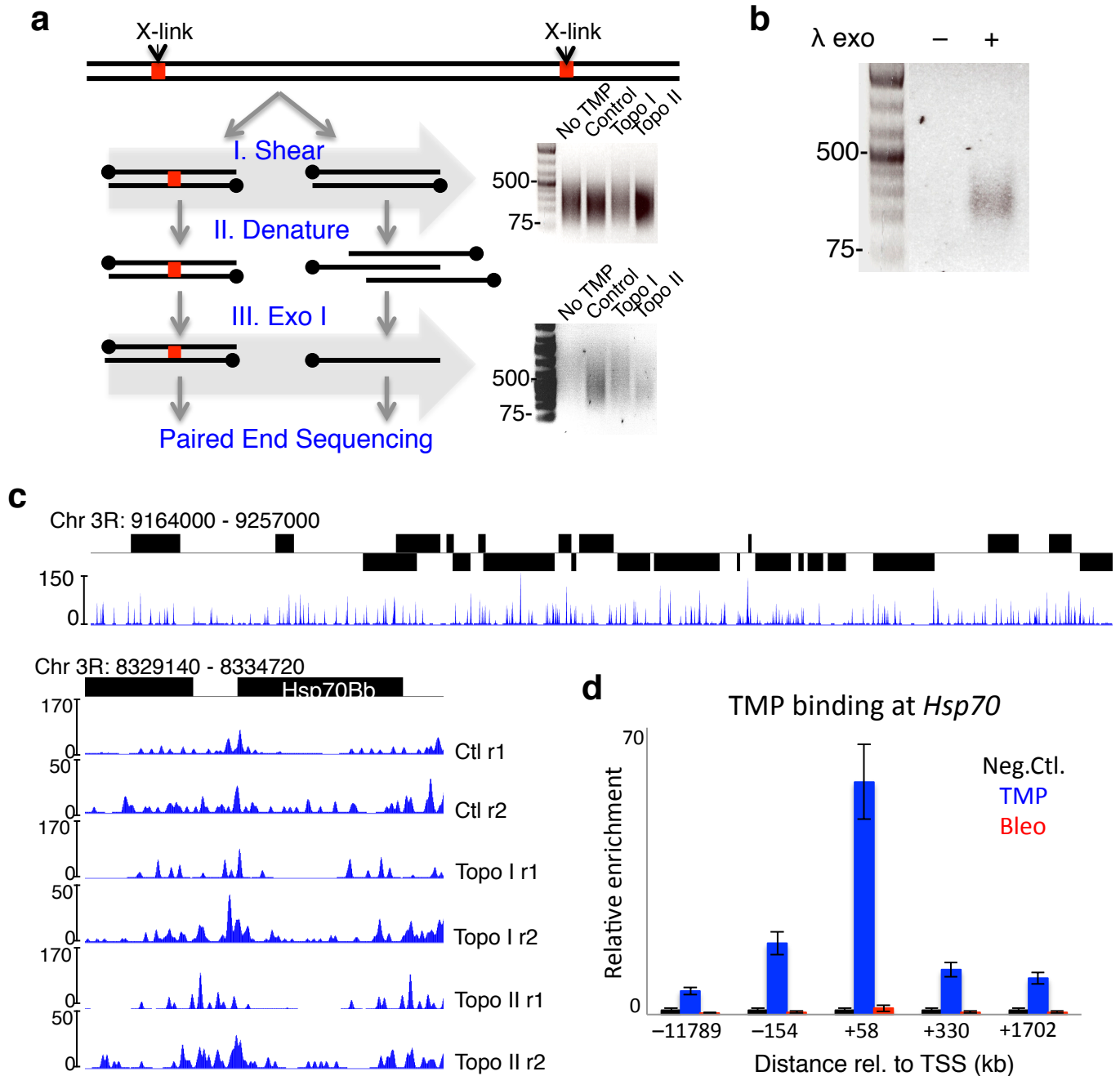


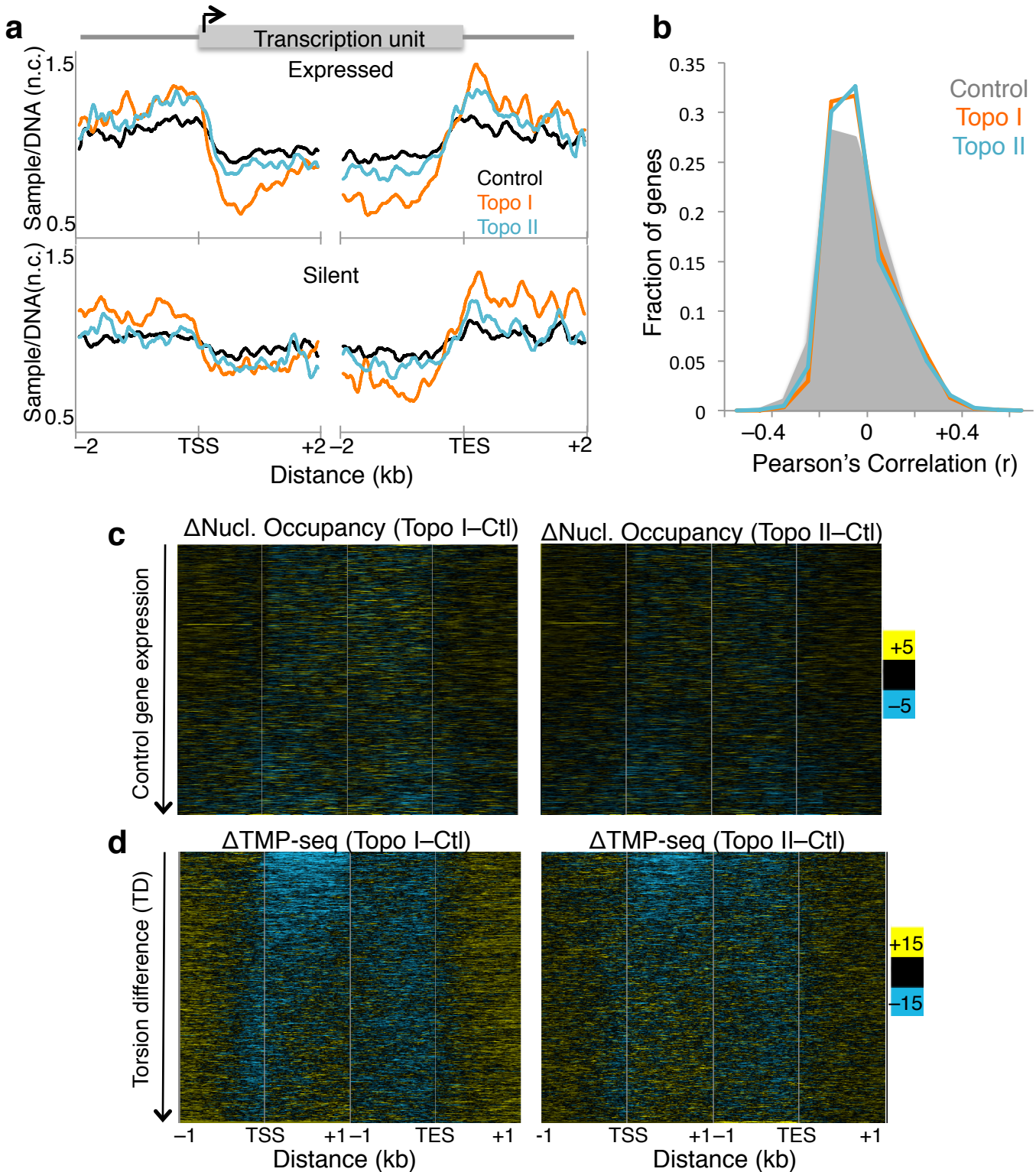
Supplementary Information

## **Transcription-generated torsional stress destabilizes nucleosomes**

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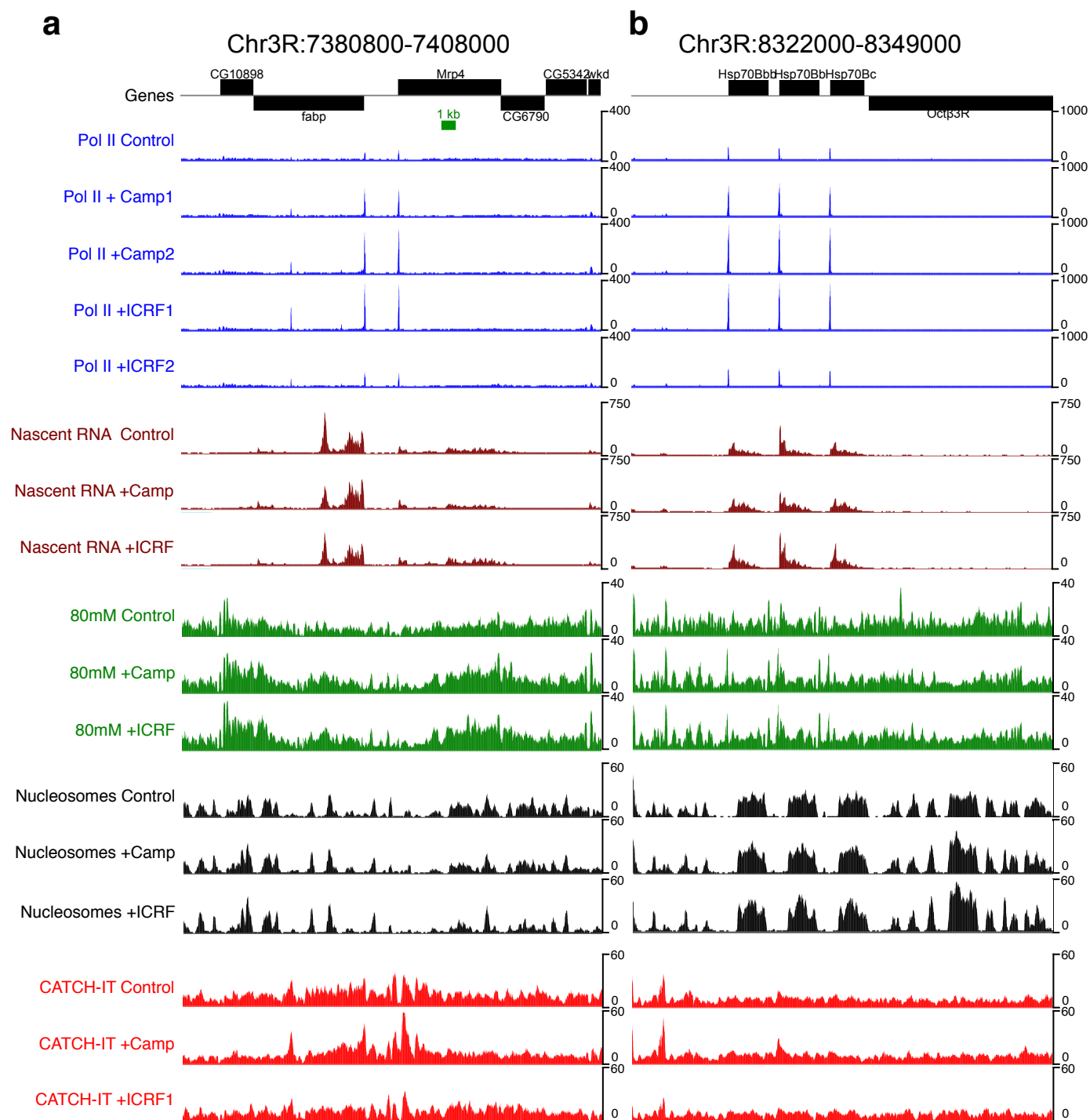


**Supplementary Figure 1: TMP-seq detects torsion changes near the TSS.** (a) Enriching for TMP-crosslinked fragments. DNA is sheared to an average size of 250 bp (top gel). Samples were denatured in boiling water bath for 10 minutes, snap-cooled in ice water, and digested with Exo I. This process was repeated until the sample non-crosslink DNA have been fully digested (bottom agarose gel). (b) Adapter ligated samples (see Fig. 1) were split into 2 aliquots, with and without 3' strand resection using  $\lambda$  Exo, followed by primer extension reaction. Without  $\lambda$  Exo digestion, no single-stranded products were detected. (c) The first sequenced nucleotide after the CCC overhang of controls is mapped onto the genome and fitted with a Kernel density estimator function (Gehring et al., 2009). A 93 kb representative region in the chromosome 3R is shown at the top. The *Hsp70C* gene locus is shown with replicates for control, Topo I-, and Topo II-inhibited samples. (d) Primers spanning the TSS of *Hsp70* were used to validate TMP-seq data. Bleomycin was used to introduce nicks on chromatin prior to TMP-treatment to release torsion. Neg. Ct. negative control



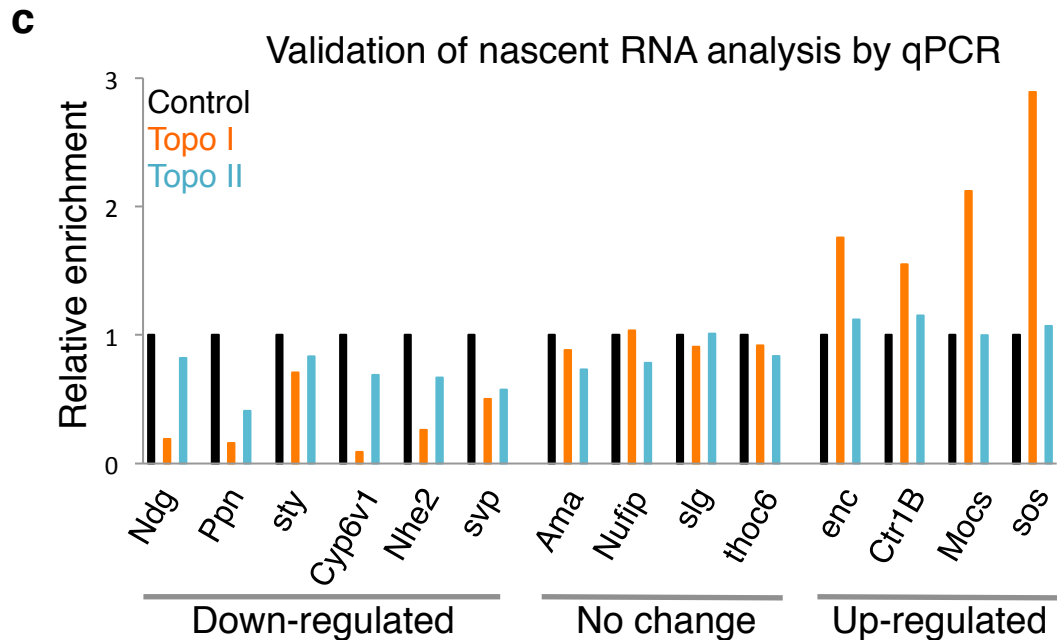
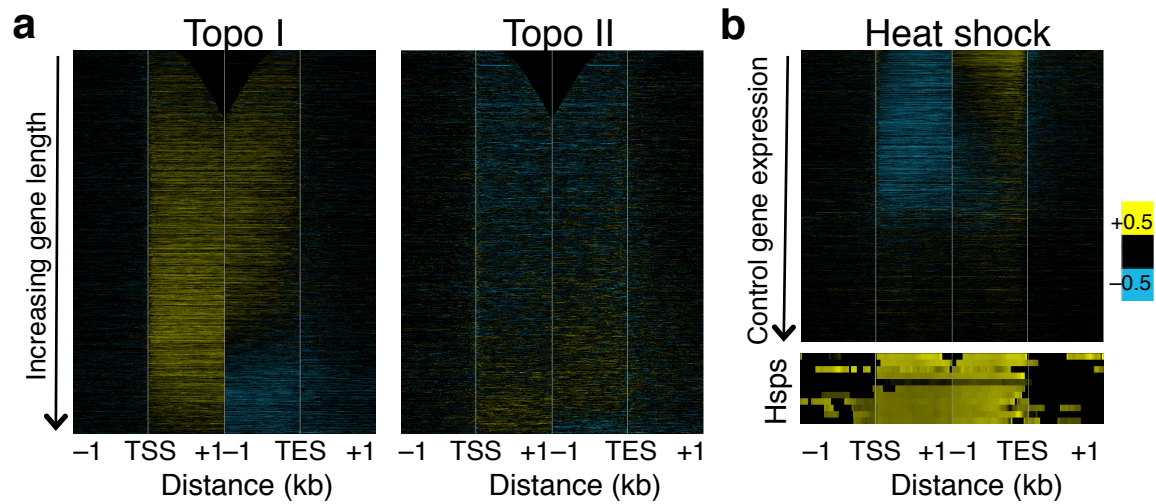
**Supplementary Figure 2: Topoisomerase inhibition does not alter nucleosome occupancy**

(a) TMP-seq data were normalized for sequence bias and the average normalized signals surrounding the TSS and TES were determined for expressed (top) and silent (bottom) genes. (b) For each gene and for each corresponding sample, the Pearson's correlation ( $r$ ) was determined for the 1 kb region surrounding the TSS between TMP-seq and nucleosome occupancy data, and the distribution of the correlation was plotted. (c) The changes in nucleosome occupancy were determined by subtracting the MNase-seq values of control from Topo I- (left) and Topo II- (right) inhibited samples, and were displayed as heat maps with genes ordered by decreasing gene expression in control samples. Contrast = 5. (d) Heat map as in main Fig. 2b with genes arranged by torsion difference (TD). Ctl: control

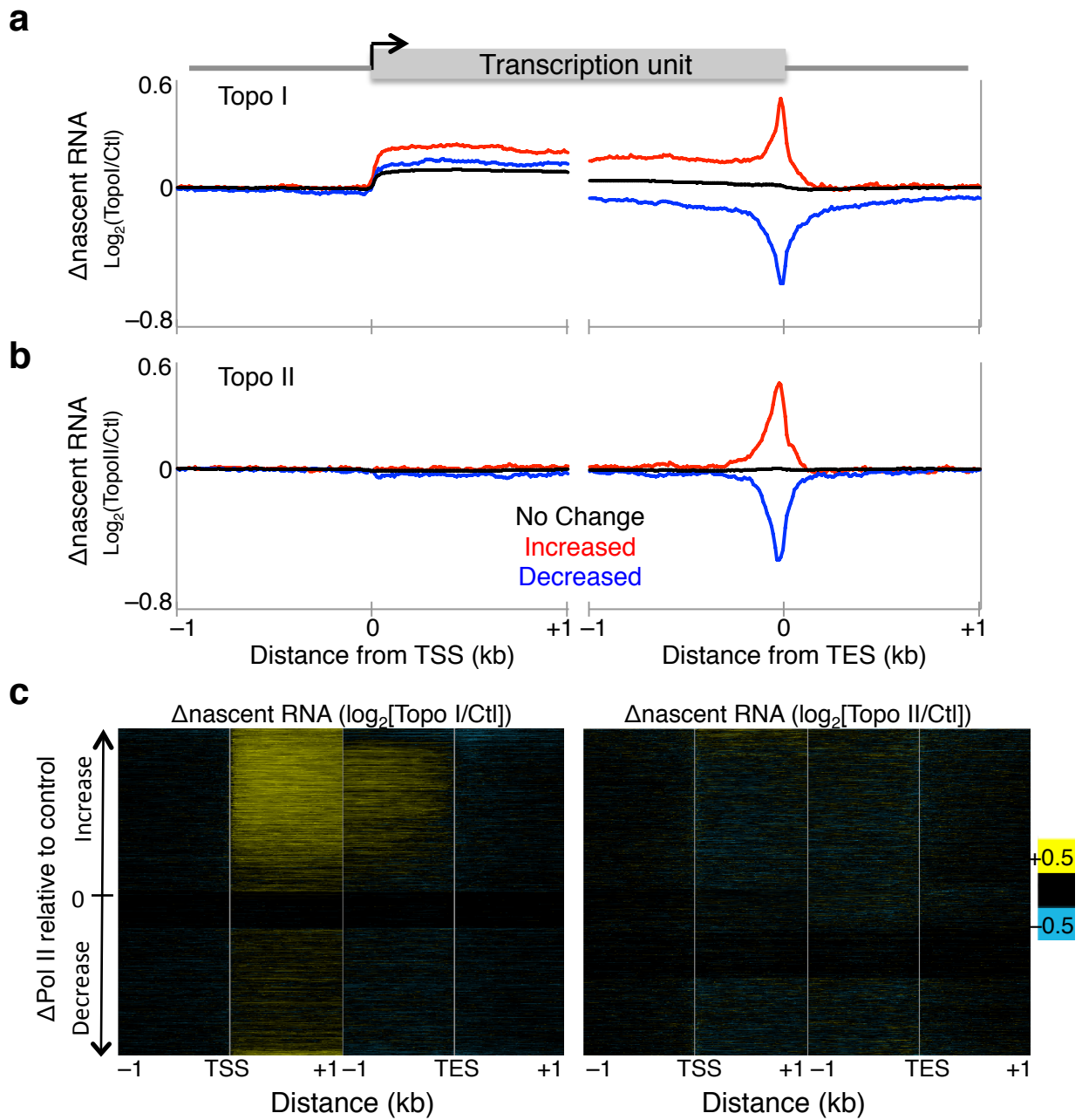


**Supplementary Figure 3: Gene tracks of various data sets.** Sequenced reads from stalled Pol II ChIP (blue), nascent RNA-seq (brown), low-salt soluble chromatin (80 mM) (green), total nucleosomes (black), and CATCH-IT (red) are mapped onto the genome. Gene tracks for each data set in two regions of chromosome 3R are shown, (a) the three-gene cluster of *Hsp70*, and (b) a representative region. Camp: camptothecin, ICRF: ICRF-193

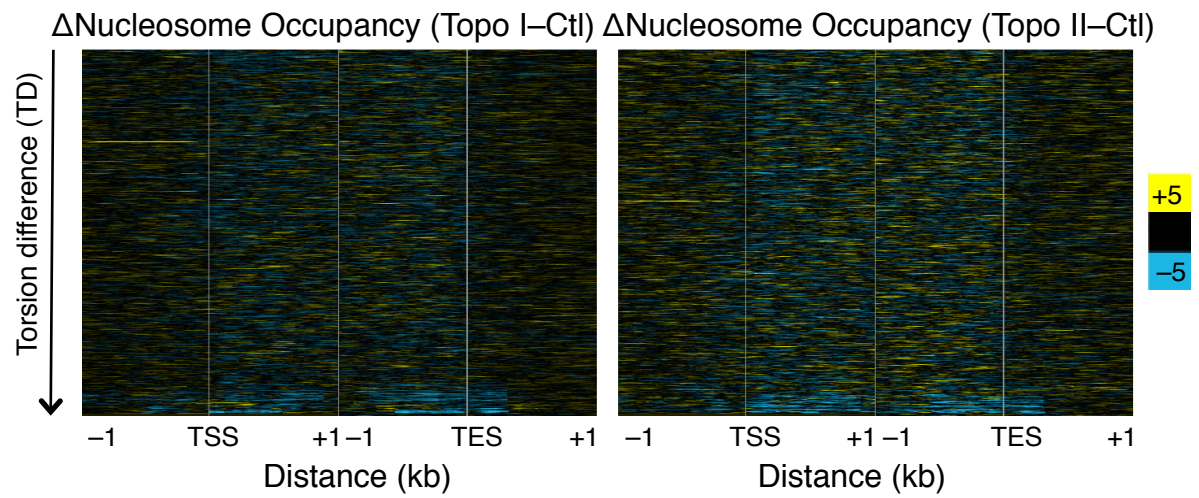




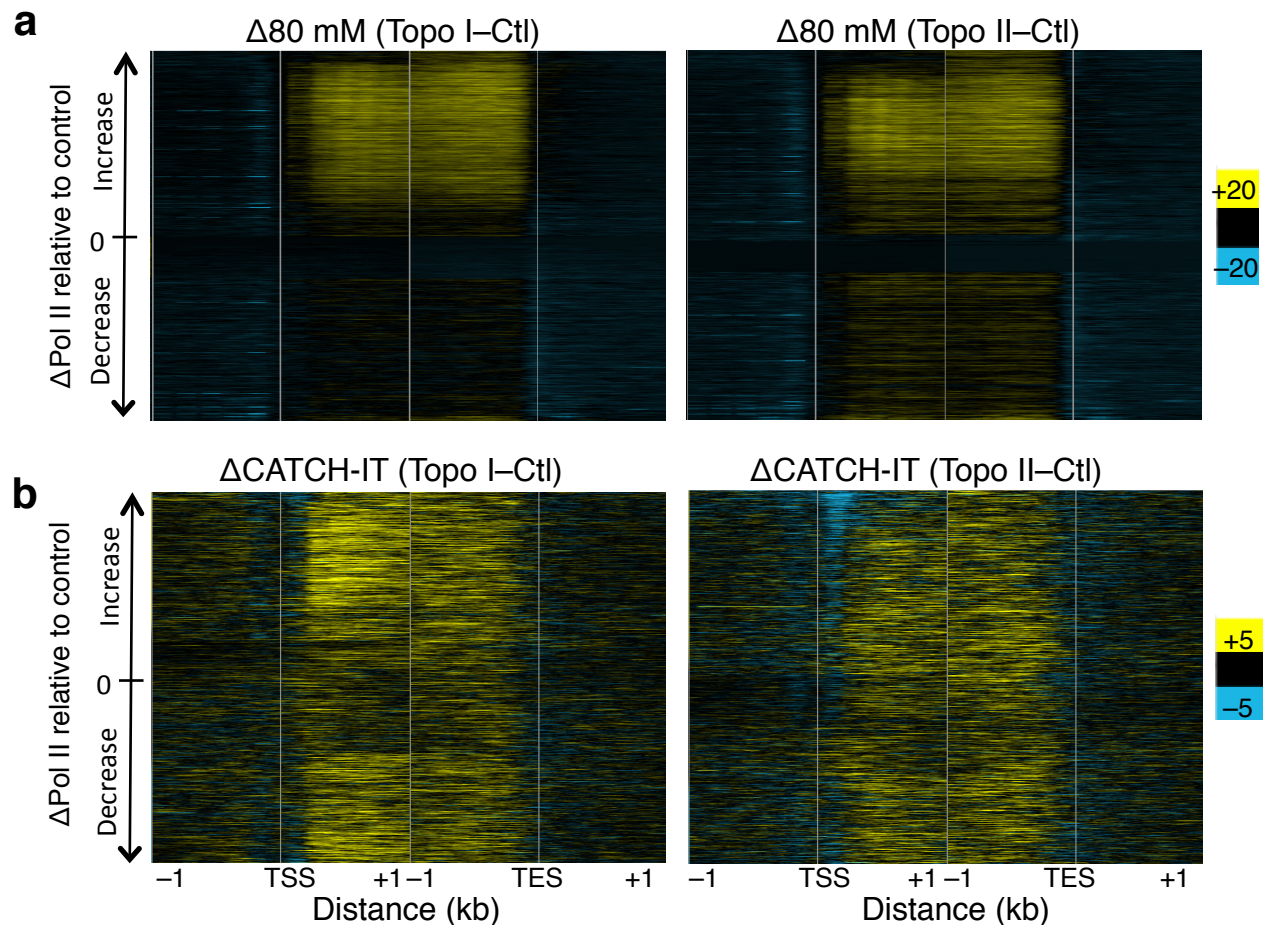
**Supplementary Figure 4: Validation of nascent RNA-seq.** (a) Heat maps of nascent RNA-seq are displayed for Topo I- (left) and Topo II-inhibited (right) samples as performed in Fig. 4b but with genes arranged by increasing gene length. The 3' end of long genes experiences a decrease in nascent RNA after Topo I inhibition. (b) Heat shocked samples were processed for nascent RNA and sequencing in parallel with untreated control and topoisomerase inhibited samples. The log ratio of heat shocked over control was calculated and displayed as heat map for regions 2 kb surrounding the TSS and TES with all genes arranged in decreasing expression of control samples (top). As expected, the nascent RNA levels decreased for most transcribed genes following 15 minutes of heat shock. Also as expected, the highly induced Hsp genes showed increased expression (bottom). (c) Based on the sequencing results, 3 groups of genes were selected: down-regulated, no change, and up-regulated after Topo I inhibition. Primers were designed for each of the genes and were used to perform qPCR analysis on replicate nascent RNA preparation. qPCR results show similar trends with sequencing data.



**Supplementary Figure 5: Nascent RNA-seq reveals Pol II kinetics.** (a – b) Genes were separated by increased (red), decreased (blue), or no change (black) in expression relative to control samples after Topo I (a) or II (b) inhibition, and the average change in nascent RNA surrounding the TSS and TES relative to control is plotted. Despite the increase in nascent RNA near the TSS after Topo I inhibition, only a fraction of genes increase in expression as measured by the increased nascent RNA levels at the TES of genes. (c) Heat map of the change in nascent RNA relative to control samples for Topo I (left) and II (right) with genes arranged by change in stalled Pol II. Topo I inhibition results in altered Pol II initiation and elongation kinetics. Ctl: control



**Supplementary Figure 6: Effects of topoisomerase inhibition on chromatin.** (a) The change in nucleosome occupancy is displayed as heat maps with genes arranged by TD (see Methods). No change in nucleosome occupancy is seen due to torsional stress. Ctl: control.



**Supplementary Figure 7: Nucleosome solubility and turnover as a function of Pol II kinetics.** (a) Heat maps of 80 mM salt soluble nucleosomes are displayed for Topo I- (left) and Topo II-inhibited (right) samples as performed in main Fig. 5c but with genes arranged by change in stalled Pol II. Nucleosomes of genes that increase in stalled Pol II after topo inhibition have increased low-salt solubility. (b) Heat maps of CATCH-IT are displayed for Topo I- (left) and Topo II-inhibited (right) samples as performed in Fig. 6b but with genes arranged by change in stalled Pol II. Genes that increase and decrease in stalled Pol II after topo inhibition results in increased nucleosome turnover. Ctl: control

**Supplementary Table 1. qPCR primers**

Gene	Forward primer	Reverse primer
Ppn	CATGGGCAAACACTTGACAC	CGAGGAACAGGAGCAGAAAC
Ndg	CCGAATTGAGAGCCTACTGC	GCCGCGTGCTATTTCTCTAC
Lar	CCACTTCCGTTGACCACTTT	CGTCAGATTGCTGAAAACA
sty	AAAAGACGAAGCGAAACGAA	GAATTTGCAAGTCGCCTCTC
svp	ATTAGGGTGCCCACTCACAG	GGGACCCTGAACCCATCTAT
Nhe2	AACAAGCACATCAACCACCA	CGGTTTGGTTTCGTTTCAGT
Ctr1B	AGCAGCGTAGGAAGAACGAG	CAGCAGGTACGAGATGACGA
Cyp6v1	AGGAAAGTTTTCGGGCATTCT	TCCCCCAATTTGTTTCATGTT
Ama	TTCGGGACTTTCCTTGAGAA	TCGGCTCAAAAATCCGTATC
thoc6	CTGAAACGCGCTTACAACAA	TTGCGCCAAATTAAGGATTC
Nufip	AGCAAACCCCGAACACTATG	TTTCTCTCGCAAACATGTCG
Mocs1	CCCTTCCCAATGGACCTAAT	TATGTTTCCATCCGCAGTGA
slgA	ACATGGTTGAGGATGGGGTA	AAACCGAGGACAGGTGTTTG
enc	GTCAACGAAGATGCGCTGTA	CAGGTGTGTGGAACATGATGG
Sos	TCATACGCGTCTTTTCGTGAG	GTTCTCAAAGCAGCTACCG
Hsp70		
-11789	TCTCTGGCCGTTATTCTCTATTTCG	AACGAGAACAGTGCGCCGTTTA
-154	TTGTGACTCTCCCTCTTTGT	TTCGCGAACATTCGAGGCG
58	CGGAGAGTCAATTCTATTCAAACA	CTTGCACTTTATTGCAGATTGT
330	TGGGTGTCTACCAACATGGCAA	ATGAGGCGTTCCGAATCTGTGA
1702	TCGACTTGGACGCCAATGGAAT	AGAGCCGTCCCTTGTGGTTCTT