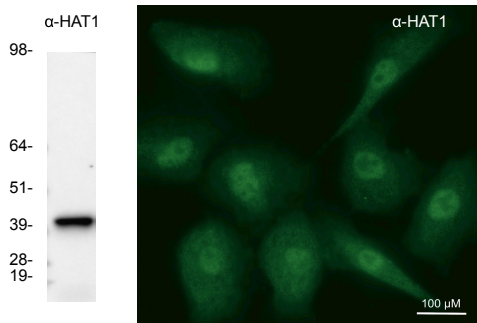
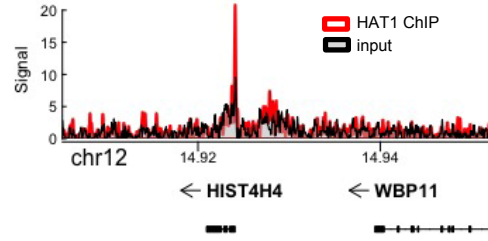


Figure S1, Related to Figure 2.

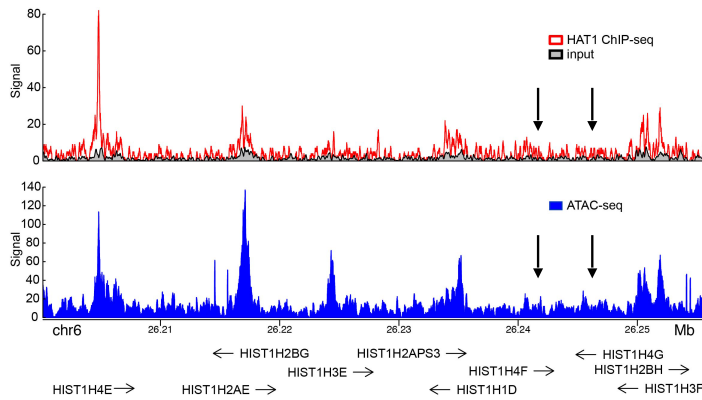
A



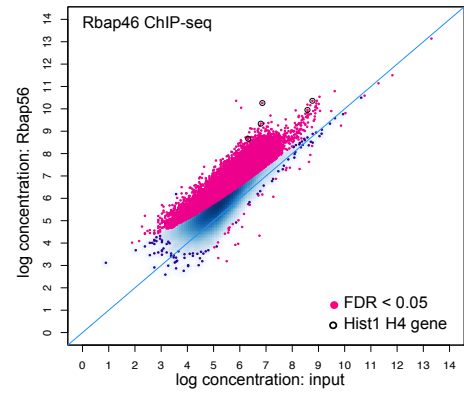
B



C



D



E

```

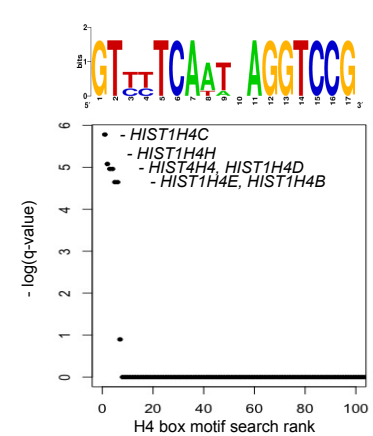
Hist1H4c 509 AAAACAATGACCAAAAT---GAAACAGTTTTCTTGCACCACCTGGT--GACCA
Hist1H4d 417 GCCTCAGCCCTCTGGCTCTCC---AGTCTGAGCAAGGGAGAAATGCACAGAAGG
Hist1H4B 425 CTCCCTCCGCTGCTTAG---GTCACTGCCCTCAGGGGGGAGCAAAAAGTGAAGGCA
Hist1H4e 510 GGGAACTGAGTCTGTGTGACGTCACTCCAGTCTGATCTGTAAGGGTAAGGCCAGCAG
Hist1H4h 556 AAAGGCTGAGTCTGTGTGACGTCACTCCAGGCTGGGTATCCGGGGTGGGCCAAGAA
consensus 601 a g aa c Ttagt gtca gagg a g agaat gga ga a

Hist1H4c 563 AATTGAATAAAAAAAAAAACCAGCCAACTCAAGTGTGTTCAAATCAGGTCCGCCAAGT
Hist1H4d 473 GGGGAACAGTGTGTGTCCGCCCTTCTCAAGAGGTTCCTCAACAGGTCCGCCAAGG
Hist1H4B 481 GC---AACGCTCCTATCCCTGCCCTTTCAGTTCCAATAGGTCCGATGTTC
Hist1H4e 570 GC---AGCACAAAGTCCCTATGCGGCTTTCAGTTCCAATAGGTCCGAAATCC
Hist1H4h 616 GC---AGGACCAAAGTCCCTATGCGGCTTTCAGTTCCAATAGGTCCGAAATCC
consensus 661 gc aa ac a aat Ccg g C c T gGT tTCAat AGGTCCG t c

Hist1H4c 623 TTGTA-----T---TAAGGAAGTGTTCAGTTCATACCCTCCACTGCG
Hist1H4d 533 TATTATAAAGTGTCTGC-GTGGCTCGCCAGACGTATCGGTTACAGATTAAACAGCTGT
Hist1H4B 536 GTGATAAAGTCTCTG---GCTTGCTTCTTTTCGCTACCTGGTTTTTGTGTCTAGC
Hist1H4e 625 CGGCATAAAGAACTACCGTCCGTGTTTTTCAGATTTTTCGGGCTATTTCGTGTGT
Hist1H4h 671 CGGCATAAAGGGCGTCTTGGCTGCGGCTTTCAGTTCCAATAGGTCCGAAATCC
consensus 721 g Ata a g GCGCTTGGCTTGGCTTTCAGTTCCAATAGGTCCGAAATCC

Hist1H4c 664 ATAGAAATCATGCTCGCCGGCCAAAGCGGAAAGGCTTGGCCAAAGGTTGGTCTTAAG
Hist1H4d 592 GGTTCCAAGATGCTCGCCGGCTAAAGGGCGAAAGGCTTGGCTAAGGTTGGCCAAAG
Hist1H4B 592 TGGTTAGACATGCTCGCCGGCCAAAGCGGAAAGGCTTGGCTAAGGTTGGTCTTAAG
Hist1H4e 685 GTGTTGGTCAATGCTCGCCGGCCAAAGCGGAAAGGCTTGGCTAAGGTTGGTCTTAAG
Hist1H4h 731 TGCATAGTCAATGCTCGCCGGCTAAAGGCGAAAGGCTTGGCTAAGGTTGGTCTTAAG
consensus 781 ttaa cATGCTCG CCgG AAAGCGGAAAGG TgGgTAAGgG GG GC AAG
    
```

F



## Figure S1, Related to Figure 2

A. (left) hTert-HME1 protein extract was fractionated by SDS-PAGE and immunoblotted with anti-HAT1 antibodies. (right) The anti-HAT1 antibodies were used to detect HAT1 by immunofluorescence in hTert-HME1 cells. See also Figure 2A.

B. ChIP-seq tracks for anti-HAT1 antibodies and input control at the Hist4H4 locus from hTert-HME1 cells. See also Figure 2B, C.

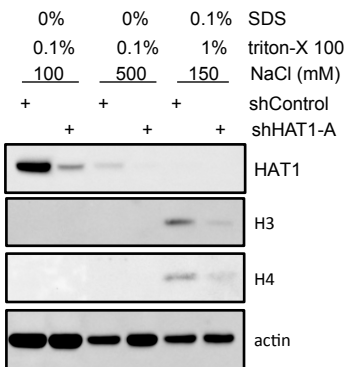
C. (upper) ChIP-seq tracks for anti-HAT1 antibodies and input control at the Hist1 locus to show peak height differences at the *HIST1H4E*, *HIST1H4F* and *HIST1H4G* loci and surrounding genes. (lower) ATAC-seq tracks. Arrows indicate location of the *HIST1H4F* and *HIST1H4G* genes that have a closed chromatin state and no HAT1 ChIP signal. See also Figure 2B, C.

D. Antibodies to Rbap46 were used for ChIP-seq and differentially bound loci were computed compared to input control. Loci significantly enriched by antibodies to Rbap46 are in red (FDR <0.05) and loci linked to Hist1 H4 genes are circled. See also Figure 2C.

E. Multiple sequence alignment of *HIST1H4* genes bound by HAT1 as determined by ChIP-seq. The H4-box is indicated with red underline. The H4 gene start codon is underlined in blue. See also Figure 2D.

F. (upper) Position-weight-matrix of the H4-box identified in the promoters of HAT1-bound H4 genes. (lower) The H4-box position-weight-matrix was used to query the accessible chromatin landscape of hTert-HME1 cells (ATAC-seq intervals) and matching loci are ranked by significance. See also Figure 2D.

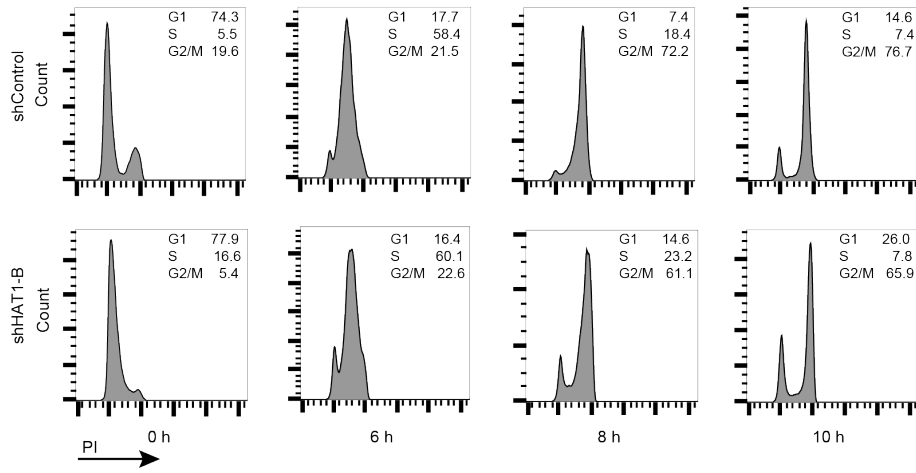
**Figure S2, Related to Figure 3.**



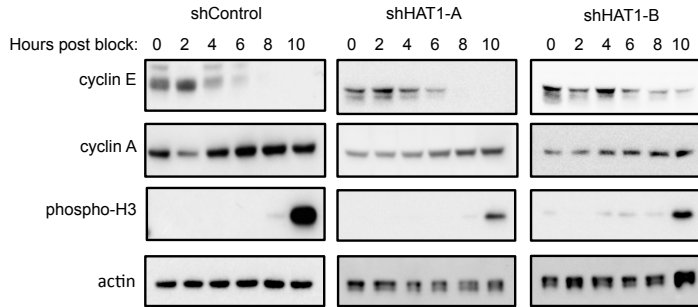
hTert-HME1 cells were infected with shControl or shHAT1-A lentiviruses and cultured for six days, then sequential extractions were performed with the indicated lysis conditions from left to right. Specifically, the first extraction was performed with 0.1% triton-X with 100 mM NaCl, then 0.1% triton-X with 500 mM NaCl, and finally RIPA (0.1% SDS, 1% triton-X and 150 mM NaCl). Recovered proteins from each extraction were fractionated by SDS-PAGE and then immunoblotted. See also Figure 3A.

**Figure S3, Related to Figure 3.**

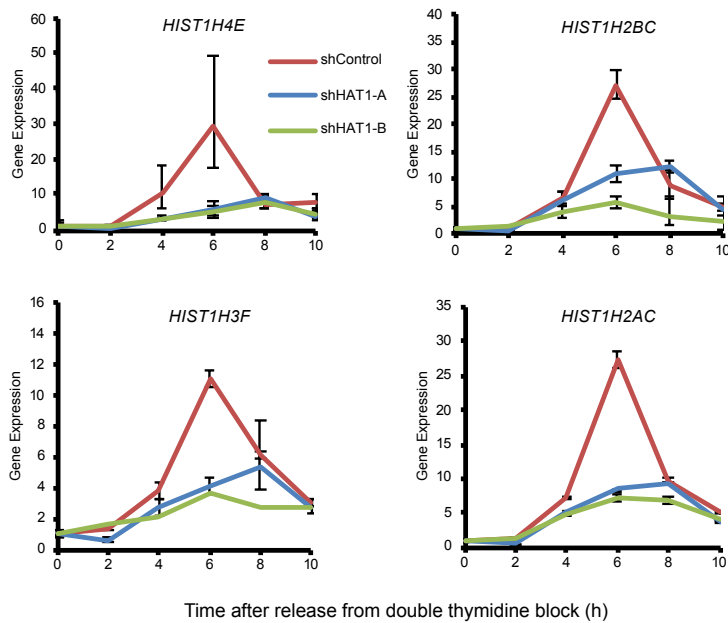
**A**



**B**



**C**



**Figure S3, Related to Figure 3.**

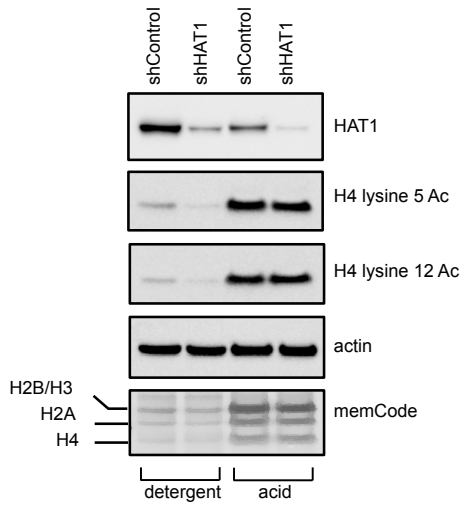
A. shRNA-containing stable cell lines were synchronized at the G1/S transition by double-thymidine block then released into S-phase and cells were harvested at the indicated time points by ethanol fixation, stained with PI and analyzed by flow cytometry. The percentage of cells in G1, S and G2/M phases of the cell cycle are indicated. See also Figure 3D.

B. shRNA-containing stable cell lines were synchronized at the G1/S transition by double-thymidine block then released into S-phase and cells were harvested at the indicated time points for protein extraction, fractionation by SDS-PAGE and immunoblotting. See also Figure 3D.

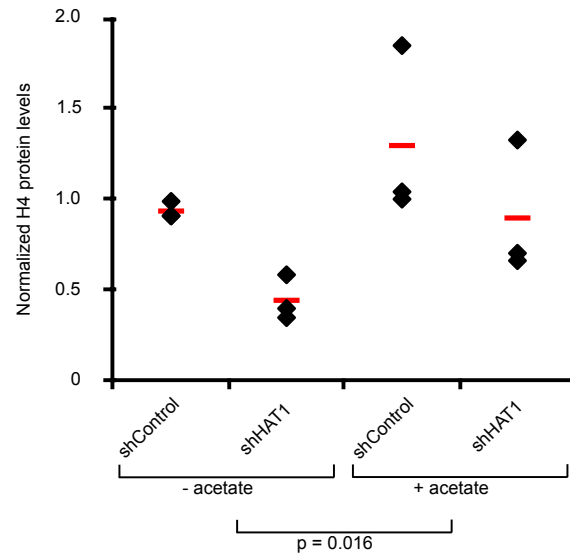
C. shRNA-containing stable cell lines were synchronized at the G1/S transition by double-thymidine block then released into S-phase and cells were harvested at the indicated time points by RNA extraction and levels of histone gene transcripts were measured by qRT-PCR. Mean +/- 95% confidence interval of three replicates is plotted. See also Figure 3.

**Figure S4, Related to Figure 4.**

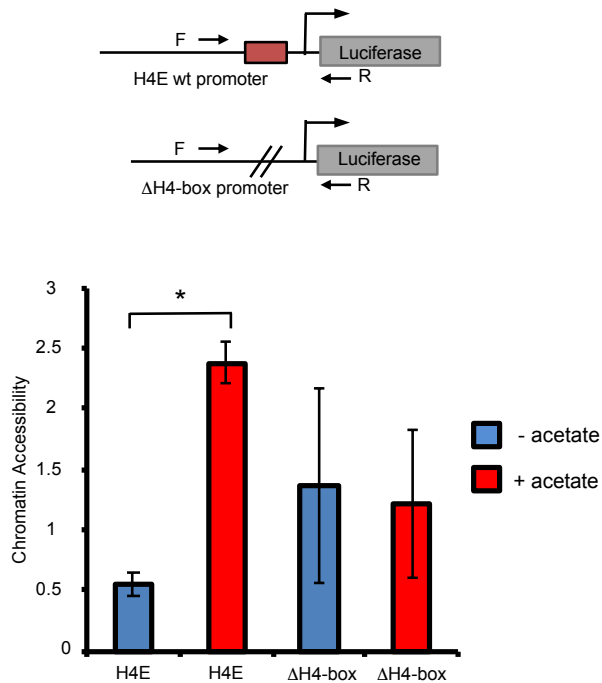
**A**



**B**



**C**



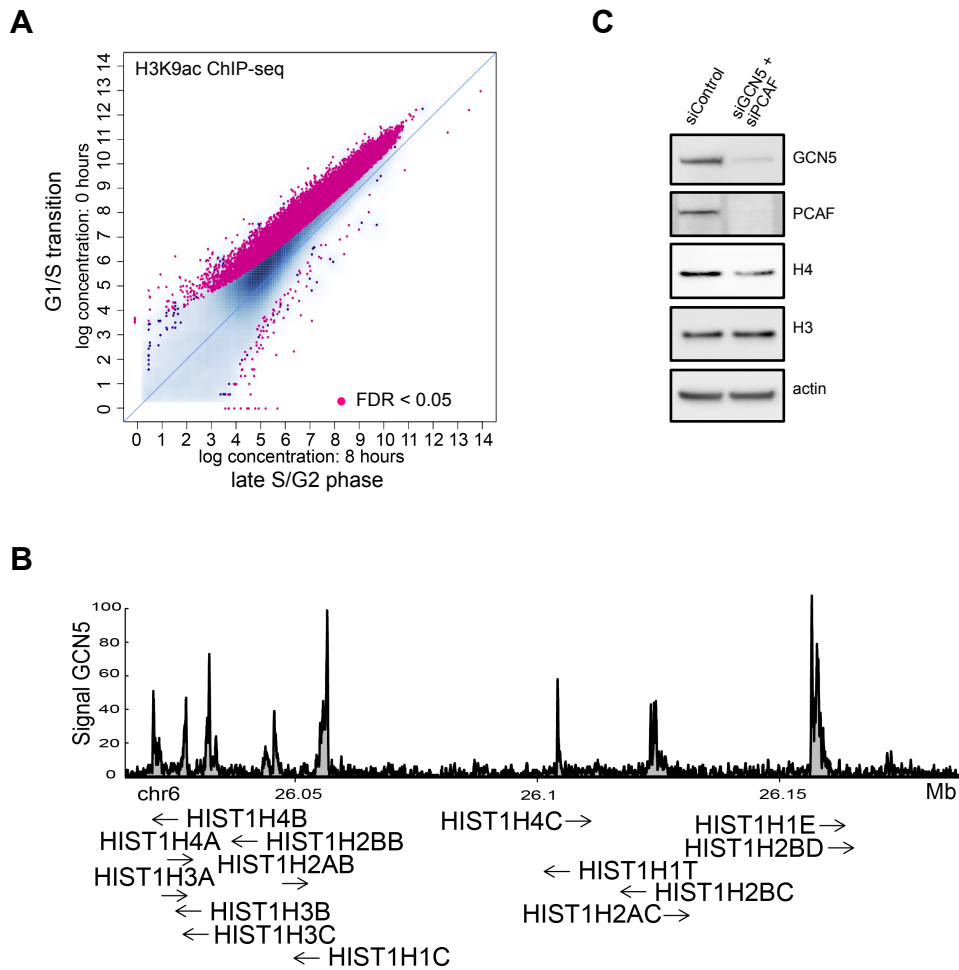
A. HAT1 is required for H4 lysine 5 and 12 acetylation on newly synthesized histones but not on chromatin-bound histones. hTert-HME1 cells were treated with lentiviral shRNAs and proteins extracted by detergent or acid then analyzed by SDS-PAGE and immunoblot or memCode blue stain. Detergent-extracted histones are newly-synthesized, whereas acid-extracted histones are chromatin-

bound. See also Figure 4B.

B. hTert-HME1 cells were treated with lentiviral shRNAs and then proteins were extracted by detergent and analyzed by SDS-PAGE and immunoblot. H4 protein quantitated by densitometry and normalized to protein loading controls (either actin or NBN). Black diamonds represent data from three biological replicates and red bar indicates mean. P-value was calculated with Student's t (paired, two-tailed). See also Figure 4E.

C. (top) Schematic of the Hist1H4E promoter luciferase reporters with or without the H4 box (red). The primer sets used to amplify the reporter are indicated. (below) The Hist1H4E promoter luciferase reporter construct was transfected to 293T cells with or without acetate treatment (5 mM). After 16 hours, chromatin accessibility was measured by Tn5 transposase activity (see methods) followed by qPCR amplification using the primer configuration indicated above. \*  $p < 0.05$ . Mean  $\pm$  SEM of 2 independent biological replicates. See also Figure 4F, G.

**Figure S5, Related to Figure 5.**



A. hTert-HME1 cells were synchronized at the G1/S transition by double-thymidine block (0 hours) or in late-S/G2 phase (8 hours release after release from double-thymidine block) and cross-linked by formaldehyde. Then ChIP-seq was performed with anti-H3K9ac antibodies. Differential peak calling was performed and significantly altered peaks (FDR < 0.05) are indicated in pink. See also Figure 5A-E.

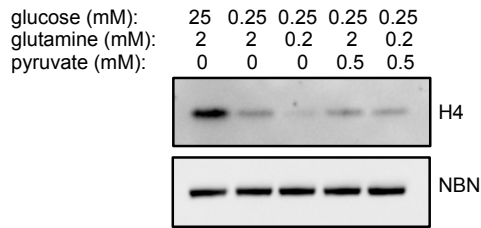
B. ENCODE ChIP-seq signal (ENCFF000XEE) for GCN5/KAT2A in HeLa-S3 cells at the Hist1 locus. See also Figure 5F.

C. hTert-HME1 were transfected with control siRNAs or pools of siRNAs to GCN5 and PCAF. After three days proteins were collected and analyzed by SDS-PAGE and immunoblot. See also Figure 5F.

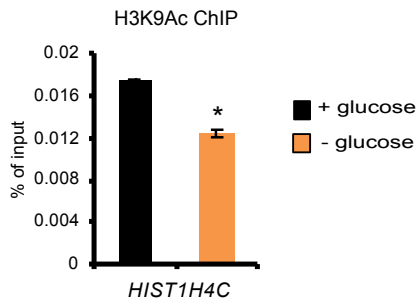


**Figure S6, Related to Figure 6.**

**A**



**B**



A. hTert-HME1 cells were grown in glucose and glutamine-free RPMI media supplemented with the indicated amounts of glucose, glutamine and pyruvate. Cells were grown in culture for three days, then harvested by detergent extraction and analyzed by SDS-PAGE then immunoblot. See also Figure 6E.

B. hTert-HME1 cells were grown in normal conditions or glucose withdrawal (1 mM) for three days, then ChIP was performed with antibodies recognizing acetylated H3 lysine 9. The Hist1H4C promoter was amplified by qPCR with specific primers. Mean +/- SD, \*  $p < 0.05$ . See also Figure 6F.