SUPPLEMENTAL INFORMATION

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Analysis of MNase-seq footprints

We re-analyzed MNase-seq footprinting data for ESCs depleted of the indicated factors that were previously published (Hainer et al., 2015) (GEO: GSE57170). Libraries were constructed from total MNase digested DNA (not size selected) and were then selected and purified with Agencourt Ampure beads, as previously described (Carone et al., 2014; Henikoff et al., 2011), to provide a range of DNA footprints up to ~200 bp. To increase the signal-noise ratio of MNase footprints, we obtained additional coverage of the same libraries on an Illumina HiSeq2000 using paired-end sequencing (100 bp) at the UMass Medical School deep sequencing core facility.

Data analysis

Paired-end reads were collapsed and adapter sequences were removed from fastq files. Reads were mapped to the mouse mm9 genome using Bowtie2, and only uniquely mapped reads with zero, one, or two mismatches were used. The read size distribution was determined for each library, and reads were sorted for small read size fragments (≤80 bp), subnucleosome size fragments (100-130 bp), and nucleosome size fragments (135-165 bp). To calculate occupancy around factor binding sites, seqMINER (Ye et al., 2011) was used to sum reads 2000 bp upstream and downstream of the factor binding site sequences obtained from

previously performed ChIP-seq experiments, binned in 20 bp intervals, and normalized to the average, genome-wide coverage. TSS reference sites were used based on mm9 TSS coordinates. Factor binding site sequences were called from: Brg1 (GEO:GSE27708), Mbd3 (GEO:GSE31690), Mtf2 (GEO:GSE19708), Pwp1 (GEO:GSE59389), Ezh2 (ENCODE), Suz12 (GEO:GSE11724), Esrrb (GEO:GSE11431), Ring1B (GEO:GSE52619), NcoA3 (GEO:GSE40193), MafK (GEO:GSE49847), MacroH2A1 (GEO:GSE35087), Yy1 (GEO:GSE25197), Nanog (GEO:GSE11724), Oct4 (GEO:GSE11724), H3.3 (GEO:GSE16893), H2A.Z (GEO:GSE34483), Nipbl (GEO:GSE22562), p300 (GEO:GSE49847), Sox2 (GEO:GSE11724), SetDB1 (GEO:GSE18371), Znf384 (GEO:GSM1003807), Hcfc1 (GEO:GSE49847), Zc3h11A (GEO:GSE49847), Lsd1 (GEO:GSE18515), Klf4 (GEO:GSE11431), Tcf3 (GEO:GSE11724), Med1 (GEO:GSE22562), Med12 (GEO:GSE22562), Smc1 (GEO:GSE22562), Smc3 (GEO:GSE22562), and p400 (GEO:GSE42209).

ChIP-seq

ChIP experiments were performed as previously described (Hainer et al., 2015). Cells from RNAi-mediated KD were fixed, washed with ice-cold PBS, and pelleted. Cell pellets were lysed through sonication in a Bioruptor (UCD-200), and supernatants were saved. 30 µL of chromatin was stored overnight at 4°C for input samples while the remainder of the chromatin was combined with antibody coupled protein A magnetic beads (NEB) and incubated at 4°C overnight with constant rotation. H3 antibody (abcam, ab1791), H2A.Z antibody (abcam, ab4174), or Klf4 antibody (kind gift from Huck Hui Ng from the Genome Institute

of Singapore, (Chen et al., 2008)) coupled protein A magnetic beads (NEB) were blocked with 5 mg/mL BSA overnight at 4°C, prior to incubation with sheared chromatin. Magnetic beads were washed, and material was eluted at 65°C on a thermomixer. Eluted material was combined to a new microfuge tube and incubated at 65°C overnight to reverse crosslinking. Input DNA was diluted with 170 µL elution buffer and treated similarly. Samples were treated with RNaseA/T1 (Ambion) and proteinase K (Ambion) and then PCI extracted. Ethanol precipitated ChIP-enriched DNA was then used for library construction.

Library construction

Single-end libraries of ChIP-enriched DNA were prepared as described previously (Chen et al., 2013). Samples were end-repaired, A-tailed, and adaptor-ligated with DNA purification over a column between each step. DNA was PCR amplified with KAPA HiFi polymerase using 16 cycles of PCR. The library was size-selected on a 1% agarose gel, its concentration determined using a NanoDrop (Thermo), and the integrity was confirmed by sequencing ~10 fragments from each library. Libraries were sequenced on an Illumina HiSeq2000 using single-end sequencing (50 bp) at the UMass Medical School deep sequencing core facility.

Data Analysis

Single-end fastq reads were collapsed, adapter sequences were removed, and reads were mapped to the mm9 genome using bowtie, allowing one mismatch. Aligned reads were processed in HOMER (Heinz 2010) by using the "annotatePeaks" command to make 20 bp bins over regions of interest and sum

the reads within each window. H3 ChIP-seq experiments were aligned over the following datasets: H2A.Z (GEO:GSE34483), Pwp1 (GEO:GSE59389), NcoA3 (GEO:GSE40193), Esrrb (GEO:GSE11431), Lsd1 (GEO:GSE18515), MafK (GEO:GSE49847), Ring1B (GEO:GSE52619), Ezh2 (ENCODE), and TSS reference sites based on mm9 TSS coordinates. H2A.Z ChIP-seq experiments were aligned over the following datasets: H2A.Z (GEO:GSE34483 (Figure 4A) and GEO:GSE39237 (Figure S3)), Pwp1 (GEO:GSE59389), Lsd1 (GEO:GSE18515), MafK (GEO:GSE49847) and TSS reference sites based on mm9 TSS coordinates.

SUPPLEMENTAL FIGURES

Supplemental Figure 1, related to Figure 1. Confirmation of efficient *Mbd3* KD and *Smarca4* KD.

(A-D) Efficient KD of *Mbd3* **(A-B)** and *Smarca4* **(C-D)** in ESCs confirmed by random primed RT-qPCR with expression levels normalized to *GAPDH* and shown relative to *EGFP* KD **(A,C)** and through Western blotting, where actin serves as a loading control **(B,D)** Asterisk (*) indicates a non-specific band. **(E)** MNase digestion on *EGFP* KD, *Mbd3* KD, and *Smarca4* KD. Asterisk (*) indicates samples used in MNase-seq experiment.

Supplemental Figure 2, related to Figures 2 and 3. esBAF regulates histone H3 occupancy over Ezh2 and Ring1B binding sites.

Aggregation plots of histone H3 ChIP-seq over Ezh2 and Ring1B binding sites in *EGFP*, *Mbd3*, and *Smarca4* KD ESCs. Asterisks (*, **) indicate p-values as described in Figure 1.

Supplemental Figure 3, related to Figure 4. Brg1 is required for H2A.Z occupancy at a subset of locations.

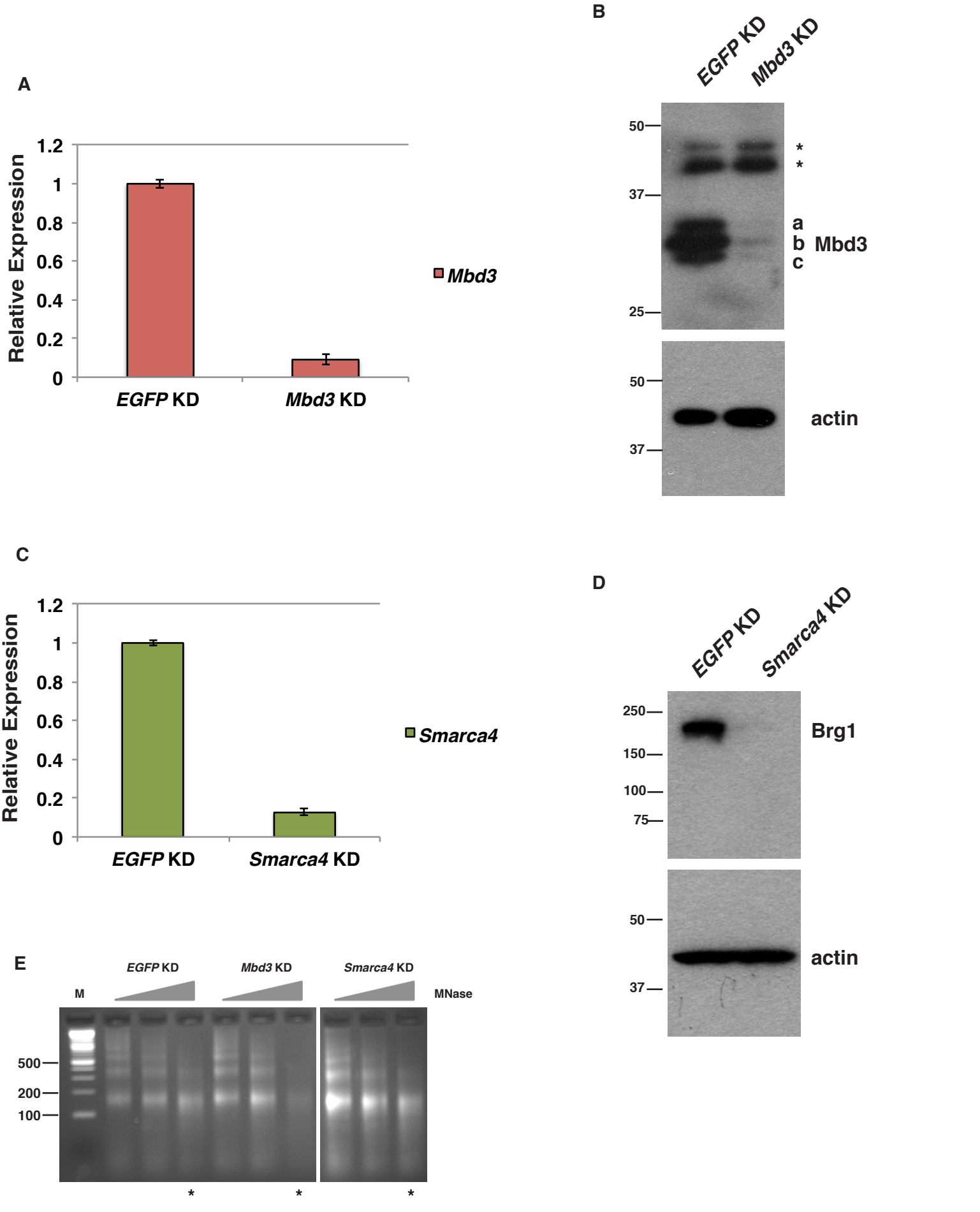
(A) Heatmaps of H2A.Z occupancy over published H2A.Z binding sites in *EGFP* (left) and *Smarca4* (right) KD ESCs. Binding sites were taken from (GEO:GSE39237). Occupancy is indicated as $\log_2$(normalized reads). **(B)** Aggregation plots of histone H2A.Z ChIP-seq over a subset of transcription factor binding sites +/− 2 kb of *EGFP* KD, *Mbd3* KD, and *Smarca4* KD ESCs. Asterisks (*, **) indicate p-values (<0.05, <0.001) reflecting statistical significance of changes in H2A.Z occupancy over the factor peak in *Mbd3* relative to *EGFP* KD (red) or *Smarca4* relative to *EGFP* KD (green).

Supplemental Data 1, related to Figures 1-3. Small reads, nucleosome size reads, and subnucleosomes size reads from MNase-seq reveal unique patterns in *Mbd3* KD and *Smarca4* KD ESCs.

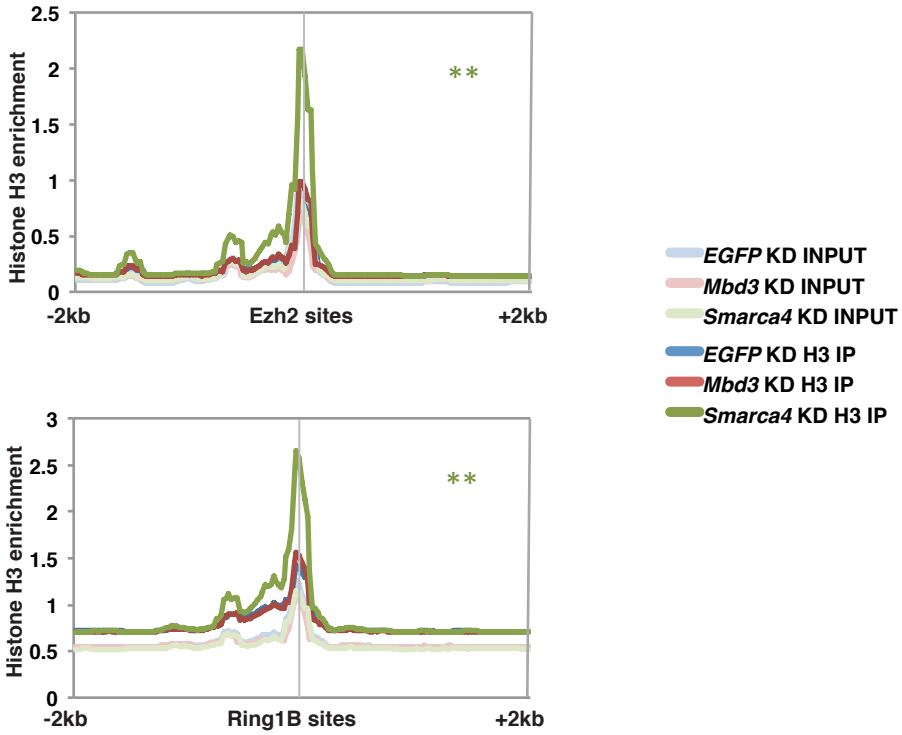
(A-C) Aggregation plots of relative factor occupancy obtained from small read size fragments (≤80 bp, **A**), mononucleosome size fragments (135-165 bp, **B**), and sub-mononucleosome size fragments (100-130 bp, **C**) of MNase-seq data upon *EGFP* KD, *Mbd3* KD, or *Smarca4* KD averaged over various factor binding sites +/− 2 kb in ESCs. Binding sites were called from previously published ChIP-

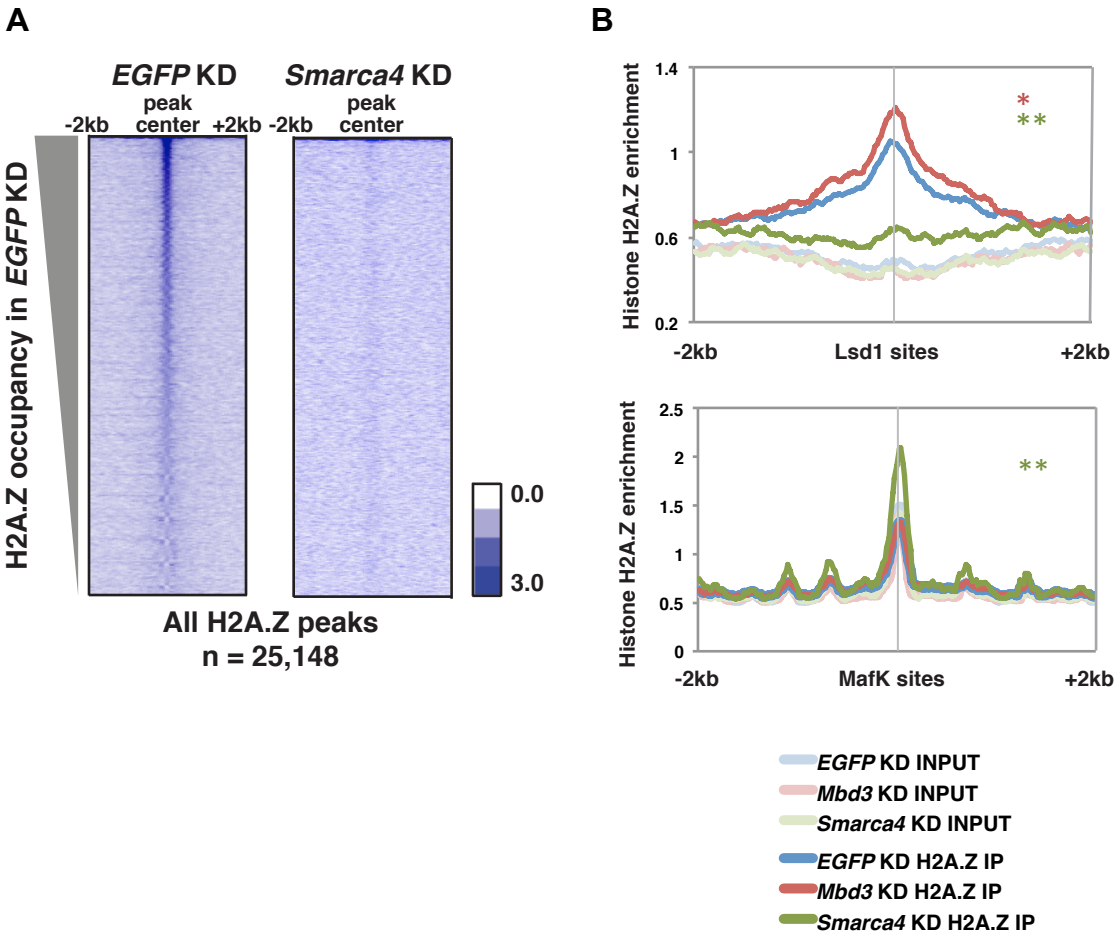
seq datasets (see Supplemental Experimental Procedures). Asterisks (*, **) indicate p-values (<0.05, <0.001) reflecting statistical significance of changes in reads of each size class over the factor peak in *Mbd3* relative to *EGFP* KD (red) or *Smarca4* relative to *EGFP* KD (green).

Supplemental Figure 1



Supplemental Figure 2




A
EGFP KD
Smarca4 KD
peak center
-2kb
+2kb
H2A.Z occupancy in EGFP KD
0.0
3.0
All H2A.Z peaks
n = 25,148
B
Histone H2A.Z enrichment
1.4
1
0.6
0.2
*
**
-2kb
Lsd1 sites
+2kb
2.5
2
1.5
1
0.5
0
MafK sites
EGFP KD INPUT
Mbd3 KD INPUT
Smarca4 KD INPUT
EGFP KD H2A.Z IP
Mbd3 KD H2A.Z IP
Smarca4 KD H2A.Z IP