Inflammasome-mediated GSDMD activation facilitates escape of Candida albicans from macrophages

Candida albicans is the most common cause of fungal sepsis. Inhibition of inflammasome activity confers resistance to polymicrobial and LPS-induced sepsis; however, inflammasome signaling appears to protect against C. albicans infection, so inflammasome inhibitors are not clinically useful for candidiasis. Here we show disruption of GSDMD, a known inflammasome target and key pyroptotic cell death mediator, paradoxically alleviates candidiasis, improving outcomes and survival of Candida-infected mice. Mechanistically, C. albicans hijacked the canonical inflammasome-GSDMD axis-mediated pyroptosis to promote their escape from macrophages, deploying hyphae and candidalysin, a pore-forming toxin expressed by hyphae. GSDMD inhibition alleviated candidiasis by preventing C. albicans escape from macrophages while maintaining inflammasome-dependent but GSDMD-independent IL-1β production for anti-fungal host defenses. This study demonstrates key functions for GSDMD in Candida’s escape from host immunity in vitro and in vivo and suggests that GSDMD may be a potential therapeutic target in C. albicans-induced sepsis.

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**Candida albicans** is the most common opportunistic fungal pathogen, and it can cause life-threatening disseminated candidiasis and sepsis in immunocompromised patients. Despite the availability of antifungal agents, there are an estimated 400,000 *Candida* bloodstream infections each year worldwide, with a mortality rate of up to 50%. Sepsis, including fungal sepsis, is generally defined as life-threatening organ dysfunction caused by dysregulated systemic host inflammatory responses to microbial infection. Sepsis arises when an overwhelming response to infection leads to tissue and organ injury. Sepsis is a major health threat and the primary cause of morbidity and mortality in critically ill patients with infection or trauma, and it is characterized by both inflammatory and immunosuppressive responses. Early during the stages of sepsis, microbes or microbe-derived molecules trigger the production and release of pro-inflammatory mediators such as TNF-α, ICAM-1, IL-6, and IL-1, also known as a "cytokine storm". Overwhelming and sustained release of pro-inflammatory mediators exaggerates systemic inflammation and leads to vascular injury and multiple organ damage in severe cases (septic shock). Patients surviving this phase become increasingly immunosuppressed and may die from secondary infections despite the use of appropriate antibiotics. Sepsis-related deaths can therefore occur at either pathophysiological stage.

While pro-inflammatory cytokines are a necessary component of the host response to invading pathogens, excessive pro-inflammatory cytokine production can cause exaggerated and deleterious tissue inflammation in sepsis. IL-1β is a prototypic pro-inflammatory cytokine and key initiator of the host inflammatory response, which itself can trigger systemic inflammation and consequence tissue and organ damage. IL-1β mediates a wide repertoire of expression programs involved in secondary inflammation, on the one hand activating monocytes, macrophages, and neutrophils and on the other inducing adaptive Th1 and Th17 cellular responses.

Caspase-1-mediated canonical and caspase-11-mediated non-canonical inflammasomes directly or indirectly control pro-IL-1β cleavage and consequent production and secretion of mature IL-1β. Mice lacking key inflammasome components such as NLRP3 or caspase-1/11 are hypersusceptible to *C. albicans*-induced sepsis. In agreement with these studies, in a mouse model of systemic candidiasis, *Casp1/11−/−* mice (Fig. S1a) were more susceptible to *C. albicans* infection, displaying higher death rates (Fig. S1b), worse clinical scores (Fig. S1c), more severe organ damage (Fig. S1d), and increased fungal burden (Fig. S1e, f) compared to wild-type (WT) mice.

Infection with *C. albicans* triggered robust cleavage of full-length GSDMD and generated the active N-terminal fragment in mouse bone marrow-derived macrophages (BMDMs), indicating direct activation of GSDMD by *C. albicans* (Fig. 1a). GSDMD cleavage occurred as early as 4 h after the infection and continued to increase thereafter (Fig. S1g). We next investigated the host response to intravenously injected *C. albicans* in Gsdmd-deficient mice. Surprisingly, as a well-known downstream target of caspase-1/11, GSDMD disruption led to the completely opposite phenotype to *Casp1/11* disruption. Gsdmd deletion, without affecting the cleavage and activation of caspase 1 (Fig. 1a), conferred partial protection from *C. albicans*-induced sepsis and disease, as shown by increased survival (Fig. 1b), alleviation of infection-induced body weight loss (Fig. 1c), and improved clinical outcomes (Fig. 1d) in Gsdmd−/− mice. WT mice infected with *C. albicans* also developed larger kidneys than Gsdmd−/− mice (Fig. 1e–g), with histopathological examination revealing large areas of necrosis and hemorrhage in *C. albicans*-infected WT kidneys (Fig. 1h). By contrast, kidneys from *C. albicans*-infected Gsdmd−/− mice showed significantly less necrosis and less severe tissue damage (Fig. 1h). Mice with hematogenous disseminated candidiasis die from progressive sepsis, and kidney fungal burden is correlated with the severity of renal failure and systemic acidosis, hallmarks of severe sepsis. We therefore investigated the fungal loads in several organs, including the kidneys. As expected, *C. albicans* organisms were mainly lodged in the kidneys. In line with resistance to disseminated candidiasis, Gsdmd−/− mice displayed a ten-fold decrease in kidney fungal burden as calculated by the total number of colony-forming units (CFUs) in kidneys on day 2 compared to WT mice (Fig. 1i). Thus, the alleviated *C. albicans* infection in Gsdmd−/− mice appears to be at least partially due to better clearance of *C. albicans* by the host defense system. Consistently, methenamine silver staining...
revealed large numbers of fungal hyphae growing in necrotic areas in the collecting ducts of kidneys from C. albicans-infected WT mice, while the number of hyphae in the kidneys was markedly decreased in Gsdmd-/- mice (Fig. 1j). Collectively, these results demonstrate that GSDMD plays a key role in C. albicans infection and may represent a potential therapeutic target for C. albicans-induced sepsis.

Casp1/11 disruption completely abolishes, while Gsdmd disruption only reduces, IL-1β production. We next explored the mechanism responsible for the discrepant outcomes of candidiasis in Casp1/11- and Gsdmd-deficient mice. IL-1β plays an important role in anti-C. albicans host defenses. The impaired host response to invading C. albicans in Casp1/11-/- mice was thought to be due to their inability to produce IL-1β38–46. Due to the critical roles of
Gsdmd facilitates *C. albicans* escape from macrophages. *Gsdmd* disruption not only failed to impair *C. albicans* clearance but also promoted host defenses against *C. albicans* infection (Fig. 1). We next explored the mechanism by which *Gsdmd* disruption alleviated *C. albicans* infection. *Gsdmd* is a key mediator of pyroptosis, a form of lytic cell death. *C. albicans*-induced macrophage lytic cell death is an important cellular event for both the fungal pathogen and the host. *C. albicans* uses this mechanism to escape from macrophages and disseminate,[45,46] while macrophages use this mechanism to secrete IL-1β to mount innate and adaptive anti-fungal immune responses.[38,39] In an in vitro phagocytosis assay, unicellular yeast-form *C. albicans* were efficiently engulfed by both the WT and *Gsdmd*Δ/Δ BMDMs (Fig. 3a). The number of yeast particles engulfed by 100 BMDMs (phagocytosis index), the percentage of BMDMs engulfing at least one yeast particle (phagocytosis index), and the percentage of *C. albicans* engulfed by BMDMs were quantified, and there were no differences between WT and *Gsdmd*Δ/Δ BMDMs (Fig. 3b). To maximize the efficiency of phagocytosis,[56–60] and most importantly to mimic the serum-containing physiological environment, we used serum-opsonised *C. albicans* in this study (Fig. S3a, b). Under this condition, the phagocytosis of *C. albicans* by BMDMs was extremely efficient, with most of the opsonized *C. albicans* engulfed within 1 h (Fig. 3b).

To further investigate *C. albicans*-macrophage interactions, we employed time-lapse live imaging to observe the behavior of *C. albicans*-infected BMDMs (Fig. 3c and Supplementary Movie 1). Engulfed *C. albicans* quickly grew hyphae inside cells, breaking the plasma membrane to escape from BMDMs. The average number of hyphae escaping from *Gsdmd*Δ/Δ BMDMs was significantly lower than that from WT BMDMs (Fig. 3d). We used propidium iodide (PI), a membrane-impermeable dye, to detect the loss of plasma membrane integrity caused by lytic cell death. The percentage of PI+ cells increased gradually after *C. albicans* infection, with *Gsdmd*Δ/Δ BMDMs displaying significantly reduced levels at each time point after 3 h (Fig. 3e).

By assessing macrophage lytic death through lactate dehydrogenase (LDH) release, *C. albicans* infection induced robust LDH release from WT BMDMs over time. LDH release was suppressed in *Gsdmd*Δ/Δ BMDMs, with a 50% reduction detected 12 h after *C. albicans* infection, again demonstrating that *C. albicans*-induced macrophage death was partially blocked in *Gsdmd*-deficient BMDMs (Fig. 3f).
Finally, we measured the effect of macrophages on the overall growth and survival of \textit{C. albicans}. First, GFP-expressing \textit{C. albicans} were grown in the presence or absence of macrophages, and the \textit{C. albicans} mass was calculated from the fluorescence intensity of each culture. Compared to macrophage-free cultures, \textit{C. albicans} mass was reduced by 25% in the presence of WT macrophages but by >50% in the presence of \textit{Gsdmd}^{-/-} macrophages, indicating that \textit{Gsdmd} disruption elevated the \textit{C. albicans} clearing capability of macrophages (Fig. 3g). Similar results were obtained when \textit{C. albicans} growth and survival were assessed by measuring the number of colony-forming units (CFUs) in culture (Fig. 3h). In summary, \textit{Gsdmd} disruption promoted macrophage survival and attenuated the escape of \textit{C. albicans} from macrophages, thereby enhancing the \textit{C. albicans} clearing capability of macrophages.

Of note, \textit{Gsdmd}^{-/-} macrophages still displayed \textit{C. albicans}-induced lytic death (Fig. 3d–f). The fact that significant macrophage death still occurred in the absence of GSDMD suggests that \textit{C. albicans}-induced macrophage death could be mediated by both GSDMD-dependent pyroptosis and GSDMD-independent mechanisms. Thus, significant amounts of IL-1β can still be released in \textit{Gsdmd}^{-/-} mice (Fig. 2) via
Fig. 2 Gsdmd disruption reduces, while Casp-1/11 disruption completely abolishes, IL-1β secretion. a C. albicans-triggered GSDMD cleavage and IL-1β production in Casp-1/11−/− mice. BMDMs from WT and Casp-1/11−/− mice were infected with C. albicans for 6 h at MOI 50. GSDMD (cleaved p30 and full-length p53), pro-IL-1β, and mature IL-1β (m-IL-1β) in the cell lysates and m-IL-1β in the supernatants were assessed by western blotting. Results are representative of data from at least three biological replicates. The intensities of protein bands were quantified using ImageJ. Comparison was made by two-way ANOVA followed by Sidak’ multiple comparisons test. Data shown are means ± SD (n = 3 biologically independent samples). p = 0.003 (cell lysate-m-IL-1β), p < 0.0001 (supernatant-m-IL-1β) vs. WT. Source data are provided as a Source Data file. b IL-1β secretion by BMDMs from WT and Casp-1/11−/− mice. BMDMs were infected with C. albicans (MOI = 10) for the indicated time periods. IL-1β in culture supernatants was measured by ELISA. Comparison was made by two-way ANOVA followed by Sidak’ multiple comparisons test. Data shown are means ± SD (n = 3 mice per group). p < 0.0001 (12 h, 16 h) vs. WT. c C. albicans-triggered IL-1β production in WT and Gsdmd−/− mice assessed by western blotting. Comparison was made by two-way ANOVA followed by Sidak’ multiple comparisons test. Data shown are means ± SD (n = 3 mice per group). p < 0.0001 vs. WT. Source data are provided as a Source Data file. d IL-1β secretion by BMDMs from WT and Gsdmd−/− mice measured by ELISA. Comparison was made by two-way ANOVA followed by Sidak’ multiple comparisons test. Data shown are means ± SD (n = 3 mice per group). p = 0.0003 (4 h), p < 0.0001 (8 h, 12 h, 16 h) vs. WT. e Gsdmd disruption did not alter the total amount of m-IL-1β produced by C. albicans-challenged BMDMs. BMDMs from WT and Gsdmd−/− mice were infected with C. albicans for 12 h at the indicated MOIs. The amounts of m-IL-1β in culture supernatants and cell lysates (intracellular) were measured by ELISA. Comparison was made by two-way ANOVA followed by Sidak’ multiple comparisons test. Data shown are means ± SD (n = 3 mice per group). p < 0.0001 vs. WT. f C. albicans-induced IL-1β production in Casp-1/11−/− mice. WT and Casp-1/11−/− mice were intravenously challenged with 1 × 10^6 CFU C. albicans, and the level of IL-1β in the serum was assessed at the indicated time points. Two-tailed unpaired Student’s t test was used for statistical comparisons. Data shown are means ± SD (n = 4 mice per group). p = 0.0022 (8 h), p < 0.0001 (16 h), p = 0.0029 (24 h) vs. WT. g C. albicans-induced IL-1β production in Gsdmd−/− mice. WT and Gsdmd−/− mice were intravenously challenged with 1 × 10^6 CFU C. albicans. Two-tailed unpaired Student’s t test was used for statistical comparisons. Data shown are means ± SD (n = 4 mice per group). p = 0.1542 (8 h), p = 0.0881 (16 h), p = 0.0003 (24 h) vs. WT. h Gsdmd−/− mice were infected with C. albicans or anakinra-treated WT and Gsdmd−/− mice. Mice were sacrificed 2 days after intravenous injection of 1 × 10^6 CFU C. albicans. Two-tailed unpaired Student’s t test was used for statistical comparisons. Data presented are the means ± SD (n = 7 for Control, n = 5 for Anakirna-treated mice) of three independent experiments. p = 0.0014 (kidney without anakinra), p = 0.8061 (kidney with anakinra) vs. WT. i Clinical score of C. albicans-challenged untreated or anakinra-treated WT and Gsdmd−/− mice. Two-tailed unpaired Student’s t test was used for statistical comparisons. Data presented are the means ± SD (n = 7 for Control, n = 10 for Anakirna-treated mice) of three independent experiments. p = 0.0009 (12 h without anakinra), p = 0.0551 (12 h with anakinra), p = 0.0001 (24 h without anakinra), p = 0.5316 (24 h with anakinra) vs. WT. j Kaplan-Meier survival plots of C. albicans-challenged untreated or anakinra-treated WT and Gsdmd−/− mice. Mice were intravenously challenged with 2 × 10^5 CFU C. albicans and monitored for 15 days. Log-rank analysis was used. p = 0.0031 (without anakinra), p = 0.4337 (with anakinra) vs. WT.

GSDMD-independent macrophage lytic death to maintain host responses to C. albicans, thereby alleviating infection in these mice. Candida infection also directly triggered TNFa and IL-6 production in macrophages. However, WT and Gsdmd−/− macrophages generated the same amount of TNFa and IL-6 after Candida infection (Fig. S3c), supporting the notion that the expression and secretion of these cytokines were GSDMD-independent. Thus, the elevated anti-fungal response observed in the GSDMD-deficiency mice was unlikely mediated by overall upregulation of pro-inflammatory cytokine production.

Hyphae formation is essential for C. albicans-induced GSDMD cleavage and macrophage death, facilitating C. albicans escape from macrophages. GSDMD-mediated pyroptosis facilitates C. albicans escape from macrophages (Fig. 3). We next explored the mechanism triggering GSDMD cleavage and activation. It has been reported that C. albicans must switch from a unicellular yeast form into a filamentous hyphal form for infringement of macrophage activation and macrophage lytic cell death.44,45 Thus, using a yeast-locked strain (cph1ΔΔ efg1ΔΔ) defective in filamentous growth and unable to accomplish yeast-to-hyphae or -pseudo-hyphae transition in response to stimuli such as serum or macrophages61, we investigated the role of hyphae in C. albicans-induced GSDMD cleavage and IL-1β processing by western blotting. Yeast-locked C. albicans failed to efficiently trigger GSDMD cleavage and IL-1β production by BMDMs (Fig. 4a, b). Consistent with previous findings, pro-IL-1β processing was significantly suppressed in mutant C. albicans-challenged BMDMs, suggesting that hyphae formation was critical for infringement of macrophage activation and consequent pro-IL-1β processing (Fig. 4a, b). We therefore measured secreted IL-1β levels in the supernatant by ELISA (Fig. 4c). WT C. albicans induced robust IL-1β release from BMDMs, which was mostly hyphae dependent, since IL-1β release was significantly blocked when macrophages were treated with yeast-locked C. albicans (Fig. 4c). Hyphae-dependent IL-1β secretion was suppressed by ~50% in Gsdmd−/− BMDMs, again indicating a critical role for GSDMD in this process (Fig. 4d). However, consistent with the data presented in Fig. 2 and 3, this result also suggested that C. albicans-induced IL-1β release might also be mediated by GSDMD-independent mechanisms, since Gsdmd−/− BMDMs could still generate a significant amount (~50%) of IL-1β (Fig. 4d). Time-lapse live imaging revealed that yeast-locked C. albicans were phagocyted as efficiently as WT C. albicans (Supplementary Movie 2, Fig. 4e). However, mutant C. albicans failed to significantly rupture macrophage membranes, as evidenced by the reduced number of PI+ macrophages (Fig. 4e, f). Similar to IL-1β release, C. albicans-induced LDH release was largely hyphae dependent (Fig. 4g) and partially mediated by GSDMD (Fig. 4h).

Finally, the overall growth and survival of C. albicans in the presence of macrophages was measured by CFU counting. Yeast-locked C. albicans displayed significantly reduced survival compared to WT C. albicans (Fig. 4i). Thus, hyphae formation is essential for C. albicans-induced GSDMD cleavage and macrophage death. Yeast-locked C. albicans were trapped in intact macrophages, preventing their escape (Fig. 4j).

GSDMD plays critical roles in both candidalysin-independent and -dependent macrophage death and C. albicans escape from macrophages. GSDMD cleavage and activation in macrophages is mediated by caspase-1/11. C. albicans-induced GSDMD cleavage was abolished in Casp1/11-deficient macrophages (Fig. 2a). Recent studies have revealed that a hyphae-associated fungal toxin, candidalysin, can trigger inflammation and caspase-1 activation in macrophages.64,65 Accordingly, we examined whether candidalysin is required for GSDMD-mediated macrophage death and C. albicans escape from macrophages during C. albicans infection. Treatment with candidalysin-deficient (ecel1ΔΔ) C. albicans still triggered GSDMD cleavage in BMDMs but at significantly lower efficiency.
(>50%) than treatment with WT C. albicans (Fig. 5a, b). Similarly, IL-1β release was significantly reduced when macrophages were treated with candidalysin-deficient C. albicans. However, candidalysin disruption in C. albicans did not completely abolish IL-1β release from BMDMs, indicating the presence of candidalysin-independent IL-1β production (Fig. 5c). IL-1β release induced by candidalysin-deficient C. albicans was significantly inhibited in Gsdmd−/− BMDMs, confirming the essential role of GSDMD in candidalysin-independent IL-1β release (Fig. 5c). Based on the amounts of IL-1β release triggered by WT and ece1ΔΔ C. albicans, we also calculated candidalysin-dependent IL-1β release. On one hand, this process was also suppressed in Gsdmd−/− BMDMs, demonstrating that GSDMD was important for both the candidalysin-independent and -dependent IL-1β release (Fig. 5d).
Fig. 3 GSDMD facilitates C. albicans escape from macrophages. a Gsdmd disruption did not affect phagocytosis of C. albicans by macrophages. WT and GsdmdΔ/Δ BMDMs were incubated with GFP-expressing C. albicans for 60 min at MOI 10. Shown are the representative images. Scale bars represent 10 µm. b Quantification of in vitro phagocytosis capacity of BMDMs. Phagocytosis efficiency was expressed as the percentage of BMDMs that engulfed at least one C. albicans. Phagocytosis index was expressed as the average number of internalized C. albicans per 100 cells. At least 200 cells were assessed for each sample. Data shown are means ± SD (n = 5 for WT 15 min, n = 6 for WT 60 min, n = 4 for GsdmdΔ/Δ biologically independent samples per group). c Escape of engulfed C. albicans from macrophages. WT and GsdmdΔ/Δ BMDMs were incubated with C. albicans at MOI 10. Dying cells were stained with propidium iodide (PI) (red) (200 ng/mL). Images were acquired every 5 min for 12 h using a 20× dry lens. Shown are representative images at the indicated time points. Scale bars represent 20 µm. Experiments were repeated three times. The related time-lapse video is included in the Supplemental Materials. d The number of escaped hyphae per macrophage. After 6 h, there were too many hyphae to be counted accurately. At least 200 cells were assessed for each sample. Data shown are means ± SD (n = 5 biologically independent samples per group). Comparisons were conducted with a two-tailed unpaired student’s t-test. p = 0.0101 (4 h), p = 0.0257 (6 h) vs. WT. e The percentage of PI-positive BMDMs calculated at each time point. At least 200 cells were assessed for each sample. Data shown are means ± SD (n = 5 biologically independent samples per group). Comparisons were conducted with a two-tailed unpaired student’s t-test. p = 0.0171 (9 h), p = 0.0306 (12 h) vs. WT. f C. albicans-induced BMDM death assessed by a lactate dehydrogenase (LDH) cytotoxicity assay. WT and GsdmdΔ/Δ BMDMs were incubated with C. albicans at MOI 10. Relative LDH release was expressed as the percentage LDH activity in supernatants of cultured cells (medium) compared with total LDH (from the medium and cells) and used as an index of cytotoxicity. All values represent mean ± SD of three independent experiments. Comparisons were conducted with a two-tailed unpaired student’s t-test. p = 0.0371 (4 h), p = 0.0070 (8 h), p = 0.0008 (12 h), p = 0.0014 (16 h) vs. WT. g Growth of GFP-expressing C. albicans (JJC2078-GFP) in the presence of WT and Gsdmd-deficient macrophages. WT and GsdmdΔ/Δ BMDMs were incubated with GFP-expressing C. albicans for 6 h at MOI 5. The relative C. albicans mass was measured as the percentage fluorescence intensity of macrophage-containing C. albicans cultures compared with C. albicans cultures in the absence of macrophages. All values represent mean ± SD of three independent experiments. Comparisons were conducted with a two-tailed unpaired student’s t-test. p = 0.0025 (16 h) vs. WT. h Growth and survival of C. albicans in the presence of WT and Gsdmd-deficient macrophages assessed by a colony formation assay. WT and GsdmdΔ/Δ BMDMs were incubated with C. albicans at MOI 2 for the indicated time periods. Relative C. albicans survival was measured as the percentage CFU in macrophage-containing C. albicans cultures compared with C. albicans cultures in the absence of macrophages. All values represent mean ± SD of three independent experiments. Comparisons were conducted with a two-tailed unpaired student’s t-test. p > 0.9999 (2 h), p = 0.0424 (4 h) vs. WT.

On the other hand, GSDMD disruption did not completely abolish either candidalysin-independent or -dependent IL-1β release, suggesting that both processes could also be mediated by GSDMD and pyroptosis-independent mechanisms.

Consistent with the attenuated IL-1β release observed in ece1AΔC. albicans-treated macrophages, time-lapse live imaging showed significantly lower numbers of escaped ece1AΔC. albicans compared to WT counterparts (Supplementary Movie 3, Fig. 6a, b). Most opsonized WT and mutant C. albicans cells were phagocytosed within 1 h, and both WT and ece1AΔC. albicans quickly grew hyphae inside cells. However, candidalysin-deficient C. albicans could not penetrate BMDM membranes as efficiently as WT C. albicans, as indicated by fewer PI-positive cells being detected in candidalysin-deficient C. albicans-treated BMDMs compared to WT C. albicans-treated BMDMs (Fig. 6c). Infection with candidalysin-deficient C. albicans also induced significantly less LDH release, again suggesting that candidalysin partially contributed to macrophage death (Fig. 6d). Similarly, C. albicans escape from macrophages and C. albicans-induced macrophage death were mediated by both candidalysin-independent and -dependent mechanisms and both were GSDMD-dependent (Fig. 6c, d).

Collectively, our results show that both candidalysin-independent and -dependent mechanisms could trigger GSDMD activation and mediate C. albicans escape from macrophages. As a pore-forming toxin, candidalysin may facilitate C. albicans-induced rupture of the plasma membrane and macrophage lytic death, which enable C. albicans to escape from host cells (Fig. 5a). Indeed, due to the impaired escape, candidalysin-deficient C. albicans grew longer hyphae (>50 µm) inside macrophages (Fig. 5b). In agreement with the in vitro results, in the mouse systemic candidiasis model, mice infected with candidalysin-deficient C. albicans displayed higher survival rates (Fig. 7a), better clinical scores (Fig. 7b), less severe kidney enlargement (Fig. 7c), less kidney damage (Fig. 7d), and decreased fungal burden (Fig. 7e, f) than mice infected with WT C. albicans. These effects were observed in both the WT and GSDMD-deficient mice (Fig. 5c–i), again suggesting that candidalysin also plays a role in GSDMD and pyroptosis-independent mechanisms.

The yeast to hyphae switch inside a macrophage can be easily observed in ex vivo Candida infection models. After being engulfed by a macrophage, C. albicans switches from yeast to hyphae, pierces the phagolysosome, and comes out of macrophage by killing it66,67. Although hyphae formation inside macrophages is considered an important factor for virulence, detecting this process in vivo in Candida-infected host is challenging. It was reported that, in a zebrafish model of C. albicans infection, formation of long hyphae inside macrophages is a relatively rare event68,69. To assess the role of candidalysin and GSDMD in controlling Candida escape in vivo, we developed an assay (Fig. S5a) in which macrophages from the kidney of Candida-infected mice were isolated and the internalized Candida cells were visualized based on their GFP expression or by staining with a fluorescent-labeled anti-Candida albicans antibody (Fig. S5b). Although GsdmdΔ/Δ mice displayed a significant decrease in kidney fungal burden as calculated by the total number of CFUs in kidneys (Fig. 1I), the percentage of kidney macrophages containing trapped Candida increased significantly compared to that in WT mice. Candidalysin-deficiency in C. albicans led to their augmented retention in macrophages (Fig. S5c–d). These results further confirm that GSDMD and candidalysin play critical roles in C. albicans escape from macrophages during Candida infection.

C. albicans-elicited GSDMD activation is mediated by K+ efflux-dependent canonical inflammasomes. GSDMD needs to be cleaved by proteases such as caspases and neutrophil elastase (ELANE) to induce lytic cell death and IL-1p secretion. C. albicans-induced GSDMD cleavage was attenuated in Casp1/11Δ/Δ compared to WT BMDMs (Fig. 2a). During C. albicans infection, Casp1/11Δ/Δ BMDMs consistently released less LDH (Fig. 8a) and showed reduced plasma membrane perturbation (Fig. 8b, c) at each time point examined, further indicating that the caspase-1/11-GSDMD axis plays a critical role in C. albicans-induced macrophage pyroptotic death. GSDMD can be cleaved by both
caspase-1-mediated canonical inflammasomes and caspase-11-mediated non-canonical inflammasomes. GSDMD cleavage was efficiently blocked by VX-765, a specific caspase-1/caspase-4 inhibitor (Fig. S6a), suggesting that C. albicans-induced GSDMD cleavage and activation were mainly mediated by canonical inflammasomes and caspase-1 (Fig. S6b). The serine protease inhibitor diisopropyl fluorophosphate (DFP) failed to inhibit C. albicans-induced GSDMD cleavage in BMDMs (Fig. S6b). As a result of caspase-1 inhibition, treatment with VX-765 specifically suppressed C. albicans-induced production of IL-1β, but not other cytokines such as IL-6, IL-10, MIP2, and TNFα (Fig. S6aC). Of note, C. albicans-induced macrophage death was only partially blocked in Casp1/11−/− BMDMs compared to untreated WT BMDMs (Fig. S6a–c and Supplementary Movie 4), suggesting...
Hyphae formation is critical for C. albicans-induced GSDMD cleavage and C. albicans escape from macrophages. a C. albicans-triggered GSDMD cleavage and IL-1β production. BMDMs from WT and GsdmdΔ/Δ mice were infected with WT or hyphae-deficient (cph1Δ/Δ, efg1Δ/Δ) C. albicans for 6 h at MOI 50. GSDMD (cleaved p30 and full-length p53), pro-IL-1β, and mature IL-1β (m-IL1-β) in the cell lysates and m-IL1-β in the supernatants were assessed by western blotting. Results are representative of data from at least three biological replicates. Source data are provided as a Source Data file. b The intensities of protein bands were measured using ImageJ. Data shown are means ± SD (n = 3 biologically independent samples). Comparisons were conducted with a two-tailed unpaired student’s t test. p = 0.0288 (m-IL1-β in lystate), p = 0.0757 (m-IL1-β in supernatant) vs. WT. c IL-1β secretion by BMDMs. BMDMs were infected (untreated BMDMs as controls) with WT or hyphae-deficient (cph1Δ/Δ, efg1Δ/Δ) C. albicans (MOI = 10) for 12 h. IL-1β in culture supernatants was measured by ELISA. Data shown are means ± SD (n = 3 biologically independent samples per group). Comparisons were conducted with a two-tailed unpaired student’s t test. p < 0.0001 vs. WT. d Hyphae-dependent IL-1β release calculated by subtracting the level of IL-1β release induced by hyphae-deficient (cph1Δ/Δ, efg1Δ/Δ) mutants from that induced by WT C. albicans. Data shown are means ± SD (n = 3 biologically independent samples per group). Comparisons were conducted with a two-tailed unpaired student’s t test. p < 0.0001 vs. WT. e Hyphae formation was critical for C. albicans escape from macrophages. BMDMs were incubated with WT or hyphae-deficient (cph1Δ/Δ, efg1Δ/Δ) C. albicans at MOI 10. Dying BMDMs were stained with PI (red). Images were acquired as described in Fig. 3c. Shown are representative images at the indicated time points. The related time-lapse video is included in the Supplemental Materials. f The percentage of PI-positive BMDMs calculated at each time point. At least 200 cells were assessed for each sample. Data shown are means ± SD (n = 3 biologically independent samples per group). Comparisons were conducted with a two-tailed unpaired student’s t test. p = 0.0199 (3 h), p = 0.0079 (6 h), p < 0.0001 (9 h), p = 0.0001 (12 h) vs. WT. g C. albicans-induced BMDM death assessed by the LDH assay. WT and GsdmdΔ/Δ BMDMs were incubated (untreated BMDMs as controls) with WT or hyphae-deficient (cph1Δ/Δ, efg1Δ/Δ) C. albicans at MOI 10 for 12 h. Relative LDH release was assessed as described in Fig. 3f. Data shown are means ± SD (n = 3 biologically independent samples per group). Comparisons were conducted with a two-tailed unpaired student’s t test. p = 0.0008 GsdmdΔ/Δ (C. albicans) vs. WT (C. albicans), p < 0.0001 (C. albicans) vs. WT (cph1Δ/Δ, efg1Δ/Δ). h Hyphae-dependent LDH release calculated by subtracting the level of LDH release induced by hyphae-deficient (cph1Δ/Δ, efg1Δ/Δ) mutants from that induced by WT C. albicans. Data shown are means ± SD (n = 3 biologically independent samples per group). Comparisons were conducted with a two-tailed unpaired student’s t test. p = 0.0025 vs. WT. i Growth and survival of C. albicans assessed by colony formation assays. BMDMs were incubated with WT or hyphae-deficient (cph1Δ/Δ, efg1Δ/Δ) C. albicans at MOI 10 for 6 h. Relative C. albicans survival was measured as described in Fig. 3h. Data shown are means ± SD (n = 6 for C. albicans, n = 7 for cph1Δ/Δ, efg1Δ/Δ). Comparisons were conducted with a two-tailed unpaired student’s t test. p < 0.0001 vs. WT. j A schematic diagram illustrating the role of hyphae in mediating macrophage death and facilitating C. albicans escape from macrophages.

The existence of inflammasome-independent death-promoting mechanisms.

Potassium (K+) efflux is a major trigger of canonical inflammasome activation. The decrease in intracellular K+, which is likely mediated by TWIK2 potassium efflux channel39, is an essential trigger for NLRP3 activation induced by ATP and other DAMPs21–23. K+ efflux has also been implicated in Candida-induced inflammasome activation and pyroptosis in macrophages74. KCl treatment reduces fungal burden, suggesting fungi can use pyroptosis to evade killing by macrophages74. Consistently, we also observed that inhibition of K+ efflux by increasing extracellular potassium concentration blocked caspase-1 activation (Fig. S7a, b) and suppressed the processing and release of IL-1β, but not TNFα, IL-10, or IL-6 (Fig. S7c). C. albicans-induced GSDMD cleavage was completely inhibited in KCl-treated BMDMs, again confirming that GSDMD cleavage is mediated by canonical inflammasome activation (Fig. 8d). C. albicans-induced macrophage death is dictated by both candidalysin-independent and -dependent mechanisms (Fig. 6). To investigate the relative contribution of K+ efflux to the two processes, we incubated untreated and KCl-treated BMDMs with WT, hyphae-deficient, and candidalysin-deficient C. albicans (Fig. 8e). C. albicans-triggered macrophage death, as assessed by LDH release, was significantly inhibited by blocking K+ efflux. Similar to as observed in Casp1/11Δ/Δ BMDMs, significant macrophage death was still detected in KCl-treated macrophages, again indicating that C. albicans-triggered macrophage death was likely mediated by both the inflammasome-dependent pyroptosis and inflammasome-independent mechanisms. Interestingly, on one hand, compared to WT C. albicans, hyphae-deficient and candidalysin-deficient C. albicans still induced less LDH release in KCl-treated macrophages, suggesting that inflammasome-independent macrophage death was also facilitated by candidalysin and hyphae formation (Fig. 8e). This may simply be mediated by the pore-forming activity of candidalysin and hyphae-driven physical peeling of the plasma membrane. On the other hand, KCl effectively inhibited macrophage death triggered by candidalysin-deficient C. albicans, suggesting that K+ efflux was critical for candidalysin-independent macrophage death (Fig. 8e). K+ efflux also appeared to be essential for candidalysin-dependent macrophage death, with 50% of candidalysin-dependent LDH release inhibited by KCl treatment (Fig. 8f). Finally, time-lapse imaging (Supplementary Movie 5 and Fig. 8g) showed that inhibition of K+ efflux did not affect engulfment of C. albicans by macrophages but reduced cell lytic death, preventing C. albicans escaping from macrophages by penetrating plasma membranes (Fig. 8h). Taken together, our results demonstrate that during C. albicans infection, K+ efflux-dependent canonical inflammasomes trigger both the candidalysin-independent and -dependent GSDMD cleavage and macrophage pyroptosis, facilitating escape of C. albicans from macrophages.

Pharmacologic inhibition of GSDMD alleviates C. albicans infection.

Our results have shown that inflammasome-mediated GSDMD activation could regulate C. albicans escape from macrophages. C. albicans infection was significantly reduced in GsdmdΔ/Δ mice. Therefore, GSDMD inhibition is likely to be a promising therapeutic strategy for fungal sepsis. Importantly, Gsdmd knockout mice are similar to their wild-type littermates in body weight, survival rate, appearance, and behavior, and differential leukocyte counts in the bone marrow and peripheral blood are also normal in Gsdmd knockout mice53. Thus, targeting GSDMD for fungal sepsis may have few off-target effects. Small molecule inhibitors are ideal for clinical application, and several inhibitors have recently been developed to target the GSDMD pathway75–80. Rathkey et al. identified necrosunomamide (NSA) as a potent inhibitor of GSDMD through direct binding to GSDMD to inhibit pyroptosis; it was also efficacious in sepsis models, suggesting that GSDMD inhibitors may be used clinically in infectious and inflammatory diseases75. In nigericin-stimulated LPS-primed BMDM cells, NSA does not inhibit other innate immune pathways and cleavage of GSDMD occurs normally, indicating that NSA does not inhibit caspase-175. Interestingly, a
recent study revealed that, in monosodium urate (MSU) crystal–stimulated LPS-primed BMDMs, NSA may also inhibit inflammasomes upstream of GSDMD, thereby preventing pyroptosis independent of GSDMD targeting. We investigated whether NSA could modulate host defense in Candida infection. Treatment with NSA blocked pro-caspase-1, pro-IL-1β and GSDMD cleavage in C. albicans-infected macrophages, consistent with the idea that it can inhibit pyroptosis upstream of GSDMD, in addition to inhibiting GSDMD p30 itself (Fig. S8a, b). Similar to what was observed in GSDMD deficient mice, NSA treatment significantly suppressed, but did not completely inhibit IL-1β production by infected macrophages (Fig. S8c). This effect was specific to IL-1β, with the production of IL-6, TNFa, and MIP2 being less affected (Fig. S8d). NSA effectively inhibited C. albicans-induced macrophage death, as indicated by fewer PI-positive cells being detected in NSA-treated BMDMs compared to untreated BMDMs (Fig. S8e, f). LDH release was also suppressed in NSA-treated BMDMs, with a 40% reduction detected 12 h after C. albicans infection, again demonstrating that C. albicans-induced macrophage death could be partially blocked by NSA treatment (Fig. S8g).

We next treated mice with NSA before intravenous injection of C. albicans and then administered it daily during the course of C. albicans infection (Fig. 9a). In the absence of infection, NSA-treated mice had similar body weights, survival, appearance, and behavior to untreated mice, with no obvious abnormalities detected. NSA-treated mice displayed enhanced resistance to C. albicans infection, as evidenced by an increased survival rate (Fig. 9b), improved clinical scores (Fig. 9c), less severe kidney enlargement (Fig. 9d, e), and decreased fungal burden (Fig. 9f) compared to untreated mice. It is well documented that NSA can efficiently inhibit GSDMD-mediated pyroptosis. Thus, these results confirmed that inhibition of GSDMD and pyroptosis can induce resistance to Candida infection. As observed in Gsdmd−/− mice, NSA-treated mice produced less IL-1β after C. albicans infection (Fig. 9g). Similarly, this modest IL-1β production appeared to be sufficient for initiating host anti-fungal immunity. Collectively, our results demonstrate that pharmacologic inhibition of GSDMD may offer a potential therapeutic strategy for C. albicans infection.

Pharmacologic inhibition of GSDMD alleviates C. albicans-induced death of human macrophages. C. albicans infection leads to lytic death of human monocyte derived macrophages (hMDMs). We next explored the role of human GSDMD (hGSDMD) in this process. Similar to what was observed in mouse macrophages, infection with C. albicans triggered robust cleavage of full-length hGSDMD and generated the active N-terminal fragment in hMDMs (Fig. 10a). NSA treatment significantly suppressed C. albicans-induced pro-caspase-1 and...
hGSDMD cleavage, as well as IL-1β production (Fig. 10a, b). In NSA-treated hMDMs, pro-IL-1β processing was not affected. In fact, more cleaved mature IL-1β was detected in hMDMs due to the inhibition of its release (Fig. 10a). Using PI to detect the loss of plasma membrane integrity caused by lytic cell death, the percentage of PI+ cells increased gradually after C. albicans infection, with NSA-treated hMDMs displaying significantly reduced levels (Fig. 10c, d). We also assessed macrophage lytic death through LDH release. Similarly, C. albicans infection induced robust LDH release from hMDMs over time. LDH release was suppressed by NSA treatment, with more than 50% reduction detected at each time point after C. albicans infection, again demonstrating that C. albicans-induced human macrophage death could be blocked by GSDMD inhibition (Fig. 10e).

Infection with candidalysin-deficient (ece1Δ/Δ) C. albicans induced significantly less hMDM death, as indicated by fewer PI-positive cells being detected in (Fig. 10f, g) and less LDH release by (Fig. 10h) candidalysin-deficient C. albicans-treated hMDMs compared to WT C. albicans-treated hMDMs (Fig. 10f). ece1Δ/Δ-induced hMDM death could be further inhibited by NSA, confirming that NSA treatment suppressed candidalysin-independent death of C. albicans infected hMDMs (Fig. 10h). Based on the amounts of LDH release triggered by WT and ece1Δ/Δ C. albicans, we also calculated candidalysin-dependent
independent samples per group). For PI

\[ p = \ldots \]

C. albicans

does not completely abolish IL-1β

\[ \beta \]

Candidalysin-dependent and -independent hMDM death (Fig. S9f) and IL-1β secretion (Fig. S9a, b), PI uptake (Fig. S9c, d), and LDH release (Fig. S9e). Similarly, IL-1β secretion induced by Candidalysin-dependent and -independent hMDMs, confirming the essential role of GSDMD in both Candidalysin-dependent and -independent IL-1β release (Fig. 10i).

Finally, similar to what was observed in mouse macrophages, C. albicans–induced hGSDMD activation and hMDM death were mediated by the caspase-1 inflammasome. Treatment with Caspase-1 inhibitor VX-765 significantly suppressed C. albicans–induced hGSDMD cleavage (Fig. S9a), IL-1β secretion (Fig. S9a,b), PI uptake (Fig. S9c,d), and LDH release (Fig. S9e). Additionally, VX-765 inhibited both Candidalysin-dependent and -independent hMDM death (Fig. S9f) and IL-1β release (Fig. S9g). However, unlike NSA, which did not affect pro-IL-1β processing, VX765 significantly suppressed pro-IL-1β cleavage, with little m-IL-1β detected in hMDMs or supernatant (Fig. S9a,b, and Fig. S9g).

Discussion

The incidence of C. albicans infections has increased dramatically over the last decades: increased prevalence of chronic illnesses, antibiotic resistance, and numbers of immunocompromised patients due to surgery, chemotherapy, and organ transplantation, have created a large vulnerable population.

Despite an expanding antifungal armamentarium, treatment unfortunately is often unsuccessful and resistance can emerge.

Given the underlying morbidity of many patients, disseminated candidiasis remains a life-threatening infection and fungal sepsis is a high clinical priority.

Nevertheless, the precise mechanisms leading to C. albicans–induced sepsis remain unclear. Despite decades of exhaustive research, no significant breakthroughs have emerged and there are no FDA-approved drugs or effective cures for sepsis.

Thus, alternative therapeutic strategies are needed. The significance of developing personalized host-modulating therapies to counteract the growing threat of antifungal resistance is well recognized, because such resistance cannot simply be overcome by using microbe-centered strategies. Here we find a key role for macrophage GSDMD in C. albicans infection and established it as a potential host-directed therapeutic target for C. albicans–induced sepsis.

Host cells are equipped with extracellular and cytoplasmic pathogen sensors such as Toll-like receptors (TLRs) and nucleotide-binding domain and leucine-rich repeat-containing family receptors (NLRs). Activation of these sensors triggers the assembly of the inflammasome, a multimeric protein complex containing active caspase-1, which converts pro-IL-1β into its biologically active form. IL-1β subsequently initiates and further amplifies innate and adaptive immune responses. Overwhelming and sustained IL-1 release contributes to exaggerated systemic inflammation and multiple organ damage in severe sepsis, making inhibition of inflammasome-mediated cytokine production a valid strategy to prevent sepsis.

Additionally, macrophage death is an important cellular host defense mechanism since it mediates the secretion of IL-1β. GSDMD is known to be a key mediator of inflammasome-mediated macrophage pyroptotic death and IL-1β secretion.

Macrophage pyroptosis, triggered by inflammasomes, caspase-1/11, and GSDMD, is a major type of macrophage lytic cell death. Thus, for bacterial sepsis where IL-1β facilitates septic shock, inhibition of GSDMD-mediated pore formation is considered an effective therapeutic strategy to prevent early shock caused by systemic hyperinflammation. GSDMD disruption indeed protects against lethal bacterial sepsis and Gsdmd-deficient mouse show significantly improved survival compared to WT mice in both LPS-induced and cecal ligation and puncture (CLP) sepsis models. 

These results agree with observations in inflammasome (e.g., NLRP3, caspase-1/11, and ASC)-deficient mice, in which IL-1β production and bacterial sepsis are also suppressed.

However, inflammasomes appear to play a different role in fungal infection. As a key component of the innate immune response, inflammasome activation has been extensively investigated in C. albicans infection. Previous studies have suggested a critical role for innate immune receptors in mediating inflammatory responses against C. albicans. Upon Candida infection, NLRP3-mediated inflammasome assembly is induced by key pattern recognition receptors and their downstream molecules, including TLR2, Dectin-1, Syk and ZBP142.

Inflammasome activation plays essential roles in host defenses against C. albicans including inducing protective antifungal Th1/Th17 responses. Mice lacking key inflammasome components such as ASC,
Fig. 7 Candidalysin-deficient C. albicans cause less severe infection and tissue damage. a Kaplan-Meier survival plots of mice infected with WT or candidalysin-deficient (ece1Δ/Δ) C. albicans. Mice were intravenously challenged with 1 x 10^6 CFU C. albicans. Survival rates were analyzed using Kaplan-Meier survival curves and log-rank testing. p = 0.0003 vs. WT. b Clinical scores of mice challenged with WT or candidalysin-deficient (ece1Δ/Δ) C. albicans. Two-tailed unpaired Student’s t test was used for statistical comparisons. Data shown are means ± SD (n = 10 mice per group). p = 0.0388 (16 h), p = 0.0483 (24 h), p = 0.0074 (30 h) vs. WT C. albicans. c Up panel, comparison of the kidneys of mice challenged with WT or candidalysin-deficient (ece1Δ/Δ) C. albicans. Mice were sacrificed 2 days after intravenous injection of 1 x 10^6 CFU C. albicans. Shown are representative images. Lower panel, kidney weights of C. albicans-challenged mice. Two-tailed unpaired Student’s t test was used for statistical comparisons. Data shown are means ± SD (n = 8 kidneys per group), p = 0.0136 (ece1Δ/Δ), p = 0.0008 (w/o C. albicans) vs. WT C. albicans. d Histopathologic assessment of the kidneys of mice challenged with WT or candidalysin-deficient (ece1Δ/Δ) C. albicans. Mice were sacrificed 2 days after intravenous injection of 1 x 10^6 CFU C. albicans. Shown are representative H&E-stained sections of kidney tissues. Experiments were repeated three times. e Fungal burden of kidneys, livers, and spleens of mice challenged with WT or candidalysin-deficient (ece1Δ/Δ) C. albicans. Mice were sacrificed 2 days after intravenous injection of 1 x 10^6 CFU C. albicans. Two-tailed unpaired Student’s t test was used for statistical comparisons. Data shown are means ± SD (n = 7 for WT C. albicans-challenged mice, n = 8 for ece1Δ/Δ-challenged mice per group). p = 0.0032 (kidney) vs. WT C. albicans. f C. albicans in the kidney were identified by Grocott methenamine silver staining. Mice were sacrificed 2 days after intravenous injection of 1 x 10^6 CFU C. albicans. Results are representative of data from at least three biological replicates.

NLRP3, and caspase-1/11 are hypersusceptible to C. albicans infection45,46,106. Of note, NLRP3-independent non-canonical inflammasome activation has also been implicated in Candida infection. Dectin-1 was identified as a key extracellular pathogen sensor for IL-1β induction and processing, directly activating a non-canonical caspase-8 inflammasome for pro-IL-1β processing and without the need for internalization of fungal pathogens47,107,108. Despite the complex mechanism, all these studies suggest that inflammasome activation confers essential protective immunity against C. albicans infection. Thus, unlike in bacterial sepsis, inhibition of inflammasome-mediated cytokine production is currently not considered a valid strategy to prevent C. albicans-induced sepsis.

Paradoxically, here we discovered that although disruption of caspase-1/11 aggravated C. albicans infection as previously reported45,46,106, disruption of GSDMD, an effector molecule downstream of caspase-1/11, protected against C. albicans-induced sepsis. What causes this discrepancy? Fine-tuning of inflammasome-mediated IL-1β production is critical for C. albicans-induced pathology. Proinflammatory cytokines, including
IL-1β are key initiators of sepsis-associated life-threatening organ dysfunction. Profound cytokine release is associated with C. albicans-induced immunopathology. However, IL-1β is also required for antifungal immunity. Inflammasome-mediated processing of pro-IL-1β is critical for the generation of mature IL-1β. C. albicans-induced IL-1β secretion is almost completely diminished in macrophages isolated from inflammasome-null mice (e.g., Nlrp3, Asc, Casp1 knockouts)45,46,106. No mature IL-1β was detected inside caspase-1/11-deficient macrophages, confirming that the lack of IL-1β production was primarily caused by complete inhibition of caspase-mediated processing of pro-IL-1β. Caspase-1/11 also play a role in pyroptosis, a key mechanism mediating IL-1β release from macrophages, by mediating GSDMD cleavage and activation. However, GSDMD depletion only partially blocked IL-1β release upon C. albicans infection, indicating that IL-1β secretion can also be driven by mechanisms independent of GSDMD-mediated pyroptosis109,110. During C. albicans infection, mature IL-1β was generated via inflammasome activation even in GSDMD-deficient macrophages. Consistently, Gsdmd-/- mice could still release significant amounts of mature IL-1β via GSDMD-independent mechanisms to mount a sufficient anti-Candida immune response without exaggerating tissue inflammation and multiorgan damage. Therefore, the aggravated C. albicans infection observed in inflammasome-deficient mice occurs as a result of abolished IL-1β production. GSDMD disruption only partially reduced IL-1β production in C. albicans infected hosts, with the modest IL-1β production appearing to be sufficient for initiating anti-fungal immunity. Indeed, in the presence of interleukin 1 receptor antagonist anakinra, the protective effect elicited by GSDMD disruption was abolished. The fact that GSDMD disruption alleviated and inflammasome disruption aggravated C. albicans infection indicates functional uncoupling of GSDMD and the inflammasome during C. albicans-induced IL-1β processing and secretion. This uncoupling is unique to C. albicans infection and was not observed in bacterial47–49,53,54 or viral infection111, where GSDMD and inflammasome disruption conferred the same phenotypes. In summary, this study demonstrates key functions for GSDMD in
host defense against Candida infection and suggests that GSDMD may be a potential therapeutic target in C. albicans-induced sepsis. GSDMD was originally identified as a key factor responsible for the inflammatory form of lytic pyroptotic death in macrophages. However, this protein is also expressed in other cell types including various immune cells other than macrophages. We recently revealed that GSDMD is highly expressed in neutrophils and is a key regulator of neutrophil death. GSDMD cleavage and activation in neutrophils can be caspase-independent and instead mediated by a neutrophil-specific serine protease, neutrophil elastase (ELANE), released from neutrophil granules into the cytosol during neutrophil death. ELANE-derived N-terminal fragment is fully active and can induce lytic cell death as efficiently as the caspase-derived N-terminal fragment. It is intriguing to see whether ELANE-mediated activation of GSDMD in neutrophils plays a role in anti-Candida host defense. Similarly, the contribution of GSDMD in NK, DC, T, and B cells also remains elusive.

GSDMD is a member of the gasdermin family. There are five parologue GSDMs (A-E) in humans, most of which have been shown to form pores that can activate pyroptosis when cleaved. While GSDMD is central to pyroptosis in myeloid cells, other gasdermins can also modulate cell death. For instance, Gasdermin E (GSDME) is turning out to be a critical GSDM in cancer cells. It is cleaved and activated to form pores by caspase-3 and classical apoptotic stimuli, and by killer lymphocyte granzyme B (GzmB) when cells are recognized as targets for immune elimination. GzmB both directly cleaves GSDME and indirectly activates GSDME because it activates caspase-3. GSDME activation converts noninflammatory apoptosis to inflammatory pyroptosis. A recent study showed that intratumoral delivery of nanoparticles conjugated with a precleaved form of mouse gasdermin A (GSDMA3) could cause selective tumor cell pyroptosis. Caspase-8, the caspase activated by the TNF death receptor to trigger apoptosis, can cleave gasdermins C and D (GSDMC, GSDMD) to convert apoptosis to pyroptosis. Gasdermin B (GSDMB) is a substrate of granzyme A (GzmA), the other abundant granzyme in killer T and NK cells. Whether gasdermin family members other than GSDMD are also involved in anti-Candida host defense needs to be further investigated.

We also identified the mechanism augmenting antifungal immunity in Gsdmd−/− mice. Host–pathogen interactions and their role in cell death are highly complex, involving a fine balance between pro- and antideath strategies for both host and pathogen. Pathogens can increase their success by hijacking host death system components. Programmed cell death is a central player in Candida infections, and membrane disruption is a key mechanism by which pathogens destroy immune cells for self-survival. C. albicans is known to trigger macrophage lytic death and use this as a mechanism to facilitate their escape from the macrophage. Pyroptosis is an inflammatory form of programmed cell death that is triggered by invading pathogens. GSDMD-mediated macrophage pyroptosis has evolved as an efficient host defense mechanism, particularly against intracellular pathogens. Thus, pyroptosis is usually an advantageous adaptation that removes the niche for intracellular pathogen replication. However, GSDMD disruption paradoxically improved host anti-C. albicans responses, suggesting that this particular pathogen may have hijacked this defense mechanism to improve its survival in infected hosts. C. albicans-triggered excessive activation of GSDMD-mediated pyroptosis led to widespread cell death, causing tissue damage, organ failure, and lethal septic shock, and this mechanism was abolished in Gsdmd−/− mice. GSDMD-deficient macrophages became more resistant to C. albicans-induced pyroptosis, preventing the escape of engulfed Candida from macrophages, thereby reducing the fungal burden and improving outcomes in C. albicans-infected hosts.

At the cellular and molecular level, C. albicans-induced GSDMD cleavage was mainly mediated by K+ efflux-dependent canonical inflammasomes and could be regulated by hyphae formation and candidalysin. C. albicans virulence is governed by its ability to readily interconvert between a singular budding yeast form and a filamentous hyphae form. While the yeast form reproduces quickly and rapidly proliferates, the hyphal form invades and destroys host cells and tissues. Using key adhesin
**Fig. 9** GSDMD antagonist NSA alleviates *C. albicans* infection in mice. 

**a** Experimental scheme for assessing the effects of pharmacologic inhibition of GSDMD on *C. albicans* infection. Mice were intravenously challenged with $2 \times 10^5$ CFU *C. albicans* 1 h after intraperitoneal injection of sesame oil alone (control) or NSA (dissolved in sesame oil, 20 mg/kg body weight). The challenged mice received daily NSA treatment (20 mg/kg body weight) and were monitored for 15 days. Controls were injected with sesame oil only during the same time period. 

**b** Kaplan-Meier survival plots of *C. albicans*-challenged mice. Survival rates were analyzed using Kaplan-Meier survival curves and log-rank testing. $p = 0.0427$ vs. control. 

**c** Clinical score of *C. albicans*-challenged untreated and NSA-treated mice. Two-tailed unpaired Student’s t test was used for statistical comparisons. Data presented are the means ± SD (n = 8 mice per data point) of three independent experiments. $p = 0.005$ vs. control. 

**d** Comparison of the kidneys of *C. albicans*-challenged untreated and NSA-treated mice. Mice were sacrificed 2 weeks after the *C. albicans* infection. Shown are the representative images from three independent experiments. 

**e** Kidney weights of *C. albicans*-challenged mice. Two-tailed unpaired Student’s t test was used for statistical comparisons. Data presented are the means ± SD (n = 6 for Vehicle, n = 7 for NSA-treated mice per data point) of three independent experiments. $p = 0.0279$ vs. control. 

**f** Fungal burden of kidneys from untreated and NSA-treated mice 2 days after *C. albicans* infection. Two-tailed unpaired Student’s t test was used for statistical comparisons. Data presented are the means ± SD (n = 8 mice per data point) of three independent experiments. $p = 0.0379$ vs. control. 

**g** *C. albicans*-induced IL-1β production in untreated and NSA-treated mice. Mice were intravenously challenged with $2 \times 10^5$ CFU *C. albicans*. Two-tailed unpaired Student’s t test was used for statistical comparisons. Data shown are means ± SD (n = 5 for uninfected, n = 7 for *C. albicans* infected mice). $p = 0.0242$ vs. control (untreated mice).
structures, hyphae strongly attach to host cells and modulate different invasion mechanisms including induced endocytosis and active intercalation\textsuperscript{130}. The latter confers an additional benefit to virulent \textit{C. albicans}, namely the ability to escape the phagolysosome and thus disrupt host defense mechanisms\textsuperscript{131}. In this study, we demonstrated that hyphae formation was also critical for \textit{C. albicans}-induced GSDMD cleavage and the subsequent death of host macrophages.

\textit{C. albicans}-induced phagocyte damage was partially attributed to its ability to physically disrupt cellular integrity through conversion from the yeast to the filamentous form. Additionally, candidalysin, a hyphae-associated cytolytic pore-forming peptide toxin, was recently identified as a \textit{C. albicans} virulence factor involved in mucosal and systemic infection\textsuperscript{63–65,132–135}. Candidalysin is both a central trigger for NLRP3 in \textit{flammasomedependent caspase-1 activation via K\textsuperscript+ eflux and a key driver of...
inflammasome-independent cytolysis of macrophages upon infection with C. albicans. Consistent with previous studies, we also found that membrane disruption and cell death caused by candidalysin could be inflammasome-independent and that IL-1β was still released in a candidalysin-dependent manner through an inflammasome-independent pathway. Candidalysin is a pore-forming toxin that causes membrane perturbations, leading to K+ efflux and subsequent inflammasome activation and pyroptosis. Candidalysin facilitates IL-1β processing and release via inflammatory caspases; however, macrophages infected with candidalysin-deficient mutants still released significant amounts of IL-1β, suggesting that other fungal components (e.g., β-D-glucans) could activate inflammasomes and process IL-1β during C. albicans infection. Additionally, we also observed significant GSDMD cleavage and cell death when macrophages were incubated with candidalysin-deficient C. albicans, supporting the existence of a candidalysin-independent death program. Presumably, during C. albicans infection, hyphal proteins other than candidalysin may facilitate GSDMD cleavage and plasma membrane perforation, leading to host cell death. Alternatively, in a candidalysin-independent manner, macrophages might be mechanically destroyed by hyphae inside the cells that physically pierce the host cell membrane. Notably, filamentation is necessary but not sufficient to trigger NLRP3 inflammasome-mediated pyroptosis, indicating that C. albicans-mediated macrophage damage is not solely due to hyphae-induced physical disruption of cellular integrity.

Collectively, our results suggest that the host response to C. albicans infection is complex and mediated by multiple factors and pathways (Fig. S10). First, both inflammasome-dependent and -independent mechanisms are involved. Upstream inflammasome and caspase-1 activation are essential for processing pro-IL-1β to mature IL-1β as well as C. albicans-induced GSDMD cleavage. However, macrophage death and secretion of mature IL-1β can occur in the absence of inflammasome activation. Second, C. albicans-induced macrophage death and mature IL-1β secretion can be triggered by both the GSDMD-dependent pyroptosis and GSDMD-independent mechanisms. In the absence of GSDMD, secretion of caspase-processed mature IL-1β can still be supported by GSDMD-independent mechanisms. Thus, although inflammasome activation is absolutely required for IL-1β production and anti-Candida host defenses, significant amounts of IL-1β can still be produced in Gsdmd<sup>−/−</sup> mice to maintain host defenses against C. albicans, alleviating infection in these mice. Third, the response to C. albicans infection can be either candidalysin-dependent or -independent. Candidalysin can induce K+ efflux through plasma membrane perforation, which in turn leads to canonical inflammasome activation, GSDMD cleavage, and macrophage pyroptosis. Thus, this candidalysin- and GSDMD-mediated relay mechanism enables C. albicans to escape host immunity via progressive plasma membrane permeabilization and host immune cell lysis. In the absence of candidalysin, hyphae still trigger rupture of the plasma membrane, inflammasome activation, and GSDMD cleavage. This is likely to be mediated by mechanical piercing or other hyphal proteins. It is noteworthy that, as a pore-forming toxin, candidalysin can also induce macrophage cell death through an inflammasome and K + -independent manner. It is well documented that NLRP3-mediated pyroptosis contributes to C. albicans-induced damage of mBMDMs. Macrophage lysis is dependent on caspase-1, ASC, and NLRP3. Consistently, C. albicans-induced cell death is alleviated in NLRP3 inflammasome-deficient macrophages. However, the cell death cannot be completely inhibited by disrupting the NLRP3...
inflammasome, suggesting that Candidalysin can trigger both the inflammasome-dependent and -independent cell death. Consistent with the role of candidalysin in Candida-induce macrophage lysis and Candida escape from macrophages, in the mouse systemic candidiasis model, we found that mice infected with the candidalysin-deficient (ece1Δ/Δ) C. albicans displayed higher survival rates and decreased fungal burden compared with WT C. albicans. A previous study showed that, in an oral candidiasis model, the fungal burden was also lower in ece1Δ/Δ C. albicans infection compared with wild type63. Interestingly, in another study, Svidergall et al. detected increased fungal burden with the candidalysin-deficient C. albicans135. The discrepancy is likely caused by different experimental conditions in their compared with our experiments. We both revealed that Candidalysin promoted mortality in mouse models of systemic fungal infection. Svidergall et al. focused on neutrophil recruitment. Their data indicated that candidalysin was required for neutrophil recruitment; thus when candidalysin-deficient C. albicans (2 × 10⁴) were used to infect mice, fungal burden of the kidney at 1 day increased significantly due to reduced neutrophil accumulation. This effect was diminished 4 days post-infection. Our study focuses on the escape of C. albicans from infected macrophages, and we inoculated mice with more Candida albicans cells (1 × 10⁶) and assessed fungal burden 2 days post-infection. In this setup, mice infected with the candidalysin-deficient C. albicans displayed decreased fungal burden compared with WT C. albicans. Finally, yeast-to-hyphal transition and hyphae-associated candidalysin induced both GSDMD-dependent and -independent cell death during C. albicans infection. It is plausible that direct membrane disruption by C. albicans could help IL-1β release independent of pyroptosis. Moreover, Monteleone et al. recently showed that pro-IL-1β cleavage results in relocation of mature IL-1β to the plasma membrane and its subsequent release via both GSDMD-dependent and -independent pathways109. These mechanisms may explain the GSDMD-independent release of mature IL-1β by C. albicans-challenged macrophages.

When inflammasome and pyroptosis axis is blocked, C. albicans-induced macrophage lysis46 can not be completely inhibited, suggesting that C. albicans can escape from macrophages via both inflammasome-dependent and -independent mechanisms. A previous study suggests a defined order of candidalysin, inflammasome activation, pyroptosis, macrophage lysis, and C. albicans escape during C. albicans-macrophage interactions45. The early phase escape is likely mediated by a host-cell programmed death pathway including candidalysin, inflammasome activation, GSDMD, and pyroptosis. Inflammasome-independent macrophage lysis occurs at later time points and/or higher organism burdens46. The later phase C. albicans escape may be mediated by candidalysin-dependent but pyroptosis-independent cell death. Alternatively, the polarized growth of hyphae can directly lead to physical rupture of macrophages independent of candidalysin, by piercing of the fungal filaments through the plasma membrane. Finally, disruption of host glucose homeostasis may also contribute to massive macrophage death at the later stage136,137. Inflammasome activation can be triggered by glucose starvation in macrophages, which occurs when fungal load increases sufficiently to outcompete macrophages for glucose136. Despite the complexity of the host response to C. albicans, we clearly established GSDMD as a host component that exacerbates inflammatory responses during C. albicans-induced sepsis and can thus be targeted to combat C. albicans invasion (Fig. S10).

Methods

Materials and Resources. Most reagents used in this application (e.g. antibodies, chemicals, media, etc.) were purchased from reputable commercial vendors. As such, these reagents have passed a quality control screening process, and this information was provided to us upon purchase. Key resources used in this study are listed in Table S1.

Mouse strains. Gsdmd−/− mice (in a C57BL/6 background) were generated as previously described53. Casp1/11−/− (in a C57BL/6NJ background), C57BL/6 WT, and C57BL/6NJ WT mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Eight- to 12-week-old mice were used in all experiments. Corresponding sex- and age-matched WT mice were used as controls in all experiments performed with KO mice. The Boston Children’s Hospital Animal Care and Use Committee approved and monitored all mouse procedures.

C. albicans strains. C. albicans ATCC MYA-2876 (SC5314)38 was the wild type used in all experiments. The ece1Δ/Δ strain was a kind gift of William Fonzi189. The cph1Δ/Δ, efg1Δ/Δ strain was generated as previously described41. For construction of other strains, including the primers used in current study, please see previous study140. C. albicans were grown on YPD agar plates at 30 °C, and cells were harvested by centrifugation, washed twice, and resuspended in PBS. For in vitro experiments, C. albicans were osponized with 20% mouse serum (auto-logous) in PBS for 20 min at 37 °C before infecting BMDMs.

Primary Cell Culture. Mouse bone marrow was isolated from femurs and tibias. Bone marrow-derived macrophages (BMDMs) were prepared as described previously140. Briefly, bone marrow cells were isolated from mice in culture medium (DMEM supplemented with 10% FBS, 100 μM penicillin/ streptomycin, and 30 ng/ml recombinant mouse M-CSF). Cells were seeded at 1.5 × 10⁶/well in a 6-well plate with 2 ml culture medium per well and cultured for 6 days. The medium was changed on day 3, and floating non-adhesive cells were washed away during the medium change. Four to five million cells per mouse were routinely obtained. Microscopic examination confirmed that >95% attached cells were morphologically mature macrophages. For pharmacological treatments, the indicated chemicals or inhibitors were added to the culture medium 1 h prior to C. albicans infection. The GSDMD antagonist necrosumonamide (NSA) was dissolved in dimethyl sulfoxide (DMSO), with DMSO alone used as control.

C. albicans infection model. Mice were injected intravenously with C. albicans yeast in a 100 μl volume of sterile pyrogen-free PBS (2 × 10⁴ or 1 × 10⁵). Survival was assessed daily for 15 days. To evaluate C. albicans-induced sepsis, we used a well-established clinical scoring system141 in which the appearance, activity, response to stimulus, and eyes were assessed.

Infection of BMDMs with C. albicans. Fresh overnight cultures of C. albicans yeast were resuspended in PBS and osponized with 20% mouse serum (auto-logous) in PBS for 20 min at 37 °C. BMDMs from WT or KO mice (0.5 × 10⁵/well) were incubated with C. albicans at indicated multiplicities of infection (MOI) with intermittent shaking. After each time period, cells were lysed by adding distilled H₂O₂, and serially diluted aliquots were spread on YPD agar plates. CFUs were counted after incubating the plates overnight at 30 °C. C. albicans suspensions without any BMDMs were used as input control. In vitro C. albicans killing capibilities were reflected by the decrease in C. albicans CFUs after incubation.

Preparation and infection of human monocyte-derived macrophages (hMDMs). Human peripheral blood mononuclear cells (hPBMC) were isolated using a Ficoll density gradient medium (LymphoprepTM, Stem cell technologies; Catalog #70801) following a protocol provided by the manufacturer. Subsequently, human monocytes were isolated from hPBMCs using EasySep™ Human Monocyte Isolation Kit (Stem cell technologies: Catalog #19359) according to the manufacturer’s protocol. To differentiate monocytes into hMDMs, the isolated monocytes were resuspended in IMDM GlutaMAX Supplement culture medium (ThermoFisher: 31980-030) supplemented with 10% FBS, and 40 ng/ml recombinant human M-CSF. Cells were seeded at 1.5 × 10⁶ in a 6-well plate with 2 ml culture medium per well and cultured for 6 days. The medium was changed on day 3, and floating non-adhesive cells were washed away during the medium change. hMDMs were collected from the plate 6 days after human M-CSF treatment and the cell densities were adjusted to 5 × 10⁶ cells/ml. Finally, the cells were resuspended in 24 or 96-well plates at a final concentration of 2.5 × 10⁵ or 5 × 10⁵ cells/well, respectively in IMDM + FBS and incubated overnight. For pharmacological treatment, the indicated chemicals or inhibitors were added to the culture medium 1 h prior to C. albicans infection. Macrophage infection experiments were performed in Opti-MEM medium. Media was drawn from healthy blood donors. All protocols were approved by the Children’s Hospital Institutional Review Board and were subjected to annual review.

Immunoblotting. At each time point, BMDMs were lysed immediately with cell lysis buffer (2× Laemmli sample Buffer, Bio-Rad, Hercules, CA) containing 2-mercaptoethanol (Bio-Rad) and protease inhibitor cocktail (Cell Signaling). Protein blots were incubated with primary antibodies (1:4) and then dissolved in the same lysis buffer. Samples were denatured by boiling at 100 °C for 5 min and transferred to ice. After brief sonication (5–10 s), 15 μl of sample was
subjected to 4–15% gradient SDS-PAGE. Proteins were transferred to polyvinylidene difluoride or nitrocellulose membranes (Millipore, Bedford, MA). Blockade and antibody incubation were performed in PBS containing 0.05% Tween-20 and 5% Blotting-Grade Blocker (Bio-Rad). After blocking the membranes for 1 h, primary antibodies were added in blocking solution at the following dilutions: mouse anti-GSDMD antibody (ab209845, 1:500; Abcam, Cambridge, UK), mouse anti-IL-1β/IL-1F2 antibody (AB-401-NA, 1:1000; R&D Systems, Minneapolis, MN); anti-IL-1β antibody (DH3H12) (1:2507, 1:1000; Cell Signaling Technology, Danvers, MA), anti-cleaved-IL-1β (Asp117) antibody (52718 S, 1:1000; Cell Signaling Technology), Antimouse NLRP3/Cryopyrin antibody (ARG60539, 1:1000; Arigo), anti-mouse GAPDH antibody (5174 S, 1:1000; Cell Signaling Technology), Anti-mouse Caspase-1 (E2Z1C) (24232 S, 1:1000; Cell Signaling Technology), anti-cleaved-IL-1β (Asp446) antibody (ARG40539, 1:1000; Arigo), Anti-mouse Caspase-1 (p20) antibody (52717 S, 1:1000; Cell Signaling Technology), anti-cleaved-IL-1β (Asp512) antibody (D3A32) (89316 S, 1:1000; Cell Signaling Technology), anti-total IL-1β antibody (D16H11) antibody (5174 S, 1:1000; Cell Signaling Technology). Kidney tissue samples were stained with hematoxylin and eosin (H&E) and examined by light microscopy.

Grocott’s methenamine silver stain and Periodic Schiff-Methenamine Silver (PASM) stain. Grocott’s methenamine silver (GMS) staining was used to visually identify fungi in infected kidneys and was performed at the HSM Rodent Histology Core facility. Briefly, kidney sections were oxidised with 4% chromic acid at RT for 1 h, treated with 1% sodium metabisulphite at RT for 1 min, and stained with 60 °C silver solution for 20 min or until sections turned yellowish-brown. Fungi were stained dark brown. PASM staining was also used to identify fungi in infected kidneys and was performed using a PASM staining kit (Servicebio, G1059) following the manufacturer’s protocol. Kidney sections were treated with periodic acid-silver merialmenes (PASM) staining solution A overnight, acidified with PAS staining solution B for 15-20 mins, and stained with 60 °C silver solution until sections turned yellowish-brown. Fungi were stained black or dark brown.

Detection of macrophages containing trapped C. albicans in kidneys of infected mice. Mice (WT and Gsdmd−/−) were intravenously challenged with 3 × 10^6 CFU WT (GFP+) or candialysin-deficient (ece1A/Δ) C. albicans. Three days after C. albicans infection, kidneys were homogenized using 40 µm cell strainers. BMDMs were lysed with 1 ml ACK lysis buffer (Gibco) for 5 min at room temperature. Cells were then stained with PE-Cy7-conjugated CD45 (BioLegend), PE-conjugated F4/80 (BioLegend), and APC-conjugated CD11b (BioLegend) antibodies for 30 min. For cells isolated from WT (GFP+) C. albicans-infected mice, macrophages containing trapped C. albicans were recognized by GFP fluorescence. For cells isolated from candialysin-deficient (ece1A/Δ) C. albicans-infected mice, the samples were fixed and permeabilized (BD Biosciences) after surface marker staining, and then stained with FITC-conjugated anti-C. albicans antibody (thermosifer) for 1 h. Flow cytometry was performed on the LSRII Fortessa (BD Biosciences) instrument. Flow cytometry data were analyzed with FlowJo software.

C. albicans infection of NSA-treated mice. Mice were intravenously challenged with 2 × 10^6 CFU C. albicans 1 h after intraperitoneal injection of sesame oil alone (control) or NSA (dissolved in sesam oil, 20 mg/kg body weight). Challenged mice received daily NAS treatment (20 mg/kg body weight) and were monitored for 15 days. Controls were injected with sesame oil during the same time period. Survival rates were analyzed using Kaplan-Meier survival curves and log-rank testing. Clinical scores of untreated and NSA-treated mice were assessed 24 h after C. albicans challenge. Kidney weights and size were examined 2 weeks after C. albicans infection. Histopathologic assessment of the kidneys, GMS staining of C. albicans in the kidney, and measurement of fungal burden in the kidneys were all performed twice for each genotype or treatment group. Shown are representative H&E stained sections of kidney tissue.

C. albicans infection of anakinra-treated mice. Mice were intraperitoneally injected with 2 doses of anakinra (diluted in PBS, 100 mg/kg body weight per dose) administered 24 h apart. The first dose was applied 1 h before and the second dose was applied 23 h after C. albicans infection. Challenged mice were monitored for 15 days. Survival rates were analyzed using Kaplan-Meier survival curves and log-rank testing. Clinical scores of untreated and anakinra-treated mice were assessed 24 h after C. albicans challenge. Kidney weights and size) examination, histopathologic assessment of the kidneys, GMS staining of C. albicans in the kidney, and measurement of fungal burden in the kidneys were all conducted twice for each c. albicans infection. Shown are representative H&E stained sections of kidney tissue.

Tissue fungal burden. Kidney, lung, liver, and spleen samples were homogenized using 40 µm cell strainers, diluted with saline, and quantitatively cultured overnight on YPD agar at 30 °C. The results are expressed as log CFU per organ.

Histopathology. WT, Gsdmd−/−, and Csp-1/11−/− mice were intravenously challenged with 1 × 10^6 CFU WT or ece1A/Δ C. albicans. Mice were euthanized by asphyxiation by inhalation of CO2 2 days after C. albicans injection. Kidneys were dissected, fixed in 10% neutral buffered formalin (Sigma Aldrich), and then embedded in paraffin. Paraffin-embedded sections (~6 µm thick) were stained with hematoxylin and eosin (H&E) and examined by light microscopy.

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C. albicans infection of anakinra-treated mice. Mice were intraperitoneally injected with 2 doses of anakinra (diluted in PBS, 100 mg/kg body weight per dose) administered 24 h apart. The first dose was applied 1 h before and the second dose was applied 23 h after C. albicans infection. Challenged mice were monitored for 15 days. Survival rates were analyzed using Kaplan-Meier survival curves and log-rank testing. Clinical scores of untreated and anakinra-treated mice were assessed 24 h after C. albicans challenge. Kidney weights and size) examination, histopathologic assessment of the kidneys, GMS staining of C. albicans in the kidney, and measurement of fungal burden in the kidneys were all conducted twice for each C. albicans injection. Shown are representative H&E stained sections of kidney tissue.
were pooled and analyzed together. No samples or animals subjected to successful procedures and/or treatments will be excluded from the analysis. For survival analyses, we used survival data to generate Kaplan-Meier survival curves and performed comparisons between groups by log-rank analysis using Prism (GraphPad Software, La Jolla, CA).

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

All data needed to evaluate the conclusions in the paper, including data associated with main figures and supplementary figures, are available within the article or in the Supplementary Information. Additional data related to this paper may be requested from the authors. Source data are provided with this paper.

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