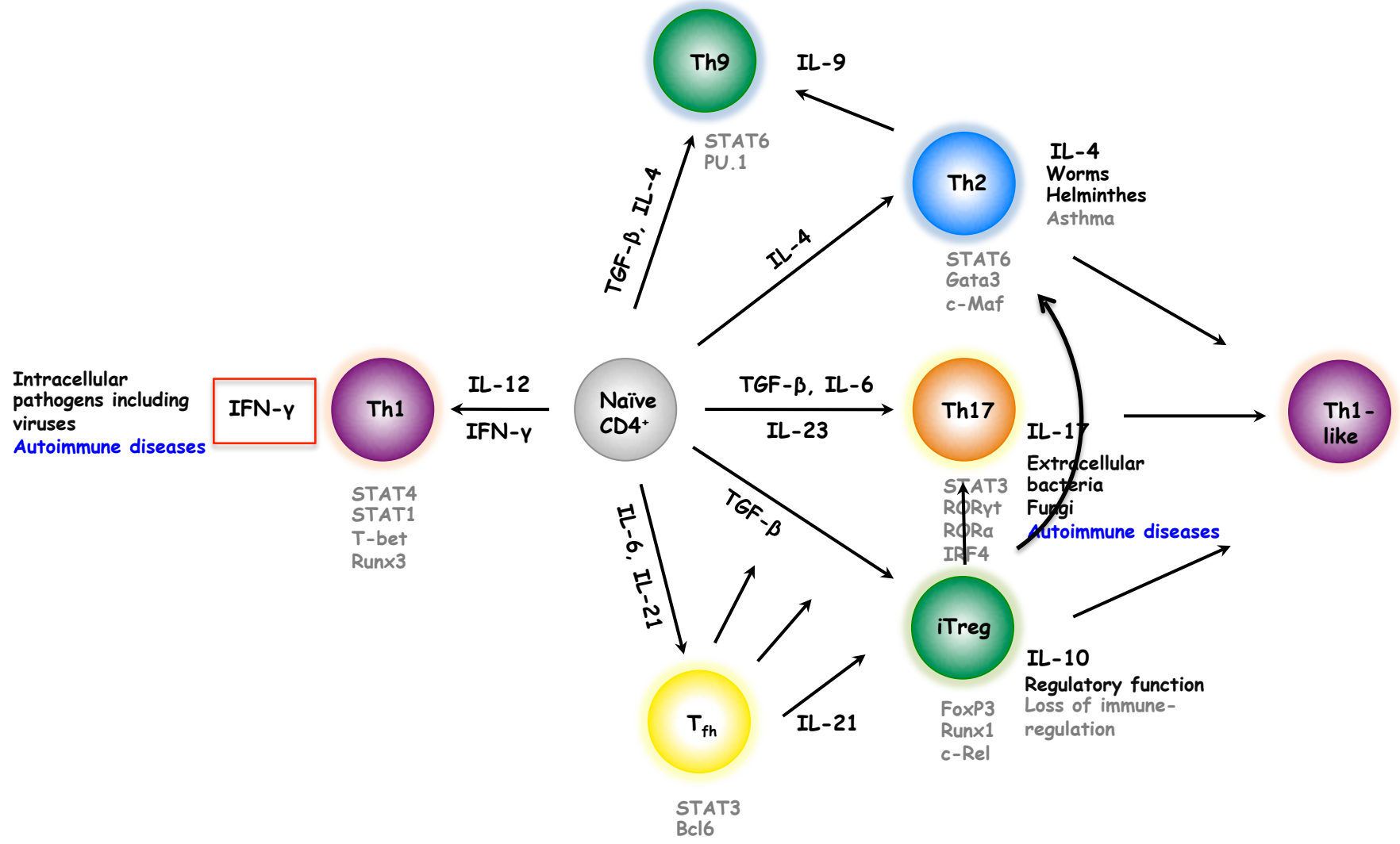
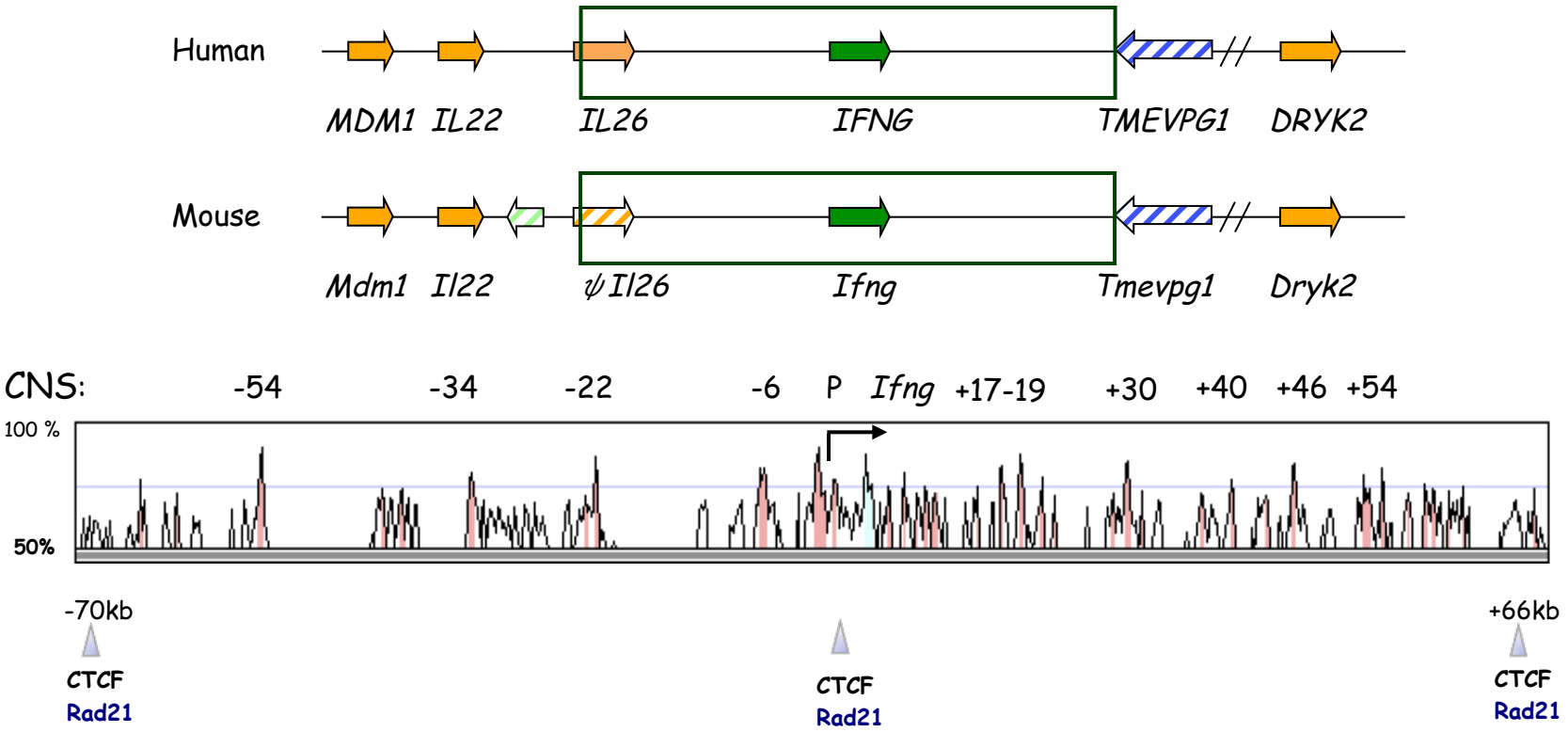
Epigenetic Control of Effector T cell Development

Robin D. Hatton, PhD
University of Alabama at Birmingham

Diversity of T Helper Cell (Th) Responses



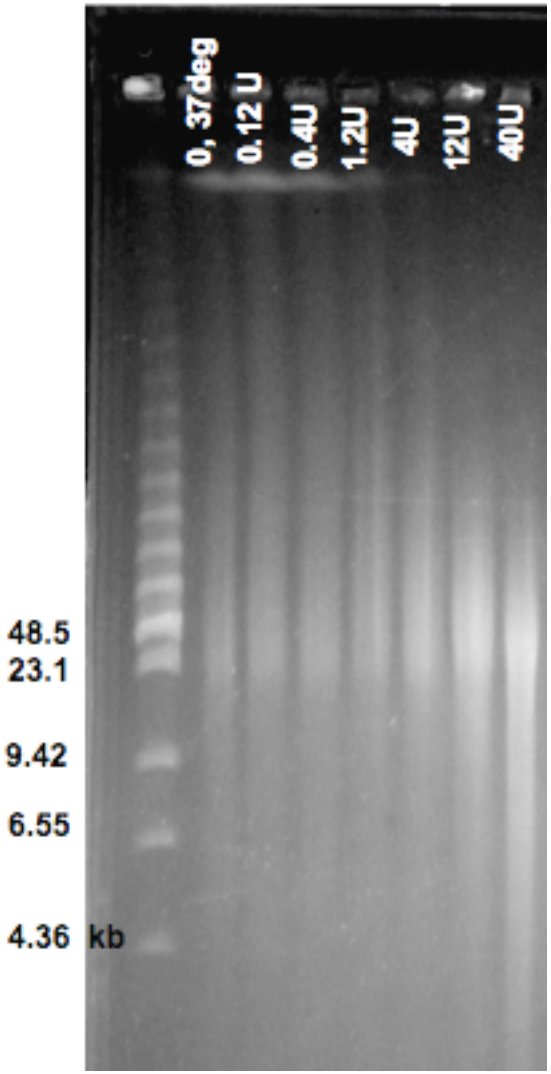
Homology-based Prediction of Regulatory Functions

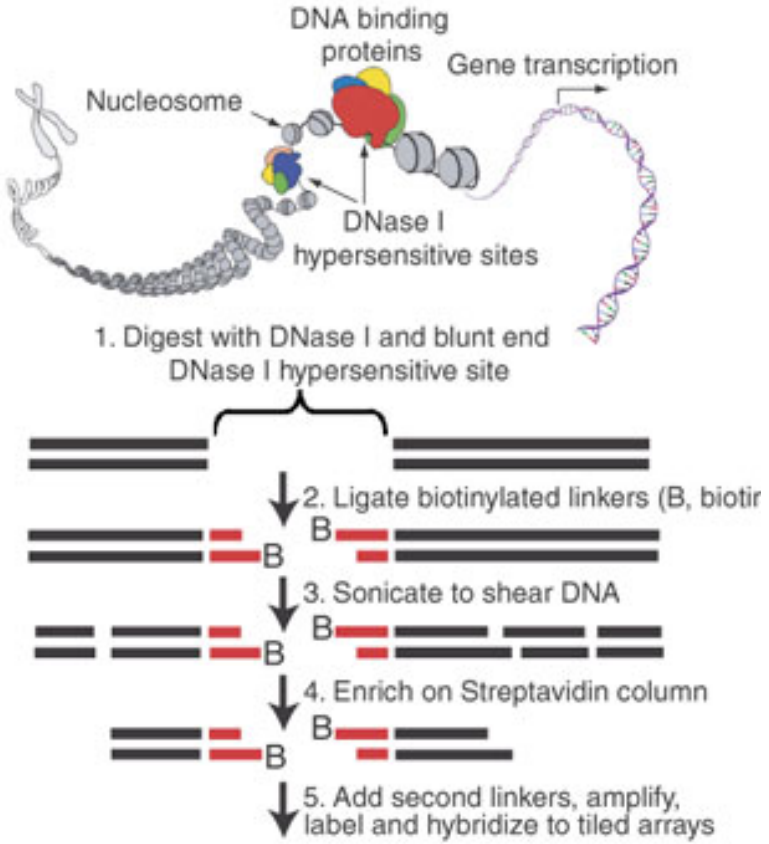


- ~ 140 kb encompasses the *Ifng* locus
- **All *Ifng* CNSs interact with the promoter** although functions of individual CNSs yet to be established

Hajdur S. et al. Nature 2009.
Sekimata M. et al. Nat. Immunol 2009.

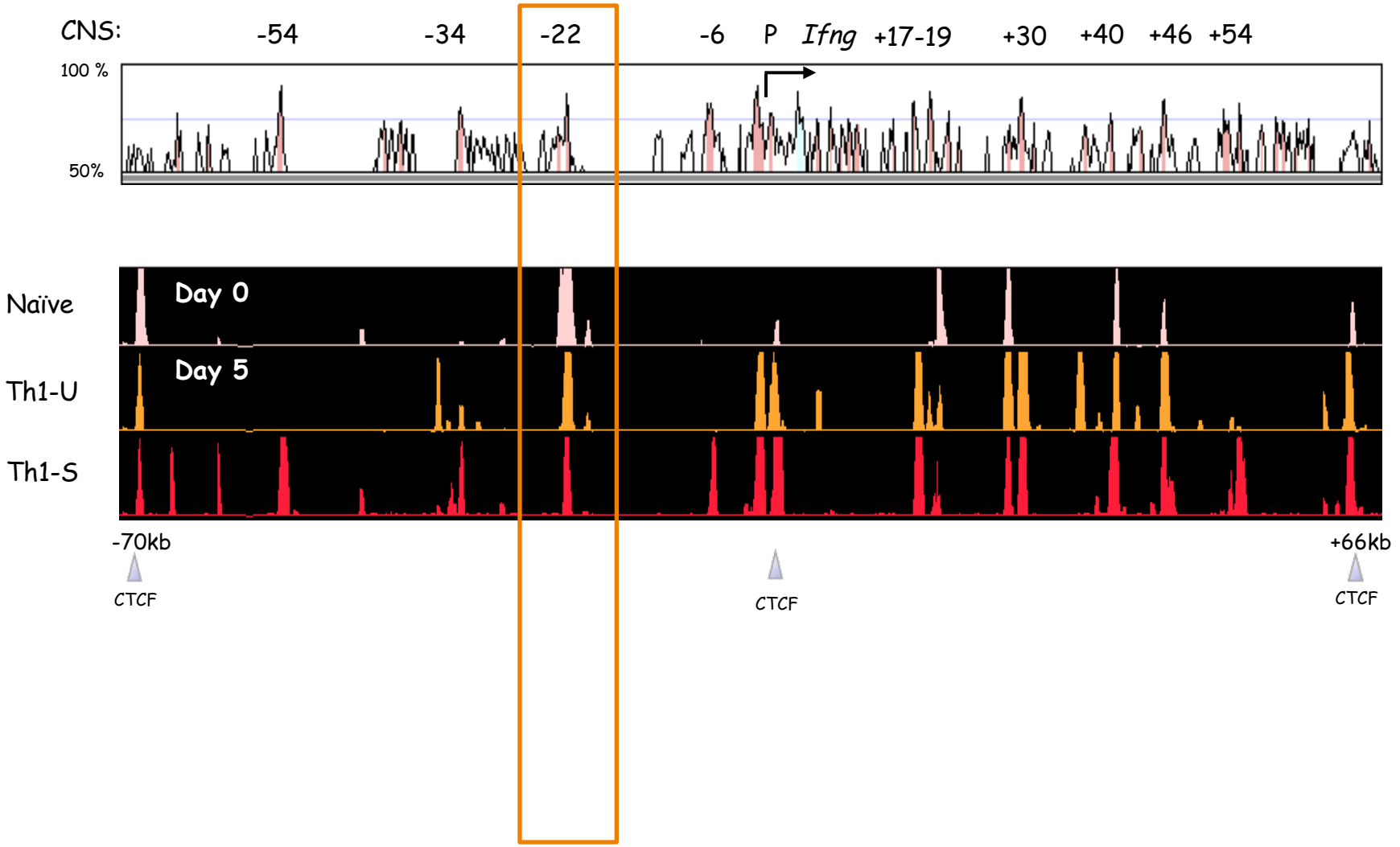
High-throughput Analyses: ChIP-chip and DNase-chip





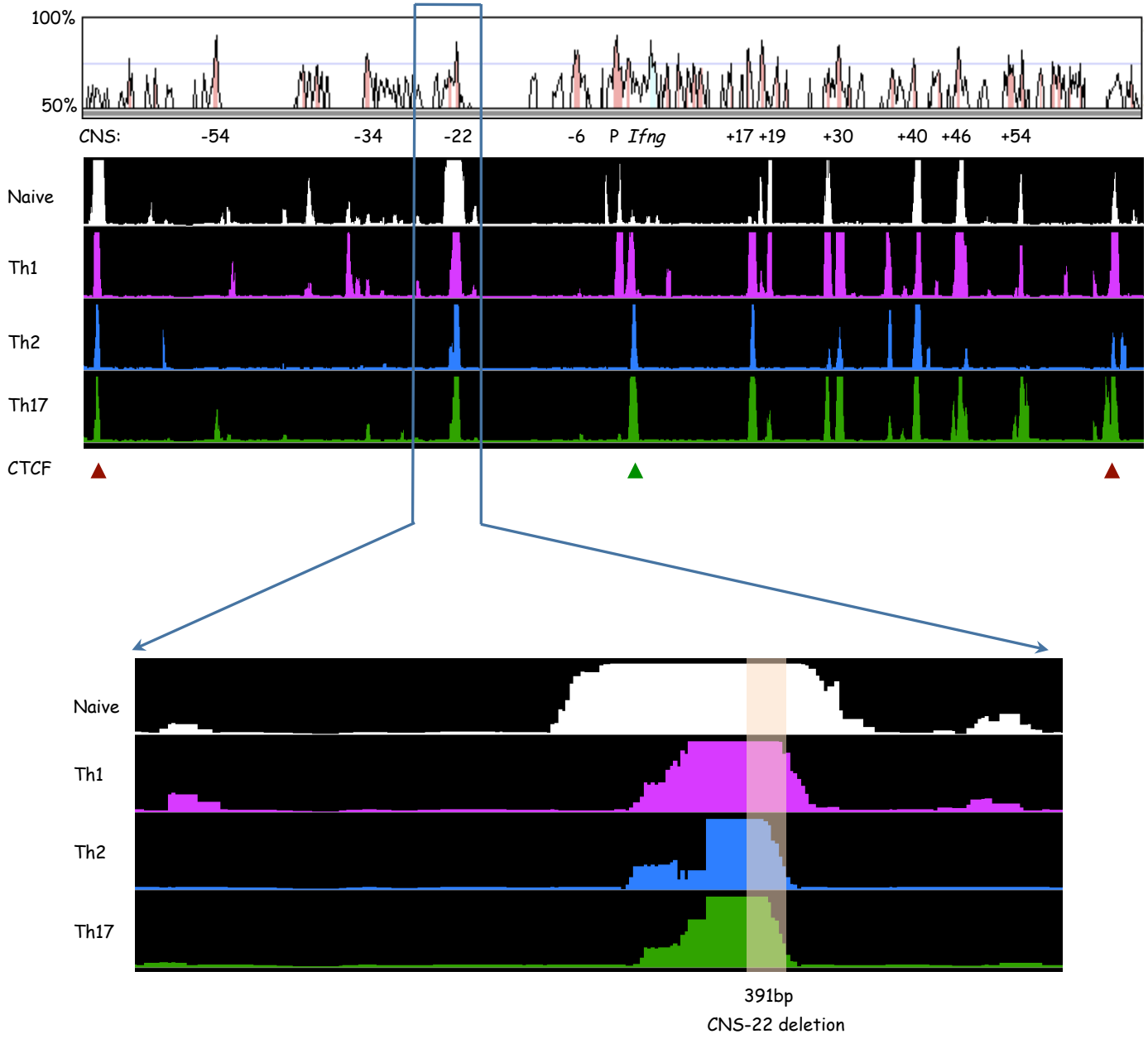
Crawford et al. *Nat. Methods* 2006

The Extended *Ifng* Locus : Th1 Specific Accessibility

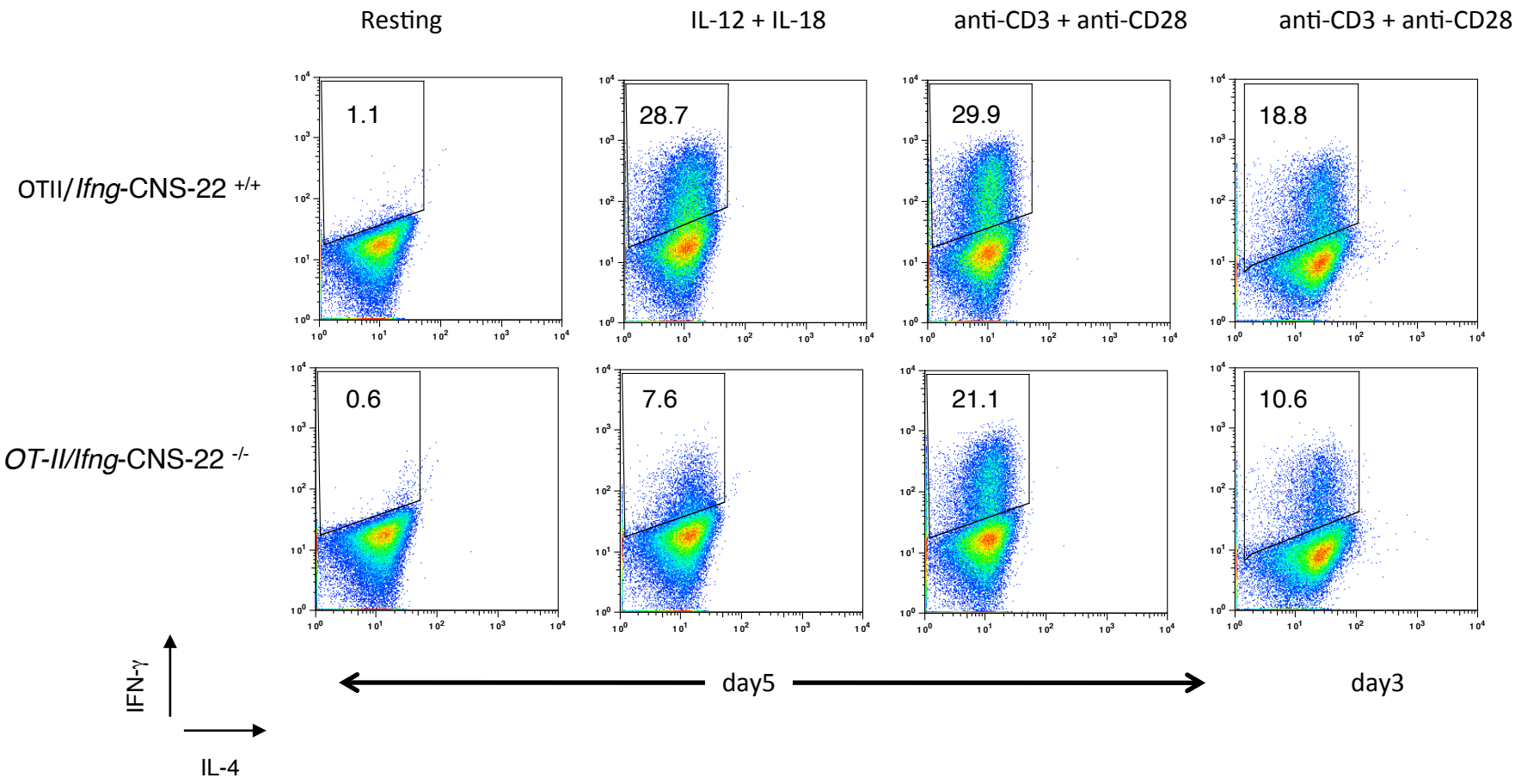


Balasubramani et al. *Immunity* 2010

Ifng CNS-22 Deletion

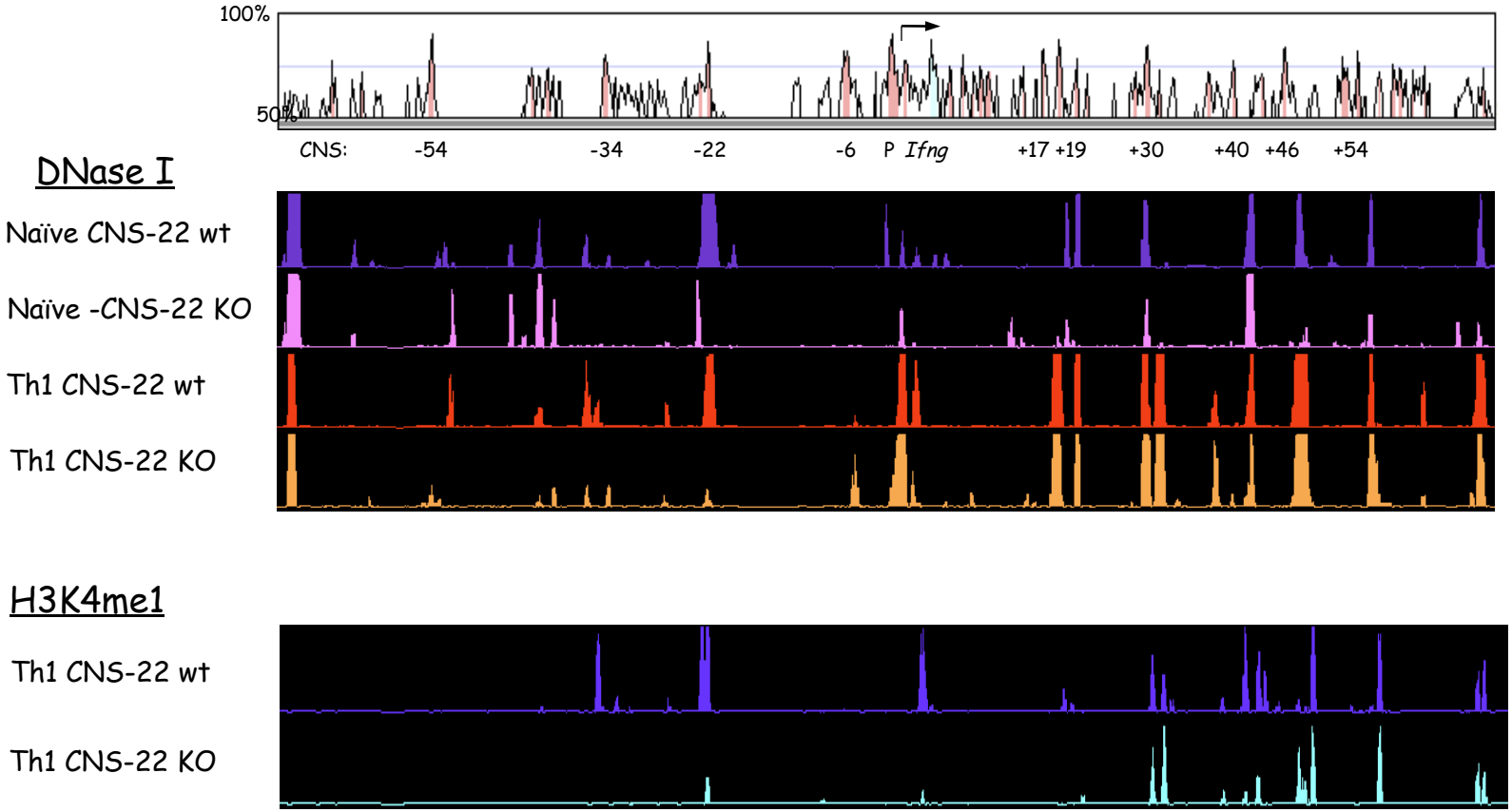


Impairment in Cytokine Driven *Ifng* Gene Transcription in CD4$^+$ T Cells



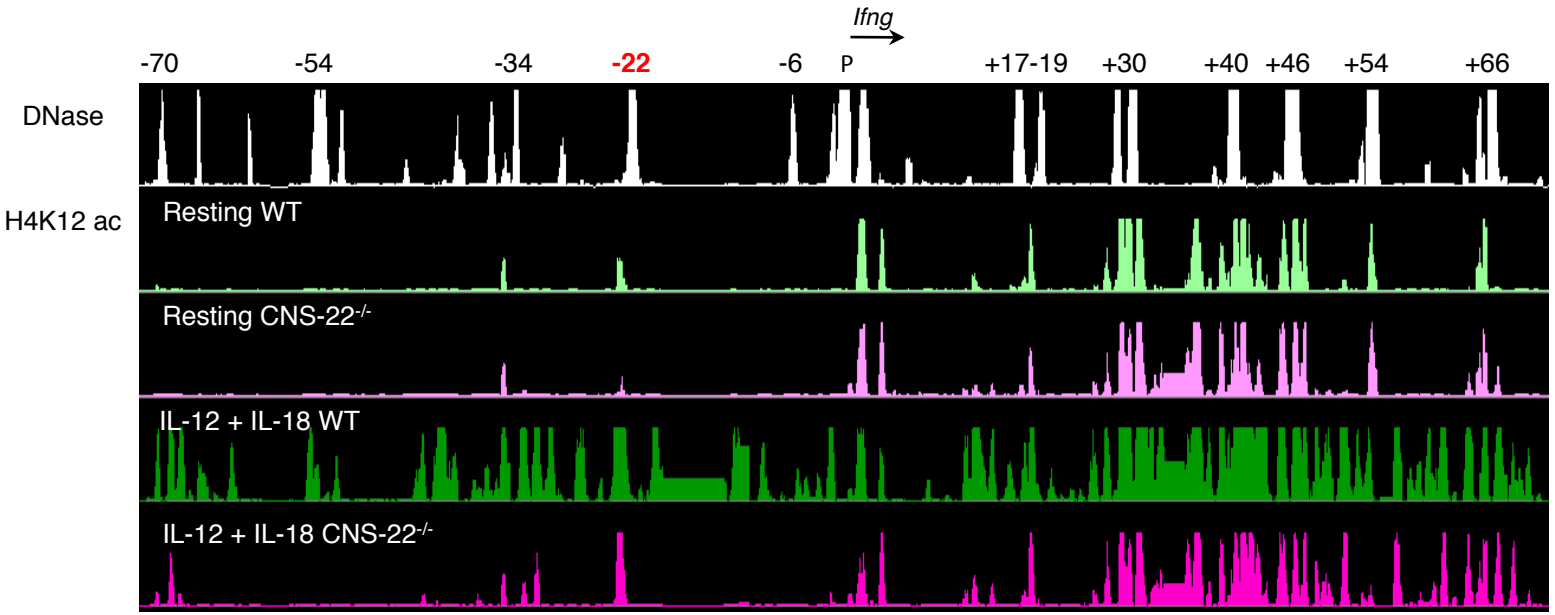
Balasubramani, A.

CNS-22 Partially Controls *Ifng* Locus Remodeling



Balasubramani, A.

Diminished Hyperacetylation in the Absence of CNS-22



Summary

- CNS-22 resides in an area of open chromatin early in T cell development and in all T cell lineages analyzed
- CNS-22 recruits factors involved in the optimal expression of IFNγ
- Deletion of CNS-22
  - Impacts IL-12 and IL-18 driven induction of *Ifng* in both T cells and NK cells
  - Impacts local chromatin structure

Acknowledgements

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Benjamin Weaver

Rita Luther

Collaborators

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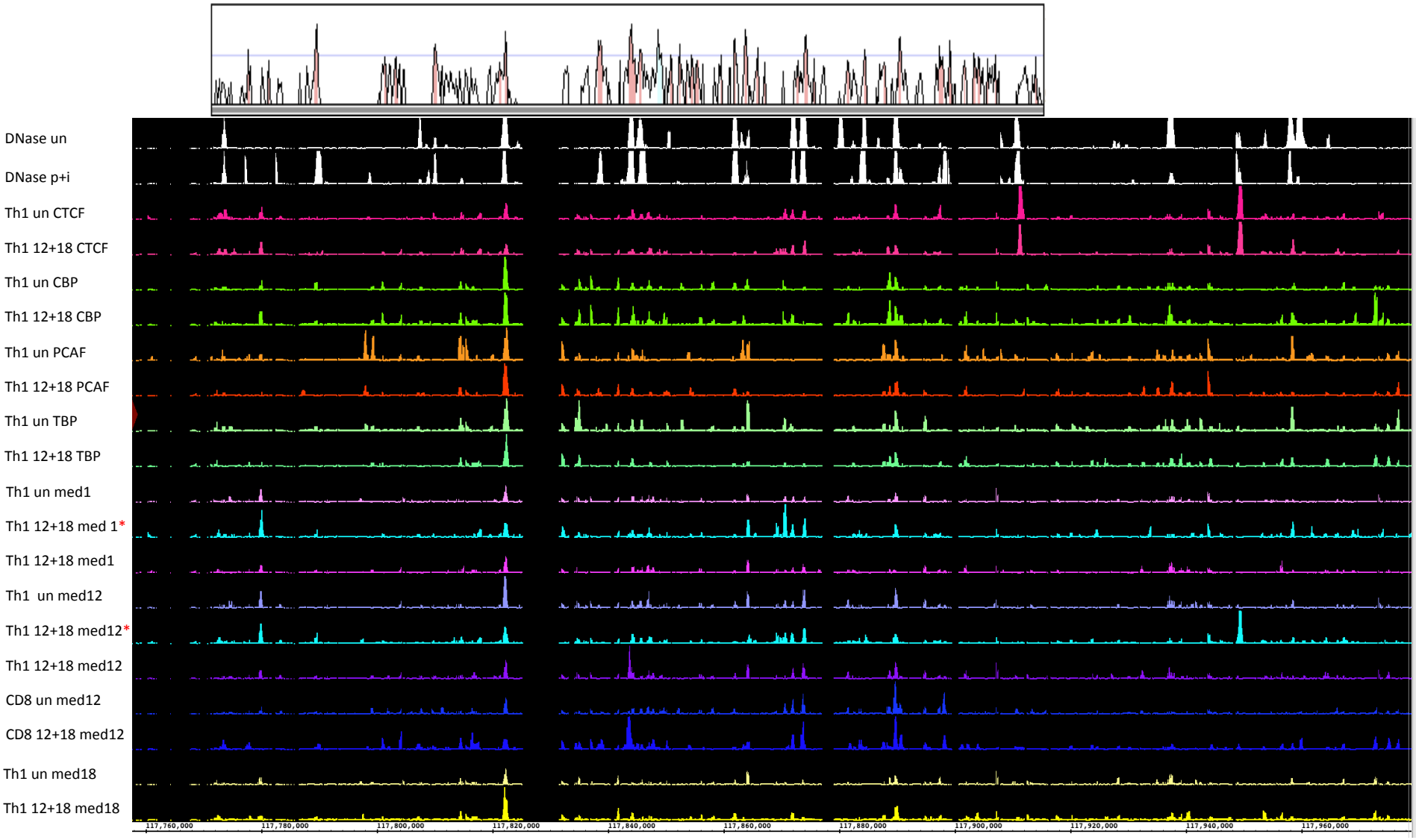
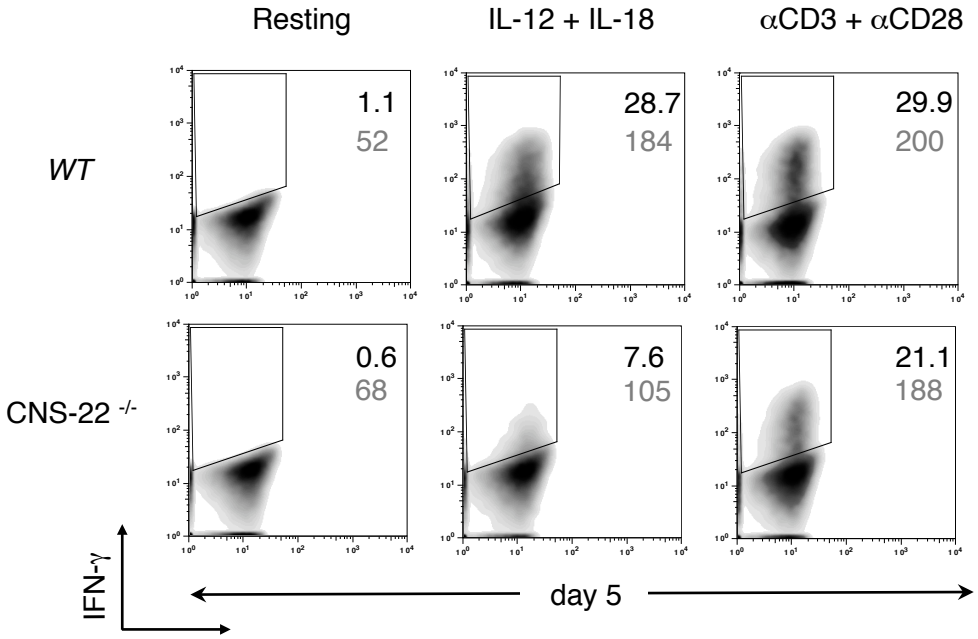
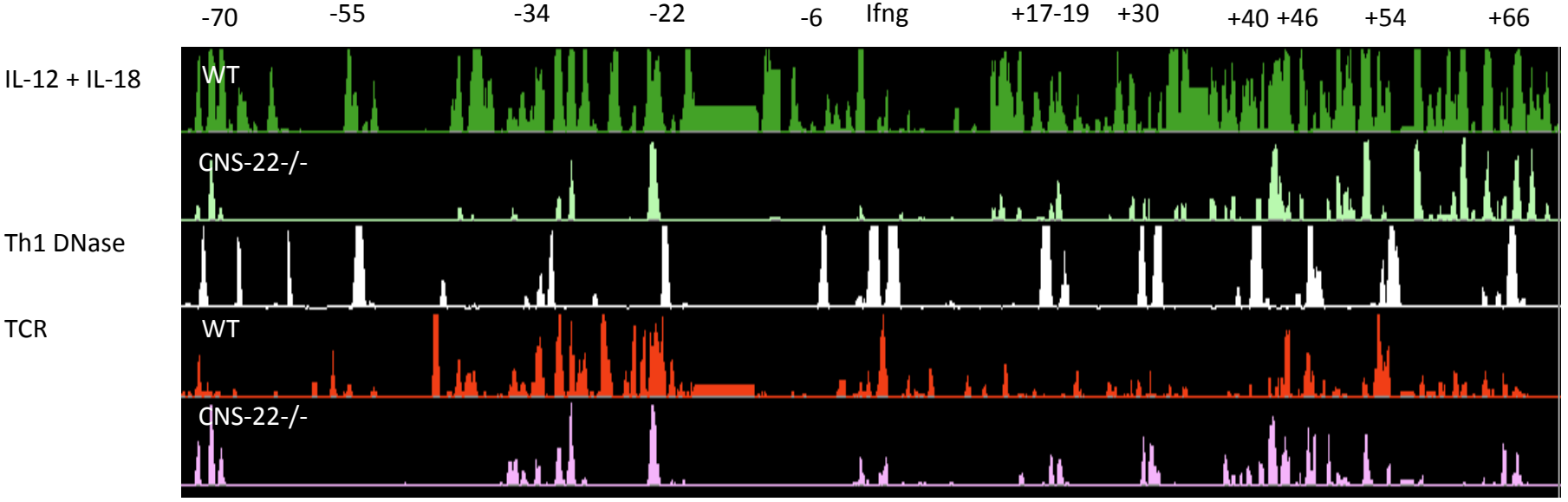
Yoichiro Shibata (Duke)

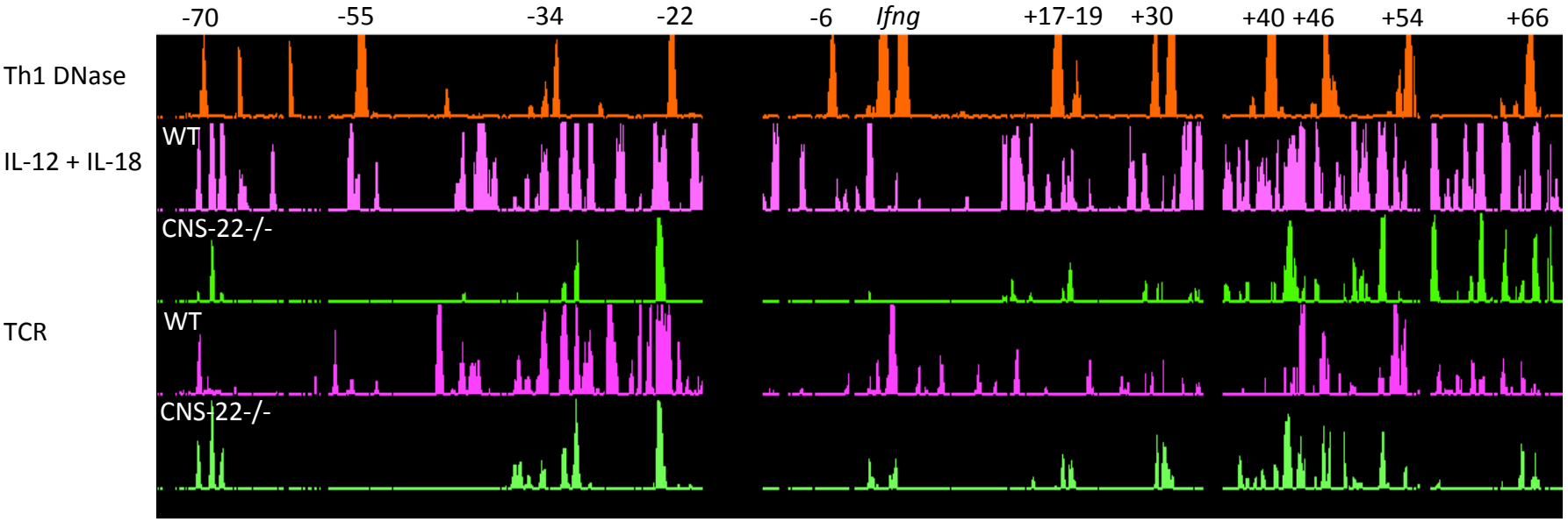


* From the first set of data for med1 and med12

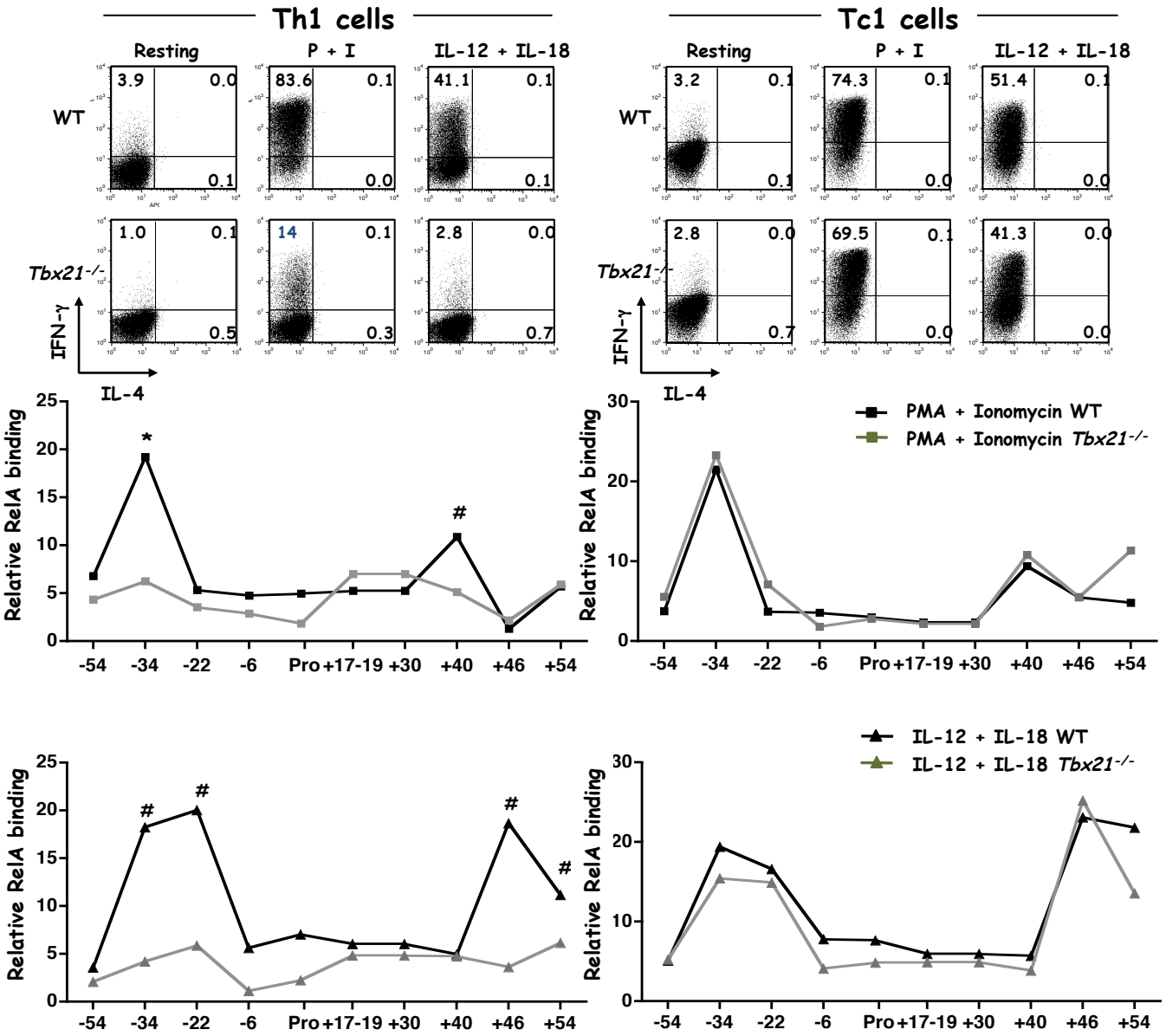


ChIP-chip datasets normalized against respective resting H4K12ac levels..

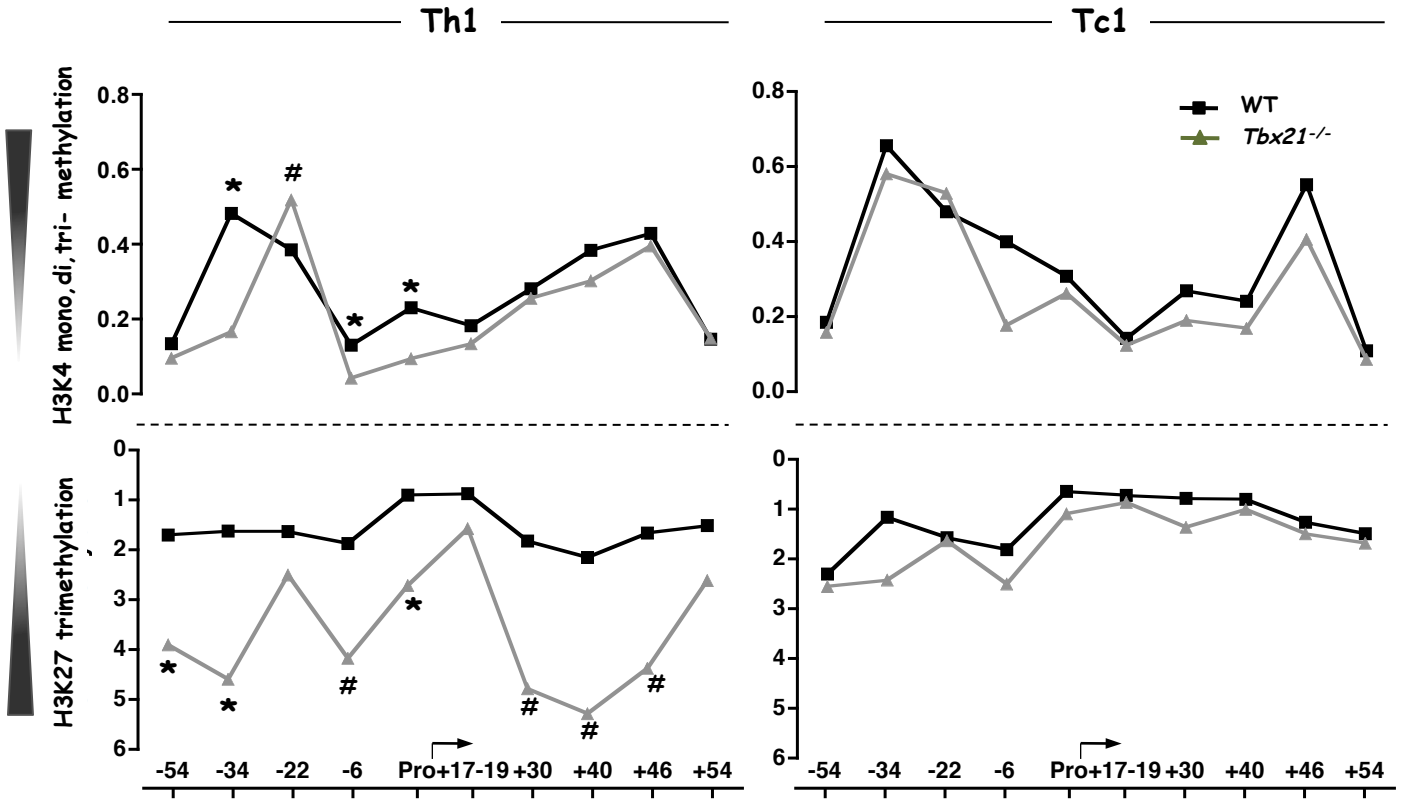



-70
-55
-34
-22
-6
Ifng
+17-19
+30
+40
+46
+54
+66
Th1 DNase
IL-12 + IL-18
WT
CNS-22-/-
TCR
WT
CNS-22-/-

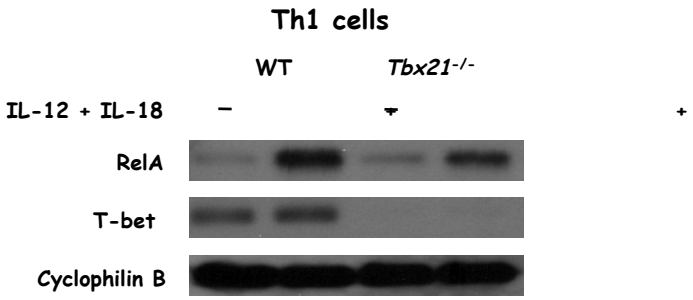
T-bet-Dependent Rel A Recruitment to *Ifng* Locus in Th1 Cells

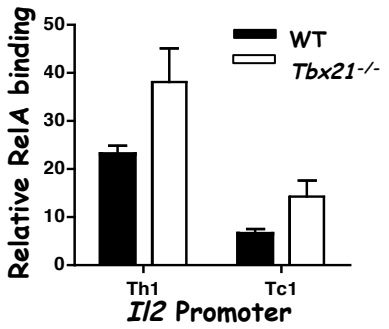


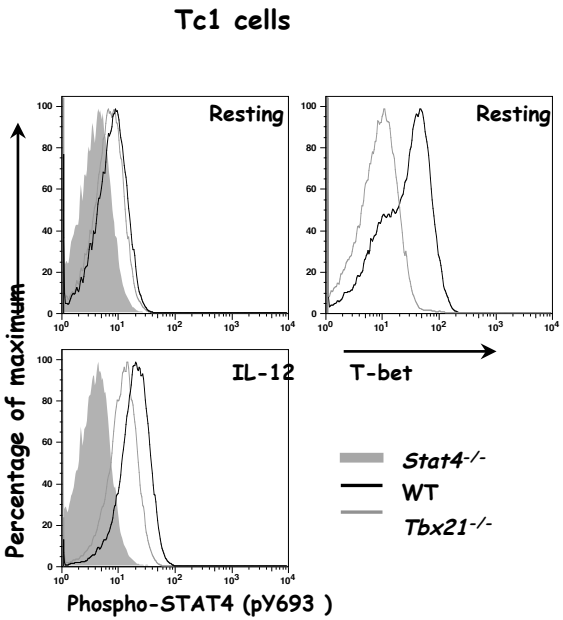
T-bet Dependent Remodeling of the *Ifng* Locus

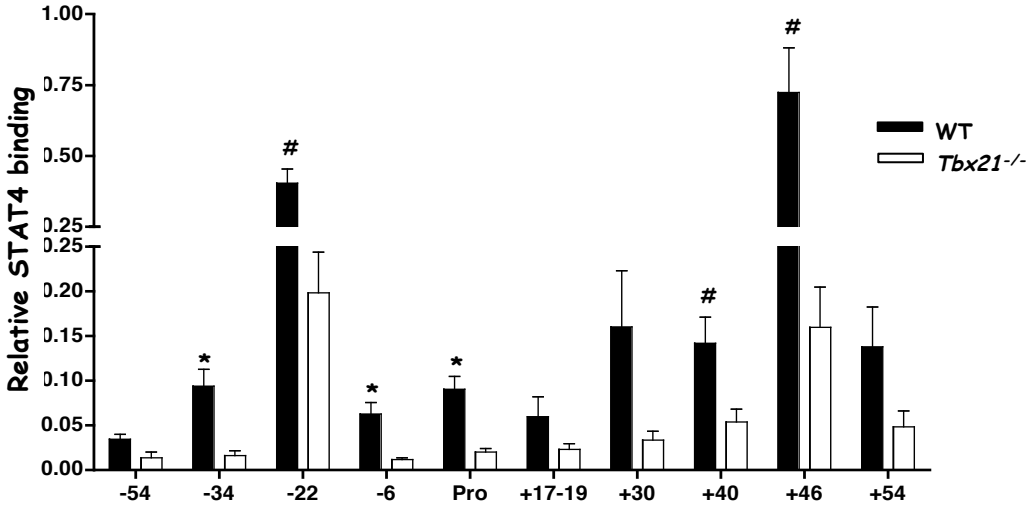


Diminished STAT4 Recruitment to *Ifng* Locus in the Absence of T-bet

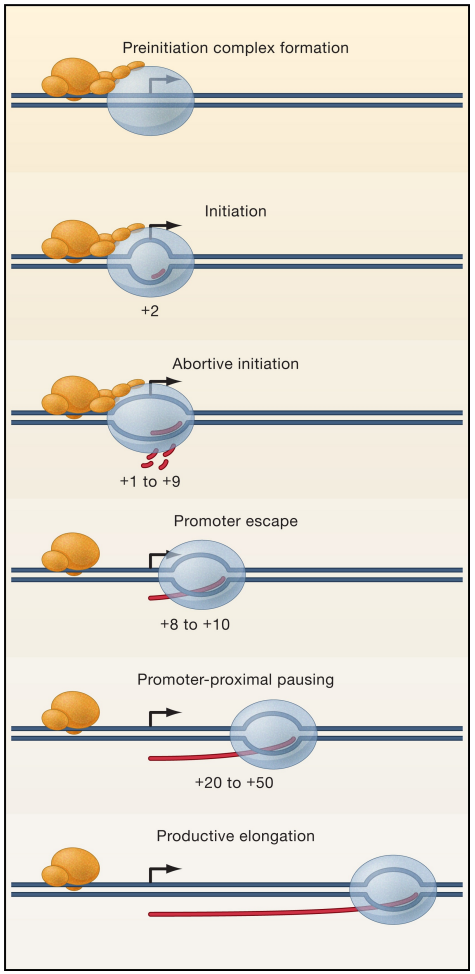


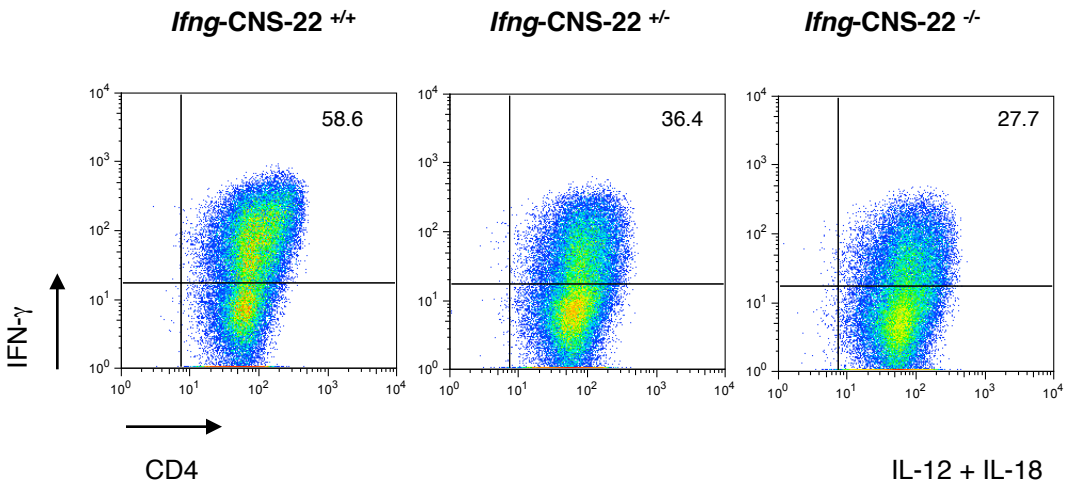






Enhancers: How Do They Drive RNA Pol II Dependent Transcription

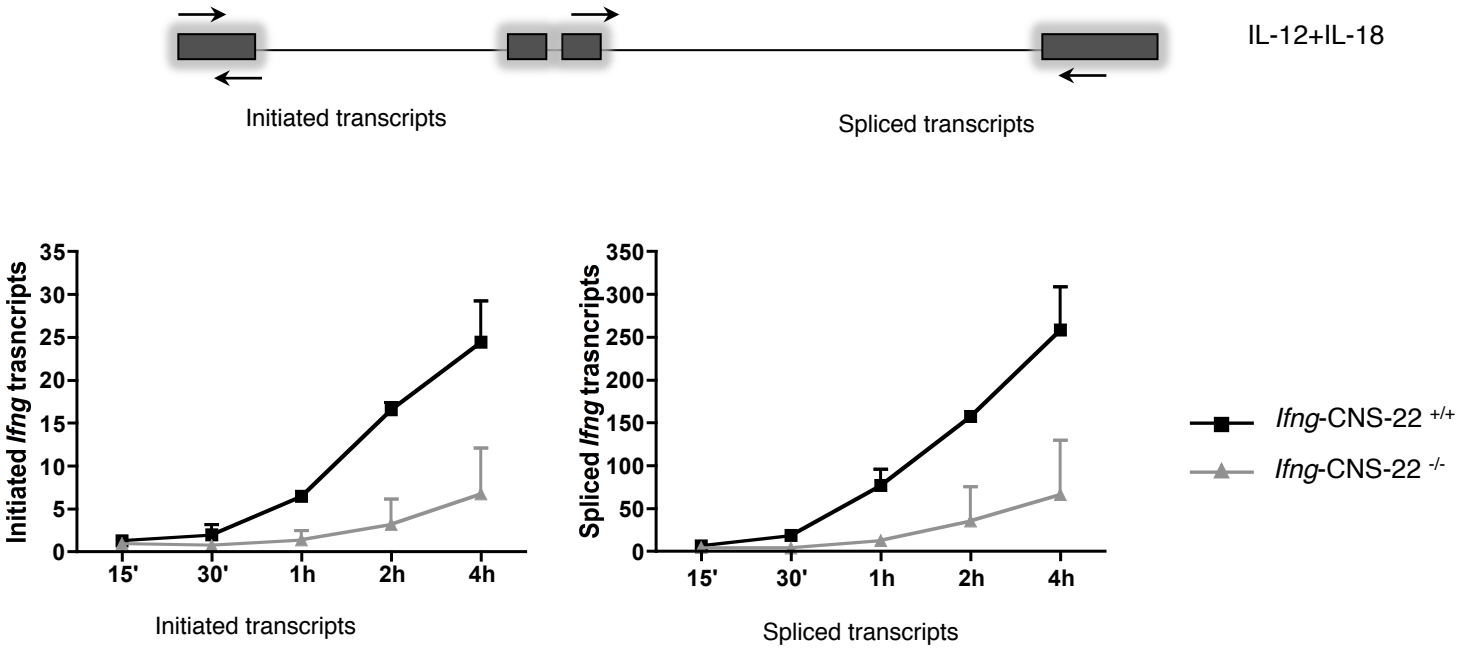




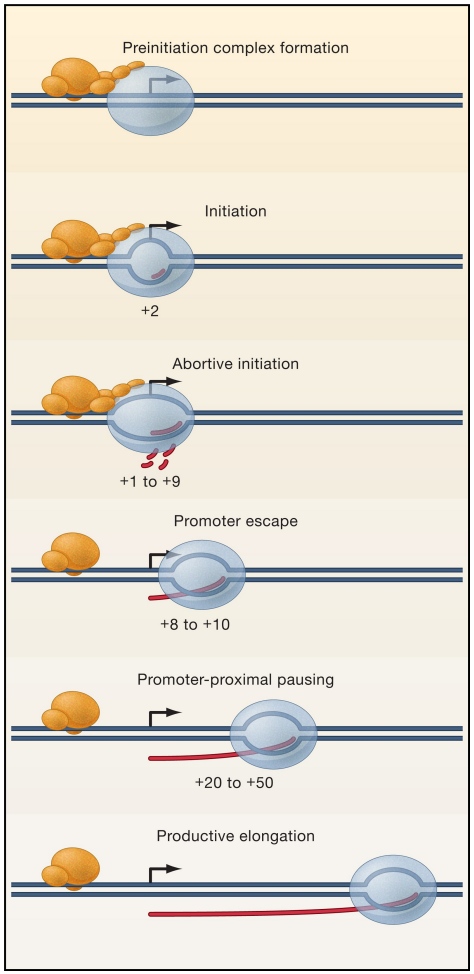
- **Pol II dynamics**
  - Abortive transcription
  - Productive elongation
- **Enhancers**
  - Permissive epigenetic remodeling
  - Push Pol II past the promoter i.e. facilitate transition from initiation to elongation: Thought to impact elongation, but dispensable for Pol II recruitment and initiation
  - Pol II has been shown to be recruited to other enhancers: even involved in generation of enhancer associated transcripts (eRNAs)

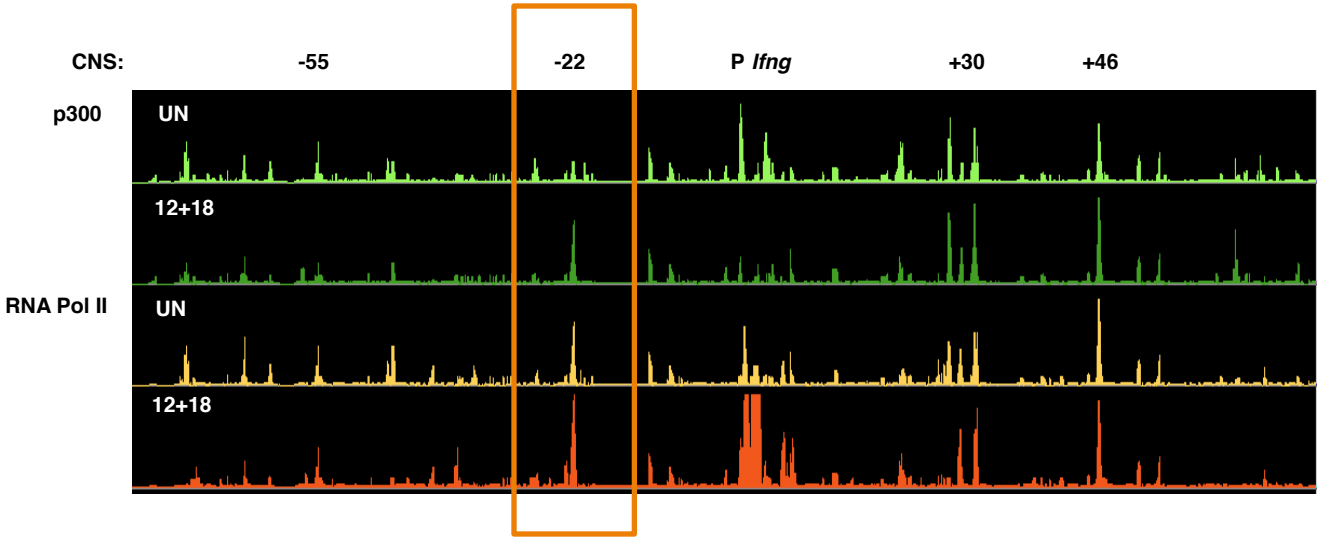
Margaritis et al. *Cell* 2008

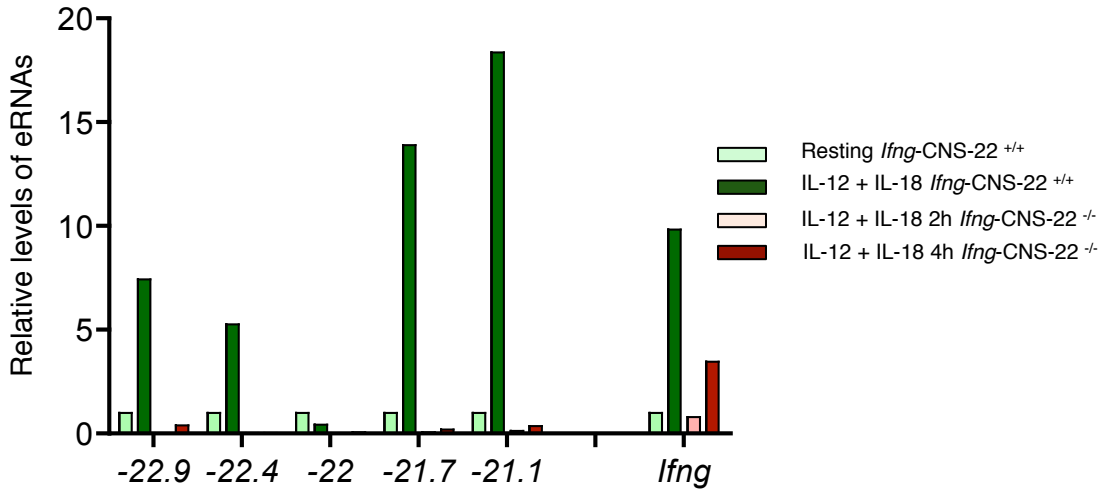
CNS-22: Transcript Initiation or Elongation



Recruitment of p300 and RNA Pol II to Distal Enhancers

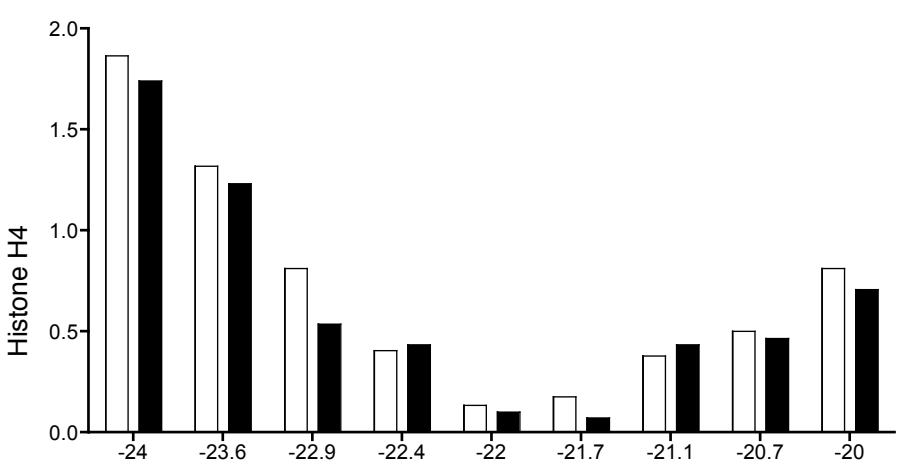


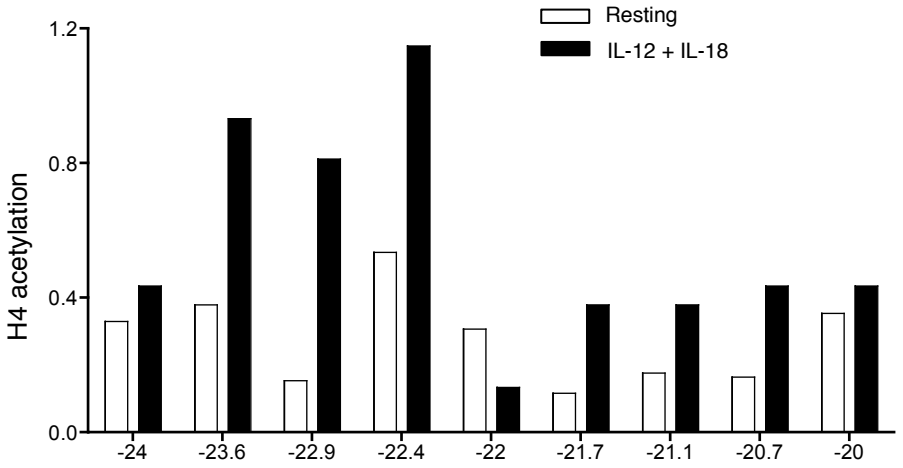


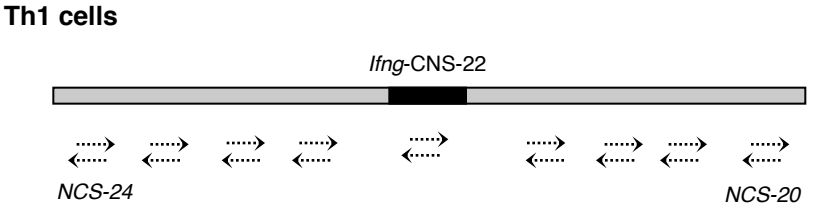


Margaritis et al. *Cell* 2008

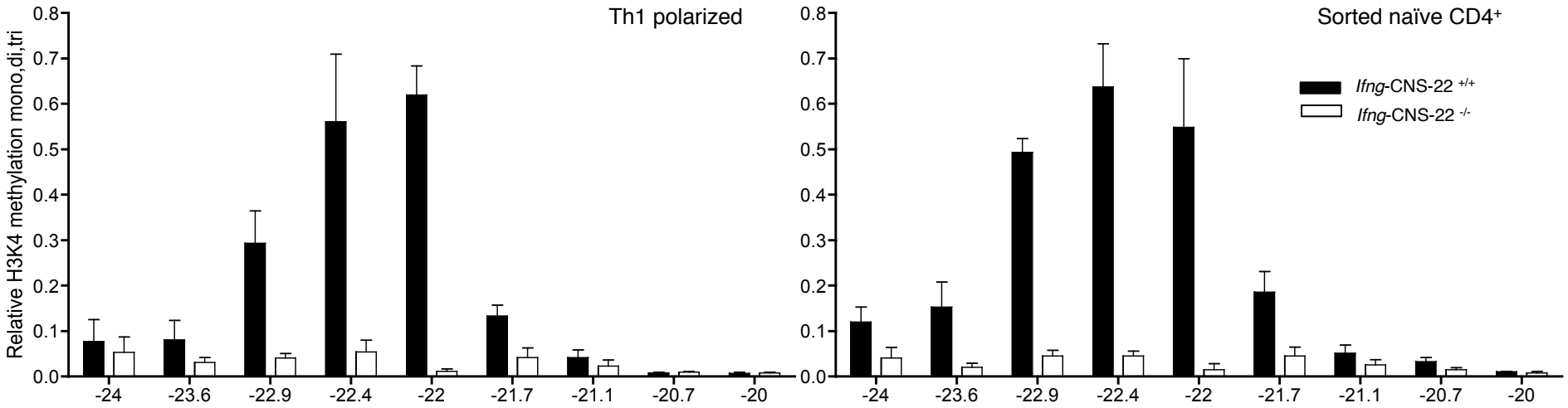
H4 Association Remains Unperturbed

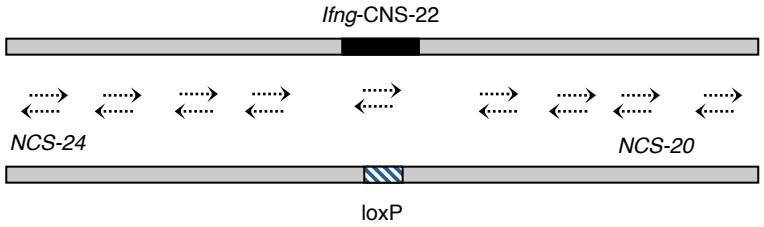






CNS-22 Dependent Local Permissive Remodeling





Development of a Novel System for Site-Specific, Single-Copy, Directional Targeting of BAC Transgenes; The HAT-BAC System

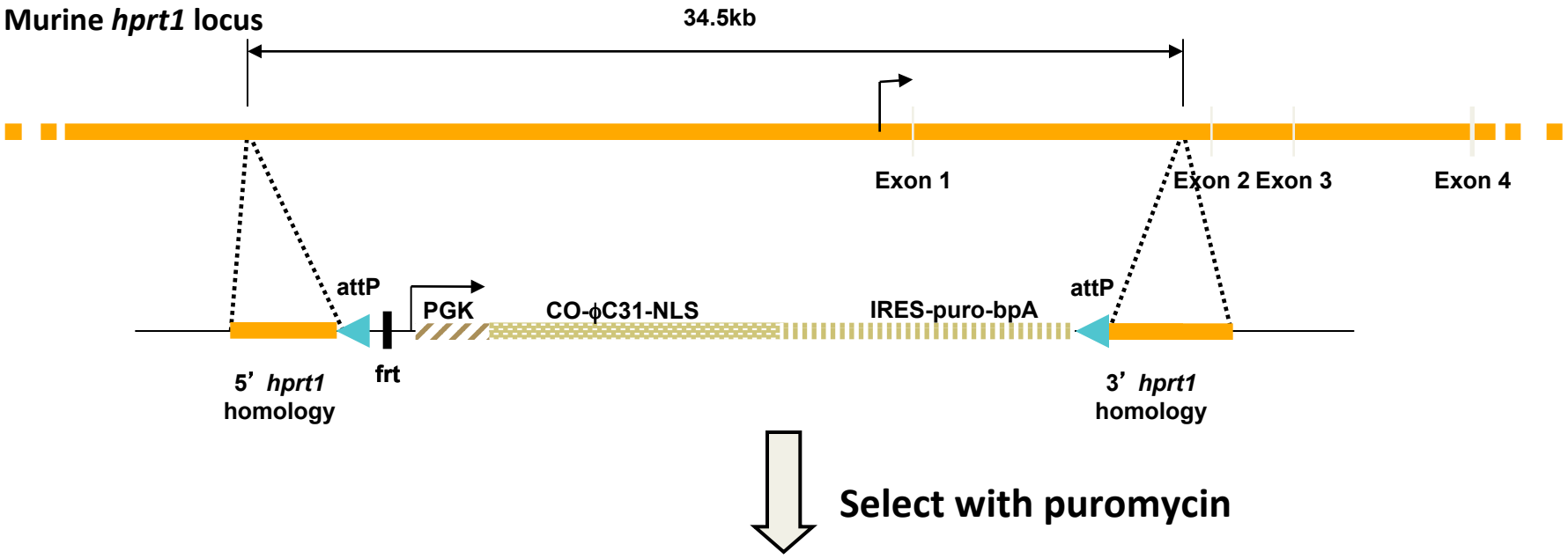
- **ES cell based**
- **Targeting to *hprt1* locus;**
  - **X-linked (single copy in ES cells)**
  - **ubiquitously expressed, provides a favorable chromatin environment for transgene expression**
  - **prior success in targeting and expressing BAC transgenes**
  - **mice derived from independent ES cell clones containing transgene expressed from the same promoter exhibit comparable levels of expression**
  - **reconstitution of hprt1 expression permits HAT selection of correctly targeted clones**
- **Implementation of a novel DNA recombinase for efficient targeting via exchange reaction**

Cre and Flp vs ϕC31 Integrase

- Cre or Flp
  - insertion of a circular DNA into the genome (*trans* event), two *cis*-positioned recognition sites are created
  - intramolecular interactions are kinetically favored over intermolecular interactions; these recombinases favor deletion rather than integration of DNA
  - transgene integration occurs at low efficiency because the reaction equilibrium is shifted in favor of excision

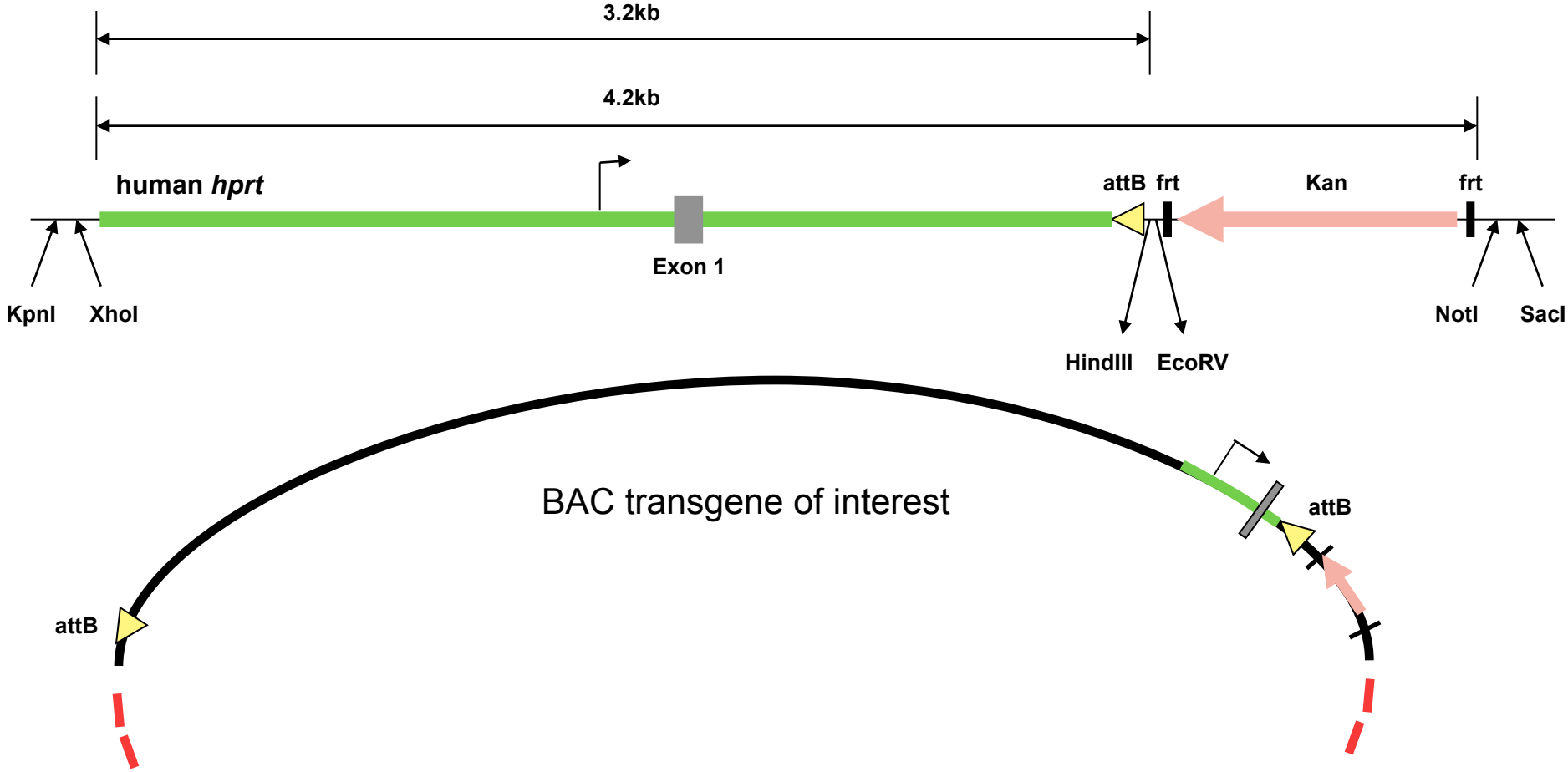
- phiC31 integrase
  - can be optimized to work well in mammalian cells (NLS, codon usage); no other phage or bacterially-encoded proteins or factors required
  - catalyzes only the attB x attP reaction and not the reverse reaction (lack of excisionase)
  - better suited for cassette exchange reactions due to its unidirectionality

Targeting of hprt1 Locus in ES Cells



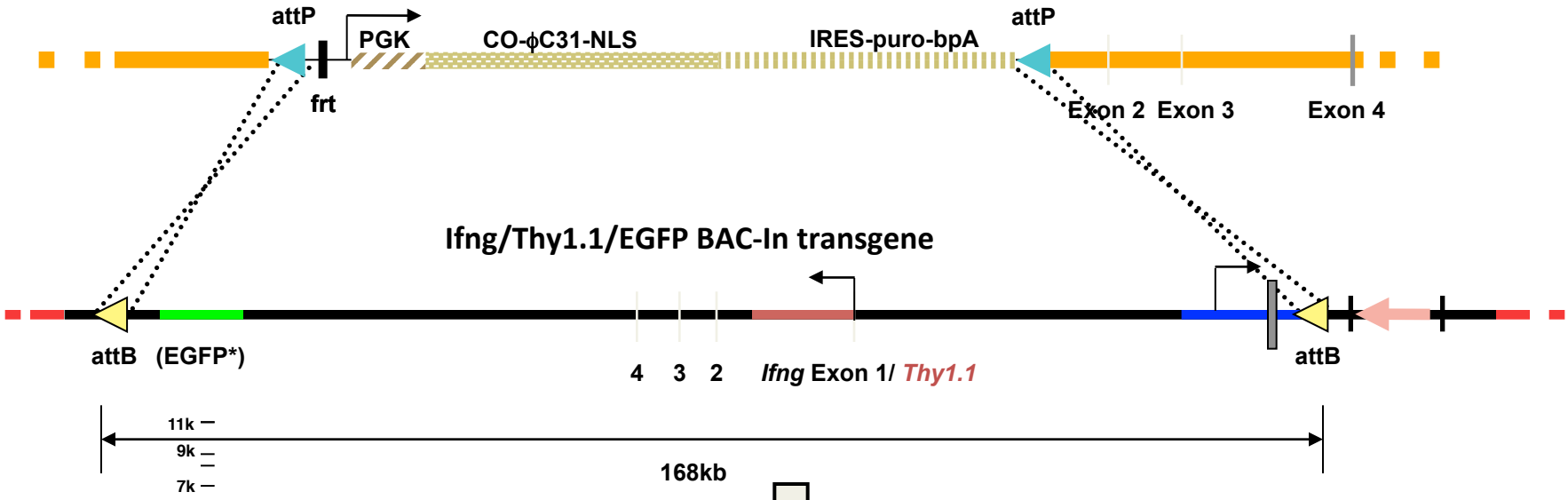
Engineering BACs for Site-Specific Recombination

- **Insert 5' attB site**
- **Insert the cassette: [human *hprt* promoter/exon 1+ second attB site]**



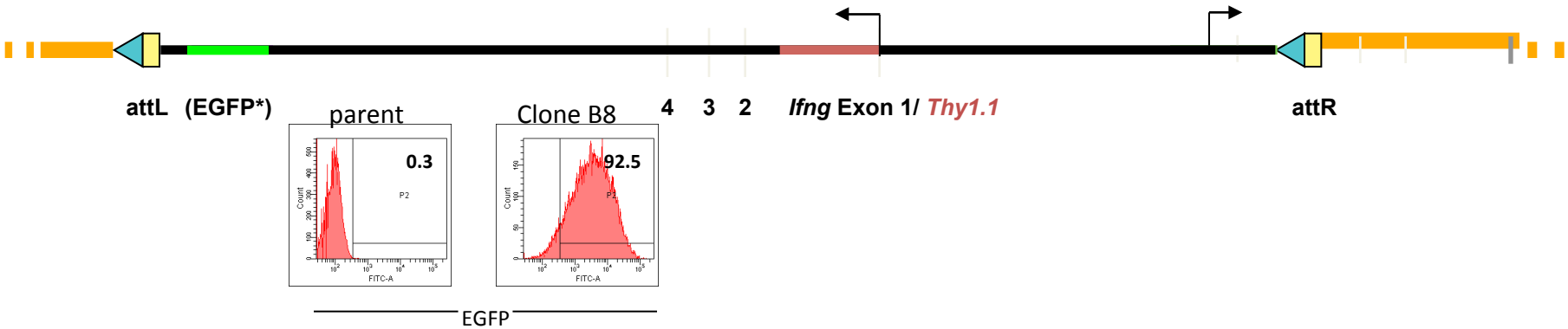
Targeting of BAC Transgene to the Docking Site in HAT-BAC ES Cells

HAT-BAC ES *hprt1* locus containing docking site



Select with HAT

Restored *hprt1* locus containing BAC transgene



Histone Modifying Enzymes

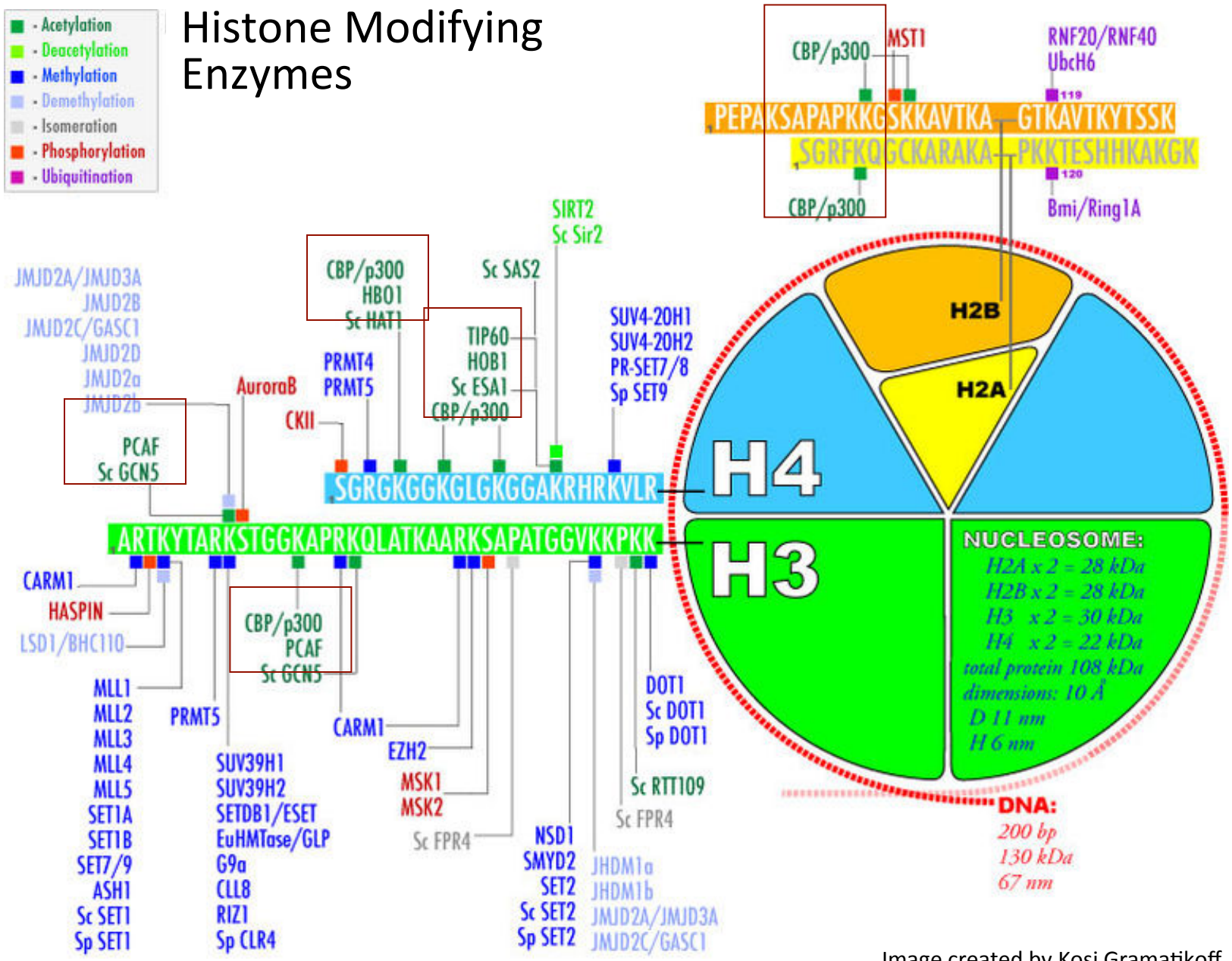
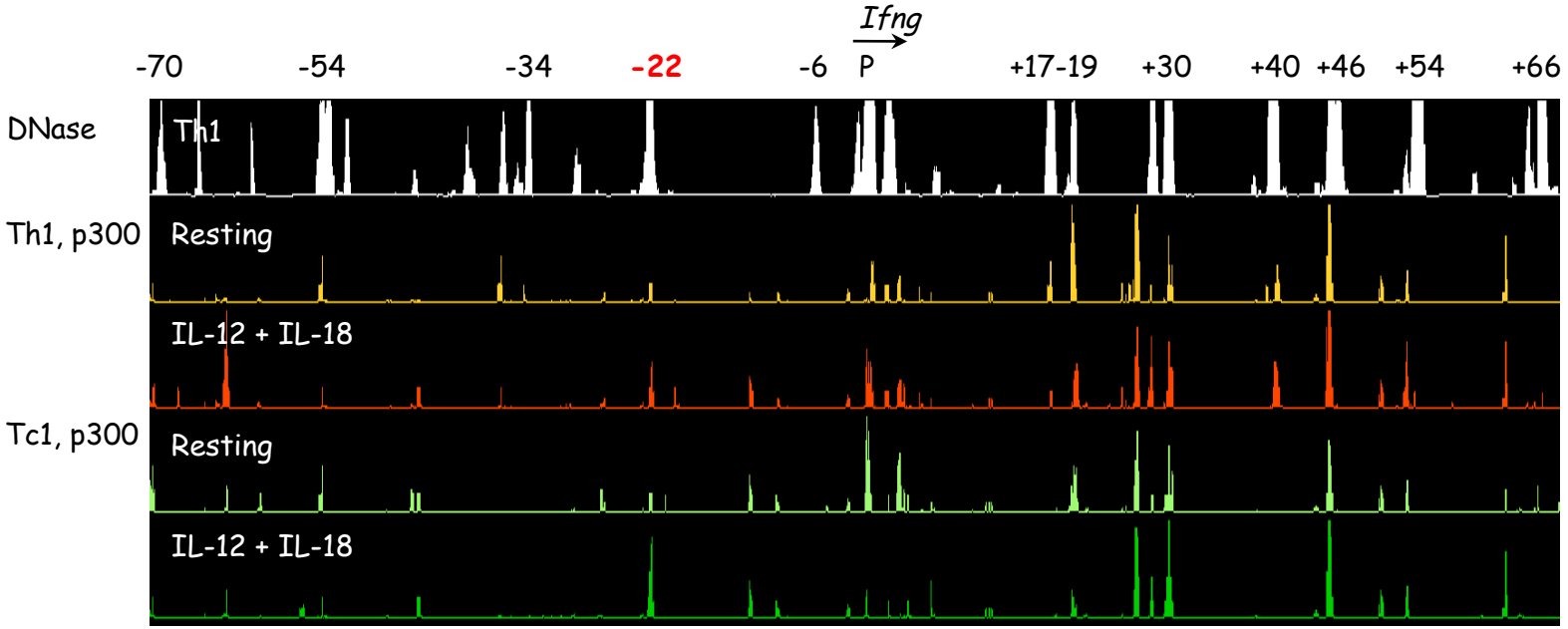
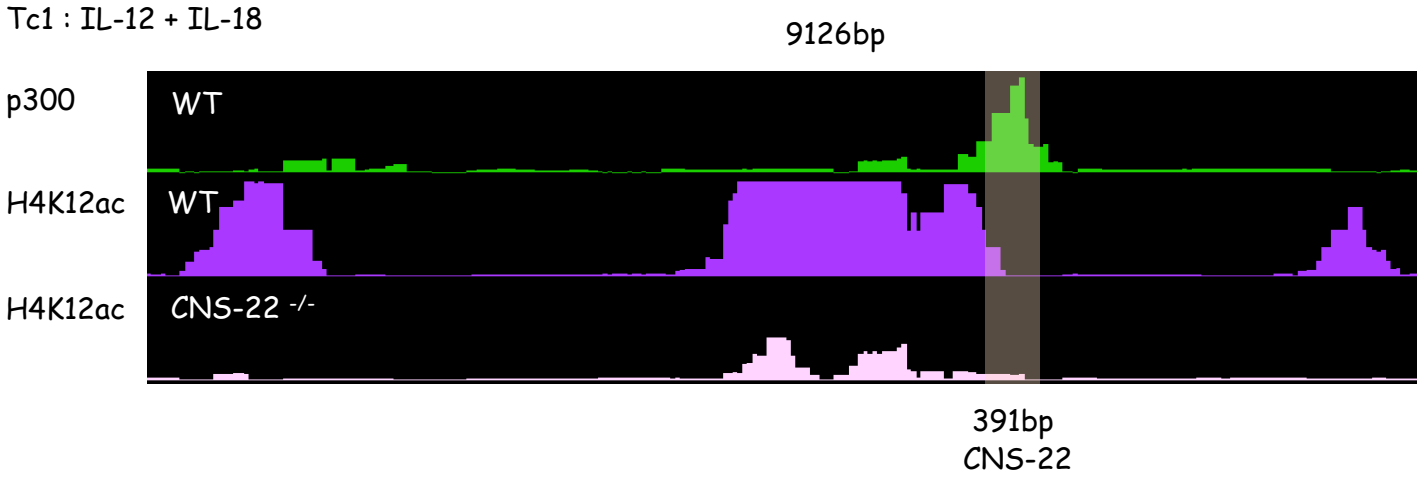


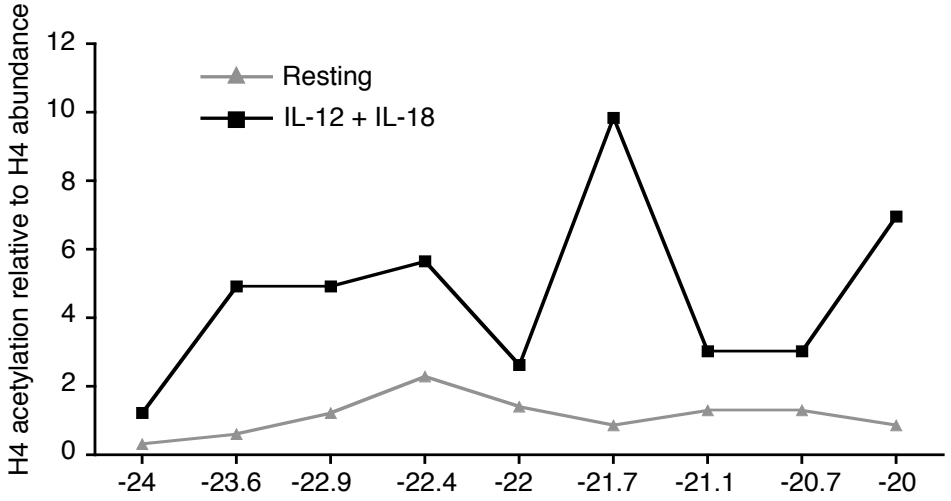
Image created by Kosi Gramatikoff

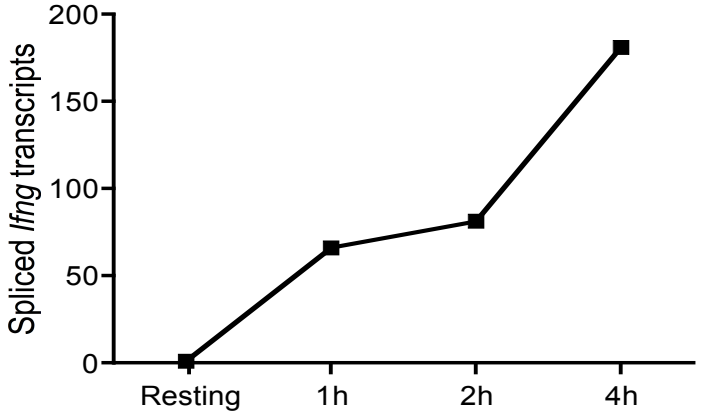
p300 Binding Maps to CNS Elements Across *Ifng* Locus

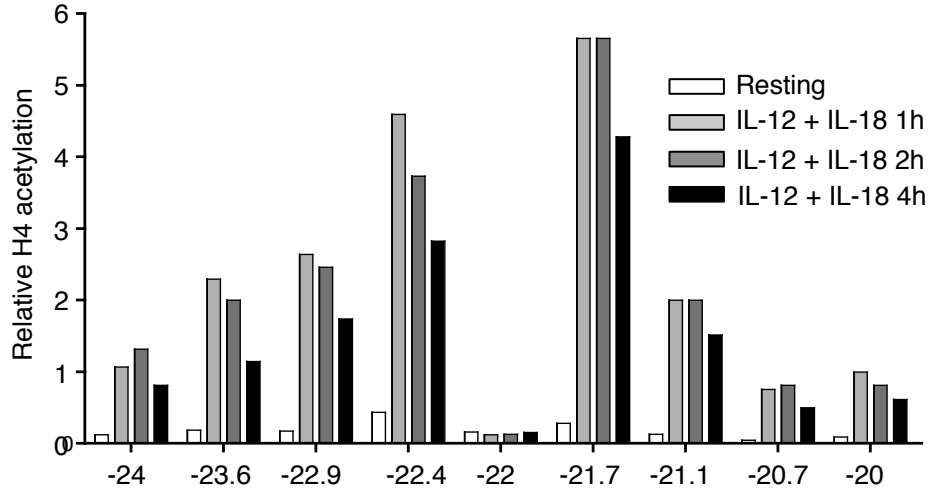




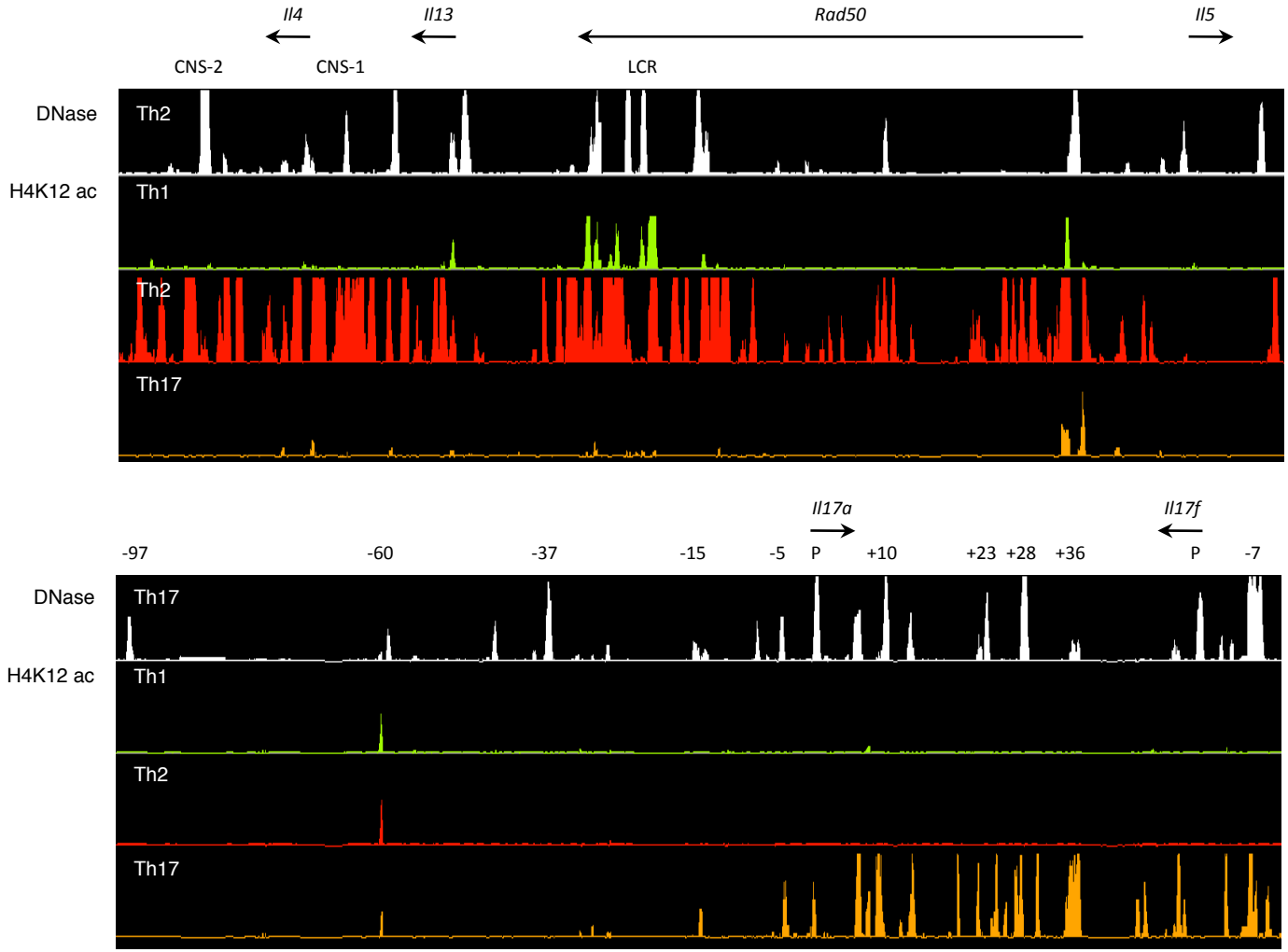
Activation-induced Hyperacetylation of CNS-22 Precedes *Ifng* Transcript Induction







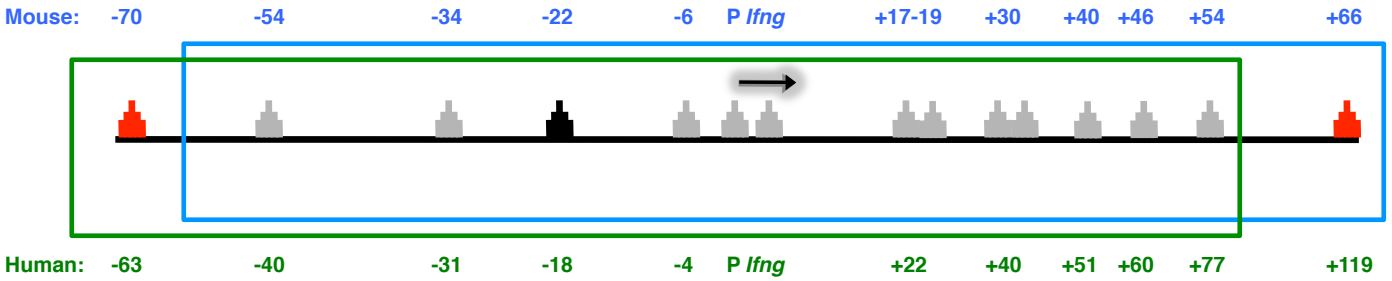
Histone Acetylation as a Measure of Transcriptional Activity



Acetylation of Lineage-specifying Genes

Gene	Expression pattern	Levels of H4K12 acetylation		
		Th1	Th2	Th17
Tbx21	Th1	+++++	+	-
Gata3	Th2	+	+++++	-
Rora	Th17	-	-	+++++
Rorc	Th17	-	-	+++++
Il21	Th17 > Th1/Th2	++	+++	+++++
Il10	Th2> Th1/Th17	++	+++++	-
Ccr6	Th17	-	-	+++++
Fasl	Th1	+++++	-	-

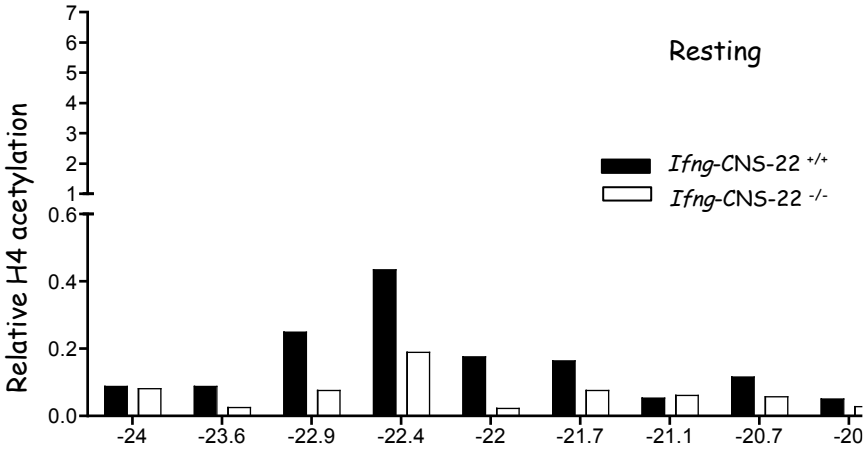
Summary II



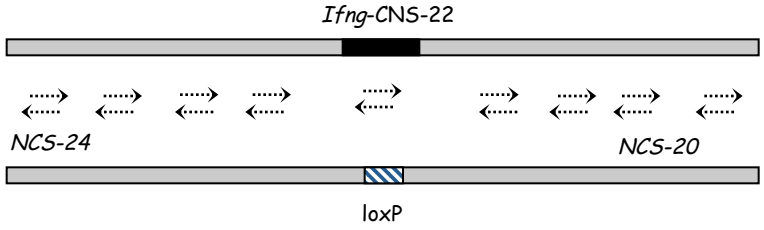
- **Deletion of CNS-22 impacts *Ifng* gene transcription**
  - 391 bp deletion in a locus that is approximately 140 kb in length.
  - First element in the *Ifng* locus whose function has been directly examined *in vivo*

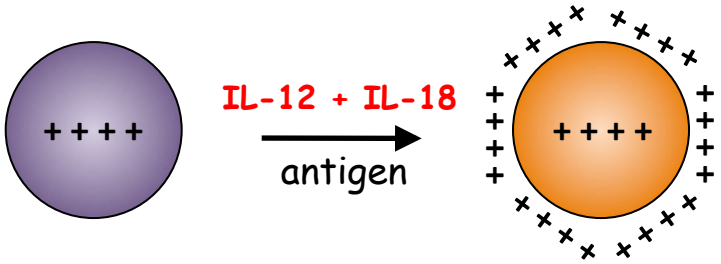
- **Original Hypothesis: CNS-22 plays an essential role in long-range remodeling of the *Ifng* locus**
  - Several differences between the BAC-transgenic and endogenous deletion of CNS-22
  - So what is the function of CNS-22?

CNS-22 Initiates Local Changes in Remodeling



Th1 cells





Th1 day 5 (Resting)

Activated : IFN-γ secreting cell