

Supplemental Material

Accumulation of RNA on chromatin disrupts heterochromatic
silencing

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Supplemental Table S1: Strains used in this study

65	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1</i>	SPY797
80	h+ leu1-32 ade6-210 ura4DS/E otrR(SphI)::ura4+ <i>clr4Δ</i> ::kanMX6	SPY815
34	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 dcr1Δ</i> ::hphMX6	
260	h+ otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 <i>ago1Δ</i> ::kanMX6	SPY418
510	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 caf1Δ</i> ::kanMX6	
521/522	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 caf1Δ</i> ::kanMX6 <i>dcr1Δ</i> ::hphMX6	
523/524	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1Δ</i> ::hphMX6 <i>caf1Δ</i> ::kanMX6	
544	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 ccr4Δ</i> ::hphMX6	
577	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 ccr4Δ</i> ::hphMX6 <i>dcr1Δ</i> ::kanMX6	
1023	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 mot2Δ</i> ::kanMX6	
997	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 dcr1Δ</i> ::hphMX6 <i>mot2Δ</i> ::kanMX6	
651	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 not2Δ</i> ::kanMX6	
654	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 not2Δ</i> ::kanMX6 <i>dcr1Δ</i> ::hphMX6	
728	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 rcd1Δ</i> ::kanMX6	
729	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 rcd1Δ</i> ::kanMX6 <i>dcr1Δ</i> ::hphMX6	
735	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 caf16Δ</i> ::kanMX6	
736	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 caf16Δ</i> ::kanMX6 <i>dcr1Δ</i> ::hphMX6	
633	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 taz1Δ</i> ::kanMX6	
636	h90 otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1 taz1Δ</i> ::kanMX6 <i>dcr1Δ</i> ::hphMX6	

588	h90, ade6-D1, his3-D1, leu1-3, ura4-D18, otr1R(SphI)::ade6 ⁺ , TAS-his3 ⁺ -tel1(L), TAS-ura4 ⁺ -tel2(L)	FY1862
591	h90, ade6-D1, his3-D1, leu1-3, ura4-D18, otr1R(SphI)::ade6 ⁺ , TAS-his3 ⁺ -tel1(L), TAS-ura4 ⁺ -tel2(L), <i>caf1Δ</i> ::kanMX6	
599	h90, ade6-D1, his3-D1, leu1-3, ura4-D18, otr1R(SphI)::ade6 ⁺ , TAS-his3 ⁺ -tel1(L), TAS-ura4 ⁺ -tel2(L), <i>dcr1Δ</i> ::hphMX6	
600	h90, ade6-D1, his3-D1, leu1-3, ura4-D18, otr1R(SphI)::ade6 ⁺ , TAS-his3 ⁺ -tel1(L), TAS-ura4 ⁺ -tel2(L), <i>caf1Δ</i> ::kanMX6, <i>dcr1Δ</i> ::hphMX6	
955	h90, ade6-D1, his3-D1, leu1-3, ura4-D18, otr1R(SphI)::ade6 ⁺ , TAS-his3 ⁺ -tel1(L), TAS-ura4 ⁺ -tel2(L) <i>caf1Δ</i> ::natMX6, <i>taz1Δ</i> ::kanMX6	
63	h ⁺ otr1R(SphI)::ura4 ⁺ ura4-DS/E leu1-32 ade6-M210	
773	h ⁺ otr1R(SphI)::ura4 ⁺ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG-3xHA- <i>caf1</i>	
848	h ⁺ otr1R(SphI)::ura4 ⁺ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG-3xHA- <i>caf1</i> <i>clr4Δ</i> ::kanMX6	
1038	h90 otr1R(SphI)::ura4 ⁺ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1</i> <i>SPNCRNA.70Δ</i> ::ade6-2xbla-hph-nmt1-5'tlh-adh1T	
1039	h90 otr1R(SphI)::ura4 ⁺ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1</i> <i>SPNCRNA.70Δ</i> ::ade6-2xbla-hph-nmt1-3'tlh-adh1T	
1040	h90 otr1R(SphI)::ura4 ⁺ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1</i> <i>SPNCRNA.70Δ</i> ::ade6-2xbla-hph-nmt1-LEU2-adh1T	
746	h90 otr1R(SphI)::ura4 ⁺ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1</i> <i>caf1Δ</i> ::kanMX6 <i>ccr4Δ</i> ::hphMX6	
530	h90 otr1R(SphI)::ura4 ⁺ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1</i> <i>exo2Δ</i> ::kanMX6	
558	h90 otr1R(SphI)::ura4 ⁺ ura4-DS/E leu1-32 ade6-M210 natMX6::3xFLAG- <i>ago1</i> <i>mlo3Δ</i> ::hphMX6	
708	h90 otr1R(SphI)::ura4 ⁺ ura4-DS/E leu1-32 ade6-M210 kanMX6::3xFLAG- <i>ago1</i> <i>cid14Δ</i> ::natMX6	
581	h90 ade6-M210 leu1-32 ura4-D18 taz1 ⁺ : HA-ura4 ⁺	SPTN327
602	h90 ade6-M210 leu1-32 ura4-D18 taz1 ⁺ : HA-ura4 ⁺ <i>caf1Δ</i> ::kanMX6 <i>dcr1Δ</i> ::hphMX6	
1073	h ⁺ otr1R(SphI)::ura4 ⁺ ura4-DS/E leu1-32 ade6-M210 <i>caf1Δ</i> ::kanMX6 <i>dcr1Δ</i> ::hphMX6	

1082	h+ otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX::caf1 promoter <i>caf1</i> , <i>dcr1Δ</i> ::hphMX6	
1084	h+ otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX::caf1 promoter <i>caf1</i> , <i>dcr1Δ</i> ::hphMX6 nat::caf1promoter- caf1D53AD243AD174A	
1141	h+ otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 natMX::caf1, <i>ccr4H664A-ccr4Terminator</i> ::hphMX6 <i>dcr1Δ</i> ::hphMX6	
1143	h+ otr1R(SphI)::ura4+ ura4-DS/E leu1-32 ade6-M210 <i>dcr1Δ</i> ::hphMX6, <i>ccr4H664A-ccr4Terminator</i> ::hphMX6, nat::caf1promoter- caf1D53AD243AD174A	

Supplemental Table S2: Oligonucleotides used in this study

192F o1	R1	GAAGTTCACTCAGTCATAATTAATTGGGTAAC	
192R o3	R1	GGGCCCAATAGTGGGGGCATTGTATTTGTG	
289F	3' <i>tlh</i>	AACCCAGACACAGAAATTCG	
289R	3' <i>tlh</i>	AGCCCATGACCTACAGTCAG	NB / RT
179F	5' <i>tlh</i>	CCAGCTCTTTTCGTTCAAGGAC	
179R	5' <i>tlh</i>	AGTTGACGCTCCTTGGAAGA	NB / RT
559F	middle <i>tlh</i>	CAGAGCACAAGAGATGGTGT	
559R	middle <i>tlh</i>	ATTGGCTTTTCAGCAAATT	NB / RT
113D	28S <i>rRNA</i>	AACACCACTTTCTGGCCATC	NB
110a F	<i>tdh1</i>	CCAAGCCTACCAACTACGA	
110a R	<i>tdh1</i>	AGAGACGAGCTTGACGAA	RT
110f F	Cen <i>dg</i>	CTGCGGTTCAACCCTTAACAT	
110f R	Cen <i>dg</i>	CAACTGCGGATGGAAAAAGT	RT
655F	Intergenic <i>SPAC212.10</i> and <i>SPAC212.09c</i>	GGACAGTCGGGAACAAC	
655R	Intergenic <i>SPAC212.10</i> and <i>SPAC212.09c</i>	CGGGCTATGCTATCCTCTAC	NB
648F	<i>etlh</i> F	TCTTCCCATTTCCTCCTA	
648R	<i>etlh</i> R	TTTTGAAGCGACTTTAGCA	
219F	<i>act1</i>	GATTCTCATGGAGCGTGGTT	
219R	<i>act1</i>	CTCATGAATACCGGCGTTTT	RT
170T		TTTTTTTTTTTTTTTTTT	RT
475F	R3	AATACAATTCGCGAGTCTTATACT	
475R	R3	AATAAGAATTATCAACCCTACCCTA	
770F	R2	TGCCTCTACCATCTTCTTCA	
770R	R2	AACAAAAGGTTGGAACTGG	

Primers used for ChIP and qRT-PCR if no specification, RT: reverse transcription, NB: Northern Blot

Supplemental Table S3: Strains used for sequencing

small RNA-seq:
wild type (65); <i>caf1Δ</i> (510); <i>caf1Δdcr1Δ</i> (521); <i>ccr4Δ</i> (544); <i>ccr4Δdcr1Δ</i> (577); <i>taz1Δ</i> (633); <i>taz1Δdcr1Δ</i> (636); <i>exo2Δ</i> (530); <i>rrp6Δ</i> (GEO: GSE3863); <i>mlo3Δ</i> (558); <i>cid14Δ</i> (GEO: GSE95821); <i>dis3-54Δ</i> (GEO: GSE19734); <i>leo1Δ</i> (GEO: GSE66940); <i>swi6Δ</i> (GEO: GSE70945)
RNA-seq: (in duplicate once total, once p(A)RNA and several qRT-PCR experiments)
total RNA-seq:
wild type (65); <i>caf1Δdcr1Δ</i> (521); <i>clr4Δ</i> (80)
p(A)RNA-seq:
wild type (65); <i>dcr1Δ</i> (34); <i>caf1Δ</i> (510); <i>caf1Δdcr1Δ</i> (521)
RNA Pol II- RNA IP-seq: (in duplicate)
wild type (65, 63); <i>caf1Δdcr1Δ</i> (521, 1073); <i>dcr1Δ</i> (1082); <i>caf1*ccr4*dcr1Δ</i> (1143)
H3 ChIP-seq: (single seq. confirmed by qRT-PCR)
wild type (65); <i>caf1Δdcr1Δ</i> (521)
H3 RNA IP-seq: (in duplicate)
wild type (65); <i>caf1Δdcr1Δ</i> (521); <i>caf1Δ</i> (510); <i>dcr1Δ</i> (34)
Caf1 ChIP-seq: (in duplicate: twice exo-ChIP, twice normal ChIP)
Untagged; 3xFLAG- <i>caf1</i> (773); 3xFLAG- <i>caf1 clr4Δ</i> (848)
Caf1 RNA IP-seq: (in duplicate)
Untagged; 3xFLAG- <i>caf1</i> (773); 3xFLAG- <i>caf1 clr4Δ</i> (848)
DRIP-seq: (in duplicate)
wild type (65) + RNase H; wild type (65) - RNase H; <i>caf1Δdcr1Δ</i> (521) + RNase H; <i>caf1Δdcr1Δ</i> (521) - RNase H
Pol II ChIP-seq: (single seq. consistent with GSM768586)

wild type (65)
H3K9me2 ChIP-seq: (single sequencing, confirmed by several qPCRs)
wild type (65); <i>caf1Δdcr1Δ</i> (521); <i>caf1Δ</i> (510); <i>taz1Δ</i> (633), <i>caf1Δtaz1Δ</i> (955)

Supplemental Table S4: Description of custom Perl scripts (Scripts.tar.bz2)

findpeaks.pl and findpeaks2_mRNA.pl : annotation of reads
findpeaks2_GTF.pl and findpeaks2_mRNA_joinCDSpeaks_norm.pl : Counting of reads for gene groups / each gene
pileup_igv_norm_CDS_strand.pl : Quantification and normalization of reads, generation of strand specificity and generation of igv files
findpeaks2_compare : Comparison of two files