

# The K27me3 histone demethylase Kdm6b (JMJD3) is necessary for hematopoietic stem-cell (HSC) self-renewal

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MSIBS '19

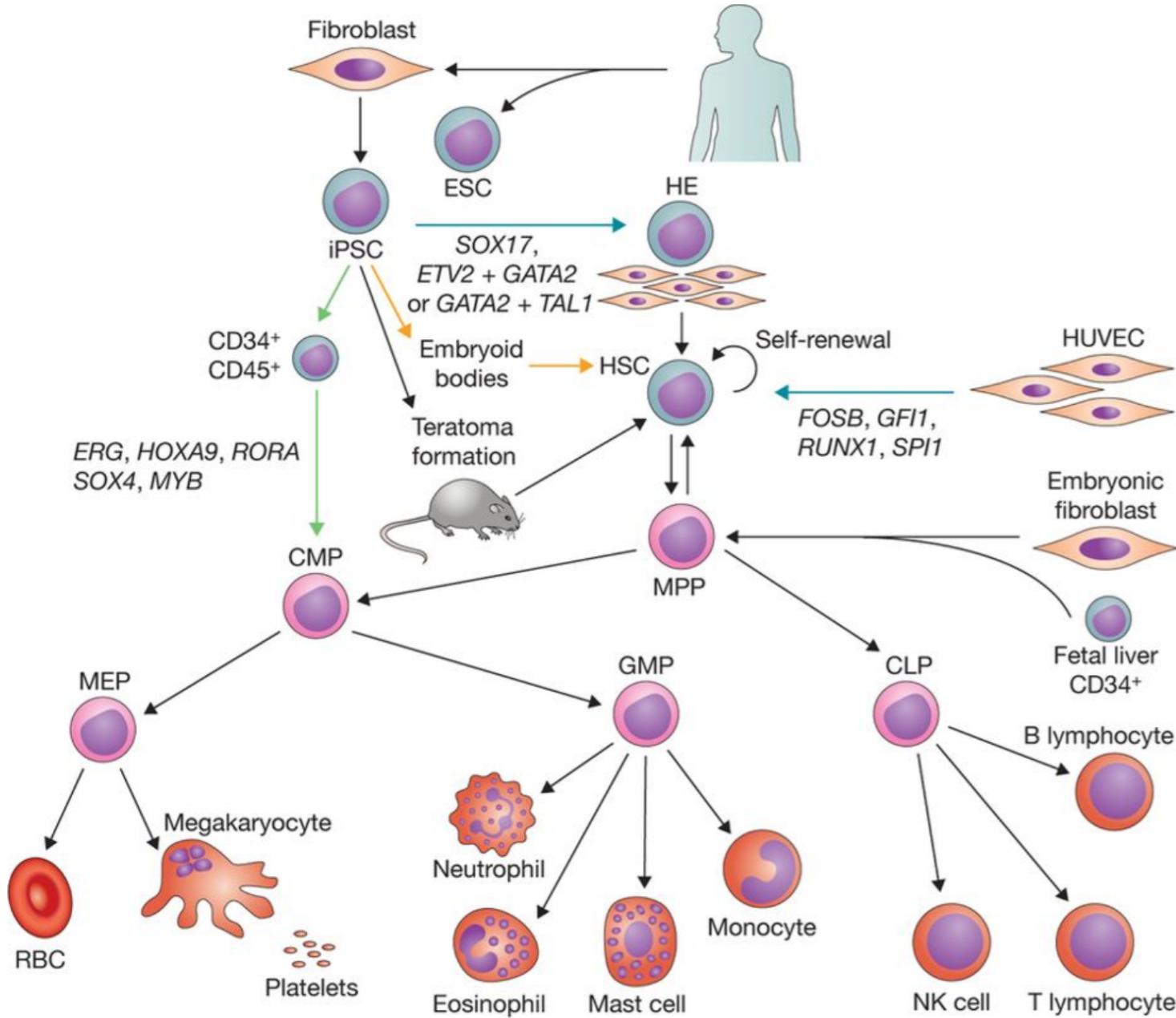
# Overview

## Background

1. Hematopoiesis
2. Possible roles of KDM6B in cell-type specific differentiation
3. KDM6B relevance to disease etymology
4. Project questions
5. Analysis
6. Conclusions

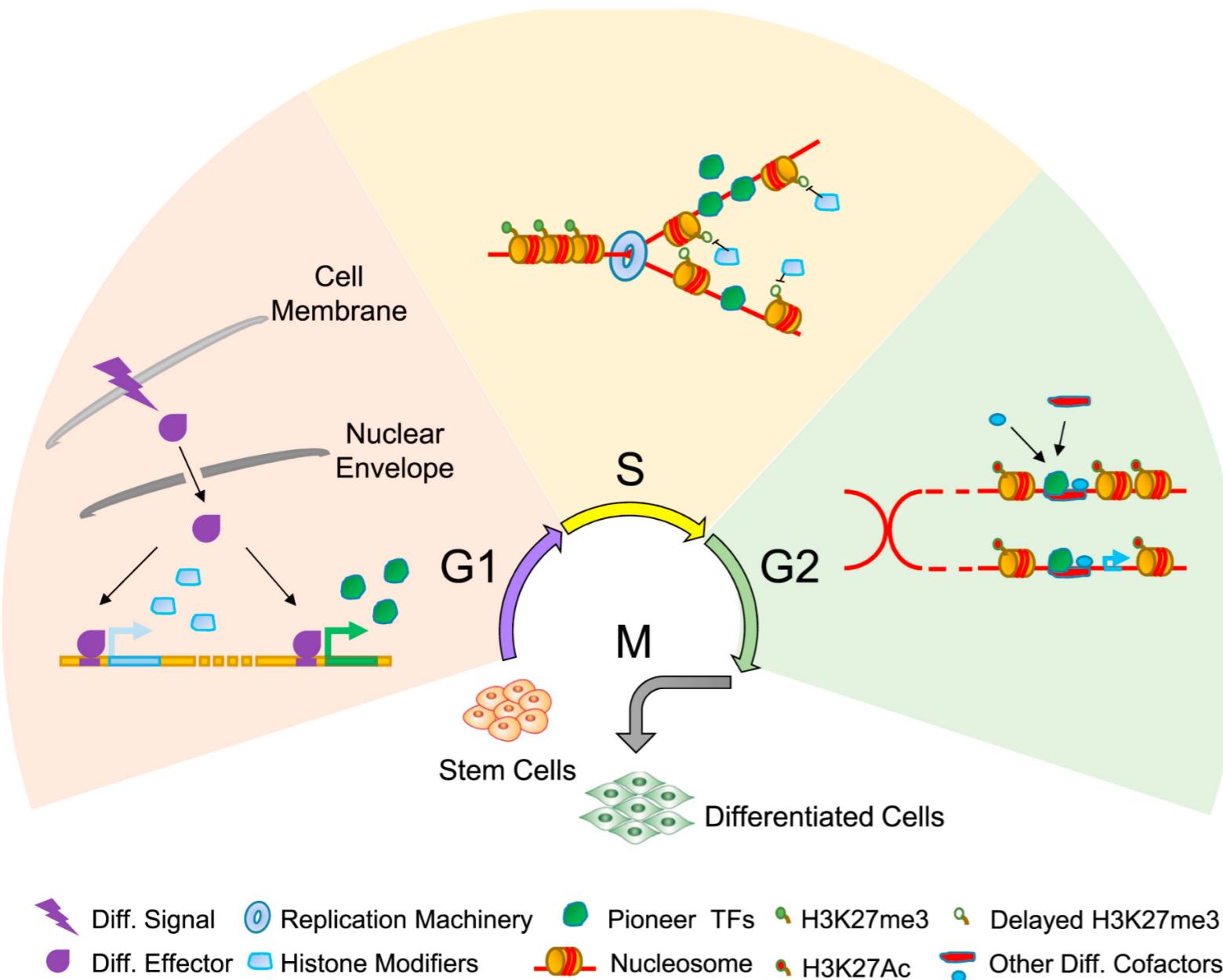
## Computational work

# Hematopoiesis



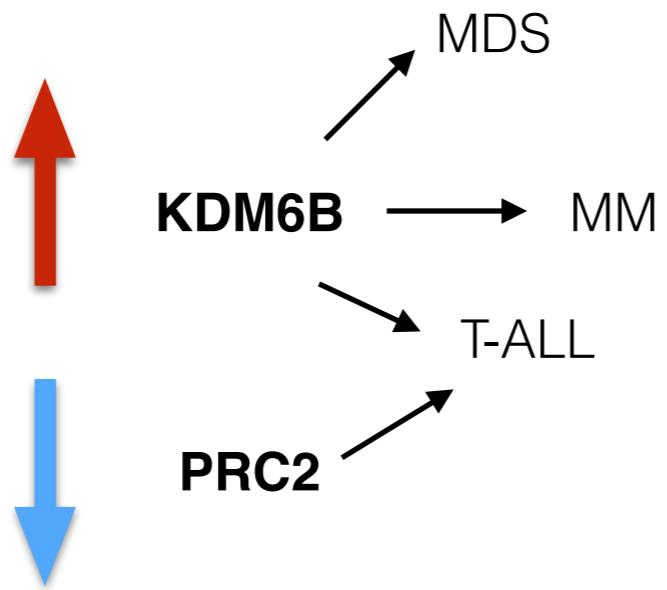
- Process by which all blood cellular components are made
- Adult hematopoietic stem cells (HSCs) possess a self-renewal capacity to regenerate or expand all erythroid, myeloid and lymphoid cell lineages

# Kdm6b (JMJD3) is a H3k27me3/me2 histone demethylase

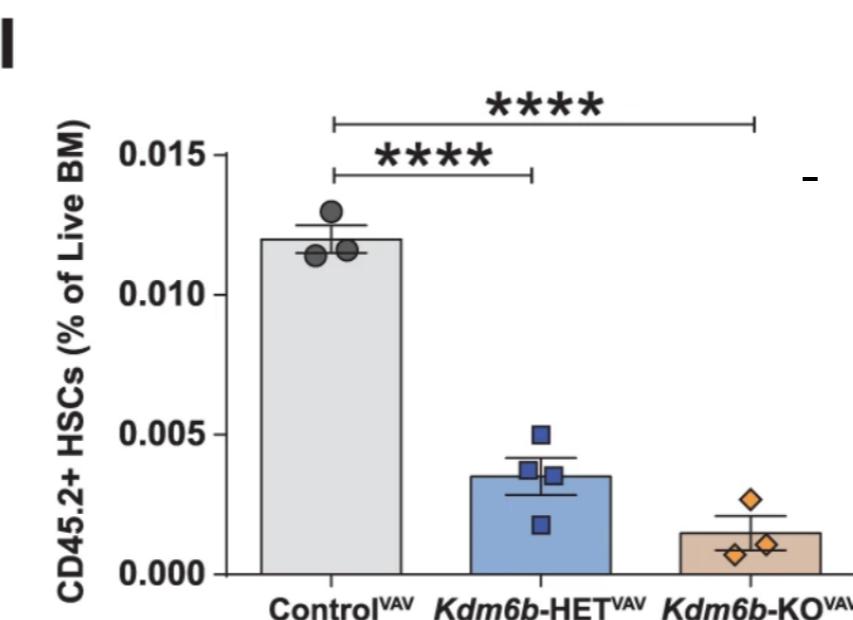
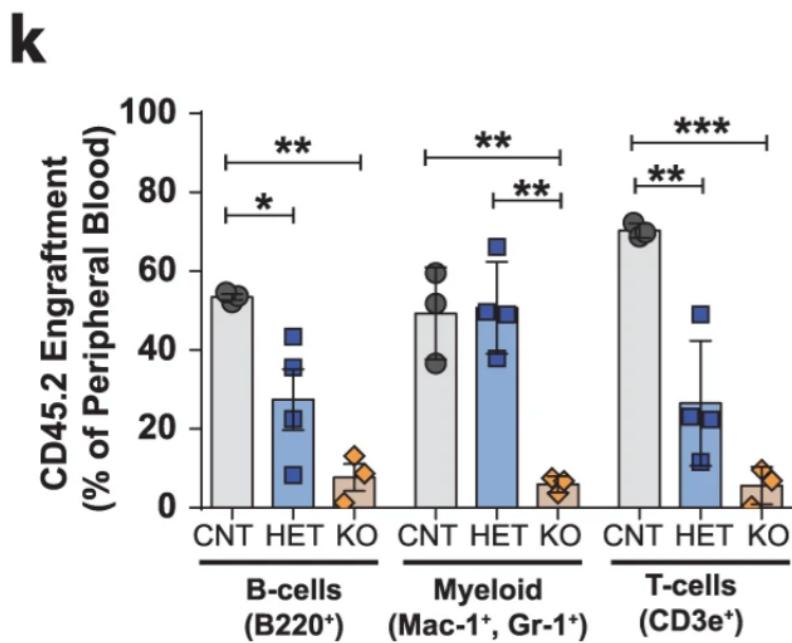


- H3K27me3 is a repressive histone mark for condensed chromatin and low accessibility to TFs
- Antagonizes Polycomb repressive complexes (PCR2) heterochromatin-formation activity by “erasing” repressive H3K27me3 marks

# Kdm6b activity is unregulated in blood disorders and necessary for HSC self-renewal



- These include myelodysplastic syndromes (MDS), Hodgkin's lymphoma (HL), multiple myeloma (MM), and T-cell acute lymphoblastic leukemia (T-ALL)



- 25% of T-ALL cases also involve inactivating mutations of PRC2 complex

# Project question

**Q:** What are the molecular mechanisms mediating self-renewal dysregulation in Kdm6b-deficient adult HSCs

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- Does loss of Kdm6B cause a global repressive environment, leading to downstream silencing of the pluripotent program?

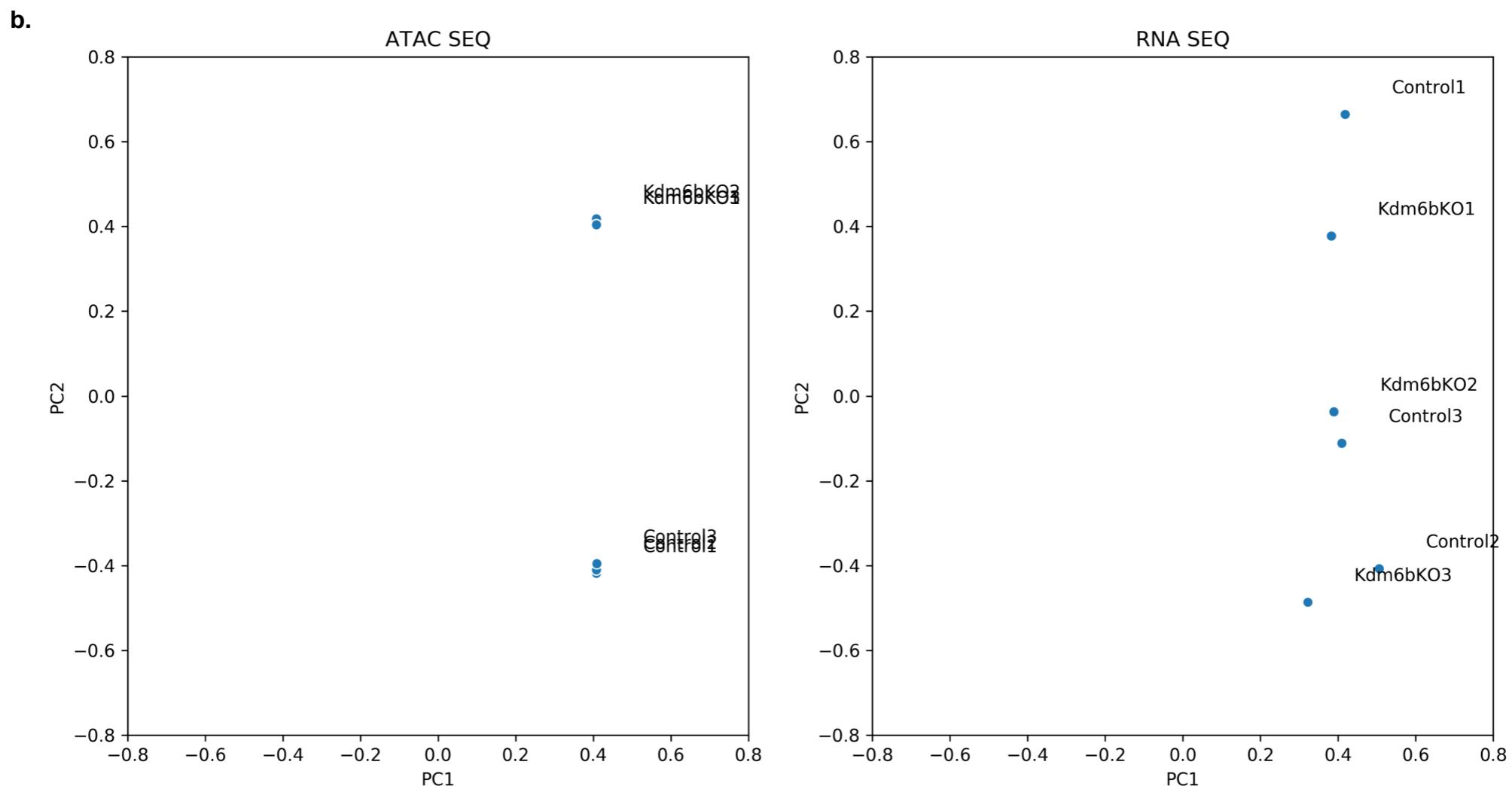
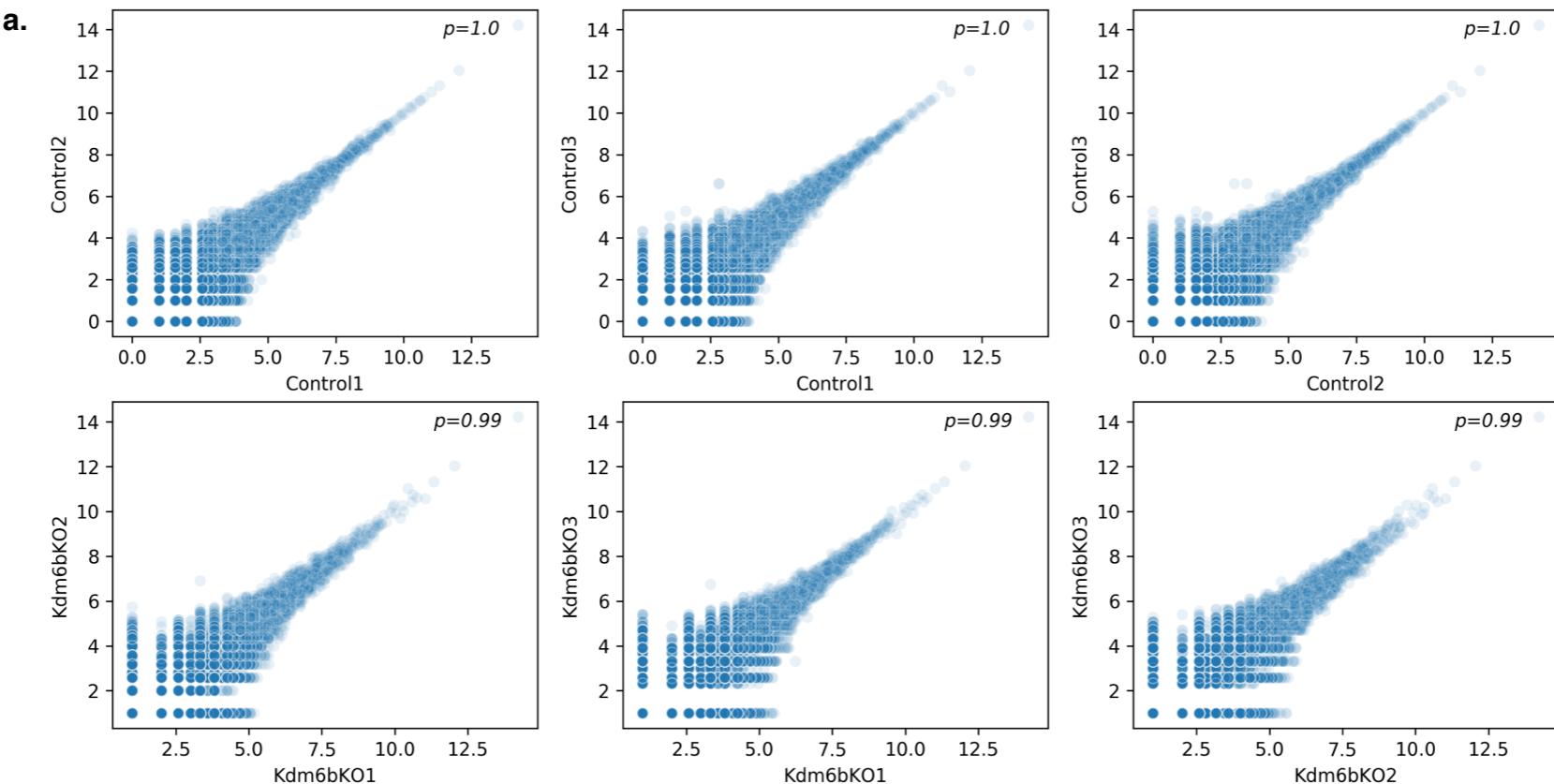
## Workflow

1. RNA-seq and ATAC-seq analysis of WT and KDM6B KO Adult HSCs
2. Differential expression
3. Differential accessible region (DAR)
  - v. non-DAR analysis
4. Differential transcription factor (TF) motif enrichment in WT and KDM6BKO
5. TF Footprinting with dnase2tf

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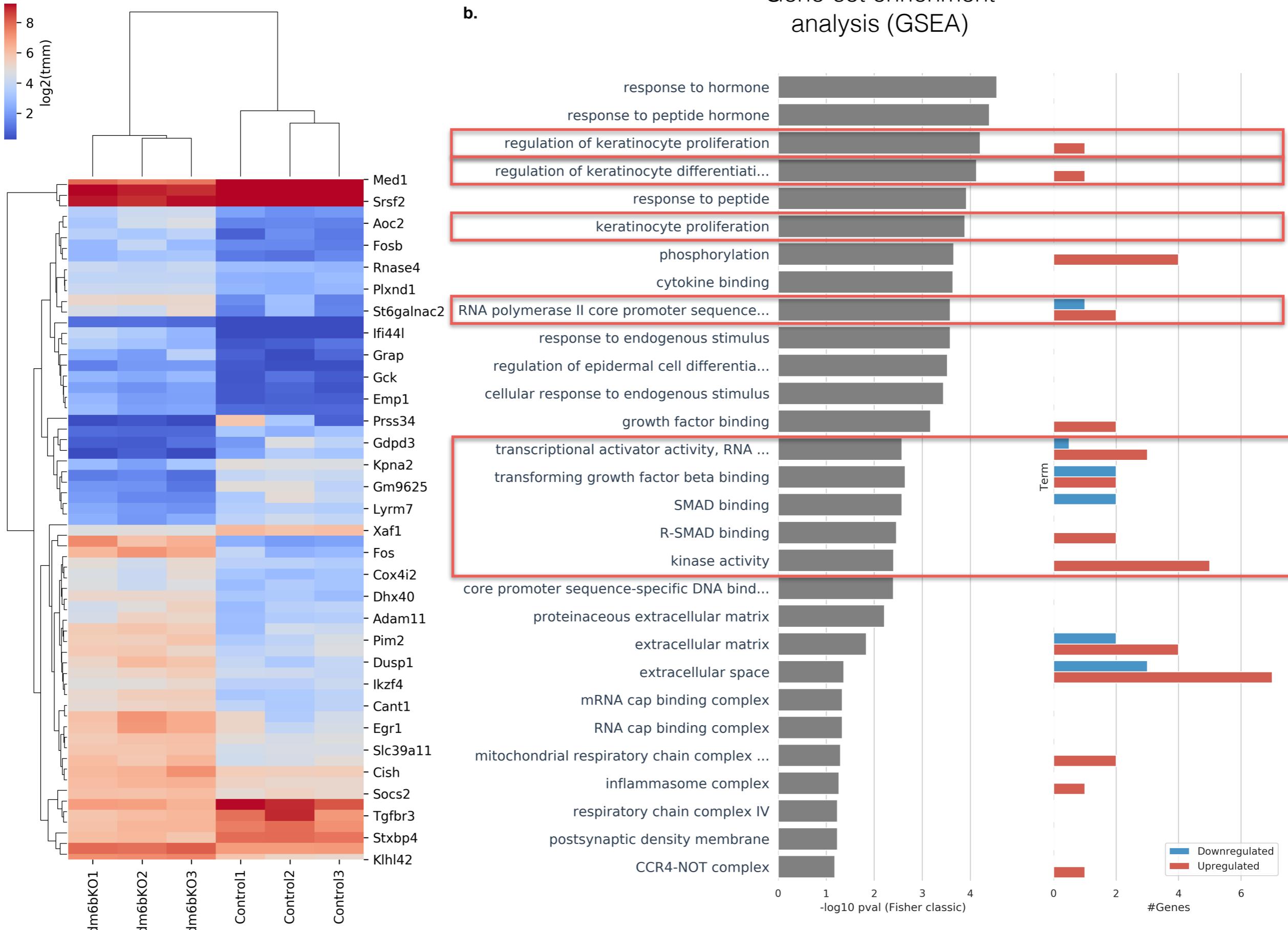
Fig.1



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Fig.2



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Fig.3

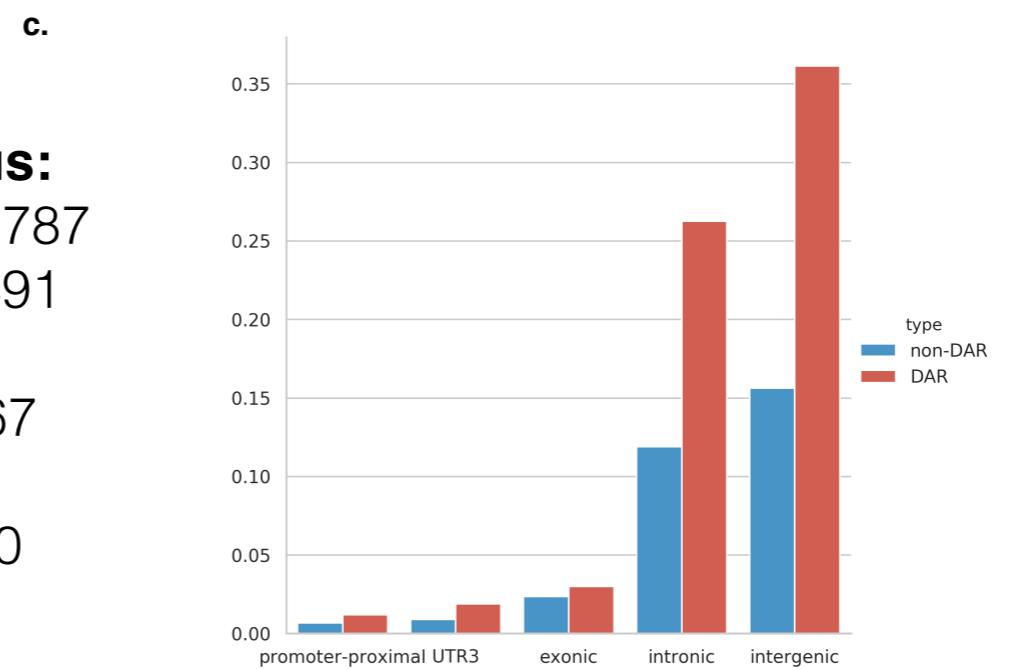
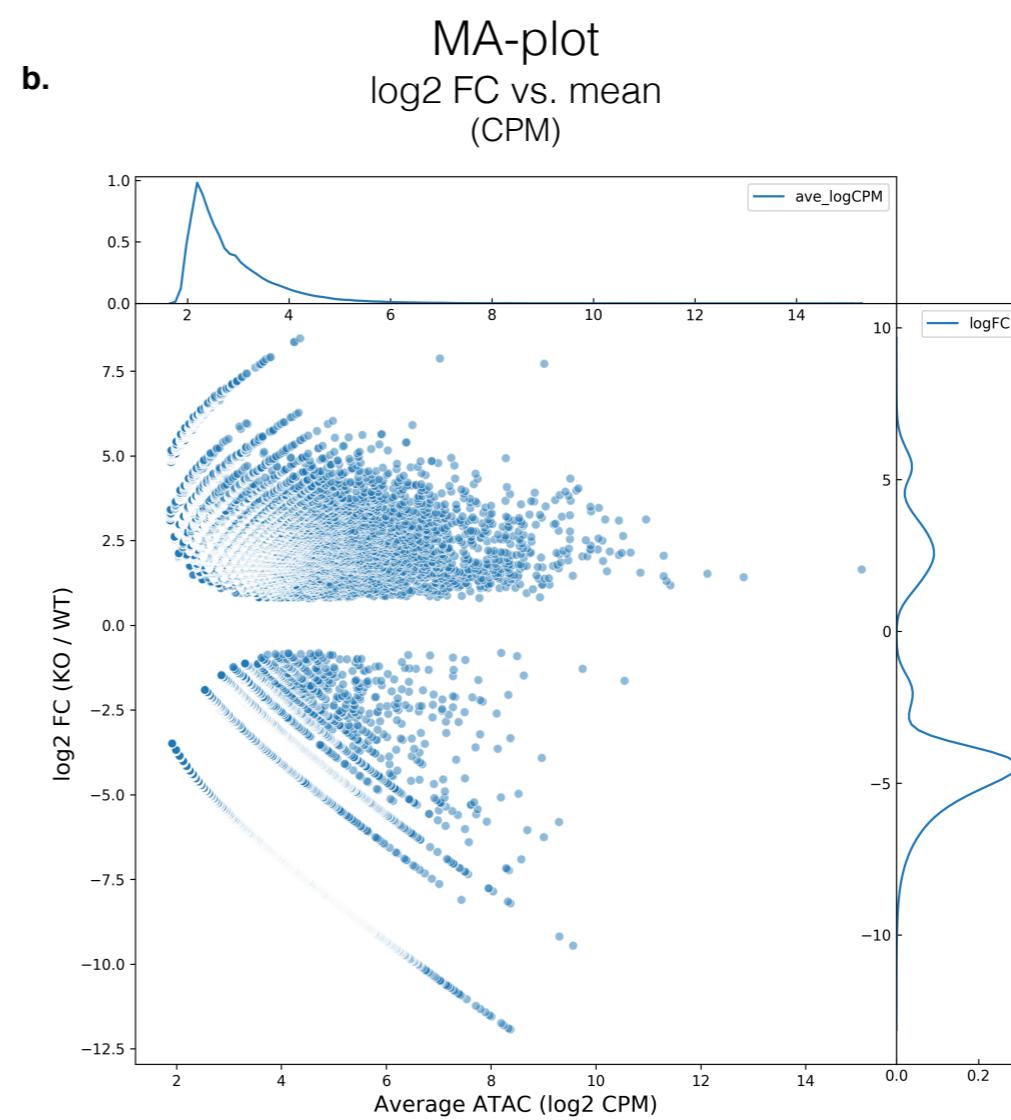
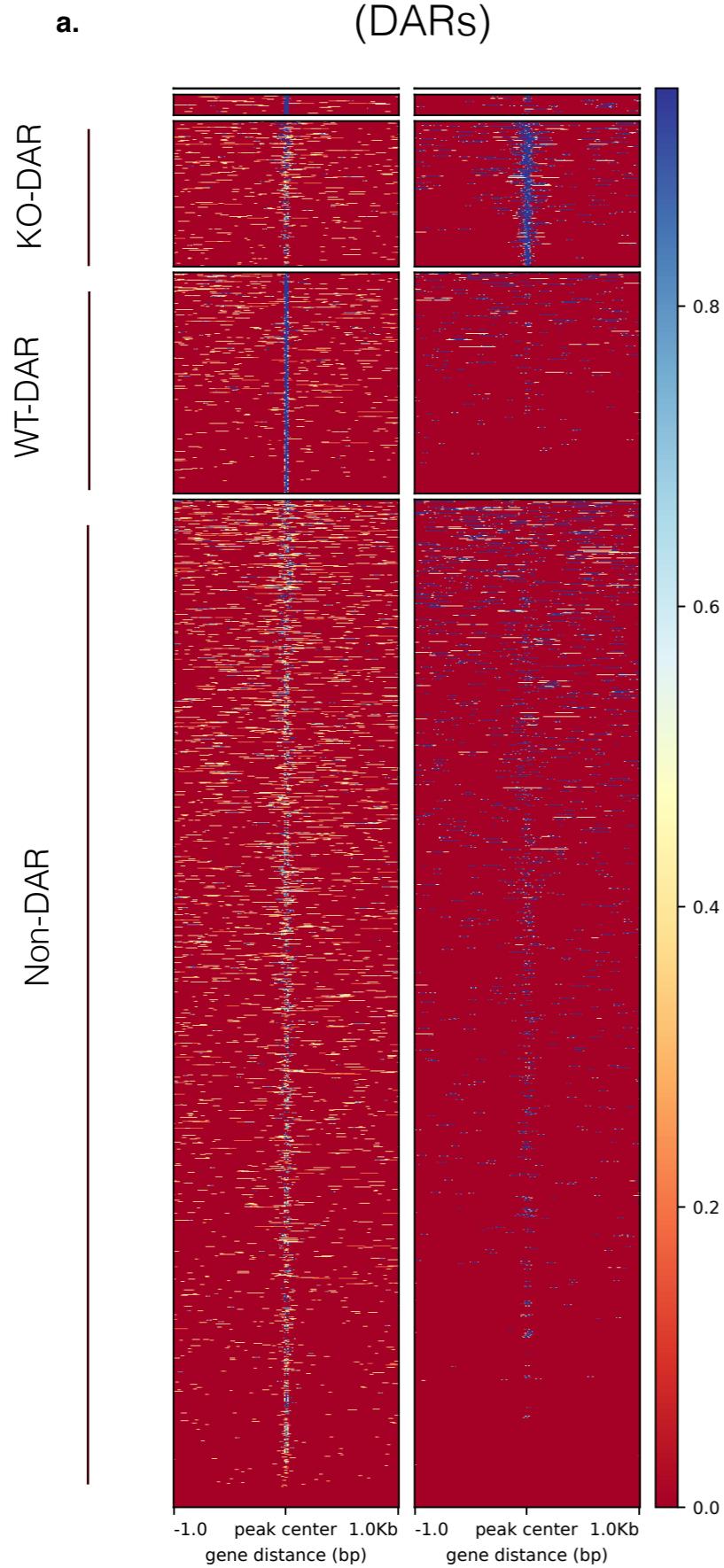
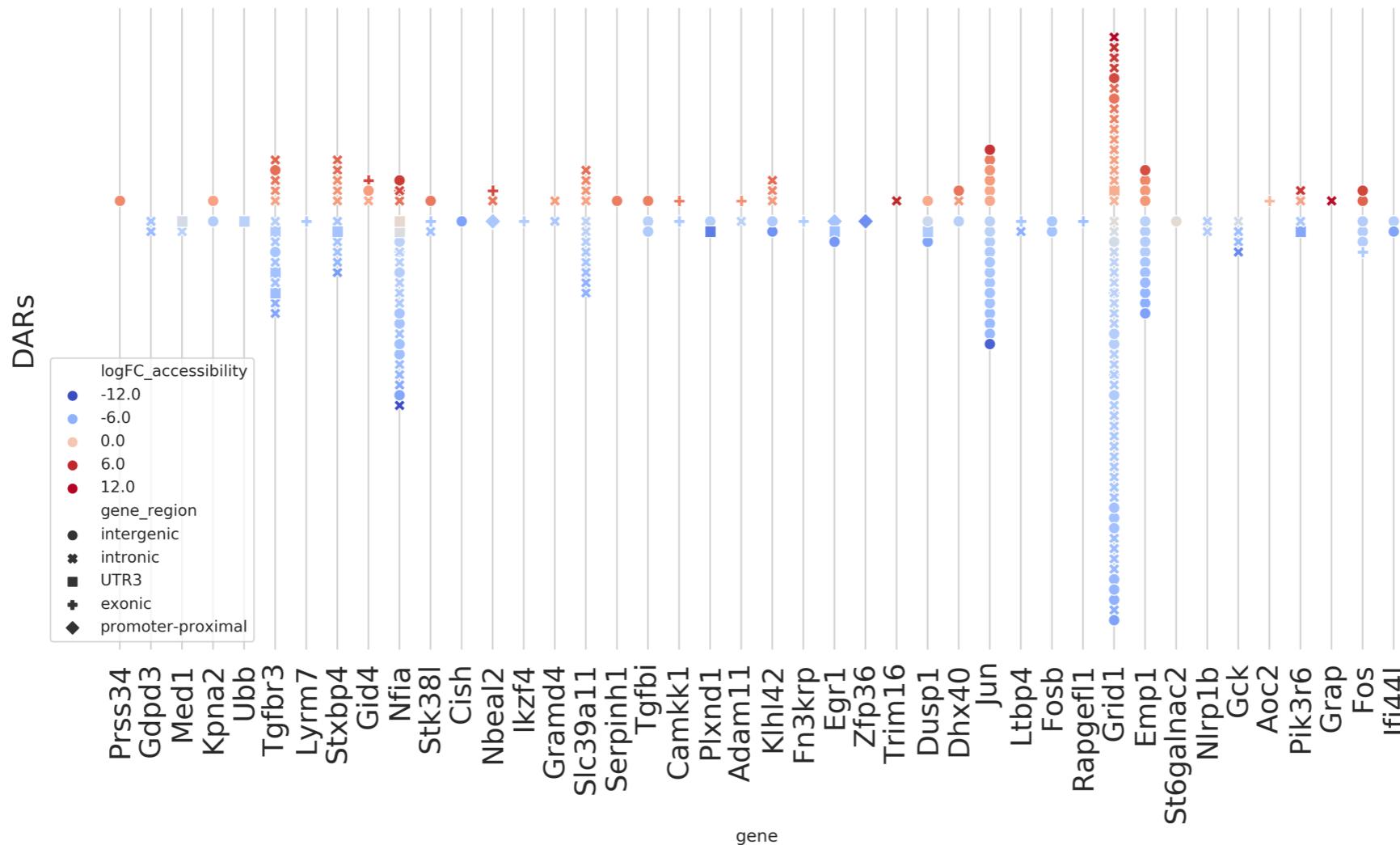
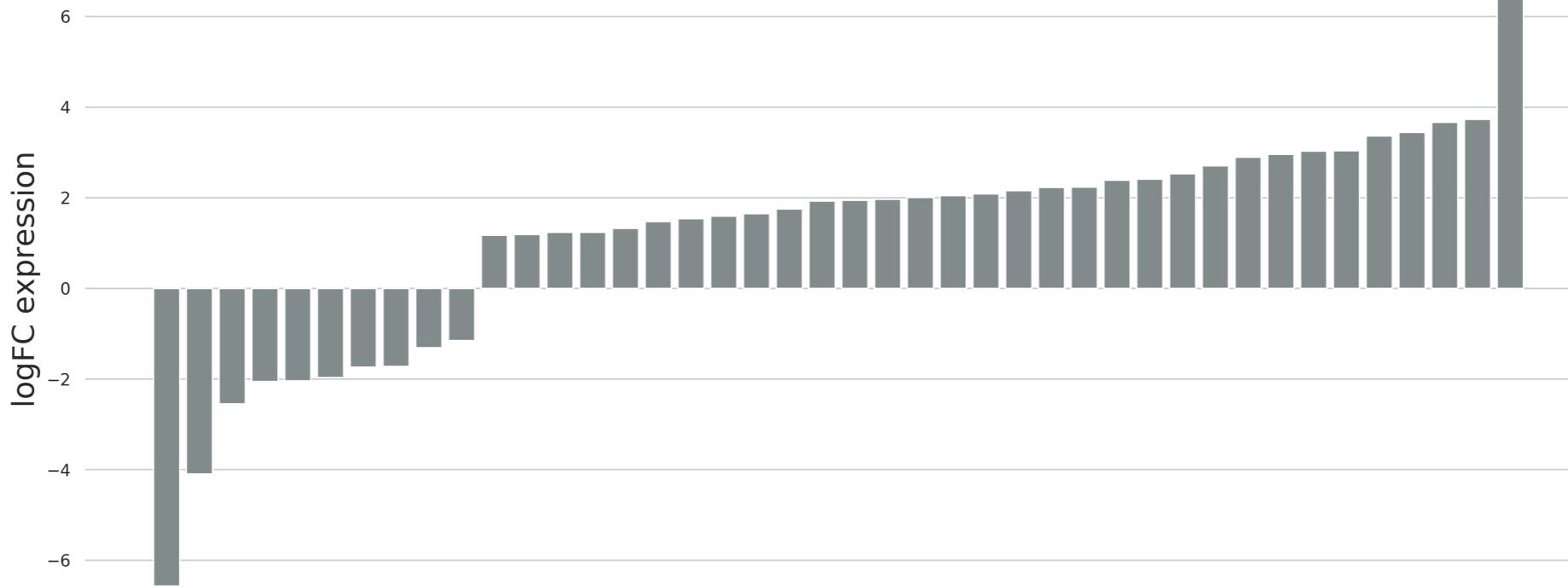
Differentially-accessible regions  
(DARs)

Fig.4

**a.**

## DARs associated with DE genes



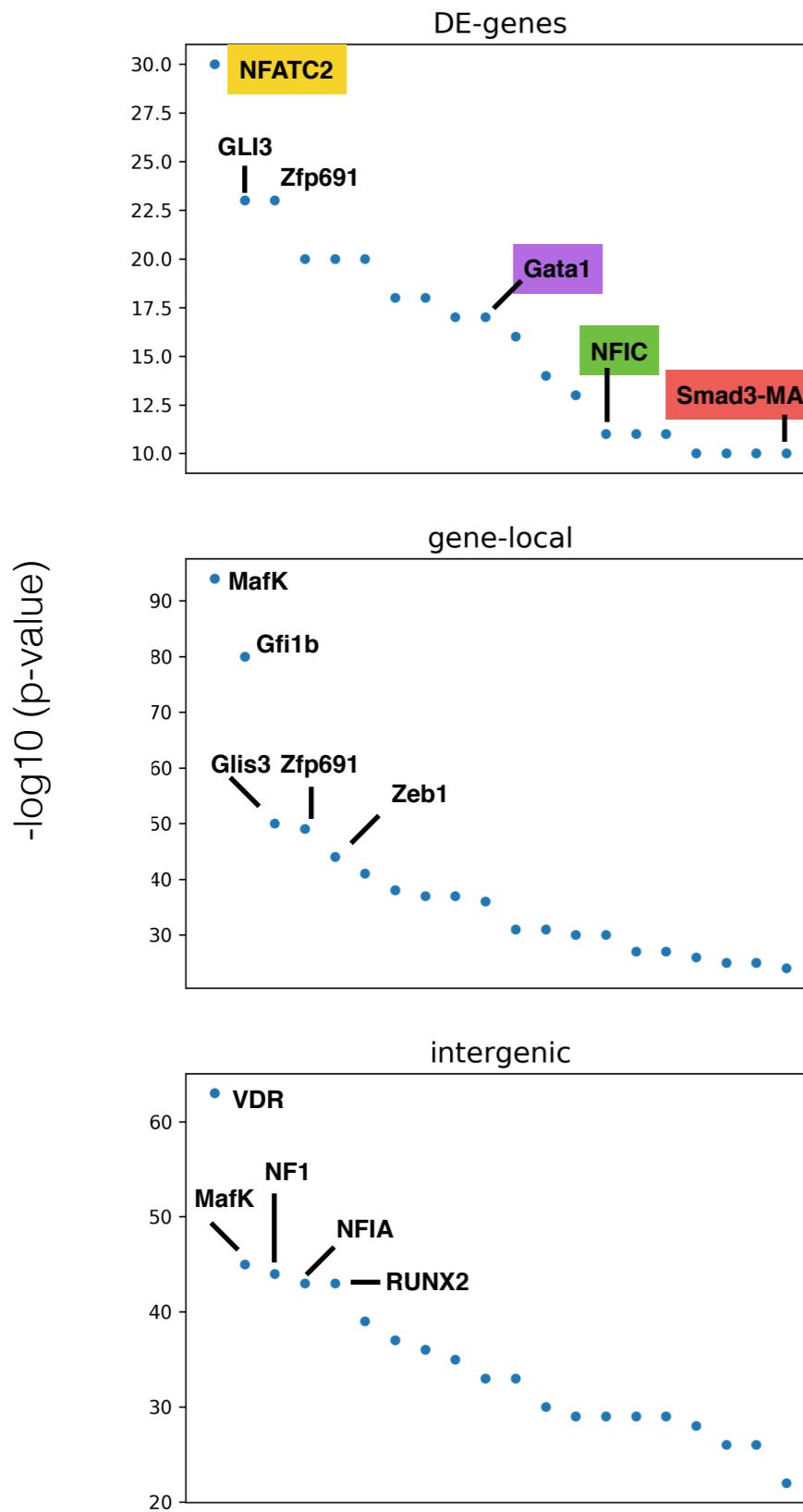
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Fig.5

a.

DAR v. Non-DAR  
denovo motif enrichment



b.

Motif density heatmap of  
DE-gene peaks

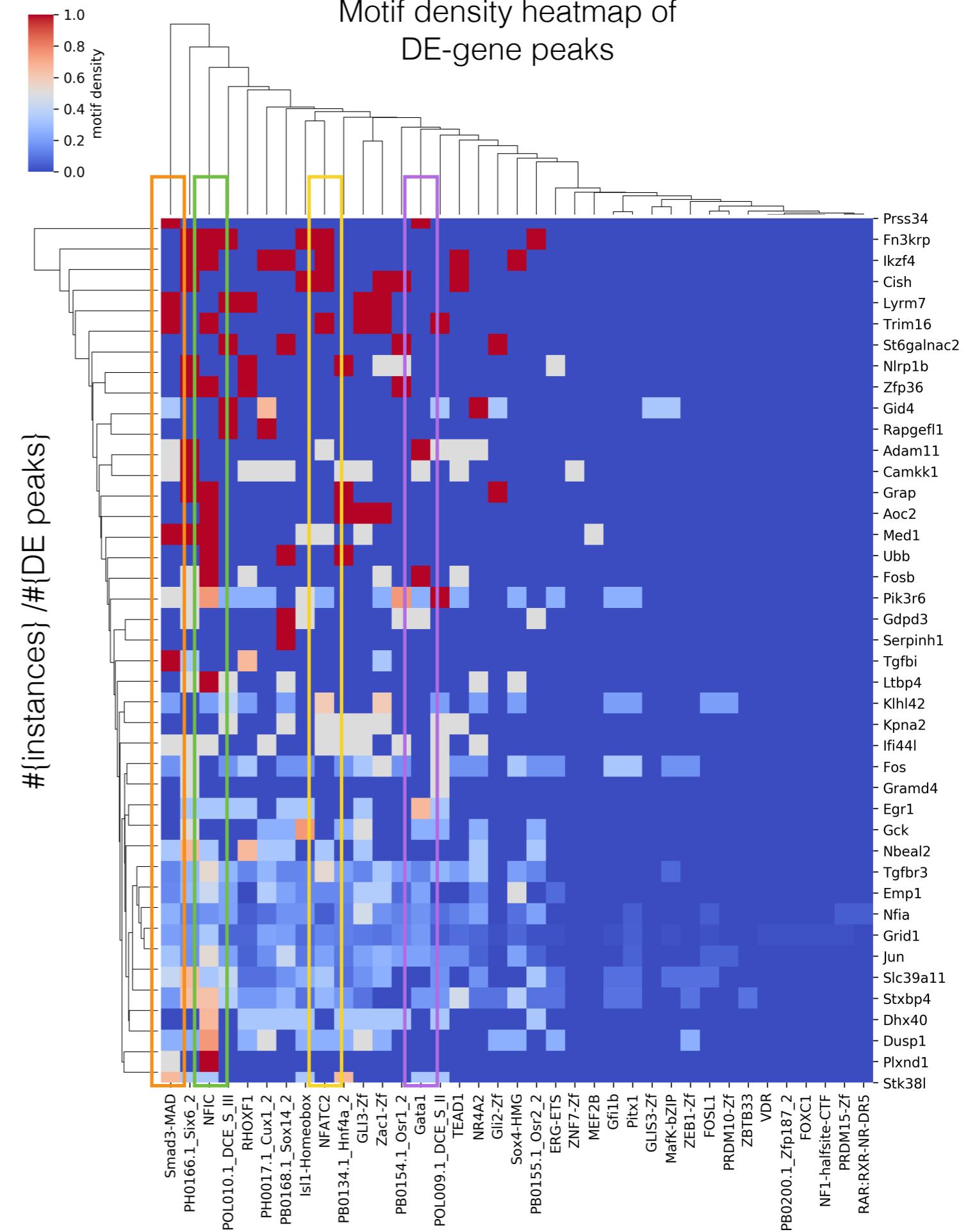
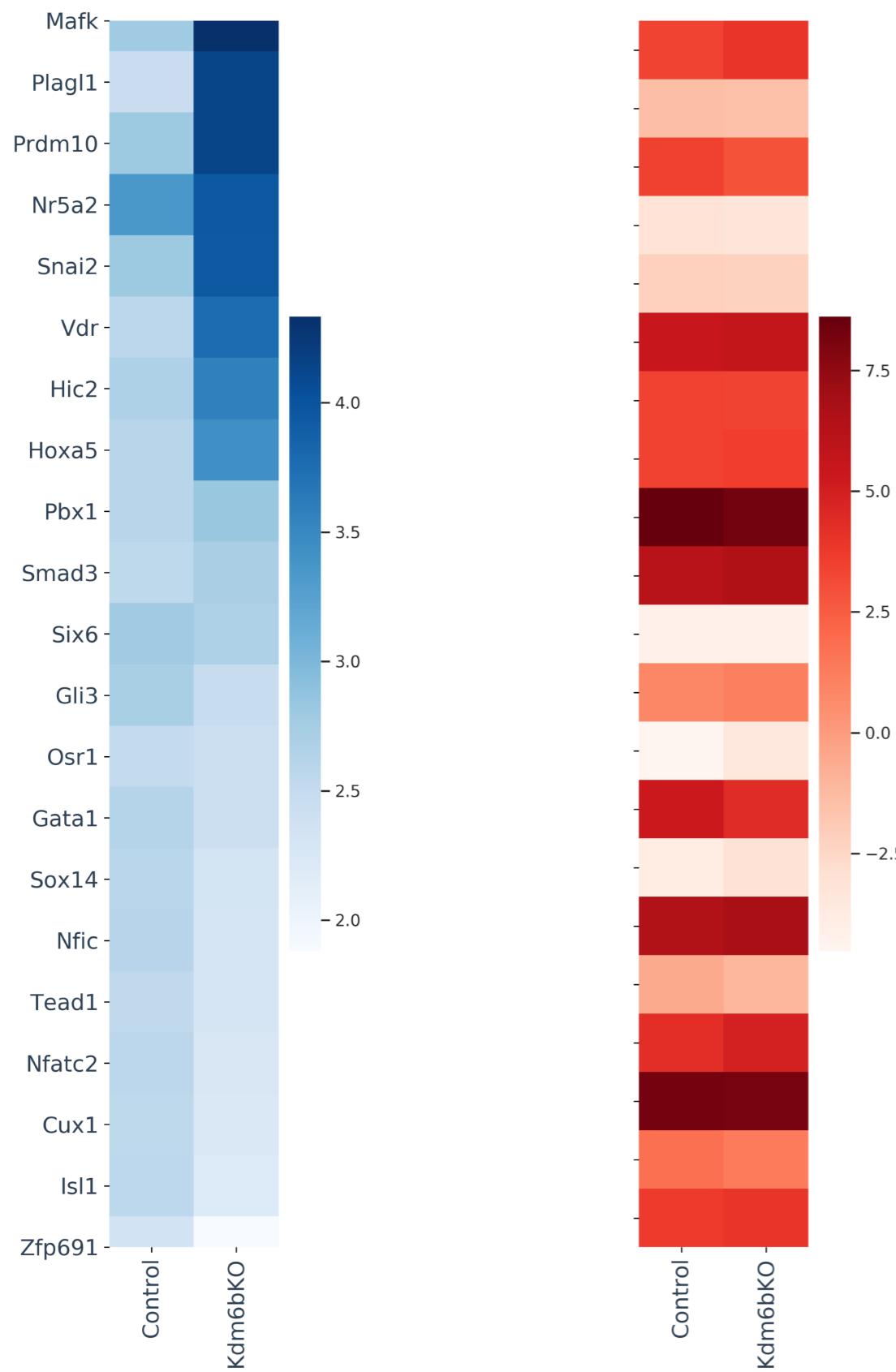


Fig.5

c.

Average motif instance  
peak-accessibility



Transcription factor (TF)  
expression

d.

Gene-set enrichment analysis  
of expressed TFs



0 2 4 6 8 10 12 14 16  
-log<sub>10</sub> pval (Fisher classic)

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Fig.6 KO-specific footprints

a.

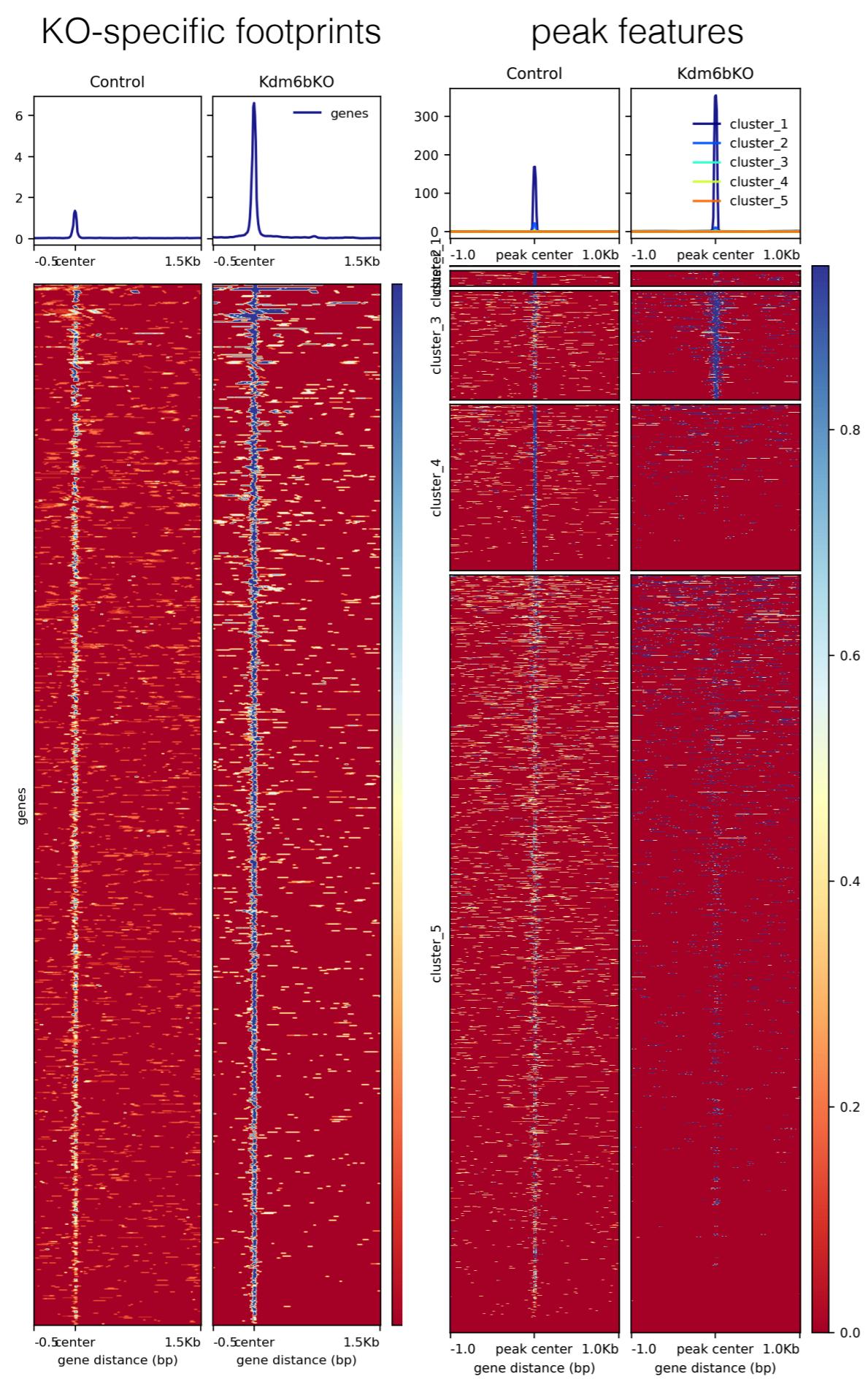
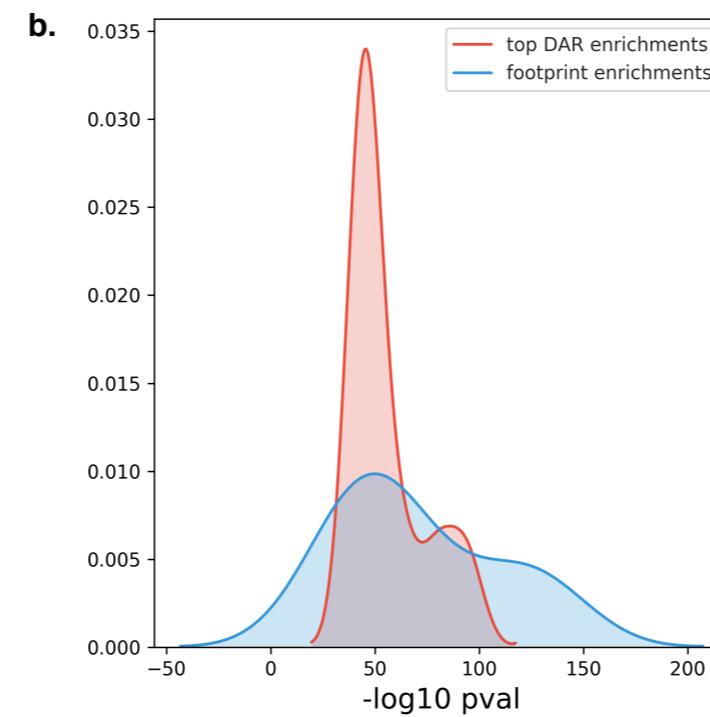
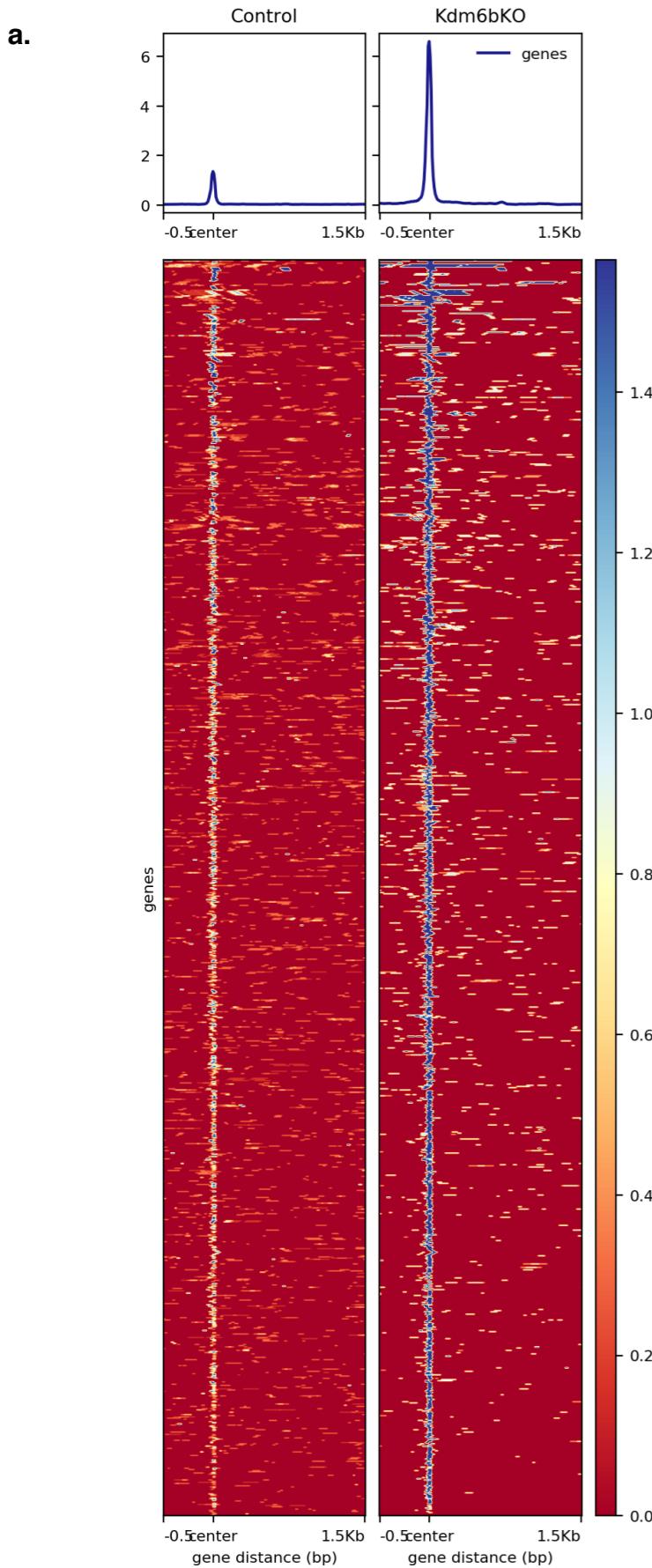


Fig.6



ZNF416(Zf)/HEK293-ZNF416.GFP-ChIP-Seq(GSE58341)/Homer

Match Rank: 1

Score: 0.78

Offset: -2



HIC1(Zf)/Treg-ZBTB29-ChIP-Seq(GSE99889)/Homer

Match Rank: 3

Score: 0.67

Offset: -1



RBPJ/MA1116.1/Jaspar

Match Rank: 2

Score: 0.71

Offset: 0



i. enrichment in footprints by group

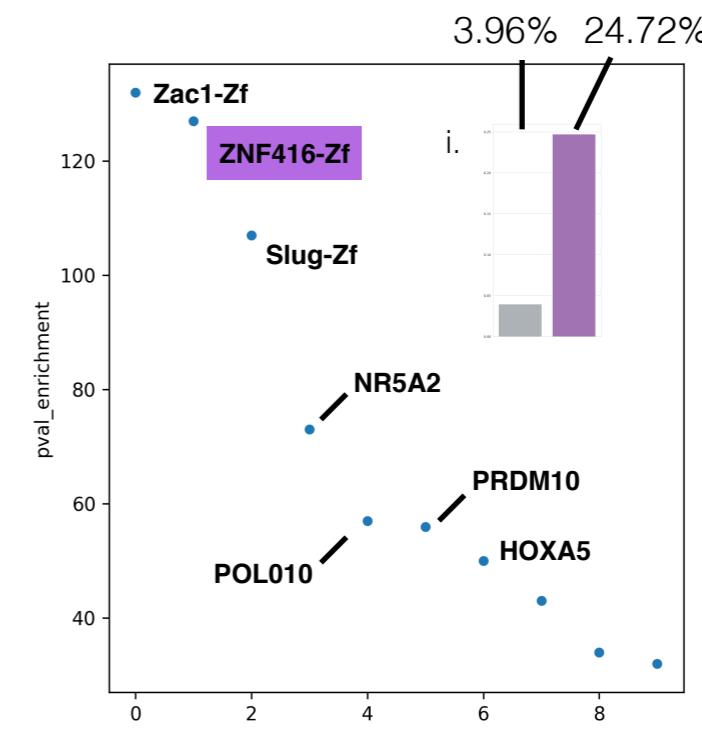
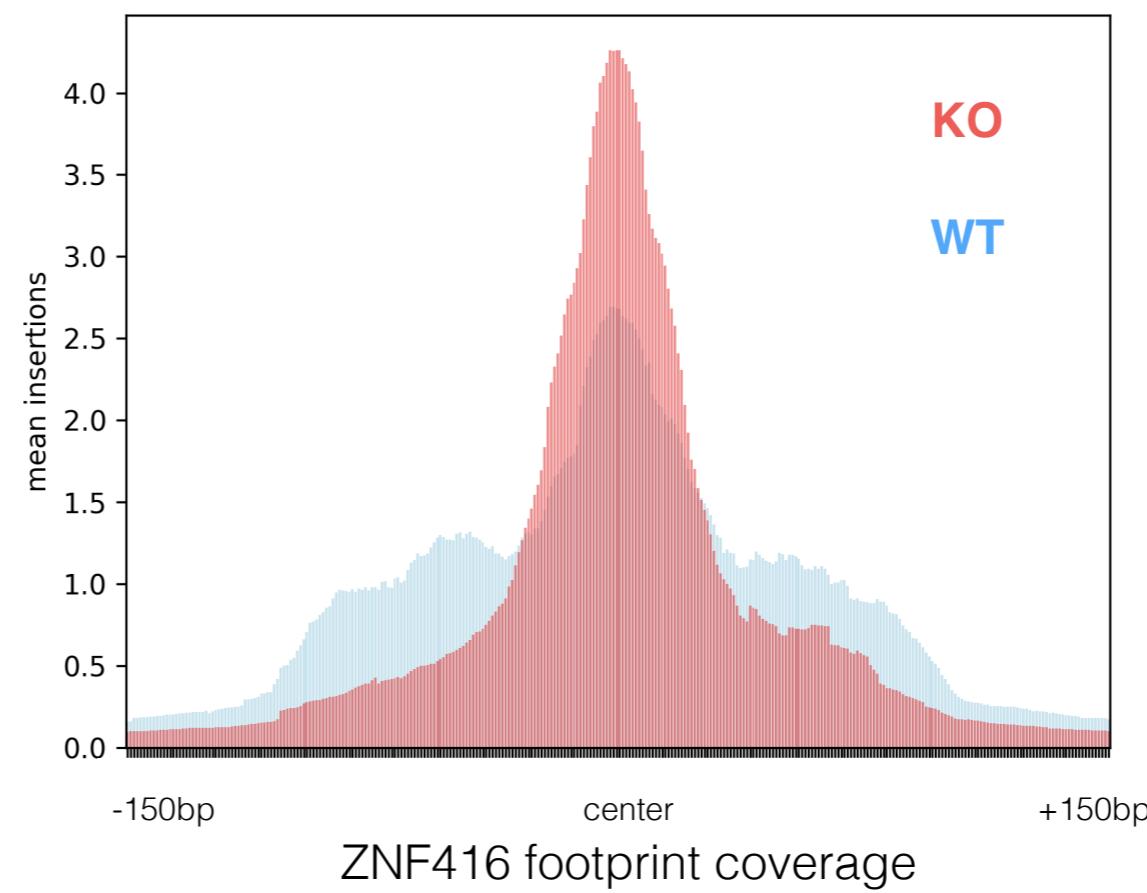
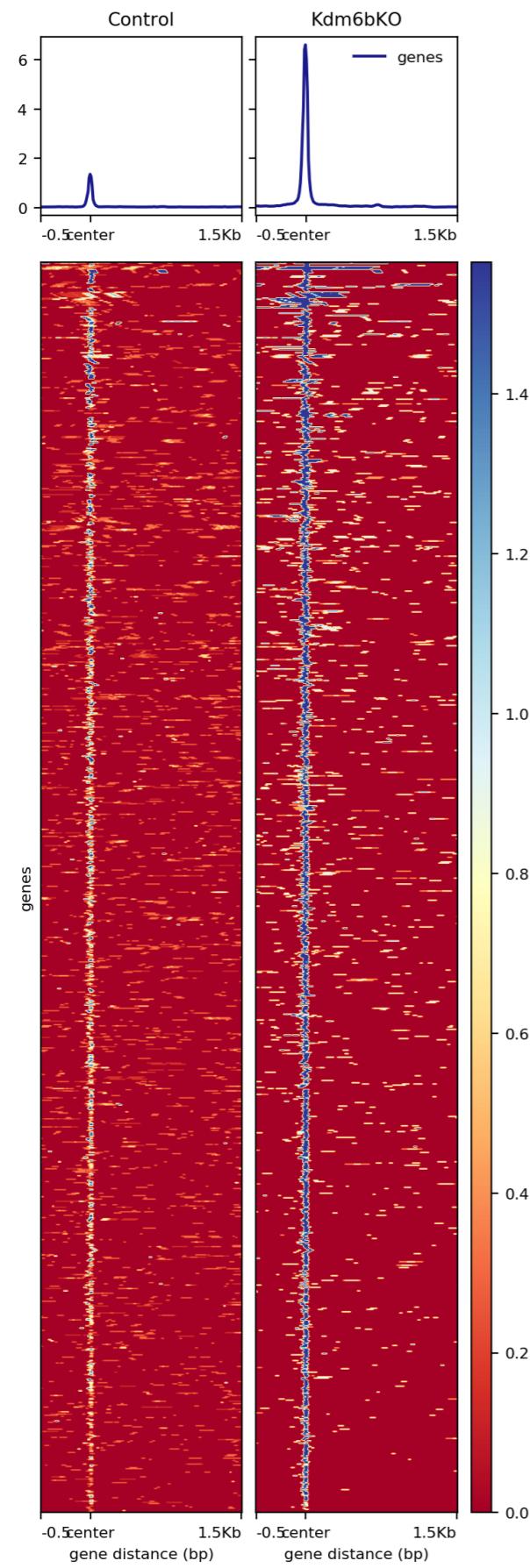
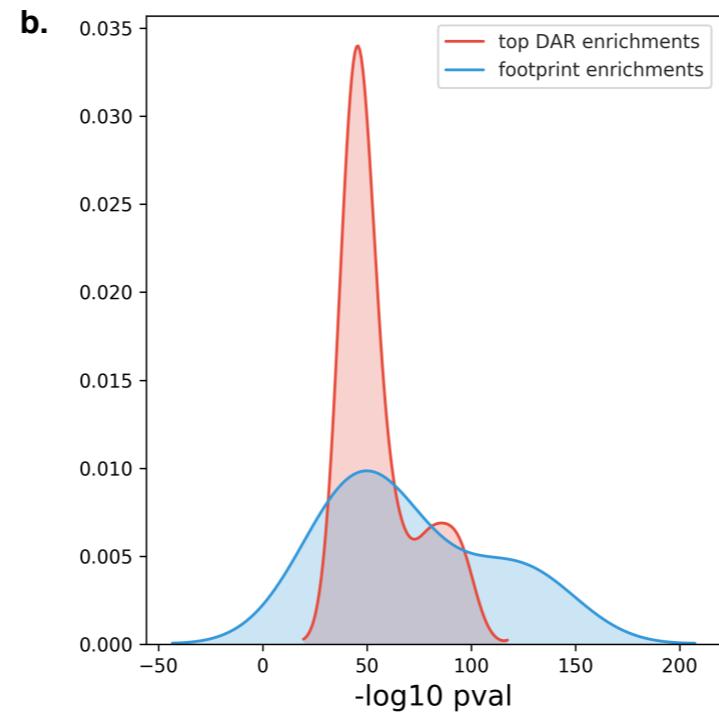
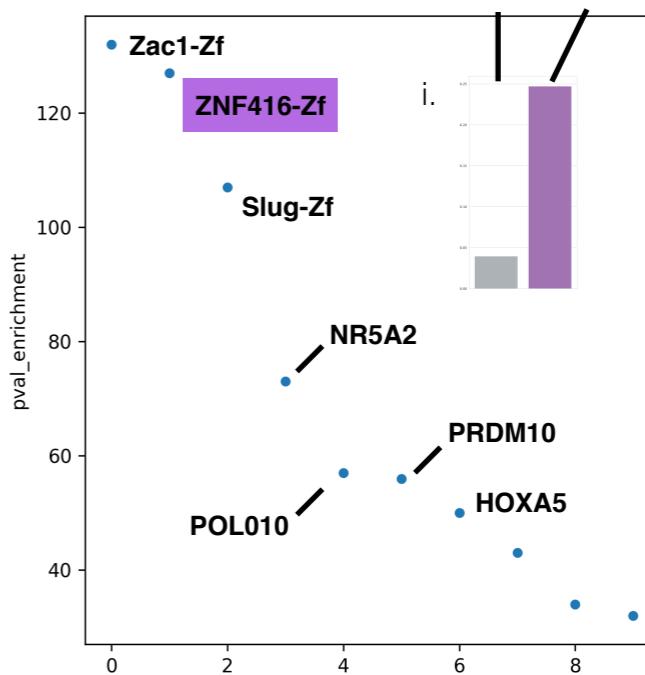


Fig.6

## i. enrichment in footprints by group

3.96% 24.72%

KO-footprints = 1076  
WT-footprints = 10,664



# Conclusions

1. KDM6B-KO causes highly-local upregulation of “pro-keratinocyte” differentiation program (downstream effects are expression of cell-type specific AP1/Fos signal transducers)
2. KDM6B Adult HSCs paradoxically show a global decrease in accessibility relative to wild-type though a greater proportion of upregulated genes
3. Lack of motif co-enrichment in KO-specific footprints suggests that very specific “differentiation factors” are involved in orchestrating loss of self-renewal

