Supporting Information

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Materials and Methods

Animals. Animal use was limited to adult laboratory mice of the C57BL/6Hsd strain. All procedures were approved by Institutional Animal Care and Use Committees of the University of California Santa Barbara and the Sanford-Burnham Medical Research Institute. Mice were provided sterile pellet food and water ad libitum. Littermates and related cohorts aged 8–16 wk were used for experimentation. Mice lacking a functional gene encoding GPIbα (1) were kindly provided by Zaverio Ruggeri (The Scripps Research Institute, La Jolla, CA). Mice bearing null mutations in the genes encoding *Asgr1* or *Asgr2* have been previously described (2, 3). All alleles were maintained through at least eight generations of meiotic recombination in the C57BL/6NHsd background. All animals were provided sterile pellet food and water ad libitum. Littermates and related cohorts aged 8–16 wk were used for experimentation.

Human Subjects. The Institutional Review Board of the Cottage Health System approved experiments to acquire anonymous human blood samples for analysis upon informed consent of healthy volunteers.

Reagents. Chemicals, biologics, and other reagents used included *N*-hydroxysuccinimidobiotin (Pierce), paraformaldehyde (Electron Microscopy Sciences), biotin and FITC-conjugated lectins including *Erythrina cristagalli* lectin (ECA) and *Ricinus communis*-1 agglutinin (RCA-I) (Vector Laboratories), CellTracker Orange (Molecular Probes), alkaline phosphatase (Sigma), streptavidin-alkaline phosphatase (BD Pharmingen), Todd-Hewitt broth (Teknova), RBC lysis buffer (eBioscience), BSA (Jackson Immunoresearch), serum-separator, and EDTA-Vacutainer tubes (Becton Dickinson). Enzymes used were *Clostridium histolyticum* collagenase (Sigma-Aldrich), *Streptococcus pneumoniae* (*SPN*) NanA neuraminidase (Seikagaku/Northstar Bioproducts), *Vibrio cholerae* (*VC*) neuraminidase (Roche Applied Science), or *Arthrobacter ureafaciens* (*AUS*) neuraminidase (EY Laboratories). Antibodies included FITC or DyLight649-conjugated rat antibodies that bind to CD42a (GPIX, clone Xia.B4), CD42b (GP1bα, clone Xia.G5), and CD42c (GPIbβ, clone Xia.C3) (Emfret Analytics), FITC- or Phycoerythrin (PE)-conjugated antibodies to CD41 (MWReg30) and a polyclonal IgG control (BD Pharmingen), FITC- or APC-conjugated anti-CD61 (2C9.G3), anti-TER119, and all isotype controls (eBiosciences), von Willebrand Factor (DAKO), antithrombin, coagulation factors I, II, and X (Hematologic Technologies), and PE-conjugated anti-Gr1 (clone RB6-8C5) and anti-CD3 (2C11) (eBioscience).

Bleeding Times. Mice were anesthetized and restricted horizontally. The tail was severed 2 mm from the tip (or additional 1 mm for subsequent time points) with a razor blade and immersed vertically 1 cm below the surface of 37 °C saline. Time until bleeding stopped was recorded. Cauterization was not required.

Coagulation and Hematology. Blood used in coagulation assays was collected by cardiac puncture. Citrated platelet-poor plasma was prepared from citrated blood by centrifugation at 22 °C. Plasma samples were aliquoted and frozen at −80 °C within 4 h of blood collection. Hematology assessment, blood coagulation time assays, platelet counts, and blood coagulation factor assays were performed as previously described (4–6). Platelets were identified using flow cytometry with either anti-CD41 or anti-CD61 antibodies.

Blood Chemistry and Cytokine Measurements. Blood was collected from the retroorbital sinus while the mouse was kept under general (inhalant) anesthesia. Blood was collected into Microtainer Serum Separator Tubes (BD) with no anticoagulant and allowed to clot for 30 min at room temperature. Sera collected after centrifugation at 13,000 rpm for 10 min. For blood chemistry, a VetScan Comprehensive Diagnostic Profile reagent rotor was used with the VetScan Chemistry Analyzer according to the manufacturers' instructions.

Neuraminidase and Anti-CD41 Treatment. For in vitro treatment, isolated whole blood was incubated with neuraminidase doses as indicated in the text, ranging from 0.001 to 0.3 U/mL for 2 h at 37 °C before analysis. For in vivo treatment, neuraminidase or antibody was i.v. injected into the retroorbital sinus or the tail vein. Neuraminidase doses ranging from 0.25 to 50 U/kg in 100 μL diluted with PBS were i.v. injected (via retroorbital sinus). Anti-CD41 or polyclonal IgG control (0.5 mg/kg) were diluted up to 100 μL in PBS and delivered i.v. into the retroorbital plexus. Serial samples were taken by retroorbital sinus bleeds after injection. Each mouse was bled up to 3 time points for analyses of coagulation factor levels or activity.

Mouse Platelet Isolation, Survival, and Fate. Whole blood was incubated in vitro with indicated neuraminidase at specified dose for 2 h at 37 °C. For the final 30 min, CellTracker red was added to 2- μM final concentration. Platelet-rich plasma was prepared by centrifugation at 268 × *g* for 8 min. Platelets were separated from platelet-rich plasma by centrifugation at 834 × *g* for 5 min and washed twice in Tyrode's buffer (137 mmol/L NaCl, 2.8 mmol/L KCl, 1 mmol/L $MgCl_2$, and 10 mmol/L Hepes, pH 7.4) to remove excess CellTracker dye. Platelet-rich plasma from donor mice were used in in vitro experiments or infused into recipient mice of indicated genotypes via retroorbital sinus in a maximum volume of 200 μL. Blood samples were obtained at indicated time points to evaluate circulatory half-life of donor platelets in recipient mice by flow cytometry. Platelets were identified by forward- and side-scatter profiles and confirmed by anti-CD41 antibody binding. The relative proportion of CellTracker-loaded infused donor platelets remaining in circulation (visible in phycoerythrin detection channel) was calculated as a percentage of that measured at the first time point collected 1 to 2 min after transfusion.

***SPN* Infection.** Wild-type (*SPN*) serotype 2 isolate D39 was used throughout the study as previously described (3). All bacterial infections were administered by i.p. injection at cfu doses indicated.

Histology. Tissues were fixed in 10% (vol/vol) buffered formalin, trimmed, processed, embedded in paraffin, sectioned at a width of 5 μm, histochemically stained, and visualized by light microscopy at magnifications indicated and as previously described (3).

1. Ware J, Russell S, Ruggeri ZM (2000) Generation and rescue of a murine model of platelet dysfunction: The Bernard-Soulier syndrome. *Proc Natl Acad Sci USA* 97(6):2803–2808.
2. Soukharev S, Miller JL, Sauer B (1999) Segmental genomic replacement in embryonic stem cells by double lox targeting. *Nucleic Acids Res* 27(18):e21.
3. Ishibashi S, Hammer RE, Herz J (1994) Asialoglycoprotein receptor deficiency in mice lacking the minor receptor subunit. *J Biol Chem* 269(45):27803–27806.
4. Grewal PK, et al. (2008) The Ashwell receptor mitigates the lethal coagulopathy of sepsis. *Nat Med* 14(6):648–655.

5. Wang Y, et al. (2001) Modeling human congenital disorder of glycosylation type IIa in the mouse: Conservation of asparagine-linked glycan-dependent functions in mammalian physiology and insights into disease pathogenesis. *Glycobiology* 11(12): 1051–1070.

6. Ellies LG, et al. (2002) Sialyltransferase ST3Gal-IV operates as a dominant modifier of hemostasis by concealing asialoglycoprotein receptor ligands. *Proc Natl Acad Sci USA* 99(15):10042–10047.

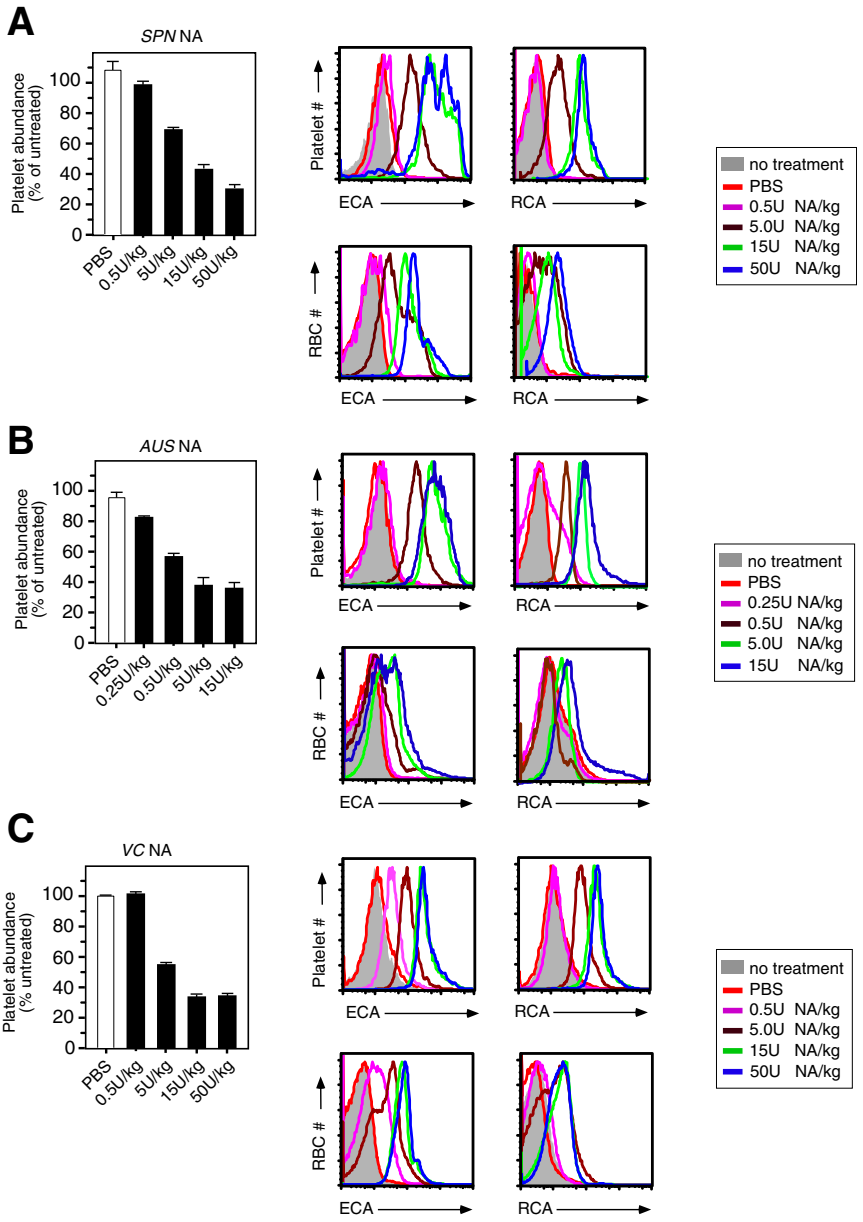

Fig. S1. Dose–response of microbial neuraminidases in platelet desialylation and the induction of thrombocytopenia. Circulating platelet sialylation and abundance in WT mice were measured by flow cytometry 2 h after i.v. administration of indicated doses of neuraminidase (NA) obtained from (*A*) *SPN*, (*B*) *AUS*, and (*C*) *VC*. Platelets were identified with anti-CD41 antibody. Red blood cells were identified with Ter-119 antibody. Exposure of galactose after desialylation was simultaneously determined with ECA and RCA-I lectins.

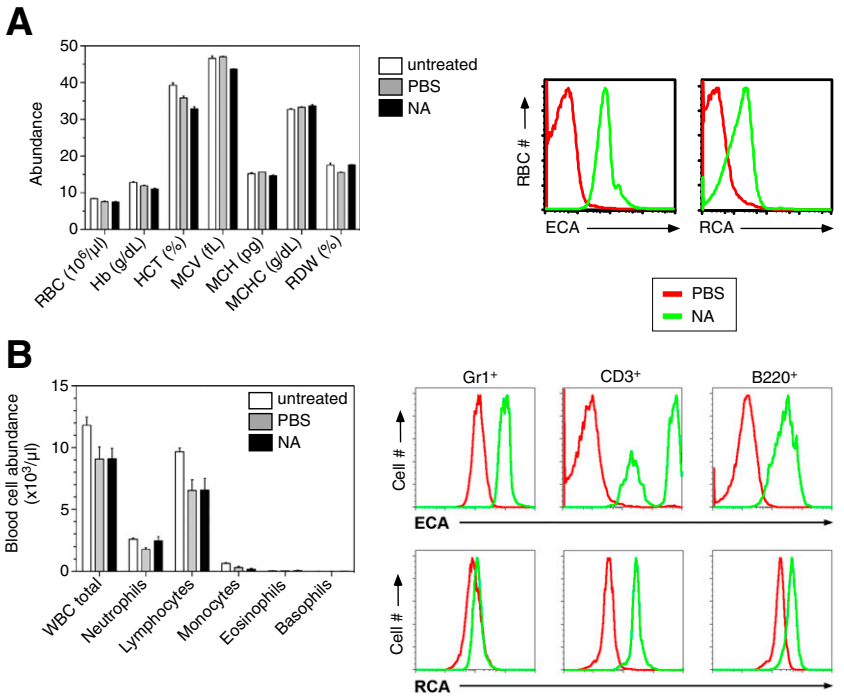

Fig. S2. Hematological measurements after i.v. neuraminidase treatment. Wild-type mice were bled 2 h after i.v. treatment with neuraminidase (NA) or PBS before measuring (*A*) red blood cell parameters and (*B*) nucleated blood cell measurements. Desialylation of blood cells representing neutrophils ($Gr1^+$), T cells ($CD3^+$), and B cells ($B220^+$) were determined by flow cytometry with measurements of ECA and RCA-I lectin binding. Similar results were observed at 24 h after treatment. HCT, hematocrit; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; RDW, red blood cell distribution width.

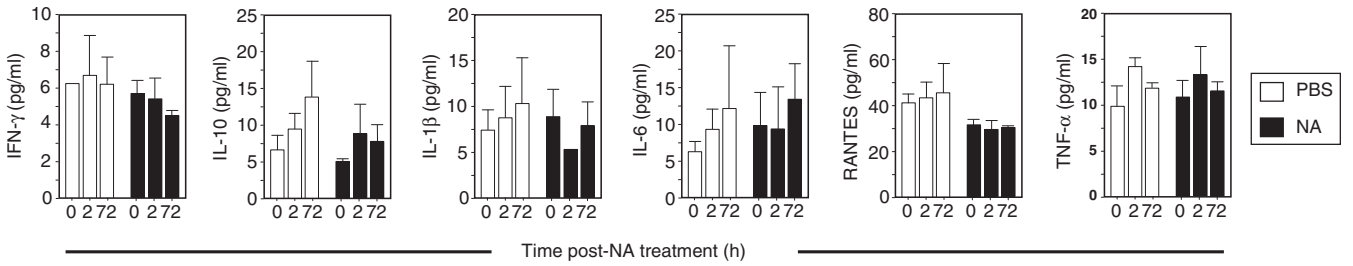

Fig. S3. Inflammation marker abundance in the blood after neuraminidase (NA) treatment. Abundance of indicated cytokines in blood sera determined using ELISA at multiple time points after i.v. administration of PBS or neuraminidase to WT mice. RANTES (regulated on activation, normal T cell expressed and secreted) is also Chemokine ligand 5 (CCL5).

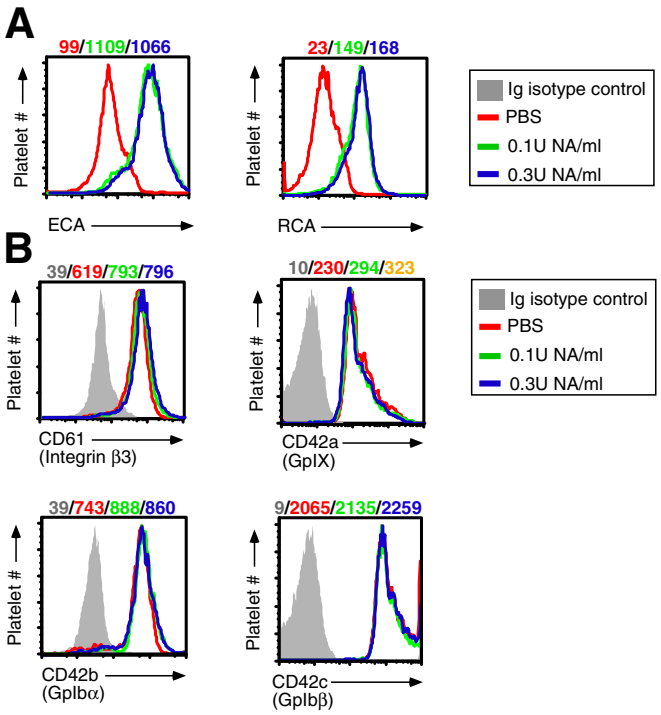

Fig. S4. Desialylation and glycoprotein abundance at the surface of platelets after neuraminidase (NA) treatment. (*A*) Platelet desialylation and (*B*) platelet glycoprotein abundance were measured in vitro 30 min after the addition of neuraminidase or PBS at the indicated dosages to isolated whole blood.

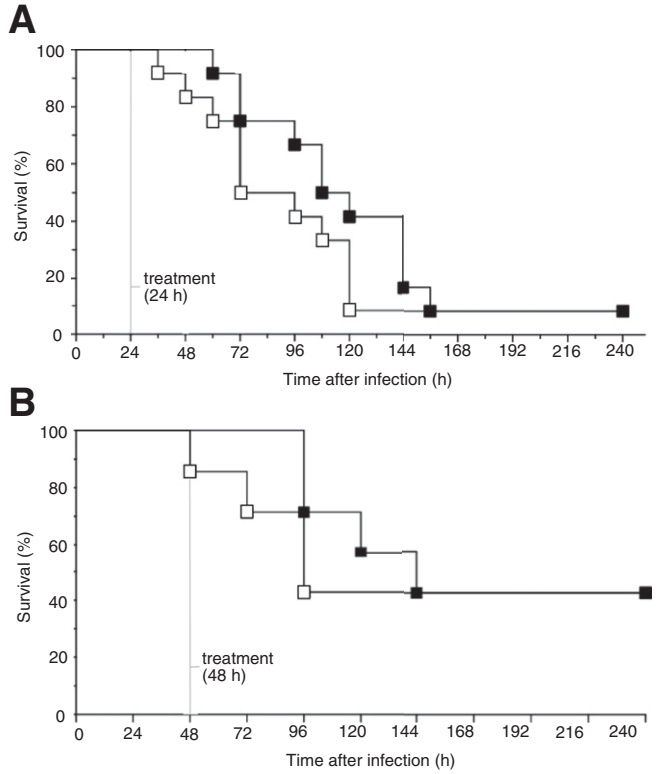

Fig. S5. Survival of mice administered i.v. neuraminidase at different times after *SPN* infection. (*A*) WT mice were infected with a lethal dose (2×10^5 cfu) of *SPN* by i.p. injection and were administered PBS or neuraminidase i.v. 24 h after infection. In parallel studies, (*B*) WT mice were infected with an LD_{50} dose (1×10^3 cfu) of *SPN* by i.p. injection and were administered PBS or neuraminidase i.v. 48 h after infection. White squares, PBS treatments; black squares, neuraminidase treatments.

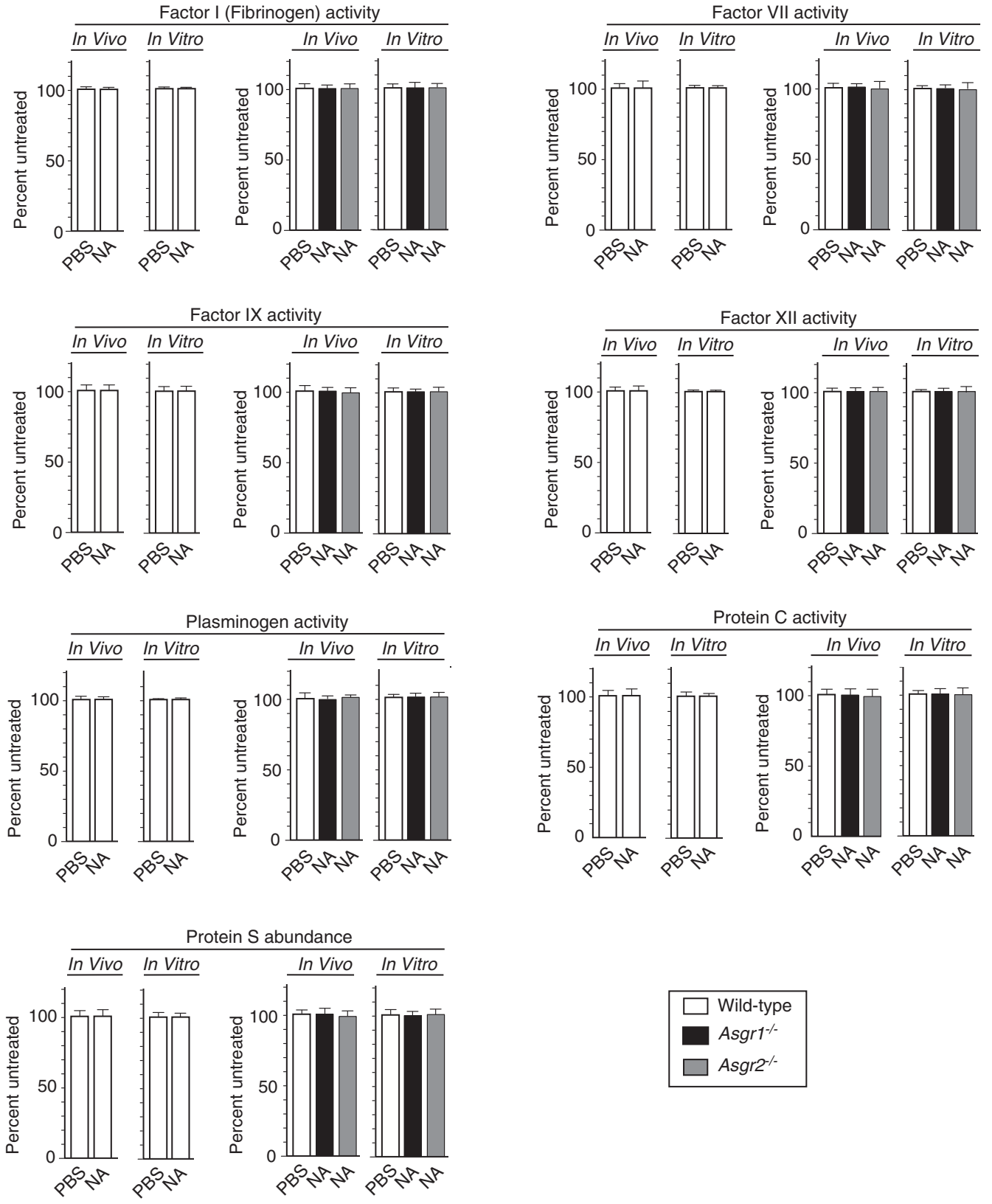

Fig. S6. Abundance or activity of blood coagulation factors by i.v. neuraminidase treatment. WT and Ashwell-Morell receptor (AMR)-deficient mice were bled 2 h after i.v. treatment with either neuraminidase (NA) or PBS. Plasma was isolated for measurements of blood coagulation factor abundance and activity as previously described (5, 6).

Table S1. Blood chemistry in WT mice after neuraminidase administration in vivo and in vitro

Analyte	In vitro PBS	In vitro NA	In vivo PBS	In vivo *SPN* NA	In vivo *AUS* NA	In vivo *VC* NA
Albumin (g/dL)	2.9 ± 0.3	2.9 ± 0.4	3.6 ± 0.2	4.2 ± 0	3.3 ± 0.1	3 ± 0.2
Alanine aminotransferase (U/L)	47.3 ± 11	48.5 ± 10.2	43.6 ± 9.5	33.5 ± 13	43.8 ± 3.5	39 ± 6.5
Amylase (U/L)	937 ± 64	921 ± 53	795.5 ± 135.3	791.5 ± 168.5	784.0 ± 145.5	850 ± 199
Total bilirubin (mg/dL)	0.2 ± 0	0.2 ± 0	0.2 ± 0.0	0.3 ± 0	0.2 ± 0.0	0.2 ± 0
Total nitrogen (mg/dL)	21 ± 1	20.3 ± 1.1	10.5 ± 2.1	24.5 ± 1	18.3 ± 6.8	15.5 ± 1.5
Calcium (mg/dL)	8.9 ± 0.1	8.7 ± 0.2	9.0 ± 0.1	9.1 ± 0	9.3 ± 0.1	9.56 ± 0.1
Phosphorus (mg/dL)	9 ± 0.4	11.2 ± 1.4	9.7 ± 0.5	8.2 ± 0.6	8.0 ± 1.4	7.8 ± 0.2
Creatinine (mg/dL)	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0.0	0.2 ± 0
Glucose (mg/dL)	191 ± 5	189.3 ± 2.6	169.5 ± 9	175.5 ± 9.5	179.0 ± 5.1	173 ± 2
Sodium (mmol/L)	151 ± 2	153 ± 0.6	151.5 ± 1.4	151 ± 1	151.1 ± 1.8	154.5 ± 0.5
Potassium (mmol/L)	4.7 ± 0.5	5.6 ± 0.5	5.2 ± 0.3	4.7 ± 0.5	5.7 ± 0.5	4.8 ± 0.2
Total protein (g/dL)	4.7 ± 0.2	4.6 ± 0.2	4.9 ± 0.2	5.1 ± 0.2	4.5 ± 0.1	4.6 ± 0.1
Globulin (g/dL)	1.7 ± 0.2	1.7 ± 0.2	1.5 ± 0.2	0.9 ± 0.2	1.6 ± 0.3	1.5 ± 0.1

In vivo analyses were accomplished among sera isolated 2 h after i.v. administeration of PBS or neuraminidase (NA). In vitro analyses were accomplished on sera prepared 30 min after addition of PBS or neuraminidase to isolated whole blood.

Table S2. Blood chemistry during *SPN* infection and NA treatment in WT and AMR-deficient (*Asgr1* or *Asgr2*) mice

Analyte	WT *PBS* *PBS*	WT *SPN* PBS	WT *SPN* NA	$Asgr1^{-/-}$ untreated	$Asgr1^{-/-}$ *SPN* PBS	$Asgr1^{-/-}$ *SPN* NA	$Asgr2^{-/-}$ untreated	$Asgr2^{-/-}$ *SPN* PBS	$Asgr2^{-/-}$ *SPN* NA
Albumin (g/dL)	3.7 ± 0.1	2.7 ± 0.2	1.5 ± 0.2	3.6 ± 0.2	3.2 ± 0.4	2.3 ± 0.2	3.6 ± 0.1	2.5 ± 0.2	2.0 ± 0.2
Amylase (U/L)	815 ± 57.2	1768 ± 331	2109 ± 459	739 ± 75.9	1128 ± 51	1367 ± 185	705 ± 49.3	1016 ± 170	1467 ± 309
Total bilirubin (mg/dL)	0.2 ± 0	0.2 ± 0.0	0.25 ± 0.02	0.2 ± 0	0.2 ± 0.0	0.25 ± 0.02	0.2 ± 0	0.3 ± 0.1	0.3 ± 0.04
Calcium (mg/dL)	9.0 ± 0.1	9.9 ± 0.2	8.0 ± 0.4	9.7 ± 0.3	9.0 ± 0.5	8.3 ± 0.4	9.4 ± 0.1	10.1 ± 0.1	8.5 ± 0.3
Phosphorus (mg/dL)	7.6 ± 0.5	7.6 ± 0.8	12.2 ± 1.7	9.7 ± 0.7	9.6 ± 0.4	15.6 ± 1.3	8.8 ± 0.4	9.3 ± 1.1	12.4 ± 2.6
Creatinine (mg/dL)	0.2 ± 0.01	0.2 ± 0.01	0.23 ± 0.02	0.2 ± 0	0.22 ± 0.02	0.28 ± 0.04	0.2 ± 0	0.2 ± 0	0.23 ± 0.03
Glucose (mg/dL)	156.9 ± 11.8	147.0 ± 2.6	167.0 ± 15.7	142.9 ± 11.9	144.7 ± 12.9	147.0 ± 14.2	163.0 ± 13	152.3 ± 6.7	146.5 ± 14.9
Sodium (mmol/L)	149.8 ± 1.1	152.3 ± 1.2	149.5 ± 0.7	154.4 ± 1.3	149.3 ± 1.2	152.7 ± 1.9	147.9 ± 1.2	145.0 ± 2.3	147.3 ± 2.3
Potassium (mmol/L)	5.0 ± 0.3	5.5 ± 0.3	5.7 ± 0.5	5.1 ± 0.1	5.4 ± 0.4	6.1 ± 0.6	5.2 ± 0.2	5.2 ± 0.2	4.9 ± 0.3
Total protein (g/dL)	4.7 ± 0.1	5.5 ± 0.1	3.6 ± 0.2	5.2 ± 0.3	5.1 ± 0.2	4.8 ± 0.2	4.9 ± 0.1	4.6 ± 0.3	4.2 ± 0.2
Globulin (g/dL)	1.2 ± 0.1	2.7 ± 0.2	2.0 ± 0.1	1.6 ± 0.1	2.8 ± 0.1	2.4 ± 0.2	1.3 ± 0.1	2.1 ± 0.3	2.3 ± 0.3

Measurements were completed on sera prepared from untreated or *SPN*-infected mice of the indicated genotypes 24 h after infection and 16 h after i.v. administration of PBS or neuraminidase (NA).

Table S3. Blood chemistry during *SPN* infection and antiplatelet antibody treatment

Analyte	$Asgr1^{-/-}$ untreated	$Asgr1^{-/-}$ *SPN* IgG	$Asgr1^{-/-}$ *SPN* anti-CD41	*Asgr-2*$^{-/-}$ untreated	$Asgr2^{-/-}$ *SPN* IgG	$Asgr2^{-/-}$ *SPN* anti-CD41
Albumin (g/dL)	3.6 ± 0.2	2.5 ± 0.3	2.2 ± 0.2	3.6 ± 0.1	2.6 ± 0.2	2.7 ± 0.2
Amylase (U/L)	739 ± 75.9	1012 ± 148	948 ± 111.8	705 ± 49.3	1528 ± 252	1071 ± 154.8
Total bilirubin (mg/dL)	0.2 ± 0	0.2 ± 0.0	0.23 ± 0	0.2 ± 0	0.5 ± 0.2	0.22 ± 0.02
Calcium (mg/dL)	9.7 ± 0.3	8.6 ± 0.7	9.9 ± 0.2	9.4 ± 0.1	9.2 ± 0.4	9.2 ± 0.3
Phosphorus (mg/dL)	9.7 ± 0.7	9.5 ± 0.8	10.1 ± 0.9	8.8 ± 0.4	12.3 ± 2.5	11.5 ± 2.3
Creatinine (mg/dL)	0.2 ± 0	0.23 ± 0.03	0.25 ± 0.1	0.2 ± 0	0.2 ± 0	0.24 ± 0.04
Glucose (mg/dL)	142.9 ± 11.9	138.3 ± 5.3	168.5 ± 25.4	163.0 ± 13	134.7 ± 16	149 ± 10.8
Sodium (mmol/L)	154.4 ± 1.3	152.3 ± 1.9	158 ± 2.3	147.9 ± 1.2	151.3 ± 1.9	146.8 ± 2.1
Potassium (mmol/L)	5.1 ± 0.1	5.6 ± 0.3	6.1 ± 0.4	5.2 ± 0.2	5.9 ± 0.5	5.8 ± 0.6
Total protein (g/dL)	5.2 ± 0.3	4.7 ± 0.3	5.0 ± 0.2	4.9 ± 0.1	4.6 ± 0.2	4.9 ± 0.2
Globulin (g/dL)	1.6 ± 0.1	2.5 ± 0.2	2.8 ± 0.1	1.3 ± 0.1	2.2 ± 0.3	2.3 ± 0.2

Measurements were completed on sera prepared from untreated or *SPN*-infected mice 24 h after infection and 16 h after i.v. treatment with anti-CD41 or polyclonal IgG antibodies.

Table S4. *N*-glycosylation consensus sites in human and mouse protein orthologs

	Homo sapiens			*Mus musculus*		
Glycoprotein	Total amino acids	No. of sites	*N*-site position	Total amino acids	No. of sites	*N*-site position
I (α-Fibrinogen)	866	3	419; 686; 831	789	2	609; 754
I (β-Fibrinogen)	491	1	**394**	481	1	**384**
I (γ-Fibrinogen)	453	1	**78**	436	1	**77**
II (Prothrombin)	622	4	**121; 143; 205; 416**	618	5	**122; 144; 207; 413;** 553
V	2,224	37	51; 55**; 239; 297; 382; 460;** 468**; 554;** 667; **741; 752; 760; 776;** 782; **821;** 938; 977; 1074; 1083; 1103; 1106; 1203; 1221; 1257; 1266; 1293; 1311; 1338; 1347; 1374; 1383; 1479; 1499; 1559; 1703; 2010; 2209	2183	26	176; **238; 296; 381; 459; 552; 740; 751; 759; 775; 820;** 841; 959; 972; 1038; 1049; 1058; 1067; 1088; 1438; 1459; 1460; 1519; 1663; 1811; 2168
VII	466	2	205; 382	446	2	186; 244
VIII	2,351	25	**60; 258; 601;** 776; 803; 847; 919; **962;** 982; **1020; 1024; 1074; 1085;** 1204; **1274; 1278; 1301; 1319;** 1403; 1431; 1461; 1531; 1704; 1829; 2137	2319	26	**61;** 233; **259;** 423; **601;** 880; **958;** 1015; **1022; 1026;** 1044; **1076; 1087;** 1136; 1161; 1192; 1255; 1268**; 1273; 1274; 1302; 1316;** 1340; 1378; 1797; 2105
IX	461	2	**203;** 213	471	4	36; **204;** 223; 316
X	488	2	**221;** 231	481	2	187; **218**
XI	625	5	**90; 126; 353; 450; 491**	624	6	**90; 126;** 297; **355; 449; 490**
XII	615	2	**249;** 433	597	4	**249;** 271; 324; 415
vWF	2,813	16	**99; 156;** 211**; 666; 857; 1231; 1515; 1574; 2223; 2290;** 2357**; 2400; 2546; 2585;** 2635; **2790**	2813	15	**99; 156; 666; 857;** 1005; **1231; 1515; 1574; 2223; 2290; 2400; 2546; 2585; 2790;** 2810
Antithrombin	464	4	**128; 167; 187; 224**	465	4	**129; 168; 188; 225**
Protein C	461	3	139; **290; 355**	460	3	214; **290; 354**
Protein S	676	3	**499; 509;** 530	675	2	**499; 509**
Plasminogen	810	1	**307**	812	2	136; **307**
α2 Antiplasmin	491	4	**126; 295; 309; 316**	491	4	**126; 295; 309; 316**

Numbers and positions of *N*-glycosylation consensus sites in each ortholog with site positions indicated relative to methionine at position 1. *N*-glycosylation consensus sites are defined as Asn-Xaa-Ser/Thr (NXS/T), where Xaa is any amino acid except proline. Numbers in bold indicate *N*-glycosylation sequons/positions conserved between human and mouse orthologs. Protein sequences were obtained from the UniProt Knowledgebase (www.uniprot.org) and analyzed using *NetNGlyc* (http://www.cbs.dtu.dk/).