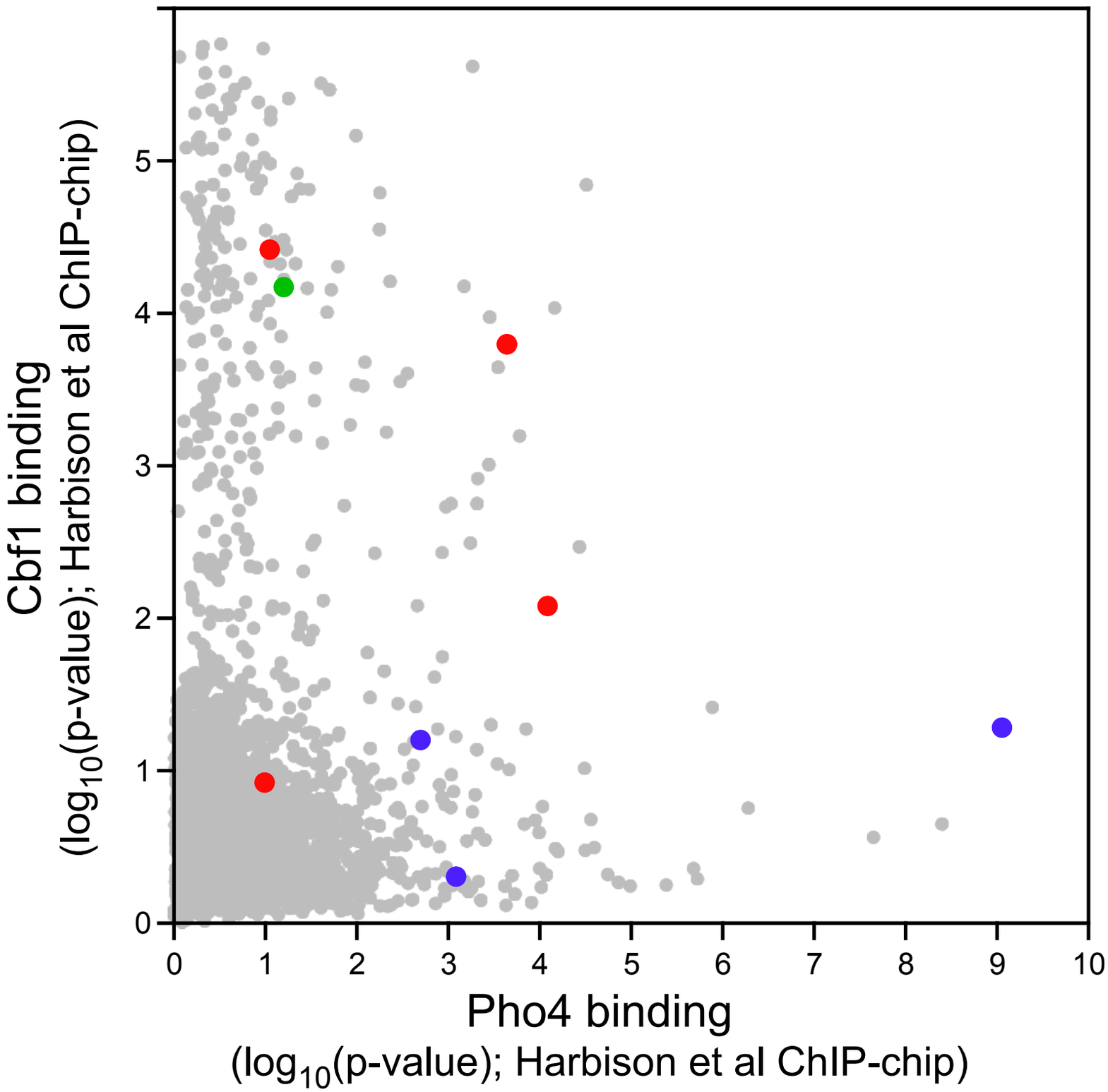
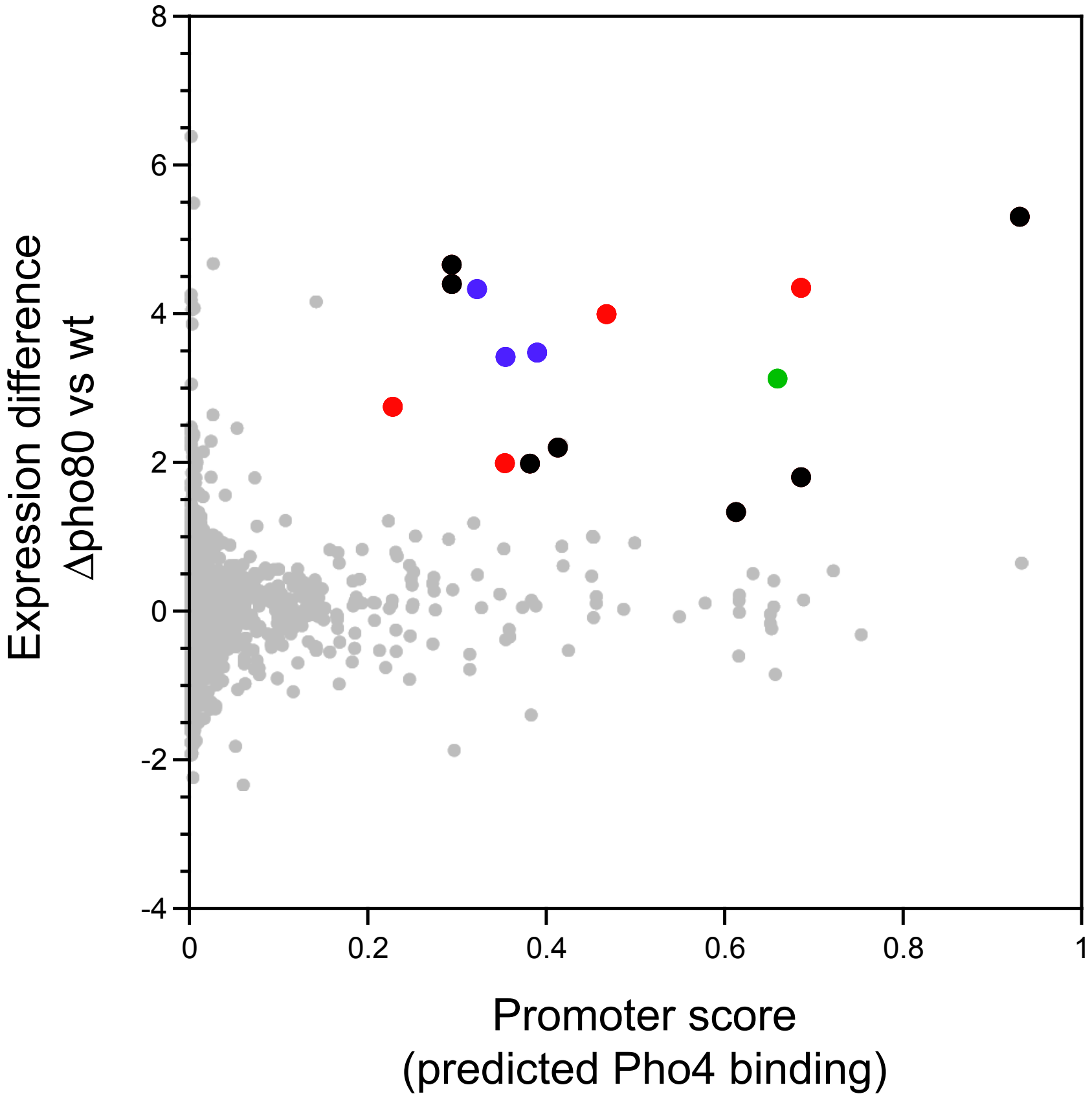
Supp. Fig. 1


Cbf1 binding
(log10(p-value); Harbison et al ChIP-chip)
0
1
2
3
4
5
0
1
2
3
4
5
6
7
8
9
10
Pho4 binding
(log10(p-value); Harbison et al ChIP-chip)

Supp. Fig. 2


Expression difference
Δpho80 vs wt
8
6
4
2
0
-2
-4
0
0.2
0.4
0.6
0.8
1
Promoter score
(predicted Pho4 binding)

Supplemental Figure 3

>VTC4 (wild-type)
ATGTTGCATTTTTATTATTCATC**CG**CACGTG**GC**TG**CA**CACGTG**AA**TGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>VTC4 mutated 3' site
ATGTTGCATTTTTATTATTCATC**CG**CACGTG**GC**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>AA
ATGTTGCATTTTTATTATTCATC**AA**CACGTG**TT**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>AC
ATGTTGCATTTTTATTATTCATC**AC**CACGTG**GT**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>AG
ATGTTGCATTTTTATTATTCATC**AG**CACGTG**CT**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>AT
ATGTTGCATTTTTATTATTCATC**AT**CACGTG**AT**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>CA
ATGTTGCATTTTTATTATTCATC**CA**CACGTG**TG**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>CC
ATGTTGCATTTTTATTATTCATC**CC**CACGTG**GG**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>CG
ATGTTGCATTTTTATTATTCATC**CG**CACGTG**CG**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>CT
ATGTTGCATTTTTATTATTCATC**CT**CACGTG**AG**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>GA
ATGTTGCATTTTTATTATTCATC**GA**CACGTG**TC**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>GC
ATGTTGCATTTTTATTATTCATC**GC**CACGTG**GC**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>GG
ATGTTGCATTTTTATTATTCATC**GG**CACGTG**CC**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>GT
ATGTTGCATTTTTATTATTCATC**GT**CACGTG**AC**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>TA
ATGTTGCATTTTTATTATTCATC**TA**CACGTG**TA**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

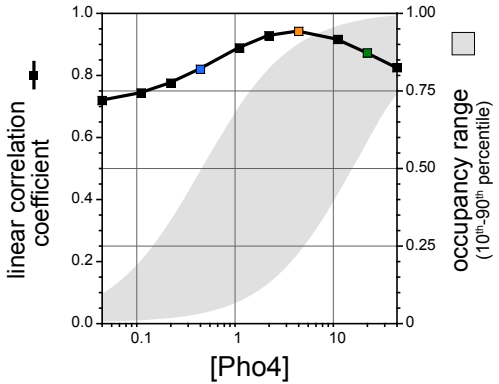
>TC
ATGTTGCATTTTTATTATTCATC**TC**CACGTG**GA**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

>TG
ATGTTGCATTTTTATTATTCATC**TG**CACGTG**CA**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

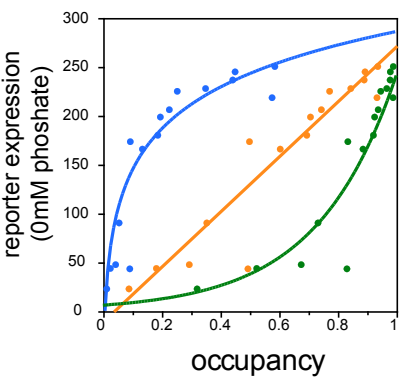
>TT
ATGTTGCATTTTTATTATTCATC**TT**CACGTG**AA**TGCACAATCGAATGAAAAAGAAGCAATACTATCTTTATATATTGAGGCAATCAGAAAGAAGTTA
CAGGCTAACAATCAAATCGGCCAATAAAAGAGCATAACAAGGCAGGAACAGCT

Supp Figure 4

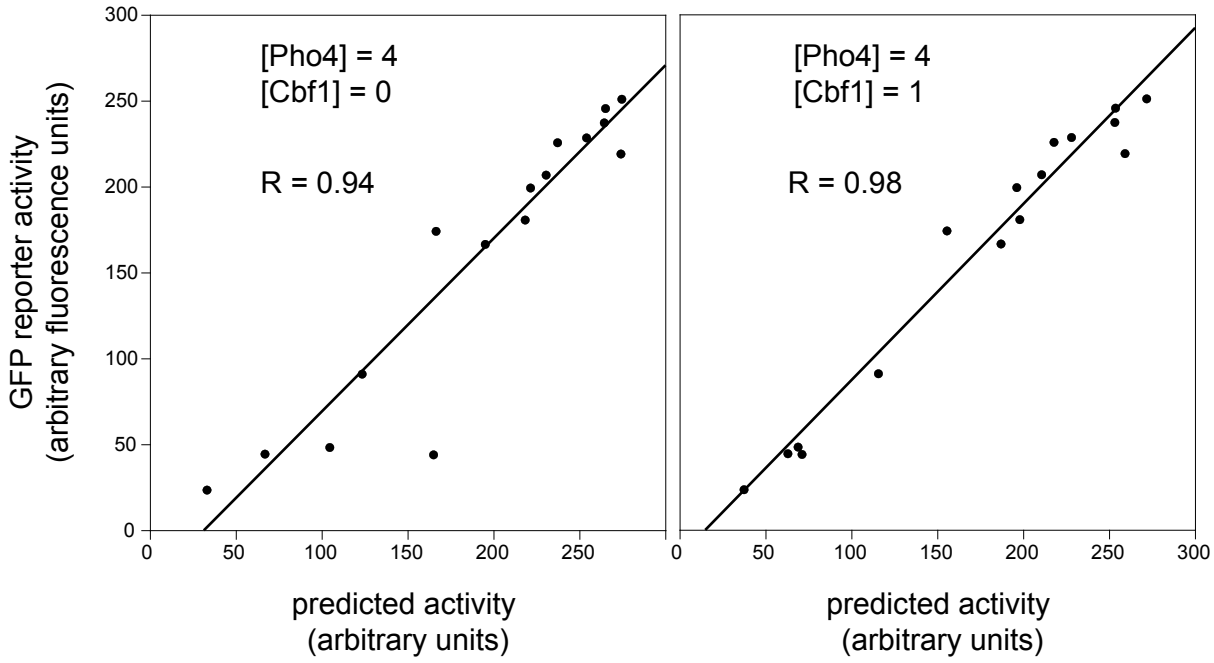
A



B

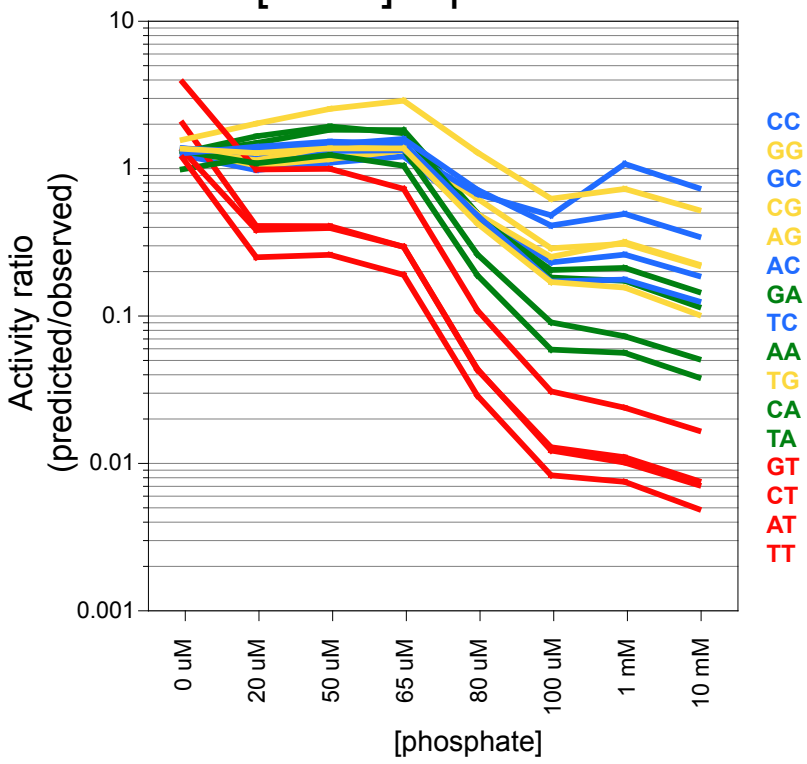


Supp Figure 5

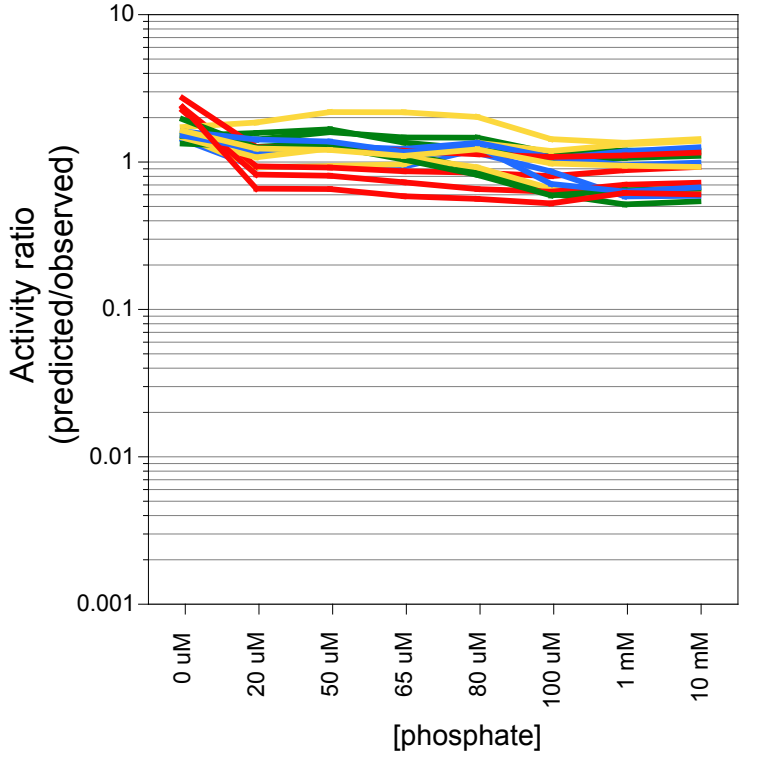


Supp Figure 6

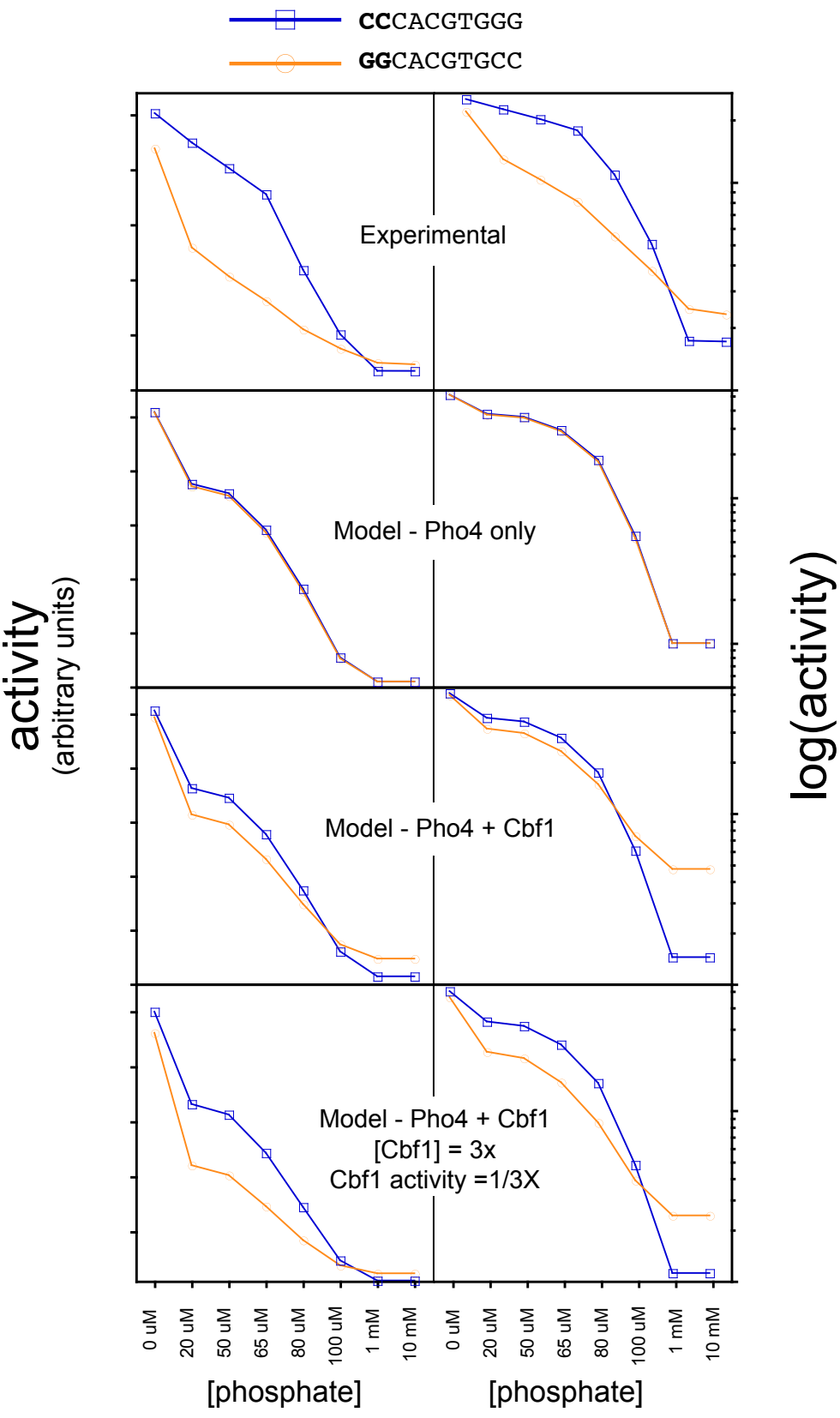
A



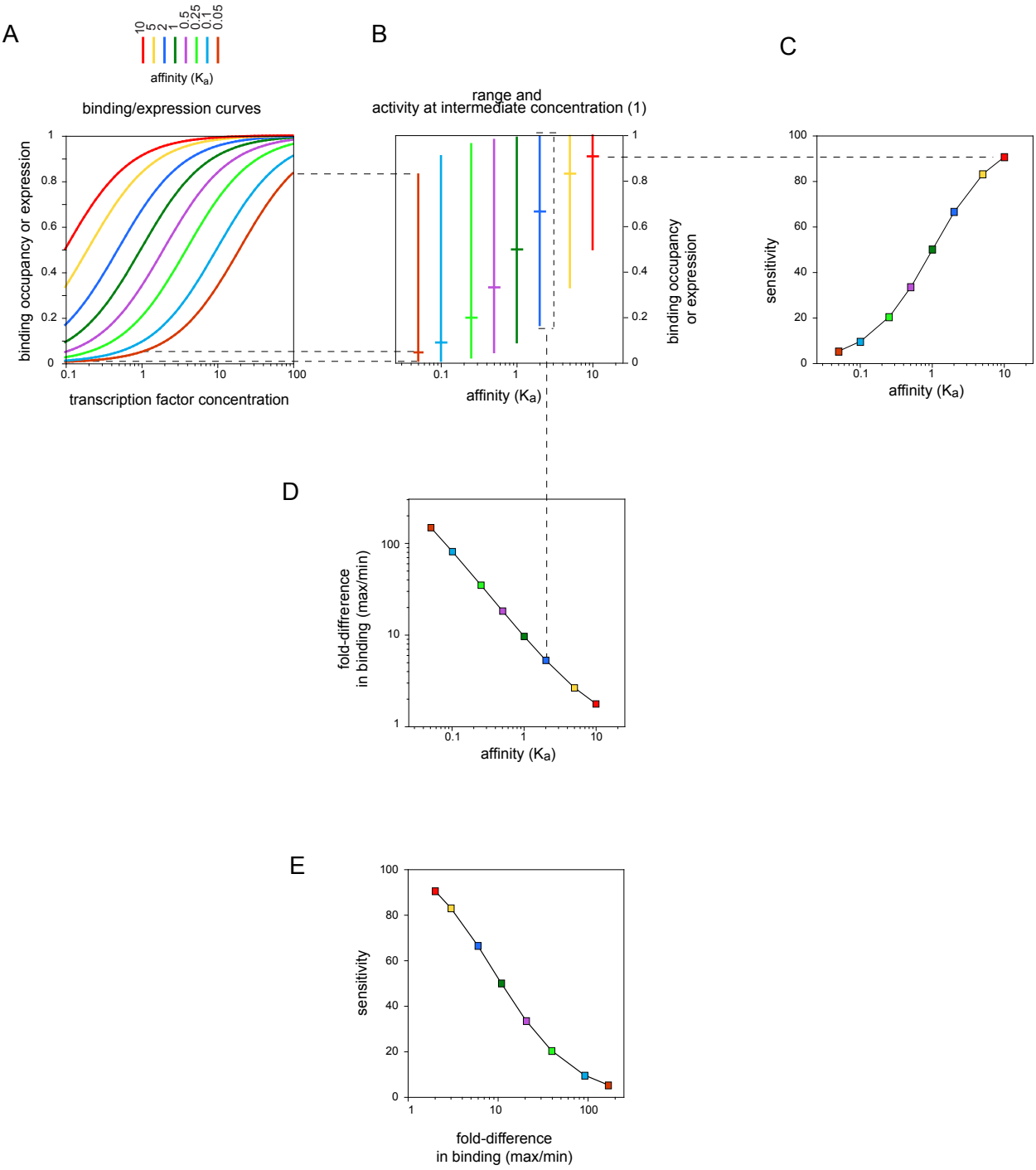
B



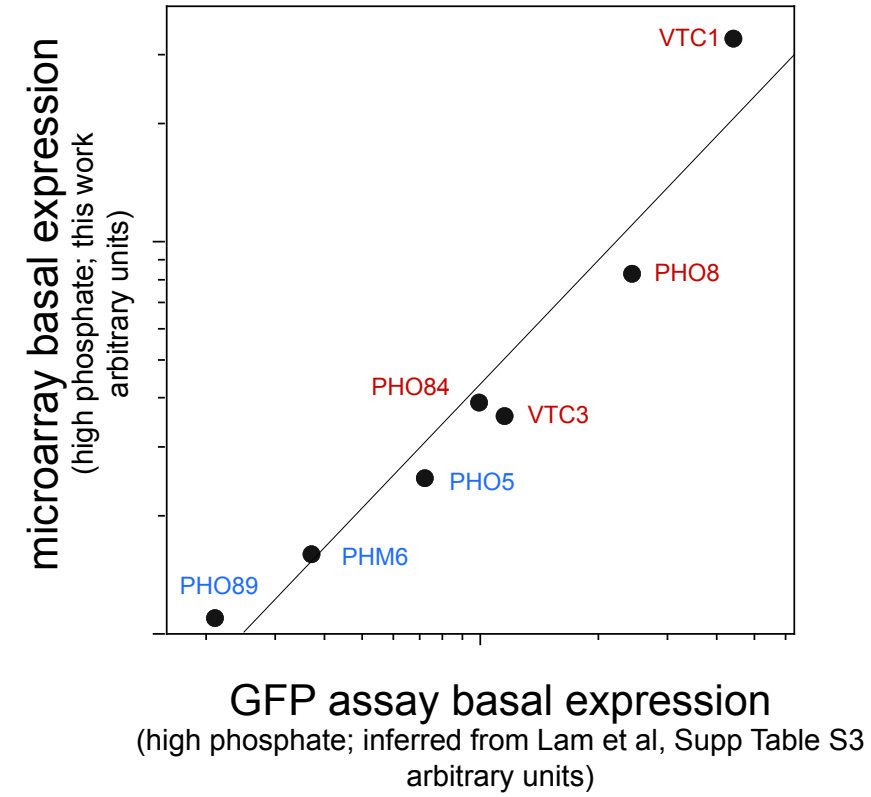
Supp Figure 7

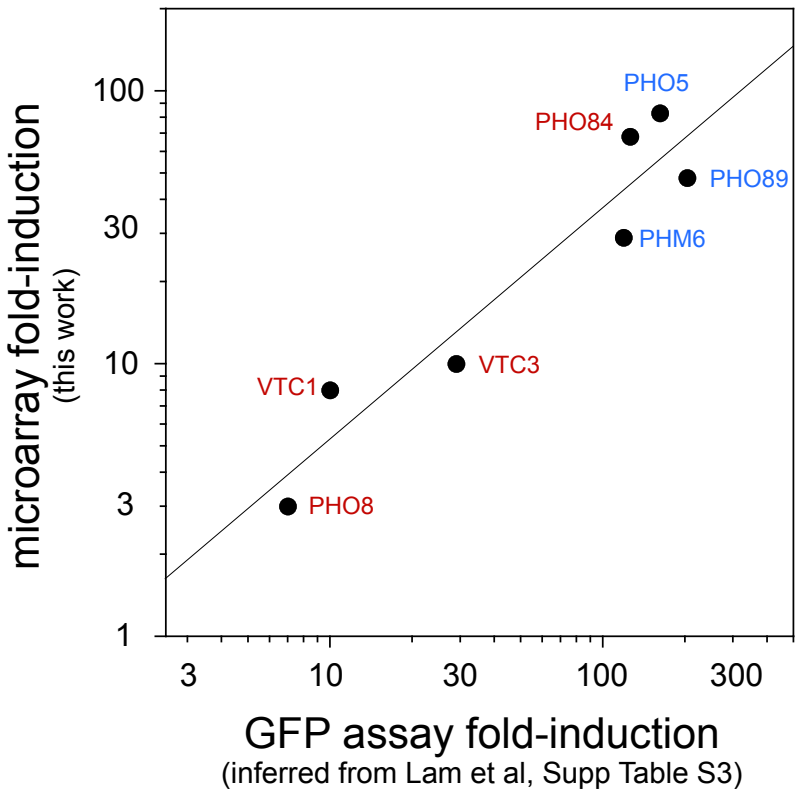


Supp Fig 8

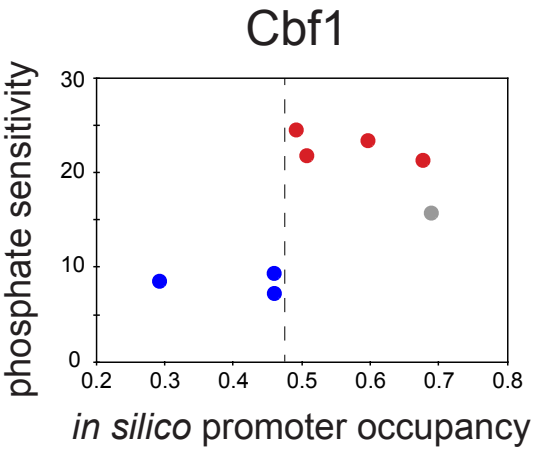

A
10
5
2
1
0.5
0.25
0.1
0.05
affinity (Ka)
binding/expression curves
binding occupancy or expression
transcription factor concentration
B
range and
activity at intermediate concentration (1)
affinity (Ka)
binding occupancy
or expression
C
sensitivity
affinity (Ka)
D
fold-difference
in binding (max/min)
affinity (Ka)
E
sensitivity
fold-difference
in binding (max/min)

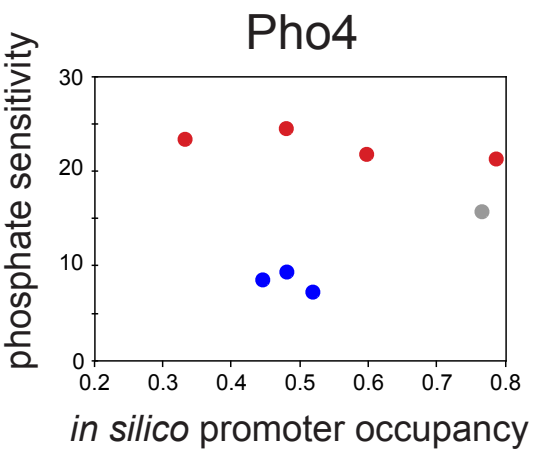
Supp Fig 9


microarray basal expression
(high phosphate; this work
arbitrary units)
VTC1
PHO8
PHO84
VTC3
PHO5
PHM6
PHO89
GFP assay basal expression
(high phosphate; inferred from Lam et al, Supp Table S3
arbitrary units)


microarray fold-induction
(this work)
100
30
10
3
1
PHO5
PHO84
PHO89
PHM6
VTC3
VTC1
PHO8
3
10
30
100
300
GFP assay fold-induction
(inferred from Lam et al, Supp Table S3)

Supp Figure 10





Supp Figure 11

Parameter sensitivity analysis for
Cbf1-mediated chromatin opening model
and its effect on induction sensitivity.

- ● parameter values used in Fig 6C of the manuscript
- ○ value used in place of the ● value in Fig 6E of the manuscript

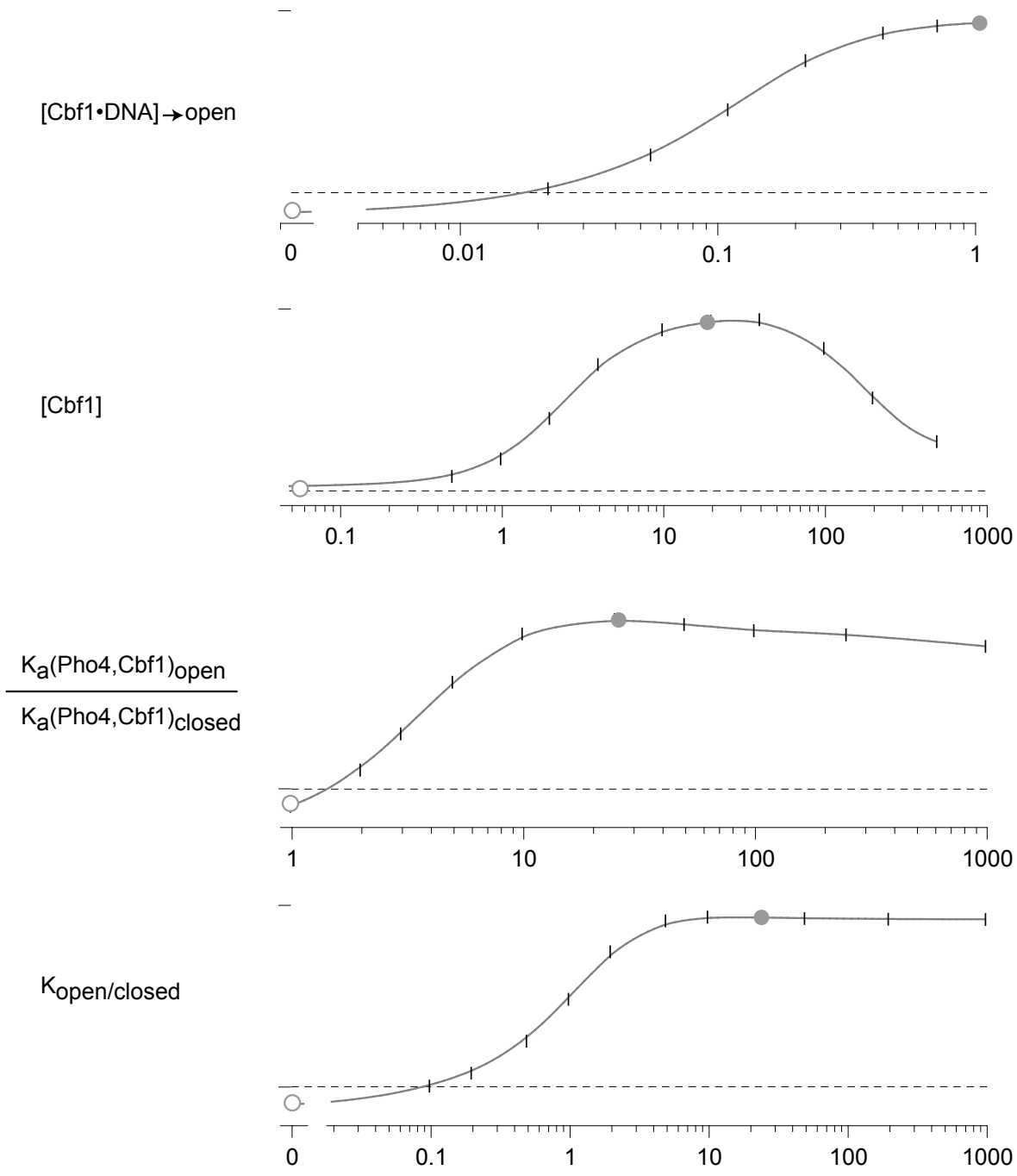


FIGURE LEGENDS (SUPPLEMENTARY INFORMATION)

Supp. Fig. 1: ChIP-chip enrichment p-values were taken from the work of Harbison et al, and include nearly all yeast promoters. (9) For Pho4, the p-value used for this plot was the most significant of two values reported, one from an experiment labeled PHO4_YPD (rich media), the other PHO4_Pi- (phosphate limited). For Cbf1, the value used was also the most significant from two experiments, one labeled CBF1_YPD (rich media), the other CBF1_SM (minimal media). Many genes show relatively significant enrichment for both Pho4 and Cbf1. Genes colored red and blue correspond to the red and blue colored genes in Figure 6 of the text, which are referred to as high and low sensitivity genes, respectively. Note that the high sensitivity genes (red) tend to have higher Cbf1:Pho4 enrichment ratios. The gene colored green is VTC4.

Supp. Fig. 2: Identification of Pho4 target genes based on differential expression in a strain with constitutive nuclear localization of Pho4 (y-axis) and high predicted binding affinity for Pho4. Expression differences are taken from Ogawa et al (19). Promoter binding occupancies were calculated using GOMER (5) and the Pho4 PMW reported in this paper. Genes indicated by large non-gray circles are ones for which a test statistic based on the ranking of the gene along each axis is significantly high (FDR <5%). (18) Genes colored red and blue correspond to the red and blue colored genes in Figure 6 of the text. The gene colored green is VTC4. Genes colored black are other genes that meet the criterion for significance.

Supp. Fig. 3: Promoter sequences of VTC4, VTC4 with the weaker Pho4 site knocked out, and the sixteen promoters containing single palindromic binding sites that were fused to GPF, integrated into the genome, and assayed for expression at various phosphate concentrations.

Supp. Fig. 4 (A) Sensitivity of linear correlation coefficient to Pho4 concentration for the correlation between single-site promoter occupancy and gene expression under fully induced conditions (squares and solid line, the colored squares cross-reference the data in panel B). The value at [Pho4]=4 corresponds to the fits shown in Fig 3B and Fig 4A in the text. The gray area represents the range in occupancy for the promoters, or more precisely the range from the 10th percentile value to the 90th. Percentile values were interpolated from the occupancy values for the 16 binding sites. (B) Examples of the expression vs. occupancy fit at concentrations above (green) and below (blue) the optimal value (orange). Colors correspond to the colored squares in panel A, which show the Pho4 concentration and the linear correlation coefficient for each fit. Concentrations above and below the optimum yield predicted occupancies that are not linearly related to expression.

Supp. Fig. 5: Reporter gene activity in 0mM phosphate plotted against predicted activity at [Pho4] = 4 and in the absence of Cbf1 (left panel) and the presence of Cbf1 (right panel; [Cbf1] = 1). Predicted activities were calculated as described in Methods, and linearly scaled so that the arbitrary values for predicted activity were similar to those for the experimental fluorescence values. (Scaling has no effect on correlation coefficients).

Supp. Fig. 6: Ratios of predicted/observed expression values as a function of phosphate concentration for each of the 16 reporter constructs. (A) no Cbf1 term. (B) [Cbf1]=1 and fixed transcriptional activation activity as described. [Pho4] was parameterized separately for each panel, maximizing the correlation coefficient for all 16 reporters; [Pho4] values are the same as those used in Fig 4B of the text (solid orange and solid black lines, respectively).

Supp. Fig. 7: Experimental and predicted expression profiles as a function of phosphate concentration for core motifs flanked by CC or GG. For each data set, expression is shown on both a linear scale (left) and a log scale (right) to accentuate different aspects of the comparisons. Note that the model that includes Pho4 only shows expression profiles that are essentially superimposed, reflecting the near identify of the Pho4 affinities for the two sites. Experimentally, however, there is a crossover point such that the GG motif has higher activity in high phosphate, while the CC motif has higher activity at intermediate phosphate. This is replicated by the model

that includes Cbf1 competition and Cbf1 transcriptional activity. The effect of modifying [Cbf1] and Cbf1 activity is shown in the bottom panel.

Supp. Fig. 8: Relationships among binding affinity, binding curves, expression sensitivity, and fold-change in transcription factor binding for a set of idealized promoters with single binding sites. (A) Binding isotherms showing fractional occupancy as a function of transcription factor concentration for sites with the Ka values indicated in the legend. Under the assumption that expression is directly related to transcription factor occupancy, the y-axis values can also be interpreted as relative expression. (B) A representation of the binding/expression values found in panel A, at three concentrations. As the dashed lines from panel A indicate, the extent of the colored lines in panel B indicate the binding occupancy at extreme concentrations (0.1 and 100) while the short horizontal line indicates the binding/expression at an intermediate concentration (arbitrarily 1). (C) The normalized expression value at the intermediate concentration is defined as the sensitivity of expression. Note that sensitivity is a direct function of affinity. (D) The fold-change in transcription factor occupancy between low and high concentrations of the factor can be obtained from the values indicated by the ends of the lines in panel B. Note that there is an inverse relationship between the affinity of the site and the fold-change in binding that can be measured. This is because high affinity sites have binding that is further along the hyperbolic binding curve at low concentrations than do the low affinity sites. Increases in concentrations therefore have smaller effects on the change in binding, not (necessarily) in terms of the absolute binding but in terms of the ratio of binding at high and low concentrations. (E) Inverse relationship between the sensitivity of gene expression and the fold change in binding. This is essentially a re-plotting of the sensitivity and fold-change values shown in panels D and E, but plotting them against each other rather than each against affinity.

Supp. Fig. 9:
Comparison of expression values obtained in our microarray analysis with values obtained by Lam et al using GFP fusions. Top panel compares values for gene expression at high values of phosphate (low levels of Pho4). Bottom panel compares the fold-induction between low and high phosphate. Both plots can be linearly fit with R=0.95.

Supp. Fig. 10: Predicted promoter occupancy scores (x-axis) for Cbf1 (top) and Pho4 (bottom). Values along y-axis are the expression sensitivity as defined in the text and shown in Fig 5. Color-coding of genes is based on expression sensitivity and is also as shown in Fig 5. Occupancy scores were obtained using the PWMs described in the text, scoring 600bp sequences 5' to the ORF with the program GOMER. (5)

Supp. Fig. 11: Sensitivity analysis for parameters that are essential to the modeling of chromatin remodeling by Cbf1 binding, and their effect on the correlation between predicted expression sensitivity and observed. The correlation coefficient values have been removed for visual clarity, but can be inferred from the dashed lines (correlation coefficient = 0) and from the gray circles, that indicate the parameter values used in the model in Fig 6D and E (correlation coefficient = 0.75). The effects of independently adjusting the model parameters are shown for four parameters: (i) the fraction of Cbf1-bound promoter that is shifted to the open conformation at the next, incrementally higher concentration of Pho4; (ii) the concentration of Cbf1; (iii) the degree to which the binding of Pho4 and Cbf1 is inhibited on 'closed' chromatin; (iv) the equilibrium constant between open and closed chromatin. This determines the fraction of non-Cbf1 bound promoters that is in each state. The short vertical line segments intersecting the curves showing the parameter values that were explored in the analysis; the curves are smoothed curves through these points.

Supp. Table 1

site name	sequence	relative Ka (Pho4)	relative Ka (Cbf1)	relative Kd (Pho4)	relative Kd (Cbf1)
AA	**AA**CACGTG**TT**	0.82	0.76	1.22	1.31
AC	**AC**CACGTG**GT**	1.15	0.67	0.87	1.50
AG	**AG**CACGTG**CT**	1.83	1.25	0.55	0.80
AT	**AT**CACGTG**AT**	0.14	3.84	7.07	0.26
CA	**CA**CACGTG**TG**	0.34	0.26	2.95	3.92
CC	**CC**CACGTG**GG**	4.81	0.27	0.21	3.76
CG	**CG**CACGTG**CG**	2.79	0.70	0.36	1.43
CT	**CT**CACGTG**AG**	0.08	0.60	13.34	1.66
GA	**GA**CACGTG**TC**	0.99	0.63	1.01	1.59
GC	**GC**CACGTG**GC**	2.70	0.66	0.37	1.52
GG	**GG**CACGTG**CC**	4.63	1.42	0.22	0.71
GT	**GT**CACGTG**AC**	0.33	13.00	3.01	0.08
TA	**TA**CACGTG**TA**	0.19	0.22	5.37	4.50
TC	**TC**CACGTG**GA**	0.52	0.19	1.93	5.29
TG	**TG**CACGTG**CA**	0.77	0.58	1.29	1.72
TT	**TT**CACGTG**AA**	0.03	0.29	31.13	3.50
VTC4 5' site	**CG**CACGTG**GC**	2.74	0.68	0.37	1.48
VTC4 3' site	**CA**CACGTG**AA**	0.10	0.27	9.57	3.70

Sixteen Pho4/Cbf1 motif variants assayed using GFP fusions, plus the two variants found in the VTC4 promoter. Relative free energies of binding were determined for dinucleotides flanking the CACGTG core by Maerkl and Quake (8). Under the assumption that the free energy effects of the two flanking regions are additive, equilibrium constants were calculated for binding to the particular motif shown relative to an equimolar mixture of all possible sites.

Supplementary Table 2

PHO4	A	C	G	T
1	0.055	-0.045	-0.195	0.280
2	0.109	-0.141	-0.216	0.459
3	0.983	-0.788	2.112	1.721
4	-0.813	2.181	1.734	1.950
5	1.995	-0.793	2.015	1.025
6	1.025	2.015	-0.793	1.995
7	1.950	1.734	2.181	-0.813
8	1.721	2.112	-0.788	0.983
9	0.459	-0.216	-0.141	0.109
10	0.280	-0.195	-0.045	0.055

Supplementary Table 3

CBF1	A	C	G	T
1	-0.128	0.272	-0.278	0.372
2	0.135	0.211	-0.039	-0.215
3	0.983	-0.788	2.112	1.721
4	-0.813	2.181	1.734	1.950
5	1.995	-0.793	2.015	1.025
6	1.025	2.015	-0.793	1.995
7	1.950	1.734	2.181	-0.813
8	1.721	2.112	-0.788	0.983
9	-0.215	-0.039	0.211	0.135
10	0.372	-0.278	0.272	-0.128