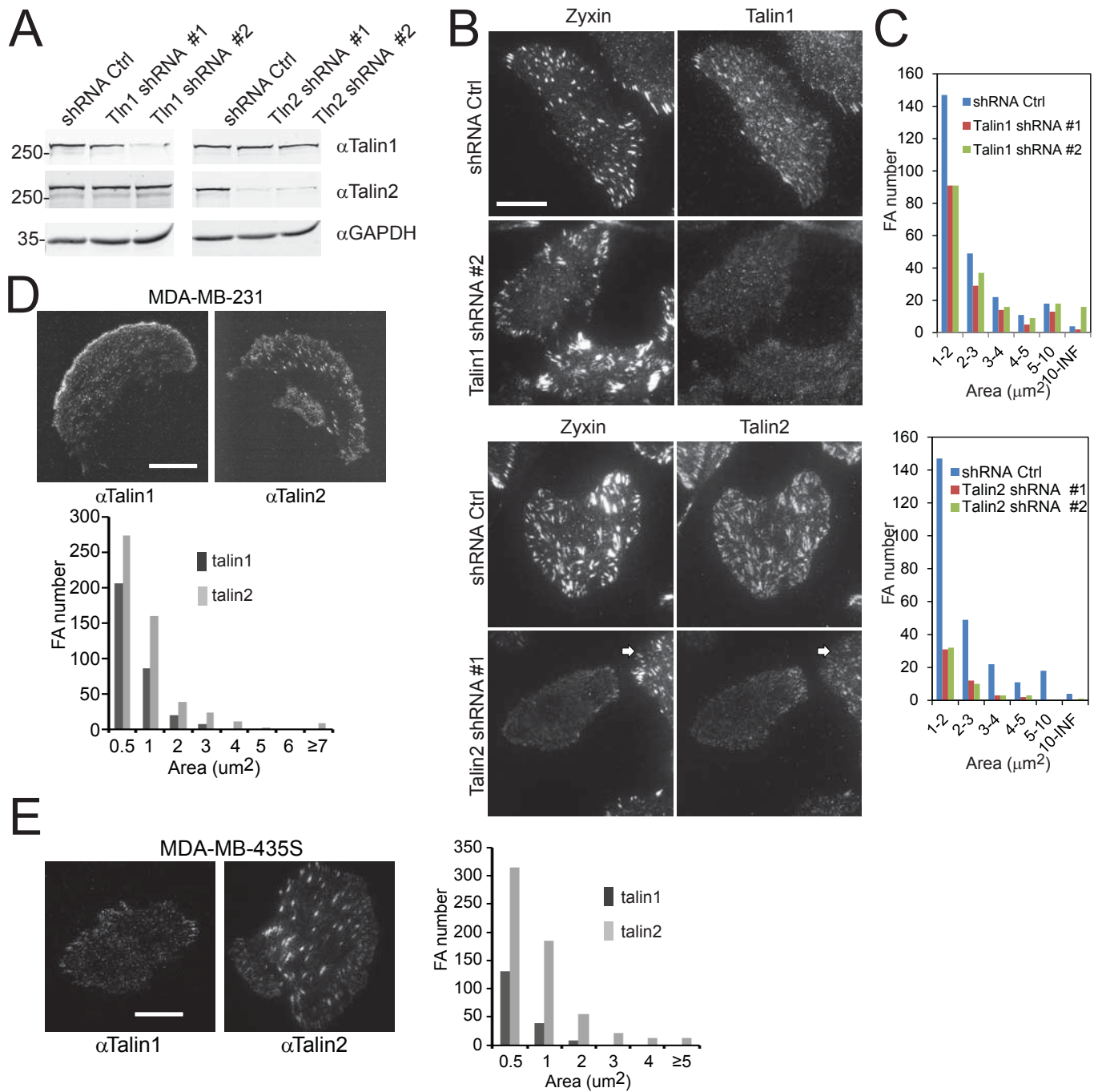


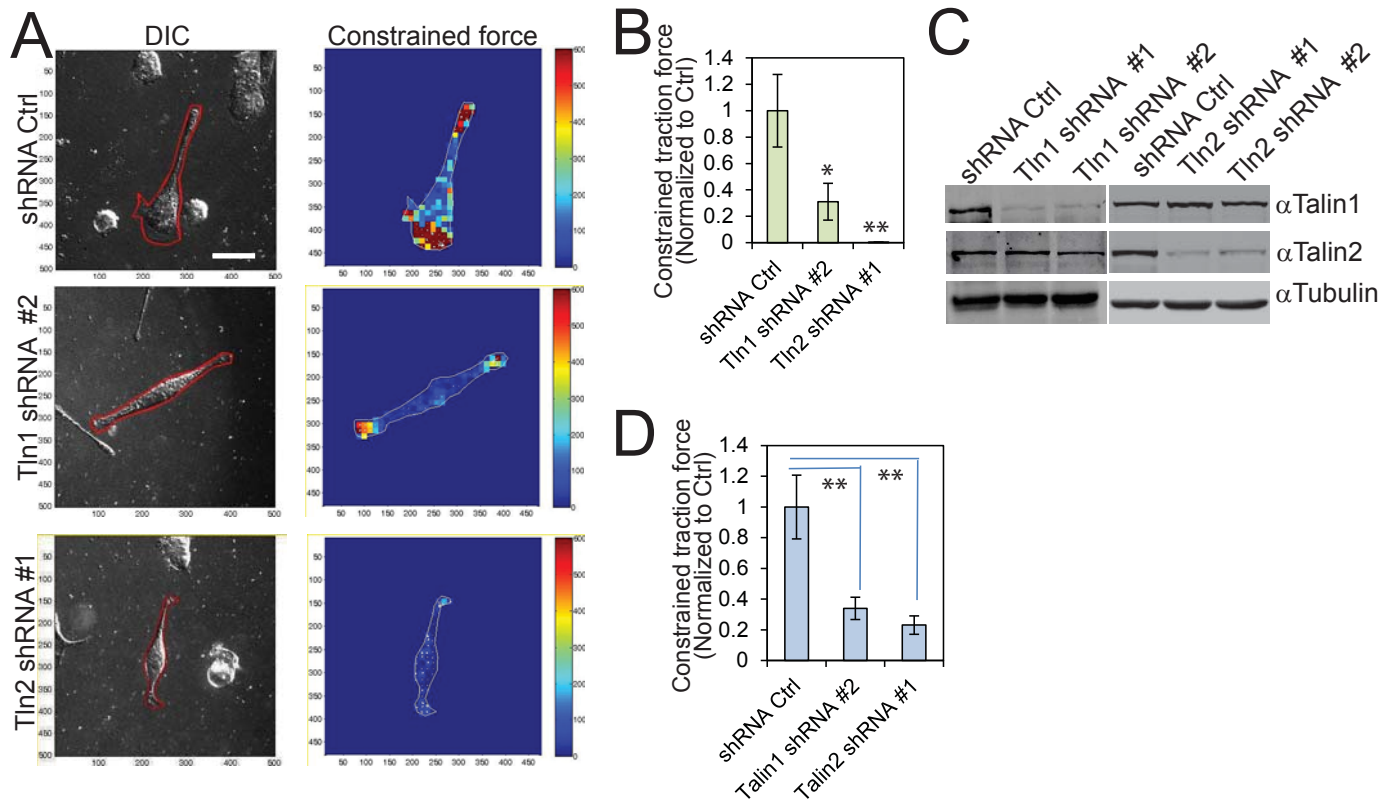
Supplementary Fig. S1. Interaction of talin1, talin2 and mutants with integrin β1A, β1D and β3A tails.

A. Binding of talin1₁₋₄₄₆ and talin2₁₋₄₄₉ to immobilized GST-β1A, -β1D and -β3A. CHO-K1 cells were transiently transfected with EGFP-talin1₁₋₄₄₆ and -talin2₁₋₄₄₉, respectively. Cell lysates were incubated with glutathione-agarose beads preloaded with GST-β1A, -β1D and -β3A, respectively. The binding was determined by Western blotting using an anti-GFP antibody. **B.** Binding of talin1₁₋₄₄₆, talin1₁₋₄₄₆^{C336A}, talin1₁₋₄₄₆^{C336G}, and talin1₁₋₄₄₆^{C336Y} to GST-β1A. **C.** Binding of EGFP-talin1₁₋₄₄₆, talin1₂₀₃₋₄₀₁, talin2₁₋₄₄₉ and talin2₂₀₆₋₄₀₃ to immobilized GST and GST-β1A tails. The interaction was detected by immunoblotting with an anti-GFP antibody.

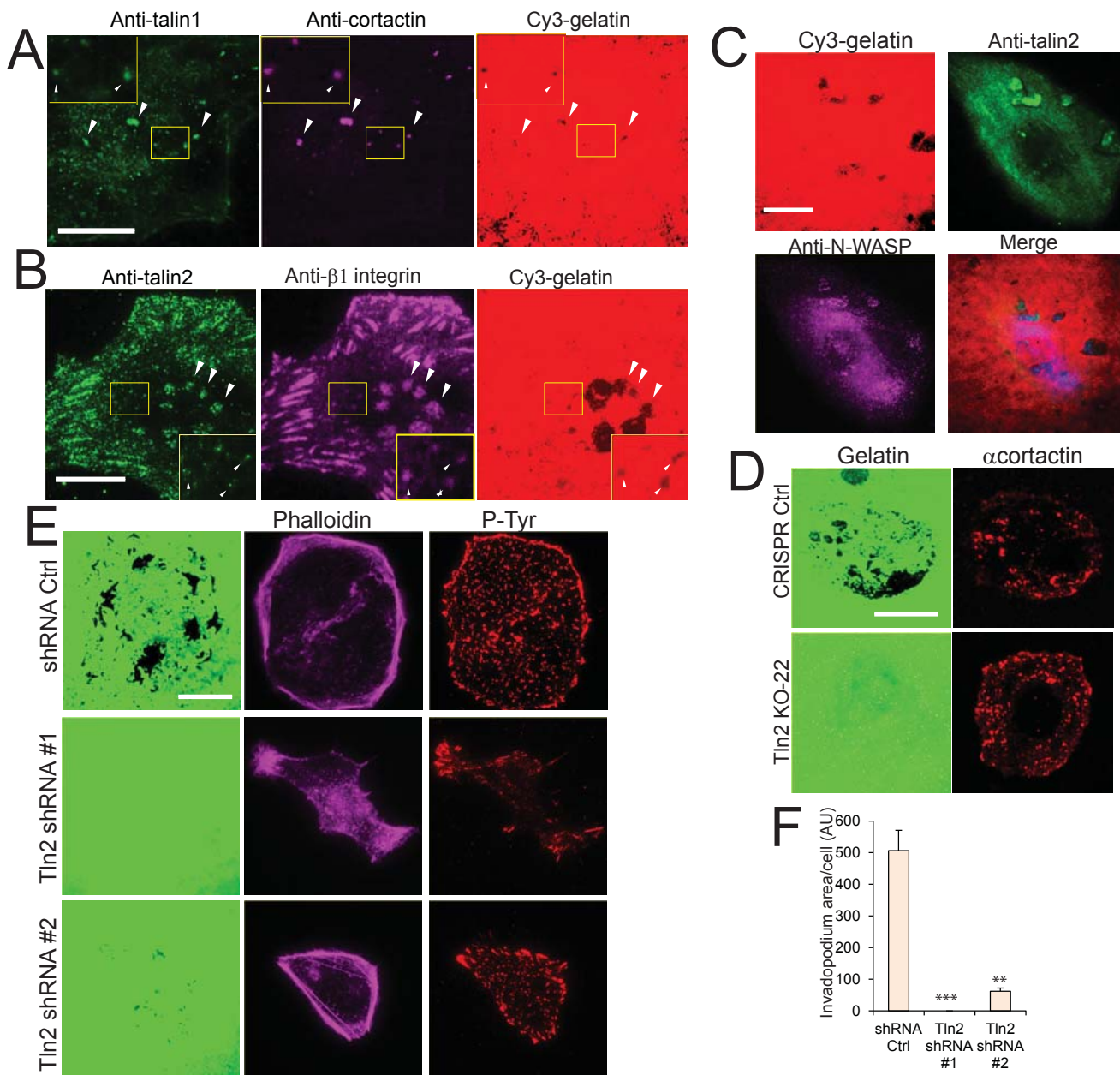


Supplementary Fig. S2. Talin1 is required for small FA formation, whereas talin2 is responsible for larger FA assembly.

A. The levels of endogenous talin1 and talin2 in talin1 or talin2-depleted U2 OS cells. **B.** The distribution of zyxin and talin1 (or talin2) in talin1- or talin2-depleted U2 OS cells. Talin1 or talin2-depleted cells were plated on fibronectin (5 $\mu\text{g}/\text{ml}$) for 4 h, fixed and co-stained for talin1 (or talin2) and zyxin. Zyxin and talin images were acquired by TIRF microscopy. Arrows point to a cell with talin2 partial knockdown. Scale bar, 20 μm . **C.** Area distribution of FAs in talin1- (Left) and talin2- (Right) depleted U2 OS cells was analyzed using Nis-Elements. For each group, FAs from 20 cells were analyzed. Data are averages from 2 independent experiments. **D, E.** Talin1 and talin2 area distribution in MDA-MB-231 and MDA-MB-435S cells. Cells were plated on fibronectin and stained for talin1 or talin2. The size and area distribution of talin1 and talin2 was analyzed using Nis-Elements. Scale bar, 20 μm .



Supplementary Fig. S3. The role of talin1 and talin2 in traction force production. **A.** Depletion of talin2 abolished traction force generation in U2 OS cells. Scale bar, 30 μ m. **B.** Quantitative constrained traction force in U2 OS cells that express shRNA control, talin1 or talin2 shRNA. Data are presented as mean \pm SEM from more than 30 cells. **C.** The levels of endogenous talin1 and talin2 in talin1 or talin2-depleted MDA-MB-231 cells. **D.** Depletion of either talin1 or talin2 inhibited traction force generation in MDA-MB231 cells. Data are presented as mean \pm SEM from more than 30 cells. * p <0.05, ** P <0.01.



Supplementary Fig. S4. The role of talin in invadopodium development. **A.** MDA-MB-231 cells were cultured on immobilized Cy3-gelatin on glass-bottom dishes, fixed, stained for talin1 plus cortactin. Arrow heads point to co-localized spots. Insets show magnified invadopodia in the box. Scale Bars: 20 μ m. **B.** U2 OS cells were cultured on immobilized Cy3-gelatin on glass-bottom dishes, fixed, stained for talin2 plus β 1 integrin. Arrow heads point to co-localized spots. Scale Bars: 20 μ m. **C.** U2 OS cells were cultured on immobilized Cy3-gelatin, stained for talin2 and N-WASP. Scale bar, 20 μ m. **D.** Talin2-null U2 OS cells were cultured on immobilized Alexa488-gelatin, stained for cortactin, using CRISPR vector cells as a control. Scale bar, 20 μ m. **E.** U2 OS cells that express shRNA control, talin2 shRNA #1 or #2 were cultured on Alexa488-gelatin immobilized on glass-bottom dishes, fixed and stained with Alexa 647-phalloidin and anti-phospho-Tyrosine antibody. Scale bar, 20 μ m. Data are presented as mean \pm SEM of 3 independent experiments. **P<0.01, ***P < 0.001.