

## Supplementary Materials for

### Rewiring of Transcriptional Regulatory Networks: Hierarchy, Rather Than Connectivity, Better Reflects the Importance of Regulators

Nitin Bhardwaj, Philip M. Kim,\* Mark B. Gerstein\*

\*To whom correspondence should be addressed. E-mail: pi@kimlab.org (P.M.K.); mark.gerstein@yale.edu (M.B.G.)

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List S1. *E. coli* transcriptional regulatory network hierarchy (PDF format).

List S2. Yeast transcriptional regulatory network hierarchy (PDF format).

List S3. *E. coli* transcriptional regulatory network (PDF format).

List S4. *S. cerevisiae* transcriptional regulatory network (PDF format).

List S5. Regulatory network based on grouping a subset of the genes in *E. coli* by operon (PDF format).

List S6. Regulatory network based on specific conditions in *S. cerevisiae* (PDF format).

Data S1 to S4. The rewiring events and effects of gene deletion or edge addition to *E. coli* and *S. cerevisiae* transcriptional networks (Microsoft Excel format).

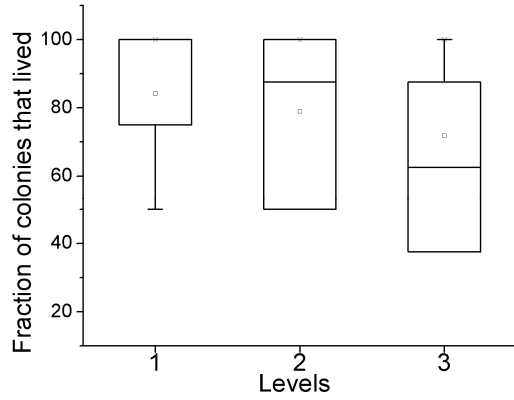
## **Description of the regulatory networks and data sets used to construct the transcriptional regulatory networks for *S. cerevisiae* and *E. coli***

The regulatory trees (List S1 and S2) were constructed from the *E. coli* transcriptional regulatory network (List S3) from RegulonDB (as of July 2009) (1), and from data from various biochemical and genetic experiments in *S. cerevisiae* (List S4) (2-5). Table S1 shows the properties of the regulatory networks.

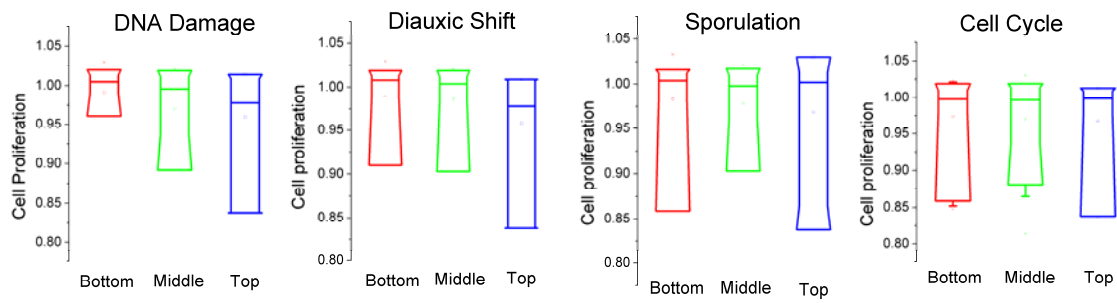
The rewiring events and effects of gene deletion or edge addition to the *E. coli* regulatory network are shown in Data S1 and Data S2, with Tables S2 and S3 explaining the data sets. The rewiring events and effects of edge addition or deletion to the *S. cerevisiae* regulatory network are shown in Data S3 and Data S4, with Tables S4 and S5 explaining the data sets.

## **Description of the data sets used to construct hierarchies from data grouped into specific subsets**

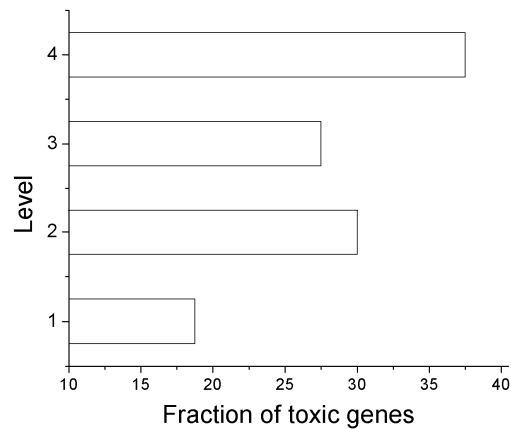
Regulatory trees were constructed from regulators involved in sensing different kinds of signals. The data was obtained from RegulonDB [<http://regulondb.ccg.unam.mx/>, (1)] (List S5). We grouped genes according to their operon organization in *E. coli* [data from RegulonDB, (1)] and rebuilt the hierarchy using the same algorithm as that described above. Because the dataset was smaller (one operon represented all the component genes), there were only 3 levels in this hierarchy (fig. S1 and List S5). Similarly, for *S. cerevisiae*, we used subnetworks that were responsive only under certain conditions to build smaller hierarchies with only 3 levels using the same method (6). We used the subset of the transcriptional networks that is active under four conditions: cell cycle, sporulation, diauxic shift, and DNA damage (List S6).



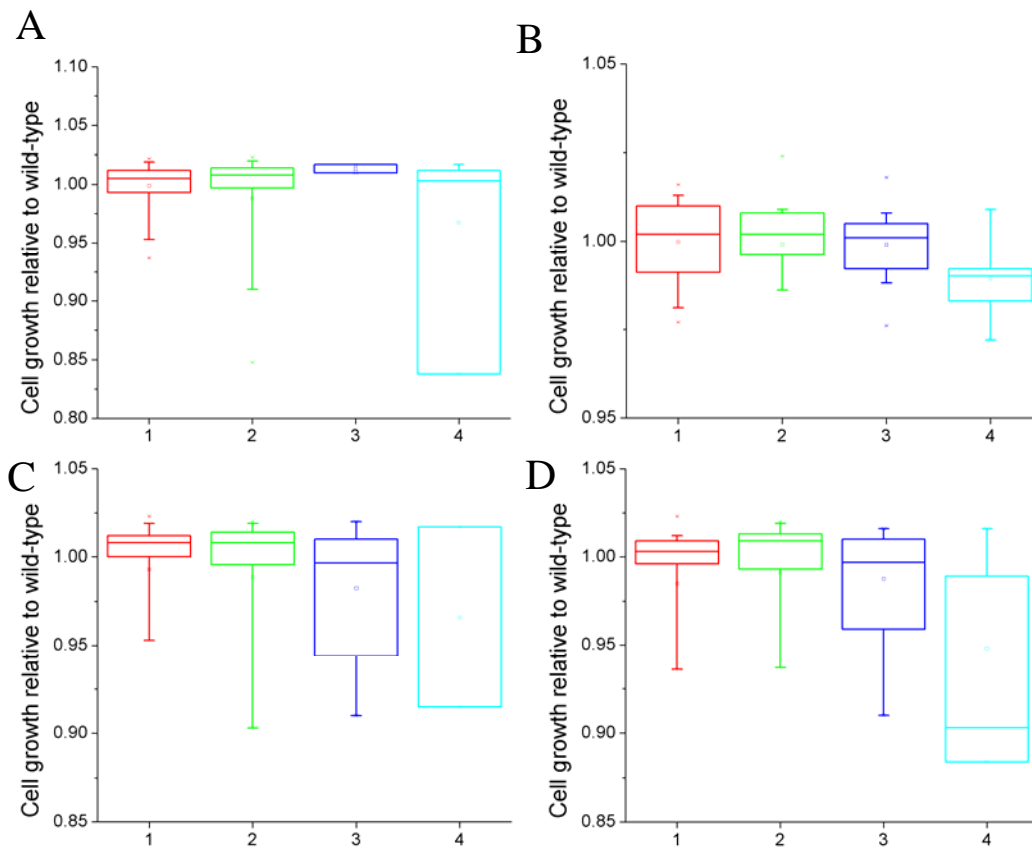
**Figure S1.** Operon-based hierarchy in *E. coli*. The data were organized into operons from the using RegulonDB (1). The operon-based networks and the hierarchies are listed in List S5.



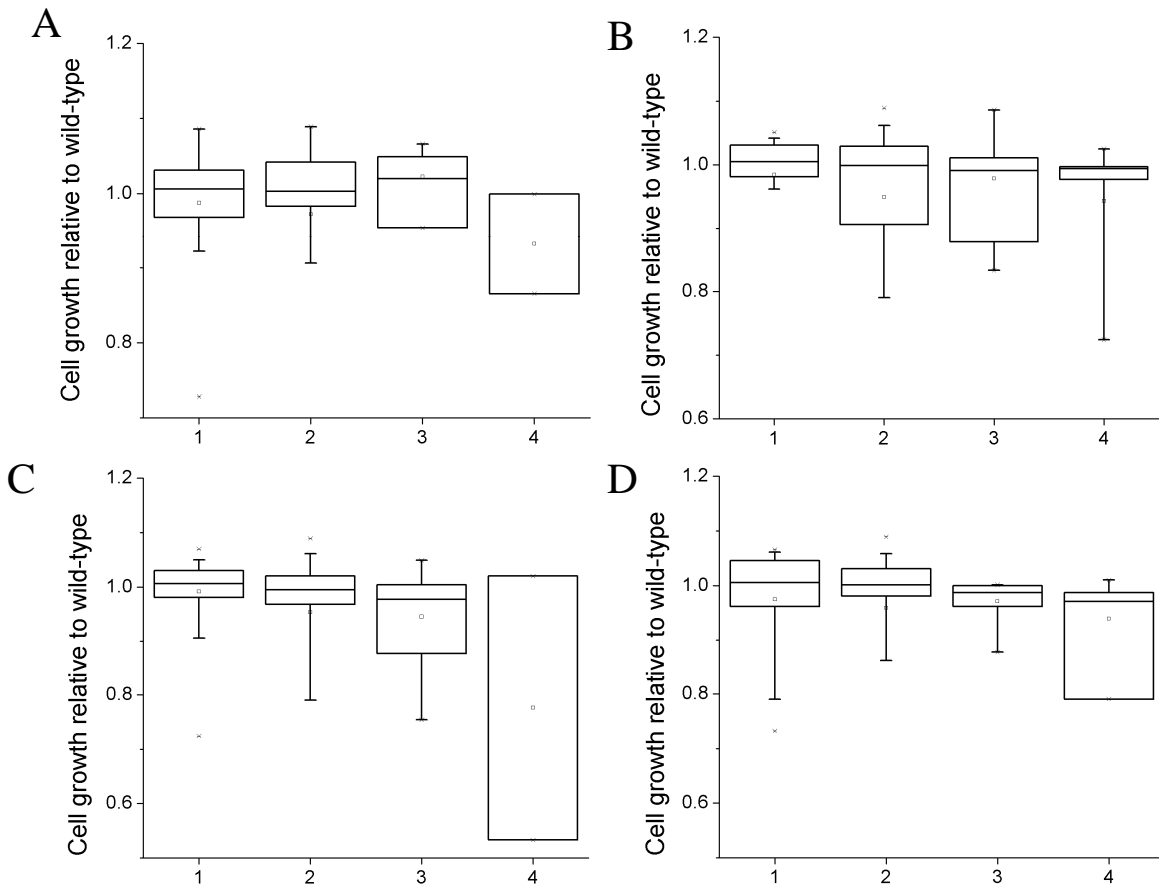
**Figure S2.** Condition-specific hierarchies in yeast. Subnetworks were obtained from (6). The networks and the hierarchies are listed in List S6.



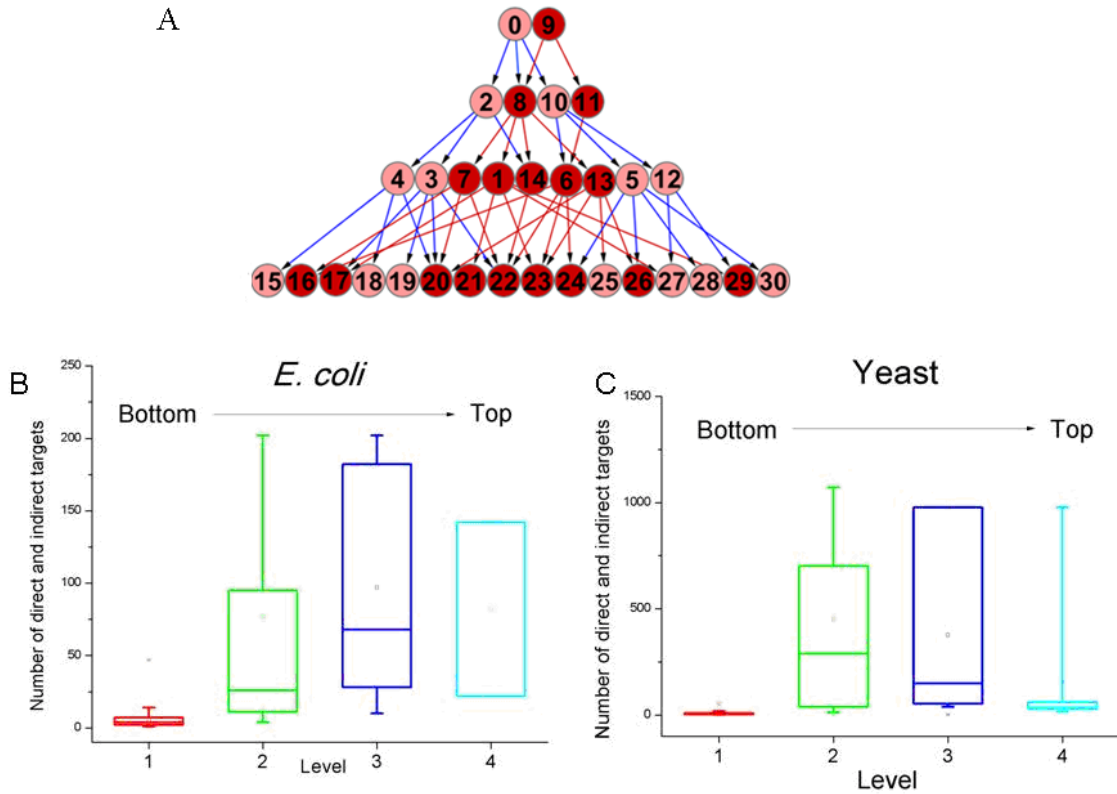
**Figure S3.** Effects of overexpression, a means of increasing the strength of regulatory interactions. Toxic genes (7) were mapped onto the yeast hierarchy (List S2).



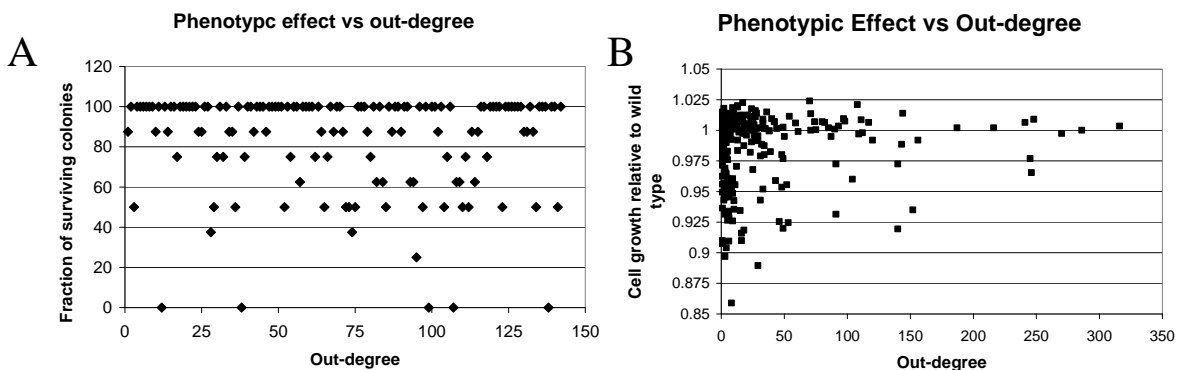
**Figure S4.** Phenotypic effect of deletion of nodes from various levels in the yeast regulatory subnetworks coming from individual studies: **(A)** Lee *et al.* (2), **(B)** Harbison *et al.* (3), **(C)** Horak *et al.*(4), **(D)** Svetlov *et al.* (5). The phenotypic data is from Deutschbauer *et al.* (8). The x-axis is levels in the hierarchy.



**Figure S5.** Phenotypic effect of deletion of nodes from various levels in yeast regulatory subnetworks coming from individual studies: **(A)** Lee *et al.* (2), **(B)** Harbison *et al.* (3), **(C)** Horak *et al.* (4), **(D)** Svetlov *et al.* (5). The phenotypic data is from Costanzo *et al.* (9). The x-axis is levels in the hierarchy.



**Figure S6.** Indirect targets and number of targets versus position in the hierarchy for *E. coli* and *S. cerevisiae*. **(A)** An example of 'indirect targets' of node 9. All the downstream nodes in the hierarchy are highlighted in red. **(B)** Number of direct and indirect targets versus position in the hierarchy (in one of the four levels) for *E. coli*. **(C)** Number of direct and indirect targets versus position in the hierarchy (in one of the four levels) for *S. cerevisiae*.



**Figure S7.** Phenotypic effect versus out-degree. Out-degree is the number of targets in the regulatory network. **(A)** Fraction of surviving colonies in *E. coli*. **(B)** Cell proliferation rate relative to wild type for yeast.

**Table S1.** Properties of the regulatory networks used. These are the characteristics of the networks shown in List S3 and List S4.

Properties	<i>S. cerevisiae</i>	<i>E. coli</i>
Number of regulatory interactions	12873	3123
Number of regulators	288	143
Number of targets	4410	1420
Number of inter-regulator interactions	632	220

**Table S2.** Description of Data S1. These data explain how *E. coli* gene deletion affected network rewiring and colony survival and is based on data from Baba *et al.* (10). The filename is "Data\_S1\_Ecoli\_changes\_hier\_baba\_et\_al.xls". Positions in the hierarchy are indicated as: Bottom=Level 1; First = Level 2; Two = Level 3; Three = Level 4. WT, wild type.

Column	Column Title	Description
A	Delete	The gene deleted
B	Current_level	The position in the wild-type hierarchy
C	Out_TF	The number of TFs this gene regulates; bottom level regulators equals 0.
D	Out_overall	The total number of targets of this gene
E	In	In-degree, the number of regulators of this gene
F	Colonies	The fraction of colonies that survived
G	Changes	The number of changes in the hierarchy upon deletion of the gene
H, K, N, Q	Gene_moved	A gene that moved its position in the hierarchy
I, L, O, R	Initial_level	The initial level of the gene in the WT hierarchy
J, M, P, S	Final_level	The final position of the gene in the rearranged hierarchy



**Table S3.** Description of Data S2. These data explain how *E. coli* edge addition affected network rewiring and growth and is based on data from Isalan *et al.* (11). The filename is "Data\_S2\_Ecoli\_changes\_hier\_Isalan\_et\_al.xls". The rewiring events are noted one row above the row with the new edge. For example, the row with the data "rpoE first two" comes above the row with "appY\_rpoE 1 7.23E-09" and means that appY\_rpoE fusion leads to 1 change: rpoE changes from the second level to the first level. Note that the first level is one above the bottom: Bottom=Level 1; First = Level 2; Second = Level 3; Third = Level 4. ORF, open reading frame; l.s.d, least squares difference.

Column	Column Title	Description
A	promoter_ORF_pair	the promoter and ORF fused together. This creates a new edge between the regulators of promoter and the ORF.
B	Number of changes	the number of changes in the hierarchy upon addition of that new edge.
C	l.s.d.	It measures the 'least square difference' between the mutant growth and the wild-type growth so a higher value indicates a slower cell growth.

**Table S4.** Description of Data S3. These data explain how deletion of genes affected the yeast network rewiring and cell fitness and is based on data from Deutschbauer *et al.* (8). The filename is "Data\_S3\_Yeast\_Changes\_hier\_Deutschbauer.xls". Positions in the hierarchy are indicated as: Bottom=Level 1; First = Level 2; Two = Level 3; Three = Level 4. Tag fitness is the cell growth rate of the deletion strain. #DIV/0! indicates that the Het and Hom tag fitness could not be calculated.

Column	Column title	Description
A	Delete	The gene deleted
B	Current_level	The position in the wild-type hierarchy.
C	Out_TF	The number of TFs this gene regulates; bottom level regulators equals 0.
D	Out_Overall	The total number of targets of this gene
E	In	In-degree, the number of regulators of this gene
F	Het	Tag fitness of the heterozygous deletion grown in YPD medium
G	Hom	Tag fitness of the homozygous deletion grown in YPD medium
H	Ave_hom_het	Average fitness of the homozygous and heterozygous deletion grown in YPD
I	Changes	The number of changes in the hierarchy upon deletion of the gene
J, M, P, S, V, Y, AB	Gene_moved	A gene that moved its position in the hierarchy
K, N, Q, T, W, Z, AC	Initial_level	The initial level of the gene in the WT hierarchy
L, O, R, U, X, AA, AD	Final_level	The final position of the gene in the rearranged hierarchy

**Table S5.** Description of Data S4. These data explain how deletion of yeast genes affected network rewiring and cell growth and is based on data from Constanzo *et al.* (9). The filename is "Yeast\_Changes\_hier\_Constanzo.xls". Positions in the hierarchy are indicated as: Bottom=Level 1; First = Level 2; Two = Level 3; Three = Level 4. For the phenotype (pheno) data, cell growth is relative to wild type with a value >1 greater than wild-type and <1 less than wild-type. The P-value data indicates the confidence in the genetic interaction (a low value reflects a high confidence that the interaction is true).

Column	Column title	Description
A	Deleted1	The first gene deleted
B	Deleted2	The second gene deleted
C	Level1	The first gene's position in the wild-type hierarchy.
D	Level2	The second gene's position in the wild-type hierarchy.
E	P-value	P-value of the genetic interaction between them
F	Pheno	Cell growth upon deletion of that pair of genes
G	Changes	The number of changes in the hierarchy upon deletion of that pair of genes
H, K, N, Q, T, W	Gene_moved	A gene that moved its position in the hierarchy
I, L, O, R, U, X	Initial_level	The initial level of the gene in the WT hierarchy
J, M, P, S, V, Z	Final_level	The final position of the gene in the rearranged hierarchy

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