Pasapera et al., http://www.jcb.org/cgi/content/full/jcb.200906012/DC1

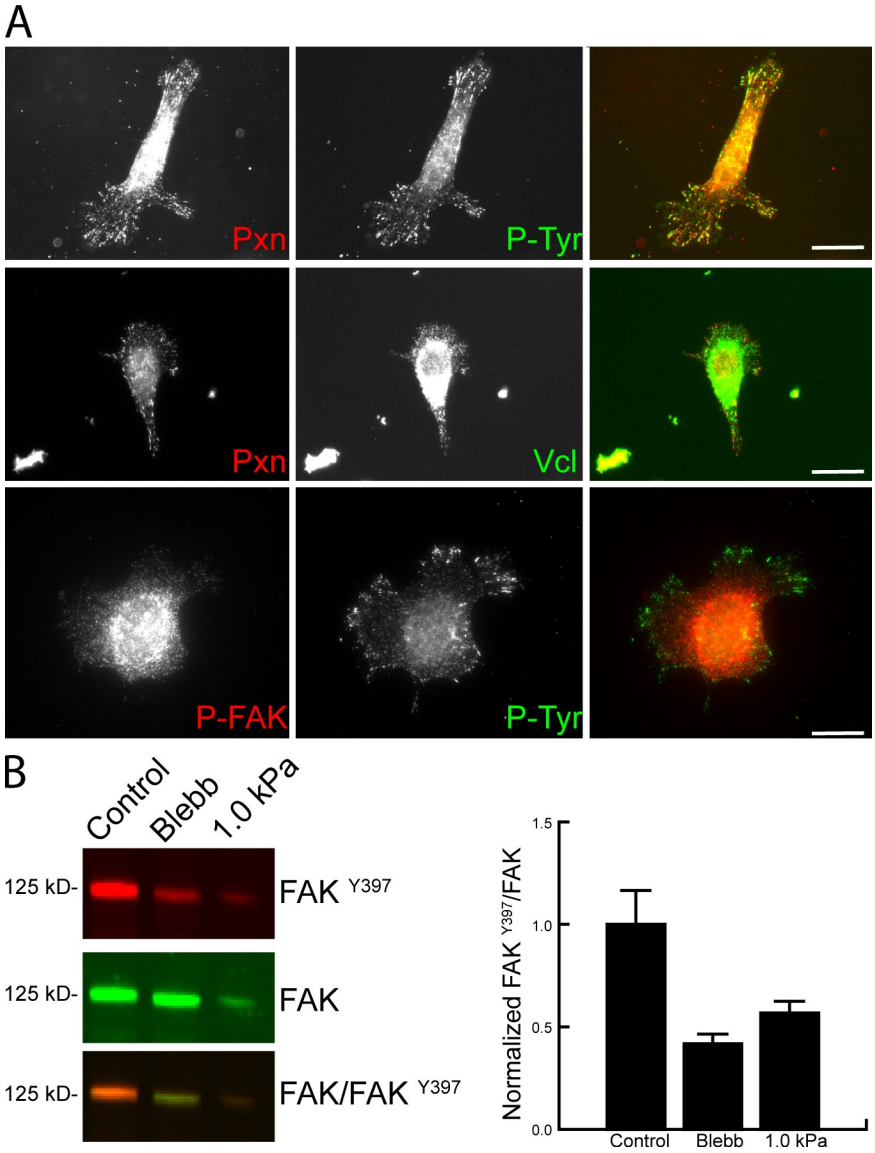


Figure S1. **Substrate compliance causes reduction of vinculin and FAK in adhesions and reduction of FAK phosphorylation.** Effects of substrate compliance on protein localization to adhesions and FAK phosphorylation in MEF cells. (A) Cells were plated on fibronectin-coupled 1.0-kPa polyacrylamide gels adhered to coverslips and processed for immunolocalization of proteins. (top) Immunolocalization of PY epitopes (P-Tyr; green) and paxillin (Pxn; red). (middle) Paxillin (red) and vinculin (Vcl; green). (bottom) FAK phosphorylated on Y397 (P-FAK; red) and PY epitopes (green). (right) Merged images are shown. Bars, 20 µm. (B) Immunoblot of lysates of untreated MEF cells (control), cells treated with 20 µM blebbistatin (Blebb), or cells plated on 1.0-kPa fibronectin-coupled polyacrylamide gels using antibodies specific to FAK or pY397 FAK (FAK^{Y397}). Error bars indicate 95% confidence interval of the mean.

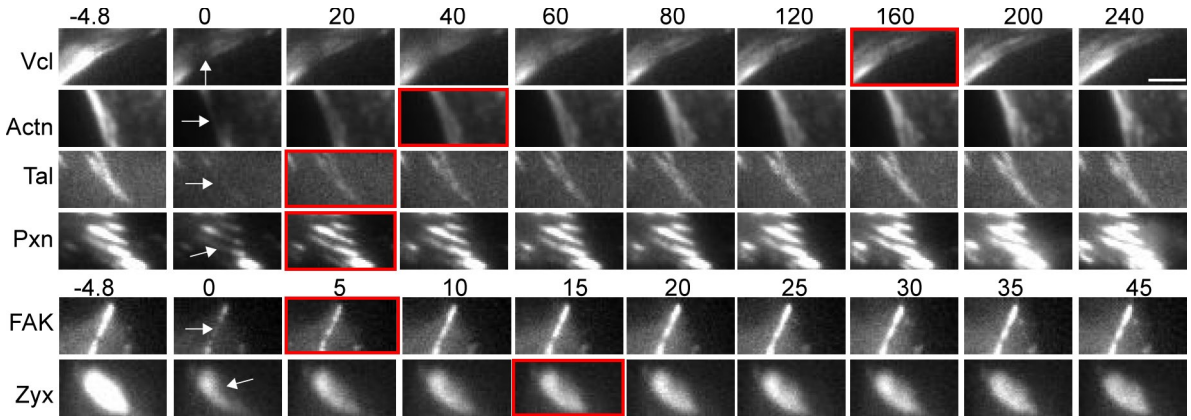


Figure S2. **FRAP analysis of EGFP-tagged adhesion proteins.** Time-lapse image series from FRAP of EGFP-tagged proteins in single adhesions in MEF cells. The adhesion at the arrows was photobleached, and recovery was from the unbleached cytosolic pool. Vcl, EGFP-vinculin; Actn, EGFP–α-actinin; Tal, EGFP–talin 1; Pxn, EGFP-paxillin; FAK, EGFP-FAK; Zyx, EGFP-zyxin. Time is shown in seconds (relative to the time of photobleaching, time = 0) along the top of each panel. Note the longer time scale of the image series in the top group versus the bottom group. The red boxes outline the frame corresponding approximately to the mean half-time of FRAP for that EGFP-tagged protein. Bar, 2 μm.

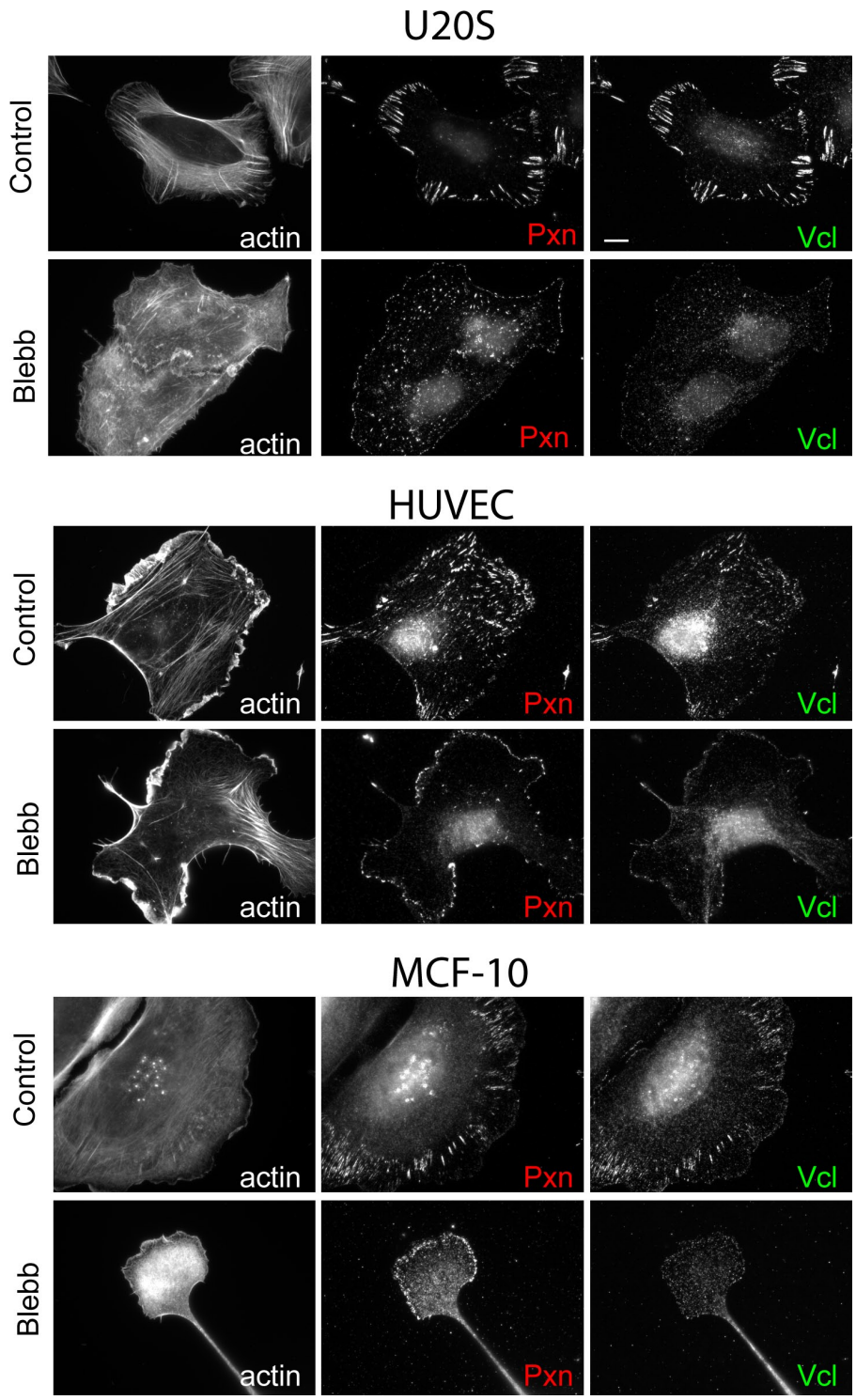


Figure S3. **Myosin II–mediated vinculin recruitment to adhesions is cell type independent.** Effects of myosin II inhibition on the localization of vinculin in adhesions of different cell types. Comparison of untreated cells (control) or cells treated for 1 h with 20 µM blebbistatin (Blebb). Human osteosarcoma cells (U2OS; top), human umbilical vein endothelial cells (HUVEC; middle), and human breast epithelial cells (MCF-10; bottom) are shown. Immunolocalization of vinculin (Vcl; right) and paxillin (Pxn; middle) and fluorescent phalloidin staining of actin filaments (left) are shown. Bar, 10 µm.

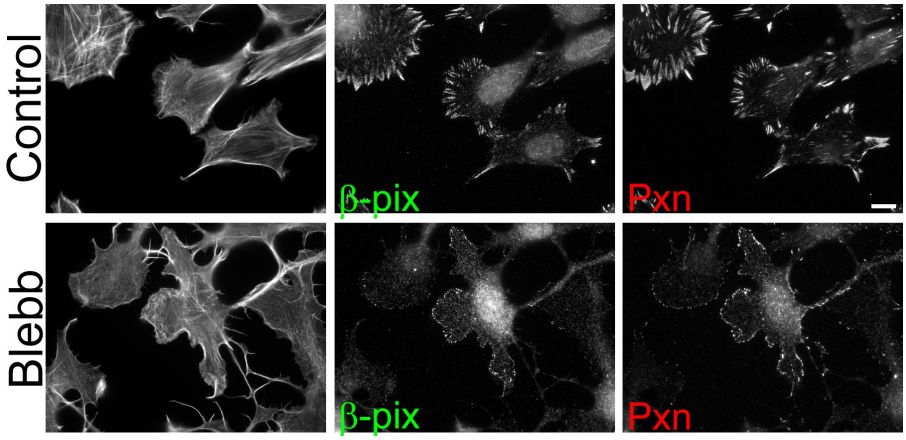


Figure S4. **β-Pix localization to adhesions is myosin II independent.** Effects of myosin II inhibition on the localization of β-Pix in adhesions. Comparison of untreated MEF cells (control) or MEF cells treated for 1 h with 20 µM blebbistatin (Blebb). Immunolocalization of paxillin (Pxn; right), β-Pix (middle), and fluorescent phalloidin staining of actin filaments (left). Bar, 10 µm.

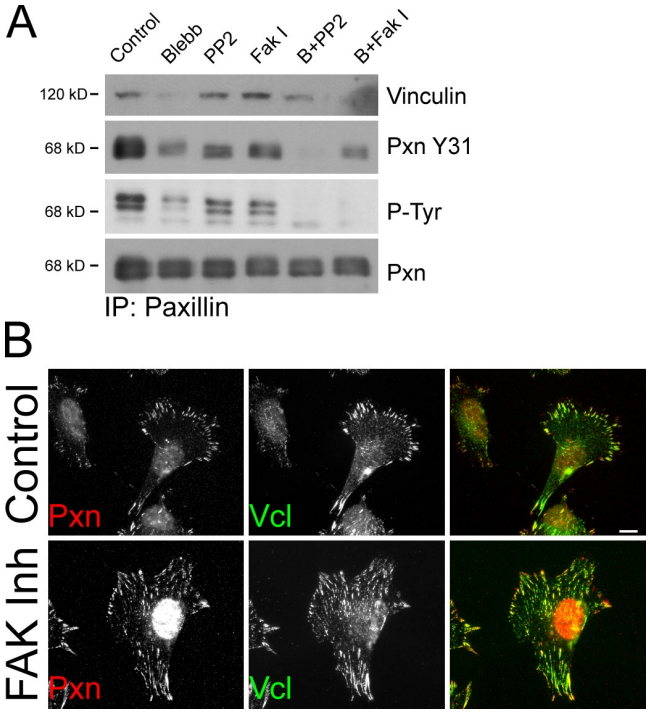
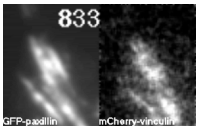


Figure S5. **Pharmacological inhibition of FAK does not inhibit vinculin association with paxillin or localization to adhesions.** (A) Western blot analysis of paxillin IPs from lysates of untreated cells (control) and cells treated with 20 µM blebbistatin (Blebb), 10 µM PP2, 100 µM FAKi (PF271), 20 µM blebbistatin and 10 µM PP2 (B + PP2), and 20 µM blebbistatin and 100 µM FAKi (B + FakI) using antibodies specific to pY31 paxillin (Pxn Y31), paxillin (Pxn), or PY (P-Tyr). (B) Comparison of MEF cells treated for 1 h with 100 µM PF271 (FAK Inh) versus control cells (control) and immunolabeled with antibodies to paxillin (red) and vinculin (Vcl; green). Bar, 10 µm.



Video 1. **Time-lapse TIRF images of EGFP-paxillin and mCherry-vinculin in an adhesion forming and growing at the leading edge of a migrating MEF cell.** Time in seconds corresponds to the same time scale as seen on the x axis of Fig. 4 F. Note that the absolute intensity of mCherry is much lower than EGFP, thus, the signal is closer to noise, and after image scaling, this results in a very noisy background.