Ye et al., http://www.jcb.org/cgi/content/full/jcb.200908045/DC1

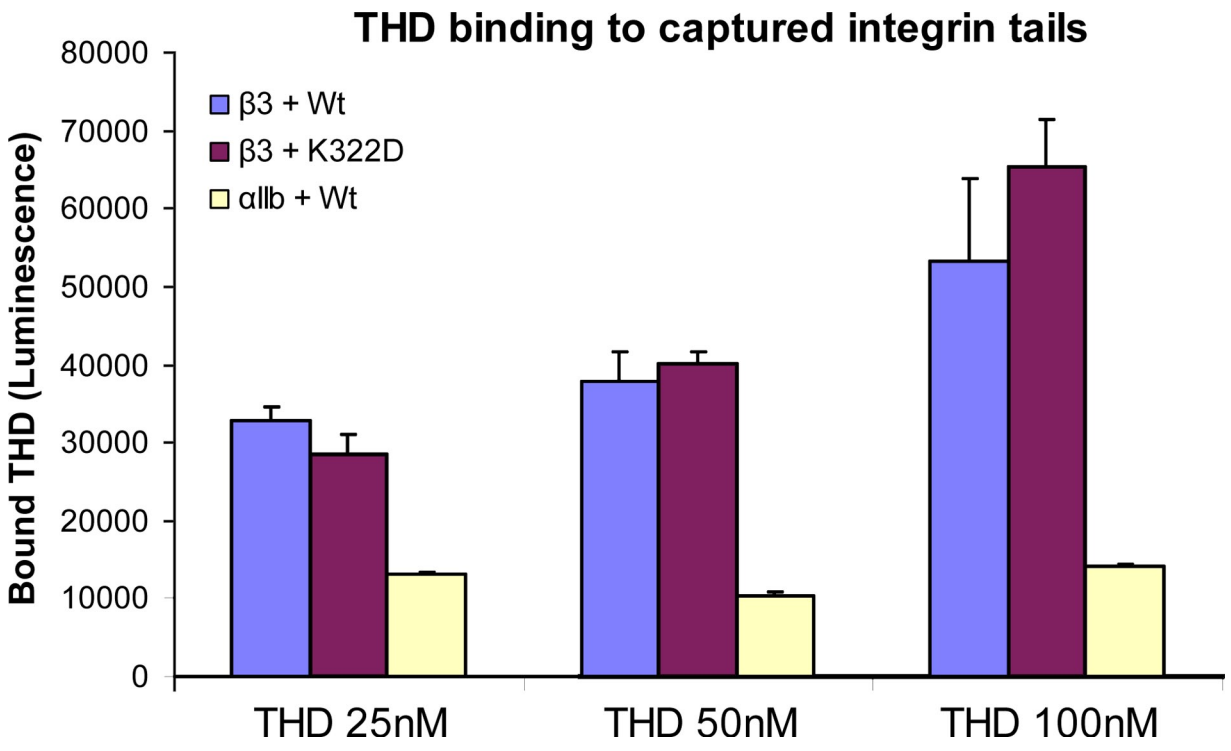

Figure S1. **THD(K322D) binds β3 tails.** The ELISA assays were performed as described in Arias-Salgado et al. (2003). In brief, biotinylated integrin tail model proteins were captured on an ELISA plate coated with neutravidin. After blocking and washing, N-terminal V5-tagged THD(wt) or THD(K322D) was added and incubated at 37°C for 1 h. The unbound proteins were washed away and bound proteins were detected with anti-V5 antibody (Invitrogen). THD(K322D) binds to β3 tail to the same extent as THD(wt).

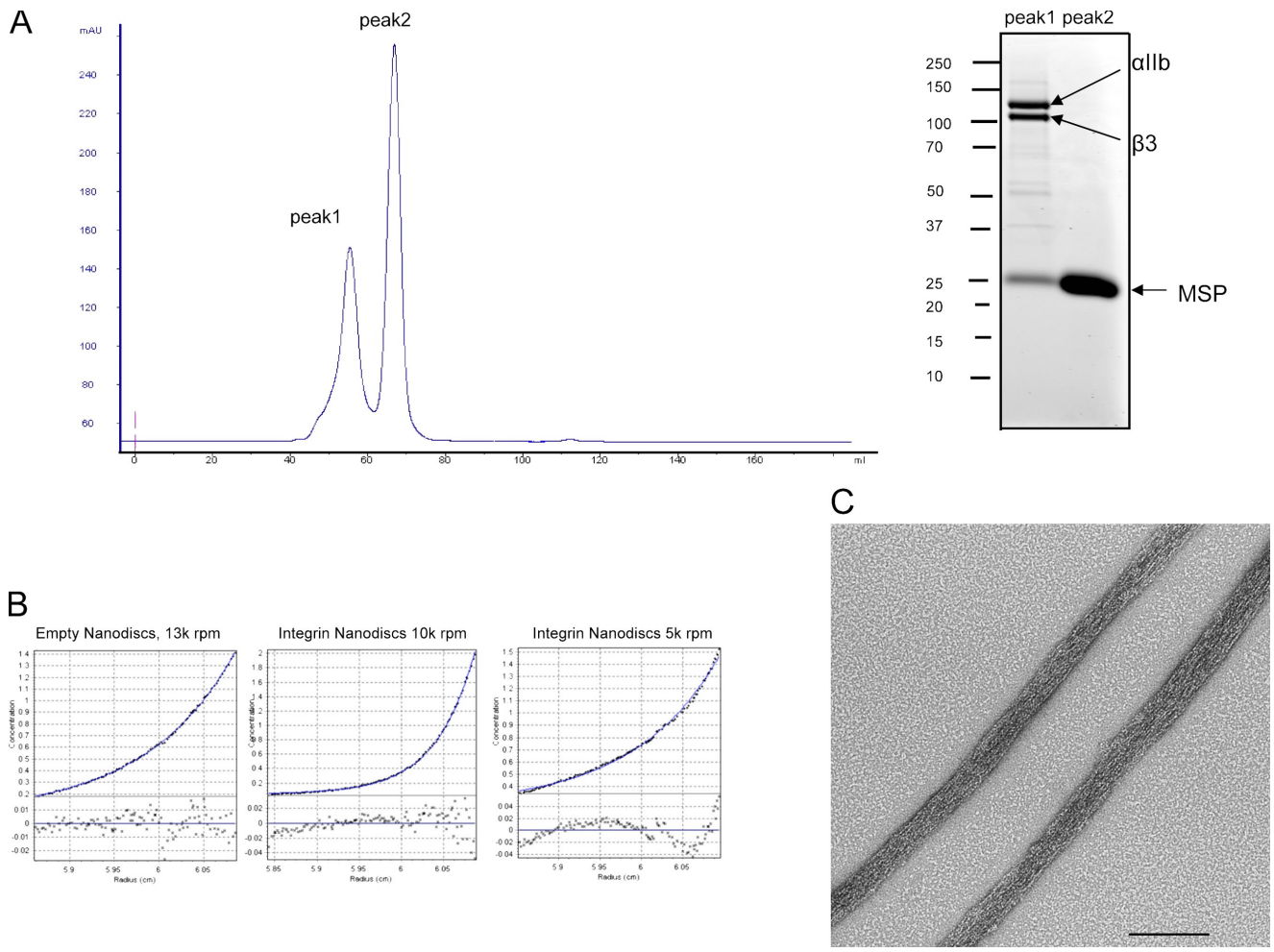

Figure S2. **Purification of integrin nanodiscs.** (A) Chromatograms of integrin nanodisc purification on a size-exclusion column. Integrin nanodiscs were assembled as described in the Materials and methods section. The two peaks were well separated. SDS-PAGE analysis confirms that peak 1 is integrin nanodiscs because it contains both integrin and MSP. Peak 2 contains empty (integrin-free) nanodiscs. (B) Measured data points and fitted curve from analytical ultracentrifugation for determination of molecular weight. From left to right are measurements for empty nanodiscs, single-integrin nanodiscs, and dual-integrin nanodiscs. The blue dots are actual measurements and the exponential-shaped curve is a theoretical fit with the determined molecular weight. The bottom of each panel shows the residuals of the curve fitting. (C) Fibrin fiber formed without addition of integrin nanodiscs. Bars, 100 nm.

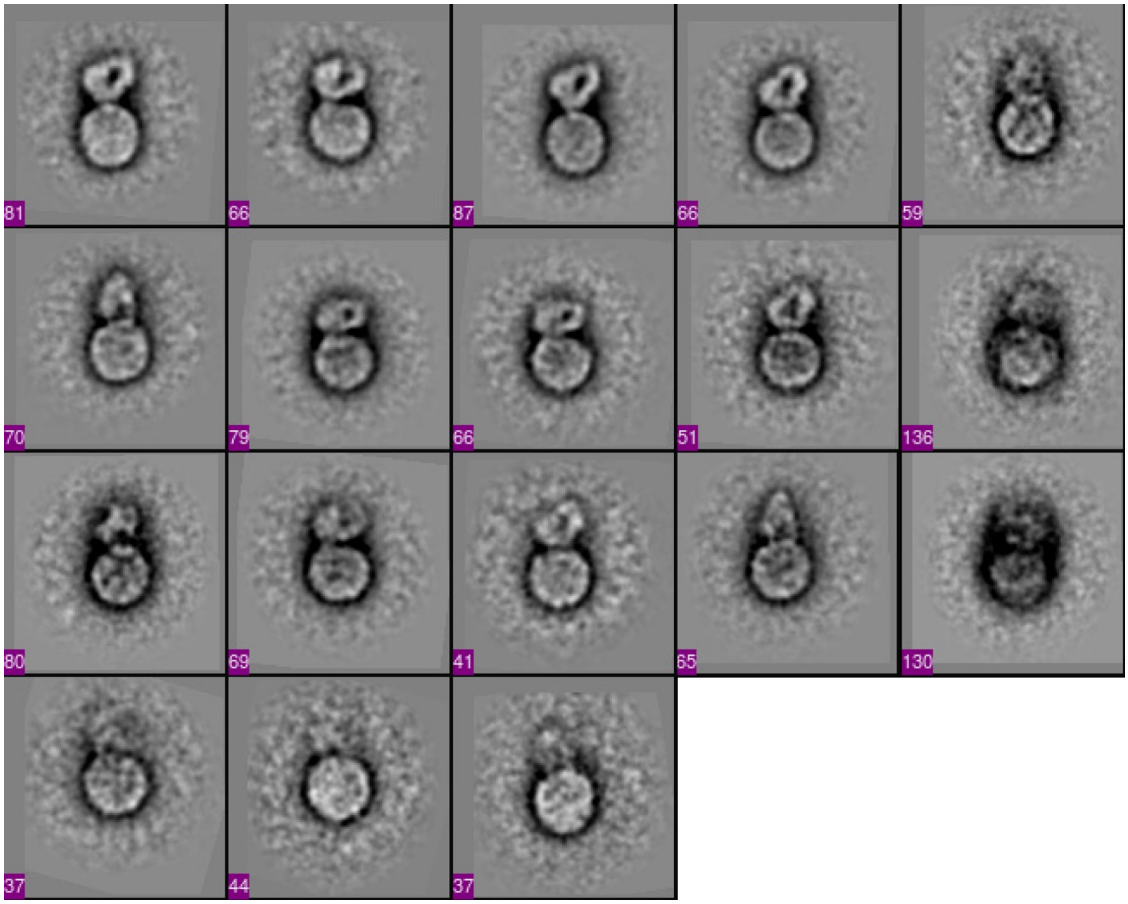

Figure S3. **Similar to Fig. 7 A, but showing the entire 18 class averages of the integrin nanodiscs.** Boxes are 50 × 50 nm in size.

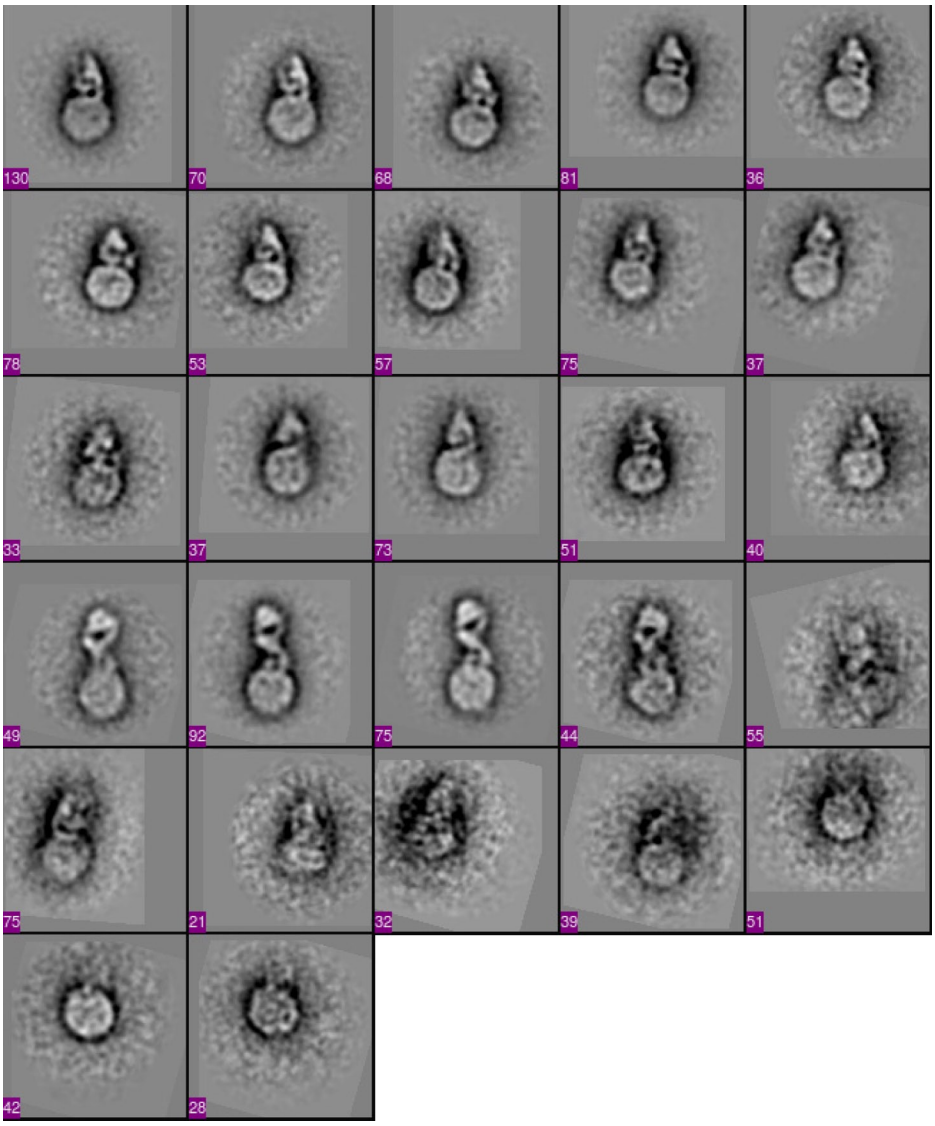

Figure S4. **Similar to Fig. 7, B and C, but showing the entire 27 class averages of integrin nanodiscs in the presence of 5 µM THD.** Boxes are 50 × 50 nm in size

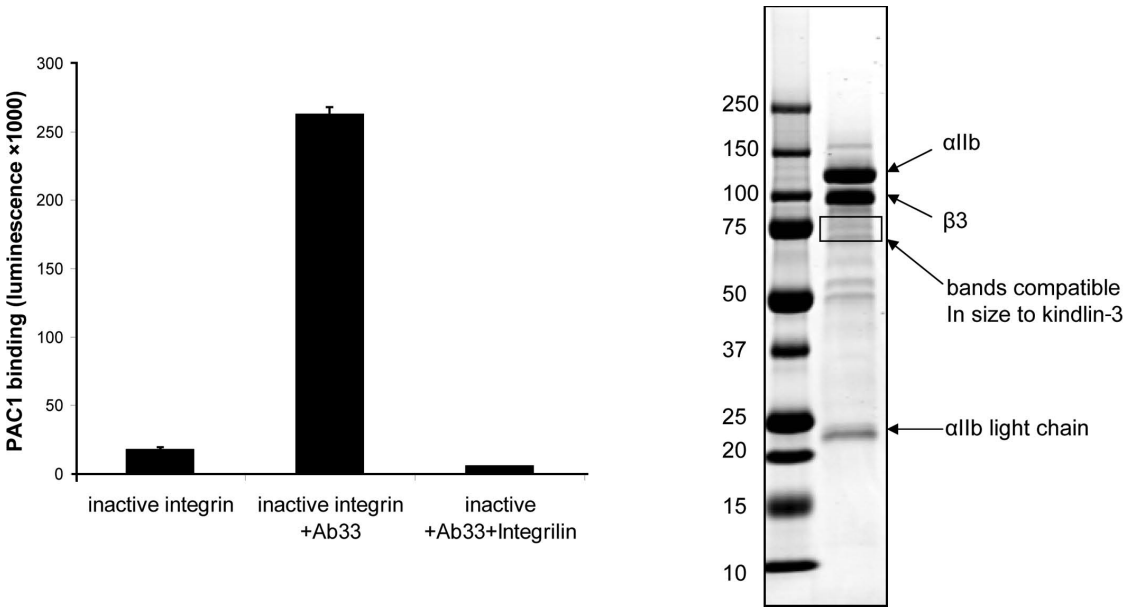

Figure S5. **Preparation and activity of inactive integrins.** Inactive integrins prepared as described in the Materials and methods section and activity of the inactive integrins were confirmed by activity assay. In brief, detergent-solubilized integrins were captured on an ELISA plate coated with AP3 antibody and activation was detected by the binding of PAC1 antibody. Experiments were also performed in the presence of anti-LIBS6 as full activation control and in the presence of 20 μM eptifibatide as negative control. Error bars represent standard error of triplicates. The activation index of the inactive integrins was 4.6% and was calculated as $100 \times (L-L_0) / (L_{max}-L_0)$, where L = luminescence intensity, L_0 = luminescence in the presence of 1 μM eptifibatide, and L_{max} = luminescence in the presence of anti-LIBS6 antibody. On the right, Coomassie stain of SDS-PAGE analysis used to determine the purity of the integrin preparation. The box in the PAGE gel indicates absence of polypeptides at mobilities compatible with kindlin-3 and was used to estimate the maximum possible abundance of kindlin-3 in the integrin preparation.

Reference

Arias-Salgado, E.G., S. Lizano, S. Sarkar, J.S. Brugge, M.H. Ginsberg, and S.J. Shattil. 2003. Src kinase activation by direct interaction with the integrin beta cytoplasmic domain. *Proc. Natl. Acad. Sci. USA.* 100:13298–13302. doi:10.1073/pnas.2336149100