

# **Epigenetic Modifiers in the Pathogenesis of Follicular Lymphoma**

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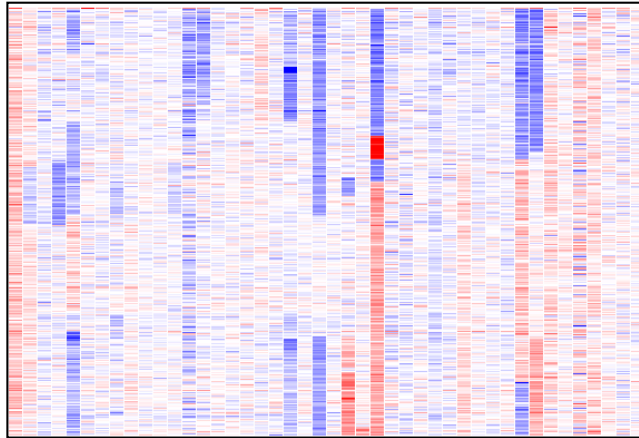
**Indolent Lymphoma Workshop**

**Bologna**

**May 2017**

# Experimental Strategy

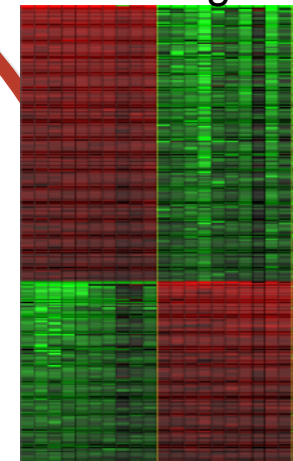
Copy Number Analysis  
(Affymetrix SNP 6.0 Array)



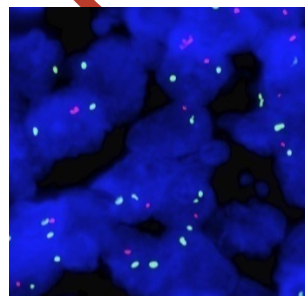
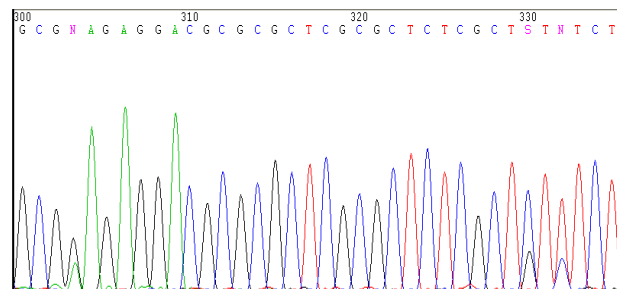
Whole Exome Capture  
& Next Gen Sequencing



Gene Expression  
Profiling



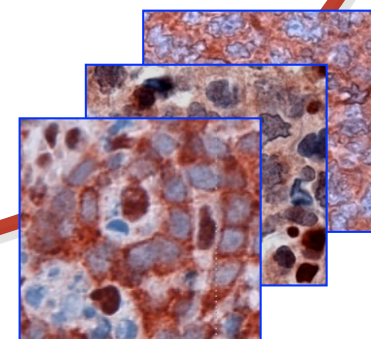
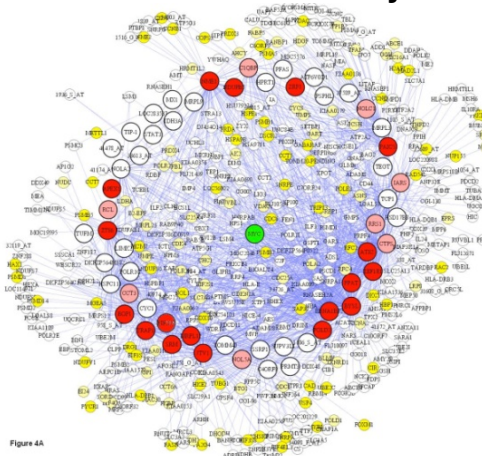
Candidate Gene Resequencing



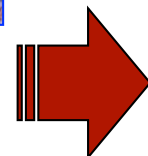
FISH analysis

**~100 DLBCL  
primary biopsies**

Bioinformatics analysis

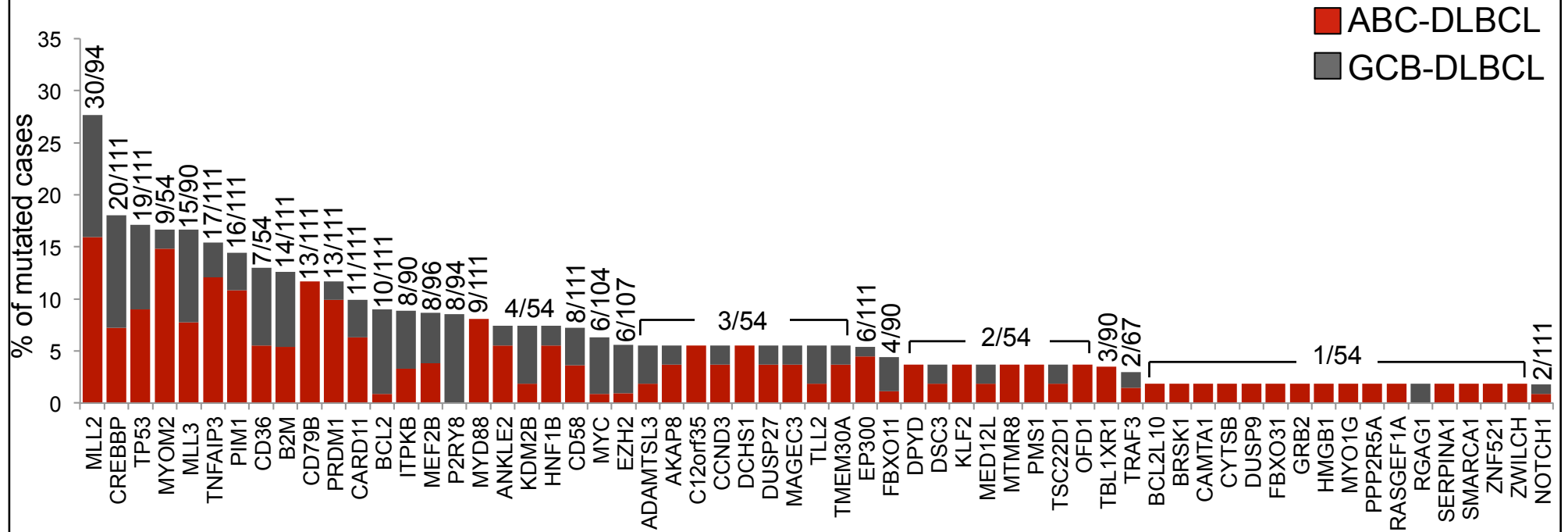


IHC analysis

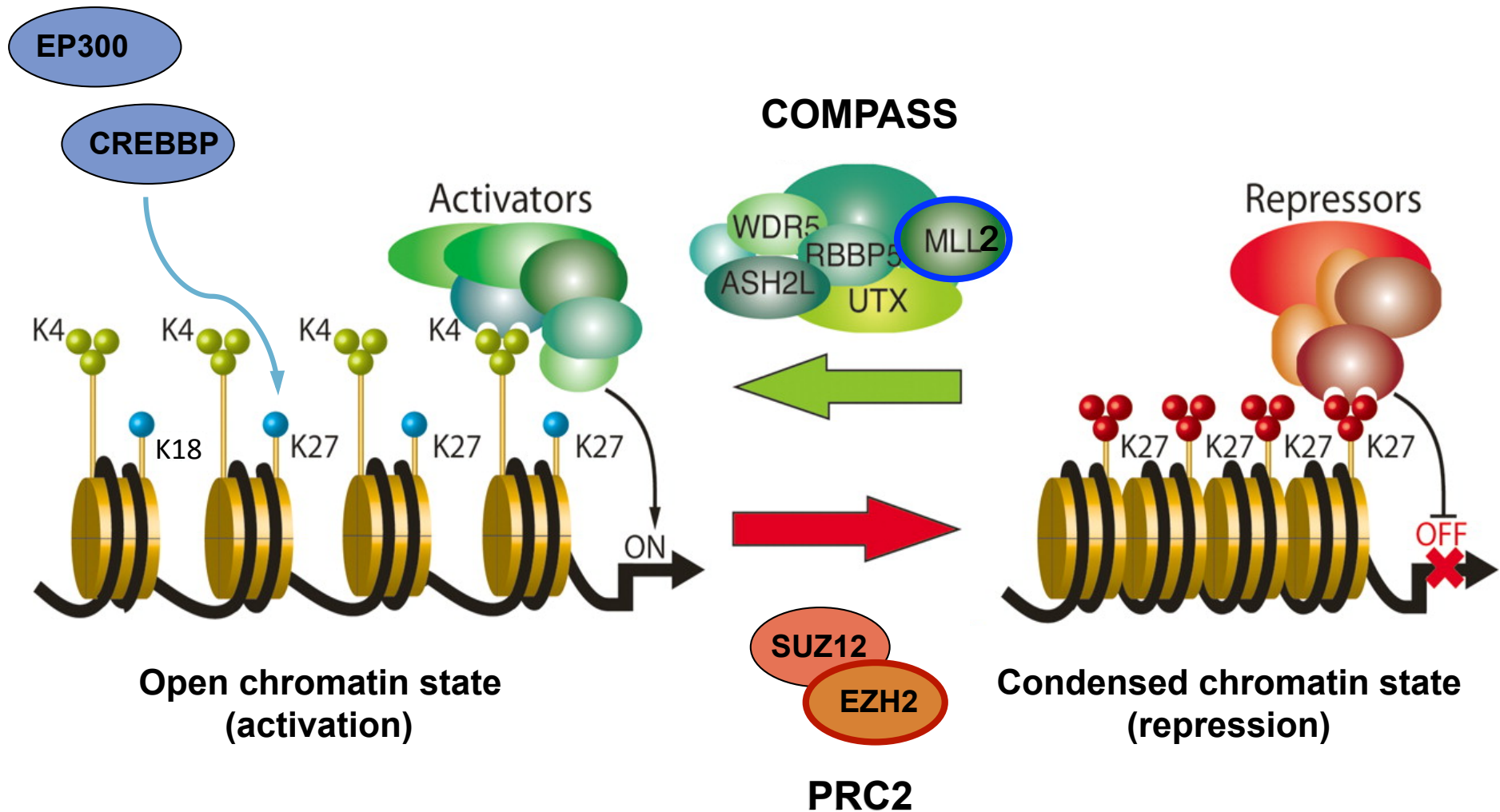


**Functional  
Readouts**

# Recurrently mutated genes in DLBCL



# Epigenetic mechanisms and transcriptional regulation

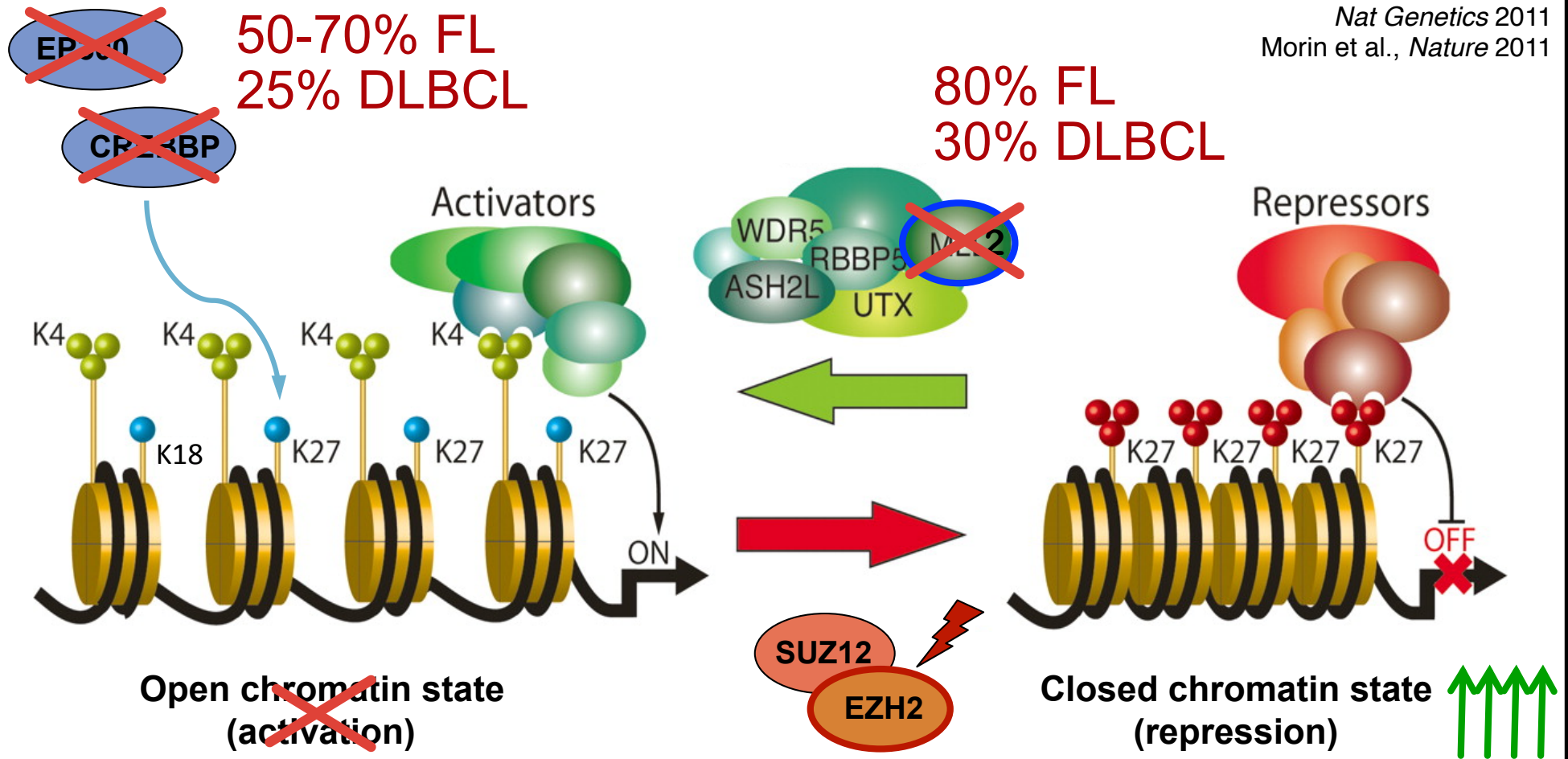


- H3K27Ac, H3K18Ac
- H3K4me1 (enhancer)/H3K4me3 (promoters)
- H3K27me3



# Genetic-based aberrant epigenetic regulation in lymphoma

Pasqualucci et al., Nature 2011  
 Nat Genetics 2011  
 Morin et al., Nature 2011

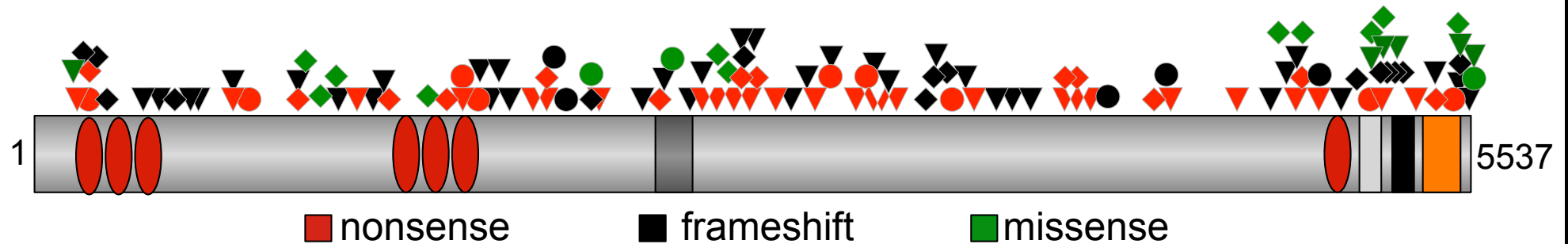


- H3K27Ac, H3K18Ac
- H3K4me1 (enhancer)/H3K4me3 (promoters)
- H3K27me3

Morin et al., Nat. Genetics 2010

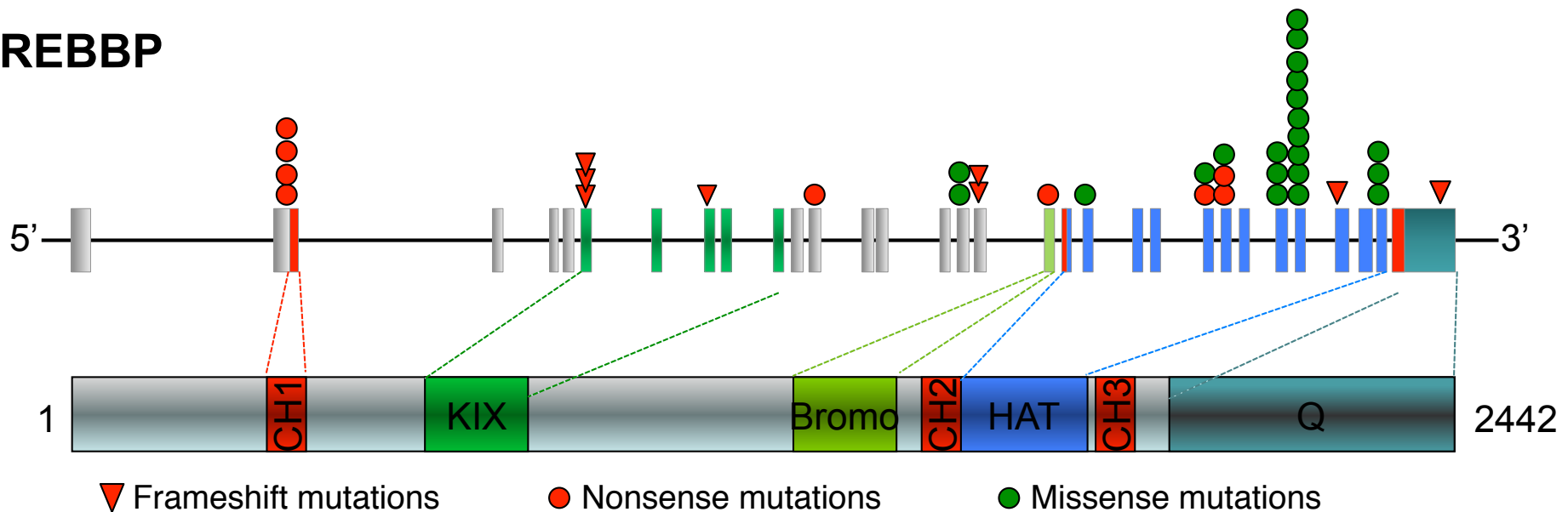
# Mutations of MLL2 and CREBBP generate truncated proteins lacking the C-terminal catalytic domains

## MLL2 (KMT2D)



Pasqualucci et al., *Nature Genetics* 2011  
Morin et al., *Nature* 2011

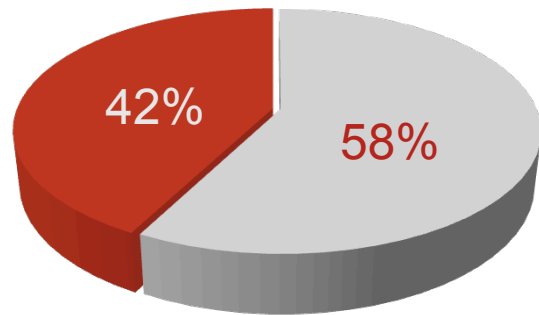
## CREBBP



Pasqualucci et al., *Nature* 2011

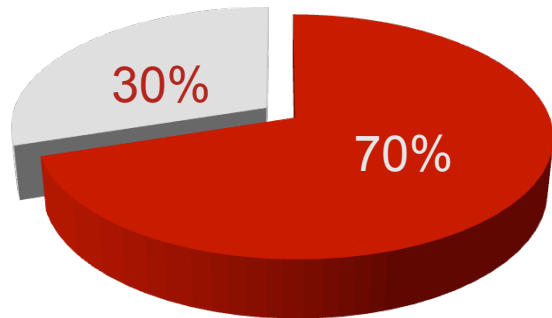
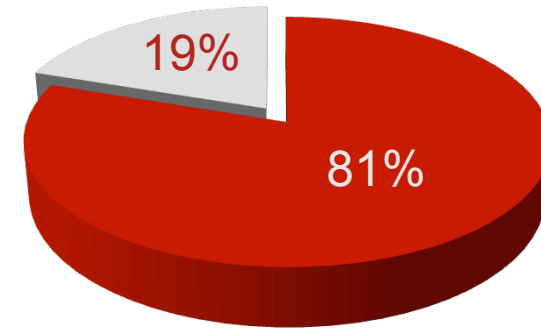
# Mutations of MLL2 and CREBBP are mostly monoallelic, suggesting a haploinsufficient tumor suppressor role

**MLL2 (KMT2D)**

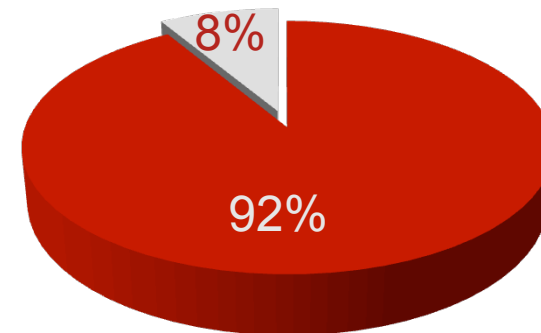


*FL/tFL*

**CREBBP**



*de novo DLBCL*



*Dose-dependent pathogenic effect in*

■ monoallelic *Kabuki syndrome*

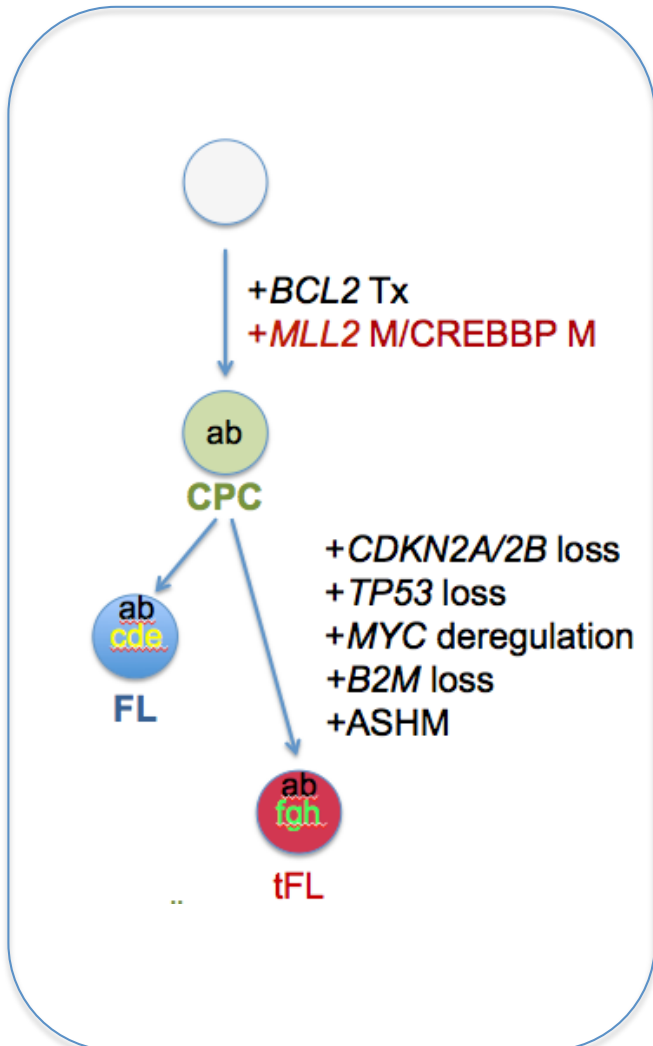
■ monoallelic *Rubinstein-Taybi syndrome*

Genetic lesions in  
Histone Methyltransferases

Genetic lesions in  
Histone Acetyltransferases

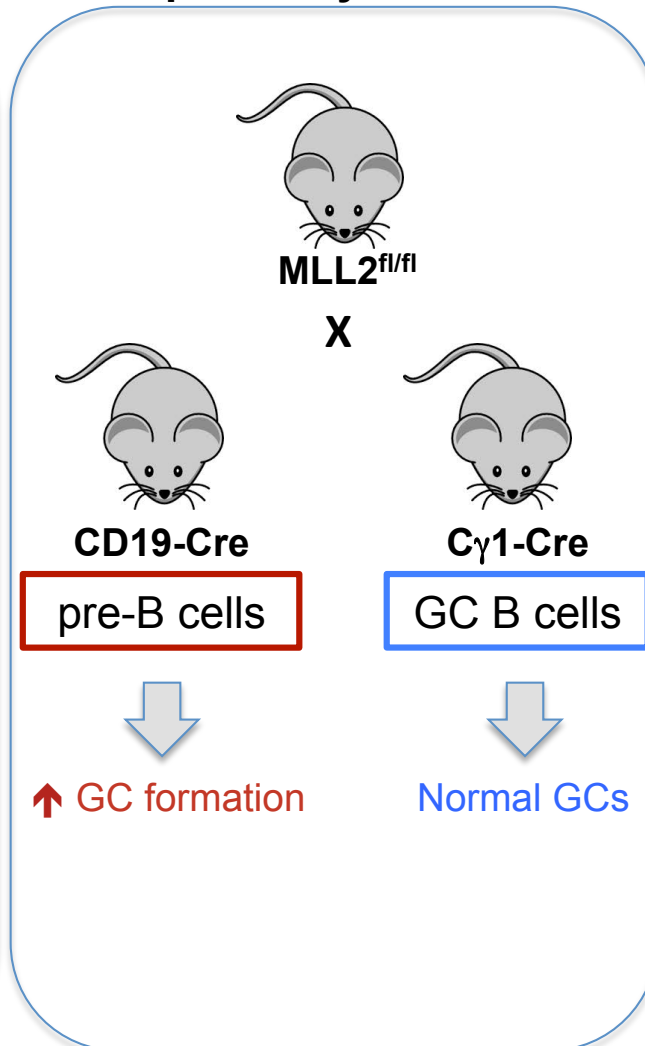
# *Mll2* loss is acquired early during clonal expansion and cooperates with BCL2 deregulation in lymphoma

## Early lesion in FL/DLBCL



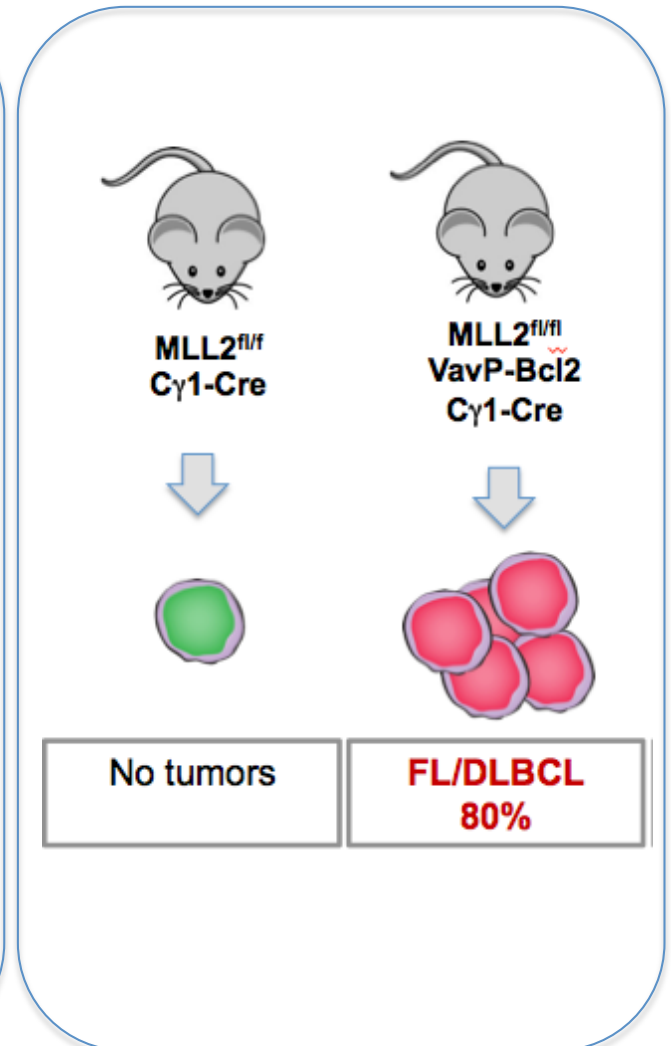
Pasqualucci et al., *Cell Reports* 2014

## Increased GC formation upon early deletion



Zhang et al., *Nature Med* 2015

## Tumor suppressor in mice



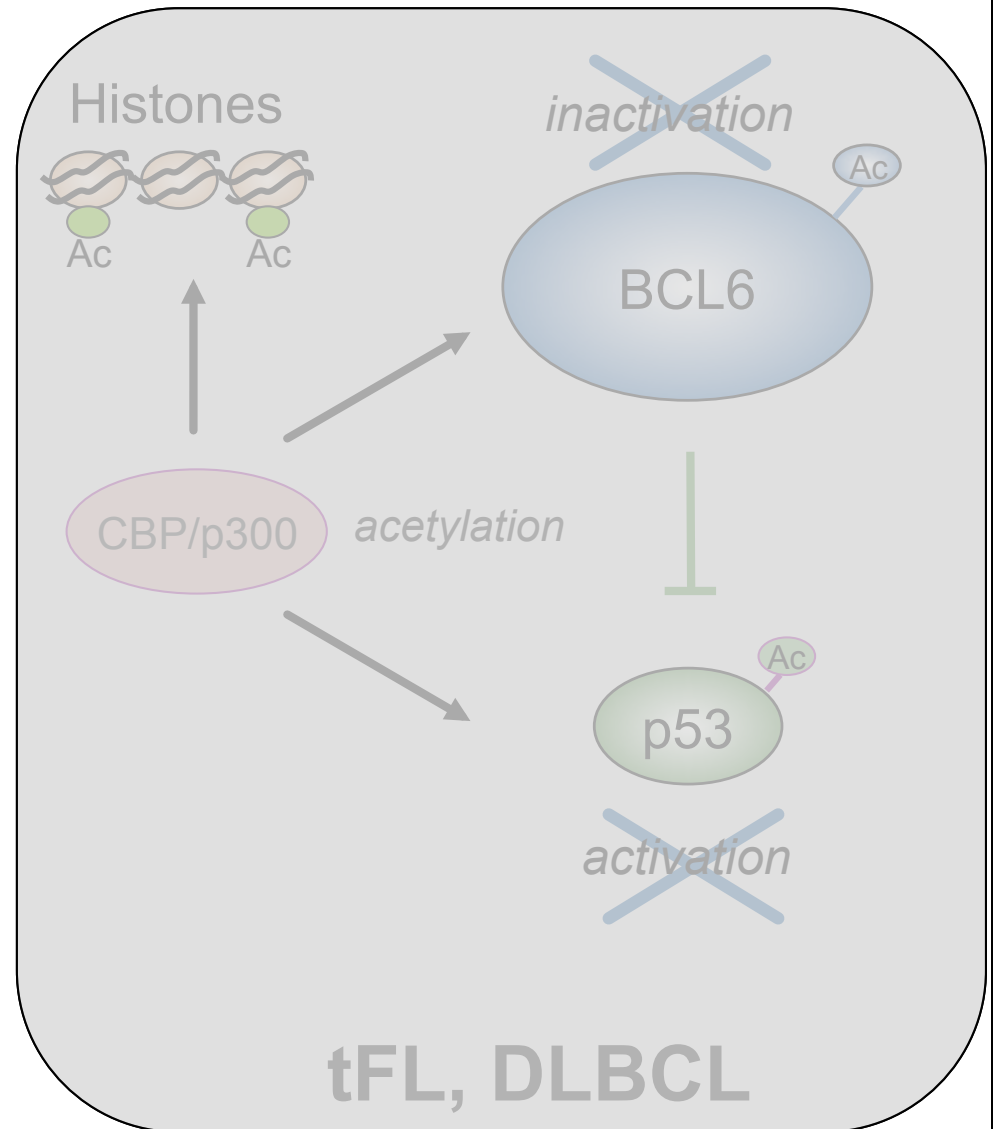
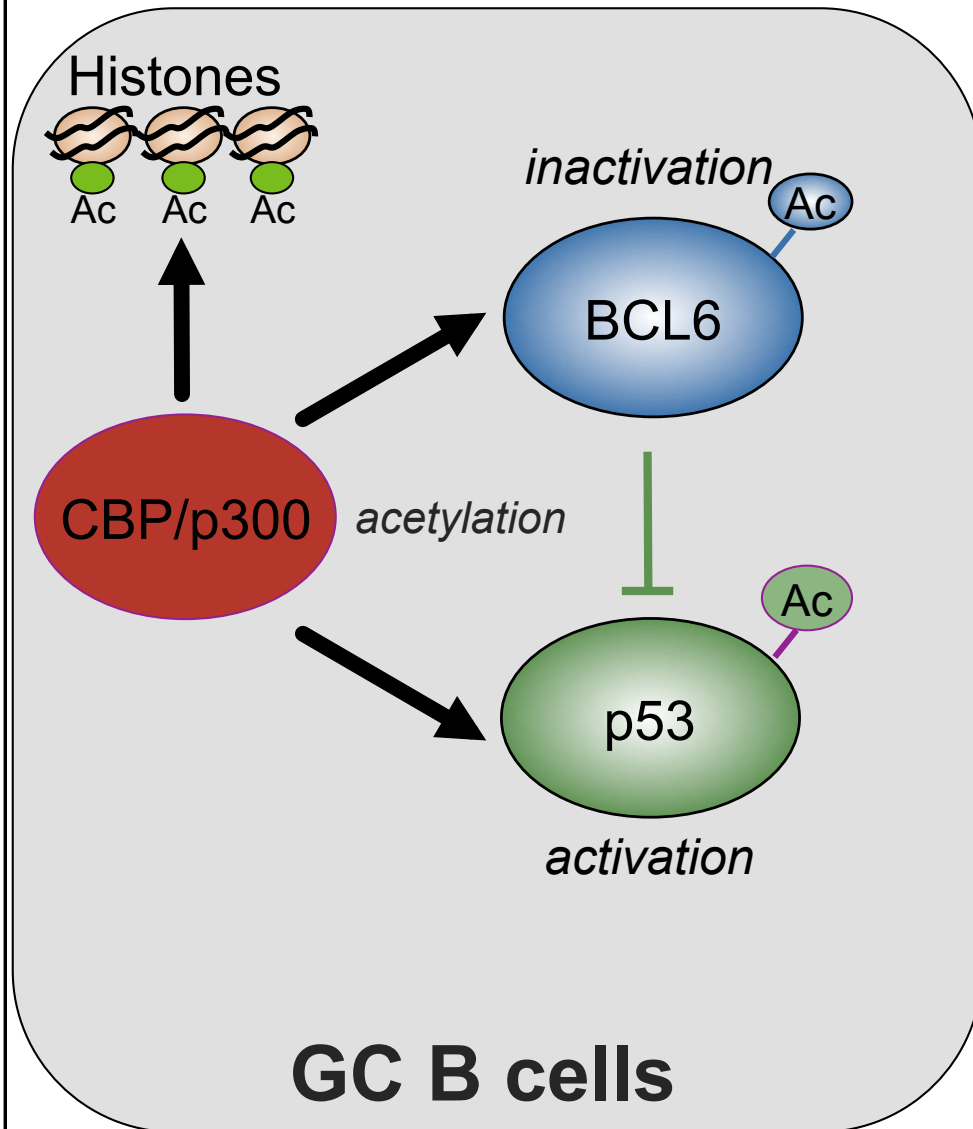
Zhang et al., *Nature Med* 2015

# Genetic lesions in Histone Methyltransferases

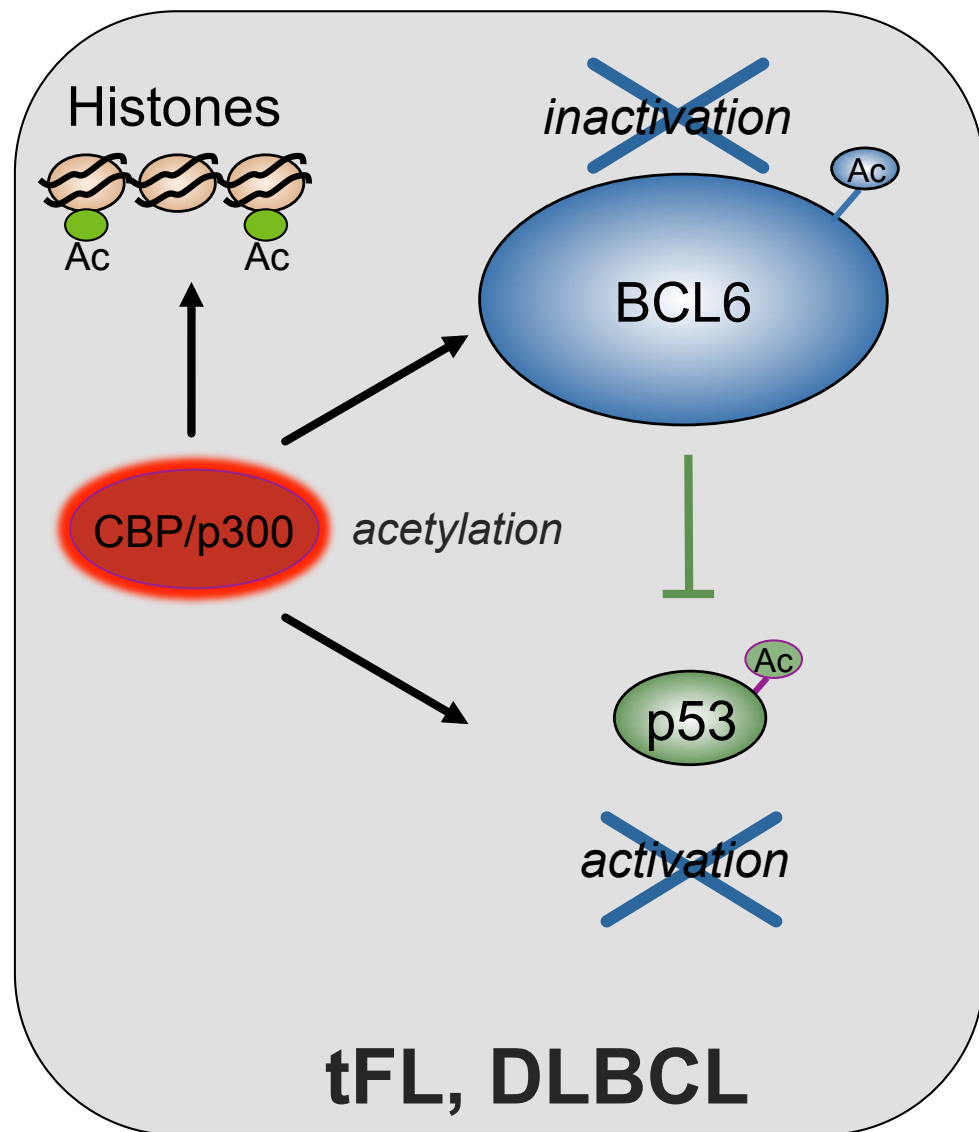
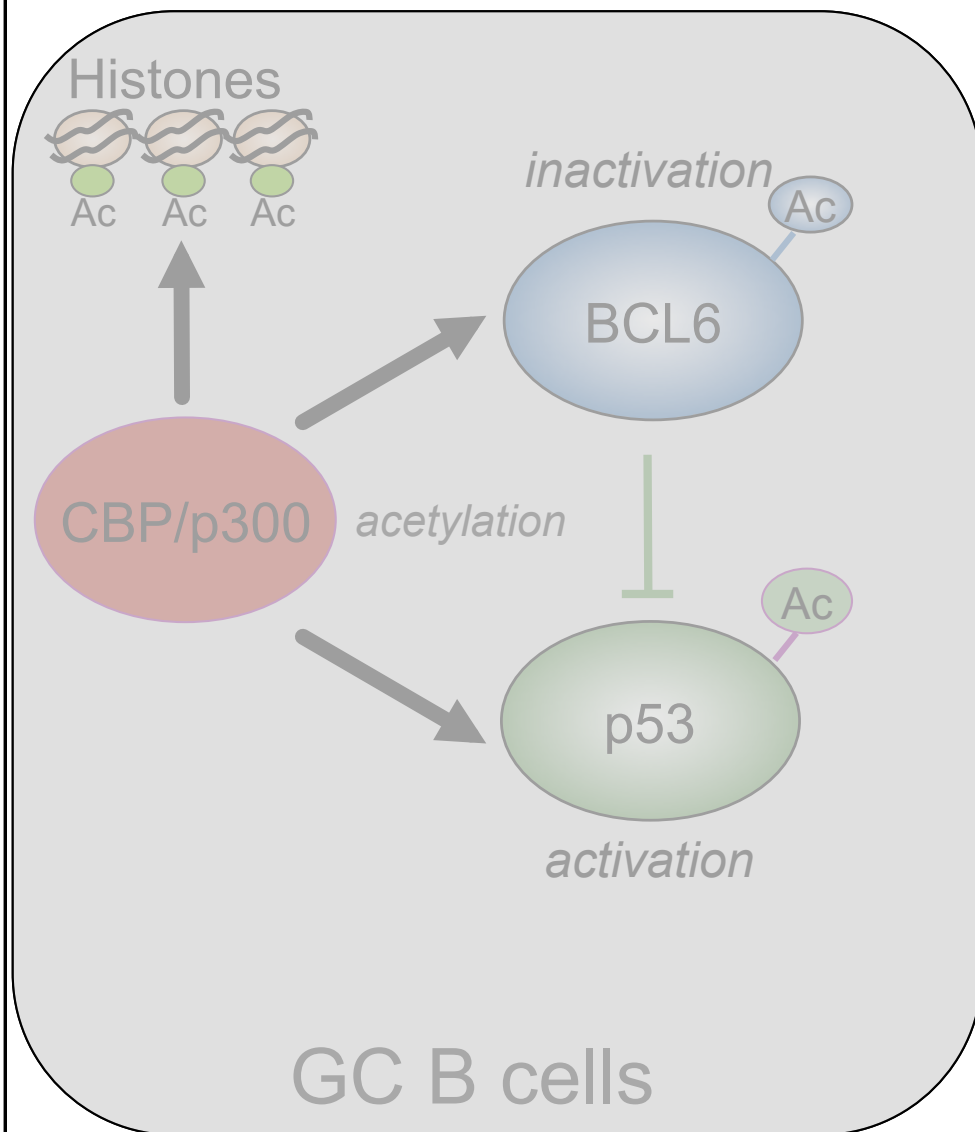
Genetic lesions in  
Histone Acetyltransferases



# Role of CREBBP inactivation in B cell lymphoma

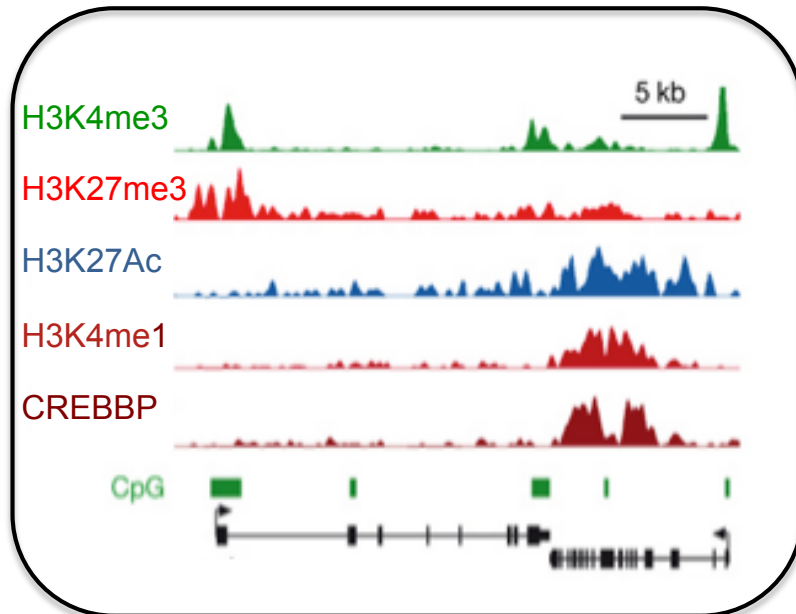


# Role of CREBBP inactivation in B cell lymphoma



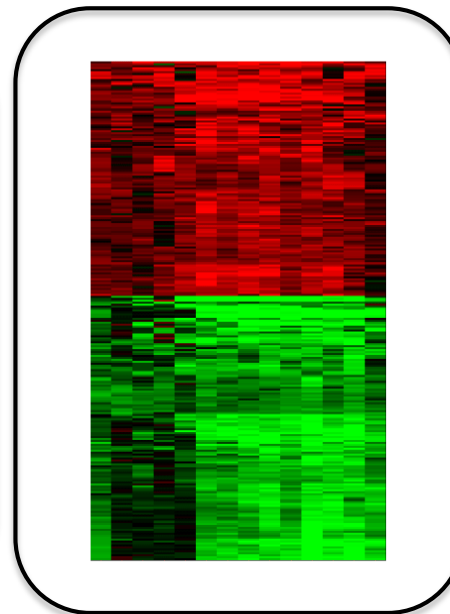
# Defining the transcriptional network regulated by CREBBP in GC B cells – Experimental Strategy

ChIP-seq



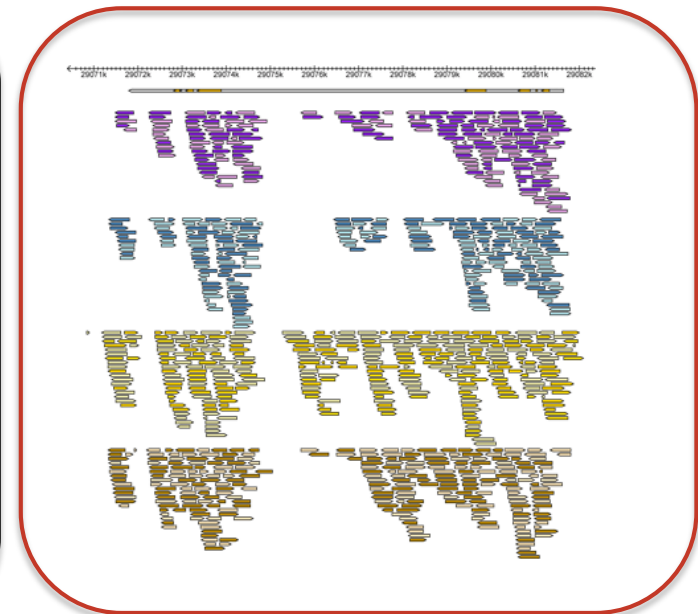
Human GC B cells

Affy U133p2

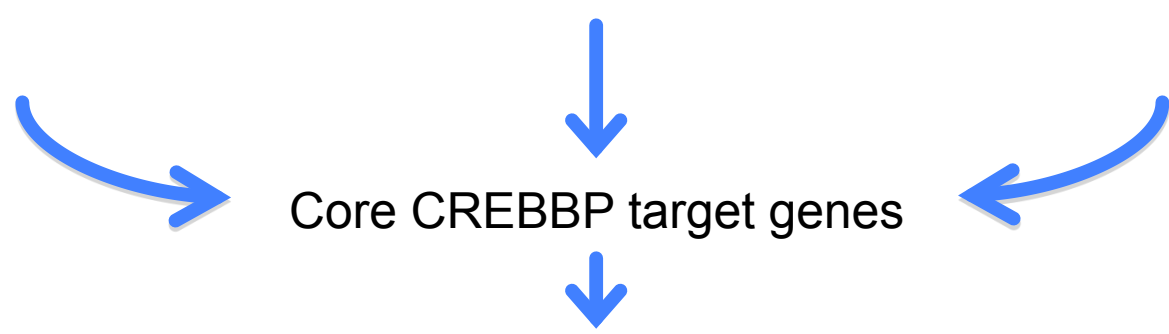


Mouse GC B cells  
(*Crebbp* WT vs KO)

RNA-Seq



Human GC B cells  
(vs Naive and Memory)

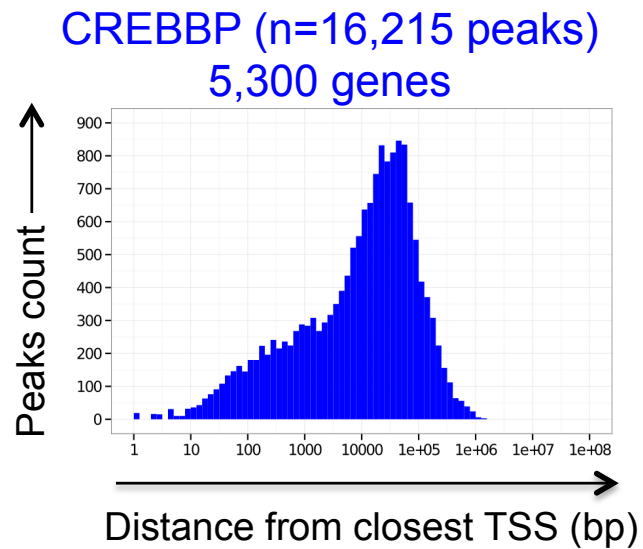


Core CREBBP target genes

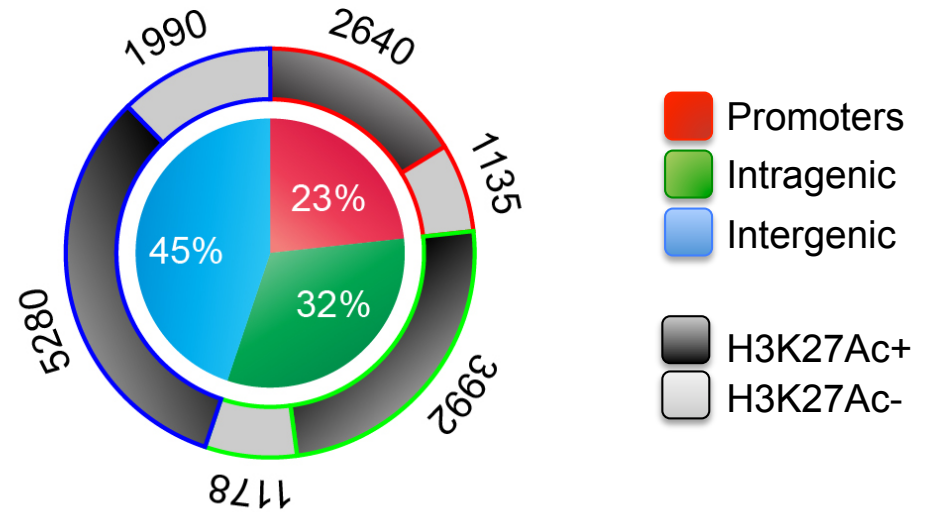
Track in human biopsies (with/without CREBBP mutations)

# CREBBP binds preferentially to TSS-distal regions decorated by H3K27Ac and H3K4me1 marks

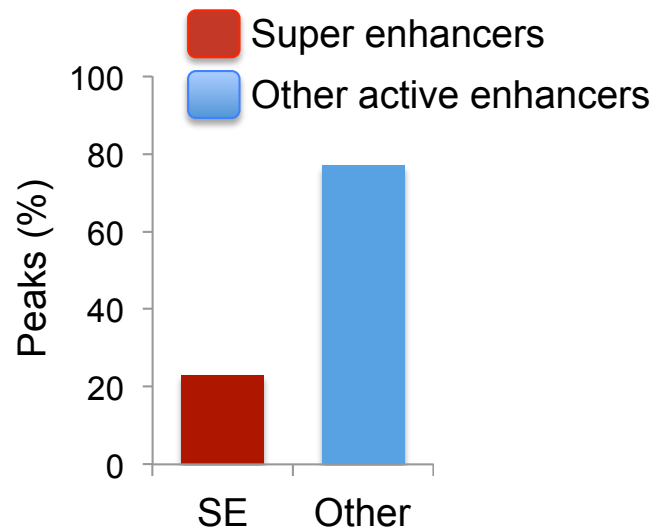
a.



b.



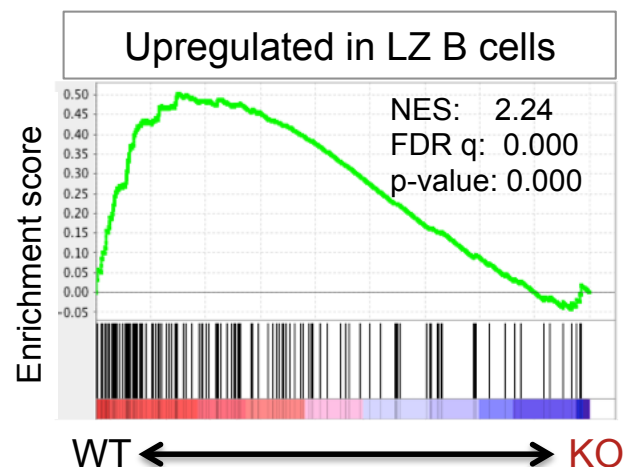
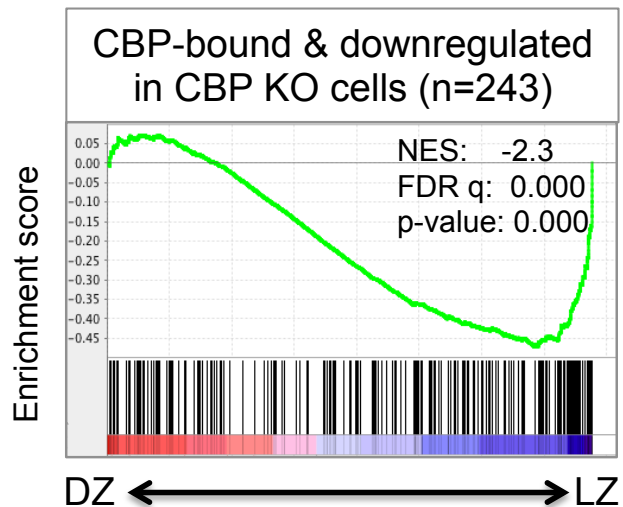
c.



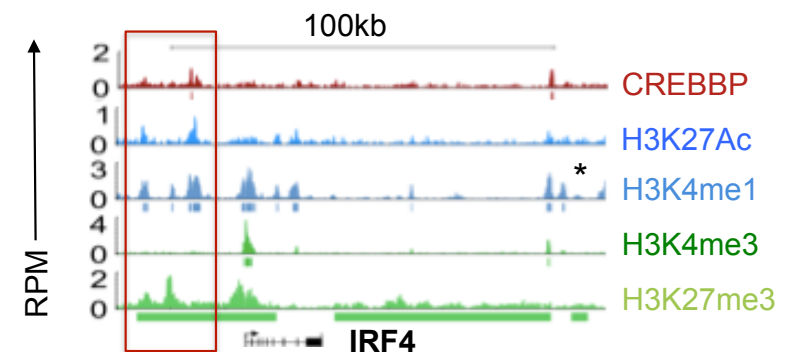
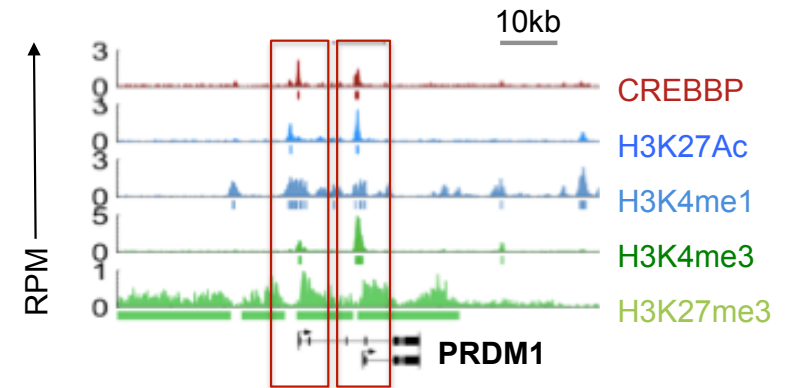
*CREBBP is a central regulator of enhancer activities in the GC*

# CREBBP-bound regions are enriched in “light-zone” upregulated genes, including master regulators of plasma cell differentiation

## a. GSEA analysis



## b. ChIP-Seq

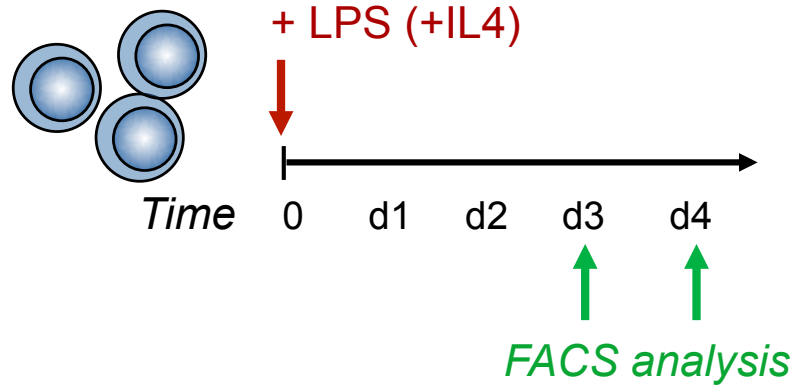


*CREBBP modulates signaling pathways that are activated in the GC light zone upon signals important for cell fate decisions*

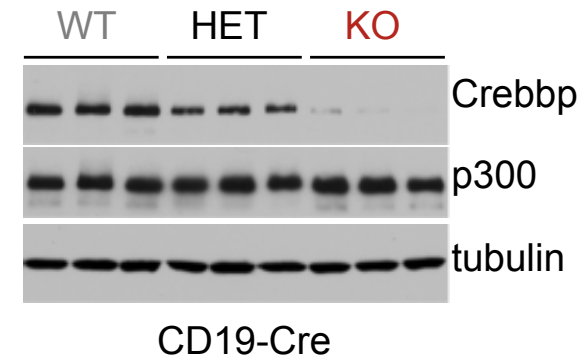
# A full complement of CREBBP is necessary for efficient plasma cell differentiation (*ex vivo*)

a.

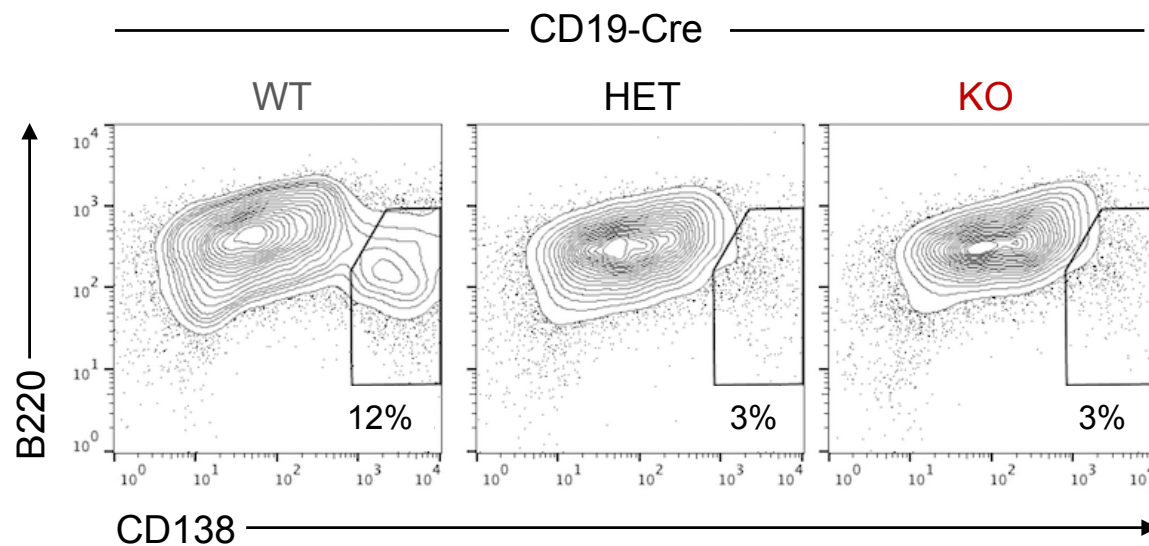
B220+ lymphocytes



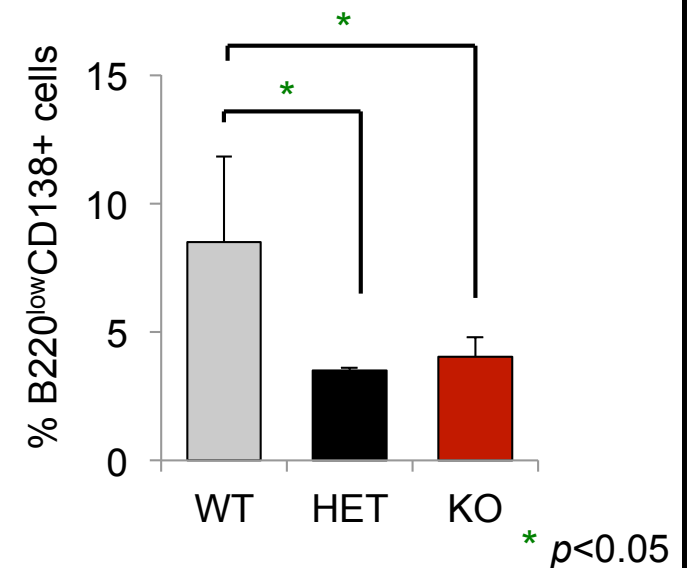
b.



c.

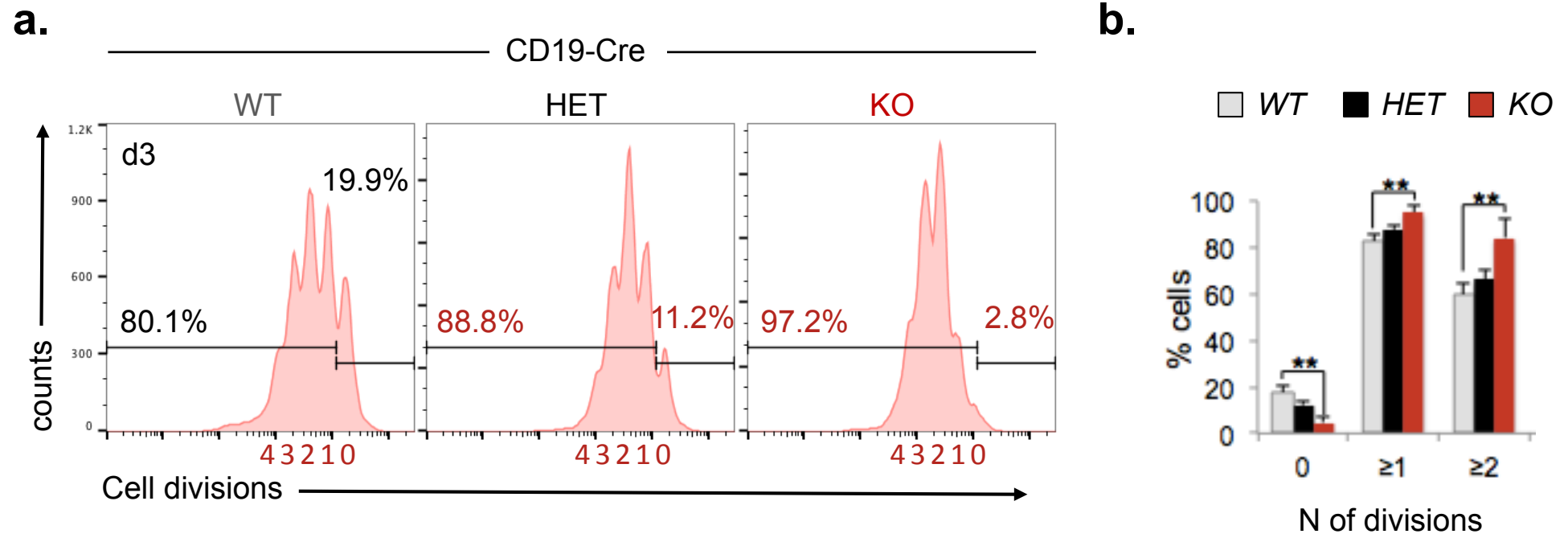


d.





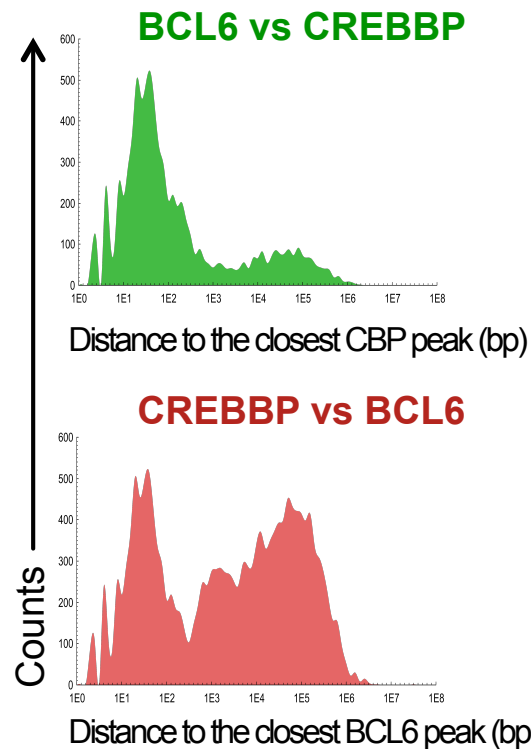
# CREBBP heterozygous and homozygous null B cells have proliferative advantage



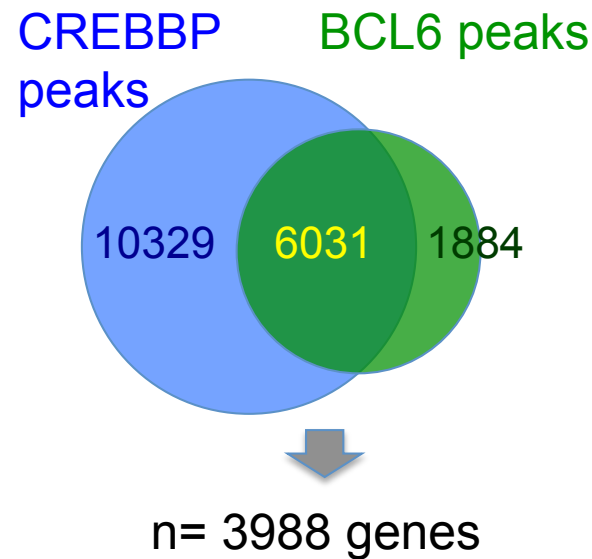
\*  $p < 0.05$ ; \*\*  $p < 0.01$

# CREBBP is involved in the activation of BCL6-repressed genes

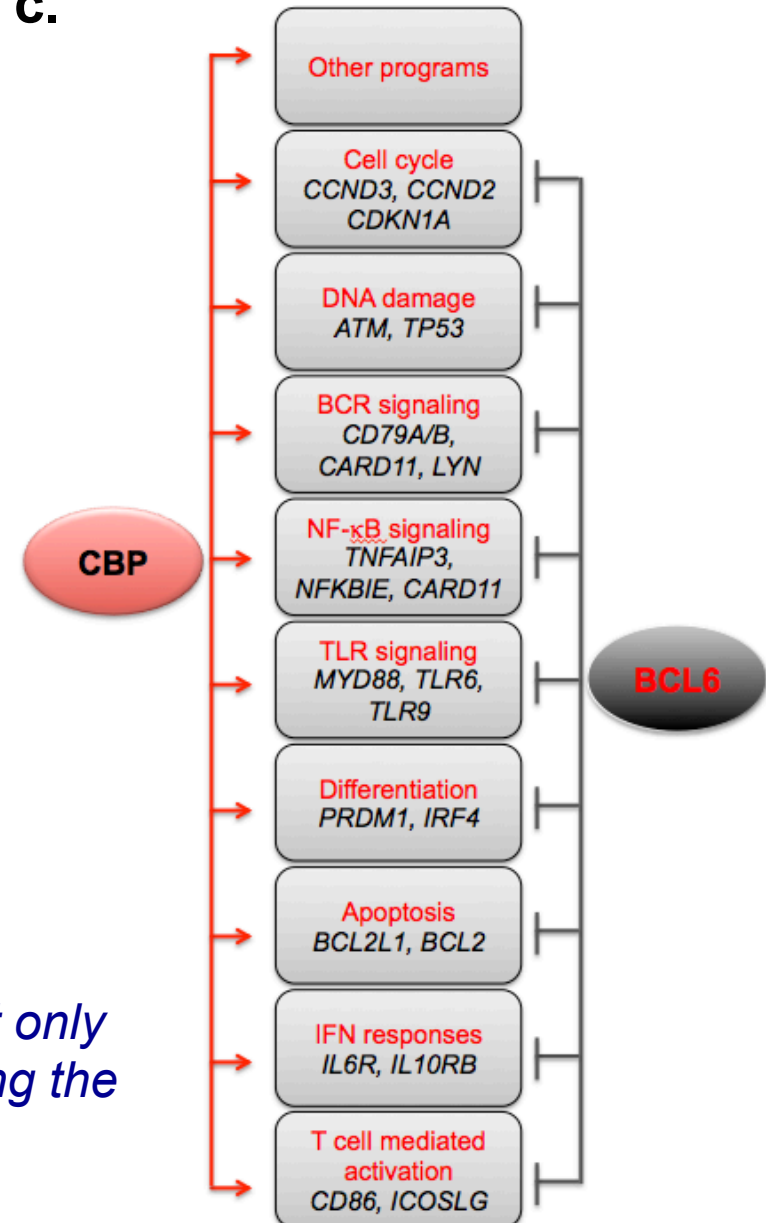
a.



b.

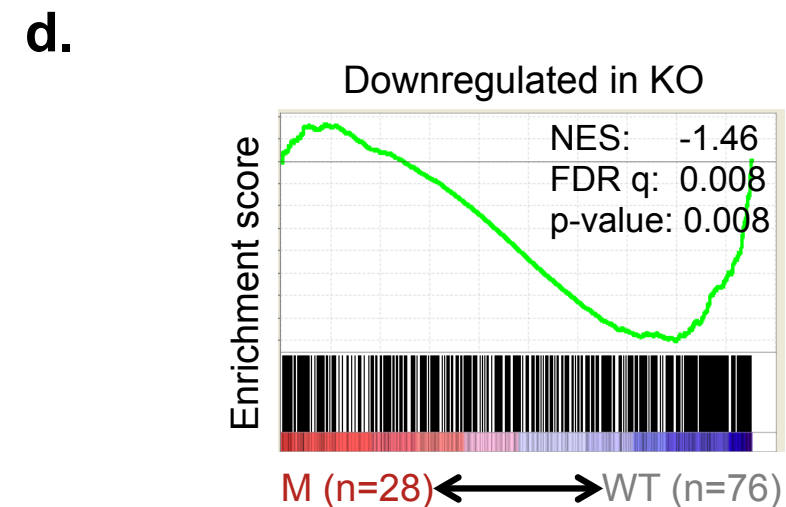
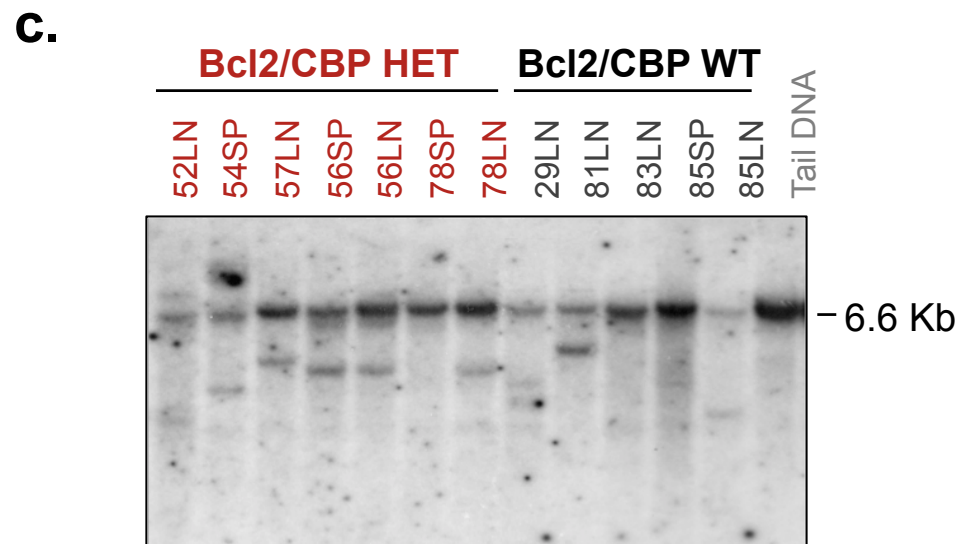
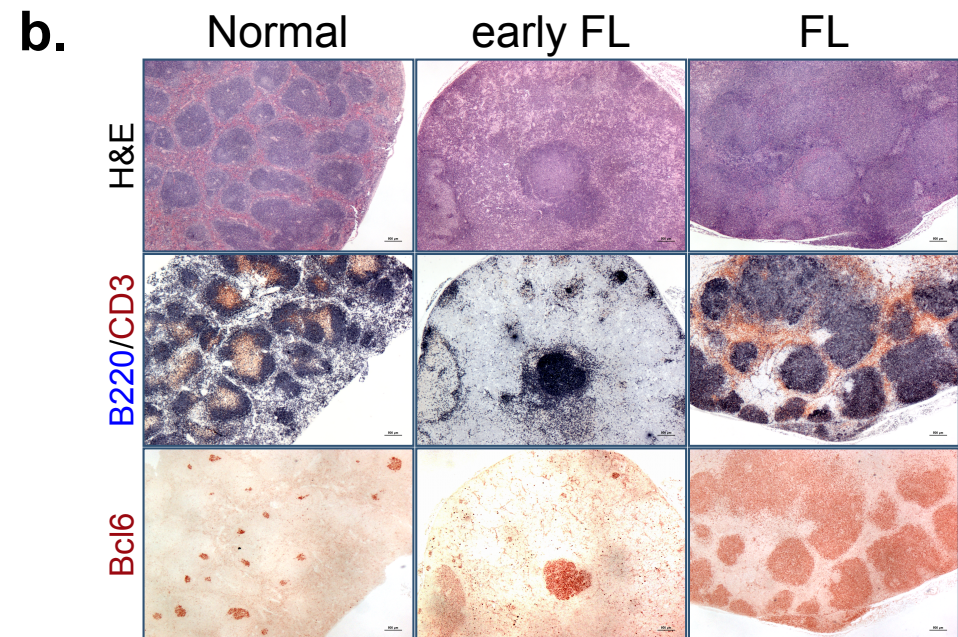
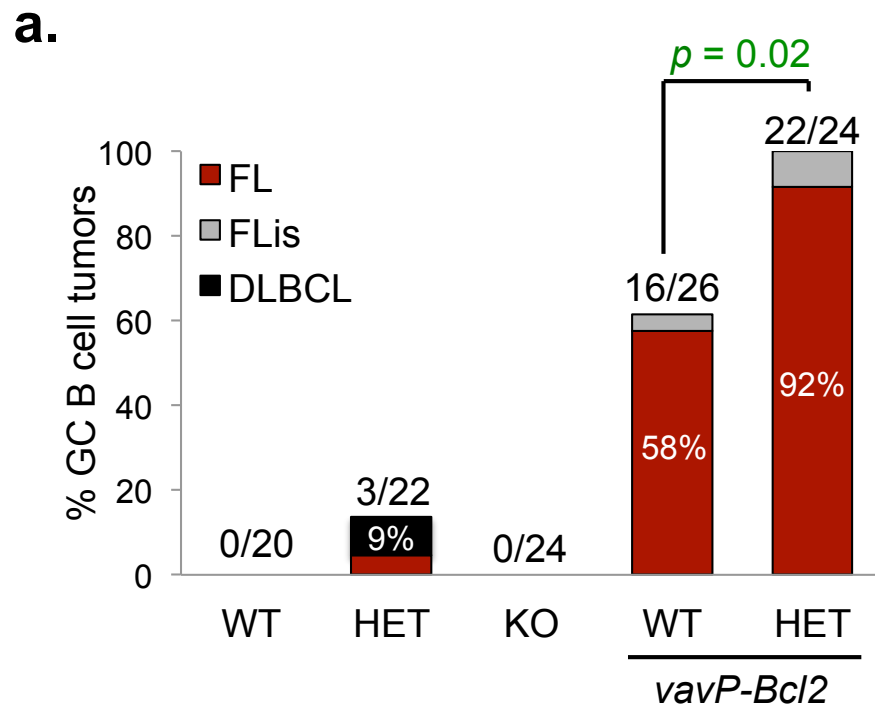


c.

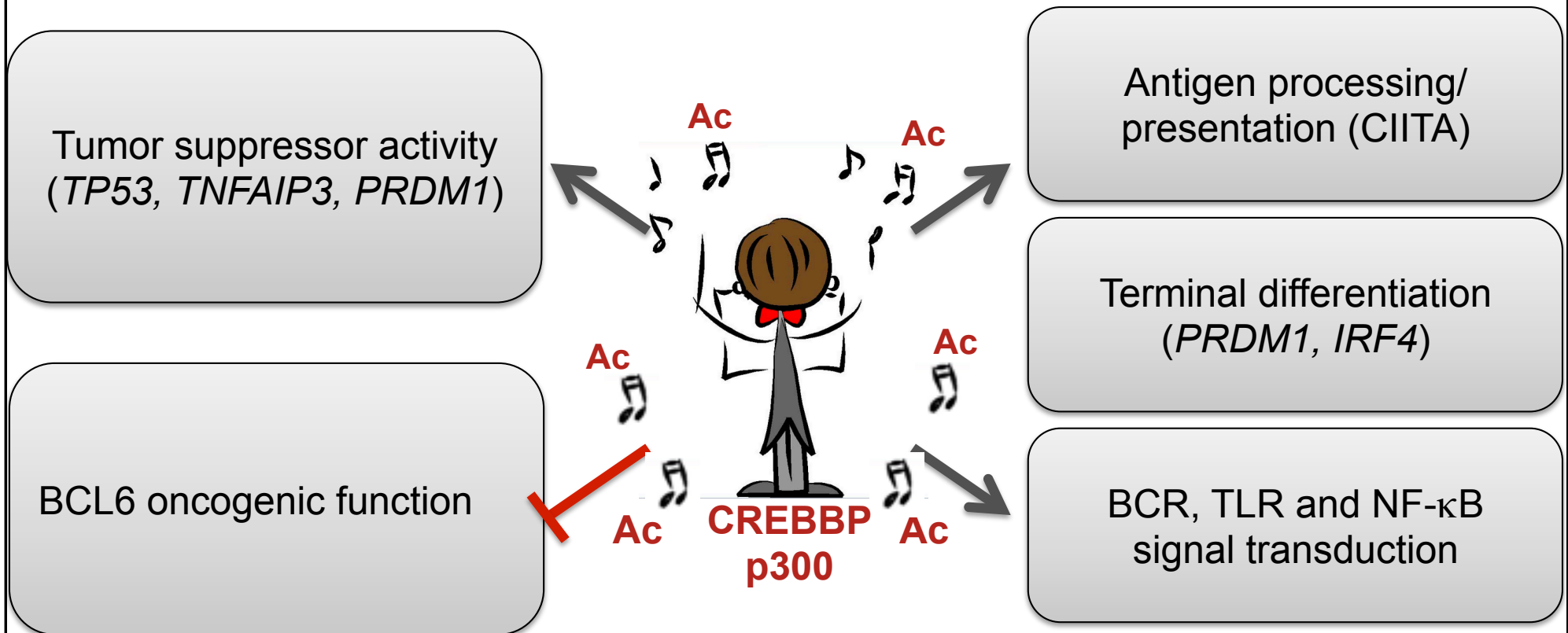


*CBP counteracts the oncogenic function of BCL6 not only by acetylating its protein but also by directly facilitating the transcription of its repressed genes*

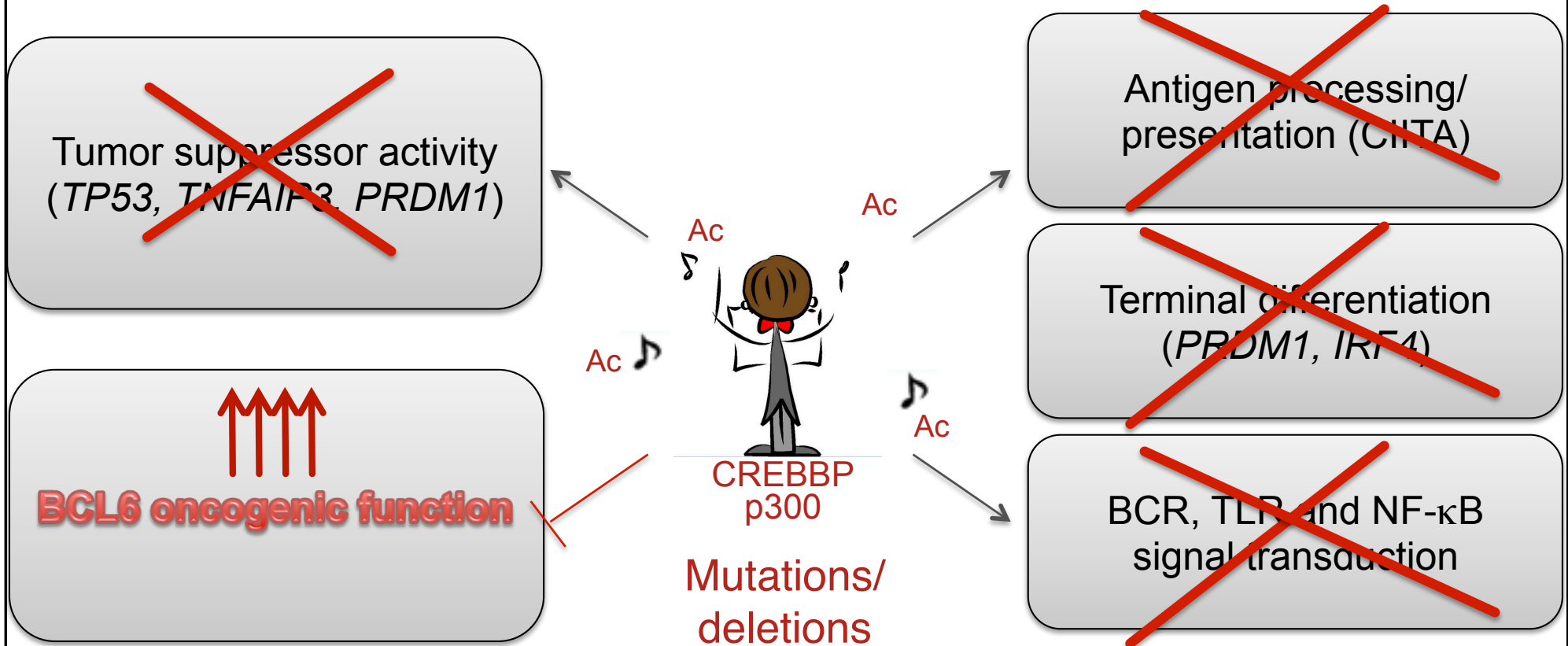
# Loss of one *Crebbp* allele facilitates BCL2-driven follicular lymphoma development



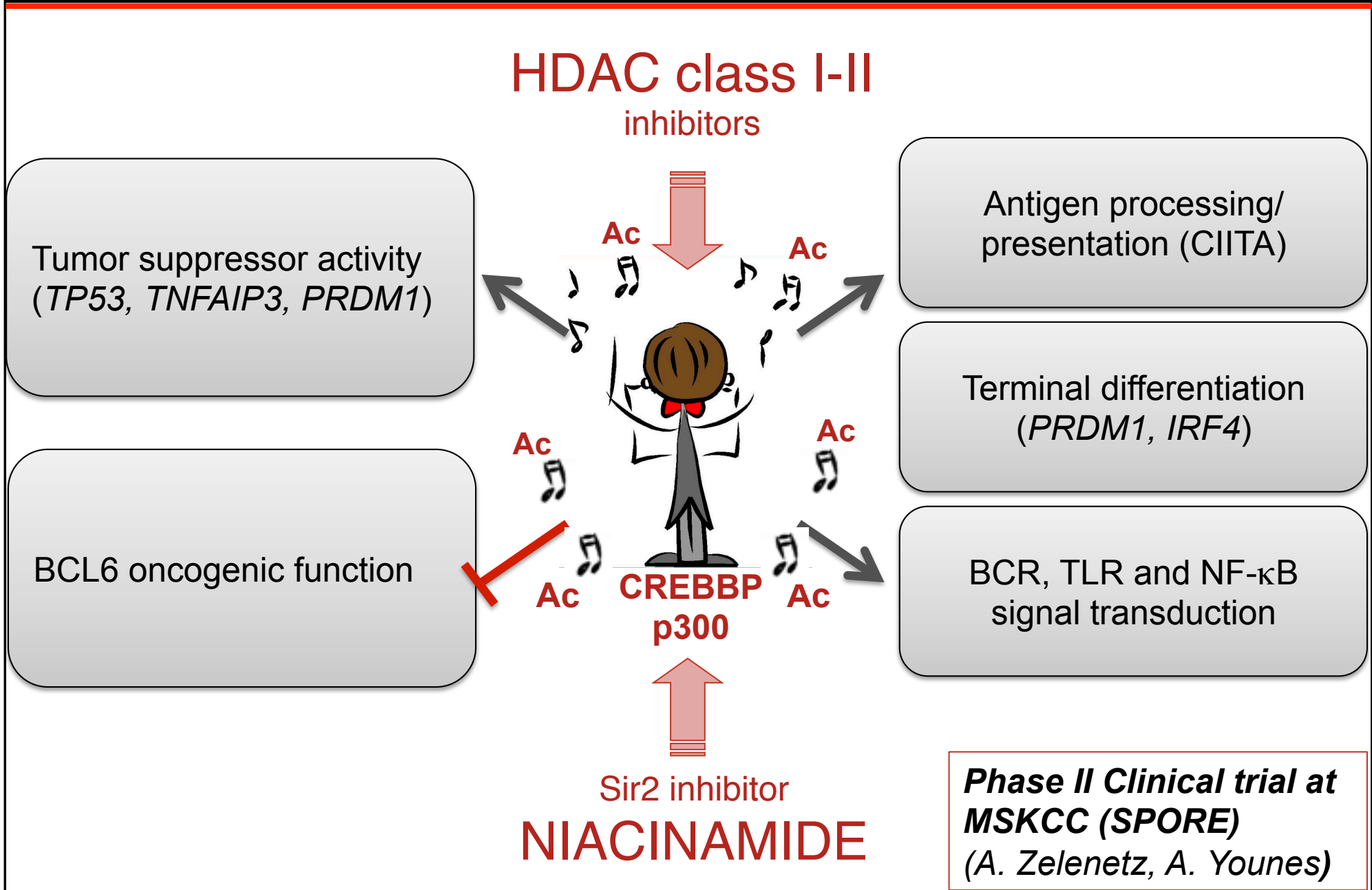
# What did we learn?



# Consequences of CREBBP inactivation in the GC



# Therapeutic targeting of the CBP network





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Shafinaz Hussein

## Northwestern University

Amy Chadburn

## European Institute of Oncology

Stefano Pileri

## CRCT Toulouse

Pierre Brousset

# Common and distinct pathways in DLBCL subtypes

## Histone/chromatin modification

Acetyl-transferases (CBP, p300); Methyl-transferases (MLL2)

Loss of DNA damage response

BCL6 deregulation

Escape from immune surveillance (CTL + NK)

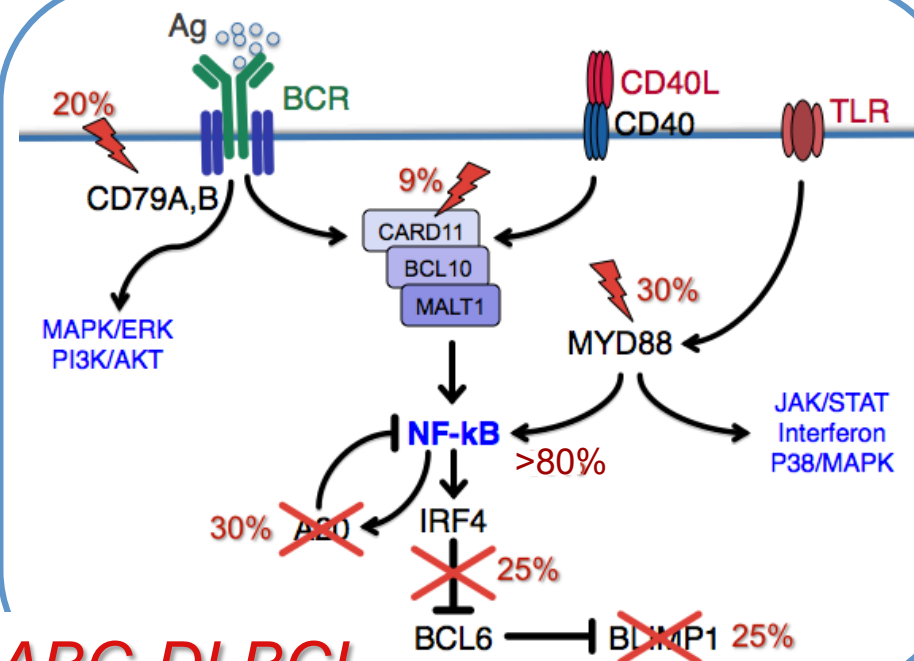
B2M, HLA-I loss, CD58

*shared*

FOXO1 deregulation/nuclear localization

- BCL2 translocations 25-30%
- MYC translocations 10%
- EZH2 mutations 22%
- GNA13/S1PR2 mut 20-30%
- TNFRSF14 mutations 15-20%

GCB-DLBCL



ABC-DLBCL