

Electronic Supplementary Materials

For: Applied Microbiology and Biotechnology

Title: SUMO expression shortens the lag phase of *Saccharomyces cerevisiae* yeast growth caused by complex interactive effects of major mixed fermentation inhibitors found in hot-compressed water-treated lignocellulosic hydrolysate

Authors: Lahiru N. Jayakody^{1,2}, Masafumi Kadowaki², Keisuke Tsuge³, Kenta Horie², Akihiro Suzuki^{1,2}, Nobuyuki Hayashi^{1,4}, and Hiroshi Kitagaki^{1,2*}

¹Department of Biochemistry and Applied Biosciences, United Graduate School of Agricultural Sciences, Kagoshima University, 1-21-24, Korimoto, Kagoshima City, Kagoshima 890-8580, Japan

²Department of Environmental Sciences, Faculty of Agriculture, Saga University, Saga 840-8502, Japan

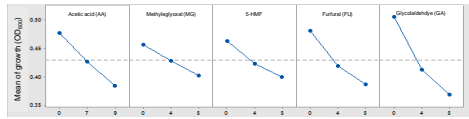
³Industrial Technology Center of Saga, 114 Yaemizo, Saga 849-0932, Japan

⁴Department of Applied Biochemistry and Food Science, Faculty of Agriculture, Saga University, Saga 840-8502, Japan

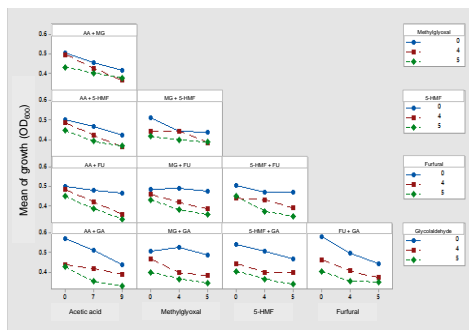
*Corresponding author; e-mail: ktgkhrs@cc.saga-u.ac.jp, phone: +81952288766, fax: +81952288709

Supplementary figures

a



b



c

Source	P-Value
Model	0.000
Linear	0.000
Acetic acid	0.000
Methylglyoxal	0.000
5-HMF	0.000
Furfural	0.000
Glycolaldehyde	0.000
2-Way Interactions	0.000
Acetic acid*Glycolaldehyde	0.014
Acetic acid*5-HMF	0.160
Acetic acid*Furfural	0.001
Acetic acid*Methylglyoxal	0.002
Glycolaldehyde*5-HMF	0.003
Glycolaldehyde*Furfural	0.030
Glycolaldehyde*Methylglyoxal	0.015
5-HMF*Furfural	0.006
5-HMF*Methylglyoxal	0.352
Furfural*Methylglyoxal	0.005
3-Way Interactions	0.057
Acetic acid*Glycolaldehyde*5-HMF	0.001
Acetic acid* Glycolaldehyde*Furfural	0.341
Acetic acid* Glycolaldehyde*Methylglyoxal	0.056
Acetic acid*5-HMF*Furfural	0.037
Acetic acid*5-HMF*Methylglyoxal	0.403
Acetic acid*Furfural*Methylglyoxal	0.801
Glycolaldehyde*5-HMF*Furfural	0.687
Methylglyoxal*5-HMF*Glycolaldehyde	0.773
Methylglyoxal*Furfural*Glycolaldehyde	0.733
5-HMF*Furfural*Glycolaldehyde	0.087
4-Way Interactions	0.211
Acetic acid*Methylglyoxal*5-HMF*Furfural	0.111
Acetic acid*Methylglyoxal*5-HMF*Glycolaldehyde	0.055
Acetic acid*Methylglyoxal*Furfural*Glycolaldehyde	0.460
Acetic acid*5-HMF*Furfural*Glycolaldehyde	0.265
Methylglyoxal*5-HMF*Furfural*Glycolaldehyde	0.889

Fig. S1 Major inhibitors in hot-compressed water-treated lignocellulose hydrolysate had interactive inhibitory effects. (a) Major effect plots. (b) Interactive effect plots. (c) Statistical significance of interactions. Red letters and values indicate statistically significant interactions ($p < 0.05$).

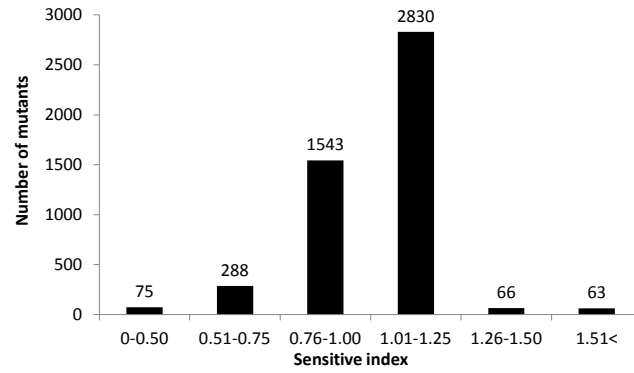
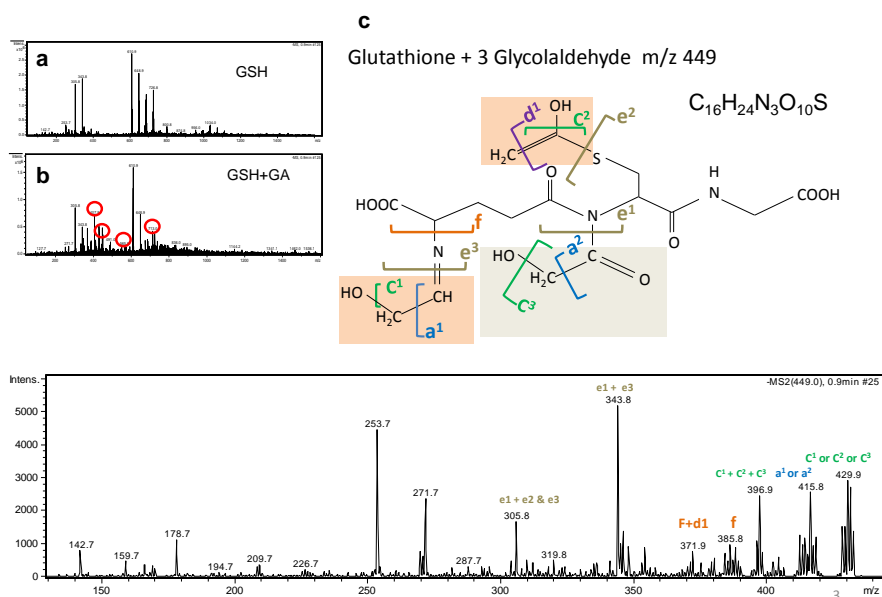


Fig. S2 Sensitivity distributions of homozygous diploid deletion strains to the combinational inhibitory effects of fermentation inhibitors. All homozygous diploid deletion strains were plotted according to their sensitivities to SYN_{actual}. Data are expressed as the averages of triplicate experiments.



2 Glutathione + 2 Glycolaldehyde m/z 713

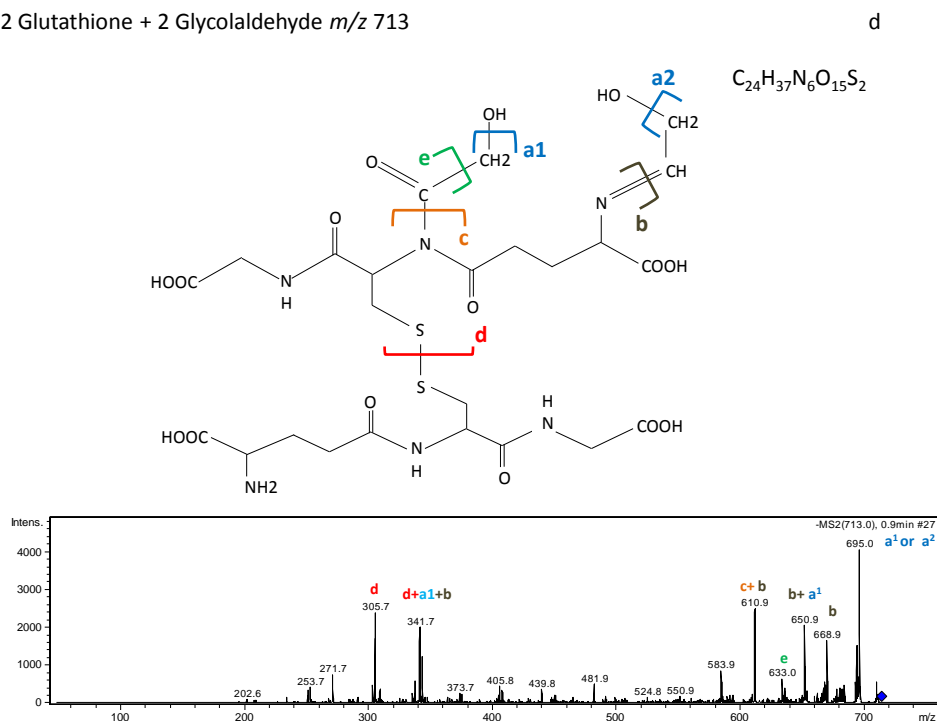


Fig. S3 Conjugation of glutathione with glycolaldehyde. ESI-MS spectra of (a) glutathione and (b) glycolaldehyde and glutathione. ESI-MS² spectra and proposed chemical structures of (c) glutathione and 3 glycolaldehyde adduct, and (d) 2 glutathione and 2 glycolaldehyde adduct.

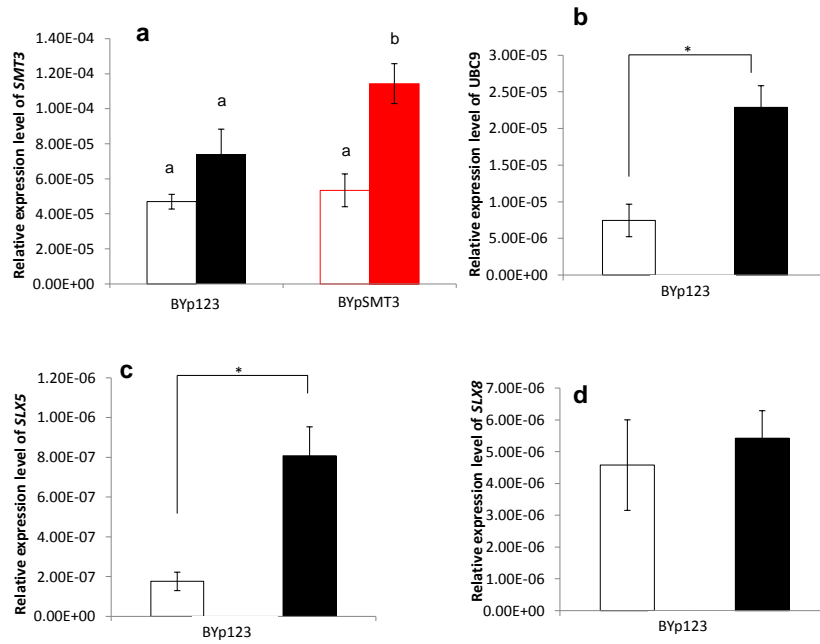


Fig. S4 Exposure to a combination of fermentation inhibitors induced the expression of genes associated with the SUMO pathway. qRT-PCR analysis was performed according to the method described in Jayakody et al. (2013) to measure (a) *SMT3*, (b) *UBC9*, (c) *SLX5*, and (d) *SLX8* expression with mRNA extracted from strains grown in 10 mL of SNY₅ medium after 4 h incubation at 30 °C. The results are expressed as means \pm SEM. Statistically significant differences between the two strains are indicated (* $p < 0.05$). Bars labelled with different letters indicate statistical significance ($p < 0.05$; one-way ANOVA followed by Tukey's post hoc honest significance difference test). BYp123 in SC medium (empty black bars), BYp123 in SYN₅ medium (filled black bars), BYpSMT3 in SC medium (empty red bar), BYpSMT3 in SYN₅ medium (filled red bar).

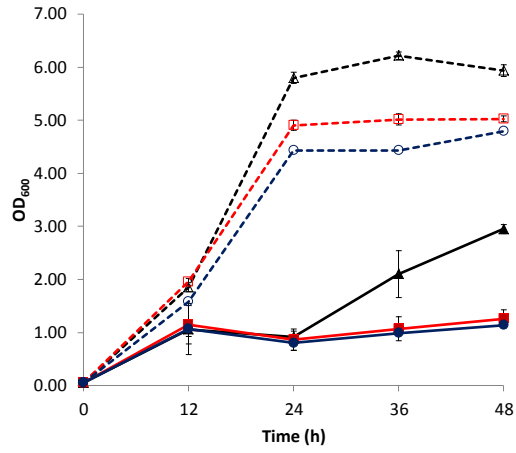


Fig. S5 A mutant defective in the Slx5-Slx8 complex was highly sensitive to the combined effect of inhibitors. (a) BY4743slx5Δ, (b) BY4743slx8Δ. Growth profiles were evaluated in strains grown in 10 mL of SNY₅ medium. The results are expressed as means ± SEM. Statistically significant differences between the two strains are indicated (* $p < 0.05$, ** $p < 0.01$). BY4743 in SC medium (empty black triangle with dashed black line), BYpADH1 in SYN₅ medium (filled black triangle with solid black line), BY4743slx5Δ in SC medium (empty red square with dashed red line), BY4743slx5Δ in SYN₅ medium (filled red square with solid red line). BY4743slx8Δ in SC medium (empty purple circle with dashed purple line), BY4743slx8Δ in SYN₅ medium (filled purple square with solid purple line).

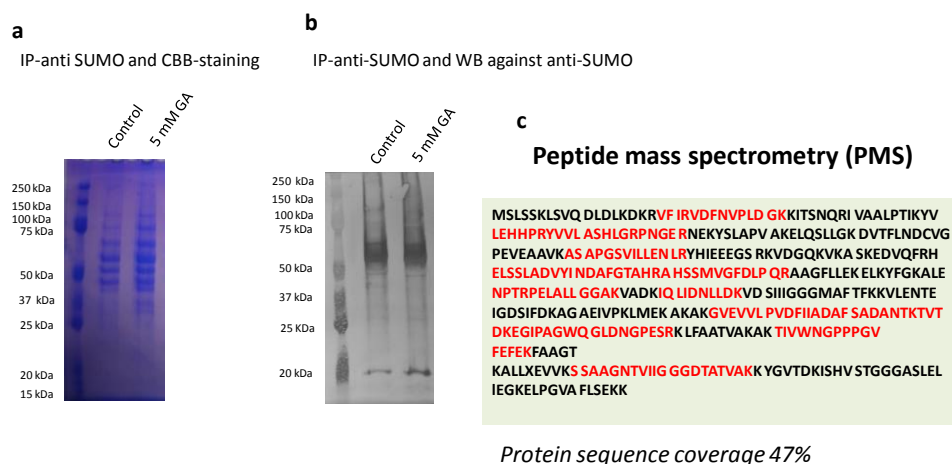


Fig. S6 Glycolaldehyde-induced SUMOylation of Pgk1. (a) CBB staining and (b) Western blot analysis of extracts immunoprecipitated with anti-SUMO antibody of glycolaldehyde-treated cells. (c) PMS analysis of Pgk1. Red letters indicate unmatched peptides in NCBI BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastp&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome).

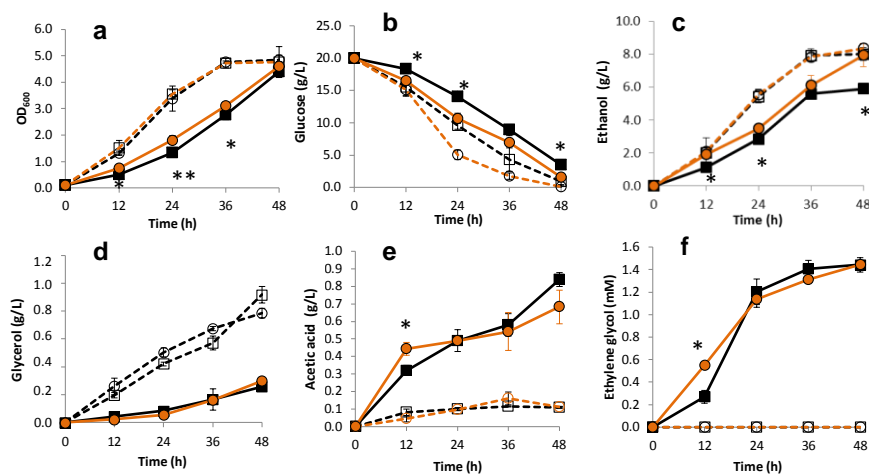


Fig. S7 Fermentation profile of the *SMT3*-expressing strain. The fermentation profile was evaluated for strains grown in 10 mL of SNY₅ medium. (A) Growth profile. (B) Glucose consumption. (C) Ethanol production. (D) Glycerol production. (E) Acetic acid production. (F) Ethylene glycol production. The results are expressed as means \pm SEM. Statistically significant differences between the two strains are indicated (* $p < 0.05$, ** $p < 0.01$). BYp123 in SC medium (empty black square with dashed black line), BYp123 in SYN₅ medium (filled black square with solid black line), BYpSMT3 in SC medium (empty black circle with dashed orange line), BYpSMT3 in SYN₅ medium (filled orange circle with solid orange line).

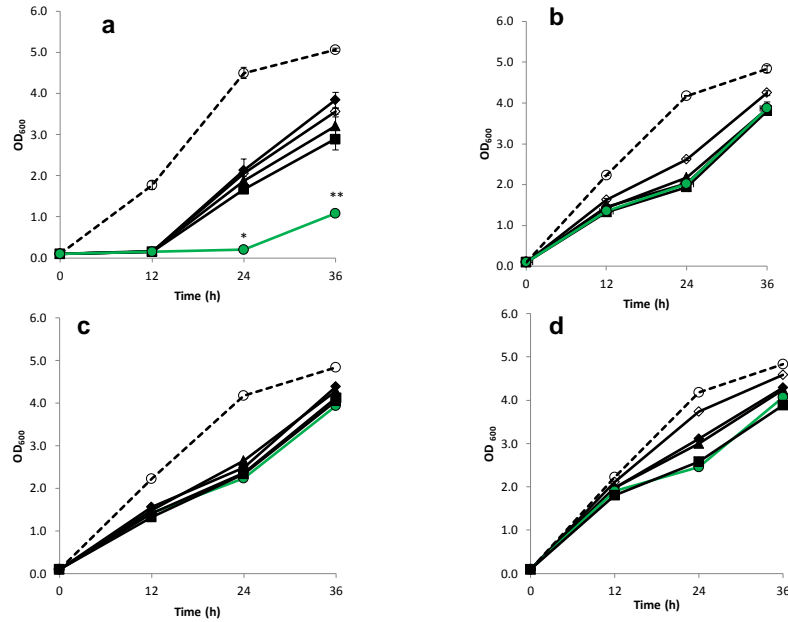


Fig. S8 Inhibition of proteasome activity induced glycolaldehyde toxicity. Growth profiles were evaluated for the BY4743*pdr1* strain grown in 200 μ L of SC medium containing 10 mM fermentation inhibitor (FI) with different concentrations of the proteasome inhibitor MG132 (PI) in 96-well plates. (a) Glycolaldehyde. (b) Furfural. (c) 5-HMF. (d) Methylglyoxal. The OD₆₀₀ values of the samples were measured with a plate reader. The results are expressed as means \pm SEM (n=5). Statistically significant differences between the two strains are indicated (* $p < 0.05$, ** $p < 0.01$). Strain in SC medium without FI or PI (empty black circle with dashed black line), 10 mM of FI (empty black diamond with solid black line), 10 mM of FI and 5 mM of PI (solid black diamond with solid black line), 10 mM of FI and 10 mM of PI (solid black triangle with solid black line), 10 mM of FI and 25 mM of PI (solid black square with solid black line), 10 mM of FI and 50 mM of PI (solid green circle with solid green line).

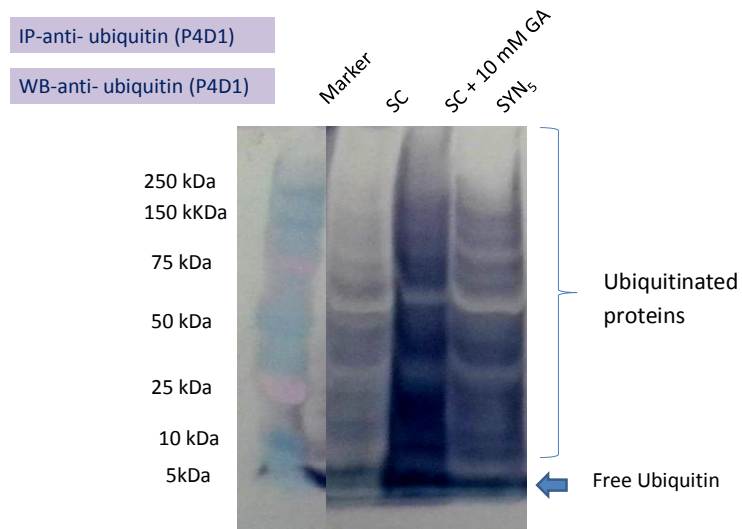


Fig. S9 Glycolaldehyde or inhibitors induce ubiquitination of proteins. Proteins were extracted from the cells grown in 10 mM of glycoaldehyde-containing SC medium or SYN₅ medium. Ubiquitinated proteins were immunoprecipitated with anti-ubiquitin antibody (P4D1), and western blot analysis was performed with anti-ubiquitin antibody (P4D1).

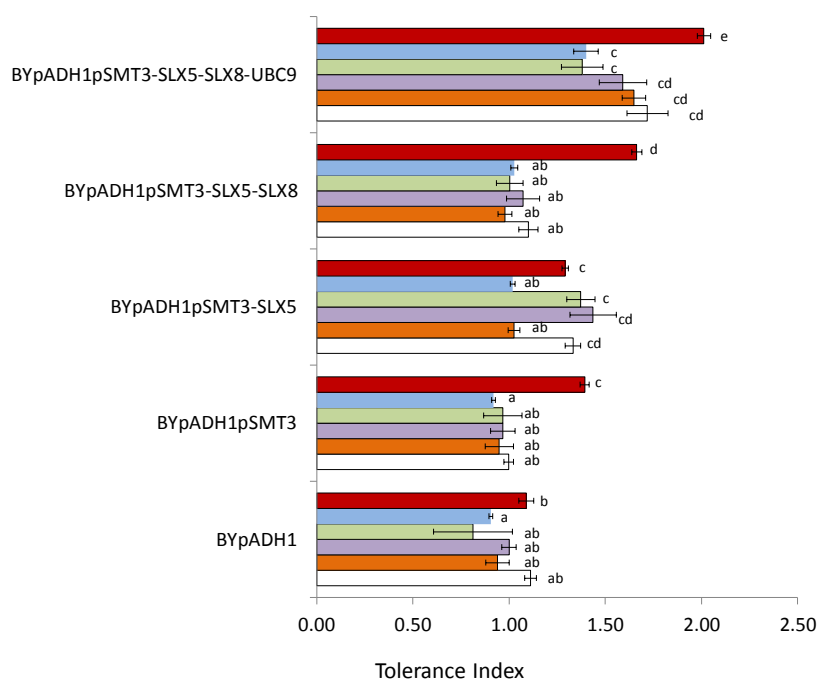


Fig. S10 Single inhibitor tolerance versus combinational inhibitor tolerance. Growth profiles of the developed strains were evaluated in 200 μ L of SC medium containing 5 mM of inhibitor or SYN₅ in 96-well plates. OD₆₀₀ values of samples were measured with a plate reader at 24 h. The tolerance index was calculated as follows: Tolerance index = (OD₆₀₀ of the gene-expressing strain in inhibitor-containing SC medium or SYN₅ medium / OD₆₀₀ of gene-expressing strain in SC medium) / (OD₆₀₀ of the control strain [BYp123] in inhibitor-containing SC medium or SYN₅ medium / OD₆₀₀ of the control strain [BYp123] in SC medium). Bars labelled with different letters indicate statistical significance ($p < 0.05$; one-way ANOVA followed by Tukey's post hoc honest significance difference test). Strain in glycolaldehyde-containing medium (*white bars*), 5-HMF-containing medium (*orange bars*), furfural-containing medium (*purple bars*), methylglyoxal-containing medium (*green bars*), acetic acid-containing medium (*blue bars*), SYN₅ medium (*red bars*).

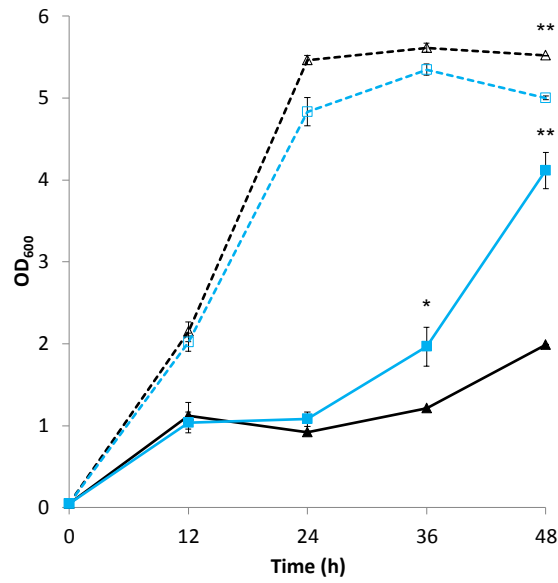


Fig. S11 Growth profile of the Taiwan industrial bioethanol yeast strain expressing components of the SUMO-dependent ubiquitination system. Growth profiles were evaluated by strains grown in 10 mL of SNY₅ medium. The results are expressed as means \pm SEM. Statistically significant differences between the two strains are indicated (* $p < 0.05$, ** $p < 0.01$). Taiwan strain in SC medium (*empty black triangle with dashed black line*), Taiwan strain in SYN₅ medium (*filled black triangle with solid black line*), Taiwan + pSMT3-SLX5-SLX8-UBC9 in SC medium (*empty blue square with dashed blue line*), Taiwan + pSMT3-SLX5-SLX8-UBC9 in SYN₅ medium (*filled blue square with solid blue line*).

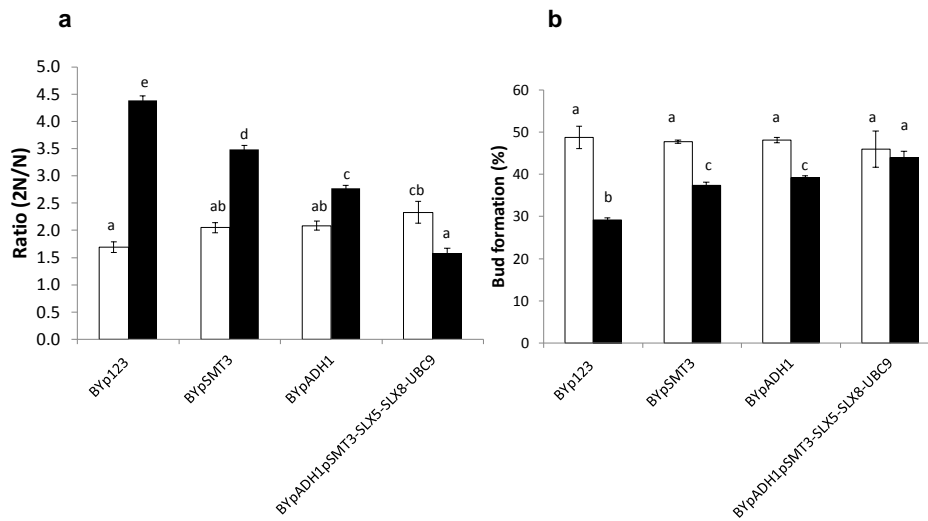


Fig. S12 Fermentation inhibitors, including glycolaldehyde, caused cell cycle arrest. (a) Ratios of 2N:N in FACS analyzed samples. The areas representing N and 2N DNA content from FACS histograms were quantified by Image J software. (b) Bud formation ratios in cells were analyzed by microscopic observation of 200 cells per sample. Results are expressed as means \pm SEM of independent triplicate experiments from respective independent starter cultures. Bars labelled with different letters indicate statistical significance ($p < 0.05$; one-way ANOVA followed by Tukey's post hoc honest significance difference test). Strains in SC medium (*empty black bars*), strains in SYN₅ medium (*filled black bars*).

Supplementary Tables

Table S1 Primers used for PCR and RT-PCR in the study.

	Gene	Forward primer	Reverse primer
PCR primers used for Infusion cloning	<i>SMT3</i> (852122)	HpaI-GAG CTC TAG AGG TTA GCC ATG CTG TTT CCA TCA	HpaI-GAA ATT CGC TTA GTT TGT GGC ACG TCG TGA AAG AAT
	<i>SLX5</i> (851549)	KpnI-GCA TAC AAT CAA CTG GTA CCT GTT CAA AGT GTC ACT TCT TC	KpnI-CTC GAG TCG ACC CGG GTA CCA ACA CCC ACA GCA GCC CA
	<i>SLX8</i> (856852)	Sall-TGG TAC CCG GGT CGA CAG GGA AAG AAA GAA CTG TT	Sall-TAG AGC TCG AGT CGA ATT CCT CGG AGT GCT TTG TT
	<i>UBC9</i> (851495)	SmaI-TGG GTG TTG GTA CCC TGC TTC ATC CAG TTT AGT GGG	SmaI-TTT CCC TGT CGA CCC CCG AAC TAA AAG AAA GAT GCC
RT-PCR primers	<i>SMT3</i> (852122)	CAA GAT CAA AAA GAC CAC TCC	ATG TCC AAA TCT TCA GGG GT
	<i>SLX5</i> (851549)	ACG CCT GAT ATT CGT AGT GCT	CCA CAC AAG CAA CAA ACA GGA
	<i>SLX8</i> (856852)	AGG GAA ACA ATG CAT ACC GAA	TCT TTG GGT GCT TCT TTG GT
	<i>UBC9</i> (851495)	AAG CTG ATG GGT CCA TGG ATT	AAA CAT ATT GTG CCA CTT GGA

Table S2 Identified mutants which show high sensitivities to combined major inhibitors present in hot-compressed water-treated cellulose hydrolyte.

	ORF	Gene	SI	SEM	T _{score}	Function
1	YHR041C (856437)	<i>SRB2</i>	0.49	0.02	0.00	Subunit of the RNA polymerase II mediator complex; associates with core polymerase subunits to form the RNA polymerase II holoenzyme; general transcription factor involved in telomere maintenance
2	YHR059W (856456)	<i>FYV4</i>	0.48	0.00	0.00	Protein of unknown function, required for survival upon exposure to K1 killer toxin (1)
3	YCR105W (850469)	<i>ADH7</i>	0.09	0.00	0.01	NADPH-dependent medium chain alcohol dehydrogenase with broad substrate specificity; member of the cinnamyl family of alcohol dehydrogenases; may be involved in fusel alcohol synthesis or in aldehyde tolerance
4	YGR219W (853134)	<i>YGR219W</i>	0.49	0.01	0.00	Dubious open reading frame unlikely to encode a protein, based on available experimental and comparative sequence data; partially overlaps the verified ORF <i>MRPL9/YGR220C</i>
5	YJL075C (853370)	<i>APQ13</i>	0.38	0.01	0.00	Dubious open reading frame, unlikely to encode a protein; not conserved in closely related <i>Saccharomyces</i> species; 85% of ORF overlaps the verified gene <i>NET1</i> ; null mutant is sensitive to sorbate
6	YKL158W (853699)	<i>APE2</i>	0.49	0.07	0.02	Aminopeptidase yscII; may have a role in obtaining leucine from dipeptide substrates; <i>APE2</i> has a paralog, <i>AAP1</i> , that arose from the whole genome duplication
7	YLR068W (850757)	<i>FYV7</i>	0.49	0.08	0.01	Essential protein required for maturation of 18S rRNA; required for survival upon exposure to K1 killer toxin
8	YLR131C (850822)	<i>ACE2</i>	0.48	0.15	0.04	Transcription factor required for septum destruction after cytokinesis; phosphorylation by Cbk1p blocks nuclear exit during M/G1 transition, causing localization to daughter cell nuclei, and also increases Ace2p activity; phosphorylation by Cdc28p and Pho85p prevents nuclear import during cell cycle phases other than cytokinesis; part of RAM network that regulates cellular polarity and morphogenesis; <i>ACE2</i> has a paralog, <i>SWI5</i> , that arose from the whole genome duplication
9	YML004C (855009)	<i>GLO1</i>	0.48	0.00	0.00	Monomeric glyoxalase I, catalyzes the detoxification of methylglyoxal (a by-product of glycolysis) via condensation with glutathione to produce S-D-lactoylglutathione; expression regulated by methylglyoxal levels and osmotic stress
10	YOR027W (854192)	<i>STH1</i>	0.30	0.01	0.00	Hsp90 cochaperone, interacts with the Ssa group of the cytosolic Hsp70 chaperones and activates Ssa1p ATPase activity; interacts with Hsp90 chaperones and inhibits their ATPase activity; homolog of mammalian Hop
11	YOR036W (854201)	<i>PEP12</i>	0.31	0.00	0.00	Target membrane receptor (t-SNARE) for vesicular intermediates traveling between the Golgi apparatus and the vacuole; controls entry of biosynthetic, endocytic, and retrograde ²⁴ traffic into the prevacuolar compartment; syntaxin
12	YOR065W (854231)	<i>CYT1</i>	0.29	0.01	0.00	Cytochrome c1, component of the mitochondrial respiratory chain; expression is regulated by the heme-activated, glucose-repressed Hap2p/3p/4p/5p CCAAT-binding complex

13	YOR080W (854247)	<i>DIA2</i>	0.50	0.02	0.00	Origin-binding F-box protein; forms an SCF ubiquitin ligase complex with Skp1p and Cdc53p; plays a role in DNA replication; plays a role in transcription; required for correct assembly of RSC complex, correct RSC-mediated transcription regulation, and correct nucleosome positioning; involved in invasive and pseudohyphal growth; required to target Cdc6p for ubiquitin-mediated destruction during G1 phase
14	YOR130C (854297)	<i>ARG11</i>	0.37	0.00	0.00	Ornithine transporter of the mitochondrial inner membrane, exports ornithine from mitochondria as part of arginine biosynthesis; human ortholog is associated with hyperammonaemia-hyperornithinaemia-homocitrullinuria (HHH) syndrome
15	YOR235W (854410)	<i>IRC13</i>	0.49	0.00	0.00	Dubious open reading frame unlikely to encode a protein, based on available experimental and comparative sequence data; null mutant displays increased levels of spontaneous Rad52 foci
16	YOL049W (854108)	<i>GSH2</i>	0.37	0.02	0.00	Glutathione synthetase, catalyzes the ATP-dependent synthesis of glutathione (GSH) from gamma-glutamylcysteine and glycine; induced by oxidative stress and heat shock
17	YPR074C (856188)	<i>TKL1</i>	0.35	0.02	0.00	Transketolase; catalyzes conversion of xylulose-5-phosphate and ribose-5-phosphate to sedoheptulose-7-phosphate and glyceraldehyde-3-phosphate in the pentose phosphate pathway; needed for synthesis of aromatic amino acids; <i>TKL1</i> has a paralog, <i>TKL2</i> , that arose from the whole genome duplication
18	YCR009C (854297)	<i>RVS161</i>	0.43	0.03	0.00	Amphiphysin-like lipid raft protein; interacts with Rvs167p and regulates polarization of the actin cytoskeleton, endocytosis, cell polarity, cell fusion and viability following starvation or osmotic stress
19	YDR025W (851589)	<i>RPS11A</i>	0.43	0.08	0.01	Protein component of the small (40S) ribosomal subunit; homologous to mammalian ribosomal protein S11 and bacterial S17; <i>RPS11A</i> has a paralog, <i>RPS11B</i> , that arose from the whole genome duplication
20	YDR049W (851618)	<i>VMS1</i>	0.47	0.01	0.00	Component of a Cdc48p-complex involved in protein quality control; exhibits cytosolic and ER-membrane localization, with Cdc48p, during normal growth, and contributes to ER-associated degradation (ERAD) of specific substrates at a step after their ubiquitination; forms a mitochondrially-associated complex with Cdc48p and Npl4p under oxidative stress that is required for ubiquitin-mediated mitochondria-associated protein degradation (MAD); conserved in <i>C. elegans</i> and humans
21	YDR057W (851627)	<i>YOS9</i>	0.46	0.16	0.04	ER quality-control lectin; integral subunit of the HRD ligase; binds to glycans with terminal alpha-1,6 linked mannose on misfolded N-glycosylated proteins and participates in targeting proteins to ERAD; member of the OS-9 protein family
22	YDR075W (851647)	<i>PPH3</i>	0.48	0.15	0.04	Catalytic subunit of protein phosphatase PP4 complex; active complex is composed of Pph3p and Psy2p, with Psy4p apparently providing additional substrate specificity in some cases; regulates recovery from the DNA damage checkpoint and also the gene conversion- and single-strand annealing-mediated pathways of meiotic double-strand break repair; involved in activation of Gln3p to alleviate nitrogen catabolite repression; Pph3p and Psy2p localize to foci on meiotic chromosomes

23	YDR153C (851731)	<i>ENT5</i>	0.47	0.01	0.00	Protein containing an N-terminal epsin-like domain involved in clathrin recruitment and traffic between the Golgi and endosomes; associates with the clathrin adaptor Gga2p, clathrin adaptor complex AP-1, and clathrin
24	YEL033W (856678)	<i>MTC7</i>	0.46	0.03	0.00	Predicted metabolic role based on network analysis derived from ChIP experiments, a large-scale deletion study and localization of transcription factor binding sites; null mutant is sensitive to temperature oscillation in a <i>cdc13-1</i> mutant
25	YBR077C (852369)	<i>SLM4</i>	0.48	0.01	0.00	Component of the EGO complex, which is involved in the regulation of microautophagy, and of the GSE complex, which is required for proper sorting of amino acid permease Gap1p; gene exhibits synthetic genetic interaction with <i>MSS4</i>
26	YBR078W (852370)	<i>ECM33</i>	0.45	0.03	0.00	GPI-anchored protein of unknown function; possible role in apical bud growth; GPI-anchoring on the plasma membrane crucial to function; phosphorylated in mitochondria; similar to Sps2p; ECM33 has a paralog, <i>PST1</i> , that arose from the whole genome duplication
27	YOL148C (854017)	<i>SPT20</i>	0.37	0.04	0.00	Subunit of the SAGA transcriptional regulatory complex; involved in maintaining the integrity of the complex; mutant displays reduced transcription elongation in the G-less-based run-on (GLRO) assay
28	YDL006W (851558)	<i>PTC1</i>	0.41	0.00	0.00	Type 2C protein phosphatase (PP2C); dephosphorylates Hog1p, inactivating osmosensing MAPK cascade; involved in Fus3p activation during pheromone response; deletion affects precursor tRNA splicing, mitochondrial inheritance, and sporulation
29	YDL013W (851549)	<i>SLX5</i>	0.45	0.00	0.00	Subunit of the Slx5-Slx8 SUMO-targeted ubiquitin ligase (STUbL) complex, stimulated by SUMO-modified substrates; contains a RING domain and two SIMs (SUMO-interacting motifs); forms SUMO-dependent nuclear foci, including DNA repair centers
30	YDR484W (852095)	<i>VPS52</i>	0.45	0.01	0.00	Component of the GARP (Golgi-associated retrograde protein) complex, Vps51p-Vps52p-Vps53p-Vps54p, which is required for the recycling of proteins from endosomes to the late Golgi; involved in localization of actin and chitin
31	YDR495C (852106)	<i>VPS3</i>	0.44	0.03	0.00	Component of CORVET tethering complex; cytoplasmic protein required for the sorting and processing of soluble vacuolar proteins, acidification of the vacuolar lumen, and assembly of the vacuolar H ⁺ -ATPase
32	YHR038W (856433)	<i>RRF1</i>	0.47	0.01	0.00	Mitochondrial ribosome recycling factor, essential for mitochondrial protein synthesis and for the maintenance of the respiratory function of mitochondria
33	YKR007W (853876)	<i>MEH1</i>	0.38	0.02	0.00	Component of the EGO complex, which is involved in the regulation of microautophagy, and of the GSE complex, which is required for proper sorting of amino acid permease Gap1p; loss results in a defect in vacuolar acidification
34	YGR285C (853202)	<i>ZUO1</i>	0.43	0.01	0.00	Ribosome-associated chaperone, functions in ribosome biogenesis and, in partnership with Ssz1p and Ssb1/2, as a chaperone for nascent polypeptide chains; contains a DnaJ domain and functions as a J-protein partner for Ssb1p and Ssb2p
35	YAL035W (851196)	<i>FUN12</i>	0.43	0.02	0.00	GTPase required for general translation initiation; promotes Met-tRNA ^{iMet} binding to ribosomes and ribosomal subunit joining; promotes GTP-dependent maturation of 18S

						rRNA by Nob1p; protein abundance increases in response to DNA replication stress; homolog of bacterial IF2
36	YLR148W (850840)	PEP3	0.46	0.00	0.00	Component of CORVET tethering complex; vacuolar peripheral membrane protein that promotes vesicular docking/fusion reactions in conjunction with SNARE proteins, required for vacuolar biogenesis
37	YLR234W (850935)	TOP3	0.49	0.01	0.00	DNA Topoisomerase III, conserved protein that functions in a complex with Sgs1p and Rmi1p to relax single-stranded negatively-supercoiled DNA preferentially, involved in telomere stability and regulation of mitotic recombination
38	YLR337C (851051)	VRP1	0.35	0.02	0.00	Proline-rich actin-associated protein involved in cytoskeletal organization and cytokinesis; related to mammalian Wiskott-Aldrich syndrome protein (WASP)-interacting protein
39	YLR338W (851050)	OPI9	0.39	0.01	0.00	Dubious open reading frame unlikely to encode a protein, based on available experimental and comparative sequence data; partially overlaps the verified ORF <i>VRP1/YLR337C</i>
40	YNL280C (855441)	ERG24	0.48	0.02	0.00	C-14 sterol reductase, acts in ergosterol biosynthesis; mutants accumulate the abnormal sterol ignosterol (ergosta-8,14 dienol), and are viable under anaerobic growth conditions but inviable on rich medium under aerobic conditions
41	YOL051W (854106)	GAL11	0.50	0.04	0.00	Subunit of the RNA polymerase II mediator complex; associates with core polymerase subunits to form the RNA polymerase II holoenzyme; affects transcription by acting as target of activators and repressors; forms part of the tail domain of mediator
42	YPL254W (855821)	HFII	0.42	0.02	0.00	Adaptor protein required for structural integrity of the SAGA complex, a histone acetyltransferase-coactivator complex that is involved in global regulation of gene expression through acetylation and transcription functions
43	YPL045W (856062)	VPS16	0.36	0.01	0.00	Subunit of the vacuole fusion and protein sorting HOPS complex and the CORVET tethering complex; part of the Class C Vps complex essential for membrane docking and fusion at Golgi-to-endosome and endosome-to-vacuole protein transport stages
44	YDR027C (851591)	VPS54	0.44	0.01	0.00	Component of the GARP (Golgi-associated retrograde protein) complex, Vps51p-Vps52p-Vps53p-Vps54p, which is required for the recycling of proteins from endosomes to the late Golgi; potentially phosphorylated by Cdc28p
45	YFR001W (850551)	LOC1	0.42	0.00	0.00	Nuclear protein involved in asymmetric localization of <i>ASH1</i> mRNA; binds double-stranded RNA in vitro; constituent of 66S pre-ribosomal particles; relocates from nucleus to cytoplasm upon DNA replication stress
46	YGL058W (852822)	RAD6	0.45	0.02	0.00	Ubiquitin-conjugating enzyme (E2), involved in postreplication repair (as a heterodimer with Rad18p), DSB repair and checkpoint control (as a heterodimer with Bre1p), ubiquitin-mediated N-end rule protein degradation
47	YGL070C (852810)	RPB9	0.48	0.06	0.01	RNA polymerase II subunit B12.6; contacts DNA; mutations affect transcription start site selection and fidelity of transcription
48	YJR090C (853552)	GRR1	0.35	0.01	0.00	F-box protein component of the SCF ubiquitin-ligase complex; Grr1p interacts with G1 cyclin Cln3p, and Grr1p and Cdc4p redundantly target Cln3p for degradation; involved in carbon catabolite repression, glucose-dependent divalent cation transport, high-affinity

						glucose transport, morphogenesis, and sulfite detoxification
49	YJL028W (853425)	YJL028W	0.40	0.02	0.00	Protein of unknown function; may interact with ribosomes, based on co-purification experiments
50	YJL184W (853255)	<i>GON7</i>	0.49	0.00	0.00	Component of the EKC/KEOPS protein complex with Kae1p, Cgi121p, Pcc1p, and Bud32p; EKC/KEOPS complex is required for t6A tRNA modification and may have roles in telomere maintenance and transcription; implicated in osmotic stress response
51	YJL189W (853250)	<i>RPL39</i>	0.47	0.01	0.00	Subunit of the SWI/SNF chromatin remodeling complex; involved in transcriptional regulation; functions interdependently in transcriptional activation with Snf2p and Snf6p; relocates to the cytosol under hypoxic conditions
52	YBR289W (852592)	<i>SNF5</i>	0.47	0.01	0.00	Subunit of the SWI/SNF chromatin remodeling complex; involved in transcriptional regulation; functions interdependently in transcriptional activation with Snf2p and Snf6p; relocates to the cytosol under hypoxic conditions
53	YCR047C (850414)	<i>BUD23</i>	0.50	0.02	0.00	Methyltransferase, methylates residue G1575 of 18S rRNA; required for rRNA processing and nuclear export of 40S ribosomal subunits independently of methylation activity; diploid mutant displays random budding pattern
54	YFR019W (850574)	<i>FAB1</i>	0.47	0.01	0.00	1-phosphatidylinositol-3-phosphate 5-kinase; vacuolar membrane kinase that generates phosphatidylinositol (3,5)P ₂ , which is involved in vacuolar sorting and homeostasis
55	YGL135W (852742)	<i>RPL1B</i>	0.50	0.03	0.00	Ribosomal 60S subunit protein L1B; N-terminally acetylated; homologous to mammalian ribosomal protein L10A and bacterial L1; RPL1B has a paralog, RPL1A, that arose from the whole genome duplication; rpl1a rpl1b double null mutation is lethal
56	YAL021C (851212)	<i>CCR4</i>	0.48	0.02	0.00	Component of the CCR4-NOT transcriptional complex, which is involved in regulation of gene expression; component of the major cytoplasmic deadenylase, which is involved in mRNA poly(A) tail shortening
57	YLR371W (851086)	<i>ROM2</i>	0.45	0.01	0.00	GDP/GTP exchange factor (GEF) for Rho1p and Rho2p; mutations are synthetically lethal with mutations in rom1, which also encodes a GEF; Rom2p localization to the bud surface is dependent on Ack1p; <i>ROM2</i> has a paralog, <i>ROM1</i> , that arose from the whole genome duplication
57	YNR052C (855788)	<i>POP2</i>	0.37	0.01	0.00	RNase of the DEDD superfamily, subunit of the Ccr4-Not complex that mediates 3' to 5' mRNA deadenylation
59	YOL145C (854020)	<i>CTR9</i>	0.43	0.00	0.00	Component of the Paf1p complex involved in transcription elongation; binds to and modulates the activity of RNA polymerases I and II; required for expression of a subset of genes, including cyclin genes; involved in <i>SER3</i> repression by helping to maintain <i>SRG1</i> transcription-dependent nucleosome occupancy; contains TPR repeats
60	YNL147W (855575)	<i>LSM7</i>	0.37	0.00	0.00	Lsm (Like Sm) protein; part of heteroheptameric complexes (Lsm2p-7p and either Lsm1p or 8p); cytoplasmic Lsm1p complex involved in mRNA decay; nuclear Lsm8p complex part of U6 snRNP and possibly involved in processing tRNA, snoRNA, and rRNA; protein

						abundance increases and forms cytoplasmic foci in response to DNA replication stress
61	YBR191W (852489)	<i>RPL21A</i>	0.44	0.00	0.00	Ribosomal 60S subunit protein L21A; homologous to mammalian ribosomal protein L21, no bacterial homolog; <i>RPL21A</i> has a paralog, <i>RPL21B</i> , that arose from the whole genome duplication
62	YDR174W (851754)	<i>HMO1</i>	0.45	0.00	0.00	Chromatin associated high mobility group (HMG) family member involved in genome maintenance; rDNA-binding component of the Pol I transcription system; associates with a 5'-3' DNA helicase and Fpr1p, a prolyl isomerase
63	YGR063C (852955)	<i>SPT4</i>	0.46	0.00	0.00	Component of the universally conserved Spt4/5 complex (DSIF complex); the complex has multiple roles in concert with RNA polymerases I and II, including regulation of transcription elongation, RNA processing, quality control, and transcription-coupled DNA repair; Spt4p also localizes to kinetochores and heterochromatin and affects chromosome dynamics and silencing; required for transcription through lengthy trinucleotide repeats in ORFs or non-protein coding regions
64	YGR092W (852984)	<i>DBF2</i>	0.33	0.00	0.00	Ser/Thr kinase involved in transcription and stress response; functions as part of a network of genes in exit from mitosis; localization is cell cycle regulated; activated by Cdc15p during the exit from mitosis; also plays a role in regulating the stability of <i>SWI5</i> and <i>CLB2</i> mRNAs; phosphorylates Chs2p to regulate primary septum formation and Hof1p to regulate cytokinesis; <i>DBF2</i> has a paralog, <i>DBF20</i> , that arose from the whole genome duplication
65	YJL175W (853263)	YJL175W	0.30	0.01	0.00	Dubious open reading frame unlikely to encode a functional protein; deletion confers resistance to cisplatin, hypersensitivity to 5-fluorouracil, and growth defect at high pH with high calcium; overlaps gene for SWI3 transcription factor
66	YER116C (856852)	<i>SLX8</i>	0.49	0.01	0.00	Subunit of Slx5-Slx8 SUMO-targeted ubiquitin ligase (STUbL) complex; stimulated by prior attachment of SUMO to the substrate; contains a C-terminal RING domain; forms nuclear foci upon DNA replication stress
67	YGL127C (852750)	<i>SOH1</i>	0.37	0.03	0.00	Subunit of the RNA polymerase II mediator complex; associates with core polymerase subunits to form the RNA polymerase II holoenzyme; involved in telomere maintenance; conserved with other metazoan <i>MED31</i> subunits
68	YBR081C (852373)	<i>SPT7</i>	0.43	0.02	0.00	Subunit of the SAGA transcriptional regulatory complex, involved in proper assembly of the complex; also present as a C-terminally truncated form in the SLIK/SALSA transcriptional regulatory complex