Supplemental Data

Supplemental Table 1 *S. cerevisiae* strains.

Name	Genotype	Reference
RMY200	MATa ade2-101$^{(och)}$ his3^{200} lys2-801$^{(amp)}$ trp1^{901} ura3-52 hht1,hhf1::LEU2 hht2,hhf2::HIS3 plus pRM200 (CEN4 ARS1 TRP1 HHT2 HHF2)	(1)
UKY403	MATa ade2-101 his3-201 leu2-3,113 lys2-801 trp1-901 ura3-52 GAL+ thr- tyr- arg4-1 hhf1::HIS3 hhf2::LEU2 plus pUK421 (AMP CEN TRP1 GAL1-HHF2)	(2)
W303-1a	MATa leu2-3,112 trp1-1 can1-100 ura3-1 ade2-1 his3-11,15	(3)
AMR51	W303-1a top1-8::LEU2	(3)
RS191	W303-1a top2-1(ts)	(3)
RS192	W303-1a top1-8::LEU2 top2-1(ts)	(3)
BY4741	MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	ATCC# 201388
YBR289W BY4741	BY4741 snf5Δ	ATCC# 4007175
Z460	MATa, ura3-52, leu2-3,112, his3D200, his4-912, lys2-128, rpb1D187::HIS3 plus pRP1-1U (AMP, CEN, LEU2, rpb1-1)	(4)

Supplemental Table 2 Primers.

Experiment	Sequence
Pol II ChIP at *PHO84*	GGGTATTGGTATCGGTGGTG

Pol II ChIP at *PHO84*	AACATGCCAACCCTAGAACG
Top2 and H3 ChIP at *PHO84*	AGGGCCCTTTCAACTCATCT
Top2 and H3 ChIP at *PHO84*	GCATAACTGCACCGATCTCA
Pol II ChIP at *PHO5*	CTTGGGACTACGATGCCAAT
Pol II ChIP at *PHO5*	TGAACAAGTTGGAACCGACA
Top2 and H3 ChIP at *PHO5*	TGGAAGTCATCTTATGTGCGCTGC
Top2 and H3 ChIP at *PHO5*	ACGTGTGAGTGCCAAGGTTGTATC

Supplemental Figure Legends:

Figure S1. A high resolution map of Top2 and nucleosome occupancy at chromosome VI.

Representative ChIP-chip data of Top2 (black) and H3 (red) are shown around the centromere of chromosome VI (top panel) and an ORF poor region adjacent to telomere VI-R (bottom panel). Images were generated using the Affymetrix Integrated Genome Browser.

Figure S2. Verification of the microarray nucleosome depletion data.

H3 ChIP-chip data were rank sorted and four genes were randomly selected from the top 100 and four genes from the bottom 100. ChIP followed by semi-quantitative PCR was then carried out at these genes to verify the microarray data. There is no significant increase in H3 binding at any of the loci from the top 100, whereas the loci from the

bottom 100 show significant histone H3 loss during H4 depletion. H3 ChIP data at each gene are normalized to the ACT1 gene and to input. The average of three independent experiments is shown. Error bars represent the standard deviation.

Figure S3. Nucleosomes are depleted and Top2 is recruited during gene activation at *PHO5*.

ChIP of histone H3, Top2, and Pol II at the promoter and ORF of the *PHO5* gene at various timepoints following shift to low phosphate media in wild type yeast (strain BY4741). ChIP analysis was performed using semi-quantitative PCR and normalized to input and the *SPS2* gene. The average of three independent experiments is shown. Error bars represent the standard deviation.

Figure S4. Top2 is depleted from the genome following shift to the non-permissive temperature.

Top2 ChIP-chip data in wild type, *top1Δ*, and *top1Δtop2-ts* strains. Average binding across the promoter and ORF is shown, separated into clusters as in Figure 4.

Figure S5. Top2 is positively correlated with transcriptional activity.

Data for Top2 (A) and H3 (B) binding from ChIP-chip analysis were classified into five groups according to previously reported transcriptional rates (5). The average profiles are analyzed as described in Figure 1.

Figure S6. Top1 binding at promoters is positively correlated with transcription.

Previously published data for Top1 ChIP-chip analysis (6) were classified into five groups according to previously reported transcriptional rates (5). Average binding in each group over the upstream activating sequence (UAS), transcription start site (TSS) and ORF is shown.

Figure S7. Gene length is not correlated with Top2 effect on transcription.

Pol II ChIP-chip in wild type (blue) and *top1Δtop2-ts* (red) strains. Experiments were performed as in Figure 4. Z-scored average log ratio binding across the promoter and ORF (-1000 bp to +3000 bp) is shown. The data have been separated into five groups based upon gene length.

Figure S8. Loss of Top1 and Top2 topoisomerase activity does not lead to increased nucleosome occupancy across the promoter or ORF.

H3 ChIP-chip binding following loss of Top1 and Top2 (RS192) activity normalized to wild type (W303-1a). Cells were grown to log phase at 25°C and then shifted to 37°C for 30 minutes prior to harvesting chromatin. ORFs were separated into 10 equal sized bins and their associated promoters were divided into five 100 bp bins. Data for 6215 ORFs was averaged. Error bars represent the 95% confidence interval. Note the very small increase in H3 occupancy, likely secondary to decreased transcription in the *top1Δtop2-ts*

strain.

Figure S9. Loss of Top1 and Top2 topoisomerase activity does not alter nucleosome density at promoters.

Top2 and H3 binding at the promoter (-500bp to 0bp from translation start site) upon Top1 and Top2 inhibition was compared as in Figure 2. R represents the Pearson correlation between the percentile-ranked samples.

Supplemental References

1. Mann RK & Grunstein M (1992) Histone H3 N-terminal mutations allow hyperactivation of the yeast GAL1 gene in vivo. *Embo J* 11(9):3297-3306.
2. Han M & Grunstein M (1988) Nucleosome loss activates yeast downstream promoters in vivo. *Cell* 55(6):1137-1145.
3. Brill SJ & Sternglanz R (1988) Transcription-dependent DNA supercoiling in yeast DNA topoisomerase mutants. *Cell* 54(3):403-411.
4. Apone LM, *et al.* (1998) Broad, but not universal, transcriptional requirement for yTAFII17, a histone H3-like TAFII present in TFIID and SAGA. *Mol Cell* 2(5):653-661.
5. Holstege FC, *et al.* (1998) Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* 95(5):717-728.
6. Venters BJ, *et al.* (2011) A comprehensive genomic binding map of gene and chromatin regulatory proteins in Saccharomyces. *Mol Cell* 41(4):480-492.

Figure S1, related to Figure 1

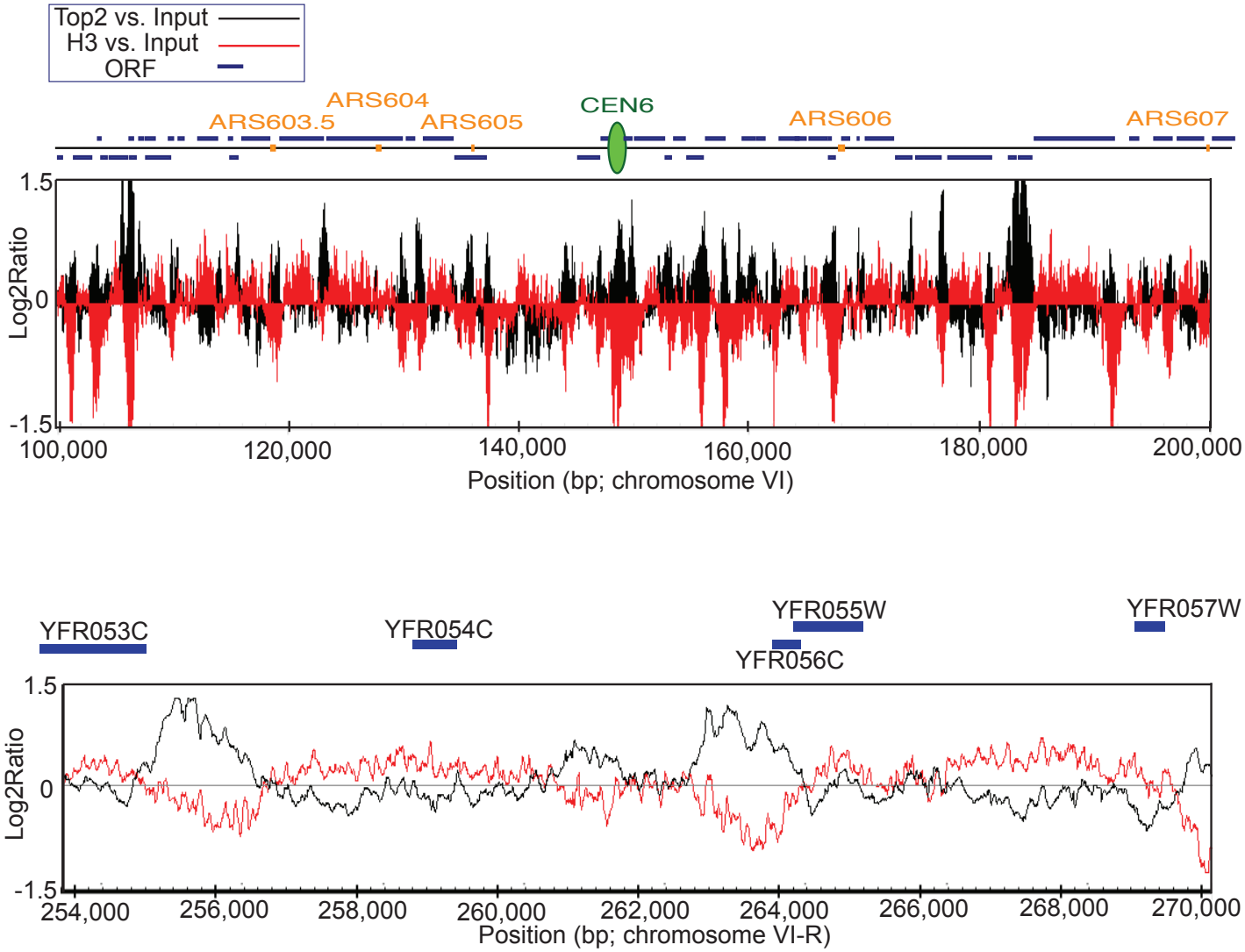


Figure S2

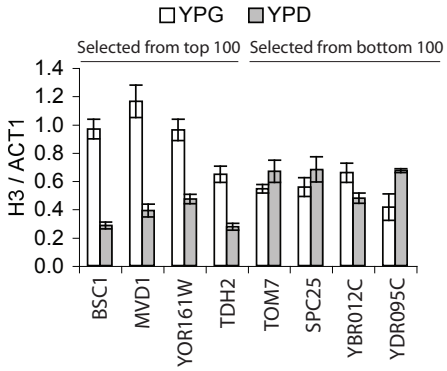

YPG
YPD
Selected from top 100
Selected from bottom 100
H3 / ACT1
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
BSC1
MVD1
YOR161W
TDH2
TOM7
SPC25
YBR012C
YDR095C

Figure S3

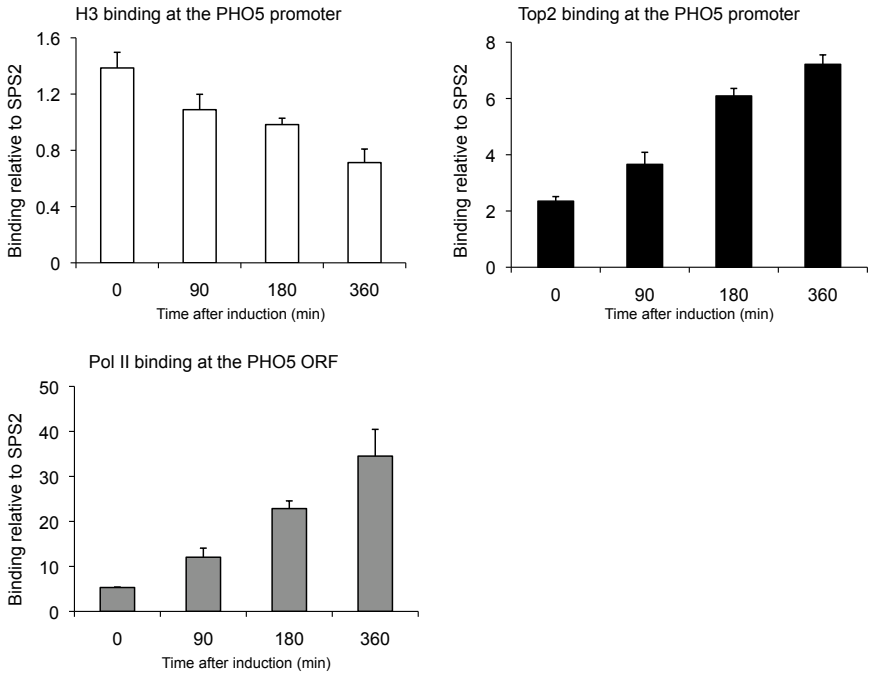
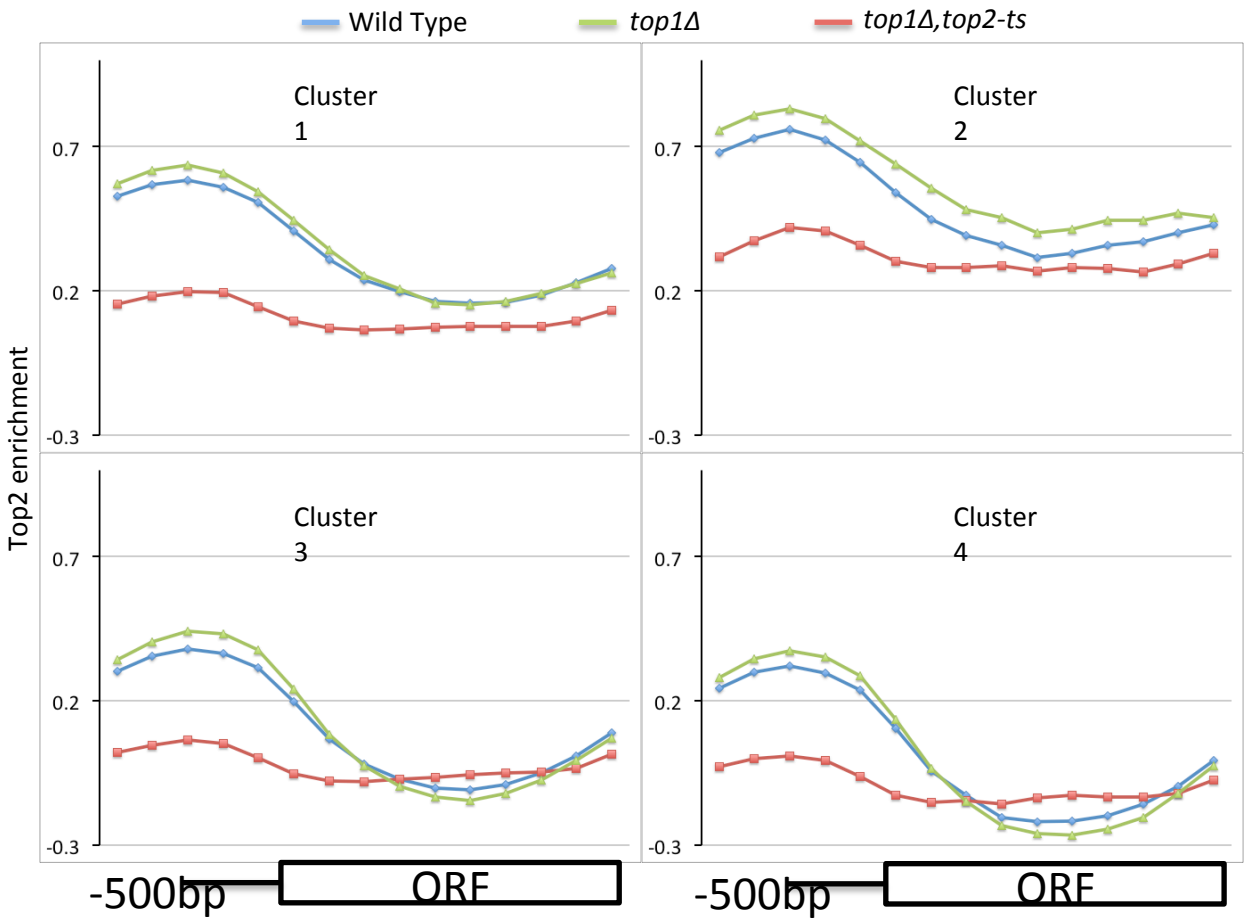

H3 binding at the PHO5 promoter
Binding relative to SPS2
1.6
1.2
0.8
0.4
0
0
90
180
360
Time after induction (min)
Top2 binding at the PHO5 promoter
Binding relative to SPS2
8
6
4
2
0
0
90
180
360
Time after induction (min)
Pol II binding at the PHO5 ORF
Binding relative to SPS2
50
40
30
20
10
0
0
90
180
360
Time after induction (min)

Figure S4


Wild Type
top1Δ
top1Δ,top2-ts
Cluster 1
Cluster 2
Cluster 3
Cluster 4
0.7
0.2
-0.3
Top2 enrichment
-500bp
ORF
-500bp
ORF

Supplemental Figure 5

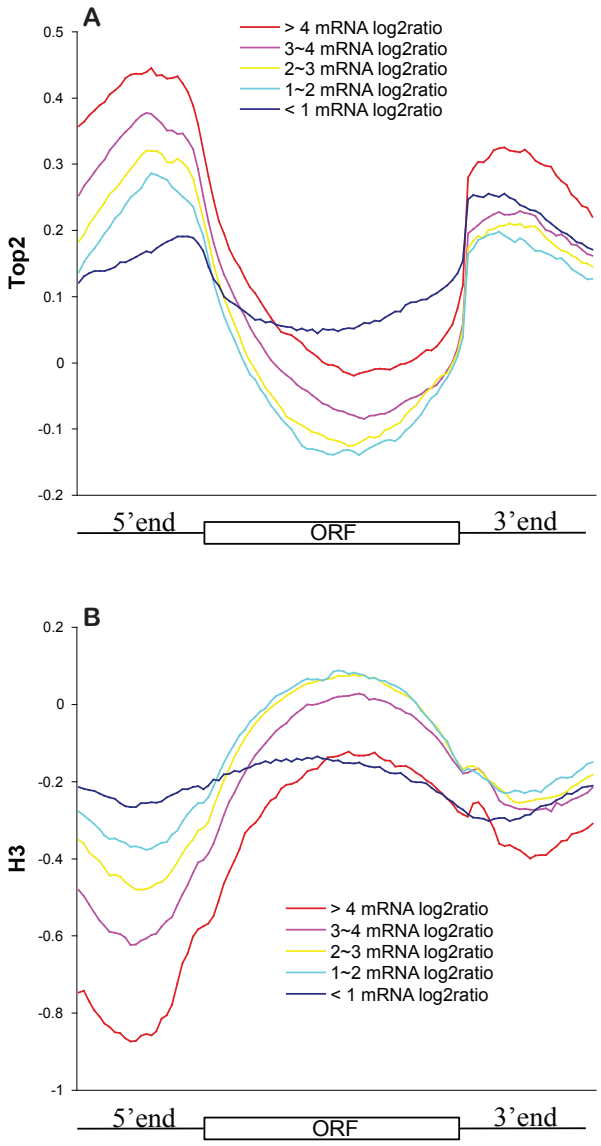


Figure S6

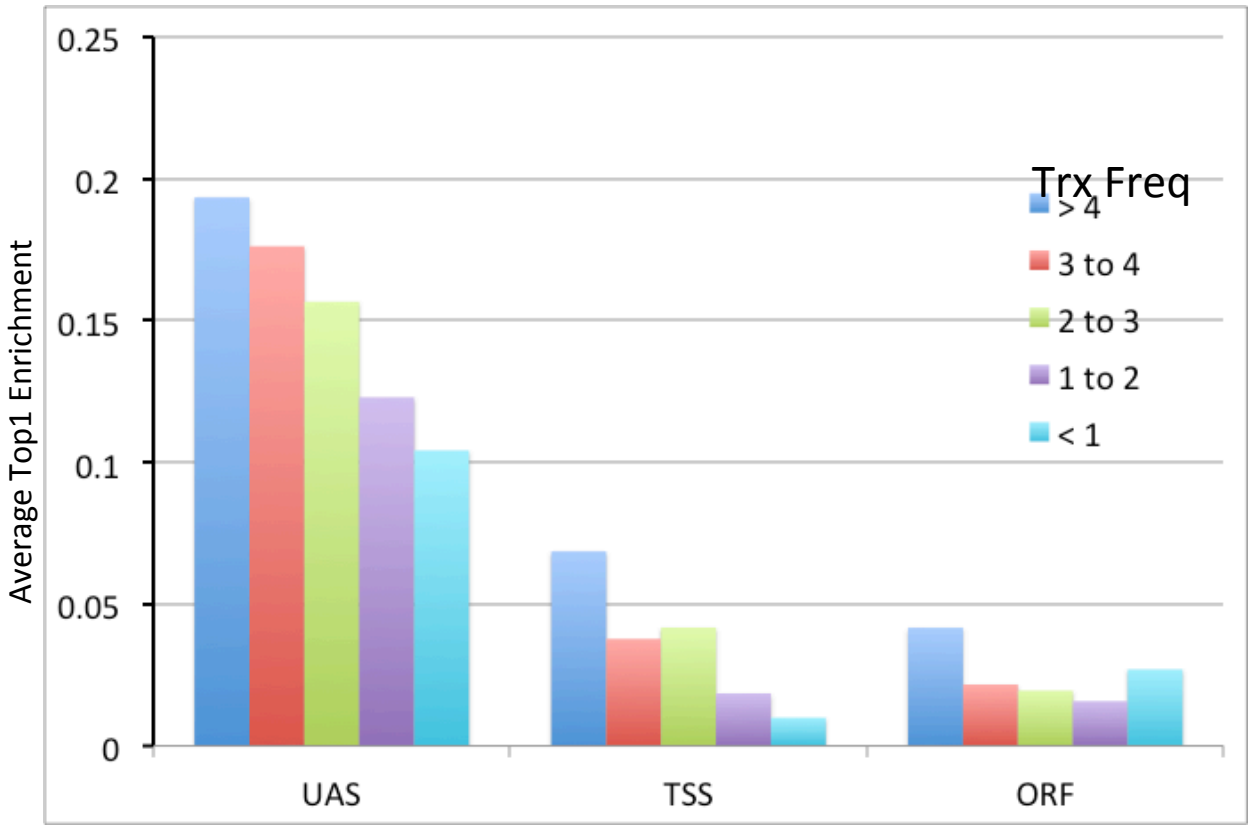

0.25
0.2
0.15
0.1
0.05
0
Average Top1 Enrichment
UAS
TSS
ORF
Trx Freq
> 4
3 to 4
2 to 3
1 to 2
< 1

Figure S7

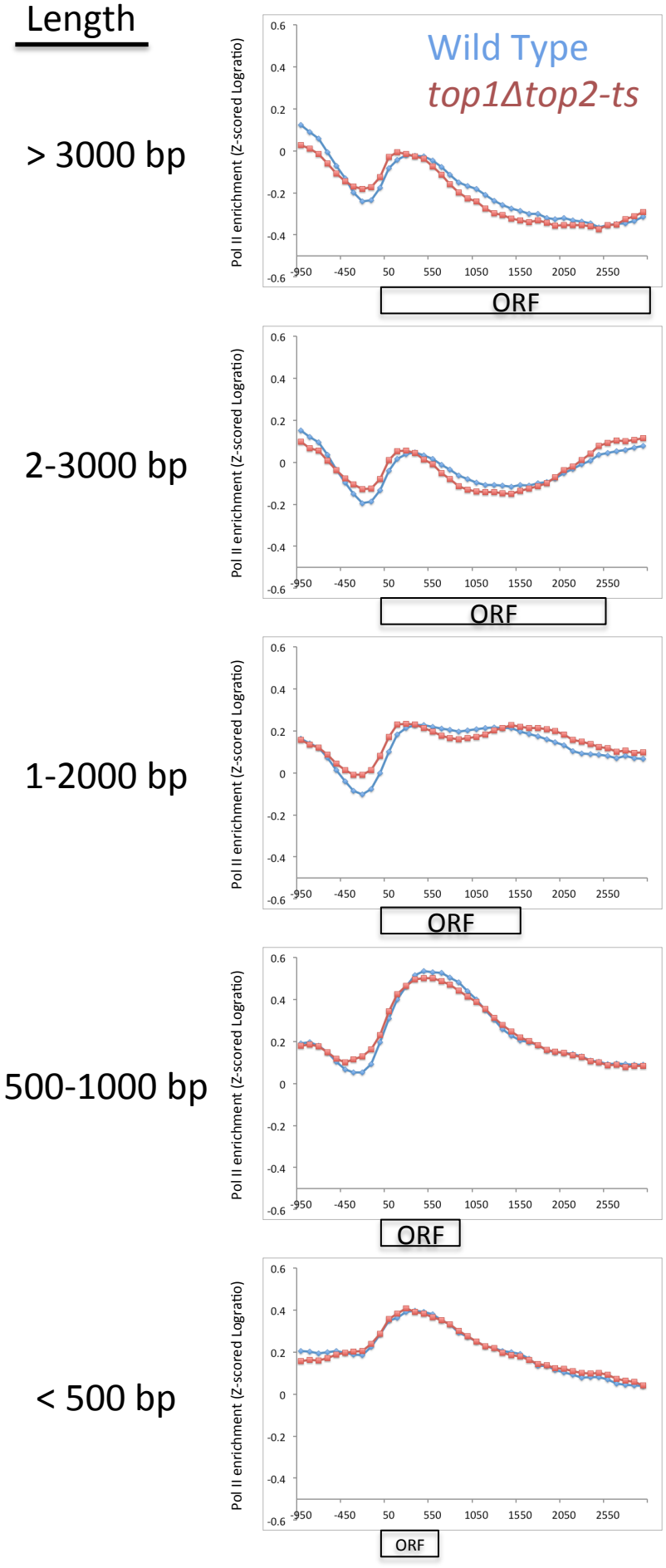

Length
> 3000 bp
2-3000 bp
1-2000 bp
500-1000 bp
< 500 bp
Wild Type
top1Δtop2-ts
Pol II enrichment (Z-scored Logratio)
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-950
-450
50
550
1050
1550
2050
2550
ORF

Figure S8

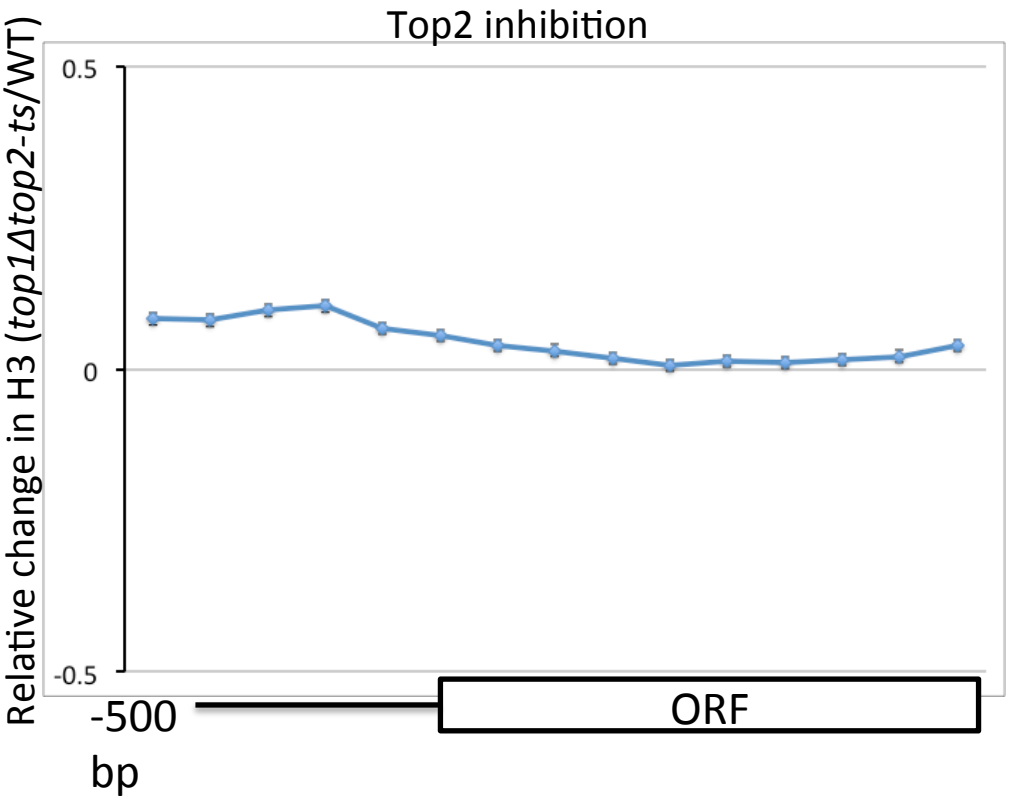
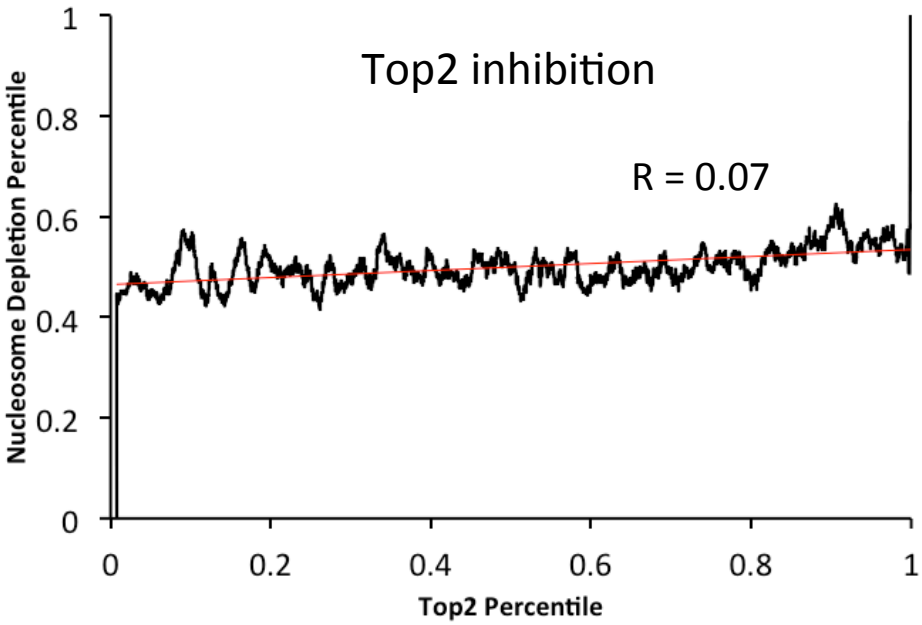

Top2 inhibition
Relative change in H3 (top1Δtop2-ts/WT)
0.5
0
-0.5
-500
bp
ORF

Figure S9


Top2 inhibition
R = 0.07
Nucleosome Depletion Percentile
Top2 Percentile
0
0.2
0.4
0.6
0.8
1