Leibniz Institute on Aging –
Fritz Lipmann Institute

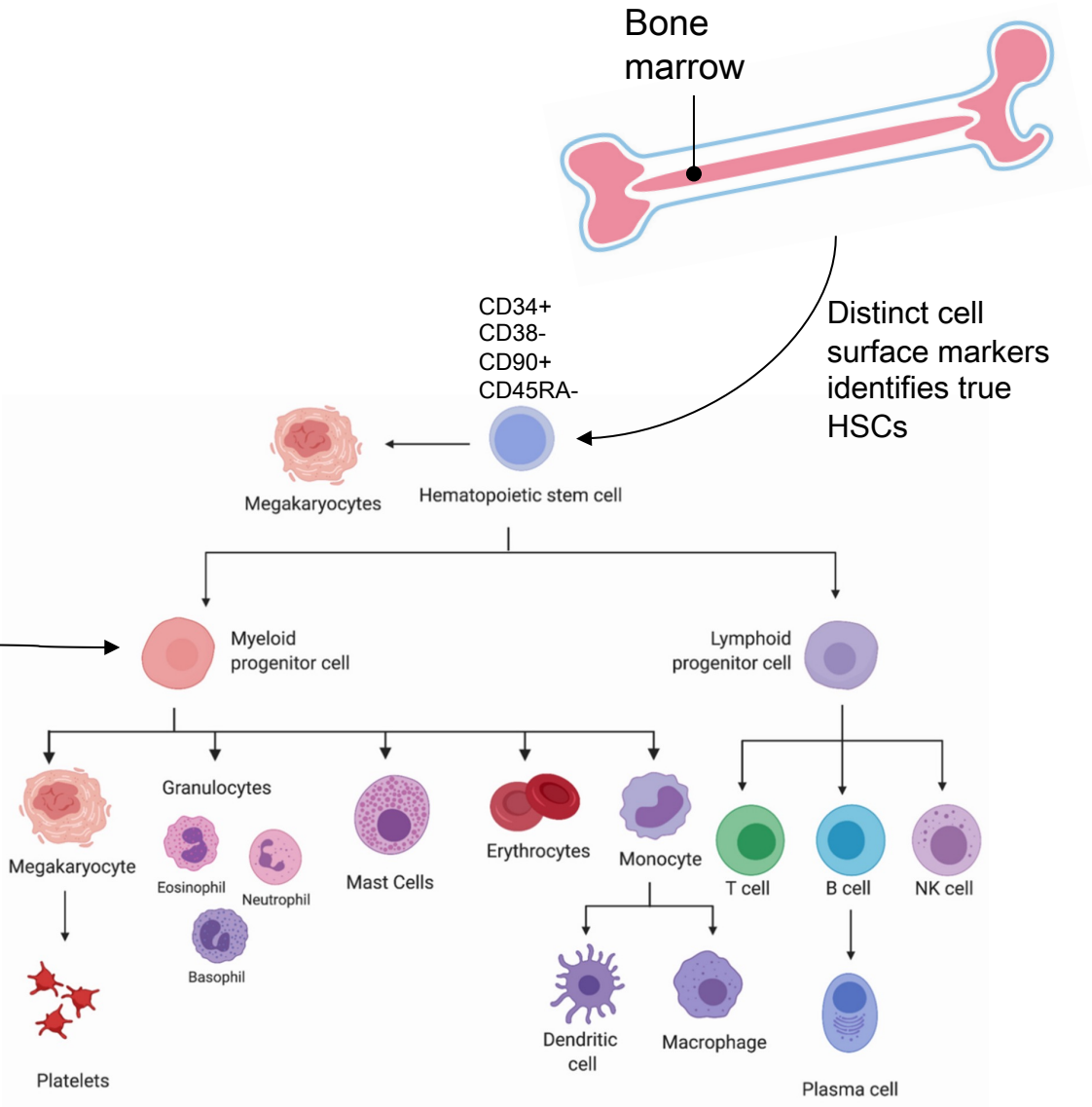
Stem Cell Aging – CUT&Tag Data Analysis

39th TBI Winterseminar in Bled
Atakan Ayden – 15.02.2024

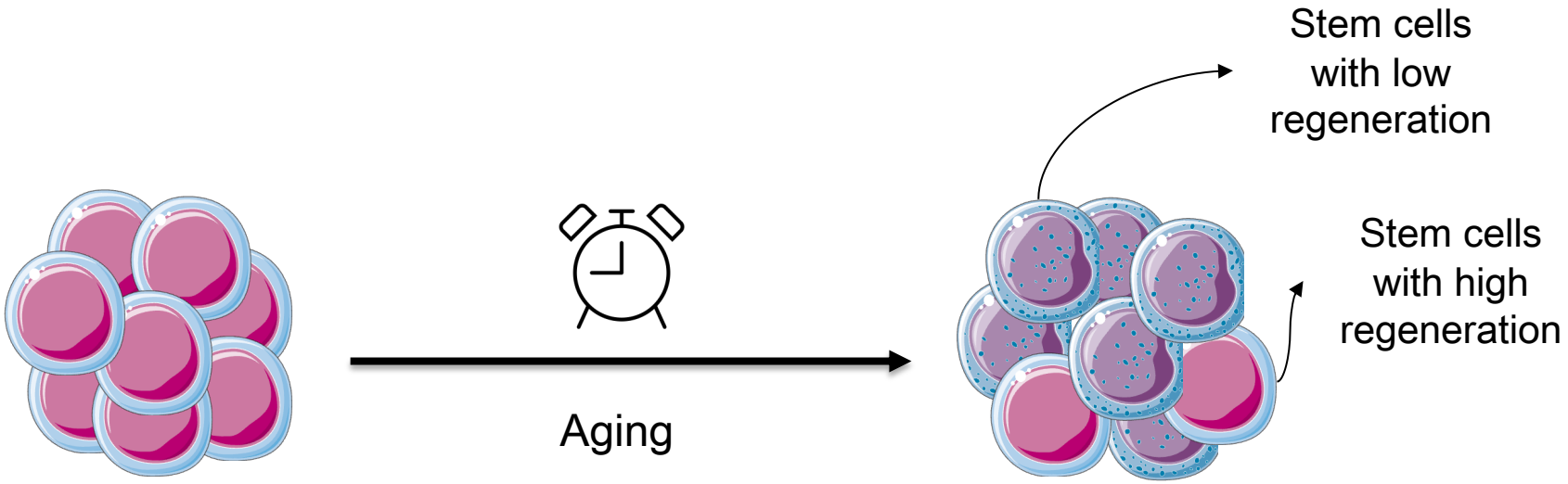
Hematopietic Stem Cells (HSCs)

- Hematopietic stem cells are multipotent:
  - they can differentiate into blood cells

HSCs go through an intermediate progenitor differentiation step



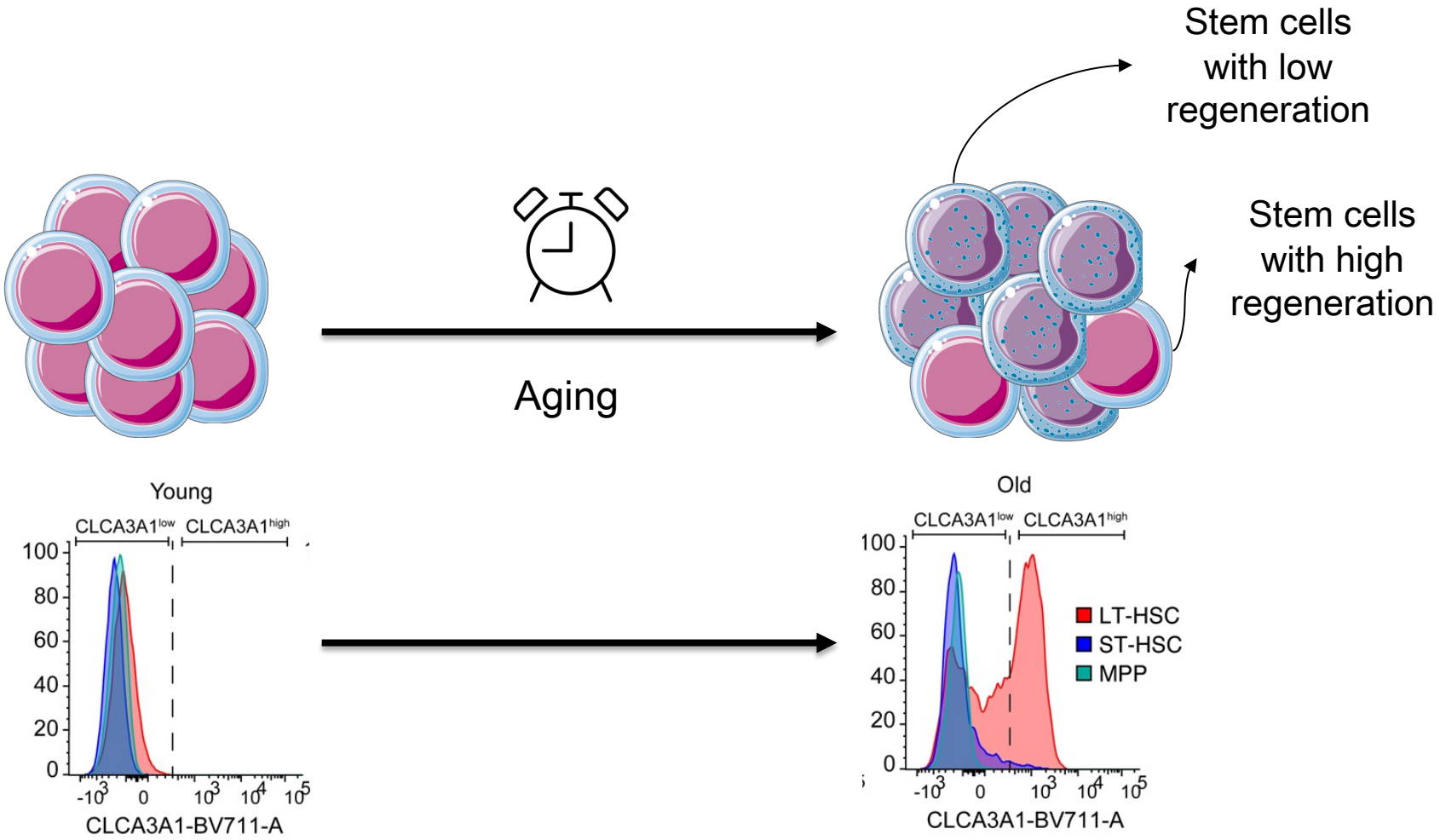
HSCs Reduce Their Regenerational Ability with Age



During aging, blood cells are derived from fewer and fewer stem cells.

"Taz protects hematopoietic stem cells from an aging-dependent decrease in PU.1 activity", Nature Communications, 2022

HSCs Reduce Their Regenerational Ability with Age



During aging, blood cells are derived from fewer and fewer stem cells.

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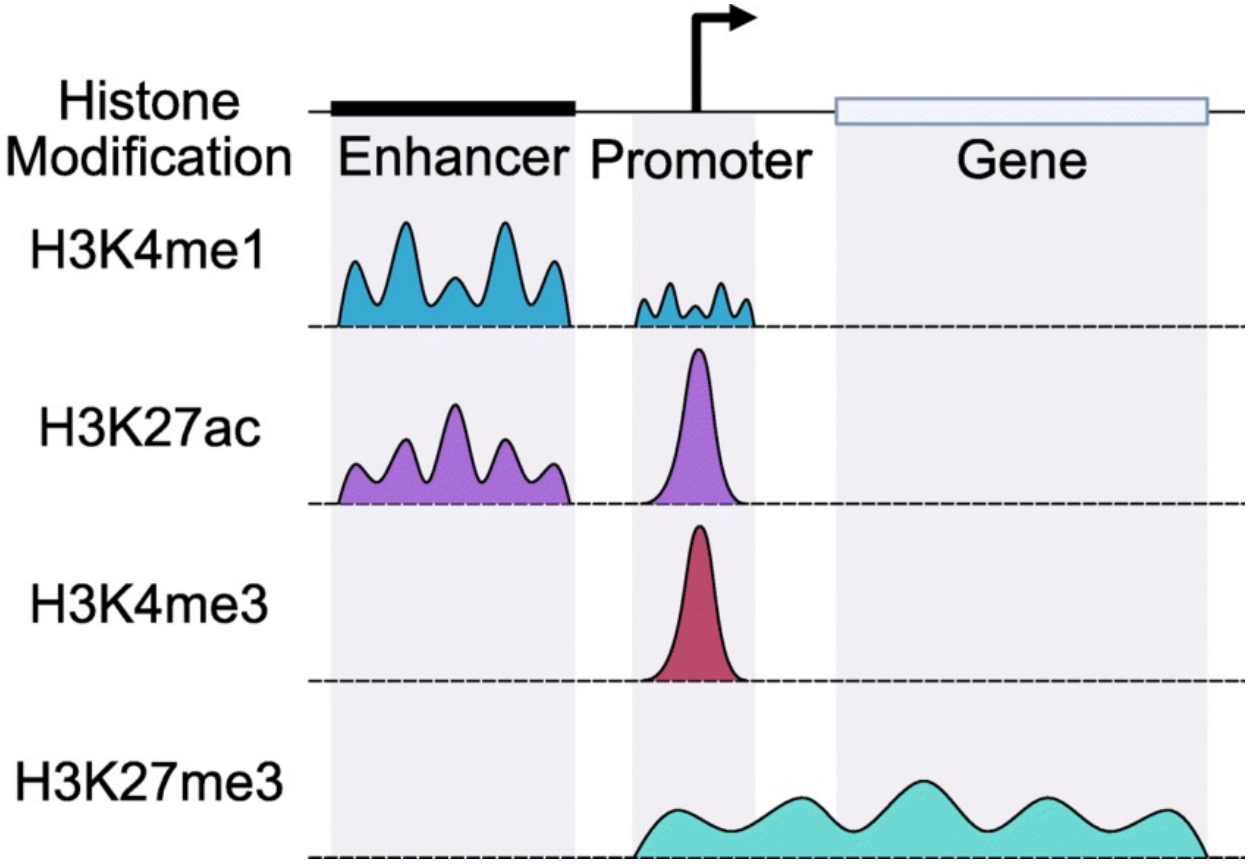
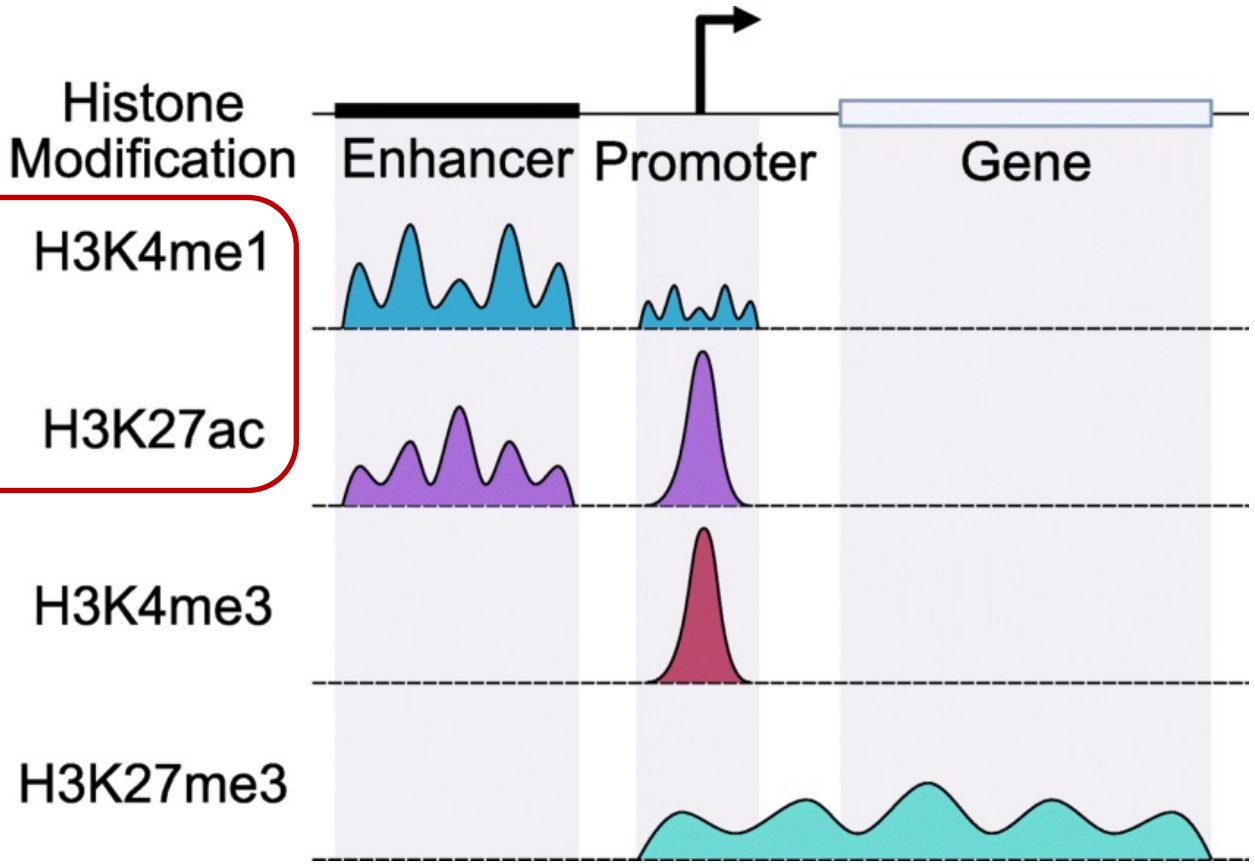
Histone Marks to Detect Active Promoters/Enhancers with Aging



GoPeaks: histone modification peak calling for CUT&Tag, Genome Biology, 2022

Histone Marks to Detect Active Promoters/Enhancers with Aging

H3K4me1 and H3K27ac are essential for enhancers to **activate gene transcription**.

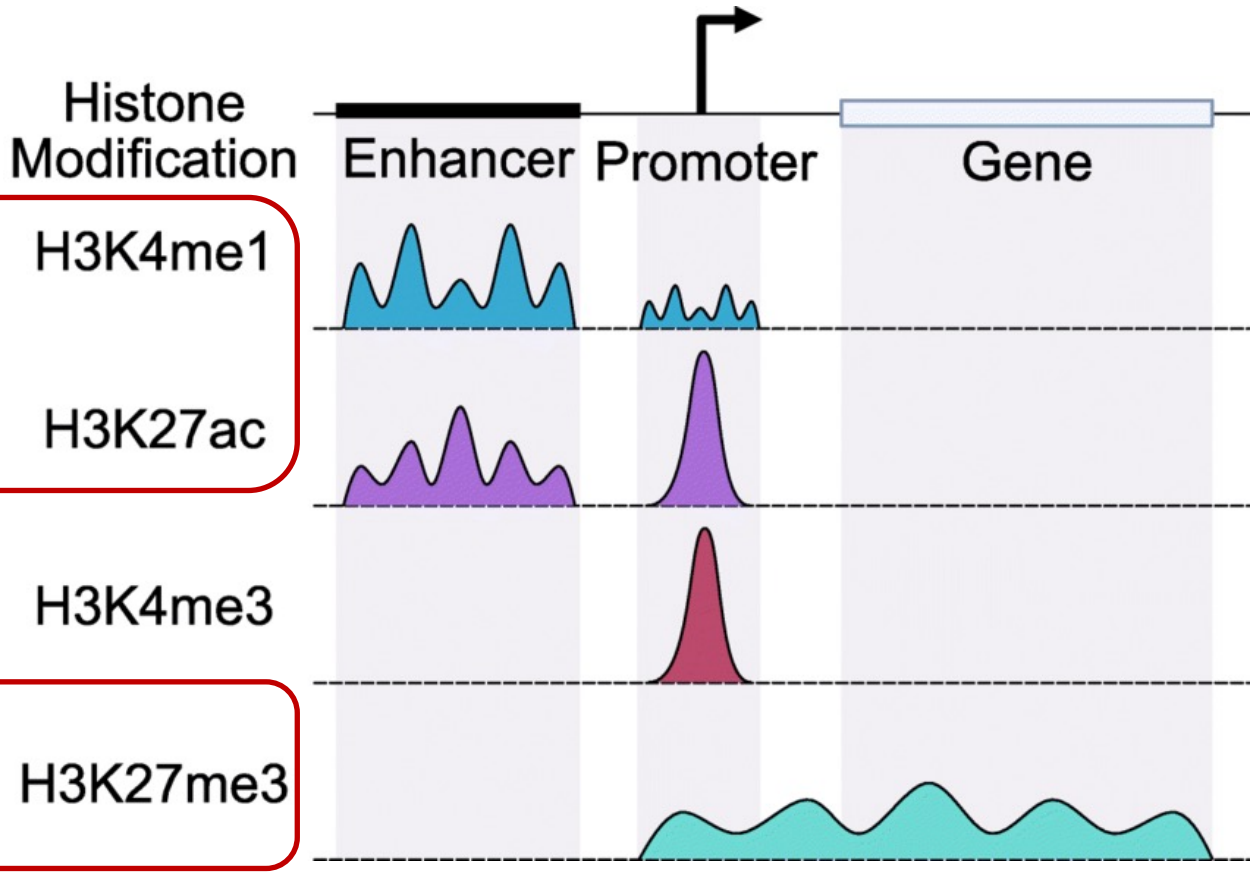


GoPeaks: histone modification peak calling for CUT&Tag, Genome Biology, 2022

Histone Marks to Detect Active Promoters/Enhancers with Aging

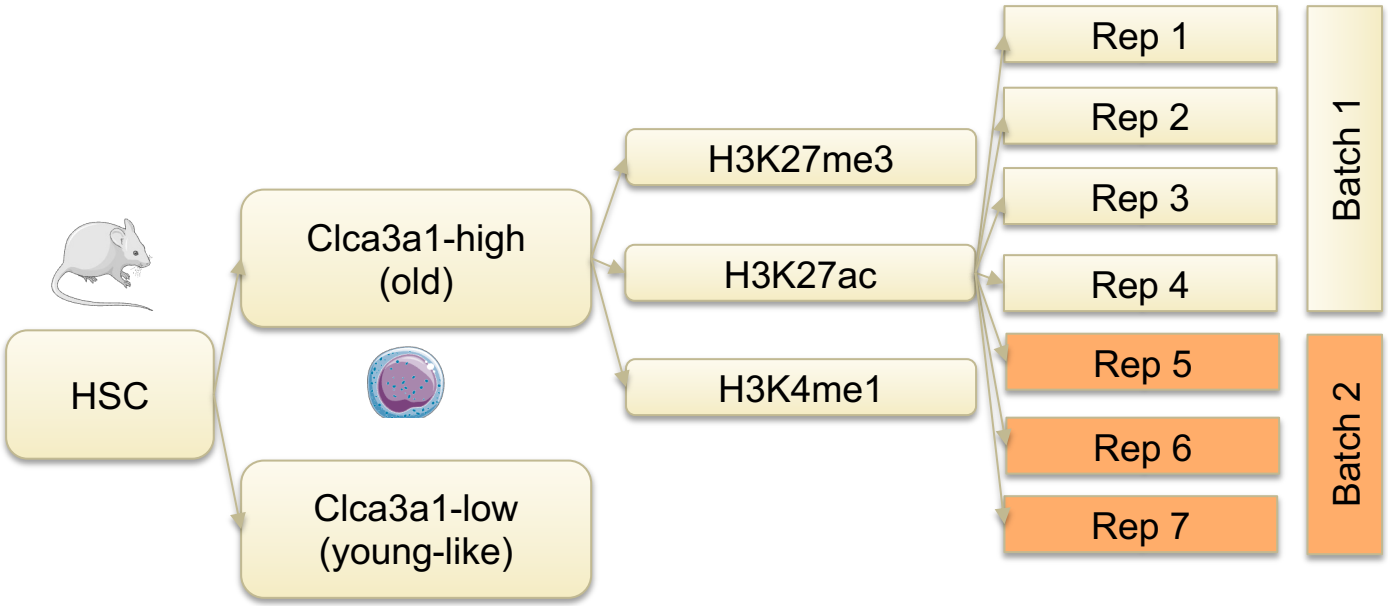
H3K4me1 and H3K27ac are essential for enhancers to **activate gene transcription**.

H3K27me3 shape gene **transcription inhibition**.



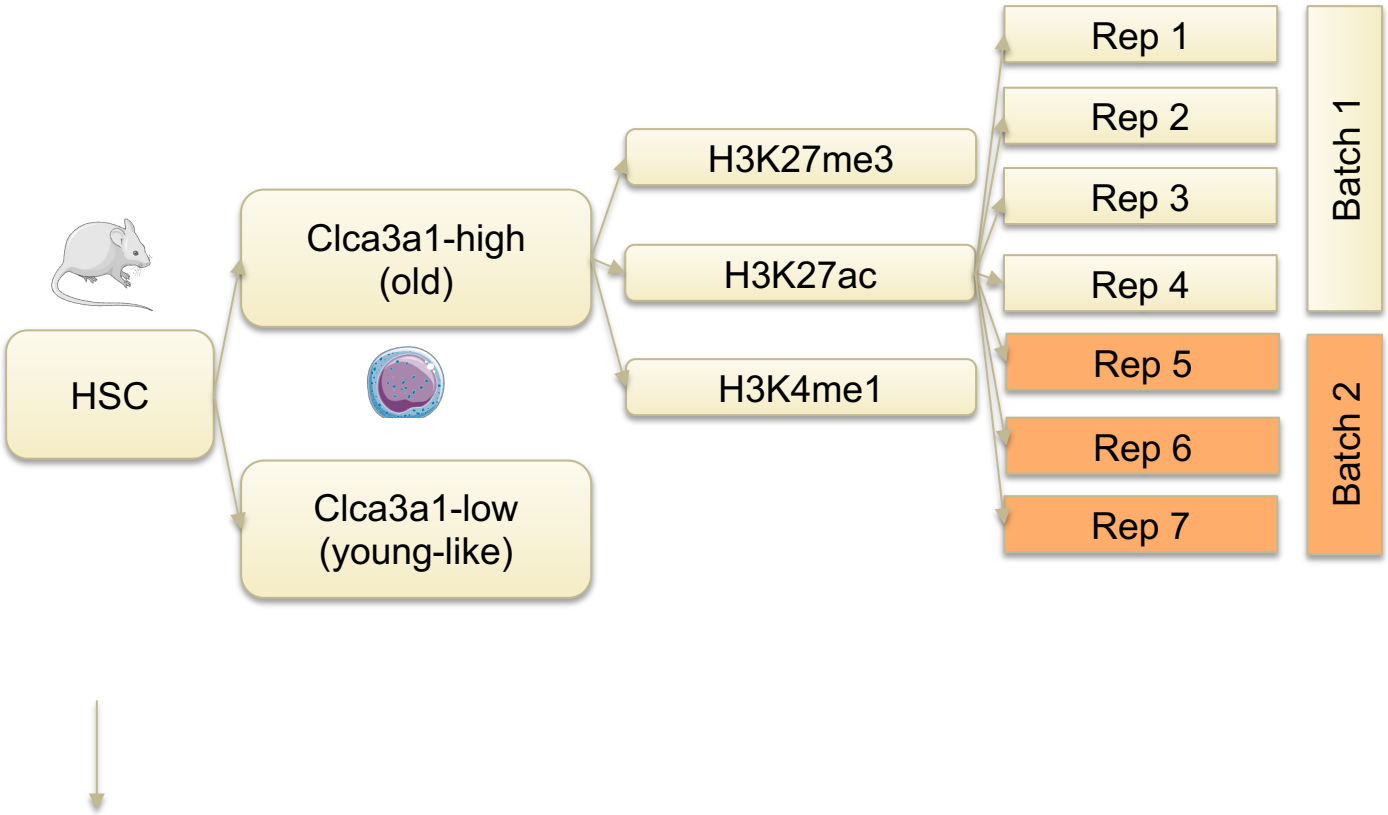
GoPeaks: histone modification peak calling for CUT&Tag, Genome Biology, 2022

Experimental Design



"CUT&Tag for efficient epigenomic profiling of small samples and single cells." Nature Communication, 2019

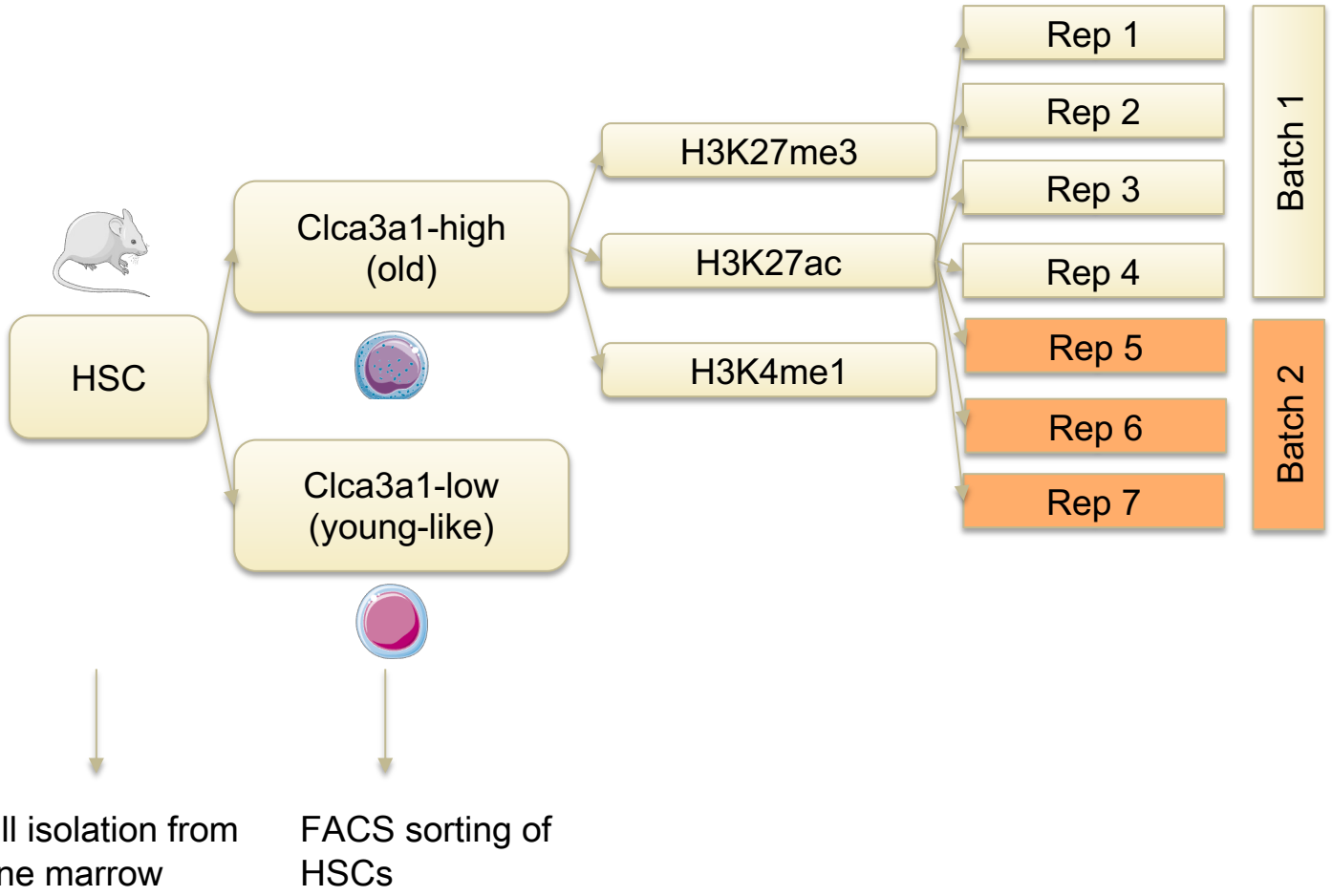
Experimental Design



Cell isolation from
bone marrow

"CUT&Tag for efficient epigenomic profiling of small samples and single cells." Nature Communication, 2019

Experimental Design



"CUT&Tag for efficient epigenomic profiling of small samples and single cells." Nature Communication, 2019

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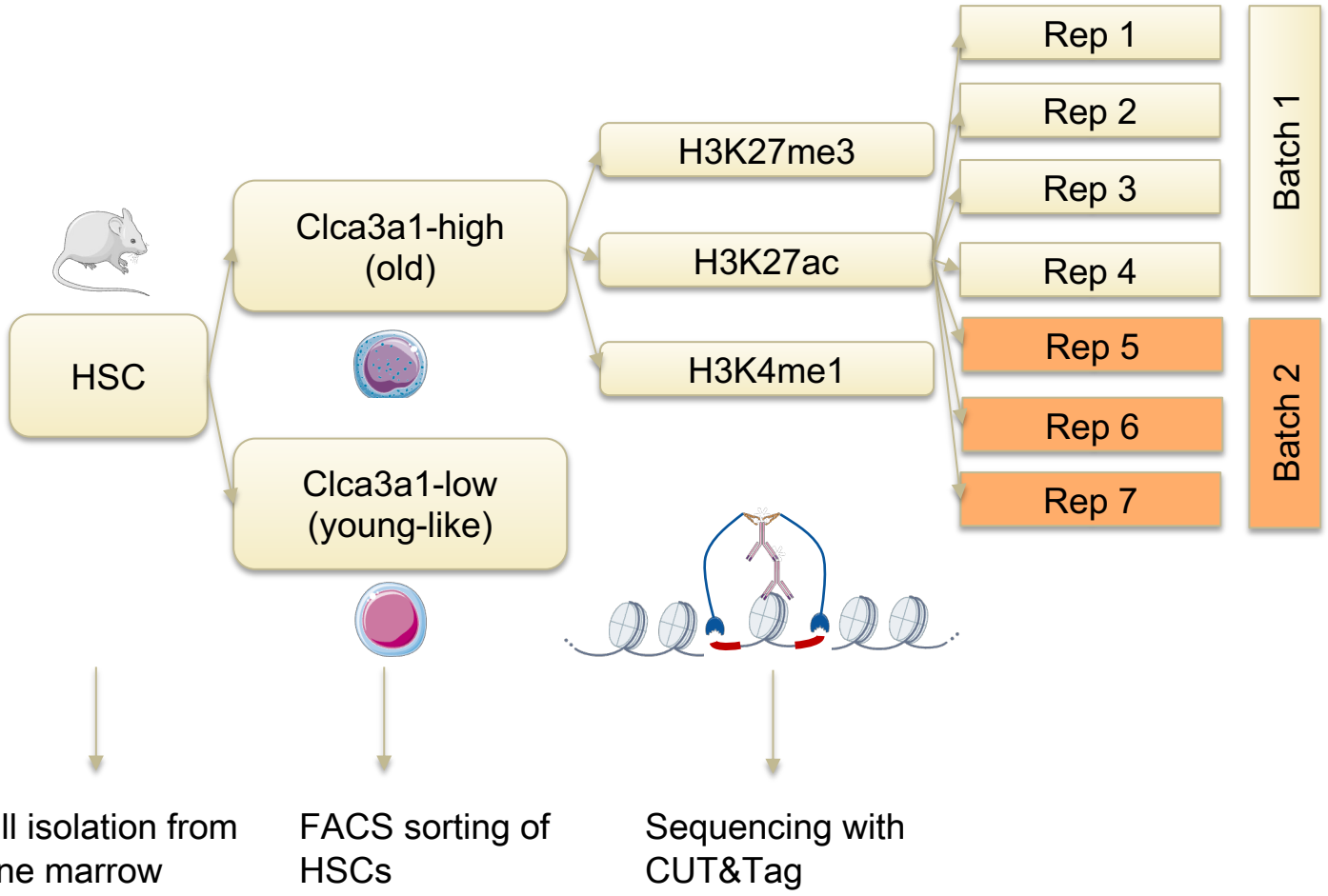
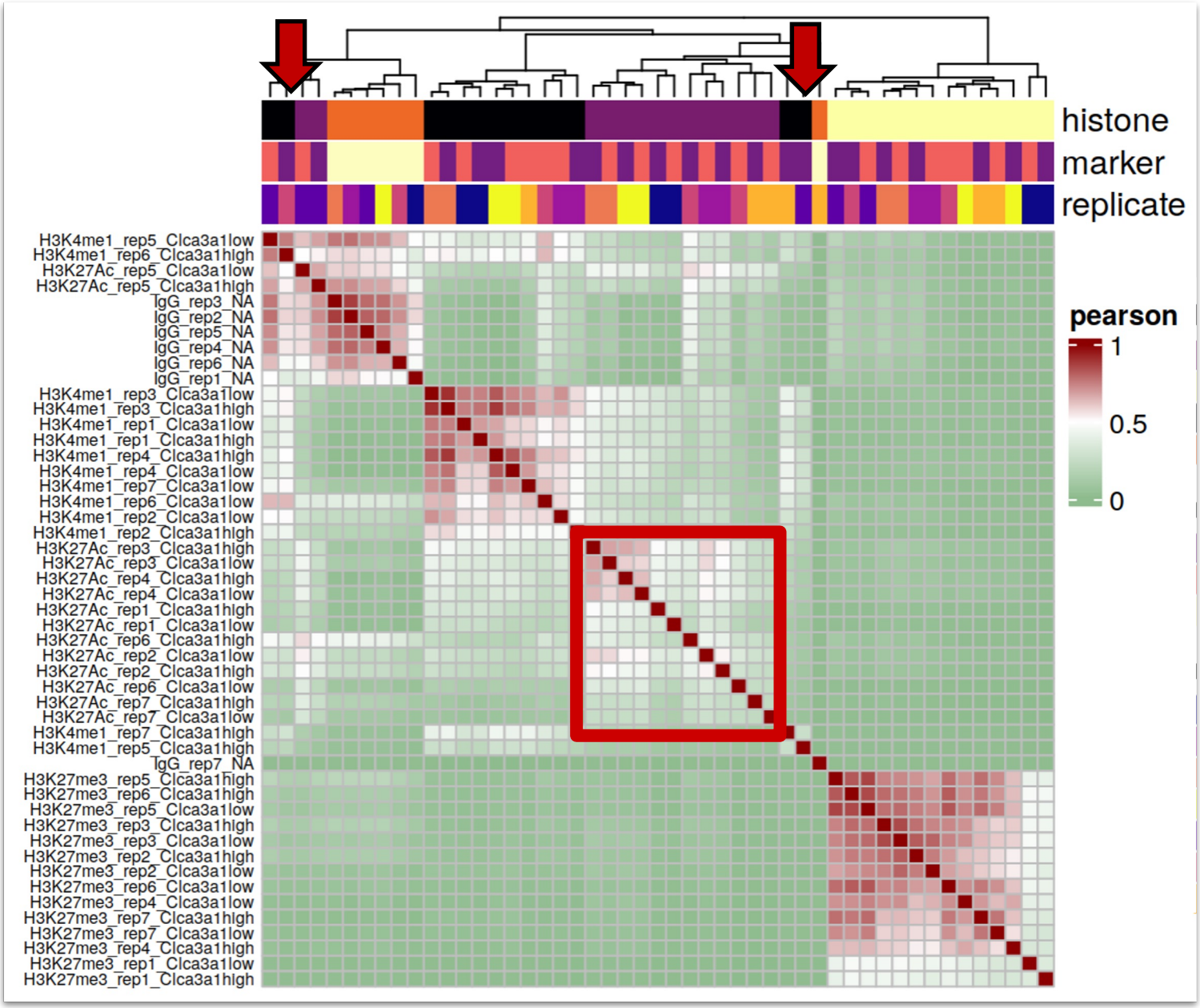


"CUT&Tag for efficient epigenomic profiling of small samples and single cells." Nature Communication, 2019

Challenges in This Project

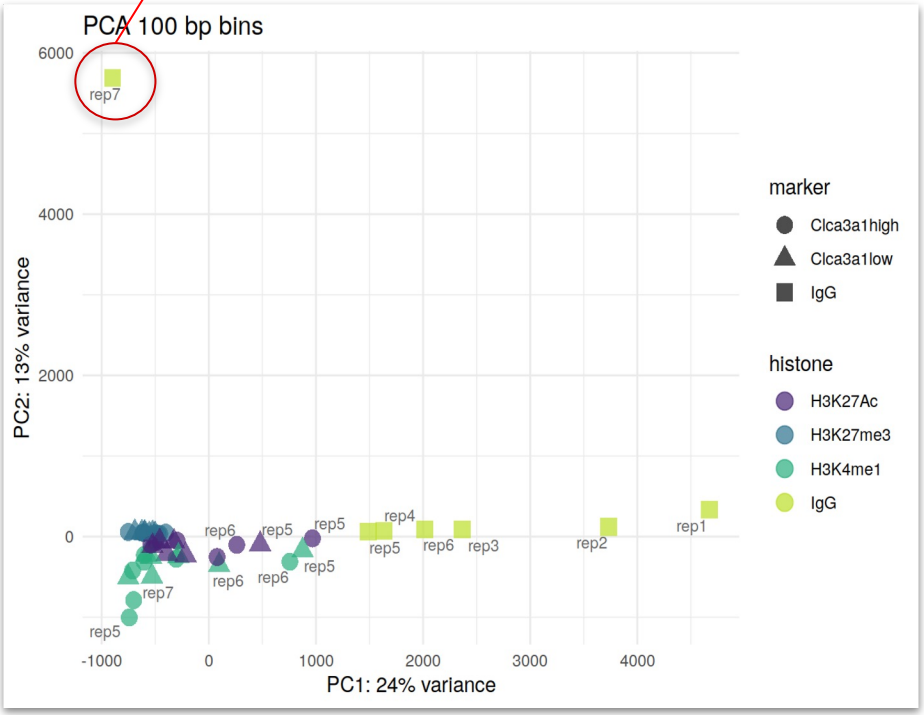
- Low input:
  - Few cells from HSCs
  - Low covarage from CUT&Tag
- Relatively new protocol:
  - CUT&Tag analysis not established well
  - **Necessity to develop better tools**
- High variability between replicates

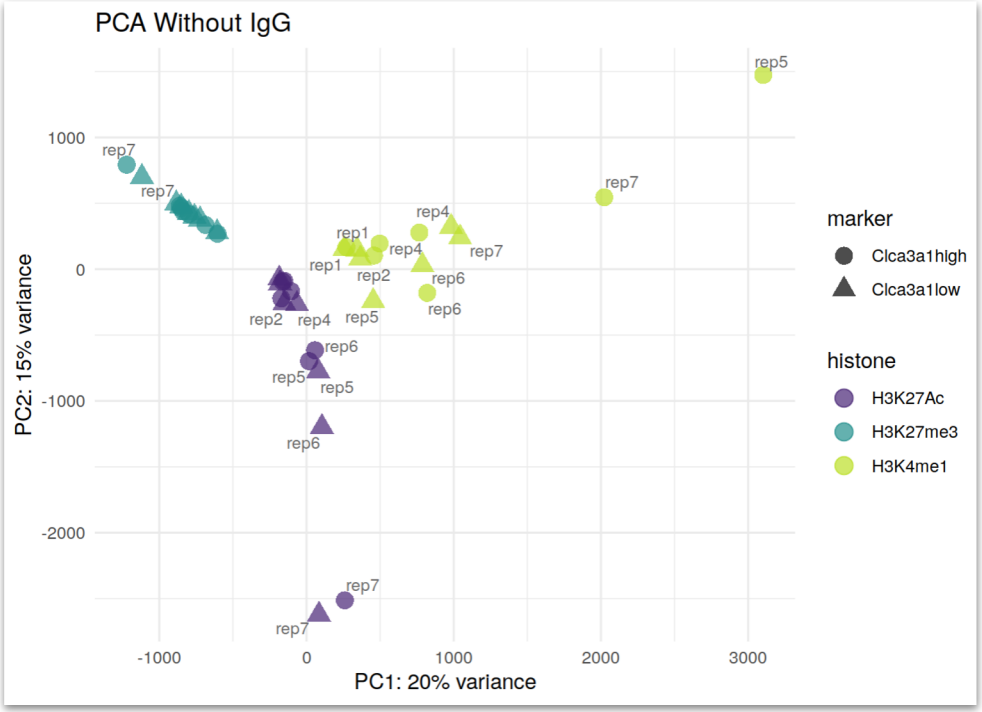
Replicate Reproducibility - Correlations



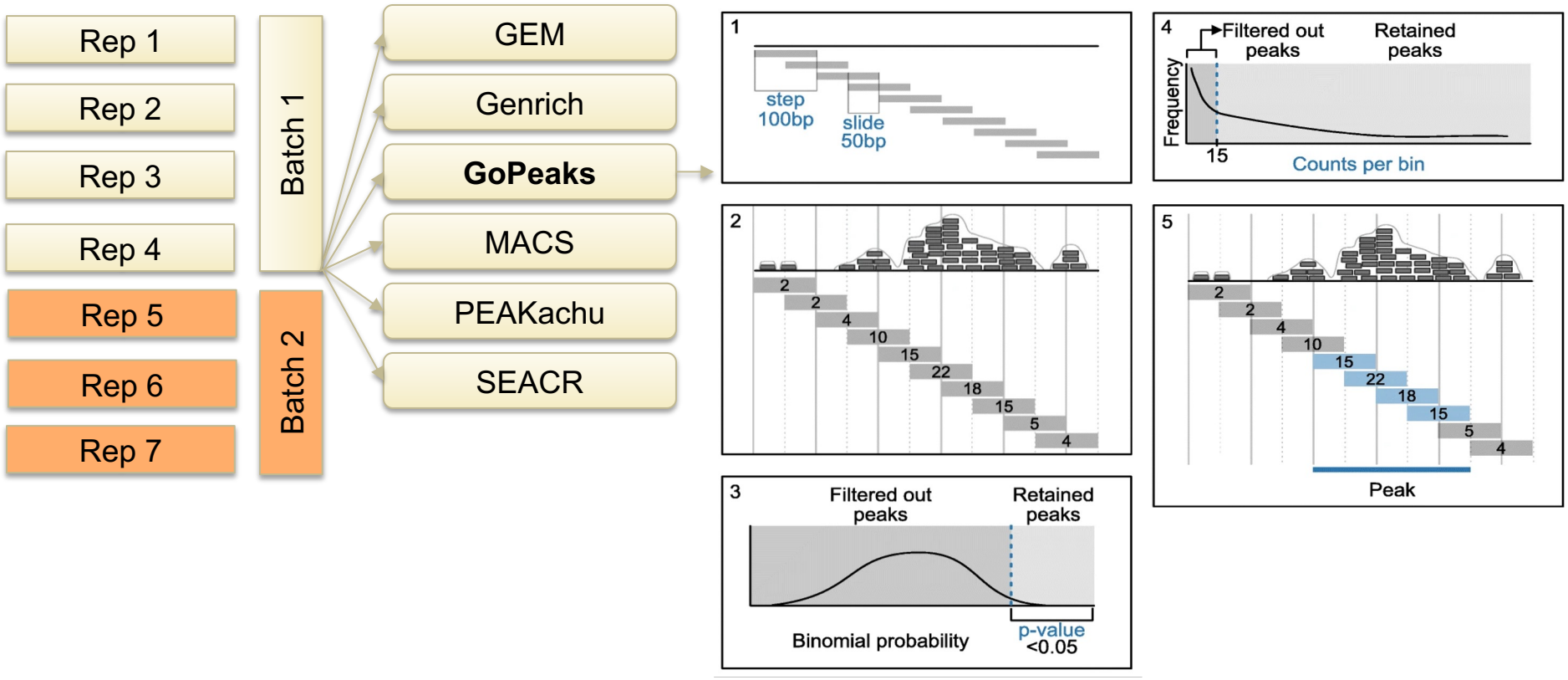
Replicate Reproducibility - PCA

Replicate 7 is IgG control





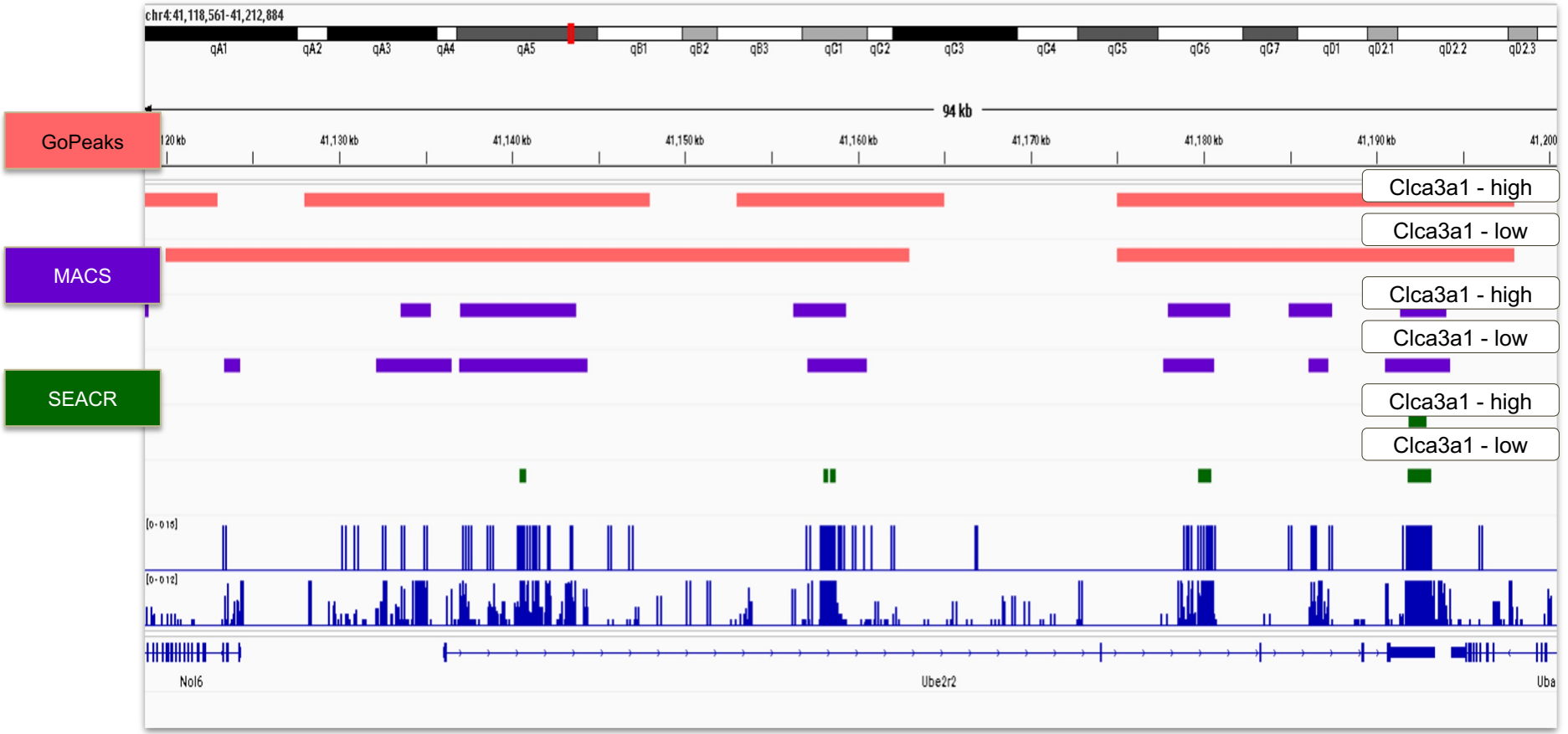
Several Peak Calling Algorithms Were Used



GoPeaks developed specifically for CUT&Tag data

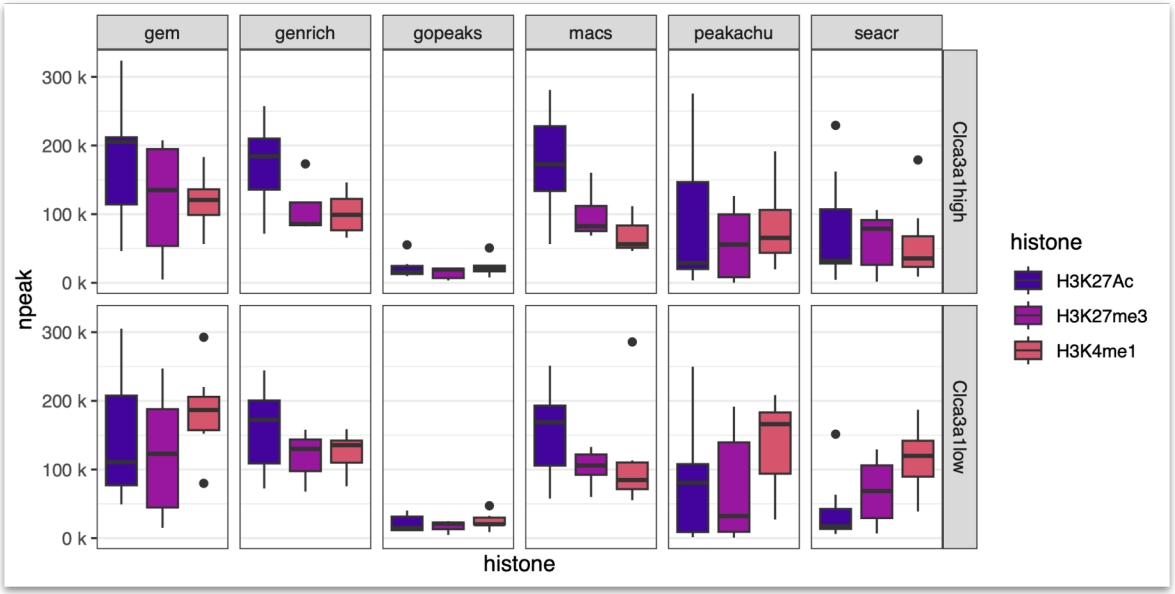
"GoPeaks: histone modification peak calling for CUT&Tag", Genome Biology, 2022

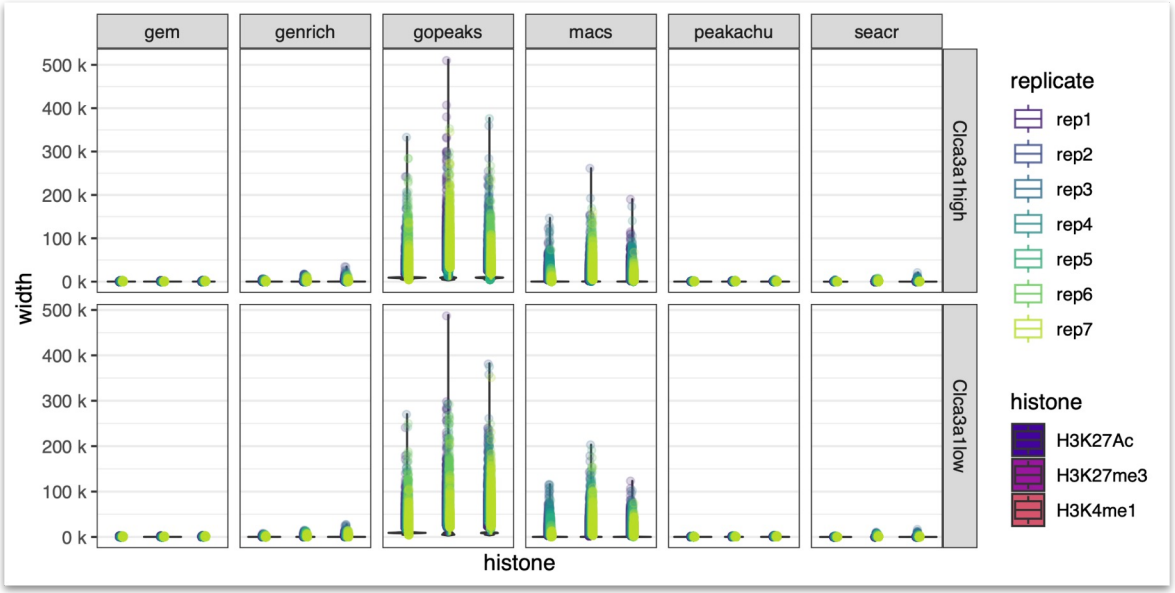
Peak Calling for CUT&Tag



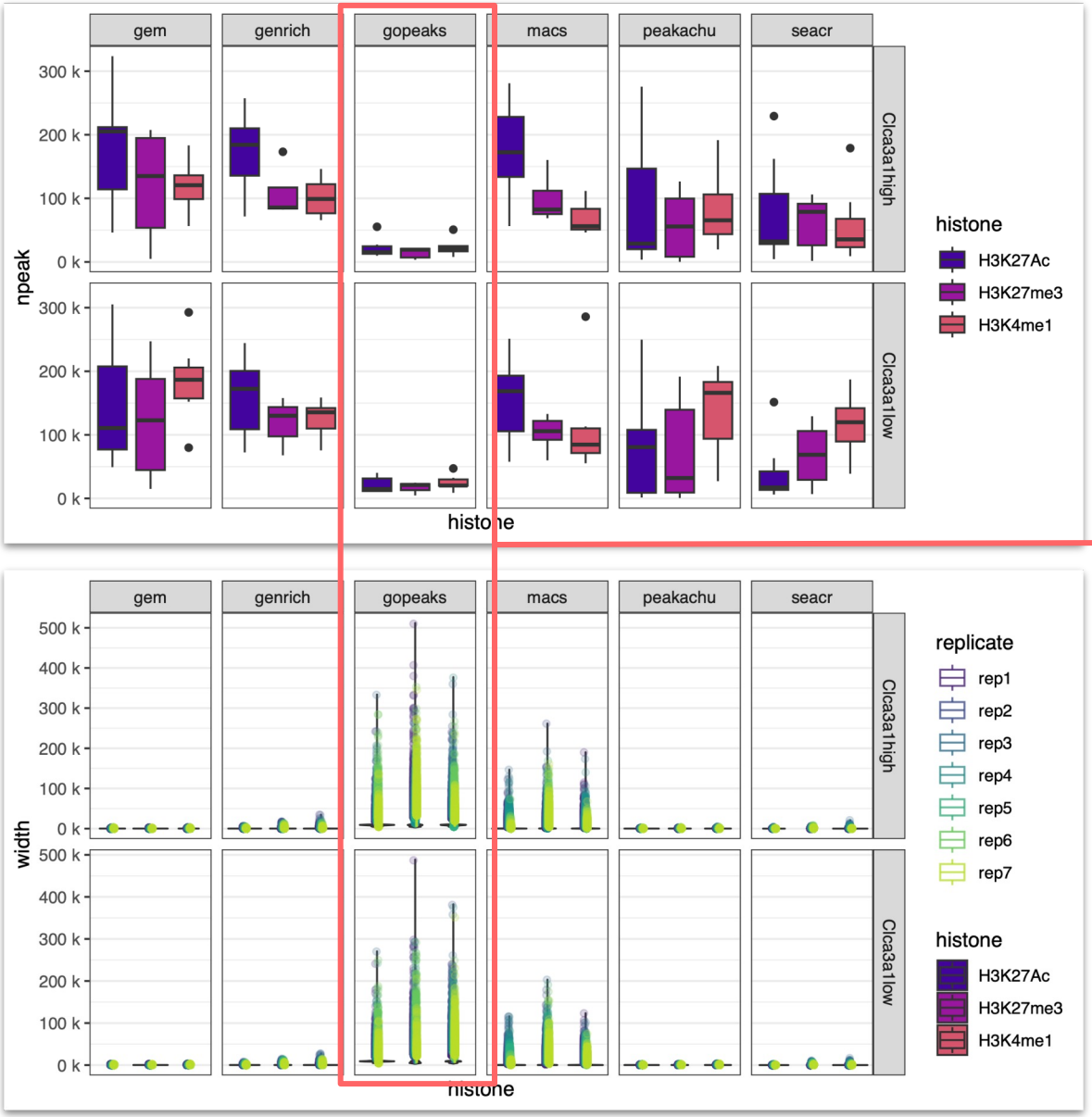
Each peak caller gave a varied set of peaks.

Peak Widths and Numbers





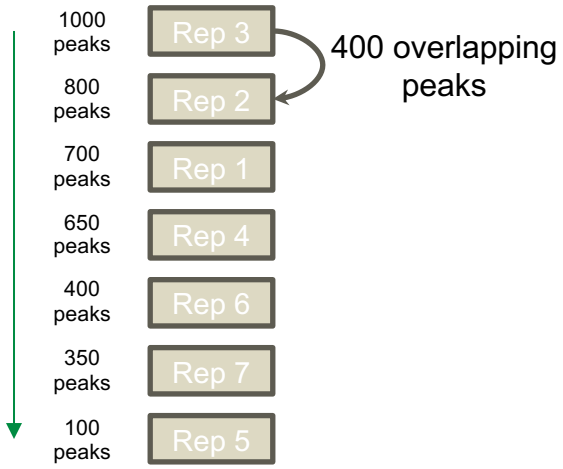
Peak Widths and Numbers



Low number and wide peaks compared to other peak callers.

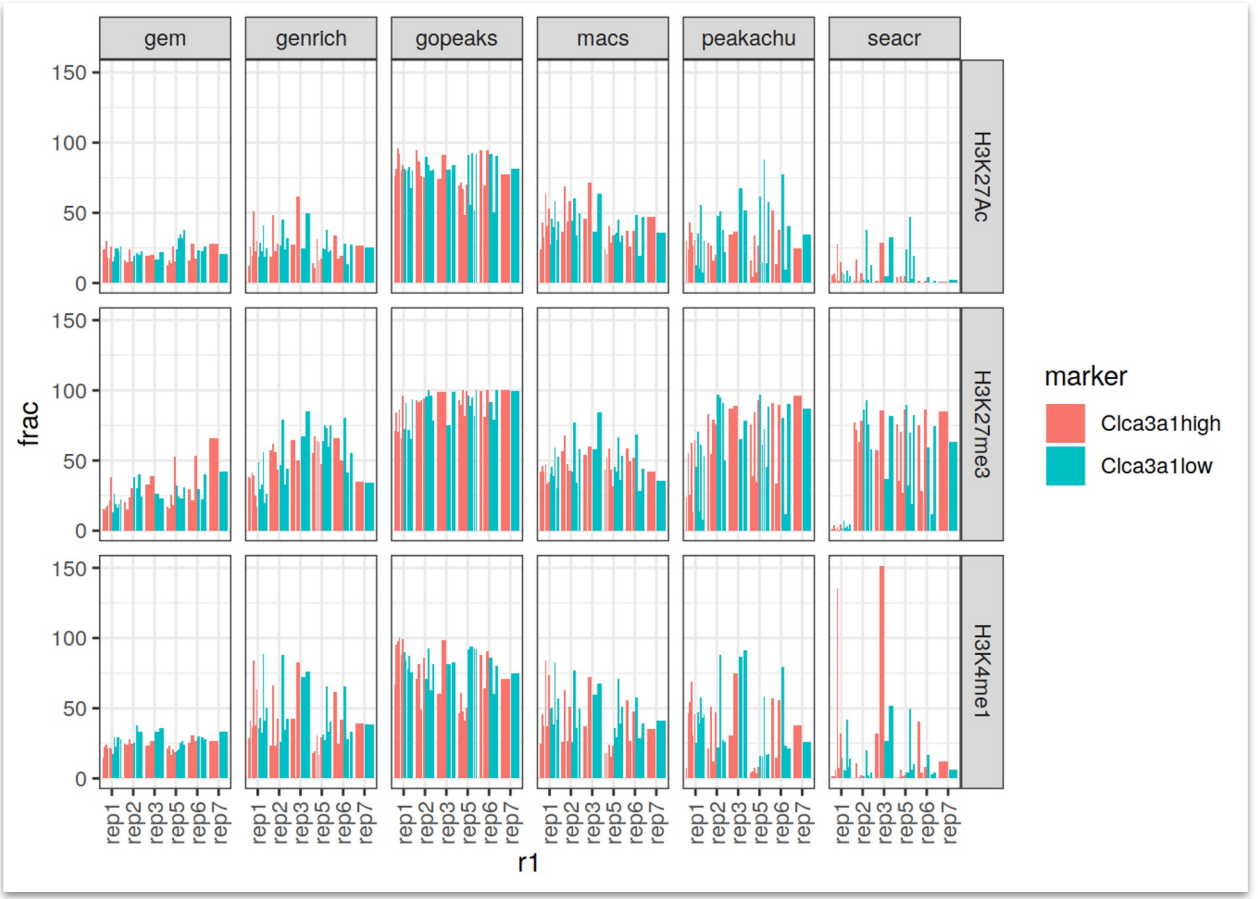
Reproducibility of the Peaks – Our (Simple) Method

Our implementation of reproducibility



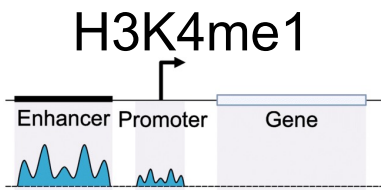
for rep 2:

n_{rep3} // n_{rep2} * 100 =
400 / 800 * 100 = **50%**



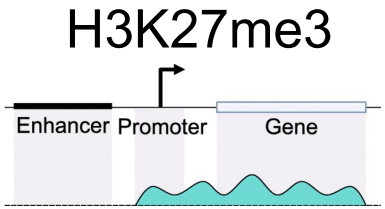
At the end, MACS2 seemed to be best caller for our data.

Genomic Annotations of the Peaks - ChromHMM



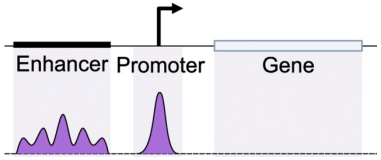
Clca3a1 low

Clca3a1 high



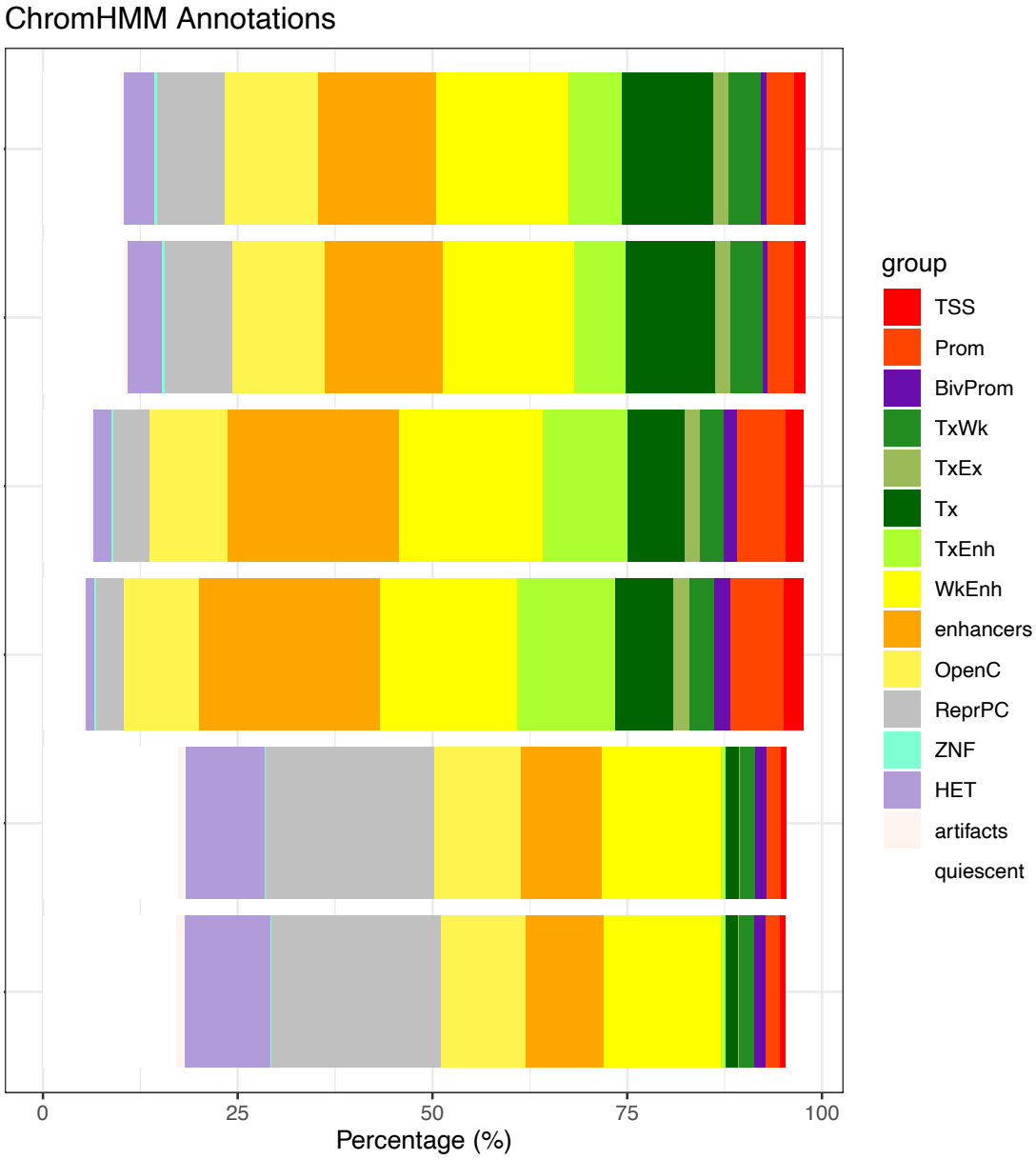
Clca3a1 low

Clca3a1 high



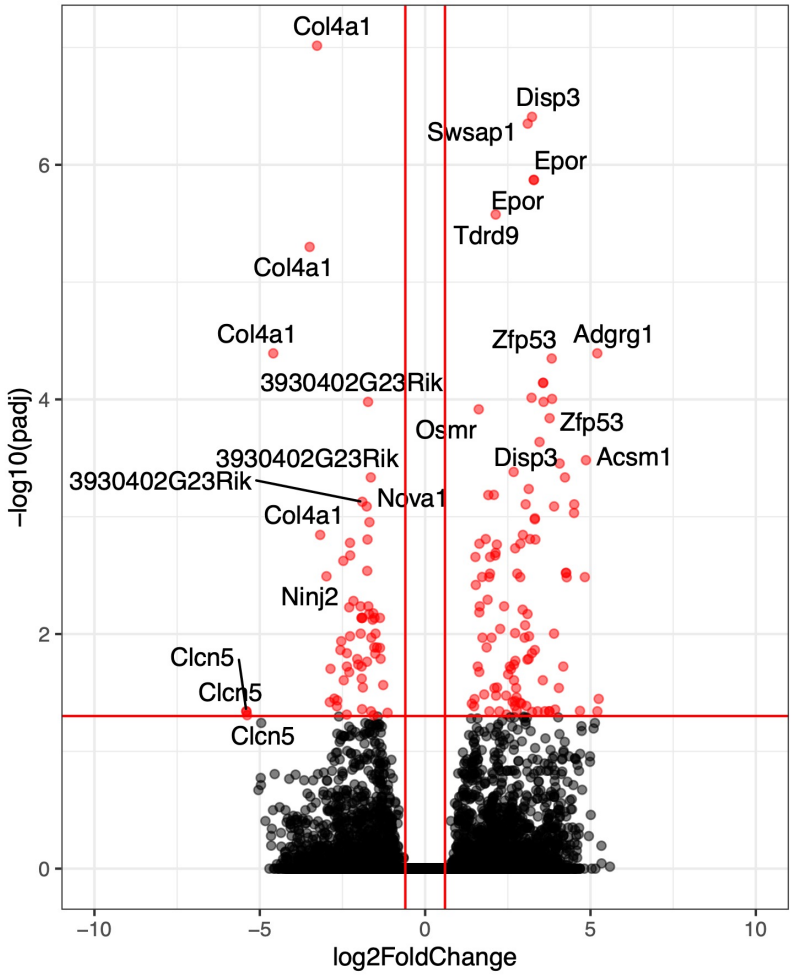
Clca3a1 low

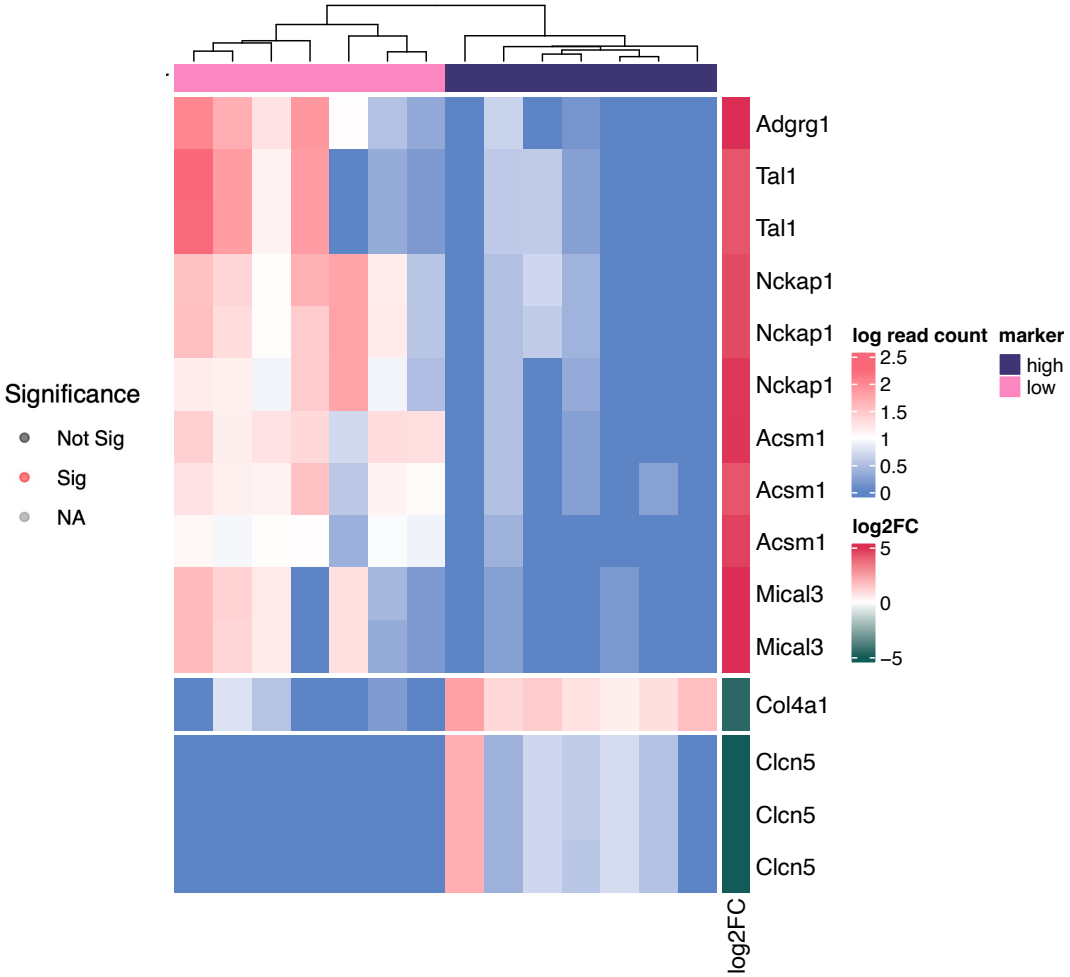
Clca3a1 high



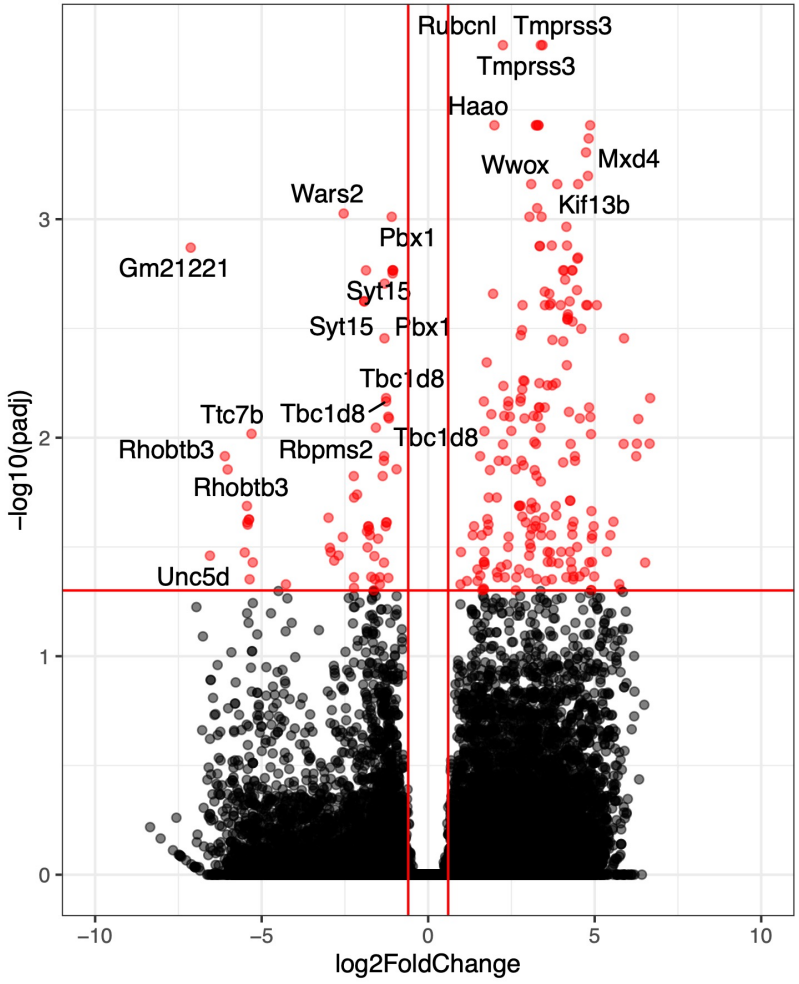
Universal chromatin state annotation of the mouse genome, Genome Biology, 2023

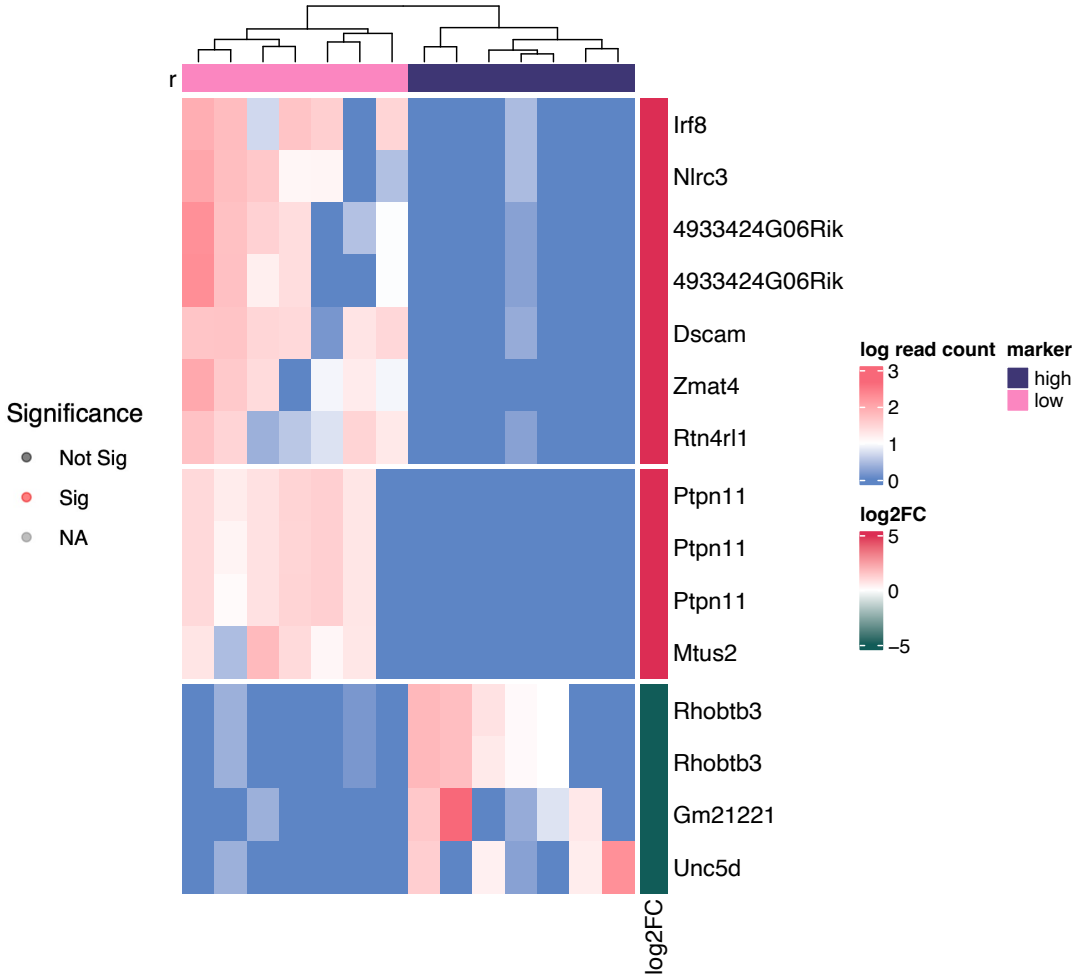
Differential Peaks Between Clca3a1^{high} vs. Clca3a1^{low} (H3K27me3)





Differential Peaks Between Clca3a1^{high} vs. Clca3a1^{low} (H3K4me1)

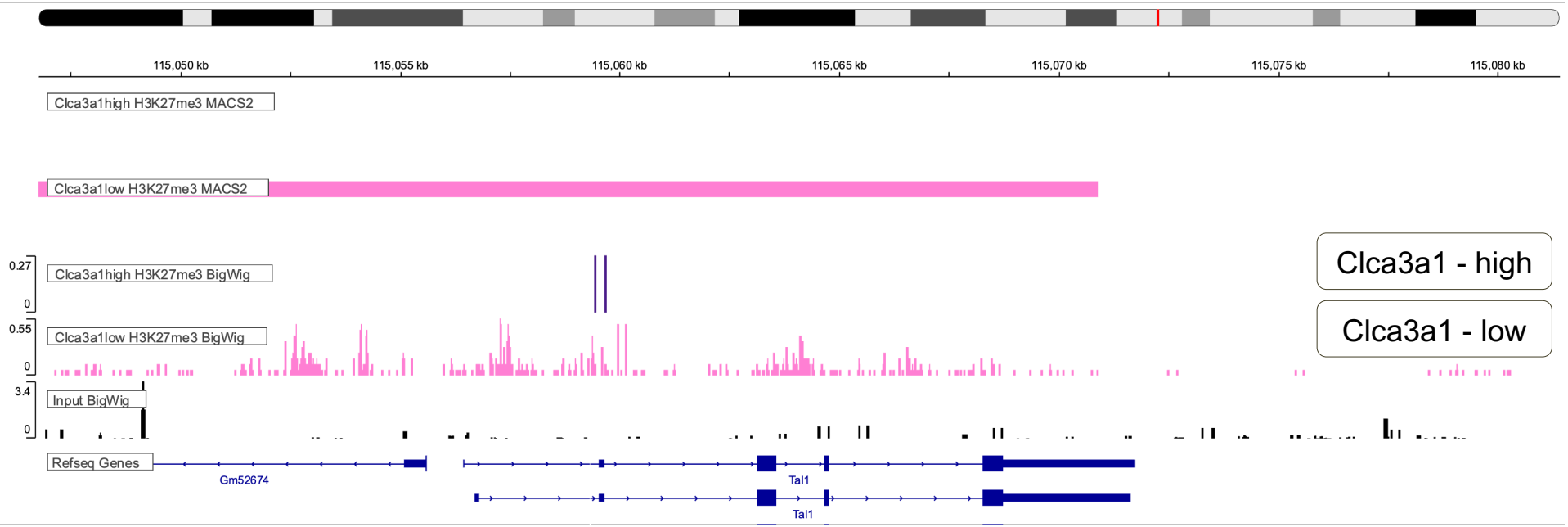




Closer Look of H3K27me3 in Tal1

Tal1

- Transcription factor
- Involved in acute lymphoblastic leukemia
- Involved in myeloid cell differentiation



Summary and Future Remarks

- We have batch effect and high variability between replicates.
- Signals are sparse through genome, which might cause differences between peak callers.
- We identified MACS as good candidates for peak calling.
- Even we have differential peaks in H3K27me3 and H3K4me1, there is no enriched GO term or pathway.

In future,

- To get more reliable peaks, we can use ATAC-seq information.
- The end goal would be integrate all epigenetic signals to make genome level atlas of aging mice.

Acknowledgements

Hoffmann Group

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Katjana Schwab
Shuping Yuan
Robert Schwarz
Niels Jahn
Silke Förste
Tycho Kirchner

Von Eyss Group

Bjoern von Eyss

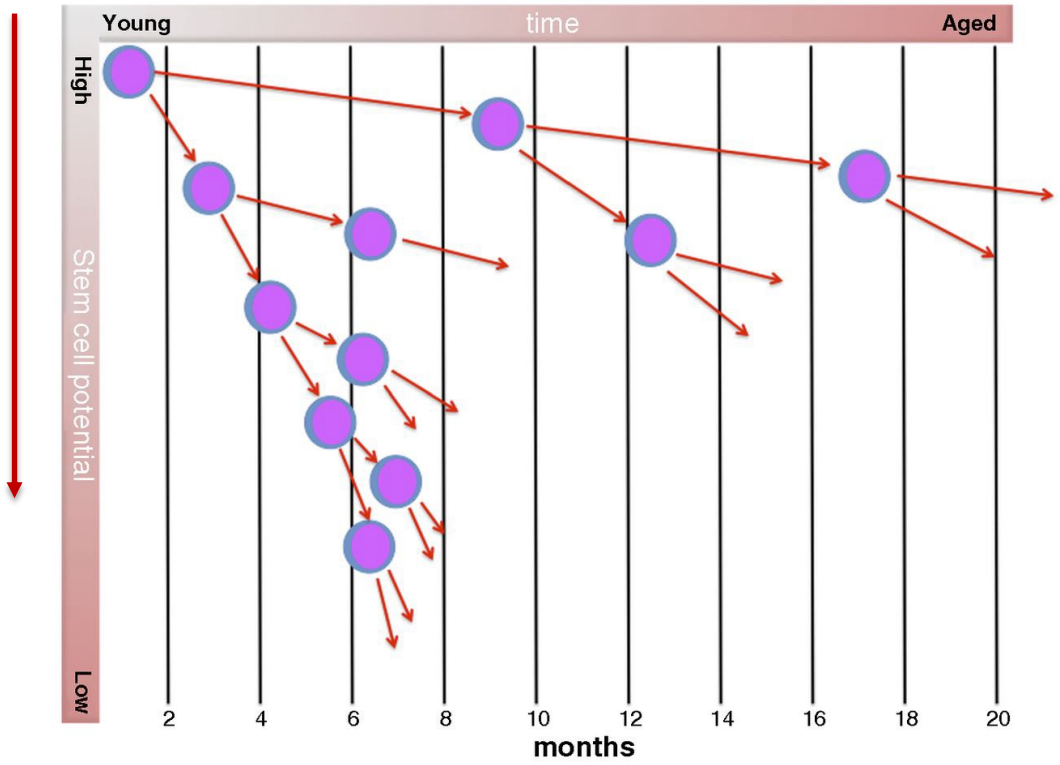
Kyung Mok Kim
Cagla Donmez

Lgsa
Leibniz Graduate School on Aging

HSC Aging

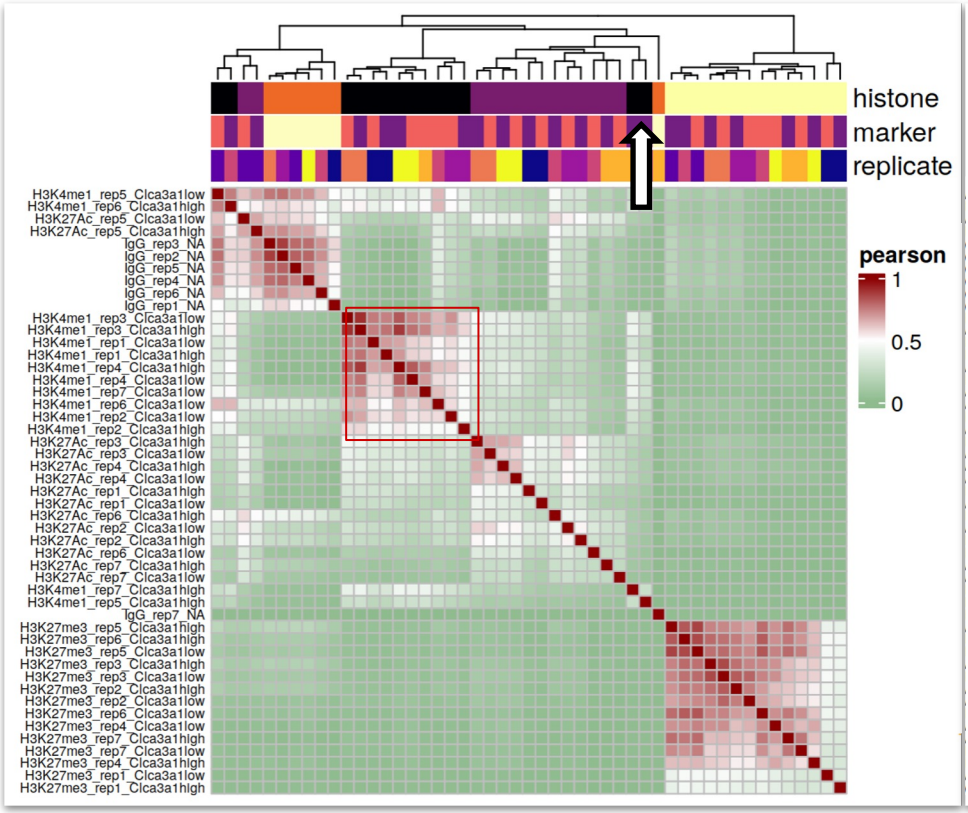
Pool of stem cells with reduced potential increases to compensate for loss of function of individual stem cells (**Clonal hematopoiesis**)

Potential of blood cell production declines with each cell division.

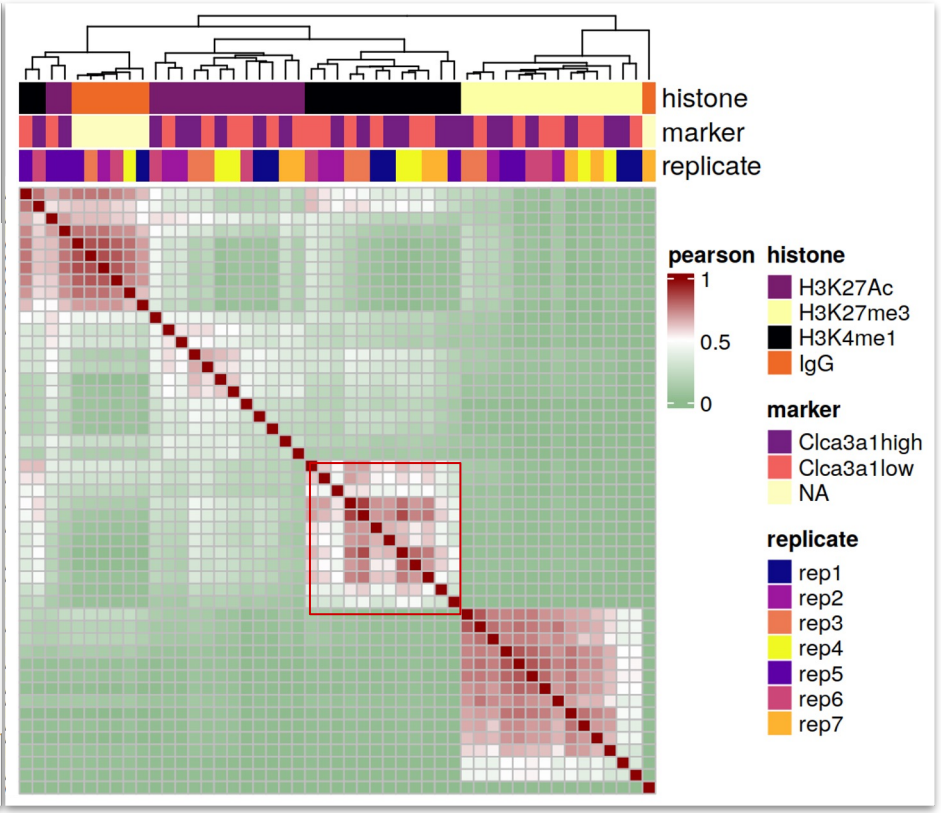


Aging of hematopoietic stem cells, Blood (2018)

Replicate Reproducibility - Correlations



Genomic bins (100bp)

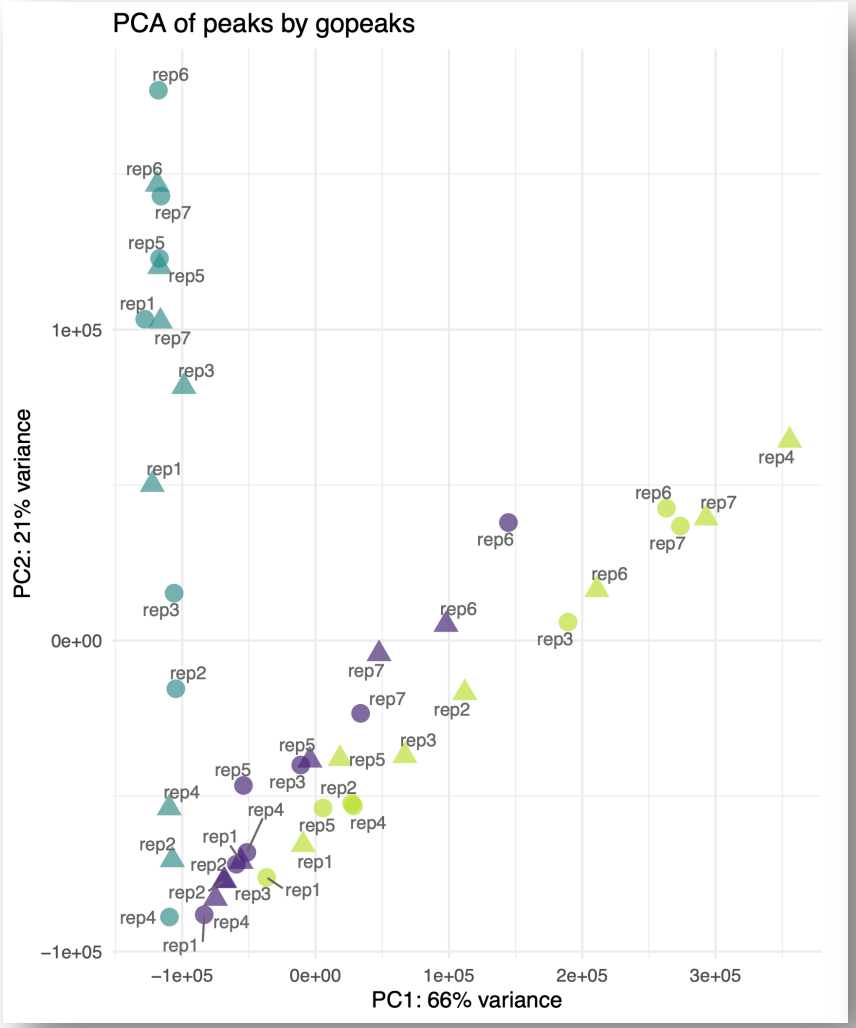


Batch correction (using COMBAT)

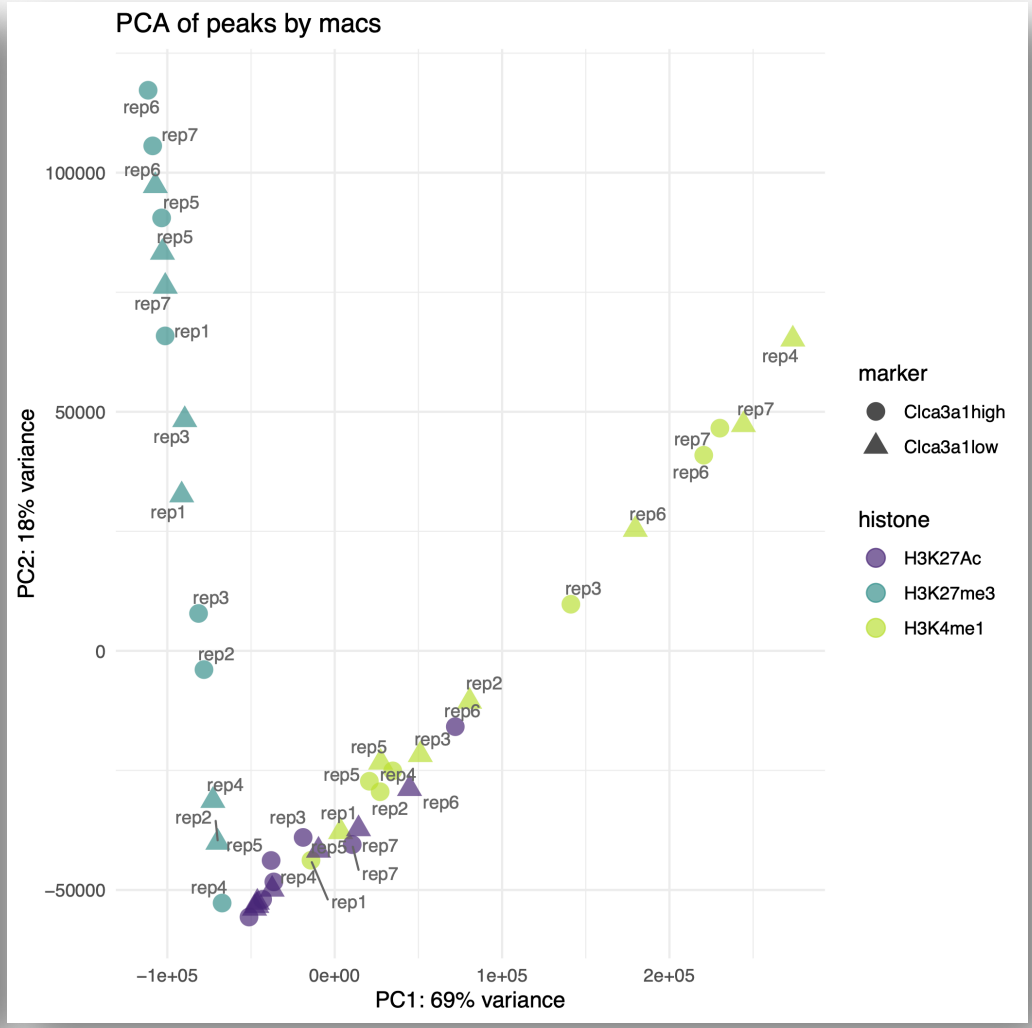
"ComBat-seq: batch effect adjustment for RNA-seq count data." NAR Genomics & Bioinformatics, 2020

PCA of the Peaks – Sample Level

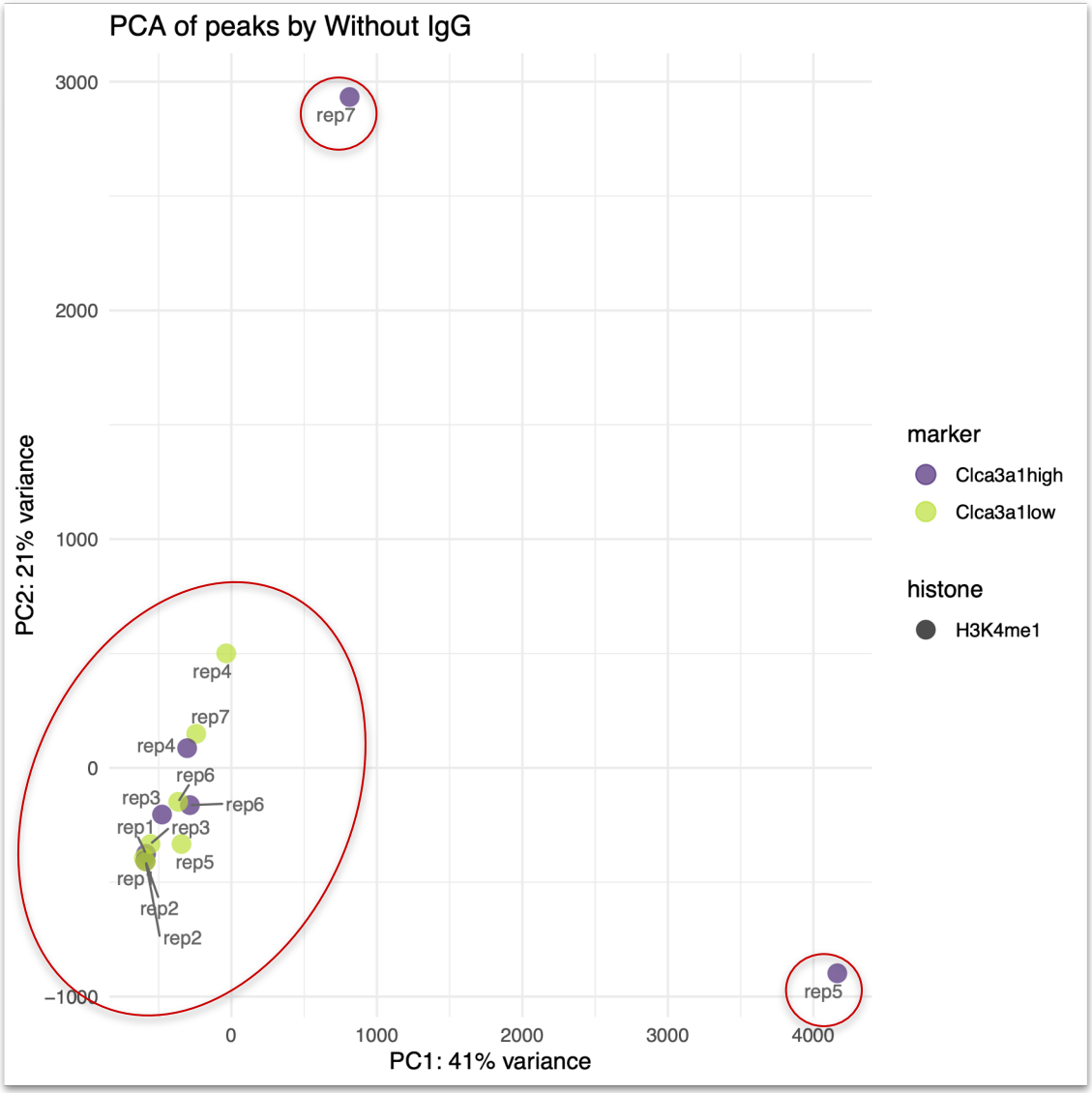
GoPeaks



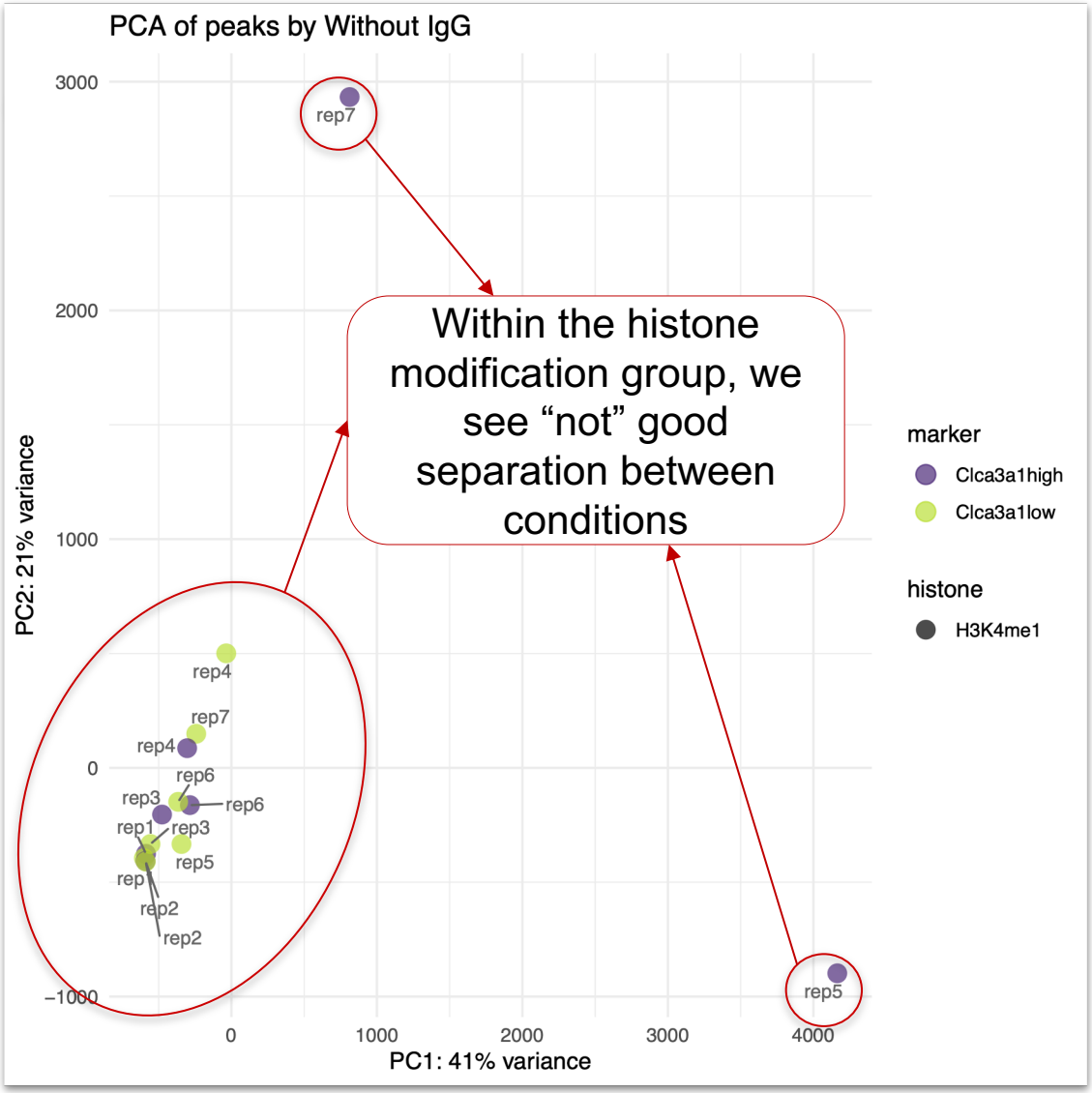
MACS



Replicate Reproducibility – Marker Level PCA (H3K4me1)

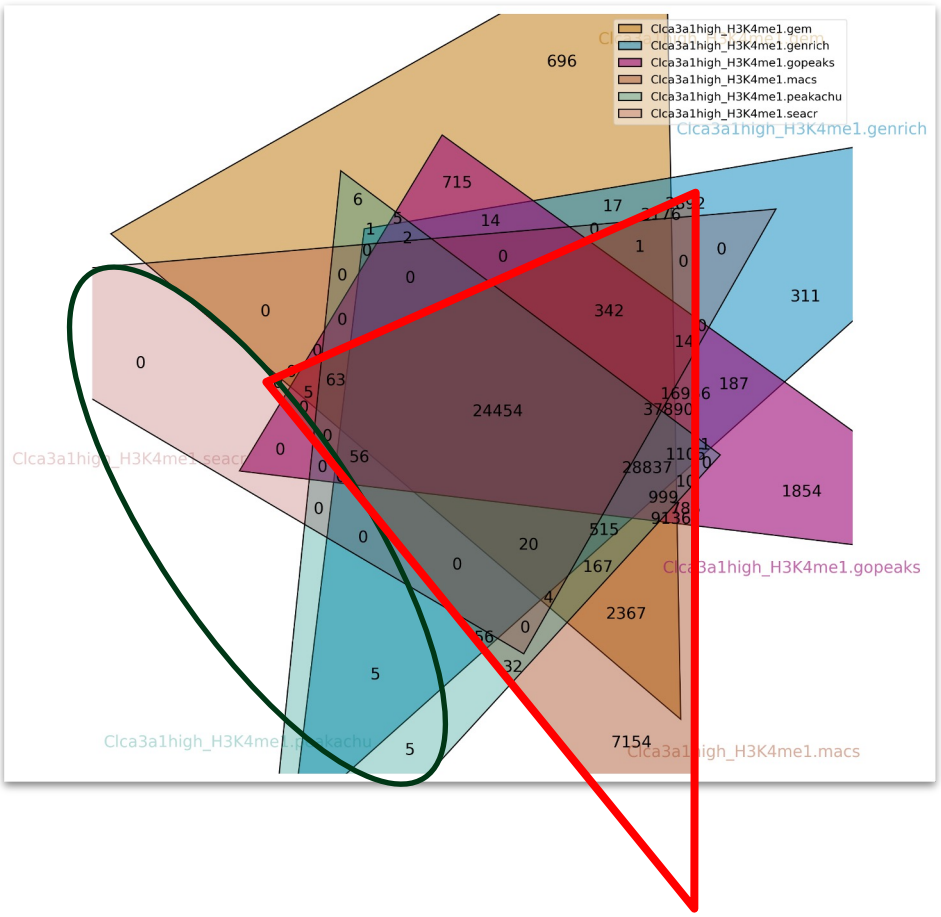


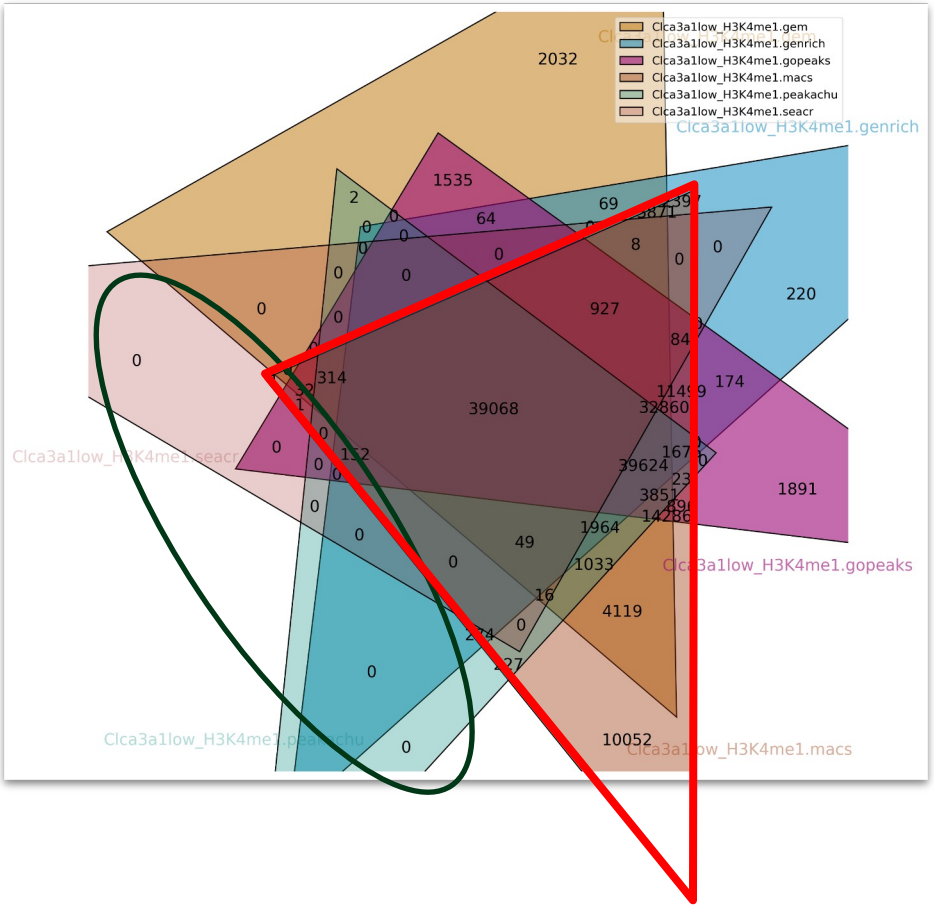
Replicate Reproducibility – Marker Level PCA



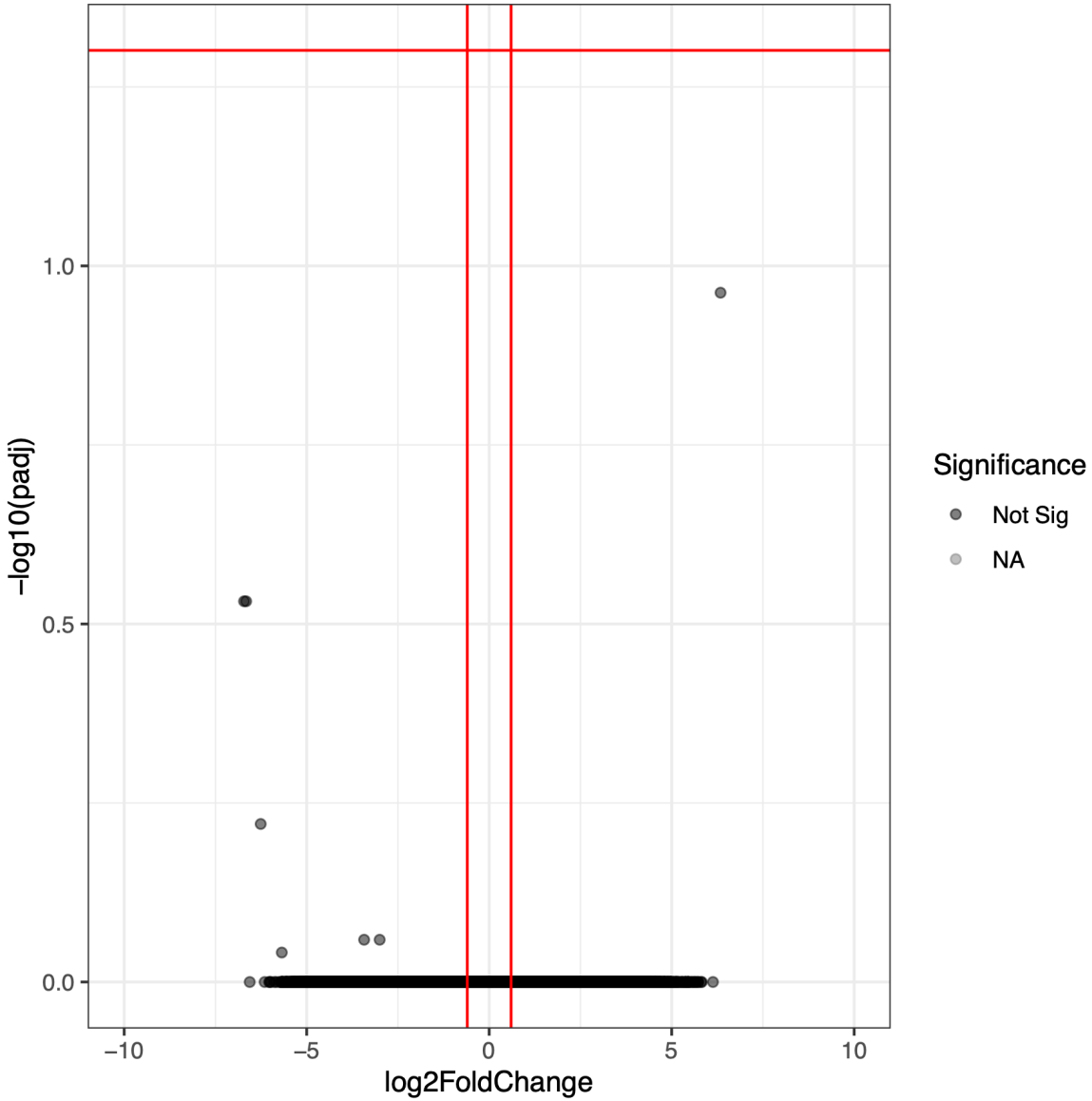
Overlap of the Peaks Between Peak Callers

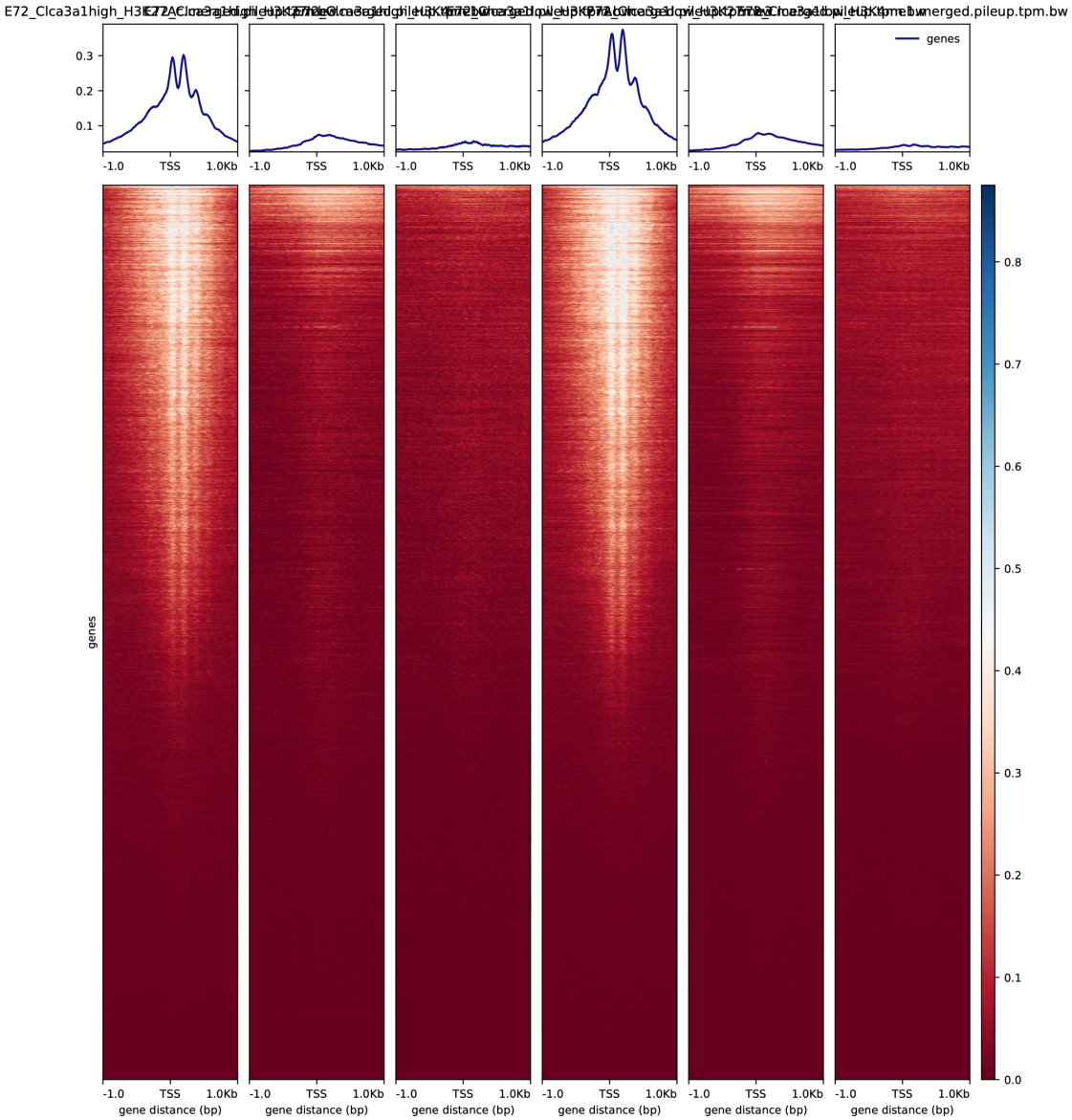
MACS covers most of the peaks also called by other tools.





Differeantial Peaks Between Clca3a1^{high} vs. Clca3a1^{low} (H3K27Ac)




E72_Clca3a1high_H3K4me1_merged.pileup.tpm.bw
genes
0.3
0.2
0.1
-1.0
TSS
1.0Kb
genes
gene distance (bp)
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0