

# Genomics of Diffuse Large B Cell Lymphoma: Role of Mutations of Non-Coding Regulatory Sequences

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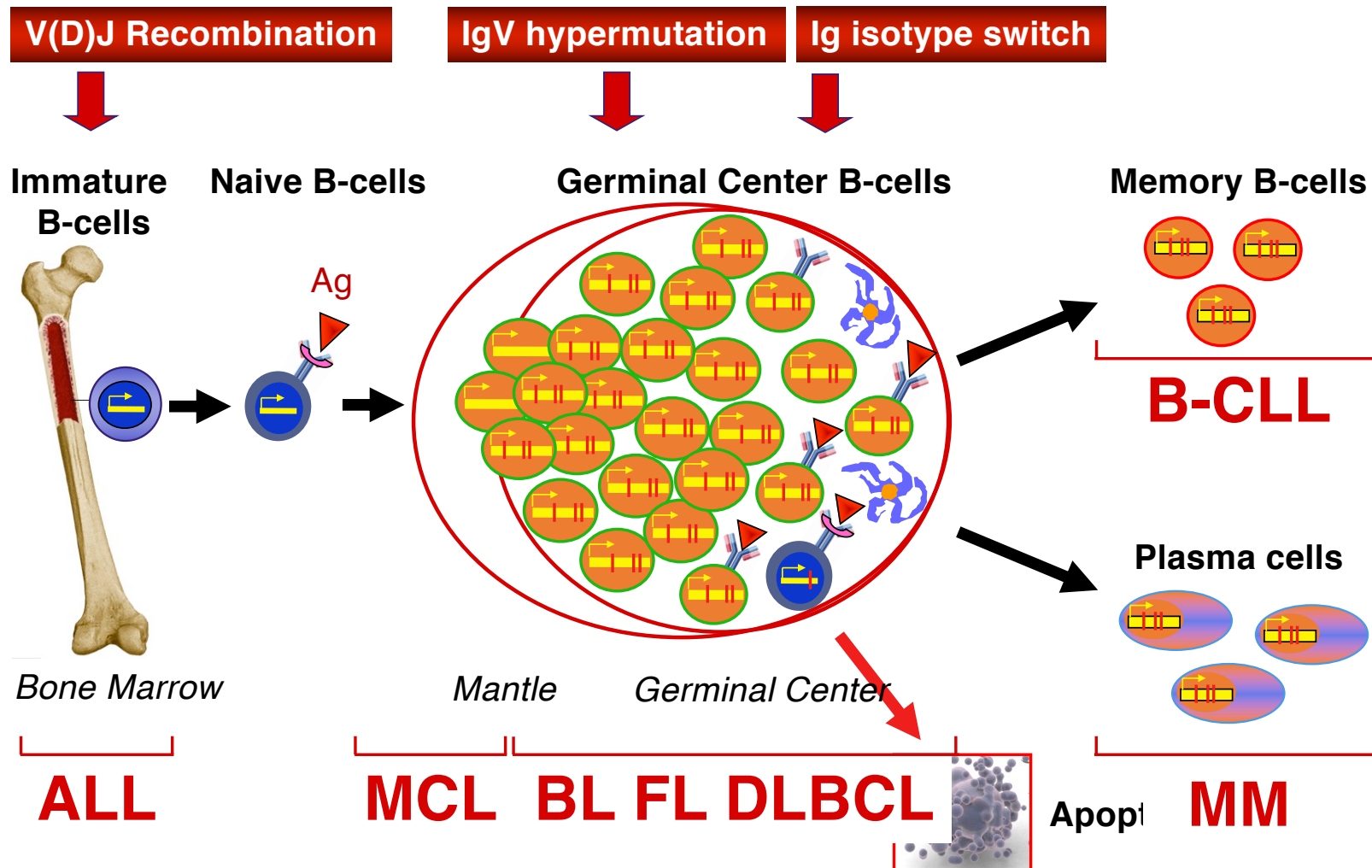
I have the following financial relationships to disclose:

Consultant for: NeoGenomics, Astra Zeneca, DiaTech

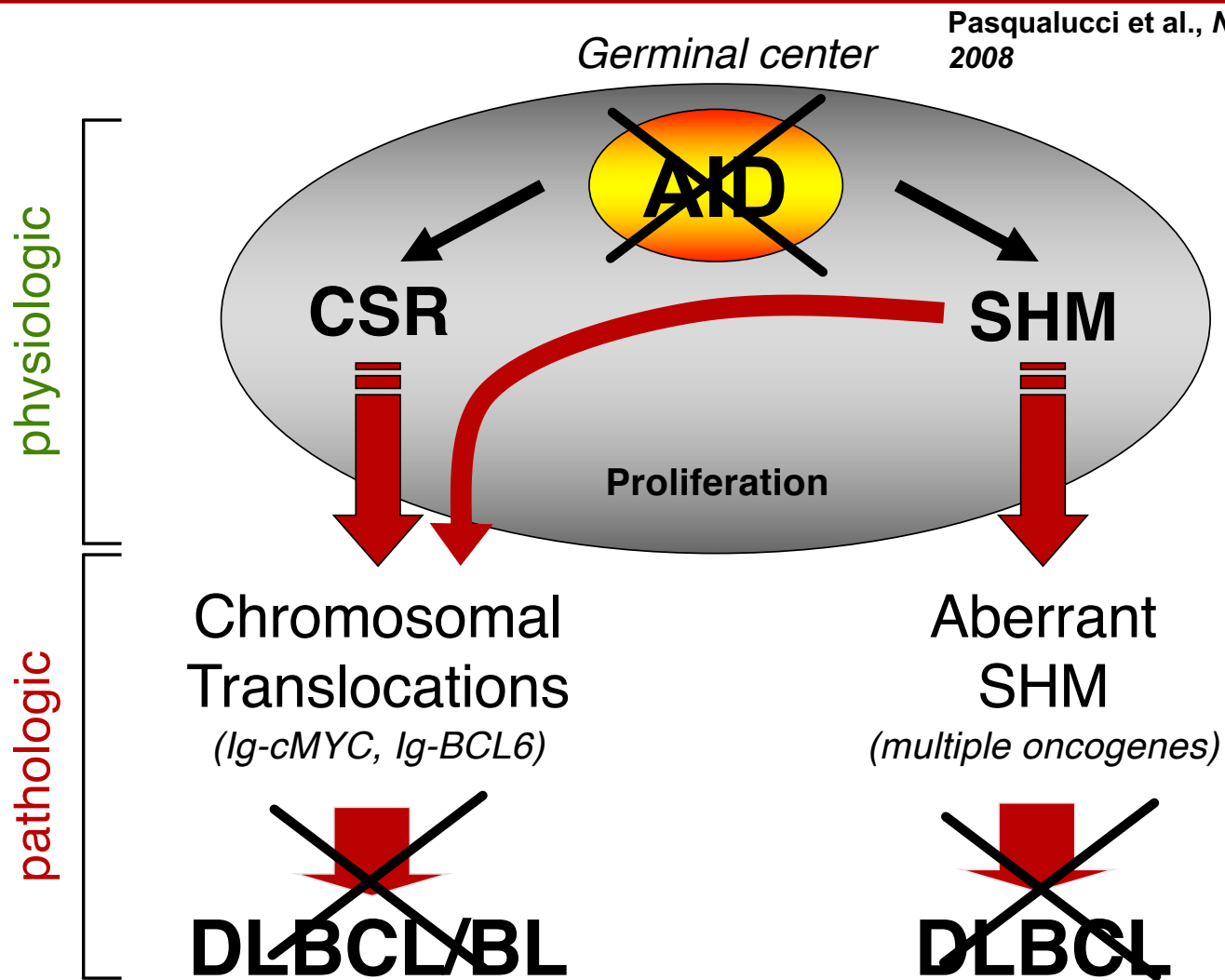
Research support: Astra Zeneca

# Germinal Centers and Lymphomagenesis

Basso & Dalla-Favera, Nat Rev Immunology, 2015



# AID-mediated hypermutation is required for lymphomagenesis



# Common and distinct targets of genetic lesion in DLBCL COO molecular subtypes

## Histone/chromatin modification

Acetyl-transferases (CREBBP, EP300); Methyl-transferases (KMT2D)

## BCL6 deregulated activity

BCL6 translocations, MEF2B mutations

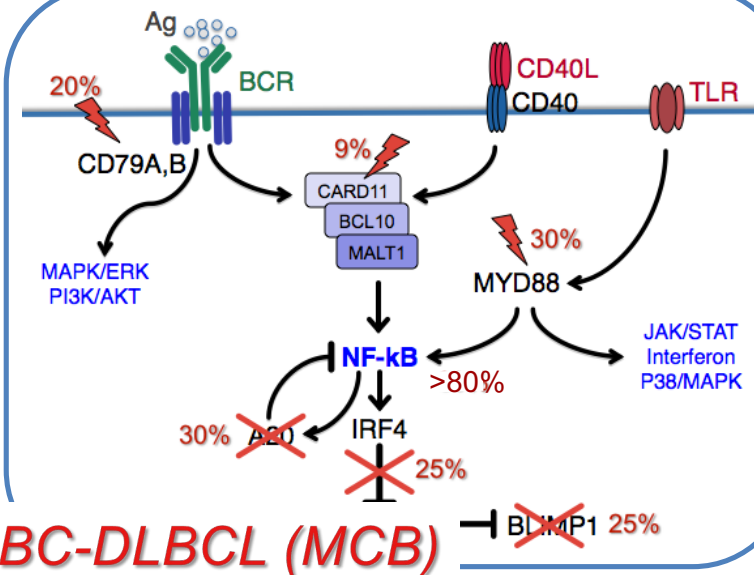
## Escape from immune surveillance (CTL + NK)

B2M mutations, HLA-I loss, CD58 mutations

Shared (EZB, BN2, A53 etc)

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

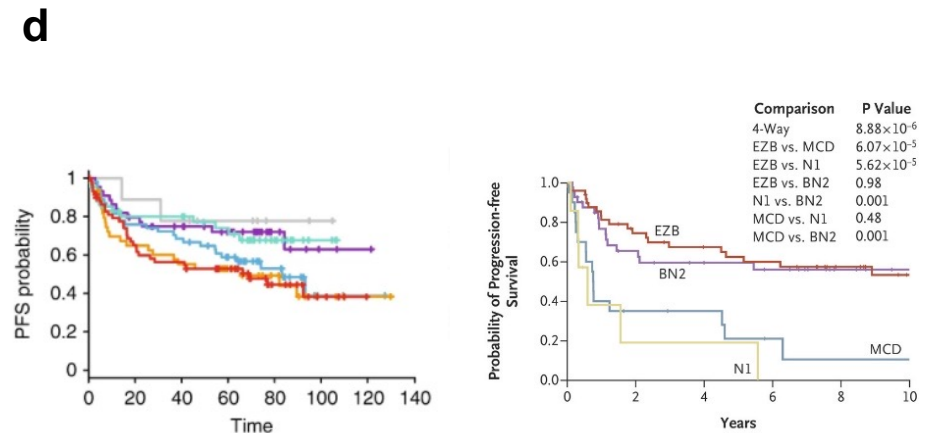
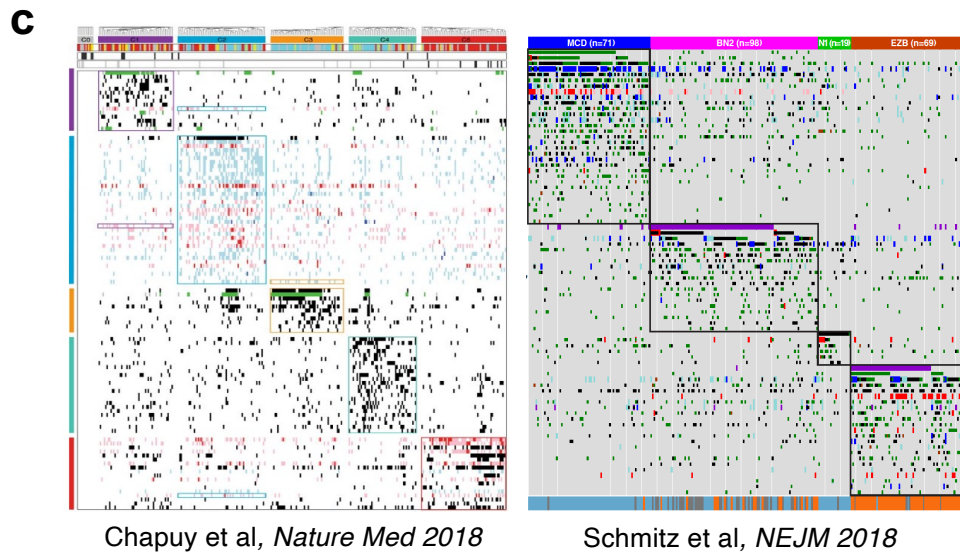
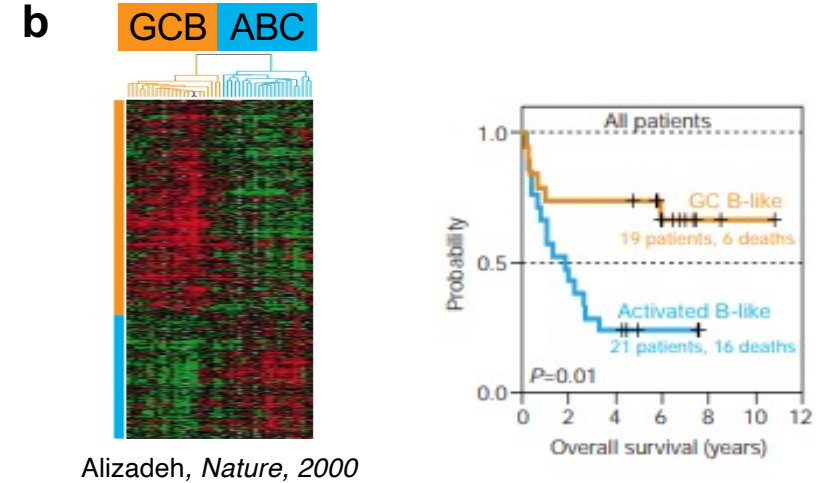
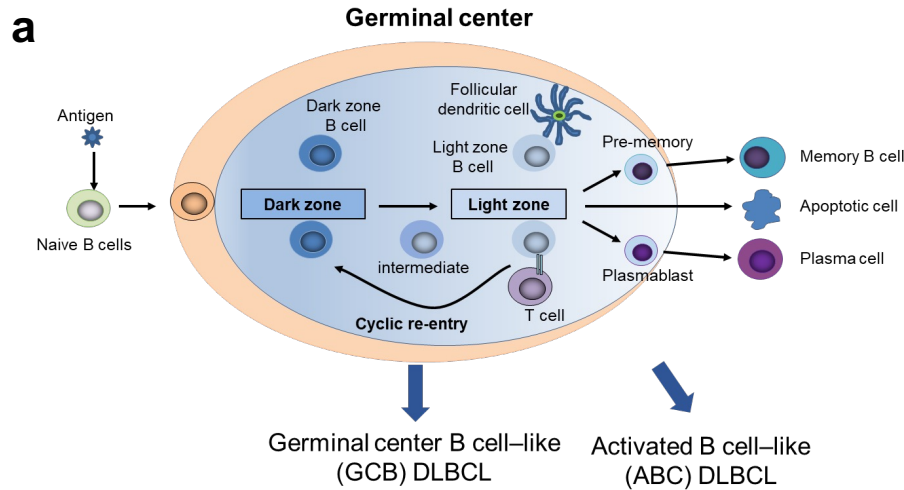
GCB-DLBCL (EZB)



ABC-DLBCL (MCB)

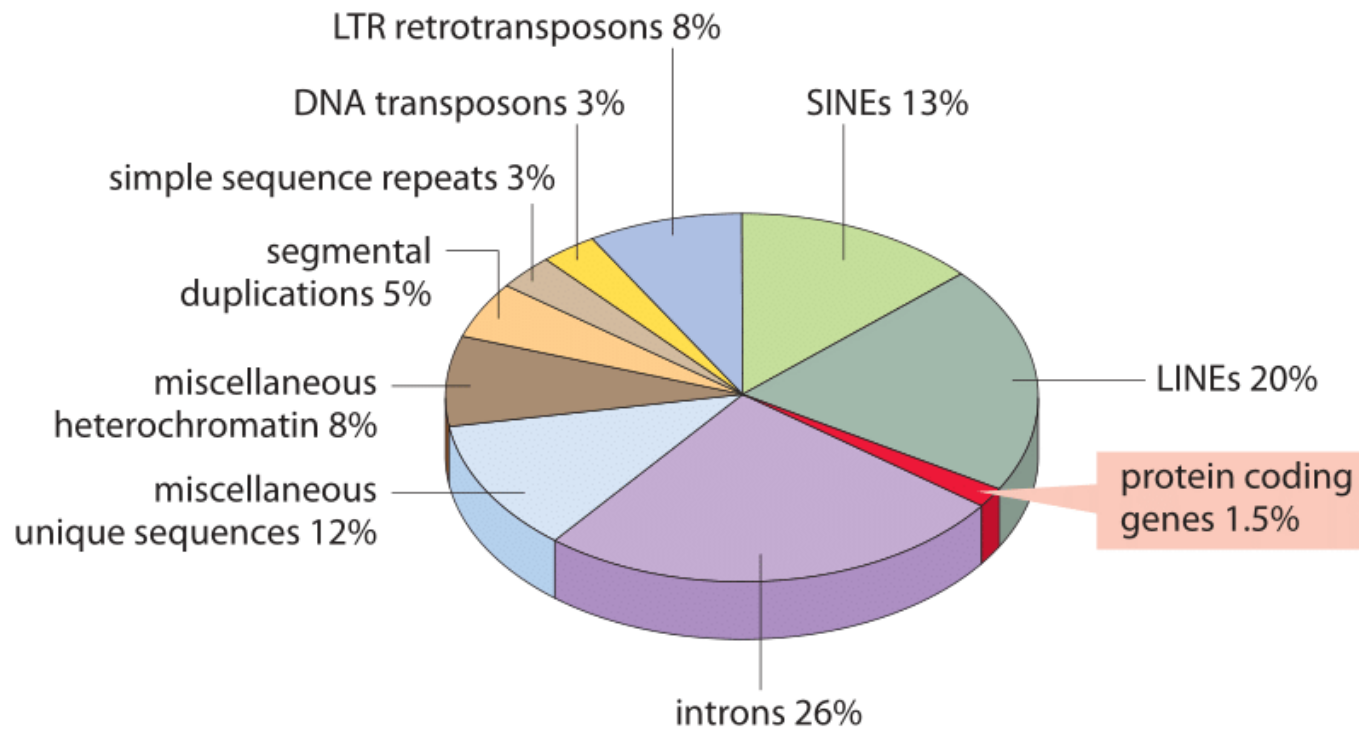
BLIMP1 25%

# Genomic classification of DLBCL



# Protein coding genes represent <2% of the human genome: what about the non-coding genome?

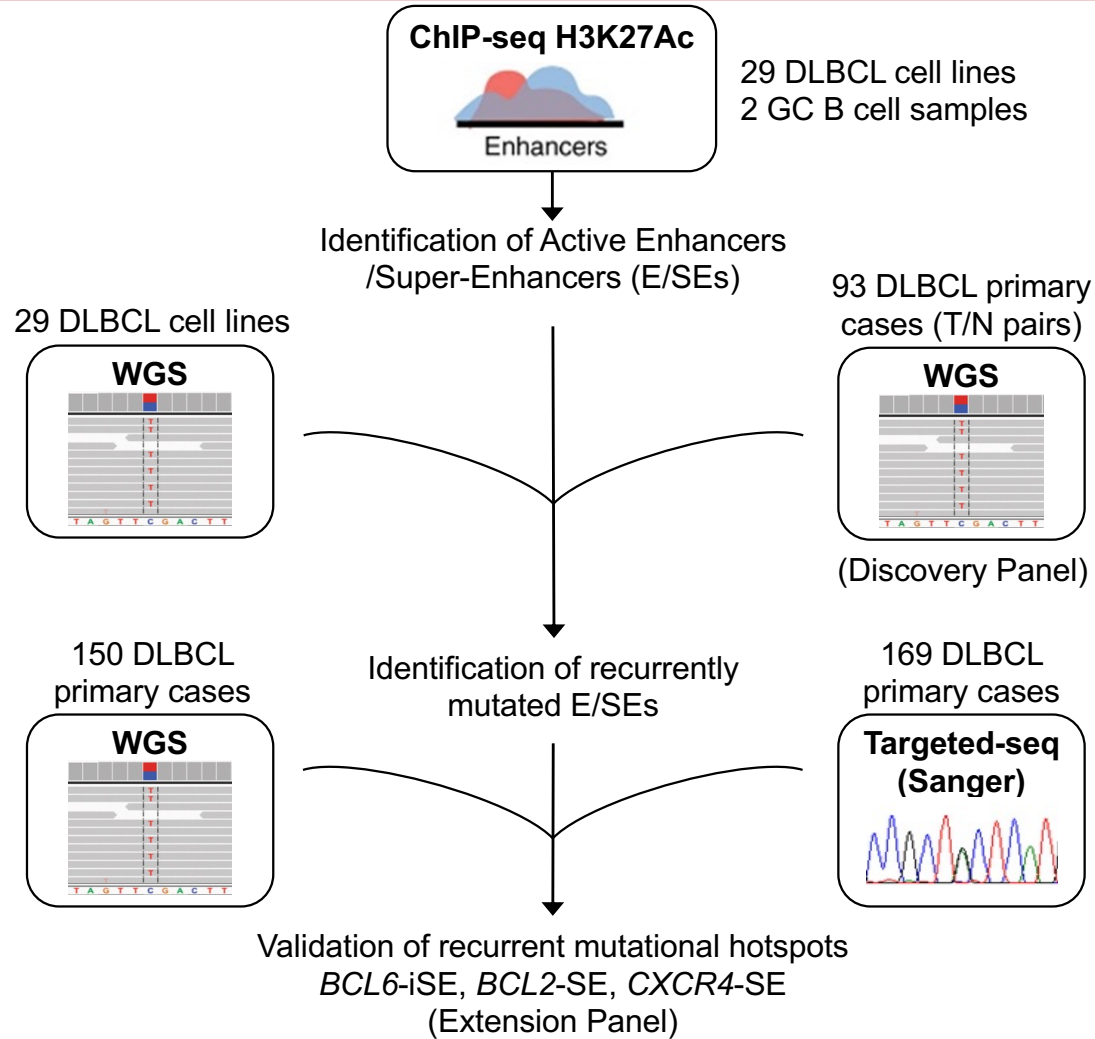
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**Genome-wide analysis of non-coding regulatory mutations in Diffuse Large B-cell Lymphoma**

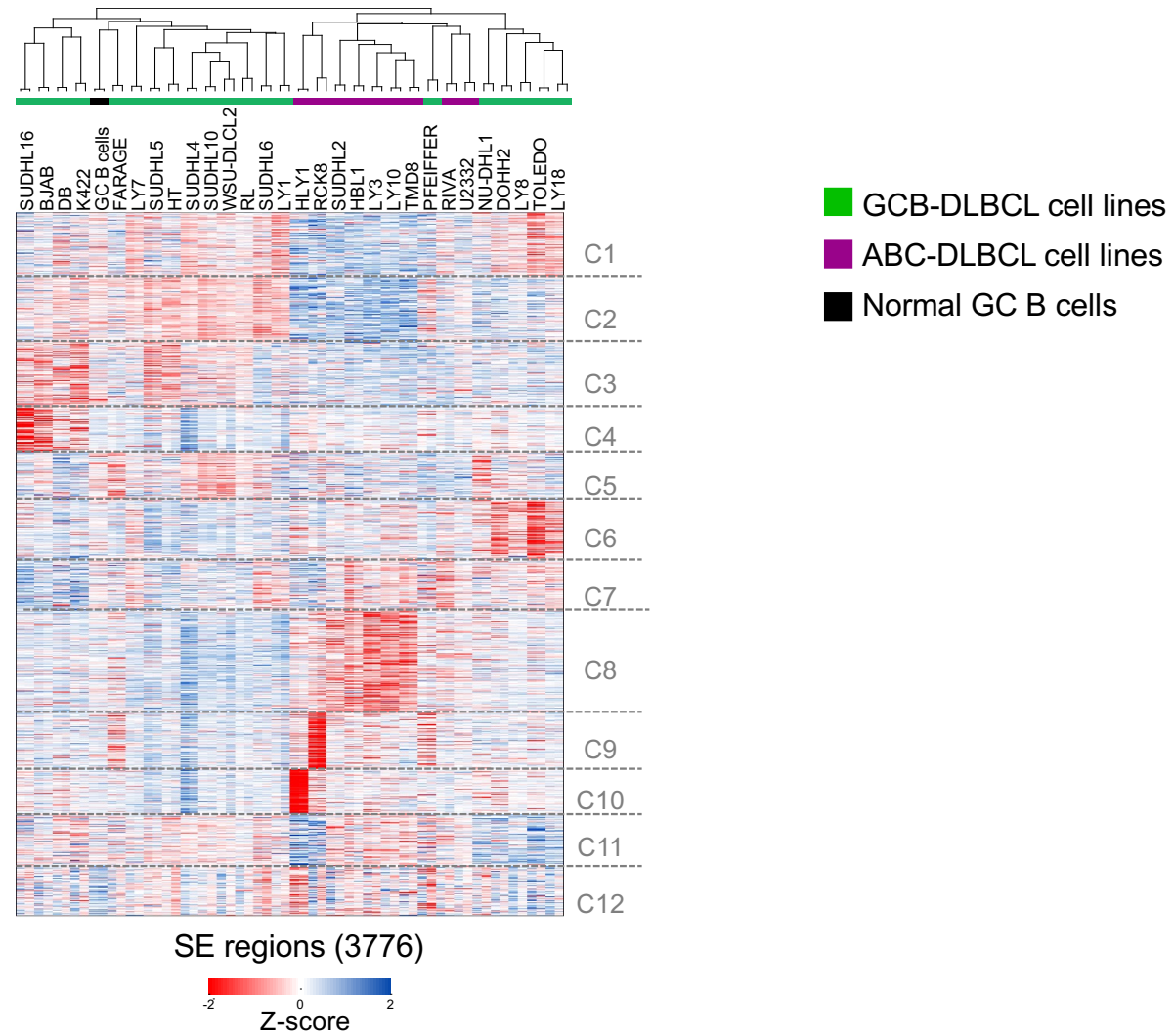


# Identification of functional non-coding mutations (enhancer/super-enhancer)

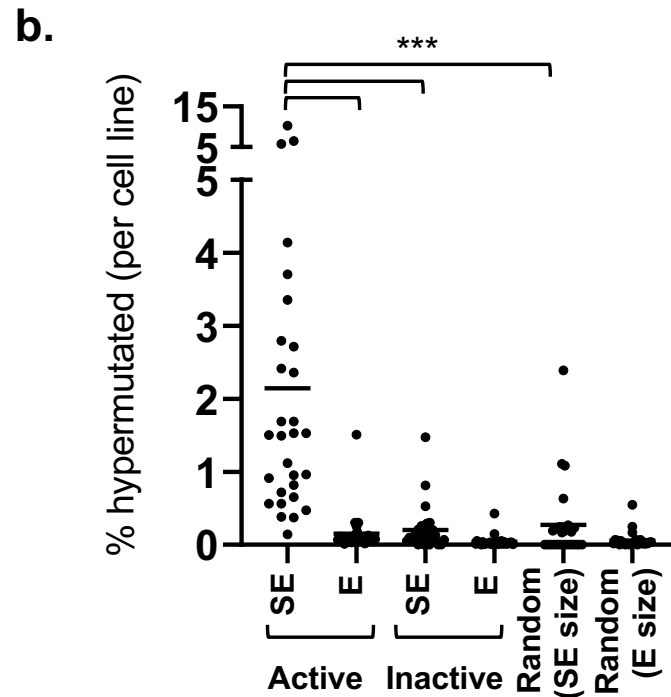
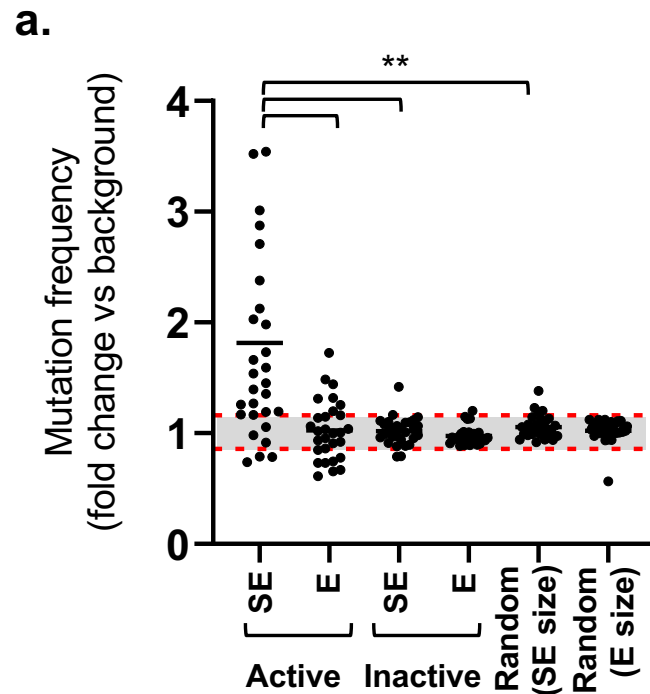


Bal et al., *Nature*, 2022

# Shared and subtype-specific SEs in DLBCL



# SE regions are hypermutated in DLBCL cell lines

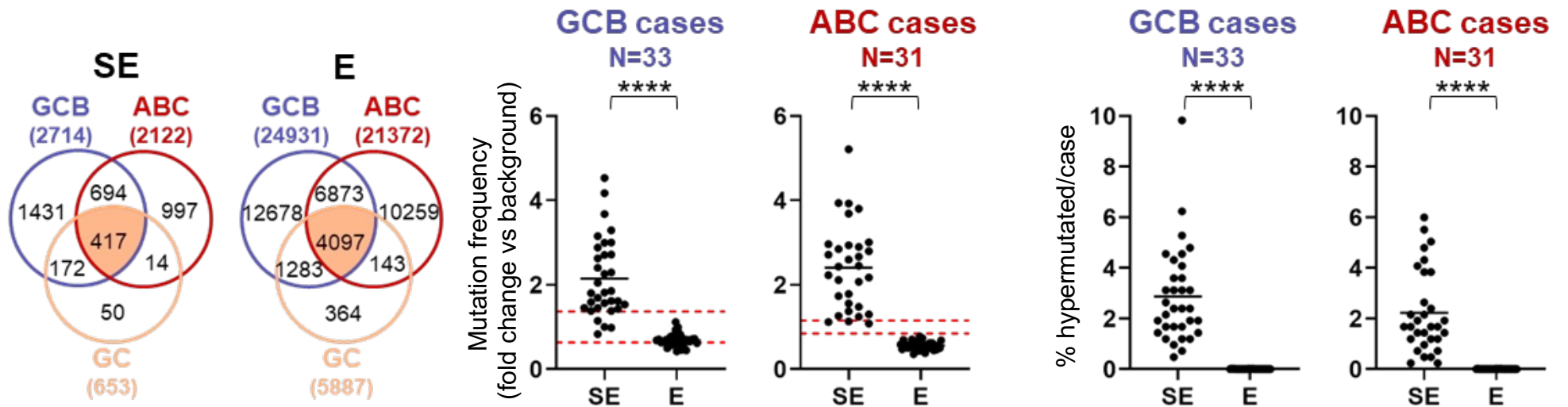


## Hypermutated E/SEs:

- $\geq 3$  mut with intermutation distance  $\leq 1$ kb
- Mutation frequency significantly higher with respect to background (mutations in the rest of the genome)

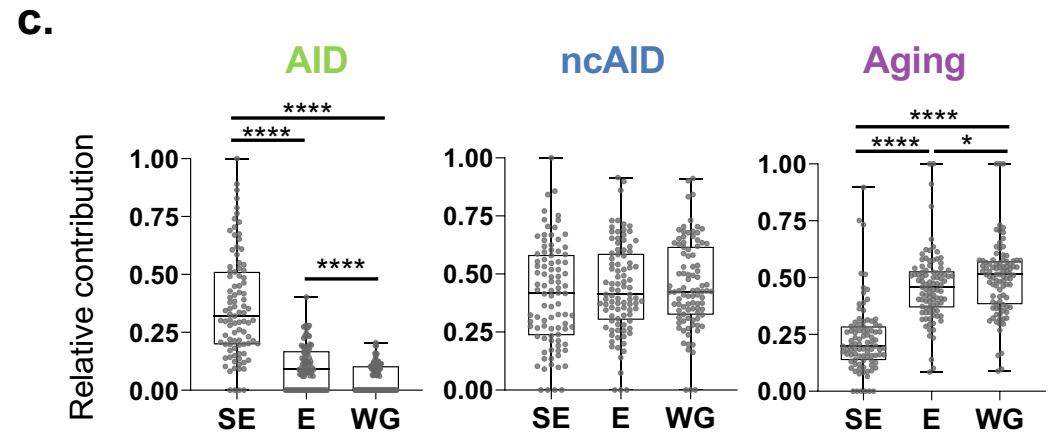
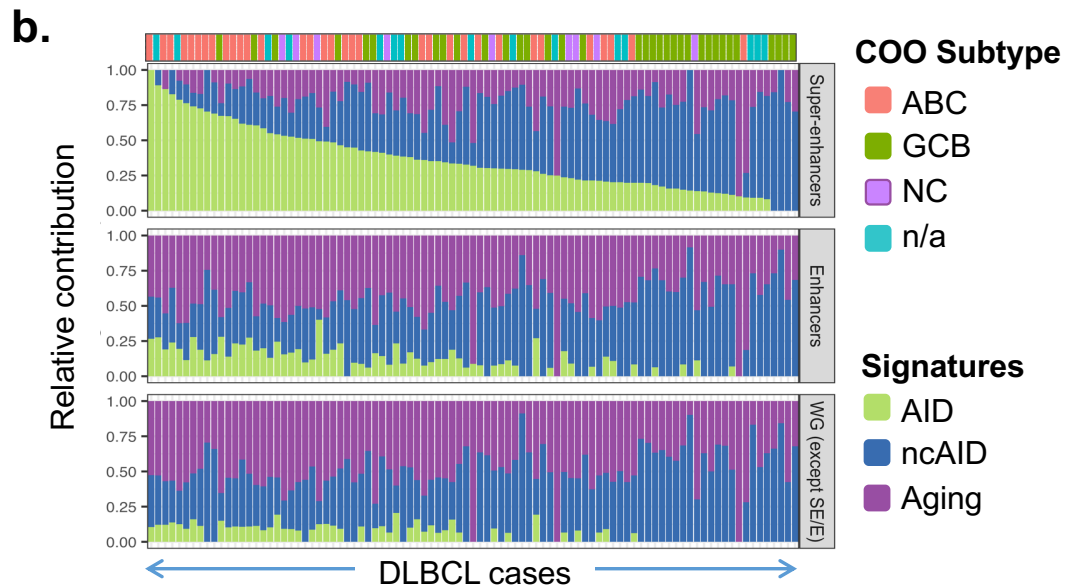
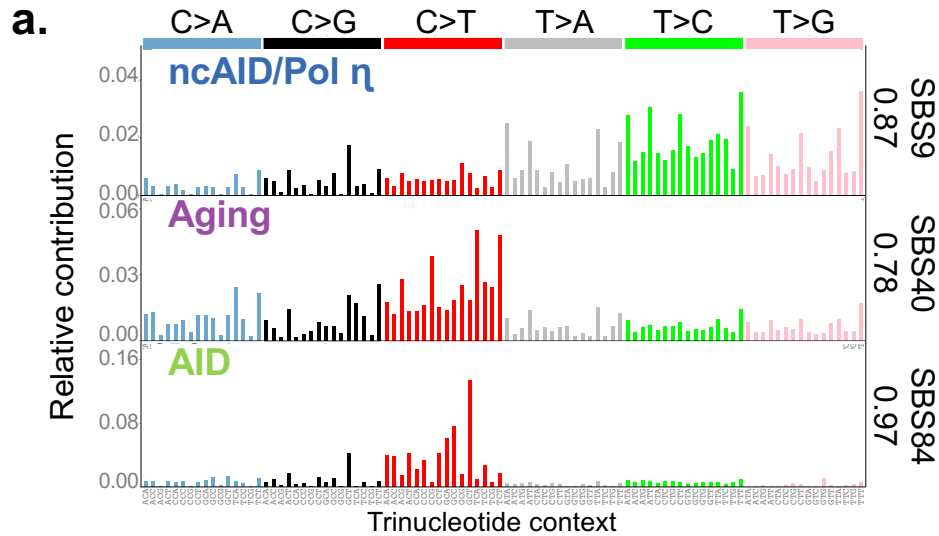
\*\*  $p < 0.01$ , paired t-test  
\*\*\*  $p < 0.001$ , paired t-test

# SEs are hypermutated in DLBCL primary cases

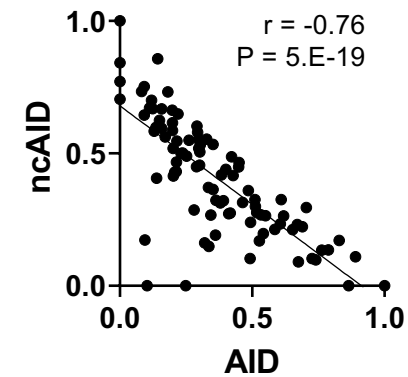


\*\*\*\* p<0.0001, paired t-test

# Mutational signatures in SEs display AID hallmarks



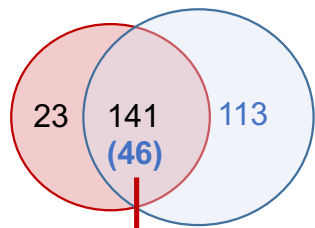
**d.** Inverse contribution of ncAID vs AID signature in SEs



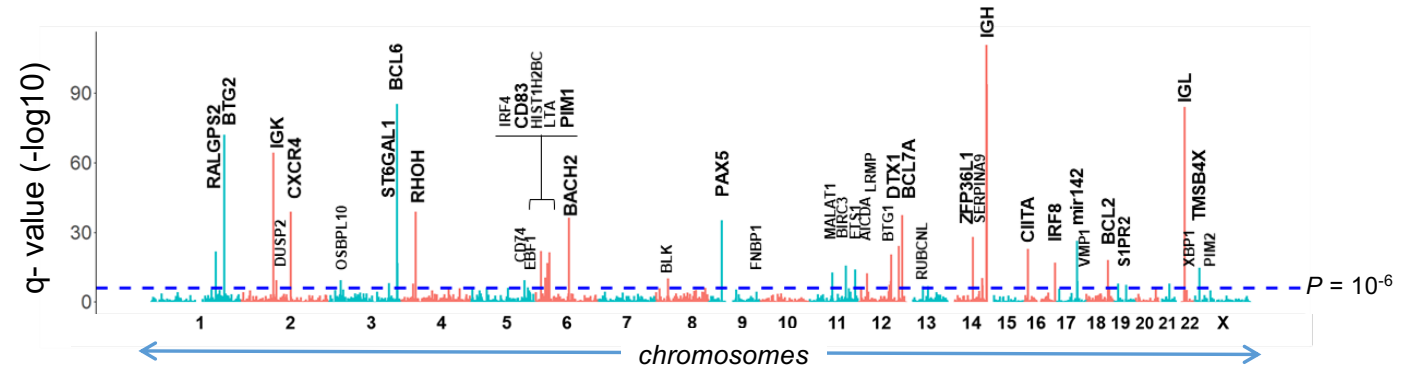
# Candidate genes linked to recurrently mutated SEs are enriched in lymphoma oncogenes

a.

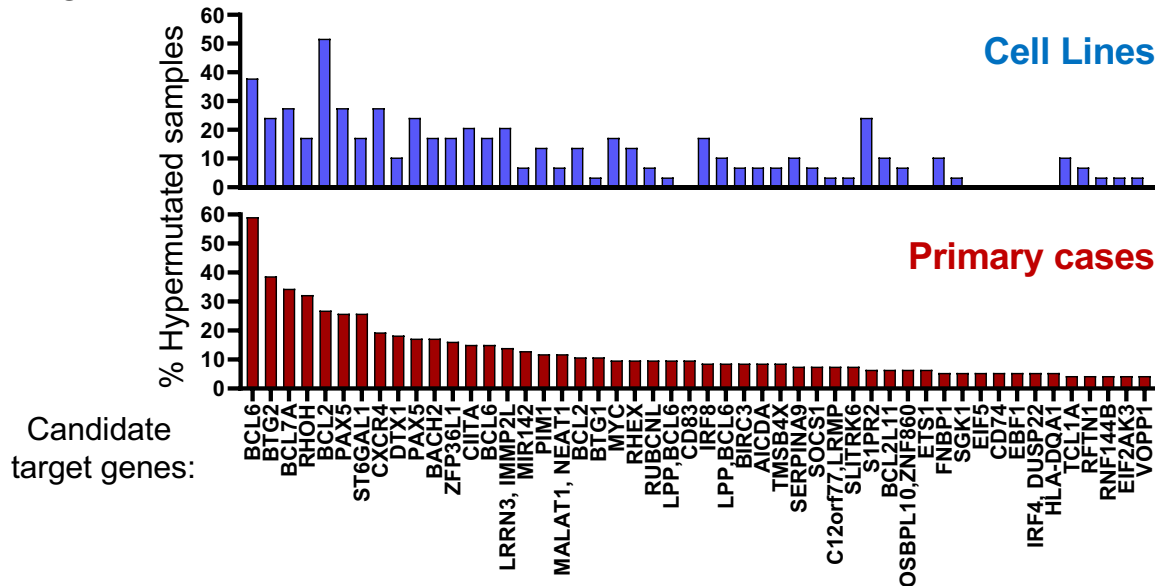
FishHook-1kb-regions (164)    Hypermutated SE  $\geq 2$  cases (159)



b.



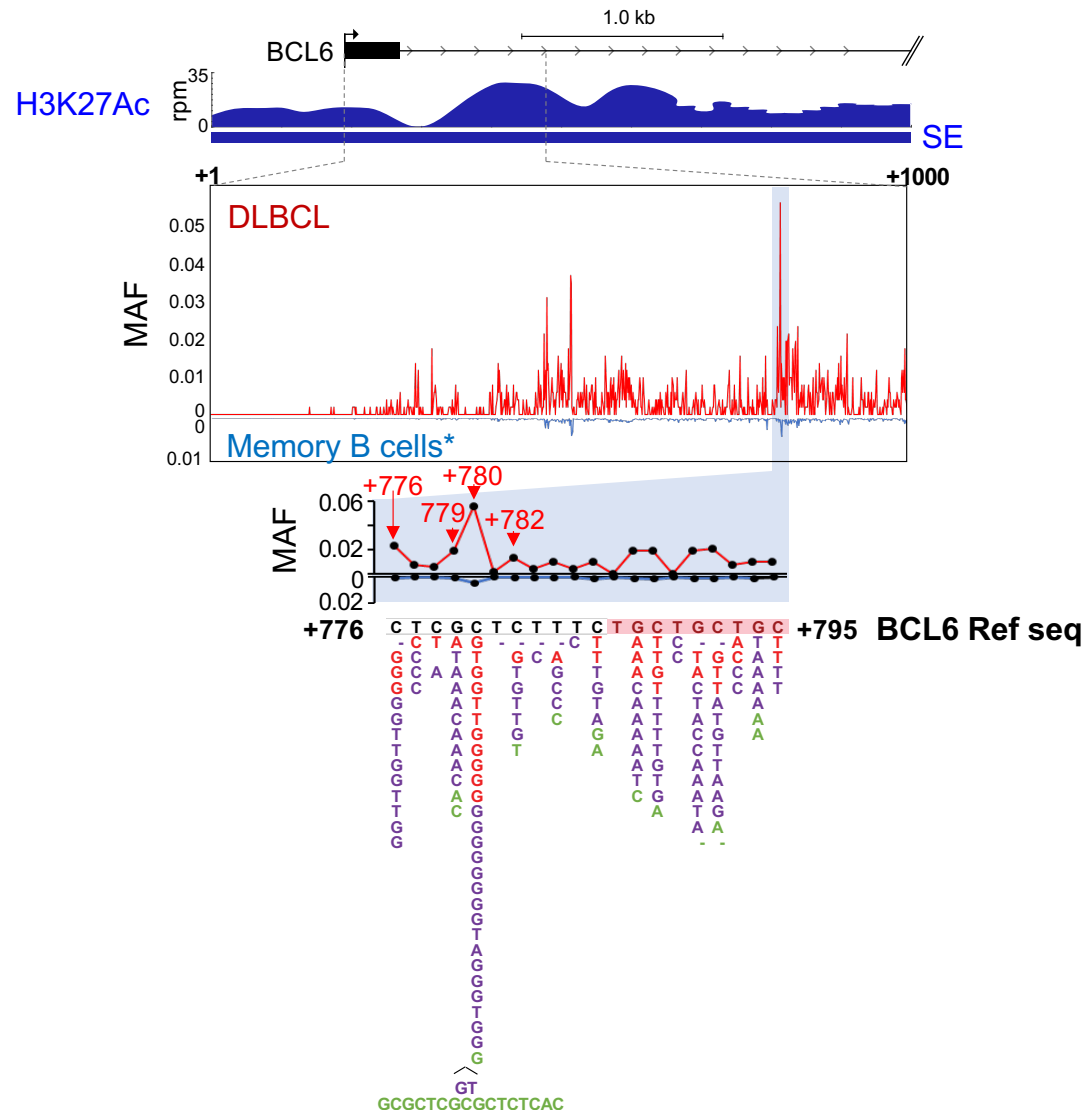
c.



FishHook

(Imielinski et al, Cell 2017)

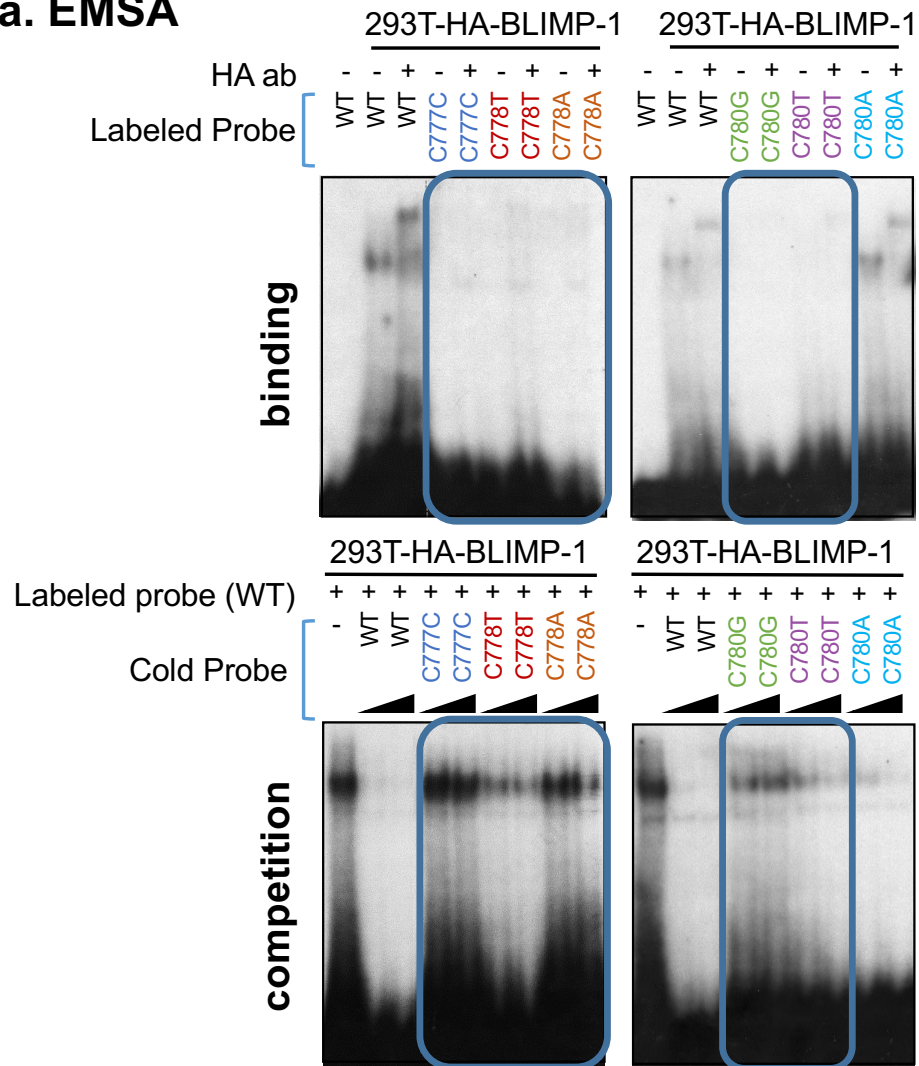
# A highly recurrent mutational hotspot in the BCL6 intragenic SE



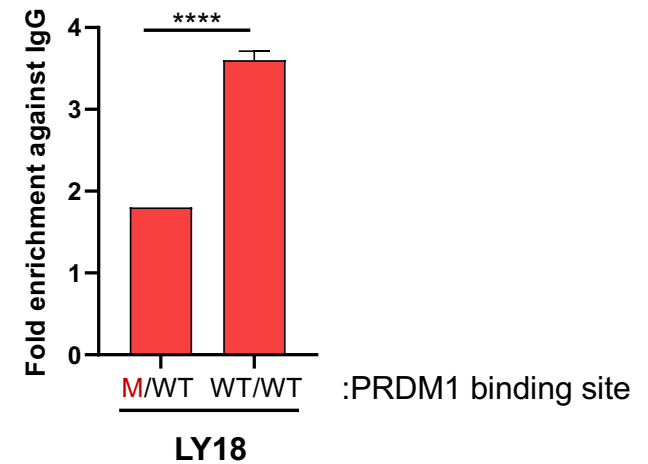
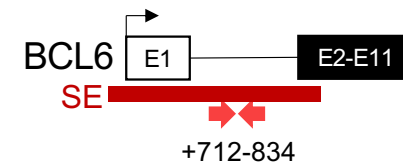
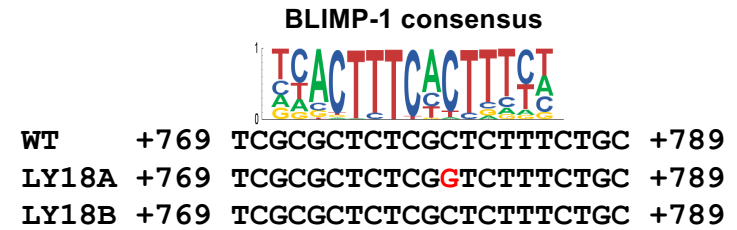
\*Shen JC et al., PNAS, 2019

# Recurrent mutations in BCL6 intragenic SE target BLIMP-1 binding site

## a. EMSA



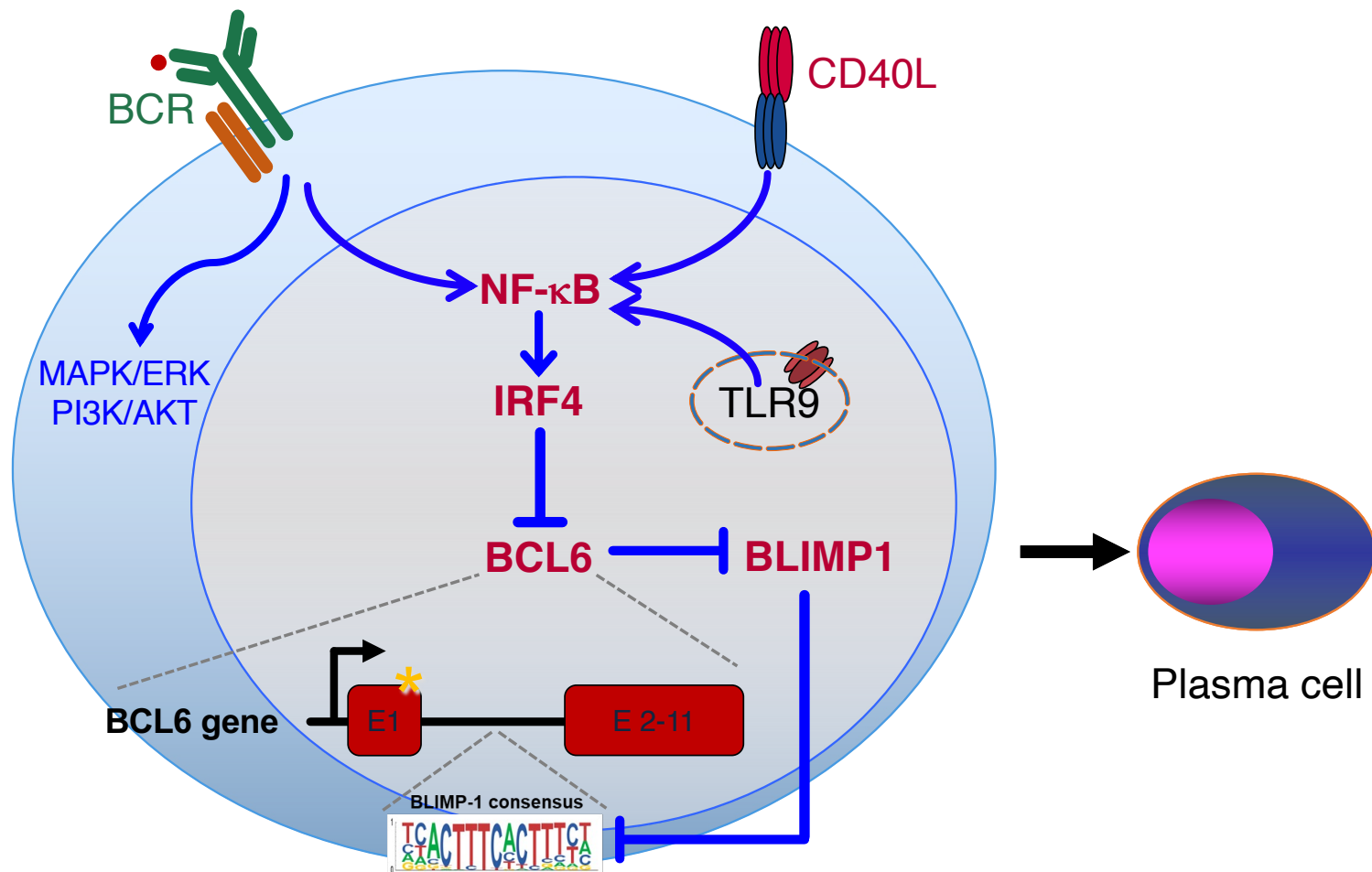
## b. ChIP-qPCR (CD40 stimulated cells)





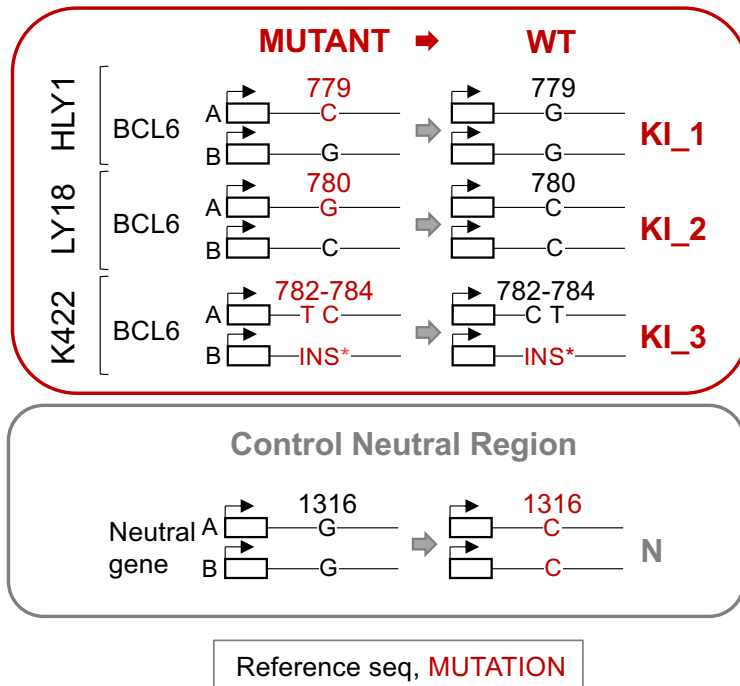
# Downregulation of BCL6 expression is required for the initiation of post-GC differentiation

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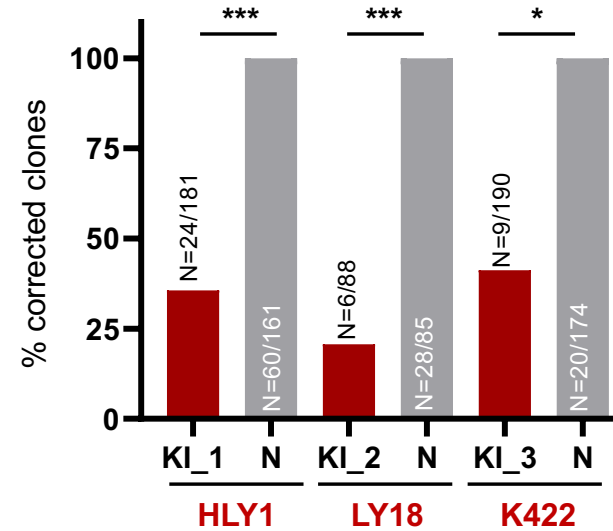


# Correction of mutations in BCL6 SE leads to counter-selection

## a. CRISPR/Cas9-editing of endogenous site



## b. Clones Recovery



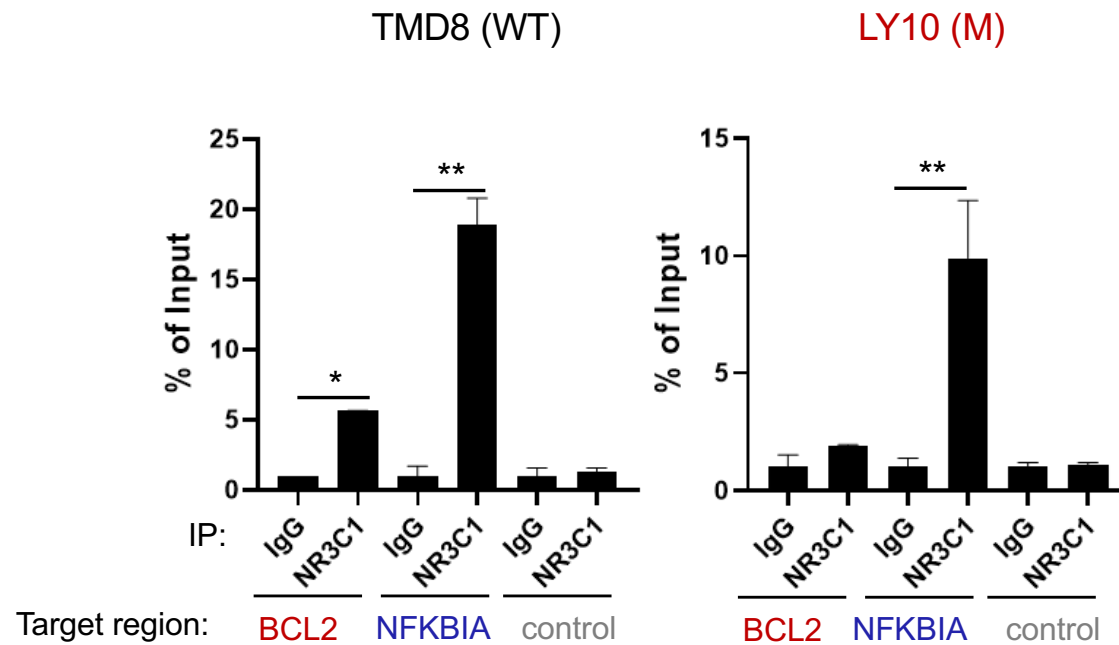
\* Ins 16bp 780\_781



# Mutations in BCL2 intragenic SE abrogate NR3C1 binding in DLBCL cells

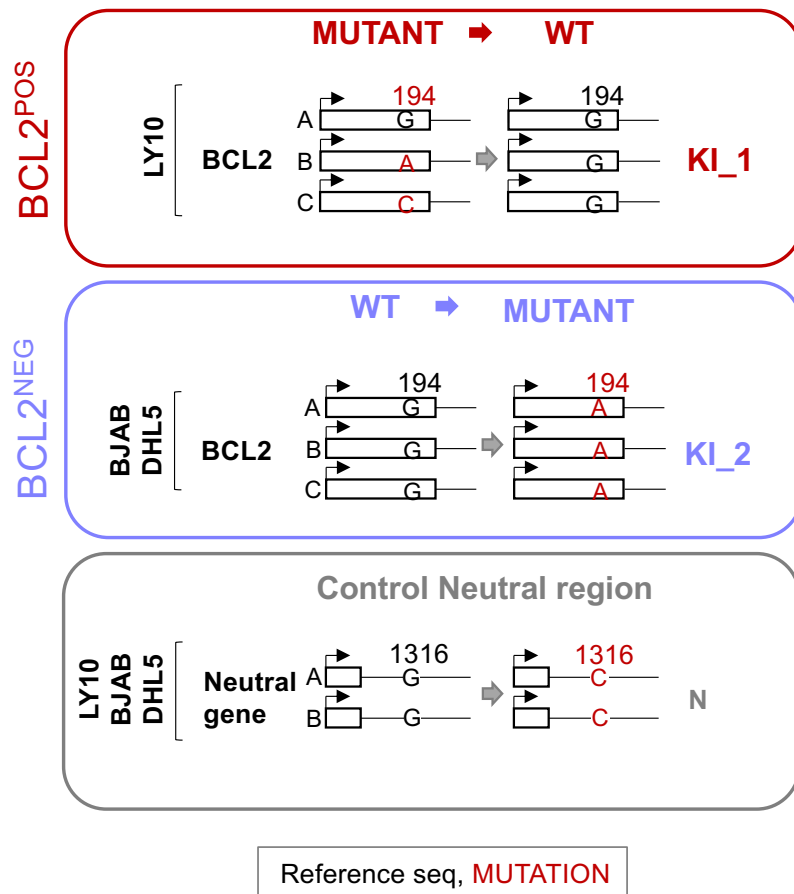
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## NR3C1 ChIP-qPCR

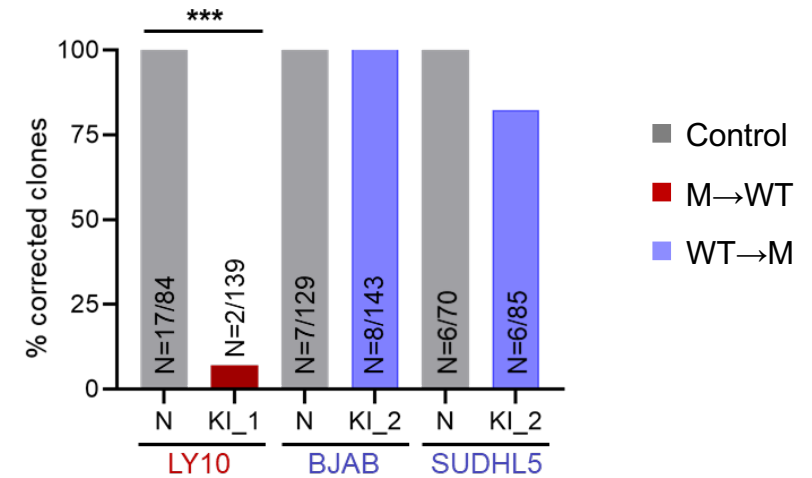


# Correction of mutations in BCL2 iSE leads to counter-selection

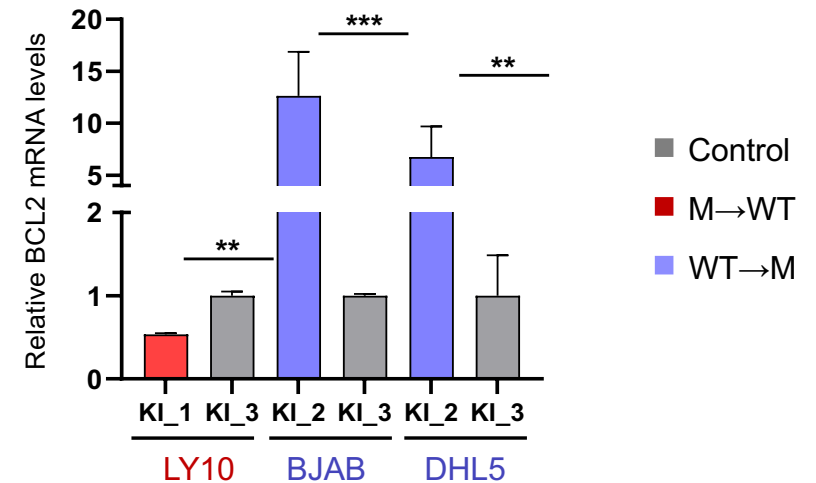
## a. CRISPR/Cas9-editing of endogenous site



## b. Clones Recovery

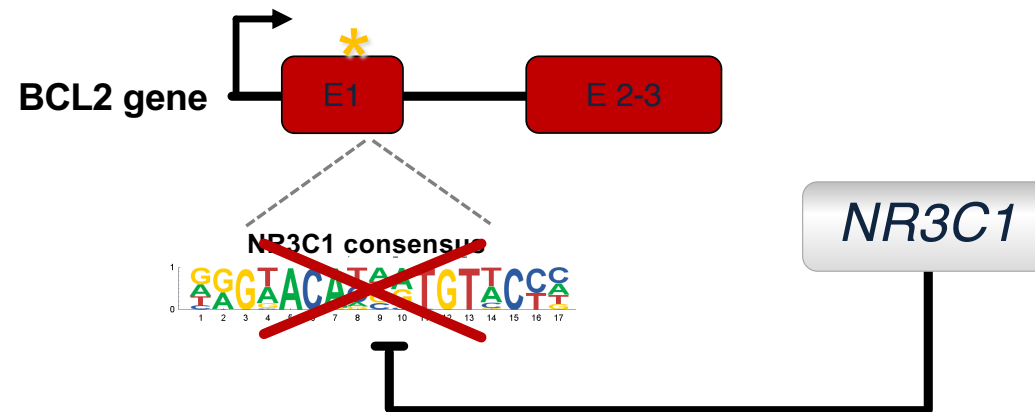
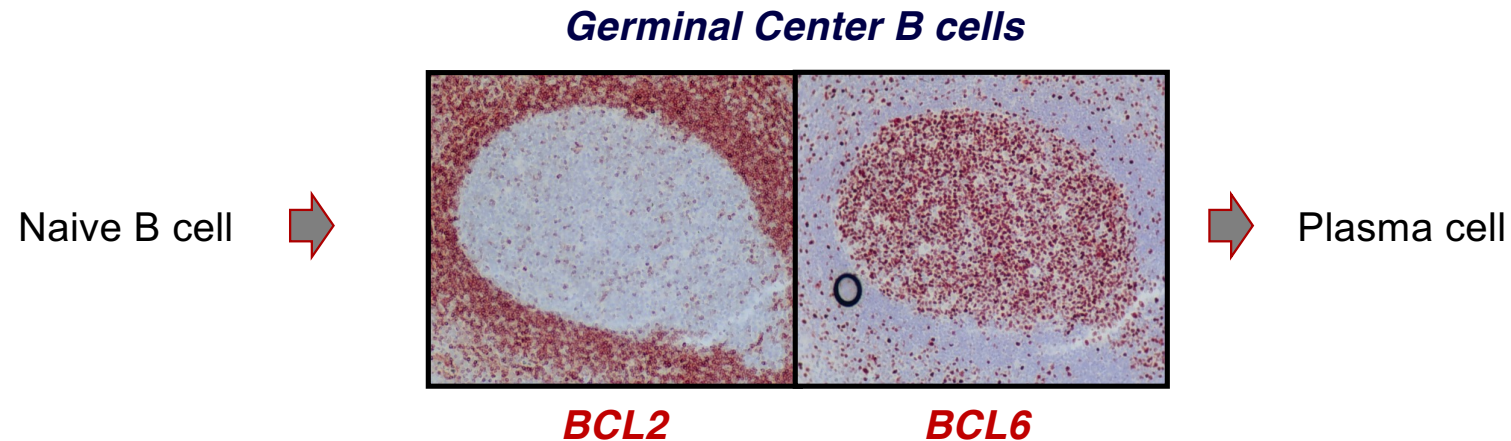


## c. BCL2 mRNA expression

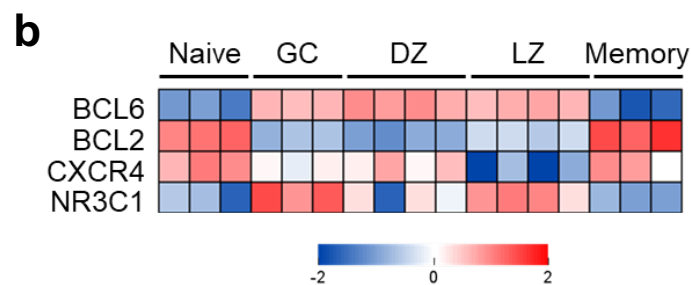
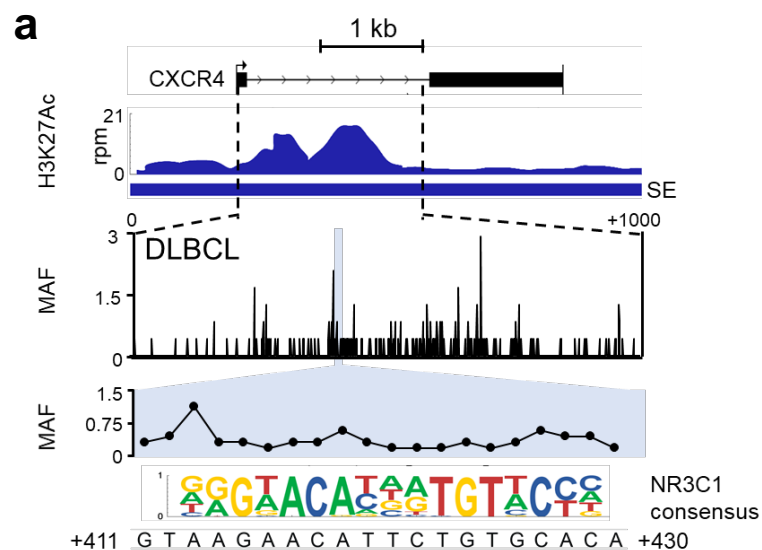


# Multiple genetic lesions contribute to BCL2 deregulation in lymphoma

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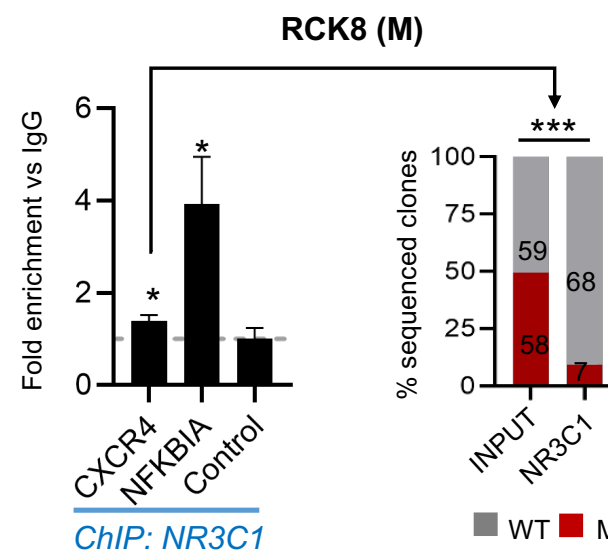
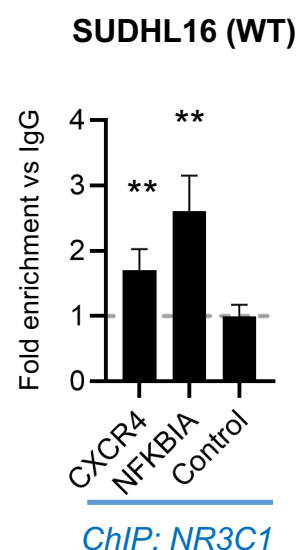
# A mutational hotspot in the CXCR4 SE abrogates DNA-binding and transcriptional activation by the glucocorticoid receptor (NR3C1)



## CXCR4

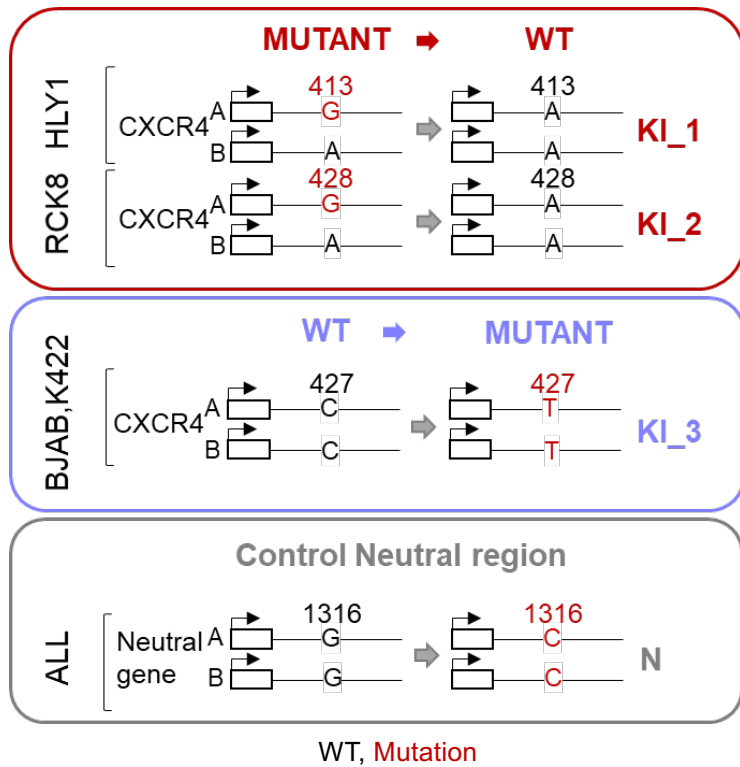
- Transmembrane receptor for the CXCL12/SDF1 chemokine
- Involved in MAPK activation and AKT signaling
- Essential role in cell migration and GC DZ/LZ organization
- Oncogenic truncating mutations in Waldenstroem Macroglobulinemia
- Coding mutations found in few DLBCL cases

**c**

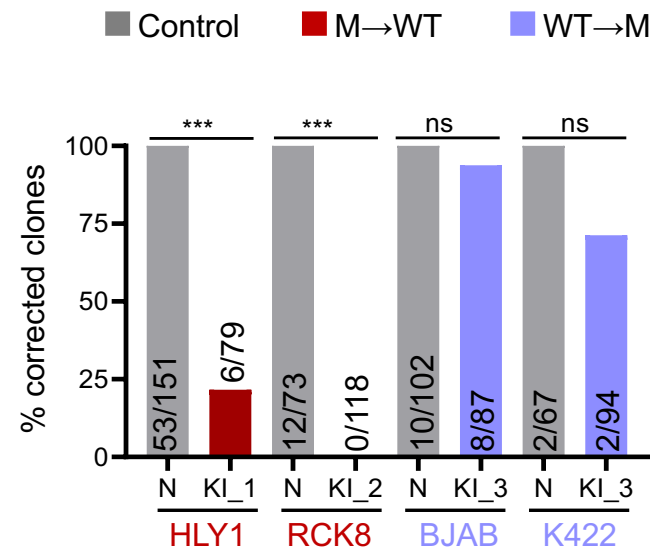


# Correction of mutations in the CXCR4 SE induces counter-selection of lymphoma cells

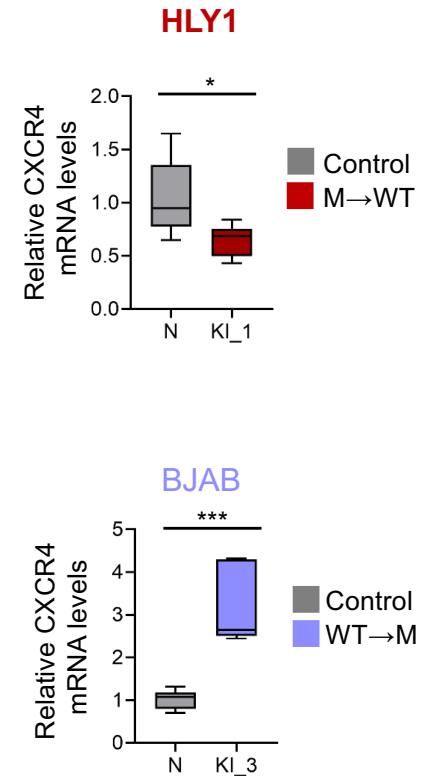
a



b

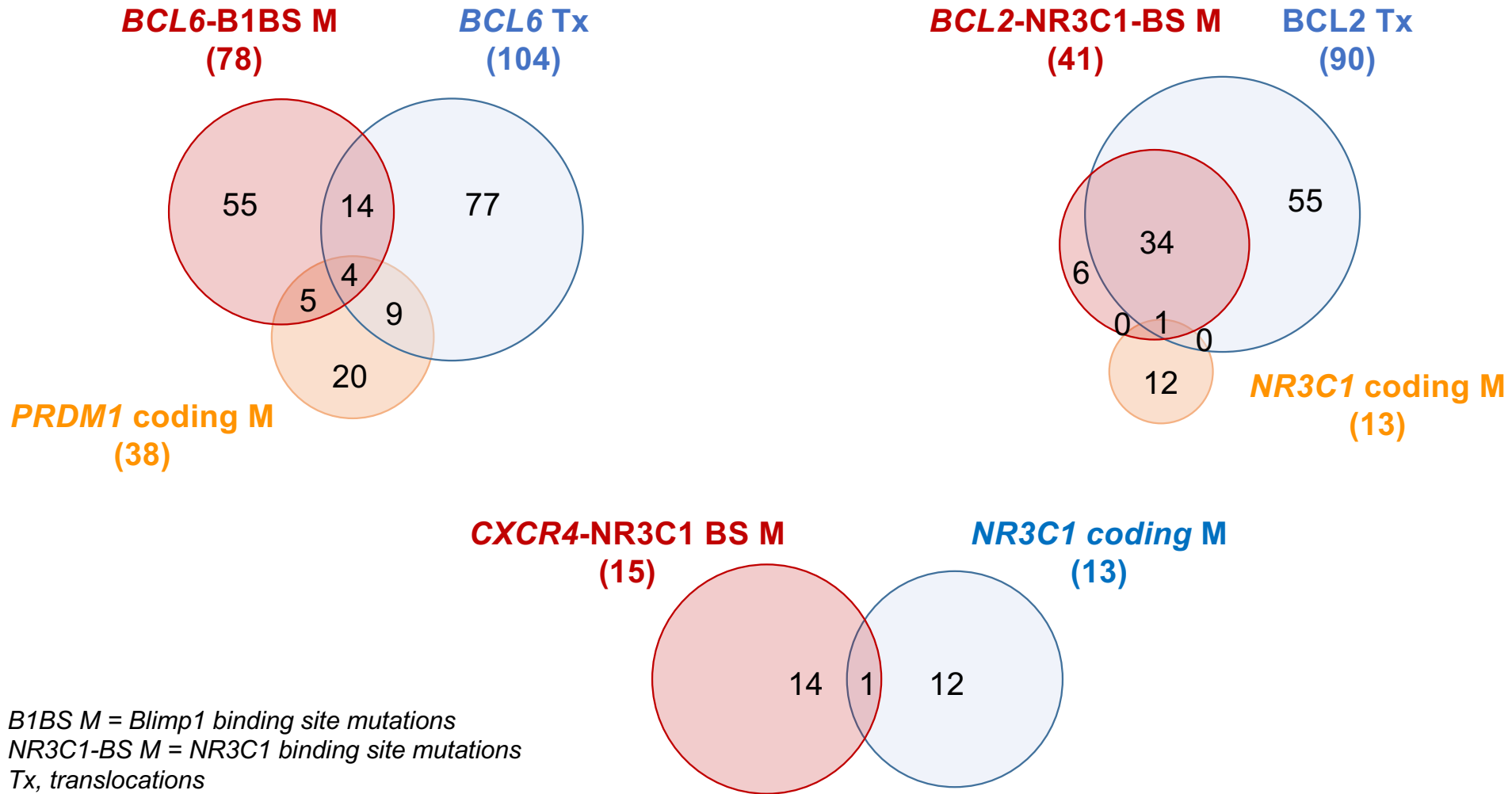


c





# SE-mutations identify complementary mechanisms deregulating target gene expression



# Summary

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- Super-enhancers (SE) are hypermutated in DLBCL
- SE Mutations are caused by AID
- Mutated SE are often linked to proto-oncogenes, potentially leading to their dysregulation
- Recurrent SE mutation hotspots in the BCL6, BCL2 and CXCR4 loci cause their dysregulated expression
- ~80 SE are mutated (3-70 per case) in 93 cases tested, which identify new altered pathways of pathogenetic and clinical relevance

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Andrea Califano, Columbia University, NY  
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# AID-mediated aberrant somatic hypermutation in DLBCL

## Hypermethylation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas

**nature**  
International journal of science

Laura Pasqualucci<sup>\*</sup>, Peter Neumeister<sup>\*</sup>, Tina Goossens<sup>†</sup>,  
Gouri Nandjogud<sup>‡</sup>, R. S. K. Chaganti<sup>‡</sup>, Ralf Küppers<sup>†\*</sup>  
& Riccardo Dalla-Favera<sup>\*</sup>

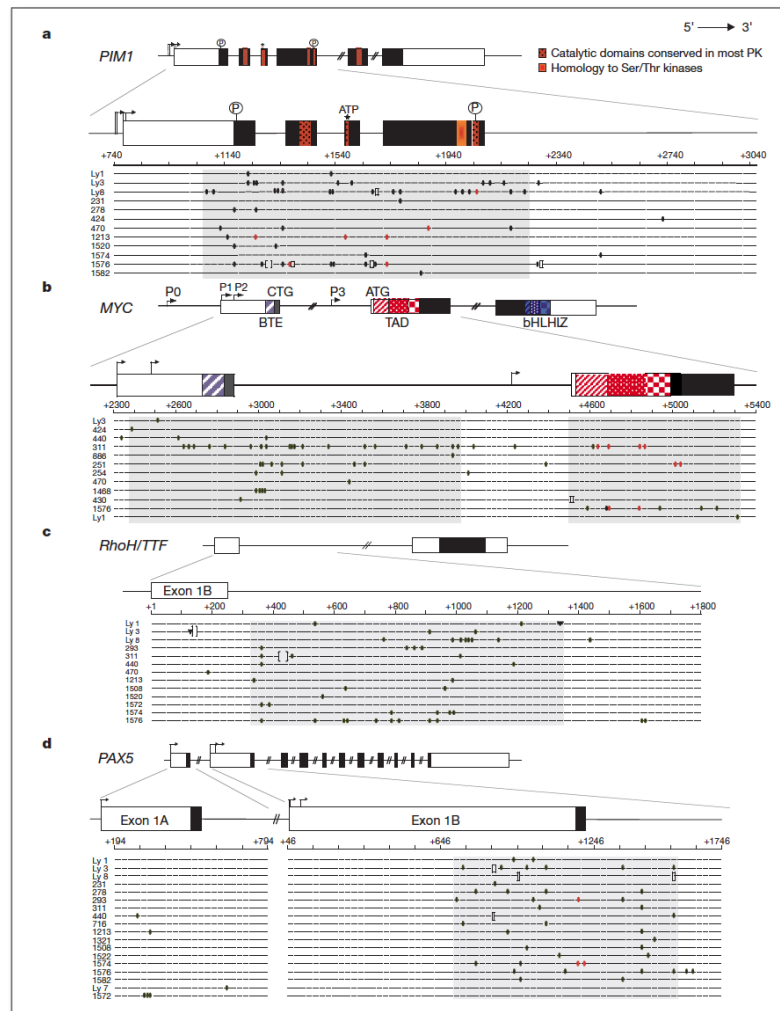
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<sup>‡</sup> Laboratory of Cancer Genetics and the Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, USA

*Nature*, 2001

- ❖ Distribution within ~2Kb from TSS
- ❖ Requires active transcription at target gene
- ❖ Requires AID
- ❖ Overlaps with translocation breakpoint regions
- ❖ Due to malfunction of physiologic SHM mechanism



Pasqualucci et al., *PNAS*, 1998  
Shen et al., *Science*, 1998  
Pasqualucci et al., *Nature*, 2001  
Pasqualucci et al., *Nat. Genetics*, 2008  
Liu et al., *Nature*, 2008  
Khodabakhshi et al., *Oncotarget*, 2012  
Arthur et al., *Nat. Communications* 2018

# Model for the generation of genetic lesions in B-NHL

Pasqualucci et al., *Nature Genetics* 2008

