Bays et al., http://www.jcb.org/cgi/content/full/jcb.201309092/DC1

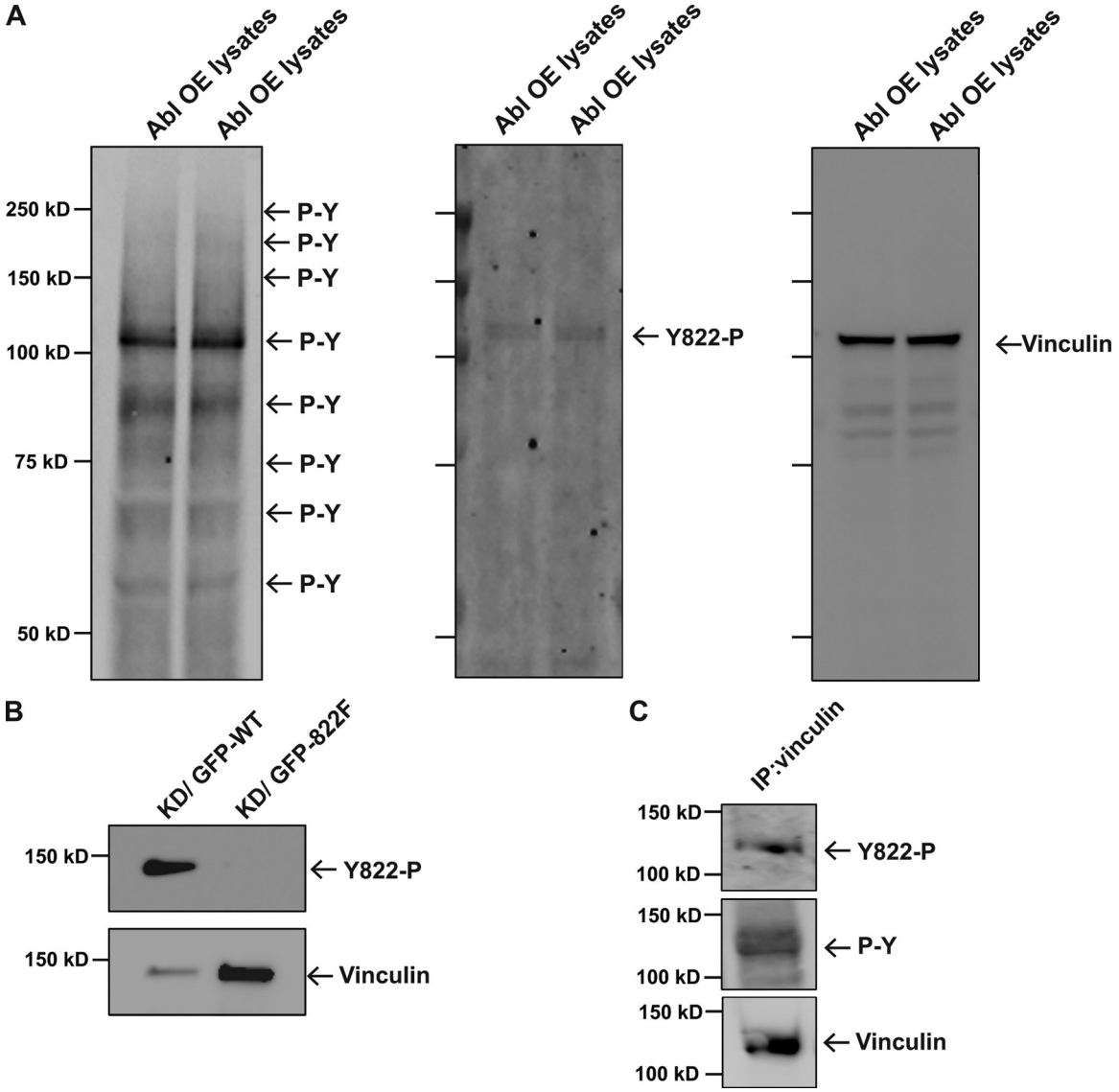

Figure S1. **Specificity of the phospho-Y822 antibody.** (A) Total cell lysates from BALB/c fibroblasts transformed by Abelson mouse leukemia virus were immunoblotted with a phosphotyrosine antibody and then stripped and reprobed with the phosphospecific Y822 antibody. The blots were reprobed a third time with hVIN1, an antibody that recognizes vinculin. Note that the phospho-Y822 antibody recognizes a single band that corresponds to the molecular weight of vinculin; the faintness of the band in the Y822 blot as compared with the others in the manuscript likely results from the stripping and reprobing of the blot. (B) GFP-vinculin or GFP-Y822F vinculin were immunoprecipitated from MCF10a cells in which endogenous vinculin was silenced. The resulting immunoprecipitates were immunoblotted with antibodies that recognize phospho-Y822 vinculin. Subsequently the blots were stripped and reprobed with antibodies against GFP to show the levels of protein present. The phosphospecific antibody does not recognize GFP-Y822F vinculin even when it is present in excess over the wild-type protein. (C) Vinculin was immunoprecipitated from Abl-overexpressing lysates and probed with the phosphotyrosine and phosphospecific Y822 antibody. The blots were stripped and reprobed for vinculin.

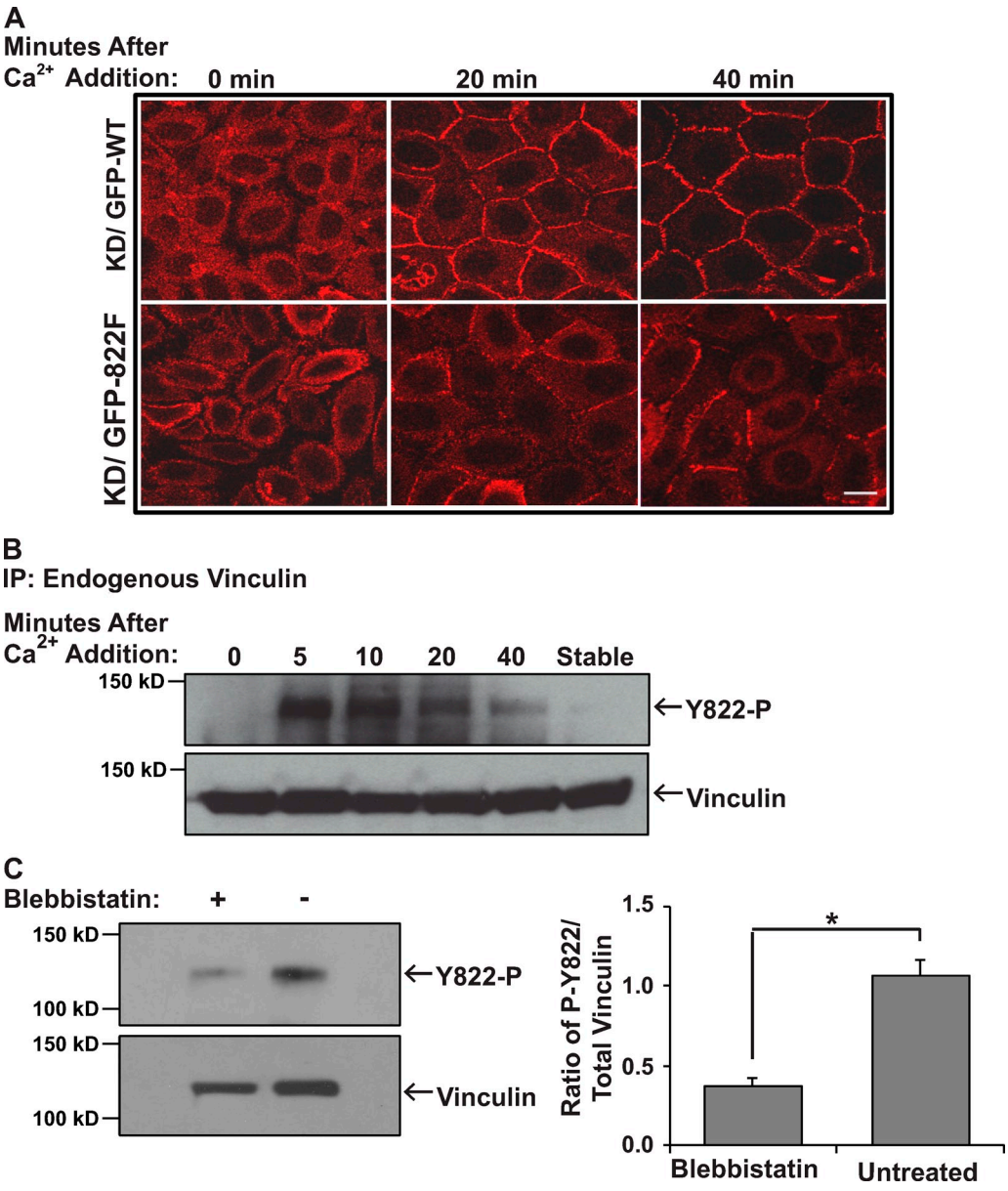

Figure S2. **Examination of vinculin phosphorylation in response to cadherin engagement and in response to application of force on E-cadherin.** (A and B) Cell junctions in confluent cells expressing indicated proteins were disassembled by incubation in calcium-free media (0 min). Calcium was then restored to the cultures for the indicated times. (A) The cells were examined by immunofluorescence staining with E-cadherin to show junction formation. Bar, 20 µm. (B) Vinculin Y822 phosphorylation is highest during junction assembly. Vinculin was immunoprecipitated from lysates of cells before treatment, after calcium was restored for the indicated times, or that were left in calcium-containing media (stable). The immunoprecipitates were immunoblotted with antibodies against pY822, stripped, and reprobed with antibodies against vinculin. (C) Blebbistatin reduces vinculin Y822 phosphorylation. Confluent, parental MCF10a cells were treated with 25 µM blebbistatin for 60 min and the levels of phospho-Y822 or total vinculin were examined. *, $P < 0.01$.

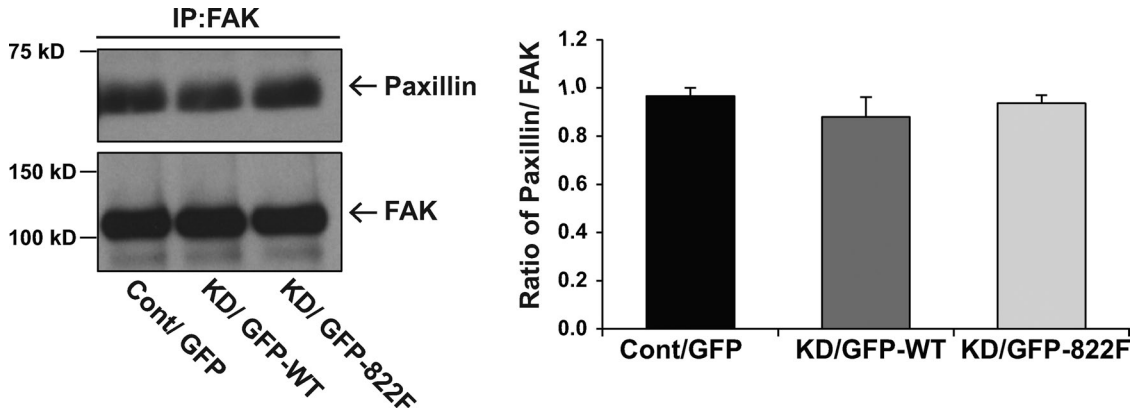

Figure S3. **Paxillin recruitment to FAK is unaltered in the Y822F-expressing cell lines.** Previous work indicated that there is increased recruitment of paxillin to FAK in cells expressing Y822F[9]. Here, FAK was immunoprecipitated from the indicated cell lines and the levels of bound paxillin were examined by immunoblotting. The blot was stripped and reprobed with antibodies against FAK to reveal the amounts of proteins recovered. The graph indicates the amount of paxillin-bound FAK in the control (Cont/GFP), WT-vinculin (KD/GFP-WT), and Y822F (KD/Y822F) rescue cell lines and shows that binding is similar among the cell lines.

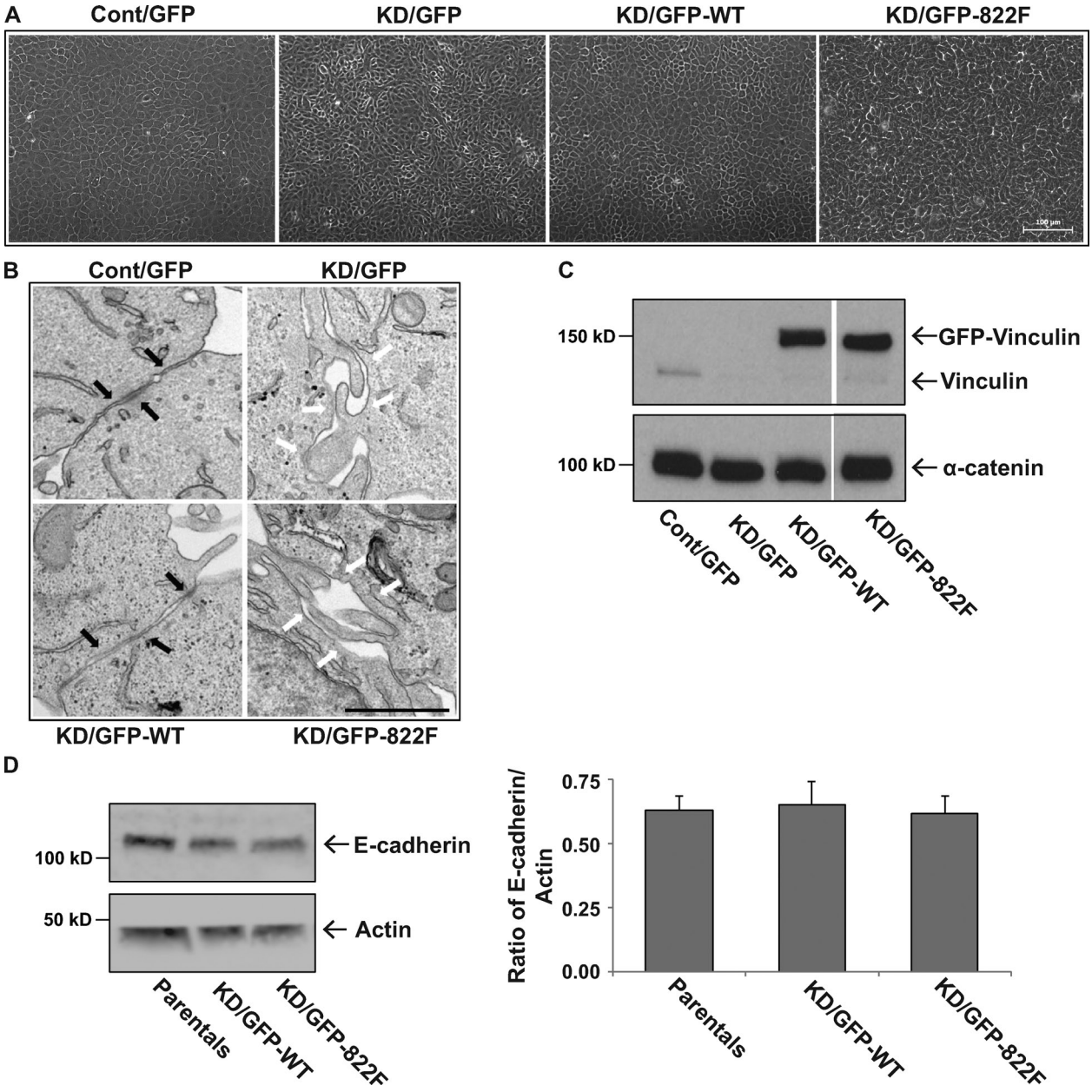

Figure S4. **Y822F does not rescue the epithelial cell–cell junction defects induced by loss of vinculin.** (A) Phase-contrast images of the indicated cell lines. Bar, 100 µm. Note that Y822F does not rescue loss of epithelial morphology induced by vinculin loss. (B) Visualization of the cell–cell junctions in the indicated cell lines using transmission electron microscopy. Black arrows indicate adherens junctions; White arrows indicate regions where adherens junctions are disrupted. Bar, 1 µm. (C) Expression levels of the various proteins in the MCF10a cells. Western blot analysis using antibodies against vinculin (VIN) or α-catenin as loading control show that the effects are not due to differences in expression levels. (D) MCF10a cells line lysates were immunoblotted for E-cadherin and actin as a load control. Quantification from two independent experiments show E-cadherin expression is not altered in GFP-WT or GFP-822F cell lines.

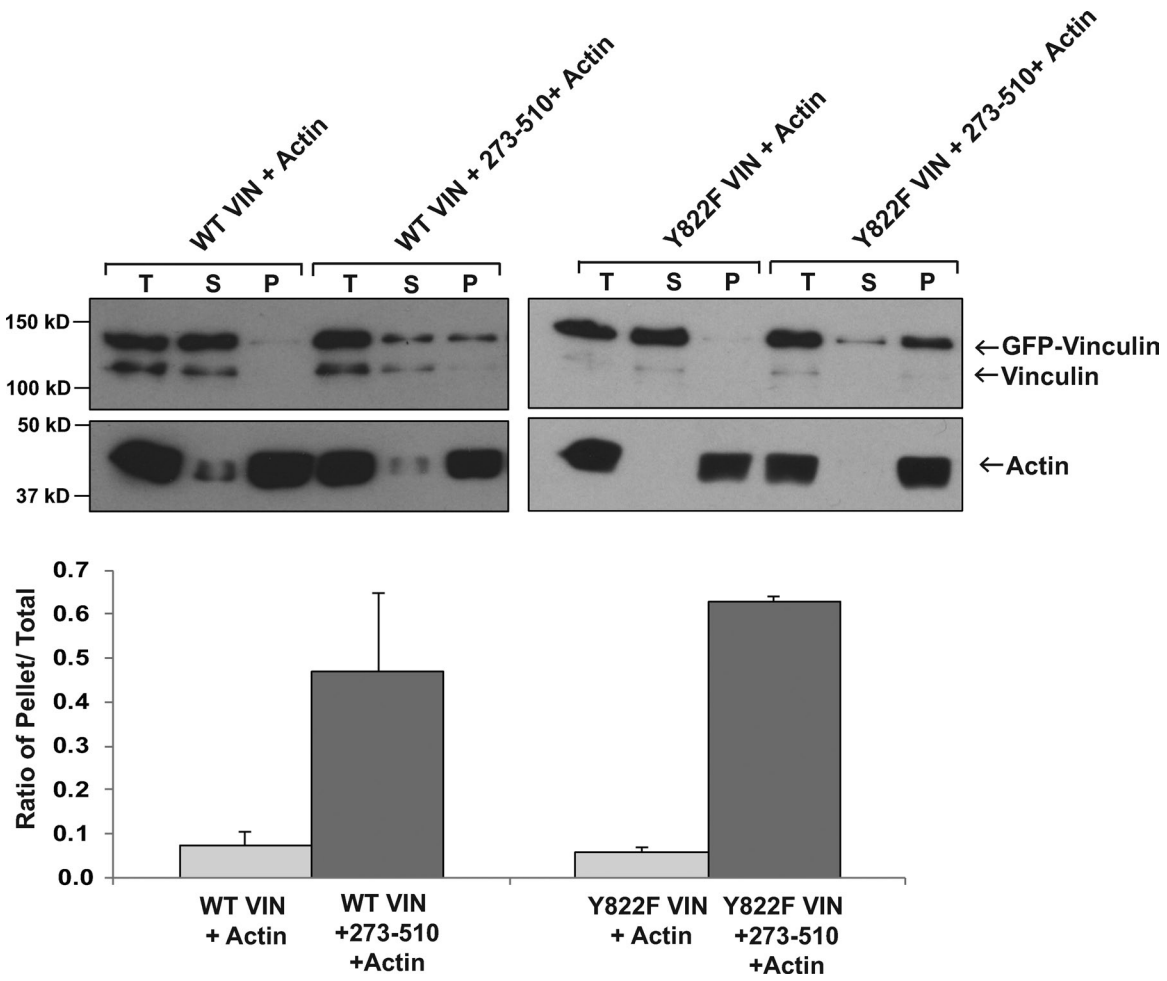

Figure S5. **WT and Y822F vinculin co-sediment with actin equally well.** HEK293 cells expressing GFP-tagged full-length vinculin (GFP-WT) or Y822F vinculin (GFP-822F) were lysed, and the lysates were clarified of endogenous actin filaments by high-speed centrifugation and then incubated with 5 μm actin filaments (WT + Actin), 5 μm actin filaments and with 10 μm α-catenin 273–510 (WT + 273–510 + Actin). The actin filaments were pelleted by centrifugation. Equivalent amounts of sample before centrifugation (T), or the supernatant (S) or the pellet (P) fractions obtained after centrifugation, were recognized with antibodies against vinculin and actin. Average amounts of bound vinculin to total vinculin from two representative experiments are shown.