Supplemental Materials

Molecular Biology of the Cell

Vaughan et al.

Figure S1. **Lipid probes mark distinct regions at the wound edge.** (A) Farnesyl-eGFP localizes to wounds by 49 s (n=9). (A') Farnesyl-eGFP accumulates where active Cdc42 is concentrated. (B) eGFP-Spo20 but not eGFP-Spo20^{mut} detects PA at wounds. (C) eGFP-PKC beta C1 but not eGFP-PKC beta C1^{mut} detects DAG at wounds. (D) PS, PA, and actin (Alexa Fluor 647) display unique localization patterns at the wound edge at 90 s. A-B, scale bar= 20 µm; C, scale bar= 10 µm. W=wound.

Figure S2. **Inhibition of PIP2-PLC or PAPH pathways does not inhibit Rho and Cdc42 activity.** (A) Cells injected with eGFP-rGBD and mRFP-wGBD were incubated in ethanol (control) or U-73122. Cells treated with U-73122 display more intense active Rho and Cdc42 zones. (B) Cells injected with eGFP-rGBD and mRFP-wGBD were incubated in methanol (control) or propranolol. Propranolol treatment did not affect Rho and Cdc42 zone activity. (C) Rho and Cdc42 zone intensity was measured in cells from A (n=12). (D) Rho and Cdc42 zone intensity was measured in cells from B. (Control, n=6; Prop, n=9). Top and bottom whiskers represent max and min values, respectively. Scale bar= 20 µm.

Figure S3. **PKC localization and overexpression.** (A) Cells injected with mRFP-wGBD and PKC beta-eGFP show overlap of the active Cdc42 zone with PKC beta. (A') A line scan from A reveals broad PKC beta enrichment with peak intensity inside the Cdc42 zone. (B) Cells injected with mRFP-wGBD and PKC eta-eGFP show PKC eta enrichment inside the Cdc42 zone. (B') A line scan from B reveals PKC eta peak intensity inside the Cdc42 zone. (C) Cells injected with mRFP-wGBD and PKC delta-eGFP show PKC delta enrichment inside the Cdc42 zone. (C') A line scan from C reveals PKC delta peak intensity inside the Cdc42 zone. (D) Cells injected with mRFP-wGBD and aPKC lambda-eGFP show no localization of PKC lambda to the wound. (E) Cells were injected with PKC beta-eGFP and PKC eta-mCherry and incubated in either DMSO (control) or D609 and wounded. D609 decreases recruitment of both PKC beta and PKC eta to the wound. (F) Fluorescence intensity was quantified in cells from E (n=13). (G) Cells were injected with eGFP-rGBD and mRFP-wGBD along with PKC delta. (H) Cells were injected with eGFP-rGBD and mRFP-wGBD along with PKC eta. Scale bar= 20 µm. W=wound.

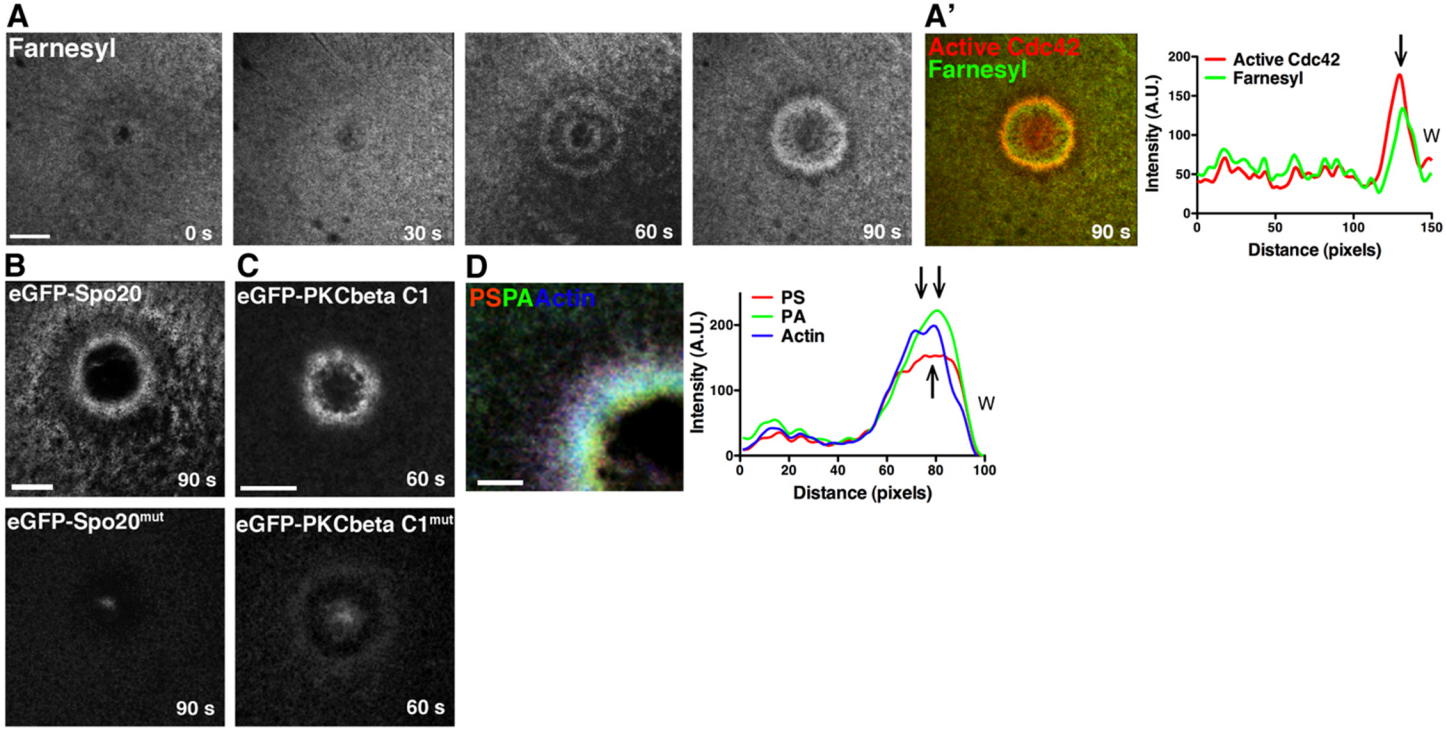
A
Farnesyl
0 s
30 s
60 s
90 s
A'
Active Cdc42
Farnesyl
90 s
Active Cdc42
Farnesyl
Intensity (A.U.)
Distance (pixels)
W
B
eGFP-Spo20
90 s
C
eGFP-PKCbeta C1
60 s
D
PSPAActin
PS
PA
Actin
Intensity (A.U.)
Distance (pixels)
W
eGFP-Spo20mut
90 s
eGFP-PKCbeta C1mut
60 s

Figure S1

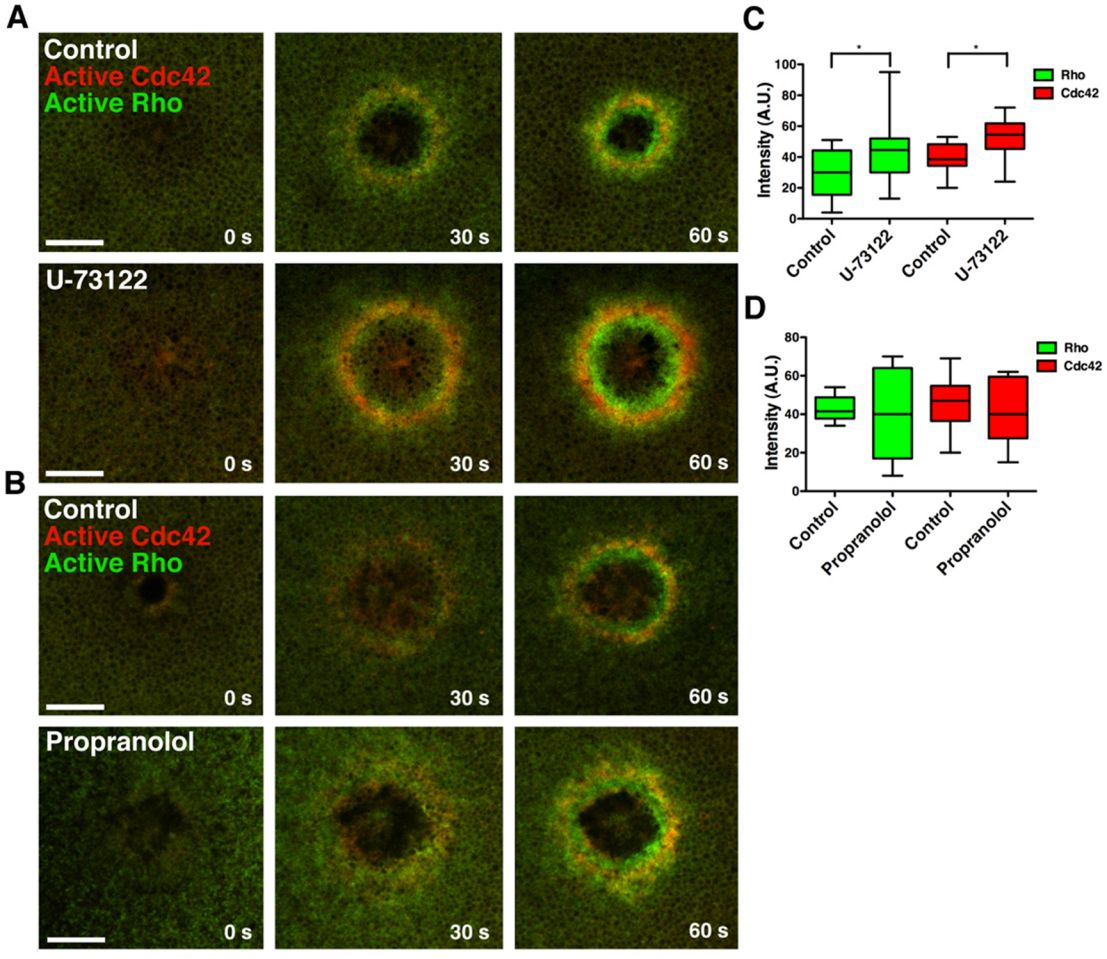
A
Control
Active Cdc42
Active Rho
0 s
30 s
60 s
U-73122
0 s
30 s
60 s
B
Control
Active Cdc42
Active Rho
0 s
30 s
60 s
Propranolol
0 s
30 s
60 s
C
Intensity (A.U.)
100
80
60
40
20
0
Rho
Cdc42
*
*
Control
U-73122
Control
U-73122
D
Intensity (A.U.)
80
60
40
20
0
Rho
Cdc42
Control
Propranolol
Control
Propranolol

Figure S2

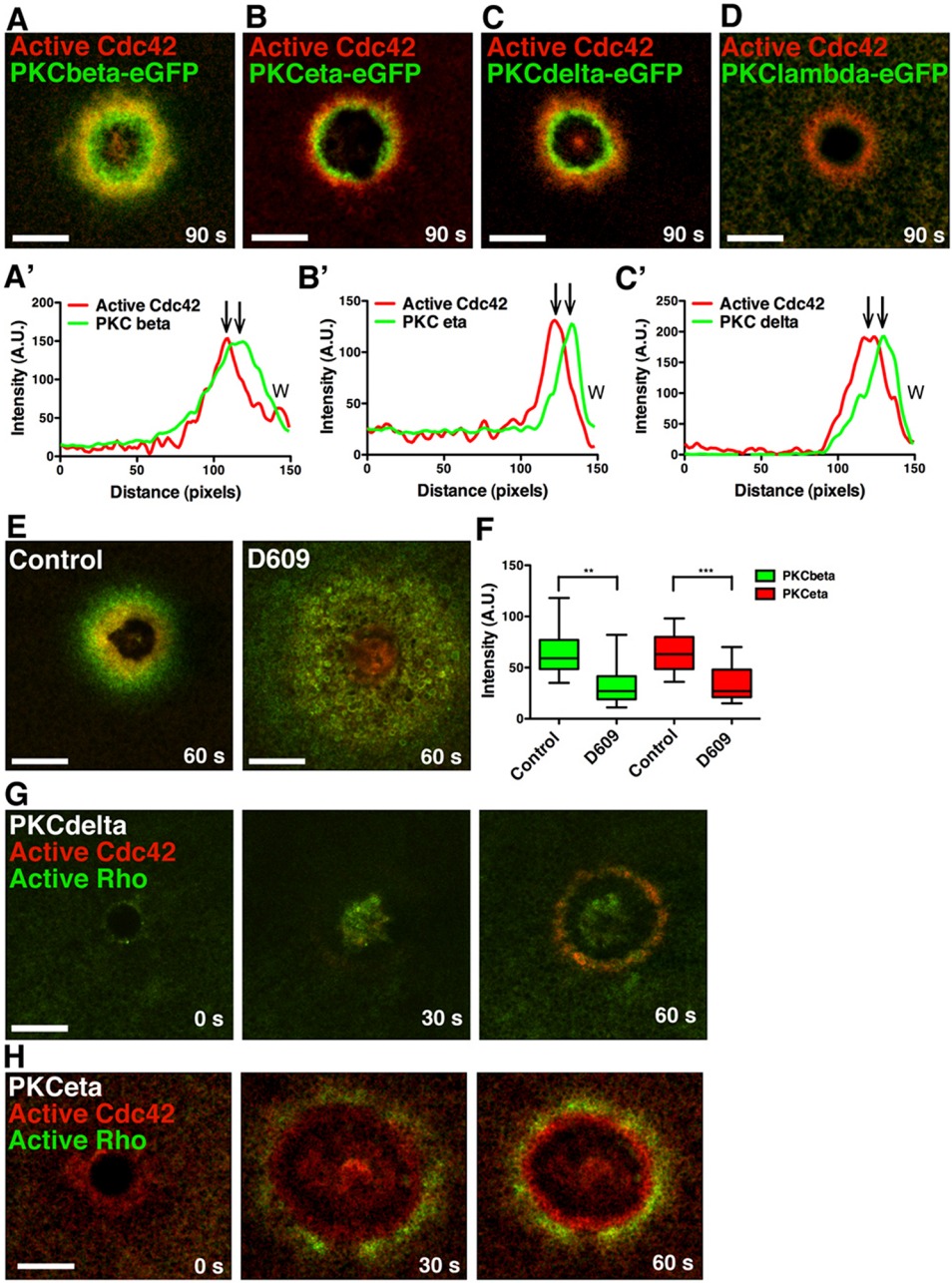
A
Active Cdc42
PKCbeta-eGFP
90 s
B
Active Cdc42
PKCeta-eGFP
90 s
C
Active Cdc42
PKCdelta-eGFP
90 s
D
Active Cdc42
PKClambda-eGFP
90 s
A'
Active Cdc42
PKC beta
Intensity (A.U.)
Distance (pixels)
W
B'
Active Cdc42
PKC eta
Intensity (A.U.)
Distance (pixels)
W
C'
Active Cdc42
PKC delta
Intensity (A.U.)
Distance (pixels)
W
E
Control
60 s
D609
60 s
F
Intensity (A.U.)
PKCbeta
PKCeta
**
***
Control
D609
Control
D609
G
PKCdelta
Active Cdc42
Active Rho
0 s
30 s
60 s
H
PKCeta
Active Cdc42
Active Rho
0 s
30 s
60 s

Figure S3