



## Eicosanoid biosynthesis influences the virulence of *Candida parapsilosis*

Tanmoy Chakraborty, Ernst Thuer, Marieke Heijink, Renáta Tóth, László Bodai, Csaba Vágvölgyi, Martin Giera, Toni Gabaldón & Attila Gácsér

To cite this article: Tanmoy Chakraborty, Ernst Thuer, Marieke Heijink, Renáta Tóth, László Bodai, Csaba Vágvölgyi, Martin Giera, Toni Gabaldón & Attila Gácsér (2018) Eicosanoid biosynthesis influences the virulence of *Candida parapsilosis*, *Virulence*, 9:1, 1019-1035, DOI: 10.1080/21505594.2018.1475797

To link to this article: <https://doi.org/10.1080/21505594.2018.1475797>



© 2018 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.



View supplementary material [↗](#)



Published online: 27 Jul 2018.



Submit your article to this journal [↗](#)



Article views: 201



View Crossmark data [↗](#)

RESEARCH ARTICLE



## Eicosanoid biosynthesis influences the virulence of *Candida parapsilosis*

Tanmoy Chakraborty<sup>a</sup>, Ernst Thuer<sup>b,c</sup>, Marieke Heijink<sup>d</sup>, Renáta Tóth<sup>a</sup>, László Bodai<sup>e</sup>, Csaba Vágvolgyi<sup>a</sup>, Martin Giera<sup>d</sup>, Toni Gabaldón<sup>b,c,f</sup>, and Attila Gácsér<sup>id a</sup>

<sup>a</sup>Department of Microbiology, University of Szeged, Szeged, Hungary; <sup>b</sup>Centre for Genomic Regulation (CRG), Barcelona Institute of Science and Technology, Barcelona, Spain; <sup>c</sup>Department of Experimental and Health Sciences, Universitat Pompeu Fabra (UPF), Barcelona, Catalonia, Spain; <sup>d</sup>Center for Proteomics and Metabolomics, Leiden University Medical Center, Leiden, The Netherlands; <sup>e</sup>Department of Biochemistry and Molecular Biology, University of Szeged, Szeged, Hungary; <sup>f</sup>Institució Catalana de Recerca i Estudis Avançats (ICREA), Barcelona, Spain

### ABSTRACT

Lipid mediators, derived from arachidonic acid metabolism, play an important role in immune regulation. The functions of bioactive eicosanoids range from modulating cytokine signaling and inflammasome formation to anti-inflammatory and pro-resolving activities. Human pathogenic fungi such as *Candida albicans*, *Candida parapsilosis*, *Cryptococcus neoformans* and *Aspergillus fumigatus* have been shown to produce such lipid mediators, associated with their virulence. To date, investigations into the molecular mechanisms of fungal eicosanoid biosynthesis in different species have revealed that several genes are associated with prostaglandin production. However, these routes remain uncharacterized in *C. parapsilosis* with early results suggesting it uses pathways distinct from those found in *C. albicans*. Therefore, we aimed to identify and characterize *C. parapsilosis* genes involved in eicosanoid biosynthesis. Following arachidonic acid treatment of *C. parapsilosis* cells, we identified several genes interfering with prostaglandin production. Out of the identified genes, homologues of a multi copper oxidase (*FET3*), an Acyl-CoA thiolase (*POT1*) and an Acyl-CoA oxidase (*POX1-3*) were found to play a significant role in prostaglandin synthesis. Furthermore, all three genes were confirmed to enhance *C. parapsilosis* pathogenicity, as the corresponding deletion mutants were cleared more efficiently by human macrophages and induced higher levels of pro-inflammatory cytokines. In addition, the mutants were less virulent than the wild-type strain in a mouse model of systemic infection. Taken together, we identified three genes that regulate eicosanoid biosynthesis in *C. parapsilosis* and impact the fungus' virulence.

### ARTICLE HISTORY

Received 12 February 2018  
Accepted 8 May 2018

### KEYWORDS

*Candida parapsilosis*; fungal eicosanoids; immunomodulation; host-pathogen interaction; virulence

## Introduction

Eicosanoids, are bioactive lipid molecules derived from the 20-carbon poly-unsaturated fatty acid, arachidonic acid. Prostaglandins and leukotrienes are two major groups of eicosanoids produced in almost all mammalian cells. Their production is initiated by phospholipases that mediate the release of arachidonic acid from the cell membrane and their activity is generally facilitated by binding to G-protein coupled receptors (GPCRs) and peroxisomal proliferator-activated receptors (PPARs) in different cells. Lipid mediators play a major role in inflammasome activation and the maintenance of cellular homeostasis and in some cases, they act as pro-resolving/anti-inflammatory immune modulators (e.g. lipoxins). Three major enzymatic pathways are known to regulate eicosanoid synthesis, namely cyclooxygenase (*COX*), lipoxygenase (*LOX*) and cytochrome P450 proteins. Several drugs can block

these enzymes and inhibit the generation of bioactive lipid mediators. For example, nonsteroidal anti-inflammatory drugs (NSAIDs), such as indomethacin or ibuprofen, target mainly the COX enzymes, thus prostaglandin biosynthesis, to alleviate inflammation [1–3]. Interestingly, the recognition of the *C. albicans* cell wall  $\beta$ -glucan by dectin-1 on the surface of macrophages enhances macrophage cytosolic phospholipase A2 activity and COX expression, thus promoting prostaglandin biosynthesis in the host [4,5]. Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is known to inhibit the Th1 response and promote Th2 immune responses [6,7]. Following *C. albicans* recognition, the induced PGE<sub>2</sub> production also enhances Th17 response [8].

Human pathogenic fungi can also produce eicosanoids, mainly PGE<sub>2</sub>, themselves [9]. However, they lack COX and LOX required for their production, which suggests the presence of alternative routes for prostaglandin biosynthesis. Such routes have been identified

**CONTACT** Attila Gácsér ✉ [gacsera@bio.u-szeged.hu](mailto:gacsera@bio.u-szeged.hu) Department of Microbiology, University of Szeged, Közép fasor 52, Szeged, 6726, Hungary  
Supplemental data for this article can be accessed [here](#).

© 2018 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

in *C. albicans* (*FET3* and *OLE2* mediated pathways) [9], *C. neoformans* (*LAC1*) [10] and *Aspergillus* (*PPO* gene family) species [11]. These fungal eicosanoids play a role in fungal pathogenesis, as deletion of *LAC1* in *C. neoformans* resulted in attenuated virulence while disruption of *PPO* genes drastically reduced asexual spore formation in *Aspergillus nidulans* and leads to a hyper-virulent phenotype in *A. fumigatus* [11]. Additionally, the yeast to hyphal transition in *C. albicans* is regulated in part by  $\text{PGE}_2$  and mature biofilms also produce this prostaglandin [12,13].

*C. parapsilosis*, is an important opportunistic human fungal pathogen, which belongs to the CTG clade of the *Ascomycetes* family. After *C. albicans*, it is the second or third leading cause of invasive candidiasis [14]. In particular, low birth weight infants are at increased risk for *C. parapsilosis* infection [15,16]. Previously, we demonstrated that *C. parapsilosis* is also able to produce immunomodulatory prostaglandins from exogenous arachidonic acid. Although, in contrast with *C. albicans*, the fatty acid desaturase *OLE2* is not involved in the process; hence, the molecular mechanisms behind eicosanoid biosynthesis remain to be elucidated [17].

In the current study, our aim was to identify and characterize genes involved in *C. parapsilosis* eicosanoid biosynthesis. Following arachidonic acid induction, we performed RNA sequencing and rigorously investigated gene function through characterizing the generated corresponding deletion mutant strains. We determined that homologues of a multi copper oxidase (*FET3*), an Acyl-CoA thiolase (*POT1*) and an Acyl-CoA oxidase (*POX1-3*) significantly impacted eicosanoid synthesis. Furthermore, according to our results, all three genes influence the virulence of *C. parapsilosis* *in vitro* and also regulate pathogenicity *in vivo*.

## Results

### Identification of differentially regulated genes following arachidonic acid induction

To identify genes involved in the biosynthesis of lipid mediators, we performed global transcriptomic analysis on *C. parapsilosis* GA1 [18] yeast cells following growth in the presence of arachidonic acid (AA). As a control [zero amount versus background exposure increase (OH)], equal amounts of cells were grown without the addition of arachidonic acid. Altogether, 151 genes showed significantly altered expression (fold change > 1.5) in the presence of arachidonic acid when compared to control. Out of the 151 genes, 68 genes were upregulated, while 83 genes showed decreased

expression levels (Table S1). Hierarchical clustering and principal component analysis (PCA) (Figure S1) showed that the three replicates from each group clustered together by condition, confirming the reliability of the obtained results.

Functional categorization of the genes by Gene Ontology (GO) term analysis using the candida genome database [19] showed, that 14% of the genes involved in lipid metabolic processes (GO:0006629) are upregulated (Figure 1(a)). The heat map indicates all of the upregulated ORFs in the induced condition (Figure 1(b)). Detailed GO term annotations of the upregulated genes can be found in supplementary Table S1. For further analyzes, we selected six upregulated genes with known lipid metabolic process regulatory homologous in *C. albicans*, (Table 1). Using these genes, we further validated the RNA sequencing data results by performing qRT-PCR analysis (Table 2). qRT-PCR confirmed that the six genes were upregulated following arachidonic acid pretreatment in both *C. parapsilosis* GA1 as well as in a second strain, CLIB 214. As five out of six genes showed higher expression levels in the CLIB 214 type strain, we subsequently used this strain for further analyzes.

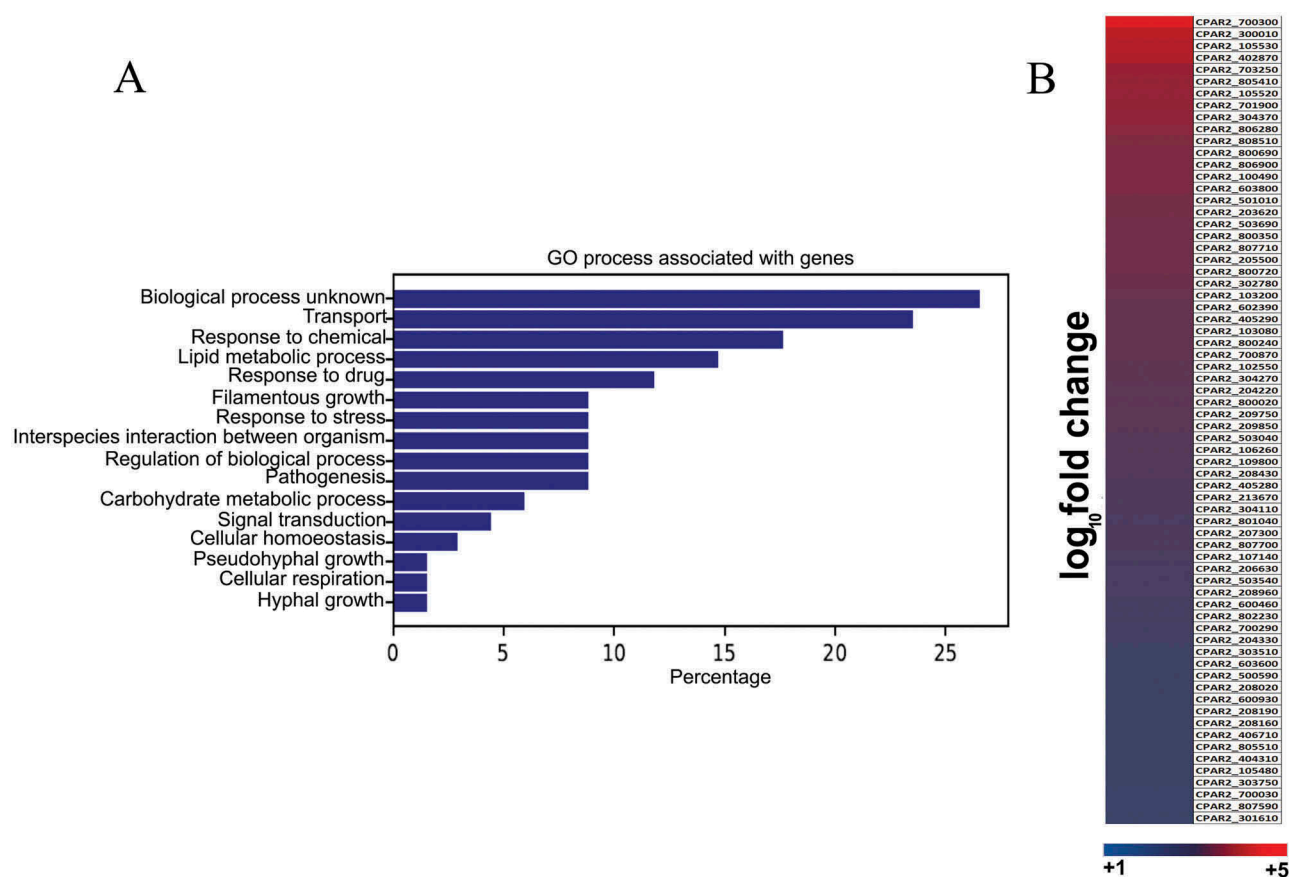
In order to determine the role of the identified genes in eicosanoid biosynthesis, we generated homozygous deletion mutant strains for each candidate gene by applying a gene disruption method previously introduced by Holland *et al* [20].

### Homozygous deletion mutants of *CPAR2\_603600*, *CPAR2\_800020* and *CPAR2\_807710* genes showed a significant reduction in extracellular lipid mediator production

Using liquid chromatography-mass spectrometry (LC/MS), we analyzed all null mutant strains for their ability to produce eicosanoids. This approach has previously demonstrated that the storage of arachidonic acid results in the production of auto-oxidation products [21,22].

Therefore, we also incubated 100  $\mu\text{M}$  arachidonic acid in PBS at 30°C for 24 hours to measure the amount of spontaneously produced eicosanoids without the presence of fungal cells.

The LC/MS data for the secretory eicosanoid analysis revealed that the deletion mutant strains of *CPAR2\_603600*, *CPAR2\_800020* and *CPAR2\_807710* produced less prostaglandin  $\text{D}_2$  ( $\text{PGD}_2$ ), although in case of *CPAR2\_807710* this reduction was not significant. Further, all three mutant strains showed a significant decrease in  $\text{PGE}_2$  production. In contrast, a reduction in 15-keto-prostaglandin  $\text{E}_2$  (15-keto- $\text{PGE}_2$ ) production was measurable only in case of *CPAR2\_807710*



**Figure 1.** RNA sequencing and data analysis. (a) Global gene expression analysis was performed on the wild type *C. parapsilosis* strain after 3 hours of growth in presence of arachidonic acid. Functional analysis of genome wide expression data suggests that lipid metabolism and transport related pathways are significantly altered in the presence of arachidonic acid. (b) Heat map shows all the *C. parapsilosis* genes upregulated in presence of arachidonic acid. For further information see supplementary table S1.

**Table 1.** Six up-regulated genes from the RNA sequencing data analysis and their homologues in *C. albicans* and their fold change expression values in *C. parapsilosis*.

CPAR2 GeneID	<i>Candida albicans</i> homologue	Fold change
CPAR2_807710	POX1-3(2)	2.48
CPAR2_205500	ECI1	2.48
CPAR2_102550	FAA21	2.19
CPAR2_800020	POT1	2.10
CPAR2_807700	POX1-3(1)	1.80
CPAR2_603600	FET3	1.63

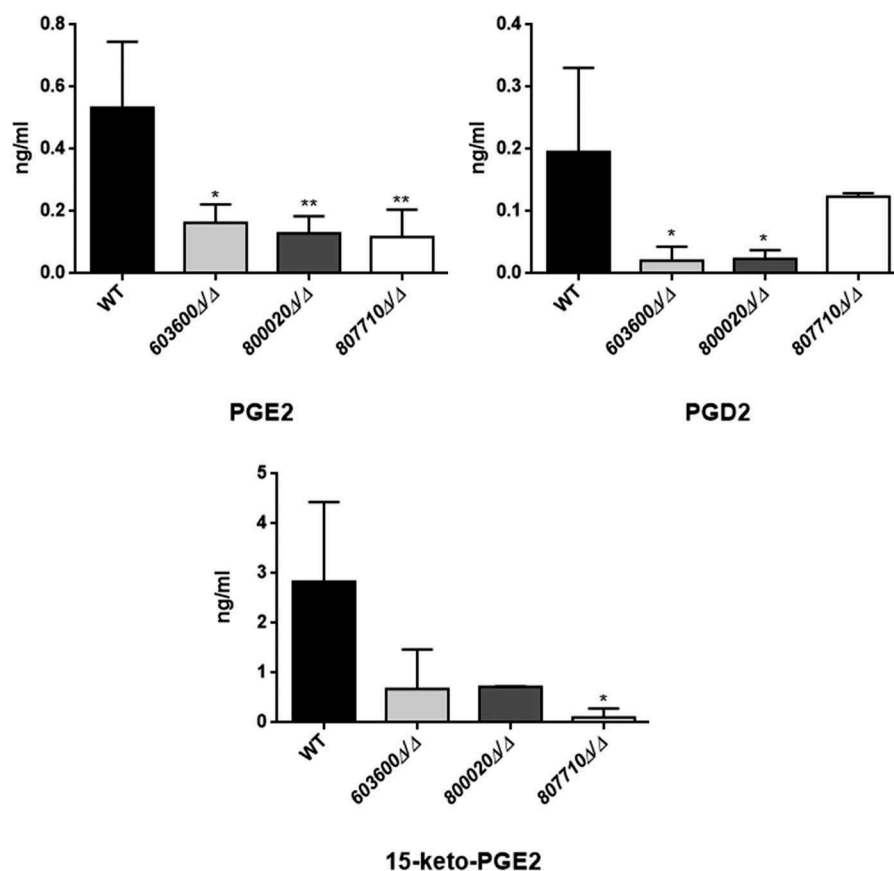
**Table 2.** Confirmation of the RNA sequencing data: fold change values of the 6 selected genes in both *C. parapsilosis* strains determined by qRT-PCR analysis.

CPAR GeneID	CLIB	GA1
CPAR2_807710	20.96	4.70
CPAR2_205500	7.27	2.41
CPAR2_102550	11.81	3.14
CPAR2_800020	3.63	2.17
CPAR2_807700	7.48	1.08
CPAR2_603600	0.67	3.21

(Figure 2). *CPAR2\_102550Δ/Δ*, *CPAR2\_205500Δ/Δ* and *CPAR2\_807700Δ/Δ* did not show any difference in PGD<sub>2</sub>, PGE<sub>2</sub> and 15-keto-PGE<sub>2</sub> production (Figure S2).

### Fungal 5-D2-isoprostane identified during LC/MS analysis

The LC/MS data for the secreted eicosanoids also revealed, that *C. parapsilosis* incubations gave rise to an uncommon isoprostane likely identified to be a 5-D2-IsoProstane which is considered an autoxidative isomer of PGD<sub>2</sub> (Figure 3). Investigating the selective ion trace  $m/z$  351  $\rightarrow$  115 and comparing retention times (RT) and tandem mass spectra we observed a signal which overlapped with an authentic standard of lipoxin A<sub>4</sub> (LXA<sub>4</sub>), however, some differences in the fragment intensities during tandem mass spectrometry prompted us to further evaluate the identity of this signal. We used a secondary solvent system for confirmatory analysis (data not shown). This analysis revealed that the signal suspected to be LXA<sub>4</sub> was indeed something else as authentic standard and analyte no longer co-eluted. In order to propose a possible structure for the obtained signal we carried out tandem mass spectrometry as well as MS<sup>3</sup> analysis of the obtained

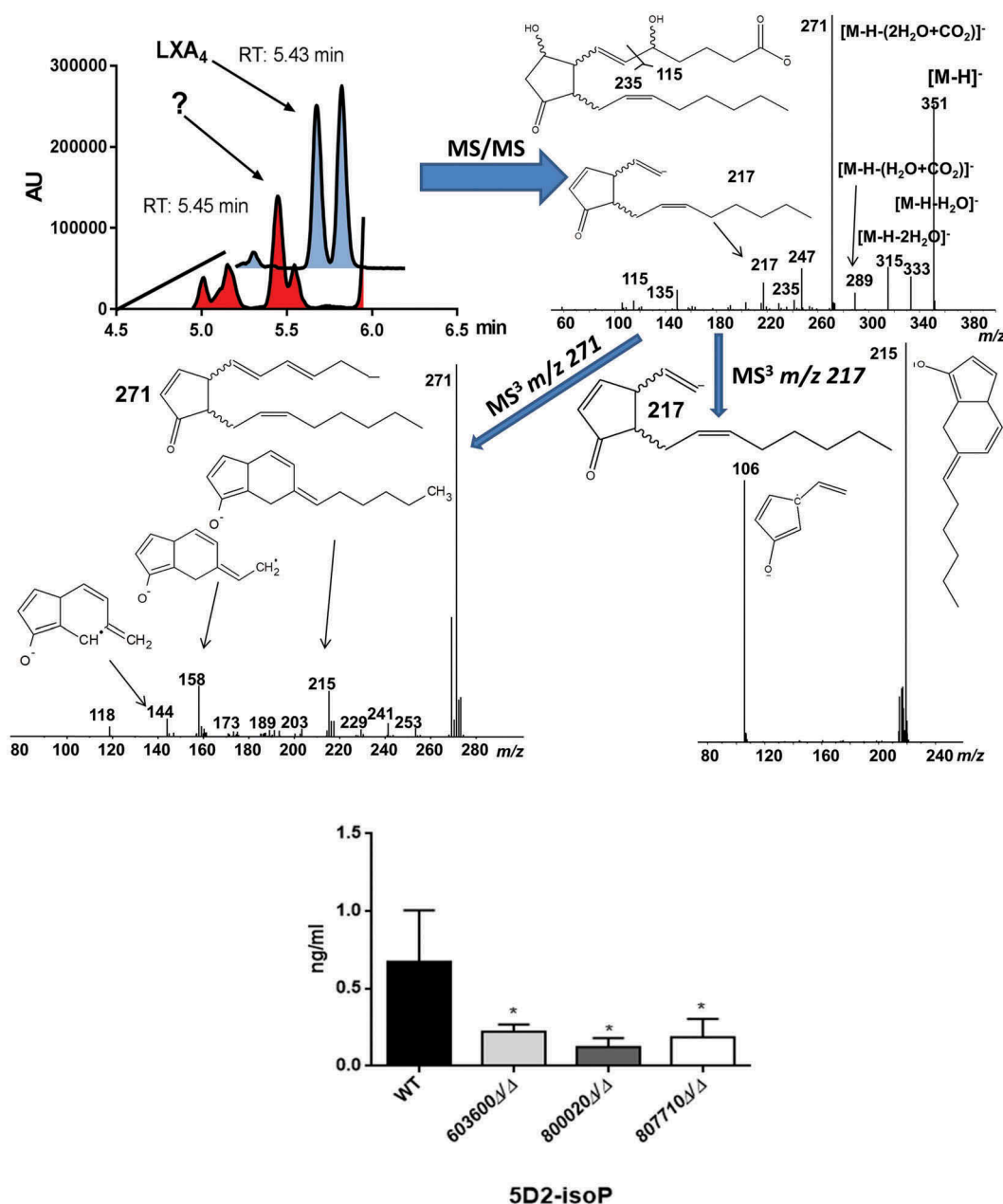


**Figure 2.** Reduced eicosanoid production by *C. parapsilosis* mutant strains. *C. parapsilosis* CLIB 214 wild type strain and three null mutant strains 603600Δ/Δ, 800020Δ/Δ and 807710Δ/Δ were grown for 24 hours at 30°C in the presence of 100 μM arachidonic acid in PBS. PBS with only arachidonic acid served as control. After sterile filtration, 100 μl of cell-free samples were analyzed with LC/MS. Among the examined eicosanoids, the three null mutants showed significant reductions in PGE<sub>2</sub> (CPAR2\_603600Δ/Δ, CPAR2\_800020Δ/Δ and CPAR2\_807710Δ/Δ) and one strain in 15-keto-PGE<sub>2</sub> (CPAR2\_807710Δ/Δ) production. Strains 603600Δ/Δ and 800020Δ/Δ had a strong trend toward a lower production of PGD<sub>2</sub> compared to the wild type strain. \**P* < 0.05, \*\**P* < 0.01.

peak. As can be seen from Figure 3, based on the mass spectrometric data, we propose the observed oxylipin to be a 5-D2-IsoP [23]. The fragment *m/z* 115 is indicative of a 5-hydroxy group, in combination with the MS/MS fragments *m/z* 217 and 271 as well as the MS<sup>3</sup> fragments *m/z* 215 and 106 we propose 5-D2-IsoP to be a likely candidate molecule for the observed signal. According to our data, besides the low production of prostaglandins, mutant strains incubations of CPAR2\_603600, CPAR2\_800020 and CPAR2\_807710 also contained less 5-D2-IsoP compared to the wild type (Figure 3). This reduction was significant in all the mutant strains. No significant differences were found in case of CPAR2\_102550Δ/Δ, CPAR2\_205500Δ/Δ and CPAR2\_807700Δ/Δ in terms of 5-D2-IsoP (Figure S2).

### Phagocytosis and killing of 603600Δ/Δ, 800020Δ/Δ and 807710Δ/Δ mutants by human macrophages

Human peripheral blood monocyte derived macrophages (PBMC-DM) were used to characterize the virulence properties of mutant strains with altered eicosanoid producing profiles. We first examined the phagocytic activity of PBMC-DM by fluorescence-activated cell sorting (FACS). *Candida* yeast cells were labeled with the fluorescent dye Alexa Fluor 488 (a succinidyl ester) and then co-incubated with PBMC-DMs for 2 hours. Our results indicated that PBMC-DMs ingested each of the mutant strains more efficiently compared to the wild type strain (Figure 4). We also examined the yeast cell killing efficiency of PBMC-DMs by comparing the recovered fungal CFUs. Our data showed that each of the mutant strains were killed more effectively by PBMC-DMs in comparison to the wild type strain (Figure 5(a)).

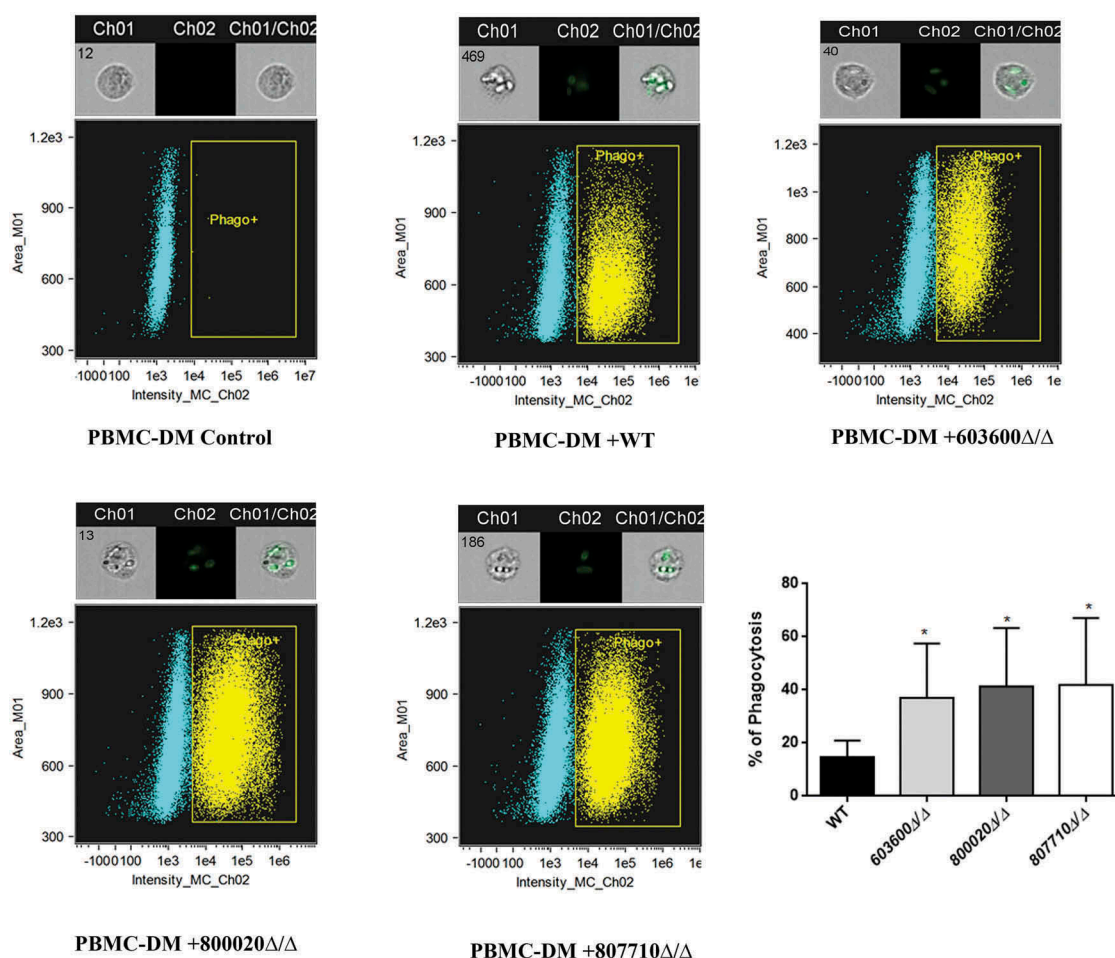


**Figure 3.** Suggestive identification and measurement of fungal 5D2-IsoP in wild type and eicosanoid mutant strains. Upper left, LC/MS analysis of cell supernatant after growing *C. parapsilosis* wild type strain in presence of 100  $\mu$ M arachidonic acid in PBS. Blue trace shows the elution of an authentic LXA<sub>4</sub> standard (1 ng/mL) in the transition  $m/z$  351→115. Red trace shows the elution of an overlapping signal in a representative *C. parapsilosis* sample. (Upper right) MS/MS spectrum of the signal co-eluting with LXA<sub>4</sub> and chemical structure of the candidate molecule 5D2-IsoP. Middle MS<sup>3</sup> spectra of  $m/z$  271 (left) and  $m/z$  217 (right). Lower panel, *CPAR2\_800020Δ/Δ* showed a significant reduction in 5D2-IsoP production, although 5D2-IsoP levels also decreased in *CPAR2\_603600Δ/Δ* and *CPAR2\_807710Δ/Δ* strains.

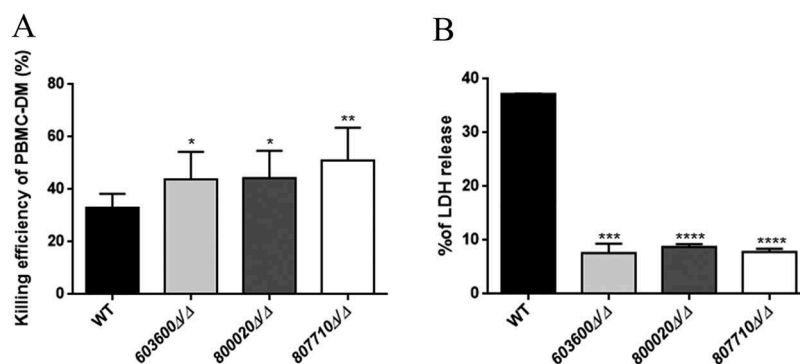
### Host cell damage is decreased by 603600Δ/Δ, 800020Δ/Δ and 807710Δ/Δ strains

Next, we examined the mutant strains' abilities to cause host cell damage by measuring the amount of LDH released by human PBMC-DMs following infection. As shown in Figure 5(b), we found that the

PBMC-DMs showed significantly lower LDH release when infected with the mutants compared to the wild type. Our results indicated that 603600Δ/Δ, 800020Δ/Δ and 807710Δ/Δ mutant strains show a lower host cell damaging capacity than wild type cells (Figure 5(b)).



**Figure 4.** Phagocytosis of wild type and eicosanoid mutant strains by human macrophages by imaging flow cytometry. The phagocytosis of wild type and *C. parapsilosis* eicosanoid mutant cells by PBMC-DMs was analyzed by an imaging flow cytometer. Yeast cells were labeled with AlexaFluor447 and co-incubated with macrophages for 2 hours at 37°C with 5% CO<sub>2</sub>. Data were obtained from three independent experiments. Representative dot plots and summarized data of the flow cytometric analysis are shown. \* $P < 0.05$ .

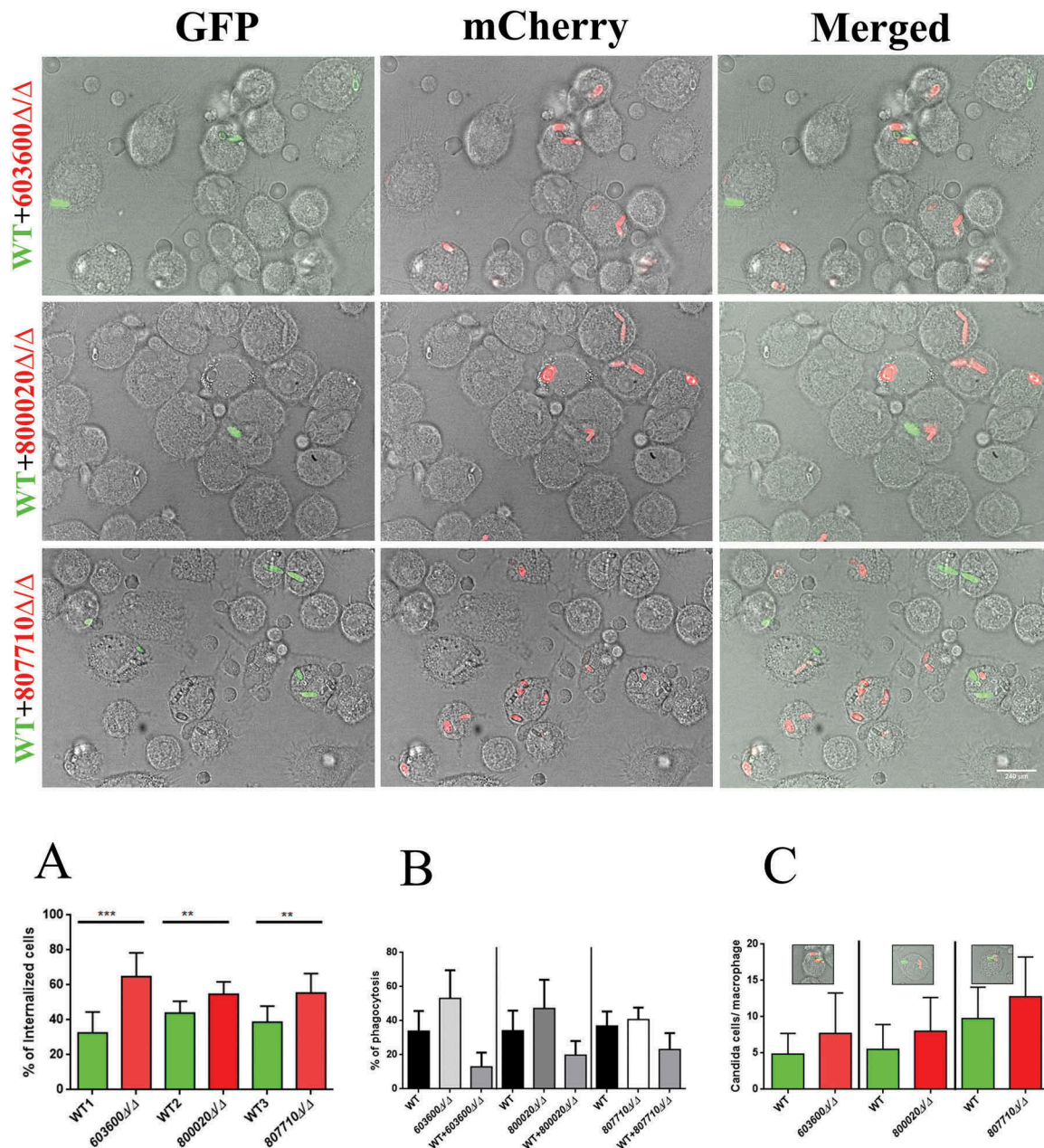


**Figure 5.** Killing of *C. parapsilosis* strains by human PBMC-DMs and host cell damage. (a) The efficiency of killing by human macrophages was analyzed by CFU determination. Experiments were performed in triplicates. The obtained data represents the killing efficiency of macrophages gained from 5 healthy donors. (b) Human PBMC-DMs were infected with wild type and *C. parapsilosis* eicosanoid mutants for 24 hours and LDH release was measured. LDH release is expressed as % of positive control. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $p < 0.002$ , \*\*\*\* $p < 0.0001$ .

### Macrophages favor the uptake of 603600Δ/Δ, 800020Δ/Δ and 807710Δ/Δ strains over the wild type

We tested the uptake efficiency of the mutants by human PBMC-DMs using a competition assay and compared them to the wild type strain. During the assay, we used differently labeled *Candida* strains: a GFP tagged wild type strain and mCherry labeled

mutant strains. PBMC-DMs were infected with 2 types of fungal cells mixed in a ratio of 1:1 and co-incubated for 2 hours. Interactions were monitored via fluorescent imaging. The percentage of internalized cells was calculated for each strain, and the values for the mutants were compared to those of the wild type's (Figure 6(a)). We calculated the percentage of phagocytosis by counting the total number of PBMC-DMs (Figure 6(b)). The number of green



**Figure 6.** Phagocytic competition assay. The competition assays were performed using combinations of GFP tagged wild type *C. parapsilosis* and mCherry tagged eicosanoid mutant strains. Equal numbers of yeast cells expressing GFP or mCherry were mixed together and added to human PBMC-DMs at the MOI of 1:2. (a) Significantly higher number of mutant cells (red) were internalized compared to the wild type cells (green). (b) Percentage of phagocytic macrophages in each case (c) Number of macrophages with both wild type and mutant strain in each case did not show any difference. \*\*P < 0.01, \*\*\*p < 0.002.

and red cells inside a single PBMC-DM which phagocytosed both type of cells was also analyzed (Figure 6(c)). Overall our results revealed that, PBMC-DMs significantly preferred the uptake of 603600 $\Delta/\Delta$ , 800020 $\Delta/\Delta$  and 807710 $\Delta/\Delta$  cells over the wild type. However, when the PBMC-DMs phagocytosed both type of cells we did not find any significant preference for the mutant cells.

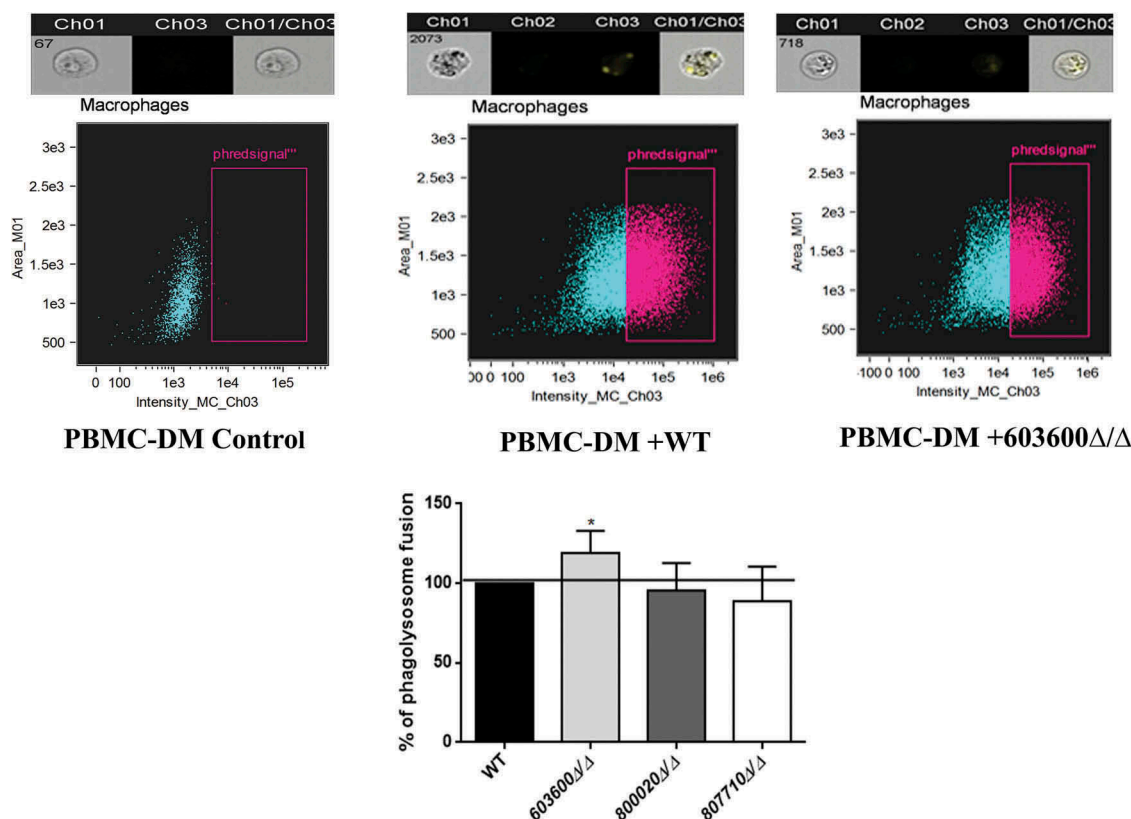
### Influence on phagosome-lysosome fusion

Next, we addressed whether the lack of the examined lipid mediators had any effect on phagosome-lysosome fusion in human PBMC-DMs. We analyzed the phagosome-lysosome co-localization after co-incubating pHrodo stained *Candida* cells with PBMC-DMs for 2 hours. Although, all three mutants are phagocytosed and killed more efficiently by PBMC-DMs, only the 603600 $\Delta/\Delta$  strain induced a higher rate of phagosome-lysosome fusion, suggesting the corresponding gene's influence on phagosome maturation (Figure 7).

### Reduction in prostaglandin production alters the cytokine response

Eicosanoid derived lipid mediators effectively regulate inflammatory responses. Multiple functions, dependent on concentration, location and timing have been described for the prostaglandins (e.g. PGD<sub>2</sub> and PGE<sub>2</sub>) [1-3,24,25]. In order to examine the immunological responses triggered by the 603600 $\Delta/\Delta$ , 800020 $\Delta/\Delta$  and 807710 $\Delta/\Delta$ , we stimulated human PBMC-DMs for 24 hours with each strain and determined the amount of cytokine and chemokine production. During the experiments we measured pro-IL1 $\beta$ , TNF $\alpha$ , Interleukin-1 receptor antagonist (IL-1ra), interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-10 (IL-10) levels.

PBMC-DMs infected with 807710 $\Delta/\Delta$  produced significantly higher amounts of the pro-inflammatory cytokine, IL-6, TNF $\alpha$ , chemokine IL-8 and IL-1ra levels compared to the wild type strain. Similarly, PBMC-DMs stimulated with 603600 $\Delta/\Delta$  and 800020 $\Delta/\Delta$  produced higher amounts of Pro-IL-1 $\beta$ , IL-1ra, IL-6 and TNF $\alpha$  compared to the reference strain, although these differences were not significant. Further, these two



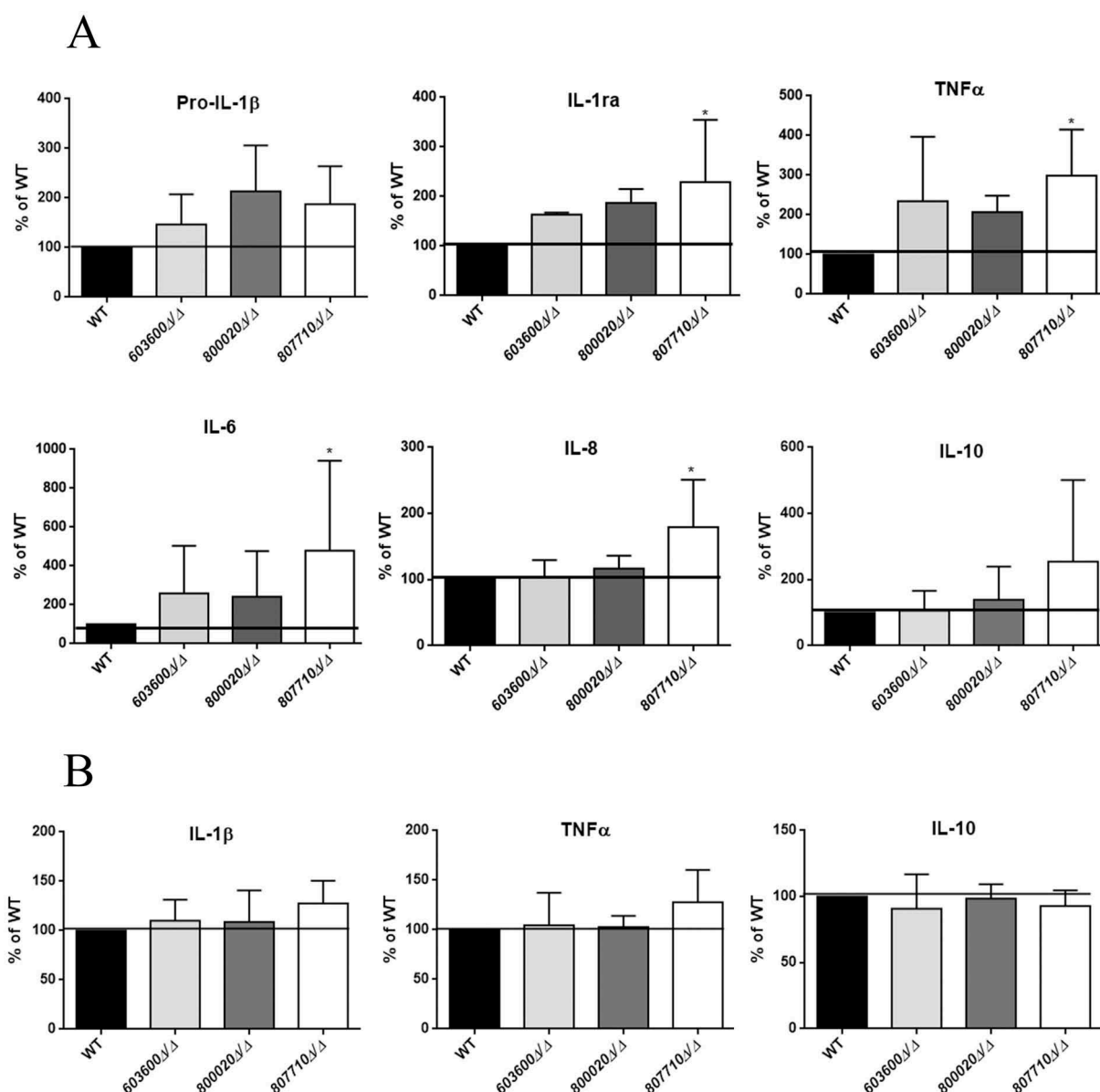
**Figure 7.** Phagosome-lysosome fusion in response to wild type and eicosanoid mutants. Phago-lysosome co-localization in human PBMC-DMs following the phagocytosis of pHrodo labeled *Candida* cells. Representative picture of a phagocytosing macrophage during quantitative imaging analysis. Ch1: brightfield image, Ch3: green fluorescence channel. Graph showing the difference between the extent of phago-lysosome fusion in case of the wild type and the mutants. \* $P < 0.05$ .

mutant strains did not induce IL-8 secretion. In terms of IL-10 production, none of the mutant strains showed a significant difference compared to the wild type ( $p > 0.05$ ), however, 807710 $\Delta/\Delta$  showed a trend towards higher levels of release (Figure 8(a)).

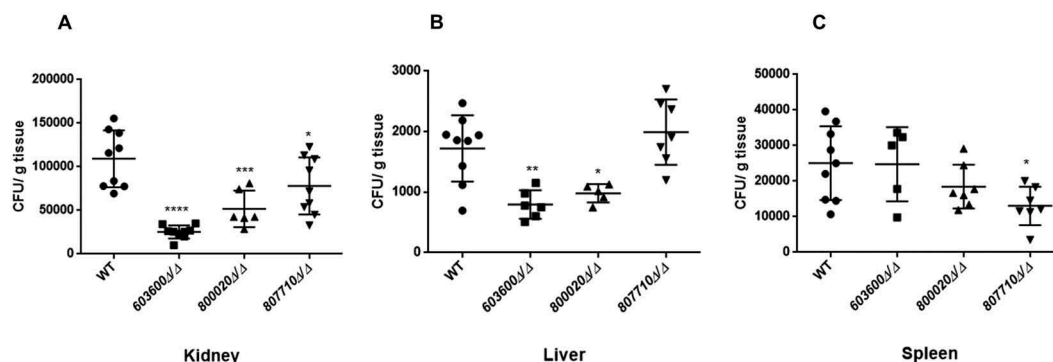
We analyzed the cytokine production by human primary PBMCs following fungal stimuli. PBMCs infected with 807710 $\Delta/\Delta$  showed a trend towards a higher IL-1 $\beta$  and TNF $\alpha$  (Figure 8(b)) release, although there was no difference in case of the other mutant strains. These data suggest that fungal eicosanoids may also influence the host cytokine response.

### 603600 $\Delta/\Delta$ , 800020 $\Delta/\Delta$ and 807710 $\Delta/\Delta$ strains show attenuated virulence *in vivo*

Following *in vitro* studies, we also aimed to examine the virulence of the 603600 $\Delta/\Delta$ , 800020 $\Delta/\Delta$  and 807710 $\Delta/\Delta$  *in vivo* using a mouse model of disseminated candidiasis. Following the intravenous infection of BALB/c mice, the fungal burdens of different organs were determined three days after the infection. After CFU recovery, we found that mice infected with 603600 $\Delta/\Delta$  showed significantly reduced fungal burdens in the liver and kidneys, while 800020 $\Delta/\Delta$



**Figure 8.** Cytokine secretion of human macrophages in response to wild type and eicosanoid mutants. (a) Pro IL-1 $\beta$ , IL-1ra, TNF $\alpha$ , IL-6, IL-8, and IL-10 levels were measured by ELISA after stimulation of PBMC-DMs with wild type or eicosanoid mutant strains for 24 hours. Data were normalized for each donor to cytokine levels induced by the wild type strain (100%) to minimize donor to-donor variability. (b) IL-1 $\beta$ , TNF $\alpha$  and IL-10 were also measured after infection of human PBMCs with the same strains. Data represent % cytokine production  $\pm$  SEM for 6 donors. \* $P < 0.05$ .



**Figure 9.** Fungal burden in organs after intravenous infection. Five mice were infected intravenously with  $2 \times 10^7/100 \mu\text{l}$  of *C. parapsilosis* wild type or eicosanoid mutant cells. CFUs recovered from kidney (a), liver (b), and spleen (c) after 3 days of the infection. The results are pooled data from two independent experiments. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $p < 0.002$ , \*\*\*\* $p < 0.0001$ .

inoculated mice also revealed lower fungal burdens in the liver and kidneys. Finally, CFUs recovered following 807710Δ/Δ injection were significantly less in the spleen and kidneys compared to those recovered from wild type-infected mice (Figure 9). These results indicate that these eicosanoid biosynthesis genes play role in *C. parapsilosis* virulence.

## Discussion

Eicosanoids, a group of bioactive mediators, are signaling molecules with diverse physiological and pathological functions known to modulate inflammatory responses. Prostaglandins, leukotrienes and lipoxins are groups of eicosanoids involved in pro-, and/or anti-inflammatory responses. During vasodilation prostaglandins and leukotrienes induce increased permeability of post capillary venules and also recruit complement components and leukocytes to the site of inflammation [2]. In contrast, lipoxins act as pro-resolving lipid mediators, as for example, LXA<sub>4</sub> inhibits the recruitment of neutrophil and eosinophil granulocytes in post capillary venules [3]. Previously, it has been hypothesized that, during fungal infection the invading fungi induce host prostaglandin biosynthesis that causes a local anti-inflammatory response, which in turn could contribute to fungal invasion [26].

In recent years, several studies have revealed that human pathogenic fungi are able to produce lipid mediators, specifically prostaglandins that might as well contribute to their virulence. This group of pathogens includes species such as *A. nidulans*, *A. fumigatus*, *C. neoformans*, *C. albicans* and *C. parapsilosis*.

Investigations have revealed several biosynthetic pathways used by some of these pathogens. As an example, *A. nidulans* and *A. fumigatus* have three dioxxygenase-encoding genes, namely *ppoA*, *ppoB* and *ppoC*

with high sequence similarity to mammalian cyclooxygenases [11]. Besides regulating sexual and asexual sporulation, all three *ppo* genes contribute to prostaglandin biosynthesis, possibly via oxygenating arachidonic acid, thereby generating the prostaglandin precursor PGH<sub>2</sub> [11].

Other studies have revealed that certain pathogenic fungi, such as *C. albicans* and *C. neoformans*, do not possess COX-like enzymes, thus they must have evolved prostaglandin biosynthetic pathways different from those of mammals [12,13]. In *C. neoformans*, a member of the multicopper oxidase family, the Lac1 laccase regulates PGE<sub>2</sub> biosynthesis, possibly by converting the prostaglandin precursor PGG<sub>2</sub> to PGE<sub>2</sub> and 15-keto-PGE<sub>2</sub> [27].

In *C. albicans* however, the fatty acid desaturase OLE2 and a multicopper oxidase FET3 play a role in prostaglandin production via novel pathways using exogenous arachidonic acid as precursor [9]. CaFET3, a laccase homolog (and also a member of the Fet family of multicopper oxidases) is suggested to regulate PGE<sub>2</sub> biosynthesis through a mechanism similar to that of LAC1 in *C. neoformans*. On the other hand, Ole2, a putative delta9 desaturase also containing a cytochrome B domain, is hypothesized to regulate PGE<sub>2</sub> production via the oxidation of exogenous arachidonic acids [9].

Notably, in these species, the identified genes are pleiotropic regulators as they also participate in mechanisms such as sporulation (*ppo* genes), cell wall homeostasis (*LAC1*) or iron uptake (*FET3*), and thus contribute to virulence in a complex manner. Nevertheless, the role of fungal prostaglandins in virulence is evident as, in all yet examined fungal species, their production has been associated with markedly altered immune responses [11,26]. To expand our knowledge about the role of fungal eicosanoids in pathogenesis, we examined lipid mediator production

in another important human fungal pathogen, *C. parapsilosis*. As the incidence of this species has increased over the past two decades and the patient group at risk includes immunosuppressed children and adults as well as neonates, understanding the pathogenesis of *C. parapsilosis* has gained increased attention [16,28]. Preliminary studies have started to elucidate the prostaglandin profile of this species [17], however, the involved biosynthetic pathways and the presence or role of other fungal eicosanoids remained elusive. Previously, we have shown that in the presence of exogenous arachidonic acid, *C. parapsilosis* is capable of producing fungal prostaglandins, although *OLE2* is not involved in the synthetic mechanisms, leaving the corresponding biosynthetic processes unexplored [17].

Therefore, in the current study, we aimed to reveal regulators involved in eicosanoid biosynthetic mechanisms and to investigate their roles in the virulence of this species. Following arachidonic acid induction, we identified three genes that significantly influence the biosynthesis of fungal prostaglandins. Our results indicate, that *CPAR2\_603600*, a homologous gene of *CaFET3* is involved in  $\text{PGE}_2$  and  $\text{PGD}_2$  production, *CPAR2\_807710*, a homologue of the acyl-coenzyme A oxidase, *ScPOX1-3*, regulates  $\text{PGE}_2$  and 15-keto- $\text{PGE}_2$  synthesis, and *CPAR2\_800020*, a homologue of 3-ketoacyl-CoA thiolase, *ScPOT1*, influences  $\text{PGE}_2$  biosynthesis. Using LC/MS analysis, we observed that the disruption of each gene led to a decrease in the corresponding eicosanoids' production.

While *CaFET3* is known to interfere with fungal prostaglandin production [9], no such role has been associated with *CaPOX1-3*, and *CaPOT1* in *C. albicans*, suggesting a novel function of the corresponding homologues in *C. parapsilosis*.

The roles of *CaFET3* and *CaPOT1* in *C. albicans*' virulence have been investigated. Deletion of *CaFET3* resulted in reduced adhesiveness to fibroblasts, although no significant differences were observed between the virulence of the wild type and the  $\Delta/\Delta\text{fet3}$  strain in a mouse model of systemic candidiasis [29]. In contrast with *C. albicans*, upon the removal of the ortholog of *FET3* in *C. parapsilosis* indeed influenced the fungi's virulence in vivo, given the lower amount of fungal burden measured. In contrast, *CaPOT1*, a 3-ketoacyl-CoA thiolase, involved in fatty acid utilization, is not required for virulence in an embryonated chicken egg infection model [30]. However, its ortholog in *C. parapsilosis* is involved in virulence regulation as represented by both our *in vitro* and *in vivo* virulence studies. Unfortunately, to date, we lack information about the hypothetical acyl-coenzyme A oxidase's (*CaPOX1-3*) role in *C. albicans* pathogenesis. Interestingly, our

results also suggest the involvement of the corresponding gene's ortholog in the virulence of *C. parapsilosis*.

These data suggest, that in contrast to *C. albicans*, the homologous genes of *FET3*, *POT1* and *POX1-3* in *C. parapsilosis* indeed contribute to fungal virulence, although the corresponding mechanisms still need to be elucidated.

Interestingly, *CPAR2\_603600* might be involved in delaying phagosome-lysosome fusion, a phenomenon not yet investigated in *C. albicans*, although additional studies are needed to confirm this mechanism hypothesis.

Fungal prostaglandins produced by *C. albicans* and *C. neoformans* alter host cytokine responses by down-regulating chemokine (IL-8) and pro-inflammatory cytokine (e.g. TNF $\alpha$ ) production while concomitantly up-regulating anti-inflammatory responses via promoting IL-10 release [26]. Our results suggest a similar effect with *C. parapsilosis* eicosanoids, as mutant strains defective in prostaglandin production induced higher pro-inflammatory cytokine responses, as shown by the increased levels of Pro-IL-1 $\beta$ , IL-6 and TNF $\alpha$  released by human PBMC-DMs. Although, stimulation of human PBMCs did not result in a significant increase of the examined cytokines in case of any of the mutant strains. These data suggest that *CPAR2\_807710*, *CPAR2\_800020* and *CPAR2\_603600* contribute unequally to the alteration of host immune responses.

This is the first study reporting the presence of 5D2-IsoP in fungal incubations that might actively contribute to fungal virulence. We have identified three *C. parapsilosis* eicosanoid biosynthesis regulatory genes, namely *CPAR2\_807710*, *CPAR2\_800020* and *CPAR2\_603600*, that are involved in the production of fungal prostaglandins. Virulence studies performed with the corresponding null mutant strains suggests that these regulatory genes also influence the fungi's virulence. Although, further investigation is needed to thoroughly understand the importance of fungal eicosanoids, our results can contribute to a better understanding of host pathogen interactions during candidiasis.

## Materials and methods

### Strains and growth conditions

All *C. parapsilosis* strains used in this study are listed in Table S2. Strains were grown in YPD (1% dextrose, 1% peptone and 0.5% yeast extract) at 30°C. For colony selection 2% agar was added in the media. Nourseothricin (NAT) resistant transformants were selected on YPD plates supplemented with 100  $\mu\text{g/ml}$

NAT. Cells transformed with *LEU2* and *HIS1* markers were selected on synthetic complete media (SC; 2% Dextrose, 0.95% yeast nitrogen base, mixture of amino acid, 2% agar) without leucine and histidine.

### RNA extraction

For RNA sequencing, the *C. parapsilosis* GA1 strain was grown overnight in 2 ml of YPD media at 30°C with continuous shaking applied at 180 rpm. The next day, cells were washed 3 times with PBS and then counted using a Burkert's chamber. Cell concentration was adjusted to  $2 \times 10^7$  cells per 10 ml PBS supplemented with 500  $\mu$ M of arachidonic acid in triplicates. As a control, cells were also grown in 10 ml PBS supplemented with ethanol only, as it is the dissolving agent used for arachidonic acid solubilization. After 3 hours of growth at 30°C, RNA was isolated using the Ribopure Yeast RNA isolation Kit (Ambion) following the manufacturer's instructions. For validating the RNA sequencing data, *C. parapsilosis* CLIB and GA1 cells were grown as described above and RNA extraction was performed using the same kit.

### RNA sequencing and data analysis

RNA-seq library preparation and sequencing, paired-end reads (Illumina Truseq V2 PolyA, not -stranded, V3-150,  $2 \times 75$ bp, 25M read) were generated from three biological replicates of induced and non-induced *C. parapsilosis* cells.

For initial quality assessment and data preprocessing we used FastQC 0.10.1 [<http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/>] quality assessment and Trimmomatic v0.32 [31] where preset conditions were applied for quality cutoff to remove low quality regions from the raw data [parameters used LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36.]. Mapping was carried out against the reference genome obtained from the Candida Genome Database [19]. We used the splice junction sensitive mapper Tophat 2.0.1.13 [32] with default settings, and mapped by applying the bowtie 2.2.4 [33] short read mapper. The counts per gene were estimated by using flux-capacitor [34]. To estimate differential expression, we used the R package Deseq2 [35] with a cutoff of log2fold change of 1.5. Gene Ontology enrichment was performed using the CGD database [19].

### Reverse transcription PCR

A total of 500 ng RNA was used for cDNA synthesis. The cDNA was synthesized using the Revert Aid first

Strand cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's instructions.

### Real time PCR

Real time PCR was performed in a final volume of 20  $\mu$ l using Maxima SYBR Green/Fluorescein qPCR Master Mix (2X) (Thermo Scientific). The reaction was performed in C1000 Thermal Cycler (Bio-Rad) using the following protocol: 95°C for 3mins, 95°C for 10 s, 60°C for 30 s, 65°C for 5 s for 50 cycles. Fold change in mRNA expression was calculated by  $\Delta\Delta C_t$  method (Real-Time PCR applications guide BIO-Rad). *TUB4* gene was used as a housekeeping gene for an internal control.

### Preparation and transformation of competent cells

All the deletion mutants and fluorescently labeled strains were constructed by chemical transformation. The parental CLIB 214 double auxotrophic strain (CPL2H1) [20] and the mutants were inoculated in 2 ml YPD and grown overnight at 30°C with shaking applied at 180 rpm. Then the culture was diluted to an OD<sub>600</sub> of 0.2 in 30 ml YPD broth and grown at 30°C to an OD<sub>600</sub> of 0.8–1. The culture was centrifuged at 4000 rpm for 5 min and the pellet was suspended in 3 ml of ice-cold water. The cells were again centrifuged at 4000 rpm for 5 min and washed with 1 ml ice cold TE-LiOAC (0.1M lithium acetate, 10 mM TRIS and 1mM EDTA). After centrifugation the final pellet was dissolved in 300  $\mu$ l of ice cold TE-LiOAC. A transformation mix was prepared with 100  $\mu$ l competent cells, 10  $\mu$ l single stranded salmon sperm DNA (10 mg/ml) and 25–30  $\mu$ l fusion PCR product and the mixture was kept at 30°C for 30 min. Finally, 700  $\mu$ l PLATE (0.1M lithium acetate, 10 mM tris, 1mM EDTA and 40% PEG 3350) was added to the transformation mix and cells were kept overnight at 30°C. The next day, cells were heat shocked at 44°C for 15 min, centrifuged and washed with 1 ml of YPD media. The cells were then re-suspended in 1 ml of YPD and incubated at 30°C at 200 rpm for 2–3 hours. After the incubation, the cells were centrifuged and dissolved in 100  $\mu$ l of YPD, then plated on the respective agar plates.

### Generation of *C. parapsilosis* mutant strains

For gene deletion, the fusion PCR technique was used, as described previously by Holland et al [20]. Target genes are listed in Table 2 (Figure 1) and the primers are used listed in TabS3. Approximately 500bp upstream and 500bp downstream sequences of the target genes were amplified with primer pairs 1,3 and 4,6 using DreamTaq

polymerase (Thermo Scientific). Selection markers *LEU2* and *HIS1* were amplified with primer pairs 2 and 6 from the plasmids pSN40 (*LEU2*) and pSN52 (*HIS1*). Finally, all the PCR products were purified using the PCR Purification kit (Qiagen) and fused with Phusion Taq polymerase (Thermo Scientific) using primers 1 and 6. The resulting disruption cassette was transformed into the double auxotrophic CLIB 214 strain.

### Construction of fluorescent tagged strains

All the *C. parapsilosis* fluorescently labeled strains were generated using the Gateway® cloning technology (Invitrogen). The fluorescent constructs were expressed under an overexpression promoter targeted in the *NEUT5* locus. The fluorescence expression was confirmed after checking the strains under fluorescent microscope.

### Sample preparation for secreted eicosanoid measurement

For eicosanoid measurement,  $2 \times 10^7$  *Candida* cells were inoculated in 10 ml of PBS+ 100  $\mu$ M arachidonic acid in triplicates and incubated at 30°C for 24 hours. Three tubes with only PBS and 100  $\mu$ M arachidonic acid were also incubated and served as control. For secreted eicosanoids, the cell supernatant was collected using sterile filtration after centrifugation. 100  $\mu$ l sample was complemented with 96  $\mu$ l MeOH and 4  $\mu$ l internal standard solution (final concentration: 1 ng/ml PGE<sub>2</sub>-d4, LTB<sub>4</sub>-d4 and 15-HETE-d8 and 10 ng/ml DHA-d5) and analyzed without further processing.

### Analysis of lipid mediators by LC/MS

Eicosanoids were analyzed using a targeted LC-MS/MS method according to published protocols with a minor modification [36]. The drying temperature was set to 450°C instead of 400°C. Table S4 shows the MRM characteristics of the monitored eicosanoids. When concentrations were determined external calibration was carried out with linear regression using a weighing factor of  $1/x^2$ . For calculating the actual concentration of the secreted eicosanoids, the amount of spontaneously generated eicosanoids (PBS+ AA control sample) were subtracted from the total amount of eicosanoids produced by each strain (data not shown). MS<sup>3</sup> analysis were carried out under identical conditions using an excitation energy (AF2 value) of either 0.5 or 0.8 (V) as collisional energy in the MS<sup>3</sup> experiments, investigating the MS/MS fragments *m/z* 217 and 271.

### Isolation and differentiation of PBMCs

Human PBMCs were isolated from buffy coats of healthy individual by density gradient centrifugation [37]. Briefly, blood samples were first diluted with PBS (1:1 dilution). The diluted blood was overlaid above the Ficoll solution (Ficoll Paque PLUS-GE Healthcare) and then centrifuged at 900 rpm for 30 mins. After centrifugation, the mononuclear cell layer was aspirated with a pipette, collected in a separate 50 ml centrifuge tube and then washed 3 times with PBS. Finally, cells were suspended in RPMI 1640 medium (Lonza) supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin. Cells were counted and the concentration was adjusted to  $5 \times 10^6$  cells/ml. For later experiments, 100  $\mu$ l of  $5 \times 10^5$  cells in RPMI were added to U-bottom 96-well plates. For macrophage differentiation, cells were added to flat bottom 24-well plates with the concentration of 1ml of  $1 \times 10^7$  cells per well and incubated for 90 mins at 37°C in the presence of 5% CO<sub>2</sub> and 100% humidity. After the incubation, RPMI was removed and cells were washed with 1 ml of PBS. Finally, the cells were re-suspended in X-VIVO 15 media (Lonza) supplemented with 100 U/ml penicillin, 100 mg/ml streptomycin and 10ng/ml recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF, Sigma-Aldrich). The media was changed every 2<sup>nd</sup> day for 7 days. During the study, PBMCs were used for cytokine measurement only.

### Infection of PBMCs and PBMC-DMs

For the infection experiments, *C. parapsilosis* strains were grown overnight in 2 ml YPD media, washed 3 times with PBS and adjusted to the proper concentrations used for each experiment. For the infection of both human PBMCs (cytokine measurement only) and PBMC-derived macrophages, the multiplicity of infection (MOI) was 1:5. *Candida* cells were dissolved in 100  $\mu$ l of either RPMI/PS (PBMC) or GM-CSF-free X-VIVO 15 (PBMC-DM) medium and were added to host cell containing wells.

### Killing assay

Human PBMC-DMs ( $5 \times 10^5$ /well) were co-incubated with *C. parapsilosis* cells in 24-well plastic culture plates at a MOI of 1:5. As a control, equal amounts of *Candida* cells were incubated in the GM-CSF free X-VIVO 15 medium only (500  $\mu$ l/well). After 3 hours of incubation, macrophages were lysed with PBS + 4% Triton X-100 solution. Control wells with yeast cells only were treated similarly. Finally, the lysates and the

cells were serially diluted, plated on YPD plates and incubated for 2 days at 30°C. After incubation, the number of CFUs was determined and the recovered CFUs from each strain were compared. The killing efficiency was calculated as follows: (number of live *Candida* cells in control wells – number of live *Candida* cells in co-cultures)/number of live *Candida* cells in control wells  $\times$  100. The experiments were performed with PBMC-DMs derived from five independent donors with triplicates.

### Phagocytosis assay using flow cytometry

For the phagocytosis assays, *Candida* cells were labeled with the fluorescent dye Alexa Fluor 447 carboxylic acid succinidyl ester (Invitrogen) and measured using the FlowSight Imaging flow cytometer (Amnis). For labeling, the yeast cell suspension (100  $\mu$ l containing  $10^9$  cells) was treated with 11  $\mu$ l of  $\text{Na}_2\text{CO}_3$  (1 M, pH 10) and 2  $\mu$ l Alexa Fluor 447 (1 mg/ml in DMSO) was added followed by incubation for 1 hour at room temperature, carefully protected from light. After the incubation, the cells were washed four times with PBS and adjusted to the proper concentration of  $2.5 \times 10^6$  (MOI of 1:5). Human PBMC-DMs ( $5 \times 10^5$ /well) were infected with labeled *Candida* cells and kept for 2 hours at 37°C with 5%  $\text{CO}_2$ . Then, the non-phagocytosed *Candida* cells were washed with PBS and the yeast containing macrophages were detached from the cell culture plates with TrypLETM Express solution (Gibco). Finally, macrophages were collected in FACS buffer (0.5% FBS in PBS), centrifuged and dissolved in 200  $\mu$ l PBS and measurement using the flow cytometer. The obtained raw data was analyzed by the IDEAS Software (Amnis). The experiments were performed with PBMC-DMs derived from five independent donors.

### Phagocytosis competition assay

GFP (wild type) and mCherry (mutant strains) expressing *C. parapsilosis* strains were grown overnight in YPD at 30°C at 200 rpm. Next day, the cells were harvested by centrifugation at 3000 rpm for 5 mins and washed with PBS. PBMC-DMs were infected with a mixture of fungal cells (wild type and a mutant strain, mixed in a ratio of 1:1), and were co-incubated at 37°C for 2 hours. Then the co-culture was incubated and then washed 2–3 times with PBS before visualized under the fluorescent microscope [38]. At least 300 macrophages and 150 yeast cells were counted for each experiment and the experiments were performed with macrophages derived from three independent donors.

### LDH measurement

We determined the release of lactate dehydrogenases from the supernatant, as an indicator of host cell death, by the LDH cytotoxicity detection kit (Takara) according to the manufacturer's instructions. Cytotoxicity was calculated after subtracting the absorbance value of the infected control from all the test samples: cytotoxicity (%) is calculated as follows:  $(\text{OD}_{\text{Experimental value}} / \text{OD}_{\text{Positive control}}) \times 100$ . Supernatant collected from the cells treated with 1% TritonX-100 was used as positive control. The experiments were performed with PBMC-DMs generated from five independent donors.

### Analysis of phagosome lysosome fusion by FACS

For phagosome-lysosome fusion analysis by quantitative imaging (Amnis Flow Sight), the *Candida* cells were labeled with the fluorescent dye pHrodo® (Invitrogen). First, the yeast cell suspension (100  $\mu$ l containing  $10^9$  cells) was treated with 11  $\mu$ l of  $\text{Na}_2\text{CO}_3$  (1 M, pH 10) and then 2  $\mu$ l pHrodo (1 mg/ml in DMSO) was added followed by incubation for 1 hr at room temperature in the dark. After the incubation, cells were washed 4 times with HBSS buffer (Hank's Balanced Salt Solution) (LONZA) and adjusted to the proper concentration (see phagocytosis assay protocol). Human PBMC-derived macrophages were infected with labeled *Candida* cells at a 1:5 ratio and kept for 2 hrs to allow phagocytosis. Then, the non-phagocytosed *Candida* cells were washed with 1xPBS and macrophages were detached from cell culture plates by TrypLETM Express solution (Gibco). Finally, macrophages were collected in FACS buffer (0.5% FBS in 1xPBS), centrifuged and dissolved in 1xPBS. Phagolysosome fusion was then measured by Amnis FlowSight. Data were analyzed by the IDEAS Software (Amnis).

### Cytokine measurement by ELISA

The concentration of different cytokines was analyzed by commercial ELISA kits according to the manufacturer's instructions. The IL-1 $\beta$ , IL-1ra, IL-6, TNF $\alpha$ , IL-10 and IL-8 ELISA kits were obtained from R&D Systems (Abingdon, United Kingdom). For the pro-IL1 $\beta$  measurement, macrophages were lysed by repeated freeze thaw cycles and then the supernatant was collected [39]. The experiments were performed with both PBMCs and macrophages derived from peripheral blood mononuclear cells (PBMC-DMs) were used from at least five independent donors.

## Mouse model of systemic *Candida* infection and fungal burden

For the experiments, a non-lethal experimental mouse model of disseminated candidiasis was used [40]. Briefly, groups of five 8–12-weeks-old female Balb/c mice (22 – 27 g. of weight) were infected with  $2 \times 10^7$ /100  $\mu$ l *C. parapsilosis* cells via the lateral tail vein using a syringe with a 32-gauge needle. A group of control mice was injected with 100  $\mu$ l of sterile PBS. Two independent experiments were performed. The mice were maintained with sterile water and food *ad libitum*. Three days following infection, animals were euthanized, and their livers, kidneys and spleens were aseptically removed. The organs were weighed and homogenized in sterile PBS with a tissue grinder. CFUs were determined after 48 hours of incubation at 30°C, and CFU/g tissue was calculated.

## Ethics statement

The *in vivo* mouse infection experiments were performed according to National (1998. XXVIII; 40/2013) and European (2010/63/EU) animal ethics guidelines. The experimental protocols were approved by the Animal Experimentation and Ethics Committee of the Biological Research Centre of the Hungarian Academy of Sciences and the Hungarian National Animal Experimentation and Ethics Board (clearance number: XVI./03521/2011.). The University of Szeged granted permissions XII./00455/2011 and XVI./3652/2016 to work with mice.

For PBMC isolation, blood was collected from healthy individuals. The Institutional Human Medical Biological Research Ethics Committee of the University of Szeged gave approval for the procedure and the respective consent documents. Healthy individuals provided written informed consent. The experiments were performed in accordance with guidelines and regulations of the Ethics Committee of University of Szeged and the experimental protocols were approved by the same institutional committee.

## Statistical analysis

Unpaired t-tests were used to determine differences between groups in case of phagocytosis assay, killing assay, LDH assay and phagocytic competition assay. Differences were considered statistically significant at  $P \leq 0.05$ . One-way ANOVA with Dunnett's Post-Hoc test was used to determine the differences between groups in case of eicosanoid analysis, cytokine analysis and *in vivo* experiment. Graph Pad Prism 6 software was used to perform the statistical analysis.

## Acknowledgments

We thank Christophe D'Enfert for sharing the plasmids for the Gateway system and Judith Berman for providing us with the GFP and mCherry plasmids. We also thank Tibor Németh for giving us the GFP expressing wild type strain for the phagocytic competition assay. We are thankful to Ferenc Jankovics from Biological Research Centre, Szeged for helping with the confocal microscopy. We would like to thank Csaba Papp for assisting with the confocal microscopy for the phagocytic competition assay; Katalin Csonka, Erik Zajta, Dharendra N. Singh for helping with the *in vivo* mouse experiment. We thank Prof. Dr. Wilfried Niessen (hyphen Mass Spec) for his help with interpreting the MS/MS and MS/MS/MS spectra of the candidate molecule 5D2-IsoP. We are also thankful to Joshua D. Nosanchuk for critically reading the manuscript and give valuable suggestions.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## Funding

TC was supported the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreements FP7-PEOPLE-2013-ITN-606786 'ImResFun' and from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No H2020-MSCA-ITN-2014-642095. AG was funded by NKFIH NN 113153, by GINOP-2.3.2-15-2016-00035, by GINOP-2.3.3-15-2016-00006 and by OTKA-NKFIH K123952. RT was supported by TÁMOP 4.2.4. A/2-11-1-2012-0001 National Excellence Program - Elaborating and operating an inland student and researcher personal support system convergence program. MH and MG lab was supported by the Leiden University Medical Centre, Leiden, The Netherlands. ET was supported the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreements FP7-PEOPLE-2013-ITN-606786 'ImResFun' and from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No H2020-MSCA-ITN-2014-642095. TG group acknowledges support of the Spanish Ministry of Economy and Competitiveness grants, 'Centro de Excelencia Severo Ochoa 2013-2017' SEV-2012-0208, and BFU2015-67107 cofounded by European Regional Development Fund (ERDF); from the CERCA Programme/ Generalitat de Catalunya; from the European Union and ERC Seventh Framework Programme (FP7/2007-2013) under grant agreement FP7-PEOPLE-2013-ITN-606786, and a grant from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No H2020-MSCA-ITN-2014-642095.

## ORCID

Attila Gácsér  <http://orcid.org/0000-0003-2939-9580>

## References

1. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science*. 2001 Nov 30;294(5548):1871–1875. PubMed PMID: 11729303.
2. Dennis EA, Norris PC. Eicosanoid storm in infection and inflammation. *Nat Rev Immunol*. 2015 Aug;15(8):511–523. PubMed PMID: 26139350; PubMed Central PMCID: PMC4606863.
3. Lawrence T, Willoughby DA, Gilroy DW. Anti-inflammatory lipid mediators and insights into the resolution of inflammation. *Nat Rev Immunol*. 2002 Oct;2(10):787–795. PubMed PMID: 12360216.
4. Suram S, Brown GD, Ghosh M, et al. Regulation of cytosolic phospholipase A2 activation and cyclooxygenase 2 expression in macrophages by the beta-glucan receptor. *J Biol Chem*. 2006 Mar 3;281(9):5506–5514. PubMed PMID: 16407295.
5. Suram S, Gangelhoff TA, Taylor PR, et al. Pathways regulating cytosolic phospholipase A2 activation and eicosanoid production in macrophages by *Candida albicans*. *J Biol Chem*. 2010 Oct 1;285(40):30676–30685. PubMed PMID: 20643646; PubMed Central PMCID: PMC2945562.
6. Shibata Y, Henriksen RA, Honda I, et al. Splenic PGE2-releasing macrophages regulate Th1 and Th2 immune responses in mice treated with heat-killed BCG. *J Leukoc Biol*. 2005 Dec;78(6):1281–1290. PubMed PMID: 16204627.
7. Romani L. Immunity to fungal infections. *Nat Rev Immunol*. 2011 Apr;11(4):275–288. PubMed PMID: 21394104.
8. Smeekens SP, Van De Veerdonk FL, van der Meer JW, et al. The *Candida* Th17 response is dependent on mannan- and beta-glucan-induced prostaglandin E2. *Int Immunol*. 2010 Nov;22(11):889–895. PubMed PMID: 21059767.
9. Erb-Downward JR, Noverr MC. Characterization of prostaglandin E2 production by *Candida albicans*. *Infect Immun*. 2007 Jul;75(7):3498–3505. PubMed PMID: 17470538; PubMed Central PMCID: PMC1932954.
10. Erb-Downward JR, Noggle RM, Williamson PR, et al. The role of laccase in prostaglandin production by *Cryptococcus neoformans*. *Mol Microbiol*. 2008 Jun;68(6):1428–1437. PubMed PMID: 18410494; PubMed Central PMCID: PMC3973538.
11. Tsitsigiannis DI, Bok JW, Andes D, et al. *Aspergillus* cyclooxygenase-like enzymes are associated with prostaglandin production and virulence. *Infect Immun*. 2005 Aug;73(8):4548–4559. PubMed PMID: 16040966; PubMed Central PMCID: PMC1201276.
12. Erb-Downward JR, Huffnagle GB. Role of oxylipins and other lipid mediators in fungal pathogenesis. *Future Microbiol*. 2006 Aug;1(2):219–227. PubMed PMID: 17661667.
13. Fischer GJ, Keller NP. Production of cross-kingdom oxylipins by pathogenic fungi: an update on their role in development and pathogenicity. *J Microbiol*. 2016 Mar;54(3):254–264. PubMed PMID: 26920885; PubMed Central PMCID: PMC5107414.
14. Brown GD, Denning DW, Gow NA, et al. Hidden killers: human fungal infections. *Sci Transl Med*. 2012 Dec 19;4(165):165rv13. PubMed PMID: 23253612.
15. Trofa D, Gacser A, Nosanchuk JD. *Candida parapsilosis*, an emerging fungal pathogen. *Clin Microbiol Rev*. 2008 Oct;21(4):606–625. PubMed PMID: 18854483; PubMed Central PMCID: PMC2570155.
16. Van Asbeck EC, Clemons KV, Stevens DA. *Candida parapsilosis*: a review of its epidemiology, pathogenesis, clinical aspects, typing and antimicrobial susceptibility. *Crit Rev Microbiol*. 2009;35(4):283–309. PubMed PMID: 19821642.
17. Grozer Z, Toth A, Toth R, et al. *Candida parapsilosis* produces prostaglandins from exogenous arachidonic acid and *OLE2* is not required for their synthesis. *Virulence*. 2015;6(1):85–92. PubMed PMID: 25654274; PubMed Central PMCID: PMC4603437.
18. Gacser A, Trofa D, Schafer W, et al. Targeted gene deletion in *Candida parapsilosis* demonstrates the role of secreted lipase in virulence. *J Clin Invest*. 2007 Oct;117(10):3049–3058. PubMed PMID: 17853941; PubMed Central PMCID: PMC1974868.
19. Skrzypek MS, Binkley J, Binkley G, et al. The *Candida* Genome Database (CGD): incorporation of Assembly 22, systematic identifiers and visualization of high throughput sequencing data. *Nucleic Acids Res*. 2017 Jan 4;45(D1):D592–D596. PubMed PMID: 27738138; PubMed Central PMCID: PMC5210628.
20. Holland LM, Schroder MS, Turner SA, et al. Comparative phenotypic analysis of the major fungal pathogens *Candida parapsilosis* and *Candida albicans*. *PLoS Pathog*. 2014 Sep;10(9):e1004365. PubMed PMID: 25233198; PubMed Central PMCID: PMC4169492.
21. Brose SA, Baker AG, Golovko MY. A fast one-step extraction and UPLC-MS/MS analysis for E2/D2 series prostaglandins and isoprostanes. *Lipids*. 2013 Apr;48(4):411–419. PubMed PMID: 23400687; PubMed Central PMCID: PMC3608832.
22. Galano JM, Lee YY, Oger C, et al. Isoprostanes, neuroprostanes and phytoprostanes: an overview of 25 years of research in chemistry and biology. *Prog Lipid Res*. 2017 Oct;68:83–108. PubMed PMID: 28923590.
23. Korner A, Schlegel M, Theurer J, et al. Resolution of inflammation and sepsis survival are improved by dietary Omega-3 fatty acids. *Cell Death Differ*. 2018 Feb;25(2):421–431. PubMed PMID: 29053142; PubMed Central PMCID: PMC5762854.
24. FitzGerald GA. BIOMEDICINE. Bringing PGE(2) in from the cold. *Science*. 2015 Jun 12;348(6240):1208–1209. PubMed PMID: 26068834.
25. Laan LC, Williams AR, Stavenhagen K, et al. The whipworm (*Trichuris suis*) secretes prostaglandin E2 to suppress proinflammatory properties in human dendritic cells. *FASEB J*. 2017 Feb;31(2):719–731. PubMed PMID: 27806992; PubMed Central PMCID: PMC5240662.
26. Noverr MC, Phare SM, Toews GB, et al. Pathogenic yeasts *Cryptococcus neoformans* and *Candida albicans* produce immunomodulatory prostaglandins. *Infect Immun*. 2001 May;69(5):2957–2963. PubMed PMID: 11292712; PubMed Central PMCID: PMC98248.

27. Erb-Downward JR, Huffnagle GB. *Cryptococcus neoformans* produces authentic prostaglandin E2 without a cyclooxygenase. *Eukaryot Cell*. 2007 Feb;6(2):346–350. PubMed PMID: 17158733; PubMed Central PMCID: PMC1797952.
28. Chow BD, Linden JR, Bliss JM. *Candida parapsilosis* and the neonate: epidemiology, virulence and host defense in a unique patient setting. *Expert Rev Anti Infect Ther*. 2012 Aug;10(8):935–946. PubMed PMID: 23030332; PubMed Central PMCID: PMC3498909.
29. Eck R, Hundt S, Hartl A, et al. A multicopper oxidase gene from *Candida albicans*: cloning, characterization and disruption. *Microbiology*. 1999 Sep;145(Pt 9):2415–2422. PubMed PMID: 10517594.
30. Otzen C, Bardl B, Jacobsen ID, et al. *Candida albicans* utilizes a modified beta-oxidation pathway for the degradation of toxic propionyl-CoA. *J Biol Chem*. 2014 Mar 21;289(12):8151–8169. PubMed PMID: 24497638; PubMed Central PMCID: PMC3961645.
31. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 2014 Aug 1;30(15):2114–2120. PubMed PMID: 24695404; PubMed Central PMCID: PMC4103590.
32. Kim D, Pertea G, Trapnell C, et al. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol*. 2013 Apr 25;14(4):R36. PubMed PMID: 23618408; PubMed Central PMCID: PMC4053844.
33. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods*. 2012 Mar 4;9(4):357–359. PubMed PMID: 22388286; PubMed Central PMCID: PMC3322381.
34. Montgomery SB, Sammeth M, Gutierrez-Arcelus M, et al. Transcriptome genetics using second generation sequencing in a Caucasian population. *Nature*. 2010 Apr 1;464(7289):773–777. PubMed PMID: 20220756; PubMed Central PMCID: PMC3836232.
35. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15(12):550. PubMed PMID: 25516281; PubMed Central PMCID: PMC4302049.
36. Jonasdottir HS, Brouwers H, Kwekkeboom JC, et al. Targeted lipidomics reveals activation of resolution pathways in knee osteoarthritis in humans. *Osteoarthritis Cartilage*. 2017 Jul;25(7):1150–1160. PubMed PMID: 28189826.
37. Schlenke P, Kluter H, Muller-Steinhardt M, et al. Evaluation of a novel mononuclear cell isolation procedure for serological HLA typing. *Clin Diagn Lab Immunol*. 1998 Nov;5(6):808–813. PubMed PMID: 9801339; PubMed Central PMCID: PMC96206.
38. Keppler-Ross S, Douglas L, Konopka JB, et al. Recognition of yeast by murine macrophages requires mannan but not glucan. *Eukaryot Cell*. 2010 Nov;9(11):1776–1787. PubMed PMID: 20833894; PubMed Central PMCID: PMC2976302.
39. Gross O, Poeck H, Bscheider M, et al. Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence. *Nature*. 2009 May 21;459(7245):433–436. PubMed PMID: 19339971.
40. Ifrim DC, Bain JM, Reid DM, et al. Role of Dectin-2 for host defense against systemic infection with *Candida glabrata*. *Infect Immun*. 2014 Mar;82(3):1064–1073. PubMed PMID: 24343653; PubMed Central PMCID: PMC3957982.