Osmani et al., http://www.jcb.org/cgi/content/full/jcb.201003091/DC1

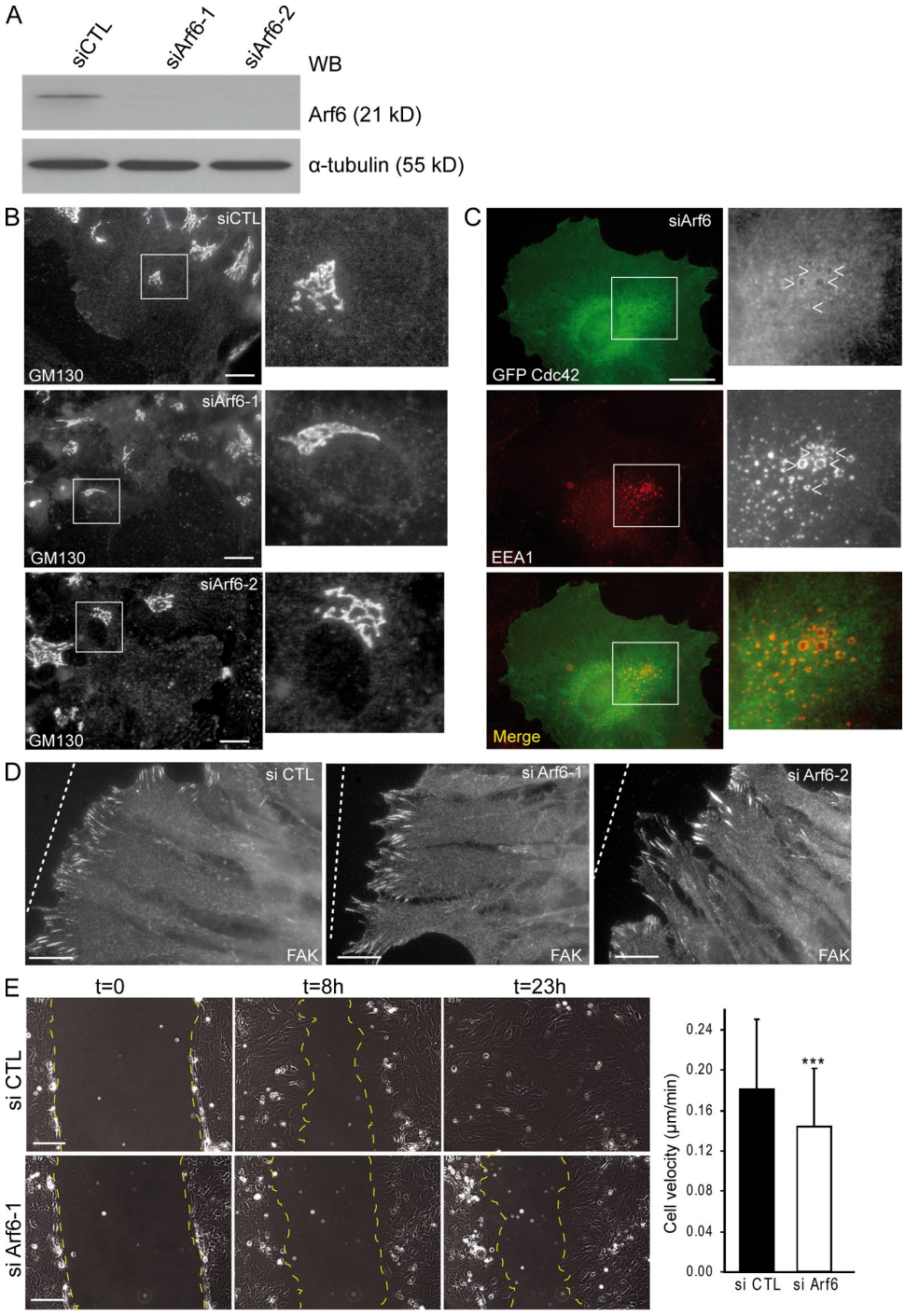


Figure S1. **Arf6 depletion by siRNA alters Cdc42 localization but does not affect the polarized distribution of focal adhesions.** Cells were nucleofected with the indicated siRNA and incubated for 3 d before wounding. (A) Cell lysates were analyzed by anti-Arf6 and anti–α-tubulin Western blotting. (B) Immunofluorescence images showing GM130 staining of the cis-Golgi apparatus. (C) After wounding, wound-edge cells were microinjected with the GFP-Cdc42 construct. 4 h later, cells were fixed and stained with an anti-EEA1 antibody. (B and C, right) Higher magnification images of the boxed regions are shown. (D) Vinculin staining showing the polarized distribution of focal adhesions in control and Arf6-depleted astrocytes at the wound edge. White dotted lines indicate the direction of the wound. Bars, 10 µm. (E, left) Phase-contrast images showing the same wound region over time (0 , 8, or 23 h after wounding). The wound edges are highlighted with dashed yellow lines. Bar, 100 µm. (right) Histogram showing the mean velocity of migrating astrocytes. ***, $P < 0.001$. Data are mean ± SEM of three independent experiments totalizing >100 cells.

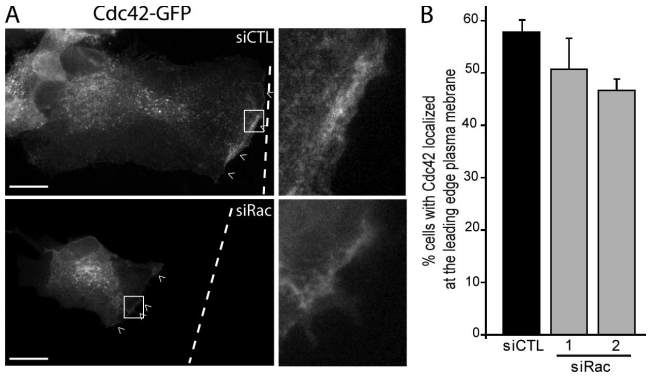


Figure S2. **Inhibition of Rac does not prevent Cdc42 recruitment to the leading edge.** Astrocytes were nucleofected with the indicated siRNA 3 d before wounding. Just after wounding, cells were microinjected with the GFP-Cdc42 construct. (A) GFP-Cdc42 fluorescence images are shown. Higher magnification images (right) of the boxed areas show Cdc42-GFP enrichment in siCTL- and siArf6-1–transfected cells. Dotted lines indicate the direction of the wound. Arrowheads point to regions of the leading edge showing an accumulation of GFP-Cdc42. Bars, 10 µm. (B) Histogram showing the percentage of wound-edge astrocytes with a Cdc42 accumulation at the wound edge. Data are mean ± SEM of two independent experiments totalizing >50 cells.

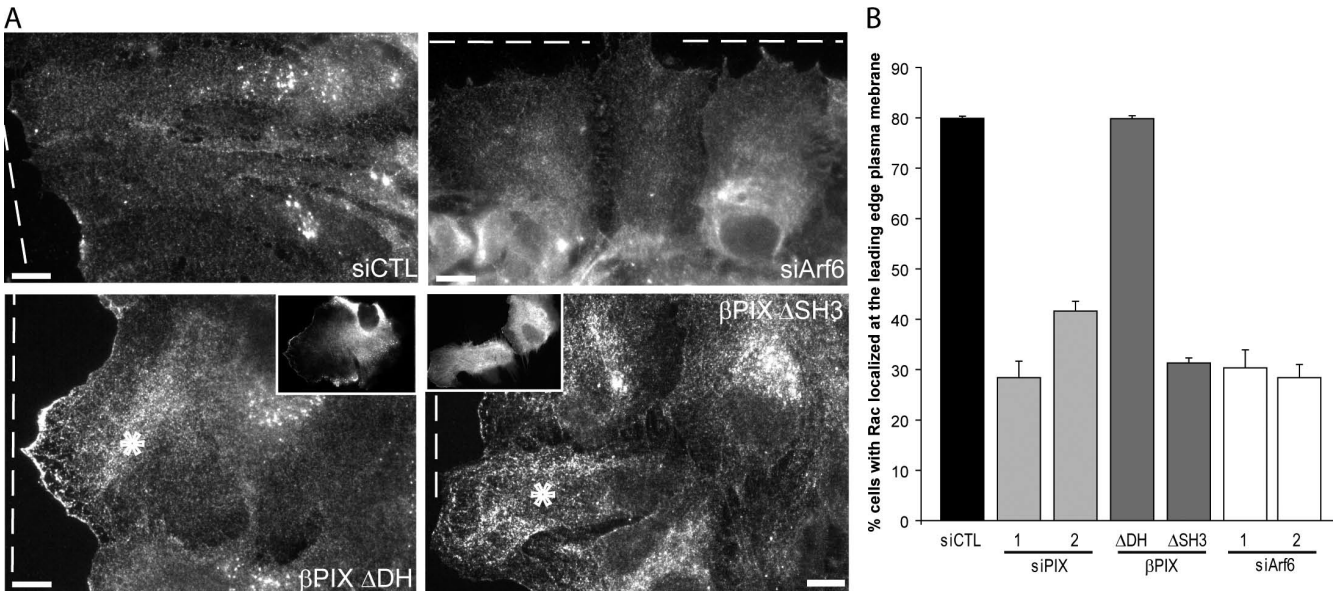
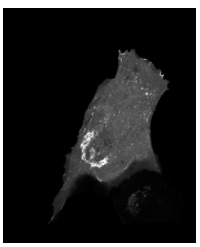
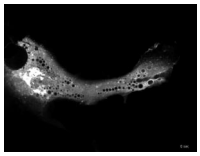


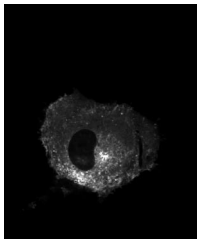
Figure S3. **Role of βPIX and Arf6 in the recruitment of Rac to the cell leading edge.** Astrocytes have been nucleofected with the indicated siRNA (control siRNA, siCTL, βPIX siRNA1 and -2, and Arf6 siRNA1 and -2) or microinjected with the indicated βPIX constructs. (A) Immunostaining of Rac in migrating astrocytes 4 h after wounding. Insets show the expression of the microinjected construct. Dotted lines indicate the direction of the wound. Bars, 10 µm. (B) Histogram showing the percentage of wound-edge cells with a leading edge accumulation of Rac at the leading edge. Data are shown as mean ± SEM of three independent experiments totalizing >300 cells.



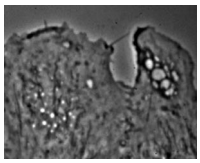
Video 1. **Cdc42 dynamics visualized in migrating astrocytes expressing GFP-Cdc42.** After wounding of the cell monolayer, astrocytes of the wound edge were microinjected with a GFP-Cdc42 construct and imaged 4 h later. Real-time duration was 267 s. Frame size, 43 × 53 µm.



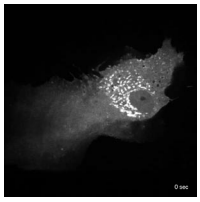
Video 2. **Cdc42 dynamics visualized in migrating astrocytes expressing GFP-Cdc42.** After wounding of the cell monolayer, astrocytes of the wound edge were microinjected with a GFP-Cdc42 construct and imaged 8 h later. Real-time duration was 495 s. Frame size, 47 × 36 μm.



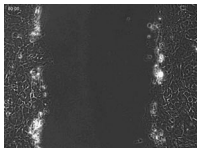
Video 3. **Cdc42 dynamics visualized in nonmigrating astrocyte expressing GFP-Cdc42.** Confluent astrocytes were microinjected with a GFP-Cdc42 construct and imaged 4 h later. Real-time duration was 65 s. Frame size, 43 × 53 μm.



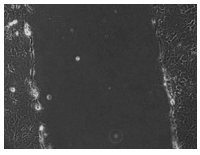
Video 4. **Phase-contrast videomicroscopy showing membrane dynamics at the leading edge of migrating astrocytes.** Astrocyte migration was induced by wounding of the cell monolayer. Contrast-phase images showing the dynamics of macropinosomes in the protrusion of migrating astrocytes 4 h after wounding. Real-time duration was 10 min. Frame size, 35 × 28 μm.



Video 5. **Cdc42 dynamics visualized in Arf6-depleted migrating astrocyte expressing GFP-Cdc42.** Astrocytes were nucleofected with siArf6-1 and grown to confluence for 3 d. After wounding of the cell monolayer, astrocytes of the wound edge were microinjected with a GFP-Cdc42 construct and imaged 8 h later. Real-time duration was 100 s. Frame size, 43 × 43 μm.



Video 6. **Phase-contrast videomicroscopy showing the migration of control astrocytes.** Astrocytes were nucleofected with control siRNA and grown to confluence for 3 d. Migration was induced by wounding of the cell monolayer at time 0. Real-time duration was 23 h. Frame size, 898 × 671 μm.



Video 7. **Phase-contrast videomicroscopy showing the migration of Arf6-depleted astrocytes.** Astrocytes were nucleofected with siArf6-1 and grown to confluence for 3 d. Migration was induced by wounding of the cell monolayer at time 0. Real-time duration was 23 h. Frame size, 898 × 671 μm.