Supporting Information

Roca-Cusachs et al. 10.1073/pnas.1220723110

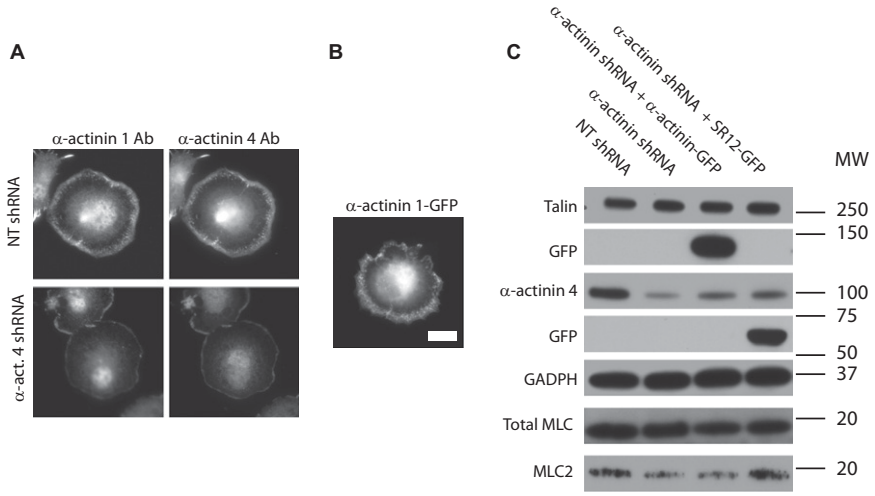


Fig. S1. Distribution of α-actinin 1 and 4 and effect of α-actinin depletion and rescues on talin and myosin light chain (MLC) expression. (*A*) Images showing cells transfected with nontargeting (NT) or α-actinin 4 shRNA stained with antibodies against α-actinin 1 (*Left*) and α-actinin 4 (*Right*). The distributions for both types of α-actinin are very similar, and both antibodies show a marked decrease in intensity after α-actinin 4 depletion. As the shRNA sequence used was in the untranslated region of the α-actinin 4 mRNA and has no homology with α-actinin 1, this shows that the α-actinin 1 antibody is unspecific and also recognizes α-actinin 4. This antibody has therefore been labeled as "total α-actinin" in Fig. 1. (*B*) Cell expressing α-actinin 1 GFP, showing a very similar distribution to that of the antibodies. (Scale bar, 20 μm.) (*C*) Western blot of cells transfected with NT or α-actinin shRNA and rescued with full-length (FL) α-actinin-GFP or SR12-GFP. No differences were observed in talin or total MLC expression, whereas a certain decrease in MLC2 was observed upon α-actinin depletion.

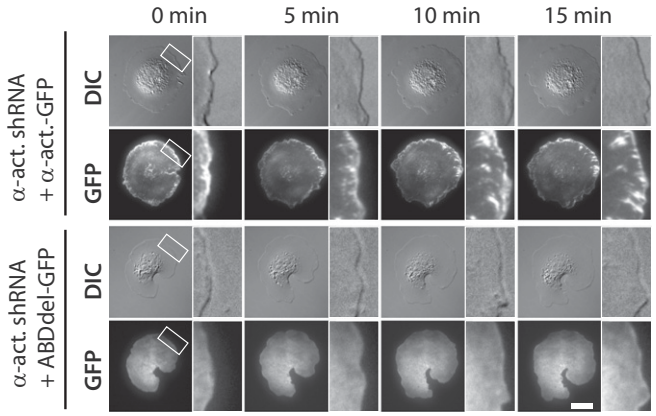


Fig. S2. Lamellipodial rim of FL but not of actin binding domain-deleted (ABDdel) α-actinin-GFP matures into elongated adhesions. Time sequence in differential interference contrast (DIC) and total internal reflection fluorescence (TIRF) GFP channels of spreading of cells transfected with α-actinin shRNA and rescued with FL or ABDdel α-actinin-GFP. Expanded views amplify the areas marked with a white rectangle. (Scale bar, 20 μm.)

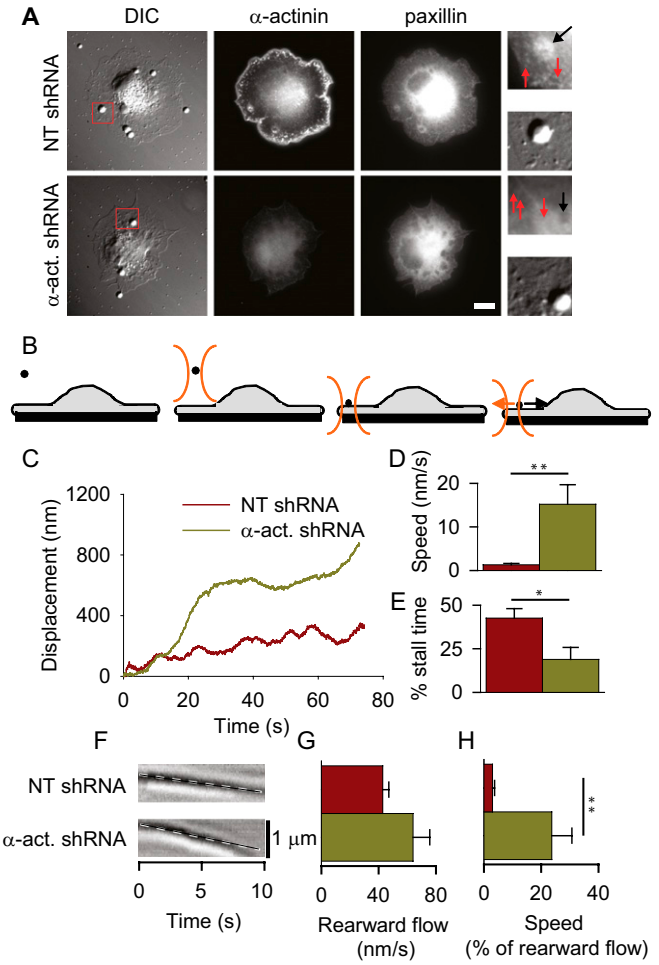


Fig. S3. α-Actinin interferes with initial talin-dependent adhesion formation. (*A*) Cells transfected with NT or α-actinin shRNA stained for α-actinin and paxillin after adhering to 3-μm and 0.5-μm fibronectin-coated silica beads. Expanded view shows the position of 3-μm beads (black arrows) and 0.5-μm beads (red arrows) in paxillin staining. Only 3-μm beads in NT cells show paxillin recruitment. (*B*) Optical tweezers assay (from left to right). Diffusing small 0.5-μm fibronectin-coated silica beads are captured with an optical trap and moved to the lamellipodium of a spread cell. The cell then attaches to the bead and attempts to transport it with the rearward moving actin cytoskeleton (black arrow). As the bead moves from the trap, it experiences a force proportional to the distance from the center of the trap (100 pN/μm, orange arrow). The ability of the cell to pull the bead away from the trap is then monitored. (*C*) Sample traces of 0.5-μm fibronectin-coated silica beads in NT and α-actinin shRNA cells as they escape a 100-pN/μm optical trap. (*D*) Corresponding average escape speed from the trap. (*E*) Percentage of time that beads were not moving away from the trap (stall time) ($n > 20$ beads from ≥ 9 cells measured on 3 different days). (*F*) Kymographs (images of a line of pixels as a function of time) showing the rearward flow of cytoplasmic markers in NT and α-actinin shRNA cells. (*G*) Corresponding quantification of rearward flow. No significant differences were observed ($n > 17$ markers from ≥ 9 cells measured on 3 different days). (*H*) Bead speeds expressed as percentage of rearward flow. $^{*}P < 0.05$, $^{**}P < 0.01$.

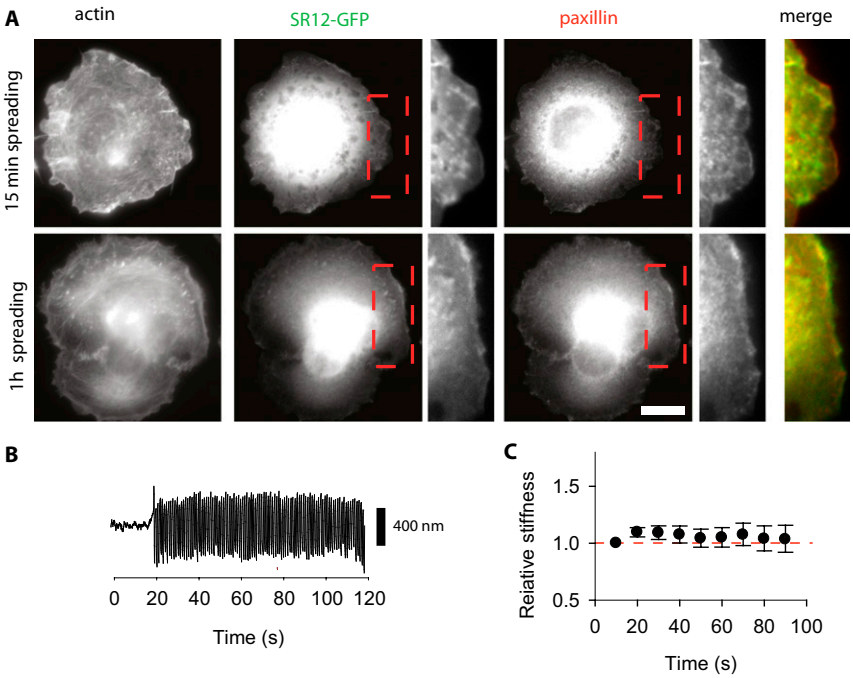


Fig. S4. SR12-GFP does not rescue focal adhesion formation or mechanotransduction in α-actinin–depleted cells. (*A*) Cells transfected with α-actinin shRNA and rescued with SR12-GFP stained after 15 min or 1 h for F-actin and paxillin. Expanded views amplify the areas marked with a red rectangle. (Scale bar, 20 μm.) Merge expanded view shows colocalization (in yellow) between SR12-GFP (green) and paxillin (red). (*B* and *C*) Example bead traces (*B*) and average relative stiffness (*C*) as a function of time of fibronectin-coated beads submitted to a 1-nN force pulsating at 1 Hz attached to cells transfected with α-actinin shRNA and rescued with SR12-GFP. The sample traces show the oscillation of the beads as a function of time in response to the applied force (which begins at the 20-s time point). No reinforcement was observed (n = 33 beads from 11 cells measured on 2 different days).

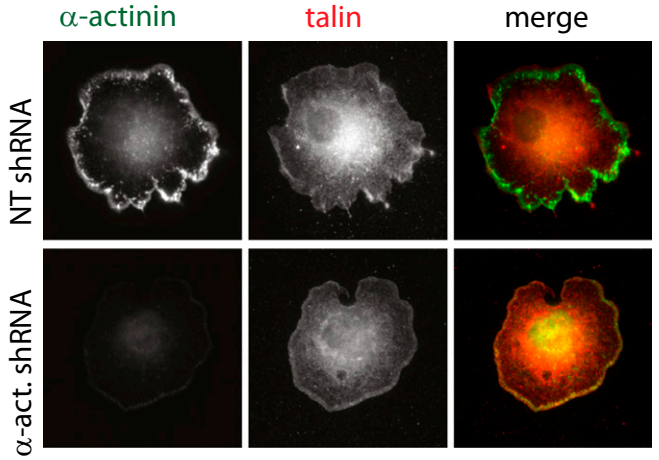


Fig. S5. Effect of α-actinin depletion on talin distribution. Cells transfected with NT or α-actinin shRNA stained after 15 min of spreading for α-actinin and talin (green and red, respectively, in merged image). (Scale bar, 20 μm.)

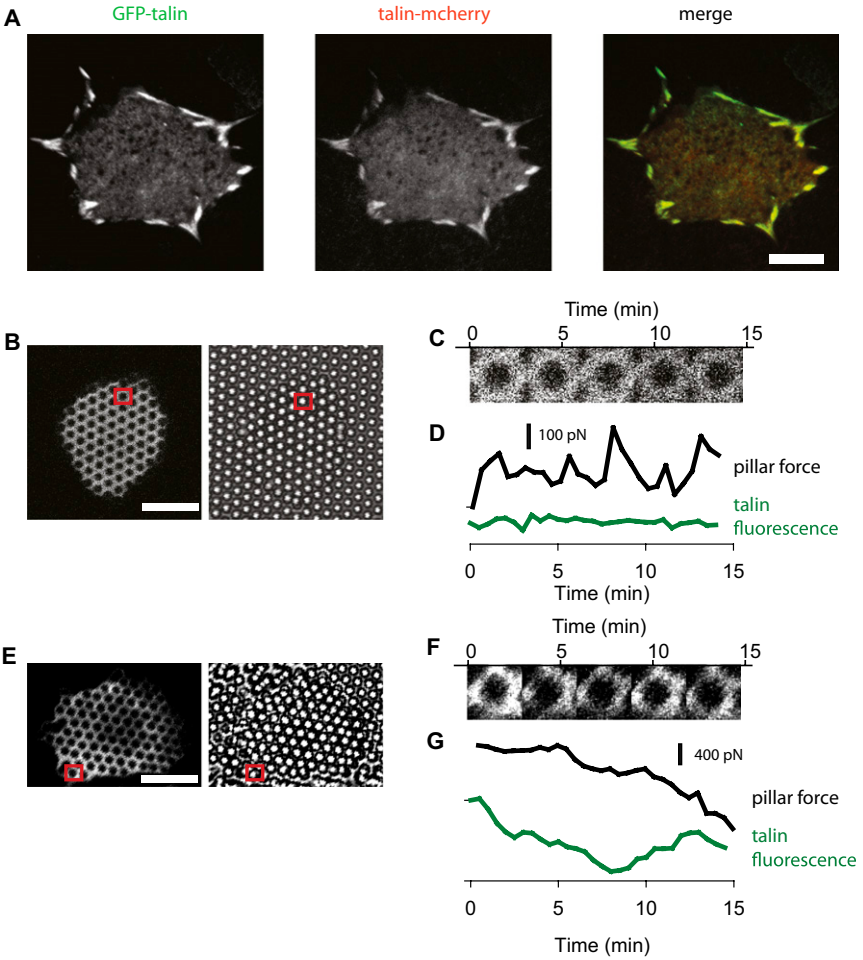


Fig. S6. Talin-mCherry localizes and behaves like GFP-talin. (*A*) GFP (*Left*), mCherry (*Center*), and merged (*Right*) TIRF images of a cell cotransfected with GFP-talin and talin-mCherry. Localization was identical. (Scale bar, 20 mm.) As the GFP-talin construct was previously reported to accurately report talin localization and to rescue function in talin-depleted cells (1, 2), the identical localization of mCherry-talin indicates that it is an equally valid reporter. (*B*) Images of a cell plated on fibronectin-coated pillars transfected with GFP-talin showing GFP signal (*Left*) and bright-field pillar image (*Right*). (Scale bar, 10 μm.) (*C*) Sequence showing evolution of GFP fluorescence of the pillar marked in red in *B*. (*D*) Traces depicting the change with time of pillar force and talin fluorescence. Both signals were not correlated, and talin fluorescence was stable, as previously observed with talin-mCherry (Fig. 5). (*E–G*) Same data as in *B–D* shown for a different pillar in another cell. To evaluate whether stable talin fluorescence levels could hide small-scale correlations with force, pillars with visible fluctuations in both force and talin fluorescence were specifically searched. No correlation was observed (see traces in *G*).

1. Zhang X, et al. (2008) Talin depletion reveals independence of initial cell spreading from integrin activation and traction. *Nat Cell Biol* 10(9):1062–1068.
2. Roca-Cusachs P, Gauthier NC, Del Rio A, Sheetz MP (2009) Clustering of α(5)β(1) integrins determines adhesion strength whereas α(v)β(3) and talin enable mechanotransduction. *Proc Natl Acad Sci USA* 106(38):16245–16250.

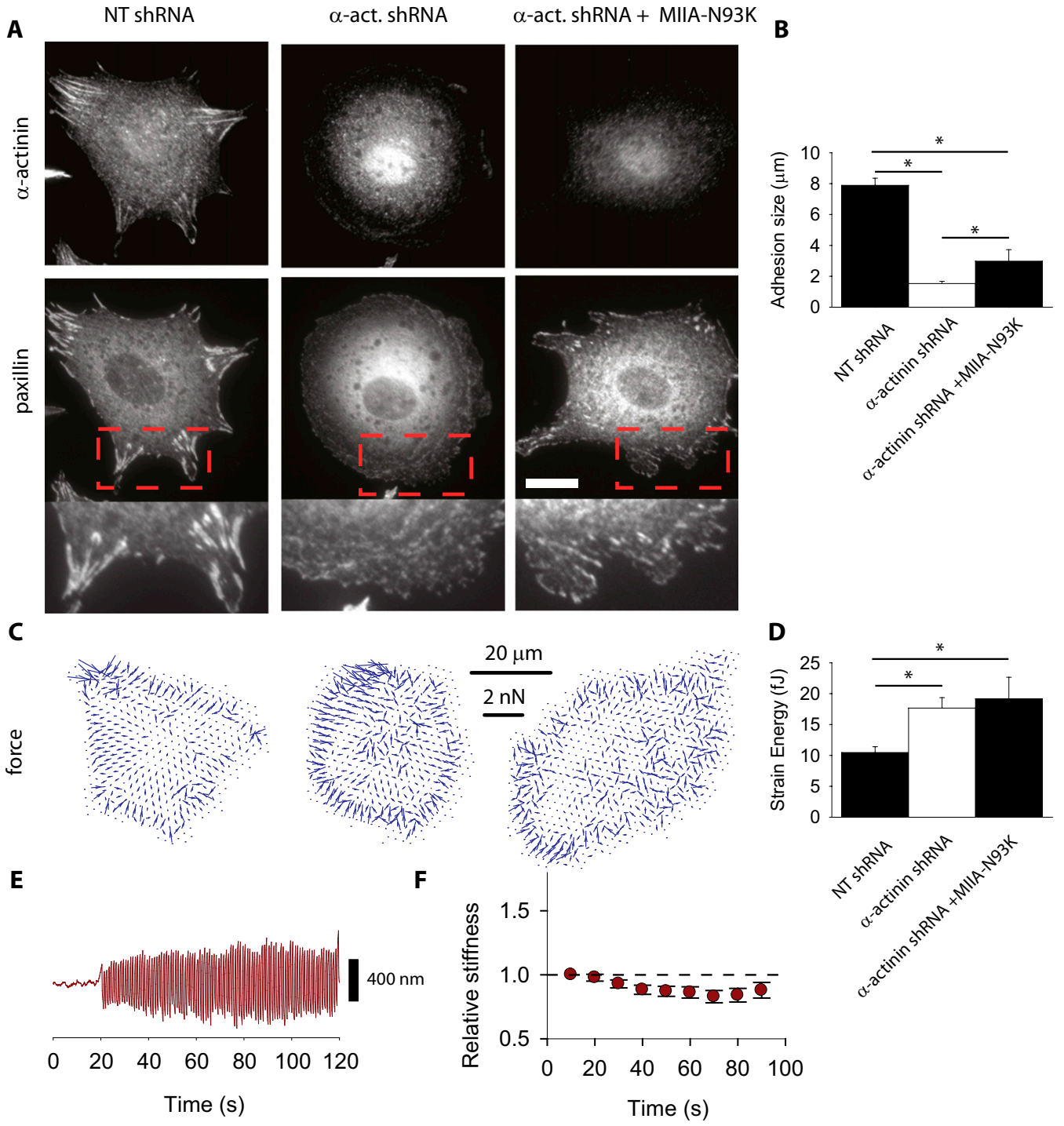


Fig. S7. Actin cross-linking does not rescue α-actinin depletion. (*A*) Cells transfected with either NT or α-actinin shRNA and rescued with GFP-MIIA-N93K plated on fibronectin for 1 h. Cells were stained for α-actinin (*Upper*) and paxillin (*Lower*). Expanded view shows a magnification of the red square in the paxillin image. (Scale bar, 20 μm.) (*B*) Quantification of paxillin adhesion length (*$P < 0.05$, $n \geq 5$ cells measured on 2 different days). (*C*) Vector plots with arrows depicting the magnitude and direction of forces exerted on 1-μm pillar arrays coated with fibronectin. Cell transfections are the same as in *A*. (Scale bar, 20 μm; force scale bar indicates the length of a force arrow of 2 nN.) (*D*) Corresponding quantification of average forces (strain energy) exerted by cells. *$P < 0.05$, $n \geq$ 11 cells measured on 2 different days. (*E* and *F*) Example bead trace (*E*) and average relative stiffness (*F*) as a function of time of fibronectin-coated beads submitted to a 1-nN force pulsating at 1 Hz attached to cells transfected with α-actinin shRNA and rescued with GFP-MIIA-N93K ($n = 34$ beads from 12 cells measured on 2 different days). No reinforcement was observed.

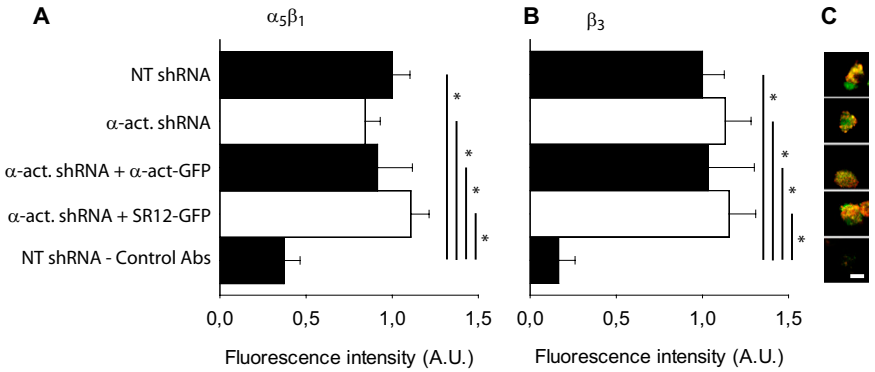
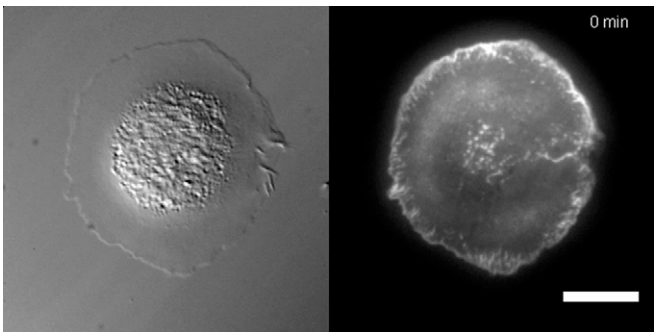
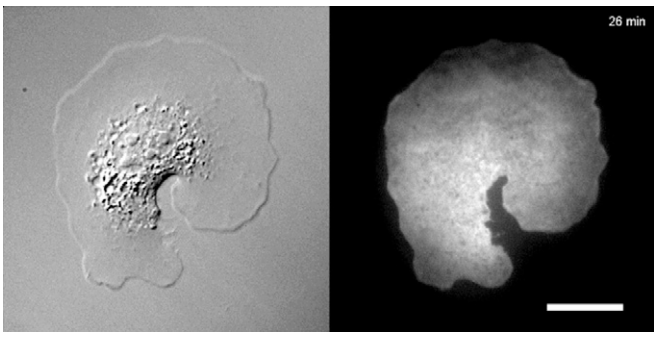


Fig. S8. α-Actinin depletion and mutants do not affect integrin expression on the membrane before spreading. Cells transfected with NT or α-actinin shRNA and rescued with FL α-actinin-GFP or SR12-GFP were trypsinized and incubated for 30 min in suspension in Ringer buffer with inhibitory antibodies against $\alpha_5\beta_1$ and β_3 integrins. Cells were then fixed, incubated with secondary antibodies, and observed in the microscope. The bar labeled "NT shRNA - Control Abs" corresponds to cells transfected with NT shRNA and incubated with an antibody against Trinitrophenol (which is not expressed in mouse cells) and secondary antibodies. (*A*) Quantification of $\alpha_5\beta_1$ fluorescence levels. (*B*) Quantification of β_3 fluorescence levels. (*C*) Examples of stained cells (green, $\alpha_5\beta_1$; red, β_3). (Scale bar, 20 μm.) Cells stained with control Abs had significantly lower fluorescence levels than all other cells, showing the specificity of the staining procedure. Integrin levels in all other cases were not significantly altered, showing that the expression of both integrins on the membrane before spreading was not affected by α-actinin depletion and mutants. $n \geq 12$ cells from two different experiments. $^*P < 0.05$.



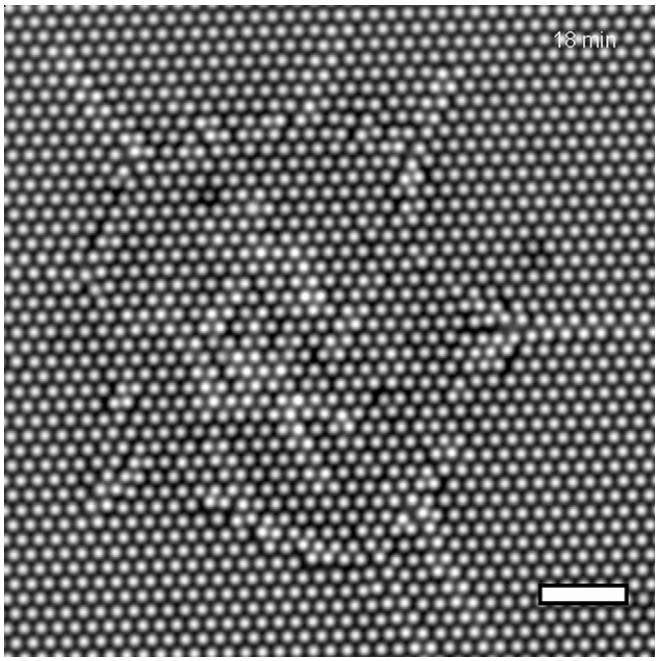
Movie S1. Related to Fig. 2. DIC (*Left*) and TIRF GFP (*Right*) sequence of spreading of a cell transfected with α-actinin shRNA and rescued with FL α-actinin-GFP. (Scale bar, 20 μm.)

Movie S1



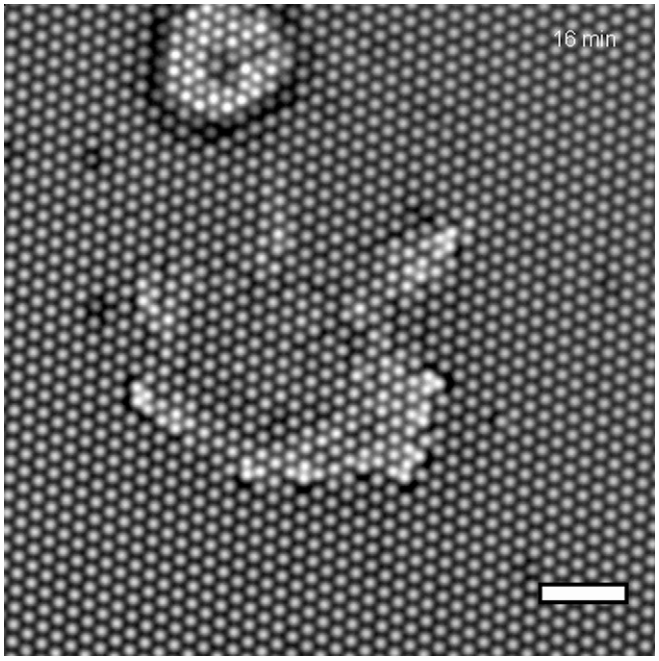
Movie S2. Related to Fig. 2. DIC (*Left*) and TIRF GFP (*Right*) sequence of spreading of a cell transfected with α-actinin shRNA and rescued with ABDdel α-actinin-GFP. (Scale bar, 20 μm.)

Movie S2



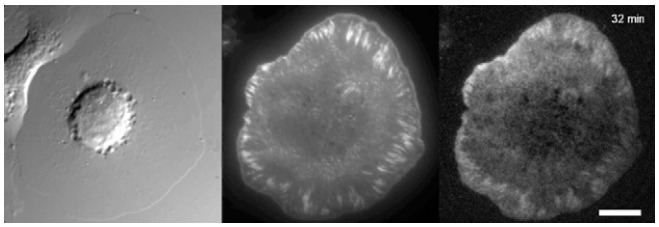
Movie S3. Related to Fig. 4. Sequence of a cell transfected with NT shRNA spreading on fibronectin-coated pillars. (Scale bar, 10 μm.)

Movie S3



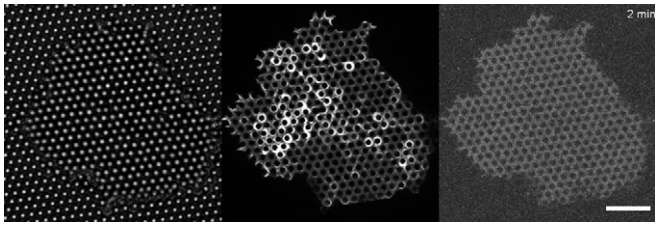
Movie S4. Related to Fig. 4. Sequence of a cell transfected with α-actinin shRNA spreading on fibronectin-coated pillars. (Scale bar, 10 μm.)

Movie S4



Movie S5. Related to Fig. 5. Time sequence of cells cotransfected with α-actinin-GFP and talin-mCherry showing DIC images (*Left*), α-actinin images (*Center*), and talin images (*Right*). (Scale bar, 20 μm.)

Movie S5



Movie S6. Related to Fig. 5. Time sequence of cells plated on fibronectin-coated pillars and cotransfected with α-actinin-GFP and talin-mCherry. Pillar images are shown (*Left*), α-actinin images (*Center*), and talin images (*Right*). (Scale bar, 10 μm.)

Movie S6