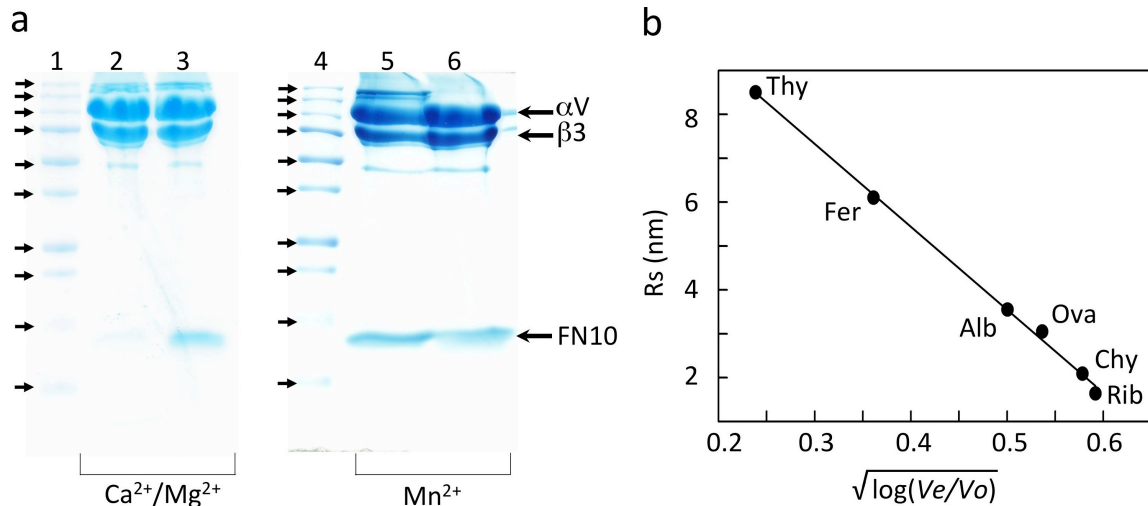
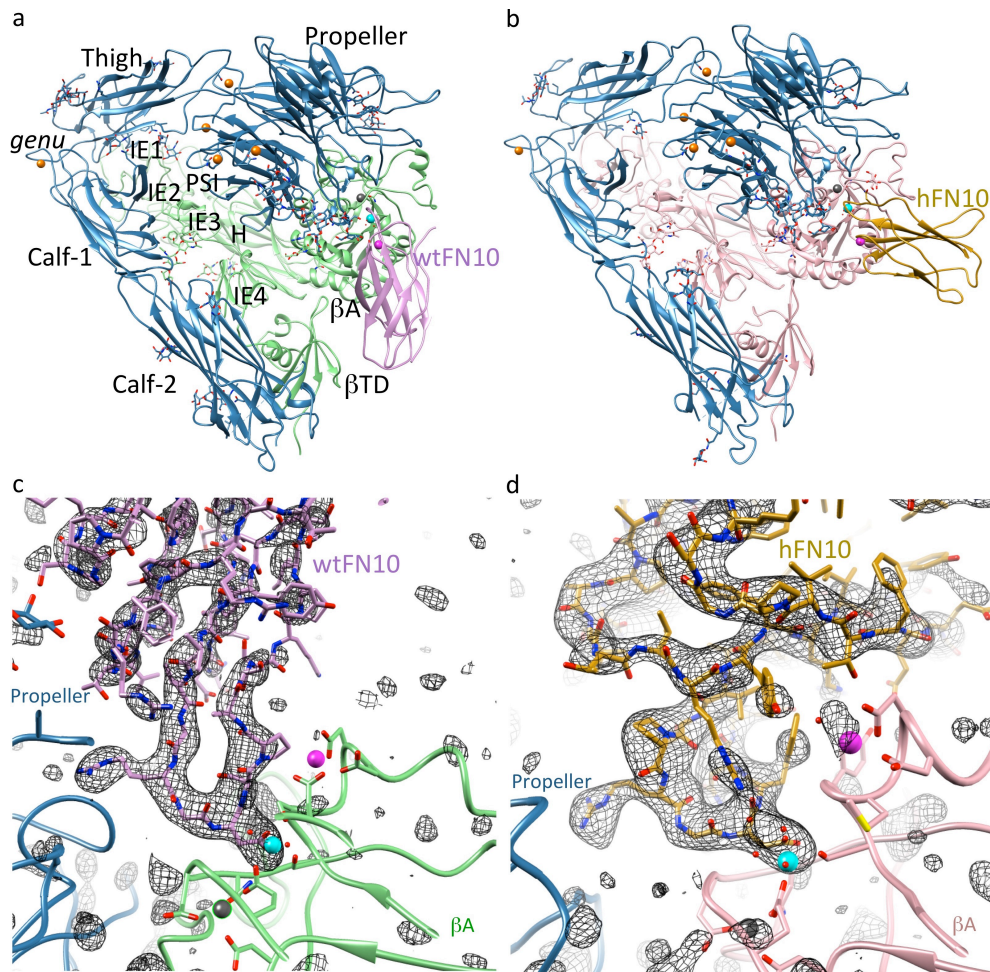


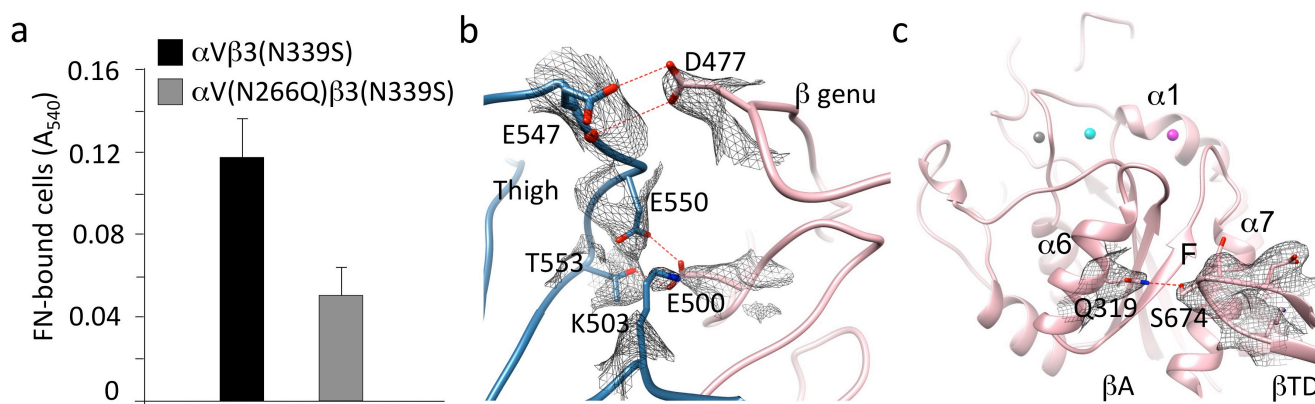
## Supplementary Figures



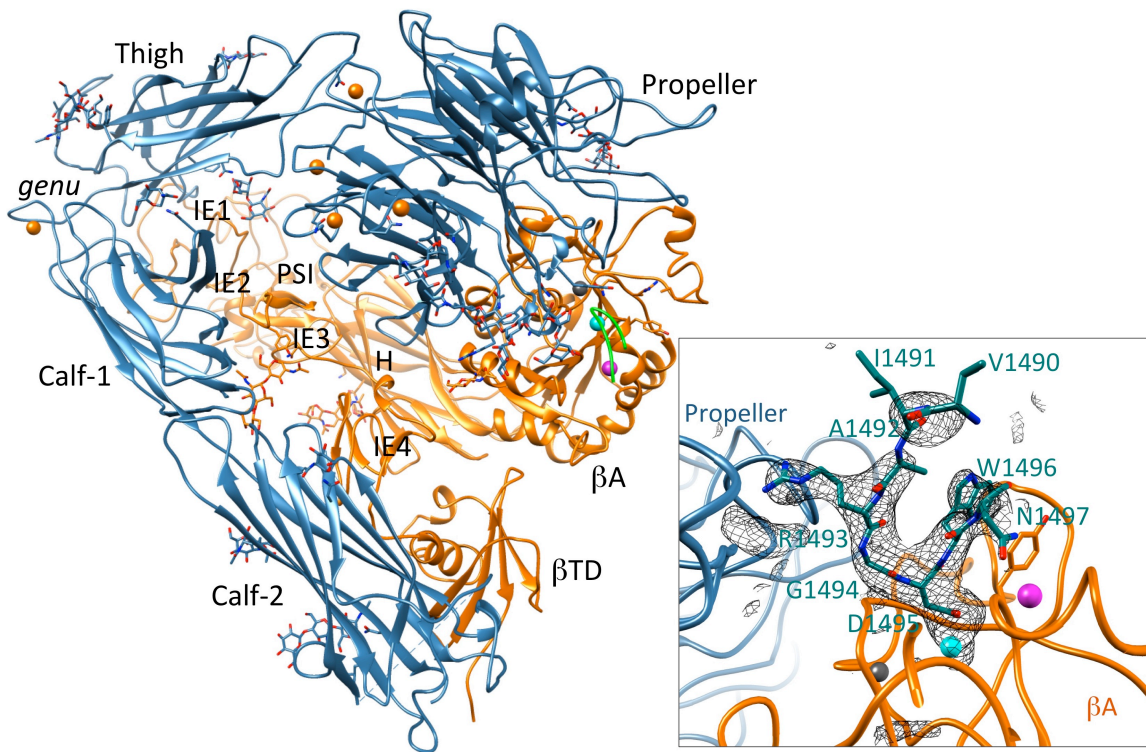
**Supplementary Figure 1. Formation of  $\alpha\text{V}\beta\text{3}$ -FN10 complexes and fitted standard curve used to measure their Stokes radii.** (a) Coomassie-stained reduced SDS-PAGE gels of size-fractionated  $\alpha\text{V}\beta\text{3}$  following its incubation with wFN10 (lanes 2, 5) or hFN10 (lanes 3, 6) in 1mM each of  $\text{Ca}^{2+}/\text{Mg}^{2+}$  or 1mM  $\text{Mn}^{2+}$ . 40 $\mu\text{g}$  of protein mixtures were loaded in lanes 2, 3, 5, and 6. Lanes 1, 4 molecular mass markers (small arrows from top to bottom: 250, 150, 100, 75, 50, 37, 25, 20, 15, 10 kDa).  $\alpha\text{V}\beta\text{3}$  formed a stable complex with hFN10 in  $\text{Ca}^{2+}/\text{Mg}^{2+}$  or  $\text{Mn}^{2+}$  but with wtFN10 only in  $\text{Mn}^{2+}$ . (b) Protein standards were Thyroglobulin (Thy), Ferritin (Fer), Albumin (Alb), Ovalbumin (Ova), chymotrypsinogen A (Chy), and ribonuclease A (Rib). Elution volumes were expressed as the square root of the log ratio of elution volume ( $V_e$ )/Void volume ( $V_o$ ). Linear regression curve fit is plotted.



**Supplementary Figure 2. Crystal structures and omit maps of  $\alpha$ V $\beta$ 3-FN10 complexes.** Ribbon diagrams of  $\alpha$ V $\beta$ 3 ectodomains bound to wtFN10 (a) or hFN10 (b). Both integrins are in the same orientation.  $\alpha$ V chain is in blue and  $\beta$ 3 chain is in light green (a) or pink (b). The four- $\alpha$ V domains (Propeller, Thigh, Calf-1 and Calf-2) and eight domains of  $\beta$ 3 (PSI,  $\beta$ A, Hybrid [H], IE1-4 and  $\beta$ TD) are marked in (a). Orange spheres represent the five  $Mn^{2+}$  ions at the base of the Propeller and at the genu of  $\alpha$ V.  $Mn^{2+}$  ions at LIMBS (gray), MIDAS (cyan) and ADMIDAS (magenta) are shown as spheres. Glycan carbons are indicated in the respective chain color. (c, d)  $\sigma$ A weighted *F<sub>o</sub>-F<sub>c</sub>* omit electron density (ED) maps of the ligand-binding site and surrounding area for  $\alpha$ V $\beta$ 3-wtFN10 (c) and  $\alpha$ V $\beta$ 3-hFN10 (d) structures contoured at 4.0  $\sigma$ , with FN10 protein omitted from the map calculation in each case.

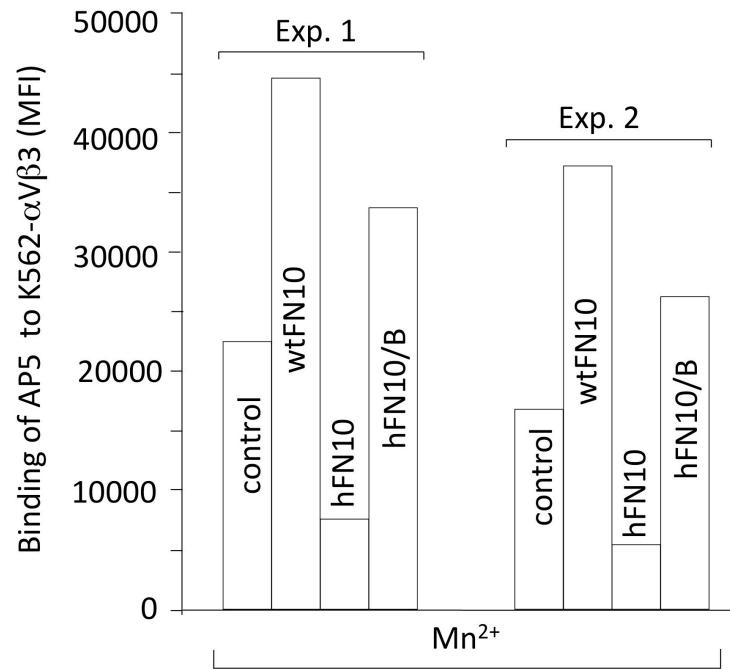


**Supplementary Figure 3. Significance of FN-glycan interaction in  $\alpha V\beta 3$ -wtFN10 structure and IE2/Thigh and  $\beta TD/\beta A$  contacts found in the  $\alpha V\beta 3$ -hFN10 structure.** (a) Adhesion (mean $\pm$ SD, n=3 independent experiments) of HEK293T cells expressing  $\alpha V\beta 3(N339S)$  or  $\alpha V(N266Q)\beta 3(N339S)$  to immobilized full-length FN in presence of  $Ca^{2+}/Mg^{2+}$ .  $A_{540}$ : absorbance at 540 nm. (b) Ribbon diagram showing the ionic and electrostatic interactions between  $\beta 3$ 's EGF-like domain 2 (IE2)(in pink) and  $\alpha V$ 's Thigh domain (in blue).  $\sigma A$  weighted  $2Fo-Fc$  map (gray, contoured at 1.0  $\sigma$ ) around the interacting residues is shown. The carboxyl oxygens of the  $\beta$ -genu residue Asp477 in  $\beta 3$ -subunit IE2 H-bond to a carboxyl and carbonyl oxygens of Glu547 in the Thigh domain. OE1 and OE2 of the IE2 residue Glu500 H-bond to Glu550 OD1 and to Thr553 OG, respectively, with OE2 also forming a salt bridge with Lys503 of Thigh domain. (c) Ribbon diagram showing the intrachain H-bond between Ser674 in the CD loop of the  $\beta TD$  and Gln319 in the  $\alpha 6$  helix of the  $\beta A$  domain.  $\sigma A$  weighted  $2Fo-Fc$  maps around Gln319 and the Asp672-Lys676 sequence is shown in gray contoured at 1.0  $\sigma$ .  $\alpha$  helices 1, 6 and 7 and strand F- $\alpha 7$  loop are labeled. The metal ions are colored as in supplementary Fig. 2.



**Supplementary Figure 4. Crystal structure of  $\alpha$ V $\beta$ 3-hFN10/B complex.** Ribbon diagram of the  $\alpha$ V $\beta$ 3 ectodomain bound to hFN10/B (in light green). Integrin domains are labeled. Orange spheres represent the five  $Mn^{2+}$  ions at the base of the Propeller and at the  $\alpha$ V genu. Glycan carbons are indicated in the respective chain color. Inset,  $\sigma_A$  weighted *Fo-Fc* omit map for of the ligand-binding site and surrounding area for the  $\alpha$ V $\beta$ 3-hFN10/B structure contoured at 4.0  $\sigma$ , with hFN10/B protein omitted from map calculation.  $Mn^{2+}$  ions at LIMBS, MIDAS and ADMIDAS are colored as in supplementary Fig. 2.





**Supplementary Figure 5. Expression of the AP5 epitope on FN10-M/B-bound  $\alpha V\beta 3$ .** Histograms showing binding of fluoresceinated AP5 mAb to K562- $\alpha V\beta 3$  cells in absence (control) or presence of wtFN10, hFN10 or hFN10/B in 1mM  $MnCl_2$ . Histograms show the results of two independent experiments (Exp).