

➤ Exploring the repertoire of regulatory regions on bovine blood cells methylome

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➤ A bit of context...

---> From farm to fork: boosting animal production

- Population growth (9.7 billion by 2050, rising to 11.2 billion by 2100)¹
- Produce more healthy food with fewer natural resources; reduce environmental impact; preserve biodiversity and adjusting to societal expectations¹
- Changes in breeding strategies > improved ability to accurately use genotype to predict phenotype (for example, disease resistance and adaptation)¹

---> Infectious diseases: a major concern

- Infectious diseases significantly impact cattle production worldwide²
- Many complex traits with genetic basis detangled; lack of information regarding the mechanism behind immune response²
- Increasing focus on the potential relevance of non-genetic variation impact on cattle phenotypes²



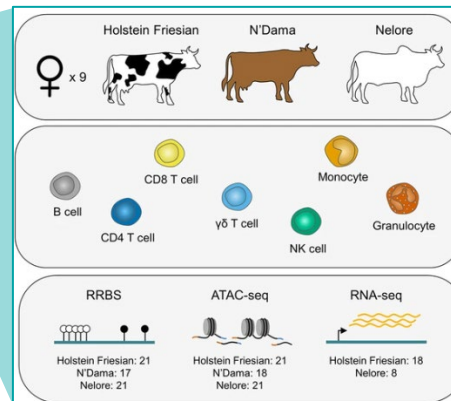
Research | [Open access](#) | Published: 22 May 2023

Profiling the immune epigenome across global cattle breeds

Jessica Powell , Andrea Talenti, Andressa Fisch, Johanneke D. Hemmink, Edith Paxton, Philip Toye, Isabel Santos, Beatriz R. Ferreira, Tim K. Connelley, Liam J. Morrison & James G. D. Prendergast

Genome Biology 24, Article number: 127 (2023) | [Cite this article](#)

4025 Accesses | 7 Citations | 15 Altmetric | [Metrics](#)



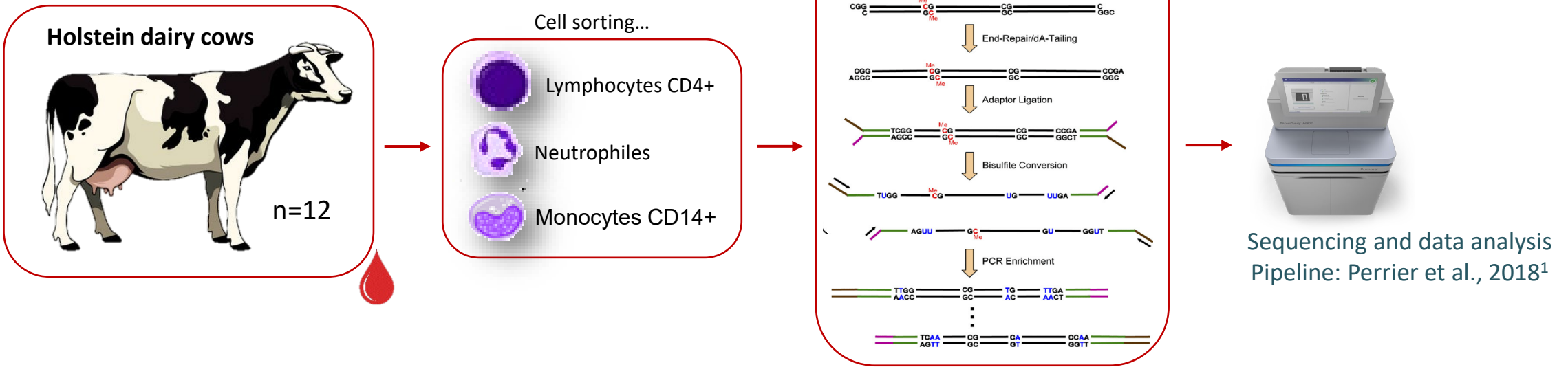
Epigenetic markers in distal regions (outside of gene promoters) better define cell type identity

What about the regulatory landscape of immune/blood cells?



➤ Exploring the repertoire of regulatory regions on bovine blood cells methylome

---> Experimental design (blood cells methylome)



---> Functional annotation

- Mazdak et al., 2023: <https://doi.org/10.1093/g3journal/jkad108> (CAGE experiment – TSS and TSS enhancer; 24 tissue types)
- Kern et al., 2021: <https://doi.org/10.1038/s41467-021-22100-8> (ATAC- and ChIP-Seq - chromatin states; 8 tissues)

BovReg
Understanding cattle genomes

G3
Genes | Genomes | Genetics

G3, 2023, 13(6), jkad108
<https://doi.org/10.1093/g3journal/jkad108>
Advance Access Publication Date: 22 May 2023
Investigation

Improving the annotation of the cattle genome by annotating transcription start sites in a diverse set of tissues and populations using Cap Analysis Gene Expression sequencing

Mazdak Salavati¹, Richard Clark², Doreen Becker³, Christa Kühn^{3,4}, Graham Plastow⁵, Sébastien Dupont⁶, Gabriel Costa Monteiro Moreira⁶, Carole Charlier^{6,7}, Emily Louise Clark⁶, on behalf of the BovReg consortium

nature COMMUNICATIONS

Functional annotations of three domestic animal genomes provide vital resources for comparative and agricultural research

Colin Kern¹, Ying Wang¹, Xiaolin Xu¹, Zhangyuan Pan¹, Michelle Halstead¹, Ganrea Chanthavixay¹, Perot Saelao¹, Susan Waters¹, Ruidong Xiang^{2,3}, Amanda Chamberlain³, Ian Korf⁴, Mary E. Delany¹, Hans H. Cheng⁵, Juan F. Medrano¹, Alison L. Van Eenennaam¹, Chris K. Tuggle⁶, Catherine Ernst⁷, Paul Flicek⁸, Gerald Quone⁹, Pablo Ross¹⁰ & Huaijun Zhou¹⁰

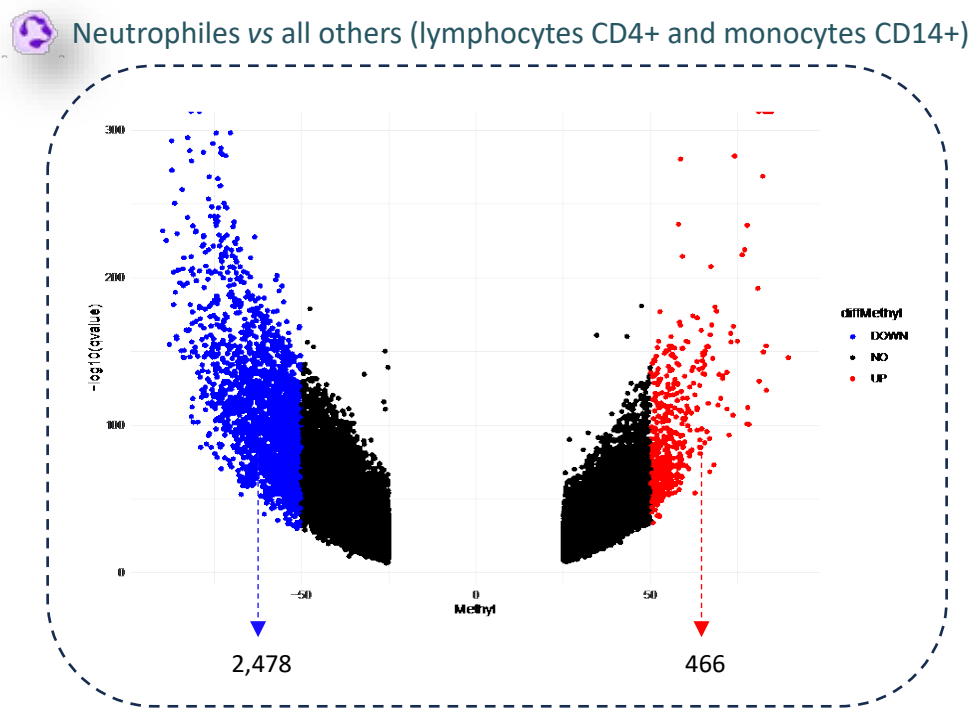
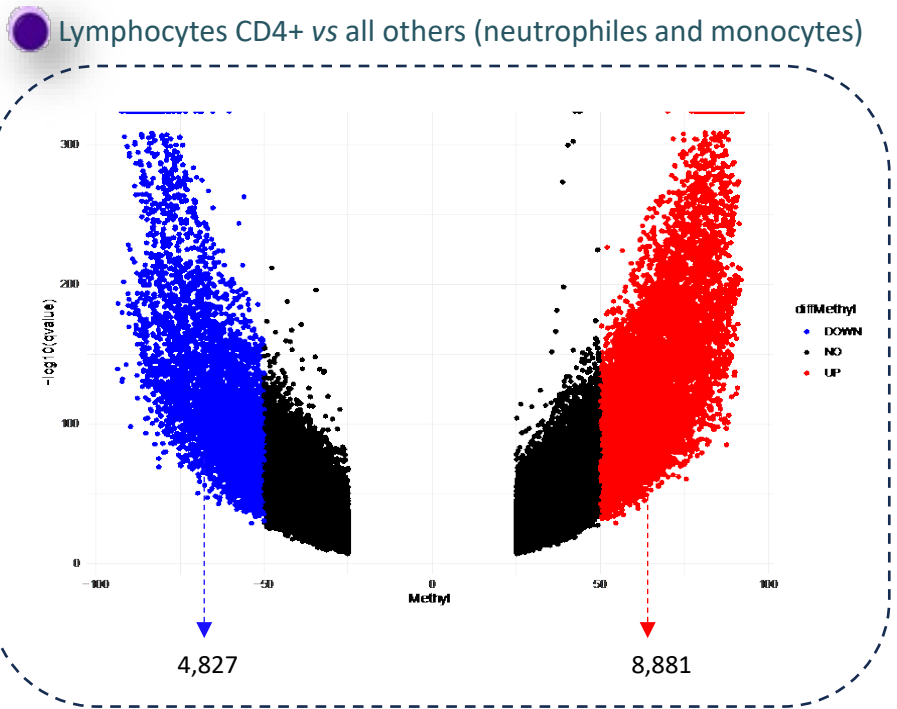
> Exploring the repertoire of regulatory regions on bovine blood cells methylome

→ Differentially methylated sites (DMC)



Pipeline: Perrier et al., 2018¹
- commonly presented in all the animals
- no overlap with SNPs (1,000 Bulls file)
- methylKit ($\Delta\text{meth} \geq 25\%$; $\text{padj} < 1\%$)

Dr. Valentin Costes



- Hypermethylation in Lymphocytes (CD4+) compared to neutrophils and monocytes (CD14+)
- Hypomethylation in neutrophils compared to lymphocytes(CD4+) and monocytes(CD14+)

¹ Perrier et al., 2018: <https://doi.org/10.1186/s12864-018-4764-0>

> Exploring the repertoire of regulatory regions on bovine blood cells methylome

---> Differentially methylated sites (DMC)

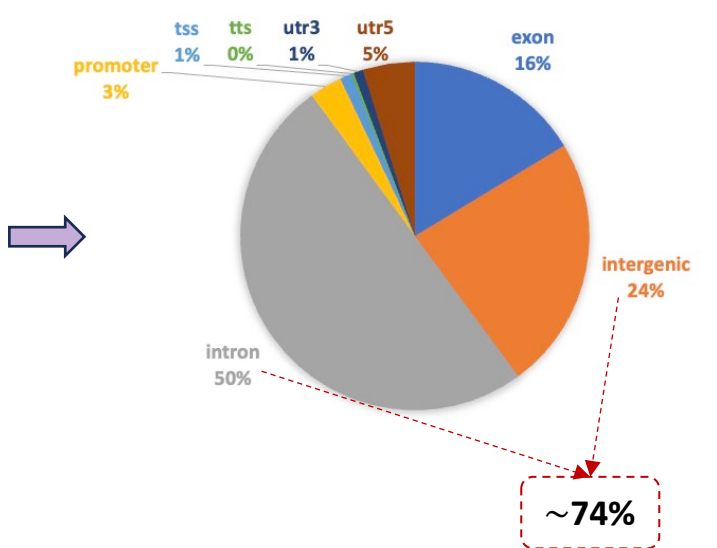
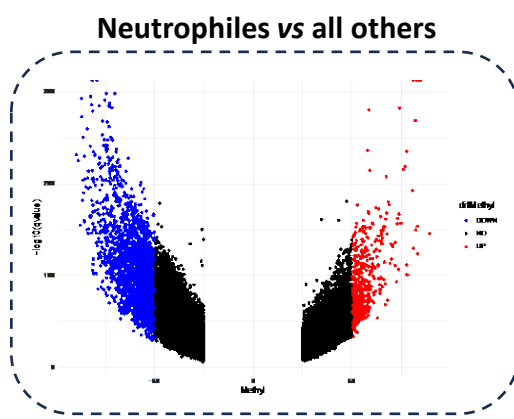
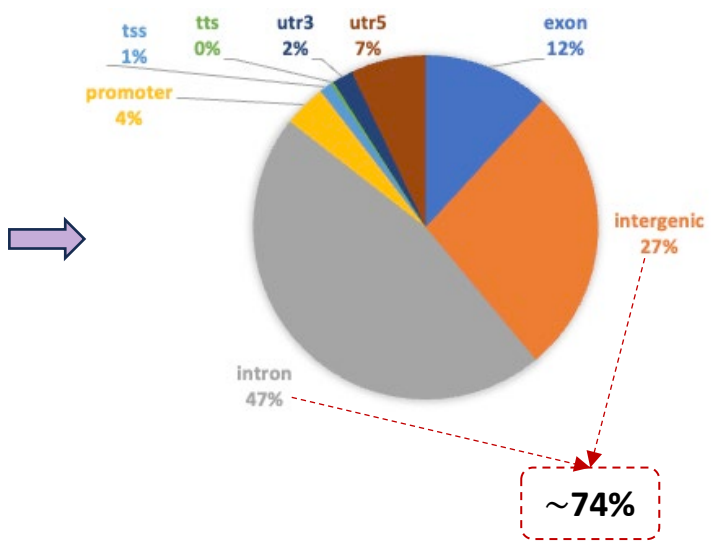
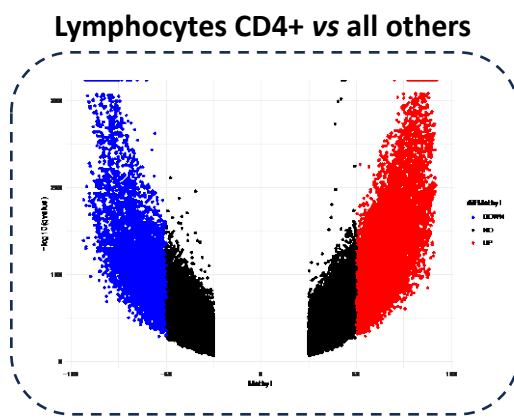


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Dr. Valentin Costes

In agreement with the literature,
~74% of the DMCs are located in
intronic/intergenic regions which
can harbour regulatory elements!

*What about the regulatory regions in
bovine genome?*



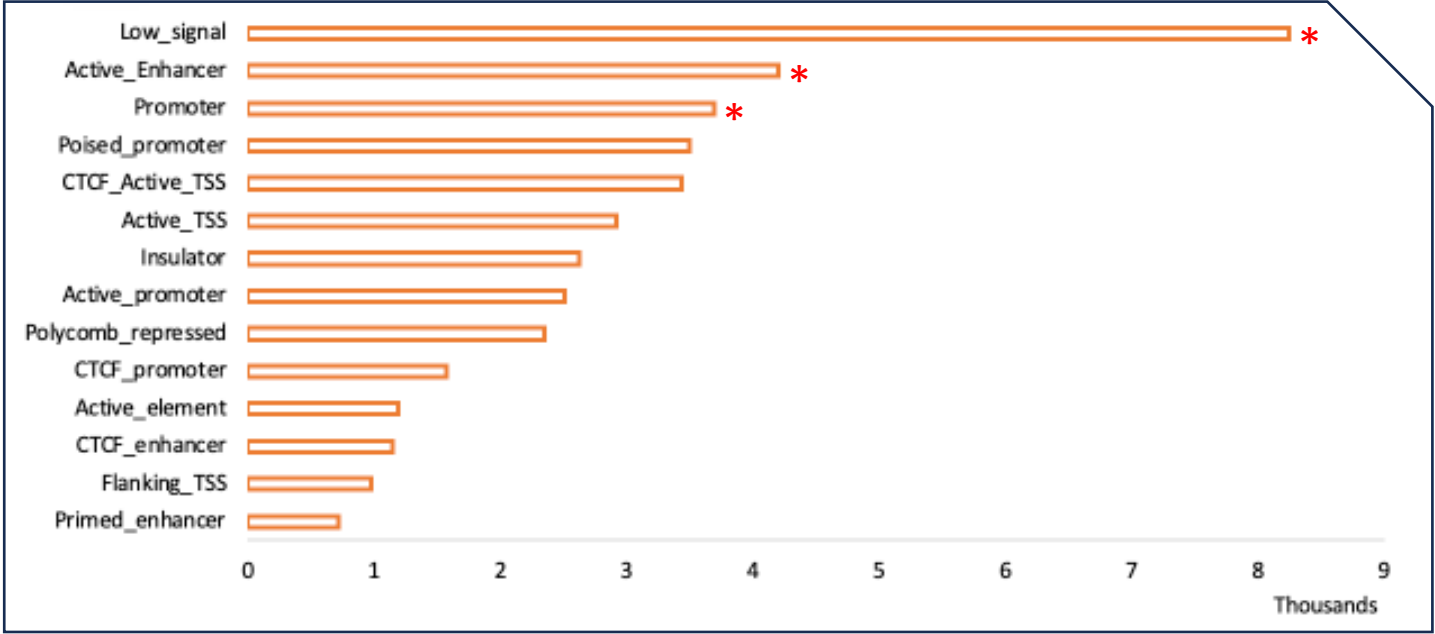
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September 3th, 2024

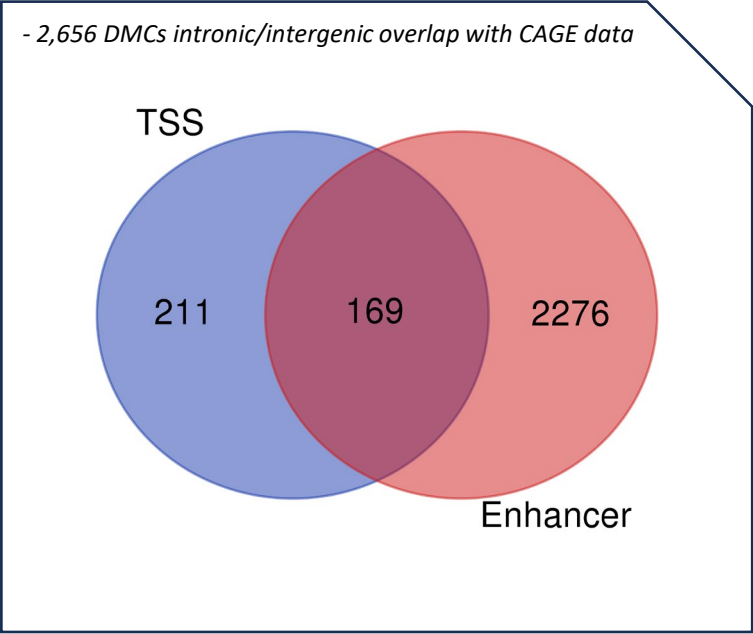
¹ Perrier et al., 2018: <https://doi.org/10.1186/s12864-018-4764-0>

➤ Exploring the repertoire of regulatory regions in lymphocytes CD4+

 Lymphocytes CD4+ vs all others (10,078 DMCs intronic/intergenic - 74%)



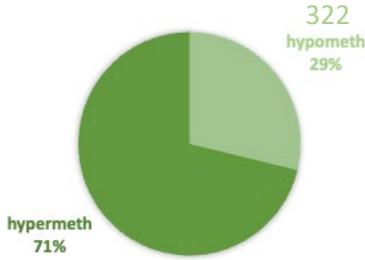
Kern et al., 2021¹



BovReg CAGE; Mazdak et al., 2023²

- Intronic/intergenic DMCs overlapping promoter associated states/CAGE tags: unannotated genes/isoforms?
- Intronic/intergenic DMCs with a pronounced overlap with active enhancer annotation (are they hypomethylated in Lymphocytes?)

1,115 intronic/intergenic DMCs overlapped active enhancers supported by both datasets



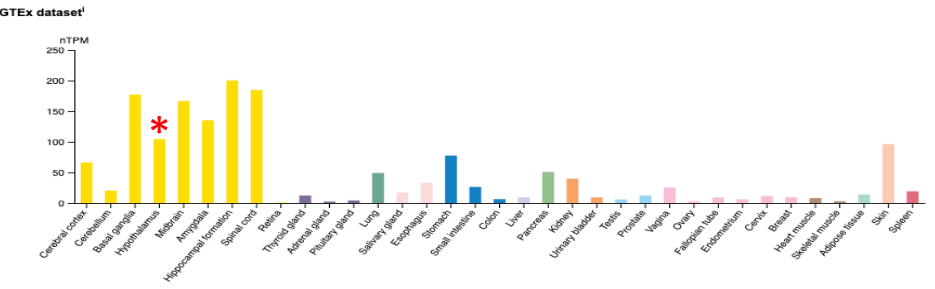
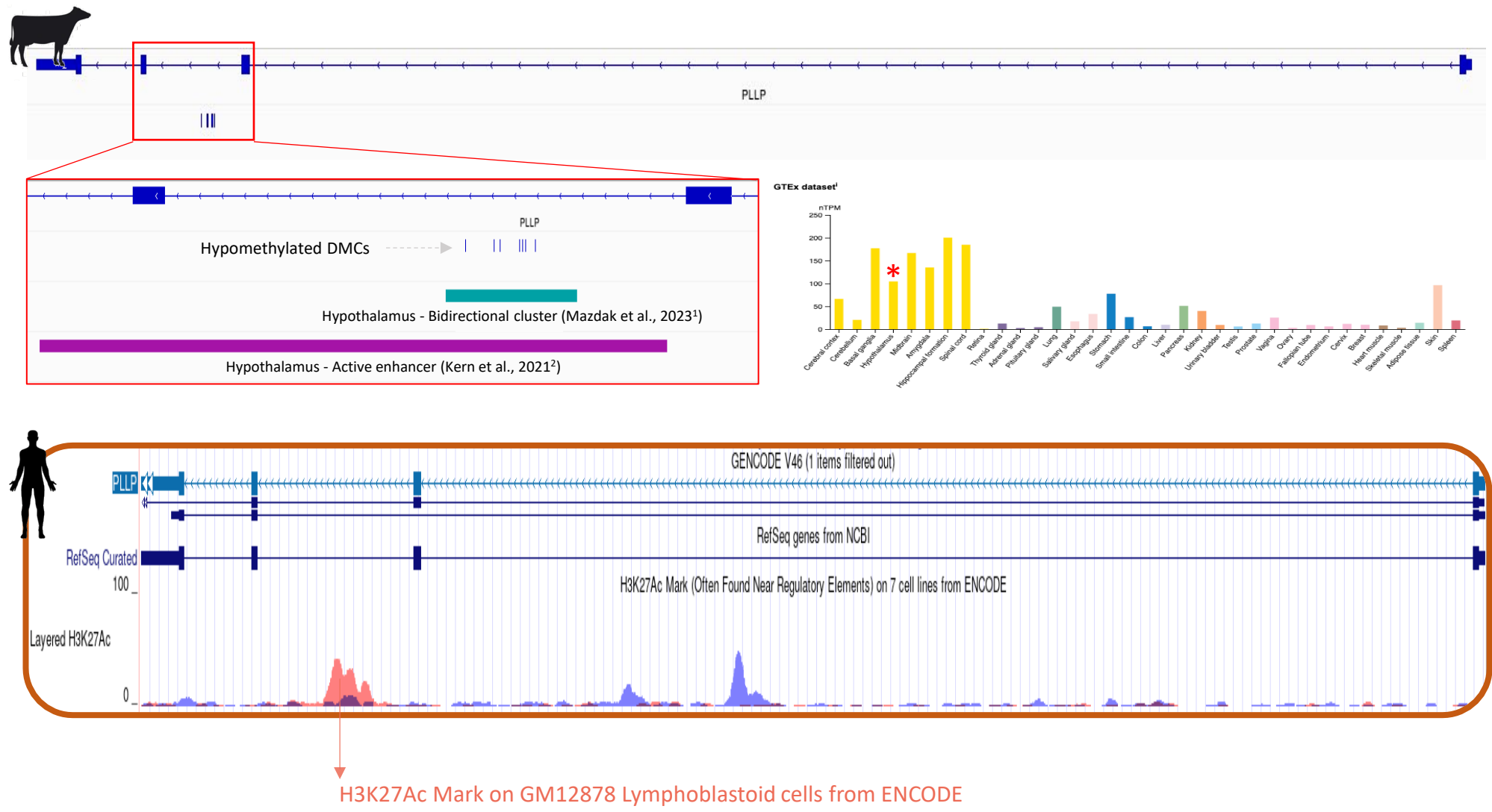
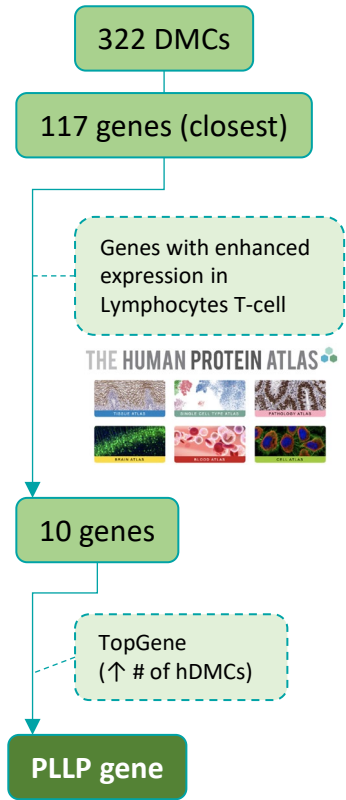
Are they close to genes with expression enriched in lymphocytes?



¹ Kern et al., 2021: <https://doi.org/10.1038/s41467-021-22100-8>; ² Mazdak et al., 2023: <https://doi.org/10.1093/g3journal/jkad108>

> Exploring the repertoire of regulatory regions in lymphocytes CD4+

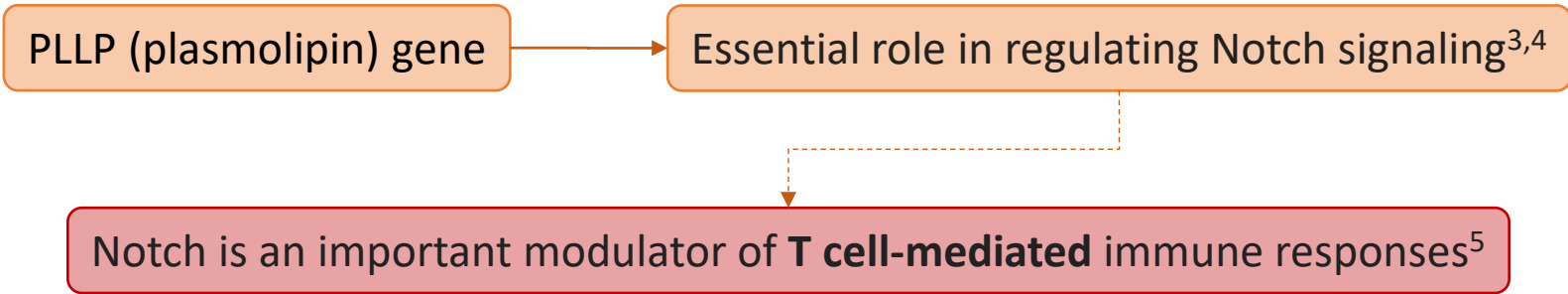
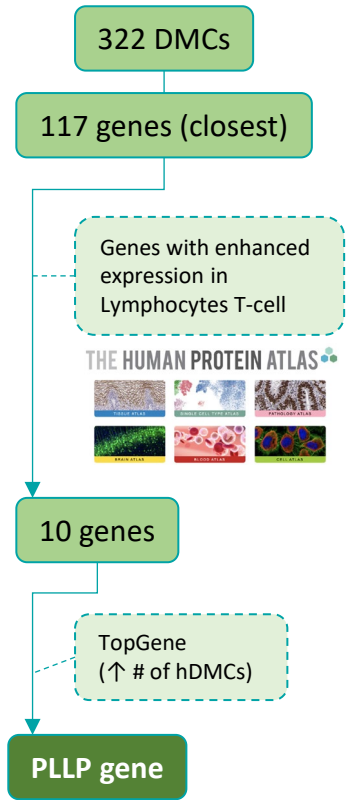
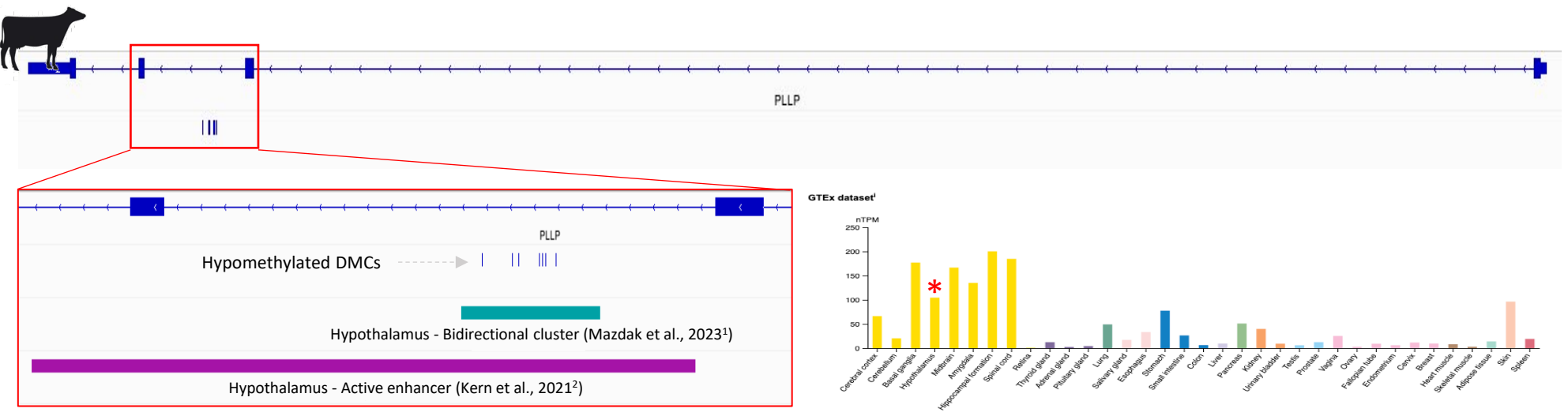
 Lymphocytes CD4+ vs all others (**322** intronic/intergenic hypomethylated DMCs)



> Exploring the repertoire of regulatory regions in lymphocytes CD4+



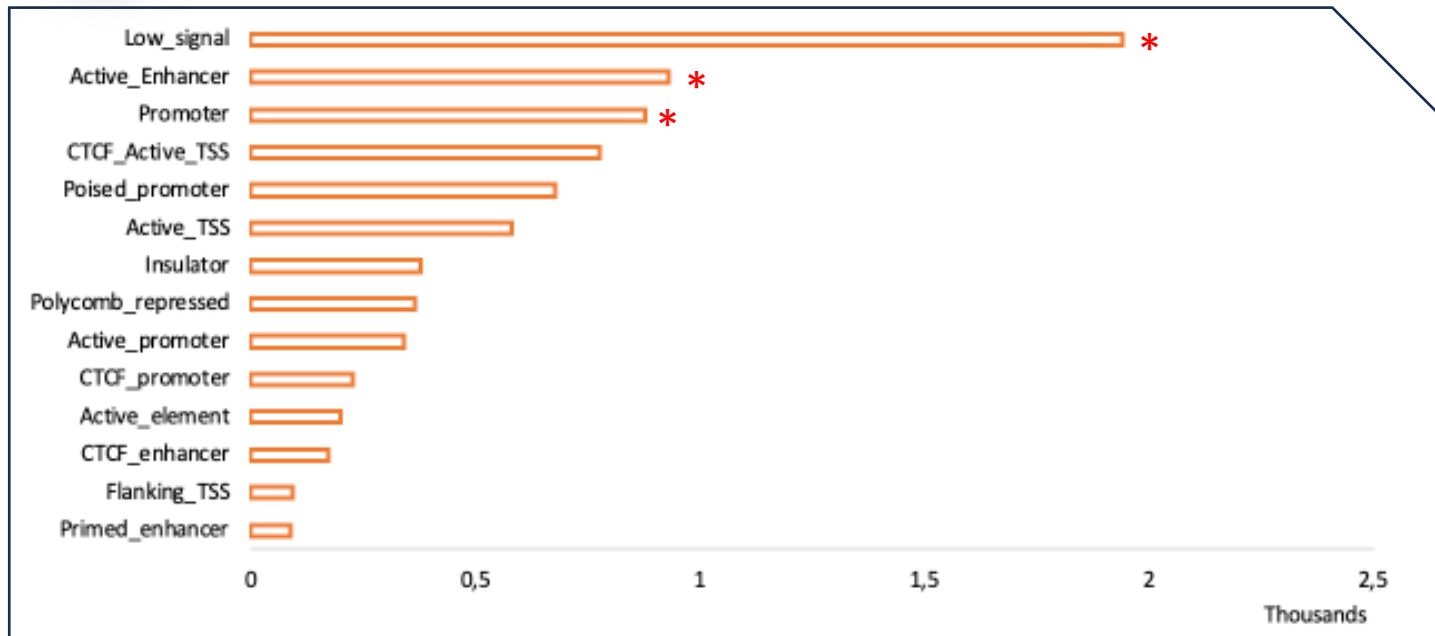
Lymphocytes CD4+ vs all others (**322** intronic/intergenic hypomethylated DMCs)



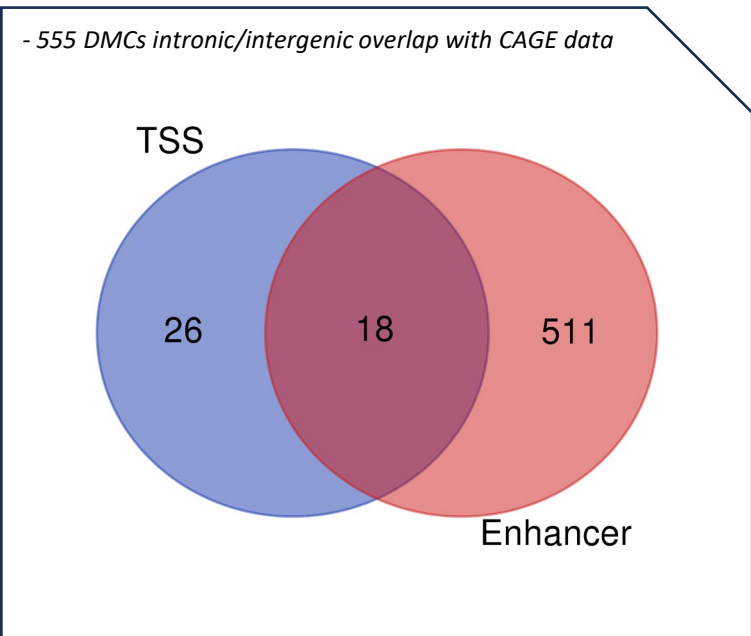
> Exploring the repertoire of regulatory regions in neutrophils



Neutrophils vs all others (2,166 DMCs intronic/intergenic - 74%)



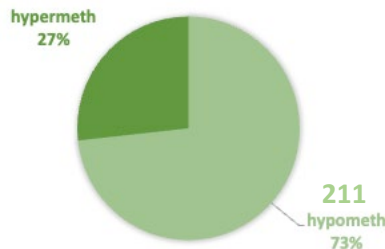
Kern et al., 2021¹



BovReg CAGE; Mazdak et al., 2023²

- Intronic/intergenic DMCs overlapping promoter associated states/CAGE tags: unannotated genes/isoforms?
- Intronic/intergenic DMCs with a pronounced overlap with active enhancer annotation (are they hypomethylated in Neutrophils?)

288 intronic/intergenic DMCs overlapped active enhancers supported by both datasets



Are they close to genes with expression enriched in neutrophils?



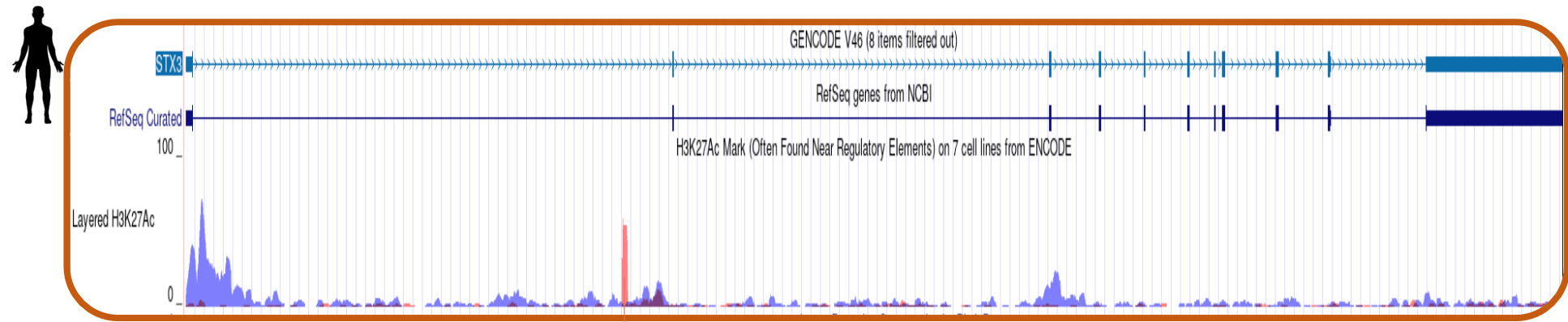
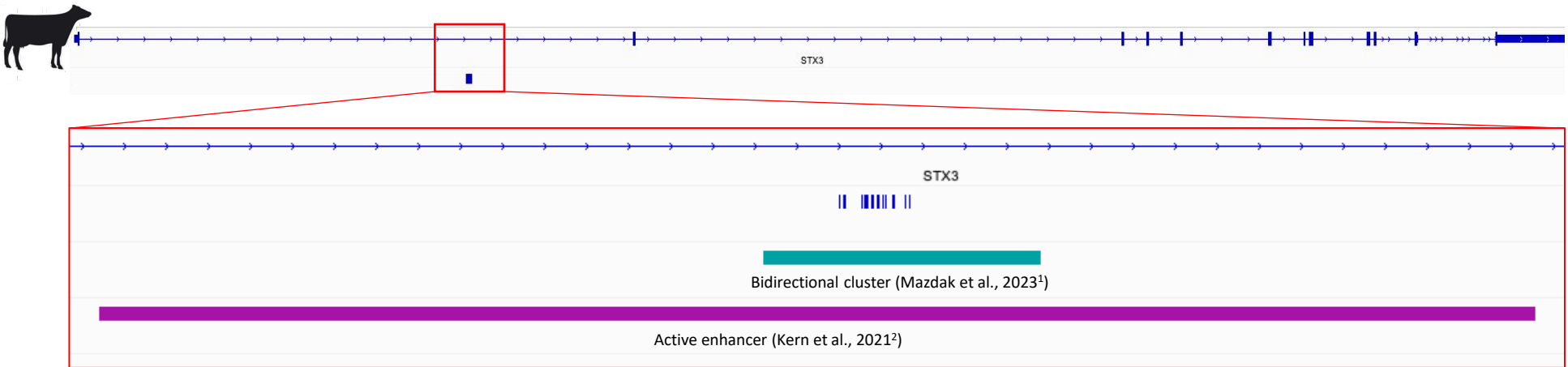
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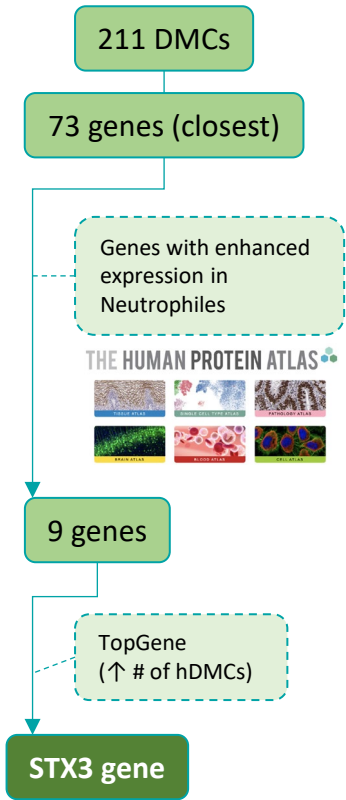
> Exploring the repertoire of regulatory regions in neutrophils



Neutrophils vs all others (**211** intronic/intergenic hypomethylated DMCs)



H3K27Ac Mark on GM12878 Lymphoblastoid cells from ENCODE

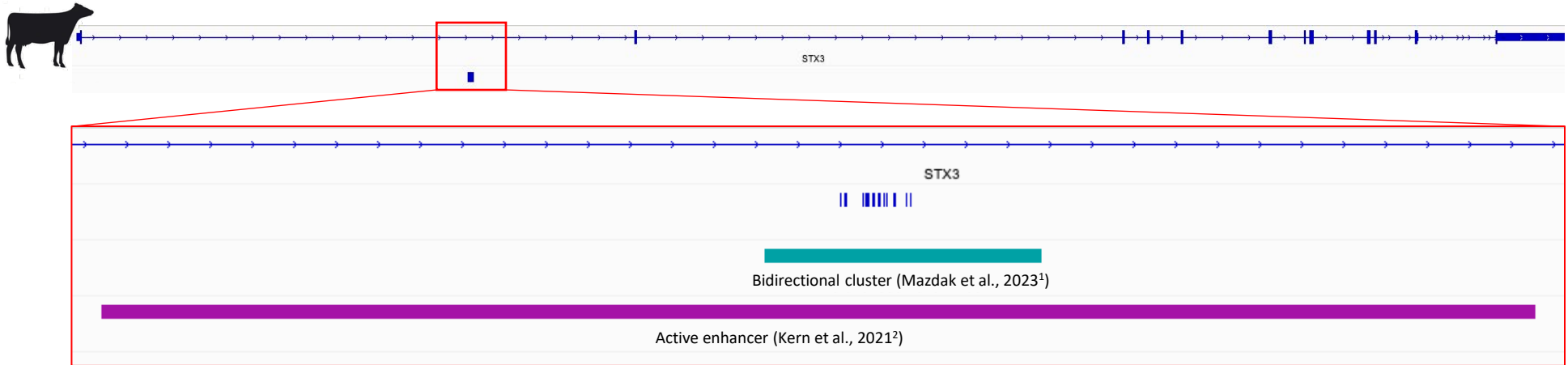


¹ Mazdak et al., 2023: <https://doi.org/10.1093/g3journal/jkad108>; ² Kern et al., 2021: <https://doi.org/10.1038/s41467-021-22100-8>

> Exploring the repertoire of regulatory regions in neutrophils



Neutrophils vs all others (**211** intronic/intergenic hypomethylated DMCs)



STX3 (Syntaxin 3) gene

Essential role in trafficking pathways of cytokines in neutrophil granulocytes³

Neutrophil-derived cytokines: immunoregulatory cytokines⁴

211 DMCs

73 genes (closest)

Genes with enhanced expression in Neutrophils

THE HUMAN PROTEIN ATLAS



9 genes

TopGene (↑ # of hDMCs)

STX3 gene



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¹ Mazdak et al., 2023: <https://doi.org/10.1093/g3journal/jkad108>; ² Kern et al., 2021: <https://doi.org/10.1038/s41467-021-22100-8>;

³ Naegelen et al., 2015: 10.1189/jlb.3A0514-254RR; ⁴ Tecchio et al., 2014: <https://doi.org/10.3389/fimmu.2014.00508>

> Exploring the repertoire of regulatory regions on bovine blood cells methylome

---> Take home messages

- Epigenetic markers in distal regions (outside of gene promoters) help to better define cell type identity
- Integration of functional annotation helps to refine the detection of marks reflecting cell type identity
- Multi-omics approach: how the genome adapts? Uncovering mechanism behind immune response...

SPOILER ALERT!!!

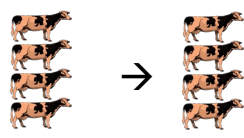


Heat stress induces a loss of DNA methylation at specific loci of genome

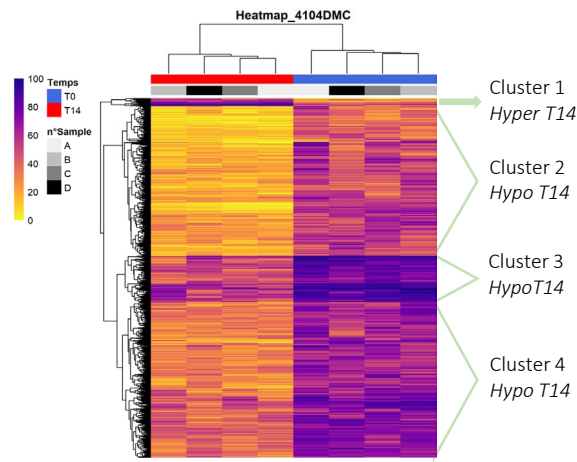
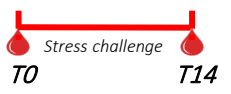
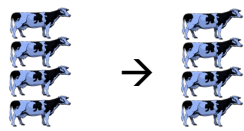
Gabriel Costa Monteiro Moreira, Alexis Ruiz González, Mélodie Joigner, Valentin Costes, Francesca Ali, Hélène Kiefer, Hélène Jammes, Daniel Rico



THI 73-82



THI 61-64



- > ~4k DMCs hypomethylated
- > Hypomethylation of important regulatory regions (e.g. enhancers)
- > **Changes in methylation profile of cell type specific regulatory regions**
- > Impact on immune system!

> Exploring the repertoire of regulatory regions on bovine blood cells methylome

→ Take home messages

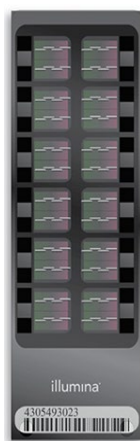
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Interrogating methylome in large cohort animals! It includes probes that interrogates regulatory elements!

RumiGen

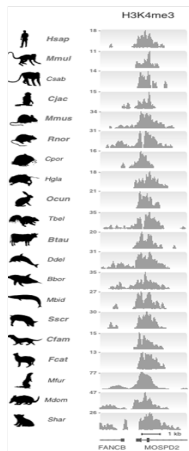
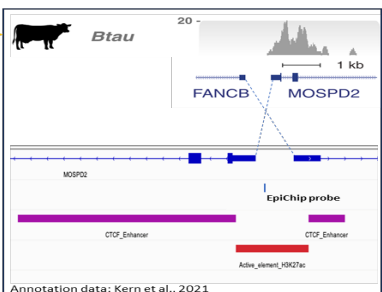
Coming soon
(March 2025)



EpiChIP



Cell **Article**
Enhancer Evolution across 20 Mammalian Species
Diego Villar,^{1,2} Camille Berthelot,^{1,2} Sarah Aldridge,^{1,2} Tim F. Rayner,¹ Margus Luik,¹ Miguel Pignatelli,² Thomas J. Park,¹ Robert Deaville,¹ Jonathan T. Erichsen,² Anna J. Jasinska,¹ James M.A. Turner,¹ Mads F. Bortelsen,¹ Elizabeth P. Murchison,¹ Paul Flicek,^{1,2} and Duncan T. Odum,^{1,2}



Functional epigenetics!!
Epigenome editing...

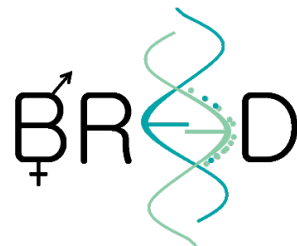
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Thank you for your attention!

MECP2 team

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