

# **Supplemental Materials**

*Molecular Biology of the Cell*

Pfanzagl et al.

**Figure S1.** Domains of Msn2 related proteins. Selected parts of a multiple sequence alignment of the identified Msn2 and Msn4 homologues shows the conservation of the 4 domains and the consensus sequence.

**Figure S2.** Multiple sequence alignment of 22 protein sequences related to *Saccharomyces cerevisiae* Msn2 and Msn4.

**Figure S3.** Localization effects of Msn2 phosphomimetic mutations. (A) W303 *msn2Δmsn4Δ* cells expressing either Msn2A5-GFP or Msn2D5-GFP were exposed to glucose starvation and different stress conditions. Pictures were taken 10 minutes after induction. (B) W303 *msn2Δmsn4Δcdc35Δpde2Δ* cells expressing either Msn2D5-GFP or Msn2D6-GFP were grown in presence of 3mM cAMP to exponential phase and shifted to medium lacking cAMP. PKA activity was re-induced by addition of 3mM cAMP.

**Figure S4.** mRNA level changes of genes encoding key enzymes of the (A) glycolysis gluconeogenesis, and (B) pentosephosphate cycle. Log<sub>2</sub> values of expression change are indicated.

**Figure S5.** The Msn2A6 expression profile resembles glucose-starved cells. The expanded compendium shows distinct expression pattern signatures for mutants similar to high and low glucose growth.

**Data file S1.** Sheet Expression levels: Gene expression changes induced by Msn2A6 after 90 minutes induction of expression by 17-β-estradiol addition (6nM). Selected fold change (log<sub>2</sub>) and signal difference values of up and downregulated genes are listed. Sheet Carbon Pathways: Expression levels of genes of the primary carbon and energy pathways as derived from KEGG database (<https://www.genome.jp/kegg/pathway.html>)

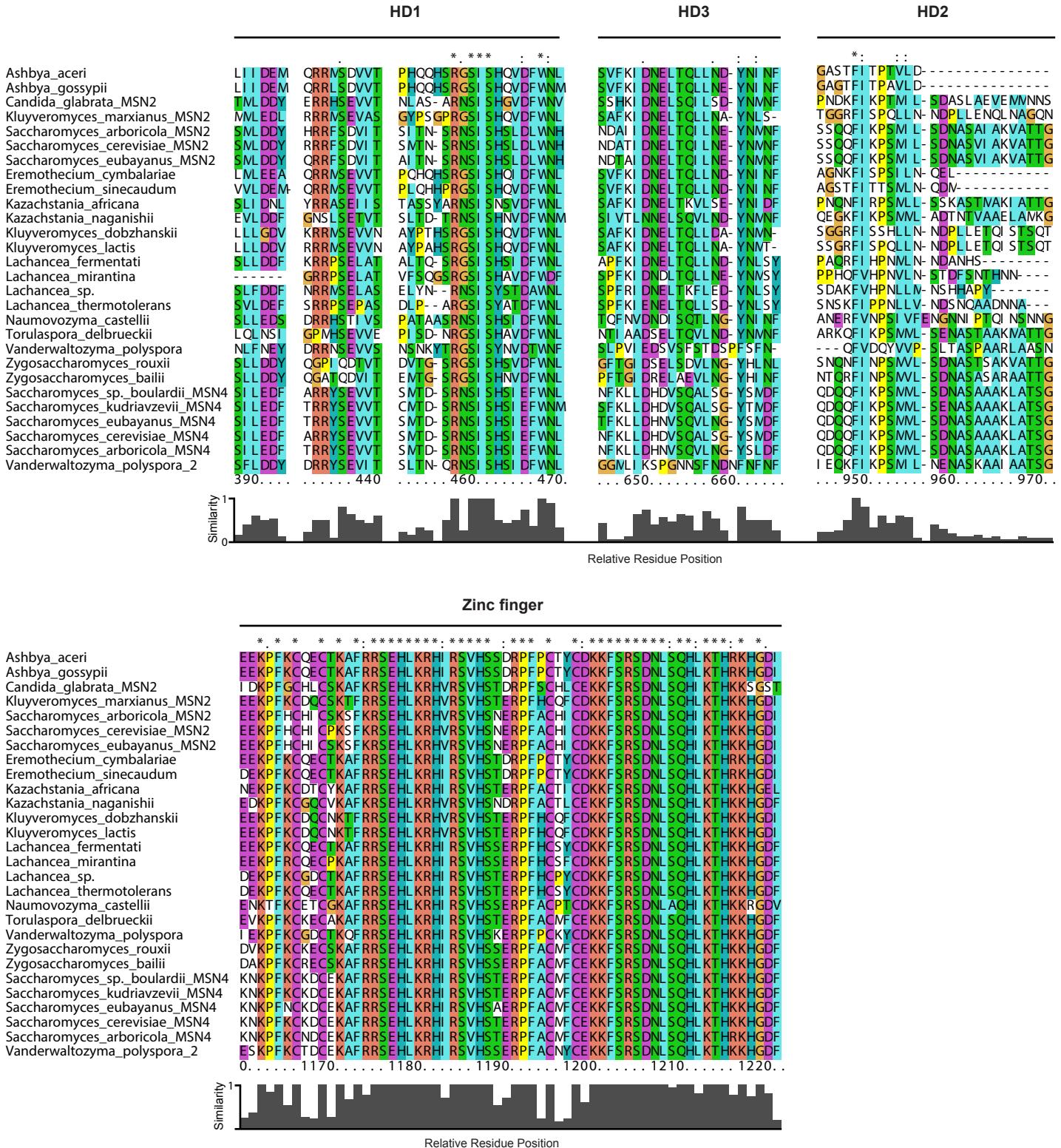
**Data file S2.** Values to reconstruct the heatmap of Figure 3 and Figure S4 for visualization with treeview.

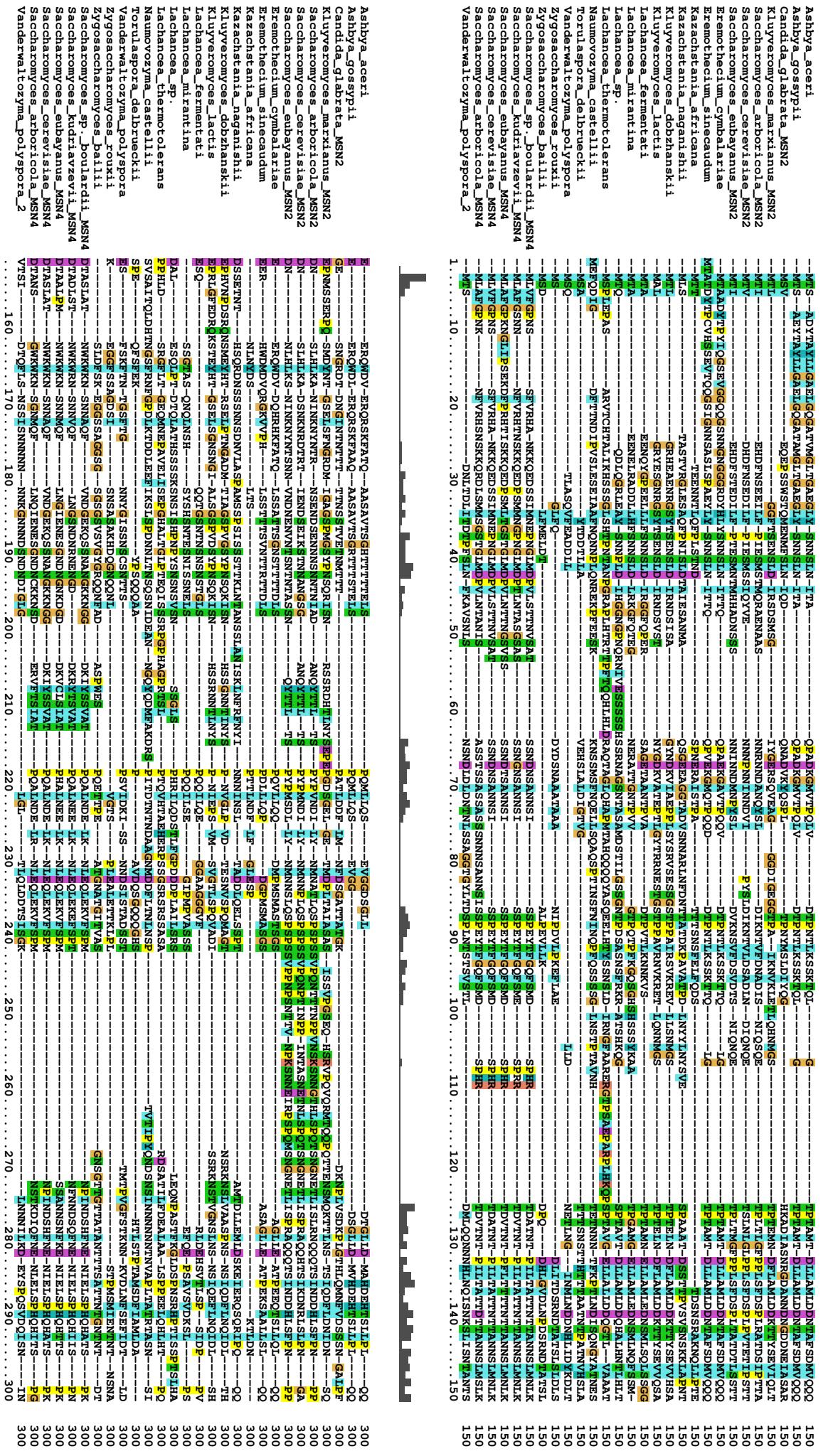
**Data file S3.** Data of metabolite measurements.

**Data file S4.**List of protein sequences used for multiple sequence alignment

**Data file S5.**Multiple sequence alignment of *Saccharomyces cerevisiae* Msn2

## Figure S1

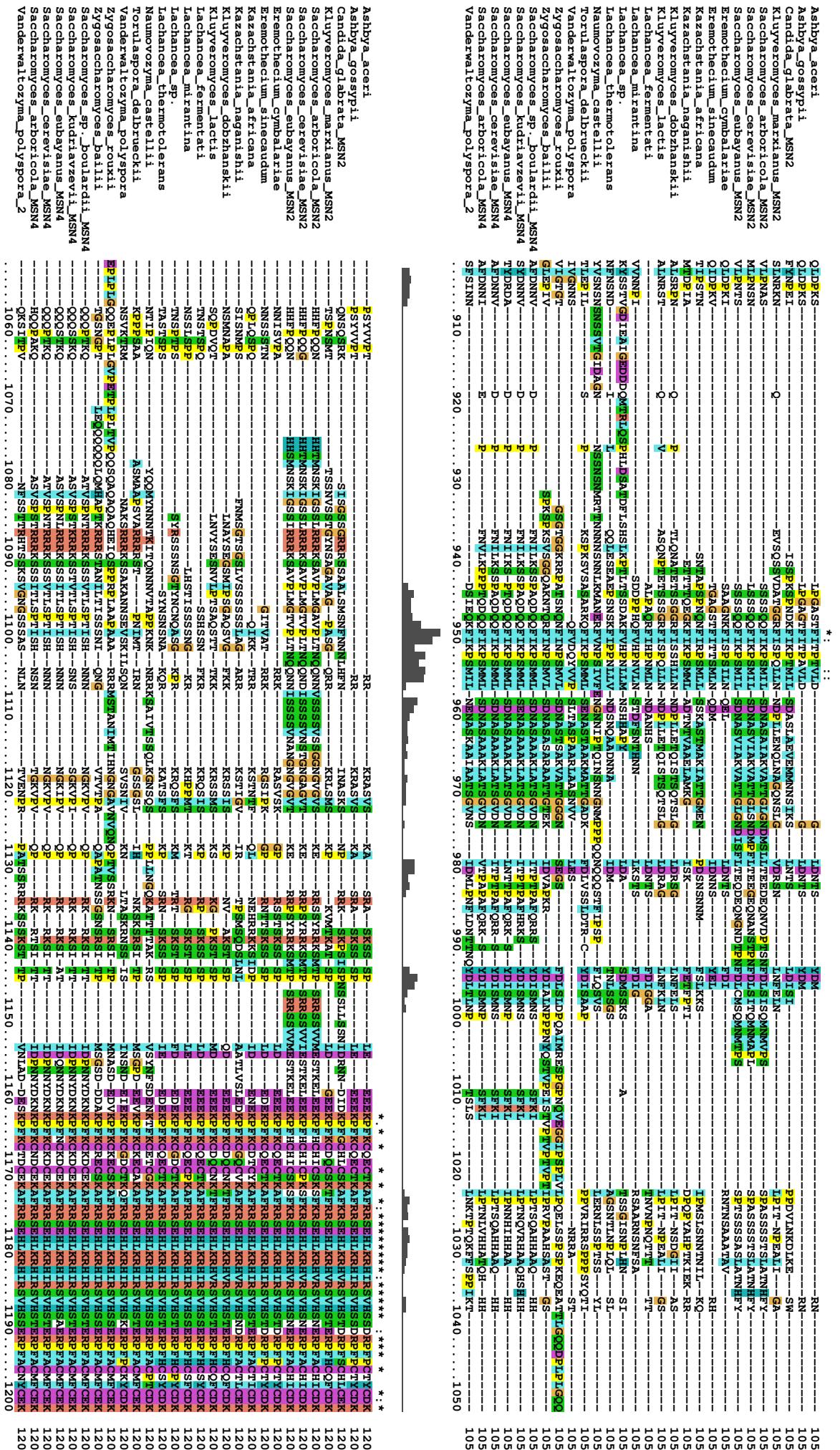






<i>Ashbya aceri</i>	-TASSHNSITAV--GNAVUFKTDNLQVLD--YINP	-NAPSKTA	-TRURAGCINPGSAPTSASGSN-	750
<i>Ashbya goossypii</i>	--TASSHNSITAV--GVUPVFKEELNLQVLD--YINP	-NAPSKTA	-TRURAGCINPGSAPTSASGSN-	750
<i>Candida glabrata</i> MSN2	--RATNGGTEKHLDSDLSDI SHKDNELQD--YINP	-KPIGEESP	-TRURAGCINPGSAPTSASGSN-	750
<i>Saccharomyces arboricola</i> MSN2	--ANPNTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Saccharomyces cerevisiae</i> MSN2	--PNTTNAVNNTGIAHNDAI AGPKNNDNAEELQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Saccharomyces eubayanus</i> MSN2	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Eremothecium cymbalariae</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Eremothecium sinclairum</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Kazachstania naganishii</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Kluyveromyces dozhanski</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Kluyveromyces lactis</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Lachancea fermentati</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Lachancea mirantina</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Lachancea sp.</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Lachancea thermotolerans</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Namurozyma castellii</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Namurozyma sp. bouardii</i> MSN2	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Namurozyma sp. kudriavzevi</i> MSN4	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Vanderwaltozyma polyspora</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Zygosaccharomyces bailii</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Zygosaccharomyces castellii</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Zygosaccharomyces delbrueckii</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Zygosaccharomyces dubiosporae</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Zygosaccharomyces rouxii</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Zygosaccharomyces bailii</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Saccharomyces castellii</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Saccharomyces kudriavzevi</i> MSN4	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Saccharomyces cerevisiae</i> MSN4	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Saccharomyces eubayanus</i> MSN2	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
<i>Vanderwaltozyma polyspora</i>	--NPTTNAVNNTGIAHNDAI DNLQD--YINP	-DNLSA SVA	-TRURAGCINPGSAPTSASGSN-	750
	..... 610 .....	620 .....	630 .....	640 .....
	..... 650 .....	660 .....	670 .....	680 .....
	..... 690 .....	700 .....	710 .....	720 .....
	..... 730 .....	740 .....	750 .....	750 .....

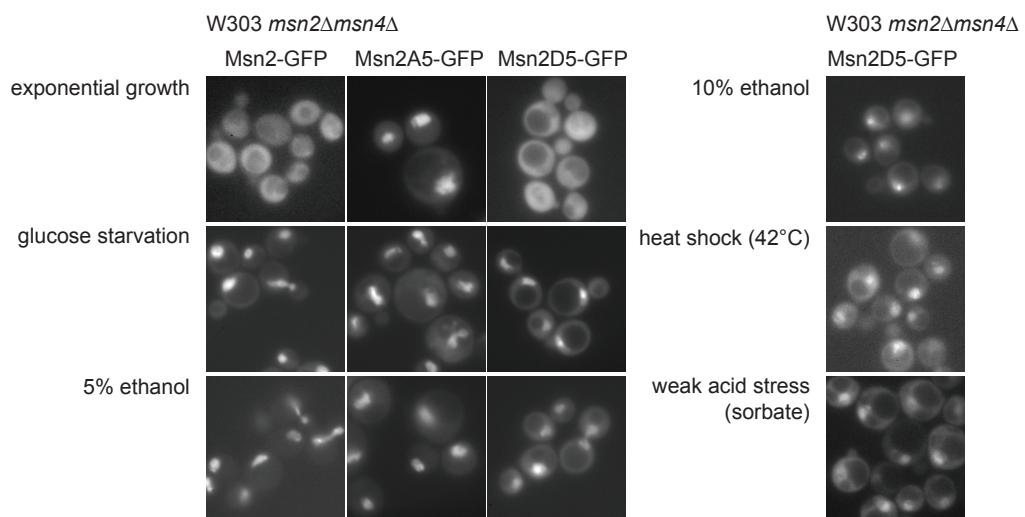
<i>Ashbya aceri</i>	--ISSAMVA SRSNVSEPH--VOK	-HMSVYDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Ashbya goossypii</i>	--ISSAMVA SRSNVSEPH--VOK	-HMSVYDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Candida glabrata</i> MSN2	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Kluyveromyces marxianus</i> MSN2	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Saccharomyces arboricola</i> MSN2	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Saccharomyces cerevisiae</i> MSN2	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Saccharomyces eubayanus</i> MSN2	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Eremothecium cymbalariae</i>	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Eremothecium sinclairum</i>	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Kazachstania naganishii</i>	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Kluyveromyces dobhanskii</i>	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Kluyveromyces lactic</i>	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Lachancea fermentati</i>	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Lachancea malintana</i>	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Lachancea sp.</i>	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Lachancea thermotolerans</i>	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Namurozyma castellii</i>	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
<i>Vanderwaltozyma polyspora</i>	--DVSNSAGQSIRPFKRP	-HRASMSVTDGA--NPVMDLY	-GDN--KVVKPO	900
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	..... 800 .....	810 .....	820 .....	830 .....
	..... 840 .....	850 .....	860 .....	870 .....
	..... 880 .....	890 .....	900 .....	900 .....



<i>Ashbya aceri</i>	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Ashbya gosupii</i>	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Candida glabrata</i> , MSN2	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Kluyveromyces marxianus</i> , MSN2	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Saccharomyces arboricola</i> , MSN2	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Saccharomyces cerevisiae</i> , MSN2	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Saccharomyces eubayanus</i> , MSN2	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Eremothecium cymbaliae</i>	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
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<i>Kazachstanica africana</i>	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
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<i>Kluveromyces dozhanskii</i>	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Kluveromyces lactis</i>	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
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<i>Lachancea milantini</i>	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Lachancea sp.</i>	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Lachancea thermotolerans</i>	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Naumovoxys castellani</i>	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Torulaspora delbrueckii</i>	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Vanderwaltomyza polypora</i>	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Zygosaccharomyces rouxi</i>	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Zygosaccharomyces bailii</i>	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Saccharomyces kudriavzevii</i> , MSN4	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Saccharomyces eubayanus</i> , MSN4	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Saccharomyces cerevisiae</i> , MSN4	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Saccharomyces arboricola</i> , MSN4	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337
<i>Vanderwaltomyza polypora</i> , 2	KFSSDDILSQHLKTHRGDKDPPTTKG-----	1337

**Figure S3**

**A**



**B**

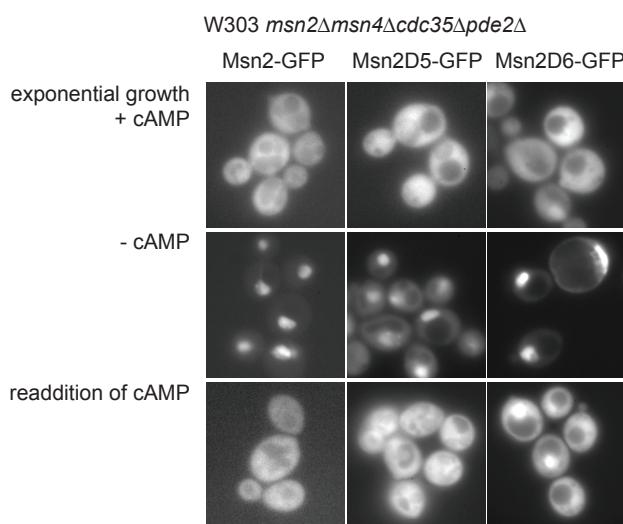
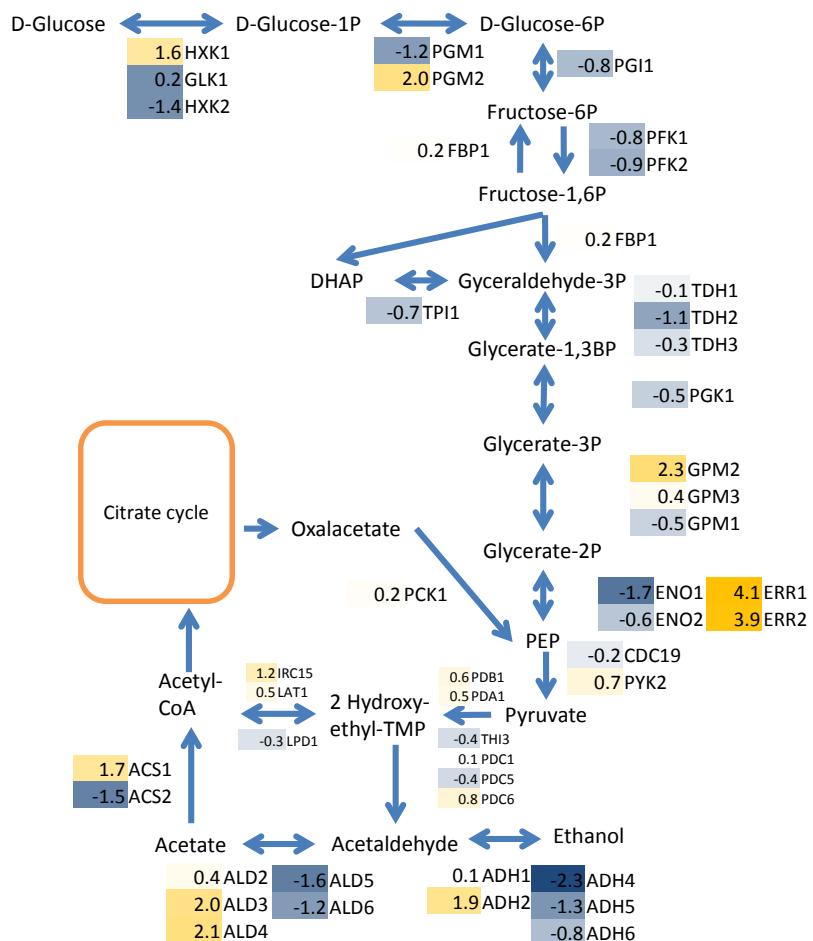


Figure S4

A



B

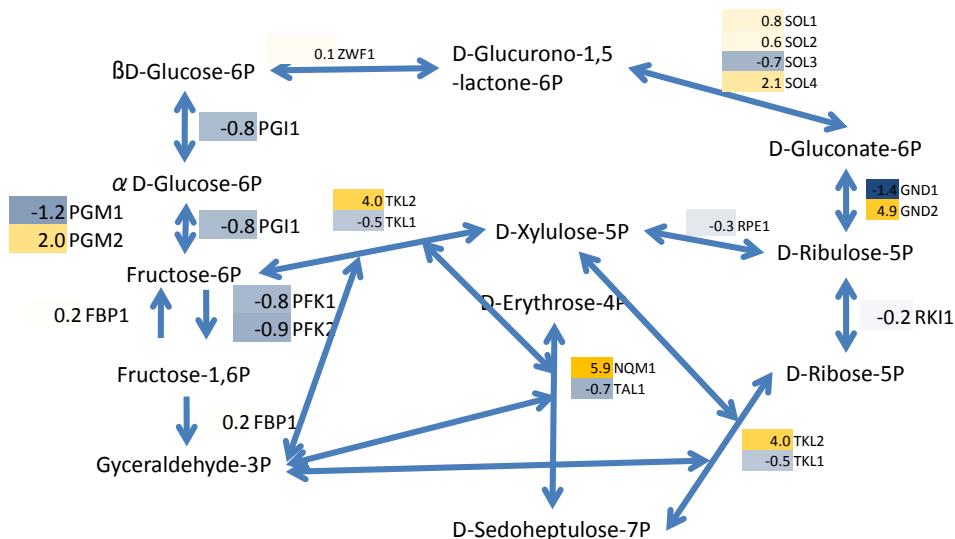
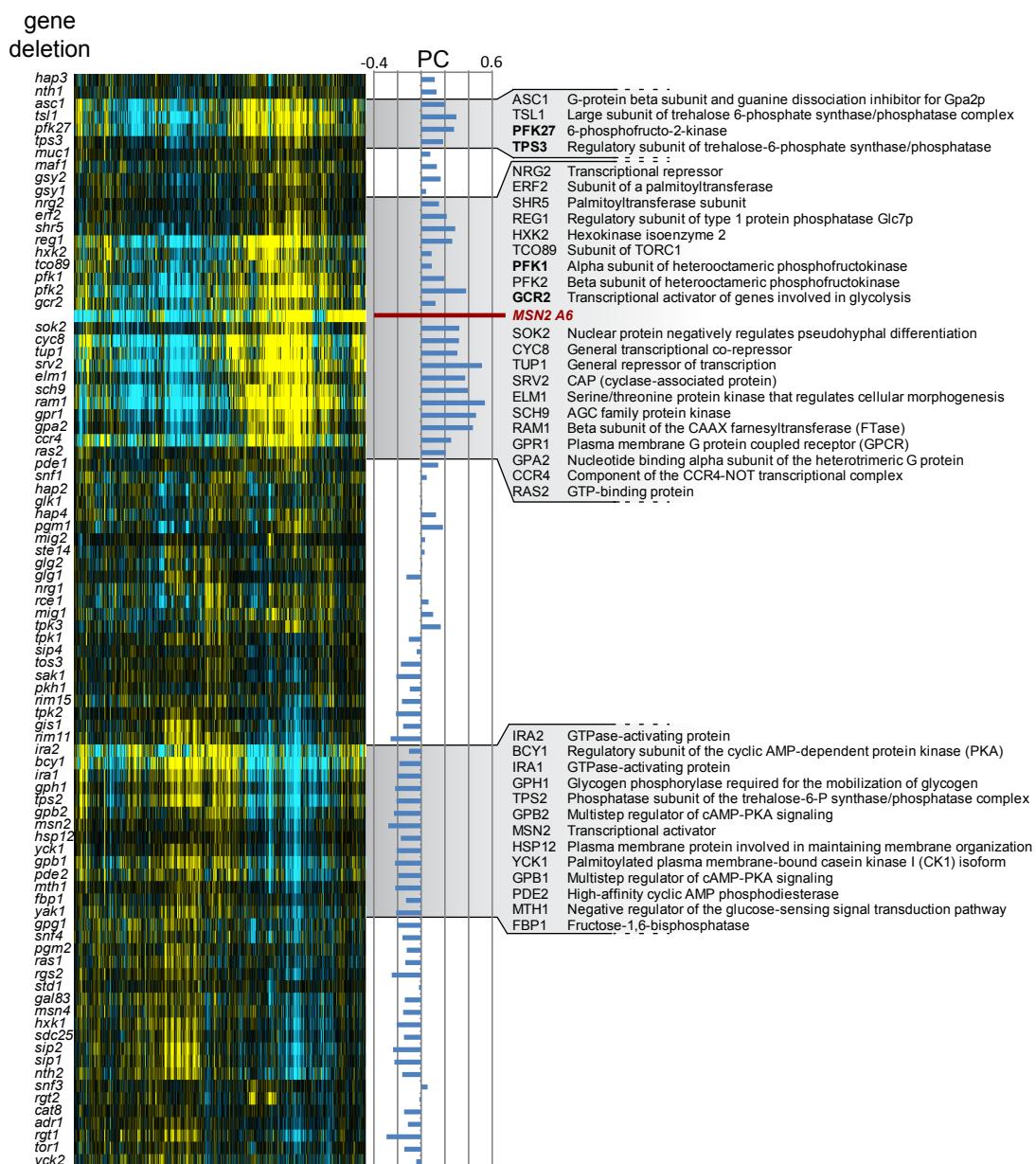


Figure S5



Uniprot ID for protein sequences similar to ScMsn2

R9X9G1  
Q75DD8  
Q6FU62  
WOTE20  
J8PK04  
A2Q129  
A0A0L8RDU3  
G8JQ41  
A0A120K2A0  
H2AMSO  
J7S9G1  
A0A0A8LDU0  
Q6CIG0  
A0A1G4MF34  
A0A1G4J958  
A0A1G4IX78  
C5DGW1  
G0V8Q0  
G8ZQC6  
A7TH69  
C5DWZ7  
CDH11741.1  
A0A0L8VLY4  
J6EQA0  
A0A0L8RFN9  
P33749  
J8LLC8  
A7TTK9

**Table S2:** Oligonucleotides used in the study.

Name	sequence
O6	5`-TGGCCGTTACGGTCGACCATG-3`
O18	5`-GATGATGATGTCGACACTAA TTTATCACGCCG-3` ,
O19	5`-GTCGA CGTTCTAGTGATCTCTTATTG-3` ,
O4	5`-AGCCATGGT ATGGTGACCCCTGCTGTGGG-3` .
O12	5`-TTTGGATCCTCCTCTGTCA AAAATGGC-3`
O13	5`-AAAGGATCCAAAATCGGTTCTTCCCTTCGG-3`
O1	5`-CTAAAATGACGGTCGACCATG-3`
O15	5`-TTTGGATCCTATGGTAGCGT CATTGTTTCGGG-3`
O16	5`-TTTGGATCCGTAGAATTGGAGTT GAATTAGAAATGGAG-3`
O17	5`TTTGAATT CCTATTGCTGGATTAATTTCGGATGGTTCAAAGGT CCAAAGAGTGAGAAATAGAGTTCCCTCG-3`