Supplemental materials

The phosphatase Rtr1 regulates global levels of serine 5 RNA Polymerase II C-terminal domain phosphorylation and cotranscriptional histone methylation

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Supplemental methods

ChIP - Quantitative PCR

S.*cerevisiae* strains were derived from BY4741. The WT, *rtr1Δ,* and *rtr2Δ* strains were purchased from yeast knockout collection (GE Dharmacon) (1) and genotypes were confirmed by PCR. Chromatin solutions were prepared as described previously (2), and IP was performed as described previously (3) but with 2µl of 3E8 rat monoclonal antibody (Millipore). The abundance of IP and input DNA was analyzed by qPCR on Mastercycler RealPlex^2 Thermocycler (Eppendorf) and Sybergreen Master Mix (Roche). Primers relative to the ATG on the *PMA1* gene were as follows: PMA1 -73, 5'- CCCCAGCTAGTTAAAGAAAAT CATTGAA - 3'; PMA1 +71, 5'- TCTTGAGTTGGCTGATGAGCTGAA – 3'; PMA1 +1010, 5' - GTTTGCCAG CTGTCGTTACCACCA C – 3'; PMA1 +1250, 5' – TTCTTCTTTCTGGAAGCAGCCAAAC -3'; PMA1 +2018, 5' - CTATTATTGATGCTTTGAAGACCTCCAG – 3'; PMA1 +2290, 5' - TGCCCAAAATAATAG ACATACCCCATA A – 3'. The real time PCR parameters were as follows: 95°C for 3min for one cycle, 95°C 15 sec, 54°C for 45 sec, 72°C for 25 sec for 40 cycles, followed by one cycle of 95°C for 15 sec, 55°C for 15 sec, and 95°C for 15 sec. To calculate the amount of DNA in each sample, a standard curve of known concentrations of yeast genomic DNA was amplified with each PCR primer set. Fold enrichment was calculated by dividing the percent IP of the indicated regions over the percent IP of a control region on chromosome V as performed previously (4,5).

ChIP-exo

ChIP-exo analysis was performed as described in Fox *et al* 2015 (6) according to methods developed by the Pugh lab (2,7). In brief, WT and *rtr1Δ* strains were transformed with a 3X-FLAG tagging cassette containing the URA3 marker for selection of integration following homologous recombination. Strains were confirmed by western blot, affinity purification mass spectrometry, and sequencing. ChIP was performed using FLAG affinity resin (Sigma) to isolate RNAPII complexes and their associated DNA from both strain backgrounds. Exonuclease treatment of the isolated complexes and SOLID library construction was performed as described (2,6,7). SOLID sequencing data processing and alignment was also performed as previously reported (6).

ChIP-exo normalization

The Rpb3-FLAG ChIP-exo data was used for comparison with the serine 5-phosphorylated RNAPII ChIP on chip data. Since the two datasets were obtained using a different analytical platform, the average gene analysis for total RNAPII (Rpb3-FLAG) was not used to directly normalize the Ser5-P RNAPII data. To use a similar approach for data normalization, the Rpb3-FLAG data from wild-type and *rtr1*Δ strains was processed as follows. The read counts per nucleotide in the yeast genome were summed for each microarray probe region. This resulted in a lower resolution dataset than what was experimentally measured however this transformation was required for proper comparison to the ChIP-chip dataset. As shown in Figure S1, the reduction in the total resolution for the Rpb3-FLAG ChIP-exo data did not result in any significant changes in the total RNAPII occupancy at the *PMA1* gene. Following data binning to the microarray probe coordinates, the dataset was median normalization as previously performed for the ChIP-chip datasets. The resulting values were then log transformed ($\log_2$) as shown in Figure 2B in the main text. For direct normalization of the Ser5-P RNAPII levels at *PMA1* and *LEU1* shown in Figure 5, we calculated the percent of maximum RNAPII occupancy at each gene across the wild-type and *rtr1*Δ Rpb3-FLAG binned data. Using these values, we were able to normalize the levels of Ser5-P RNAPII by the relative amounts of total RNAPII occupancy across the genes to adjust for any changes in total RNAPII levels. For reference, the percent maximum calculations are shown as Figure S2.

Table S1: Gene ontology enrichment analysis of the RNAPII target genes in each of the five Ser5-P RNAPII clusters.

Figure S1: Examples of ChIP-exo data processing to match Agilent 1x244k microarray probe density. A. Raw read count data for Rpb3-FLAG ChIP-exo data at PMA1 and LEU1 region on chromosome 7. **B**. Raw read count data for the same region as in A following binning of the read count data to match the probe density on the Agilent 1x244k array.

Figure S2: Percent maximum read count data at *PMA1* and *LEU1*. Data for the same region shown in Figure S1 and Figure 5 following binning of the data, median normalization of the dataset, and calculation of percent maximum total RNAPII occupancy.

Figure S3: Rtr2 does not affect Ser5-P dephosphorylation at *PMA1.* In S. *cerevisiae*, Rtr2 is a protein of unknown function and is a paralog of Rtr1 that arose during genome duplication (8). Previous studies have shown that Rtr1 causes changes in Ser5-P patterns (3,5,9). However, given ~30% sequence identity between the two proteins, and presumably similar amounts of Rtr2 protein present in yeast when compared to Rtr1, the question arises as to whether Rtr2 also displays phosphatase activity. Our previous attempts to purify Rtr2 have revealed that the protein is insoluble when expressed in bacteria (data not shown). To determine the effects of Rtr2 deletion on the levels of Ser5-P *in vivo*, we performed ChIP-qPCR analysis to reveal the levels of Ser5-P RNAPII on the *PMA1* gene from *rtr1Δ* and *rtr2Δ* strains and compared to WT (Figure S1). *PMA1* is a highly expressed gene in yeast that has previously been used as a prototypical gene to study the localization of different forms of RNAPII phosphorylation (4). A schematic of the *PMA1* gene and the primers used for PCR amplification are shown in Figure S1A. We found a significant increase in Ser5-P RNAPII levels in the *rtr1Δ* strain in the midpoint of the gene body (Figure S1B). In contrast, the levels of Ser5-P observed in the *rtr2Δ* strain were consistent with WT levels at all three regions. These data support previous findings that Rtr1 is the phosphatase that targets Ser5-P RNAPII during transcription elongation. Interestingly, Rtr2 does not appear to contribute to RNAPII dephosphorylation at *PMA1*. Overall, these data suggest that Rtr2 is not a regulator of Ser5-P CTD modification at RNAPII target genes.

Table S1: Statistically overrepresented GO terms within each cluster (p<0.01 cutoff)

Cluster 1		P-Value
GO:0016491	Oxidoreductase Activity	9.82E-11
GO:0016209	Antioxidant Activity	1.43E-05
GO:0004601	Peroxidase Activity	0.000118
GO:0016684	Oxidoreductase Activity	0.000118
GO:0006790	Sulfur metabolism	0.000668
GO:0015891	Siderophore Transport	0.000873
GO:0005381	Iron Ion Transmembrane Transporter	0.00111
GO:0004364	Glutathione Transferase Activity	0.00142
GO:0046519	Sphingoid Metabolic Process	0.00228
GO:0042221	Response to Chemical Stimulus	0.00296
GO:0006672	Ceramide Metabolism	0.00431
GO:0033212	Iron Assimilation	0.00431
GO:0000041	Transition Metal Ion Transport	0.00434
GO:0005740	Mitochondrial Envelope	0.00434
GO:0030503	Regulation of Cell Redox	0.00434
GO:0045454	Cell Redox Homeostasis	0.00434
GO:0006979	Response to Oxidative Stress	0.00537
GO:0051186	Cofactor Metabolism	0.00764
GO:0030001	Metal Ion Transport	0.00944
GO:0006827	High Affinity Iron Ion Transport	0.00974
GO:0015343	Siderophore - Iron Transmembrane Transport Activity	0.00974
GO:0042927	Siderophore Transporter Activity	0.00974
Cluster 2		P-Value
GO:0006464	Protein Modification	0.00956
Cluster 3		P-Value
GO:0003964	RNA-directed DNA Polymerase Activity	0.000663
GO:0003887	DNA - directed DNA Polymerase Activity	0.00123
GO:0007015	Actin Filament Organization	0.00176
GO:0044464	Cell Part	0.00217
GO:0016772	Phospho-transferase Activity	0.00274
Cluster 4		P-Value
GO:0005730	Nucleolus	6.60E-37
GO:0022613	Ribonucleoprotein Complex Biogenesis & Assembly	1.46E-36
GO:0043228	Non-membrane Bound Organelle	2.11E-36
GO:0043232	Intracellular Non-membrane Bound Organelle	2.11E-36
GO:0010467	Gene Expression	3.40E-36
GO:0042254	Ribosome Biogenesis & Assembly	8.42E-36
GO:0016072	rRNA Metabolic Process	1.31E-30
GO:0006364	rRNA Processing	5.48E-30
GO:0044452	Nucleolar Part	8.68E-30

GO:0032991	Macromolecular Complex	9.81E-28
GO:0030529	Ribonucleoprotein Complex	4.62E-27
GO:0043170	Macromolecular Metabolic Complex	1.22E-24
GO:0006412	Translation	1.75E-22
GO:0031981	Nuclear Lumen	6.77E-22
GO:0005732	Small Nucleolar Ribonucleoprotein Complex	4.22E-21
GO:0019843	rRNA Binding	1.24E-19
GO:0005829	Cytosol	1.27E-19
GO:0000154	rRNA Modification	8.68E-19
GO:0031428	snoRNP Complex	8.68E-19
GO:0044422	Organelle Part	1.45E-18
GO:0044446	Intracellular Organelle Part	1.45E-18
GO:0044428	Nuclear Part	7.98E-18
GO:0030555	RNA Modification	7.29E-17
GO:0030556	rRNA Modification	7.29E-17
GO:0044445	Cytosolic Part	8.57E-17
GO:0009987	Cellular Process	1.27E-16
GO:0009059	Macromolecule Biosynthetic Process	1.73E-16
GO:0044238	Primary Metabolic Process	2.05E-16
GO:0005830	Cytosolic Ribosome	2.44E-16
GO:0006996	Organelle Organization	2.44E-16
Cluster 5		P-Value
GO:0060090	Molecular Adaptor	3.84E-14
GO:0030533	Triplet Codon-amino acid adaptor	3.84E-14
GO:0006414	Translation Elongation	3.84E-14
GO:0043284	Biopolymer Biosynthetic Process	1.02E-13
GO:0000943	Retrotransposon Nucelocapsid	3.90E-09
GO:0032197	RNA - mediated Transposition	6.02E-09
GO:0005829	Cytosol	2.33E-08
GO:0006412	Translation	2.33E-08
GO:0032196	Transposition	1.98E-07
GO:0009059	Macromolecular Biosynthetic Process	5.63E-07
GO:0044260	Cellular Macromolecule Metabolic Process	2.07E-05
GO:0019538	Protein Metabolic Process	2.40E-05
GO:0044267	Cellular Protein Metabolic Process	2.40E-05
GO:0010467	Gene Expression	0.000207
GO:0044249	Cellular Biosynthetic Process	0.00575

Figure S1

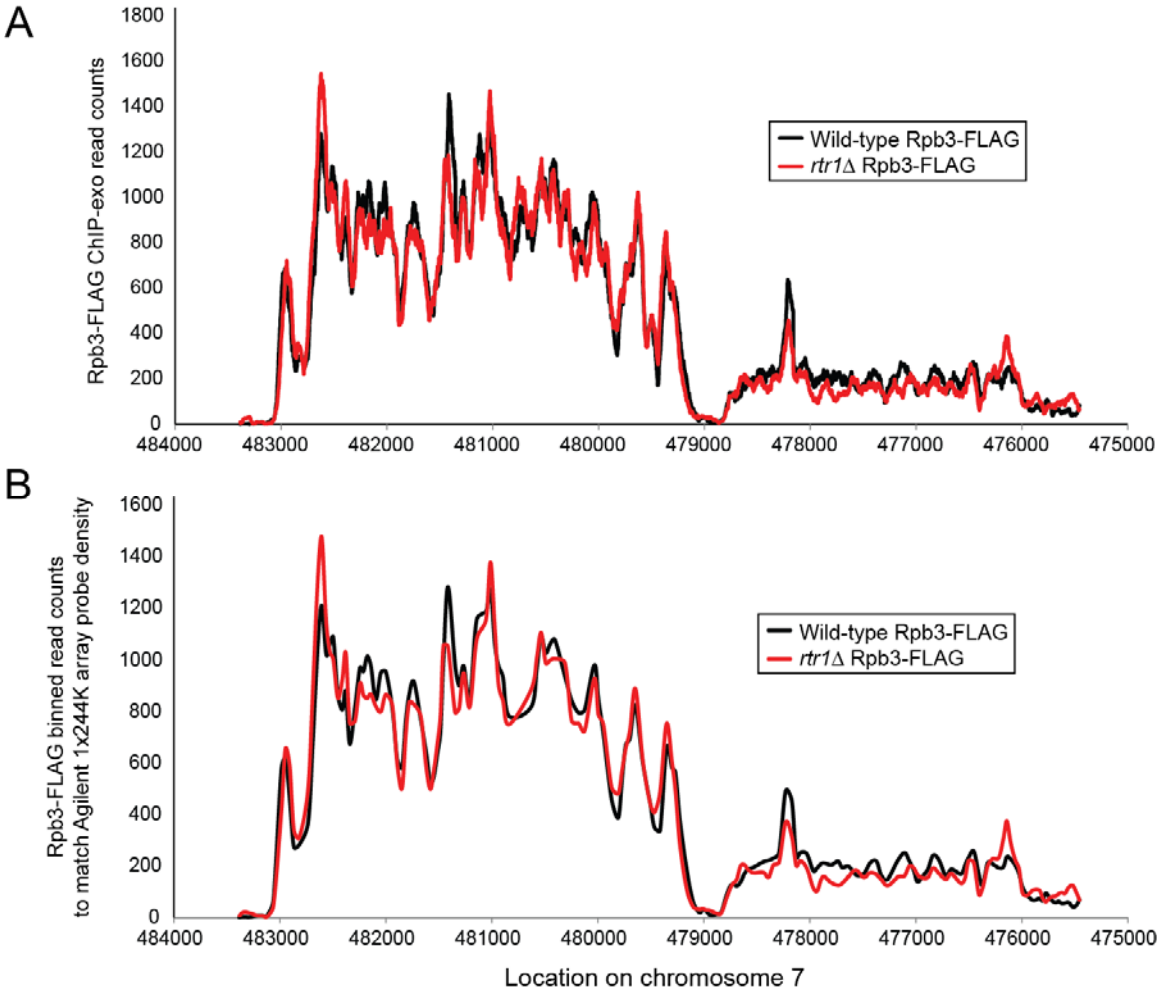

A
Rpb3-FLAG ChIP-exo read counts
1800
1600
1400
1200
1000
800
600
400
200
0
Wild-type Rpb3-FLAG
rtr1Δ Rpb3-FLAG
484000
483000
482000
481000
480000
479000
478000
477000
476000
475000
B
Rpb3-FLAG binned read counts
to match Agilent 1x244K array probe density
1600
1400
1200
1000
800
600
400
200
0
Wild-type Rpb3-FLAG
rtr1Δ Rpb3-FLAG
484000
483000
482000
481000
480000
479000
478000
477000
476000
475000
Location on chromosome 7

Figure S2

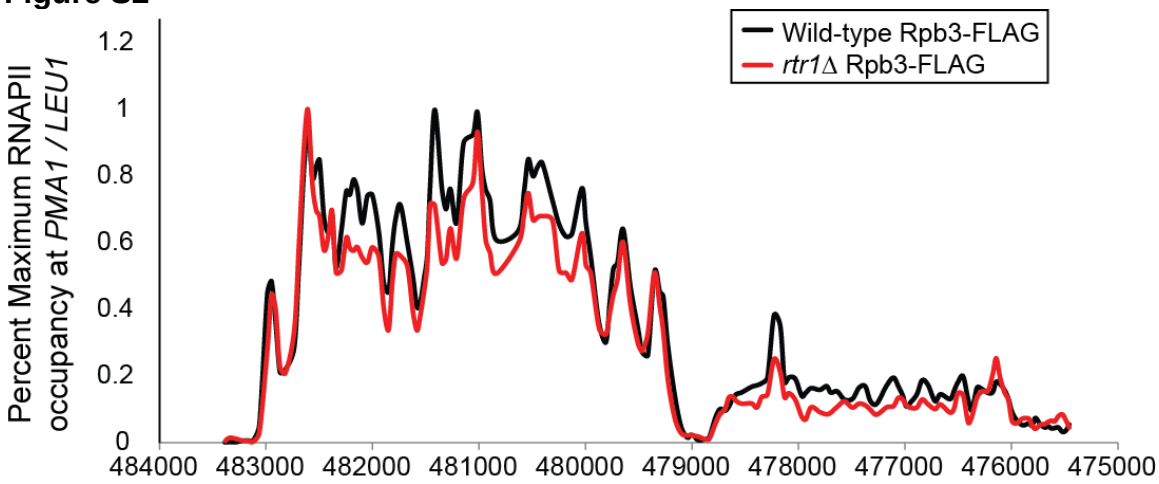
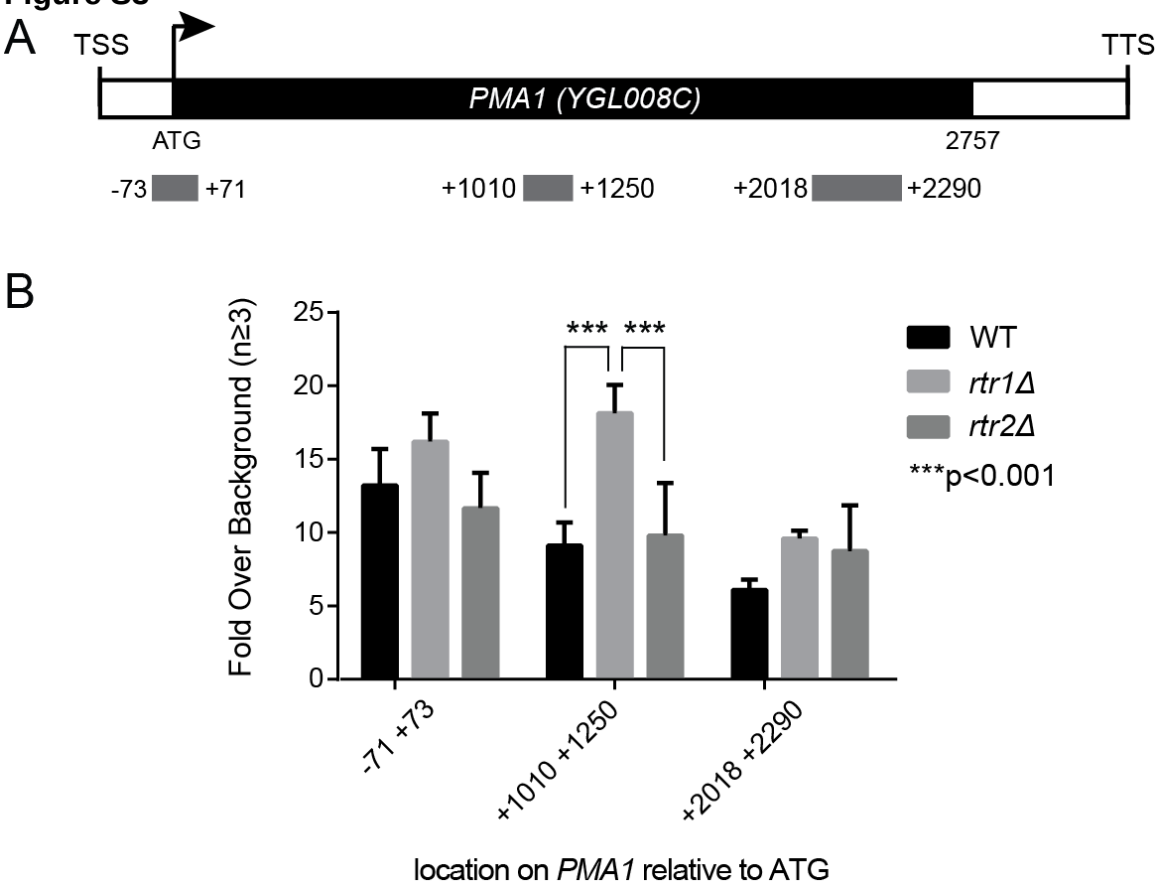

Wild-type Rpb3-FLAG
rtr1Δ Rpb3-FLAG
Percent Maximum RNAPII occupancy at PMA1 / LEU1
1.2
1
0.8
0.6
0.4
0.2
0
484000
483000
482000
481000
480000
479000
478000
477000
476000
475000

Figure S3


A
TSS
TTS
PMA1 (YGL008C)
ATG
2757
-73
+71
+1010
+1250
+2018
+2290
B
Fold Over Background (n≥3)
25
20
15
10
5
0
*** ***
WT
rtr1Δ
rtr2Δ
***p<0.001
-71 +73
+1010 +1250
+2018 +2290
location on PMA1 relative to ATG

References

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