Supplementary Figures

A nucleosome turnover map reveals that the stability of histone H4 Lys20 methylation depends on histone recycling in transcribed chromatin

J Peter Svensson, Manu Shukla, Victoria Menendez-Benito, Ulrika Norman-Axelsson, Pauline Audergon, Indranil Sinha, Jason C Tanny, Robin C Allshire, Karl Ekwall

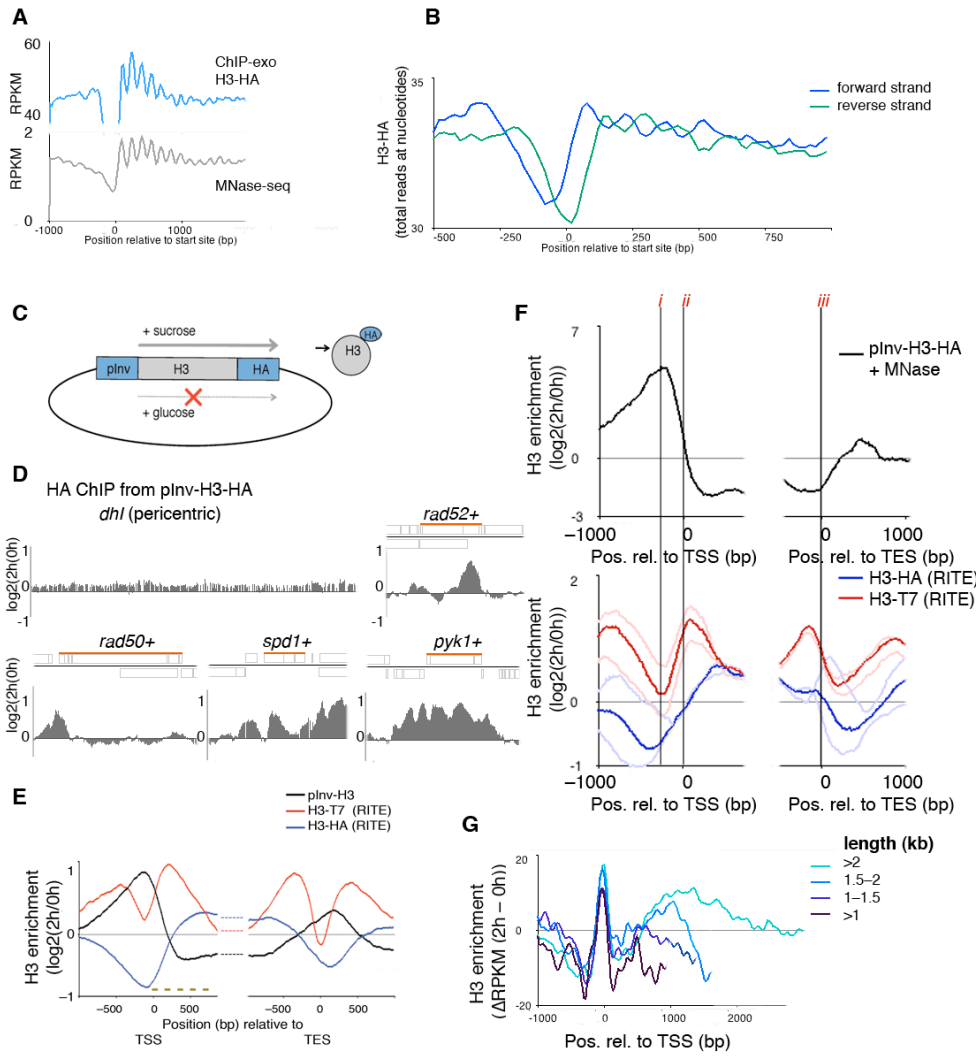

Fig. S1. Comparison between alternative techniques. (**A**) Nucleosome spacing as revealed by ChIP-exo for H3-HA and MNase-seq from GSM1201968. (**B**) Alignment of single basepair data (**C**) Model for the plasmid based pInv system to measure histone turnover. (**D**) Genome browser view of new H3 in a strain with pInv-H3-HA, 2h after induction of the invertase promoter. (**E**) Metagene analysis using ChIP-tiling array for pInv-H3-HA, H3-HA (RITE) and H3-T7 (RITE). All annotated genes (*n*=5136) are used. Approximate nucleosome positions are marked by brown lines. (**F**) Metagene analysis of all 96 genes in the dataset GSM1103380 (partial genome). Genes are aligned at TSS (left) or TES (right). Three vertical lines indicate *i*) mid-5´ NDR, *ii*) TSS and *iii*) TES. For bottom panel, the same genes are used with the RITE (ChIP-tiling array protocol). (**G**) Meta-gene analysis of histone enrichment for H3-HA (2h/0h) at different lengths of genes.

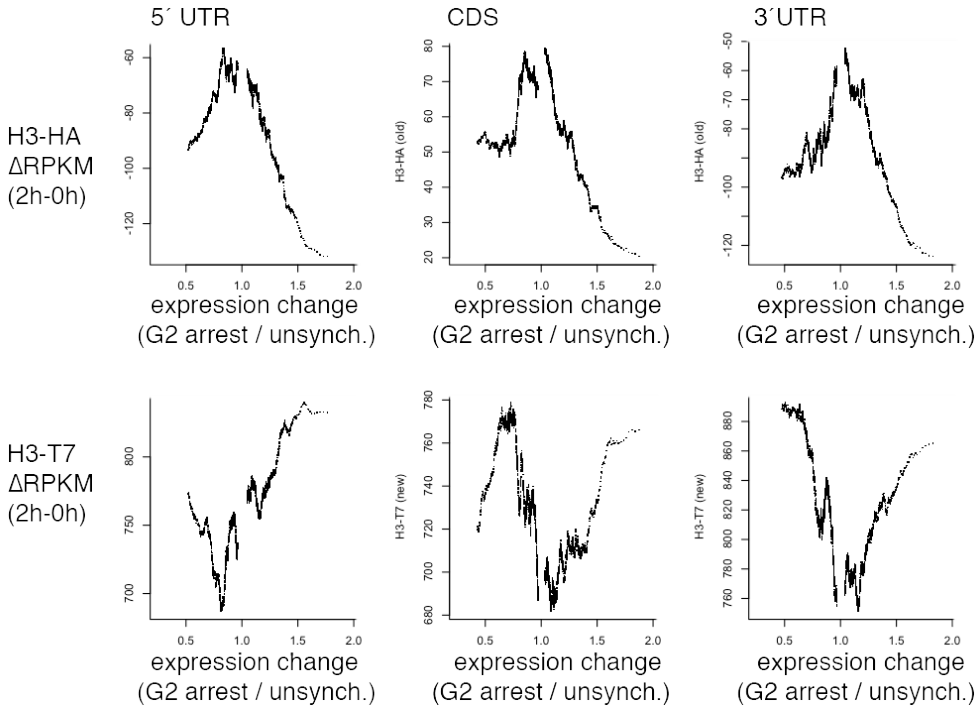

Fig. S2. Transcription induction and repression confers changes in nucleosome turnover. Histone H3 exchange at UTRs and CDS of genes that change their expression in G2 relative to unsynchronized cells. Running averages (window 500) are calculated separately for expression change ratios <1 and >1.

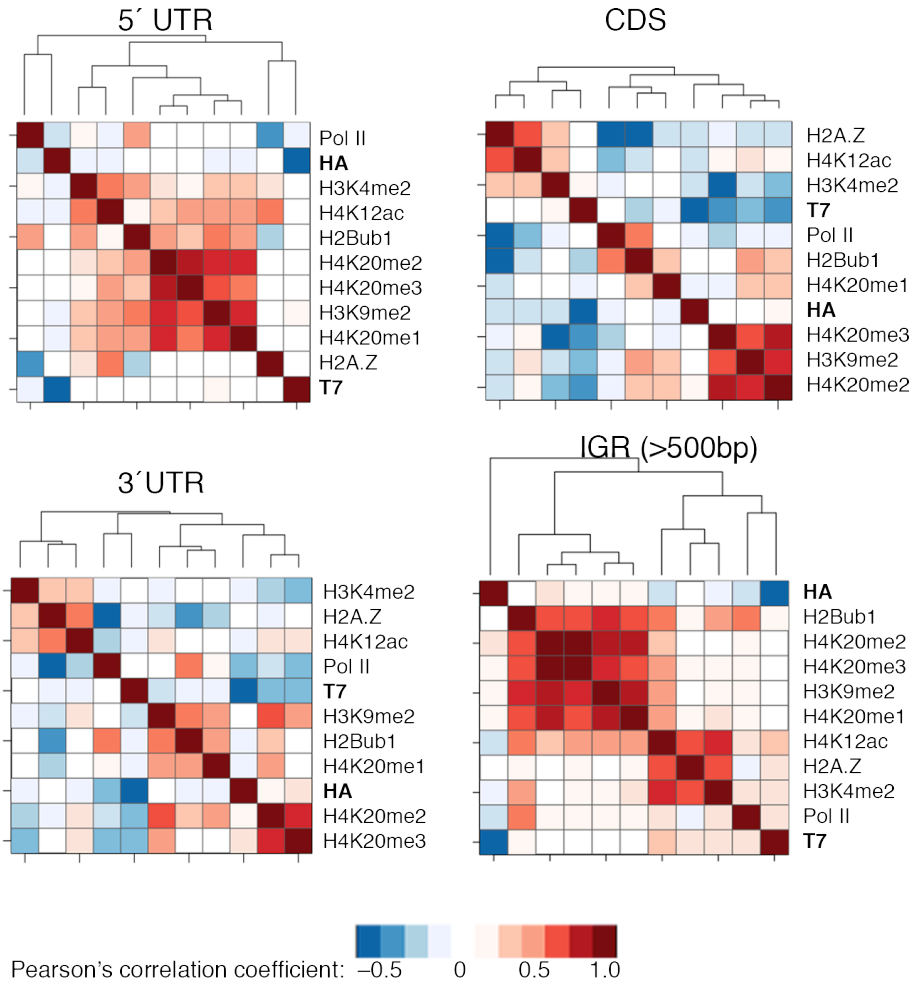

Fig. S3. Correlation between chromatin modifications and nucleosome turnover in regions relative to transcripts. Hierarchical clustering of correlation coefficients from ChIP signals for 5´ UTR, CDS, 3´ UTR and intergenic regions of >500bp. All ChIP-microarray samples were normalized to input (log2). In the ChIP-exo turnover data, i.e. H3-HA (RITE) and H3-T7 (RITE), data from the 2h samples are relative to the 0h timepoint.

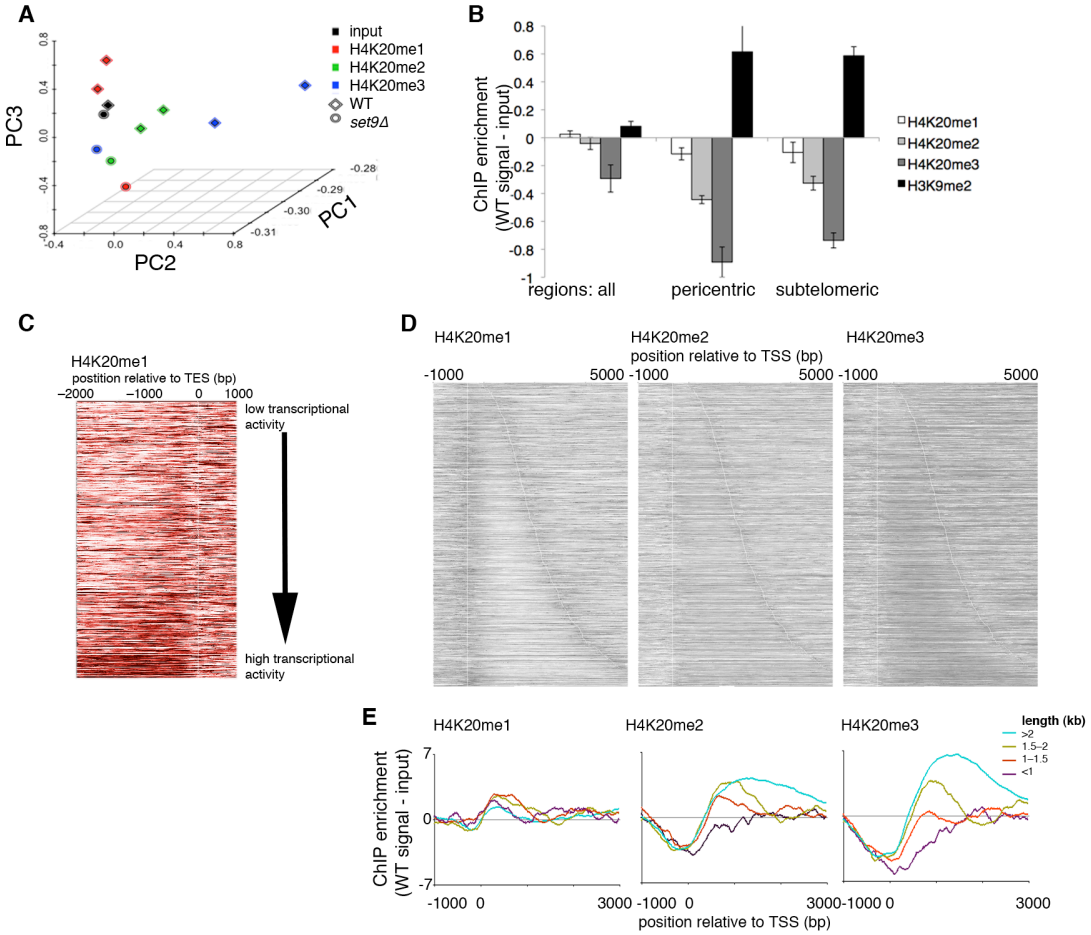

Fig. S4. Characterization of H4K20 methylation. (A) PCA (principal component analysis) plot of the different methylation states of H4K20 in WT and *set9*Δ. (**B**) Levels of methylated H4K20, and heterochromatin mark H3K9me2 at all transcribed regions, pericentric regions and subtelomeric regions (<25kb from telomeric repeats), as taken from the ChIP-microarray data. Error bars show the range of two independent experiments. **(C)** Heatmap of H4K20me1 aligned at the TES, where transcribed units are ordered from low (top) to high (bottom) expression. Long genes (>2000) and non-expressed genes (<1 transcript/cell) are not included (**D)** Heatmaps of the three methylations of H4K20 as normalized to H4 levels. (**E**). Meta-gene analysis of H3K20me1/me2/me3 using subsets of genes with different length.

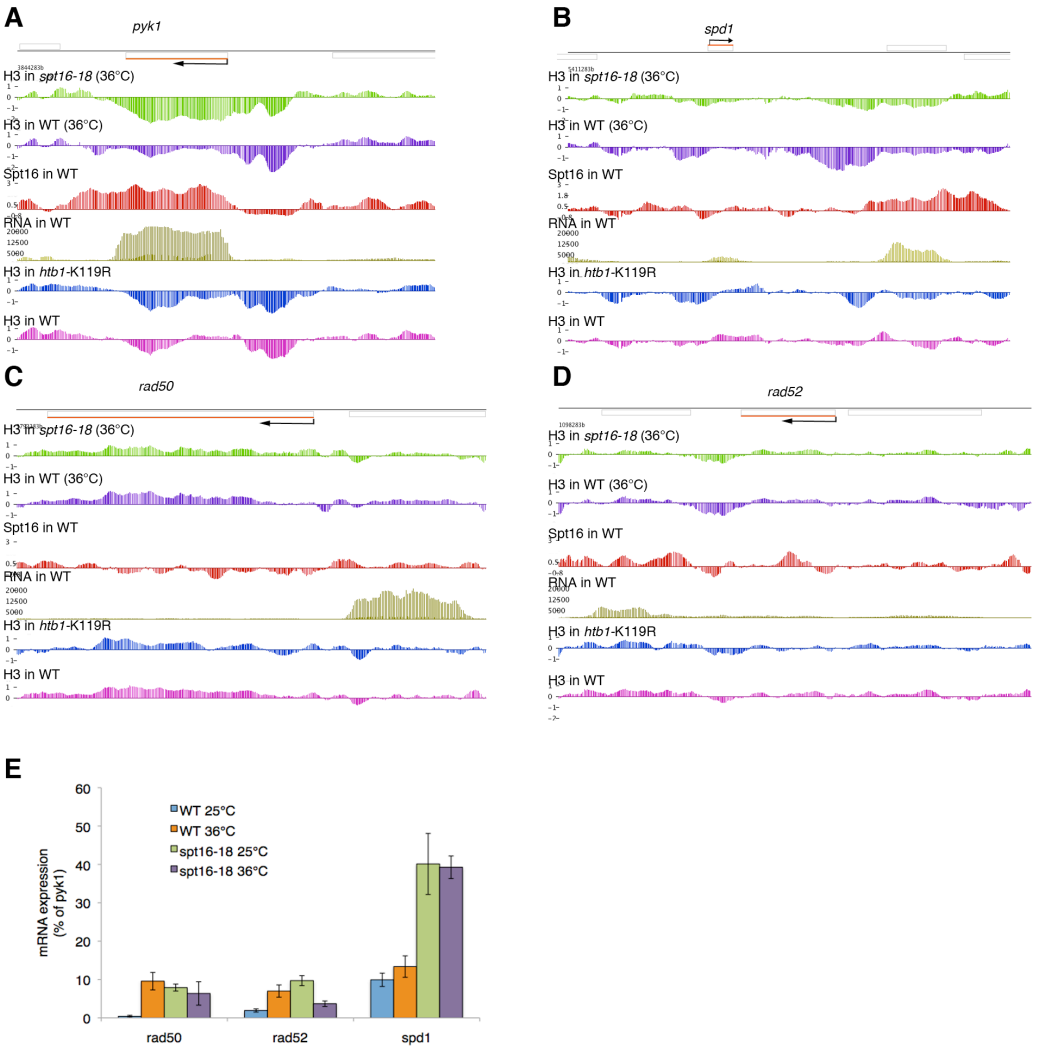

Fig. S5. Expression and nucleosome density in the genomic regions of four genes. (**A–D**) Genome browser views of nucleosome density (H3), Spt16 and expression levels over four loci. Unless otherwise stated, experiments were conducted at 30°C. (**A**) *pyk1*$^{+}$, (**B**) *spd1*$^{+}$, (**C**) *rad50*$^{+}$, (**D**) *rad52*$^{+}$. The arrows show the direction of transcription. (**E**) Expression levels of genes in WT and *spt16-18* cells at 25°C or 36°C. Bars and errorbars represent the average and the range of two independent experiments.

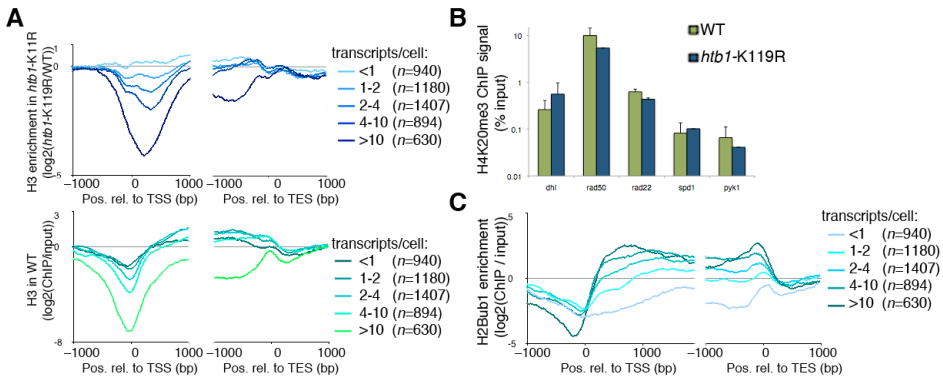

Fig. S6. H2B monoubiquitination has minor effects on nucleosome density and H4K20me3. (**A**) Changes in histone H3 density in *htb1*-K119R cells relative to WT (top) and histone H3 density WT (bottom) grown at permissive temperature (25°C) followed by 1h at the restrictive temperature (36°). Data stratified after the average number of transcript/cell during vegetative growth phase. (**B**) ChIP-qPCR of H4K20me3 at five genomic loci in *htb1*-K119R cells and WT. Bars show averages of three independent experiments, error bars depict s.e.m. (**C**) Metagene analysis of H2Bub1 levels in WT cells.