Integrative Analysis Reveals Histone Demethylase LSD1 Regulates RNA Polymerase II Pausing

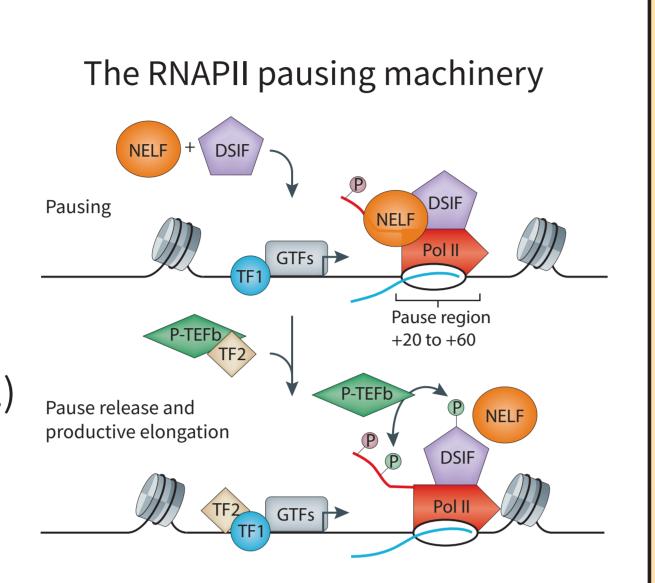
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Background

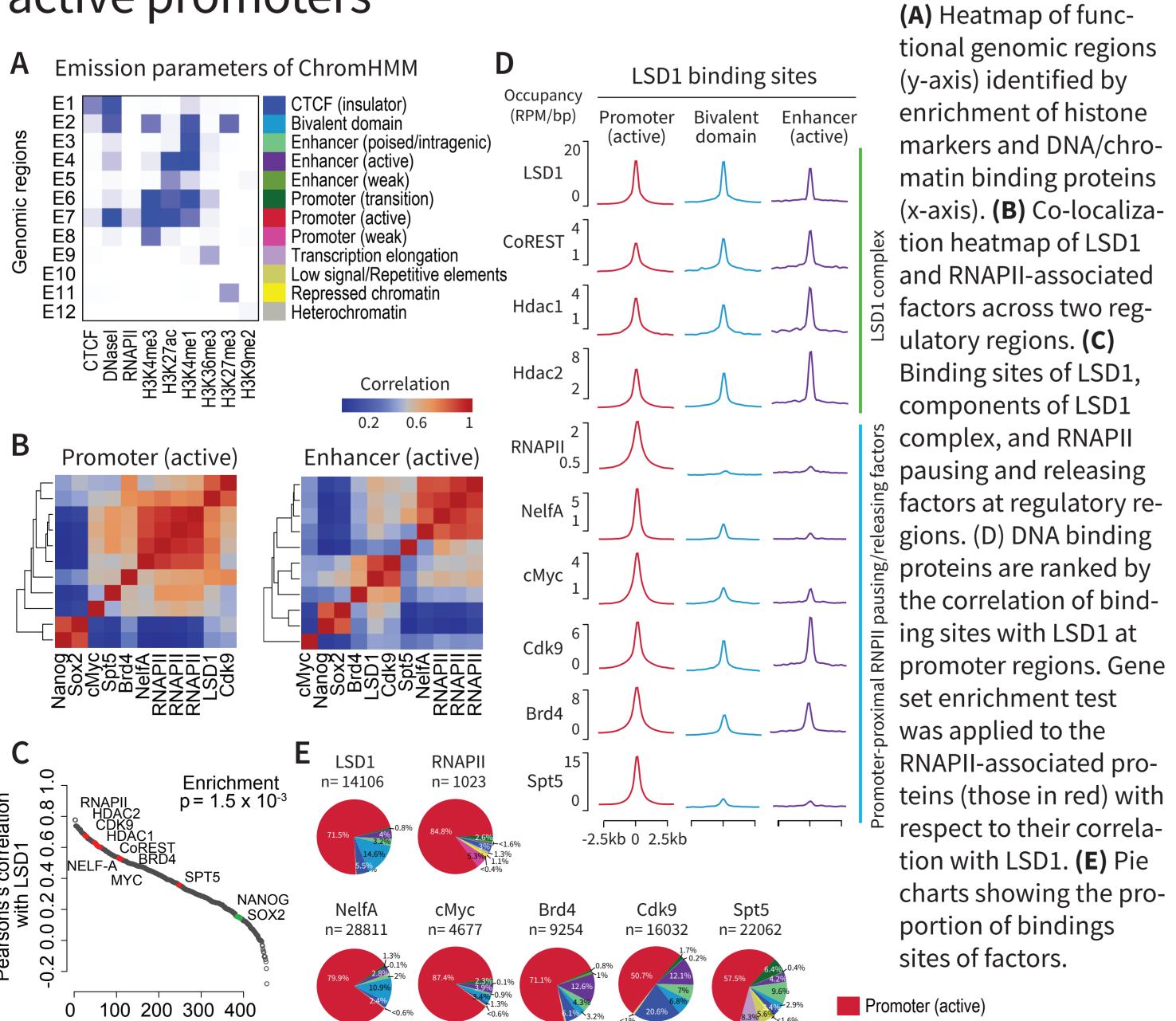
RNA polymerase (RNAPII) pausing at the promoter of genes is a rate-limiting step in transcriptional regulation [1]. The coordinated actions of pausing and releasing factors collectively modulate RNAPII pausing. The involvement of chromatin remodellers such as the histone lysine-specific demethylase 1 (LSD1) in RNAPII pausing has not been well documented. Whilst LSD1 is well-known for its role in decommissioning enhancers during ESC differentiation in mouse (mESC) [2], its role at promoters of genes remains largely unknown.



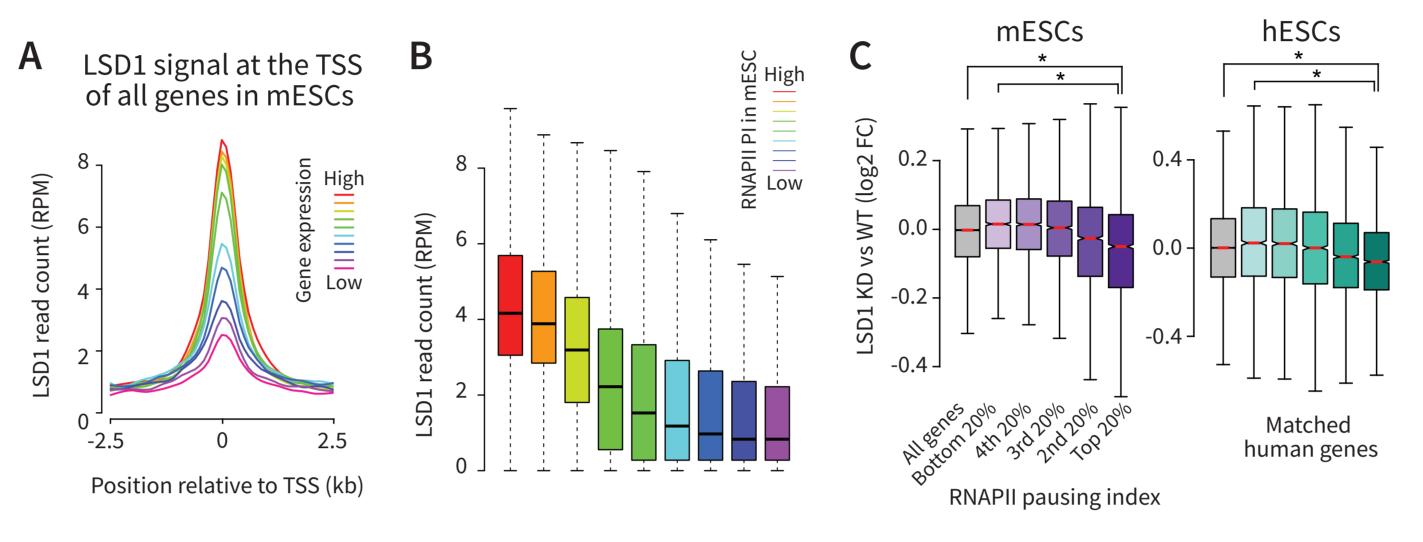
Schematic illustration of pausing and release of RNAP II (adapted from [3]).

Here, we perform an integrative analysis to present evidence for a previously unanticipated role of LSD1 as a regulator of RNAPII pausing through.

LSD1 co-localizes with RNAPII pausing machinery at active promoters

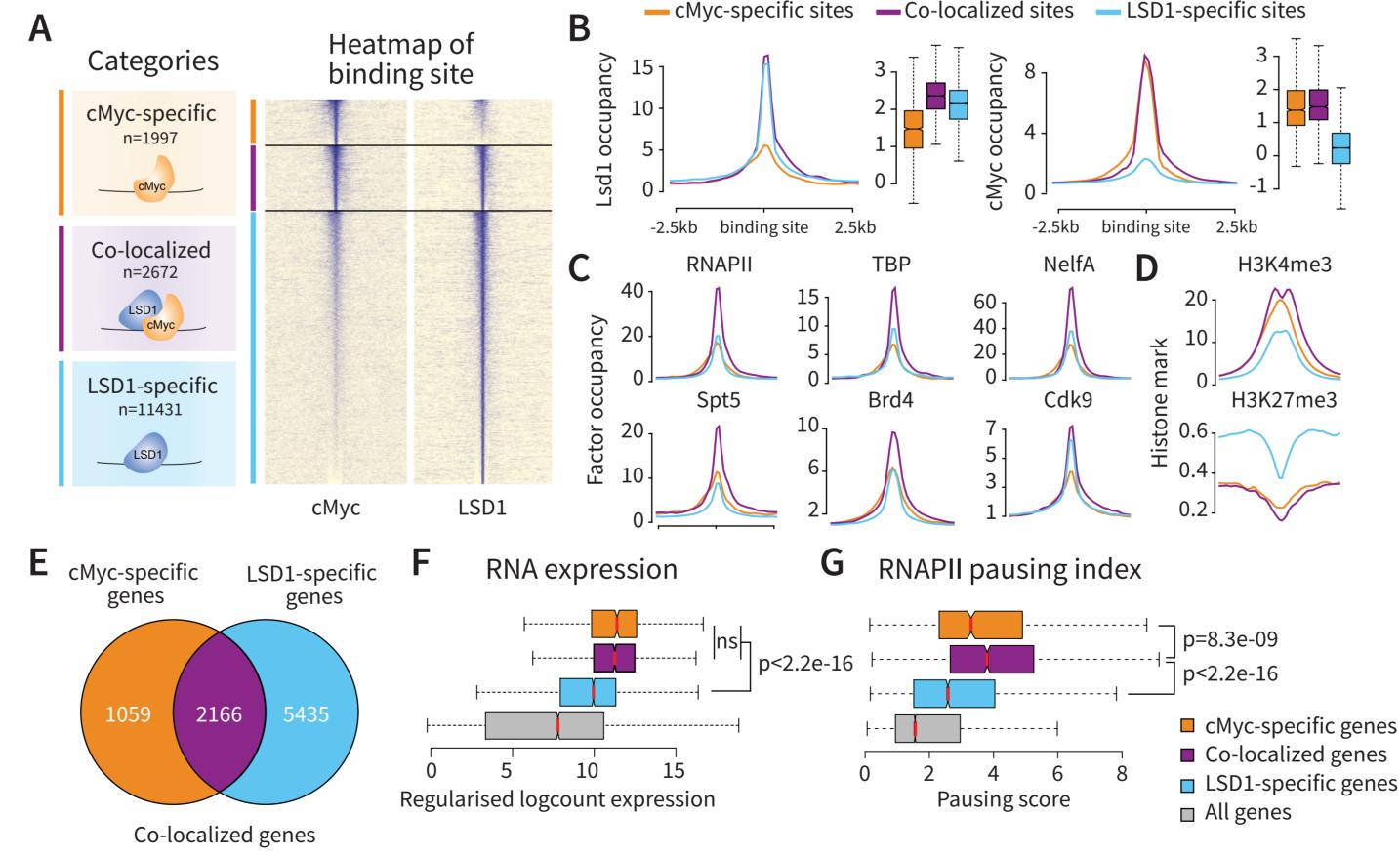


LSD1 knockdown affects genes with higher RNAPII pausing than those with lower pausing



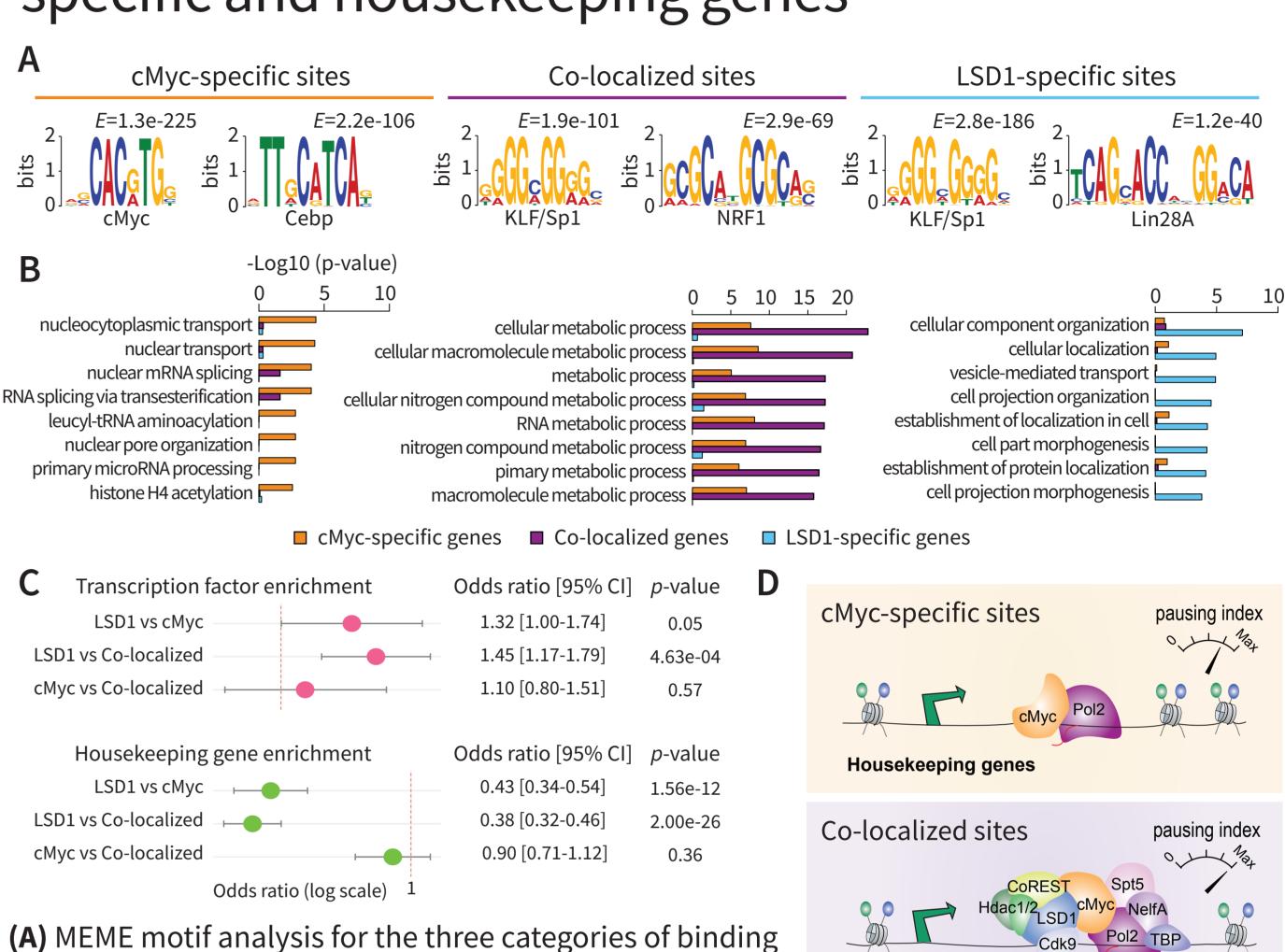
(A) Gene sets were paritioned by expression level in mESC and the level of LSD1 signal (RPM; +/- 1kb around the transcription start stite [TSS]) [2] was plotted for each gene set. (B) Boxplot of LSD1 signal (RPM) at gene promoters grouped according to RNAPII pausing index (PI). (C) Boxplot of log2 fold-change in gene expression after LSD1 knockdown for gene sets grouped by pausing index in mESCs [4] (left) and human ESCs [5] (right). * denotes statistical significance (p < 0.05)

LSD1 and cMyc co-occupied sites are enriched for RNAPII pausing factors



(A) Heatmap showing the overlap in binding sites by the enrichment of either cMyc or LSD1. (B) Density plots and boxplots of cMyc and LSD1 occupancy (RPM) at the three genomic categories. The lines colored by category denote the averaged signal across sites from each category. Density plots of (C) RNAPII machinery and pause/release factors and (D) hsitone marks. (E) Venn diagram showing the overlap of genes by presence of either cMyc or LSD1. Boxplots of (F) regularised and log-transformed RNA expression and (G) RNAPII pausing by each gene set. Statistical analyses were performed using the Wilcox rank-sum test.

LSD1 sites are differentially enriched for cell-type specific and housekeeping genes



sites. The y-axis denotes sequence similarity. **(B)** Over representation analysis of gene set enrichment of the three gene sets. Y-axis dnotes the degree of enrichment in terms of the negative log10 p-value. **(C)** Test of non-independent overlap (Fisher's exact test) of the gene sets with transcription factors (top) and housekeeping genes (bottom). **(D)** Schematic of the differential regulation of genes either bound by LSD1 or cMyc or bound by both.

LSD1-specific sites

Corest
Hdac1/2
LSD1
Po

Cell type-specific genes

H3K4me3
H3K27ac

Housekeeping genes (metabolic genes)

pausing index

Conclusions

Our integrative analysis implicates LSD1 as a regulator of RNAPII pausing at the promoter of genes in mESCs. We propose that LSD1 may modulate the release of paused RNAPII through its association with key pause release factors, Cdk9 and cMyc, and demonstrate the functional enrichment of transcription factors and housekeeping genes by LSD1 and cMyc occupany.

Acknowledgements and References

[1] Core, L. & Adelman, K. Promoter-proximal pausing of RNA polymerase II: A nexus of gene regulation. Genes and Development (2019). [2] Whyte, W. A. et al. Enhancer decommissioning by LSD1 during embryonic stem cell differentiation. Nature (2012). [5] Adelman, K. & Lis, J. T. Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. Nat. Rev. Genet. 13, 720–731 (2012). [6] Foster, C. T. et al. Lysine-Specific Demethylase 1 Regulates the Embryonic Transcriptome and CoREST Stability. Mol. Cell. Biol. (2010). [7] Adamo, A. et al. LSD1 regulates the balance between self-renewal and differentiation in human embryonic stem cells. Nat. Cell Biol. (2011).

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