Discovery of enzymatic targets of transcriptional activators via in vivo covalent chemical capture

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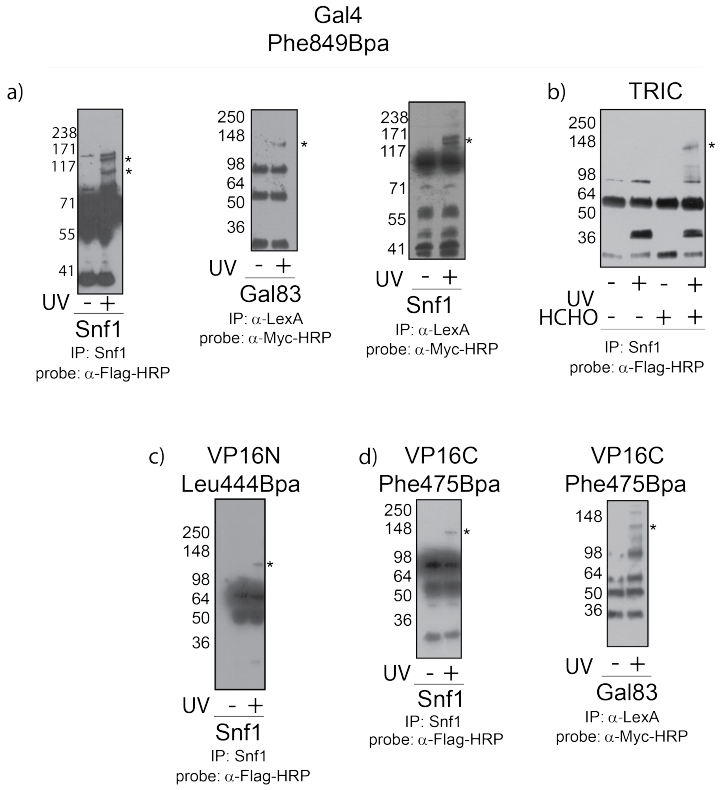
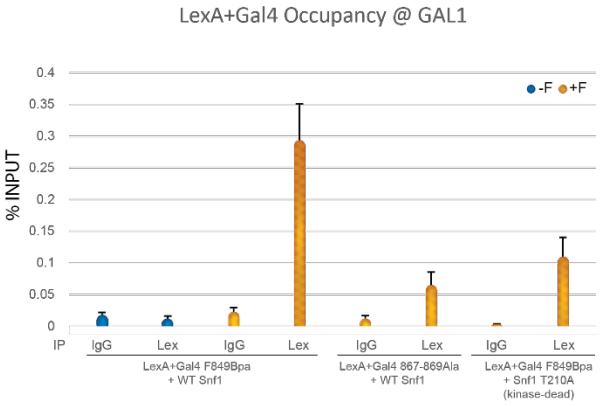
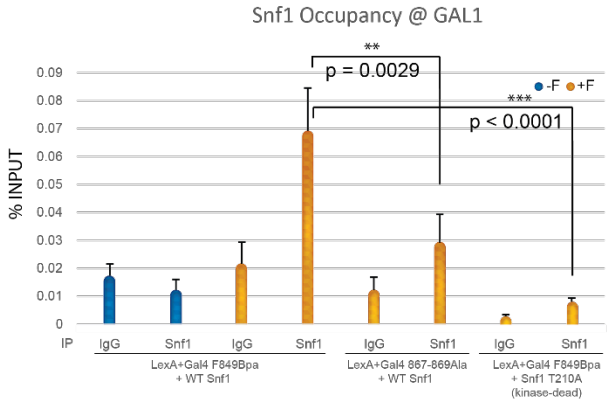
Supplementary Figures and Text

Supplementary Figure 1. Gal4 and VP16 crosslink to Snf1 and Gal83 in vivo. Full Western blots are displayed for Figure 4 in the main text. In vivo covalent chemical capture was carried out in yeast expressing either Gal4 Phe849Bpa, VP16N L444Bpa or VP16C F475Bpa and, in some cases, a myc-tagged version of the Snf1 and Gal83 subunits. Crosslinked species are marked with an asterisk.The resulting yeast lysates were immunoprecipitated with either a Snf1 or LexA antibody as indicated and the immunoprecipitated complexes were analyzed by Western blot with either a Flag-HRP or myc-HRP antibody to detect covalently bound complexes. (a) Gal4 directly contacts the catalytic subunit Snf1 and the exchangeable subunit Gal83 in live yeast. Free Snf1-6xmyc migrates at ~100 kDa with a Gal4-Snf1 complex migrating at ~130 kDa. Free Gal83-6xMyc appears to migrate at about 85 kDa with a crosslinked Gal4-Gal83 complex observed at around 120 kDa. The molecular weight of LexA+Gal4-flag-6HIS is approximately 30 kDa. (b) Tandem reversible and irreversible crosslinking (TRIC) was performed to investigate the interaction of DNA bound Gal4 with Snf1 at the Gal1 promoter. (c,d) The amino- and carboxy-terminal subdomains of the amphipathic activator VP16 were tested for crosslinking to the catalytic subunit of the Snf1 kinase complex as well as a myc-Gal83 construct in yeast.

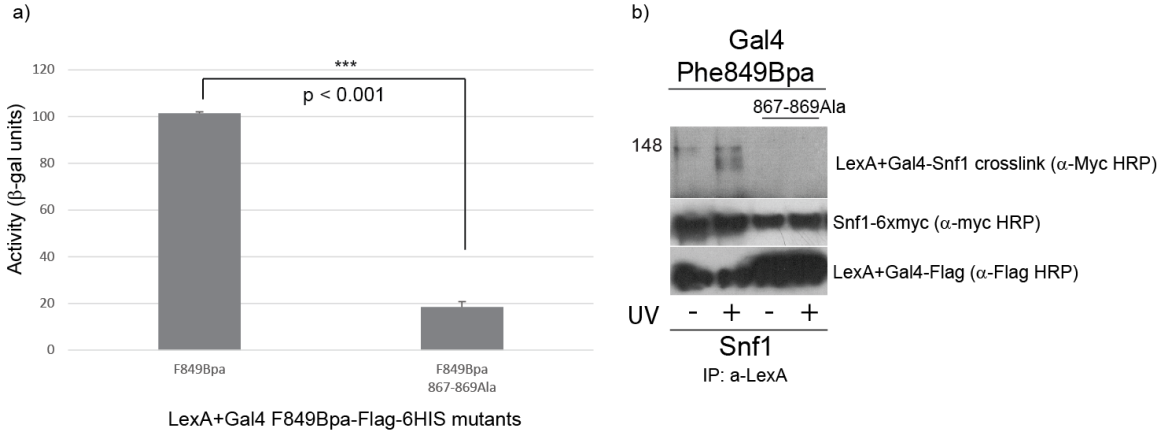


Supplementary Figure 2. LexA+Gal4 and Snf1 occupancy at GAL1-LacZ gene. Chromatin immunoprecipitation (ChIP) was performed as described in Methods and Materials section of main text. All experiments were carried out in a yeast strain lacking Snf1 (ΔSnf1) and either WT Snf1-6xmyc or a kinase dead Snf1-6xmyc T210A mutant complemented back in. With the exception of the Gal4 867-869 triple alanine mutant, all ChIP studies were done with the Gal4 F849Bpa construct. Cultures were grown to mid-log phase in glucose and then induced with galactose prior to treatment with formaldehyde. Each bar on the graph represents the % input reported as the mean and standard error from at least three independent experiments. Compiled data and data analysis can be found in Supplementary Table 3. (Left) ChIP analysis of Gal4 occupancy at the GAL1-LacZ gene as determined by immunoprecipitation with an antibody for LexA. (right) ChIP analysis of Snf1 occupancy at the GAL1-LacZ gene as determined by immunoprecipitation with an antibody for Snf1 protein.

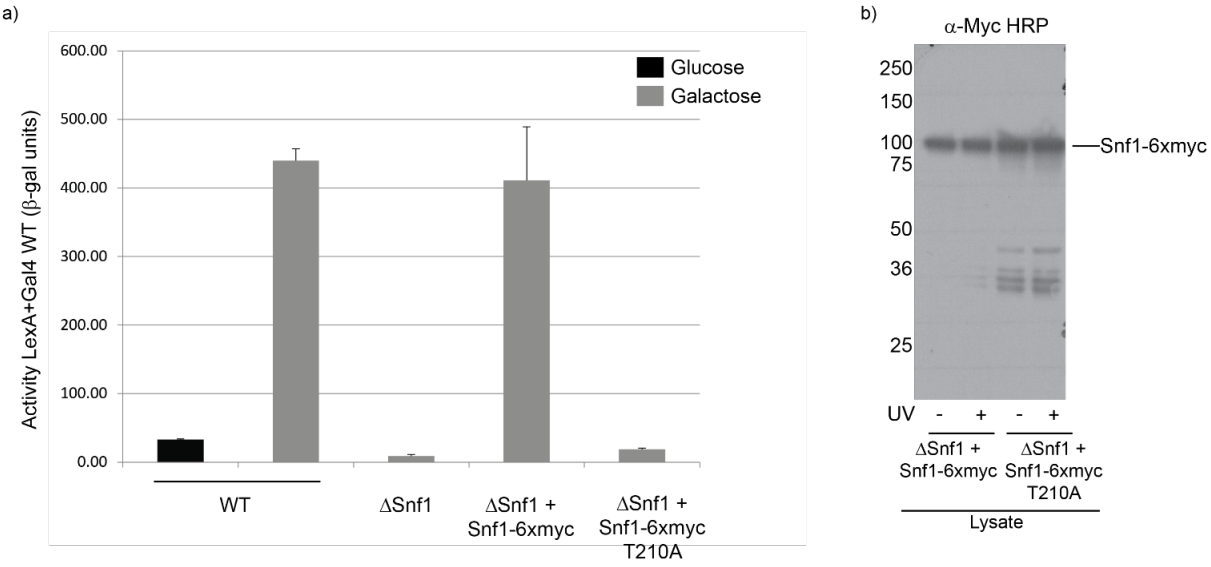




Supplementary Figure 3. Activity and expression of LexA+Gal4 F849Bpa mutants. β-galactosidase assays were conducted as described previously[1] (a) Bars represent the mean of at least three biological replicates. Error bars are the standard error of the mean (SEM). p-values were obtained by running an unpaired t-test between the two sample sets. (b) Expression and crosslinking of LexA+Gal4 F849Bpa mutants. In vivo covalent chemical capture using LexA+Gal4 F849Bpa or its cognate 867-869 triple alanine mutant was carried out as described previously.[1] Yeast lysates were immunoprecipitated with a-LexA antibody and the blots were probed with a-Myc HRP antibody (top and middle panels) and then stripped and reprobed with a-Flag HRP antibody (bottom panel). The top panel shows crosslinking between Gal4 F849Bpa and Snf1 as described in Figure 4 of the main text. The middle panel shows free Snf1-6xmyc in yeast lysate input lanes. The bottom panel shows levels of Gal4 F849Bpa and its cognate mutant after immunoprecipitation with LexA antibody. Both mutants express in yeast, indicating that the loss of activity observed in (a) is not due to lack of mutant protein expression.

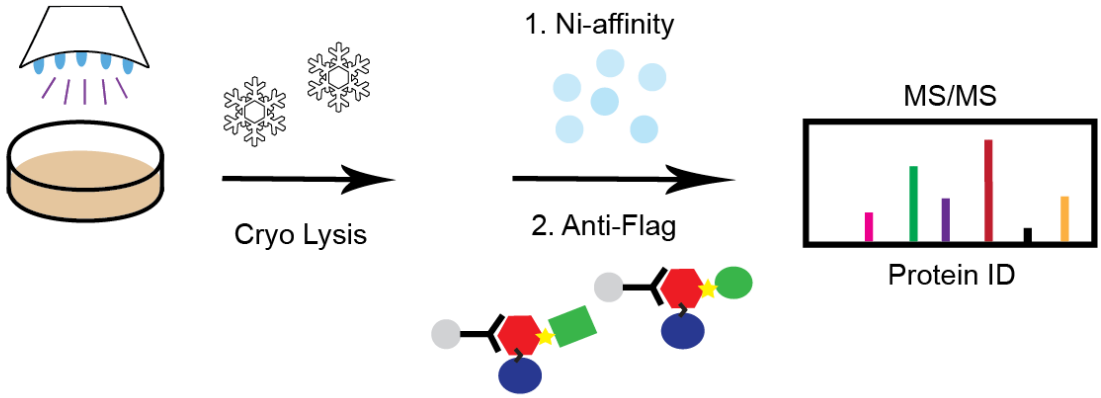


Supplementary Figure 4. Activity and expression of Snf1 kinase mutants. (a) Beta-galactosidase assays were carried out for LexA+Gal4 WT in the indicated yeast strains as previously described.[2] Cultures were grown in glucose to mid-log phase and then induced with galactose prior to measuring activation potential. Data labeled 'WT' were generated in a yeast strain (LS41) that expresses endogenous Snf1 kinase. All other data was generated in a yeast strain that has had Snf1 kinase deleted (ΔSnf1) and either WT Snf1-6xMyc or a kinase dead Snf1-6xMyc T210A mutant co-expressed alongside the activator. (b) Expression of WT Snf1-6xMyc and Snf1-6xMyc T210A mutant. An aliquot of whole cell lysate from cultures expressing either the WT or kinase-dead Snf1-6xMyc protein was separated on SDS-PAGE and analyzed via Western blot with a Myc-HRP antibody. The free Snf1 protein runs at approximately 100 kDa, with both constructs expressing in the ΔSnf1 yeast strain.



Supplementary Figure 5. Covalent Chemical Capture – MudPIT workflow and Snf1 Spectral Count Data. (a) Large scale yeast cultures were subjected to gentle irradiation under UV light to activate Bpa-protein crosslinking. Following crosslinking, the yeast were lysed under cryogenic conditions with a Retsch planetary ball mill and the covalent species were purified from yeast lysate using Ni-affinity pulldown under denaturing conditions followed by a milder Flag-affinity purification. The purified protein was then analyzed via MudPIT MS. (b) The Snf1 kinase protein contains a polyhistidine sequence on its amino terminus that results in its purification during the Ni-affinity pulldown. Accordingly, in (c) the Snf1 protein is highly enriched under both UV and non-UV treated conditions.

a)



b) **Sequence of Snf1 protein**

NH_2-MSSNNNTNTAPANANSS**HHHHHHHHHHHHHH**GHGGSNSTLNNPKSSLADGAHI
GNYQIVKTLGEGSFGKVKLAYHTTTGQKVALKIINKKVLAKSDMQGRIEREISYLRLLR
HPHIIKLYDVIKSKDEIIMVIEYAGNELFDYIVQRDKMSEQEARRFFQQIISAVEYCHRHK
IVHRDLKPENLLLDEHLNVKIADFGLSNIMTDGNFLKTSCGSPNYAAPEVISGKLYAGPE
VDVWSCGVILYVMLCRRLPFDDESIPVLFKNISNGVYTLPKFLSPGAAGLIKRMLIVNPL
NRISIHEIMQDDWFKVDLPEYLLPPDLKPHPEEENENNDSKKDGSSPDNDEIDDNLVNI
LSSTMGYEKDEIYESLESSEDTPAFNEIRDAYMLIKENKSLIKDMKANKSVSDELDTFLS
QSPPTFQQQSKSHQKSQVDHETAKQHARRMASAITQQRTYHQSPFMDQYKEEDSTV
SILPTSLPQIHRANMLAQGSPAASKISPLVTKKSKTRWHFGIRSRSYPLDVMGEIYIALKN
LGAEWAKPSEEDLWTIKLRWKYDIGNKTNTNEKIPDLMKMVIQLFQIETNNYLVDFKFDG
WESSYGDDTTVSNISEDEMSTFSAYPFLHLTTKLIMELAVNSQSN-COOH

c)

Snf1 MudPIT data		
-UV Average SC	+UV Average SC	p-value
696.1 ± 294.2	703.8 ± 43.8	0.96

Supplementary Table 1. Spectral Count Data and Analysis for LexA+Gal4 F849Bpa in Glucose. Table uploaded as separate file.

Supplementary Table 2. Spectral Count Data and Analysis for LexA+Gal4 F849Bpa in Galactose. Table uploaded as separate file.

Supplementary Table 3. ChIP Data and Analysis – Snf1 and LexA+Gal4F849Bpa. ChIP data from at least three biological replicates is displayed. Details regarding procedure, data analysis, and formulas used can be found in Supplementary Methods section.

	% Input values													
	Biological replicate 1			Biological Replicate 2			Biological Replicate 3			Biological Replicate 4				
Condition	*A1*	*A2*	*A3*	*B1*	*B2*	*B3*	*C1*	*C2*	*C3*	*D1*	*D2*	*D3*	**Average % Input**	**SEM**
849 -F IgG	0.000	0.001	0.001	0.024	0.038	0.047	0.018	0.016	0.020	0.001	0.001	0.004	0.014	0.005
849 -F Lex	0.001	0.001	0.001	0.016	0.013	0.018	0.002	0.001	0.001	0.001	0.001	0.000	0.005	0.002
849 -F Snf1	0.001	0.000	0.000	0.030	0.031	0.046	0.020	0.021	0.015	0.001	0.001	0.000	0.014	0.005
849 +F IgG	0.008	0.005	0.011	0.040	0.052	0.064	0.004	0.004	0.006	*	*	*	0.022	0.008
849 +F Lex	0.331	0.383	0.349	0.454	0.445	0.462	0.075	0.077	0.062	*	*	*	0.293	0.057
849 +F Snf1	0.065	0.038	0.044	0.148	0.128	0.105	0.039	0.025	0.030	*	*	*	0.069	0.015
AAA +F IgG	0.006	0.003	0.004	0.005	0.006	0.009	0.00	0.01	0.00	*	*	*	0.005	0.001
AAA +F Lex	0.127	0.121	0.101	0.135	0.132	0.133	0.00	0.01	0.00	*	*	*	0.085	0.020
AAA +F Snf1	0.031	0.026	0.023	0.006	0.017	0.010	0.00	0.01	0.00	*	*	*	0.014	0.004
T210A +F IgG	0.003	0.002	0.002	0.003	0.004	0.004	0.006	0.007	0.003	0.001	0.000	0.001	0.003	0.001
T210A +F Lex	0.263	0.257	0.311	0.096	0.098	0.108	0.036	0.050	0.028	0.022	0.022	0.024	0.110	0.031
T210A +F Snf1	0.019	0.014	0.014	0.006	0.004	0.007	0.012	0.005	0.004	0.002	0.002	0.002	0.008	0.002

p-value	0.00287632	849 v AAA, IP Snf1
	5.42802E-05	849 v T210, IP Snf1

Supplementary Table 4. Table of Plasmids

Plasmid ID	Function	Vector	Yeast Marker
LexA+Gal4 F849Bpa-Flag-6HIS	Expresses LexA(1-202)+Gal4(840-881)+1xFlag tag + 6x HIS tag with Bpa replacing Phe849 in the Gal4 activation domain. ADH1 promoter drives expression of activator	pCLexA	HIS
LexA+Gal4 F849Bpa-3xFlag-6HIS	Expresses LexA(1-202)+Gal4(840-881)+3xFlag tag + 6x HIS tag with Bpa replacing Phe849 in the Gal4 activation domain. ADH1 promoter drives expression of activator	pCLexA	HIS
LexA+Gal4 F849Bpa-AAA-Flag-6HIS	Expresses LexA(1-202)+Gal4(840-881)+1xFlag tag + 6x HIS tag with Bpa replacing Phe849 in the Gal4 activation domain and a triple alanine mutation at residues 867-869 (Y867A, L868A, F869A). ADH1 promoter drives expression of activator	pCLexA	HIS
pSNR-tRNA-pBpaRS	Expresses pBpa tRNA under the control of the SNR52 promoter and also expresses the synthetase (BpaRS) specific for charging the pBpa tRNA. SNR52 promoter drives expression of tRNA, GPD promoter drives expression of the synthetase	pSNR	TRP
pSNR-tRNA-pBpaRS	Expresses pBpa tRNA under the control of the SNR52 promoter and also expresses the synthetase (BpaRS) specific for charging the pBpa tRNA. Used only in experiments in ΔSnf1 strain. SNR52 promoter drives expression of tRNA, GPD promoter drives expression of the synthetase	pGADT7	LYS
LexA+VP16N L444Bpa-Flag	Expresses LexA(1-202)+VP16 (413-456)+FLAG tag with Bpa replacing Leu444 in VP16 ADH1 promoter drives expression of activator	pCLexA	HIS
LexA+VP16C F475Bpa-Flag	Expresses LexA(1-202)+VP16 (457-490)+FLAG tag ADH1 promoter drives expression of activator	pCLexA	HIS
Snf1-6xMyc	Expresses full length Snf1 fused to a 6x c-Myc tag on C-terminus of protein. ADH1 promoter drives expression of Snf1	pGADT7	LEU
Snf1-6xMyc T210A	Expresses full length Snf1 T210A fused to a 6x c-Myc tag on C-terminus of protein. ADH1 promoter drives expression of Snf1	pGADT7	LEU
Gal83-6xMyc	Expresses full length Gal83 fused to a 6x c-myc tag on C-terminus of protein. ADH1 promoter drives expression of Gal83	pGADT7	LEU
Mas5-6xmyc	Expresses full length Mas4 fused to a 6x c-myc tag on the C-terminus of the protein. ADH1 promoter drives expression of Mas5	pGADT7	LEU
Sti1-6xmyc	Expresses full length Sti1 fused to a 6x c-myc tag on the C-terminus of the protein. ADH1 promoter drives expression of Sti1	pGADT7	LEU
Sgt2-6xmyc	Expresses full length Sgt2 fused to a 6x c-myc tag on the C-terminus of the protein. ADH1 promoter drives expression of Sgt2	pGADT7	LEU
Nog1-6xmyc	Expresses full length Nog1 fused to a 6x c-myc tag on the C-terminus of the protein. ADH1 promoter drives expression of Nog1	pGADT7	LEU

Rrp5-6xmyc	Expresses full length Rrp5 fused to a 6x c-myc tag on the C-terminus of the protein. ADH1 promoter drives expression of Rrp5	pGADT7	LEU

(a) **Supplementary Table 5**. Table of Primers

Primer ID	Sequence	Description
P1	5’ GTACATCTCGAGATGAGCAGTAACAACAACACAAAC 3’	XhoI-Snf1 Fwd
P2	5’ GCTACGCATATGATTGCTTTGACTGTTAACGGCTAATTC 3’	NdeI-Snf1 Rev
P3	5’ GACTTAAAGCTTATGGCTGGCGACAACCCTGA 3’	HindIII-Gal83 Fwd
P4	5’ GCTACGCATATGATTACTGATCTGAGACTTTTG 3’	NdeI-Gal83 Rev
P5	5’ CATTACCTCGAGATGTCAGCATCAAAAGAAGAAATTGCTGCCC 3’	XhoI-Sgt2 Fwd
P6	5’ GTAATGGGATCCCTATTGCTTGTTCTCATTGTCTGGTGTTTCATCTG 3’	BamHI-Sgt2 Rev
P7	5’ CATTACCTCGAGATGTCACAAGACGCTGCTATTGCAGAGC 3’	Xho1-Hxt6 Fwd
P8	5’ GTAATGGGATCCTTTGGTGCTGAACATTCTCTTGTACAATGGC 3’	BamHI-Hxt6 Rev
P9	5’ CATTACCTCGAGATGGTTAAAGAAACTAAGTTTTACGATATTCTAGGTG 3’	XhoI-Mas5 Fwd
P10	5’ GTAATGGGATCCTCATTGAGATGCACATTGAACACCTTCG 3’	BamHI-Mas5 Rev
P11	5’ CATTACCTCGAGATGTCATTGACAGCCGATGAATACAAACAACAAGG	XhoI-Sti1 Fwd
P12	5’ GTAATGGGATCCTTAGCGGCCAGTCCGGATGATACCAG 3’	BamHI-Sti1 Rev
P13	5’ CATTACCTCGAGATGCAACTTTCATGGAAGGATATCCCTACTGTCGCTCC 3’	XhoI-Nog1-Fwd
P14	5’ GTAATGGGATCCTCAACGGAAATCTGTCTTACCGACACCACGCTTACCAC 3’	BamHI-Nog1-Rev
P15	5’ CATTACGGATCCATGGTAGCTTCCACCAAAAGAAAGAGAGATGAAGATTTTCC 3’	BamHI-Rrp5-Fwd
P16	5’ GTAATGCCCGGGTTCGTCTGCTTTTTGAGATTCATGGCTAGCGAC 3’	XmaI-Rrp5 Rev
P17	5' GACTGATGGTAATTTCTTAAAGGCTTCTTGTGGTTCTCCCAATTATGCG 3'	SDM Snf1 T210A Fwd
P18	5' CGCATAATTGGGAGAACCACAAGAAGCCTTTAAGAAATTACCATCAGTC 3'	SDM Snf1 T210A Rev
P19	5' CAATGGATGATGTATATAACGCTGCTGCTGATGATGAAGATACCCC 3'	SDM Gal4 867-869 AAA Fwd
P20	5' GGGGTATCTTCATCATCAGCAGCAGCGTTATATACATCATCCATTG 3'	SDM Gal4 867-869 AAA Rev
P21	5' CAAACCCAAAAAAAGAGGATTACAAGGATCACGATGGTGATTACAAGGA TCACGATATTGACTATAAAGACGACGAC 3'	SDM 3xFlag insert Fwd
P22	5' GTCGTCGTCTTTATAGTCAATATCGTGATCCTTGTAATCACCATCGTGAT CCTTGTAATCCTCTTTTTTTGGGTTTG 3'	SDM 3xFlag insert Rev
P23	5' CTATAAAGACGACGACGACAAACATCATCATCATCATTAAGGATCCG TCGACCTGCAG 3'	SDM 6HIS Fwd
P24	5' CTGCAGGTCGACGGATCCTTAATGATGATGATGATGTTTGTCGTCG TCGTCTTTATAG 3'	SDM 6HIS Rev
P25	5' GTAACAAGTTTTGCTACACTCCCTTAATAAAGTCAACATGTCTGTTATTAA TTTCACAGGTAGTTCTGGTCC 3'	5' Snf1-TRP1 cassette Fwd
P26	5'CATAAAAAAAAGGGAACTTCCATATCATTCTTTTACGTTCCACCATTATTT CTTAGCATTTTTGACGAAATTTGC 3'	3' Snf1-TRP1 cassette Rev
P27	5' CTGCGCATTCGTGTCCAAACAGTCATTCAGG 3'	gSnf1 +210 seq forward

P28	5' CATAGAGCGTGAAATTTGCTTTTCATCCGAAG 3'	gSnf1 -193 seq Rev
P29	5' GCCTACATCTTTACCTCAGATCCACAGAGC 3'	Snf1 int. fwd seq
P30	5' GCCTATGGCAGTACTCGACGGCAC 3'	Snf1 int. Rev seq
P31	5' CTGCATGGAGATGAGTCGTGGC 3'	Trp1 int. fwd seq
P32	5' GCCACGACTCATCTCCATGCAG 3'	Trp1 int. rev seq
P33	5' CCTTCTCTTTGGAACTTTCAGTAATACGCTTAACTGC 3'	GAL1-LacZ Fwd
P34	5' GGGCGATCGGTGCGGGCCTCTTCGC 3'	GAL1-LacZ Rev

Supplementary Methods

(b) Construction of plasmids

pLexAVP16N Leu444Bpa, pLexAVP16C Phe475Bpa, and pSNRtRNA-pBpaRS plasmids were constructed as previously described.[1,2] Briefly, the LexA fusion genes were cloned into vector pCLexA, which has a 2μ origin of replication and an ADH1 promoter that drives activator expression. Importantly, in our previous work we demonstrate that maximal Bpa incorporation is about 20% compared to the wild-type protein expression. Thus, while the vector used in these studies is high copy, the expression of Bpa-containing activators is likely much lower than that achieved with canonical protein expression. pLexA+Gal4 Phe849Bpa-Flag-6HIS was created by modifying previously described pLexA+Gal4 Phe849Bpa to include a 6HIS tag 3' to the Gal4-Flag open reading frame.[1] Insertion of the 6HIS tag was achieved by site-directed mutagenesis with primers P23/P24 and Qiagen Quikchange two-step SDM protocol. For TRIC experiments, a 3x Flag tag was additionally inserted using site directed mutagenesis with primers P21 and P22 (Qiagen Quikchange).
To create p6xmyc-Snf1, primers P1 and P2 were used to clone out the Snf1 gene from yeast genomic DNA. The purified PCR product was then digested with XhoI and NdeI and ligated into XhoI and NdeI digested pGADT7-6xmyc backbone using standard molecular cloning techniques. To create Gal83-6xMyc, primers P3 and P4 were used to clone out the Gal83 gene from yeast genomic DNA. The purified Gal83 PCR product was then digested with HindIII and NdeI and ligated into a HindIII/NdeI digested pGADT7-6xmyc backbone using standard molecular cloning techniques. Similar procedures were used to generate Sgt2-6xmyc (P5/P6), Hxt6-6xmyc (P7/P8), Mas5-6xmyc (P9/P10), Sti1 (P11/P12), Nog1-6xmyc (P13/P14) and Rrp5-6xmyc (P15/P16). Restriction enzymes and T4 DNA ligase were purchased from NEB. Snf1-6xMyc T210A was made using the Qiagen Quikchange two-step mutagenesis protocol with primers P17 and P18. All sequences were verified by the University of Michigan DNA Sequencing Core.

Construction of Snf1 delete strain
ΔSnf1 LS41 was constructed using a PCR-mediated gene deletion strategy with a cassette containing the full TRP1 open reading frame and ~40 bp complementarity to the 5' and 3' flanking regions of genomic Snf1. The SNF1::Trp1 deletion cassette was transformed into LS41 using a lithium acetate transformation protocol and transformants were selected on plates lacking uracil and tryptophan.[3] Colonies were screened using polymerase chain reaction (PCR) with PCR products visualized on a 1% agarose gel stained with ethidium bromide.
Primers P25 and P26 were used to clone out the Trp1 gene off pFA6a-TRP1-PGAL1-HBTH (Addgene, plasmid # 26898). The PCR product was verified on 1% agarose gel stained with ethidium bromide and the product was gel purified (QIAquik gel extraction kit and protocol, Qiagen). The purified Snf1-Trp1 cassette was transformed into LS41 yeast using standard lithium acetate/PEG transformation methods and plated on plates containing 2% glucose and lacking uracil and tryptophan for selection.[3] Transformants were screened via colony PCR using primer combinations P27/P28, which are specific for the Snf1 locus, and colonies showing a PCR product of ~750 bps were screened further using primer combinations P27/P30, P28/P29, P27/P32, and P28/P31 to verify that Snf1 was definitively replaced by the TRP1 gene at the Snf1 locus. Further confirmation was obtained by submitting the P27/P28 gel purified PCR product for sequencing, which returned a TRP1 sequence.

Gal4-Gal80 in vivo covalent chemical capture

For all covalent chemical capture experiments, Bpa purchased from Chem Impex International (cat.# 05110) was used. To perform in vivo cross-linking, an individual colony expressing pLexA+Gal4 F849TAG-Flag-6HIS was inoculated in 10 mL SC media containing 2% Raffinose and lacking histidine, tryptophan, and uracil (H-W-U-) for selection. This culture was incubated overnight at 30 °C with agitation and used to inoculate 1L cultures of H-W-U- SC media containing either 2% Glucose or 2% Raffinose and 2% Galactose. To each 1 L culture, 10 mL of 100 mM Bpa dissolved in 1M NaOH and 10 mL 1M HCl were added. Cultures were incubated overnight at 30 °C with agitation and grown to mid-log phase OD_{660} (~0.8). Yeast were harvested by centrifugation at 6000 rcf at 4°C for 15min (Beckman Avanti J-201, JLA 8.1000). Cell pellets were washed with SC media lacking histidine and tryptophan and resuspended in 2mL H-W-U- SC media with either 2% Raffinose and 2% Galactose or 2% Glucose. Yeast were transferred to cell culture dishes and subjected to UV irradiation at 365 nm light (Eurosolar 15 W UV lamp) with cooling for 0.5 h. Irradiated cells were then harvested by centrifugation and stored at -80°C until lysis.

In vivo crosslinking to capture Gal4 targets

To obtain each MS data set, 10 L yeast cultures were grown and irradiated as described previously but on larger scale.[1] Briefly, yeast were grown in SC media containing 2% raffinose, 2% galactose with 1 mM Bpa but lacking histidine, tryptophan, and uracil (H-W-U-) for selection and grown at 200 rpm, 30 °C with constant air bubbling using a bioreactor (New Brunswick, Bioflo 300) to mid-log phase OD_{660} (~0.8). Yeast were harvested by centrifugation at 6000 rpm for 15 min and resuspended in SC media with 2% raffinose and galactose but lacking histidine, tryptophan, uracil (H-W-U-), Bpa and irradiated for 1 h at 365 nm with cooling using an Euro Solar UV lamp. Control samples, were no irradiation was to be performed were directly taken to the next step. After irradiation, the yeast were centrifuged and washed 2X with 100 mM PBS and resuspended in ~ 40 mL MS Lysis Buffer (100 mM PBS pH 7.0, 150 mM NaCl, 10% glycerol, 0.5% NP-40 and 10 mM beta-mercaptoethanol) containing 2x protease inhibitor (complete mini EDTA free protease inhibitor tablets, Roche) and flash frozen with liquid Nitrogen and stored at -80 °C until lysis.

MudPIT Sample Preparation and Analysis

Cryolysis procedure for MS samples

Cryolysis was performed using a Retsch planetary ball mill PM 200. All equipment was cooled in liquid nitrogen prior to use. Frozen yeast cells were crushed in a chilled mortar and pestle with liquid nitrogen added. Ground cells were added to the chilled lysis chambers and loaded into the ball mill. PM-200 was run three times for two minutes/cycle at 500 rpm with liquid nitrogen cooling performed between each cycle. Chambers were removed from machine after final spin and the frozen lysed yeast were recovered from the chambers and placed in a 50 mL centrifuge tube on dry ice.

Affinity purification of crosslinked samples for MS analysis

Lysed yeast were allowed to thaw in a cold water bath and then centrifuged for 30 minutes at 4 °C, 9299 rcf (Beckman Coulter Allegra X-22R, fixed rotor). While cells were spinning, 300-500 mL Ni-agarose bead slurry was washed three times with 1 mL chilled PBS. Supernatant from lysed yeast was transferred to a clean 50 mL centrifuge tube and the Ni-agarose beads (resuspended in 1 mL chilled PBS) were added to the supernatant. Beads and supernatant were incubated for 1 hour on rotating carousel in 4 °C cold room. After incubation, beads were spun down (low rpm) and the supernatant was poured off. Ni-agarose beads were then resuspended in the residual supernatant and washed in 5 mL chilled Nickel Wash Buffer (100 mM PBS pH 7.0, 150 mM NaCl, 10% glycerol, 3M Guanidine-HCl, 30 mM imidazole, 0.2% Tween-20, 10 mM Beta-mercaptoethanol) in a 15 mL centrifuge tube. Washed beads were centrifuged at 2500 rpm for 2 minutes at 4 °C (Beckman Coulter Allegra X-22R, bucket rotor) and the supernatant decanted. This procedure was repeated 4 more times for a total of five-5 mL washes. The beads were then washed twice with 5 mL cold 100 mM PBS. PBS was decanted and the beads were resuspended in 500 μL 100 mM PBS and transferred to a chilled 1.75 microcentrifuge flip cap tube.

Residual beads on the 15 mL tube were washed with about 500 μL 100 mM PBS and transferred to a microcentrifuge tube. Beads were spun briefly at low rpm (Eppendorf 5417C microfuge).
Beads were resuspended in 500 μL chilled Nickel Elution Buffer (100 mM PBS pH 7.0, 500 mM Imidazole, 0.5% Tween-20) and mixed gently for 1 minute by inversion and then re-centrifuged. The supernatant (eluant) was pipetted off and collected in a separate, clean 1.75 mL microcentrifuge tube. Another 500 μL chilled Nickel Elution Buffer was added to the beads and mixed by inversion for 10 minutes, 4 ^{o}C in a cold room. During the second elution, a 10K microcentrifuge tube concentrator (Vivascience 10K MWCO) was washed with 100 mM PBS. The first elution was centrifuged to remove any lingering Ni-agarose and the eluant was pipetted out and added to the concentrator. Concentrator was spun at 15,000xG for 5 minutes at 4 ^{o}C. Elutions were repeated for a total of 3 elutions, each elution added to the concentrator after centrifugation to remove residual Ni-agarose. 500 μL of 100 mM chilled PBS was added to the protein and then run through the concentrator. This was repeated for a total of 3 PBS buffer exchanges to get a final volume of 1000-1500 μL of protein.
400 μL of Flag-agarose (Sigma, M2) was washed three times with 1 mL 100 mM PBS pH 7.0,, Concentrated sample was added to the Flag-agarose and incubated for 2 hours at 4 ^{o}C, with rotation. Flag-agarose was then washed ten times with 1 mL/wash Flag Wash Buffer (100 mM PBS pH 7.0, 150 mM NaCl, 0.5% NP-40), spinning between washes (5000 rpm, 30 sec, Eppendorf 5417C). Next, Flag-agarose was washed twice with 100 mM PBS to dilute out the NP-40. Flag Elution Buffer (100 mM PBS pH 7.0, 350 mM NaCl, 0.1% NP-40) containing 10 mg/mL flag peptide was used for elution. 333 μL Flag elution buffer was added to washed Flag-agarose and incubated for 30 minutes, 4 ^{o}C, with rotation. Beads were briefly centrifuged and the elution transferred to a new 1.75 mL Eppendorf tube. Elution was quick frozen in liquid nitrogen and stored in -80 ^{o}C freezer. Second elution was performed in the same fashion and the final 333 μL was added to beads for a third elution that was allowed to run overnight. The next morning, the two frozen elutions were thawed and spun down to remove residual Flag-agarose. Elutions were transferred to new tubes and then centrifuged again to remove any chance of residual beads remaining. These first two elutions, cleared of all beads, were then concentrated in a 10K concentrator at 15,000 xG until down to 100 μL. The overnight elution was then added and centrifuged down to 100 μL. 500 μL of ammonium bicarbonate (50 mM) was then added to the concentrator for a total of 4 buffer exchange cycles with the protein. Concentrated sample was then transferred to a clean microcentrifuge tube. Sample was quick frozen in liquid nitrogen, packed in dry ice, and shipped for mass-spectrometry analysis.

MudPIT Analysis
All MudPIT analyses were performed at the Center for Physiological Proteomics in La Jolla, California. For both the glucose-grown and galactose-grown cultures, three biological replicates were analyzed for UV-treated samples and two biological replicates were analyzed for non-UV control samples. Spectral counting was used for identifying hits. Crosslinked products were denatured by adding urea to a final concentration of 8 M, in 50 mM Tris (pH 8.0). The protein was then reduced with 10 mM Tris(2-carboxyethyl) phosphine HCl (TCEP, Sigma) for 30 minutes at room temperature. The proteins were subsequently alkylated with fresh 12.5 mM iodoacetamide (IAA; Sigma) and the concentration of urea was reduced to 2 M by adding 50 mM Tris (pH 8.0). Next, crosslinked proteins were digested overnight with 1 mM $CaCl_2$ and 2 μg Trypsin at 37 ^{o}C. Digested peptides were acidified to a final concentration of 5% formic acid and centrifuged at 17,000xg for 15 minutes. Half of the digested mixture was pressure loaded onto a biphasic strong cation exchange/reverse phase capillary column and separated by 2D liquid chromatography and tandem MS using an 11 step gradient on an LTQ mass spectrometer. Full MS spectra were acquired with a mass range of 400-1,800 followed by 7 MS/MS scans. All MS/MS spectra were collected using a normalized collision energy of 35% and an isolation window of 2 Da. One microscan was applied for all experiments in the LTQ. Spray voltage was set to 2.50 kV, and the flow rate through the column was 0.20 uL/min. The data was analyzed against the entire yeast genome using the SEQUEST algorithm and the analysis was performed using the DTASelect software package. For both the glucose-grown and galactose-grown cultures, three biological replicates were analyzed for UV-treated samples and two biological replicates were analyzed for non-UV control samples. Spectral counting was used for identifying hits. Hits from each replicate were compiled and the mean spectral counts for each protein were calculated. Proteins with less than two average spectral counts were removed from further analysis. UV-treated and non-UV samples within a single carbon

source condition were compared to determine enrichment values (Average +UV sc/average –UV sc). Proteins were considered significant if the enrichment ratio (+UV/-UV) was greater than or equal to 5 and if a t-test comparing UV-treated and non-UV data for a protein generated a p-value of less than or equal to 0.05. Hits with unknown function and proteins already identified as targets of Gal4 were not displayed in Figure 3b. The complete galactose MS data set and analysis can be found in Supplementary Table 2.

MudPIT data analysis and filtering
To avoid zero values in the denominator, all spectral count values were adjusted to contain an additional 0.1. Spectral counts for each protein were averaged within the non-UV (N=2) and +UV (N=3) datasets. Proteins with a spectral count average less than 2 in both the –UV and +UV datasets were removed from further analysis. Enrichment ratios were calculated for proteins with at least two average spectral counts in either condition (Average +UV s.c./Average non-UV s.c.) and t-tests were carried out in Excel to generate p-values for the enrichment ratios. Proteins with an enrichment ratio of five or greater and a p-value of 0.05 or less were considered to be significant hits. Those with an average of at least five spectral counts were considered high-scoring hits and those with an average between 2 and 5 spectral counts were considered moderate-scoring hits. Proteins that were previously identified as Gal4 targets were excluded from the list (Gal80) as were proteins with no characterized function.

(c) β-Galactosidase assays
To evaluate the ability of each LexA+Gal4 F849Bpa-6HIS-flag to activate transcription in the presence or absence of glucose, saturated cultures (SC media + 2% raffinose) of yeast expressing Gal4 Phe849Bpa were used to inoculate 5 mL SC media containing either 2% glucose or 2% raffinose + 2% galactose but lacking histidine and tryptophan for selection. The cells were grown to an OD_{660} of 0.8-1 and harvested. The activity of each construct was monitored using β-galactosidase assays as previously described.[1]

For activity of LexA+Gal4 WT in ΔSnf1strains (Supplementary Figure 4a), a single colony of each construct was inoculated in 5 mL yeast dropout media containing 2% glucose and grown overnight at 30°C. These cultures were then used to inoculate additional 5 mL cultures of dropout media containing 2% glucose and 1 mM Bpa. Cultures were grown to mid-log phase, harvested via centrifugation, washed with dropout media, and, with the exception of the glucose control culture, were then resuspended in media containing 2% raffinose, 2% galactose, and 1 mM Bpa. Cultures were induced for 3 hours at 30°C before being harvested. The activity of each construct was monitored using β-galactosidase assays as previously described.[2]

ChIP assays
All ChIP experiments were conducted in ΔSnf1 LS41 strain with pGADT7-Snf1-6xmyc complemented back in. An individual colony of pLexA+Gal4 Phe849Bpa-Flag-6HIS or its cognate triple alanine mutant was grown in 5 mL SC media containing 2% glucose but lacking histidine, tryptophan, leucine, lysine and uracil for selection. The culture was incubated overnight at 30 °C with agitation. This culture was used to inoculate 100 mL cultures of SC media containing 2% glucose, 1 mL 100 mM pBpa dissolved in 1M NaOH and 1 mL 1M HCl. The cultures were incubated overnight at 30 °C with agitation to an OD_{660} of ~0.8. Cultures were harvested via centrifugation, washed with media, and added to induction media composed of SC media lacking HWKLU and containing 2% raffinose, 2% galactose and 1 mM Bpa. Cells were induced for 3 hours prior to formaldehyde treatment. Non-formaldehyde treated cultures were harvested by centrifugation at 3901 rcf (Beckman Coulter Allegra X-22R, 4 deg C) and washed with 25 mL media lacking his, trp, lys, leu, and ura. Cell pellets were transferred to a 1.75 mL tube, re-centrifuged, and the pellet frozen and stored at -80 ^{o}C until lysis. Formaldehyde treated samples were equipped with a stirbar on a stir plate. To each culture, 2.9 mL 37% formaldehyde solution was then added and allowed to stir for 5 minutes at room temperature, at which point the reaction was quenched with excess 2M glycine for 5 minutes with stirring. The treated culture was harvested via centrifugation, washed with 50 mL media lacking his, trp, lys, leu and ura, transferred to 1.75 mL tube and the cell pellet frozen and stored at -80 ^{o}C until lysis.
Cell pellets were thawed on ice and lysed as previously described.[2] After mechanical shearing and whole cell lysate separation, the lysate and insoluble pellet were resuspended by pipetting and each sample was sonicated at a setting of 10% (double-step microtip, Fisher Scientific Dismembrator Model 500) for 2

minutes on ice with 30 sec pulse on/off. Samples were then centrifuged at 4 ^{o}C for 20 minutes at max speed. Soluble chromatin was separated from the pellet and 10 μL removed as an input sample. The remaining soluble chromatin was split equally between three 1.75 mL tubes. Immunoprecipitation with either 2 μg Snf1 antibody (sc-15621, Santa Cruz Biotechnologies), LexA antibody (sc-1725, Santa Cruz Biotechnologies), or control IgG (sc-2027, Santa Cruz Biotechnologies) proceeded for 2 hours, 4 ^{o}C followed by incubation for 1 h with ~40 μL of prewashed protein G magnetic Dynabeads slurry (Life Technologies). Beads were washed twice with 1X ChIP Lysis Buffer ((50 mM Hepes-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na-Deoxycholate and 2X Complete Mini, EDTA Free Protease Inhibitor (Roche)), twice with 1X ChIP Lysis buffer containing 0.5M NaCl, twice with 1x ChIP Wash Buffer (10 mM Tris-HCl pH 8.0, 250 mM LiCl, 0.5% NP-40, 1% Na-Deoxycholate and 1 mM EDTA), and twice with TE Buffer (10 mM Tris-HCl, 1 mM EDTA). Immunoprecipitated complexes were eluted in 50 μL elution buffer (50 mM Tris-HCl, 10 mM EDTA, 1% SDS) at 65 ^{o}C for 30 minutes. The eluate was transferred to a new 1.75 mL tube to which 120 μL of TE-SDS (10 mM Tris-HCl, 1 mM EDTA, 0.1% SDS) buffer was added and 90 μL TE-SDS was added to the 10 μL of input. Formaldehyde crosslinks were reversed overnight in a 65 °C water bath. Samples were purified using Qiagen PCR Clean protocol and eluted in 58 μL Buffer EB. qPCR on all samples and inputs was run using Promega GoTaq qPCR master mix (A6001, Promega) using primers P33 and P34 on an Applied Biosystems StepOnePlus instrument. At least three independent biological replicates were run for each condition, with each biological replicate run in triplicate for qPCR quantitation. PCR amplification with **primer** P33 and P34 yielded a single band around 450 bps. This band was gel purified and submitted for sequencing. Results returned a sequence for the GAL1-LacZ gene, as expected (below).

Sequence of GAL1-LacZ PCR amplified product
*5'*TGCATAANCCACTTTAACTAATACTTTCAACATTTTCGGTTTGTATTACTTCTTATTCAAATGTAATAA
AAGTATCAACAAAAAATTGTTAATATACCTCTATACTTTAACGTCAAGGAGAAAAAACTATAATGACTA
AATCTCATTCAGAAGAAGTGATTGTACCTGAGTTCAATTCTAGCGCAAAGGAATTACCAAGACCATTG
GCCGAAAAGTGCGGAATTCCAAGCTTGGCCAAGCCCGGATCCGGAGCTTGGCTGTTGCCCGTCTCA
CTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGAT
TCATTAATGCAGCTGGCACGACAGGTTTCCCGACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCA
GCTGGCGTAATAGCGAAGAGGCCCGCACCG 3'

ChIP Data Analysis
Input C_T values for a single biological replicate were averaged and $\log_2(1/60)$ was subtracted from this value to generate a normalized Input value. The individual C_T values generated for a set of technical triplicates were subtracted from the respective normalized input values to generate a ΔC_T value (negative number) for each measurement. The ΔC_T value was then log transformed ($100*2^{(\Delta C_T)}$) to generate individual % input values which are displayed in Supplementary Table 3. The % input values generated across at least three biological replicates were averaged and the standard error of the mean calculated for graphical display. p-values were calculated using an unpaired t-test.

(d) Crosslinking to Snf1 and Gal83.
An individual colony expressing either pLexA+Gal4 Phe849Bpa-6HIS-Flag, pLexA+VP16 Leu444Bpa-Flag, or pLexA+VP16C Phe475Bpa-Flag was inoculated in 5 mL SC media containing 2% raffinose but lacking histidine, tryptophan, and uracil (H-W-U-) for selection. This culture was incubated overnight at 30 °C with agitation and then used to inoculate 100 mL cultures of H-W-U- SC media containing 2% raffinose and 2% galactose. To each culture, 1 mL of 100 mM pBpa dissolved in 1M NaOH and 1 mL 1M HCl were added. Cultures were incubated overnight at 30 °C with agitation and grown to mid-log phase OD_{660} (~0.8). Yeast were harvested by centrifugation at 3901 rcf at 4^{o}C for 5min (Beckman-Coulter Allegra X-22R). Cell pellets were washed with H-W-U- SC media and resuspended in 2mL H-W-U- SC media containing 2% raffinose and 2% galactose. Yeast were transferred to cell culture dishes and subjected to UV irradiation at 365 nm light (Eurosolar 15 W UV lamp) with cooling for 0.5 h. Irradiated cells were then harvested by centrifugation and stored at -20^{o}C until lysis. For lysis, cell pellets were resuspended in 600 μL Lysis buffer (50 mM HEPES-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na-Deoxycholate and 2X Complete Mini, EDTA Free Protease Inhibitor (Roche)) and lysed

using glass beads by vortexing at 4 °C. The lysate was pelleted and the supernatant incubated with 10 µL (2 ng) of Snf1 antibody (sc-15621, Santa Cruz Biotechnologies) for 2 h at 4 °C for immunoprecipitation. The protein bound to the antibody was isolated by incubation for 1 h with 40 µL of prewashed protein G magnetic Dynabeads (Life Technologies) at 4 ^{o}C. After immunoprecipitation, the beads were washed 6 times with 1 mL Wash Buffer (10 mM Tris-HCl pH 8.0, 250 mM LiCl, 0.5% NP-40, 0.1% Na-Deoxycholate and 1 mM EDTA) and stored dry at -20 ^{o}C until elution. The crosslinked sample was eluted from the beads by heating at 95 ^{o}C for 10 min in Laemmli 2x Buffer (BioRad) containing 250 mM DTT and run on a 4-20% Tris-Glycine TGX gel (BioRad). The separated proteins were then transferred to PVDF membrane and probed using Western Blot analysis with a 1:1000 dilution of anti-FLAG (M2) antibody (Sigma) in 5% milk PBST.
Crosslinking to Gal83 was carried out in the same way, except that p6xmyc-Gal83 was co-expressed alongside each activator construct in yeast. Cells were additionally grown in media lacking histidine, tryptophan, leucine and uracil for selection and lysates were immunoprecipitated with a LexA antibody (sc-1725, Santa Cruz Biotechnologies). Resulting Western blots were probed with a 1:1000 dilution of myc-HRP antibody (sc-40 HRP, Santa Cruz Biotechnologies) in 5% milk PBST.

Tandem Reversible and Irreversible Crosslinking (TRIC) at GAL1-LacZ

An individual colony of pLexA+Gal4 Phe849Bpa-6HIS-3xFLAG was grown in 5 mL SC media containing 2% Raffinose but lacking histidine, tryptophan and uracil for selection. The culture was incubated overnight at 30 °C with agitation. Following incubation, this culture was used to inoculate 100 mL cultures of SC media containing 2% Raffinose and 2% Galactose. For Bpa incorporation, 1 mL 100 mM Bpa dissolved in 1M NaOH and 1 mL 1M HCl were added to the above cultures. The cultures were incubated overnight at 30 °C with agitation to an OD_{660} of ~0.8. When cultures reached the appropriate OD_{660}, the cells were treated in the following manner:
The cultures receiving only UV treatment were centrifuged at 3901 rcf, 4 ^{o}C (Beckman Coulter Allegra X-22R), washed with SC media lacking histidine, tryptophan and uracil, and recentrifuged. Cell pellets were resuspended in 2mL SC media lacking histidine, tryptophan and uracil and containing 2% Raffinose, 2% Galactose. Cells were transferred to a small cell culture dish and subjected to UV irradiation at 365 nm light (Eurosolar 15 W UV lamp) with cooling for 0.5 h.
The cultures receiving formaldehyde treatment were equipped with a stirbar and placed on a stirplate in a fume hood. 2.9 mL of 37% formaldehyde was added directly to the culture and allowed to stir for 5 minutes before being quenched with 15 mL of 2M Glycine for 5 minutes. Cells were then centrifuged and cell pellets were washed with 50 mL H-W-U- media and recentrifuged. Samples intended to additionally receive UV crosslinking were then resuspended in 2 mL H-W-U- media containing 2% Raffinose, 2% Galactose and transferred to a small cell culture dish and subjected to UV irradiation at 365 nm UV light (Eurosolar 15 W UV lamp with cooling for 0.5h.
For lysis, cells were resuspended in 600 µL Lysis buffer (50 mM HEPES-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na-Deoxycholate and 2X Complete Mini EDTA Free Protease Inhibitor (Roche) and lysed using glass beads and vortexing at 4 °C. We found in these studies that complete cellular lysis is necessary to eliminate background signal caused by cell lysis during sonication. Subsequent lysates were removed and the remaining pellet was then washed 2x with “Harsh” ChIP buffer (50 mM HEPES-KOH pH 7.5, 1M NaCl, 1 mM EDTA, 1% Triton X-100, 1% Na-Deoxycholate) followed by 2 washes with regular ChIP buffer (50 mM HEPES-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na-Deoxycholate and 2X Complete Mini EDTA Free Protease Inhibitor (Roche)). Pellets were resuspended in 600 µL ChIP lysis buffer containing protease inhibitor and sonicated at a setting of 10% (double-step microtip, Fisher Scientific Dismembrator Model 500) for 2 minutes on ice with 30 sec pulse on/off. Samples were then centrifuged at 4 ^{o}C for 20 minutes at max speed. Soluble chromatin was then removed from the pellet and immunoprecipitated with Snf1 antibody (sc-15621, Santa Cruz Biotechnologies) for 2 hours, 4 ^{o}C. The protein bound to the antibody was isolated by incubation for 1 h with ~50 µL of prewashed protein G magnetic Dynabeads (Life Technologies). After immunoprecipitation, the beads were washed 6 times with 1 mL Wash Buffer (10 mM Tris-HCl pH 8.0, 250 mM LiCl, 0.5% NP-40, 0.1% Na-Deoxycholate and 1 mM EDTA) and stored dry at -20 ^{o}C until elution. The crosslinked sample was eluted from the beads and formaldehyde crosslinks reversed by heating at 95 ^{o}C for 20 min in NuPAGE 4x LDS Sample buffer (Life Technologies) containing 250 mM DTT. Samples were run on a 4-20% Tris-Glycine TGX gel (Bio-Rad) and transferred to a PVDF

membrane. Western Blot analysis was carried out using a 1:1000 dilution of anti-FLAG (M2) antibody (Sigma) in 5% PBST.

References

(1) Majmudar, C. Y.; Lee, L. W.; Lancia, J. K.; Nwokoye, A.; Wang, Q.; Wands, A. M.; Wang, L.; Mapp, A. K. *J Am Chem Soc* **2009**, *131*, 14240.

(2) Krishnamurthy, M.; Dugan, A.; Nwokoye, A.; Fung, Y.; Lancia, J.; Majmudar, C. Y.; Mapp, A. K. *ACS Chem Biol* **2011**, *6*, 1321.

(3) Gietz, R. D.; Schiestl, R. H. *Nat Protocols* **2007**, *2*, 31.