Supplemental Materials
Molecular Biology of the Cell
Thevathasan et al.
Supplementary Information

Supplemental material
Supplementary information includes 5 supplementary figures and 13 movies.

Figure S1. (A) Time-lapse imaging of cells transfect with CFKBP, LDR and YFP-akt-PH. Addition of Rapa leads to no detectable activation of PI3K or protrusion activity. (B,C) 3-PIs and PR maps corresponding to the time series shown in (A). Time is in min and sec. scale bar: 10μm.

Figure S2. (A,B) Snapshot of a time series showing the YFP-akt-PH/CF-iSH ratio (A) and protruded (red) and retracted (blue) areas (B). To generate the 3-PI map, the average ratio along each angle α (relative to the cell centroid) is measured for each time point (background is excluded from these measurements). The 3-PI map displays this ratio as a function of angle and time (C). To quantify the local and persistent nature of the 3-PI signals, the map was devided in 20 angular bins and the sum of ratio values was calculated for each bin, from a time point immediately after Rapamacin addition (grey bar) to the end of the time series (D). 3-PI ratio values for the three adjacent bins with the highest total PI3K activity were then added and divided by the sum of PI3K activities in all the 20 bins. The 3-PI polarity index was further normalized as described in methods. A similar approach was employed to generate the PR map. Here, the protruded (red) and retracted (blue) pixels were summed along each angle α and converted to protrusion or retraction velocities as described in methods. Velocities were then plotted as a function of angle and time to yield the PR map (E). (F) The polarity index for protrusion velocity was then calculated based on the same three adjacent bins used to define the 3-PI polarity index.

Figure S3 (A,E) knockdown of NRas and KRas using two independent siRNA sequence for each Ras isoform and revealed by immunoblotting against NRas (A) or PanRas (E). The upper band in (E) is KRas, which migrates at a higher apparent molecular weight than H- and NRas

(Omerovic *et al.*, 2008). (B,F) NRas or KRas silencing has no effect on bulk 3-PI production in response to synthetic activation of PI3K. (C,D,G,H) Examples of 3-PI and PR maps obtained from NRas- (C,D) or KRas-silenced cells (G,H).

Figure S4. NIH3T3 cells were transfected with CFP-HRas, YFP-RBD, mcherry-FRB-iSH and Lyn-FKBP and exposed to 100nM Rapa. FRET hot spots appear after synthetic activation of PI3K and are confined to protruding domains (colored in red in the bottom row).

Figure S5. (A) Immunoblot showing knockdown of Rac1 using two different siRNA sequences. We only used Rac1 siRNA #1, since it caused a strong reduction in Rac1 levels. (B) Comparison of basal migration rates (recorded over 4 hrs) in serum-starved cells transfected with scramble, HRas, NRas, KRas or Rac1 #1 siRNAs. (n = 30 for each condition). No significant reduction in basal migration was observed for each of these pertubations.

movie 1. Time-lapse imaging of Rapa-induced PI3K activation. Both the CF-iSH and YFP-akt-PH channels are shown. Movie played at 20 frames per sec (fps). (sped up ~450x).

movie 2. Time-lapse YFP-akt-PH/CF-iSH ratio imaging in Rapa-treated cells. Movie played at 20 fps. The frame at which Rapa is added is highlighted.

movie 3. Time-lapse imaging of protrusion events. Movie played at 20 fps.

movie 4. Time-lapse imaging of Rapa-induced PI3K activation in HRas-silenced cells. Both the CF-iSH and YFP-akt-PH channels are shown. Movie played at 20 fps.

movie 5. Time-lapse YFP-akt-PH/CF-iSH ratio imaging in HRas-silenced cells. Movie played at 20 fps.

movie 6. Time-lapse imaging of protrusion events in HRas-silenced cells. Movie played at 20 fps.

movie 7. Ras activity monitored by the FRET probe Raichu-HRas in Rapa-stimulated cells. Movie played at 20 fps.

movie 8. Time-lapse imaging of PI3K activation in PDGF-stimulated cells. Both the Lyn-CFP and YFP-akt-PH channels are shown. The frame at which PDGF is added is highlighted. Movie played at 20 fps. (sped up ~850 x).

movie 9. Time-lapse YFP-akt-PH/Lyn-CFP ratio imaging in PDGF-treated cells. Movie played at 20 fps.

movie 10. Time-lapse imaging of protrusion events in PDGF-stimulated cells. Movie played at 20 fps.

movie 11 Time-lapse imaging of PI3K activation in HRas-silenced and PDGF-stimulated and cells. Both the Lyn-CFP and YFP-akt-PH channels are shown. The frame at which PDGF is added is highlighted. Movie played at 20 fps. (sped up ~850 x).

movie 12 YFP-akt-PH/LynCFP ratio imaging in HRas-silenced and PDGF-stimulated cells. Movie played at 20 fps.

movie 13 Protrusion activity in HRas-silenced and PDGF-stimulated cells. Movie played at 20 fps.

Supplementary reference.

Omerovic, J., Hammond, D.E., Clague, M.J., and Prior, I.A. (2008). Ras isoform abundance and signalling in human cancer cell lines. Oncogene *27*, 2754-2762.

Figure S1

Thevathasan et al.,

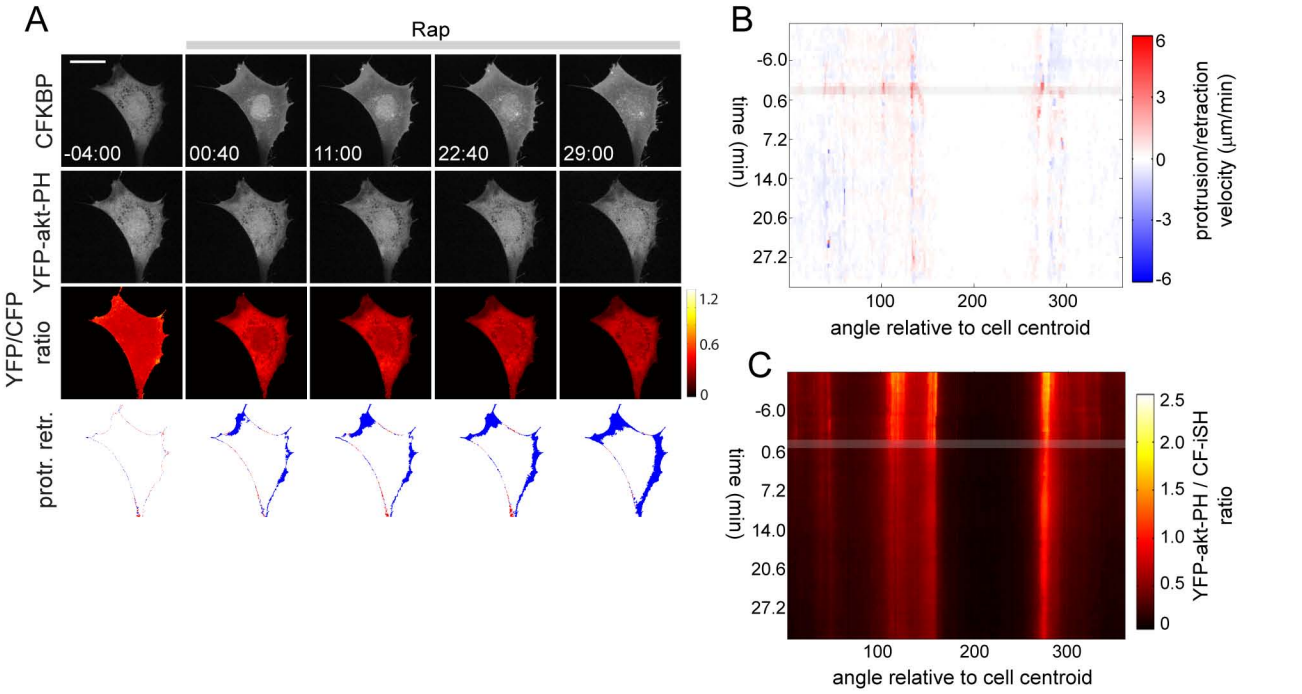
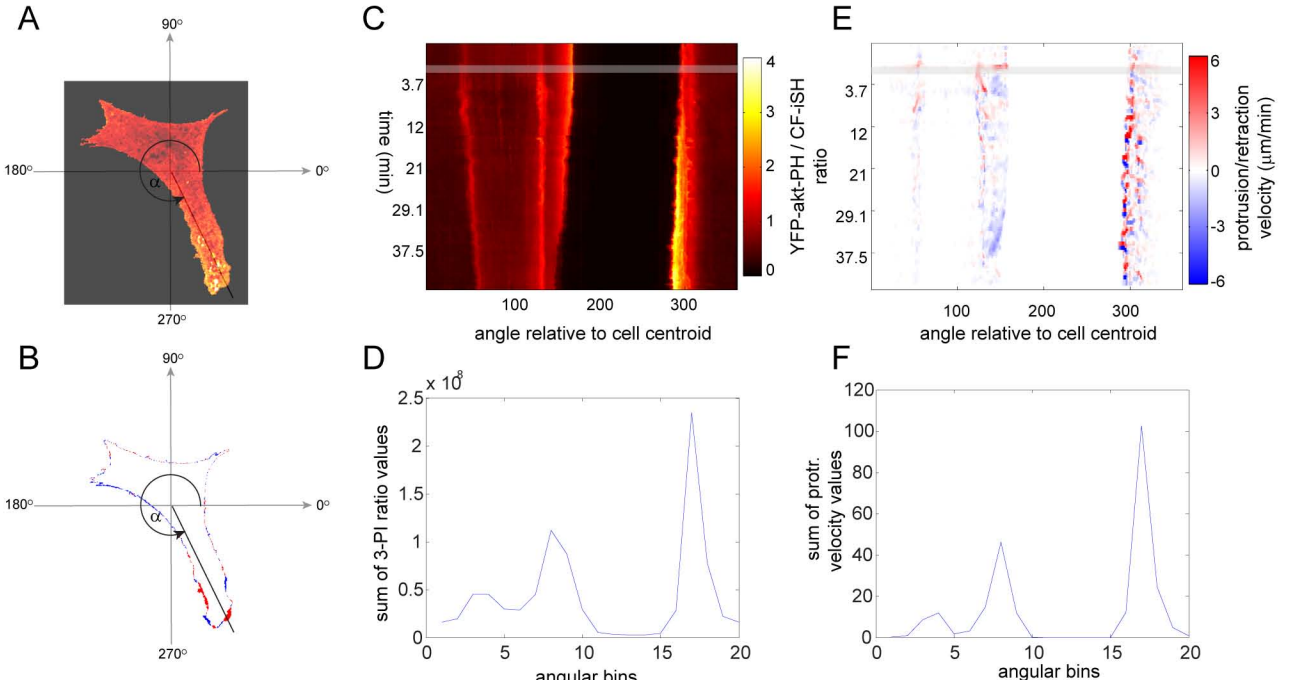


Figure S2

Thevathasan et al.,



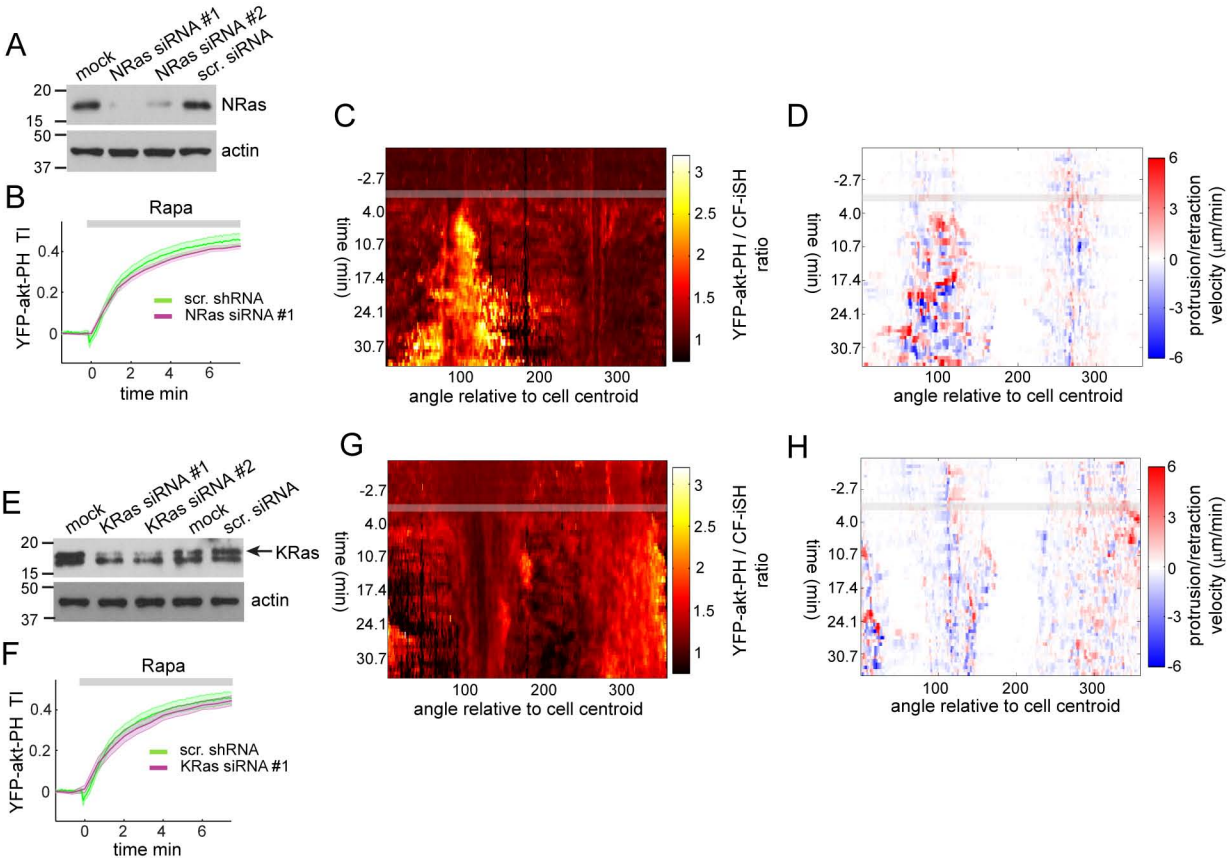
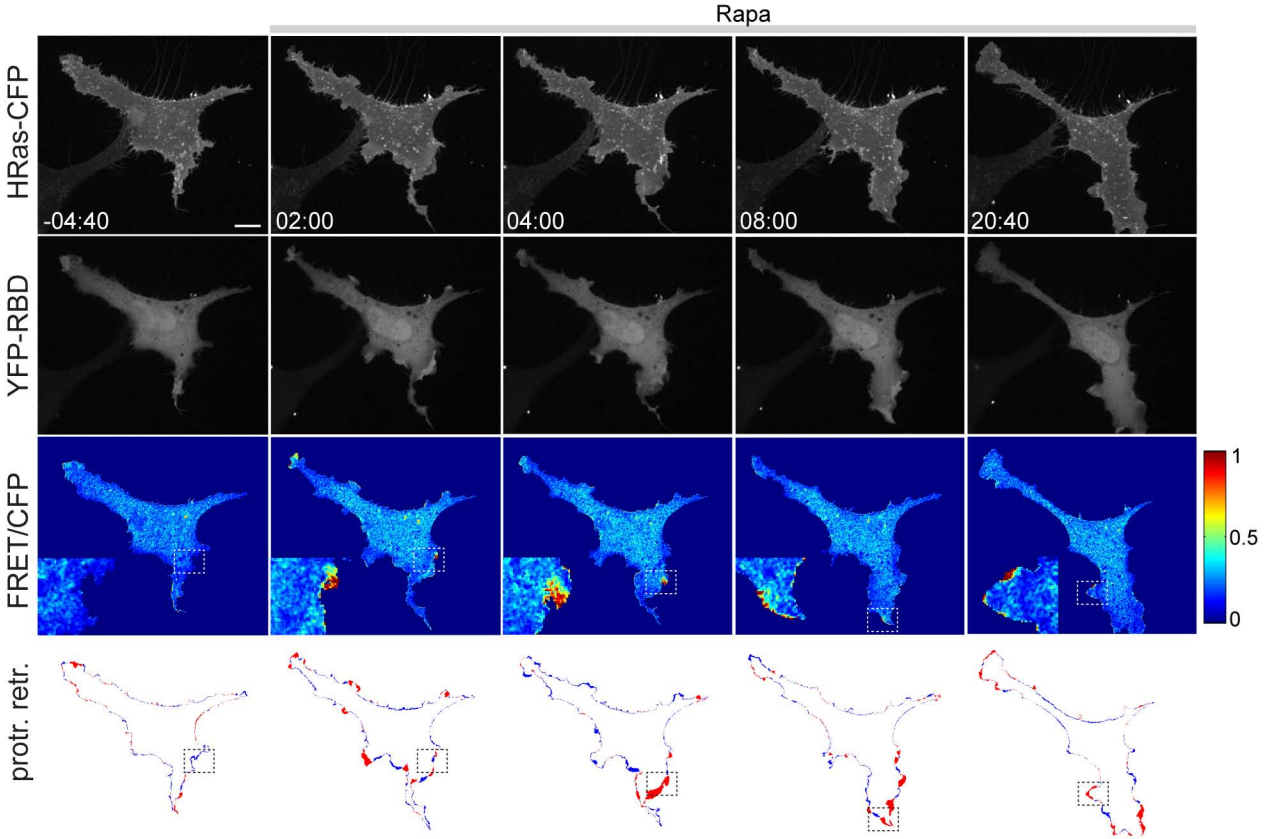
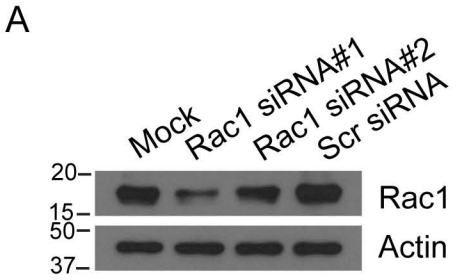
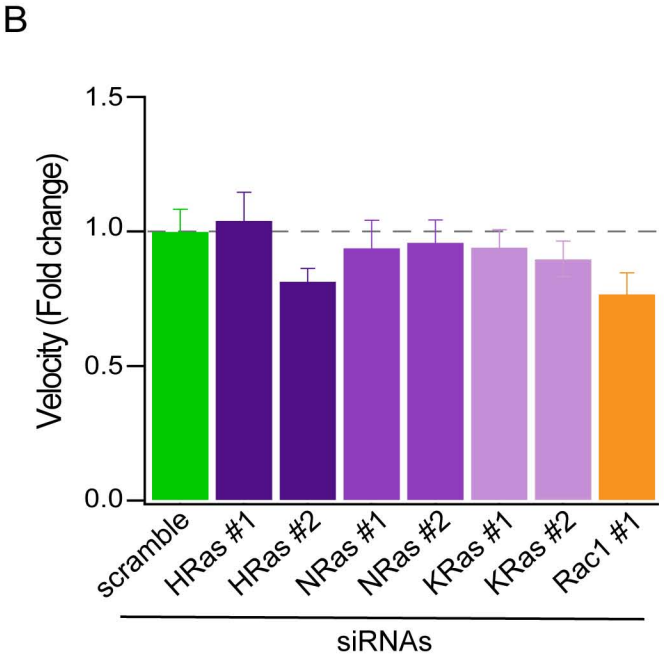

A
mock
NRas siRNA #1
NRas siRNA #2
scr. siRNA
20
15
50
37
NRas
actin
B
Rapa
YFP-akt-PH TI
0.4
0.2
0
scr. shRNA
NRas siRNA #1
0 2 4 6
time min
C
-2.7
4.0
10.7
17.4
24.1
30.7
time (min)
100 200 300
angle relative to cell centroid
3
2.5
2
1.5
1
YFP-akt-PH / CF-iSH ratio
D
6
3
0
-3
-6
protrusion/retraction velocity (μm/min)
E
mock
KRas siRNA #1
KRas siRNA #2
mock
scr. siRNA
KRas
actin
F
Rapa
scr. shRNA
KRas siRNA #1
G
H

Figure S4

Thevathasan et al.,




A
Mock
Rac1 siRNA#1
Rac1 siRNA#2
Scr siRNA
20
15
50
37
Rac1
Actin


B
Velocity (Fold change)
1.5
1.0
0.5
0.0
scramble
HRas #1
HRas #2
NRas #1
NRas #2
KRas #1
KRas #2
Rac1 #1
siRNAs