**Supplemental Data**

**Antibody characterization**

The specificity of each antibody in western blot was characterized by comparing the histone signal in wild-type (WT) relative to substitution mutant strains. The signal obtained with each antibody, including five anti-H4ac antibodies and the H4S1ph antibody, was greatly decreased when the targeted amino acid is mutated to a non-modifiable mimic (Fig. 1B and 1C).

The antibody specificity was also determined in the ChIP experimental format. The H4K12ac antibody was highly specific in ChIP, as the signal decreased by more than 10-fold when H4K12 is substituted to arginine (Fig. 2A). The H4S1ph ChIP signal strictly parallels the appearance of H4S1ph by western blot (not shown), and was significantly decreased in an H4S1A strain (Fig. 2B). In addition, previous analyses show that binding of these antibodies is not altered by coincident H4 acetylation and H4S1ph on the same tail (2,3).

**Supplemental Material and Methods**

**Sample preparation for massive parallel sequencing**

DNA purified from chromatin immunoprecipitation was processed using “DNA processing for ChIP samples” kit and instructions, as recommended by instructions from the manufacturer (Illumina). DNA sequences were obtained on a Solexa G2 Genome Analyzer. All reads were aligned to the yeast genome allowing up to two mismatches. If the best match was unique, then the read was retained for further analysis. The number of reads are presented in Supp. Table 1.

**Bioinformatic analysis**

**Data Management**

The positions of the uniquely-aligning reads were loaded into a mysql database for further processing and visualization. Additional data such as SGD genes (from UCSC) and nucleosome positioning data (4) where loaded into the database for visualization and analysis.
Estimating DNA fragment length

We estimated the DNA fragment length for a DNA sample by computing the average strand-specific profiles of read ends at a 5bp resolution +/-2KB from the start of all genes and identifying the shift that yields the maximum correlation. A strand-specific profile is an average profile of the number of starts (for plus strand reads) or end (for minus strand reads) that align at locations relative to gene TSSs. These profiles should have the same shape, but be shifted relative to each other by the average DNA fragment length. We shifted the plus-strand profile relative to the negative-strand profile and computed the correlation between the two. The shift that yielded of highest correlation was used. Only H4Ac had a relatively sharp, unambiguous, correlation maximum; the other two data sets had broader correlation peaks that spanned the H4Ac peak. The estimates were 220bp (from 165-280bp for H4), 180 (from 175-180bp for H4Ac) and 170 (from 130-345 for H4S1ph). Because the data all came from the same initial sonicated sample, we used the H4Ac peak value (180bp) value of all lanes in most further processing.

Computing profiles at TSS

To compute the profiles of histone occupancy or modification across the starts of genes we extended each read to 180bp total length, then determined the total number of centers of the extended spans that fell in each 20bp bin positioned relative to the TSS.

Computing profiles at Genomic Profiles

To compute ratios of modifications to H4 occupancy, we used a sliding window of 200bp positioned every 10bp. We tallied the number of overlapping reads (per million) and added 1 before taking the ratio. Using reads per million ensures that the ratio is not affected by differences in the total number of reads acquired for each track. Adding the pseudocounts ensures that the ratios are not too sensitive to low values in the H4 (denominator) data.

Measuring enrichment of marks over regions

We computed enrichment ratios at 50bp intervals over the whole genome of the number of reads per million reads per KB that overlap each 50bp window of H4Ac and H4S1ph versus H4 with a pseudo-count of 1. To compute the average ratio on an interval relative to a gene, we took the average value of
the 50bp bins that overlap the interval. For H4Ac we used the region -200 to 750bp and for H4S1ph, -200 to 300bp. In this analysis reads were extended to 200bp which should not make any significant difference from the 180bp used elsewhere.

Gene Set Enrichment Analyses

We used the GSEA program to compute enrichment of gene expression modules in the modification data (5,6). The definitions of the Barkai modules were downloaded from http://barkai-serv.weizmann.ac.il/Modules then reformatted to a GMT file format for GSEA (7). Similar steps were taken for the Esposito gene expression data. Yeast genes and their histone modification log2 ratios were formatted as a presorted rank (RNK) file for input to GSEA. We used 1000 permutations, the weighted scoring scheme, and set size limits of 15 and 200.

Data availability

All genome-wide data are available in the ArrayExpress database (http://www.ebi.ac.uk/microarray-as/ac/). Submission is currently in process and an ID number will soon be available.
Supplemental Figure Legends

Supp. Figure 1. Genome-wide analysis of H4S1ph and H4K12ac distribution

(A) Histone H4 distribution of a representative region. ChIP-SEQ reads are presented along a portion of chromosome VIII. Genes ORF are annotated from SGD database and represented by oriented black arrows, labeled with systematic gene names. Nucleosome-free regions localized the promoter of each gene (black arrows) and match nucleosomes positions previously identified (black boxes, 4).

(B) H4S1ph and H4K12ac distribution of the same region as panel A. The number of reads for each modification has been normalized to total number of reads for the H4 sample. H4 data are presented in grey (same as Panel A). The 5’ regions of each gene appear enriched in H4S1ph and H4K12ac compared to H4 levels (asterisks).

(C) H4S1ph and H4K12ac enrichment, normalized to the local abundance in H4. Data are presented in Log2 scale.

Supp. Figure 2. Histone occupancy is not affected by a H4S1A mutation

Histone H4 occupancy in the IME2 promoter and ORF is not decreased by the introduction of an H4S1A mutation. This phenotype is similar to previous observations in an sps1Δ strain, which can not phosphorylated H4S1. Increased ChIP signal is likely due to the lack of chromatin compaction observed in the H4S1A and sps1Δ mutants (1).

Supplemental Table 1. Summary of the massive parallel sequencing data statistics

Supplemental Table 2. Primers lists

Supplemental Table 3. List of all GSEA modules and their gene composition

Supplemental Table 4. List of GSEA modules enriched for H4S1ph

Supplemental Table 5. List of GSEA selected genes enriched in H4S1ph

Supp. Tables 1 and 2 are in this document. Supp Tables 3, 4 and 5 are independent files.
Supp. Bibliography

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### Supp. Table 1. Summary of massive parallel sequencing data statistics

| ChIP sample | H4     | H4S1ph | H4K12ac |
|-------------|--------|--------|---------|
| Total number of reads | 8,743,611 | 6,227,404 | 8,976,606 |
| Number of unique reads aligned on the genome (%) | 5,225,048 (60%) | 4,395,046 (71%) | 6,242,056 (70%) |
### Supp. Table 2. Primers lists

Primers used for ChIP

| Set   | Forward                      | Reverse                       |
|-------|------------------------------|-------------------------------|
| Ime2  |                              |                               |
| Promoter 1 | AGCGGGACAACTGCTACGTC       | ATGGGCTCTCCTCTTTGGTACTC       |
| Promoter 2 | ATAAAGACAATGATGACAAAACTGCAT | GACCCTCGTATAATCCTGGGAGTGT     |
| Promoter 3 | TGGTAACCCAGATACCTATTCTCTCA  | TGCGGAAGTCTTGGGTGAACAG        |
| ORF 4  | GCCTAAAATGTTCAACAAAAAATATAT | GCCAATACACCTTTGTTCTTTTTTTT    |
| ORF 5  | CCCATCCAGACCAAGCAAT         | TTTGGTCTCTTGGGCTTTGGT        |
| ORF 6  | GGAATGTATGGAGCAAACCTATAC    | CGGCGCCTTTATATCTTTTCA        |

ACS1  CACAACGGCCCATCTCAGAT  TGGCGTTGCAGAAGATGAAC
ADY2  CCCGTCACAGTTGGAGAAT  GGCTTTGCTTAAAGTCGACCTTTCA

Primers used for RT-qPCR

| Forward                      | Reverse                       |
|------------------------------|-------------------------------|
| Ime1 | GGTGGTGTATTTTTCAACCTCAAGGC   | TTATAGATGACCTCAGTTGCCCCTCC   |
| Ime2 | CAAGCATTGATTTTTTGAGGTTATA   | CTGCGGCAATTGTTGGA            |
| Smk1 | CCAACCAACGGTATAGTAAAGTCCGTAG | CGACGATAAGATTTCCTGCAAGATGCAC |
| Ssp1 | AAAAGTAGGCAAAAGGCAAGGGA      | CAGTTCTCTAGCTTCTTGCCCT       |
| Dit2 | TCGTGAAAATGGGAGGCC          | TTGGAAACTACAAATGTCGAC        |
| Nup85 | CAGGAAAAGGTCTGCTACATGCAGG   | ATTTAACACTCTGTCATCCTAAAATTCAGG |