Supplemental information

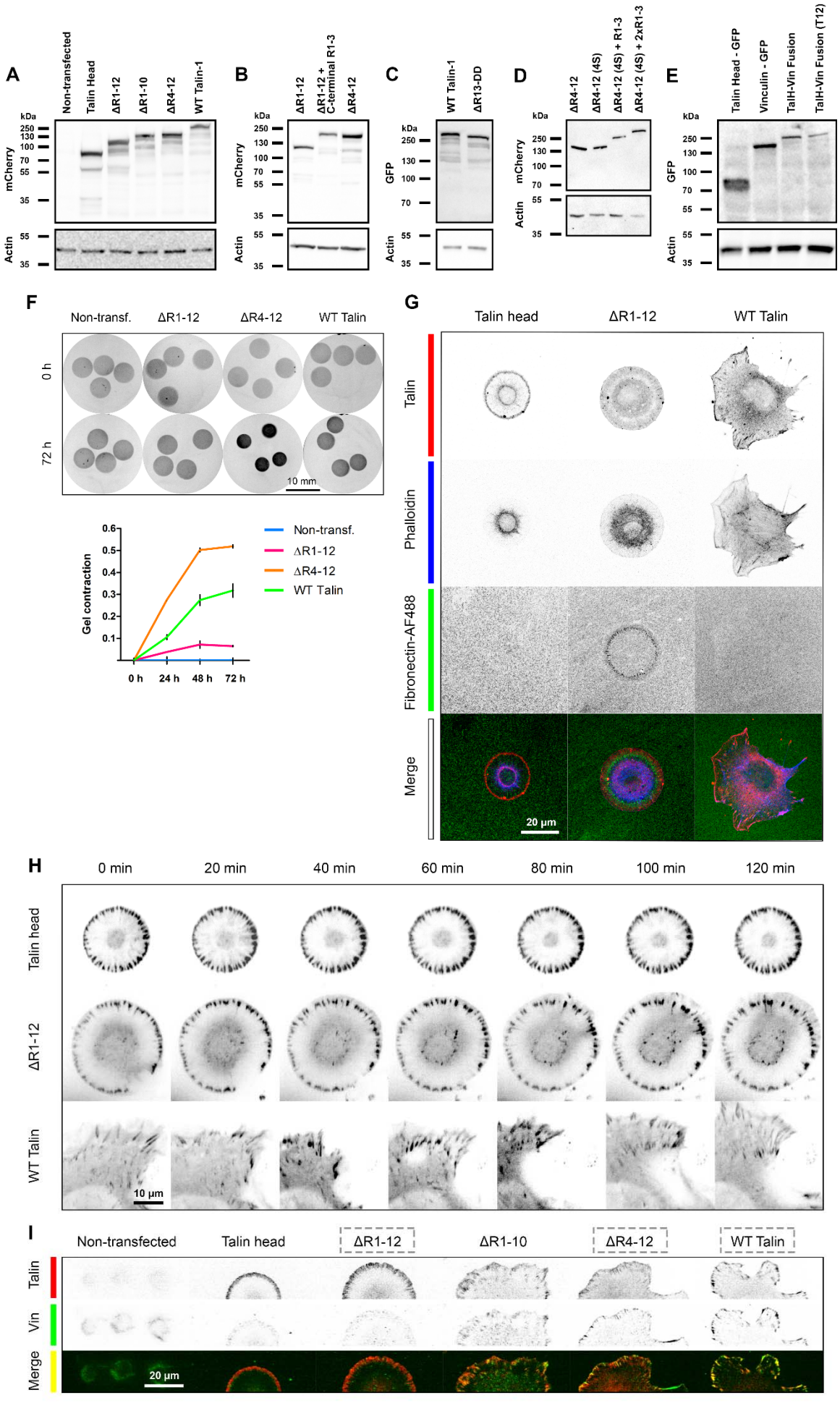


Figure S1, Related to Figure 1. (A - E) Western blot analysis for different talin forms. To confirm the correct expression of modified talin proteins at comparable expression levels, lysates of transiently transfected *Tln1-/-Tln2-/-* cells were analyzed by Western blotting with anti-mCherry, anti-GFP and anti-actin. (F) The macroscopic contraction of collagen-I matrices embedded with cells expressing different talin forms was followed over 72 hours. Note how both wild type talin and the ΔR4-12 protein were able to macroscopically contract the collagen-I matrix, while the ΔR1-12 protein did not allow comparable gel contraction. (G) Fibronectin reorganization assay. *Tln1-/-Tln2-/-* cells expressing the talin head domain, ΔR1-12 or wild type talin were plated on glass coverslips coated with AlexaFluor488 labeled fibronectin and fixed after 120 minutes. In the inversed coloring of the AlexaFluor488-fibronecting image, increased accumulation of fibronectin is indicated by a darker color. Note how the ΔR1-12 protein is able to mediate the relocation of fluorescent fibronectin from the distal end of the adhesion sites to the central end of these adhesions. (H) Time-lapse image series for cells shown in Figure 1I. *Tln1-/-Tln2-/-* cells expressing Talin-GFP fusion proteins were imaged for 120 min using spinning disk confocal microscope. (I) Representative images of vinculin immunostaining. Close-ups of the samples marked with dashed boxes and quantification of vinculin intensity are presented in Figure 1J - M.

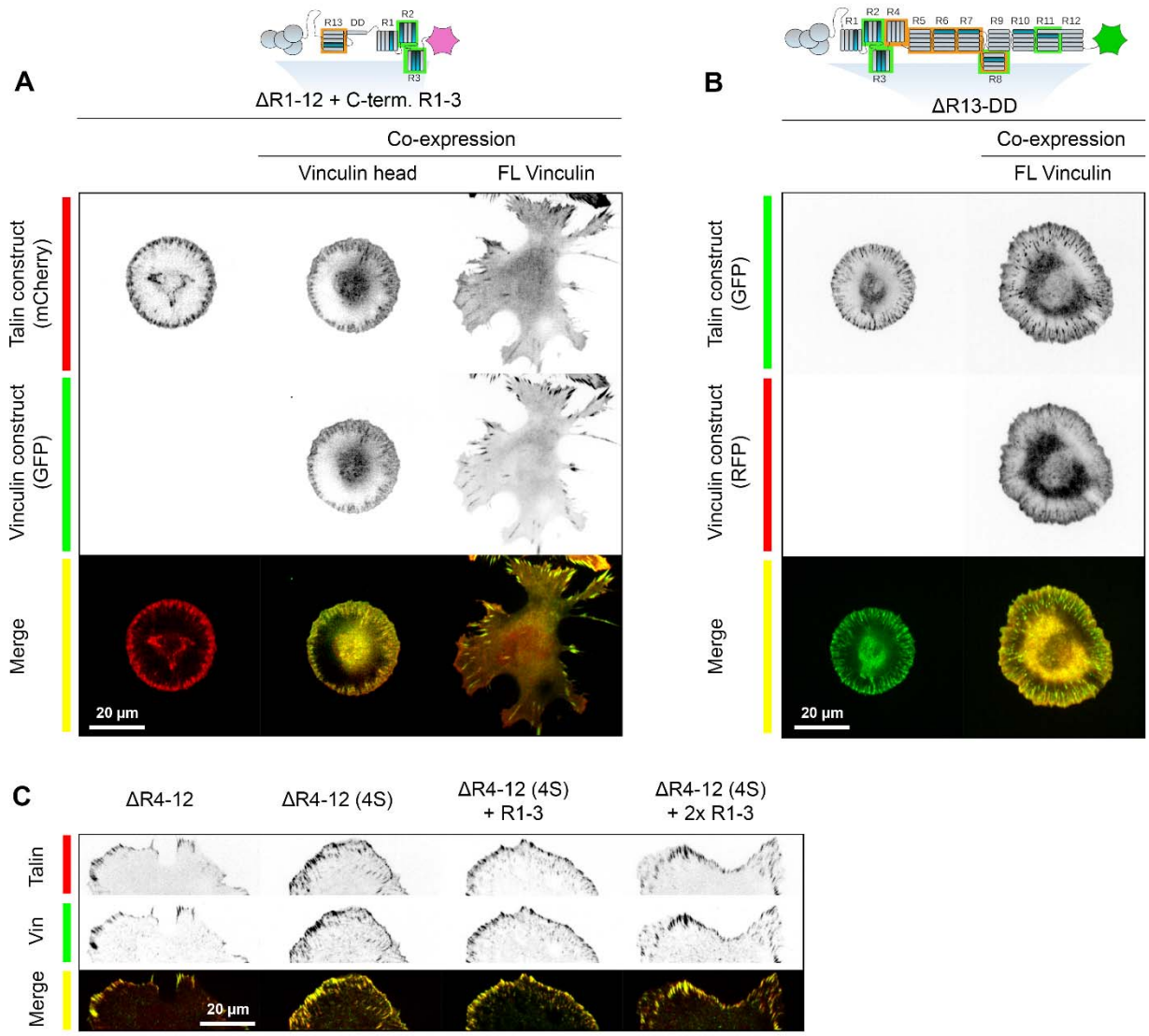


Figure S2, Related to Figure 2. (A) Co-expression of full-length vinculin, but not its head domain, facilitates the polarization of cells expressing the ΔR1-12 + C-term. R1-3 talin form. *Tln1-/-Tln2-/-* cells were co-transfected with the ΔR1-12 + C-term. R1-3 and either full-length vinculin or vinculin head domain (residues 1 - 255) tagged with GFP. The co-expressed vinculin head domain co-localized with the talin protein, but was unable to facilitate cell polarization. Co-expression of either full-length vinculin or its auto-inhibition deficient mutant T12 (not shown) resulted in cell polarization. (B) Co-expression of vinculin tagged with RFP with talin ΔR13-DD protein tagged with GFP. (C) Representative images of vinculin immunostaining of cells expressing destabilized talin proteins.

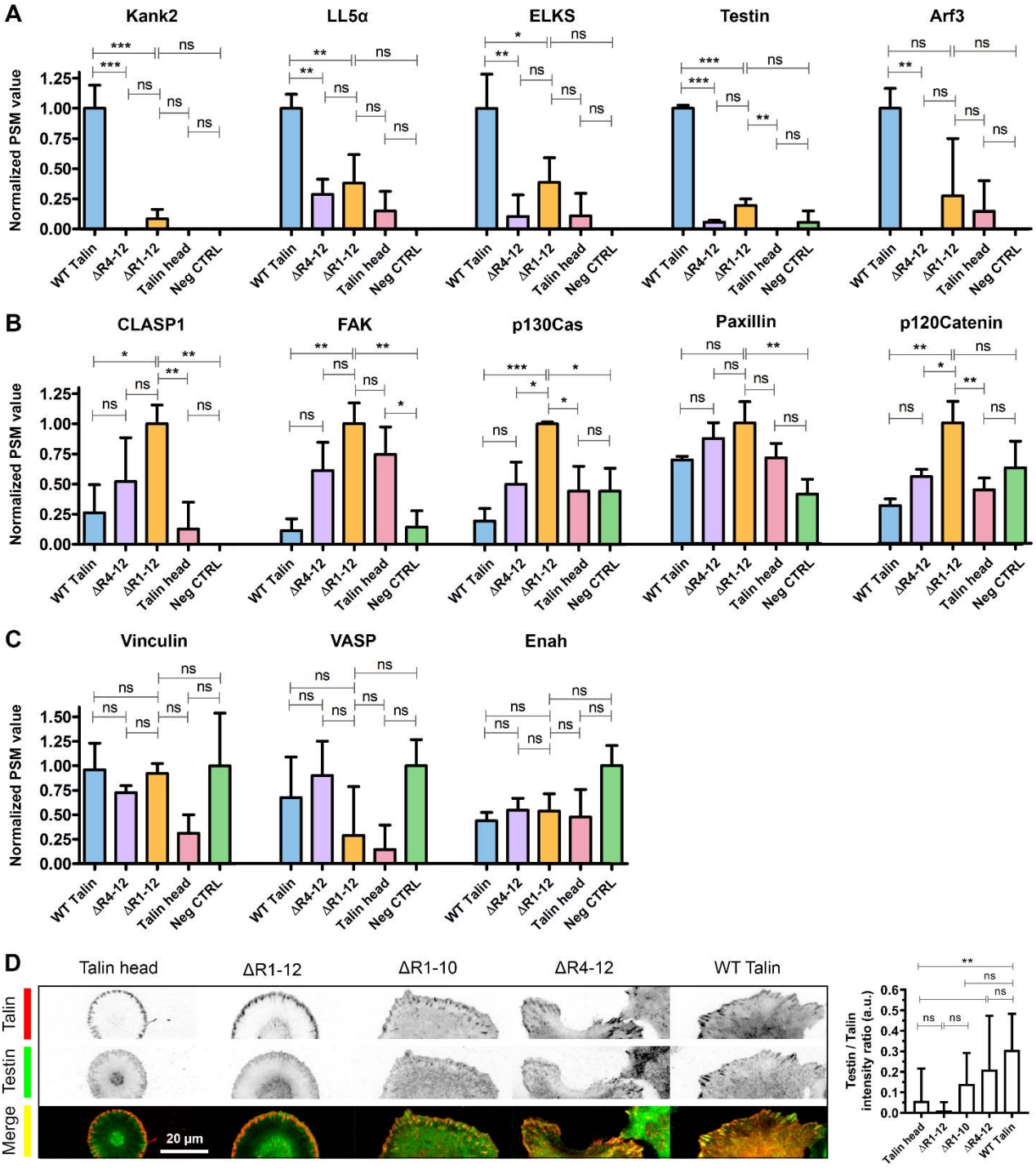


Figure S3, Related to Figure 3. BioID analysis for cells expressing modified talin proteins. (A) Normalized peptide spectrum match (PSM) values for proteins with the highest signal in cells expressing wild type talin. (B) Normalized PSM values for proteins showing the increased signals in cells expressing interactions with the ΔR1-12 protein as compared to the other talin forms. (C) Normalized PSM values for adhesion proteins Vinculin, VASP and Enah. In each graph, PSM values were normalized to the highest value. Bars represent mean PSM values of three replicate MS/MS runs ± standard deviation. (D) Testin immunostaining for *Tln1-/-Tln2-/-* cells expressing different talin proteins. n = 8-13 cells for each talin protein. * = p<0.05, ** = p<0.01, *** = p<0.001

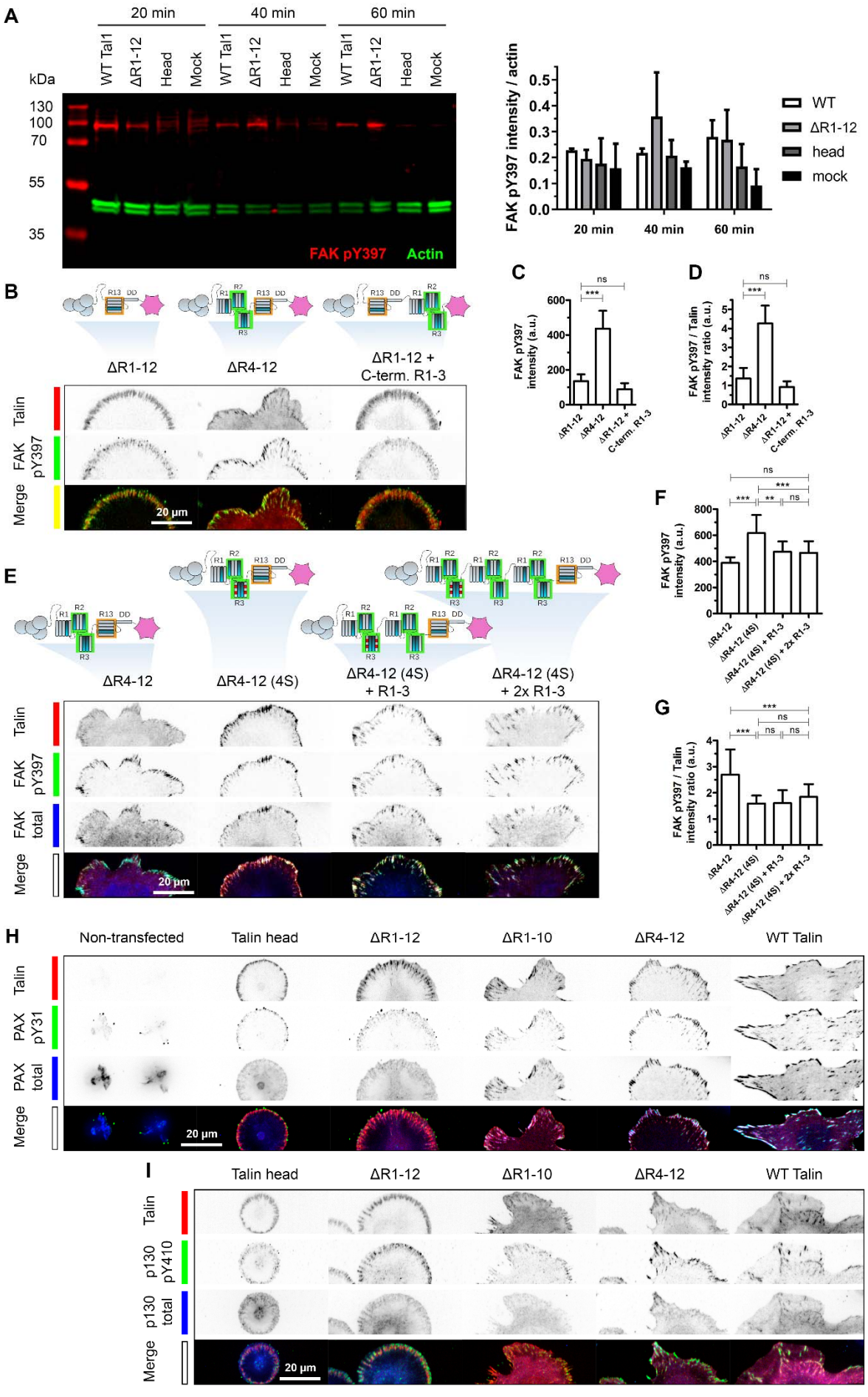


Figure S4, Related to Figure 4. (A) Western blot analysis for FAK activation at different time points after plating cells. Bars represent mean ± standard deviation from two independent experiments. (B - D) Representative images and quantification of FAK pY397 immunostaining for *Tln1-/-Tln2-/-* cells expressing ΔR4-12 or ΔR1-12 + C-term. R1-3 proteins. n = 4 - 22 cells for each talin protein. In C, FAK pY397 intensity in adhesion sites was normalized by the average talin intensity in adhesion sites in each cell. (E – G) Representative images and quantification of FAK pY397 immunostaining for *Tln1-/-Tln2-/-* cells expressing destabilized talin proteins. n = 8 - 16 cells for each talin protein. (H) Representative images of paxillin and paxillin pY31 immunostaining in cells expressing talin different talin proteins. (I) Representative images of p130Cas and p130Cas pY410 immunostaining in cells expressing different talin proteins. In all graphs, bars represent mean ± standard deviation. The statistical significance of all results was analyzed by one-way ANOVA and Bonferroni test, ns = not significant, * = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.

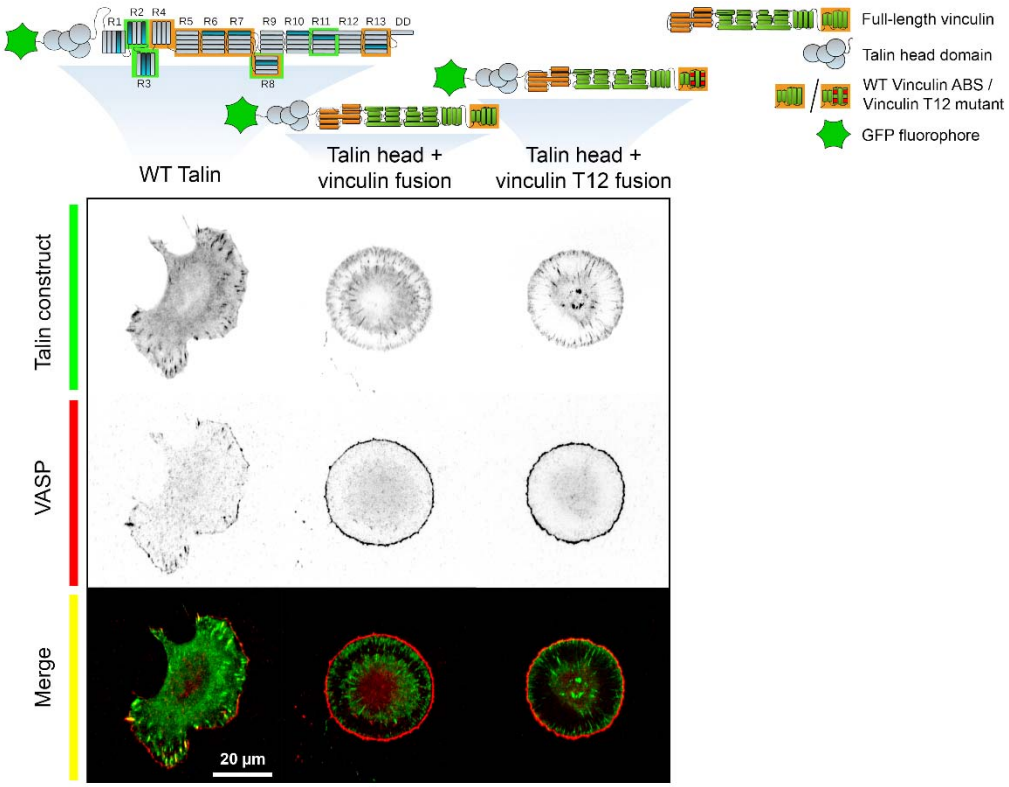
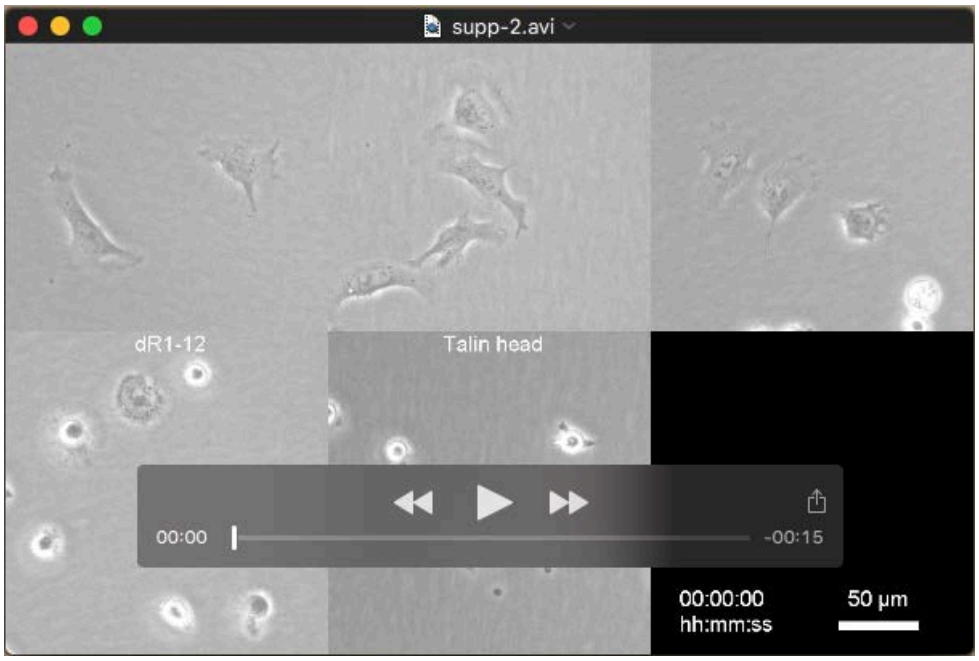
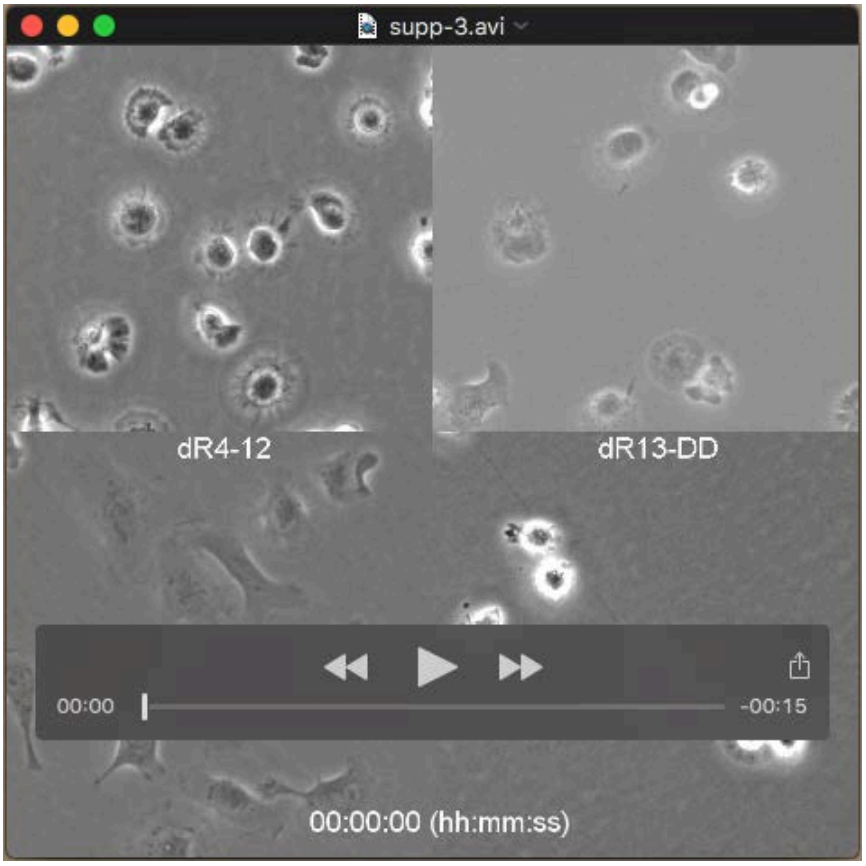


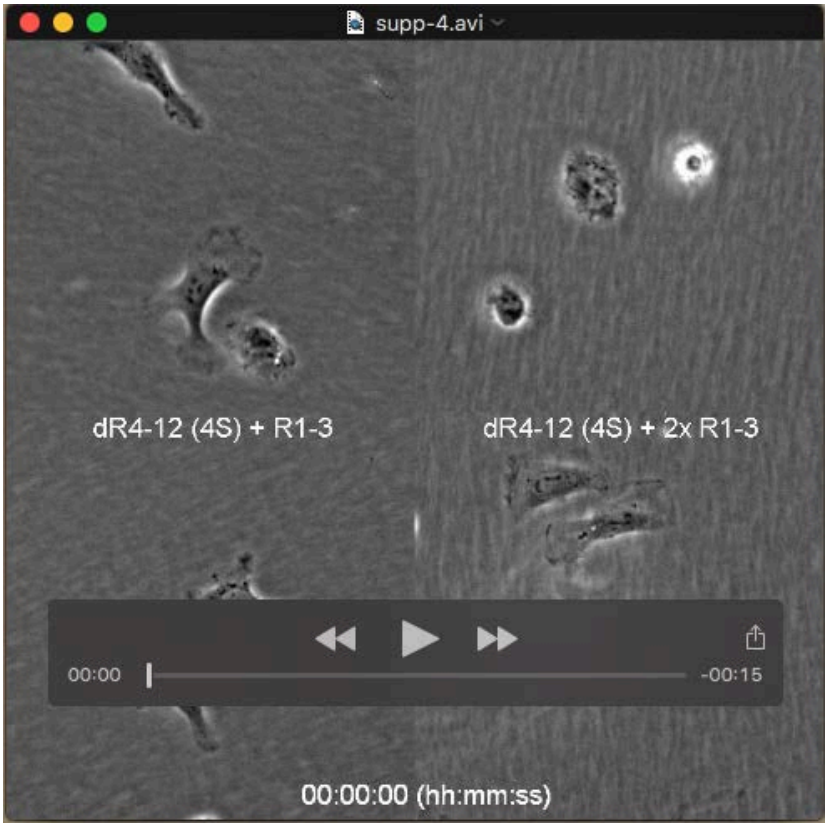
Figure S5, Related to Figure 5. Representative confocal microscope images of *Tln1-/-Tln2-/-* cells expressing wild-type talin or the talin head domain + vinculin fusion proteins. Vinculin (T12) mutant is autoinhibition deficient and has a constitutively active F-actin binding domain. The talin-vinculin fusion protein localized into radial adhesion sites and induced isotropic cell spreading, but did not recruit any VASP into adhesion sites. Instead, VASP strongly localized to the edge of the cell lamellipodium.



Movie 1, Related to Figure 1E. Time-lapse video of *Tln1-/-Tln2-/-* cells expressing the truncated talin proteins. Cells expressing different talin proteins were plated on a fibronectin-coated polystyrene plate and allowed to attach to the surface for 120 minutes before imaging. Time-lapse series was collected at a 360 seconds interval for 12 hours.



Movie 2, Related to Figure 2A, E. Time-lapse video for *Tln1-/-Tln2-/-* cells expressing the reorganized talin proteins or the ΔR13-DD protein. Cells expressing different talin proteins were plated on a fibronectin-coated polystyrene plate and allowed to attach to the surface for 120 minutes before imaging. Time-lapse series was collected at a 120 seconds interval for 12 hours. The size of each field is 200 μm x 200 μm. Note how the cells expressing the ΔR1-12 + C-terminal R1-3 protein have a non-polarized phenotype identical to cells expressing the ΔR1-12 form.



Movie 3, Related to Figure 2I. Time-lapse video for *Tln1-/-Tln2-/-* cells expressing the destabilized and tandem talin proteins presented. Cells expressing different talin proteins were plated on a fibronectin-coated polystyrene plate and allowed to attach to the surface for 120 minutes before imaging. Time-lapse series was collected at a 360 seconds interval for 12 hours. The size of each field is 200 μm x 200 μm. Note how the destabilization of the talin R3 subdomain by point mutations (ΔR4-12 (4S) talin mutant) inhibits cell spreading, polarization and migration. However, this effect is rescued by the addition of non-destabilized talin rod subdomains R1-3 into a tandem configuration (ΔR4-12 (4S) + R1-3 and ΔR4-12 (4S) + 2xR1-3 tandem proteins).

Table S1. Related to Figure S3. Complete list of all proteins identified in the proximity biotinylation analysis. Normalized peptide spectrum match (PSM) values for proteins reliably identified in at least one of the samples. Each sample was analyzed in three replicate MS/MS runs. All PSM values were normalized to the PSM value for the SH-Tag in each run.

Click here to Download Table S1

Table S2, Related to Figure 3. Complete list of all phosphorylated sites reliably identified in at least one of the four studied samples (WT talin, Δ1-12, Talin head, Negative control). Phosphorylated sites identified in at least two out of the three experimental replicates were considered reliable. For each of these sites, the mean intensity of the three experimental replicates was calculated. Detected sites with undetermined intensity values were given an intensity value of zero. #DIV/0 denotes that the given site was not detected in this sample.

Click here to Download Table S2

Table S3, Related to Figure 3. List of phosphorylated sites detected in each sample. Only sites reliably identified in at least one of the four samples are included in the list (see Table S2). Also sites that were detected but whose intensity could not be determined are included.

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Table S4, Related to Figure 3. Detected phosphorylated sites unique for each sample and common for all samples. Comparison based on sites presented in the Table S3.

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Table S5, Related to Figure 3. Enrichment analysis for the functional clustering terms of differentially phosphorylated proteins. Enrichment analysis for the functional clustering terms (Gene ontology term Biological Processes) of proteins with one or more differentially phosphorylated sites. See Table S4 for the complete lists of phosphorylated sites.

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Table S6, Related to Figure 3. Lists of differently phosphorylated proteins identified in the pairwise analysis of phosphorylation sites. List of proteins differently phosphorylated at one or more residues. The mean intensities of each site were compared in two pairs, WT talin vs. ΔR1-12 and ΔR1-12 vs. talin head. A 5-fold increase in the determined intensity was considered to be significant. Sites undetected in one sample and with undetectable signal intensity in the other sample were filtered out. Finally, duplicate hits within each list were filtered out.

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Table S7, Related to Figure 3. List of the differentially phosphorylated residues in the adhesion proteins presented in Figure 3B. For palladin the exact phosphorylation site could not be determined.

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