Supplemental Methods and Figures

Critical care monitoring

Respiration and heart rate, temperature and mean systemic arterial pressure (MSAP) were monitored with a Cardell Max-12 HD Duo monitor. These physiologic data were recorded every 30 minutes, and blood samples were collected at T-0.5, 0, +2, +4, +6, +8, +24, +48, +72 and +168 hours. The total amount of blood that was withdrawn over the first 24 hours period was less than 10% of the animals' calculated blood volume (70 ml/kg).

Custom sandwich ELISAs for protease-inhibitor complexes

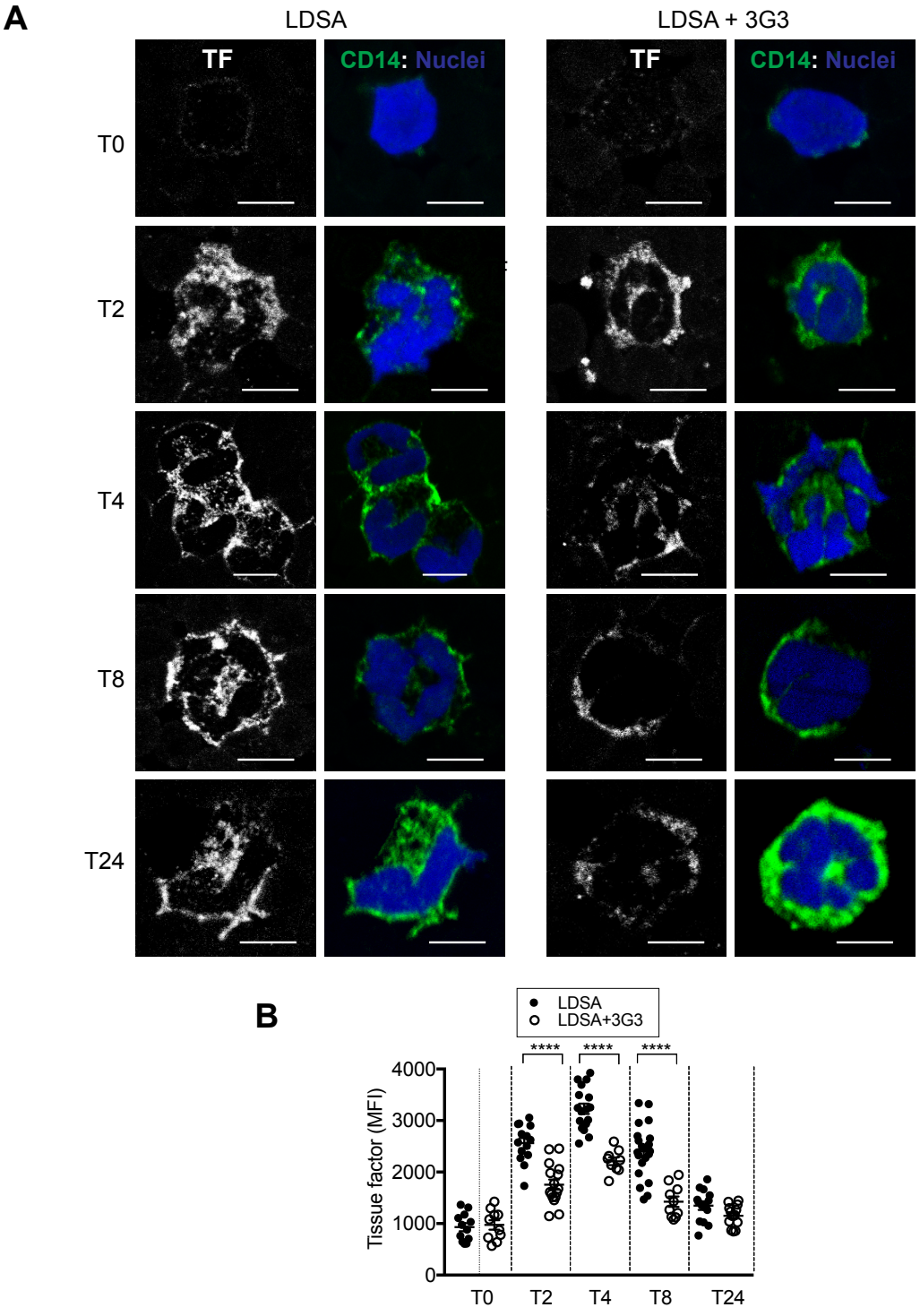
96-well plates were coated overnight at 4°C with one of the following affinity-purified antibodies (100 µl/well): (i) goat anti-human FXII (2 µg/ml); (ii) sheep anti-human FXI (2 µg/ml); (iii) sheep anti-human FIX (2 µg/ml); (iv) sheep anti-human FX (2 µg/ml); (v) sheep anti-human thrombin (2 µg/ml), all from Affinity Biologicals; or (vi) goat anti-human FVII (0.3 µg/ml, R&D Systems, Minneapolis, MN, USA). The standards for FXIIa-AT, FXIa-AT and FIXa-AT complexes were serial dilutions of dextran sulfate (0.1 mg/ml) treated, anticoagulated baboon plasma (recalcified and lepirudin treated). For FVIIa-AT standard curve, baboon plasma was incubated with tissue factor-phospholipids vesicles (PT reagent). After each step plates were washed with PBS containing 0.1% Tween-20 then blocked with the same buffer containing 1%BSA. EDTA plasma samples were incubated for 60 minutes in PBS containing 1% BSA, 5mM EDTA, 5mM Benzamidine hydrochloride, 0.1% Tween-20. Biotinylated affinity purified sheep anti-human antithrombin (0.5µg/ml, Affinity Biologicals) was used for detection followed by streptavidin-HRP (400 ng/ml, Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and ortho-phenylenediamine (OPD) as substrate. The absorbance values were recorded at 492nm. FXIIa-C1inhibitor and kallikrein-C1inhibitor complexes were measured by ELISA utilizing the mouse monoclonal anti-human C1inhibitor (clone KOK12, gift from Dr. S. Zeerleder, Sanquin

B.V., Amsterdam, Netherlands, 2 µg/ml) for capture and HRP conjugated affinity purified anti FXII (0.5 µg/ml, Affinity Biologicals) or anti prekallikrein (0.5 µg/ml, Affinity Biologicals) antibodies as detection antibody, as described[1]. The percentage of protease-serpin complexes was calculated by equating the amount at T0 as 100%.

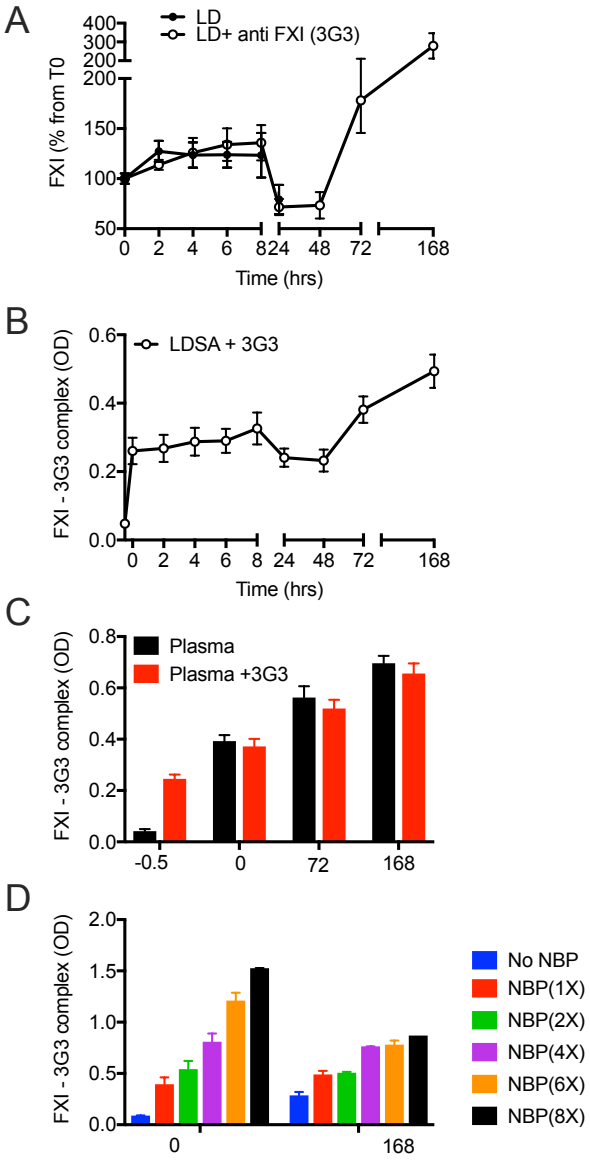
Characterization of the availability of the 3G3 antibody and dynamics of in vivo FXI inhibition. Total FXI protein in plasma was detected with a sandwich ELISA from Affinity Biologicals. FXI-3G3 complexes in plasma were detected with a custom sandwich ELISA using a sheep polyclonal antibody anti human FXI for capture and a donkey anti human IgG-HRP conjugate for detection. To determine the FXI that is potentially not bound to 3G3, plasma samples collected before (T0) and two time-points after challenge (72 and 168 hours) were incubated in vitro with an additional amount of 3G3 (25 µg/ml) and the change in amount of FXI-3G3 complexes was determined as above. Plasma collected before infusion of 3G3 (T-0.5h) was used as baseline reference. Addition of exogenous 3G3 did not increase FXI-3G3 levels at any time point, suggesting that all FXI in plasma was already in complex with 3G3. To determine the amount of 3G3 available to inhibit FXI, increasing concentrations of normal baboon plasma were added to diluted plasma samples collected from two animals treated with 3G3, before (0) and at 168 hours post-challenge. The results show that 3G3 was at least 8 fold excess over FXI at T0 (before challenge) and four fold at the end of the experiment (168h).

References

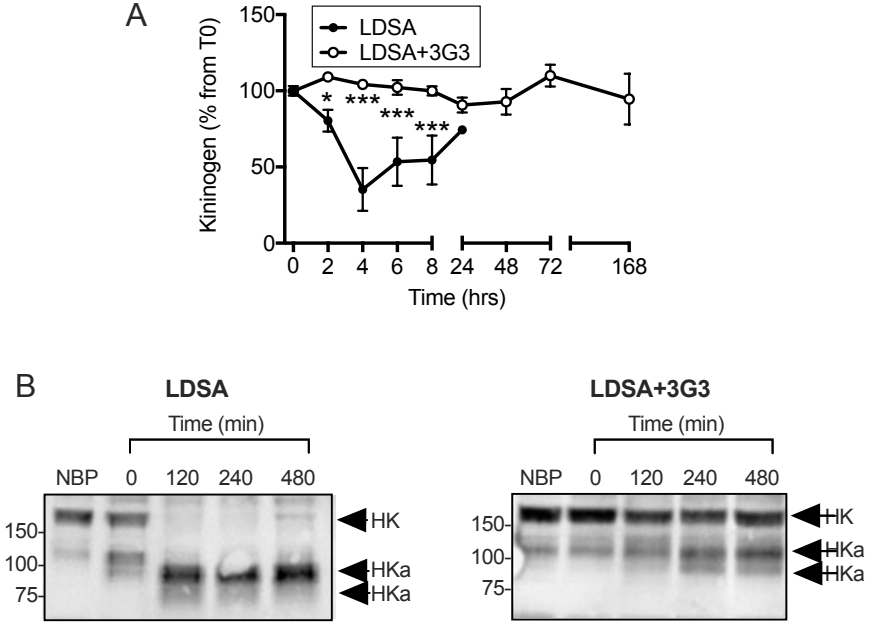
1. Popescu NI, Silasi R, Keshari RS, et al. Peptidoglycan induces disseminated intravascular coagulation in baboons through activation of both coagulation pathways. *Blood*. 2018;132(8):849-860.



Supplemental Figure 1. Semiquantitative analysis of tissue factor expression on blood cells.
(A): Immunostaining for tissue factor and CD14 on blood smears collected during the time course of *S. aureus* challenge, without (LDSA) or with 3G3 treatment (LD100+3G3). Serial blood smears were methanol fixed, sequentially stained with biotinylated IgG anti-human TF (HTF-1) and monoclonal anti human CD14 (23G4), followed by detection with Cy3-labeled streptavidin (Jackson ImmunoResearch, West Grove, PA) and donkey anti mouse FITC. Confocal images were acquired on a Nikon Eclipse TE2000-U inverted microscope equipped with a Nikon C1 scanning head using EZ-C1 software. Representative micrographs are shown. To better differentiate the changes in fluorescence intensity, TF staining is shown as grayscale; CD14 is shown in green and nuclear counterstaining in blue. Magnification bar: 10μm.
(B): Quantitation of TF immunostaining on serial blood smears depicted in panel A. Mean fluorescence intensity (MFI) of monocyte TF staining was quantified in at least 10 individual fields from each time point. Same time-point data are compared between untreated and 3G3-treated groups using two-tailed Student's t-test. ****P<0.0001.



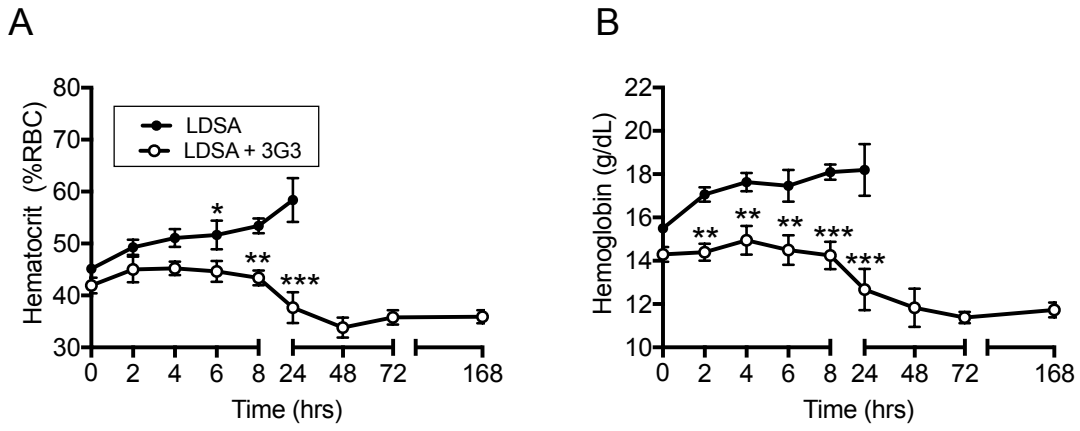
Supplemental Figure 2. Dynamics of FXI inhibition by 3G3 during the time course of sepsis.
(A): Total Factor XI antigen determined by a sandwich ELISA. FXI concentration at T0 is shown as 100%.Data are shown as mean±SEM.
(B): Factor XI-3G3 complexes in plasma measured by a sandwich ELISA using anti FXI antibodies for capture and anti human IgG-HRP for detection. Data are shown as mean±SEM of optical density (OD).
(C): To determine the FXI that is potentially not bound to 3G3, plasma samples collected from treated baboons, before (T0) and two time-points after bacteria challenge (72 and 168 hrs) were incubated in vitro with additional 3G3 (25 µg/ml) and the change in amount of FXI-3G3 complexes was determined as above. Plasma collected before infusion of 3G3 (T-0.5h) was used as baseline reference. Data are shown as mean±SEM of optical density (OD).
(D): To determine the amount 3G3 available to inhibit FXI, increasing concentrations of normal baboon plasma (NBP) were added to 1:100 diluted plasma samples collected from two animals treated with 3G3, before (0) and at 168 hours post-challenge. The results show that 3G3 vs. FXI was at least 8 fold excess at T0 (before challenge) and four fold at the end of the experiment (168h). Data are shown as mean±SEM of optical density (OD).



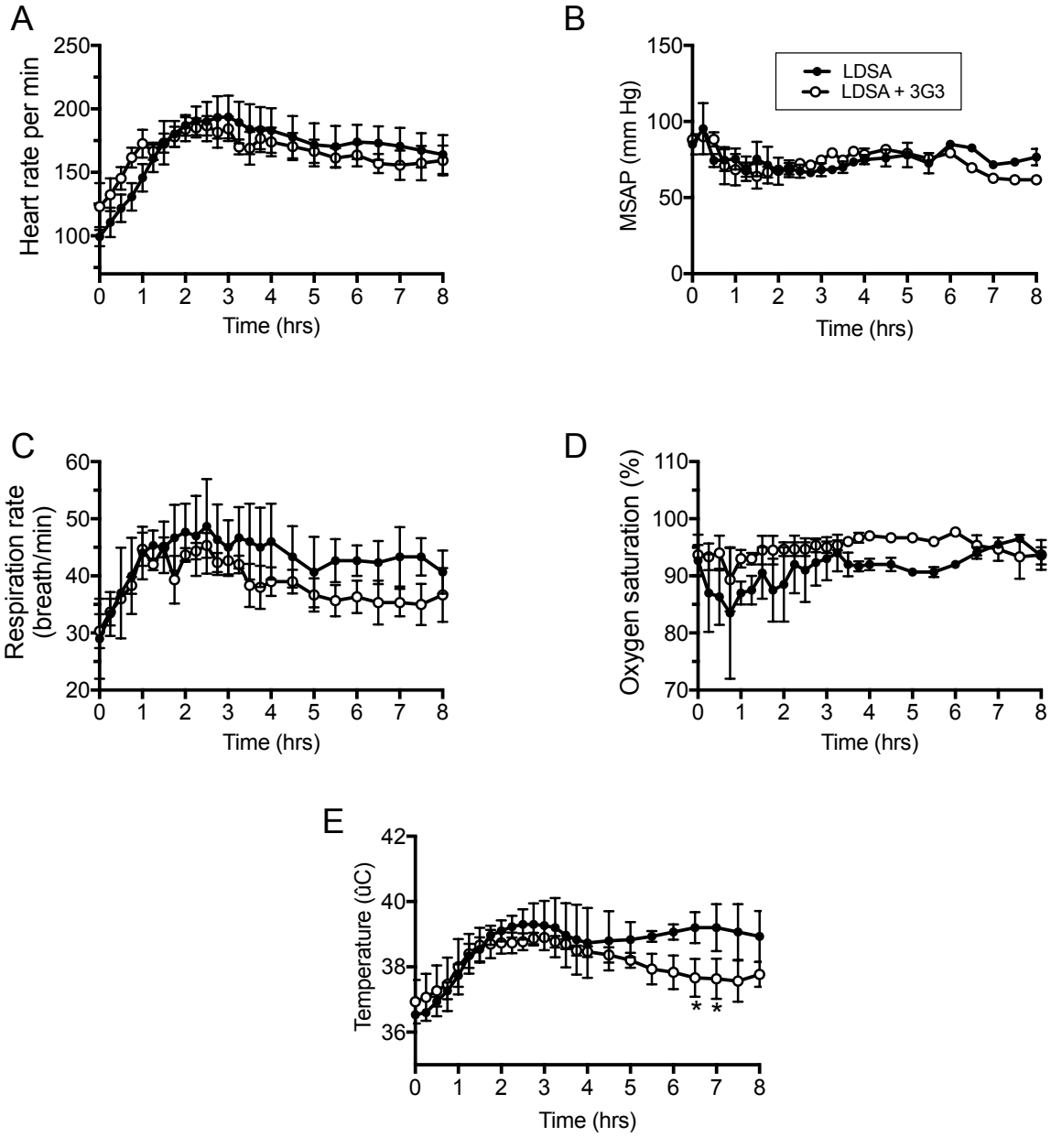
Supplemental Figure 3. Effect of pretreatment with 3G3 on plasma kininogen following the challenge with heat inactivated *S. aureus* in baboons.

(A): Time-course changes of total kininogen measured using an immunoassay that detects both high molecular kininogen and its degradation forms. Data are represented as mean±SEM. Same time-points are compared between LD-challenged (LDSA) and LD challenged, 3G3 pretreated (LDSA+3G3) animals using two-tailed Student's test. *p<0.05; ***p<0.001.

(B): Western blot detection of high-molecular weight kininogen cleveage products in plasma collected from representative animals challenged with a lethal dose of heat-inactivated S. aureus (LDSA) without or with 3G3 treatment (LDSA+3G3).

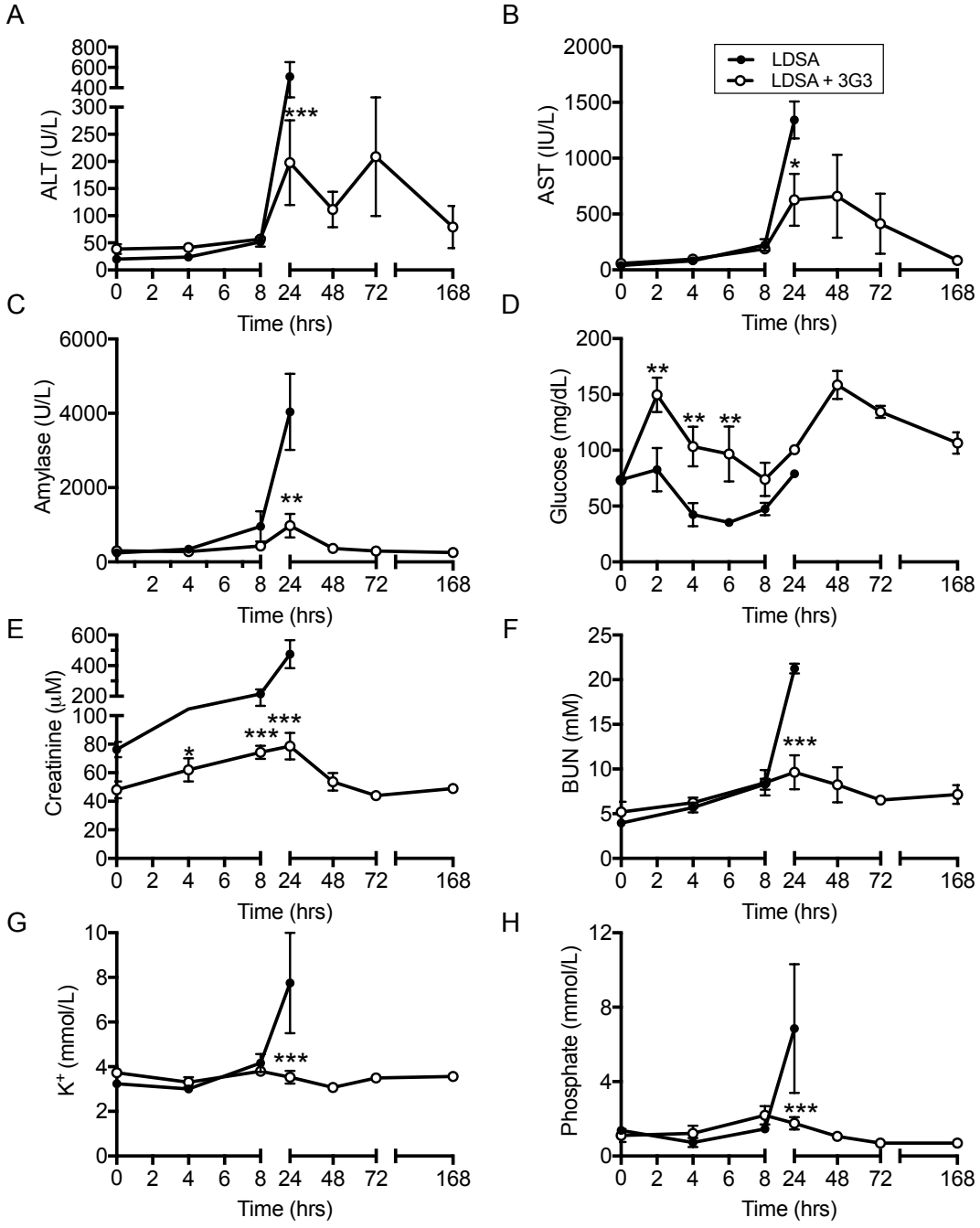


Supplemental Figure 4. Effect of pretreatment with 3G3 on the time course of changes in hematocrit and hemoglobin levels following a lethal dose of heat-inactivated *S. aureus* injection.
Data are represented as mean ± SEM. Same time-points are compared between untreated and 3G3-treated animals using two-tailed Student's t test. ***P< 0.001.



Supplemental Figure 5. Effect of pretreatment with 3G3 on changes in vital signs following a lethal dose of heat-inactivated *S. aureus* injection.

(A) Heart rate, (B) mean systemic arterial pressure (MSAP), (C) respiration rate, (D) oxygen saturation, (E) body temperature. Data are presented as mean ± SEM. Same time-points are compared between untreated and 3G3-treated animals using two-tailed Student's t test. *P < 0.05, **P < 0.01, ***P < 0.001.



Supplemental Figure 6. Effect of pretreatment with 3G3 on changes in liver and kidney damage and function parameters following a lethal dose of heat-inactivated *S. aureus* injection.
Time course evaluation of plasma biomarkers of: Liver injury: (A) alanine aminotransferase [ALT] and (B) aspartate aminotransferase [AST]; Liver function: (D) glucose; Pancreas injury: (C) amylase; Pancreas function (D) glucose ; Kidney function: (E) creatinine, (F) blood urea nitrogen, (G) potassium and (H) phosphate. Data are presented as mean ± SEM. Same time-points are compared between untreated and 3G3-treated animals using two-tailed Student's t test.: *P < 0.05, **P< 0.01, **P< 0.001.