

Supplemental Materials

Molecular Biology of the Cell

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Supplementary Information

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A Mechanism of Leading Edge Protrusion in the absence of Arp2/3

Complex

Supplementary Information

Supplementary figure legends

Figure S1: Geometry and mechanical stability of actomyosin assemblies. (A) Geometric parameters of the arc. The radial angle φ parameterizes the radius R and length L of the arc; l represents the distance between the arc ends. (B). Plots of the contractile pressure P as function of the radial angle φ for two values of the myosin strength (blue curve for strong and green for weak myosin). The constant membrane tension is shown with black line. The pressure and tension are shown in arbitrary units; angle is in radian. The plots are shown for $l = L_0/2$. (C) The dynamics of the arc radii during the recovery process after the blebbistatin wash-out. For each cell (N=3) few representative arcs were selected for the analysis and computed mean (blue curve) and SD (error bars) are shown. In the plot the arc radius values were normalized to 1 at the first time point immediately after the blebbistatin wash-out.

Figure S2. Signalling and chemotaxis response to PDGF and EGF.

(A) Phospho-tyrosine (P-Tyr) quantification of ARPC3^{+/+} and ARPC3^{-/-} fibroblasts in response to stimulation with 25ng/ml PDGF. Left panels show representative cell images;

Box plots on the right show quantification of whole-cell p-Tyr levels. Small box shows the mean, line shows the median, large box the SEM and whiskers show SD. (B) Left: Immunoblot analysis of S536-phosphorylated NF- κ B p65 (a readout of NF- κ B activation) and actin loading control) in ARPC3^{+/+} and ARPC3^{-/-} cells. Right: quantification of P-p65 levels after normalization to the loading control; histograms show average and SEM from 3 experiments. (C) Cell trajectories shows ARPC3^{+/+} cells migrating in EGF gradient in the presence of ARPC3^{-/-} cell-conditioned media. Histograms on the right show percentages (average and SEM from 3 experiments) of tracked cells migrating either up or down the EGF gradient.

Supplementary Movies:

Movie 1. ARPC3^{+/+} fibroblast cell spreading in the presence of DMSO. Total length of the movie is 2 hr with frames every 2 min. Scale Bar represents 25 μ m.

Movie 2. ARPC3^{+/+} cell spreading in the presence of SMIFH2. Total length of the movie is 2 hr with frames every 2 min. Scale Bar represents 25 μ m.

Movie 3. ARPC3^{-/-} cell spreading in the presence of DMSO. Total length of the movie is 2 hr with frames every 2 min. Scale Bar represents 25 μ m

Movie 4. ARPC3^{-/-} cell spreading in the presence of SMIFH2. Total length of the movie is 2 hr with frames every 2 min. Scale Bar represents 25 μ m.

Movie 5. ARPC3^{-/-} cell spreading after SMIFH2 washout. The same cells are shown as those in Movie 4. Total length of the movie is 2 hr with frames every 2 min. Scale Bar represents 25µm.

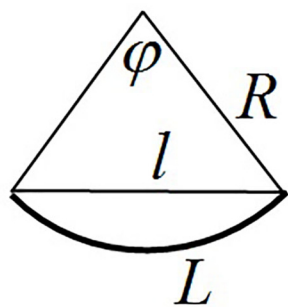
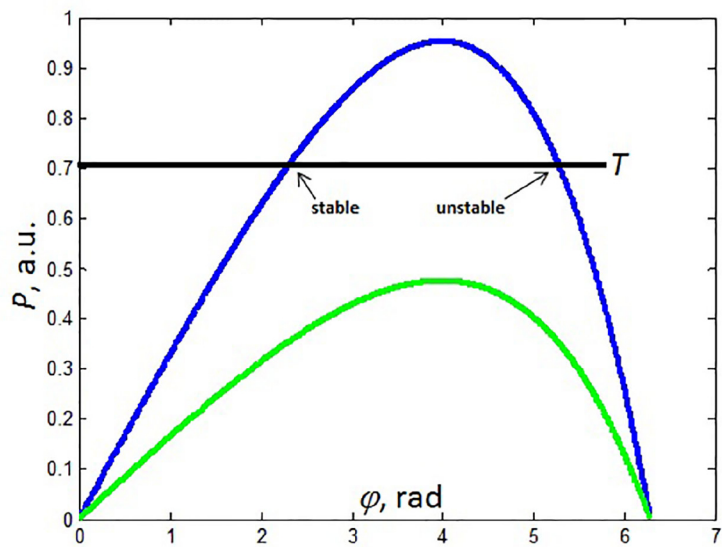
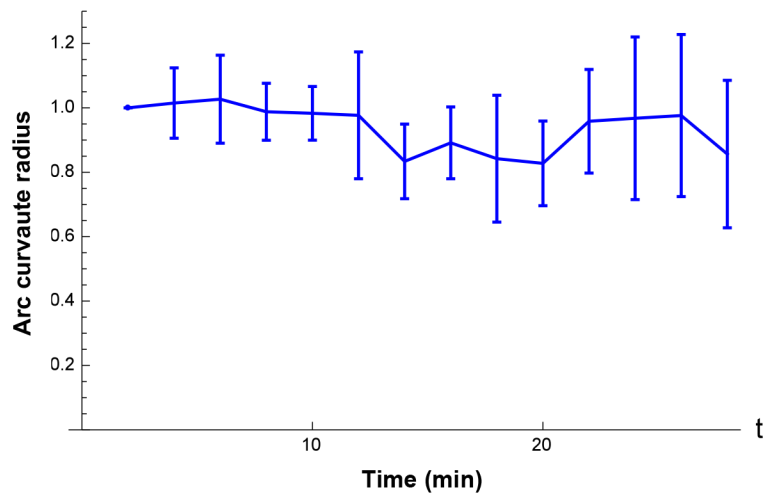
Movie 6. ARPC3^{-/-} cell undergoing randomly migrating before (left) and after (right) of SMIFH2 addition. Total length of each movie is 90 min with frames every 2 min. Scale Bar: 25µm.

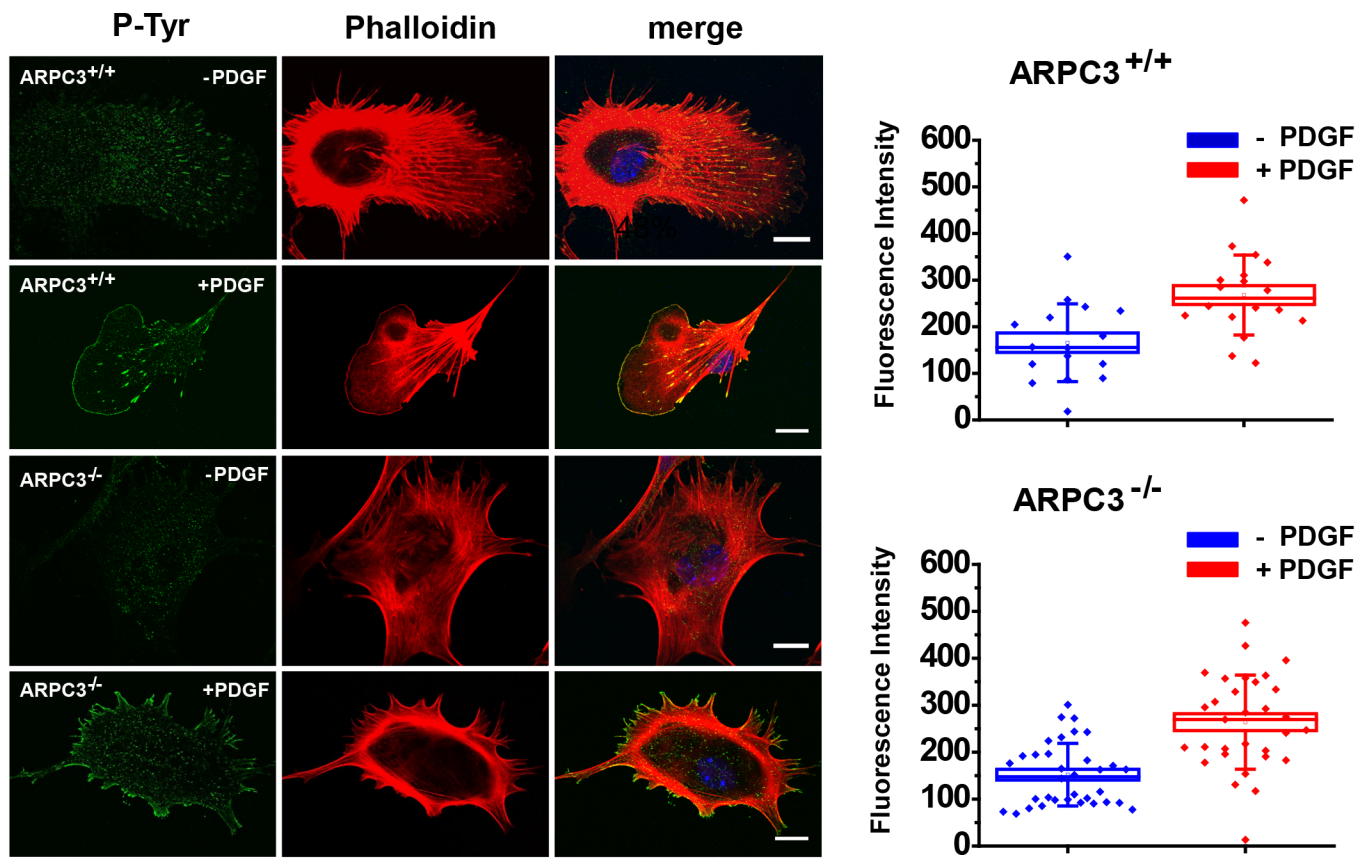
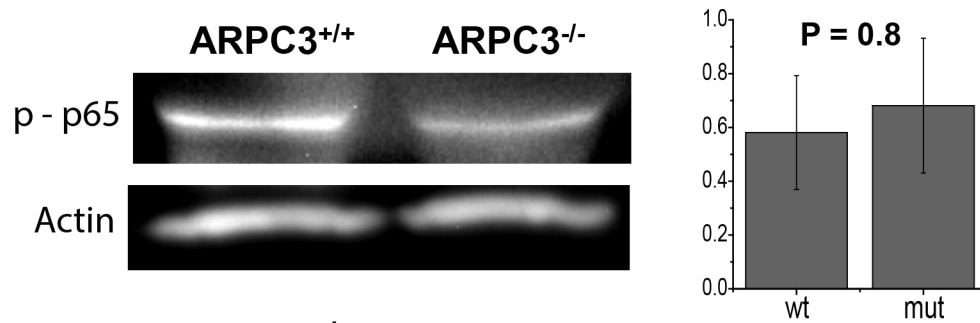
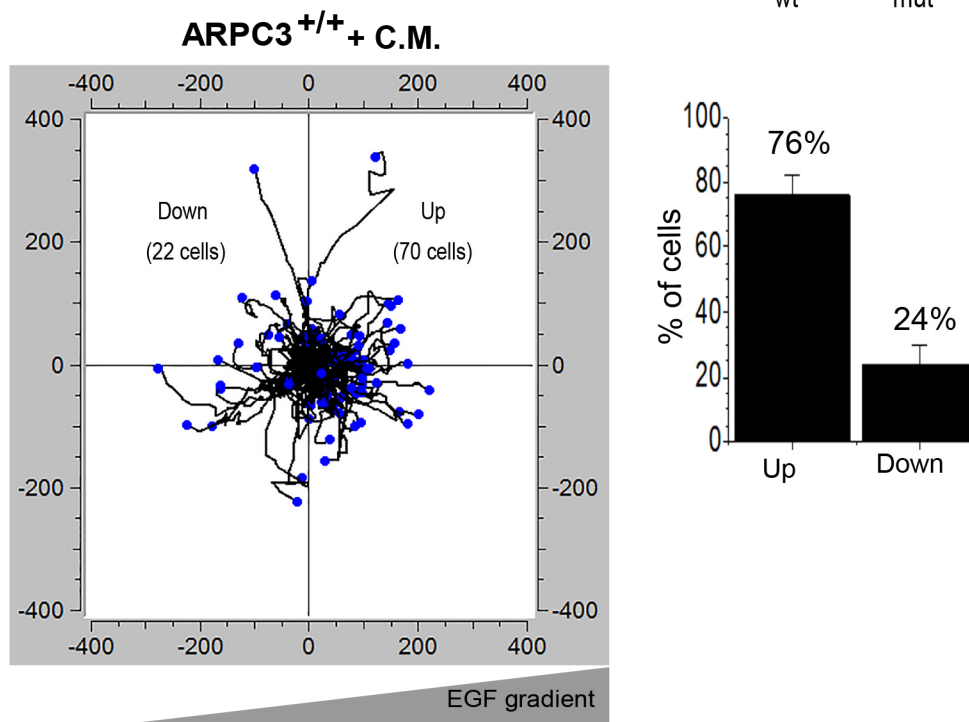
Movie 7. ARPC3^{+/+} cell spreading in the presence of blebbistatin. Total length of the movie is 2 hr with frames every 2 min. Scale Bar represents 25µm.

Movie 8. ARPC3^{+/+} cell spreading after blebbistatin washout. The same cells are shown as those in Movie 6. Total length of the movie is 2 hr with frames every 2 min. Scale Bar represents 25µm.

Movie 9. ARPC3^{-/-} cell spreading in the presence of blebbistatin. Total length of the movie is 2 hr with frames every 2 min. Scale Bar represents 25µm.

Movie 10. ARPC3^{-/-} cell spreading after blebbistatin washout. The same cells are shown as those in Movie 8. Total length of the movie is 2 hr with frames every 2 min. Scale Bar represents 25µm.

A**B****C****Figure. S1**

A**B****C****Figure. S2**