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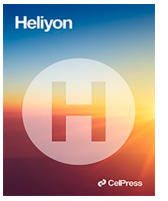

Research article

Effect of the *Sho1* gene on the pathogenicity of *Candida albicans* and immune function *in vivo*

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ABSTRACT

Objectives: Sho1, a ubiquitous membrane protein in fungi, plays a pivotal role in various physiological processes, such as osmotic stress, oxidative stress, temperature response, and virulence regulation across different fungal species. This study aimed to investigate the effect of the *Sho1* gene on the pathogenicity of *Candida albicans* and its immune function *in vivo*.

Materials and methods: Ninety-nine clinical strains from various infection sites were collected to investigate the expression levels of the *Sho1* gene compared to its levels in the standard strain (SC5314). *Sho1*-knockout strains (*Sho1Δ/Δ*) were constructed to investigate the impact of the *Sho1* gene deletion on the biofilm formation, adhesion, and flocculation abilities of *C. albicans*. A mouse model of systemic infection was established to evaluate the impact of *Sho1* deletion on survival, organ pathology, and immune cell function, as assessed by flow cytometry.

Results: The expression level of the *Sho1* gene was found to be higher in clinical strains derived from sterile fluids, sputum, and secretions compared to that in the standard strains. Deletion of the *Sho1* gene diminished the biofilm-formation capacity of *C. albicans*, leading to a sparse structure and reduced thickness, as well as diminished adhesion and flocculation abilities. Deletion of the *Sho1* gene prolonged mouse survival; decreased the fungal load in the liver, kidney, and spleen; and reduced inflammatory cell infiltration into the kidney. In the spleens of mice injected with the *Sho1Δ/Δ* strain, a decrease was observed in the percentage of M1-type macrophages and an increase in M2-type macrophages, resulting in a decreased M1/M2 macrophage ratio. Additionally, an increase was observed in the number of Th1 cells and a decrease in the number of Th2 and Th17 cells, leading to an increased Th1/Th2 ratio.

Conclusion: The *Sho1* gene significantly contributes to the pathogenesis of *C. albicans* by influencing its biological behaviour and immune response *in vivo*.

1. Introduction

Candida albicans is a prevalent opportunistic pathogenic fungus in clinical settings. It can cause superficial colonisation and deep-

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seated invasive infections [1]. It is frequently transmitted through the bloodstream, leading to severe systemic infectious diseases, with a mortality rate of up to 50 % [2,3]. Changes in the structure of the microbial flora, such as reduced diversity and density or compromised immune systems, can cause *C. albicans* to change from a symbiotic to pathogenic fungus. This transition is accompanied by a shift from yeast to mycelial forms, facilitating invasion and systemic dissemination *via* the bloodstream, resulting in sepsis [4]. The pathogenesis of *C. albicans* infection is multifaceted and influenced by factors, such as the immune status of the patient and production of body fluids and secretions [5]. In recent years, advancements in modern molecular biology technologies have revealed the impact of genomic alterations in *C. albicans* on its pathogenic potential.

Sho1 (signalling high osmolarity protein) is a tetra transmembrane protein ubiquitously present in fungi. It comprises a cytoplasmic SH3 domain at the C-terminus and four transmembrane segments at the N-terminus [6,7]. The *Sho1* protein is intricately linked to biological behaviours, such as filamentous growth, osmotic stress, oxidative stress, temperature response, and virulence regulation across various fungi, including *C. albicans* [8,9], *Saccharomyces cerevisiae* [10], *Aspergillus fumigatus* [11], *Cryptococcus neoformans* [12], and *Fusarium granulosa* [13]. Additionally, in *C. albicans*, this gene is regarded as a pivotal upstream factor in glycolysis, and it may be involved in cell wall biogenesis and oxidative stress processes during growth [6,14]. However, its effect on the pathogenicity of *C. albicans* and its immune function *in vivo* remain unclear.

Macrophages are pivotal components of the host innate immunity against infections and can be categorised into two types based on their phenotype and function: M1-type and M2-type macrophages [15]. The balance of M1/M2 macrophages is influenced by various factors and can subsequently affect the degree of inflammation and tissue damage in organs and tissues [16,17]. T helper (Th) cells are vital immune cells involved in initiating, regulating, and executing immune responses within the body, and they play critical roles in resistance against fungal infections. Based on the secretion of cytokines, Th cells are primarily categorised into Th17, Th1, and Th2 types [18], with the balance of Th1/Th2 being crucial for maintaining the normal immune state in the body [19]. An imbalance in this ratio can disrupt the local immune function and compromise the resistance of the body to external pathogens.

This study aimed to investigate the effect of the *Sho1* gene on the pathogenicity of *C. albicans* and its immune function *in vivo*. Initially, the expression level of the *Sho1* gene was compared between strains to ascertain whether it was elevated in clinical strains that exhibited greater pathogenicity. Subsequently, an *Sho1*-knockout strain was generated using homologous recombination, and its impact on the mycelial morphology, biofilm structure, thickness, adhesion, and flocculation ability of *C. albicans* was investigated through relevant experiments. Finally, a mouse model of systemic bloodstream infection was established to assess the alterations in mouse survival duration and fungal load in various organs. The effects of the *Sho1* gene on tissue injury and immune function were investigated through histological observations and flow cytometry to elucidate the pathogenic mechanism of *C. albicans* and lay a theoretical and scientific foundation for the development of future clinical therapeutic interventions.

2. Materials and Methods

2.1. Sample collection

Samples from 99 clinical strains were collected from the clinical laboratory of the 980th Hospital of the PLA Joint Logistics Support Force between 2021 and 2023, comprising 45 vaginal secretions, 45 sputum samples, and 9 sterile body fluid samples. This study adhered to the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of the 980th Hospital of the PLA Joint Logistics Support Force (2023-KY-93, 2023-6-26). The clinical strains used in this study were collected with the verbal informed consent of the patients and were approved by the Ethics Committee of the 980th Hospital of PLA Joint Logistics Support Force. The standard *C. albicans* strain, SC5314, was generously provided by Prof. Zhengxin He from the Central Laboratory of the 980th Hospital of the PLA Joint Logistics Support Force.

2.2. RNA extraction and reverse transcription-quantitative polymerase chain reaction

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was used to quantify the expression levels of the *Sho1* gene in various clinical strains and the standard strain of *C. albicans* (SC5314). Total RNA was extracted from the strains using the M5 EASY spin yeast RNA Rapid Extraction Kit (Mei5 Biotechnology Co., Ltd., Beijing, China), and the concentration and purity of RNA in each sample were assessed using a spectrophotometer. Reverse transcription was carried out using the Trans Script® First-Strand cDNA Synthesis Super Mix Kit (Transgene Biotech Co., Ltd., Beijing, China). qPCR was conducted using the Trans Script® Green qPCR Super Mix Kit (Transgene Biotech Co., Ltd.). The reaction conditions included an initial denaturation at 94 °C for 30 s, followed by 40 cycles of denaturation at 94 °C for 5 s, annealing at 57 °C for 15 s, and extension at 72 °C for 10 s [20,21]. The relative mRNA expression level was determined using the $2^{-\Delta\Delta CT}$ method. The primer sequences are listed in Supplementary Table 1.

2.3. Construction of Sho1-gene-knockout strain of C. albicans

The detailed experimental procedures for constructing the *Sho1*-gene-knockout strain of *C. albicans* (Sho1Δ/Δ) [22] are provided in Supplementary File 1. The primer sequences used in the construction of *Sho1Δ/Δ* are provided in Supplementary Table 2.

2.4. Determination of growth curves

The activated *C. albicans* solution was suspended in phosphate-buffered saline (PBS), and both the test (*Sho1Δ/Δ*) and control group

(SN152) were cultured at 35 °C on a shaking table at 120 rpm. Absorbance values were measured at various time-points [23].

2.5. Detection of biofilm formation ability

The concentration of *C. albicans* was adjusted to 1×10^7 colony-forming units (CFU)/mL, and it was incubated in a 5 % CO_2 incubator at 35 °C for 24 h. Following staining with crystal violet, the samples were dissolved and decolourised in 33 % (v/v) glacial acetic acid for 30 min, after which the absorbance values were measured [21].

2.6. Detection of adhesion capacity

C. albicans adhesion was assessed using a polystyrene plate model [24]. The *C. albicans* solution was incubated in an incubator at 37 °C for 4 h, after which non-adherent *C. albicans* was washed off with PBS. After staining with crystal violet, the samples were dissolved and decolourised in 33 % (v/v) glacial acetic acid for 15 min, and the absorbance was measured.

2.7. Observation of the morphology of mycelia and colonies

A suspension of *C. albicans* was added to a slide, allowed to dry naturally, and fixed. Mycelial morphology was then observed after Gram staining. The morphology of individual colonies was observed in solid yeast extract-peptone-dextrose (YPD) medium. The *C. albicans* suspension was evenly coated onto the medium using the dilution coating method and cultured at 35 °C and 5 % CO_2 for 7 days until dry.

2.8. Detection of the flocculation ability

The *C. albicans* suspension was placed in a glass tube, filling no more than 1/3 of the tube, and cultured in an air shaker at 35 °C and 200 rpm for 6 h. Subsequently, the tube was transferred to a vortex shaker and vigorously shaken for 30 s. The degree of flocculation in each tube was measured while at rest [25].

2.9. Scanning electron microscopy

After adjusting the concentration of *C. albicans* to 1×10^7 CFU/mL, the fungus was added to the slide and incubated at 37 °C for 48 h. Subsequently, the slide was rinsed slowly with PBS, fixed with 2.5 % glutaraldehyde overnight, and dehydrated with an ethanol gradient of 50, 70, 80, 90, and 100 %, for 10 min per concentration. After dehydration, the slides were removed and dried at room temperature [26]. Finally, gold was sprayed using an ion coater and the samples were observed using a scanning electron microscope (SEM; SU8100; Hitachi, Tokyo, Japan).

2.10. Laser-scanning confocal microscopy

A *C. albicans* suspension with a concentration of 2×10^6 CFU/mL was added to a confocal dish and incubated at 37 °C for 24 h. Non-adherent *C. albicans* cells on the surface of the dish were gently washed with PBS buffer. The FUN-1 solution was diluted with GH solution to a concentration of 25 μM. Subsequently, freshly prepared FUN-1 dye was added and the mixture was incubated for 30 min at room temperature in the dark. The biofilm was scanned layer-by-layer along the z-axis using laser-scanning confocal microscopy (LSCM; LSM900; Carl Zeiss AG, Oberkochen, Germany), and the thickness of the biofilm was measured using ImageJ software [27].

2.11. Construction of a systemic infection model

Six-to eight-week-old female SPF BALB/c mice were used in this study. The mice were divided into three groups: a test group (injected with *Sho1Δ/Δ*), a control group (injected with SN152), and a normal saline group (injected with normal saline). After a 1-week adaptation period, 100 μL of *C. albicans* solution or normal saline was injected into the tail vein to induce systemic infection [28]. For survival, fungal organ load determination and histological observations, the injected concentration was 1×10^6 CFU/mL. For flow cytometric analysis, the concentration was 5×10^5 CFU/mL.

2.12. Observation of survival time and determination of organ fungal load

The survival period of mice in each group (n = 10) was monitored for 15 days, and deaths were recorded daily. After establishing the systemic infection model for 48 h, the mice (n = 3) were euthanised and the target organs (liver, kidney, and spleen) were removed. The organs were then homogenised in PBS using an ultrasonic mill, evenly spread onto plates with solid YPD medium, and incubated at 35 °C with 5 % CO_2 for 48 h. Finally, the colonies were observed and counted [28].

2.13. Histological observation

After establishing the systemic infection model for 48 h, the mice (n = 3) were euthanised and the kidneys were removed. The

kidney tissue was fixed in formalin for 48 h, embedded in paraffin, and sliced into continuous sections with a thickness of 4 μm. Haematoxylin and eosin (HE) and periodic acid-Schiff (PAS) staining were performed [26].

2.14. Flow cytometry

After establishing the systemic infection model, the mice were euthanised at various time-points (the group of experimental animals is shown in Supplemental Table 3). The spleen was removed, and spleen tissue was filtered through a 70 μm cell strainer to obtain a single-cell suspension. The cells were centrifuged at 1300 rpm for 10 min and the supernatant was discarded. Mononuclear cells were isolated using a mononuclear cell separation solution (Tianjin Haoyang Huaoke Biotechnology Co., Ltd., Tianjin, China) and the cell concentration was adjusted to 1×10^7/mL. Following the instructions of the manufacturer, the membranes were incubated with anti-CD16/CD32 antibodies (MultiSciences Biotech Co., Ltd., Hangzhou, China) to prevent nonspecific binding. A single positive tube was used as a compensation. The antibody colour scheme is presented in Supplementary Table 4 [29,30]. Flow cytometry (CANTO; BD Biosciences, Franklin Lakes, NJ, USA) was used to detect the cells, and the results were analysed using FlowJo v.10.8.1_CL software (FlowJo, LLC, Ashland, OR, USA).

2.15. Statistical analyses

All statistical analyses were conducted using SPSS 26.0 software (IBM, Armonk, NY, USA). For normally distributed data, Student's *t*-tests were used to compare two groups, and one-way analysis of variance (ANOVA) was used for comparisons among multiple groups. For non-normally distributed data, a two-tailed Mann-Whitney *U* test was used to compare the two groups. Survival curves were assessed using the log-rank test. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Higher expression level of the Sho1 gene in clinical isolates compared to the standard strain

The RT-qPCR results indicated that the expression level of the *Sho1* gene was significantly higher in clinical isolates (sterile fluid, secreta, and sputum) than in the standard strain (SC5314) ($p < 0.05$). Additionally, no significant difference was observed in the expression level of the *Sho1* gene among sterile fluids, secreta, and sputum ($p > 0.05$) (Fig. 1).

3.2. Successful construction of Sho1Δ/Δ strain

Verification experiments showed that the growth of the defective strain SN152 matched the theoretical expectations (Supplemental Table 5). *His*- and *Leu*-gene- knockouts were confirmed by 1 % agarose gel electrophoresis (Fig. 2A–C). Successful transformations were confirmed by growth on MM-100 μg/mL R plates, with no growth in the control group (Fig. 2D). Single colonies, labelled Sho1-H-L-1 to −6, were cultured overnight in MM-100 μg/mL Arg liquid medium. The *His*, *Leu*, and *Sho1* gene regions were identified (Fig. 2E–G). Sequencing revealed that Sho1-H-L-1, -2, and -4 matched the template. Supplementary Fig. 4 shows the His and Leu results for Sho1-H-L-1, which was chosen for further experiments.

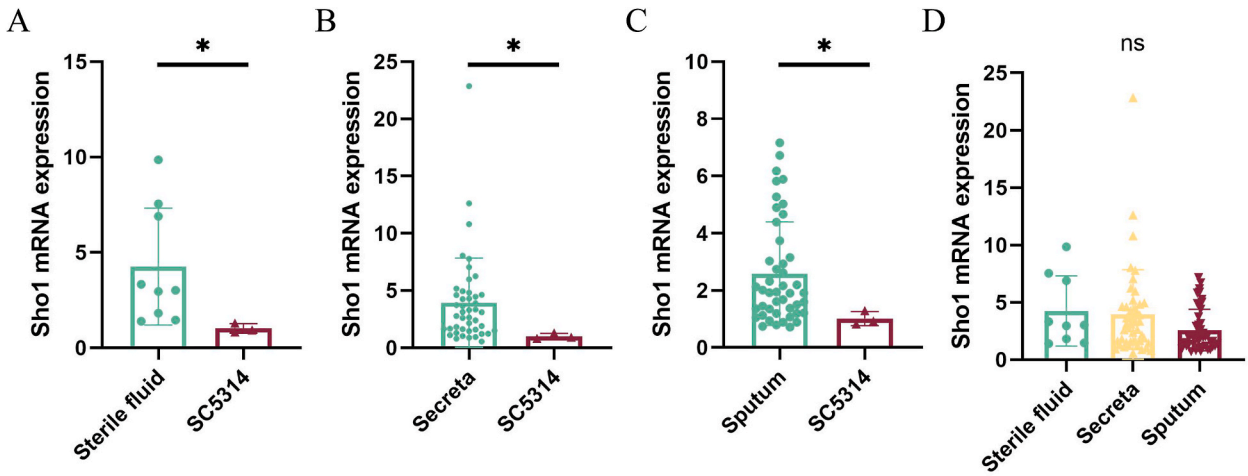

Fig. 1. (A–C) Expression of the *Sho1* gene in clinical and standard strains, *$p < 0.05$ by unpaired Student's *t*-test; (D) Comparison of the *Sho1* gene expression levels in sterile fluids (n = 9), secreta (n = 45), and sputum (n = 45); ns, no significant difference by one way analysis of variance (ANOVA).

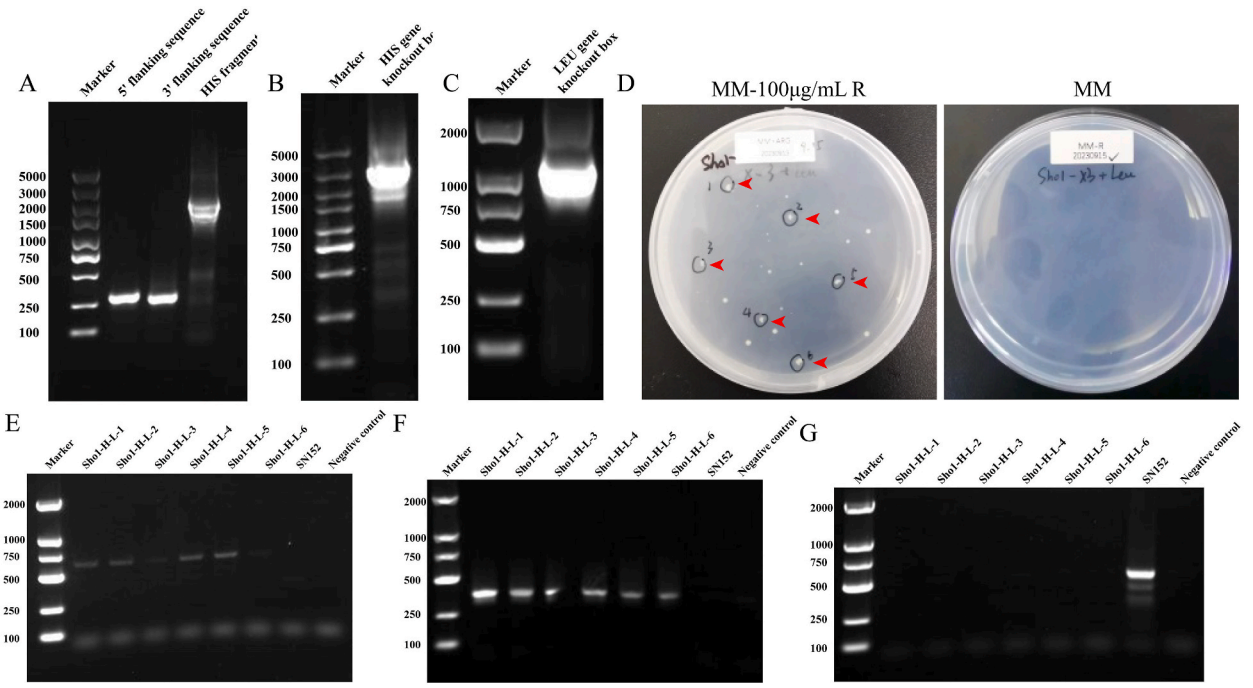

Fig. 2. (A) Agarose gel electrophoresis identification of the *His* gene knockout box; (B) Amplified *His* knockout box; (C) Agarose gel electrophoresis identification of the *Leu* gene knockout box; (D) Results of electrotransfer of the *His* and *Leu* gene knockout box. Left figure shows the growth results of the *His*- and *Leu*-gene-knockout box on the MM-100 μg/mL R plate, and right figure shows the growth results on the MM plate; Identification of *His*-gene-knockout box upstream (608 bp); (E) *Leu* gene knockout box upstream (361 bp) (F) and *Sho1* (487 bp) (G), with water as the negative control.

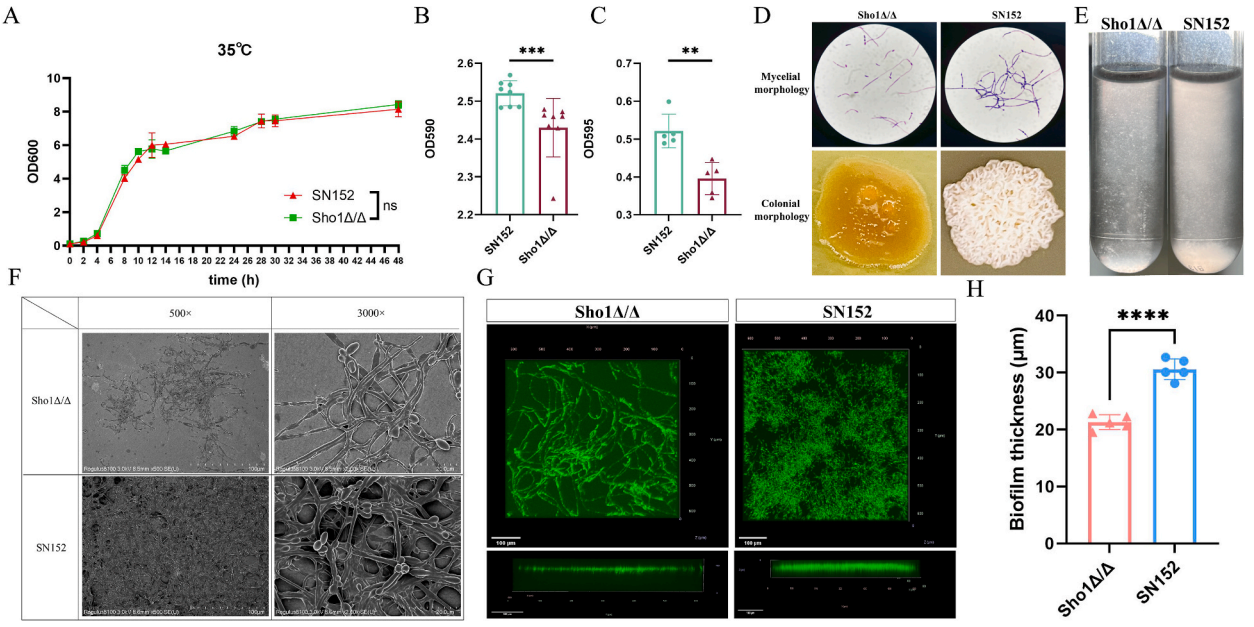

Fig. 3. (A) Determination of the growth curve of *C. albicans*, n = 3; (B) Detection of biofilm formation ability of *C. albicans*, n = 8; (C) Detection of adhesion ability of *C. albicans*, n = 5; (D) Observation of mycelia and colony morphology of *C. albicans*; (E) Flocculation experiments of *C. albicans*; (F) Scanning electron microscopy observation (bar = 100 μm for 500 × ; bar = 20 μm for 3000 ×); (G) Laser-scanning confocal microscopy observation (bar = 100 μm); (H) Statistical analysis of *Sho1Δ/Δ* and SN152 biofilm thickness, n = 5. (ns, no significant difference, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ by unpaired Student's *t*-test).

3.3. Sho1 influenced the phenotype of C. albicans

The growth curve results indicated that knocking out *Sho1* did not significantly affect the growth rate of *C. albicans* compared to that of the control strain SN152 (Fig. 3A). However, the deletion of *Sho1* resulted in decreased biofilm formation and adhesion ability of *C. albicans* (Fig. 3B and C). Gram staining showed that *Sho1Δ/Δ* predominantly exhibited a yeast or pseudomycelium state, while SN152 mostly displayed a mycelium state. The morphology of individual colonies revealed that the surface of *Sho1Δ/Δ* colonies was smooth, whereas SN152 colonies appeared rough (Fig. 3D). Deletion of the *Sho1* gene resulted in a decreased flocculation ability of *C. albicans* (Fig. 3E). SEM results revealed that the SN152 biofilm was intricate and tightly arranged, with numerous mycelia forming a solid structure, whereas the *Sho1Δ/Δ* biofilm was sparse, with significantly fewer mycelia (Fig. 3F). Additionally, the biofilm thickness decreased significantly in *Sho1Δ/Δ* compared to SN152 (Fig. 3G and H).

3.4. Sho1Δ/Δ had prolonged survival and reduced fungal load in organs compared to SN152

A systemic infection model of *C. albicans* was established using tail vein injections. The survival results showed that mice injected with SN152 had higher mortality rates on days 1 and 2, while those injected with *Sho1Δ/Δ* remained alive beyond the observation period, with a statistically significant difference between the two groups ($p < 0.05$) (Fig. 4A). The results of organ fungal load measurements showed that the number of colonies in the liver, kidney, and spleen was significantly reduced in mice injected with *Sho1Δ/Δ* compared to those injected with SN152 ($p < 0.05$) (Fig. 4B–D).

3.5. Sho1 Knockdown in the SN152 strain reduced its inflammatory effects in vivo

The HE staining revealed a large number of red blood cells in the kidneys of mice injected with SN152, with noticeable blood oozing. The number of inflammatory cells in the kidneys of mice was significantly reduced in those injected with *Sho1Δ/Δ* compared to those injected with SN152 (Fig. 4E). PAS staining revealed a significant reduction in the number of *C. albicans* cells in the kidneys of mice injected with *Sho1Δ/Δ* (Fig. 4F). These results demonstrated that deletion of the *Sho1* gene reduced the virulence of *C. albicans in vivo*.

3.6. Sho1 Knockdown in the SN152 strain reduced the M1/M2 macrophage ratio in spleens of mice

Flow cytometry was used to analyse the changes in the number of macrophages in the spleens of mice injected with different strains. The results showed that the proportion of M1-type macrophages decreased compared to that at days 1, 5, and 7 after *Sho1Δ/Δ* injection compared to SN152 injection (Fig. 5A and B), while the proportion of M2-type macrophages was higher after *Sho1Δ/Δ*

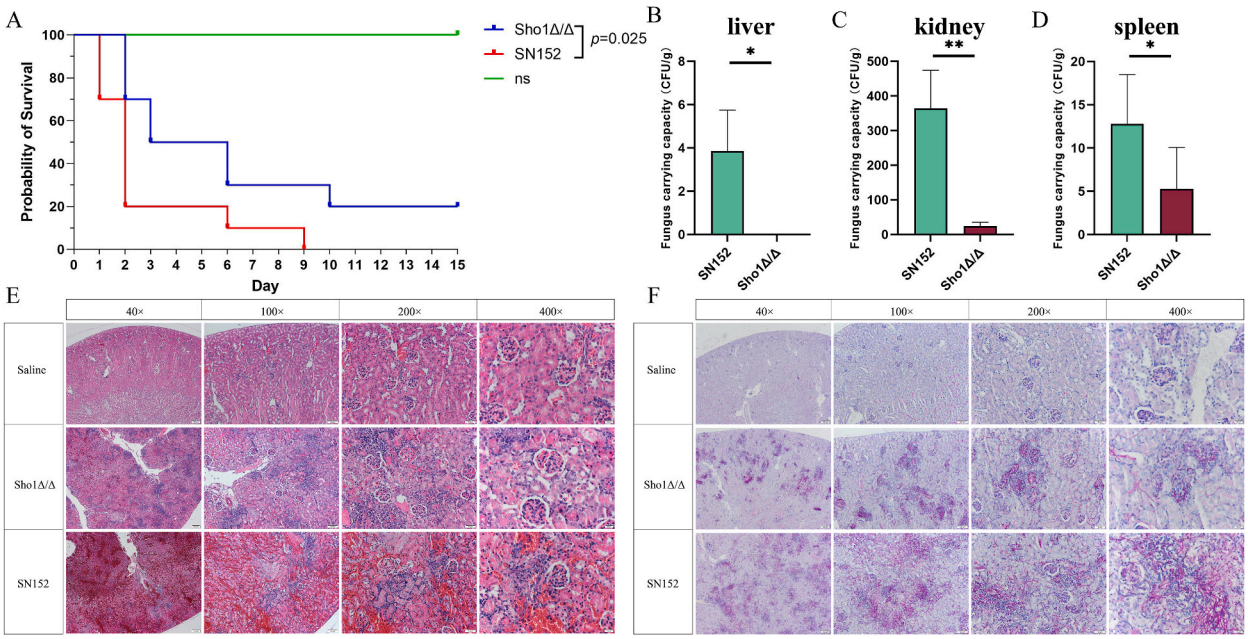

Fig. 4. (A) Survival curves of mice after caudal vein injection of *C. albicans Sho1Δ/Δ* and SN152, n = 10; (B–D) Results of fungal load determination in liver, kidney, and spleen of mice, n = 3, *$p < 0.05$ and **$p < 0.01$ by two-tailed Mann-Whitney *U* test (B and D) or unpaired Student's *t*-test (C); Haematoxylin and eosin staining (E) and periodic acid-Schiff staining (F) of kidney tissue (40 × , bar = 200 μm; 100 × , bar = 100 μm; 200 × , bar = 50 μm; 400 × , bar = 20 μm).

injection than after SN152 injection at all time-points (Fig. 5C and D). The ratio of M1/M2 cells decreased after *Sho1Δ/Δ* injection compared to after SN152 injection (Fig. 5E). The flow cytometry analysis strategies for the macrophages are shown in Supplementary Fig. 1.

Sho1Δ/Δ promoted an increase in the ratio of Th1/Th2 cells and the number of Th17 cells in spleens of mice.

Flow cytometry was used to analyse the changes in Th cells in spleens of mice after injecting different strains. Initially, compared with saline injection, SN152 injection reduced the proportion of Th1 cells and increased the proportion of Th2 and Th17 cells in spleens of mice (Supplementary Fig. 3). The results showed that the proportion of Th1 cells in spleens of mice was higher on days 1, 3, and 7 after *Sho1Δ/Δ* injection than after SN152 injection (Fig. 6A and B), and the proportion of Th2 cells was lower in mice injected with *Sho1Δ/Δ* than those injected with SN152 at all time-points (Fig. 6C and D). The ratio of Th1/Th2 cells was higher after *Sho1Δ/Δ* injection than after SN152 injection (Fig. 6E). Additionally, the proportion of Th17 cells in spleens of mice decreased on days 1, 3, and 7 after *Sho1Δ/Δ* injection compared to after SN152 injection (Fig. 7A and B). The flow cytometry analysis strategies for Th cells are shown in Supplementary Fig. 2.

4. Discussion

C. *albicans* is the most common opportunistic pathogen in systemic blood-borne infections, accounting for 70–90 % of fungal infections [5]. *C. albicans* infection can affect multiple tissues or organs throughout the body, resulting in lesions in various anatomical sites, including oral candidiasis [31], cutaneous candidiasis [32], candidal vaginitis [33], cystitis [34], and candidemia [35]. Furthermore, the virulence of *C. albicans* infection varies depending on the site and is influenced by factors, such as the immune status of the patient, body fluids, and secretions.

Sho1, a membrane protein commonly found in fungi, serves as an upstream regulator of glycolysis and is involved in regulating the Ras1-cAMP signalling pathway [36]. Notably, Sho1 is closely associated with microcolony formation and glycolysis [14]. Moreover, Sho1 significantly promotes the growth and development of *C. neoformans* mycelia [37], indicating that it may influence the pathogenicity of *C. albicans*. Therefore, we collected 99 clinical strains of *C. albicans* from different infection sites and confirmed by RT-qPCR that the expression level of the *Sho1* gene in these clinical strains was higher than that in standard strains, indicating that the *Sho1* gene may be related to the clinical pathogenicity or virulence of *C. albicans*.

Gene knockout refers to the technique of silencing or deleting a specific gene in a controlled manner, resulting in the loss of its corresponding gene function, using methods such as CRISPR-Cas9 and homologous recombination technology [22]. Previously, CRISPR-Cas9 technology was used to construct gene-knockout strains of *C. albicans*. However, this method is challenging, complex,

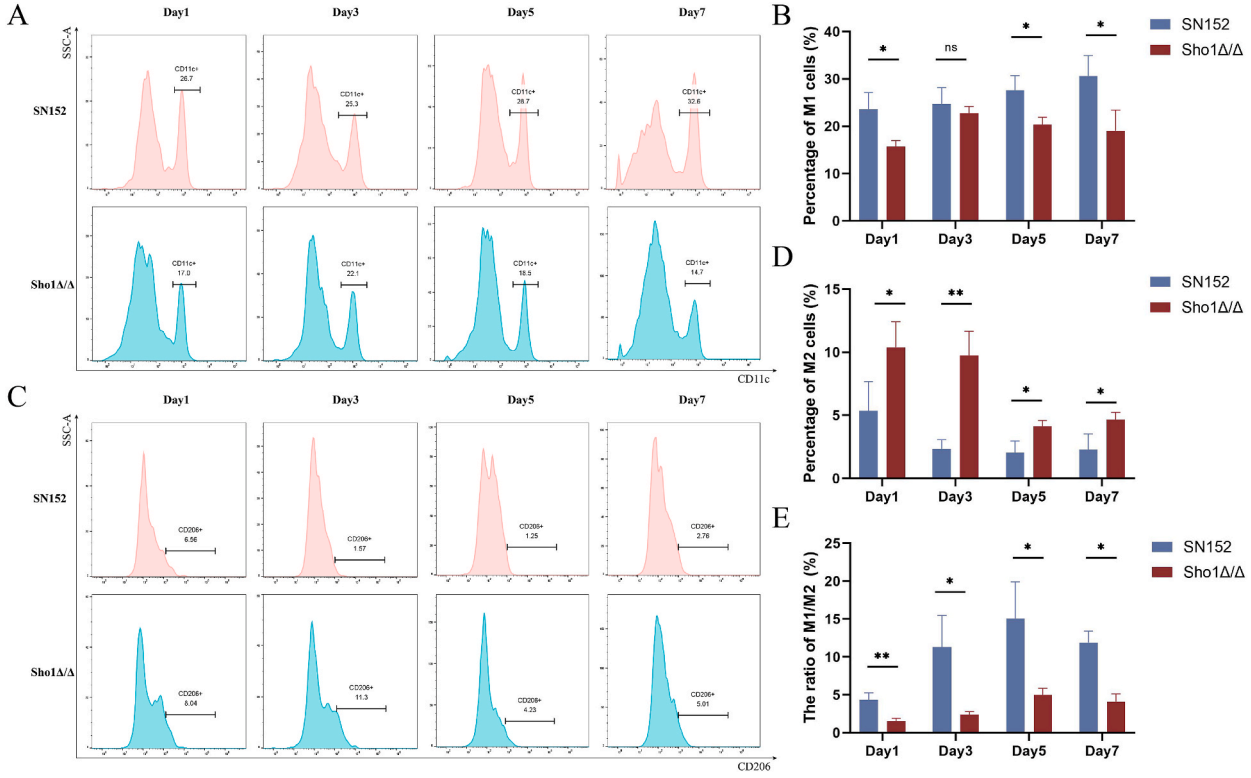

Fig. 5. (A–B) Analysis of the proportion of M1 macrophages, n = 5; (C–D) Analysis of the proportion of M2 macrophages, n = 5; (E) Ratio of M1/M2 macrophages, n = 5, ns, no significant difference, *$p < 0.05$ and **$p < 0.01$ by unpaired Student's *t*-test.

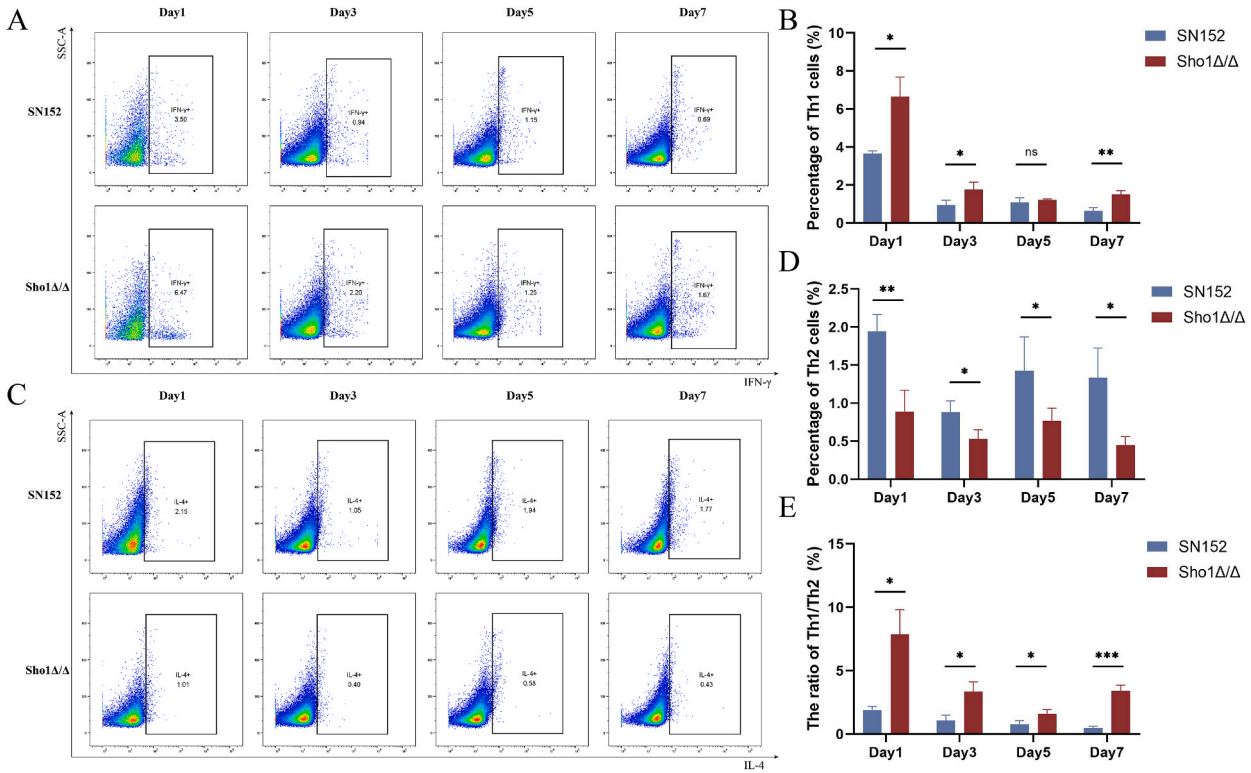

Fig. 6. (A–B) Analysis of the proportion of Th1 cell, n = 5; (C–D) Analysis of the proportion of Th2 cells, n = 5; (E) Ratio of Th1/Th2 cells, n = 5, ns, no significant difference, $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ by unpaired Student's *t*-test.

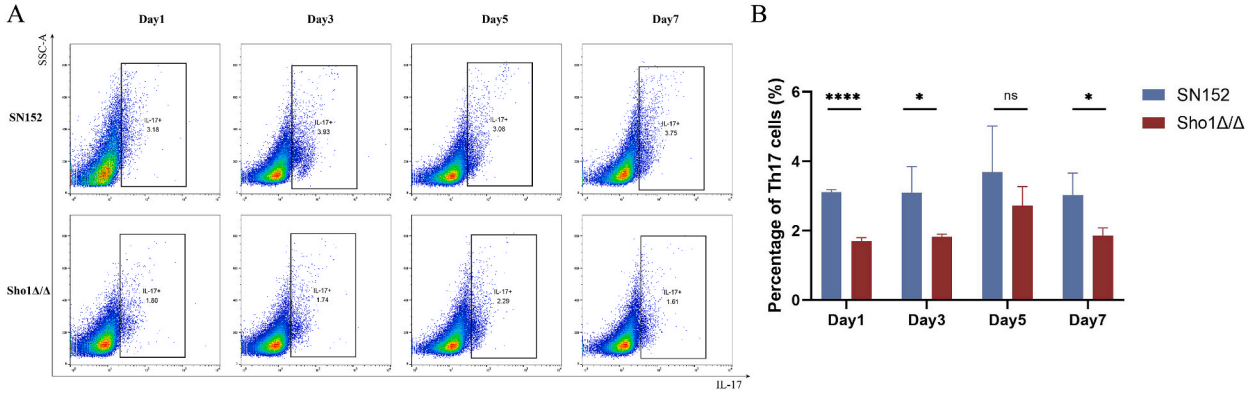

Fig. 7. Results of Th17 cell proportion analysis, n = 5, ns, no significant difference, $*p < 0.05$ and $****p < 0.0001$ by unpaired Student's *t*-test.

and prone to off-target effects [38]. Therefore, in this study, homologous recombination technology was adopted. The defective strain SN152 was used as the parent strain, *His* and *Leu* were used as nutritional screening marker genes, and two alleles of *C. albicans Sho1* were knocked out. After nutritional selection, PCR, and sequencing identification, we successfully obtained the *Sho1*-defective mutant strain, namely *Sho1Δ/Δ*, laying the foundation for further exploration of the effect of the *Sho1* gene on *C. albicans*.

The formation of *C. albicans* biofilm is closely related to mycelial formation, adhesion, and drug resistance [39]. Biofilms are typically found on inert materials, but can also occur in living tissues. The biofilm formation process is slow, and antibiotics are often ineffective at completely removing it, leading to recurrent infections during clinical treatment [40–42]. Examination of the biofilm formation and adhesion abilities of *C. albicans* revealed that deletion of the *Sho1* gene decreased the biofilm formation ability of *C. albicans*. Additionally, the results indicated that *Sho1Δ/Δ* exhibited decreased flocculation ability compared to SN152, suggesting that the *Sho1* gene influenced the mutual recognition and adhesion ability of *C. albicans*. Subsequently, SEM and LSCM were used to further observe the effects of the *Sho1* gene on the structure and thickness of *C. albicans* biofilms. The results demonstrated that deletion of the *Sho1* gene led to the inability of *C. albicans* to form biofilms with tight and thick structures, thereby affecting its

pathogenicity.

Subsequently, we established a systemic infection model of *C. albicans* to further investigate the impact of the *Sho1* gene on pathogenicity and immune function. Deletion of the *Sho1* gene extended the survival time of mice infected with *C. albicans* and significantly reduced fungal load in the liver, kidney, and spleen. Histological observations revealed that the *Sho1Δ/Δ*-infected group exhibited reduced renal inflammation and *C. albicans* burden.

Macrophages are crucial components of the innate immune system. M1-type macrophages play a pro-inflammatory role by producing cytokines, such as IL-β, IL-12, and IL-23, while M2-type macrophages are involved in anti-inflammatory responses and tissue repair [15]. Macrophages can undergo phenotypic and functional changes under microenvironmental regulation [16,17]. The balance between M1 and M2 macrophages reflects the extent of inflammation and tissue damage. Our results revealed a decrease in the number of M1-type macrophages, an increase in the number of M2-type macrophages, and a resultant decrease in the M1/M2 ratio in the *Sho1Δ/Δ*-infected group compared to the SN152-infected group.

Th cells are immune cells that play a crucial role in combating fungal infections [18,19]. Th1 cells play a crucial role in activating mononuclear macrophages and neutrophils, regulating immune responses, and combating infections caused by pathogens such as *Staphylococcus aureus*, *S. epidermidis*, and *C. albicans* [19,43]. Th2 cells inhibit Th1 cells, potentially compromising their immune function. An increase in Th2 cells may impair the immune defence of the body against *C. albicans* infection [44]. The IL-17/Th17 ratio are often associated with *C. albicans* infections, particularly mucocutaneous infections, such as oral and cutaneous candidiasis [45–47]. Th17 cells, also known as inflammatory T helper cells, are frequently implicated in proinflammatory responses [48]. Additionally, Th1 and Th17 cells have been implicated in chronic inflammatory and autoimmune diseases [49]. Under normal circumstances, the balance between Th1 and Th2 cells is crucial for maintaining normal immune function [50]. Disruption of this balance can disturb local immune function, thereby compromising the ability of the body to resist fungal infections. Compared to the SN152-infected group, the *Sho1Δ/Δ*-infected group exhibited an increase in the number of Th1 cells and a decrease in the number of Th2 and Th17 cells, resulting in an elevated Th1/Th2 ratio. This suggested that the virulence of *C. albicans* and the level of inflammation decreased *in vivo* following *Sho1* deletion.

5. Conclusions

In summary, this study revealed that the expression level of the *Sho1* gene was higher in clinical strains of *C. albicans* than in standard strains. Next, we constructed *Sho1Δ/Δ* and demonstrated its impact on the formation of *C. albicans* biofilm. *Sho1* deletion resulted in a sparse biofilm structure, reduced thickness, and diminished adhesion and flocculation abilities. In a systemic infection model, deletion of the *Sho1* gene prolonged the survival of mice and reduced the fungal load in the liver, kidney, and spleen. Histological observations revealed that both *C. albicans* and inflammatory cells decreased in the *Sho1Δ/Δ* group. Additionally, deletion of the *Sho1* gene affected immune function *in vivo*, resulting in a decrease in the ratio of M1/M2 macrophages. The proportion of Th1 cells increased, whereas the proportions of Th2 and Th17 cells decreased, leading to an increased Th1/Th2 ratio. Further research into the *Sho1* gene might be helpful to reveal the exact pathogenic mechanism of *C. albicans* and to develop relevant therapeutic strategies.

Data availability statement

No data were generated by the research described in the article.

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Ethics statement

Studies involving human participants were reviewed and approved by the Ethics Committee of the 980th Hospital of the PLA Joint Logistics Support Force (2023-KY-93). The clinical strains used in this study were collected with the verbal informed consent of the patients and were approved by the Ethics Committee of the 980th Hospital of the PLA Joint Logistics Support Force.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

CRediT authorship contribution statement

Mengyan Li: Writing – original draft, Visualization, Validation, Supervision, Software, Methodology, Investigation, Formal

analysis, Data curation. **Hua Wang:** Writing – original draft, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Huihai Zhao:** Visualization, Validation, Resources, Methodology, Formal analysis. **Mengyu Jiang:** Visualization, Validation, Software, Formal analysis, Data curation. **Mengge Cui:** Visualization, Validation, Methodology, Investigation. **Keran Jia:** Writing – review & editing, Resources, Investigation, Funding acquisition, Conceptualization. **Daxin Lei:** Writing – review & editing, Methodology, Formal analysis, Conceptualization. **Fukun Wang:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at bhttps://doi.org/10.1016/j.heliyon.2024.e38219.

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