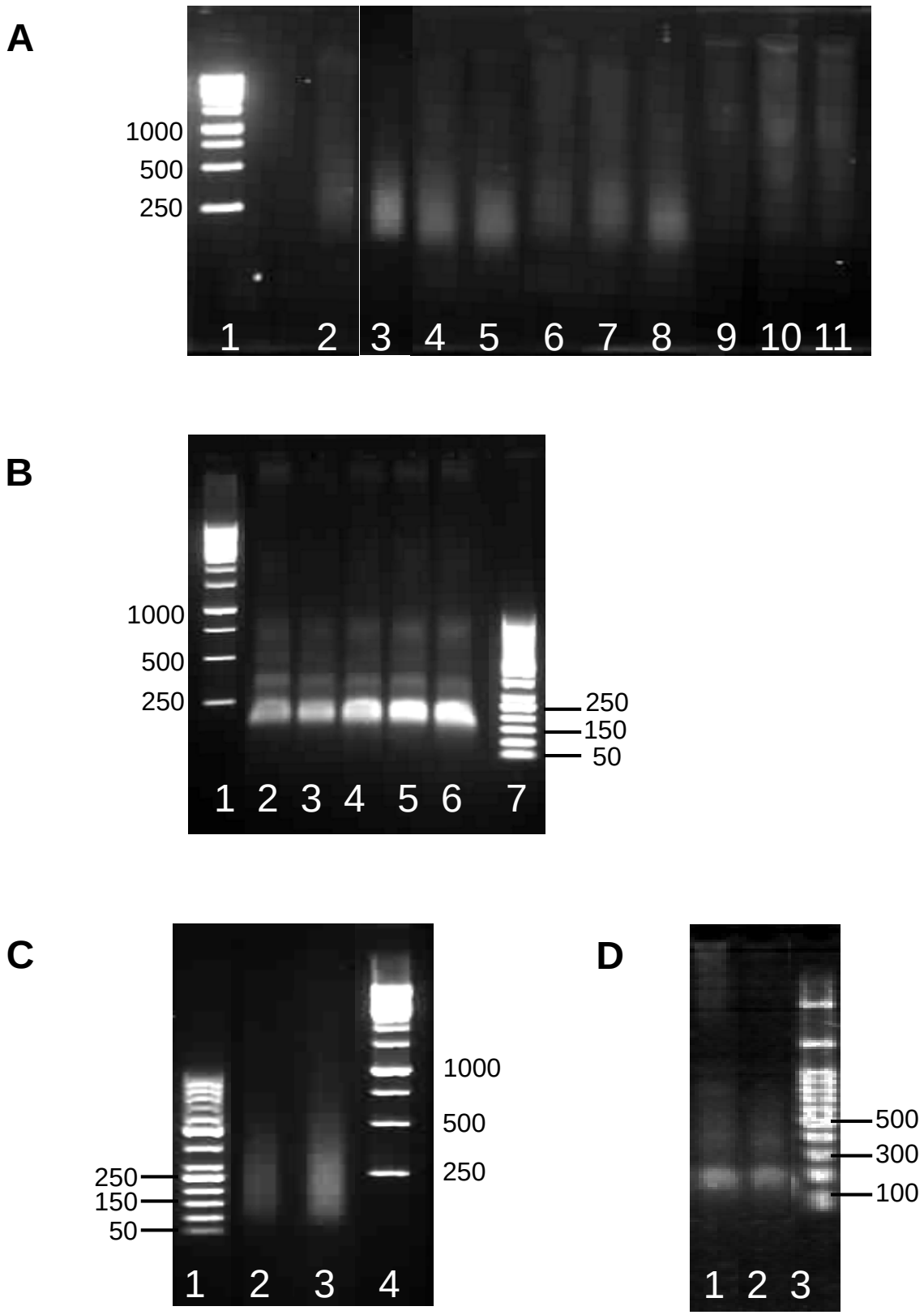
Additional file 1

Figure S1: Setup of chromatin preparation conditions. (A) Gel electrophoresis (1% agarose stained with GelRed, Biotium, Fremont, USA) of cross-linked and sonicated chromatin. Cross-linking was reversed before migration. Lane 1: GeneRuler 1 kb DNA Ladder, ref. SM0311, Thermo-Scientific, Waltham, USA. Lanes 2 to 5: FA 1 % for 5 min followed by respectively 2 x 5, 3 x 5, 4 x 5 or 6 x 5 min of sonication. Lanes 6 to 8: FA 1 % for 15 min followed by respectively 2 x 5, 4 x 5 or 6 x 5 min of sonication. Lanes 9 to 11: FA 2 % for 30 min followed by respectively 2 x 5, 4 x 5, 6 x 5 min of sonication. (B) Gel electrophoresis (1% agarose stained with GelRed) of Mnase digested chromatin. Lane 1: GeneRuler 1 kb DNA Ladder. Lane 2: 10 U Mnase for 7 min. Lane 3: 10 U Mnase for 10 min. Lane 4: 20 U Mnase for 10 min. Lane 5: 10 U Mnase for 15 min. Lane 6: 20 U Mnase for 15 min. Lane 7: GeneRuler 50 bp DNA Ladder, ref. SM0371, Thermo-Scientific, Waltham, USA. (C) Gel migration (1% agarose stained with GelRed) of muscle X-ChIP chromatin samples used for the sequencing. Lane 1: GeneRuler 50 bp DNA Ladder. Lane 2: Replicate 1 (X_R1). Lane 3: Replicate 2 (X_R2). Lane 4: GeneRuler 1 kb DNA Ladder. (D) Gel migration (1% agarose stained with GelRed) of muscle N-ChIP chromatin samples used for the sequencing. Lane 1: Replicate 1 (N_R1). Lane 2: Replicate 2 (N_R2). Lane 3: 100 bp DNA marker ref. 523002, Ready-to-use DNA Markers: 100 bp, Dominque Dutscher, Brumath, France.



David et al.: Additional File 1_Figure S1

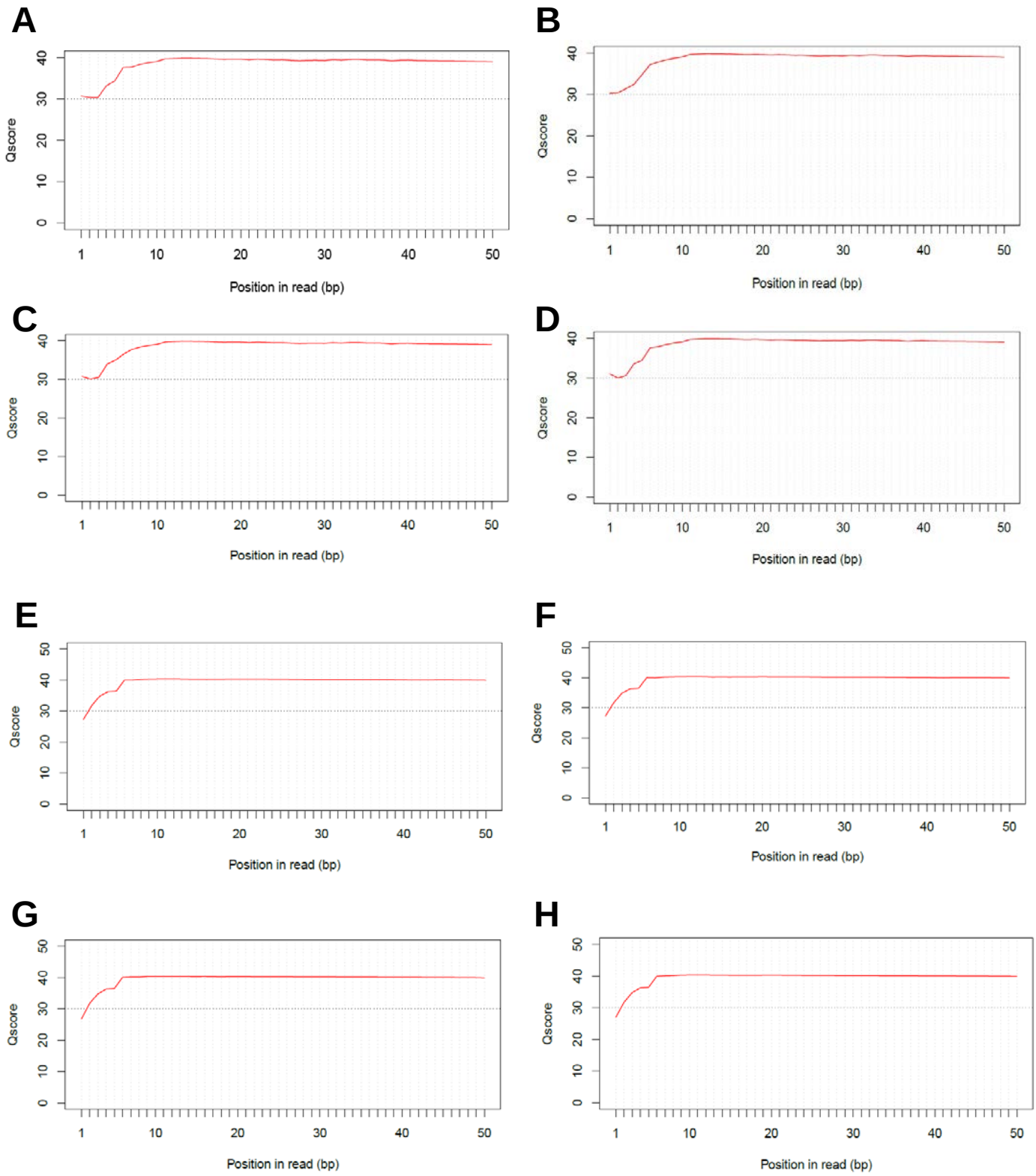
Table S1: ChIP-qPCR primers

Primer name	Forward primer	Reverse primer	Product size (bp)
PAX5	5'-GCATTTGAGTTGTCAGTCTATTC-3'	5'-GTGCTACCCGTCCGAATTAC-3'	158
SOX2	5'-AACGGATCGCCTACCTACAG-3'	5'-GAATGAGACGAGGAGGTGACTAC-3'	193
GAPDH	5'-AGACAGGAACAAGGCGTAGTG-3'	5'-CCTTGAAGTGTCCGTGTGTAG-3'	131
Common peak 1	5'-GGTGGGTGGGATGGTTATC-3'	5'-GCTGTCTGTGTACAGGGAATTAAAC-3'	103
Common peak 2	5'-AGCCTGCGGGTAGGAAATAG-3'	5'-GCAGAACAAGACGCCTACAG-3'	96
Common peak 3	5'- TCCAGGTGTTCCCTTTGAGTAG-3'	5'-GGTCTCTTCCAATCTGGGATAC-3'	91
Common peak 4	5'-TCCCATTTCCAGCTCTTTAATAC-3'	5'-CTTCGCACATGAGCCACTAC-3'	162

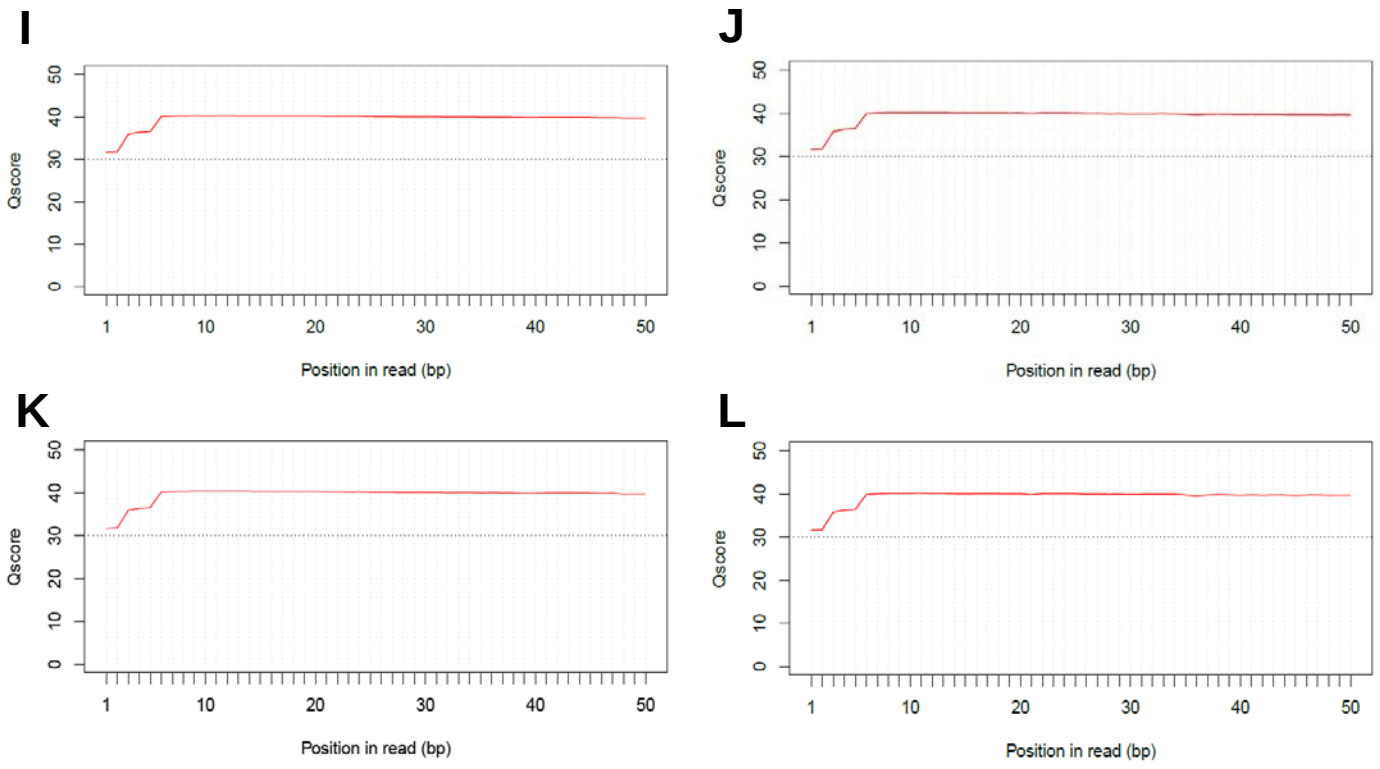
Table S2: Quantity of chromatin and DNA used throughout the protocol. Quantification of chromatin kept for input or used for immunoprecipitation was estimated by spectrophotometry (NanoDrop ND-1000 Spectrophotometer). For all subsequent steps, DNA concentrations were determined by Qubit Fluorometric Quantitation (Qubit dsDNA Assay Kit, Thermo Scientific, Waltham, USA).

Protocol	X-ChIP				N-ChIP			
Sample	X_R1		X_R2		N_R1		N_R2	
Fraction	input	H3K27me3	input	H3K27me3	input	H3K27me3	input	H3K27me3
Chromatin kept for input or used for IP (µg)	1.8	6	1.8	6.4	2.5	5	2.5	5
DNA obtained after reverse-crosslink and purification or IP (ng)	604.42	59.19	746.68	96.27	301.86	26.1	477.31	41.83
DNA used for library preparation (ng)	100.17	10.14	100.25	10.18	100.62	10.09	100.77	10.23
DNA obtained from library preparation (ng)	129.74	84.27	126.60	65.43	177.48	129.30	143.85	174.37

Figure S2: Quality assessment of the sequencing reads. Quality was determined by the FastQC q30 value (Babraham Bioinformatics, version 0.11.2, arguments --nogroup –casava). q30 is the percentage of bases above a Qscore of 30. (A) H3K27me3 X_R1 reads from muscle, q30: 94.05 %; (B) Input X_R1 reads from muscle, q30: 93.98 %; (C) H3K27me3 X_R2 reads from muscle, q30: 93.98 %; (D) Input X_R2 reads from muscle, q30: 94.16 %; (E) H3K27me3 N_R1 reads from muscle, q30: 97.21 %; (F) Input N_R1 reads from muscle, q30: 97.31 %; (G) H3K27me3 N_R2 reads from muscle, q30: 97.15 %; (H) Input N_R2 reads from muscle, q30: 97.22 %; (I) H3K27me3 HT_X_R1 reads, q30: 97.28 %; (J) Input HT_X_R1 reads, q30: 97.39 %; (K) H3K27me3 HT_X_R2 reads, q30: 96.89 %; (L) Input HT_X_R2 reads, q30: 96.84 %.

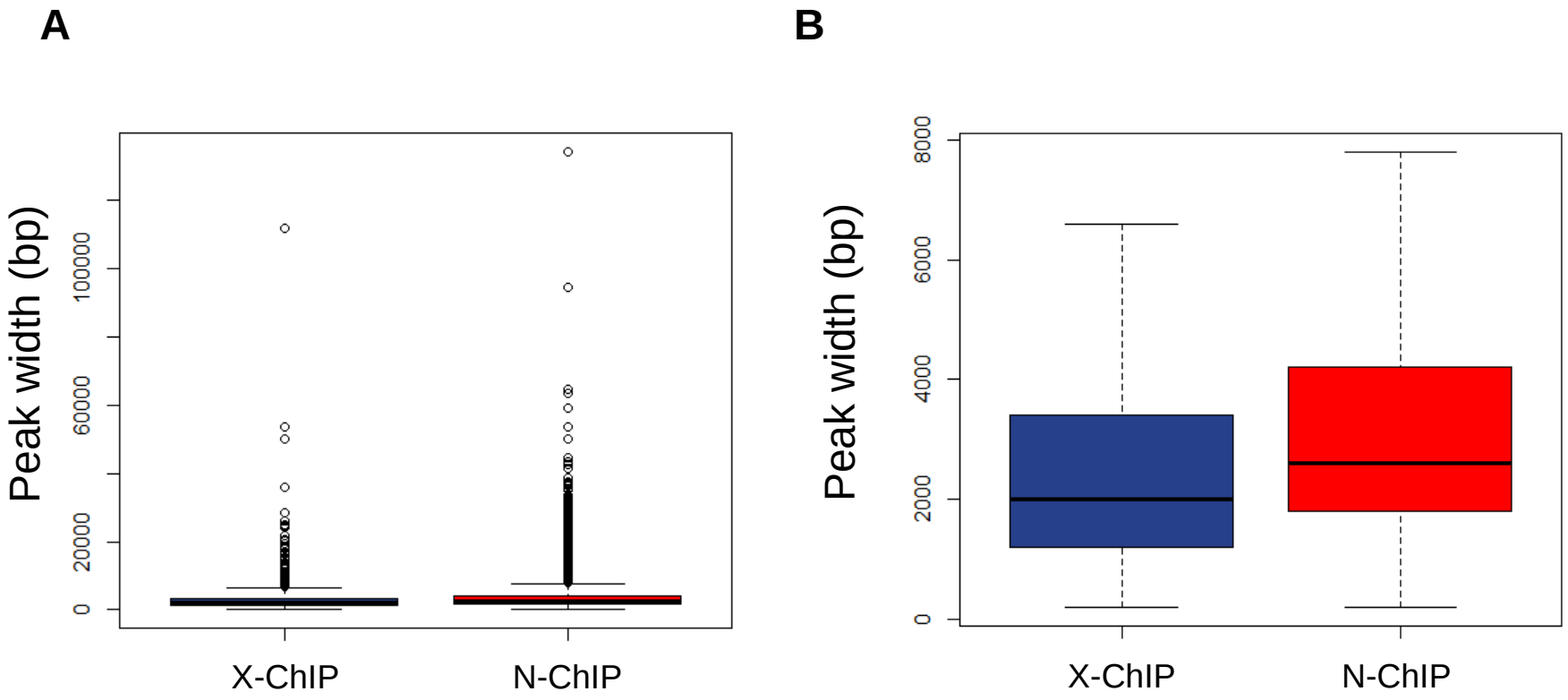


David et al.: Additional File 4_Figure S2



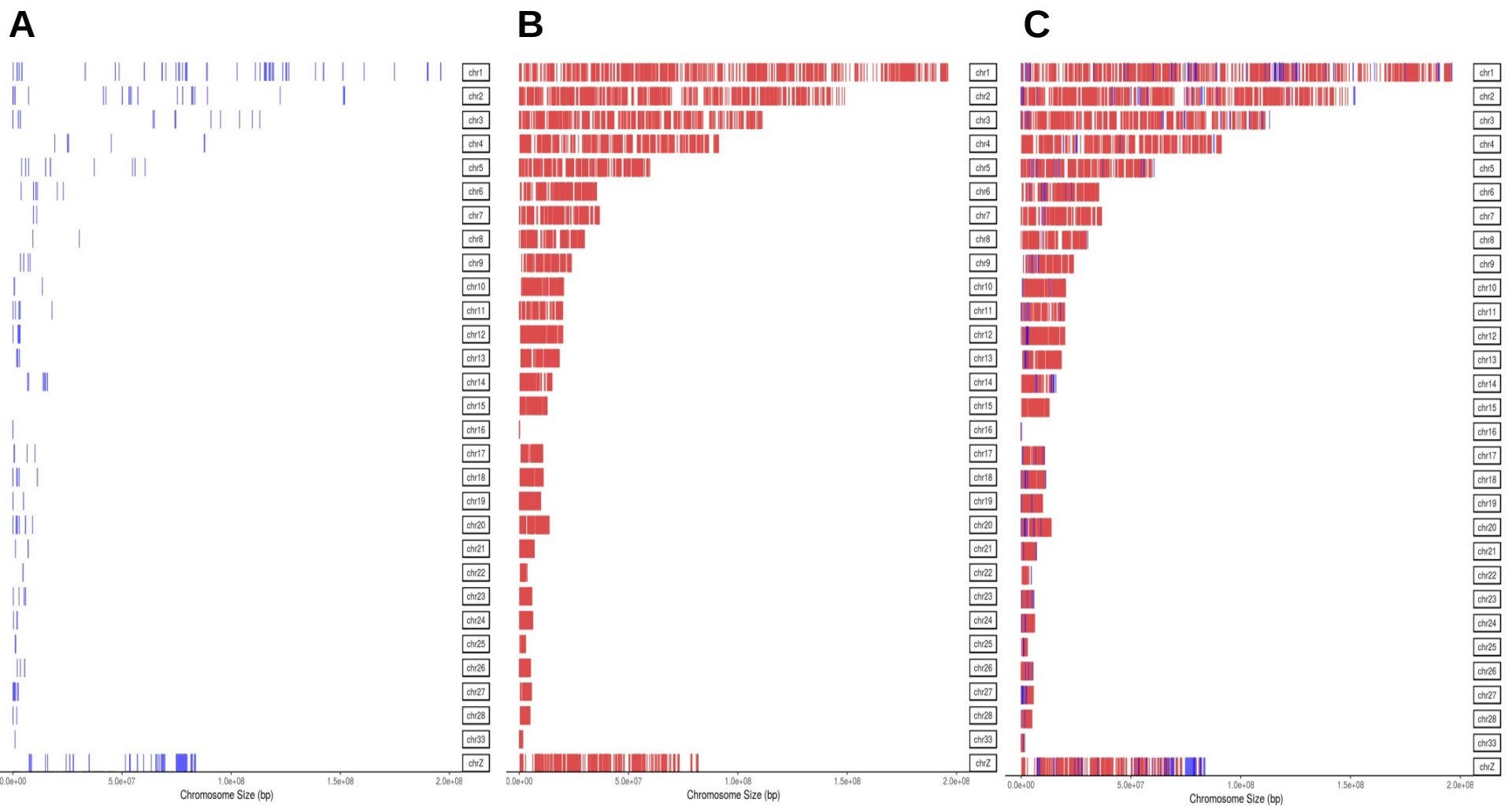
David et al.: Additional File 4_Figure S2

Figure S3: Boxplot representing the distribution of the 40M peaks lengths. Boxplots of peak width (A) with or (B) without outliers (using the argument outline=F) were determined using the R boxplot function on 40M datasets. Common peaks between X-ChIP-seq replicates are shown in blue (median width: 2000 bp), N-ChIP-seq common peaks in red (median width: 2600 bp).



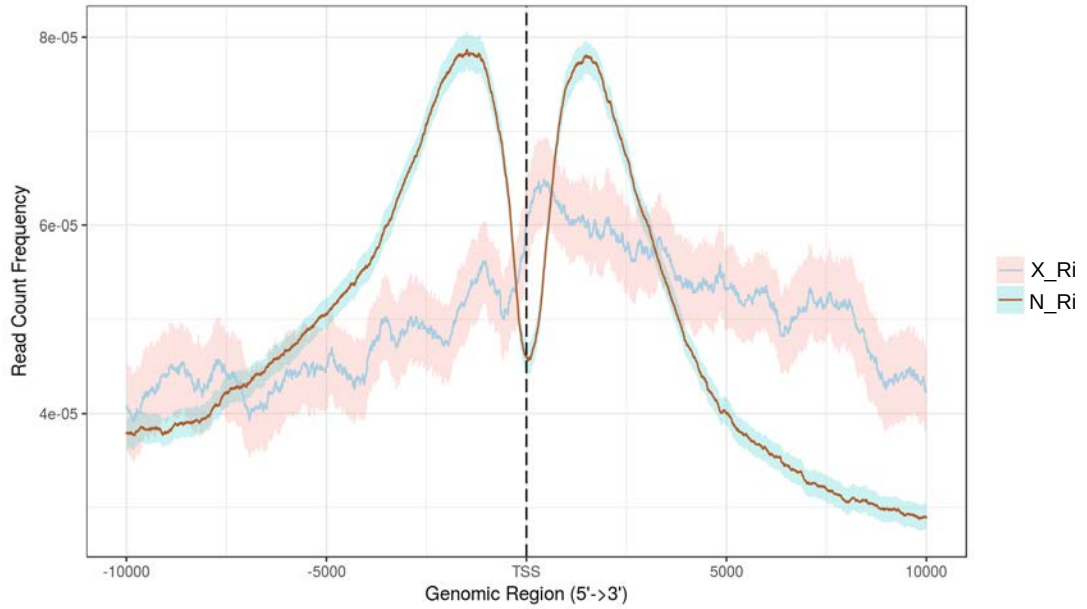
David et al.: Additional File 5 Figure S3

Figure S4: X-ChIP-seq and N-ChIP-seq peaks analysis. (A-C) Covplots were calculated with ChIPseeker on 40M datasets (Yu G, Wang L and He Q, 2015). (A) Covplot of X-ChIP-seq common peaks; (B) Covplot of N-ChIP-seq common peaks; (C) Covplot of Merged covplot of both X-ChIP-seq peaks (blue) and N-ChIP-seq peaks (red); (D) Profile of common peak data around the TSS (+/- 10 Kb of the TSS) obtained using the plotAvgProf command of ChIPseeker. X_Ri: common X-ChIP-seq peaks, N_Ri: common N-ChIP-seq peaks; (E) Correlation analysis of H3K27me3 peak localization with relative expression value. Transcriptome data from Loyau T *et al.* (2016) was used. Expression values were computed from microarray data (8 x 60 K) corresponding to male control animals of the same age (35d), line (Cobb500) and tissue (*P. major*) for all probes. Probe sequences were aligned against the Galgal5 genome (NCBI) using blastn (default parameters) and the probes displaying 100% of identity were kept, corresponding to 13965 unique genes with expression values. The intersection of expression values and peaks was performed in R (R Development Core Team, 2008) using Rstudio (RStudio Team, 2015) based on the following criteria: presence of a peak within the gene coordinates (5'UTR-3'UTR). The CRAN package ggplot2 was used to generate the density plot (left) and the boxplot (right) of mean expression values for all genes and the genes that contained a peak from the N-ChIP-seq (N-Ri) or X-ChIP-seq (X-Ri) analysis (only common peaks between replicates were used).

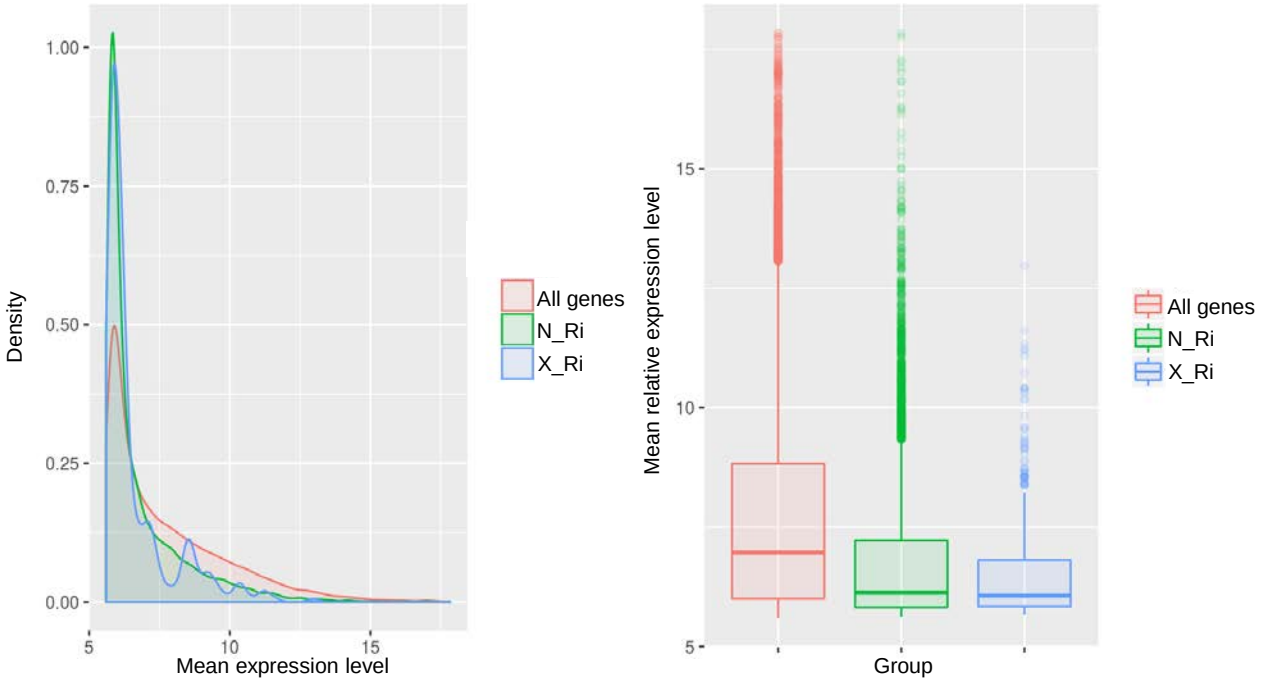


David et al.: Additional File 6 Figure S4

D



E



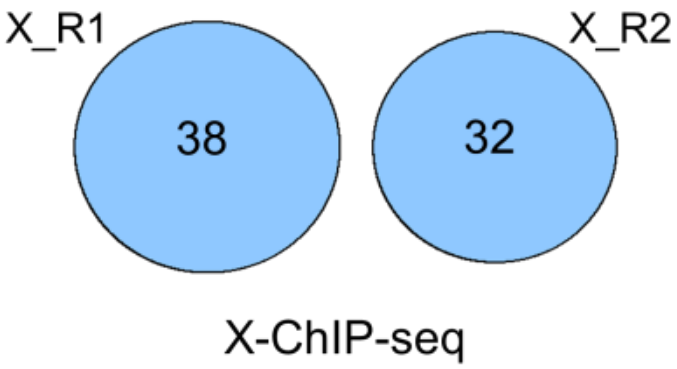
David et al.: Additional File 6 Figure S4

Table S3: Broad peak detection analysis by MACS2 for both H3K27me3 X-ChIP-seq and N-ChIP-seq experiments. 40 million of unique reads per sample were randomly selected among the uniquely mapped reads for each replicate and broad peak detection was performed with the peak caller MACS2 (MACS2_callpeak 2.1.0.20140616, bandwidth 300, q-value 0.05, build model OFF, broad peaks ON).

Method	X-ChIP		N-ChIP	
Sample	X_R1	X_R 2	N_R 1	N_R 2
Peak number	38	32	17113	17591
Genome coverage (Mb)	0.0186	0.0154	36.60	35.36

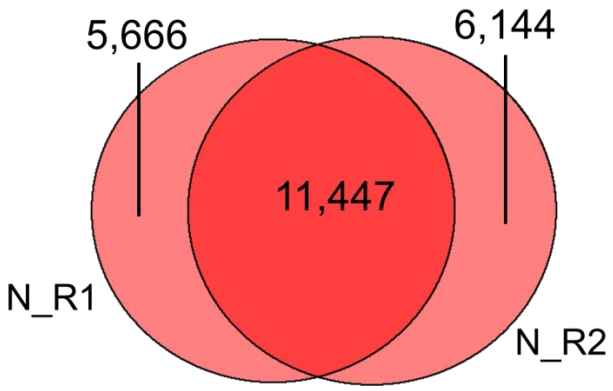
Figure S5: Comparison of H3K27me3-enriched peak regions detected by MACS2 and between MACS2 and epic. (A) Venn diagram representing the intersection between broad peak regions detected by MACS2 for both X-ChIP-seq replicates. No common regions were found. (B) Venn diagram representing the overlap between broad peak regions detected by MACS2 for N-ChIP-seq replicates. (C) Venn diagram representing the intersection between broad peak regions common to both N-ChIP-seq replicates detected by epic and MACS2.

A



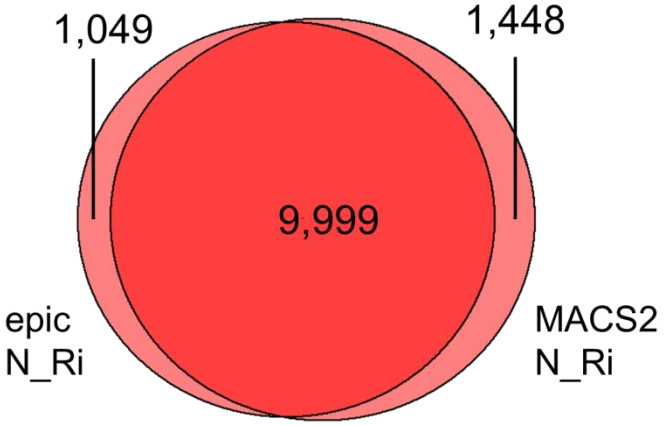
X-ChIP-seq

B



N-ChIP-seq

C



N-ChIP-seq : epic *vs* MACS2

Supplemental methods: X-ChIP-seq protocol for hypothalamus tissue samples

Hypothalamus tissues were snap frozen at sampling. Samples were ground in a mortar with a pestle cooled with liquid nitrogen then resuspended in 900 µL of ice-cold PBS-C and transferred to 1.5 mL TPX tubes. 100 µL of crosslink buffer was added (100 mM NaCl, 1 mM EDTA pH 8, 0.5 mM EGTA pH 8, 50 mM HEPES pH 8, 1 % formaldehyde) and tubes were incubated 5 min at room temperature under shaking. The reaction was stopped by adding glycine to a final concentration of 0.125 M for 5 min at room temperature under shaking. Pellets were washed once in PBS-C and then incubated 10 min in 300 µL of SDS lysis buffer (50 mM Tris-HCl pH 8, 20 mM EDTA pH 8, 1 % SDS, 1 X Complete™ protease inhibitors). Samples were vortexed and incubated 10 min on ice, then sonicated 4 x 5 min using the Bioruptor (high – 30 s ON / 30 s OFF). Samples were centrifuged 5 min at 10600 *g* and the supernatant containing the chromatin was collected and used fresh or stored at -80 °C.

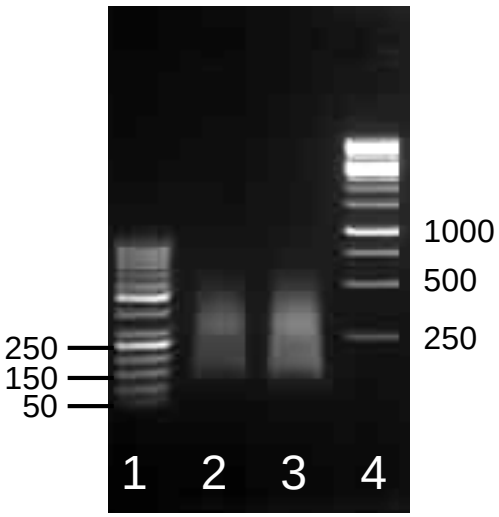
Chromatin quantification, quality assessment, immunoprecipitation and Illumina sequencing were performed as described for the muscle (2.45 ng for HT_X_R1 and 1.1 ng for HT_X_R2 of IP DNA and 100 ng of input DNA were used to prepare the sequencing libraries).

Table S4: Sequencing results of hypothalamus X-ChIP-seq analyzed using epic. Two biological replicates were sequenced (HT_X_R1 and HT_X_R2). Reads were mapped against the Galgal5 chicken genome.

Method	X-ChIP			
Sample	HT_X_R1		HT_X_R2	
Fraction	Input	H3K27me3	Input	H3K27me3
Total number of uniquely mapped reads (M)	42.44	94.64	29.23	112.77
Total peak number	13133		12531	
Total genome coverage (Mb)	59.70		59	
Number of common peaks between replicates	11308			

Figure S6: Fragmentation assessment of chromatin before immunoprecipitation for hypothalamus samples and X-ChIP results at three control loci. (A) 1% agarose stained with GelRed was used for the chromatin migration. Lane 1: GeneRuler 50 bp DNA Ladder, ref. SM0371, Thermo-Scientific, Waltham, USA. Lane 2: Replicate 1 (HT_X_R1). Lane 3: Replicate 2 (HT_X_R2). Lane 4: GeneRuler 1kb DNA Ladder, ref. SM0311, Thermo-Scientific, Waltham, USA. (B-D) IGV visualization of H3K27me3 40M unique reads signal in the hypothalamus tissue at control loci. H3K27me3 enrichments were normalized to the input [log2(IP/input)]. The green boxes represent broad peaks detected by epic. (B) *SOX2* (chr9: 16918111-16919468); (C) *PAX5* (chrZ: 81789479-81896738); (D) GAPDH (chr1: 76950864-76956805) loci.

A



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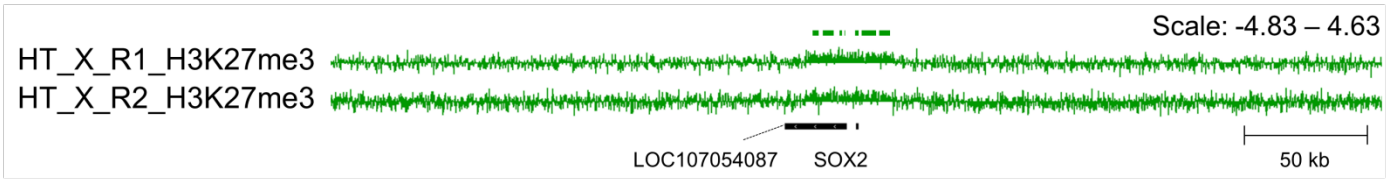

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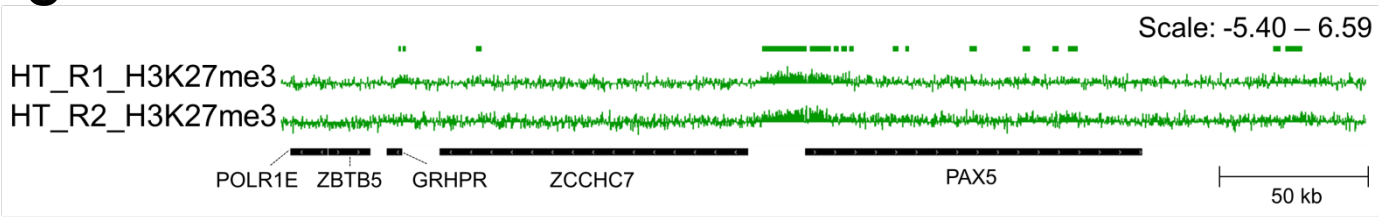

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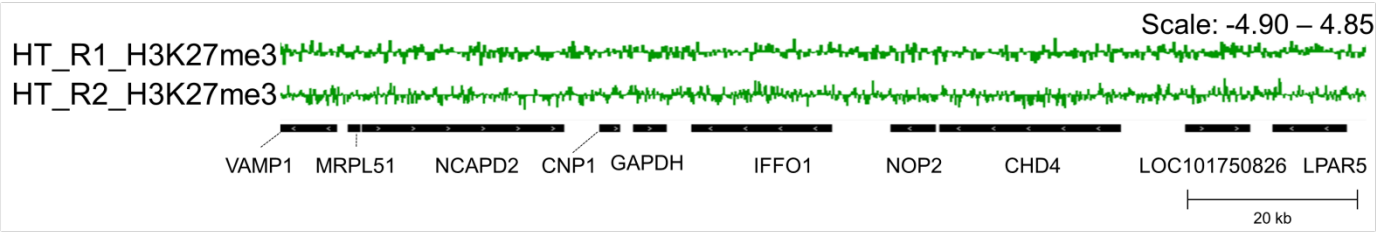

David et al.: Additional File 8_Figure S6