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## 1. Extended Data

Figure #	Figure title One sentence only	Filename This should be the name the file is saved as when it is uploaded to our system. Please include the file extension. i.e.: <i>Smith_ED_Fig1.jpg</i>	Figure Legend If you are citing a reference for the first time in these legends, please include all new references in the Online Methods References section, and carry on the numbering from the main References section of the paper.
Extended Data Fig. 1	Transcriptomic profiling PBMCs stimulated with live <i>C. albicans</i> or <i>C. auris</i> and respective cell wall components $\beta$ -glucans and mannans for 4 and 24 hours.	Ext_data_figure1.ep s	<b>a</b> , Principal component analysis (PCA) performed on normalized count data (normTransform, DESeq2) demonstrates the main component introducing variance in the dataset is time (40%), as indicated by a clear split between the early 4-hour host induced response (left, triangle) and the late 24-hour response (right, circle). To a lower extent (15%), the second component introducing variance appears to be inherent to the stimulus (color). <b>b</b> , At 4 hours, PCA reveals a clear donor clustering (shape) irrespective of stimulus (color), indicating the main variance in the early host response reflects inter-individual differences (left). PCA on the late response, 24 hours, is predominantly influenced by the respective stimulus (38%, color), and to a lower extent by the donors (19%, shape), indicated by the scattering of stimuli together with a rough clustering amongst donors. <b>c</b> , Pathway enrichment plot displaying the top 20 enriched pathways for both <i>C. albicans</i> live and <i>C. auris</i> live (color) at 24 hours. Enrichment determined using Consensus PathDB, including pathways as defined by KEGG and Reactome (shape), considering a p-adjusted value < 0.01 (indicated as 'q-value') significant. Size of the geometric points indicates the amount of DEG in relation to the pathways' size. <a href="#">The exact q values and the data used to make this figure can be found in Source Data Extended Data Fig.1.</a>
Extended Data Fig. 2	Comparative LDH secretion,	Ext_data_figure2.ep	<b>a</b> , Assessment of <i>Candida</i> induced cell death of PBMCs after 24 hours

	<p>LDH cytokine gene expression and phagocytosis dynamics between <i>C. albicans</i> and <i>C. auris</i>.</p>	<p>s</p>	<p>stimulation without (RPMI; negative control) or with <i>C. albicans</i>, several <i>C. auris</i> strains originating from all five geographical clades or a positive control (dead cells). Lactate dehydrogenase (LDH) was detected as measure of cell death (Mean <math>\pm</math> SEM, n=6, pooled from two independent experiments). <b>b</b>, Log<sub>2</sub>Fold Change (Log<sub>2</sub>FC) of <i>IL-6</i>, <i>IL-1<math>\beta</math></i>, and <i>IL-1RN</i> (encoding for IL-1Ra) gene expression in PBMCs from 3 donors stimulated for 24 hours with <i>C. albicans</i> (1006110) and <i>C. auris</i> (KCTC17810, clade II) and their respective cell wall components, <math>\beta</math>-glucans (left) and mannans (right). Graphs represent Log<sub>2</sub>FC from DEG analysis. * <math>p &lt; 0.05</math>, ** <math>p &lt; 0.01</math>, *** <math>p &lt; 0.001</math>, 1-way ANOVA with correction for multiple comparison. <b>c</b>, The BMDM phagocytic capacity of Thimerosal-fixed <i>C. albicans</i> or <i>C. auris</i> strains in the course of 3-hours. BMDM engulfment depicted as the percentage of macrophages having phagocytosed at least one fungal cell (left), and the phagocytic index, here considered as the number of fungal cells engulfed per 100 macrophages (right); graphs represent mean, n=9, pooled from at least two independent experiments. <b>d</b>, BMDM phagocytic capacity of Thimerosal-fixed <i>C. albicans</i> or <i>C. auris</i> strains after 1 hour. Engulfment is depicted as the percentage of macrophages having phagocytosed at least one fungal cell; graphs represent mean <math>\pm</math> SEM, n = 9, pooled from at least two independent experiments. <b>e</b>, BMDM phagocytic capacity of live <i>C. albicans</i> or <i>C. auris</i> strains after 1 hour. Engulfment is depicted as the percentage of macrophages having phagocytosed at least one fungal cell. Graphs represent mean <math>\pm</math> SEM, n = 9 (n=7 for <i>C. auris</i> 10051893), pooled from at least two independent experiments. * <math>p &lt; 0.05</math>, ** <math>p &lt; 0.01</math>, *** <math>p &lt; 0.001</math>, <b>d</b> 1-way ANOVA with a Holm-Sidak's multiple comparison test, <b>e</b> Kruskal Wallis test with two-sided Dunn's multiple comparison. <b>f</b>, Distribution of phagocytosed Thimerosal-fixed fungal cells per macrophage in a 3-hour period, n<math>\geq</math>100 observations per condition. <a href="#">Data used to make this figure can be found in Source Data</a></p>
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			<a href="#">Extended Data Fig. 2.</a>
Extended Data Fig. 3	Relative <i>C. auris</i> induced ROS production and heat-sensitivity of the cell wall components responsible for the <i>C. auris</i> induced cytokine production.	Ext_data_figure3.ep s	<p><b>a</b>, Neutrophil ROS release after 1-hour stimulation without (RPMI; negative control) or with heat-killed <i>C. albicans</i>, <i>C. auris</i> strains or zymosan (positive control), depicted in relative light units (RLU) either as time-course (left) or as area under the curve (AUC, right), n=9. <b>b</b>, PBMC ROS release after 1-hour stimulation without (RPMI; negative control) or with heat-killed <i>C. albicans</i>, <i>C. auris</i> strains or zymosan (positive control), depicted in RLU either as time-course (left) or as AUC (right), n=6. <b>c</b>, TNF-<math>\alpha</math>, IL-6, IL-1<math>\beta</math>, and IL-1Ra levels in the supernatant of PBMCs after stimulation without (RPMI; negative control) or with heat-killed <i>C. albicans</i> and <i>C. auris</i> from all five geographical clades for 24 hours, n=8. <b>d</b>, PBMC production of cytokines IFN-<math>\gamma</math> (n=10; n=7 for <i>C. auris</i> 10051895), IL-10 (n=6), IL-17 (n=6), and IL-22 (n=14; n=6 for <i>C. auris</i> 10051893; n=11 for <i>C. auris</i> 10051895) after stimulation without (RPMI; negative control) or with heat-killed <i>C. albicans</i> and <i>C. auris</i> for 7 days.</p> <p>Graphs represent mean <math>\pm</math> SEM, data are pooled from at least two independent experiments. * p &lt; 0.05, ** p &lt; 0.01, *** p &lt; 0.001, **** p = 0.001, <b>a-b</b> Time curves (left panels) were assessed for statistical differences between <i>C. auris</i> strains and <i>C. albicans</i> by a two-way ANOVA, Area Under curve (AUC) means (right panels) were compared using the two-sided Wilcoxon signed rank test, <b>c-d</b> two-sided Wilcoxon matched pairs signed-rank test comparing respective <i>C. auris</i> strains with <i>C. albicans</i> as control or reference species. <a href="#">Data used to make this figure can be found in Source Data Extended Data Fig. 3.</a></p>
Extended Data Fig. 4	Transcriptional changes induced by purified cell wall components and their respective exposure on <i>C. albicans</i> and <i>C. auris</i>	Ext_data_figure4.ep s	<p><b>a</b>, Heatmap displaying the Log<sub>2</sub>Fold change (color scale) of the top 50 DEG of <i>C. albicans</i> live, for both <i>Candida</i> species and their cell wall components, <math>\beta</math>-glucan and mannan, at 4 hour (left panel) and 24 hours (right panel). <b>b</b>, Flow cytometry plot based on forward scatter component (FSC) and side scatter component (SSC), demonstrating <i>C.</i></p>

	surface.		<p><i>auris</i> strains are slightly smaller and of higher complexity than <i>C. albicans</i>. <b>c</b>, Flow cytometry-based comparison of cell wall components of <i>C. albicans</i> and <i>C. auris</i> strains. Mean fluorescent intensity (MFI) of thimerosal-fixed <i>Candida</i> cells stained for Fc-Dectin-1, a marker for <math>\beta</math>-glucan (left), and ConA, a marker for mannans (right). Graphs represent mean <math>\pm</math> SEM of the 3 means, each performed with three replicates in three independent measurements, * <math>p &lt; 0.05</math>, Kruskal Wallis test with two-sided Dunn's multiple comparison test was performed comparing the respective <i>C. auris</i> strains with the two <i>C. albicans</i> reference strains. <a href="#">Data used to make this figure can be found in Source Data Extended Data Fig. 4.</a></p>
Extended Data Fig. 5	Evaluation of cytokine production upon <i>C. albicans</i> and <i>C. auris</i> mannan stimulation.	Ext_data_figure5.ep s	<p><b>a</b>, PBMC production of cytokines TNF-<math>\alpha</math>, IL-6, IL-1<math>\beta</math>, and IL-1Ra after 24 hours stimulation without (RPMI; negative control) or with purified mannans from <i>C. albicans</i> and <i>C. auris</i> strains in the presence of 10% heat-inactivated human serum, n=7. <b>b</b>, PBMC production of cytokines IFN-<math>\gamma</math> (n=6), IL-17 (n=9), and IL-22 (n=9) after 7 days hours stimulation without (RPMI; negative control) or with purified mannans from <i>C. albicans</i> and <i>C. auris</i> strains in the presence of 10% human serum. Graphs represent mean <math>\pm</math> SEM, data pooled from at least two independent experiments. * <math>p &lt; 0.05</math>, two-sided Wilcoxon matched pairs signed-rank test, comparing respective <i>C. auris</i> strains with <i>C. albicans</i> as control or reference species. <a href="#">Data used to make this figure can be found in Source Data Extended Data Fig. 5.</a></p>
Extended Data Fig. 6	Cytokine levels in plasma and organ homogenates from <i>C. albicans</i> and <i>C. auris</i> -infected mice.	Ext_data_figure6.jp g	<p><b>a</b>, IL-6 production in plasma and supernatants from liver homogenates. <b>b</b>, IFN-<math>\gamma</math> production in supernatants from kidney and spleen homogenates. <b>c-e</b>, IL-1<math>\beta</math> (c), IL-17 (d), and IL-10 (e) production in plasma and supernatants from liver, kidney, and spleen homogenates. Mice have been infected i.v. with <math>1 \times 10^6</math> CFU of <i>C. albicans</i> or <i>C. auris</i>. Graphs represent mean <math>\pm</math> SEM, n=6 per group per time-point pooled from two independent experiments. <a href="#">Data used to make this figure can be found in Source Data Extended Data Fig. 6.</a></p>

Extended Data Fig. 7	Applied gating strategies across flow cytometry experiments.	Ext_data_figure7.eps	<p><b>a</b>, Gating strategy for FITC-labelled <i>Candida</i> in PBMCs (linked to Figure 2b). All events were plotted based on forward scatter (FS) and side scatter (SS) characteristics. In the upper plot (2.1) the region of cells positive for FITC-<i>Candida</i> was highlighted (green gate) while in the bottom plot (2.2) CD14 positive cells are represented (red gate) gated within the total PBMCs population (1). Within the CD14+ cells selection, the amount of phagocytosed FITC positive <i>Candida</i> was examined by plotting (3) the FITC signal against the CD14-PB450 signal (blue gate) and the percentage of cells and mean fluorescent intensity (MFI) were used for analysis. <b>b</b>, Gating strategy for Thimerosal-fixed <i>Candida</i> cells stained for either <math>\beta</math>-glucan using Fc-Dectin-1 or ConA as marker for mannans (linked to Figures S4c).</p>
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6 **2. Supplementary Information:**

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8 **A. Flat Files**

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Item	Present?	Filename This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. The extension must be .pdf	A brief, numerical description of file contents. i.e.: <i>Supplementary Figures 1-4, Supplementary Discussion, and Supplementary Tables 1-4.</i>
Supplementary Information	Yes	SI_Supplementary_tables.pdf	Supplementary Tables 1-4

Reporting Summary	Yes	Complete_Bruno_reporting_summary.pdf
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14 **B. Additional Supplementary Files**

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Type	Number If there are multiple files of the same type this should be the numerical indicator. i.e. "1" for Video 1, "2" for Video 2, etc.	Filename This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. i.e.: <i>Smith_Supplementary_Video_1.mov</i>	Legend or Descriptive Caption Describe the contents of the file
Supplementary Video	1	S1.mp4	<i>C. auris</i> is able to multiply within phagosomes.
Supplementary Video	2	S2.mp4	<i>C. auris</i> accumulates in high numbers within macrophages and does not induce macrophage lysis.
Supplementary Video	3	S3.mp4	<i>C. auris</i> cells are taken up extensively into a subpopulation of macrophages.
Supplementary Video	4	S4.mp4	Phagocytosis of <i>C. albicans</i> SC5314, macrophage lysis after 3 hours.

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18 **3. Source Data**

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Figure	Filename This should be the name the file is saved as when it is uploaded to our system, and should include the file extension. i.e.: <i>Smith_SourceData_Fig1.xls</i> , or <i>Smith_Unmodified_Gels_Fig1.pdf</i>	Data description i.e.: Unprocessed Western Blots and/or gels, Statistical Source Data, etc.
Source Data Fig. 1	SD_Main_Fig1.xlsx	Supporting data for Fig. 1
Source Data Fig. 2	SD_Main_Fig2.xlsx	Supporting data for Fig. 2
Source Data Fig. 3	SD_Main_Fig3.xlsx	Supporting data for Fig. 3
Source Data Fig. 5	SD_Main_Fig5.xlsx	Supporting data for Fig. 5
Source Data Fig. 6	SD_Main_Fig6.xlsx	Supporting data for Fig. 6
Source Data Extended Data Fig. 1	SD_Ext_Data_Fig1.xlsx	Supporting data for Extended Data Fig. 1.
Source Data Extended Data Fig. 2	SD_Ext_Data_Fig2.xlsx	Supporting data for Extended Data Fig. 2.
Source Data Extended Data Fig. 3	SD_Ext_Data_Fig3.xlsx	Supporting data for Extended Data Fig. 3.
Source Data Extended Data Fig. 4	SD_Ext_Data_Fig4.xlsx	Supporting data for Extended Data Fig. 4.
Source Data Extended Data Fig. 5	SD_Ext_Data_Fig5.xlsx	Supporting data for Extended Data Fig. 5.
Source Data Extended Data Fig. 6	SD_Ext_Data_Fig6.xlsx	Supporting data for Extended Data Fig. 6.

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**Host immune response against the emerging fungal pathogen *Candida auris*:  
transcriptional and functional insights**

Mariolina Bruno<sup>#1</sup>, Simone Kersten<sup>#1, 2</sup>, Judith M. Bain<sup>3</sup>, Martin Jaeger<sup>1</sup>, Diletta Rosati<sup>1</sup>,  
Michael D. Kruppa<sup>4</sup>, Douglas W. Lowman<sup>4</sup>, Peter J. Rice<sup>5</sup>, Bridget Graves<sup>4</sup>, Ma Zuchao<sup>4</sup>, Y. Ning  
Jiao<sup>4</sup>, Anuradha Chowdhary<sup>6</sup>, George Renieris<sup>7</sup>, Frank L. van de Veerdonk<sup>1, 8</sup>, Bart-Jan  
Kullberg<sup>1, 8</sup>, Evangelos J. Giamarellou-Bourboulis<sup>7</sup>, Alexander Hoischen<sup>1, 2</sup>, Neil A. R. Gow<sup>3, 9</sup>,  
Alistair J. P. Brown<sup>3, 9</sup>, Jacques F. Meis<sup>8, 10, 11, \*</sup>, David L. Williams<sup>4, \*</sup>, Mihai G. Netea<sup>1, 12, \*</sup>

<sup>1</sup> Department of Internal Medicine, Radboud Institute for Molecular Life Sciences, Radboud  
University Medical Center, Nijmegen, the Netherlands

<sup>2</sup> Department of Human Genetics, Radboud Institute for Molecular Life Sciences, Radboud  
University Medical Center, Nijmegen, the Netherlands

<sup>3</sup> Medical Research Council Centre for Medical Mycology, University of Aberdeen, Foresterhill,  
Aberdeen, UK

<sup>4</sup> Departments of Surgery, Biomedical Sciences and Center of Excellence in Inflammation,  
Infectious Disease and Immunity, Quillen College of Medicine, East Tennessee State University,  
Johnson City, TN, USA

<sup>5</sup> Department of Clinical Pharmacy, Skaggs School of Pharmacy and Pharmaceutical Sciences,  
University of Colorado, Aurora, CO, USA

<sup>6</sup> Department of Medical Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, New  
Delhi, India

<sup>7</sup> 4th Department of Internal Medicine, National and Kapodistrian University of Athens,  
Medical School, Athens, Greece.

<sup>8</sup> Center of Expertise in Mycology, Radboud University Medical Center and Canisius Wilhelmina  
Hospital, Nijmegen, the Netherlands

<sup>9</sup> MRC Centre for Medical Mycology, University of Exeter, Geoffrey Pope Building, Stocker  
Road, Exeter EX4 4QD, UK.

<sup>10</sup> Bioprocess Engineering and Biotechnology Graduate Program, Federal University of Paraná,  
Curitiba, PR, Brazil

<sup>11</sup> Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital,  
Nijmegen, the Netherlands

<sup>12</sup> Department for Genomics & Immunoregulation, Life and Medical Sciences Institute (LIMES),  
University of Bonn, Germany

<sup>#</sup>These authors contributed equally

<sup>\*</sup>These authors share senior authorship

**Corresponding author:**

Mihai G. Netea

Department of Internal Medicine (463) and Radboud Center for Infectious Diseases (RCI),  
Radboud University Nijmegen Medical Centre, Geert Grooteplein 8, Nijmegen 6500 HB, the  
Netherlands. mihai.netea@radboudumc.nl

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65 **ABSTRACT (MAX 150 words)**

66 *Candida auris* is amongst the most important emerging fungal pathogens, yet mechanistic  
67 insights in its immune recognition and control are lacking. Here, we integrate transcriptional  
68 and functional immune cell profiling to uncover innate anti-*C. auris* defense mechanisms. *C.*  
69 *auris* induces a specific transcriptome in human mononuclear cells, a stronger cytokine  
70 response compared to *C. albicans*, but a lower macrophage lysis capacity. *C. auris*-induced  
71 innate immune activation is mediated through recognition of C-type lectin receptors, mainly  
72 elicited by structurally unique *C. auris* mannoproteins. In *in-vivo* experimental models of  
73 disseminated candidiasis, *C. auris* was less virulent than *C. albicans*. Collectively, these results  
74 demonstrate that *C. auris* is a strong inducer of innate host defense and identify possible  
75 targets for adjuvant immunotherapy.

76

## 77 INTRODUCTION

78 *Candida auris* is an important emerging fungal pathogen that was first described in 2009, and has,  
79 since then, spread across six continents as a causative microorganism of hospital-acquired infections <sup>1</sup>.  
80 For several reasons, *C. auris* is among the most challenging of emerging human pathogens identified  
81 in the last decade. It is highly resistant to many of the commonly used antifungal drugs <sup>1</sup> and, within a  
82 few years, it has rapidly spread worldwide <sup>2,3</sup> through the nearly simultaneous (but independent)  
83 emergence of four distinct phylogeographical clades <sup>4</sup>. Recently, a potential fifth clade has been  
84 described in Iran <sup>5</sup>. Every major clade except for clades II and V has been linked to outbreaks with  
85 invasive infection <sup>6</sup>. On the contrary, clade II generally shows antifungal susceptibility and has a  
86 propensity for ear infections. Similar to clade II, clade V isolate was recovered from ear. Clade III is  
87 associated with bloodstream infections and, together with clade II, tends to form large cell aggregates  
88 <sup>7</sup>. This has been linked with a reduced virulence in a *Galleria mellonella* infection model <sup>8</sup>.

89 *C. auris* poses difficulties in routine microbiological identification <sup>9,10</sup> and it is challenging to  
90 eradicate in healthcare settings <sup>11-14</sup>. This is due to its strong capacity to colonize skin, its  
91 transmittance *via* patient-to-patient route or contaminated fomites, and its high survival capacity on  
92 plastic surfaces and in the hospital environment <sup>15</sup>. The risk factors for *C. auris* infections are generally  
93 similar to those for other types of *Candida* infections, such as prolonged hospitalization, use of central  
94 venous catheters, abdominal surgery and exposure to broad-spectrum antibiotics or antifungals <sup>16</sup>.  
95 However, due to its acquired resistance to many antifungal drugs, the overall crude mortality rate of  
96 *C. auris* candidemia is high, ranging from 30% to 60%, with infections typically occurring several weeks  
97 (10-50 days) after admission <sup>4,12,17,18</sup>. Echinocandins are currently recommended by the CDC as empiric  
98 treatment of *C. auris* infections, although resistance has been reported. Several new therapeutic  
99 alternatives, such as fosmanogepix <sup>19,20</sup>, ibrexafungerp <sup>21-23</sup> and rezafungin <sup>24,25</sup> are currently under  
100 clinical investigation.

101 Considering the importance of *C. auris* as an emerging human pathogen, it is imperative to  
102 understand the host defense mechanisms. This is particularly true given the high resistance of this

103 fungus to anti-mycotic drugs, which makes it a prime candidate for the development of host-directed  
104 therapy (i.e. immunotherapy). However, almost nothing is known regarding the host immune  
105 response against *C. auris*. Host defense against *Candida* species is dependent on a finely tuned  
106 interplay of innate and adaptive immune responses. A first physical barrier consists of the skin and  
107 mucosa. The second barrier, represented by the innate immune system, is largely dependent on the  
108 recognition of evolutionarily conserved fungal cell wall components (pathogen-associated molecular  
109 patterns, PAMPs) by innate immune cells such as monocytes, macrophages and neutrophils. In turn,  
110 the release of proinflammatory cytokines, combined with antigen-presentation activity of myeloid  
111 cells, is crucial for shaping the **adaptive immunity**, representing a third, long-term barrier against  
112 fungal infection <sup>26</sup>.

113         The *Candida* cell wall is divided into an outer layer of highly mannosylated proteins  
114 (mannoproteins) and an inner layer, mainly comprised of  $\beta(1\rightarrow3)$  and  $\beta(1\rightarrow6)$ -glucans and chitin <sup>27</sup>.  
115 These PAMPs are recognized by various pattern recognition receptor (PRRs) on the surface of immune  
116 cells: C-type lectin receptors (CLRs) such as Dectin-1, Dectin-2, macrophage mannose receptor (MMR),  
117 Mincle (macrophage-inducible C-type lectin), DC-SIGN (dendritic cell specific intercellular adhesion  
118 molecule-3-grabbing non-integrin) and Toll-like Receptors (TLRs), especially TLR2 and TLR4 <sup>28</sup>.  
119 Coordinated engagement of PRRs results in the activation of innate immune effector mechanisms  
120 such as phagocytosis, reactive oxygen species (ROS) release and production of pro- and anti-  
121 inflammatory cytokines. In turn, together with the antigen-presentation activity of myeloid cells, the  
122 release of pro-inflammatory cytokines shapes the adaptive immune response <sup>26</sup>.

123         While the antifungal host defense mechanisms have been extensively studied for *C. albicans*,  
124 little is known about the host immune response against *C. auris*. Almost all multi-drug resistant *C.*  
125 *auris* strains are susceptible to killing by the salivary antimicrobial peptide Histatin 5 (Hst-5) <sup>29</sup>, while  
126 Johnson and colleagues showed that neutrophil recruitment and formation of neutrophils  
127 extracellular traps (NETs) were lower for *C. auris* than *C. albicans* <sup>30</sup>. Recently it was reported that *C.*  
128 *albicans*, *C. tropicalis*, *C. guilliermondii*, *C. krusei* and *C. auris* differentially stimulate cytokine

129 production in peripheral blood mononuclear cells (PBMCs)<sup>31</sup>, but little is known regarding the  
130 particularities of these responses and the mechanisms mediating them. Considering the knowledge  
131 gap in our understanding of anti-*C. auris* host defense mechanisms, we set out to comprehensively  
132 assess the mechanisms through which innate immune cells recognize *C. auris*, initiate innate  
133 antifungal immune responses, and protect the host against *C. auris* infection. This mechanistic insight  
134 into *C. auris* host interactions is instrumental for the development of novel host-directed approaches  
135 for the treatment of severe *C. auris* infections and, thereby, improve patient outcomes.

136

## 137 RESULTS

### 138 Common and specific transcriptome signatures induced by *C. albicans* and *C. auris* in human immune 139 cells

140 To gain a broad overview of the host immune response against *C. auris*, RNA sequencing was  
141 performed on PBMCs from three healthy donors that were exposed to either live *C. albicans* or *C.*  
142 *auris* for 4 or 24 hours. Due to the high genome-wide nucleotide identity across *C. auris* clades I-IV  
143 (98.7%)<sup>32</sup>, the analysis of the *C. auris* reference strain KCTC171810 (clade II) is expected to provide  
144 valuable insight into generic *C. auris* induced host responses. This *C. auris* reference strain was  
145 compared to *C. albicans* 10061110, which, to date, remains the most common cause of mucosal and  
146 systemic candidiasis<sup>33</sup>.

147 Principle component analysis (PCA) of the normalized PBMC RNAseq dataset revealed that the  
148 majority of the variance in the experiment as a whole was time-dependent, as demonstrated by a  
149 clear separation of the 4-hour and 24-hour stimulation time points (Extended Data Fig. 1a).

150 Comparison of the stimulated and non-stimulated samples at the 4-hour time point indicates that a  
151 limited response was induced early on. Moreover, stimulus clustering at 4 hours suggests this short-  
152 term response was similar in *C. auris* and *C. albicans* (Extended Data Fig. 1a). At the 4-hour time point,  
153 clustering of the donors irrespective of stimulus indicates that inter-individual differences underpin

154 the observed variance (**Extended Data Fig. 1b, left**). As PBMC donors were considered biological  
155 replicates, comparison of the average PBMC response to their control condition revealed significant  
156 overlap in the 4-hour host response between *C. albicans* and *C. auris*. With 71 differentially expressed  
157 genes (DEG; fold change  $\geq 2$  and p-adjusted value  $< 0.01$ ) upregulated by both *Candida* species, the  
158 respective overlap ranges from 67% of the total number of DEG for *C. albicans* (71 / 109) to 95% of  
159 the total number of DEG for *C. auris* (71 / 79).

160 In contrast, at 24 hours, the response is primarily stimulus-driven (**Extended Data Fig. 1b,**  
161 **right**), as indicated by the scattering of donor responses dependent on pathogen exposure. After 24h  
162 of stimulation, the common *C. auris* and *C. albicans*-induced host response increased to 243 DEG  
163 (**Figure 1a**), in turn accounting for 55% of the total number of DEG for *C. albicans* (243 / 442) and 50%  
164 of the total number of DEG for *C. auris* (243 / 484). This late shared response between both *Candida*  
165 species was consistent with the observation that the 24-hour induced PBMC transcriptomes were  
166 more stimulus-specific (**Extended Data Fig. 1b**). Pathway enrichment analysis revealed that the 4-hour  
167 *Candida* intrinsic response was delineated by a common activation of the CC and CXC chemokines  
168 (**Supplementary Table 1**). In contrast, the 24-hour PBMC transcriptomic response was characterized by  
169 a broader upregulation of chemokines, interleukins (IL), tumor necrosis factor and their receptors  
170 (**Figure 1a** and **Supplementary Table 2**).

171 The substantial activation of glucose, fructose and mannose metabolism was unique to the *C.*  
172 *albicans*-induced transcriptional response of PBMCs at 24 hours. Conversely, the DEG that were more  
173 strongly induced upon PBMC exposure to *C. auris* appeared to be linked to type I and II interferons, as  
174 well as antiviral mechanisms triggered via IFN-stimulated genes, including the ISG15 immune  
175 mechanisms (**Figure 1a** and **Supplementary Table 2**). Collectively, these data show that *C. albicans* and  
176 *C. auris* are potent activators of the host immune system, and they are not only able to activate  
177 common transcriptional responses, but also induce pathways specific to each pathogen.

178

179 ***C. auris* is a more potent inducer of host immune response compared to *C. albicans***

180 One-third of the top 15 enriched pathways, based on those DEG that were unique for *C. auris*, were  
181 also found to be enriched in the common *Candida* response (**Figure 1a**). This was not the case for *C.*  
182 *albicans*, indicating that *C. auris* has the ability to upregulate more genes in these pathways compared  
183 to *C. albicans*. Most pronounced within these unique DEG were distinct interleukins such as *IL1RN*  
184 (encoding for *IL-1Ra*), *IL10*, *IL19*, *IL26* and *IL27*, as well as interferon (IFN) associated genes, e.g. *STAT2*,  
185 *DDX58*, *EIF2AK2*, *OAS2*, *OAS3*, *IFIT2*, *IFIT3*, *IFIT35* and *IFITM1* (**Supplementary Table 2**). Furthermore,  
186 DEG were more potently induced in response to *C. auris* than *C. albicans* (Student's T-test, p-value of  
187 0.003). An additional pathway enrichment analysis on all the upregulated DEG confirmed that the  
188 total number of DEG for mutually enriched pathways was higher when the PBMCs were stimulated  
189 with *C. auris* rather than with *C. albicans* (**Extended Data Fig. 1c**). Collectively, the broader and  
190 stronger induction of DEG by *C. auris* resulted in higher enrichment scores (q-value) for corresponding  
191 pathways in comparison to *C. albicans*, suggesting that *C. auris* is a more potent trigger of the host  
192 response.

193 With the transcriptomic analysis suggesting cytokine signaling to be at the core of the host  
194 response, we aimed to verify these observations at the protein level. For this, the cytokine production  
195 ability of PBMCs isolated from healthy volunteers was assessed following 24-hour exposure to three  
196 different live clinical isolates for each *Candida* species, all cultured under similar conditions. As a  
197 measure of *Candida* induced cytotoxicity, the detection of lactate dehydrogenase (LDH) release  
198 revealed that PBMC viability after 24 hours was not impacted (**Extended Data Fig. 2a**). With the  
199 exception of the anti-inflammatory cytokine *IL-1Ra*, PBMCs exposed for 24 hours to both clinical  
200 isolates of *C. auris* produced significantly higher amounts of the pro-inflammatory cytokines TNF $\alpha$ , IL-  
201 6, and IL-1 $\beta$  compared to the *C. albicans*-stimulated PBMCs (**Figure 1b**). Moreover, to test whether this  
202 observation is clade-dependent or reflects the general *C. auris* response, cytokine production was  
203 assessed upon 24-hour stimulation with different *C. auris* strains originating from the five different  
204 clades. While showing variation between *C. auris* strains in cytokine production (**Figure 1c**), a similar

205 pattern between pro-inflammatory cytokines was observed. Of note, *C. auris* clade V induced similar  
206 levels of pro-inflammatory cytokines and significantly lower IL-1Ra levels compared to *C. albicans*. *C.*  
207 *auris* clades II and III induced cytokine production moderately, but this was still significantly higher  
208 compared to *C. albicans*. *C. auris* clades I and IV present as extremely potent inducers of pro-  
209 inflammatory cytokines. Regardless of this clade dependent variability, all *C. auris* clades except for  
210 Clade V were observed to drive a significantly enhanced pro-inflammatory cytokine response  
211 compared to *C. albicans* (**Figure 1c**). However, at the transcriptional level, PBMCs stimulated with *C.*  
212 *auris* showed only a trend towards a stronger induction of *IL-6* ( $\text{Log}_2\text{FC } 8.41 \pm \text{lfcSE } 1.4$ ) and *IL-1 $\beta$*   
213 ( $\text{Log}_2\text{FC } 6.45 \pm \text{lfcSE } 1.29$ ) expression levels than upon exposure to *C. albicans* ( $\text{Log}_2\text{FC} = 7.58 \pm \text{lfcSE}$   
214  $1.37$  for *IL-6*;  $\text{Log}_2\text{FC } 5.59 \pm \text{lfcSE } 1.31$  for *IL-1 $\beta$* ) (ns, **Extended Data Fig. 2b**).

215

216 ***C. auris* replicates faster than *C. albicans* in vivo, leading to altered multiplicity of infection (MOI), but**  
217 **does not cause macrophage lysis**

218 Killing of *Candida* by professional phagocytes of the innate immune system, such as monocytes,  
219 macrophages or dendritic cells, is an important line of defense at the site of infection. In order to  
220 study the differences in phagocytosis dynamics of professional phagocytes between *C. auris* and *C.*  
221 *albicans*, we employed live-cell video microscopy coupled with dynamic image analysis of bone  
222 marrow-derived macrophages (BMDMs). Phagocytosis capacity was assessed by combining BMDMs  
223 with live and thimerosal-killed *C. auris* and *C. albicans* strains at an intended MOI of 3:1, yeast cells per  
224 macrophage. By including fixed yeasts, we were able to assay phagocytosis in the absence of rapid  
225 adaptive changes in the composition of the *Candida* cell wall. Results are expressed as percentage of  
226 phagocytic BMDM (% uptake), indicating the percentage of macrophages having phagocytosed at  
227 least one fungal cell. In addition, we assayed the Phagocytic Index which is defined as the number of  
228 fungal cells engulfed and fully inside the phagosome per 100 macrophages (by excluding the fungal  
229 cells adhering but not internalized). No significant differences in *C. auris* and *C. albicans* phagocytosis  
230 (% uptake) were observed for fixed *Candida* (**Extended Data Fig. 2c**). There was a trend towards a

231 higher phagocytic index in BMDMs at later time points (after the second hour) for both of the fixed *C.*  
232 *auris* strains compared to *C. albicans* strains (Extended Data Fig. 2c), possibly because fungal cells tend  
233 to be phagocytosed in clusters. However, *C. auris* strain 10051893 had a lower phagocytosis efficiency  
234 after 1 hour compared to *C. albicans* SC5314 (Extended Data Fig. 2d). Focusing on live strains, we  
235 observed that the phagocytic index of both *C. auris* strains gradually increases in time, to a greater  
236 extent than the *C. albicans* strains (Figure 2a). In order to evaluate the phagocytosis dynamics using  
237 human cells, we incubated human PBMCs stimulated for 15, 30 and 120 minutes (2 hours) with  
238 thimerosal-fixed FITC-labeled *C. albicans* 10061110 and *C. auris* KCTC17810, and then calculated the  
239 percentage of FITC-positive cells as well as the mean fluorescence intensity (MFI) in the CD14<sup>+</sup>  
240 population. *C. auris* showed a significantly higher rate of phagocytosis and MFI at all the time points  
241 measured compared to *C. albicans* (Figure 2b).

242           Using real-time live cell microscopy, live *C. auris* cells were observed to bud repeatedly outside  
243 the macrophages, with a doubling time of ~1 hour. The *C. auris* budding rate decreased following  
244 phagocytic engulfment, although cells continued to multiply within phagosomes (Supplementary Video  
245 1). Of interest, *C. auris* 10051895 accumulated in high numbers within macrophages, indicating that  
246 the starting MOI had exceeded the intended initial 3:1 ratio (Supplementary Video 2). This triggered  
247 our interest to estimate the actual MOI ratio at the start of image acquisition. Due to the time elapsed  
248 between the counting of *Candida* in each sample and the image acquisition, the starting 3:1 ratio for  
249 *C. auris* rose up to 7:1, presumably due to ongoing budding, despite the fact that samples were stored  
250 at 4°C in PBS until the live imaging commenced. In contrast, the MOIs for live *C. albicans* as well as for  
251 the fixed strains remained around the desired target of 3:1 yeast:macrophage.

252           The elevated MOIs for *C. auris* may be a contributing factor to the trend for higher phagocytic  
253 index achieved at 3 hours for live strain 10051895 (Figure 2a). However, an elevated starting MOI for  
254 *C. auris* 10051893 did not enhance phagocytosis, as the live form of this strain was poorly recognized  
255 by BMDMs, showing a significantly lower percentage of uptake as compared to *C. albicans* 10061110

256 (Extended Data Fig. 2e). From the representative movies recorded, we quantified the distribution of  
257 yeast per individual macrophage after 3 hours and found that for both *C. auris* strains, there was a  
258 tendency for some macrophages to engulf many fungal cells, yet for other macrophages to engulf  
259 none. This phenomenon was less surprising for the *Candida* experiments using live microorganisms  
260 (Figure 2c), because *C. auris* continues to divide prior to and during the phagocytosis experiment.  
261 However, the fixed strains also displayed this varied distribution, with both strain of *C. auris* being  
262 phagocytosed in large numbers by some macrophages (Extended Data Fig. 2f). Supplementary Video 3  
263 shows that *C. auris* cells are taken up extensively into a subpopulation of macrophages, but despite  
264 the vast burden, these phagocytes continue to move around in pursuit of further fungal targets  
265 (Supplementary Video 3).

266 Finally, macrophage lysis was determined following engulfment of live *Candida* and it emerged  
267 that the *C. auris* strains examined were significantly less able to kill macrophages after 3 hours than *C.*  
268 *albicans* 10061110, but not compared to *C. albicans* SC5314 (Figure 2d, Supplementary Video 4),  
269 despite having a comparable (or greater, in the case of *C. auris* 10051895) phagocytic index. These  
270 findings demonstrate that *C. auris* is differentially recognized by phagocytic BMDMs and internalized  
271 with a higher phagocytic index compared to *C. albicans* but is not able to induce lysis of the phagocytic  
272 cells.

273

#### 274 **Host immune response upon *C. auris* exposure is mediated by heat-sensitive cell wall** 275 **components.**

276 Variability in the cell surface structures, such as differentially expressed mannoproteins or altered  $\beta$ -  
277 glucan exposure between the *C. auris* and *C. albicans* cell walls, could account for the differential  
278 cytokine responses triggered by these pathogens. To elucidate whether this might account for the  
279 observed differences in cytokine induction, the *C. auris* and *C. albicans* strains were subjected to heat-  
280 killing. This approach facilitates disruption of the outer layer of the *Candida* cell wall<sup>34</sup>, in turn  
281 enhancing  $\beta$ -glucan exposure<sup>35,36</sup>. The heat-killed strains were used to stimulate PBMCs for 24 hours

282 and 7 days. Since the production of reactive oxygen species (ROS) can positively contribute to immune  
283 responses<sup>37</sup>, in addition to PBMCs cytokine production, ROS release in both neutrophils and PBMCs  
284 was assessed during 1 hour stimulation with serum-opsonized heat-killed *C. auris* and *C. albicans*  
285 strains. The area under the curve (AUC) was calculated to examine quantitative differences in ROS  
286 release.

287 In neutrophils both the ROS release over time and the total amount of production (AUC) were  
288 significantly lower following *C. auris* stimulation, compared to *C. albicans* (Extended Data Fig. 3a). In  
289 PBMCs, although the time-course luminescence was significantly decreased in *C. auris*-stimulated  
290 cells, only *C. auris* 10051893 induced a significantly lower total ROS production than *C. albicans*  
291 (Extended Data Fig. 3b).

292 Unexpectedly, compared to *C. albicans*, the cytokine response was almost completely  
293 abrogated after PBMC stimulation with heat-killed *C. auris* for 24 hours (Figure 2e). This phenomenon  
294 was observed for all *C. auris* clades (Extended Data Fig. 3c), subsequently marking this characteristic  
295 for the general *C. auris* induced host immune response. In addition, after 7 days, the production of  
296 IFN- $\gamma$  and IL-17 by PBMCs stimulated with heat-killed *C. auris* was significantly lower compared to  
297 heat-killed *C. albicans* (Extended Data Fig. 3d). Hence, we reasoned that a heat sensitive component of  
298 the cell wall might be responsible for most of the increased cytokine induction by *C. auris*.

299

### 300 Mannans drive the host response to *C. auris*

301 We attempted to unravel the contribution of the different fungal cell wall components to the  
302 activation of host responses by *C. auris*, compared to *C. albicans*. PBMCs were exposed to the purified  
303 cell wall components,  $\beta$ -glucans and mannans, from both *Candida* species, and the transcriptional  
304 responses of the stimulated immune cells were assessed by RNA sequencing. The species-specific cell  
305 wall contribution was assessed by comparison of the number of shared DEG upon exposure to the  
306 different cell wall components and expressed as proportion of the respective live stimulus.

307           The early 4-hour host response was predominantly induced by  $\beta$ -glucan, which was sufficient  
308 to explain around 82% and 57% of the respective live responses of *C. albicans* (89 / 109) and *C. auris*  
309 (45 / 79) (**Figure 3a**). The  $\beta$ -glucans from each species resulted in similar PBMC gene expression  
310 profiles (**Extended Data Fig. 4a**). Although the relative contribution of *C. albicans*  $\beta$ -glucan decreases to  
311 approximately 13% (55 / 442) in the late phase, 24 hours after stimulation, they remain able to  
312 upregulate several *C. albicans*' top 50 DEG, leaving them a main contributor of the evoked response in  
313 the live setting (**Figure 3a** and **Extended Data Fig. 4a**). In contrast,  $\beta$ -glucans from *C. auris* failed to elicit  
314 a response analogous to the live *C. auris* exposure, explaining only a mere 2% (10 / 484) of the live *C.*  
315 *auris*-induced response. Conversely, however, mannans from *C. auris* stimulated 28% (136 / 484) of  
316 the evoked transcriptional response to live *C. auris* cells. Moreover, *C. auris* mannan seemed to  
317 outperform *C. albicans*  $\beta$ -glucan in relation to the top 50 DEG of *C. albicans*, displaying an induction  
318 pattern similar to its live setting (**Extended Data Fig. 4a**). Overall, these results indicate that the host  
319 recognition and subsequent initiation of downstream responses against *C. albicans* is mainly  
320 dependent on  $\beta$ -glucan. For *C. auris*, early 4-hour stimulation of PBMCs is mainly mediated by  $\beta$ -  
321 glucan, whereas mannans are fundamental for orchestrating the *C. auris*-specific host response at  
322 later time points (24 hours).

323           Our data suggest a stronger *C. auris* host immune response and a differential role in gene  
324 expression between *C. albicans* and *C. auris* cell wall components. Therefore, to investigate the  
325 differences in cell wall structure between *C. auris* and *C. albicans*, we first compared forward (FSC)  
326 and side (SSC) light scatter of fungal cells using flow cytometry. In line with what has previously been  
327 described<sup>23</sup>, we found that the *C. auris* strains have a smaller average cell size compared to *C.*  
328 *albicans*. Of the *C. auris* strains, strain 10051893 shows more complexity/granularity (higher SSC) than  
329 strain 10051895 (**Extended Data Fig. 4b**).

330           Next, we measured  $\beta$ -glucan exposure on the fungal cell surface by flow cytometry on  
331 thimerosal-fixed *Candida* cells stained with Fc-Dectin-1. *C. auris* 10051893 displayed significantly  
332 reduced exposure of  $\beta$ -glucan as compared to *C. albicans* SC5314 (**Extended Data Fig. 4c**). At the gene

333 expression level, *C. auris*  $\beta$ -glucan ( $\text{Log}_2\text{FC } 2.9 \pm \text{lfcSE } 0.6$ ) tended to induce less effectively IL-1Ra as  
334 compared to  $\beta$ -glucan isolated from *C. albicans* ( $\text{Log}_2\text{FC } 4.5 \pm \text{lfcSE } 0.6$ ) (ns, [Extended Data Fig. 2b](#)). At  
335 the cytokine level, though large variation between different strains was observed, no significant  
336 differences in cytokine production of PBMCs stimulated with purified  $\beta$ -glucans from *C. auris*  
337 compared to *C. albicans* were found for TNF $\alpha$  and IL-1 $\beta$ , while there was significantly lower IL-6  
338 production in response to  $\beta$ -glucan from *C. auris* 10031160, *C. auris* 10051256, *C. auris* 10051263, *C.*  
339 *auris* 10051522 and *C. auris* 10051252 when compared to *C. albicans*  $\beta$ -glucan. Interestingly,  $\beta$ -glucan  
340 from *C. auris* strains, except for *C. auris* 10051522, *C. auris* 10051244 and *C. auris* 10051252, induced  
341 a significantly lower IL-1Ra production compared to *C. albicans*  $\beta$ -glucan (**Figure 3b**). Moreover,  
342 similarly to *C. albicans*  $\beta$ -glucan, *C. auris*  $\beta$ -glucan synergistically boosted Pam3Cys (TLR2 agonist)-  
343 induced IL-1 $\beta$  production in PBMCs, as well as TNF $\alpha$  and IL-6 production (**Figure 3c**).

344         Having ruled out a major role for  $\beta$ -glucans in explaining the difference in cytokine stimulation  
345 induced by *C. auris* and *C. albicans*, we assessed the role of glycosylated mannoproteins from the  
346 fungal cell wall<sup>34</sup>. Examination of mannan exposure, by staining thimerosal-fixed *Candida* cells with  
347 Concanavalin A (ConA), revealed a relatively low level of exposure of surface mannans in *C. auris*  
348 strains. This difference was significant for *C. auris* 10051893 as compared with *C. albicans* 10061110  
349 ([Extended Data Fig. 4c](#)). However, *C. auris* mannans were observed to significantly induce the gene  
350 expression of pro-inflammatory cytokines *IL-6* ( $\text{padj} = 0.0001$ ) and *IL-1 $\beta$*  ( $\text{padj} = 0.0003$ ) compared to  
351 those of *C. albicans* ([Extended Data Fig. 2b](#)).

352         In line with these observations, mannans from all eight *C. auris* strains induced a significantly  
353 higher cytokine production than *C. albicans* mannans 24 hours after stimulation of PBMCs, both pro-  
354 inflammatory and anti-inflammatory (**Figure 3d**). Except for IL-1Ra production, opsonization by human  
355 serum was necessary for mannan-induced production of cytokines ([Extended Data Fig. 5a](#)). After 7  
356 days of stimulation, no significant differences between mannan from *C. auris* and *C. albicans* as well  
357 with the unstimulated cells have been observed ([Extended Data Fig. 5b](#)).

358

359 **Unique structure of *C. auris* mannans**

360 Nuclear magnetic resonance (NMR) spectroscopy analyses of respective cell wall components for both  
361 *Candida* species revealed no structurally unique features in  $\beta$ -glucans isolated from *C. auris*. However,  
362 the distance between side-chain branching points (average number of  $\beta$ -linked glucosyl repeat units),  
363 was larger for *C. auris*  $\beta$ -glucans than for *C. albicans*  $\beta$ -glucans (Supplementary table 3). In contrast,  
364 these side chains were much shorter for *C. auris* than for *C. albicans*  $\beta$ -glucans. For *C. auris* mannans,  
365 the acid stable portion was similar across all clinical isolates, revealing long side chains with varying  
366 lengths containing linked  $\alpha$ -1,2-mannose,  $\alpha$ -1,3-mannose and  $\beta$ -1,2-mannose in varying amounts  
367 (Figure 4). Strikingly, the acid labile portion of *C. auris* mannans revealed two distinct M- $\alpha$ -1-  
368 phosphate side chains (Figure 4), marking a unique structural feature that has not been observed in  
369 the fungal kingdom before. Subsequent multi-detector gel permeation chromatography highlighted *C.*  
370 *auris* mannans as extremely small biopolymers with a molecular weight (MW) ranging from 6.1 to 16.1  
371  $\times 10^3$  Da among the clinical isolates. This represents a major difference from *C. albicans* mannan,  
372 which has a MW of  $500 \times 10^3$  D (Supplementary table 3).

373 We assessed the effects of these distinct and unique structural features of *C. auris* mannans  
374 on their binding capacity with rhDectin-2 and rhMannose receptors, compared to *C. albicans*  
375 mannans. Variability in binding affinities was observed amongst mannans from the different clinical *C.*  
376 *auris* isolates, ranging from an equilibrium dissociation constant ( $K_D$ ) of 1.0 to 6.0  $\mu$ M for rhDectin-2  
377 and 2.1 to 6.3  $\mu$ M for rhMannose. Although differentially recognized by both receptors, their overall  
378 binding affinities were an order of magnitude lower than those observed for *C. albicans* mannans  
379 (Supplementary table 3). Moreover, of the examined structural features, the affinity of *C. auris*  
380 mannans for the rhDectin-2 receptor was solely associated with a higher MW ( $r^2=0.4488$ ,  $p=0.034$ ). A  
381 slightly lower association was observed for rhMannose ( $p=0.096$ ) yet displaying a similar trend.  
382 Collectively, our data suggest that the double M- $\alpha$ -1-phosphate side chains and small molecular size,

383 represent highly unique physicochemical properties of *C. auris* mannans that probably contribute to  
384 the decreased recognition efficiency by two important anti-fungal recognition receptors.

385

### 386 **The CLRs complement receptor 3 (CR3) and MMR contribute to the *C. auris*-induced cytokine** 387 **production**

388 Next, we investigated the downstream effects of the reduced binding affinity of *C. auris* mannans on  
389 intracellular signaling pathways and the subsequent activation of the immune system. The spleen  
390 tyrosine kinase, Syk, is an important mediator downstream of several CLRs<sup>38</sup>. CLR signaling from  
391 Dectin-1 and other lectins also involves the serine/threonine-protein kinase Raf-1<sup>39</sup>. Inhibition of Syk  
392 and Raf-1 decreased TNF $\alpha$ , IL-6, and IL-1  $\beta$  production in response to *C. auris* stimulation (**Figure 5a**),  
393 indicating the involvement of these two signaling pathways in cytokine production.

394         Due to the importance of Syk and Raf-1 pathways in CLR pathway mediation, we subsequently  
395 hypothesized a role for these receptors in *C. auris* recognition. Therefore, we pre-incubated PBMCs  
396 with neutralizing antibodies against important *Candida* CLRs (e.g., Dectin-1, Dectin-2, Mincle, DC-SIGN,  
397 MMR, CR3, and their control isotypes), one hour prior to stimulation with live *C. albicans* or *C. auris*.  
398 As expected, blocking Dectin-1 significantly decreased TNF $\alpha$  production upon *C. albicans* stimulation  
399 but, surprisingly, increased TNF $\alpha$  upon *C. auris* stimulation. (**Figure 5b**). We observed a significant  
400 reduction in *C. auris*-induced IL-6 and IL-1Ra production upon blocking of MMR (**Figure 5b**).  
401 Interestingly, neutralization of Dectin-1 and DC-SIGN led to a significant increase of IL-6 induced by *C.*  
402 *auris* compared to IgG2b isotype control. Moreover, blockade of CR3 led to a significant reduction in  
403 IL-1 $\beta$  production and an increase in IL-1Ra production (**Figure 5b**). We conclude that CR3 and MMR  
404 signaling promotes cytokine production in response to *C. auris*, while blocking Dectin-1 functionality  
405 perturbs this cytokine production.

406

### 407 ***C. auris* is less virulent than *C. albicans* in an experimental model of murine disseminated candidiasis**

408 To evaluate the virulence of *C. auris* *in vivo*, immunocompetent C57BL/6J mice were injected

409 intravenously (i.v.) with either  $10^7$  Colony Forming Unit (CFU) of *C. auris* 10051895 (n=10) or *C.*  
410 *albicans* 10061110 (n=11). Their survival was monitored over the course of 14 days. Significantly more  
411 immunocompetent mice survived infection with *C. auris* than with *C. albicans* (3/11 deaths for *C. auris*  
412 and 11/11 deaths for *C. albicans* over 14 days; Chi square=21.42;  $p < 0.0001$ , Mantel-Cox test) (**Figure**  
413 **6a**). In order to evaluate the differential organ invasion capacity between *C. auris* and *C. albicans*  
414 infection, we injected intravenously either  $10^6$  CFU of *C. auris* or *C. albicans* and sacrificed mice at day  
415 3 (n=5 with *C. albicans*, n=5 with *C. auris*) and day 7 (n=4 with *C. albicans*, n=5 with *C. auris*) for CFU  
416 counting both in liver and kidneys. Although after day 3 there weren't any significant differences, a  
417 significantly lower fungal burden has been found at day 7 in **the kidneys of *C. auris*** infected mice  
418 compared to *C. albicans*-infected (**Figure 6b**). To confirm whether the *ex vivo* higher pro-inflammatory  
419 response towards *C. auris* found in human PBMCs **holds in vivo**, we measured myeloperoxidase (MPO)  
420 in organs and several cytokines both in plasma and organs of mice infected with either  $10^6$  CFU of *C.*  
421 *albicans* or *C. auris*. Except for a significantly lower Keratinocyte chemoattractant (KC) at day 7 (**Figure**  
422 **6c**), no significant differences between cytokine levels have been found between *C. auris* and *C.*  
423 *albicans*-infected mice (**Figure 6d** and **Extended Data Fig 6a-e**). To understand the inflammatory  
424 cytokine induction after the same load of *Candida* CFU, we normalized the inflammatory cytokine  
425 production with the actual remaining CFU in organs by calculating the ratio of the mean MPO/CFU and  
426 KC/CFU. MPO and KC production per remaining *C. auris* CFU were higher **than** the remaining *C.*  
427 *albicans* CFU count (**Figure 6f**), supporting our in vitro findings in human PBMCs. In conclusion,  
428 **improved survival and better clearance** of invasive *C. auris* infection as compared to *C. albicans* is  
429 ensured by an adequate immune response in immunocompetent mice.

430

431 DISCUSSION

432

433 In the current study, we investigated the transcriptional and functional responses of human PBMCs  
434 and murine BMDMs to the rapidly emerging fungal pathogen *C. auris*. *C. auris*-induced host responses  
435 were compared to those elicited by *C. albicans*, as this species remains the most frequent cause of  
436 nosocomial fungal infections in humans to date<sup>33</sup>. A broad assessment of various clinical strains, as  
437 well as further verification amongst the five *C. auris* clades, revealed that, with the exception of clade  
438 V, *C. auris* induces a stronger immune response than *C. albicans in vitro*. Functional and structural  
439 assessment of  $\beta$ -glucans and mannans highlighted the presence of small and structurally unique *C.*  
440 *auris* mannans, which were crucial for immune recognition. Compared to *C. albicans*, *C. auris* isolates  
441 examined in this study were more efficiently phagocytosed by immune cells, induced lower levels of  
442 macrophage lysis, and displayed lower virulence in a murine model of disseminated infection.

443 *C. auris* induced robust transcriptional changes in human PBMCs. These included both  
444 common pathways induced by *C. albicans* as well, but also more robust specific IFN-dependent  
445 transcriptional programs and explicit cytokine responses. This conclusion is supported by a recent  
446 study by Mora-Montes and colleagues<sup>31</sup>. Secondly, *C. auris* appear to induce stimulation of immune  
447 cells by sequential engagement of different components of the cell wall. The early (4 hour) responses  
448 are mainly induced by  $\beta$ -glucans, and this initial phase of the response is largely similar to that induced  
449 by *C. albicans*. This is probably explained by the similar structure of *C. auris* and *C. albicans*  $\beta$ -glucans.  
450 In contrast, the late transcriptomic responses (24 hours) induced in PBMCs by *C. auris* display  
451 significant differences and broader upregulation of immune genes compared with those induced by *C.*  
452 *albicans*. These late responses are mainly mediated by *C. auris* mannoproteins with a specific  
453 structure that includes a unique M- $\alpha$ -1-phosphate side chain in the acid labile portion of *C. auris*  
454 mannans, which has not been observed in the fungal kingdom to date. In line with the results of Yan *et*  
455 *al.* reporting that *C. auris* mannans strongly bind to serum IgG and mannose-binding lectin (MBL)<sup>40</sup>,

456 we showed that opsonization by human serum was necessary for *C. auris* mannan-induced cytokine  
457 production.

458 By comparing *C. albicans* induced cytokine production with the different *C. auris* clades, we  
459 observed variability that is probably linked to their distinct phenotypic, epidemiological, and drug  
460 resistance properties. In particular, clade V was the least immunogenic, while clades I and IV were the  
461 strongest inducers of cytokine production. The amount of cytokines induced by the various strains  
462 might be correlated to clade-specific characteristics which, in turn, might influence the level of  
463 colonization/persistence in the host. When a healthy host encounters *C. auris* from clade I or IV, it  
464 responds with a prompt high pro-inflammatory cytokine response which is protective. Although it is  
465 also linked with invasive infections, clade III isolates show a lower *in vitro* cytokine production  
466 compared to isolates from other clades. This may be linked to their tendency to form aggregates,  
467 which might make innate immune recognition challenging. Finally, the fact that Clade II has a relatively  
468 lower cytokine production, despite a higher phagocytosis rate as compared with *C. albicans*. This  
469 might be related to its simpler mannan structure compared to isolates from Clade I and Clade IV  
470 (Figure 4).

471 An important question concerns the PRRs responsible for the recognition of *C. auris*. Our  
472 experiments using neutralizing antibodies revealed a significant role for the CLRs, especially MMR and  
473 CR3, in the induction of cytokines by *C. auris*. The role of these receptors in the recognition of  
474 mannans is well known<sup>41</sup>. However, our binding affinity data show that r-MMR binds *C. auris* mannans  
475 with a low affinity, and the *in vitro* neutralization of MMR led to only partial loss of cytokine  
476 production, arguing that additional mannan-recognizing receptors contribute to anti-*C. auris* host  
477 defense. On the other hand, the blocking of Dectin-1 significantly increased TNF $\alpha$  and IL-6 upon *C.*  
478 *auris* stimulation for 24 hours. This interesting observation could be explained by differences in  
479 relative  $\beta$ -glucan abundance. However, recently Navarro-Arias *et al.*<sup>31</sup> quantified the abundance of  
480 different cell wall components and found that total  $\beta$ -glucan and mannans in *C. auris* were  
481 comparable to *C. albicans*. We suggest that this phenomenon might be due to a combination of two

482 factors: i) the different exposure of *C. auris*  $\beta$ -glucan as compared to *C. albicans* (Extended data Fig.  
483 4C); ii) the differential and variable cell wall adaptation of *C. auris* strains during the interaction with  
484 the host which determines Dectin-1 dependence of *C. auris* host response. Such phenomenon occurs  
485 *in vivo* during *C. albicans* infection in a strain-specific way and the differences in the levels of cell-wall  
486 chitin influence the role of Dectin-1<sup>42</sup>. Interestingly, Navarro-Arias *et al.* have reported significantly  
487 more cell wall chitin in *C. auris* compared to *C. albicans*<sup>31</sup>. Since high chitin levels reduce the  
488 dependence on Dectin-1 recognition, we speculate that differences in *C. auris* cell wall adaptation to  
489 the host, variations in chitin content (higher chitin in *C. auris*) and differences in cell wall structure  
490 (less exposure of beta glucan in *C. auris* and structurally unique mannoproteins) might provide an  
491 explanation, at least in part, for the *C. auris* Dectin-1 (in)dependency.

492 Cytokine induction is important upon pathogen recognition, but the induction of phagocytosis  
493 is also crucial<sup>43</sup>. We observed a higher phagocytic index for *C. auris* compared to *C. albicans*. This is  
494 likely due to a better recognition of *C. auris* mannans by immune cells, as cell wall glycosylation is  
495 critically important for the recognition and ingestion of *C. albicans* by macrophages<sup>44</sup>. Therefore, to  
496 shed more light on these processes, future investigations might examine the phagocytosis dynamics of  
497 *C. auris* mutant strains that are defective in their cell wall architecture. Interestingly, when the fate of  
498 the fungus was assessed through video time-lapse microscopy, it was also clear that the continued cell  
499 division of *C. auris* leads to altered MOI that are greater than *C. albicans* and this may also contribute  
500 to the stronger stimulation of inflammation. However, this did not result in the death of the  
501 phagocytes, most likely due to the lack of hyphae formation by engulfed *C. auris* cells. In line with  
502 previous studies pointing to glucose competition as the main effector mechanism through which *C.*  
503 *albicans* induce macrophage death<sup>45</sup>, a clear enrichment of glucose, fructose and mannose  
504 metabolism pathways in the host were observed unique for 24-hour *C. albicans* exposure (as seen in  
505 Figure 1A and Supplementary Table 2). Conversely, the respective absence of the induction of such a  
506 metabolic shift by *C. auris* suggests that this may be a likely mechanism to explain the lower  
507 macrophage lysis induced by phagocytosed *C. auris*, and subsequently may explain the lower

508 virulence. In addition, this could represent an important difference of the paths followed by different  
509 *Candida* species as survival mechanisms.

510         The stronger induction of cytokines and lower macrophage lysis after 3 hours of phagocytosis  
511 might have been expected to lead to lower virulence of *C. auris in vivo* compared with *C. albicans*. In  
512 line with this hypothesis, experiments in a model of murine disseminated candidiasis demonstrate  
513 that *C. auris* is less virulent compared to *C. albicans*, a conclusion supported by recent additional  
514 studies<sup>46,47</sup>. Neutrophils are considered one of the most important host immune response to fungi  
515 through phagocytosis and intracellular killing, or by releasing NETs<sup>48</sup>. In a recent study<sup>30</sup>, human  
516 neutrophils were poorly recruited to sites of *C. auris* infection, were less able to kill *C. auris* compared  
517 to *C. albicans* and failed to form NETs in response *C. auris*. However, neutrophils are important  
518 contributors to the host defense against *Candida* species<sup>49</sup>. Our *in vivo* results show that MPO  
519 production in *C. auris*-infected mice is similar to *C. albicans*, indicating that neutrophil activation is  
520 comparable. In addition, the comparable innate and adaptive cytokine production in mice infected  
521 with *C. albicans* and *C. auris*, as well as the similar or even better organ clearance, suggest that the  
522 immune response toward *C. auris* is fully functional in an immunocompetent host. When the cytokine  
523 levels were linked to the remaining organ CFU count by a cytokine/CFU ratio, we observed that there  
524 was a higher cytokine production per singular *C. auris* CFU cultured from the organ, as compared to *C.*  
525 *albicans*. This is in line with the potent pro-inflammatory response observed in human PBMCs  
526 stimulated with *C. auris*. Future studies are warranted to dissect the relative importance of  
527 neutrophils and macrophages in the host defense against *C. auris*.

528         In conclusion, we performed a first comprehensive assessment of the innate host defense  
529 mechanisms against the rapidly emerging human pathogen *C. auris*. The overall conclusion is that the  
530 host defense mechanisms induced by *C. auris* are generally classical antifungal mechanisms, but  
531 important specific responses are also triggered by unique *C. auris*-specific mannoprotein structures.  
532 The ensuing immune responses are effective and lead to an effective elimination of the fungus. Our  
533 study argues that the intrinsic virulence of *C. auris* is not higher than other *Candida* species circulating

534 in the patient population, but it is rather the infection control problem of this pathogen and its high  
535 resistance to antifungal drugs that make it dangerous. The challenges that need to be pursued in the  
536 coming years are to identify in even more detail the most effective components of the anti-*C. auris*  
537 host defense, and to design and test novel host-directed therapies to enhance these pathways and  
538 improve the outcome to the infection. In this respect, based on our results highlighting the peculiarity  
539 of *C. auris* mannoprotein structures, one promising therapeutic possibility could be fosmanogepix  
540 (APX001A), a novel agent that targets the fungal protein Gwt1 (glycosylphosphatidylinositol-anchored  
541 wall transfer protein 1), thereby inhibiting the maturation and localization of GPI-anchored  
542 mannoproteins in the cell wall<sup>20</sup>. Several studies reported higher survival rates and lower fungal  
543 burden in *C. auris* infected mice treated with this novel drug<sup>20,50</sup>. One of the reasons for this higher  
544 efficacy could be the crucial role of mannans for *C. auris* pathogenicity in the host. In addition to being  
545 the first comprehensive study of the host immune response to *C. auris*, our data provide further  
546 support from an immunological and microbiological perspective for the development of drugs  
547 potentially targeting mannan synthesis as new and efficient anti-fungal drugs for *C. auris*.

548

549

550 **METHODS**

551 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

552

553 **Ethics statement for ex vivo human PBMC stimulations**

554

555 *Ex vivo/in vitro* human PBMC stimulations - Inclusion of healthy controls was approved by the local  
556 institutional review board (CMO region Arnhem-Nijmegen, #2299 2010/104) and conducted according  
557 to the principles of the International Conference on Harmonization–Good Clinical Practice guidelines.  
558 Buffy coats from healthy donors were obtained after written informed consent (Sanquin, Nijmegen,  
559 the Netherlands).

560

561 **Ethics statement for in vivo mice studies**

562 All animal experiments were conducted in the unit of animals for medical scientific purposes of  
563 University General Hospital 'Attikon' (Athens, Greece) according to EU Directive 2010/63/EU for  
564 animal experiments and to the Greek law 2015/2001, which incorporates the Convention for the  
565 Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes of the Council  
566 of Europe (code of the facility EL 25BIO014, approval no. 1853/2015). All experiments were licensed  
567 from the Greek veterinary directorate under the protocol number 7467/24-12-2013. All animal  
568 experiments were reported using the ARRIVE guidelines.

569

570 **PBMCs isolation and stimulation**

571 Venous blood from the antecubital vein of healthy volunteers was drawn in EDTA tubes after  
572 obtaining written informed consent. PBMC isolation was performed as previously described<sup>51</sup>. Briefly,  
573 the PBMC fraction was obtained using density centrifugation in Ficoll-Paque (Pharmacia Biotech,  
574 Piscataway, USA). Cells were then washed twice in PBS and re-suspended in RPMI-1640+ medium  
575 (RPMI1640 Dutch modification supplemented with 50µg/mL gentamicin, 2mM L-glutamine and 1mM

576 pyruvate; Gibco, Invitrogen, Breda, The Netherlands). Afterwards, PBMCs were counted and re-  
577 suspended in a concentration of  $5 \times 10^6$  cells /mL.  $5 \times 10^5$  PBMCs were added in 100  $\mu$ L to round-bottom  
578 96-well plates (Greiner, Alphen Aan Den Rijn, NL) and incubated with 50  $\mu$ L of stimulus (RPMI, live, 4%  
579 paraformaldehyde (PFA) or heat killed *Candida albicans* yeast  $1 \times 10^6$  /mL or *Candida auris*  $1 \times 10^6$  /mL;  
580 100  $\mu$ g/mL purified *C. albicans* or *C. auris* mannan; 10  $\mu$ g/mL purified *C. albicans* or *C. auris*  $\beta$ -glucan)  
581 and 50  $\mu$ L of eventual inhibitor or medium with or without 10% human serum. Serum was either  
582 complement active, if not otherwise indicated, or heat inactivated by incubation for 30 minutes at  
583 56°C in a water bath according to a commonly used protocol. After 1 hour of pre-incubation with  
584 inhibitor or medium, stimuli or medium was added. In detail, for receptor blockade experiments,  
585 before stimulation with *C. albicans* or *C. auris*, PBMCs were pre-incubated for 1 hour with 5  
586  $\mu$ g/mL anti-DC SIGN antibody 10  $\mu$ g/mL, anti-Dectin-1, 10  $\mu$ g anti Mincle and 10  $\mu$ g/mL control  
587 IgG2b; 10  $\mu$ g/mL anti-Dectin-2 antibody and 10  $\mu$ g/mL of its control IgG1]); 10  $\mu$ g/mL anti-CR3  
588 antibody and control IgG (R&D), 10  $\mu$ g/mL MR-blocking antibody and 10  $\mu$ g/mL Goat IgG  
589 isotype control. After 1 hour, cells were stimulated with  $10^6$  heat-killed *C. albicans* and *C.*  
590 *auris*. For the intracellular pathways blockade experiment 50 nM Syk inhibitor, 1  $\mu$ M Raf-  
591 inhibitor or the same concentration of vehicle (DMSO) has been used. Concentrations of  
592 inhibitors were selected as being the highest non-cytotoxic concentrations. All supernatants  
593 were stored at -20°C until analyzed.

<b>Antibodies</b>		
<i>Product</i>	<i>Source</i>	<i>Article Number</i>
DC-SIGN Monoclonal Antibody (clone 120507)	Fisher Scientific	MA1-25615
Human Dectin-1/CLEC7A Allophycocyanin Mab (Clone 259931)	Bio-Techne/R&D	MAB1859
Anti-hMincle-IgG	Invivogen	mabg-hmcl
Mouse IgG2B Isotype Control	Bio-Techne/R&D	MAB004
Human Dectin-2/CLEC6A Antibody	Bio-Techne/R&D	MAB3114

IgG1 Isotype Control	Bio-Techne/R&D	MAB002
Human MMR/CD206 Antibody	Bio-Techne/R&D	AF2534
anti-hIntegrin beta2 - hIntegrin b2 Affinity Purified Goat IgG	Bio-Techne/R&D	AF1730

594

595 **Cytokine and lactate measurements**

596 All cytokine levels were measured in the cell culture supernatants using commercially  
597 available ELISA assays according to the protocol supplied by the manufacturer. For human  
598 cytokines, IL-1 $\beta$ , TNF $\alpha$ , IL-6, IL-1Ra, and IL-10 were measured after 24 hours, and IL-17, IL-22,  
599 and IFN- $\gamma$  were measured after 7 days of stimulation. For mouse cytokines, KC, IL-1 $\beta$ , IL-6, IL-  
600 10, IL-17, and IFN- $\gamma$  were measured after 3 and 7 days. Lactate was measured by a Lactate  
601 Fluorometric Assay Kit (Biovision, Milpitas, USA).

602

<b>ELISA assays</b>		
<i>Product</i>	<i>Source</i>	<i>Article Number</i>
Human IL-1 $\beta$ ELISA	R&D Systems	DY201
Human TNF $\alpha$ ELISA	R&D Systems	DY210
Human IL-17 ELISA	R&D Systems	D1700
Human IL-22 ELISA	R&D Systems	D2200
Human IL-6 ELISA	R&D Systems	DY206
Human IL-8 ELISA	Sanquin	M1918
Human IL-10 ELISA	R&D Systems	D1000B
Human IFN- $\gamma$ ELISA	Sanquin	M1933
Mouse KC ELISA	R&D Systems	MKC00B
Mouse IL-1 $\beta$ ELISA	R&D Systems	MLB00C
Mouse IL-6 ELISA	R&D Systems	M6000B
Mouse IL-10 ELISA	R&D Systems	M1000B
Mouse IL-17 ELISA	R&D Systems	M1700
Mouse IFN- $\gamma$ ELISA	R&D Systems	MIF00

603

604 **Cytotoxicity assay**

605 5x10<sup>5</sup> PBMCs were added in 100 µL to flat-bottom 96-well plates (Greiner, Alphen Aan Den Rijn, NL)  
 606 and incubated with 50 µL of stimulus (RPMI, live *Candida albicans* yeast 1x10<sup>6</sup>/mL or different strains  
 607 of live *Candida auris* 1x10<sup>6</sup>/mL) for 24 hours. Cell viability was assessed using CytoTox 96 non-  
 608 radioactive cytotoxicity assay (Promega, Leiden, the Netherlands) according to the  
 609 manufacturers' instructions. Released lactate dehydrogenase (LDH), a stable cytosolic enzyme  
 610 released upon cell lysis, was measured in the supernatant with a 30-minute coupled  
 611 enzymatic assay. The color intensity, as a result of the conversion of tetrazolium salt (INT) into  
 612 ref formazan, is proportional to the number of lysed cells. As positive control, cells were lysed  
 613 with 0.5% Triton-X reflecting maximal LDH release.

614

615 ***Candida* strains**

616 *C. auris* strains from five clades have been used (clade I, South Asia; clade II, East Asia; clade  
 617 III, South Africa; clade IV, South America; clade V, Iran). *C. albicans* and *C. auris* strains were  
 618 prepared by growing cells for 24h in either a Sabouraud broth or on Sabouraud plates at 30°C.  
 619 Unless otherwise indicated, experiments were performed using *C. albicans* CWZ10061110, *C.*  
 620 *auris* KCTC17810 reference, *C. auris* CWZ10051893 and *C. auris* CWZ10051895. Stimulations  
 621 were performed using either live, heat-killed (12 hours at 56°C) or 4% PFA-killed  
 622 microorganisms. Heat killing caused the disruption of the outer layer of the *Candida* cell wall  
 623 <sup>34</sup> in turn enhancing β-glucan exposure <sup>35,36</sup>. While *Candida* killing *via* heat treatment disrupts  
 624 the outer layer causing exposure of β-glucan in the cell wall, with PFA fixation and thimerosal  
 625 fixation the cell wall structure remains intact <sup>34,52,53</sup>.

Fungal Strains	from	name(s)
<i>C. albicans</i>	ATCC	ATCC MYA-3573/UC820
<i>C. albicans</i>	ATCC	SC5314 / ATCC MYA-2876

<i>C. albicans</i>	J. Meis (Clinical blood isolate)	CWZ 10061110
<i>C. albicans</i>	J. Meis (Clinical blood isolate)	CWZ 10070679
<i>C. auris</i> (Clade I)	A. Chowdhary	CWZ 10051893 (Clade I)
<i>C. auris</i> (Clade I)	A. Chowdhary	CWZ 10051895 (Clade I)
<i>C. auris</i> (Clade II)	J. Meis	KCTC17810 (Clade II)
<i>C. auris</i> (Clade III)	J. Meis	CDC AR 383/B11221 (Clade III)
<i>C. auris</i> (Clade IV)	J. Meis	CWZ 10051522 (Clade IV)
<i>C. auris</i> (Clade V)	J. Meis	CDC AR 1097/ IFRC 2086/CWZ 10111018 (Clade V)

626

627

628

629  **$\beta$ -glucan and mannan isolation from *C. auris* strains**

630 For the cell wall experiments, a total of 8 different *C. auris* clinical strains were used for  
631 glucan and mannan extraction, originating from three different clades (I, II and IV). Isolates  
632 were grown in 25 mL YPD (1% yeast extract, 2% dextrose, 2% peptone) at 30°C for 48 hours  
633 before parallel isolation of respective cell wall components under identical conditions. Aiding  
634  $\beta$ -glucan and mannan collection, cell walls were disrupted by 3 cycles of freeze/thawing (-  
635 20°C) and consecutive harvesting of cell pellets. Of each culture a small aliquot was plated on  
636 YPD media to exclude the presence of viable cells, after which the cell pellets were suspended  
637 in 10 mL of 0.75 N NaOH to a final concentration of ~3 mg/mL. Suspensions were heated to  
638 105°C for 15 minutes, cooled and separated by centrifugation (10 minutes x 863 g). The  
639 supernatant, containing the mannans, were harvested and dialyzed against 300 volumes of 18  
640 MOhm water (2000 MWCO). After subsequent harvesting, the pH was confirmed to neutral,  
641 followed by freezing and lyophilization to dryness. For  $\beta$ -glucans, the centrifuged extract was

642 transferred, treated with 10 mL of 1.0N H<sub>3</sub>PO<sub>4</sub> and heated 105°C for 15 minutes. After cooling  
643 down, pellets were collected by centrifugation (10 minutes x 863 g) and extracted once more  
644 with 10 mL 100% ethanol containing 1% (v/v) H<sub>3</sub>PO<sub>4</sub> at 90°C for 15 minutes. β-glucans were  
645 harvested by centrifugation and purified by washing 3x with 18 MOhm water, after which the  
646 final pellet was frozen and lyophilized to dryness.

647

#### 648 ***C. albicans* mannan isolation**

649 Mannan isolation was performed using a modification of the Fehling method as previously  
650 described<sup>54</sup>. Briefly, yeast cells, grown O/N at 30°C, were delipidated by suspending cell  
651 pellets in 100 mL of acetone and incubation for 20 minutes. The supernatant was decanted  
652 after 5-minute centrifugation at 500 rpm and pellets were air-dried for 30 minutes.

653 Facilitating mannan extraction, pellets were suspended in 200 mL of distilled H<sub>2</sub>O (dH<sub>2</sub>O) and  
654 supplemented with an equivalent amount of glass beads 0.5μ to pellet weight and followed  
655 by bead beating (3x 30 second pulses). Cell extracts were autoclaved for two hours and  
656 subsequently centrifuged for 5 minutes at 5,000 rpm. Respective supernatant was split into  
657 two; one half was left untreated while the other was treated with 500 mg of pronase for 16  
658 hours at 37°C to abolish glycosidic activity. Ensuring the suppression of bacterial growth  
659 during pronase treatment sodium azide was added at a final concentration of 50 nM. The  
660 pronase-treated and untreated samples were 1:1 diluted with freshly prepared Fehling  
661 solution and allowed to mix for 1 hour at RT, followed by a 20-minutes incubation allowing  
662 precipitation of the mannan-copper complexes. After decantation, complexes were treated  
663 with 5 mL HCl. When dissolved, 100 mL of a 1:8 mixture of glacial acetic acid:methanol  
664 (GAE:MEOH) was added for mannan precipitation. After 4 hours, samples were washed  
665 repeatedly with GAE:MEOH until the remaining precipitate appeared colorless (lack of any

666 blue/green) and followed by 3x methanol washes. The final precipitate was dissolved in 100  
 667 mL of dH<sub>2</sub>O and dialyzed against 300 volumes of dH<sub>2</sub>O over 48 hours and for 12 hours against  
 668 200 volumes of ultrapure dH<sub>2</sub>O to remove any salts, acid, methanol and other low molecular  
 669 weight contaminants remaining from the extraction protocol. Dialyzed mannans were frozen  
 670 and lyophilized for 48 – 72 hours and stored at -20°C.

<b>Cell wall components</b>		
<b>Name</b>	<b>from</b>	<b>Fungal strains</b>
<i>C. auris mannans 1</i>	David Williams	KCTC17810 (Clade II)
<i>C. auris mannans 2</i>	David Williams	CWZ 10031160 (2012) (Clade I)
<i>C. auris mannans 3</i>	David Williams	CWZ 10031163 (2012) (Clade I)
<i>C. auris mannans 4</i>	David Williams	CWZ 10051256 (2013) (Clade I)
<i>C. auris mannans 5</i>	David Williams	CWZ 10051263 (2013) (Clade I)
<i>C. auris mannans 6</i>	David Williams	CWZ 10051522 (2014) (Clade IV)
<i>C. auris mannans 7</i>	David Williams	CWZ 10051244 (2014) (Clade I)
<i>C. auris mannans 8</i>	David Williams	CWZ 10051252 (2014) (Clade I)
<i>C. albicans mannans</i>	David Williams	SC5314
<i>C. auris β-glucans 1</i>	David Williams	KCTC17810 (Clade II)
<i>C. auris β-glucans 2</i>	David Williams	CWZ 10031160 (2012) (Clade I)
<i>C. auris β glucans 3</i>	David Williams	CWZ 10031163 (2012) (Clade I)
<i>C. auris β -glucans 4</i>	David Williams	CWZ 10051256 (2013) (Clade I)
<i>C. auris β glucans 5</i>	David Williams	CWZ10051263 (2013) (Clade I)
<i>C. auris β glucans 6</i>	David Williams	CWZ10051522 (2014) (Clade IV)
<i>C. auris β glucans 7</i>	David Williams	CWZ 10051244 (2014) (Clade I)
<i>C. auris β glucans 8</i>	David Williams	CWZ 10051252 (2014) (Clade I)
<i>C. albicans β glucans</i>	David Williams	SC5314

671

672 **RNA purification**

673 PBMCs from three healthy donors, with a concentration of  $5 \times 10^6$  cells/mL, were stimulated  
 674 in flat-bottom 12-well plates (Corning, NY, USA) with freshly counted live *C. albicans* ( $1 \times 10^6$ /mL)  
 675 and *C. auris* ( $1 \times 10^6$ /mL), and purified cell wall components β-glucans (10 μg/mL) and mannans  
 676 (10 μg/mL) isolated from both *Candida* species as described above. Of note, the time between  
 677 *Candida* cell count and experiment start did not exceed 15 minutes. PBMCs were cultured in  
 678 the presence of 10% human pooled serum. At 4 and 24 hours cells were lysed with RLT

679 buffer. Prior to subjection to the RNeasy Mini Kit (Qiagen), lysates were homogenized using a  
680 1 mL syringe with a 0.8 x 15 mm needle. RNA was subsequently extracted following  
681 manufacturers' protocol, including an on-column DNase digestion using the RNase-Free  
682 DNase set (Qiagen). Quantification and quality assessment of extracted RNA was performed  
683 using the Qubit RNA HS assay (Thermo Fisher Scientific) and Agilent 2200 TapeStation (RNA  
684 HS Screentape, Agilent), respectively. The majority of samples subjected to quality  
685 assessment revealed an RNA integrity number (RIN<sup>e</sup>) of  $\geq 8$ .

### 686 **QuantSeq 3' mRNA sequencing**

687 Libraries were generated from the extracted RNA using the QuantSeq 3' mRNA-Seq Library  
688 Prep Kit-FWD from Lexogen (Lexogen, Vienna, Austria) in accordance to the manufacturers'  
689 protocol. Three separate preparations were performed, split by PBMC donor, in turn limiting  
690 the number of samples to 14 to 18 samples per prep. RNA input was normalized to 100 ng for  
691 donor A, and to 250 ng for donors B and C. An aliquot (1:10) of double stranded cDNA  
692 libraries was used for quantitative PCR, in turn indicating 17 – 18 cycles as optimal for  
693 endpoint PCR (17- donor B; 18 - donors A, C). Accurate quantification and assessment of  
694 quality of the generated libraries was performed using Qubit dsDNA HS assay (Thermo Fisher  
695 Scientific, Waltham, USA) and Agilent 2200 TapeStation (HS-D1000 ScreenTape, Agilent, Santa  
696 Clara, USA). The cDNA concentration and average fragment size were used to determine the  
697 molar concentration of the individual libraries. Consequently, libraries were pooled equimolar  
698 to 100 fmol. After a final dilution of the pool to a concentration of 4 nM, the libraries were  
699 sequenced on a NextSeq 500 instrument (Illumina, San Diego, USA), with 75 cycle (i.e. 75bp  
700 single-end sequence reads), high output kit with a 1.1 pM final loading concentration.

## 701 Differential gene expression analysis

702 Quality of the acquired sequencing data was controlled using FastQC tool v0.11.5 (Babraham  
703 Bioinformatics) and subsequently followed by the removal of adapter sequences and poly(A)  
704 tails with Trim Galore! v.0.4.4\_dev (Babraham Bioinformatics) and Cutadapt v1.18<sup>55</sup>. On  
705 average ~ 6 million reads per individual library were retrieved. Filtered and trimmed reads  
706 were mapped to the human reference genome (hg38/GRCh38) using the STAR aligner v2.6.0a  
707 ([Supplementary Table 4](#))<sup>56</sup>. Less than 1% of all reads were comprised of overrepresented  
708 sequences and were uniquely mapped with a median of 4 million reads (74.1%). After  
709 generating gene level count data using the HTSeq-count tool v0.11.0<sup>57</sup>, an additional filtering  
710 step was performed ensuring the exclusion of several non-coding RNAs, i.e. mtRNA, lincRNA,  
711 snRNA, tRNA, miscRNA and snoRNA, in our dataset. Given the absence of sample replicates,  
712 PBMC donors were considered biological replicates. Hence, in the differential gene expression  
713 analysis using DESeq2 v1.22.0, including logFold Shrinkage and apeglm<sup>58</sup>, the average PBMC  
714 donor response to the different stimuli were compared to their control condition, RPMI.  
715 Genes with a fold change of  $\geq 2$  and a p-adjusted value  $< 0.01$  were considered differentially  
716 expressed genes (DEG). Allowing identification of the main principle components  
717 underpinning the majority of the variance, a PCA analysis was performed on DESeq2  
718 normalized counts (normTransform).

719

## 720 Pathway enrichment analysis

721 In order to distinguish between the responses triggered by both *Candida* species, DEG were  
722 compared between species for the analogous stimulations (live, mannan and  $\beta$ -glucan), and  
723 corresponding time-points. In turn resulting a group of DEG that overlap between the two  
724 species, and DEG that were uniquely attributed to either one of the *Candida* species.

725 Overrepresentation analysis were performed on all groups per stimulation (and time-point)  
726 using Consensus PathDB<sup>59</sup>, including pathways as defined by pathway databases Kyoto  
727 Encyclopedia of Genes and Genomes KEGG<sup>60</sup> and Reactome<sup>61</sup>. Minimum overlap in input  
728 was set at 2, together with a p-value cut-off of 0.01. For downstream analysis, pathways were  
729 considered enriched with a corrected p-value <0.01 (indicated as 'q-value').

730

### 731 **Structural characterization of mannans by NMR spectroscopy**

732 To gain insight into the mannan structure of both *Candida* species, isolated mannans were  
733 subjected to solution-state 1D <sup>31</sup>P-coupled and <sup>31</sup>P-decoupled <sup>1</sup>H NMR spectroscopy and 2D  
734 COSY NMR spectroscopy. Data acquisition and subsequent analysis were based on methods  
735 described by Lowman et al.<sup>62</sup>. In short, <sup>1</sup>H NMR spectra were collected using a Bruker Avance  
736 III 600 NMR spectrometer operating at 331°K (58°C). Roughly 10 mg of mannan was dissolved  
737 in 600 mL of dH<sub>2</sub>O. Chemical shift referencing was accomplished relative to TMSP at 0.0 ppm.  
738 Proton 1D NMR spectra were collected with 2 dummy scans, 256 scans, 65,536 data points,  
739 20 ppm sweep width centered at 6.2 ppm, and 1 second pulse delay. For the 1D <sup>31</sup>P  
740 decoupled <sup>1</sup>H NMR experiment, spectra were collected at 333° K (60° C) with 2 dummy scans,  
741 1024 scans, 65,536 data points, 21 ppm sweep width centered at 6.2 ppm and the <sup>31</sup>P  
742 decoupling pulse centered at 3.0 ppm. All 1D spectra were processed using exponential  
743 apodization with 0.3 Hz line broadening. COSY spectra were collected using 2048 by 128 data  
744 points, 8 dummy scans, 32 scans, and 6.0 ppm sweep width centered at 3.0 ppm and  
745 processed with sine apodization in both dimensions and zero-filled to 1024 data points in f1.  
746 Processing was accomplished with the JEOL DELTA (version 5.0.4.4) and Bruker TopSpin  
747 (version 4.0.6) software packages.

748

749 **Molecular weight measurements**

750 To determine the MW of mannans, isolates from *C. albicans* and *C. auris* strains were  
751 subjected to high performance gel permeation chromatography (GPC) as previously described  
752 <sup>63</sup>. Using a Viscotek/Malvern GPC system, consisting of a GPCMax autoinjector fitted to a TDA  
753 305 detector (Viscotek/Malvern, Houston, TX). System calibration was achieved using  
754 Malvern pullulan and dextran standards. Mannan isolates, ranging between 3 to 6 mg/mL,  
755 dissolved in mobile phase (50 mM of sodium nitrate, pH 7.3) were subjected to a 60-minute  
756 incubation at 60°C, followed by sterile filtration (0.2 µm) and injected into the GPC (100-200  
757 µL). Samples were analyzed in duplicate or triplicate and data analysis performed with  
758 Viscotek OmniSec software (version 4.7.0.406).

759

760 **Binding interaction of mannans with PRRs rhDectin-2 and rhMannose**

761 Assessment of mannan binding interactions to the recombinant Dectin-2 and Mannose  
762 receptors (R&D systems) were carried out on an Octet K2 BLI instrument (ForteBio) in 10X  
763 Kinetics Buffer (pH 7.4) at 30°C and 1000 rpm. Increasing concentration of the respective  
764 ligands (3.125-400 µg/mL) were used to generate respective saturation curves, after which  
765 the binding affinities were calculated for mannans isolated from *C. albicans* and *C. auris*  
766 strains as previously described <sup>64</sup>. The Ni-NTA biosensor was subjected to a 3-minute  
767 equilibration prior to 10 minutes of exposure 0.1 ug/mL HIS-tagged rhDectin-2 or rhMannose  
768 receptor proteins and final a 10-minute dissociation in 10X kinetics buffer for measuring the  
769 BLI signal, consistently 20 seconds after transferring. Subsequently followed by a series 8  
770 similar 5-minute exposure to an increasing concentration (2-fold) of carbohydrate. To control  
771 for receptor dissociation during the experiment, a parallel biosensor with the immobilized  
772 receptors was placed in the 10X kinetics buffer without respective carbohydrate exposure.

773 Data analysis was performed using the GraphPad Prism 7.0 software and the dissociation  
774 constant  $K_D$  is presented as mean value with a 95% confidence interval.

775

#### 776 **ROS assay**

777 The induction of ROS was measured by oxidation of luminal (5-amino-2,3, dihydro-1,4-  
778 phtalazinedione) and determined in an automated LB96V Microlumat plus luminometer (EG & G  
779 Berthold, Bad Wildbad, Germany). Briefly, PBMCs ( $5 \times 10^5$  per well) or neutrophils ( $2.5 \times 10^5$ ) per well  
780 were seeded into white 96-well plates and incubated in medium containing either RPMI, Zymosan  
781 ( $100 \mu\text{g}/\text{mL}$ ), heat-killed opsonized *C. albicans* or *C. auris* yeast ( $10^7$  CFU/mL). 20  $\mu\text{L}$  of 1 mM Luminol  
782 was added to each well in order to start the chemiluminescence reaction. Each measurement was  
783 carried out at least in duplicate. Chemiluminescence was determined every 145 seconds at  $37^\circ\text{C}$  for 1  
784 hour. Luminescence was expressed as relative light units (RLU) per second. The RLU/sec within the  
785 area under the curve (AUC) were plotted against time and analyzed by using Graphpad Prism v.7.0.

786

#### 787 **FITC-labelling of *Candida***

788 To label cells with fluorescein isothiocyanate (FITC, CAS Number: 3326-32-7),  $1 \times 10^8/\text{mL}$  of  
789 thimerosal fixed cells were sonicated and resuspended in 0.1 mg/mL of FITC in 0.1 M  
790 carbonate-bicarbonate buffer (pH 9.6). After incubation for 30 minutes on a tube roller at  $4^\circ\text{C}$   
791 in the dark, unbound FITC was washed away by centrifugation at 3000 rpm  $4^\circ\text{C}$  for 10 minutes  
792 three times in PBS. Before use, cells were resuspended to a concentration of  $1 \times 10^7/\text{mL}$  in PBS,  
793 aliquoted and stored in the dark at  $-20^\circ\text{C}$ .

794

#### 795 **Phagocytosis assay in human cells**

796 To test *Candida* strains uptake by human monocytes,  $4 \times 10^6$  cells/mL of thimerosal-killed  
797 FITC-labelled *C. albicans* and *C. auris* were pre-opsonized with 20% human pooled serum for 1

798 hour at 37°C, 5% and subsequently incubated with  $2 \times 10^5$  PBMCs/well (MOI 2:1,  
799 fungal:human cells) for either 30 minutes or 2 hours at 37°C, + 5% CO<sub>2</sub>. After the incubation  
800 period, cells were gently washed with PBS (1% BSA) and then stained in a total volume of 50  
801 µL using CD14 monoclonal antibody (Mouse-anti-Human CD14 Pacific Blue, Beckman coulter,  
802 clone RMO52, dilution 1:20) for 30 minutes at 4°C on ice in the dark. Afterwards, cells were  
803 washed, and the fluorescence signal of extracellular non-phagocytosed *Candida* was  
804 quenched with 0.1% Trypan blue solution (Sigma, St. Louis, USA; CAS Number 72-57-1). Cells  
805 were subsequently measured on a CytoFLEX flow cytometer (Beckman coulter, Pasadena,  
806 USA) and the data were analyzed using the Kaluza Analysis software version 2.1. To determine  
807 the uptake of *C. albicans* and *C. auris* by human monocytes, the percentage of CD14 positive  
808 cells which had phagocytosed FITC positive *Candida* (percentage of FITC-positive cells in the  
809 CD14-positive population) was calculated. For the detailed gating strategy see Extended data  
810 Fig. 7a.

811

#### 812 **Phagocytosis assay in BMDMs**

813 Bone marrow was extracted from femurs and tibias of eight-week-old male C57BL/6 mice and  
814 differentiated for 7 days with RPMI Medium 1640 Glutamax (Gibco) supplemented with 10%  
815 heat-inactivated foetal calf serum, 100 U/mL Penicillin/Streptomycin and 15% L929 cell-  
816 conditioned medium at 37°C with 5% CO<sub>2</sub>. BMDM were added to 8 well u-slide (ibidi,  
817 Gräfelfing, Germany) at  $0.5 \times 10^5$  cells per well to adhere overnight. *C. albicans* and *C. auris*  
818 strains were prepared by growing cells for 24 hours in Sabouraud broth at 30°C and followed  
819 by 3 washes in PBS. Fixed *Candida* cells were prepared by incubating the Sabouraud-grown  
820 yeast overnight at room temperature in 50 Mm thimerosal (Sigma, St. Louis, USA) followed by  
821 5 wash steps in PBS. Phagocytosis dynamics were determined following the addition of 3:1;

822 yeast: BMDM. The intended Multiplicity of Infection (MOI) was calculated using cell count  
823 (hemocytometer) and the actual MOI was observed from videos. Live imaging of macrophage  
824 interactions with live or fixed *C. albicans* and *C. auris* were performed using a Nikon Ti Eclipse  
825 microscope with objective 20x magnification set to acquire images at 1-minute intervals using  
826 Volocity software (Version 6.3, PerkinElmer, Waltham, USA), with thanks to the University of  
827 Aberdeen Microscopy Core Facility. Movies generated from 3 hour interactions were  
828 analyzed to determine over time the proportion of macrophages phagocytosing yeast (%  
829 uptake), the number of yeast phagocytosed per 100 macrophages (phagocytic index), the  
830 proportion of macrophage death after 3 hours (macrophage lysis) and the distribution of  
831 yeast contained within individual macrophages. Experiments were performed on 3 occasions,  
832 with a total of 9 movies generated per condition. Phagocytic index data are based on yeasts  
833 fully inside macrophages. Yeasts adhering but not internalized would not be included in cell  
834 counts for phagocytic index. Statistical analyses were performed by ANOVA using GraphPad  
835 Prism (v 7.0).

836

### 837 **Cell wall staining**

838 Fixed *Candida* yeast were stained for exposed cell wall  $\beta$ -glucans using Fc-Dectin-1 (a gift from  
839 Gordon Brown, University of Aberdeen) and secondary F(ab')<sub>2</sub> anti-human IgG AlexaFluor 488  
840 conjugate (Life Technologies). ConA-Texas Red conjugate (Life Technologies, Carlsbad, USA)  
841 was used to detect cell wall mannans. Cells were counted, and  $2.5 \times 10^6$  yeast were combined  
842 with FACS wash (1% bovine serum albumin and 5 mM EDTA in PBS) with either Fc-Dectin-1 at  
843 1  $\mu$ g/mL or ConA at 25  $\mu$ g/mL. After a 30 min incubation on ice, cells were washed twice in  
844 FACS wash, then incubated with secondary F(ab')<sub>2</sub> (for Fc-Dectin-1 only) on ice for 45  
845 minutes, with a further 2 wash steps. Flow cytometry was performed on an LSR Fortessa

846 cytometer (BD, Macquarie Park, Australia) with thanks to the University of Aberdeen IFCC  
847 Core Facility. Data were analysed using FlowJo software (version 10.0.8). For the detailed  
848 gating strategy see [Extended Data Fig. 7b](#).

849

### 850 ***In vivo* experimental model of disseminated candidiasis**

851 For the *in vivo* experiments *C. auris* strain 10051895 was selected as representative of Clade I,  
852 which was the first *C. auris* clade identified and associated with bloodstream infections and  
853 high mortality rates<sup>17</sup>. Experiments were conducted with a total of 200 C57Bl6 male mice, 7-8  
854 weeks old, which were purchased from Pasteur Institute (Athens, Greece, EL 25 BIObr 011).  
855 Mice were allowed to acclimate for seven days before start of the experiments. Animals were  
856 housed in cages, with no more than 5 mice per cage, under constant temperature (21°C) and  
857 humidity with a 12-h light/dark cycle. All animals had ad libitum access to food and water.  
858 Analgesia was achieved with paracetamol suppositories. Other analgesics were avoided in  
859 order to avoid interactions with the immune system. Healthy mice were i.v. challenged via the  
860 tail vein with  $1 \times 10^7$  CFU/mouse log-phase inoculum<sup>46,47</sup> of *C. albicans* 10061110 (n=11) and *C.*  
861 *auris* 10051895 (n=10) following slight anesthesia with methoxyflurane (2, 2-dichloro-1,1  
862 difluoroethyl methyl-ether in butylated hydroxytoluene 0.01% w/w). Mice were split into  
863 groups via a randomization table. Survival was recorded for 14 days; three- and seven-days  
864 post challenge mice were sacrificed by the intramuscular injection of ketamine. For evaluating  
865 the fungal burden at day 3 and day 7 from the inoculation of  $1 \times 10^6$  CFU/mice (n=4-5 mice per  
866 group per timepoint), we removed kidneys and livers were weighted and homogenized. The  
867 number of fungal counts were measured via serial dilutions 1:10 at 0.9% saline and expressed  
868 as log<sub>10</sub> CFU/g. For collecting the organs homogenates, at day 3 and day 7 from the injection

869 of  $1 \times 10^6$  *Candida* CFU/mice (n=5-6 mice per group per timepoint), after a midline incision  
870 under aseptic conditions, the entire spleen was removed and segments of the right kidney  
871 and of the liver were cut and put into separate sterile containers.

872

### 873 **Statistical analysis**

874 Statistical analysis, except when otherwise indicated, was performed using the Graphpad  
875 Prism 7 software. All experiments were performed at least in duplicate. In experiments with a  
876 sample size <4, no statistical testing was performed due to a small sample size. Datasets with  
877 a sample size >8 were tested for normality via the D'Agostino-Pearson omnibus normality  
878 test, and when normally distributed subjected to a 1-way ANOVA including Holm-Sidak's  
879 multiple comparison test, as specified in corresponding method section and figure captions.  
880 Given the experimental set-up, where the same donors were used, yet exposed to different  
881 *Candida* species and strains (stimulations), measurements were considered  
882 paired/dependent. Hence, the Wilcoxon signed-rank test for non-parametric matched data  
883 was applied to non-normally distributed data. To enhance insight in the *C. auris*' induced host  
884 response, all statistical analysis was performed comparing respective *C. auris* strains with *C.*  
885 *albicans*, in the current study considered the control species. In the case of non-normally  
886 distributed data and comparison to multiple control strains, a Kruskal Wallis test including  
887 Dunn's multiple comparison was applied. For statistical testing of *IL-6*, *IL-1B*, and *IL-1RN*  
888 expression levels, an ordinary 1-way ANOVA was performed comparing the Log2 Fold  
889 Changes and corresponding lfcSEs between the respective *C. auris* and *C. albicans* (live,  $\beta$ -  
890 glucans and mannans) conditions. Moreover, *in vivo* mice data presents an exception, with a

891 Log rank-test for survival assessment and Mann Whitney U test to assess fungal burden due  
892 sample independence. In all cases, a p-value of  $<0.05$  was considered significant.

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### 1073 DATA AVAILABILITY

1074 Requests for materials should be addressed to the corresponding autor (M.G.N.). The

1075 datasets generated from this study are accessible through GEO Series accession number

1076 [GSE154911 \(https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE154911\)](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE154911). Source data  
1077 is provided for main and extended data figures.

1078

#### 1079 **COMPETING INTERESTS**

1080 The authors declare no competing interests.

1081

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#### 1096 **AUTHOR CONTRIBUTIONS**

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1103 [contributed equally to this work. J.F.M., D.W.L. and M.G.N. share senior authorship.](#)

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1105

## 1106 [Main Figure Legends](#)

1107 **Figure 1 | Comparative analysis of the general and clade-specific *C. auris* induced host response with *C.***  
1108 ***albicans* at 24 hours. a,** Venn diagram representing the number of DEG of both *Candida* species and  
1109 their relative overlap, reveals substantial overlap between the *C. albicans* (10061110) and *C. auris*  
1110 (KCTC17810, clade II) live induced host-response at 24 hours. DEG were subjected to a pathway  
1111 enrichment analysis, in turn revealing the top 15 *Candida* intrinsic (overlapping DEG, middle panel)  
1112 and species specific (DEG unique for *C. albicans*, left panel; DEG unique for *C. auris*, right panel)  
1113 pathways. Enrichment determined using Consensus PathDB, including pathways as defined by KEGG  
1114 (red) and Reactome (pink), considering a p-adjusted value < 0.01 (indicated as 'q-value') significant.  
1115 Size of the geometric points indicates the amount of DEG in relation to the pathways' size. [The exact q](#)  
1116 [values and DEG in pathways can be found in Supplementary Table 2.](#) **b-c,** *C. auris* is a more potent  
1117 inducer of the immune system in comparison to *C. albicans*. **b,** TNF- $\alpha$ , IL-6, IL-1 $\beta$ , and IL-1Ra levels in  
1118 supernatants of PBMCs after stimulation without (RPMI; negative control) or with live *C. albicans* and  
1119 *C. auris* for 24 hours (n=12). **c,** TNF- $\alpha$ , IL-6, IL-1 $\beta$ , and IL-1Ra levels in supernatants of PBMCs after  
1120 stimulation without (RPMI; negative control) or with live *C. albicans* and *C. auris* from all five  
1121 geographical clades for 24 hours (n=8). Graphs represent mean  $\pm$  SEM, pooled from at least two  
1122 independent experiments. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001, two-sided Wilcoxon matched pairs  
1123 signed-rank test was performed comparing respective *C. auris* strains with *C. albicans* as control or  
1124 reference species. [The data used to make this figure can be found in Source Data Fig.1.](#)

1125

1126 **Figure 2 | Evaluation of *C. auris* phagocytosis dynamics by human and murine host immune cells and**  
1127 **heat-sensitivity of the cell wall component responsible for *C. auris* induced cytokine production. a,** The  
1128 BMDM phagocytic capacity of live *C. albicans* or *C. auris* strains in a 3-hour period. BMDM engulfment,  
1129 depicted as the percentage of macrophages having phagocytosed at least one fungal cell (left).  
1130 Phagocytic index was considered the number of fungal cells engulfed per 100 macrophages (right);  
1131 graphs represent mean (n=9), pooled from at least two independent experiments. **b,** Phagocytosis  
1132 assay in human PBMCs: percentage of FITC-positive cells in the CD14<sup>+</sup> population. Phagocytosis  
1133 efficiency assessed as percentage of CD14<sup>+</sup> cells that engulfed FITC-labelled *Candida* (left) and  
1134 corresponding mean fluorescence intensity (MFI) of the total CD14<sup>+</sup> population (right). Graphs  
1135 represent mean  $\pm$  SEM (n=6), pooled from two independent experiments, \* p < 0.05, two-sided

1136 Wilcoxon matched pairs signed-rank test, comparing respective *C. auris* strains with *C. albicans* as  
1137 control or reference species. **c**, Distribution of phagocytosed live fungal cells per macrophage in a  
1138 period of 3 hours,  $n \geq 100$  observations per condition. **d**, Killing capacity of live *C. albicans* and *C. auris*,  
1139 depicted as the percentage of lysed macrophages (BMDM) after 3 hours of exposure.  
1140 Yeast:Macrophage ratio (MOI) was 3:1. Graphs represent mean  $\pm$  SEM,  $n = 9$  ( $n=10$  for *C. auris*  
1141 10051895), pooled from at least two independent experiments, \*  $p < 0.05$ , Kruskal Wallis test with  
1142 two-sided Dunn's multiple comparison between the two *C. auris* strains and the two *C. albicans*. **e**,  
1143 TNF- $\alpha$  ( $n=10$ ), IL-6 ( $n=13$ ), IL-1 $\beta$  ( $n=13$ ), and IL-1Ra ( $n=9$ ) levels in supernatants of PBMCs after  
1144 stimulation without (RPMI; negative control) or with heat-killed *C. albicans* or *C. auris* for 24 hours.  
1145 Graphs represent mean  $\pm$  SEM, data are pooled from at least two independent experiments. \*  $p <$   
1146  $0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , two-sided Wilcoxon matched pairs signed-rank test, comparing  
1147 respective *C. auris* strains with *C. albicans* as control or reference species. [Data used to make this](#)  
1148 [figure can be found in Source Data Fig. 2.](#)

1149

1150 **Figure 3 | Mannans are fundamental for orchestrating the *C. auris* induced late host response.**  
1151 **a**, Split Venn diagrams indicating the number of DEG upon *C. albicans* 10061110 (left) and *C. auris*  
1152 KCTC17810 (clade II; right) live stimulation on the left, with its respective overlap between exposure to  
1153 the purified cell wall components  $\beta$ -glucan and mannan. Left split Venn diagram visualizes the early, 4-  
1154 hour response, and the right split Venn diagram reflects the late, 24-hour response. **b**, PBMC  
1155 production of cytokines TNF- $\alpha$ , IL-6, IL-1 $\beta$ , and IL-1Ra after 24 hours stimulation without (RPMI;  
1156 negative control) or with purified  $\beta$ -glucans from *C. albicans* and *C. auris* strains in the presence of  
1157 10% human serum,  $n=6$  ( $n=3$  for *C. auris*  $\beta$ -glucan 10051244). **c**, PBMC production of TNF- $\alpha$ , IL-6, IL-  
1158 1 $\beta$ , and IL-1Ra after 24-hour stimulation without (RPMI; negative control) or with Pam3cys and/or  
1159 purified  $\beta$ -glucans from different *C. albicans* and *C. auris* strains in the presence of 10% human serum,  
1160  $n=6$ . **d**, PBMC production of cytokines TNF- $\alpha$ , IL-6, IL-1 $\beta$ , and IL-1Ra after 24 hours stimulation without  
1161 (RPMI; negative control) or with purified mannans from *C. albicans* and *C. auris* strains in the presence  
1162 of 10% human serum,  $n=10$ . Graphs represent mean  $\pm$  SEM, pooled from at least two independent  
1163 experiments. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , two-tailed Wilcoxon matched pairs signed-rank  
1164 test and was performed comparing both cell wall components extracted from *C. auris* strains with *C.*  
1165 *albicans* as control or reference species (SC5314). [Data used to make this figure can be found in](#)  
1166 [Source Data Fig. 3.](#)

1167 **Figure 4 | Structural characterization of *C. auris* mannans.** (A) 2D COSY Nuclear magnetic resonance  
1168 (NMR) spectroscopy analysis of mannans purified from various *C. auris* clinical strains, originating from  
1169 clades I, II and IV, and *C. albicans*. Although NMR reveals varying side-chain lengths containing  $\alpha$ -1,2-  
1170 mannose,  $\alpha$ -1,3-mannose and  $\beta$ -1,2-mannose across clinical isolates, characteristic for *C. auris*  
1171 mannans are the two distinct M- $\alpha$ -1-phosphate side chains.

1172 **Figure 5 | Exploration of PRR and signaling pathways involved in the *C. auris* induced host cytokine**  
1173 **production.**

1174 **a**, PBMC production of cytokines TNF- $\alpha$ , IL-6, IL-1 $\beta$ , and IL-1Ra after 24 hours stimulation without  
1175 (RPMI; negative control) or with PFA-fixed *C. albicans* and *C. auris* strains, subjected to vehicle (DMSO)  
1176 ( $n=9$ ) or a 1-hour pre-incubation with Syk ( $n=6$ ) and Raf-1 ( $n=9$ ) inhibitors. **b**, PBMC production of

1177 cytokines TNF- $\alpha$ , IL-6, IL-1 $\beta$ , and IL-1Ra after 24 hours stimulation without (RPMI; negative control) or  
1178 with live *C. albicans* and *C. auris* strains, subjected to a 1 hour pre-incubation with the isotype  
1179 antibodies IgG2b, Goat IgG and IgG1, or DC-SIGN, Dectin-1, Mincle, MMR, CR3 and Dectin-2 blocking  
1180 antibodies, n=6. Graphs represent mean  $\pm$  SEM, data pooled from at least two independent  
1181 experiments, \* p < 0.05, \*\* p < 0.01, **a** two-sided Wilcoxon matched pairs signed-rank test comparing  
1182 (within each *Candida* strain) the respective inhibitor with its vehicle, **b** two-sided Wilcoxon matched  
1183 pairs signed-rank test comparing (within each *Candida* strain) the neutralizing antibodies with the  
1184 correspondent isotype controls. [Data used to make this figure can be found in Source Data Fig. 5.](#)

1185 **Figure 6 | *C. auris* is less virulent than *C. albicans* in an experimental model of murine disseminated**  
1186 **candidiasis. a**, Survival curve of immunocompetent mice i.v. challenged with *C. albicans* (n=11) or *C.*  
1187 *auris* (n=10). Mice were i.v. injected with  $1 \times 10^7$  CFU of the respective *Candida* strain and monitored  
1188 daily. **b**, Fungal burden of immunocompetent mice i.v. challenged with  $1 \times 10^6$  CFU of *C. albicans* (day 3,  
1189 n=5; day 7, n=4) or *C. auris* (day 3, n=5; day 7, n=5) in the liver and kidney at 3 (top) and 7 (bottom)  
1190 days post injection. **c-d**, KC (**c**) and MPO (**d**) production in supernatants from liver, kidney and spleen  
1191 homogenates (n=6 per group per time-point). **e**, KC production in plasma of mice (n=6 per group per  
1192 time-point) infected i.v. with  $1 \times 10^6$  CFU of *C. albicans* or *C. auris*. Graphs represent mean  $\pm$  SEM, data  
1193 pooled from at least two independent experiments, \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001, **a**, Mantel–  
1194 Cox two-sided log-rank test, **b-e** two-sided Mann-Whitney U test.  
1195 **f**, Ratio of the mean MPO or KC production (log scale, data from Figure 6d, 6c) to the mean of fungal  
1196 burden (log scale, data from Figure 6b) in kidney (blue bars) and liver (white bars) of mice infected  
1197 with *C. albicans* or *C. auris*. Data are represented as the ratio of the mean log MPO and KC values (n=6  
1198 per group per time-point), to the mean log CFU (*C. albicans*: day 3, n=5; day 7, n=4. *C. auris*: day 3,  
1199 n=5; day 7, n=5). [Data used to make this figure can be found in Source Data Fig. 6.](#)

1200