Characterization of a novel Integrin binding protein that is essential for $\alpha_{\text{Iib}}\beta_3$ outside-in signaling and hemostasis

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No Conflict-of-Interest to disclose
Integrin activation involves proteins binding to the cytoplasmic domain of its β subunit
Identify integrin binding proteins

Proteins that are known to bind to integrin pulled down by the GST-β₃CD beads:

- Talin 1 and 2,
- Integrin-linked protein kinase,
- Kindlin 2 and 3,
- c-Src,
- α-actinin, etc.

Novel proteins pulled down by the GST-β₃CD beads:

- VPS33B, etc.

VPS33B is a member of the Sec-1 domain family, which plays a role in vesicle-mediated protein trafficking to lysosomal compartments and in membrane docking/fusion reactions of late endosomes/lysosomes.
Colocalization of VPS33B with $\alpha_{IIb}\beta_3$
GST-β integrin cytoplasmic domains (GST-βCD) pull down VPS33B from cell lysates
Mapping VPS33B binding site in β integrin

Pull down:

- GST
- GST-β3 716-730
- GST-β3 729-762
- GST-β3 744-762
- Input

IB: FLAG
GST-β integrin cytoplasmic domains (GST-βCD) pull down VPS33B from platelet lysates.
Overexpression of VPS33B in CHO cells potentiates \( \alpha_{IIb}\beta_3 \)-dependent cell spreading.
Generation of platelet-specific VPS33B conditional knockout mice
Platelets lacking VPS33B are defective in spreading on fibrinogen
Platelets lacking VPS33B fail to support clot retraction.
VPS33B is involved in $\alpha$-granule biosynthesis

**ARC syndrome:**
Arthrogryposis,
Renal dysfunction,
Cholestasis

Infection,
Hemorrhage,
No $\alpha$ granules in platelets
Platelet aggregation and ATP release are reduced in VPS33B knockout platelets
Tail bleeding times are prolonged in VPS33B knockout mice
Conclusion

- VPS33B is a novel binding protein of β integrins and is required for α_{IIb}β_3 outside-in signaling.
- VPS33B contributes to α-granule biosynthesis.
- VPS33B plays a role in platelet activation and hemostasis.
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GST-β integrin cytoplasmic domains (GST-βCD) pull down purified VPS33B
VPS33B is not required for dense granule and lysosome biosynthesis