Figure S1. Reads aligning to annotated open and closed chromatin. The location of reads of different insert sizes from our K562 WCE data was compared to the ENCODE annotation of the genome using BEDTools [1]. Reads with smaller insert sizes, indicating that they originate from smaller DNA fragments, mapped to transcription start sites (open chromatin) at a higher rate than those of longer insert sizes. An opposite relationship was observed for closed chromatin. Note, however, that the Y axis is truncated to show this effect and the change in the percentage of reads tracking to repressed regions is small. Reads below and above the displayed insert sizes were excluded due to low read coverage and associate noise.

Figure S2: Read coverage across the genome. Images of Tiled Data Files (TDFs) generated by the IGV browser [2, 3] displaying the density tracks of reads aligned across the genome. The tracks show the correspondence in read coverage in monoclonal and polyclonal antibodies (from ENCODE and Mouse ENCODE) over representative genomic loci. A-C GM12878; D-F mES. A. Chromosome 19:41,792,290-41,912,756 (about 120Kb) for H3K4me1, H3K4me3 (two monoclonal lots) and H3K27ac. B. Chromosome 20:61,503,810-62,032,504 (about 500Kb) for H3K27me3. C. Chromosome (about 250Kb) for H3K9me3. D. Chromosome 6:125,035,929-125,153,426 (about 120Kb) for H3K4me1, H3K4me3 (two monoclonal lots) and H3K27ac. E. Chromosome 6:51,949,208-52,399,454 (about 450Kb) for H3K27me3. F. Chromosome 8:65,216,638-65,604,437 (about 390Kb) for H3K9me3.

Figure S3: Saturation curve showing the number of bases called as being in peaks as a function of sequencing depth for H3K9me3 and H3K27me3. The final dataset of the merged technical replicates was randomly down sampled to 20 different read depths and peaks were called in each dataset using HOMER. For these datasets, curves do not show the expected pattern and do not appear to reach saturation.

Figure S4: Mapping of peaks and reads to canonical chromatin regions of the genome as defined by the ENCODE mappings. These plots display the percentage of reads that map to each canonical genome region. The canonical genome regions were defined by the combined ENCODE mapping and are abbreviated as follows: CTCF-enriched elements (CTCF), promoter flanking regions (PF), transcription start sites (TSS), transcribed regions (T), enhancers (E), weak enhancers (WE), and repressed regions (R). Reads were normalized by random downsampling.

Figure S5. Correlation between monoclonal and polyclonal antibodies across the genome. Scatter plots (Loglog) show counts of reads per bin in non-overlapping 2000 bp bins tiled throughout the genome in replicates of the monoclonal antibody (left), the polyclonal antibody (right), and polyclonal antibody versus monoclonal antibody (center). The reads for each dataset were normalized by A. insert size or by B. random downsampling.

Figure S6: Experimental quality control. While we generated four technical replicates of the H3K27me3 polyclonal antibody, one of the replicates (Replicate number 4) did not pass our quality control. Points on the graph represent the number of reads falling into each variable-sized bin defined by the canonical chromatin regions of the genome as defined by the ENCODE. A. A comparison of Replicate 3 to the summed read counts of Replicates 1 and 2. This is in line with our expectations. B. The same comparison to Replicates 1 and 2, this time using Replicate 4. Here we see that Replicate 4 has a systematically reduced read count in transcription start sites. No other classes of genomic regions were shifted. We concluded that Replicates 4 did not pass our quality control.

Figure S7: Validation of the polyclonal antibody targeting H3K4me1 by peptide array. The details are presented in the same manner as the publically available validations of the other antibodies we used in the study (Table 1), and include the details of the antibody and validation protocol, the row signal of the
peptide array (bottom left); reactivity with the synthetic peptides on the array (top right), and the cross reactivity other modifications of the same peptide.

Supplemental References


Figure S1.
Figure S2.

A.

Data range:

H3K4me1 mono

H3K4me1 poly (ENCODE)

lot 8

H3K4me3 mono

lot 9

H3K4me3 poly (ENCODE)

H3K27ac mono

H3K27ac poly (ENCODE)

RefSeq Genes

HNRNPUL1 CCDC97 TGFBI B9D2 TEME91 EXOSC3 BCKDHA

B.

H3K27me3 mono

H3K27me3 poly (ENCODE)

RefSeq Genes

DDO1 GTXI BHE103 HBE13 MR1245 CAB79358 CAB8044

C.

H3K9me3 mono

H3K9me3 poly (ENCODE)

RefSeq Genes

H3K9me3 mono

H3K9me3 poly (ENCODE)
D.

Data range:

H3K4me1
- mono
- poly (mouse ENCODE)

lot 8
lot 9

H3K4me3
- mono
- poly (mouse ENCODE)

H3K27me3
- mono
- poly (mouse ENCODE)

WCE
RefSeq Genes

E.

Data range:

H3K27me3
- mono
- poly (mouse ENCODE)

WCE (mouse ENCODE)
RefSeq Genes

F.

Data range:

H3K9me3
- mono
- poly (mouse ENCODE)

WCE (mouse ENCODE)
RefSeq Genes

Spock3

Chd4
Nop2
If01
Gapdh
Ncap2
Mrpl51

Hoxa1
Hoxa3
Hoxa9
5730457N03Rik
Figure S3
Figure S4.
Figure S5.
Figure S6.
Figure S7.

Antibody: H3-K4me1
Vendor: Active Motif
Part number: 39297
Lot number: 1714002

Test Date: 4-1-2016
User: RI

Primary antibody:
Primary ab dilution: 1:20,000
Incubation: O/N at 4°C

Secondary antibody:
Vendor: Active Motif
Part number: 100612
Lot number: 6012051
Concentration: 1:2500
Incubation: 1 hour at room temperature

Protocol:
- immerse array in 4 mL of blocking solution containing TBST containing 5% non-fat dried milk
- incubate on a rotator for 4 hours at room temperature
- carefully pour off buffer
- perform a quick rinse (30 seconds) with TBST buffer
- wash 3 x 5 minutes in TBST
- dilute primary antibody in 4 mL of Blocking solution
- incubate on a rotator overnight at 4°C
- carefully pour off buffer
- perform a quick rinse (30 seconds) with TBST buffer
- wash 3 x 5 minutes in TBST
- dilute secondary antibody in 4 mL of Blocking solution
- incubate on a rotator for 1 hour at room temperature
- carefully pour off buffer
- perform a quick rinse (30 seconds) with TBST buffer
- wash 3 x 5 minutes in TBST
- detect using FluorChemQ

Cross inhibition of reactivity by other modifications of the same peptide
Graph is scaled so that saturation of signal = 1

Grided image of the array