

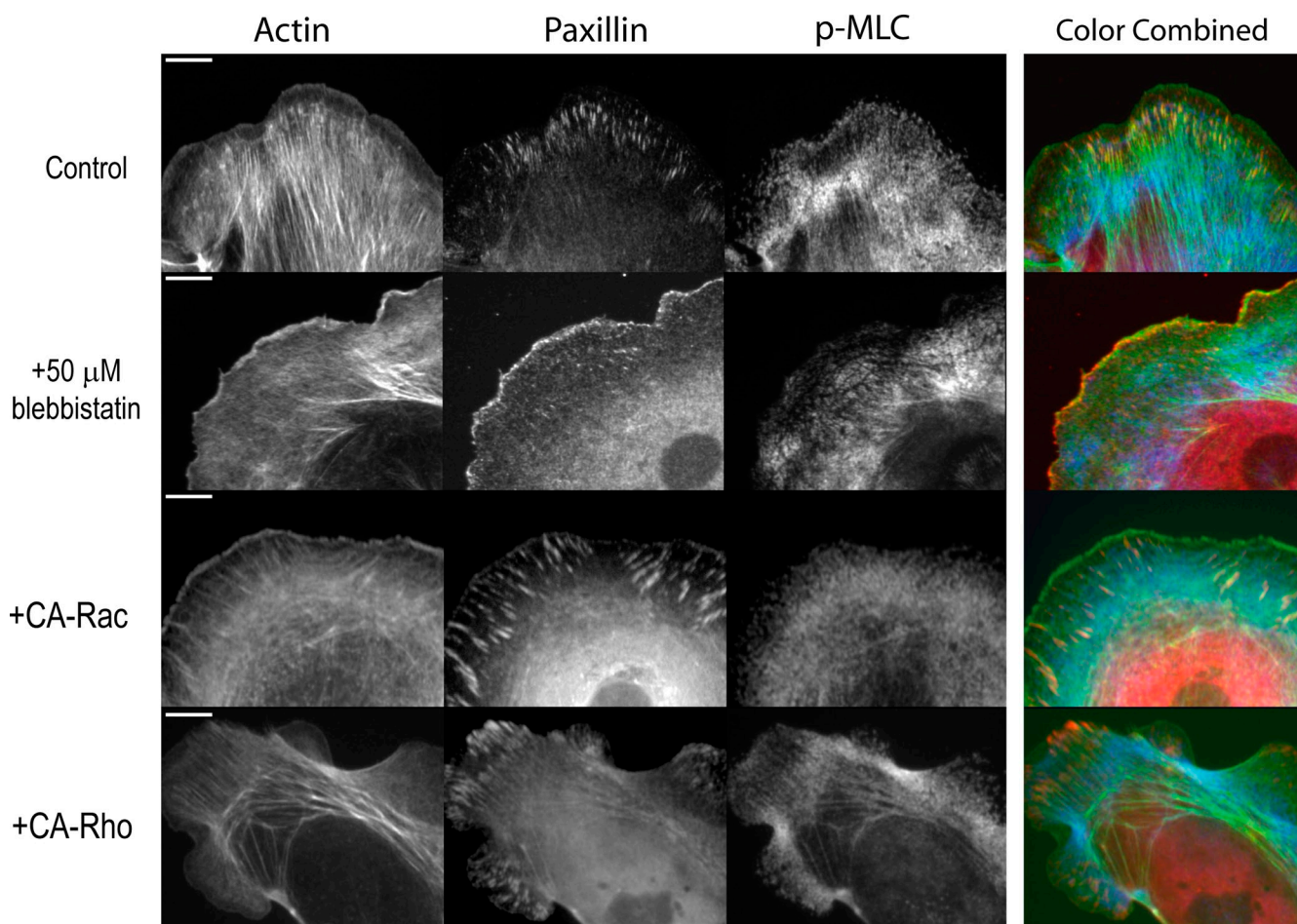
Gardel et al., <http://www.jcb.org/cgi/content/full/jcb.200810060/DC1>

Figure S1. **Immunofluorescence of F-actin, paxillin, and serine-19-phosphorylated myosin II light chain.** Immunofluorescence of F-actin, paxillin, and serine-19-phosphorylated myosin II light chain (pMLC) in control cells, cells treated with 50 mM blebbistatin, and cells expressing either constitutively active Rac1 (CA-Rac) or constitutively active RhoA (CA-Rho). Under all conditions, there exists a narrow band of actin meshwork that is absent of myosin II; this is termed the lamellipodium. Within and proximal to the lamellipodium, focal adhesions, as visualized by paxillin immunostaining, initiate. Inhibition of myosin II abrogates actomyosin bundles and reduces FA size. Overexpression of CA-Rac prevented the disassembly of adhesions and they appear as long bands throughout the lamella and reduced actomyosin bundles. Overexpression of CA-Rho promotes FA maturation and turnover, FAs are dense and localized closer to the cell edge, and actomyosin bundles are prominent. In all conditions pMLC is found within the lamellum. Bars, 15 μ m.

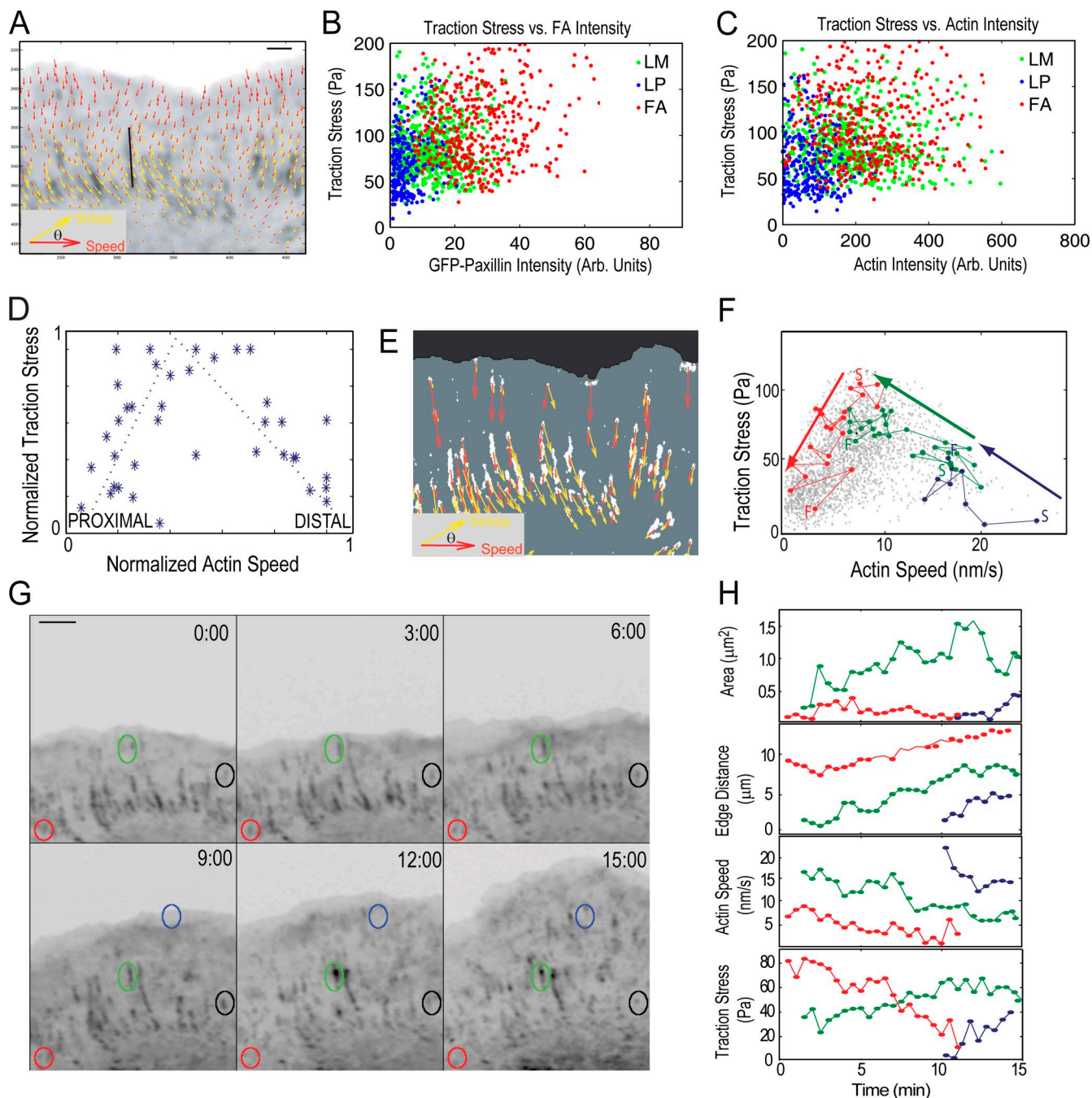


Figure S2. Traction stress versus paxillin and actin intensity. (A) Illustration of traction stress and interpolated F-actin velocity vector fields. To determine the correlation in traction stress and F-actin speed across the entire leading edge of the cells, the F-actin speed vectors (red) were interpolated onto the origin of the traction stress vectors (yellow). These data are plotted in B and C. The black line represents an example of a line scan used to obtain plots of the traction stress–F-actin speed relationship across single FAs as in D. (B and C) The traction stress versus GFP-paxillin intensity (A) or x-rhodamine actin intensity (B) measured at each grid point across the lamellipodia and lamella do not show strong correlations. Traction stress and GFP-paxillin intensity or x-rhodamine F-actin intensity were grouped into three distinct regions based on proximity to cell edge and location within segmented FAs. The lamellipodium (LP; blue) was defined as points within 2.7 mm from the cell edge outside of segmented FAs. The focal adhesions (FA; red) were defined as data points within segmented FAs that were within 2.7–8 μm away from the cell edge. Lamellum (LM) data are located within the same range of distances as FAs, but outside segmented FAs. (D) Biphasic relationship between traction stress and F-actin speed across single FA. Traction stress versus F-actin speed within single FA obtained from averaging 5–10 line scans across individual FAs. Stress, speed, and distance along FAs were normalized relative to their maximum values for each FA to allow comparison of speed and stress variation across FAs that differ in absolute stress speed and size magnitude. (E) Determination of mean traction stresses and F-actin speeds within segmented FAs. We obtained the centroid of the regions demarcated by segmented FAs, and the traction stress and F-actin velocity vectors that originated within the masked regions were interpolated onto this centroid to give a single value for these parameters for each FA. (F and H) Centroids were linked over time to obtain FA “time trajectories.” (F–H) Evolution of F-actin speed and traction stress in single FAs. (F) Traction stress versus F-actin speed plotted for points only within segmented FAs. (gray points) All data in segmented FAs for a 25-frame video. (colored points) Evolution of FAs in three different states: initiation (blue), strengthening (green), and weakening (red). The FAs analyzed are highlighted by circles of similar color in the montage of GFP-Paxillin images in G (inverted contrast). Bar, 3 μm . The start (S) and finish (F) time points of each evolution are indicated. (H) Time dependence of FA area, FA distance from cell edge, FA stress, and local F-actin speed for the three FAs delineated by the red, green, and blue ovals, respectively, in G. The black oval in G indicates the FAs analyzed in Fig. 3 D.

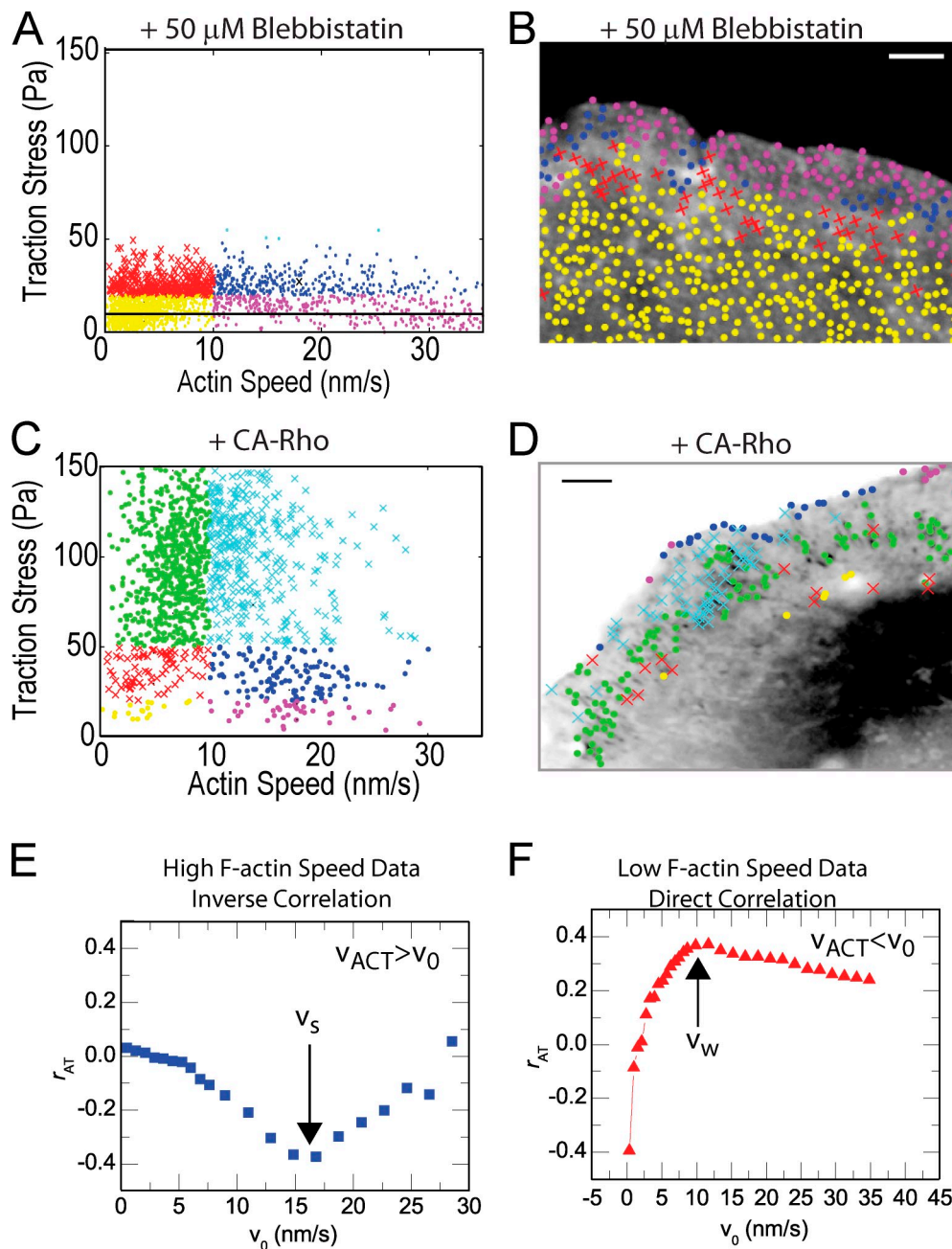


Figure S3. **Traction stress versus F-actin speed.** (A–D) Effect of altering myosin II ATPase (A and B) and RhoA activity (C and D) on the relationship between traction stress and F-actin speed. (A) Traction stress versus F-actin speed for all data points in a 14-frame video of a cell treated with 50 μ M blebbistatin for 1 h, plotted on the same scale as Fig. 3 B and Fig. 4 A. Resolution limit of traction stress is indicated by the solid black line. (B) Image of x-rhodamine actin in a 50 μ M blebbistatin-treated cell, with spatial location of stress/speed data points plotted and colored as in A. Bar, 3 μ m. (C) Traction stress versus F-actin speed for data points within segmented FAs in a 20-frame video of a cell expressing CA-Rho, plotted on the same scale as Fig. 3 B and Fig. 4 A. (D) Image of GFP-paxillin (inverted contrast) in a cell expressing CA-Rho, with spatial location of stress/speed data points plotted and colored as in C. Bar, 3 μ m. (E and F) Calculation of correlation coefficient between traction stress and F-actin speed at high and low F-actin speeds. To characterize the extent of a linear correlation between traction stress and F-actin speed, all data points where $v_{ACT} > v_0$ were considered high F-actin speed data and data points where $v_{ACT} < v_0$ were considered low F-actin speed data. We calculated the correlation coefficients for a series of two variables, x and y , as $r(x,y) = \text{cov}(x,y)/(\sigma_x \sigma_y)$, where $\text{cov}(x,y)$ is the covariance matrix and σ_x and σ_y are the standard deviations. A correlation coefficient of $r_{AT} = 1$ indicates a perfect direct correlation, $r_{AT} = -1$ indicates a perfect inverse correlation, and $r_{AT} = 0$ indicates no correlation. (E) Correlation coefficient for all data where $v_{ACT} > v_0$; typical values of r_{AT} range from -0.35 to -0.56 . We successively decreased v_0 to increase the range of F-actin speeds used in calculating the correlation and observed a minimum at the F-actin speed v_s . This speed, v_s , was calculated for three cells in each perturbation condition and describes the extent of where the inverse correlation between stress and speed is maximized and is indicated by blue data points in Fig. 5 (A–D). A linear regression was calculated for all data with $v_{ACT} > v_0$. (F) For the low F-actin speeds ($v_{ACT} < v_0$; red symbols), $r_{AT} > 0$, indicating a direct relationship, typically range from 0.34 to 0.5 . To characterize the direct relationship we observed at low speeds we calculated the correlation coefficient for all speeds lower than v_0 and successively increased v_0 to find where r_{AT} was maximized at the F-actin speed v_w . Speeds lower than v_w were used to calculate a linear regression to the dataset and are indicated by the red data points in Fig. 5 (A–D).