

sample array. Positive binding interactions are shown as red spots where only the printing control (green) is visible for negative interactions.

Figure 2. Antibody binding to histone peptide microarrays. Results of two independent arrays consisting of 24 independent spots for each peptide are depicted as heatmaps of the normalized mean intensity and plotted on a scale from 0 to 1 with 1 (yellow) being the most significant (see Methods). **(A)** Interactions of H3K4- and H3K79-specific antibodies with methylated peptides derived from the N-terminus of histone H3 (antibodies used are given in **Table S1** and further information in **Figure S1 and S3**). **(B)** Recognition of histone H3 acetyllysine peptides by H3K14ac antibodies. **(C)** Alignment of sequence surrounding H3K14 and H3K16. **(D)** Western blot of yeast whole cell extract probed with H3K14ac antibody preincubated with various concentrations of histone H3 peptides.

Figure 3 Effect of neighboring modifications on histone antibody recognition. Results of two independent arrays consisting of 24 independent spots for each peptide are depicted as heatmaps of the normalized mean intensity and plotted on a scale from 0 to 1 with 1 (yellow) being the most significant (see Methods). **(A)** Heatmap of neighboring modification effect on H3K4me3-specific antibody recognition. **(B)** Recognition of H3S10 phosphorylation by mono- and dual-specific PTM antibodies. **(C)** bar graph of data in **(B)**. Differences in intensities were compared using two-way ANOVA analyses and confidence intervals (* 95% and ** 99%) are indicated for individual comparisons. Further information is available in **Figure S3**.

Figure 4 Chromatin-associating domain binding to histone peptide arrays. **(A)** (*top*) Molecular representation of the Rag2 PHD domain binding to an H3K4me3-containing peptide (PDB accession 2V83). (*bottom*) Heatmap of Rag2 PHD domain binding to histone H3 peptides. **(B)** (*top*) Molecular representation of the BPTF PHD domain binding to an H3K4me3-containing peptide (PDB accession 2F6J). (*bottom*) Heatmap of Rag2 PHD-Bromo domain binding to histone H3 peptides. **(C)** (*top*) Molecular representation of the CHD1 chromodomain binding to an H3K4me3-containing peptide (PDB accession 2B2W). (*bottom*) Heatmap of CHD1 chromodomain binding to histone H3 peptides. All models were constructed using PyMol software. Additional information is also contained in Figures S2 and S4.

Supplementary Figure S1 Comparison of H3K4 methyllysine-specific antibodies for different methylation states (H3K4me1 – Millipore 07-436, H3K4me2 – Active Motif 39142, H3K4me3 –

Active Motif 39160). Data are plotted as the mean with SEM for the indicated peptide from a single array. The results of two independent arrays are shown. Differences in intensities were compared using two-way ANOVA analyses and confidence intervals (* 95% and *** 99.9%) are indicated for individual comparisons.

Supplementary Figure S2. Effect of neighboring acetylation on BPTF binding. Data are plotted as the mean with SEM for the indicated peptide from a single array. Differences in intensities were compared using two-way ANOVA analyses and confidence intervals (* 95%, ** 99% and *** 99.9%) are indicated for comparisons to the H3K4me3 peptide with no other modifications (left).

Supplementary Figure S3. Heat map of all experimental antibody data. Data was normalized to the strongest interaction plotted on a scale from 0 to 1 with 1 (yellow) being the most significant.

Supplementary Figure S4. Scatter plots comparing two arrays for Rag2, BPTF, and CHD1.