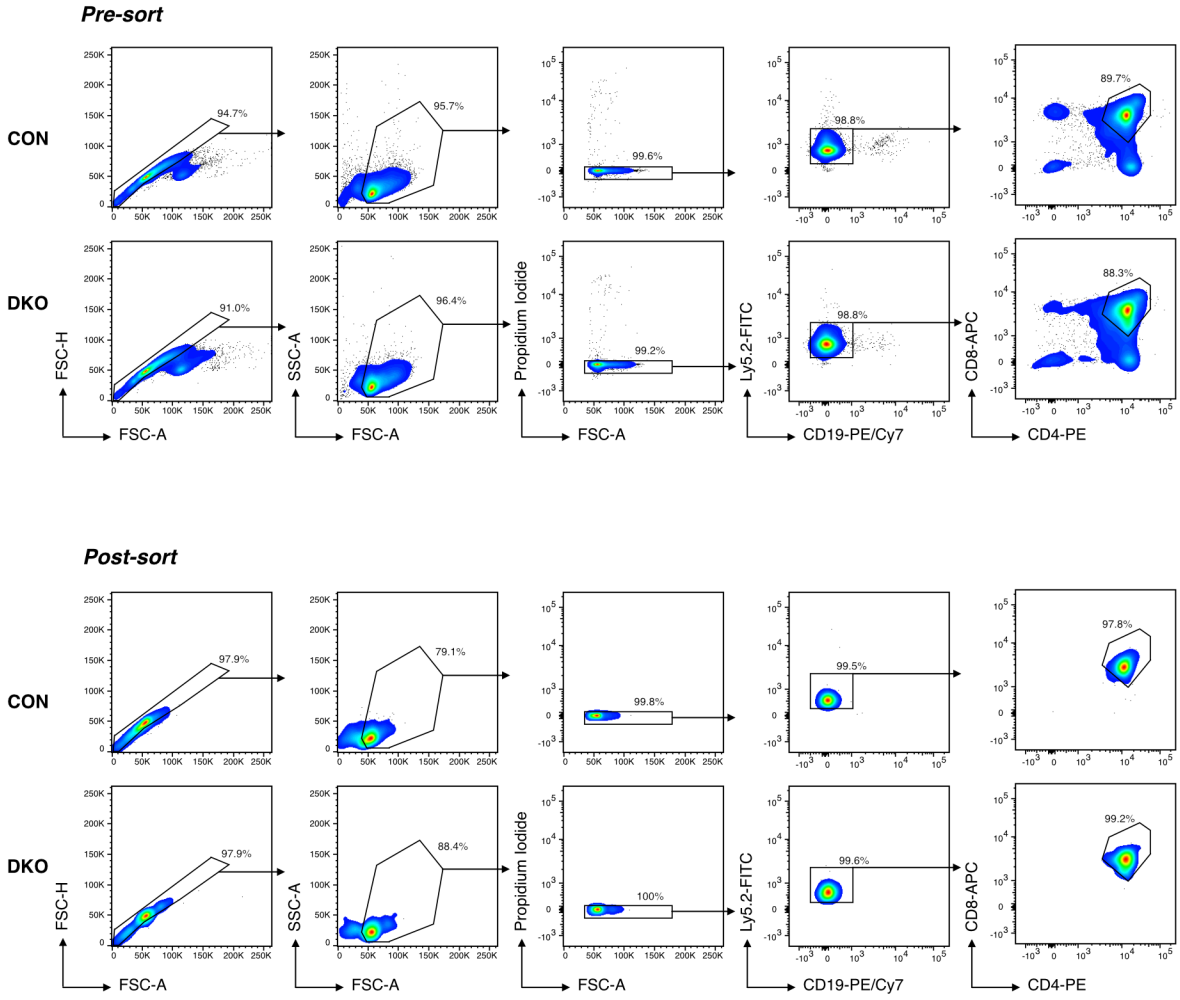
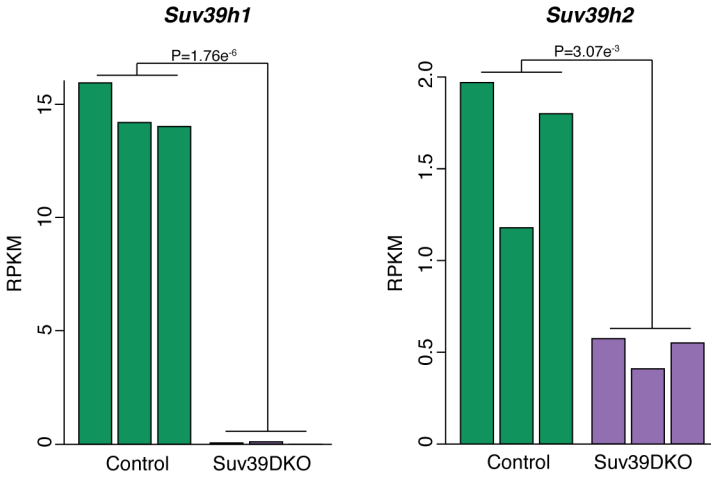
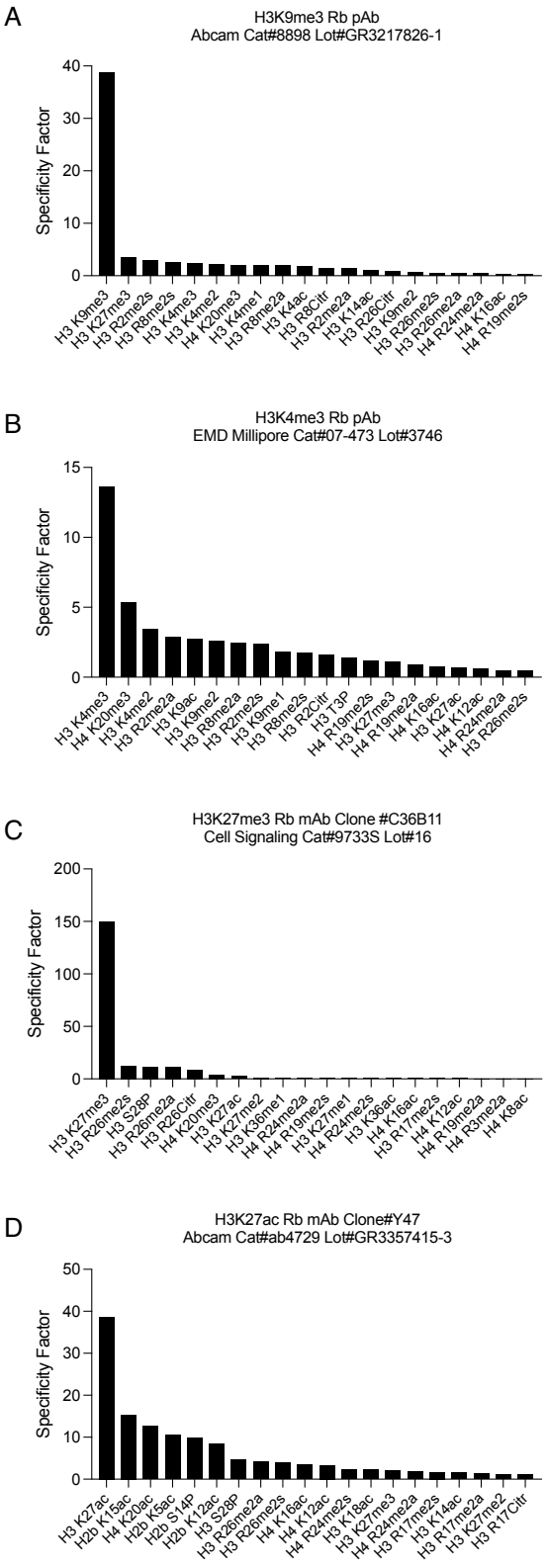


Supplemental Figure S1: Representative flow cytometric gating of double-positive (DP) thymocytes
Primary DP thymocytes ($CD4^+CD8^+CD19^-$) were flow-sorted from total thymic cells from Suv39DKO and control chimeric mice. The $CD45.2^+$ congenic mark was used to identify donor cells of appropriate genotype as opposed to residual $CD45.1^+$ white blood cells from the irradiated host.

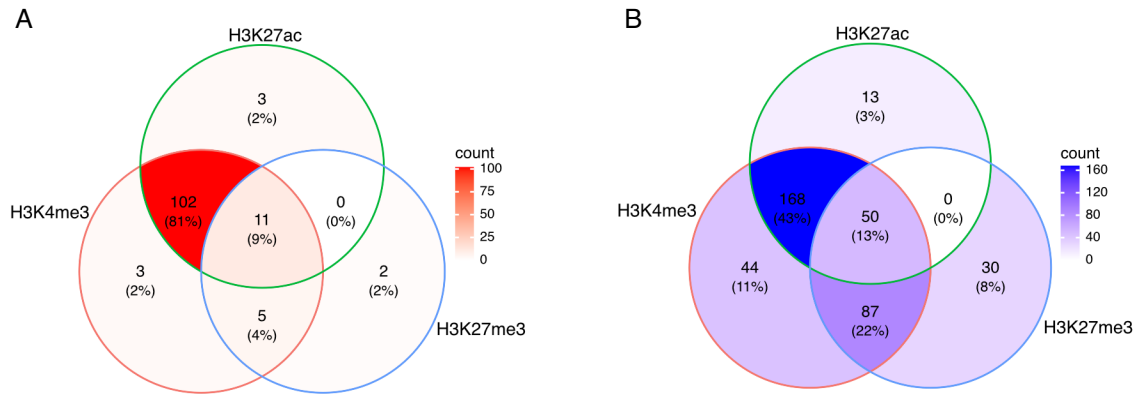


Supplemental Figure S2: *Suv39h1* and *Suv39h2* expression are both significantly reduced in Suv39DKO cells (related to Figure 1).

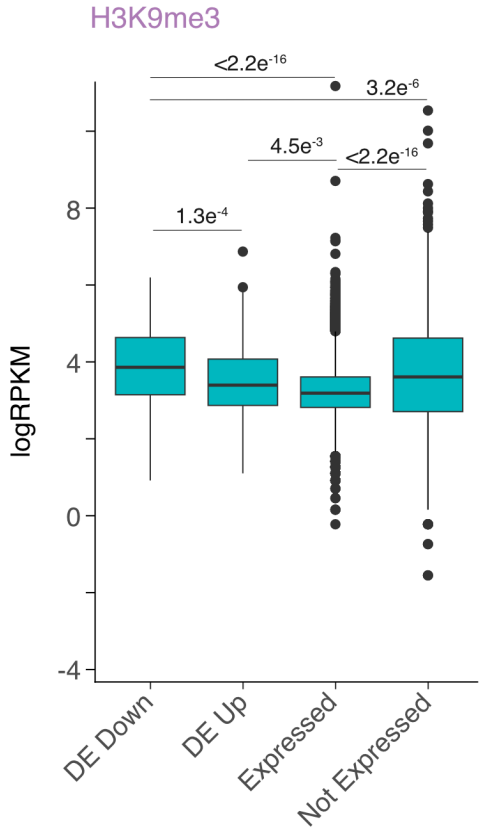
Suv39h1 and *Suv39h2* mRNA expression shown as RPKM in individual RNA-seq replicates from control and Suv39DKO cells. Annotated P values shown are the adjusted p-value from *edgeR* analysis.



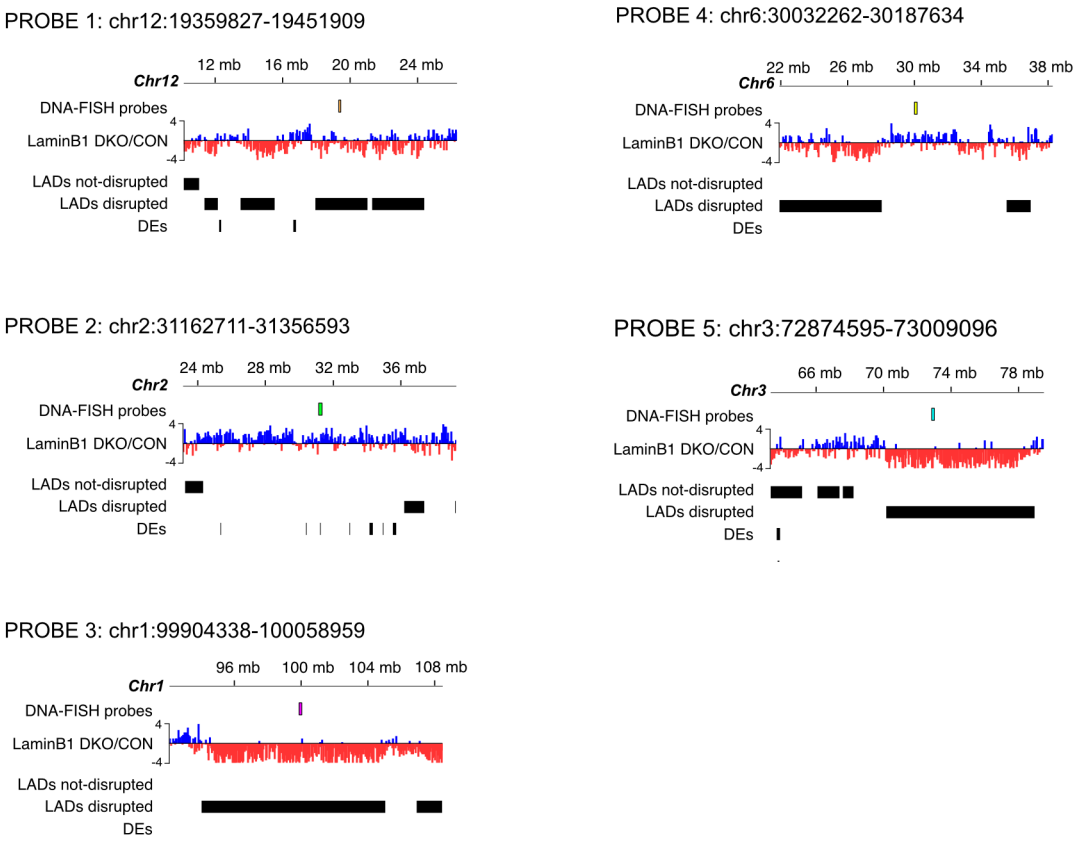
Supplemental Figure S3: Antibody specificity peptide array (related to Figure 2).
Antibodies used for ChIP-seq were tested in the Active Motif MODified Peptide Array as per manufacturer's instructions. The top 20 modifications for each individual antibody are plotted.



Supplemental Figure S4: Venn diagrams showing proportion of DE gene promoters overlapping H3K4me3, H3K27ac and H3K27me3 ChIP-seq peaks in Suv39DKO cells (related to Figure 2).
Up-regulated genes shown in **(A)** and down-regulated genes shown in **(B)**. Promoters defined as 2kp upstream and 500bp downstream of the gene transcription start site (TSS).

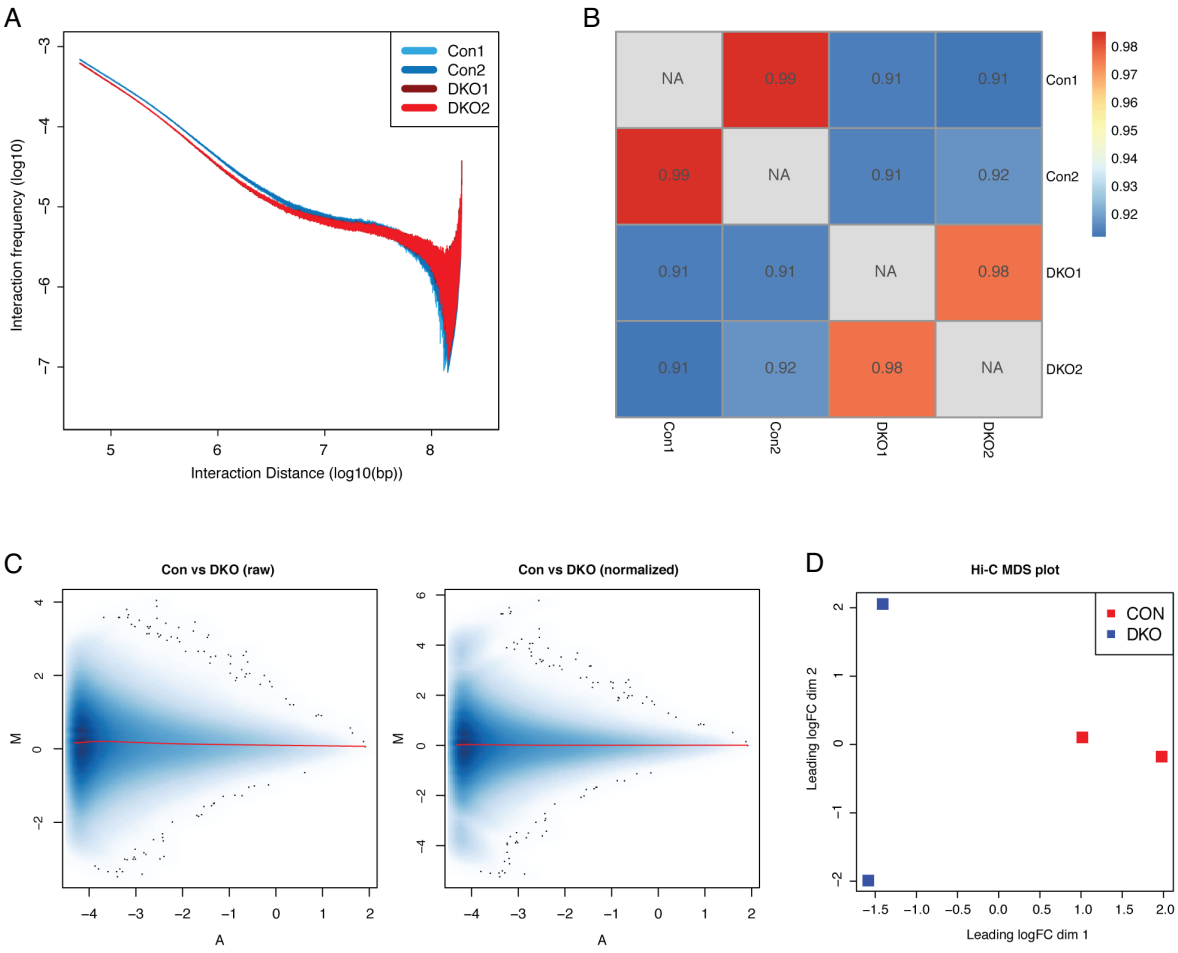


Supplemental Figure S5: Distribution of H3K9me3 in gene promoters (related to Figure 1)
Distributions of H3K9me3 logRPKM across up-regulated (DE Up), down-regulated (DE Down) and non-DE (divided into expressed and non-expressed genes based on expression level in control cells) from control double-positive thymocytes. Promoters are defined as 2kb upstream to 500bp downstream the transcription start site (TSS) of a given gene. Box plots depict the interquartile range (IQR) ± 1.5xIQR with median annotated. Distributions were compared by Wilcoxon rank sum test with continuity correction.

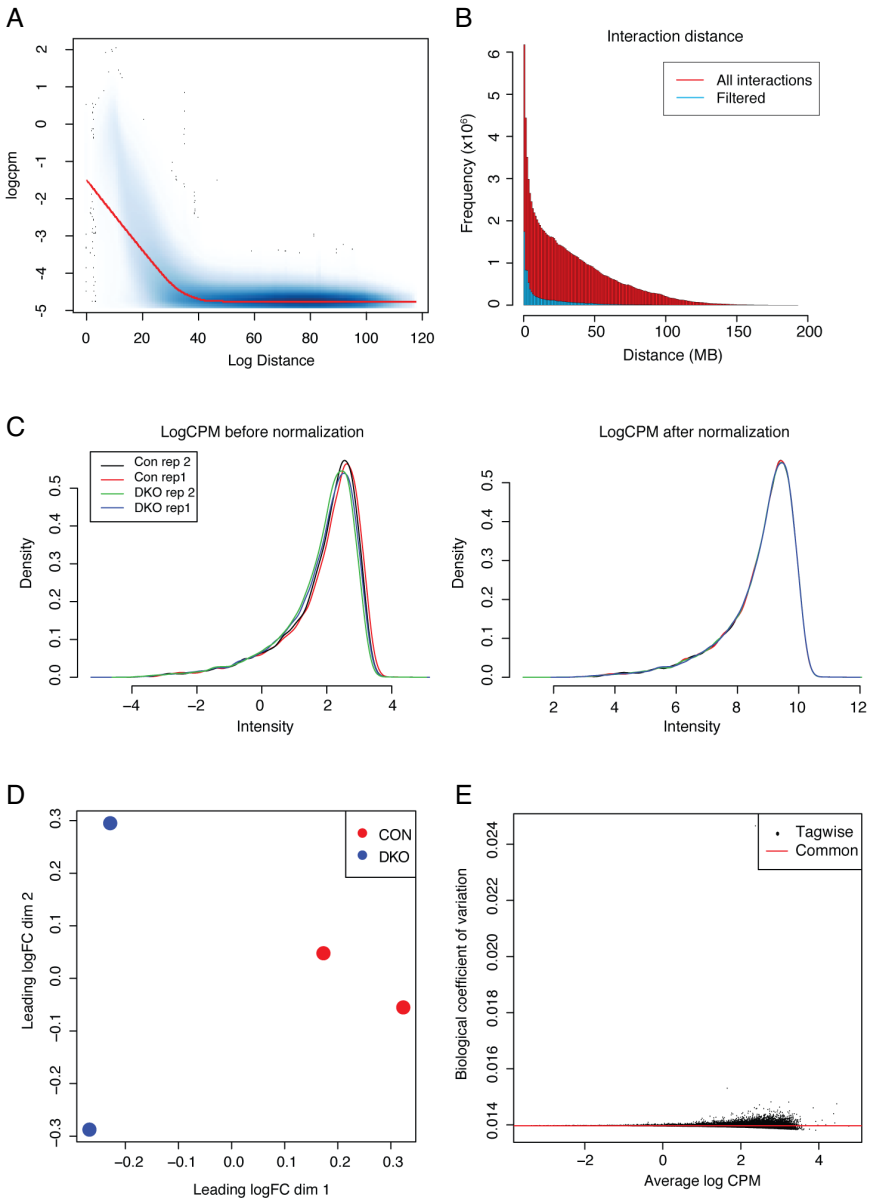


Supplemental Figure S6: Genomic coordinates for DNA-FISH probes used to validate LaminB1 ChIP-seq data (related to Figure 2).

LaminB1 ChIP-seq shown as a ratio of DKO to CON signal. LADs not-disrupted, LADs disrupted, as well as differentially expressed (DE) gene loci are also annotated.



Supplemental Figure S7: Normalization and reproducibility of HiC data (related to Figure 3).
A) Interaction decay curves of libraries of the read-pair interaction frequency (log10) as a function of the interaction distance (log10). **B)** Heatmap of the median reproducibility score (stratum adjusted correlation coefficient) across all chromosomes between replicate libraries at 50 kbp. **C)** Mean-abundance (MA) plot of the Con (Rep 1) library versus DKO (Rep 1) library before and after normalization. Plotted on the y-axis is the log-fold change of the interacting bin-pair counts between libraries and plotted on the x-axis is the log-intensity averages. **D)** Multidimensional-scaling plot (MDS) of the filtered and normalized logCPM values of bin-pairs for each library. The distance between each pair of samples was the 'leading log fold change', defined as the root-mean-square average of the 5000 largest $\log_2$ fold changes between that pair of samples.



Supplemental Figure S8: Normalization and reproducibility of gene-centric interactivity analysis (related to Figure 5).

A) Smoothscatter plot of the average log2 counts per million (log2CPM) for all interactions as a function of distance$^{0.25}$. A fitted loess curve in red and the minimum log2CPM value required as a function of distance in purple. **B)** Frequency of interactions at anchor distance before and after interaction filtering. **C)** LogCPM of samples before and after normalization. **D)** Multidimensional-scaling plot (MDS) of the filtered and normalized logCPM values of each promoter for each library. The distance between each pair of samples was the 'leading log fold change', defined as the root-mean-square average of the 500 largest $\log_2$ fold changes between that pair of samples. **E)** A plot of the tagwise biological coefficient of variation for each promoter versus the average log2-count per million (CPM) and common dispersion (red line). The dispersions were estimated with estimateDisp from the edgeR package with robust=TRUE and trend.method="none".