



# Dynamic changes in chromatin accessibility and Runx transcription factor interactions in T cell development



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Victoria Tobin(1), Boyoung Shin(2), Ellen Rothenberg(2)

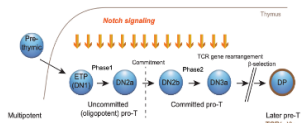
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### Introduction

During T cell development, hematopoietic precursors migrate to the thymus where they acquire T cell identity. In "Phase1" of this process, progenitor cells retain multipotentiality; they progress to "Phase2" as they commit to the T lineage and give up alternate options. Expressed throughout development, Runx1 and Runx3 play a crucial role in T lineage specification by activating the T cell program and repressing alternative lineage potentials in a stage-specific manner. Our goal is to explore changes in chromatin accessibility and Runx-DNA binding during T development through ChIP-seq and ATAC-seq analysis.

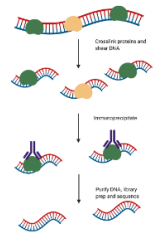
### Overview of T cell development



When hematopoietic progenitor cells enter the thymus, they are still multipotent and express both multipotency and non-T associated genes despite the fact that they are destined to become T cells. In these early stages (ETP and DN2a), we consider the cells to be in "Phase1", and they progress to

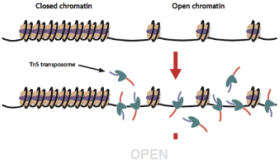
### Methods

#### Runx1 and Runx3 ChIP-seq in early T cell development



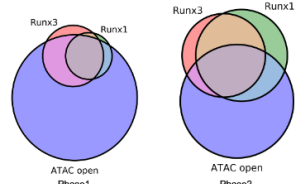
ChIP-seq allows you to sequence DNA binding regions of a protein of interest. We analyzed ChIP-seq data generated in the Rothenberg lab for Runx1 and Runx3 binding in both Phase1 and Phase2 developing T cells.

#### ATAC-seq across early T cell development



### Results

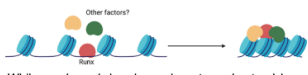
#### Runx factors mainly bind open chromatin in Phase1, and both open and closed chromatin in Phase2



When we compared Runx binding sites to regions of open chromatin in the corresponding developmental stage, we found Runx binding sites in Phase1 were focused in open chromatin. However, we found that in Phase2, Runx factors bound to both open and closed chromatin. As Runx increasingly binds more closed chromatin across development, we hypothesized that Runx may be repressing genes associated with alternative lineages by closing the associated chromatin.

Runx dependent genes are highly correlated with regions of Runx binding both open and closed chromatin states

### A possible mechanism of Runx repression



While much work has been done to understand how transcription factors activate their target genes, much less is known about how they can repress. We found evidence that Runx factors may be repressing genes associated with alternate cell lineages during early T development by closing the associated regions of chromatin. While a detailed mechanism is still unknown, one hypothesis is that Runx may be recruiting other factors, such as chromatin remodelers, to these sites.

### Conclusions and Acknowledgements

#### Conclusions

- Runx dependent genes (eg. *Bcl11b*, *Cd3*, and *Tcf7*) were highly correlated with regions of Runx binding in both open and closed chromatin states
- Runx repressed genes (eg. *Id3*, *Pou2af1*) were correlated with Runx binding regions that were open in Phase 1 but closed in Phase 2
- Runx may be repressing genes through a mechanism that closes the associated chromatin

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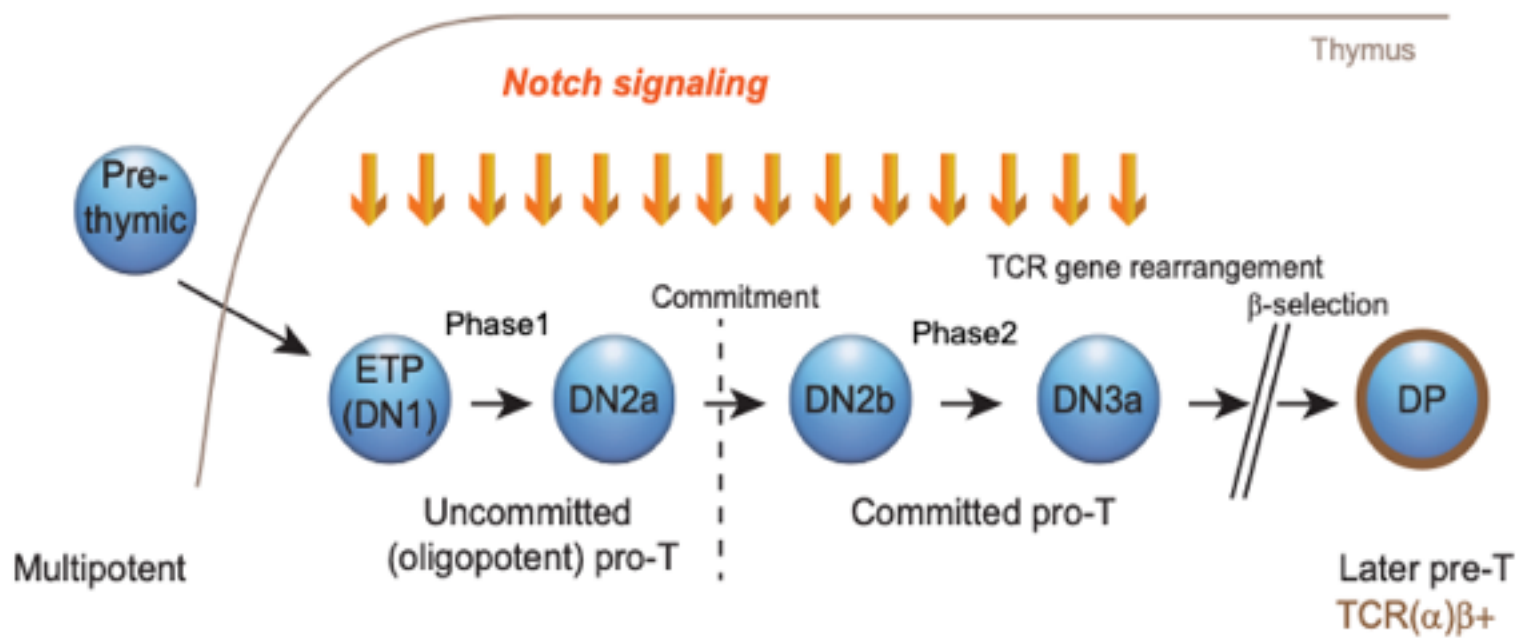




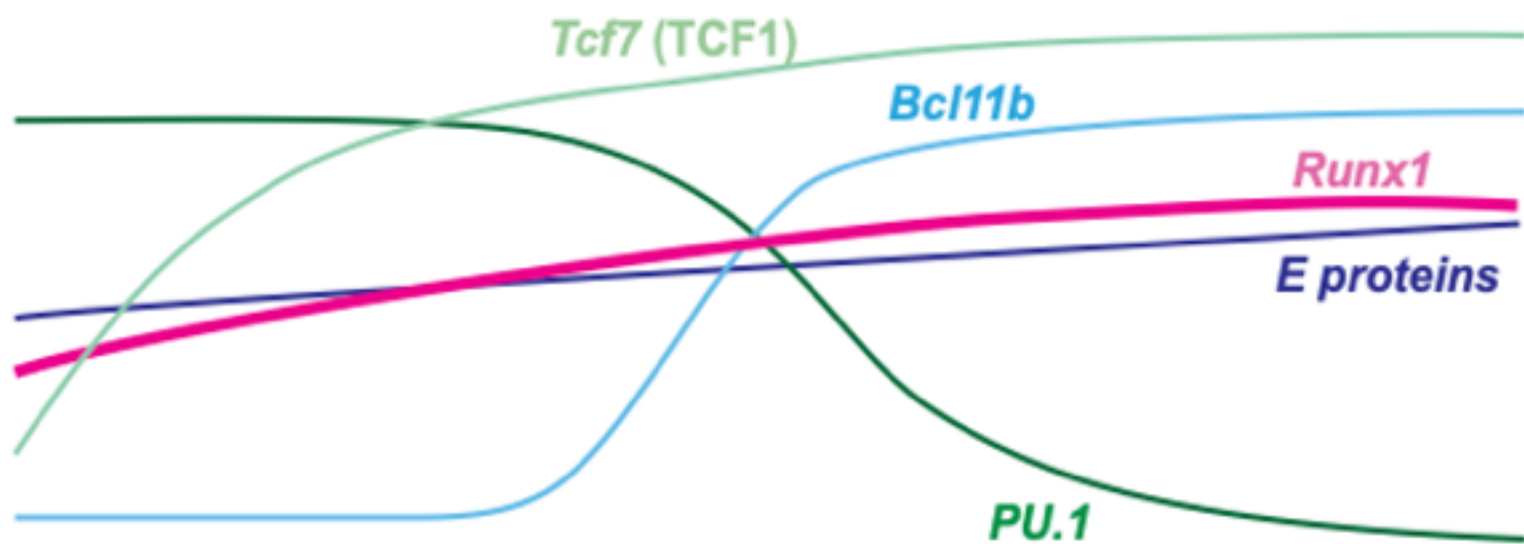
## INTRODUCTION

During T cell development, hematopoietic precursors migrate to the thymus where they acquire T cell identity. In "Phase1" of this process, progenitor cells retain multipotentiality; they progress to "Phase2" as they commit to the T lineage and give up alternate options. Expressed throughout development, Runx1 and Runx3 play a crucial role in T lineage specification by activating the T cell program and repressing alternative lineage potentials in a stage-specific manner. Our goal is to explore changes in chromatin accessibility and Runx-DNA binding during T development through ChIP-seq and ATAC-seq analysis.

# OVERVIEW OF T CELL DEVELOPMENT



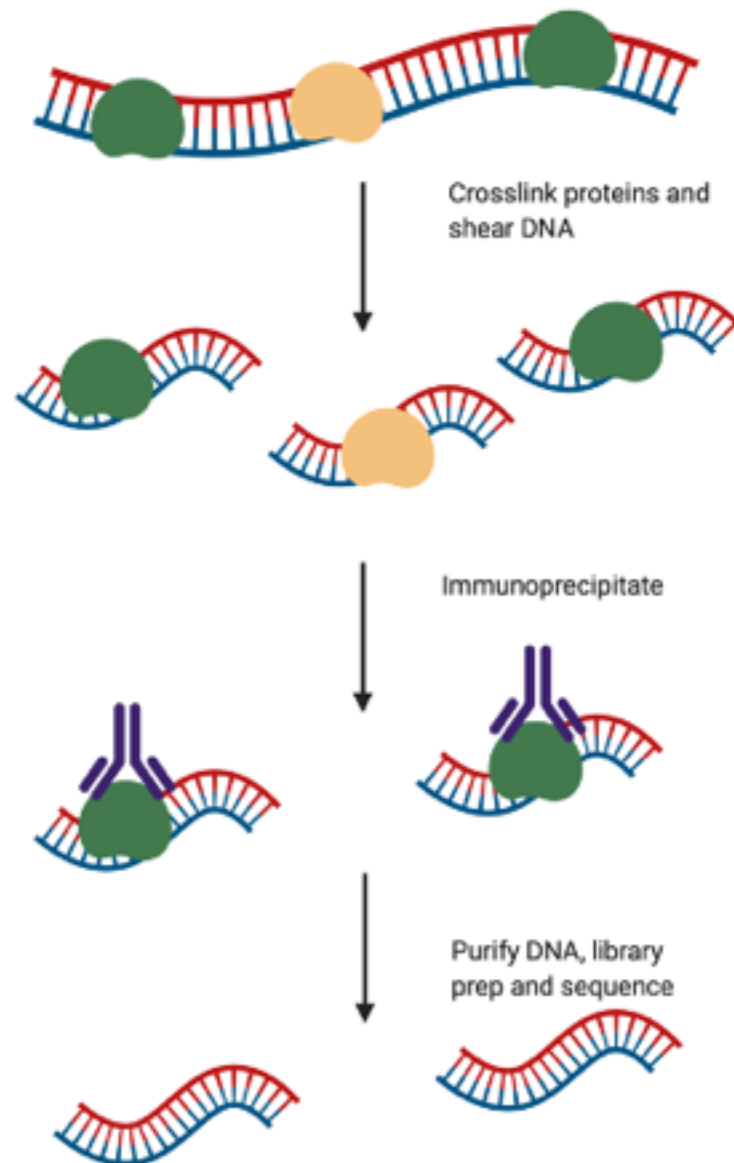
When hematopoietic progenitor cells enter the thymus, they are still multipotent and express both multipotency and non-T associated genes despite the fact that they are destined to become T cells. In these early stages (ETP and DN2a), we consider the cells to be in "Phase1", and they progress to "Phase2" as they commit to the T cell fate (DN2b and DN3).



How these cells commit to the T lineage and give up alternative options involves a highly specialized control gene network with many players involved. Certain factors, for instance PU.1 are expressed in Phase1, while others, for instance Bcl11b, are not expressed until Phase2. Interestingly, Runx factors (Runx1 and Runx3) are expressed across early development. Runx factors are known to both activate T cell genes and repress genes associated with alternate lineages. Despite constant expression, Runx factors play different roles in Phase1 vs Phase2, and our goal is to understand how they mediate these phase specific roles.

## METHODS

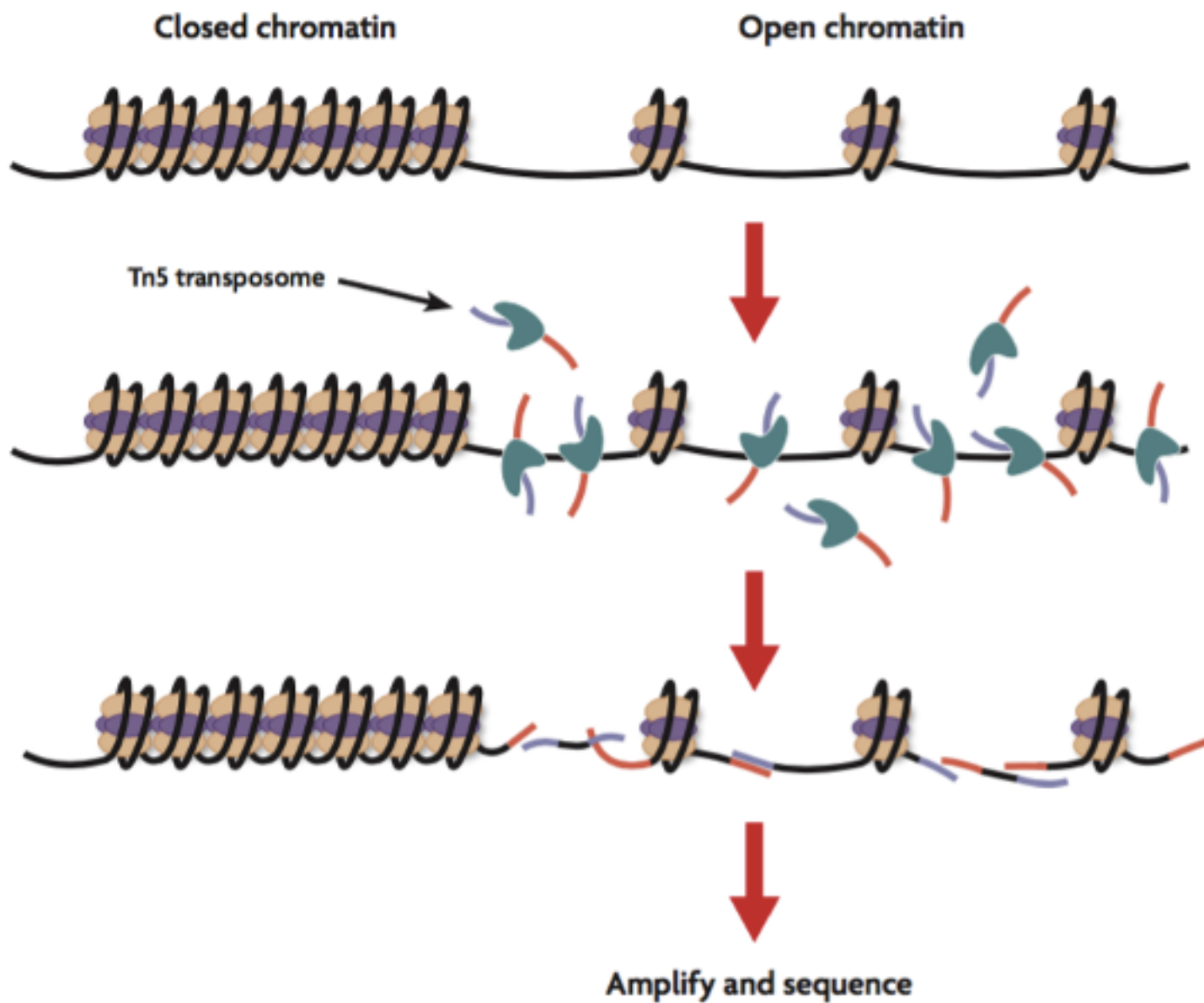
# Runx1 and Runx3 ChIP-seq in early T cell development



ChIP-seq allows you to sequence DNA binding regions of a protein of interest. We analyzed ChIP-seq data generated in the Rothenberg lab for Runx1 and Runx3 binding in both Phase1 and Phase2 developing T cells.



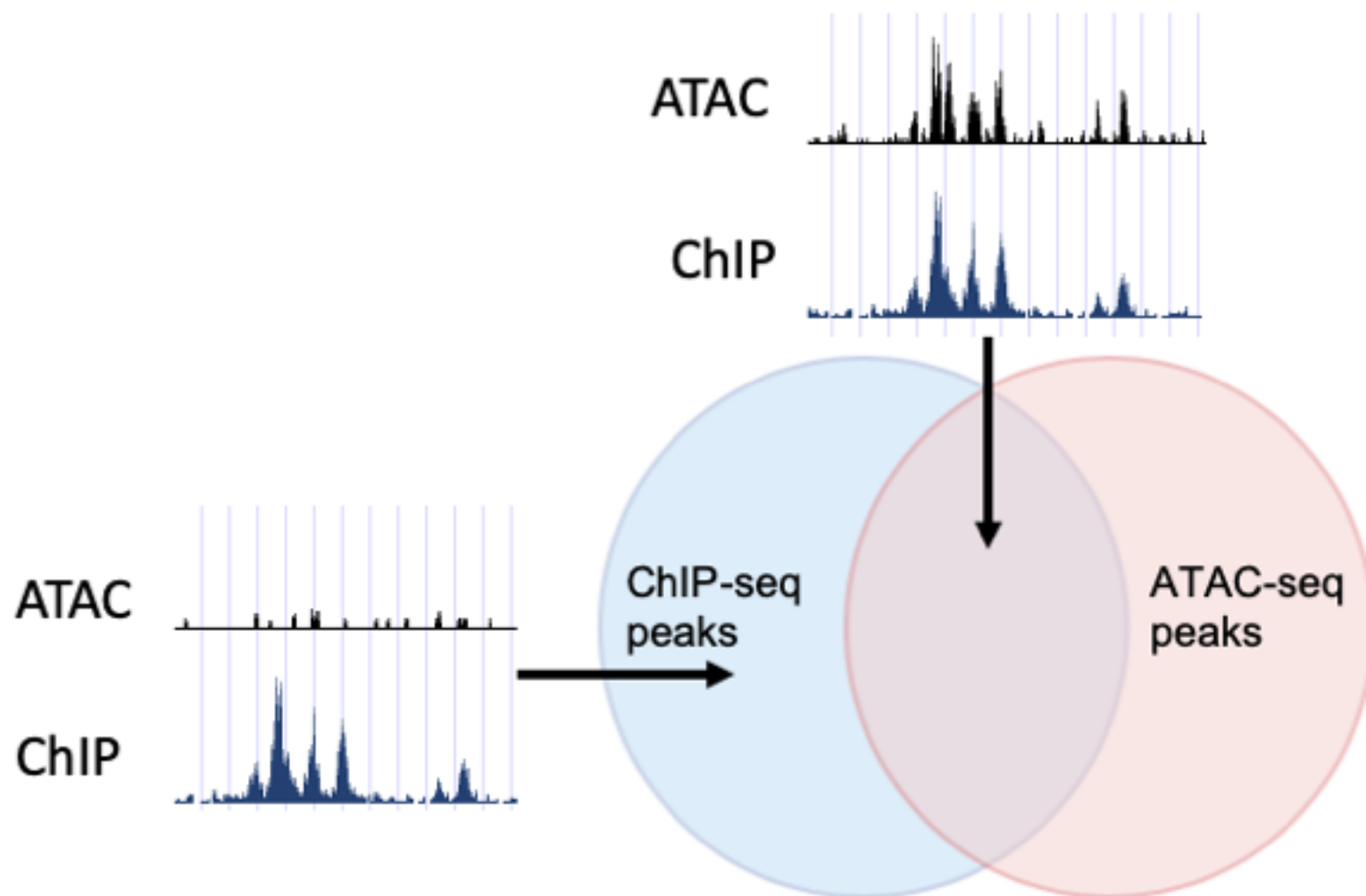
# ATAC-seq across early T cell development



<https://www.activemotif.com/blog-atac-seq>

ATAC-seq allows you to sequence regions of open chromatin through use of a transposase that cleaves and adds adapter sequences to open sites. We analyzed ATAC-seq data from the Immunological Genome Project (ImmGen) for Phase1 and Phase2 developing T cells.

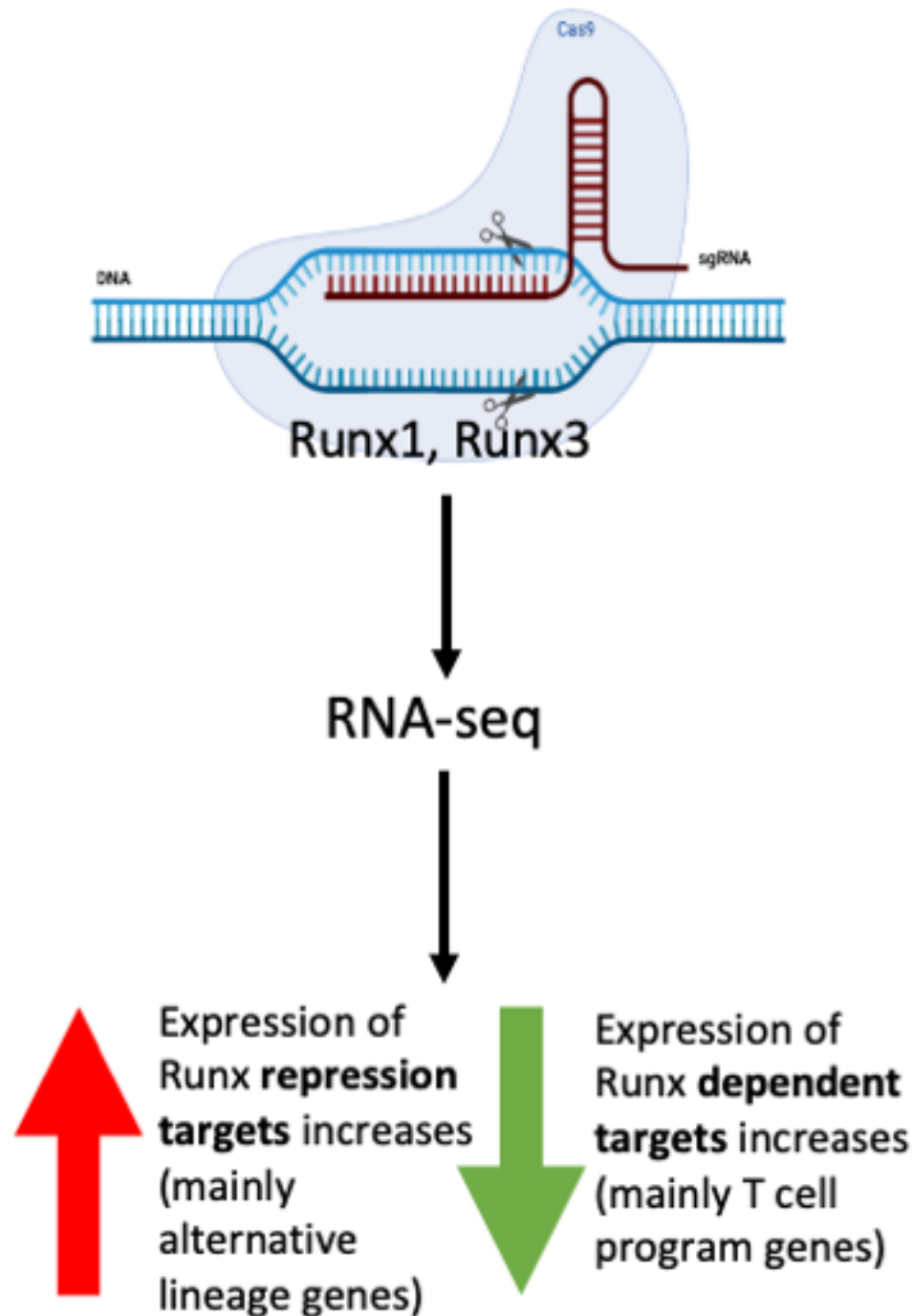
# Comparing ChIP-seq and ATAC-seq data



We can directly compare Runx ChIP-seq data with ATAC-seq data for the corresponding developmental stage to determine sites where Runx is binding open chromatin vs closed chromatin. In regions where ATAC and ChIP peaks overlap, Runx is binding open chromatin, whereas in regions where ChIP peaks don't overlap with ATAC peaks, Runx is binding closed chromatin.



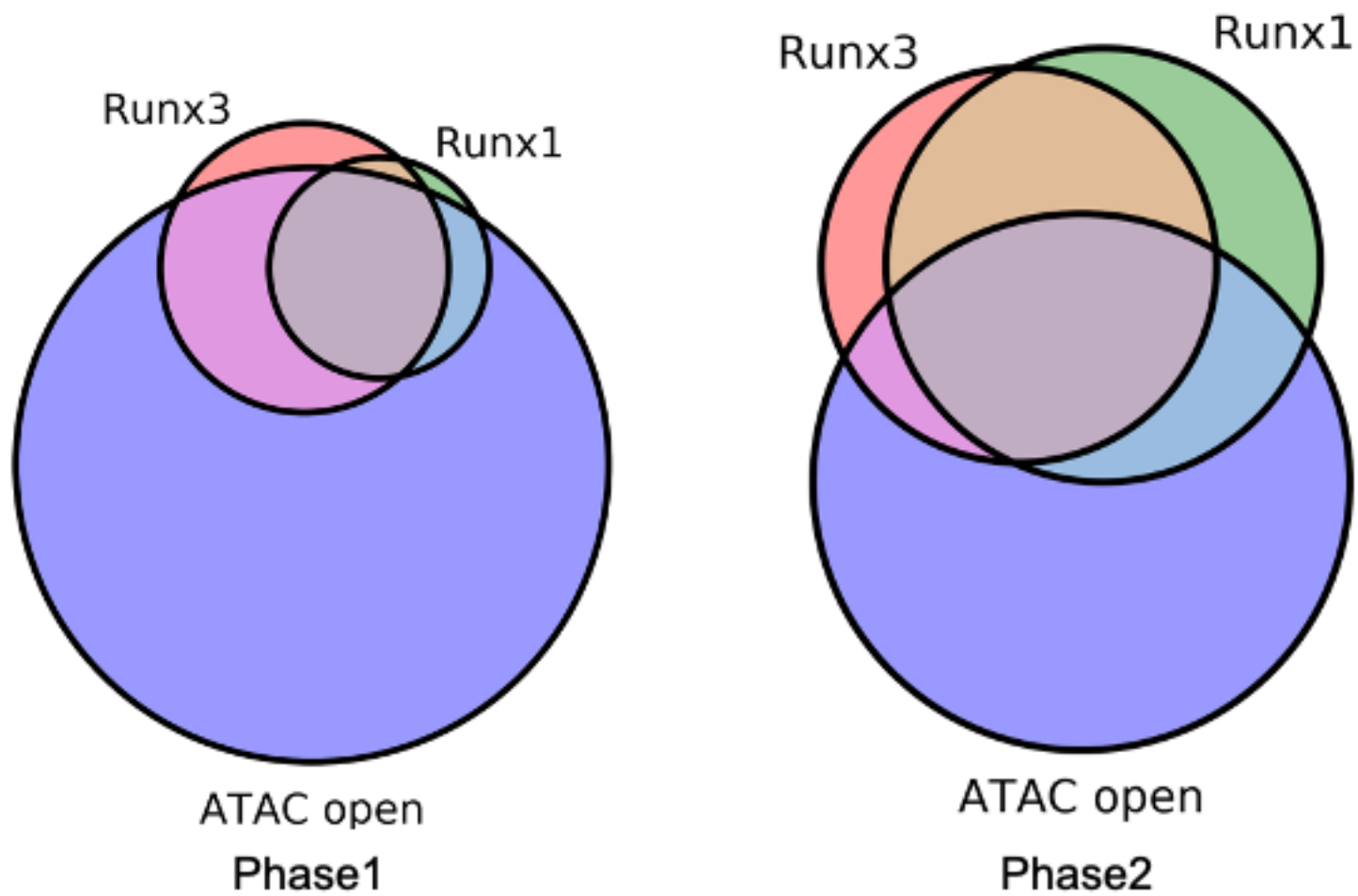
# RNA-seq to define Runx differentially expressed genes (DEGs)



Runx DEGs were determined by deleting Runx1 and Runx3 in pro-T cells with CRISPR, performing RNA-seq and looking for changes in expression when compared to normal developing T cells.

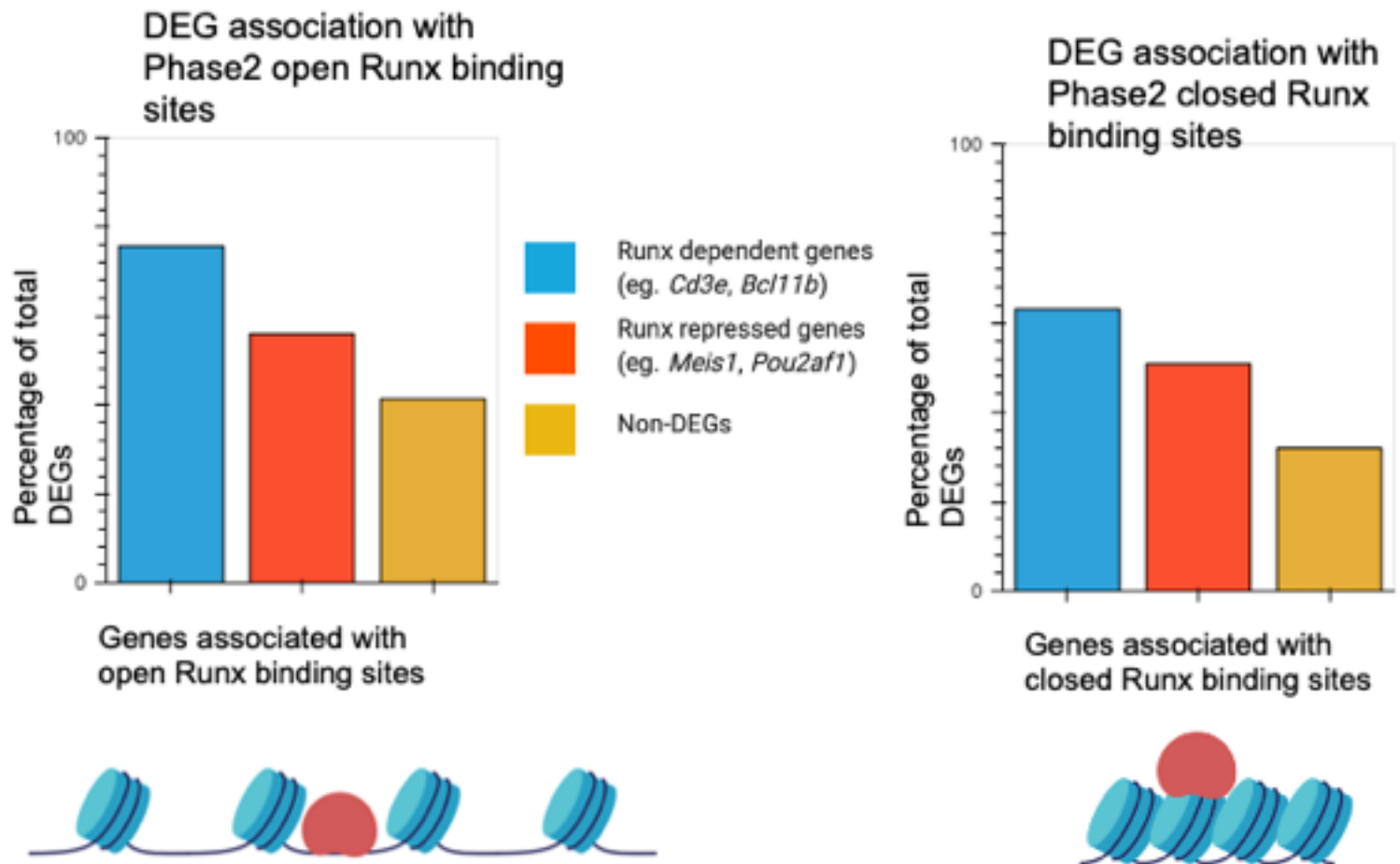
# RESULTS

Runx factors mainly bind open chromatin in Phase1, and both open and closed chromatin in Phase2



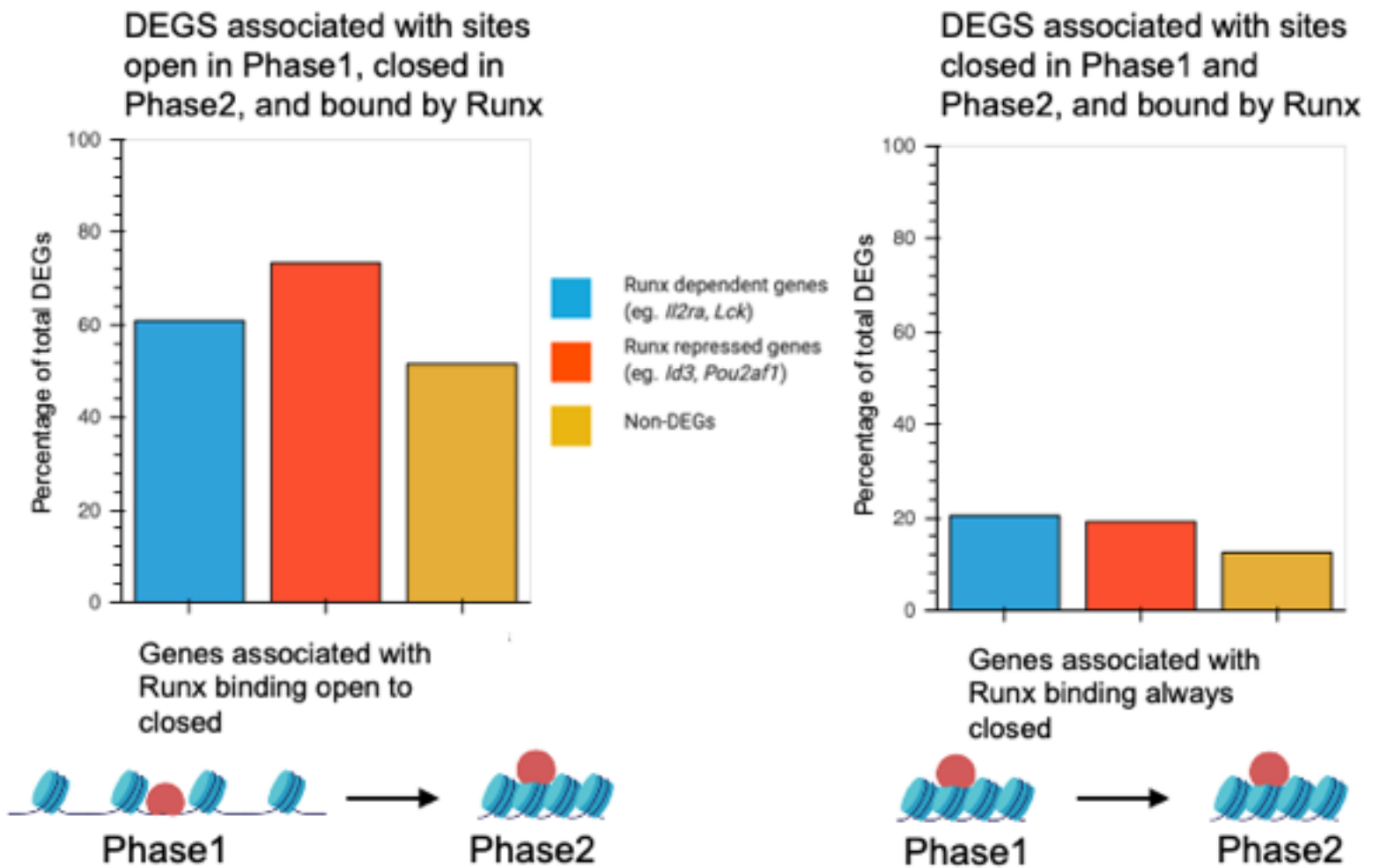
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# Runx dependent genes are highly correlated with regions of Runx binding both open and closed chromatin states



We identified genes associated with regions of Runx binding sites using GREAT, a tool that associates genomic regions to their putative target genes. We then compared these genes to Runx DEGs. We found that regions where Runx binds in open chromatin in Phase2 are highly associated with Runx dependent genes. Based on our hypothesis, we thought we might see a higher correlation with Runx repression targets in regions where Runx binds closed chromatin, but in this analysis we still saw a higher association with Runx dependent targets.

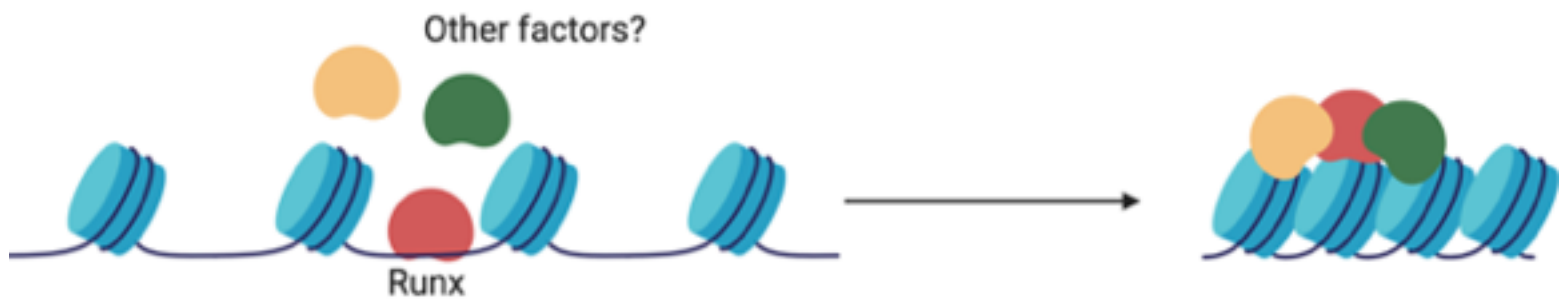
# Runx binding sites that go from open in Phase1 to closed in Phase2 are correlated with Runx repressed genes



We focused in on the only the sites we thought Runx might be closing as a mechanism of repression by analyzing only sites that were open in Phase1, closed in Phase2, and bound by Runx. We also filtered DEGs to remove indirect Runx targets as well as genes from contaminating cell lineages. We then uncovered a correlation between genes associated with these actively closing regions and Runx repressed DEGs. As a control, we analyzed sites bound by Runx that remain closed across development, and no longer saw this correlation.



## A POSSIBLE MECHANISM OF RUNX REPRESSION



While much work has been done to understand how transcription factors activate their target genes, much less is known about how they can repress. We found evidence that Runx factors may be repressing genes associated with alternate cell lineages during early T development by closing the associated regions of chromatin. While a detailed mechanism is still unknown, one hypothesis is that Runx may be recruiting other factors, such as chromatin remodelers, to these sites.

## CONCLUSIONS AND ACKNOWLEDGEMENTS

### Conclusions

- Runx dependent genes (eg. *Bcl11b*, *Cd3*, and *Tcf7*) were highly correlated with regions of Runx binding in both open and closed chromatin states
- Runx repressed genes (eg. *Id3*, *Pou2af1*) were correlated with Runx binding regions that were open in Phase 1 but closed in Phase 2
- Runx may be repressing genes through a mechanism that closes the associated chromatin
- Despite constant expression throughout T cell development, Runx mediates phase specific roles by interacting with genomic loci undergoing dynamic changes in accessibility

### Acknowledgements

This work was funded by NIH R01 AI135200 and the Cancer Research Institute/ Irvington Postdoctoral Fellowship CRI.SHIN. Student support was provided by NIH T35 OD010956