Electronic Supplementary Material (ESI) for Molecular Omics.

Supplementary Figures and Tables

A QUANTITATIVE AND TEMPORAL MAP OF PROTEOSTASIS DURING HEAT SHOCK IN *SACCHAROMYCES CEREVISIAE*

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Methods

Detailed description of protein significance testing procedure

In order to determine statistically significantly changing proteins with respect to time point T0 we used the MSstats package [1]. Protein identities, conditions, biological replicates and intensities from MaxQuant were uploaded, retaining protein ID information from the 'proteinGroups.txt' file, conditions and biological replicates from the 'annotation.csv' file, and intensities from the 'evidence.txt' file. Data normalization was performed using the 'equalizeMedians' option and summarization using the 'Tukey's median polish' option. Following this, a condition comparison was performed using the 'groupComparison' option. Log_2 fold changes and adjusted *p values* were obtained for the WT and *ssb1*Δ strains. .

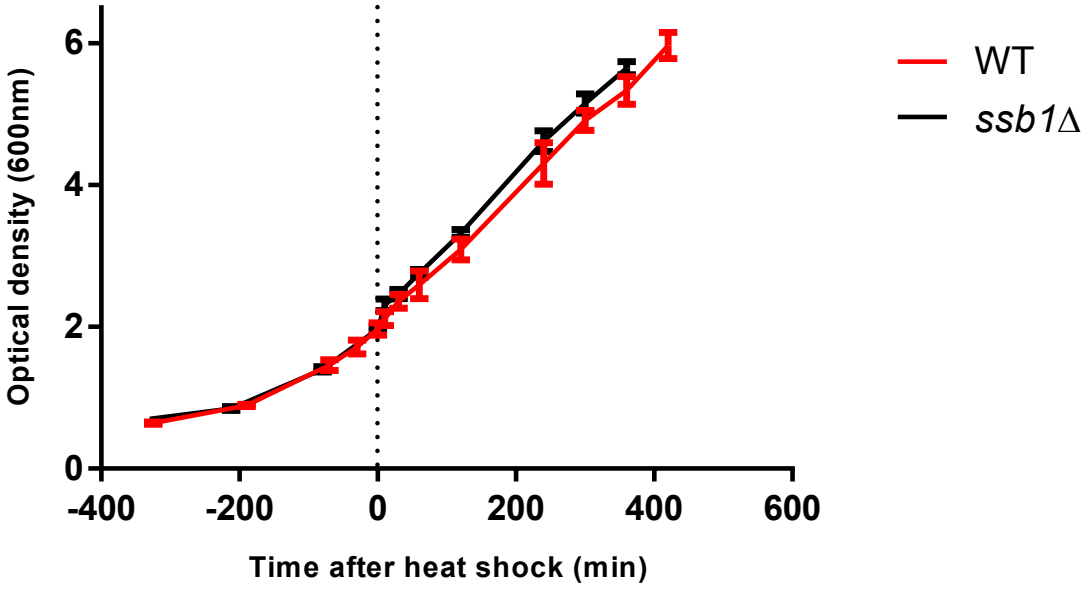
Additionally, we defined empirical thresholds for the minimum magnitude of change a protein had to display in order to be considered significantly regulated. This was based on the technical variance in each block of LC-MS analysis. To that end, a distribution of associated fold changes was generated by calculating ratios between all permutations of the QC sample pairs in either WT-QC or *ssb1*Δ-QC (e.g. QC1 vs QC2, QC1 vs QC3, …, QC2 vs QC3, etc.) (**Supplementary Figure 3**). The 2.5^{th} and 97.5^{th} percentile values of the resulting Gaussian distributions were then assigned as down- and up-regulation thresholds respectively. The thresholds were 0.69<log2FC<-0.958 for the WT strain and 0.634<log2FC<-0.714 for the ssb1Δ mutant. Only proteins with fold changes outside these thresholds and an associated FDR corrected *p-value* < 0.05 were considered significantly changing.

Results

Supplementary Table 1.Transcription factors with experimental evidence of binding to YBR085C-A

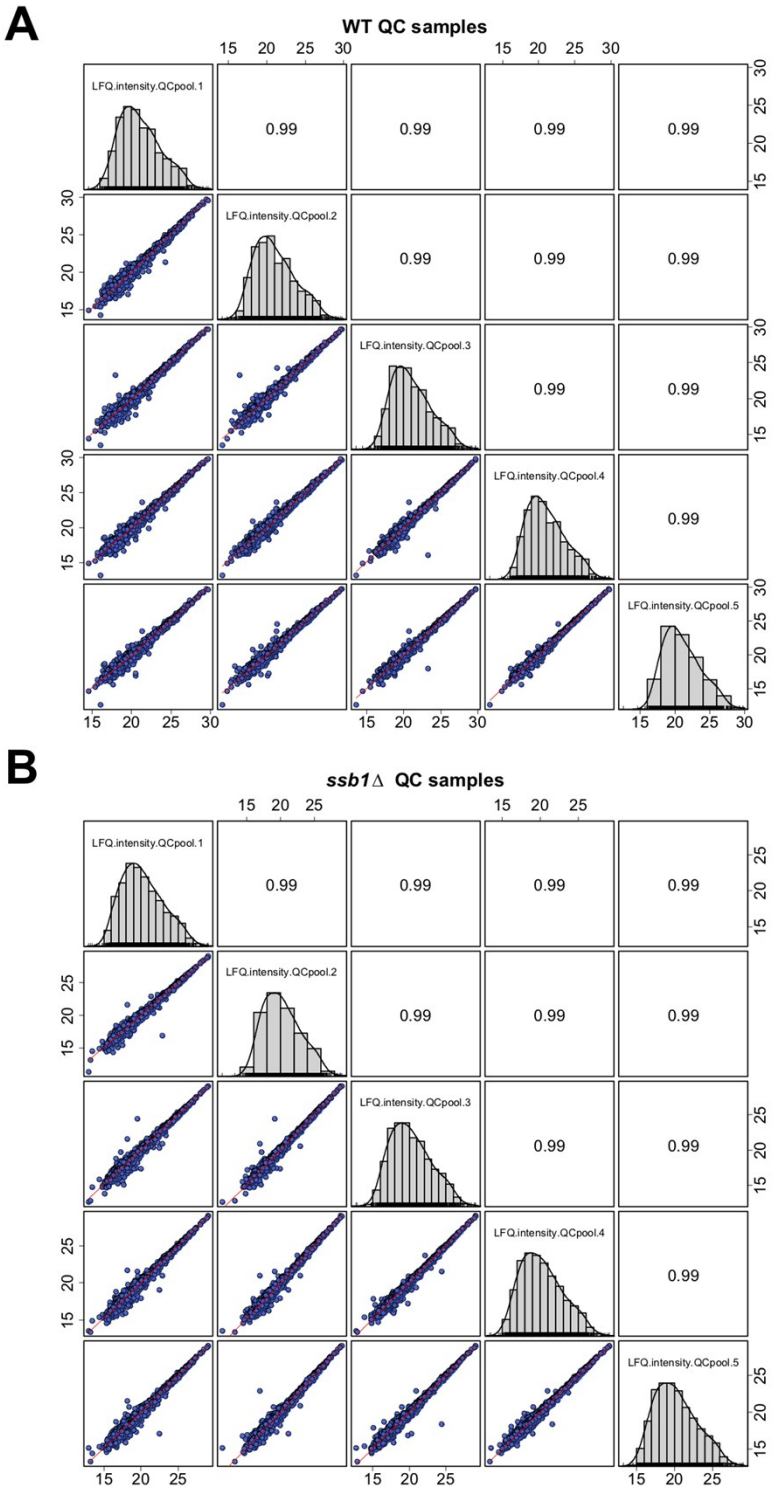
Transcription Factor	Study	Experiment type
Hsf1p	Harbison et al. [2]	ChIP-on-chip
Ino4p	Workman et al. [3]	ChIP-on-chip
Msn2p	Harbison et al. [2]	ChIP-on-chip
Pho2p	Harbison et al. [2]	ChIP-on-chip
Skn7p	Harbison et al. [2] and Ni et al. [4]	ChIP-on-chip
Sok2p	Borneman et al. [5]	ChIP-on-chip
Fhl1p	Kasahara et al. [6]	ChIP-on-chip
Sko1p	Capaldi et al. [7] and Ni et al. [4]	ChIP and ChIP-on-chip
Ste12p	Lefrancois et al. [8]	ChIP-seq
Yap1p	Tan et al. [9]	ChIP-on-chip
Gcn4p	Ernst et al. [10]	ChIP-on-chip
Hmo1p	Kasahara et al. [6]	ChIP-on-chip
Cin5p	Ni et al. [4]	ChIP-on-chip
Yap6p	Ni et al. [4]	ChIP-on-chip
Abf1p	Schlecht et al. [11]	ChIP-on-chip

Figures



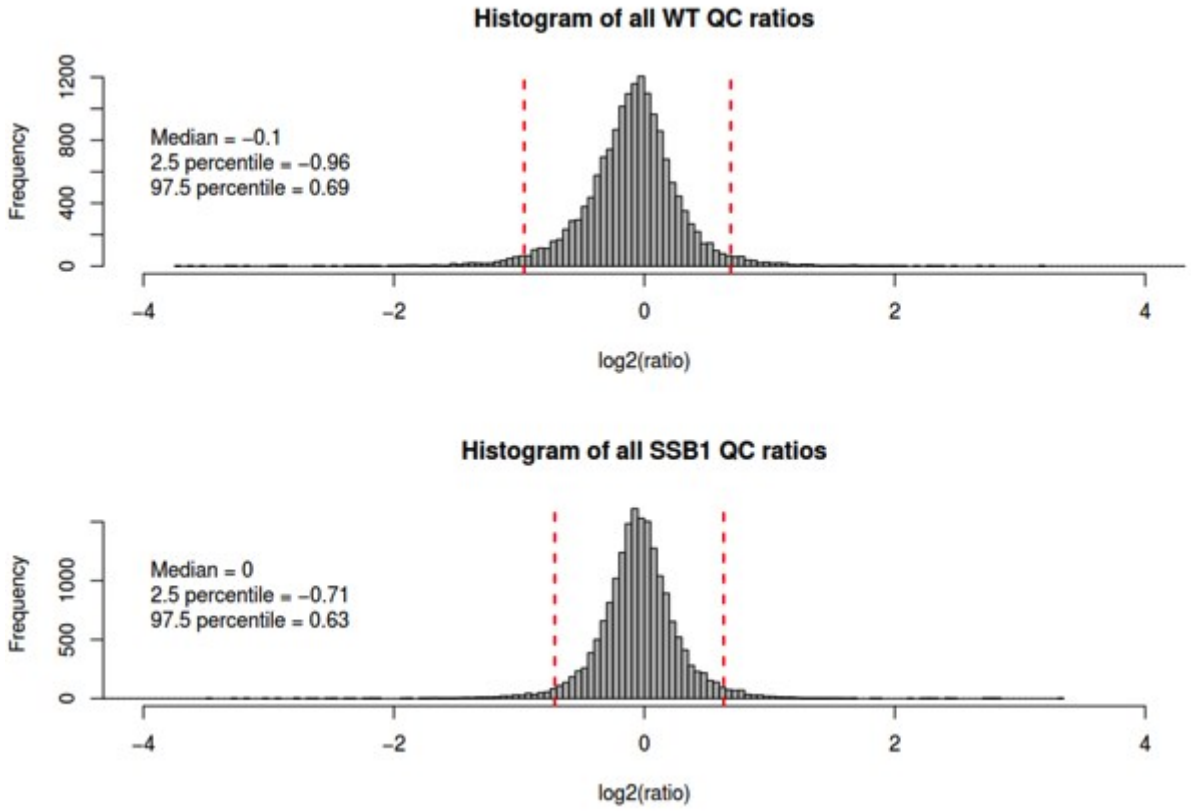
Supplementary Figure 1 Evaluation of WT and ssb1Δ cell growth before and during heat stress.

Optical density (at 600 nm) of the cultures was measured and results are shown as the mean ± standard deviation of the four biological replicates. Dashed line indicates transfer from 30˚C to 37˚C.



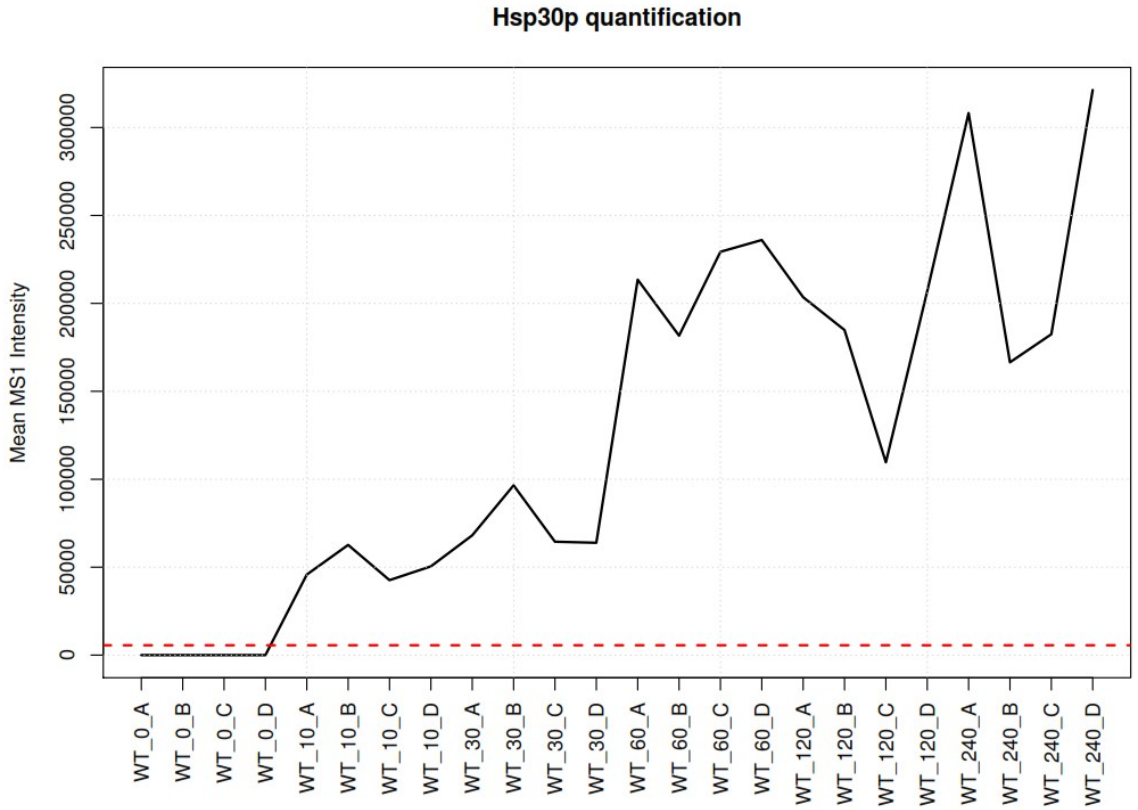
Supplementary Figure 2 Quantification repeatability from mass spectrometry acquisition.

The scatterplot matrix below the diagonal shows a comparison of label free protein quantifications (log2) between the five QC samples acquired through the mass spectrometry run. On the diagonal, histograms of intensity distributions in each QC sample are displayed. The number shown above the diagonal is the Pearson correlation coefficient between the two relevant replicates. B) A corresponding scatterplot for SSB1 data.



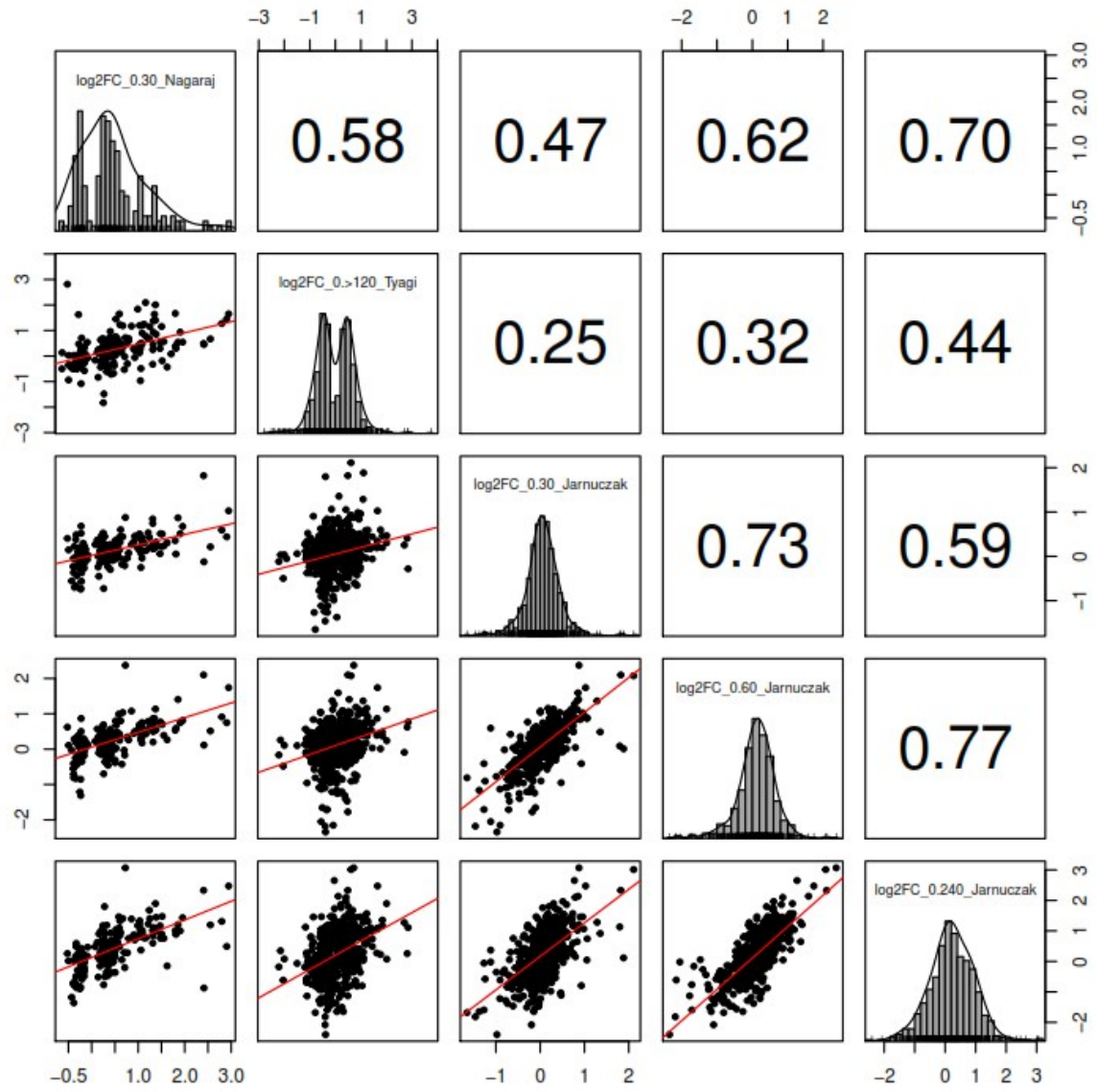
Supplementary Figure 3 Definition of protein fold change thresholds.

The histograms show the distribution of fold changes between all permutations of the QC sample pairs. The dashed lines show the 2.5 and 97.5 percentile cut-offs that were used for determining the magnitude of a fold change at which a protein was considered to be significantly regulated (in addition to passing the FDR-corrected p-value threshold < 0.05). The cut-offs equated to -0.96 (lower bound) and 0.69 (upper bound) for the WT cells, and -0.71 (lower bound) and 0.63 (upper bound) for the mutant cells.

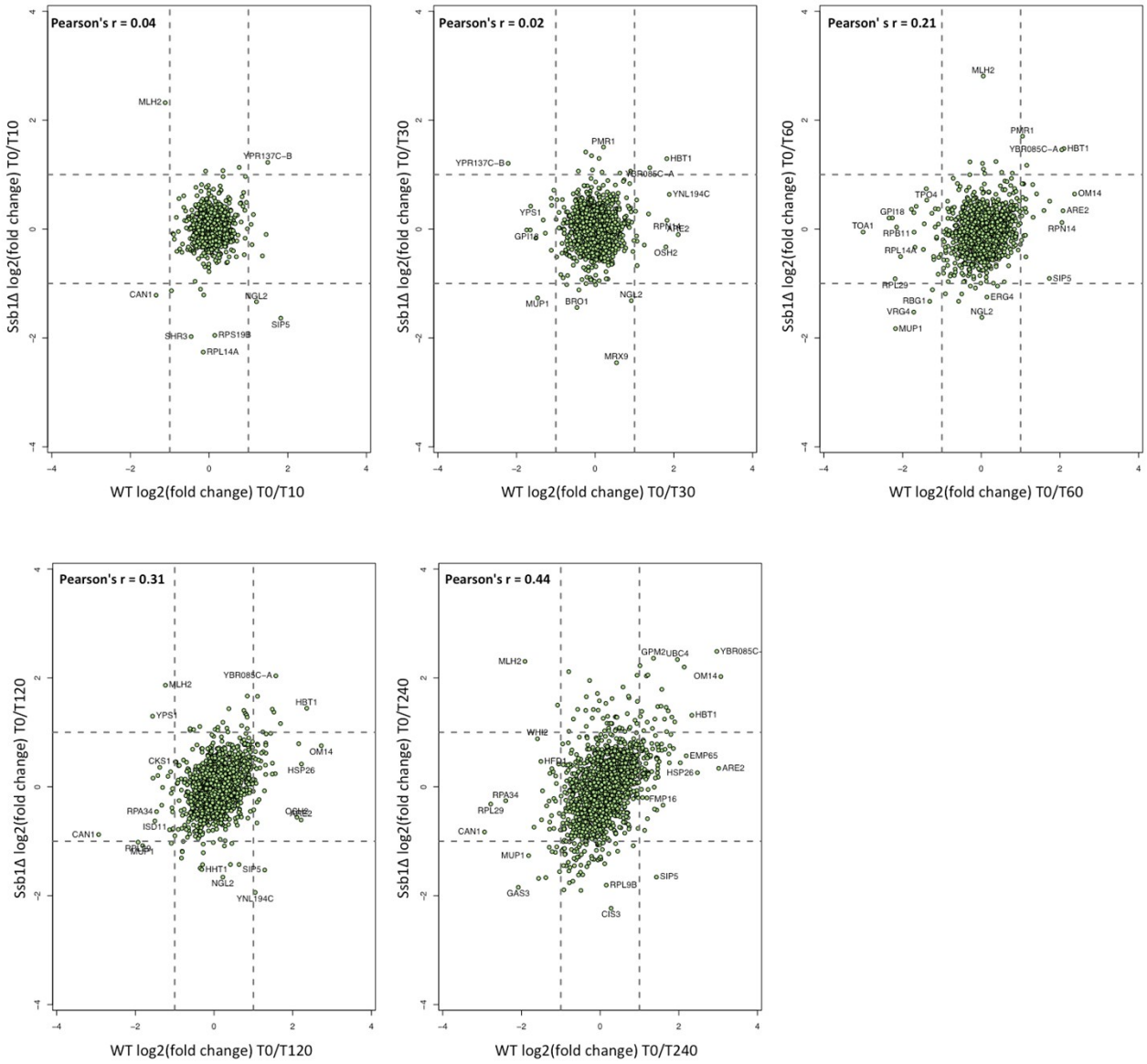


Supplementary Figure 4 Quantification of Hsp30p in the WT samples.

Hsp30p (YCR021C) did not have any detectable peptides in the T0 samples, but was detected and quantified in all later time-points based on three unique peptide sequences: ASGETAIHEPEPEAEQAVEDTA, SITGEVPGIR and LSLTGGFSHHHATDDVEDAAPETK. The solid line represents the average intensity of these three peptides at each time point. The red dashed line represents, for comparison, the intensity value of the lowest quantifiable peptides in this experiment.

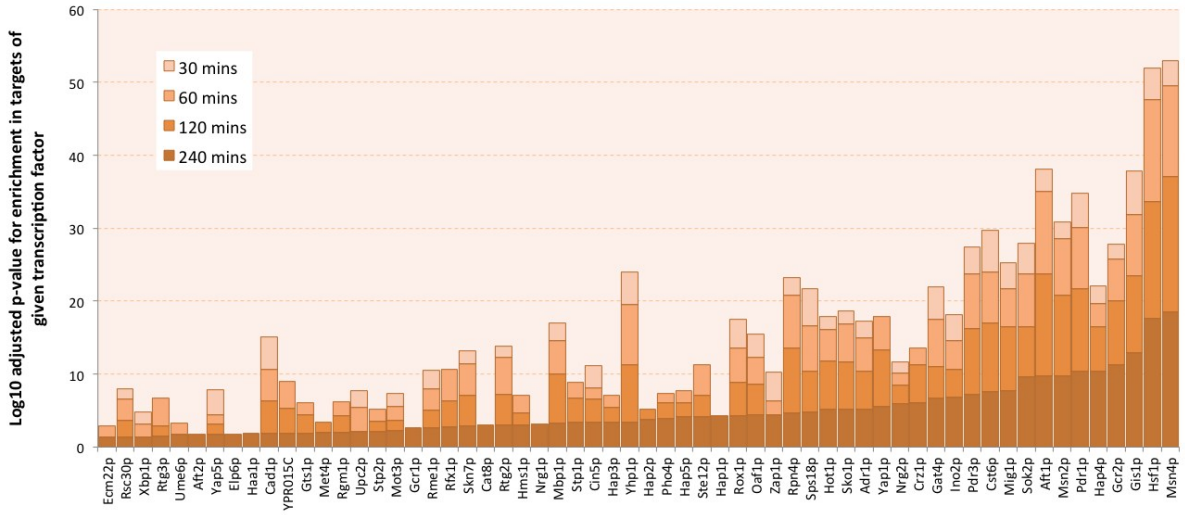


Supplementary Figure 5 Correlation of protein fold changes between this and two other yeast heat shock studies: Nagaraj [12] and Tyagi [13].



Supplementary Figure 6. Comparison of protein abundance fold changes between wold type and mutant yeast strains at matched time points after heat shock.

Pairwise comparisons between protein fold changes in the two yeast strains are shown at matched time points after heat shock. Each plot compares the fold change with respect to T0 for each protein in both conditions. The early time points show relatively little remodelling of the proteome has occurred which increases over time post-stress. We note that the correlations between the two strains are modest and effectively non-existant at early time points but increase with time. The data clearly shows a discordance in response between the two strains, whilst some key heat shock response proteins such as Hsp26p remain relatively unaffected in the mutant strain even after 2 or more hours. Interestingly, the novel heat shock responder YBR085C-A is strongly up-regulated in both strains.



Supplementary Figure 7. Enrichment of transcription factor targets in the upregulated proteome over time.

Enrichment in the up-regulated proteome sets for individual transcription factors was calculated using the GeneCodis [14] website taking the sets of proteins deemded to be up-regulated by MSStats at each time point using an adjusted p-value of < 0.05 cutoff. The enrichment in these datasets was then estimated by GeneCodis, which uses Yeastract [15] data on known transcription factor-target relationships, against a background total of 1740 proteins observed at least once across all our experiments. Too few proteins were detected with significant increased abundance at 10 mins, and enrichment p-values were therefore only estimated for 30, 60, 120 and 240 minutes after the initial stress. They were then converted to positive integers for convenience ($-\log_{10}$ p-value), prior to plotting as a stacked-histogram above, ordered from left to right on increasing significance for the 240 time point. The presence of significant enrichments for targets of many key stress response transcription factors is noted, in particular Hsf1p, Msn2p, Msn4p targets which are well reported heatshock transcription factors.

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