

Supplementary material

Residues outside of the 14-3-3 motif regulate Adr1 binding to and inhibition by Bmh proteins

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Running title: Insights into Bmh-Adr1 interactions

Keywords: Transcription regulation, transcription activator, protein-protein interaction,
14-3-3

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Supplementary Table S1: Plasmid				
Plasmid	Marker	Backbone	Cassette	Reference
pHZ18 ⁺	URA3		<i>UAS_{GAL10}-TATA_{CYC1}-lacZ</i>	(1)
pOBD2	<i>TRP1-CEN3</i>		<i>ADH1p-GBD</i>	(2)
pBFBMH1	<i>TRP1-2μ</i>	pBF339	<i>ADH1_p-HA-BMH1</i>	(3)
pGEX-3X-BMH1	<i>Amp^r</i>	pGEX3X	<i>tac_p-GST-BMH1</i>	(3)
pGBDA13	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 215-310</i>	(4)
pGBDA14	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 215-260</i>	(4)
pGBDA26	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 241-280</i>	(4)
pKP03	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 215-250</i>	This study
pKP04	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 215-240</i>	This study
pKP06	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 221-260</i>	This study
pKP07	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 221-240</i>	This study
pKP09	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-260</i>	This study
pKP10	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240</i>	This study
pKP14	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 215-310 (Δ254-260)</i>	This study
pKP15	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 215-310 (Δ298-303)</i>	This study
pKP16	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 215-310 (Δ254-260) and (Δ298-303)</i>	This study
pKP27	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 215-420/Δ241-259</i>	This study
pKP29	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-420/Δ241-259</i>	This study
pKP39	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240/R228A</i>	This study
pKP40	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240/R228K</i>	This study
pKP41	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240/A229P</i>	This study
pKP42	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240/S230A</i>	This study
pKP43	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240/S230D</i>	This study

pKP46	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240/F231A</i>	This study
pKP47	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240/F231S</i>	This study
pKP48	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240/S232P</i>	This study
pKP50	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240/S232R</i>	This study
pKP51	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240/A233G</i>	This study
pKP52	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240/A233T</i>	This study
pKP55	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240/Y239C</i>	This study
pKP56	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240/Y239N</i>	This study
pKP59	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (R228K)</i>	This study
pKP60	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (A229P)</i>	This study
pKP61	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (S230A)</i>	This study
pKP62	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (S230D)</i>	This study
pKP65	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (F231S)</i>	This study
pKP68	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (S232R)</i>	This study
pKP69	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (A233G)</i>	This study
pKP70	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (A233T)</i>	This study
pKP71	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (Y239C)</i>	This study
pKP72	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (Y239N)</i>	This study
pKP73	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (WT)</i>	This study
pKP74	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-250 (WT)</i>	This study
pKP75	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-250 (R227L)</i>	This study
pKP76	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-250 (R228K)</i>	This study
pKP77	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-250 (A229P)</i>	This study
pKP78	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-250 (S230A)</i>	This study
pKP79	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-250 (F231S)</i>	This study
pKP80	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-250 (A233T)</i>	This study
pKP81	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-250 (Y239N)</i>	This study
pKP85	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (Q234A)</i>	This study
pKP94	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (S235A)</i>	This study
pKP100	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (A236G)</i>	This study
pKP104	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (S238A)</i>	This study
pKP106	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-460/Δ241-260 (Y239F)</i>	This study
pKP128	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240-(GS)₃-261-271 (Q234A)</i>	This study
pKP129	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240-(GS)₃-261-271 (S235A)</i>	This study
pKP130	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240-(GS)₃-261-271 (A236G)</i>	This study
pKP131	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240-(GS)₃-261-271 (S238A)</i>	This study
pKP132	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240-(GS)₃-261-271 (Y239F)</i>	This study
pKP133	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 226-240-(GS)₃-261-271 (Y239W)</i>	This study
pKP159	pKP127	pOBD2	<i>ADR1 226-240-(GS)₃-261-271 (S237A)</i>	This study
pKP162	pKP127	pOBD2	<i>ADR1 226-240-(GS)₃-261-271 (Y239A)</i>	This study
pKP86	<i>TRP1-2μ</i>	pBF339	<i>ADH1_p-HA-BMH1DDM</i>	This study

pKP92	<i>Amp^r</i>	pGEX3X	<i>tac_p-GST-BMH1DDM</i>	This study
pKP134	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 215-260/NTH1</i>	This study
pKP135	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 215-260/FIN1</i>	This study
pKP136	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 215-260/MIG1</i>	This study
pKP137	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 215-260/REG1</i>	This study
pKP138	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 215-260/SFL1</i>	This study
pKP139	<i>TRP1-CEN4</i>	pOBD2	<i>ADR1 215-260/MSN2</i>	This study

Supplementary Table S2: Primer			
Name	Sequence (5'-3')	Target	References
ADR1-215F	CCAAAAAAGAGATCGAATTCCAGGCTAATGTAAACG TAAGTACTTG	ADR1	This study
ADR1_221_F	CCAAAAAAGAGATCGAATTCCAGTACTTGAAAAAACT GACGCGCA	ADR1	This study
ADR1_226_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGGCTTC ATTTAGCG	ADR1	This study
ADR1_240_R	CGACGGATCCCCGGGAATTGCCATGTCAAGCATAGCTGG ATGCTGATTGTGC	ADR1	This study
ADR1_250_R	CGACGGATCCCCGGGAATTGCCATGTCAATGTTGTTCTA GCGAAGATTGGTC	ADR1	This study
ADR1-260R	CGACGGATCCCCGGGAATTGCCATGTCAAGGCGTAGAA AATTTAACAC	ADR1	This study
ADR1-310R	CGACGGATCCCCGGGAATTGCCATGTCAATAATCCAAAT TCATTGTTG	ADR1	This study
ADR1-420R	CGACGGATCCCCGGGAATTGCCATGTCAAGGTTGGTTGT CTGATACCT	ADR1	This study
ADR1_460_R	CGACGGATCCCCGGGAATTGCCATGTCAGGCGGTAGCA CTTACGCTATCAT	ADR1	This study
PP_ADR1- dBMHa_f	CTAGAACAACATCCAAAGGATGAATTAGTTCCACTTGAC TTG	ADR1	This study
PP_ADR1- dBMHa_r	CAAGTCAAGTGGAACTAATTCATCCTTTGGATGTTGTTT TAGCG	ADR1	This study
PP_ADR1- dBMHb_f	CCAATTTCAATATAGCATTAACTCAACAATGAATTTGG ATTATAAATTGC	ADR1	This study
PP_ADR1- dBMHb_r	CAATTTATAATCCAAATTCATTGTTGAGTTTAATGCTATA TTGAAATTGGAATC	ADR1	This study
ADR1_R227L_F	CCAAAAAAGAGATCGAATTCCAGACGTTGAGGGCTTC ATTTAGCG	ADR1	This study
ADR1_R228K_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAAGGCTTC ATTTAG	ADR1	This study
ADR1_R228A_F	CCAAAAAAGAGATCGAATTCCAGACGCGCGCTGCTTC ATTTAGCG	ADR1	This study
ADR1_A229P_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGCCTTC ATTTAGCG	ADR1	This study
ADR1_S230A_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGGCTGC ATTTAGC	ADR1	This study
ADR1_S230L_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGGCTTT ATTTAGCG	ADR1	This study

ADR1_S230D_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGGCTGATTTTAGCG	ADR1	This study
ADR1_S230E_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGGCTGATTTTAGCG	ADR1	This study
ADR1_F231A_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGGCTTAGCTAGCGC	ADR1	This study
ADR1_F231S_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGGCTTAGCTAGCGCA	ADR1	This study
ADR1_S232R_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGGCTTAGTTAGAGCACAA	ADR1	This study
ADR1_S232A_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGGCTTAGTTGCTGCACAA	ADR1	This study
ADR1_S232I_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGGCTTAGTTATCGCACAA	ADR1	This study
ADR1_S232P_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGGCTTAGTTCCAGCACAA	ADR1	This study
ADR1_A233P_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGGCTTAGTTAGCCACAATC	ADR1	This study
ADR1_A233G_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGGCTTAGTTAGCGGACAATC	ADR1	This study
ADR1_A233T_F	CCAAAAAAGAGATCGAATTCCAGACGCGCAGGGCTTAGTTAGCACACAATC	ADR1	This study
ADR1_Y239C_R	CGACGGATCCCCGGGAATTGCCATGTCAAGCACAGCTGGATGCTGATTGTGC	ADR1	This study
ADR1_Y239N_R	CGACGGATCCCCGGGAATTGCCATGTCAAGCATTGCTGGATGCTGATTGTGC	ADR1	This study
Q234A-F	CATTTAGCGCAGCATCAGCATCCAGCTAT	ADR1	This study
Q234A-R	ATAGCTGGATGCTGATGCTGCGCTAAATG	ADR1	This study
S235A-F	CATTTAGCGCACAAGCAGCATCCAGCTAT	ADR1	This study
S235A-R	ATAGCTGGATGCTGCTTGTGCGCTAAATG	ADR1	This study
A236G-F	TAGCGCACAATCAGGATCCAGCTATGCT	ADR1	This study
A236G-R	AGCATAGCTGGATCCTGATTGTGCGCTAA	ADR1	This study
S237A-F	GCACAATCAGCAGCCAGCTATGCTGGCT	ADR1	This study
S237A-R	AGCCAGCATAGCTGGCTGCTGATTGTG	ADR1	This study
S238A-F	ACAATCAGCATCCGCCTATGCTGGCTCT	ADR1	This study
S238A-R	AGAGCCAGCATAGGCGGATGCTGATTGT	ADR1	This study
Y239A-F	AATCAGCATCCAGCGCTGCTGGCTCTGGCTCT	ADR1	This study
Y239A-R	AGCCAGAGCCAGCAGCGCTGGATGCTGATT	ADR1	This study
Y239F-F	AATCAGCATCCAGCTTTGCTGGCTCTGGCTC	ADR1	This study
Y239F-R	AGCCAGAGCCAGCAAAGCTGGATGCTGATT	ADR1	This study
Y239W-F	AATCAGCATCCAGCTGGGCTGGCTCTGGCTCT	ADR1	This study
Y239W-R	AGAGCCAGAGCCAGCCAGCTGGATGCTGATT	ADR1	This study
ADR1_240_260_F_Fus	TATGCTGGCTCTGGCTCTGGCTCTCCTGAATTAGTTCCACTTGACTTG	ADR1	This study
ADR1_240_260_R_Fus	TTCAGGAGAGCCAGAGCCAGAGCCAGCATAGCTGGATGCTGATTGTGC	ADR1	This study
ADR1_R_240_Fus_261	TAATTCAGAGCCAGAGCCAGAGCCAGCATAGCTGGATGCTGATTG	ADR1	This study
ADR1_R_Y239C_Fus_261	TAATTCAGAGCCAGAGCCAGAGCCAGCACAGCTGGATGCTGATTGTGC	ADR1	This study
ADR1_R_Y239N_Fus_261	TAATTCAGAGCCAGAGCCAGAGCCAGCATTGCTGGATGCTGATTGTGC	ADR1	This study
ADR1_F_261_F	TGCTGGCTCTGGCTCTGGCTCTGAATTAGTTCCACTTGAC	ADR1	This study

us	TTGAAG		
BMH1_D7R_F	CCAAAAAAGAGATCGAATTCCAGATGTCAACCAGTCG TGAACGTTCTGTGTAC	BMH1	This study
BMH1_L14AE_ Q14NK_mpR	CATTTCTTCATAACGTTCCGGCCTGTTTATTCTGCTTGGCT AGGTACAC	BMH1	This study
BMH1_L14AE_ Q14NK_mpF	GTGTACCTAGCCAAGCAGAATAAACAGGCCGAACGTTA TGAAGAAATG	BMH1	This study
BMH1_Y87N_K 90N_E94Q_mpR	CGGAGATCTTAGTTAGTTGGGTCTCAATGTTCGAACGGT TCGAACAAATCAACTCG	BMH1	This study
BMH1_Y87N_K 90N_E94Q_mpF	CGAGTTGATTTGTTTCGAACCGTTTCGAACATTGAGACCCA ACTAACTAAGATCTCCG	BMH1	This study
BMH1_R	CGACGGATCCCCGGGAATTGCCATGTCACTTTGGTGCTT CACCTTCGGCGGCAG	BMH1	This study
BMH1_D7R_F_ BF339	CGGGATCCTGTCAACCAGTCGTGAACG	BMH1	This study
BMH1_R_BF33 9	CGGGATCCTTACTTTGGTGCTTCACCTTCG	BMH1	This study
REG1-F2	GTGACGTCAAGCCACAAGAAAATGGAAATGACAGCAGT CGGATCCCCGGGTTAATTA	REG1	This study
REG1-R1	ATTTTCTCTTCATGTTGACTTCAAAATTCTTTCTTCTAGA ATTCGAGCTCGTTTAAAC	REG1	This study
REG1-F	GTAAACGTAAGTACTTGAAAAAACTGACGAGAACAAG ATCAATGGGCCT	REG1	This study
REG1-R	GTTGTTCTAGCGAAGATTGGTTCGGGCAAAGCATATTCAT CAAGAAGGC	REG1	This study
NTH1-F	GTAAACGTAAGTACTTGAAAAAACTGACGAGACGTGG TTCTGAGGATGA	NTH1	This study
NTH1-R	GTTGTTCTAGCGAAGATTGGTTCGGGCAAAGCATAGGTGT CATCCTCAG	NTH1	This study
SFL1-F	GTAAACGTAAGTACTTGAAAAAACTGACGCGAAAGAA TTCTAGTAATC	SFL1	This study
SFL1-R	GTTGTTCTAGCGAAGATTGGTTCGGGCAAAGCGTAGTTTT GATTACTAGAAT	SFL1	This study
MIG1-F	GTAAACGTAAGTACTTGAAAAAACTGACGAGATCCACT AGTGGTACGAA	MIG1	This study
MIG1-R	GTTGTTCTAGCGAAGATTGGTTCGGGCAAAGCATACCCCA AAGTGTGCAA	MIG1	This study
MSN2-F	GTAAACGTAAGTACTTGAAAAAACTGACGAGGCAAAG AGCTTCTTACC	MSN2	This study
MSN2-R	GTTGTTCTAGCGAAGATTGGTTCGGGCAAAGCGTAGCTTA GTGAATCAT	MSN2	This study
FIN1-F	GTAAACGTAAGTACTTGAAAAAACTGACGAGAAGGAT TATGTCGCCGGA	FIN1	This study
FIN1-R	GTTGTTCTAGCGAAGATTGGTTCGGGCAAAGCATAGCCCT TCAGACATTCC	FIN1	This study
GEX_sF	GGCAAGCCACGTTTGGTG	pGEX-3X	(5)
OBDsF	CTATCTATTCGATGATGA	pOBD-2	(5)
OBDsR	CTTGCGGGGTTTTTCAGT	pOBD-2	(5)

Supplementary Table S3: Are high throughput Bmh-targets enriched for the consensus sequence pattern?

	Binding	no Binding	SUMS
with consensus sequence	204 ^d	3104	3308 ^a
without consensus sequence	66	2512	2578
SUMS	270 ^b	5616	5886 ^c

^aNumber of unique sequence entries hit for the sequence pattern, RX₂₋₃[pS/T]X₄₋₁₀Y.

^bNumber of high throughput targets found by Kakiuchi et al., 2007 (6).

^cNumber of protein sequences searched in SGD.

^dNumber of targets among 270 were enriched with the sequence motif.

$$p - value = \frac{3308! 2578! 270! 5616!}{204! 3104! 66! 2512! 5886!}$$

Fisher's Exact Test for Count Data

data: data

p-value = 1.667e-11

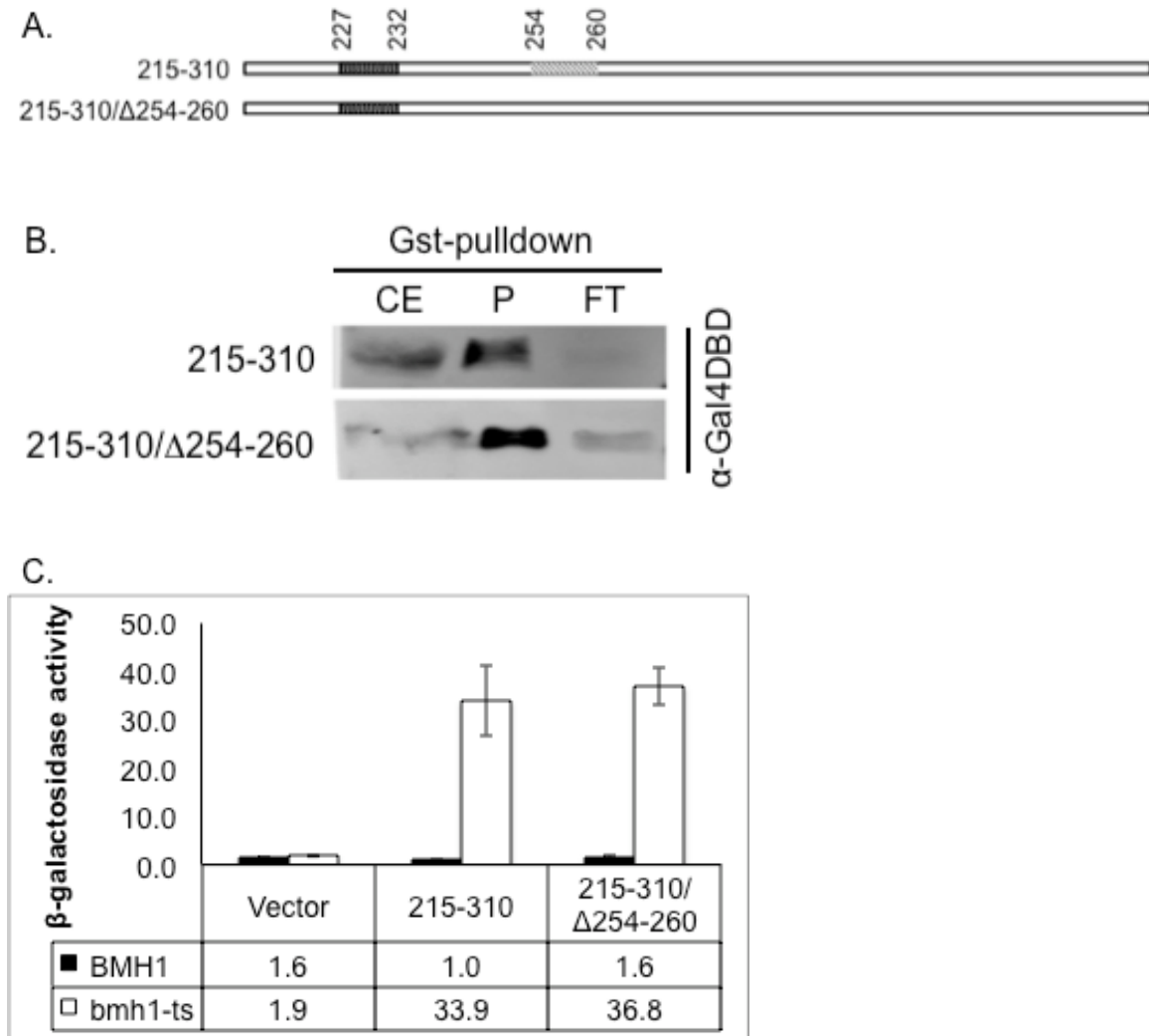
alternative hypothesis: true odds ratio is not equal to 1

95 percent confidence interval: 1.875953 3.370684

sample estimates: odds ratio 2.500961

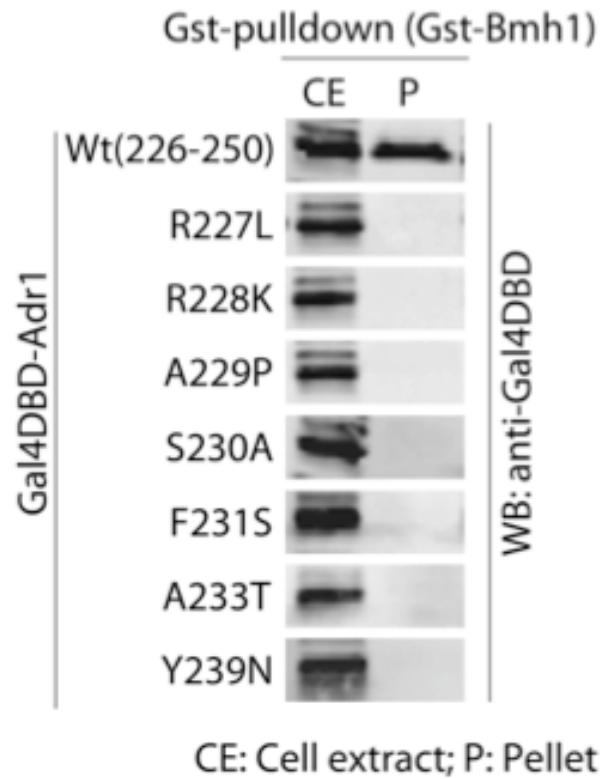
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Supplementary Fig 1



Supplementary Fig 1: The minimal Bmh-binding region (226-240 amino acids) of Adr1 is sufficient and important to show Bmh-dependent activity regulation of the latter. [A] Diagram shows the Gal4DBD-fused Adr1 constructs expressed in yeast and used for binding and activity assays. [B] Gst-pulldown profile of the Gal4DBD-Adr1 variants using *E. coli* expressed Gst-Bmh1 and yeast extract (CE) containing Gal4DBD-Adr1 variant. “FT”- Flow through and ‘P’- Pellet. [C] β -galactosidase activity assays from the reporter cassette (*GAL10p-CYC1-lacZ*) in *BMH1* WT and *bmh1-ts* mutant strains harboring either empty vector or plasmid having above-mentioned Gal4DBD-Adr1 fusion construct. Error bars represent the standard deviation in three separate experiments using three different biological replicates.

Supplementary Fig 2



Supplementary Fig 2: Gst-pulldown results of Gal4DBD-Adr1 fusion variants encompassing 226-250 residues of Adr1. Pulldown was done using yeast cell extract (CE) containing Gal4BDD-Adr1 fusion variant having indicated mutation and *E. coli* expressed Gst-Bmh1. After extensive washing beads were used as pellet (P) fraction. Proteins were visualized by western blotting using anti-Gal4DBD antibody.

Supplementary Fig 3

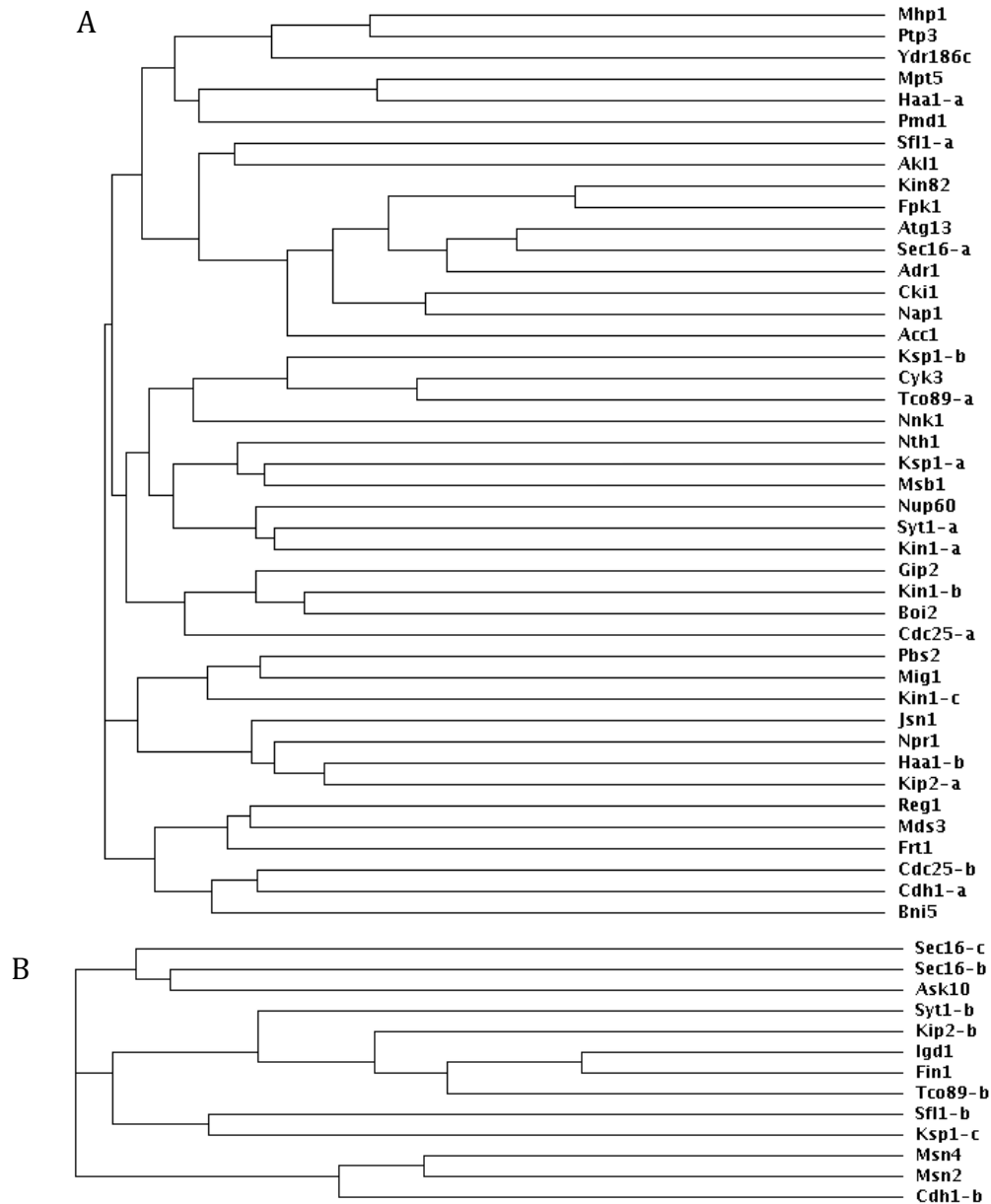


Figure3: Probing of Bmh-binding sequence of Adr1 to understand the yeast 14-3-3 motif(s). Sequence alignment followed by generation of phylogenetic tree using ClustalW2 of the matched sequence patterns indicated in the Table 2. Fig A shows the tree for peptides having $(R_x2[pS/T]_{x4-10}Y)$ sequence pattern and Fig B containing sequences with $(R_x3[pS/T]_{x4-10}Y)$ pattern (x is any amino acids, p indicates phosphorylated residue and subscript values indicate the number of residues). Dark circle marked peptide sequences were picked up for binding assays shown in Fig 5B.