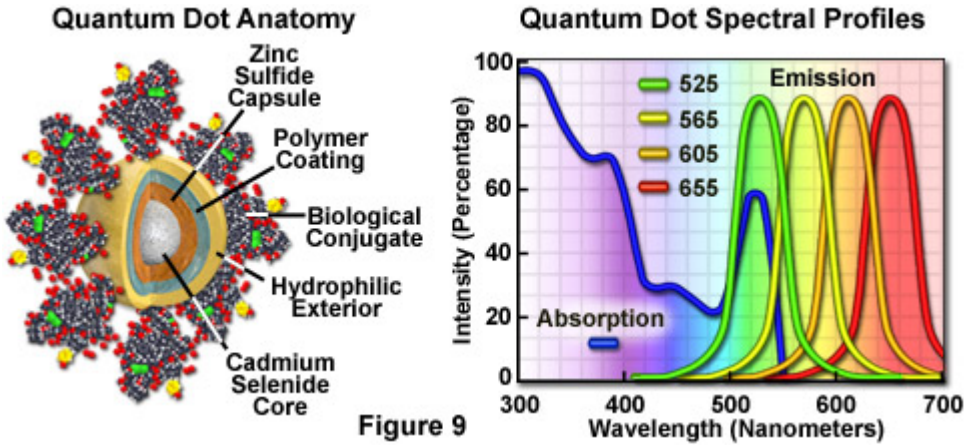
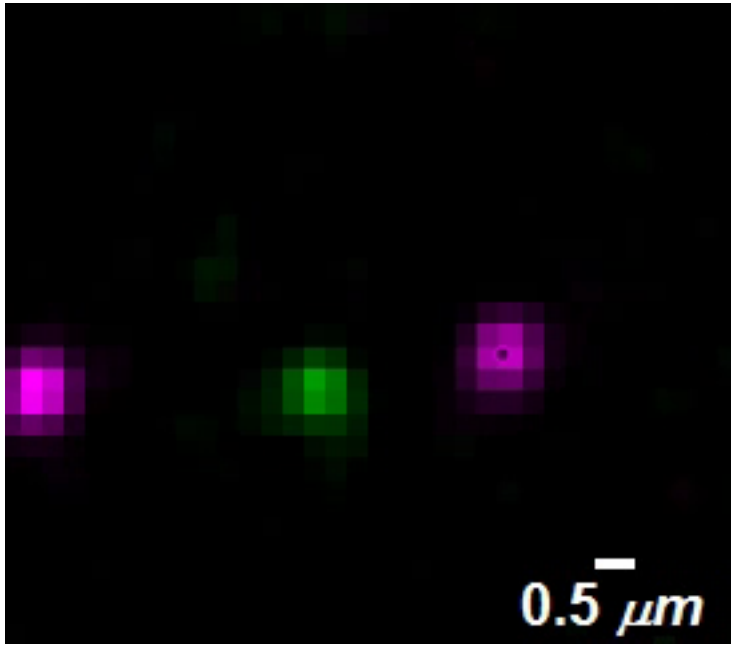


Figure 9

Art NANOLAB UCLA

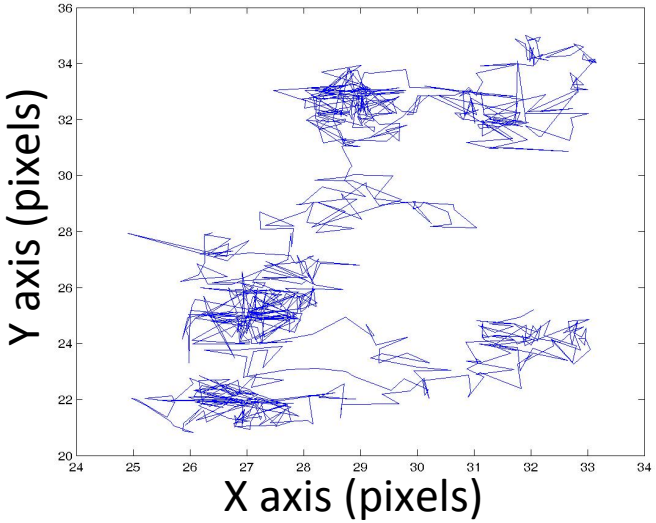
Single Particle Tracking

- Wide field microscope
- Mercury lamp excitation

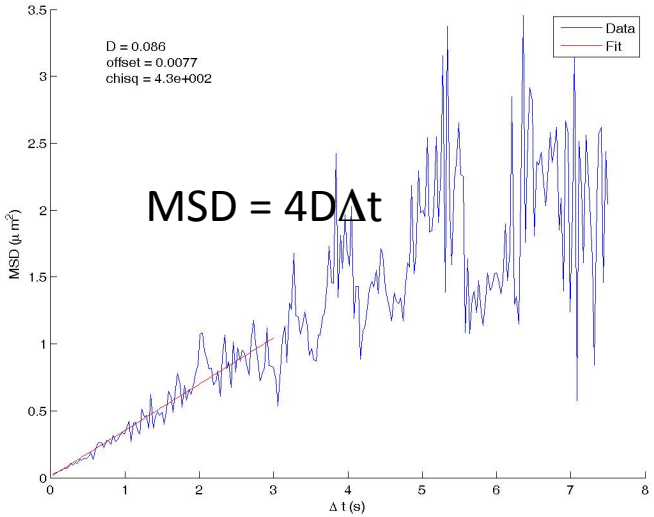


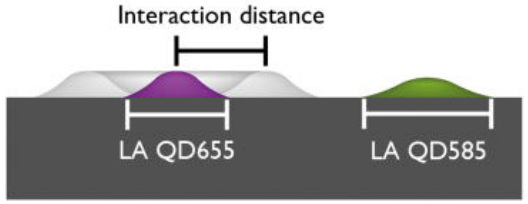
Imaged 30 frames/sec
Playback 1x

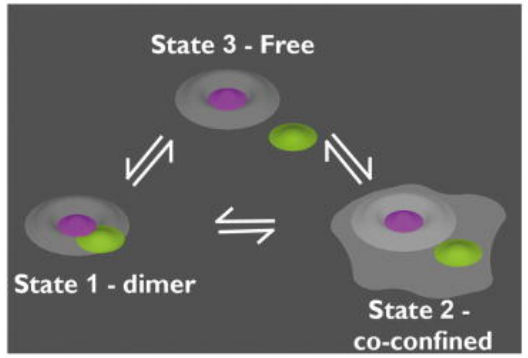
Track spatial movement of receptors

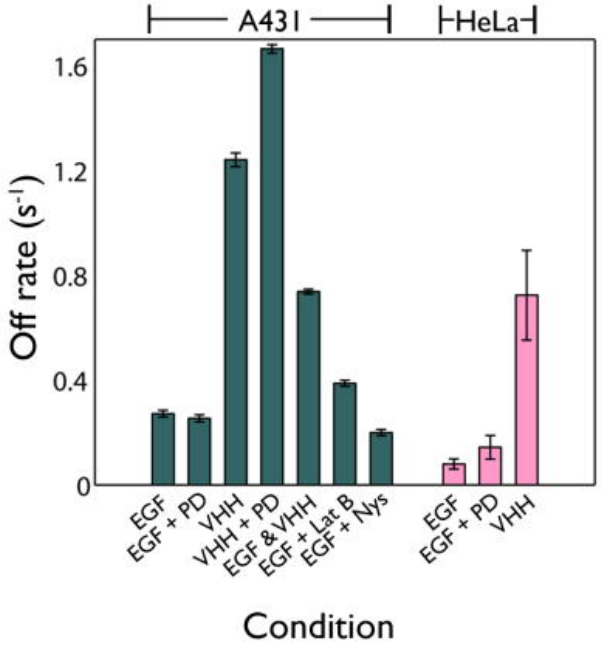


Determine the diffusion of receptors

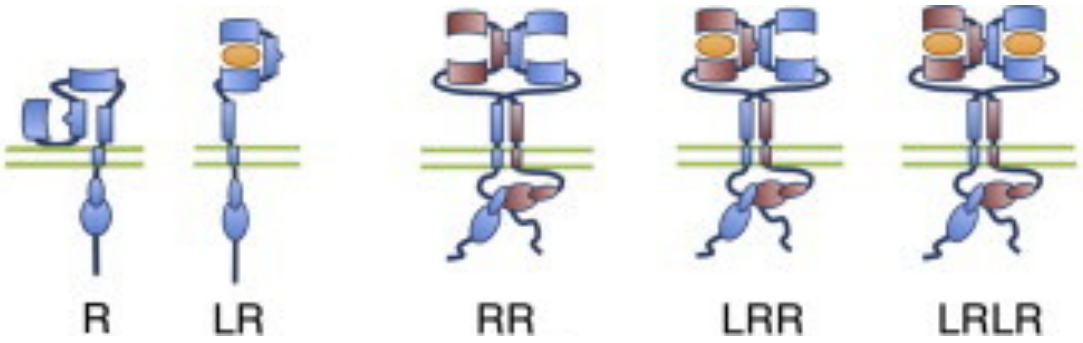


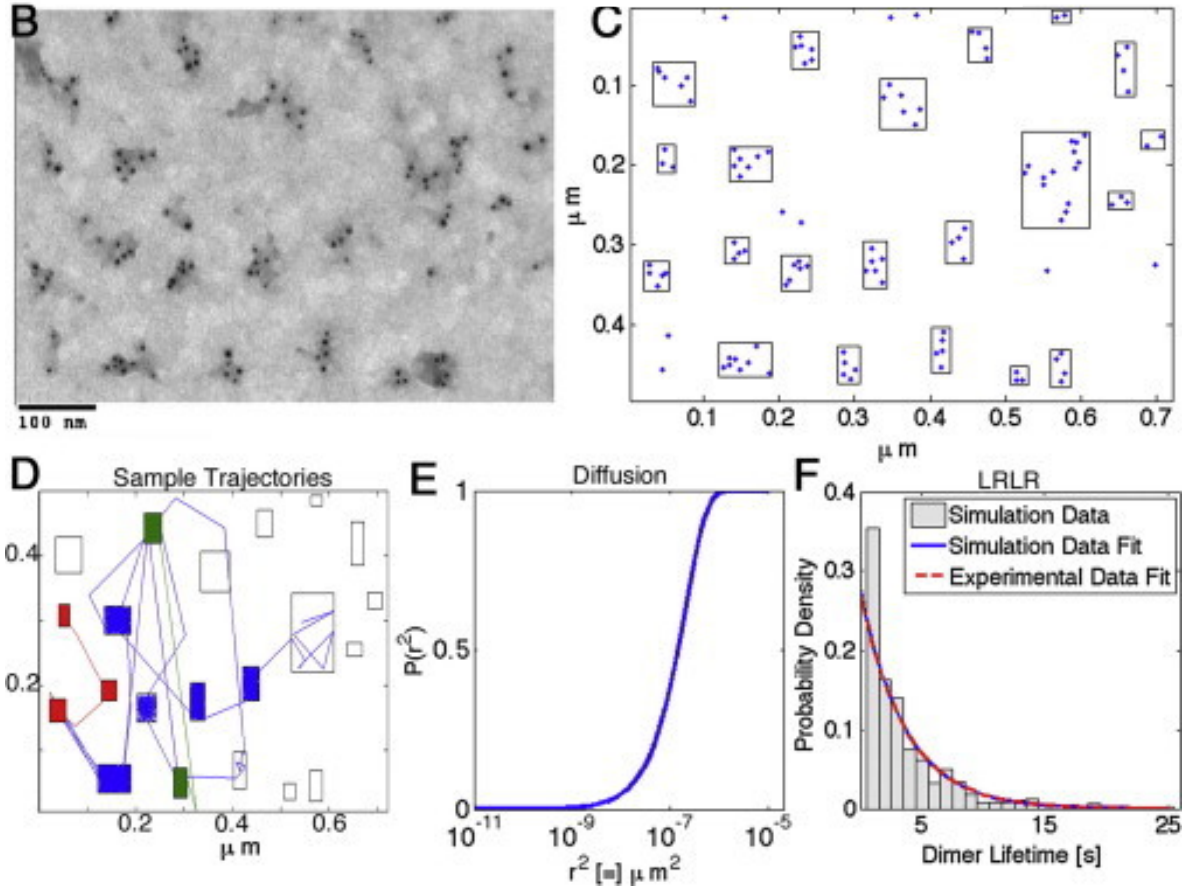






Can use data calculated from SPT trajectories (state dependent diffusion, dimer off rates) to parameterize spatial stochastic models





Biophys J. 2013 Sep 17; 105(6): 1533–1543.

The Players

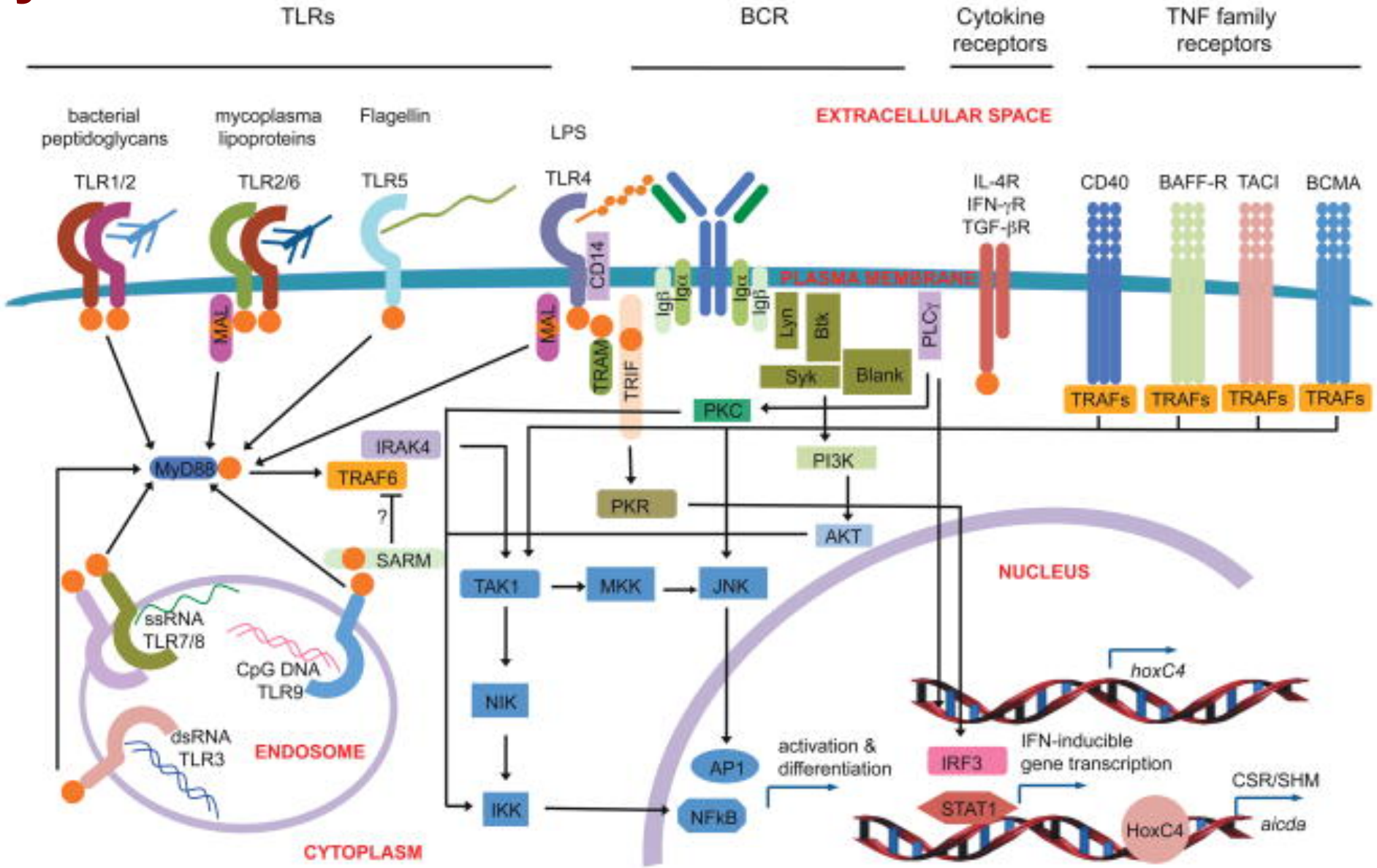
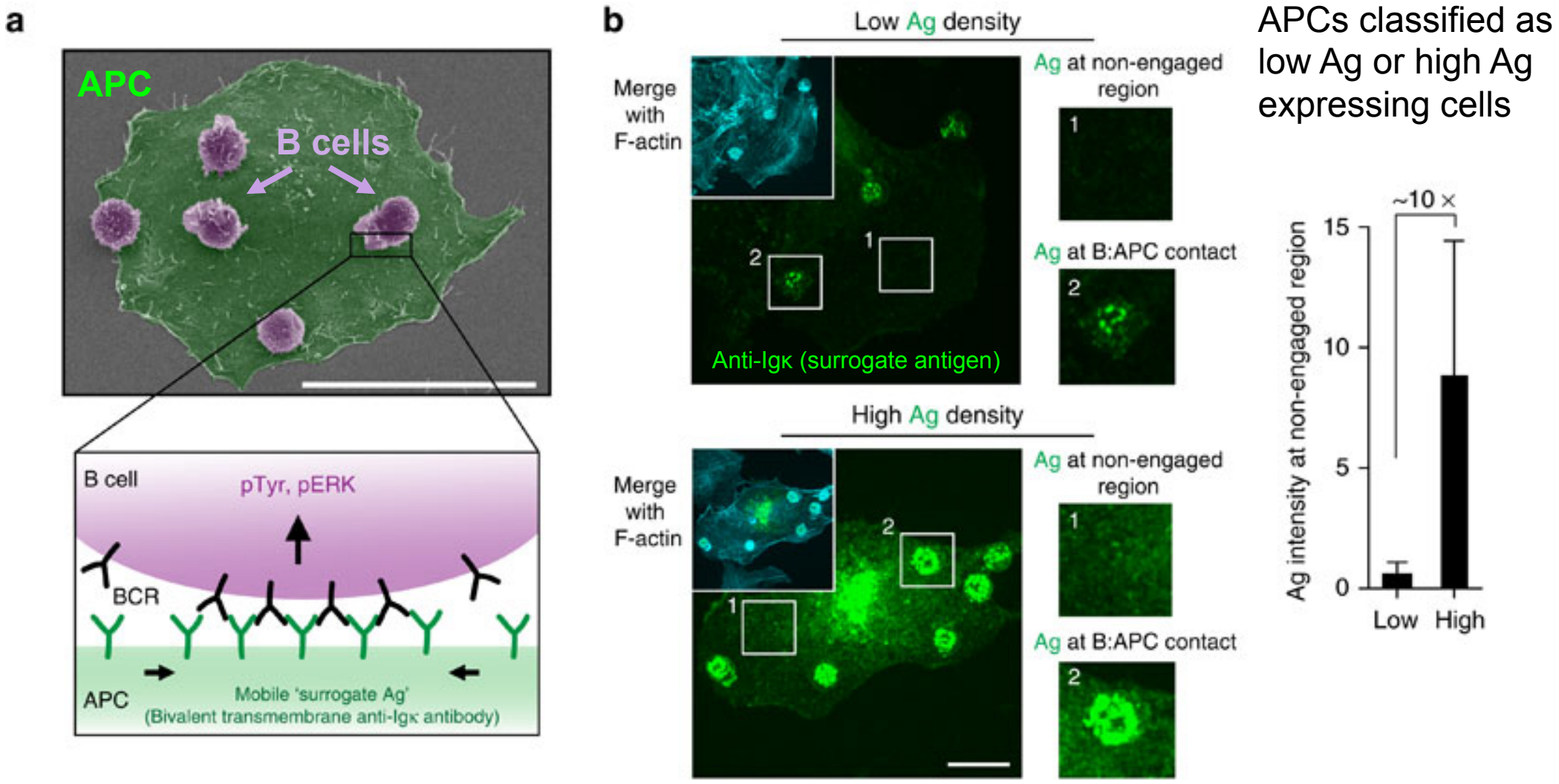
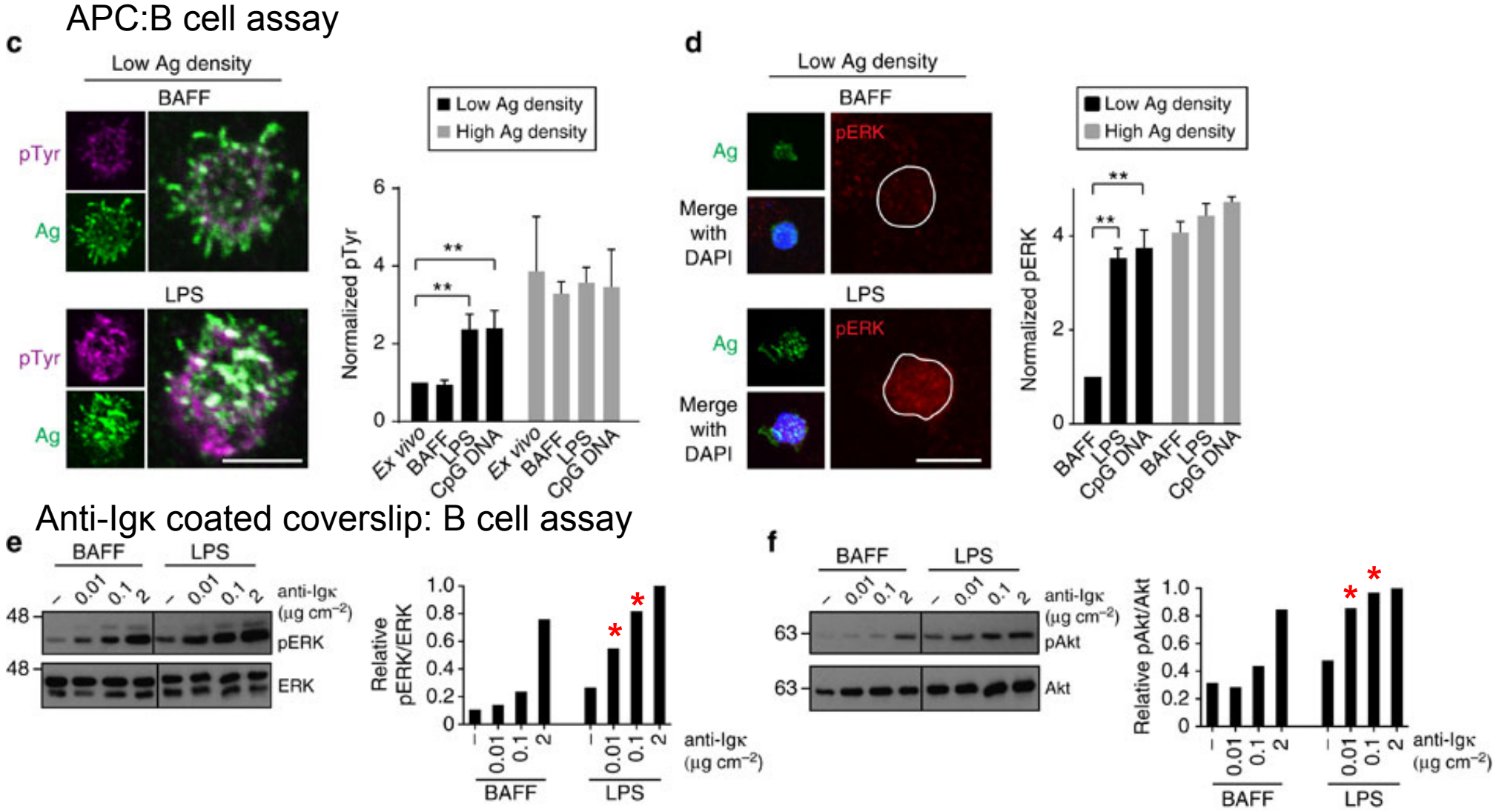

TLRs
BCR
Cytokine receptors
TNF family receptors
EXTRACELLULAR SPACE
bacterial peptidoglycans
mycoplasma lipoproteins
Flagellin
LPS
TLR1/2
TLR2/6
TLR5
TLR4
IL-4R
IFN-γR
TGF-βR
CD40
BAFF-R
TACI
BCMA
PLASMA MEMBRANE
CD14
MAL
TRAM
TRIF
Igβ
Igα
Igε
Igβ
Lyn
Btk
Syk
Blank
PLCγ
TRAFs
PKC
PI3K
AKT
PKR
IRAK4
MyD88
TRAF6
?
SARM
TAK1
MKK
JNK
NIK
IKK
AP1
NFkB
activation & differentiation
NUCLEUS
hoxC4
IRF3
IFN-inducible gene transcription
STAT1
HoxC4
CSR/SHM
aicda
ssRNA TLR7/8
CpG DNA TLR9
dsRNA TLR3
ENDOSOME
CYTOPLASM

Figure 1: TLR priming increases the sensitivity of B cells to membrane-bound antigens.



Antigen presenting cells (APCs) expressing anti-Igκ antibody bind B Cell Receptors (BCRs) on B cells .

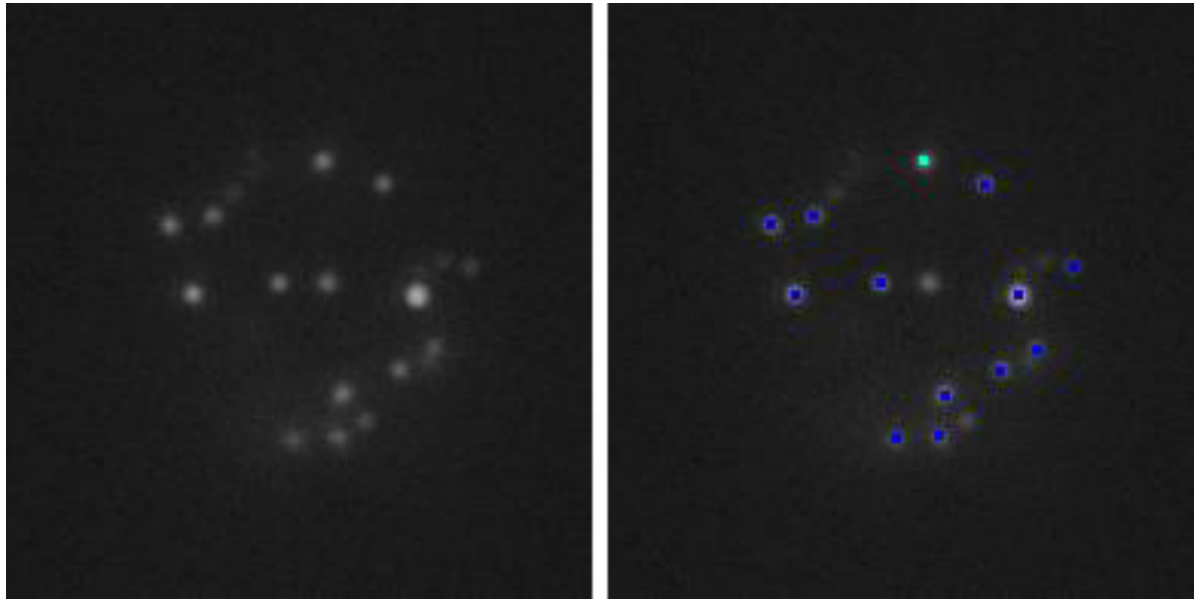
Figure 1: TLR priming increases the sensitivity of B cells to membrane-bound antigens.



Activation of the TLRs increased BCR signaling (pTyr, pErk and pAKt) at low Ag density.

Tracking mIgM-QD labeled BCRs on B cells

Control B cells
(BAFF)



LPS-treated B cells

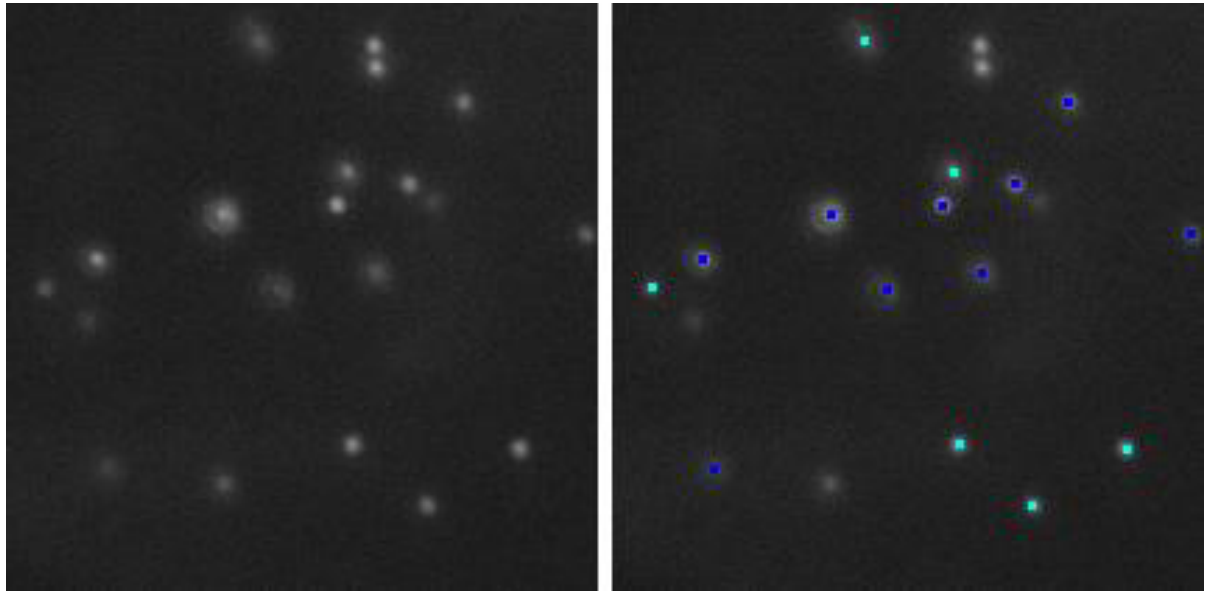
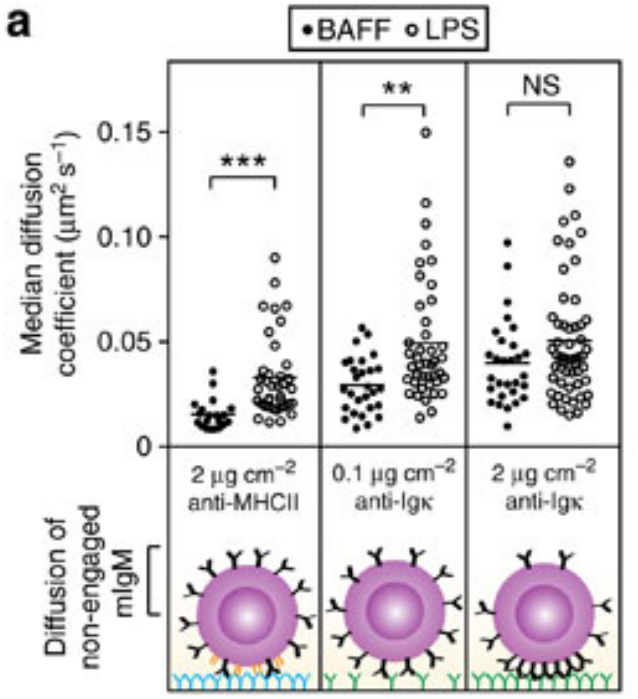


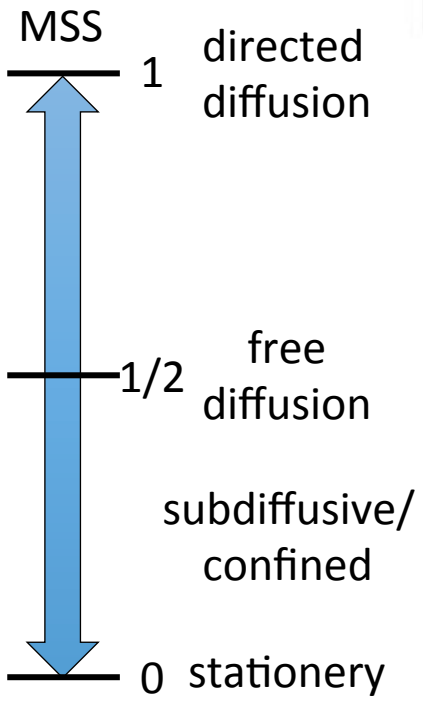
Figure 2: TLR ligands increase BCR mobility and decrease BCR confinement.

B-cells incubated O/N with LPS to activate TLRs then plated on coverslips with anti-MHCII (no actovation) or antigen (activation)

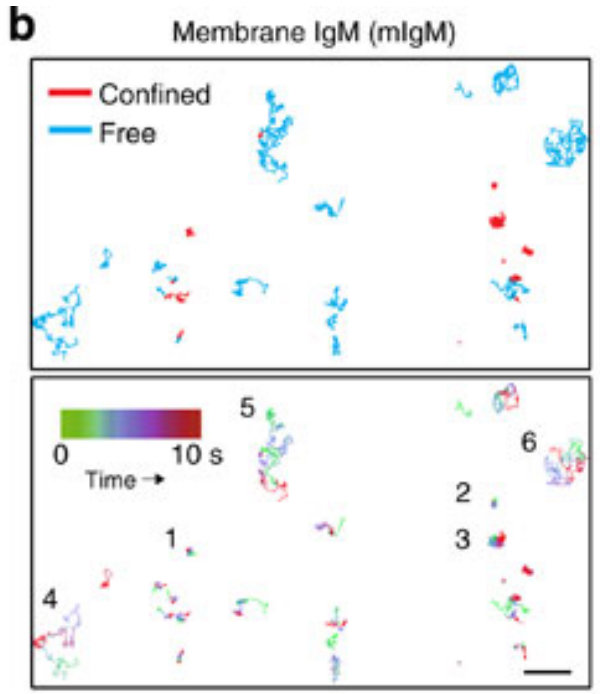
Tracking non-engaged BCRs with IgM Fab-QD that binds to the BCR

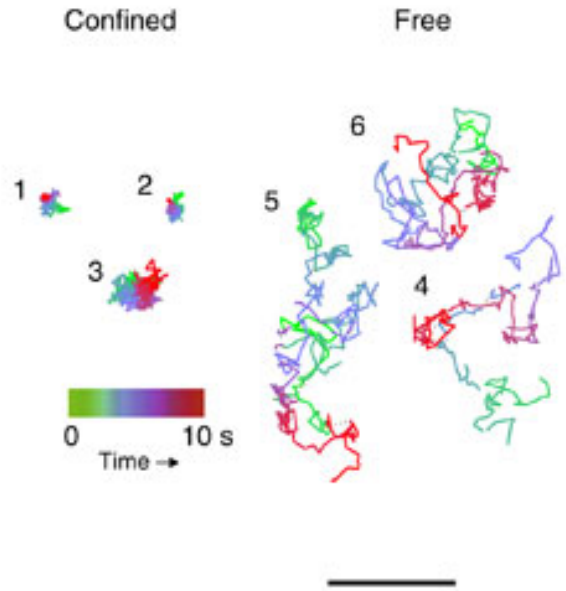
Localization error ~ 10 nm





B-cells + LPS





Tracked receptors can be classified as confined or free based on moment scaling spectrum (MSS) analysis

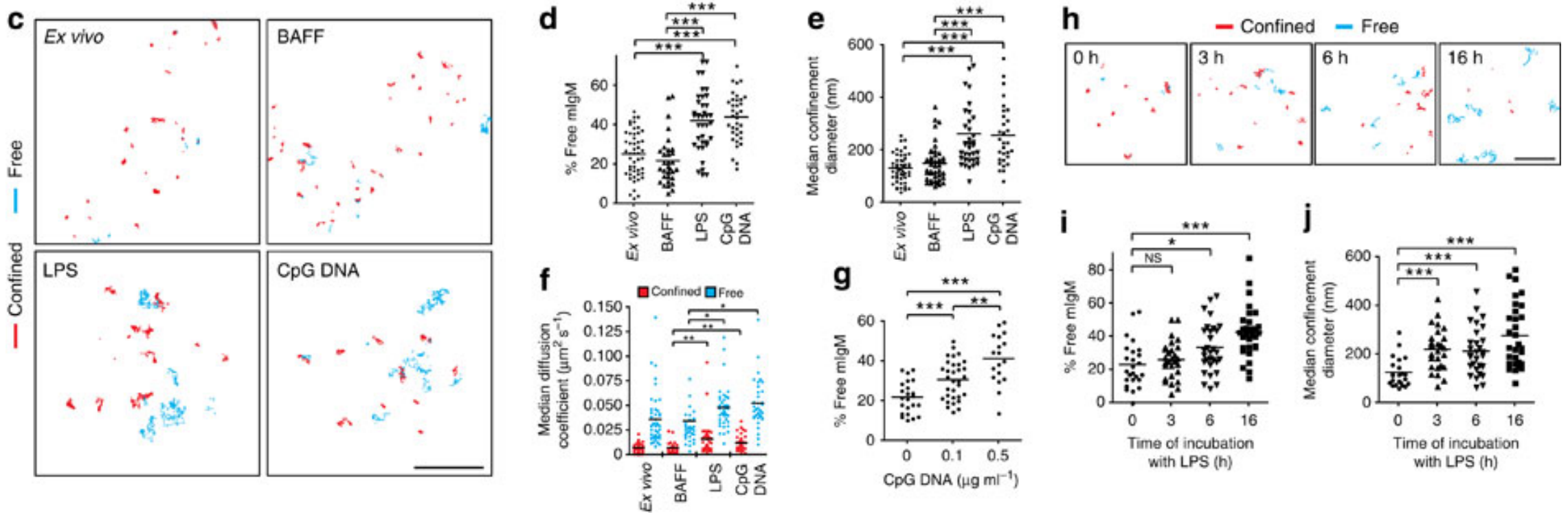
Hidden Markoff Model divides tracks into confined vs free segments and allows the measurement of how quickly receptors switch from confined to free ($k_{slow} \rightarrow k_{fast}$) or from free to confined ($k_{fast} \rightarrow k_{slow}$).

Condition	# of tracks analyzed	D_{slow} (10^{-3} µm^2 s^{-1})	D_{fast} (10^{-2} µm^2 s^{-1})	$k_{slow \rightarrow fast}$ (s^{-1})	$k_{fast \rightarrow slow}$ (s^{-1})	K_{eff} = $k_{slow \rightarrow fast} / k_{fast \rightarrow slow}$
Fig. 2h-j						
Control (BAFF only)	1430	**1.97** [1.94 – 2.00]	**4.66** [4.59 – 4.73]	**2.96** [2.87 – 3.06]	**3.86** [3.70 – 4.02]	**0.768** [0.739 – 0.797]
LPS, 3 h	955	**4.09** [3.96 – 4.22]	**6.49** [6.37 – 6.61]	**3.40** [3.26 – 3.56]	**3.19** [3.02 – 3.40]	**1.06** [1.01 – 1.13]
LPS, 6 h	877	**2.85** [2.78 – 2.93]	**5.12** [5.05 – 5.21]	**3.59** [3.45 – 3.74]	**2.88** [2.73 – 3.03]	**1.25** [1.19 – 1.31]
LPS, 16 h	1902	**5.29** [5.18 – 5.37]	**7.71** [7.61 – 7.79]	**2.56** [2.48 – 2.66]	**2.12** [2.03 – 2.22]	**1.21** [1.16 – 1.26]

TLR signaling makes it more likely for the BCRs to switch from confined to free (Keff > 1).

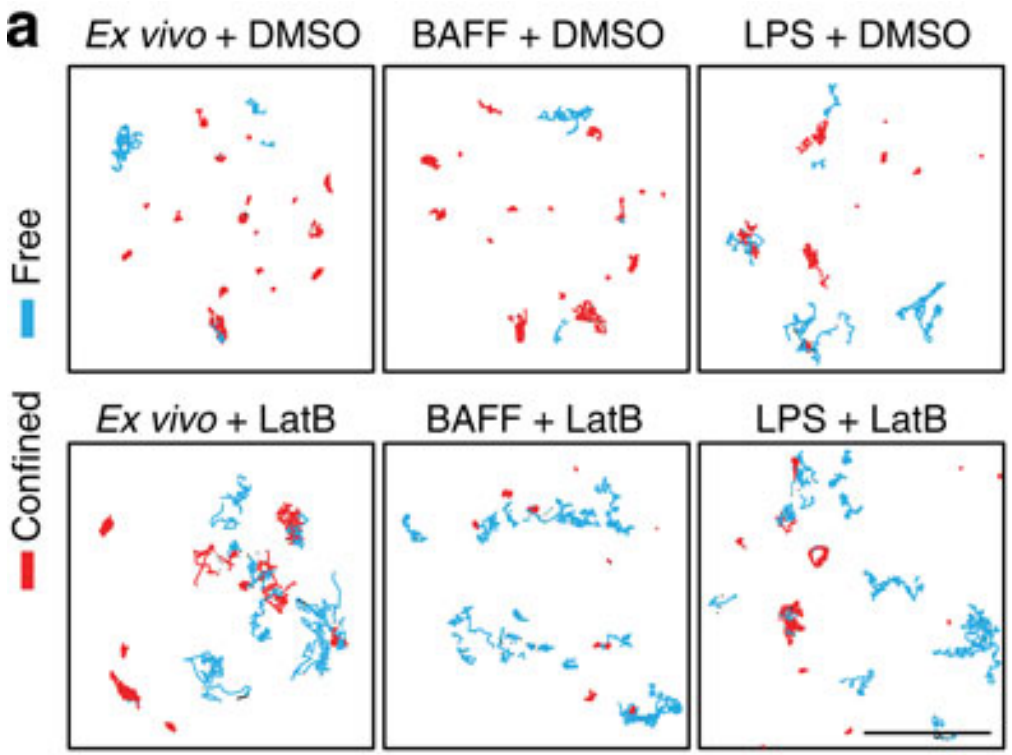
Figure 2: TLR ligands increase BCR mobility and decrease BCR confinement.

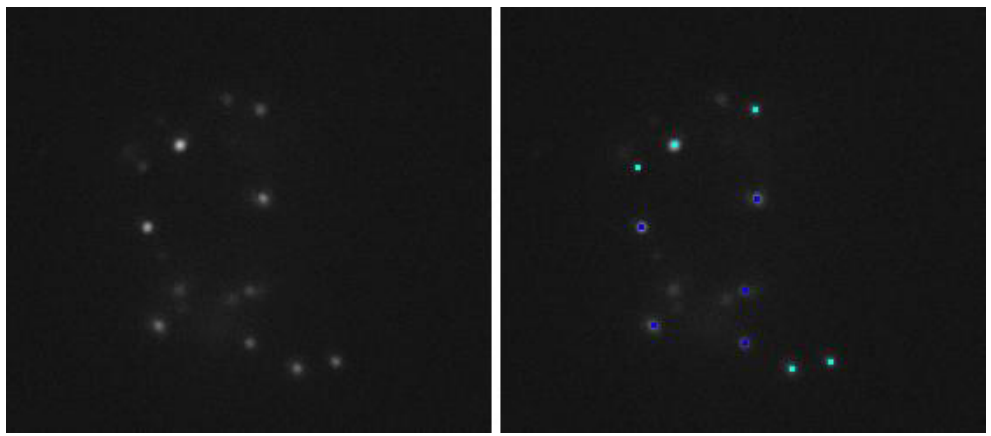
Stimulation of TLR signaling by LPS or CpG DNA increases the # of freely diffusing BCR

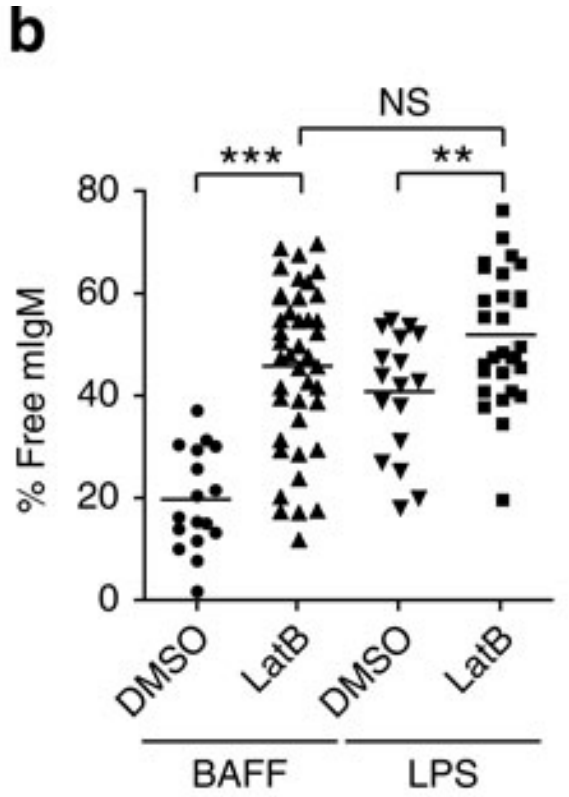


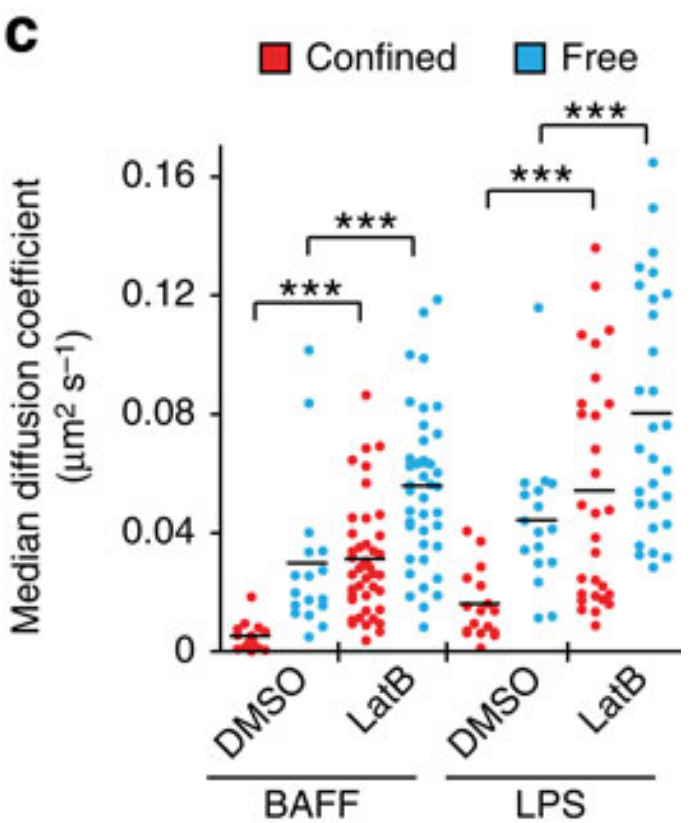
The affect of TLR signaling on BCR diffusion is concentration and time dependent.

Figure 3: BCR confinement and diffusion are controlled by the actin cytoskeleton.









Disrupting the actin cytoskeletion with latrunculin treatment also increases BCR free diffusion

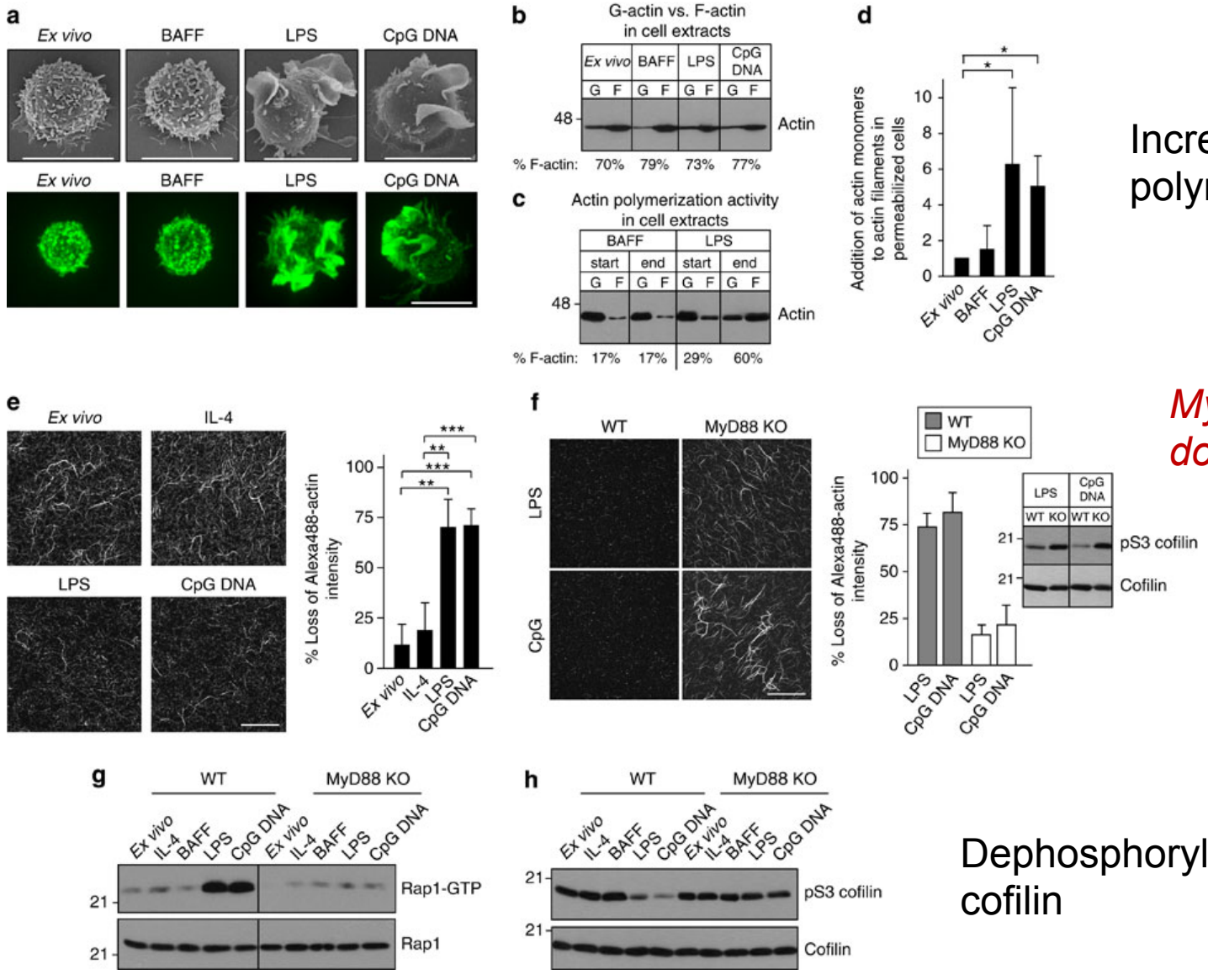
Figure 4: TLR signaling enhances actin dynamics and activates cofilin.

After TLR activation:

Increased F-actin rich cell ruffling after TLR activation

Increased F-actin severing activity. (Fluorescent F-actin)

Increased GTP-bound Rap1 that increases actin dynamics



Increased de novo actin polymerizing activity

MyD88 links TLR to downstream signaling

Dephosphorylation/activation of cofilin

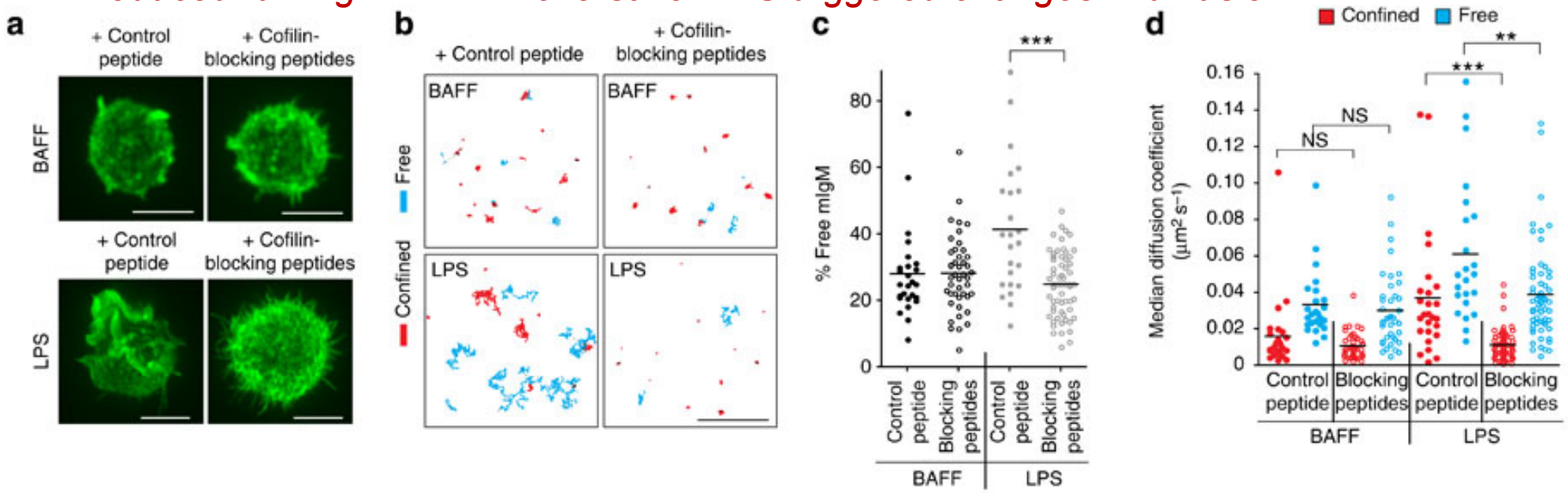
TLR activation creates more dynamic F-actin networks. Rapid turnover and reassembly leads to more transient actin barriers.

Figure 5: TLR enhancement of BCR mobility is dependent on actin severing.

Blocking peptides prevent binding of cofilin to F-actin

Reduced ruffling

Reversal of LPS-triggered changes in diffusion



TLR signaling primes the BCR for activation by increasing F-actin severing

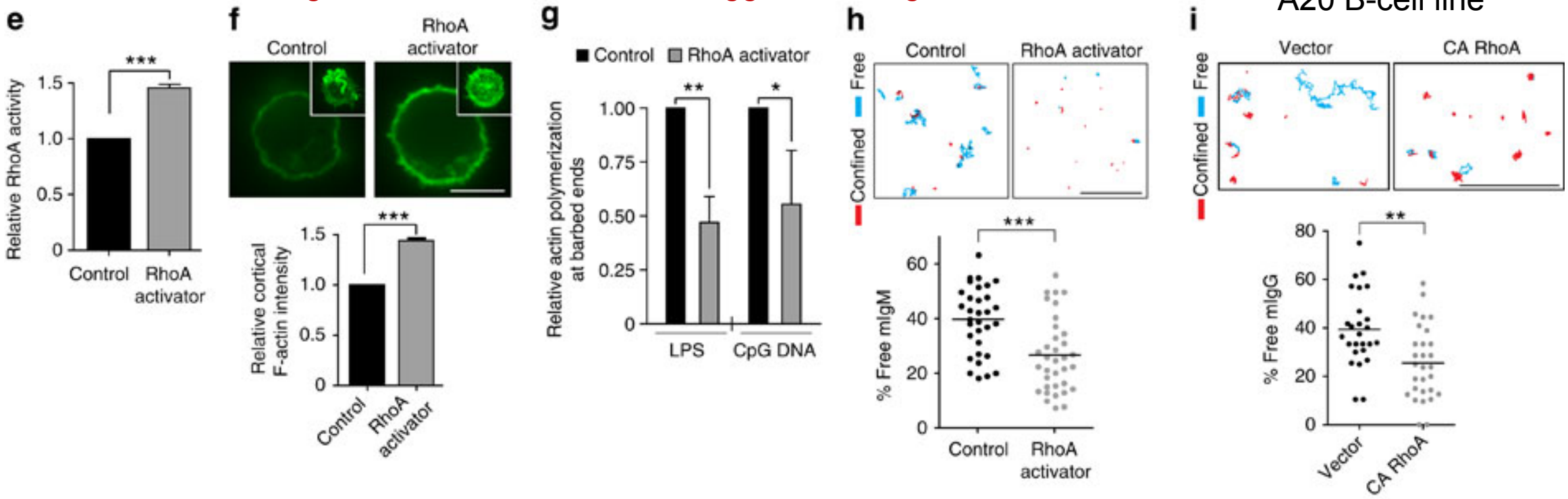
Figure 5: TLR enhancement of BCR mobility is dependent on actin severing.

Treatment with cell-permeant cytotoxic necrotizing factor activates RhoA and stabilizes actin filaments

Reduced ruffling

Reversal of LPS-triggered changes in diffusion

A20 B-cell line

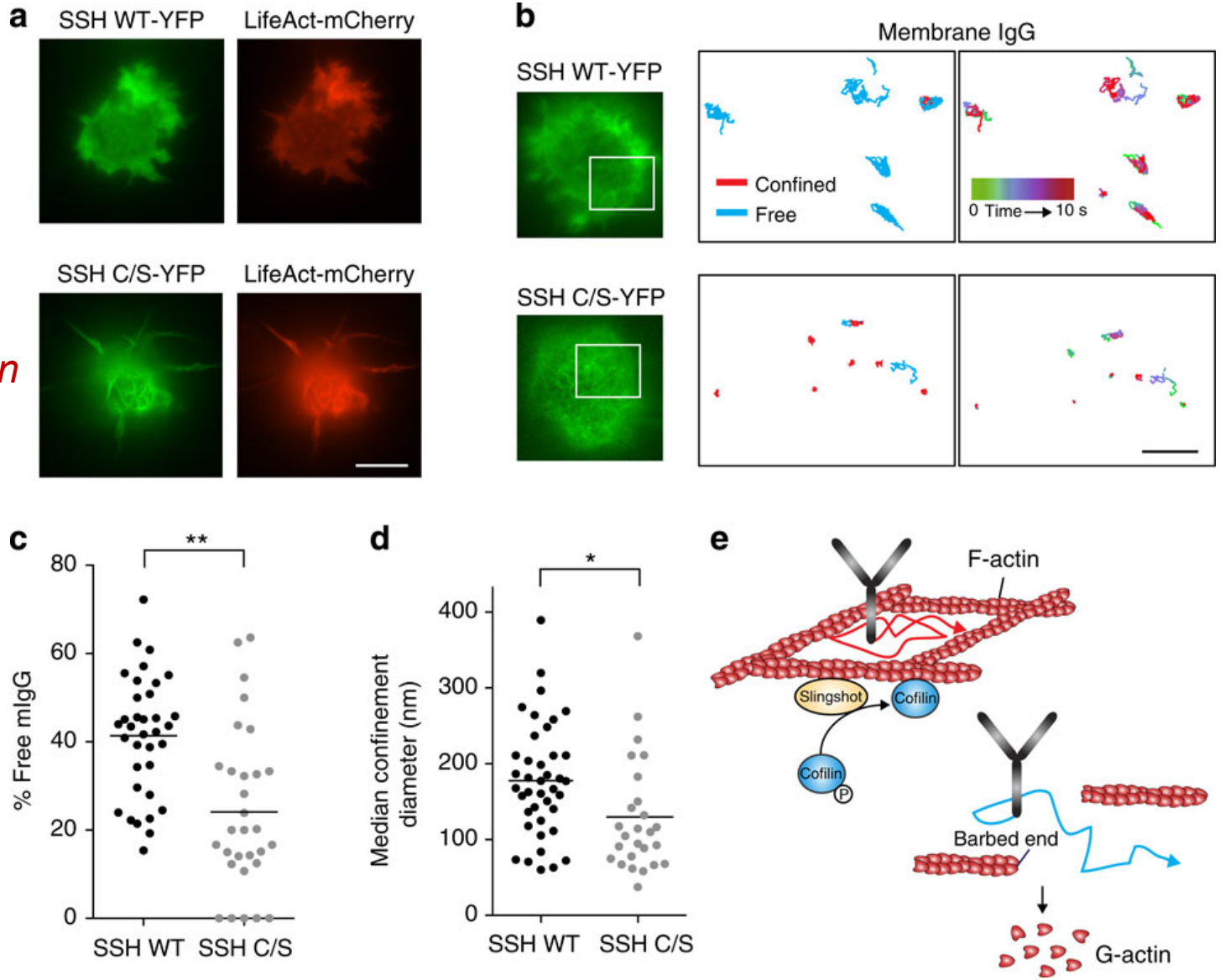


Either blocking peptides that limit actin severing or stabilizing actin filaments by activating Rho A reverses LPS-triggered changes in cell ruffling and BCR diffusion.

Figure 6: Cofilin activity controls BCR confinement.

SSH (Slingshot) is the phosphatase that activates cofilin

Abnormal actin filaments



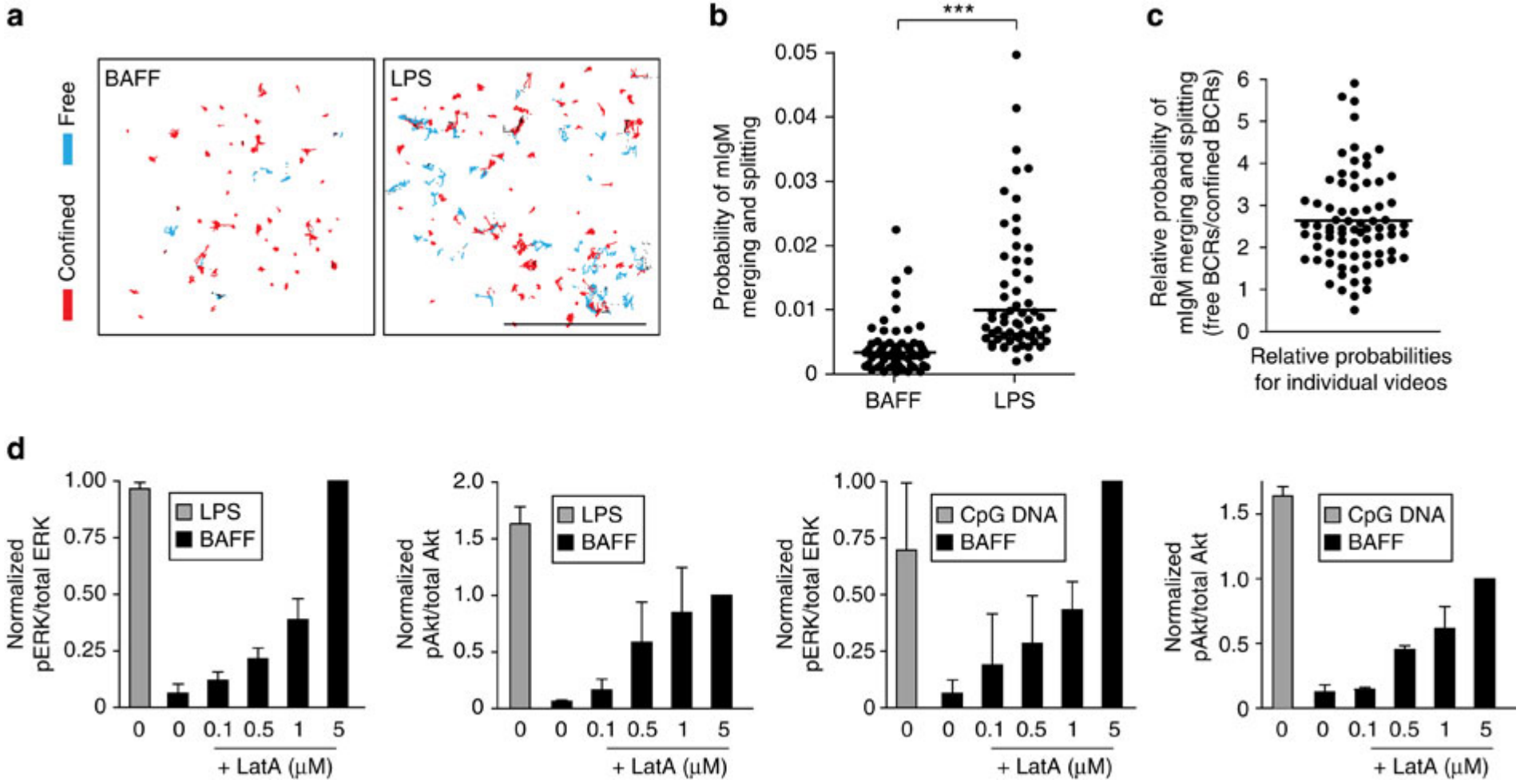
Decreased mobility/ increased confinement

Slingshot is needed to release BCR from confinement zones.

Figure 7: TLR ligands increase BCR–BCR collisions and antigen independent tonic signalling.

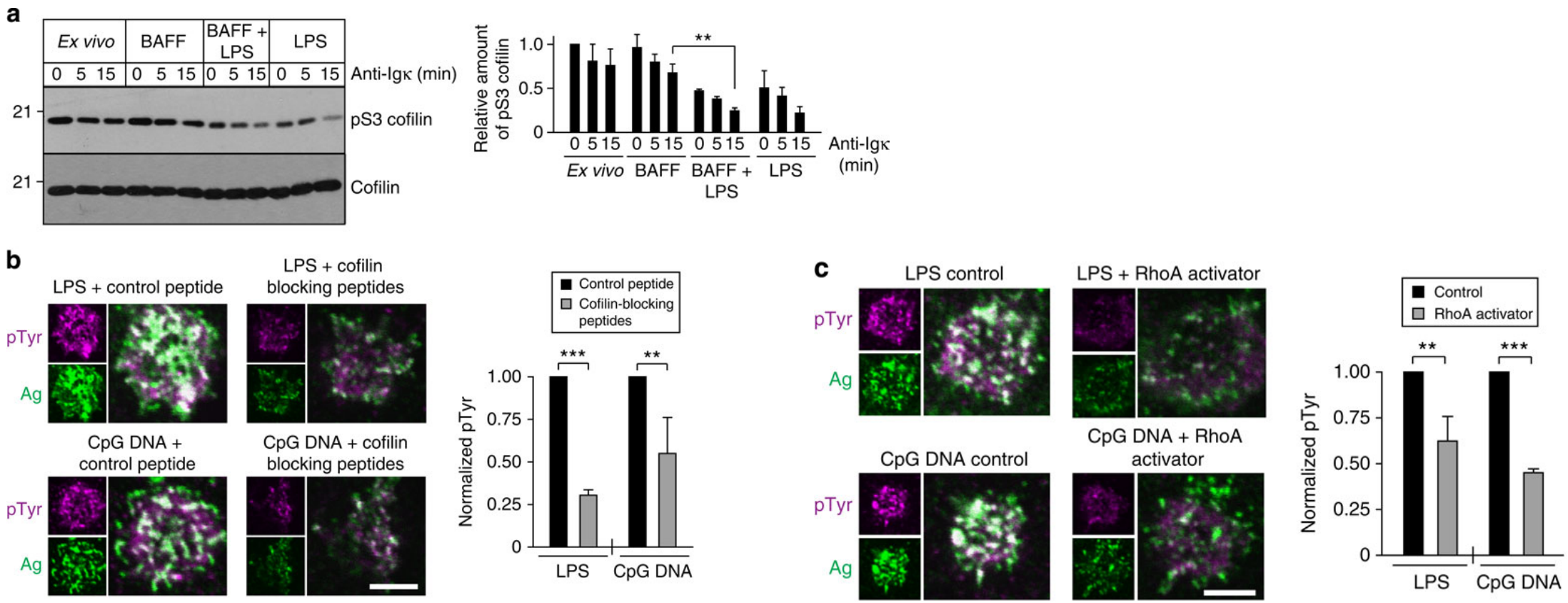
B-cell survival requires tonic, antigen independent BCR signaling.

BCR-BCR collisions detected with merge/split algorithms



TLR activation may increase BCR collisions and increase tonic signaling

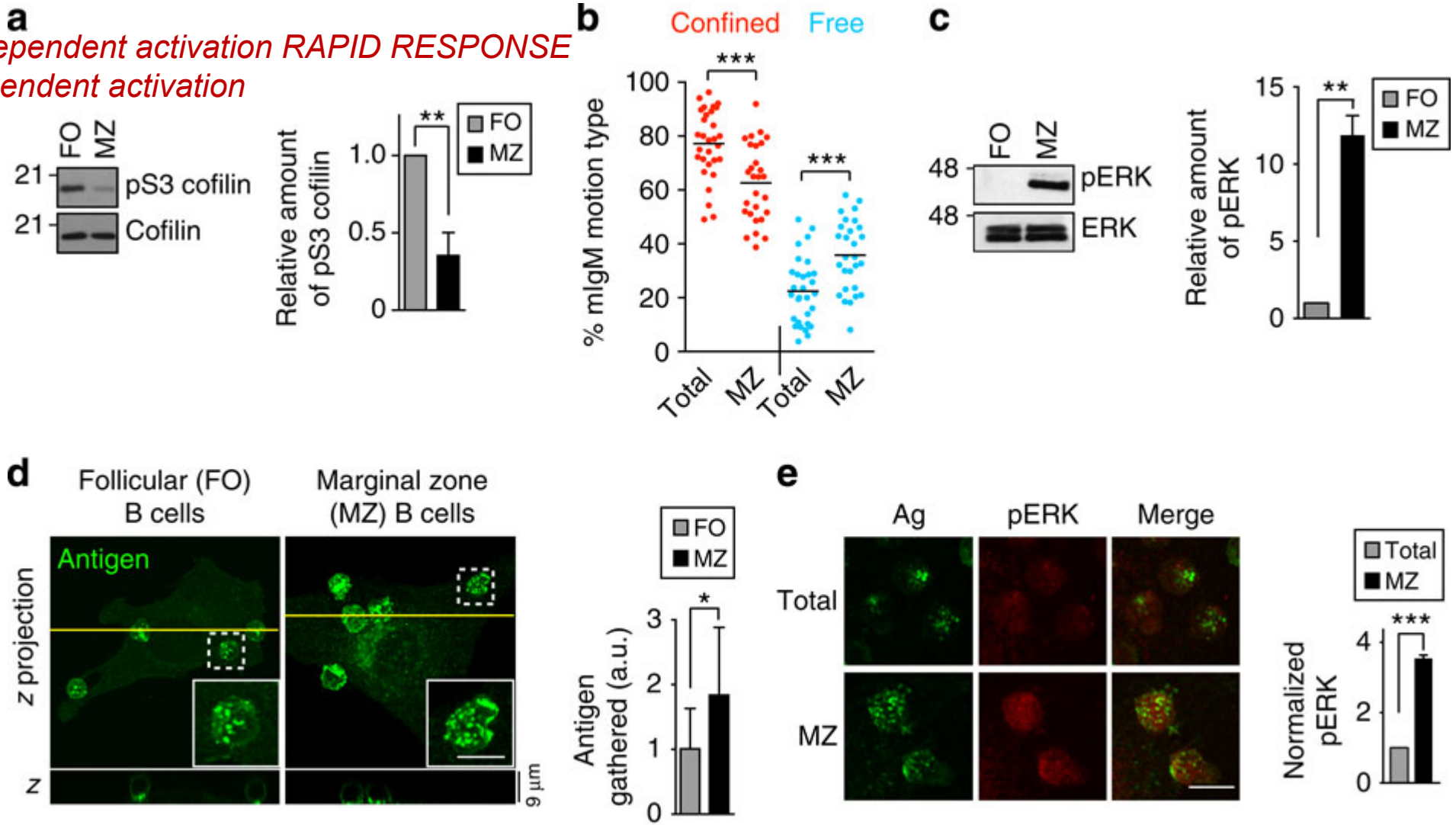
Figure 8: B-cell responses to APC-bound antigens are dependent on actin dynamics that correlate with cofilin activation.



Inhibiting actin dynamics limits B-cell response to APCs expressing low levels of antigen.

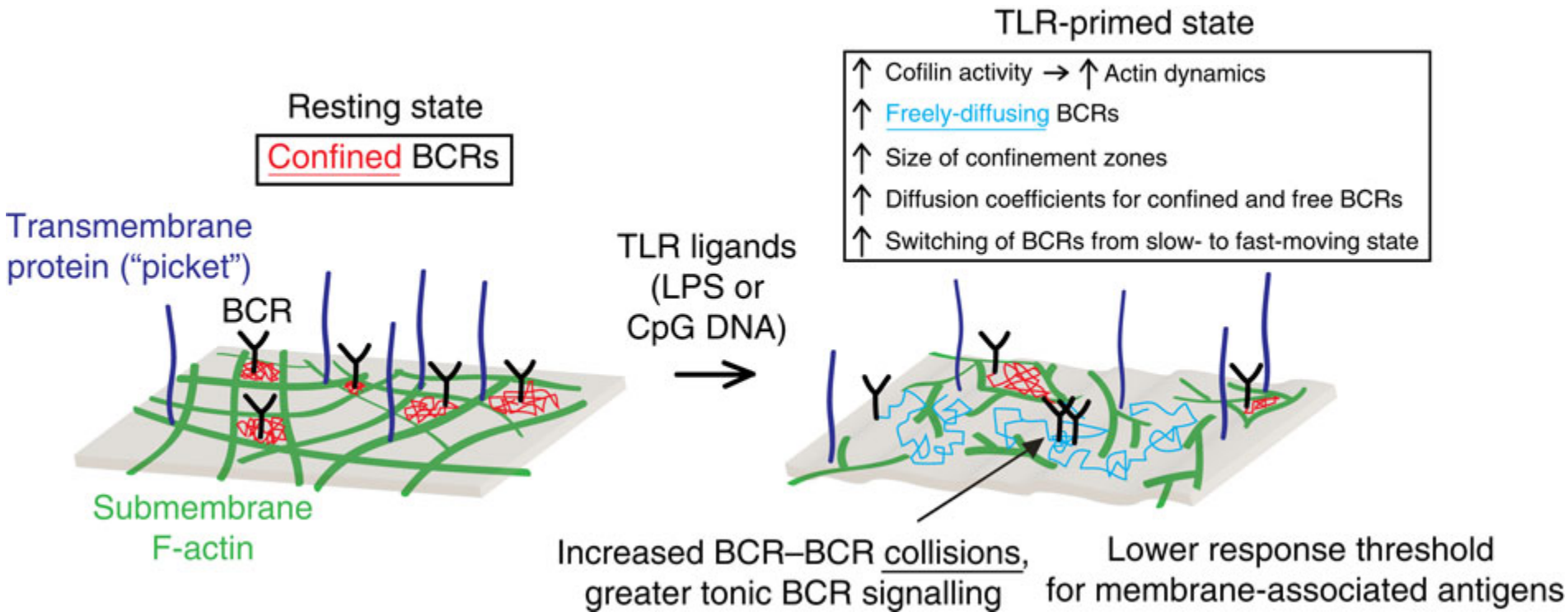
Figure 9: Marginal Zone (MZ) B cells have greater cofilin activation, BCR mobility and BCR signalling than Follicular (FO) B cells.

MZ B cells → T cell independent activation RAPID RESPONSE
FO B cells → T cell dependent activation



TLR activation is may be important in priming MZ Bcells for activation.

Figure 10: Model for TLR-induced transition of B cells to a primed state.



Cross talk of the TLR and BCR pathways involves changes in membrane dynamics, priming cells for BCR clustering and signaling. Different B-cell types may have a more or less stable actin cytoskeleton creating differentially sensitive B-cells.

This mechanism may also be important for other interacting pathways.