Supporting Information

Sialylation on O-glycans protects platelets from clearance by liver Kupffer cells

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SI Materials and Methods

Mice.

To generate mice lacking hematopoietic *C1galt1*, *C1galt1$^{f/f}$;Tie2Cre* mice, which lack *C1galt1* in both endothelial and hematopoietic cells, were bred with endothelial cell–specific *T-syn* transgenic mice (*Tie2-T-syn* Tg) that express *C1galt1* specifically in endothelial cells as described previously (1). The resulting mice (HC *C1galt1*$^{-/-}$) lack *O*-glycans in mature hematopoietic cells including platelets, since *Tie2-T-syn* rescues *O*-glycan expression in endothelial cells but not in differentiated hematopoietic cells. Mice were of C57BL/6J congenetic background and kept in a specific pathogen-free facility. Mice lacking asialoglycoprotein receptor 1 (*Asgr1*$^{-/-}$) were crossed with HC *C1galt1*$^{-/-}$ mice to generate HC *C1galt1*$^{-/-}$;*Asgr1*$^{-/-}$ mice. Mouse genotypes were determined by PCR of DNA from tail biopsies. Sex- and age-matched wild-type (WT) littermate mice were used for controls.

Platelet preparation

Mouse blood was collected via cardiac puncture with a heparinized syringe with 21G needle, transferred into a 1.5 ml tube containing 200 μL of Tris-buffered saline/heparin (TBS/Hep, 20 mM Tris/HCl; 137 mM NaCl, pH 7.3, 20 U/mL heparin), then 1:1 diluted with modified Tyrode's buffer (134 mM NaCl, 0.34 mM Na2HPO4, 2.9 mM KCl, 12 mM NaHCO3, 20 mM Hepes, pH 7.0, 5 mM glucose, 0.35% BSA). Blood was centrifuged at 100*g* for 8 minutes, and the supernatant was transferred to a new tube. Platelet-rich plasma (PRP) was prepared by centrifuging the supernatant at 200*g* for 6 min. PRP was centrifuged at 800*g* for 10 min to obtain a platelet pellet. The platelet pellet was washed twice in 1 mL Tyrode's buffer with 0.5 μM prostacyclin (Sigma).

Flow cytometry

Cell staining was performed in fluorescence-activated cell sorting (FACS) buffer (PBS with 0.5% FBS) with appropriate monoclonal antibodies (mAbs) and isotype-matched controls or lectins. mAbs included phycoerythrin (PE)-conjugated anti–mouse CD41 (MWReg30; Biolegend), PE- or PerCP/Cy5.5-conjugated anti-mouse F4/80 (BM8; Biolegend), biotinylated anti-Tn (Ca3638; mouse IgM), and anti-sTn (3F1; mouse IgG, SBH Sciences), anti-mouse CLEC4F (R&D systems), anti-Asgr1, and FITC-conjugated anti-goat IgG (Abcam). For resialylation of Tn on HC *C1galt1*$^{-/-}$ platelets, 50 μL of total reaction mixture containing 4×10^8/ml of HC *C1galt1*$^{-/-}$ platelets in modified Tyrode's buffer and 2 mM cytidine-5'-monopho-N-acetylneuraminic acid sodium salt (CMP-SA) (EMD Millipore) were incubated with 9 μg/ml of recombinant human alpha-N-acetylgalactosaminide α-2,6-sialyltransferase 1 (ST6GalNAcI) (SBH Science) at 37°C for 30 min, and then the platelets were stained by anti-sTn antibody to detect sialy-Tn by flow cytometry. Lectins were biotinylated Sambucus Nigra lectin (SNA), Maackia Amurensis lectin II (MAL II), and Ricinus Communis agglutinin I (RCA I), Vicia Villosa lectin (VVL), as well as fluorescein-labeled VVL, fluorescein-labeled concanavalin A (Con A), which were purchased from Vector Laboratories. In some experiments, 2×10^7 WT platelets in Tyrode's buffer were treated with 2 μl PNGase F (New England Biolabs) at 37°C for 18 h, treated with α2-3, 6, 8 neuraminidase (New England Biolabs, 2,000 μ/ml or Roche, 0.3 U/ml) at 37°C for 5 min, or treated with 30 μg/ml *O*-sialoglycoprotein endopeptidase (OSGE, Cedarlane) at 37^{o}C for 30 min. And then the platelets were washed with Tyrode's buffer and stained by MAL II or PE-conjugated antibody to mouse GPIbα (clone Xia.G5). All samples were analyzed by a FACSCalibur (BD Bioscience).

Platelet count, survival, and clearance assays

Platelets were counted by flow cytometry using PE anti–mouse CD41 staining and Polybead® microspheres 3.00 μm (Polysciences) as internal standards. To detect reticulated platelets in circulation, 4 μl whole blood was diluted in 1:30 in FACS buffer, then incubated with 2 μg/ml PE anti–mouse CD41 and 10 ug/ml thiazole orange (Sigma-Aldrich) for 20 min at room temperature in dark. Percentage of reticulated platelets was defined as PE-anti-CD41 and Thiazole Orange positive cells in 10,000 platelets analyzed (2). For survival assay, isolated platelets from donor mice were biotinylated by incubation with 1 mg sulfo-NHS-biotin (Pierce, Rock-ford, IL) in 1 mL PBS (pH 8.0) for 30 minutes at room temperature. 10^8 biotinylated platelets were then injected into recipient mice via the retro-orbital vessel plexus. Also, we performed in vivo biotin labeling method as previously described (3). Briefly, 0.6 mg of sulfo NHS-biotin was injected into WT or HC *C1galt1*$^{-/-}$ mice. After transfusion of biotin-labeled platelets or biotin reagent, 2 μl blood was collected from tail vein at various time points, and mixed 1:50 with buffer (116 mM NaCl, 13.6 mM tri-sodium citrate, 8.6 mM Na_2HPO_4, 1.6 mM KH_2PO_4, 0.9 mM EDTA, 11.1 mM glucose), followed by washing twice in Hank's balanced salt solution (HBSS) containing 0.5% FBS and 1 mM EDTA. The percentage of biotinylated platelets was determined by flow cytometry via double staining with streptavidin-PE (BD Biosciences) and FITC anti–mouse CD41 (BD Biosciences).

For competitive survival assay, 5×10^7 CellTracker Orange CMRA (red fluorescence, Life Technologies)-labeled WT platelets and 5×10^7 CMFDA (green fluorescence, Life Technologies)-labeled HC *C1galt1*$^{-/-}$ platelets were competitively transfused to WT or *Asgr1*$^{-/-}$ recipient mice. After transfusion, 2 μl blood was collected from tail vein at various time points and CMRA or

CMFDA labeled platelet survival was detected by flow cytometry. Fluorescent platelet survival rate at 2 min after transfusion was set as 100%. 120 min later, recipients were sacrificed and subsequently subjected to vascular perfusion. The liver, spleen and other control organs were then collected for confocal imaging analysis.

Desialylation and flow cytometry assay to evaluate platelet uptake by Kupffer cells

To prepare desialylated platelets, WT platelets (10^9/ml) were treated with α2-3, 6, 8 neuraminidase (New England Biolabs, 2,000 u/ml) at 37°C for 5 min. Platelets were then washed twice in HBSS containing 0.5% FBS and 1 mM EDTA and resuspended in Tyrode's buffer with 0.5 μM prostacyclin. RCA I, which binds to desialylated galactose, was used to confirm the desialylation. Treated, mock-treated, and HC *C1galt1*$^{-/-}$ platelets were labeled with CMFDA and then infused into WT or *Asgr1*$^{-/-}$ recipients, respectively (5×10^7 platelets/mouse, 8 - 10 week old). 90 min later, recipients were euthanized and then perfused through systemic circulation with 10 ml HBSS buffer (without $Ca^{2+}Mg^{2+}$) with 20 mM HEPES. The liver and spleen were collected for confocal imaging analysis. Meanwhile, 1.5 g liver from each recipient was minced into small pieces, dispersed in 10 ml perfusion buffer containing 30 μg/ml liberase (Roche) and 5 mM $CaCl_2$ (Sigma), and incubated at 37°C for 30 min. 10 ml perfusion buffer with 2% FBS and 5 mM EDTA was then added to stop digestion and the lysates were filtered through a 100 μm cell strainer. Non-parenchymal cells were isolated after mixing with 33% percoll by centrifugation for 30 min at 800*g* at room temperature. Percentage of fluorescent platelet associated Kupffer cells was defined as CMFDA and F4/80 double positive population based on flow cytometry analysis.

Macrophage depletion

Mice were injected intravenously with 200µl clodronate liposome (FormuMax) per 20 - 25 g body weight 2 days before platelet transfusions based on our published methods (4). Control mice were injected with liposome control. Depletion efficiency was confirmed by flow cytometry analysis of peripheral monocytes and staining for macrophages using F4/80 antibody + anti rat Alexa Flour 488 in liver sections of treated mice.

For clearance assay, 5×10^7 CMRA labeled WT platelets and 5×10^7 CMFDA labeled HC *C1galt1*$^{-/-}$ platelets were competitively transfused to WT or *Asgr1*$^{-/-}$ recipient mice with or without macrophage depletion. 2 µl blood was collected from tail vein at various time points after transfusion and the ratio of transfused HC *C1galt1*$^{-/-}$:WT platelets was detected by flow cytometry.

Measurement of platelet binding to Kupffer cell and analyzing platelet ingestion by HepG2 cell

Isolation of non-parenchymal cells from liver was describes previously in this paper. Non-parenchymal cells were cultured in 6 well-plate. After 2 h, non-adherent cells were removed and adherent cells were kept to be cultured, of which more than 90% were F4/80 and CLEC4F double positive Kupffer cells. For siRNA knockdown of CLEC4F, nearly 40-50% confluent Kupffer cells were transfected with scramble or CLEC4F specific short interfering RNA (OriGene) using siTran siRNA transfection reagent (OriGene). After 72 h, the transfected Kupffer cells were harvested using cell dissociation solutions (Sigma). Reduction of surface expression of CLEC4F was analyzed by flow cytometry. $5x10^5$ Kupffer cells were incubated

with PKH26 (Red fluorescence dye, Sigma)-labeled mock-treated or desialylated WT, or HC *C1galt1*$^{-/-}$ platelets ($1x10^7$) with gentle agitation at 37^{o}C. After 30 min, the incubation was stopped washing HBSS without calcium. For competitive inhibition assay, Kupffer cells were pre-incubated with or without 100 μg/ml of Gal polymer or GalNAc polymer (Glycotech) 30 min at room temperature before adding labeled mock-treated or desialylated WT, or HC *C1galt1*$^{-/-}$ platelets respectively. Platelet binding to Kupffer cell was measured and shown as fluorescence intensity by a FACSCalibur (BD Biosciences).

For platelet ingestion by hepatocyte, we followed the published method (5). Briefly, human hepatocyte cells (HepG2 cell line, 1×10^5/well, ATCC) were incubated with CMFDA (green)-labeled WT or HC *C1galt1*$^{-/-}$ platelets (10^7/well) with gentle agitation at 37°C. After 30 min, the incubation was stopped by washing with HBSS without calcium. The cells were detached by gentle scratching and, stained with PE anti-mouse CD41. HepG2 cells with ingested platelets acquire green fluorescence. HepG2 cells with platelets on their surfaces were stained with PE anti-mouse CD41. Ingested platelets by HepG2 were identified as CMFDA single positive events. Data were analyzed by LSRII flow cytometer (BD Biosciences).

Western blot

Isolation of bone marrow cells was described previously (6). Isolated bone marrow cells, hepatocytes, and Kupffer cells were lysed by the lysis buffer, 1% Triton X-100, 125 mM NaCl, 50 mM Tris, pH 7.4, 10 mM EDTA, 2 mM PMSF, 0.1 % SDS with a protease inhibitor cocktail (1:100; Thermo Fisher Scientific). The lysates were analyzed by Western blotting using rabbit Ab against C1galt1 (Thermo Fisher) or Asgr1 (Sino Biological), or mouse Ab against GAPDH

(Thermo Fisher) followed by HRP-conjugated goat anti-rabbit IgG or HRP-conjugated goat anti-mouse IgG, respectively.

Histology, immunostaining, and confocal imaging

Histology was analyzed by hematoxylin and eosin (H&E) staining of paraffin-embedded tissues. Immunohistochemical staining was performed based on our published methods.[23] For immunofluorescence imaging, the following combinations were used: 9 μm cryosections of tissues were stained with chicken anti-albumin (Abcam) + donkey anti-chicken Cy5 (Jackson ImmunoResearch) or hamster anti-mouse CD31 + donkey anti-hamster DyLight 649 (5 μg/ml); liver cryosections from WT and HC *C1galt1*$^{-/-}$ were stained for platelets (rabbit anti-thrombocyte serum + anti-rabbit AF488), Kupffer cells (rat anti-F4/80 + anti rat Dylight 550, Abcam) and hepatocytes (chicken anti-albumin + anti chicken Cy5), and nuclei (TO-PRO3, ThermoFisher). In some experiments, thick WT and HC *C1galt1*$^{-/-}$ liver cryosections (50 μm thickness) were stained for confocal imaging analysis. Samples were analyzed by a confocal microscope (Zeiss 710 Microscope System). Z-stack images were collected at 2-μm steps with sequential laser excitation to eliminate bleed-through and with confocal parameters selected to minimize the thickness of the calculated optical section with Zeiss software ZEN. Volume images from the confocal tif image datasets were further processed with IMARIS software (Bitplane AG) and presented as maximum intensity projections of the z stacks or for three-dimensional views. Total number of bound platelets in the liver was counted using 20x magnification confocal image. To define association of the platelets with the Kupffer cells, and inside or outside the Kupffer cells, 40x 3D z stacked image was reconstructed and rotated to view the platelet location through various angles, and orthogonal projection images of z stacks were used.

Spinning-disk confocal intravital microscopy

Liver spinning-disk confocal intravital microscopy was performed following previously published methods (7, 8). Under anesthesia, mouse body temperature was maintained at 37°C by a heating pad (Physi-temp Instrument, Inc). An abdominal midline incision was made, the liver was mobilized carefully onto a custom-made stage, and superfused with thermo-controlled (37°C) bicarbonate-buffer saline. WT, HC *C1galt1*$^{-/-}$, or desialylated WT platelets were labeled with CellTracker Deep Red (Life Technologies). 5×10^7 labeled platelets were injected through a cannulated carotid artery to the WT recipients. For AMR blocking experiments, 10 mg fetuin or asialofetuin was intraperitoneally injected into recipient mice 30 min prior to platelet infusion. Kupffer cells were stained by PE anti-F4/80 mAb. The adhesion of the platelets to the sinusoids was visualized at 20 s (early phase) and 30 min (late phase) after platelets were infused to recipients using a Nikon ECLIPS E600-FN upright microscope, equipped with an Olympus 20x/1.00W XLUM Plan FL water immersion objective lens, coupled to a confocal light path based on a modified Yokogawa CSU-X1 head (Yokogawa Electric Corporation) (6). 488, 561, and 642-nm excitation lasers were sequentially controlled and merged into a single optic cable, and introduced into the CSU-X head. Fluorescence signals were detected through three emission filters of ET525/50, ET605/52, and ET700/75 controlled by an ASI FW-1000 Filterwheel (Applied Scientific Instrumentation), and captured using a 512 x 512 pixel back-thinned EMCCD camera (C9100-13; Hamamatsu). The National Institute of Health acquisition software Micromanager controlled the spinning-disk confocal microscope, and captured the images/video clips. The results were analyzed with ImageJ software.

Gene expression profiling in Kupffer cells

Non-parenchymal cells were isolated from liver as described, and then F4/80 positive Kupffer cells were sorted by Moflo XDP cell sorter (Beckman Coulter). Total RNA from Kupffer cells, WT and *Asgr1*$^{-/-}$ livers was extracted with the RNeasy mini kit (Qiagen) according to the manufacturer's protocol. Expression of Kupffer cell-specific genes *Asgr1*, *EMR1*, *CD163* and *CD68* as well control *GAPDH* were analyzed by real-time PCR using primers below.

Gene name	Forward primer sequence	Reverse primer sequence
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC
Asgr1	CTCCTTAGCCTGGGCTCTTC	CCGGAGTTGGGAATTTTGGGA
EMR1	GTGACTCACCTTGTGGTCCT	CAGACACTCATCAACATCTGCG
CD163	CCTCCGTGGTCATCCACTTT	GGACAAACCTTTTACAACCAGGA
CD68	ACTTCGGGCCATGTTTCTCTT	GGGGCTGGTAGGTTGATTGT

Platelet glycan analysis

Platelets isolated from WT or HC *C1galt1*$^{-/-}$ mice were dissolved in Ambic buffer (50 mM ammonium bicarbonate). The samples were denatured by heating for 5 min at 100°C and then digested with trypsin (37°C, overnight). After tryptic digestion, the *N*-glycans were released using PNGase F (New England BioLabs). The *N*-linked glycan fraction was eluted through a C^{18} reversed phase cartridge. Then the *O*-glycans were released by β-elimination. Both *N*-glycans and *O*-glycans were permethylated and analyzed by Matrix-assisted laser-desorption time-of-flight mass spectrometry (MALDI-TOF)-MS/MS in the reflector positive ion mode.

To estimate the total sialic acids on the human or murine platelet surface glycoproteins, seven representative glycoproteins on platelets, i.e., glycoproteins Ibα, Ibβ, αIIbβ3, VI, IX, and V (9, 10), were analyzed. Known and potential *N*- or *O*-glycosites of each glycoprotein (G_i) were

searched by Uniprot database or predicted according to online glycosylation site prediction servers (NetNGlyc 1.0 and NetOGlyc 4.0). Based upon glycan structures by MALDI-TOF-MS/MS analysis, the maximum of three and two non-reducing ends of *N*- and *O*-glycans could be sialylated respectively. Based on previously reported copy numbers of each glycoprotein on mouse platelets (10, 11). The estimation of total sialic acids on *N*- and *O*-glycans per platelet was measured using the following formulas:

$$S_i = C_i \times G_i \times E_i$$

$$S = \Sigma S_i$$

where S is the total sialic acids on *N*- or *O*-glycans per platelet, and S_i is the sialic acids on *N*- or *O*- glycans of glycoprotein I, C_i and G_i are the copies and *N*- or *O*- glycosites on glycoprotein i, respectively, and E_i is 3 for *N*-glycans and 2 for *O*-glycans. For human platelets, the relative levels of glycoproteins were calculated respectively by log 10 normalized iBAQ intensity based on LC-MS/MS analysis according to Proteomics DB (12).

Statistics

The unpaired Student *t* test was used to determine *P* values as indicated in the figures.

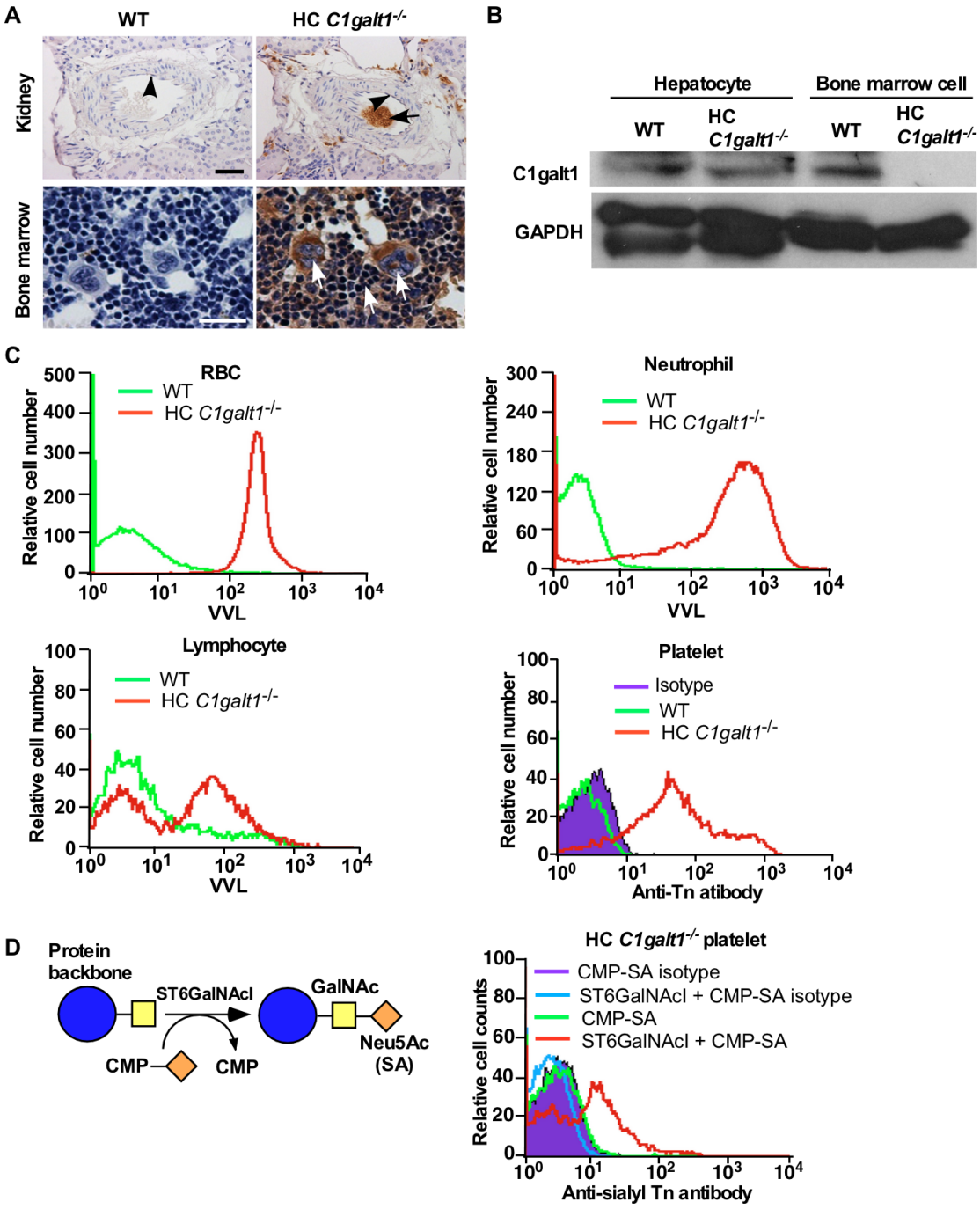

Fig. S1. Characterization of the specificity and efficiency of C1galt1 deletion in HC *C1galt1*$^{-/-}$ mice. (A) Immunohistochemical staining of WT and HC *C1galt1*$^{-/-}$ paraffin tissue sections with anti-Tn mAb. Brown indicates positive staining. Arrows marks Tn-positive blood cells including megakaryocytes of HC *C1galt1*$^{-/-}$ mice. Arrowheads indicate Tn-negative endothelial cells in the

kidney vessels. Scale bar, 50 μm. (B) Western blot analysis of expression of C1galt1 using WT or HC *C1galt1*$^{-/-}$ hepatocytes or bone marrow (BM) cells. GAPDH is a loading control. (C) Flow cytometric analysis of Tn expression on peripheral red blood cells (RBC), neutrophils, and lymphocytes defined by FSC x SSC (size profile) on FACScan, and platelets were defined by anti-CD41. The Tn antigen was detected by either VVL (Vicia Villosa lectin), a lectin that recognizes Tn-antigen specifically, or an anti-Tn antibody. (D) Flow cytometric analysis of sialyl Tn expression by anti-sialyl Tn antibody on HC *C1galt1*$^{-/-}$ platelets with or without in vitro sialylation. Addition of sailic acids to the GalNAc on *C1galt1*$^{-/-}$ platelets was achieved by incubating CMP-sialic acids with alpha-N-acetylgalactosaminide α-2,6-sialyltransferase 1 (ST6GalNAcI). The control group was HC *C1galt1*$^{-/-}$ platelets incubated with CMP-sialic acids without ST6GalNAcI.

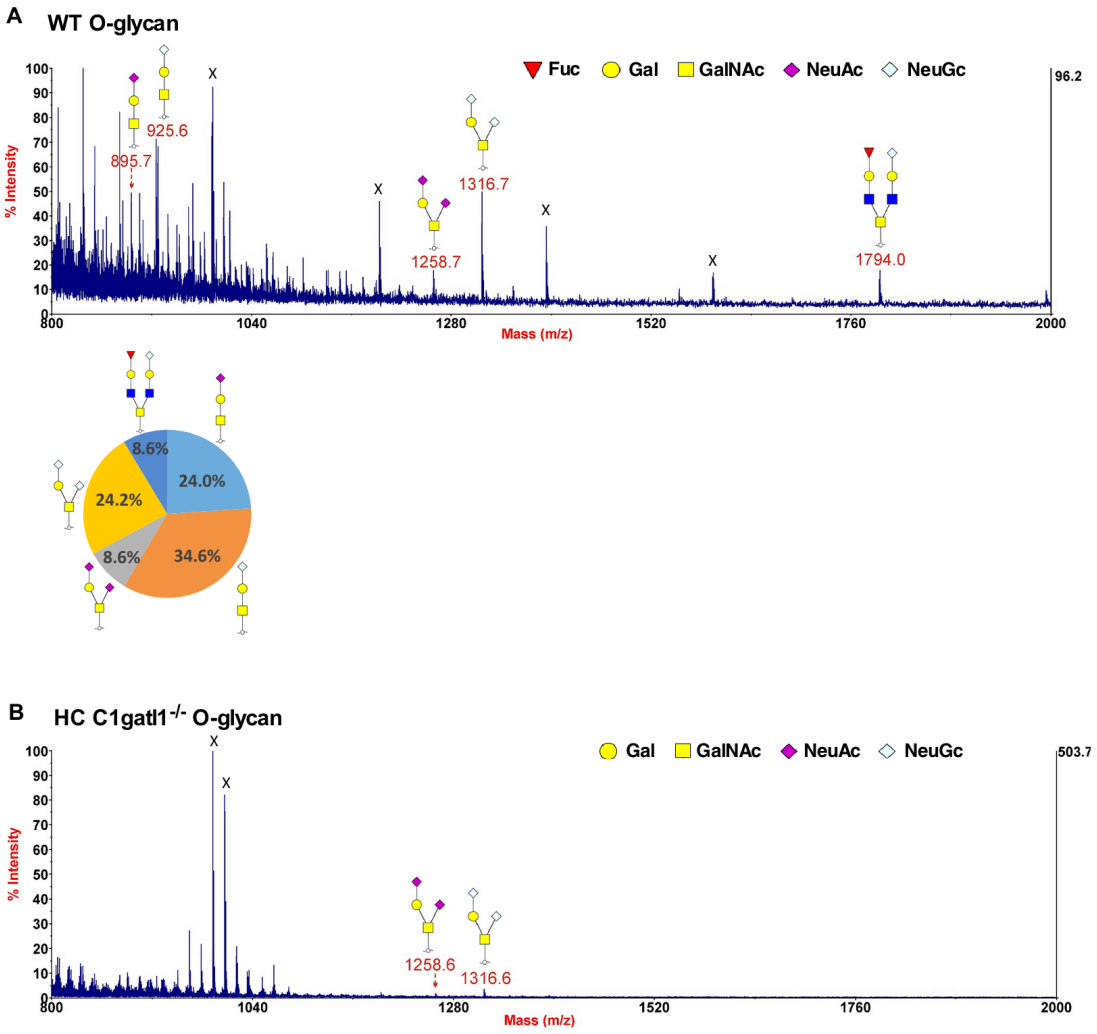

Fig. S2. MALDI-TOF/TOF-MS analysis of *O*-glycan structures in (A) WT and (B) HC *C1galt1*$^{-/-}$ platelets. Annotated MALDI-TOF mass spectrum of permethylated *O*-glycans from sample WT or HC C1galt1$^{-/-}$ platelets was positive mode. All annotated ions were $[M+Na]^+$. The number indicated above the peak in the spectra is the m/z value of the ion that has been detected by the mass spectrometer. Each section in the pie chart indicates the percentage of corresponding glycan, the percentage was obtained by comparing the peak height of this glycan to that of all glycans in this sample. "X" Additional signals were verified that these were not derived from carbohydrates and did not produce any typical fragmentations representative of permethylated oligosaccharides .

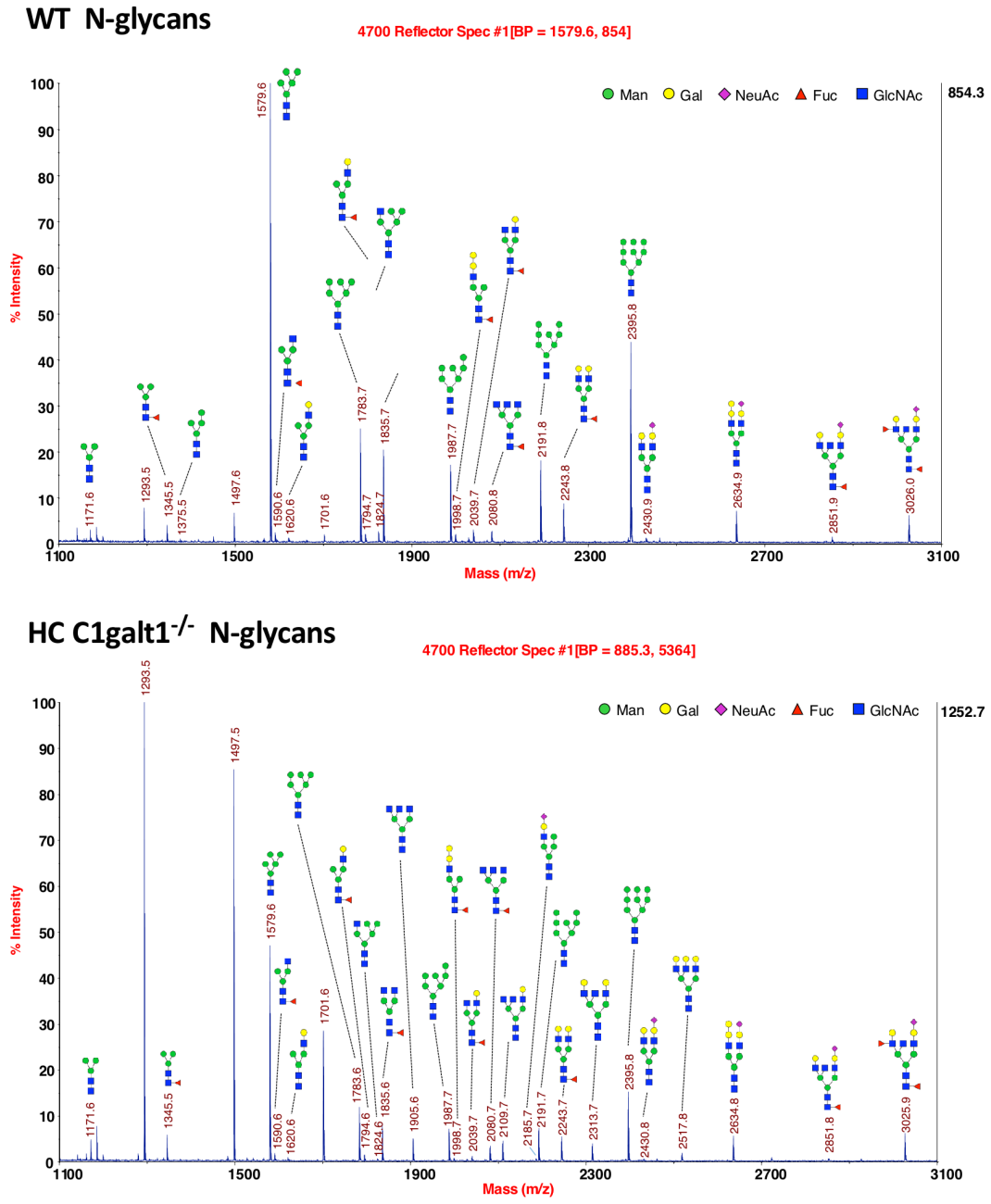

Fig. S3. MALDI-TOF/TOF-MS analysis of N-glycan structures in WT and HC *C1galt1*$^{-/-}$ platelets.

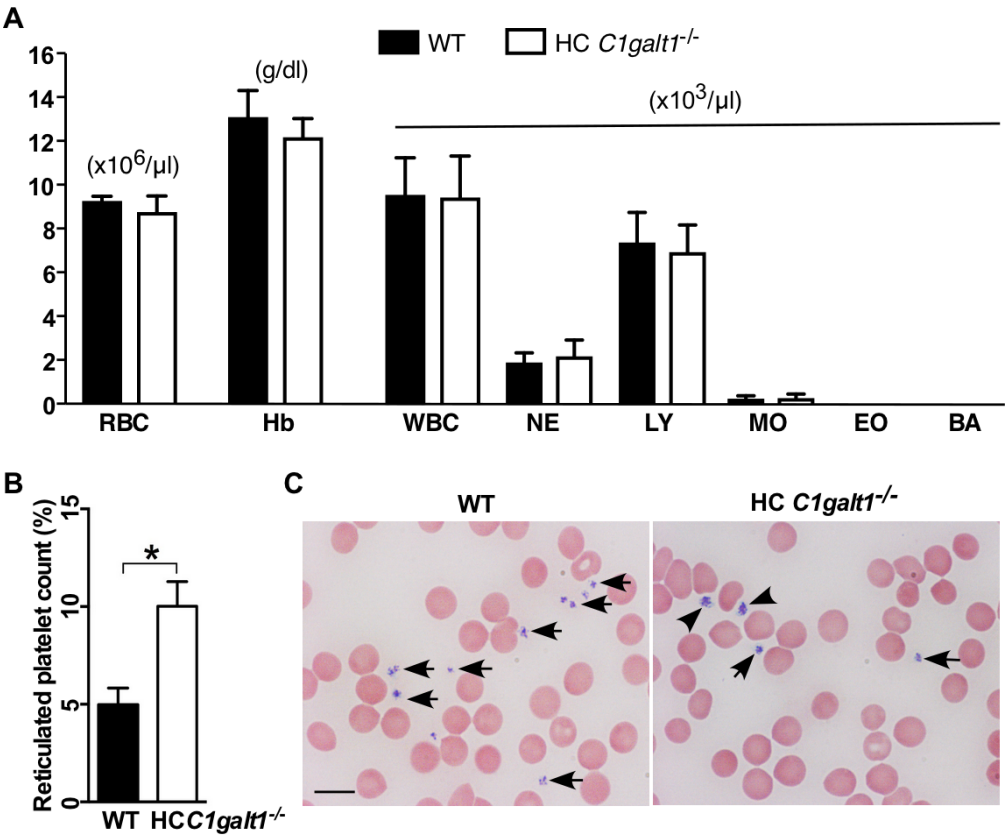

Fig. S4. Analysis of peripheral blood cells in HC *C1galt1*$^{-/-}$ mice. (A) Peripheral blood count. RBC, red blood cell; Hb, hemoglobin; WBC, white blood cell; NE, neutrophil; LY, lymphocyte; MO, monocyte; EO, eosinophil; BA, basophil. Means ± SD, n = 10 mice per genotype. (B) Peripheral reticulated platelet (%) in WT and HC *C1galt1*$^{-/-}$ mice. Means ± SD are shown for numbers of platelets and reticulocytes, n = 5 mice per genotype. **P* < .05. (C) Wright-Giemsa stained peripheral blood smears from WT and HC *C1galt1*$^{-/-}$ mice. Arrow indicates platelets with normal size, and arrowhead marks giant platelets. Scale bar, 10 μm.

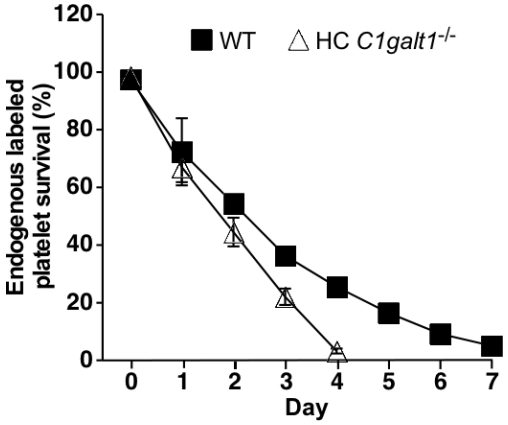

Fig. S5. Endogenous platelet clearance in WT or HC *C1galt1*$^{-/-}$ mice. Platelet clearance was measured by in vivo biotin labeling method. Sulfo NHS-biotin was intravenously injected (0.6 mg/mouse) into WT or HC *C1galt1*$^{-/-}$ mouse. 2 μl blood was then collected from tail vein at indicated time points and analyzed by flow cytometry for biotinylated platelets (gated on anti-CD41 staining). Platelet survival rate was determined by the ratio of the number of biotinylated platelets at a given time to the initial number of biotinylated platelets (day 0). Means ± SD are shown for percentage of survival platelets in each genotype. n = 3 mice per genotype.

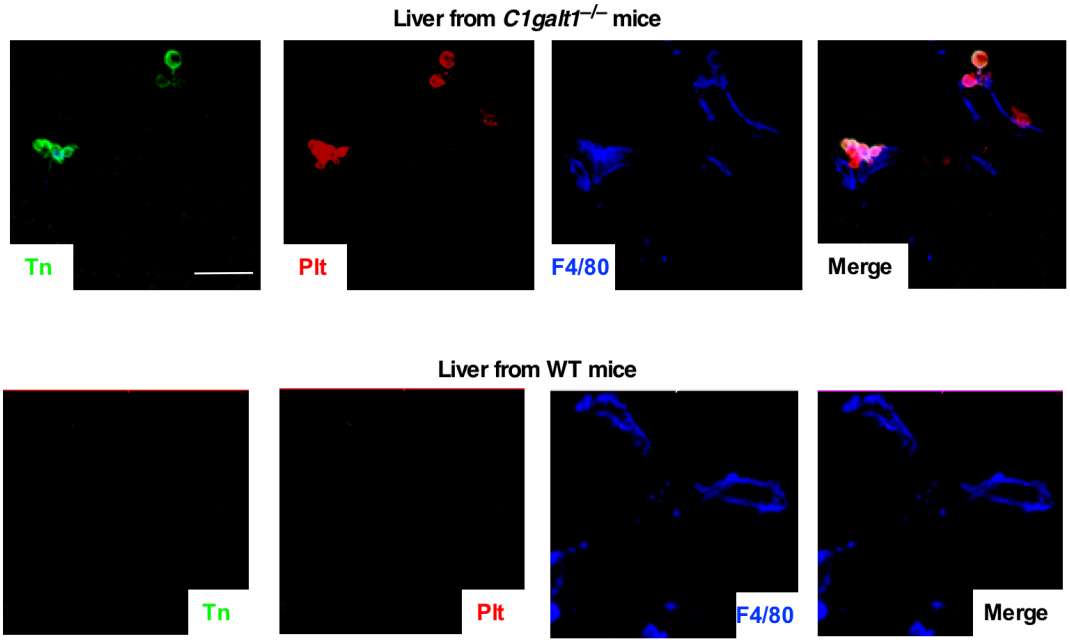

Fig. S6. Representative images of immunostaining of liver sections from HC *C1galt1*$^{-/-}$ or WT mice. The tissue sections were stained by biotin anti-Tn (Tn), rabbit anti-thrombocyte serum (Plt), and rat anti-F4/80 (Kupffer cell), followed by Alexa 488 streptavidin, Dylight 550 anti-rabbit, or Dylight 649 anti-rat, respectively. Scale bar, 10 μm

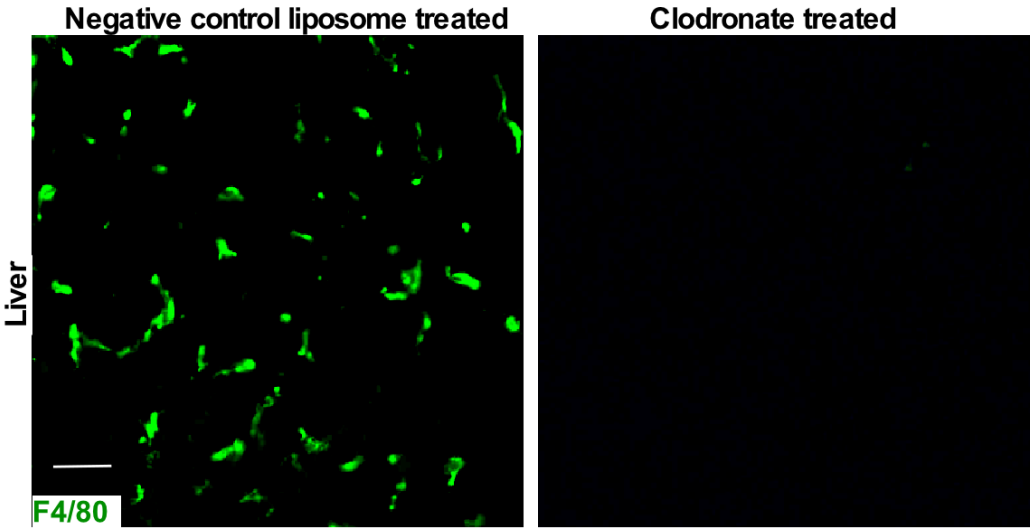

Fig. S7. Representative confocal images of immunostaining of liver sections of WT mice treated with the macrophage depletion reagent Clodronate and the negative liposome. F4/80, macrophages/Kupffer cell marker. Scale bar, 50 μm.

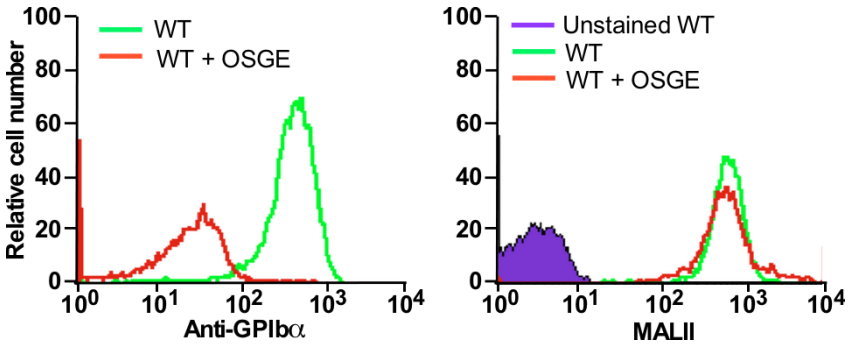

Fig. S8. Flow cytometric analysis of anti-GPIbα (left) or MALII (right) binding to WT platelets with or without OSGE treatment.

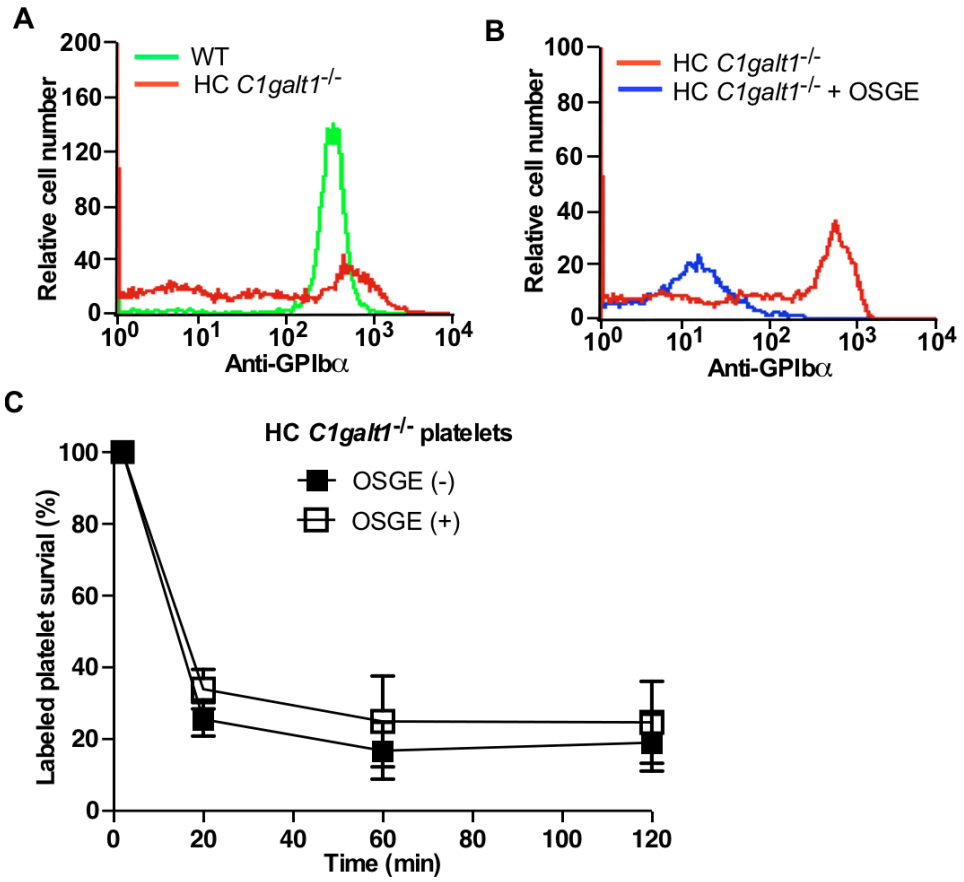

Fig S9. Flow cytometric analysis of anti-GPIbα binding to WT or HC *C1galt1*$^{-/-}$ platelets (A), or to HC *C1galt1*$^{-/-}$ platelets with or without OSGE treatment (B), and transfused HC *C1galt1*$^{-/-}$ platelet survival rate with or without OSGE treatment (C). 5×10^7 of non-treated CMRA-labeled (red) platelets and 5×10^7 of OSGE-treated CMFDA-labeled platelets (green) were injected to WT mice. The percentage of labeled platelets was measured by flow cytometry in recipient's peripheral blood at different time points (2 min, 20 min, 60 min and 120 min). Platelet survival rate (%) was determined by the ratio of the percentage of labeled platelets at a given time to the percentage of labeled platelets at 2 min (n = 3).

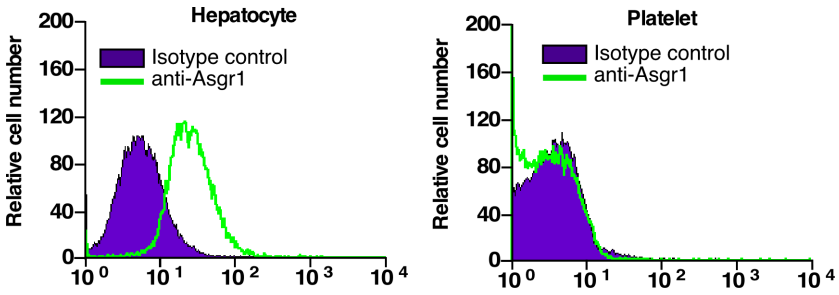

Fig. S10. Flow cytometric analysis of AMR (Asgr1) on WT hepatocytes or platelets.

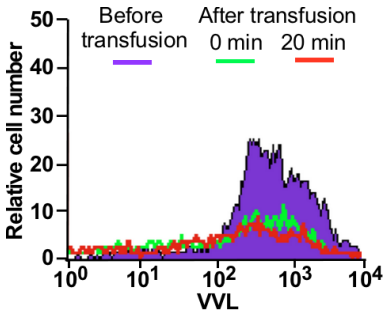

Fig. S11. Tn expression on HC *C1galt1*$^{-/-}$ platelets before and after transfusion analyzed by flow cytometry. Biotinylated HC *C1galt1*$^{-/-}$ platelets (6 x 10^7) were transfused into *Asgr1*$^{-/-}$ mice (n = 3) to determine if expression of Tn antigen (GalNAc) on HC *C1galt1*$^{-/-}$ platelets was altered by potential glycosylation during circulation. Blood samples were collected at 0 min and 20 min after transfusion. Biotinylated platelets were stained by FITC-VVL and PE-streptavidin before and after transfusion.

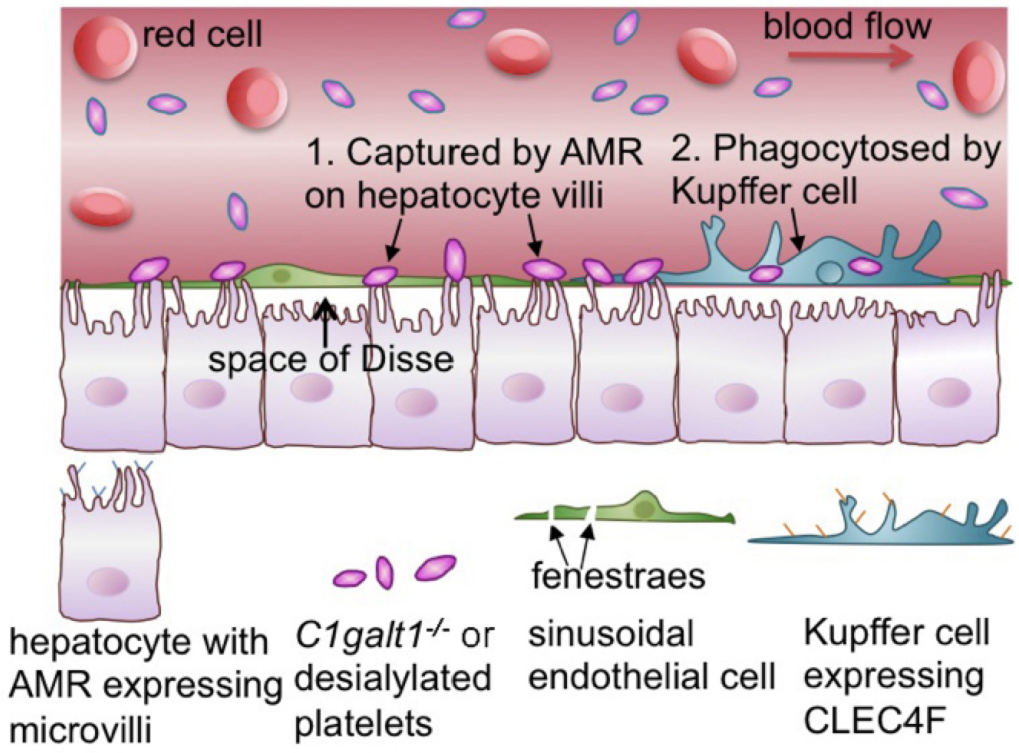

Fig. S12. Diagram depicting a two-step process for desialylated or HC *C1galt1*$^{-/-}$ platelets to be captured by the AMR on hepatocytes under dynamic flow conditions and subsequently phagocytosis by the Kupffer cell mediated by the CLEC4F in the liver.

Table S1. Estimated number of sialic acids per platelet from mouse

Protein name	Copies/platelet (10^4)[a]	Potential glycosites[b]		Total sialic acids (10^4)	
		N-	O-	N-	O-
GP1bα	2.5	0	120	0	600.0
GP1bβ	2.5	1	1	7.5	5.0
Itgb3	4.0–8.0	6	16	72.0	128.0
Itga2b	4.0–8.0	5	10	60.0	80.0
GPVI	0.4	2	13	2.2	9.6
GPIX	2.5	1	1	7.5	5.0
GPV	1.3	7	3	26.3	7.5
Sum				175.5	835.1

Note: [a] Copy numbers of each glycoprotein were based on previously reported on mouse platelets (10, 11). [b] Known and potential *N*- or *O*-glycosites of each glycoprotein were searched by Uniprot database or predicted according to online glycosylation site prediction servers (NetNGlyc 1.0 and NetOGlyc 4.0, http://www.cbs.dtu.dk/services/).

Table S2. Estimated number of sialic acids per human platelet

Protein name	Log 10 normalized iBAQ intensity[a]	Potential glycosites[b]		Total sialic acids	
		N-	*O*-	*N*-	*O*-
GP1bα	6.85	4	70	82.2	959.0
GP1bβ	6.85	1	3	20.6	41.1
Itgb3	7.02	7	13	147.4	182.5
Itga2b	7.42	5	4	111.3	59.4
GPVI	5.44	1	11	16.3	119.7
GPIX	6.68	1	2	20.0	26.7
GPV	6.88	8	9	165.1	123.8
Sum				562.9	1512.2

Note: [a] The relative levels of glycoproteins were calculated respectively by log 10 normalized iBAQ intensity based on LC-MS/MS analysis according to Proteomics DB (12). [b] Known and potential *N*- or *O*-glycosites of each glycoprotein were searched by Uniprot database (http://www.uniprot.org/) or predicted according to online glycosylation site prediction servers (NetNGlyc 1.0 and NetOGlyc 4.0, http://www.cbs.dtu.dk/services/).

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