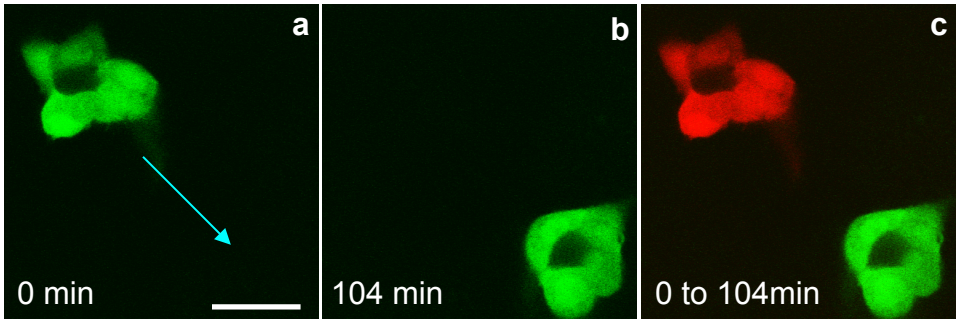
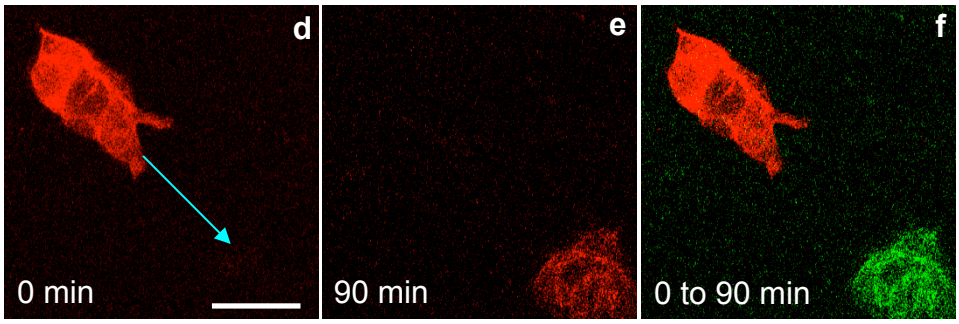
Figure S1

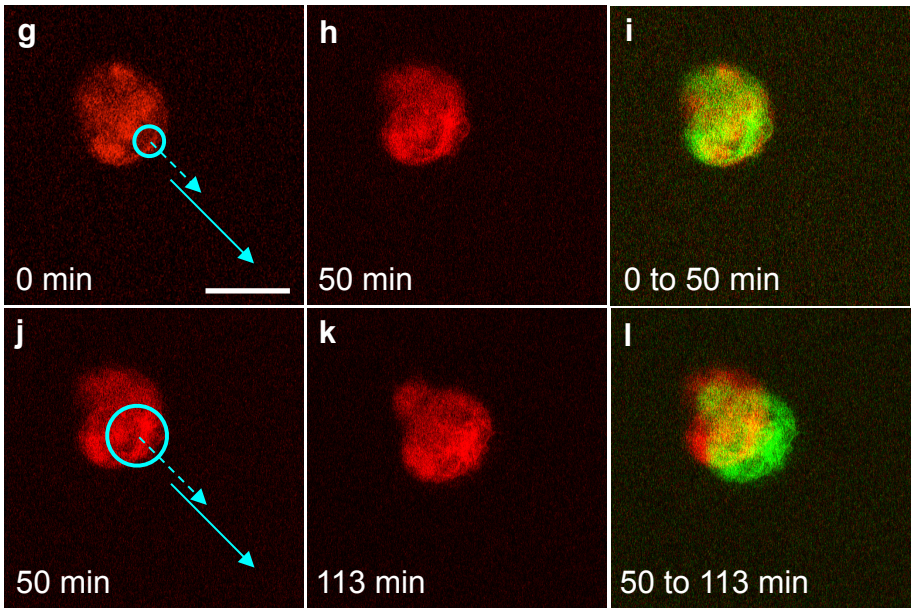
Slbo-Gal4;UAS-MCD8GFP



Slbo-Gal4; UAS-mCherry-PA-RacQ61L



Slbo-Gal4;UAS-mCherry-PA-RacQ61L; UAS-RacT17N



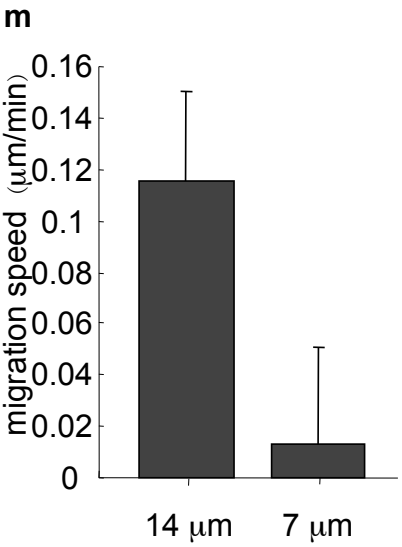
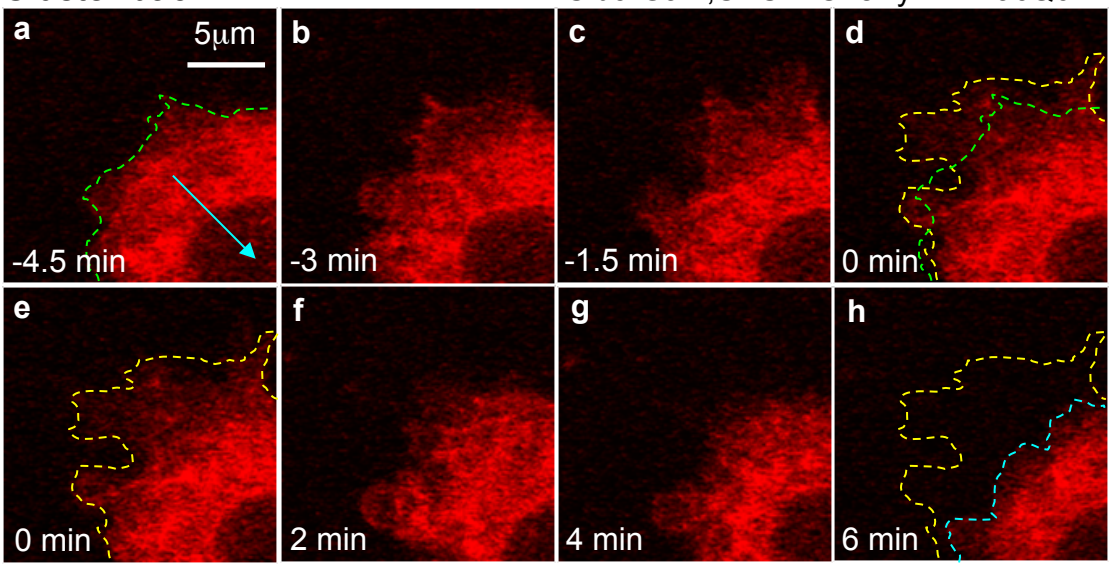
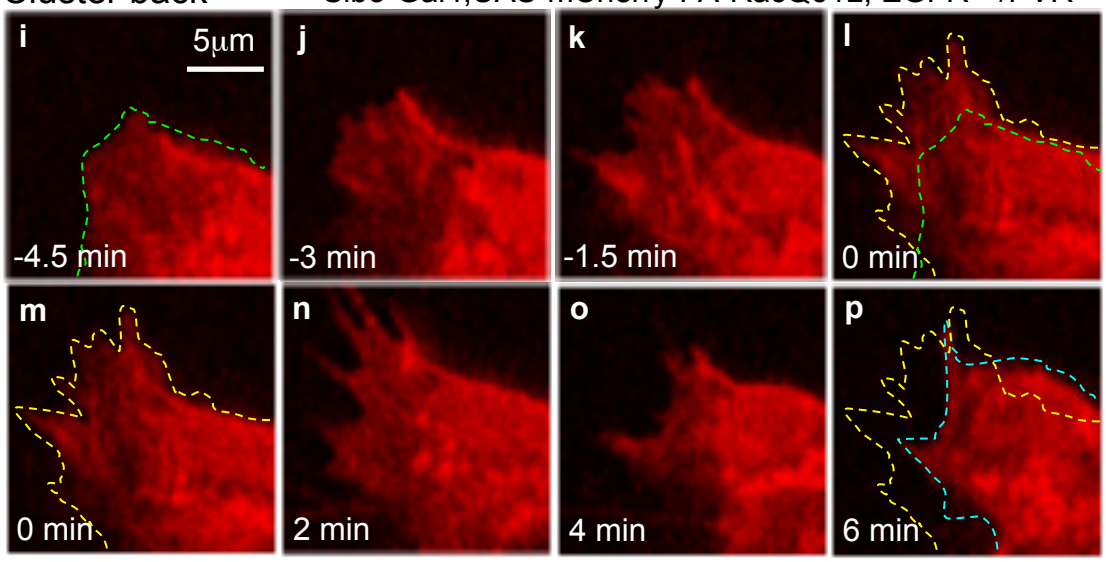


Figure S2

Cluster back — Slbo-Gal4;UAS-mCherry-PA-RacQ61L



Cluster back — Slbo-Gal4;UAS-mCherry-PA-RacQ61L; EGFR^{DN}/PVR^{DN}



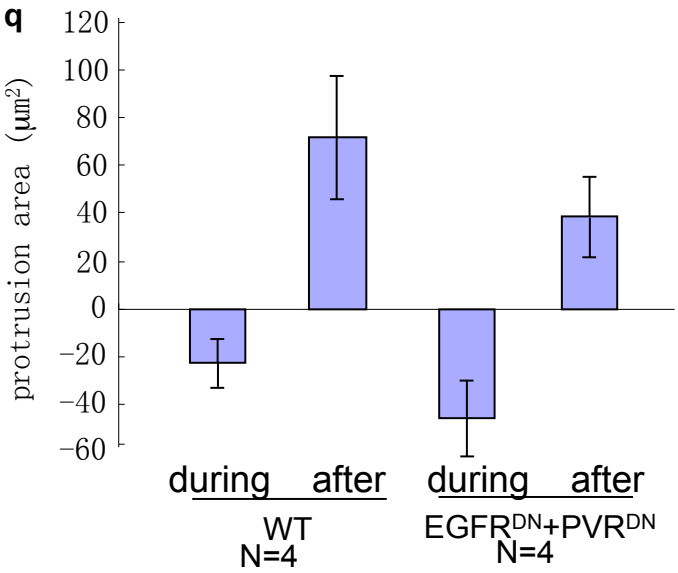
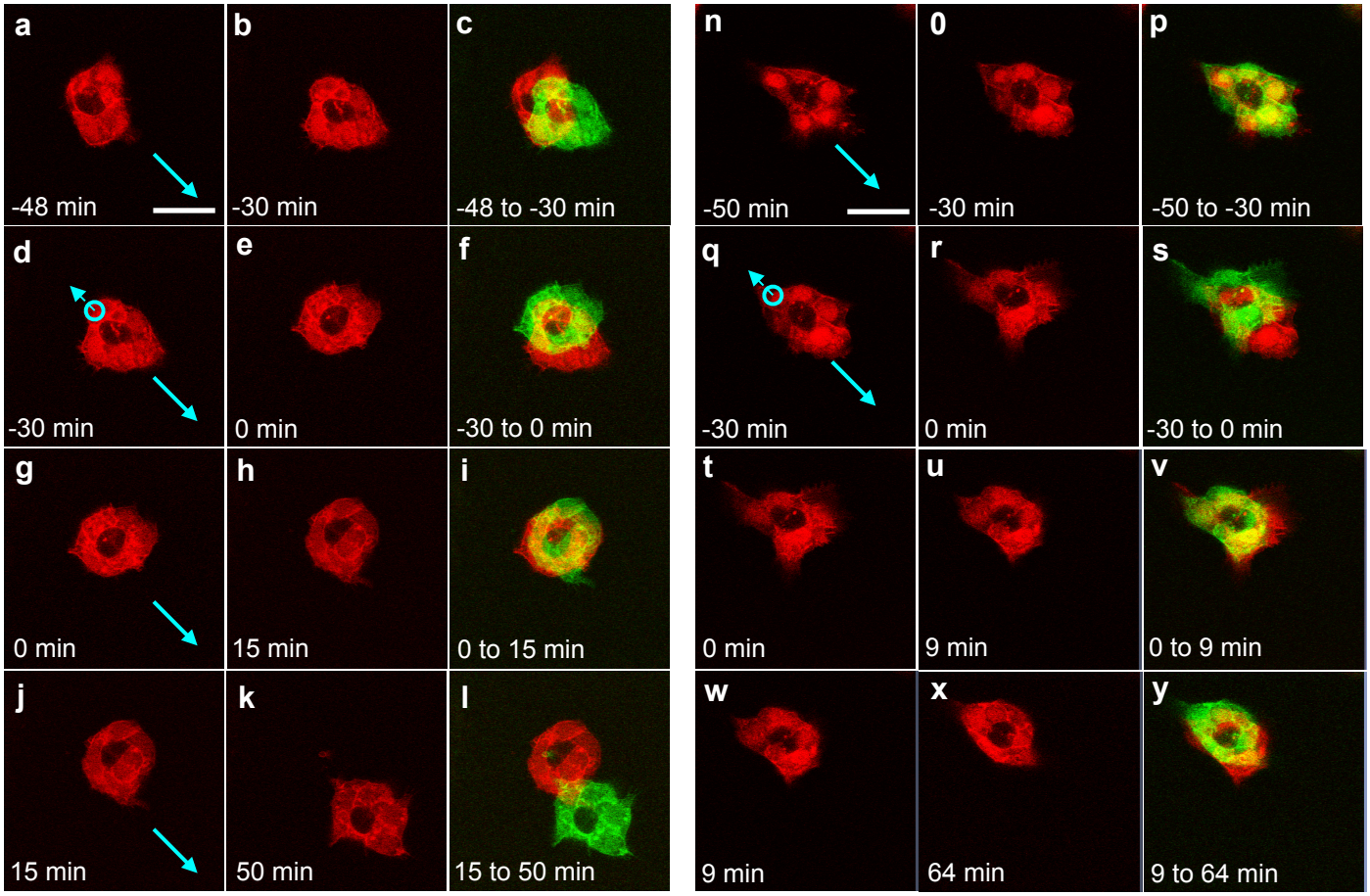
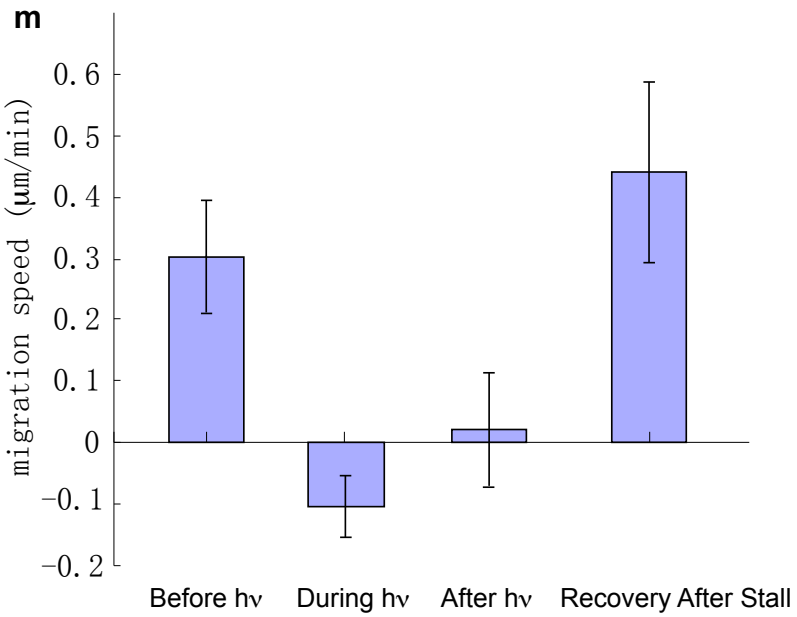


Figure S3



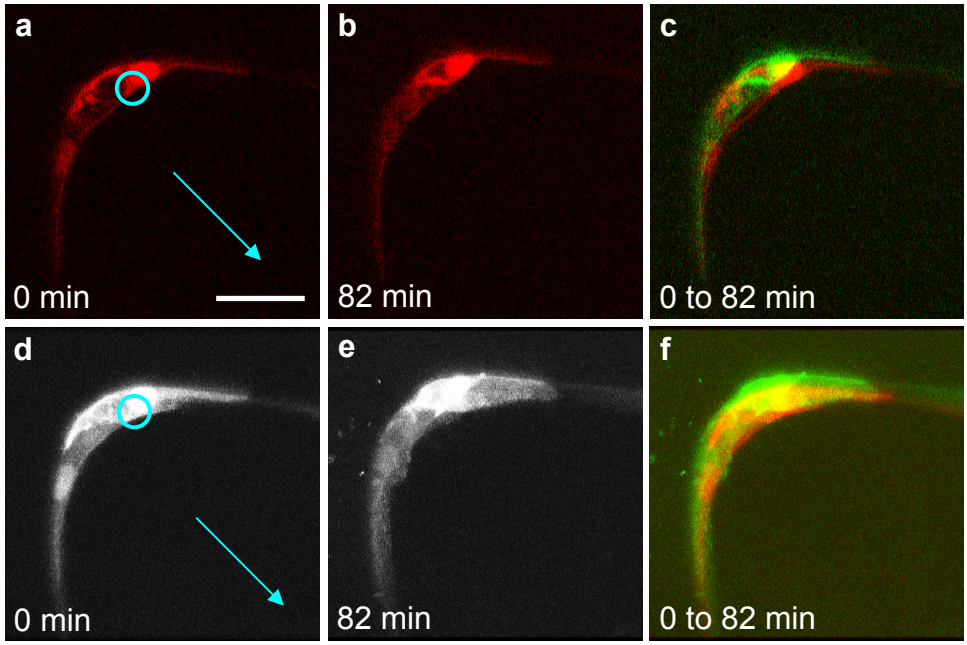
Slbo-Gal4;UAS-mCherry-PA-RacQ61L

Slbo-Gal4;UAS-mCherry-PA-RacQ61L; EGFR^{DN}/PVR^{DN}

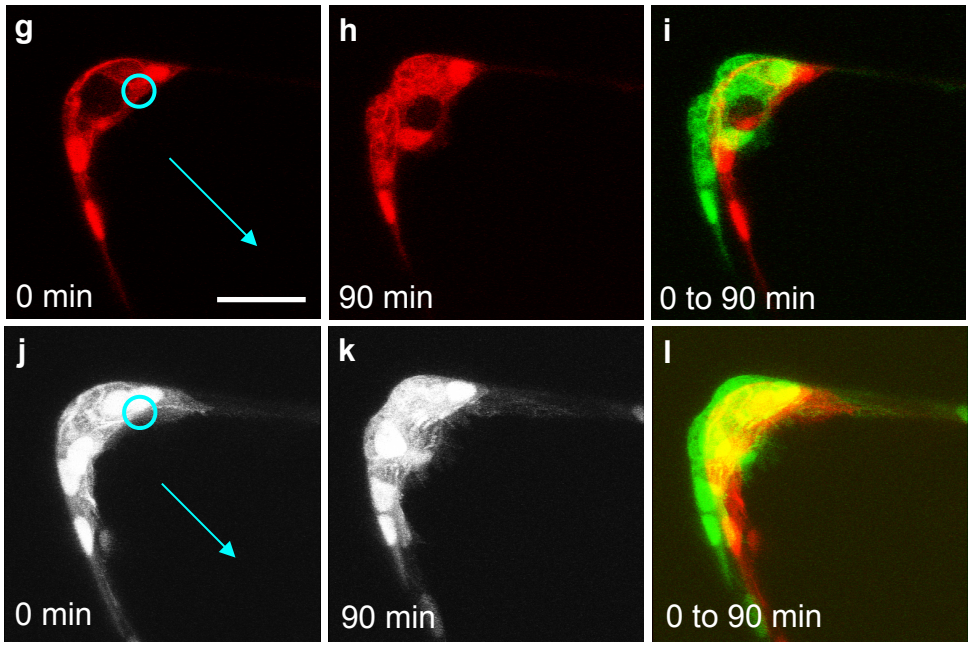


Lag time is from 9 to 48 mins in 7 independent experiment
Average lag time: 24+/-12 mins

Figure S4

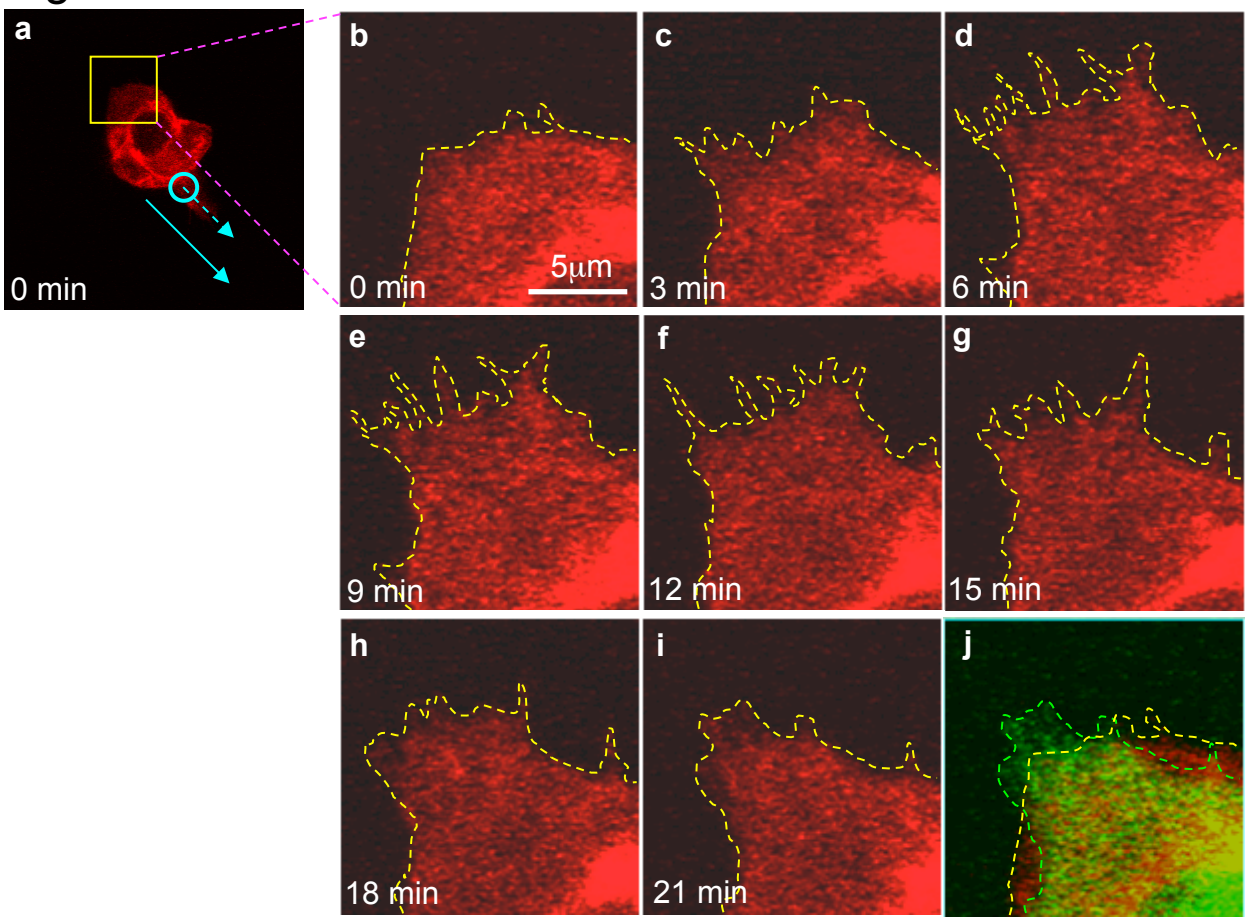


Slbo-Gal4;UAS-mCherry-PA-RacQ61L; UAS-dome$^{\Delta CYT}$

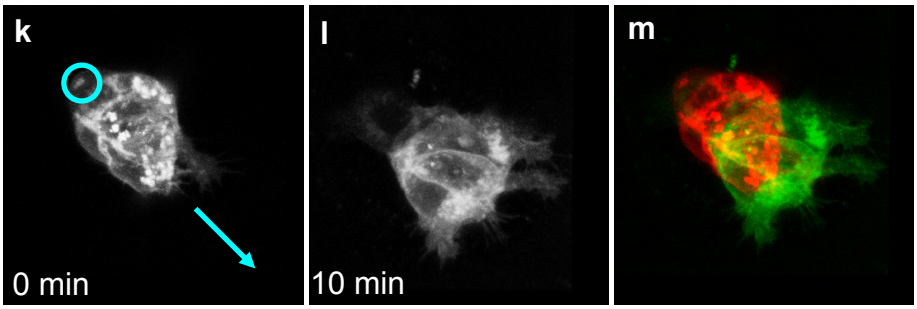


Slbo-Gal4;UAS-mCherry-PA-RacQ61L; Slbo^{PZ1310}/Slbo^{e7b}

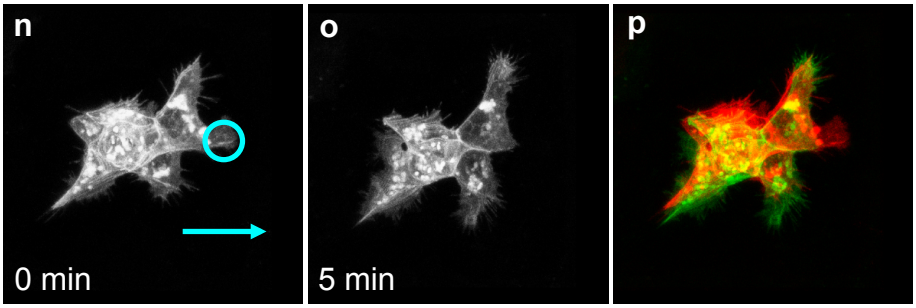
Figure S5



Slbo-Gal4;UAS-mCherry-PA-RacT17N



Slbo-Gal4;UAS-mCherry-PA-RacT17N



Slbo-Gal4;UAS-mCherry-PA-RacQ61L; $EGFR^{DN}/PVR^{DN}$

Figure S6

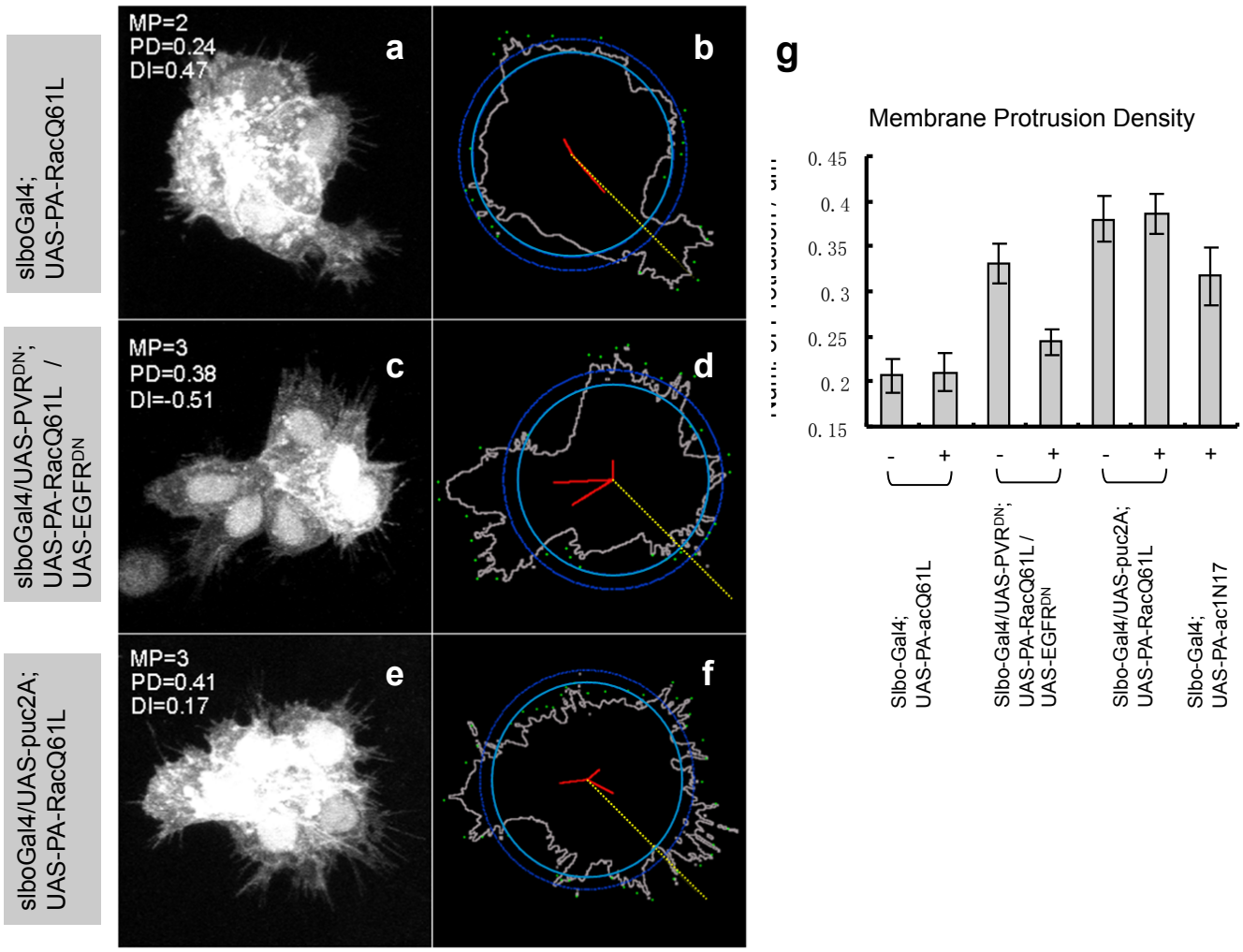
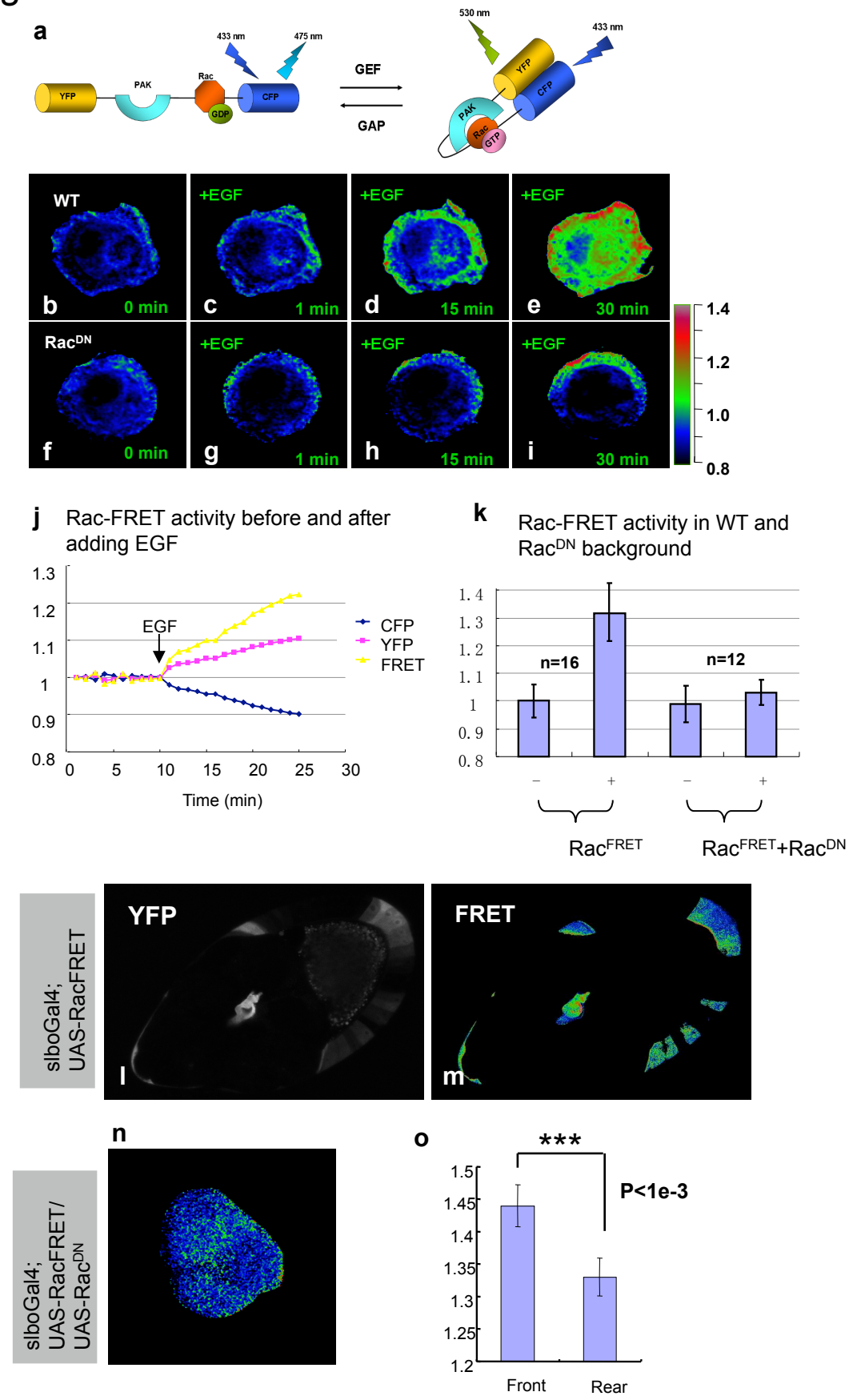

slboGal4;
UAS-PA-RacQ61L
slboGal4/UAS-PVR^{DN};
UAS-PA-RacQ61L /
UAS-EGFR^{DN}
slboGal4/UAS-puc2A;
UAS-PA-RacQ61L
MP=2
PD=0.24
DI=0.47
a
b
MP=3
PD=0.38
DI=-0.51
c
d
MP=3
PD=0.41
DI=0.17
e
f
g
Membrane Protrusion Density
0.45
0.4
0.35
0.3
0.25
0.2
0.15
-
+
-
+
-
+
+
Slbo-Gal4;
UAS-PA-acQ61L
Slbo-Gal4/UAS-PVR^{DN};
UAS-PA-RacQ61L /
UAS-EGFR^{DN}
Slbo-Gal4/UAS-puc2A;
UAS-PA-RacQ61L
Slbo-Gal4;
UAS-PA-ac1N17

Figure S7


a
YFP
PAK
Rac
GDP
CFP
433 nm
475 nm
GEF
GAP
530 nm
433 nm
YFP
CFP
PAK
Rac
GTP
WT
b 0 min
+EGF
c 1 min
+EGF
d 15 min
+EGF
e 30 min
RacDN
f 0 min
+EGF
g 1 min
+EGF
h 15 min
+EGF
i 30 min
1.4
1.2
1.0
0.8
j Rac-FRET activity before and after adding EGF
EGF
CFP
YFP
FRET
Time (min)
k Rac-FRET activity in WT and RacDN background
n=16
n=12
RacFRET
RacFRET+RacDN
slboGal4; UAS-RacFRET
YFP
l
FRET
m
slboGal4; UAS-RacFRET/ UAS-RacDN
n
o
***
P<1e-3
Front
Rear