**Supplemental Materials**

**Supplemental Figure 1 (Figure S1) Rac1-T17N and Rac2-T17N specifically inhibit the activation of Rac1 and Rac2.** (A) 1 μM Rac1-T17N pretreated human neutrophils in suspension were stimulated by $1x10^{-7}$ M fMLP. Active Rac1 and Rac2 were detected by PBD pull-down assay (see Materials and Methods). The ratio of active Rac at each time point as compared to time zero was described as [Active Rac (time X)/Total Rac (time X)] / [Active Rac (0 min)/Total Rac (0 min)]. Therefore, if the ratio of active Rac to the zero time is 1, it means no activation of Rac. If the ratio is more than 1, it means activation of Rac, and if the ratio is less than 1, it means inhibition of Rac activation. Rac1-T17N treatment decreased Rac1 activation but not Rac2, suggesting Rac1-T17N specifically inhibits Rac1 activation in human neutrophils. (B) 2 μM Rac2-T17N pretreated human neutrophils in suspension were stimulated by $1x10^{-7}$ M fMLP. The active Rac1 or Rac2 were detected by PBD pull-down assay, and presented as described in (A) above. Rac2-T17N treatment decreased Rac2 activation significantly, but not Rac1 activity, suggesting Rac2-T17N specifically inhibits Rac2 activation in human neutrophils. These concentrations of Tat-GTPases were shown to give optimal inhibition in preliminary dose-response studies, and were used in all chemotaxis experiments. (The result is the representive of two independent experiments)

**Supplemental Figure 2 (Figure S2) Determination of fMLP gradient fields.** (A) A series of fluorescein solutions of known concentration were imaged. (B) Based on the

linear relationship between the concentrations of fluorescein and the fluorescence intensity, a standard curve of fluorescein concentration vs fluorescence intensities was constructed. (C) A sample image of the gradient field from the micropipette containing $1x10^{-4}$ M fMLP is shown at 5 min after the addition to the chamber at the top of the panel, and a line scan across the gradient field, as indicated by the red arrow, is shown at the bottom of the panel. (D) A sample image of the gradient field from the micropipette containing $1x10^{-5}$ M fMLP is shown at 5 min after the addition to the chamber at the top of the panel, and a line scan across the gradient field, as indicated by the red arrow, is shown at the bottom of the panel.

**Supplemental Figure 3 (Figure S3) The specificity of the Rac1 and Rac2 antibodies.**

10 ng of recombinant Rac1 or Rac2 were run in separate lanes on a 12% SDS-PAGE gel and then immunobloted using either Rac1-selective antibody 23A8 (Upstate) or Rac2-specific antibody R786 (in house). (The result is representive of two independent experiments)

**Supplemental Movie 1-Human neutrophils in high fMLP gradient**

Control, Rac1-T17N and Rac2 T17N treated neutrophils were stimulated with a high fMLP gradient created by a micropipette containing $3x10^{-4}$ M fMLP. The movie was recorded at 20 s interval.

Note: The micropipette was added at 2 min.

**Supplemental Movie 2-Human neutrophils in low fMLP gradient**

Control, Rac1-T17N and Rac2 T17N treated neutrophils were stimulated with a low fMLP gradient created by a micropipette containing $3x10^{-5}$ M fMLP. The movie was recorded at 20 s interval.

Note: The micropipette was added at 2 min.

**Supplemental Movie 3 –Behavioral similarity of human neutrophils in gradients of fMLP and uniform fMLP**

The morphological changes of sample human neutrophils, which were subjected to the stimulation of either gradients of fMLP or uniform fMLP, were animated and overlaid with their cell tracks by using DIAS.

**Supplemental Movie 4-Morphological changes of control, Rac1-T17N and Rac2-T17N treated neutrophils in high uniform concentration fMLP**

The morphological changes of control and Rac1-T17N or Rac2-T17N treated neutrophils in high uniform concentration of fMLP were animated and overlaid with their cell tracks by using DIAS.

**Supplemental Movie 5-Morphological changes of control, Src and PI3K inhibitor treated neutrophils in high uniform concentration fMLP**

The morphological changes of control and Src or PI3K inhibitor treated neutrophils in high

uniform concentration of fMLP were animated and overlaid with their cell tracks by using DIAS.

Figure 1

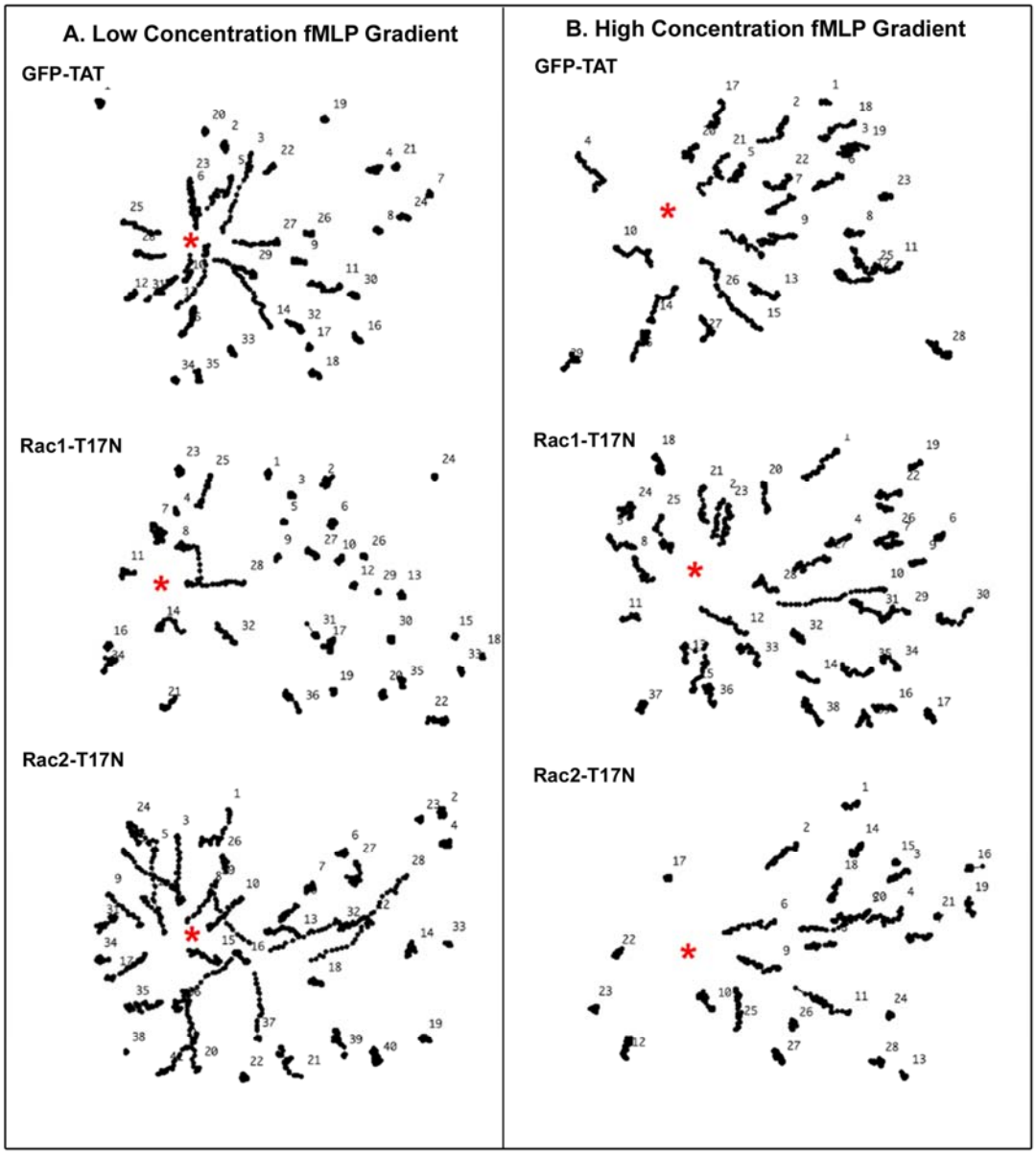

A. Low Concentration fMLP Gradient
B. High Concentration fMLP Gradient
GFP-TAT
GFP-TAT
Rac1-T17N
Rac1-T17N
Rac2-T17N
Rac2-T17N

Figure 2

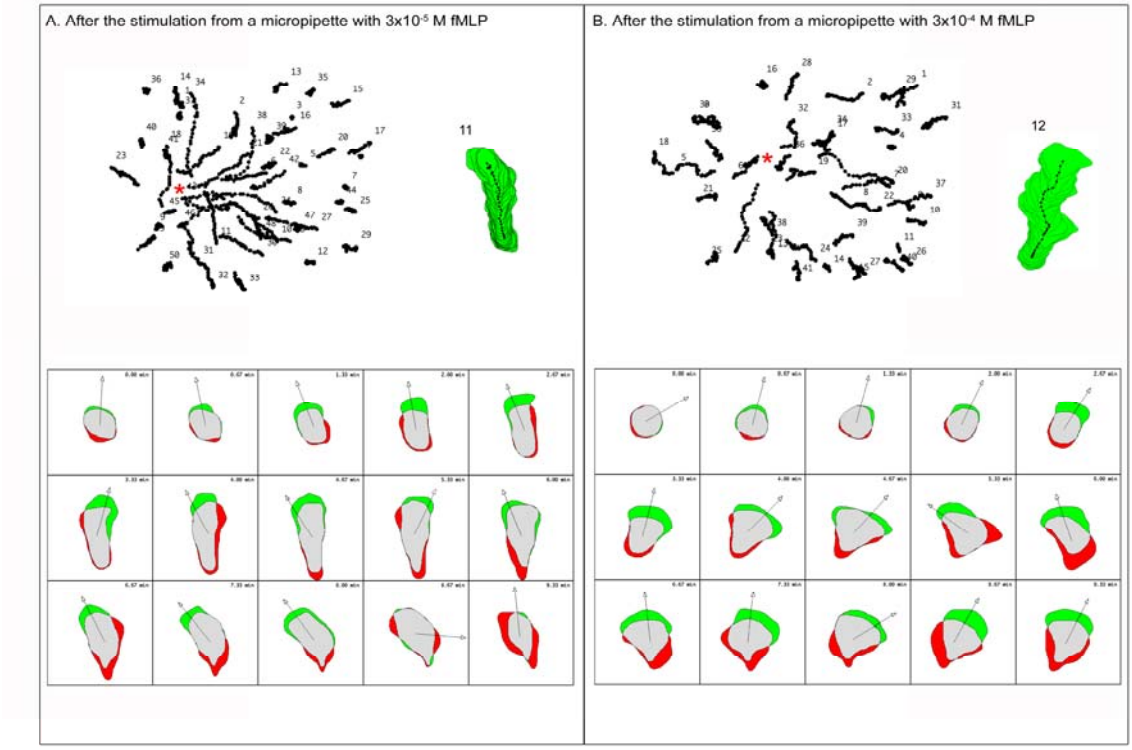

A. After the stimulation from a micropipette with 3x10-5 M fMLP
B. After the stimulation from a micropipette with 3x10-4 M fMLP
11
12

Figure 3

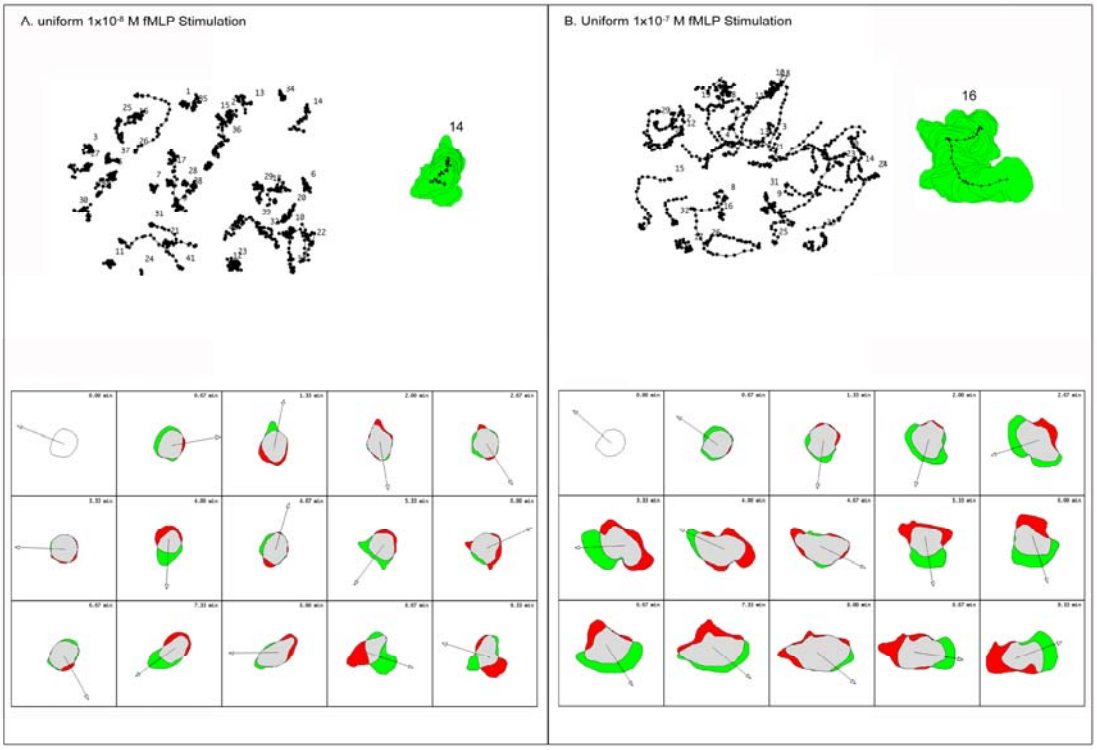

A. uniform 1x10-8 M fMLP Stimulation
14
B. Uniform 1x10-7 M fMLP Stimulation
16

Figure 4

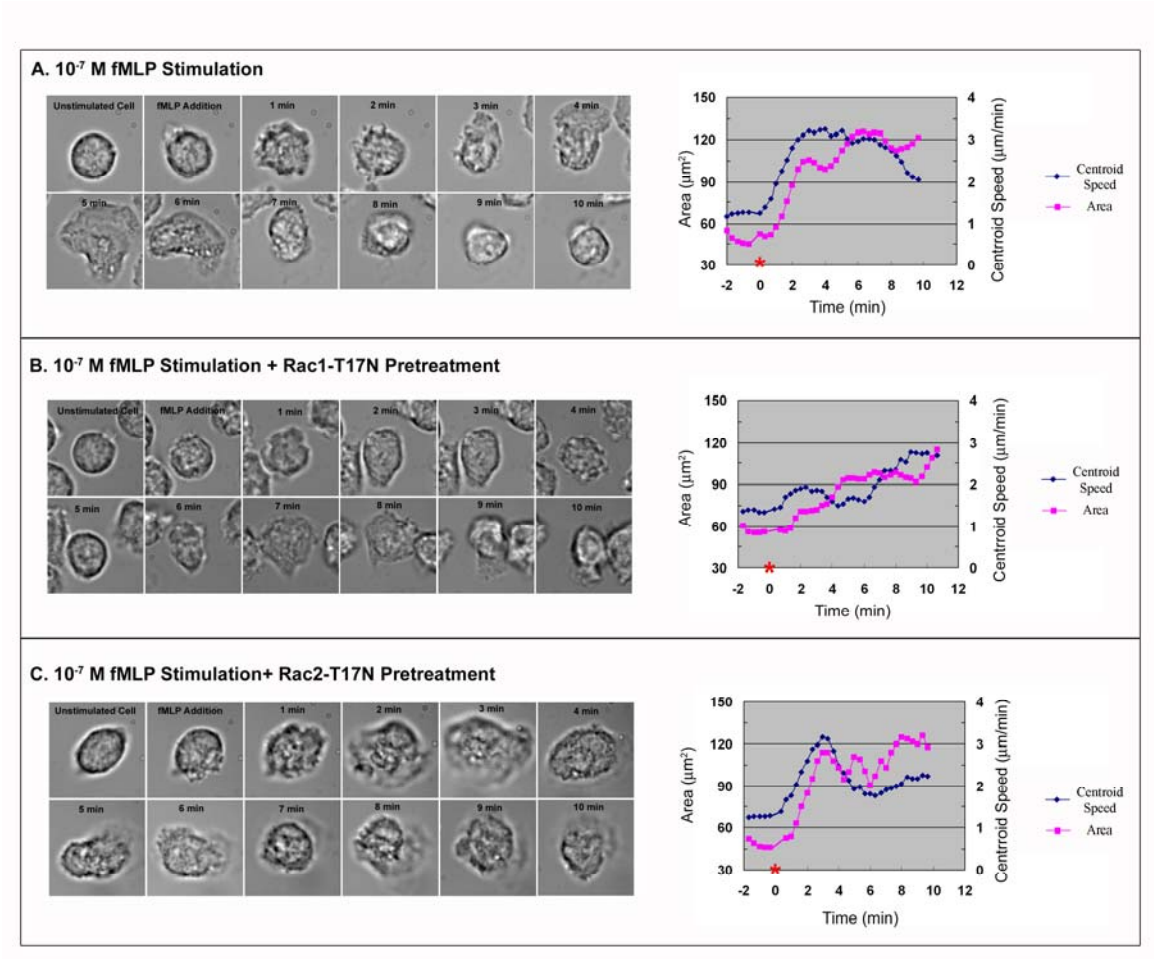

A. 10-7 M fMLP Stimulation
Unstimulated Cell
fMLP Addition
1 min
2 min
3 min
4 min
5 min
6 min
7 min
8 min
9 min
10 min
Area (μm²)
Centroid Speed (μm/min)
Time (min)
Centroid Speed
Area
B. 10-7 M fMLP Stimulation + Rac1-T17N Pretreatment
C. 10-7 M fMLP Stimulation+ Rac2-T17N Pretreatment

Figure 5

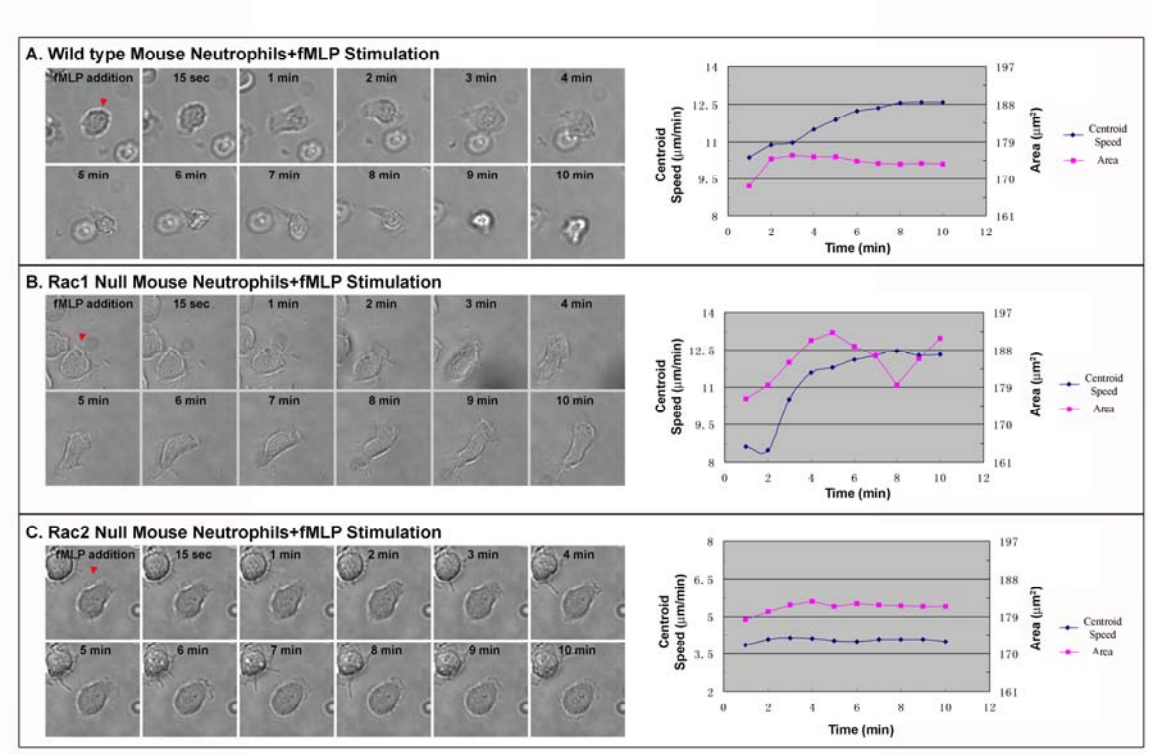

A. Wild type Mouse Neutrophils+fMLP Stimulation
fMLP addition
15 sec
1 min
2 min
3 min
4 min
5 min
6 min
7 min
8 min
9 min
10 min
Centroid Speed (μm/min)
Area (μm²)
Time (min)
Centroid Speed
Area
B. Rac1 Null Mouse Neutrophils+fMLP Stimulation
C. Rac2 Null Mouse Neutrophils+fMLP Stimulation

Figure 6

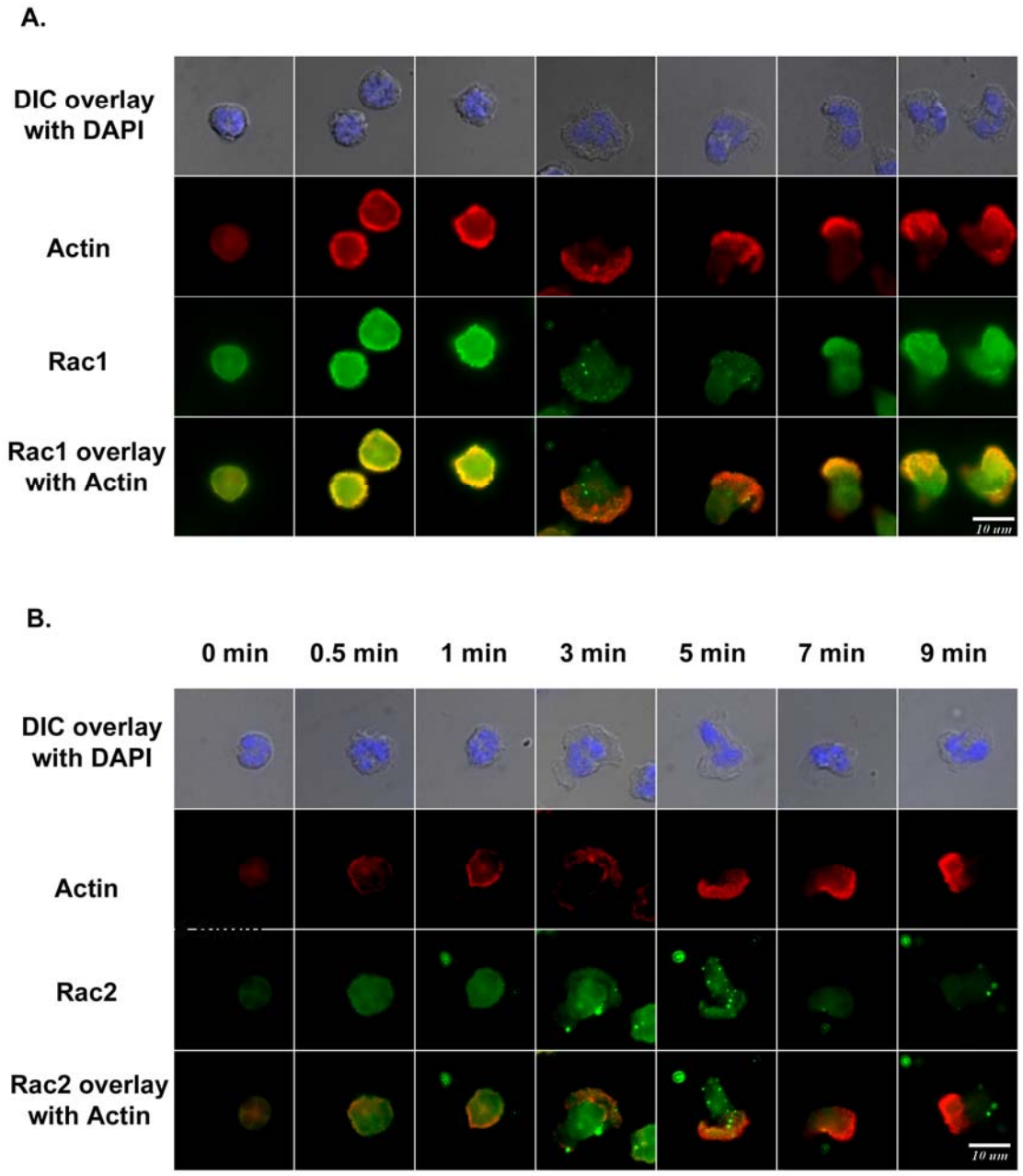

A.
DIC overlay with DAPI
Actin
Rac1
Rac1 overlay with Actin
10 um
B.
0 min
0.5 min
1 min
3 min
5 min
7 min
9 min
DIC overlay with DAPI
Actin
Rac2
Rac2 overlay with Actin
10 um

Figure 7

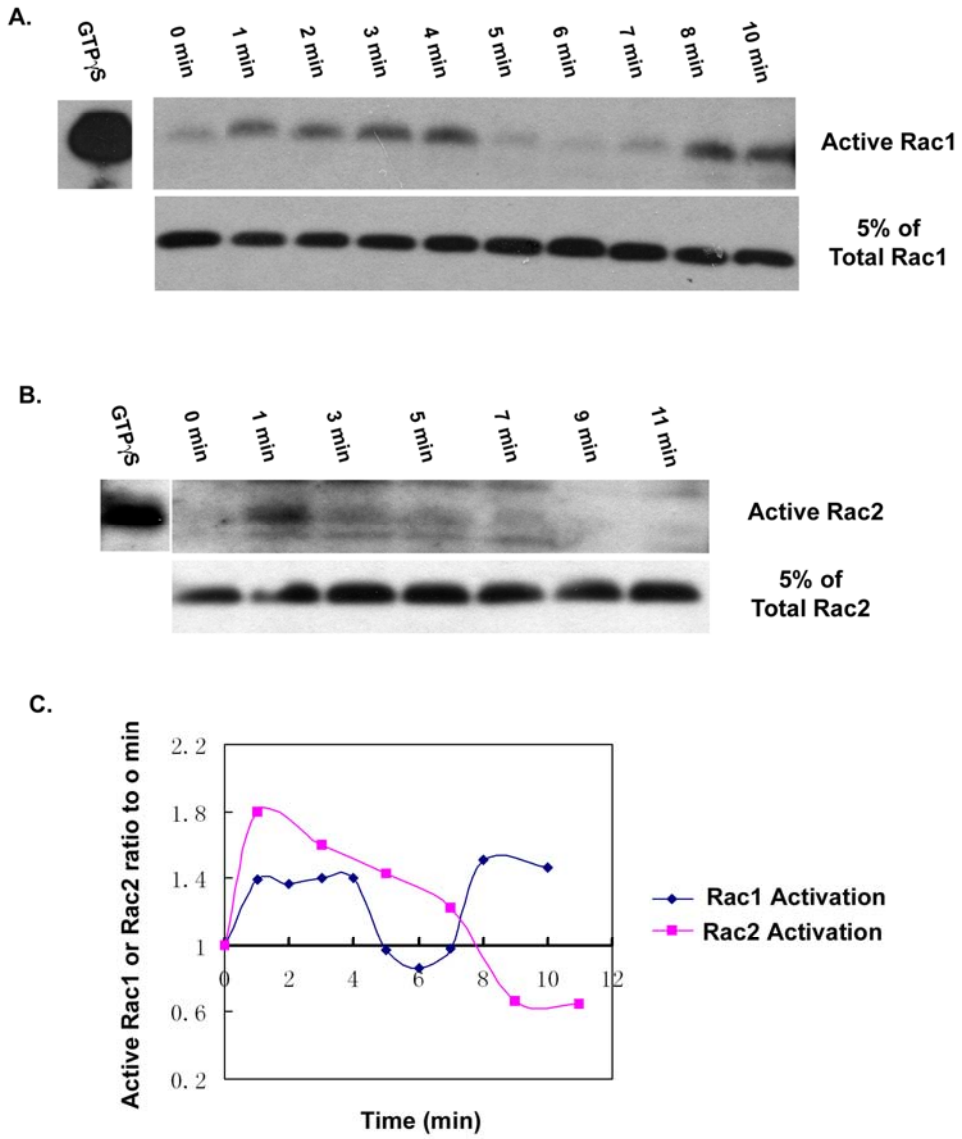

A.
GTPγS
0 min
1 min
2 min
3 min
4 min
5 min
6 min
7 min
8 min
10 min
Active Rac1
5% of Total Rac1
B.
GTPγS
0 min
1 min
3 min
5 min
7 min
9 min
11 min
Active Rac2
5% of Total Rac2
C.
Active Rac1 or Rac2 ratio to o min
2.2
1.8
1.4
1
0.6
0.2
0
2
4
6
8
10
12
Time (min)
Rac1 Activation
Rac2 Activation

Figure 8

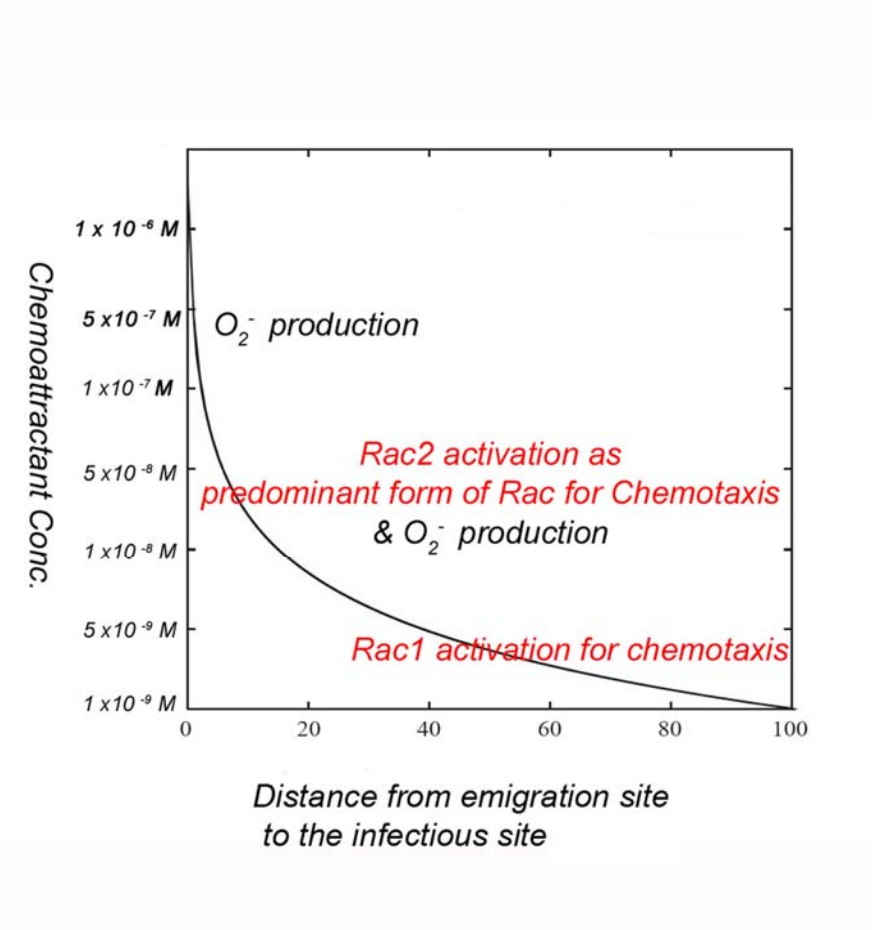

1 x 10 -6 M
5 x10 -7 M
1 x10 -7 M
5 x10 -8 M
1 x10 -8 M
5 x10 -9 M
1 x10 -9 M
Chemoattractant Conc.
O2- production
Rac2 activation as
predominant form of Rac for Chemotaxis
& O2- production
Rac1 activation for chemotaxis
0
20
40
60
80
100
Distance from emigration site
to the infectious site

Figure S1

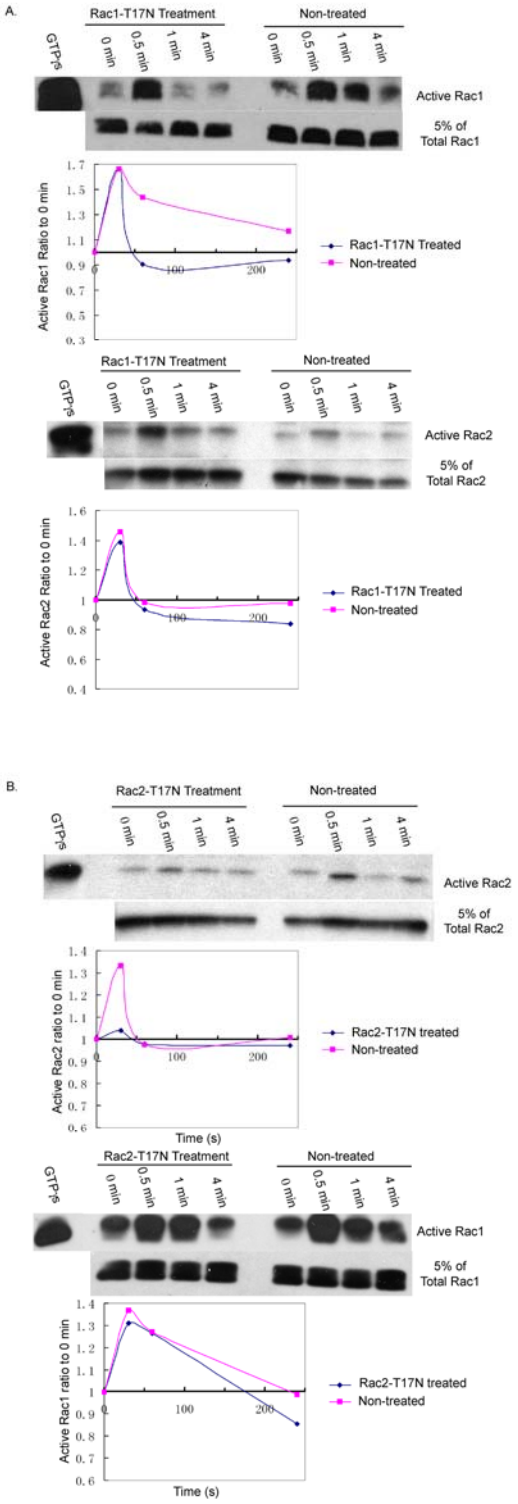

A.
Rac1-T17N Treatment
Non-treated
GTP-γS
0 min
0.5 min
1 min
4 min
Active Rac1
5% of Total Rac1
Active Rac1 Ratio to 0 min
Rac1-T17N Treated
Non-treated
Active Rac2
5% of Total Rac2
Active Rac2 Ratio to 0 min
B.
Rac2-T17N Treatment
Active Rac2 ratio to 0 min
Rac2-T17N treated
Time (s)
Active Rac1
Active Rac1 ratio to 0 min

Figure S2

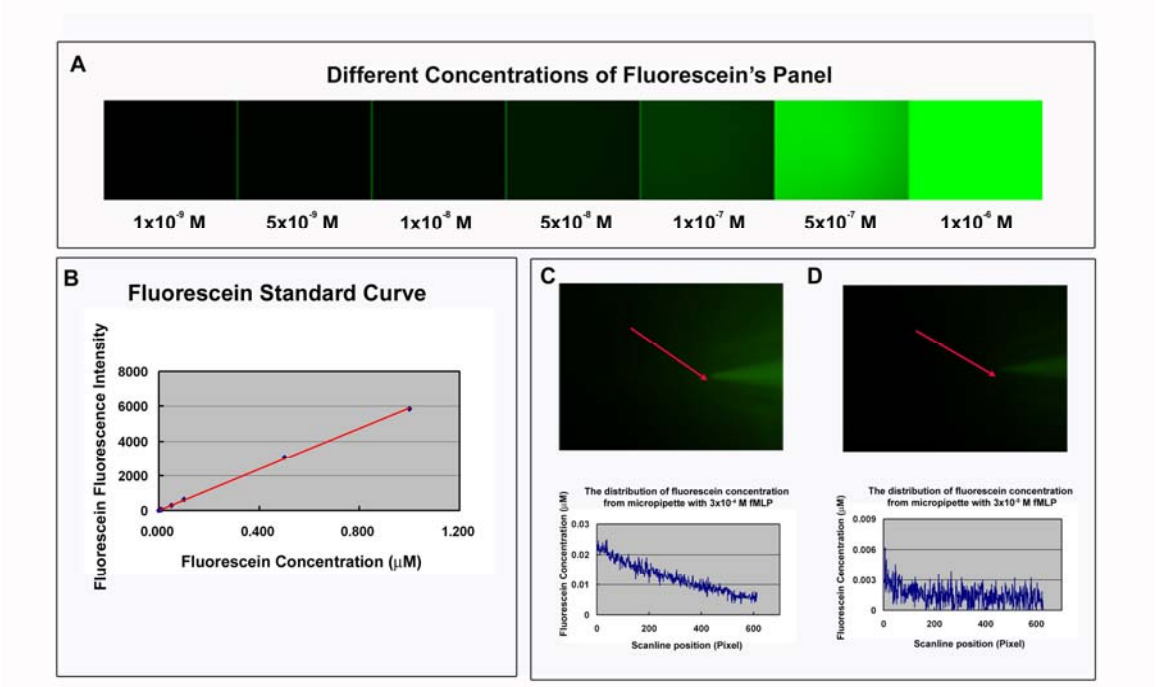
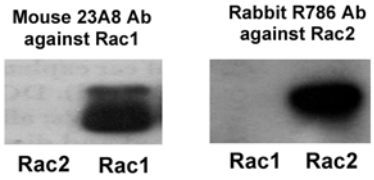

A
Different Concentrations of Fluorescein's Panel
1x10-9 M
5x10-9 M
1x10-8 M
5x10-8 M
1x10-7 M
5x10-7 M
1x10-6 M
B
Fluorescein Standard Curve
Fluorescein Fluorescence Intensity
8000
6000
4000
2000
0
0.000
0.400
0.800
1.200
Fluorescein Concentration (μM)
C
D
The distribution of fluorescein concentration from micropipette with 3x10-6 M fMLP
Fluorescein Concentration (μM)
0.03
0.02
0.01
0
0
200
400
600
Scanline position (Pixel)
The distribution of fluorescein concentration from micropipette with 3x10-6 M fMLP
Fluorescein Concentration (μM)
0.009
0.006
0.003
0
0
200
400
600
Scanline position (Pixel)

Figure S3


Mouse 23A8 Ab against Rac1
Rac2
Rac1
Rabbit R786 Ab against Rac2
Rac1
Rac2