Supporting Information (SI) Appendix for

Using Heparan Sulfate Octadecasaccharide (18-mer) as a Multi-target Agent to Protect Against Sepsis

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Materials and Methods

Synthesis of Heparan Sulfates

The syntheses of 6-mer, 12-mer, 18-mers were completed according to the method published previously (1). Briefly, PmHS2 (heparan synthase 2 from P. multocida) was used with UDP-sugars to elongate the monosaccharide, GlcA-pNP (p-nitrophenyl glucuronide, from Carbosyn), to the desired size of backbones. The backbone was then subjected to modification with N-sulfotransferase (NST), C_5-epimerase (C_5-epi), and 2-O-sulfotransferase (2-OST). Four primary steps were involved in the synthesis, including step elongation-1 to add GlcNTFA, step elongation-2 to add GlcA, step N-sulfation, and step of epimerization/2-O-sulfation (Supplementary Fig. S1A). These steps were repeated to prepare the final products. Finally, the products were purified using a Q-Sepharose column (GE Health) by anion-exchange chromatography with an overall yield of ~4%, ~17% and ~48% for 18-mer, 12-mer and 6-mer, respectively. Using the chemoenzymatic method, we also synthesized both NAc 18-mer and NS6S 18-mer. For the synthesis of NAc18-mer, only elongation steps were involved; for the synthesis of NS6S 18-mer, elongation, *N*-sulfation and 6-*O*-sulfation steps were involved. ESI-MS was used to measure the molecular weight and prove the structures of the products.

Step elongation-1 was to add a GlcNTFA residue. In the example of synthesizing an 8-mer (GlcNTFA-GlcA-GlcNS-GlcA-GlcNS-IdoA2S-GlcNS-GlcA-pNP) intermediate from a 7-mer (GlcA-GlcNS-GlcA-GlcNS-IdoA2S-GlcNS-GlcA-pNP), the reaction mixture included NaAcO (50 mM, pH 6), $MnCl_2$ (5 mM), 13 g of 7-mer, pmHS2 (90 μg/ml) and UDP-GlcNTFA (24 mM) in a total volume of 500 mL, then incubated at 37°C overnight. A Q-Sepharose column (0.4 L) was used for purification with a gradient elution from 0.2 M to 0.8 M NaCl in 20 mM sodium acetate pH 5 in 240 min; the flow rate was 4 mL/min.

Step elongation-2 was to add a GlcA residue. In the example of converting the 8-mer (GlcNTFA-GlcA-GlcNS-GlcA-GlcNS-IdoA2S-GlcNS-GlcA-pNP) intermediate to a 9-mer intermediate (GlcA-GlcNTFA-GlcA-GlcNS-GlcA-GlcNS-IdoA2S-GlcNS-GlcA-pNP), the reaction mixture included NaAcO (50 mM, pH 6), $MnCl_2$ (5 mM), 14 g of 8-mer intermediate, pmHS2 (135 μg/ml) and UDP-GlcA (10 mM) in a total volume of 1.3 L, then incubated at 37°C overnight. A Q-Sepharose column (0.4 L) was used for purification with gradient elution from 0.2 M to 0.8 M NaCl in 20 mM sodium acetate pH 5 in 240 min; the flow rate was 4 mL/min.

Step N-sulfation converted a $GlcNH_2$ residue to a GlcNS residue using N-sulfotransferase (NST). In one example, 13 g of $GlcNH_2$-GlcA-GlcNS-GlcA-GlcNS-IdoA2S-GlcNS-GlcA-pNP was incubated with NST (45 μg/ml) and PAPS (2 equivalence of free amino groups) in a solution containing 2-(N-morpholino) ethanesulfonic acid (MES, 50 mM, pH 7.0) and at 37°C overnight in a reaction volume of 0.7 L. A Q-Sepharose column (0.4 L) was used for purification with gradient elution from 0.2 M to 0.8 M NaCl in 20 mM sodium acetate pH 5 in 240 min; the flow rate was 4 mL/min.

Step epimerization/2-*O*-sulfation was to convert an internal GlcA residue to an IdoA2S residue using both C5-epi and 2-OST. For example, 13 g of GlcNS-GlcA-GlcNS-GlcA-GlcNS-IdoA2S-GlcNS-GlcA-pNP (8-mer) was incubated in a solution containing Tris (50 mM) buffer (pH 7.5) and semi-purified C5-epi (6 μg/ml), 2-OST (13 μg/ml) and PAPS (2 equivalence of start 8-mer material amount) at 37°C overnight in a total volume of 1 L. A Q-Sepharose column (0.4 L) was used for purification with a gradient elution from 0.4 M to 0.8 M NaCl in 20 mM sodium acetate pH 5 in 320 min; the flow rate was 4 mL/min.

The saccharide backbones were purified using a C18-column (3 × 15 cm, or 120 g, Biotage) with a gradient elution method (0–80% methanol in H_2O, 0.1% trifluoroacetic acid, 5

mL/min). All sulfated saccharide products were purified using a Q-Sepharose column and were eluted with a linear gradient of 0.2 -1 M NaCl in 20 mM NaOAc-HAcO, pH 5.0 in 3-6 hours. Different sizes of Q-Sepharose columns were chosen by the binding affinity of the product and reaction scale. At the end of the synthesis, we obtained 6-mer (13 g, purity 99.5%), 12-mer (12 g, purity 99.2 %), and 18-mer (2.4 g, purity 90.2%). Here are the ESI-MS results: 6-mer measured MW = 1455.56 Da (theoretical MW=1455.18 Da): 12-mer measured MW = 2925.53 Da (theoretical MW = 2925.35 Da); 18-mer measured MW = 4456.90 Da (theoretical MW = 4455.55 Da); NAc18-mer measured MW = 3555.5 Da (theoretical MW = 3553.97) and NS6S18-mer measured MW = 4616.0 Da (theoretical MW = 4615.7).

At every synthesis step, the products were monitored by Shimadzu HPLC equipped with a Propac column (Propac PA1, 10 μm, 9 mm × 250 mm, Thermo Fisher). Buffer A was 20 mM NaAcO (pH 5.0), and buffer B was 2 M NaCl with 20 mM NaAcO (pH 5.0). A linear gradient was used to separate the start material and product. Different gradients were chosen by the sulfation degree of compounds. The UV detector was set at 310 nm to monitor the oligosaccharides. The structures of the intermediates from each step were characterized by electrospray ionization mass spectrometry (ESI-MS).

ESI-analysis were performed at a Thermo LCQ-Deca in negative ionization mode. A syringe pump (Harvard Apparatus) introduces the sample by direct infusion (50 μL/min). The sample (2-5 μg) was diluted in 200 μL of 10 mM ammonium bicarbonate with the electrospray source set to 3 kV and 150 °C. The automatic gain control was 1×10^7 for full scan MS. The MS data were acquired and processed using Xcalibur 1.3.

Endotoxin removal for HS

Endotoxin-free oligosaccharides(6-mer, 12-mer, 18-mer and NAc 18-mer) were prepared for the animal studies based on a previously published method (2). Briefly, a sterile 50 mL centrifuge tube (Amicon Ultra-15, Ultracel-100k; Merck Millipore) was used to remove endotoxin in 6-mer, 12-mer, and 18-mers by centrifugation of the oligosaccharide solution at 4,000 rpm for 10 min with four repeated washes to collect all HS oligosaccharides. The filtered solutions containing oligosaccharides were collected and dried. The level of endotoxin in the purified oligosaccharides was confirmed by the Limulus Amebocyte Lysate (LAL) kit (sensitivity = 0.125 EU/mL, Associates of Cape Cod Inc.) according to manufacturer's protocol.

Determination of the in vitro and ex vivo anti-FXa and anti-IIa activity

An anti-Xa activity assay was performed on a previously published method (2). Briefly, the dilution was prepared as follows: human FXa (Enzyme Research Laboratories) to 25 U/mL with PBS; chromogenic substrate S-2765 (Diapharma) to 1 mg/mL in water; unfractionated heparin (UFH), fondaparinux (FPX), 6-mer, 12-mer, 18-mer to 5 μg/mL in PBS. The reaction mixture with 60 μL of human plasma (Sigma-Aldrich) and 60 μL of solution containing saccharides at different concentrations was incubated for 2 min at room temperature. FXa (100 μL) was added for another 4 min incubation at room temperature. S-2765 substrate (30 μL) was added, and the reaction was measured at 405 nm continuously for 3 min. The absorbance values were plotted against the reaction time.

An anti-IIa activity assay was performed based on a previously published method (2). Briefly, the dilution was prepared as follows: thrombin from bovine plasma (Sigma-Aldrich) to 20 U/mL in PBS with bovine serum albumin (1 mg/mL); chromogenic substrate S-2238 (Diapharma) to 1 mg/mL in water; UFH, FPX, 6-mer, 12-mer, 18-mer to 5 μg/mL in PBS. The

reaction mixture with 60 μL of antithrombin (35 μg/mL, Cutter biologics) and the saccharide solution (60 μL) was incubated for 2 min at room temperature. Thrombin was added and incubated for 4 min at room temperature before adding S-2238 substrate (30 μL) and measured at 405 nm continuously for 5 min. The absorbance values were plotted against the reaction time.

Heparanase digestion of 18-mers

Recombinant heparanase was expressed in insect cells using the baculovirus expression system and purified by a heparin-Sepharose column, as previously described (3). 18-mer or NS6S 18-mer (200 μg/mL) was incubated with heparanase (20 μg/mL) at 37 ℃ in 50 mM MOPS at pH 6. After 24 hours of incubation, the reaction was inactivated by heating at 95℃ for 5 min.

LC-MS and HPLC analysis of heparanase digested 18-mer and NS6S 18-mer

LC-MS/MS analysis of heparanase digested oligosaccharides was implemented on a Vanquish Flex UHPLC system (Thermo Fisher, Waltham, MA) coupled with a TSQ Fortis triple-quadrupole mass spectrometer. The ACQUITY Glycan BEH Amide column (1.7 μm, 2.1 × 150 mm; Waters) was used at 60°C. The mobile phase A was 50 mM ammonium formate in water (pH 4.4) and mobile phase B was acetonitrile. The elution gradient and flow rate consisted of 0-6 min 83% B, flow rate 0.3 mL/min; 6.1-45 min 83-5% B, flow rate 0.25 mL/min; 45-55 min 5% B, 0.25 mL/min; 55.1-65 min 83% B, flow rate 0.3 mL/min. On-line triple-quadrupole mass spectrometry was used as the detector. ESI-MS analysis was operated in the negative-ion mode using the following parameters: Negative ion spray voltage at 3.0 kV, sheath gas at 55 Arb, aux gas 25 arb, ion transfer tube temperature at 250°C and vaporizer temperature at 400°C.

TraceFinder software was applied for data processing. A total of 0.5-1 μg of heparanase digested oligosaccharides in 2 μL was injected per run.

The reaction of heparanase digestion was also monitored using an ion exchange HPLC column (ProPac PA1) with a gradient elution of buffer B (2M NaCl, 20 mM sodium acetate, pH 5.0) from Buffer A (20 mM sodium acetate, pH 5.0) at a flow rate of 1 ml/min. A linear gradient of 40-50% B in 40 minutes was used for the analysis of 18-mer, and a linear gradient of 70-85% B in 40 minutes was used for the analysis of NS6S18-mer. The eluent was detected by an on-line UV detector at 310 nm.

Cecal ligation and puncture (CLP) mouse model

All studies have followed the protocols approved by the University of North Carolina (UNC) at Chapel Hill Institutional Animal Care and Use Committee (IACUC ID:20-009). CLP was performed on a previously published method (4) with male 10 to 12 weeks old C57/BL6 mice (the Jackson Laboratory) with body weights of around 25 to 30 grams to induce polymicrobial sepsis. Mice were housed in the animal facility for at least two weeks before the experiment to ensure the fecal flora stability. Mice were anesthetized with 3% isoflurane for hair removal and the following surgery to perform CLP injury. The mouse abdomen was sterilized with betadine and ethanol, and a 1-cm midline incision was made. The mouse cecum was externalized, ligated 1-cm from the distal end of the cecum with a 4:0 silk suture, punctured through-and-through with a 21-gauge needle, and gently squeezed to extrude a small amount of feces from the perforation sites. The cecum was reinternalized before the double-layer closure of the peritoneum and skin with 5:0 nylon sutures and the AutoClip system, respectively. Sham mice received a similar process as CLP mice described above, without the cecum externalized and reinternalized but not ligated or punctured. After the surgery, each mouse was given 500 μL of fluid in total after the

CLP surgery to prevent dehydration. A 500 μL of fluid included saline, meloxicam (2mg/kg), with or without the 18-mer (20 mg/kg). Mice were recovered on a warm blanket until they could raise themselves. In the biomarker analysis study, each mouse was given 10 μL/g at 0, 6, and 12 hours, with or without the 18-mer. For example, the treated mice at 25 g were administered 250 μL of 18-mer (2 mg/mL) or saline. At 24 hours after CLP, mice were anesthetized with 3% isoflurane for blood, peritoneal lavage, and organ collection. Blood was collected through inferior vena cava with 50 μL of 3.8% sodium citrate; peritoneal lavage was collected by flushing 1 mL sterile PBS in the peritoneal space. After sample collection, mice were sacrificed with cervical dislocation. In the survival study, mice were administered subcutaneously with 20 mg/kg of 18-mer (or 6-mer) at 0, 6, 12, 24, 36, and 52 hours after CLP injury. Mice were frequently checked at least every 6 hours until sacrificed at 72 hours after CLP. Mice were promptly euthanized during the experiment if they met the humane endpoint.

Hematologic analysis

After blood collection from mice in the CLP model, complete blood count was determined in whole blood by Element HT5 veterinary complete blood count analyzer (Heska).

Counts of colony forming units (CFU)

After peritoneal lavage collection from mice in the CLP model, the diluted peritoneal lavage (1:100, 1:1000, 1:10000) in sterile PBS were plated on agar plates (1.5% agarose, Fisher; 2% Difco LB broth, BD) and incubated under aerobic conditions at 37 °C. Bacterial colonies were counted after 24 hours. Results were specified as CFU/mL in log10.

Pull-down assay/Immunoblot

Biotinylated LPS (Invivogen, USA) (5 mg/kg) with or without HS (6-mer, 12-mer, 18-mer at 5 mg/kg or 50 mg/kg) was injected into male 10 to 12 weeks old C57/BL6 mice intraperitoneally (i.p.). After 2 hours, the mouse was sacrificed, and the mouse peritoneum was lavaged with 1 mL of sterile PBS. Peritoneal lavage was collected and frozen immediately for pull-down assay. In each pull-down reaction, peritoneal lavage (100 μL) was incubated with 50 μL of Pierce high-capacity streptavidin agarose (Thermo Fisher) at room temperature for 1 hour to isolate biotinylated LPS bound complexes. After washing with PBS five times, samples were eluted with lithium dodecyl sulfate (LDS) sample buffer and 50 mM of tris[2-carboxyethyl] phosphine–HCl (TCEP) solution (Thermo Fisher). Preload (15 μL of peritoneal lavage without streptavidin agarose incubation) and eluted samples were separated using NuPAGE 4-12% Bis-Tris protein gels (Invitrogen), transferred to nitrocellulose membranes (Bio-Rad), and detected by Ponceau S solution (Sigma). Membranes were rinsed and then cut for Western blot staining with anti-HMGB1 (1:10000, Abcam) and ApoA-I (1:4000, Novus Biologicals), followed by anti-rabbit HRP (1:10000, Cell Signaling Technology).

In a competitive binding assay, 1-10 μg/mL of recombinant ApoA-I (Sino Biological) or 100 μg/mL of HS oligosaccharide was incubated with collected peritoneal lavage overnight at 4℃ on a rotator before incubating with streptavidin agarose.

Proteomic analysis

UNC Michael Hooker Proteomics Center performed the proteomic analysis for LPS-binding protein. Briefly, the peritoneal lavage from biotinylated LPS injected mice was eluted by

streptavidin agarose for electrophoresis as described above. Using Coomassie staining, the band around 25 kDa was identified and cut for proteomic analysis (Supplementary Fig. S9). To prepare for LC/MS/MS analysis, half of the sample volume (25 μL) was denatured with 8 M urea, followed by reduction with dithiothreitol (DTT) and alkylation with iodoacetic acid (IAA). The sample was digested with trypsin overnight at 37℃ before acidification with 0.5% trifluoroacetic acid (TFA) and cleaned by a C18 ziptip. The LC/MS/MS analysis was performed on a Thermo Easy nLC1200-PHCO QEHF system, 45 min method. The data was searched against reviewed contaminants and Uniport mouse databased using Sequest within Proteome Discoverer 2.4. Only targets with high confidence peptides and proteins (false discovery rate (FDR) < 1%) and proteins with >1 peptide are shown in the result (Supplementary Table S1), ranked by peptide spectrum match (PSM). As ApoA-I was demonstrated with the most abundant PSM, the LPS-bound protein around 25 kDa was confirmed as ApoA-I.

Histone induced lethality *in vivo*

According to the previous report(5, 6), calf thymus histones (75 mg/kg, Sigma) was injected retro-orbitally into the anesthetized mice by 3% inhaled isoflurane with or without 18-mer or NAc 18-mer (75 mg/kg). The mice were monitored for 300 minutes (5 hours) after injection for the survival experiment. After 300-minute monitoring, mice were euthanized by cervical dislocation under isoflurane (3%).

Histone induced cytotoxicity *in vitro*

EA.hy926 cells were obtained from Dr. Rafal Pawlinski's lab (6). Cells cultured in less than 20 passages were seeded in 12-well plates (3×10^5 cell/ well) in DMEM medium (Gibco) with

10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin. When reaching 90% confluence, cells fasted for 18 hours of incubation in a serum-free DMEM medium. Cells were next incubated in serum-free DMEM containing 30 μg/mL histone H3 (Roche, Germany) with varying concentrations of HS (0 – 50 μg/mL of 6-mer, 12-mer, 18-mer). After 1 hour of incubation, PBS was used to wash cells twice before adding 0.05% trypsin-EDTA (Invitrogen) for detachment. Next, cells were collected by centrifugation (300 × g for 3 min) and resuspended in 1 mL PBS buffer containing 10 μg/mL propidium iodide (Sigma). After 10 min incubation, protected from light, cells were subjected to flow cytometry on Becton Dickinson LSRFortessa to evaluate cell viability. Appropriated unstained and untreated samples were used to calibrate gating and sorting conditions.

LPS induced inflammation in macrophages

Raw264.7 cells were purchased from ATCC (American Type Culture Collection). Cells cultured in less than 20 passages were seeded in 48-well plates (1×10^5 cell/ well) in DMEM medium (Gibco) with 10% FBS and 1% Penicillin-Streptomycin (Invitrogen). When reaching 90% confluence, cells were fasted by overnight incubation in a serum-free DMEM medium. To test HMGB1/LPS mediated inflammation, recombinant HMGB1 isolated from HEK 293 cells (15) was incubated with LPS (Escherichia coli serotype O111:B4, Sigma, USA) at 37℃ for 30 minutes in serum-free DMEM before adding to the cells. Cells were then treated with HMGB1/LPS complex with or without 18-mer for 16 hours, and the supernatants were harvested for analysis by ELISA. To test the effect of HDL on LPS, varying concentrations of human HDL (5 – 500 μg/mL, purchased from Athens Research and Technology, USA) were used to treat the

cells for 6 hours. Then, the HDL-treated cells were incubated with 10 ng/mL of LPS for 16 hours in serum-free DMEM before harvesting cell supernatants for analysis by ELISA.

Biomarker analysis

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Total RNA was isolated from different mice organs, including lung, kidney, liver, and heart, with TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. 1-5 μg of total RNA was reverse transcribed into cDNA using SuperScript II Reverse Transcriptase (Thermo Fisher Scientific, Inc.). mRNA expression of pro-inflammatory cytokines (IL-6), neutrophil markers (MCP-1), adhesion molecules (iCAM-1), and internal control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was evaluated by qPCR using the PowerUp SYBR Green Master Mix (Applied Biosystems). Primer pair sequences are summarized in **Table S2**, designed based on literature and ordered from Integrated DNA Technologies, Inc or Eton Bioscience. The amplification condition was as follows: 50˚C (2 min), 95˚C (2 min), followed by 40 cycles at 95˚C (15 s), 55˚C (15 s), 72˚C (1 min). The melting curve was tested after the amplification, with the condition as follows: 95˚C (15 s), 60˚C (1 min), 95˚C (15 s). The experiment was done using the QuantStudio™ 6 Flex Real-Time PCR System (Thermo Fisher). mRNA levels were quantified with the 2-ΔΔCt method and normalized to the internal control gene GAPDH. The results are shown relative to the expression level of the sham group.

Western blotting

Mouse plasma was collected 24 hours after CLP or sham surgery. Plasma containing 40 μg total protein (measured by Bradford assay) was denatured at 80 ˚C 10 min before electrophoresis at

130 V with NuPAGE 4-12% Bis-Tris protein gels (Invitrogen) for one hour. The gel was transferred at 30 V for 40 min to the nitrocellulose membrane (Bio-Rad). After blocking with 5% BSA in PBS for 2 hours at room temperature, the membrane was incubated overnight with an anti-histone H3 antibody (1:1000, Abcam). Anti-rabbit HRP (1:10000, Abcam) was incubated for one hour at room temperature before being detected by SuperSignal West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific).

Enzyme-linked immunosorbent assay (ELISA)

Mouse plasma and peritoneal lavage collected from CLP or LPS model or cell supernatant from LPS treated Raw264.7 were diluted and analyzed for inflammation markers IL-6, MCP-1, iCAM-1 (kits obtained from Thermo Fisher Scientific, Inc.), HMGB1 (kit obtained from Tecan Group Ltd.), and ApoA-I (kits obtained from Abcam, USA). The measurement was performed according to the manufacturer's protocols.

Multiplex analysis

Mouse plasma collected from CLP model were diluted in 1:2 and/or 1:50 and analyzed for sepsis related inflammation and immunological markers by Luminex Discovery Assay, Mouse premixed multi-analyte kit (R&D Systems) and Bio-Plex 200 system. We selected 21 markers based on literatures and our interest (7), including G-CSF, GM-GSF, IFNg, IL-1α, IL-5, IL-6, IL-10, IL-12 p70, IL-13, IL-17, KC (CXCL1), MIP-2 (CXCL2), IP-10 (CXCL10), MCP-1 (CCL2), RANTES (CCL5), TNFα, P-selectin, KIM-1, iCAM-1, RAGE, Syndecan-1. The measurement was performed by UNC advanced analytics core according to the manufacturer's protocols.

Kidney injury markers

Creatinine and BUN assay was performed with mice plasma collected 24 hours after CLP surgery (kits obtained from Crystal Chem and Invitrogen, respectively). The measurement was performed according to the manufacturer's protocols.

HDL measurement

HDL was measured in plasma or peritoneal lavage collected from mice that underwent CLP surgery or LPS injection (cholesterol assay kit from Abcam, USA). The measurement was performed according to the manufacturer's protocols. In short, mice plasma or peritoneal lavage was mixed with an equal volume of 2 x precipitation buffer and centrifuged at 5,000 rpm for 10 min to separate HDL from LDL/VLDL fraction. Then, according to the manufacturer's protocols, the diluted peritoneal lavage (2 x) and plasma (50 x) were incubated with cholesterol assay buffer, cholesterol probe, and enzyme mix. The Cholesterol reaction mix was incubated at 37℃ for 60 min in the dark and measured at OD 570 nm.

LPS quantification

Mouse plasma or peritoneal lavage was collected from mice receiving 5 mg/kg i.p. of biotinylated LPS with or without 50 mg/kg of 18-mer for 2 hours. LPS levels in the samples were evaluated by using Pierce LAL chromogenic endotoxin quant kit (Thermo Fisher Scientific) based on the manufacturer's instruction. Briefly, 50 μL of plasma (diluted 100-1000x) and peritoneal lavage (diluted 500-5000 x) were added to a sterile 96-well plate along with endotoxin standard in serial dilution. Amebocyte lysates (50 μL) were freshly prepared and added to each well. After 8-min incubation, 100 μL of the chromogenic substrate was pre-warmed and added to each well for 6-min incubation. The readout was measured at OD 405nm after adding 50 μL of 25% acetic acid solution to stop the reaction. All reactions were maintained sterile at 37℃.

Determination of 18-mer binding to LPS

18-mer (60 μM) was incubated with various doses of LPS (0 – 100 μM) in 500 μL PBS buffer for 30 min at room temperature. The mixtures were transferred to the 100 kDa centrifugal filter (Millipore) and centrifuged for 10 min at 4,000 pm. The binding ability of LPS determines by decreasing absorbance value of 18-mer after passing through the filter at UV 310 nm. LPS was measured by the Limulus Amebocyte Lysate (LAL) kit (Associated of Cape Cod Inc.) following the manufacturer's protocol. LPS did not pass through a 100 kDa centrifugal filter under this condition.

The method was validated in the previous study (6). Briefly, the positive and negative control experiments were performed with antithrombin (AT) to HS hexasaccharide: GlcNS6S-GlcA-GlcNS3S6S-IdoA2S-GlcNS6S-GlcA-pNP (6-mer AXa) and GlcNS2S-GlcA-GlcNS2S-GlcA-GlcNS2S-GlcA-pNP (NS 6-mer). 6-mer AXa binds to AT but not NS 6-mer. To validate the method, different concentrations of AT (0 – 0.2 mM) were incubated with 0.2 mM of NS 6-mer or 6-mer AXa in 1mL PBS buffer for 30 min at room temperature. The binding mixtures were transferred in a 30 kDa centrifugal filter (Millipore) and centrifuged at 4,000 rpm for 10 min. The binding ability of AT determines by decreasing the concentration of 6mer or 6-mer AXa after passing through the filter at UV 310 nm.

Determination of the binding of HS oligosaccharides to H3 by SPR

SPR measurements were performed on a T200 SPR (Cytiva). Research grade sensor CM5 chip and HBS-EP+ buffer were from Cytiva. To obtain kinetic data for interactions of H3 and HS oligosaccharides, H3 was immobilized on a CM5 chip according to the standard amine coupling protocol. Briefly, carboxymethyl groups on the CM5 chip surface were first activated using an injection pulse of 35 μL (flow rate, 5 μL/min) of an equimolar mix of N-ethyl-N-(dimethyl

aminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) (final concentration 0.05 M, mixed immediately prior to injection). Following activation, histone H3 was diluted to 200 μg/mL in 100 mM sodium acetate (pH 5.5) buffer and injected over the activated biosensor surface for immobilization. Excess unreacted sites on the sensor surface were deactivated with a 35 μL injection of 1M ethanolamine. Next, a reference flow cell was prepared using an injection pulse of 35 μL (flow rate, 5 μL/min) of an equimolar mix of EDC and NSH, followed by a 35 μL injection of 1M ethanolamine.

Oligosaccharide samples were diluted in HBS-EP+ buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.05% surfactant P20, pH 7.4). Different dilutions of oligo samples were injected at a flow rate of 30 μL/min. At the end of the sample injection, the same buffer flowed over the sensor surface to facilitate dissociation. After a 3 min dissociation time, the sensor surface was regenerated by injecting 30 μL of 2M NaCl to get the fully regenerated surface. The binding response was monitored as time (sensorgram) function at 25 °C. The sensorgrams were fit globally to obtain apparent on (k_a) and off (k_d) rates and binding equilibrium dissociation constant: K_D ($K_D = k_d/k_a$) using the T200 Evaluation software and assuming a 1:1 Langmuir model.

Dissociation of HDL by HS

Human HDL (Athens Research and Technology) (4 μg) was incubated with 1 μg of 6-mer, 12-mer, or 18-mer in 50 mM sodium acetate buffer (pH 5.0) for 1 h at 37℃. Samples were centrifuged at 13,000 rpm at 4℃ for 30 min after incubation. The supernatant and pellet were separated, mixed with LDS sample buffer and TCEP solution (Thermo Fisher), and denatured at 95℃ for 5 min. In Western blot analysis, samples were separated by electrophoresis with a

similar setting described above. The gel was stained in Coomassie blue to visualize ApoA-I (protein band migrated to around 25 kDa).

Histological Analysis

Animals were humanely euthanized with 3% isoflurane using CO_2 followed by cervical dislocation. Kidneys were collected from all animals and fixed in 10% Neutral Buffered Formalin (Thermo Fisher Scientific). Kidneys were then routinely processed to paraffin, sectioned to 5 µm, and stained with hematoxylin and eosin. Tissues were evaluated microscopically in a masked fashion by a board-certified veterinary pathologist (RSS).

Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). Statistical significance between experimental and control groups was analyzed by two-tailed unpaired Student t-test and between multiple groups by one-way ANOVA followed by Dunnett's multiple comparison tests. In addition, Kaplan-Meier survival curves in the CLP study were analyzed by log-rank (Mantel-Cox) test. All statistical analysis was performed using GraphPad Prism software (ver. 8.3.1; GraphPad Software, Inc.).

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Accession	Description	Coverage [%]	# PSMs	# Peptides	# Unique Peptides	# AAs	MW [kDa]	calc. pI
Q00623	Apolipoprotein A-I OS=Mus musculus OX=10090 GN=Apoa1 PE=1 SV=2	70	396	38	38	264	30.6	5.73
P35908v2	SWISS-PROT:P35908 Tax_Id=9606 Gene_Symbol=KRT2 Keratin, type II cytoskeletal 2 epidermal	91	189	64	51	639	65.4	8
P04264	SWISS-PROT:P04264 Tax_Id=9606 Gene_Symbol=KRT1 Keratin, type II cytoskeletal 1	59	132	52	45	644	66	8.12
P13645	SWISS-PROT:P13645 Tax_Id=9606 Gene_Symbol=KRT10 Keratin, type I cytoskeletal 10	58	127	38	32	593	59.5	5.21
P13647	SWISS-PROT:P13647 Tax_Id=9606 Gene_Symbol=KRT5 Keratin, type II cytoskeletal 5	61	77	49	30	590	62.3	7.74
P02533	SWISS-PROT:P02533 Tax_Id=9606 Gene_Symbol=KRT14 Keratin, type I cytoskeletal 14	68	76	38	15	472	51.6	5.16
P00761	Trypsin - Sus scrofa (Pig).	40	72	7	7	231	24.4	7.18
P35527	SWISS-PROT:P35527 Tax_Id=9606 Gene_Symbol=KRT9 Keratin, type I cytoskeletal 9	55	62	28	27	623	62.1	5.3
P04259	SWISS-PROT:P04259 Tax_Id=9606 Gene_Symbol=KRT6B Keratin, type II cytoskeletal 6B	56	61	38	3	564	60	8
P02538	SWISS-PROT:P02538 Tax_Id=9606 Gene_Symbol=KRT6A Keratin, type II cytoskeletal 6A	57	61	38	5	564	60	8
P08779	SWISS-PROT:P08779 Tax_Id=9606 Gene_Symbol=KRT16 Keratin, type I cytoskeletal 16	59	55	30	14	473	51.2	5.05
P07724	Serum albumin OS=Mus musculus OX=10090 GN=Alb PE=1 SV=3	56	42	29	27	608	68.6	6.07
Q04695	SWISS-PROT:Q04695 Tax_Id=9606 Gene_Symbol=KRT17 Keratin, type I cytoskeletal 17	31	32	19	3	432	48.1	5.02
Q8K0E8	Fibrinogen beta chain OS=Mus musculus OX=10090 GN=Fgb PE=1 SV=1	53	28	18	18	481	54.7	7.08
Q8VCM7	Fibrinogen gamma chain OS=Mus musculus OX=10090 GN=Fgg PE=1 SV=1	44	25	19	19	436	49.4	5.86
P99024	Tubulin beta-5 chain OS=Mus musculus OX=10090 GN=Tubb5 PE=1 SV=1	52	22	18	5	444	49.6	4.89
E9Q557	Desmoplakin OS=Mus musculus OX=10090 GN=Dsp PE=1 SV=1	8	22	19	19	2883	332.7	6.8
P21812	Mast cell protease 4 OS=Mus musculus OX=10090 GN=Mcpt4 PE=1 SV=1	49	21	11	11	246	27.2	9.48
P11276	Fibronectin OS=Mus musculus OX=10090 GN=Fn1 PE=1 SV=4	10	20	18	18	2477	272.4	5.59
P68372	Tubulin beta-4B chain OS=Mus musculus OX=10090 GN=Tubb4b PE=1 SV=1	45	20	16	2	445	49.8	4.89

Table S1. Potential match of the protein (~M.W. 25kda) bound to LPS.

After Coomassie staining, the band at ~M.W. 25kda in suppl fig. S4 was sent to proteomic analysis. The protein was determined as apolipoprotein A-I (apoA1) based on PSM. PSMs: Peptide spectrum match; AAs: amino acids.

Gene	Sequence (5’ to 3’)	Reference (8)
GAPDH	ATGTGTCCGTCGTGGATCTG	10.1111/jcmm.13831
	CCTCAGTGTAGCCCAAGATG	
IL-6	ACCACTCCCAACAGACCTGTC	
	ACTCCAGGTAGCTATGGTACTC	
iCAM-1	TTCACACTGAATGCCAGCTC	
	GTCTGCTGAGACCCCTCT TG	
MCP-1	CTTCTGGGCCTGCTGTTCA	10.1159/000492589
	CCAGCCTACTCATTGGGATCA	

Table S2. Primer sequence used in the qPCR analysis for kidney samples.

Pg/mL	**Sham 1**	**Sham 2**	**Sham 3**	**CLP 1**	**CLP 2**	**CLP 3**	**18m 1**	**18m 2**	**18m 3**
IL-6	0.26	0	47.92	37912.22	36056.99	26818.47	228.82	358.29	39.725
IL-10	0	0	0	3388.46	4762.71	20817.35	37.5	79.6	0
IL-13	0	0	0	7.2	11.555	14.65	0	0	0
IL-17	0	22.6	3.25	70.805	38.69	175.79	0	39.75	24.77
IL-12 p70	0	22.72	8.43	83.59	99.37	117.81	0	53.285	22.72
TNFa	0	0	0	72.94	71.77	63.49	6.07	4.68	0
IFNg	8.84	1.66	7.18	90.12	92.385	626.965	119.66	104.99	31.305
MCP-1	167.03	245.99	110.955	8879.34	29486.95	67285.34	354.14	680.89	278.51
KC	87.52	166.635	158.19	71553.47	70430.46	494969.7	3528.035	1843.37	475.43
IP-10	0	0	0	2525.34	3611.82	4484.9	0	53.125	0
GM-GSF	19.645	0	0	19.63	23.245	72.605	9.44	6.825	12.34
P-selectin	25570.05	27636.68	24965.68	60733.93	65317.49	199417.1	45918.9	11055.34	32955.07
TIM-1	0	0	0	192.385	202.1	197.25	0	0	0
RANTES	86.435	134.9	77.14	1668.175	1617.73	3775.015	412.705	389.92	162.01
MIP-2	5.665	3.44	4.84	20743.44	20886.22	30000	205.34	190.99	9.135
G-CSF	162.08	93.55	172.41	161249.5	151777.1	200000	52006.95	70039.09	1346.295
iCAM-1	32425.2	35284.14	42505.35	101890.7	108826.3	126471.5	68095.01	88021.33	57872.47
IL-1a	2009.42	1440.94	894.355	488.61	1135.015	1200.705	1089.12	559.24	854.765
IL-5	149.76	16.535	25.02	33.695	30.505	106.79	0	1.445	59.21
RAGE	7.62	4.165	42.92	0	79.025	0	0	0	13.365
Syndecan1	3961	4264.05	3850.46	10957.92	13903.62	22976.27	3382.51	3955.785	4357.475

Table S3. Original value of multiplex assay.

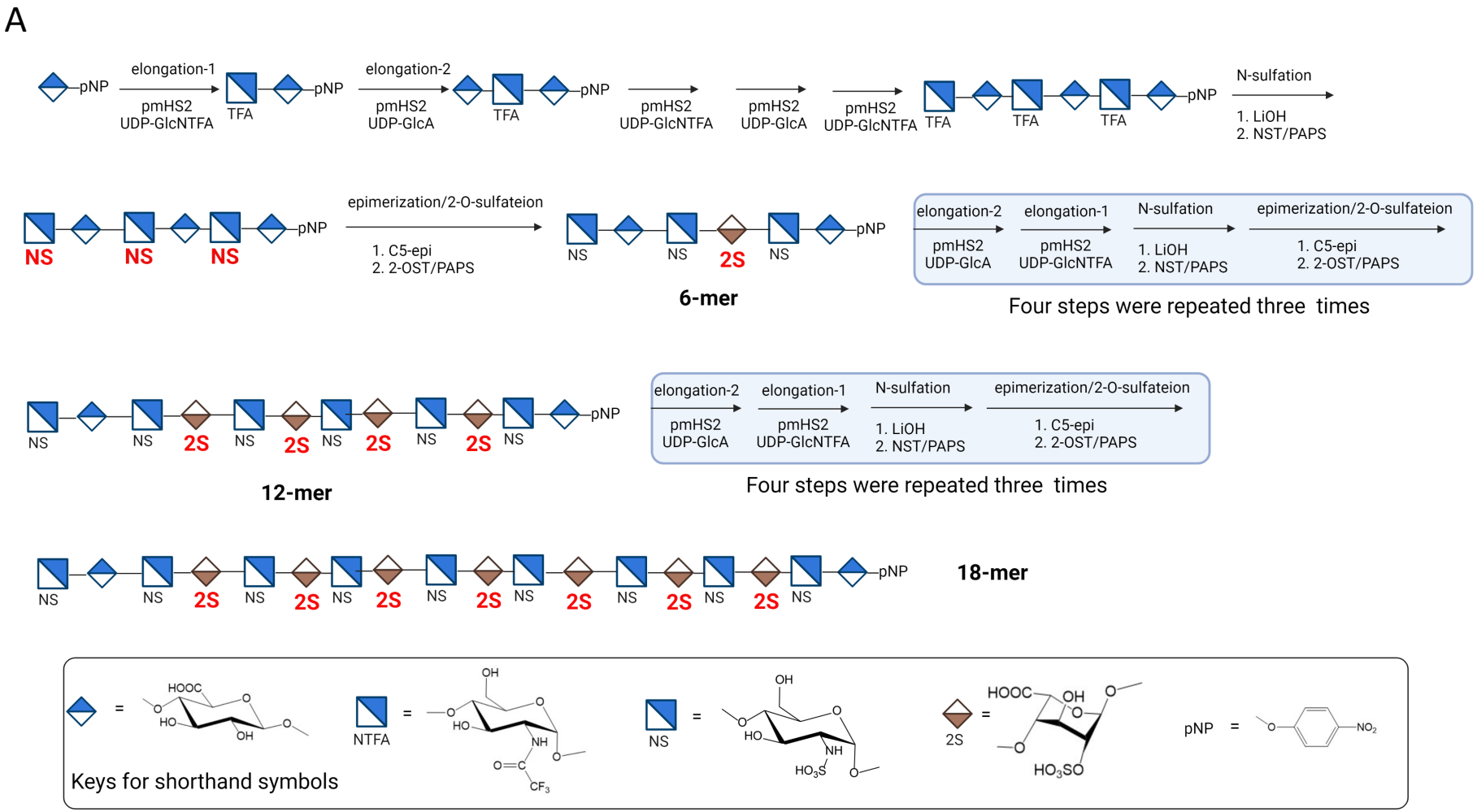


Fig. S1 Chemoenzymatic synthesis of 6-mer, 12-mer and 18-mer. (A) The synthesis is initiated from *para*-nitrophenyl glucuronide (GlcA-pNP) momosaccharide. It took eight enzymatic modification steps to obtain 6-mer, 18 steps to obtain 12-mer, and 30 steps to obtain 18-mer. Abbreviations: pmHS2, heparosan synthase 2 from *Pasteurella multocida*; NST, *N*-sulfotransferase; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; C_5-epi, C_5-epimerase; 2-OST, 2-*O*-sulfotransferase. **(B, C)** The anticoagulant activity was evaluated by testing the inhibition effect of 6-mer, 12-mer, 18-mer, unfractionated heparin (UFH), and fondaparinux (FPX) to FXa and FIIa. UFH and FPX were used as controls in this experiment. All HS (UFH, FPX, 6-mer, 12-mer, 18-mer) was tested at 5 μg/mL. The data are expressed as mean (n=3) ± SEM.

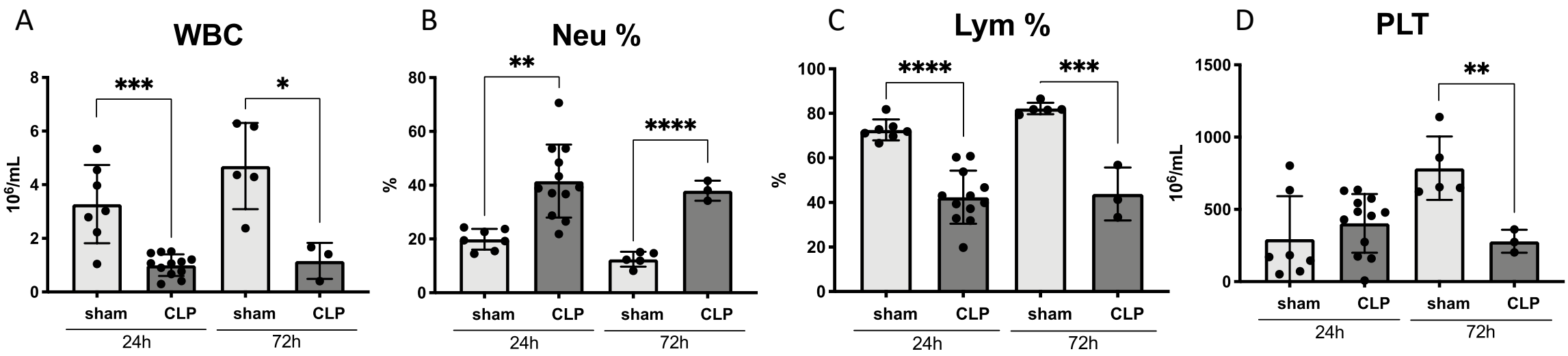


Fig. S2 Hematologic analysis of CLP induced septic mice. Plasma collected from mice receiving sham or CLP surgery were used to analyze hematologic changes. Data expressed as mean ± SEM and analyzed by unpaired student's t-test, n = 7-12 mice in the 24-hour group, n = 3-5 mice in the 72-hour group.*p<0.05; **p<0.01; ***p<0.001, ****p<0.0001. Abbreviations: WBC, white blood cell; Neu, neutrophil; Lym, lymphocyte; PLT, platelet.

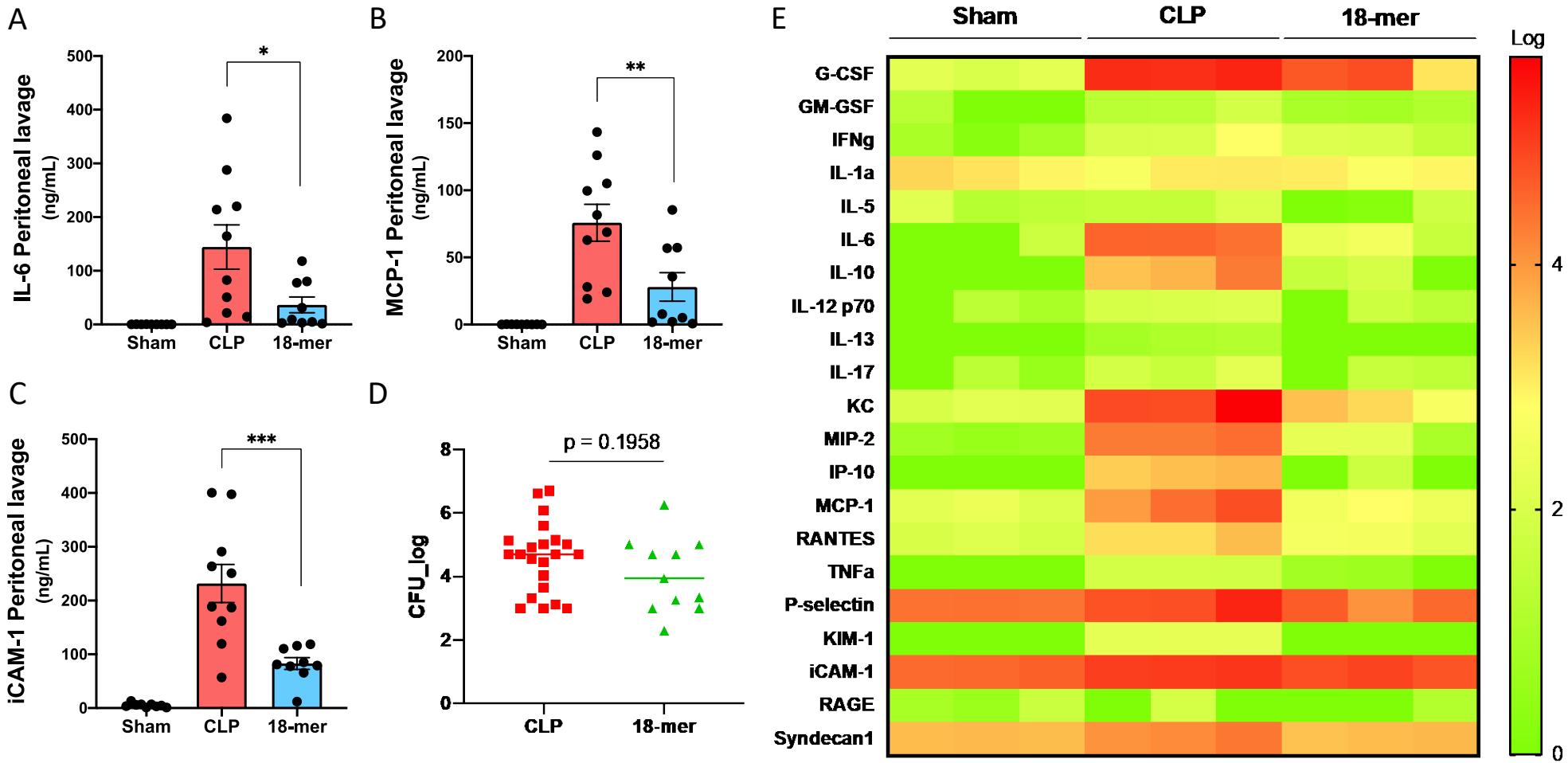


Fig. S3 18-mer reduces CLP-induced systemic inflammation independent of infection. Mice were subjected to a sham procedure or CLP and sacrificed 24 hours after the sham or CLP surgery. In the 18-mer group, mice were administered 18-mer (S.Q., 20mg/kg) at 0, 6, and 12 hours after CLP. **(A-C)** The protein level of IL-6, MCP-1, and iCAM-1 were tested from mouse peritoneal lavage. Data expressed as mean±SEM and analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. n = 6-10 male and 3-5 female mice. *p<0.05; **p<0.01; ***P<0.001. **(D)** Colony forming units (CFU) count (Log10) in the peritoneal lavage harvested from CLP or 18-mer treated CLP mice 24 hours after CLP surgery. **(E)** Heatmap analysis of cytokine and chemokine measurements performed by multiplex analysis for sham, CLP, and 18-mer groups. Plasma was evaluated with samples taken 24 hours after the sham or CLP procedure. Heat map showing the evaluation of cytokine and chemokine values in Log10. IL, Interleukin; TNF, tumor necrosis factor; IFNg, Interferon gamma; KC, Keratinocyte-derived chemokines; IP, Interferon gamma-induced protein; GM-CSF, Granulocyte macrophage colony-stimulating factor; KIM-1, kidney injury molecule 1; RANTES, Regulated on activation, normal T cell expressed and secreted; MIP, Macrophage inflammatory protein; G-CSF, Granulocyte-colony stimulating factor; RAGE, Receptor for advanced glycation end products.

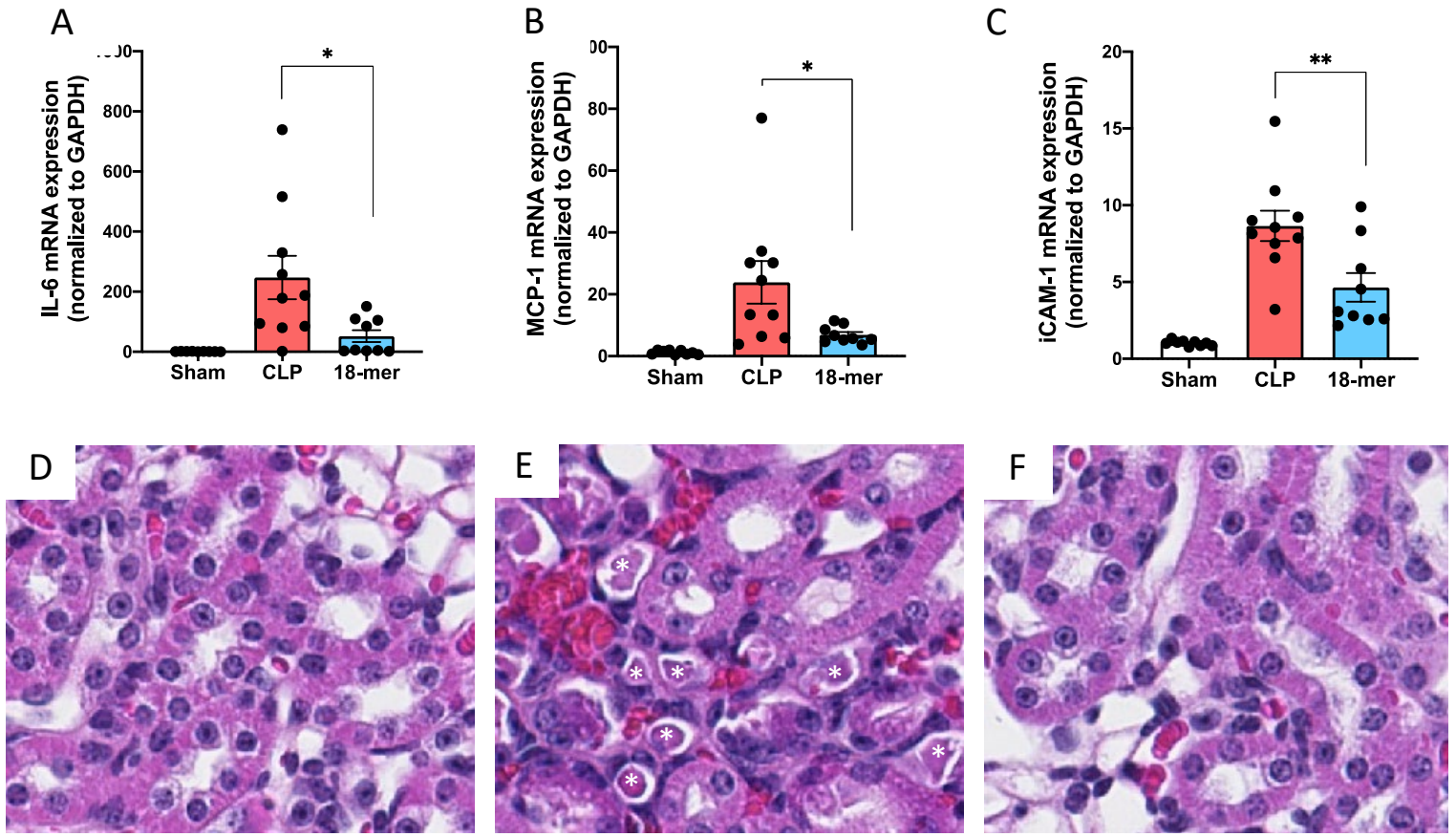


Fig. S4 18-mer reduces CLP-induced kidney inflammation and damage. Mice were subjected to a sham procedure or CLP. In the 18-mer group, mice were administered 18-mer (S.Q., 20mg/kg) at 0, 6, 12 hours and sacrificed 24 hours after CLP. **(A-C)** The mRNA levels of IL-6, MCP-1, and iCAM-1 were tested from mouse kidney homogenates. Data expressed as mean±SEM and analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. n = 6-10 male and 3-5 female mice. *$p<0.05$; **$p<0.01$. **(D-F)** Represented images of H&E stained inner stripe of the outer medulla (ISOM) from sham **(D)**, CLP **(E)**, and 18-mer **(F)** mice. Eosinophilic material and epithelial degeneration in the thin segment and thick ascending limb of Henle's (asterisks).

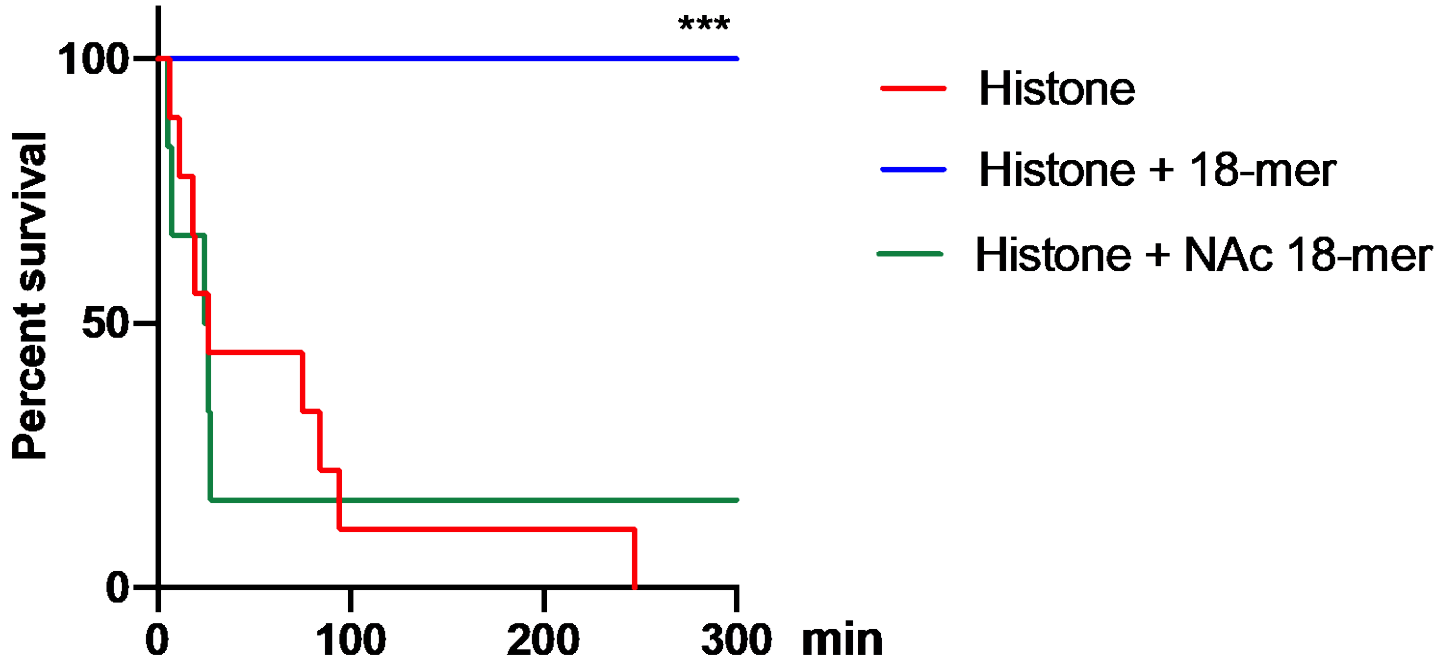


Fig. S5 18-mer, but not NAc 18-mer, protects against histone-induced lethality. Mice are adminitered with histone (75 mg/kg) alone or with 18-mer or Nac 18-mer (75 mg/kg) by retro-orbital injection. Both male and female mice were tested (histone, n=8 male and 2 female ; histone + 18-mer, n=5 male and 2 female ; histone + NAc 18-mer, n = 10 male and 1 female). Data analyzed by log-rank test; overall, p = 0.0016; Histone vs. Histone + 18-mer, p=0.0003; Histone vs. Histone + NAc 18-mer, p= 0.1709.

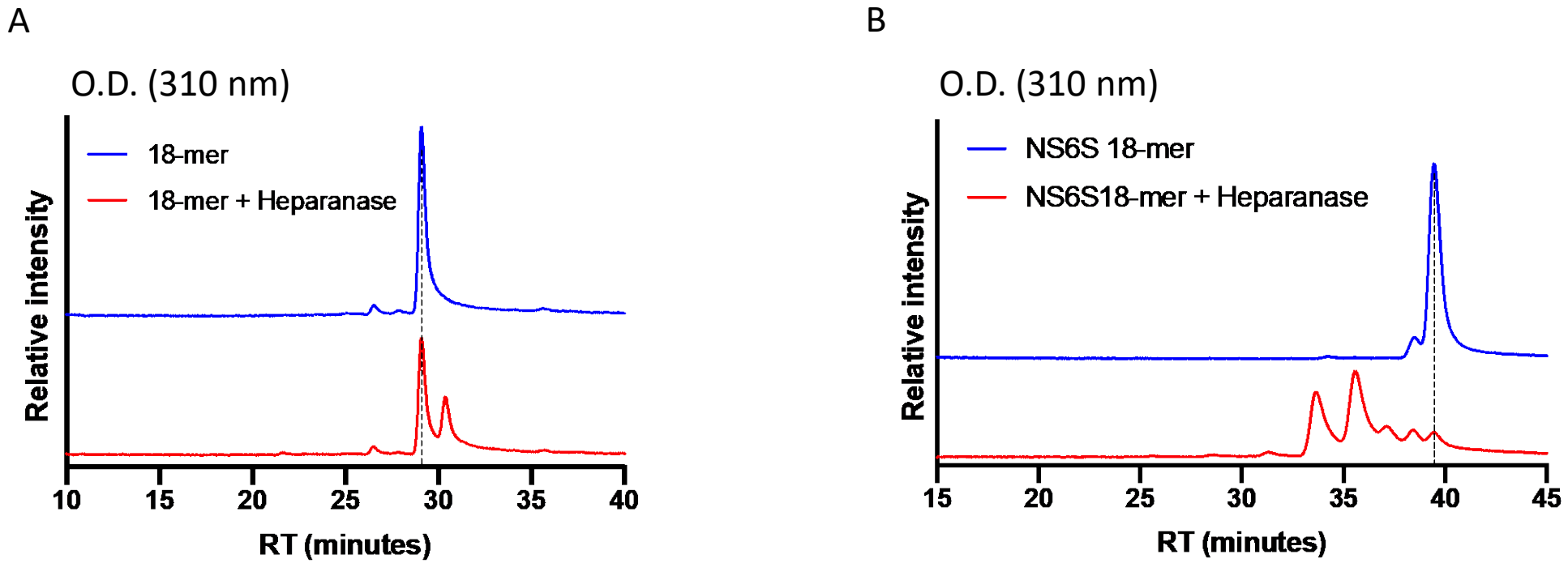
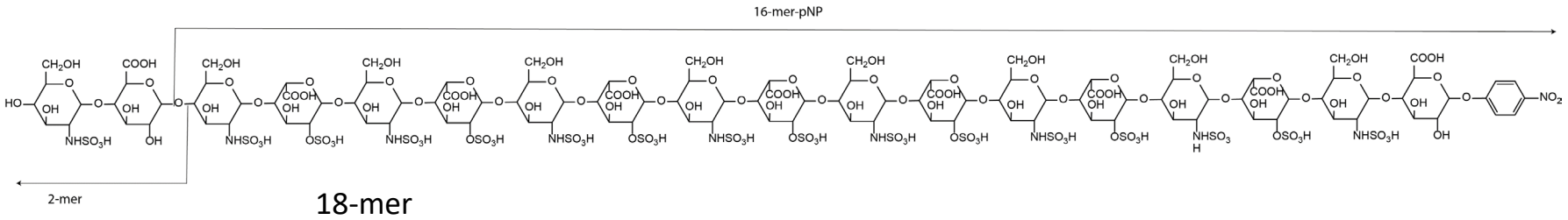


Fig. S6 18-mer is partially resistant to heparanase digestion as determined by HPLC. (A) 18-mer or (B) NS6S 18-mer (200 μg/mL) were incubated with heparanase (20 μg/mL) at 37 ℃ in 50 mM MOPS at pH 6. After 24 hours of incubation, the reaction was inactivated by heating at 95℃ 5 min and analyzed by HPLC. A dashed line in (A) and (B) indicates the eluted position of undigested 18-mer and NS6S 18-mer. Under this condition, NAc 18-mer was totally resistant to heparanase digestion.

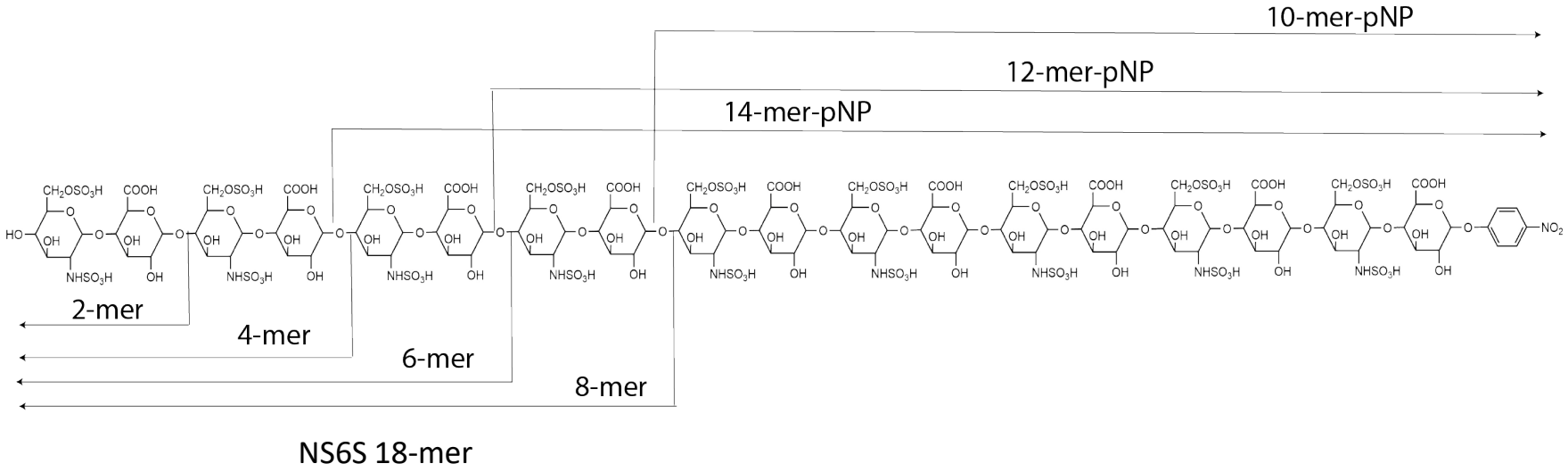
A



B

Detected products	Calculated MW	Measured MW
2-mer	435.07	435.08 ± 0.1
16-mer-pNP	4035.19	4037.28 ± 1.14
18-mer-pNP	4452.24	4454.67± 2.09

C



D

Detected products	Calculated MW	Measured MW
2-mer	515.03	515.09 ± 0.11
4-mer	1012.04	1012.00 ± 0.12
6-mer	1509.05	1509.11± 0.03
8-mer	2006.09	2006.65 ± 0.18
10-mer-pNP	2624.10	2624.46 ± 0.71
12-mer-pNP	3121.11	3121.82 ± 0.26
14-mer-pNP	3618.13	3619.32 ± 0.24

Fig. S7 Structural analysis of the products 18-mer or NS6S 18-mer after heparanase digestion by LC/MS. (A) 18-mer or (C) NS18-mer show the degradation sites after heparanase digestion. The digested products were subjected to LC-MS analysis. The digested oligosaccharide fragments from 18-mer (C) and NS6S 18-mer (D) were identified by LC/MS.

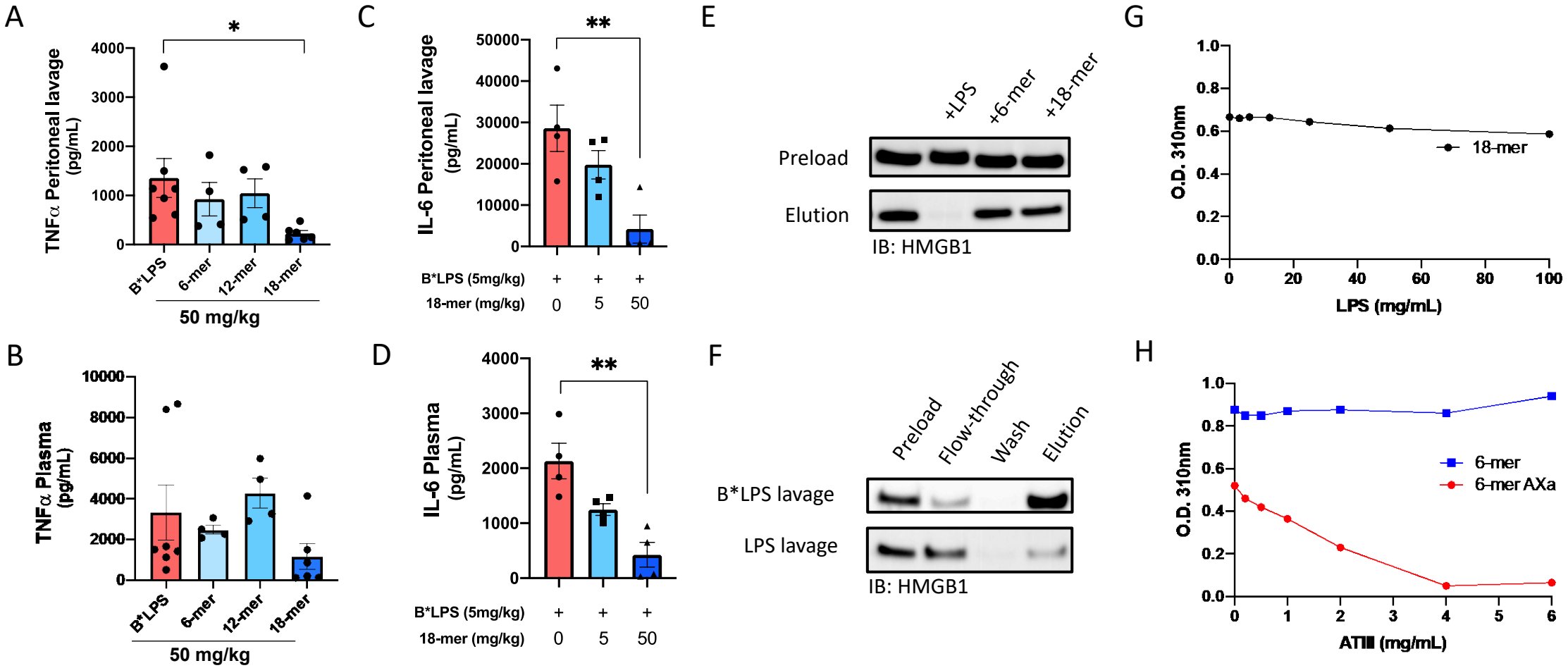


Fig S8. Evaluation of 18-mer effects on HMGB1/LPS induced inflammation. **(A,B)** Plasma and peritoneal lavage were collected from mice 2 hours after biotinylated LPS (B*LPS) administration (i.p., 5 mg/kg) with or without HS treatment (n = 4-7). The protein level of TNF-α was evaluated by ELISA. (**C**,**D**) The level of IL-6 in peritoneal lavage and plasma (n = 4) from endotoxemia mice with or without 18-mer treatment. **(E)** Peritoneal lavage was collected from mice 2 hours after B*LPS administration (i.p., 5 mg/kg) and incubated with LPS, 6-mer, or 18-mer (10mg/mL) for 1h at room temperature. The B*LPS/HMGB1 complex in the lavage. **(F)** Peritoneal lavage was collected from mice 2 hours after B*LPS or LPS administration (i.p., 5 mg/kg). The B*LPS/HMGB1 complex in the lavage was isolated by affinity pull-down using streptavidin resin following immunoblot of HMGB1. Samples are labeled as "Preload": prior to the affinity-pull down experiment; "Flow-through": flow-through lavage after incubation with streptavidin; "Wash": PBS buffer incubated with streptavidin, and "Elution" : elution buffer incubated with streptavidin, after denatured at 95°C. The full image is presented in ***SI Appendix,* Fig. S14**. **(G,H)** The complex of 18-mer and LPS cannot be captured using an ultracentrifugal approach (**G**). The ultracentrifugal approach was validated by binding to antithrombin (AT III) using 6-mer AXa as positive control and 6-mer as negative control (**H**). Presence of HS was detected by absorbance at O.D. 310nm with triplicates.

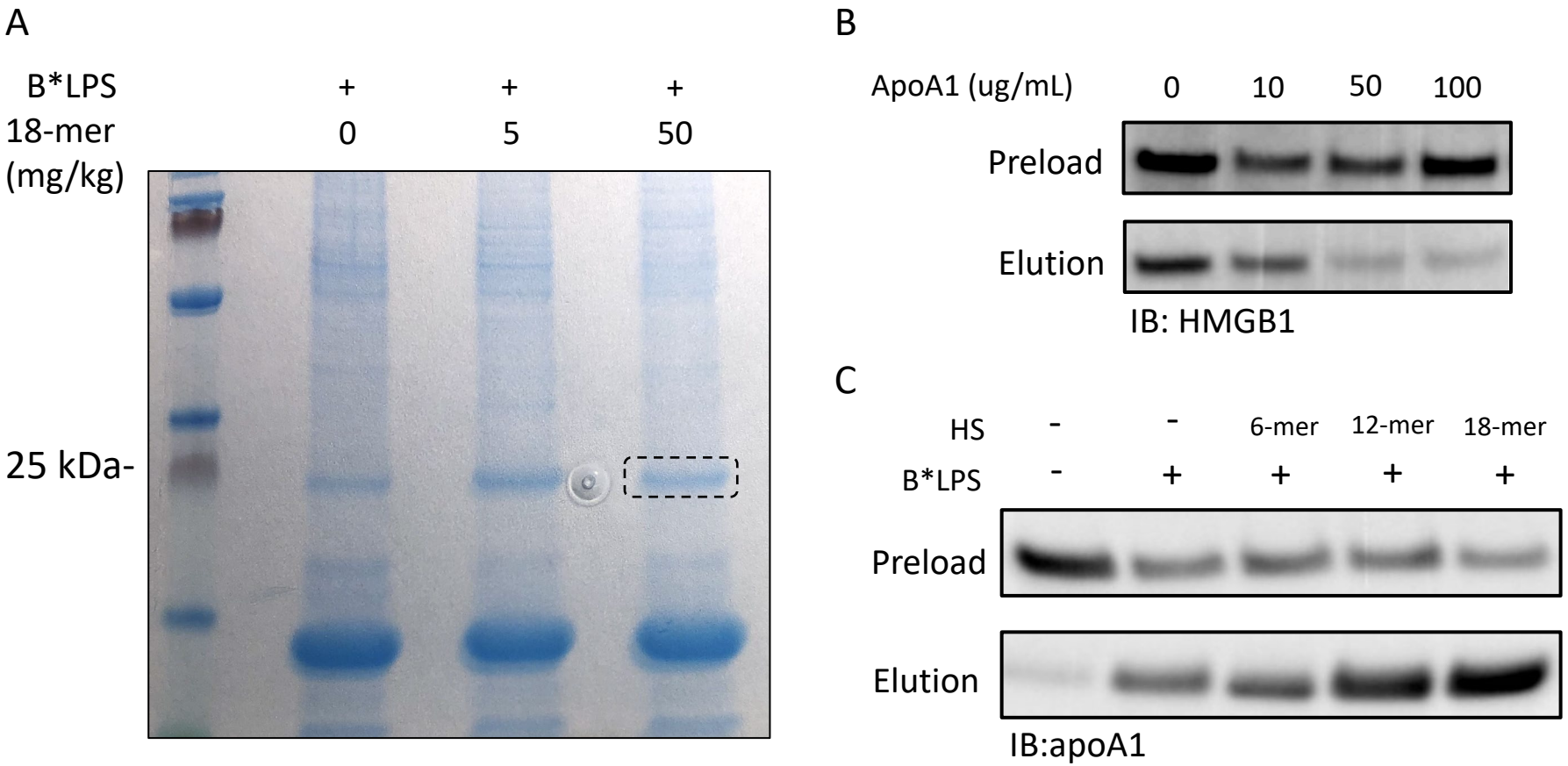


Fig S9. Investigation of the apoA-I/LPS complex. (A) Peritoneal lavage were collected from mice 2 hours after biotinylated LPS (B*LPS) administration (i.p., 5 mg/kg) with or without 18-mer treatment. The SDS-PAGE profile of the peritoneal lavage was analyzed by Coomassie staining after the pull-down assay using streptavidin resin. A band around 25kDa was sent to proteomic analysis and identified as apoA-I. **(B)** Peritoneal lavage were collected from mice 2 hours after B*LPS administration and incubated with different concentration of recombinant apoA-I. The presence of B*LPS/HMGB1 complex are shown by affinity pull-down assay and immunoblot of HMGB1. **(C)** Peritoneal lavage were collected from mice 2 hours after B*LPS administration (i.p., 5 mg/kg) with 6-mer, 12-mer, and 18-mer treatment. The presence of B*LPS/HMGB1 complex are shown by affinity pull-down assay and immunoblot of HMGB1. Samples before and after passing streptavidin resin are labeled "preload" and "elution", respectively. The full image is presented in ***SI Appendix,* Fig. S16.**

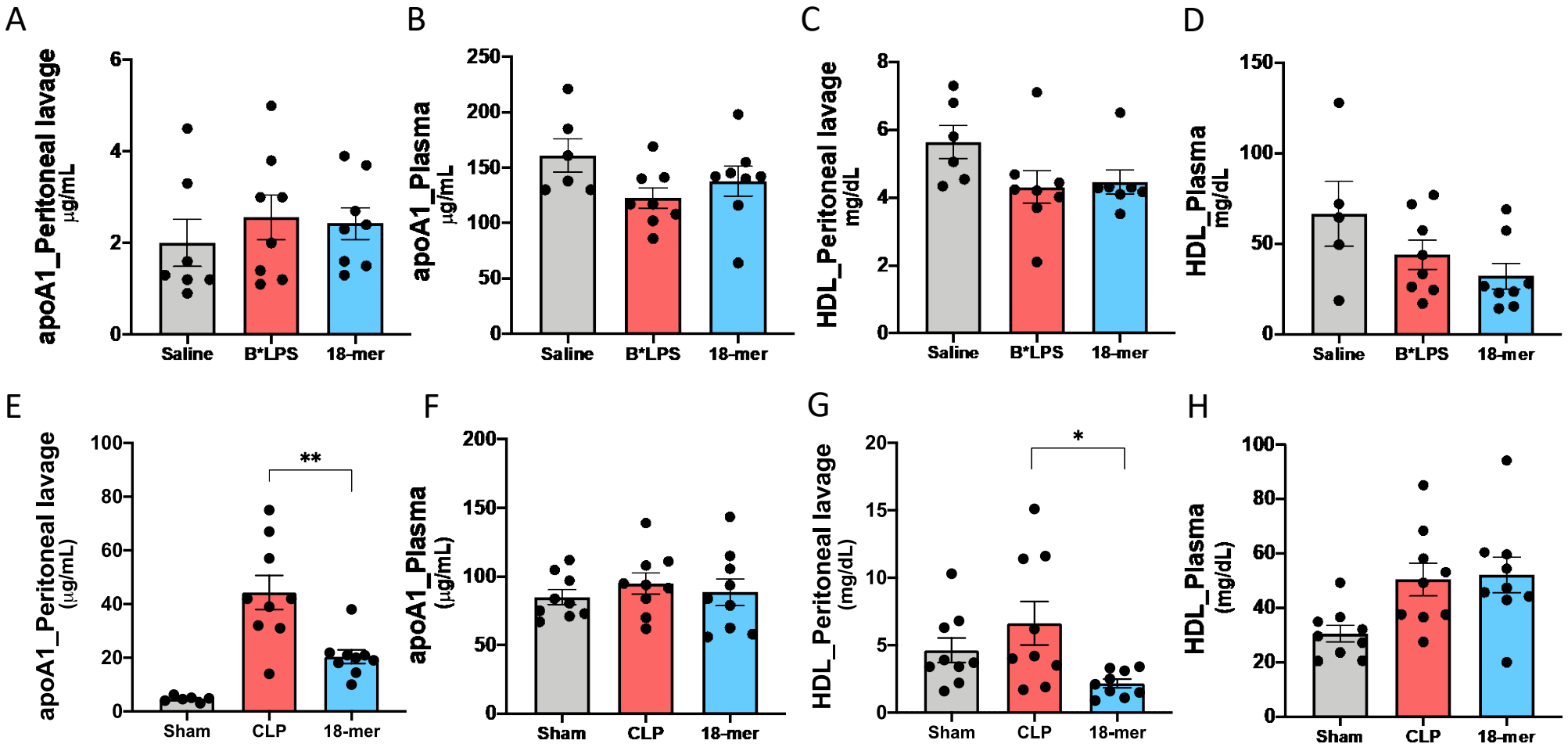


Fig S10. Concentration of apoA-I and HDL in plasma and peritoneal lavage from Biotinylated LPS (B*LPS) injected mice and CLP mice. (A-D) Concentration of apoA-I and HDL were measured by ELISA in plasma and peritoneal lavage that obtained at 2 hours after I.P. injection of biotinylated LPS (B*LPS, 5mg/kg) and 18-mer (50 mg/kg) /B*LPS co-injected mice**. (E-H)** Concentration of apoA-I and HDL were measured by ELISA in plasma and peritoneal lavage that obtained at 24 hours after sham or CLP surgery with or without 18-mer treatment (S.Q., 0, 6, 12 hours after CLP surgery) (n = 6-9). Data expressed as mean ± SEM and analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. *$p<0.05$; **$p<0.01$.

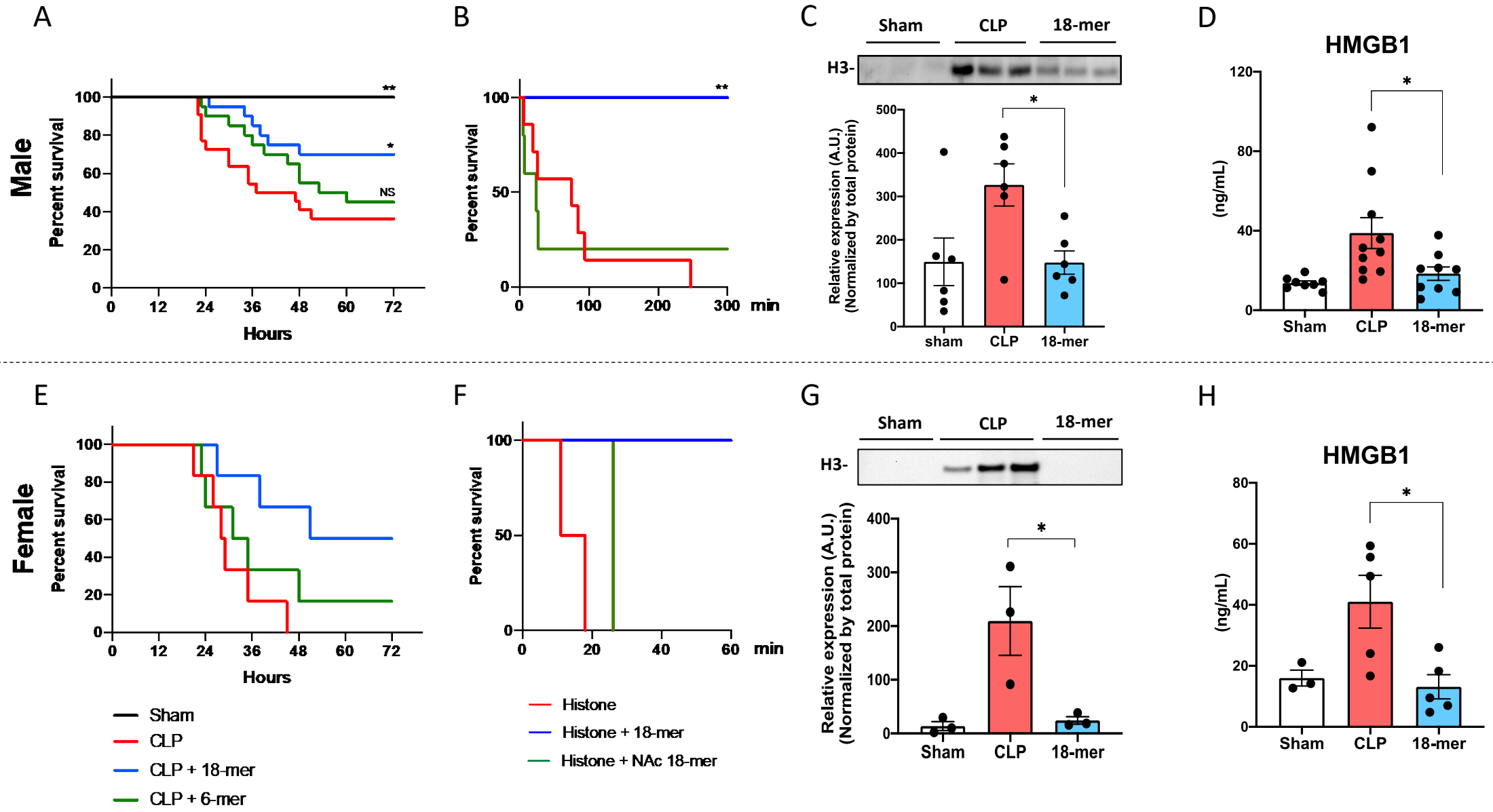

A
Male
Percent survival
100
80
60
40
20
0
0 12 24 36 48 60 72
Hours
**
*
NS
B
Percent survival
100
50
0
0 100 200 300 min
**
C
Sham CLP 18-mer
H3-
Relative expression (A.U.)
(Normalized by total protein)
500
400
300
200
100
0
sham CLP 18-mer
*
D
HMGB1
(ng/mL)
120
80
40
0
Sham CLP 18-mer
*
E
Female
Percent survival
100
80
60
40
20
0
0 12 24 36 48 60 72
Hours
Sham
CLP
CLP + 18-mer
CLP + 6-mer
F
Percent survival
100
50
0
0 20 40 60 min
Histone
Histone + 18-mer
Histone + NAc 18-mer
G
Sham CLP 18-mer
H3-
Relative expression (A.U.)
(Normalized by total protein)
400
300
200
100
0
Sham CLP 18-mer
*
H
HMGB1
(ng/mL)
80
60
40
20
0
Sham CLP 18-mer
*

Fig. S11 Comparison of survival studies and DAMPs levels in plasma between male (A-D) and female (E-H) mice. (A, E) The 72-hour survival in CLP mice was administered (s.q.) with saline, 6-mer, and 18-mer at 0, 6, 12, 24, 36, and 52 hours. **(A)** sham, n=8; CLP, n=22; CLP + 18-mer, n=20 , CLP + 6-mer, n=20. Data analyzed by log-rank test; overall, p = 0.0065; CLP vs. Sham, p=0.0053; CLP vs. CLP + 18-mer, p= 0.0134; CLP vs. CLP + 6-mer, p= 0.1842. **(E)** n=6. Data analyzed by log-rank test; overall, p = 0.0495; CLP vs. CLP + 18-mer, p= 0.1353. **(B, F)** The 300-min survival in histone (75 mg/kg, r.o.) injected mice were administered (r.o.) with 18-mer or NAc 18-mer (75 mg/kg, r.o.). **(B)** Histone, n=8; Histone + 18-mer, n=5, Histone + NAc 18-mer, n=10. Data analyzed by log-rank test; overall, p = 0.0079; Histone vs. Histone + 18-mer, p=0.0011; Histone vs. Histone + NAc 18-mer, p= 0.5473. **(F)** Histone, n=2; Histone + 18-mer, n=2, Histone + NAc 18-mer, n=1. Data analyzed by log-rank test; overall, p = 0.0775. **(C, D, G, H)** Mice were subjected to a sham procedure or CLP and sacrificed 24 hours after the sham or CLP surgery. In the 18-mer group, mice were administered 18-mer (S.Q., 20 mg/kg) at 0, 6, and 12 hours after CLP. The protein level of H3 (**C**, Male; **G**, Female) and HMGB1 (**D**, Male; **H**, Female)were tested from mouse plasma. Data expressed as mean±SEM and analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. n = 6-10 male and 3-5 female mice. *p<0.05.

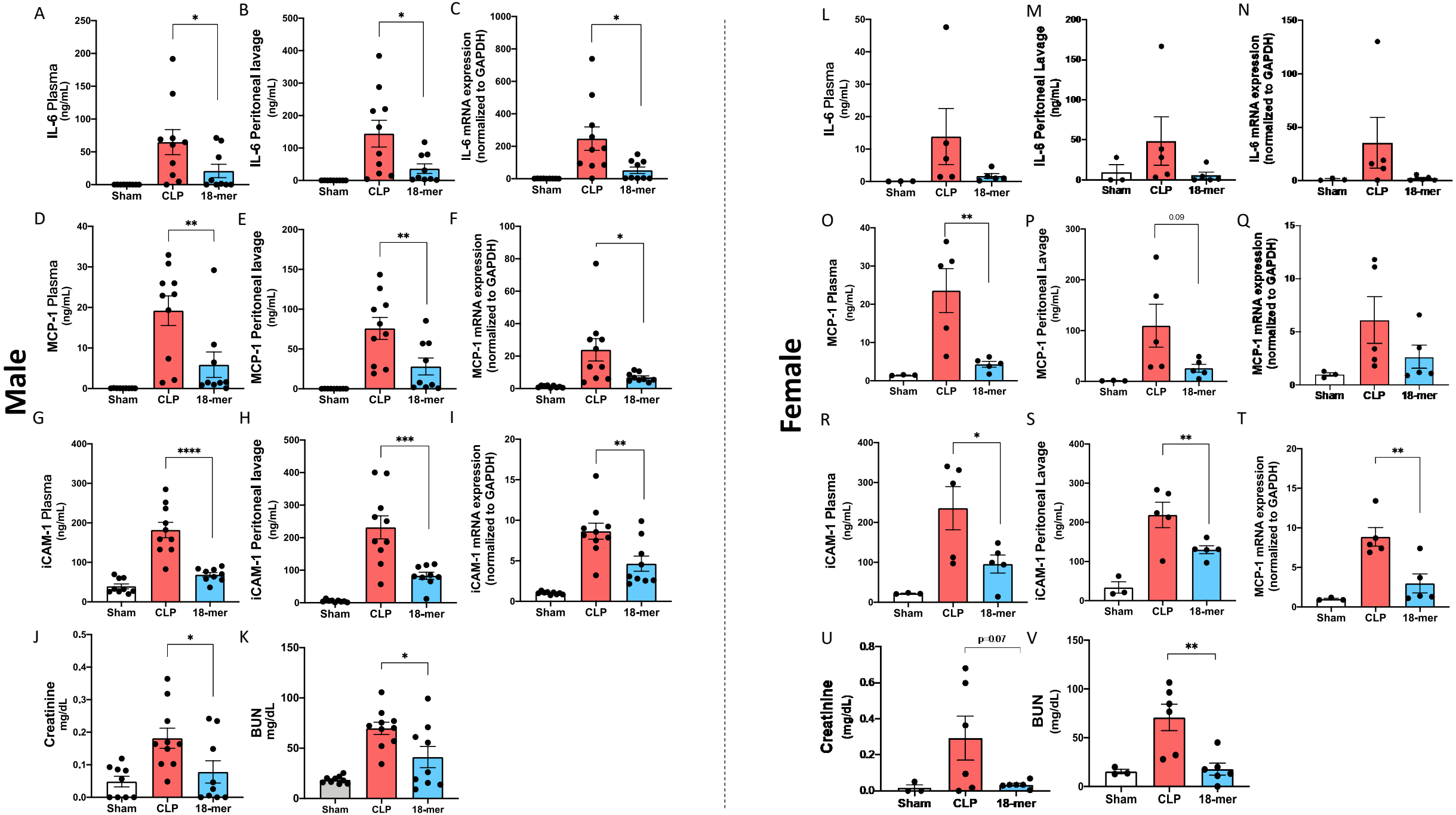

Male
Female
A
IL-6 Plasma (ng/mL)
B
IL-6 Peritoneal lavage (ng/mL)
C
IL-6 mRNA expression (normalized to GAPDH)
D
MCP-1 Plasma (ng/mL)
E
MCP-1 Peritoneal lavage (ng/mL)
F
MCP-1 mRNA expression (normalized to GAPDH)
G
iCAM-1 Plasma (ng/mL)
H
iCAM-1 Peritoneal lavage (ng/mL)
I
iCAM-1 mRNA expression (normalized to GAPDH)
J
Creatinine mg/dL
K
BUN mg/dL
L
IL-6 Plasma (ng/mL)
M
IL-6 Peritoneal Lavage (ng/mL)
N
IL-6 mRNA expression (normalized to GAPDH)
O
MCP-1 Plasma (ng/mL)
P
MCP-1 Peritoneal Lavage (ng/mL)
0.09
Q
MCP-1 mRNA expression (normalized to GAPDH)
R
iCAM-1 Plasma (ng/mL)
S
iCAM-1 Peritoneal Lavage (ng/mL)
T
MCP-1 mRNA expression (normalized to GAPDH)
U
Creatinine (mg/dL)
p=0.07
V
BUN (mg/dL)
Sham
CLP
18-mer

Fig. S12 Comparison of inflammatory markers levels and kidney damage markers in plasma, peritoneal lavage, and kidney between male (A-K) and female (L-V) mice. Mice were subjected to a sham procedure or CLP and sacrificed 24 hours after the sham or CLP surgery. In the 18-mer group, mice were administered 18-mer (S.Q., 20mg/kg) at 0, 6, and 12 hours after CLP. The protein or mRNA levels of IL-6 (**A-C**, Male; **L-N**, Female), MCP-1 (**D-F**, Male; **O-Q**, Female), and iCAM-1(**G-I**, Male; **R-T**, Female) were tested from mouse plasma , peritoneal lavage, or kidney homogenates. Creatinine and BUN (**J-K**, Male; **U-V**, Female) were analyzed from mouse plasma. Data expressed as mean±SEM and analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. n = 6-10 male and 3-5 female mice. *p<0.05, **p<0.01; ***p<0.001, ****p<0.0001.

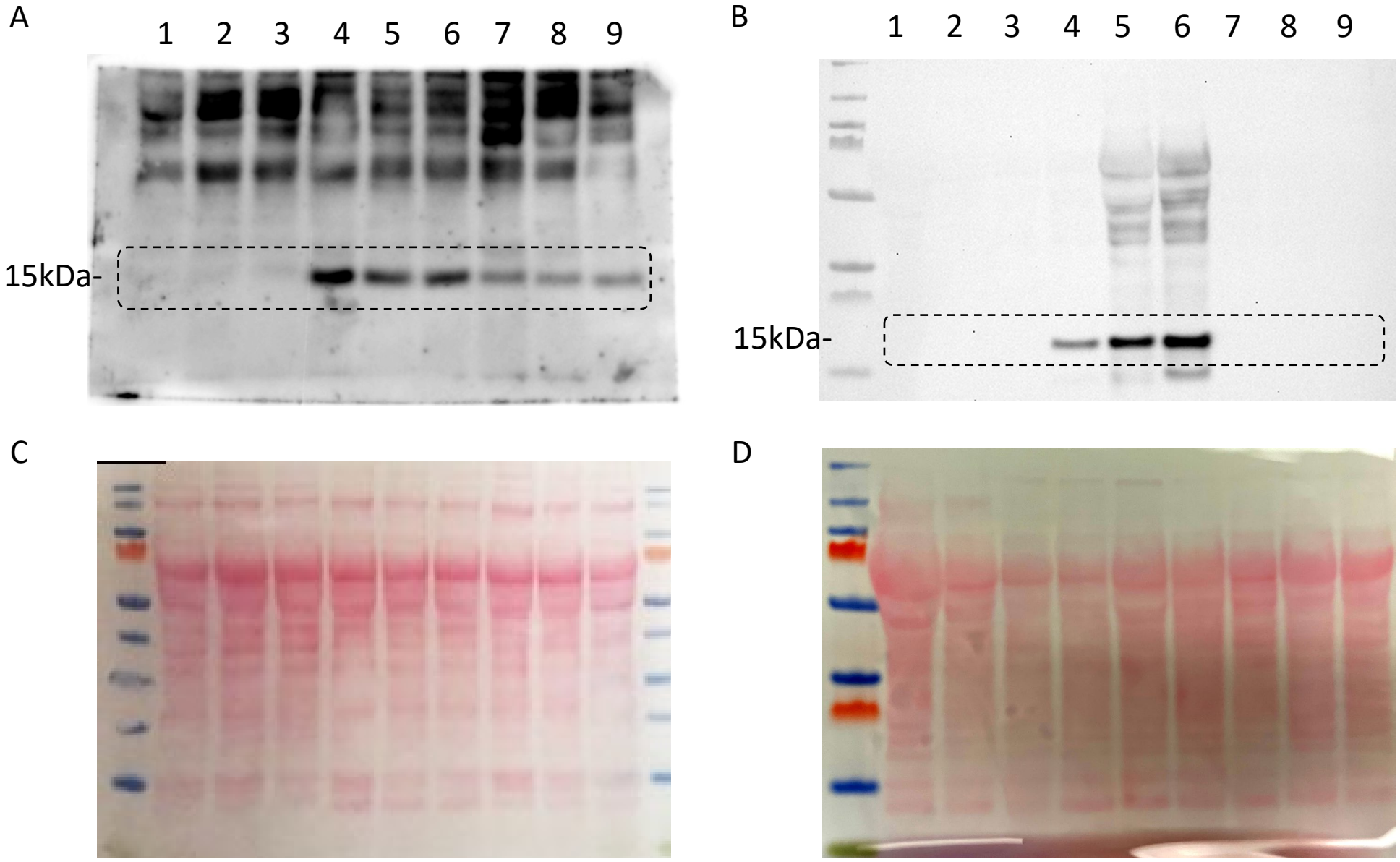


Fig S13. Full western blot and Ponceau S images from H3 plasma from CLP mice (Fig. 1B, S11C, S11G). The nitrocellulose membrane was stained by primary antibody anti-H3 (**A**, Male; **B**, Female) following staining by Ponceau S (**C**, Male; **D**, Female). Sample input were plasma collected at 24 hours after: lane 1-3: sham; lane 4-6: CLP; lane 7-9: CLP + 18-mer.

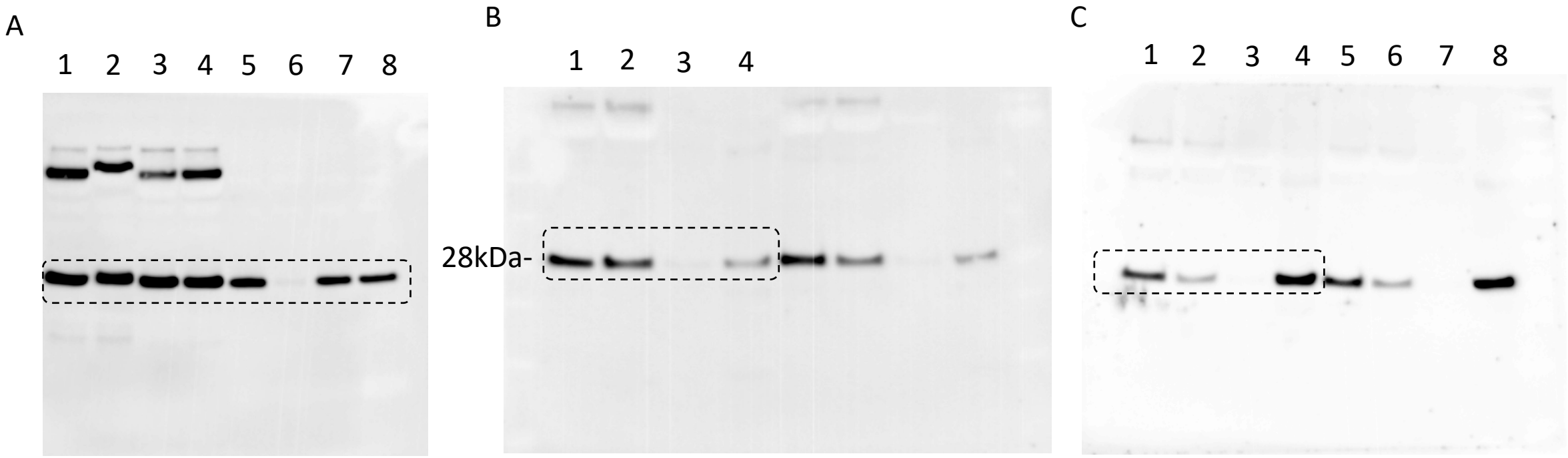


Fig S14. Full western blot of LPS pull-down assay with *ex vivo* competitive binding (Fig. S8E, F). (A)Pull-down assay with peritoneal lavage from B*LPS injected mice incubated with LPS, 6-mer or 18-mer was shown. Lane 1-4: preload from peritoneal lavage incubated with PBS (1), LPS (2), 6-mer(3), 18-mer (4); lane 5-8: elution from peritoneal lavage incubated with PBS (5), LPS (6), 6-mer(7), 18-mer (8). **(B,C)** Pull-down assay followed by immunoblot of HMGB1 with LPS I.P. injection (B) biotinylated LPS (B*LPS) I.P. injection (C) . Sample input: lane 1: preload; land 2: flow-through; lane 3: wash; lane 4: elution. Pull-down assay with peritoneal lavage from B*LPS injected mice incubated with 18-mer was shown in lane 5-8: lane 5: preload; land 6: flow-through; lane 7: wash; lane 8: elution.

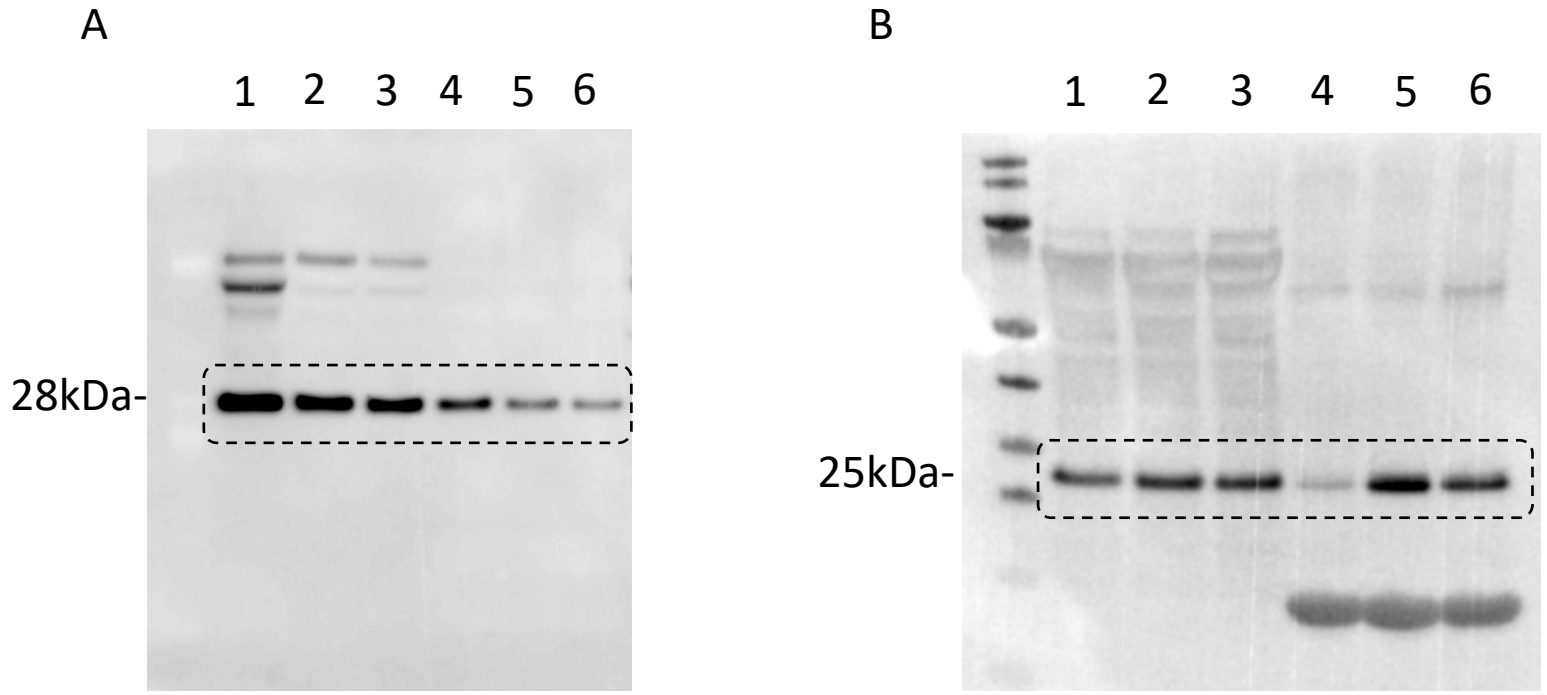


Fig S15. Full western blot of pull-down assay with 18-mer/B*LPS co-injection (Fig.3B). Pull-down assay with biotinylated LPS (B*LPS) and 18-mer (5 or 50 mg/kg) co-i.p. injection, immunoblot with HMGB1 **(A)** and apoA-I **(B)**. Sample input: lane 1-3: preload from peritoneal lavage from mice injected B*LPS (1), B*LPS + 18mer (5 mg/kg) (2), B*LPS + 18mer (50 mg/kg) (3). Lane 4-6: elution from peritoneal lavage from mice injected B*LPS (4), B*LPS + 18mer (5 mg/kg) (5), B*LPS + 18mer (50 mg/kg) (6).

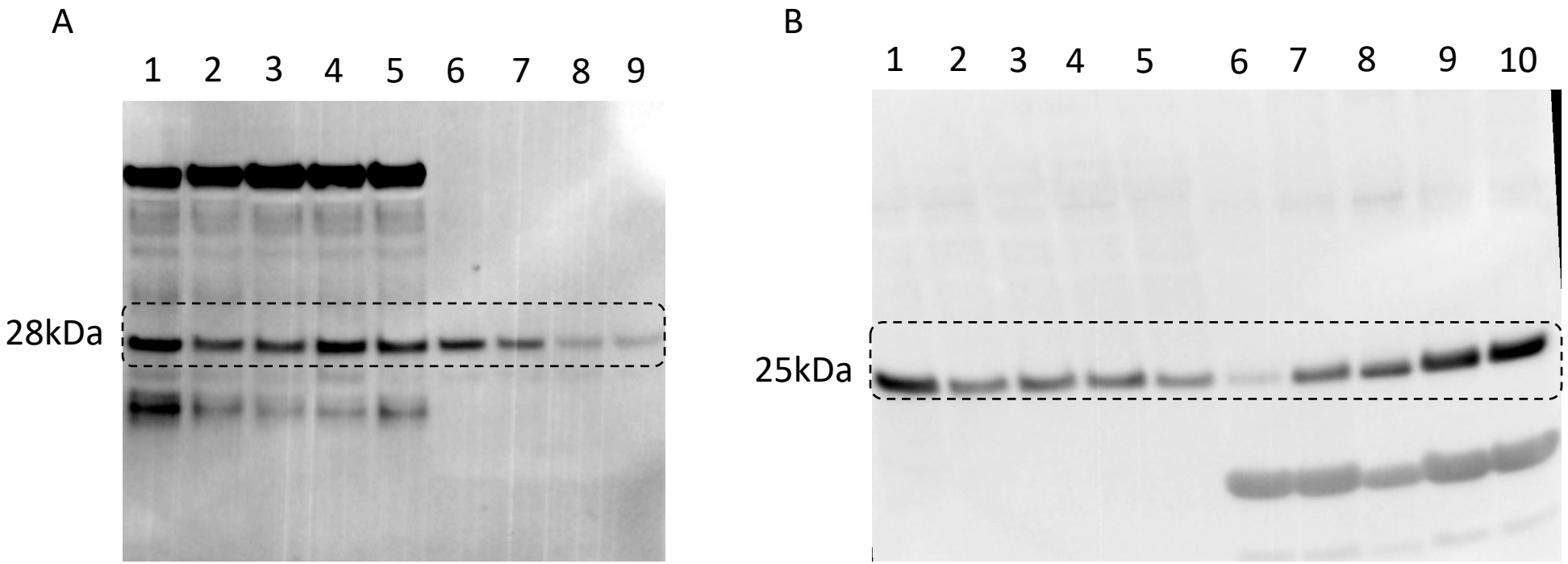


Fig S16. Full western blot of pull-down assay with apoA1 ex vivo competition and HS/B*LPS co-injection (Fig.S9B, C). (A) Pull-down assay with peritoneal lavage from B*LPS injected mice incubated with recombinant apoA1 (10, 50, 100 μg/mL) was shown. Lane 2-5: preload from peritoneal lavage incubated with PBS (2), apoA-I (10μg/mL) (3), apoA-I (50μg/mL) (4), apoA-I (100μg/mL) (5); lane 6-9: elution from peritoneal lavage incubated with PBS (6), apoA-I (10μg/mL) (7), apoA-I (50μg/mL) (8), apoA-I (100μg/mL) (9). Lane 1 is peritoneal lavage without incubation of other solution. **(B)** Pull-down assay with B*LPS and 6-mer, 12-mer or 18-mer co-i.p. injection, immunoblot with apoA1. Sample input: lane 1-5 were preload from peritoneal lavage from mice injected sterile saline (1), B*LPS (2), B*LPS + 6-mer (3), B*LPS + 12-mer (4), B*LPS + 18-mer (5). Lane 6-10 were elution from peritoneal lavage from mice injected sterile saline (6), B*LPS (7), B*LPS + 6-mer (8), B*LPS + 12-mer (9), B*LPS + 18-mer (10).