**ATAC seq**

It is good to take into account that the QC is performed beforehand. Where the TSS enrichment and HQAA values were to low according to ENCODE protocol ([https://www.encodeproject.org/atac-seq/#standards](https://eur03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.encodeproject.org%2Fatac-seq%2F%23standards&data=05%7C02%7Ce.l.f.van_horssen%40lumc.nl%7C84e73be291f241670e0308dd2fe37769%7Cc4048c4fdd544cbd80495457aacd2fb8%7C0%7C0%7C638719376186479383%7CUnknown%7CTWFpbGZsb3d8eyJFbXB0eU1hcGkiOnRydWUsIlYiOiIwLjAuMDAwMCIsIlAiOiJXaW4zMiIsIkFOIjoiTWFpbCIsIldUIjoyfQ%3D%3D%7C0%7C%7C%7C&sdata=%2BJjm47WTt%2B0dsxe4DZgrww5nvKpDJsq8f4QOtLObKvo%3D&reserved=0)). In the PCA (see email) no clustering of the conditions could be seen, we still wanted to do the DiffBind performance on this dataset. When this gives no insight, maybe combinding ATAC with RNAseq gives more valuable information.

The workflow was as followed: first we load in our data into the Genome Browser and looked at our different conditions and genes of interest. To say something about the differences (if they were differentially expressed) we used the package DiffBind in R. This package.

is used for analyzing differential binding in for example ATAC-seq data. It compares DNA binding patterns across different conditions to identify regions of the genome where differential binding occurs, such as changes in chromatin accessibility. DiffBind includes normalizing data, visualizing differential binding, and performing statistical tests to determine significant differences in binding across conditions (Deseq and EdgeR).

After this we searches for the genome regio’s of our genes of interest and looked if they showed overlap in our differential expressed chromatin peaks. We performed this analysis on the whole dataset as on the dataset with only the conditions/samples of interest.

Whole dataset:

In total there were 36 comparisons, with 842 differential binding sites.

*Genes of interest:*

For our genes of interest 6 differential binding sites occurs in the following comparisons:

SLC2A1: Unstim vs aCD3+aCD27+a4-1BB   
SLC2A1: aCD3 vs aCD3+aCD27+a4-1BB   
SLC2A1: aCD3+aCD27 vs aCD3+aCD27+a4-1BB   
SLC2A1: aCD3+a4-1BB vs aCD3+aCD27+a4-1BB   
SLC2A1: aCD3+aCD28 vs aCD3+aCD28+aCD27+a4-1BB   
SLC2A1: aCD3+aCD28+aCD27 vs aCD3+aCD27+a4-1BB   
SLC2A1: aCD3+aCD28+a4-1BB vs aCD3+aCD27+a4-1BB   
SLC2A1: aCD3+aCD28+aCD27+a4-1BB vs aCD3+aCD27+a4-1BB   
ACACA: Unstim aCD3+aCD28+aCD27+a4-1BB   
ACACA: Unstim vs aCD3+aCD27+a4-1BB   
ACACA: aCD3 vs aCD3+aCD28   
ACACA: aCD3 vs aCD3+aCD28+aCD27+a4-1BB   
ACACA: aCD3 vs aCD3+aCD27+a4-1BB   
ACACA: aCD3+aCD27 vs aCD3+aCD28   
ACACA: aCD3+aCD27 vs aCD3+aCD27+a4-1BB   
ACACA: aCD3+a4-1BB vs aCD3+aCD28+aCD27+a4-1BB   
ACACA: aCD3+aCD28 vs aCD3+aCD28+a4-1BB   
ACACA: aCD3+aCD28 vs aCD3+aCD28+aCD27+a4-1BB   
ACACA: aCD3+aCD28+aCD27 vs aCD3+aCD27+a4-1BB   
ACACA: aCD3+aCD28+a4-1BB vs aCD3+aCD27+a4-1BB   
ACACA: aCD3+aCD28+aCD27+a4-1BB vs aCD3+aCD27+a4-1BB   
NR4A1: Unstim vs aCD3+aCD28   
NR4A1: Unstim vs aCD3+aCD28+aCD27   
NR4A1: Unstim aCD3+aCD28+aCD27+a4-1BB   
NR4A1: aCD3 vs aCD3+aCD28   
NR4A1: aCD3 vs aCD3+aCD28+aCD27   
NR4A1: aCD3 vs aCD3+aCD28+aCD27+a4-1BB   
NR4A1: aCD3+aCD27 vs aCD3+aCD28   
NR4A1: aCD3+aCD27 vs aCD3+aCD28+aCD27   
NR4A1: aCD3+aCD27 vs aCD3+aCD28+aCD27+a4-1BB   
NR4A1: aCD3+a4-1BB vs aCD3+aCD28   
NR4A1: aCD3+a4-1BB vs aCD3+aCD28+aCD27   
NR4A1: aCD3+aCD28+aCD27 vs aCD3+aCD28+a4-1BB   
NR4A1: aCD3+aCD28+aCD27 vs aCD3+aCD27+a4-1BB   
ESPL1: aCD3+aCD27 vs aCD3+aCD28+aCD27   
ESPL1: aCD3+aCD28+aCD27 vs aCD3+aCD27+a4-1BB   
TOP2A: Unstim vs aCD3+aCD27+a4-1BB   
TOP2A: aCD3 vs aCD3+aCD27+a4-1BB   
TOP2A: aCD3+aCD27 vs aCD3+aCD27+a4-1BB   
TOP2A: aCD3+a4-1BB vs aCD3+aCD27+a4-1BB   
TOP2A: aCD3+aCD28 vs aCD3+aCD28+aCD27+a4-1BB   
TOP2A: aCD3+aCD28+aCD27 vs aCD3+aCD27+a4-1BB   
TOP2A: aCD3+aCD28+a4-1BB vs aCD3+aCD27+a4-1BB   
TOP2A: aCD3+aCD28+aCD27+a4-1BB vs aCD3+aCD27+a4-1BB   
CDKN1B: Unstim vs aCD3+aCD27+a4-1BB   
CDKN1B: aCD3 vs aCD3+aCD27+a4-1BB   
CDKN1B: aCD3+a4-1BB vs aCD3+aCD27+a4-1BB   
CDKN1B: aCD3+aCD28+aCD27 vs aCD3+aCD27+a4-1BB   
CDKN1B: aCD3+aCD28+a4-1BB vs aCD3+aCD27+a4-1BB   
CDKN1B: aCD3+aCD28+aCD27+a4-1BB vs aCD3+aCD27+a4-1BB

Dataset of interest:

In total there were 21 comparisons, with 801 differential binding sites.

*Genes of interest:*

For our genes of interest 3 differential binding sites occurs in the following comparisons

|  |
| --- |
| ACACA: aCD3 vs aCD3+aCD28 |
| ACACA: aCD3+aCD27 vs aCD3+aCD28 |
| NR4A1: Unstim vs aCD3+aCD28  NR4A1: Unstim vs aCD3+aCD28+aCD27  NR4A1: aCD3 vs aCD3+aCD28  NR4A1: aCD3 vs aCD3+aCD28+aCD27  NR4A1: aCD3+aCD27 vs aCD3+aCD28  NR4A1: aCD3+aCD27 vs aCD3+aCD28+aCD27  NR4A1: aCD3+a4-1BB vs aCD3+aCD28  NR4A1: aCD3+a4-1BB vs aCD3+aCD28+aCD27  NR4A1: aCD3+aCD28+aCD27 vs aCD3+aCD28+a4-1BB |
| ESPL1: aCD3+aCD27 vs aCD3+aCD28+aCD27 |

We looked again at the Genome Browser, to our differential expressed regio’s and differential expressed genes. An example is added in the attachment of this email for Gene: NR4A1