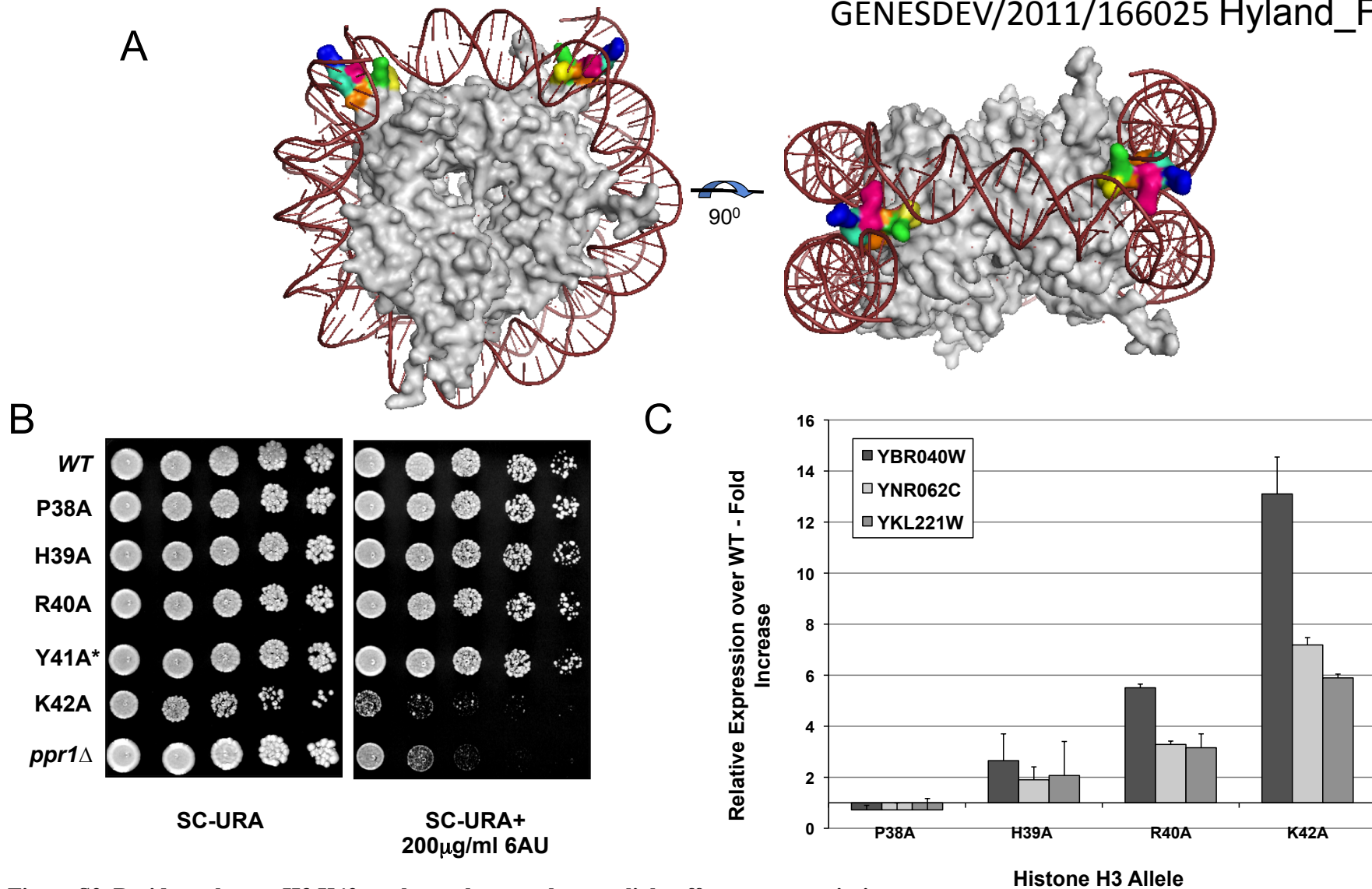
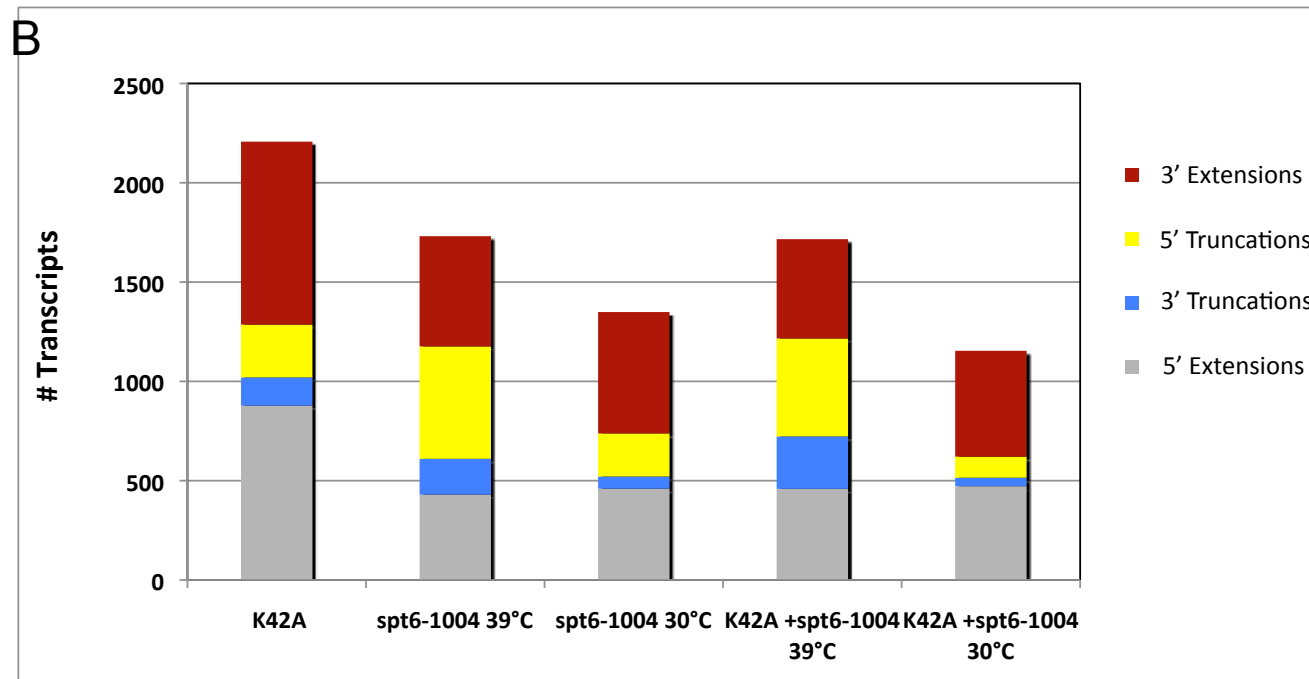
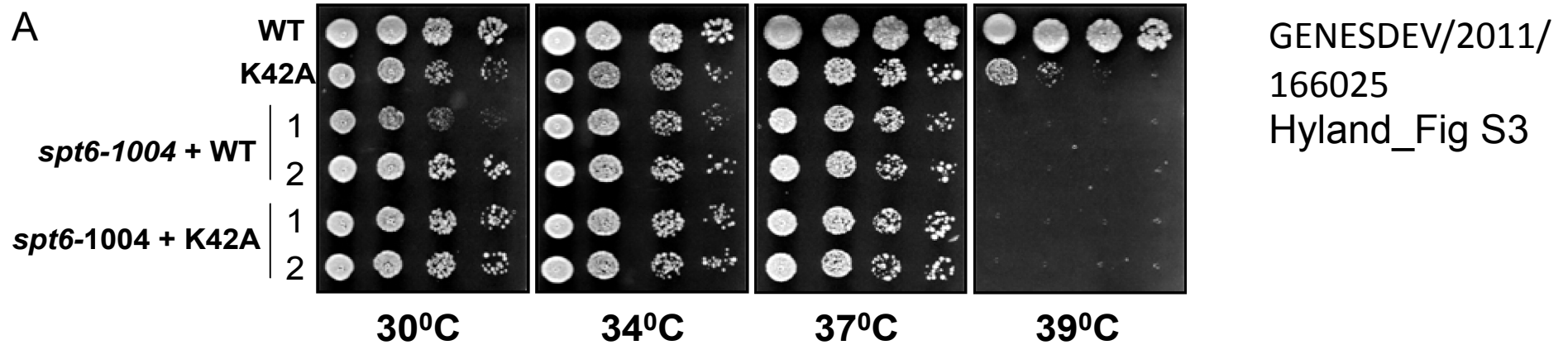


**Figure S1.** The classification of genes up-regulated by K42A as a percentage of all genes affected in broad gene ontology (GO) terms. The entire yeast genome is similarly analyzed as shown as a comparison.

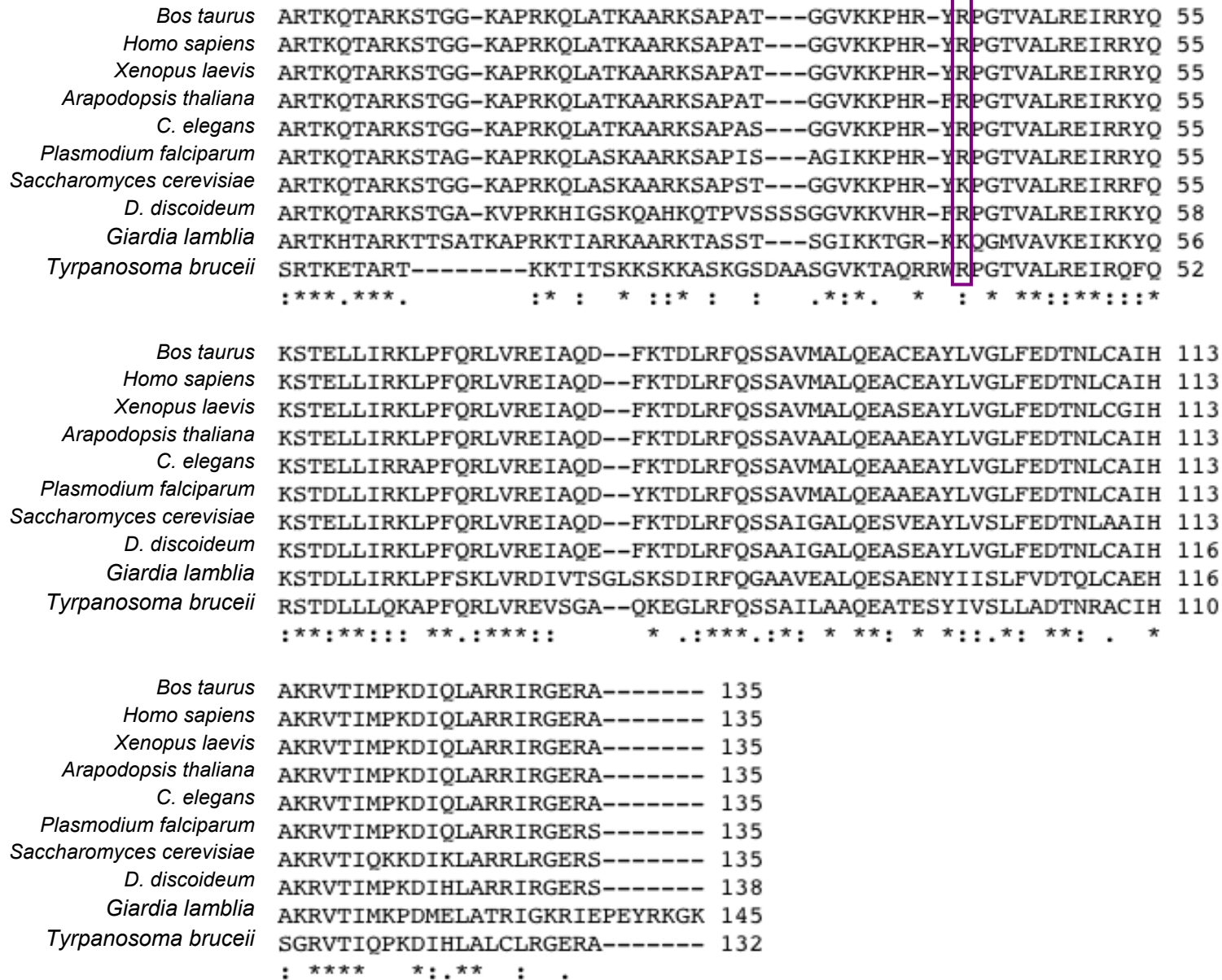


**Figure S2. Residues close to H3 K42 on the nucleosome have a slight effect on transcription**

**A** The crystal structure of the *S. cerevisiae* crystal nucleosome (White *et al* 2001 *EMBO* **20** 5207-5218) highlighting histone H3 residues positioned at the DNA entry and exit points. (Pro38=blue, His39=cyan, Arg40=magenta, Tyr41=orange, Lys42=green, Pro43=yellow) Generated using PYMOL. **B** Strains harboring wither wild-type (WT) or the indicated mutation in histone H3 were plated with an initial OD A600 0.5, and serially diluted five fold on SC-URA as a growth control and SC+200μg/ml 6AU to detect transcriptional defects. *ppr1*Δ is used as a positive control in this assay. \*in the presence of WT histone H3. **C** Quantitative RT-PCR analysis of the expression of 3 genes in strains expressing the indicated H3 mutation. Data are presented as fold change over wild-type.

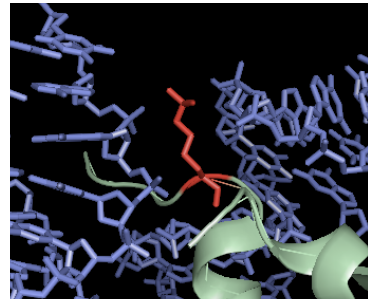


**Figure S3. Lack of a genetic interaction between *spt6-1004* and H3 K42A.** (A) Strains with the indicated genotype were plated on YPD as in figure 2b and incubated at 4 different temperatures as indicated. Plates at 37°C and 39°C were incubated for 3 and 4 days respectively. (B) Detection of mRNA length changes. RNA was extracted from strains with the indicated genotype at the indicated temperature, and used to probe a DNA tiling array of the yeast genome. mRNA length changes were categorized into four classes, those arising from 3' extensions, 5' truncations, 3' truncations and 3' extensions events and the number of transcripts in each class is shown.

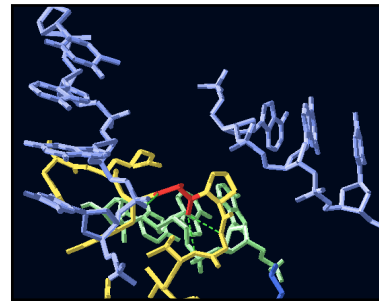


**Figure S4. Multiple Histone H3 sequence alignment**

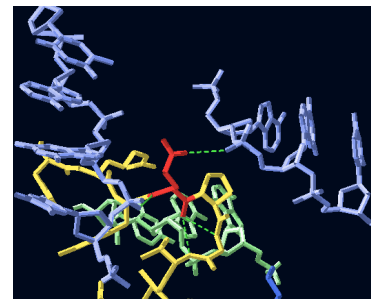
Core histone H3 amino acid sequence alignment indicating the lack of conservation of lysine at position 42 in higher eukaryotes.



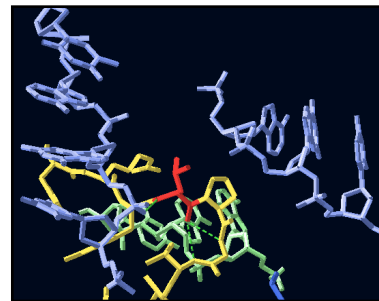
K42me2



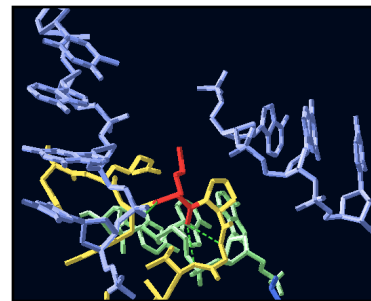
K42A (single rotamer)



K42Q (8 rotamers)

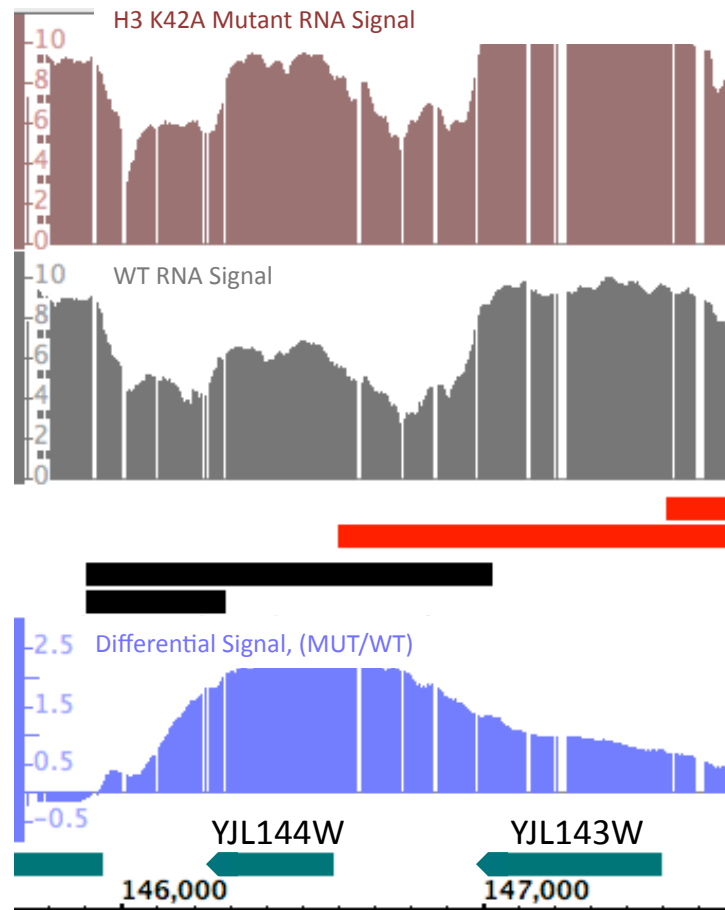


K42L (22 rotamers)



K42M (10 rotamers)

**Figure S5.** 42 = red, DNA = blue. Histone H3 = green/yellow) Shown for each is the rotamer with the greatest energy minimization out of the total number of rotamers possible for the substitution. All graphics were generated using Swiss PDB viewer except that displaying histone H3 K42me2 which was generated in PYMOL. The nucleosome pdb file 1ID3 was amended to include the dimethyl group at H3 K42, courtesy of Michael S. Cosgrove.



**Figure S6 Analysis of RNA length changes in H3 K42A cells spanning two loci.** Integrated genome browser screen shot of  $\text{Log}_2$  WT (grey) and H3 K42A (maroon) RNA levels, as well as the differential RNA levels ( $\text{Log}_2$  K42A/WT) (blue) for a segment of chromosome 10 spanning *YJL144W* and *YJL143W*. The red and black bars represent RNA length change calls made by the method. In this particular bridging event, the differential signal could represent both 5' extensions (red segment) and 3' extensions (black segment) events at both loci.