Supplementary Materials for

Antibody efficacy in bacterial sepsis is rescued by targeting macrophage PAMP-STAT1-FcRn axis

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The PDF file includes:

Materials and Methods

Mice

All immunization and infection experiments were conducted in sex-matched mice, including the C57BL/6J, ICR (procured from GemPharmatech), and various genetically modified strains. With the exception of the humanized FcRn (hFcRn, C57BL/6N) and A129 mice, all genetically modified mice were maintained on a C57BL/6J background. Mice with C57BL/6J or C57BL/6N background were housed under specific pathogen-free conditions with *ad libitum* access to water and standard chow. Specifically, FcRn knockout (KO) (*Fcgrt*$^{-/-}$; #NM-KO-00133) was kindly provided by Dr. Jingren Zhang at the Tsinghua University (*43*) and bred in our animal facility. STAT1 KO (*Stat1*$^{-/-}$; #NM-KO-200611) and *Fcgrt* floxed (#NM-CKO-200094) mice were obtained from the Model Organisms Center. The *LsyM-Cre* (#004781) strain was sourced from Jackson Laboratory. The IgM & IgD double KO (DKO; #C001342) and hFcRn (#C001701) mice were acquired from Cyagen. The TLR4 KO (*TLR4*$^{-/-}$; #N000192) mice were purchased from GemPharmatech. The A129 mice were a generous gift from Dr. Yang Liu within our institution.

Bacteria

The *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10* mutant used in this study is maintained in our laboratory and was originally provided by Dr. Dominique Missiakas (The University of Chicago). The pathogenic *E. coli* BSI099 strain was a kind gift from Dr. Guobao Tian (Sun Yat-sen University), and the isogenic *lpxC* mutant was previously constructed in the *A. baumannii* ATCC 17978 background (*16*). For routine culture, *S. aureus* was grown in tryptic soy broth (TSB) or on TSB agar, while both *E. coli* and *A. baumannii* were cultivated in Luria broth (LB) or on LB agar; all strains were incubated at 37°C.

Antibodies

Commercial intravenous immunoglobulin (IVIG), composed of >95% human IgG (hIgG) with a specified 1-3% human IgM (hIgM) content, was purchased from Chengdu Rongsheng Pharmaceuticals (#S20237008). Monoclonal mouse IgG2b and IgM antibodies specific for Keyhole Limpet Hemocyanin (KLH) were purchased from Absea Biotechnology (Cat. No. K06036M09G11C and K06036M01E09C, respectively). The hIgG1 used in this study is a recombinant human monoclonal antibody (mAb) targeting SpA_{KKAA}, a variant of the *S. aureus* protein A (SpA) encoded by the *spa* gene (*44*, *45*). The hIgG1^{YTE} was engineered to contain M252Y/S254T/T256E (YTE) mutations in the Fc portion of hIgG1. Primers 5'-TACATCACACGGGAGCCTGAGGTCACATGCGTGGT-3' and 5'-CTCCCGTGTGATGTAGAGGGTGTCCTTGGGTTTTG-3' were used to generate the YTE variant in the previously constructed pVITRO1 plasmid encoding hIgG1 (*45*). The variable heavy and light chain genes of 3E9 and anti-FcRn antibody (formerly known as SYNT001) were synthesized from published sequences (*20*, *46*) by GENERAL BIOL (Chuzhou, China) and swapped into the pVITRO1-102.1F10-IgG1/λ plasmid (Addgene, #50366). Plasmids were transfected into HEK-293F cells using polyethylenimine, followed by selection with hygromycin B (400 μg/mL) and expansion of stable transfectants. Antibodies were affinity purified from supernatants of expanded cultures on protein G resin (GenScript) and dialyzed against PBS as described (*13*). The anti-FcRn antibody was labeled with Elab Fluor® 647 (EF647) NHS ester (succinimidyl ester; Elabscience, #E-LK-E006C) according to the manufacturer's instructions.

Additional antibodies used for flow cytometry or Western blot are described in the following sections.

Mouse sepsis model

A monomicrobial murine sepsis model was established. Briefly, *S. aureus* was cultured to an OD_{600} of 0.45, while *E. coli* and *A. baumannii* were grown to an OD_{600} of 1.0. Bacterial cells were then pelleted, washed once with PBS, and resuspended in PBS at the desired CFU/ml. A 100 µl aliquot of the suspension was administered *via* the retro-orbital plexus (*S. aureus* and *E. coli*) or intraperitoneally (*A. baumannii*) to anesthetized mice. Animals were monitored daily for clinical signs and body weight changes. At designated time points, serum and tissue samples (liver, kidney, and spleen) were collected. Serum was analyzed for concentrations of endogenous and exogenous antibodies; tissues were weighed, homogenized, serially diluted, and plated on agar to count bacterial burden in tissues (CFU/g of tissue). Survival was monitored to evaluate the protective efficacy of the antibodies.

Antibody concentration assay

Antibody concentrations were determined by enzyme-linked immunosorbent assay (ELISA) as follows. To quantify endogenous hIgG in human serum or exogenous hIgG in mouse serum, microtiter plates were coated overnight with 200 ng/well goat anti-hIgG-Fcγ (Jackson ImmunoResearch, #109-005-008) and blocked. The wells were then incubated with a 1:2,500,000 dilution of human serum or a 1: 50,000 dilution of serum from mice treated with 500 µg IVIG, followed by an HRP-conjugated goat anti-hIgG-$F(ab')^2$ antibody (Jackson ImmunoResearch, #109-035-006). For measuring endogenous mouse IgG and IgM, microtiter plates were coated overnight with 30,000× (for mIgG) or 5,000× (for mIgM) dilution of serum sample. After blocking, the plates were incubated with HRP-conjugated goat anti-mIgG (Jackson ImmunoResearch, #115-035-003) or -mIgM (Invitrogen, #62-6820). To measure the pharmacokinetics of injected antibodies (hIgG1, $hIgG1^{YTE}$, mIgG2b, or mIgM), mice were injected into the peritoneal cavity with 100 µg test mAb. Serum was collected at indicated time points post-injection, and antibody concentrations were detected using antigen-coated plates (SpA_{KKAA} or KLH) and matched HRP-conjugated secondary antibodies (Promega, #W403B; Jackson ImmunoResearch, #115-035-003; Invitrogen, #62-6820). For tissue antibody quantification, samples were processed under perfused and non-perfused conditions. Intracardiac perfusion was performed in anesthetized mice via the left ventricle at a constant rate of 4 ml/min using PBS until the tissues appeared visibly pale and the lungs turned white. Both perfused and non-perfused tissues, as well as mouse fecal samples, were weighed and homogenized in PBS containing 0.1% Triton X-100 using a Tissuelyser II (Qiagen). The homogenates were centrifuged, and the supernatants were appropriately diluted and applied to SpA_{KKAA}-coated microtiter plates. All antibody concentrations were calculated from standard curves ranging from 0.1 ng/ml to 10 µg/ml. Human IgM levels were determined using a commercial ELISA kit (Mabtech, #3880-1AD-6). All plates were developed using TMB Chromogen Solution (Sangon Biotech). Recombinant SpA_{KKAA} was purified from *E. coli* as described (*44*), and KLH was purchased from ThermoFisher (#77600).

Flow cytometric analysis of FcRn, FcγRs, and CRs expression

Single-cell suspensions were prepared from mouse spleen, liver, lung, and blood. Spleens were harvested and gently mashed through a 70-µm cell strainer in RPMI-1640 medium. The resulting

splenocytes were treated with red blood cell (RBC) lysis buffer (Biosharp, #B541001-0100) for 2 minutes on ice. After lysis, cells were washed and resuspended in PBS containing 2 mM EDTA for subsequent use. Liver non-parenchymal cells (NPCs) were isolated using a modified collagenase-DNase digestion protocol (*47*). Briefly, mice were euthanized, and livers were perfused *via* the portal vein with PBS, excised, and mechanically dissociated in digestion buffer (RPMI 1640 with 0.5 mg/ml collagenase IV, 20 μg/ml DNase I, and 0.5 mM $CaCl_2$) using a tissue dissociator (RWD Life Science). The homogenate was filtered through a 70-μm strainer and washed. The cell pellet was treated with RBC lysis buffer on ice for 3 minutes, and the reaction was quenched with RPMI 1640 medium. The suspension was centrifuged at 60 ×g for 2 minutes to pellet hepatocytes, and the supernatant containing enriched NPCs was collected. Lung tissues were excised and subjected to mechanical and enzymatic dissociation in digestion buffer (RPMI 1640 with 0.5 mg/ml collagenase IV, 50 μg/ml DNase I, and 0.5 mM $CaCl_2$) using a tissue dissociator (RWD Life Science). The homogenate was filtered through a 70-μm strainer, washed, and subjected to RBC lysis. Finally, the cells were resuspended in PBS with 2 mM EDTA. Blood was collected via the retro-orbital plexus. After RBC lysis, immune cells were washed and resuspended in PBS with 2 mM EDTA. Prior to staining, single-cell suspensions were labeled with a fixable viability dye (ThermoFisher, #eF506) and blocked with an anti-CD16/32 antibody (Biolegend, #156604). Surface staining was performed sequentially, starting with anti-Ly-6G/6C antibodies, followed by a cocktail of other surface markers. For intracellular FcRn staining, cells were fixed, permeabilized (ThermoFisher, #88-8824-00), and stained with an EF647-conjugated anti-FcRn antibody (made in-house). For FcγR staining, single-cell suspensions were directly incubated with antibodies against FcγRs (FcγRI-BV605, Biolegend, #139323; FcγRII-APC, Biolegend, #156406; FcγRIII-PE/Cy7, Biolegend, #158016; FcγRIV-BV421, Biolegend, #149521) without prior Fc blocking. After incubation, cells were stained with antibodies (CD45-RB705, BD Pharmingen, #570291 or CD45-BUV395, Biolegend, #103192; CD31-BV421, Biolegend, #102424; CD11b-APC/Cy7, Biolegend, #101225; F4/80-PE, Biolegend, #123110; Tim-4-PE/Cy7, Biolegend, #130010 or Tim-4-PerCP-eFluor™ 710, ThermoFisher, #46-5866-82; Ly6C-BV785, Biolegend, #128041; Ly6G-FITC, Biolegend, #127605; CD11c-BUV496, BD Pharmingen, #117348) in Brilliant Stain buffer (ThermoFisher, #00-4409-42) for the identification of cell populations and complement receptors (CRs). Viable cell populations were gated as Kupffer cells ($CD45^+CD31^-CD11b^{low}F4/80^{high}Tim\text{-}4^+$), macrophages ($CD45^+CD31^-CD11b^{low}F4/80^{high}$), monocytes ($CD45^+CD31^-CD11b^+Ly6C^{high}Ly6G^-$), neutrophils ($CD45^+CD31^-CD11b^+Ly6G^{high}$), and endothelial cells ($CD45^-CD31^+$). All data were acquired on a CytoFLEX LX flow cytometer (Beckman Coulter) and analyzed with CytExpert software.

Bone marrow-derived macrophages (BMDMs)

Bone marrow-derived macrophages (BMDMs) were generated as follows. Bone marrow cells were isolated from the femurs and tibias of 6- to 8-week-old mice by flushing with ice-cold RPMI 1640 medium. The cell suspension was gently dissociated by pipetting, filtered through a 70-μm strainer, and treated with RBC lysis buffer. After washing, the cells were cultured for 7 days in RPMI 1640 medium supplemented with 10% endotoxin-free FBS (Sigma, F8318) and 30% L929-conditioned medium to induce macrophage differentiation. The L929-conditioned medium, serving as a source of M-CSF, was prepared by harvesting supernatant from L929 cells cultured for 5 days in DMEM with 10% FBS, followed by filtration through a 0.22-μm filter. After differentiation, BMDMs were subjected to one of the following treatments: infection with

S. aureus or *E. coli* at the defined MOI (multiplicity of infection); stimulation with LPS (InvivoGen, #tlrl-b5lps) or PGN (InvivoGen, #tlrl-pgns2) at the indicated concentrations; pretreatment with inhibitor, BAY11-7082 (Selleck, #S2913) for 1 hour in serum-free medium or fludarabine (TargetMol, #T1038) for 24 hours in medium containing 10% FBS, followed by stimulation with LPS or PGN. All treatments were performed for the indicated durations in medium containing 2% FBS. The identity of BMDMs was confirmed by flow cytometry, with >95% of the cells being $CD11b^{+}F4/80^{+}$. For intracellular FcRn detection, cells were then fixed, permeabilized, and stained with an EF647-conjugated anti-FcRn antibody (made in-house).

RNA extraction and qPCR analysis

Total RNA was isolated from tissue macrophages, BMDMs, or human umbilical vein endothelial cells (HUVECs) using TRIzol reagent (Invitrogen, #15596018CN) and reversely transcribed by StarScript III All-in-One gDNA Removal Mix (GenStar, #A230-10) according to the manufacturer's protocol. Quantitative PCR (qPCR) was performed with 2 × RealStar Green Fast Mixture (GenStar, #A301-10) and gene-specific primers (mouse *Fcgrt*: 5'-AGCTCAAGTTCCGATTCCTG-3' and 5'-GATCTGGCTGATGAATCTAGGTC-3'; human *Fcgrt*: 5'-GAAACCTGGAGTGGAAGGAG-3' and 5'-CGGAGGGTAGAAGGAGAAGG-3'; mouse *Tnf-α*: 5'-ATGAGAAGTTCCCAAATGGCC-3' and 5'-TCCACTTGGTGGTTTGCT ACG-3'; mouse *Ifn-β*: 5'-CCACCAGCAGACAGTGTTTC-3' and 5'-GAAGATCTCTGCTCGGACCA-3'; mouse *Ifn-γ*: 5'-TGAACGCTACACACTGCATCTTGG-3' and 5'-CGACTCCTTTTCCGCTTCCTGAG-3'). Relative mRNA levels were calculated via the 2^(-ΔΔCt) method with normalization to *Gapdh* (mouse: 5'-GAGAAACCTGCCAAGTATGATGAC-3' and 5'-ATCGAAGGTGGAAGAGTGGG-3'; human: 5'-CATCAAGAAGGTGGTGAAGCAG-3' and 5'-TTCATTGTCGTACCAGGAAATGAG-3').

Bulk RNA sequencing and analysis

Bulk RNA sequencing was performed on Kupffer cells and splenic macrophages. Kupffer cells were isolated by preparing a liver single-cell suspension from the liver, followed by direct fluorescence-activated cell sorting (FACS). Splenic macrophages were first enriched using anti-F4/80 magnetic beads (Stemcell, #100-0659) and subsequently purified by FACS to obtain a highly pure population. Cells were pooled from 3-7 mice for RNA extraction. Total RNA was extracted from the sorted cells using TRIzol reagent, and samples meeting quality thresholds (RIN/RQN ≥ 7.0; 28S/18S ratio ≥ 1.0) were selected for library construction. Sequencing was conducted on the DNBSEQ-T7 platform to generate 150 bp paired-end reads (PE150). For bioinformatic analysis, raw reads were processed with SOAPnuke to obtain high-quality clean data. Clean reads were then aligned to the mouse reference genome (GRCm39) using HISAT2. Gene expression levels were quantified with RSEM using the FPKM metric. Genes with a mean TPM > 0.5 across the group were considered expressed and retained for subsequent analysis. Differential expression analysis was performed using DESeq2, identifying genes with an absolute $\log_2$ fold change greater than 0.5 at a false discovery rate (FDR) of < 0.05 as statistically significant. Transcription factor (TF) activities were inferred from the shared differentially expressed genes using the decoupleR R package (v2.16.0) (*48*). High-confidence mouse regulons (confidence levels A-C) were obtained from the DoRothEA database (v1.22.0) (*49*). Sample-specific TF activity scores were estimated using the Univariate Linear Model (ULM) algorithm.

For visualization, the top 20 TFs ranked by the mean absolute activity score across all samples are shown.

Western blotting

Whole-cell lysates were prepared from cells using RIPA lysis buffer supplemented with protease and phosphatase inhibitor cocktails (MedChemExpress, MCE). The lysates were denatured in 1× SDS loading buffer, separated by SDS-PAGE, and transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with QuickBlock^{TM} blocking buffer (Beyotime, #P0252) and subsequently probed overnight at 4°C with the following primary antibodies: anti-phospho-STAT1 (Tyr701) (Abclonal, #AP0054), anti-phospho-STAT1 (Ser727) (Abclonal, #AP1000), anti-STAT1 (Abclonal, #A19563), and anti-β-actin (Abclonal, #AC050). After washing with TBST, the membranes were incubated with an HRP-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch, #111-035-003) for 2 hours at room temperature. Protein bands were visualized using an Omni-ECL^{TM} Ultra-Sensitive Chemiluminescence Detection Kit (Epizyme Biotech, #SQ201) and detected with a chemiluminescence imaging system.

ELISA for antibody binding

Binding measurements were performed in microtiter plates coated with 1×10^7 CFU of *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10* or *E. coli* BSI099 bacteria in 0.1 M carbonate buffer (pH 9.5) at 4°C overnight or at 37°C for 2 hours. Wells were blocked by 2% (w/v) bovine serum albumin (BSA) before incubation with serial concentrations of test antibodies or indicated dilutions of mouse serum. Binding capacity was quantified following incubation with HRP-conjugated secondary antibodies.

Public dataset analysis

The prognostic value of FCGRT expression was assessed through analysis of public GEO datasets. The primary cohort, GSE65682 (n=478 with 28-day outcomes), was analyzed with the "maxstat" R package to determine an optimal cutoff for *Fcgrt* expression, thereby stratifying patients into high- and low-expression groups. Survival analysis was performed using Kaplan-Meier curves in GraphPad Prism 10, with statistical significance determined by the log-rank test ($p < 0.05$). For independent validation, the GSE63042 cohort (n=106) was utilized, with patients dichotomized at the median *Fcgrt* expression level. Additionally, datasets GSE28750 and GSE33341 were integrated to compare *Fcgrt* expression levels between septic patients and healthy controls. To validate our experimental findings in human macrophages, we analyzed dataset GSE13670 focusing on *Fcgrt* expression at 8, 24, and 48 hours after *S. aureus* infection.

Isolation and differentiation of primary human monocytes (HMs)

Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of healthy donors and septic patients by Ficoll density gradient centrifugation. $CD14^+$ monocytes were purified from PBMCs using magnetic microbeads (STEMCELL, #19359) according to the manufacturer's instructions. The monocytes were then differentiated into human monocyte-derived macrophages (hMDMs) by culturing for 7 days in RPMI-1640 medium supplemented with 10% FBS and 50 ng/mL human M-CSF (Biolegend, #574806) at 37°C with 5% CO_2. The medium was replaced every 3 days. For stimulation experiments, hMDMs or PBMCs were either treated with specified concentrations of LPS or PGN, or infected with bacteria at a defined MOI in medium containing 2% FBS; all treatments were carried out for the indicated durations. For inhibition, hMDMs were

pretreated for 1 hour with either Ruxolitinib (RUX; 1 μM; MCE, #HY-50856) (*50*) or Adezmapimod (ADE/SB203580; 600 nM; MCE, #HY-10256) (*51*) to inhibit STAT1 phosphorylation at Tyr701 or Ser727, respectively. Subsequently, the cells were co-stimulated with LPS (1 μg/mL) and PGN (10 μg/mL) for 24 hours. For immunostaining, cells were first incubated with TruStain FcX™ (Biolegend, #422302) and a fixable viability dye (ThermoFisher, #eF506). Surface antigens were stained with CD11b-APC/Cy7 (Biolegend, #101225) and CD14-PE (Biolegend, #301805). Cells were then fixed, permeabilized, and stained intracellularly with antibodies against FcRn (EF647, made in-house) and CD68-FITC (Biolegend, #333806). Viable monocytes ($CD11b^+CD14^+$) and macrophages ($CD11b^+CD14^+CD68^+$) were gated for flow cytometric analysis.

Antibody transcytosis assay

BMDMs and hMDMs were seeded in low-attachment 24-well plates at a density of 10^5 cells/well and treated with a PAMP (LPS, 1 μg/ml or PGN, 10 μg/ml) for 24 hours. After stimulation, medium was removed, and cells were then incubated with either FITC-dextran (100 μg/ml) or Alexa Fluor 647-labeled hIgG1(5 μg/ml) diluted in fresh medium containing the respective PAMP for indicated durations. Following incubation, cells were washed twice with PBS containing 0.1% BSA and resuspended in a residual volume of 50 μl. An equal volume of IC fixation buffer (ThermoFisher, #88-8824-00) was added for fixation at room temperature for 30 mins. Cells were washed once with 0.01% BSA-PBS and resuspended for flow cytometry (*52*, *53*).

Evaluation of antibody protection in mice

The efficacy of IVIG and 3E9 mAb was evaluated in a monomicrobial murine sepsis model. Mice received an intraperitoneal injection of the indicated antibody dose 12-16 hours before bacterial challenge. The STAT1 inhibitor carnosic acid (CA) was administered *via* intraperitoneal injection. The inhibitor was given at a dose of 20 mg/kg, dissolved in a solution (5 % DMSO, 30 % PEG300, 10 % tween 80, 55 % H_2O), twice daily for 3 days, with the initial dose administered 2 hours prior to bacterial challenge. Under anesthesia, mice were inoculated via the retro-orbital venous plexus with 100 μl of a bacterial suspension in PBS at a concentration of 5×10^8 CFU/ml (*S. aureus* Δ*spa*Δ*sbi*Δ*ssl10*) or 3×10^9 CFU/ml (*E. coli* BSI099). Following infection, survival was monitored 2 to 3 times daily.

Statistical analysis

Data are presented as mean ± SEM. For comparisons between two groups, an unpaired, two-tailed Student's *t* test was applied. When three or more groups were compared under a single independent variable, a one-way ANOVA with multiple comparisons test was conducted. To assess the effects of two independent variables, data were analyzed by two-way ANOVA with multiple comparisons test. Mouse survival was statistically compared using the log-rank (Mantel-Cox) test. All data were analyzed by Prism 10 (GraphPad Software), and statistical significance was indicated as follows: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

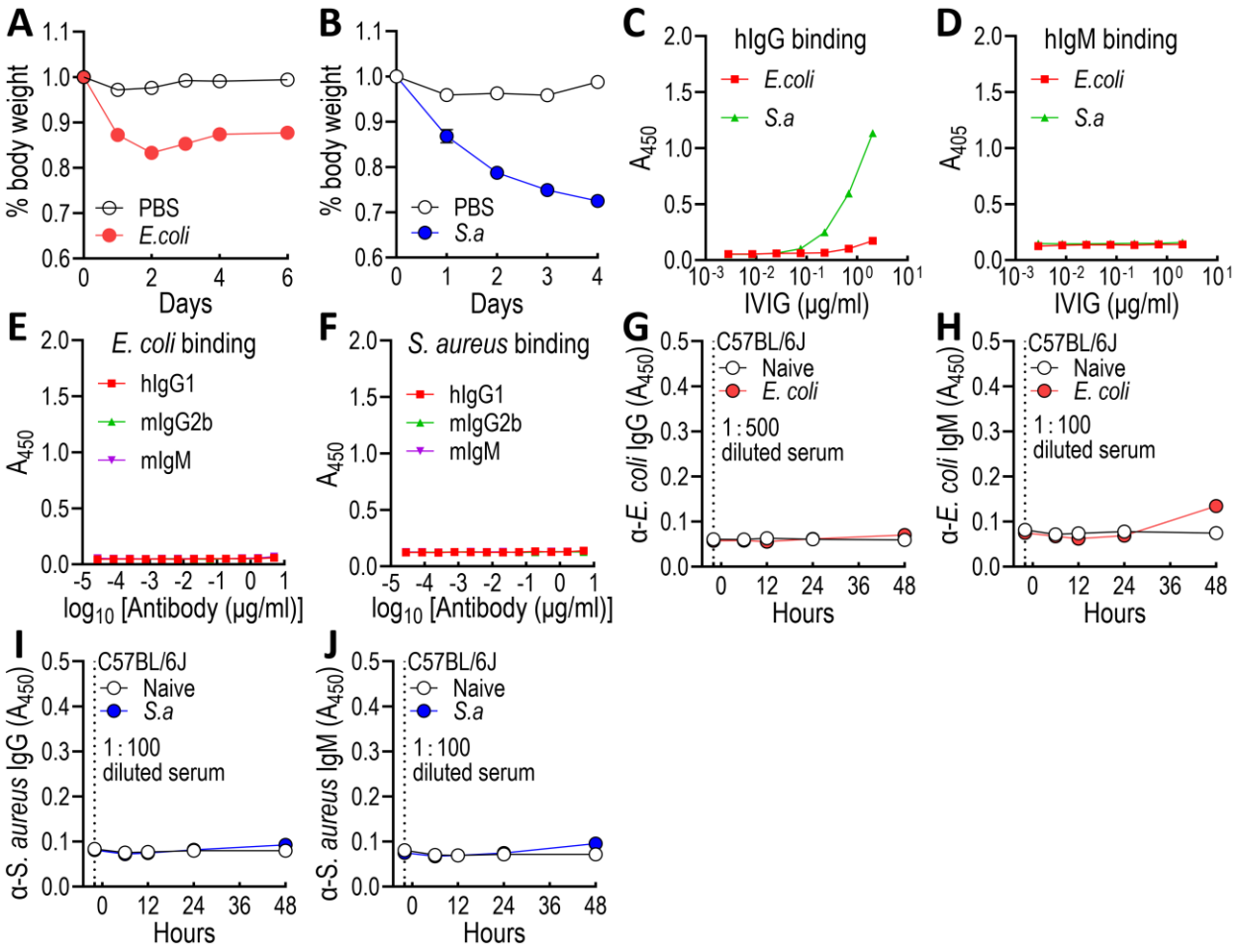

Fig. S1.

Loss of endogenous and exogenous IgG in septic mice. (A and B) Relative body weight in C57BL/6J mice (n = 6) with pathogenic *E. coli*- (A) or *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10* (B)-induced sepsis. (C and D) ELISA analysis (n = 3) of IVIG binding to *E. coli* or *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10*, detecting bound hIgG (C) and hIgM (D). (E and F) ELISA analysis (n = 3) of hIgG1, mIgG2b, and mIgM mAb binding to *E. coli* (E) or *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10* (F). (G to J) Detection of serum IgG (G and I) and IgM (H and J) antibodies against *E. coli* (G and H) or *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10* (I and J) in mice (n = 5) at indicated times after septic challenge with respective bacterium. Data are presented as mean ± SEM.

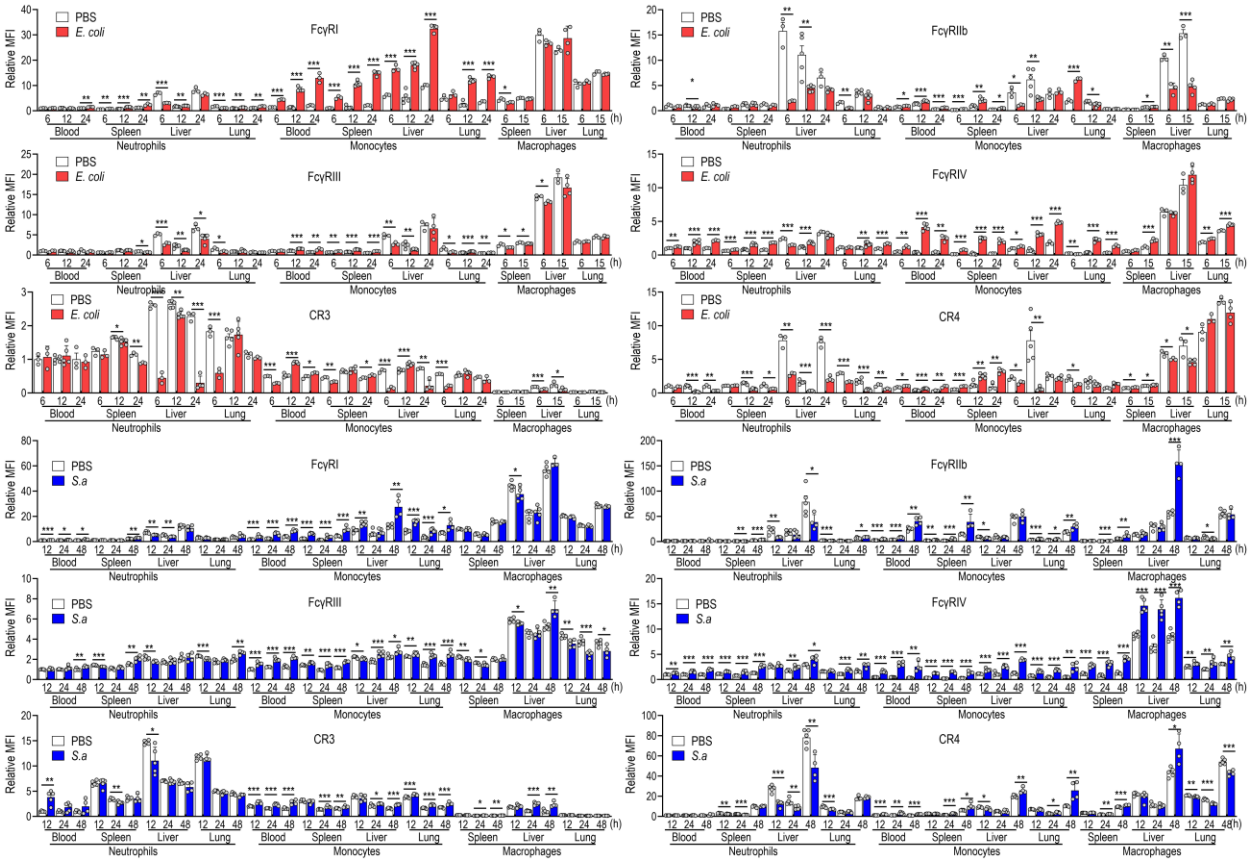

Fig. S2.

Expression of FcγR and complement receptor (CR) in immune cells from septic mice. Four FcγRs (FcγRI, FcγRIIb, FcγRIII, and FcγRIV) and two CRs (CR3 and CR4) were quantified in neutrophils, monocytes, and macrophages from blood and different tissues by flow cytometry analysis. The blood and tissue samples were collected from C57BL/6J mice (n = 3 to 5) challenged with PBS, pathogenic *E. coli*, or *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10*. Data are presented as mean ± SEM. Significant differences (*p < 0.05, **p < 0.01, and ***p < 0.001) were identified by two-tailed Student's *t* test.

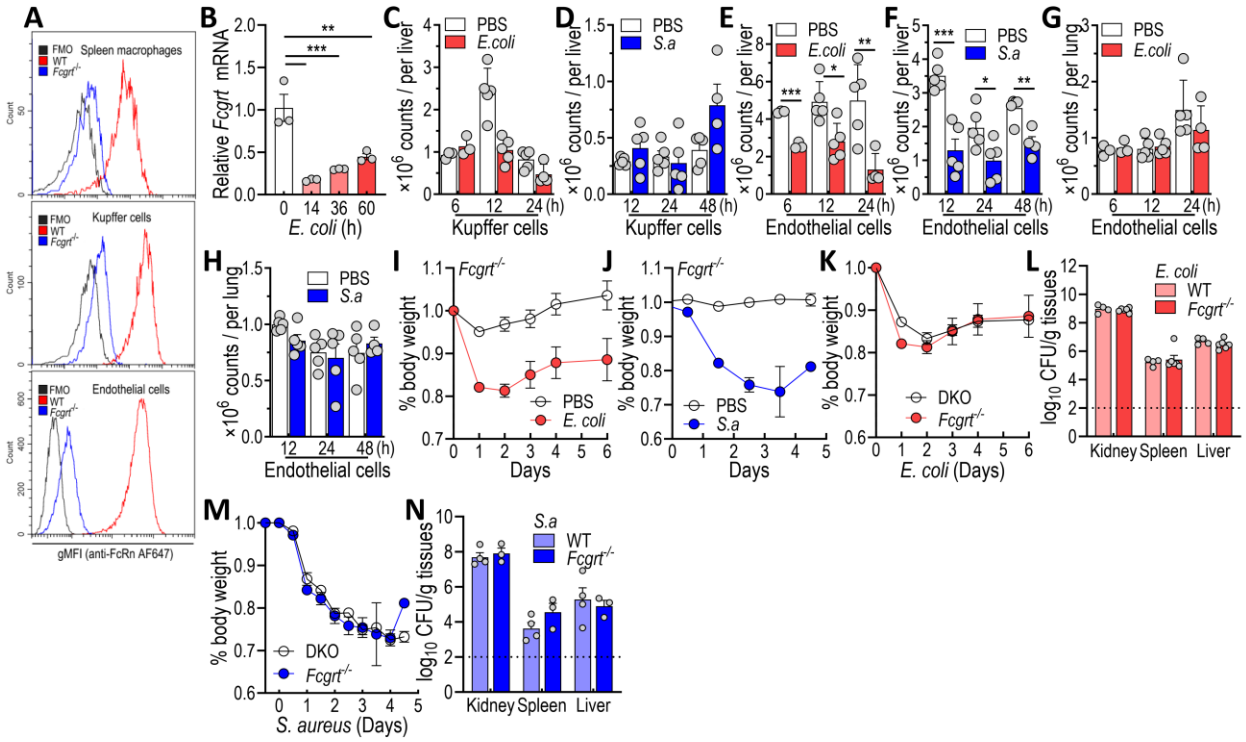

Fig. S3.

Macrophage FcRn reduction-mediated loss of circulating IgG in septic mice. (A) Confirmation of specificity of anti-FcRn mAb in tissue macrophages from WT and *Fcgrt*$^{-/-}$ mice by flow cytometry analysis. (B) qPCR analysis of *Fcgrt* expression in splenic macrophages (n = 3) isolated from mice during sepsis induced by *E. coli*. (C to H) The numbers of Kupffer cells (C and D), liver endothelial cells (E and F), and lung endothelial cells (G and H) in mice (n = 3 to 5) after septic challenge with *E. coli* or *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10*. (I and J) Relative body weight in *Fcgrt*$^{-/-}$ C57BL/6J mice (n = 3 to 5) with pathogenic *E. coli*- (I) or *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10* (J)-induced sepsis. (K and M) Comparison of relative body weight between DKO (n = 5) and *Fcgrt*$^{-/-}$ (n = 6) mice after septic challenge with *E. coli* (K) or *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10* (M). (L and N) Comparison of bacterial loads in tissues between DKO and *Fcgrt*$^{-/-}$ mice (n = 4 to 6) at 96 hours after septic challenge with *E. coli* (L) or *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10* (N). Data are presented as mean ± SEM. Significant differences (*p < 0.05, **p < 0.01, and ***p < 0.001) were identified in B by one-way ANOVA analysis.

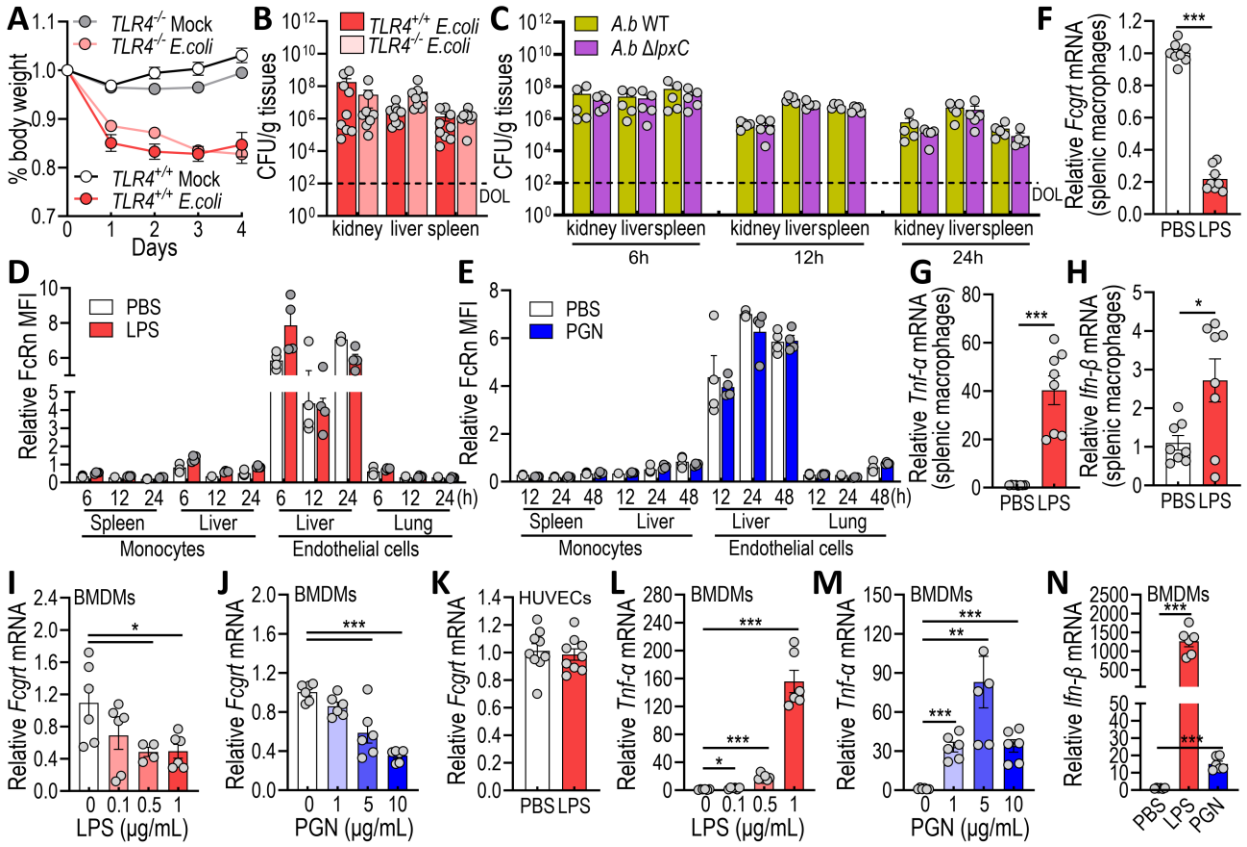

Fig. S4.

Bacteria-derived PAMPs as a cause of sepsis-associated IgG loss in mice. (A) Relative body weight in *TLR4*$^{+/+}$ and *TLR4*$^{-/-}$ C57BL/6J mice (n = 10) with pathogenic *E. coli*-induced sepsis. (B) Bacterial loads in tissues from *TLR4*$^{+/+}$ and *TLR4*$^{-/-}$ C57BL/6J mice at 96 hours after septic challenge with pathogenic *E. coli*. (C) Bacterial loads in tissues from mice at indicated times after septic challenge with *A. baumannii* WT or Δ*lpxC*. (D and E) Flow cytometry analysis of relative FcRn expression in monocytes and endothelial cells isolated from murine tissues (n = 3 to 5) at indicated times after LPS (D, 5 mg/kg) or PGN (E, 10 mg/kg) challenge. (F to H) qPCR analysis of *Fcgrt* (F), *Tnf-α* (G), and *Ifn-β* (H) expression in splenic macrophages from PBS- or LPS-treated C57BL/6J mice (n = 8). (I and J) qPCR analysis of *Fcgrt* expression in BMDMs (n = 5 to 6) treated with indicated doses of LPS (I) or PGN (J) for 6 h. (K) qPCR analysis of *Fcgrt* expression in HUVECs (n = 9) treated with LPS (1 μg/ml) for 12h. (L to N) qPCR analysis of *Tnf-α* (L and M) and *Ifn-β* (N) expression in BMDMs (n = 5 to 6) treated with indicated doses of LPS or PGN for 6 h. Data are presented as mean ± SEM. Significant differences (*p < 0.05, **p < 0.01, and ***p < 0.001) were identified in F-H by two-tailed Student's t test and in I, J, L, M, and N by one-way ANOVA analysis.

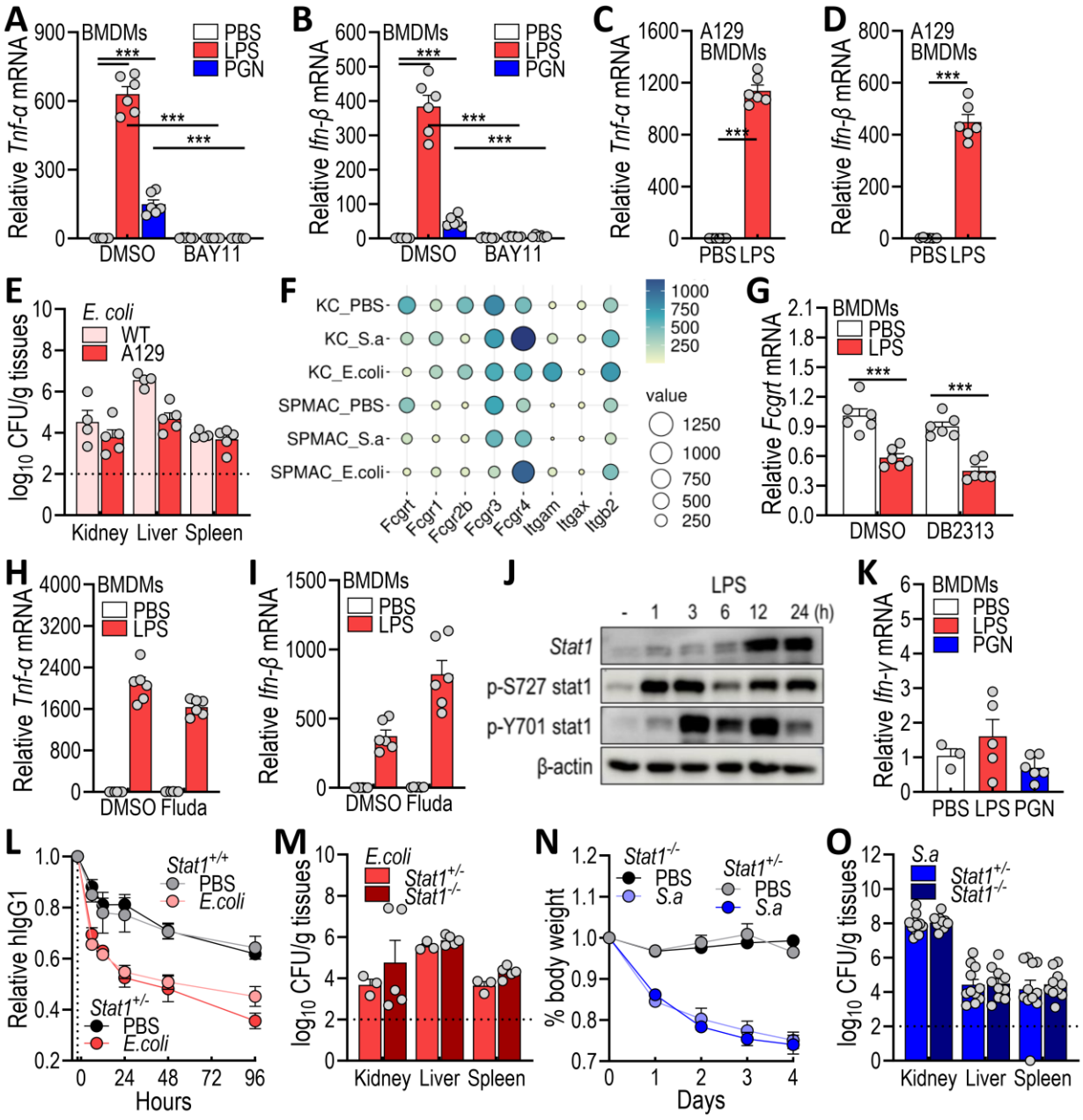

Fig. S5.

STAT1 activation-mediated FcRn reduction and IgG loss in septic mice. (A and B) Effect of NF-κB inhibitor BAY11-7082 (BAY11, 10 μM) on *Tnf-α* (A) and *Ifn-β* (B) expression in BMDMs (n = 6) treated with LPS (1 μg/ml) or PGN (10 μg/ml) for 12h. (C and D) qPCR analysis of *Tnf-α* (C) and *Ifn-β* (D) expression in A129 mouse-derived BMDMs (n = 6) treated with LPS (1 μg/ml) for 12h. (E) Bacterial loads in tissues from A129 mice at 96 hours after septic challenge with pathogenic *E. coli*. (F) Expression levels of FcRn, FcγRs, and CRs in kupffer cells (KC) and splenic macrophages (SPMAC) from septic mice. Transcriptomic data were derived from mice with sepsis induced by pathogenic *E. coli* or *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10*. (G) qPCR analysis of *Fcgrt* expression in BMDMs (n = 6) treated with PU.1 (encoded by the *Spi1* gene) inhibitor DB2313 (300 nM) and stimulated with LPS (1 μg/ml) for 12h. (H and I)

Effect of STAT1 inhibitor Fludarabine (Fluda, 50 μM) on *Tnf-α* (H) and *Ifn-β* (I) expression in BMDMs ($n = 6$) treated with LPS (1 μg/ml) for 12h. (J) Western blotting analysis of STAT1 phosphorylation in BMDMs treated with 1 μg/ml LPS. β-actin was used as the loading control. (K) qPCR analysis of *Ifn-γ* expression in BMDMs ($n = 3$ to 6) treated with LPS (1 μg/ml) or PGN (10 μg/ml) for 12h. (L) Serum hIgG1 kinetics in *Stat1*$^{+/-}$ and *Stat1*$^{+/+}$ C57BL/6J mice ($n = 5$) following its administration and subsequent septic challenge with pathogenic *E. coli*. (M and O) Bacterial loads in tissues from *Stat1*$^{+/-}$ and *Stat1*$^{-/-}$ C57BL/6J mice at 96 hours after septic challenge with *E. coli* (M, $n = 3$ to 5) or *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10* (O, $n = 11$). (N) Relative body weight in *Stat1*$^{+/-}$ and *Stat1*$^{-/-}$ C57BL/6J mice ($n = 10$) with pathogenic *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10*-induced sepsis. Data are presented as mean ± SEM. Significant differences (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$) were identified in A, B, and G by one-way ANOVA analysis and in C and D by two-tailed Student's *t* test.

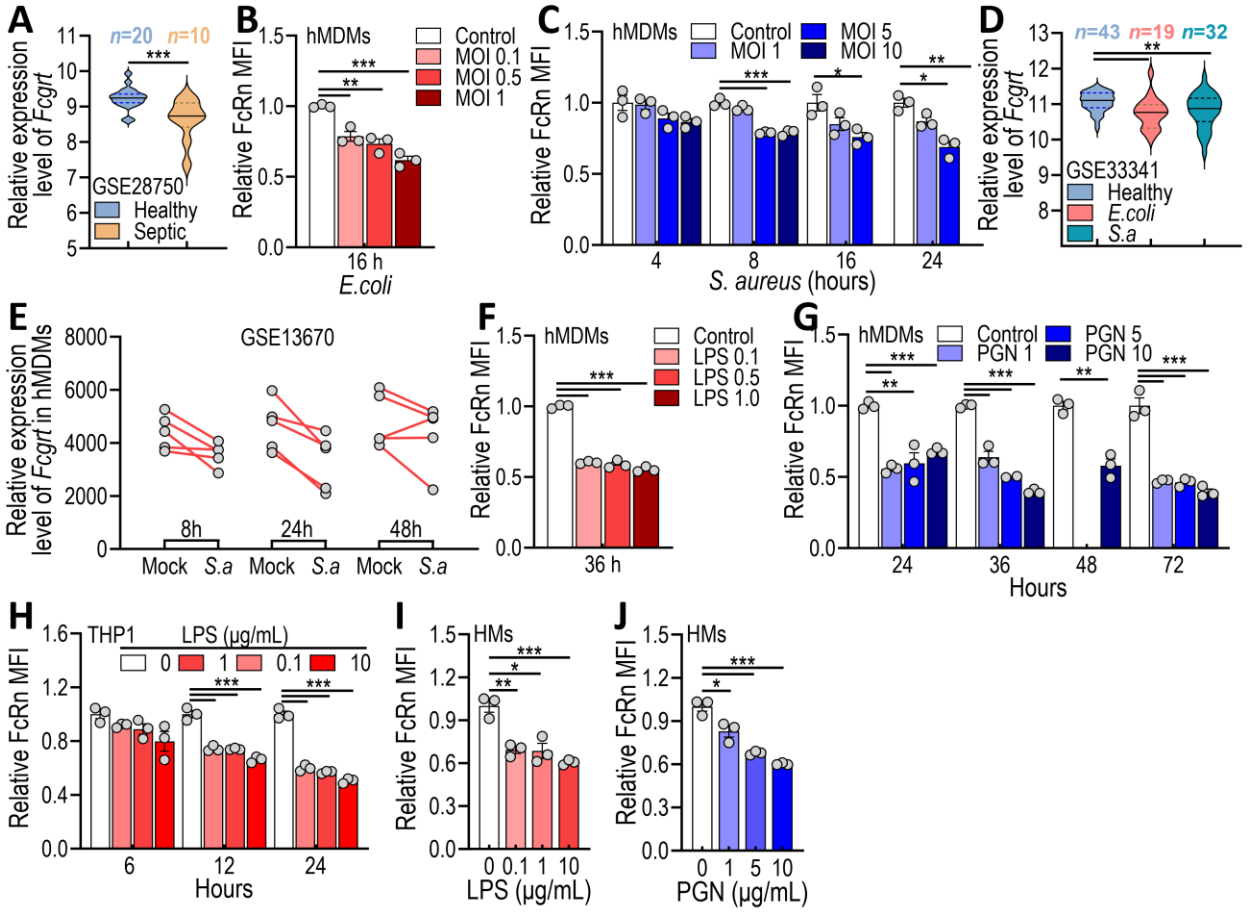

Fig. S6.

Conservation of the bacteria-PAMP-STAT1 pathway driving FcRn reduction in humans. (A) Analysis of *Fcgrt* expression in a public dataset (GSE28750) of whole-blood leukocytes from healthy donors and septic patients. (B and C) Flow cytometry analysis of FcRn expression in hMDMs ($n = 3$) infected with *E. coli* (B) or *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10* (C) at indicated MOIs and times. (D) Analysis of *Fcgrt* expression in a public dataset (GSE28750) of whole-blood leukocytes from healthy donors and septic patients with infections caused by *E. coli* or *S. aureus*. (E) Analysis of *Fcgrt* expression in a public dataset (GSE13670), featuring hMDMs infected with *S. aureus* at indicated times. (F and G) Flow cytometry analysis of FcRn expression in hMDMs ($n = 3$) stimulated with LPS (F) or PGN (G) at indicated concentrations and times. (H to J) Flow cytometry analysis of FcRn expression in THP1 cells (H, $n = 3$) and human CD14+ monocytes (I and J, $n = 3$) stimulated with LPS (H, I) or PGN (J) at indicated concentrations and times. Data are presented as mean ± SEM. Significant differences (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$) were identified in A by two-tailed Student's *t* test and in B, C, D, F, G, H, I, and J by one-way ANOVA analysis.

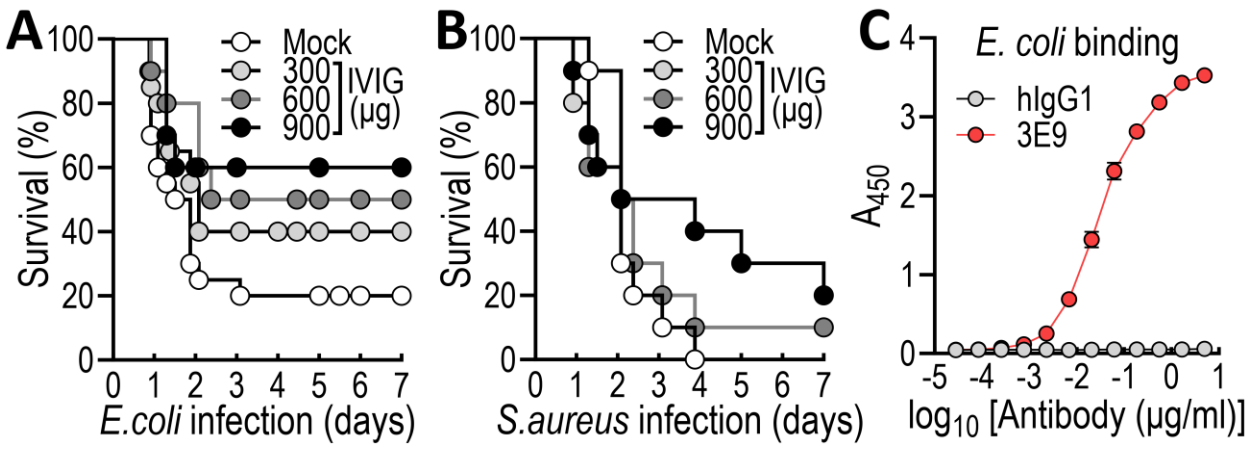

Fig. S7.

Antibody efficacy in mice with bacterial sepsis. (A and B) Survival of mice (n = 10) after IVIG administration at indicated doses, followed by septic challenge with *E. coli* (A) or *S. aureus* Δ*spa*Δ*sbi*Δ*ssl10* (B). (C) ELISA analysis (n = 3) of hIgG1 and 3E9 mAb binding to *E. coli*. Data are presented as mean ± SEM.

References and Notes

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