Supplementary Figures, Figure legends, and Supplementary Table 1 for

The AMP-activated protein kinase Snf1 regulates transcription factor binding, RNA Polymerase II activity and mRNA stability of glucose repressed genes in *Saccharomyces cerevisiae*

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*Running title:
Snf1 promotes Adr1 binding, RNA Pol II transcription, and mRNA stability

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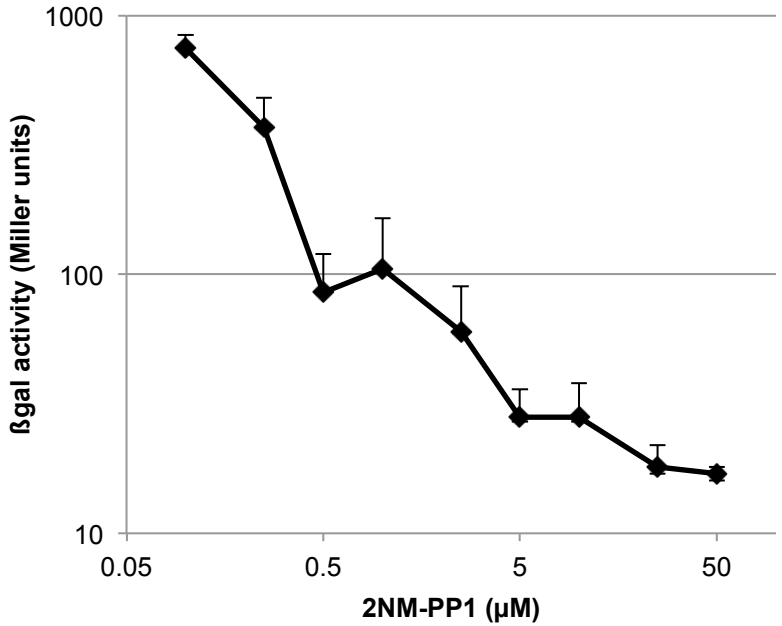
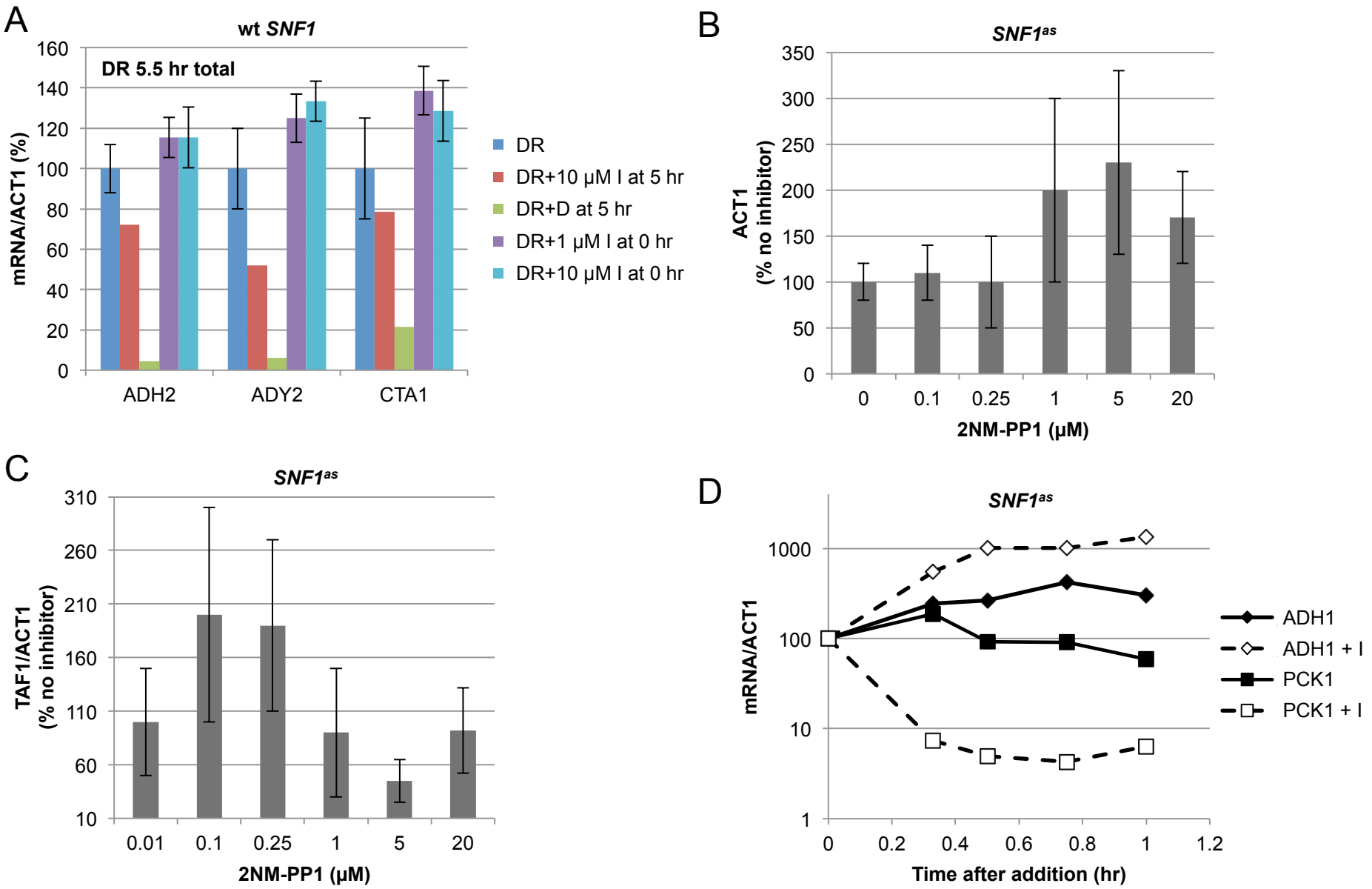
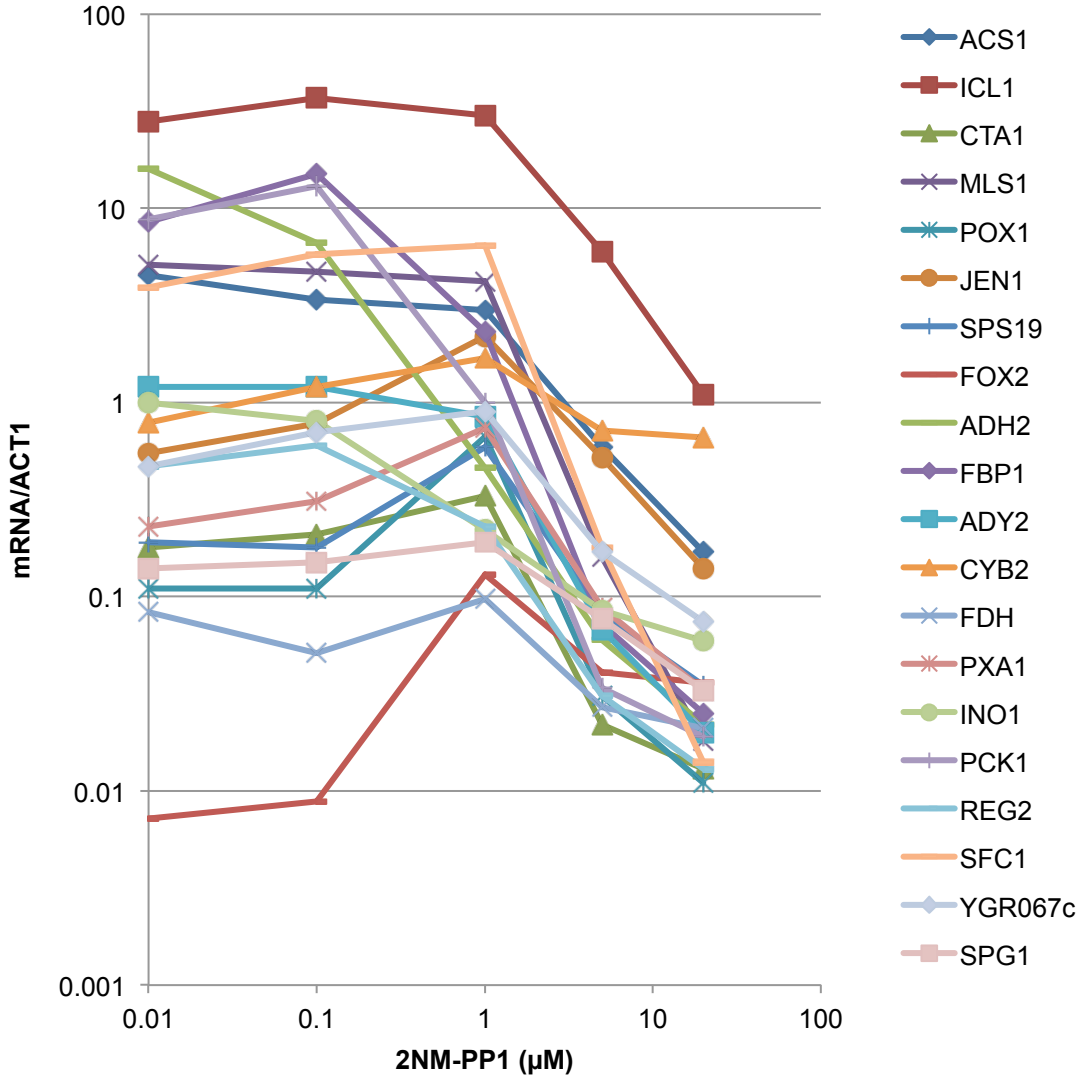
Supplementary Figure 1. Inhibition of ADH2-lacZ expression by 2NM-PP1.



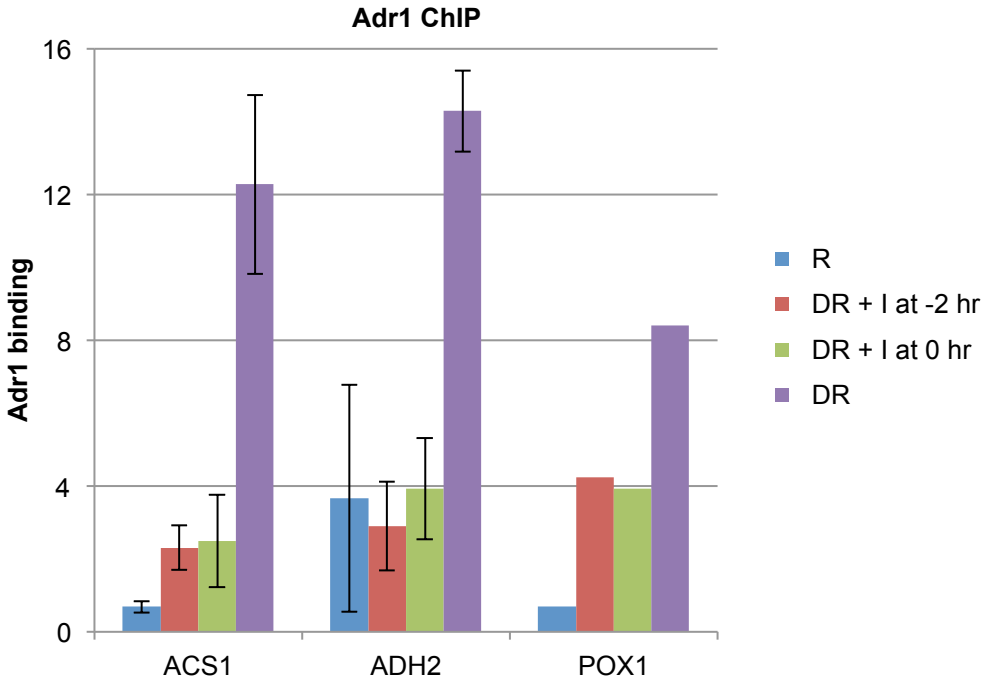
Supplementary Figure 2. 2NM-PP1 does not inhibit Snf1-independent gene expression and is not inhibitory in a wild-type *SNF1* strain.



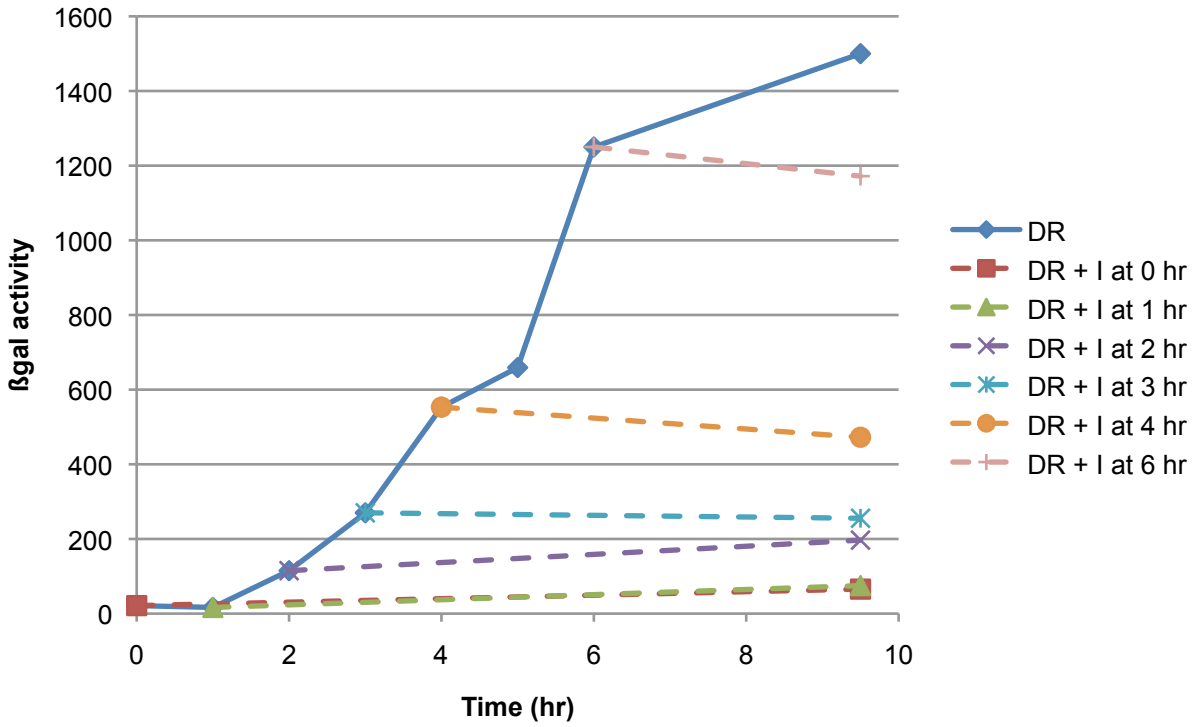
Supplementary Figure 3. Gene expression at 4 hr in the presence of 2NM added before derepression.



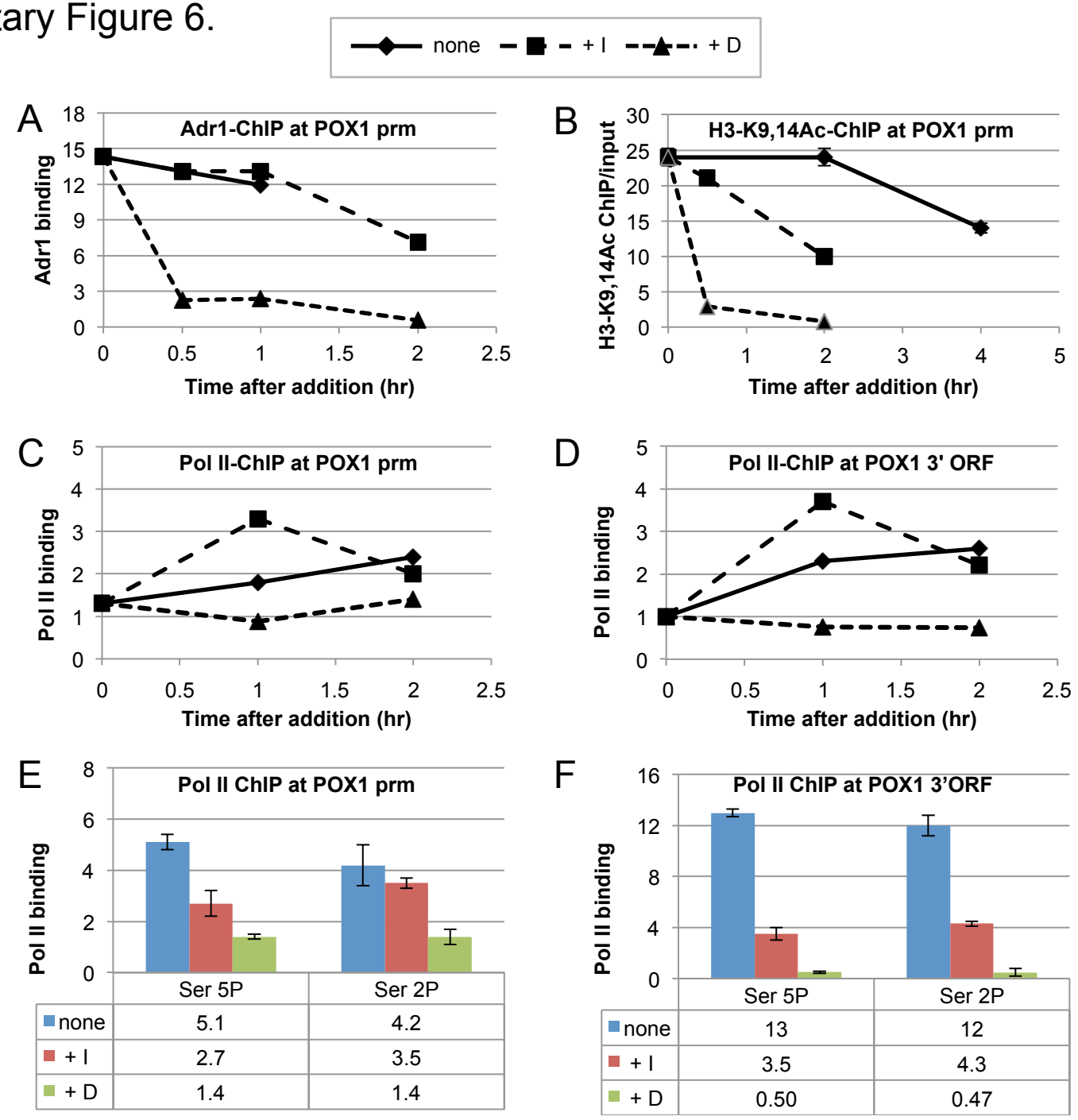
Supplementary Figure 4. Adr1 binding after inhibiting Snf1^{as} before and at time of glucose depletion.



Supplementary Figure 5. *ADH2*-lacZ expression in TYY923 (*SNF1*^{as}).



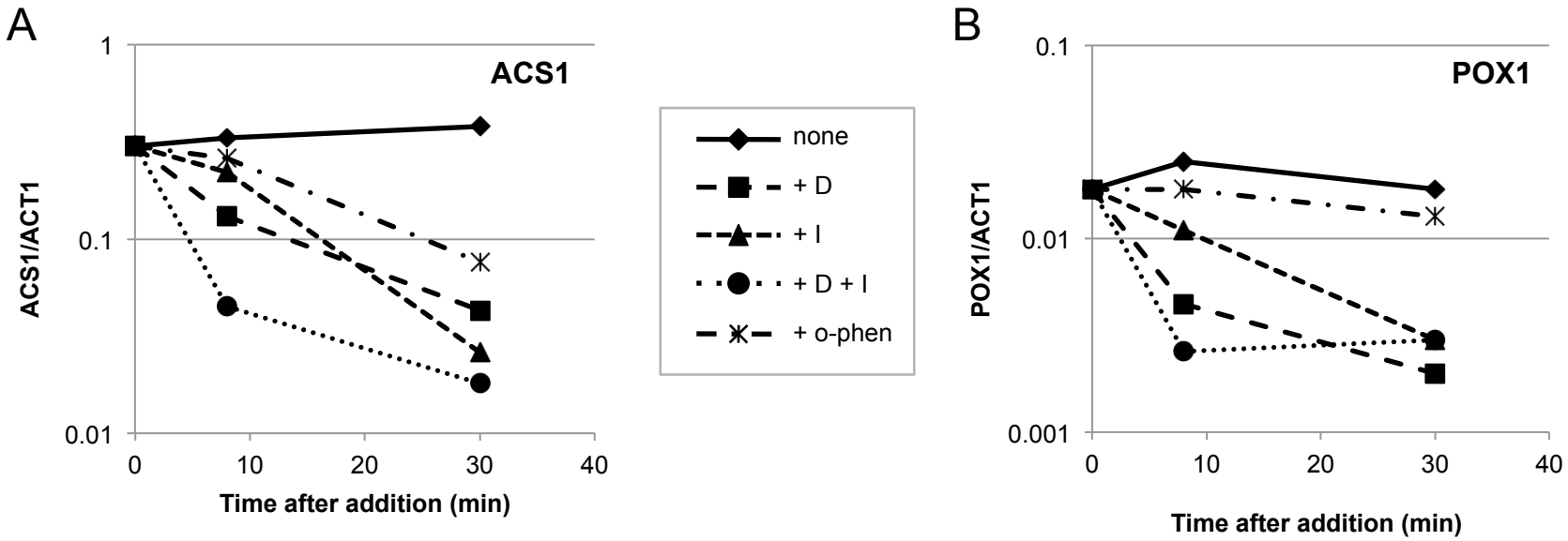
Supplementary Figure 6.



	Ser 5P	Ser 2P
none	5.1	4.2
+ I	2.7	3.5
+ D	1.4	1.4

	Ser 5P	Ser 2P
none	13	12
+ I	3.5	4.3
+ D	0.50	0.47

Supplementary Figure 7. mRNA turnover in a reg1Δ strain.



Supplementary Figure legends

Supplementary Figure 1. Inhibition of *ADH2*-lacZ expression by 2NM-PP1. Strain TYY923 carrying plasmid pBMG23 (*CEN3-TRP1 ADH2-lacZ*) was grown at 30°C in SM containing 3% glucose, 0.2% casamino acids, uracil, and adenine. At an A600~1 the cells were pelleted at 3000 RPM in a Sorvall RC3B and resuspended in derepressing medium with the same additions except glucose was present at 0.05% final concentration. 2NM-PP1 (or DMSO) was added at the time of derepression and the cultures were incubated with vigorous agitation at 30°C. Four hr after glucose was depleted triplicate samples were assayed for β-galactosidase activity (expressed in Miller units).

Supplementary Figure 2. 2NM-PP1 specifically inhibits Snf1^{as}. A. Strain CKY19 (*SNF1*) was grown in repressing conditions and the cells were pelleted and resuspended in derepressing medium. The culture was then divided into three portions. 2NM-PP1 was added to final concentrations of 1 and 10 μM to cultures A (1 μM I at 0 hr) and B (10 μM I at 0 hr), respectively. Nothing was added to culture C. After five hours of derepressed growth culture C was divided into three aliquots. 2NM-PP1 (10 μM final concentration; DR+I at 5 hr)) was added to one, glucose (3% final concentration) was added to another (DR+D), and DMSO (0.05% final concentration) was added to the third (DR). After thirty min further growth duplicate 10 ml samples were removed from all five cultures, RNA was prepared and RT-qPCR was performed to measure RNA levels for the genes indicated. mRNA levels are expressed relative to *ACT1* mRNA and are displayed as the percentage of the mRNA levels in the control, DMSO-treated culture. **B., C.** Strain TYY1077 (*SNF1^{as}*) was grown in high glucose and shifted to derepressing conditions in the presence of the indicated amount of 2NM-PP1. RNA was isolated after 4 hours of derepression and assayed for *ACT1* (B) or TAF1 (C) mRNA. **D.** *ADH1* and *PCK1* mRNA expression after inhibiting Snf1^{as} after 4 hours of growth in derepressing conditions. Strain TYY1077 (*SNF1^{as}*) was grown in high glucose and shifted to derepressing conditions for 4 hours. 2NM-PP1 or DMSO (control) was added to final concentrations of 10 μM and 0.05%, respectively. Samples were collected for RNA analysis at the times indicated.

Supplementary Figure 3. Dose-response curve of glucose-repressible, *SNF1*-dependent mRNAs in the presence of different concentrations of the Snf1^{as} inhibitor 2M-PP1. Cultures of three isogenic *SNF1^{as}* strains, TYY923, TYY924, and TYY925 were grown in YPD (3% glucose) and derepressed by pelleting the cells and resuspending them in YP with 0.05% glucose in the presence of the indicated concentrations of 2NM-PP1. RNA was isolated and specific mRNAs were quantified by RT-qPCR. The glucose-repressible mRNA levels are expressed relative to *ACT1* mRNA which did not change when 2NM-PP1 was present (**Supp. Figure 2**). The values are the average of three biological replicates and the standard deviation was about 20%. The experiment was performed twice with similar results. The results of a single experiment are shown. The lowest inhibitor concentration was 0.1μM. The "no inhibitor" value on the X-axis is represented as 0.01μM to display that axis on a log scale.

Supplementary Figure 4. Adr1-myc binding after inhibiting Snf1^{as} before and at the time of glucose depletion. Triplicate cultures of Snf1^{as} strain TYY1077 were grown in YPD containing 3% glucose. At an A_{600}=0.5 the culture was divided into four portions. One portion received 2NM-PP1 (Inhibitor (I)) at a final concentration of 10 μM (DR I at -2 hr). Nothing was added to the other three cultures. After two hours of continued incubation in repressing conditions the cells of all four cultures were collected by centrifugation. The cells from one untreated culture

(repressed, R) were immediately processed for RNA analysis. The other three portions were resuspended in 50 ml of YP with 0.05% glucose. 2NM-PP1 was added to a final concentration of 10 μM to two of these (DR +I at -2 hr and DR +I at 0 hr) and DMSO was added to the control culture (DR). After 4 hours of vigorous agitation the cells were collected from the DR cultures and RNA was prepared for RT-qPCR analysis of mRNA levels for *ACS1*, *ADH2*, and *POX1*.

Supplementary Figure 5. Inhibition of *ADH2*-lacZ expression by 2NM-PP1 added during derepression. Duplicate samples were withdrawn and β-galactosidase activity was measured at the times indicated. At the same times a portion of the culture was added to a flask containing 2M-PP1 (10 μM final concentration) and the flask was agitated until 9.5 hours post-glucose depletion when β-galactosidase activity was determined in duplicate aliquots of each inhibited culture.

Supplementary Figure 6. Continuous Snf1 activity is not needed during derepression for Adr1 and RNA Pol II binding, histone H3-K9,14 acetylation or RNA Pol II CTD phosphorylation

(A) Adr1-myc binding after inhibiting Snf1^{as} and adding glucose. Adr1-Myc binding in strain TYY1077 was measured by ChIP after derepression as described in the legend to Experimental ProcedureThe cultures were derepressed for 2 hours in low glucose medium. The final concentration of 2NM-PP1 was 10 μM. Duplicate 60 samples were removed at the times indicated. A 10 ml portion of this sample was used for RNA preparation and analysis. The remaining 50 ml was used for ChIP analysis for Adr1-Myc13 as described in Experimental Procedures. The data are expressed as binding (ChIP/input) for *POX1prm* relative to ChIP/input at the TEL region used as a reference. **(B)** Histone H3-K9,14 acetylation after inhibiting Snf1^{as} or adding glucose. Strain TYY1077 was grown and treated as described above. ChIP for K9,14 acetylated histone H3 was performed using anti-histone H3-K9,14 antisera from Santa Cruz as described in Experimental Procedures. The data are expressed as the ratio of ChIP/input without normalizing to the level of acetylation at the *TEL* region which changed less than 2-fold with any of the treatments. **(C, D)** RNA Pol II remains bound to the *POX1* transcription start site (tss) and ORF after inhibiting Snf1^{as}. Growth and treatment of the cells is described above. RNA Pol II binding was measured by ChIP as described in Experimental Procedures using ChIP-grade Abcam anti-Pol II antibody 8WG16. The data are expressed as binding (ChIP/input) for *POX1tss* relative to ChIP/input at the *TEL* region used as a reference. **(E,F)** RNA Pol II CTD associated with *POX1* is phosphorylated on Ser2 and Ser5 after Snf1^{as} inhibition. The cultures for ChIP analysis were derepressed for 6 hr (none) and 2NM-PP1 (+I) or glucose (+D), was added after 2 hours of derepression. Abcam anti-pSer5 and anti-pSer2 polyclonal antisera were used as described in Experimental Procedures. The data are expressed as binding (ChIP/input) for *POX1tss* and ORF relative to ChIP/input at the *TEL* region used as a reference.

Supplementary Figure 7. Active Snf1^{as} protects glucose repressible mRNAs from glucose-induced rapid decay. Strain TYY1085 (*reg1*Δ *SNF1^{as}*) was grown in YPD medium and derepressed in YP containing 0.05% glucose as described in Experimental Procedures. Additions were made 4 hours after initial glucose depletion and samples were removed for RNA preparation 7.5, 30 and 60 min later. The additions were 2NM-PP1 (10 μM final concentration), glucose (3% final concentration), 2NM-PP1 plus glucose (10 μM and 3%, final concentrations, respectively), 1,10-o-phenanthroline (100 μg/ml final concentration) or DMSO (0.05% final concentration). Messenger RNAs were quantified by RT-qPCR as described in Experimental Procedures. Triplicate zero-min samples were prepared and their average was used as the initial time-point. The data are expressed relative to *ACT1* mRNA levels.

Supplementary Table 1. mRNA half-lives (min) for Snf1-dependent, glucose-repressible genes.

Gene	Growth condition*			
	Glucose	2NM-PP1	*rpb1-1*	1,10-o-phenanthroline
Adr1-dependent genes				
ACS1	1.5	4	10	10
ADH2	2.5	5	16	16
ADY2	3	5	10	10
ALD4	3	5	12	12
ARO9	8	9	8	13
CIT2	3	4	8	10
CTA1	4	7	9	16
CYB2	2	5	8	14
FAA2	2	7	9	16
FOX2	4	8	10	25
FDH1/2	5	6	60	20
GUT1	4	6	12	9
GUT2	2	10	14	21
ICL2	3	8	9	13
PIP2	4	20	7	24
POT1	3	5	16	15
POX1	3	6	40	10
PUT4	3	5	7	9
PXA1	2	5	10	45
SPG1	2	5	45	80
SPS19	5	10	14	40
YIL057C	3	3	16	20
Cat8-dependent genes				
FBP1	3	3	12	5
ICL1	2	4	4	20
JEN1	2	4	4	6
MDH2	2	3	8	6
MLS1	2	4	7	7
PCK1	3	4	22	7
REG2	2	4	10	9
SFC1	2	5	7	7
YGR067C	2	5	7	7
Average:	3.0	5.9	14	17
SD:	1.3	3.2	12	14
Non- Snf1-dependent genes				
ADH1	increase	increase	>60	>60
TAF1	no change	>100	30	>100
ACT1	no change	no change	16	16

Growth conditions: 3% glucose, 10μM 2NM-PP1, 36.5^{o}C or 100 μg/ml 1,10-o-phenanthroline added after 4 hours of DR. Cells harvested at 5, 10, 20, 40, and 60 min.