Supplementary Information

Synthetic gene expression perturbation systems with rapid, tunable single-gene specificity in yeast

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Synthetic promoters

We used the *GAL1* promoter as a template of the synthetic promoters driving expression of a target gene by Z_4EV, GEV, and Z_3EV. The modified *GAL1* promoter with modified UAS_{GAL} region highlighted in green and Xba1 and Not1 restriction sites in red is shown below. The 3 CGG-N_{11}-GCC sequences are underlined.

>*Modified GAL1 promoter*

TTATATTGAATTTTCAAAAATTCTTACTTTTTTTTTGGATGGACGCAAAGAAGTTTAATAATCATATTACA
TGGCATTACCACCATATACATATCCATATCTAATCTTACTTATATGTTGTGGAAATGTAAAGAGCCCCATT
ATCTTAGCCTAAAAAAACCTTCTCTTTGGAACTTTCAGTAATACGCTTAACTGCTCATTGCTATATTGAAG
TGCGGCCGCGAAGCCGCCGAGCGGGCGACAGCCCTCCGACGGAAGACTCTAGACCGTGCGTCCTCGTCT
TCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTCGCGCCGCACTGCTCCGAACAATAAAGATTCTACAAT
ACTAGCTTTTATGGTTATGAAGAGGAAAAATTGGCAGTAACCTGGCCCCACAAACCTTCAAATTAACGAAT
CAAATTAACAACCATAGGATGATAATGCGATTAGTTTTTTAGCCTTATTTCTGGGGTAATTAATCAGCGAA
GCGATGATTTTTGATCTATTAACAGATATATAAATGGAAAAGCTGCATAACCACTTTAACTAATACTTTCA
ACATTTTCAGTTTGTATTACTTCTTATTCAAATGTCATAAAAGTATCAACAAAAAATTGTTAATATACCTC
TATACTTTAACGTCAAGGAGAAAAAACTATA

In Z_4EVpr, the Xba1-Sequence-Not1 region was replaced by the following sequence where the Z_4EV binding sequence GCGGCGGAGGAG is underlined:

GCGGCCGCGGCGGAGGAGTGCGGCGGAGGAGGAGCGGCGGAGGAGTGCGGCGGAGGAGGAGCGGCGGAGGA
GTGCGGCGGAGGAGTCTAGA

In the Z_3EVpr , the Xba1-Sequence-Not1 region was replaced by the following sequence where the Z_3EV binding sequence GCGTGGGCG is underlined.

GCGGCCGCGTGGGCGTGCGTGGGCGGGCGTGGGCGTGCGTGGGCGGGCGTGGGCGTGCGTGGGCGTCTAGA

As written above, the optimal Zif268 binding sequence is GCGTGGGCG. The non-canonical binding sites (in order of decreasing strength) are GCGTGGGTG, GCGTGGGAG, GCGTGGGGCC, and GCGTGGGGG.

Artificial Transcription Factors

The sequences of GEV, Z_4EV, and Z_3EV are shown below. The DNA binding domains are shown in red; the estrogen receptor is in green; VP16 is in blue. The linker between the Gal4dbd and ER is highlighted in pink and yellow.

>*GEV*

ATGAAGCTACTGTCTTCTATCGAACAAGCATGCGATATTTGCCGACTTAAAAAGCTCAAGTGCTCCAAAGA
AAAACCGAAGTGCGCCAAGTGTCTGAAGAACAACTGGGAGTGTCGCTACTCTCCCAAAACCAAAAGGTCTC
CGCTGACTAGGGCACATCTGACAGAAGTGGAATCAAGGCTAGAAAGACTGGAACAGCTATTTCTACTGATT
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ccctgcagctgcgtcgactctagaggatccatctgctggagacatgagagctgccaacctttggccaagcc
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>Z_4EV

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> Z_3EV

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Plasmid sequences for pMN9, KanMX-ZEV promoter template

>pMN9

CTAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACC
AATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCA
GTTTGGAACAAGAGTCCACTATTAAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATCAGGG
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CGCCGCGCTTAATGCGCCGCTACAGGGCGCGTCCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGC
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Plasmid sequences for pMN10, KanMX-ZEV promoter template

pMN10

CTAAATTGTAAGCGTTAATATTTTGTTAAAATTCGCGTTAAATTTTTGTTAAATCAGCTCATTTTTTAACC
AATAGGCCGAAATCGGCAAAATCCCTTATAAATCAAAAGAATAGACCGAGATAGGGTTGAGTGTTGTTCCA
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GACACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTC
TCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGA
AAAGTGCCAC

B1H sequences for determining Z_4 binding motif

The following sequences were recovered from the B1H procedure and used to identify the Z_4 recognition motif:

GCGGCGGAGGAG
GCGGCGGAGGAG
GCGGCTGAGGAG
GCGGCGGAGCCG
GCGGGTGAGGCG
GCGGCGGAGGAG
GCGGCGGCGGAG
GCGGCGGAGGAG
GCGGCTGAGACG
GCGGCTGAGAAG
GCGGCGGCGGCG
GCGGCTGCGGAG
GCGCCGGCGGAT
GCGGCGGCGGAG
GCGGCGGAGGCG
GCGGCTGAGGCG
GCGCCTGAGCAT
GCGGCGGAGGAG
GCGGGTGAGGCG
GCGGCGGAGGCG

Table S1: Yeast strains constructed in this study.
All strains are s288c with a repaired *HAP1* allele.

Strains	Description
yMN1/DBY12098	ura3Δ, leu2Δ0::ACT1pr-GEV-NatMX, Matα
yMN2/DBY12394	ura3Δ, leu2Δ0::ACT1pr-Z_3EV-NatMX, Matα
yMN3/DBY12395	ura3Δ, leu2Δ0::ACT1pr-Z_4EV-NatMX, Matα
yMN4	yMN1+pMN1
yMN5	yMN1+ pMN2
yMN6	yMN2+ pMN1
yMN7	yMN2+ pMN3
yMN8	yMN2+ pMN4
yMN9	yMN2+ pMN5
yMN10	yMN2+ pMN6
yMN11	yMN2+ pMN7
yMN13	yMN3+ pMN1
yMN14	yMN3+ pMN8
DBY12396	ura3Δ, leu2Δ0::ACT1pr-Z_4EV-NatMX, can1Δ::Z_4EVpr-GFP, Matα
DBY12397	leu2Δ0::ACT1pr-Z_4EV-NatMX, Matα
DBY12398	leu2Δ0::ACT1pr-Z_4EV-NatMX, Mata
DBY12399	ura3Δ, leu2Δ0::ACT1pr-Z_4EV-NatMX, Mata
DBY12400	leu2Δ0::ACT1pr-Z_3EV-NatMX, Mata
DBY12401	ura3Δ, leu2Δ0::ACT1pr-Z_3EV-NatMX, Mata
DBY12402	ura3Δ, leu2Δ0::ACT1pr-Z_3EV-NatMX, can1Δ::Z_3EVpr-GFP, Matα
DBY12406	leu2Δ0::ACT1pr-Z_3EV-NatMX, Matα
DBY12412	ura3Δ, leu2Δ0::ACT1pr-Z_4EV-NatMX, KanMX-Z_4EVpr-*GCN4*
DBY12416	leu2Δ0::ACT1pr-Z_4EV-NatMX, ybr032wΔ::LEU2pr$_{(-314 \searrow -1)}$-*LEU2*, Matα
DBY12417	leu2Δ0::ACT1pr-Z_4EV-NatMX, ybr032wΔ::LEU2pr$_{(-314 \searrow -1)}$-*LEU2*, Mata
DBY12418	leu2Δ0::ACT1pr-Z_3EV-NatMX, ybr032wΔ::LEU2pr$_{(-314 \searrow -1)}$-*LEU2*, Mata
DBY12419	leu2Δ0::ACT1pr-Z_3EV-NatMX, ybr032wΔ::LEU2pr$_{(-314 \searrow -1)}$-*LEU2*, Matα
DBY12423/ yRSM257	leu2Δ0::ACT1pr-Z_4EV-NatMX, ybr032wΔ::LEU2pr$_{(-314 \searrow -1)}$-*LEU2*, KanMX-Z_4EVpr-*GCN4, Matα*

Table S2: Plasmids used in this study.

Plasmid	reporter	Promoter	Binding site	Marker	Notes:
pMN1	Gfp	-	-	*URA3*	empy vector
pMN2	Gfp	Modified GAL1	CGG-N11-GCC	*URA3*	
pMN3	Gfp	Modified GAL1	GCGTGGGCG	*URA3*	wt
pMN4	Gfp	Modified GAL1	GCGTGGGTG	*URA3*	2-fold less affinity
pMN5	Gfp	Modified GAL1	GCGTGGGAG	*URA3*	5-fold less affinity
pMN6	Gfp	Modified GAL1	GCGTGGGCC	*URA3*	9-fold less affinity
pMN7	Gfp	Modified GAL1	GCGTGGGGG	*URA3*	20-fold less affinity
pMN8	Gfp	Modified GAL1	GCGGCGGAGGAG	*URA3*	-
pMN9	-	KanMX-Z_3EVpr	-		For making inducible genomic alleles
pMN10	-	KanMX-Z_4EVpr	-		For making inducible genomic alleles

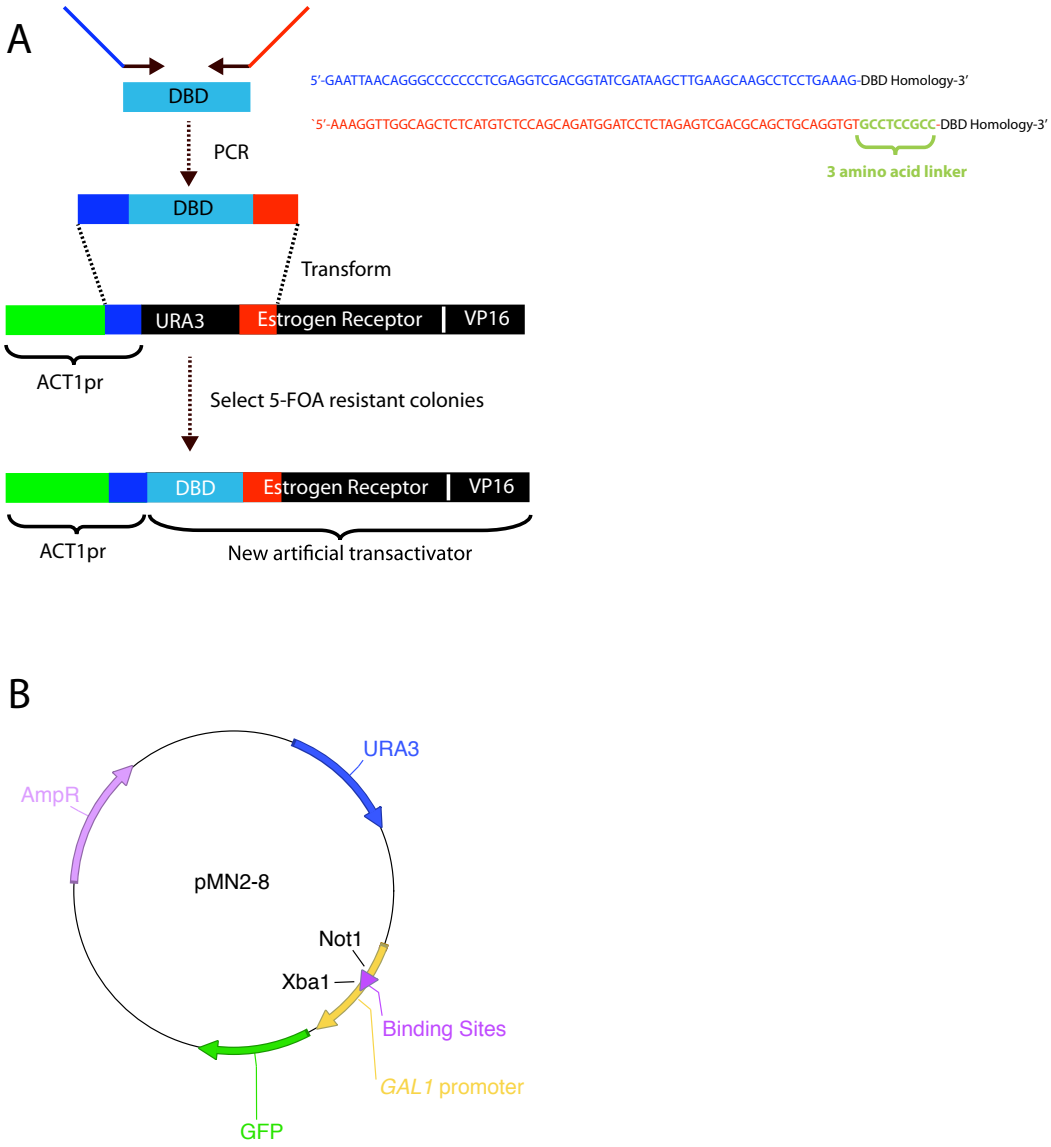

Figure S1: (A) To construct Z_3EV and Z_4EV, the Gal4 DNA binding domain of a chromosomally integrated GEV was replaced with *URA3*. DBDs (Zif268 and Z_4 array) were PCR amplified with homology to the ACT1 promoter (blue) and estrogen receptor (red). Linear PCR fragments were transformed into yeast, which were selected on 5-FOA. Successful transformants were confirmed by PCR. (B) Schematic of reporter plasmids pMN2-8. pMN1 contains GFP but not the *GAL1* promoter.

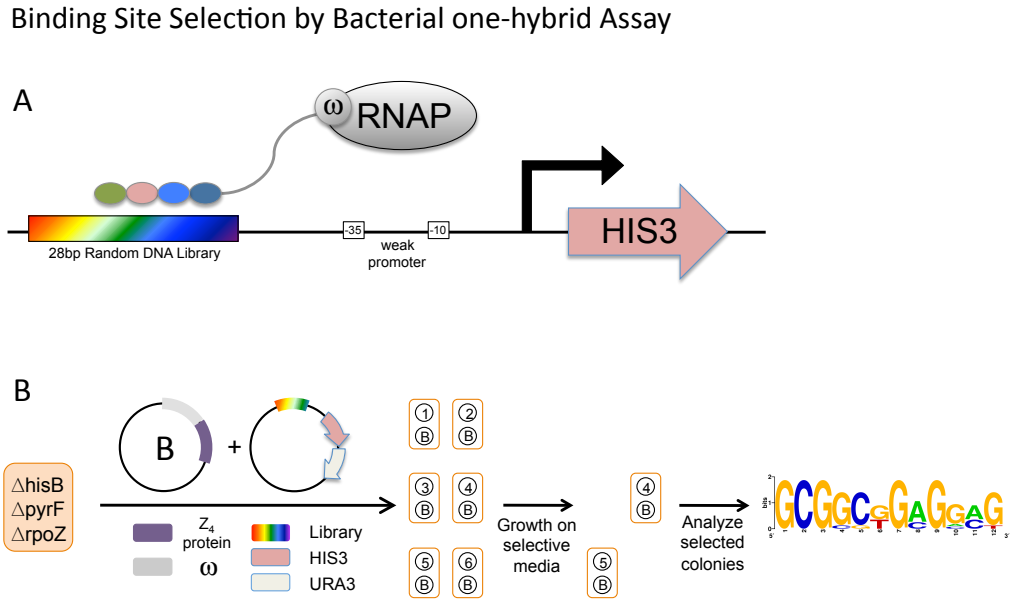

Figure S2: Binding site selection by bacterial one-hybrid assay. (A) Cartoon of reporter activation in B1H system. Here a 4-fingered protein, where each finger is depicted as a different color oval, is expressed as a fusion to the omega subunit of RNA polymerase. If the protein is able to bind a sequence in the library (rainbow box) upstream of the promoter that regulates the *HIS3* gene, it will recruit RNAP and activate the reporter. When grown on selective media, functional protein-DNA interactions can be assayed by screening for survival on media lacking histidine. (B) In practice, the Z_4 expression vector and the library reporter vector are transformed into a selection compatible *E. coli* strain. Transformants are plated on selective media. Those that survive are sequenced in the library window and MEME is used to recover an over represented motif that represents the DNA-binding specificity of the protein, which we have depicted as a sequence logo.

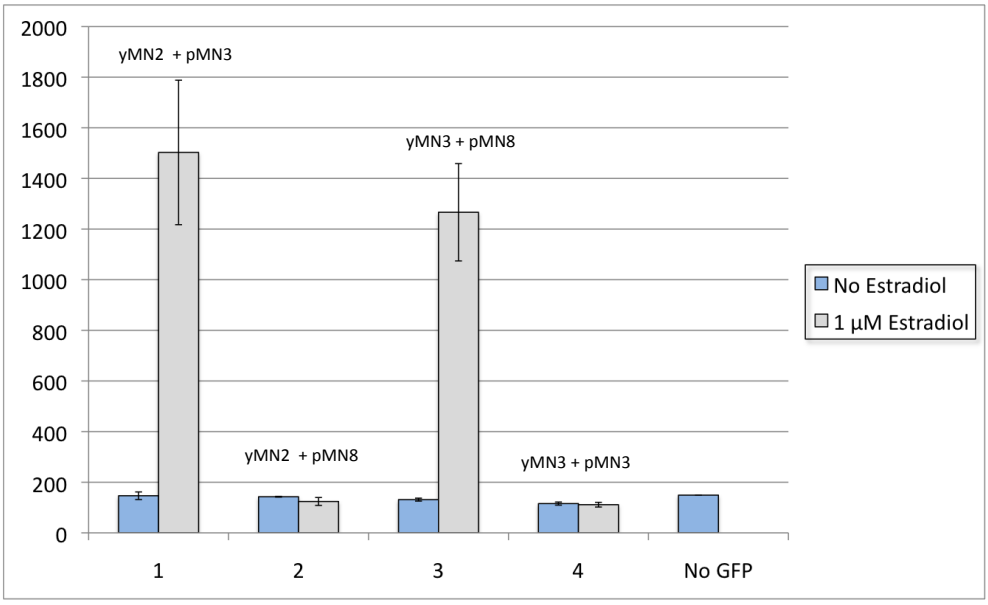

Figure S3: There is no crosstalk between Z_3EV and Z_4EV. Strains contain either Z_3EV (labeled 1 and 2; strain = yMN2) or Z_4EV (labeled 3 and 4; strain = yMN3). Strains contain either plasmid pMN3 (containing the Z_3EV-GFP reporter) or pMN8 (containing Z_4EV-GFP reporter). (1) yMN2 + pMN3. (2) yMN2 + pMN8. (3) yMN3 + pMN8. (4) yMN3 + pMN3. A yeast strain lacking GFP is shown to the right as a negative control. Cultures were grown to mid-log phase in SC-URA and then 1 µM β-estradiol was added to the medium. Following 12 hours of induction, the mean and standard deviation of three replicates was quantified by flow cytometry.

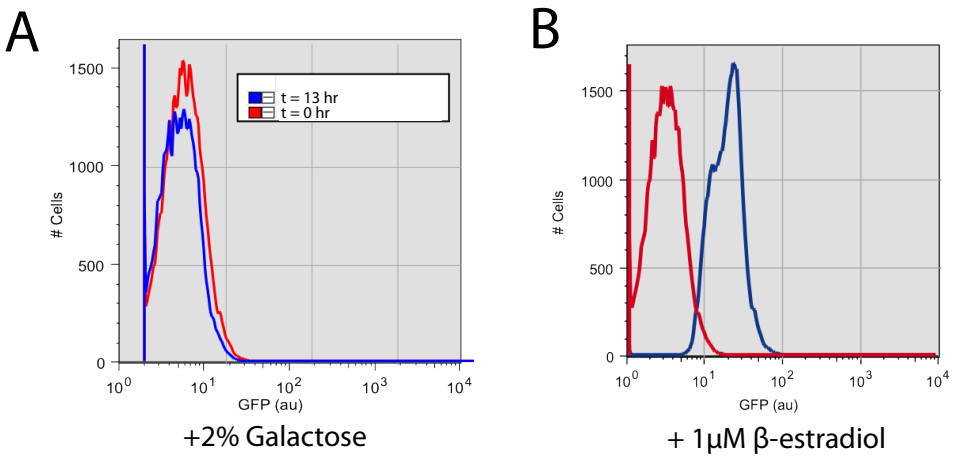

Figure S4: The Z_4EV responsive promoter is not induced by Gal4p. DBY12396 was grown to early-log phase in rich medium with 2% raffinose as a carbon source. Growth medium was then supplemented with either (A) 2% galactose or (B) 1 μM β-estradiol for 12 hours.

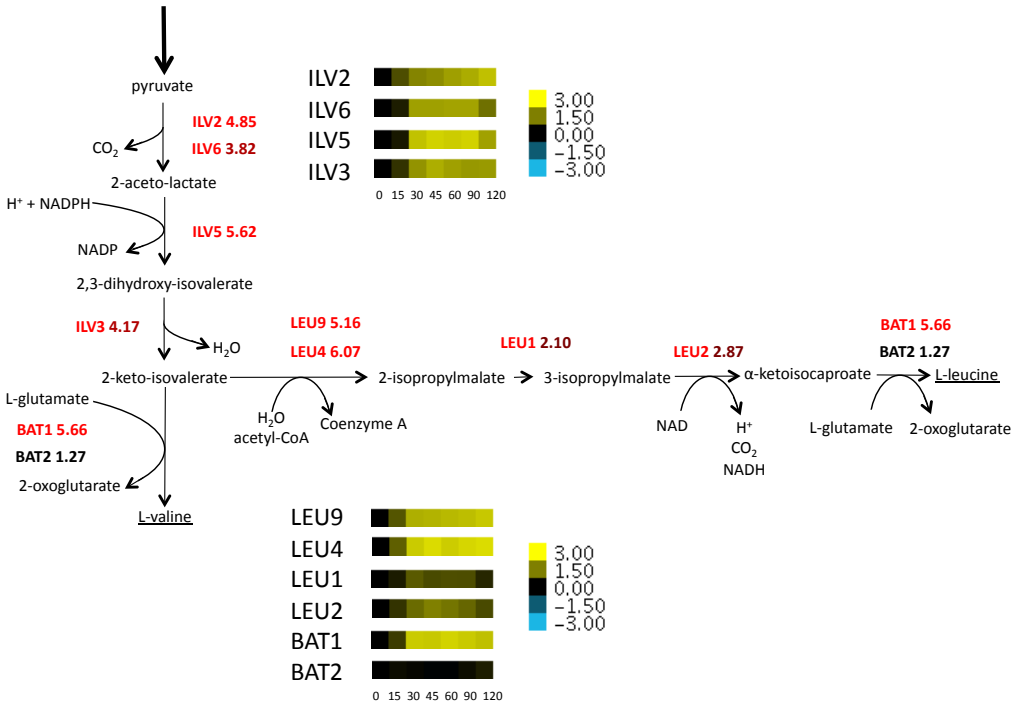

Figure S5: The transcriptional response of leucine and valine biosynthesis genes to Gcn4p induction. The maximum fold-change in expression is indicated next to each gene. The heatmap colorbar is in units of $\log_2$ fold-change in expression.

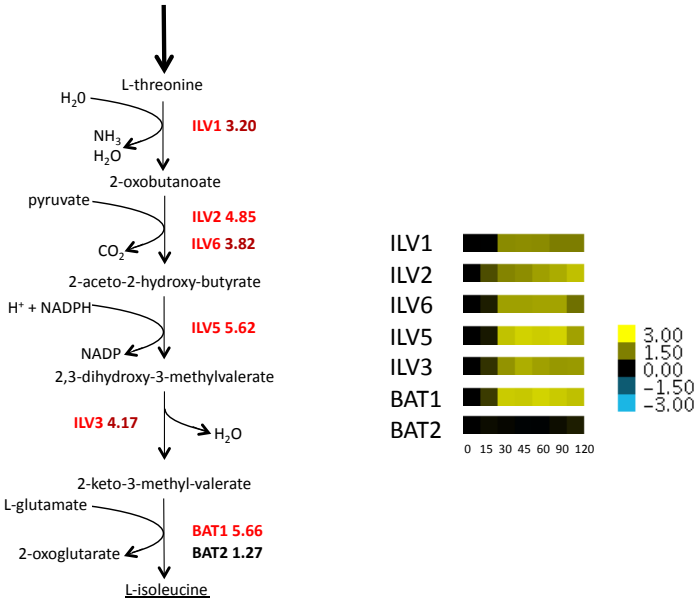

Figure S6: The transcriptional response of isoleucine biosynthesis genes to Gcn4p induction. The maximum fold-change in expression is indicated next to each gene. The heatmap colorbar is in units of $\log_2$ fold-change in expression.

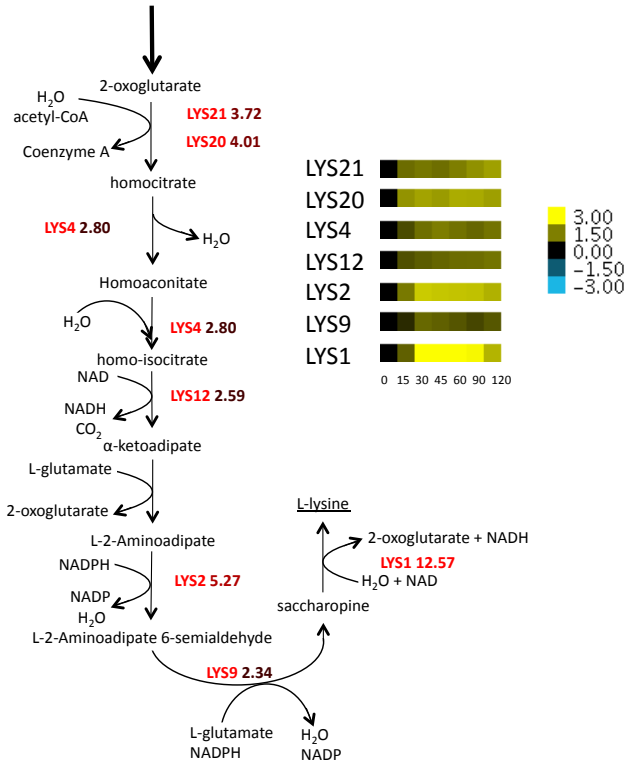

Figure S7: The transcriptional response of lysine biosynthesis genes to Gcn4p induction. The maximum fold-change in expression is indicated next to each gene. The heatmap colorbar is in units of $\log_2$ fold-change in expression.

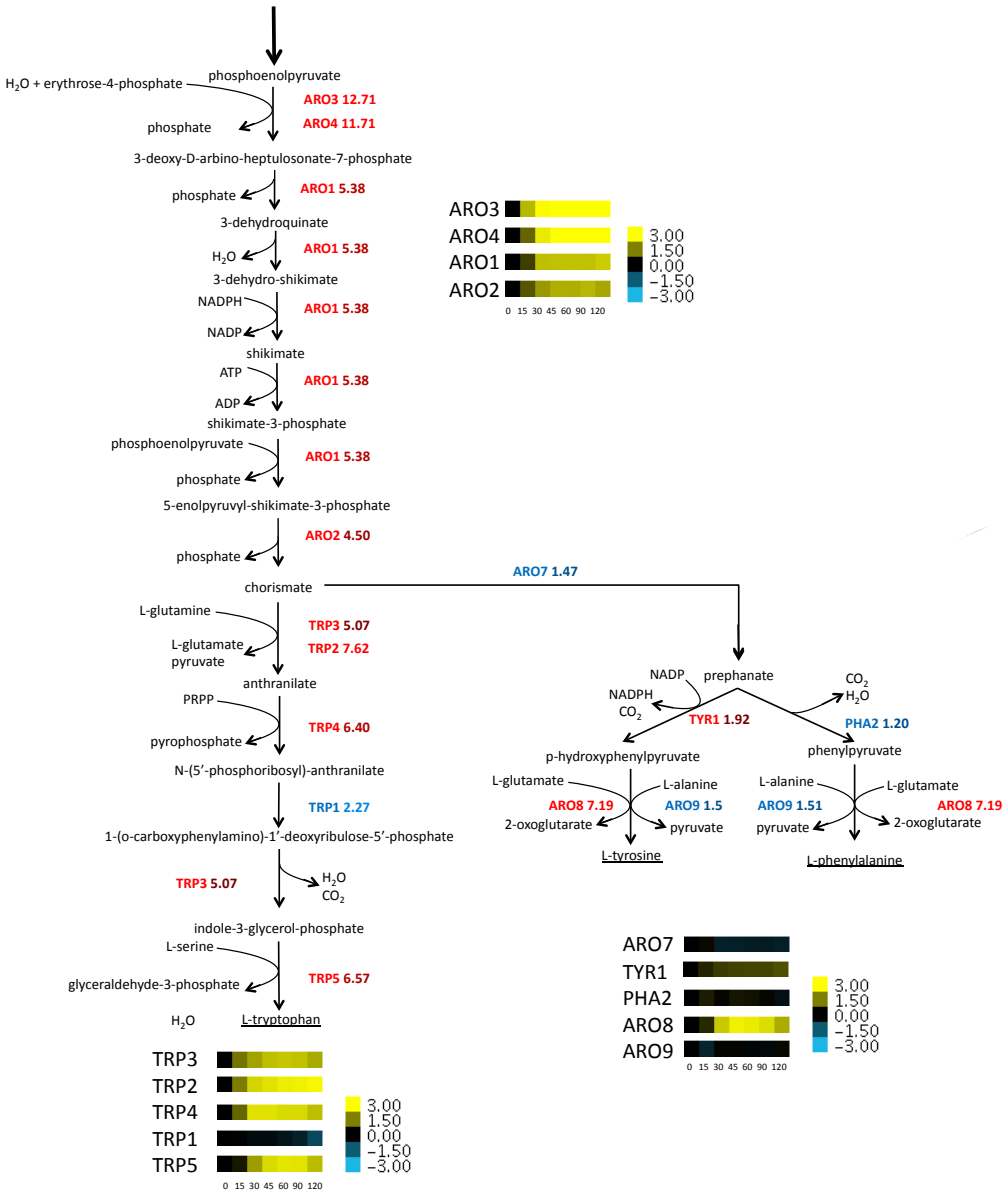

Figure S8: The transcriptional response of tryptophan, phenylalanine, and tyrosine biosynthesis genes to Gcn4p induction. The maximum fold-change in expression is indicated next to each gene. The heatmap colorbar is in units of $\log_2$ fold-change in expression.

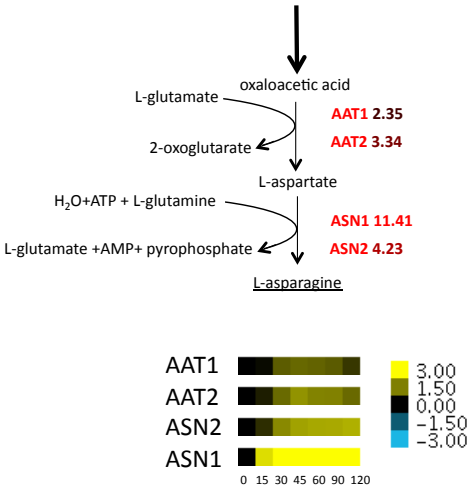

Figure S9: The transcriptional response of asparagine biosynthesis genes to Gcn4p induction. The maximum fold-change in expression is indicated next to each gene. The heatmap colorbar is in units of $\log_2$ fold-change in expression.

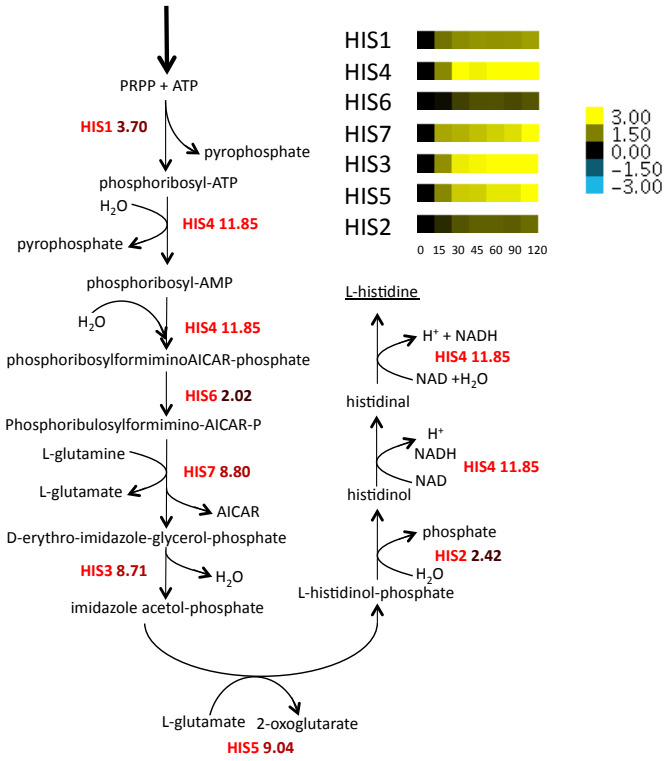

Figure S10: The transcriptional response of histidine biosynthesis genes to Gcn4p induction. The maximum fold-change in expression is indicated next to each gene. The heatmap colorbar is in units of $\log_2$ fold-change in expression.

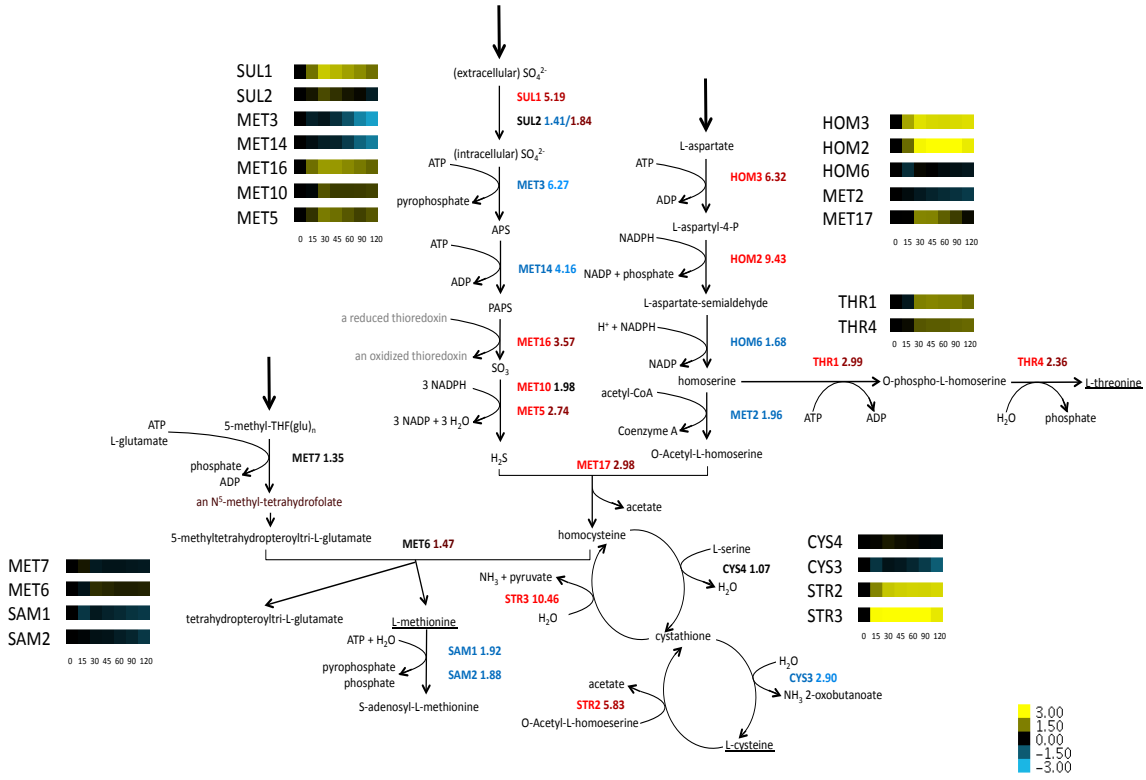

Figure S11: The transcriptional response of methionine and cysteine biosynthesis genes to Gcn4p induction. The maximum fold-change in expression is indicated next to each gene. The heatmap colorbar is in units of $\log_2$ fold-change in expression.

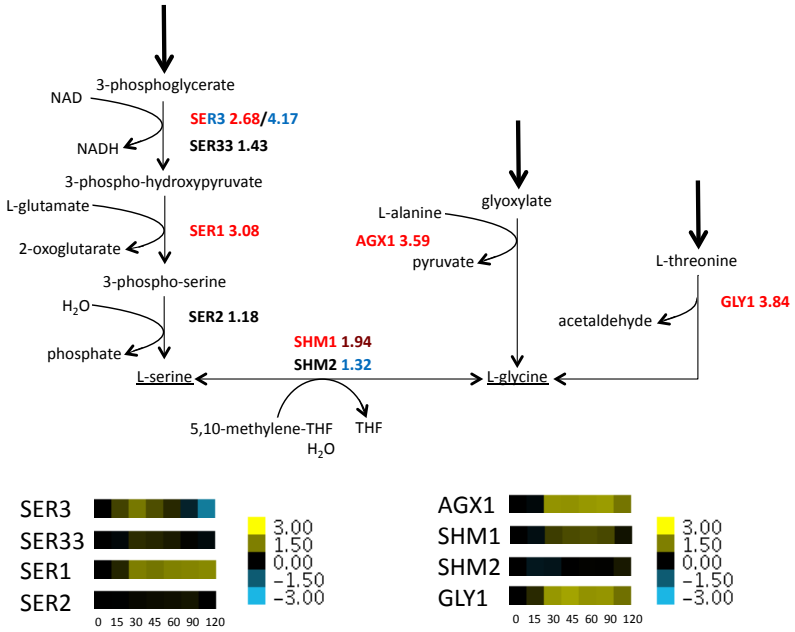

Figure S12: The transcriptional response of serine and glycine biosynthesis genes to Gcn4p induction. The maximum fold-change in expression is indicated next to each gene. The heatmap colorbar is in units of $\log_2$ fold-change in expression.

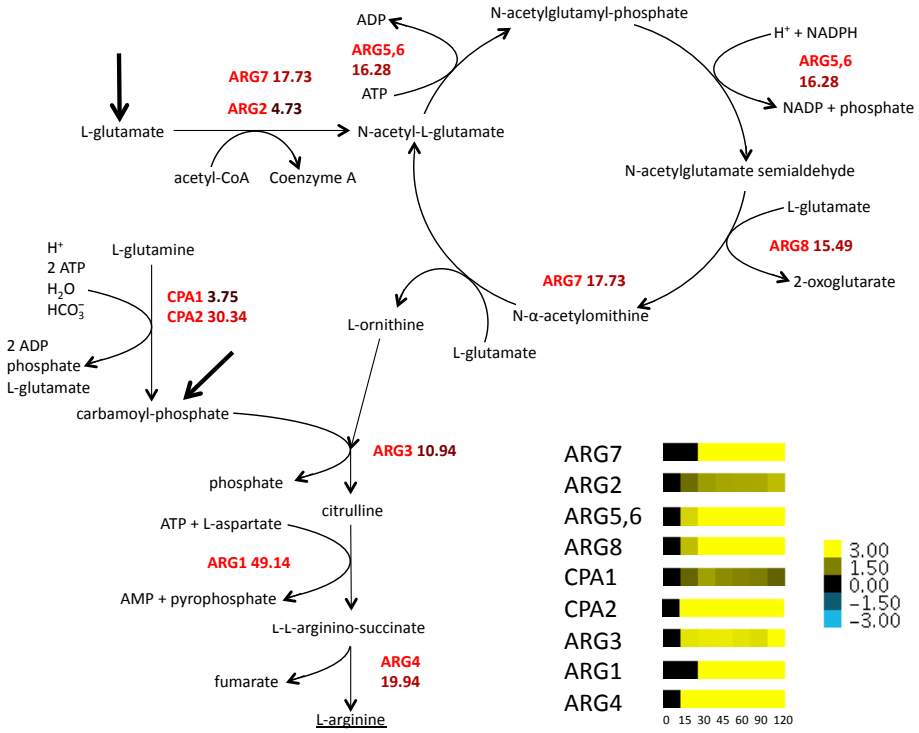

Figure S13: The transcriptional response of arginine biosynthesis genes to Gcn4p induction. The maximum fold-change in expression is indicated next to each gene. The heatmap colorbar is in units of $\log_2$ fold-change in expression.

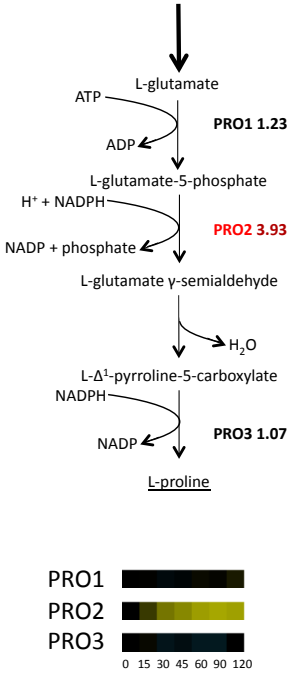

Figure S14: The transcriptional response of proline biosynthesis genes to Gcn4p induction. The maximum fold-change in expression is indicated next to each gene. The heatmap colorbar is in units of $\log_2$ fold-change in expression.

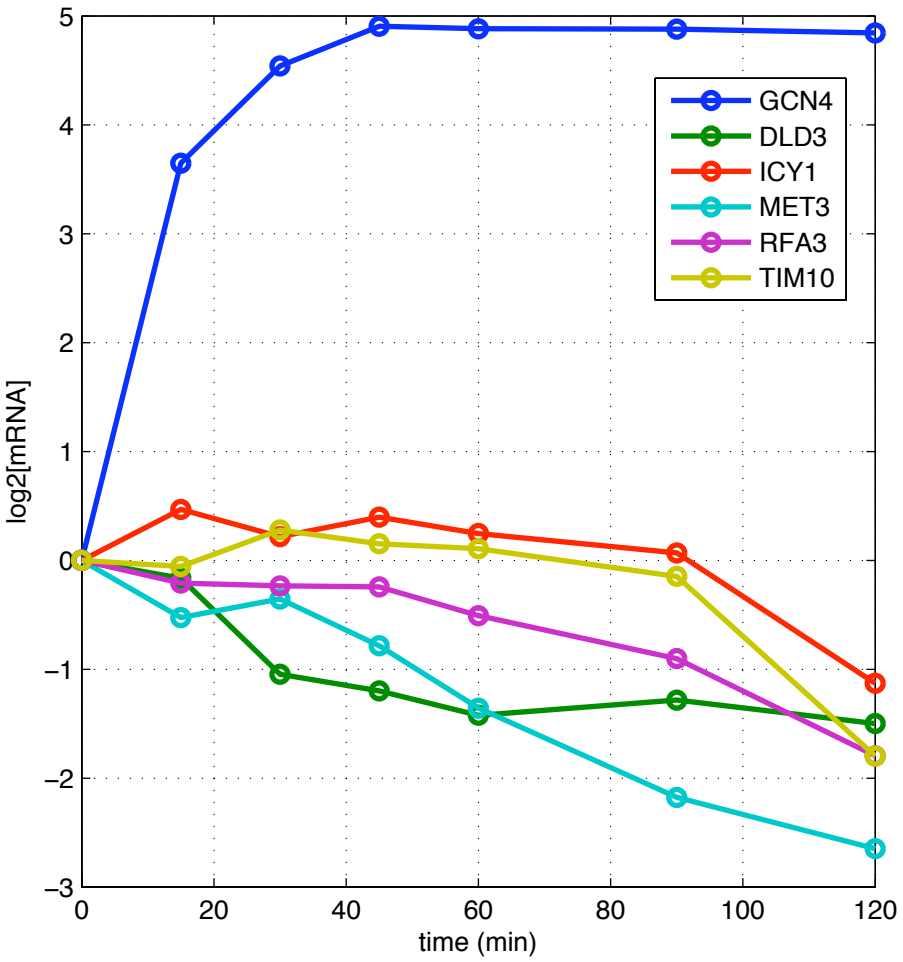

Figure S15: Transcriptional response of 5 previously determined Gcn4p targets (MacIsaac *et al*; p-value < 0.005) that are repressed >2-fold at 120 minutes in response to Gcn4p induction.

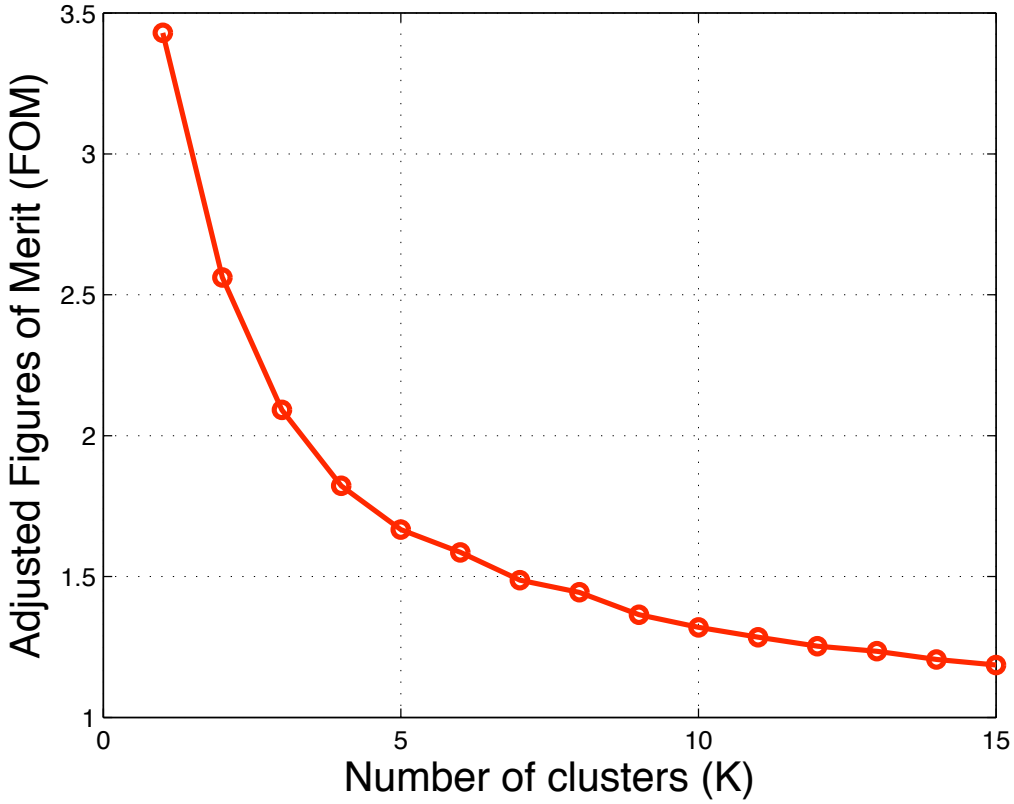

Figure S16: Figures of merit analysis of gene expression data in Figure 7D.