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Loss of Gst1 enhances resistance to MMS by reprogramming the transcription of DNA damage response genes in a Rad53-dependent manner in *Candida albicans*

Huaxin Cai[1†], Yuting Feng[1,2†], Jia Wang[1], Zhenyu Cao[1], Rui Lv[1] and Jinrong Feng[1*]

Abstract

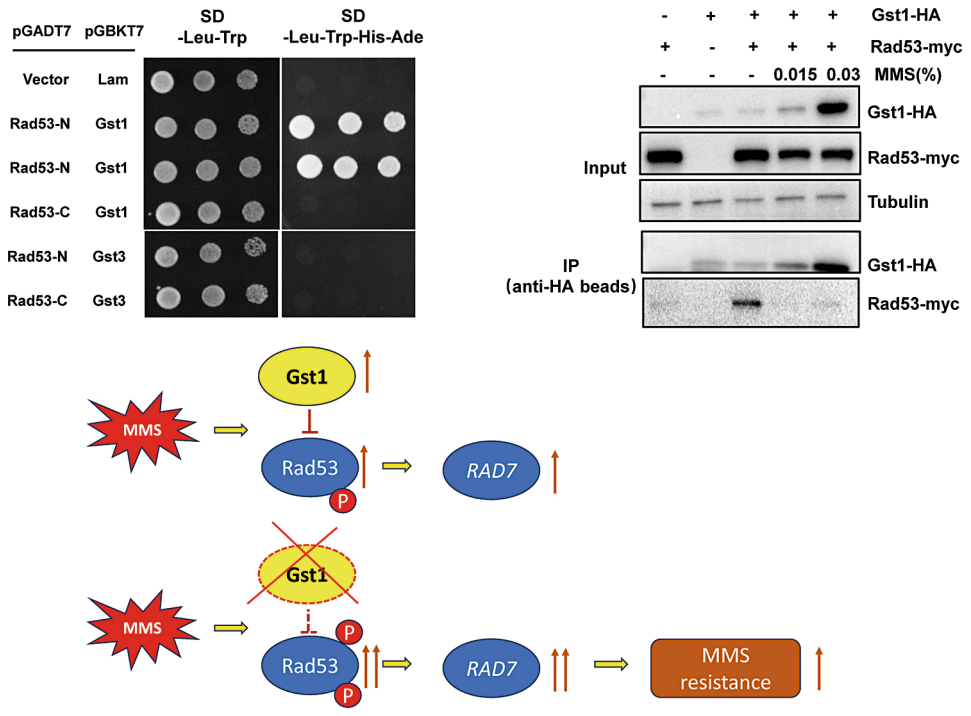
The DNA damage response is a highly conserved protective mechanism that enables cells to cope with various lesions in the genome. Extensive studies across different eukaryotic cells have identified the crucial roles played by components required for response to DNA damage. When compared to the essential signal transducers and repair factors in the DNA damage response circuitry, the negative regulators and underlying mechanisms of this circuitry have been relatively under-examined. In this study, we investigated Gst1, a putative glutathione transferase in the fungal pathogen *Candida albicans*. We found that under stress caused by the DNA damage agent MMS, *GST1* expression was significantly upregulated, and this upregulation was further enhanced by the loss of the checkpoint kinases and DNA repair factors. Somewhat counterintuitively, deletion of *GST1* conferred increased resistance to MMS, potentially via enhancing the phosphorylation of Rad53. Furthermore, overexpression of *RAD53* or deletion of *GST1* resulted in upregulated transcription of DNA damage repair genes, including *CAS1*, *RAD7*, and *RAD30*, while repression of *RAD7* transcription in the *GST1* deletion reversed the strain's heightened resistance to MMS. Finally, Gst1 physically interacted with Rad53, and their interaction weakened in response to MMS-induced stress. Overall, our findings suggest a negative regulatory role for *GST1* in DNA damage response in *C. albicans*, and position Gst1 within the Rad53-mediated signaling pathway. These findings hold significant implications for understanding the mechanisms underlying the DNA damage response in this fungal pathogen and supply new potential targets for therapeutic intervention.

†Huaxin Cai and Yuting Feng contributed equally to this work.

*Correspondence:
Jinrong Feng
jinrong532@163.com

Full list of author information is available at the end of the article

Graphical Abstract



Keywords *Candida albicans*, DNA damage response, Glutathione transferase, Rad53, Gst1

Introduction

Glutathione transferases (GSTs) are a crucial group of multifunctional enzymes that play a vital role in detoxifying exogenous chemicals resulting from environmental pollutants [1, 2]. GSTs catalyze the conjugation of glutathione (GSH) to electrophilic substrates, leading to the formation of less reactive and more soluble compounds [3, 4], and thereby facilitating their removal from cells through membrane-based glutathione conjugate pumps [5]. Soluble cytoplasmic glutathione transferases, commonly referred to as prototypical GSTs, play a crucial role in shielding cells from oxidative stresses and facilitating detoxification mechanisms. These enzymes exist in aerobic organisms as either homologous or heterodimeric entities.

Because of the crucial roles of GSTs in antioxidation and detoxification across plants, microorganisms, and mammals, it is common for GST-encoding genes to exhibit significant induction in cells exposed to oxidizing agents. In the bacterium *Proteus mirabilis*, exposure to various oxidative stresses led to increased transcription of glutathione S-transferase B1-1 (PmGST B1-1) [6]; the null mutant of PmGST B1-1 exhibited heightened sensitivity to oxidative stress and several antimicrobial drugs, highlighting its essential roles in protecting against oxidative stress and detoxifying antimicrobial agents [6]. Furthermore, three genes encoding glutathione S-transferases (*gst1*$^+$, *gst2*$^+$, and *gst3*$^+$) in *Schizosaccharomyces pombe* are all induced by hydrogen peroxide, while their deletion mutants display increased sensitivity to fluconazole, suggesting the potential involvement of GSTs in antifungal drug detoxification in fungi [7]. Based on their biological role as antioxidants in humans, GSTs are potentially implicated in the pathogenesis and progression of various diseases, including asthma, basal cell carcinoma, and different types of cancers [8–11].

GST-encoding genes can be induced by various stresses [12], including DNA damage. Methyl methanesulfonate (MMS) is a common mutagenic agent which alters guanine (to 7-methylguanine) and adenine (to 3-methlyladenine), leading to base mispairing and replication blocks, respectively [13]. In *S. cerevisiae*, Caba et al. reported a dramatic 84-fold increase in the transcription of *GTT2*, which encodes a glutathione S-transferase, following exposure to 0.12% MMS for 120 min compared to that in the untreated group. Moreover, under the same condition, there was also a substantial induction of the endoplasmic reticulum GST gene *GTT1* [14]. Similarly, Jelinsky et al. reported the induction of *GTT1*, *GTT2*, and *GDT1* following MMS treatment [15]. However, no direct involvement of these GST-encoding genes has been found in DNA damage response. In the plant pathogenic fungus *Fusarium oxysporum*, 6 GST genes, including *FOXG_01307*, *FOXG_01536*, *FOXG_11476*, and

FOXG_14789, were found to be inducible by MMS [16]. In the fungal pathogen *Candida albicans*, we demonstrated that 3 glutathione S-transferase-encoding genes, *GST1*, *GST2*, and *GST3*, were induced by MMS, with *GST1* showing the most significant upregulation [17]. Overall, the DNA-damage-stress-based upregulation of GST-encoding genes across these model organisms suggests a conserved response pattern.

The MMS-induced upregulation of GSTs in these organisms may indicate a role in the DNA damage response. In HEK-293T cells, the glutathione S-transferase Pi (GSTpi) provides support for this hypothesis, as overexpression of GSTpi resulted in a reduction in DNA damage following treatment with MMS or adriamycin (ADR). Furthermore, upon DNA damage, the phosphorylation of GSTpi at Ser184 enhances NBS1 nuclear translocation, activating the ATM-Chk2-p53 signaling cascade and inducing cell cycle arrest at the G2/M phase to allow more time for DNA damage repair [18]. Additionally, an investigation involving 388 healthy adult volunteers revealed a correlation between GST activity and oxidized bases as well as base excision repair (BER) capacity. Notably, among those exposed to asbestos and the corresponding reference group, those with a homozygous GSTT1 deletion exhibited significantly lower BER rates, suggesting that GST polymorphisms and activity may influence DNA stability and repair mechanisms for oxidized bases [19].

Currently in fungi, the mechanisms underlying the induction of GST genes by DNA damage stress and their potential connection to DNA damage repair remain poorly understood. Given the significant upregulation of *GST1* in *C. albicans* upon exposure to MMS, we focused on exploring its function in the DNA damage response. We observed that deleting *GST1* confers increased resistance to MMS, a surprising result given the transcriptional activation of *GST1* in response to MMS exposure. This suggests that the relationship between MMS treatment and glutathione S transferase function in *C. albicans* is complex, and we investigated this relationship in depth to clarify our findings. This research provides significant insights into the role of Gst1 in Rad53-mediated DNA damage response in *C. albicans* and supplies potential targets for therapeutic intervention in this fungal pathogen.

Materials and methods

Strains, media, and reagents

C. albicans strains were cultured in YPD media supplemented with 50 mg of uridine per liter, as previously described [20]. The *C. albicans* cells were inoculated into liquid YPD media and cultured on an orbital shaker (200–220 rpm) at 30 °C. The plates with *C. albicans* cells were kept in an incubator at 30 °C. The strains and primers used in this study are listed in Tables S1 and S2, respectively. MMS was purchased from Sigma (USA). The reagents and amino acids used for the media were purchased from Sangon (China). The solid media contained 2% agar.

DNA manipulation

To construct a *GST1* deletion strain, both alleles of *GST1* in the *C. albicans* strain BWP17 were replaced with a *HIS1* marker using a transient CRISPR/Cas9 system [17]. The correct knockout strain for *GST1* was confirmed by PCR using primers GST1-Te-F and GST1-Te-R. Similarly, the CRISPR/Cas9 system was used to delete *GST1* in homozygous single gene deletion strains for *RAD18*, *RAD14*, *RAD53*, and *PPH3* to create double gene deletions.

To overexpress the *GST1* gene, the ORF and terminator of *GST1* were amplified by PCR and cloned into the *Kpn* I site in the CIP10-ADH1 plasmid [17], generating CIP10-ADH1-GST1. Subsequently, CIP10-ADH1-GST1 was linearized by *Stu* I to direct integration at RP10 and transformed into the BWP17 strain, selecting on SD-Ura plates to generate a *GST1*-overexpression strain.

To determine the expression of *GST1*, a DNA fragment containing an HA tag was amplified from the pFA-HA-URA3 plasmid and integrated downstream of the open reading frame (ORF) of the *GST1* gene in the wild-type strain, as well as the *RAD53* deletion strain and *DUN1* deletion strain. In addition, a GFP tag was integrated downstream of the ORF of the *GST1* gene in the wild-type strain to determine the intracellular localization of Gst1.

To suppress the transcription of *RAD7*, *CAS1*, *RAD16*, *NTG1* and *RAD30* in the *GST1* deletion strain, a dCas9-mediated interference system was utilized. In brief, the interference plasmid for the specific gene was generated by amplifying the pRS159 plasmid [21] with a pair of primers containing the sgRNA sequence, generating a series of pRS159-sgRNA plasmids. Subsequently, the interference plasmid carrying sgRNA was linearized with *Pac* I and transformed into the wild-type strain and the *GST1* deletion strain. Transformants were selected on YPD plates supplemented with 200 μg/ml nourseothricin and used for the spot assay.

To confirm the effect of decreasing *RAD7* in the *GST1* deletion strain, a *MET3* promoter was also employed to repress the transcription of *RAD7*. In general, the endogenous promoter of *RAD7* was replaced by a *MET3* promoter using a transient CRISPR/Cas9 system as previously described [17]. The repair DNA, containing the *MET3* promoter, was amplified from the pFA-MET3-ARG4 plasmid by PCR and transformed into the wild-type strain and the *GST1* deletion strain together with the Cas9 gene and sgRNA. Transformants were selected and purified on SD-Arg plates. Similarly, *RAD52* was repressed by the *MET3* promoter in the wild-type strain

and the *GST1* deletion strain. To repress the transcription of MET3p-driven *RAD7* and *RAD52*, 10 mM methionine (Met) and cysteine (Cys) were added to liquid YPD overnight culture.

Real-time PCR (RT–qPCR)

The overnight cells were diluted in fresh YPD at a ratio of 1/10 and treated with MMS for 90 min. Total RNA was extracted using an RNA-easy isolation reagent from Vazyme, China (R701), and cDNA was synthesized using a cDNA synthesis kit from Vazyme, China (R212) containing DNase for removing residual DNA from the template. RT−qPCR was performed using ChamQ SYBR qPCR Master Mix (Vazyme, China) according to the manufacturer's protocol. The specific primers used for the target genes and the GAPDH primers used as controls were listed in Table S2. The transcription level of a specific gene to GAPDH in the control group was normalized as 1. The different transcription of selected genes in treated and control groups was compared using a paired *t* test by GraphPad Prism 8.0.1 software. The transcription data for each gene were averaged from at least three independent experiments.

Protein extraction and Western blot analysis

To assess the expression of Gst1, a strain carrying an HA tag at the C-terminus of Gst1 was used [22]. The log phase cells were treated with MMS for 90 min before harvesting. Protein extraction was carried out as previously described, utilizing cell lysis buffer supplemented with protease inhibitors from Beyotime, China (P0013), and 10 mM phenylmethanesulfonyl fluoride (PMSF) was added before use. Protein concentrations were determined using a Bradford protein assay kit from Generway Biotech, China (GK5011). Western blot analysis was carried out as previously described [22]. To detect the expression of Gst1-HA or Rad53-HA, a rabbit monoclonal anti-HA antibody (Thermo, USA; 1:2000) and a goat anti-rabbit IgG secondary antibody (Proteintech, China;1:2500) were used. To assess the phosphorylation of H2A, a histone H2A (phospho S129) antibody (Abcam, UK; 1:2500) was used.

RNA preparation and RNA-seq assay

Two colonies of the *C. albicans* wild-type BWP17 strain, the *GST1* deletion strain, and the *GST1* overexpression strain were inoculated into 3 mL of liquid YPD and incubated overnight at 30 °C on a shaker at 200 rpm. The overnight cultures were diluted to an OD600 of 0.1 in 10 mL of YPD media and grown to an OD600 of approximately 0.8 at 30 °C with shaking. Subsequently, the cells were treated with 0.015% MMS for 90 min before being harvested for RNA extraction. Total RNA was extracted using a TRIzol reagent kit (Invitrogen, USA) according to the manufacturer's protocol. RNA quality was assessed on an Agilent 2100 Bioanalyzer (Agilent Technologies, USA) and checked using RNase-free agarose gel electrophoresis. RNA library sequencing was performed on the Illumina NovaSeq 6000 platform by Gene Denovo Biotechnology Co., Ltd. (Guangzhou, China). *C. albicans* SC5314 downloaded from NCBI (https://www.ncbi.nlm.nih.gov/genome/21?genome_assembly_id=294796) was used as a reference genome. The raw data have been deposited in the NGDC GSA database (CRA014118).

The analysis of RNA differential expression was conducted using the DESeq2 software to compare two distinct groups. Transcripts meeting the criteria of a false discovery rate (FDR) below 0.05 and an absolute fold change of ≥2 were identified as differentially expressed transcripts (DEGs). Gene Ontology (GO) enrichment analysis identified GO terms significantly enriched in DEGs compared to the genomic background, focusing on DEGs associated with specific biological functions. Subsequently, all DEGs were mapped to GO terms in the Gene Ontology database (http://www.geneontology.org/). The number of genes per term was computed, and enriched GO terms in DEGs relative to the genomic background were determined using a hypergeometric test. The resulting p-values underwent FDR correction, with significance defined at FDR≤0.05. GO terms meeting this criterion were classified as significantly enriched GO terms.

Yeast two-hybrid assay

For the yeast two-hybrid assay, the ORFs of *GST1* and *GST3* were amplified and cloned into the *Eco*R I and *Xho* I sites in the pGBKT7 plasmid using a one-step cloning kit (Yeasen, China). The pGADT7 plasmid, harboring either the N-terminal or C-terminal region of the Rad53 protein from a previous investigation, was employed [23]. Prey and bait plasmids were co-transformed into the *S. cerevisiae* strain AH109 as previously described [23]. Transformants were selected on SC-Leu-Trp plates and tested on SC-Leu-Trp-Ade-His plates. The interaction between pGADT7 and pGBKT7-Lam served as the negative control in the experiment. The growth observed on SC-Leu-Trp-Ade-His plates indicated a positive interaction.

Coimmunoprecipitation (Co-IP)

For the Co-IP assay, Rad53 was tagged with a myc tag at its C-terminus in the wild-type strain following previously described methods. The wild-type strain carrying the Gst1-HA construct or both the Gst1-HA and Rad53-myc constructs was incubated overnight and transferred to fresh YPD media for 3–4 h. Subsequently, the cells were treated with 0.015% or 0.03% MMS for 120 min prior to being harvested for total protein extraction. In

general, approximately 5 mg of protein extract was incubated with 15 µL of anti-HA magic beads (MCE, China) in 750 µL of extraction buffer at 4 °C overnight. The beads were washed three times using 1 mL of washing buffer (50 mM Tris-HCl, 150 mM NaCl, 0.5% Tween-20, pH 7.4), followed by elution of proteins through incubation at 100 °C for 10 min in protein loading buffer. Finally, immunoblot analysis was performed using an anti-HA antibody (Abclonal, China;1:2000) to detect Gst1-HA or an anti-myc antibody (Thermo, USA;1:2000) to detect Rad53-myc.

Results

MMS induced the expression of *GST1* in *C. albicans*

In a previous study [17], we obtained two sets of transcriptional data during the response to DNA damage stress in *C. albicans* and observed the upregulation of glutathione S transferase-encoding genes (including *GST1*, *GST2*, and *GST3*), indicating potential roles in the DNA damage response. Notably, a significant upregulation of *GST1* was observed, prompting us to focus on this gene and further investigate its potential association with the DNA damage response. Utilizing RT-qPCR analysis, we verified a dose-dependent enhancement in the transcription levels of *GST1* following MMS exposure, with a 2.8-fold increase at 0.005% MMS, a 7.7-fold enhancement at 0.01% MMS, and an 11-fold upregulation at 0.015% MMS concentration (Fig. 1A). The expression of *GST1* was further validated through Western blot analysis; under normal conditions, only minimal basal levels of Gst1 were observed, but exposure to a low concentration of MMS (0.015%) resulted in a notable elevation in protein abundance (Fig. 1B, Up panel). In addition, a high MMS dose (0.03%) amplified the Gst1 protein level even further (Fig. 1B, Up panel). Moreover, the short exposure to a high dose of MMS (0.03%) resulted in a significant increase in the protein level of Gst1 (Fig. S1). Notably, treatment with H_2O_2, but not NaCl, fluconazole, or rapamycin, induced an upregulation of Gst1 expression in *C. albicans* (Fig. 1B, down panel). In order to investigate the relationship between MMS-induced Gst1 expression and oxidative stress, we employed N-acetylcysteine to mitigate the potential oxidative stress induced by MMS, and assessed the expression of Gst1. Our results revealed that treatment with N-acetylcysteine only marginally attenuated the expression of Gst1 (Fig. S2A), suggesting the upregulation of Gst1 was primarily DNA damage-associated.

The DNA damage checkpoint kinases Rad53 and Dun1 play a crucial role in coordinating DNA damage response. Therefore, we examined the transcription levels of *GST1* following the deletion of checkpoint genes in *C. albicans* using RT-qPCR analysis. Intriguingly, deletion of *RAD53* or *DUN1* resulted in a 3.4 or 3.3-fold increase in *GST1* transcription levels in the absence of MMS (Fig. 1C). Upon exposure to MMS stress, deletion of *DUN1* notably heightened the transcription level of *GST1* (Fig. 1C, left panel). A comprehensive assessment of Gst1 protein levels was then performed in strains lacking *RAD53* or *DUN1*. Under normal conditions, Gst1-HA expression was virtually imperceptible; however, Gst1-HA expression increased upon deletion of either *RAD53* or *DUN1* (Fig. 1C, right panel). Under MMS stress conditions, the Gst1 level increased in the wild-type strain and in the strains lacking checkpoint kinases. However, the protein levels of Gst1 exhibited a notable increase in strains lacking *RAD53* and *DUN1* compared to the level in the wild-type strain, in response to low (0.015%) or high dose (0.03%) of MMS (Fig. 1C, right panel).

Given the notable increase resulting from the removal of checkpoint kinases, we proceeded to examine the transcription of *GST1* in strains lacking *RAD52*, *MMS22*, and *RAD18*, which are involved in DNA damage repair. Consistently, the transcription of *GST1* was upregulated upon the loss of Rad52, a key player in homologous recombination repair, moreover, under MMS stress conditions, the transcription of *GST1* was further increased in the *RAD52* deletion strain compared with the wild-type strain (Fig. 1D). Similar upregulation of *GST1* was observed in the *MMS22* deletion strain. Collectively, exposure to MMS or defects in DNA damage repair induce the expression of Gst1, hinting at Gst1's role as a signal transducer in DNA damage response in *C. albicans*.

Deletion of *GST1* conferred increased resistance to MMS in *C. albicans*

The MMS-induced expression of *GST1* suggested a potential involvement of *GST1* in the DNA damage response. To investigate a possible role of *GST1* in the DNA damage response in *C. albicans*, we deleted *GST1* and assessed the phenotype under MMS stress. Deletion of *GST1* unexpectedly increased cellular resistance to MMS stress (Fig. 2A). Reintroduction of an ectopic *GST1* gene nearly decreased MMS resistance to the level of the wild-type strain. Additionally, the *GST1* deletion strain exhibited no discernible response to H_2O_2 or other stresses, such as UV (Fig. 2A). We also used N-acetylcysteine to inhibit the potential oxidative stress triggered by MMS, and evaluated the phenotype post *GST1* deletion. Our findings demonstrated that the administration of N-acetylcysteine minimally reduced the heightened resistance resulting from *GST1* deletion (Fig. S2B), highlighting the involvement of Gst1 in DNA damage response. To monitor the enhanced resistance to MMS conferred by deleting *GST1*, we assessed the survival of the *GST1* mutant under MMS stress conditions. With 0.05% MMS, the WT strain exhibited an average survival of 27%,

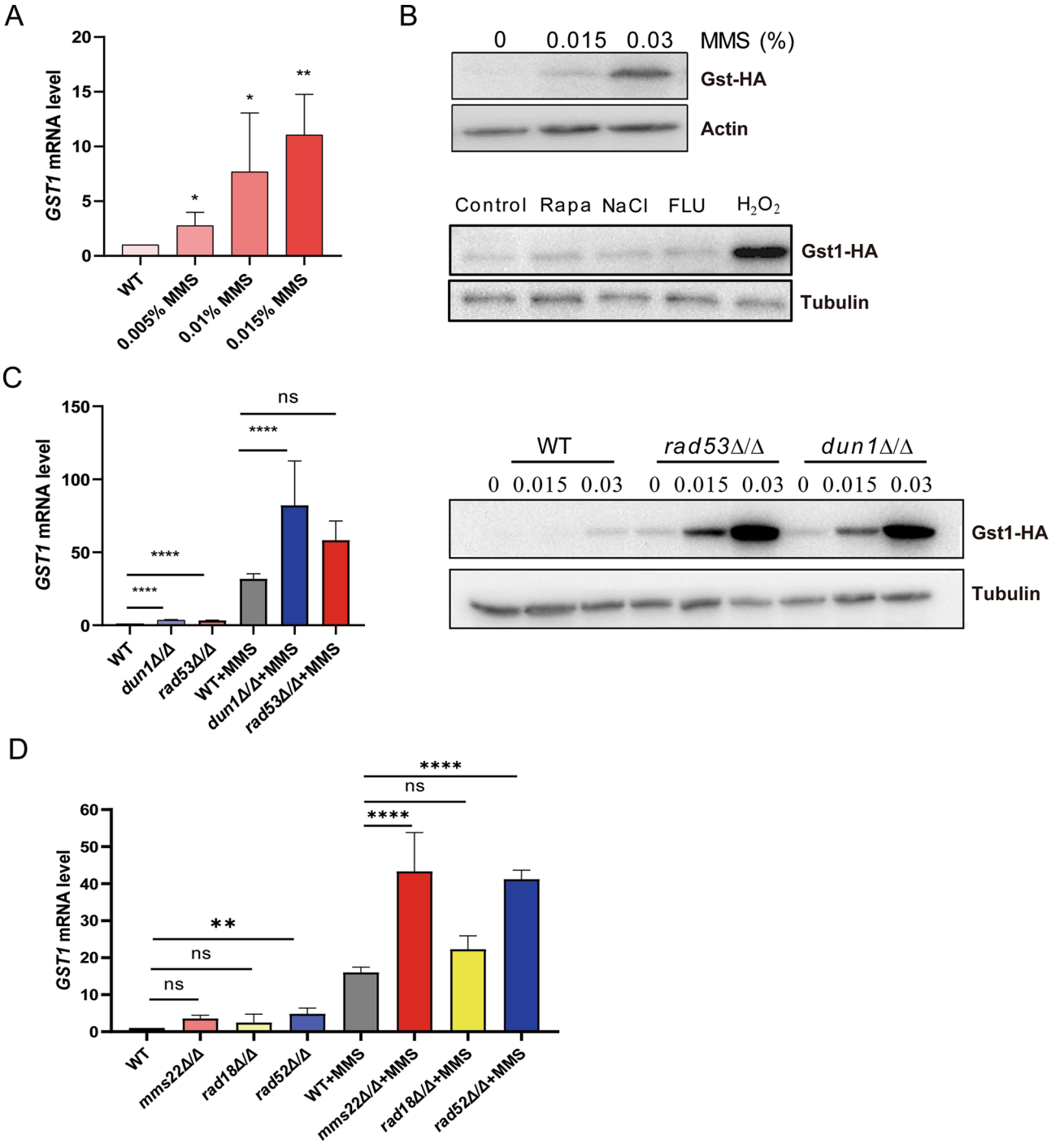


Fig. 1 The expression of *GST1* in *C. albicans* was stimulated by exposure to MMS-induced stress or the deletion of DNA damage response genes. (**A**) The transcription of *GST1* in response to MMS treatment was examined by RT-qPCR. The transcription of *GST1* in the wild-type strain with MMS treatment was compared to that without MMS stress conditions, using a paired *t* test with GraphPad Prism 8.0.1 software. * represents $p < 0.05$, ** represents $p < 0.01$. (**B**) The protein level of Gst1 was assessed using Western blot. Wild-type cells harboring the Gst1-HA fusion were treated with 0.015% or 0.03% MMS (up panel), 3 mM H_2O_2, 1.5 M NaCl, 10 nM rapamycin (Rapa), or 5 μg/ml fluconazole (Flu) (down panel) for 90 min. (**C**) The expression of *GST1* in the *RAD53* or *DUN1* deletion strains was examined by RT-qPCR and Western blot. For RT-qPCR assay, *C. albicans* cells were treated with 0.015% MMS for 90 min. For Western blot, log phase cells were treated with 0.015% or 0.03% MMS for 90 min. Untreated cells served as the control group. (**D**) The relative transcription of *GST1* in the wild-type strain (SN148) and the strains lacking *RAD52*, *RAD18*, and *MMS22* was measured using RT–qPCR. Log phase cells were treated with 0.02% MMS for 90 min. The transcription of *GST1* in the deletion strains was compared to that in the WT group under normal or MMS stress conditions, using one-way ANOVA/Tukey tests with GraphPad Prism 8.0.1 software. ** represents $p < 0.01$ and **** represents $p < 0.0001$. NS represents no significant difference

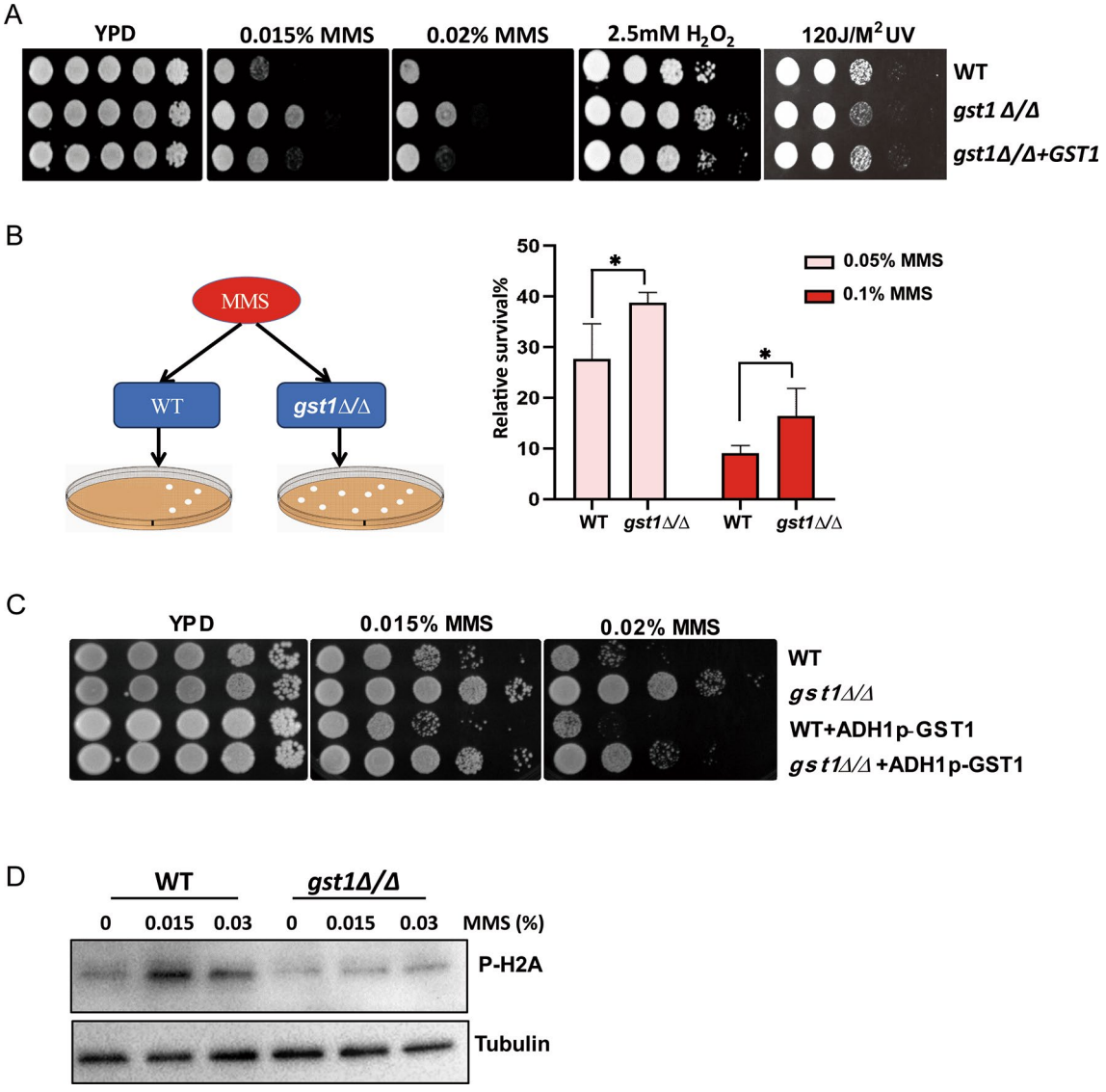


Fig. 2 Deletion of *GST1* increased resistance to MMS in *C. albicans*. (**A**) Phenotypic assay of the *GST1* deletion strain. The wild-type strain, the *GST1* deletion strain, and the complemented strain containing linearized CIP10-GST1 were utilized. (**B**) Survival assay of the *GST1* deletion strain under MMS stress. Differences between groups were compared using the paired *t* test with GraphPad Prism 8.0.1 software. * represents $p < 0.05$. (**C**) Phenotypic assay of the *GST1*-overexpressing strain. The wild-type and *GST1* deletion strains transformed with the ADH1p-driven *GST1* construct were used for the spot assay, and their parental strains were utilized as controls. (**D**) The phosphorylation of histone H2A after deleting *GST1* was checked by Western blot. Log phase cells were treated with 0.015% or 0.03% MMS for 90 min. An H2A (S129) antibody was used to determine the phosphorylation level of histone H2A

while the *GST1* deletion strain displayed a significantly increased survival rate of 38% (Fig. 2B). Similarly, under 0.1% MMS stress, the survival of the *GST1* deletion strain was greater than that of the WT strain.

Given that the deletion of *GST1* conferred resistance to MMS, we investigated the impact of *GST1* overexpression on MMS resistance in *C. albicans*. An ADH1p-driven *GST1* construct was introduced into both the wild-type and the *GST1* deletion strain; overexpression of *GST1* resulted in a marginal reduction in MMS resistance in both strains (Fig. 2C). Notably, even with ADH1p-driven *GST1*, the *GST1* deletion strain still exhibited greater MMS resistance than the wild-type strain carrying ADH1p-driven *GST1*, providing further evidence supporting the role of Gst1 in mediating MMS resistance.

Histone H2AX phosphorylation is considered as a marker for DNA damage in a wide range of eukaryotic organisms [24, 25]. To investigate the impact of *GST1* deletion on the genome, we examined the phosphorylation status of histone H2A in *C. albicans*. Using an antibody specific for histone H2A (phospho S129), we observed an increase in histone H2A phosphorylation under MMS-induced stress, consistent with previous

findings [26]. However, in the *GST1* deletion strain, a reduced level of histone H2A phosphorylation was evident compared to that in the wild-type controls (Fig. 2D). Importantly, even under MMS stress conditions, there was no significant increase in histone H2A phosphorylation in the *GST1* deletion strain; instead, H2A phosphorylation in the *GST1* deletion strain remained significantly lower than that observed in the wild-type strain (Fig. 2D & Fig. S3). These observations suggest a decreased occurrence of DNA damage events in the absence of *GST1*.

Profiling the transcriptional changes affected by Gst1

To elucidate how Gst1 regulates cellular activities, particularly the DNA damage response, we conducted RNA-seq analysis on the *GST1* deletion strain. In total, 6120 transcripts were detected, with 187 transcripts showing significant changes according to a log2-fold cutoff of 1.0 (Fig. 3A, Table S3). Among these differentially expressed genes, 92 were upregulated, including *Orf19.5033*, encoding a potential ubiquitin-like modifier involved in autophagy; *IFA14*, encoding a putative LPF family protein; and *IFD6*, encoding an aldo-keto reductase (Fig. 3B). In general, deleting *GST1* did not cause dramatic changes in the upregulated genes; however, *Orf19.4476*, encoding a protein with an NADP-dependent oxidoreductase domain, showed the most significant upregulation (log2 = 2.78). In addition, 95 genes were downregulated, including *ASG7*, which encodes an α-cell-specific protein, and *CFL4*, which encodes a potential ferric reductase that exhibited strong downregulation (Fig. 3B). Moreover, two virulence-specific genes, *ECE1* and *HWP1*, were significantly downregulated, suggesting a potential involvement of Gst1 in the virulence of *C. albicans*.

To further determine the role of Gst1, we performed GO analysis of the DEGs after deleting *GST1* (Fig. 3C). Among the top 20 GO terms, several oxidation pathways were enriched, including oxidoreductase activity (GO:0016491), oxidoreductase activity acting on CH or CH2 groups with disulfide as acceptor (GO:0016728), D-threo-aldose 1-dehydrogenase activity (GO:0047834), oxidoreductase activity acting on NAD(P)H (GO:0016651), oxidoreductase activity acting on CH or CH2 groups (GO:0016725), methylenetetrahydrofolate dehydrogenase ($NADP^+$) activity (GO:0004488) and oxidoreductase activity acting on NAD(P)H, quinone or similar compound as acceptor (GO:0016655). In particular, 41 genes were enriched in oxidoreductase activity terms (Fig. 3D); *ADH2*, encoding an alcohol dehydrogenase, and *SOD1*, encoding a cytosolic copper- and zinc-containing superoxide dismutase, were significantly upregulated, while *CFL4*, encoding a potential ferric reductase; *AOX2*, encoding an oxidase; and *SOD3*, encoding a cytosolic manganese-containing superoxide dismutase, were significantly downregulated. The enrichment of oxidation-related GO terms suggested that Gst1 may play a role in antioxidation in *C. albicans*. In addition, several genes were enriched for oligopeptide transporter activity (GO:0015198), metal ion transmembrane transporter activity (GO:0046873), substrate-specific transporter activity (GO:0022892), copper ion transmembrane transporter activity (GO:0005375), ion transmembrane transporter activity (GO:0015075), transporter activity (GO:0005215) and transition metal ion transmembrane transporter activity (GO:0046915). Therefore, Gst1 may have extensive roles in transport. Moreover, differential transcription was observed for several genes involved in the DNA damage response; *DBP3*, *BMT9*, *Orf19.810*, *Orf19.3021*, and *Orf19.4522* exhibited upregulation (Fig. 3D), which is consistent with the enhanced resistance to MMS after *GST1* depletion.

Gst1 affected the transcription of DNA damage response genes in *C. albicans*

Compared with the deletion of *GST1*, the overexpression of *GST1* had the opposite effect on resistance to MMS, as evidenced by our phenotypic data. Therefore, we performed RNA-seq analysis to elucidate the impact of Gst1 on the DNA damage response in the presence of a *GST1* overexpression strain. Upon comparison of the transcriptional patterns between the *GST1* deletion strain and the *GST1* overexpression strain, a total of 881 genes displayed notable alterations, surpassing a log2-fold threshold of 1.0 (Fig. 4A, Table S4). Among these DEGs, 765 genes were upregulated; notably, the upregulated genes included *HAK1* and *BMT9*. In contrast, only 116 genes were downregulated, including *CFL1*, *ECE1* and *HWP1*.

Given the observed heightened resistance to MMS upon deletion of *GST1*, we were intrigued by alterations in genes involved in the DNA damage response. When compared to the *GST1* overexpression strain, a group of 20 genes involved in the DNA damage response showed increased transcription in response to MMS-induced stress. Notable members include *RAD7*, responsible for encoding a nucleotide excision repair factor; *RNA14*, contributing to DNA damage checkpoint signaling; and *NTG1*, involved in the synthesis of DNA repair glycosylases(Fig. 4B).

The transcription of the selected DNA damage response genes was evaluated using RT–qPCR. In the *GST1* deletion strain, the transcription of *RAD7* increased by 1.9-fold compared to that in the wild-type strain under non-MMS stress conditions. Under MMS stress conditions, the transcription of *RAD7* in the wild-type strain increased by 6.8-fold and further increased to 12.7-fold in the *GST1* deletion strain, demonstrating a significant difference compared to that in the wild-type strain (Fig. 4C). Similarly, *RAD16*, *RAD30*, *PSO2*, *CAS1*, *NTG1*, *RNA15*, and *RAD52* all showed significantly

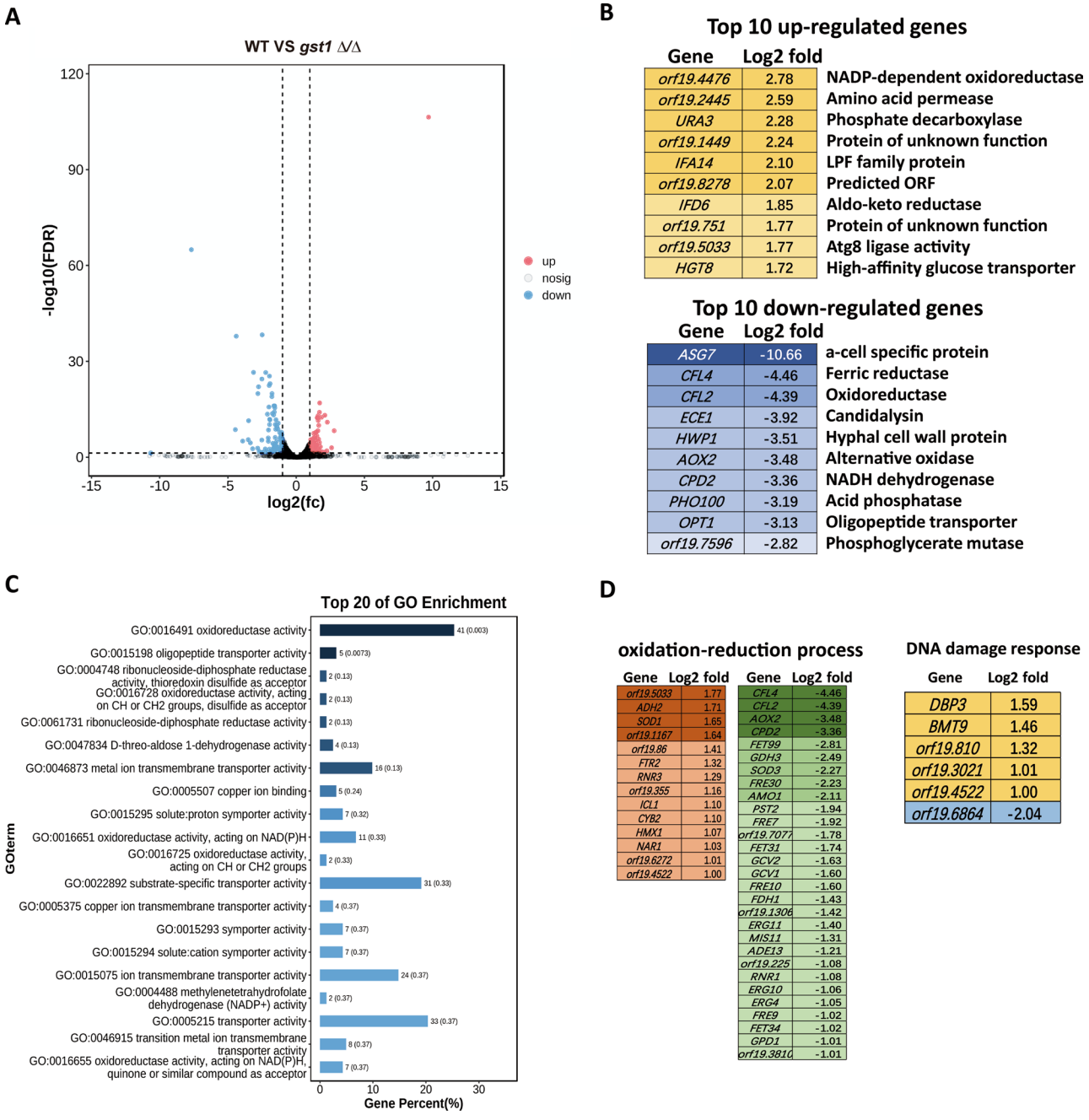
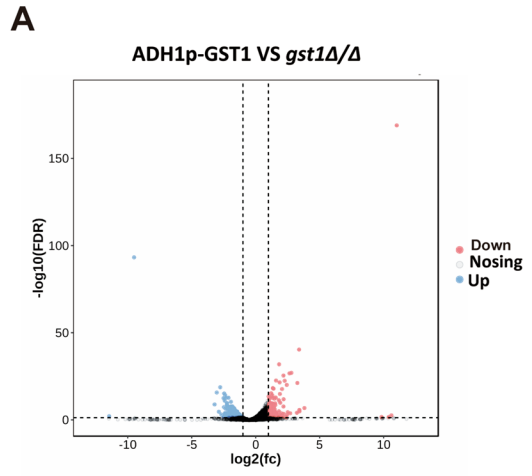


Fig. 3 Profiling transcriptional changes affected by deleting *GST1* in *C. albicans*. (**A**) Volcano plot of genes affected by deleting *GST1*. (**B**) The top 10 upregulated and downregulated genes after deleting *GST1*. (**C**) GO term analysis of genes showed significant differences by deleting *GST1*. (**D**) Oxidation-reduction and DNA damage response-related genes affected by Gst1. The log2-fold change in expression of each gene is shown

upregulated transcription upon deletion of *GST1* under both normal and MMS stress conditions (Fig. 4C). However, deletion of *GST1* led to an increase in the transcription levels of *RAD2* and *RAD24* only under normal conditions but not under MMS stress conditions. Additionally, we noted a reversal in the heightened transcription levels of *RAD7* and *RAD52* in a *GST1* complemented strain (Fig. S4). Therefore, deleting *GST1* may lead to transcriptional reprogramming and thus affect the expression patterns of *RAD7* and other DNA damage response genes in *C. albicans.*

RAD7 served as a potential downstream effector gene affected by Gst1 in the DNA damage response in *C. albicans*

In order to delve deeper into whether the increased resistance to MMS due to *GST1* deletion can be attributed to the upregulated expression of DNA damage response



B

Gene	Log2 fold	
RNA15	1.88	DNA damage checkpoint signaling
NTG1	1.59	DNA repair glycosylases
CAS1	1.49	Transcription factor
PSO2	1.41	Putative nuclease
SAE2	1.39	Double-strand break repair
RAD24	1.35	DNA damage checkpoint signaling
RTT101	1.32	Involved in response to DNA damage
TEL1	1.30	Protein serine/threonine kinase activity
RAD32	1.30	DNA repair
RAD16	1.25	Recognize and bind damaged DNA
RNA14	1.22	DNA damage checkpoint signaling
MAG1	1.15	DNA-3-methyladenine glycosylase activity
RAD2	1.15	Nucleotide excision repair nuclease
PAN2	1.12	Postreplication repair
CDC9	1.12	DNA ligase activity
HSM3	1.11	Mismatch repair
RAD7	1.11	Nucleotide Excision Repair Factor 4
AHC1	1.08	Histone acetyltransferase activity
DNA2	1.04	DNA replication factor involved in DNA repair
RAD52	1.02	Homologous DNA recombination

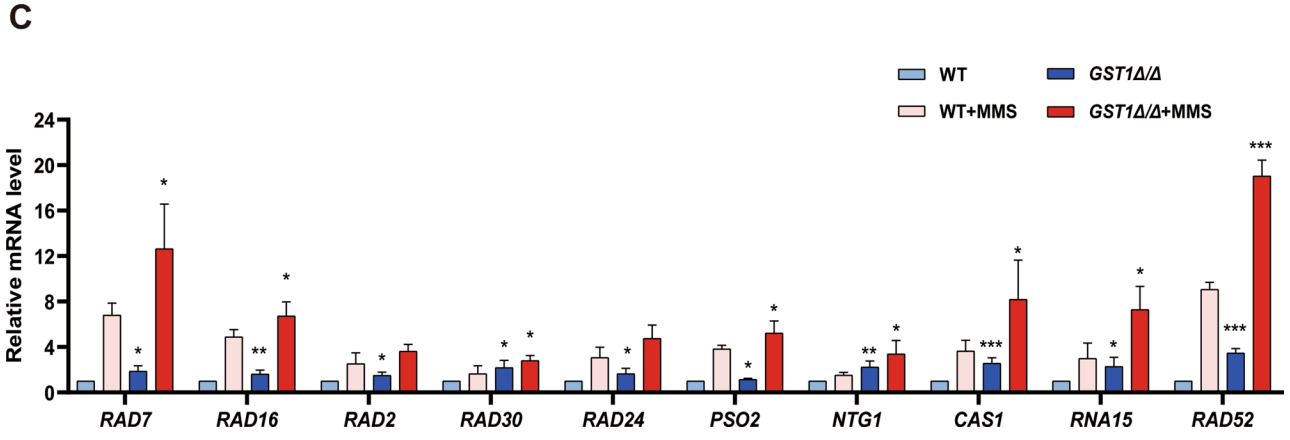


Fig. 4 Transcriptional changes in DNA damage response genes mediated by Gst1 in *C. albicans*. (**A**) Volcano plot of genes affected by the overexpression of *GST1*. (**B**) DNA damage response genes affected by deleting *GST1*. The transcription of the indicated genes in the *GST1* deletion strain was compared to that in the *GST1* overexpression strain. (**C**) DNA damage response genes whose transcription increased in the *GST1* deletion strain were checked by RT–qPCR. Log phase cells were treated with 0.02% MMS for 90 min. The levels of specific DDR genes in the *GST1* deletion strain were compared to those in the wild-type strain using a paired *t* test with GraphPad Prism 8.0.1 software. * represents $p < 0.05$, ** represents $p < 0.01$ and *** represents $p < 0.001$

(DDR) genes, we employed a dCas9-mediated gene interference system to suppress the transcription of selected DDR genes in the *GST1* deletion strain [21]. Suppression of *RAD16*, *RAD30*, *CAS1*, and *PSO2* in the *GST1* deletion strain did not result in a change to the MMS phenotype, but suppression of *RAD7* effectively decreased the MMS resistance caused by deleting *GST1* (Fig. 5A).

To further validate the role of Rad7 in mediating MMS resistance resulting from *GST1* deletion, we replaced its native promoter with a *MET3* promoter in both the wild-type and *GST1* deletion strains. Initially, we assessed the transcriptional level of *RAD7* and observed that upon the addition of 10 mM Met and Cys, its transcription significantly decreased to less than 1% of that in the untreated group (Fig. 5B). The cells were incubated in liquid YPD plus 10 mM Met and Cys and then spotted on YPD plates containing different concentrations of MMS. The *GST1* deletion strain still exhibited clear MMS resistance; however, when carrying the *MET3p-RAD7* construct, it displayed reduced MMS resistance similar to that of the wild-type strain (Fig. 5C). Nevertheless, the wild-type strain containing the *MET3p-RAD7* construct showed nearly no visible reduction in MMS resistance.

Recognizing the pivotal function of Rad52 in homologous recombination (HR), we proceeded to inhibit the transcription of *RAD52*, employing it as a control (Fig. 5B). In the wild-type strain, the repression of *RAD52* notably reduced resistance to MMS (Fig. 5C). Similarly, in the *GST1* deletion strain, the repression of *RAD52* lowered its MMS resistance, although it remained above that of the wild-type strain (Fig. 5C). Consequently, although the suppression of *RAD52* resulted in an overall decline in resistance to MMS, *RAD7* might serve as

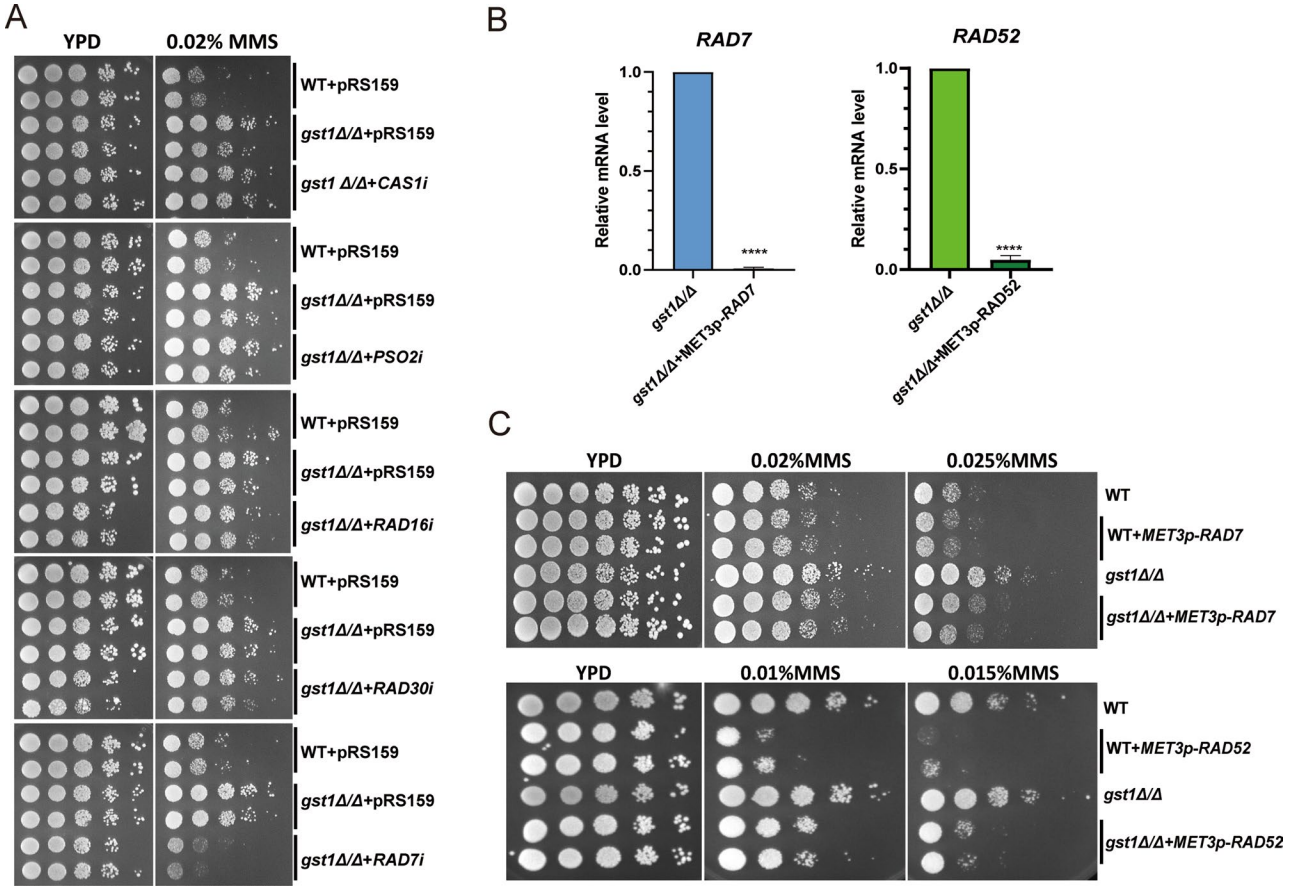


Fig. 5 The role of Rad7 in the Gst1-mediated DNA damage response in *C. albicans*. (**A**) Phenotypic assay of the *GST1* deletion strain carrying interference plasmids. A dCas9 interference system was used to suppress the transcription of *RAD7*, *RAD16*, *RAD30*, *CAS1*, and *PSO2*. (**B**) The transcription level of *RAD7* and *RAD52* was assessed by RT–qPCR. Gene repression was conducted by using the *MET3* promoter-mediated gene suppression system. **** represents $p < 0.0001$. (**C**) Phenotypic assay of the *GST1* deletion strain after interference with the transcription of *RAD7* and *RAD52*. The indicated strains were cultured in liquid YPD media supplemented with 10 mM Met and Cys before being harvested for RNA extraction or spot assays

a critical downstream effector gene for modulating the DNA damage response mediated by deleting *GST1.*

The increased resistance to MMS post-deleting *GST1* implicated the participation of Rad53

Deletion of *GST1* increased the resistance to MMS and induced the transcription of DNA damage response genes; thus, we investigated whether it directly or indirectly governs the DNA damage response. Initially, we examined the subcellular localization of Gst1 by integrating a GFP tag into Gst1. Under normal conditions, the Gst1-Gfp fusion protein distributed throughout the entire cell; similarly, under MMS-induced stress conditions, no discernible nuclear translocation of the Gst1-Gfp fusion protein was detected (Fig. 6A). Therefore, Gst1 may indirectly regulate the transcription of DNA damage response genes.

To investigate the specific contribution of Gst1 to the DNA damage response, we tested a number of genetic interactions based on several DNA damage repair genes. Deletion of *RAD14*, or *RAD18* resulted in heightened sensitivity to MMS, while the additional deletion of *GST1* failed to rescue MMS sensitivity, although deletion of the *GST1* single gene in the wild-type increased MMS resistance (Fig. 6B). Rad53 and Pph3 were chosen to represent the DNA damage response signaling pathway [27]. The deletion of *PPH3* caused strong MMS sensitivity, while the double deletion with *GST1* showed similar MMS sensitivity to that of the *PPH3* deletion strain (Fig. 6B). Additionally, deletion of *RAD53* led to pronounced sensitivity to MMS, but the double deletion of *RAD53* with *GST1* further augmented susceptibility to MMS (Fig. 6B). Therefore, the participation of Rad53 may be essential for the heightened resistance to MMS observed upon *GST1* deletion.

Considering that our genetic epistatic assay assigned *GST1* to a Rad53-related pattern, we investigated whether it modulates the transcription of potential targets in a checkpoint-related way. To assess the impact of *GST1* deletion on Rad53, we conducted Western blot analysis and observed that Rad53 phosphorylation was induced by MMS treatment in the wild-type strain

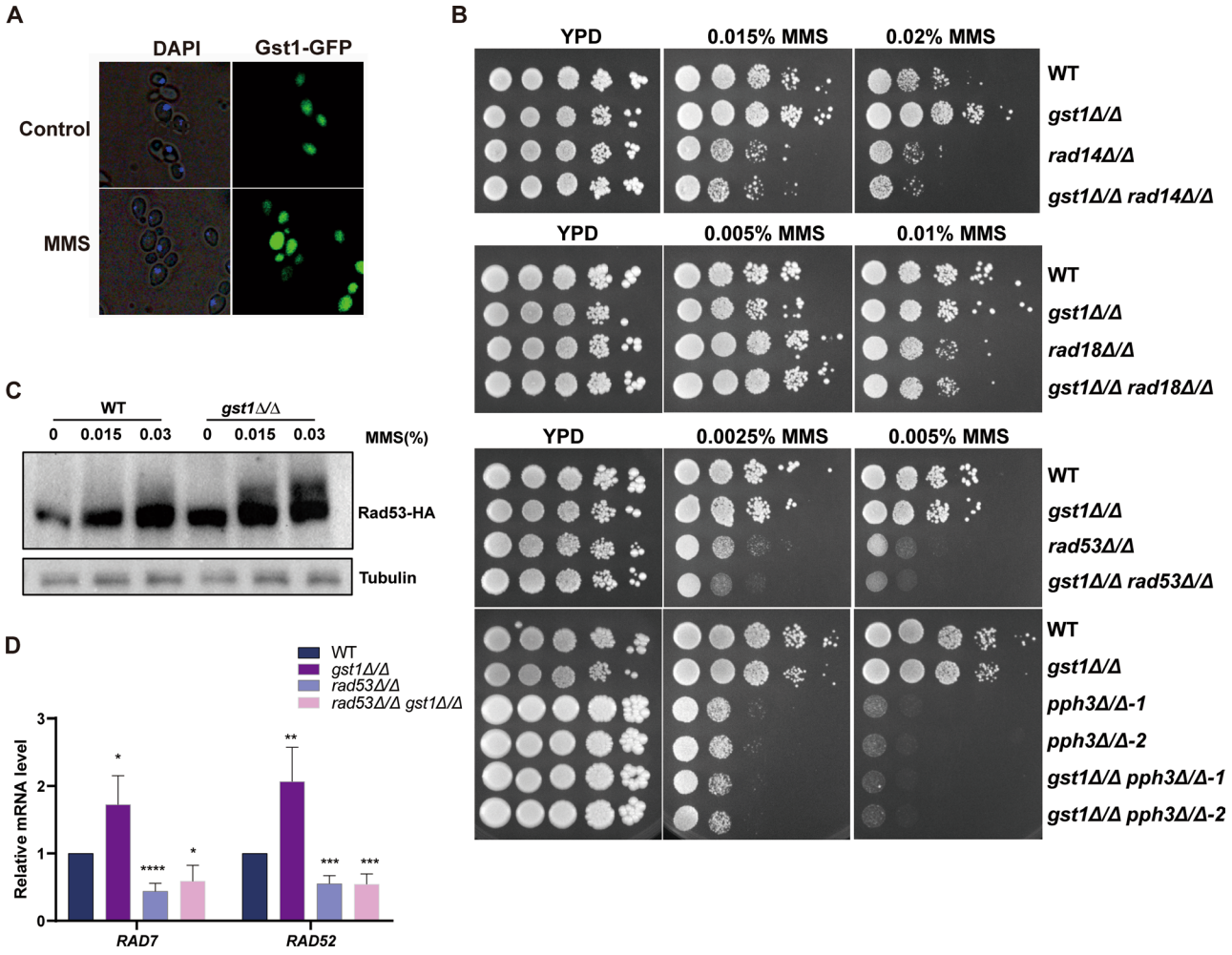


Fig. 6 The increased resistance to MMS resulting from *GST1* deletion entailed the involvement of Rad53 in *C. albicans*. (**A**) The cellular localization of the Gst1-GFP fusion. The log phage-transformed cells were either treated with or without 0.02% MMS for 90 min and stained with DAPI. (**B**) Phenotypic assay of the double deletion of *GST1* with *RAD14*, *RAD18*, *RAD53*, and *PPH3* under MMS stress. (**C**) The phosphorylation of Rad53 after deleting *GST1* was examined by Western blot. The wild-type strain and *GST1* deletion strain carrying an HA tag were used. (**D**) The transcription level of *RAD7* and *RAD52* was assessed by RT–qPCR. The transcription of *RAD7* or *RAD52* was compared to that in the WT group under MMS stress conditions using a paired *t* test with GraphPad Prism 8.0.1 software. * represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$ and **** represents $p < 0.0001$

(Fig. 6C). Interestingly, in the *GST1* deletion strain, Rad53 phosphorylation appeared to be greater than that in the wild-type strain, as evidenced by the slower migration of the Rad53 band, suggesting increased phosphorylation (Fig. 6C).

To further validate the role of Rad53 in facilitating the heightened resistance associated with *GST1* deletion, we checked the transcription of DNA damage repair genes in the *RAD53* deletion strain. Upon *GST1* deletion, there was an upregulation in the transcription of *RAD7* and *RAD52*, whereas deletion of *RAD53* led to a downregulation in their transcription levels (Fig. 6D). As predicted, the transcriptional levels of *RAD7* and *RAD52* exhibited a significant decrease in the *GST1 RAD53* double deletion strain, mirroring those observed in the *RAD53* deletion mutant (Fig. 6D). Consequently, the augmented resistance to MMS post-*GST1* deletion implicates the involvement of Rad53.

Gst1 regulated the DNA damage response in a checkpoint-dependent manner

Checkpoints play critical roles in coordinating the cellular response to DNA damage. Given that deletion of *GST1* resulted in enhanced phosphorylation of Rad53, we hypothesized that the increase in MMS resistance observed might be attributed to enhanced Rad53 functionality. To investigate this prediction, we overexpressed *RAD53* in the wild-type strain and assessed its impact on MMS sensitivity. Consistent with our speculation, overexpression of *RAD53* significantly augmented resistance to MMS in *C. albicans* (Fig. 7A), suggesting a potential

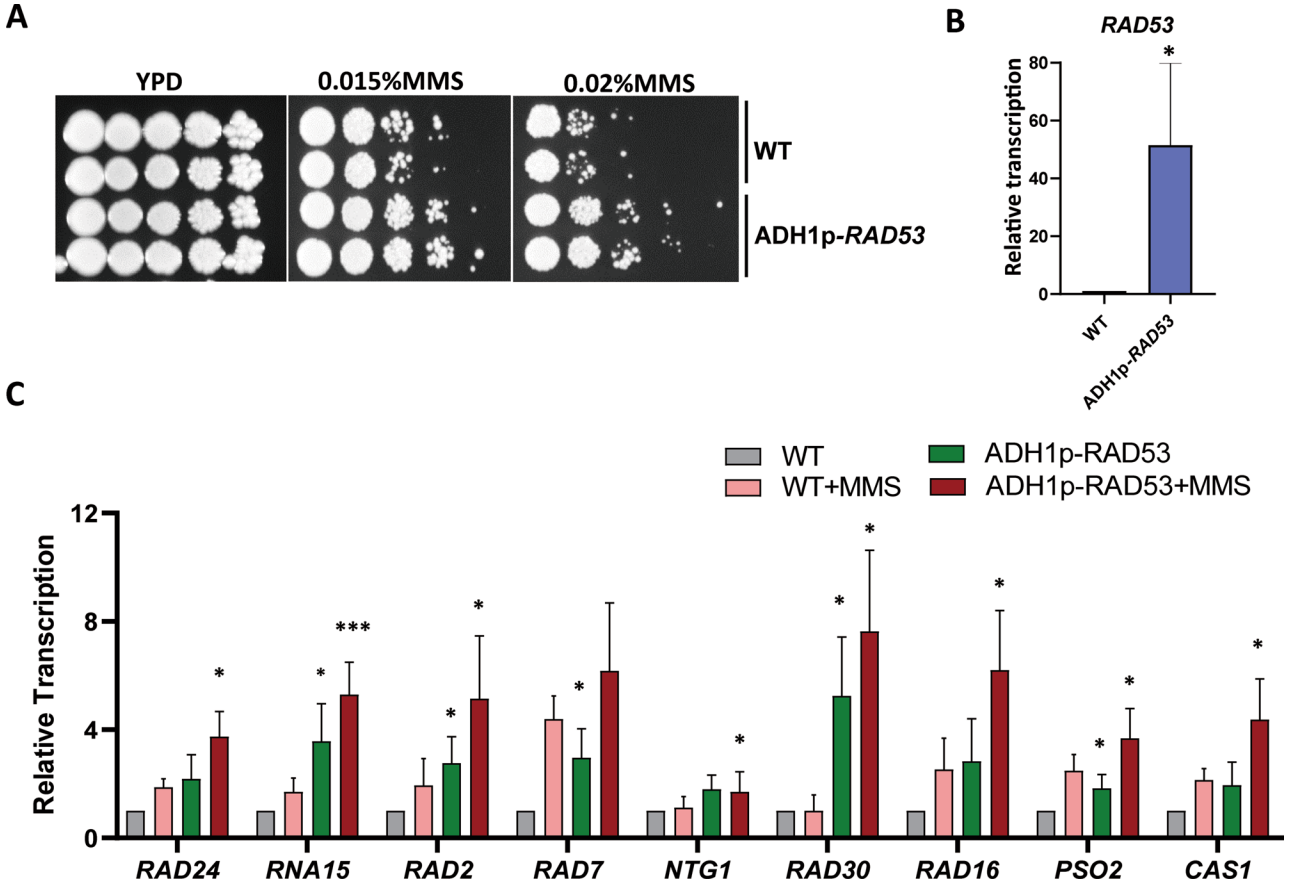


Fig. 7 Overexpression of *RAD53* increased the resistance to MMS and the transcription of DDR genes. (**A**) Phenotypic analysis of the *RAD53*-overexpressing strain. A wild-type strain carrying an *ADH1p-RAD53* construct was used. The transcription of *RAD53* (**B**) and DNA damage response genes (**C**) in the *RAD53* overexpression strain was checked by RT–qPCR. The wild-type and *RAD53*-overexpressing strains were treated with 0.02% MMS for 90 min before being harvested for RNA extraction. The transcription of the indicated genes in the *GST1*-overexpressing strain was compared to that in the WT group under normal or MMS stress conditions using paired *t* tests with GraphPad Prism 8.0.1 software. * represents $p < 0.05$, and *** represents $p < 0.001$

mechanistic link between Gst1 and checkpoint-mediated regulation involving Rad53.

According to our transcriptional profile, deletion of *GST1* promoted the transcription of multiple DNA damage response genes and resulted in increased phosphorylation of Rad53, generating heightened resistance to MMS. A potential mechanism may involve the phosphorylation of Rad53, which promotes the transcription of target DNA damage response genes regulated by Gst1. In order to explore this hypothesis, we assessed the transcriptional levels of these DDR genes in the *RAD53* overexpression strain; under untreated conditions, *RAD53* exhibited a 51.5-fold upregulation compared to the wild-type strain (Fig. 7B). Consistently, overexpression of *RAD53* resulted in the upregulation of *RNA15*, *RAD2*, *RAD30*, and *PSO2* under both normal and MMS stress conditions (Fig. 7C). Moreover, overexpression of *RAD53* enhanced the transcription of *RAD7* under normal conditions and increased the transcription of *RAD24*, *NTG1*, *RAD16*, and *CAS1* under MMS-induced stress conditions. Taken together, our results indicate that the deletion of *GST1* enhances the phosphorylation of Rad53, which subsequently amplifies the transcriptional activation of DNA damage response genes, ultimately leading to increased sensitivity to MMS.

Gst1 physically interacted with Rad53

Given the impact of Gst1 on regulating gene transcription in a Rad53-related manner, we examined its potential interaction with Rad53. Here, we used two truncated versions of Rad53 to test their possible interaction with Gst1 through a yeast two-hybrid assay. This choice was based on a previous observation that only the N-terminal segment, rather than the entire Rad53, exhibited an interaction with Psy2 [23]. We observed that Gst1 interacts specifically with the N-terminus of Rad53, while no discernible interaction was detected with its C-terminus (Fig. 8A). In contrast, Gst3, a glutathione transferase, showed no clear interaction with either the N- or C-terminus of Rad53.

Moreover, coimmunoprecipitation (Co-IP) assays conducted under normal conditions validated the direct

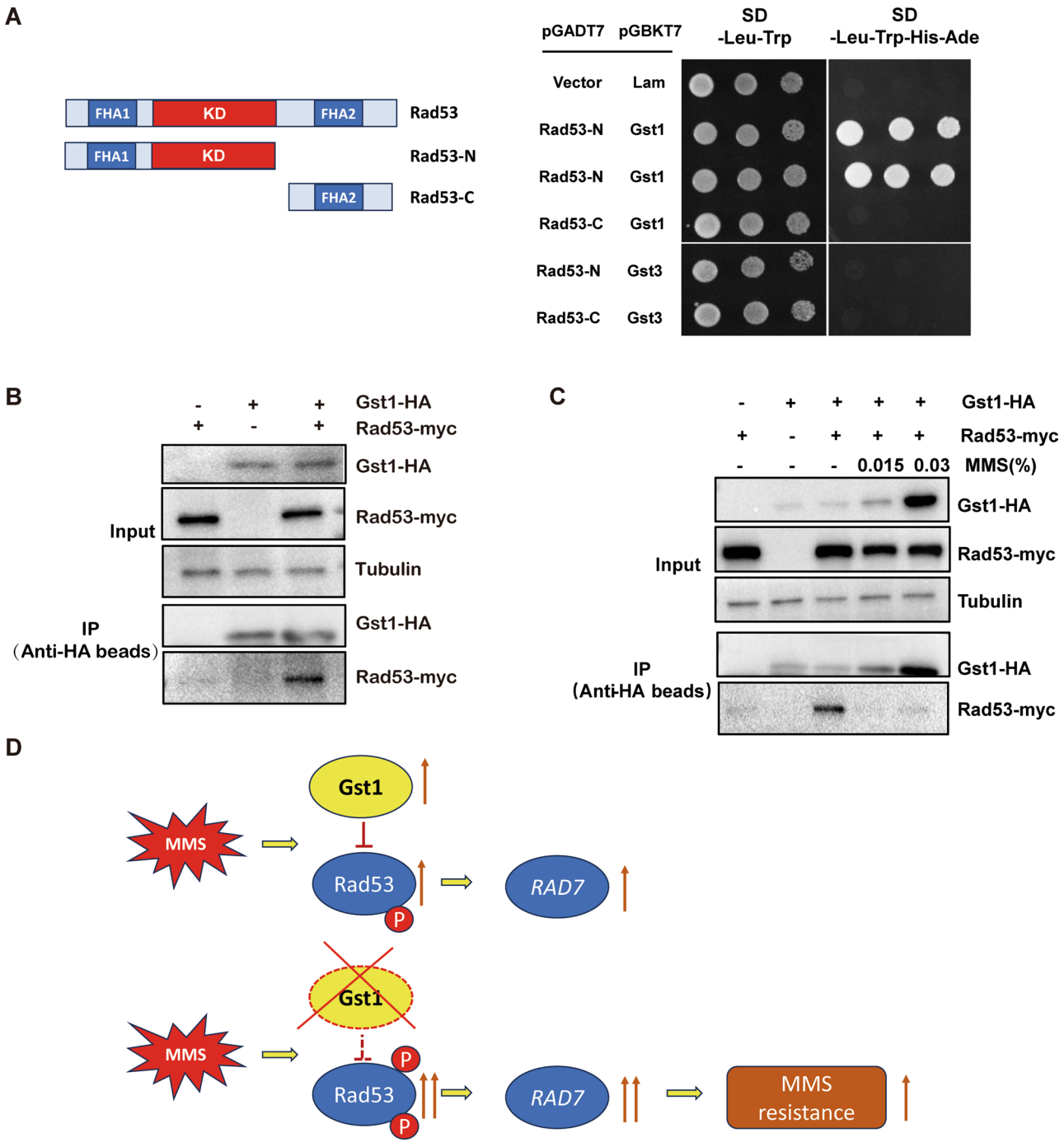


Fig. 8 Gst1 interacted with Rad53 in *C. albicans*. The interaction between Rad53 and Gst1 was examined by a yeast two-hybrid assay (**A**) and Co-IP (**B** & **C**). For the Co-IP assay, wild-type strains carrying Gst1-HA, Rad53-myc, or both were used. In panel C, cells carrying Gst1-HA and Rad53-myc tags were either treated with or without 0.015% and 0.03% MMS. Anti-HA beads were used to pull down Gst1. (**D**) Potential working model for the interaction between Gst1 and Rad53. Under normal conditions, Gst1 interacts with Rad53 and partially blocks its phosphorylation, affecting the transcription of *RAD7* and other DNA damage response genes. By removing Gst1, the phosphorylation of Rad53 increases and promotes the transcription of DDR genes, thereby increasing the DNA repair efficiency

interaction between Gst1 and Rad53 (Fig. 8B). Interestingly, when exposed to MMS-induced stress, the binding between Gst1 and Rad53 showed a marked decrease (Fig. 8C). Overall, our findings suggest a specific interaction between Gst1 and Rad53.

Discussion

In this study, we investigated the role of Gst1, a putative glutathione transferase in *C. albicans*, in the DNA damage response. Exposure to MMS or defects in DNA damage repair induced the expression of Gst1; deletion of *GST1* conferred increased resistance to MMS, while overexpression of *GST1* resulted in slight decreased resistance to MMS. The function of Gst1 in the DNA damage response is associated with the checkpoint kinase Rad53, as deletion of the *GST1* gene promoted the phosphorylation of Rad53 and consequently enhanced the transcription of DDR genes, part of the response leading to MMS resistance.

The DNA damage response is applied for cells to cope with unexpected DNA modifications upon DNA damage stress. The transcriptional regulation of DNA damage-responsive genes may be altered to facilitate efficient repair. In a previous study, we identified global transcriptional changes induced by MMS stress and observed significant upregulation of *GST1* in two independent datasets [17]. This upregulation was confirmed at both the mRNA and protein levels; the significant upregulation induced by MMS treatment may be attributed to its undetectable expression under normal conditions. Generally, glutathione transferases are involved in antioxidation; in *S. pombe*, *gst1*$^+$, *gst2*$^+$, and *gst3*$^+$ are induced by H_2O_2 treatment [7]. Similarly, studies have shown that MMS treatment rapidly suppresses respiration and boosts reactive oxygen species (ROS) generation in *S. cerevisiae* [28]. Therefore, the heightened ROS levels could potentially stimulate the transcription of *GST1* and other genes encoding glutathione transferases in *C. albicans*, given their likely participation in antioxidative defense mechanisms. Within this investigation, the introduction of N-acetylcysteine under MMS-induced stress only slightly mitigated the expression of Gst1, hinting at a tight transcriptional correlation associated with DNA damage-induced stress. Furthermore, the transcription of *GST2* and *GST3* was also upregulated under the stresses of MMS, suggesting a common regulatory mechanism for the GST-encoding genes. These findings partially align with results from the glutathione S-transferase-encoding genes *GTT2*, *GTT1*, and *GTD1* in *S. cerevisiae* [14, 15], as well as *FOXG_13646* and *FOXG_13780* in the fungal pathogen *Fusarium oxysporum* under MMS stress [16]. Notably, the expression of Gst1 was significantly elevated upon depletion of the DNA damage checkpoint kinases, such as Rad53 and Dun1, as well as crucial DNA repair factors, such as Rad52. It is likely that exposure to MMS generates DNA damage stress and potential oxidative stress, and the lack of these DNA repair factors exacerbates the DNA damage stress, potentially enhancing the expression of Gst1. This increased level of Gst1 in response to MMS-induced stress may signify its direct involvement in coping with damaged DNA or its indirect function as a signal transducer of DNA damage response pathways. Delving deeper into the specific mechanism by which MMS treatment induces the expression of *GST1* necessitates additional exploration. Overall, our findings suggest that the induction of glutathione S-transferases in response to MMS represents a conserved mechanism across eukaryotic cells.

In the present study, the deletion of *GST1*, but not *GST2* or *GST3* (Fig. S5) resulted in increased resistance to MMS and decreased phosphorylation of H2A. Despite the application of N-acetylcysteine to mitigate the oxidative stress induced by MMS, the *GST1* deletion still showed increased resistance to MMS. These findings suggest that Gst1 is not essential for DNA damage repair but rather negatively regulates the DNA damage response. The increased resistance to genotoxic stress caused by deleting *GST1* is partially supported by the results in *S. pombe*; *gst1*$^+$ deletion leads to increased resistance to HU, cycloheximide, tunicamycin, and other stress-inducing agents [29]. An efficient DNA damage response requires the involvement of DNA damage repair and signal transducers. However, timely deactivation or inhibition of the DNA damage response is also crucial for cells to restore normal development and thus involves negative regulators of the DNA damage response. In *S. cerevisiae*, a series of genes have been reported to be involved in the negative regulation in response to DNA damage stress. Through analysis of the genomic data of *S. cerevisiae*, we observed 65 mutants exhibiting increased resistance to MMS due to classical gene deletion or mutation, indicating their negative roles in the MMS-induced DNA damage response. The transcriptional response of *GST1* under MMS stress was consistent with that of *MOH1*, which encodes a pro-apoptotic factor in *S. cerevisiae*. Although *MOH1* is induced by MMS, its deletion results in increased resistance to MMS and other genotoxic stresses, similar to that of the *GST1* deletion strain [30]. Moreover, a series of studies revealed that glutathione S-transferase is correlated to apoptosis; glutathione-S-transferase omega 1 (GSTO1-1) acts as a mediator of signaling pathways involved in aflatoxin B1-induced apoptosis [31]. Therefore, in view of the MMS-mediated transcription and increased resistance to MMS by deleting *GST1*, it is possible that Gst1 may influence apoptosis or other cell death pathways in *C. albicans*. Additionally, the expression of ribonucleotide-diphosphate reductase-encoding genes, including *RNR2*, *RNR3*, and *RNR4*, is

induced by MMS stress; however, mutation of the allosteric activity site in RNR leads to elevated dNTP pools, thereby enhancing resistance to MMS and UV radiation [32]. Nevertheless, deletion of *DOT1* enhances resistance to MMS in *S. cerevisiae*; Dot1 regulates H3K79 methylation, which modulates resistance toward MMS through ubiquitylation of PCNA at lysine 164 [33, 34]. To explore the involvement of Gst1 in the DNA damage response, it would be necessary to construct double mutants of *GST1* and other potential negative regulators of the DNA damage response and perform a genetic interaction assay in the future. Collectively, these findings indicate that Gst1 may function as a negative regulator in the MMS-induced DNA damage response in *C. albicans*.

Current results assign Gst1 to the checkpoint kinase Rad53-related signaling pathway. Deletion of *GST1* increased MMS resistance and enhanced the phosphorylation of Rad53. Consistent with these findings, overexpression of Rad53 increased the resistance to MMS. While in *S. cerevisiae*, the heightened MMS resistance resulting from the deletion of *DOT1* was partially alleviated by the overexpression of *RAD53* from a high-copy plasmid, this discrepancy may stem from the functional variances between these two species [33]. In line with this, we have demonstrated that overexpression of *DUN1* similarly confers enhanced resistance to MMS (Fig. S6). Moreover, overexpression of *RAD53* increased the transcription of Gst1's potential targets identified in our study. Therefore, it is reasonable to speculate that Gst1 may participate in the phosphorylation of Rad53 and thus promote the transcription of DDR genes, generating increased MMS resistance. A previous report suggested that glutathione transferase is correlated with checkpoint kinases; phosphorylation at Ser184 of GSTpi inhibits the ubiquitination-mediated degradation of NBS1, and phosphorylated GSTpi can further enhance NBS1 nuclear translocation to activate the ATM-Chk2-p53 signaling pathway [18]. Although Gst1 showed an inverse role compared to that of GSTpi in the DNA damage response, this finding provides evidence for a relationship between glutathione transferases and checkpoint kinases. Given that Gst1 is not a phosphatase, its role in regulating phosphorylation may be indirect. Our protein interaction assay revealed a physical interaction between Gst1 and Rad53, potentially mediated through the catalytic domain of Rad53. Furthermore, AlphaFold3 modeling predicted a potential interaction between Gst1 and Rad53, as illustrated in Figure S7 [35]. However, no interaction was detected between Gst3 and Rad53 despite the induction of *GST3* with MMS treatment (Fig. 8B). Therefore, the regulation on the phosphorylation of Rad53 may be specific to Gst1 rather than glutathione transferase activity. A possible working model could be that the interaction of Gts1 with Rad53 partially inhibits the complete phosphorylation of Rad53; depletion of Gst1 unmasks the hidden sites in Rad53, triggering its complete activation and enhancing the transcription of DDR genes and DNA repair efficiency (Fig. 8C). The attenuated association between Rad53 and Gst1 under MMS-induced stress, as demonstrated in our CoIP analysis, might support this model. Moreover, *GST1* deletion amplifies the phosphorylation of Rad53 but diminishes the phosphorylation of H2A, a key target of Rad53. This phenomenon may stem from heightened repair efficacy post-*GST1* deletion, leading to decreased DNA damage and subsequently diminished H2A phosphorylation.

GST1 deletion or *RAD53* overexpression both induce the transcription of DNA damage response genes, including *RAD7*. In *S. cerevisiae*, *RAD7* encodes a nucleotide excision repair protein responsible for repairing base damage caused by exposure to ultraviolet (UV) light [36]. Our earlier research revealed that MMS treatment induced the transcription *RAD7* in *C. albicans*, indicating its response to MMS-induced stress [17]. Furthermore, NER factors are important for regulating tolerance against alkylation damage induced by MMS in fission yeast, supporting the significance of Rad7 in the Gst1-mediated response to MMS [37]. Given the direct interaction between Gst1 and Rad53, it is possible that Gst1 regulates the transcription of DNA damage response genes in a manner related to checkpoint kinases. A comprehensive study in *S. cerevisiae* revealed several transcription factors involved in the response to DNA damage stress. The checkpoint kinase Rad53 directly regulates the downstream transcription factors Msn4, Mbp1, Swi4, and Swi6 or modulates the functionality of transcription factors Gcn4, Rfx1, Fkh2, Ndd1, and Mcm1 through its downstream kinase Dun1 [38]. This regulation affects the expression of genes related to arginine metabolism, nucleotide biosynthesis, and cell cycle regulation. For example, transcription factors Gcn4 and Fkh2 are implicated in regulating the expression of *RAD7* in *S. cerevisiae* [39, 40]. Moreover, emerging evidence indicates that Rad53 directly interacts with gene promoters, playing a role in regulating gene transcription in *S. cerevisiae* [41]. Therefore, the extensive upregulation of DNA damage genes resulting from *RAD53* overexpression suggests a potential direct regulatory role for Rad53 in the transcriptional control of DDR genes (Fig. 8C). Overall, the Gst1-Rad53 signaling axis may regulate the transcription of DNA damage response genes through a transcription factor-dependent mechanism. Additional research is required to explore the disparity between the transcription of DDR genes and the Gst1-Rad53 signaling cascade in *C. albicans*.

In general, we have observed the increased expression of the glutathione transferase Gst1 in response to MMS-induced stress, with its levels further amplified following

the depletion of checkpoint kinases and DNA repair factors. Deletion of *GST1* prompts augmented resistance to MMS and reduced phosphorylation of histone H2A, indicating Gst1's negative influence on DNA damage response in *C. albicans*. Additionally, Gst1 directly interacts with Rad53; removal of *GST1* triggers heightened Rad53 kinase phosphorylation levels, subsequently stimulating Rad53-dependent gene transcription and bolstering resistance against MMS. Our findings offer valuable insights into Gst1's role in negatively modulating the Rad53-dependent signaling pathway during DNA damage response in *C. albicans*.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12964-024-01865-7.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5 Fig. S1 The expression of Gst1 induced by a high dose of MMS was checked by Western blot. The log phase cells were treated with 0.03% MMS for 0, 15, 30, 45, or 60 minutes.

Supplementary Material 6 Fig. S2 The expression of Gst1 induced by MMS and increased MMS resistance post-deleting *GST1* was partially inhibited by the addition of N-acetylcysteine. (A) The expression of Gst1 under the treatment of MMS and N-acetylcysteine (NAC) was examined by western blot. (B) Phenotypic assay of the *GST1* deletion strain under the treatment of MMS and NAC.

Supplementary Material 7 Fig. S3 The phosphorylation of histone H2A after deleting *GST1* was checked by Western blot. Log phase cells were treated with 0.03% MMS for 45 or 90 minutes. An H2A (S129) antibody was used to determine the phosphorylation level of histone H2A.

Supplementary Material 8 Fig. S4 Transcription of *RAD7* and *RAD52* in the *GST1* complemented strain. The transcription of *RAD7* and *RAD52* was examined by RT-qPCR and compared to the level in the *GST1* deletion strain. The difference was compared using a paired t test with GraphPad Prism 8.0.1 software. ** represents p<0.01 and **** represents p<0.0001

Supplementary Material 9 Fig. S5 Phenotypic assay of the single deletion strains for *GST1*, *GST2*, or *GST3*.

Supplementary Material 10 Fig. S6 Phenotypic assay of the *DUN1* overexpression strain. The wild-type strain carrying an *ADH1p-DUN1* construct was used.

Supplementary Material 11 Fig. S7 Potential interaction between Gst1 and Rad53 predicted by AlphaFold 3. Rad53 is depicted in green, while Gst1 is represented in orange, with a predicted interaction region shown on the right.

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Author contributions
HC, YF, JW, ZC, and RL contributed to strain constructon and phenotype assay. YF perforemd RNAseq assay. HC, YF, and JW performed Western blot and RT-qPCR assay. HC performed the protein interaction assay. JF contributed to conception and design, analysis and interpretation of the data. JF wrote and revised the manuscript. All authors reviewed the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Pathogen Biology, School of Medicine, Nantong University, Nantong, Jiangsu, China
[2]Department of Clinical Laboratory, Kunshan Hospital of Chinese Medicine, Kunshan, China

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