Calprotectin modulates inflammatory collateral tissue damage during intraperitoneal origin systemic candidiasis

Authors Madhu Shankar[#1], Nathalie Uwamahoro[#1], Sandra Holmberg[2], Thomas Vogl[3], Johannes Roth[3], Constantin F. Urban[1*]

Affiliations

[1]Department of Clinical Microbiology, Umeå Centre for Microbial Research (UCMR) & Molecular Infection Medicine Sweden (MIMS), Umeå University, Umeå 90185, Sweden.

[2]Department of Medical Chemistry and Biophysics, Umeå University, Umeå 90187, Sweden.

[3]Institute of Immunology, Universitätsklinikum Münster, Germany

[#] contributed equally to this work

Address correspondence to: Constantin Urban, constantin.urban@umu.se

Abstract

Peritonitis is a leading cause of severe sepsis in surgical intensive care units, as over 70% of patients diagnosed with peritonitis develop septic shock. A critical role of the immune system is to return to homeostasis after combating infection. S100A8/A9 (calprotectin) is an antimicrobial, pro-inflammatory protein used as a marker for diagnosis of inflammatory diseases. Here we describe the role of calprotectin on inflammatory collateral tissue damage (ICTD). WT and calprotectin-deficient *in vivo* *Candida albicans* disseminated peritonitis mice model, and primary macrophages were treated with recombinant calprotectin and the anti-inflammatory compound, paquinimod. The effects on ICTD and fungal clearance were investigated. Calprotectin-deficient mice developed less ICTD than wildtype mice. Injection of recombinant calprotectin resulted in increased ICTD and fungal clearance comparable to wildtype levels. Treatment with paquinimod, a specific inhibitor of calprotectin, abolished ICTD. The data indicated that calprotectin controls ICTD levels and host antimicrobial modulation at a systemic level during intra-abdominal candidiasis (ICA).

IMPORTANCE: Fungal pathogens like *C. albicans* pose a significant burden on health at a global level comparable to malaria and tuberculosis. Despite the increasing antifungal drug resistance, fungi remain underestimated as pathogens. Fungal dissemination to organs during severe fungal systemic inflammation is often lethal for immunocompromised patients, particularly during intraabdominal surgeries where disseminating infections in patients can become life-threatening conditions with a matter of hours. This study utilizes *in vivo* techniques for recombinant protein therapy and pharmacological inhibition (paquinimod) to modulate activity of a systemic

antimicrobial protein demonstrating that calprotectin controls the severity of fungal sepsis and hence provides an option for pharmacological intervention.

Introduction

Peritonitis frequently results in severe sepsis in surgical patients, especially in the intensive care unit (ICU),(1), as more than 70% of patients often succumb to death within 72h,(2). Hence, we require treatments that extend life expectancy to allow proper treatment.(3). Peritonitis is characterized by inflammation of the membrane in the abdominal cavity, the peritoneum. Peritonitis often occurs upon disruption of physical barriers or can become spontaneous in severe organ failure, causing alterations of the physiologic flora residing in the gastrointestinal tract (GIT),(1, 4). These alterations prompt an inflammatory response that targets the removal of contaminants from the peritoneal cavity into circulation. There, pathogens prompt activation of host immune responses and the release of pro-inflammatory mediators including Interleukin-6 (IL6), macrophage inflammatory protein -1α (MIP-1α) and tumor necrosis factor –α (TNFα) to recruit phagocytes to the peritoneum,(5). The attempt to restrict infection promotes abscess development through production of fibrinous exudate. Failure to confine peritonitis may lead to organ failure, coma and death,(6).

Interestingly, inflammation can be described as a spectrum degrees of deviation from homeostasis, characterized by outcomes that are either intentional (pathogen clearance) or collateral (pathological side effect, called inflammatory collateral tissue damage -ICTD),(7). The focus of most studies on detection and elimination of pathogens has neglected host tolerance to disease through the resolution of collateral outcomes of inflammation in an attempt to establish homeostasis, (8).

Acute or systemic inflammatory pathological states are often associated with intra-abdominal surgery,(9). Surgical intervention disrupting the natural barriers of the gastrointestinal tract (GIT) lead to deep-seated microbial infection by gut colonizing

65 organisms and frequently *Candida albicans* infection. As a result, the most common
66 non-mucosal fungal diseases among hospitalized patients in the developed world are
67 *Candida* peritonitis, also referred to as intra-abdominal candidiasis (IAC). IAC is
68 challenging to diagnose and hence results in high mortality rates ranging from 25% -
69 60%, (9).

70 The resolution of infection is an active process involving reprogramming of
71 cells and modulation of mediators,(10). Included among these mediators are
72 multifunctional proteins, such as the S100A8/A9 heterodimeric complex (calprotectin).
73 Here, we used an experimental model for IAC to study the contribution of calprotectin
74 to ICTD.

75 Participating as a signaling molecule that binds toll-like receptor-4 (TLR4) to
76 induce pro-inflammatory chemokines and cytokines, the heterodimer calprotectin is the
77 physiologically relevant and is the bioactive form that is secreted by activated, stressed
78 or necrotic cells. Under high calcium conditions, such as present in the extracellular
79 milieu and culture medium, S100A8/A9 heterodimers quickly tetramerize, thereby
80 losing TLR4 signaling capacity,(11). Thus, S100A8 homodimers, which cannot
81 tetramerize, are widely accepted experimental stimuli to mimic heterodimer activity.

82 Furthermore, calprotectin binds micronutrients including zinc, manganese, and
83 calcium, and is often deployed by leukocytes as a mechanism to either deprive microbes
84 of the nutrients or poison the microbes in high quantities,(12). Neutrophils deploy the
85 heterodimer against *C. albicans* during the formation of neutrophil extracellular
86 traps,(13). In addition to the antimicrobial benefit, calprotectin is used in diagnostics to
87 monitor neutrophil elevation in various inflammatory diseases, though the exact

mechanisms on calprotectin modulation to resolve inflammatory homeostasis remains elusive,(14).

One treatment currently under clinical trials is paquinimod, an immunomodulatory compound that prevents the binding of S100A9 to TLR4, designed to target chronic inflammatory diseases,(15–20). Using a murine peritonitis model with *C. albicans*, we show that inflammatory responses failed to contain the pathogen in the peritoneum, which in turn led to detrimental ICTD dependent on the presence of calprotectin. Interestingly, treatment of mice with paquinimod abrogated calprotectin-induced ICTD suggesting paquinimod as promising adjunct therapy option during severe ICA.

Results

A disseminated fungal peritonitis model to determine roles of S100A8/A9

During ICA, fungal cells dissemination reach the liver and other organs via lymphatics or bloodstream,(6). Liver tissue damage and leukocytosis are hallmarks of deep-seated and systemic *C. albicans* infection,(21). To establish a fungal ICA model and determine the role of calprotectin in systemic inflammation, we obtained the previously characterized *S100A9*-deficient mouse (*S100A9*$^{-/-}$) that can induce the *S100A8* transcripts but lacks the S100A8 protein,(22). S100A8 in S100A9$^{-/-}$ mice is rapidly degraded in the proteasome, avoiding systemic overwhelming immune responses. However, under chronic TNF conditions, this degradation process is insufficient leading to severe phenotypes in artificial mouse strains (TTP$^{-/-}$/S100A9$^{-/-}$ or itghTNF/S100A9$^{-/-}$) indicating that heterodimer activity must be regulated tightly to restrict operating range,(23).

Using wildtype (WT) and *S100A9*$^{-/-}$ mice, *C. albicans* intraperitoneal injections were administered, liver tissues collected after 24 hours of infection and indicators of sepsis were measured. The infection was determined to be systemic using phenotypic analysis of mice and histopathological analysis from hematoxylin-eosin stained (H & E stain) liver sections after 24 hours. The development of eye exudates (Figure 1a) was an indication of fungal dissemination from the peritoneal cavity to other loci in the mouse. WT liver sections indicated higher numbers of leukocytic infiltration zones compared to *S100A9*$^{-/-}$ sections (Figure 1b, top panel). Conversely, there was visual evidence of more fungal cells in the *S100A9*$^{-/-}$ livers compared to WT (Figure 1b, bottom panel). The inflammatory score (Figure 1c). showed a large number of inflammatory cell infiltrates

(level 3) in WT *C. albicans* infected samples compared to lack of inflammation (level 1) observed in *S100A9*$^{-/-}$ mice.

Blood alanine aminotransferase (ALT) levels in the blood are an indicator of liver damage,(1, 24). *C. albicans* infected WT mice (101.6U/L) showed elevated levels compared to *S100A9*$^{-/-}$ mice (37.2U/L) which showed similar ALT levels to uninfected mice, suggesting lack of systemic tissue damage in the calprotectin-deficient animals (Figure 2a). The ability of the host to clear the infection or organ microbial load is related to the colony-forming units (CFUs),(25). *S100A9*$^{-/-}$ mice had a significantly higher fungal load (2.5 fold of average levels) compared to WT mice (Figure 2b).

Macrophages are sentinels for immune signaling that leads to leukocytosis but may cause problems when uncontrolled,(26). The pro-inflammatory cytokine response of WT and *S100A9*$^{-/-}$ was determined using bone marrow-derived macrophages (BMDMs) to monitor the ability of primary macrophages to induce TNFα (Figure 2c). Higher levels of TNFα (3.1 fold of average levels measured) were induced by WT BMDMs (570.7 pg/ml) compared to *S100A9*$^{-/-}$ BMDMs (185.5 pg/ml), suggesting a defect in TNFα induction in cells lacking calprotectin.

S100A8/A9 is responsible for ICTD during disseminated peritonitis

The active role of calprotectin on systemic candidiasis is currently unknown. Further, the implications on the use of calprotectin in recombinant protein therapy is also unknown. A recombinant therapy approach was employed to determine the role calprotectin in sepsis,(27). Murine *S100A8* was expressed in *E. coli*, purified and verified by mass spectrometry to obtain functional and structurally stable homodimers

as previously reported,(28). For completion, we also purified *S100A9,* as a control, although purified protein dimerization does not occur and renders the protein inactive,(23). To determine the effects of recombinant S100A9 and S100A8 proteins (rS100A8 and rS100A9) on disseminated *C. albicans* infection Purified calprotectin monomers (Figure 3a) of predicted sizes (S100A8 (~10 kDa) and S100A9 (~14 kDa)) were purified and injected (100µl (100 µg/ml) protein per WT or *S100A9*$^{-/-}$) in *C. albicans* infected mice. We did not use the recombinant heterodimer (S100A8/A9) in our experiments as these quickly form tetramers with predominantly anti-inflammatory effects *in vivo*,(23).

S100A9$^{-/-}$ mice showed no difference when treated with rS100A9 (Figure 3b). Treatment of *S100A9*$^{-/-}$ mice with rS100A8 showed a higher level of ALT (5.9 fold of average levels measured) compared to untreated with levels not significantly different to WT suggesting that constitutively active S100A8 homodimers mimic heterodimers to cause WT levels of liver damage (Figure 3b) and that the calprotectin-mediated effect on ICTD is direct rather than indirect. calprotectin is an antimicrobial protein against *C. albicans*,(13). In this context, the fungal clearance defect observed in *C. albicans* infected *S100A9*$^{-/-}$ mice was remedied, reducing fungal load after treatment with rS100A8 (Figure 3c). To determine whether the S100A8 activity would aid the *S100A9*$^{-/-}$ mutant in eliciting an appropriate cytokine response, BMDMs infected with *C. albicans* were treated with rS100A8. Induction (7.5 fold of average levels) of TNFα levels was obtained in *S100A9*$^{-/-}$ BMDMs treated with rS100A8 (Figure 3d) comparable to WT levels upon *C. albicans* infection (Figure 2c). *In vivo, S100A9*$^{-/-}$ BMDMs infected with *C. albicans* showed higher levels of various cytokines compared to rS100A8 treated BMDMs (Figure 3e-3j). Furthermore, the macrophage chemoattractant

MIP1α (*CCL3*) and *MIP1β* (*CCL4*), crucial for the recruitment of various leukocytes and expression of inflammatory cytokines including IL6 and TNFα,(29), were both significantly elevated (3.9 and 3.4 fold respectively) upon rS100A8 treatment (Figure 3g and 3h). In agreement, *C. albicans* infected *S100A9*$^{-/-}$ BMDMs treated with rS100A8 produced higher levels of IL6 and TNFα than untreated BMDMs (Figure 3e and 3f). Also monitored was the induction of the anti-inflammatory cytokine IL10, which showed low but significant levels induced when mice were treated with rS100A8, while CXCl-1 levels declined (Figure 3i and 3j). Induction of pro-inflammatory cytokines and modulation of tissue damage by rS100A8 suggest that calprotectin heterodimers are main contributors of inflammatory responses during *C. albicans* infection. The induction of lower levels of the anti-inflammatory IL-10 suggests that calprotectin also affects anti-inflammatory cytokines.

Paquinimod therapy nullifies ICTD induced by rS100A8

Targeted deletion of *S100A9* improves survival in mouse models of bacterial-induced sepsis,(30). Higher lethality of a systemic infection is often due to the inability to contain sepsis-induced organ and tissue damage by the host,(1). There are no specific therapies against sepsis, and often management focuses on containing the infection through source control and antibiotics or antifungals plus organ function support,(31). We hypothesized that using an anti-inflammatory drug in WT mice may mimic the beneficial aspect (lack of tissue damage) observed in the *S100A9*$^{-/-}$ mutant might extend host survival from fungal sepsis.

The activity of paquinimod, a novel anti-inflammatory compound initially developed against systemic Lupus Erythematosus (SLE) that targets liver, lung, heart and skin S100A9,(32), was tested *in vitro* and *in vivo*. Supplementary figure 1 shows the

194 percentage of dead cells (propidium iodide positive cells) using flow cytometry analysis
195 (FACS) on treatments of WT BMDMs with various drug concentrations of paquinimod.
196 Among the concentrations used, no significant toxic effects were observed
197 (Supplementary figure 1). However, there was significant TNFα, and CCL-3/MIP-1α
198 reduction at 300 µg/ml and 930 µg/ml paquinimod concentrations compared to
199 untreated *C. albicans* infected BMDMs (Figure 4a and 4c). The anti-inflammatory
200 cytokine IL10 (Figure 4b), was also reduced albeit, initial levels (BMDMs + Ca) were
201 too low to discern systemic effects compared to levels observed under rS100A8
202 treatment **(**Figure 3i). Treatment of mice with the drug had a higher fungal burden in
203 infected WT mice, while and the higher levels of the fungal load was consistently high
204 in *S100A9*$^{-/-}$ mice compared to treated *S100A9*$^{-/-}$ mice (Figure 4d). Paquinimod did not
205 have significant effects on fungal clearance in *S100A9*$^{-/-}$ mice (Figure 4e), and complete
206 elimination of liver tissue damage was observed in *C. albicans* infected *S100A9*$^{-/-}$ mice
207 with rS100A8 upon treatment with paquinimod (Figure 4f).

208 To determine whether paquinimod could be used to alleviate sepsis derived from
209 fungal peritonitis, infected wildtype mice were treated with paquinimod every 24 hours
210 with paquinimod (Figure 5a). Although the drug did not prevent mice from succumbing
211 to systemic infection, treated mice survived longer compared to untreated mice,
212 particularly between 24 and 48 hours (Figure 5b). Despite the similar weight loss
213 observed between treated and untreated mice (figure 5c), treated mice presented an
214 inability to clear fungal exudates in the eyes (Figure 5d), consistent the observed
215 decreased fungal clearance in the liver by S100A9 mutant mice (Figure 2b). In addition
216 to this, treated mice were generally more active during *C. albicans* infection, despite the
217 higher fungal burden.

Discussion

This work establishes the first study to characterize the role of immune-modulating calprotectin on host resolution of inflammation from a peritoneal-derived disseminated fungal infection. The gastrointestinal commensal nature of *C. albicans* requires that mucosal damage and neutropenia are achieved for *C. albicans* dissemination,(33). Unfortunately, most experimental models studying systemic fungal diseases use intravenous (IV) injection of fungal cells even though this route primarily bypasses mucosal host defenses that require breach before the development of sepsis,(34). This study utilized an IAC model to induce systemic inflammation mimicking a severe clinical concern of postoperative *Candida* peritonitis,(35). Similar to IV, IP-induced infection allows rapid blood dissemination of pathogens with exposure to an active population of phagocytes, complement cascade and the potential for abscess formation in the peritoneal cavity,(35). The peritonitis infection model presented here aimed to breach the immune barriers in the peritoneal cavity, and this was phenotypically clear in the dissemination of *C. albicans* in murine eyes (figure 1a). This method was superior in that; it is more representative of a systemic fungal infection that occurs in peritonitis, compared to the standard blood IV infection route that efficient to bypass many absorption barriers and metabolic mechanisms,(36). The IP injection route allows pathogen exposure to active phagocyte populations in the peritoneum and the potential for the host to contain the infection through the formation of abscesses,(6).

WT liver fungal clearance was ~3 times more efficient when compared to the calprotectin mutant (Fig 2b). Notably, the *S100A9*$^{-/-}$ mutant used, lacks the S100A8 protein as well, despite the presence of S100A8 transcripts as previously reported,(23,

37). The various leucocyte infiltration zones observed in WT liver tissues compared to *S100A9*$^{-/-}$ mice (Fig 1b, c) indicated that the recruitment of higher levels of leukocytes coincides with fungal clearance in WT mice. These findings suggested that the presence of calprotectin is required for fungal clearance during disseminated infection, and supports our previous findings that implicated antimicrobial properties of calprotectin during candidiasis,(13). Notably, presence of calprotectin subjected WT mice to nearly ~3 times more TNFα levels and tissue damage compared to *S100A9*$^{-/-}$ mice as indicated by ALT levels (Fig 2 b, c), implicating that the induction of S100A8/A9 inflammatory responses modulates processes which contribute to tissue damage. These findings support the association of the S100 family of proteins with inflammatory disorders,(23), and our data indicates that calprotectin activity depends on strict modulation of both antimicrobial and inflammatory activity. These insights would especially be critical in the case of immunocompromised patients.

Despite constitutive expression (6.5% of transcriptome) and abundance (~ 45% of cytosol) of S100A9 and S100A8 in neutrophils Elgazzar, 2015; Niemiec et al., 2017, these proteins are additionally induced through appropriate stimulants and are often strongly expressed in acute and chronic inflammatory lesions and degenerative disorders such as Alzheimer disease,(38), suggesting potentially systemic roles by calprotectin. Indeed, the IP treatment of *C. albicans S100A9*$^{-/-}$ with rS100A8 resulted in *S100A9*$^{-/-}$ fungal clearance similar to WT. Depending on the $Ca^{2+}$and Zn^{2+}-binding properties of calprotectin, various protein conformation and oligomerization states are obtained, including self-assembly into tetramers and possibly larger oligomers,(38). Experimental limitation did not allow us to determine the exact site of recombinant

265 protein activity if either direct *C. albicans* targeting in the peritoneal cavity, intra-organ
266 fungal clearance or both.

267 Since rS100A8 showed significant *in vivo* activity, we used *in vivo S100A9*$^{-/-}$ and
268 the *S100A9*$^{-/-}$ primary macrophages in a *C. albicans ex vivo* infection assay, and showed
269 that rS100A8 mediates macrophage activation as indicated by the higher levels of the
270 chemokines Cxcl-1, CCL-3 and CCL-4 (Fig 2e), pro-inflammatory cytokines as well as
271 the anti-inflammatory cytokine IL10. The induction of IL10 by rS100A8 is consistent
272 with homeostatic management of systemic inflammation,(39). Balancing of pro- and
273 anti-inflammatory cytokines is a common theme of host factors with local and system-
274 wide effects as exemplified by interferon signaling pathways during viral infections,
275 (40). Though rS100A8 showed efficient fungal clearance, in circumstances where the
276 immune system is compromised, the activity of S100A8 has the potential to induce dire
277 effects on host-mediated tissue damage.

278 Our data not only demonstrate the potential of recombinant protein therapy of
279 calprotectin but also showed a new therapeutic potential for paquinimod. The chemical
280 inhibition of S100A9 by paquinimod is used to treat chronic inflammatory diseases,(15,
281 18). As a specific binder for S100A9, we found paquinimod targeting the inflammatory
282 effects of S100A8, potentially indirectly affecting the active dimer site. Paquinimod
283 alleviated the tissue-damaging effects of rS100A8 (Fig 3d, e). No toxicity was observed
284 against bone marrow-derived macrophages (BMDMs) at the concentration used on mice
285 trials and paquinimod activity affected the inflammatory modulators as observed in the
286 reduction of CCL-3 and IL-10. Hence, paquinimod represents a potential adjuvant
287 therapeutic option that targets the host responses instead of the pathogen, in particular,
288 since the compound had no adverse effect on fungal clearance. Although only 12 hours

were added to the survival time of paquinimod treated mice between 24 and 48 hours, the rate of drug metabolism and therefore depletion in mice are unknown, and this information would be required to fine-tune treatment. However, an extra 12 hours to a patient with a 72-hour survival/diagnostic window might be valuable.

This study showed the dilemma of the protective and harmful roles of calprotectin in *Candida albicans*-induced fungal peritonitis (Figure 6). Fungal peritonitis that is not contained by host defenses leads to pathogen infiltration of host organs (Figure 6.1). Fungal-host interaction induces the systemic release of calprotectin,(13) for fungal clearance and ALTs into the systemic circulation (Figure 6.2-6.3). Local innate immune cells (represented in this study as primary macrophages respond with a cytokine 'storm' of pro-inflammatory cytokines (Figure 6.4- 6.5), that leads to increased leukocyte infiltrates systemically (Figure 6.6), and promote increased tissue damage in the effort of fungal clearance (Figure 6.7). To our knowledge, this is the first study to present both the potential for a systemically functional protein, evidence for potential antimicrobial recombinant protein therapy, and adjuvant treatment as paquinimod-mediated inhibition of calprotectin showed reduction of ICTD (Figure 6.vi).

The significant improvement of ICTD in mice with experimental IAC upon paquinimod treatment supports the idea that the detrimental effects of inflammation may indeed influence the increased mortality associated with invasive fungal infections,(41). Therefore, more insights in the activity of this compound will provide options that might be utilized to increase the time required for proper diagnosis of infection related to systemic inflammation. Considering the high mortality (>50%) due

312 to do fungal sepsis,(1), future adjuvant therapies similar to paquinimod could be the key

313 against peritonitis and other severe inflammatory malignancies.

314

Materials and Methods

Ethical statement. Animal experiments and isolation of cells were carried out following the recommendations in the Guide for the Care and Use of Laboratory Animals, conformed to Swedish animal protection laws and applicable guidelines (djurskyddslagen 1988:534; djurskyddsförordningen 1988:539; djurskyddsmyndigheten DFS 2004:4) in a protocol approved by the local Ethical Committee (Umeå djurförsöksetiska nämnd, Permit Number A79-14.

Statistical analysis. Statistical analysis was conducted using Graphpad Prism 6 software and *P* values less than 0.5 were considered significant. All two-group comparisons in *Candida* CFU data, inflammatory score, ALT data, and ELISA data were conducted using the unpaired, two-tailed student's *t*-test. Comparisons of WT and *S100A9*$^{-/-}$ mice data were performed using One-way ANOVA multiple comparisons analysis as specified in figure legends. In all comparisons, the sample size is specified in figure legends, and a $P<0.05$ was considered significant. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

Yeast strains and growth conditions. *C. albicans* clinical isolate strain - SC5314 was cultured overnight in YPD (1% yeast extract, 2% bacto-peptone and 2% glucose) at 30°C. The Candida cells were washed three times in PBS prior use in all assays. Cell numbers were calculated using Vi-CELL Cell Viability Analyzer (Beckman Coulter AB).

Animal infections and isolation of bone marrow-derived macrophages, and tissue analyses. All mice were maintained according to a previous report,(42) at Umeå Centre for Comparative Biology (UCCB), Umeå University, Umeå, Sweden.

Primary macrophages (BMDMs) cells and differentiated as described in a previous report,(43). BMDMs flow cell cytometry (FACs) was conducted using BD LSR II flow cytometer (BD Biosciences, San Jose, CA), using propidium iodide,(43), on paquinimod treated cells at indicated concentrations.

For tissue damage and fungal load, analyses were conducted according to a previous report,(44), with 3×10^6 *C. albicans* cells intraperitoneal injection per g of mouse after 24 hours of infection,(11). Blood ALT levels were measured using vetscan VS2^{TM} (SCIL animal care company) as previously reported,(24, 45, 46).

Histological preparations and inflammatory score analyses were conducted as in previous reports, (13, 47), For the inflammatory score, Whole sections were analyzed for inflammation and scored under the supervision of a specialized animal pathologist. The sections from each animal were scored as zero if they had no inflammatory cells present in the tissue, one for a few inflammatory cells (1–20 cells), two for moderate cell infiltration (21–40 cells), three for a large number of inflammatory cells (41–60 cells), and four if inflammation was spread all over in the tissue (61 cells),(47).

Cytokine and chemokine quantification. BMDMs were seeded at 1×10^5 cells per well in 96-well microplates and then infected with *C. albicans* at an MOI of 1 for 24 hours. Cell-free culture supernatants were harvested after *C. albicans* infection. Supernatants were analyzed for indicated cytokines of chemokines by ELISA (Biolegend-ELISA MAX™ 9727 Pacific Heights Blvd, San Diego, CA, USA), or Pro-Mouse cytokine BioPlex® 200 multiplex (Bio-Rad Laboratories) according to the manufacturer's instructions.

Generation of recombinant S100A8 and S100A9. The S100A8 and S100A9 gene encoding for monomers of the mouse dimer calprotectin (UniProt P27005, P31725) were codon-optimized and synthesized using DNA2.0, GST tagged and cloned in *E. coli* BL2. Proteins were fractionated and purified using Superdex 75 16/600 column (GE Healthcare Life Sciences, UK) The primary sequence, the intact mass and the presence of product were confirmed by mass spectrometry using an ABI 4800 MALDI tandem time-of-flight mass spectrometry. Recombinant proteins were screened for endotoxin and LPS contamination and levels were below 0.1EU/ mL, as previously recommended,(48).

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Author contributions

CFU provided funding and together with MS and NU designed the study, MS and NU collected and analyzed the data. TV and JR contributed to the conceptualization of the study. Manuscript drafting by NU, MS and CFU. SH contributed preparation of histological sections. All authors contributed to data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agree to be accountable for all aspects of the work

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Competing interests, none declared.

Ethics approval

Animals, conformed to Swedish animal protection laws and applicable guidelines (djurskyddslagen 1988:534; djurskyddsförordningen 1988:539;

393 djurskyddsmyndigheten DFS 2004:4) in a protocol approved by the local Ethical

394 Committee (Umeå djurförsöksetiska nämnd, Permit Number A79-14.

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References

1. Fiore M, Maraolo AE, Leone S, Gentile I, Cuomo A, Schiavone V, Bimonte S, Pace MC, Cascella M. 2017. Spontaneous peritonitis in critically ill cirrhotic patients: a diagnostic algorithm for clinicians and future perspectives. Ther Clin Risk Manag 13:1409–1414.

2. Paoli C, Reynolds M, Sinha M, Gitlin M. 2018. 1486: CURRENT BURDEN, OUTCOMES, AND COSTS OF SEPSIS MANAGEMENT IN UNITED STATES HOSPITALS. Crit Care Med 46.

3. Denning DW, Perlin DS, Muldoon EG, Colombo AL, Chakrabarti A, Richardson MD, Sorrell TC. 2017. Delivering on Antimicrobial Resistance Agenda Not Possible without Improving Fungal Diagnostic Capabilities. Emerg Infect Dis J 23:177.

4. de Jong PR, González-Navajas JM, Jansen NJG. 2016. The digestive tract as the origin of systemic inflammation. Crit Care 20:279.

5. Yao V, Platell C, Hall JC. 2003. Role of peritoneal mesothelial cells in peritonitis. BJS (British J Surgery) 90:1187–1194.

6. Skipworth RJE, Fearon KCH. 2008. Acute abdomen: peritonitis. Surg 26:98–101.

7. Wang A, Huen SC, Luan HH, Yu S, Zhang C, Gallezot J-D, Booth CJ, Medzhitov R. 2016. Opposing Effects of Fasting Metabolism on Tissue Tolerance in Bacterial and Viral Inflammation. Cell 166:1512-1525.e12.

8. Hamidzadeh K, Christensen SM, Dalby E, Chandrasekaran P, Mosser DM. 2017. Macrophages and the Recovery from Acute and Chronic Inflammation. Annu Rev Physiol 79:567–592.

9. Lichtenstern C, Schmidt J, Knaebel HP, Martin E, Büchler MW, Weigand MA. 2007. Postoperative Bacterial/Fungal Infections: A Challenging Problem in Critically Ill Patients after Abdominal Surgery. Dig Surg 24:1–11.

10. Netea MG, Balkwill F, Chonchol M, Cominelli F, Donath MY, Giamarellos-Bourboulis EJ, Golenbock D, Gresnigt MS, Heneka MT, Hoffman HM, Hotchkiss R, Joosten LAB, Kastner DL, Korte M, Latz E, Libby P, Mandrup-Poulsen T, Mantovani A, Mills KHG, Nowak KL, O'Neill LA, Pickkers P, van der Poll T, Ridker PM, Schalkwijk J, Schwartz DA, Siegmund B, Steer CJ, Tilg H, van der Meer JWM, van de Veerdonk FL, Dinarello CA. 2017. A guiding map for inflammation. Nat Immunol 18:826–831.

11. Vethanayagam RR, Almyroudis NG, Grimm MJ, Lewandowski DC, Pham CTN, Blackwell TS, Petraitiene R, Petraitis V, Walsh TJ, Urban CF, Segal BH. 2011. Role of NADPH Oxidase versus Neutrophil Proteases in Antimicrobial Host Defense. PLoS One 6:e28149.

12. Zackular JP, Chazin WJ, Skaar EP. 2015. Nutritional Immunity: S100 Proteins at the Host-Pathogen Interface. J Biol Chem2015/06/08. 290:18991–18998.

13. Urban CF, Ermert D, Schmid M, Abu-Abed U, Goosmann C, Nacken W, Brinkmann V, Jungblut PR, Zychlinsky A. 2009. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. PLoS Pathog2009/10/30. 5:e1000639–e1000639.

14. Manolakis AC, Kapsoritakis AN, Tiaka EK, Potamianos SP. 2011. Calprotectin, Calgranulin C, and Other Members of the S100 Protein Family in Inflammatory Bowel Disease. Dig Dis Sci 56:1601–1611.

15. Fransén Pettersson N, Deronic A, Nilsson J, Hannibal TD, Hansen L, Schmidt-Christensen A, Ivars F, Holmberg D. 2018. The immunomodulatory quinoline-3-carboxamide paquinimod reverses established fibrosis in a novel mouse model for liver fibrosis. PLoS One 13:e0203228–e0203228.

16. Kraakman MJ, Lee MK, Al-Sharea A, Dragoljevic D, Barrett TJ, Montenont E, Basu D, Heywood S, Kammoun HL, Flynn M, Whillas A, Hanssen NM, Febbraio MA, Westein E, Fisher EA, Chin-Dusting J, Cooper ME, Berger JS, Goldberg IJ, Nagareddy PR, Murphy AJ. 2017. Neutrophil-derived S100 calcium-binding proteins A8/A9 promote reticulated thrombocytosis and atherogenesis in diabetes. J Clin Invest2017/05/15. 127:2133–2147.

17. Masouris I, Klein M, Dyckhoff S, Angele B, Pfister HW, Koedel U. 2017. Inhibition of DAMP signaling as an effective adjunctive treatment strategy in pneumococcal meningitis. J Neuroinflammation 14:214.

18. Schelbergen RF, Geven EJ, van den Bosch MHJ, Eriksson H, Leanderson T, Vogl T, Roth J, van de Loo FAJ, Koenders MI, van der Kraan PM, van den Berg WB, Blom AB, van Lent PLEM. 2015. Prophylactic treatment with S100A9 inhibitor paquinimod reduces pathology in experimental collagenase-induced osteoarthritis. Ann Rheum Dis 74:2254 LP – 2258.

19. van den Bosch MH, Blom AB, Schelbergen RF, Koenders MI, van de Loo FA, van den Berg WB, Vogl T, Roth J, van der Kraan PM, van Lent PL. 2016. Alarmin S100A9 Induces Proinflammatory and Catabolic Effects Predominantly in the M1 Macrophages of Human Osteoarthritic Synovium. J Rheumatol 43:1874 LP – 1884.

20. Yun J, Xiao T, Zhou L, Beuerman RW, Li J, Zhao Y, Hadayer A, Zhang X, Sun D, Kaplan HJ, Shao H. 2018. Local S100A8 Levels Correlate With Recurrence of Experimental Autoimmune Uveitis and Promote Pathogenic T Cell Activity. Invest Ophthalmol Vis Sci 59:1332–1342.

21. Fiore M, Cascella M, Bimonte S, Maraolo AE, Gentile I, Schiavone V, Pace MC. 2018. Liver fungal infections: an overview of the etiology and epidemiology in patients affected or not affected by oncohematologic malignancies. Infect Drug Resist 11:177–186.

22. Manitz M-P, Horst B, Seeliger S, Strey A, Skryabin B V, Gunzer M, Frings W, Schönlau F, Roth J, Sorg C, Nacken W. 2003. Loss of S100A9 (MRP14) results in reduced interleukin-8-induced CD11b surface expression, a polarized microfilament system, and diminished responsiveness to chemoattractants in vitro. Mol Cell Biol 23:1034–1043.

23. Vogl T, Stratis A, Wixler V, Völler T, Thurainayagam S, Jorch SK, Zenker S, Dreiling A, Chakraborty D, Fröhling M, Paruzel P, Wehmeyer C, Hermann S, Papantonopoulou O, Geyer C, Loser K, Schäfers M, Ludwig S, Stoll M, Leanderson T, Schultze JL, König S, Pap T, Roth J. 2018. Autoinhibitory regulation of S100A8/S100A9 alarmin activity locally restricts sterile inflammation. J Clin Invest2018/04/03. 128:1852–1866.

24. Vatsalya V, Avila D, Frimodig JC, Barve SS, McClain CJ, Gobejishvili L. 2016. Liver Injury Assessment by Vetscan VS2 Analyzer and Most Frequently Used ALT/GTP Reagent. Gastroenterol Hepatol (Bartlesville, Okla)2016/04/13. 4:107.

25. Bayer AS, Edwards JE, Guze LB. 1981. Experimental intraabdominal candidiasis in rabbits: therapy with low-total-dose intravenous amphotericin B. Antimicrob Agents Chemother 19:179 LP – 184.

26. Grainger JR, Konkel JE, Zangerle-Murray T, Shaw TN. 2017. Macrophages in gastrointestinal homeostasis and inflammation. Pflugers Arch2017/03/10. 469:527–539.

27. Gazzar M El. 2015. Immunobiology of S100A8 and S100A9 Proteins and Their Role in Acute Inflammation and Sepsis. Int J Immunol Immunother 2.

28. Futami J, Atago Y, Azuma A, Putranto EW, Kinoshita R, Murata H, Sakaguchi M. 2016. An efficient method for the preparation of preferentially heterodimerized recombinant S100A8/A9 coexpressed in Escherichia coli. Biochem Biophys reports 6:94–100.

29. Palomino DCT, Marti LC. 2015. Chemokines and immunity. Einstein (Sao Paulo) 13:469–473.

30. Kingsley MK, Bhat BV, Badhe BA, Dhas BB, Parija SC. 2020. Narciclasine improves outcome in sepsis among neonatal rats via inhibition of calprotectin and alleviating inflammatory responses. Sci Rep 10:2947.

31. Rello J, Valenzuela-Sánchez F, Ruiz-Rodriguez M, Moyano S. 2017. Sepsis: A Review of Advances in Management. Adv Ther2017/10/11. 34:2393–2411.

32. Li X, Zhu L, Wang B, Yuan M, Zhu R. 2017. Drugs and Targets in Fibrosis. Front Pharmacol 8:855.

33. Koh AY, Köhler JR, Coggshall KT, Van Rooijen N, Pier GB. 2008. Mucosal Damage and Neutropenia Are Required for Candida albicans Dissemination. PLOS Pathog 4:e35.

34. Hohl TM. 2014. Overview of vertebrate animal models of fungal infection. J Immunol Methods2014/04/04. 410:100–112.

35. Hall* JC, Heel* KA, Papadimitriou‡ JM, Platell* C. 1998. The pathobiology of peritonitis. Gastroenterology 114:185–196.

36. Bardal SK, Waechter JE, Martin DS. 2011. Chapter 2 - Pharmacokinetics, p. 17–34. *In* Bardal, SK, Waechter, JE, Martin, DSBT-AP (eds.), . Content Repository Only!, Philadelphia.

519 37. Korndörfer IP, Brueckner F, Skerra A. 2007. The Crystal Structure of the Human
520 (S100A8/S100A9)2 Heterotetramer, Calprotectin, Illustrates how Conformational
521 Changes of Interacting α-Helices Can Determine Specific Association of Two
522 EF-hand Proteins. J Mol Biol 370:887–898.

523 38. Vogl T, Gharibyan AL, Morozova-Roche LA. 2012. Pro-inflammatory S100A8
524 and S100A9 proteins: self-assembly into multifunctional native and amyloid
525 complexes. Int J Mol Sci2012/03/05. 13:2893–2917.

526 39. Shouval DS, Ouahed J, Biswas A, Goettel JA, Horwitz BH, Klein C, Muise AM,
527 Snapper SB. 2014. Interleukin 10 receptor signaling: master regulator of
528 intestinal mucosal homeostasis in mice and humans. Adv Immunol 122:177–210.

529 40. Majer O, Bourgeois C, Zwolanek F, Lassnig C, Kerjaschki D, Mack M, Müller
530 M, Kuchler K. 2012. Type I interferons promote fatal immunopathology by
531 regulating inflammatory monocytes and neutrophils during Candida infections.
532 PLoS Pathog2012/07/26. 8:e1002811–e1002811.

533 41. Poutsiaka DD, Davidson LE, Kahn KL, Bates DW, Snydman DR, Hibberd PL.
534 2009. Risk factors for death after sepsis in patients immunosuppressed before the
535 onset of sepsis. Scand J Infect Dis 41:469–479.

536 42. Hobbs JAR, May R, Tanousis K, McNeill E, Mathies M, Gebhardt C, Henderson
537 R, Robinson MJ, Hogg N. 2003. Myeloid cell function in MRP-14 (S100A9) null
538 mice. Mol Cell Biol 23:2564–2576.

539 43. Uwamahoro N, Verma-Gaur J, Shen H-H, Qu Y, Lewis R, Lu J, Bambery K,
540 Masters SL, Vince JE, Naderer T, Traven A. 2014. The Pathogen <span
541 class="named-content genus-species" id="named-content-
542 1">Candida albicans Hijacks Pyroptosis for Escape from
543 Macrophages. MBio 5:e00003-14.

544 44. Lopes JP, Stylianou M, Backman E, Holmberg S, Jass J, Claesson R, Urban CF.
545 2018. Evasion of Immune Surveillance in Low Oxygen Environments Enhances
546 Candida albicans Virulence. MBio 9:e02120-18.

547 45. Parasuraman S, Raveendran R, Kesavan R. 2010. Blood sample collection in
548 small laboratory animals. J Pharmacol Pharmacother 1:87–93.

549 46. Röhm M, Grimm MJ, D'Auria AC, Almyroudis NG, Segal BH, Urban CF. 2014.
550 NADPH oxidase promotes neutrophil extracellular trap formation in pulmonary
551 aspergillosis. Infect Immun2014/02/18. 82:1766–1777.

552 47. Bieghs V, Vlassaks E, Custers A, Van Gorp PJ, Gijbels MJ, Bast A, Bekers O,
553 Zimmermann LJI, Lütjohann D, Voncken J-W, Gavilanes AWD, Kramer BW,
554 Shiri-Sverdlov R. 2010. Chorioamnionitis Induced Hepatic Inflammation and
555 Disturbed Lipid Metabolism in Fetal Sheep. Pediatr Res 68:466–472.

556 48. Schwarz H, Schmittner M, Duschl A, Horejs-Hoeck J. 2014. Residual endotoxin
557 contaminations in recombinant proteins are sufficient to activate human CD1c+
558 dendritic cells. PLoS One 9:e113840–e113840.

559

Figure Legends

Figure 1. Intraperitoneal fungal peritonitis mouse model depicting the necessity of calprotectin in inflammation and fungal clearance. A) Fungal eye exudate (pus) as evidence for peritoneal cavity failure to contain *C. albicans* infection after 24 hours intraperitoneal (IP) infection in a calprotectin inhibited mouse (more details in figure 5). Shown is the clear eye of uninfected (PBS) compared to the white pus and loss of vision in infected wild type (WT) mice. **B)** Representative photomicrographs of hematoxylin-eosin (H&E) stained liver sections of uninfected and infected wildtype and s100A9 mutant used in quantifications in **C**. Zoomed images show one of many observed increased inflammatory cell infiltrates in WT infected cells (top panel) and arrows indicate yeast or hyphal cells (bottom panel) at 20x magnification. CV: central vein, PV: portal vein. **C)** An inflammatory score of H&E-stained liver sections of uninfected and infected WT and *S100A9*$^{-/-}$ mutant mice (hence, also S100A8 protein-deficient). A score of 2 = moderate cell infiltration, >3 = large number of infiltrates, 4 = full tissue inflammatory infiltration. Error bars indicate standard deviation and ****p< 0.0001

Figure 2. Calprotectin is required for liver inflammatory induced collateral tissue damage (CTD) and fungal clearance. A) *Alanine transferase levels (ALTs) used as an indicator of liver tissue damage as a result of C. albicans infection***.** Plasma ALT levels were analyzed from 100µl of plasma in a vetscan rotor 24 h post-infection. WT and *S100A9*$^{-/-}$ mice were infected with 3 x 10^6 cells per g of mice. n = 7 (WT and *S100A9*$^{-/-}$), and n= 10 (WT and *S100A9*$^{-/-}$ + Ca) total mice. **** *p*-value =<0.0001. **B)** *Organ levels of colony-forming units (CFUs) as a measure of host fungal burden. C. albicans* CFUs in homogenized infected livers of indicated mice were quantified. **A-B)** Box- and whisker plots show the smallest observation, lower quartile, mean, upper quartile and largest observation. **** *p*-value =<0.0001. **C)** *C. albicans infection induces the production of less pro-inflammatory cytokines in S100A9*$^{-/-}$ *mice.* WT and *S100A9*$^{-/-}$ bone marrow-derived macrophages (BMDMs) were infected with *C. albicans* (1 *C. albicans*: 1 macrophage). *TNFα* levels were measured from supernatants at 24-hour post-infection. ** *p*-value =<0.01, n = 3.

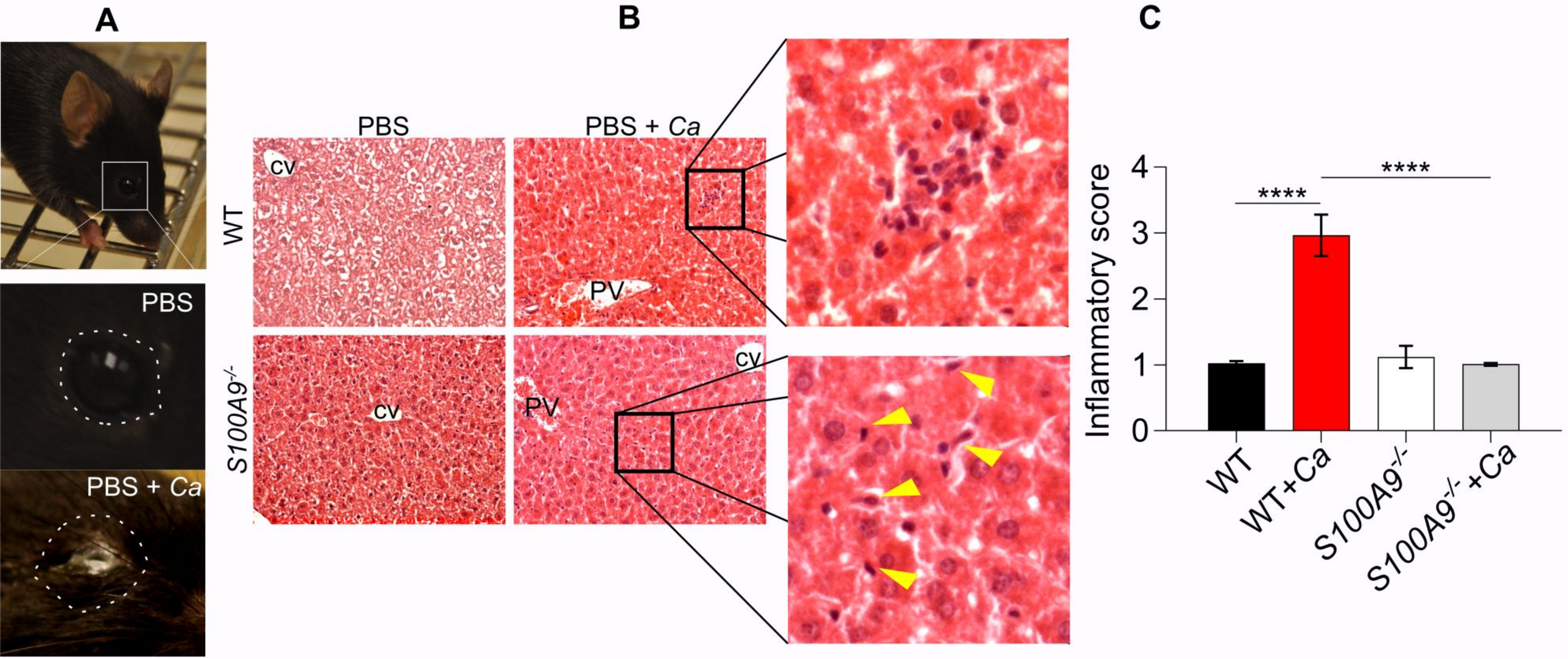
Figure 3. The calprotectin *S100A8* monomer is required to induce pro-inflammatory cytokines and liver tissue damage. A) *The purity of murine recombinant S100A8 and S100A9 proteins expressed in E. coli used in* **B - I**. Shown is a representative blot of each fractionated protein using size exclusion chromatography, confirmed by mass spectrometry. **B).** *Effect of calprotectin monomers on C. albicans induced collateral tissue damage.* S100A8 (r S100A8) and S100A9 (r S100A9) murine recombinant soluble proteins were intraperitoneally administered (100µg/ml) to WT and *S100A9*$^{-/-}$ mice and shown are plasma ALT levels. **C)** *Effect of calprotectin on host*

599 *fungal clearance.* Shown are CFUs in homogenized infected livers of indicated mice **B-**
600 **C**) Data are presented as a box- and whisker plots showing the smallest observation,
601 lower quartile, mean, upper quartile and largest observation, statistical significance was
602 analyzed by one-way Anova (n = 5 mice per group in 3 separate experiments), **** *p*-
603 value =<0.0001. (**D-I**) *Recombinant calprotectin can restore WT levels of pro-*
604 *inflammatory cytokines in S100A9$^{-/-}$ mice. In vitro* cytokine levels in bone marrow-
605 derived macrophages (BMDMs) infected with *C. albicans* (1 *C. albicans*: 1
606 macrophage) were measured from supernatants, 24-hour post-infection. a Pro-Mouse
607 cytokine BioPlex® 200 multiplex array (Bio-Rad, Hercules, CA) was used to detect and
608 quantify *S100A9$^{-/-}$* mouse cytokines infected with *C. albicans* and treated with r
609 S100A8. n=3, **p*-value =<0.05 ****p*-value =<0.0001.
610
611 **Figure 4. Paquinimod reverses the tissue-damaging effects of *S100A8*. A-C)** *Effect*
612 *of the anti-inflammatory drug paquinimod (Paq) on anti/pro-inflammatory cytokines in*
613 *C. albicans infected BMDMs.* **A)** BMDM infected with *C. albicans* (1 *C. albicans*: 1
614 macrophage) were treated with indicated concentrations of paquinimod. **B-C)** BMDM
615 were infected and treated with Paq (930µg/ml). Cytokines were measured from
616 supernatants 24 h post-infection. n=3. **D-F)** *In vivo effects of Paq on rS100A8 activity*
617 *against fungal burden and tissue damage.* **D-E)** Shown are liver CFUs of *C. albicans*
618 from infected, and then Paq (30mg/kg) treated WT and *S100A9$^{-/-}$* mice. Data are mean ±
619 SD from n =3. **E)** S100A8 murine recombinant soluble proteins were intraperitoneally
620 administered (100µg/ml) to *C. albicans* infected WT and *S100A9$^{-/-}$* mice and treated
621 with Paq. **F).** *Effect of Paq on recombinant calprotectin induced collateral tissue*
622 *damage during C. albicans infection.* Note that **F** contains WT and *S100A9$^{-/-}$* controls

623 initially presented in figure **3B** as experiments were conducted together. Data are
624 presented as a box- and whisker plots showing the smallest observation, lower quartile,
625 mean, upper quartile and largest observation, statistical significance was analyzed by
626 student t-test n = 5 mice per group, **p*-value =<0.05, **** *p*-value =<0.0001.
627

628 **Figure 5. Inhibition of calprotectin by paquinimod significantly increases host**
629 **survival from *C. albicans* infection A-D)** WT mice infected with *C. albicans* were
630 treated with Paq (30mg/kg) at 24-hour intervals up to 5 days, and mice weight and
631 survival were monitored. **A)** Paq mice treatment strategy. **B-C)** Survival and sequential
632 body weight of WT mice infected at the indicated timeline in **A**. **B)**. *Paq treated*
633 *infected mice survived 12 hours longer than untreated mice after 24 hours of C.*
634 *albicans infection*. **C**) *Paq does not affect mice weight loss during C. albicans infection.*
635 Shown are the grams lost over time. **p-value <0.01. Shown are mean and SD. **D)**
636 p*aquinimod-treated infected WT mice showing decreased eye fungal clearance.* Shown
637 are the pictures of mice eyes. Healthy mice are black, open and clear of debris.
638 Unhealthy mice have eye exudates/pus, squinty or shut due to *C. albicans* infiltration of
639 the eyes.
640
641 **Figure 6. Study summary.** Using a systemic fungal peritonitis mouse model **(1)**,
642 findings implicate calprotectin as a systemically essential protein **(2-6, i-vi))** for the
643 control of cytokine and chemokine modulation **(4-5)**, organ leukocytes recruitment **(6)**,
644 host tissue damage **(7)** and fungal clearance **(vi)**. In systemic intra-abdominal
645 originating candidiasis, the cost of fungal clearance (antimicrobial/active calprotectin
646 signaling) a feedback-loop that increases tissue damage, while the cost of decreased
647 tissue damage (anti-inflammatory/ inactive calprotectin signaling) is a high fungal
648 burden (Figures 1-5). Both are lethal if not controlled (Figure 5b). Future studies should
649 focus on fine-tuning recombinant therapy in cases where active calprotectin signaling is
650 inadequate, and anti-inflammatory treatments like paquinimod, in conjunction with
651 antifungal treatments to increase host survival time to aid treatment.

Supplementary figure 1. A) Paquinimod at tested concentrations was not cytotoxic. Bone marrow-derived macrophages (BMDMs) were treated with indicated concentrations of paquinimod (Paq), stained with propidium iodide (pI) and analyzed using FACs live cell analysis. Shown are representative histograms and percent cell death due to paquinimod.


A
PBS
PBS + Ca
B
PBS
PBS + Ca
WT
S100A9-/-
cv
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0
1
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WT
WT+Ca
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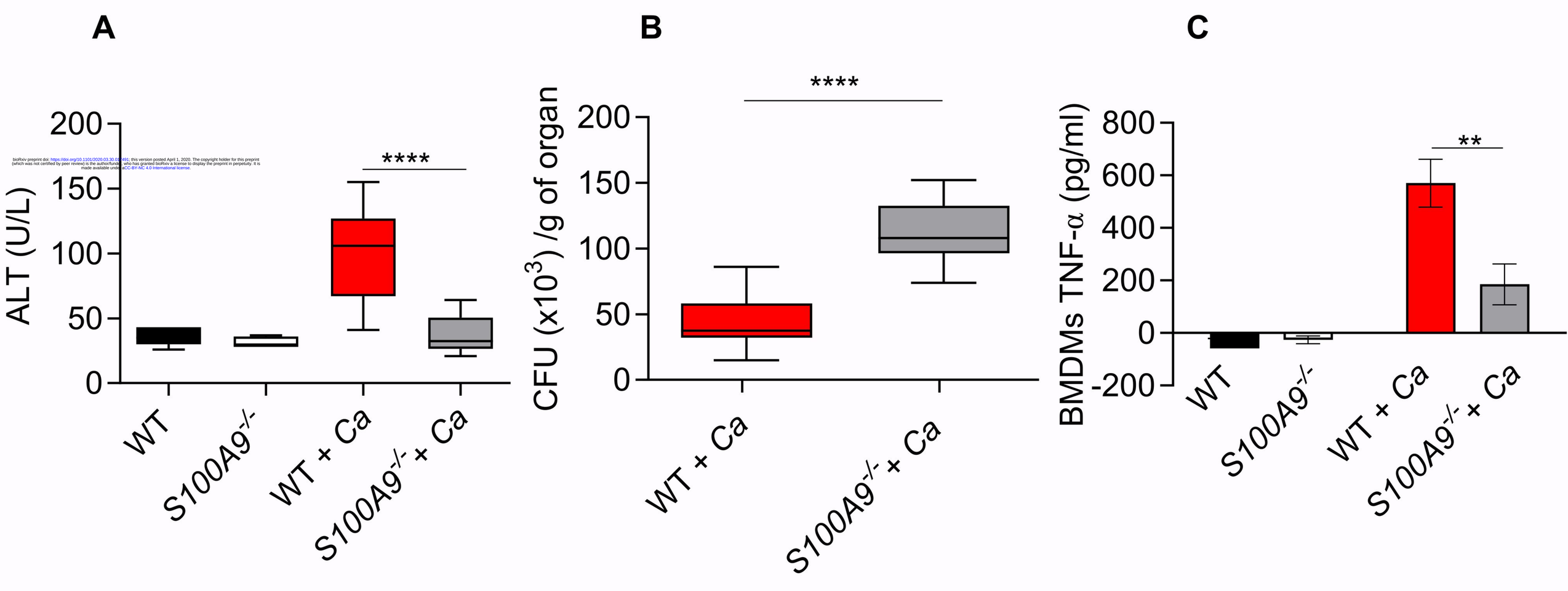
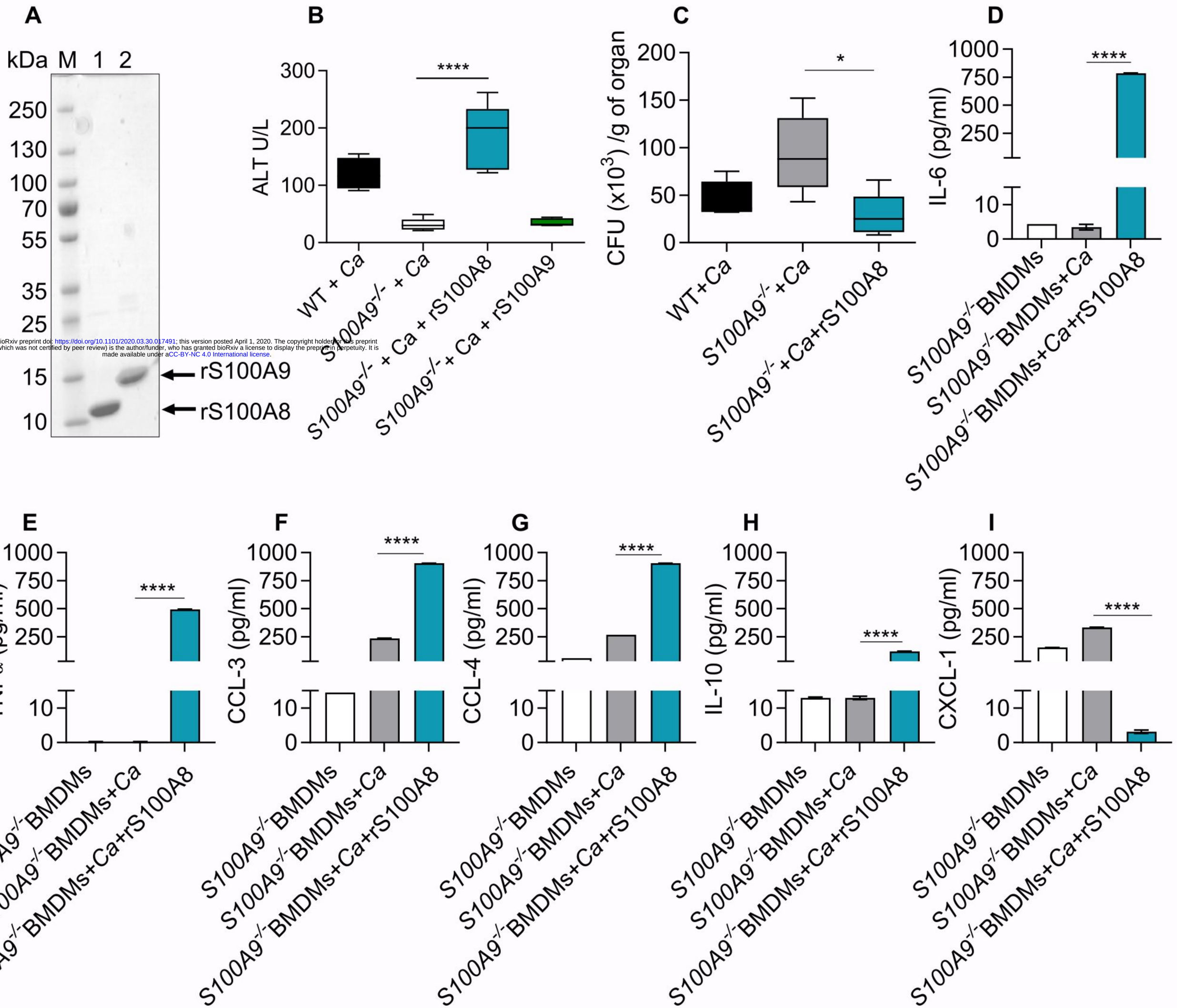
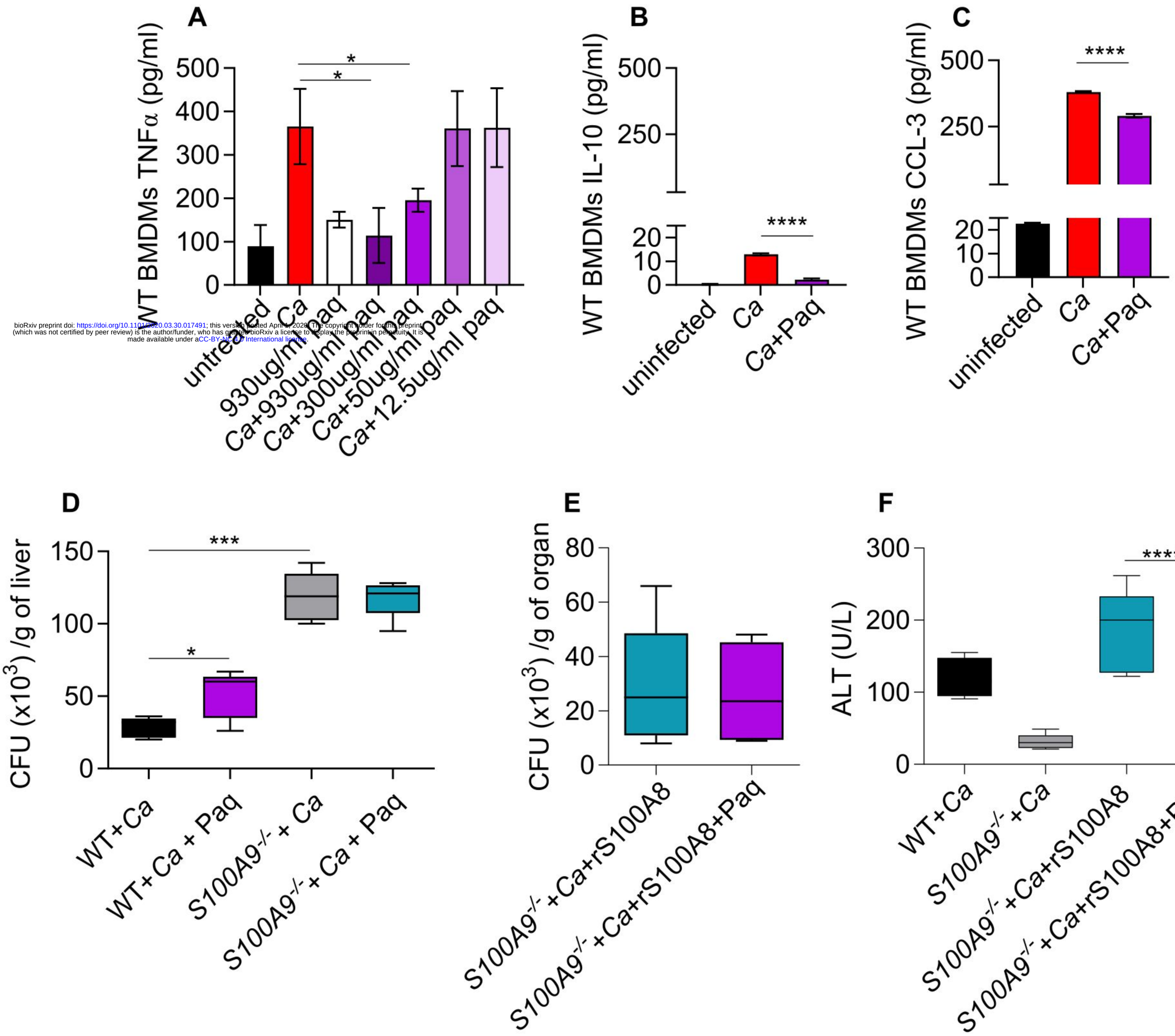
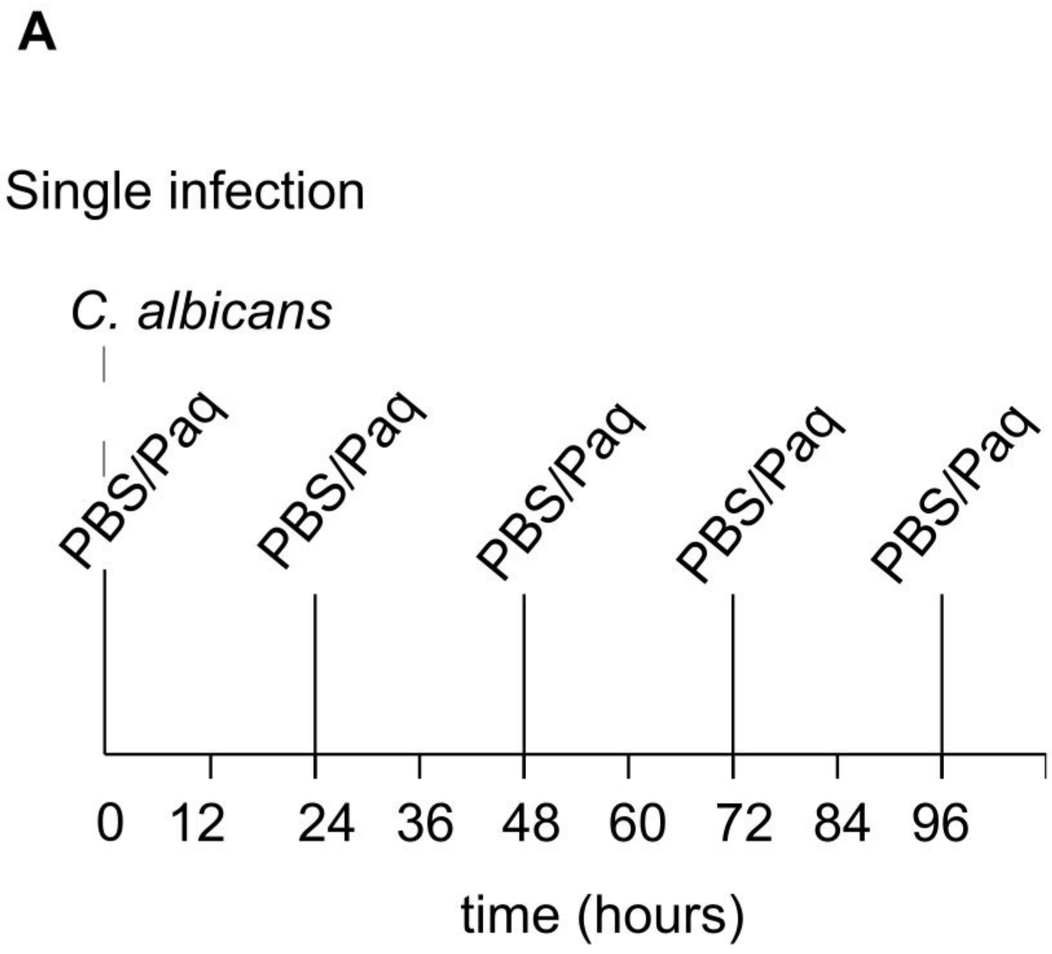
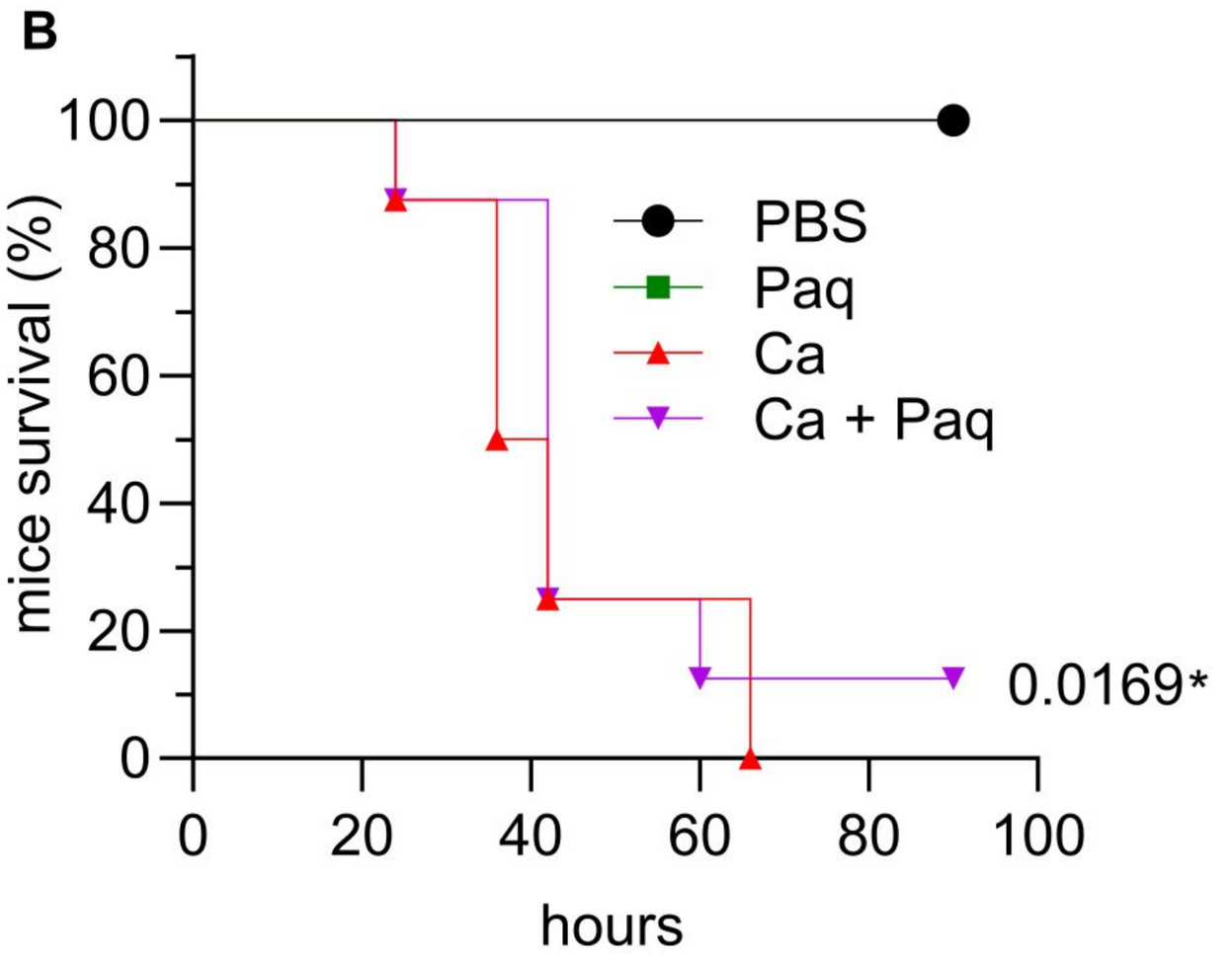
S100A9-/- +Ca

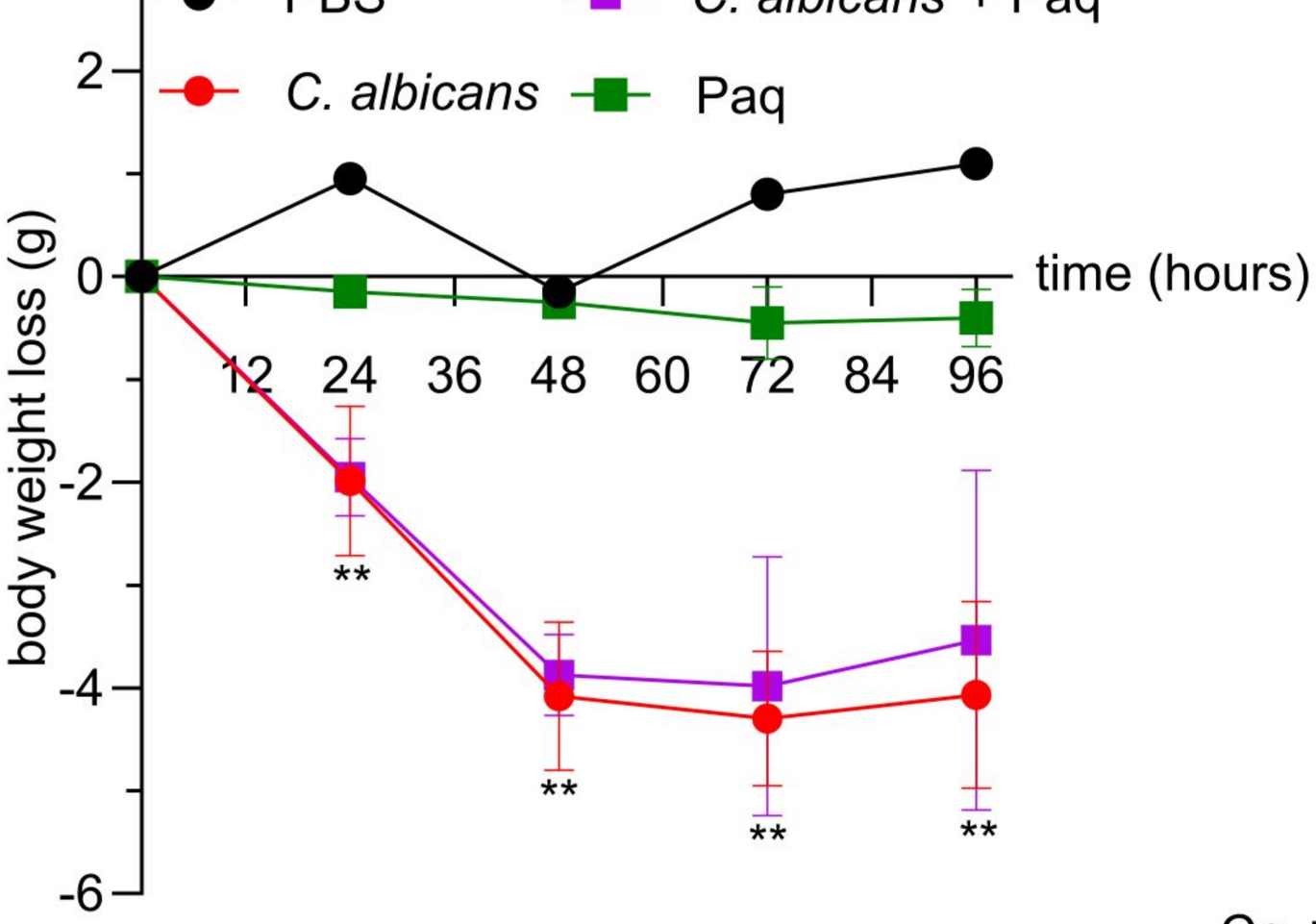


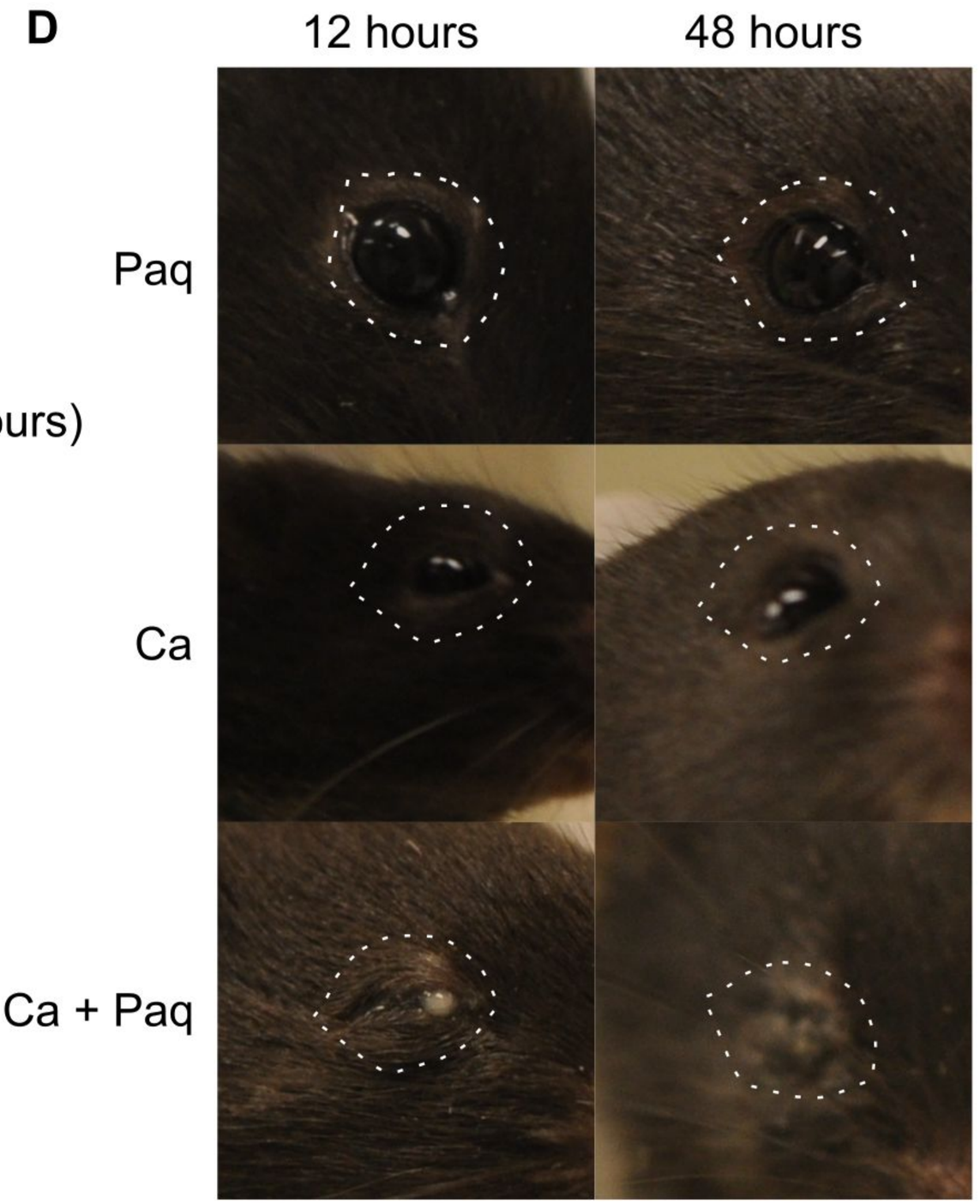












C. albicans

1
IP systemic
peritonitis infection model

host
circulation

fungal
infected
tissue

2

necrotic cell

3
release of calprotectin &
pro/inflammatory factors

infiltrating leucocytes
(increase inflammatory score)

6
recruitment of
peripheral leukocytes

5
'cytokine
storm'

CCL3 CXCL1 CXCL2 TNFα *IL6* ALTs ALTs

Active calprotectin signaling

7
ICTD

4
Increased levels of
pro-inflammatory
factors

TLR4 ALTs ALTs ALTs

ii)

iii)
inhibition of
calprotectn activity

TLR4 X

i)

Calprotectin inhibitor

iv)
basal cytokine &
chemokine levels

Inactive calprotectin signaling

high fungal burden

vi)

v)
low infiltrating leucocytes
(basal inflammatory score)

ALTs Alanine transferases (ALTs)

Toll-like receptor 4

monocytes

PMNs

necrotic hepatocytes with
Inflammatory collateral tissue damage (ICTD)

-*S100A8*
-*S100A9 calprotectin*

paquinimod