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Supplemental Methods

Mass Spectrometry Analysis. Proteins were digested with recombinant trypsin (Trypzean; Sigma-Aldrich T3568) at a 1:100 enzyme:protein ratio for 18 hours at 37°C. Peptides were extracted twice with acetonitrile, pooled, and desalted with Sep-Pak Vac 3cc (500 mg) C18 columns (Waters, Milford, MA, USA; WAT020805). Dried peptide samples were resuspended in 2% acetonitrile, 0.1% TFA immediately prior to HPLC-ESI-MS/MS analysis using a NanoLC 400 HPLC system with a cHiPLC-nanoflex module (Eksigent, Dublin, CA, USA) connected to a TripleTOF 5600 mass spectrometer (AB Sciex, Framingham, MA, USA). Peptides were resolved using ChromXP C18-CL trapping (200 µm x 0.5 mm) and analytical (75 µm x 15 cm) cartridges (3 µm particle size, 120 Å pore size; Eksigent 5015839 and 804-00001) at a flow rate of 300 nL/min with a 90 min gradient from 5% to 35% acetonitrile in 0.1% TFA (total run time of 120 min). A data-dependent acquisition method was used with cycles of 250 millisec MS survey scans between 400–1600 m/z followed by up to 50 MS/MS scans (50 milliseconds each) of the most abundant ions (limited to +2 to +4 charge, intensity >150, 6 sec dynamic exclusion at 100 ppm width) using a collision energy (CE) of 10 and a collision energy spread (CES) of 5. Proteins in each sample were identified using the Mascot MS/MS ions database search tool (Matrix Science, Inc.). Search parameters included trypsin specificity, 10 ppm peptide and 0.6 Da fragment ion tolerances, up to 2 missed cleavages, and oxidized Met and acetylated N-terminus as variable modifications. Results were filtered to include peptides with a false detection rate of 1%. All hits of interest were additionally confirmed by manual inspection of fragment ion spectra.

RNA-sequencing Analysis. Nine RNA samples were barcoded, pooled and sequenced using the Illumina HiSeq2500 using the rapid run mode. More than 6.7 million 2x100bp reads were sequenced. The reads were quality trimmed using FastX-Toolkit v. 0.0.13.2 (http://hannonlab.cshl.edu/fastx_toolkit/download.html) with maximum length set at 151, a trim length of 50, and a trimscore of 30. Quality trimmed reads were next aligned to the R64-1-1 Ensembl *Saccharomyces cerevisiae* genome release 82 (S288C) using Tophat2 version 2.0.7 (1,2). The number of mismatches allowed was 1, max-intron-length was set to 560, min-intron-length was set to 60, and due to the strand-specificity of the library, the library-type was set to "fr-firststrand". HTSeq v.0.6.1 (3) was run to count the number of reads mapping to each gene, using Biopython v.2.7.3 in the analysis and htseq-count was run in "intersection-nonempty" mode. We used EdgeR software, release-3.1, to identify genes that were differentially expressed (4). Filtered data contained genes that were identified in at least three samples sets with at least one count per million which included 5,900 genes. All statistical analyses were performed using R-3.2.1 by applying the (GLM) function. PCA and volcano scatter plot analysis were generated in R-3.2.1 using the DEGs identified in the EdgeR analysis. The volcano scatter plots were generated by graphing the FDR versus the FC for the comparison between WT aerobic and WT hypoxic datasets or the WT hypoxic and ***set4*****Δ** hypoxic datasets. Genewise dispersion estimates were used in the analysis, which allows each gene to have a different dispersion parameter, providing flexibility and power in the model. A false discovery rate (FDR) of 0.01 was used as a cutoff for differential expression. The GOSLIM mapper by the *Saccharomyces* genome database was used to determine and categorize the biological pathways that correlated with each differentially expressed gene identified.

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References

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