CELLULAR ANALYSIS OF VID24 KNOCKOUT STRAIN UNDER 5-HYDROXYMETHYLFURFURAL STRESS IN *SACCHAROMYCES CEREVISIAE*

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Abstract. The use of microorganisms for efficient conversion of 5-hydroxymethylfurfural (HMF) to more valuable chemical materials has received extensive attention. However, HMF can seriously impede the growth of microorganisms. In order to overcome the problem, it is of great significance to explore the mechanism of microbial tolerance to HMF. A total of 294 genes has been found to be associated with HMF tolerance in *Saccharomyces cerevisiae* by whole gene knockout library scanning and SGAtool analysis. In this study, KEGG and GO enrichment analysis on Cytoscape identified *ASP2, GRH1, PIB1, PSD2, VID24, VMA21, VPS21, VPS51* in cytoplasmic vesicles pathway as HMF tolerance-related genes. Among them, *VID24* is involved in the tolerance regulation of HMF based on spot-test validation. According to the subcellular structure observation, after 60 Mm HMF treatment for 3 h, the proportion of damaged endoplasmic reticulum (ER) cells, damaged vacuole mitochondrial cells in *VID24* gene knockout strain respectively increased by 3.92% and 29.83% compared with standard strain BY4741. In summary, gene *VID24* plays an important role in protecting ER in cellular level and maintaining the stability of vacuole structures. These mechanisms provide a theoretical basis for HMF modification in strains.

Keywords: *Saccharomyces cerevisiae, gene knockout library, SGAtools, subcellular structures, tolerance*

Introduction

Previous studies have found that biomass has many benefits, the primary one being that it cannot be depleted like fossil fuels. With its abundance on earth, biomass could be a renewable and sustainable alternative to fossil fuel to alleviate the current energy crisis (Abo et al., 2019). As one of the most abundant biomass resources, lignocellulose is mainly composed of cellulose, hemicellulose and lignin (Chundawat et al., 2011).

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Although it is a potential sustainable alternative to petroleum, lignocellulose requires pretreatment prior to bioconversion of it (Liu et al., 2019). Due to the method and intensity of pretreatment, a large number of toxic byproducts are formed during hydrolysis, among which 5-hydroxymethylfurfural (HMF) is the most representative compound (Barakat et al., 2012). As an essential multi-purpose bioderived furanic platform compound, 5-hydroxymethylfurfural (HMF) can be effectively transformed into a variety of value-added derivatives due to its rich chemistry, which are raw materials for the production of plastics and drugs (Román-Leshkov and Dumesic, 2009). Currently, HMF is widely used in the preparation of plastics, polyamides, metal-organic frameworks (MOF), drugs, plasticizers etc. (Yang et al., 2017). In general, the application of HMF will have an important impact on human beings, both in areas where it is being utilized at present and in areas where it will be discovered and applied in the future.

HMF possesses versatile chemical activity, making it possible to be further transformed into high-value biochemical products through a series of physical or chemical methods, but the key obstacle in scalable industrial production lies in meeting the cost-effective demand for it is an energy-consumption process. The *bacterium Cupriavidus basilensis* was found to metabolize many of the toxic constituents of lignocellulosic hydrolysate including HMF (Koopman et al., 2010). Later, Koopman et al. introduced the gene *hmf*H encoding the oxidoreductase of HMF into *pseudomonas putida S12*, and deployed the resulting whole-cell biocatalyst to produce FDCA from HMF, reaching 30. 1 g/L FDCA and a production of 97% (Koopman et al., 2010). Therefore, the microbial degradation of HMF is by far the most effective and low-cost method. Although degradation of HMF can create value-added chemicals for human beings, HMF is a highly toxic compound inhibiting microbial production of FDCA and cell metabolism.

Studies have found that HMF induces the enhancement of cellular permeability, making it easier for HMF to enter cells through membrane and inhibiting the activities of enzymes participating in glycolysis and tricarboxylic acid (TAC) cycle (Petersson et al., 2006; Zheng et al., 2015). Therefore, tapping into HMF tolerance mechanism is crucial to microorganism modification in the hope to improve their tolerance to HMF as well as their production capacity. In addition, the enhancement of cellular tolerance can also be achieved by regulating redox homeostasis and consequently increasing NADP, NADPH-dependent dehydrogenases (Ma and Liu, 2010).

Previous studies have found that the up-regulation of *HSF1, YAP1, PDR1, PDR3, RPN4* and other genes are related to HMF tolerance (Ma and Liu, 2010), but the related genes need to be further explored. Therefore, we used the whole gene knockout library of *S. cerevisiae* to screen genes related to HMF tolerance in the presence of 60 mM HMF. Through SGAtool software and KEGG and GO enrichment, we have found that HMF toleration-related genes are enriched in the cytoplasmic vesicles pathway. These genes are *ASP2, GRH1, PIB1, PSD2, VID24, VMA21, VPS21, VPS51*. This paper will verify the tolerance of the above gene knockout strains to HMF, and analyze the subcellular damage of these gene knockout strains. All these efforts aim to clarify the underlying HMF tolerance mechanism of the mentioned genes enriched in cytoplasmic vesicles pathway.

Experimental materials and methods

Experimental materials

The non-essential gene knockout strain bank of *S. cerevisiae* used in this experiment was donated by Professor Beidong Liu of the University of Gothenburg; 5-

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hydroxymethylfurfural (HMF), AGAR, peptone, glucose, yeast leachate powder, Genomycin (G418), sodium chloride, dye (2'7'-DCF diacetate, Mito Tracker Green FM, Vacuole Membrane Marker MDY-64, diaminophenylindole (DAPI), ER-Tracker Red dye) were purchased from Chengdu Vanke Co., LTD.

Main instruments used were the followings: -80°C refrigerator (DW-86L728J, Qingdao Haier Special Electrical Appliances Co., Ltd), high-speed low-temperature centrifuge (Sorvall ST 16R, Thermo Fisher Scientific (China) Co., Ltd), Inverted fluorescence microscope (DMIL LED Fluo, Leica Microsystems CMS GmbH), enzyme label instrument (MOLECULAR DEVICES, SpectraMax 190), super evolution table (HR40-IIA2, Qingdao Haier Special Electrical Appliances Co., Ltd), constant temperature shaker oscillator (GY2019-SW, Zhengzhou Shengyuan Instrument Co., Ltd), oven (DHG-2150B, Zhengzhou Shengyuan Instrument Co., Ltd), autoclave cooker (GI54DS, Zhiwei (Xiamen) Instrument lnc), 4°C refrigerator (HYC-940, Qingdao Haier Special Electrical Appliances Co., Ltd).

Experimental methods

Enrichment of cytoplasmic vesicles pathways and functional analysis of related genes.

KEGG and GO enrichment analysis of differentially expressed genes in *S. cerevisiae* BY4741 under HMF stress was conducted using Cytoscape software. 5-hydroxymethylfurfural (HMF tolerance or sensitivity screening score ≥ 0.2 or ≤ -0.2) was used as the criterion for tolerance or sensitivity (Cao et al., 2022). If the value is ≥ 0.2, the knockout gene is a sensitive gene; If the value is ≤ -0.2, the knockout gene is a tolerance gene. The gene number and score of the enriched genes were queried and listed in SGA data. A comparative analysis was performed to verify which knockout gene contributed most to HMF tolerance.

Spot test experiment

Preparation of YPD solid medium: 1% mother extract powder, 2% peptone, 2% glucose and 2% AGAR. YPD + G418 medium: add G418(final concentration of 100 mg/L) into YPD medium. Strains (knockout strains or complementation strains) were streaked on to YPD + G418 plate to obtain colonies. Next, the single colony was inoculated into a 100 mL triangular flask containing 30 mL of YPD + G418 liquid medium. The cultured bacterial solution was incubated at 30°C and on a rotary shaker at 200 r/min for 18-24 h. Calibrate the cell density (optical density at 600 nm wavelength, OD_{600}) of the cultured bacterial solution to 1.0 and make a serial ten-fold dilution to obtain bacterial solution of different concentrations. 5 μL of diluted bacterial solution was transferred on to YPD + G418 solid medium containing inhibitors of different concentrations using a different concentrations multichannel pipette. Observe the strains and take photos after 3 to 4 days' culture.

Observation of subcellular structure

Standard strains of *S. cerevisiae* (BY4741 and candidate gene knockout strains) were isolated into YPD liquid medium and cultured overnight at 30°C and 200 r/min, and then they were transferred into centrifuge tubes ($OD_{600} = 0.8$) and continued to be cultured at 30°C and 200 r/min for 3 h. Those cultured for 0 h were taken and set as pre-treatment group while those cultured for 3 h as post-treatment group (cultured in liquid

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medium containing 60 mM HMF for 3 h), and both groups were stained to observe their subcellular structure, which was done under previous technical guidance (Madeo et al., 1999; Wang et al., 2020). The bacterial solution was transferred to 1.5 ml EP tube (OD_{600} = 1.0) and dyes (thawed in advance) 2'7'-DCF diacetate, Mito Tracker Green FM, Vacuole Membrane Marker MDY-64, Diaminophenylindoles (DAPI, ER-Tracker Red dye) were separately added into the centrifuged bacterial solution. In the end, ROS accumulation, chromatin disorder, mitochondrial membrane damage, endoplasmic reticulum damage, and vesicle membrane damage were observed at subcellular level under a fluorescence microscope with filters for DIC, GFP, Rhod, DAPI and the proportions of different morphology were calculated.

Statistical method and the applied software

The average of the three-sample data is adopted in the experiment, and the difference between the samples is tested through T-test. In the T-test, * indicates a significant difference ($p < 0.05$), ** indicates a very significant difference ($p < 0.01$), and *** indicates an extremely significant difference ($p < 0.001$). This experiment mainly used Graphpad Prism 8 software for plotting and statistical data analysis.

Results and analysis

Inhibitor concentration screening

In this experiment, HMF was selected as the inhibitor of this trial, and its toxic and harmful properties were used to screen out the genes associated with HMF tolerance. Since inhibitor tolerance is a high-throughput screening, it is necessary to find the optimal inhibitory concentration. The optimal concentration for plaque detection is the semi-inhibitory concentration of *Saccharomyces cerevisiae* at which *Saccharomyces cerevisiae's* growth recovers to half of that in the inhibitor-free medium. Therefore, based on plaque detection, we finally figured out the optimal inhibitory concentration from a gradient of 50 mM, 60 mM, 70 mM HMF. It can be seen from the *Figure 1* that after the treatment with 50 mM, 60 mM and 70 mM HMF, four *Saccharomyces cerevisiae* (BY4741) colonies recovered at a concentration of 50 mM, indicating this concentration performed weak inhibition while only two colonies recovered at a concentration of 70 mM, indicating that the inhibition effect was too strong. Three colonies recovered at a concentration of 60 mM, indicating that this concentration was the optimal HMF screening concentration.

Cytoplasmic vesicles pathway enriched genes and their sensitivity or tolerance screening and function

In prior studies, whole gene knockout libraries of *S. cerevisiae* were screened under HMF treatment. Cytoscape software, KEGG and enrichment of the GO analysis were used to identify sensitivity or tolerance to HMF in accordance with colony growth and eight related genes were found on the cytoplasmic vesicles pathway--*ASP2, GRH1, PIB1, PSD2, VID24, VMA21, VPS21, VPS51* (*Fig. 2*). As is also shown in *Figure 2*, four genes of *GRH1, VMA21, APS2* were involved in the cellular processes of some cytoplasmic vesicles, vesicle membranes, cytoplasmic vesicle membranes and coated vesicle membranes on this pathway, while *VID24* was found to be associated with the first three processes. In addition, *PIB1, PSD2, VPS21, VPS51* genes were involved in

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the cellular process of endosomal membrane and part of vesicle membrane on this pathway. Generally, eight genes above were involved in different responses in the cytoplasmic vesicles pathway. Based on SGA data analysis (*Table 1*), preliminary analysis found that the score of the eight cytoplasmic vesicles pathway genes above were all greater than 0.2, indicating that the knockout of these genes was highly likely to induce strain's tolerance to HMF in its growth. In particular, the weight of *PSD2* gene reached 0.53 (well above 0.2), indicating that the knockout of this gene probably leads to the improvement of tolerance to HMF stress in *S. cerevisiae*.

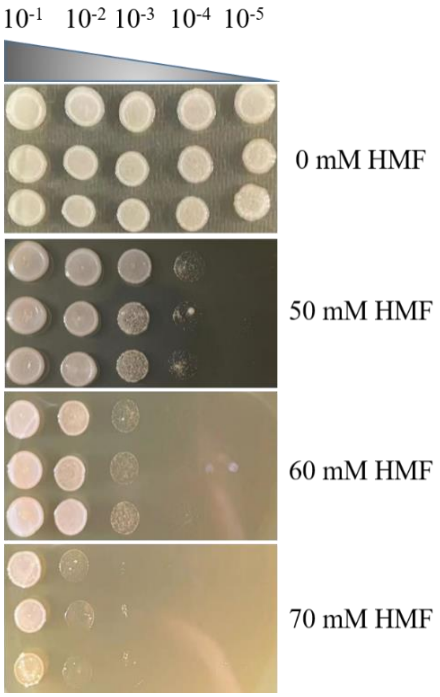

Figure 1. *Screening of Saccharomyces cerevisiae at different inhibitory concentrations*

Table 1. *Key genes of HMF tolerance in cytoplasmic sac pathway*

NO	AN	AO	NC size (EX)	NC std. (EX)	NC size (CN)	NC std. (CN)	Score	Score stdev	p-Value
1	*APS2*	*YJR058C*	1.20	0.00	0.87	0.01	0.33	0.01	0.00
2	*GRH1*	*YDR517W*	1.22	0.01	0.95	0.02	0.27	0.01	0.00
3	*PIB1*	*YDR313C*	1.30	0.01	0.95	0.01	0.35	0.01	0.00
4	*PSD2*	*YGR170W*	1.29	0.04	0.77	0.05	0.53	0.04	0.00
5	*VID24*	*YBR105C*	0.95	0.01	0.71	0.00	0.24	0.01	0.00
6	*VMA21*	*YGR105W*	1.05	0.02	0.83	0.02	0.22	0.02	0.00
7	*VPS21*	*YOR089C*	1.29	0.02	0.93	0.01	0.36	0.02	0.00
8	*VPS51*	*YKR020W*	1.27	0.03	1.05	0.01	0.23	0.03	0.00

NO: Number; AN: Array Name; AO: Array ORF; NC size: Normalized colony size; NC std.: Normalized

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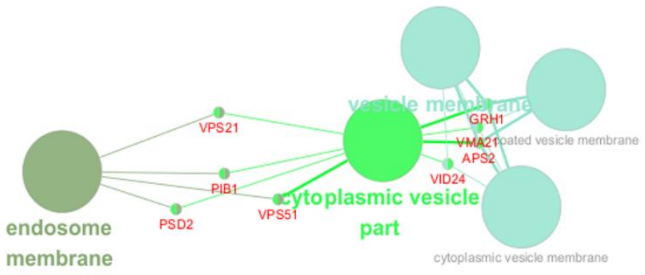

Figure 2. *KEGG enrichment analysis of HMF tolerance gene in cytoplasmic vesicles pathway*

HMF sensitive strain screening

In this study, the toxic properties of 5-hydroxymethylfurfural (HMF), a representative inhibitor of furan substances, were used to screen the tolerance genes related to the cytoplasmic vesicles pathway. Since the SGA database provides only a preliminary screening of tolerance or sensitivity genes, spot test validation are used to verify their authenticity. *VPS21* is an essential gene, for its deletion causes cell death, so *VPS21* knockout strain as well as *ASP2*, *GRH1*, *PIB1*, *PSD2*, *VID24*, *VMA21* and *VPS21* knock-out strains was separately spotted in the medium containing 60 mM HMF, and a spot plate assay without 60 mM HMF was used as control. *Figure 3* showed that all 5 concentrations of the 7 strains above and BY4741 grew on solid YPD without 60 mM HMF. In the spot plate experiment of solid YPD containing 60 mM HMF, it was found that the 4 concentrations (10^{-1} to 10^{-4}) of *APS2* and *PIB1* knockout strains all grew out colonies compared with BY4741, indicating that the *APS2* and *PIB1* knockout strains were neither tolerant nor sensitive to HMF. In addition, it was also found that colonies of *PSD2*, *VMA21*, *VPS21* knockout strains and BY4741 were present in 10^{-1}, 10^{-2} and 10^{-3} dilutions in both groups, indicating that *PSD2*, *VMA21*, *VPS21* gene knockout had little effect on altering the sensitivity or tolerance of the strains to 60 mM HMF. It is noteworthy that although when treated with 60 mM HMF, colonies of both BY4741 and the *GRH1* knockout strain were detected on the 10^{-1}, 10^{-2} and 10^{-3} dilution plates, the colonies of the *GRH1* knockout strain were not as dense as those of BY4741 on the 10^{-3} dilution plates, indicating that *GRH1* gene knockout strain was slightly sensitive to HMF. In the point plate assay of the knockout strain of *VID24*, it can be noted that the colonies of BY4741 were present on the first 4 dilutions, namely 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, while the colonies of *VID24* knockout strain appeared on the first two dilutions with very few on the 10^{-4} plate, indicating that the *VID24* gene knockout strain exhibits significant sensitivity to HMF. Given that, the subcellular structure of *VID24* knockout strain will be analyzed subsequently in this paper.

Subcellular structure observations

Reactive oxygen species

As shown in *Figure 4A*, intracellular reactive oxygen species (ROS) accumulation changes the status of the cell. We used 2,7′-Dichlorofluorescein diacetate staining to detect reactive oxygen (ROS) intermediates and quantify the overall oxidative stress in cells. As shown in *Figure 4B*, three replicates were set for each strain, and the number of cells which fluoresced green of BY4741 and *VID24* gene knockout strains were counted. The

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percentages of cells which fluoresced green for BY4741 and *VID24* gene knockout strains were 22.48% and 23.79% when cultured at 30°C for 0 h in the 60 mM HMF-absent solution. The percentages of cells which fluoresced green in the total cells were 18.61%, 20.77%, 21.99% for the three duplicate samples of strain BY474 and the figures for the *VID24* gene knockout duplicates were 17.96%, 19.87% and 23.54% respectively. Based on the above results, it can be concluded that the level of reactive oxygen species in cells of BY4741 and *VID24* gene knockout strains remained relatively consistent before and after the 60 mM HMF treatment, indicating that BY4741 and *VID24* gene knockout strains were capable of metabolizing the reactive oxygen species induced by HMF.

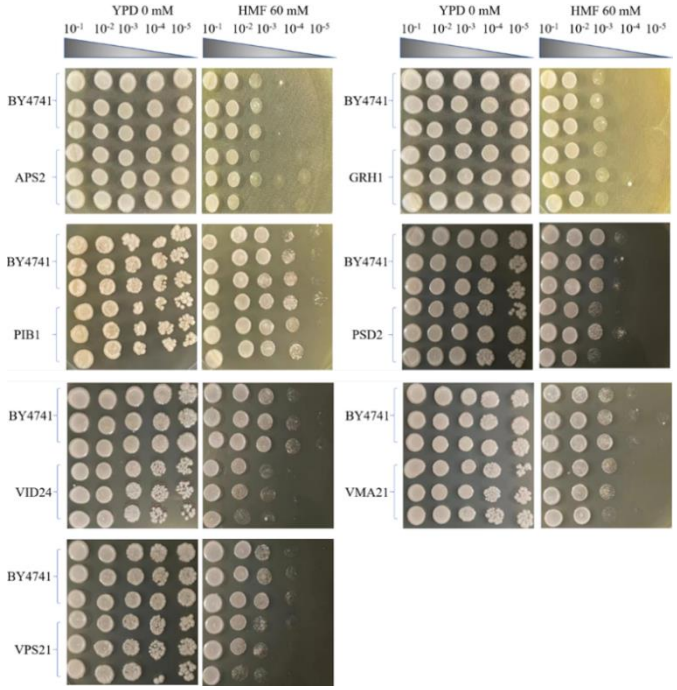

Figure 3. *Spot test verification of candidate genes*

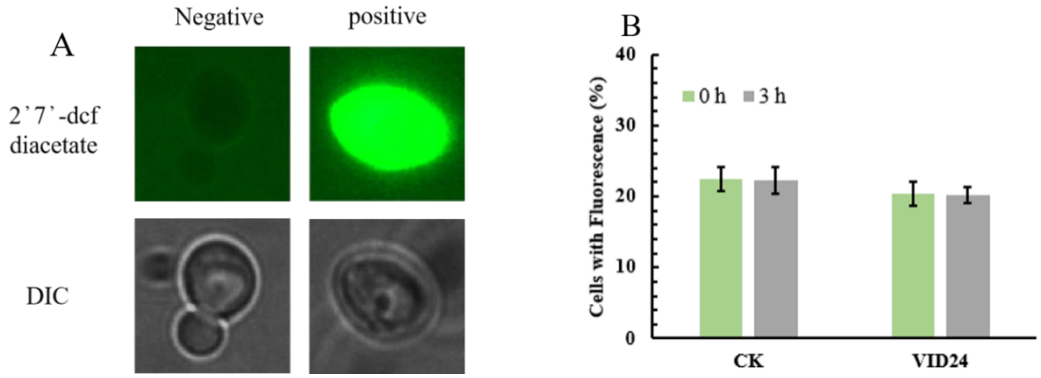

Figure 4. *Reactive oxygen species accumulation in Saccharomyces cerevisiae. A, accumulation of reactive oxygen species in cells. B, the proportion of cells that contained reactive oxygen species after 0 mM and 60 mM HMF treatment for 0 and 3 h. 2'7'-DCF diacetate (top column): reactive oxygen species indicator dye. DIC (down column): differential interference microscope. The data represent averages of three experiments. At least 100 cells were examined on each bright-field image*

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Mitochondria

As shown in *Figure 5A,* exposed to 60 mM HMF, necrotic cells of *S. cerevisiae* strongly fluoresced green when stained by Mito Tracker Green FM (while normal cells emit weak green fluorescence). As shown in *Figure 5B,* mitochondria-damaged cells in BY4741 and *VID24* gene knockout strains accounted for 8.39% and 7.81% of total cells in HMF-free medium HMF at 30°C for 0 h, while the percentages for each climbed to 10.95% and 11.02% after 3 h, treatment of 60 mM HMF. The above results demonstrated that BY4741 and *VID24* knockout strains suffered from comparable mitochondrial damage when HMF was absent, however, when exposed to 60 mM HMF for 3 h, both BY4741 and *VID24* gene knockout strains showed increased cell damage. This suggested that *S. cerevisiae* strain exposure to 60 mM HMF can cause increased mitochondrial damage.

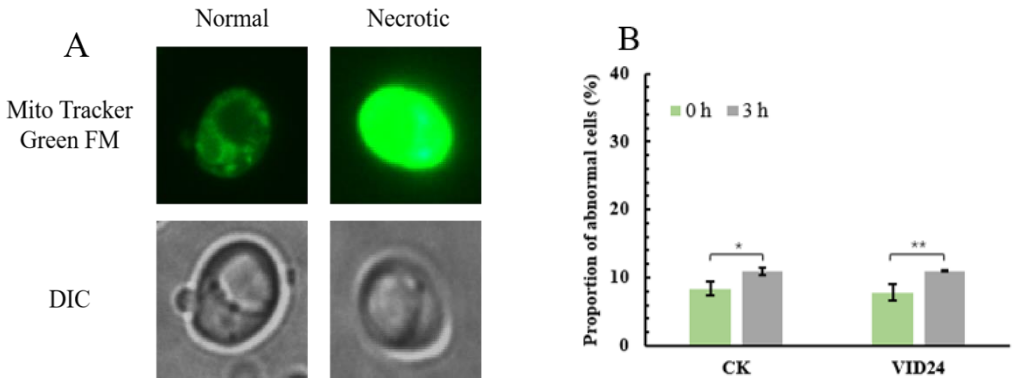

Figure 5. *Mitochondrial morphological changes in Saccharomyces cerevisiae. A, different morphology of mitochondria in cells. B, the proportion of cells that displayed abnormal mitochondria after 0 mM and 60 mM HMF treatment for 0 and 3 h. Mito Tracker Green FM (top column): the mitochondria-specific dye. DIC (down column): differential interference microscope. *p < 0.05; **p < 0.01 indicates significant differences. The data represent averages of three experiments. At least 100 cells were examined on each bright-field image*

Vacuole

As shown in *Figure 6A,* after 60 mM HMF treatment, Vacuole Membrane Marker MDY-64 staining distinguished the abnormal cells with single large vacuoles from normal (damaged) ones with multiple medium-sized vacuoles. According to *Figure 6B,* when incubated in the medium without HMF for 0 h, the percentages of cells with abnormal vacuole for BY4741 and *VID24* gene knockout strains were 20.59% and 51.87%, respectively. After 3 h of 60 mM HMF treatment. The percentages of cells with abnormal vacuoles were 20.1% and 49.93%, respectively. The *VID24* knockout strain had a very high percentage of cells with abnormal vesicles both before and after HMF treatment, suggesting that the gene *VID24* is closely related to the maintenance of vesicle stability.

Chromatin

As shown in *Figure 7A,* chromatin in some cells could experience anomalous diffusion under 60 mM HMF stress. From *Figure 7B,* the percentages of chromatin damaged cells in untreated BY4741 and *VID24* gene knockout strains stood at 1.66%

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and 1.36%, respectively. Yet 3 h of 60 mM HMF treatment did not exacerbate the damage, leaving the figures at 1.66% and 1.41%, respectively. The insignificant difference in chromatin damage between BY4741 and *VID24* knockout strains in both pre-treatment and post-treatment comparisons indicates that *VID24* knockdown does not cause chromatin damage and 60 mM HMF has no toxicity on cellular chromatin and does not affect the chromosome structure of the above strains.

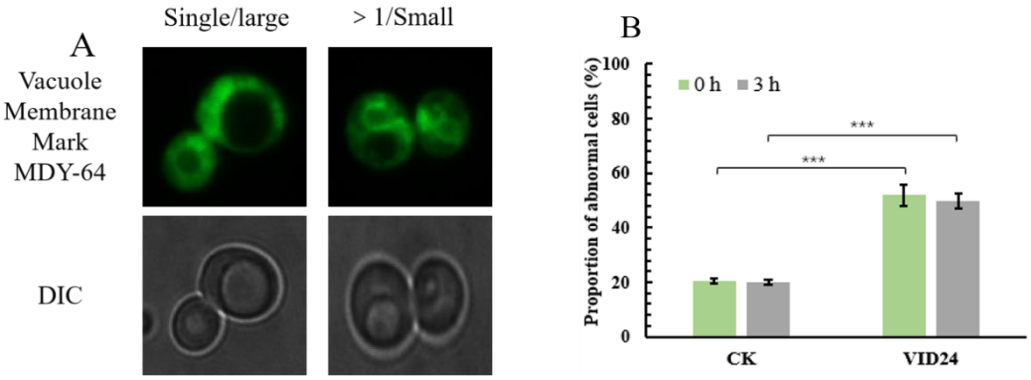

Figure 6. *Morphological changes of vacuoles in Saccharomyces cerevisiae. A, different morphology of vacuoles in cells. B, the proportion of cells that displayed abnormal vacuole after 0 mM and 60 mM HMF treatment for 0 and 3 h. Vacuole Membrane Marker MDY-64 (top column): vacuole specific dye. DIC (down column): differential interference microscope. Single/large: single large vacuole. > 1/Small: more than single small vacuole. ***p < 0.001 indicates significant differences. The data represent averages of three experiments. At least 100 cells were examined on each bright-field image*

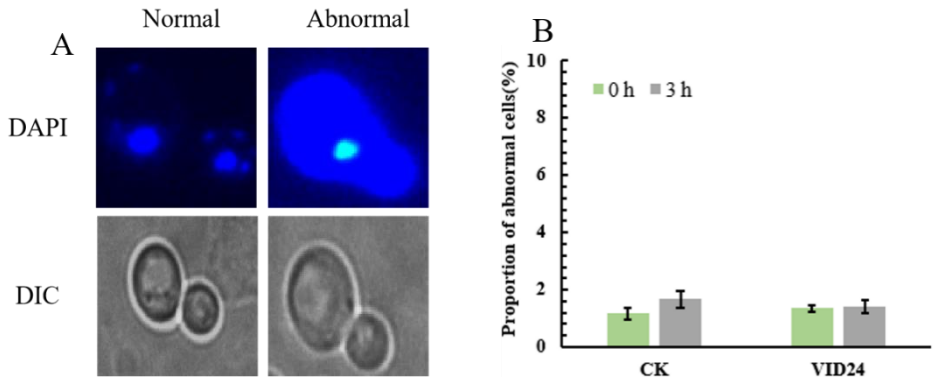

Figure 7. *Morphological changes of chromatin in Saccharomyces cerevisiae. A, different morphologies of chromatin in cells. B, the proportion of cells that displayed abnormal chromatin after 0 mM and 60 mM HMF treatment for 0 and 3 h. Diaminophenylindole (DAPI) (top column): DNA specific dye. DIC (down column): differential interference microscope. The data represent averages of three experiments. At least 100 cells were examined on each bright-field image*

Endoplasmic reticulum

As shown in *Figure 8A*, the endoplasmic reticulum cells before and after 60 mM HMF treatment were observed under red fluorescence. The cells showed two types:

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normal and injured. As shown in *Figure8B*, the percentages of damaged ER cells BY4741 and *VID24* gene knockout strains under 60 mM HMF stress for 3 h were 17.32% and 21.24% respectively in contrast to 11.3% and 11.89% in the control group. From the data, there was no significant difference in the ER damage between untreated BY4741 and *VID24* knockout strains. At 3 h treatment, both BY4741 and *VID24* gene knockout strains were affected by 60 mM HMF, resulting in ER damage of normal cells, and a subsequent rise in cell necrosis. These results showed that 60 mM HMF caused damage to both of the two strains, especially to the *VID24* knockout strain.

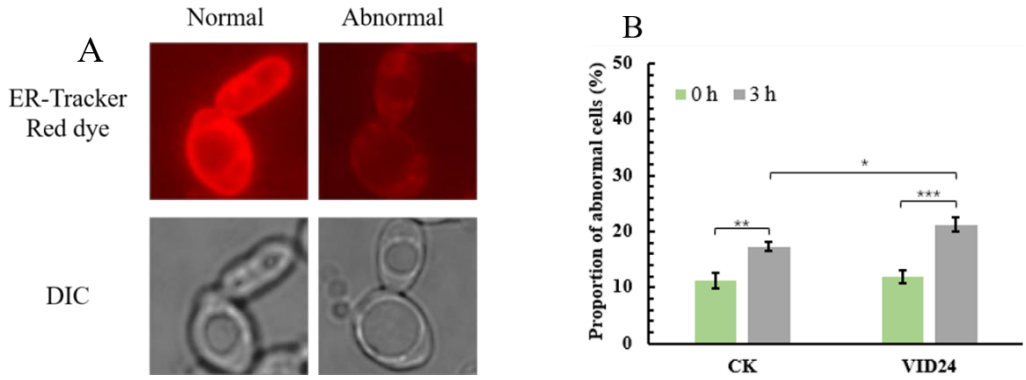

Figure 8. *Morphological changes of endoplasmic reticulum in Saccharomyces cerevisiae. A, different morphologies of the ER in cells. B, the proportion of cells that displayed abnormal ER after 0 mM and 60 mM HMF treatment for 0 and 3 h. ER-Tracker Red dye (top column): endoplasmic reticulum specific dye. DIC (down column): differential interference microscope. *p < 0.05; **p < 0.01; ***p < 0.001 indicates significant differences. The data represent averages of three experiments. At least 100 cells were examined on each bright-field image*

Discussion

In this study, eight key genes of cytoplasmic vesicles pathway *ASP2*, *GRH1*, *PIB1*, *PSD2*, *VID24*, *VMA21*, *VPS21* and *VPS51* obtained through enrichment analysis were preliminarily identified as up-regulate genes according to the whole gene knockout database of *Saccharomyces cerevisiae* under HMF stress. Among the above eight genes, however, the *VID24* gene knockout strain exhibited a growth impaired phenotype when exposed to the 60 mM HMF in contrast to the standard strain, indicating that *VID24* gene was a HMF-tolerance gene. *DDI1* and *ATG8* genes have been previously found to be related to HMF tolerance in the cytoplasmic vesicles pathway, involved in SNARE binding protein and autophagosome and Cvt vesicles components of DNA damage induction (Ma and Liu, 2010). This experiment identified novel genes on the cytoplasmic vesicle pathway related to HMF tolerance. According to the subcellular analysis of this experiment, the knockout of *VID24* gene has a great influence on the vacuoles. According to NCBI inquiry, *VID24* gene is mainly involved in the process of protein catabolism, protein targeting of vacuoles and external components of membranes, etc., which is consistent with the finding that gene *VID24* plays an important role in maintaining the stability of vacuoles. It is exciting to point out 60 mM HMF treatment increases the severity of ER damage.

At present, the tolerance mechanism of furfural in *S. cerevisiae* has been thoroughly investigated and understood, but the study on HMF tolerance is not enough. Since both of them are furfural derivatives, with toxic aldehyde group and furan ring, HMF and

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furfural share similar inhibitory mechanisms. Many pathways have been found related to furfural, but no cytoplasmic vesicles-related pathway has been reported, which may be explained by our assumption that cytoplasmic vesicles pathway is specific to HMF-tolerance. It was found that *VID24* promoted the degradation of fructose-1,6-phosphate to fructose-6-acid by FFBPase, which was involved in glycolysis, gluconogenesis, pentose phosphate and other metabolic pathways (Regelmann et al., 2003). Catalyzing fructose-1, 6-phosphoric acid to fructose-6-acid, FBPase is a pathway for the reverse of glycolysis. In the absence of *VID24*, as FBPase synthesis is enhanced, fructose-1,6-bisphosphate synthesis is reduced accordingly, as a result glycolysis is suppressed. We speculate a slowdown in growth of the *VID24* knockout strain of *S. cerevisiae*. However, in this phenotypic verification, there was no significant difference in the growth of *VID24* knockout strain and BY4741 strain on the inhibitor-free YPD plate. It is likely that the deficiency of glycolysis is complemented by other energy metabolic pathways in *S. cerevisiae*, which renders the growth-inhibition effects less obvious than anticipated. Nevertheless, when exposed to 60 mM HMF, the growth of *VID24* knockout strain was significantly inhibited, suggesting that there are other mechanisms affecting the normal growth of the strain under 60 mM HMF stress. Previous studies have found that one of the resistance mechanisms of *S. cerevisiae* to HMF is the degradation of HMF catalyzed by NADH and aldehyde reductase. When NADH is absent, the detoxification of HMF is significantly affected and the growth of *S. cerevisiae* is restrained. However, *S. cerevisiae* can rapidly detoxify HMF (Liu et al. 2008; Liu 2011) with the presence of NADH in large amount, which can be produced by glycolysis where Glyceraldehyde-3-phosphate is converted into 1,3-bisphosphoglycerate (Wahlbom and Hahn-Hägerdal, 2002; Liu, 2018; Hoffmann et al., 2021). when *VID24* is knocked out, Glycerate 1,3-P2 (D-Glycerate 1,3-diphosphate) synthesis via the glycolysis pathway is affected to the extent that less NADH is generated. Therefore, the decreased 60 mM HMF detoxification as a result of the drop in NADH is most likely what makes the *VID24* knockout strain sensitive to 60 mM HMF stress.

Previous studies have found that after *VID24* gene knockout, cytoplasmic vesicles cannot transport normally and its ubiquitin phosphorylation is inhibited so that accumulation of unfolded proteins in the endoplasmic reticulum causes ER stress (Imai et al., 2000; Teusink et al., 2000; Santt et al., 2008; Menssen et al., 2012). These findings suggest that *VID24* is the central hub that turns on vesicle transport and regulates ubiquitination. As it is suggested based on our subcellular observation that the damage of endoplasmic reticulum was more severe after 60 mM HMF treatment. We hypothesize that vesicles in *VID24* knockout strains are unable to move properly so HMF-induced damaged or unfolded proteins fail to be transported and further degraded by ubiquitinate. As a result, all proteins accumulate in the endoplasmic reticulum and cause ER stress. This explains why the ER damage of *VID24* knockout strain was more serious than that of standard strain when *S. cerevisiae* was treated with 60 mM HMF.

Conclusion

In this study, the whole gene knockout library was used to verify the subcellular structure of *S. cerevisiae*. Combined with bioinformatics analysis, we identified that the gene *VID24* is closely associated with 5-hydroxymethylfurfural tolerance. During

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the following phenotype validation of *VID24* by spot plate assay and subcellular structure observation, we discovered that the *VID24* gene on the cytoplasmic vesicles pathway plays an important role in keeping endoplasmic reticulum free from HMF damage and maintaining vacuole stability. Thus, this study lays a theoretical foundation for modifying *Saccharomyces cerevisiae* strains to improve tolerance to HMF, which is important for mitigating HMF formation or removing HMF in mass production.

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APPENDIX

Appendix 1. *SGAtool analyzed the scores of each knockout strain under HMF conditions*

No	Array ORF	Array name	Normalized colony size (EXPERIMENT)	Normalized colony std. dev. (EXPERIMENT)	Normalized colony size (CONTROL)	Normalized colony std. dev. (CONTROL)	Score	Score std. dev.	p-Value
1	*YMR283C*	*RIT1*	0.38	0.01	1.04	0.03	-0.66	0.01	0.00
2	*YDR049W*	*VMS1*	0.38	0.00	0.98	0.02	-0.60	0.00	0.00
3	*YJR075W*	*HOC1*	0.69	0.01	1.21	0.02	-0.52	0.01	0.00
4	*YBL047C*	*EDE1*	0.40	0.02	0.92	0.01	-0.52	0.02	0.00
5	*YDR068W*	*DOS2*	0.42	0.04	0.94	0.02	-0.52	0.04	0.00
6	*YGL105W*	*ARC1*	0.27	0.01	0.78	0.00	-0.51	0.01	0.00
7	*YHR030C*	*SLT2*	0.80	0.05	1.29	0.05	-0.48	0.05	0.00
8	*YOR191W*	*ULS1*	0.65	0.02	1.11	0.03	-0.46	0.02	0.00
9	*YMR272C*	*SCS7*	0.61	0.07	1.06	0.02	-0.45	0.07	0.00
10	*YJL124C*	*LSM1*	0.43	0.02	0.88	0.03	-0.45	0.02	0.00
11	*YDR360W*	*YDR360W*	0.62	0.02	1.05	0.00	-0.43	0.02	0.00
12	*YNL032W*	*SIW14*	0.72	0.02	1.12	0.12	-0.42	0.02	0.00
13	*YML035C*	*AMD1*	0.51	0.01	0.93	0.04	-0.41	0.01	0.00
14	*YBR025C*	*OLA1*	0.55	0.01	0.96	0.01	-0.41	0.01	0.00
15	*YDR378C*	*LSM6*	0.29	0.02	0.69	0.03	-0.40	0.02	0.00
16	*YNR051C*	*BRE5*	0.64	0.00	1.05	0.02	-0.40	0.00	0.00
17	*YLR217W*	*YLR217W*	0.94	0.02	1.35	0.03	-0.39	0.02	0.00
18	*YJL121C*	*RPE1*	0.37	0.05	0.75	0.05	-0.39	0.05	0.00
19	*YMR275C*	*BUL1*	0.66	0.03	1.02	0.04	-0.37	0.03	0.00
20	*YKL023W*	*YKL023W*	0.64	0.04	1.01	0.03	-0.36	0.04	0.00
21	*YNR005C*	*YNR005C*	0.74	0.01	1.09	0.06	-0.35	0.01	0.00
22	*YLR085C*	*ARP6*	0.67	0.00	1.03	0.04	-0.34	0.00	0.00
23	*YML041C*	*VPS71*	0.63	0.04	0.98	0.04	-0.34	0.04	0.00
24	*YJR059W*	*PTK2*	0.49	0.04	0.83	0.02	-0.34	0.04	0.00
25	*YNL136W*	*EAF7*	0.45	0.02	0.78	0.03	-0.33	0.02	0.00
26	*YJL136C*	*RPS21B*	0.60	0.06	0.92	0.02	-0.32	0.06	0.00
27	*YLR330W*	*CHS5*	0.85	0.03	1.16	0.04	-0.32	0.03	0.00
28	*YCR053W*	*THR4*	0.65	0.02	0.97	0.01	-0.31	0.02	0.00
29	*YNL099C*	*OCA1*	0.78	0.01	1.09	0.03	-0.31	0.01	0.00
30	*YGL084C*	*GUP1*	0.51	0.01	0.80	0.01	-0.30	0.01	0.00
31	*YDL078C*	*MDH3*	0.91	0.03	1.21	0.04	-0.30	0.03	0.00
32	*YOR006C*	*TSR3*	0.86	0.05	1.16	0.06	-0.30	0.05	0.00
33	*YDR067C*	*OCA6*	0.73	0.02	1.03	0.03	-0.30	0.02	0.00
34	*YER151C*	*UBP3*	0.73	0.01	1.02	0.01	-0.29	0.01	0.00
35	*YNL229C*	*URE2*	0.69	0.02	0.98	0.01	-0.29	0.02	0.00
36	*YPR134W*	*MSS18*	0.55	0.02	0.84	0.00	-0.29	0.02	0.00
37	*YPR024W*	*YME1*	0.56	0.01	0.84	0.02	-0.28	0.01	0.00
38	*YKL046C*	*DCW1*	0.94	0.09	1.22	0.05	-0.28	0.09	0.00
39	*YHR086W*	*NAM8*	0.75	0.04	1.02	0.03	-0.28	0.04	0.00
40	*YDL050C*	*YDL050C*	0.74	0.00	1.01	0.01	-0.27	0.00	0.00
41	*YML008C*	*ERG6*	0.50	0.02	0.77	0.02	-0.27	0.02	0.00
42	*YNL147W*	*LSM7*	0.51	0.05	0.78	0.04	-0.27	0.05	0.00

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No	Array ORF	Array name	Normalized colony size (EXPERIMENT)	Normalized colony std. dev. (EXPERIMENT)	Normalized colony size (CONTROL)	Normalized colony std. dev. (CONTROL)	Score	Score std. dev.	p-Value
43	*YGR038W*	*ORM1*	0.93	0.01	1.20	0.03	-0.26	0.01	0.00
44	*YNL254C*	*RTC4*	0.82	0.05	1.08	0.02	-0.26	0.05	0.00
45	*YPL183C*	*RTT10*	0.73	0.00	0.99	0.02	-0.26	0.00	0.00
46	*YMR026C*	*PEX12*	0.78	0.03	1.02	0.05	-0.25	0.03	0.00
47	*YBL094C*	*YBL094C*	0.71	0.01	0.96	0.07	-0.25	0.01	0.00
48	*YDR485C*	*VPS72*	0.67	0.01	0.92	0.00	-0.25	0.01	0.00
49	*YJL062W*	*LAS21*	0.74	0.02	0.99	0.01	-0.25	0.02	0.00
50	*YOR012W*	*YOR012W*	0.76	0.02	1.00	0.01	-0.25	0.02	0.00
51	*YHR078W*	*YHR078W*	0.85	0.03	1.09	0.01	-0.24	0.03	0.00
52	*YNL008C*	*ASI3*	0.95	0.03	1.19	0.05	-0.24	0.03	0.00
53	*YFL023W*	*BUD27*	0.64	0.03	0.88	0.03	-0.24	0.03	0.00
54	*YJR082C*	*EAF6*	0.78	0.02	1.02	0.02	-0.24	0.02	0.00
55	*YNL072W*	*RNH201*	0.76	0.03	1.00	0.02	-0.24	0.03	0.00
56	*YMR282C*	*AEP2*	0.78	0.02	1.01	0.04	-0.23	0.02	0.00
57	*YLR452C*	*SST2*	0.99	0.02	1.23	0.02	-0.23	0.02	0.00
58	*YBR001C*	*NTH2*	0.74	0.02	0.97	0.00	-0.23	0.02	0.00
59	*YNL089C*	*YNL089C*	0.85	0.02	1.08	0.02	-0.23	0.02	0.00
60	*YMR031W-A*	*YMR031W-A*	0.59	0.03	0.83	0.03	-0.23	0.03	0.00
61	*YLR332W*	*MID2*	0.92	0.05	1.14	0.04	-0.23	0.05	0.00
62	*YHR066W*	*SSF1*	0.77	0.01	1.00	0.01	-0.23	0.01	0.00
63	*YBL007C*	*SLA1*	0.64	0.01	0.87	0.03	-0.23	0.01	0.00
64	*YJR074W*	*MOG1*	0.64	0.05	0.87	0.03	-0.23	0.05	0.00
65	*YDR304C*	*CPR5*	0.88	0.01	1.11	0.03	-0.23	0.01	0.00
66	*YLR098C*	*CHA4*	0.89	0.01	1.12	0.04	-0.23	0.01	0.00
67	*YIL132C*	*CSM2*	0.91	0.07	1.13	0.03	-0.22	0.07	0.00
68	*YLR070C*	*XYL2*	0.94	0.06	1.17	0.05	-0.22	0.06	0.00
69	*YMR318C*	*ADH6*	0.81	0.02	1.03	0.02	-0.22	0.02	0.00
70	*YDR532C*	*KRE28*	0.57	0.01	0.79	0.02	-0.22	0.01	0.00
71	*YJR097W*	*JJJ3*	0.70	0.03	0.93	0.03	0.22	0.03	0.00
72	*YJL211C*	*YJL211C*	0.89	0.04	1.10	0.05	0.22	0.04	0.00
73	*YGR051C*	*YGR051C*	0.97	0.02	1.19	0.11	0.22	0.02	0.00
74	*YFL013C*	*IES1*	0.87	0.03	1.09	0.05	0.22	0.03	0.00
75	*YDR334W*	*SWR1*	0.72	0.01	0.94	0.03	0.22	0.01	0.00
76	*YHR110W*	*ERP5*	0.90	0.02	1.12	0.00	0.22	0.02	0.00
77	*YHL024W*	*RIM4*	0.92	0.01	1.13	0.01	0.21	0.01	0.00
78	*YGR055W*	*MUP1*	0.62	0.02	0.84	0.04	0.21	0.02	0.00
79	*YCL009C*	*ILV6*	0.94	0.04	1.15	0.04	0.21	0.04	0.00
80	*YLL046C*	*RNP1*	0.98	0.00	1.21	0.10	0.21	0.00	0.00
81	*YCR085W*	*YCR085W*	0.81	0.02	1.01	0.02	0.21	0.02	0.00
82	*YHL023C*	*NPR3*	0.83	0.01	1.04	0.03	0.21	0.01	0.00
83	*YJL141C*	*YAK1*	0.84	0.10	1.04	0.03	0.21	0.10	0.00
84	*YCR062W*	*YCR062W*	0.86	0.03	1.08	0.03	0.21	0.03	0.00
85	*YPL213W*	*LEA1*	0.67	0.01	0.88	0.02	0.20	0.01	0.00
86	*YGL059W*	*PKP2*	0.97	0.04	1.18	0.01	0.20	0.04	0.00
87	*YBL024W*	*NCL1*	0.77	0.02	0.97	0.00	0.20	0.02	0.00
88	*YKR084C*	*HBS1*	0.67	0.03	0.88	0.02	0.20	0.03	0.00
89	*YPL105C*	*SYH1*	0.89	0.01	1.09	0.01	0.20	0.01	0.00
90	*YGR122C-A*	*YGR122C-A*	0.98	0.02	1.19	0.04	0.20	0.02	0.00
91	*YDL051W*	*LHP1*	0.80	0.02	1.00	0.01	0.20	0.02	0.00
92	*YMR163C*	*INP2*	0.81	0.01	1.01	0.02	0.20	0.01	0.00
93	*YDL091C*	*UBX3*	1.21	0.03	1.01	0.01	0.20	0.03	0.00
94	*YKR019C*	*IRS4*	1.19	0.05	0.99	0.01	0.20	0.05	0.00

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95	*YGL014W*	*PUF4*	1.24	0.05	1.04	0.01	0.20	0.05	0.00
96	*YOR108W*	*LEU9*	1.15	0.05	0.94	0.01	0.20	0.05	0.00
97	*YDR032C*	*PST2*	1.17	0.03	0.97	0.02	0.20	0.03	0.00
98	*YJR014W*	*TMA22*	1.11	0.01	0.90	0.02	0.20	0.01	0.00
99	*YOL081W*	*IRA2*	1.03	0.08	0.82	0.02	0.20	0.08	0.00
100	*YIL085C*	*KTR7*	1.03	0.01	0.83	0.01	0.20	0.01	0.00
101	*YER066W*	*RRT13*	1.17	0.05	0.97	0.01	0.20	0.05	0.00
102	*YGR183C*	*QCR9*	1.07	0.01	0.86	0.04	0.20	0.01	0.00
103	*YGL180W*	*ATG1*	1.22	0.06	1.01	0.03	0.21	0.06	0.00
104	*YLR177W*	*YLR177W*	1.14	0.01	0.93	0.02	0.21	0.01	0.00
105	*YCR009C*	*RVS161*	1.05	0.03	0.84	0.01	0.21	0.03	0.00
106	*YKR031C*	*SPO14*	1.23	0.02	1.02	0.00	0.21	0.02	0.00
107	*YML026C*	*RPS18B*	1.04	0.02	0.84	0.02	0.21	0.02	0.00
108	*YLR407W*	*YLR407W*	1.08	0.00	0.87	0.03	0.21	0.00	0.00
109	*YJR010C-A*	*SPC1*	1.24	0.06	1.03	0.01	0.22	0.06	0.00
110	*YBL104C*	*SEA4*	1.11	0.01	0.89	0.01	0.22	0.01	0.00
111	*YDL232W*	*OST4*	0.91	0.05	0.70	0.01	0.22	0.05	0.00
112	*YIL138C*	*TPM2*	1.27	0.02	1.06	0.03	0.22	0.02	0.00
113	*YGR105W*	*VMA21*	1.04	0.02	0.83	0.02	0.22	0.02	0.00
114	*YGR161C*	*RTS3*	1.12	0.01	0.90	0.01	0.22	0.01	0.00
115	*YBR141C*	*YBR141C*	1.19	0.01	0.97	0.01	0.22	0.01	0.00
116	*YKL146W*	*AVT3*	1.15	0.02	0.94	0.02	0.22	0.02	0.00
117	*YLR179C*	*YLR179C*	1.26	0.04	1.05	0.04	0.22	0.04	0.00
118	*YBR082C*	*UBC4*	1.14	0.02	0.92	0.01	0.22	0.02	0.00
119	*YEL057C*	*YEL057C*	1.16	0.02	0.94	0.01	0.22	0.02	0.00
120	*YCL036W*	*GFD2*	1.20	0.04	0.98	0.01	0.22	0.04	0.00
121	*YDR209C*	*YDR209C*	1.30	0.01	1.08	0.03	0.22	0.01	0.00
122	*YKL073W*	*LHS1*	0.97	0.23	0.72	0.15	0.22	0.23	0.00
123	*YKL048C*	*ELM1*	0.92	0.06	0.70	0.02	0.22	0.06	0.00
124	*YML004C*	*GLO1*	1.21	0.01	0.98	0.01	0.22	0.01	0.00
125	*YLR371W*	*ROM2*	1.15	0.04	0.93	0.01	0.22	0.04	0.00
126	*YOR183W*	*FYV12*	1.10	0.01	0.87	0.01	0.23	0.01	0.00
127	*YLR176C*	*RFX1*	1.27	0.03	1.05	0.01	0.23	0.03	0.00
128	*YAL022C*	*FUN26*	1.17	0.09	0.92	0.07	0.23	0.09	0.00
129	*YKR020W*	*VPS51*	1.28	0.03	1.05	0.01	0.23	0.03	0.00
130	*YHR162W*	*MPC2*	1.02	0.04	0.79	0.02	0.23	0.04	0.00
131	*YKL174C*	*TPO5*	1.21	0.03	0.98	0.02	0.23	0.03	0.00
132	*YLR384C*	*IKI3*	1.10	0.02	0.87	0.00	0.23	0.02	0.00
133	*YOR193W*	*PEX27*	1.18	0.01	0.95	0.01	0.23	0.01	0.00
134	*YNL171C*	*YNL171C*	1.05	0.01	0.82	0.02	0.23	0.01	0.00
135	*YDR508C*	*GNP1*	1.26	0.02	1.03	0.01	0.23	0.02	0.00
136	*YPR018W*	*RLF2*	1.12	0.04	0.89	0.02	0.23	0.04	0.00
137	*YIR019C*	*FLO11*	1.25	0.10	1.03	0.04	0.23	0.10	0.00
138	*YLR443W*	*ECM7*	1.14	0.00	0.92	0.03	0.23	0.00	0.00
139	*YNL170W*	*YNL170W*	0.90	0.03	0.66	0.02	0.23	0.03	0.00
140	*YML097C*	*VPS9*	1.17	0.01	0.93	0.02	0.23	0.01	0.00
141	*YGR078C*	*PAC10*	1.10	0.03	0.88	0.05	0.23	0.03	0.00
142	*YOR182C*	*RPS30B*	1.12	0.01	0.88	0.01	0.23	0.01	0.00
143	*YMR145C*	*NDE1*	1.23	0.07	0.99	0.02	0.23	0.07	0.00
144	*YDL076C*	*RXT3*	1.19	0.00	0.96	0.04	0.24	0.00	0.00
145	*YKR047W*	*YKR047W*	1.22	0.02	0.97	0.04	0.24	0.02	0.00
146	*YMR029C*	*FAR8*	1.19	0.04	0.96	0.02	0.24	0.04	0.00

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147	*YBR095C*	*RXT2*	1.11	0.02	0.87	0.00	0.24	0.02	0.00
148	*YNR029C*	*YNR029C*	1.10	0.01	0.86	0.04	0.24	0.01	0.00
149	*YMR271C*	*URA10*	1.21	0.07	0.96	0.04	0.24	0.07	0.00
150	*YLR327C*	*TMA10*	1.16	0.03	0.91	0.03	0.24	0.03	0.00
151	*YGL149W*	*YGL149W*	1.21	0.02	0.98	0.02	0.24	0.02	0.00
152	*YKL164C*	*PIR1*	1.16	0.06	0.93	0.05	0.24	0.06	0.00
153	*YML128C*	*MSC1*	1.24	0.02	1.00	0.02	0.24	0.02	0.00
154	*YDR156W*	*RPA14*	1.34	0.03	1.10	0.03	0.24	0.03	0.00
155	*YDL074C*	*BRE1*	0.97	0.00	0.73	0.03	0.24	0.00	0.00
156	*YMR039C*	*SUB1*	1.28	0.08	1.04	0.02	0.24	0.08	0.00
157	*YBR105C*	*VID24*	0.95	0.01	0.71	0.00	0.24	0.01	0.00
158	*YPL086C*	*ELP3*	1.04	0.04	0.80	0.00	0.25	0.04	0.00
159	*YDR469W*	*SDC1*	1.09	0.01	0.85	0.01	0.25	0.01	0.00
160	*YKL092C*	*BUD2*	1.20	0.03	0.95	0.01	0.25	0.03	0.00
161	*YPL071C*	*YPL071C*	1.04	0.02	0.80	0.01	0.25	0.02	0.00
162	*YLR287C-A*	*RPS30A*	1.17	0.04	0.92	0.03	0.25	0.04	0.00
163	*YOR078W*	*BUD21*	1.04	0.01	0.79	0.01	0.25	0.01	0.00
164	*YBR181C*	*RPS6B*	1.18	0.00	0.93	0.01	0.25	0.00	0.00
165	*YKR023W*	*YKR023W*	1.21	0.01	0.97	0.01	0.25	0.01	0.00
166	*YLR015W*	*BRE2*	1.16	0.03	0.91	0.00	0.25	0.03	0.00
167	*YMR312W*	*ELP6*	1.07	0.02	0.82	0.02	0.25	0.02	0.00
168	*YMR152W*	*YIM1*	1.16	0.05	0.91	0.01	0.25	0.05	0.00
169	*YGL179C*	*TOS3*	1.22	0.02	0.97	0.01	0.25	0.02	0.00
170	*YFL030W*	*AGX1*	1.12	0.03	0.86	0.03	0.26	0.03	0.00
171	*YKL109W*	*HAP4*	1.11	0.05	0.86	0.01	0.26	0.05	0.00
172	*YHR200W*	*RPN10*	1.16	0.06	0.90	0.01	0.26	0.06	0.00
173	*YIL154C*	*IMP2'*	1.23	0.01	0.97	0.01	0.26	0.01	0.00
174	*YLR024C*	*UBR2*	1.21	0.04	0.95	0.03	0.26	0.04	0.00
175	*YNL224C*	*SQS1*	1.19	0.02	0.93	0.03	0.26	0.02	0.00
176	*YML035C-A*	*YML035C-A*	1.24	0.02	0.98	0.02	0.26	0.02	0.00
177	*YHR194W*	*MDM31*	0.93	0.01	0.67	0.02	0.26	0.01	0.00
178	*YDR459C*	*PFA5*	1.14	0.02	0.88	0.04	0.26	0.02	0.00
179	*YMR067C*	*UBX4*	1.08	0.04	0.82	0.02	0.26	0.04	0.00
180	*YLR320W*	*MMS22*	1.07	0.01	0.81	0.01	0.26	0.01	0.00
181	*YDR466W*	*PKH3*	1.17	0.01	0.91	0.02	0.26	0.01	0.00
182	*YHR059W*	*FYV4*	1.02	0.05	0.76	0.01	0.26	0.05	0.00
183	*YDL189W*	*RBS1*	1.26	0.01	1.00	0.03	0.26	0.01	0.00
184	*YDR096W*	*GIS1*	1.33	0.01	1.07	0.03	0.26	0.01	0.00
185	*YIL141W*	*YIL141W*	1.26	0.01	0.99	0.03	0.26	0.01	0.00
186	*YPL196W*	*OXR1*	1.25	0.02	0.98	0.03	0.26	0.02	0.00
187	*YML051W*	*GAL80*	1.25	0.08	0.98	0.05	0.26	0.08	0.00
188	*YHR096C*	*HXT5*	1.32	0.04	1.06	0.01	0.27	0.04	0.00
189	*YMR223W*	*UBP8*	1.27	0.00	1.00	0.02	0.27	0.00	0.00
190	*YGR200C*	*ELP2*	1.04	0.00	0.76	0.03	0.27	0.00	0.00
191	*YGL250W*	*RMR1*	1.27	0.03	1.00	0.00	0.27	0.03	0.00
192	*YJL169W*	*YJL169W*	1.12	0.06	0.85	0.03	0.27	0.06	0.00
193	*YDR517W*	*GRH1*	1.22	0.01	0.95	0.02	0.27	0.01	0.00
194	*YJR108W*	*ABM1*	1.22	0.06	0.95	0.01	0.27	0.06	0.00
195	*YPR132W*	*RPS23B*	1.16	0.03	0.90	0.02	0.27	0.03	0.00
196	*YDR374C*	*YDR374C*	1.03	0.01	0.76	0.02	0.27	0.01	0.00
197	*YKL026C*	*GPX1*	1.14	0.05	0.86	0.01	0.27	0.05	0.00
198	*YNL319W*	*YNL319W*	1.07	0.07	0.80	0.03	0.28	0.07	0.00

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199	*YBR189W*	*RPS9B*	1.15	0.01	0.88	0.02	0.28	0.01	0.00
200	*YPL181W*	*CTI6*	1.27	0.05	0.99	0.03	0.28	0.05	0.00
201	*YIL077C*	*YIL077C*	1.18	0.04	0.89	0.00	0.28	0.04	0.00
202	*YKL179C*	*COY1*	1.24	0.01	0.96	0.03	0.28	0.01	0.00
203	*YNR064C*	*YNR064C*	1.22	0.09	0.95	0.04	0.28	0.09	0.00
204	*YPR151C*	*SUE1*	1.20	0.04	0.92	0.02	0.29	0.04	0.00
205	*YGL181W*	*GTS1*	1.20	0.11	0.91	0.09	0.29	0.11	0.00
206	*YOL053W*	*AIM39*	1.02	0.05	0.73	0.05	0.29	0.05	0.00
207	*YMR315W*	*YMR315W*	1.20	0.04	0.90	0.01	0.29	0.04	0.00
208	*YLR264W*	*RPS28B*	1.15	0.03	0.86	0.02	0.29	0.03	0.00
209	*YGL244W*	*RTF1*	0.93	0.02	0.63	0.03	0.29	0.02	0.00
210	*YMR100W*	*MUB1*	1.15	0.03	0.87	0.04	0.30	0.03	0.00
211	*YJR011C*	*YJR011C*	1.03	0.01	0.74	0.01	0.30	0.01	0.00
212	*YLR172C*	*DPH5*	1.09	0.06	0.79	0.00	0.30	0.06	0.00
213	*YML102W*	*CAC2*	1.27	0.02	0.97	0.01	0.30	0.02	0.00
214	*YGR239C*	*PEX21*	1.17	0.05	0.87	0.02	0.30	0.05	0.00
215	*YOL007C*	*CSI2*	1.26	0.00	0.96	0.02	0.30	0.00	0.00
216	*YKR074W*	*AIM29*	1.28	0.02	0.97	0.02	0.30	0.02	0.00
217	*YMR219W*	*ESC1*	1.32	0.05	1.02	0.02	0.30	0.05	0.00
218	*YDR458C*	*HEH2*	1.22	0.08	0.91	0.01	0.30	0.08	0.00
219	*YOR139C*	*YOR139C*	1.28	0.00	0.97	0.00	0.30	0.00	0.00
220	*YHR111W*	*UBA4*	1.21	0.02	0.90	0.02	0.31	0.02	0.00
221	*YBR275C*	*RIF1*	1.38	0.00	1.07	0.02	0.31	0.00	0.00
222	*YDL093W*	*PMT5*	1.18	0.02	0.87	0.02	0.31	0.02	0.00
223	*YER067C-A*	*YER067C-A*	1.18	0.05	0.86	0.01	0.32	0.05	0.00
224	*YOL059W*	*GPD2*	1.23	0.02	0.92	0.04	0.32	0.02	0.00
225	*YKR103W*	*NFT1*	1.32	0.03	1.00	0.00	0.32	0.03	0.00
226	*YIL153W*	*RRD1*	1.28	0.04	0.96	0.02	0.32	0.04	0.00
227	*YOL080C*	*REX4*	1.33	0.01	1.01	0.01	0.32	0.01	0.00
228	*YDL187C*	*YDL187C*	1.28	0.03	0.96	0.02	0.32	0.03	0.00
229	*YLR004C*	*THI73*	1.23	0.03	0.91	0.02	0.32	0.03	0.00
230	*YHR034C*	*PIH1*	1.18	0.04	0.85	0.01	0.33	0.04	0.00
231	*YLR412W*	*BER1*	1.14	0.01	0.82	0.01	0.33	0.01	0.00
232	*YBR009C*	*HHF1*	1.25	0.08	0.92	0.03	0.33	0.08	0.00
233	*YJR058C*	*APS2*	1.20	0.01	0.87	0.01	0.33	0.01	0.00
234	*YLR131C*	*ACE2*	1.27	0.03	0.93	0.00	0.33	0.03	0.00
235	*YOR301W*	*RAX1*	1.20	0.02	0.86	0.01	0.34	0.02	0.00
236	*YNL206C*	*RTT106*	1.19	0.01	0.86	0.01	0.34	0.01	0.00
237	*YDL083C*	*RPS16B*	1.19	0.03	0.85	0.02	0.34	0.03	0.00
238	*YNL045W*	*LAP2*	1.20	0.03	0.86	0.00	0.34	0.03	0.00
239	*YDR313C*	*PIB1*	1.30	0.01	0.95	0.01	0.35	0.01	0.00
240	*YBR095C*	*RXT2*	1.31	0.01	0.97	0.01	0.35	0.01	0.00
241	*YJR087W*	*YJR087W*	1.38	0.12	1.03	0.03	0.35	0.12	0.00
242	*YDR257C*	*RKM4*	1.34	0.06	0.98	0.04	0.35	0.06	0.00
243	*YDL129W*	*YDL129W*	1.44	0.03	1.08	0.01	0.36	0.03	0.00
244	*YOR089C*	*VPS21*	1.29	0.02	0.93	0.01	0.36	0.02	0.00
245	*YOR135C*	*YOR135C*	0.97	0.04	0.61	0.02	0.36	0.04	0.00
246	*YLR423C*	*ATG17*	1.29	0.01	0.92	0.05	0.36	0.01	0.00
247	*YOR045W*	*TOM6*	1.30	0.02	0.94	0.01	0.36	0.02	0.00
248	*YBR082C*	*UBC4*	1.29	0.03	0.91	0.03	0.37	0.03	0.00
249	*YKL213C*	*DOA1*	1.26	0.03	0.89	0.01	0.37	0.03	0.00
250	*YLR418C*	*CDC73*	0.92	0.02	0.55	0.02	0.37	0.02	0.00

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251	*YPL032C*	*SVL3*	1.16	0.03	0.79	0.03	0.37	0.03	0.00
252	*YPL139C*	*UME1*	1.28	0.03	0.91	0.01	0.37	0.03	0.00
253	*YMR252C*	*YMR252C*	1.21	0.03	0.83	0.01	0.37	0.03	0.00
254	*YLR048W*	*RPS0B*	1.15	0.03	0.77	0.01	0.38	0.03	0.00
255	*YMR124W*	*YMR124W*	1.20	0.00	0.82	0.02	0.38	0.00	0.00
256	*YMR274C*	*RCE1*	1.29	0.05	0.91	0.01	0.38	0.05	0.00
257	*YLR174W*	*IDP2*	1.34	0.05	0.94	0.03	0.39	0.05	0.00
258	*YLR388W*	*RPS29A*	1.20	0.03	0.81	0.00	0.39	0.03	0.00
259	*YAR002W*	*NUP60*	1.32	0.01	0.93	0.01	0.39	0.01	0.00
260	*YHR048W*	*YHK8*	1.33	0.05	0.93	0.04	0.40	0.05	0.00
261	*YOR007C*	*SGT2*	1.14	0.03	0.73	0.00	0.40	0.03	0.00
262	*YMR073C*	*IRC21*	1.27	0.01	0.87	0.01	0.41	0.01	0.00
263	*YMR025W*	*CSI1*	1.44	0.09	1.02	0.03	0.41	0.09	0.00
264	*YOL103W*	*ITR2*	1.17	0.05	0.75	0.02	0.42	0.05	0.00
265	*YIL008W*	*URM1*	1.31	0.06	0.85	0.04	0.45	0.06	0.00
266	*YML048W-A*	*YML048W-A*	1.24	0.04	0.79	0.03	0.45	0.04	0.00
267	*YML010C-B*	*YML010C-B*	1.37	0.08	0.92	0.01	0.46	0.08	0.00
268	*YKL066W*	*YKL066W*	1.47	0.01	1.00	0.02	0.47	0.01	0.00
269	*YKR072C*	*SIS2*	1.43	0.04	0.97	0.01	0.47	0.04	0.00
270	*YLR218C*	*COA4*	1.35	0.06	0.88	0.02	0.48	0.06	0.00
271	*YOR312C*	*RPL20B*	1.46	0.11	0.97	0.01	0.48	0.11	0.00
272	*YML102C-A*	*YML102C-A*	1.31	0.02	0.83	0.01	0.48	0.02	0.00
273	*YMR105C*	*PGM2*	1.45	0.01	0.96	0.03	0.49	0.01	0.00
274	*YOL068C*	*HST1*	1.35	0.02	0.87	0.03	0.49	0.02	0.00
275	*YKL037W*	*AIM26*	1.12	0.02	0.62	0.00	0.50	0.02	0.00
276	*YHR003C*	*YHR003C*	1.42	0.02	0.92	0.01	0.50	0.02	0.00
277	*YKL074C*	*MUD2*	1.41	0.01	0.90	0.03	0.50	0.01	0.00
278	*YIL108W*	*YIL108W*	1.41	0.05	0.91	0.04	0.50	0.05	0.00
279	*YCR063W*	*BUD31*	1.26	0.01	0.75	0.03	0.51	0.01	0.00
280	*YGR201C*	*YGR201C*	1.55	0.04	1.04	0.03	0.51	0.04	0.00
281	*YGR170W*	*PSD2*	1.29	0.04	0.77	0.05	0.53	0.04	0.00
282	*YCL037C*	*SRO9*	1.37	0.01	0.82	0.01	0.55	0.01	0.00
283	*YAL013W*	*DEP1*	1.36	0.09	0.80	0.02	0.57	0.09	0.00
284	*YDR348C*	*PAL1*	1.55	0.02	0.98	0.01	0.57	0.02	0.00
285	*YGR202C*	*PCT1*	1.51	0.04	0.94	0.03	0.58	0.04	0.00
286	*YNL040W*	*YNL040W*	1.58	0.05	1.00	0.03	0.59	0.05	0.00
287	*YPL182C*	*YPL182C*	1.61	0.07	1.01	0.02	0.59	0.07	0.00
288	*YJL016W*	*YJL016W*	1.44	0.04	0.84	0.02	0.60	0.04	0.00
289	*YKL015W*	*PUT3*	1.58	0.15	0.95	0.01	0.63	0.15	0.00
290	*YKL076C*	*YKL076C*	1.54	0.06	0.90	0.01	0.64	0.06	0.00
291	*YOL004W*	*SIN3*	1.39	0.02	0.73	0.01	0.66	0.02	0.00
292	*YKL069W*	*YKL069W*	1.36	0.02	0.70	0.01	0.66	0.02	0.00
293	*YKR082W*	*NUP133*	1.37	0.02	0.68	0.02	0.68	0.02	0.00
294	*YMR106C*	*YKU80*	1.69	0.00	0.96	0.02	0.74	0.00	0.00

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