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The zinc cluster transcription factor Czf1 regulates cell wall architecture and integrity in *Candida albicans*

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Abstract

The fungal cell wall is essential for the maintenance of cellular integrity and mediates interactions of the cells with the environment. It is a highly flexible organelle whose composition and organization is modulated in response to changing growth conditions. In the pathogenic yeast *Candida albicans*, a network of signaling pathways regulates the structure of the cell wall, and mutants with defects in these pathways are hypersensitive to cell wall stress. By harnessing a library of genetically activated forms of all *C. albicans* zinc cluster transcription factors, we found that a hyperactive Czf1 rescued the hypersensitivity to cell wall stress of different protein kinase deletion mutants. The hyperactive Czf1 induced the expression of many genes with cell wall-related functions and caused visible changes in the cell wall structure. *C. albicans czf1*Δ mutants were hypersensitive to the antifungal drug caspofungin, which inhibits cell wall biosynthesis. The changes in cell wall architecture caused by hyperactivity or absence of Czf1 resulted in an increased recognition of *C. albicans* by human neutrophils. Our results show that Czf1, which is known as a regulator of filamentous growth and white-opaque switching, controls the expression of cell wall genes and modulates the architecture of the cell wall.

KEYWORDS
Candida albicans, cell wall, protein kinases, zinc cluster transcription factor

1 | INTRODUCTION

The fungal cell wall is essential for the maintenance of cellular integrity and is also the interface of the cell with the environment. In pathogenic fungi such as *Candida albicans*, components of the cell wall mediate interactions with the host and are recognized by the immune system (Gow & Hube, 2012). The *C. albicans* cell wall comprises an inner layer composed of a network of β-1,3-glucan, β-1,6-glucan, and chitin, and an outer fibrillar layer consisting of extended mannan chains of heavily glycosylated mannoproteins (Lenardon et al., 2020). It is a highly flexible structure whose actual composition and organization depend on the growth conditions (Childers et al., 2020a; Hall, 2015). A variety of signaling pathways, including different protein kinases and transcription factors, regulate cell wall architecture and remodeling in response to environmental signals (Alonso-Monge et al., 1999, Ballou et al., 2016, Blankenship et al., 2010, Bruno et al., 2006, Eisman et al., 2006, Navarro-Garcia et al., 1998, Pradhan et al., 2018). Mutants with defects in these signaling pathways exhibit increased sensitivity to cell wall-damaging compounds because they cannot appropriately respond to the imposed cell wall stress.

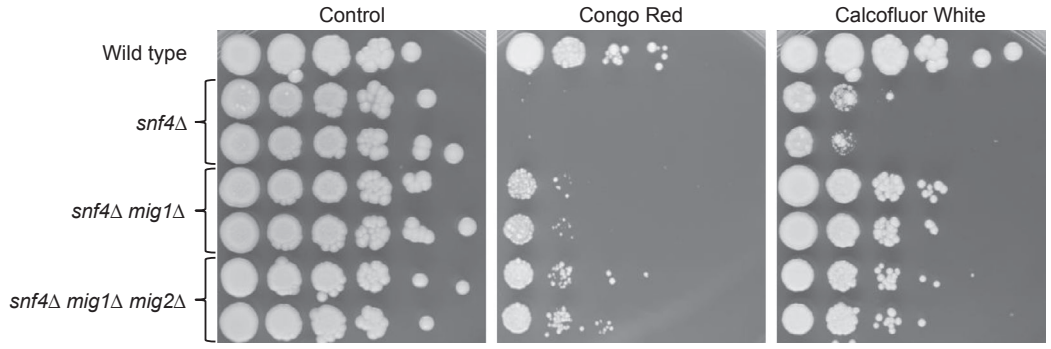

FIGURE 1 Deletion of *MIG1* and *MIG2* remediates the hypersensitivity of *snf4*Δ mutants to cell wall stress. YPD overnight cultures of the wild-type strain SC5314, *snf4*Δ single mutants, *snf4*Δ *mig1*Δ double mutants, and *snf4*Δ *mig1*Δ *mig2*Δ triple mutants were diluted to an optical density (OD_{600}) of 2.0. Serial 10-fold dilutions were spotted on YPD plates without (control) or with 50 μg/ml Congo Red or 10 μg/ml Calcofluor White and incubated for 4 days at 30°C. Both independently generated series of mutants are shown in each case

A key regulator of metabolic adaptation is the heterotrimeric SNF1 kinase, a member of the highly conserved family of AMP-activated kinases (Hedbacker & Carlson, 2008). In *C. albicans*, the SNF1 complex consists of the catalytic α-subunit Snf1, the regulatory γ-subunit Snf4, and one of the two β-subunits Kis1 and Kis2 (Corvey et al., 2005). The functionality of the SNF1 complex also requires the upstream activating kinase Sak1, which phosphorylates the catalytic subunit Snf1 at Thr208 (Ramírez-Zavala et al., 2017). Until recently, it was thought that the catalytic subunit Snf1 is essential in *C. albicans*, because no deletion mutants were obtained in several attempts by different research groups, but it has now been demonstrated that *snf1*Δ mutants are viable and can grow when conditions are optimal (Enloe et al., 2000, Mottola et al., 2020, Petter et al., 1997, Ramírez-Zavala et al., 2017). In previous work, we, therefore, used *snf4*Δ and *sak1*Δ mutants, in which SNF1 function is severely compromised, to investigate the role of this kinase in the biology of *C. albicans* (Mottola & Morschhäuser, 2019, Mottola et al., 2020, Ramírez-Zavala et al., 2017). These studies showed that SNF1 is required for the utilization of carbon sources other than glucose, similar to its role in the model yeast *Saccharomyces cerevisiae*. The mutants are also highly sensitive to cell wall stress, which may at least partially be due to their metabolic defects that prevent the maintenance of cell wall integrity under stressful conditions even when grown on glucose.

In *S. cerevisiae*, an important downstream target of SNF1 is the repressor Mig1, which together with the functionally related repressor Mig2 inhibits the expression of genes that enable the utilization of alternative carbon sources (Lutfiyya & Johnston, 1996; Nehlin & Ronne, 1990). When the preferred carbon source glucose is not available or limiting, Snf1 phosphorylates and thereby inactivates Mig1, resulting in derepression of its target genes (Ostling & Ronne, 1998). Interestingly, *C. albicans* Mig1 lacks the consensus Snf1 phosphorylation sites, indicating that the SNF1 signaling pathway is different in the two yeast species (Zaragoza et al., 2000). Nevertheless, it was recently found that deletion of *MIG1* and *MIG2* restores the ability of *C. albicans* mutants with a defective SNF1 to utilize alternative carbon sources (Lagree et al., 2020).

In our present study, we sought to identify SNF1 downstream transcription factors that ensure the maintenance of cell wall integrity under stressful conditions. Hypothesizing that a kinase-independent activation of such a transcription factor could bypass the requirement for SNF1 to withstand an otherwise lethal cell wall stress, we harnessed a library of artificially activated zinc cluster transcription factors, the largest family of transcriptional regulators in *C. albicans*, for this purpose. This approach identified Czf1, a transcription factor that has no homologue in *S. cerevisiae* and promotes filamentous growth and white-opaque switching in *C. albicans* (Brown et al., 1999; Vinces & Kumamoto, 2007; Zordan et al., 2007). The hyperactive Czf1 induced the expression of many genes with cell wall-related functions and rescued the hypersensitivity of various protein kinase deletion mutants to cell wall stress, although none of the kinases was essential for Czf1 phosphorylation. In a wild-type background, Czf1 was required for basal levels of tolerance to caspofungin, an antifungal drug that inhibits cell wall biosynthesis, and to limit the activation of host immune cells in the presence of the fungus. Our study establishes Czf1 as a regulator of cell wall architecture and integrity in *C. albicans*.

2 | RESULTS

2.1 | Deletion of *MIG1* and *MIG2* only partially overcomes the hypersensitivity of *snf4*Δ mutants to cell wall stress

The transcriptional repressors Mig1 and Mig2 have overlapping functions in the regulation of genes involved in the utilization of alternative carbon sources, which are repressed when glucose is available (Lagree et al., 2020). The SNF1 kinase is required to relieve cells from this repression and enable growth on carbon sources other than glucose (Lagree et al., 2020, Mottola et al., 2020, Ramírez-Zavala et al., 2017). Deletion of *MIG1* and *MIG2* restores growth of *snf4*Δ mutants on alternative carbon sources, albeit not to wild-type levels (Mottola et al., 2020). We tested if inactivation of these repressors would also overcome the hypersensitivity of

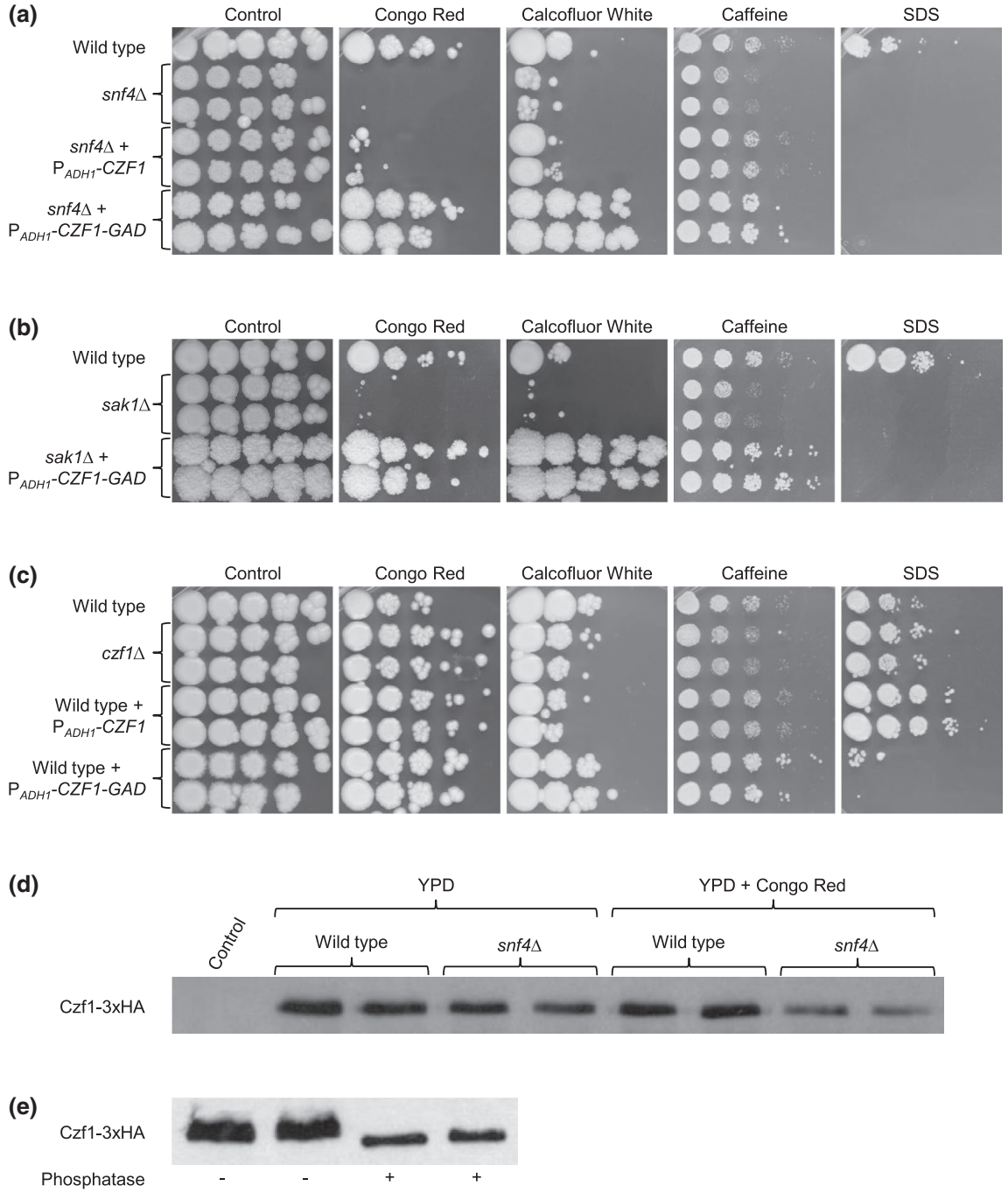

FIGURE 2 An activated form of Czf1 overcomes the hypersensitivity of SNF1 complex mutants to cell wall stress. (a–c) YPD overnight cultures of the wild-type strain SC5314 and derivatives expressing wild-type *CZF1* or the hyperactive *CZF1-GAD* allele from the *ADH1* promoter in *snf4*Δ (A), *sak1*Δ (b), or wild-type (c) backgrounds were diluted to an optical density (OD_{600}) of 2.0. Serial 10-fold dilutions were spotted on YPD plates without (control) or with 50 μg/ml Congo Red, 40 μg/ml Calcofluor White, 15 mM caffeine, or 0.04% SDS and incubated for 4 days at 30°C. Both independently generated series of mutants are shown in each case. (d) Detection of 3xHA-tagged Czf1 in wild-type and *snf4*Δ mutant backgrounds by Western blotting with an anti-HA antibody. YPD overnight cultures of the strains were diluted in fresh YPD medium and grown for 1 hr at 30°C, followed by two additional hours in the absence or presence of 50 μg/ml Congo Red. Two independent transformants expressing the HA-tagged *CZF1* allele were tested in each case; the untagged wild-type strain SC5314 served as negative control. (e) Wild-type samples were left untreated (–) or treated with phosphatase (+)

*snf4*Δ mutants to cell wall stress. As shown in Figure 1, deletion of both *MIG1* and *MIG2* improved the growth of *snf4*Δ mutants in the presence of the cell wall-damaging agents Congo Red and Calcofluor White; nevertheless, the triple mutants were still hypersensitive to these compounds. It has recently been demonstrated that deletion of *MIG1* and *MIG2* in *C. albicans* wild-type cells also results in hypersensitivity to cell wall inhibitors, probably through their impact on carbon physiology (Lagree et al., 2020). Therefore, a well-controlled

activity of Mig1 and Mig2 seems to be important for an adequate response of *C. albicans* to cell wall stress.

2.2 | An activated form of Czf1 suppresses the hypersensitivity of *snf4*Δ and *sak1*Δ mutants to cell wall stress

The maintenance of cell wall integrity in response to different types of stress is regulated by various signaling pathways involving many different protein kinases and transcription factors (see "Introduction" section). We reasoned that a kinase-independent activation of a downstream transcription factor might bypass the requirement of the kinase to react to an inducing signal. The largest family of transcription factors in *C. albicans* are the zinc cluster transcription factors (ZCFs), many of which can be artificially activated by fusing the Gal4 activation domain (GAD) to their C-terminus (Schillig & Morschhäuser, 2013). To investigate if an activated form of any of the 82 ZCFs of *C. albicans* could revert the hypersensitivity of mutants with a defective SNF1 kinase complex to cell wall stress, we introduced a previously described *ZCF-GAD* library (Schillig & Morschhäuser, 2013) into a *C. albicans snf4*Δ mutant and selected for transformants that could grow on plates with Congo Red (see materials and methods for details). One recovered transformant that could grow well on these plates contained the *CZF1-GAD* fusion. To confirm that the hyperactive Czf1, and not an unspecific suppressor mutation, was responsible for the increased Congo Red resistance, we integrated the *CZF1-GAD* allele into two independently constructed *snf4*Δ mutants. As can be seen in Figure 2a, the hypersensitivity of the *snf4*Δ mutants to Congo Red was rescued by the *CZF1-GAD* allele. Since *snf4*Δ mutants are also hypersensitive to other agents causing cell wall/membrane stress (Ramírez-Zavala et al., 2017), we compared the sensitivity of the strains to several such compounds. The sensitivity of the *snf4*Δ mutants to Calcofluor White and caffeine was also reverted; the strains containing the *CZF1-GAD* allele were even more resistant than the wild-type strain SC5314 (Figure 2a). In contrast, the strains remained hypersensitive to SDS. Since the *CZF1-GAD* allele was expressed from the strong *ADH1* promoter, we tested whether overexpression of a wild-type *CZF1* copy from the same promoter would have a similar effect. However, while the slight hypersusceptibility of the *snf4*Δ mutants to caffeine was reverted by overexpression of wild-type *CZF1*, their sensitivity to Congo Red and Calcofluor White was only marginally decreased. These results indicate that strongly increased activity of Czf1 is required to rescue the hypersusceptibility of *snf4*Δ mutants to cell wall stress.

Mutants lacking the Snf1-activating kinase Sak1 and *snf4*Δ mutants display similar phenotypes (Ramírez-Zavala et al., 2017). Therefore, we expected that the activated Czf1 would also rescue the sensitivity of *sak1*Δ mutants to cell wall stress. Indeed, the introduction of the *CZF1-GAD* fusion into two independently generated *sak1*Δ mutants restored growth on plates containing Congo Red, Calcofluor White, and caffeine, whereas the strains remained hypersensitive to SDS (Figure 2b). We then tested if Czf1 was required for normal cell wall stress tolerance of *C. albicans* and if overexpression of wild-type or hyperactive *CZF1* in a wild-type background would increase the resistance of the cells. Deletion of *CZF1* in strain SC5314 did not result in increased susceptibility to Congo Red, Calcofluor White, and SDS in these assays (Figure 2c), indicating that Czf1 is dispensable for the basal resistance of *C. albicans* to these agents, but the *czf1*Δ mutants were slightly hypersensitive to caffeine. Interestingly, overexpression of *CZF1* increased the resistance of wild-type cells to SDS, whereas the hyperactive Czf1 made them more susceptible (Figure 2c). Of note, the hyperactive Czf1 also caused a filamentous colony phenotype on control plates, in line with the known ability of Czf1 to promote filamentation of *C. albicans* under some conditions (Brown et al., 1999). However, Czf1-induced filamentation did not seem to be the cause of the increased resistance of *snf4*Δ mutants to cell wall stress, since a hyperactive *UME6-GAD* allele, which also promotes filamentous growth (Schillig & Morschhäuser, 2013), did not rescue the sensitivity of the *snf4*Δ mutants to Congo Red and Calcofluor White and even further increased their sensitivity, and also that of the wild type, to caffeine and SDS (Figure S1).

2.3 | The activated Czf1 remediates the hypersensitivity of various protein kinase deletion mutants to cell wall stress

The fact that *czf1*Δ mutants were not hypersensitive to the cell wall-damaging agents Congo Red and Calcofluor White argued that the SNF1 kinase does not ensure normal cell wall stress resistance by activating Czf1. In line with this, we did not observe an altered electrophoretic mobility of HA-tagged Czf1 in *snf4*Δ mutants or upon treatment of the cells with Congo Red (Figure 2d), although phosphatase-treatment of wild-type samples resulted in faster migration of the HA-tagged Czf1 (Figure 2e). The hyperactive Czf1 might therefore not specifically overcome cell wall defects of cells lacking a functional SNF1 kinase but also in other mutants. To test this possibility, we introduced the *CZF1-GAD* allele into 10 other protein kinase deletion mutants that exhibited hypersensitivity to Congo Red in a phenotypic screening of a protein kinase deletion mutant library generated in our laboratory (Ramírez-Zavala et al., 2017 and unpublished results). Indeed, the hyperactive Czf1 (partially) restored the resistance of many, albeit not all of these mutants to Congo Red and/or Calcofluor White (Figure 3). The hypersensitivity of *mck1*Δ, *nik1*Δ, *pan3*Δ, *ssn3*Δ, and *cla4*Δ mutants to both of these agents was remediated, whereas the *mkc1*Δ and *mkk2*Δ mutants regained wild-type resistance to Calcofluor White but remained sensitive to Congo Red (Figure 3a). In contrast, the activated Czf1 did not improve the growth of *ire1*Δ, *kin2*Δ, and *yck2*Δ mutants under these conditions (Figure 3b). Some of the kinase mutants were also more sensitive than the wild type to caffeine, and this hypersensitivity was alleviated in the case of the *cla4*Δ, *mkc1*Δ, *mkk2*Δ, and *kin2*Δ mutants, but not for the *ssn3*Δ and *ire1*Δ mutants.

Interestingly, while the activated Czf1 caused hypersensitivity to SDS in most mutant backgrounds, similar to its effect in wild-type cells, it did not further increase SDS sensitivity in *ssn3*Δ mutants and even restored growth of the *kin2*Δ mutants in the presence of SDS.

To investigate if Czf1 levels or phosphorylation status were altered in one or several of these other protein kinase mutants, we introduced the HA-tagged *CZF1* allele into the mutants for comparison with the wild type. No change in the electrophoretic mobility

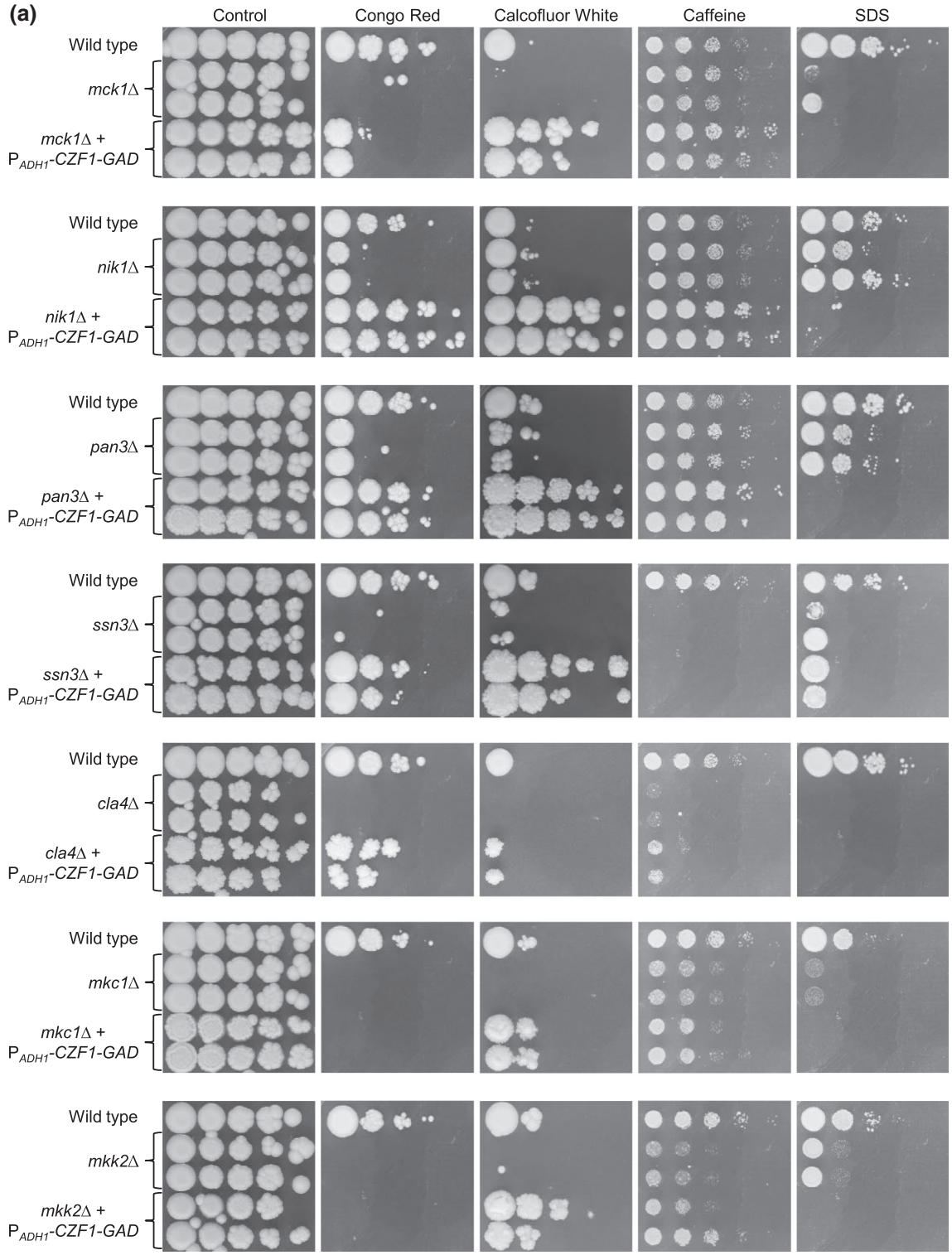

FIGURE 3 An activated form of Czf1 remediates the hypersensitivity of various protein kinase deletion mutants to cell wall stress. YPD overnight cultures of the wild-type strain SC5314, protein kinase mutants, and derivatives expressing the hyperactive *CZF1-GAD* allele from the *ADH1* promoter were diluted to an optical density (OD_{600}) of 2.0. Serial 10-fold dilutions were spotted on YPD plates without (control) or with 50 μg/ml Congo Red, 20 μg/ml Calcofluor White, 15 mM caffeine, or 0.04% SDS and incubated for 4 days at 30°C. Both independently generated series of mutants are shown in each case. (a) Kinase mutants whose hypersensitivity to cell wall stress was (partially) rescued by the activated Czf1. (b) Kinase mutants that remained hypersensitive to cell wall stress

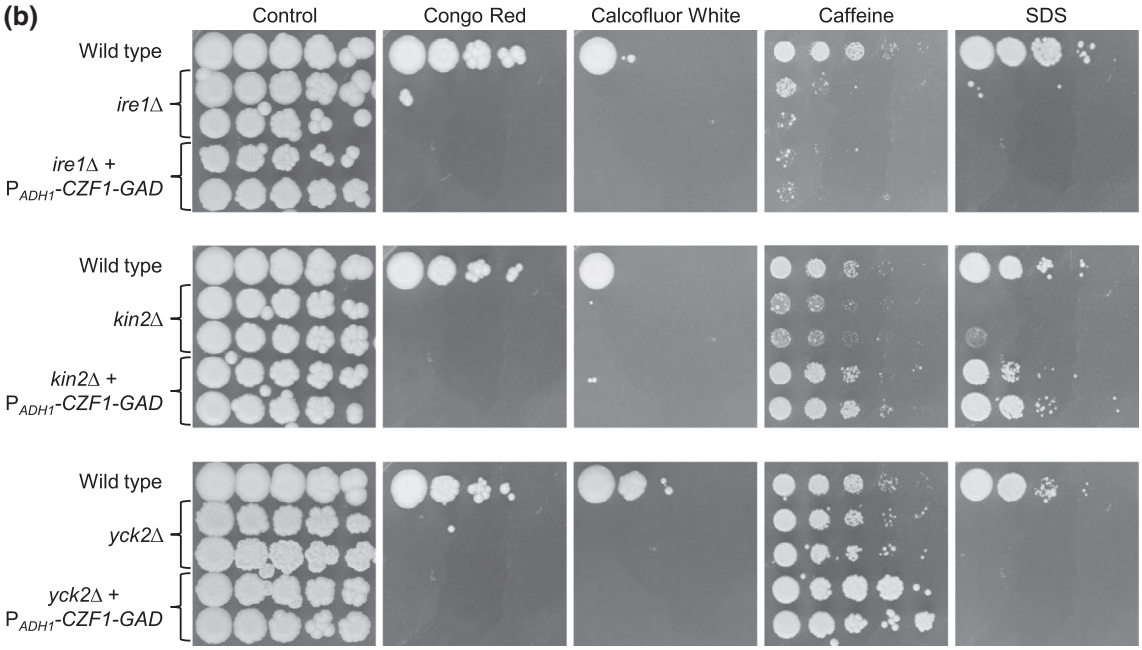

FIGURE 3 (*Continued*)

of Czf1 was detected in any of the kinase mutants, indicating that none of these kinases is alone responsible for Czf1 phosphorylation (Figure 4a). Czf1 protein levels were reduced in most of the mutants, but this did not correlate with the ability of the hyperactive Czf1 to restore growth in the presence of cell wall-damaging agents (compare with Figure 3). For example, Czf1 levels in the *mck1*Δ and *nik1*Δ mutants were comparable to those in the wild type, indicating that the cell wall stress sensitivity of these mutants was not caused by Czf1 deficiency, although the hyperactive Czf1 restored cell wall stress resistance in the same mutants. On the other extreme, Czf1 was undetectable in the *yck2*Δ mutants (Figure 4a), pointing to a deficiency in Czf1 production or stability, but constitutive expression of the hyperactive *CZF1-GAD* allele, which was confirmed by Western blot analysis (Figure 4b), did not rescue the growth defect of the mutants (Figure 3). Altogether, these results indicate that the hyperactive Czf1 caused alterations that remediated the sensitivity of different mutants to cell wall stress, depending on the (undefined) nature of the cell wall defect of the mutants, but simultaneously rendered the cells more sensitive to the membrane-damaging agent SDS.

2.4 | The hyperactive Czf1 alters the structure of the cell wall

To understand how Czf1 might overcome the hypersensitivity of the different protein kinase deletion mutants to cell wall stress, we determined the alterations in gene expression caused by the hyperactive transcription factor in a wild-type background. RNA-seq experiments identified 194 genes that were upregulated by a $\log_2$ value > 2 in both independently constructed derivatives of strain SC5314 containing the *CZF1-GAD* fusion (Table S1). Gene ontology term analysis showed that the most highly enriched terms associated with these 194 genes were "cell surface" (32 genes, 16.5%, $p = 8.18e^{-14}$) and "cell wall" (24 genes, 12.4%, $p = 7.3e^{-11}$). Furthermore, several additional genes in this list encode mannosyltransferases with a known or putative role in cell wall mannosylation (*BMT7*, *MNN22*, *MNN45*) or other cell wall proteins (*SCW4*, orf19.675, orf19.7214), but which are not annotated with these cellular component GO terms in the *Candida* Genome Database (Table S1). The upregulation of these genes by the hyperactive Czf1 may alter the structure of the cell wall in such a way that defects of the *snf4*Δ and other protein kinase deletion mutants are at least partially remedied.

As shown in Figure 1, deletion of *MIG1* and *MIG2* also partially relieved the hypersensitivity of *snf4*Δ mutants to cell wall stress. Lagree et al. (2020) found that 488 genes were significantly upregulated in *mig1*Δ *mig2*Δ mutants compared to a wild-type strain when grown in YPD medium. Interestingly, 58 of these were among the 194 genes that were upregulated by the hyperactive Czf1, a highly significant overlap ($p = 3.23e^{-21}$), and enriched for GO terms related to sugar transport (Table S2). These observations further point to a linkage between carbohydrate metabolism and cell wall biogenesis and the regulation of these processes by both Czf1 and Mig1/Mig2.

We investigated if the increased sensitivity of the *snf4*Δ and other protein kinase mutants to cell wall stress and the alterations caused by the hyperactive Czf1correlated with visible changes in the cell wall. To this aim, we examined the strains by transmission electron microscopy (TEM) and determined the thickness of the inner and outer wall layers (we note that the technique used provided only limited resolution of the outer fibrillar layer). Representative photographs are shown in Figure 5, and quantitative data are presented in Figure 6. In the wild-type background, the width of the outer mannoprotein layer was increased by the activated Czf1, while no significant change was detected in the inner layer. Except for the *snf4*Δ mutants, all

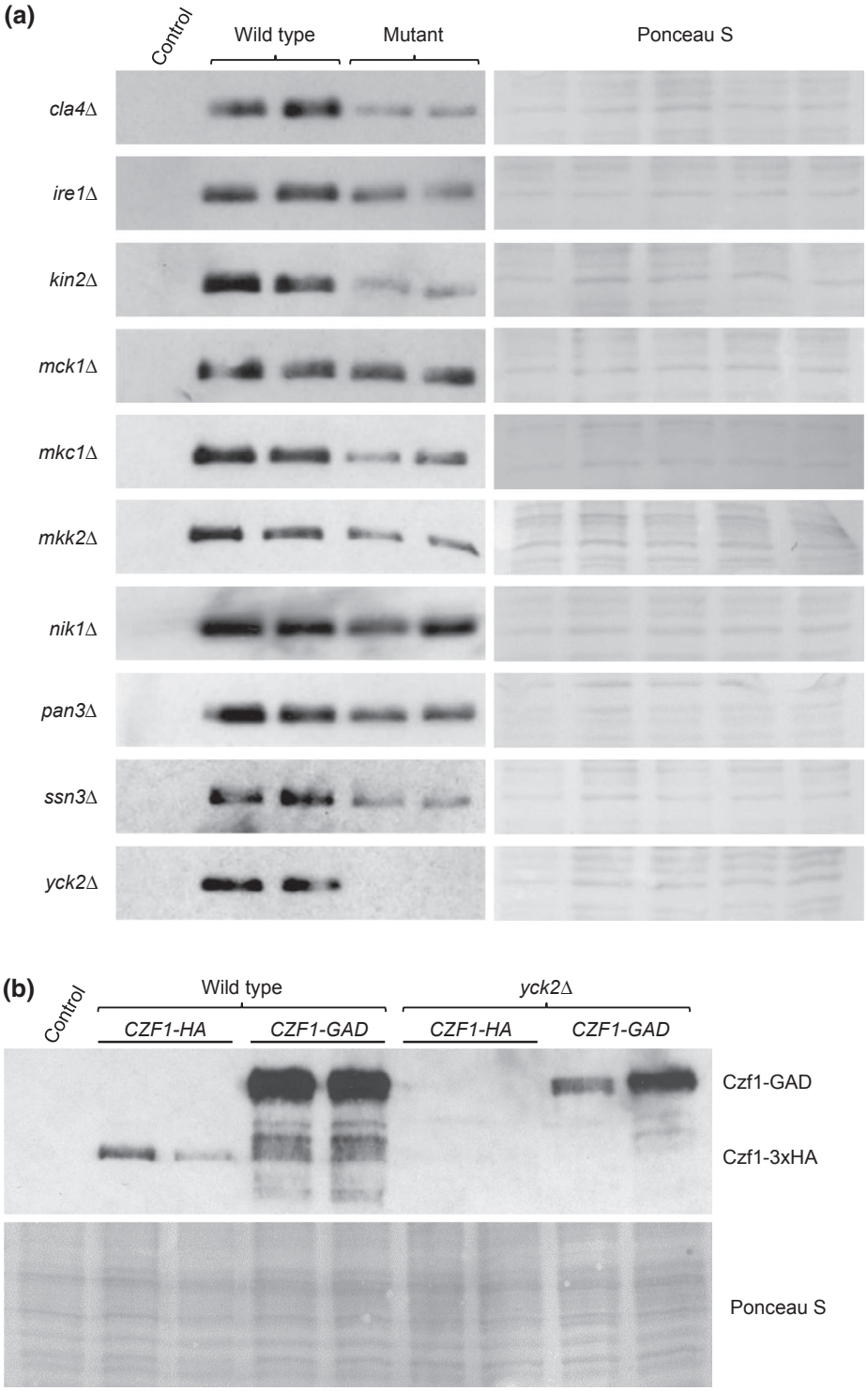

FIGURE 4 Detection of 3xHA-tagged Czf1 in wild-type and protein kinase deletion mutant backgrounds by Western blotting with an anti-HA antibody. YPD overnight cultures of the strains were diluted in fresh YPD medium and grown for 3 hr at 30°C. Two independent transformants expressing the HA-tagged *CZF1* allele were tested in each case; the untagged wild-type strain SC5314 served as negative control. Separate Western blots were performed for the comparison of the different protein kinase mutants with the wild type. (a) Strains expressing the HA-tagged wild-type *CZF1* from its endogenous promoter; (b) strains expressing the hyperactive *CZF1-GAD* allele, which also contains the 3xHA tag, from the *ADH1* promoter. Parts of the Ponceau S-stained blots are shown as loading controls

kinase mutants exhibited differences compared to the wild type. The thickness of the outer cell wall was decreased in the *cla4*Δ, *ire1*Δ, and *yck2* mutants and increased in the *mck1*Δ, *mkc1*Δ, *mkk2*Δ, *nik1*Δ, and *pan3*Δ mutants. The thickness of the inner wall layer was reduced in the *mkk2*Δ, *nik1*Δ, and *yck2*Δ mutants, while it was increased in the *cla4*Δ, *ire1*Δ, *kin2*Δ, *mck1*Δ, *pan3*Δ, and *ssn3*Δ mutants (some of the

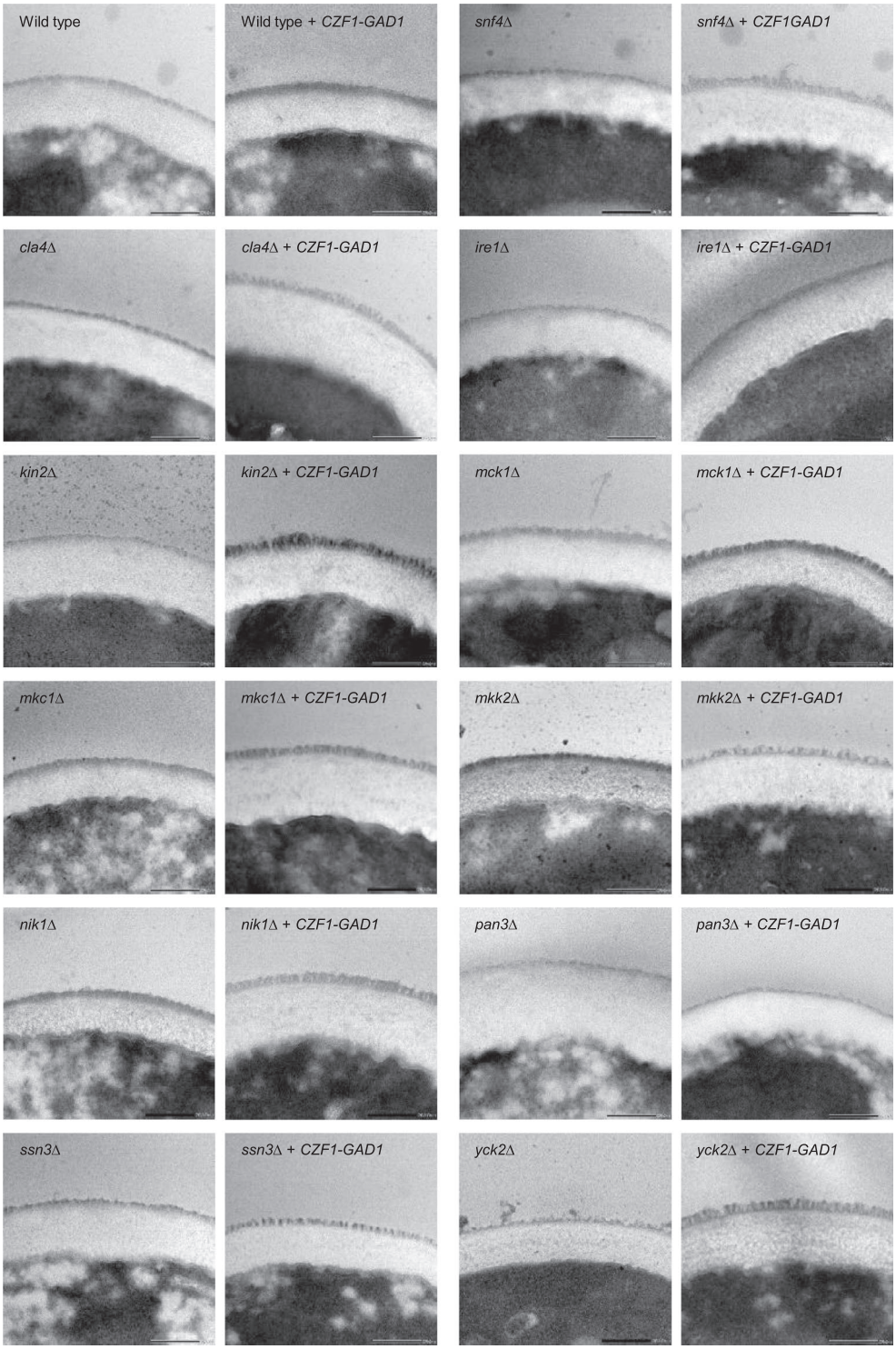

FIGURE 5 Transmission electron micrographs of the wild-type strain SC5314, protein kinase mutants, and derivatives with the hyperactive *CZF1-GAD* allele. Scale bars: 200 nm. Due to the TEM technique used in our experiments, the outer fibrillar wall layer appears thinner than in studies using high-pressure freeze substitution TEM

differences were minor, albeit statistically significant). The hyperactive Czf1 caused cell wall alterations in all kinase mutants. The thickness of both outer and inner layers was increased in the *snf4*Δ, *cla4*Δ, *kin2*Δ, *mkc1*Δ, *nik1*Δ, and *yck2*Δ mutants, but reduced in the *pan3*Δ mutants. In other mutants, opposite changes were observed for the inner and outer layers (*mkk2*Δ, *ssn3*Δ) or only one of the two layers was measurably affected (*ire1*Δ, *mck1*Δ). Altogether, these results show that the hyperactive Czf1 caused alterations in the cell wall of all strains tested. Interestingly, both increased (e.g. *snf4*Δ, *cla4*Δ) and decreased (*pan3*Δ) wall thickness could rescue the hypersensitivity of mutants to cell wall stress, depending on the affected kinase and the stress-inducing agent (compare with Figure 3).

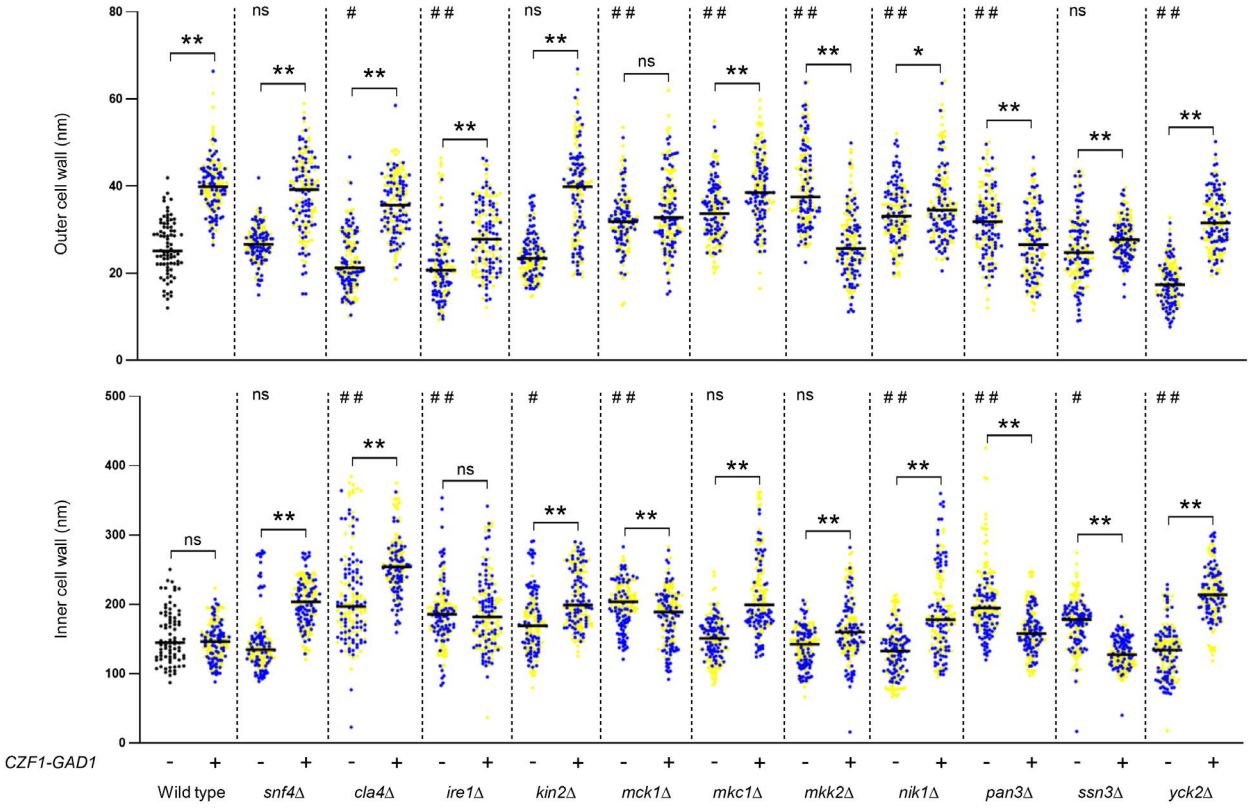

FIGURE 6 Thickness of the outer and inner cell wall layers of the wild-type strain SC5314, protein kinase mutants, and derivatives with the hyperactive *CZF1-GAD* allele. For each strain, 10 cells were measured at eight different locations. Data for the two independently generated series of strains are distinguished by blue and yellow dots and were combined for the statistical evaluation. The black bars show the means of the 80 (wild type) and 160 (kinase mutants) measurements. Statistically significant differences between protein kinase mutants and the wild-type parental strain (#) and between presence (+) and absence (–) of the *CZF1-GAD* allele in each background (*) are indicated with symbols. # # and **$p < .001$; # and *, $p < .01$; ns, not significant ($p > .01$)

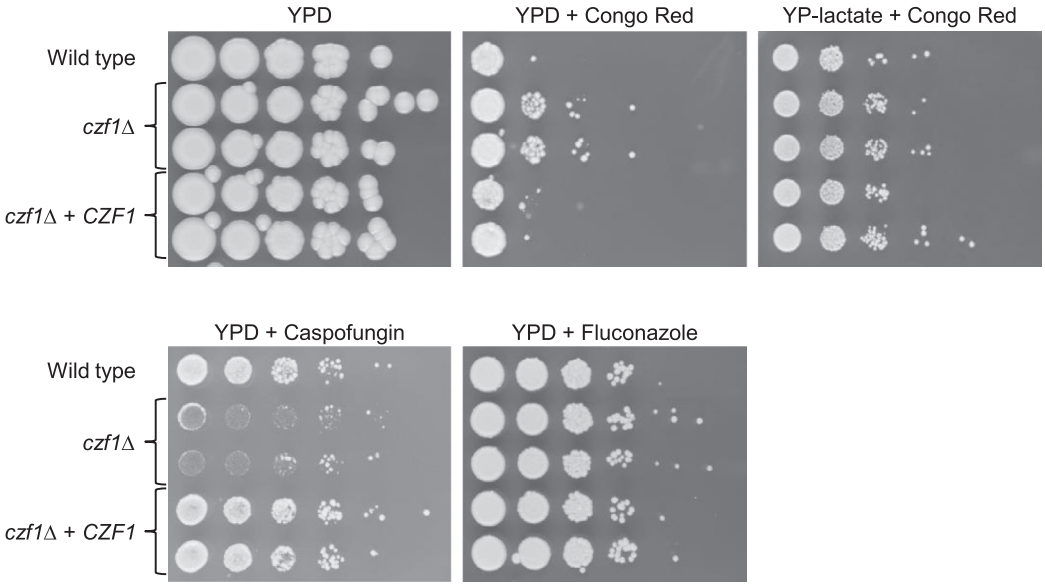

FIGURE 7 *C. albicans**czf1*Δ mutants are hypersensitive to caspofungin. YPD overnight cultures of the wild-type strain SC5314, homozygous *czf1*Δ mutants (*czf1*Δ), and complemented strains (*czf1*Δ + *CZF1*) were diluted to an optical density (OD_{600}) of 2.0. Serial 10-fold dilutions were spotted on YPD or YP-lactate agar plates without or with 50 μg/ml Congo Red, 100 ng/ml caspofungin, or 5 μg/ml fluconazole and incubated for 3 days at 30°C. Both independently generated series of strains are shown

In a separate experiment, we also tested if deletion of *CZF1* caused visible alterations in the cell wall structure. As can be seen in Figure S2, the *czf1*Δ mutants exhibited a thinner inner cell wall layer, but no detectable changes in the outer cell wall layer.

2.5 | Conditional cell wall stress susceptibility of *czf1*Δ mutants

In a very recent paper, Childers et al. reported that Czf1 is involved in the masking of β-1,3-glucan in the cell wall of *C. albicans* upon exposure to lactate (Childers et al., 2020b). We, therefore, reasoned that the role of Czf1 in the tolerance of *C. albicans* wild-type cells to cell wall stress, which was not obvious in our previous assays (Figure 2c), might become evident under other conditions that induce cell wall remodeling. Indeed, we found that the *czf1*Δ mutants were hypersensitive to caspofungin, an antifungal drug that inhibits β-1,3-glucan biosynthesis (Figure 7). We also tested Congo Red sensitivity on plates containing lactate instead of glucose. Interestingly, in this set of experiments we observed a slightly increased resistance of the *czf1*Δ mutants to Congo Red on YPD plates, which was not clearly evident in our previous assays (Figure 2c), but no difference to the wild type when lactate was the carbon source. We did not observe the previously reported hypersensitivity of *czf1*Δ mutants to fluconazole, which inhibits ergosterol biosynthesis and thereby membrane function (Dhamgaye et al., 2012). Of note, these authors also observed increased resistance of a *czf1*Δ mutant to Congo Red.

Since the *czf1*Δ mutants exhibited increased sensitivity to caspofungin, we tested if the hyperactive Czf1 would ameliorate the caspofungin hypersensitivity of protein kinase mutants with cell wall defects. In line with a previous report (Blankenship et al., 2010), most of the tested protein kinase mutants were hypersensitive to caspofungin, but except for the *mck1*Δ mutants the hyperactive Czf1 further increased rather than decreased their sensitivity (Figue S3). Interestingly, we found that deletion of *MIG1* and *MIG2* also further increased the sensitivity of *snf4*Δ mutants to caspofungin (Figure S3), in contrast to the improved resistance to Congo Red and Calcofluor White (compare with Figure 1), highlighting the different effect of the antifungal drug on fungal cells compared with other cell wall-damaging agents. Altogether, our results indicate that Czf1 is involved in the regulation of cell wall architecture, but its impact on cell wall integrity and resistance to cell wall stress depends on the conditions.

2.6 | Czf1-mediated cell wall changes affect the recognition of *C. albicans* by neutrophils

Since the cell wall architecture determines the recognition of *C. albicans* by the host immune system, we reasoned that the cell wall alterations caused by *CZF1* deletion or increased Czf1 activity would impact immune responses. Therefore, we compared the formation of reactive oxygen species (ROS), also known as oxidative burst, and IL-8 release in primary human neutrophils exposed to *C. albicans* wild-type cells, *czf1*Δ mutants, and fungal cells with a hyperactive Czf1. Figure 8 shows that both types of mutants stimulated a stronger oxidative burst and IL-8 secretion than the wild type, indicating that the observed cell wall changes increased the recognition of the fungal cells by the neutrophils and induced a stronger pro-inflammatory activation.

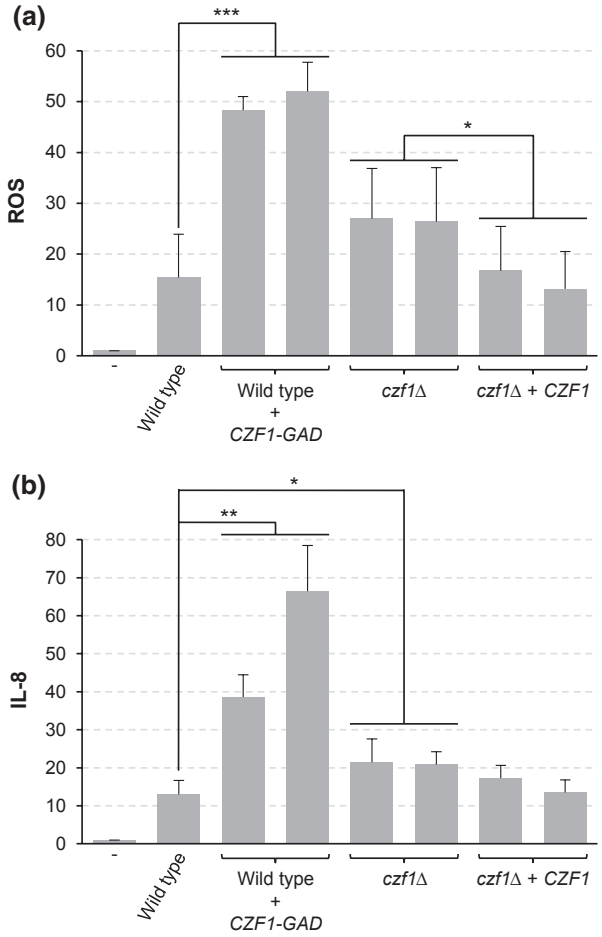

FIGURE 8 ROS production and IL-8 secretion by PMNs in the absence (–) or presence of the *C. albicans* wild-type strain SC5314, derivatives containing the hyperactive *CZF1-GAD* allele, *czf1*Δ mutants, and complemented strains. Control values were set to 1. Results for both independently generated series of strains are shown separately; data were combined for statistical analysis. Significant differences from the wild type are indicated by stars. ***$p < .001$; **$p < .01$; *, $p < .05$. The increased ROS induction by the *czf1*Δ mutants compared to the wild type did not reach statistical significance, but ROS levels induced by the *czf1*Δ mutants were significantly higher than those induced by the complemented strains

3 | DISCUSSION

The initial goal of this study was to identify a target transcription factor of the SNF1 kinase that mediates SNF1-dependent adaptations to cell wall stress. A kinase-independent, constitutively active form of such a transcription factor might rescue the hypersensitivity

of cells with a defective SNF1 kinase to cell wall-damaging agents. Indeed, by harnessing a library of artificially activated zinc cluster transcription factors, we found that a hyperactive form of Czf1 improved the growth of *snf4*Δ mutants, but also of other mutants lacking protein kinases required for cell wall integrity, under cell wall stress. However, our experiments did not establish Czf1 as a downstream target of any of these kinases, since the electrophoretic mobility of Czf1, which was increased after phosphatase treatment, was not detectably altered in the mutants.

A previous *C. albicans* phosphoproteome analysis found that Czf1 is phosphorylated at five serine residues, S34, S278, S280, S281, and S282 (Willger et al., 2015). If these residues are phosphorylated by different kinases, an alteration of the electrophoretic mobility of Czf1 may not be detectable in single mutants lacking one of these kinases. It is therefore possible that one or more of the protein kinases whose cell wall defects were rescued by the genetically activated Czf1 may be involved in Czf1 phosphorylation. Furthermore, many additional protein kinase deletion mutants are hypersensitive to cell wall stress (Blankenship et al., 2010; Cheetham et al., 2007; Eisman et al., 2006; Liu et al., 2014; Rauceo et al., 2008; Roman et al., 2009; Song et al., 2008 and own unpublished results) and may act on Czf1. Whether phosphorylation is involved in the activation of Czf1 remains to be determined, since in our assays the HA-tagged Czf1 was already phosphorylated in cells grown in YPD medium (Figure 2e). So far, Czf1 activity is known to be regulated by controlling its expression and binding to target promoters in cooperation with other transcription factors (Hernday et al., 2013; Vinces et al., 2006; Vinces & Kumamoto, 2007; Zordan et al., 2007).

Two previous studies hinted to a role for Czf1 in cell wall structure and integrity. Dhamgaye et al. (2012) observed that *CZF1* was overexpressed in many fluconazole-resistant *C. albicans* isolates and found that a *czf1*Δ mutant was hypersensitive to different inhibitors, including drugs that act on ergosterol biosynthesis (fluconazole, terbinafine), protein synthesis (anisomycin), calcineurin (FK506), and the cell membrane (SDS). In contrast, the *czf1*Δ mutant exhibited increased resistance to Congo Red, and the authors found that *GSL1*, encoding a β-1,3-glucan synthase, was upregulated in the mutant. Based on the latter observation, the authors suggested that Czf1 acts as a repressor of β-1,3-glucan synthesis and negatively regulates cell wall integrity. In our assays, *czf1*Δ mutants were not hypersensitive to fluconazole or SDS, and the expression of *GSL1* or other β-1,3-glucan synthase genes was not altered by the hyperactive Czf1 in our RNA-seq experiments. Our results rather indicate that Czf1 affects the production of other cell wall proteins, such as mannosyl transferases, which are required for normal cell wall mannan. In line with this model, Childers et al. (2020b) recently reported that the reduction in β-1,3-glucan exposure at the cell surface, which is induced in wild-type cells in the presence of lactate, did not occur in a *czf1*Δ mutant, suggesting a role for Czf1 in β-1,3-glucan masking. Our finding that *czf1*Δ mutants induced a stronger neutrophil response than wild-type cells supports this function of Czf1. Of note, both absence and hyperactivity of Czf1 increased recognition by the neutrophils, demonstrating that Czf1 activity must be well controlled to establish an appropriate cell wall architecture. Childers et al. also found that the major exoglucanase Xog1 contributes to β-1,3-glucan masking by removing it from the cell surface. Interestingly, *XOG1* is one of the genes that were upregulated by the hyperactive Czf1 in our present study (Table S1) and may therefore contribute to the alterations in the structure of the cell wall observed in these strains. However, despite the increased expression of *XOG1*, the overall altered architecture of the cell wall that was caused by the hyperactive Czf1 resulted in increased recognition of the fungal cells by human neutrophils.

Czf1 has long been known to regulate morphological transitions in *C. albicans*, inducing filamentous growth under some environmental conditions and the switch to the mating-competent opaque cell form (Brown et al., 1999; Vinces & Kumamoto, 2007; Zordan et al., 2007). These changes in cell morphology involve restructuring of the cell wall, and the findings of our study suggest that ensuring an appropriate architecture of the cell wall may be a major function of Czf1 in *C. albicans* morphogenesis and in response to environmental changes.

4 | EXPERIMENTAL PROCEDURES

4.1 | Strains and growth conditions

The *C. albicans* strains used in this study are listed in Table S3. All strains were stored as frozen stocks with 17.2% glycerol at −80°C and subcultured on YPD agar plates (10 g yeast extract, 20 g peptone, 20 g glucose, 15 g agar per liter) at 30°C. Strains were routinely grown in YPD liquid medium at 30°C in a shaking incubator. For the selection of nourseothricin-resistant transformants, 200 μg/ml nourseothricin (Werner Bioagents, Jena, Germany) was added to YPD agar plates. To obtain nourseothricin-sensitive derivatives in which the *SAT1* flipper cassette was excised by FLP-mediated recombination, transformants were grown overnight in YCB-BSA-YE medium (23.4 g yeast carbon base, 4 g bovine serum albumin, 2 g yeast extract per liter, pH 4.0) without selective pressure to induce the *SAP2* promoter controlling *caFLP* expression. Appropriate dilutions were plated on YPD agar plates and grown for 2 days at 30°C. Individual colonies were picked and streaked on YPD plates as well as on YPD plates with 100 μg/ml nourseothricin to confirm nourseothricin sensitivity.

4.2 | Plasmid constructions

Plasmids containing artificially activated forms of all *C. albicans* zinc cluster transcription factor genes under the control of the *ADH1* promoter, such as pCZF1-GAD1, have been described previously (Schillig & Morschhäuser, 2013). To express wild-type *CZF1* from the *ADH1* promoter, a SalI-BglII fragment from plasmid pTET1-CZF1 containing the *CZF1* coding sequence (Ramírez-Zavala et al., 2008) was inserted instead of the *UPC2* coding region in plasmid pUPC2E1 (Schillig & Morschhäuser, 2013), resulting in pCZF1E1. To tag Czf1 with a 3xHA epitope, part of the *CZF1* coding region was amplified

from genomic DNA of strain SC5314 with primers CZF1-10 (5′-CGGACTCGAGTTCTGAAACACCACAGG-3′) and CZF1-11 (5′-GAGAGGATCCTTTACTTCTGTATTCAACAATACC-3′). The latter primer introduced a *Bam*HI site, encoding a Gly-Ala linker, instead of the *CZF1* stop codon. The *CZF1* downstream region was amplified with primers CZF1-8 (5′-AGTACTGCAGCTTCTCTGTGTTGGAGGG-3′) and CZF1-9 (5′-ATCACCGCGGTTGTAGAACTCAAATACCC-3′) (introduced restriction sites are underlined). The PCR products were digested with *Xho*I/*Bam*HI and *Pst*I/*Sac*I, respectively, and substituted for the flanking *MRR1* sequences in plasmid pZCF36DBH1 (Schubert et al., 2011), resulting in pCZF1H1.

4.3 | Strain constructions

C. albicans strains were transformed by electroporation (Köhler et al., 1997) with the following gel-purified linear DNA fragments: The insert from plasmid pCZF1E1 was used to express wild-type *CZF1* from the *ADH1* promoter in strain SC5314 and *snf4*Δ mutants. The insert from plasmid pCZF1H1 was used to introduce a C-terminal 3xHA tag into either of the endogenous *CZF1* alleles in strain SC5314 and mutant derivatives. The insert from pCZF1-GAD1 was used to express the artificially activated *CZF1* from the *ADH1* promoter in various protein kinase deletion mutants. The *CZF1* deletion construct contained in plasmid pCZF1M2 (Ramírez-Zavala et al., 2008) was used to sequentially delete both *CZF1* alleles of strain SC5314 with the help of the *SAT1* flipper cassette. A complementation cassette from pCZF1K1 containing the *CZF1* coding region and upstream sequences (Ramírez-Zavala et al., 2008) was used to reintroduce an intact *CZF1* copy into the *czf1*Δ mutants, followed by recycling of the *SAT1* flipper cassette.

4.4 | Isolation of genomic DNA and Southern hybridization

Genomic DNA from *C. albicans* strains was isolated as described previously (Reuß et al., 2004). The DNA was digested with appropriate restriction enzymes, separated on a 1% agarose gel, transferred by vacuum blotting onto a nylon membrane, and fixed by UV crosslinking. Southern hybridization with enhanced chemiluminescence-labeled probes was performed with the Amersham ECL™ Direct Nucleic Acid Labelling and Detection System (GE Healthcare UK Limited, Little Chalfont Buckinghamshire, UK) according to the instructions of the manufacturer.

4.5 | Screening of the *ZCF-GAD* library for *snf4*Δ suppressors

The plasmids containing the 82 *ZCF-GAD* fusion constructs were divided into nine pools, each containing a different subset of the library (see Table S4). The expression cassettes contained in pools 1 to 7 were excised from the vector backbone by digestion with *Apa*I/*Sac*II, and those from pool 8 by digestion with *Kpn*I/*Sac*II. For two plasmids (pool 9), partial digests with KpnI/SacII were performed to obtain intact expression cassettes. The digested plasmid DNAs were then used to transform the *snf4*Δ mutant SCSNF4M4A, followed by plating on YPD agar with 200 μg/ml nourseothricin. After 2 days of growth at 30°C, transformant colonies were washed off the plates and suspended in 5 ml water. One hundred microliters of each pool were then spread on YPD agar plates with 50 μg/ml Congo Red and incubated for 2 days at 30°C. One colony from pool 7 grew on this plate and stably maintained the resistant phenotype upon restreaking. Southern hybridization showed that the strain contained an expression cassette insertion at the *ADH1* locus. The inserted *ZCF* gene was amplified by PCR with primers binding in the *ADH1* upstream region (5′-TGATAGAGACCCAATGCAAAGCC-3′) and in the *GAD* sequence (5′-GTTATGAGGTTCGGACCGTTGC-3′), which are common to all *ZCF-GAD* fusions. The size of the PCR product corresponded to that expected for *CZF1* in this pool, and sequencing confirmed that the strain contained the *CZF1-GAD* fusion.

4.6 | Growth assays

The sensitivity of strains to cell wall/membrane stress was tested by dilution spot assays on solid media. For this, YPD overnight cultures were adjusted to an OD_{600} of 2.0 and serial tenfold dilutions prepared in a 96-well microtiter plate. Ca. 5 μl of the cell suspensions were transferred with a replicator onto YPD or YP-lactate (10 g yeast extract, 20 g peptone, 20 g lactate, 15 g agar per liter) plates without or with inhibitors as indicated in the figure legends. The plates were incubated for 3 to 4 days at 30°C and photographed.

4.7 | Western blotting

Overnight cultures of the strains were diluted 10^{-2} in 50 ml fresh YPD medium and grown for 1 hr at 30°C, followed by 2 hr of growth in the presence or absence of 50 μg/ml Congo Red. Cells were collected by centrifugation, washed in 50 ml H_2O, and resuspended in 500 μl breaking buffer (100 mM triethylammonium bicarbonate buffer [TEAB], 150 mM NaCl, 1% SDS, cOmplete EDTA-free Protease Inhibitor Cocktail and PhosStop Phosphatase Inhibitor Cocktail [Roche Diagnostics GmbH, Mannheim, Germany]) supplemented with protease and phosphatase inhibitors. An equal volume of 0.5-mm acid-washed glass beads was added to each tube. Cells were mechanically disrupted on a FastPrep-24 cell-homogenizer (MP Biomedicals, Santa Ana, USA) with three 40-s pulses, with 5 min on ice between each pulse. Samples were centrifuged at 13,000 rpm for 15 min at 4°C, the supernatant was collected, and the protein concentration was quantified using the Bradford protein assay. Equal amounts of protein of each sample were mixed with one volume of 2× Laemmli buffer, heated for 5 min at 95°C, and separated on an SDS-9% polyacrylamide gel. Separated proteins were transferred

onto a nitrocellulose membrane with a mini-Protean System (Bio-Rad, Munich, Germany) and stained with Ponceau S to control for equal loading. Membranes were blocked in 5% BSA-TBST (Tris-buffered saline with Tween 20) at room temperature for 1 hr and subsequently incubated overnight at 4°C with rat monoclonal anti-HA-Peroxidase antibody (Roche Diagnostics GmbH). Membranes were washed in TBST and signals detected with the ECL chemiluminescence detection system (GE Healthcare Bio-Sciences GmbH, Munich, Germany). For phosphatase treatment, the cell extracts were prepared as described above, except that the phosphatase inhibitor cocktail was omitted in the breaking buffer. Cell extracts were incubated with λ Protein Phosphatase (New England Biolabs, Ipswich, USA) at 30°C for 30 min.

4.8 | RNA-seq experiments

Triplicate overnight cultures of the wild-type strain SC5314 and derivatives containing the *CZF1-GAD* allele (strains SCCZF1GAD1A and -B) were diluted 10^{-2} in fresh YPD medium and grown for 3 hr at 30°C. Cells were harvested by centrifugation and washed once in water. Cells were broken using hot phenol and RNA was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Samples were treated with TURBO DNase (Invitrogen GmbH, Darmstadt, Germany) to remove any residual genomic DNA, and remaining RNA was cleaned by a second purification using the RNeasy Mini kit. Further steps to prepare for and conduct RNA sequencing were done by Eurofins Genomics (Konstanz, Germany). Such steps included purification of poly(A) mRNA molecules, mRNA fragmentation, preparation of strand-specific cDNA libraries, adapter ligation, and Illumina (HiSeq 2,500) sequencing (single reads, 1 × 50 base pair length). Between 35 and 48 million reads per sample were obtained as FASTQ files. Low-quality reads were eliminated and adapter sequences trimmed using the Trimmomatic tool (v0.36) (Bolger et al., 2014) using default settings for single-end reads. High-quality reads were mapped to the reference genome (SC5314 Assembly 22) using STAR (v 2.6.1b) (Dobin et al., 2013). Read counts per ORF were extracted from the resulting BAM files using HTSeq (v 0.6.1) (Anders et al., 2015). Normalization and statistical comparisons of data were conducted using RStudio (v 1.1.456) with the DESeq2 package (v 1.14.1) (Love et al., 2014). The RNA-seq data have been submitted to the NCBI Gene Expression Omnibus (GEO) database under accession number GSE165091. Gene ontology analysis was performed using the GO enrichment tool (GO term finder) in the *Candida* Genome Database (http://www.candidagenome.org/cgi-bin/GO/goTermFinder), excluding computationally predicted annotations (evidence codes IEA, RCA).

4.9 | Transmission electron microscopy

Single colonies grown on YPD for 2 days at 30°C were collected and fixed in fixation buffer (2.5% glutaraldehyde, 2% formaldehyde, 0.1 M cacodylate pH 7.2) overnight at 4°C, followed by several washing steps in 50 mM cacodylate buffer (pH 7.2). Cells were further fixed in 2% buffered osmium tetraoxide for 90–120 min, followed by several washing steps in water. Cells were stained overnight with 0.5% uranyl acetate dihydrate for contrasting and washed several times in water. Samples were dehydrated using increasing concentrations of ethanol, followed by ethanol removal using propylene oxide. Cells were then embedded in epon at 60°C for 48 hr. Ultrathin sections were cut using a microtome and imaged using a JEOL JEM-1400 Flash transmission electron microscope (120 kV) and a Matataki Flash digital camera. To determine the average thickness of the inner and outer cell wall, 10 representative cells were imaged and measured at 8 locations each using SightX TEM Imaging Viewer version 2.1.13. Statistical comparisons were conducted and data plotted with GraphPadPrism version 9.0, using one-way ANOVA, plus Dunnett's multiple comparison correction where appropriate.

4.10 | Neutrophil assays

Human neutrophils were purified from venous blood of healthy volunteers using the MACSxpress Whole Blood Neutrophil Isolation Kit (Miltenyi Biotec, Bergisch-Gladbach, Germany) according to the manufacturer's instructions. Immune cell fractions were re-suspended in RPMI 1,640 (Gibco) containing 5% (vol/vol) heat-inactivated fetal calf serum (FCS) (Biochrom GmbH, Berlin, Germany). *C. albicans* strains were grown overnight in modified YPD medium (2% glucose, 1% peptone, 0.5% yeast extract) at 30°C, reseeded in the same medium, grown to midlog phase, and harvested in Hank's Balanced Salt Solution (HBSS). The oxidative burst was determined by the 2′,7′-dichlorofluorescein (DCF) assay: Neutrophils were pre-incubated with 2 μM 2′,7′-dichlorofluorescein diacetate (DCF-DA) (Sigma-Aldrich, Taufkirchen, Germany) at room temperature for 30 min. In a 96-well flat-bottom black optical plate (Greiner), 4 x 10^6 DCF-DA-treated neutrophils/ml were confronted with 2 × 10^6 *C. albicans* cells/ml (multiplicity of infection [MOI] = 0.5) in RPMI 1,640 with 5% (vol/vol) heat-inactivated FCS. Neutrophils in media alone served as a negative control. Fluorescence was measured in a Tecan Infinite 200 plate reader at 37°C with excitation at 485 nm and emission at 535 nm over a period of 3 hr. Release of IL-8 by neutrophils was measured in supernatants from the respective samples after 3 hr of confrontation using Luminex technology (ProcartaPlex multiplex immunoassay, Thermo Fisher Scientific, Waltham, MA) according to the manufacturer's instructions. Three independent replicates using cells from non-identical donors were used. Data are presented as arithmetic means and standard deviations. In each case, data for the two independently generated strains were combined for statistical analysis. Statistical significance was calculated using a two-sided *t*-test for unpaired data.

Human peripheral blood was collected from healthy volunteers with written informed consent. All protocols were approved by the ethics committee of the University Hospital Jena (permit number: 273-12/09).

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REFERENCES

Alonso-Monge, R., Navarro-Garcia, F., Molero, G., Diez-Orejas, R., Gustin, M., Pla, J. et al (1999) Role of the mitogen-activated protein kinase Hog1p in morphogenesis and virulence of *Candida albicans*. *Journal of Bacteriology*, *181*, 3058–3068. https://doi.org/10.1128/JB.181.10.3058-3068.1999

Anders, S., Pyl, P.T. & Huber, W. (2015) HTSeq–a Python framework to work with high-throughput sequencing data. *Bioinformatics*, *31*, 166–169. https://doi.org/10.1093/bioinformatics/btu638

Ballou, E.R., Avelar, G.M., Childers, D.S., Mackie, J., Bain, J.M., Wagener, J. et al (2016) Lactate signalling regulates fungal β-glucan masking and immune evasion. *Nature Microbiology*, *2*, 16238.

Blankenship, J.R., Fanning, S., Hamaker, J.J. & Mitchell, A.P. (2010) An extensive circuitry for cell wall regulation in *Candida albicans*. *PLoS Pathogens*, *6*, e1000752. https://doi.org/10.1371/journal.ppat.1000752

Bolger, A.M., Lohse, M. & Usadel, B. (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Brown, D.H. Jr, Giusani, A.D., Chen, X. & Kumamoto, C.A. (1999) Filamentous growth of *Candida albicans* in response to physical environmental cues and its regulation by the unique *CZF1* gene. *Molecular Microbiology*, *34*, 651–662. https://doi.org/10.1046/j.1365-2958.1999.01619.x

Bruno, V.M., Kalachikov, S., Subaran, R., Nobile, C.J., Kyratsous, C. & Mitchell, A.P. (2006) Control of the *C. albicans* cell wall damage response by transcriptional regulator Cas5. *PLoS Pathogens*, *2*, 204–210. https://doi.org/10.1371/journal.ppat.0020021

Cheetham, J., Smith, D.A., da Silva Dantas, A., Doris, K.S., Patterson, M.J., Bruce, C.R. et al (2007) A single MAPKKK regulates the Hog1 MAPK pathway in the pathogenic fungus *Candida albicans*. *Molecular Biology of the Cell*, *18*, 4603–4614.

Childers, D.S., Avelar, G.M., Bain, J.M., Larcombe, D.E., Pradhan, A., Budge, S. et al (2020a) Impact of the environment upon the *Candida albicans* cell wall and resultant effects upon immune surveillance. *Current Topics in Microbiology and Immunology*, *425*, 297–330.

Childers, D.S., Avelar, G.M., Bain, J.M., Pradhan, A., Larcombe, D.E., Netea, M.G. et al (2020b) Epitope shaving promotes fungal immune evasion. *mBio*, *11*, e00984-20.

Corvey, C., Koetter, P., Beckhaus, T., Hack, J., Hofmann, S., Hampel, M. et al (2005) Carbon source-dependent assembly of the Snf1p kinase complex in *Candida albicans*. *The Journal of Biological Chemistry*, *280*, 25323–25330. https://doi.org/10.1074/jbc.M503719200

Dhamgaye, S., Bernard, M., Lelandais, G., Sismeiro, O., Lemoine, S., Coppee, J.Y. et al (2012) RNA sequencing revealed novel actors of the acquisition of drug resistance in *Candida albicans*. *BMC Genomics*, *13*, 396. https://doi.org/10.1186/1471-2164-13-396

Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S. et al (2013) STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, *29*, 15–21. https://doi.org/10.1093/bioinformatics/bts635

Eisman, B., Alonso-Monge, R., Roman, E., Arana, D., Nombela, C. & Pla, J. (2006) The Cek1 and Hog1 mitogen-activated protein kinases play complementary roles in cell wall biogenesis and chlamydospore formation in the fungal pathogen *Candida albicans*. *Eukaryotic Cell*, *5*, 347–358. https://doi.org/10.1128/EC.5.2.347-358.2006

Enloe, B., Diamond, A. & Mitchell, A.P. (2000) A single-transformation gene function test in diploid *Candida albicans*. *Journal of Bacteriology*, *182*, 5730–5736. https://doi.org/10.1128/JB.182.20.5730-5736.2000

Gow, N.A. & Hube, B. (2012) Importance of the *Candida albicans* cell wall during commensalism and infection. *Current Opinion in Microbiology*, *15*, 406–412. https://doi.org/10.1016/j.mib.2012.04.005

Hall, R.A. (2015) Dressed to impress: impact of environmental adaptation on the *Candida albicans* cell wall. *Molecular Microbiology*, *97*, 7–17.

Hedbacker, K. & Carlson, M. (2008) SNF1/AMPK pathways in yeast. *Frontiers in Bioscience*, *13*, 2408–2420. https://doi.org/10.2741/2854

Hernday, A.D., Lohse, M.B., Fordyce, P.M., Nobile, C.J., DeRisi, J.L. & Johnson, A.D. (2013) Structure of the transcriptional network controlling white-opaque switching in *Candida albicans*. *Molecular Microbiology*, *90*, 22–35.

Köhler, G.A., White, T.C. & Agabian, N. (1997) Overexpression of a cloned IMP dehydrogenase gene of *Candida albicans* confers resistance to the specific inhibitor mycophenolic acid. *Journal of Bacteriology*, *179*, 2331–2338. https://doi.org/10.1128/JB.179.7.2331-2338.1997

Lagree, K., Woolford, C.A., Huang, M.Y., May, G., McManus, C.J., Solis, N.V. et al (2020) Roles of *Candida albicans* Mig1 and Mig2 in glucose repression, pathogenicity traits, and *SNF1* essentiality. *PLoS Genetics*, *16*, e1008582. https://doi.org/10.1371/journal.pgen.1008582

Lenardon, M.D., Sood, P., Dorfmueller, H.C., Brown, A.J.P. & Gow, N.A.R. (2020) Scalar nanostructure of the *Candida albicans* cell wall; a molecular, cellular and ultrastructural analysis and interpretation. *The Cell Surface*, *6*, 100047. https://doi.org/10.1016/j.tcsw.2020.100047

Liu, Y., Solis, N.V., Heilmann, C.J., Phan, Q.T., Mitchell, A.P., Klis, F.M. et al (2014) Role of retrograde trafficking in stress response, host cell interactions, and virulence of *Candida albicans*. *Eukaryotic Cell*, *13*, 279–287. https://doi.org/10.1128/EC.00295-13

Love, M.I., Huber, W. & Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*, 550. https://doi.org/10.1186/s13059-014-0550-8

Lutfiyya, L.L. & Johnston, M. (1996) Two zinc-finger-containing repressors are responsible for glucose repression of *SUC2* expression. *Molecular and Cellular Biology*, *16*, 4790–4797. https://doi.org/10.1128/MCB.16.9.4790

Mottola, A. & Morschhäuser, J. (2019) An intragenic recombination event generates a Snf4-independent form of the essential protein kinase Snf1 in *Candida albicans*. *mSphere*, *4*, e00352-19.

Mottola, A., Schwanfelder, S. & Morschhäuser, J. (2020) Generation of viable *Candida albicans* mutants lacking the "essential" protein kinase Snf1 by inducible gene deletion. *mSphere*, *5*, e00805-20.

Navarro-Garcia, F., Alonso-Monge, R., Rico, H., Pla, J., Sentandreu, R. & Nombela, C. (1998) A role for the MAP kinase gene *MKC1* in cell wall construction and morphological transitions in *Candida albicans*. *Microbiology (Reading)*, *144*(Pt 2), 411–424. https://doi.org/10.1099/00221287-144-2-411

Nehlin, J.O. & Ronne, H. (1990) Yeast MIG1 repressor is related to the mammalian early growth response and Wilms' tumour finger proteins. *EMBO Journal*, *9*, 2891–2898. https://doi.org/10.1002/j.1460-2075.1990.tb07479.x

Ostling, J. & Ronne, H. (1998) Negative control of the Mig1p repressor by Snf1p-dependent phosphorylation in the absence of glucose. *European Journal of Biochemistry*, *252*, 162–168. https://doi.org/10.1046/j.1432-1327.1998.2520162.x

Petter, R., Chang, Y.C. & Kwon-Chung, K.J. (1997) A gene homologous to *Saccharomyces cerevisiae SNF1* appears to be essential for the viability of *Candida albicans*. *Infection and Immunity*, *65*, 4909–4917. https://doi.org/10.1128/IAI.65.12.4909-4917.1997

Pradhan, A., Avelar, G.M., Bain, J.M., Childers, D.S., Larcombe, D.E., Netea, M.G., Shekhova, E., Munro, C.A., Brown, G.D., Erwig, L.P., Gow, N.A.R. & Brown, A.J.P. (2018) Hypoxia promotes immune evasion by triggering β-glucan masking on the *Candida albicans* cell surface via mitochondrial and cAMP-protein kinase A signaling. *mBio*, *9*, e01318-18.

Ramírez-Zavala, B., Mottola, A., Haubenreißer, J., Schneider, S., Allert, S., Brunke, S. et al (2017) The Snf1-activating kinase Sak1 is a key regulator of metabolic adaptation and *in vivo* fitness of *Candida albicans*. *Molecular Microbiology*, *104*, 989–1007.

Ramírez-Zavala, B., Reuß, O., Park, Y.-N., Ohlsen, K. & Morschhäuser, J. (2008) Environmental induction of white-opaque switching in *Candida albicans*. *PLoS Pathogens*, *4*, e1000089. https://doi.org/10.1371/journal.ppat.1000089

Rauceo, J.M., Blankenship, J.R., Fanning, S., Hamaker, J.J., Deneault, J.S., Smith, F.J. et al (2008) Regulation of the *Candida albicans* cell wall damage response by transcription factor Sko1 and PAS kinase Psk1. *Molecular Biology of the Cell*, *19*, 2741–2751.

Reuß, O., Vik, Å., Kolter, R. & Morschhäuser, J. (2004) The *SAT1* flipper, an optimized tool for gene disruption in *Candida albicans*. *Gene*, *341*, 119–127. https://doi.org/10.1016/j.gene.2004.06.021

Roman, E., Cottier, F., Ernst, J.F. & Pla, J. (2009) Msb2 signaling mucin controls activation of Cek1 mitogen-activated protein kinase in *Candida albicans*. *Eukaryotic Cell*, *8*, 1235–1249. https://doi.org/10.1128/EC.00081-09

Schillig, R. & Morschhäuser, J. (2013) Analysis of a fungus-specific transcription factor family, the *Candida albicans* zinc cluster proteins, by artificial activation. *Molecular Microbiology*, *89*, 1003–1017.

Schubert, S., Popp, C., Rogers, P.D. & Morschhäuser, J. (2011) Functional dissection of a *Candida albicans* zinc cluster transcription factor, the multidrug resistance regulator Mrr1. *Eukaryotic Cell*, *10*, 1110–1121. https://doi.org/10.1128/EC.05100-11

Song, Y., Cheon, S.A., Lee, K.E., Lee, S.Y., Lee, B.K., Oh, D.B. et al (2008) Role of the RAM network in cell polarity and hyphal morphogenesis in *Candida albicans*. *Molecular Biology of the Cell*, *19*, 5456–5477.

Vinces, M.D., Haas, C. & Kumamoto, C.A. (2006) Expression of the *Candida albicans* morphogenesis regulator gene *CZF1* and its regulation by Efg1p and Czf1p. *Eukaryotic Cell*, *5*, 825–835. https://doi.org/10.1128/EC.5.5.825-835.2006

Vinces, M.D. & Kumamoto, C.A. (2007) The morphogenetic regulator Czf1p is a DNA-binding protein that regulates white opaque switching in *Candida albicans*. *Microbiology*, *153*, 2877–2884. https://doi.org/10.1099/mic.0.2007/005983-0

Willger, S.D., Liu, Z., Olarte, R.A., Adamo, M.E., Stajich, J.E., Myers, L.C. et al (2015) Analysis of the *Candida albicans* phosphoproteome. *Eukaryotic Cell*, *14*, 474–485. https://doi.org/10.1128/EC.00011-15

Zaragoza, O., Rodriguez, C. & Gancedo, C. (2000) Isolation of the *MIG1* gene from *Candida albicans* and effects of its disruption on catabolite repression. *Journal of Bacteriology*, *182*, 320–326. https://doi.org/10.1128/JB.182.2.320-326.2000

Zordan, R.E., Miller, M.G., Galgoczy, D.J., Tuch, B.B. & Johnson, A.D. (2007) Interlocking transcriptional feedback loops control white-opaque switching in *Candida albicans*. *PLoS Biology*, *5*, e256. https://doi.org/10.1371/journal.pbio.0050256

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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