Chemistry & Biology *14*

Supplemental Data

Cofilin Activity Downstream of Pak1 Regulates Cell Protrusion Efficiency by Organizing Lamellipodium and Lamella Actin Networks

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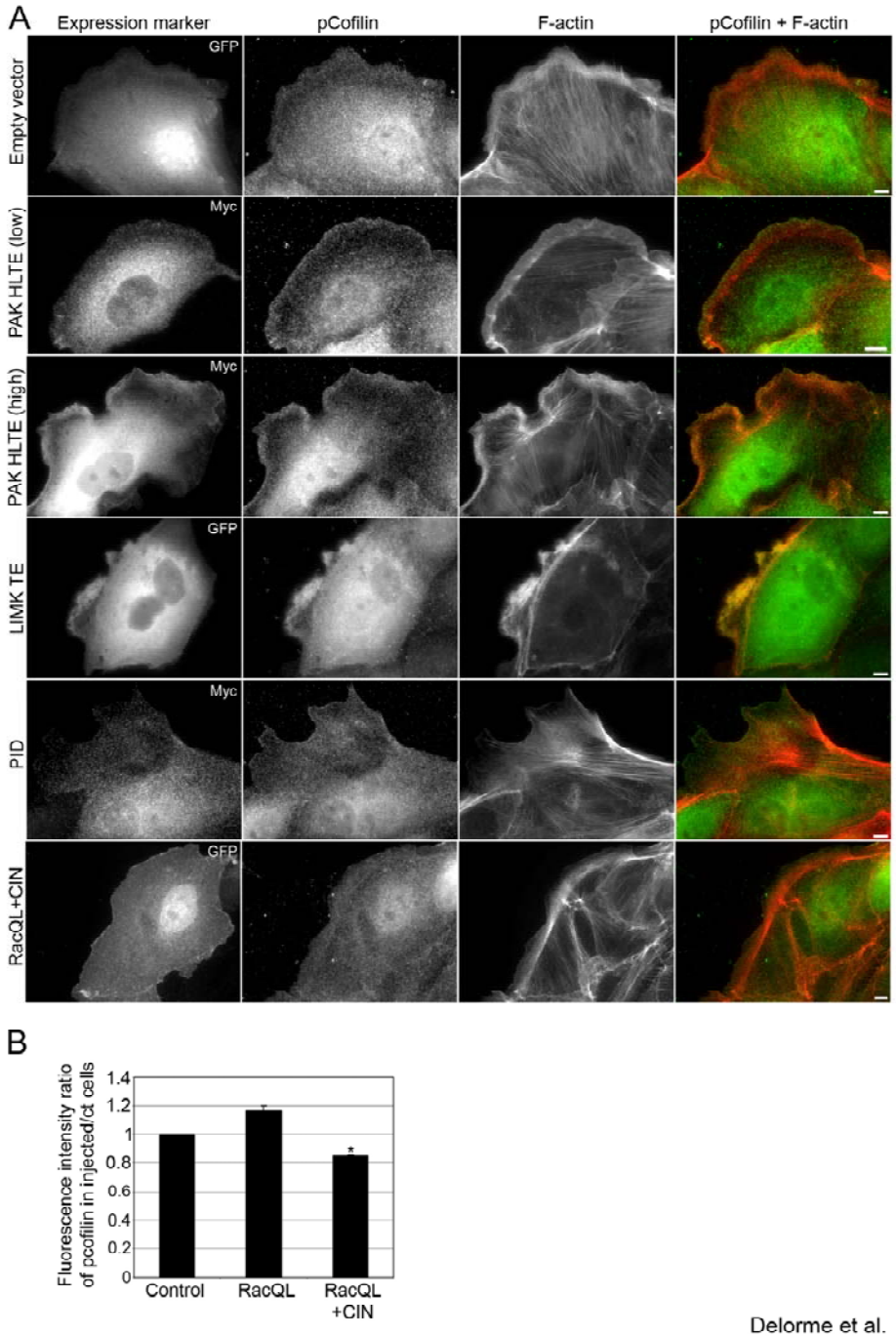

Figure S1. PAK1 Regulates Cofilin Phosphorylation at the Cell Leading Edge

(A) Immunolocalization of phosphorylated-cofilin (pcofilin, green) and F-actin phalloidin staining (red) in cells expressing the control empty vector, active PAK1 (PAK HLTE),

active LIMK (LIMK TE), PID or RacQL in combination with CIN (RacQL+CIN). Bar = 10 µm. Of note, cells expressing a low amount of active PAK1 display the same phenotype as an active Rac1-expressing cell, i.e. a rounded protrusion with increased phosphorylated cofilin at the leading edge.

(B) Fluorescence intensity ratio of pcofilin in injected/control cells at the cell edge (± SEM). The experiment was repeated at least three times; n cells ≥ 22 for each condition. *, $P < 0.05$ compared to RacQL-expressing cells.

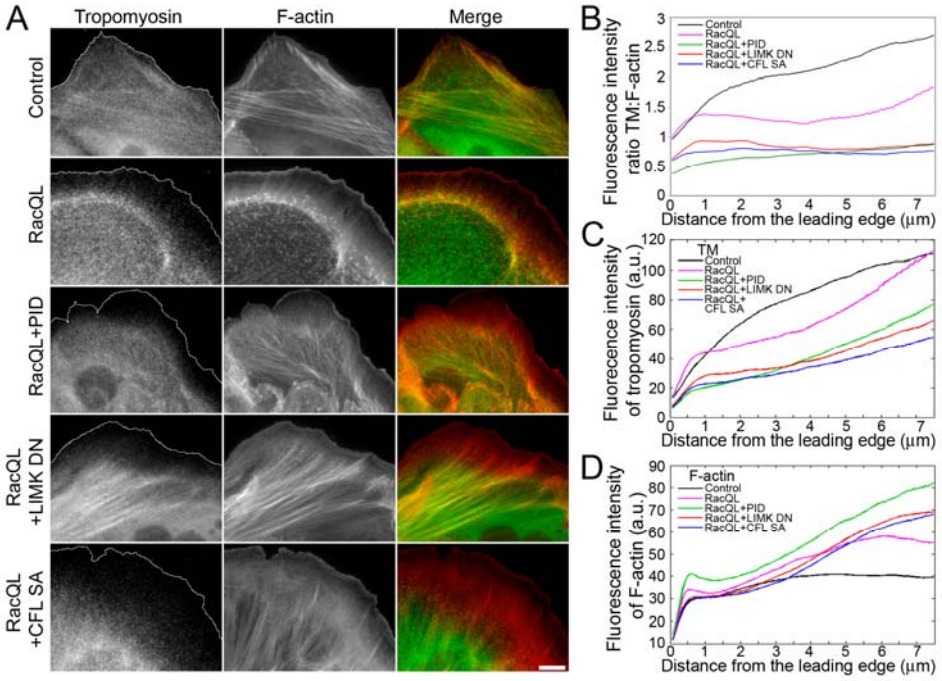

Figure S2. Active Cofilin Displaces High Molecular Weight Tropomyosin from the Leading Edge

(A) Immunolocalization of tropomyosin (TM) (green) and F-actin phalloidin staining (red) in control cell, cell expressing RacQL alone or in combination with PID, LIMK DN or CFL SA. Bar = 10 µm. The F-actin staining allows the detection of the leading edge (white lines in TM panel).

(B) Tropomyosin/F-actin fluorescence intensity ratio in control (black), RacQL (pink), RacQL+PID (green), RacQL+LIMK DN (red) and RacQL+CFL SA (blue) cells, measured from the cell edge (0 µm) into the cell center (7.5 µm).

(C, D) Fluorescence intensity of tropomyosin (C) and F-actin (D) in injected cells (control: black, RacQL: pink, RacQL+PID: green, RacQL+LIMK DN: red and RacQL+CFL SA: blue), measured from the leading edge (0 µm) into the cell center (7.5 µm). In B-D, data shown represent one experiment and are averaged from n ≥ 7 cells for each condition. The experiment has been repeated at least three times.

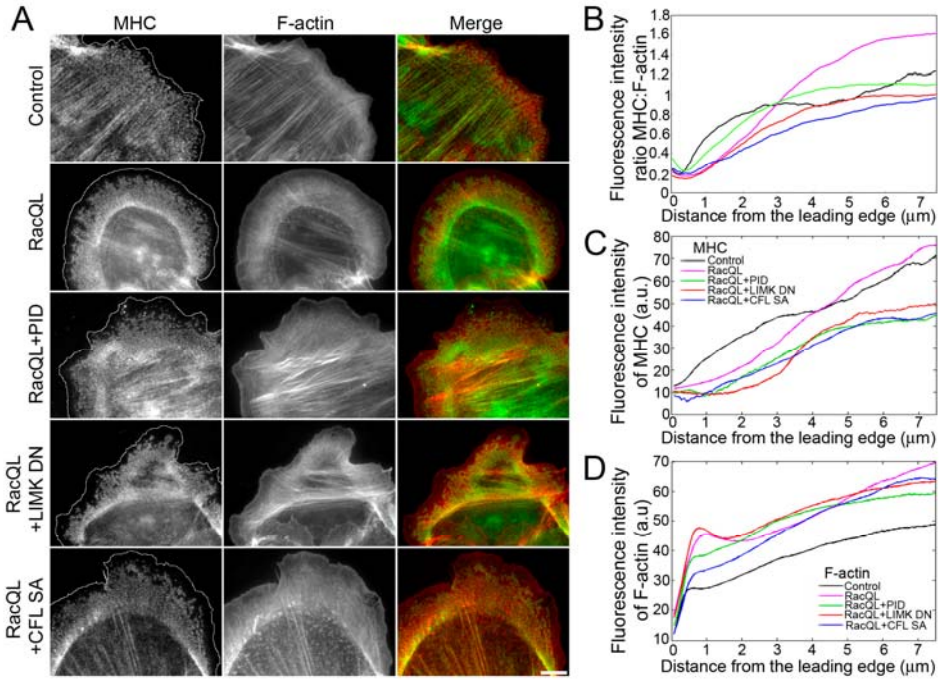

Figure S3. Active Cofilin Displaces Myosin II from the Leading Edge

(A) Immunolocalization of myosin IIA heavy chain (MHC) (green) and F-actin phalloidin staining (red) in control cell, cell expressing RacQL alone or in combination with PID, LIMK DN or CFL SA. Bar = 10 µm. The F-actin staining allows the detection of the leading edge (white lines in MHC panel).

(B) MHC/F-actin fluorescence intensity ratio in control (black), RacQL (pink), RacQL+PID (green), RacQL+LIMK DN (red) and RacQL+CFL SA (blue) cells, measured from the cell edge (0 µm) into the cell center (7.5 µm).

(C, D) Fluorescence intensity of MHC (C) and F-actin (D) in injected cells (control: black, RacQL: pink, RacQL+PID: green, RacQL+LIMK DN: red and RacQL+CFL SA: blue), measured from the leading edge (0 µm) into the cell center (7.5 µm). In B-D, data shown represent one experiment and are averaged from n ≥ 12 cells for each condition. The experiment has been repeated at least three times.

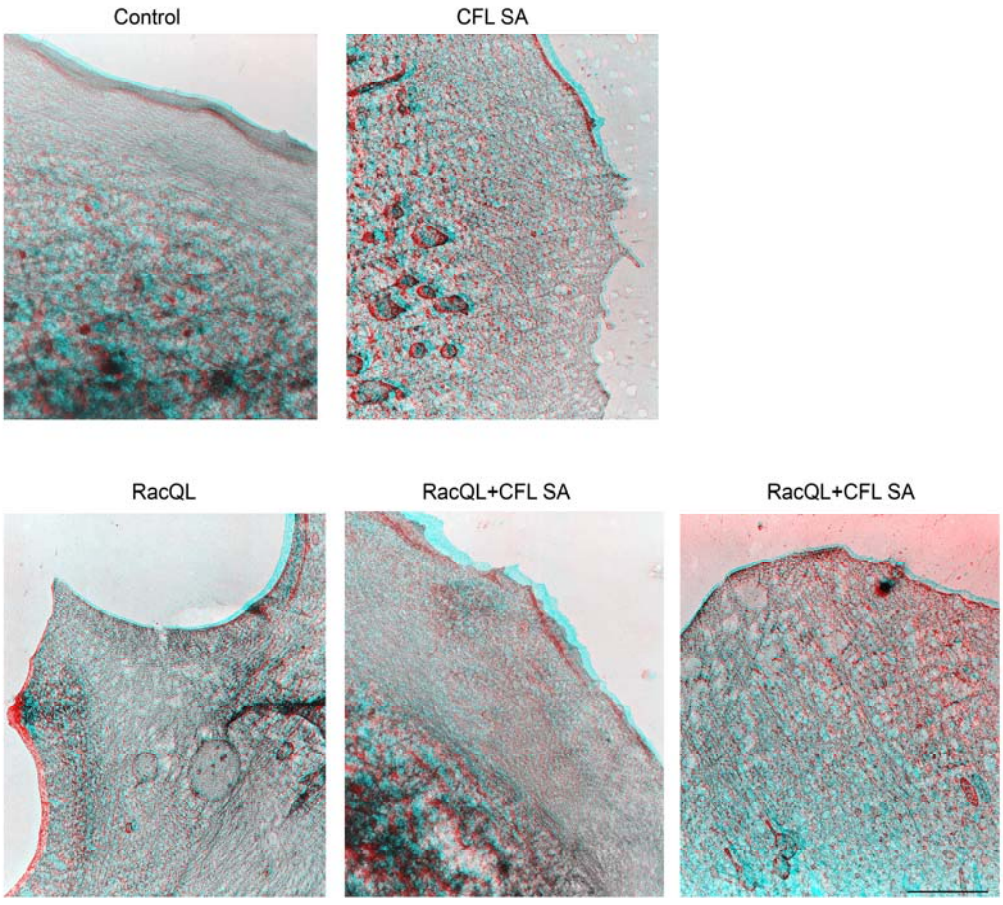

Figure S4. Active Cofilin Regulates Lamellipodium and Lamella Network Organization-Stereo Views

Anaglyph views of column B in Figure 8, showing the actin network organization at the leading edge of control cells, PtK1 cells expressing RacQL, RacQL+CFL SA or CFL SA. Bar = 2.4 µm. Anaglyph images are used to provide a stereoscopic three-dimensional effect, when viewed with 2 color glasses (red/cyan color).

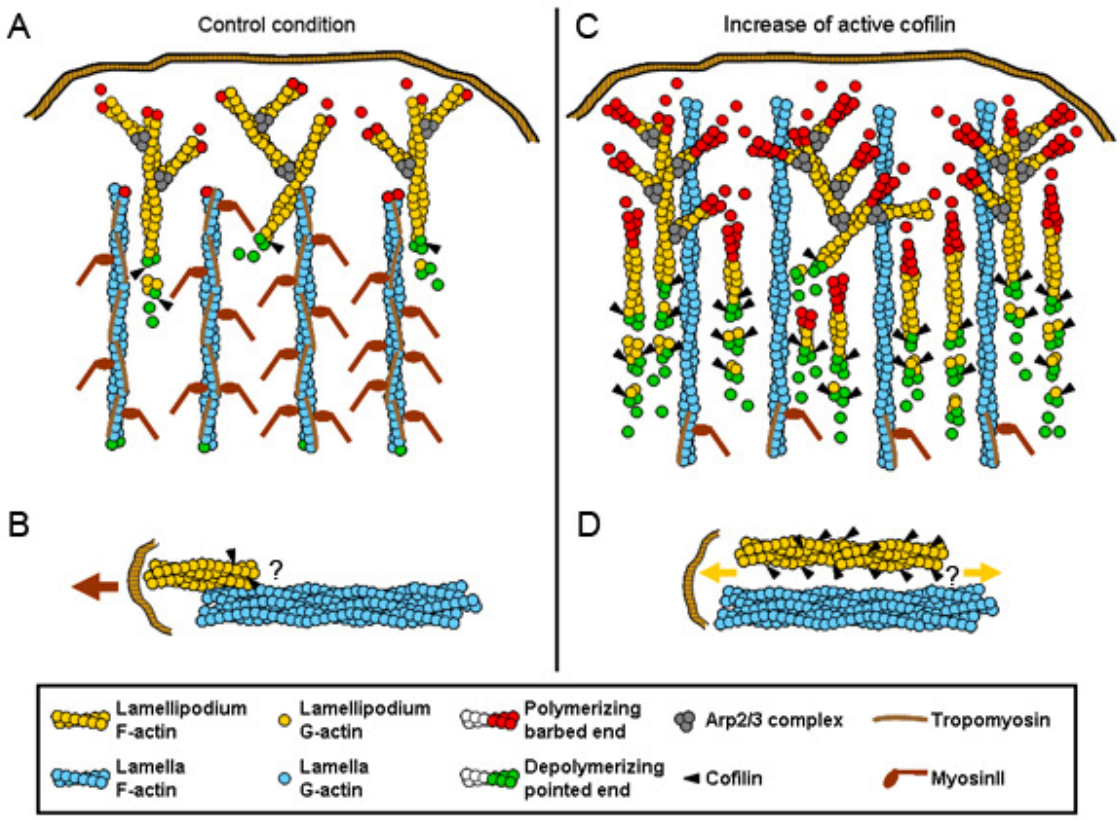

Figure S5. Model of Cofilin-Based Regulation of Lamellipodium/Lamella Organization

(A, B) In control conditions, lamellipodium and lamella networks present little overlap within the tip of the lamella, adjacent to the cell edge (A). Coordination between these two actin networks leads to efficient protrusion (B).

(C, D) Enhancement of cofilin activity widens the lamellipodium and accelerates its F-actin treadmilling rate. The lamella, completely covered by the lamellipodium, extends to

the leading edge (C). We propose that increased cofilin activity decouples the two modules. By this mechanism, enhanced cofilin activity reduces the dynamics of edge movements and net protrusion rates (D).

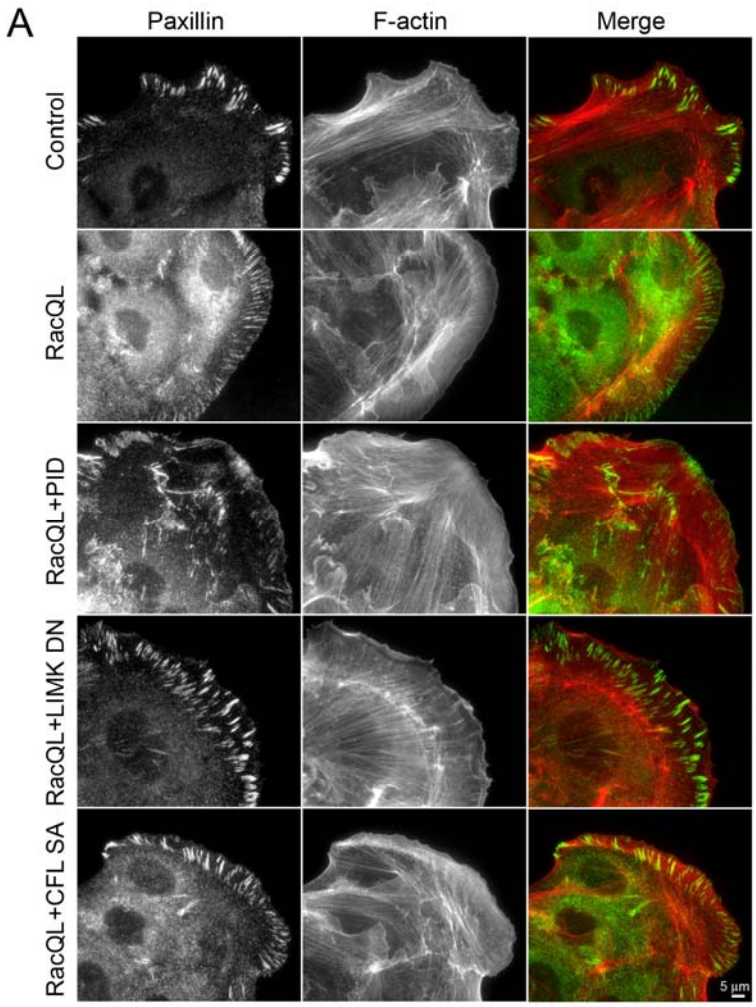

Figure S6. Substrate Adhesions Form Close to the Leading Edge in Cells with Enhanced Cofilin Activity

(A) Immunolocalization of paxillin (green) and F-actin phalloidin staining (red) in control cell, cell expressing RacQL alone or in combination with PID, LIMK DN or CFL SA. Bar = 5 µm.

(B) Frequency histogram of the distance of the distal-most border of paxillin foci from the leading edge in control cells (black, n = 33 cells), cells expressing RacQL alone (pink, n = 41 cells) or in combination with PID (green, n = 47 cells), LIMK DN (red, n = 30 cells) or CFL SA (blue, n = 41 cells) (55 measurements per cell per condition).