Supplemental material

Gingras et al., https://doi.org/10.1083/jcb.201810061

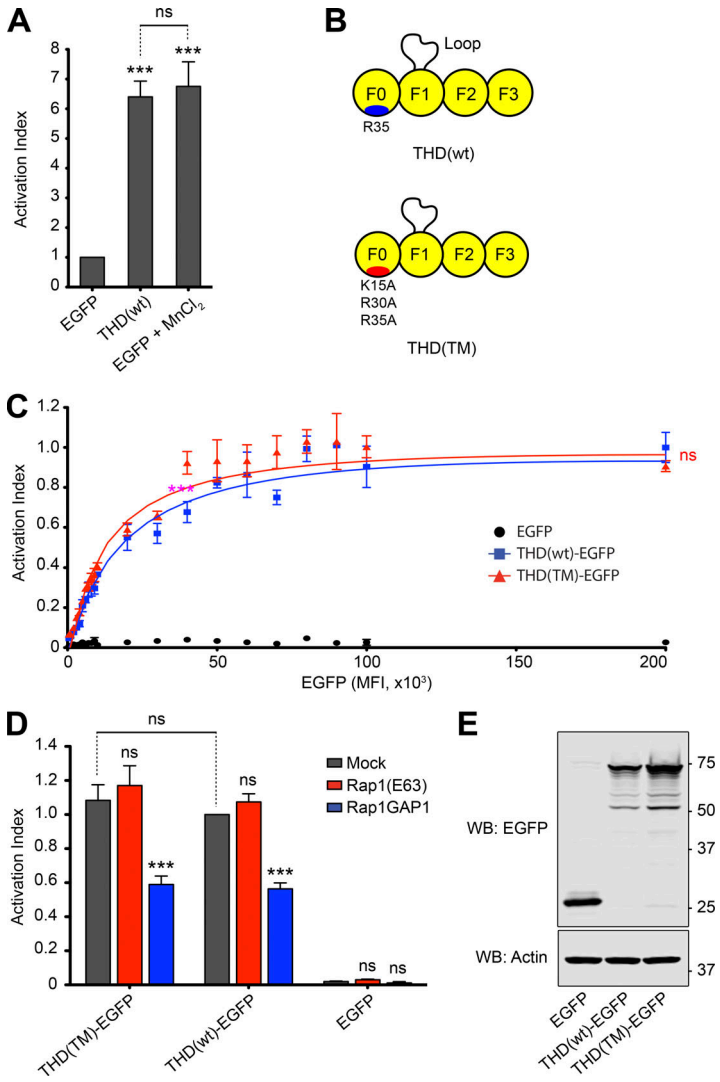

Figure S1. **THD(wt)-EGFP, THD(TM)-EGFP, and $MnCl_2$ show similar levels of integrin activation. (A)** THD and Mn++ activate integrin αIIbβ3 to a similar extent. A5 cells stably expressing αIIbβ3 integrin were transfected with cDNA encoding EGFP or THD-EGFP. Integrin activation was assayed by binding of PAC1 to EGFP-positive cells in the presence or absence of 1 mM $MnCl_2$. Bar graphs represent mean ± SEM of three independent experiments normalized to the EGFP condition. Two-way ANOVA with Bonferroni post-test. Each condition was compared with the EGFP control. ***, P < 0.001. **(B)** THD constructs used in C–E. **(C and D)** Talin1(K15A,R30A,R35A) triple mutation (TM) does not impede αIIbβ3 integrin activation in A5 cells. A5 cells stably expressing αIIbβ3 integrin were transfected with cDNA encoding THD-EGFP alone (C) or in combination with Rap1(Q63E) or Rap1GAP1 (D). Integrin activation was assayed by binding of PAC1 to EGFP-positive cells. Transfection of EGFP alone was used as control. **(C)** Activation indices were normalized to the maximum value of THD(wt)-EGFP and plotted as a function of EGFP MFI. Graphs represent mean ± SEM of three independent experiments. Curve fitting was performed using the total one site-binding model in Prism 5.0. Two-way ANOVA with Bonferroni post-test. Each mutant was compared with THD. **(D)** Bar graphs represent mean ± SEM of three independent experiments normalized to THD(wt)-EGFP + mock. Two-way ANOVA with Bonferroni post-test. Each condition was compared with the respective mock control. ***, P < 0.001. No significant differences between WT and the triple mutant were detected. **(E)** Expression of the THD(TM)-EGFP triple mutant was assayed by WB.

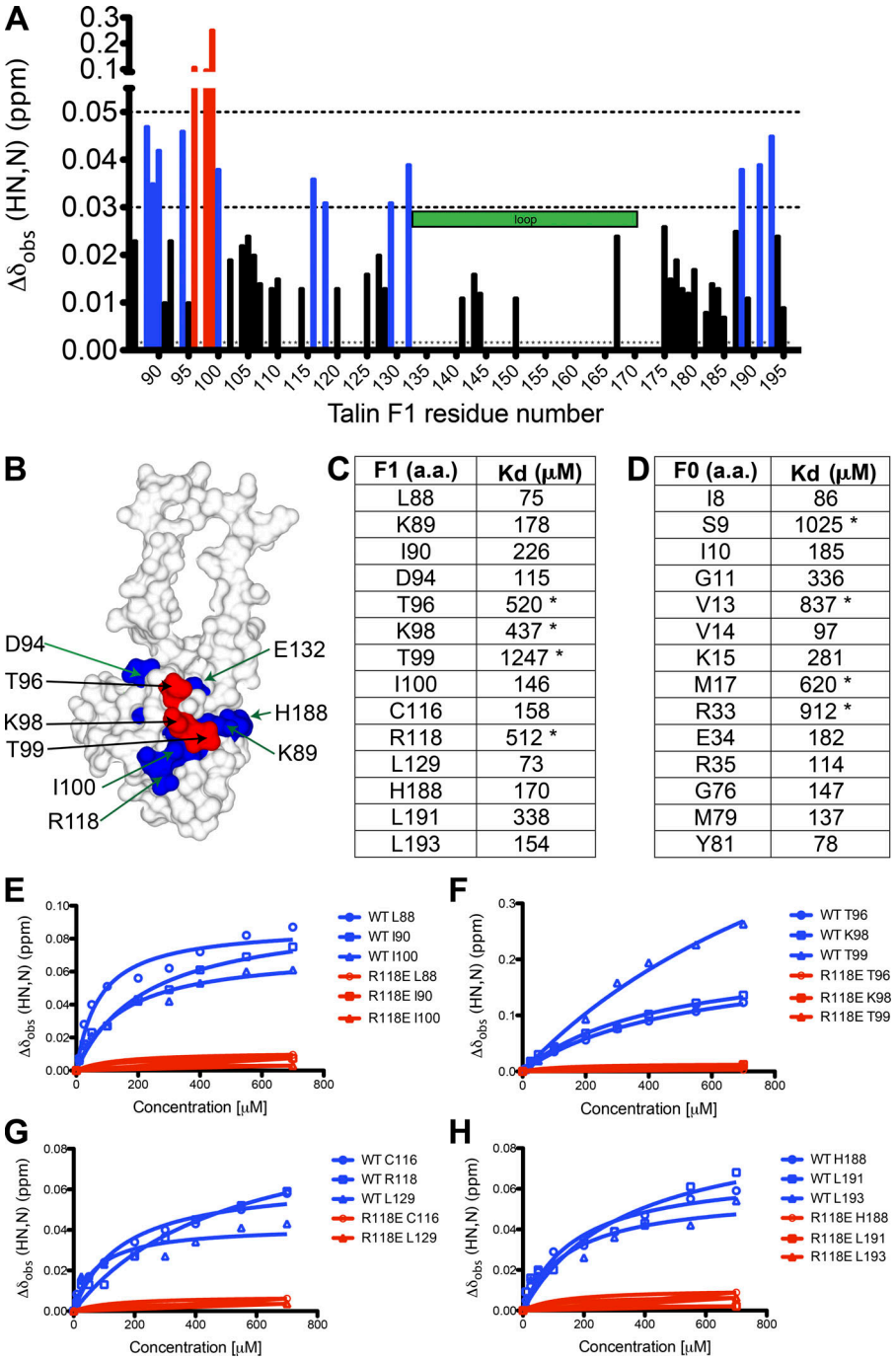

Figure S2. **Characterization of Rap1 binding to talin F1 subdomain by NMR. (A)** Summary of chemical shift changes as a plot against the residue number of F1 for assigned residues with high confidence using the previously assigned spectra from (Goult et al., 2010). **(B)** Mapping of the significant changes on the F1 structure with the same color code as in A. **(C and D)** The calculated apparent K_d values using amino acid backbone amide chemical shift perturbations for the talin1 F1 (C) and F0 domains (D). The apparent K_d values are observed in similar micromolar ranges, suggesting similar affinities of F1 and F0 for Rap1b. The asterisks highlight the larger values. **(E–H)** Titration curves for the interaction of talin1 F1 with Rap1b for residues assigned with confidence using the previously published assignment (Goult et al., 2010).

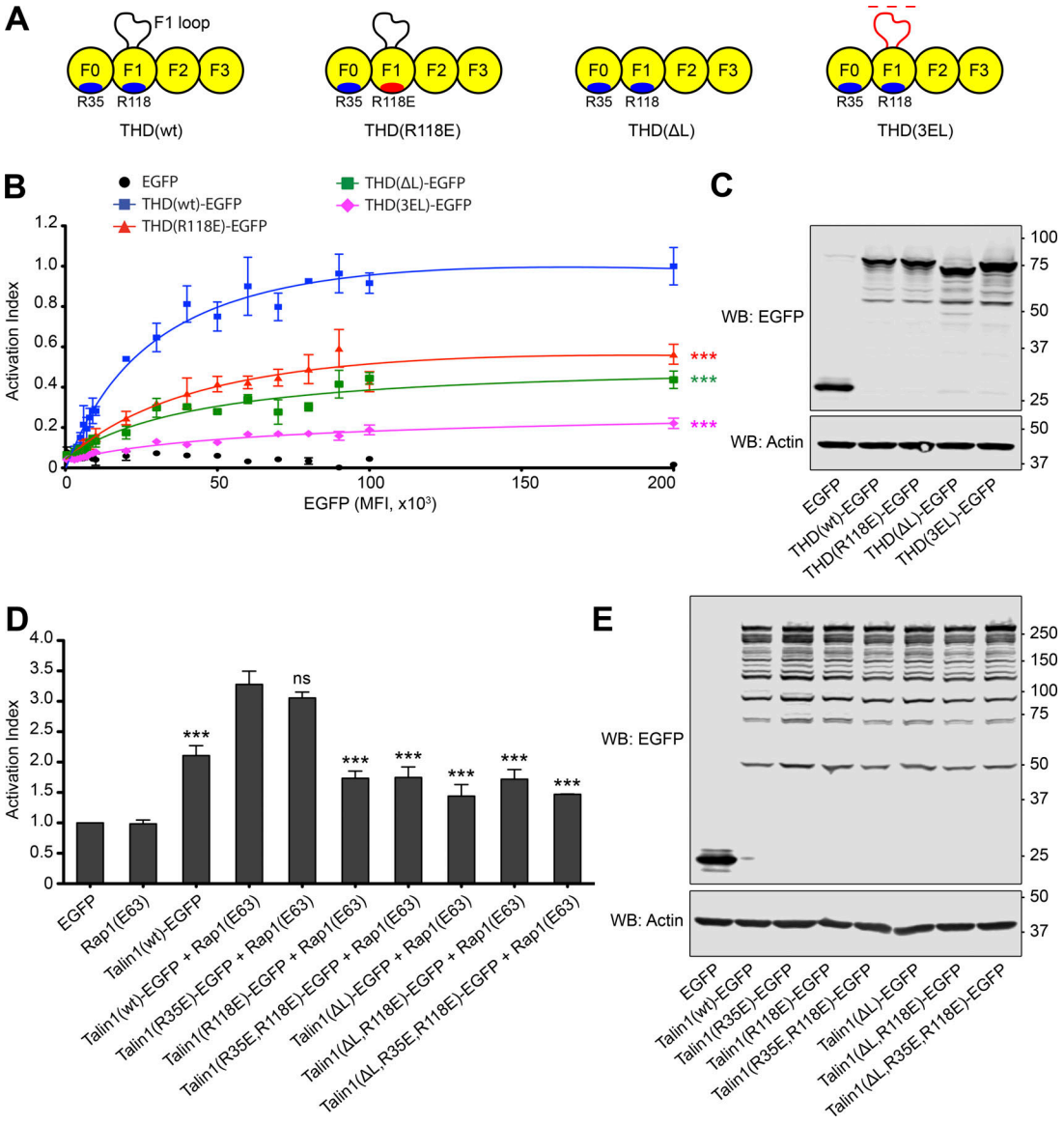

Figure S3. **The role of talin1 basic residues of the F1 loop and full-length talin1 mutants in integrin activation. (A)** THD constructs used in B and C. **(B)** A5 cells stably expressing αIIbβ3 integrin were transfected with cDNA encoding THD-EGFP. Integrin activation was assayed by binding of PAC1 to EGFP-positive cells. Transfection of EGFP alone was used as control. Activation indices were normalized to the maximum value of THD(wt)-EGFP and plotted as a function of EGFP MFI. Graphs represent mean ± SEM of three independent experiments. Curve fitting was performed using the total one-site binding model in Prism 5.0. Two-way ANOVA with Bonferroni post-test. Each mutant was compared with THD. ***, $P < 0.01$. **(C)** WB of THD-EGFP expression. Actin was used as a loading control. **(D)** A5 cells stably expressing αIIbβ3 integrin were transfected with cDNA encoding full-length talin1 fused to EGFP in combination with Rap1(Q63E). Integrin activation was assayed by binding of PAC1 to EGFP-positive cells. Bar graphs represent mean ± SEM of three independent experiments normalized to talin1(wt)-EGFP alone. One-way ANOVA with Bonferroni post-test. Each condition was compared with the talin1(wt)-EGFP alone control. ***, $P < 0.001$. **(E)** WB of talin1-EGFP expression. Actin was used as a loading control.

Reference

Goult, B.T., M. Bouaouina, P.R. Elliott, N. Bate, B. Patel, A.R. Gingras, J.G. Grossmann, G.C. Roberts, D.A. Calderwood, D.R. Critchley, and I.L. Barsukov. 2010. Structure of a double ubiquitin-like domain in the talin head: a role in integrin activation. *EMBO J.* 29:1069–1080. https://doi.org/10.1038/emboj.2010.4