Supplemental Information

Materials and Methods:

Plasmids:

The plasmid, pRS404 (1), was used in the PCR-based disruption of *SAN1.* The plasmid, pFA6a-13Myc-KanMX6 (2), was used for Myc epitope-tagging of San1, Spt16 and Pob3. For N-terminal HA epitope-tagging at the chromosomal loci of Spt16 and Pob3, the plasmid pFA6a-KanMX6-PGAL1-3HA (2) was used. The plasmid, pFA6a-3HA-TRP1 (2), was used for genomic HA epitope-tagging of Spt16 in Myc-tagged San1 strain. The plasmid, pRS406 (1), was used for growth analysis in the *Δsan1* and wild type strains in solid SC-uracil (plus 2% dextrose) with or without 100 μg/ml 6-AU. The plasmid (pUB221) expressing hexahistidine-tagged ubiquitin was obtained from the Finley laboratory (Daniel Finley, Harvard Medical School).

Strains:

The W303-1a strain that contains ~8 kb long YLR454W coding sequence under the control of *GAL1* promoter was obtained from the Struhl laboratory (Harvard Medical School; 3). The strain, RSY52, was generated by knocking out *SAN1* in W303-1a, using the pRS403 plasmid. *SAN1* was deleted in W303a to generate RSY51, using the pRS406 plasmid. Multiple Myc epitope tags were added at the original chromosomal loci of *SAN1*, *SPT16* and *POB3* in W303a to generate the RSY44, NSY16 and RSY55 strains, respectively. Likewise, multiple Myc epitope tags were added to the original chromosomal loci of *SPT16* and *POB3* in RSY51 to generate RSY53 and RSY54, respectively. The RSY41 strain was generated by PCR-based deletion of *SAN1* in the RSY40 strain (with Myc-tagged Yra1 and HA-tagged Sub2 in W303a background). The RSY42 and RSY43 strains were generated by introducing the pRS406 plasmid into the RSY40 and RSY41 strains, respectively. *PDR5* was deleted in the NSY16 strain (using the pRS403 plasmid) to generate RSY66. Multiple HA epitope tags were added to the original chromosomal locus of *SPT16* in RSY44 to generate RSY58. *SAN1* was deleted in NSY16 (using the pRS404 plasmid) to generate RSY60. The plasmid pUB221 for *CUP1*-inducible expression of hexahistidine-tagged ubiquitin was transformed into the

NSY16 and RSY60 strains to generate RSY59 and RSY61 strains, respectively. Multiple Myc epitope tags were added at the C-terminal of Spt16 in the *rpt4*-ts and wild type strains to generate RSY67 and RSY68, respectively. Similarly, multiple Myc epitope tags were added to the original chromosomal locus of *SAN1* in the *rpb1*-ts mutant strain to generate RSY49. *SAN1* was deleted in the yeast strain bearing Flag-tagged histone H2B (YKH045) to generate JFY8.

ChIP assay:

The ChIP assay was performed as described previously (4, 5). Briefly, yeast cells were treated with 1% formaldehyde, collected and resuspended in lysis buffer. Following sonication, cell lysate (400 µl lysate from 50 ml of yeast culture) was precleared by centrifugation, and then 100 µl lysate was used for each immunoprecipitation. Immunoprecipitated protein–DNA complexes were treated with proteinase K, the cross-links were reversed, and DNA was purified. Immunoprecipitated DNA was dissolved in 20 µl TE 8.0 (10 mM Tris-HCl and 1 mM EDTA, pH 8.0), and 1 µl of immunoprecipitated DNA was analyzed by PCR. The PCR reactions (a total of 23 cycles) contained [α-^{32}P]dATP (2.5 µCi for each 25-µl reaction), and the PCR products were detected by autoradiography after separation on a 6% polyacrylamide gel. As a control, “input” DNA was isolated from 5 µl of lysate without going through the immunoprecipitation step and suspended in 100 µl of TE 8.0. To compare the PCR signal arising from the immunoprecipitated DNA with that from the input DNA, 1 µl of input DNA was used for PCR analysis. Serial dilutions of input and immunoprecipitated DNA samples were used to assess the linear range of PCR amplification.

For ChIP analysis of San1 and Spt16, the above ChIP protocol was modified as described previously (4). Briefly, a total of 800 µl lysate was prepared from 100 ml of yeast culture. Following sonication, 400 µl lysate was used for each immunoprecipitation (using 10 µl of anti-HA or anti-Myc antibody and 100 µl of protein A/G plus agarose beads from Santa Cruz Biotechnology, Inc.), and immunoprecipitated DNA sample was dissolved in 10 µl TE 8.0 of which 1 µl was used for PCR analysis (a total of 23 cycles). In parallel, PCR analysis for input DNA was performed using 1 µl

DNA that was prepared by dissolving purified DNA from 5 µl lysate in 100 µl TE 8.0. The primer pairs used for PCR analysis were as follows:

ADH1 (UAS):	5′-GTTTCCGGGTGTACAATATGG-3′
	5′-CTATTGTATATCTCCCCTCCGC-3′
ADH1 (Core):	5′-GGTATACGGCCTTCCTTCCAGTTAC-3′
	5′-GAACGAGAACAATGACGAGGAAACAAAAG-3′
ADH1 (ORF1):	5′-CTGGTTACACCCACGACGGTTCTT-3′
	5′-GCAGACTTCAAAGCCTTGTAGACG-3′
ADH1 (ORF2):	5′-CGGTAACAGAGCTGACACCAGAGA-3′
	5′-ACGTATCTACCAACGATTTGACCC-3′
PGK1 (Core):	5’-GAATCGTGTGACAACAACAGCCTG-3’
	5’-CTTGCATTGACCAATTTATGC-3’
PGK1 (ORF1):	5’-GAATTGTTGCTGCTTTGCCAACCAT-3’
	5’-GCAATTCCTTAGCAACTGGAGCC-3’
PGK1 (ORF2):	5’AGACGAAGTTGTCAAGAGCTCTGC3’
	5’GAAAGCAACACCTGGCAATTCCT3’
PMA1 (Core):	5’-TCGATGGTGGGTACCGCTTAT-3’
	5’-GATGTTAGACGATAATGATAGGAC-3’
PMA1 (ORF1):	5’-ATCTTCTGACGATGACGATATCG-3’
	5’-TTCTGGAACTGGTCTAGCTTCA-3’
PMA1 (ORF2):	5’TCGGTGCTATGAACGGTATTATG3’
	5’ACATGGTAGCGATGATGTCGACAG3’.
PYK1 (Core):	5’-CTCGCCATCAAAACGATATTCG-3’
	5’-TAACTTTGAAAGGGGACCATG-3’
PYK1(ORF1),	5′-CAGAAAGTCCGAAGAATTGTACCCA-3′
	5′-TACTTGTCATCGGTGGTGAAGATCA-3′;
PYK1 (ORF2):	5’-AAGTTTCCGATGTCGGTAACGCTAT-3’
	5’-TTGGCAAGTAAGCGATAGCTTGTTC-3’
GAL1 (Core):	5′-ATAGGATGATAATGCGATTAGTTTTTTAGCCTT-3′
	5′-GAAAATGTTGAAAGTATTAGTTAAAGTGGTTATGCA-3′

GAL1 (ORF1):	5′-CAGTGGATTGTCTTCTTCGGCCGC-3′
	5′-GGCAGCCTGATCCATACCGCCATT-3′
GAL1 (ORF2):	5′-CAGAGGGCTAAGCATGTGTATTCT-3′
	5′-GTCAATCTCTGGACAAGAACATTC-3′
GAL10 (Core):	5′-GCTAAGATAATGGGGCTCTTTACAT-3′
	5′-TTTCACTTTGTAACTGAGCTGTCAT-3′
GAL10 (ORF1):	5′-CTACGAGATTCCCAAATATGATTCC-3′
	5′-TAACGCAAGATAGCAAACTTCCAAC-3′
GAL10 (ORF2):	5′-TTAATGCGAATCATAGTAGTATCGG-3′
	5′-TTACCAATAGATCACCTGGAAATTC-3′
GAL7 (Core):	5′-CTATGTTCAGTTAGTTTGGCTAGC-3′
	5′-TTGATGCTCTGCATAATAATGCCC-3′
GAL7 (ORF1):	5’-AAAGTGCAATCTGTGAGAGGCAATT-3’
	5’-TTTTCTCTTGCTTCTCTGGAGAGAT-3’
GAL7 (ORF2):	5′-TGAGACCTTGGTCATTTCAAAGAAG-3′
	5′-ATGGATACCCATTGAGTATGGGAAA-3′
YLR454W (5’ORF):	5’-ATGGTGGGGGCTTCATTTCTAAG-3’
	5’-TCAGCAATGATGGTCTGGCCAT-3’
YLR454W (3’ORF):	5’-GAGGGTCACAGATCTATTACTTGC-3’
	5’-AGTTCTTCAGGCTCCGTGTAGGA-3’
Chromosome-V:	5′-GGCTGTCAGAATATGGGGCCGTAGTA-3’
	5′-CACCCCGAAGCTGCTTTCACAATAC-3′

Autoradiograms were scanned and quantitated by the National Institutes of Health image 1.62 program. Immunoprecipitated DNAs were quantitated as the ratio of immunoprecipitate to input, and represented as ChIP signal. The average ChIP signal of the biologically independent experiments is reported with standard deviation (S.D.; Microsoft Excel 2003). The Student's t test of Microsoft Excel 2003 (with tail = 2 and types = 3) was used to determine the p values for statistical significance of the change in the ChIP signals. The changes were considered to be statistically significant at $p < 0.05$. ORF, open reading frame; UAS, upstream activating sequence; Core, core

promoter; and ORF1 and ORF2, two different locations in the coding sequence towards the 5'- and 3'-ends, respectively.

Total RNA preparation:

Total RNA was prepared from yeast cell culture following the standard protocol. Briefly, 10 ml yeast culture of a total OD_{600} of 1.0 in YPD was harvested, and then suspended in 100 µl RNA preparation buffer (500 mM NaCl, 200 mM Tris–HCl, 100 mM Na_2EDTA, and 1% SDS) along with 100 µl phenol/chloroform/isoamyl alcohol and 100 µl volume equivalent of glass beads (acid washed; Sigma). Subsequently, yeast cell suspension was vortexed with a maximum speed (10 in a VWR mini-vortexer; cat. no. 58816-121) five times (30 s each). The cell suspension was placed in ice for 30 s between pulses. After vortexing, 150 µl RNA preparation buffer and 150 µl phenol/chloroform/isoamyl alcohol were added to the yeast cell suspension followed by vortexing for 15 s with maximum speed on a VWR mini-vortexer. The aqueous phase was collected following 5 min of centrifugation at maximum speed in a microcentrifuge machine. The total RNA was isolated from the aqueous phase by precipitation with ethanol.

RT-PCR analysis:

RT-PCR analysis was performed according to the standard protocols. Briefly, total RNA was prepared from 10 ml of yeast culture. Ten micrograms of total RNA was used in the reverse transcription assay for both wild type and mutant strains. RNA was treated with RNase-free DNase (M610A, Promega) and then reverse-transcribed into cDNA using oligo(dT) as described in the protocol supplied by Promega (A3800, Promega). PCR was performed using synthesized first strand as template and the primer pairs targeted to the *ADH1, PGK1, PYK1*, *PMA1, GAL1, GAL7* and *GAL10* ORFs as well as 18S rRNA. RT-PCR products were separated by 2.2% agarose gel electrophoresis and visualized by ethidium bromide staining. The primer pairs used in the PCR analysis were as follows:

ADH1: 5'-CGGTAACAGAGCTGACACCAGAGA-3'

	5’-ACGTATCTACCAACGATTTGACCC-3’
PYK1:	5’-AAGTTTCCGATGTCGGTAACGCTAT-3’
	5’-TTGGCAAGTAAGCGATAGCTTGTTC-3’
PGK1:	5’-AGACGAAGTTGTCAAGAGCTCTGC-3’
	5’-GAAAGCAACACCTGGCAATTCCT-3’
PMA1:	5’-TCGGTGCTATGAACGGTATTATG-3’
	5’-ACATGGTAGCGATGATGTCGACAG-3’
GAL1:	5′-CAGAGGGCTAAGCATGTGTATTCT-3′
	5′-GTCAATCTCTGGACAAGAACATTC-3′
GAL7:	5′-TGAGACCTTGGTCATTTCAAAGAAG-3′
	5′-ATGGATACCCATTGAGTATGGGAAA-3′
GAL10:	5′-TTAATGCGAATCATAGTAGTATCGG-3′
	5′-TTACCAATAGATCACCTGGAAATTC-3′
18S rRNA:	5′-GAGTCCTTGTGGCTCTTGGC-3′
	5′-AATACTGATGCCCCCGACC-3′

The RT-PCR experiments were carried out three times. These experiments are biologically independent. The average signal of these biologically independent experiments is reported with S.D. (Microsoft Excel 2003). The Student's t test (with tail = 2 and types = 3) was used to determine p values for statistical significance of the change in the RT-PCR signals. The changes were considered to be statistically significant at $p < 0.05$.

Growth analysis of the *Δsan1* and wild type strains in the presence of 6-AU, HU and MMS:

The growth of the *Δsan1* and wild type cells was analyzed on solid SC-uracil (plus 2% dextrose) media with or without 100 μg/ml 6-AU. Both wild type and *Δsan1* strains were transformed with a low copy number plasmid (pRS406) expressing the *URA3* gene, inoculated in liquid SC-uracil medium (with 2% dextrose), and grown up to an OD_{600} of 0.2 at 30 ^{0}C. Subsequently, yeast cells were suspended in fresh liquid SC-uracil medium (with 2% dextrose), and grown up to an OD_{600} of 0.4 prior to spotting (3

μl) on solid SC-uracil medium (plus 2% dextrose) with or without 100 μg/ml 6-AU. Yeast cells were spotted with serial dilutions, grown at 30 °C, and photographed after 2 or 3 days. Growth analysis was carried out in biological triplicates, and consistent results were obtained. One representative set is included in Figure 1F.

The growth of the *Δsan1* and wild type cells was analyzed on solid YPD media with or without 0.026% MMS (129925–5G; Sigma). Yeast cells were inoculated in liquid YPD and grown up to an OD_{600} of 0.2 at 30 °C. Subsequently, yeast cells were suspended in fresh YPD medium and grown up to an OD_{600} of 0.4 at 30 °C prior to spotting (3 μl) on solid medium with serial dilutions. Yeast cells were grown at 30 °C and photographed after 2, 3 or 4 days. Growth analysis was carried out in biological duplicates, and consistent results were obtained. One representative set is included in Figure 6G.

The growth of the *Δsan1* and wild type cells was analyzed on solid YPD media with or without 100 mM HU. Yeast cells were inoculated in YPD and grown up to an OD_{600} of 0.2 at 30 °C. Subsequently, yeast cells were suspended in fresh YPD medium and grown up to an OD_{600} of 0.4 at 30 °C prior to spotting (3 μl) on solid medium with serial dilutions. Yeast cells were grown at 30 °C and photographed after 2, 3 or 4 days. Growth analysis was carried out in biological duplicates, and consistent results were obtained. One representative set is included in Figure 6G. Similarly, experiments were carried out using yeast strain expressing Spt16 under the *GAL1* promoter in solid YPG media with or without 6-AU, MMS and HU.

Ubiquitylation assay:

The ubiquitylation assay was performed as described previously (6, 7). Briefly, the expression of hexahistidine-tagged ubiquitin from plasmid pUB221 was induced for 6 hr by addition of $CuSO_4$ to a final concentration of 0.1 mM. Cells were harvested, suspended in buffer A2 (6 M guanidine-HCl, 100 mM Na_2HPO_4/NaH_2PO_4 at pH 8.0, 10 mM imidazole, 250 mM NaCl, 0.5% NP-40), and lysed by glass beads. Cell lysate was clarified by centrifugation, and supernatant was incubated with Ni^{2+}-NTA agarose resin (Qiagen) for 2 h at room temperature. Following incubation, Ni^{2+}-NTA resin was washed twice by buffer A2, twice by buffer A2/T2 (1 volume of buffer A2 and 3 volumes of buffer

T2), and twice by buffer T2 (50 mM Na_2HPO_4/NaH_2PO_4 at pH 8.0, 250 mM NaCl, 20 mM imidazole, 0.5% NP-40). Subsequently, the resin was washed by buffer T2 containing 50 mM histidine to reduce the level of nonspecific binding to the resin. Finally, hexahistidine-tagged ubiquitin/ubiquitylated-proteins were eluted with 2X SDS loading buffer containing 250 mM imidazole and analyzed by Western blot assay.

Formaldehyde-based *in vivo* cross-linking and co-IP assay:

The co-IP assay was performed as described previously (8). Briefly, yeast strain carrying Myc-tagged San1 and HA-tagged Spt16 was grown in YPD up to an OD_{600} of 1.0, and then cross-linked by formaldehyde. WCE was prepared by lysing and sonicating the cross-linked yeast cells. Immunoprecipiation was performed using an anti-Myc antibody and protein A/G plus agarose beads. Anti-Flag was used as a non-specific antibody. After immunoprecipitaion, the agarose beads were washed as in the ChIP assay. The washed A/G plus agarose beads were boiled in the SDS-PAGE loading buffer, and supernatant was analyzed by SDS-PAGE and western blot. An anti-HA-peroxidase antibody was used in the western blot analysis. The input is 1.25% of the lysate that was used for immunoprecipitation.

Immunopurification of Spt16 and Pob3:

The yeast strain expressing HA-tagged Spt16 or Pob3 was grown in 100 ml YPD up to an OD_{600} of 1.0, and then was harvested. Subsequently, 800 µl WCE was prepared from the culture of each strain. Immunoprecipitation was performed using anti-HA antibody and protein A/G plus agarose beads for 4 h at 4°C. 400 µl WCE, 100 µl protein A/G plus agarose beads (~50 µl bed volume) and 10 µl anti-HA antibody were used for each immunoprecipitation. The agarose beads following immunoprecipitation were washed under high stringent washing conditions as in the ChIP assay (4, 5), but 0.5 M NaCl, instead of 1 M NaCl, was used in the second and third washes of the beads. Then the beads were equilibrated by buffer E (50 mM Tris-HCl, 250 mM NaCl, 1% NP-40, 1 mM EDTA; pH 7.5) before elution of HA-tagged protein by HA peptide. The immobilized protein (HA-tagged Spt16 or Pob3) on A/G plus agarose beads was eluted by incubating the beads in two bed volumes (100 µl) of buffer E containing HA peptide

with a final concentration of 1 mg/ml and aprotinin (10 µg/ml). The beads were incubated for 30 min at 25°C on rotor. Elution was performed three times. Buffer E (100 µl) containing HA peptide and aprotinin was used for each elution.

Figure Legends:

Supplementary Figure S1: Growth analysis of Myc epitope-tagged strains in solid **(A)** and liquid **(B-D)** YPD growth media at 30 ^{o}C.

Supplementary Figure S2: San1 associates with the coding sequence of *ADH1* to enhance the engagement of elongating RNA polymerase II into transcription. **(A)** ChIP analysis of San1 association with *ADH1*. Yeast cells expressing Myc-tagged San1 were grown in YPD at 30° up to an OD_{600} of 1.0 prior to formaldehyde-based *in vivo* crosslinking. Immunoprecipitated DNA was analyzed by PCR using the primer pairs targeted to different locations of *ADH1*. **(B)** ChIP analysis at the *ADH1* core and ORF2 in the yeast strain that does not bear Myc or HA-tagged proteins, using anti-Myc and anti-HA antibodies. **(C)** ChIP analysis of association of Rpb1 with *ADH1* in the *∆san1* and wild type strains, using 8WG16 antibody (Covance) against the carboxy-terminal domain of Rpb1. **(D)** Western blot analysis for Rpb1 and Actin in the wild type and *∆san1* strains, using 8WG16 antibody (Covance) against the carboxy-terminal domain of Rpb1 and an anti-actin antibody. **(E)** ChIP analysis of association of TBP with the *ADH1* core promoter in the *∆san1* and wild type strains, using an anti-TBP antibody against TBP (obtained from Michael R. Green; University of Massachusetts Medical School). **(F)** RT-PCR analysis of *ADH1* mRNA levels in the *∆san1* and wild type strains. **(G)** Growth analysis of the *∆san1* and wild type strains in liquid YPD medium at 30 ^{0}C.

Supplementary Figure S3: Both increased and decreased abundances of Spt16 are associated with reduced association of RNA polymerase II with *ADH1*, and hence transcription. **(A and B)** ChIP analysis of Rpb1 association with *ADH1* core promoter and coding sequence (ORF2) following overexpression of Spt16 (that is under the promoter of *GAL1*; P_{GAL1}-*SPT16*) in YPG. **(C)** RT-PCR analysis of *ADH1* mRNA following overexpression of Spt16 in YPG. **(D and E)** ChIP analysis of Rpb1 association

with the *ADH1* coding sequence (ORF2) and core promoter following underexpression of Spt16 (that is under the promoter of *GAL1*; P_{GAL1}-*SPT16*) in YPD. **(F)** RT-PCR analysis of *ADH1* mRNA following underexpression of Spt16 in YPD.

Supplementary Figure S4: San1 promotes chromatin reassembly at the coding sequence of *GAL1*. **(A)** ChIP analysis of histone H3 levels at the *GAL1* coding sequence (toward the 3'-end) in the *Δsan1* and wild type strains after switching off transcription in dextrose-containing growth medium. **(B)** ChIP analysis of histone H3 levels at the *GAL1* coding sequence in the *Δsan1* and wild type strains after switching on transcription in galactose-containing growth medium. **(C)** ChIP analysis of histone H3 levels at the *GAL1* core promoter in the *Δsan1* and wild type strains after switching off transcription in dextrose-containing growth medium. **(D)** ChIP analysis of histone H3 levels at the *GAL1* core promoter in the *Δsan1* and wild type strains after switching on transcription in galactose-containing growth medium.

References:

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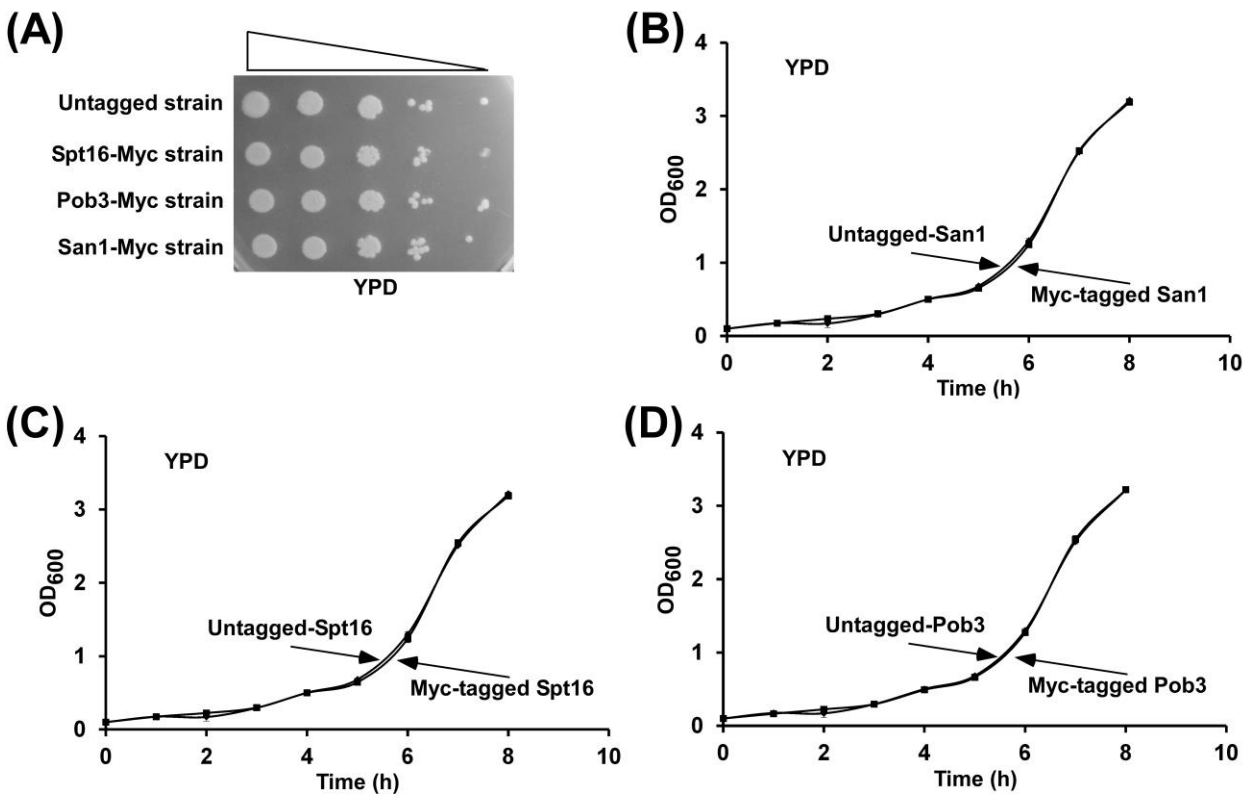
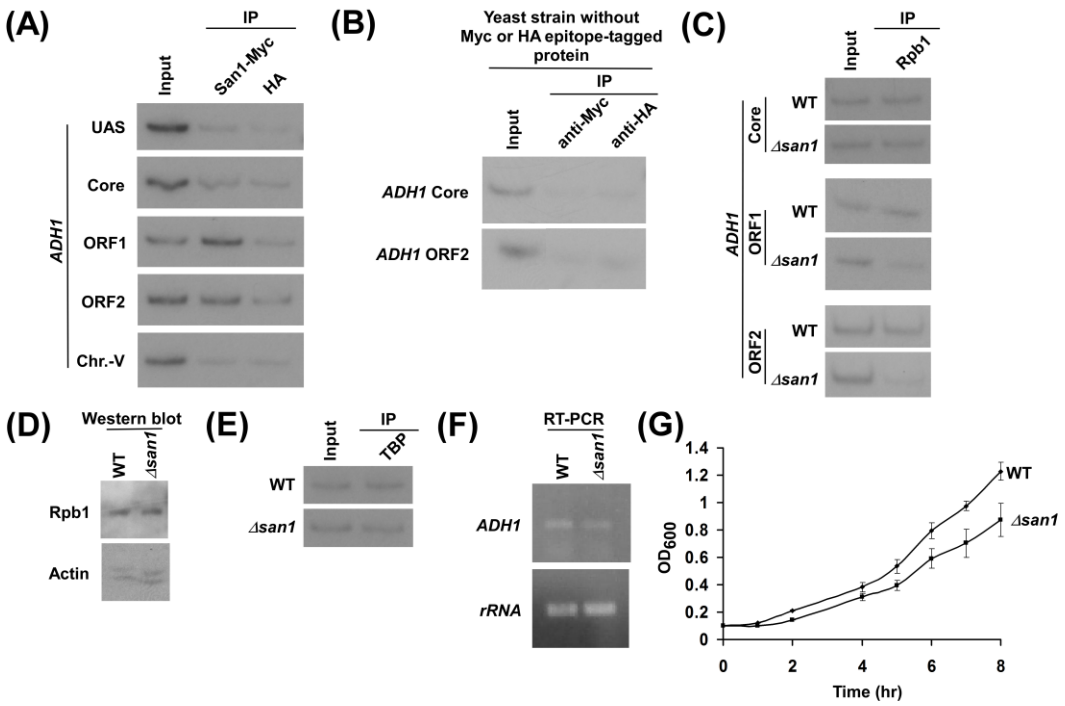
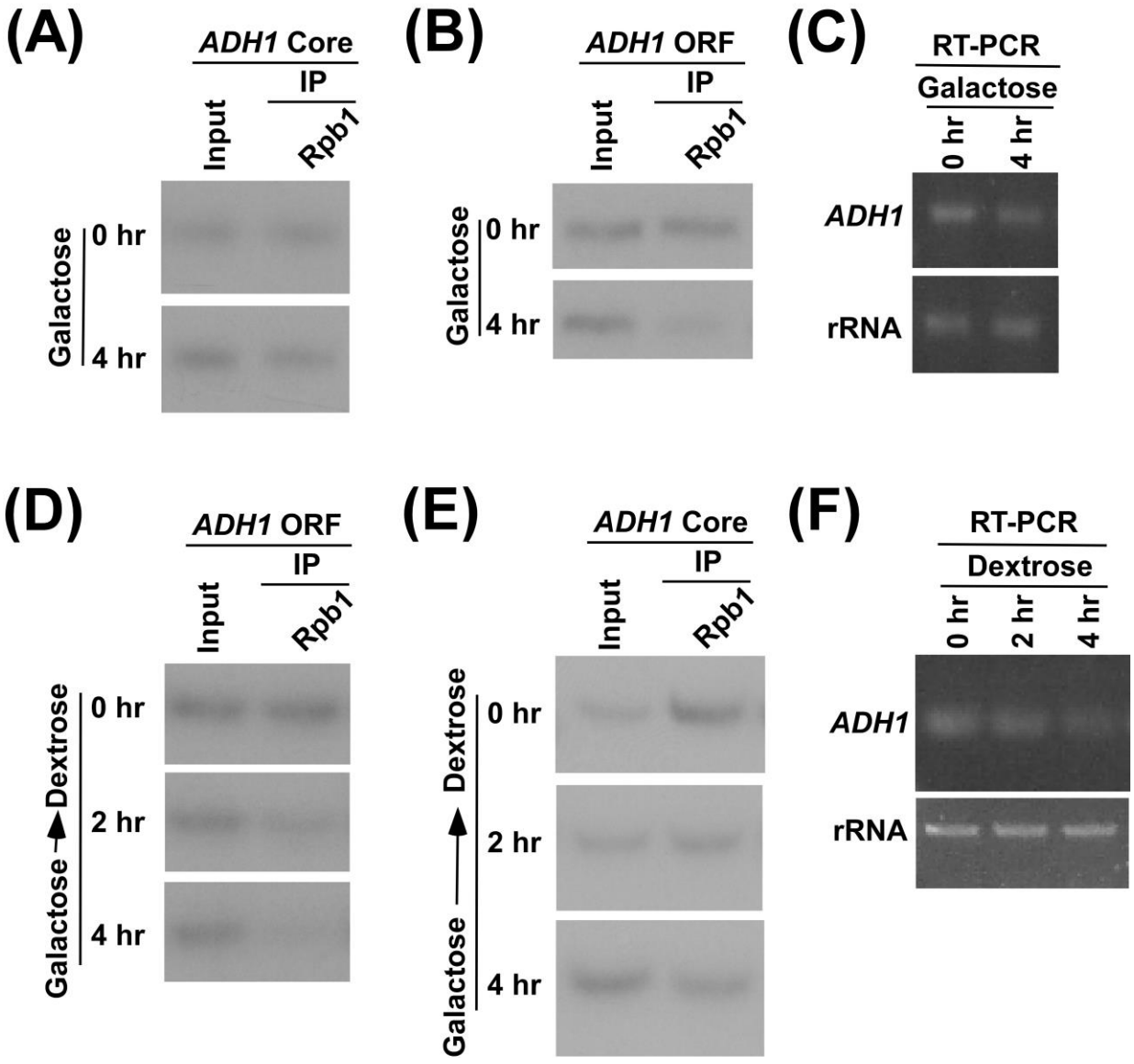
Supplementary Figure S1:



Supplementary Figure S2:



Supplementary Figure S3:



Supplementary Figure S4: