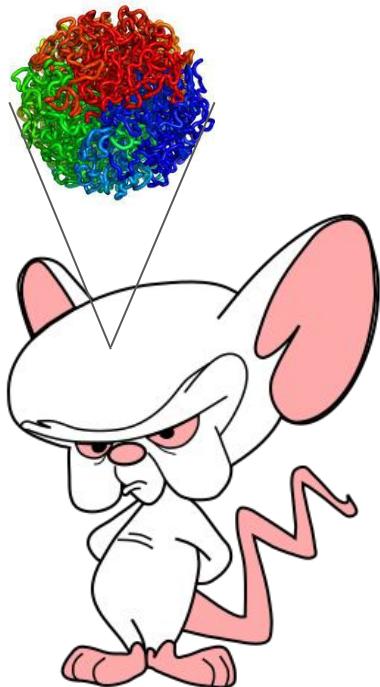


Mouse Brain Epigenome



Students:

*Ekaterina Kashuk
Alisa Fedorenko
Leonid Sidorov
Ksenia Kubenko*

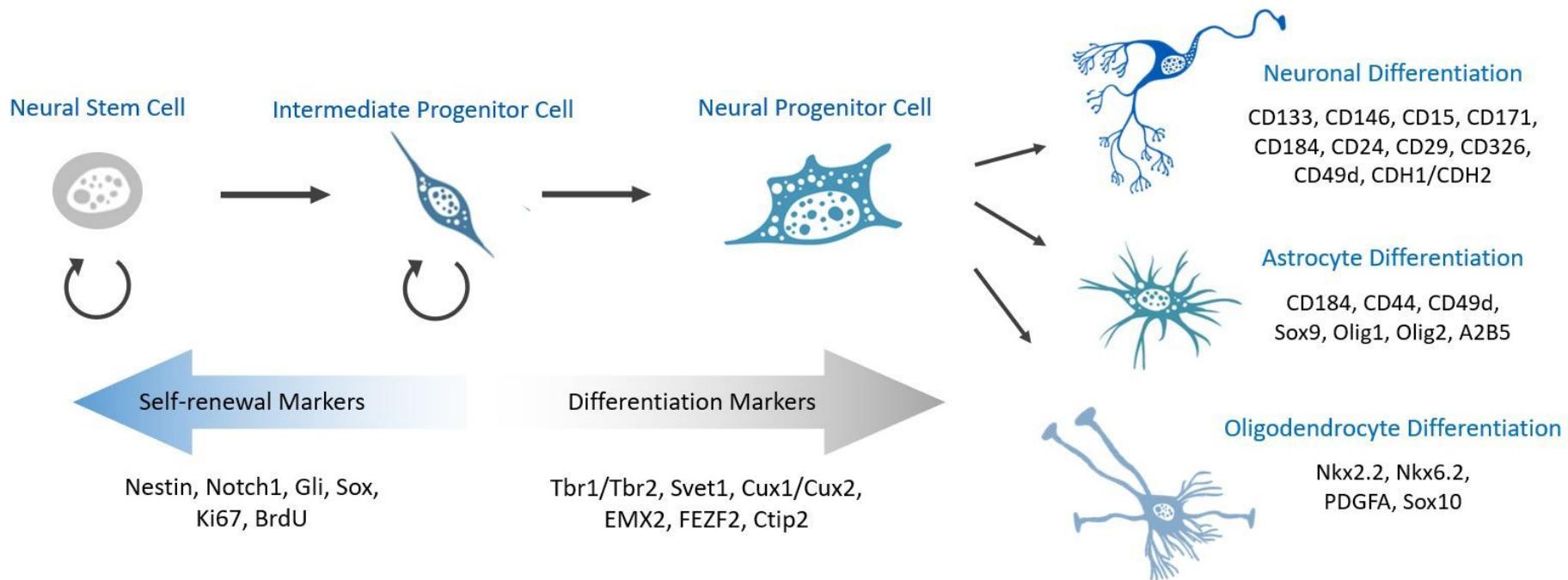
TA - Anna Kononkova

Goals of our project

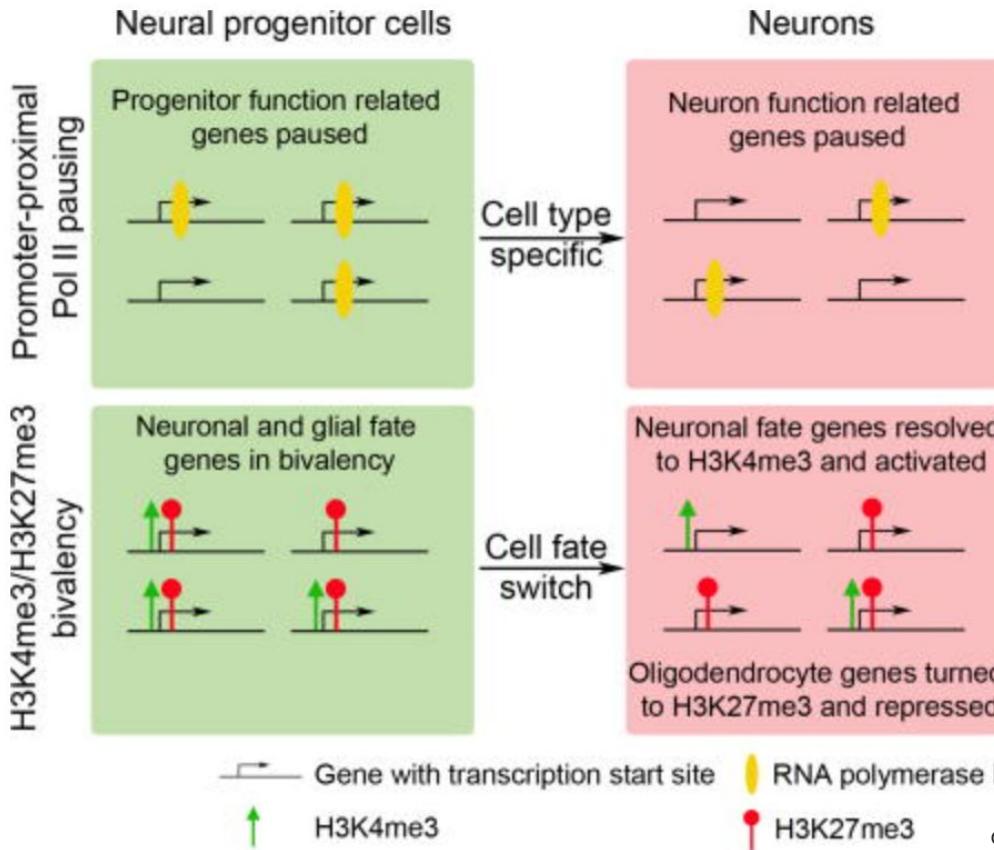
1. To compare neuronal and glial **H3K9me3 track around the H3K27me3 regions** in neurons, to investigate **Ring1B (PRC1) linkage with H3K27me3 marks** - *Alisa Fedorenko*;
2. To detect **significantly interacting regions** (fithic), to identify H3K27ac track for open chromatin, to compare this track around the H3K27me3 - *Leonid Sidorov*;
3. To find out a **correlation between compartments, TADs and gene expression** - *Ksenia Kubenko*;
4. To explore **chromatin difference in neuronal (NeuN+)** and **non-neuronal (NeuN-)** nuclei (TADs density, loops prominence, compartment changes) - *Ekaterina Kashuk*.

Chip-seq analysis

Neural and Glial development

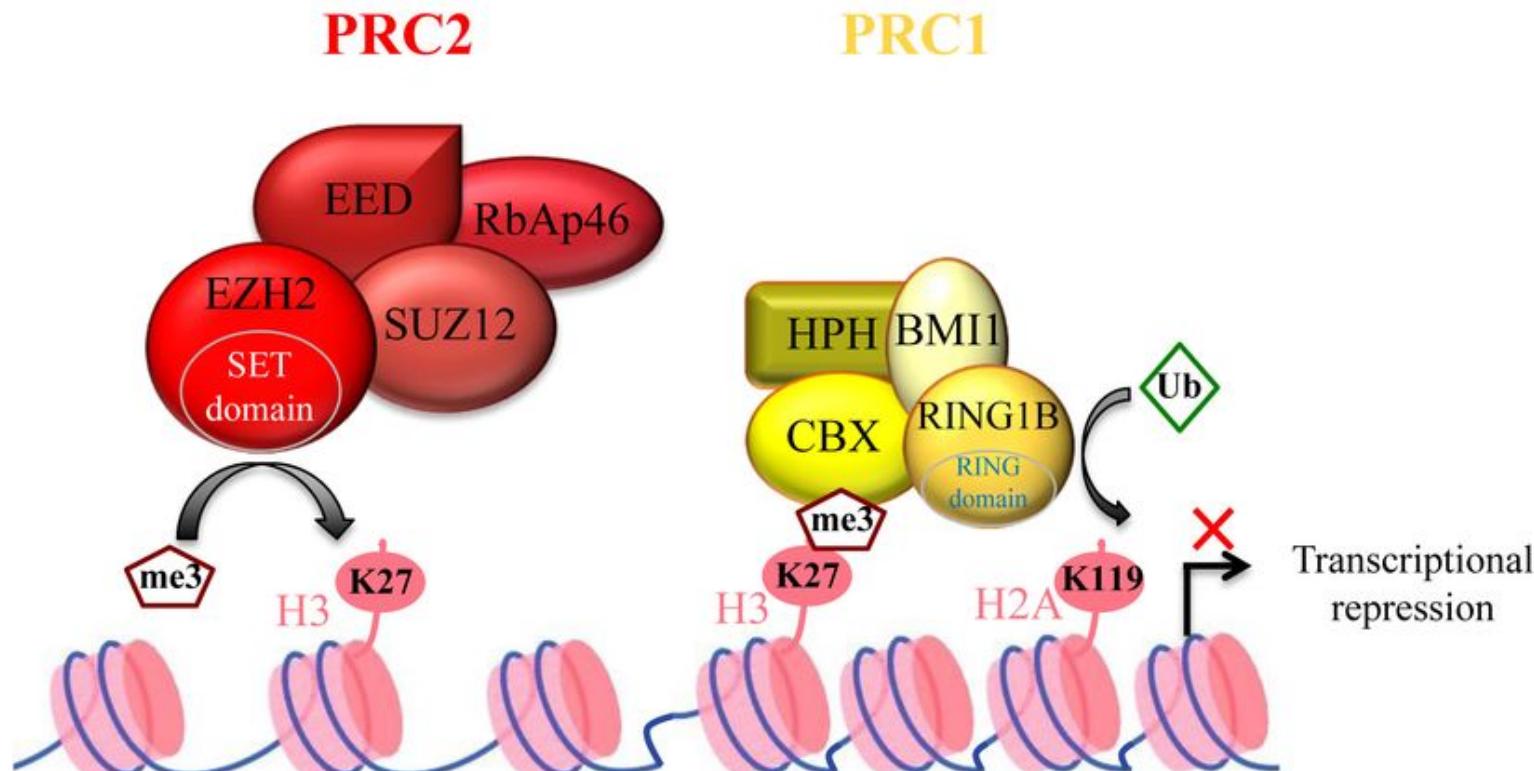


H3K27me3 role in neural and glial development



- H3K27me3 signal in promoters and enhancers distinguishes the progenitors from the differentiated cells
- H3K27me3 identifies the differentiation path of the neural stem cells (NSCs) to the glial cells

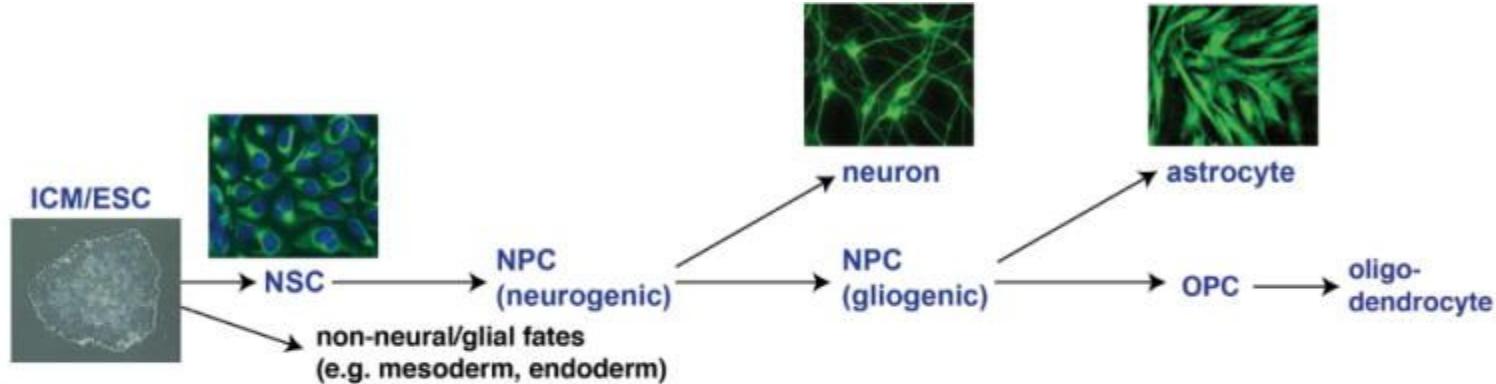
Polycomb association with H3K27me3



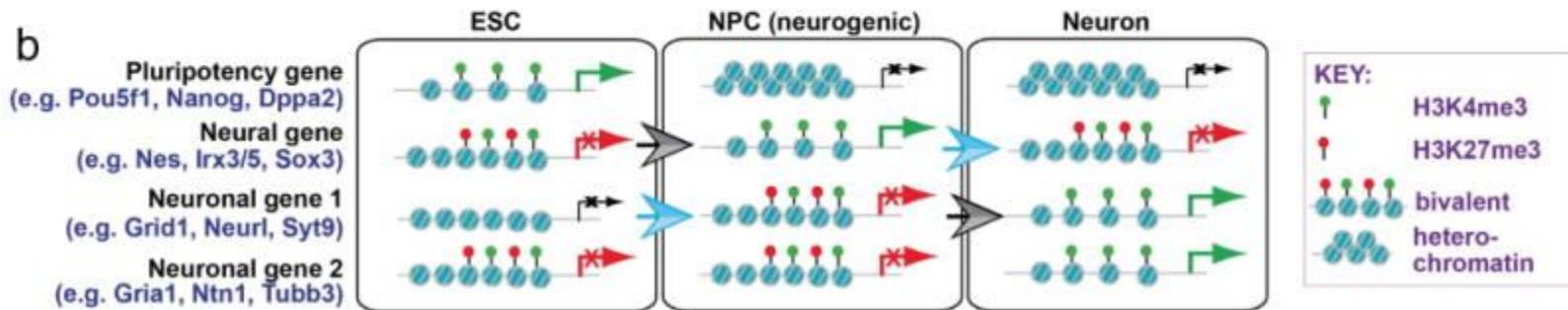
Polycomb group (PcG) proteins are central components of epigenetic regulation

H3K27ac and its role here

a



b



ChiP-seq data

Sorted neural cells (NeuN+) and non-neural (NeuN-) derived from cortical tissue of mice

- H3K9me3 mark
- H3K27ac mark

Differentiated neural progenitor cells (NPC) and cortical neurons (CN) *in-vitro* of mice

- H3K27me3 mark
- Ring1B mark

no glial cells!

ChiP-seq workflow

Tracks:

H3K27me3
H3K27ac
Ring1B

deepTools



unaligned reads
FASTQ files

```
GATCGCTTAATACCTCAGAAGCATGCTC  
GCTCATTAACTCAGAAGCATGCTCGGT  
GCATGCTGATTGCGTTACCTCAGG
```

- ✧ multiBamSummary
- ✧ computeGCBias
- ✧ correctGCBias
- ✧ bamPEFragmentSize

aligned reads
SAM/BAM files

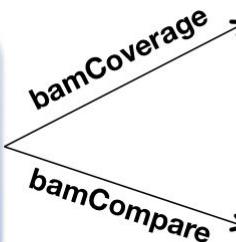
perhaps filtered &
bias-normalized

bowtie,
BWA,
STAR,
...

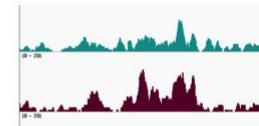
MACS2

BAM

Peak Calling

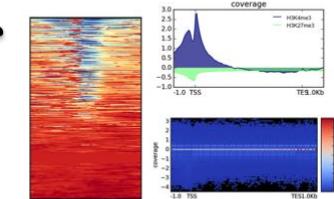


- ✧ plotPCA
- ✧ plotCorrelation



DOWNSTREAM ANALYSES

- ✧ multiBigwigSummary
- ✧ bigWigCompare
- ✧ computeMatrix



- ✧ plotHeatmap
- ✧ plotProfile

nf-core/chipseq



Peaks:

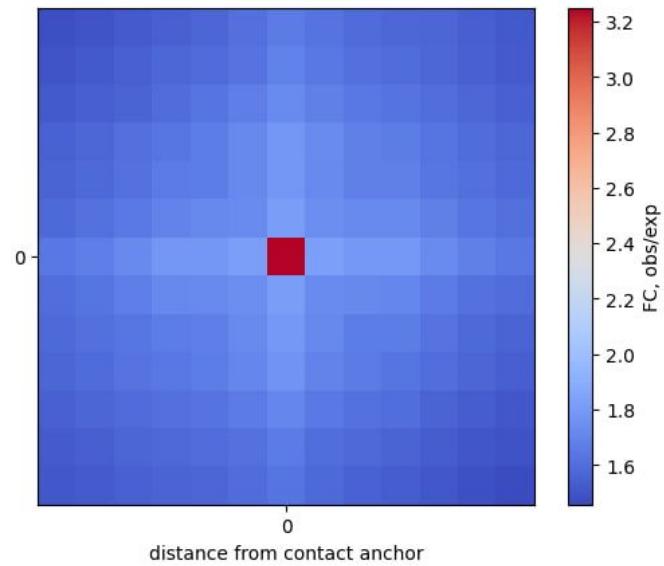
CN_H3K27me3 (filtered to sig. contacts)

Tracks:

H3K9me3 (the ratio of NeuN+ to NeuN-)

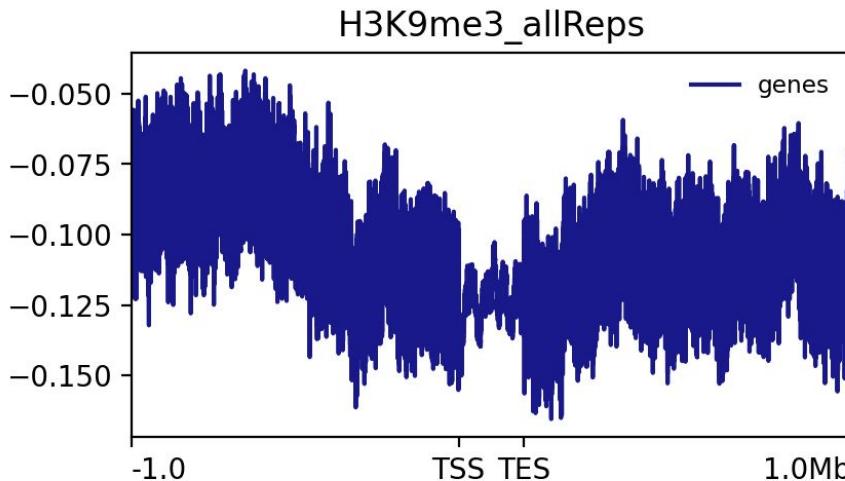
Significantly interacting chromatin regions

- Fit-Hi-C2 tools was used to obtain these significantly interacting chromatin regions;
- Threshold: **q-value** < 0.01e-5;
- In total, **25782** number of significant contacts were obtained;
- After that peaks for H3K27me3 were intersected with sig. contacts, resulting in **2043** peaks from 2651.



a) *The enrichment of significant contacts*

H3K9me3 track around CN H3K27me3 peaks:



H3K9me3_allReps track is the ratio of NeuN+ to NeuN-

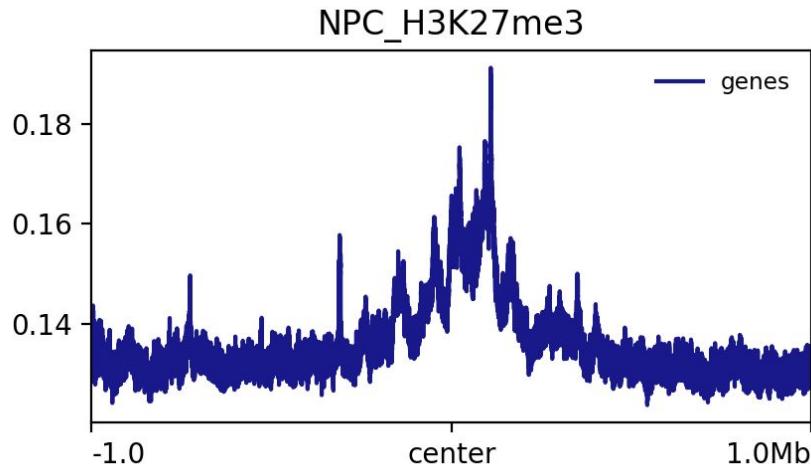
Hypothesis:

- The regions of glial cells have H3K9me3 repressive marks instead of H3K27me3 as neural cells have.

Result:

- Enrichment of H3K9me3 mark in neurons **around** the peak of H3K27me3

NPC H3K27me3 track around CN H3K27me3 peaks:



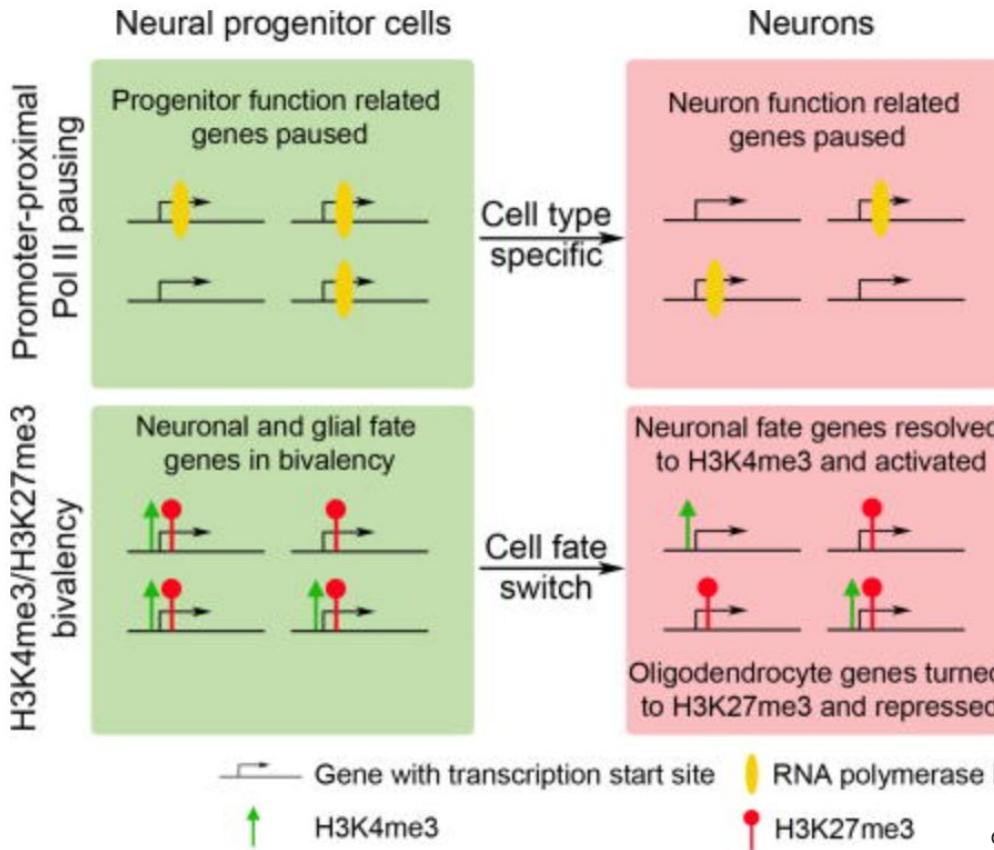
Point of interest:

- To compare H3K27me3 mark in NPC and CN

Result:

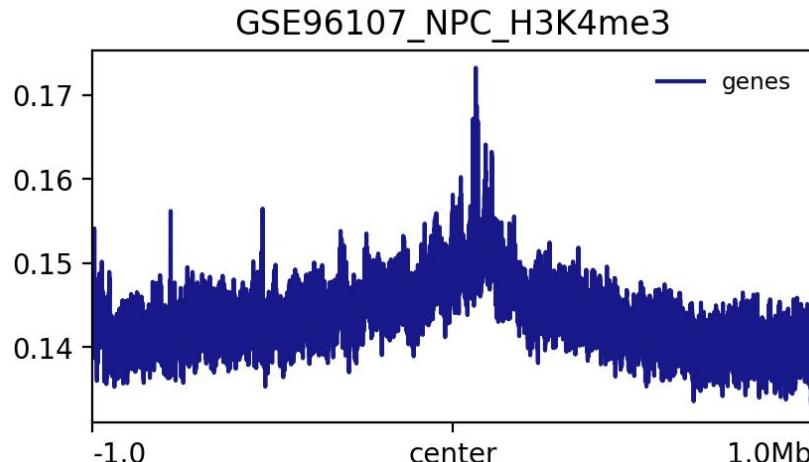
- The peak of H3K27me3
- The enrichment of H3K27me3 marks in neurons around the peak of H3K27me3

H3K27me3 role in neural and glial development



- H3K27me3 signal in promoters and enhancers distinguishes the progenitors from the differentiated cells
- H3K27me3 identifies the differentiation path of the neural stem cells (NSCs) to the glial cells

NPC H3K4me3 track around CN H3K27me3 peaks:



The bivalent genes are thought to play key roles in embryonic development, cell differentiation and tumorigenesis.

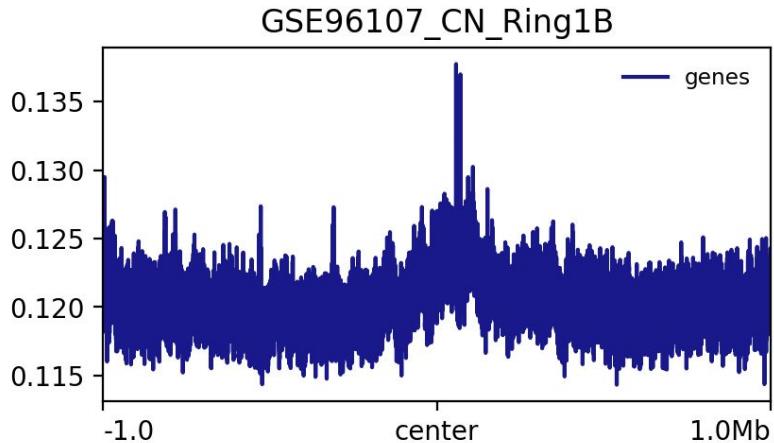
Point of interest:

- To see the presence of H3K4me3 active marks in NPC (bivalent state)

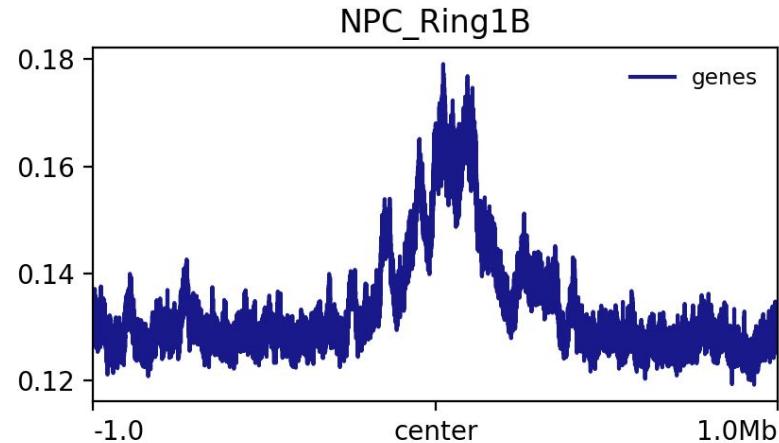
Result:

- The high peak of H3K4me3
- The enrichment of H3K9me3 marks in neurons around the peak of H3K27me3

Ring1B CN and NPC track around CN H3K27me3 peaks:

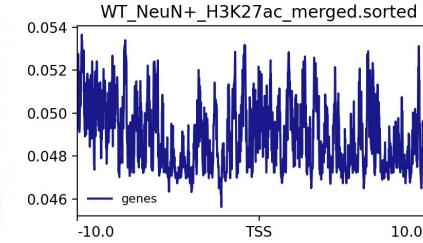
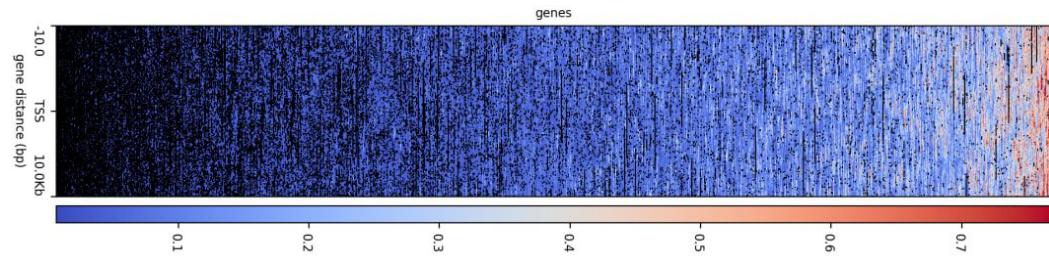


The regions enriched with H3K27me3 marks are linked with Ring1B (the polycomb protein)

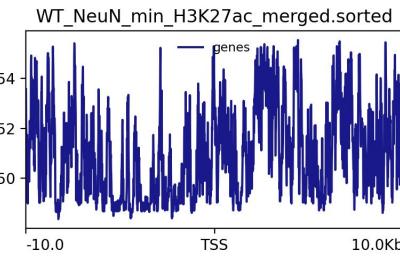
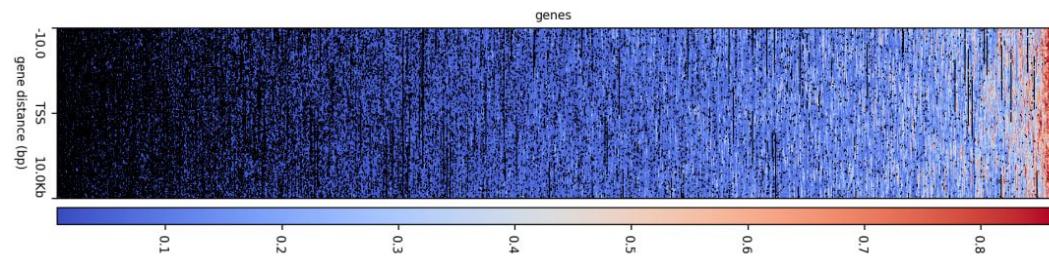


The regions enriched with H3K27me3 marks are also linked with Ring1B but less

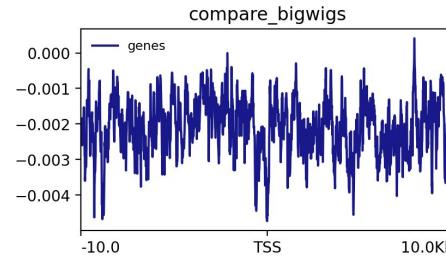
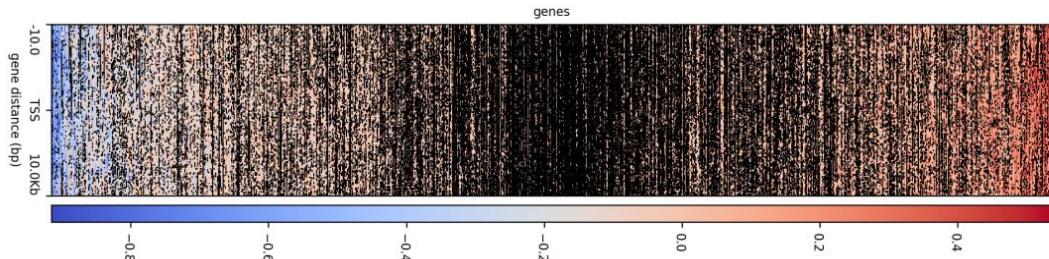
Neuronal and glial H3K27ac marks around filtered H3K27me3 track



a) Neuronal
H3K27ac



b) Glial
H3K27ac



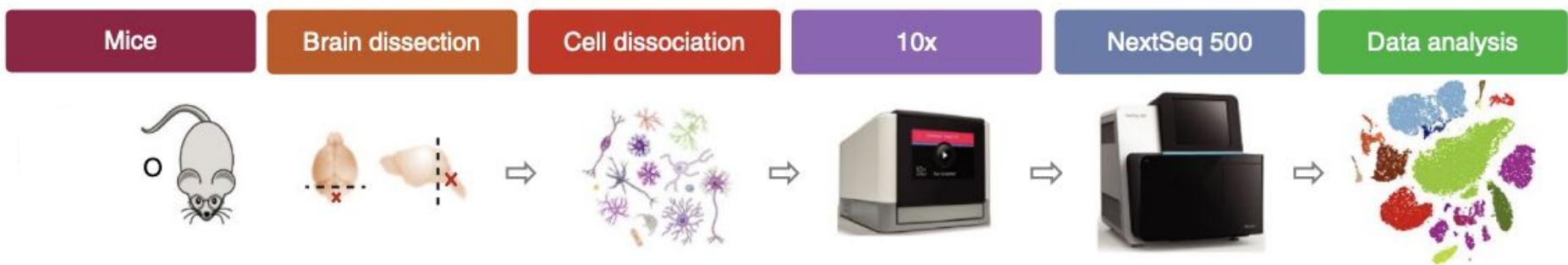
c) Compared
neuronal/glial
H3K27ac

RNA-seq analysis

The aim was to find correlation between chromatin structures (TADs, compartments) and gene expression.

Data

Single-cell RNA-seq data was taken from *Ximerakis et al., 2019*.

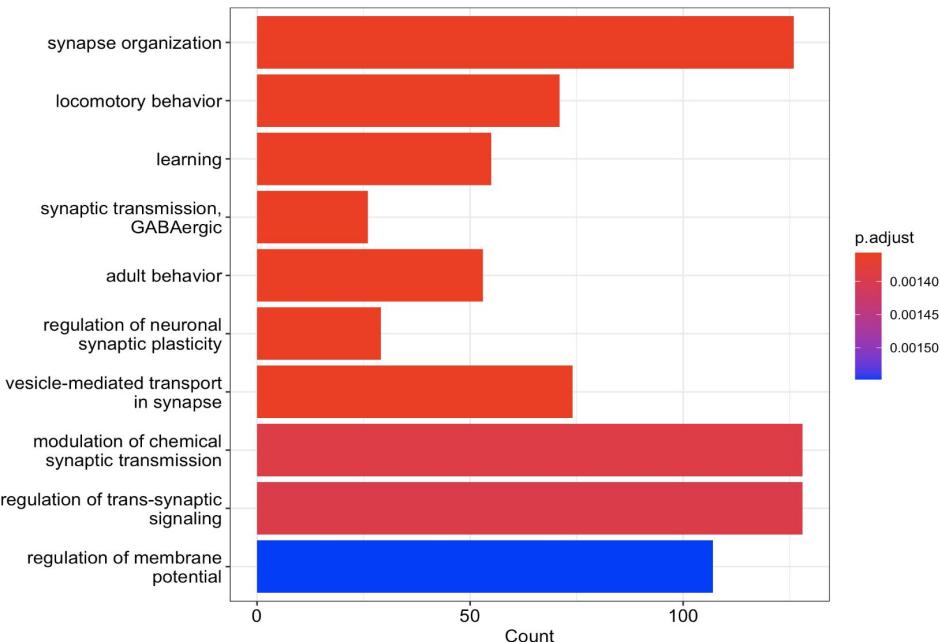


DE analysis

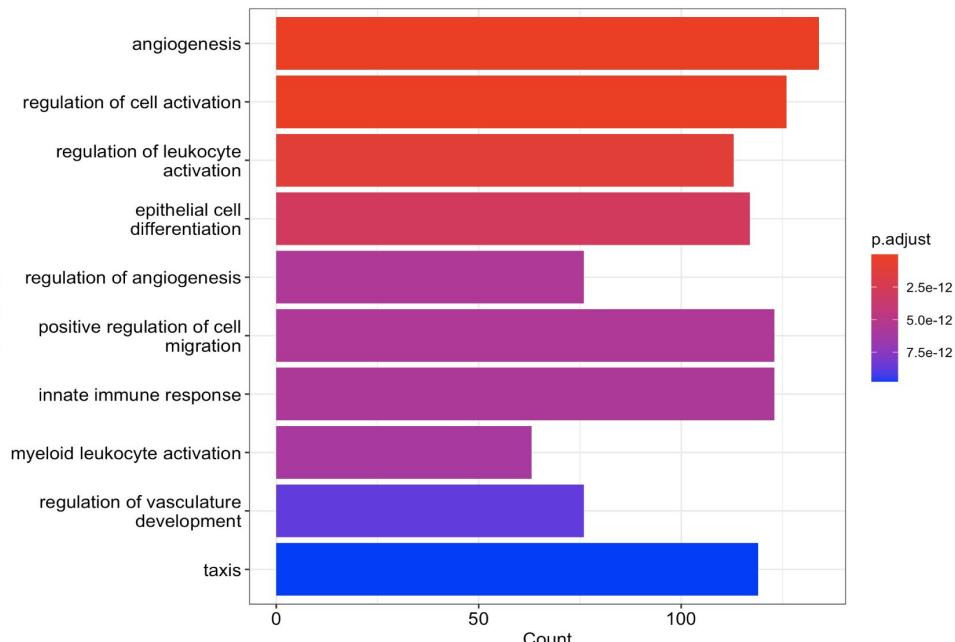
- Mann-Whitney test, multiple test correction (fdr).
13981 genes (p-value < 0.05).
- Neuronal de genes: 2748 genes (fold change > 1.1),
- Glial de genes: 1800 genes (fold change < 0.9).

Functional analysis

Neuronal DE genes



Glial DE genes



DE genes at TAD borders

In neurons neuronal DE genes are more often found at TAD borders.

In glia glial DE genes are less often found at TAD borders.

Same pattern is observed for common TAD borders.

Neuronal DE genes

	Border	Other
DE	983	1765
not DE	3913	8034

p-value < 0.05

Glial DE genes

	Border	Other
DE	324	1476
not DE	2612	10283

p-value < 0.05

DE genes and compartments

A(neurons) -> B(glia)

Neuronal DE genes are more often found in sites that changed compartments from A in glia to B in neurons.

Neuronal DE genes

	Change	Other
DE	185	2563
not DE	674	11273

p-value < 0.05

Glial DE genes

	Change	Other
DE	324	1476
not DE	2612	10283

p-value > 0.05

DE genes and compartments

A(glia) -> B(neurons)

Neuronal DE genes are more often found in sites that changed compartments from A in glia to B in neurons.

Neuronal DE genes

	Change	Other
DE	321	2427
not DE	1209	10738

p-value < 0.05

Glial DE genes

	Change	Other
DE	186	1614
not DE	1344	11551

p-value > 0.05

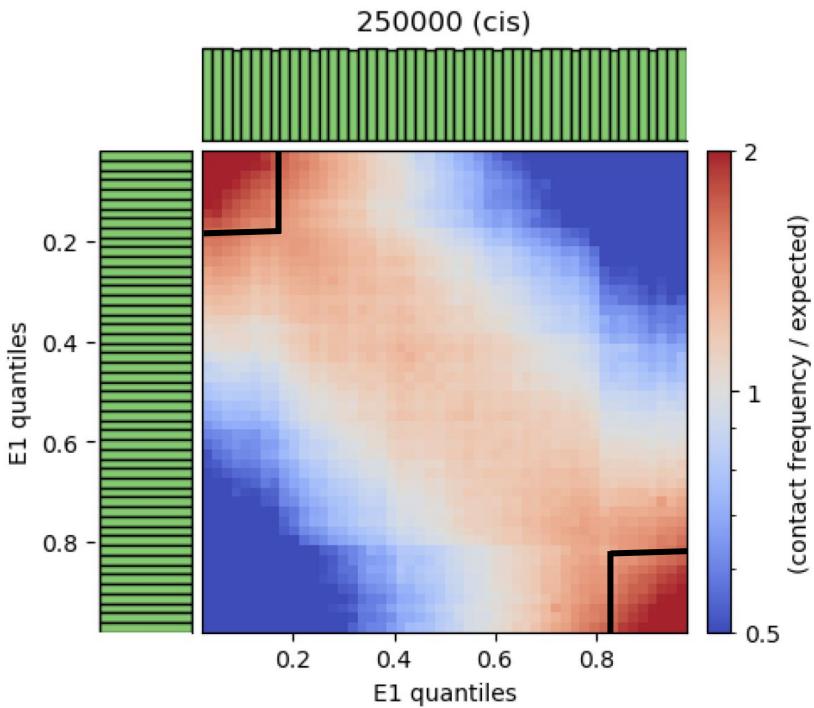
Hi-C analysis

The aim was to find differences between NeuN+ and NeuN- for:

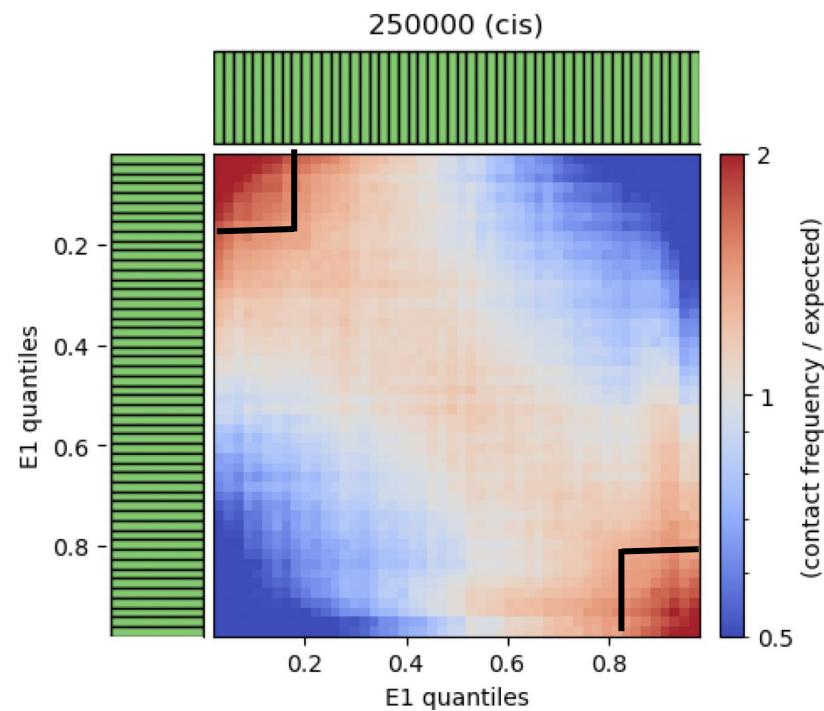
1. TADs density
2. loops prominence
3. compartment changes

Compartment calling

Glia



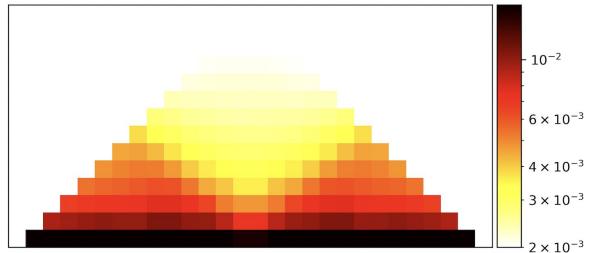
Neurons



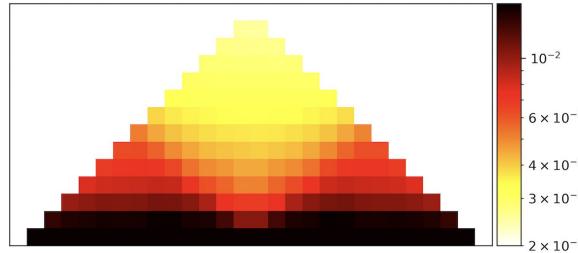
Glia

TADs

Neurons



10 kb resolution

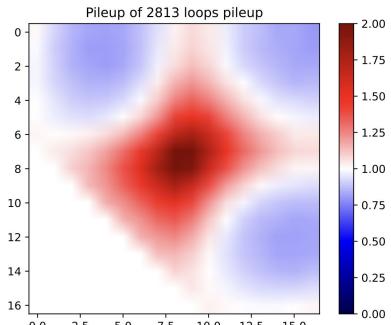


Distance vs. counts

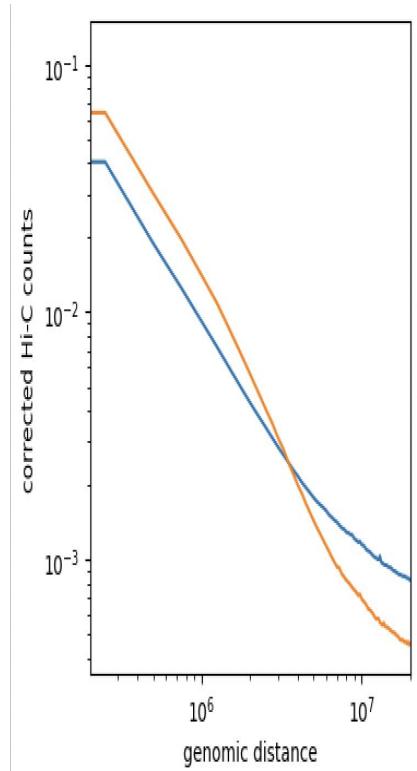
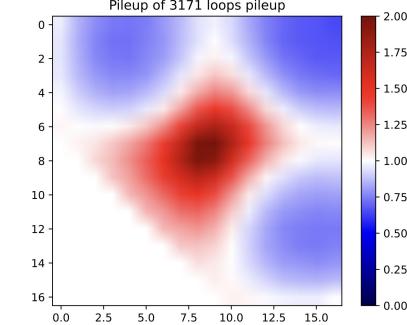
Loops

Glia

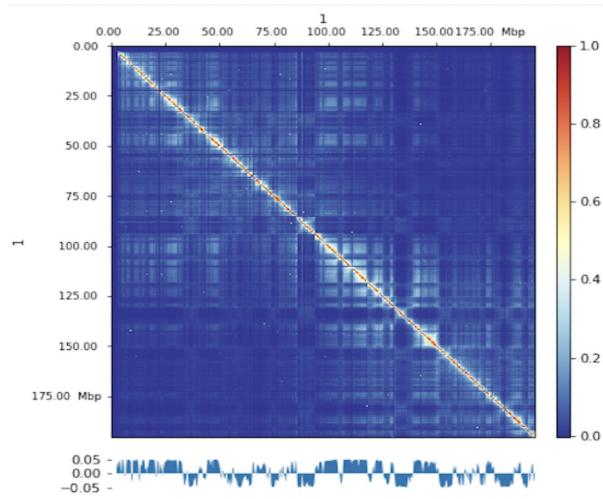
Neurons



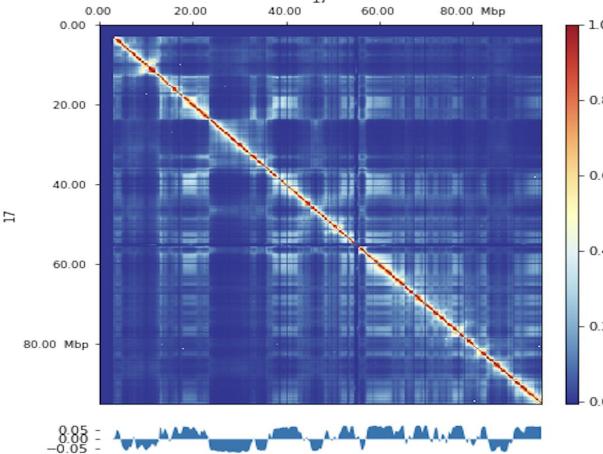
25 kb resolution



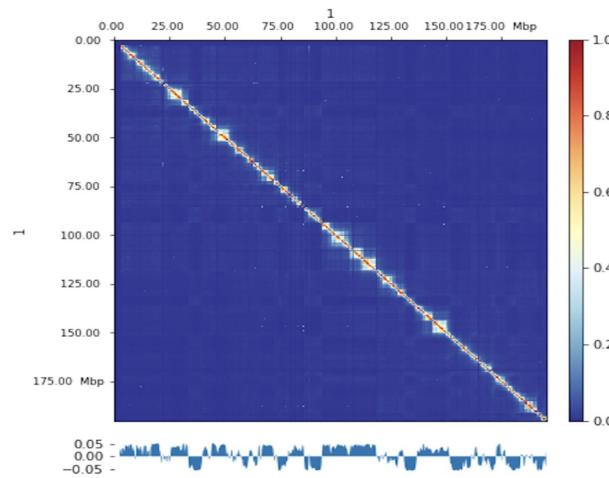
Visualization



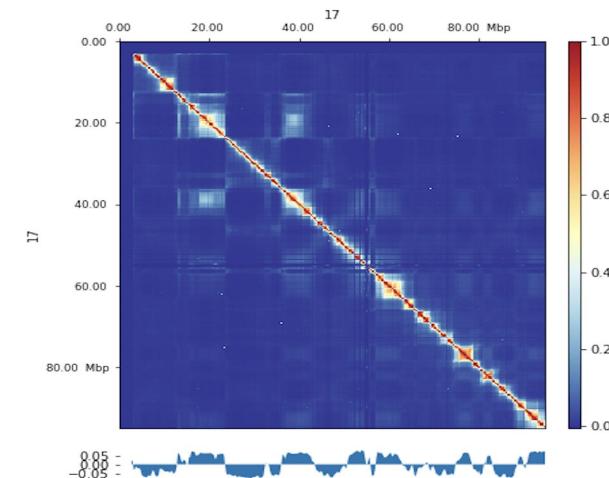
Glia,
chr.1



Glia,
chr.17

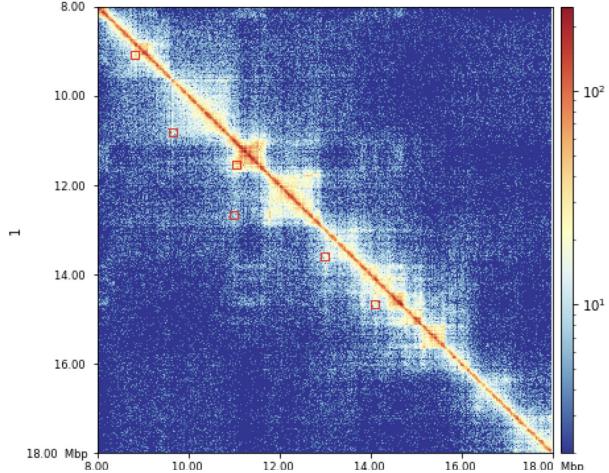


Neurons,
chr.1

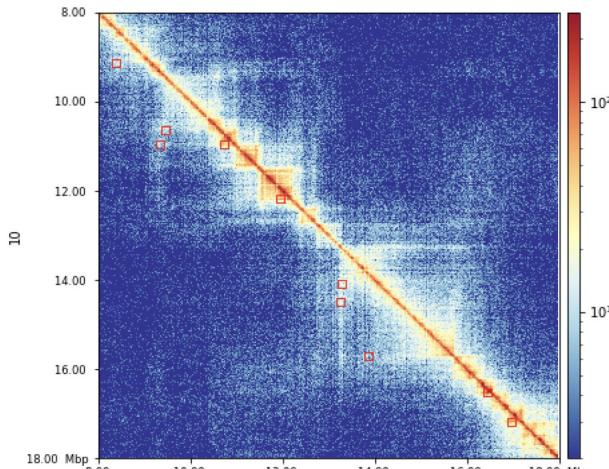


Neurons,
chr.17

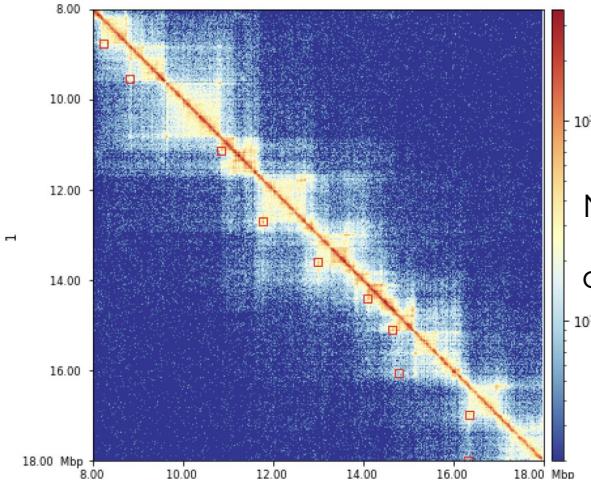
Loop visualization



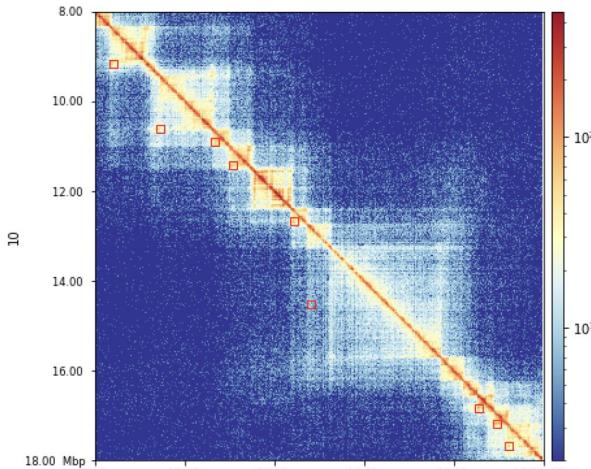
Glia,
chr.1: 8-18 Mb



Glia,
chr.10: 8-18 Mb



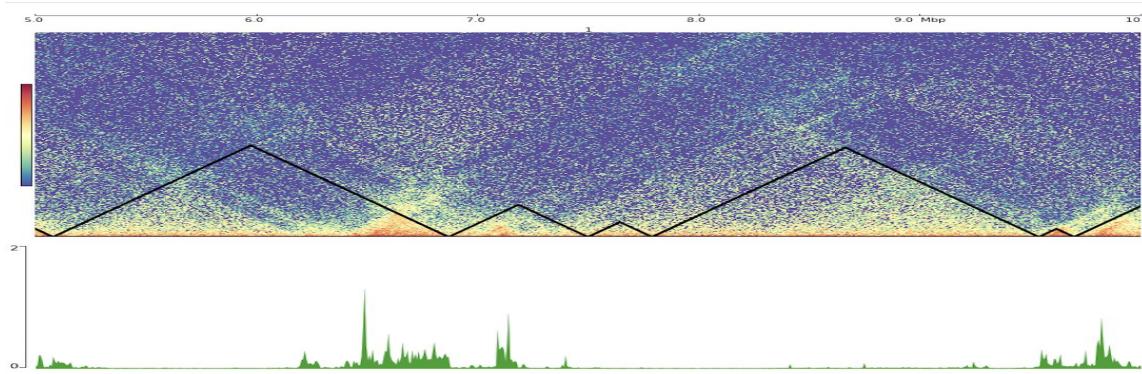
Neurons,
chr.1: 8-18 Mb



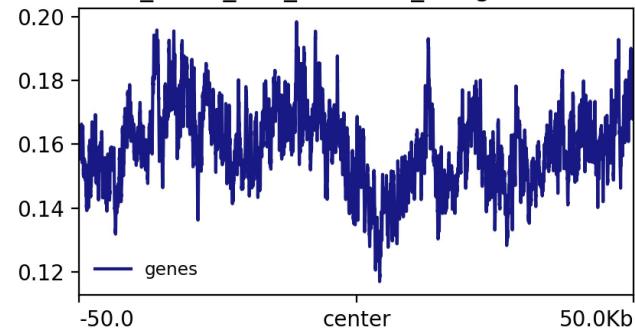
Neurons,
chr.10: 8-18 Mb

H3K27ac

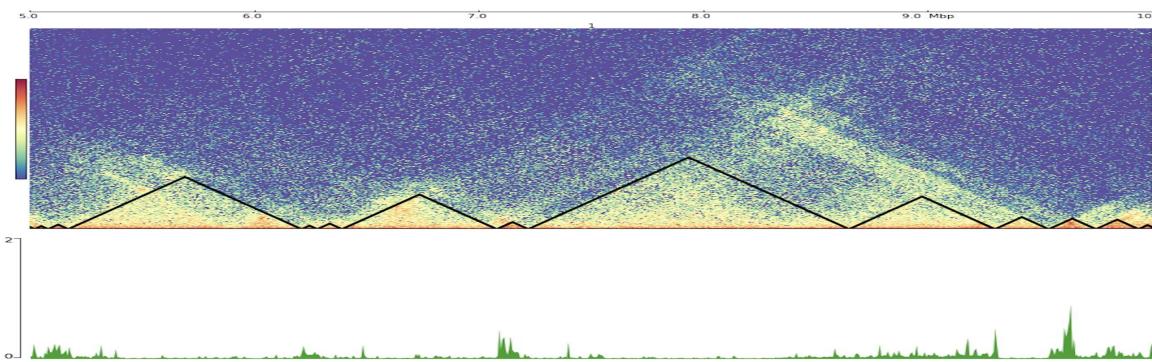
Glia



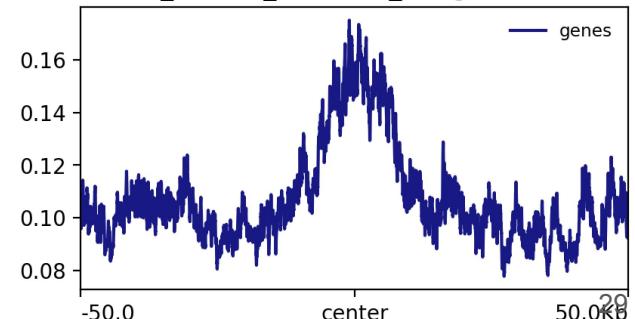
WT_NeuN_min_H3K27ac_merged.sorted



Neurons

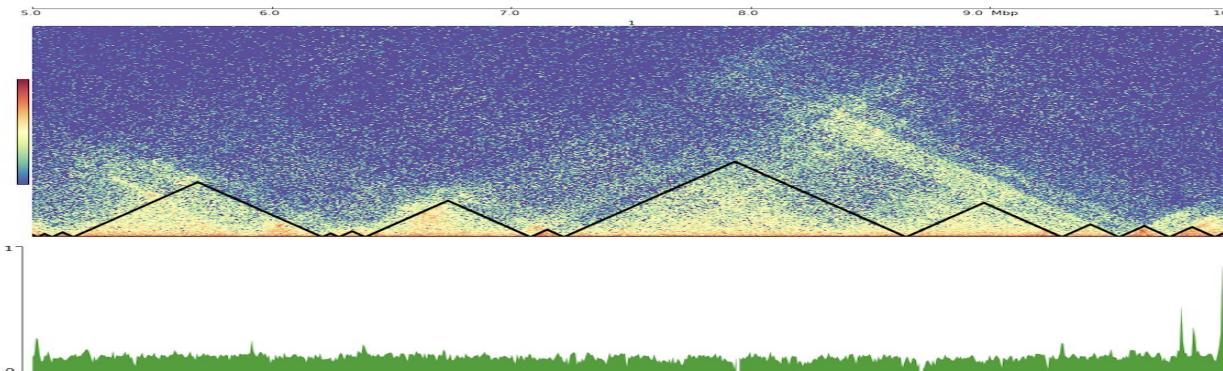


WT_NeuN+_H3K27ac_merged.sorted

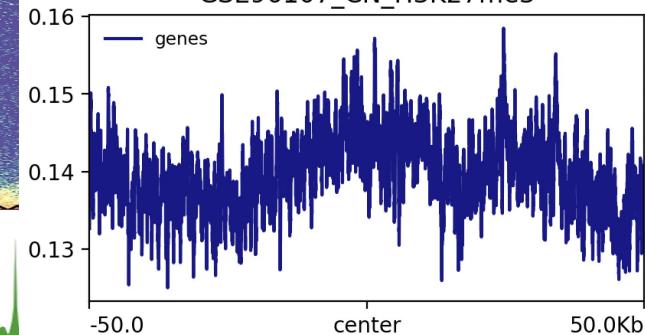


H3K27me3

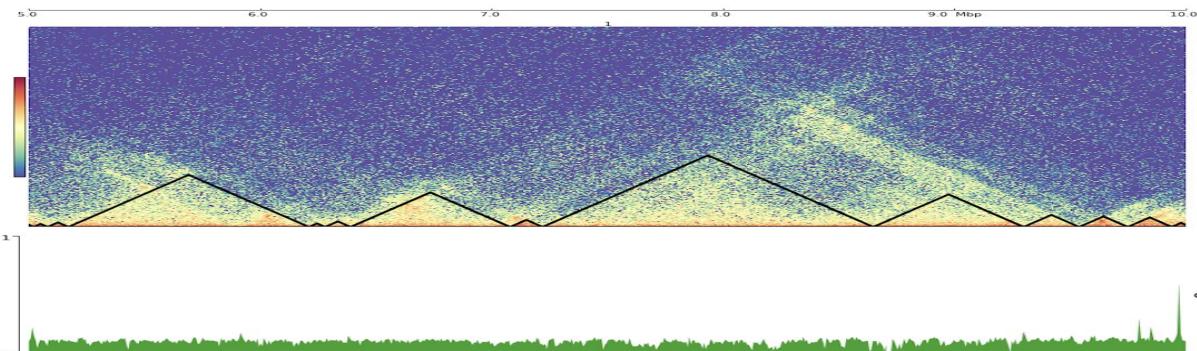
Cortical neurons



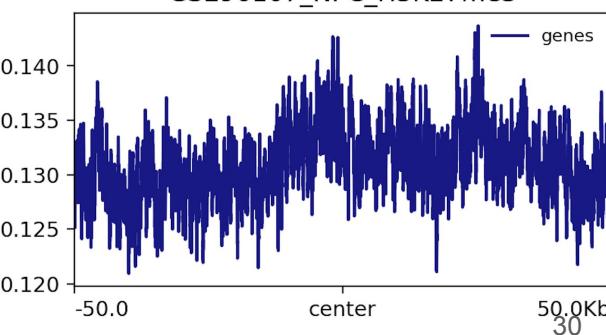
GSE96107_CN_H3K27me3



Neuronal progenitor cells

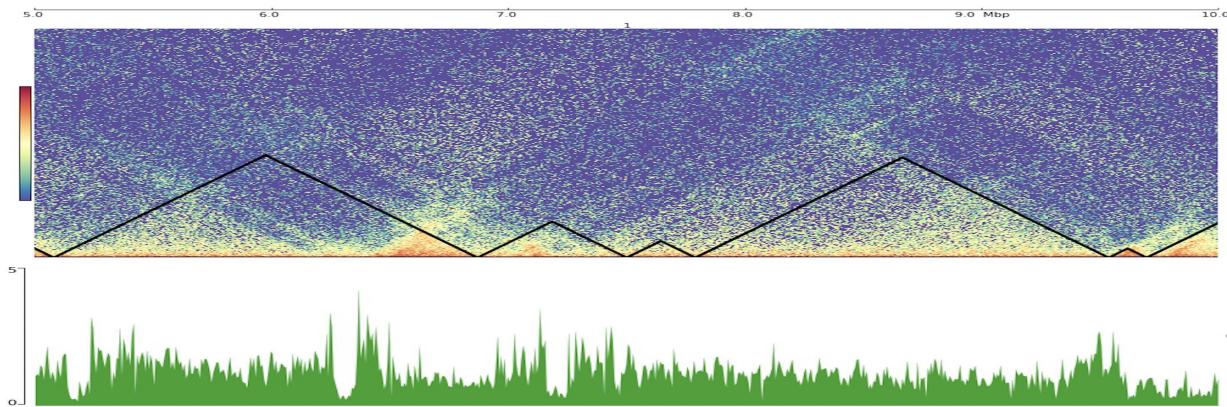


GSE96107_NPC_H3K27me3

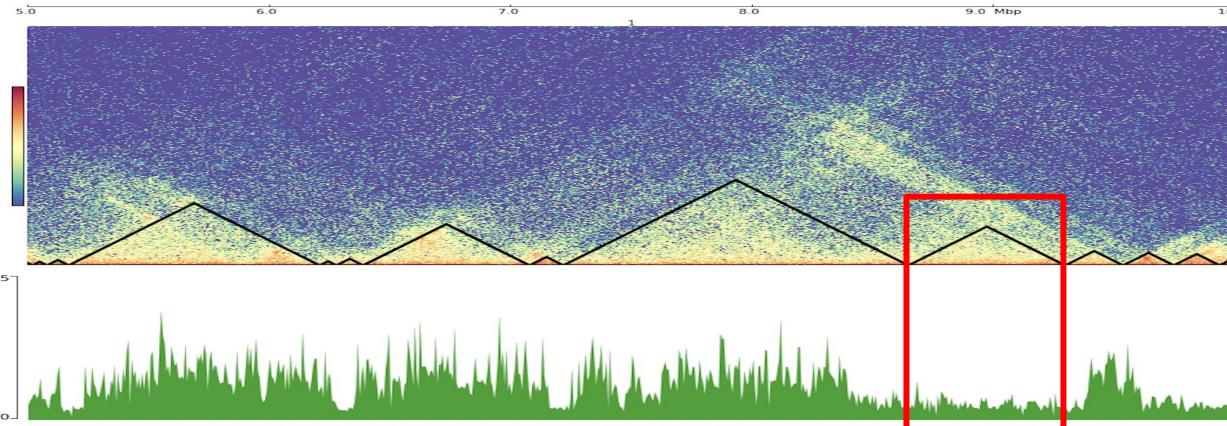


H3K9me3

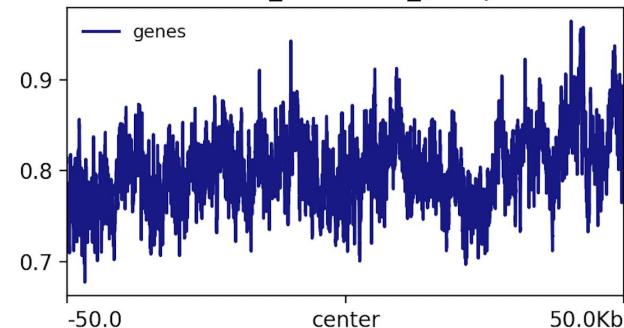
Glia



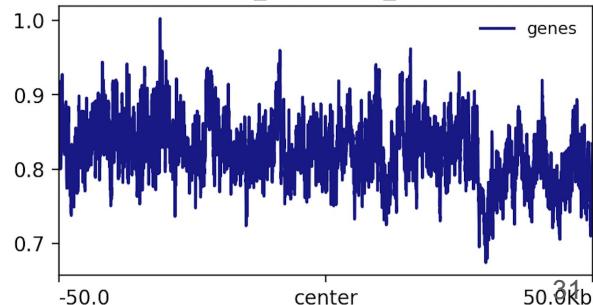
Neurons



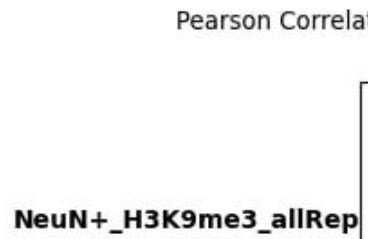
NeuN- _H3K9me3_allReps



NeuN+ _H3K9me3_allReps



1. The presence of H3K9me3 marks are differ in neurons and glia. We suspect the H3K9me3 more in glial cells;
2. The level of H3K27ac around the H3K27me3 approximately is the same in neuronal and glial cells, although acetylation is stronger in glial cells;
3. Neuronal DE genes are more often found at TAD borders, glial - less often;
4. Neuronal DE genes are more often found in sites that changed compartments;
5. Amount of loops is almost the same for both neurons and glia;
6. Glia Hi-C data displays stronger compartment pattern than Hi-C data of neurons;
7. Hi-C data of neurons demonstrates stronger TADs pattern than glia Hi-C data;
8. TAD boundaries of neurons are enriched with H3K27ac
9. TADs of both glia and neurons are enriched with H3K9me3



NeuN-_{H3K9me3_allReps.bw}

