Supporting Information

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SI Materials and Methods

Mouse Genetics. To generate *Cosmc*$^{-/y}$ in murine megakaryocytes (MC *Cosmc*$^{-/y}$), *Cosmc*$^{flox/flox}$ female mice were crossed with male C57BL/6-Tg (*Pf4-cre*) Q3Rsko/J, http://jaxmice.jax.org/strain/008535.html. *Pf4-cre* transgenic mice express a codon-improved Cre recombinase under the control of the mouse Pf4 (platelet factor 4), or Cxcl4, promoter. Cre recombinase expression is detected in the majority of megakaryocytes. *Pf4-cre* alleles were identified using PCR with specific primers (Table S3).

RT-PCR and PCR. The mRNA from platelets was isolated using FastTrack MAG mRNA Isolation Kit (Invitrogen), RT-PCR was performed with 5 ng of mRNA as template with a SuperScriptTM First-strand Synthesis System for RT-PCR (Invitrogen) according to the manufacturer's protocol. PCR was carried out with Phusion High-Fidelity PCR kit (New England Biolabs) in a 25-μL reaction. For cDNA of Cosmc, GPIbα, GPIIb, GPV, GATA-1, P-selectin, c-mpl, Pf4, VWF, and β-Actin, PCR was performed with primers (Table S2), and the PCR products were analyzed on a 1.5% (wt/vol) Tris·acetate EDTA agarose gel.

Flow Cytometry. Binding of von Willebrand factor (VWF) to endothelial/hematopoietic cells (EHC) *Cosmc*$^{+/y}$ and EHC *Cosmc*$^{-/y}$ platelets was analyzed as previously described (1, 2). Briefly, washed EHC *Cosmc*$^{+/y}$ and *Cosmc*$^{-/y}$ platelets (1.0×10^7/mL) in modified Tyrodes buffer were incubated with purified VWF (20 μg/mL; Stago) and botrocetin (1.0 μg /mL; Centerchem) at 22 °C for 30 min. After washing once, the platelets were incubated with 10 μg/mL FITC-labeled anti-VWF antibody (SZ-29, a kind gift from Changgeng Ruan, Jiangsu Institute of Hematology, Soochow University, Suzhou, Jiangsu, China) at 22 °C for 30 min, fixed with 1% paraformaldehyde (PFA), and analyzed by flow cytometry (Becton Dickinson FACScan). As negative controls, platelets were incubated with botrocetin in the absence of VWF. GPIbα expression on the platelet surface was evaluated using standard methods with FITC-conjugated rat anti-mouse GpIbα antibody (Emfret Analytics). For flow cytometry experiments with anti-Tn antibody, the anti-Tn antibody, a kind gift from Georg F. Springer (deceased), was directly FITC-labeled.

Platelet Spreading on VWF or Fibrinogen. Platelet spreading on immobilized VWF or fibrinogen after stimulation with botrocetin and bovine thrombin was performed as previously described (3). Briefly, chamber slides with microtiter wells (Nalge Nunc) were coated with 10 μg/mL purified VWF or 30 μg/mL fibrinogen (Haematologic Technologies). Washed platelets (1×10^7/mL) in modified Tyrode's buffer were stimulated with 1 μg/mL botrocetin, or 0.01 U/mL bovine thrombin and allowed to adhere and spread on VWF or fibrinogen-coated wells, at 37 °C for 90 min. The chamber slides were washed three times with PBS, cells were fixed, permeabilized, and stained with fluorescein-labeled phalloidin (Invitrogen) (4).

Transmission Electron Microscopy and Scanning Electron Microscopy. For transmission electron microscopy examination of platelets, washed murine platelets as described in *Materials and Methods* were resuspended in modified Tyrode's buffer. Platelets were fixed overnight in 2.5% (vol/vol) glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) at 4 °C. Platelets were washed with the same buffer and postfixed in 1% buffered osmium tetroxide, dehydrated through a graded ethanol series to 100%, and embedded in Eponate 12 resin (Ted Pella). Ultrathin sections were cut on a Leica UC6rt ultramicrotome (Leica Microsystems) at 70–80 nm, and counter-stained with 4% (wt/vol) aqueous uranyl acetate and 2% (vol/vol) lead citrate. Sections were examined using a Hitachi H-7500 transmission electron microscope (Hitachi High Technologies of America) equipped with a Gatan BioScan CCD camera.

For scanning electron microscopy examination, microscope cover glass (Fisherbrand) were coated with 30 μg/mL fibrinogen overnight then blocked with 5% (wt/vol) BSA. Washed platelets (1×10^6/mL) in modified Tyrode's buffer were stimulated with 0.01 U/mL bovine thrombin and allowed to adhere and spread on fibrinogen-coated wells at 37 °C for 30 min. The platelets were fixed in the same fixative for an additional 30 min then postfixed and dehydrated in the same manner and transferred into hexamethyldisilazane (HMDS). After air-drying of HMDS, cells on the cover glass were coated in a sputter coater with a gold target and then examined using a Topcon DS150 field emission scanning electron microscope.

Peanut Agglutinin and *Helix pomatia* Agglutinin Blotting. Washed platelets (1×10^7/mL) were resuspended in lysis buffer and extracts were obtained by adding 1% Nonidet P-40 and centrifuged. Then the GPIIb and GPVI were immunoprecipitated with its specific monoclonal antibody, respectively. After boiling the beads, the supernatants containing immunoprecipitated GPIIb or GPVI were treated with or without neuraminidase, analyzed by SDS/PAGE and transferred to nitrocellulose membranes. Western blotting with peanut agglutinin-HRP (1 μg/mL), or biotin labeled-*Helix pomatia* agglutinin (1 μg/mL) (Vector Laboratories) was performed as previously described (5).

1. Englund GD, Bodnar RJ, Li Z, Ruggeri ZM, Du X (2001) Regulation of von Willebrand factor binding to the platelet glycoprotein Ib-IX by a membrane skeleton-dependent inside-out signal. *J Biol Chem* 276:16952–16959.
2. Bodnar RJ, Xi X, Li Z, Berndt MC, Du X (2002) Regulation of glycoprotein Ib-IX-von Willebrand factor interaction by cAMP-dependent protein kinase-mediated phosphorylation at Ser 166 of glycoprotein Ib(beta). *J Biol Chem* 277:47080–47087.
3. Yin H, et al. (2008) Src family tyrosine kinase Lyn mediates VWF/GPIb-IX-induced platelet activation via the cGMP signaling pathway. *Blood* 112:1139–1146.
4. Gu M, Xi X, Englund GD, Berndt MC, Du X (1999) Analysis of the roles of 14-3-3 in the platelet glycoprotein Ib-IX-mediated activation of integrin alpha(IIb)beta(3) using a reconstituted mammalian cell expression model. *J Cell Biol* 147:1085–1096.
5. Wang Y, et al. (2010) Cosmc is an essential chaperone for correct protein O-glycosylation. *Proc Natl Acad Sci USA* 107:9228–9233.

Fig. S1. Abnormal expression of the O-glycan Tn antigen in platelet glycoproteins, instead of normal extended O-glycans, can lead to altered glycoprotein expression and function. O-glycosylation of proteins begins in the Golgi apparatus, where the T-synthase adds galactose form UDP-Gal to the common precursor GalNAcα1-Ser/Thr (Tn antigen) to generate a core 1 O-glycan Galβ1-3GalNAcα1-Ser/Thr. This product is then extended by addition of other sugars to generate normal O-glycans. In the absence of function of the molecular chaperone Cosmc, which is required for formation of active T-synthase, O-glycans on glycoproteins will not be elongated and they will express the Tn antigen, which can lead to glycoprotein misfolding, abnormal oligomerization, instability, proteolysis, loss-of-function, and abnormal recognition by glycan binding proteins (GBPs).

Fig. S2. RT-PCR of relative gene expression in platelets from three different mouse genotypes. The gene expression of GPIbα, GPIIb, GPV, GATA-1, Pf4, P-selectin, VWF, and c-mpl do not change compared with wild-type control. β-Actin is used as an internal control. See Table S2 for primer list.

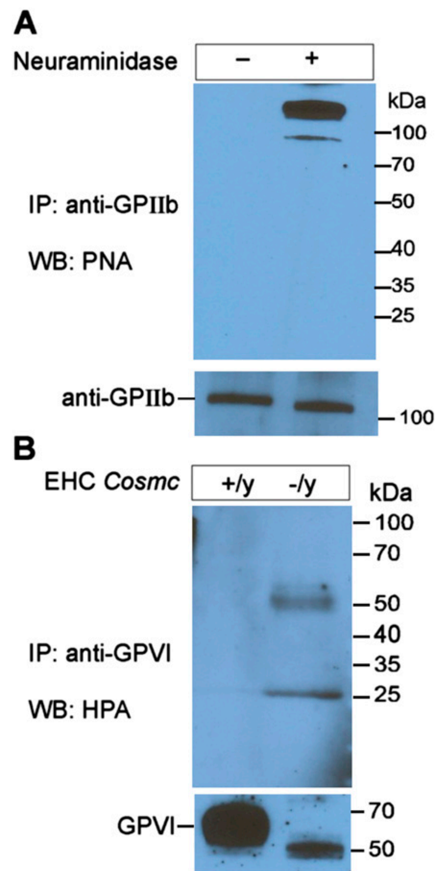

Fig. S3. GPIIbα and GPVI are O-glycosylated glycoproteins. (*A*) Peanut agglutinin (PNA) binds to wild-type GpIIb after neuraminidase treatment. In lower panel, GPIIbα is used as an internal control and appeared in both neuraminidase-treated and untreated materials. (*B*) *Helix pomatia* agglutinin (HPA) binds to GpVI of EHC *Cosmc*$^{-/y}$ platelets. Each lane represents a different genotype. (*Lower*) GPVI is used as an internal control and appeared in both EHC *Cosmc*$^{+/y}$ and EHC *Cosmc*$^{-/y}$ platelets.

Fig. S4. (*A*) Genotyping of *Pf4-cre* and *Cosmc*$^{flox/y}$ mice. Each lane represents a different mouse; + or – indicates the resulting genotype. (*Upper*) Tests for *Cosmc*$^{flox/y}$ and *Cosmc*; and (*Lower*) tests for *Pf4-cre*. See Table S3 for primer list. (*B*) RT-PCR of Cosmc and G3PDH in *Pf4-cre Cosmc*$^{flox/y}$ mice. Each lane represents a different mouse; + or – indicates the resulting genotype. (*C*) Western blot of T-synthase in *Pf4-cre Cosmc*$^{flox/y}$ mice. Each lane represents a different mouse; + or – indicate the resulting genotype.

Table S1. Complete blood counts in different genotypes of mice

Mouse	WBC (10^3/mm^3)	LYM (%)	GRA (%)	MON (%)	RBC (10^6/mm^3)	PLT (10^3/mm^3)	MPV (μm^3)
EHC *Cosmc*$^{+/y}$ (*n* = 7)	13.13 ± 3.7	75.69 ± 3.8	15.14 ± 2.8	9.17 ± 1.38	9.3 ± 1.14	787.43 ± 124.1	6.8 ± 0.07
EHC *Cosmc*$^{+/-}$(*n* = 6)	12.47 ± 1.1	81.15 ± 8.5	12.62 ± 5.3	6.83 ± 3.6	9.8 ± 1.6	497.88 ± 48* (E)	6.5 ± 0.2
EHC *Cosmc*$^{-/y}$ (*n* = 6)	15.8 ± 7.0	39.27 ± 27.5	51.53 ± 23.3	9.2 ± 4.3	7.8 ± 1.6	165.57 ± 57** (E)	7.2 ± 0.3*** (E)

E, equal variance; GRA, granulocytes; LYM, lymphocytes; MON, monocytes; MPV, mean platelet volume; PLT, platelets; RBC, red blood cells; WBC, white blood cells. *P = 0.00023, **P = 0.0000002, ***P = 0.0055. Values are ± 1 SD.

Table S2. RT-PCR primers for gene expression (See Figure S2).

Protein	Gene	Primer
Cosmc	*Cosmc(C1GALT1C1)*	5′-ATC ACT ATG CTA GGC CAC ATT AGG ATT GGA-3′
		5′-GGA GGT AAG AAA ACC AAT GCA TCA TTG AAA A-3′
GPIbα	*GP1BA*	5′-CCT GGA AGA AGC TCT GTT CCT CC-3′
		5′-CAT TGG TCT GCA GGC TCG TC-3′
GPIIb	*ITGA2B*	5′-AGG CAG AGA AGA CTC CGG TA-3′
		5′-TAC CGA ATA TCC CCG GTA AC-3′
GPV	*GP5*	5′-TGC CTA CGA ACC TCA CAC ACA TC-3′
		5′-GCT TAA CTT GAG CCC CAA GCA G-3′
GATA-1	*GATA1*	5′-AAA GAT GGA ATC CAG ACG AGG-3′
		5′-GTC AAG GCT ATT CTG TGT ACC-3′
P-selectin	*SELP*	5′-GTG CAG AGC GGT CAA ATG C-3′
		5′-CTG AGA GCT TTC TTA GCA GAG-3′
C-mpl	*C-MPL*	5′-CCC ACC TGG GAG AAA TGT GAA GAG-3′
		5′-CCG GTG TAG GTC TGG AAG CGA GGG-3′
Pf4	*PF4*	5′-CTC TTG ACA TGA GCG TCG CTG CGG-3′
		5′-CTT GAT CAC GTC CAG GCA GGT GAA-3′
VWF	*VWF*	5′-AAT TAC AAC GGC AAC AAG GGA GAC GAC-3′
		5′-CAA GAC AGA CTT CAC TGC ATT CTT CAT CC-3′
β-Actin	*ACTB*	5′-GTG GGC CGC TCT AGG CAC CAA-3′
		5′-CTC TTT GAT GTC ACG CAC GAT TTC-3′

Table S3. PCR primers for mouse genotyping

Genotype	Primer
Tie2-cre	5′-CAT CTG CCA CCA GCC AGC-3′
	5′-GGT CCA GCC ACC AGC TTG C-3′
Pf4-cre	5′-CCC ATA CAG CAC ACC TTT TG-3′
	5′-TGC ACA GTC AGC AGG TT-3′
Floxed Cosmc	5′-GCA ACA CAA AGA AAC CCT GGG-3′
	5′-TCG TCT TTG TTA GGG GCT TGC-3′