Kumar et al., http://www.jcb.org/cgi/content/full/jcb.201510012/DC1

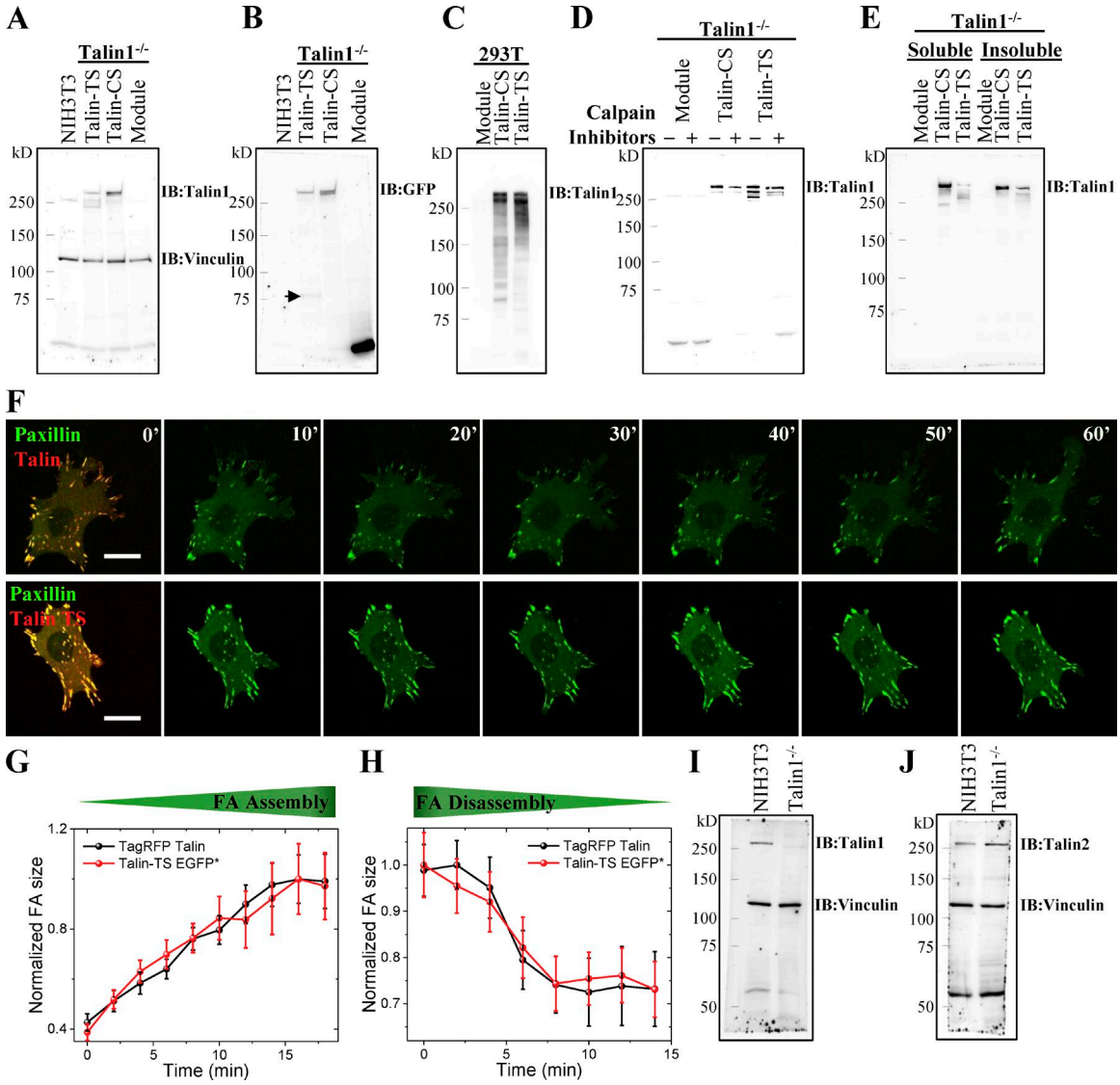

Figure S1. **Additional characterization of talin sensors in talin1$^{-/-}$ cells.** (A and B) Western blot for talin-TS and talin-CS in talin1$^{-/-}$ cells, probed for talin1 and vinculin (A) and stripped and reblotted with anti-GFP (B). The arrow indicates an ∼85-kD band. (C) Western blot of whole cell lysate of talin-CS– and talin-TS–transfected 293T cells, probed using talin1 antibody. (D) Western blot for talin-CS and talin-TS in talin1$^{-/-}$ cells without and with calpain inhibitors, probed with anti-talin1. (E) Western blot of soluble and insoluble protein after mild detergent extraction, probed with talin1 antibody. (F) Representative time-lapse images of talin1$^{-/-}$ cells cotransfected with paxillin EGFP and tagRFP talin (top) or tagRFP talin-TS (with nonfluorescent EGFP; bottom). (G and H) Plot of normalized FA size with time in assembling (n = 29 and 40; G) and disassembling (n = 37 and 47; H) FAs. Cells were freshly plated on fibronectin-coated dishes for ∼30 min before imaging. Low laser power was used, and only paxillin was imaged for time lapse to avoid phototoxicity. Cells were imaged for at least 60 min. Error bars represent SEM. Asterisks indicate mutations that destroy the fluorescence. (I and J) Western blot for talin1$^{-/-}$ cell lysate (control lane, NIH3T3 cell lysate) probed with talin1 (I) and talin2 antibodies (J). Bars, 20 µm. IB, immunoblot.

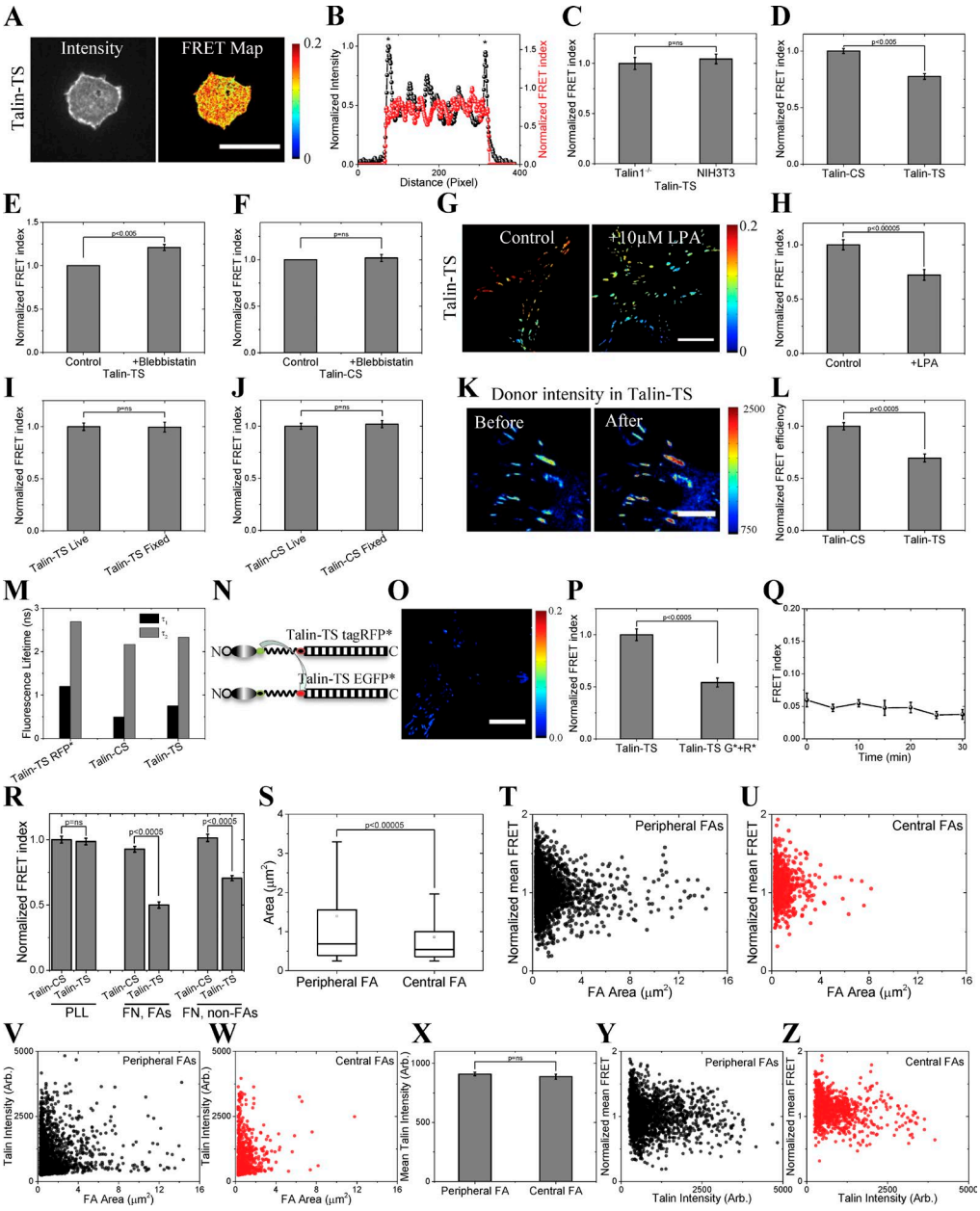

Figure S2. **Additional controls for talin-TS and differential tension on talin in peripheral and central FAs.** (A) Representative intensity and FRET map image of a talin-TS–transfected talin1$^{-/-}$ cell plated on poly-L-lysine; sections through the middle of the cell well above the substrate were analyzed. (B) Typical intensity line profile and FRET index profile across the cell. Asterisks denote plasma membrane–localized talin-TS. (C–F) Normalized FRET index of talin-TS in talin1$^{-/-}$ (n = 25) and NIH 3T3 cells (n = 25; C), talin-CS (n = 15) and talin-TS (n = 21) in NIH 3T3 cells (D), talin-TS before and after 5 µM blebbistatin treatment for 15 min (n = 6; E), and talin-CS before and after 5 µM blebbistatin treatment for 15 min (n = 14; F). (G) Representative FRET map of talin-TS in control and LPA-treated (10 µM for 30 min) cells serum starved in 0.2% FBS for 20 h. (H) Normalized FRET index for control (n = 25) and LPA-treated cells (n = 27). (I) Comparison of FRET index for talin-TS within FAs in live (n = 35) and fixed (n = 35) cells. (J) Comparison of FRET index for talin-CS within FAs in live (n = 29) and fixed (n = 28) in talin1$^{-/-}$ cells. (K) Pseudocolor map of donor intensity within FAs for talin-TS before and after acceptor photobleaching. (L) Normalized FRET efficiency for talin-CS (n = 30) and talin-TS (n = 44) within FAs measured by acceptor photobleaching. (M) Two-component fluorescence lifetime (τ_1 and τ_2) analysis of time domain data in talin-TS with nonfluorescent mutated tagRFP, talin-CS, and talin-TS. To fit the data, a biexponential model was used for all datasets, and the weighted mean lifetime was used for comparison. The global fitting method was used, in which the two lifetimes are kept constant across the members of a particular dataset, and the fractional contribution from each is allowed to vary between members of the same dataset, resulting in a spatially dependent weighted mean lifetime distribution. Hence, there are no error bars. (B and M) n > 60. (N) Schematic showing FRET between two talin-TS molecules containing mutated nonfluorescent EGFP and tagRFP. (O) Intermolecular FRET map of talin-TS within FAs. (P) Normalized intermolecular FRET index for talin-TS (n = 15 each). Asterisks indicate nonfluorescent mutants. (Q) Effect of 5 µM blebbistatin on intermolecular FRET within FAs (n = 5). (R) Normalized FRET index of cells expressing talin-CS and talin-TS, plated on poly-L-lysine (n = 20 and 15 for talin-CS and talin-TS, respectively). Alternatively, cells were plated on fibronectin, and the FRET index was measured within FAs (n = 29 and 35 for talin-CS and talin-TS, respectively) or in non-FA regions of the basal surface (n = 30 and 35 for talin-CS and talin-TS, respectively). (S) Plot of peripheral and central FA area. (T and U) Correlation between FA area and FRET index in peripheral (T) and central (U) FAs. (V and W) Correlation between FA size and talin intensity in FAs for peripheral (V) and central (W) FAs. (X) Mean talin intensity in peripheral and central FAs. (Y and Z) Correlation between normalized mean FRET index and talin intensity in peripheral (Y) and central (Z) FAs. (S–Z) n = 32. Bars: (A, G, and O) 20 µm; (k) 10 µm. Arb., arbitrary units. PLL, poly-L-lysine. FN, fibronectin. Error bars represent SEM, except for in S, where bottom and top error bars represent 5th and 95th percentiles, respectively.

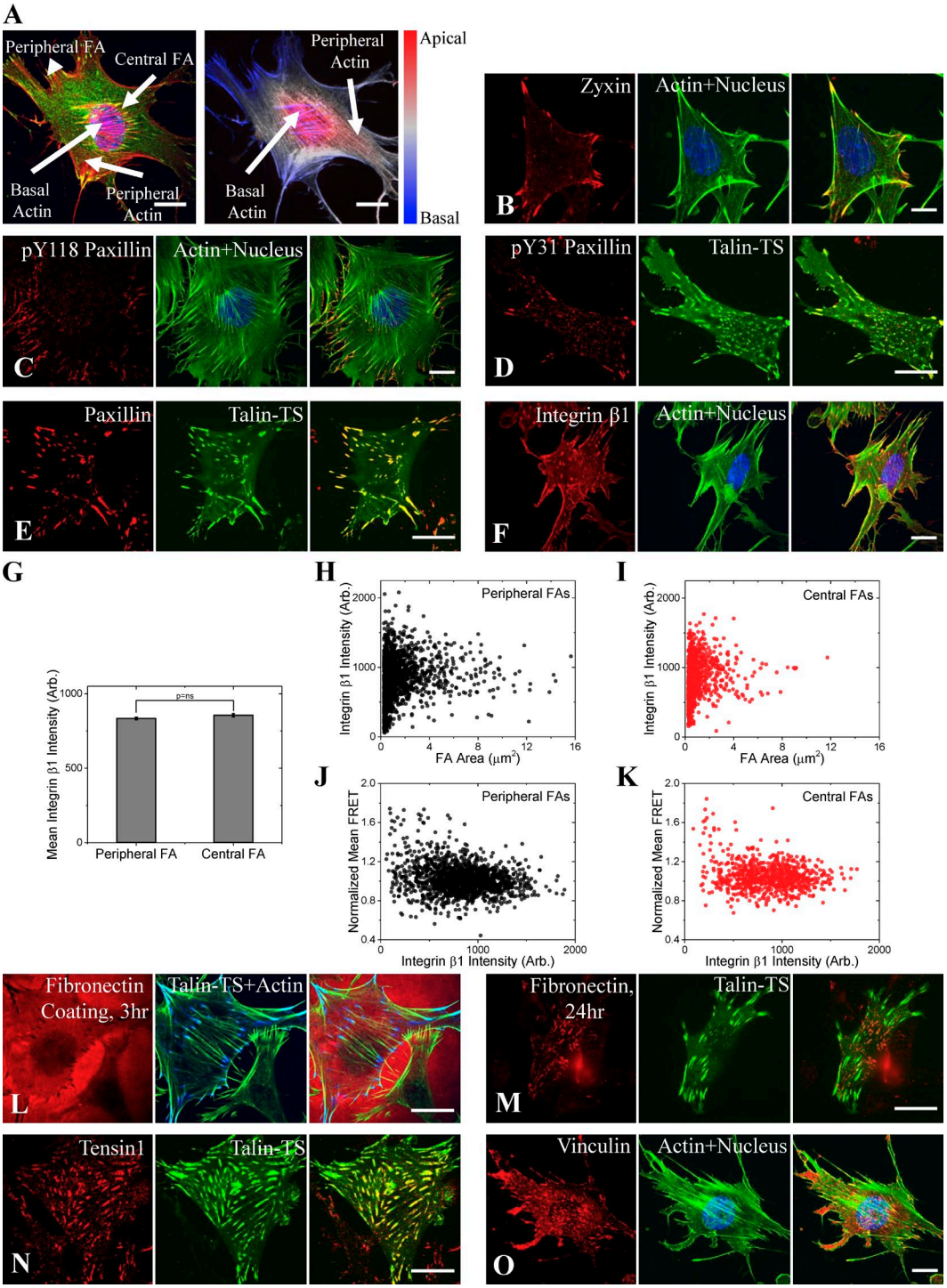

Figure S3. **Compositional difference in central and peripheral FAs.** (A) Central and peripheral actin and FAs, in which the z axis is color coded (right). (B–F) Immunofluorescence staining of zyxin (B), phospho-paxillin Y118 (C), phospho-paxillin Y31 (D), paxillin (E), and integrin β1 (F). (G) Mean intensity of integrin β1 in peripheral and central FA. *n* = 25 cells each. (H and I) Correlation between integrin β1 expression and FA area in peripheral (H) and central (I) FAs. *n* = 25 cells each. (J and K) Correlation between talin FRET index and integrin β1 expression in peripheral (J) and central (K) FAs. *n* = 25 cells each. (L–O) Immunofluorescence staining of fibronectin at 3 h after plating cells on fibronectin-coated dishes (L), fibronectin after 24 h of plating on an uncoated dish (M), tensin1 (N), and vinculin (O). Bars, 20 μm. Arb., arbitrary units.

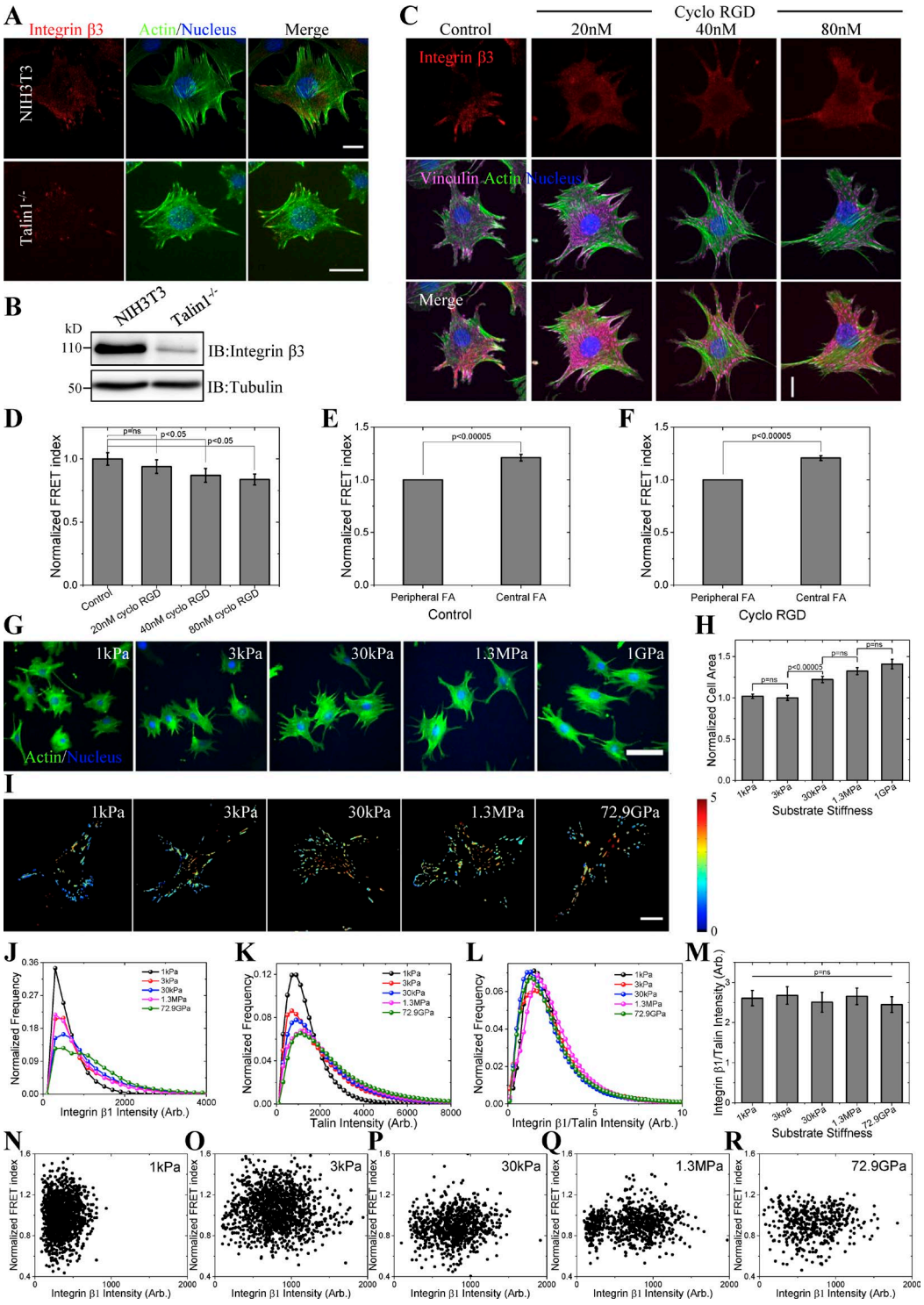

Figure S4. **Integrin β3 in central and peripheral FAs and amount of integrin β1 in FAs on substrates of different stiffness.** (A) Representative image of NIH 3T3 and talin1$^{-/-}$ cells immunostained for integrin β3 (red). Actin and nuclei are labeled in green and blue using phalloidin and Hoechst 33342, respectively. (B) Western blot for integrin β3. IB, immunoblot. (C) Representative image of integrin β3 immunostaining in NIH3T3 cells with cyclic RGD peptide (0 [control], 20, 40, and 80 nM) cyclo (Arg-Gly-Asp-D-Phe-Val). Vinculin, purple; actin, green; nucleus, blue. (D) Normalized FRET index of talin-TS in talin1$^{-/-}$ cells after cyclo RGD treatment (n = 25 in each case). (E and F) Normalized FRET index of talin-TS in peripheral and central FAs for talin1$^{-/-}$ cells in the control (n = 10; E) and treated with 80 nM cyclo RGD (n = 25; F). (G) Representative images of NIH 3T3 fibroblasts plated for 3 h on substrates of different stiffness that were coated with 10 µg/ml fibronectin (overnight) and then fixed and stained for actin and nucleus using Alexa Fluor 488–conjugated phalloidin and Hoechst 33342, respectively. (H) Plot of normalized cell area on these substrates (n = 126, 104, 93, 80, and 78 cells for 1 kPa, 3 kPa, 30 kPa, 1.3 MPa, and ∼1 GPa [plastic], respectively). (I) Representative ratio image of integrin β1/talin-TS intensity on substrates of different stiffness. (J–L) Pixel-wise intensity histogram for integrin β1 (J), talin-TS (K), and integrin β1/talin-TS intensity (L). (M) Mean integrin β1/talin-TS intensity (n = 22, 22, 25, 19, and 25 cells for 1 kPa, 3 kPa, 30 kPa, 1.3 MPa, and 72.9 GPa [glass], respectively). (N–R) Correlation between mean integrin β1 intensity and normalized talin FRET index on PDMS substrates of different stiffness. n = 9, 15, 9, 8, and 7 cells for 1 kPa (N), 3 kPa (O), 30 kPa (P), 1.3 MPa (Q), and glass (72.9 GPa; R), respectively. Bars: (A, C, and I) 20 µm; (G) 100 µm. Arb., arbitrary units.

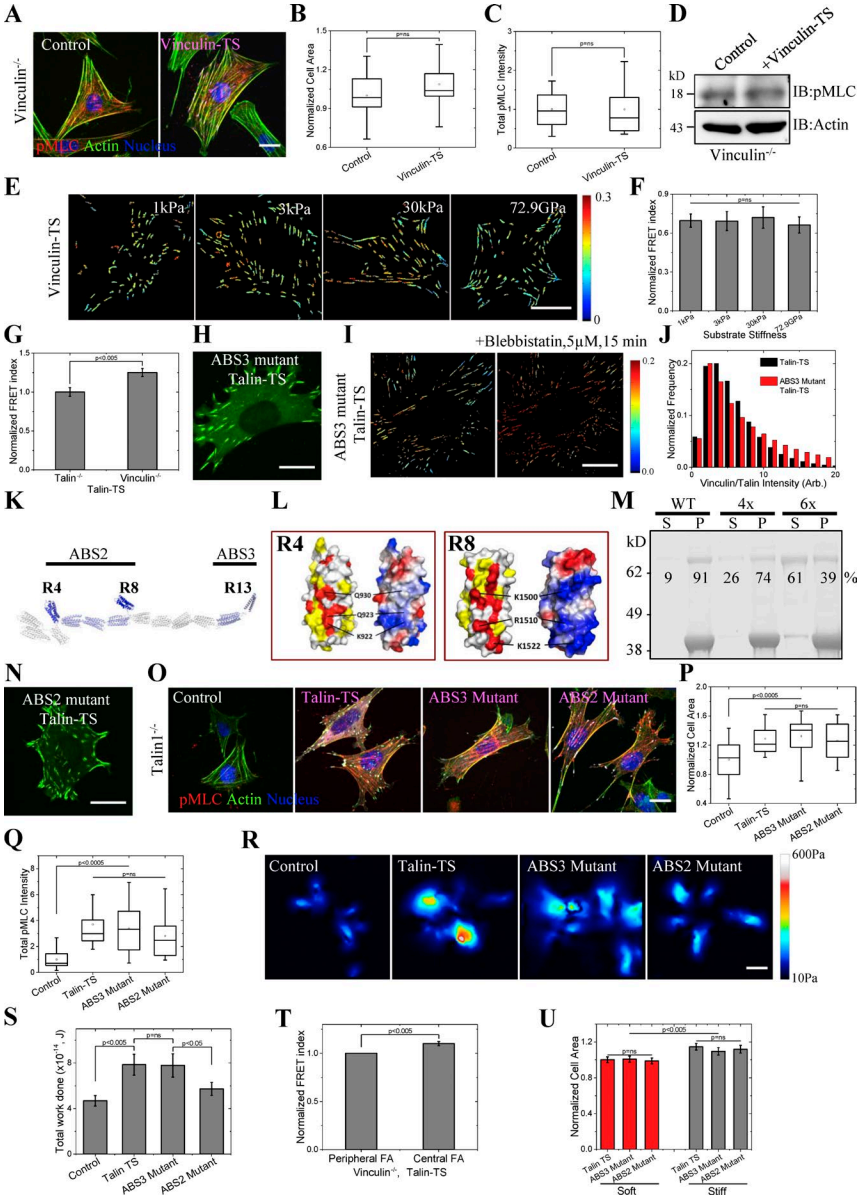

Figure S5. **Effect of stiffness on tension in vinculin and characterization of ABS2 mutant talin.** (A) Representative images of vinculin$^{-/-}$ cells, control and stably transfected with vinculin-TS (shown in purple). Cells were fixed and immunostained for phospho–myosin regulatory light chain (MLC; serine19; red). Actin (green) and nucleus (blue) were labeled using Alexa Fluor 568–phalloidin and Hoechst 33342, respectively. (B and C) Plot of normalized cell area (n = 24 and 25; B) and total phospho-MLC calculated from z-stack images captured with 0.5-μm z separation (n = 20 and 22; C) for control and vinculin-TS cells. (D) Western blot for phospho-MLC in the two cell lines. IB, immunoblot. (E) Representative FRET map of vinculin-TS stably transfected in vinculin$^{-/-}$ cells and plated on substrates of different stiffness. (F) Plot of mean FRET index for vinculin-TS, normalized by vinculin-TL for the corresponding substrate stiffness (n = 20, 20, 20, and 9 for 1 kPa, 3 kPa, 30 kPa, and 72.9 GPa [glass], respectively). (G) Normalized FRET index for talin-TS in talin1$^{-/-}$ (n = 25) and vinculin$^{-/-}$ (n = 25) cells. (H) Representative image of ABS3 mutant talin-TS in talin1$^{-/-}$ cells. (I) Representative FRET index map of ABS3 mutant talin-TS in talin1$^{-/-}$ cells before and after 5 μM blebbistatin treatment for 15 min. (J) Pixel-wise histogram for vinculin/talin ratio for talin-TS (n = 25) and ABS3 mutant talin-TS (n = 25) within FAs. Arb., arbitrary units. (K) Schematic diagram showing the location of the two ABSs (ABS2, residues 913–1653; and ABS3, residues 2300–2541) in the talin rod. The two R13 domains of the ABS3 dimer bind along one face of a single actin filament. The topologies of the R4 and R8 domains in ABS2 are similar to ABS3, and ABS2 may also wrap around single actin filaments. (L) Conservation (red is invariant and yellow is conservative substitutions) and surface charge characteristics of talin rod domains R4 and R8. The residues mutated in the 4× and 6× mutants are labeled. (M) Actin cosedimentation of talin ABS2. Talin fragments were incubated with F-actin, the actin was pelleted, and supernatants (S) and pellets (P) were analyzed by SDS-PAGE. WT, wild type. (N) Representative image of ABS2 mutant talin-TS transfected in talin1$^{-/-}$ cells. (O) Representative image of talin1$^{-/-}$ cells: control (untransfected), transfected with talin-TS, and ABS3 and ABS2 mutant talin-TS (shown in purple). Cells were fixed and immunostained for phospho-MLC (serine19; red). Actin (green) and nucleus (blue) were labeled using Alexa Fluor 568–phalloidin and Hoechst 33342, respectively. (P and Q) Plot of normalized cell area (n = 36, 20, 21, and 19; P) and total phospho-MLC calculated from z-stack images captured with 0.5-μm z separation (n = 36, 22, 23, and 25; Q) for untransfected control and talin-TS– and ABS3 and ABS2 mutant talin-TS–transfected talin1$^{-/-}$ cells. (R and S) Representative images of traction force field map (R) and plot of total work done (n = 15, 11, 14, and 11; S) for untransfected control and talin-TS– and ABS3 and ABS2 mutant talin-TS–transfected talin1$^{-/-}$ cells. (T) Normalized FRET index for talin-TS within central and peripheral FAs of vinculin$^{-/-}$ cells (n = 28). (U) Plot of normalized cell area on soft ~3-kPa (red bar; n = 50, 52, and 38) and stiff ~30-kPa (gray bar; n = 53, 40, and 33) substrate for talin-TS– and ABS3 and ABS2 mutant talin-TS–transfected talin1$^{-/-}$ cells. Bars, 20 μm. (B, C, P, and Q) Bottom and top error bars represent 5th and 95th percentiles, respectively. Small squares inside the box plots are the means. Box limits denote 25th and 75th percentiles. (F, G, and S–U) Error bars represent SEM.