CXCL5-mediated recruitment of neutrophils into the peritoneal cavity of *Gdf15*-deficient mice protects against abdominal sepsis

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Abstract

Sepsis is a life-threatening organ dysfunction condition caused by a dysregulated host response to an infection. Here we report that the circulating level of growth-differentiation factor-15 (GDF15) is strongly increased in septic shock patients and correlate with mortality. In mice, we find that Gdf15-deficient animals are protected against abdominal sepsis due to increased chemokine CXC ligand 5 (CXCL5)-mediated recruitment of neutrophils into the peritoneum leading to better local bacterial control.

Introduction

Sepsis is the leading cause of death in intensive care units with over 5 million annual deaths worldwide attributable to this condition in addition to its heavy financial burden on health care systems around the world[1,2]. Anorexia and weight loss are key features of the wasting syndrome that often accompanies late-stages of cancer and where GDF15 is known to play a critical role[3]. These features, including long-lasting loss of muscle mass, are also observed in severe sepsis[4]. These similarities led us to investigate whether GDF15 is increased in sepsis and if it plays a role in its pathophysiology.

Results and Discussion

To begin addressing these questions, we measured the level of GDF15 in 40 serum samples from severe septic patients and compared the results with those of 130 healthy controls (HC) and 33 patients with a histological diagnosis of acute appendicitis (AA, 20 diagnosed with acute phlegmonous appendicitis and 13 with acute gangrenous appendicitis) before surgery. We found significant differences in GDF15 levels both when comparing AA with HC ($p<0.0001$) and AA with sepsis patients ($p<0.0001$, Fig 1a). Severe septic patients had

substantially higher values than the other groups (Fig.1a), in good agreement with previous recent observations[5]. We then compared survival of sepsis patients 28 days after diagnosis and found that survivors had on average substantially lower levels of GDF15 on the day of diagnosis (Fig. 1b). Importantly, patients with less than 10ng/mL GDF15 at the day of diagnosis showed a low mortality rate (5.8%) 28 days post-diagnosis, whereas those with higher GDF15 levels had a high mortality rate (39%; Fig.1c). None of the clinical or analytical severity parameters of AA patients correlated with GDF15 levels (data not shown). When divided based on mortality, sepsis patients did not differ in terms of age (W=191, p=0.2054) or gender (p=1) (Supplementary Table I). Survivors had a lower SAPS II (W=244.5, p=0.0033) and APACHE II score (W=108, p=0.0174), but did not significantly differ on the SOFA score at day 1 (W=188, p=0.0702) as compared to non-survivors.

To explore the role of GDF15 in sepsis, we first measured its levels in the peripheral blood of mice challenged with i.p. injection of *E. coli* and compared them with vehicle-treated mice 24 h after injections (Fig. 1d). We found that similarly to TNF in serum at 2 hr after challenge (Fig. 1e), the levels of GDF15 were significantly increased (Fig. 1d), in agreement with the findings in sepsis patients (Fig. 1a). We then used a panel of microbial pattern recognition agonists to challenge bone marrow derived macrophages (BMDM) from wild-type (WT) C56BL/6 mice and found that TLR2 agonists were the strongest inducers of GDF15 expression and secretion (Extended Data Fig. 1a) in contrast with the broad pattern of TNF secretion measured in the same conditioned media (Extended Data Fig. 1b). Based on these results, we chose peptidoglycan (PGN) from *Bacillus subtilis* for further experiments (Fig. 1f). In contrast with either *Escherichia coli*, or growing concentrations of lipopolysaccharide (LPS) that robustly induce the secretion of TNF (Extended Data Fig. 1c) but do not induce the secretion of GDF15, PGN from *B. subtilis* strongly induces the secretion of GDF15 in a concentration dependent manner (Fig. 1f). We then asked which pathway senses PGN leading to the secretion of GDF15. In comparison with BMDM from WT C56BL/6, we found that the TLR2/MyD88 pathway is required for GDF15 secretion while both NOD1 and NOD2 are dispensable (Fig. 1g). Depending on the dose and time of challenge with PGN, TLR4/MyD88 is also capable of sensing PGN to induce GDF15 secretion (Fig. 1g and data not shown).

We then turned to the mechanistic role of GDF15 in sepsis as the strongly increased secretion could either mean this factor is involved in a compensatory response to a stress (infection) challenge or that it plays an active part in increasing the severity of infection. To distinguish between these two possibilities, we compared the survival rates of WT and Gdf-15-deficient (*Gdf15*$^{-/-}$) mice in response to a polymicrobial peritonitis using the cecum ligation and puncture (CLP) model of sepsis. We consistently observed that *Gdf15*$^{-/-}$ animals are protected against sepsis and survive for considerably longer periods (Fig. 2a). Sickness behavior is defined as a group of signs and symptoms, including anorexia, lethargy, fever, altered sleep patterns, lack of grooming, and social withdrawal in response to an infection. In line with this, *Gdf15*$^{-/-}$ mice showed reduced temperature loss as compared to WT mice, both 8h and 24h post-CLP (Fig. 2b). *Gdf15*$^{-/-}$ mice have lower scores for sickness behavior, as detailed in Extended Data Fig. 2. All tested cytokines had lower levels in *Gdf15*$^{-/-}$ animals (Extended Data Fig. 3), which reached statistically significance in the cases of Interleukin (IL)-1β and IL-12 (Extended Data Fig. 3c and f) suggesting a better control of infection in *Gdf15*$^{-/-}$ animals. We found no relevant differences in the levels of serologic markers of organ dysfunction or damage (including creatinine, LDH, CK, AST and ALT) at 24hrs, suggesting

organ damage was similar in both strains (Extended Data Fig. 4a, b, c, d and e). This result is in agreement with minor histological differences between both genotypes at 24 h after CLP (Extended Data Fig. 4f).

We then measured the bacterial load in the peripheral blood of WT and *Gdf15*$^{-/-}$ animals in independent experiments and found consistently a statistically significant lower bacterial burden in *Gdf15*$^{-/-}$ mice at 8hrs after CLP (Fig. 2c), suggesting that *Gdf15*$^{-/-}$ better control the initial local infection. Together, the results for cytokines, serologic markers of organ lesion, histopathology and bacterial burden, suggest that *Gdf15*$^{-/-}$ mice are more resistant to infection, without affecting tissue damage. To investigate further this possibility, we analyzed and compared the peritoneal lavage contents of WT and *Gdf15*$^{-/-}$ mice after 8 h of CLP. We found bacterial levels on the peritoneal lavage to be on average 10-fold lower (p=0.0159) in *Gdf15*$^{-/-}$ mice (Fig. 2d), which was in good agreement with substantially elevated relative (Fig. 2e, p=0.0159) and absolute (Fig. 2f, p=0.0159) numbers of neutrophils. The increased number of neutrophils, rather than their differential activity between genotypes, is likely to be responsible for a better local control of the initial infection as we did not observe increased phagocytic activity (data not shown). These findings are consistent with previous observations in a mouse model of myocardial infarction where *Gdf15*$^{-/-}$ mice recruited more neutrophils to the injury site[6]. In this model, mice had a worse outcome as the excess number of neutrophils exacerbated the myocardial lesion[6]. An excess recruitment of neutrophils has also been observed in *Gdf15*$^{-/-}$ mice in a model of CCl_4-induced liver fibrogenesis[7], which contributed to lesion exacerbation. In contrast, in the present case of the CLP model the increased number of neutrophils lead to the better early control of the local infection.

To identify mechanistic basis for the different numbers of neutrophils at the site of infection, we measured the main chemokine factors for neutrophil attraction, including the CCR1 ligands CCL3 (Extended Data Fig. 5 Panel a), CCL4 (Panel b) and CCL5 (Panel c) and the CXCR2 ligands MIP-2/CXCL-2 (Extended Data Fig. 5 Panel d), KC/CXCL-1 (Panel e) and CXCL5 (Fig. 2g). Only LIX/CXCL5 showed a strong and significant increase in *Gdf15*$^{-/-}$ mice (p=0.0002), while all the other chemokines were either very similar or even marginally decreased. GDF15 was increased systemically only in WT animals and completely absent in knockout animals (Extended Data Fig. 5 Panel a). IL-6 and MCP-1, a chemokine for macrophages, were very similar suggesting that CLP procedure was made with similar severity in both genotypes (Extended Data Fig. 5 Panels b and c respectively).

In conclusion, our results suggest that *Gdf15*$^{-/-}$ mice are better able to control the initial infection by locally secreting higher levels of the CXCL5 chemokine, known to be involved in neutrophil recruitment through the activation of the CXCR2 receptor[8].

1. Singer, M. *et al.* The third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA - J. Am. Med. Assoc.* **315,** 801–810 (2016).
2. Van Vught, L. A. V. *et al.* Incidence, risk factors, and attributable mortality of secondary infections in the intensive care unit after admission for sepsis. *JAMA - J. Am. Med. Assoc.* **315,** 1469–1479 (2016).
3. Johnen, H. *et al.* Tumor-induced anorexia and weight loss are mediated by the TGF-β superfamily cytokine MIC-1. *Nat. Med.* **13,** 1333–1340 (2007).
4. Gordon, B. S., Kelleher, A. R. & Kimball, S. R. Regulation of muscle protein synthesis and the effects of catabolic states. *Int. J. Biochem. Cell Biol.* **45,** 2147–2157 (2013).
5. Buendgens, L., Yagmur, E., Bruensing, J., Herbers, U. & Baeck, C. Growth

Differentiation Factor-15 (GDF-15) is a predictor of mortality in critically ill patients with sepsis. *Dis. Markers* **15,** (2017).
6. Kempf, T. *et al.* GDF-15 is an inhibitor of leukocyte integrin activation required for survival after myocardial infarction in mice. *Nat. Med.* **17,** 581–588 (2011).
7. Chung, H. K. *et al.* GDF15 deficiency exacerbates chronic alcohol- and carbon tetrachloride-induced liver injury. *Sci. Rep.* **7,** 1–13 (2017).
8. Walz, A., Strieter, R. M., Schnyder, S., Bern, C.- & Arbor, A. Neutrophil-activating peptide ena-78. 129–130
9. Rittirsch, D., Huber-Lang, M. S., Flierl, M. A. & Ward, P. A. Immunodesign of experimental sepsis by cecal ligation and puncture. *Nat Protoc* **4,** 31–36 (2009).
10. Kolmogorova, D., Murray, E. & Ismail, N. Monitoring Pathogen-Induced Sickness in Mice and Rats. *Curr. Protoc. Mouse Biol.* **7,** 65–76 (2017).

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Competing interests
The authors declare that they have no competing interests.

Author contributions
IS and HGC performed all mouse experiments with the help of ES, AB and TV. ANC and DP performed all experiments in BMDM. HGC, ANC, DP, NC, SW and DP measured biochemical parameters in patients and mouse serum, mouse peritoneal lavage, and BMDM supernatants. ES analyzed mouse peritoneal lavage cells by flow cytometry. RM performed phagocytic assays in BMDM. AB did the statistical analysis of the data. HSY and MS provided reagents and advised the project. LFM conceived the study, coordinated all experiments and wrote the paper with input from all authors.

Figure legends

Fig. 1 | GDF15 is induced by infection in humans and mice. **a,** Serum levels of GDF15 in acute appendicitis (AA) and severe sepsis compared with healthy controls (HC). **b,** Serum levels of GDF15 in septic patients at day 1 of admission to intensive care unit (ICU) comparing survival outcome at 28 days after admission to ICU. **c,** Comparison of mortality rates in patients with GDF15 serum levels lower than 10ng/mL and higher than 10ng/mL at day 1 of admission to ICU. **d** and **e**, GDF15 and TNF serum levels, respectively, in mice infected with *E. coli i.p.* (n=10) or injected with vehicle (n=10), quantified at the peak of cytokine detection. **f,** GDF15 levels in conditioned media following incubation of wild-type BMDMs for 24h without stimulus or with the TLR agonists: PFA-fixed *E. coli* at a ratio of 20 bacteria per cell; lipopolysaccharide (LPS) from *E. coli* at 0.2 to 10µg/mL; and peptidoglycan (PGN) from *B. subtilis* at 0.2 to 10µg/mL. **g,** GDF15 levels quantified as in F, following incubation of BMDMs from wild-type (WT) or knockout animals (*Myd88*$^{-/-}$, *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$, *Nod1*$^{-/-}$, *Nod2*$^{-/-}$ and *Ripk2*$^{-/-}$), for 16 h with PGN 2.5µg/mL. F and G show averages and standard deviations for three replicate wells in 96-well plates; one representative experiment is shown and at least three independent experiments were performed and quantified by ELISA.

Extended Data Fig. 1 | TLR agonists induce GDF15.
Quantification by ELISA of GDF15 and TNF secretion induced by TLR agonists in BMDMs. **a,** GDF15 levels in conditioned media following incubation of BMDMs from wild-type (WT) or knockout animals (*Tlr4*$^{-/-}$, *Tlr2*$^{-/-}$ and *Myd88*$^{-/-}$) for 24 h without stimulus or with a series of TLR agonists (details below). **b,** and **c**, quantification of TNF levels in the same conditions as in A. TLR agonists and concentrations: PFA-fixed *E. coli* at 20 bacteria per cell; PFA-fixed *C. albicans* at 20 yeast per cell; LPS from *Escherichia coli* at 200ng/mL; *Listeria monocytogenes* at 100 million bacteria/mL; peptidoglycan (PGN) from a variety of microbial sources at 10, 5 and 2.5 μg/mL; Pam3CSK4 at 300ng/mL; flagellin from *S. typhimurium* at 1μg/mL; FSL-1 (Pam2CGDPKHPKSF) at 100ng/mL; ssRNA40 at 2.5μg/mL; Poly(I:C) HMW at 10μg/mL; Poly(I:C) LMW at 10μg/mL; and CpG oligonucleotide (ODN) at 1.5μM. Averages and standard deviations are shown for three replicate wells in 96-well plates; one representative experiment is shown and at least three independent experiments were performed and quantified.

Fig. 2 | *Gdf15*$^{-/-}$ mice better control local infection due to CXCL5-mediated neutrophil influx into the peritoneum. a, Survival to cecal ligation and puncture (CLP) comparing wild-type (WT, n=20) and *Gdf15*$^{-/-}$ animals (n=19). **b,** Rectal temperature in WT (n=28) and *Gdf15*$^{-/-}$ (n=26) animals 8 and 24hr after CLP. **c,** Colony-forming units (CFUs) cultured from peripheral blood of WT (n=6) and *Gdf15*$^{-/-}$ (n=6) animals 8hr after CLP. **d,** Colony-forming units (CFUs) cultured from peritoneal lavage of WT (n=5) and *Gdf15*$^{-/-}$ (n=4) mice 8 h after CLP. **e,** Percentage and **f,** total number of neutrophils in the peritoneal lavage of WT (n=4) and *Gdf15*$^{-/-}$ (n=5) mice 8 h after CLP. **g,** Quantification by ELISA of CXCL5 in the peritoneal lavage 8hr after CLP in WT and *Gdf15*$^{-/-}$ mice without (non-infected, NI) or 8 h after CLP.

Extended Data Fig. 2 | GDF15 modulates sickness behavior. A five-point scale to evaluate the development of sickness behaviors in WT (**a**) and *Gdf15*$^{-/-}$ mice (**b**). At specific time points following CLP, animals were examined by two observers who independently scored the presence of specific signs of sickness. In each animal, the following four signs were evaluated: (1) Piloerection, (2) Ptosis, (3) Lethargy and (4) Huddling. All animals were observed inside their cages. Each parameter was score as presence (1) or absence (0). Graphs represent the distribution of sickness behavior presented at each timepoint. "0" = no sickness behavior; "4" = all four signs of sickness behavior.

Extended Data Fig. 3 | Cytokine quantification in peripheral blood of animals after CLP. a, Quantification by ELISA of TNF at 4 h, **(b)** TNF at 24 h, **(c)** IL-1β at 24 h, **(d)** IL-10 at 24 h, **(e)** IL-6 at 24 h and **(f)** IL-12 at 24 h after CLP in WT or *Gdf15*$^{-/-}$ mice.

Extended Data Fig. 4 | WT and *Gdf15*$^{-/-}$ mice have similar degrees of tissue damage after CLP. a, Colorimetric quantification of serum levels of creatinine, (**b**) LDH, (**c**) CK, (**d**) AST and (**e**) ALT 24 h after CLP in WT and *Gdf15*$^{-/-}$ mice. **f**, Histology analysis of HE stains of liver, lung and kidney from WT and *Gdf15*$^{-/-}$ mice 24hr after CLP.

Extended Data Fig. 5 | Cytokine and chemokine quantification in the peritoneal lavage and peripheral blood from mice after CLP. Quantification by ELISA of the CCR1 ligands (**a**) CCL3, (**b**) CCL4, (**c**) CCL5 and the CXCR2 ligands (**d**) MIP-2/CXCL-2 and (**e**) KC/CXCL-1 in the peritoneal lavage of WT and *Gdf15*$^{-/-}$ mice 8hr after CLP. Quantification by ELISA of **(f)** GDF15, (**g**) IL-6 and (**h**) MCP-1 in serum samples from WT and *Gdf15*$^{-/-}$ mice 8 h after CLP.

Online Methods

Human studies

Human sepsis samples were included under a project approved by The French Ethical committee (''Comité de Protection des Personnes'' Ile de France IV; 2010-A0004039). Samples from healthy persons and patients with acute appendicitis patients were included under a research project approved by the Ethics Committee of Garcia de Orta Hospital, Portugal (Reference 05/2015). Written informed consent was obtained from either each enrolled patient or their families. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). The authors declare no conflict of interest.

Mice

All animal studies were performed in accordance with Portuguese regulations and approved by the Instituto Gulbenkian de Ciência ethics committee. GDF15 null mice were obtained from M. Shong (Chungnam National University School of Medicine, Daejeon, South Korea). *Gdf15*$^{-/-}$, *Myd88*$^{-/-}$, *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$ and C57BL/6J control mice were bred and maintained under specific-pathogen free conditions at the Instituto Gulbenkian de Ciência with 12 h light/12 h dark cycle, humidity 50–60%, ambient temperature 22 ± 2°C and food and water *ad libitum*.

Cecal ligation and puncture

Bedding of C57BL/6J and *Gdf15*$^{-/-}$ mice was mixed two weeks before the start of the experiments to mitigate differences in microbiome composition. Polymicrobial sepsis was induced in mice by cecal ligation and puncture (CLP), as previously described[9]. Briefly, male mice (8-12 weeks old) were anesthetized with an intraperitoneal injection of 100 µg/g ketamine and 15 µg/g xylazine, the peritoneal cavity opened and the cecum exteriorized. Cecum was ligated at approximately 50% of its length and perforated through and through with a 19-G needle. The cecum was relocated into the abdominal cavity. Peritoneum and abdominal muscles were closed with surgical sutures and skin with metallic clips. Fluid resuscitation was performed by injecting 1 mL PBS subcutaneously immediately after surgery. Rectal temperature was measured at the indicated time-points after surgery using a rodent thermometer (Bioseb, France). For survival experiments, mice were monitored at least three times a day for one week and moribund mice were euthanized. For blood collection and peritoneal lavage experiments, mice were euthanized with CO_2, blood was collected by cardiac puncture and 5 mL sterile PBS were injected into the peritoneal cavity and subsequently aspirated.

Sickness behavior assay

The Sickness behavior assay used in this report has been described in[10]. Briefly, mice were observed by two researchers and rated for the presence (1) or absence (0) of each of the following symptoms: piloerection, ptosis, lethargy, and huddling. Score was presented as the sum of all four parameters.

E. coli challenge in mice

Escherichia coli K12 MG1655 was grown in Luria-Bertani broth at 37°C, 180 rpm for 2.5hrs until late exponential phase was reached (OD_{600nm}=0.8-1.0). The culture was then centrifuged at 4400 xg for 5 minutes at room temperature, washed in the same volume of PBS and centrifuged again. The bacterial pellet was finally resuspended in PBS to obtain an approximately $3x10^9$ CFU/mL. This bacterial suspension was immediately injected intraperitoneally in mice (200 µL/mouse).

Colony Forming Units assay
Colony forming units (CFU) were determined in blood and peritoneal lavage by serially diluting in sterile PBS and plating in trypticase soy agar plates with 5% sheep blood. Four dilutions were plated per condition. CFU were counted after incubating plates at 37 °C for 16h.

Bone marrow derived macrophage (BMDM) differentiation
BMDMs were differentiated from adult (typically 8-week-old) C57BL/6J mice. After euthanasia by CO_2 inhalation, the mouse skin was sterilized with 70% ethanol and femurs and tibia of hind limbs were removed, stripped of muscle and rinsed in RPMI medium. Bone marrow cells were flushed from cut bones using an insulin syringe with a 30G needle into 10 mL of RPMI medium. Cells were then pelleted by centrifugation at 450 g for 5 min and the cell pellet resuspended in 10 mL of RPMI supplemented with 10% (v/v) FBS and 0.2 % (v/v) penicillin/streptomycin. Cells were counted and plated at a density of 3 x 10^6 cells (including red blood cells) per 10 mL of RPMI medium supplemented with 10% FBS and 0.2 % penicillin/streptomycin, with 30% of L929-conditioned medium. After three days, an equal volume of fresh medium with 30% of L929-supernatant was added to the cells. After four additional incubation days, the medium was replaced by 10 mL of fresh medium with 30% of L929-supernatant. 24 h-48h afterwards, cells were scraped from plates, counted and seeded in C10 medium.

Media
Medium for BMDM differentiation: RPMI 1640, FBS 10% (v/v), Penicillin-streptomycin 0.2% (v/v) and added 30 % of L929-conditioned medium. L929-conditioned medium: L929 cells were cultured in T175 flasks, in 40 mL of DMEM medium with 10% (v/v) FBS and 1% (v/v) Penicillin/Streptomycin and grown to confluency. The culture medium was left unchanged for 5 days, for good production of M-CSF. Cells were then centrifuged at 290 g for 5 minutes and the supernatant was collected and filter-sterilized. C10: RPMI medium 1640 supplemented with:10% (v/v) Fetal Bovine Serum, 1% (v/v) Penicillin-Streptomycin, 1% (v/v) Pyruvate, 1% (v/v) LGlutamine, 1% (v/v) Non-essential aminoacids, 1% (v/v) Hepes buffer, 0.05 M of 2-Mercaptoethanol.

BMDM treatments
BMDMs were plated in supplemented RPMI 1640 medium (Gibco) and treated 16h later for the indicated times. The agonists used were from the mouse TLR1-9 Agonist kit (Invivogen tlrl-kit1mw) and peptidoglycan (PGN) was obtained from a variety of sources (all PGN reagents are from Sigma-Aldrich and from a series of microbial origins with the following catalog numbers: from *Bacillus subtilis*, 69554; from *Staphylococcus aureus*, 77140; from *Micrococcus luteus*, 53243; from *Streptomyces* sp., 79682; from *Methanobacterium* sp. 78721; from *Saccharomyces cerevisiae*, 72789). PFA-fixed *Escherichia coli* were incubated with the BMDMs at a ratio of 20 bacteria per cell for the indicated times.

Biochemical assays in serum, peritoneal lavage, and supernatant from BMDMs
Levels of human GDF15 were quantified in the serum of patients using the ELISA kit DuoSet human *Gdf15*$^{-/-}$ (R&Systems, catalog number DY957) according to the company's protocol. Whole mouse blood (obtained by cardiac puncture) was centrifuged at 1600 x*g* for 5 minutes at 4 °C, serum was collected and stored at -80 °C before analysis. Peritoneal lavage was centrifuged at 300 x*g* for 5 minutes at 4 °C, the supernatant was collected and stored at -80 °C before analysis. For neutrophil identification we used the following antibodies: CD11b, Ly6G, Ly6C, GR1 and anti-neutrophil (7/4). To calculate the absolute number of cells by flow cytometry, we used Perfect-Count Microspheres. The absolute number of the cell population of interest is determined by dividing the number of cells of interest acquired by the number of Perfect-Count Microspheres acquired and multiplying this result by the microsphere

concentration. Cytokine and chemokine levels were determined using the following ELISA kits, according to the manufacturer's instructions: mouse TNF-α (#430902, Biolegend), IL-1β (#432601, Biolegend), IL-6 (#431302, Biolegend), IL-10 (#431411, Biolegend), IL-12/IL-23 (p40) (#431602, Biolegend), CKCL-5/LIX (#DY443, R&D Systems), MCP-1 (#432701, Biolegend), CCL3/MIP-1α (#DY450, R&D Systems), Mouse CCL4/MIP-1β (#DY451, R&D Systems), Mouse CCL5/RANTES (#DY478, R&D Systems), CLCX-1/KC (#DY453, R&D Systems), CXCL-2/MIP-2 (#DY452, R&D Systems), mouse GDF-15 (#DY6385, R&D Systems). Serological makers of organ damage/dysfuntion were determined using the following colorimetric kits, according to the manufacturer's instructions: QuantiChrom Creatinine (#DICT, Bioassay Systems), QuantiChrom Lactate Dehydrogenase (#D2DH, Bioassay Systems), EnzyChrom Creatine Kinase (#ECPK, Bioassay Systems), EnzyChrom Alanine Transaminase (#EALT, Bioassay Systems), EnzyChrom Aspartate Transaminase (#EALT, Bioassay Systems). All absorbance readings were performed in 96-well plates using an Infinite M200 plate reader (Tecan).

Flow cytometry

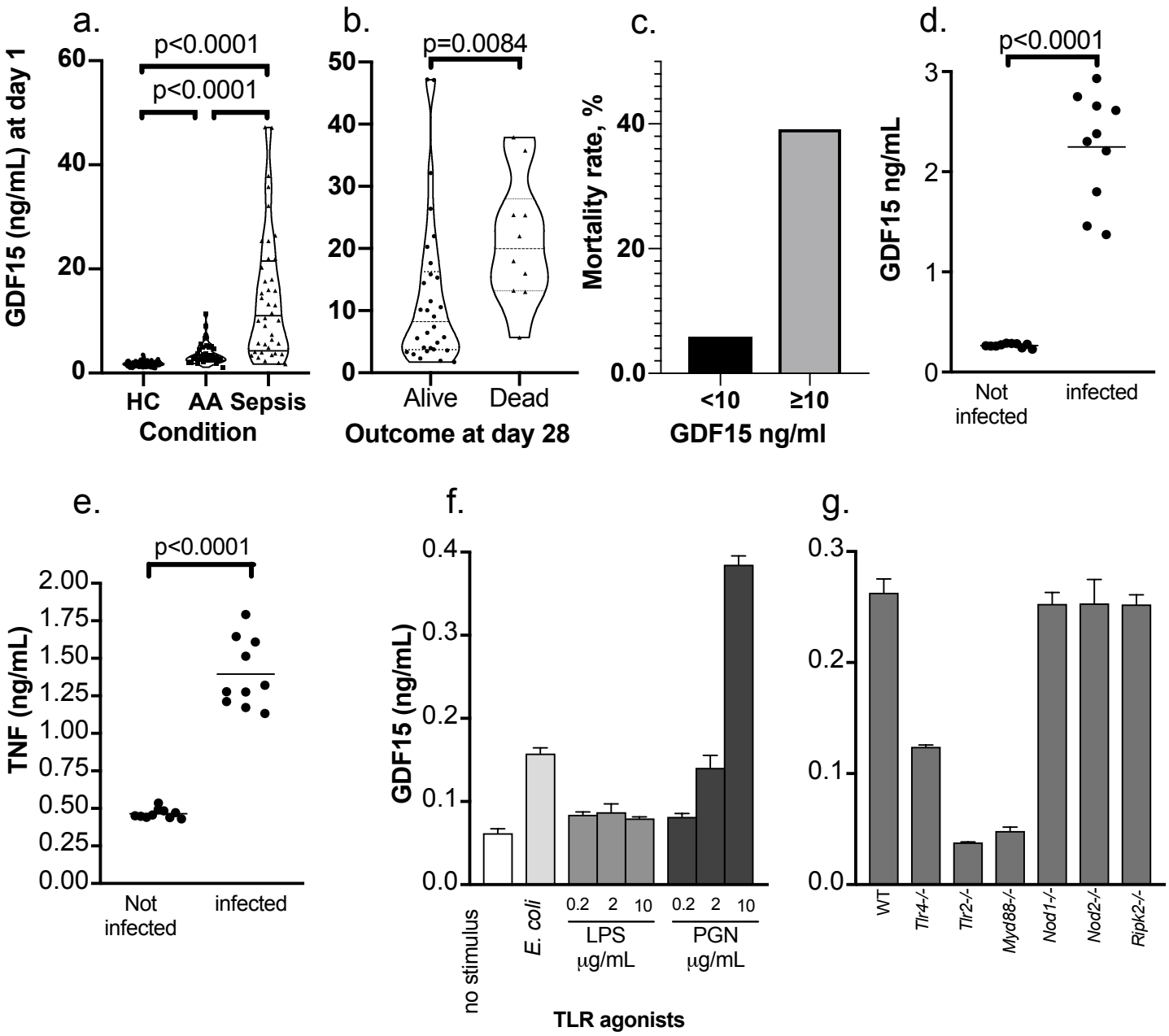
Cells were incubated with different antibodies diluted in PBS containing 2% FBS and 0.01% NaN_3. To prevent non-specific binding, cells were incubated at 4°C for 20 minutes with CD16/CD32 to block Fc receptors. Afterwards, cells were incubated for 30 minutes at 4°C with the following fluorescent labelled antibodies: anti-CD49b-PE, anti-CD3-PE, anti-B220-PE, anti-Ly6C-APC/Cy7, anti-Ly6G-APC, anti-F4/80-FITC, anti-CD11b-PerCp, anti-CD11b-BV785, anti-F4/80-A647, anti-GR1-PerCp/Cy5.5, anti-Neutrophil (7/4)-FITC, Sytox Blue. All antibodies are from Biolegend except anti-Neutrophil (7/4) which is from Abcam and B220, CD49b and GR1 which are from BD Biosciences. Flow cytometry data were acquired on LSR Fortessa X20 (Becton Dickinson) and analyzed using the FlowJo software package (Tree Star).

Histopathology

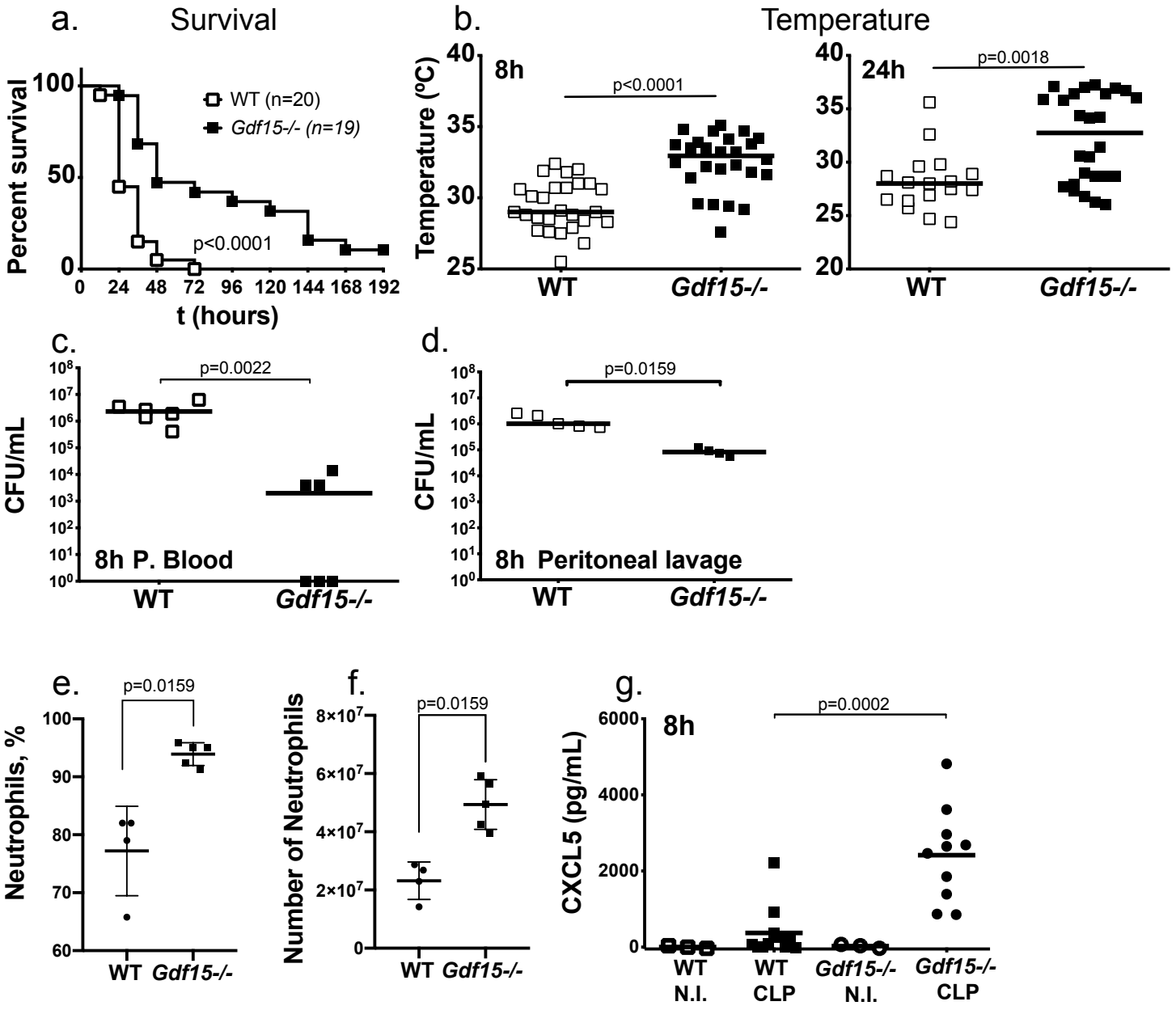
Mouse liver, lung, and kidney were collected 24h after CLP and immediately fixed in 10% buffered formalin. Samples were then embedded in paraffin, sectioned (3 μm) and stained for hematoxilin and eosin according to standard procedures. Histopathology analysis was performed by a trained pathologist, blinded to the mice strain and infection challenge, at the Instituto Gulbenkian de Ciência Histopathology Unit. Tissues were scored for damage, namely necrosis and leukocyte infiltration.

Statistical analysis

Mann-Whitney test and Mantel-Cox test (for survival curves) were used for statistical analysis, using Graphpad Prism 6.0 (GraphPad Software). A p-value <0.05 was considered statistically significant.



Santos et al. Figure 1



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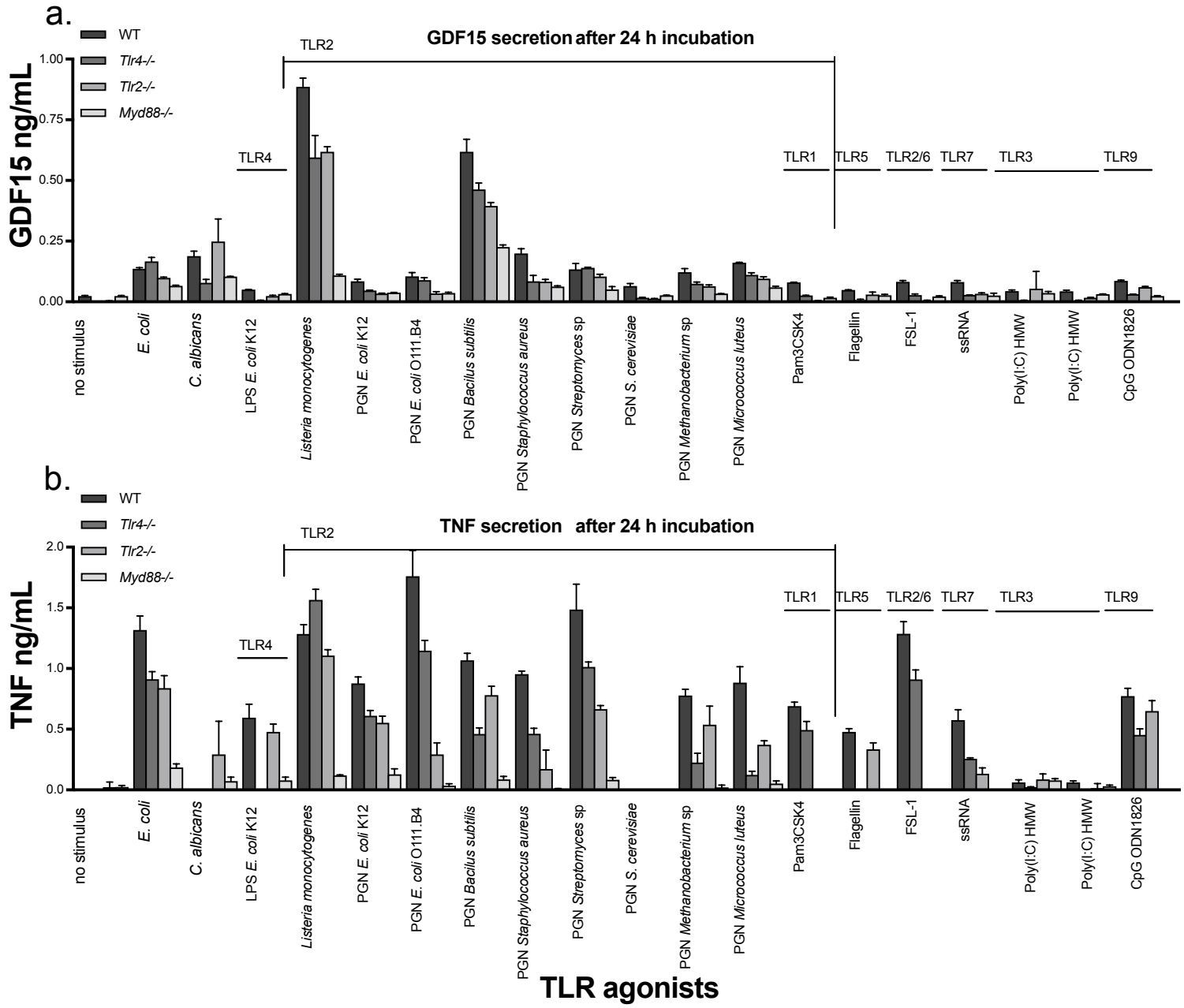
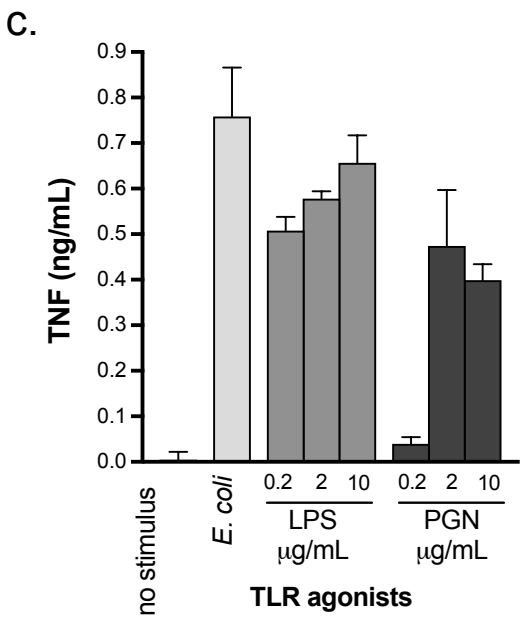
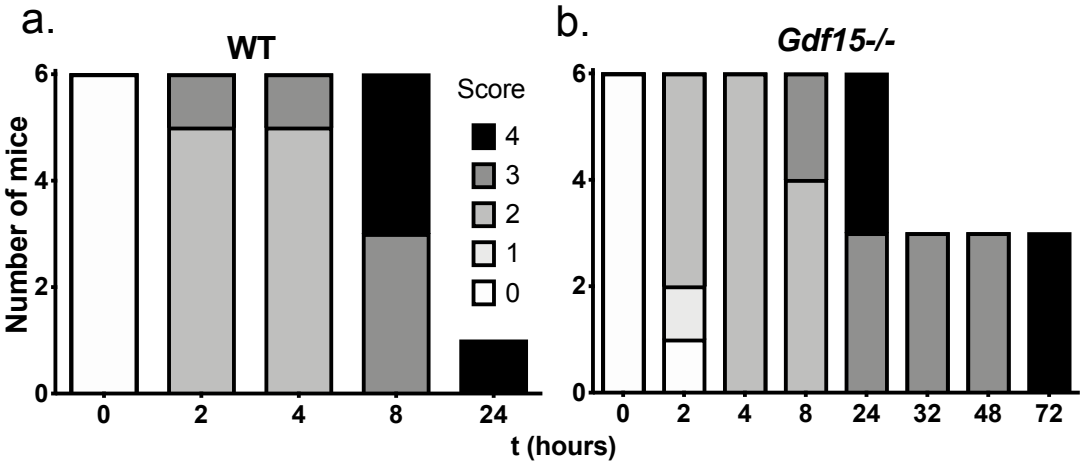
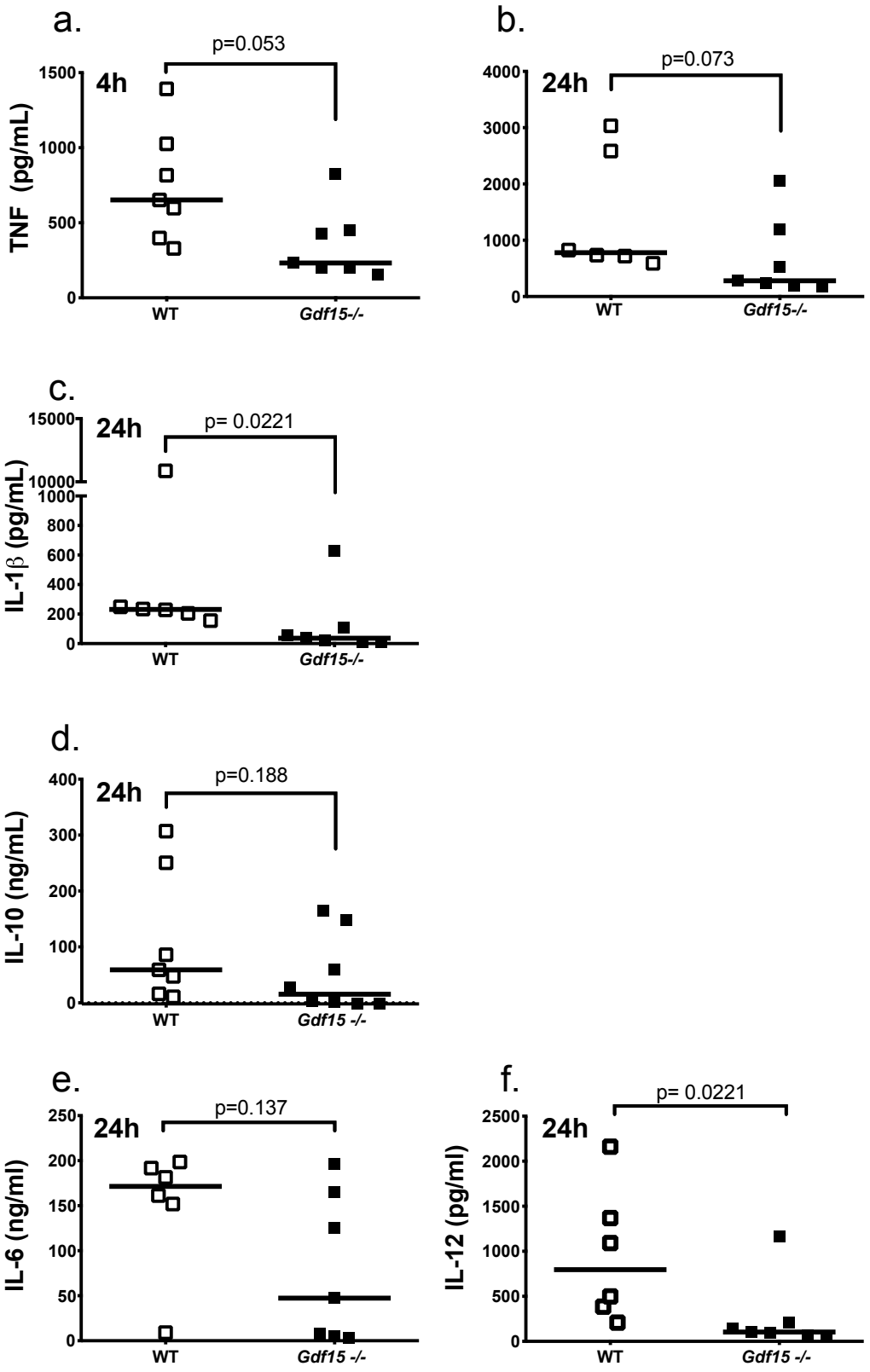




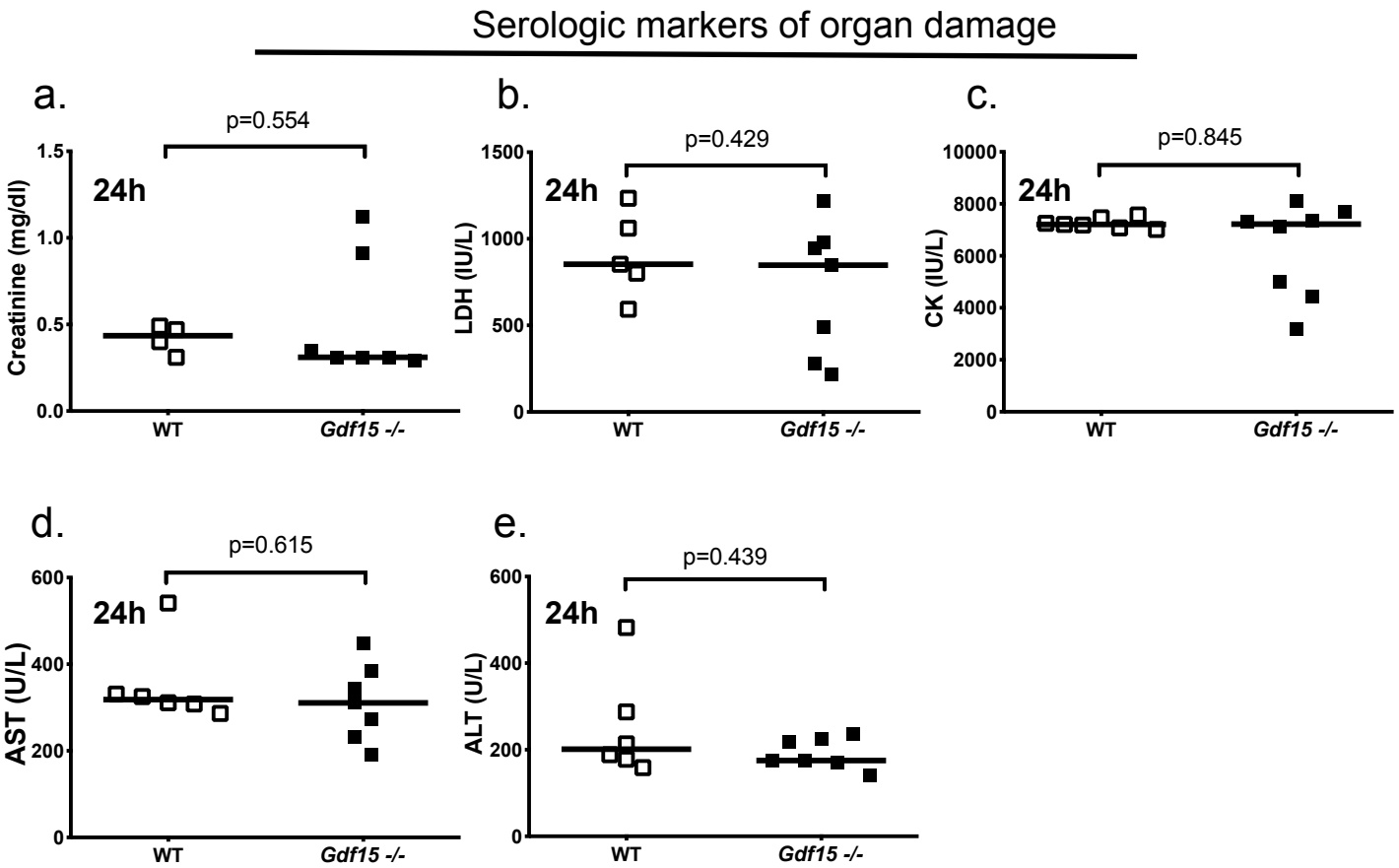
Santos et al. Extended Data Figure 1

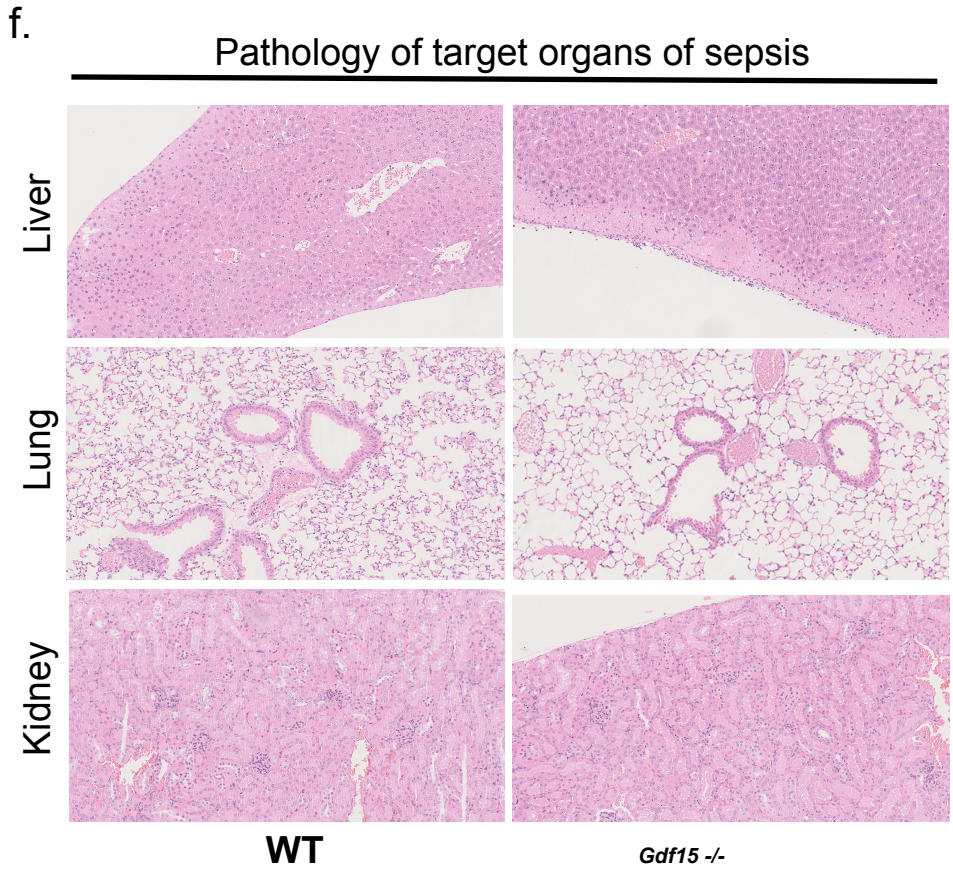


Santos et al. Extended Data Figure 2

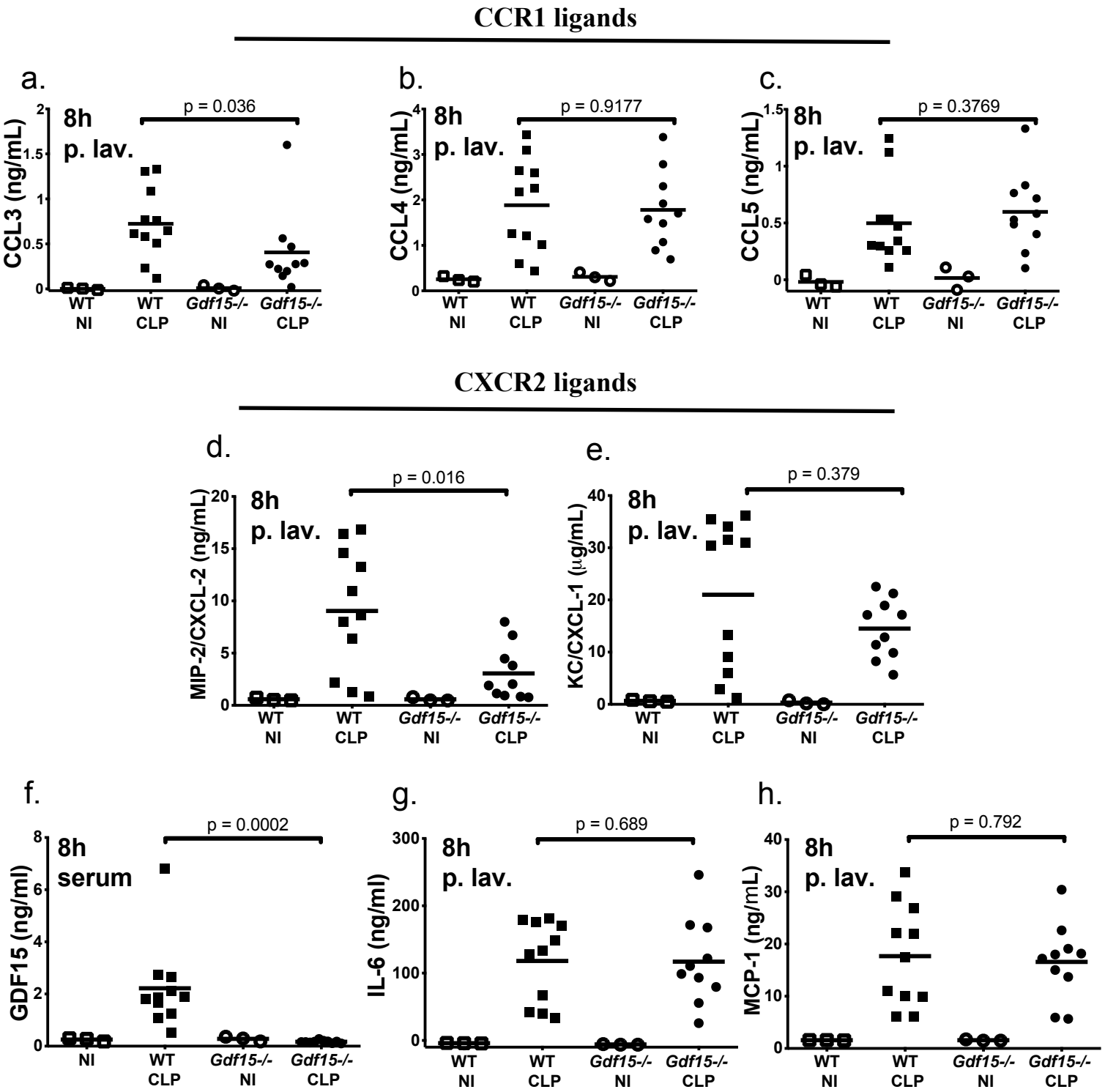


Santos et al. Extended Data Figure 3





Santos et al. Extended Data Figure 4



Santos et al. Extended Data Figure 5

Supplementary Table I
Sepsis cohort characterization. Separation based on mortality at day 28.

Variable	**Non-survivors** (n=10)	**Survivors** (n=30)	*Statistical Testing*
Age	75.8 ± 13.39; 77 (72; 85.5)	65.73 ± 20.79; 71 (58.5; 80)	W = 191 p = 0.2054
Gender	4 Male : 6 Female	14 Male : 16 Female	Fisher Exact Test; p=1
SAPS II	64.40 ± 11.18; 65.5 (60.25; 70.50)	50.6 ± 13.15; 53 (43.50; 58.50)	W = 244.5 p = 0.003289
APACHE II	31.71 ± 9.11 (3 NA); 34 (28; 35.50)	23.79 ± 4.84 (11 NA); 25 (21.5; 27)	W = 108 p = 0.01741
SOFA_D1	10.42 ± 3.53; 11.5 (8.25; 12.75)	8.33 ± 2.72 (3 NA); 8 (7; 10.5)	W = 188 p = 0.07023