

Supplemental Tables for

14-3-3 (Bmh) proteins regulate combinatorial transcription following RNA Pol II recruitment by binding at Adr1-dependent promoters in *Saccharomyces cerevisiae*

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Running title:
Bmh inhibits promoter-bound Adr1

Supplemental Table 1. Oligos used in RT-qPCR and ChIP qPCR.

Gene	Position ^a	Oligo	Sequence
ACS1 promoter	-242	ACS1-C	CACTCACCTGTTGACATGCC
		ACS1-B	TATTTGCCACCGAGGAACTGT
ACS1	+2014/2142	KB36-ACS1.f	ATCAAGAAGCATTGTTGCTTTACTG
		KB37-ACS1.r	TGCCAGGGTTTGACAATGTAG
ACT1	+916/2437	KB15-ACT1.f2	TCCCCAGGTATTGCCGAAAG
		KB16-ACT1.r2	CCAATCCAGACGGAGTACTTTC
ADH2 promoter	-235	ADH2pQ1	ACCATCCACTTCACGAGACTGA
		ADH2pL	AAAAGTCGCTACTGGCACTC
ADH2	+741/1047	LL5-ADH2QA	GTTAGCGCAGTCGTTAAGGC
		LL6-ADH2QB	CAGTACCGTTCCGCCCTACAG
FBP1 promoter	-452	CTO FBP1 A	ACCCATCAAACCTGCATGGTCC
		CTO FBP1 B	TTGGCTCTTACGCCCTTAAC
FBP1	+760/1047	LL11-FBP1QA	ACCCTCTACTGGAACGAGACTATAAG
		LL12-FBP1QB	TCTTCTTGTCGCAAGGGTATG
GAL1-10 promoter	-408	KB1-GAL1.f1	TGTTCCGGAGCAGTGC GGCCG
		KB2-GAL1.r1	ACGCTTAACTGCTCATTGCT
GAL1	+1233/1587	KB9-GAL1.f2	CGTTCATCAAGGCACCAAAT
		KB10-GAL1.r2	TCAGAGGGCTAAGCATGTGT
GAL7 promoter	-305	KB3-GAL7.f1	TTTTTCCCCTTTATTTTGTTC
		KB4-GAL7.r1	GCTCAACAGTGCTCCGAAGT
GAL7	+865/1101	KB13-GAL7.f2	GGACCTCGCCTCGATTTTA
		KB14-GAL7.r2	AATTCATCACCAGTCGCATTC
POX1 promoter	-196	POX1-CQF	TAAACTCCGAAGCGAAAGGA
		POX1-CQR	TGAGCGGAATGAGGAAAAC
POX1	+2031/2247	GL189	GCCAATTGTGAGGAAAGACTGTATAG
		GL190	TGTGATAGATATCGCCATCAAAGTAAC
TEL-VI-R		tel55	GCGTAACAAAGCCATAATGCCTCC
		tel56	CTCGTTAGGATCACGTTTCAATCC

^a The position represents the midpoint of the qPCR product and the numbers listed are relative to the ATG of the gene listed. Those products that are within the ORF are listed with the position of the product followed by the total length of the ORF.

Supplemental Table 2. Data for additional genes or stains assayed in experiments shown in Figures 1, 2, 4 and 6.

A. Presence of Bmh-binding domain in GBD-Adr1 does not affect promoter binding.

GBD-ChIP

Gene	GBD-Adr1	Promoter/ TEL-VI-R	<i>BMH1</i>	<i>bmh1-ts</i>
<i>GAL1-10</i>	215-260	RATIO	22	12
		STDEV	7.9	7.2
	255-420	RATIO	65	13
		STDEV	5.6	1.7
<i>GAL7</i>	215-260	RATIO	54	200
		STDEV	30	106
	255-420	RATIO	39	40
		STDEV	46	36

RT-qPCR

Gene	GBD-Adr1	mRNA/ <i>ACT1</i>	<i>BMH1</i>	<i>bmh1-ts</i>
<i>GAL1</i>	215-260	RATIO	0.0036	0.0072
		STDEV	0.0013	0.0016
	255-420	RATIO	0.78	1.0
		STDEV	0.081	0.23
<i>GAL7</i>	215-260	RATIO	0.0084	0.051
		STDEV	0.0065	0.0082
	255-420	RATIO	4.5	9.4
		STDEV	0.83	1.3

B. Loss of Bmh1 activity in *hdacΔ* mutants has differing effects on glucose-repressed genes.

RT-qPCR; Repressed

Gene	mRNA/ <i>ACT1</i>	WT (<i>bmh2</i>)	<i>bmh1-ts</i> <i>bmh2</i>	<i>hda1 rpd3</i>	<i>bmh1-ts</i> <i>bmh2 hda1</i> <i>rpd3</i>	<i>bmh1-ts</i> <i>bmh2 rpd3</i>	<i>bmh1-ts</i> <i>bmh2 hda1</i>	<i>bmh2 hda1</i> <i>rpd3</i>
<i>ACS1</i>	RATIO	0.00016	0.00021	0.0013	0.0083	0.0040	0.00030	0.00067
	STDEV	0.00015	6.3E-05	0.00048	0.0061	0.0012	7.0E-05	0.00053
<i>ADH2</i>	RATIO	0.0075	0.11	0.024	0.62	0.29	0.081	0.020
	STDEV	0.00014	0.011	0.0076	0.056	0.02	0.021	1.5E-03
<i>ADY2</i>	RATIO	0.0022	0.0028	0.019	0.074	0.011	0.0037	0.023
	STDEV	0.00045	0.00074	0.0014	0.018	0.00025	0.00089	0.00013
<i>CIT3</i>	RATIO	0.0017	0.0016	0.0035	0.0069			
	STDEV	4.4E-05	0.00081	0.00063	0.00096			
<i>CTA1</i>	RATIO	0.0015	0.00077	0.0027	0.0038	0.0015	0.00065	0.0016
	STDEV	0.00022	3.8E-05	0.00057	1.2E-05	0.00052	4.2E-06	4.1E-05
<i>FBP1</i>	RATIO	0.0012	0.00093	0.0046	0.0048	0.0013	0.00083	0.0025

	STDEV	0.00015	0.00050	0.00075	0.0011	0.00032	3.2E-05	0.00014
FOX2	RATIO	0.0054	0.0030	0.015	0.011	0.010	0.0024	0.011
	STDEV	0.00041	0.00077	0.0029	0.00014	0.00036	3.6E-05	0.0027
ICL1	RATIO	0.019	0.0098	0.055	0.034	0.013	0.010	0.020
	STDEV	0.0020	0.00017	0.0070	0.0041	0.0025	0.00052	0.0023
ICL2	RATIO	0.025	0.023	0.062	0.071			
	STDEV	0.0069	0.0058	0.00022	0.0052			
MLS1	RATIO	0.00051	0.00026	0.0010	0.0023	0.00069	0.00043	0.00070
	STDEV	7.3E-05	3.4E-05	0.0013	0.00039	0.00082	0.00012	1.3E-05
POX1	RATIO	0.00091	0.0010	0.0019	0.0025	0.0024	0.00059	0.0014
	STDEV	7.6E-05	0.00021	0.00067	0.00018	0.00016	6.2E-06	7.1E-05

C. Adr1, not Cat8, is required for activation of Adr1-dependent genes in the *bmh hdac* strain.

RT-qPCR; Repressed

Gene	<i>ADR1 CAT8</i> genotype	mRNA/ <i>ACT1</i>	WT	<i>bmh1-ts bmh2</i>	<i>had1 rpd3</i>	<i>bmh1-ts bmh2 had1 rpd3</i>
ACS1	<i>ADR1 CAT8</i>	RATIO	0.0096	0.014	0.068	0.21
		STDEV	0.0030	0.0030	0.023	0.024
	<i>adr1 CAT8</i>	RATIO	0.013	0.013	0.047	0.090
		STDEV	0.0030	0.0021	0.0024	0.0077
	<i>ADR1 cat8</i>	RATIO	0.0060	0.017	0.094	0.30
		STDEV	0.0012	0.00067	0.0076	0.049
FBP1	<i>ADR1 CAT8</i>	RATIO	0.0018	0.0024	0.0090	0.0076
		STDEV	0.00011	0.00068	0.0023	0.0020
	<i>adr1 CAT8</i>	RATIO	0.0017	0.0025	0.0052	0.011
		STDEV	0.00020	0.00059	0.0012	0.00066
	<i>ADR1 cat8</i>	RATIO	0.0013	0.0021	0.0060	0.0046
		STDEV	0.00060	0.00051	0.00046	0.00058
FOX2	<i>ADR1 CAT8</i>	RATIO	0.0050	0.0046	0.015	0.015
		STDEV	0.00075	0.00038	0.0018	0.0026
	<i>adr1 CAT8</i>	RATIO	0.0018	0.0011	0.0028	0.0026
		STDEV	0.00022	5.9E-05	0.00030	0.00040
	<i>ADR1 cat8</i>	RATIO	0.0029	0.0027	0.0081	0.014
		STDEV	0.0016	0.00043	0.0011	0.0032

D. Adr1^C (S230A) does not enhance transcription in a *bmh hdac* strain.

RT-qPCR; Repressed

Gene	<i>ADR1</i> plasmid	mRNA/ <i>ACT1</i>	<i>adr1</i>	<i>adr1 bmh1-ts bmh2</i>	<i>adr1 had1 rpd3</i>	<i>adr1 bmh1-ts bmh2 had1 rpd3</i>
ACS1	<i>ADR1</i>	RATIO	0.024	0.024	0.13	0.63

	<i>ADR1^c</i>	STDEV	0.014	0.0031	0.013	0.096
		RATIO	0.019	0.042	0.28	0.79
		STDEV	0.0019	0.0092	0.036	0.099
<i>ADY2</i>	<i>ADR1</i>	RATIO	0.0026	0.0031	0.035	0.097
		STDEV	0.00076	0.00081	0.0064	0.012
	<i>ADR1^c</i>	RATIO	0.0043	0.0051	0.079	0.14
		STDEV	0.0012	0.0017	0.0072	0.029
<i>FBP1</i>	<i>ADR1</i>	RATIO	0.0050	0.0074	0.017	0.024
		STDEV	0.00053	0.00081	0.0046	0.0046
	<i>ADR1^c</i>	RATIO	0.0052	0.0069	0.018	0.023
		STDEV	0.00061	0.0018	0.0044	0.0033
<i>FOX2</i>	<i>ADR1</i>	RATIO	0.0032	0.0024	0.0066	0.014
		STDEV	0.00038	0.00041	0.0033	0.0047
	<i>ADR1^c</i>	RATIO	0.0035	0.0033	0.010	0.018
		STDEV	0.00021	0.0015	0.0030	0.0059

E. *Cat8* is not required for transcription of *Adr1*-dependent genes in the absence of *Bmh* activity.

RT-qPCR; Derepressed

Gene	% WT expression relative to <i>ACT1</i>	WT	<i>bmh1-ts bmh2</i>	<i>cat8</i>	<i>bmh1-ts bmh2 cat8</i>
<i>ADY2</i>	RATIO	100	530	110	820
	STDEV	4	22	20	120
<i>ATO3</i>	RATIO	100	1300	47	360
	STDEV	4	200	8.3	63
<i>FDH</i>	RATIO	100	1000	69	390
	STDEV	14	180	12	19
<i>ICL1</i>	RATIO	100	52	1.1	1.4
	STDEV	4	4.6	0.1	0.65
<i>ICL2</i>	RATIO	100	120	48	420
	STDEV	10	30	6	52
<i>JEN1</i>	RATIO	100	220	15	84
	STDEV	5	15	1	8.5
<i>POX1</i>	RATIO	100	400	70	1300
	STDEV	21	64	8.5	180

Supplementary Table 3. *CAT8*-dependence of gene expression.

<i>CAT8</i> Genotype	<i>ADR1</i>	R	DR	<i>cat8Δ/CAT8</i> (%) (DR)
Directly <i>Cat8</i>-, <i>Adr1</i>-dependent				
ACS1				
wt	ADR1-wt	1.1	280	3.3
cat8Δ	ADR1-wt	1.0	9.2	
wt	ADR1 ^c	2.2	360	42
cat8Δ	ADR1 ^c	1.7	150	
ADH2				
wt	ADR1-wt	1.0	1200	1.0
cat8Δ	ADR1-wt	1.0	12	
wt	ADR1 ^c	20	1600	75
cat8Δ	ADR1 ^c	15	1200	
ADY2				
wt	ADR1-wt	3.9	810	1.0
cat8Δ	ADR1-wt	1	8	
wt	ADR1 ^c	5.3	1500	20
cat8Δ	ADR1 ^c	2.5	300	
ATO3				
wt	ADR1-wt	0.85	11	6.9
cat8Δ	ADR1-wt	1.0	0.78	
wt	ADR1 ^c	0.90	11	89
cat8Δ	ADR1 ^c	1.0	10	
JEN1				
wt	ADR1-wt	1.7	122	7.2
cat8Δ	ADR1-wt	1.0	8.8	
wt	ADR1 ^c	1.6	150	80
cat8Δ	ADR1 ^c	0.9	120	
<i>Cat8</i>-, <i>Adr1</i>-dependent				
ALD4				
wt	ADR1-wt	0.86	19	46
cat8Δ	ADR1-wt	1.0	8.5	
wt	ADR1 ^c	1.9	24	190
cat8Δ	ADR1 ^c	2.8	45	
ALP1				
wt	ADR1-wt	1.0	21	25
cat8Δ	ADR1-wt	1.0	5.1	
wt	ADR1 ^c	1.2	22	83
cat8Δ	ADR1 ^c	0.61	26	
CIT3				
wt	ADR1-wt	0.94	15	27
cat8Δ	ADR1-wt	1.0	4.1	

wt	ADR1 ^c	1.1	19	79
cat8Δ	ADR1 ^c	0.79	15	
		CYB2		
wt	ADR1-wt	1.0	8.3	25
cat8Δ	ADR1-wt	1.0	6.2	
wt	ADR1 ^c	1.3	7.3	61
cat8Δ	ADR1 ^c	0.96	12	
Cat8-independent, Adr1-dependent				
		FDH		
wt	ADR1-wt	1	7.9	14
cat8Δ	ADR1-wt	1	0.55	
wt	ADR1 ^c	1.6	57	42
cat8Δ	ADR1 ^c	1	24	
		FOX2		
wt	ADR1-wt	1.1	8.1	33
cat8Δ	ADR1-wt	1.0	2.7	
wt	ADR1 ^c	1	10	130
cat8Δ	ADR1 ^c	0.52	13	
		GUT1		
wt	ADR1-wt	0.74	11	42
cat8Δ	ADR1-wt	1.00	4.9	
wt	ADR1 ^c	0.72	12	150
cat8Δ	ADR1 ^c	0.69	18	
		ICL2		
wt	ADR1-wt	1.3	13	16
cat8Δ	ADR1-wt	1.0	2.1	
wt	ADR1 ^c	0.88	14	48
cat8Δ	ADR1 ^c	0.22	6.8	
		POT1		
wt	ADR1-wt	1.1	11	140
cat8Δ	ADR1-wt	1.0	16	
wt	ADR1 ^c	1.6	14	340
cat8Δ	ADR1 ^c	1.4	53	
		POX1		
wt	ADR1-wt	1	100	15
cat8Δ	ADR1-wt	1	15	
wt	ADR1 ^c	14	210	81
cat8Δ	ADR1 ^c	11	170	
		SPG1		
wt	ADR1-wt	2.7	9.0	110
cat8Δ	ADR1-wt	1.0	10	
wt	ADR1 ^c	1.9	45	210
cat8Δ	ADR1 ^c	2.8	96	
		SPS19		

wt	ADR1-wt	1.9	13	38
cat8Δ	ADR1-wt	1.0	5.1	
wt	ADR1 ^c	4.5	21	130
cat8Δ	ADR1 ^c	3.2	27	
		YIL057c		
wt	ADR1-wt	1.6	2400	23
cat8Δ	ADR1-wt	1.0	560	
wt	ADR1 ^c	2.8	1500	73
cat8Δ	ADR1 ^c	1.5	1100	
Cat8-dependent, Adr1-independent				
		FBP1		
wt	ADR1-wt	2.4	330	3.7
cat8Δ	ADR1-wt	1.0	12	
wt	ADR1 ^c	1.7	160	10
cat8Δ	ADR1 ^c	0.24	16	
		ICL1		
wt	ADR1-wt	2	150	4
cat8Δ	ADR1-wt	1	6	
wt	ADR1 ^c	1	95	3.9
cat8Δ	ADR1 ^c	0.20	4	
		MDH2		
wt	ADR1-wt	1	110	4.3
cat8Δ	ADR1-wt	1	4.7	
wt	ADR1 ^c	1.2	90	9.8
cat8Δ	ADR1 ^c	1.2	8.8	
Cat8-independent, Adr1-independent				
		ADR1		
wt	ADR1-wt	1.5	5.4	43
cat8Δ	ADR1-wt	1.0	2.3	
wt	ADR1 ^c	0.7	3.7	89
cat8Δ	ADR1 ^c	0.9	3.3	

* mRNA levels normalized to ACT1 and expressed relative to wild-type ADR1 in repressed CKY23 (adr1cat8) with wild type Adr1 activator.

** Gene expression was considered directly dependent on Adr1 and Cat8 if both factors bound to the respective promoters in a genome wide ChIP-chip study (1).

REFERENCES

1. **Tachibana, C., J. Y. Yoo, J. B. Tagne, N. Kacherovsky, T. I. Lee, and E. T. Young.** 2005. Combined global localization analysis and transcriptome data identify genes that are directly coregulated by Adr1 and Cat8. *Mol Cell Biol* **25**:2138-2146.