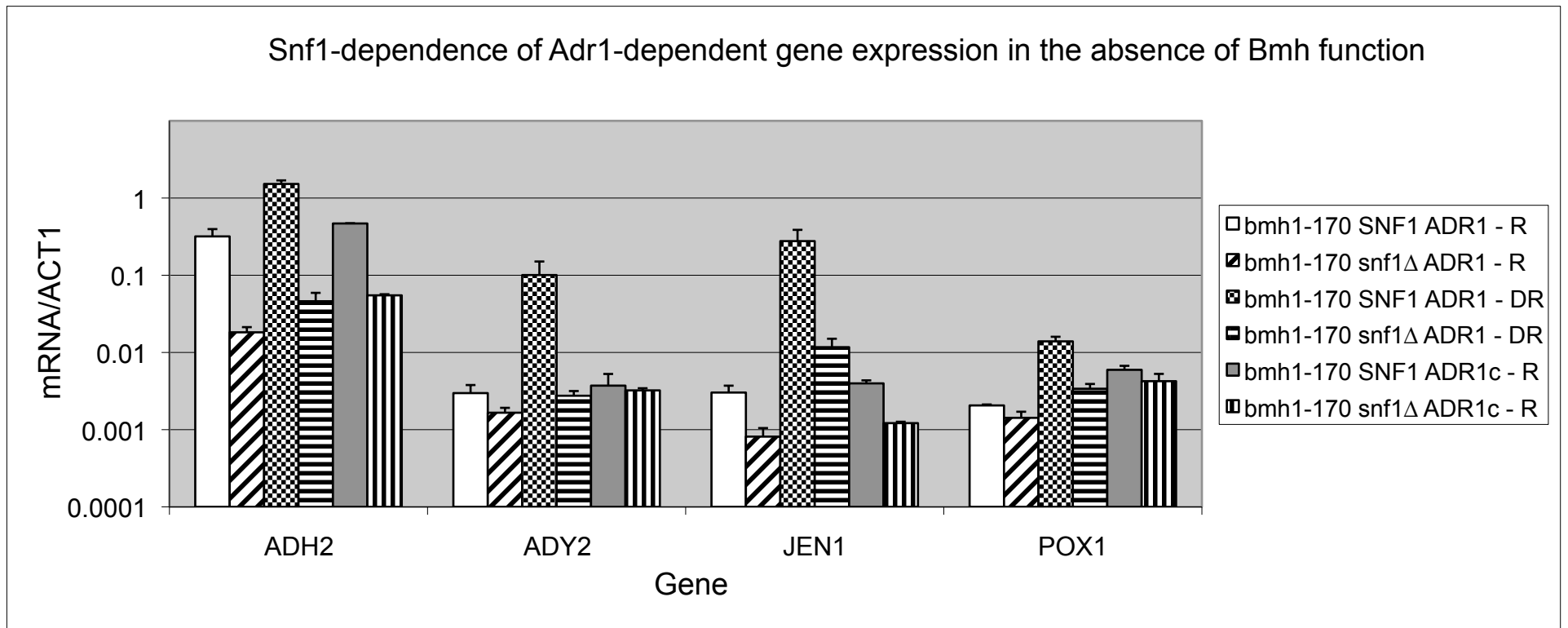


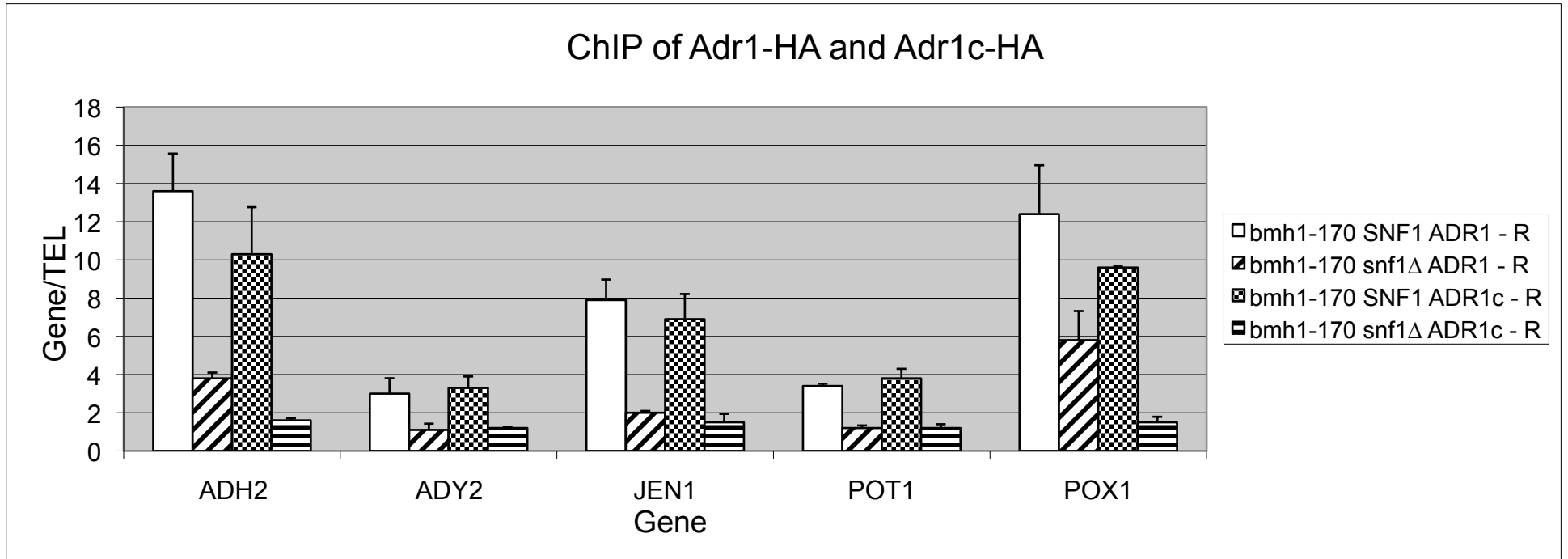
Supp
Figure 1A



Supplementary Figure S1A. Snf1-dependence of Adr1-dependent gene expression and Adr1 binding.

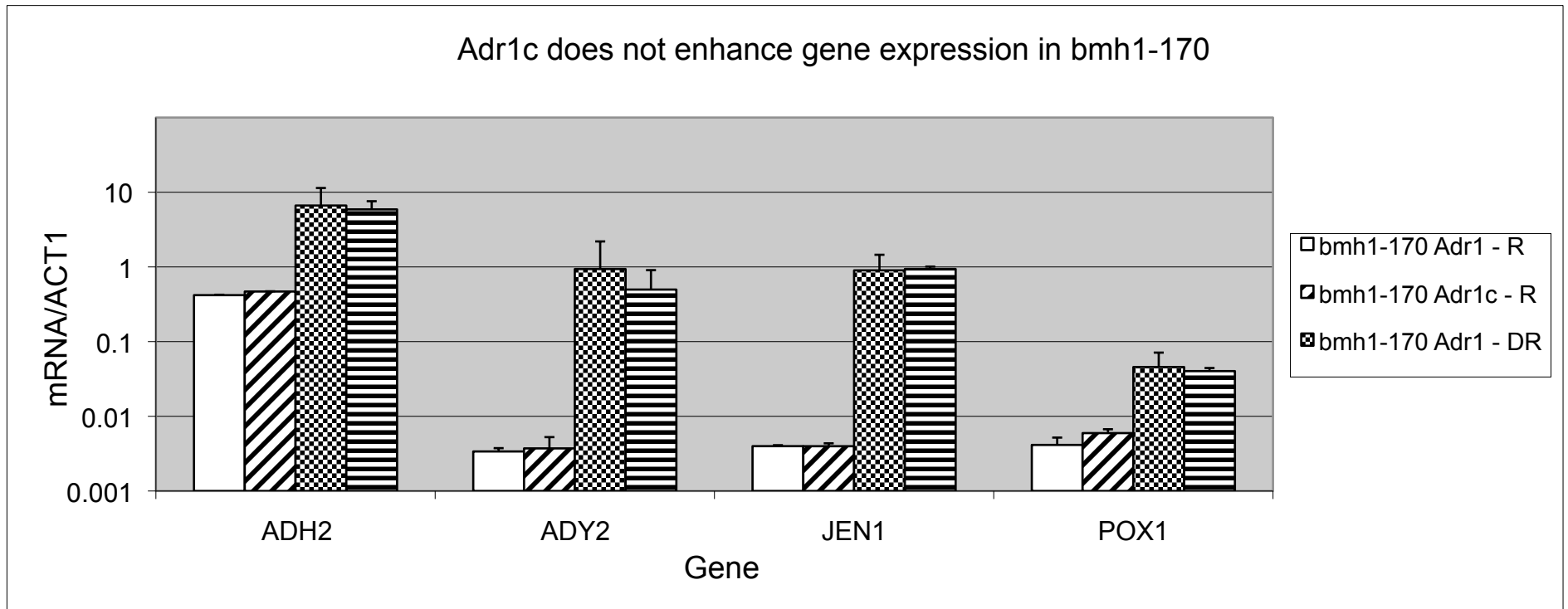
A. Strains YLL1087 *bmh2Δ bmh1-170 SNF1* and SRY69 (*snf1Δ::natmx bmh2Δ bmh1-170*) were grown at 30°C in complete medium with 3% glucose to an $A_{600} \sim 1$. A portion of the culture was collected and the remainder of the culture was centrifuged and the pelleted cells were resuspended in derepressing (DR) medium containing 0.05% glucose and shaken at 30°C for 6 hours (DR). After 6 hours cells were collected, RNA was isolated, cDNA was prepared and quantitative RT-PCR (qRT-PCR) was performed and analyzed as described in Materials and Methods.

Supp
Figure 1B

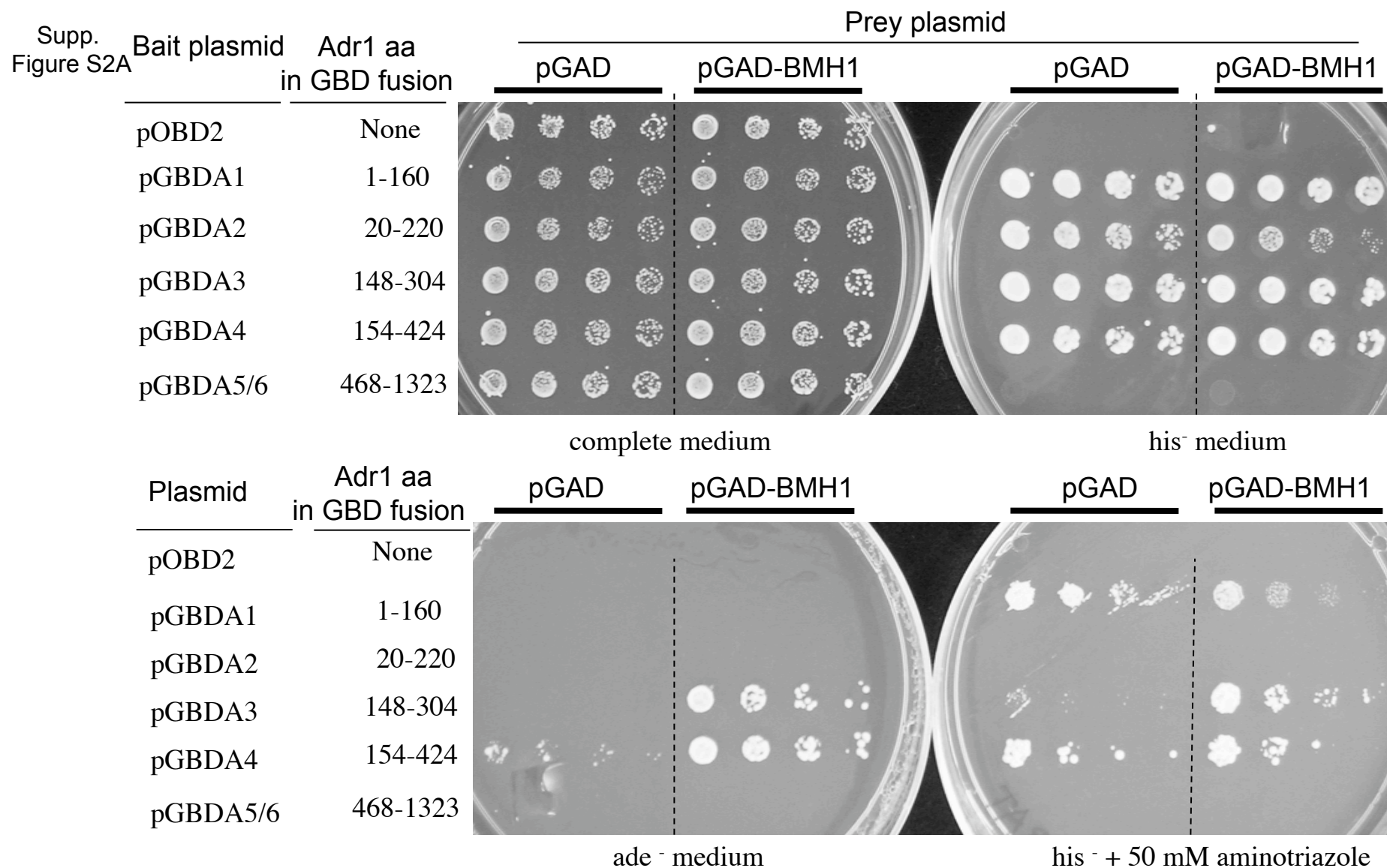


Supp. Fig. S1B. Chromatin immunoprecipitation (ChIP) of Adr1-HA and Adr1 (S230A)-HA in *SNF1* and *snf1Δ* strains. Strains EAY25 (*adr1Δ bmh1-170 SNF1*) and EAY29 (*adr1Δ bmh1-170 snf1Δ*) carrying pKD16-HA (*ADR1*) and pKD14-HA (*ADR1-S230A*) were grown in repressing medium selective for the *TRP1* plasmids. ChIP was performed as described in Materials and Methods.

Supp Figure 1C

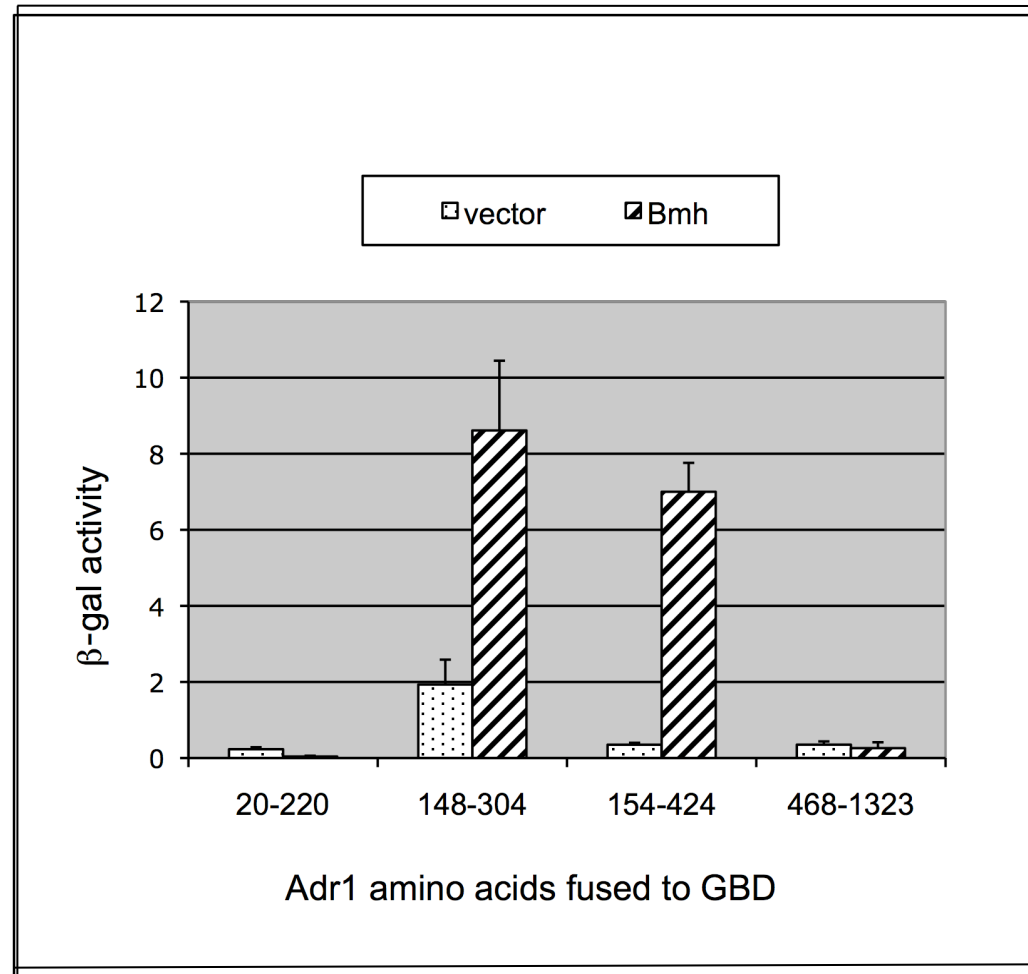


Supp. Fig. S1C. *ADH2* expression is not enhanced by *Adr1^c* in a strain lacking *Bmh* activity. Strain YLL1087 (*bmh2Δ bmh1-170*) transformed with either pKD16 (WT *ADR1*) or pKD14 (*ADR1^c*) (Supp. Table 2) was grown in synthetic medium containing 3% glucose and 0.2% casamino acids to an OD₆₀₀~1. Cells were collected, RNA was isolated, cDNA was prepared and quantitative RT-PCR (qRT-PCR) was performed as described in Materials and Methods.



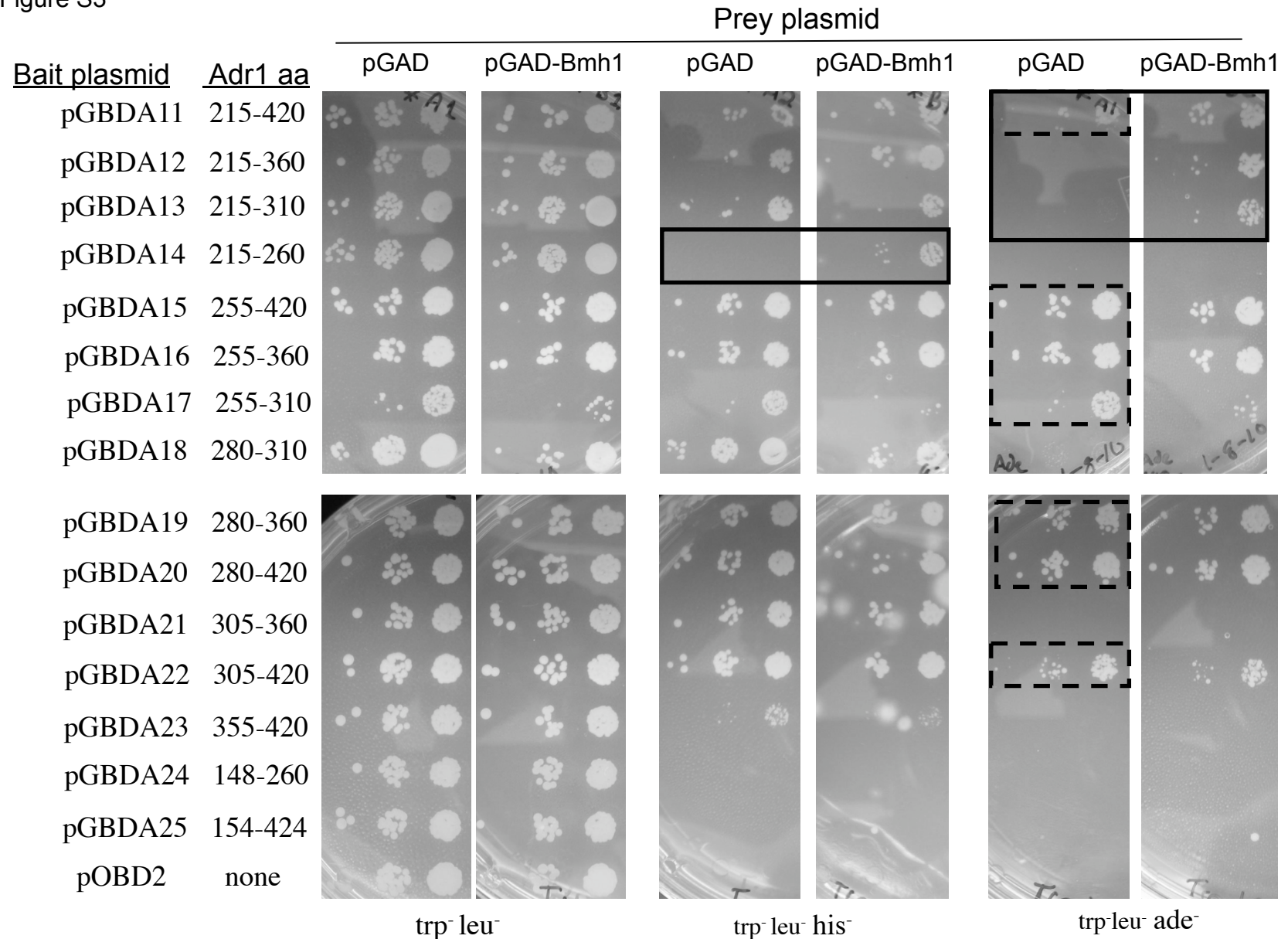
Supp. Figure S2A. Two-hybrid interaction of GBD-Adr1 with GAD-Bmh1. Yeast two-hybrid assays were performed using strain TYY304 (*GAL1-HIS3 GAL2-ADE2 GAL7-lacZ*) with one of the pGBDA plasmids (TRP⁺) and either pGAD (LEU⁺) or pGAD-BMH1 (*pKD134*, LEU⁺). Yeast were spotted onto the following selective plates to assess interactions between GBD-Adr1 and GAD-Bmh1: trp⁻ leu⁻ (complete), trp⁻ leu⁻ his⁻ (his⁻), trp⁻ leu⁻ ade⁻ (ade⁻), and trp⁻ leu⁻ his⁻ containing 50 mM 3-aminotriazole (his⁻ + 50 mM aminotriazole).

Supp. Figure S2B





Supp. Figure S2B. Two-hybrid interactions assayed by expression of GAL7-lacZ. The strains described in Supp Figure 1A were assayed for β -galactosidase activity and the results are plotted in Miller units ± 1 SD.

Supp. Figure S3



Supp. Figure S3. Two-hybrid assays using strain TYY304 expressing GBD-Adr1 fusion proteins (pGBDA) and either GAD-Bmh1 (pKD134) or GAD (pGAD-C1). Strains that activate transcription in the absence of GBD-Bmh1 are enclosed in boxes with dashed lines and strains with a two-hybrid interaction with GAD-Bmh1 are enclosed in boxes with solid lines.

Supp. Figure S4.

Protein	Yeast Species	Location	Conserved Region 1	Conserved Region 2
Rsf2	<i>S. cerevisiae</i>	332-395	TPSSMHKTKRHASFSASSAMTYMSSS-	NFELVEDAPHQVGFSTPQMTAKQLMESVSE
YML081W	<i>S. cerevisiae</i>	221-282	---IPTKSKRHASFSASSAFTYSSDN-	ELQ--ESIPHQVGFSTPQLTAQQLIENAIE
643.20	<i>S. bayanus</i>	222-285	---IPTKYKRHASFSASSAFTYSSDN-	ELQ--ESVPHQVGFSTPQLTAQQLIENAIE
703.23	<i>S. castellii</i>	249-318	SVTAATISKRHASFSAASTYVQDNN	EFQFPADIPHQVGFSTPQLSAQQLIEKVTE
B14894	<i>Z. rouxii</i>	223-283	--APPKRRKRHASFSASSAFSYVNS-	EME-PQEGPHQVGFSTPQLTAQQLMEKAVE
H04213	<i>C. glabrata</i>	219-288	LSVPASRNKRHASFSASSAFTYFPDNI	IEDLGEGIPHLVGFSTPQLSAQELIRKVMQ
16621	<i>K. waltii</i>	232-292	AEERHHKRRHASFSASSSITYTQAK-	EIP---DIPHQVGFSTPQLSAQELVEKALE
D18062	<i>K. thermotolerans</i>	189-245	LEERQQKRRHASFSASSSITYTQAK-	DIP---DIPHQVGFSTPQLSAQELVEKALE
G18062	<i>S. kluyveri</i>	220-279	-RDKPRKRKRHASFSASSALTYTQAK-	EMA---DIPHQVGFSTPQLTAQGLMEKAMM
B04477	<i>K. lactis</i>	239-295	ASNQPKKRRHASFSASTNVSYTQKK-	ELN---DIPHQVGFSTPQLTAQELFDKALE
AER159C	<i>A. gossypii</i>	216-275	ESKAPK-RKRHASFSAAHTLSYVQEK-	EMS---DIPHQVSFSTPQMTAQQLFERATK
1001.1	<i>K. polysporus</i>	168-257	LKPPVQKRRHASFSASNAFTYRPNNV	NQDLSQDVPHQVGFSTPQLSANRLLEKIFN
719.68	<i>S. castellii</i>	224-288	FASSTRGRQRHASFSAGSYTYSSTN-	EPSPSTEAPHQVGFSTPQLTTKQLMDKAME
			:*****: :*	** *.*****::: :*
				

Supp. Figure S4. Conserved motifs in the regulatory domain of Rsf2. Rsf2 orthologs were recovered from the Gene Order Browser (31) and aligned using ClustalW 2.0 WWW service at the European Bioinformatics Institute (<http://www.ebi.ac.uk/Tools/clustalw2/>; 37, 45). Amino acid consensus logos were created using WebLogo 3.0 (<http://weblogo.threeplusone.com/create.cgi>) (16).