Supplementary Figure Legends

Figure S1. Comparison of mutants with the original wild-type data produces similar results to those obtained with the estimated data from the median of the different mutants. The analyses in **Fig. 2c** and **3a** were repeated with the original wild-type data (Tirosh *et al*, 2009) and are shown in **(a)** and **(b)**, respectively.

Figure S2. Enrichment of increased expression differences upon deletion of each of the nine regulators. The analysis in Fig. 2b was repeated for each of the nine regulators.

Figure S3. P-values for the enrichment of increased expression differences at multiple thresholds. For each regulator deletion we calculated the P-value for enrichment of increased expression differences (over decreased expression differences), with different fold-change thresholds for what constitutes a change in differential expression. P-values were calculated with a binomial test, assuming that among the genes that change in differential expression, the probabilities for increased and decreased differential expression are both equal to 0.5.

Figure S4. Significance and reproducibility of changes in differential expression.

(a) Distribution of expression fold-changes between *S. cerevisiae* and *S. paradoxus*.

(b) Reproducibility of increased and decreased differential expression. For each threshold of fold-change (1, 1.1, .. , 2) we identified all genes whose differential expression increases at least by that threshold in the first biological repeat of each mutant strain. To examine the reproducibility of these increased expression differences (buffered variations) we then asked what is the percentage of these genes that also show increased differential expression (fold-change>1) in the second (dye-swapped) biological repeat. Similar analysis was performed for decreased differential expression in the mutant strains.

Figure S5. Fitness and regulatory effects of the deletion mutants are not correlated with the preferential increase of inter-species expression differences.
(a) The growth-rate effect of each of the mutants in *S. cerevisiae* and *S. paradoxus* was estimated by the increase in their doubling time, compared with the wild-type. Briefly, each mutant (and the wild-type) was grown over night in YPD medium and diluted to OD_{600nm} of 0.05-0.1. OD measurements were then taken every hour; the linear slope of the $\log_2$(OD) is an estimate for the growth rate and 1/(growth rate) is the cells doubling time. The doubling times of the mutants are shown for *S.cerevisiae* and *S. paradoxus* on the horizontal and vertical axes, respectively.
(b-e) The enrichment of increased expression differences ($\log_2$-ratio of the number of increased divided by decreased expression differences) is shown for regulators (filled circles) and metabolic enzymes (empty circles, except for in (c)) as a function of: **(b)** growth-rate effects in *S.cerevisiae*; **(c)** differential growth-rate effects among the two species (*S.cerevisiae* minus *S.paradoxus*); **(d)** number of *S.cerevisiae* genes whose expression is affected by each deletion (at least 1.5-fold change); and **(e)** the number of genes whose expression is differentially affected by each deletion (at least 1.5-fold change). Correlations between the two axes are not significant ($P>0.05$) in each of the four analyses, indicating that the preferential increase of inter-species expression differences is independent of the magnitude of the fitness and regulatory effects and of the difference in fitness and regulatory effect among the two species.

The metabolic enzyme deletions were profiled with a new microarray that lacks ~20% of the genes from the microarray used for profiling regulator deletions. To avoid this difference, the number of affected genes in (d,e) include only those that were examined in both analysis.

Figure S6. Increased versus decreased cis-differences between the two species for all deletion strains. The analysis in Fig. 2c was repeated only with the cis component of expression differences.

Figure S7. Buffered trans-differences are more correlated with the response to environmental changes than to the effects of particular transcription regulators (TFs). For each TF, we examined the percentage of correlated buffered trans-differences (both higher in *S. cerevisiae* or both higher in *S. paradoxus*) among pairs of genes which are co-regulated upon deletion of that factor but not among environmental conditions (grey), and the percentage of correlated trans-differences among pairs of genes which are not co-regulated upon deletion of that factor but are co-regulated among environmental conditions (black). Pairs of genes were regarded as co-regulated by a particular factor if they were both up-regulated or both down-regulated by at least 2-fold upon deletion of that factor and were both among the 300 genes whose expression is most affected by that deletion (Hu *et al*, 2007). Pairs of genes were regarded as correlated among environmental perturbations if the correlation between their expression profiles from a large set of environmental conditions (Ihmels *et al*, 2002) was higher than 0.4.

Figure S8. Increased expression variability between two E. coli lines upon deletion of the CRP regulator. Previous work (Cooper *et al*, 2008) examined the effects of deleting CRP (cAMP receptor protein, a key hub in the *E. coli* transcriptional network) on two *E. coli* lines that independently evolved from a common ancestor for 20,000 generations in minimal medium. Shown are the numbers of genes in which the expression difference between the two *E. coli* lines is higher (green) or lower (blue) among the CRP- strains compared with the CRP+ strains. This analysis shows that CRP buffers expression variations in these evolved strains. Cooper et al. also measured the expression of a CRP- ancestral *E. coli* line. However, we did not include this line in the analysis because in contrast to previous studies (Zheng *et al*, 2004) and to the evolved lines which show widespread effects of CRP, the ancestral CRP- line was highly similar to the ancestral CRP+ line (Pearson correlation of 0.98 between the genome-wide expression of CRP- and CRP+ lines, with only 20 genes having significant expression differences of at least 2-fold).

Cooper TF, Remold SK, Lenski RE, Schneider D (2008) Expression profiles reveal parallel evolution of epistatic interactions involving the CRP regulon in Escherichia coli. *PLoS Genet* **4:** e35.

Hu Z, Killion PJ, Iyer VR (2007) Genetic reconstruction of a functional transcriptional regulatory network. *Nat Genet* **39:** 683-687.

Ihmels J, Friedlander G, Bergmann S, Sarig O, Ziv Y, Barkai N (2002) Revealing modular organization in the yeast transcriptional network. *Nat Genet* **31:** 370-377.

Tirosh I, Reikhav S, Levy AA, Barkai N (2009) A yeast hybrid provides insight into the evolution of gene expression regulation. *Science* **324:** 659-662.

Zheng D, Constantinidou C, Hobman JL, Minchin SD (2004) Identification of the CRP regulon using in vitro and in vivo transcriptional profiling. *Nucleic Acids Res* **32:** 5874-5893.

Supplementary Figures

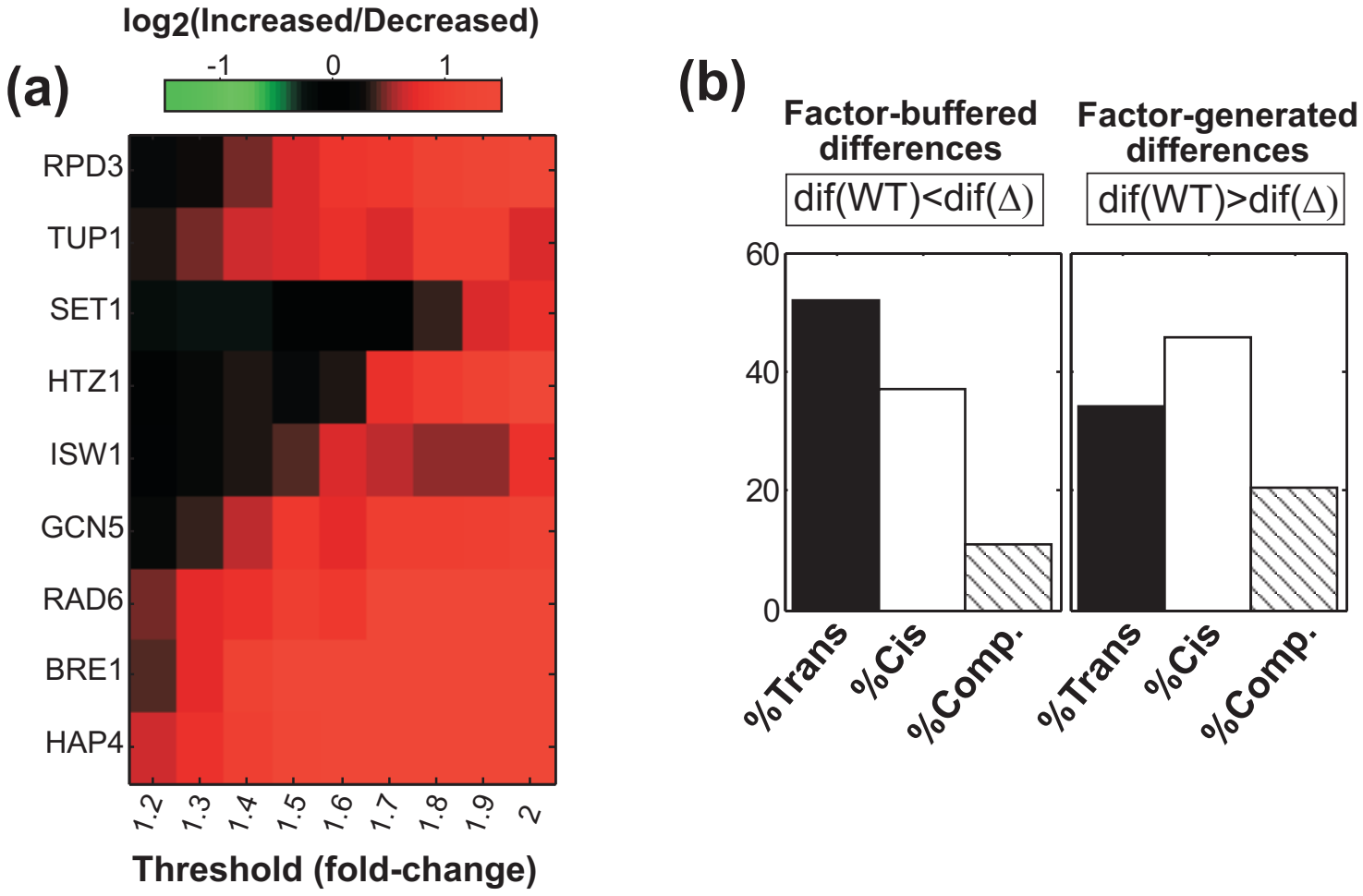

Figure S1

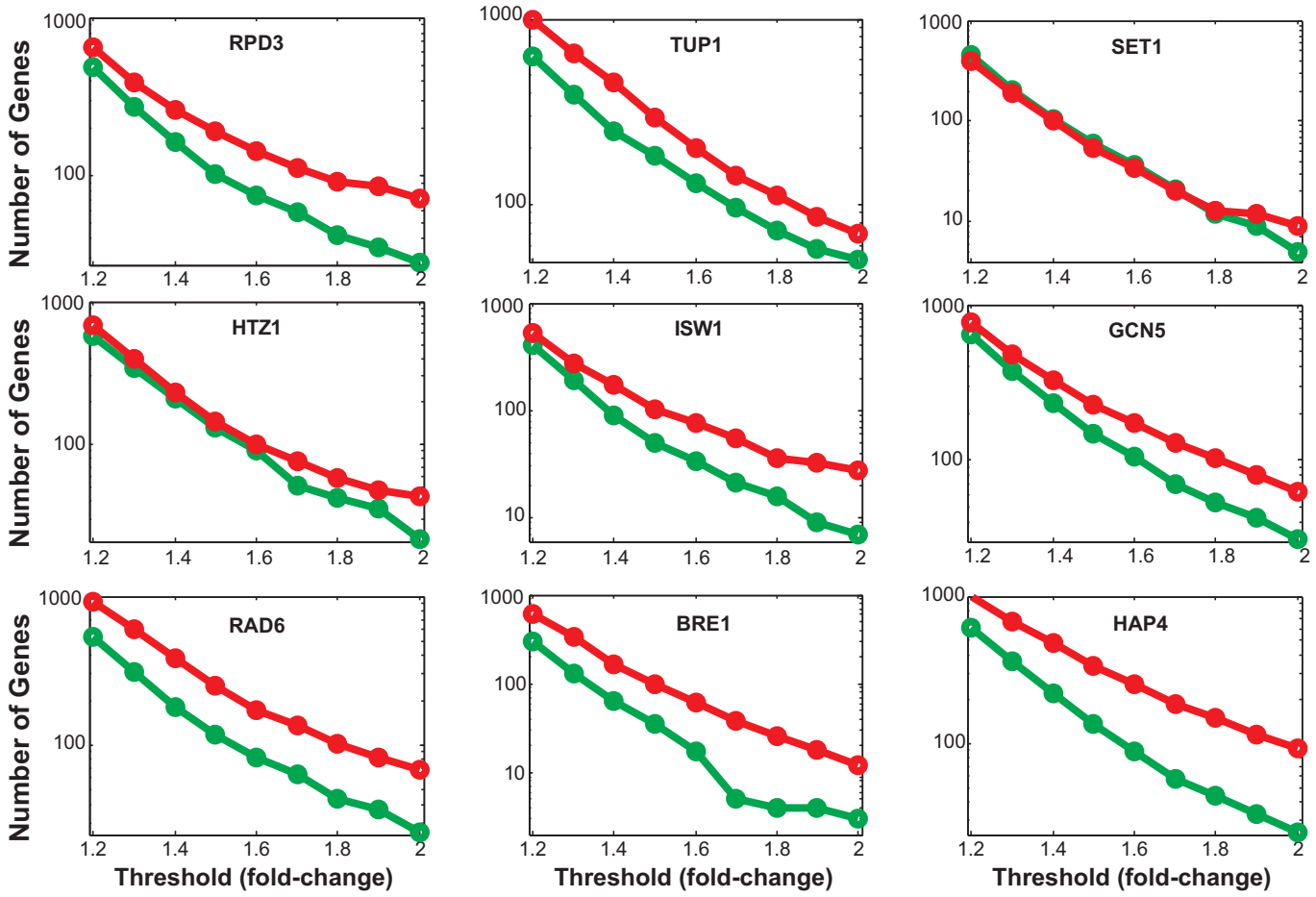

Figure S2

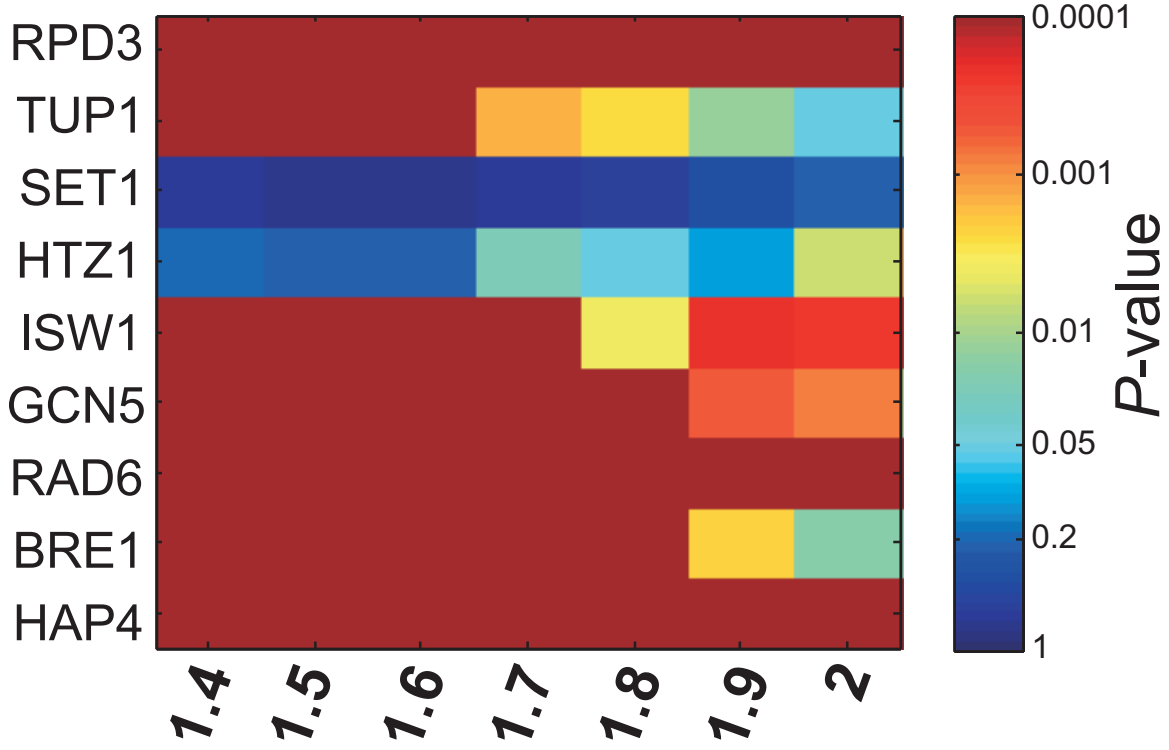

Figure S3

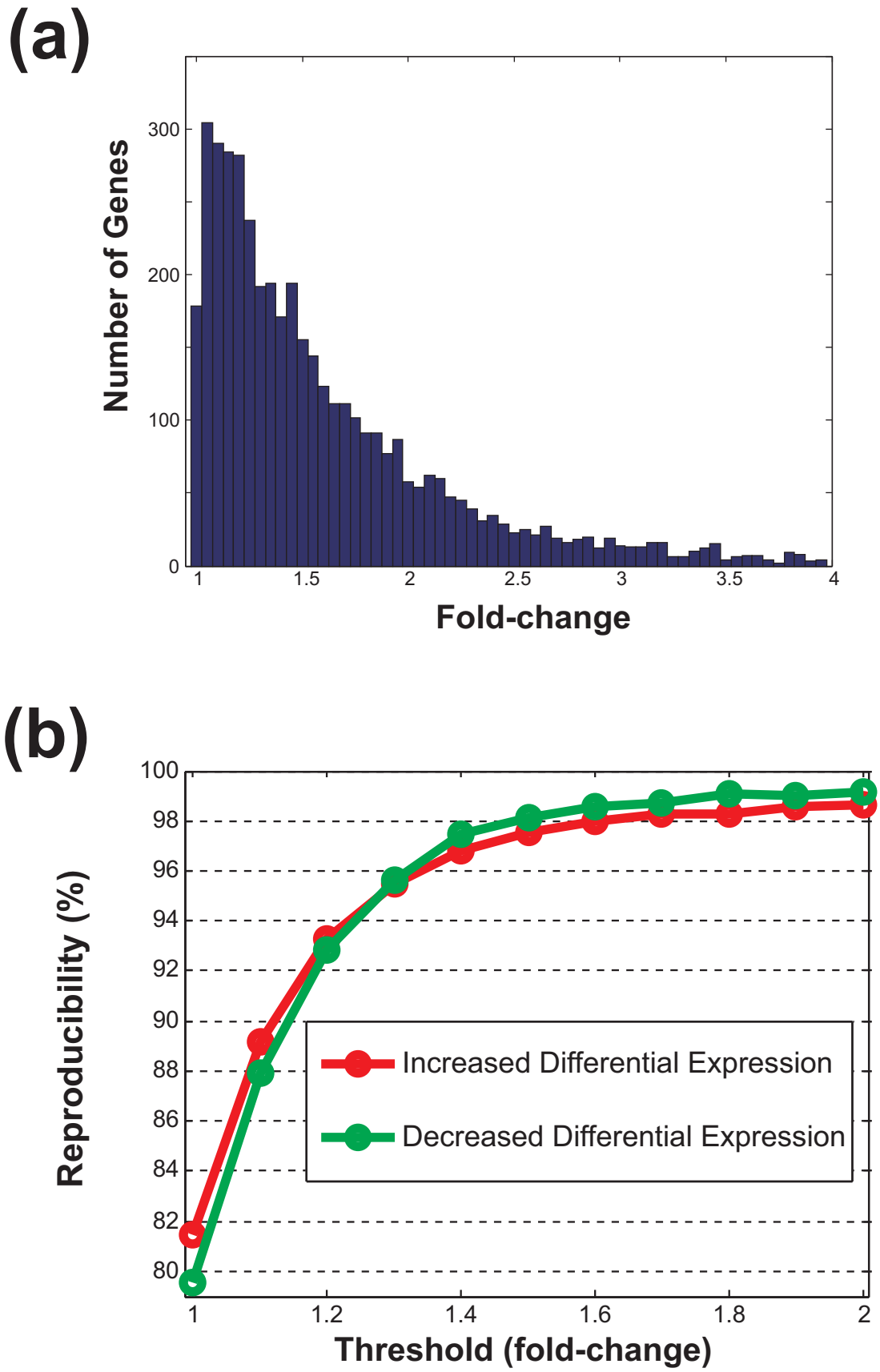
(a)
Number of Genes
300
200
100
0
1
1.5
2
2.5
3
3.5
4
Fold-change
(b)
Reproducibility (%)
100
98
96
94
92
90
88
86
84
82
80
1
1.2
1.4
1.6
1.8
2
Threshold (fold-change)
Increased Differential Expression
Decreased Differential Expression

Figure S4

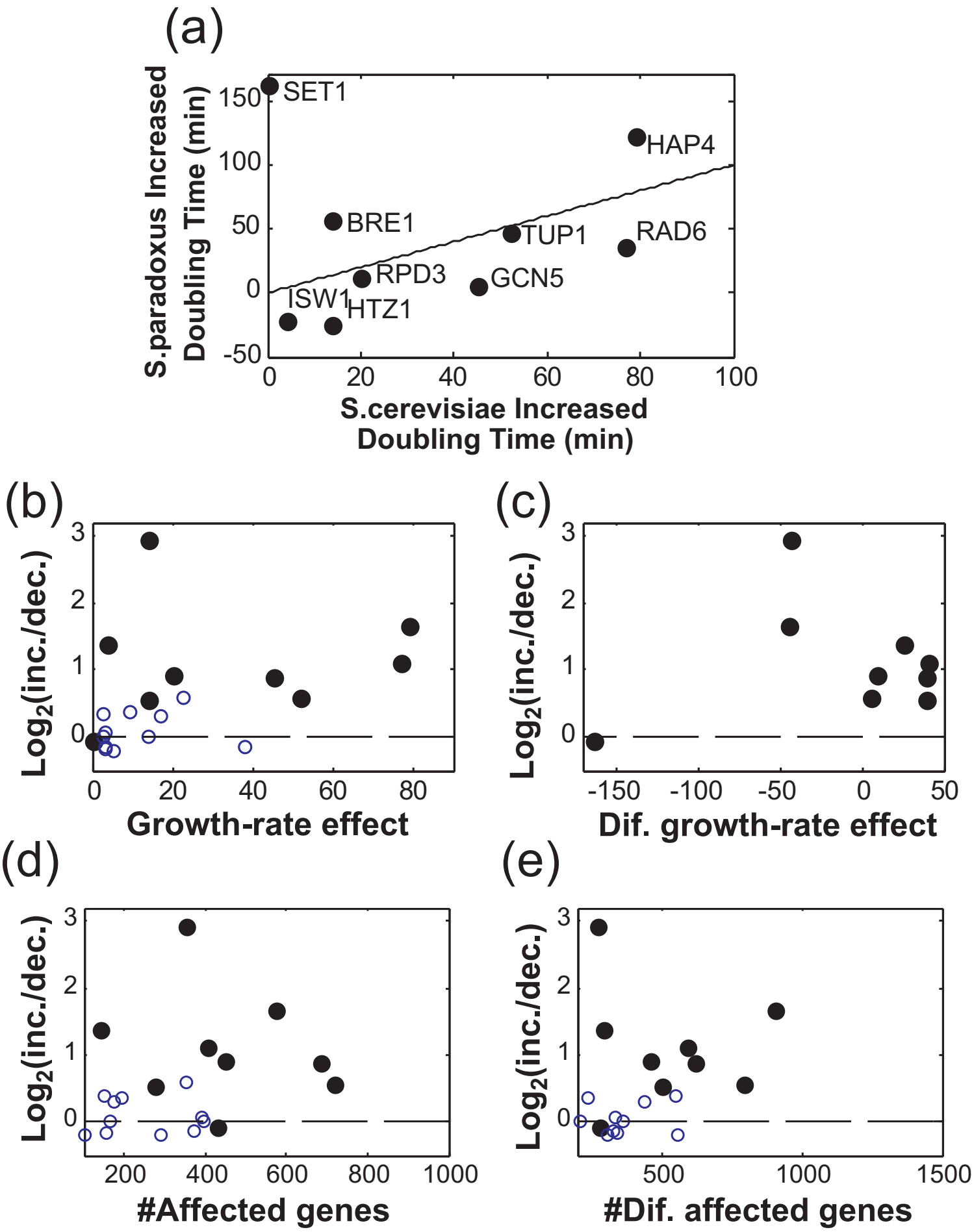
(a)
S.paradoxus Increased Doubling Time (min)
S.cerevisiae Increased Doubling Time (min)
SET1
HAP4
BRE1
TUP1
RAD6
RPD3
GCN5
ISW1
HTZ1
150
100
50
0
-50
0
20
40
60
80
100
(b)
Log2(inc./dec.)
Growth-rate effect
(c)
Log2(inc./dec.)
Dif. growth-rate effect
(d)
Log2(inc./dec.)
#Affected genes
(e)
Log2(inc./dec.)
#Dif. affected genes

Figure S5

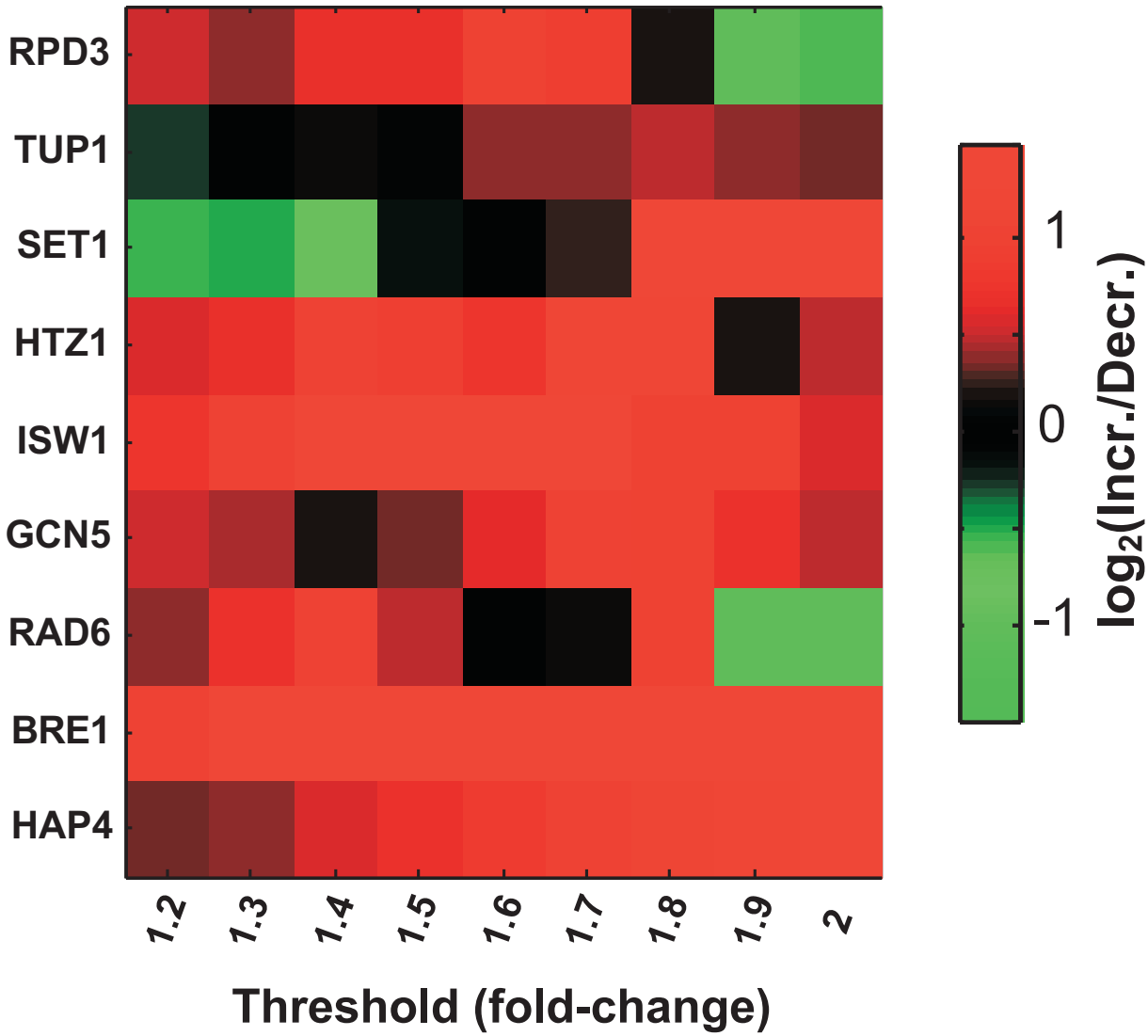
RPD3
TUP1
SET1
HTZ1
ISW1
GCN5
RAD6
BRE1
HAP4
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2
Threshold (fold-change)
1
0
-1
log2(Incr./Decr.)

Figure S6

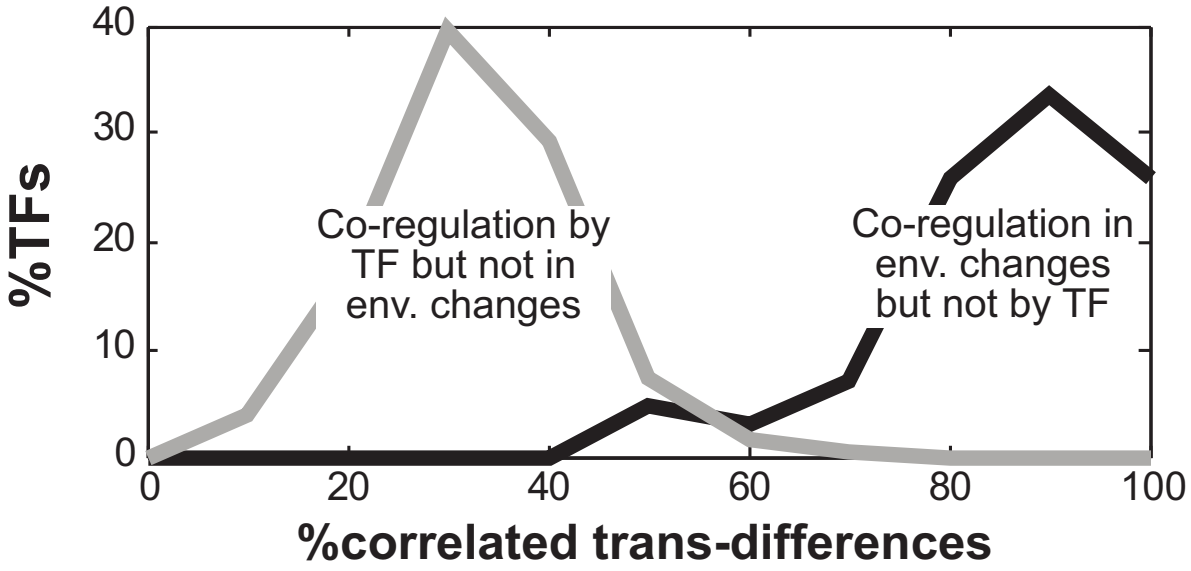

Figure S7

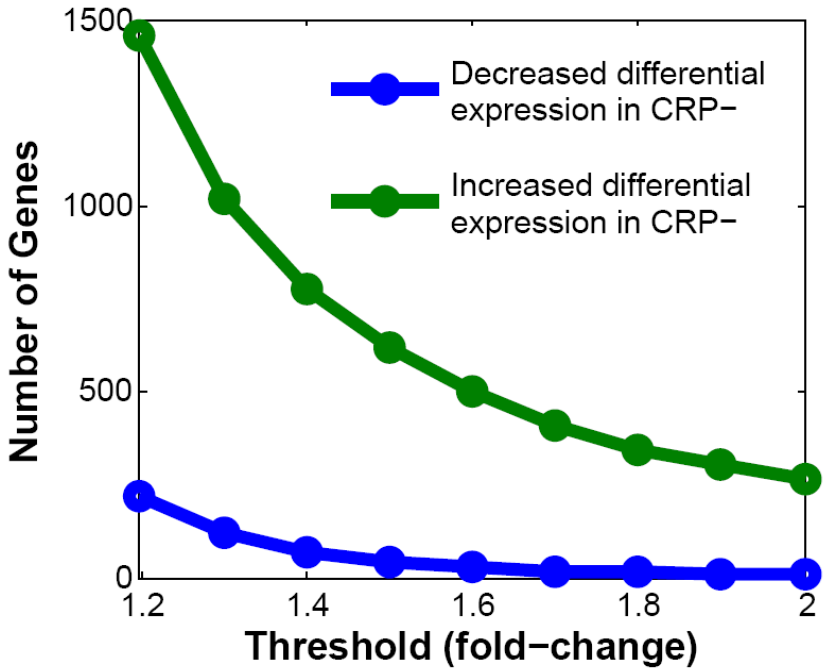

Figure S8