Supplementary Data

Supplementary Figure S1 Chemical shift perturbation experiments with integrin tails. (A) $^1$H-$^{15}$N HSQC spectra of 0.05 mM $^{15}$N-labelled $\beta$3 tail with increasing concentrations of talin1 F3 domain: 0 mM (red), 0.025 mM (tomato), 0.05 mM (orange), 0.1 mM (yellow), 0.2 mM (green), 0.4 mM (cyan), 0.6 mM (blue), 0.8 mM (purple), 1 mM (magenta). (B) $^1$H-$^{15}$N HSQC spectra of 0.05 mM $^{15}$N-labelled $\beta$1D tail with increasing concentrations of talin2 F3 domain: 0 mM (red), 0.0125 mM (tomato), 0.025 mM (orange), 0.05 mM (yellow), 0.075 mM (green), 0.1 mM (cyan), 0.2 mM (blue), 0.5 mM (purple), 1 mM (magenta). A few peaks broaden out due to intermediate exchange, but many of these can still be traced when the contour levels are taken lower. (C) Binding curves used for $K_d$ calculation. Peaks were tracked through HSQC spectra of $^{15}$N-labelled $\beta$ tail acquired with increasing concentrations of talin F3 domain. For each trackable peak, the change in chemical shift was normalized to the change at 1 mM talin. Note that while $K_d$ values were determined by fitting several curves simultaneously, for clarity each value plotted here shows the average of several peaks ± standard error.

Supplementary Figure S2 Comparisons of the talin2 and talin1 structures. (A) Asymmetric unit of the crystal structure of talin2 F2-F3 bound to the $\beta$1D integrin tail, shown in two orthogonal orientations. The integrin tail is shown in red, the talin2 F3 domain in yellow, and the talin2 F2 domain in cyan or magenta. (B) Structure of talin2 F2-F3 (yellow) aligned with the F2-F3 domains of talin1 from PDB 1MK9 (cyan) (Garcia-Alvarez et al, 2003). (C) Structure of $\beta$1D (red) bound to talin2 F2-F3 (yellow) aligned to the structure of the $\beta$3/PIPK1$\gamma$ peptide (magenta) bound to talin1 F3 (cyan) from PDB 2H7E (Wegener et al, 2007). The alignment used the backbone of the talin1 F3 domain. It is shown in two orthogonal views, and key residues are highlighted.

Supplementary Figure S3 Electron Density Maps From the $\beta$1D/Talin2 Crystal Structure. (A) The NPxY motif of $\beta$1D, showing distinct electron density (sigma 1.2). (B) A portion of the $\beta$1D membrane-proximal helix, showing distinct electron density (sigma 1.2). (C) The membrane-proximal salt bridge, showing distinct electron density (sigma 1.2). The side chain nitrogen of talin2 K327 and the side chain oxygen of $\beta$1D D759 are separated by 3.70 Å. Electron density map images were generated in Coot (Emsley & Cowtan, 2004).

Supplementary Figure S4 Alignment of talin head domain sequences from different organisms. The amino acid sequence of talin isoforms from various organisms were aligned using ClustalW (Larkin et al, 2007). Only the sequence of the N-terminal head domain is shown. Residues located in the membrane orientation patch (MOP) in the F2 domain or involved in a key talin/integrin salt bridge are highlighted and labelled with vertebrate talin1 numbering. These residues are conserved in all talin sequences tested.

Supplementary Figure S5 Representative integrin activation assay raw data. (A) A set of raw FACS data from one of the three independent experiments conducted for Figure 1c. Dot plots correlate integrin activation (PAC1 antibody signal) with GFP-talin F1-F2-F3 expression in CHO cells expressing $\alpha$IIb$\beta$3. This correlation is pronounced for talin wt, but mutations that disrupt the talin membrane orientation patch (MOP)
diminish this effect. See Materials and Methods for full experimental details. (B) Western blot showing expression levels of constructs tested.

**Supplementary Figure S6** Talin F2 mutants do not affect talin integrity or integrin binding. (A) 1D NMR spectra of 1 mM talin1 F2-F3 wt and the 4E mutant in which K256, K272, K274, and R277 in F2 were substituted with glutamates. Both spectra are indicative of a folded protein and do not display major differences. (B) Chemical shift perturbation maps for 0.05 mM β3 titrated with 1 mM talin1 F3 wt, F2-F3 wt, and F2-F3 4E. K_d values for the interactions are shown. No major differences were observed between the different constructs.

**Supplementary Figure S7** Mutations that abrogate talin binding to the membrane-proximal region of the β integrin tail. (A) Chemical shift perturbation maps for 15N-labelled wt and mutant β3 tail (0.05 mM) titrated with 1 mM talin1 F3 domain. Mutants of β3 tested were D723R, D723A, and FF727/730AA. (B) As in panel A but with β1A wt, D723R and FF763/766AA.

**Supplementary Figure S8** Disrupting the membrane-proximal salt bridge between β1A and talin1. (A) Weighted shift maps of perturbations observed in 1H-15N HSQC spectra of the β1A tail upon the addition of talin1 F3. Experiments were performed on β1A wt with talin1 wt, β1A D759R with talin1 wt, and β1A wt with talin1 K324D. Grey bars correspond to residues that could not be tracked due to exchange broadening. (B) Chemical shift perturbations in β1A upon binding to talin1 F3 wt domain mapped onto the β1D/talin2 structure (largest shifts in blue, smallest in red). (C) As in panel B but with β1A D723R.

**Supplementary Movie S1** A top view of integrin activation. This movie shows a proposed mechanism of integrin activation by talin, viewed looking down through the membrane. Talin binds to the β integrin tail and disrupts the α/β inner membrane clasp. It then tilts the integrin tail by about 20° to maximize contact between the positively charged membrane orientation patch on the F2 domain, further disrupting the α/β interaction and causing tail separation. Atoms in the α and β integrins located within 4 Å of an atom on the other integrin have been highlighted, allowing the breaking of α/β contacts to be visualized as integrin activation proceeds.

**Supplementary Movie S2** A side view of integrin activation. Same as Supplementary Movie S1, but viewed from the side, along the cell membrane.
**Supplementary Table I** Backbone RMSD values for alignment of talin2 with various talin1 structures

<table>
<thead>
<tr>
<th>Talin1 structure</th>
<th>F2-F3 (209-398)</th>
<th>F2 (209-304)</th>
<th>F3 (311-398)</th>
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<tr>
<td>1MK9</td>
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<tr>
<td>1MK7</td>
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<td>0.697</td>
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<tr>
<td>1MIX</td>
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<td>0.638</td>
<td>1.858</td>
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<tr>
<td>2H7E</td>
<td>-</td>
<td>-</td>
<td>1.056</td>
</tr>
</tbody>
</table>

RMSD values are given in Å and were calculated with MOLMOL (Koradi et al, 1996).

**Supplementary Table II** $K_d$ values for the interaction of talin1 F3 with the $\beta$3 integrin tail For wild type proteins and salt bridge-breaking mutants

<table>
<thead>
<tr>
<th></th>
<th>$K_d$ (µM)$^a$</th>
<th>$\Delta\Delta G$ (kJ/mol)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>273 ± 6</td>
<td>-</td>
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<tr>
<td>$\beta$3 D723R</td>
<td>970 ± 26</td>
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</tr>
<tr>
<td>talin1 K324D</td>
<td>800 ± 14</td>
<td>2.7</td>
</tr>
</tbody>
</table>

$^a$K$_d$ values are given in µM, ± standard error.

$^b$Δ$G$ was calculated from $K_d$ for each interaction, and ΔΔ$G$ was calculated by subtracting Δ$G$ of that interaction from Δ$G$ of the interaction involving the wild type tail (a positive value denotes a decrease in affinity).
Supplementary References


Supplementary Figure S2
Supplementary Figure S6

A

B

K_d = 273 ± 6 μM
K_d = 360 ± 13 μM
K_d = 299 ± 11 μM
Supplementary Figure S7

A

β3 wt

β3 D723R

β3 D723A

β3 F727/730AA

β3 residue

B

β1A wt

β1A D723R

β1A FF763/766AA

β1A residue
Supplementary Figure S8

A

\[ \Delta \delta^{(HN, N)} \text{(ppm)} \]

\( \beta_{1A} \text{ wt} \)

\( \beta_{1A} \text{ D723R} \)

\( \text{talin1 K324D} \)

B

C