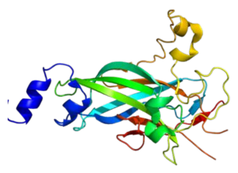
Downregulation of different genes connected with the removal of EBF1

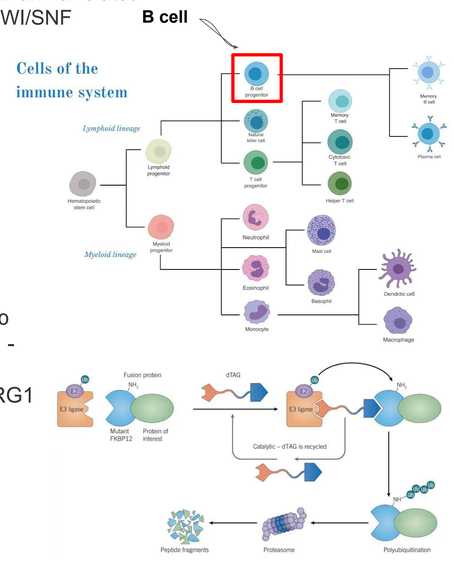
Introduction

EBF1 (Early B-Cell "pioneering" Transcription Factor 1) is a protein coding gene. **EBF1** controls the expression of key proteins required for B cell differentiation, signal transduction and function. EBF1 is a key determinant of a transcriptional regulatory network involved in establishing the B cell fate by activating lineage-specific gene expression and repressing genes. Here EBF1 acts as a pioneer TF that is able to bind naïve progenitor chromatin and establish local chromatin accessibility by recruiting BRG1 (Brahma-related gene 1), a key component of the ATP-dependent chromatin-remodelling SWI/SNF complex and has been implicated in regulating gene expression.

But we want to consider the consequences of the dTAG-induced degradation of EBF1 in B cells. **Mutation of EBF1** leads to changes in chromatin accessibility at EBF1-binding sites, accompanied by altered target gene expression and a rapid loss of BRG1 recruitment. We replaced EBF1 by EBF1-FKBPF36V in pro-B cells, promoting rapid degradation by adding the degradation TAG13 (dTAG13) dimerizer.



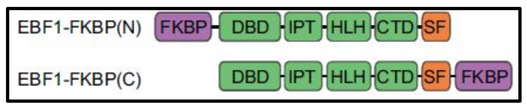
EBF1 crystal structure



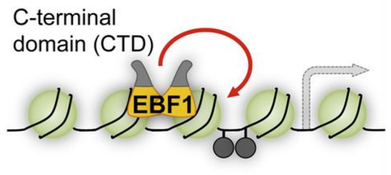
Введение

EBF1 (Early B-Cell Transcription Factor 1) контролирует экспрессию генов, необходимых для дифференцировки В-лимфоцитов. EBF1 - это пионерный транскрипционный фактор, который связывает закрытый хроматин в предшественниках В-клеток, привлекает BRG1 и способствует открытию хроматина.

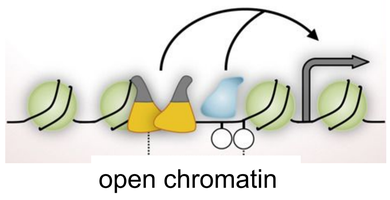
EBF1-FKBP constructs



Pioneering activity



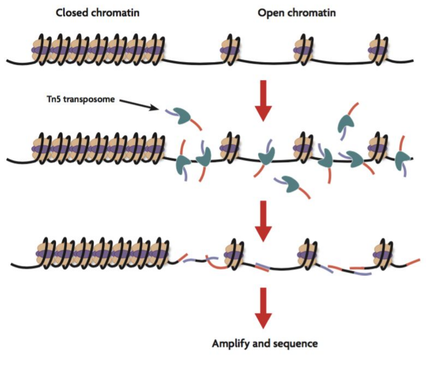
Shaping chromatin



open chromatin

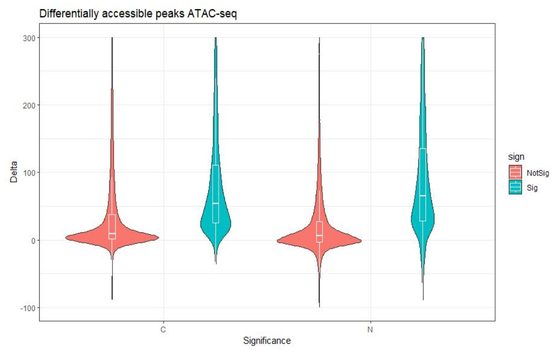
dTAG protein degradation system

ATAC-seq

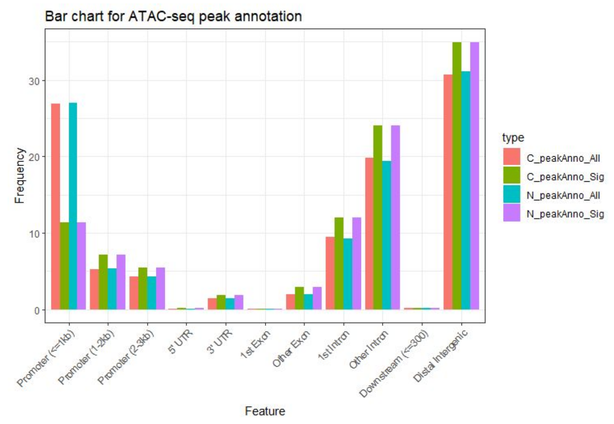


ATAC sequencing

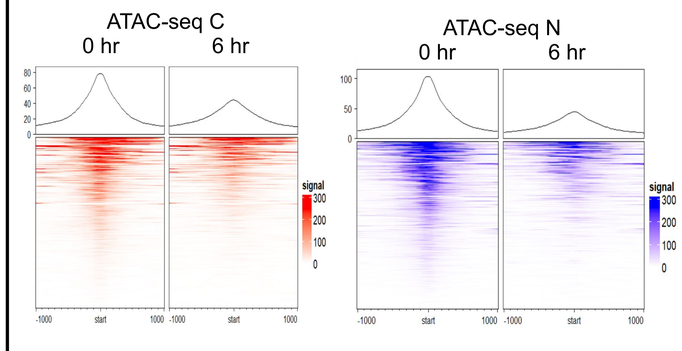
Genome-wide chromatin accessibility



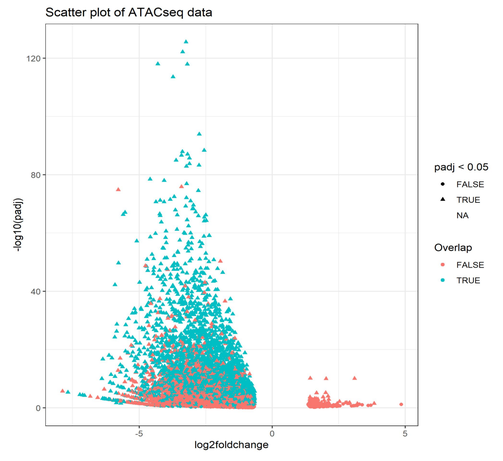
The distribution of differences (delta) between 0hr and 6hr timepoints during EBF1 degradation, split by C and N terminus dTAGs and significant versus non-significant differences.



We classified the genomic regions that are most enriched for differentially accessible (DA) peaks. We found that promoter regions were depleted for DA peaks while introns and distal intergenic regions were enriched for DA peaks.



In order to observe the effect of dTag addition to EBF1 binding site, we used ChIPseeker and tried to determine EBF1 binding at 0hr and 6hr timeframes. In addition, we used bigwig files to read coverage from ATAC-seq experiments. On the heatmap above, we can see peak signals for ATAC-seq during 0hr and 6hr - both for C and N terminus at the EBF1 ChIP-seq peaks.

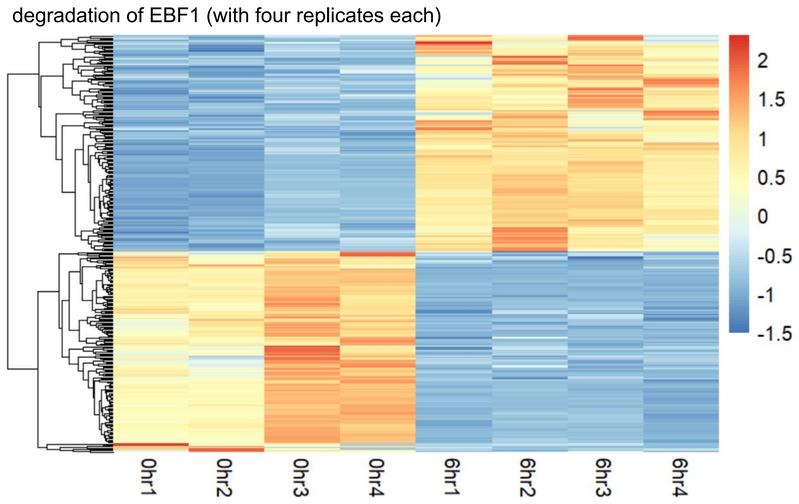


We used DESeq to identify differentially accessible (DA) ATAC-seq peaks and classified these peaks based on overlap with EBF1 binding sites. As expected, at no EBF1 binding sites was there an increase in chromatin accessibility.

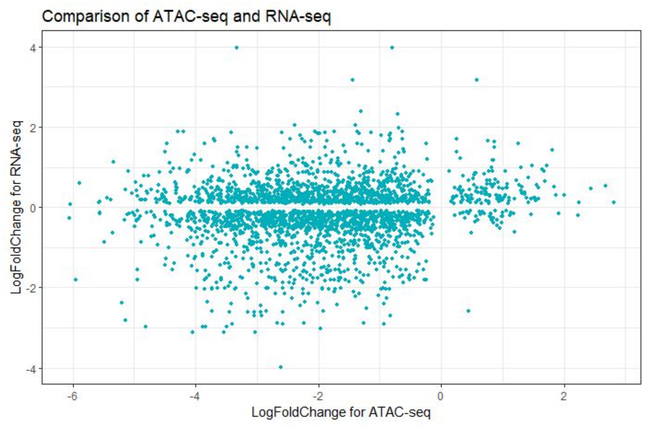
RNA sequencing

Gene expression of entire transcriptome

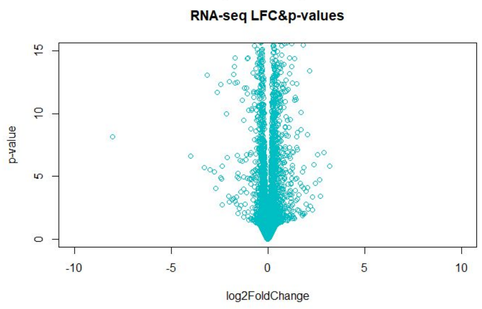
Distribution of exonic reads at 0hr and 6hr after dTAG-induced degradation of EBF1 (with four replicates each)



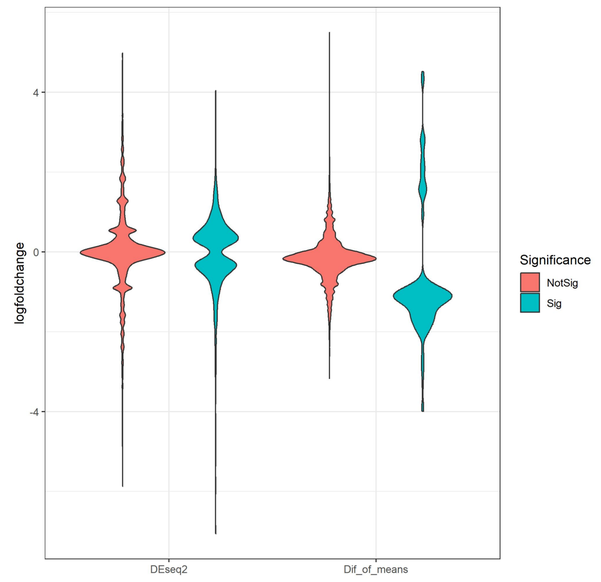
We show genes that are significantly differentially expressed between time points based on DESeq results with p value<0.05 and absolute log2 fold-change > 1. Depletion of EBF1 leads to upregulated as well as downregulated gene expression.



The dependence of RNA-seq signal on ATAC-seq signal. It is shown that stronger closure of chromatin can be associated with both upregulated and downregulated genes.



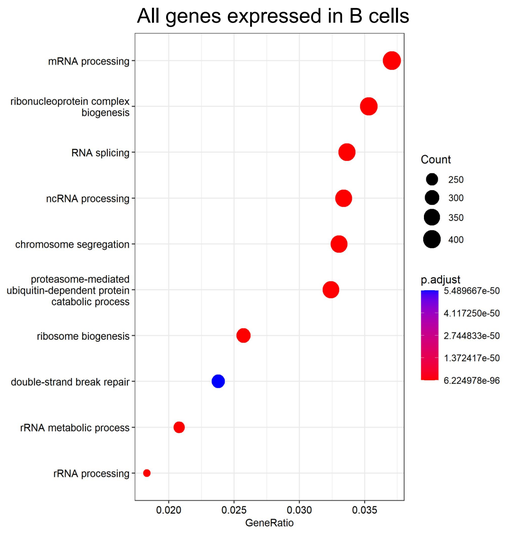
Here we show the relationship between -log10 p-value and log2 fold-change across genes from RNA-seq, demonstrating that genes are both upregulated and downregulated upon EBF1 degradation.



Comparison of two methods (DESeq2 and difference of means) to evaluate the significant changes and log2foldchange of the genes in a same dataset of RNA seq. One could see, that DEseq2 provides a result that is more similar to the normal distribution.

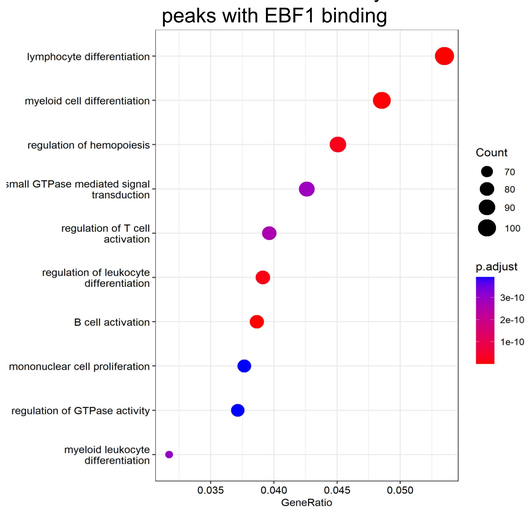
Gene Ontology (GO) analysis

All genes expressed in B cells



We analyzed expressed genes in B cells and identified biological processes associated with them using gene ontology (GO) enrichment analysis. As expected, the nearby genes play roles in generic biological functions associated with transcription.

Genes near differentially accessible peaks with EBF1 binding



We linked ATAC-seq peaks to nearest genes, and performed gene ontology enrichment analysis. Where differentially accessible peaks overlap EBF1 binding events, measured by ChIP-seq, the nearby genes are much more likely to play roles in biological functions associated with immune cell regulation.

Conclusions

- At sites of EBF1 binding, chromatin closes after the degradation of EBF1 caused by addition of dTAG.
- Chromatin inaccessibility does not necessarily lead to downregulation of transcription.
- Diminished chromatin accessibility correlated with altered gene expression. Continuous activity of EBF1 is required for the stable maintenance of the transcriptional and epigenetic state of B-cells.
- EBF1 is responsible for differentiation of cells into mature B cells and thus plays a significant role in immune system.

Выводы

- На сайтах связывания EBF1 хроматин закрывается в результате деградации EBF1 вызванной добавлением dTAG.
- Недоступность хроматина не приводит к обязательному замедлению транскрипции.
- Снижение доступности хроматина коррелирует с изменением экспрессией генов. Таким образом, непрерывная активность EBF1 необходима для стабильного поддержания транскрипционной активности и эпигенетического состояния В-клеток.
- EBF1 ответственен за дифференциацию клеток в сформированные В-клетки и, как следствие, является очень важным белком для иммунитета.