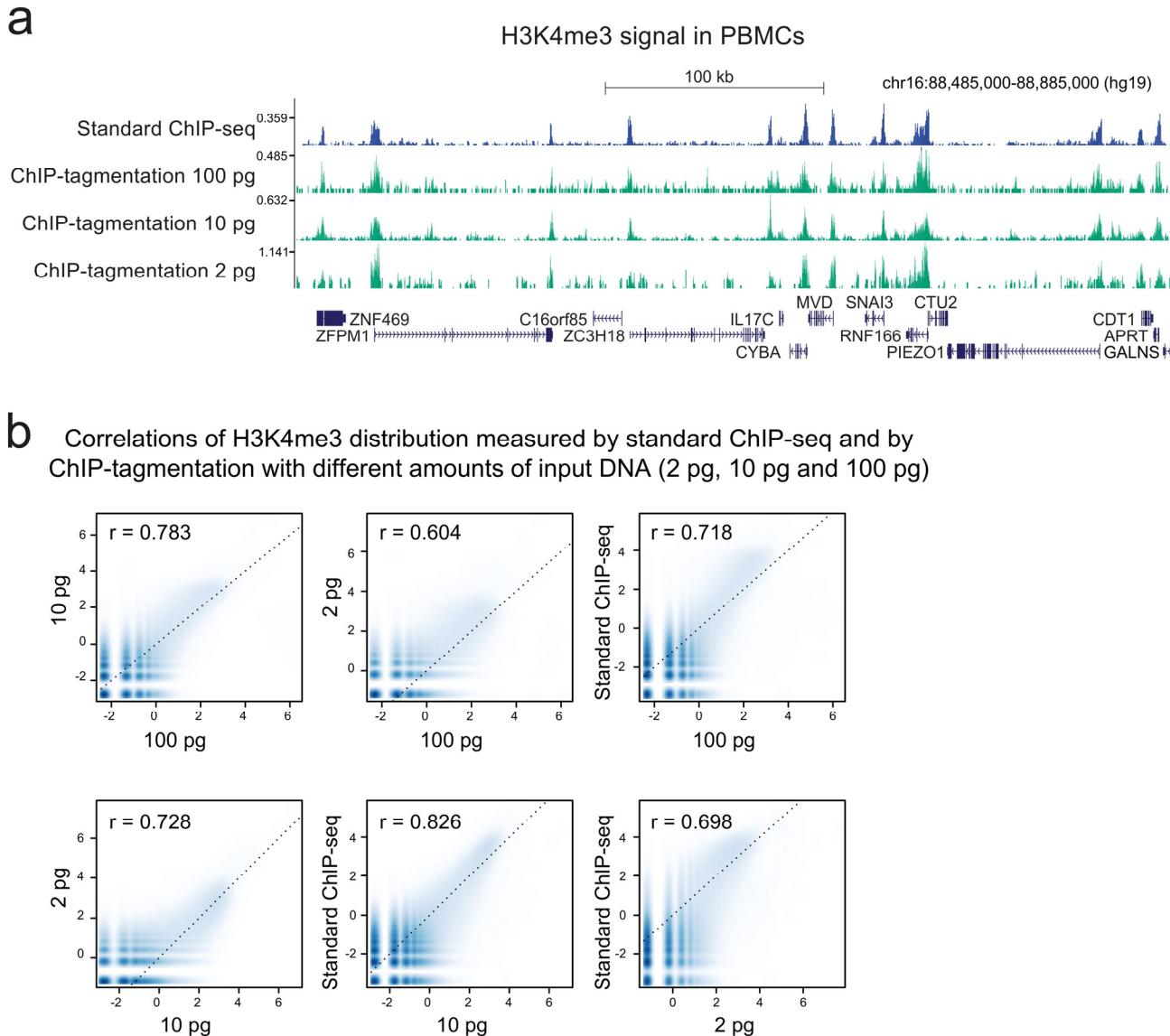


Supplementary Figure 1

Schematic overview of standard ChIP-seq, ChIP-tagmentation and ChIPmentation

Workflow of ChIPmentation as compared to standard ChIP-seq and ChIP-tagmentation with purified ChIP DNA. All three protocols start by fixing cells with formaldehyde, followed by cell lysis, sonication of chromatin, and immunoprecipitation with a specific

antibody bound to beads. For standard ChIP-seq (left), reverse-crosslinking is followed by purification of ChIP DNA, which is then subjected to library preparation in a multi-step procedure comprising end repair, purification, A-tailing, adapter ligation, and size selection. ChIP-fragmentation (center) uses purified ChIP DNA for fragmentation-based library preparation. In ChIPmentation (right), the sequencing adapters are introduced in a single step by fragmentation of bead-bound chromatin.



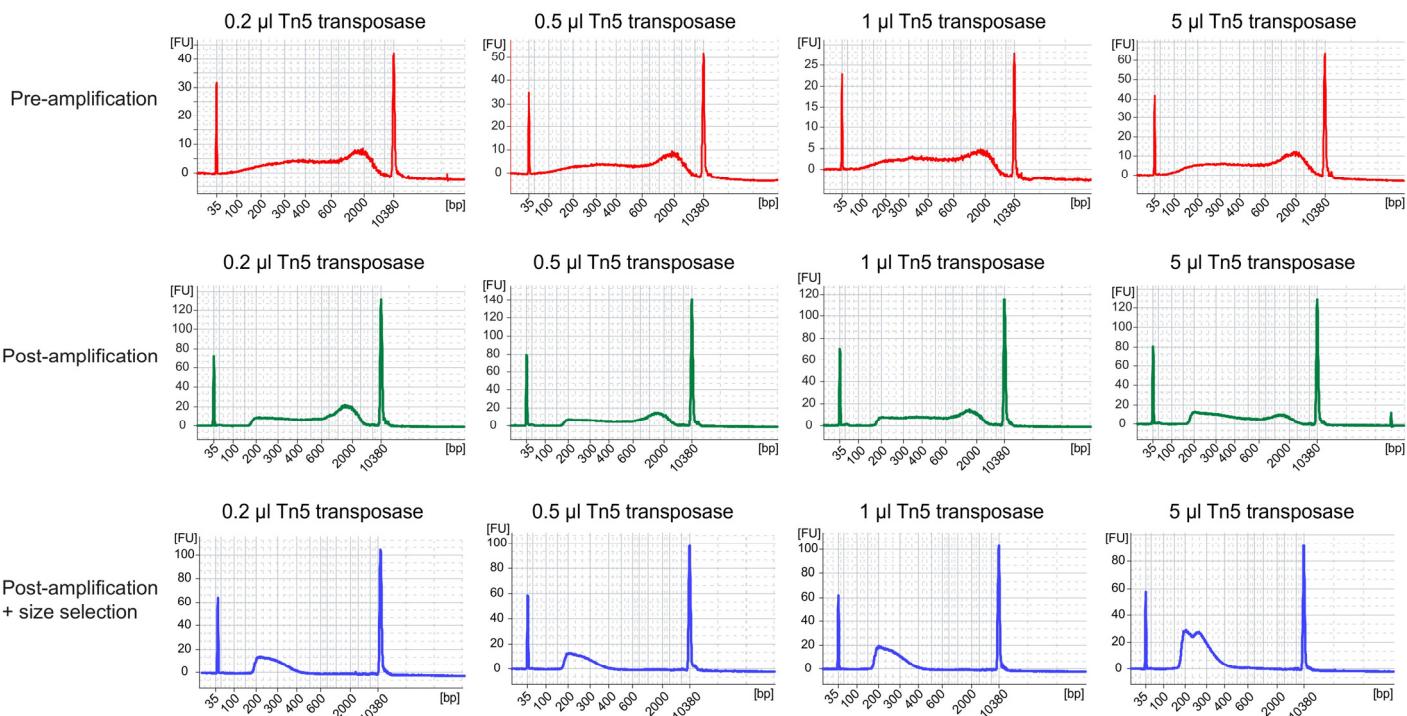
Supplementary Figure 2

Sequencing results for libraries prepared by ChIP-fragmentation starting from purified ChIP DNA as compared to standard ChIP-seq

(a) Representative UCSC Genome Browser screenshot of ChIP-fragmentation profiles for H3K4me3 in peripheral blood mononuclear cells (PBMCs) using different amounts of purified ChIP DNA as starting material. Data obtained by standard ChIP-seq for the same cell type are also included as reference.

(b) Pairwise scatterplots comparing H3K4me3 signal in peripheral blood mononuclear cells (PBMCs) between standard ChIP-seq obtained from 10 million cells and ChIP-fragmentation using different amounts of purified DNA as starting material.

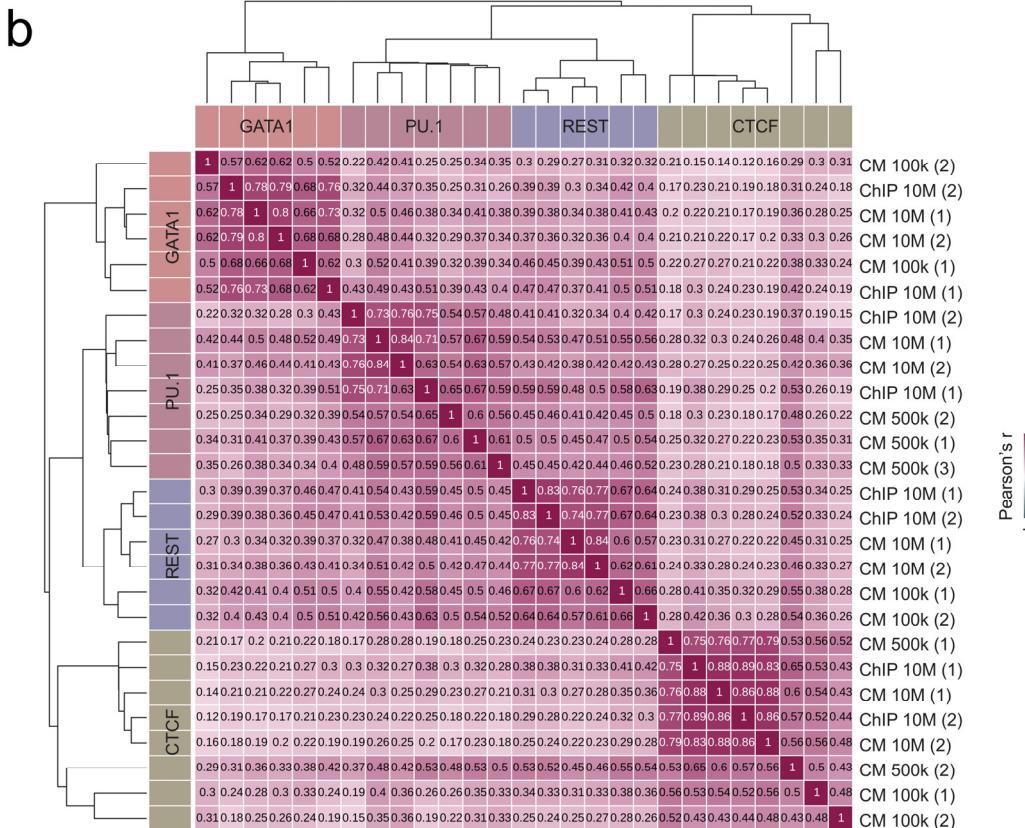
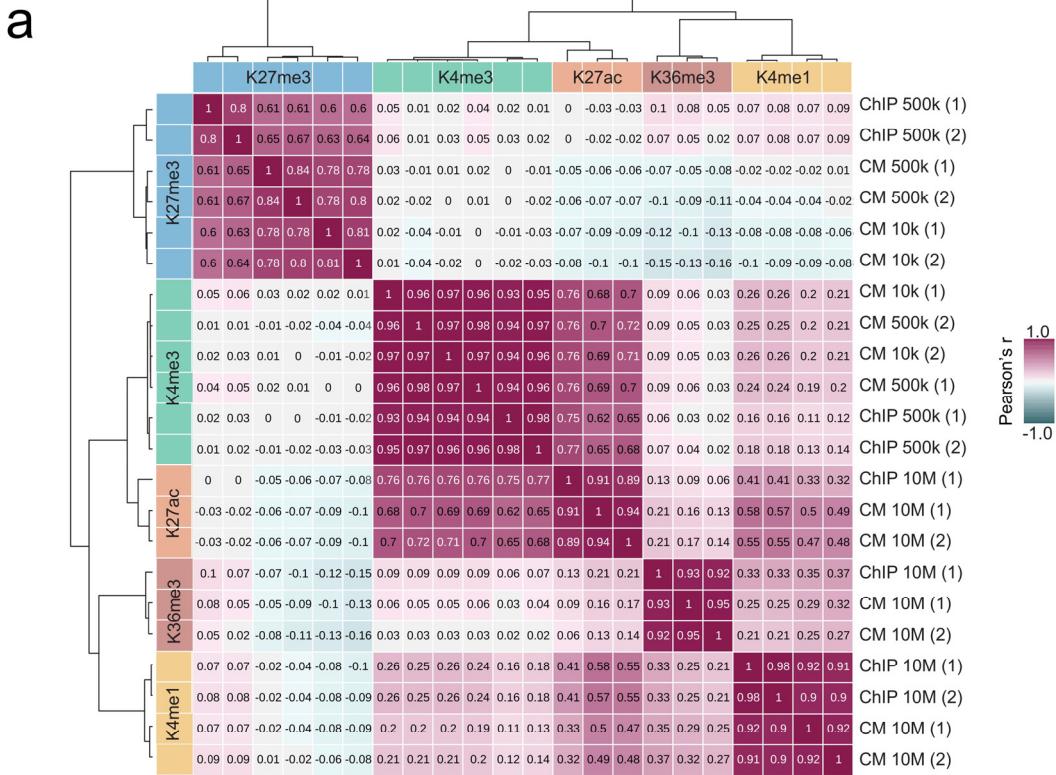
Fragment size distribution before and after PCR of ChIPmentation for H3K4me3 libraries prepared with different amounts of Tn5 transposase enzyme



Supplementary Figure 3

Effect of tagmentation enzyme concentration on ChIPmentation library size distributions

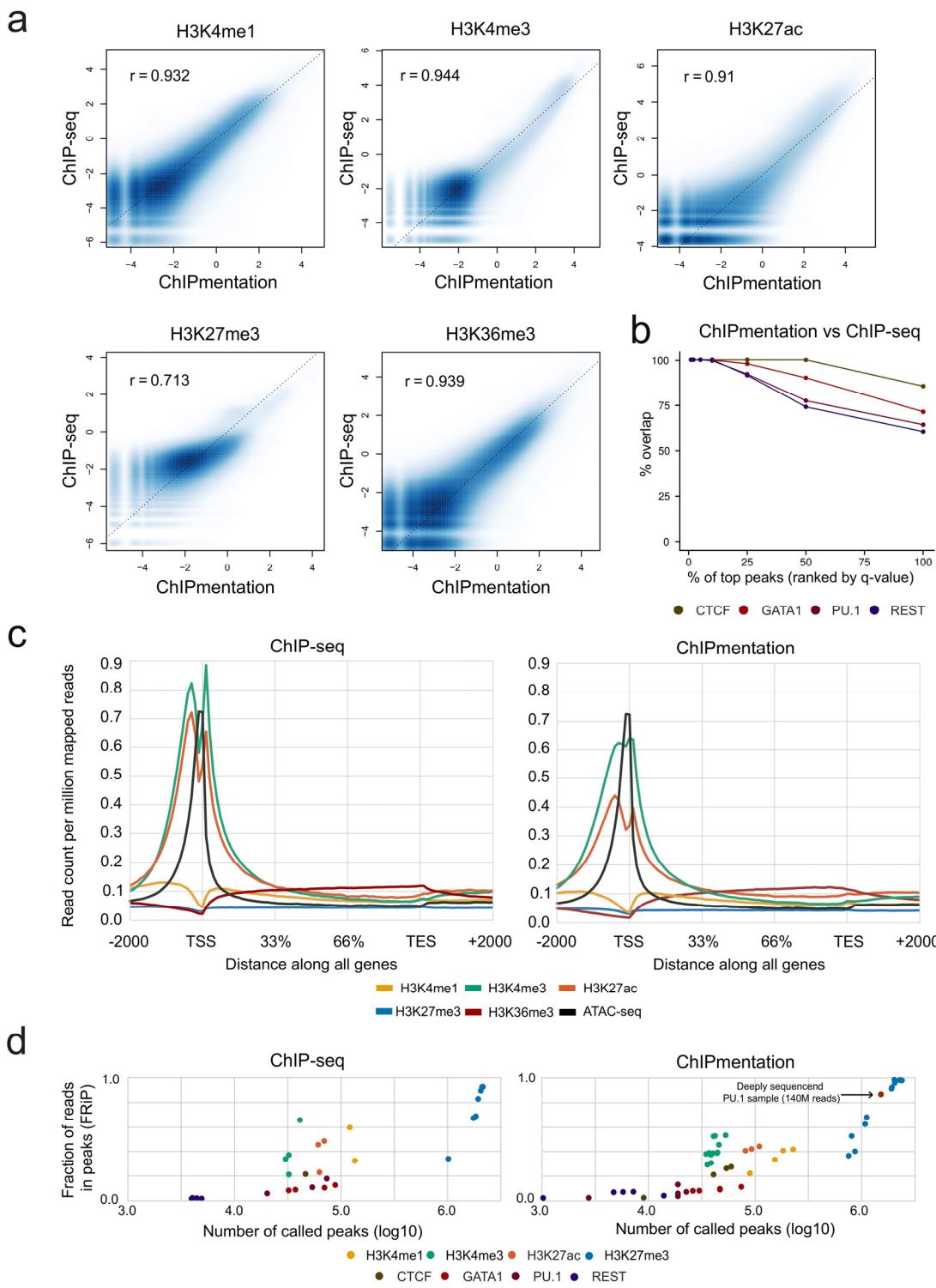
DNA fragment size distribution of ChIPmentation libraries for H3K4me3 that were prepared with different amounts of Tn5 transposase (0.2 μ l to 5 μ l enzyme from the Illumina Nextera DNA library preparation kit). Fragment size distributions after reverse-crosslinking but before library enrichment are shown in red, fragment size distributions after enrichment PCR are shown in green, and fragment size distributions of the size-selected final libraries are shown in blue.



Supplementary Figure 4

Genome-wide correlations for all ChIPmentation and ChIP-seq samples

(a) Genome-wide correlations (1,000 bp windows) for standard ChIP-seq (“ChIP”) and ChIPmentation (“CM”) data across different histone marks and different cell input amounts. (b) Genome-wide correlations (1,000 bp windows) for standard ChIP-seq and ChIPmentation data across different transcription factors and different cell input amounts.

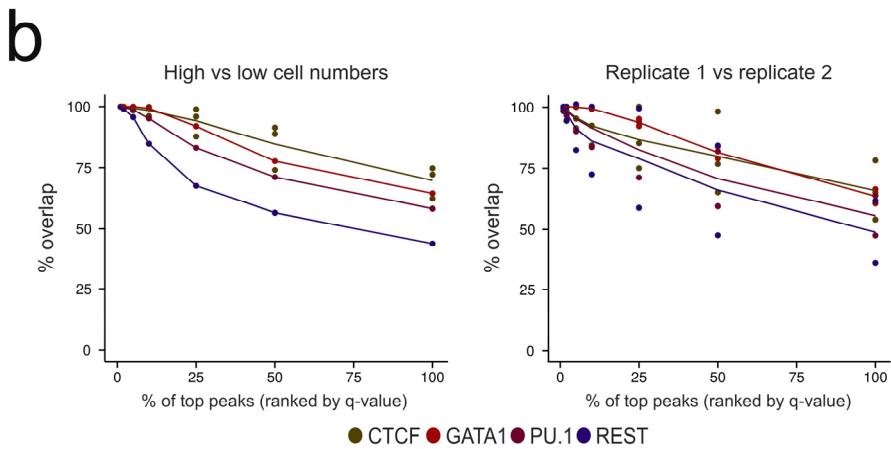
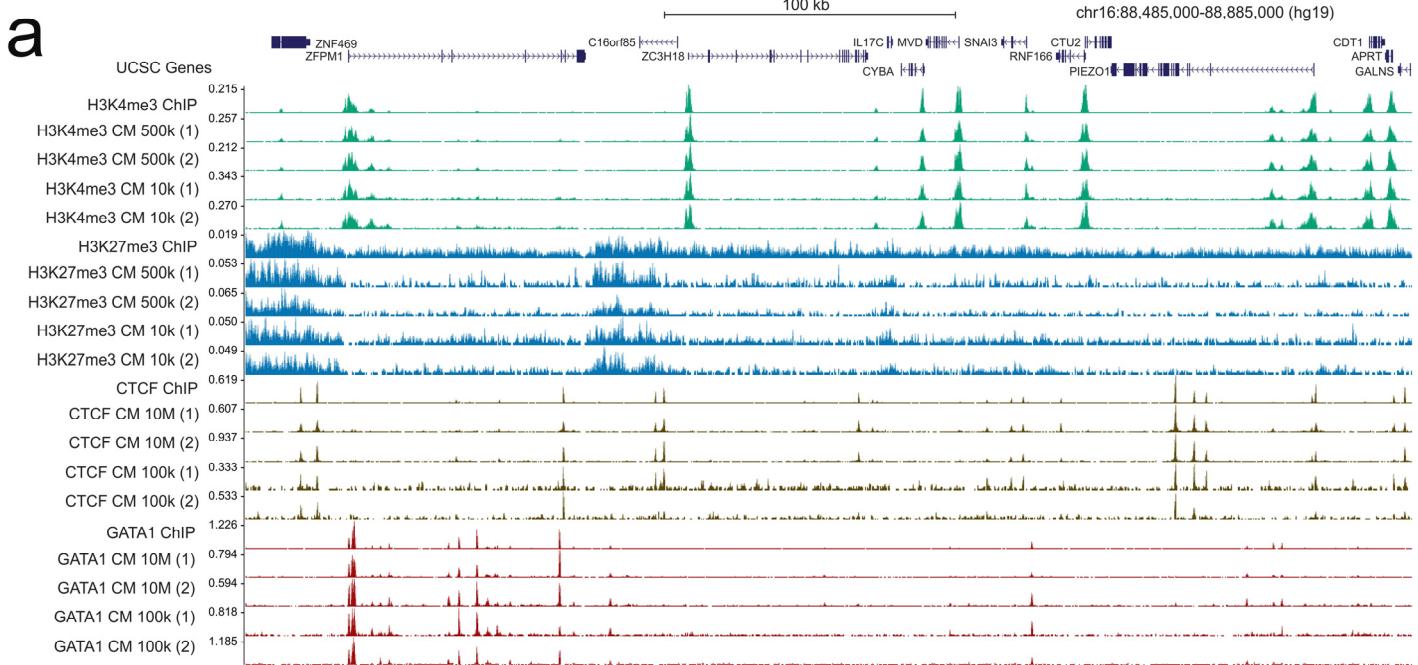


Supplementary Figure 5

Global comparison of standard ChIP-seq and ChIPmentation data

(a) Pairwise scatterplots comparing standard ChIP-seq and ChIPmentation for H3K4me1, H3K4me3, H3K27ac, H3K27me3, and

H3K36me3. (b) Peak overlap calculated as the percentage of top-X% peaks in one method that overlap with peaks in the other method. (c) Composite plot for the distribution of histone marks along all genes, shown separately for standard ChIP-seq (left) and ChIPmentation (right). Chromatin accessibility obtained by ATAC-seq is shown in black. (d) Fraction of reads in peaks (FRiP) and number of peaks called from standard ChIP-seq (left) and ChIPmentation (right) data for all sequenced libraries. Note that the sequencing depth varies between replicates (**Supplementary Table 1**).

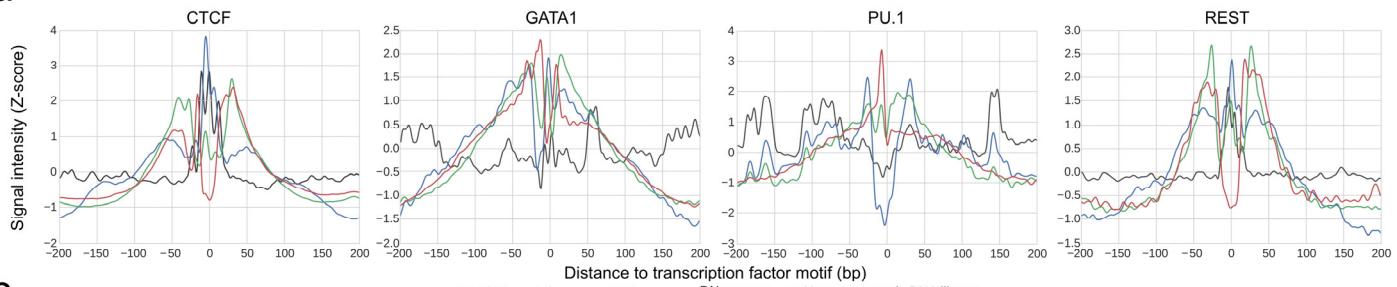
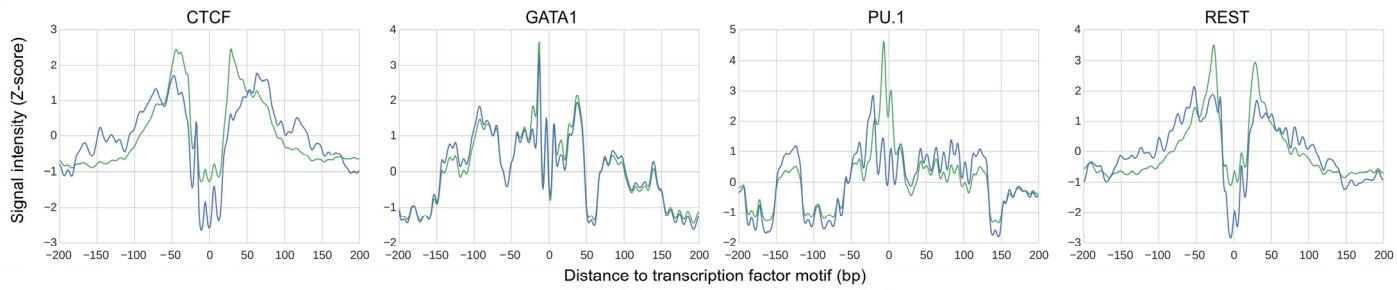
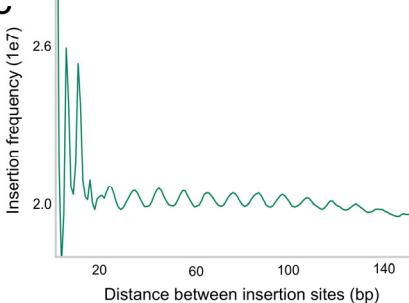
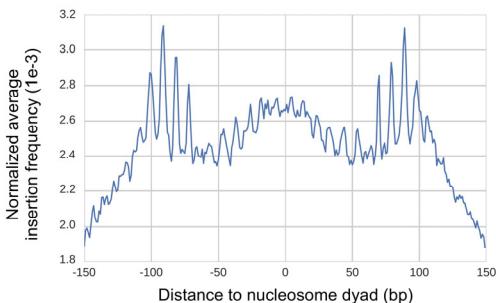


Supplementary Figure 6

Performance of ChIPmentation for low-input samples

(a) Genome browser screenshot showing ChIPmentation (“CM”) data for individual biological replicates and different cell input amounts (i.e., 10M, 500k, 100k, and 10k cells). Standard ChIP-seq (“ChIP”) data obtained from 500k (H3K4me3, H3K27me3) or 10 million cells (CTCF, GATA1) are shown as a reference.

(b) Peak overlap (fraction of top-X% peaks in one condition that overlap peaks in the other condition) between ChIPmentation samples obtained for different cell numbers (left) and between biological replicates with the same cell number (right). Lines represent the mean of all points of one factor at one x-axis position.

a**b****c****d**

Supplementary Figure 7

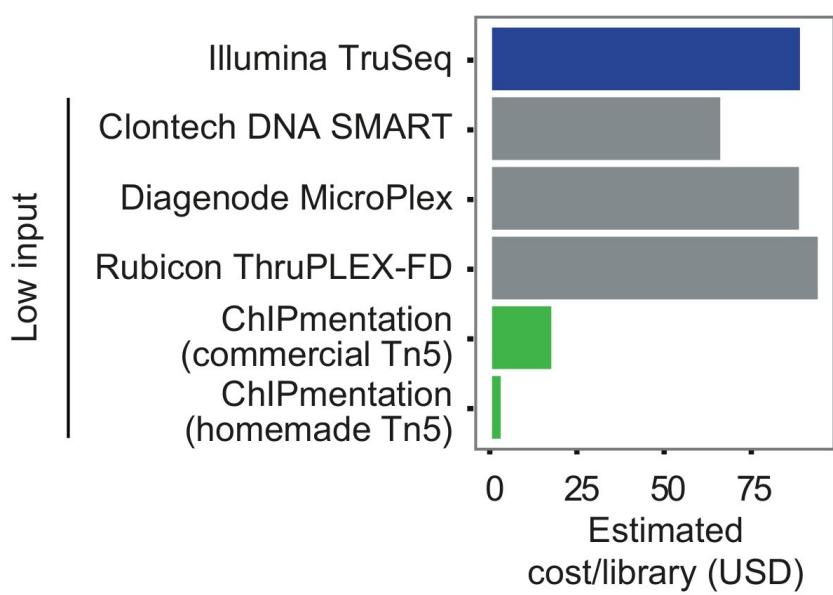
Evidence of high-resolution patterns in ChIPmentation data

(a) Tn5 transposase insertion frequencies for ChIPmentation (blue), ATAC-seq signal (green), and DNase-seq signal (red) at ChIP-seq peaks for CTCF, GATA1, PU.1, and REST, centered on the corresponding binding motifs. Signal from fragmented genomic DNA (Nextera genomic DNA library) is displayed in black, indicating inherent sequence bias of the fragmentation enzyme. Signals were averaged over all peaks, smoothed with a 20 bp Hanning window, and Z score transformed for better comparability.

(b) Normalized ChIPmentation (blue) and ATAC-seq (green) signal at ChIP-seq peaks for CTCF, GATA1, PU.1, and REST, centered on the corresponding binding motif. ChIPmentation and ATAC-seq signals were normalized against the signal observed for fragmentation of genomic DNA (Nextera genomic DNA library), in order to correct for the sequence bias of the fragmentation enzyme. Signals were averaged over all peaks, smoothed with a 20 bp Hanning window, and Z score transformed for better comparability.

(c) Frequency of pairwise distances between insertion events (5' position of reads) in ChIPmentation data for H3K4me3.

(d) Average signal intensity (insertion frequencies) for H3K4me1 ChIPmentation data around centers of nucleosomes (dyads) positioned using the NucleoATAC software (<https://github.com/GreenleafLab/NucleoATAC>) with ATAC-seq data from GM12878 cells. Note the structured pattern with higher and periodical insertions at the nucleosome borders.



Supplementary Figure 8

Comparison of reagent costs for standard ChIP-seq and ChIPmentation

Comparison of reagent costs for standard ChIP-seq (dark blue), commercially available low-input library preparation kits (grey), and ChIPmentation (green). Cost estimates were calculated for library preparation including amplification and indexing, but excluding reagents for size selection, reaction purifications, and the final quality control step prior to sequencing.

Supplementary Note: Chromatin immunoprecipitation protocols

ChIPmentation was tested and validated in combination with three different chromatin immunoprecipitation (ChIP) protocols that are described below.

ChIP version 1 (used for H3K4me3 and H3K27me3)

Cells were washed once with PBS and fixed with 1% paraformaldehyde in up to 1 ml PBS for 5 minutes at room temperature. Glycine was added to stop the reaction. Cells were collected at 500 x g for 10 minutes at 4°C (subsequent work was performed on ice and used cool buffers and solutions unless otherwise specified) and washed twice with up to 1 ml ice-cold PBS supplemented with 1 µM PMSF. The pellet was lysed in Cell Lysis Buffer (50 mM HEPES/KOH pH 7.4, 140 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 10% Glycerol, 0.5% NP-40, 0.25% Triton X-100, 1x protease inhibitors (Sigma)) for 10 minutes on ice. Nuclei were isolated by spinning the lysed cells for 10 minutes at 1,000 x g at 4°C, the supernatant was discarded, and the pellet was resuspended in Sonication Buffer (10 mM Tris-HCl pH 7.6, 1 mM EDTA, 0.1% SDS) and sonicated in a 130 µl microTUBE (for up to 3 x 10⁶ cells) on a Covaris S220 for 12 minutes until most of the fragments were 200-700 base pairs long (settings: duty cycle 2%, peak incident power 105 Watts, cycles per burst 200). Lysates were centrifuged at full speed for 5 minutes at 4°C and the supernatant was transferred to a new tube. The lysate was adjusted to 200 µl per IP with a buffer composition of 20 mM HEPES, 0.1% SDS, 1% Triton X-100, 150 mM NaCl, 1 mM EDTA, 0.5 mM EGTA and incubated with an antibody against H3K4me3 (1 µg/IP, Diagenode pAb-003-050) or H3K27me3 (1 µg/IP, Diagenode pAb-195-050) overnight at 4°C on a rotator. 20 µl of Protein A (or Protein G, dependent on the antibody used) magnetic beads were blocked overnight with 0.1% BSA in PBS and added to the IP the next day for 2 hours on a rotator at 4°C to capture the immunoprecipitated fragments. The immunoprecipitated chromatin was washed subsequently with WB1 (20 mM HEPES, 150 mM NaCl, 0.1% SDS, 0.1% DOC, 1% Triton X-100, 1 mM EDTA, 0.5 mM EGTA) (twice), WBII (20 mM HEPES, 500 mM NaCl, 0.1% SDS, 0.1% DOC, 1% Triton X-100, 1 mM EDTA, 0.5 mM EGTA) (once), WBIII (20 mM HEPES, 250 mM LiCl, 0.5% DOC, 0.5% NP-40, 1 mM EDTA, 0.5 mM EGTA) (once), and WBIV (20 mM HEPES, 1 mM EDTA, 0.5 mM EGTA) (twice). Beads were then incubated with 70 µl elution buffer (0.5% SDS, 300 mM NaCl, 5 mM EDTA, 10 mM Tris-HCl pH 8.0) containing 2 µl of Proteinase K (NEB) for 1 hour at 55°C and 8 hours at 65°C to revert formaldehyde crosslinking, and supernatant was transferred to a new tube. Another 30 µl of elution buffer was added to the beads for 1 minute, and eluates were combined and incubated with another 1 µl of Proteinase K for 1 hour at 55°C. Finally, DNA was purified with SPRI AMPure XP beads (sample-to-beads ratio 1:2) or Qiagen MinElute columns.

ChIP version 2 (used for H3K4me1, H3K36me3, and REST)

Cells were washed once with PBS and fixed with 1% paraformaldehyde in up to 1.5 ml PBS for 10 minutes at room temperature. Glycine was added to stop the reaction. Cells were collected at 500 x g for 10 minutes at 4°C (subsequent work was performed on ice and used cool buffers and solutions unless otherwise specified) and washed twice with up to 1 ml ice-cold PBS supplemented with 1 µM PMSF. The pellet was lysed in RIPA buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, 140 mM NaCl, 1% Triton x-100, 0.1% SDS, 0.1% DOC, 1x protease inhibitors (Sigma)) and sonicated in a 1 ml milliTUBE in a Covaris S220 for 30 minutes until most of the fragments were 200-700 base pairs long (settings: duty cycle 5%, peak incident power 140 Watts, cycles per burst 200). Lysates were centrifuged at full speed for 5 minutes at 4°C, and the supernatant containing the sonicated chromatin was transferred to a new tube. In parallel, 50 µl (10 µl for low-input ChIPmentation) magnetic Protein A or Protein G beads (dependent on the antibody used) were blocked and conjugated to an antibody by washing and resuspending twice in PBS, 0.5% BSA, 0.5% Tween-20. The antibody was added and bound to the beads by rotating >1 hour at room temperature. Used antibodies were H3K4me1 (1 µg/IP, Diagenode pAb-194-050), H3K36me3 (1 µg/IP, Diagenode pAb-192-050), and REST (10 µg/IP, Millipore 07-579). Blocked antibody-conjugated beads were then placed on a magnet, supernatant was removed, and the sonicated lysate was added to the beads followed by incubation for 3 hours at 4°C on a rotator. Beads were washed subsequently with 150 µl RIPA (twice), RIPA-500 (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, 500 mM NaCl, 1% Triton x-100, 0.1% SDS, 0.1% DOC,) (twice), RIPA-LiCl (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, pH 8.0, 250 mM LiCl, 1% Triton X-100, 0.5% DOC, 0.5% NP40), and TE pH 8.0 (twice). Beads were then incubated with 70 µl elution buffer (0.5% SDS, 300 mM

NaCl, 5 mM EDTA, 10 mM Tris-HCl pH 8.0) containing 2 μ l of Proteinase K (NEB) for 1 hour at 55°C and 8 hours at 65°C to revert formaldehyde crosslinking, and supernatant was transferred to a new tube. Finally, DNA was purified with SPRI AMPure XP beads (sample-to-beads ratio 1:2) or Qiagen MinElute columns.

ChIP version 3 (used for H3K27ac, PU.1, CTCF, and GATA1)

Cells were washed once with PBS and fixed with 1% paraformaldehyde in up to 1.5 ml PBS for 5-10 minutes at room temperature. Glycine was added to stop the reaction. Cells were collected at 500 x g for 10 minutes at 4°C (subsequent work was performed on ice and used cool buffers and solutions unless otherwise specified) and washed twice with up to 1 ml ice-cold PBS supplemented with 1 μ M PMSF. The pellet was lysed in buffer L3B (10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% Na-Deoxycholate, 0.5% N-lauroylsarcosine, 1x protease inhibitors (Sigma)) and sonicated in a 1ml milliTUBE in a Covaris S220 for 20 minutes until most of the fragments were 200-700 base pairs long (settings: duty cycle 5%, peak incident power 140 Watts, cycles per burst 200). Lysates were supplemented with 1% Triton-X-100 and centrifuged at full speed for 5 minutes at 4°C, and the supernatant containing the sonicated chromatin was transferred to a new tube. In parallel, beads were blocked and conjugated to an antibody by washing them twice in PBS with 0.5% BSA and resuspending 50 μ l (10 μ l beads for low-input ChIPmentation) of magnetic Protein A or Protein G beads (dependent on the antibody used) per IP in 200 μ l of PBS with 0.5% BSA. The antibody was added and bound to the beads by rotating >1 hour at room temperature. Used antibodies were H3K27ac (2 μ g, Diagenode pAb-196-050), PU.1 (5 μ g/IP, Santa Cruz sc-352), CTCF (10 μ l/IP, Millipore 07-729), and GATA1 (4 μ g/IP and 2 μ g for low-input, Abcam ab11852). Blocked antibody conjugated magnetic beads were added to the tube containing the chromatin and incubated for 3 hours at 4°C. Beads were washed subsequently with 150 μ l TF-WBI (20 mM Tris-HCl/pH 7.4, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 2 mM EDTA) (twice), TF-WBIII (250 mM LiCl, 1% Triton X-100, 0.7% DOC, 10 mM Tris-HCl, 1 mM EDTA) (twice), and TET (0.2% Tween-20, 10 mM Tris-HCl/pH 8.0, 1 mM EDTA) (twice). Beads were then incubated with 70 μ l elution buffer (0.5% SDS, 300 mM NaCl, 5 mM EDTA, 10 mM Tris HCl pH 8.0) containing 2 μ l of Proteinase K (NEB) for 1 hour at 55°C and 8 hours at 65°C to revert formaldehyde crosslinking, and supernatant was transferred to a new tube. Another 30 μ l of elution buffer was added to the beads for 1 minute and eluates were combined and incubated with another 1 μ l of Proteinase K for 1 hour at 55°C. Finally, DNA was purified with SPRI AMPure XP beads (sample-to-beads ratio 1:2) or Qiagen MinElute columns.