

Engineering of synthetic bacterium surface for glucan nanofilm assembly against invasive fungal infection

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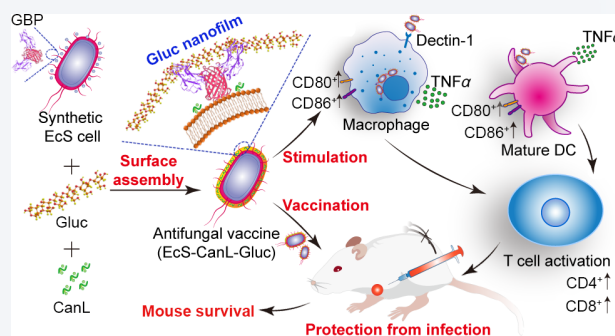
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ABSTRACT: Invasive fungal infections induced by *Candida* species are becoming an urgent threat to global health. While a series of antifungal vaccines have been developed, their efficiency is frequently compromised by the failure of immune stimulation. In this study, a novel antifungal vaccine was constructed by assembling β -glucan with the *Candida albicans* antigen candidalysin on the surface of the genetically engineered *Escherichia coli* strain EcS. This co-assembly was mediated by the cell surface-exposed artificial glucan-binding protein CipC-mCherry-CipC, leading to the formation of the surface glucan nanofilm with a thickness of ~ 80 nm. Upon co-incubation with macrophages, the vaccine efficiently escapes from the lysosomes, leading to enhanced production of reactive oxygen species (ROS) and macrophage maturation. After mouse immunization, the vaccine persists longer in lymph nodes than free candidalysin or the glucan-free complex, and induces higher levels of CD4⁺, CD8⁺, TNF α /CD4⁺ and IFN γ /CD4⁺ T cells in the spleen. In a murine model of invasive fungal infection, immunization with this vaccine remarkably increases the survival rate, reduces the kidney fungal burdens and attenuates inflammatory responses. This bioengineering strategy offers a potentially viable approach for developing co-assembled whole-cell vaccines and can be extended to leverage bacterial carriers for surface antigen display. The vaccine acts not only as antigens but also as adjuvants, thereby eliciting strong and specific protective immunity against invasive fungal infections.



KEYWORDS: synthetic biology, glucan nanofilm, cell-surface engineering, antifungal vaccine, bacterial biotherapy

1 Introduction

Although invasive fungal infections are increasingly prevalent, they have not been sufficiently studied [1–3]. According to the World Health Organization's 2022 fungal priority pathogens list, invasive fungal infections claim > 1.5 million lives annually, with *Candida albicans* accounting for 40% of cases in immunocompromised patients [4]. Even though the pathogenic mechanisms of the most prevalent *Candida* species are becoming increasingly clear [5–8], invasive candidiasis remains a significant global burden [9–11], largely because of the rising number of immunocompromised

individuals [12, 13]. Moreover, the currently available antifungal drugs are inadequate, owing to escalating antifungal resistance, constraints on their modes of administration, and their exorbitant cost [14, 15].

Thus, vaccines against fungal diseases would be highly desirable, and efforts at developing such vaccines are gaining more and more research attention [16–19]. Fungal vaccines promise to enable protection of high-risk groups against invasive fungal infections by using fungal protein antigens, and three fungal vaccines are currently in a Phase 1 clinical trial [20–24]. However, none of the fungal vaccines tested to date have shown efficacy in immunocompromised hosts, owing to poor antigen persistence and weak T cell priming [11]. Consequently, attention has been focused on developing glucan-conjugated protein vaccines, a platform that increases the immunogenicity of protein antigens [25–28]. Nevertheless, the conjugation of glucan and proteins is complicated

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and high-cost at present. This study aims to easily assemble the two kinds of antigens in a cell platform.

Herein, we report a genetically engineered antifungal vaccine against systemic fungal infection. This was accomplished by using the synthetic *Escherichia coli* strain EcS, which expresses the artificial glucan-binding protein (GBP) CipC-mCherry-CipC [29]. The CipC domains function as carbohydrate-binding modules (CBMs) derived from *Clostridium cellulolyticum* and specifically recognize β -glucan polysaccharides through carbohydrate-protein interactions. The tandem arrangement of two CipC domains provides multivalent glucan-binding capacity, thereby enhancing the stability and avidity of glucan assembly on the bacterial surface. Our previous study demonstrated that the CipC-based synthetic bacterial system efficiently recruits glucan-containing polysaccharides through strong surface glucan-binding activity [29]. Unlike glucan-conjugated protein vaccines, our EcS-based vaccine leverages bacterial bio-interfaces for co-assembly of an immune-stimulating β -glucan (Gluc) and the *C. albicans* peptide antigen candidalysin (CanL) to form surface glucan nanofilm, mimicking pathogen-associated molecular patterns (PAMPs) to combine innate and adaptive immunity. CanL was selected in the present

study because it is a key virulence-associated peptide toxin secreted during *Candida albicans* hyphal formation and plays important roles in fungal invasion, epithelial damage, and host immune activation. EcS binds to CanL, which is positively charged, via electrostatic interactions. Meanwhile, the synthetic EcS cells efficiently bound Gluc via surface-exposed GBP, thereby strongly stimulating the immune response by targeting the Dectin-1 receptor in both macrophages and dendritic cells (DCs). T helper 1 (Th1) and T helper 17 (Th17) cells are critical for antifungal immunity. This stimulation further activates spleen T cells, leading to high levels of both CD4⁺ and CD8⁺ cells. Unlike the fungus-infected mice without vaccination suffering from severe kidney inflammation and tissue damage, the infected mice after antifungal vaccination may transport Th1 cells and the fungal antibodies into the kidneys, efficiently clearing fungal cells for host survival (Fig. 1). This is the first genetically engineered bacterium to show promising utility for fungal vaccine design, and our results may facilitate the development of polysaccharide-containing conjugate vaccines that can be used to reduce mortality due to systemic invasive fungal infections.

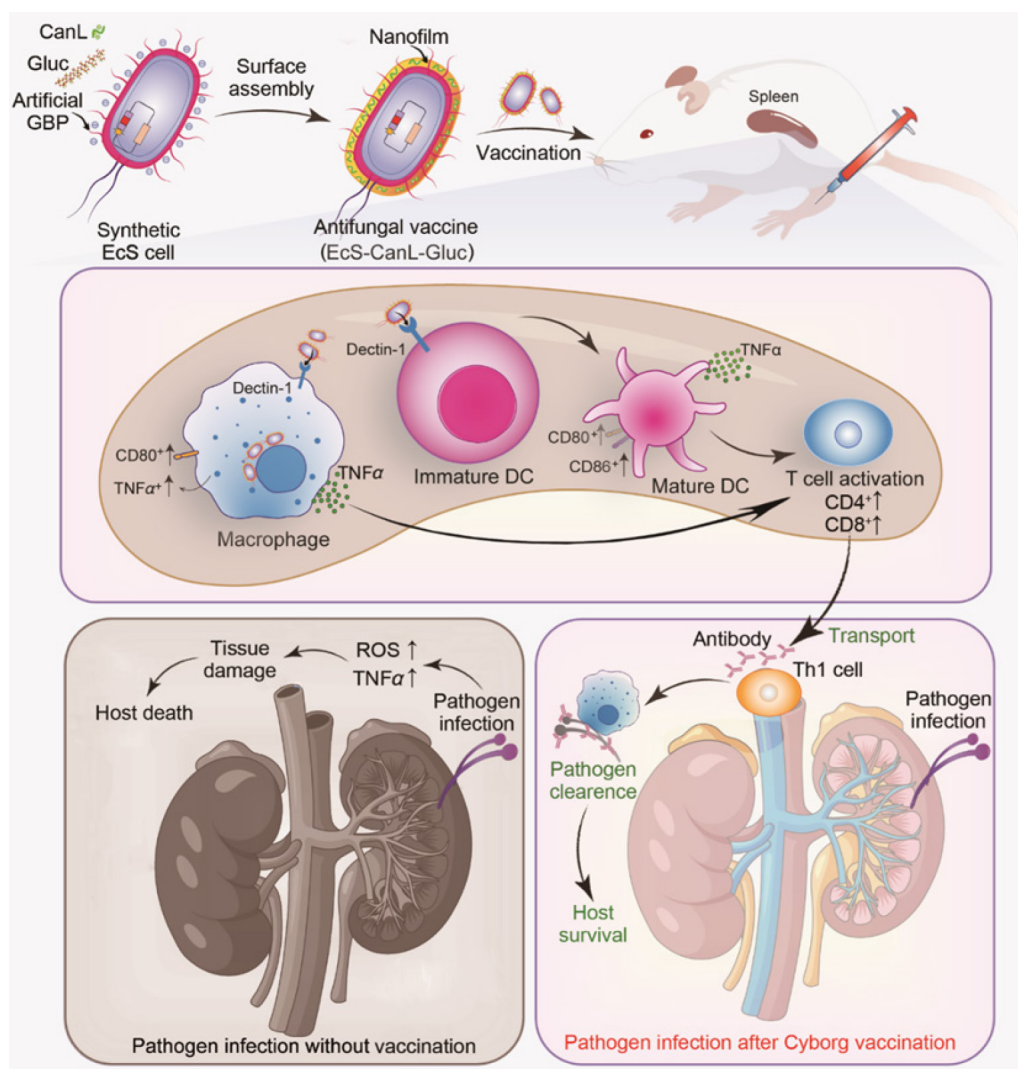


Figure 1 Schematic illustration of the construction of the EcS-CanL-Gluc antifungal vaccine for activation of antigen-presenting DCs and macrophages, and prevention of invasive fungal infections. EcS, the synthetic *E. coli* cells genetically engineered by the plasmid containing the artificial CipC-mCherry-CipC gene. GBP is encoded by CipC-mCherry-CipC.

2 Experimental

2.1 Materials and strains

β -Glucan (Gluc, Cat# G5011), 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) (Cat# D6883) and propidium iodide (PI) (Cat# P4170) were purchased from Sigma-Aldrich. Cell Counting Kit-8 (CCK-8) (Cat# CA1210) was purchased from Solarbio. LysoTracker Green DND-26 (Cat# L7526) was from Thermo Fisher Scientific. 4',6-Diamidino-2-phenylindole (DAPI) (Cat# C1002) was from Beyotime, China. The candidalysin (CanL) peptide and its fluorescein isothiocyanate (FITC)-labeled version (FITC-CanL) were synthesized by ChinaPeptides, China. *E. coli* Ec0 (Control) and *E. coli* EcCMC (EcS, engineered) were from laboratory stock (Yin et al., 2022). The pathogenic *C. albicans* SC5314 strains were clinically isolated from the Third Central Hospital of Tianjin, China. All antibodies, including PE-anti-CD11b, PE-anti-CD11c, PE-anti-CD8, FITC-anti-CD4, FITC-anti-CD80, FITC-anti-F4/80, FITC-anti-Ly6G, APC-anti-CD86, APC-anti-IFN γ , APC-anti-TNF α and APC-anti-NF- κ B p65 were all purchased from BioLegend, USA. The mRNA extraction kit and reverse transcription kit were obtained from TransGen Biotech Co., Ltd. (Beijing, China). All reagents were used without further purification.

2.2 Characterization of bacterial cells and vaccine formulations

The constructed synthetic *E. coli* cells (Ec0 or EcS) were cultured overnight in Luria-Bertani (LB) broth medium with kanamycin (50 μ g/mL) and with shaking at 37 °C. Cells were harvested by centrifugation (5000g, 10 min), washed twice with phosphate-buffered saline (PBS) (pH 7.4). Subsequently, CanL was electrostatically adsorbed onto EcS by incubating with 20 μ g/mL CanL for 5 min at room temperature, then remove the free peptides by centrifugation. For glucan coating, β -glucan (Gluc, 100 μ g/mL) was added to the bacterial suspension and incubated with shaking at 37 °C for 30 min. The mixture was centrifuged and washed three times with PBS in order to remove unbound glucan.

For scanning electron microscopy (SEM), bacterial samples were fixed with 3% formaldehyde at room temperature for 2 h, followed by dehydration through a series of graded ethanol series (30%, 50%, 70%, 90%, and 100%). The samples were then critical-point dried, sputter-coated with gold, and observed under an SEM. Energy-dispersive X-ray spectroscopy (EDS) elemental mapping was conducted to analyze the distribution of elements. Bacterial diameters were measured from SEM images using ImageJ software (NIH).

For fluorescence labeling, Gluc was conjugated with FITC. Labeled Gluc was incubated with Ec0 or EcS, and the binding capacity was assessed by flow cytometry and confocal microscopy. Nuclei were counterstained with DAPI (5 mg/L).

2.3 Cell culturing

The macrophages (RAW264.7) and dendritic cells (DC2.4) were purchased from the Cell Resource Center (CRC). Both kinds of cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (HyClone) containing 10% (v/v) fetal bovine serum (FBS) (HyClone), 100 U/mL Penicillin, and 100 μ g/mL Streptomycin. All cells were incubated under standard conditions (37 °C, 5% CO $_2$, and humidified) and they were cultured into T25 flasks (Corning) once they reached approximately 80% confluency.

2.4 Cell viability assays

DC2.4 cells were seeded and cultured for 24 h in DMEM supplemented with 10% FBS. To evaluate the toxicity, the cultures were treated with designated concentrations of free CanL, Gluc, EcS, CanL+Gluc, EcS-CanL, EcS-Gluc, or EcS-CanL-Gluc for a further 12 h. Cell viability was assessed using a CCK-8 assay kit (Solarbio, China).

2.5 Confocal microscopy

To evaluate the macrophage-targeting capacity of EcS, we incubated RAW264.7 cells with Ec0 and EcS (optical density at 600 nm (OD $_{600}$) = 0.1) for 12 h. Then the cells were stained with DAPI (5 mg/L, Beyotime, China) for 30 min, washed twice with PBS to remove free-floating Ec0 and EcS. Then the cells were observed by confocal laser scanning microscopy (CLSM) (A1+, NIKON, Japan), and the intensities of FITC and mCherry fluorescence were determined by flow cytometry (BD, USA).

To observe vaccine uptake by macrophages, we incubated RAW264.7 cells for 12 h with free FITC-CanL (10 mg/L), FITC-CanL (10 mg/L) + Gluc (100 mg/L), or EcS-FITC-CanL-Gluc. After washing twice with PBS, the cells were stained with DAPI (5 mg/L) for 30 min. They were then imaged by CLSM, and the FITC intensity was quantified by flow cytometry.

To investigate the antigen-presenting ability of the vaccine, we added EcS-CanL, EcS-Gluc, and EcS-CanL-Gluc to RAW264.7 cells. The cells were cultured for 12 h and then stained with LysoTracker Green for observation by CLSM. To analyze the quantitative co-localization, images were processed using ImageJ software.

2.6 In vitro activation of immune cells

The immune-activating effects of the vaccine on dendritic cells and macrophages were assessed by means of ROS assays, TNF α assays, and cell maturation assays. DC2.4 and RAW264.7 cells were each incubated for 12 h with CanL, Gluc, EcS, CanL+Gluc, EcS-CanL, EcS-Gluc, or EcS-CanL-Gluc, and then subjected to flow cytometry. To assess ROS levels, treated cells were incubated with DCFH-DA (10 mg/L) for 10 min, followed by twice washes with PBS.

To evaluate tumor necrosis factor alpha (TNF α) secretion, cells were fixed with 4% paraformaldehyde (PFA), permeabilized with Triton X-100, and washed with PBS. Then cells were stained with allophycocyanin (APC)-anti-TNF α for 30 min and washed twice with PBS.

For assessment of cell maturation, DC2.4 cells were stained with PE-anti-CD11c, FITC-anti-CD80, and APC-anti-CD86. RAW264.7 cells were stained with APC-anti-CD11b, FITC-anti-CD80 and APC-anti-CD86. Following PBS washes, fluorescence intensity was analyzed for all samples.

2.7 RNA-seq analysis

RAW264.7 cells were maintained for 24 h in DMEM with 10% FBS. Following this, the cultures were treated with PBS, Ec0, Ec0-CanL-Gluc, EcS, or EcS-CanL-Gluc and incubated for an additional 12 h. Following microscopic examination to confirm cellular integrity, cells were washed twice with ice-cold 1 $\times$ PBS prepared with diethyl pyrocarbonate (DEPC)-treated water. An appropriate volume of Trizol Reagent (Takara, Cat# 9108) was then added to the cells. The cells were thoroughly lysed by repeated pipetting. The resulting lysate was transferred to a 1.5 mL nuclease-free microcentrifuge tube and stored at -80 °C. Then the entire

workflow, including gel bead-in-emulsion (GEM) generation, complementary DNA (cDNA) amplification, library preparation, and sequencing, was carried out by Personal Biotechnology Co., Ltd. (Shanghai, China). Gene set enrichment analysis (GSEA), heatmap visualization, Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment were performed using the personal cloud platform. RNA sequencing (RNA-seq) was used to analyze transcriptomic changes.

2.8 RT-qPCR analysis

To detect expression of *Cxcl3*, *iNos*, and *Acod1*, cell total mRNAs were extracted using mRNA extraction kits. Total cDNAs were prepared by a reverse transcription kit. Expressions of *Cxcl3*, *iNos*, and *Acod1* were detected by real-time quantitative polymerase chain reaction (RT-qPCR) in an Eppendorf real-time PCR detection system (Realplex2, Eppendorf, USA), using the primers *Cxcl3*-5RT (CAACGGTGTCTGGATGTGTCT), *Cxcl3*-3RT (CCGCCAGCCAAGGAATACTG), *iNos*-5RT (TGGTGAAGGGACTGAGCTGT), *iNos*-3RT (TGCCACCTTTTGACAGTGATG), *Acod1*-5RT (GGATGGAGCCACCTTCACAGATC), and *Acod1*-3RT (AGGACAGCATCTTGGAGGCATTG). *Gapdh* served as the reference gene, using the primers *Gapdh*-5RT (CATCACTGCACCCAGAAGA) and *Gapdh*-3RT (ATCCACGACGGACACATTGG).

2.9 In vivo imaging and assessment of vaccine biosafety in mice

For *in vivo* imaging of immunized mice, vaccines were prepared using FITC-labeled CanL (the final concentration of FITC-labeled CanL was 20 µg/mL) by means of the procedure described above. FITC-CanL, FITC-CanL+Gluc, EcS-FITC-CanL, or EcS-FITC-CanL+Gluc was administered via subcutaneous injection to mice (at a dose of 100 µL per animal). At designated time points post-injection, the mice were anesthetized using ether. The spatial distribution of FITC fluorescence in the mice was captured using a small-animal imaging system (Xenogen, Corp., USA). Upon completion of the prime-boost immunization, major organs (including heart, liver, spleen, lungs, and kidneys) were harvested, and tissue sections were prepared for hematoxylin and eosin (H&E) staining to evaluate biosafety.

2.10 Assays of vaccine-induced spleen T-cell activation

For evaluation of the role of EcS-CanL-Gluc in activating spleen T cells, mice were primed and boosted on days -14 and -7 with PBS, CanL, CanL+Gluc, EcS-CanL, or EcS-CanL-Gluc. Then the spleen samples were collected, homogenized, digested into single cells, washed with PBS, and resuspended for staining. For immunophenotyping, cells were stained with APC-anti-CD3, FITC-anti-CD4, and PE-anti-CD8. The proportion of CD4⁺CD8⁺ cells in CD3⁺ T cells was determined by flow cytometry. To evaluate cytokine production, cells were stained with FITC-anti-CD4 followed by intracellular staining using APC-anti-TNFα or APC-anti-IFNγ antibodies. The percentages of TNFα⁺ and IFNγ⁺ cells among CD4⁺ T cells were analyzed by flow cytometry.

2.11 Induction of systemic *C. albicans* infection mouse model

To assess the therapeutic efficacy of EcS-CanL-Gluc, mice were primed and boosted on days -14 and -7 with PBS (Control), CanL,

CanL+Gluc, EcS-CanL, or EcS-CanL-Gluc. On day 0, all mice were intravenously challenged with *C. albicans* SC5314. Survival, body weight, and kidney fungal burden were monitored from day 0 to 14. On day 3 post infection, kidneys from three mice per group were collected and weighed. Tissue homogenates were prepared using Dounce homogenizers, and fungal loads were quantified by plating serial dilutions on yeast extract peptone dextrose (YPD) agar for colony-forming unit (CFU) enumeration.

2.12 Histopathological and immunofluorescence analysis of tissue sections

On day 7 post-intravenous infection with *C. albicans*, kidneys and spleens of mice were collected and fixed in 4% PFA at 4 °C for 24 h. Tissues were then dehydrated through a graded ethanol series (70%, 80%, 95%, and 100% ethanol, twice; 1 h per step) cleared in xylene (three times, 45 min each) and embedded in paraffin using standard metal molds. After solidification at 4 °C, blocks were trimmed and sectioned at 5 µm thickness with a rotary microtome (Leica, RM2245). Sections were floated on a 45 °C water bath, transferred onto poly-L-lysine-coated slides (Genview, GV-MS-W), and dried overnight at 37 °C prior to staining. For histopathological evaluation, sections were stained with H&E following standard protocols and examined under a light microscope. For immunofluorescence, antigen retrieval was performed by microwave heating in citrate buffer (pH 6.0). After cooling to room temperature, slides were washed three times with PBS (5 min each). Tissues were then incubated with the primary antibody (or dye) diluted 1:500 in PBS, followed by appropriate secondary reagents when needed. After 30 min of incubation in the dark, slides were washed, mounted with antifade medium, and imaged using a CLSM (NIKON A1+).

2.13 Statistical analysis

Data were presented as mean ± standard deviation (SD). Group differences were analyzed using an unpaired two-tailed Student's *t*-test for comparisons between two groups, and one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for comparisons among multiple groups. All statistical analyses were performed with GraphPad Prism (Version 9.0; GraphPad Software, Inc., San Diego, CA, USA). The difference between two groups was considered statistically significant for *P* < 0.05. All data are compared with the control group.

3 Results

3.1 Formation of a genetically engineered antifungal vaccine based on EcS

To co-assemble Gluc and CanL, we used the *E. coli* strain EcS and the control strain Ec0, which express the glucan-binding protein CipC-mCherry-CipC and mCherry (Fig. 2(a)), respectively, to bind Gluc. SEM revealed that the control Ec0 cells did not show an increase in cell diameter. In contrast, the EcS cells were uniformly coated with a glucan layer, with the cell diameter increasing from 400 to 560 nm, indicating the formation of a nanofilm on the EcS surface with a thickness of ~ 80 nm (Figs. 2(b) and 2(c)). EDS elemental mapping further revealed a decrease in the sulfur content on the bacterial surface by 68.8% (Fig. S1 in the Electronic Supplementary Material (ESM)), indicating that the glucan molecules masked the sulfur-containing surface proteins. Flow

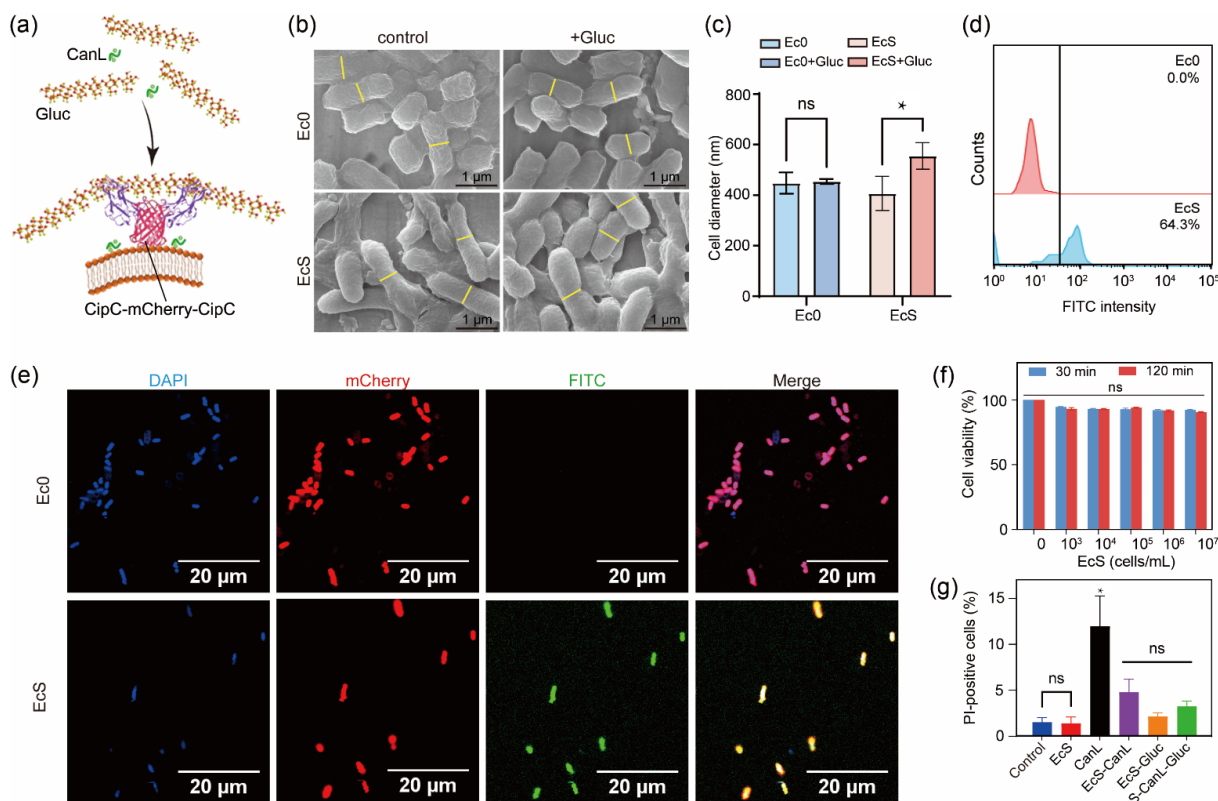


Figure 2 Characterization of Ec0, EcS, and EcS-CanL-Gluc. (a) Schematic illustration of Gluc nanofilm assembly and CanL inclusion on the engineered EcS surface. The EcS cells express and expose CipC-mCherry-CipC for recruitment of glucan, leading to the formation of a glucan nanofilm on the bacterial surface. (b) SEM images of Ec0 and EcS cells and (c) cell diameters before and after incubation with Gluc. (d) FITC intensity and (e) CLSM images of Ec0 and EcS cells after incubation with FITC-labeled Gluc. (f) Viability of DC2.4 dendritic cells after treatment with inactivated EcS at different doses for 24 h. (g) Cell death of DC2.4 cells after incubation with free EcS, CanL, EcS-CanL, EcS-Gluc, or EcS-CanL-Gluc for 24 h. Data are represented as mean $\pm$ SD, ns: not significant, * P < 0.05 versus the Control group by one-way ANOVA with Tukey's post-hoc test. Error bars indicate standard deviations (n = 3).

cytometry and CLSM confirmed that the EcS cells bound strongly to FITC-labeled Gluc, whereas negligible fluorescence was observed when Ec0 was incubated with FITC-glucan (Figs. 2(d) and 2(e), and Fig. S1 in the ESM). Taken together, these results suggested that EcS effectively interacted with Gluc via the surface-exposed CipC-based glucan-binding proteins. The CipC domains function as carbohydrate-binding modules capable of specifically recognizing β -glucan chains through multivalent polysaccharide-protein interactions, thereby enabling stable glucan nanofilm formation on the bacterial surface.

To construct an antifungal vaccine capable of eliciting robust and sustained immune activation, we leveraged the dual functionality of EcS, which not only recruits β -glucan via surface-displayed carbohydrate-binding modules but also binds to the positively charged fungal peptide CanL through electrostatic forces. We determined the cytotoxicity of different formulations by using CCK-8 assays and PI staining for flow cytometry. The CCK-8 assays revealed that the viability of DC2.4 dendritic cells was not impaired by EcS cells, even at a bacterial dose of 1×10^7 CFU/mL. Moreover, neither CanL at 0–10 mg/L nor Gluc at 0–100 mg/L obviously affected DC2.4 viability (Fig. 2(f) and Fig. S2 in the ESM). Thus, the antifungal vaccine EcS-CanL-Gluc was constructed by co-assembly of 20 μ g/mL CanL and 100 μ g/mL Gluc on the surface of EcS [30, 31]. Although the proportion of PI-positive cells was 12.5% after 24 h of incubation with CanL, the proportion was < 5% after incubation with EcS-CanL-Gluc (Fig. 2(g)). This finding indicates that EcS-CanL-Gluc had better biosafety than CanL alone. SEM

confirmed that the structural integrity of EcS-CanL-Gluc was unaffected by antigen loading (Fig. S3 in the ESM). These data demonstrate that EcS-CanL-Gluc may function as a safe antifungal vaccine capable of delivering both toxic peptide antigens and immune-stimulating polysaccharides.

3.2 Enhancement of antigen uptake and presentation by the antifungal vaccine

We propose that the glucan-binding proteins and the captured Gluc displayed on the EcS surface facilitated recognition by macrophages, thereby promoting uptake of the antifungal vaccine via receptor-mediated endocytosis (Figs. S4(a)–S4(c) in the ESM). To confirm the role of EcS in macrophage targeting and uptake promotion, FITC-CanL was used to track antigen uptake by macrophages. While FITC-CanL alone or the antigen with the mCherry-labeled Ec0 cells scarcely entered macrophages, both mCherry-labeled EcS and FITC-CanL were abundantly co-localized in the macrophages (Fig. 3(a)). This observation was confirmed by flow cytometry. EcS-FITC-CanL-Gluc led to higher levels of FITC-positive macrophages than FITC-CanL-Gluc and EcS-FITC-CanL (46.6% versus 0.4%–26.3%, Fig. 3(b)). These results revealed the enhanced antigen uptake by the antifungal vaccine.

Moreover, we evaluated the ability of the EcS-CanL-Gluc antifungal vaccine to take in and present antigens in macrophages by using lysosomal fluorescence staining. In the EcS-CanL-Gluc group, the mCherry fluorescence was partially co-localized with the

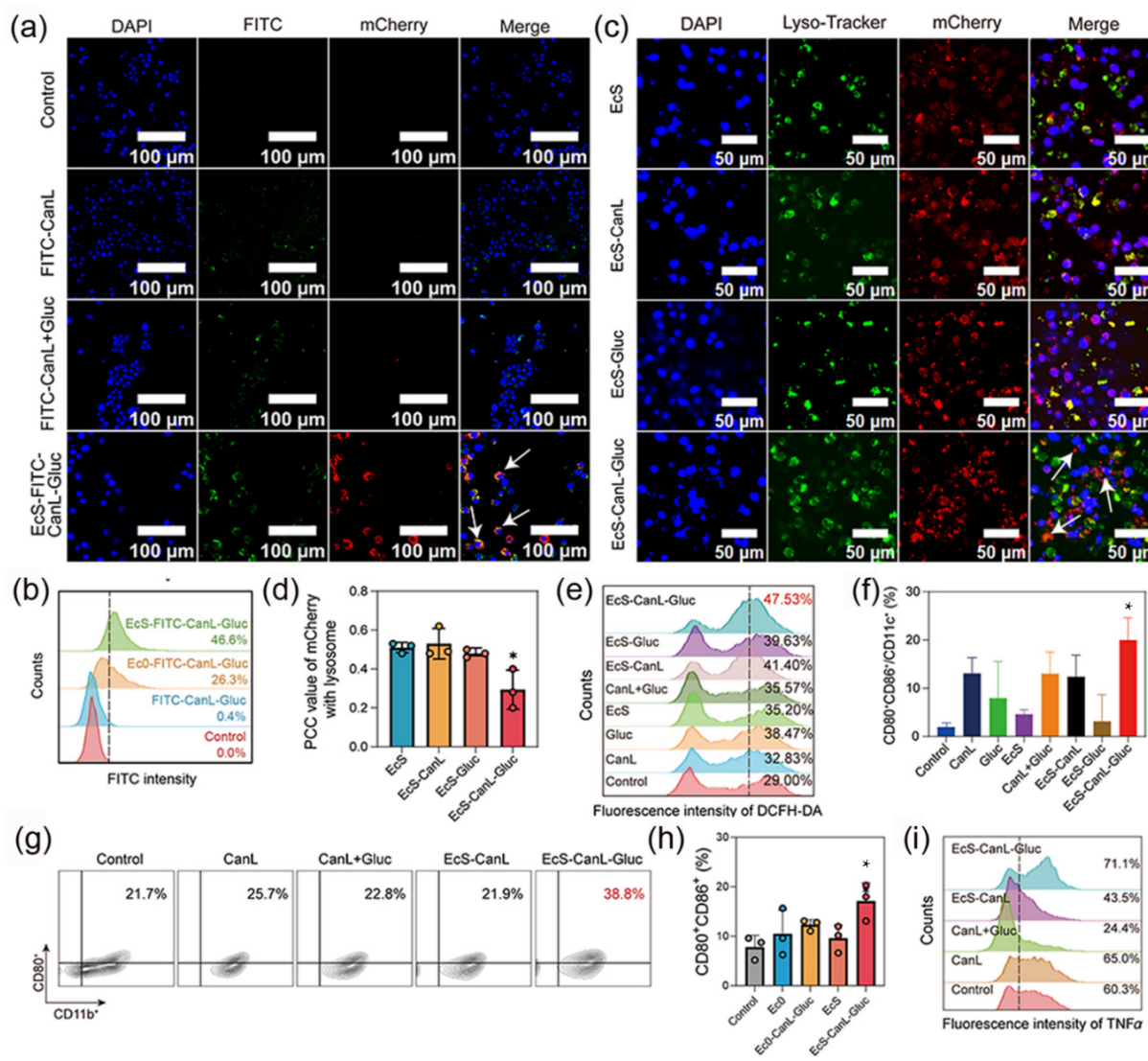


Figure 3 Promotion of antigen uptake and activation of antigen-presenting cells by the antifungal vaccine. (a) CLSM images and (b) flow cytometry analysis of RAW264.7 cells treated with FITC-CanL, FITC-CanL+Gluc, or EcS-FITC-CanL-Gluc for 12 h. Scale bars: 100 μ m. The white arrows indicate representative intracellular regions showing co-localization of EcS cells and FITC-CanL signals within macrophages. (c) CLSM images of RAW264.7 cells after 12 h of treatment with EcS, EcS-CanL, EcS-Gluc, or EcS-CanL-Gluc. The white arrows indicate antigen that was not co-localized with LysoTracker Green. Scale bars: 50 μ m. (d) Pearson's correlation coefficient (PCC) of mCherry to LysoTracker FITC. (e) ROS level of treated DC2.4 cells. (f) Percentage of CD80⁺CD86⁺ cells in DC2.4 cells after incubation with various vaccine formulations for 12 h. (g) The proportion of CD80⁺ and (h) CD80⁺CD86⁺ cells in macrophages. (i) Intracellular TNF α levels in the treated macrophages. Data are represented as mean $\pm$ SD, ns: not significant, * P < 0.05 versus the Control group by one-way ANOVA with Tukey's post-hoc test. Error bars indicate standard deviations (n = 3).

LysoTracker fluorescence (Fig. 3(c)), and the Pearson's correlation coefficient was found to be lower than the coefficients of the other three groups (0.28 versus 0.49–0.53, Fig. 3(d)). This finding suggests that the antifungal vaccine facilitated antigen escape from lysosomes for further processing.

3.3 Stimulation of antigen-presenting cell maturation and activation by the antifungal vaccine

Exogenous substances are known to stimulate the generation of ROS in immune cells [32–37]. To determine whether this occurred in our system, we quantified intracellular ROS levels in DC2.4 dendritic cells exposed to various vaccine components and formulations using DCFH-DA staining. Notably, exposure to EcS-CanL-Gluc resulted in higher ROS levels than exposure to free CanL or free Gluc (Fig. 3(e)). Meanwhile, the higher proportion of

CD80⁺CD86⁺ positive cells in EcS-CanL-Gluc treated DC2.4 cells also indicated the activation of antigen-presenting ability (Fig. 3(f)). Furthermore, flow cytometry revealed that treatment of macrophages with EcS-CanL-Gluc led to an increase in the percentage of mature CD11b⁺CD80⁺ macrophages from 21.7% to 38.8% (Fig. 3(g)), CD80⁺CD86⁺ double-positive cells increased by approximately 13% (Fig. 3(h) and Fig. S5(a) in the ESM) and an increase in the percentage of TNF α -positive macrophages from 60.3% to 71.1% (Fig. 3(i)). Of note, relatively high TNF α signals were also observed in the Control and CanL groups under the current experimental conditions because of the intrinsic inflammation in RAW264.7 macrophages, while candidalysin itself may directly induce inflammatory cytokine production as a virulence-associated fungal peptide toxin. Therefore, TNF α expression was interpreted together with ROS generation, antigen

uptake and maturation marker expression to comprehensively evaluate the immunomodulatory effects of the engineered vaccine system. Moreover, the percentage of NF- κ B (p65) positive cells in EcS-CanL-Gluc treated RAW264.7 cells was significantly increased (9.38% versus 0.62%–2.03%, Fig. S5(b) in the ESM). Together, these results indicated effective macrophage acquisition of the critical co-stimulatory function and revealed that the antifungal vaccine markedly enhanced the activation and maturation of macrophages and dendritic cells. To preliminarily evaluate whether the engineered bacterial platform could potentially interact with additional carbohydrate-derived molecules, cyclodextrin-containing EcS formulations were prepared and incubated with dendritic cells. Compared with the control group, the cyclodextrin-treated formulations induced moderate immune activation effects, although the activity remained lower than that of the β -glucan-containing system (Fig. S6 in the ESM). Considering the structural differences between cyclic α -1,4-linked cyclodextrins and β -glucan polysaccharides, the detailed interaction mechanism requires further investigation.

3.4 Remodeling of macrophage transcriptomic profiling by the antifungal vaccine

To further characterize the molecular and functional profiles of macrophages primed with EcS-CanL-Gluc, we determined differentially expressed genes (DEGs) of RAW264.7 cells between EcS-CanL-Gluc and control or DEGs between EcS-CanL-Gluc and Ec0-CanL-Gluc. RNA-seq analysis uncovered the unique and specific reprogramming of macrophage gene expression induced by the antifungal vaccine (Fig. 4 and Fig. S6 in the ESM). Venn diagram analysis identified a core set of DEGs not elicited by the control formulations (Fig. S6(a) in the ESM). Volcano plot analysis confirmed widespread transcriptional changes in the vaccine group (Fig. 4(a) and Fig. S6(b) in the ESM). Notably, the upregulation of key chemokines, including *Cxcl3* and *Ccl5*, suggests the immediate capacity of the antifungal vaccine-treated macrophages to recruit T cells, natural killer (NK) cells and neutrophils. Moreover, the upregulation of *Vcam1* indicates enhanced adhesion and infiltration of lymphocytes and monocytes. The significant induction of *Acod1* is consistent with the activation of the Dectin-1-spleen tyrosine kinase (SYK) signaling axis by the surface glucan, leading to itaconate production. This represents the potential for metabolic remodeling and epigenetic reprogramming induced by training immunity. Concurrently, the coordinated upregulation of *H2-K1* and *Tap1* suggests the activation of the major histocompatibility complex class I (MHC-I) antigen presentation pathway to potentiate a robust CD8⁺ cytotoxic T lymphocyte response crucial for eliminating intracellular pathogens.

Clustering and enrichment analyses of the vaccine-specific DEGs provided a systems-level view of these responses, indicating the activation of the IL-17 and TNF signaling pathways, the complement and coagulation cascades (Fig. 4(b)). GO enrichment analysis revealed significant involvement in biological processes such as immune response, defense response, chemokine-mediated signaling, cell migration, and immune system regulation (Fig. 4(c)). KEGG pathway analysis further corroborated these findings, highlighting significant enrichment in key signaling pathways including IL-17, TNF, chemokine, Toll-like receptor, cytokine-cytokine receptor interaction, and NF- κ B pathways (Fig. 4(d) and Figs. S6(c)–S6(e) in the ESM). Finally, the mRNA expression levels of *Cxcl3*, *iNos*, and *Acod1* were validated by RT-qPCR, and the

relative expression of these genes was all significantly upregulated in the vaccine group (Figs. 4(e)–4(g)). These changes all indicate that the Gluc on the vaccine surface, acting as a pattern recognition molecule, triggered the SYK/NF- κ B signaling pathway via the Dectin-1 receptor which drives macrophages to turn to the M1 type and stimulated the secretion of chemokines, thereby recruiting effector cells. Concurrently, CanL as a PAMP activates pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) and NOD-like receptors (NLRs) on immune cells. This activation promotes antigen-specific Th1 and Th17 immune responses through pathways such as inflammasome activation. The enrichment of the NOD-like receptor pathway may be associated with immune recognition of bacterial cell components during the macrophage processing of engineered bacterial particles. This synergistic effect strengthens the host's innate and adaptive immune defense networks. The immune-regulatory effects of the current vaccine system likely arise from synergistic interactions among the engineered bacterial biointerface, fungal antigen incorporation, and surface glucan nanofilm assembly.

3.5 Activation of spleen T cells by the antifungal vaccine

Based on the promising *in vitro* performance of the antifungal vaccine, we conducted *in vivo* vaccination studies. Initially, free FITC-CanL, FITC-CanL+Gluc, EcS-FITC-CanL, or EcS-FITC-CanL-Gluc was subcutaneously injected into the axillary and inguinal regions of mice. Antigen migration was tracked at 1 and 24 h post-injection using an *in vivo* imaging system. As shown in Fig. 5(a), fluorescence signals from free FITC-CanL were detected in the lymph nodes 1 h after injection, but the signal intensities had decreased substantially by 24 h after injection, indicating rapid elimination of the majority of free antigen from the body. On the contrary, 24 h after vaccine injection, the majority of the antigen was retained in the lymph node regions. Fourteen days after injection, mice in all the treatment groups had normal heart, liver, spleen, lung, and kidney structures, similar to those of the control mice (Fig. 5(b)). Flow cytometry showed that mice treated with the vaccine had the highest proportion of CD3⁺ T cells (42.2% of all spleen cells, Fig. 5(c)), with the highest proportions of CD4⁺ and CD8⁺ cells in CD3⁺ T cells (Figs. 5(d)–5(f)). Moreover, the vaccine induced the highest proportions of TNF α -positive and IFN γ -positive cells in CD4⁺ T cells (Figs. 5(g) and 5(h)), indicating that the vaccine strongly activated spleen T cells. Interestingly, on day 3 after prime-boost vaccination, the EcS-CanL-Gluc group displayed approximately 3-fold higher inguinal lymph node weight compared to other groups (Fig. 5(i)) along with the highest percentages of CD4⁺ and CD8⁺ T cells in lymph nodes (Fig. 5(j)). These findings may be attributable to the engineered glucan nanofilm biointerface, which forms a vaccine system with reduced rapid diffusion and clearance compared with free antigen. In addition, the surface glucan nanofilm may enhance interactions with antigen-presenting cells through glucan-recognition pathways, thereby promoting cellular uptake, lymph node trafficking, and prolonged antigen retention within draining lymph nodes. During the prime-boost vaccination period from day –14 to 0, the engineered glucan nanofilm vaccine likely promoted sustained antigen presentation, antigen-presenting cell activation, and T-cell priming prior to fungal challenge. The prolonged retention of the vaccine within draining lymph nodes may further facilitate the establishment of protective antifungal immune responses before infection.

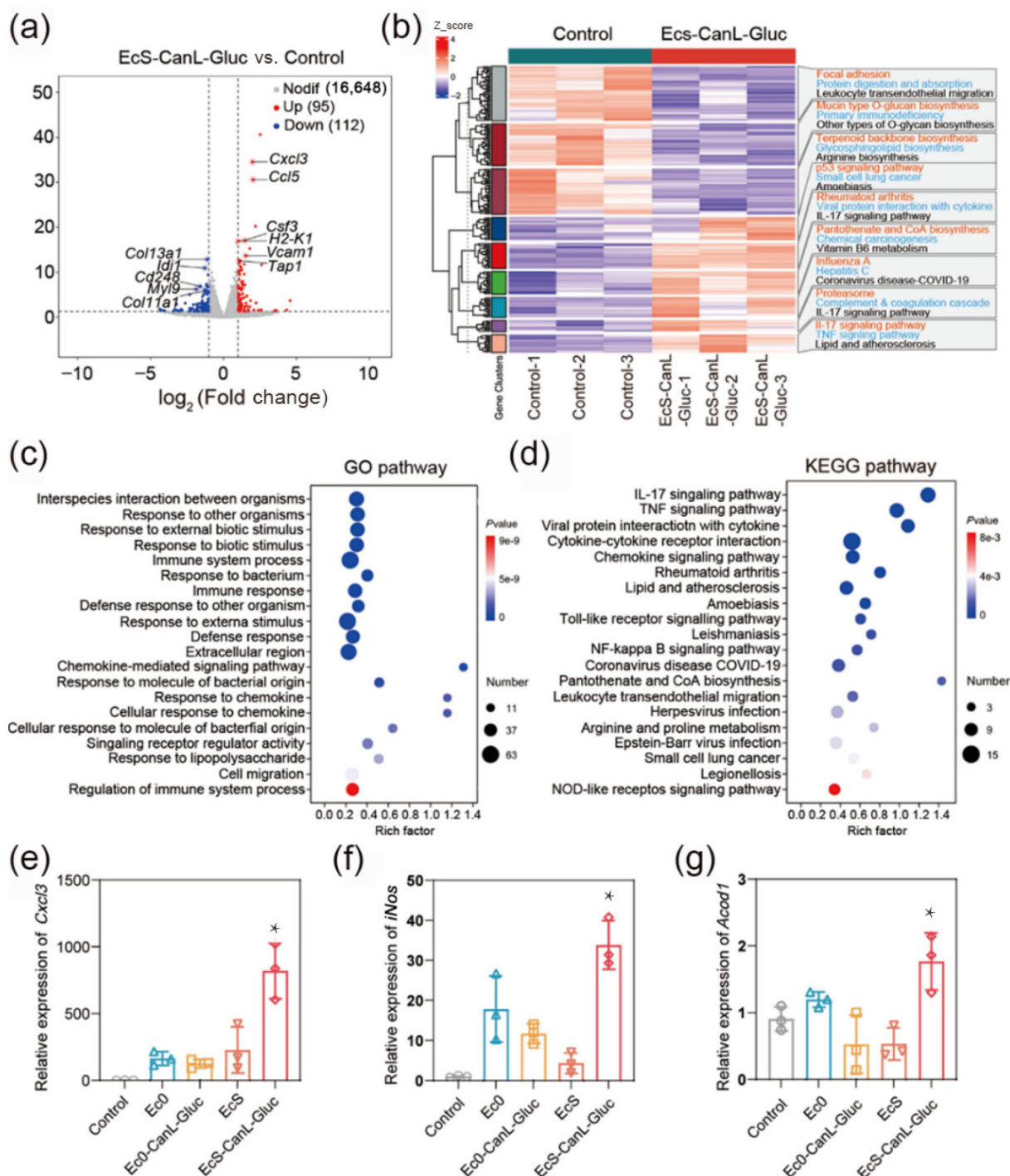


Figure 4 Remodeling of macrophage transcription profiling by the antifungal vaccine. (a) Volcano plots display DEGs from the comparison of the vaccine group (Ecs-CanL-Gluc) versus the control group. (b) Heatmap and representative enriched pathway for the vaccine-specific DEGs. Bubble plots of (c) GO Biological Process and (d) KEGG pathway enrichment analysis performed on the upregulated DEGs by Ecs-CanL-Gluc. The relative expression of (e) *Cxcl3*, (f) *iNos* and (g) *Acod1* detected by RT-qPCR (*Gapdh* as the reference). Data are represented as mean $\pm$ SD, ns: not significant, * $P < 0.05$ versus the Control group by one-way ANOVA with Tukey's post-hoc test. Error bars indicate standard deviations ($n = 3$).

3.6 Prevention of systemic *C. albicans* infection by the antifungal vaccine

We used the fungal pathogen *C. albicans* to evaluate the efficacy of EcS-CanL-Gluc as a vaccine against systemic infection after a standard prime-boost vaccination procedure (Fig. 6(a)). Specifically, mice were immunized on day -14 and again on day -7 with CanL,

CanL+Gluc, EcS-CanL, or EcS-CanL-Gluc and were infected with *C. albicans* on day 0. On days 3 and 7, kidney fungal burdens, immune cells and cytokine levels were assessed. We found that approximately 67% of the mice immunized with EcS-CanL-Gluc were alive 14 days after infection (Fig. 6(b)). In all groups, the body weights of the mice decreased during the first 5 days after fungal infection and all the mice other than those immunized with EcS-

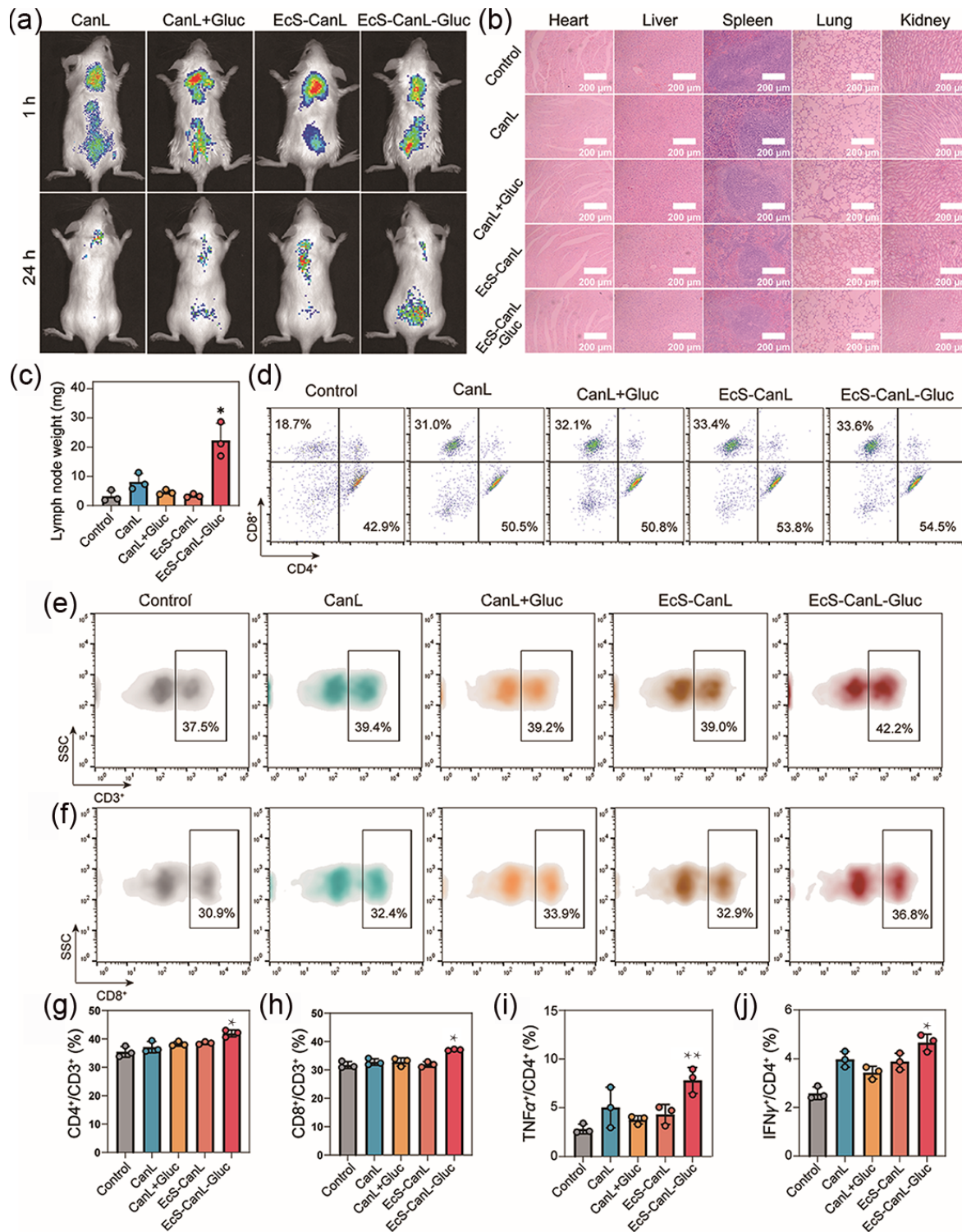


Figure 5 *In vivo* distribution and immune-stimulating activity of the antifungal vaccine in mice. (a) Distribution of FITC-CanL *in vivo* 1 and 24 h after injection. (b) H&E staining of heart, liver, spleen, lungs, and kidneys on day 3 after prime-boost vaccination. Scale bars: 200 μ m. (c) Weights of inguinal lymph nodes in vaccinated mice. (d) Flow cytometry analysis of CD8⁺ and CD4⁺ T cells in lymph nodes. Percentages of (e) CD3⁺, (f) CD8⁺ T cells in spleens. The ratio of (g) CD4⁺/CD3⁺ and (h) CD8⁺/CD3⁺ T cells in spleens. (i) Percentages of TNF α -producing CD4⁺ T cells. (j) Percentages of IFN γ -producing CD4⁺ T cells. Data are represented as mean $\pm$ SD, ns: not significant, * P < 0.05 versus the Control group by one-way ANOVA with Tukey's post-hoc test. Error bars indicate standard deviations (n = 3).

CanL-Gluc which likely recovered from the infection had died by day 9 (Fig. 6(c)). On day 3 after infection, we assessed kidney weights, fungal burdens, and histopathological structure. The control group experienced pronounced fungal invasion and

inflammation, marked by the highest kidney weight (333 mg/mouse, Fig. 6(d) and Fig. S7(a) in the ESM) and highest fungal burden (2.55×10^5 CFU/g kidney, Fig. 6(d) and Fig. S7(a) in the ESM). In contrast, the mice in the EcS-CanL-Gluc group showed

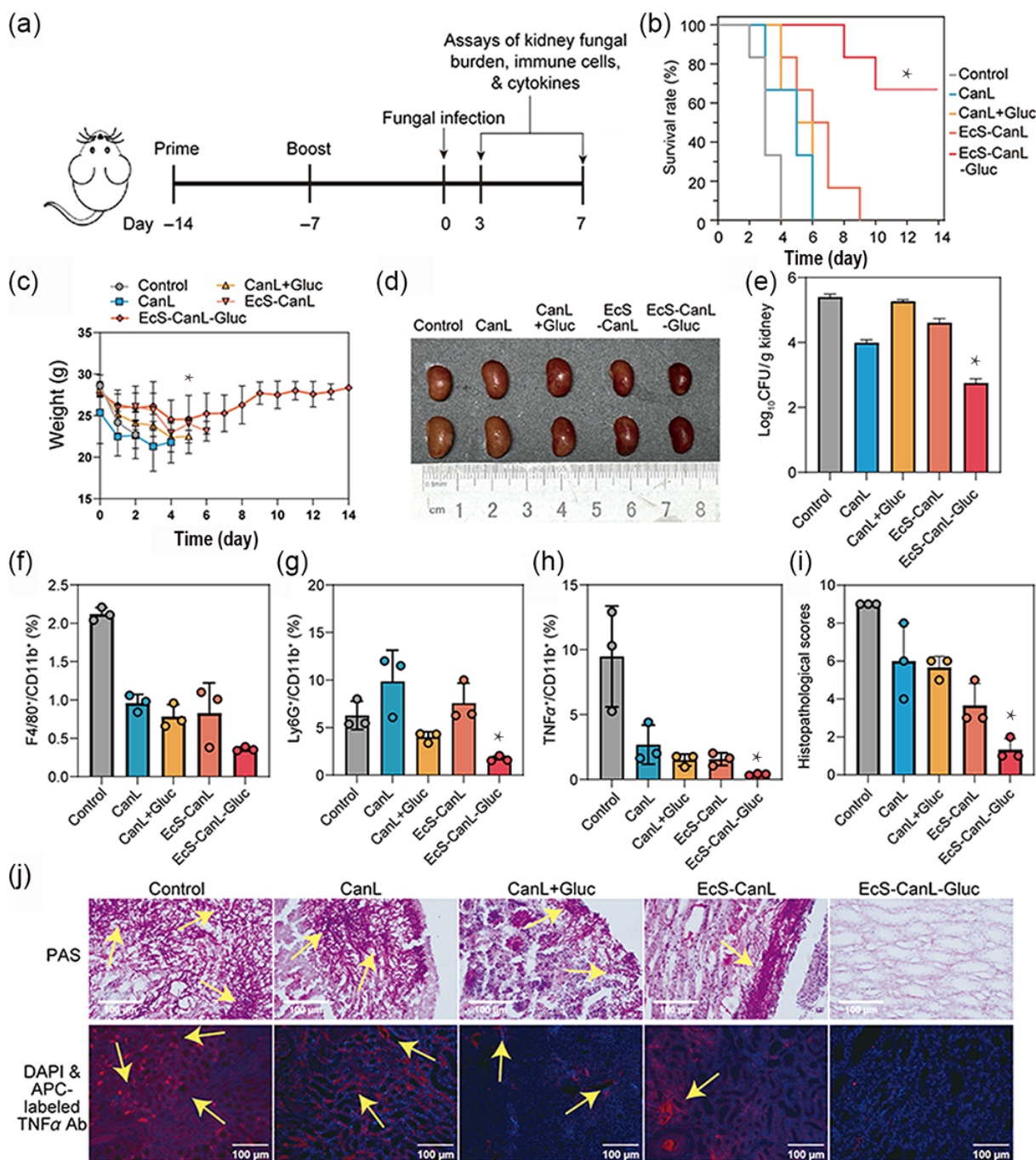


Figure 6 *In vivo* prevention of systemic *C. albicans* infection by the antifungal vaccine. (a) Schematic illustration of vaccination procedure, fungal infection, and assays of immune response in mice. (b) Survival rates. (c) Temporal dependence of body weight after infection with *C. albicans*. (d) Kidney photos and kidney weights on day 3 ($n = 3$). (e) Kidney fungal burdens ($n = 3$). Levels of (f) F4/80⁺/CD11b⁺, (g) Ly6G⁺/CD11b⁺, and (h) TNFα⁺/CD11b⁺ cells in kidney tissues ($n = 3$). (i) Histopathological scores of the kidneys. (j) Histopathological images of the mouse kidneys on day 3 revealed by periodic acid-Schiff (PAS) staining and immunofluorescence staining of TNFα. Yellow arrows indicate *C. albicans* hyphae invading the kidney tissues (PAS images, top) and the accumulation of TNFα (immunofluorescence images, bottom). Data are represented as mean $\pm$ SD, ns: not significant, * $P < 0.05$ versus the Control group by one-way ANOVA with Tukey's post-hoc test. Error bars indicate standard deviations ($n = 3$).

the lowest kidney weight (240 mg/mouse, Fig. 6(d) and Fig. S7(a) in the ESM) and lowest fungal burden (2.67×10^3 CFU/g kidney, Fig. 6(e)). Inflammation is the critical process mediating tissue damage and mouse death after fungal infection. A series of inflammation-related indicators were detected by flow cytometry and immunofluorescence staining. Consistent with the above result, flow cytometry of kidney cells showed the lowest proportion of

F4/80⁺ cells, Ly6G⁺ cells, and lowest TNFα level in CD11b⁺ cells for the EcS-CanL-Gluc group (Figs. 6(f)–6(h)). Assessment of histopathology revealed that the *C. albicans* infection caused severe kidney inflammation in mice, characterized by a high histopathological score, inflammatory cell infiltration, detachment of renal tubular epithelial cells, and diffused hyphal invasion (Figs. 6(i) and 6(j) and Fig. S7(b) in the ESM). All treatment groups

showed inflammation attenuation relative to the control, though the extent varied. While CanL alone, CanL+Gluc or EcS-CanL could partially restore renal tubules and reduce the infiltration of inflammatory cells, a high fungal burden persisted. Hyphae (deep pink filaments) were clearly observed in the histopathological images of the kidneys from the mice except the EcS-CanL-Gluc group (Fig. 6(j) and Fig. S7(b) in the ESM). Statistical analysis showed that the EcS-CanL-Gluc group had a significant decrease in the histopathological score compared to other treatment groups (Fig. 6(i)). Consistently, histopathological images of the kidney tissues by immunofluorescence staining of TNF α and H&E staining revealed that the inflammation of the kidney tissue from EcS-CanL-Gluc treated mice was relieved (Fig. 6(j) and Fig. S7(b) in the ESM).

The spleen pro-inflammatory cells were further detected by paraffin immunofluorescence and flow cytometry assays (Fig. 7). Immunofluorescence CLSM images revealed marked CD11b⁺Ly6G⁺ cell infiltration in spleen tissue from the control group (as well as the CanL, CanL+Gluc, and EcS-CanL groups, Fig. 7(a)). Notably, although fungal infection was not completely eliminated in the EcS-CanL-Gluc group, the CD11b⁺Ly6G⁺ inflammatory signals in infected tissues became substantially weaker and more localized compared with the control groups, suggesting reduced inflammatory cell infiltration and alleviated fungal infection-associated tissue inflammation after vaccination. Furthermore, flow cytometry analysis revealed that CD11b⁺Ly6G⁺ cells constituted 54% of total splenic cells in the control group, while the CanL, CanL+Gluc, and EcS-CanL groups exhibited 15.4%, 38.2%, and 48% CD11b⁺Ly6G⁺ cells, respectively (Figs. 7(b) and 7(c)). This indicates that the mice were experiencing a severe immune storm and systemic infection. In contrast, the EcS-CanL-Gluc group exhibited a CD11b⁺Ly6G⁺ cell proportion of merely 6.89%, potentially indicating that mice with the antifungal vaccine could nearly recover to normal from fungal infection. Concurrently, their CD11b⁺F4/80⁺ cell proportion (representing macrophages) was only 1.44% (distinct from the control group's 30.2%, Figs. 7(d) and 7(e)). Moreover, the EcS-CanL-Gluc group exhibited significantly lower CD4⁺IL17⁺ cell levels than the control group, indicating reduced inflammation (Figs. 7(f) and 7(g)). Therefore, engineered bacteria equipped with cell-surface glucan nanofilm effectively protected mice from invasive fungal infections.

4 Discussion

Currently, invasive fungal infection has posed a serious threat to global public health [5] while the exploration of effective strategies to prevent invasive fungal infections remains a major challenge [38]. Existing treatment of invasive fungal infections mainly relies on limited antifungal drugs such as echinocandins, triazoles, and polyenes [39], which generally face severe challenges such as delayed diagnosis, increased drug resistance, drug toxicity and the balance between efficacy and safety in immunocompromised populations [40, 41]. Vaccines are regarded as one of the most cost-effective and successful methods to eliminate infectious diseases [42]. Thus, the development of efficient fungal vaccines has profound clinical application prospects. However, although three fungal vaccines have entered the clinical trial stage, there is still no clinically approved fungal vaccine [43], and the core bottleneck lies in how to induce strong and lasting protective T cell immunity in immunocompromised hosts [44].

While polysaccharide-protein conjugate vaccines represent a

significant advance in fungal vaccines by enhancing T-cell-dependent responses, their clinical translation remains hindered by challenges in precise antigen presentation and the need for robust adjuvant systems [25, 45]. Herein, we report a synthetic bioinspired antifungal vaccine platform EcS-CanL-Gluc. Notably, the glucan assembly process relies on the surface-displayed CipC-based carbohydrate-binding modules, which specifically recognize β -glucan polysaccharides through multivalent carbohydrate-protein interactions. This engineered biointerface enables efficient glucan recruitment and stable nanofilm formation on bacterial surfaces. Such glucan recruitment is likely driven by multivalent supramolecular interactions between the CipC-based carbohydrate-binding modules and β -glucan chains, including hydrogen-bonding and hydrophobic stacking interactions, as previously reported for CBM-containing glucan-recognition systems. By co-displaying the *C. albicans* virulence factor candidalysin (CanL) and β -1,3-glucan (Gluc) on the surface of an engineered *E. coli* (EcS), we created a biomimetic bacterium that synergistically activates multiple arms of the immune system, conferring superior protection in an *in vivo* systemic candidiasis model (Fig. 1).

Studies have demonstrated that β -glucan can be recognized by specific receptors such as Dectin-1 on immune cell surfaces including macrophages, dendritic cells, and neutrophils [46–48]. The engagement of the Dectin-1 receptor on antigen-presenting cells (APCs) triggers SYK-mediated activation, which promotes ROS production and the secretion of pro-inflammatory cytokines [49–52]. In our study, the engineered bacteria EcS combined with CanL and Gluc have a better capacity to target macrophages compared with free CanL or CanL+Gluc. Meanwhile, the assembly of these three substances has no obvious cytotoxicity (Fig. 2) and promotes the maturation, activation, and antigen presentation of macrophages and dendritic cells (Fig. 3). RNA-seq results further showed that the antifungal vaccine platform potently induced ROS and upregulated *Aco1* (encoding itaconate) in macrophages (Fig. 4), which is consistent with Dectin-1/SYK pathway activation and suggests the induction of trained immunity—a metabolic and epigenetic reprogramming that enhances nonspecific host defense [53, 54]. Crucially, unlike soluble glucan or simple mixtures, the particulate presentation of Gluc on a biointerface provided a sustained danger signal and promoted efficient phagocytic uptake, as demonstrated by our CLSM and flow cytometry data.

A critical bottleneck in fungal vaccine development has been the generation of strong, antigen-specific T-helper cell responses, particularly Th1 and Th17 profiles, which are essential for clearing fungal pathogens [55]. As the first cytolytic peptide toxin secreted by *Candida albicans*, candidalysin plays a key role in mucosal and systemic infections [56]. It can strongly activate the mitogen-activated protein kinase (MAPK) signal, epidermal growth factor receptor (EGFR) pathway and NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, and stimulate the release of cytokines including IL-1 β , IL-17 and C-X-C motif chemokine ligand 1 (CXCL1) [57]. These characteristics can be used to design fusion protein vaccines or adjuvant systems to enhance the immunogenicity of vaccines [58]. At the same time, candidalysin, as a vaccine component, can drive the proliferation and IL-17 secretion of natural Th17 cells, promote the recruitment of neutrophils and induce protective immunity biased towards Th17 [59]. This is very important for antifungal protection. In this paper, the fungal antigen CanL was chosen for its role as a key cytolytic peptide and T-cell epitope. However, antigenicity alone is

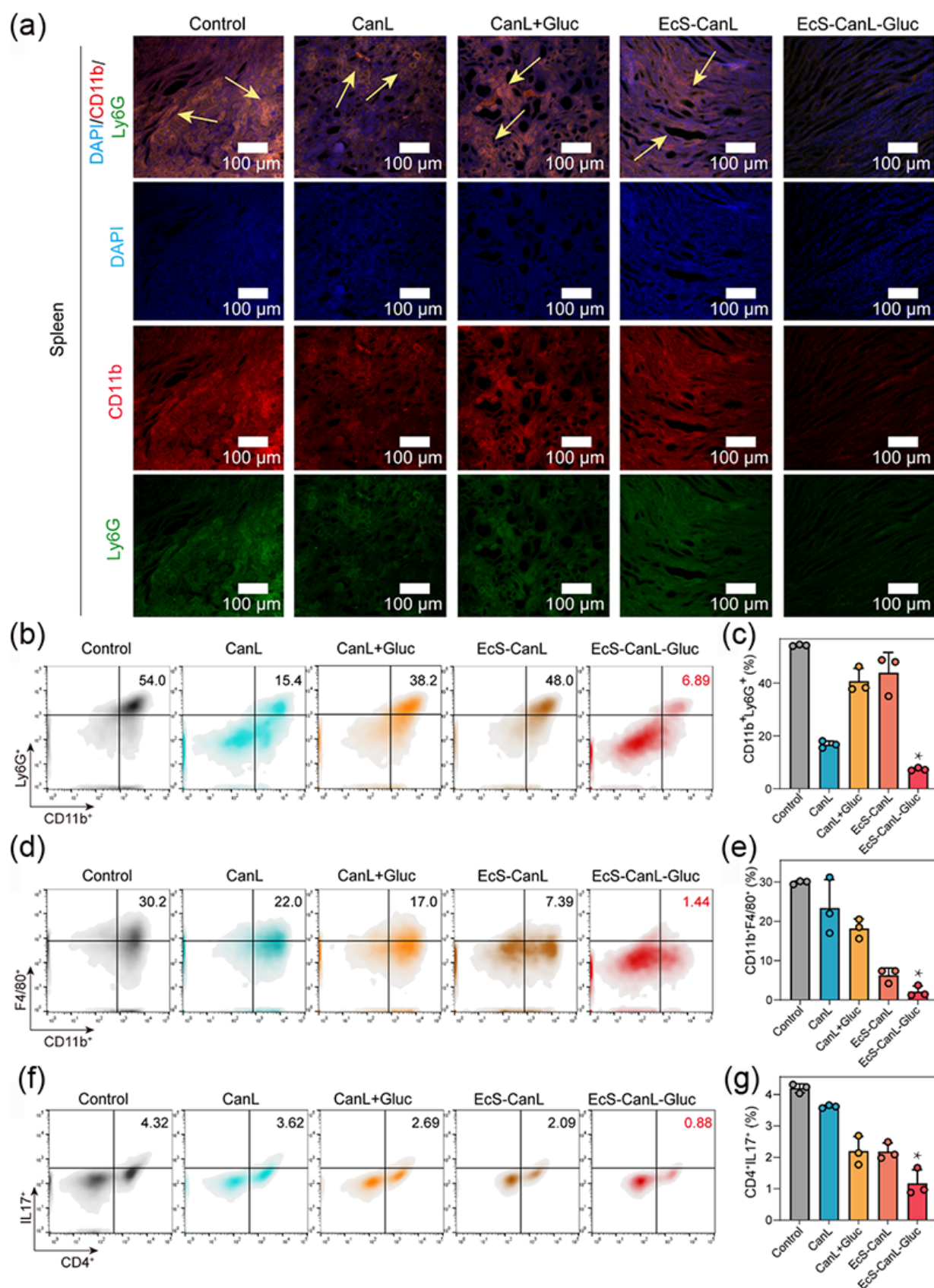


Figure 7 Paraffin immunofluorescence and flow cytometry assays of the spleen cells. (a) Immunofluorescence staining of CD11b⁺Ly6G⁺ cells in spleen cells. Yellow arrows indicate representative CD11b⁺Ly6G⁺ positive regions in infected tissues. Flow cytometry density plots of (b) CD11b⁺Ly6G⁺, (d) CD11b⁺F4/80⁺, and (f) CD4⁺IL17⁺ cells in spleen cells, and flow cytometry assays of (c) CD11b⁺Ly6G⁺, (e) CD11b⁺F4/80⁺, and (g) CD4⁺IL17⁺ cells in spleen cells. (*) indicates significant difference between the EcS-CanL-Gluc group and other groups ($n = 3$, $*P < 0.05$).

insufficient; effective cross-presentation and co-stimulation are paramount. Our platform addressed this problem by engineering a spatially co-localized delivery of antigen (CanL) and adjuvant (Gluc) on a single particle. This design likely facilitated the co-delivery of both components into the same APC cells, promoting optimal lysosomal escape as indicated by reduced LysoTracker colocalization and subsequent MHC presentation. The RNA-seq analysis of EcS-CanL-Gluc treated macrophages, showing upregulation of *Cxcl3*, *Ccl5*, and MHC-I related genes (*H2-K1*, *Tap1*), further confirmed the establishment of a microenvironment conducive to T-cell recruitment and activation (Fig. 4). Meanwhile, the *in vivo* immune results showed a dramatic enhancement in T-cell priming, evidenced by the significant expansion of CD4⁺ and CD8⁺ T cells in the lymph nodes and spleen, and a pronounced polarization toward IFN γ ⁺ and TNF α ⁺CD4⁺ T cells—a hallmark of protective Th1 immunity against *C. albicans* (Figs. 5(i) and 5(j)).

Our *in vivo* efficacy data robustly validated this immunological priming. Collectively, our results demonstrated that EcS-CanL-Gluc confers superior protection against invasive *C. albicans* infections by effectively suppressing both fungal burden and host hyperinflammatory responses. Flow cytometric analysis of kidney tissues revealed markedly reduced infiltration of F4/80⁺ macrophages, Ly6G⁺ neutrophils, and diminished TNF α production in CD11b⁺ cells, indicating alleviation of local inflammation [60, 61]. Histopathological evaluation further confirmed that, unlike other treatment groups, EcS-CanL-Gluc nearly abolished renal tissue damage, as evidenced by significantly lower histopathological scores, reduced inflammatory cell infiltration, preservation of tubular epithelial integrity, and absence of hyphal invasion (Fig. 6). Consistently, spleen analyses demonstrated that EcS-CanL-Gluc effectively mitigated systemic immune activation. While control and other treatment groups exhibited substantial CD11b⁺Ly6G⁺ cell expansion—indicative of severe inflammatory responses—this population was dramatically reduced in the EcS-CanL-Gluc group, approaching near-baseline levels. Similarly, the proportion of CD11b⁺F4/80⁺ macrophages and CD4⁺IL17⁺ cells was significantly decreased (Fig. 7).

Taken together, these findings indicate that the glucan nanofilm-engineered bacterial system not only enhances antifungal efficacy but also prevents excessive immune activation, thereby achieving a balanced host response. This dual function—simultaneous pathogen clearance and inflammation control—highlights its potential as an effective strategy for combating invasive fungal infections. Macrophages and dendritic cells may contribute to antifungal protection through distinct but complementary immunological functions. Macrophages likely participate primarily in early innate immune responses, including fungal recognition, phagocytosis, inflammatory cytokine secretion, and antifungal clearance. In contrast, dendritic cells may mainly contribute to antigen presentation, lymph node immune activation, and T-cell priming, thereby facilitating adaptive antifungal immunity. Therefore, the protective efficacy of the engineered glucan nanofilm vaccine is likely mediated through coordinated innate and adaptive immune activation rather than relying on a single immune cell population.

Compared to existing strategies, our antifungal platform offers distinct advantages. Firstly, it moves beyond traditional chemical conjugation, offering a modular, genetically programmable biointerface for antigen and adjuvant display. Secondly, the use of a

non-pathogenic, engineered bacterial vector itself provides intrinsic immune-stimulatory properties (e.g., via residual TLR agonists) and a scalable production chassis [62–64]. While live bacterial vectors have been explored [65–67], their application in antifungal vaccines, particularly as a platform for *in vitro* assembled and biomimetic PAMP complexes, is novel. Future work must address potential safety considerations for clinical translation, such as using killed engineered bacteria or further genetic detoxification, and test efficacy in immunocompromised models that more accurately reflect the target patient population. Although the current engineered bacterial biointerface was primarily optimized for β -glucan assembly, the modular nature of carbohydrate-binding module-mediated surface engineering may potentially allow extension toward additional bioactive polysaccharides through rational engineering of polysaccharide-recognition motifs. Such programmable polysaccharide assembly strategies may further broaden the applicability of engineered nanofilm-based immunomodulatory platforms.

Beyond peptide antigen incorporation, the engineered bacterial biointerface may potentially support the future development of nucleic acid-based vaccine delivery systems through rational surface engineering and incorporation of nucleic acid-binding functionalities. Such programmable multicomponent nanofilm assembly strategies may further broaden the applicability of engineered immunomodulatory platforms for the future development of antifungal, antibacterial, and antiviral vaccines. Moreover, the engineered glucan nanofilm biointerface may also possess broader immunotherapeutic implications. The intrinsic immunomodulatory properties of the glucan nanofilm and bacterial surface-associated signals may contribute to innate immune activation, antigen presentation, and immune microenvironment remodeling, thereby providing potential opportunities for future biointerface-based immunotherapies against chronic infectious diseases and tumor progression.

5 Conclusions

In summary, we developed a genetically engineered antifungal vaccine, designated EcS-CanL-Gluc, as a novel platform for protection against invasive fungal infections. The fungal antigen CanL was co-assembled with Gluc on the surface of the *E. coli* strain EcS, which has surface-exposed carbohydrate-binding modules, mimicking PAMPs to engage both innate and adaptive immunity. The vaccine not only enhances antigen uptake and presentation by antigen-presenting cells but also promotes macrophage and dendritic cell maturation, leading to robust T-cell activation and a polarized Th1/Th17 response. In a murine model of systemic candidiasis, immunization with EcS-CanL-Gluc significantly improved survival, reduced fungal burden in kidneys, and attenuated inflammatory pathology. This vaccine platform has the potential to provide significant protection against a wide range of life-threatening fungal infections, and its ability to stimulate a strong and targeted immune response makes it a promising candidate for clinical trials [25, 68–70]. These findings underscore the potential of synthetic biology-driven, bacterium-based co-assembly strategies in developing effective vaccines against invasive fungal infections. Taken together, this study established an adaptable platform for future fungal vaccine design, demonstrating promising efficacy and great prospect for clinical translation.

Electronic Supplementary Material: Supplementary material (EDS elemental mapping, cell viability and ROS levels, SEM images, uptake capacity, immune-activating capacity, venn diagram analysis, volcano plot analysis and GSEA analysis, kidney weight, and histopathological structures) is available in the online version of this article at <https://doi.org/10.26599/NR.2026.94908908>.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript and the Electronic Supplementary Material. Additional data related to this paper may be requested from the corresponding author upon request.

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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.

Author contribution statement

Y. J. G.: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing – original draft, writing – review & editing. Q. L. Y.: Conceptualization, methodology, software, validation, investigation, resources, data curation, writing – original draft, writing – review & editing, visualization, supervision, project administration, funding acquisition. Z. S. S.: Software, validation, formal analysis, investigation, resources, data curation, writing – review & editing. J. Z.: Validation, writing – review & editing. H. S.: Validation. Y. T. W.: Validation. M. S. Z.: Validation. M. C. L.: Resources, data curation, visualization, writing – review & editing.

Informed consent

Not applicable.

Ethics statement

All animal procedures were performed in strict accordance with institutional guidelines and were approved by the Animal Care and Use Committee of Nankai University (Approve number: 2024-SYDWLL-000297).

Use of AI statement

None.

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