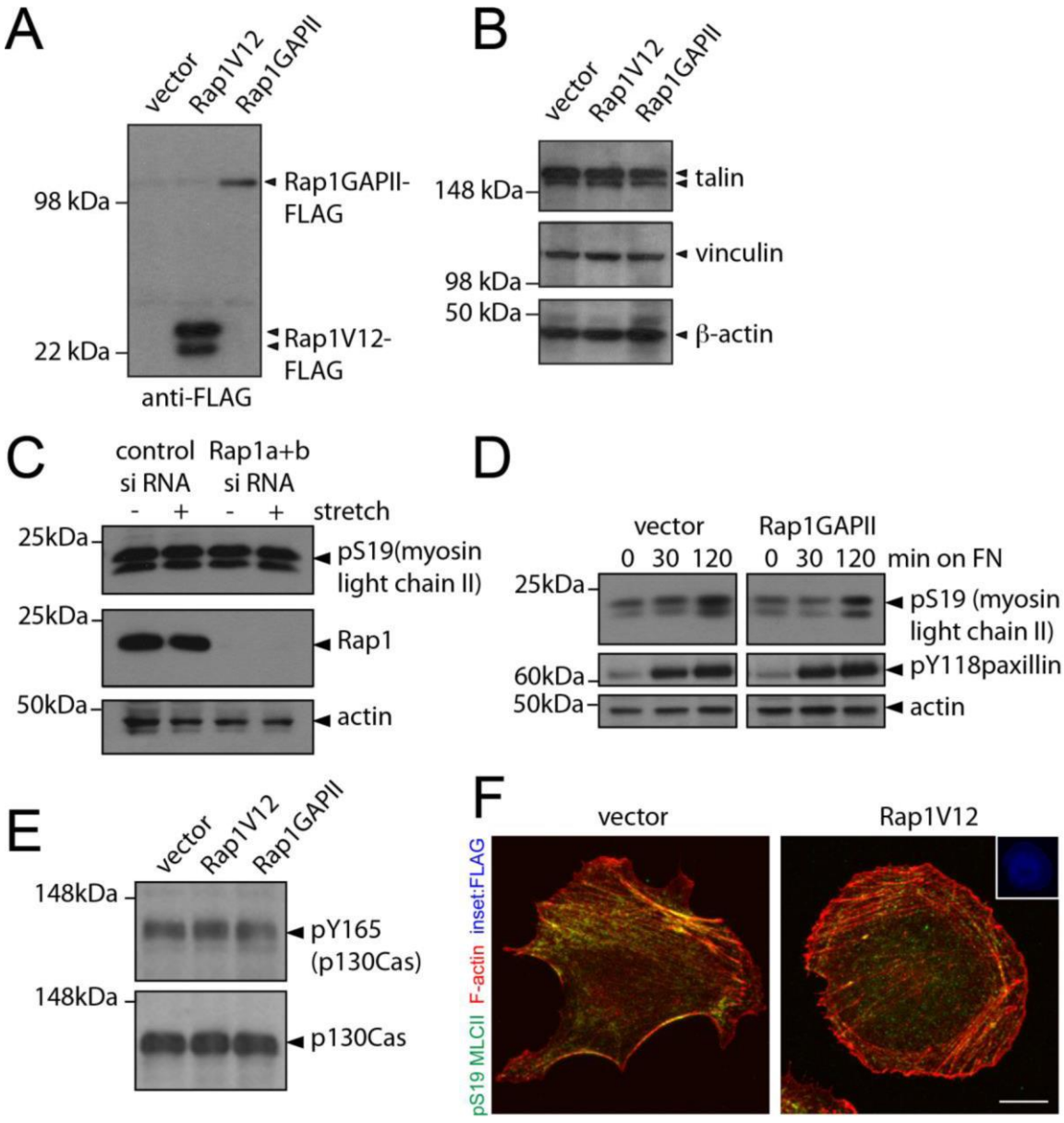


Figure S1 (relates to Figure 3 and 4). Rap activation does not alter the expression of focal adhesion proteins nor regulate the phosphorylation of myosin light chain II, paxillin, or p130Cas. A-B) B16F1 cells stably expressing vector, Rap1V12, and Rap1GAPII were lysed and probed with indicated antibodies. C) B16F1 cells were transfected with control or si RNA directed against Rap1a and Rap1b. Cells were seeded onto FN (5 μg/cm^2) coated silicone rubber plates overnight before applying 10% equibiaxial stretch to the substrata for 5 min, lysed, and probed with indicated antibodies. D) B16F1 cells expressing vector control or Rap1GAPII were seeded onto FN (5 μg/cm^2) coated tissue culture plates for indicated times, lysed, and probed with indicated antibodies. E) B16F1 cells were seeded onto FN (5 μg/cm^2) coated silicone rubber plates overnight before applying 10% equibiaxial stretch to the substrata for 5 min, lysed, and probed with indicated antibodies. F) B16F1 cells were seeded onto FN (5 μg/cm^2) coated coverglass for 2 h, fixed, and stained for pS19MLCII and F-actin. Scale bar, 10 μm.

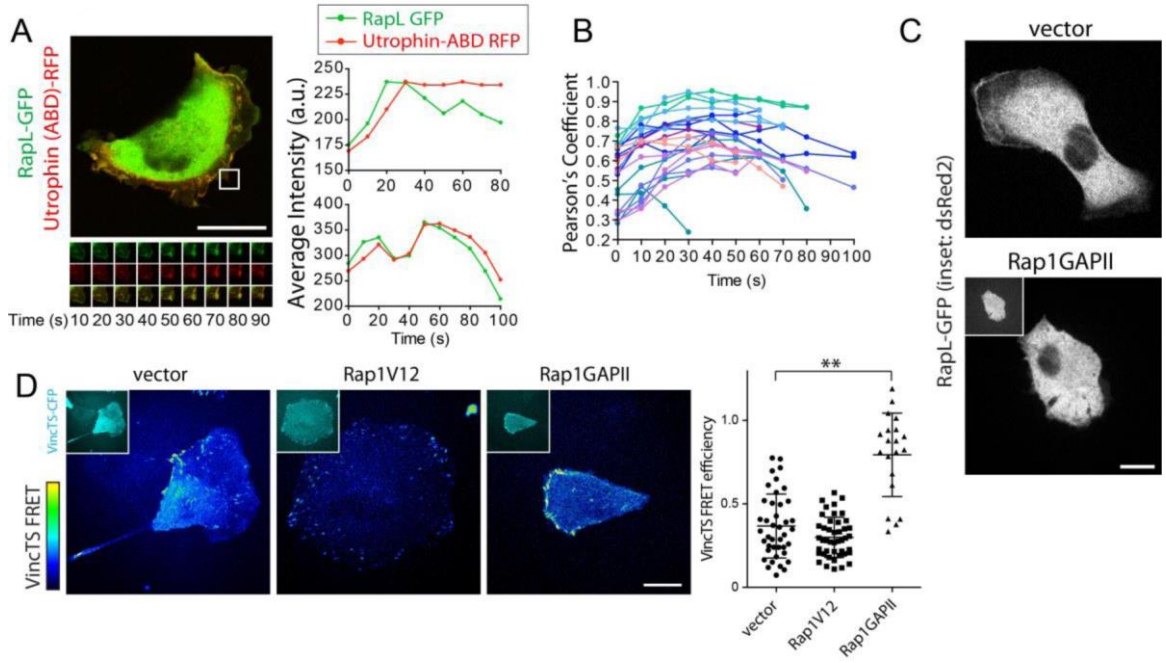
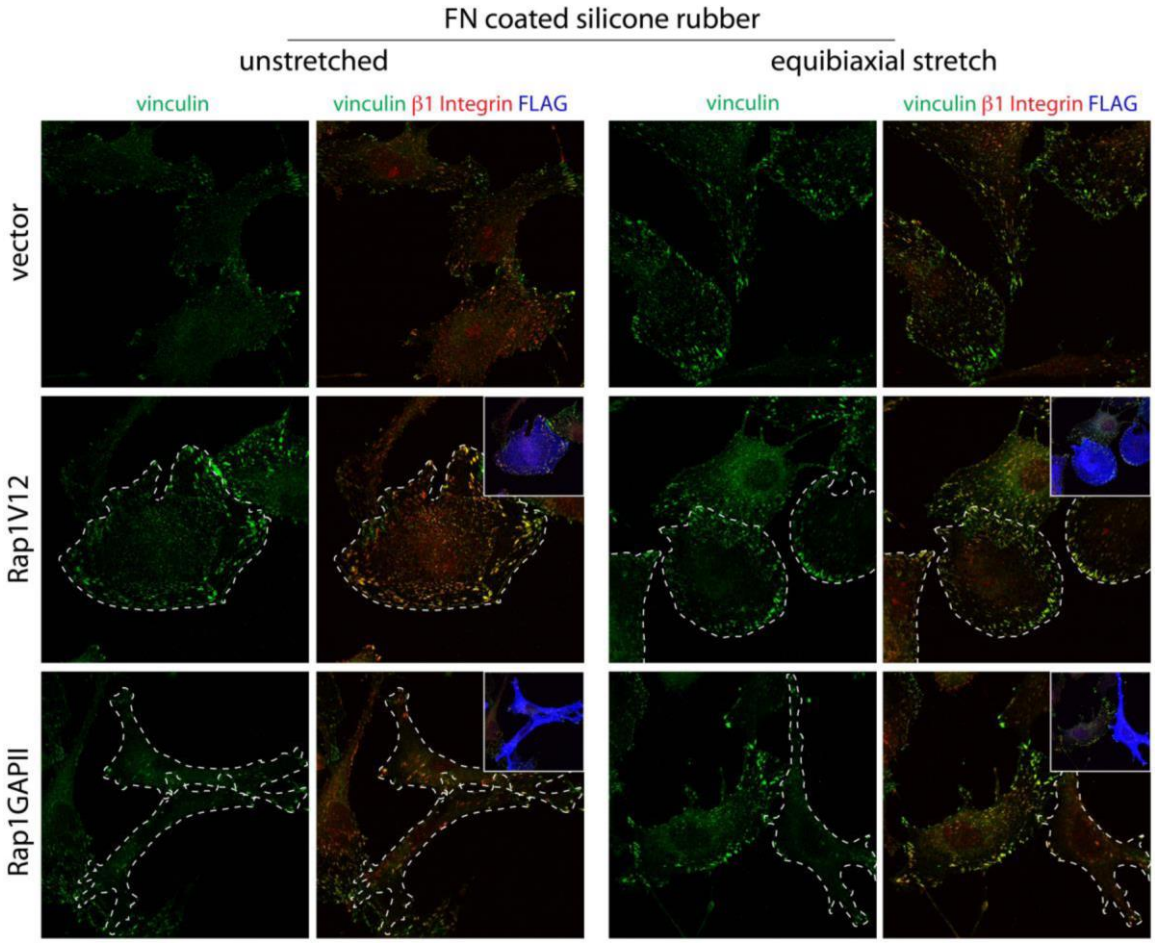
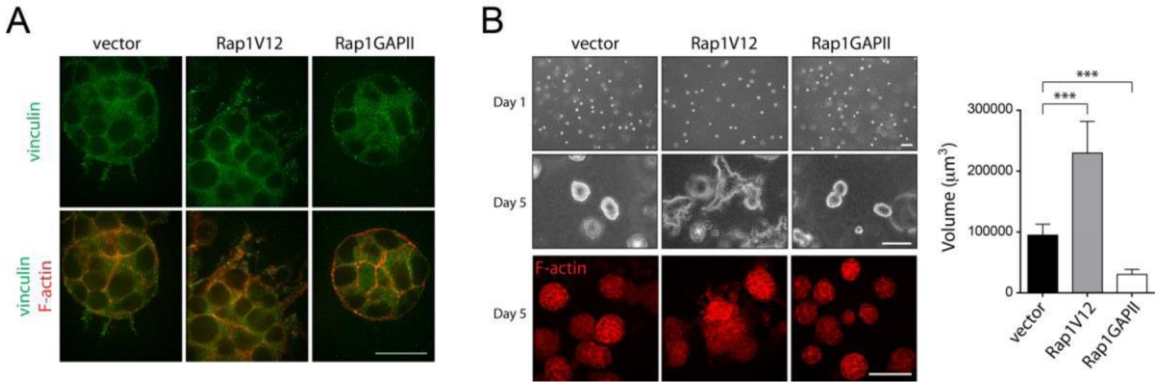


Figure S2 (relates to Figure 4). Active Rap localizes to the leading edge and regulates tension through vinculin. A-B) B16F1 cells transfected with RapL-GFP and the actin-binding domain (ABD) of utrophin fused to RFP were imaged on FN (2.5 μg cm^{-2}) coated chamber slides. As new membrane ruffling events occurred, RapL-GFP and utrophin (ABD)-RFP fluorescence were recorded. For a representative cell, time-dependent changes in RapL-GFP and utrophin (ABD)-RFP intensities within a region of interest (ROI) at the leading edge (white box) are graphed, middle panel. The Pearson's coefficient for >20 leading edge ROIs from 5 cells with active membrane ruffling are shown (B). Scale bar: 20 μm. C) B16F1 cells transfected with RapL-GFP and Rap1GAPII in a vector containing dsRed2 were imaged on FN (2.5 μg cm^{-2}) coated chamber slides. Note that the localization of RapL-GFP to the plasma membrane requires Rap activation. D) B16F1 cells were transiently transfected with vector, Rap1GAPII-FLAG, or Rap1V12 along with the vinculin tension sensor (VincTS). A ratio of the CFP signal before and after bleaching YFP for 3 frames is shown. N > 20 cells from 3 experiments. All scale bars are 20 μm. Significance was calculated using Student's unpaired two-tailed t-tests (**P < 0.01).



Supplementary Figure 3 (relates to Figure 4). Vinculin enrichment at focal adhesions in response to stretch in 2D. B16F1 cells transiently expressing vector, Rap1V12-FLAG, or Rap1GAPII-FLAG were seeded onto FN (2.5 μg cm^{-2}) coated silicone rubber plates overnight before applying 10% equibiaxial stretch to the substrata for 4 h. Cells were fixed and stained for vinculin and β1-integrin.



Supplemental Figure 4 (relates to Figure 7). Rap1-GTP regulates growth in 3D collagen gels. A-B) Single B16F1 cells stably expressing a control vector, Rap1V12, or Rap1GAPII were seeded into collagen + FN gels and grown for 5 days. A) Vinculin and F-actin were stained and the border of individual colonies with the matrix is shown. Scale bar: 100 μm. B) Phase contrast and F-actin imaging of individual fields at days 1 and 5 (left). F-actin staining was used to determine colony volume (right). Scale bar: 500 μm. *** P<0.001.