Supporting Information

Supporting Tables

Table S1. Number of genes analyzed and classified as OPN/DPN.

	S. cerevisiae	*S. kudriavzevii*	*S. bayanus*	*C. glabrata*
All analyzed genes	6399	3404	4732	4687
Genes included in DPN/OPN analysis	5652	2886	4173	3947
%classified as DPN	48.2%	53.4%	57.3%	39.8%
%classified as OPN	25.2%	18.7%	14.6%	24.1%
%unresolved classification	26.6%	27.9%	28.1%	36.2%

Gene were excluded from all analysis if they did not have a one-to-one orthology relationship with S. cerevisiae genes, if they belonged to a repeated region, or if no peak was detectable in their nucleosome profiles. Genes were excluded from the DPN/OPN analysis if their Maximal Inter-nucleosomal Distance (MID) was above 350 or below 150 bps, or if the position of the MID was more than 400 bps upsteam of the ATG.

Table S2. Conservation of OPN and DPN classifications.

	S. cerevisiae	*S. kudriavzevii*	*S. bayanus*
C. glabrata	40% (31%)	40% (32%)	40% (32%)
S. bayanus	***70% (38%)***	***68% (40%)***	
S. kudriavzevii	***66% (39%)***		

For each pair of species, the table shows the percentage of ortholog genes with the same classification to OPN or DPN, and in parenthesis the expected percentage (calculated by shuffling the genes in one of the species). Comparisons among sensu-stricto species are highlighted in bold italic.

Table S3. Estimated fragment length.

	S. cerevisiae	*S. kudriavzevii*	*S. bayanus*	*C. glabrata*
1st experiment	Not used	107 bp	124 bp	122 bp
2nd experiment	126 bp (149 bp)	Not used	125 bp (152 bp)	116 bp (148 bp)

The estimated average length of sequenced mono-nucleosome DNA fragments is shown for each sample, as calculated by the distances between consecutive peaks from different strands. Numbers in parenthesis indicate the fragment length estimated by the Agilent Bioanalyzer high sensitivity chip.

Supporting Figure Legends

Figure S1. Shift of TFBS positions.
(a) Distribution of selected transcription factor binding sites (TFBSs) relative to the ATG, for the four species analyzed. TFBS motifs were selected if the peak of their distribution was between -250 to the ATG in *S. cerevisiae* and in *C. glabrata*.
(b) Same as (a) but when positions are calculated relative to the TSS instead of the ATG, demonstrating that the shift in TFBS positions is larger than that of TSS positions. Species color code as in (a) and only *S. cerevisiae* and *C. glabrata* are presented since we have no TSS information for the other species

Figure S2. The maximal distance between promoter nucleosomes is correlated with expression levels for DPN genes (green) but not for OPN genes (red), both in *S. cerevisiae* and in *C. glabrata*.

Figure S3. Percentage of TATA box per promoter class.
TATA-containing genes were defined for the *sensu-stricto* species as in Basehoar et al. which combined conservation among the *sensu-stricto* species with experimental data in *S. cerevisiae*. For *C. glabrata* TATA-containing genes were defined as all promoters containing the motif 'TATAWAWR' between -200 and the start codon. Note that the *C. glabrata* definition does not include conservation among species, nor experimental data and is thus less accurate than the *sensu-stricto* definition, which might account for the lower enrichment seen in *C. glabrata* OPN genes. Indeed, if we use the same approach to define TATA-containing genes in *S. cerevisiae* (without conservation and experimental data) the enrichment in *S. cerevisiae* OPNs drops dramatically and becomes comparable to that observed in *C. glabrata*.

Figure S4. Same as fig. 3a,c but for comparison of *S. cerevisiae* with *S. kudriavzevii* instead of *S.bayanus*.

Figure S5. Controls for local shifts when calculating the correlation between nucleosome patterns of orthologous genes.

(a) Centering nucleosome profiles at the +1 nucleosome and excluding genes with local sequence shifts (total length of alignment gaps within coding regions larger than 50 bp) eliminates the global shift between the *sensu-stricto* species and *C. glabrata*. TSS is the transcription start site of *S. cerevisiae* and serves as basis for the +1 alignment, as explained in the material and methods.
(b) If local shifts are considerably reducing the correlations of nucleosome patterns, then we would expect these correlations to be highly dependent on gaps in sequence alignments. In contrast, similar distributions of nucleosome pattern correlations are obtained when comparing *S. cerevisiae-C. glabrata* orthologs with different total length of gaps in coding region alignements, indicating that low correlations are obtained even for genes with relatively high sequence similarity and fewer local shifts.

Figure S6. Correlation between the expression response to stress (log_2-ratio) of *S. cerevisiae* genes and their *C.glabrata* orthologs, averaged across several comparable stress conditions as defined by Roetzer et al. (including osmotic stress, oxidative stress, glucose starvation and heat shock).

Figure S7. Weak correlations between differences in nucleosome patterns (top - degree of promoter depletion, bottom - one minus the correlation in nucleosome occupancy patterns) and differences in gene expression (left - expression levels, right - expression stress response) between orthologs from *S. cerevisiae* and *C. glabrata*. For all measures (promoter depletion, expression levels and expression response) the difference was quantified as log_2 of the ratio between *S. cerevisiae* and *C. glabrata*, such that positive values reflect higher values (nucleosome-depletion, expression level or expression response) in *S. cerevisiae* and negative values reflect higher values in *C. glabrata*.

Figure S8. Analysis of functional gene-sets reveals higher conservation of promoter nucleosome depletion and higher correlation with expression changes, compared to analysis of individual genes.
(a) Conservation of the promoter depletion score of functional gene-sets. Functional gene-sets were defined as the *S. cerevisiae* GO-SLIM, and promoter depletion scores were averaged over each gene-set. We observed a high correlation between the averaged promoter depletion scores of *S. cerevisiae* and *C. glabrata* (r=0.54), which is much higher than that observed over individual genes (r=0.2, Fig. 3b).
(b) Correlation between nucleosome and expression changes. Differences in promoter depletion scores and differences in expression levels (in both cases, log_2 of *S. cerevisiae* divided by *C. glabrata*) were each averaged over functional gene-sets. We observed a considerable correlation between the averaged differences of promoter depletion and expression levels (r=-0.35), which is much higher than that observed over individual genes (r=-0.096, Fig. S7a).
(c,d) same as (a,b) but for random gene-sets of the same sizes, demonstrating that the correlations in (a,b) are not simply due to the averaging over many genes but rather reflects the coherent evolutionary changes that occurred among functionally related genes.

Figure S9. Weak correlations between overall divergence of promoter nucleosome depletion and divergence of gene expression between S. cerevisiae and C. glabrata (a, our data) or C. albicans (b, Tsankov et al. data), and correlated changes among respiration genes for *C. albicans* but not for *C. glabrata*. Shown are heatmaps of the number of genes with different combinations of the values for differential promoter nucleosome depletion and differential expression level at rich media. White circles represent the region where half of the genes are contained (in the analysis of all genes). The Pearson correlation coefficients over all genes are -0.1 and -0.13 for (a) and (b), respectively.

Supporting Figures

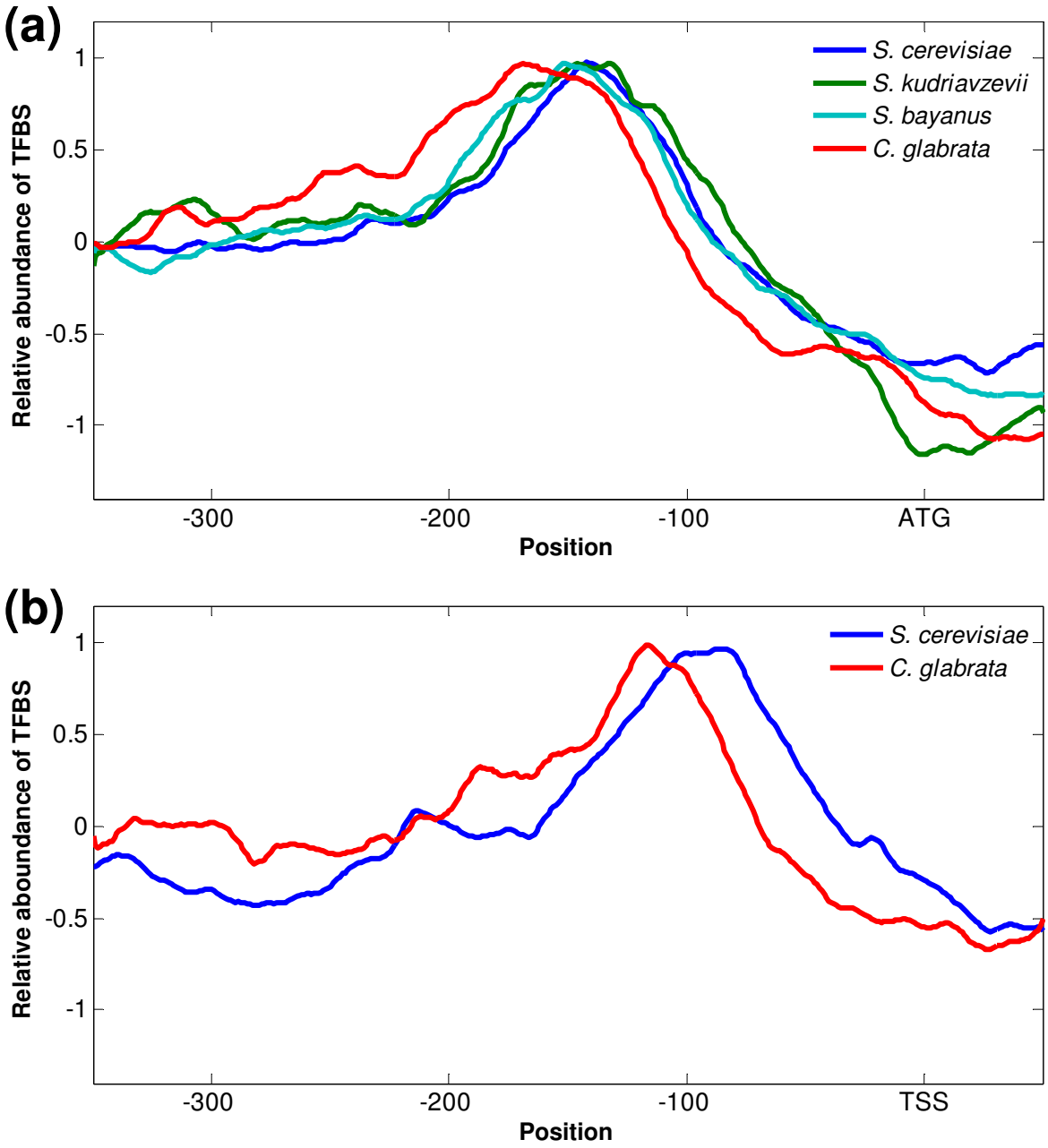

Figure S1

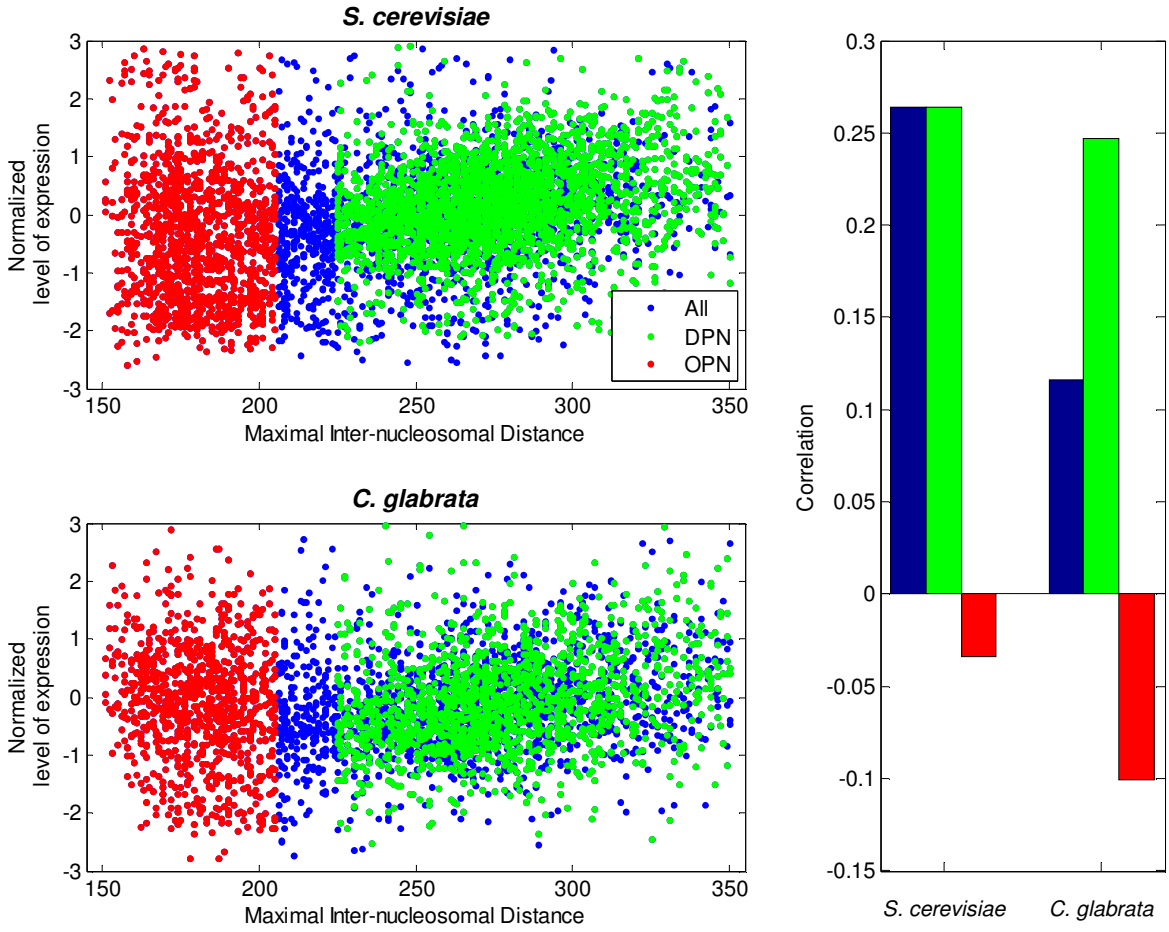
S. cerevisiae
Normalized level of expression
Maximal Inter-nucleosomal Distance
All
DPN
OPN
C. glabrata
Normalized level of expression
Maximal Inter-nucleosomal Distance
Correlation
S. cerevisiae
C. glabrata

Figure S2

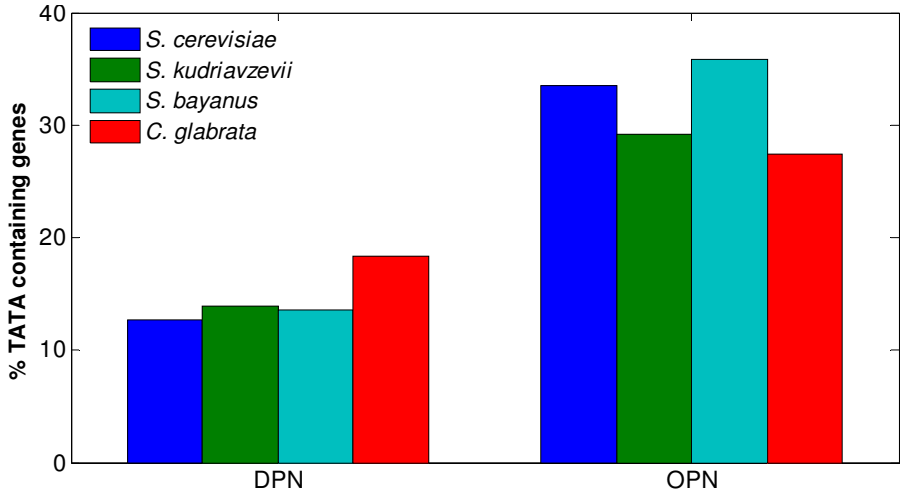
40
30
20
10
0
% TATA containing genes
S. cerevisiae
S. kudriavzevii
S. bayanus
C. glabrata
DPN
OPN

Figure S3

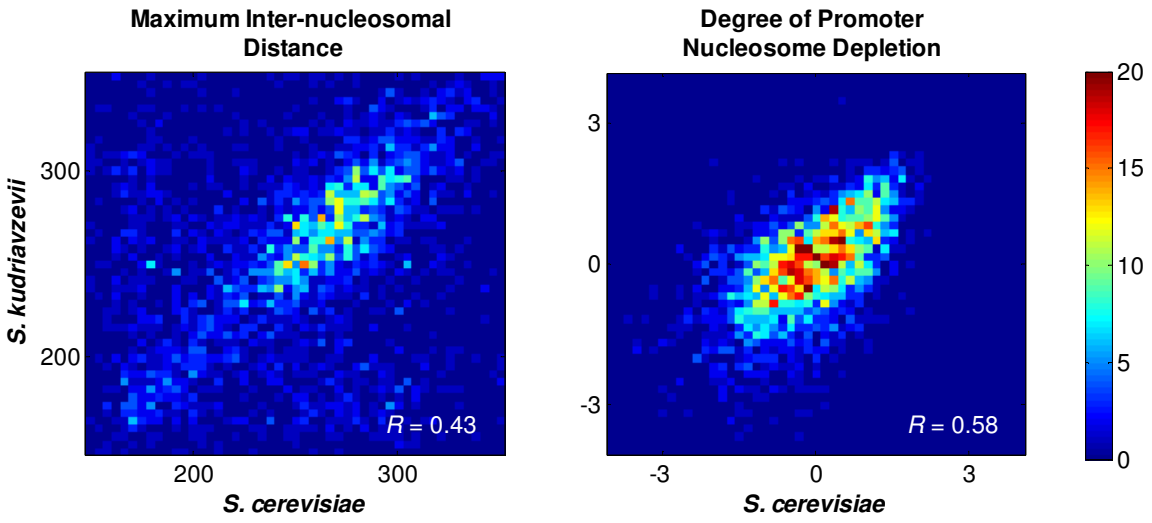
Maximum Inter-nucleosomal Distance
Degree of Promoter Nucleosome Depletion
S. kudriavzevii
300
200
R = 0.43
200
300
S. cerevisiae
3
0
-3
R = 0.58
-3
0
3
S. cerevisiae
20
15
10
5
0

Figure S4

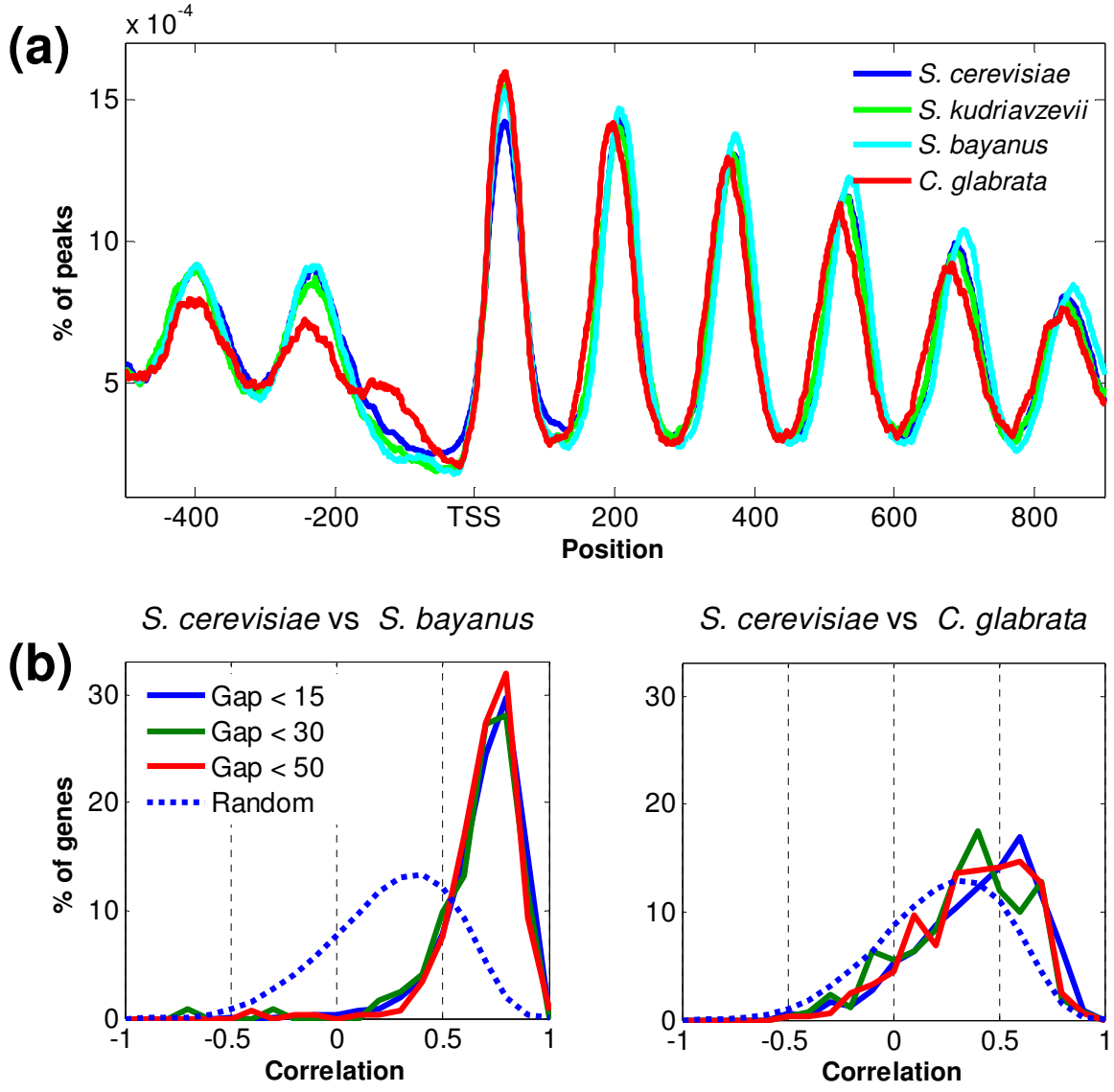
(a)
x 10^-4
15
10
5
% of peaks
S. cerevisiae
S. kudriavzevii
S. bayanus
C. glabrata
-400
-200
TSS
200
400
600
800
Position
(b)
S. cerevisiae vs S. bayanus
S. cerevisiae vs C. glabrata
Gap < 15
Gap < 30
Gap < 50
Random
30
20
10
0
% of genes
-1
-0.5
0
0.5
1
Correlation

Figure S5

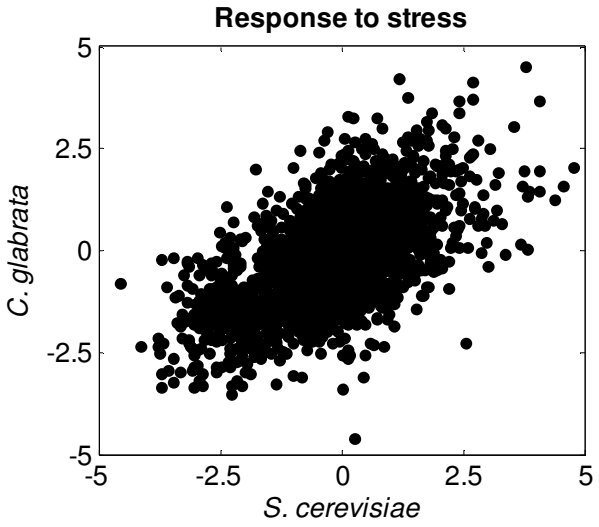
Response to stress
5
2.5
0
-2.5
-5
C. glabrata
-5
-2.5
0
2.5
5
S. cerevisiae

Figure S6

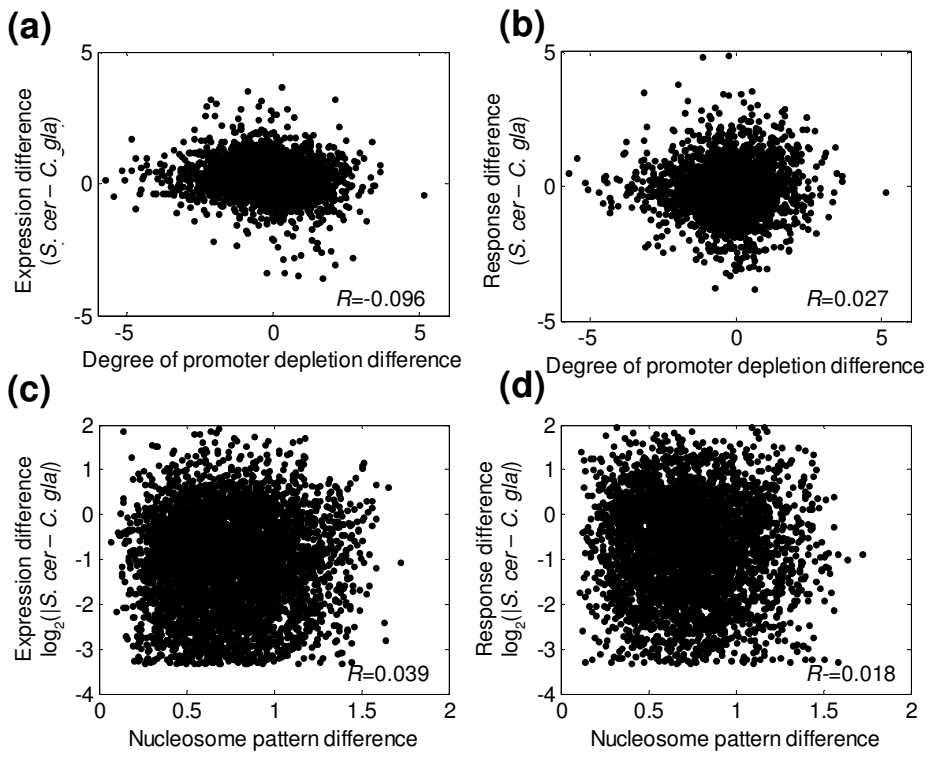
(a)
Expression difference
(S. cer – C. gla)
R=-0.096
Degree of promoter depletion difference
(b)
Response difference
(S. cer – C. gla)
R=0.027
Degree of promoter depletion difference
(c)
Expression difference
log2(|S. cer – C. gla|)
R=0.039
Nucleosome pattern difference
(d)
Response difference
log2(|S. cer – C. gla|)
R=-0.018
Nucleosome pattern difference

Figure S7

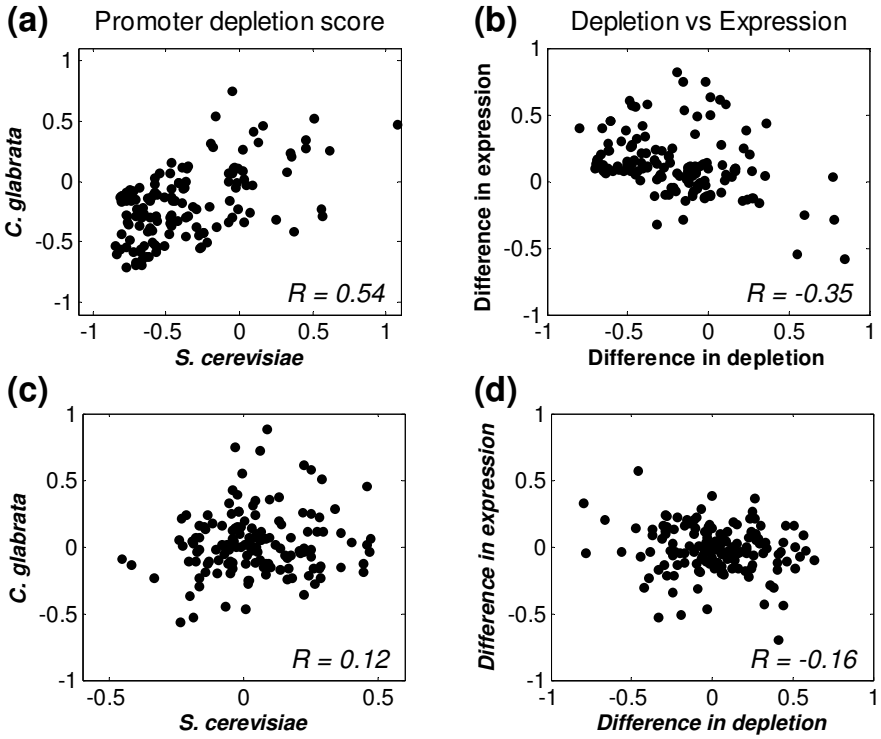
(a)
Promoter depletion score
C. glabrata
S. cerevisiae
R = 0.54
(b)
Depletion vs Expression
Difference in expression
Difference in depletion
R = -0.35
(c)
C. glabrata
S. cerevisiae
R = 0.12
(d)
Difference in expression
Difference in depletion
R = -0.16

Figure S8

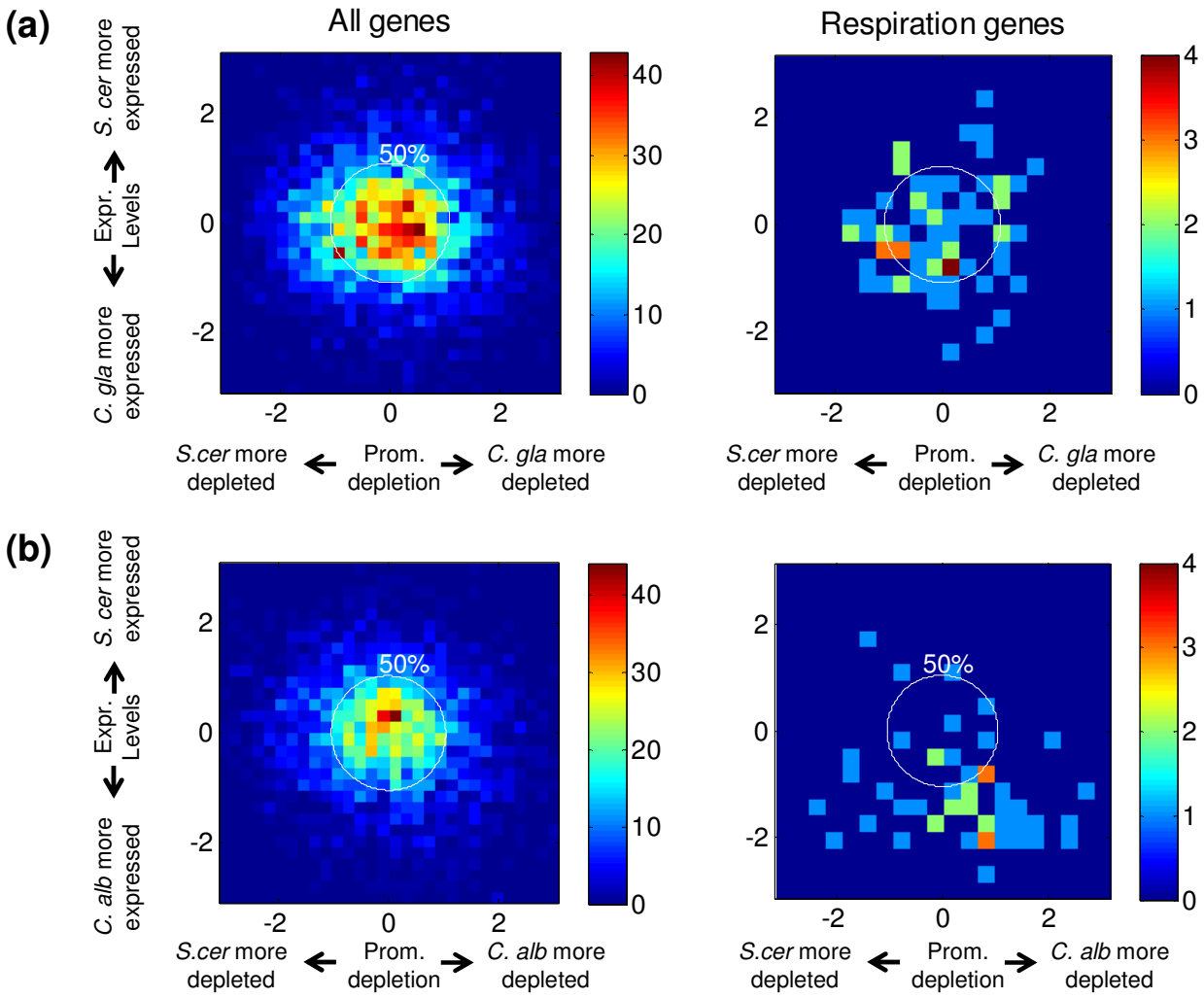
(a)
All genes
Respiration genes
S. cer more expressed
Expr. Levels
C. gla more expressed
50%
S.cer more depleted
Prom. depletion
C. gla more depleted
(b)
C. alb more expressed
C. alb more depleted

Figure S9