Dupin et al., http://www.jcb.org/cgi/content/full/jcb.200812034/DC1

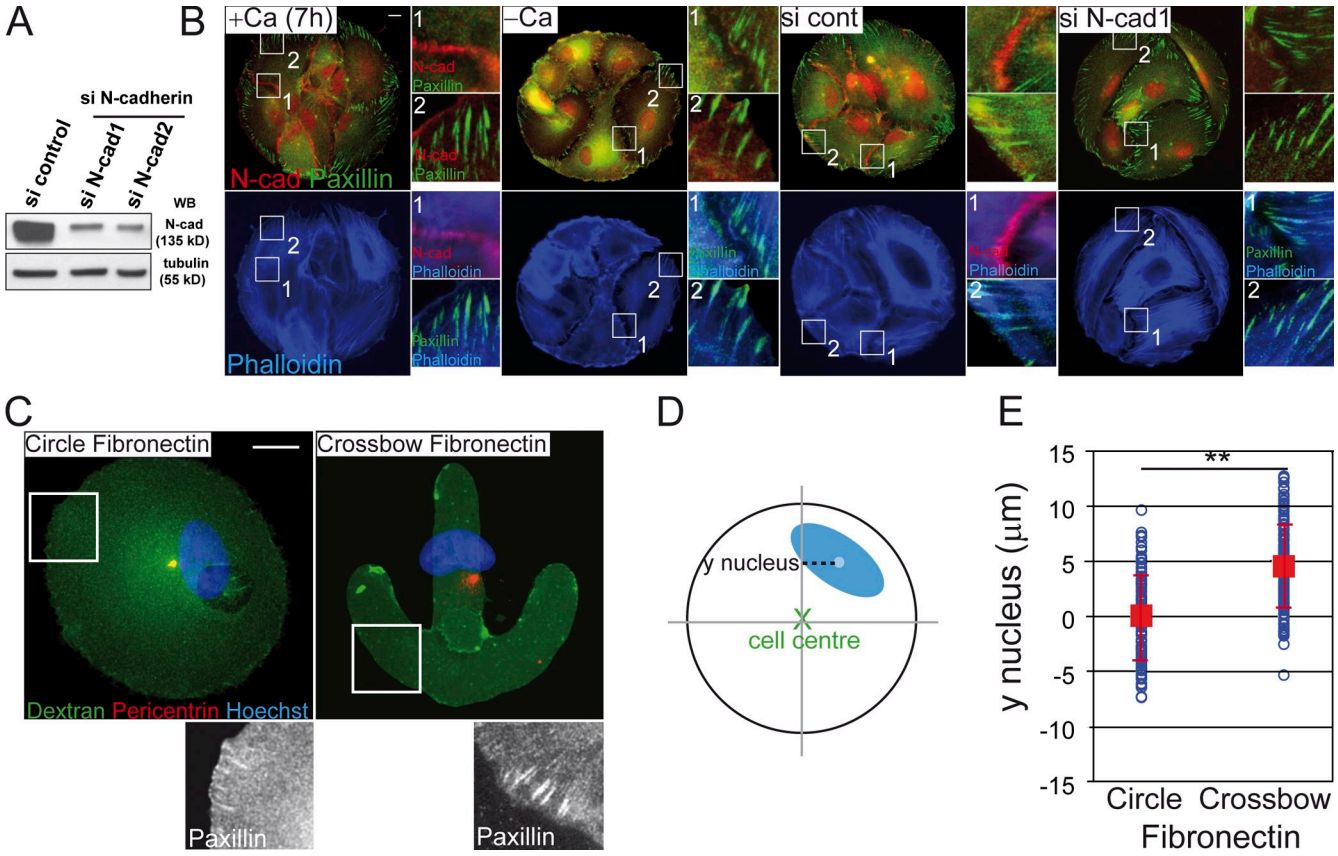


Figure S1. **Asymmetric cell–cell contacts restrict cell–matrix interactions in the free cell edge to promote nucleus off-centering.** (A) Depletion of siRNA-targeted protein was analyzed by Western blotting using the N-cadherin antibody. Membranes were successively blotted with anti-tubulin antibodies to show equal loading. (B–E) Astrocytes were plated on large fibronectin micro-patterns (B) or small circle or crossbow micropatterns (C). (B) 7 h later, control cells and calcium or N-cadherin–depleted cells were fixed and stained with N-cadherin (red), anti-paxillin (green), and phalloidin (blue). Panels on the right show high magnification of N-cadherin–paxillin, N-cadherin–phalloidin, or phalloidin-paxillin staining in the regions of cell–cell contacts (1) or free cell edge (2). Bar, 10 µm. (C) 7 h later, cells were fixed and stained with anti-pericentrin (red), anti-paxillin (white), and Hoechst (blue). Dextran fluorescence (green) represents the micropattern. Bottom panels show high magnification of paxillin staining (indicated by the boxed regions). Bar, 10 µm. (D) Schematics defining the measured parameter: the distance "y nucleus" (µm) between the nucleus centroid and the cell center along the symmetry axis (y axis) of the pattern. The cell center (green cross) and the nucleus center (blue dot) are shown. (E) Y nucleus position (blue circles) relative to the cell center for each condition. The red boxes and bars show mean values ± SD. *, $P < 0.05$; **, $P < 0.005$.

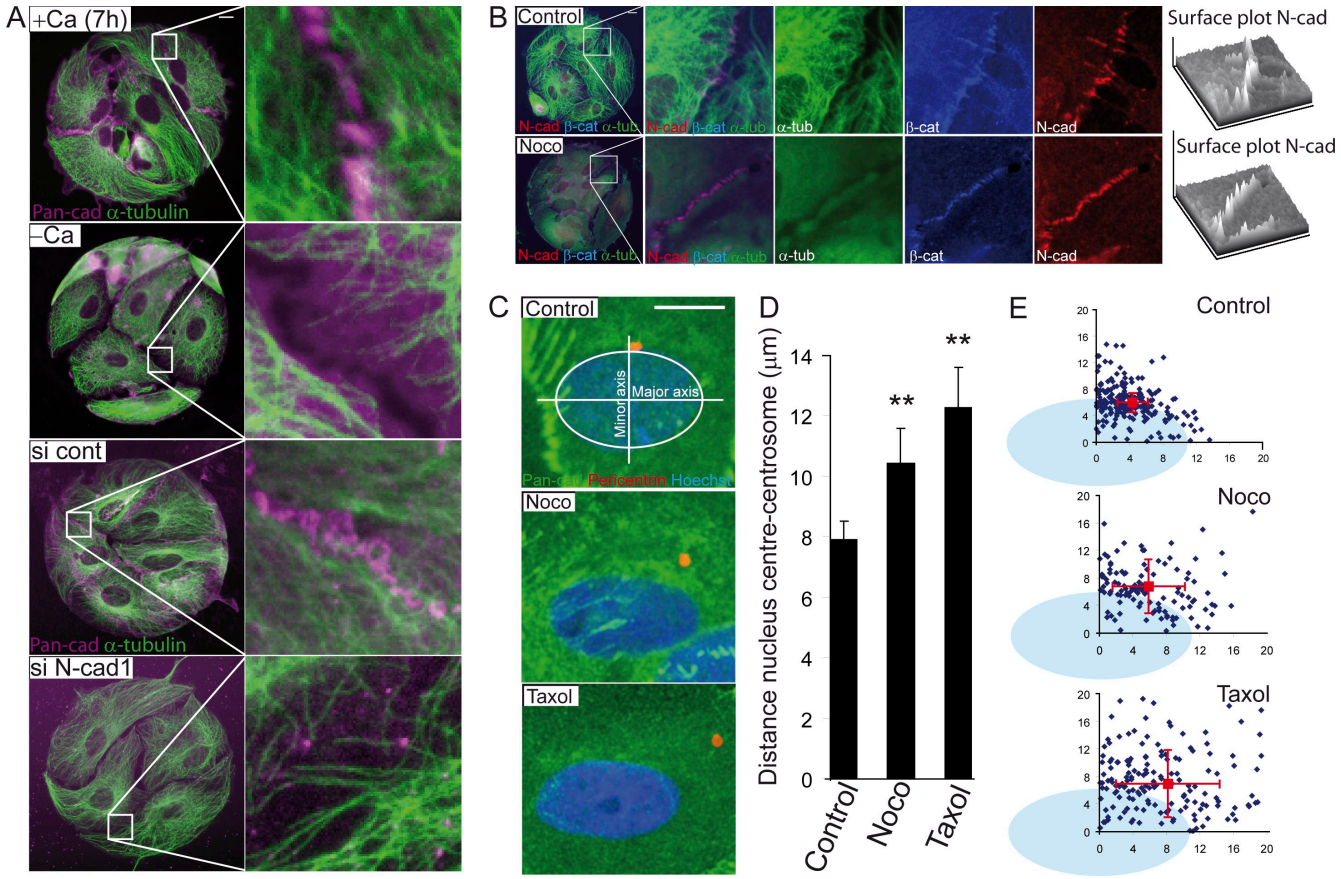
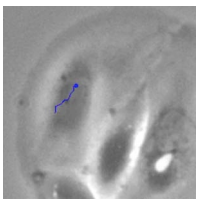


Figure S2. **Microtubules play a role in cell polarity.** (A–C) Astrocytes were plated onto fibronectin-printed micropatterns. (A) 7 h later, control cells and calcium- or N-cadherin–depleted cells were fixed and stained with anti–pan-cadherin (purple) and anti-tubulin (green). Panels on the right show high-magnification views of N-cadherin–tubulin staining. (B and C) 4 h after plating, cells were either treated with 20 µM nocodazole (Noco) or with 10 µM taxol (Taxol) for another 3 h. (B) Cells were then fixed and stained with anti–N-cadherin (red), anti-tubulin (green), and β-catenin (blue). Panels on the right show a three-dimensional graph of the intensities of the N-cadherin image. (C) Cells were stained with anti–pan-cadherin (green), anti-pericentrin (red), and Hoechst (blue). The thresholded fluorescent signal from the nucleus was fitted by an ellipse defined by its major and minor axes (white). (D) Distance between the nucleus center (black bars) and the centrosome. Data are given as mean ± SD of three independent experiments and a total of at least 120 cells. Statistical differences between control cells and cells treated with nocodazole or taxol are indicated. *, $P < 0.05$; **, $P < 0.005$. (E) Plots showing centrosome position (blue diamonds) relative to the nucleus (blue ellipse) in control and drug-treated cells. x and y axes represent, respectively, the major and the minor axes of the nucleus. Distances are indicated in micrometers. The red cross shows centrosome mean position ± SD. Bars, 10 µm.

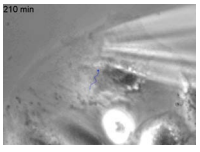
Table S1. **Mean spreading area of astrocytes plated onto fibronectin micropatterns under different conditions**

Conditions	Mean area ± SD
	μm^2
Cells engaged in cell–cell contacts (free edge)	2,040 ± 130
Cells engaged in cell–cell contacts (no free edge)	1,960 ± 150
Migrating cells	2,300 ± 120
Isolated cells (circle)	2,160 ± 70
Isolated cells (quadrant)	2,550 ± 70
−Calcium (astrocytes)	1,990 ± 170
+Calcium (astrocytes)	2,160 ± 120
−Calcium (JEG3 epithelial cells)	1,900 ± 120
+Calcium (JEG3 epithelial cells)	2170 ± 60
si control	2,160 ± 340
si N-cad1	1,990 ± 140
si N-cad2	2,120 ± 150
U373 astrocytoma cells	2,110 ± 130
si N-cad1 (migrating cells)	2,600 ± 210
si N-cad2 (migrating cells)	3,930 ± 250
GFP–N-cad	2,210 ± 130
si N-cad1 + GFP–N-cad	2,220 ± 120
E-cad–GFP	2,190 ± 60
si N-cad1 + E-cad–GFP	2,150 ± 370
Circle N-cad–Fc	2,150 ± 40
"U" N-cad–Fc	1,870 ± 60
Control	2,040 ± 130
1 µM CytoD	2,050 ± 130
20 µM nocodazol	2,120 ± 200
1 µM CytoD + 20 µM nocodazole	1910 ± 130
Enucleation: with nucleus	1,920 ± 130
Enucleation: without nucleus	1,830 ± 150
Enucleation: without nucleus + 20 µM nocodazole	2,050 ± 130

Data are given as mean ± SD of at least three independent experiments and a total of at least 90 cells.



Video 1. **Calcium depletion induces nucleus migration toward the cell center.** Astrocytes were plated onto fibronectin-printed micropatterns in the presence of calcium 3 h before being changed to calcium-free medium (t = 0). Phase contrast images were recorded every 5 min during 3 h. A blue dot corresponding to a nucleole was tracked over time with the Manual Tracking plugin (ImageJ software). Bar, 20 µm.



Video 2. **Local actin depolymerization at the free cell edge induces nucleus migration toward the cell center.** Astrocytes were plated onto fibronectin-printed micropatterns. 5 h later, cytochalasin D was injected at a constant flow rate from a micropipette placed on top of the free cell edge (t = 0). Phase contrast images were recorded every 10 min during 220 min. A blue dot corresponding to the border of the nucleus was tracked over time with the Manual Tracking plugin (ImageJ software). Bar, 10 µm.