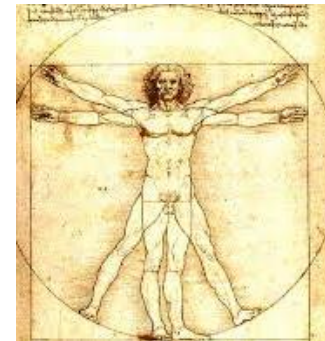
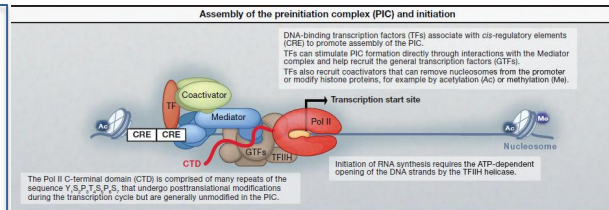
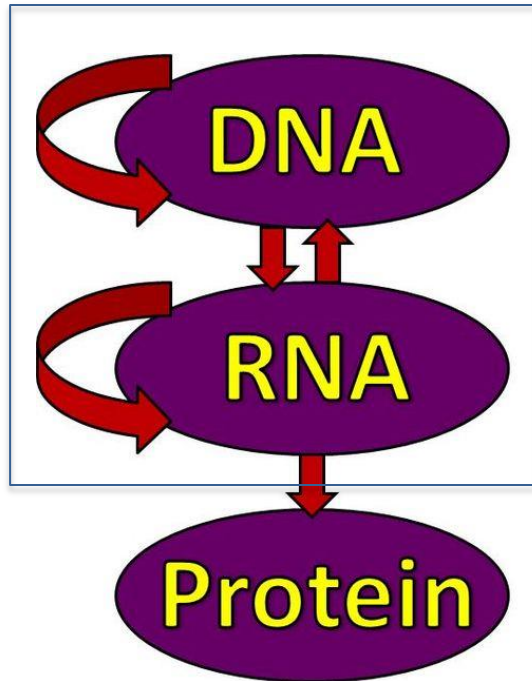


# **Chromosome Dynamics in T cells**

**Lark Kyun Kim, Ph.D.**

Assistant Professor  
Severance Biomedical Science Institute  
Yonsei University College of Medicine

# Our Ultimate Goal



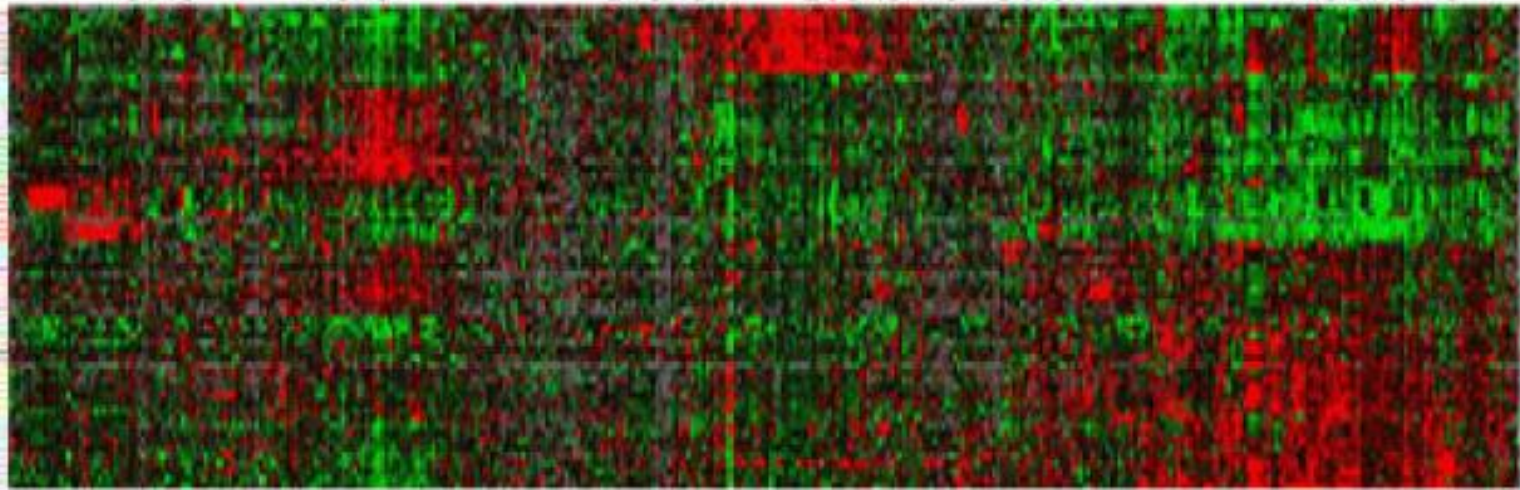
Mechanism



Biological phenomenon  
(in vivo and physiologically)

target genes  
biomarker

# Genome-wide studies



## Genome-wide sequencing

- RNA seq
- Exome seq
- ChIP seq
- Gro-seq
- Mi-seq
- LincRNA-seq

## Bioinformatics

Top 20 (or whatever number)  
Up- or down- regulated gens

# Question (I)

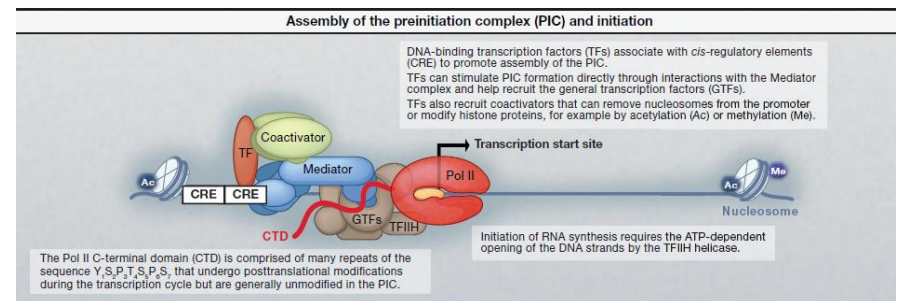
How many genes are being transcribed simultaneously in a given time point?

## Things to think about

1. The number of transcription machinery such as RNA polymerase II and TBP is unlimited?
2. Transcription can undergo anywhere in the nucleus?

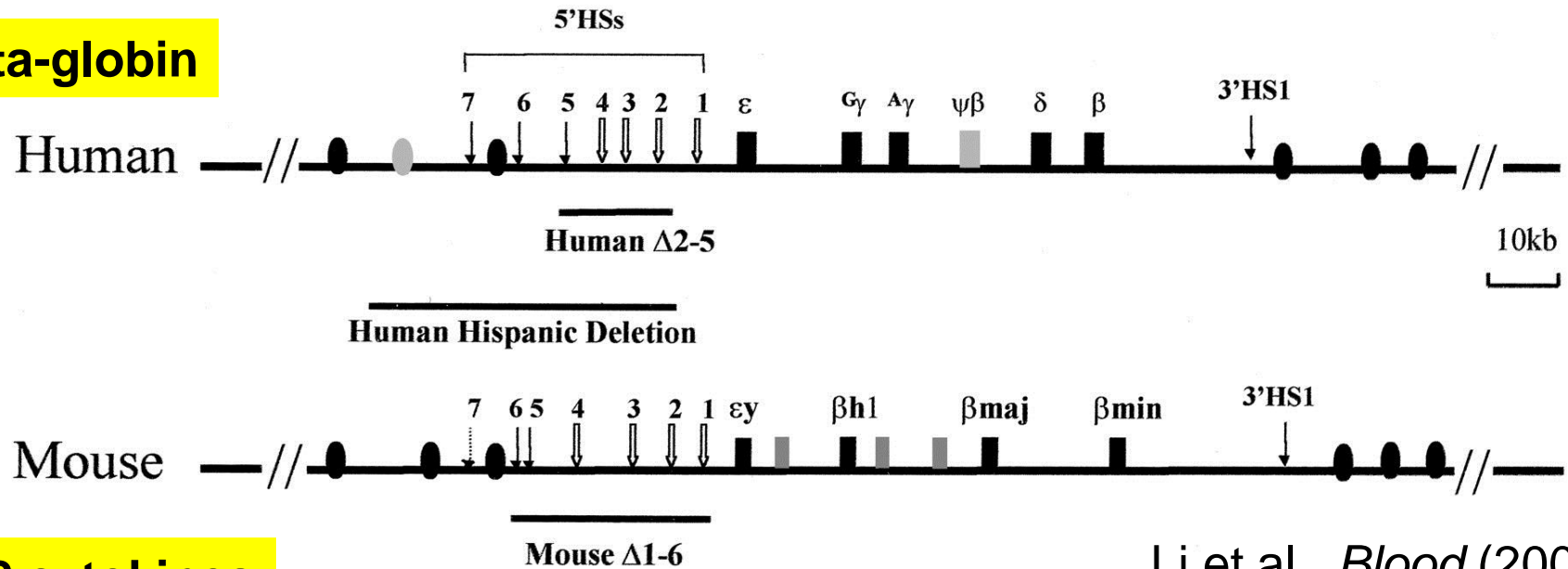
## Question (II)

Each gene is regulated individually  
only via its own promoter and/or enhancer  
or  
a kind of control tower oversees  
the transcription globally?



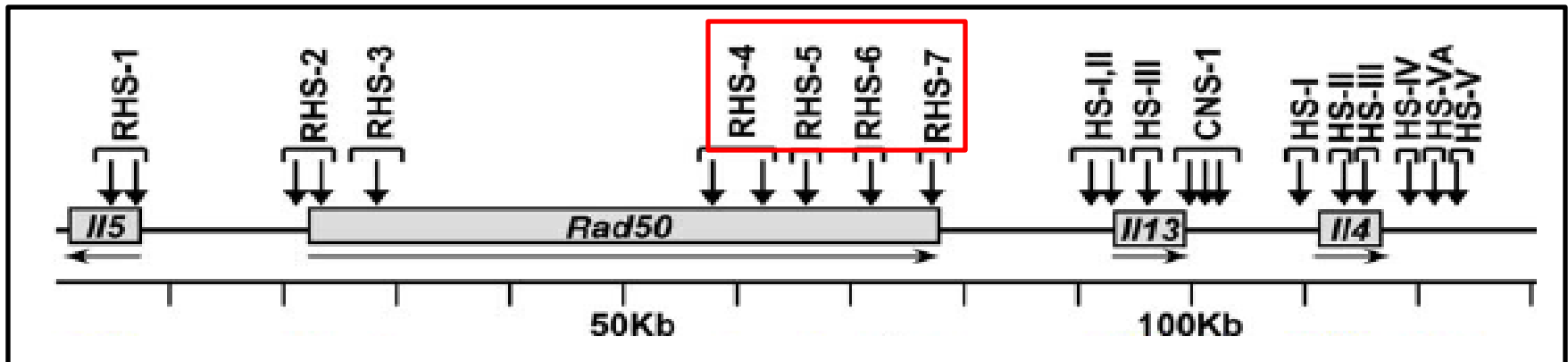
# Locus control region (LCR) - Beta-globin & Th2 cytokine genes-

## Beta-globin



Li et al., *Blood* (2002)

## Th2 cytokines



Spilianakis et al., *Nat Immunol* (2004)

## Question (III)

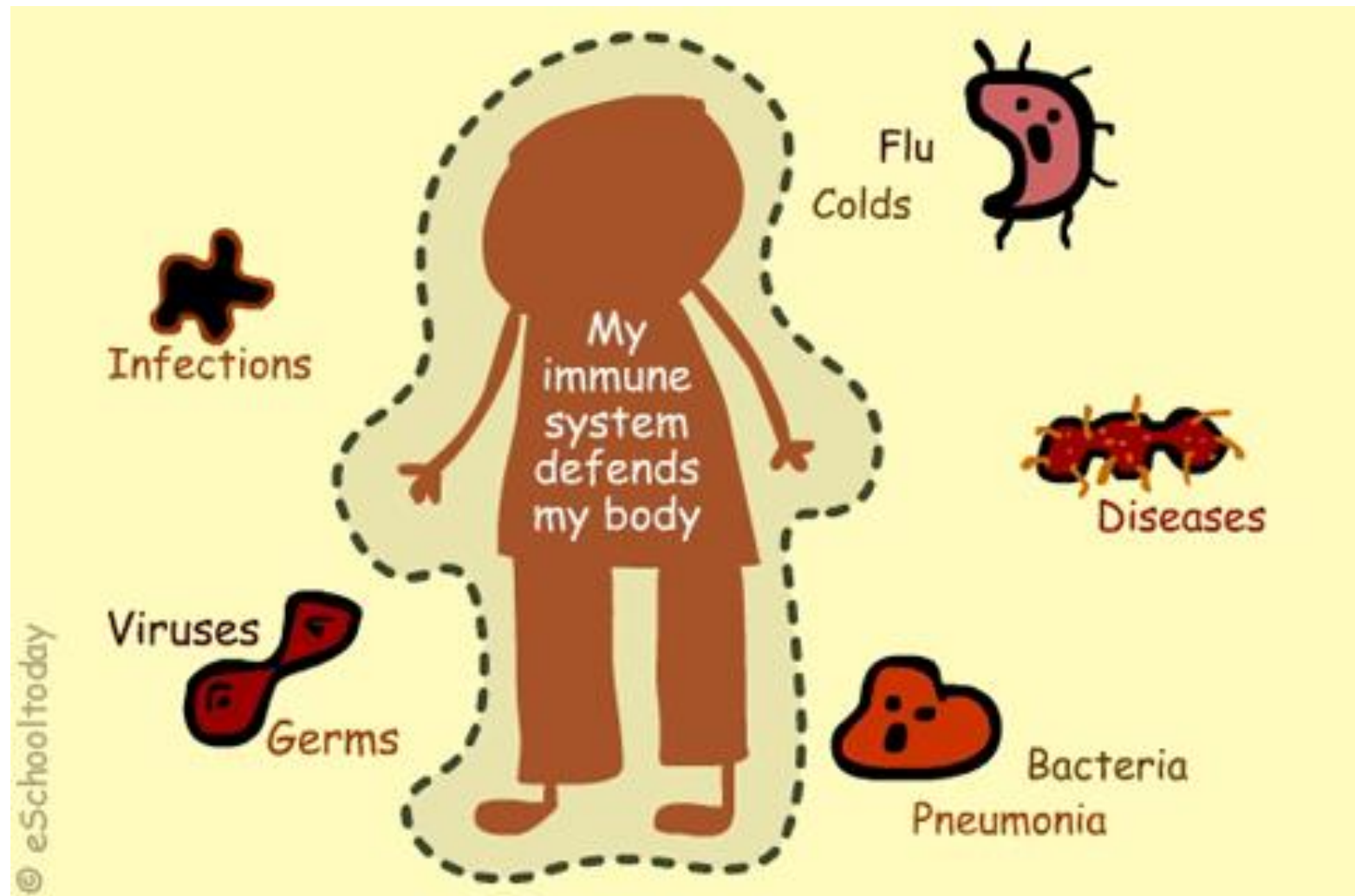
If there is a control tower which regulates  
a series of genes located closely  
at the same time,

what happens?

in case of the relevant genes are located on  
the different chromosomes

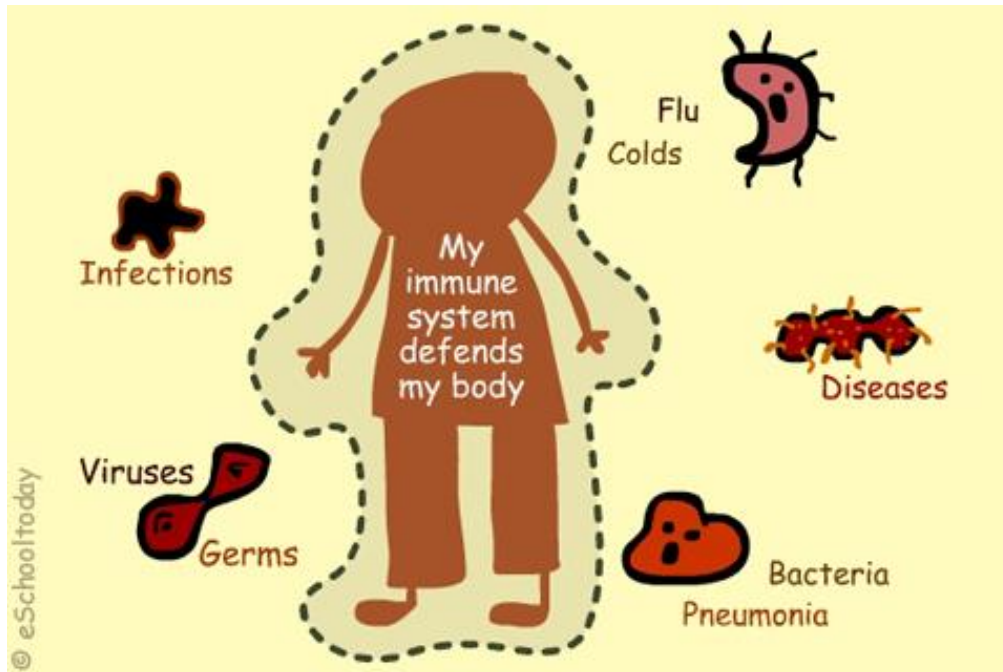
and moreover, one gene should be selected  
and the other should not

# Immune System





# Pathogens can be classified into 3 groups



## Intracellular pathogens

Virus  
Listeria  
Salmonella  
Legionella  
Mycobacterium

## Extracellular pathogens

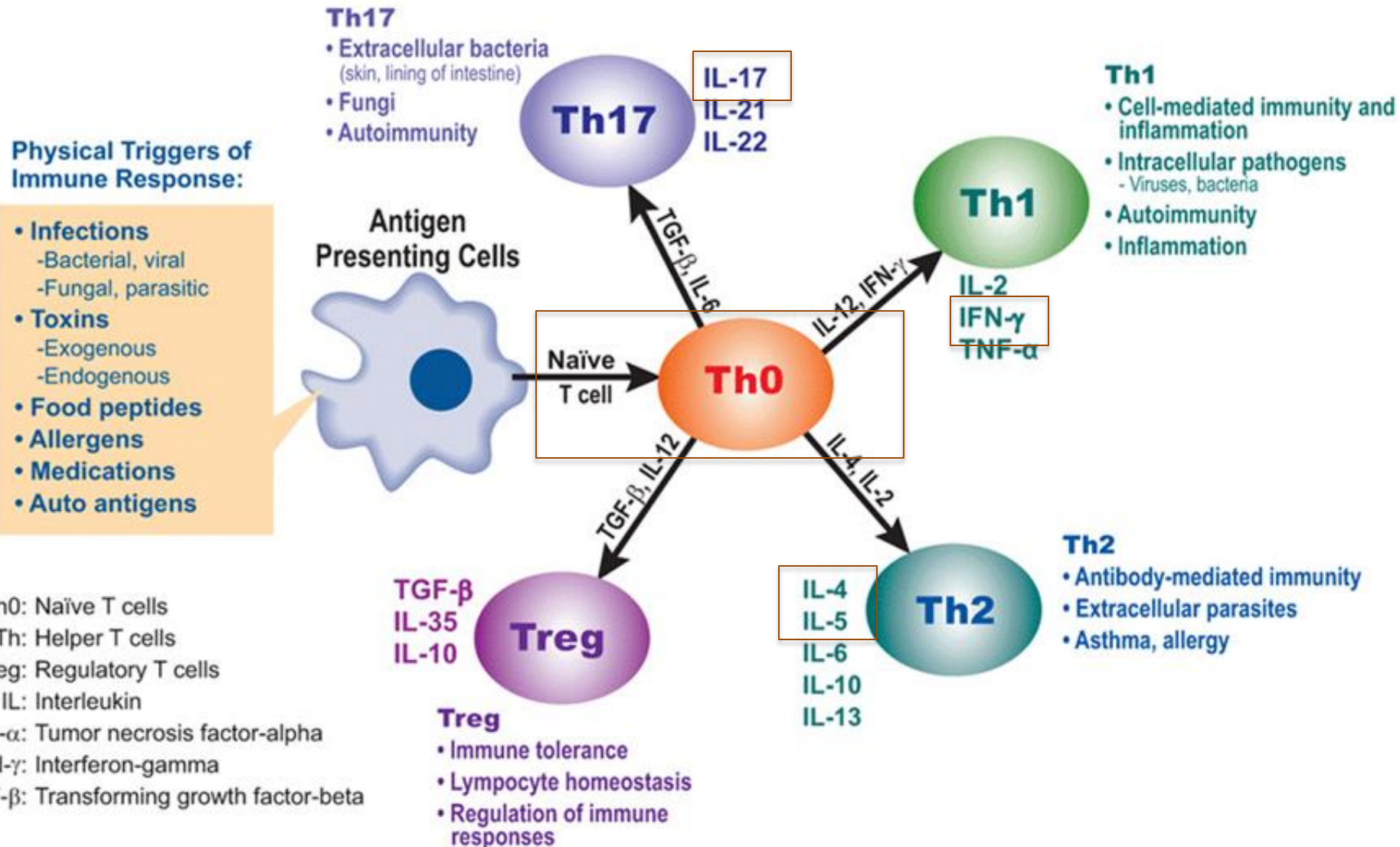
Bacillus  
Mycoplasma  
Pseudomonas  
Vibrio  
Streptococcus

## Allergy

Food allergy  
Atopic dermatitis  
Asthma

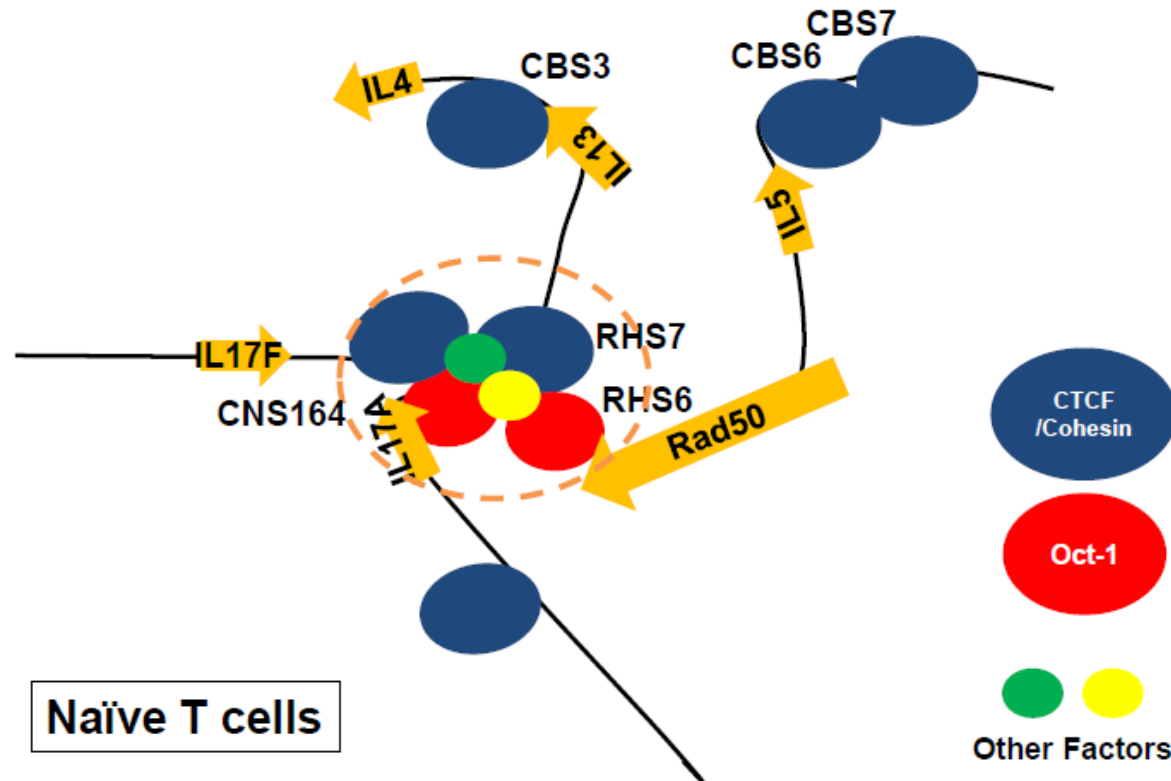
Helminth

# T cells are key regulators of immune systems

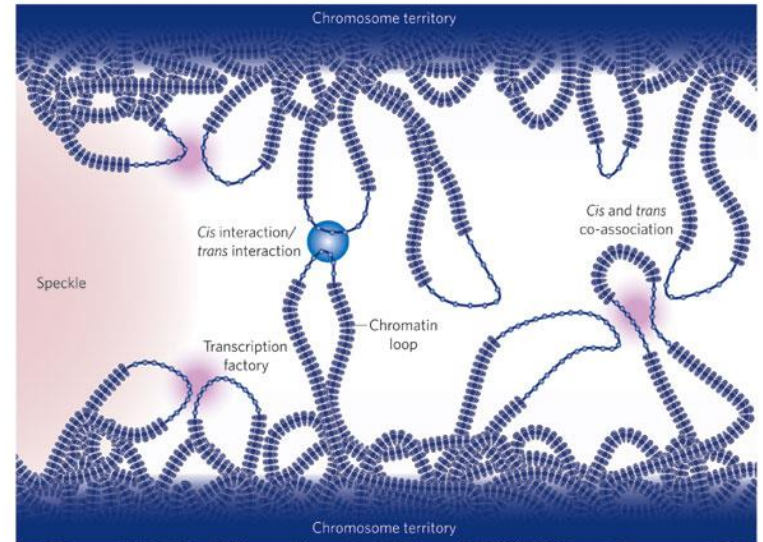
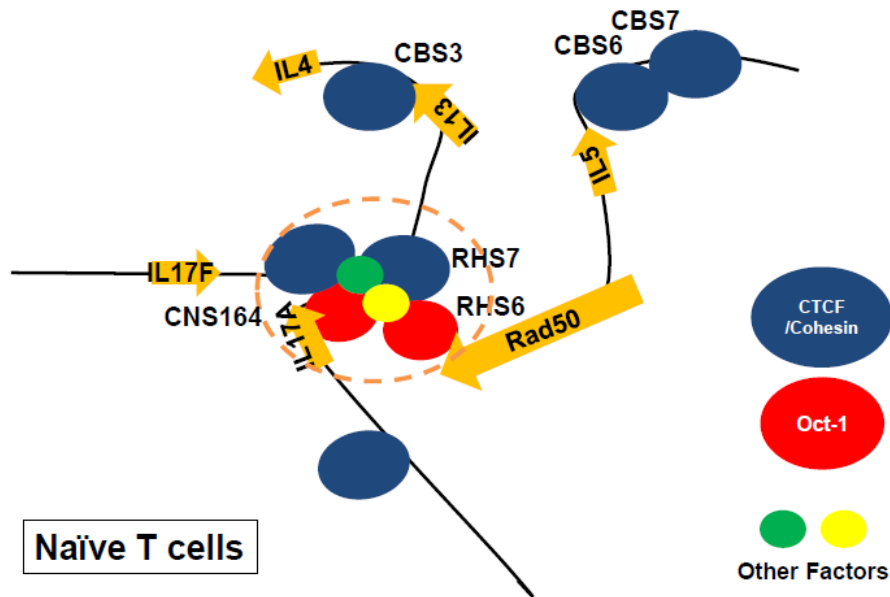


Naïve T cells have a potential to express IFN $\gamma$ , IL4 or IL17  
These genes are located on different chromosomes

# Interchromosomal association of each cytokine loci in naïve T cells

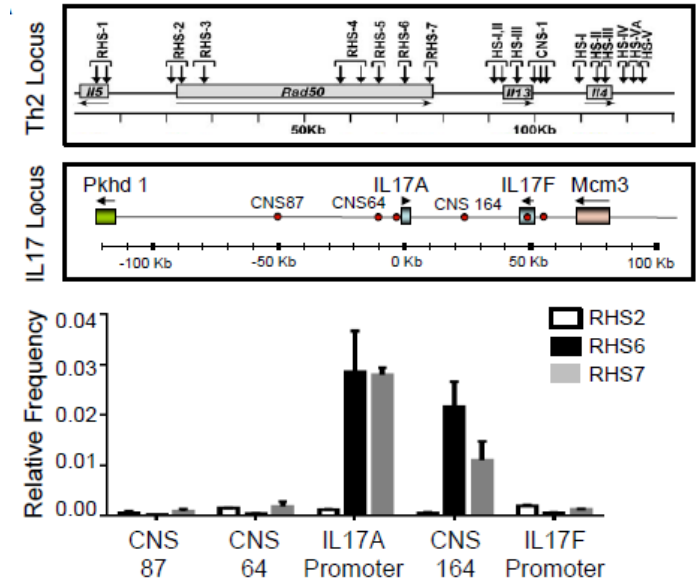
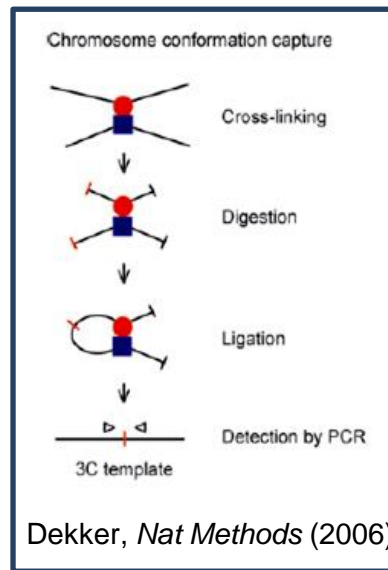
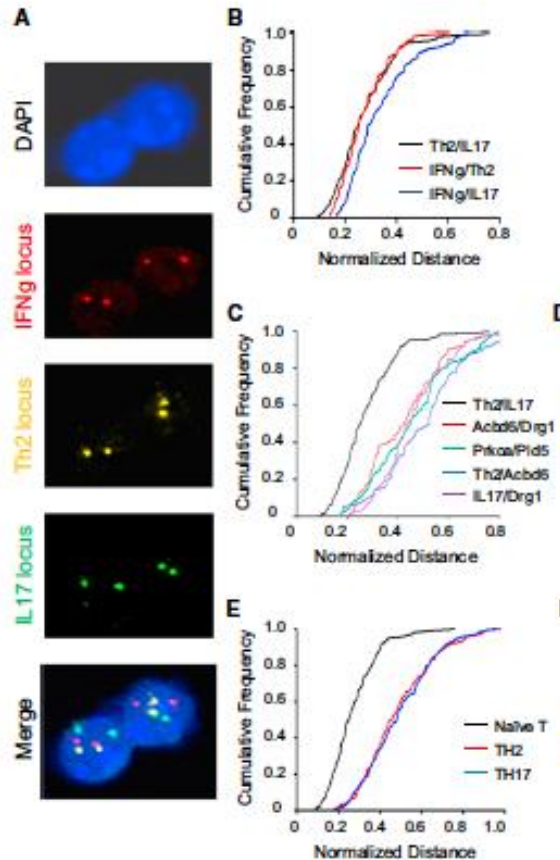


# Interchromosomal association of each cytokine loci in naïve T cells



Fraser & Bickmore, *Nature* (2007)

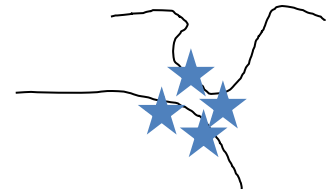
# Interchromosomal association of each cytokine loci in naïve T cells



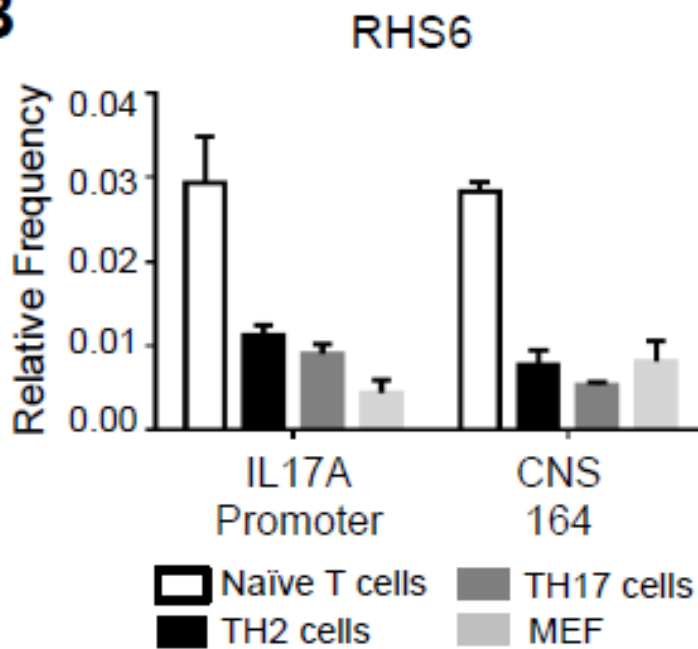
3C /4C Hi-C

DNA FISH

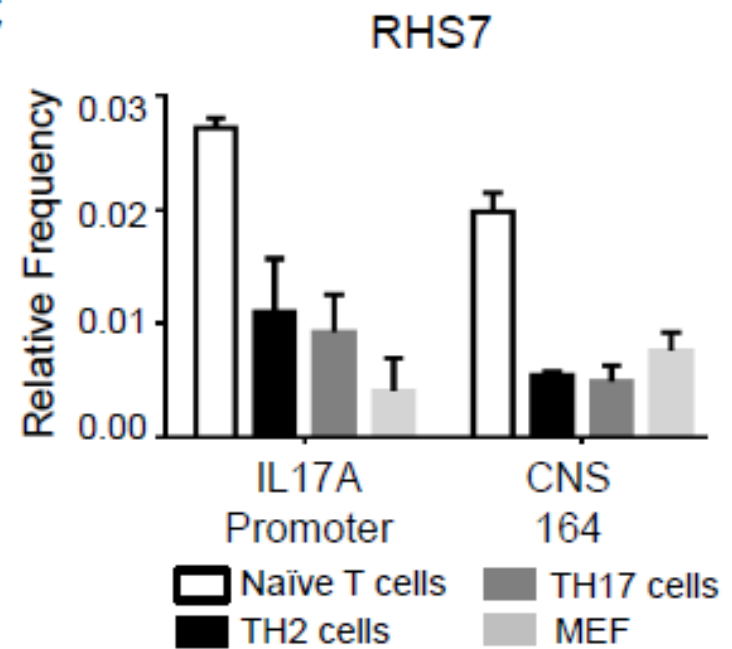
# 3C qPCR b/w Th2 locus and IL17 locus



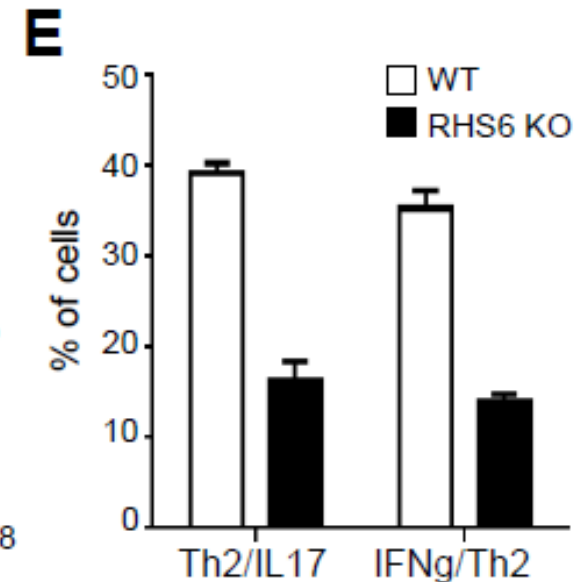
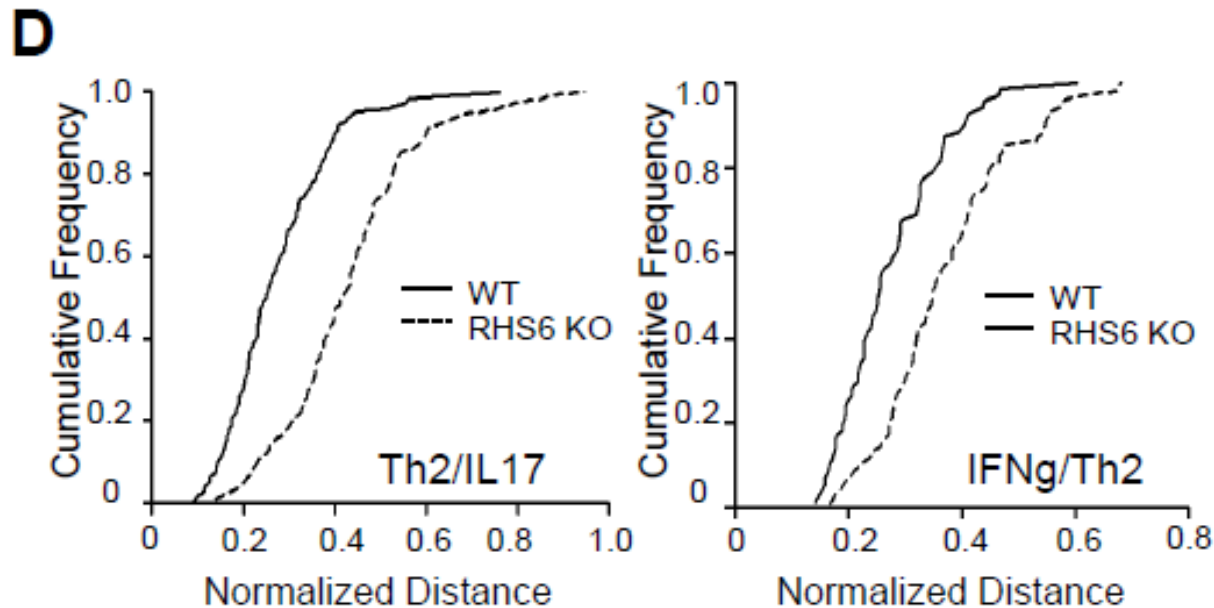
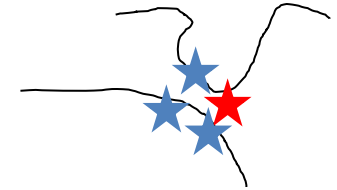
**B**



**C**



# Interchromosomal association of cytokine loci in the absence of RHS6 in naïve T cells



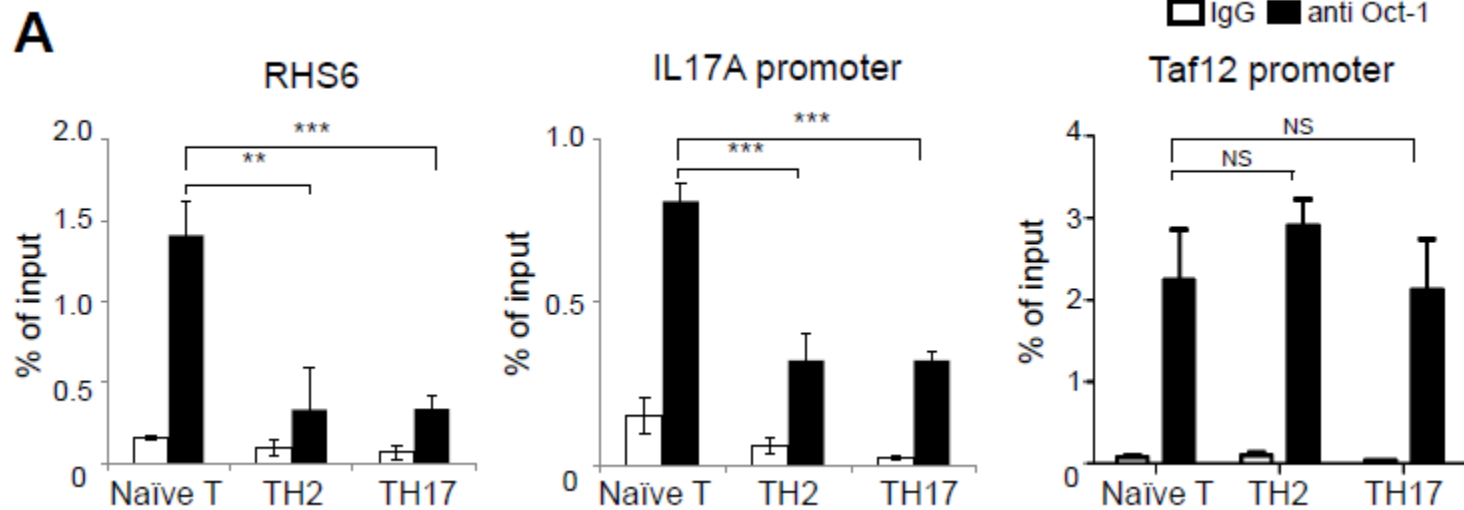
# Binding proteins on interest regions

RHS6 of Th2 locus and promoter of IL-17 gene: Oct-1

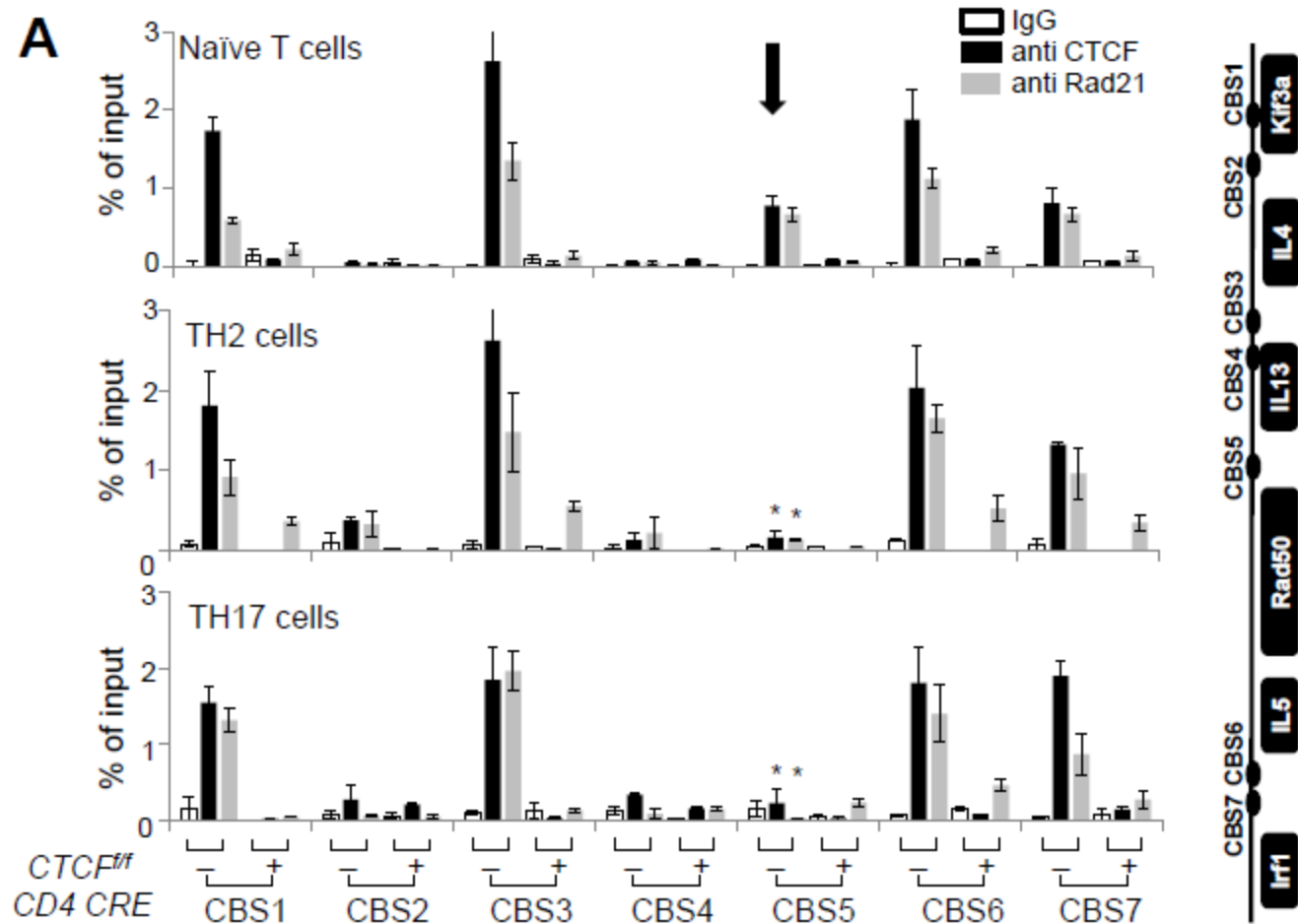
RHS7 of Th2 locus and CNS164 of IL-17 locus: CTCF



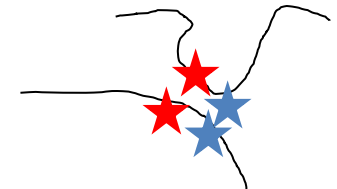
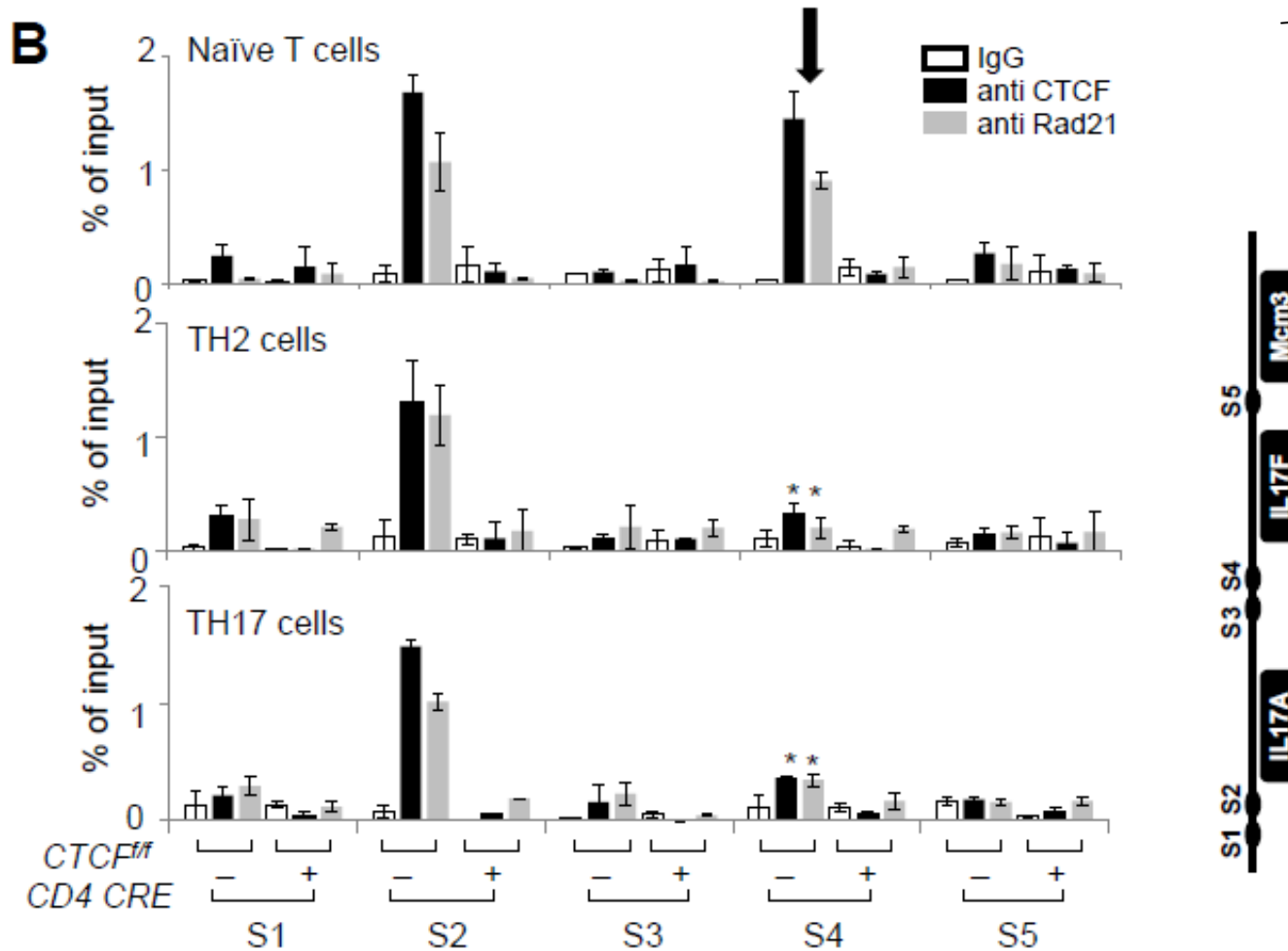
# The binding of Oct-1 at RHS6 and IL-17 promoter in naïve T cell is reduced after differentiation



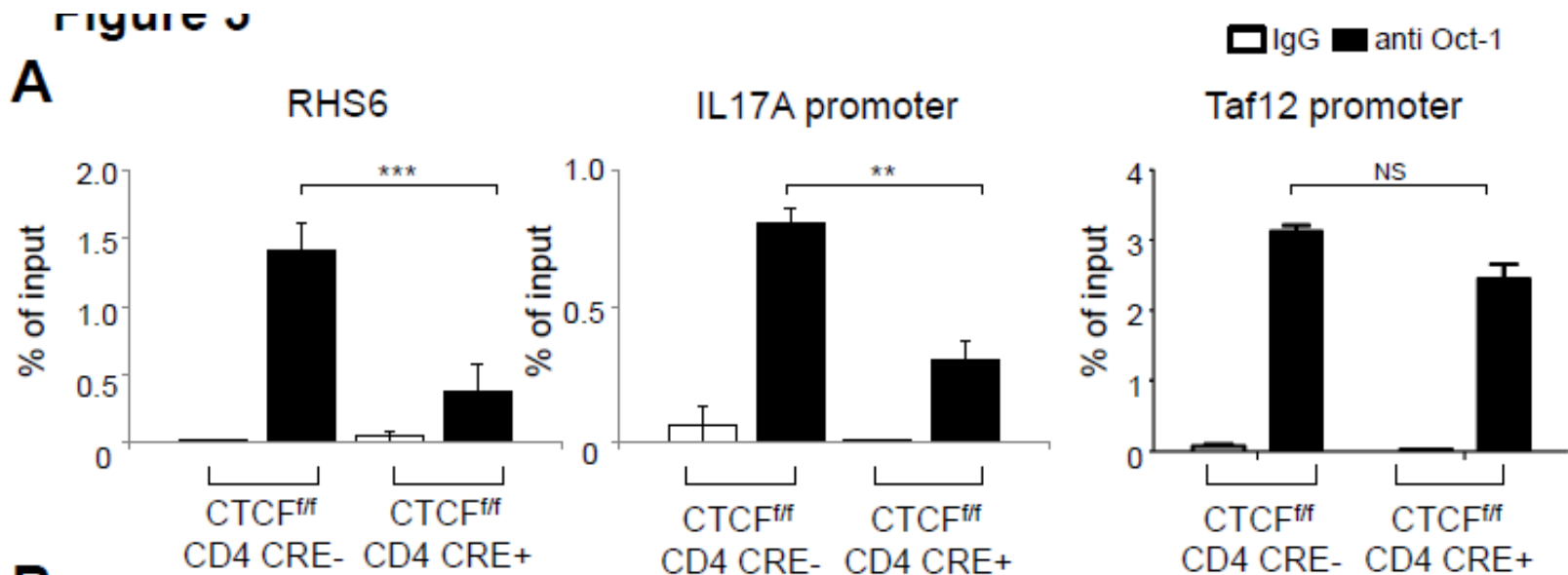
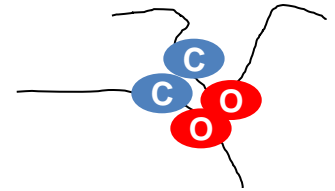
# The binding of CTCF in the Th2 locus during T cell differentiation



# The binding of CTCF in the IL-17 locus during T cell differentiation

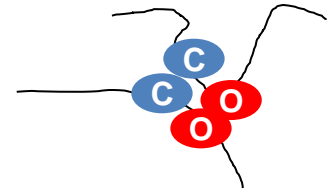
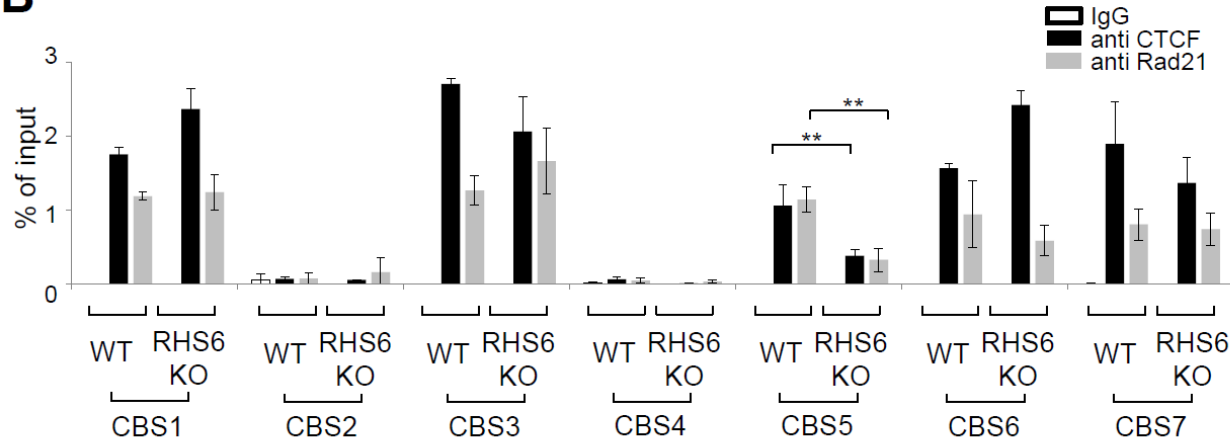


# The binding of Oct-1 at RHS6 and IL-17 promoter in the absence of CTCF

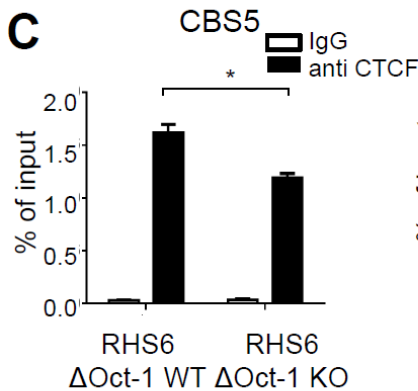


# The binding of CTCF at RHS7 and CNS164 in the absence of RHS6

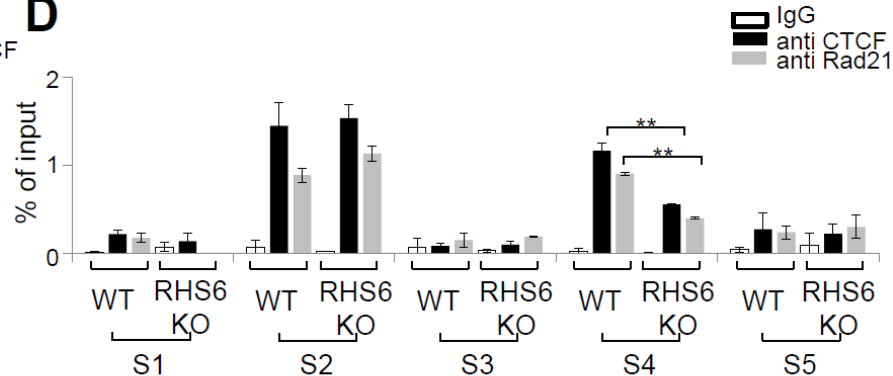
**D**



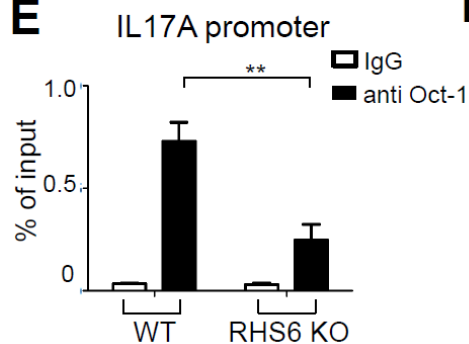
**C**



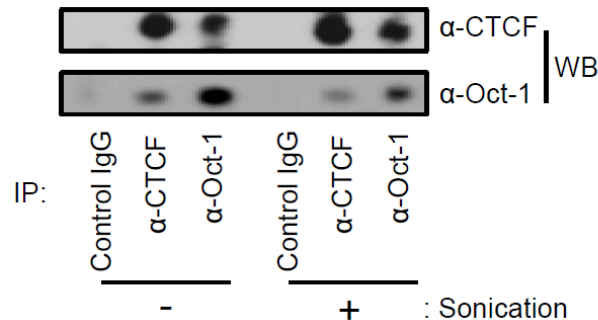
**D**



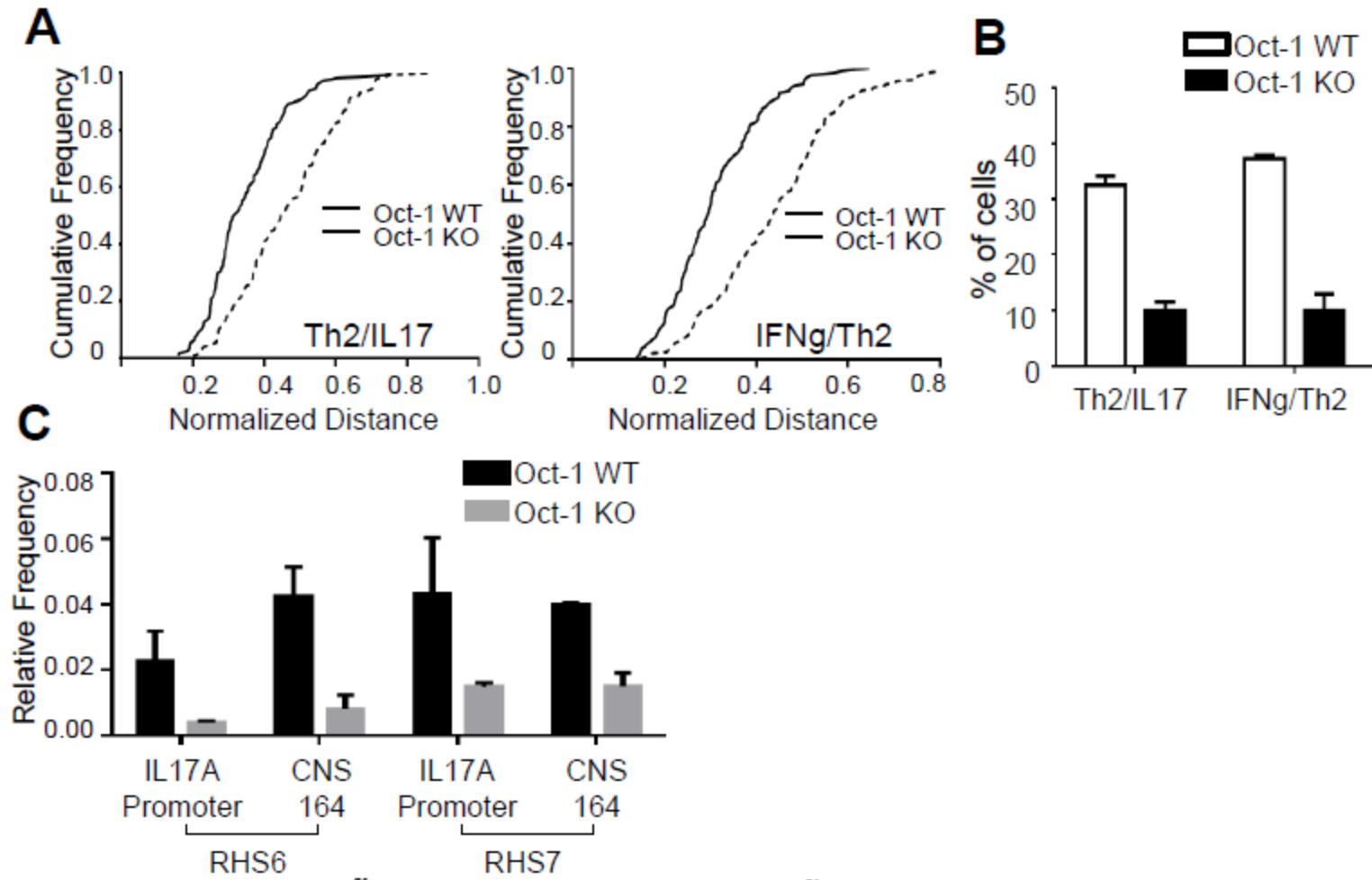
**E**



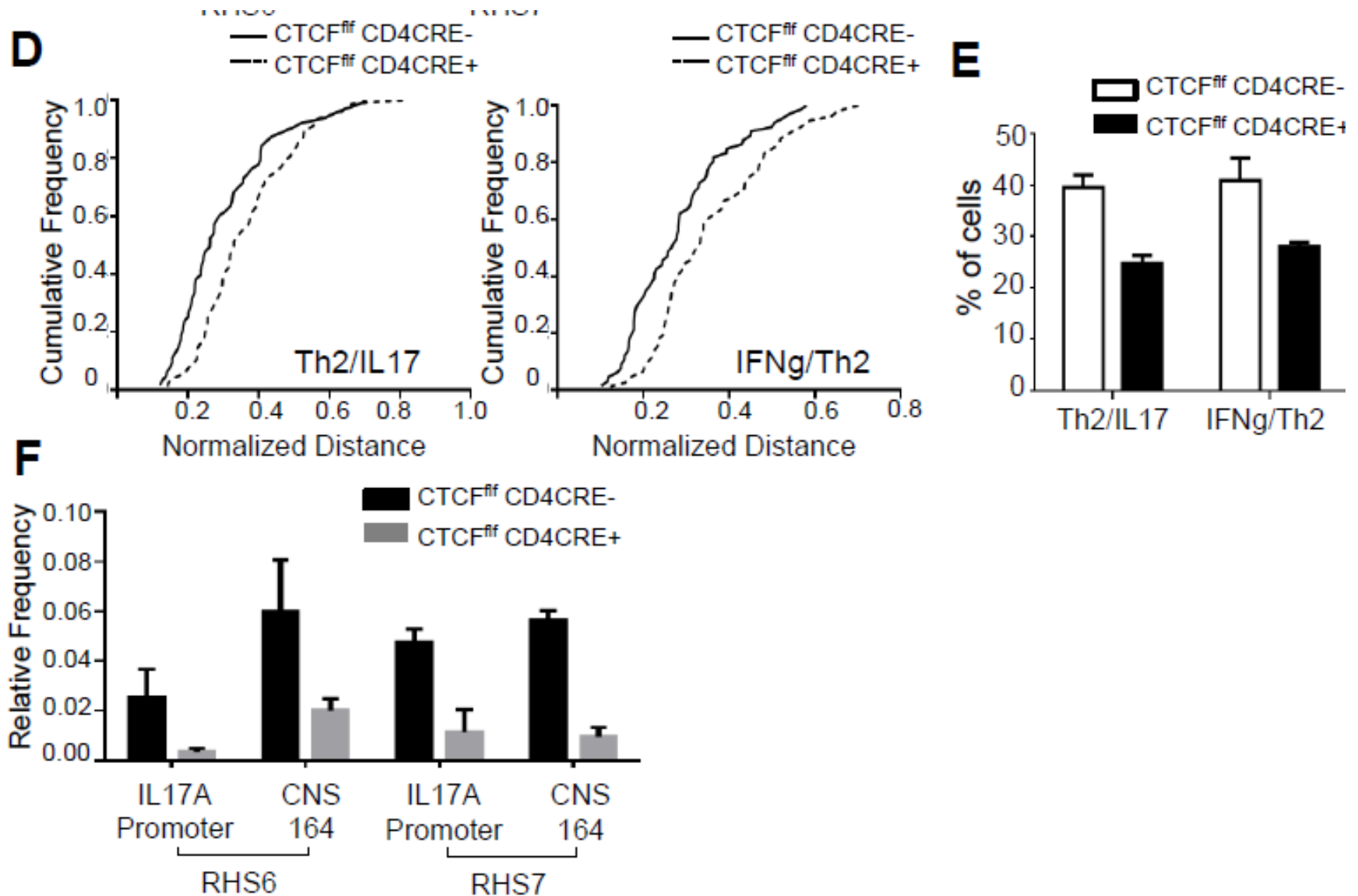
**F**



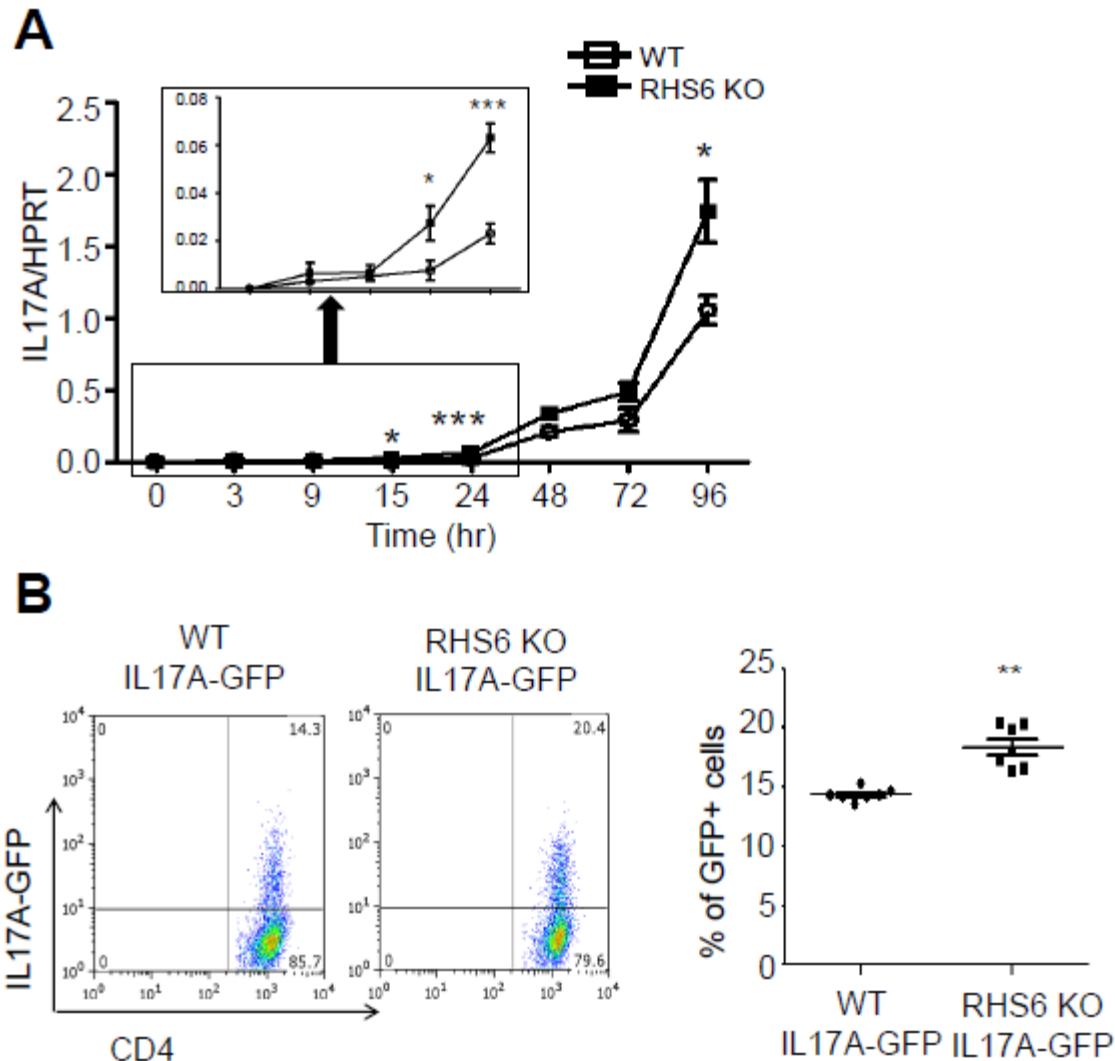
# Interchromosomal association of cytokine loci in the absence of Oct-1



# Interchromosomal association of cytokine loci in the absence of CTCF

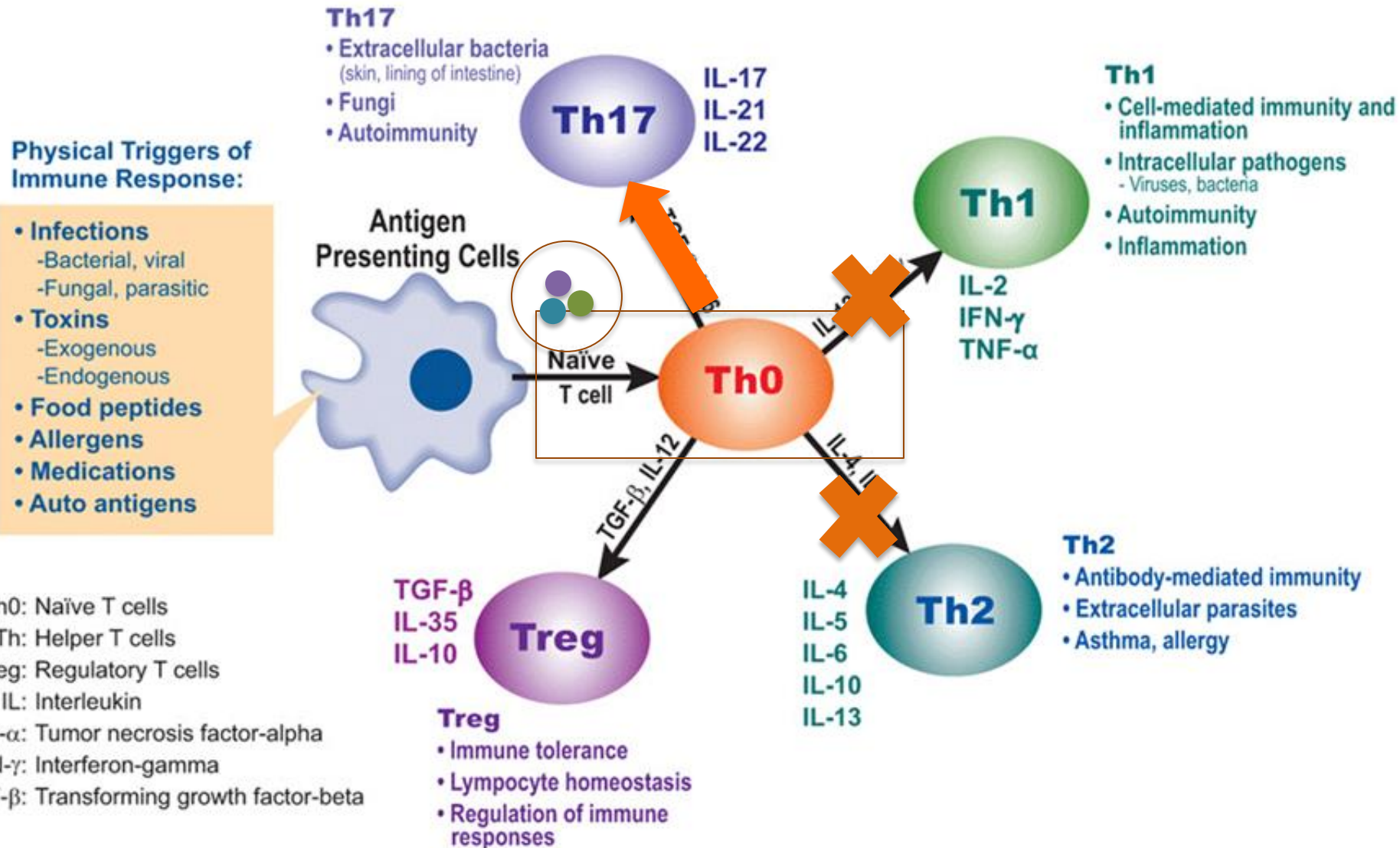


# More Th17 cells generation in the absence of interchromosomal association

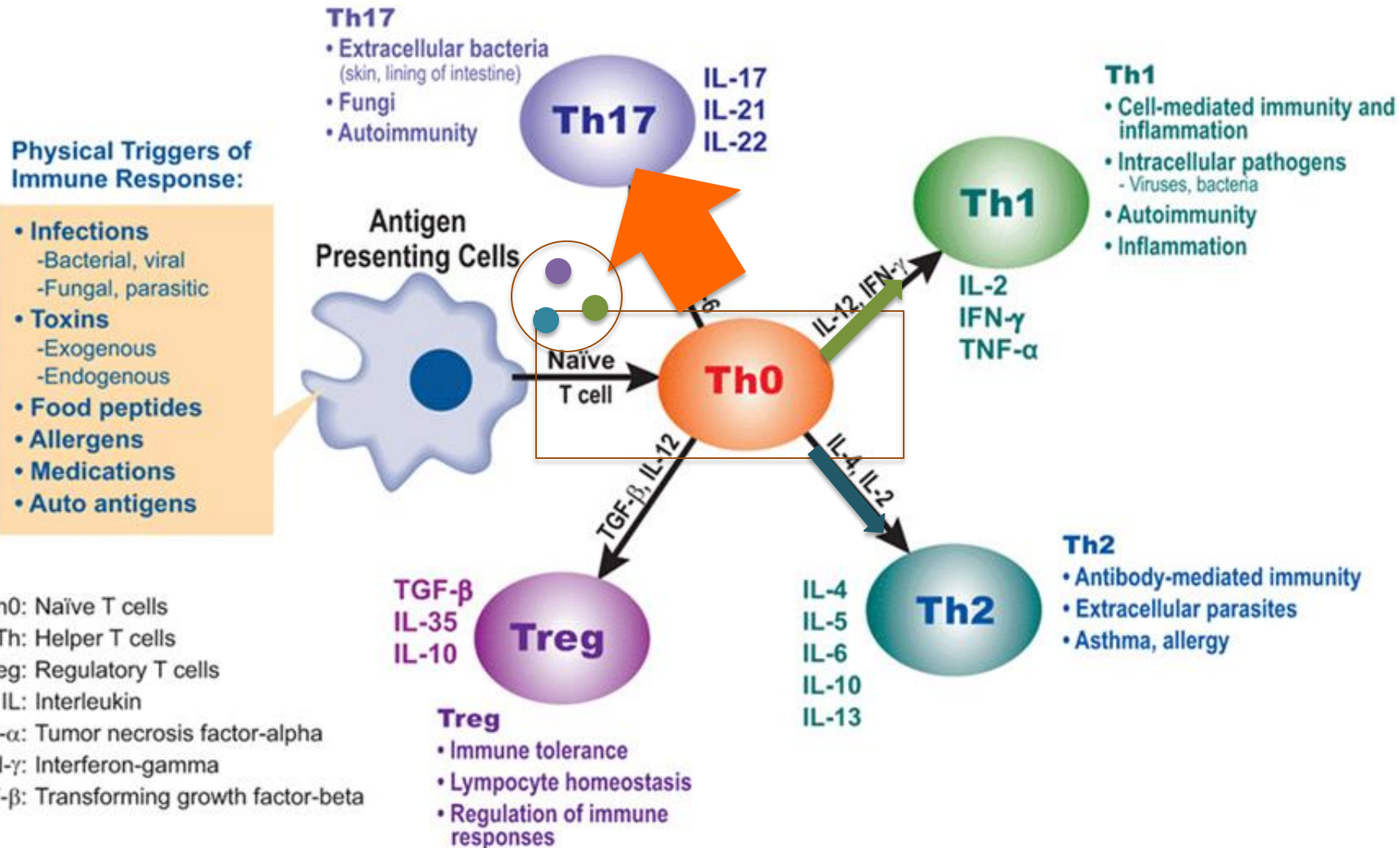




# Aberrant production of effector T cells in the absence of interchromosomal association

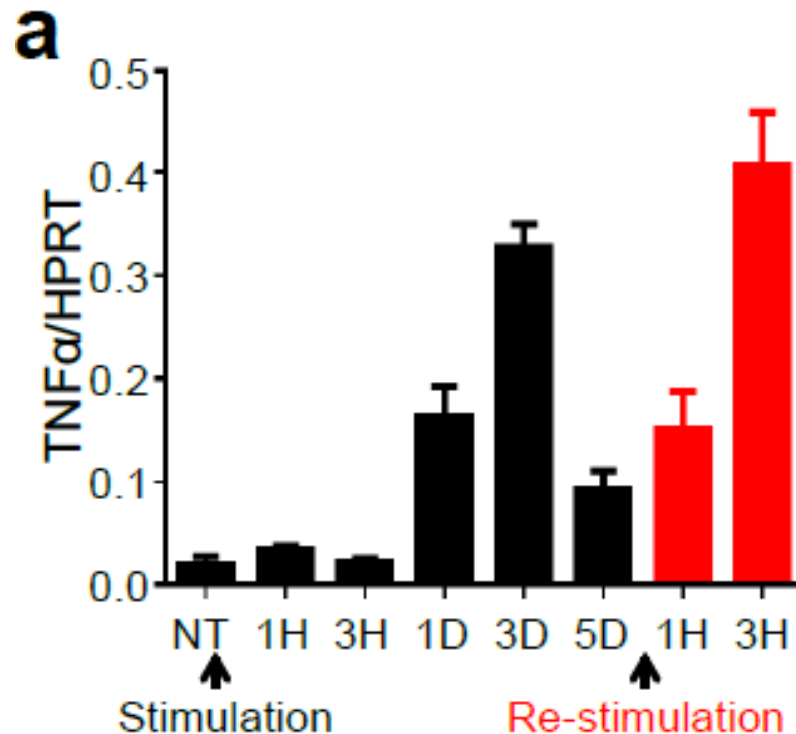


# Aberrant production of effector T cells in the absence of interchromosomal association



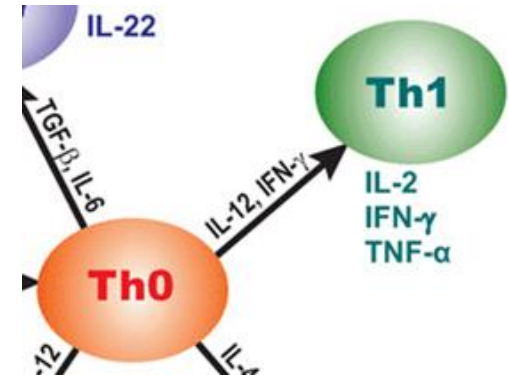
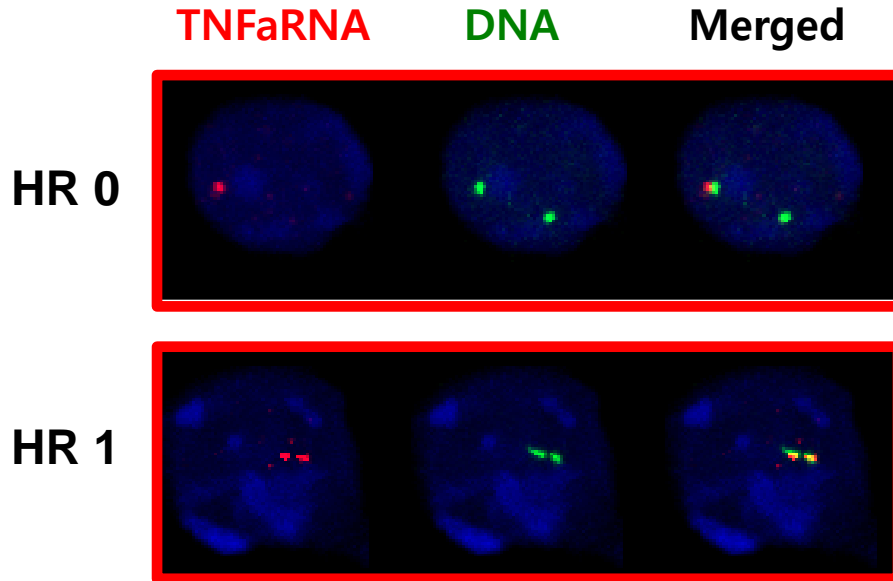
Another study on  
spatiotemporal regulation of transcription

# Immunological memory is a key feature of immunity but molecular basis of this remains unclear

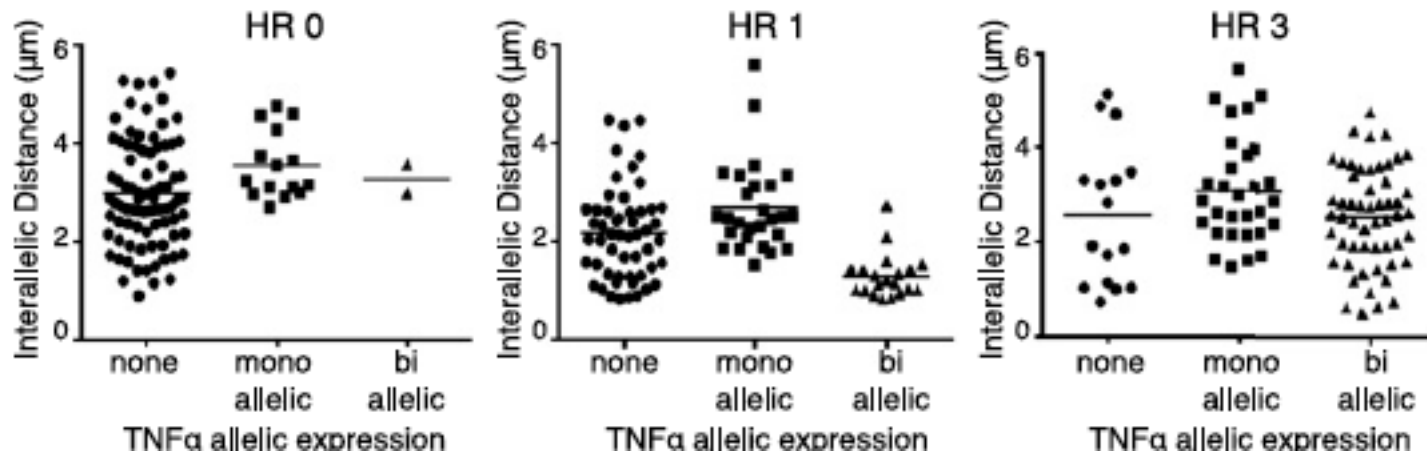


The resting Th1 cells can express TNF alpha rapidly and more efficiently upon previously encountered challenge

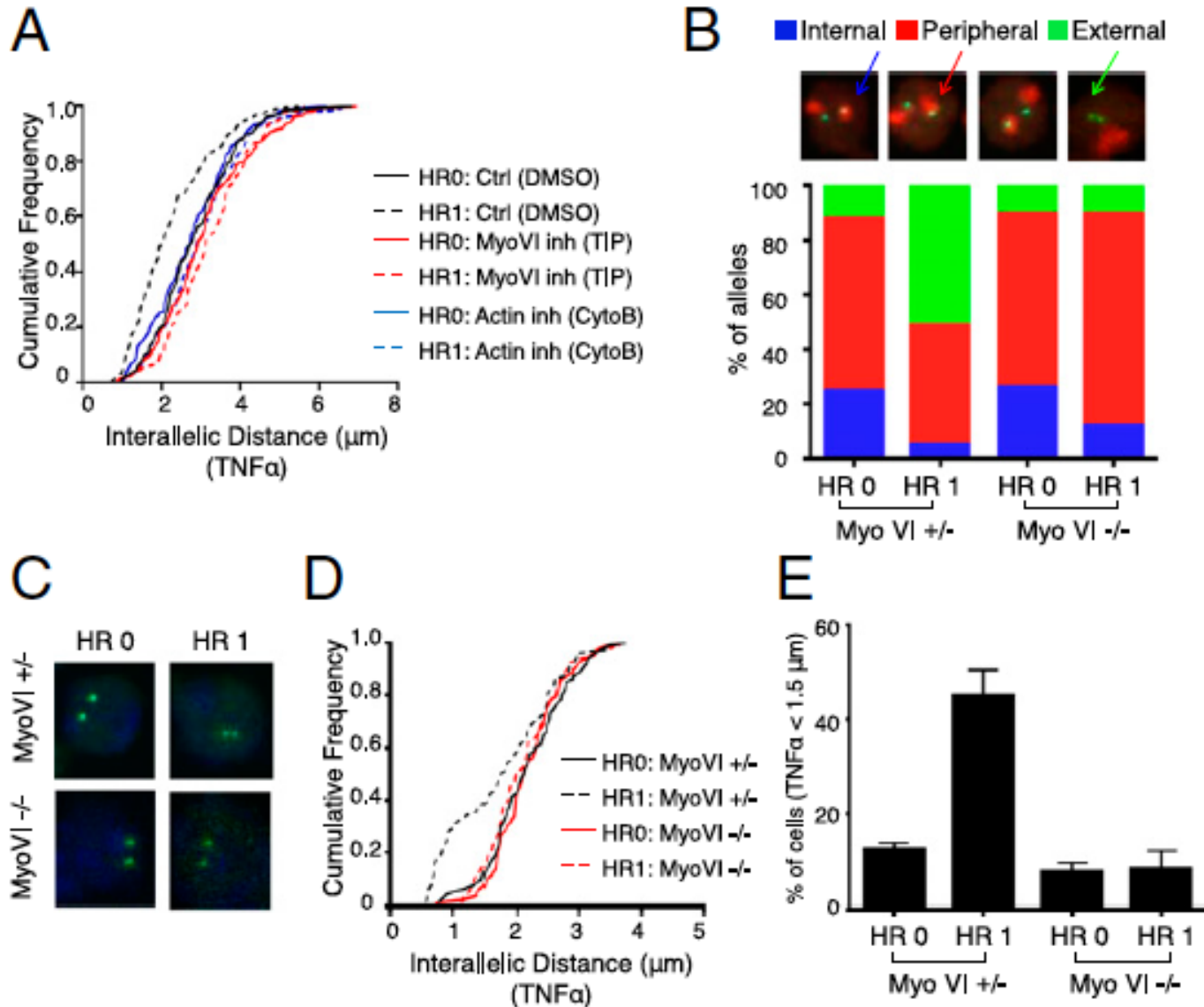
# Allelic pairing of TNF loci is required for biallelic expression



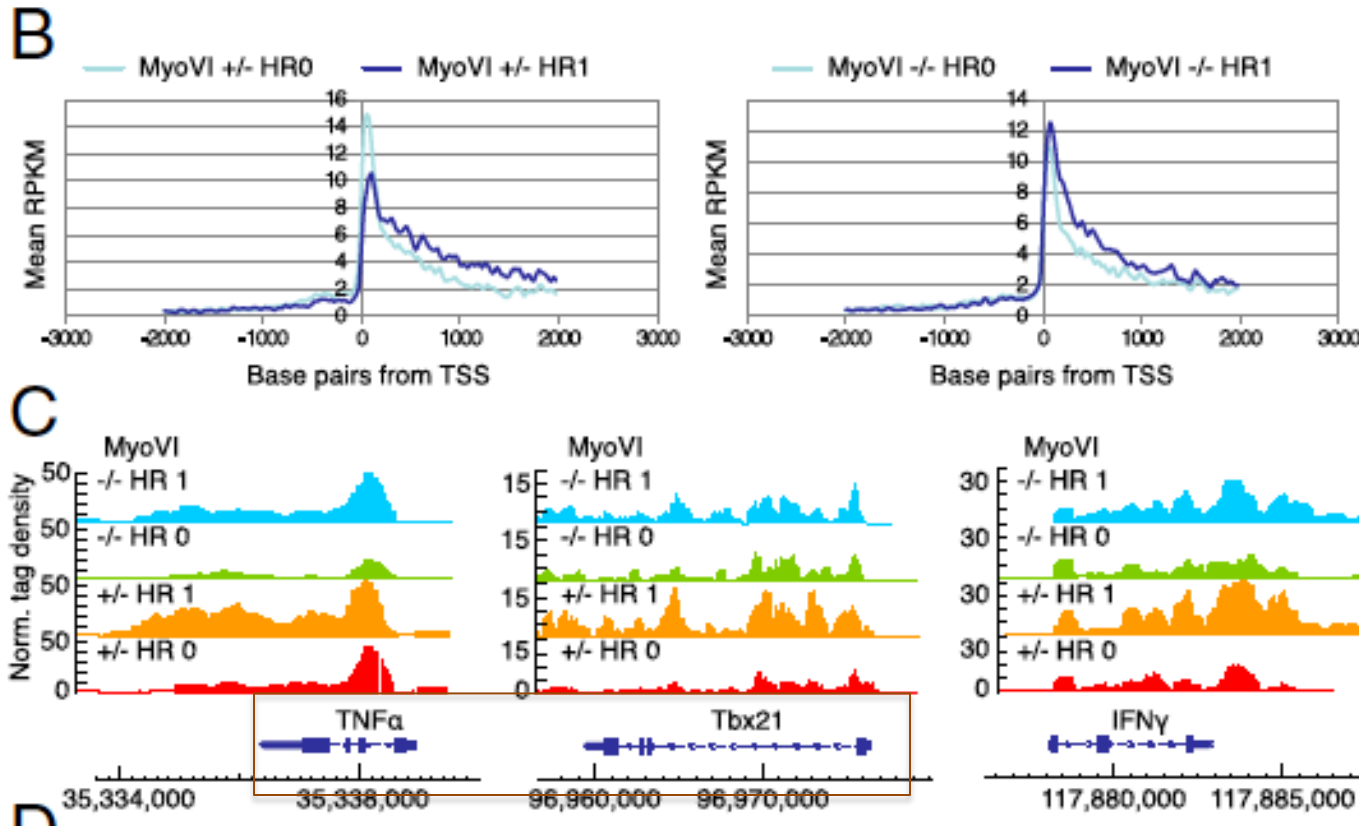
DNA FISH  
RNA FISH



# Myosin VI is a motor protein that drives allelic pairing



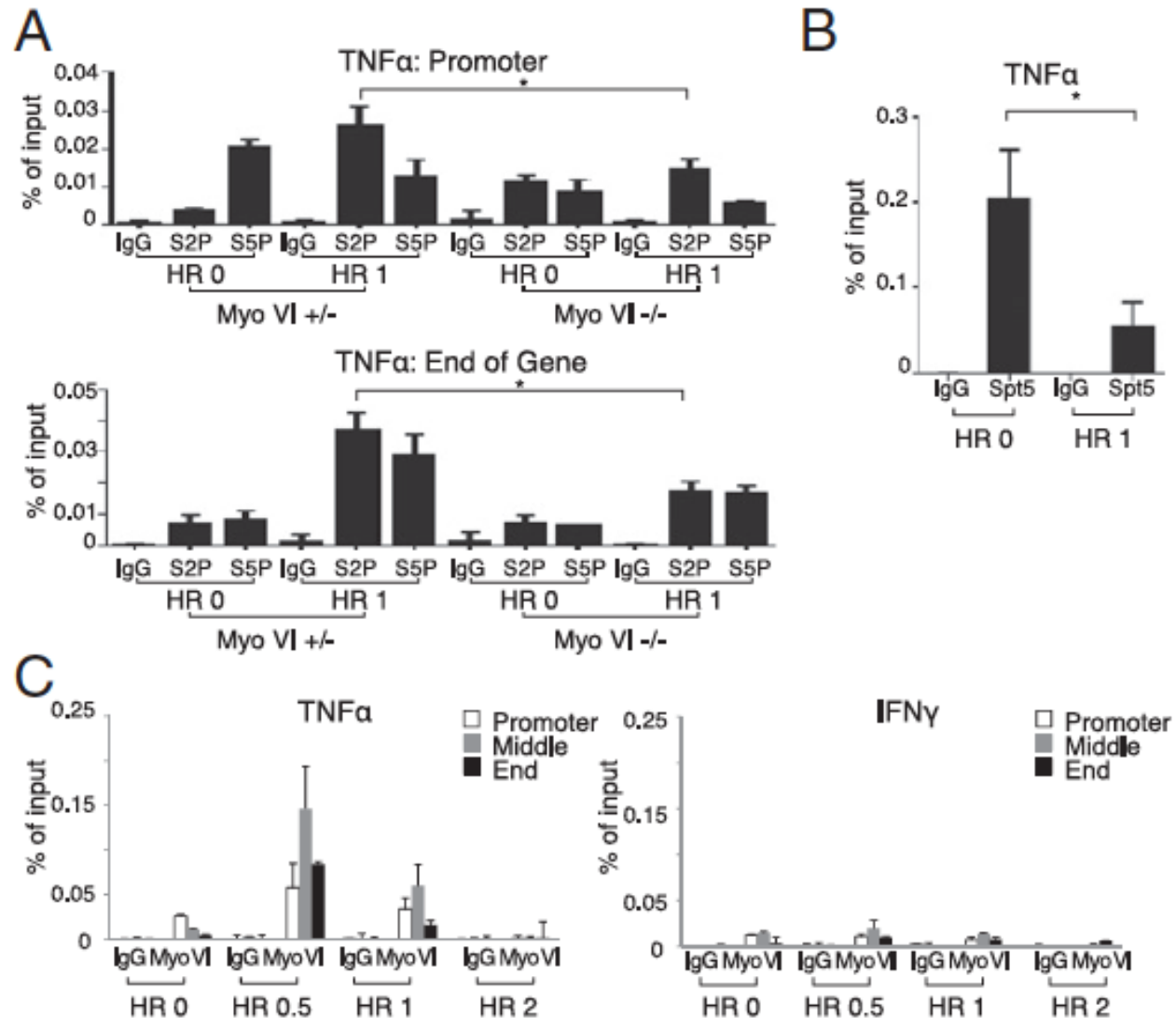
# Pol II is paused at the promoter and waits for re-challenge



Gro-seq  
(Global run-on sequencing)

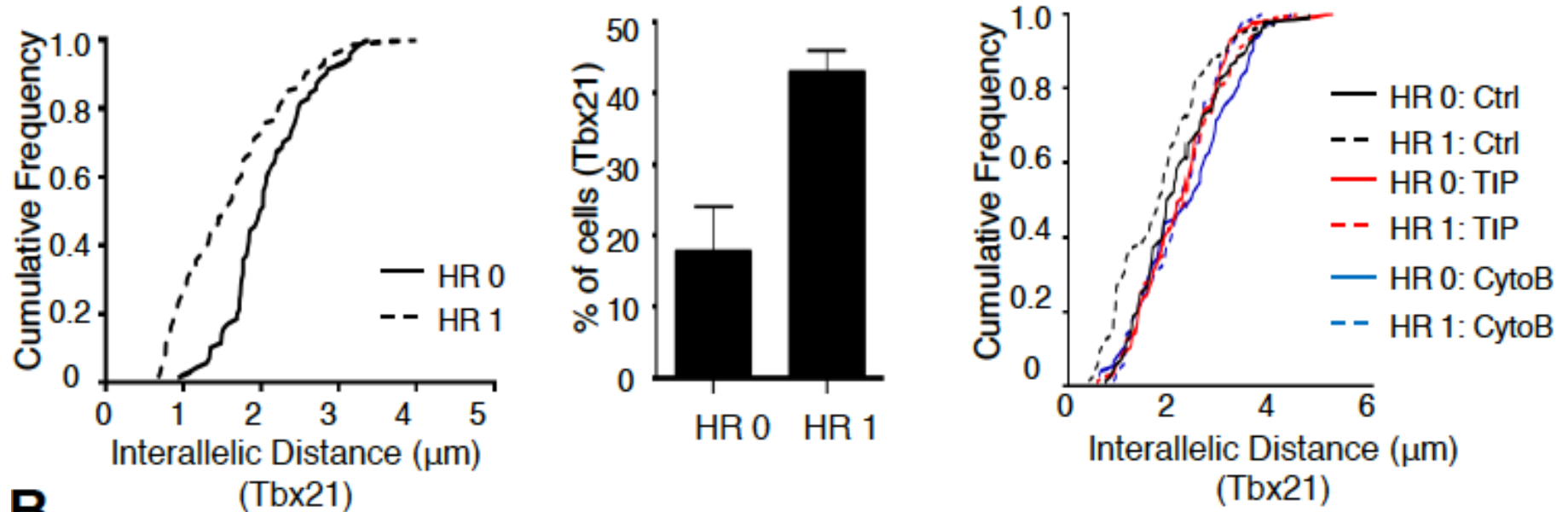


# Initiation Pol II is already bound on the promoter of TNF alpha





# T-bet also shows a homologous pairing in Th1 cells after re-stimulation

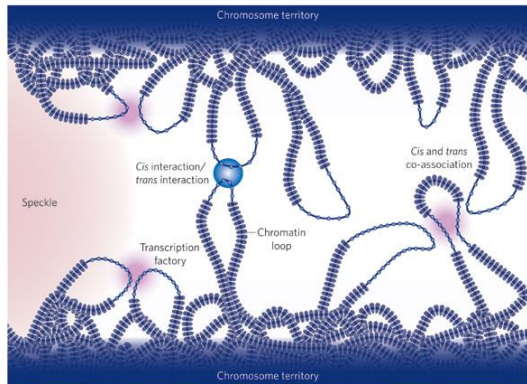


# Current knowledge in the field

Nucleus is a well-organized structure.

Chromosome territories

There has been a big advance of spatiotemporal regulation of transcription recently including us but most studies are done using cell lines and artificial system unlike ours.



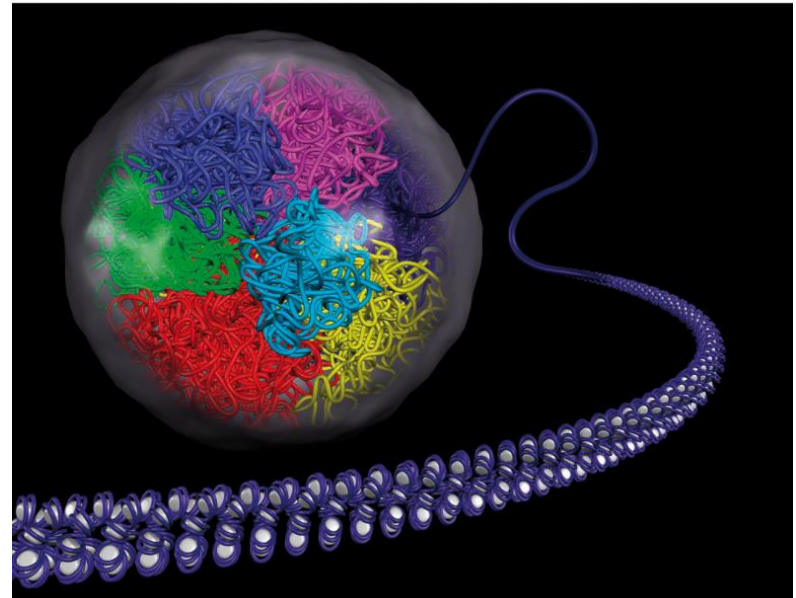
Fraser & Bickmore, *Nature* (2007)

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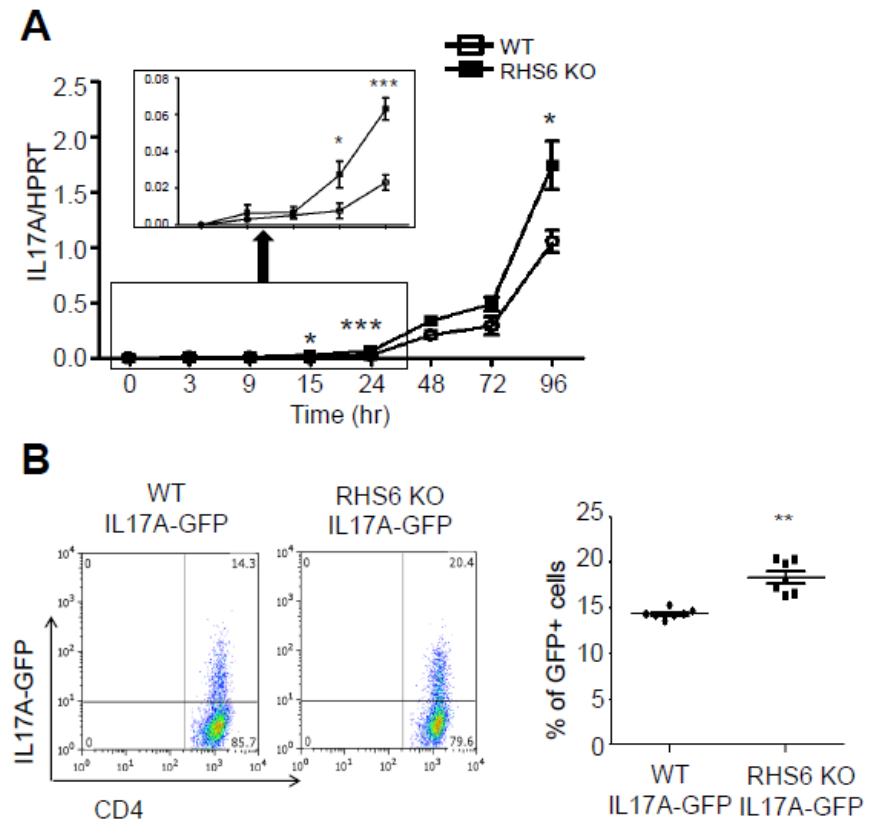
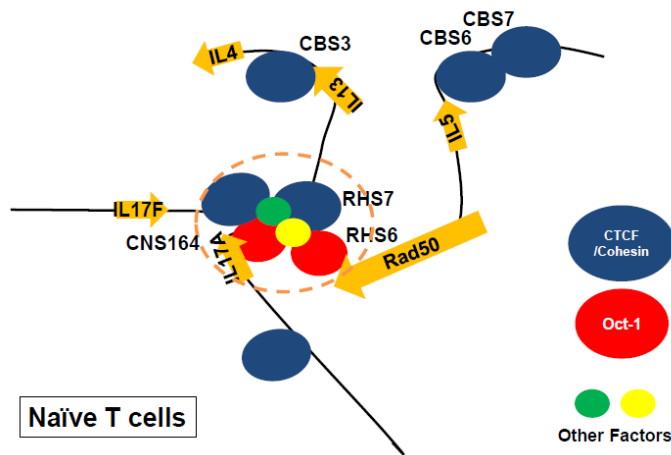
# First demonstration of the relevance between interchromosomal associations and gene expression in primary cells

Cell  
PRESS

Molecular Cell  
Article

## Oct-1 Regulates IL-17 Expression by Directing Interchromosomal Associations in Conjunction with CTCF in T Cells

Lark Kyun Kim,<sup>1,2</sup> Enric Esplugues,<sup>1,6</sup> Cornelia E. Zorca,<sup>1,3</sup> Fabio Parisi,<sup>4</sup> Yuval Kluger,<sup>4</sup> Tae Hoon Kim,<sup>3</sup> Niels J. Galjart,<sup>5</sup> and Richard A. Flavell<sup>1,2,\*</sup>



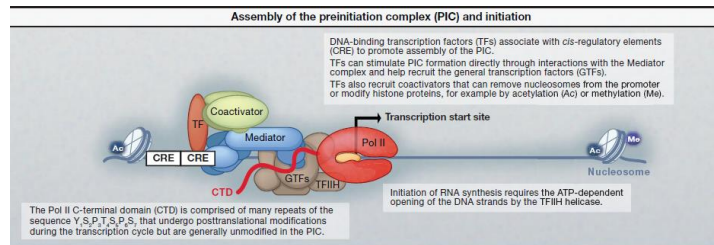
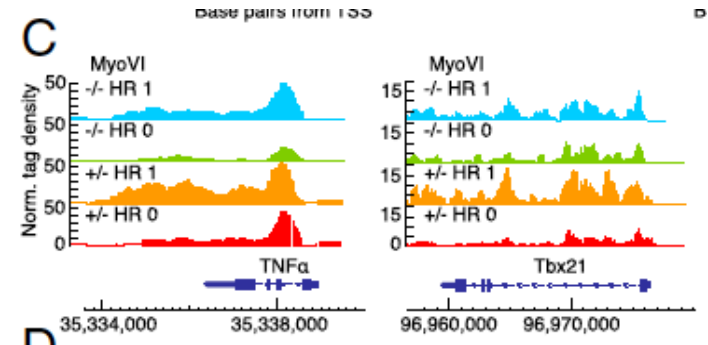
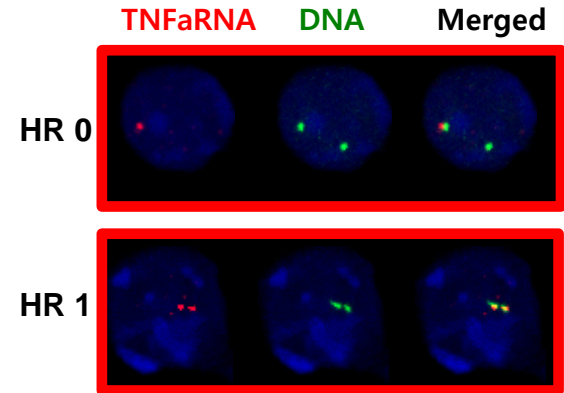
Weakness → significant but not dramatic changes!!

# Current knowledge in the field

Homolog pairing by Myosin VI and  
biallelic expression  
: novel and hot

TNF: first coming cytokines  
Tbet: master regulator of Th1 cells

Pol II pausing  
(rapid induced and developmentally  
Significant genes)



# Current challenges

1. Direct evidence
2. More mechanism
3. Another examples

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Yoon Jung Kim

All other lab members

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Jack Elias

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HHMI



Korea Research Foundation

