

Figure S1. Phylogenetic analysis of AKRs including the putative ERs *GCY1*, *YPR1*, *GRE3*, *ARA1*, *YJR096W*, and *YDL124W* of *S.cerevisiae*, *S. kudriavzevii* and *S.uvarum*; the described ERs from *Magnaporthe grisea*, *Yarrowia lipolytica*, *Trichosporonoides megachiliensis*, *Candida magnoliae* and the aldehyde reductase of *Sporidiobolus salmonicolor*.

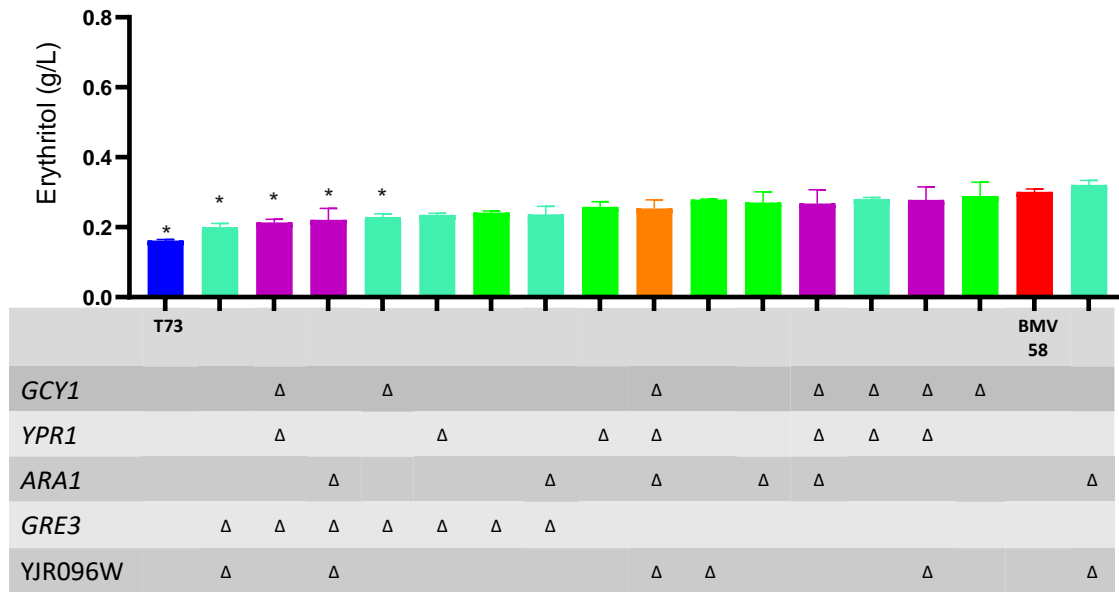


Figure S2. Concentration of erythritol produced by *S. cerevisiae* T73 (dark blue), *S. uvarum* BMV58 (red) as WT, the single mutants (green), double mutants (light blue), triple mutants (purple), quadruple mutant (orange) and quintuple mutant, with all selected genes deleted (black). This concentration was measured after fermentation on SM with 200 g/L glucose + fructose and 200 mg/L YAN. Standard deviation is shown, and the asterisk represents significant differences at the level of 0.05 in comparison with the WT (BMV58).

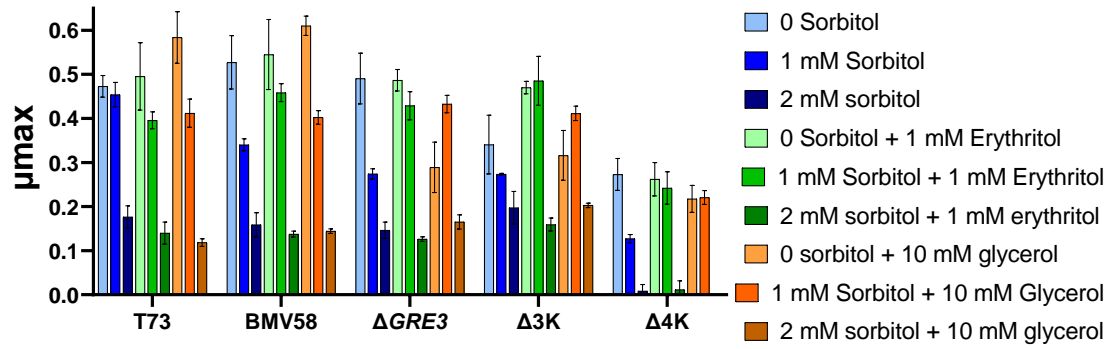


Figure S3. μ_{\max} (log. OD/h) of *S. cerevisiae* (T73), *S. uvarum* (BMV58), BMV58 mutants $\Delta GRE3$, $\Delta YJR096W$, $\Delta YPR1\Delta GCY1\Delta GRE3$ ($\Delta 3K$), and $\Delta YPR1\Delta GCY1\Delta ARA1\Delta YJR096W$ (4K) in YNB at 25°C without sorbitol and with different concentrations of sorbitol, as well as sorbitol and the addition of erythritol or glycerol. Values represents media of biological triplicates and standard deviation is shown.

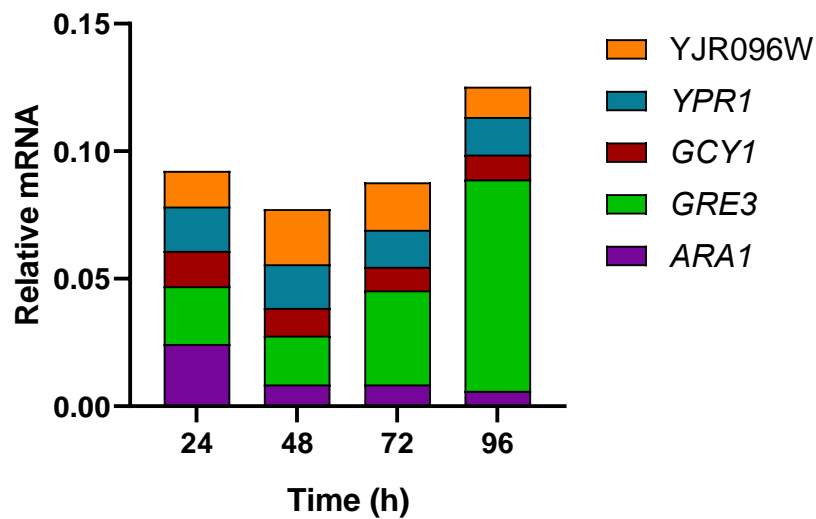


Figure S4. Representation of the level of expression of the selected genes (*YJR096W*, *YPR1*, *GCY1*, *GRE3* and *ARA1*) at 24, 48, 72 and 96 hours of fermentation (SM with 240 g/L sugars and 300 mg/L YAN) by *S. uvarum* BMV58. The values represent media of biological and technical triplicates.

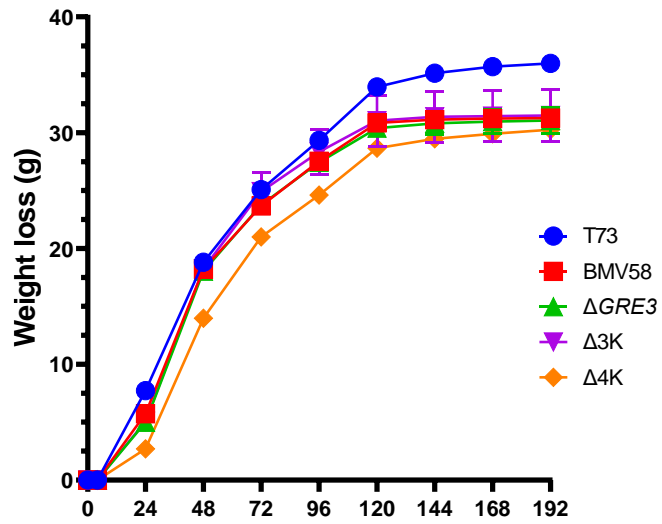


Figure S5. Growth of the strains *S. cerevisiae* T73 (blue), *S. uvarum* BMV58 (red), $\Delta GRE3$ (green), $\Delta YPR1\Delta GCY1\Delta GRE3$ ($\Delta 3K$, pink), and $\Delta YPR1\Delta GCY1\Delta ARA1\Delta YJR096W$ (4K, orange) along the fermentation on 240 g/L and 300 YAN.

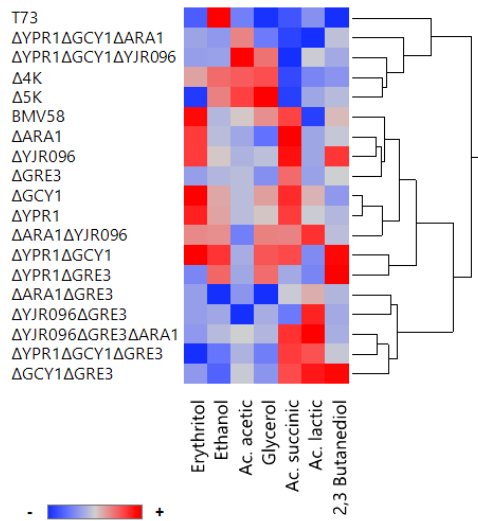


Figure S6. Heat map of *S. cerevisiae* T73, *S. uvarum* BMV58 and BMV58 mutants showing the media of the concentration of the main metabolites produced after fermentation on SM with 240 g/L glucose + fructose and 300 YAN. The colours goes from blue (low concentration) to red (high concentration).

Table S1. Primers used to knock out the selected genes by CRISPR-Cas9 in BMV58 and the primers used to prove the deletion.

Primer name	Sequence (5' to 3')	Utilization
gRNA-GCY1-BMV58_Fw	TGATATAGTTTGGATCTAAGGTTTTAGAGCTAGAAATAGCAAGTTAA AATAAGG	gRNA to guide Cas9 for deletion of YOR120W in BMV58
gRNA-GCY1-BMV58_Rv	CTTAGATCCAAACTATATCAGATCATTATCTTTCACTGCGGAG	gRNA to guide Cas9 for deletion of YOR120W in BMV58 (reverse)
DR-GCY1-BMV58	CTCAAGCTAGAAGTTAATATAGCACTATCACAAACTTAGAAAATAG TCGTACCTATGTTTTCGAAACAACCAAGACATTTAGCTAA	Donor DNA for YOR120W deletion
DR-GCY1-BMV58_Rv	TTAGCTAAATGTCTTGGTTGTTTCGAAAACATAGGTACGACTATTTTC TAAGTTTTGTGATAGTGCTATATTAACCTTCTAGCTTGAG	Reverse complement of previous single DNA strand
Check_GCY1-BMV58_Fw	ATTGGCATGCTTTCACCATA	Forward control PCR for YOR120W-BMV58 deletion
Check_GCY1-BMV58_Rv	CTCGTTGCTCGACATTTTCAT	Reverse control PCR for YOR120W-BMV58 deletion
gRNA-YPR1-BMV58_Fw	TCGACGTTGACACCAAGGAAGTTTTAGAGCTAGAAATAGCAAGTTA AAATAAGG	gRNA to guide Cas9 for deletion of YDR368W in BMV58
gRNA-YPR1-BMV58_Rv	TTCCTTGGTGTCAACGTCGAGATCATTATCTTTCACTGCGGAG	gRNA to guide Cas9 for deletion of YDR368W in BMV58 (reverse)
DR-YPR1-BMV58	CATTGATTTTCATTGCTCAAAGTACGCTTATATAGTAATTGACCTGAAC AAGCTACGTCACAGCAGATAGCTTGAATTTCTTTACT	Donor DNA for YDR368W deletion
DR-YPR1-BMV58_Rv	AGTAAAGGAAATTCAGCTATCTGCTGTGACGTAGCTTGTTCAGGTC AATTACTATATAAGCGTACTTTGAGCAATGAAATCAATG	Reverse complement of previous single DNA strand
Check_YPR1-BMV58_Fw	TGATGCTTTCATGCTTGCT	Forward control PCR for YDR368W-BMV58 deletion
Check_YPR1-BMV58_Rv	GGACGGCTTTGAATTGGTTA	Reverse control PCR for YDR368W-BMV58 deletion
gRNA-Gre3-BMV58_Fw	GGTGCAGAGGACGACAAGAGGTTTTAGAGCTAGAAATAGCAAGTT AAAATAAGG	gRNA to guide Cas9 for deletion of YHR104W in BMV58
gRNA-Gre3-BMV58_Rv	CTCTTGTCGTCCTCTGCACCGATCATTATCTTTCACTGCGGAG	gRNA to guide Cas9 for deletion of YHR104W in BMV58 (reverse)
DR-Gre3-BMV58	TCGTAGCTAGCATACCCCAAAAAAAAAAAAAAAAAAGAGAAATCAAATAA TTAACCAATGACGGATATGAGCATACCTCCCTTACTTC	Donor DNA for YHR104W deletion
DR-Gre3-BMV58_Rv	GAAGTAAAGGGAGGTATGCTCATATCCGTCATTGGGTTAATTATTTG AATTTCTCTTTTTTTTTTTTTGGGGTATGCTAGCTACGA	Reverse complement of previous single DNA strand
Check_Gre3-BMV58_Fw	TTGTCAACCATAACAACAGCA	Forward control PCR for YHR104W-BMV58 deletion
Check_Gre3-BMV58_Rv	GGAAACAGCACATTCAACACTT	Reverse control PCR for YHR104W-BMV58 deletion
gRNA-Ara1-BMV58_Fw	AGAGGGTCTATCAAAGGGGTTTTAGAGCTAGAAATAGCAAGTTA AAATAAGG	gRNA to guide Cas9 for deletion of YBR149W in BMV58
gRNA-Ara1-BMV58_Rv	CCCTTTTGATAGAACCCTCTGATCATTATCTTTCACTGCGGAG	gRNA to guide Cas9 for deletion of YBR149W in BMV58 (reverse)
DR-Ara1-BMV58	TTCAATTTGATAAAAAAAAAAGTTTGCTCTCAATCAACATCATCTAGG GTCCTTGAGACTCAGTTTATATCTATCGACCTTATTTT	Donor DNA for YBR149W deletion

DR-Ara1-BMV58_Rv	AAAATAAAGGTCGATAGATATAAACTGAGTCTCAAGGACCCTAGATGATGTTGATTGAGAGCAAACCTTTTTTTTATCAAATTGAA	Reverse complement of previous single DNA strand
Check_Ara1-BMV58_Fw	GTGCATCTGGAACTCTTCTGTA	Forward control PCR for YBR149W-BMV58 deletion
Check_Ara1-BMV58_Rw	CCAAAAAGGCCTGAGAAACAAT	Reverse control PCR for YBR149W-BMV58 deletion
gRNA-YJR096W-BMV58_Fw	GTCACAGCTGAAACATAAGCGTTTTAGAGCTAGAAATAGCAAGTTAAATAAGG	gRNA to guide Cas9 for deletion of YJR096W in BMV58
gRNA-YJR096W-BMV58_Rv	GCTTATGTTTCAGCTGTGACGATCATTTATCTTTCCTGCGGAG	gRNA to guide Cas9 for deletion of YJR096W in BMV58 (reverse)
DR-YJR096W-BMV58	GTTTTAAAGTAAAGGAAAAAGGACTAAGTCAGCATCTCTAATTAAAGCGGAACATTGGACCTTGTGTAAACTTATAAACTTTTC	Donor DNA for YJR096W deletion
DR-YJR096W-BMV58_Rv	GAAAAAGTTTATAAGTTTACACAAGGTCCAATGTTCCGCTTTAATTAGAGATGCTGACTTAGTCTTTTTCTTTACTTTAAAAAC	Reverse complement of previous single DNA strand
Check_YJR096W-BMV58_Fw	TTCATTTCTATCCCTCGCCATC	Forward control PCR for YJR096W-BMV58 deletion
Check_YJR096W-BMV58_Rw	CCGGGTAACAGGTTCTACGA	Reverse control PCR for YJR096W-BMV58 deletion

Table S2. Primers used to quantify the expression of the selected genes involved in erythritol and glycerol.

Primer name	Sequence (5' to 3')	Utilization
Gcy1-Qp_Su_F	AAGAAGATGGCTCTCGTCCA	qPCR YOR120W S.u
Gcy1-Qp_Su_R	CAACCGCCCTCGTCTTATTA	qPCR YOR120W S.u
Ypr1-Qp_Su_FW	GAGTGCCAATGCTCCGTTAT	qPCR YDR368W S.u
Ypr1-Qp_Su_RV	CGTAGCCTCTTTGGATGCTC	qPCR YDR368W S.u
Ara1-Qp_Su_FW	TTGATACCGCTTGGGCTTAC	qPCR YBR149W S.u
Ara1-Qp_Su_RV	CCCATAAGACAGGCCACACT	qPCR YBR149W S.u
Gre3-Qp_Su_FW	GGACGACAAGAGGGGTCATA	qPCR YHR104W S.u
Gre3-Qp_Su_RV	AACCAAGCTCCCTTGGAAAT	qPCR YHR104W S.u
YJR096W-Qp_Su_FW	TCGTAATCCGGGTCAAGTTC	qPCR YJR096W S.u
YJR096W-Qp_Su_RV	TTTCATCTGGTCATCCGACA	qPCR YJR096W S.u
Gcy1-Qp_Sc_F	AGGATGGTTCTCGTGCAGTG	qPCR YOR120W S.c
Gcy1-Qp_Sc_R	TCGACTTGGTTAGCAGCTGG	qPCR YOR120W S.c
Ypr1-Qp_Sc_FW	GTGTTGGGTTTCGGCACTTG	qPCR YDR368W S.c
Ypr1-Qp_Sc_RV	TGTTCCGTACCCCAAAGCTT	qPCR YDR368W S.c
Ara1-Qp_Sc_FW	CAGCACTGGGTTTGGGGAC	qPCR YBR149W S.c
Ara1-Qp_Sc_RV	CTCTGTCTCGTAGGCCCAAG	qPCR YBR149W S.c
Gre3-Qp_Sc_FW	AACGGTCTGAAAATGCCCTT	qPCR YHR104W S.c
Gre3-Qp_Sc_RV	GGCTTTCCTGATACCTTCACCA	qPCR YHR104W S.c
YJR096W-Qp_Sc_FW	TCAAACGGCTTCAAATCCCAA	qPCR YJR096W S.c
YJR096W-Qp_Sc_RV	ACCATCGCCAACCTTCCTTCT	qPCR YJR096W S.c
GPD1-qPCR_F	TGTGGTGCTTTGAAGAACG	qPCR GPD1 S.c, S.u
GPD1-qPCR_R	GTTTCTTCTCTAGATTCTGG	qPCR GPD1 S.c, S.u
GPD2-qPCR_F	GTTCCACAGACCWTACTTCC	qPCR GPD2 S.c, S.u
GPD2-qPCR_R	CCATCCCATACTTCTACG	qPCR GPD2 S.c, S.u
FPS1-qPCR_F	GTTTTGYGTTTTTCCAAAGC	qPCR FPS1 S.c, S.u
FPS1-qPCR_R	TGATAAGCCATRGARGCATT	qPCR FPS1 S.c, S.u
STL1-qPCR_F	GCTTATTGGATTGATTTTGGG	qPCR STL1 S.c, S.u
STL1-qPCR_R	TGTTAACAGCATCGTGAAGC	qPCR STL1 S.c, S.u
ACT1-qPCR_F	CATGTTCCAGGTATTGCCG	qPCR ACT1 S.c, S.u
ACT1-qPCR_R	GCCAAAGCGGTGATTTCTT	qPCR ACT1 S.c, S.u
18S-qPCR_F	TTGCGATAACGAACGAGACC	qPCR 18S S.c, S.u
18S-qPCR_R	CATCGGCTTGAAACCGATAG	qPCR 18S S.c, S.u

Table S3. *S. cerevisiae* genes with homology to YALI0F18590g ER27 in *Y. lipolytica*.

Description	Max Score	Total Score	Query Cover	E value	Per Ident	Main functions
Gcy1p/ YOR120W	233	233	86%	4e ⁻⁷⁴	45.49%	Glycerol dehydrogenase and mRNA binding activity (Jung et al. 2012).
Ypr1p/ YDR368W	229	229	89%	7e ⁻⁷³	45.85%	Reduces multiple substrates including 2-methylbutyraldehyde and D, L-glyceraldehyde (Chang et al. 2007).
Gre3p/ YHR104W	176	176	88%	7e ⁻⁵¹	33.97%	Involved in methylglyoxal, D-xylose, arabinose, and galactose metabolism (Träff, Jönsson, and Hahn-Hägerdal 2002).
Ara1p/ YBR149W	160	160	92%	2e ⁻⁴⁵	34.06%	Arabinose dehydrogenase dependent of NADP+. It is involved in carbohydrate metabolism (Amako et al. 2006).
YJR096Wp	155	155	88%	2e ⁻⁴⁴	36.03%	Xylose and arabinose reductase and involved in the response to DNA damage (Chang et al. 2007).
YDL124Wp	153	153	88%	2e ⁻⁴³	36.60%	Catalyses the NADPH-dependent reduction of aromatic α -keto amides and α -keto esters (Ishihara et al. 2004).

Table S4. List of the strain names used and the genes that are deleted on BMV58.

	<i>GCY1</i>	<i>YPR1</i>	<i>ARA1</i>	<i>GRE3</i>	<i>YJR096W</i>
ΔGCY1	Δ				
ΔYPR1		Δ			
ΔARA1			Δ		
ΔGRE3				Δ	
ΔYJR096					Δ
ΔARA1ΔGRE3			Δ	Δ	
ΔYJR096ΔGRE3				Δ	Δ
ΔARA1ΔYJR096			Δ		Δ
ΔYJR096ΔGRE3ΔARA1			Δ	Δ	Δ
ΔYPR1ΔGCY1	Δ	Δ			
ΔGCY1ΔGRE3	Δ			Δ	
ΔYPR1ΔGRE3		Δ		Δ	
Δ3K	Δ	Δ		Δ	
ΔYPR1ΔGCY1ΔARA1	Δ	Δ	Δ		
ΔYPR1ΔGCY1ΔYJR096	Δ	Δ			Δ
Δ4K	Δ	Δ	Δ		Δ
Δ5K	Δ	Δ	Δ		Δ

Table S5. Erythritol concentrations after fermentation on SM with different sugar and YAN concentrations produced by BMV58, $\Delta GRE3$, and $\Delta YPR1\Delta GCY1\Delta GRE3$. Values represent media of biological triplicates by total amount (g/L), yield per gram of glucose (g/g) and percentage of erythritol production of the mutants compared with the WT.

240 g/L glucose and fructose, 300 mg/L YAN			
	Erythritol (g/L)	Erythritol (g/g)	% Erythritol
WT (BMV58)	0,73 ± 0,02	0,0032 ± 0,0001	
$\Delta GRE3$	0,40 ± 0,01	0,0018 ± 0,0002	55,23
$\Delta YPR1\Delta GCY1\Delta GRE3$	0,21 ± 0,03	0,0010 ± 0,0007	29,85
240 g/L glucose and fructose, 200 mg/L YAN			
	Erythritol (g/L)	Erythritol (g/g)	% Erythritol
WT (BMV58)	0,55 ± 0,02	0,0024 ± 0,0001	
$\Delta GRE3$	0,40 ± 0,04	0,0017 ± 0,0002	73,98
$\Delta YPR1\Delta GCY1\Delta GRE3$	0,30 ± 0,01	0,0013 ± 0,00001	55,97
260 g/L glucose and fructose, 200 mg/L YAN			
	Erythritol (g/L)	Erythritol (g/g)	% Erythritol
WT (BMV58)	0,65 ± 0,03	0,0029 ± 0,0001	
$\Delta GRE3$	0,45 ± 0,04	0,0020 ± 0,0002	69,01
$\Delta YPR1\Delta GCY1\Delta GRE3$	0,35 ± 0	0,0016 ± 0	54,41
200 g/L glucose and fructose, 200 mg/L YAN			
	Erythritol (g/L)	Erythritol (g/g)	% Erythritol
WT (BMV58)	0,30 ± 0,009	0,0015 ± 0	
$\Delta GRE3$	0,24 ± 0,01	0,0012 ± 0	79,98
$\Delta YPR1\Delta GCY1\Delta GRE3$	0,21 ± 0,01	0,0011 ± 0	69,99
20 g/L glucose, 1.25 g/L eritrosa			
	Erythritol (g/L)	Erythritol (g/g)	% Erythritol
WT (BMV58)	0,466 ± 0.03	0,023 ± 0.001	
$\Delta GRP3$	0,453 ± 0.02	0,023 ± 0.001	97,21

Table S6. Metabolite concentrations after fermentation on SM with 240 g/L glucose + fructose and 300 YAN by all the strains. Standard deviation is depicted and significant differences are shown with letters.

	Erythritol	Ethanol	Ac. Acetic	Glycerol	Ac. Succinic	Ac. L-lactic	2,3-Butanediol
T73	0,29 ± 0,01 ^B	13,49 ± 0,45 ^F	0,46 ± 0,02 ^A	6,89 ± 0,07 ^A	3,99 ± 0,41 ^A	0,37 ± 0,02 ^{A, B, C, D}	0,47 ± 0,07 ^A
BMV58	0,72 ± 0,02 ^{F, G}	11,86 ± 0,68 ^{A, B, C, D, E}	0,62 ± 0,04 ^{A, B, C}	8,08 ± 0,65 ^{B, C}	14,88 ± 0,78 ^{E, F, G}	0,22 ± 0,01 ^A	1,22 ± 0,05 ^{B, C}
ΔGCY1	0,75 ± 0,01 ^G	12,33 ± 0,36 ^{B, C, D, E, F}	0,57 ± 0,04 ^{A, B}	7,99 ± 0,27 ^{A, B, C}	15,56 ± 0,55 ^{E, F, G}	0,53 ± 0,02 ^{C, D, E}	0,92 ± 0,14 ^B
ΔYPR1	0,69 ± 0,03 ^{E, F}	12,36 ± 0,33 ^{B, C, D, E, F}	0,57 ± 0,01 ^{A, B}	7,81 ± 0,25 ^{A, B, C}	15,09 ± 0,19 ^{E, F, G}	0,48 ± 0,05 ^{C, D}	1,05 ± 0,32 ^B
ΔARA1	0,66 ± 0,04 ^E	11,91 ± 0,41 ^{A, B, C, D, E}	0,53 ± 0,04 ^{A, B}	7,24 ± 0,69 ^{A, B}	17,29 ± 0,35 ^G	0,41 ± 0,01 ^{B, C, D}	1,12 ± 0,36 ^B
ΔGRE3	0,4 ± 0,01 ^C	11,85 ± 0,31 ^{A, B, C, D, E}	0,57 ± 0,02 ^{A, B, C}	7,4 ± 0,19 ^{A, B}	13,77 ± 0,3 ^{E, F}	0,4 ± 0,01 ^{B, C, D}	1,15 ± 0,07 ^B
ΔYJR096W	0,66 ± 0 ^E	12,13 ± 0,32 ^{B, C, D, E}	0,55 ± 0,02 ^{A, B}	7,66 ± 0,18 ^{A, B}	16,35 ± 0,21 ^{F, G}	0,41 ± 0,01 ^{B, C, D}	1,58 ± 0,01 ^{C, D}
ΔARA1ΔGRE3	0,4 ± 0,02 ^C	10,67 ± 0,47 ^A	0,5 ± 0,01 ^A	6,87 ± 0,19 ^A	10,47 ± 0,21 ^{C, D}	0,54 ± 0,03 ^{D, E, F}	1,04 ± 0,02 ^B
ΔYJR096WΔGRE3	0,4 ± 0,01 ^C	11,72 ± 0,16 ^{A, B, C, D}	0,32 ± 0,28 ^A	7,56 ± 0,09 ^{A, B}	6,04 ± 3,34 ^B	0,75 ± 0,03 ^G	0,99 ± 0,02 ^B
ΔARA1ΔYJR096W	0,57 ± 0,02 ^D	12,49 ± 0,14 ^{C, D, E, F}	0,46 ± 0,05 ^A	8,14 ± 0,01 ^{B, C}	13,04 ± 0,7 ^{D, E}	0,73 ± 0,04 ^{F, G}	1,08 ± 0,09 ^B
ΔYJR096WΔGRE3ΔARA1	0,39 ± 0,01 ^C	11,88 ± 0,15 ^{A, B, C, D, E}	0,6 ± 0,01 ^{A, B, C}	7,61 ± 0,05 ^{A, B}	15,38 ± 0,17 ^{E, F, G}	0,83 ± 0,01 ^G	1,01 ± 0,15 ^B
ΔYPR1ΔGCY1	0,75 ± 0,01 ^G	13,06 ± 0,06 ^{E, F}	0,54 ± 0,01 ^{A, B}	8,35 ± 0,09 ^{B, C}	14,64 ± 0,39 ^{E, F, G}	0,36 ± 0,01 ^{A, B, C}	1,71 ± 0,01 ^D
ΔGCY1ΔGRE3	0,39 ± 0,03 ^C	11,12 ± 0,7 ^{A, B}	0,59 ± 0,04 ^{A, B, C}	7,43 ± 0,4 ^{A, B}	14,66 ± 0,33 ^{E, F, G}	0,77 ± 0,02 ^G	1,71 ± 0,01 ^D
ΔYPR1ΔGRE3	0,36 ± 0,02 ^C	12,74 ± 0,35 ^{D, E, F}	0,53 ± 0,02 ^{A, B}	8,24 ± 0,05 ^{B, C}	8,67 ± 0,24 ^{B, C}	0,35 ± 0,01 ^{A, B, C}	1,78 ± 0,02 ^D
ΔYPR1ΔGCY1ΔGRE3	0,21 ± 0,03 ^A	11,28 ± 0,21 ^{A, B, C}	0,55 ± 0,04 ^{A, B}	7,3 ± 0,12 ^{A, B}	15,1 ± 0,2 ^{E, F, G}	0,68 ± 0,06 ^{E, F, G}	1,12 ± 0,05 ^B
ΔYPR1ΔGCY1ΔARA1	0,41 ± 0,01 ^C	11,59 ± 0,41 ^{A, B, C, D}	0,75 ± 0,2 ^{B, C, D}	7,28 ± 1,08 ^{A, B}	3,04 ± 0,18 ^A	0,19 ± 0,23 ^{A, B}	1,09 ± 0 ^B
ΔYPR1ΔGCY1ΔYJR096W	0,4 ± 0,02 ^C	11,66 ± 0,48 ^{A, B, C, D}	1,04 ± 0,06 ^E	8,21 ± 0,29 ^{B, C}	1,93 ± 0,32 ^A	0,48 ± 0,04 ^{C, D}	0,99 ± 0,1 ^B
Δ4K	0,54 ± 0,02 ^D	12,71 ± 0,22 ^{D, E, F}	0,83 ± 0,01 ^{C, D, E}	8,39 ± 0,16 ^{B, C}	3,34 ± 0,01 ^A	0,35 ± 0 ^{A, B, C}	0,9 ± 0,04 ^B
Δ5K	0,23 ± 0,01 ^A	12,57 ± 0,23 ^{D, E, F}	0,88 ± 0,03 ^{D, E}	8,86 ± 0,06 ^C	2,81 ± 0,31 ^A	0,41 ± 0,04 ^{B, C, D}	1,06 ± 0,01 ^B