

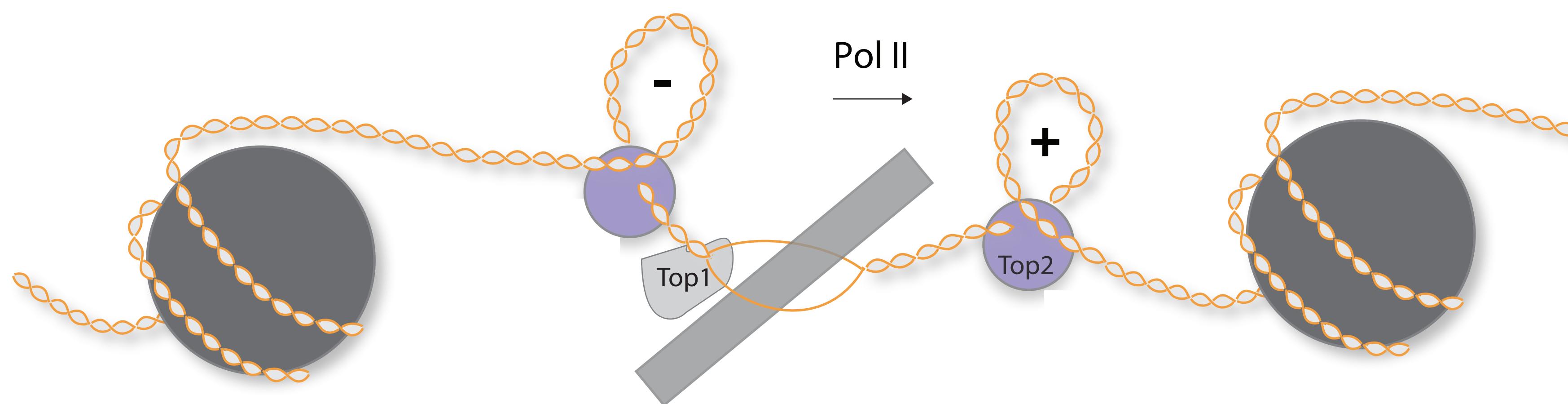
metaanalysis of topoisomerase data in yeast

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introduction

- DNA exists as a double stranded helix
- many cellular processes e.g. transcription require access to unwound DNA
- unwinding creates topological stress in DNA
- Topoisomerase enzymes relieve stress accumulated during DNA access
- interestingly, a recent report (Pederson et al. 2012) showed that, in yeast, only 15% of genes require topoisomerase activity for faithful transcription
- it is currently unclear what properties of genes necessitate topoisomerase activity for proper transcription



- to this end, we chose to examine several properties that could explain differential topoisomerase requirements:
 - gene function (Figure 2)
 - gene length (Figure 3)
 - gene locus architecture (Figure 4)
 - gene expression (Figure 5)
 - steady-state topoisomerase abundance (Figure 6)

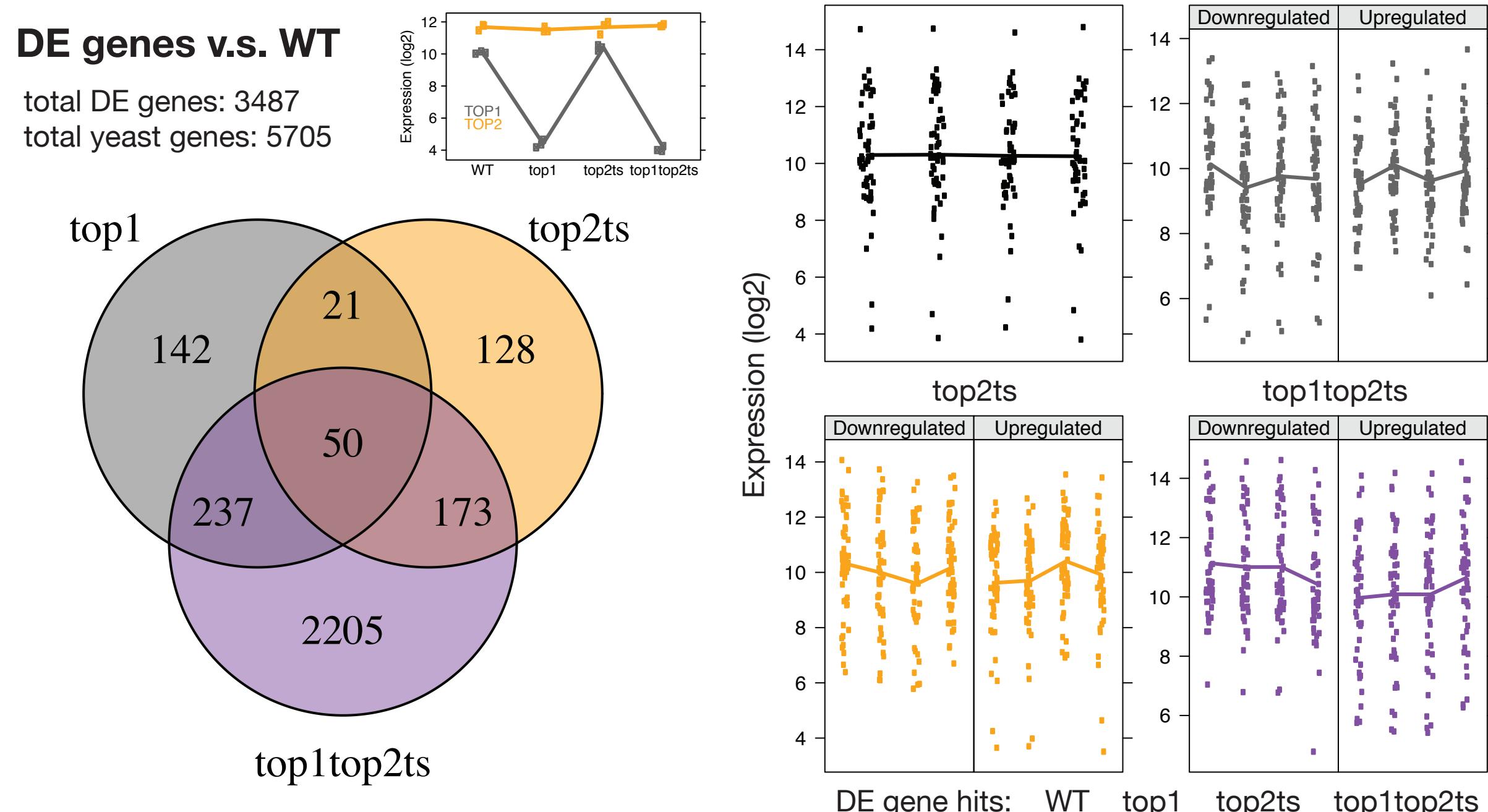


Figure 1: Identification of topoisomerase sensitive genes by differential expression analysis of topoisomerase mutants.
Genes differentially expressed in each topoisomerase mutant (top1, top2ts, top1top2ts) were identified by limma analysis of Affymetrix Genechip Yeast 2.0 microarrays (Left). Raw data was background corrected, normalized, and summarized using RMA, quantiles, and median polish algorithms, respectively. Limma hits were thresholded at a false discovery rate of 5% ('Benjamini-Hochberg multiple testing correction). Gene expression profiles for Topoisomerase I and II are shown for each genetic background (Left inset). Average gene expression profiles in each genetic background are shown for randomly selected genes ($n = 50$) from each differential expression category (Right).

GO Term	Annotated	Significant	Expected	p-value	Avg. logFC	Ontology*
nucleolus	278	1	0.88	1.7e-05	-0.15	CC
glycogen biosynthetic process	20	0	0.06	2.1e-04	-0.50	BP
glutathione cycle	9	0	0.03	5.9e-04	-0.45	BP
hydrogen sulfide biosynthetic process	6	2	0.02	5.9e-04	-0.90	BP
transmembrane transport	330	4	1.04	7.7e-04	-1.05	BP
polyphosphate metabolic process	10	0	0.03	8.7e-04	-0.32	BP
ethanol metabolic process	15	0	0.05	1.8e-03	-0.46	BP
double-strand break repair via break-ind...	26	0	0.08	1.9e-03	0.15	BP
UDP-glucosyltransferase activity	13	0	0.04	2.1e-03	-0.53	MF
sulfate assimilation	10	2	0.03	2.2e-03	-0.60	BP
cytochrome c oxidase activity	11	0	0.06	3.7e-05	0.28	MF
lysine biosynthetic process via aminoadi...	10	0	0.05	5.9e-05	0.47	BP
mitochondrial electron transport, ubiqui...	10	0	0.05	7.6e-05	0.35	BP
mitochondrial electron transport, cytoch...	9	0	0.05	2.4e-04	0.23	BP
trehalose biosynthetic process	7	1	0.08	8.0e-04	-0.36	BP
negative regulation of protein kinase ac...	15	1	0.08	1.2e-03	-0.31	BP
polyphosphate metabolic process	10	0	0.05	1.6e-03	-0.51	BP
peptide catabolic process	7	0	0.04	1.8e-03	0.00	BP
ubiquinol-cytochrome-c reductase activit...	8	0	0.04	1.8e-03	0.40	MF
integral to plasma membrane	24	0	0.12	2.0e-03	0.02	CC
proteasome storage granule	26	14	2.77	2.0e-11	1.04	CC
proteasomal ubiquitin-independent proteo...	14	8	1.49	2.4e-09	0.97	MF
threonine-type endopeptidase activity	14	8	1.49	2.4e-09	0.97	MF
extracellular region	91	32	9.68	1.6e-07	1.97	CC
response to stress	738	92	78.54	3.2e-07	1.76	BP
anchored to membrane	60	22	6.39	2.2e-06	1.23	CC
fungi-type cell wall	96	30	10.22	2.3e-06	1.35	CC
protein-DNA complex, beta-subunit co...	7	4	0.74	3.5e-05	0.00	CC
oxidation-reduction process	433	68	46.09	3.9e-05	0.40	BP
proteasome core complex, alpha-subunit c...	7	4	0.74	5.0e-05	0.92	CC

Figure 2: Gene set enrichment analysis of topoisomerase mutant differentially expressed genes.
Enrichment testing (topGO) was conducted using KS tests and a mixed weighting-elimination algorithm that weights enrichment scores of parent nodes relative to their children. Children nodes with higher enrichment scores than parents receive extra weighting and those with a lower enrichment score receive a reduction in weighting. Additionally, genes mapped to significant nodes are eliminated from higher-level nodes to prevent the inclusion of extraneous general GO terms (Alexa et al. 2006).

analysis overview

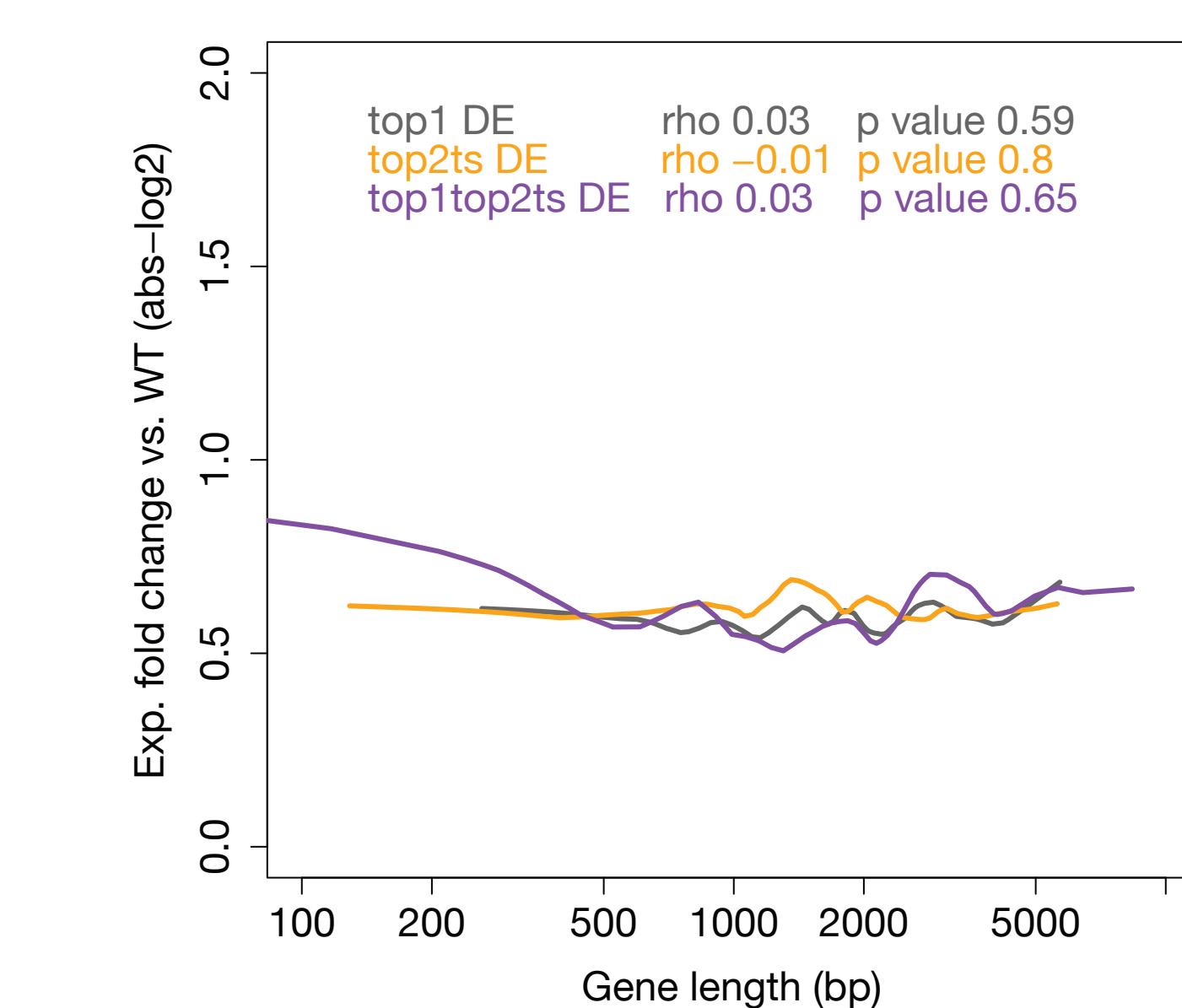
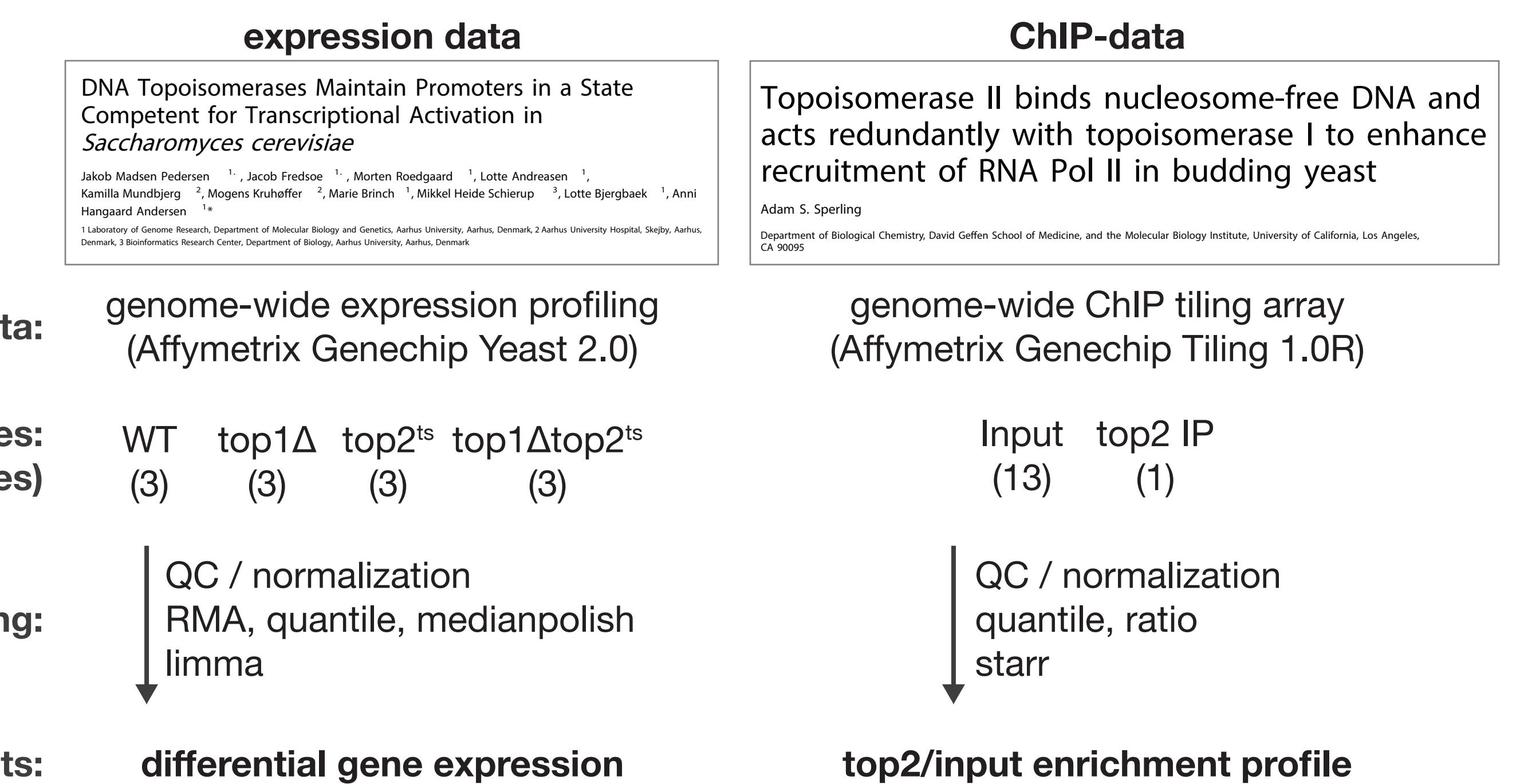


Figure 3: Requirement for topoisomerase activity does not correlate with gene length.
The fold change in gene expression for topoisomerase differentially expressed genes is plotted against gene length (loess smoothing, span = 0.2). Plots are constructed from genes sampled from the total differentially expressed set ($n = 315$ per genotype). Spearman tests were used to assess variable correlation (ρ) and statistical significance (p value).

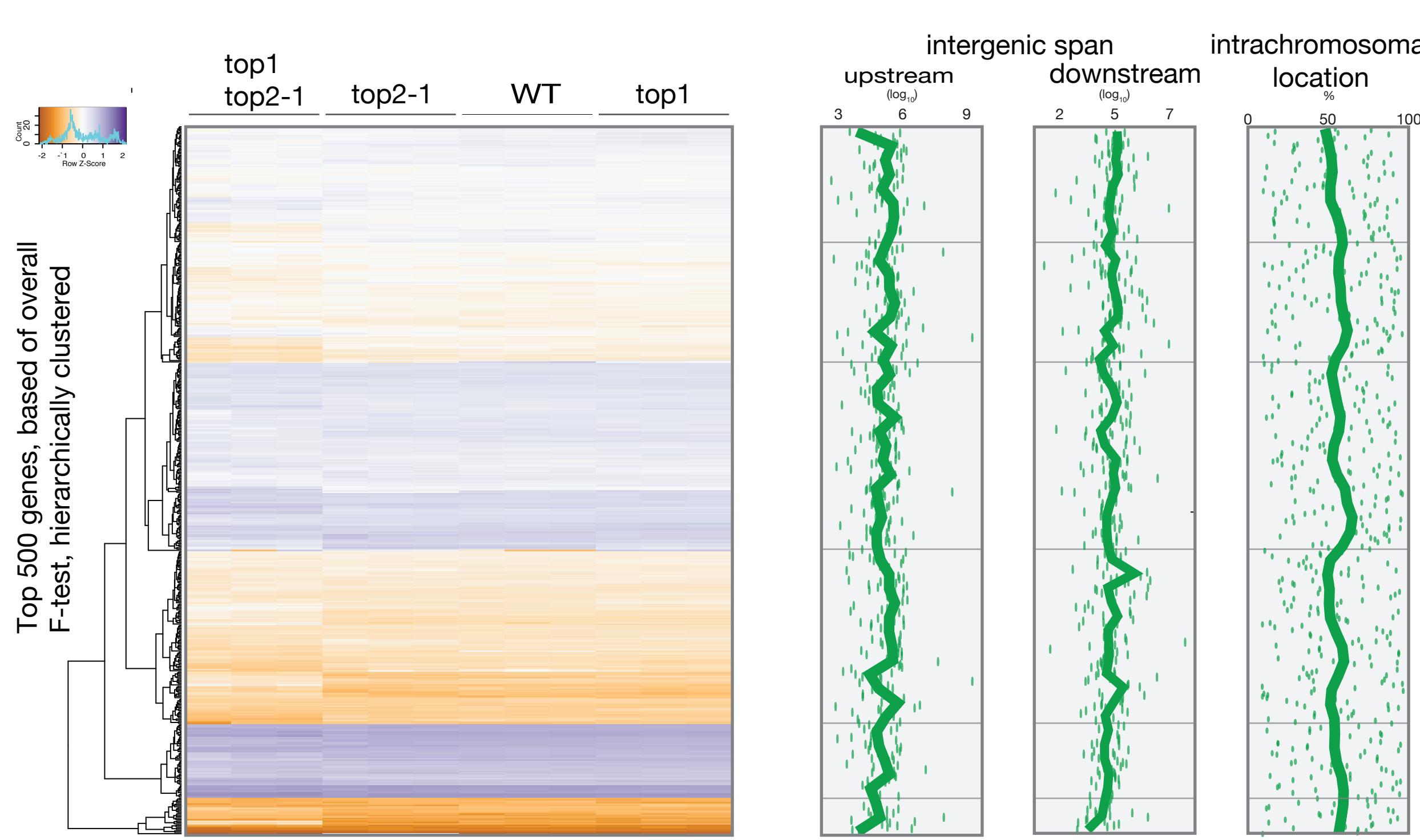


Figure 4 : Intergenic span and intrachromosomal location are not global determinants of topoisomerase activity requirement.
An overall F-test was conducted to specify differentially expressed genes across all genotypes with B.H.-corrected F.D.R. < 0.0001. The resulting top 500 genes (based on adj. P.value) were clustered hierarchically with row-scaled z-scoring and displayed in a divergent heatmap. For each gene present in the heatmap we annotated the upstream and downstream intergenic region lengths with a lowess-smoothed line over-fit (span = 0.2 ; log10 scales). We also annotated the intrachromosomal distance by plotting the percentile resulting from dividing the gene midpoint by the size of its residing chromosome.

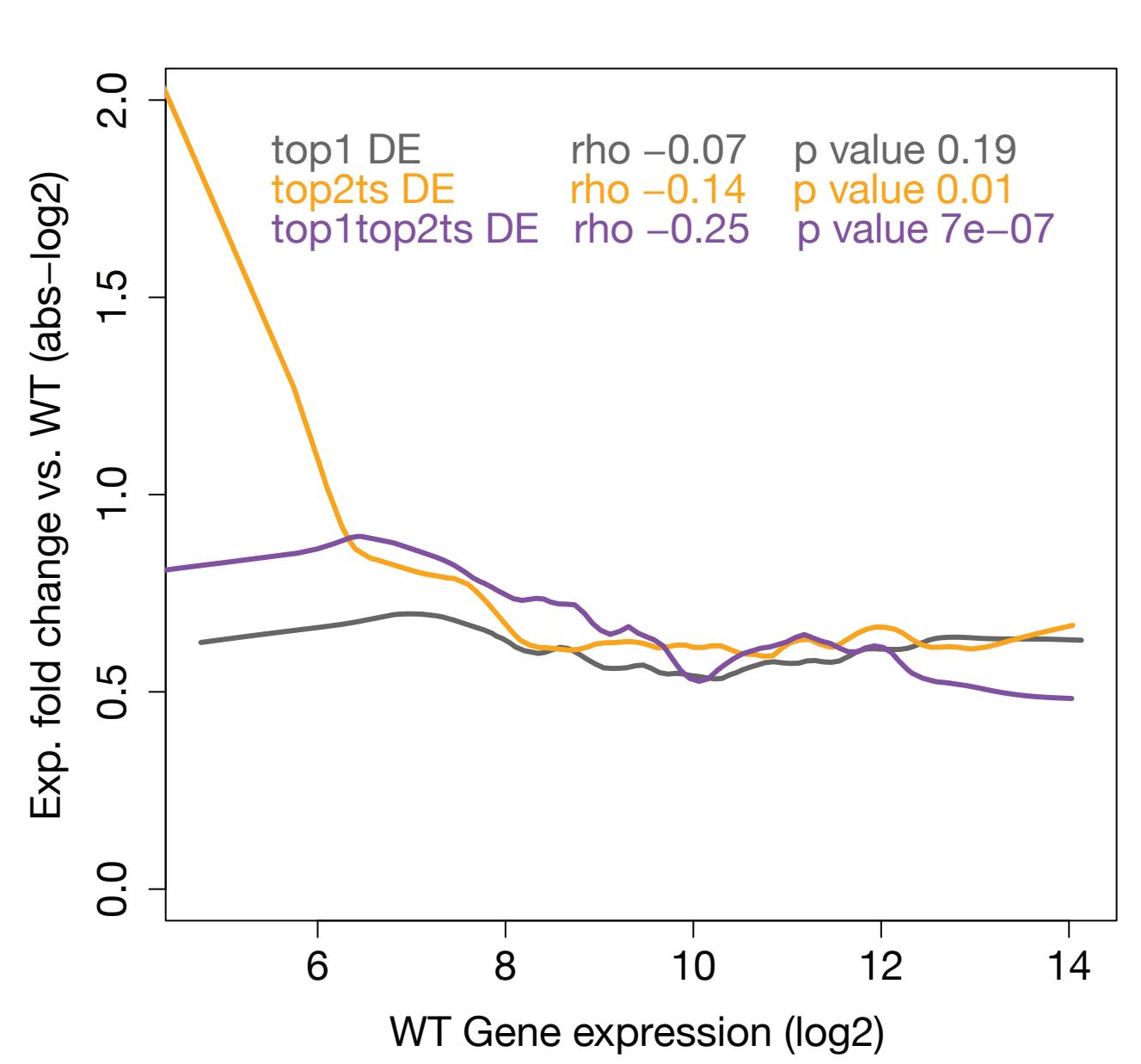


Figure 5: Requirement for topoisomerase activity modestly correlates with gene expression.
The fold change in gene expression for topoisomerase differentially expressed genes is plotted against WT gene expression (loess smoothing, span = 0.2). Plots are constructed from genes sampled from the total differentially expressed set ($n = 370$ per genotype). Spearman tests were used to assess variable correlation (ρ) and statistical significance (p value).

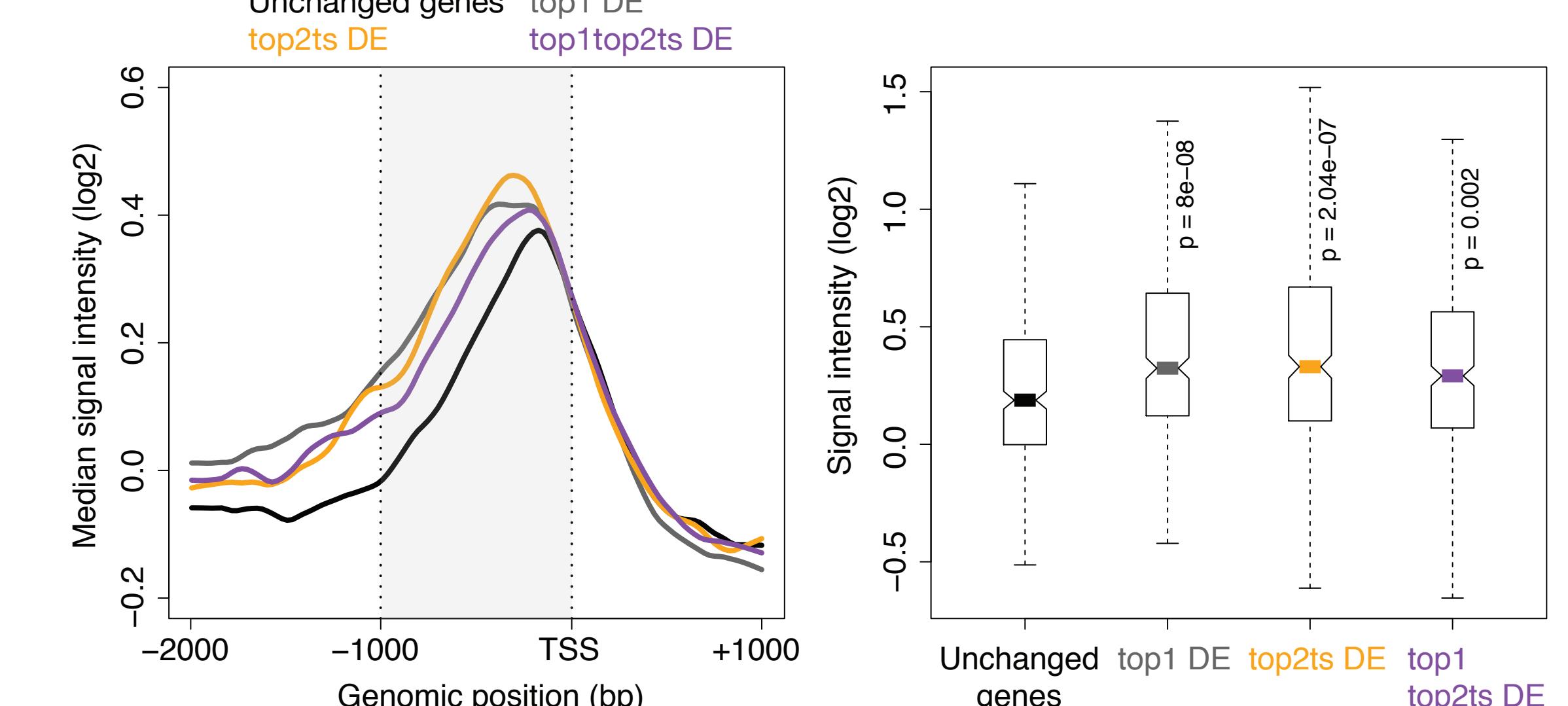


Figure 6: Steady-state Topoisomerase II occupancy is increased at genes differentially expressed in topoisomerase mutants.
Binding profiles for Top2 at gene promoters were extracted from ChIP-Chip data. Median signal intensity ($n = 350$ genes per genotype) is plotted against genomic position relative to the transcriptional start site (Left). Profiles are presented for genes identified in differential expression analysis of topoisomerase mutants. Average signal intensity (-1000 bp to TSS) is plotted for each differential expression category (Right). P-values were calculated by an unpaired, two-sample t-test assuming equal variances.