

TableS1. List of Strains

Strain number/Name	Genotype	Source
124-67: $\Delta grx3/4$ shuffle	$MAT\alpha \Delta grx3::kanMX, \Delta grx4::kanMX$ [pRS416-URA3-GRX3]	This work
123-40: Gal-Grx3	$MAT\alpha his3\Delta 1, leu2\Delta 0, lys2\Delta 0, ura3\Delta 0,$ $\Delta grx4::KanMX$, [His3MX6-PGAL1-3HA- Grx3]	This work
125-28: $\Delta grx3/4$	$\Delta grx3::KanMX4, \Delta grx4::kanMX$	This work
Suppressors 1-4	$MAT\alpha \Delta grx3/4$ suppressors	This work

Table S2. 10 x spore amino acid mix

Amino acid	wt. in mg/g per 500 ml
Adenine	200 mg
Uracil	200 mg
Tyrosine	200 mg
Histidine	100 mg
Leucine	100 mg
Lysine	100 mg
Tyropophan	100 mg
Methionine	100 mg
Arginine	100 mg
Phenylalanine	500 mg
Threonine	1.75 g
Deionized water	500 mL

TableS3. List of plasmids

Plasmids	Features	Source
YEp13	2μ <i>S. cerevisiae</i> AB320 high copy genomic library	ATCC 37323
pRS416-Grx3	This shuffling plasmid is used to cover Grx3 deletion	This work
pRS426-GPDprom-IRP1	2μm <i>URA3</i> , GPD promoter driven IRP1	This work
pRS316-RPL25eGFP	<i>CEN/ARS URA3</i>	A gift from Eduard Hurt (PMID: 18625724)

Table S4. List of primers

Name	Sequence (5'-3')
Primers used for PCR Analysis of Grx3/4	
Grx3 PF	CGGCCTTCCCTAGCTGAATAC
Grx3 PR	CATAAATAACATTACCGGGCGCGG
Grx4 PF	CCGGAACTTCCACCAACACCA
Grx4 PR	GCATCACAGGTGCAGCTTGTAC
Kan PR	TCGCAGTGGTGAGTAACCATGC
Primers used for sequencing <i>S. cerevisiae</i> AB320 genomic library in YEpl3 <i>E. coli</i>	
P1	CAGTCCTGCTCGCTTCGCTA

P2	GATATAGGCGCCAGCAACCG
ESL2-PF	GGTGGCGGCCGCTCTAGAACTAGTGAGATGAGGCCTTATTACTCCC
Name	Sequence (5'-3')
Primers used for cloning genes of interest into pRS425 at <i>Bam H1</i> restriction site by Gibson Assembly	
ESL2-PR	GATATCGAATT CCTGCAGCCC GGGGAAA ACTTGCTAGTCTAGCCG
PCK1-PF	GGTGGCGGCCGCTCTAGAACTAGTGAGGCTGCTAACATTATGGAT
PCK1-PR	GATATCGAATT CCTGCAGCCC GGGGTT GTTGATCCAGTT CAGTTATAAAAAAAA
RCR2-PF	GGTGGCGGCCGCTCTAGAACTAGTGACTTGGTCATGGAGATTAG
RCR2-PR	GATATCGAATT CCTGCAGCCC GGGGCGATGAATT GACTGTT CTGGAC
RAD57-PF	GGTGGCGGCCGCTCTAGAACTAGTGGAACGCTTCGACTCGGTCC
RAD57-PR	GATATCGAATT CCTGCAGCCC GGGGACAAT ATTAT ATTACTAATTGAACACTTAGCGA
MAF1-PF	GGTGGCGGCCGCTCTAGAACTAGTGAAATCGTGGCAGTT GCGATA
MAF1-PR	GATATCGAATT CCTGCAGCCC GGGG CATAAAGGTACATAGTT GAAAAAGGG
SOK1-PF	GGTGGCGGCCGCTCTAGAACTAGTGATTGGTCTCTGCCGTGCG
SOK1-PR	GATATCGAATT CCTGCAGCCC GGGG TACATAATGTGTCTACATT TATAGCTG
TRP1-PF	GGTGGCGGCCGCTCTAGAACTAGTGGAAAAATCAACGGTTAACGA

	CAT
TRP1-PR	GATATCGAATT CCTGCAGCCC GGGGGAGATAAGTGTGATAAAAGTTTT TACAGC
Name	Sequence (5'-3')
SFP1-PF	GGTGGCGGCCGCTCTAGAACTAGTGTTCGCTTATAAAGAGAAGGA AAG
SFP1-PR	GATATCGAATT CCTGCAGCCC GGGGGATCAGAACAGAACAGGAAGTAAG TAAAG
SEI1-PF	GGTGGCGGCCGCTCTAGAACTAGTGAAATTAAATTCAATATCAATAATA ATATACTATAAGTAACTAAAAAG
SEI1-PR	GATATCGAATT CCTGCAGCCC GGGGGATCATGGGGAGTAAACTATAT CA
AHK1-PF	GGTGGCGGCCGCTCTAGAACTAGTGCGATGGTTGATCAAAGTACG
AHK1-PR	GATATCGAATT CCTGCAGCCC GGGGGATCCTTATGCCCTACCTAAATA TAAAC
YET3-PF	GGTGGCGGCCGCTCTAGAACTAGTGATCAAAATGCTTTGCTCCT
YET3-PR	GATATCGAATT CCTGCAGCCC GGGGGTTACTGTCGGCATGAACCTAC
BDF2-PF	GGTGGCGGCCGCTCTAGAACTAGTGAGCACACAGACTTTAATAATA AAGC
BDF2-PR	GATATCGAATT CCTGCAGCCC GGGGGATGTTACAAATCTTTTATCCC CAT
CBS1-PF	GGTGGCGGCCGCTCTAGAACTAGTGAACTGACCGAACCTGACCAC

CBS1-PR	GATATCGAATTCTGCAGCCCGGGTATAACAAAAATAAGAAATAAT TGTTTTACGTACTTAT
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Name	Sequence (5'-3')
ESL1-PF	GGTGGCGGCCGCTCTAGAACTAGTGAATCAATCCTAAAGTGAGAAA GAACA
ESL1-PR	GATATCGAATTCCCTGCAGCCCGGGAATTGTAGAATGGAGTGTTCAA AAAAA
Primers used for sequence analysis of clones constructed on pRS425	
pRS425-P1	CAGGAAACAGCTATGACCATGA
pRS425-P2	TGTAAAACGACGCCAGT
Primers used for transcriptional analysis of target genes for the wild type and suppressors by qRT-PCR	
BDF2-PF	TTACTGGCAGCACCAACAGAG
BDF2-PR	TCGATACTTACCGCGCTGAC
SOK1-PF	TCTTCAACAGGTCCGAGTGC
SOK1-PR	GCAATCGCATTGCCAGTAGG
SFP1-PF	GGGTGGTGTTCATGGGGAT
SFP1-PR	ACCGTTGCTGATGCAGGTA
ESL2-PF	GTCCCGAACAGCAACAAACAC
ESL2-PR	AGCCTCCATGACGTTTCGT
ESL1-PF	GCGGGTTAACACAGTGCATT
ESL1-PR	TCTCCAATGCAGGCTCAAG

Table S5. The sequencing results of the genomic regions contained in the plasmids isolated from positive hits clones for $\Delta grx3/4$ bypass.

Screen no	Suppressor colony no	Yeast chromosome no	Genes	Gene(s) responsible for $\Delta grx3/4$ bypass
01	P1	XI (626728-632950)	ESL2, PCK1	ESL2
01	P4	IV (454206-462595)	RCR2, RAD57, MAF1, SOK1, TRP1	SOK1
02	4A	XII (924141 - 929708)	SFP1, ESI1	SFP1
02	8B	XI (625890 - 632950)	ESL2, PCK1	ESL2
02	13D	IV (325378 - 334774)	BRE1, AHK1, YET3, BDF2, CBS1	BDF2
02	15B	IV (452990 - 462595)	YRB1, RCR2, RAD57, MAF1, SOK1, TRP1	SOK1
02	19D	IX (57093-63174)	ESL1, MCM10	ESL1

Real-time fluorescence quantitative PCR analyses

Total RNAs were extracted from 1-3 mL cultures ($\sim 1.2 \times 10^8$ cells from each culture) using RNAprep Pure Plant Kit (TIANGEN Beijing) and diluted to 500 ng/ μ L. 1 μ g RNA was reverse transcribed in a 20 μ L of reaction mixture containing 0.5 μ g random primers and the GoScript Reverse Transcription System according to the manufacturer's recommended protocol (Promega, USA). Obtained cDNA can be frozen at -80 °C for long-term storage. Prior to real-time fluorescence quantitative PCR (qPCR), the reverse transcriptase was inactivated at 70°C for 15 min. In a typical qPCR reaction, 20 μ L of reaction mixture contained 4 μ L of 5 \times diluted cDNA, 0.4 μ L of 10 μ M gene-specific forward and reverse primers and 10 μ L of 2 \times Universal SYBR Green Fast qPCR Master Mix, then qPCRs were performed on a Roche LightCycle 96. The primers listed in Table S4 were designed using primer3plus (<http://www.primer3plus.com>). All reagents and consumables for the experiments were RNase-free.

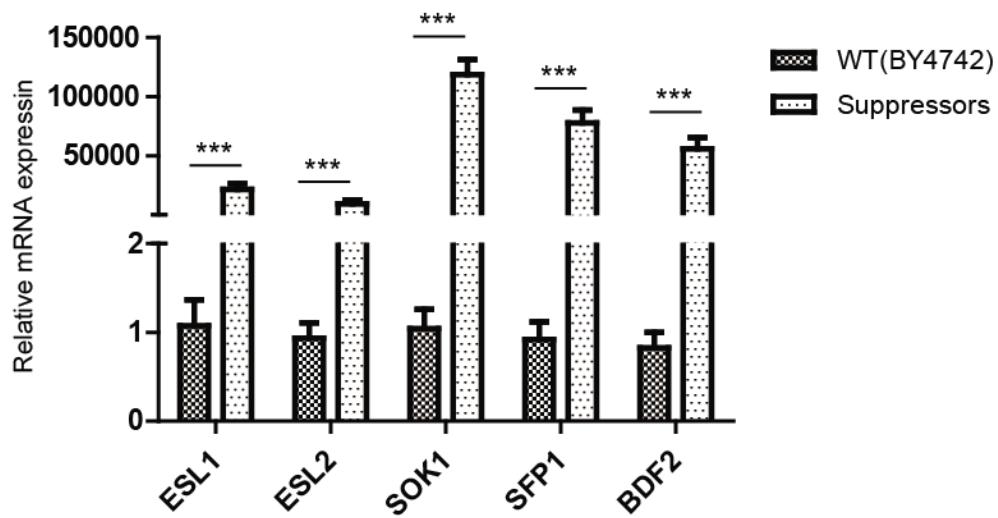


Figure S1. qRT-PCR quantitation of the expression levels of suppressor genes in suppressor strains in relative to the wild type strain. Data are expressed as mean \pm SEM (N=3 per group). ***P<0.001 versus WT(BY4742).

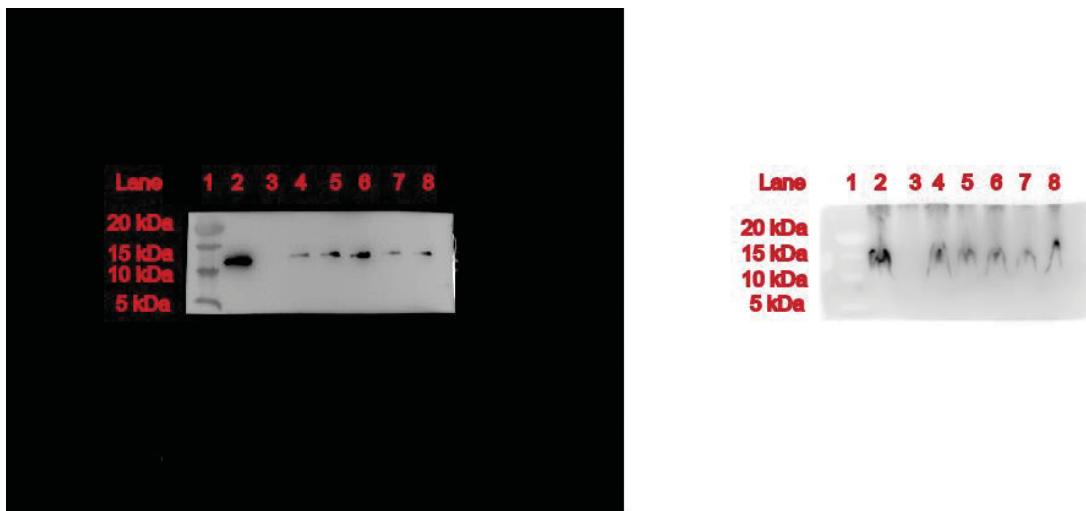


Figure S2. The original images of the CytC western blot. Lane 1-8 correspond to the protein markers, BY4742 (WT), Δ grx3/4, and suppressors with overexpression of Esl1, Esl2, Sok1, Sfp1 and Bdf2 respectively. All lanes have been used to build Figure 8.