

## SUPPLEMENTAL TABLE

**Supplemental Table 1:** Statistics for data collection and structure refinement

	Kindlin-3A'	Kindlin-3A
<b>Data Collection</b>		
Space group	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Unit cell, <i>a</i> , <i>b</i> , <i>c</i> (Å)	67.73, 129.17, 134.55	67.50, 131.49, 134.15
Wavelength (Å)	0.9785	0.9785
Resolution (Å)	46.74-2.35 (2.43-2.35)	50.00-2.40 (2.49-2.40)
Rmerge (%) <sup>*†</sup>	5.3 (71.4)	7.5 (59.4)
No. of reflections, measured/unique	220848/49776	448625/48090
I/sigma I <sup>*</sup>	20.7 (2.1)	28.1 (3.2)
Completeness (%) <sup>*</sup>	99.64 (99.78)	99.6 (99.5)
Redundancy <sup>*</sup>	4.4 (4.6)	9.3 (9.1)
<b>Refinement</b>		
Resolution(Å)	46.74-2.35 (2.43-2.35)	46.95-2.38(2.44-2.38)
Unique reflections, work/free	49774/2098	47727/2001
R <sub>work</sub> (%) <sup>*‡</sup>	21.4 (29.6)	20.3 (27.2)
R <sub>free</sub> (%) <sup>*‡</sup>	24.5(34.0)	25.7 (32.9)
No.of atoms, protein/water	6777/221	6835/181
Molecules per asymmetric unit	2	2
Average B factor (Å <sup>2</sup> )		
All atoms	56.6	61.1
Protein	56.9	61.4
Water	48.7	50.7
r.m.s.d-bond lengths (Å)	0.008	0.004
r.m.s.d-bond angles (°)	1.28	0.71
Ramachandran plot	97.4/2.6/0.0	97.2/2.8/0.0
Favored/allowed/outliers (%)		
PDB code	6V9G	6V97

PDB, Protein Data Bank; r.m.s.d, root mean square deviation

\*The highest-resolution shell is shown in parentheses.

<sup>†</sup> $R_{\text{merge}} = \frac{\sum_h \sum_i |I_i - \langle I \rangle|}{\sum_h \sum_i I_i}$ , where  $I_i$  is the observed intensity of the  $i$ -th measurement of reflection  $h$ , and  $\langle I \rangle$  is the average intensity of that reflection obtained from multiple observations.

<sup>‡</sup> $R = \frac{\sum ||F_o| - |F_c||}{\sum |F_o|}$ , where  $F_o$  and  $F_c$  are the observed and calculated structural factors, respectively, calculated for all data.  $R_{\text{free}}$  is the  $R$  value obtained for a test set of reflections consisting of a randomly selected ~5% subset of data excluded from refinement.

## **SUPPLEMENTAL LEGENDS**

**Supplemental Figure 1:** Assessment of monomer-oligomer states of kindlin-3 $\Delta$  by packing quality. (A) The molecule pair of Kindlin-3 $\Delta$  in one asymmetric unit. (B) All the molecular pairs of kindlin-3 $\Delta$  that can form interface with the reference pair (green) are shown in different colors. (C) The interface areas formed between the reference kindlin-3 $\Delta$  and the surrounding ones were analyzed in PISA.

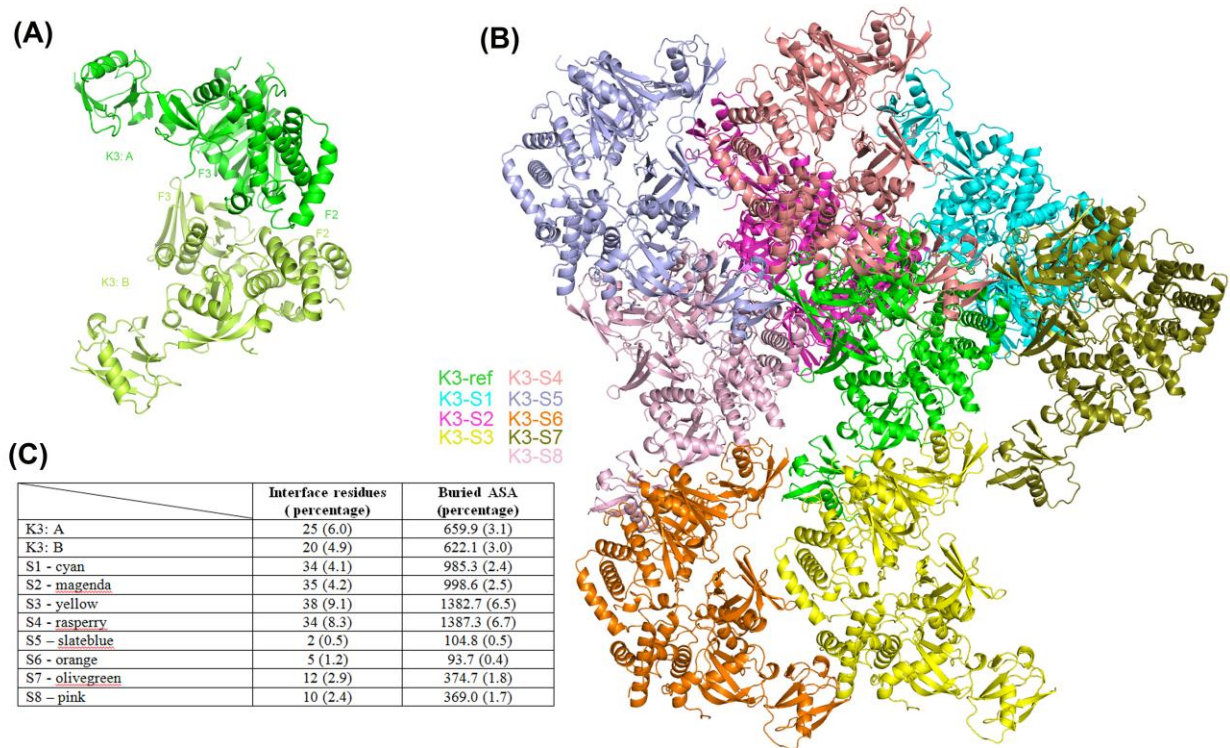
**Supplemental Figure 2:** Expression of DsRed-fused talin head domain and EGFP-fused kindlins in CHO- $\alpha$ IIB $\beta$ 3 cells. CHO- $\alpha$ IIB $\beta$ 3 cells were transiently co-transfected with DsRed-fused talin head domain (DsRed-TH) and EGFP-fused kindlins (EGFP-K1, EGFP-K2 and EGFP-K3). 24 hours after transfection, the cells were collected and lysed for SDS-PAGE and Western blotting.

**Supplemental Figure 3:** GST-EGFP-kindlin-3 forms dimers. For performing native PAGE, ~ 20  $\mu$ g of CHO- $\alpha$ IIB $\beta$ 3 cell lysate transiently expressing GST-EGFP-kindlin-3 (GST-EGFP-K3) was loaded into 4-16% Bis-Tris gels (Thermo Fisher Scientific). Gels were further transferred onto the PVDF membrane followed by Western blotting.

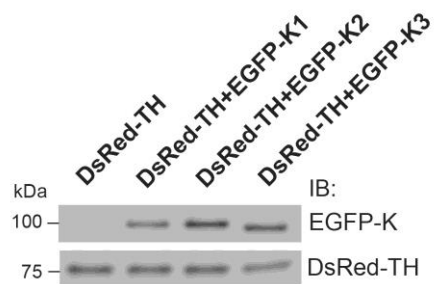
**Supplemental Figure 4:** Expression of EGFP-fused kindlin-3 in platelets. Washed platelets isolated from bone marrow transplanted mice were lysed for SDS-PAGE and Western blotting to examine the expression of EGFP-kindlin-3 (EGFP-K3) and the mutant carrying the E629AR640A mutations (EGFP-K3-E629AR640A). A homemade polyclonal anti-kindlin-3

antibody was used for Western blotting, as described previously [1]. Platelets (Kindlin-3<sup>-/-</sup>) isolated from poly(I:C) treated Kindlin-3<sup>fl/fl</sup>Mx1-Cre mice were used as a control.

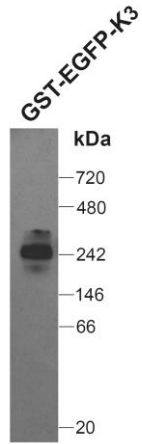
## SUPPLEMENTAL FIGURES



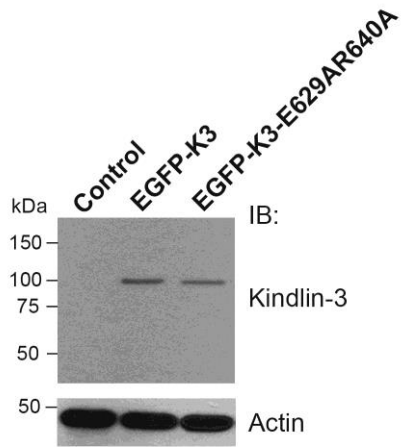
Supplemental Figure 1



Supplemental Figure 2



**Supplemental Figure 3**



**Supplemental Figure 4**

[1] Gao J, Huang M, Lai J, Mao K, Sun P, Cao Z, et al. Kindlin supports platelet integrin  $\alpha$ IIb $\beta$ 3 activation by interacting with paxillin. *J Cell Sci.* 2017;130:3764-75.