Thermal manipulation during embryogenesis impacts H3K4me3 and H3K27me3 histone marks in chicken hypothalamus

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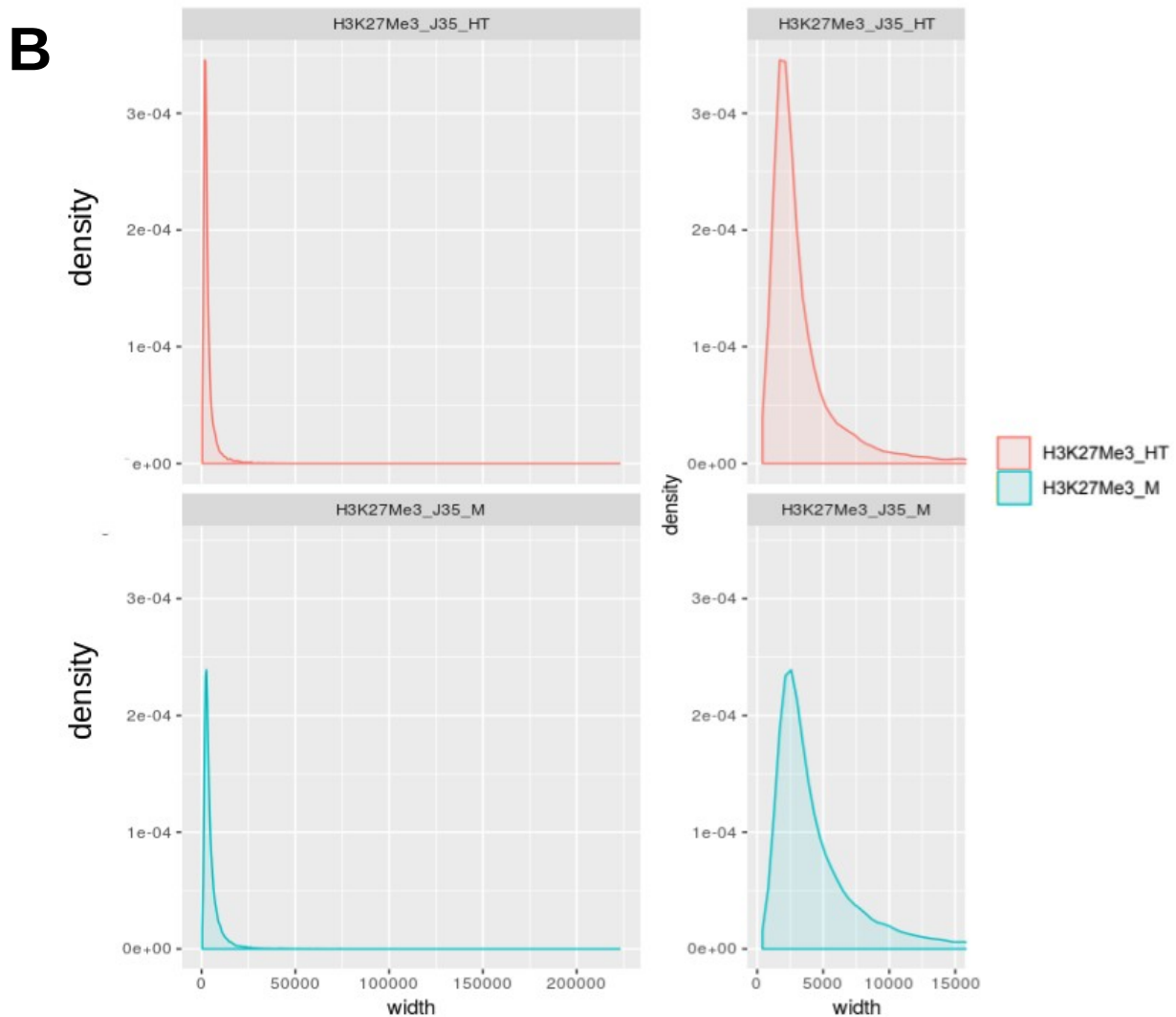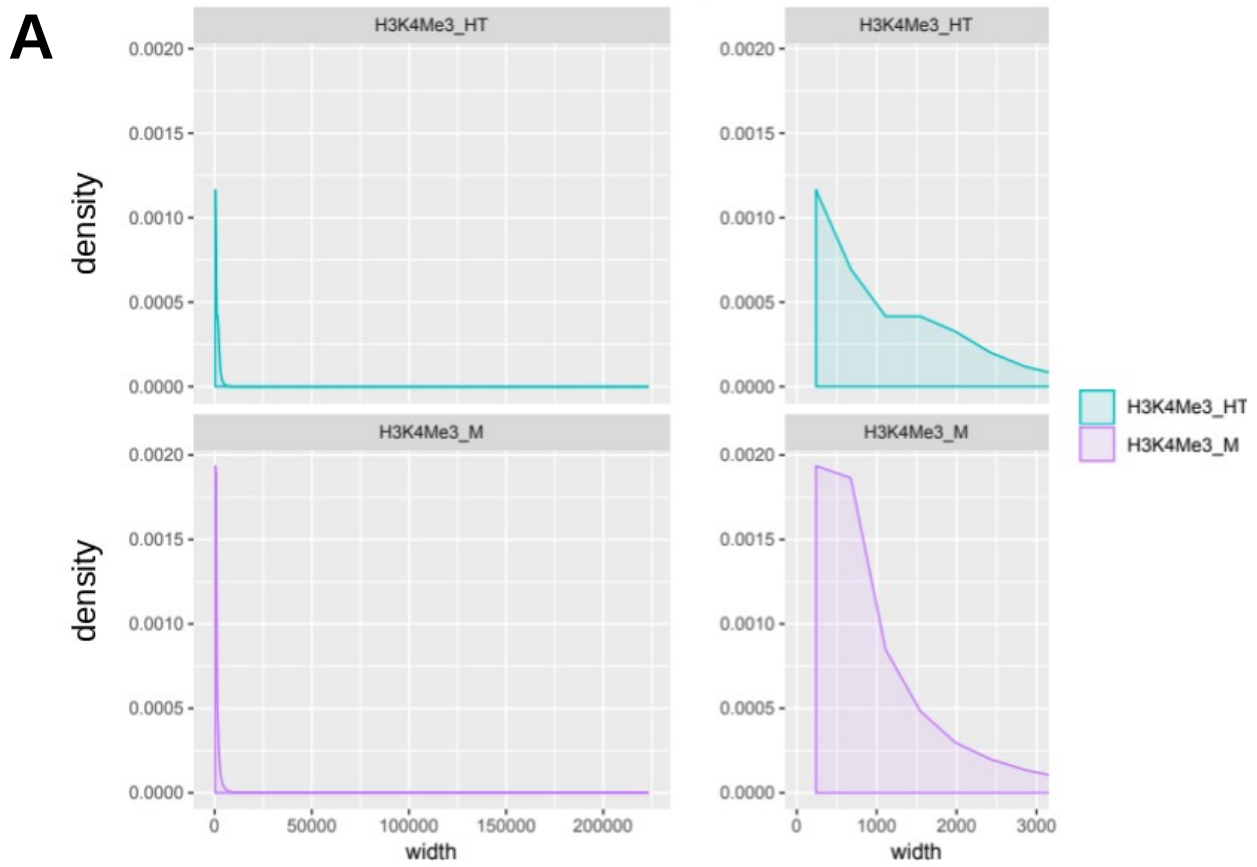Thierry Bordeau[1], Yves Bigot[2], Anne Collin[1] and Vincent Coustham[1]*

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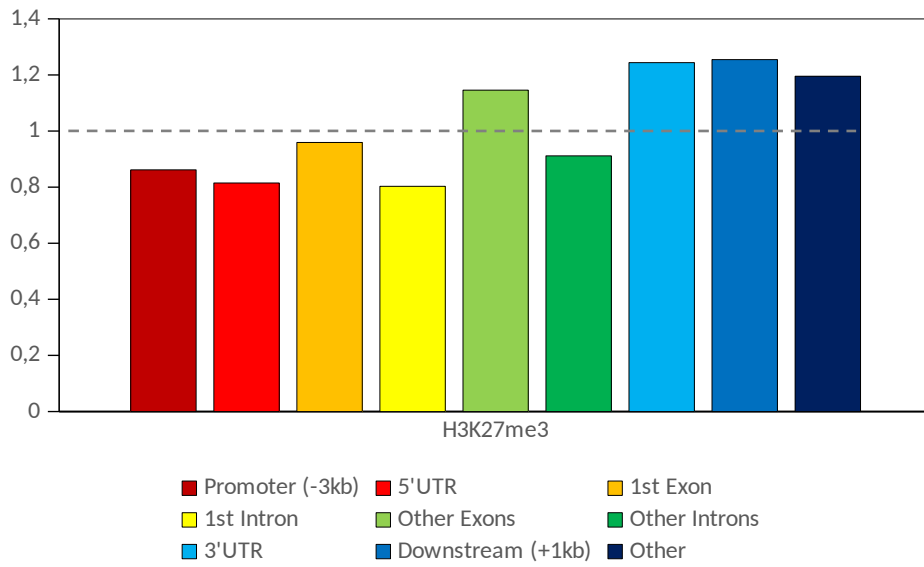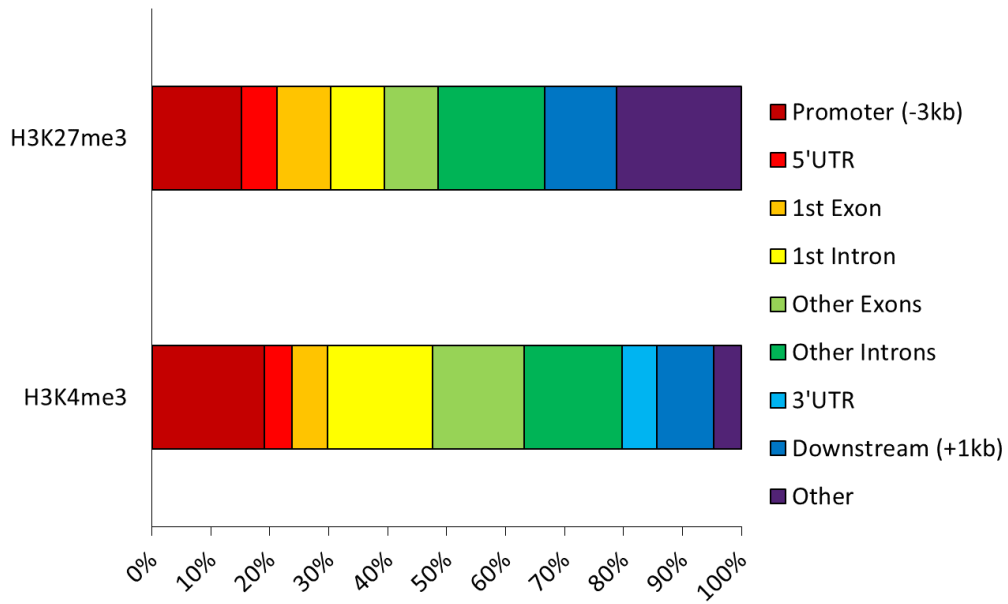[2]PRC, CNRS, IFCE, INRA, Université de Tours, 37380, Nouzilly, France

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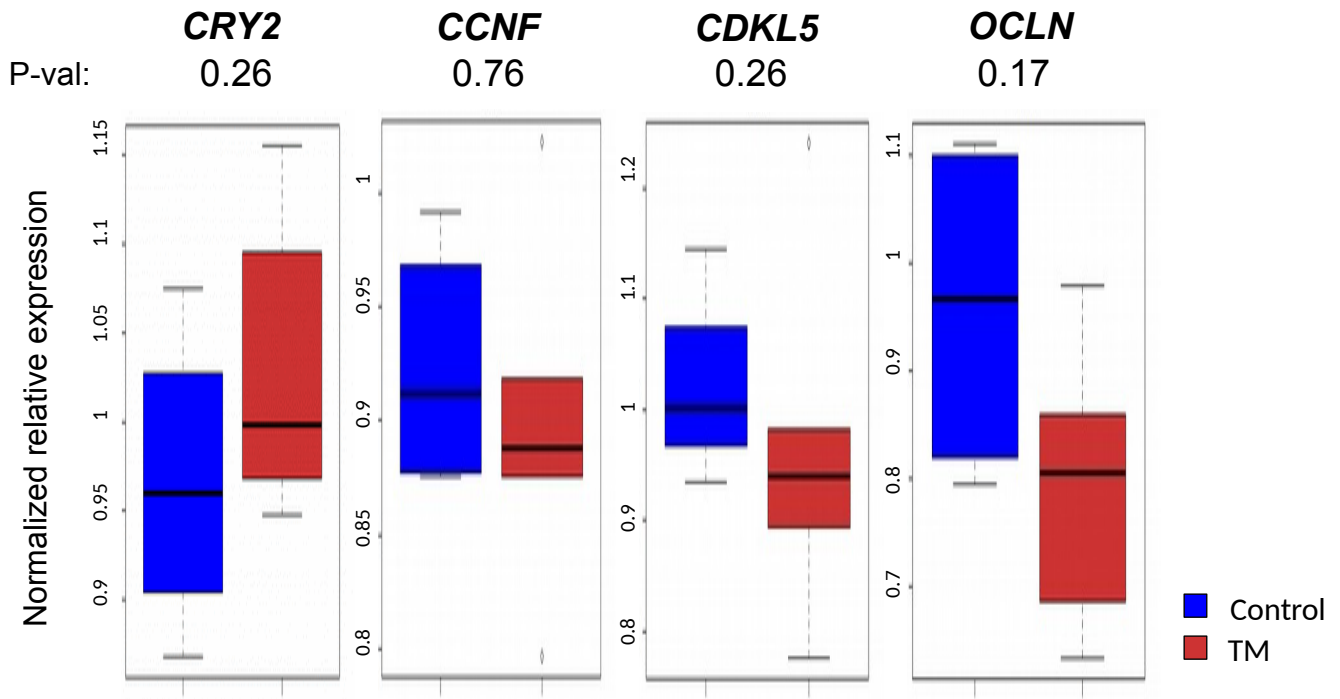*Corresponding author

Supplementary Figures

Fig. S1: Peak sizes. Density plots of (**A**) H3K4me3 and (**B**) H3K27me3 peaks width. Columns on the right correspond to a zoom on the density peaks. HT: hypothalamus; M: *Pectoralis major* muscle.

Fig. S2: Analysis of DP distribution. (A) Ratio of hypothalamus H3K27me3 DP/all analyzed peaks. (B) Distribution of muscle DP.

**Fig. S3: Relative expression of candidate genes (*CRY2*, *CCNF*, *CDKL5* and *OCLN*) that contained a differential H3K4me3 peak.** Control replicates are shown in blue, TM in red. Box boundaries represent the first and third quartiles. The median is indicated by the bold horizontal line dividing the interquartile range. Upper and lower ticks indicate the 10th and 90th percentiles.

Wang GOterms distance clustering heatmap plot
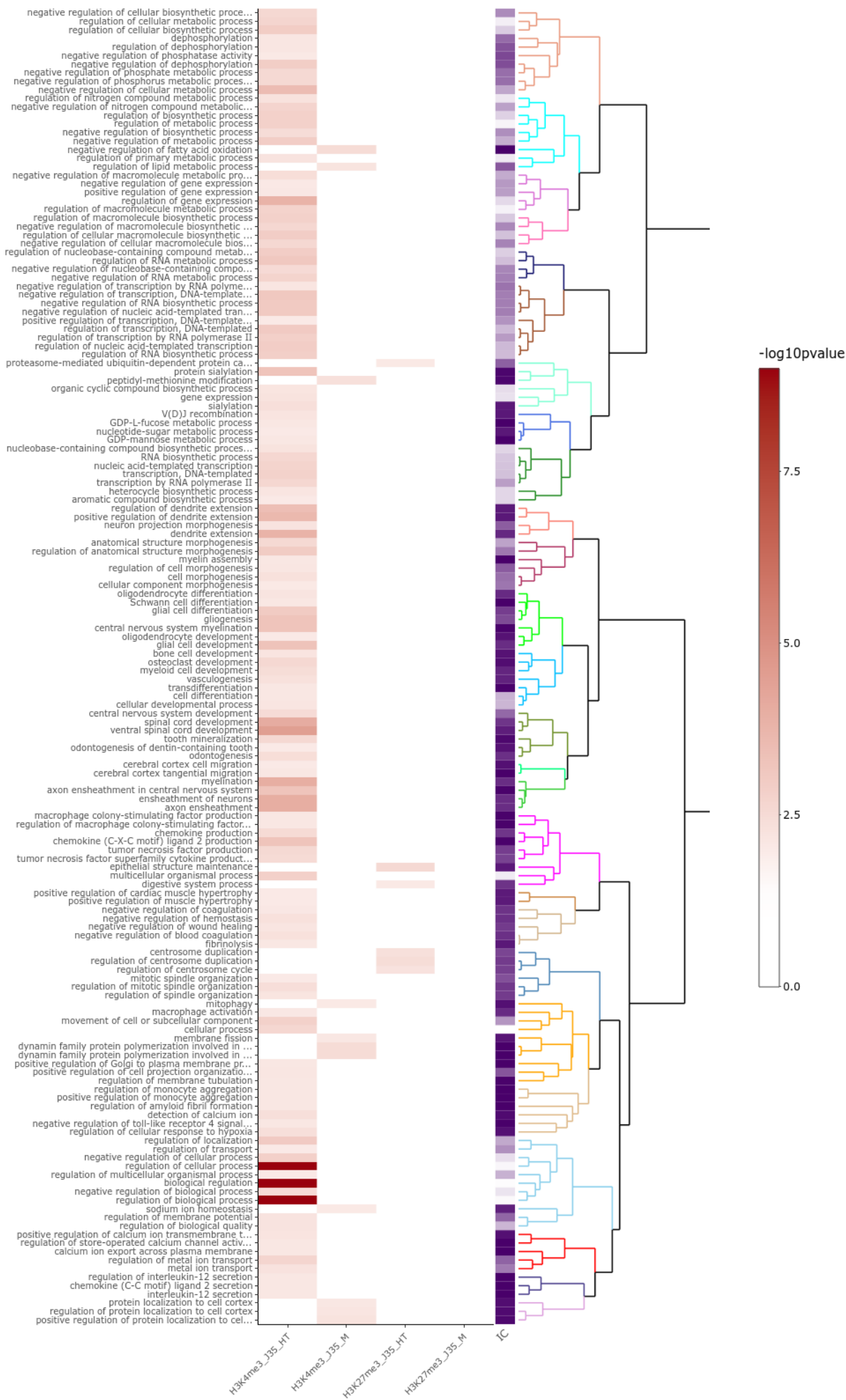
Fig. S4: Functional analysis of hypothalamus and muscle genes bearing H3K4me3 and H3K27me3 differential peaks using a clustering heatmap plot from the ViSEAGO package. From left to right: heatmap of -log10(p-value) of GO terms from functional enrichment tests for each mark and tissue, information content (IC) and dendrogram based on Wang's semantic similarity distance and Ward's clustering criterion. ViSEAGO package is available at https://bioconductor.org/packages/ViSEAGO
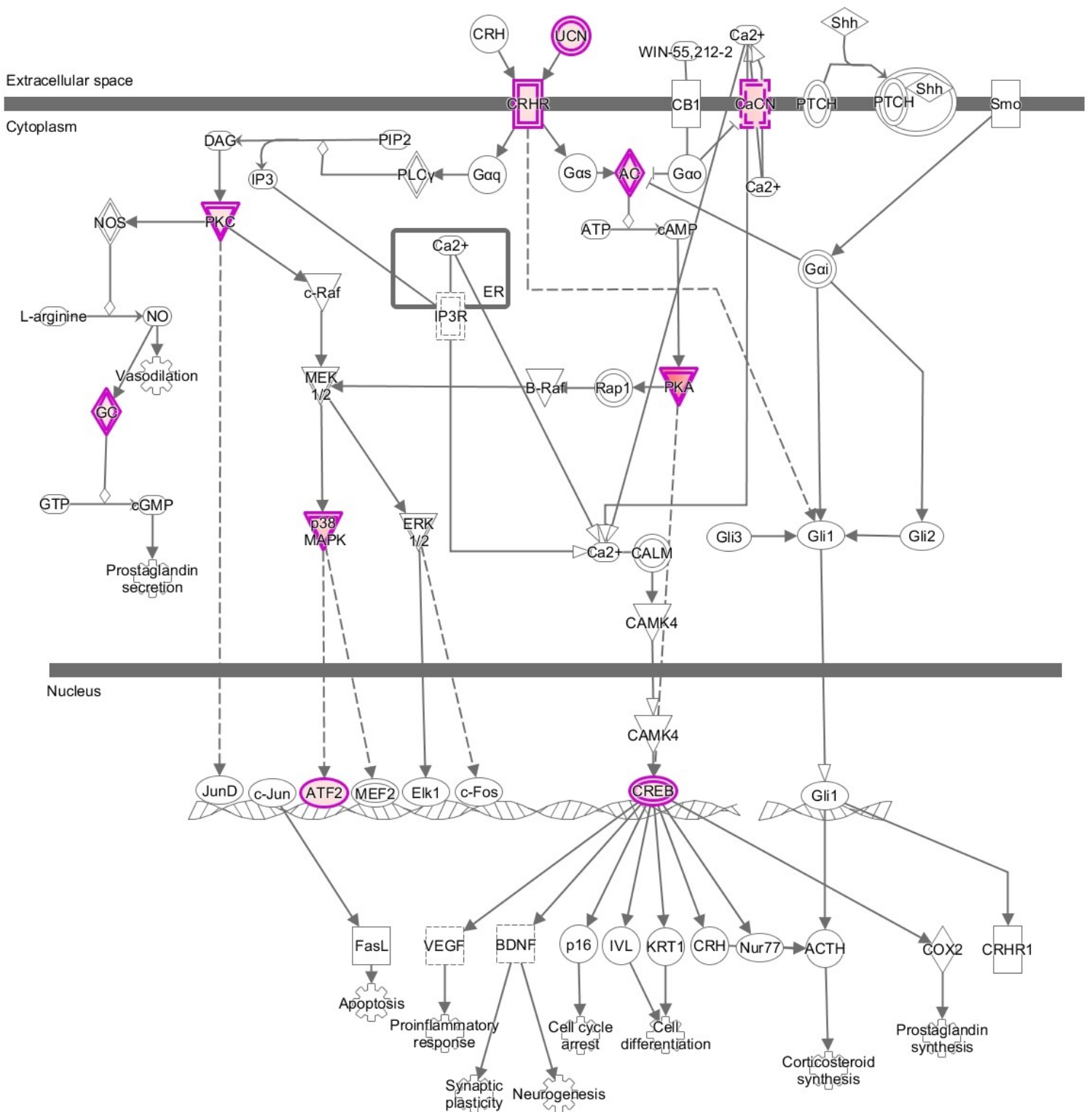
**Fig. S5: IPA canonical pathway of CRH signalling.** Factors underlined in purple correspond to candidates containing DP identified in this study. Pink colouring correspond to an increase in TM condition. Double lines correspond to complexes. Triangles correspond to kinases, ovals to transcription factors, upright rectangles to ion channels, diamond shapes to enzymes, dashed squares to growth factors. Plain arrows correspond to activation/causation, dashed arrows to an indirect interaction. IPA legend can be found at https://mbio.asm.org/content/mbio/suppl/2014/12/11/mBio.02113-14.DCSupplemental/mbo006142092st4.pdf
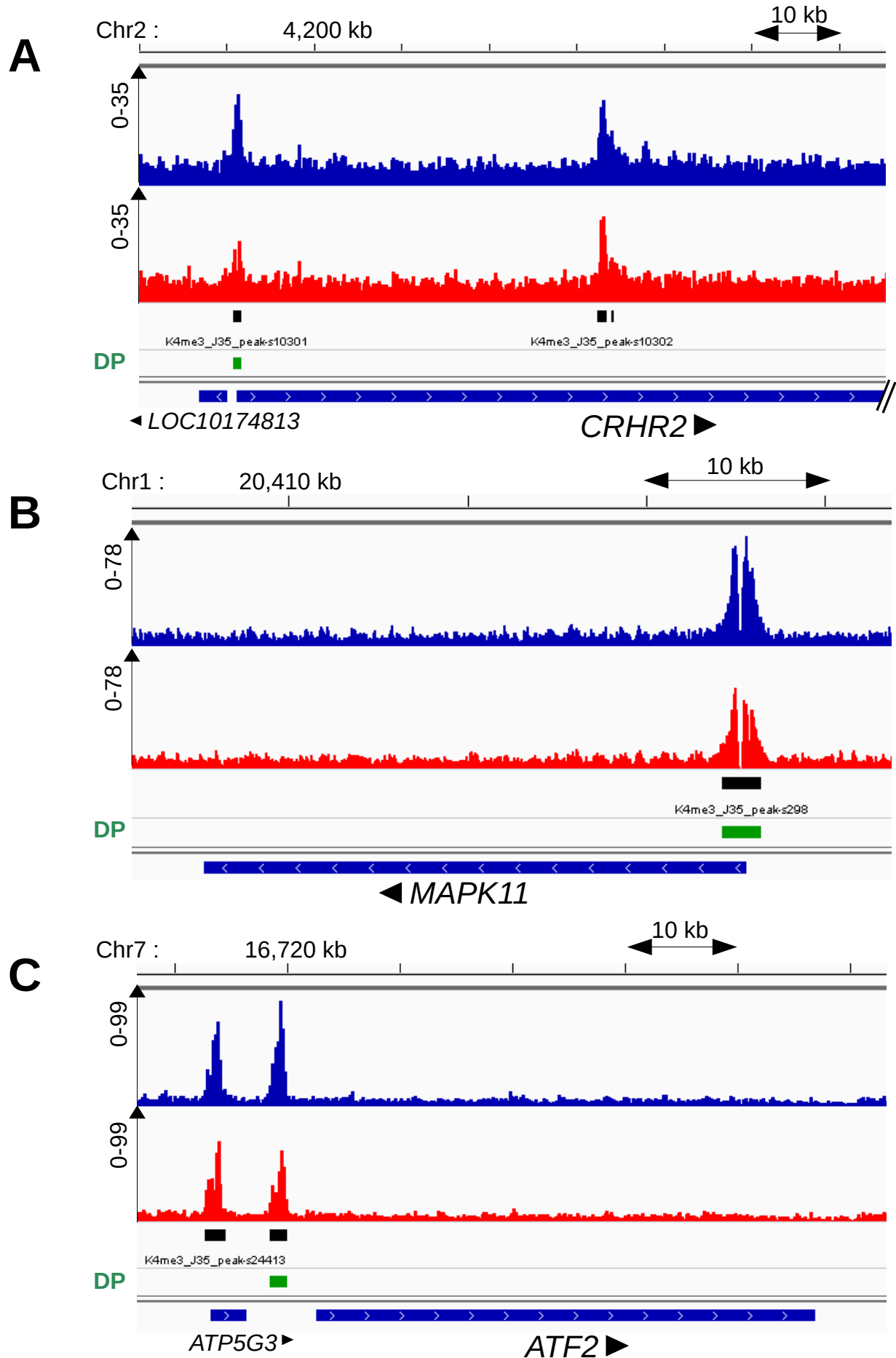
Fig. S6: Chromosome snapshots of hypothalamus H3K4me3 ChIP-seq data at (A) *CRHR2*, **(B)** *MAPK11* **and (C)** *ATF2* **loci using Integrative Genomics viewer (IGV; Broad Institute).** Tracks correspond to normalized and averaged BigWig data. Signal from H3K4me3 (blue for C, red for TM) ChIP-seq is shown. Scales have been grouped for each region using the IGV group autoscale option. Black boxes below the tracks indicate H3K4me3 peak coordinates used in the differential analysis. Green boxes indicate differential peaks (DP).
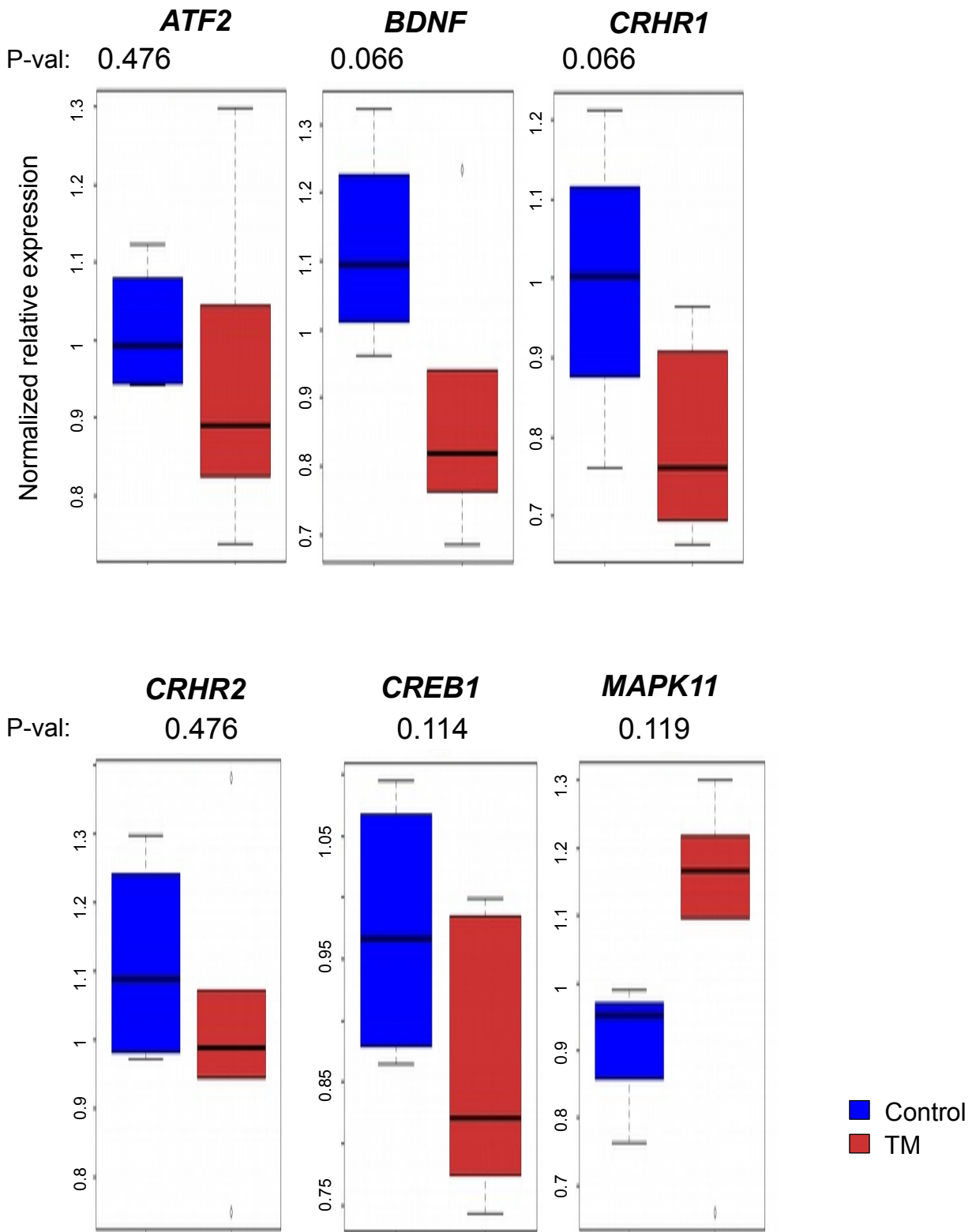
Fig. S7: Relative expression of candidate genes (*ATF2*, *BDNF*, *CRHR1*, *CRHR2*, *CREB1* and *MAPK11*) from the CRH signalling pathway. Control replicates are shown in blue, TM in red. Box boundaries represent the first and third quartiles. The median is indicated by the bold horizontal line dividing the interquartile range. Upper and lower ticks indicate the 10th and 90th percentiles. Outliers are indicate by circles.

MUSCLE   HYPOTHALAMUS

50 kb

ChrZ : 81,400 kb

0-71   0-71   0-96   0-71   0-98   0-98   0-133   0-133

13

12

11
10
9
8
7
6
5

4
3

2

1
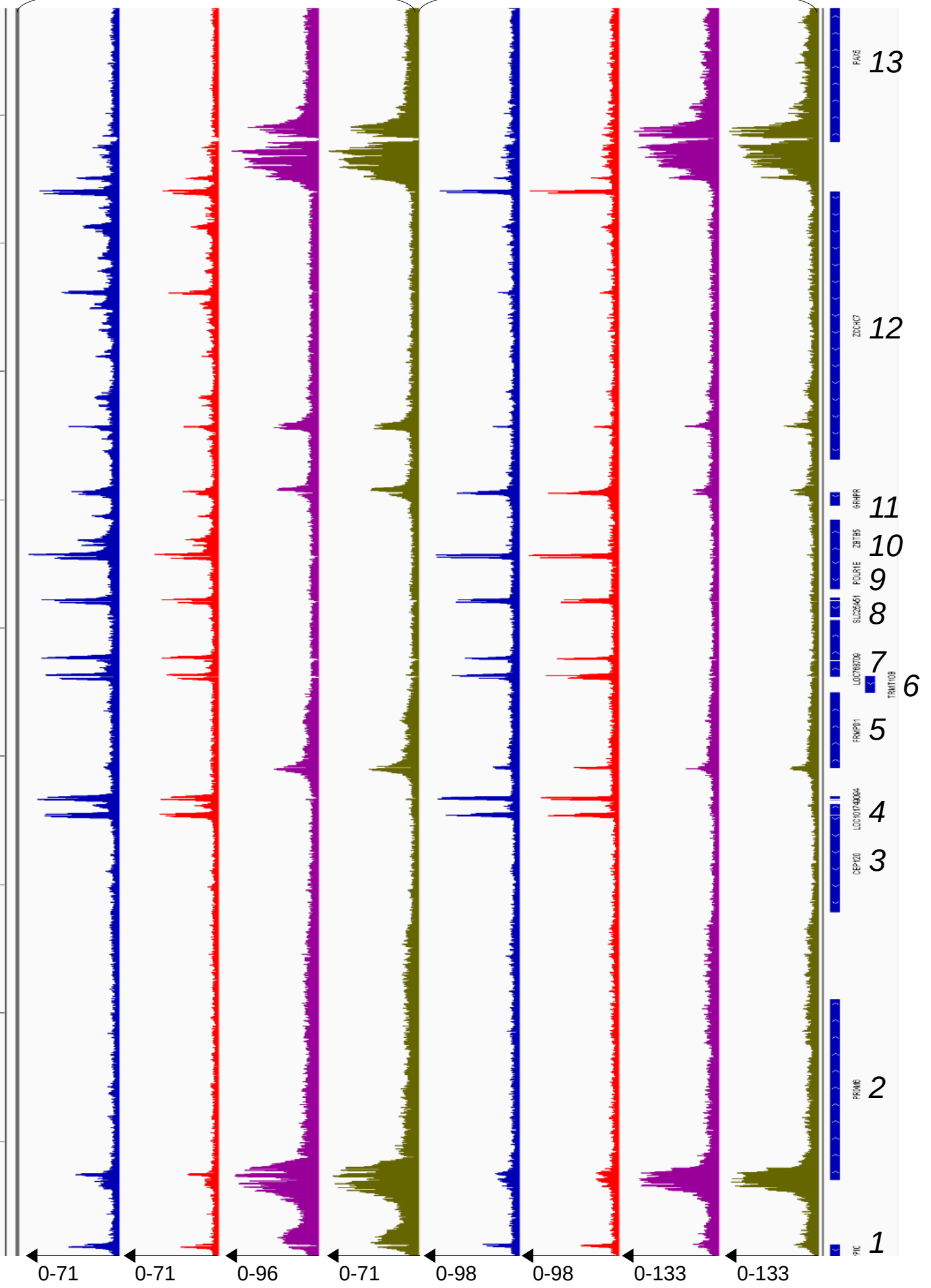
Fig. S8: Chromosome Z snapshot of muscle and hypothalamus H3K4me3 and H3K27me3 ChIP-seq data (chrZ:81,341,460-81,857,046) obtained using Integrative Genomics viewer (IGV; Broad Institute). Tracks correspond to normalized and averaged BigWig data. Signal from H3K4me3 (blue for C, red for TM) and H3K27me3 (purple for C, brown for TM) ChIP-seq is shown. Muscle tracks are shown on the left, hypothalamus tracks on the right. Scales have been grouped for each mark and tissue using the IGV group autoscale option. The plain blue boxes below the tracks indicate genes, correspondence is as following: *1: PPIC; 2: PRDM6; 3: CEP120; 4: LOC101749094; 5: FRMPD1; 6: TRMT10B; 7: LOC768709; 8: SLC25A51; 9: POLR1E; 10: ZBTB5; 11: GRHPR; 12: ZCCHC7; 13: PAX5*. In this region, only one H3K4me3 differential peak is present at the *LOC768709* promoter.