

## Supplemental Information (Li et al.)

### Supplemental Table S1. Genes closest to Sir2-dependent condensin peaks.

(See Excel file).

### Supplemental Table S2. Yeast Strains

Strains	Genotype	Source
ML1	<i>MATa his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167</i>	[1]
ML25B12	<i>MATα his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167</i>	This study
ML25	<i>MATa his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167 sir2Δ::kanMX4</i>	[1]
ML26	<i>MATa his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167 sir3Δ::kanMX4</i>	[1]
ML27	<i>MATa his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167 sir4Δ::kanMX4</i>	[1]
ML28	<i>MATα his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167 sir2Δ::kanMX4</i>	This study
ML149	<i>MATa his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167 BRN1::13xMyc-kanMX4</i>	[2]
ML152	<i>MATa his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167 SMC4::13xMyc-kanMX4</i>	[2]
ML160	<i>MATa his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167 SMC4::13xMyc-kanMX4 sir2Δ::natMX4</i>	[2]
ML161	<i>MATa his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167 BRN1::13xMyc-kanMX4 sir2Δ::natMX4</i>	[2]
ML195	<i>MATa his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167 sir2Δ::kanMX4 [pRS315]</i>	This study
ML196	<i>MATa his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167 sir2Δ::kanMX4 [pRS315-SIR2]</i>	This study
ML197	<i>MATa his3Δ200 leu2Δ1 met15Δ0 trp1Δ63 ura3-167 sir2Δ::kanMX4 [pRS315-sir2-H364Y]</i>	This study
ML275	ML1 deleted for the 100bp Sir2/condensin binding site (100bpΔ)	This study
ML279	ML275 made <i>sir2Δ::kanMX4</i>	This study
ML286	ML25B12 deleted for the 100bp Sir2/condensin binding site (100bpΔ)	This study
ML322	ML149 deleted for the 100bp Sir2/condensin binding site (100bpΔ)	This study

ML337	<i>MAT<math>\alpha</math> his3<math>\Delta</math>200 leu2<math>\Delta</math>1 met15<math>\Delta</math>0 trp1<math>\Delta</math>63 ura3-167 BRN1::13xMyc-kanMX4</i>	This study
ML339	<i>MAT<math>\alpha</math> his3<math>\Delta</math>200 leu2<math>\Delta</math>1 met15<math>\Delta</math>0 trp1<math>\Delta</math>63 ura3-167 SMC4::13xMyc-kanMX4</i>	This study
ML341	<i>MAT<math>\alpha</math> his3<math>\Delta</math>200 leu2<math>\Delta</math>1 met15<math>\Delta</math>0 trp1<math>\Delta</math>63 ura3-167 dps2<math>\Delta</math></i>	This study
ML343	<i>MAT<math>\alpha</math> his3<math>\Delta</math>200 leu2<math>\Delta</math>1 met15<math>\Delta</math>0 trp1<math>\Delta</math>63 ura3-167 sir2<math>\Delta</math>::kanMX4 hml<math>\Delta</math>::LEU2</i>	[1]
ML344	<i>MAT<math>\alpha</math> his3<math>\Delta</math>200 leu2<math>\Delta</math>1 met15<math>\Delta</math>0 trp1<math>\Delta</math>63 ura3-167 hml<math>\Delta</math>::LEU2</i>	[1]
ML350	<i>MAT<math>\alpha</math> his3<math>\Delta</math>200 leu2<math>\Delta</math>1 met15<math>\Delta</math>0 trp1<math>\Delta</math>63 ura3-167 BRN1::13xMyc-kanMX4 hml<math>\Delta</math>::LEU2</i>	This study
ML351	<i>MAT<math>\alpha</math> his3<math>\Delta</math>200 leu2<math>\Delta</math>1 met15<math>\Delta</math>0 trp1<math>\Delta</math>63 ura3-167 BRN1::13xMyc-kanMX4 sir2<math>\Delta</math>::kanMX4 hml<math>\Delta</math>::LEU2</i>	This study
ML419	<i>MAT<math>\alpha</math> his3<math>\Delta</math>200 leu2<math>\Delta</math>1 met15<math>\Delta</math>0 trp1<math>\Delta</math>63 ura3-167 RDT1::13xMyc-kanMX4</i>	This study
ML432	ML419 deleted for the 100bp Sir2/condensin binding site (100bp $\Delta$ )	This study
ML433	<i>MAT<math>\alpha</math> his3<math>\Delta</math>200 leu2<math>\Delta</math>1 met15<math>\Delta</math>0 trp1<math>\Delta</math>63 ura3-167 RDT1::13xMyc-kanMX4</i>	This study
ML440	ML1 [pGAL-HO-URA3]	This study
ML444	ML149 [pGAL-HO-URA3]	This study
ML523	<i>MAT<math>\alpha</math> his3<math>\Delta</math>200 leu2<math>\Delta</math>1 met15<math>\Delta</math>0 trp1<math>\Delta</math>63 ura3-167 BRN1::13xMyc-kanMX4 dps2<math>\Delta</math></i>	This study
ML557	XW652 sir2 $\Delta$ ::kanMX4	This study
XW652	<i>MAT<math>\alpha</math> ho ade3::GAL::HO HML<math>\alpha</math>RE HMR<math>\alpha</math>-B ura3-52 lys5 leu2-3,112 trp1::hisG</i>	[3]
XW676	<i>MAT<math>\alpha</math> ho ade3::GAL::HO HML<math>\alpha</math>RE<math>\Delta</math>::URA3 HMR<math>\alpha</math>-B ade1 leu2 trp1 ura3-52</i>	[3]
SY742	XW652 deleted for the 100bp Sir2/condensin binding site (100bp $\Delta$ )	This study
NBY8	<i>MAT<math>\alpha</math> ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1<math>\Delta</math> lys2<math>\Delta</math></i>	[4]
NBY316	<i>MAT<math>\alpha</math> ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1<math>\Delta</math> lys2<math>\Delta</math> ycs4-1</i>	[4]
NBY585	<i>MAT<math>\alpha</math> ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1<math>\Delta</math> lys2<math>\Delta</math> hml<math>\Delta</math>::LEU2</i>	[4]
NBY319	<i>MAT<math>\alpha</math> ura3-1 leu2-3,112 his3-11 trp1-1 ade2-1 can1-100 bar1<math>\Delta</math> lys2<math>\Delta</math> hml<math>\Delta</math>::LEU2 ycs4-1</i>	[4]
RF15	<i>MAT<math>\alpha</math> his3<math>\Delta</math>200 leu2<math>\Delta</math>1 met15<math>\Delta</math>0 trp1<math>\Delta</math>63 ura3-167[pRPL25NLS-GFP]</i>	This study
RF25	<i>MAT<math>\alpha</math> his3<math>\Delta</math>200 leu2<math>\Delta</math>1 met15<math>\Delta</math>0 trp1<math>\Delta</math>63 ura3-167 sir2<math>\Delta</math>::kanMX4[pRPL25NLS-GFP]</i>	This study
MD25	<i>MAT<math>\alpha</math> leu2<math>\Delta</math>1 met15<math>\Delta</math>0 trp1<math>\Delta</math>63 his3<math>\Delta</math>200::pGPD1-Os TIR-</i>	This study

	<i>HIS3 BRN1-3V5-AID2:KanMx6</i> [pGAL-HO-URA3]	
MD27	XW652 made <i>leu2Δ1::pGPD1-Os TIR-LEU2 BRN1::3V5-AID2-KanMX6</i>	This study

**Supplemental Table S3. Oligonucleotides**

Oligo name	Oligo description	DNA sequence
JS301	<i>MAT</i> $\alpha$ PCR primer-1	AGTCACATCAAGATCGTTTATGG
JS302	<i>MAT</i> $\alpha$ PCR primer-2	GCACGGAATATGGGACTACTTCG
JS467	<i>KanMX</i> 5'-out detection	TACGGGCGACAGTCACATCATG
JS1896	<i>SPBI</i> ORF (FW)	CATCGAAGTTAAGGACGACGC
JS1897	<i>SPBI</i> ORF (RV)	TCGCGCTTGACATTTAGACG
JS1898	Sir2 binding site 100 bp (FW)	TGTTTGCAAGATGGTGCTTTTT
JS1899	Sir2 binding site 100 bp (RV)	AGGAGCAGAAACGTGGCAAT
JS1909	<i>HML-I</i> silencer (ARS302) (FW)	AACATGAAAGCCCGACGTTT
JS1910	<i>HML-I</i> silencer (ARS302) (RV)	AATAATCGGGTGAAAAGAGGATAT
JS2127	<i>BRNI</i> _Degron_FW	AGTGAATTATGAGGATCTAGCGACAACACAGGCAGCG TCACGGATCCCCGGGTTAATTA
JS2128	<i>BRNI</i> _Degron_RV	GCACAAAAAAAAAAAAAAAAAAAAAAAAAAGATCA TCAAGAATTCGAGCTCGTTTAAAC
JS2167	3C <i>PDCI</i> intergenic FWD	GCCGACAGTCTGTTGAATTGG
JS2168	3C <i>PDCI</i> intergenic REV	GAAGCGGACCCAGACTTAAGC
JS2342	Sir2 binding site 100bp $\Delta$ pCORE (FW)	GACTTACAAGCACACCTTTGAATTATTTTTGTTCTCTAT GTCCTTACCATTAAGTTGATC
JS2343	Sir2 binding site 100bp $\Delta$ pCORE (RV)	TATATAGCTATTCATCAATTGAAATATTCATTTTATAAG T GAGCTCGTTTTTCGACACTGG
JS2444	pCORE 100bp $\Delta$ replacement (FW)	GACTTACAAGCACACCTTTGAATTATTTTTGTTCTCTAT GACTTATAAAATGAATATTTCAATTGATGAATAGCTAT ATA
JS2445	pCORE 100bp $\Delta$ replacement (RV)	TATATAGCTATTCATCAATTGAAATATTCATTTTATAAG TCATAGAGAACAAAATAATTCAAAGGTGTGCTTGTA AGTC
JS2494	<i>RDTI</i> upstream (-258 bp) (FW)	CGCGTTTAAAGACTTACAAGCAC
JS2496	<i>RDTI</i> upstream	TTAAATACATGCTGCAGTTTTTCG

	(-44 bp) (RV)	
JS2517	<i>RDT1</i> upstream (-19 bp) (FW)	AAAACCTGCAGCATGTATTTAATCG
JS2518	<i>RDT1</i> downstream (+19 bp) (RV)	TGCTTTCGATTATTTCTGGTTCT
JS2574	Yalpha105F	GCCCACTTCTAAGCTGATTTCAATCTCTCC
JS2575	MATdist-4R	CCTGTTCTTAGCTTGTACCAGAGG
JS2583	<i>RDT1</i> -13xMyc_FW	AATTCTATTTGTCCAGCAATCCGGCGCAAAGAAGACTA CCGGATCCCCGGGTTAATTAA
JS2584	<i>RDT1</i> -13xMyc_RV	TTTCGATTATTTCTGGTTCTAGAAATTTTCAATACCCT GAATTCGAGCTCGTTTAAAC
JS2585	<i>RDT1</i> detection	TCTATTTGTCCAGCAATCCG
JS2656	3C <i>HindIII</i> <i>HML</i>	TTCCGAAAACCACGACGAACCAG
JS2658	3C <i>HindIII</i> <i>HMR</i>	ATGGGTCATTCTAGGTCATTCTAC
JS2665	<i>SCR1</i> ORF (33 bp) (FW)	CGTTGAGAATTCTGGCCGAG
JS2666	<i>SCR1</i> ORF (468 bp) (RV)	GTAAATCCTGATGGCACCGC
JS2669	KanC3	CCTCGACATCATCTGCCAGAT
JS2703	<i>HO</i> cut-site 600 bp (FW)	TTGGATCTTAACAAACCGTAAAGGT
JS2704	<i>HO</i> cut-site 600 bp (RV)	GGTAACTAGCAAACAAAGGAAAGTCA
JS2712	MATa detection (FW)	TTGCAACAACCTTCTTCTCCTCA
JS2715	Alpha2 ORF_FW	TTGGTTTGCAAAGAACATCG
JS2716	Alpha2 ORF_RV	CTTCTTTGCCAGAGGCTCAC
JS2777	R1S/R1L ncRNA (FW)	CGTGCAAACAGTATTCCGGC
JS2778	R1L/R1S ncRNA (RV)	GCGCTGGTTGTTATTGGCAA

## Supplemental Figure Legends

**Fig S1. *MATa*-specific transcription of *RDT1* is repressed by Sir2 and Hst1.** (A) IGV screenshot of compiled raw RNA-seq read data from BY4741 (*MATa*) and BY4742 (*MAT $\alpha$* ) strains. The top two blue peaks represent Smc4-myc and Sir2-myc ChIP-seq reads. (B) Quantitative ChIP assay showing additional SIR complex subunit enrichment at the *RDT1* promoter. (C) RT-qPCR showing effects of deleting *SIR2* and/or *HST1* on *RDT1* expression when HML is present or deleted (\* $p < 0.05$ , \*\* $p < 0.005$ ).

**Fig S2. Deletion of Sir2 or the *RDT1* promoter Sir2/condensin binding site does not affect protein levels of Sir2 or Myc-tagged condensin subunits.** (A) Western blot showing steady state Sir2 protein levels in WT (ML1), *sir2 $\Delta$*  (ML25), and 100bp $\Delta$  (ML275) strains. (B) Western blot with anti-Myc detection of Brn1-13xMyc or Sir2 in WT (ML149), *sir2 $\Delta$*  (ML161), and 100bp $\Delta$  (ML322) strains. (C) Western blot with anti-Myc detection of Smc4-13xMyc or Sir2 in WT (ML152), *sir2 $\Delta$*  (ML160), and 100bp $\Delta$  version.

**Fig S3. The *RDT1*-proximal Mcm1/a2 binding site (DPS2) is important for Sir2 and condensin recruitment.** (A) Schematic diagram depicting the location of the DPS2 sequence deletion relative to other elements with the RE, with the deleted chromosome III coordinates indicated in red. (B) Quantitative ChIP of native Sir2 in WT and *dps2 $\Delta$*  strains. (C) Quantitative ChIP of Brn1-Myc in WT and *dps2 $\Delta$*  strains. @*RDT1* promoter indicates enrichment at the Sir2/condensin peak (\*\* $p < 0.005$ ).

**Fig S4. Deleting the Sir2/condensin binding site within the RE (100bp $\Delta$ ) does not alter Sir2 function at *HML $\alpha$* . (A)** Quantitative mating assay for WT (ML1) and 100bp $\Delta$  (ML275) strains. **(B)** Quantitative ChIP assay showing Sir2 enrichment at *HML-I* in WT (ML1) and 100bp $\Delta$  (ML275) strains. (\*\*p<0.005).

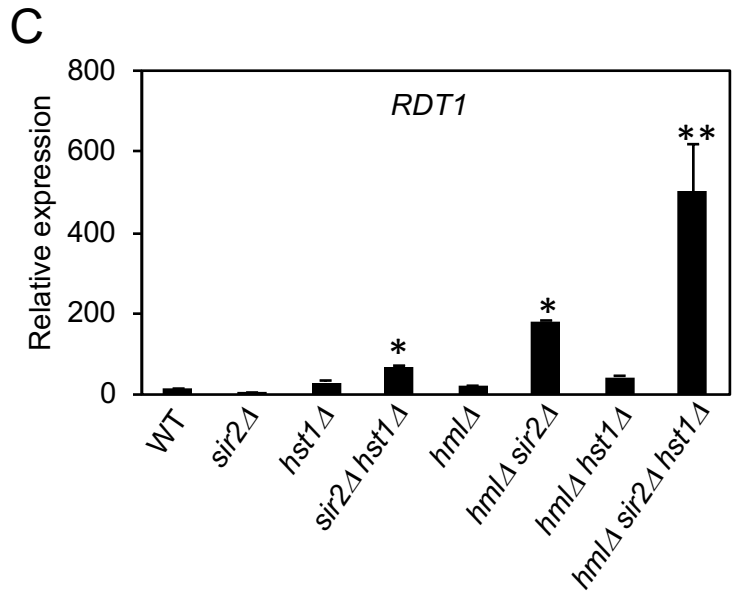
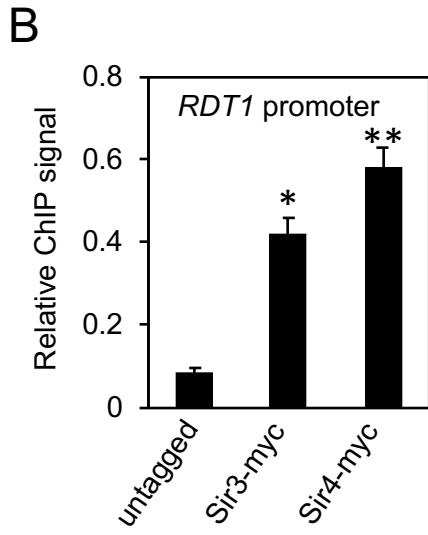
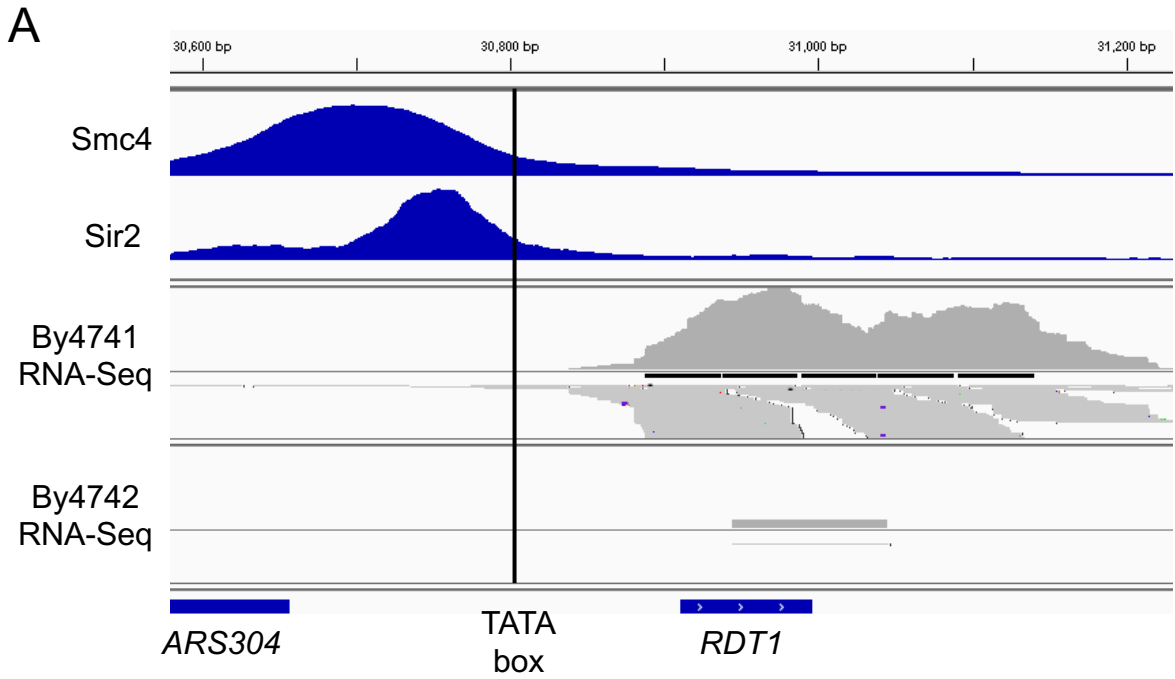
**Fig S5. Auxin inducible degron (AID)-mediated depletion of Brn1 does not derepress *RDT1* or *HML $\alpha$* . (A)** Western blot time course of auxin induced degradation of Brn1::V5-AID. Time indicates minutes after addition of auxin. **(B)** RT-qPCR of *RDT1* expression following 30 or 60 minutes of Brn1 depletion by auxin. **(C)** RT-qPCR of *HMLALPHA2* expression following 30 or 60 min of Brn1 depletion by auxin.

## SUPPLEMENTAL REFERENCES

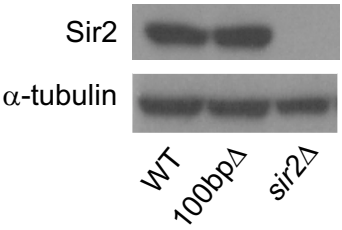
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2. Li M, Valsakumar V, Poorey K, Bekiranov S, Smith JS. Genome-wide analysis of functional sirtuin chromatin targets in yeast. *Genome Biol.* 2013;14(5):R48. PMID: 23710766.
3. Li J, Coic E, Lee K, Lee CS, Kim JA, Wu Q, et al. Regulation of budding yeast mating-type switching donor preference by the FHA domain of Fkh1. *PLoS Genet.* 2012;8(4):e1002630. PMID: 22496671.

4. Bhalla N, Biggins S, Murray AW. Mutation of YCS4, a budding yeast condensin subunit, affects mitotic and nonmitotic chromosome behavior. *Mol Biol Cell*. 2002;13(2):632-45. PMID: 11854418.

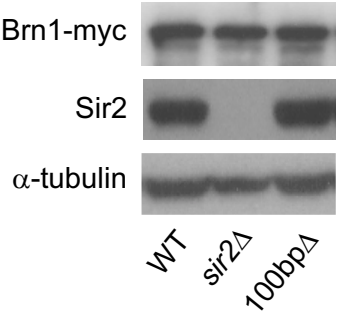




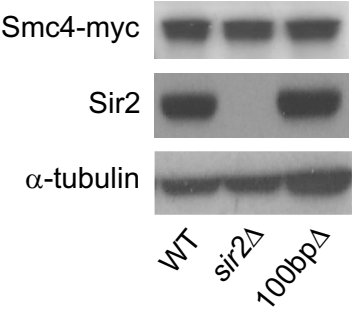
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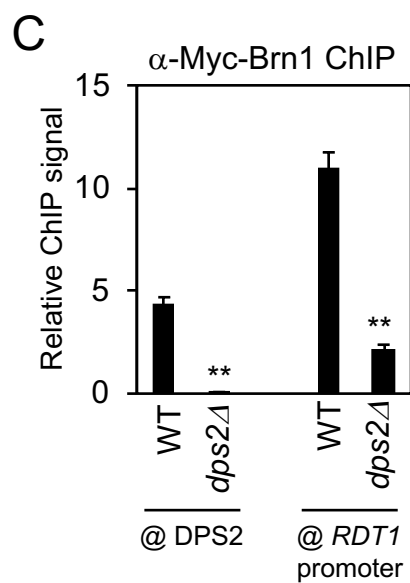
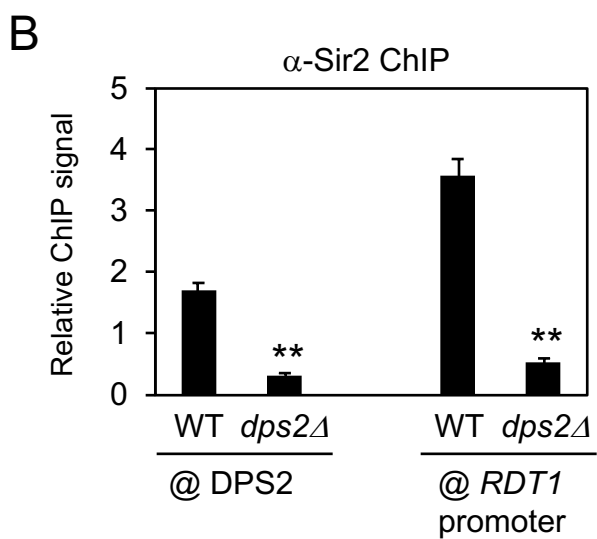
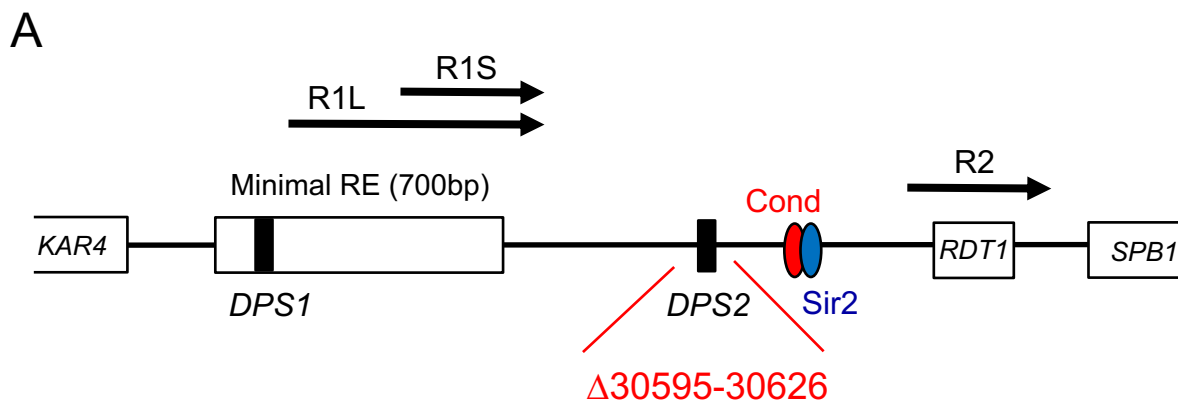


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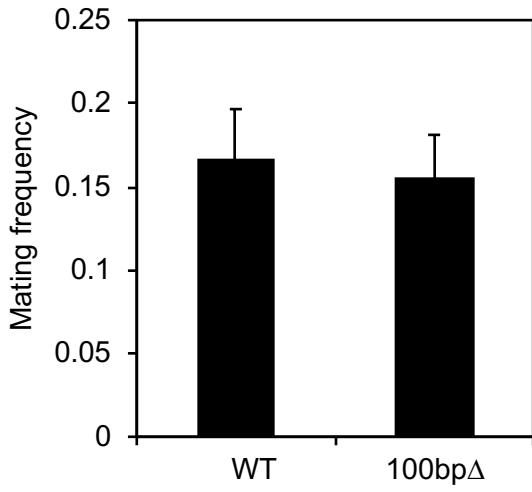


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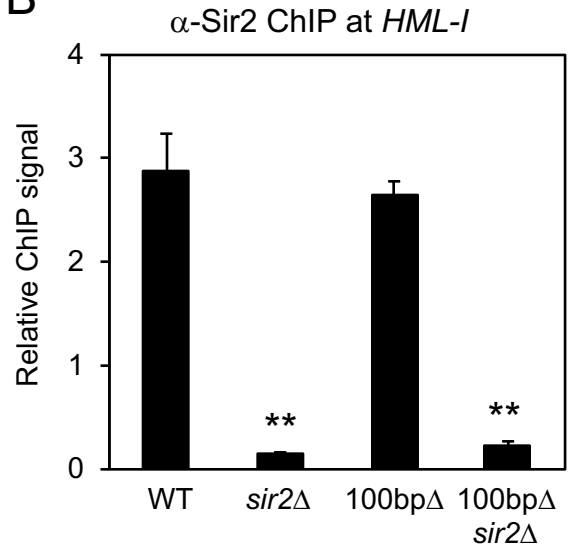




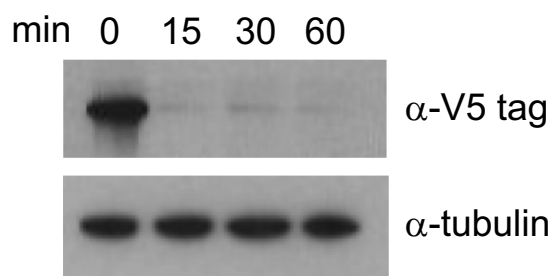
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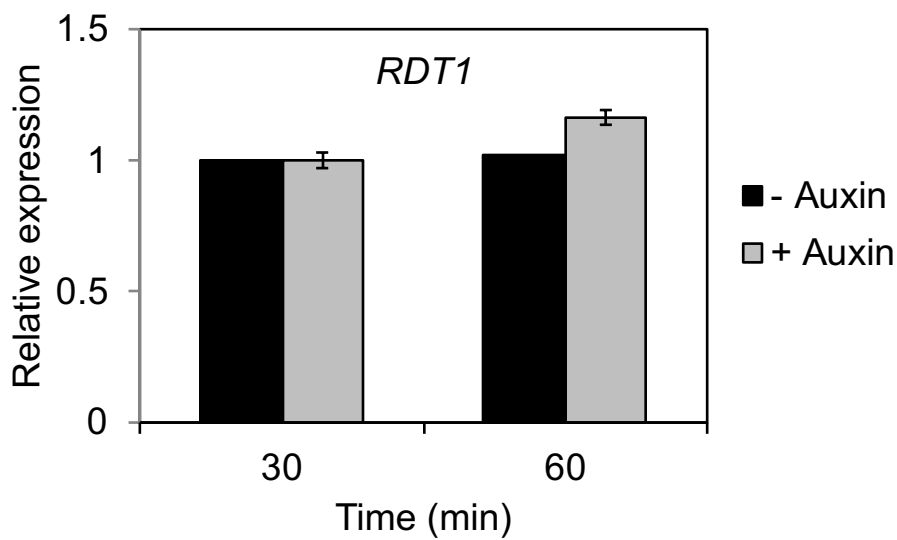
B



A



B



C

