

Supplemental material

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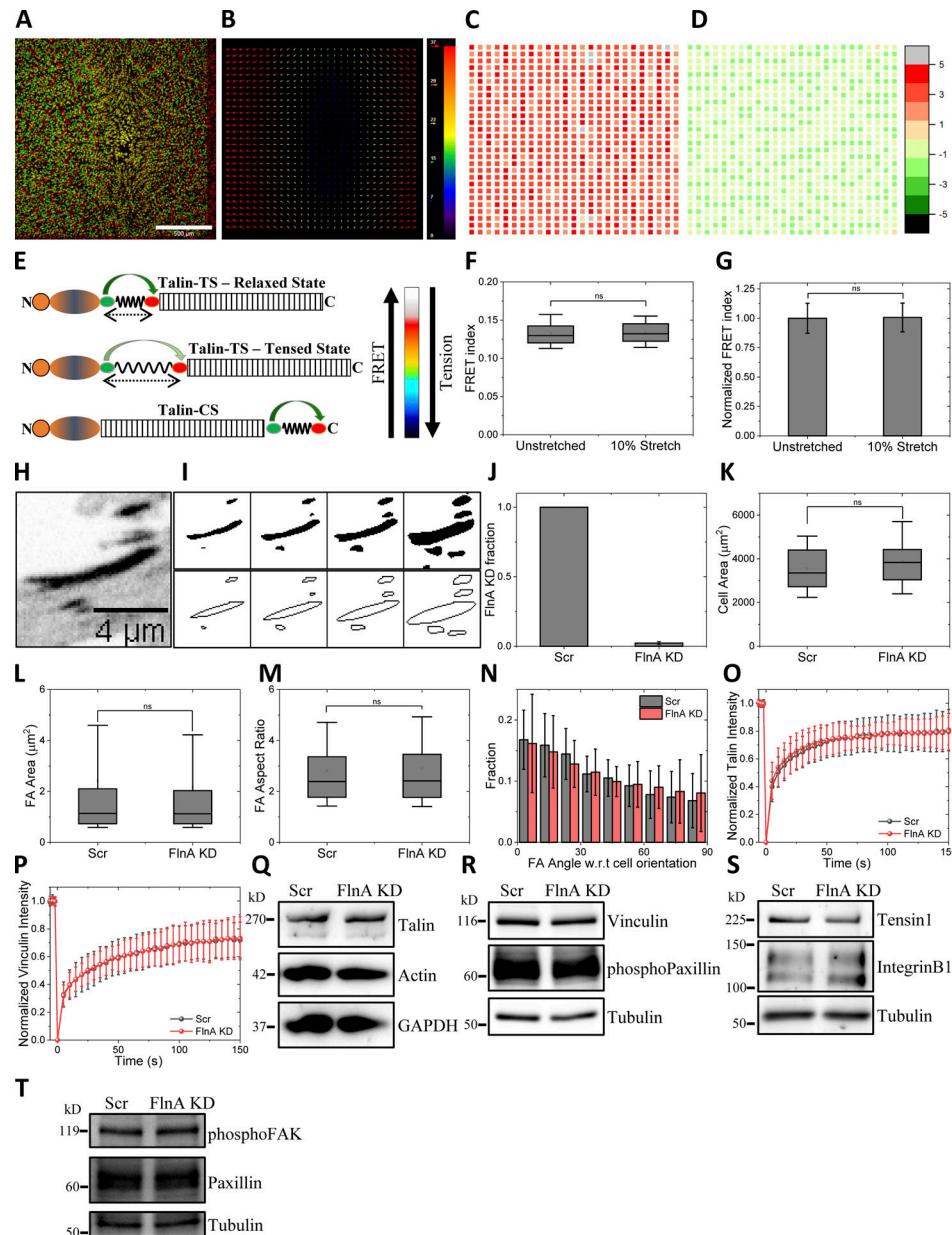


Figure S1. Stretcher calibration and controls. **(A–D)** Calibration of the stretch device. **(A)** Representative image of 2- μ m fluorescent beads before (red) and after (green) 10% uniaxial stretch along the x axis. Images from before and after stretch were aligned in the center using a 250 pixel \times 250 pixel region. Scale bar, 500 μ m. **(B)** Relative bead displacements from the image in A obtained using PIV analysis. Scale bar shows color-coded magnitude and corresponding arrow length in pixel units. 1 pixel = 1.785 μ m. Absolute cosine/parallel (C) and sine/perpendicular (D) component of vector magnitude with respect to stretch direction (x axis). Scale bar shows color-coded magnitude in pixel units. On an average, a 10% stretch results in compression by $3.3 \pm 0.2\%$ in the perpendicular direction in the central region of the PDMS membrane that was used for imaging cells. **(E)** Schematic of the relaxed (top) and tensed (middle) state of talin-TS and the talin-CS (bottom), a zero-tension control. Color bar shows FRET index/tension scale. High FRET index corresponds to low tension and vice versa. **(F and G)** FRET index box plot (F) and normalized FRET index bar graph (G) of control talin-CS in unstretched and 10% stretch conditions. $n = 15$ cells for both unstretched and 10% stretch. Error bars are SDs. **(H)** Representative inverted intensity (tagRFP) image of talin-TS transfected cells showing FA. Scale bar, 4 μ m. **(I)** Threshold image/mask (top) at decreasing cut-off (left to right) for the image shown in H and corresponding fit to an ellipse (bottom) to determine the error in orientation of individual FAs. **(J)** Quantification of FlnA KD fraction from WBs. $n = 4$. Error bars are SD. Box plot of cell area (K), FA area (L), and FA aspect ratio (M) for scr and FlnA KD cells. $n = 86$ and 78 cells for scramble and FlnA shRNA-treated cells, respectively, for M and O. Box and whiskers show 25–75th and 10–90th percentiles, respectively. Small rectangles and horizontal lines in the box plots indicate mean and median values, respectively. **(N)** Histogram of FA angle relative to cell orientation for scr and FlnA KD NIH3T3 cells ($n = 22$ cells each). Differences are not significant, obtained using paired Student's *t* test. **(O and P)** Talin (O) and vinculin (P) fluorescence intensity recovery after photobleaching a circular region of interest of diameter 1 μ m within FAs in NIH3T3 cells transiently transfected with EGFP-tagged talin and vinculin, respectively. Talin FRAP curves: for scr control, $n = 60$ from seven cells; for FlnA KD, $n = 40$ from six cells. Vinculin FRAP curves: for scr control, $n = 31$ from five cells; for FlnA KD, $n = 34$ from five cells. Error bars represent SD. **(Q–T)** Whole-cell lysate WB for talin, actin, and GAPDH (Q); vinculin, phosphorylated (Y31) paxillin, and tubulin (R); tensin1, integrin β 1, and tubulin (S); and phosphorylated (Y397) FAK, paxillin, and tubulin (T) in scr and FlnA KD NIH3T3 cells. Molecular weights (kD) are shown to the left of each band. ns, not significant.

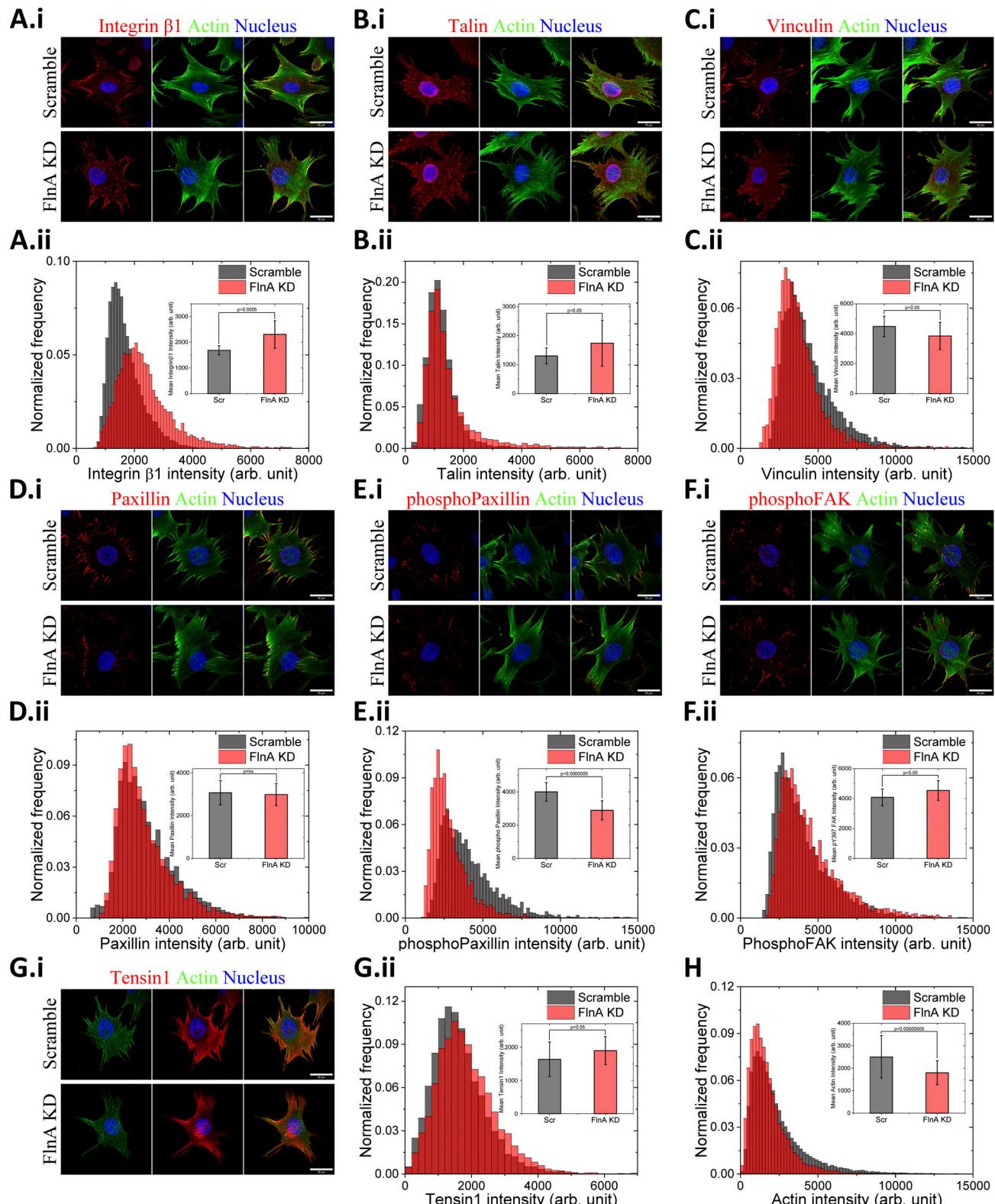


Figure S2. Talin tension images and histograms. Representative image showing localization (A–G, i) and corresponding quantifications displayed as histograms of mean intensity (A–G, ii; and H) of the indicated proteins (red) in FAs from scr and FlnA KD NIH3T3 cells. F-Actin shown in green and nuclei in blue. Insets in A–G, ii, and H show means \pm SD (per cell) for corresponding histograms. For integrin $\beta 1$, $n = 20$ cells each with 7,227 (scr) and 6,261 (FlnA KD) FAs; for talin, $n = 20$ cells each with 5,264 (scr) and 3,326 (FlnA KD) FAs; for vinculin, $n = 25$ cells each with 3,984 (scr) and 3,678 (FlnA KD) FAs; for paxillin, $n = 25$ cells each with 4,320 (scr) and 4,985 (FlnA KD) FAs; for phospho(Y31)paxillin, $n = 25$ cells each with 3,400 (scr) and 3,075 (FlnA KD) FAs; for phosphoY397FAK, $n = 25$ cells each with 3,847 (scr) and 3,646 (FlnA KD) FAs; for tensin1, $n = 20$ cells each with 7,454 (scr) and 11,260 (FlnA KD) FAs; for actin, $n = 140$ cells each with 28,042 (scr) and 24,971 (FlnA KD) FAs. Scale bar, 30 μ m. arb., arbitrary.

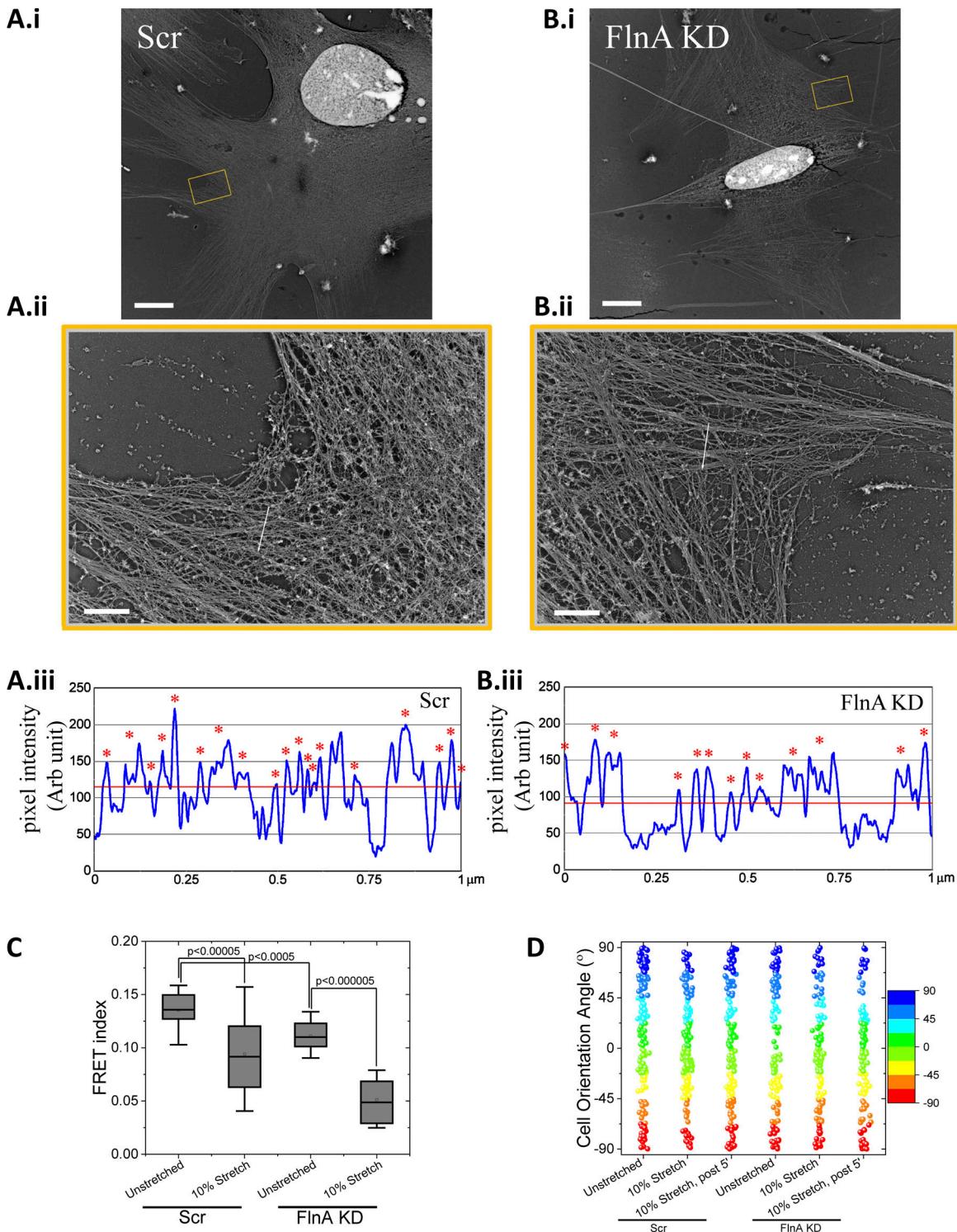


Figure S3. PREM images and analysis. **(A and B)** Another example of PREM image of scr (A, i) and FlnA KD (B, i) cells. Respective zoomed-in images (A, ii; and B, ii) are shown for the region marked in orange in A, i; and B, i. Scale bars, 10 μm (A, i; and B, i) and 1 μm (A, ii; and B, ii) are shown in the respective images at the left bottom corner. Examples of line scans (white lines in A, ii; and B, ii) with the peaks marked as red asterisks for (A, iii) scr and (B, iii) FlnA KD cells. Total length of line scan is 1 μm . Red horizontal line is the average pixel intensity line. **(C)** Box plot of talin-TS FRET index in unstretched and 10% stretch condition in scr and FlnA KD cells. $n = 25$ and 40 (scr) and 20 and 41 (FlnA KD) cells for unstretched and 10% stretch, respectively. Box and whiskers show 25–75th and 10–90th percentiles, respectively. Small rectangles and horizontal lines in the box plots indicate mean and median values, respectively. **(D)** Orientation of scr and FlnA KD cells before, immediately after, and 5 min after 10% stretch. Each dot represents one cell. Color bar represent the orientation between -90° and $+90^\circ$, with 0° being stretch direction. $n = 159$ (0%, 0 min), 172 (10%, 0 min), and 148 (10%, 5 min) for scr, and 118 (0%, 0 min), 116 (10%, 0 min), and 118 (10%, 5 min) for FlnA KD cells. No significant difference between different conditions. Both scr and FlnA KD cells are randomly oriented in unstretched, stretched (10%, immediate, and after 5 min). arb, arbitrary.