Supplementary Information

Fine Tuning of the Msn2-Mediated Yeast Stress Responses as Revealed by Systematic Deletion of Msn2/4 Partners

Amit Sadeh[1], Natalia Movshovich[2], Misha Volokh[1], Leah Gheber[2,3] and Amir Aharoni[1,4*]

[1]Departments of Life Science, [2]Clinical Biochemistry and [3]Chemistry and [4]The National Institute for Biotechnology in the Negev (NIBN), Ben-Gurion University of the Negev, Be'er Sheva 84105, Israel.

[*] Correspondence should be addressed to A.A. (aaharoni@bgu.ac.il)

Corresponding Author:

Dr. Amir Aharoni

Department of Life Sciences

Ben-Gurion University of the Negev

Be'er Sheva 84105, Israel

e-mail: aaharoni@bgu.ac.il

Tel: +972-8-6472645

Fax: +972-8-6479218

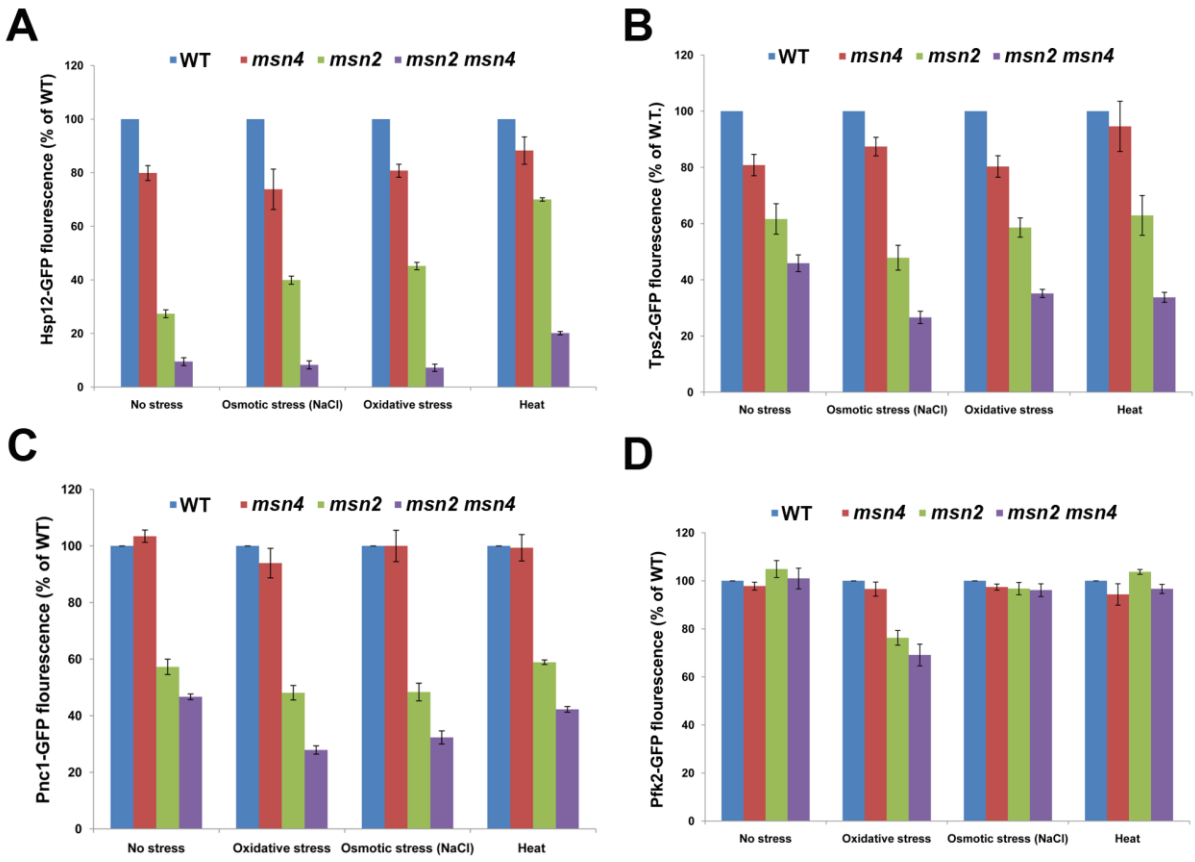

Fig. S1: The effects of *msn2* and *msn4* single deletions and *msn2 msn4*-double deletion on the expression levels of Hsp12-GFP (A), Tps2-GFP (B), Pnc1-GFP (C) and Pfk2-GFP (D) following yeast exposure to diverse stress conditions. Yeast strains were exposed to 40 min of osmotic stress (0.5 M NaCl), oxidative stress (0.6 mM H_2O_2), and heat stress (temperature shift from 30 to 37°C). Fluorescence levels were determined using flow cytometry (see Material and Methods) and normalized to levels obtained with the WT strain.

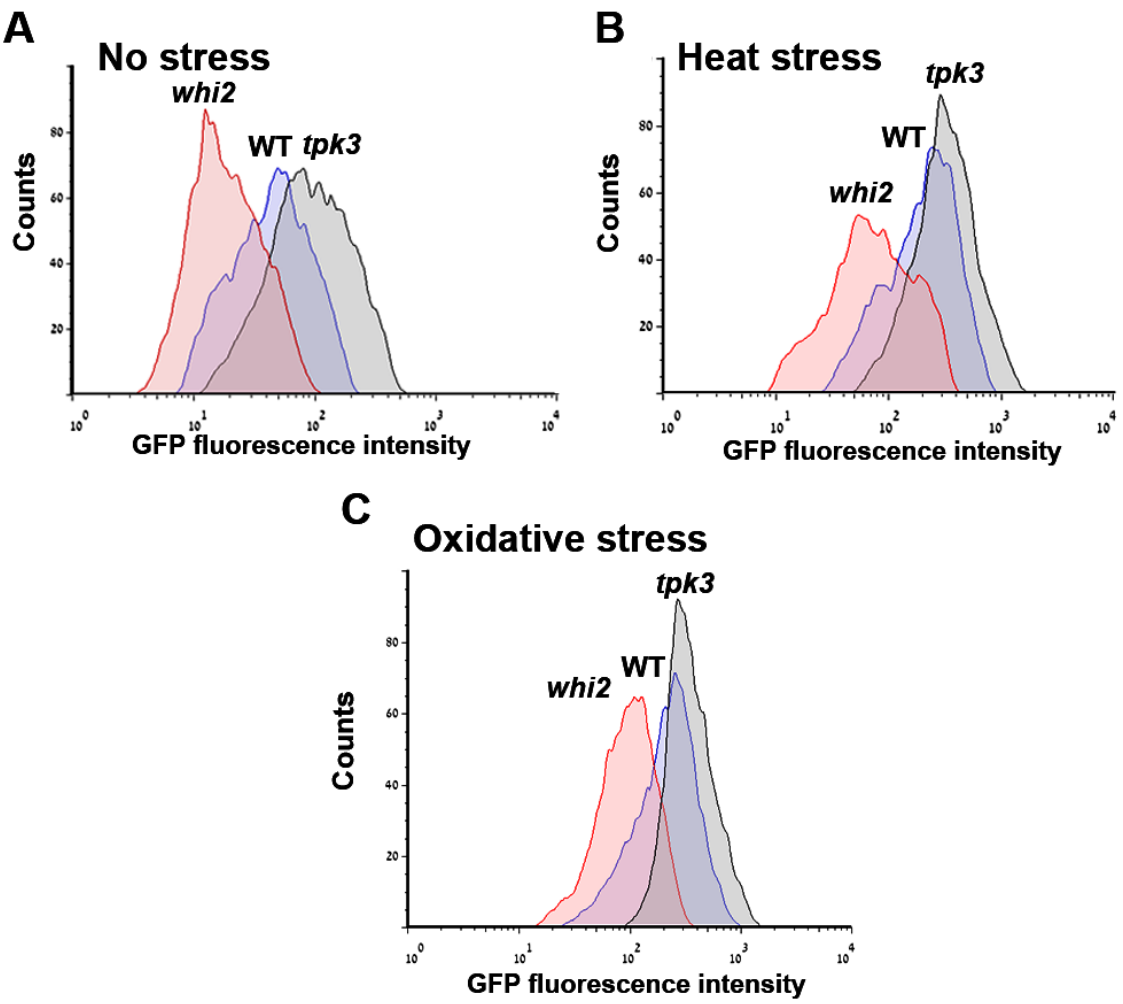

Fig. S2: Flow cytometry histogram analysis of Hsp12-GFP expression levels in WT and *whi2*- or *tpk3*-deleted strains under normal growth conditions (**A**) or following exposure to heat (37°C, **B**) or oxidative (3 mM of H_2O_2, **C**) stress. Deletion of *WHI2* or *TPK3*, respectively, results in significant decrease or increase in Hsp12-GFP expression levels following yeast exposure to different environmental conditions, respectively.

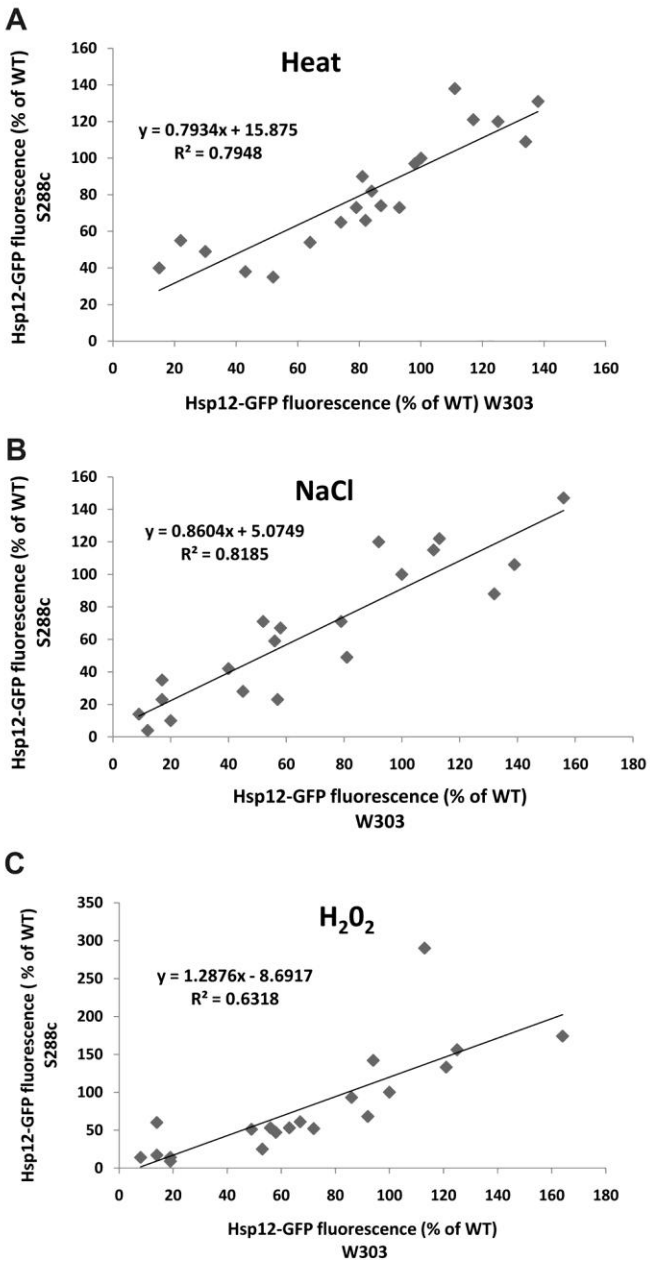

Fig. S3: Correlations between the Hsp12-GFP reporter gene expression levels in the S288c- and W303- derived strains following exposure of the deletion strains to heat (**A**), osmotic (0.5 M of NaCl, **B**) and oxidative (3 mM of H_2O_2, **C**) stress. Each point represents the expression level of Hsp12-GFP in a specific deletion strain (comprehensive data is presented in Fig. 2-3 main text). The high correlation between the expression levels of the different strains indicates that Msn2 activity is affected similarly in these strains as a result of lost interactions with missing partners.

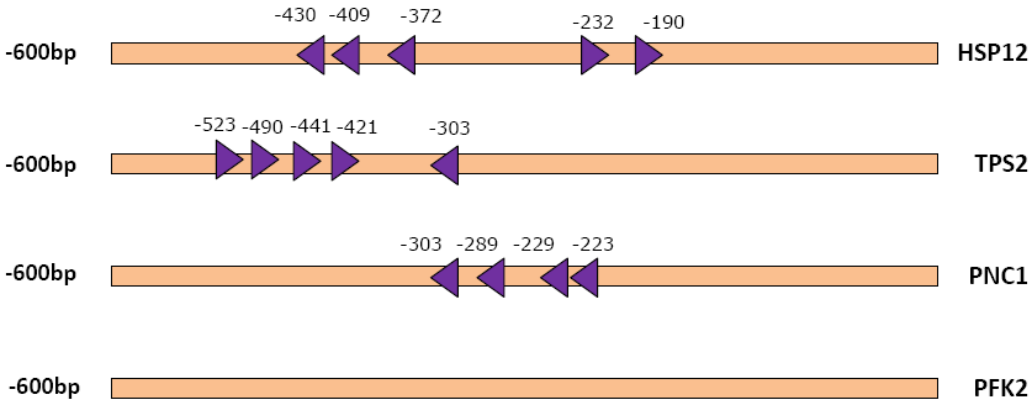

Fig. S4: Location of stress response elements (STRE) in the promoter region of the four reporter genes used for the analysis of Msn2/4 transcriptional activity. STRE locations were obtained using the YESTRACT web site (http://www.yeastract.com/) that allows searching for regulatory sites within the yeast genome.

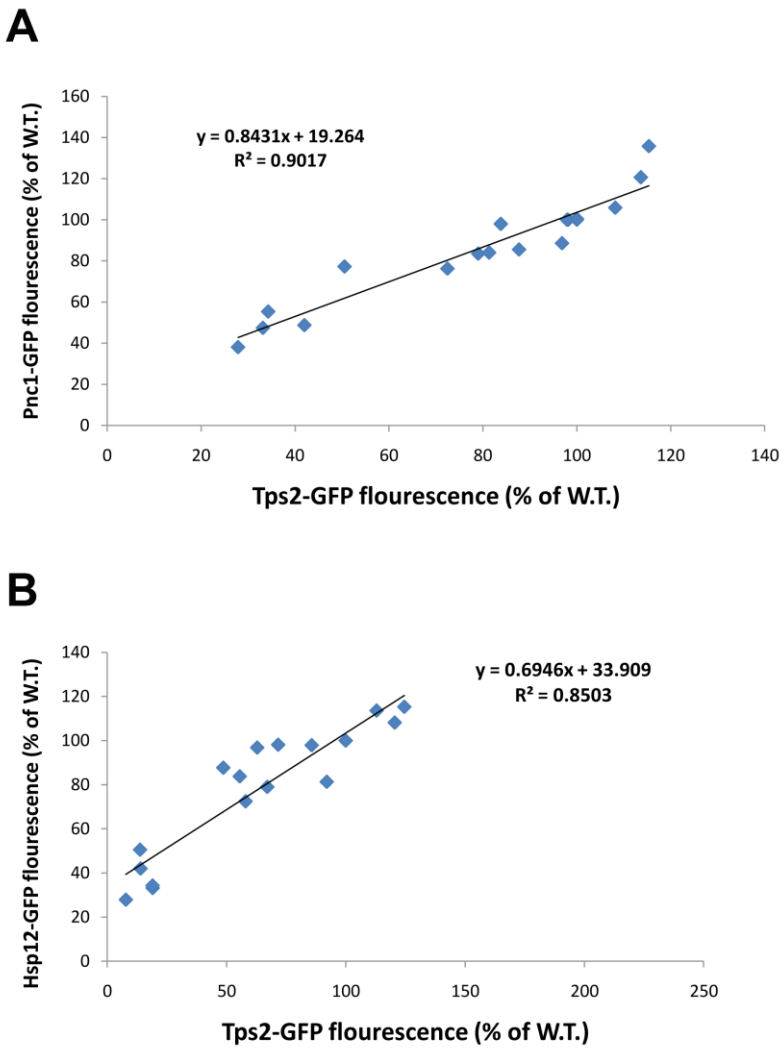

Fig. S5: Examples of correlation between the different reporter gene expression levels following exposure of the deletion strains to oxidative stress. Each point represents the expression levels of two reporter genes in a specific deletion strain (comprehensive data is presented in Fig. 3 and Table S3). The high correlation between the expression levels of the different strains indicates that Msn2 activity is affected in these strains as a result of lost interactions with missing partners.

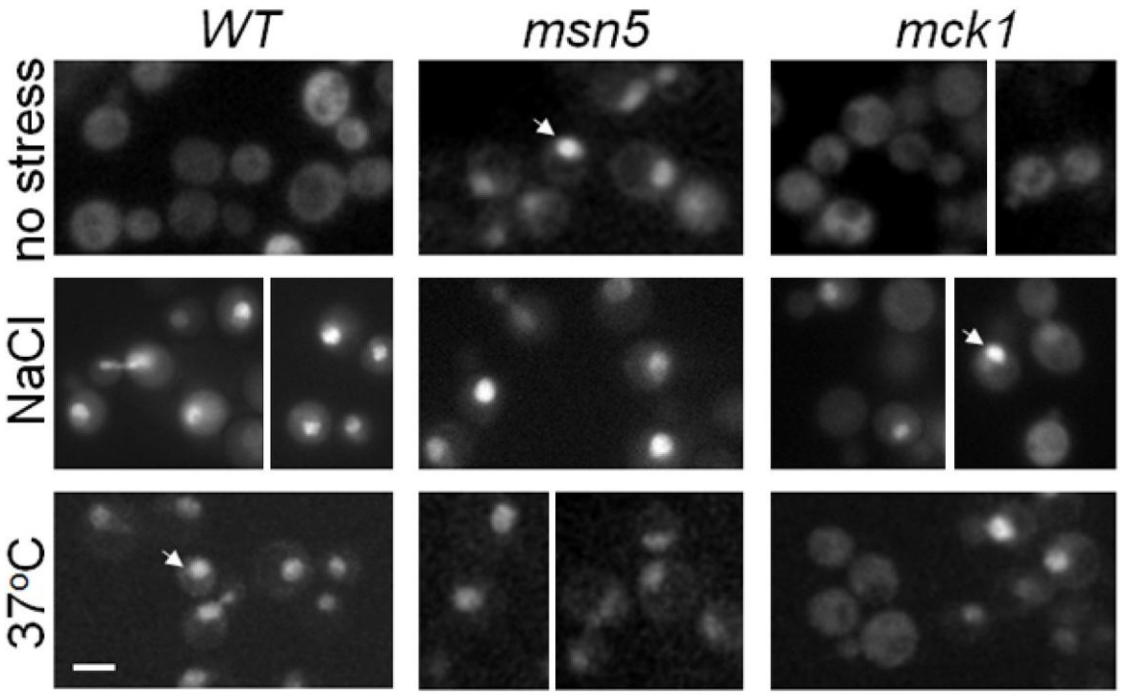

Fig. S6: Nuclear localization of Msn2-GFP is significantly reduced in *mck1*-deleted strains following exposure to heat or osmotic stress. Yeast cells expressing Msn2-GFP in WT or *msn5*-, *mck1*-deleted strains were exposed for 15-20 min to NaCl (0.5 M), elevated temperature (37^{o}C) or no stress conditions. The *msn5*-deleted strain serves as a positive control. The percentage of cells containing nuclear-localized Msn2-GFP is 86, 89 and 33 in the WT and the *msn5*- or *mck1*-deleted strains, respectively, following exposure to heat stress. Similar results were obtained following exposure to osmotic stress (99, 94 or 14 in the WT, *msn5*- or *mck1*-deleted strains, respectively). For each strain, 200 cells were analyzed and the percentage of cells containing at least a two-fold increase in nuclear fluorescence was counted as positive. Arrows indicate representative nuclear localization of Msn2-GFP. The scale bar corresponds to 5 μ.

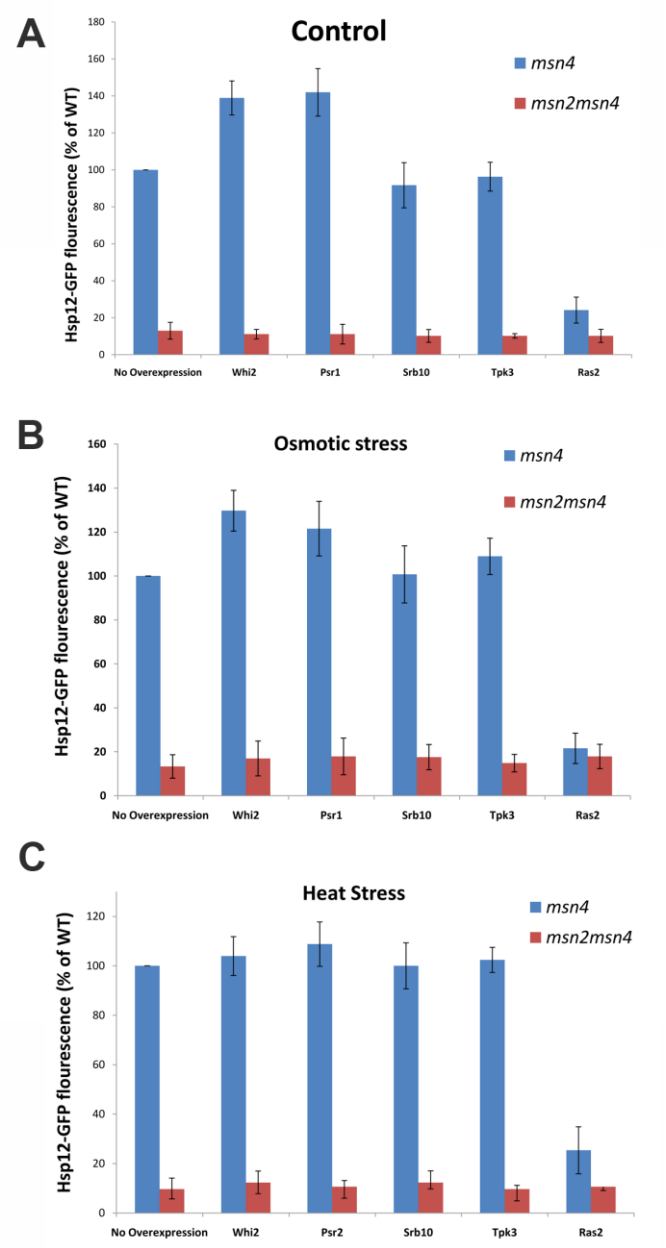

Fig. S7: Overexpression of Whi2, Psr1, Srb10, Tpk3 and Ras2 in W303 *msn4*-deleted or *msn2 msn4*-double deleted strains. Overexpression of Whi2 and Psr1 results in increased Hsp12-GFP expression following exposure to osmotic or heat stress, as well as in the absence of stress, indicating an increase in Msn2 activity. In contrast, overexpression of Ras2 results in decrease in Hsp12-GFP expression, indicating a strong suppression of Msn2 activity. These results are consistent with our analysis on the effects of deleting these genes on Msn2 activity.

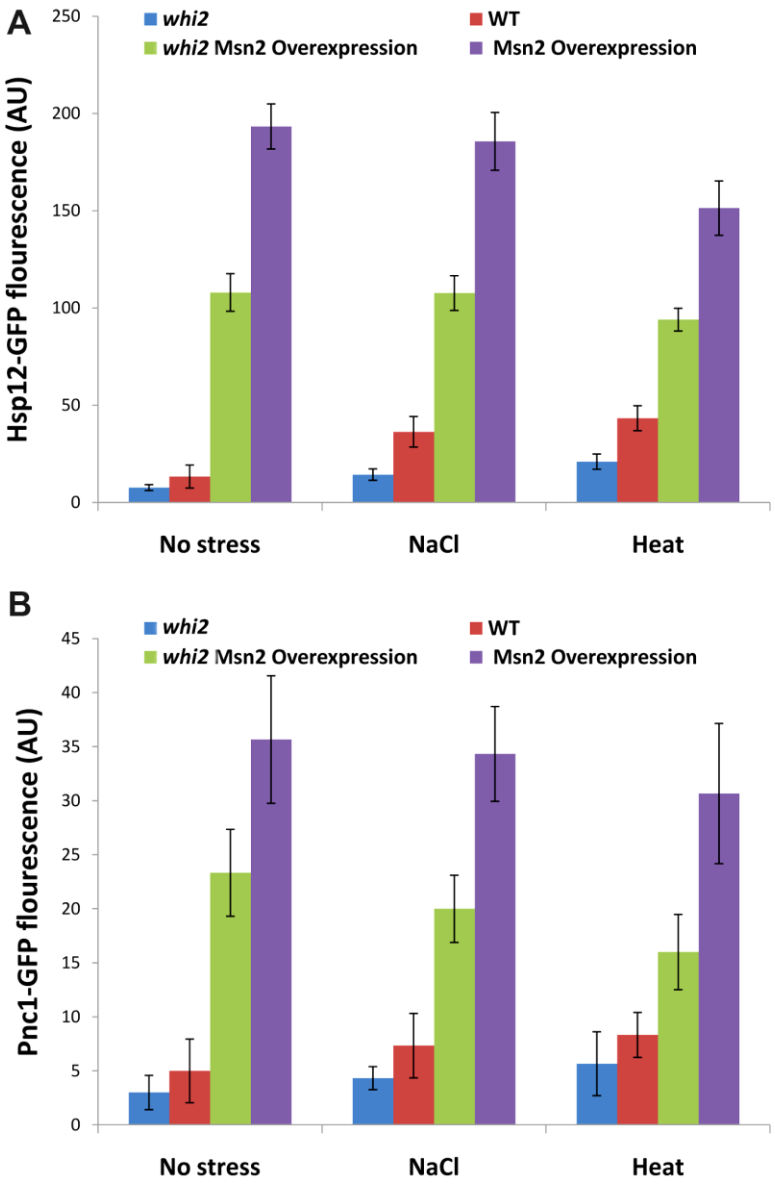

Fig. S8: The effect of overexpression of Msn2 on the background of the *whi2*-deleted S288c-derived strain and the WT strain on the expression of Hsp12-GFP (A) or Pnc1-GFP (B) reporter genes. The fluorescence levels of the different strains were examined following exposure to osmotic stress (NaCl), heat stress or in the absence of stress. Msn2 overexpression completely restores Hsp12-GFP or Pnc1-GFP fluorescence, leading to much higher fluoresence than seen in the WT strain.

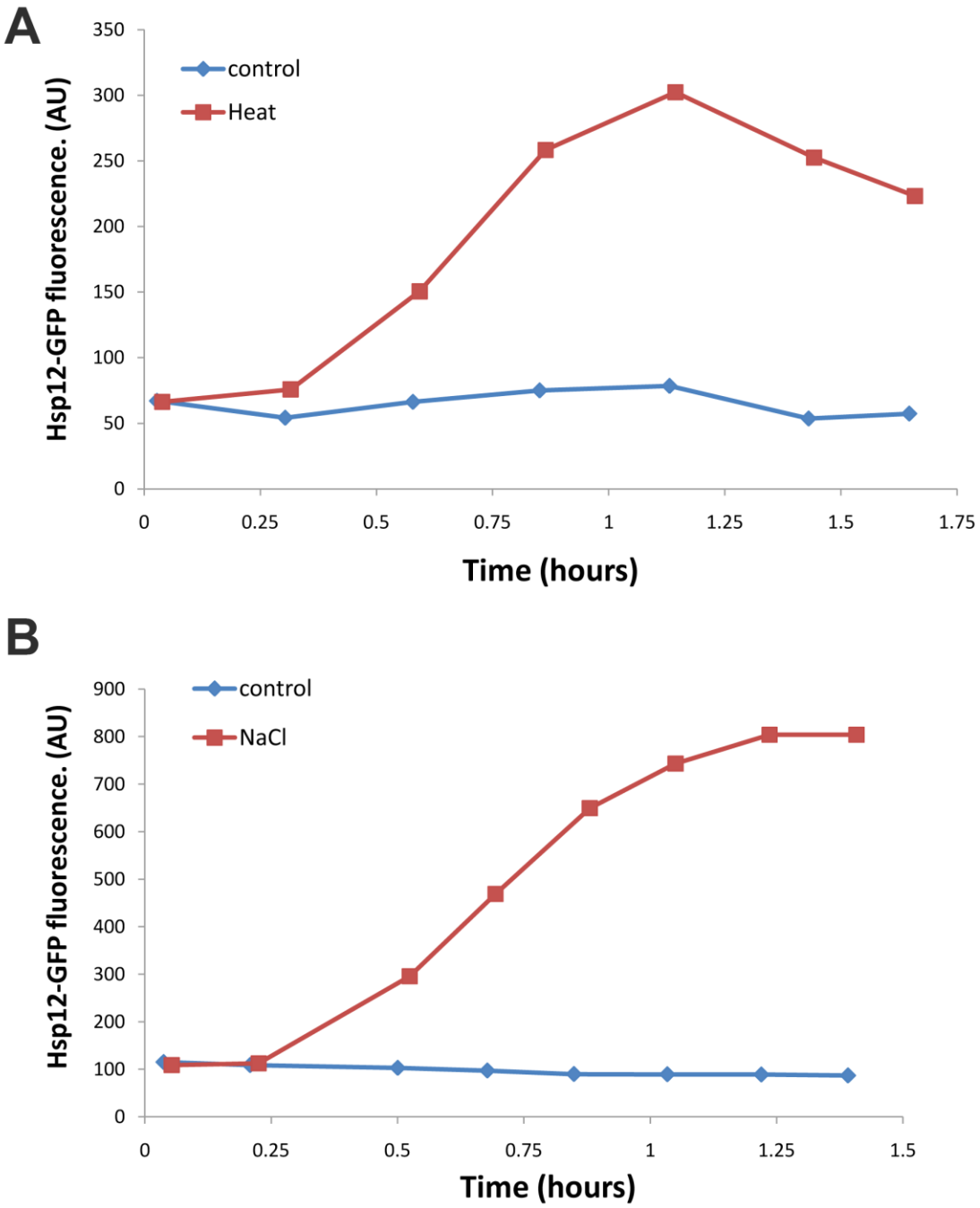

Fig. S9: Increase in fluorescence levels of W303 strains containing Hsp12-GFP following application of heat stress (A) or osmotic stress (B). Similar results were obtained with other reporter genes and with S288c strains containing Hsp12-GFP (data not shown).

Table S1: Msn2 partners analyzed in this study

Protein[a]	Systematic name	Description	Cellular localization	Null phenotype	Findings in this study
Glc7 (P)	YER133W	Type 1 serine/threonine protein phosphatase catalytic subunit, involved in many processes (eg: glycogen metabolism, sporulation, mitosis).	Bud neck Nucleus	inviable	activator of Msn2: deletion causes 10-40% reduction in activity.
Psr2 (G)	YLR019W	Functionally redundant Psr1p homolog, a plasma membrane phosphatase involved in the general stress response; required with Psr1p and Whi2p for full activation of STRE-mediated gene expression, possibly through dephosphorylation of Msn2p	Plasma membrane	viable	activator of Msn2: deletion causes 10-40% reduction in activity.
Psr1 (G)	YLL010C	Plasma membrane associated protein phosphatase involved in the general stress response; required along with binding partner Whi2p for full activation of STRE-mediated gene expression, possibly through dephosphorylation of Msn2p	Plasma membrane	viable	activator of Msn2: deletion causes 10-40% reduction in activity.
Tor1 (G)	YJR066W	PIK-related protein kinase and rapamycin target; subunit of TORC1, a complex that controls growth in response to nutrients by regulating translation, transcription, ribosome biogenesis, nutrient transport and autophagy; involved in meiosis	cytoplasm Nucleus Plasma membrane	Decreased sensitivity to: heat , oxidative stress Increased sensitivity to: cycloheximide, metals	Repressor of Msn2: deletion causes 10-100% increase in activity.
Tpk2 (G)	YPL203W	cAMP-dependent protein kinase catalytic subunit; promotes vegetative growth in response to nutrients via the Ras-cAMP signaling pathway; partially redundant with Tpk1p and Tpk3p.	cytoplasm Nucleus	Increased Sensitivity to: acid PH	activator of Msn2: deletion causes 10-40% reduction in activity.
Tpk1 (G)	YJL164C	cAMP-dependent protein kinase catalytic subunit; promotes vegetative growth in response to nutrients via the Ras-cAMP signaling pathway; inhibited by regulatory subunit Bcy1p in the absence of	Cytoplasm Nucleus	Increased Sensitivity to: hyper osmotic stress, ethanol	Repressor of Msn2: deletion causes 10-100% increase in activity.

		cAMP; partially redundant with Tpk2p and Tpk3p			
Tpk3 (G)	YKL166C	cAMP-dependent protein kinase catalytic subunit; promotes vegetative growth in response to nutrients via the Ras-cAMP signaling pathway; partially redundant with Tpk1p and Tpk2p.	Cytoplasm Nucleus		Strong Repressor of Msn2: deletion causes 100-200% increase in activity.
Mck1 (G)	YNL307C	Protein serine/threonine/tyrosine (dual-specificity) kinase involved in control of chromosome segregation and in regulating entry into meiosis; related to mammalian glycogen synthase kinases of the GSK-3 family	Soluble fraction	Increased Sensitivity to: UV, heat, hyper osmotic stress, oxidative stress, hydroxyurea.	Strong activator of Msn2: deletion causes 40-90% reduction in activity.
Mrk1 (G)	YDL079C	Glycogen synthase kinase 3 (GSK-3) homolog; one of four GSK-3 homologs in S. cerevisiae that function to activate Msn2p-dependent transcription of stress responsive genes and that function in protein degradation	Cellular component unknown	viable	No visible effect on Msn2 activity was observed
Rim11 (G)	YMR139W	Protein kinase required for signal transduction during entry into meiosis; promotes the formation of the Ime1p-Ume6p complex by phosphorylating Ime1p and Ume6p; shares similarity with mammalian glycogen synthase kinase 3-beta	cytoplasm	viable	No visible effect on Msn2 activity was observed
Ygk3 (G)	YOL128C	Protein kinase related to mammalian glycogen synthase kinases of the GSK-3 family; GSK-3 homologs (Mck1p, Rim11p, Mrk1p, Ygk3p) are involved in control of Msn2p-dependent transcription of stress responsive genes and in protein degradation	Cellular component unknown	Viable	No visible effect on Msn2 activity was observed
Rim15 (G)	YFL033C	Glucose-repressible protein kinase involved in signal transduction during cell proliferation in response to nutrients, specifically the establishment of stationary phase.	cytoplasm Nucleus	Increased Sensitivity to: heat, hyper osmotic stress.	Strong activator of Msn2: deletion causes 40-90% reduction in activity.
Snf1 (P)	YDR477W	AMP-activated serine/threonine protein kinase . required for transcription of glucose-repressed genes, thermotolerance, sporulation,	Nucleus		Strong activator of Msn2: deletion causes 40-90% reduction in

		and peroxisome biogenesis	Mitochondrion cytoplasm		activity.
Yak1 (G)	YJL141C	Serine-threonine protein kinase that is part of a glucose-sensing system involved in growth control in response to glucose availability; translocates from the cytoplasm to the nucleus and phosphorylates Pop2p in response to a glucose signal	Cytoplasm Nucleus	Increased Sensitivity to: acid PH	activator of Msn2: deletion causes 10-40% reduction in activity.
Hog1 (G)	YLR113W	Mitogen-activated protein kinase involved in osmoregulation via three independent osmosensors; mediates the recruitment and activation of RNA Pol II at Hot1p-dependent promoters; localization regulated by Ptp2p and Ptp3p	Cytoplasm	Increased Sensitivity to: hyper osmotic stress, metals	Strong activator of Msn2: deletion causes 40-90% reduction in activity.
Tor2 (G)	YKL203C	PIK-related protein kinase and rapamycin target; subunit of TORC1, a complex that regulates growth in response to nutrients and TORC2, a complex that regulates cell-cycle dependent polarization of the actin cytoskeleton; involved in meiosis	Mitochondrion Plasma membrane	inviable	Repressor of Msn2: deletion causes 10-100% increase in activity.
Srb10 (P)	YPL042C	Cyclin-dependent protein kinase, component of RNA polymerase II holoenzyme; involved in phosphorylation of the RNA polymerase II C-terminal domain; involved in glucose repression	Nucleus	Increased Sensitivity to: heat	Repressor of Msn2: deletion causes 10-100% increase in activity.
Hsp82 (G)	YPL240C	Hsp90 chaperone required for pheromone signaling and negative regulation of Hsf1p; docks with Tom70p for mitochondrial preprotein delivery.	cytoplasm	viable	No visible effect on Msn2 activity was observed
Hsp104 (G)	YLL026W	Heat shock protein that cooperates with Ydj1p (Hsp40) and Ssa1p (Hsp70) to refold and reactivate previously denatured, aggregated proteins; responsive to stresses including: heat, ethanol, and sodium arsenite.	Cytoplasm Nucleus	Increased Sensitivity to: heat	No visible effect on Msn2 activity was observed
Snf2 (P)	YOR290C	Catalytic subunit of the SWI/SNF chromatin remodeling complex	Nucleus	Increased Sensitivity to:	No visible effect on Msn2 activity

		involved in transcriptional regulation; contains DNA-stimulated ATPase activity; functions interdependently in transcriptional activation with Snf5p and Snf6p		UV. Hydroxyurea, MMS.	was observed
Gis1 (G)	YDR096W	Histone demethylase; transcription factor involved in the expression of genes during nutrient limitation.	Mitochondrion Nucleus	viable	activator of Msn2: deletion causes 10-40% reduction in activity.
Eaf7 (P)	YNL136W	Subunit of the NuA4 histone acetyltransferase complex, which acetylates the N-terminal tails of histones H4 and H2A	Nucleus	Increased Sensitivity to: acid PH, heat, hyper osmotic stress, ethanol,	Strong Repressor of Msn2: deletion causes 100-200% increase in activity.
Eaf1 (G)	YDR359C	Component of the NuA4 histone acetyltransferase complex; acts as a platform for assembly of NuA4 subunits into the native complex; required for initiation of pre-meiotic DNA replication, likely due to its requirement for expression of IME1	Nucleus	Increased Sensitivity to: heat, rapamycin, MMS, oxidative stress.	No visible effect on Msn2 activity was observed
Gcn5 (G)	YGR252W	Histone acetyltransferase, acetylates N-terminal lysines on histones H2B and H3; catalytic subunit of the ADA and SAGA histone acetyltransferase complexes; founding member of the Gcn5p-related N-acetyltransferase superfamily	Nucleus	Increased Sensitivity to: alkaline PH, heat, metals, , ethanol	activator of Msn2: deletion causes 10-40% reduction in activity.
Rpd3 (G)	YNL330C	Histone deacetylase; regulates transcription, silencing, and other processes by influencing chromatin remodeling; forms at least two different complexes which have distinct functions and members	Nucleus	Increased sensitivity to: MMS, cycloheximide	Strong activator of Msn2: deletion causes 40-90% reduction in activity.
Hda3 (G)	YPR179C	Subunit of a possibly tetrameric trichostatin A-sensitive class II histone deacetylase complex that contains an Hda1p homodimer and an Hda2p-Hda3p heterodimer; required for the activity of the complex; has similarity to Hda2p	Nucleus	Increased sensitivity to: cycloheximide	Strong activator of Msn2: deletion causes 40-90% reduction in activity.
Ras2 (G)	YNL098C	GTP-binding protein that regulates the nitrogen starvation response, sporulation, and filamentous growth; farnesylation and	Plasma membrane	Decreased sensitivity to: hyper osmotic stress,	Strong Repressor of Msn2: deletion causes 100-200% increase in

		palmitoylation required for activity and localization to plasma membrane; homolog of mammalian Ras proto-oncogenes		rapamycin.	activity.
Cdc25 (G)	YLR310C	Membrane bound guanine nucleotide exchange factor (GEF or GDP-release factor); indirectly regulates adenylate cyclase through activation of Ras1p and Ras2p by stimulating the exchange of GDP for GTP;	cytoplasm Nucleus Plasma membrane	inviable	Strong Repressor of Msn2: deletion causes 100-200% increase in activity.
Cyr1 (G)	YJL005W	Adenylate cyclase, required for cAMP production and cAMP-dependent protein kinase signaling; the cAMP pathway controls a variety of cellular processes, including metabolism, cell cycle, stress response, stationary phase, and sporulation		inviable	Repressor of Msn2: deletion causes 10-100% increase in activity.
Gal11 (P)	YOL051W	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	Nucleus	Increased Sensitivity to: heat , oxidative stress	Strong activator of Msn2: deletion causes 40-90% reduction in activity.
Tom1 (G)	YDR457W	E3 ubiquitin ligase of the hect-domain class; has a role in mRNA export from the nucleus and may regulate transcriptional coactivators; involved in degradation of excess histones	Nucleus	Increased Sensitivity to: heat, oxidative stress,	No visible effect on Msn2 activity was observed
Whi2 (P)	YOR043W	Protein required, with binding partner Psr1p, for full activation of the general stress response, possibly through Msn2p dephosphorylation; regulates growth during the diauxic shift; negative regulator of G1 cyclin expression	Cellular component unknown	Increased Sensitivity to: acid PH, rapamycin	Strong activator of Msn2: deletion causes 40-90% reduction in activity.
Ump1 (P)	YBR173C	Short-lived chaperone required for correct maturation of the 20S proteasome; may inhibit premature dimerization of proteasome half-mers; degraded by proteasome upon completion of its assembly	Cytoplasm Nucleus	Increased Sensitivity to: heat Decreased sensitivity to: MMS	Repressor of Msn2: deletion causes 10-100% increase in activity.
Bnr1 (G)	YIL159W	Formin, nucleates the formation of linear actin filaments, involved in cell	Bud neck	viable	No visible effect on Msn2 activity

		processes such as budding and mitotic spindle orientation which require the formation of polarized actin cables, functionally redundant with BNI1			was observed
Msn5 (G)	YDR335W	Karyopherin involved in nuclear import and export of proteins, including import of replication protein A and export of Swi6p, Far1p, and Pho4p; required for re-export of mature tRNAs after their retrograde import from the cytoplasm	Cytoplasm Nucleus	Increased sensitivity to: cycloheximide	Activator of Msn2: deletion causes 10-40% reduction in activity.
Sok2 (P)	YMR016C	Nuclear protein that plays a regulatory role in the cyclic AMP (cAMP)-dependent protein kinase (PKA) signal transduction pathway; negatively regulates pseudohyphal differentiation; homologous to several transcription factors	Nucleus	Increased sensitivity to: cycloheximide, hyper osmotic stress, ethanol	Deletion elevates HSP12 expression by 100-700% in a pathway only partially dependent on MSN2.

[a] Msn2 partners highlighted in red are phosphatses, partners highlighted in blue are kinases, partners highlighted in purple are chaperones, partners highlighted in green are chromatin remodelers and partners in black exhibit diverse functions. Partners that are in physical or genetic interaction with Msn2 are depicted in (P) or (G), respectively.

Table S2: Percentage of Hsp12-GFP expression levels[a] in 35 Msn2 partner-deleted strains[b] normalized to expression in the WT strain (source of data for **Fig. 2**, main text).

Gene deletion	No stress	Oxidative[b] stress	Osmotic[b] stress	Heat[b] stress
rpd3	14	8	5	34
mck1	16	9	4	35
hda3	19	21	12	20
whi2	23	14	10	38
msn2	21	14	14	40
snf1	25	25	23	54
gal11	42	17	23	55
rim15	27	47	28	65
hog1	43	60	35	49
msn5	40	51	49	66
tpk2	43	53	42	74
yak1	31	52	59	73
gcn5	41	21	54	100
gis1	46	53	71	73
psr1	53	61	67	82
psr2	70	68	71	90
glc7	58	33	109	117
msn4	67	85	72	98
tom1	79	91	96	92
snf2	72	125	73	104
eaf1	78	127	98	101
hsp104	75	104	103	111
bnr1	84	93	101	99
WT	100	100	100	100
rim11	94	96	115	96
ygk3	93	93	120	97
hsp82	103	105	95	102
mrk1	114	101	106	102
tpk1	132	133	88	121
srb10	88	156	122	138
ump1	177	142	106	131
cyr1	148	185	121	108
tor1	153	199	99	118
tor2	174	205	123	109
cdc25	195	208	122	118
eaf7	256	174	147	109
tpk3	292	290	115	120
ras2	860	532	246	356

[a] Median Hsp12-GFP expression levels normalized relative to level seen in the WT strain. All data were obtained by flow cytometry (see Fig. S2 for examples and Material and Methods).

[b] The stresses applied are oxidative stress (0.6 mM H_2O_2), osmotic stress (0.5 M NaCl) and heat stress (37^{o}C).

Table S3: Percentage of reporter gene expression levels[a] in 18 Msn2 partner-deleted strains normalized to expression in the *msn4*-deletion strain (source of data for **Fig. 3**).

	Heat stress[b]				Osmotic stress[b]				Oxidative stress[b]				No stress			
Strain[c]	***HSP12***	***TPS2***	***PNC1***	***PFK2***	***HSP12***	***TPS2***	***PNC1***	***PFK2***	***HSP12***	***TPS2***	***PNC1***	***PFK2***	***HSP12***	***TPS2***	***PNC1***	***PFK2***
msn2	15	37	42	97	9	38	32	96	8	38	28	69	19	55	47	101
mck1	52	57	57	95	12	45	47	103	19	47	33	93	24	53	52	110
whi2	43	64	59	94	20	42	51	95	19	55	34	70	32	58	56	98
hog1	30	59	69	88	17	49	74	79	14	49	42	98	22	63	65	100
gal11	22	95	49	103	17	71	58	99	14	77	51	101	63	87	92	118
rim15	74	83	75	99	45	76	67	98	58	76	72	81	44	74	72	98
tpk2	87	102	90	98	40	97	73	101	56	98	84	99	49	88	82	100
gis1	93	92	98	109	52	84	94	113	63	89	97	119	39	81	87	100
psr1	84	84	89	96	58	80	91	97	67	84	79	95	78	77	80	98
psr2	81	87	83	102	79	80	78	104	92	84	81	105	84	81	77	101
msn5	82	85	121	103	81	82	132	101	49	86	88	101	88	106	166	97
yak1	79	100	87	94	56	102	94	100	72	100	98	100	50	98	95	98
snf1	64	107	97	102	57	97	76	106	53	103	80	102	37	99	75	104
ygk3	98	95	99	97	92	100	94	100	86	100	98	102	69	95	93	99
WT*[d]	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
tpk1	117	105	111	99	132	100	100	104	121	106	108	100	166	100	126	101
srb10	111	123	112	84	113	131	129	85	125	136	115	94	130	165	150	79
tpk3	125	120	108	100	111	117	120	103	113	121	114	107	187	137	141	102
ump1	138	104	81	93	139	103	89	97	94	102	74	106	248	104	90	96
eaf7	134	104	110	93	156	96	122	96	164	99	119	94	261	95	127	92

[a] Median reporter gene expression levels normalized relative to level seen in the *msn4*-deleted strain. All data were obtained by flow cytometry (see Fig. S2 for examples and Material and Methods).

[b] The stress applied are oxidative stress (0.6 mM H_2O_2), osmotic stress (0.5 M NaCl) and heat stress (37^{o}C).

[c] All strains were generated on the background of the *msn4*-deletion strain.

[d] WT* strain is deleted of the *MSN4* gene.

Table S4: References for Msn2/4-regulated expression of *HSP12*, *TPS2*, *PNC1* and *PF2* reporter genes (data was obtained from the *Saccharomyces* genome database (SGD) site, http://www.yeastgenome.org/)

ORF/Gene	Description	Evidence for MSN2/4 regulation
HSP12	Plasma membrane-localized protein that protects membranes from desiccation; induced by heat shock, oxidative stress, osmostress, stationary phase entry, glucose depletion, oleate and alcohol; regulated by the HOG and Ras-Pka pathways.	**Direct Evidence:** PubMed Erkina et al, Mol Cell Biol, ;28(4):1207-17 (*Direct: ChIP*) PubMed Ni L et al., Genes Dev, ;23(11):1351-63 (*Direct: ChIP*) PubMed Lenssen E et al., Mol Cell Biol, 2005 Jan;25(1):488-98 (*Direct: ChIP*) **Indirect Evidence:** PubMed Chua G et al., PNAS, 2006;103(32):12045-50 (*Indirect: Microarrays - Wild type vs. TF mutant*) PubMed Melamed et al., RNA, ;14(7):1337-51 (*Indirect: Northern blotting - Wild type vs. TF mutant*) PubMed Berry and Gasch, Mol Biol Cell, ;19(11):4580-7 (*Indirect: RT-PCR - Wild type vs. TF mutant*) PubMed Garcia et al, J Biol Chem, ;0(0): (*Indirect: Microarrays - Wild type vs. TF mutant*)-1 PubMed Kelley & Ideker , PLOS Genet, ;5(5):e1000488 (*Indirect: Microarrays - Wild type vs. TF mutant*) PubMed Hazelwood et al, Appl Environ Microbiol, ;75(21):6876-6885 (*Indirect: Microarrays - Wild type vs. TF mutant*) PubMed Boy-Marcotte E et al., Mol Microbiol, 1999;3(2):274-83 (*Indirect: Proteomics - Wild type vs. TF mutant*) PubMed Causton HC et al., Mol Biol Cell, 2001;2(2):323-37 (*Indirect: Microarrays - Wild type vs. TF mutant*) PubMed Hasan R et al., Mol Microbiol, 2002;45(1):233-41 (*Indirect: Proteomics - Wild type vs. TF mutant*) PubMed Kandror O et al., Mol Cell, 2004;3(6):771-81 (*Indirect: Northern blotting - Wild type vs. TF mutant*) PubMed Treger JM et al., J Biol Chem, 1998;73(41):26875-9 (*Indirect: Northern blotting - Wild type vs. TF mutant*) **Undefined Evidence:** PubMed Martinez-Pastor MT et al., EMBO J, 1996;5(9):2227-35 (*Undef*) PubMed Schmitt AP et al., Proc Natl Acad Sci U S A, 1996;3(12):5777-82 (*Undef*)
TPS2	Phosphatase subunit of the trehalose-6-phosphate synthase/phosphatase complex, which synthesizes the storage carbohydrate, trehalose; expression is induced by stress conditions and repressed by the Ras-cAMP pathway	**Indirect Evidence:** PubMed Horan et al., Yeast, 2006;23(7):519-535 (*Indirect: Microarrays - Wild type vs. TF mutant*) PubMed Chua G et al., PNAS, 2006;103(32):12045-50 (*Indirect: Microarrays - Wild type vs. TF mutant*) PubMed Berry and Gasch, Mol Biol Cell, ;19(11):4580-7 (*Indirect: RT-PCR - Wild type vs. TF mutant*) PubMed Hazelwood et al, Appl Environ Microbiol, ;75(21):6876-6885 (*Indirect: Microarrays - Wild type vs. TF mutant*) PubMed Kandror O et al., Mol Cell, 2004;3(6):771-81 (*Indirect: Northern blotting - Wild type vs. TF mutant*) PubMed Lai LC et al., Mol Cell Biol, 2005 May;25(10):4075-91 (*Indirect: Microarrays - Wild type vs. TF mutant*) PubMed Rep M et al., J Biol Chem, 2000;75(12):8290-300 (*Indirect: Microarrays - Wild type vs. TF mutant*) PubMed Schmitt AP et al., Proc Natl Acad Sci U S A, 1996;3(12):5777-82 (*Indirect: Northern blotting - Wild type vs. TF mutant*)
PNC1	Nicotinamidase that converts nicotinamide to nicotinic acid as part of the NAD(+) salvage pathway, required for life-span extension by calorie restriction; PNC1 expression responds to all known stimuli that	**Direct Evidence:** PubMed Medvedik et al, PLOS Biol, ;5(10):e261 (*Direct: ChIP*) **Indirect Evidence:** PubMed Berry and Gasch, Mol Biol Cell, ;19(11):4580-7 (*Indirect: Microarrays - Wild type vs. TF mutant*) PubMed Kelley & Ideker , PLOS Genet, ;5(5):e1000488 (*Indirect: Microarrays - Wild type vs. TF mutant*) PubMed Boy-Marcotte E et al., Mol Microbiol, 1999;3(2):274-83 (*Indirect: Proteomics - Wild type vs. TF mutant*) PubMed Hasan R et al., Mol Microbiol, 2002;45(1):233-41 (*Indirect: Proteomics - Wild type vs. TF mutant*)

	extend replicative life-span	
PFK2	Beta subunit of heterooctameric phosphofructokinase involved in glycolysis, indispensable for anaerobic growth, activated by fructose-2,6-bisphosphate and AMP, mutation inhibits glucose induction of cell cycle-related genes	none

Table S5: List of strains used in this study

	Strain	Genotype				Generated
1	W303-1A	*MAT*a {*leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*}				Martin Kupiec, Tel-Aviv University
2	W303-1B	*MAT*α {*leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15*}				Martin Kupiec, Tel-Aviv University
3	YRG2	Mata *ura352 his3-200 ade2-101 lys2-801 trp1-901 leu2-3 112 gal4-542 gal80-538* LYS2::UAS_{GAL1}-TATA $_{GAL1}$-HIS3 URA3::$UAS_{GAL4\ 17mers(x3)}$-$TATA_{CYC1}$-*lacZ*				Stratagene
4	YMS140a	S288C his3Δ1 Δleu2 Δmet15 Δura3 Δcan::MFA1pr-leu2 Δlyp1 cyn2				Maya Schuldiner, Weizmann Institute
5	AQS1	YMS140α *HSP12::HSP12-GFP-HIS3MX6*				This study
6	AQS2	YMS140α *HSP12::HSP12-GFP-HIS3MX6 whi2:: natMX4*				This study
7	GS1*	*msn4:: hphMX4*				This study
8	GS2*	*his3::hphMX4*	*msn2::kanMX6*	*Tps2::TPS2-GFP-HIS3MX6*		This study
9	GS3*	*his3::hphMX4*	*msn2::kanMX6*	*PNC1::TPS2-GFP-HIS3MX6*		This study
10	GS4*	*his3::hphMX4*	*msn2::kanMX6*	*HSP12::HSP12-GFP-HIS3MX6*		This study
11	AYC1*			*PNC1::PNC1-GFP-HIS3MX6*		This study
12	AYC2 *		*msn2::kanMX6*	*PNC1::PNC1-GFP-HIS3MX6*		This study
13	AYC3 *	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*		This study
14	AYC4 *	*msn4:: hphMX4*	*msn2::kanMX6*	*PNC1::PNC1-GFP-HIS3MX6*		This study
15	AYC5 *			*HSP12::HSP12-GFP-HIS3MX6*		This study
16	AYC6 *		*msn2::kanMX6*	*HSP12::HSP12-GFP-HIS3MX6*		This study
17	AYC7 *	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*		This study
18	AYC8 *	*msn4:: hphMX4*	*msn2::kanMX6*	*HSP12::HSP12-GFP-HIS3MX6*		This study
19	AYC9*			*Tps2::TPS2-GFP-HIS3MX6*		This study
20	AYC10*		*msn2::kanMX6*	*Tps2::TPS2-GFP-HIS3MX6*		This study
21	AYC11*	*msn4:: hphMX4*		*Tps2::TPS2-GFP-HIS3MX6*		This study
22	AYC12*	*msn4:: hphMX4*	*msn2::kanMX6*	*Tps2::TPS2-GFP-HIS3MX6*		This study
23	AIC1*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*tpk1::natMX4*	This study
24	AIC2*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*tpk2:: natMX4*	This study
25	AIC3*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*tpk3:: natMX4*	This study
26	AIC4*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*rim11:: natMX4*	This study
27	AIC5*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*rim15:: natMX4*	This study

28	AIC6*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*gal11:: natMX4*	This study
29	AIC7*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*psr1:: natMX4*	This study
30	AIC8*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*psr2:: natMX4*	This study
31	AIC9*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*ump1:: natMX4*	This study
32	AIC10*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*sok2:: natMX4*	This study
33	AIC11*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*srb10:: natMX4*	This study
34	AIC12*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*whi2:: natMX4*	This study
35	AIC13*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*mck1:: natMX4*	This study
36	AIC14*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*snf1:: natMX4*	This study
37	AIC15*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*gis1:: natMX4*	This study
38	AIC16*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*yak1:: natMX4*	This study
39	AIC17*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*ygk3:: natMX4*	This study
40	AIC18*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*hog1:: natMX4*	This study
41	AIC19*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*msn5:: natMX4*	This study
42	AIC20*	*msn4:: hphMX4*		*HSP12::HSP12-GFP-HIS3MX6*	*eaf7:: natMX4*	This study
43	ASD1*				*whi2:: natMX4*	This study
44	ASD2*				*mck1:: natMX4*	This study
45	ASD3*				*msn5:: natMX4*	This study
46	APM1*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*tpk3:: natMX4*	This study
47	APM2*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*Eaf7:: natMX4*	This study
48	APM3*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*rim15:: natMX4*	This study
49	APM4*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*gal11:: natMX4*	This study
50	APM5*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*psr1:: natMX4*	This study
51	APM6*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*psr2:: natMX4*	This study
52	APM7*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*ump1:: natMX4*	This study
53	APM8*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*sok2:: natMX4*	This study
54	APM9*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*srb10:: natMX4*	This study

55	APM10*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*whi2:: natMX4*	This study
56	APM11*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*mck1:: natMX4*	This study
57	APM12*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*snf1:: natMX4*	This study
58	APM1*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*gis1:: natMX4*	This study
59	APM14*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*yak1:: natMX4*	This study
60	APM15*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*ygk3:: natMX4*	This study
61	APM16*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*hog1:: natMX4*	This study
62	APM17*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*ump1:: natMX4*	This study
63	APM18*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*tpk1::natMX4*	This study
64	APM19*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*tpk2:: natMX4*	This study
65	APM20*	*msn4:: hphMX4*		*PNC1::PNC1-GFP-HIS3MX6*	*msn5:: natMX4*	This study
66	ATM1*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*tpk3:: natMX4*	This study
67	ATM2*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*Eaf7:: natMX4*	This study
68	ATM3*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*rim15:: natMX4*	This study
69	ATM4*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*gal11:: natMX4*	This study
70	ATM5*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*psr1:: natMX4*	This study
71	ATM6*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*psr2:: natMX4*	This study
72	ATM7*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*ump1:: natMX4*	This study
73	ATM8*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*sok2:: natMX4*	This study
74	ATM9*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*srb10:: natMX4*	This study
75	ATM10*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*whi2:: natMX4*	This study
76	ATM11*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*mck1:: natMX4*	This study
77	ATM12*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*snf1:: natMX4*	This study
78	ATM13*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*gis1:: natMX4*	This study
79	ATM14*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*yak1:: natMX4*	This study
80	ATM15*	*msn4::*		*TPS2::TPS2-GFP-HIS3MX6*	*ygk3:: natMX4*	This study

		hphMX4				
81	ATM16*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*hog1:: natMX4*	This study
82	ATM17*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*ump1:: natMX4*	This study
83	ATM18*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*tpk1::natMX4*	This study
84	ATM19*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*tpk2:: natMX4*	This study
85	ATM20*	*msn4:: hphMX4*		*TPS2::TPS2-GFP-HIS3MX6*	*msn5:: natMX4*	This study
86	APK1#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*tpk3:: natMX4*	This study
87	APK2#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*Eaf7:: natMX4*	This study
88	APK3#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*rim15:: natMX4*	This study
89	APK4#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*gal11:: natMX4*	This study
90	APK5#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*psr1:: natMX4*	This study
91	APK6#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*psr2:: natMX4*	This study
92	APK7#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*ump1:: natMX4*	This study
93	APK8#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*sok2:: natMX4*	This study
94	APK9#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*srb10:: natMX4*	This study
95	APK10#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*whi2:: natMX4*	This study
96	APK11#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*mck1:: natMX4*	This study
97	APK12#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*snf1:: natMX4*	This study
98	APK13#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*gis1:: natMX4*	This study
99	APK14#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*yak1:: natMX4*	This study
100	APK15#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*ygk3:: natMX4*	This study
101	APK16#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*hog1:: natMX4*	This study
102	APK17#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*ump1:: natMX4*	This study
103	APK18#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*tpk1::natMX4*	This study
104	APK19#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*tpk2:: natMX4*	This study
105	APK20#	*msn4:: hphMX4*		*PFK2::PFK2-GFP-HIS3MX6*	*msn5:: natMX4*	This study

106	ASM1#	*HSP12::HSP12-GFP-HIS3MX6*	*msn2:: kanMX6*	This study
107	ASM2#	*HSP12::HSP12-GFP-HIS3MX6*	*tpk3:: kanMX6*	This study
108	ASM3#	*HSP12::HSP12-GFP-HIS3MX6*	*Eaf7:: kanMX6*	This study
109	ASM4#	*HSP12::HSP12-GFP-HIS3MX6*	*rim15:: kanMX6*	This study
110	ASM5#	*HSP12::HSP12-GFP-HIS3MX6*	*gal11:: kanMX6*	This study
111	ASM6#	*HSP12::HSP12-GFP-HIS3MX6*	*psr1:: kanMX6*	This study
112	ASM7#	*HSP12::HSP12-GFP-HIS3MX6*	*psr2:: kanMX6*	This study
113	ASM8#	*HSP12::HSP12-GFP-HIS3MX6*	*ump1:: kanMX6*	This study
114	ASM9#	*HSP12::HSP12-GFP-HIS3MX6*	*sok2:: kanMX6*	This study
115	ASM10#	*HSP12::HSP12-GFP-HIS3MX6*	*srb10:: kanMX6*	This study
116	ASM11#	*HSP12::HSP12-GFP-HIS3MX6*	*whi2:: kanMX6*	This study
117	ASM12#	*HSP12::HSP12-GFP-HIS3MX6*	*mck1:: kanMX6*	This study
118	ASM13#	*HSP12::HSP12-GFP-HIS3MX6*	*snf1:: kanMX6*	This study
119	ASM14#	*HSP12::HSP12-GFP-HIS3MX6*	*gis1:: kanMX6*	This study
120	ASM15#	*HSP12::HSP12-GFP-HIS3MX6*	*yak1:: kanMX6*	This study
121	ASM16#	*HSP12::HSP12-GFP-HIS3MX6*	*ygk3:: kanMX6*	This study
122	ASM17#	*HSP12::HSP12-GFP-HIS3MX6*	*hog1:: kanMX6*	This study
123	ASM18#	*HSP12::HSP12-GFP-HIS3MX6*	*ump1:: kanMX6*	This study
124	ASM19#	*HSP12::HSP12-GFP-HIS3MX6*	*tpk1:: kanMX6*	This study
125	ASM20#	*HSP12::HSP12-GFP-HIS3MX6*	*tpk2:: kanMX6*	This study
126	ASM21#	*HSP12::HSP12-GFP-HIS3MX6*	*msn5:: kanMX6*	This study
127	ASM22#	*HSP12::HSP12-GFP-HIS3MX6*	*gcn5:: kanMX6*	This study
128	ASM23#	*HSP12::HSP12-GFP-HIS3MX6*	*Glc7-damp:: kanMX6*	This study
129	ASM24#	*HSP12::HSP12-GFP-HIS3MX6*	*msn4:: kanMX6*	This study
130	ASM25#	*HSP12::HSP12-GFP-HIS3MX6*	*rim11:: kanMX6*	This study
131	ASM26#	*HSP12::HSP12-GFP-HIS3MX6*	*hsp82:: kanMX6*	This study
132	ASM27#	*HSP12::HSP12-GFP-HIS3MX6*	*mrk1:: kanMX6*	This study
133	ASM28#	*HSP12::HSP12-GFP-HIS3MX6*	*rim11:: kanMX6*	This study
134	ASM29#	*HSP12::HSP12-GFP-HIS3MX6*	*bnr1:: kanMX6*	This study
135	ASM30#	*HSP12::HSP12-GFP-HIS3MX6*	*hsp104:: kanMX6*	This study
136	ASM31#	*HSP12::HSP12-GFP-HIS3MX6*	*Tom1:: kanMX6*	This study
137	ASM32#	*HSP12::HSP12-GFP-HIS3MX6*	*Had3:: kanMX6*	This study
138	ASM33#	*HSP12::HSP12-GFP-HIS3MX6*	*Cyr1-damp:: kanMX6*	This study
139	ASM34#	*HSP12::HSP12-GFP-HIS3MX6*	*tor1:: kanMX6*	This study
140	ASM35#	*HSP12::HSP12-GFP-HIS3MX6*	*Tor2-damp:: kanMX6*	This study
141	ASM36#	*HSP12::HSP12-GFP-HIS3MX6*	*Cdc25-damp:: kanMX6*	This study
142	ASM37#	*HSP12::HSP12-GFP-HIS3MX6*	*Eaf7:: kanMX6*	This study
143	ASM38#	*HSP12::HSP12-GFP-HIS3MX6*	*Tpk3:: kanMX6*	This study
144	ASM39#	*HSP12::HSP12-GFP-HIS3MX6*	*Sok2:: kanMX6*	This study
145	ASM40#	*HSP12::HSP12-GFP-HIS3MX6*	*Ras2:: kanMX6*	This study
146	ASM41#	*HSP12::HSP12-GFP-HIS3MX6*	*tor1:: kanMX6 whi2:: natMX4*	This study
147	ASM42#	*HSP12::HSP12-GFP-HIS3MX6*	*Cdc25-damp::*	This study

			kanMX6 whi2:: natMX4	
148	ASM43#	*HSP12::HSP12-GFP-HIS3MX6*	*Tor2-damp:: kanMX6 whi2:: natMX4*	This study
149	ASM44#	*HSP12::HSP12-GFP-HIS3MX6*	*Tpk3:: kanMX6 whi2:: natMX4*	This study
150	ASM45#	*HSP12::HSP12-GFP-HIS3MX6*	*Ras2:: kanMX6 whi2:: natMX4*	This study

*- strains created on W303-1A background

#- strains created on S288c background