Bledzka et al., http://www.jcb.org/cgi/content/full/jcb.201501006/DC1

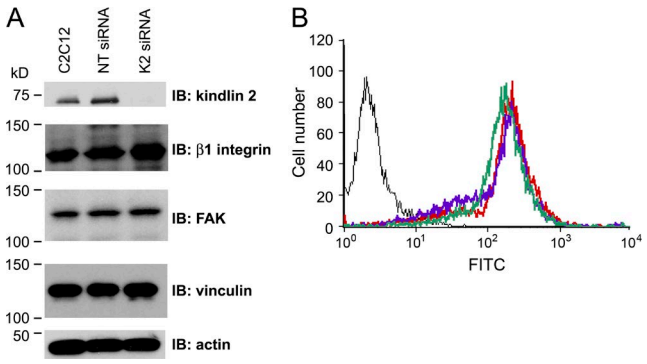

Figure S1. **Reduction of K2 expression does not affect expression levels of β_1 integrin and FA proteins.** (A) Western blot of C2C12 cells 24 h after treatment with K2 siRNA, NT siRNA, or no treatment. Immunoblots (IB) of C2C12 cell lysates were performed with anti-K2, anti–β1 integrin (clone 18/CD29 from BD), anti-FAK, anti-vinculin, or anti-actin. (B) Flow cytometry of β1 integrin (clone HA2/5 from BD) surface expression in C2C12 cells 24 h after treatment with K2 siRNA (red), NT siRNA (magenta), or no treatment (green).

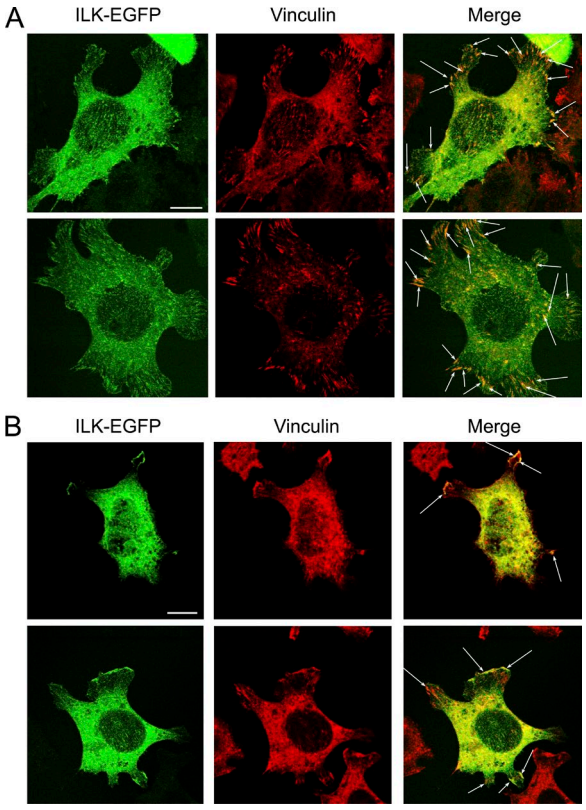

Figure S2. **Visualization of EGFP-tagged ILK localization in C2C12 cells after K2 knock-down.** C2C12 cells were transfected with nontargeting siRNA (A) or K2 siRNA (B) together with ILK. Cells were spread on fibronectin for 2 h, fixed, and stained with anti-vinculin followed by Alexa Fluor 568 secondary antibodies. Transfected cells were visualized with EGFP fluorescence. Colocalization of ILK (green) and vinculin (red) in focal adhesions is indicated in the merged panels with arrows. Bar, 10 µm.

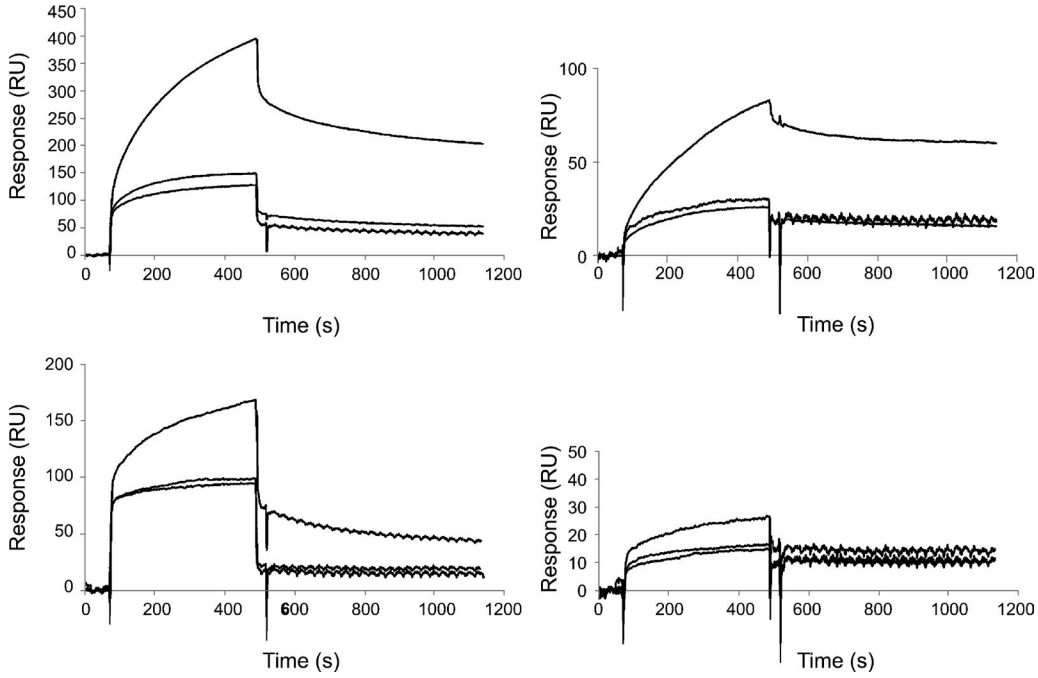

Figure S3. **Representative SPR binding profile.** 3 and 0.09 µM K2 (A) or 3 and 0.09 µM K2-LK/AA (B) were flowed over actin or BSA immobilized at a coating density of 1,000 response units (RU). The third line represents the CM5 surface activated and deactivated with no immobilized ligand (blank surface, negative control).

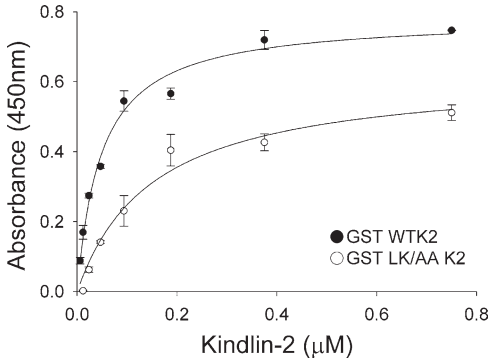

Figure S4. **Binding of K2 to actin by microtiter plate assay.** Rabbit skeletal muscle actin–coated plates were incubated with GST or GST-K2 (0–0.750 µM). Plates were washed, and bound GST proteins were detected using anti-GST mAb, peroxidase-conjugated secondary antibody, and 1-Step Ultra TMB-ELI SA as the substrate. The reaction was stopped by adding 1N HCl, and absorbance at 450 nm was measured. The value at each K2 concentration was plotted after subtraction of the nonspecific absorption (determined as the difference between the signal of GST-K2 and GST).

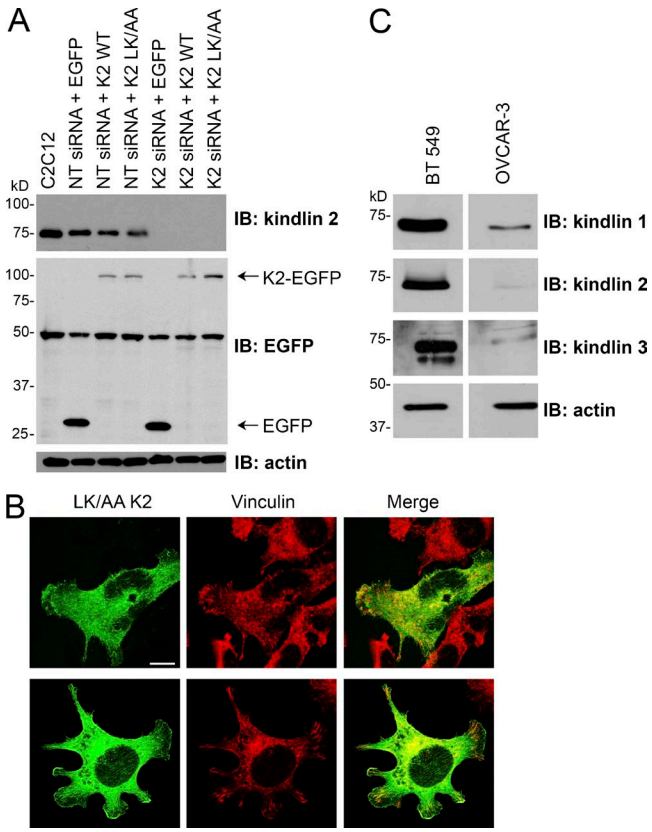

Figure S5. **Rescue of K2 expression in C2C12 cells.** (A) Western blot of C2C12 cells 24 h after treatment with K2 siRNA, NT siRNA, or no treatment and EGFP-tagged K2 constructs. Immunoblots (IB) of the cell lysates were performed with anti-K2, anti-EGFP, or anti-actin. (B) Visualization of EGFP-tagged LK/AA K2 mutant localization in C2C12 cells. C2C12 cells were spread on fibronectin for 2 h, fixed, and stained with anti-vinculin followed by Alexa Fluor 568 secondary antibodies. Transfected cells were visualized with EGFP fluorescence. Colocalization of K2 (green) and vinculin (red) in focal adhesions is indicated in the merged panels with arrows. Bar, 10 µm. (C) Kindlin proteins expression in OVCAR-3 cells. Western blot of BT-549 (a breast cancer cell line) and OVCAR-3 cells. Immunoblots of the cell lysates were performed with anti–kindlin-1, anti-K2, anti–kindlin-3, or anti-actin.