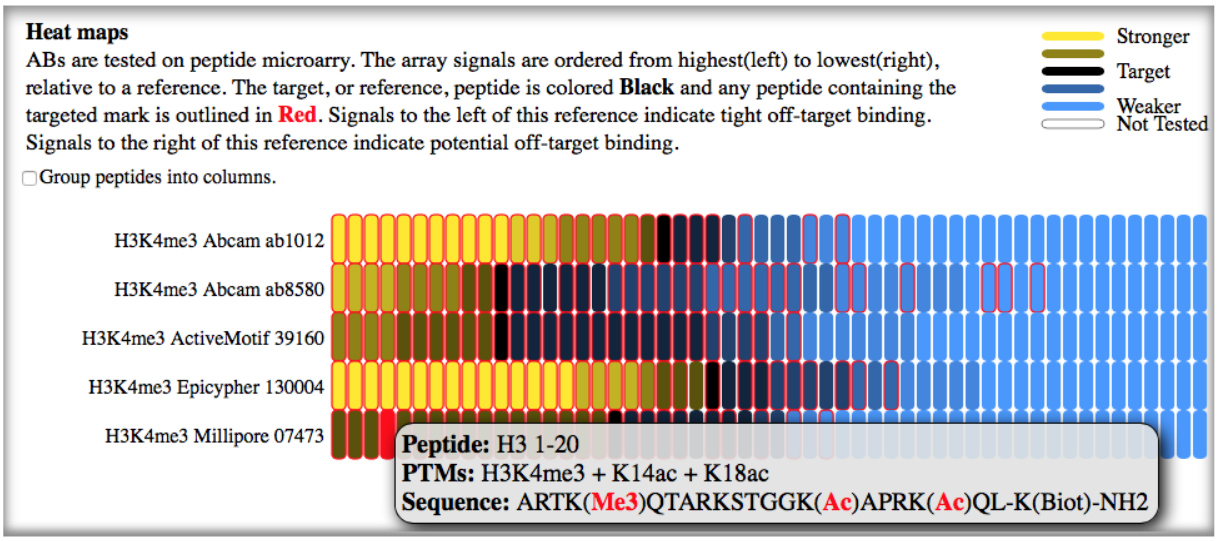
Figure S1

A


Heat maps
ABs are tested on peptide microarry. The array signals are ordered from highest(left) to lowest(right), relative to a reference. The target, or reference, peptide is colored Black and any peptide containing the targeted mark is outlined in Red. Signals to the left of this reference indicate tight off-target binding. Signals to the right of this reference indicate potential off-target binding.
Stronger
Target
Weaker
Not Tested
Group peptides into columns.
H3K4me3 Abcam ab1012
H3K4me3 Abcam ab8580
H3K4me3 ActiveMotif 39160
H3K4me3 Epicypher 130004
H3K4me3 Millipore 07473
Peptide: H3 1-20
PTMs: H3K4me3 + K14ac + K18ac
Sequence: ARTK(Me3)QTARKSTGGK(Ac)APRK(Ac)QL-K(Biot)-NH2

B

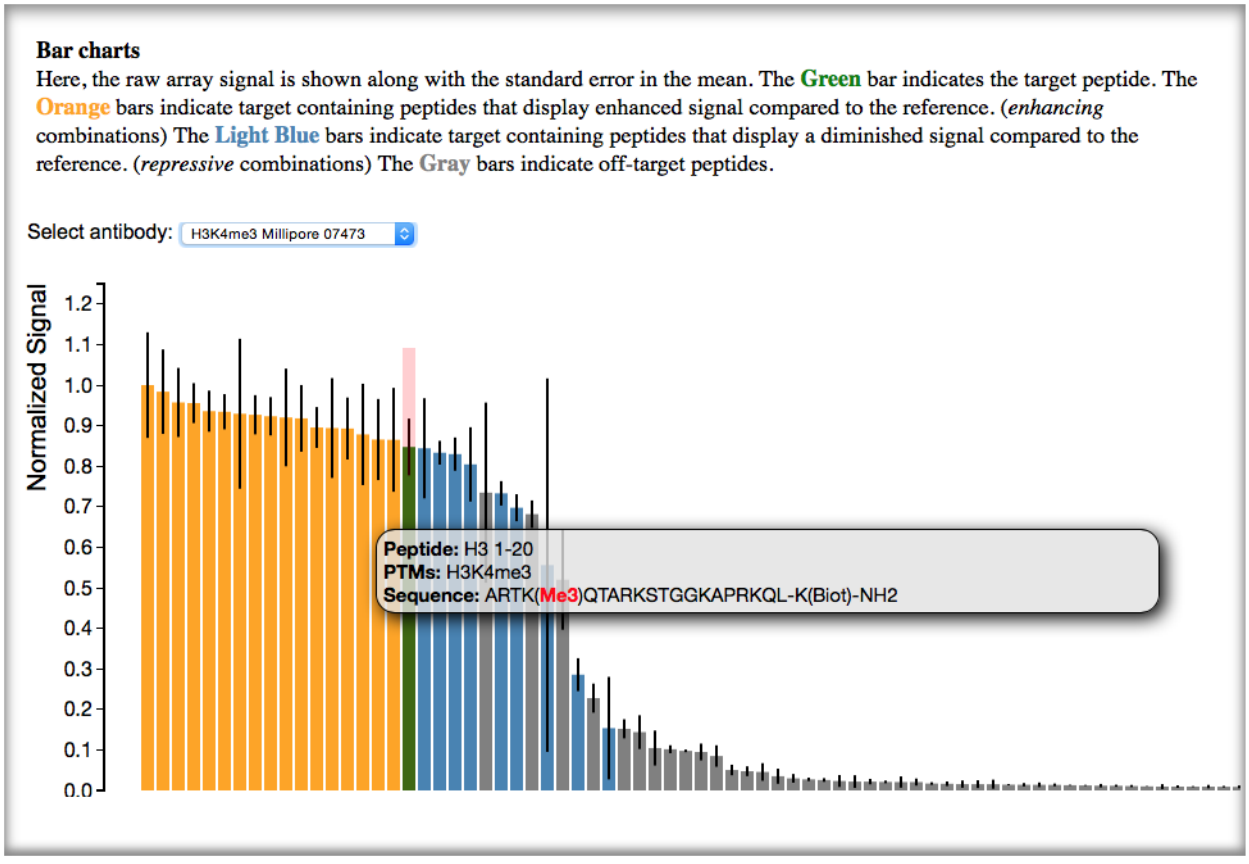

Bar charts
Here, the raw array signal is shown along with the standard error in the mean. The Green bar indicates the target peptide. The Orange bars indicate target containing peptides that display enhanced signal compared to the reference. (enhancing combinations) The Light Blue bars indicate target containing peptides that display a diminished signal compared to the reference. (repressive combinations) The Gray bars indicate off-target peptides.
Select antibody: H3K4me3 Millipore 07473
Normalized Signal
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Peptide: H3 1-20
PTMs: H3K4me3
Sequence: ARTK(Me3)QTARKSTGGKAPRKQL-K(Biot)-NH2

Figure S2

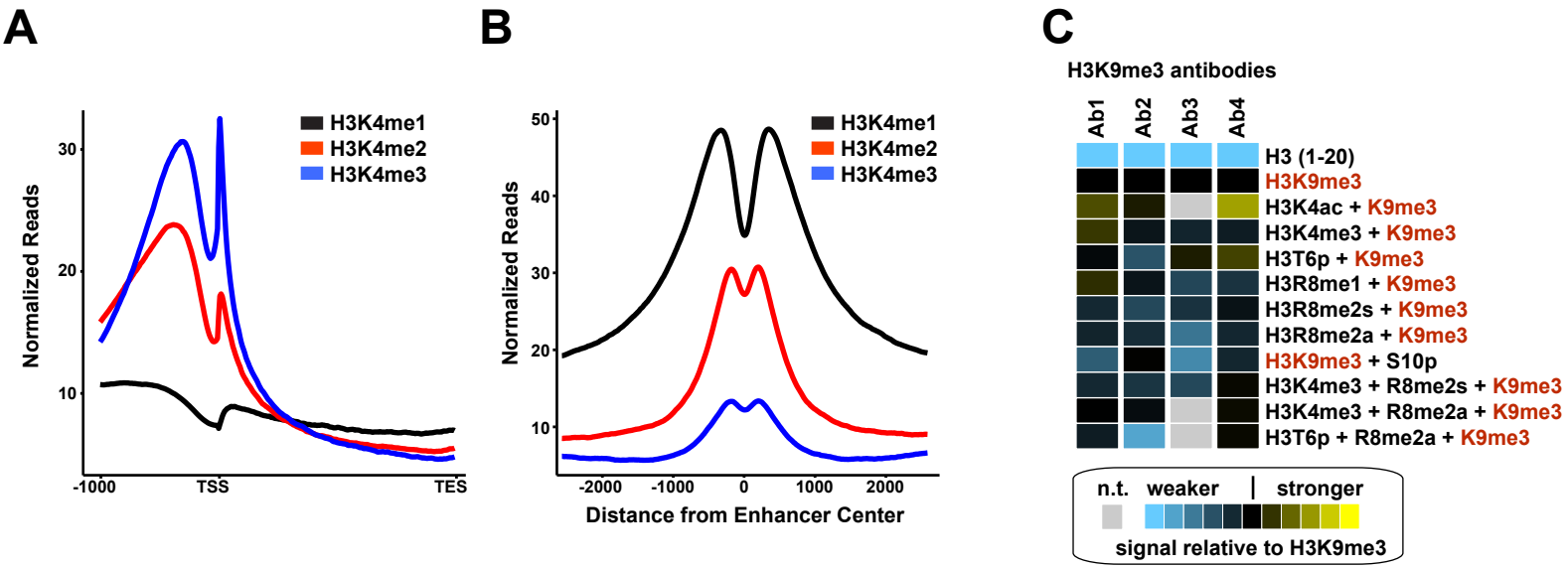

A
B
C
H3K4me1
H3K4me2
H3K4me3
Normalized Reads
30
20
10
-1000
TSS
TES
50
40
30
20
10
-2000
-1000
0
1000
2000
Distance from Enhancer Center
H3K9me3 antibodies
Ab1
Ab2
Ab3
Ab4
H3 (1-20)
H3K9me3
H3K4ac + K9me3
H3K4me3 + K9me3
H3T6p + K9me3
H3R8me1 + K9me3
H3R8me2s + K9me3
H3R8me2a + K9me3
H3K9me3 + S10p
H3K4me3 + R8me2s + K9me3
H3K4me3 + R8me2a + K9me3
H3T6p + R8me2a + K9me3
n.t.
weaker
stronger
signal relative to H3K9me3

Figure S3

H3K9me3 ab (Diagenode #C15410193)

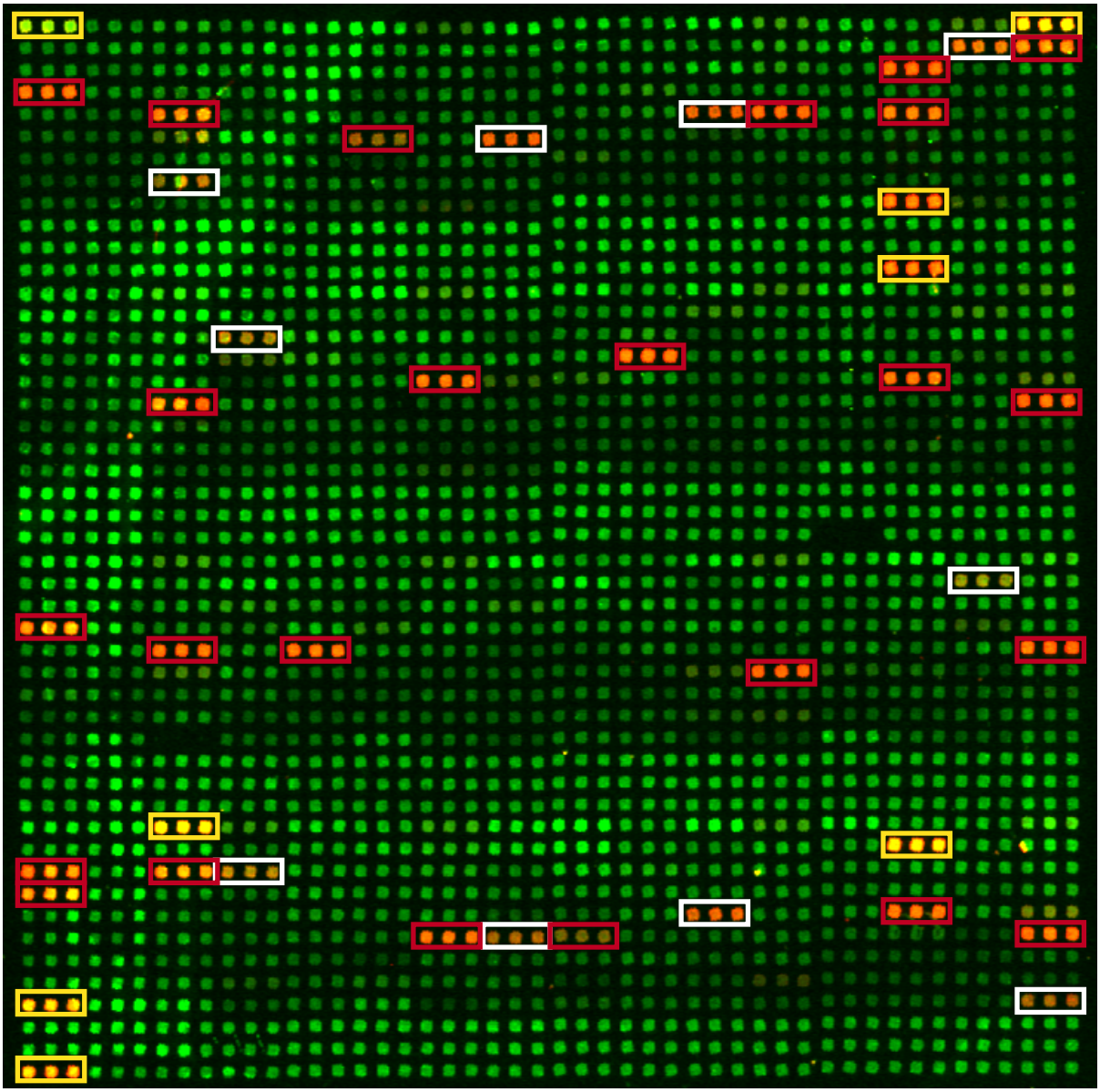
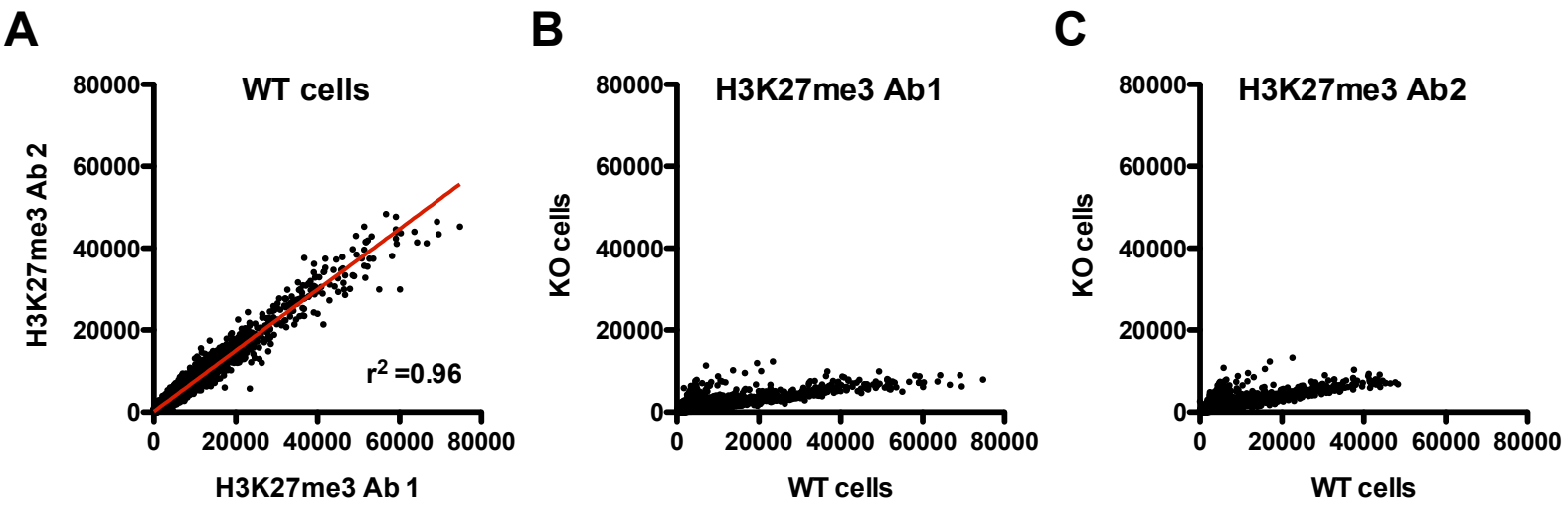


Figure S4


A
WT cells
H3K27me3 Ab 2
H3K27me3 Ab 1
r2 =0.96
0
20000
40000
60000
80000
B
H3K27me3 Ab1
KO cells
WT cells
C
H3K27me3 Ab2
KO cells
WT cells

SUPPLEMENTAL FIGURE LEGENDS

Figure S1, related to Figure 1. Representative data presentation on the Histone Antibody Specificity Database. (A) Heat maps for H3K4me3 antibodies. Array signal intensities are ordered from strongest (yellow) to weakest (blue) relative to the intended target (black). In this case, the intended target is a peptide harboring H3K4me3 (P18; see Supplemental Table 1). Peptides containing the target PTM are outlined in red. Hovering over a data point reveals a window with information on the peptide (histone and sequence context), PTMs, and a full sequence of the peptide (PTMs in red) being queried. (B) Bar graphs for H3K4me3 antibodies. Normalized array signal intensities are presented ± standard error of the mean (SEM). The green bar indicates the target peptide (see above). This target peptide serves as the reference for the bar graph color pattern. Orange bars indicate target-containing peptides with enhanced signal relative to the reference, blue bars indicate target-containing peptides with diminished signal relative to the reference, and gray bars indicate off-target peptides. Hovering over a data point reveals a window with information on the peptide (histone and sequence context), PTMs, and a full sequence of the peptide (PTMs in red) being queried.

Figure S2, related to Figure 2. Distribution of H3K4 methylation states relative to (A) transcripts and (B) enhancers. Normalized ChIP-seq signal for H3K4 methylation data were downloaded from the ENCODE project. (A) Normalized signal was calculated using deepTools (Ramirez et al., 2014) at transcripts in the human Gencode annotations V16 (~ 50,000 transcripts). Signal for each methylation state of H3K4 was plotted from -1000 base pairs (bp) away from the Transcription Start Site (TSS) through the gene body. (B) Enhancers were defined as peaks of p300 and H3K4me1 using ENCODE data in HepG2 cells, and signal for each methylation state was plotted relative to the peak center (+/- 2500 bp). (C)

Results of at least two arrays are presented as heat maps of signal intensities relative to $H3_{(1-20)}$K9me3 peptides on a scale from -1 (blue; undetectable binding) to +1 (yellow; strong binding). Gray bars indicated that a peptide was not tested (n.t.). Ab1 (Millipore 07-442); Ab2 (Diagenode C15410193); Ab3 (Active Motif 39161); Ab4 (Abcam ab8898).

Figure S3, related to Figure 2. Raw scan of a histone peptide microarray hybridized with an H3K9me3 antibody (Diagenode C15410193). All printed peptides are shown as green spots. Antibodies bound to peptides are shown as red spots. IgG control spots are outlined in yellow. H3K9me3-containing peptides recognized by this antibody are outlined in red. Off-target peptides recognized by this antibody are outlined in white.

Figure S4, related to Figure 4. Comparison of H3K27me3 ChIP-seq signals. Peaks were defined as the union of peaks called for wild type (WT) cells using both antibodies (8798 peaks). H3K27me3 reads per kilobase per million mapped reads (RPKM) was then calculated for each peak. Pair-wise comparison for (A) WT cells using each antibody, (B) WT and EED-/- cells using Ab1 and (C) WT and EED-/- cell using Ab2 are shown.

SUPPLEMENTAL TABLES

Table S1, related to Figure 1. List of arrayed peptides

Table S2, related to Figure 1. Screened histone antibodies

SUPPLEMENTAL REFERENCES

Ramirez, F., Dundar, F., Diehl, S., Gruning, B.A., and Manke, T. (2014). deepTools: a flexible platform for exploring deep-sequencing data. Nucleic acids research *42*, W187-191.