SUPPLEMENTAL INFORMATION

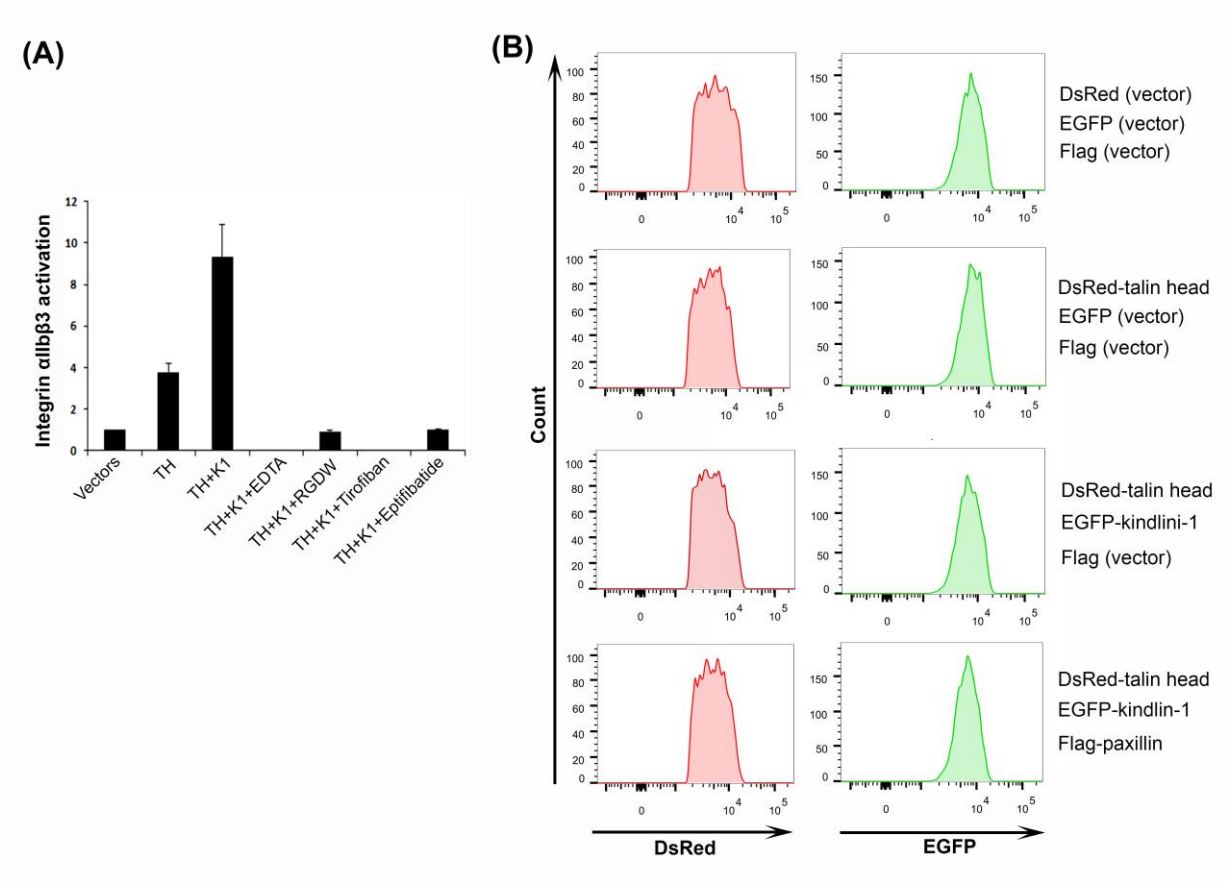

Fig. S1: Integrin αIIbβ3 activation in CHO-αIIbβ3 cells. (A) PAC-1 binding to the activated integrin αIIbβ3 induced by the talin head (TH) and kindlin-1 (K1) was inhibited by different inhibitors, including EDTA (5 mM), RGDW peptide (100 μM), tirofiban (2 μg/ml) and eptifibatide (2 μg/ml). (B) CHO-αIIbβ3 cells were transfected with different panels of plasmids to express the indicated molecules. The transfected cells were stained with 2G12 for total integrin αIIbβ3 or PAC1 specific for activated integrin αIIbβ3, followed by corresponding Alexa 633-labeled secondary antibodies. For flow cytometry analysis, cells positive for both DsRed and EGFP were gated as shown in Fig. 1B, and the histograms of DsRed and EGFP on the gated cells were shown.

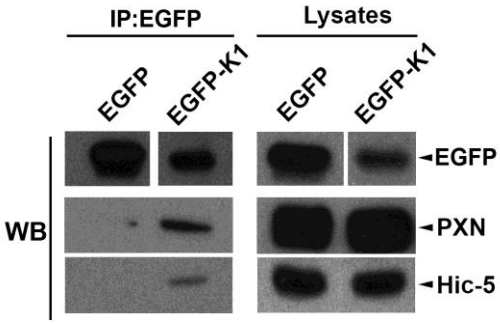

Fig. S2: Kindlin-1 interacts with the endogenous PXN in CHO-αIIbβ3 cells. CHO-αIIbβ3 cells were transfected to express either EGFP or EGFP-kindlin-1 (K1). The transfected cells were lysed for CO-IP assays using an anti-EGFP antibody. The interaction of EGFP-K1 with endogenous PXN or Hic-5 was evaluated by Western blotting.

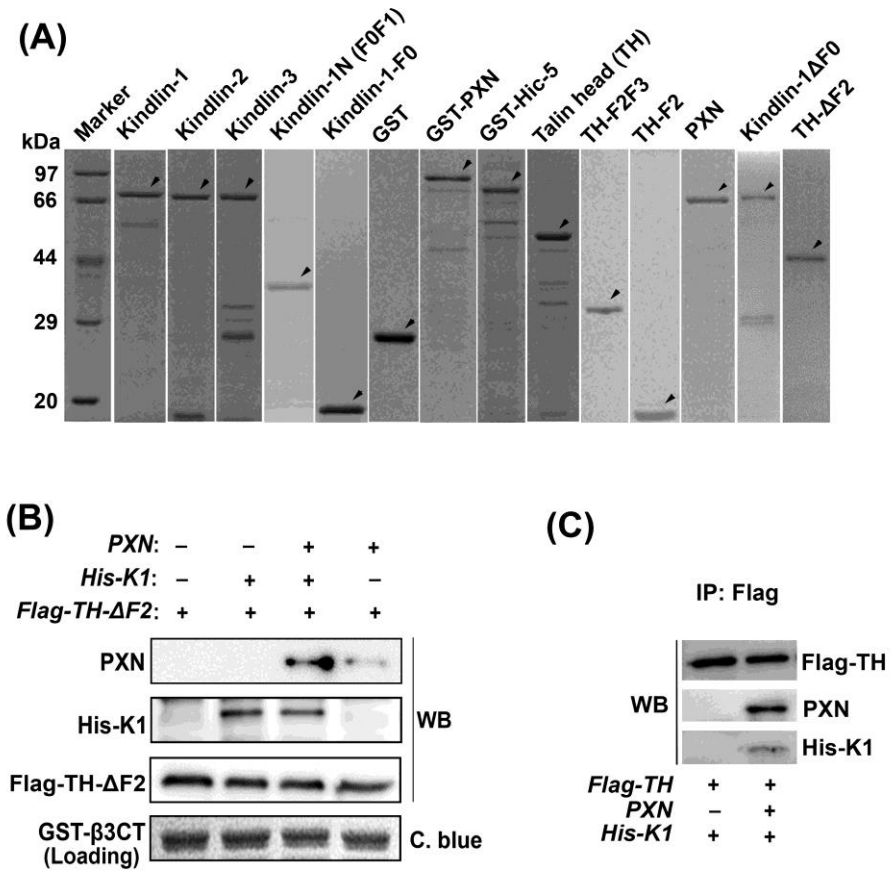

Fig. S3: Purified proteins and the formation of talin/PXN/K1 complex. (A) Purified proteins used in this study. Proteins were expressed in *E. coli* and further purified by affinity chromatography. These proteins were subjected to SDS-PAGE followed by Coomassie blue staining. The band of each protein was indicated by an arrow head. (B) GST-β3CT protein was coupled to glutathione-Sepharose beads and used for incubation with purified flag-tagged talin head with a deletion of the F2 subdomain (flag-TH-ΔF2) in the presence or absence of purified kindlin-1 (his-K1) and/or PXN. After washing, proteins precipitated with GST-β3CT were evaluated by Western blotting (WB). The loaded GST-β3CT protein on the beads was evaluated by Coomassie blue (C. blue) staining. (C) Flag-talin head (TH) protein was loaded on protein A/G agarose beads coupled with an anti-flag antibody and split into two aliquots. Equal amount of his-kindlin-1 (K1) protein was used to incubate with the beads in the presence or absence of PXN protein for overnight at 4 °C. After incubation, the beads were washed and proteins precipitated on the beads were analyzed by Western blotting (WB).

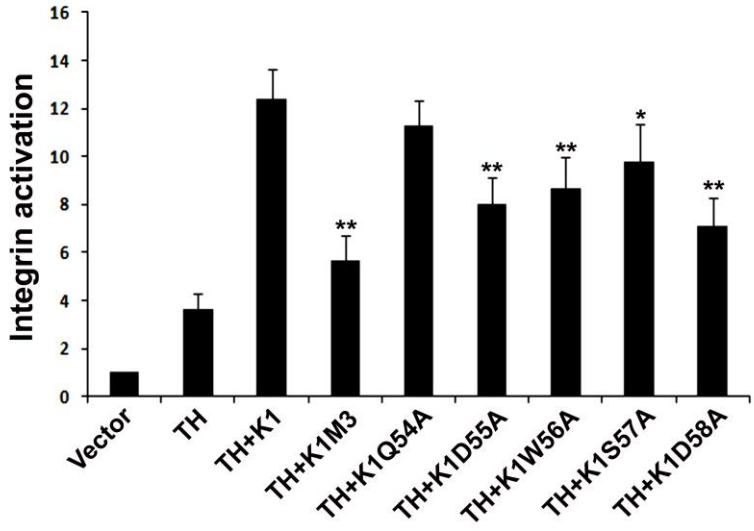

Fig. S4: The effect of each individual residue in the M3 region of kindlin-1 on integrin αIIbβ3 activation. Each of the residues in the M3 region (QDWSD) of kindlin-1 was mutated to alanine and their effects on integrin αIIbβ3 activation were evaluated in CHO-αIIbβ3 cells by the PAC1 binding assay, as described in methods. (*, $p < 0.05$; **, $p < 0.01$)

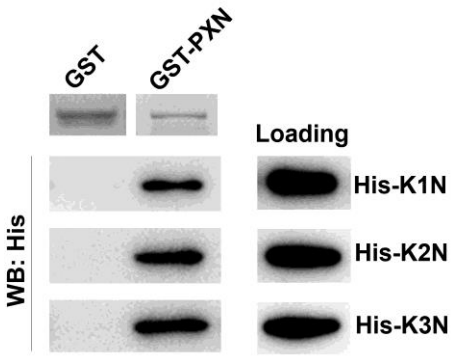

Fig. S5: PXN interacts with the N-terminal F0+F1 fragments of kindlins. GST alone and GST-tagged PXN were coupled to glutathione-Sepharose beads and used to incubate with his-tagged N-terminal fragments of kindlins that contain the F0+F1 subdomains (k1N, K2N and K3N). The precipitated proteins on the beads were evaluated by Western blotting.

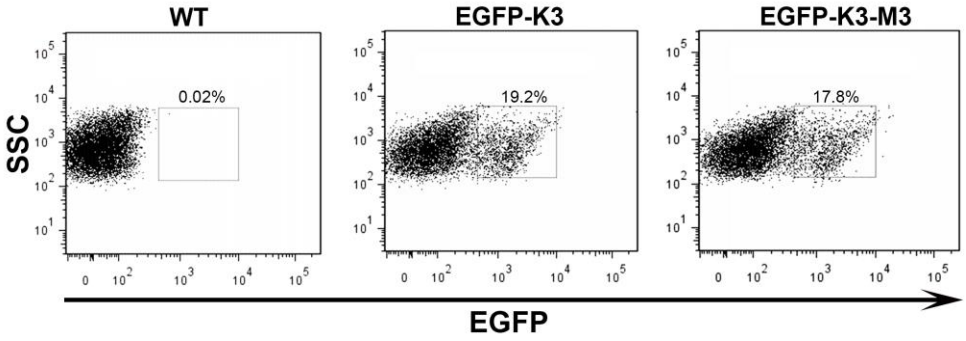

Fig. S6: EGFP-positive platelets isolated from bone marrow transplanted mice. Bone marrow cells isolated from kindlin-3-deficient mice were transduced with lentiviral particles expressing either EGFP-kindlin-3 (EGFP-K3) or the M3 mutant (EGFP-K3M3), and used to reconstitute lethally irradiated wild type mice. Platelets isolated from wild type (WT) or transplanted mice expressing either EGFP-K3 or EGFP-K3M3 were analyzed by FACS. EGFP-positive platelets were gated and used for evaluating integrin αIIbβ3 activation.