ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
DIPARTIMENTO DI
SCIENZE MEDICHE E CHIRURGICHE

POLICLINICO DI
SANT'ORSOLA

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Bologna

Aggressive Lymphoma Workshop

Bologna, Royal Hotel Carlton

May 8-9, 2023

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

R. Dalla Favera - Columbia University

President: **Pier Luigi Zinzani**

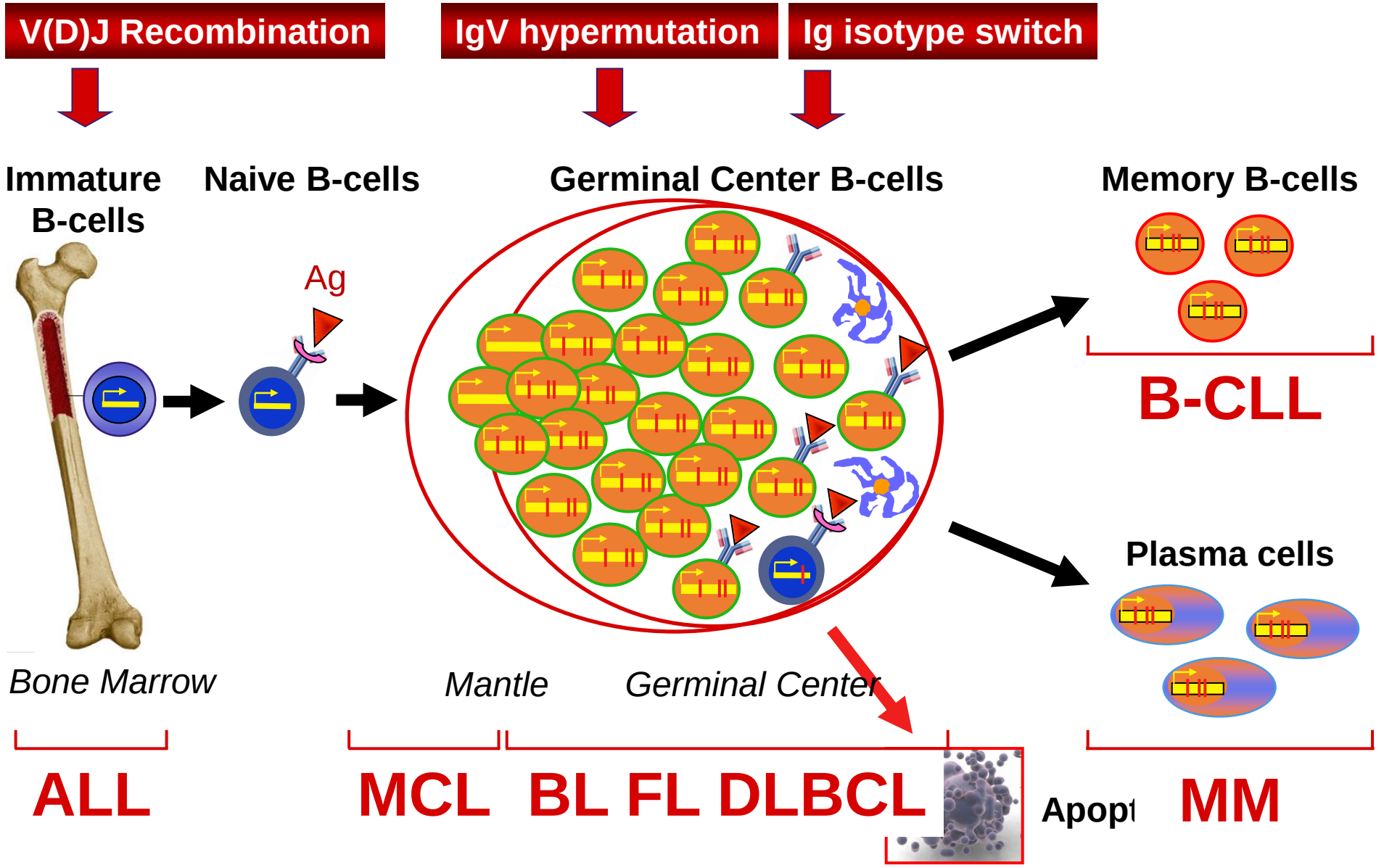
I have the following financial relationships to disclose:

Consultant for: NeoGenomics, Astra Zeneca, DiaTech

Research support: Astra Zeneca

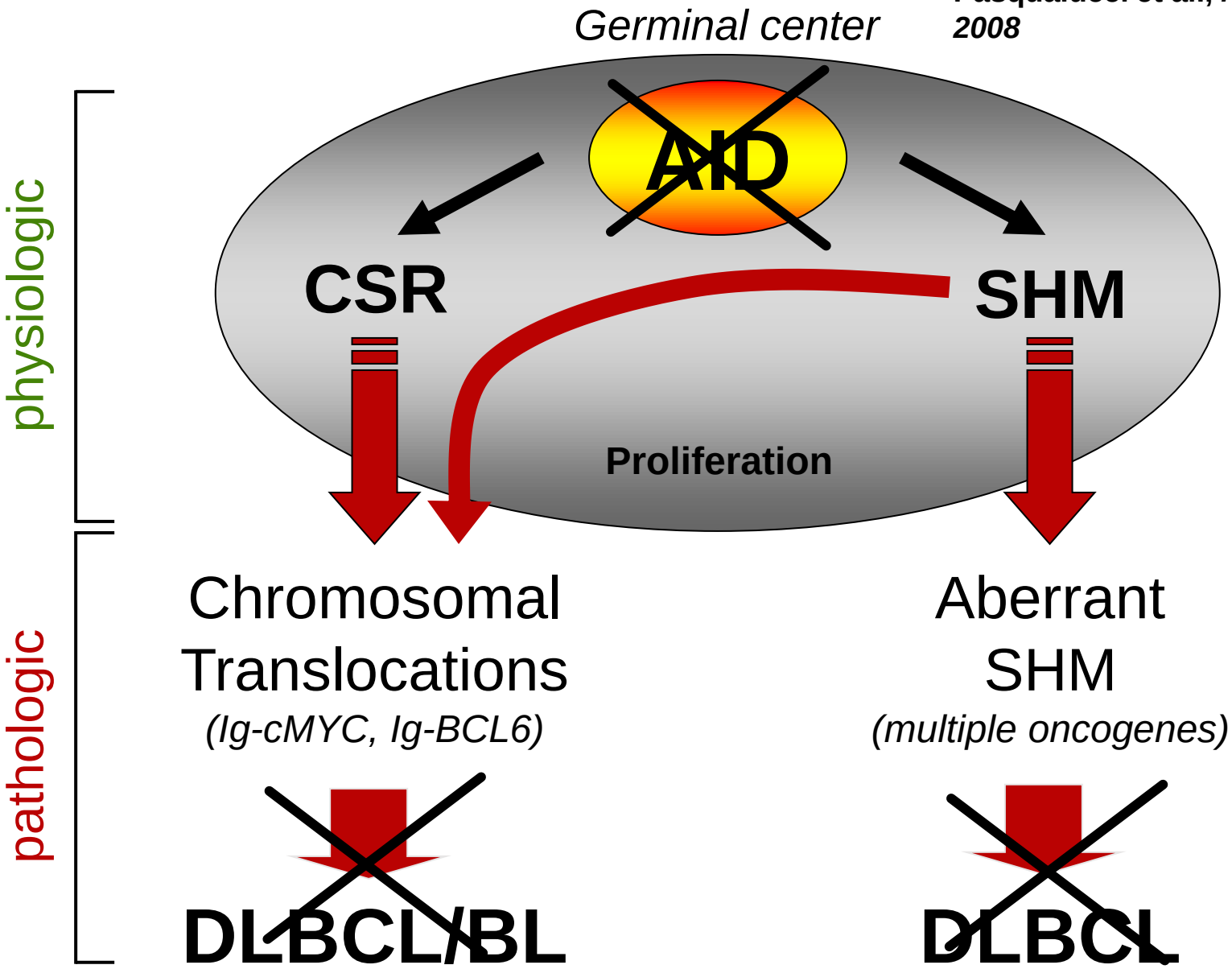
Germinal Centers and Lymphomagenesis

Basso & Dalla-Favera, Nat Rev Immunology, 2015



AID-mediated hypermutation is required for lymphomagenesis

Pasqualucci et al., *Nature Genetics* *2008*



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

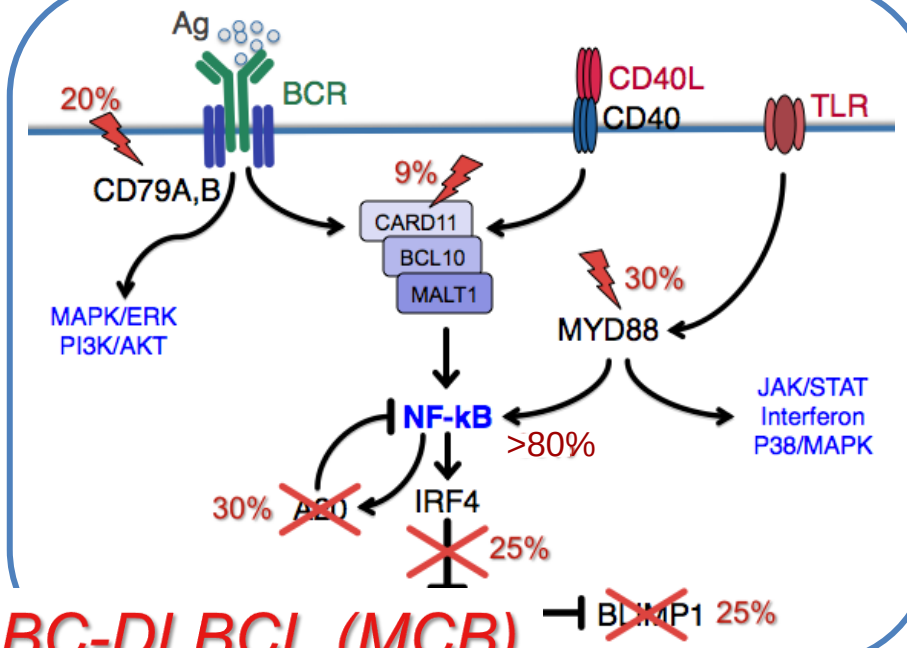
Escape from immune surveillance (CTL + NK)

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%

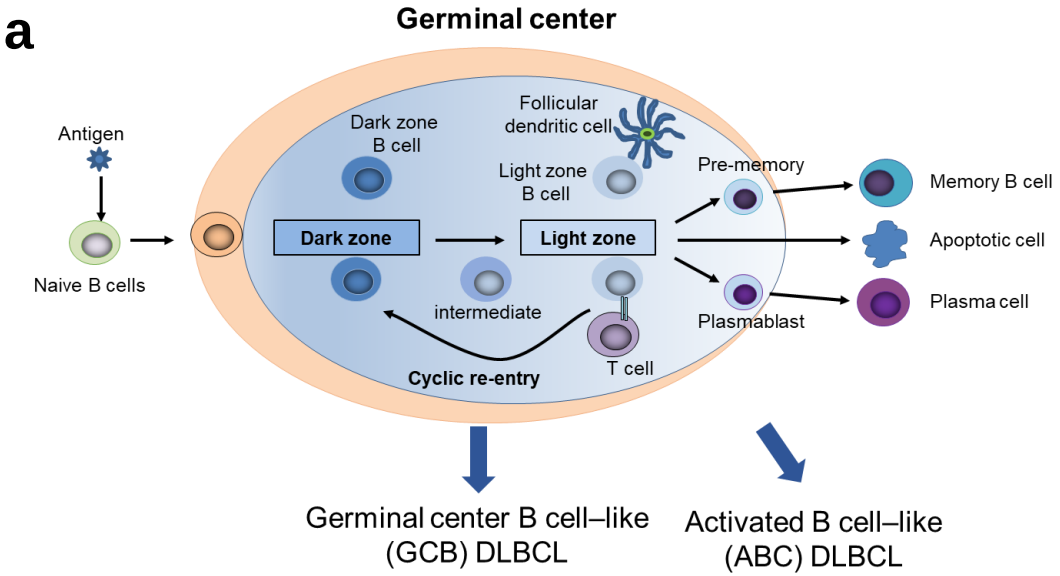
GCB-DLBCL (EZB)



ABC-DLBCL (MCB)

Genomic classification of DLBCL

a



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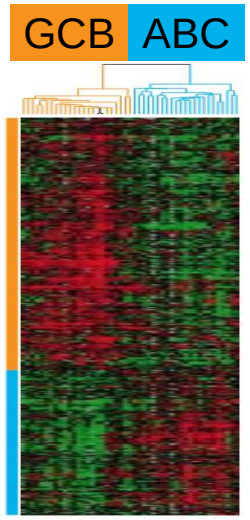
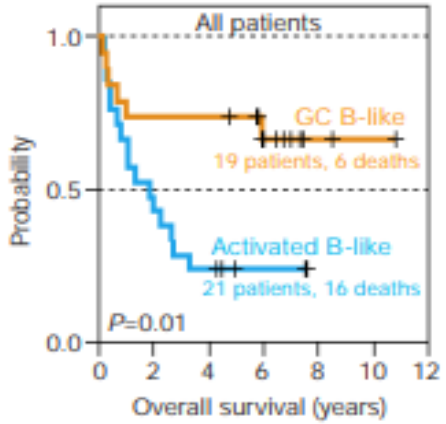


Alizadeh, *Nature, 2000*



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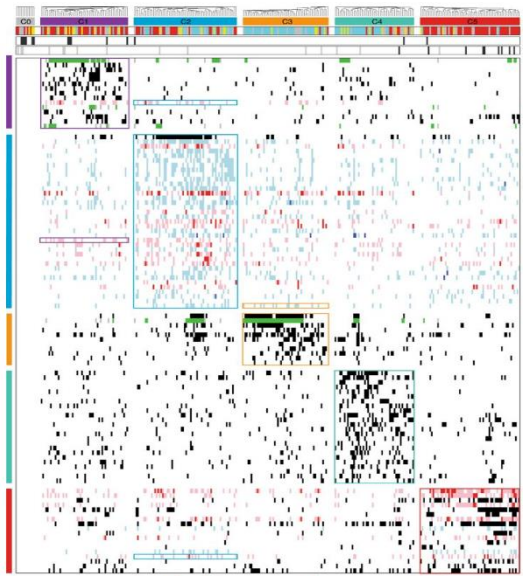
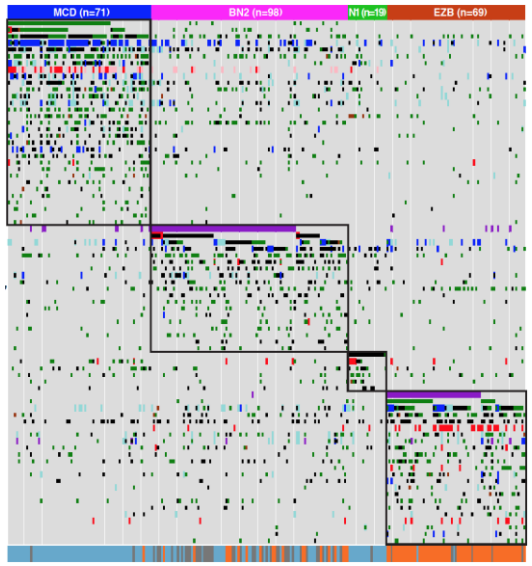
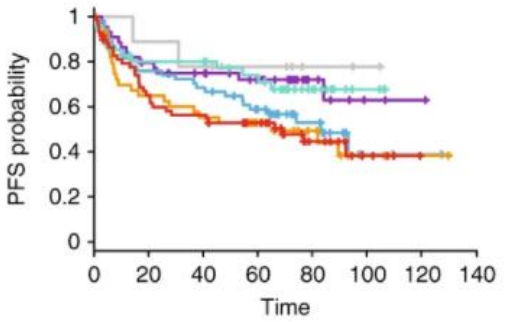


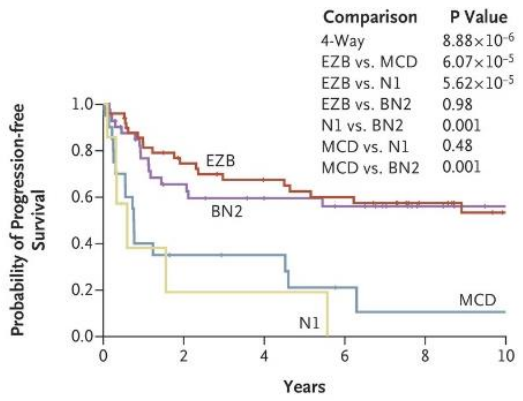
Chapuy et al, *Nature Med 2018*



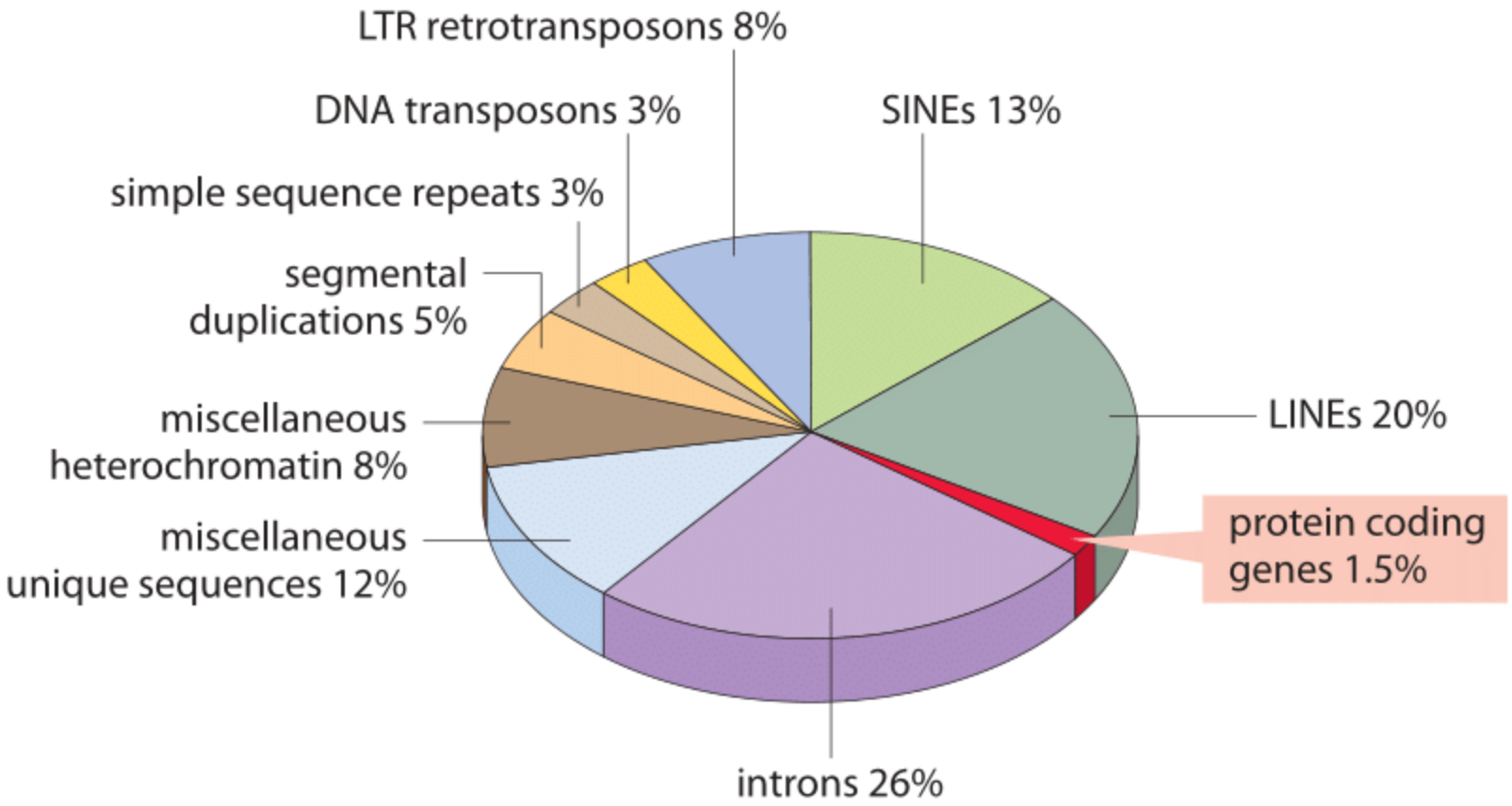
Schmitz et al, *NEJM 2018*

d



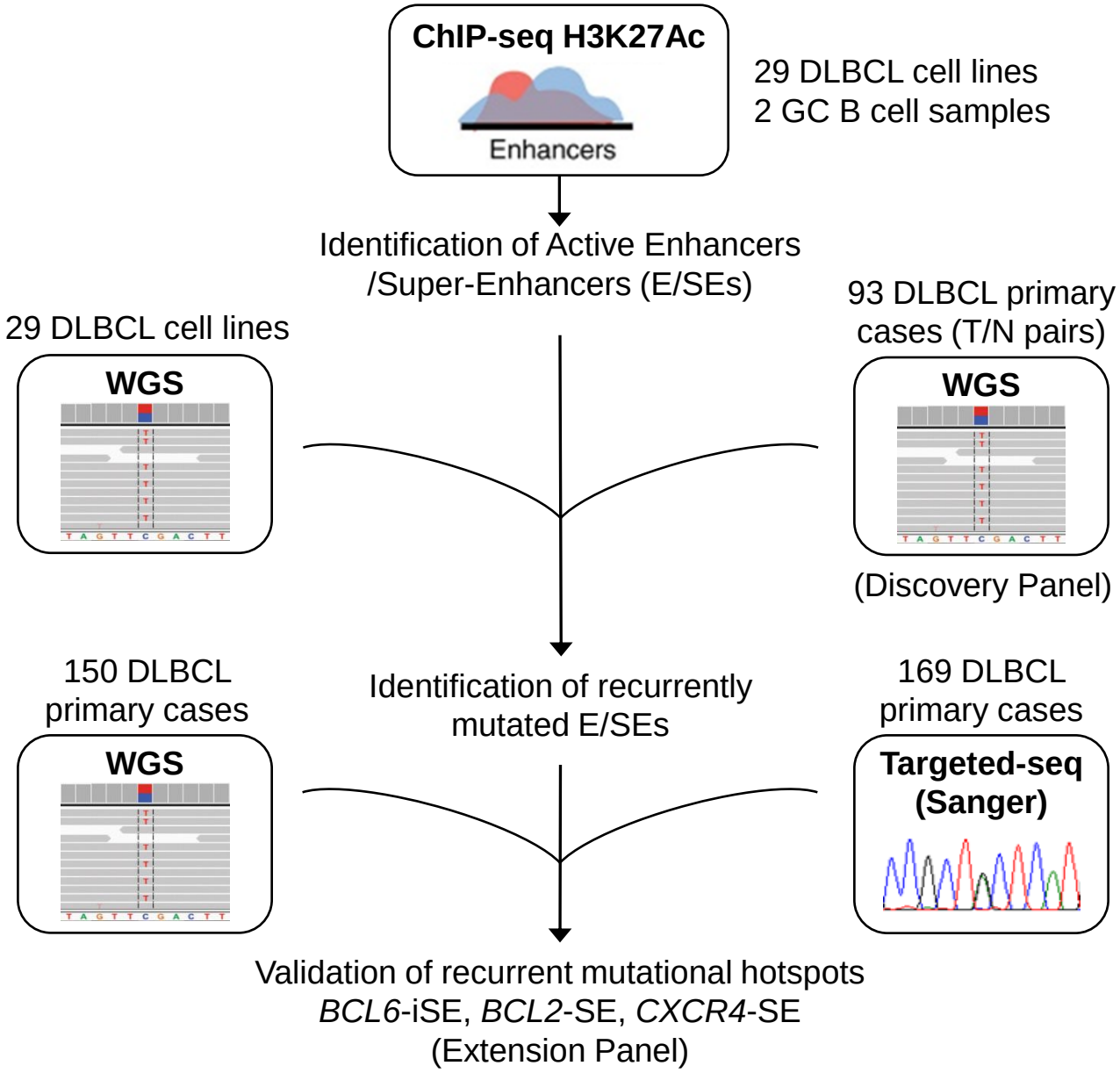


Protein coding genes represent <2% of the human genome: what about the non-coding genome?



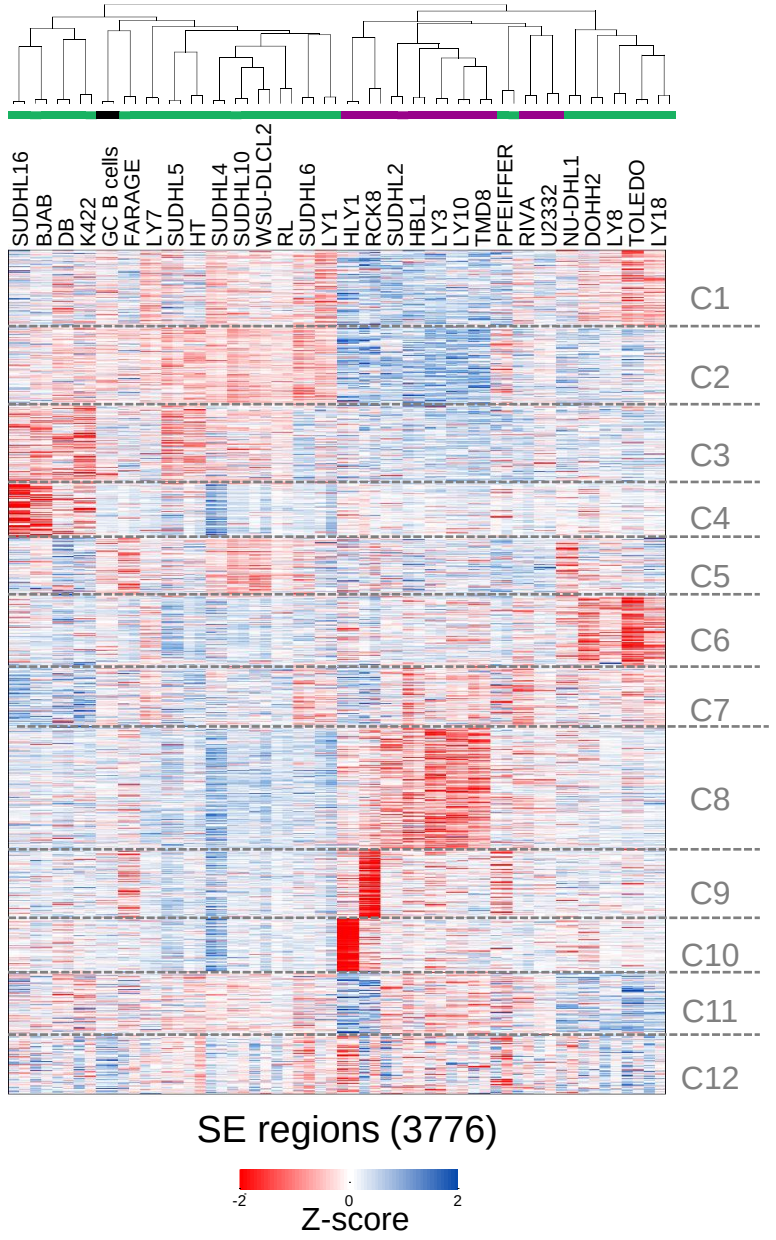
Genome-wide analysis of non-coding regulatory mutations in Diffuse Large B-cell Lymphoma

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



Bal et al., *Nature*, 2022

Shared and subtype-specific SEs in DLBCL

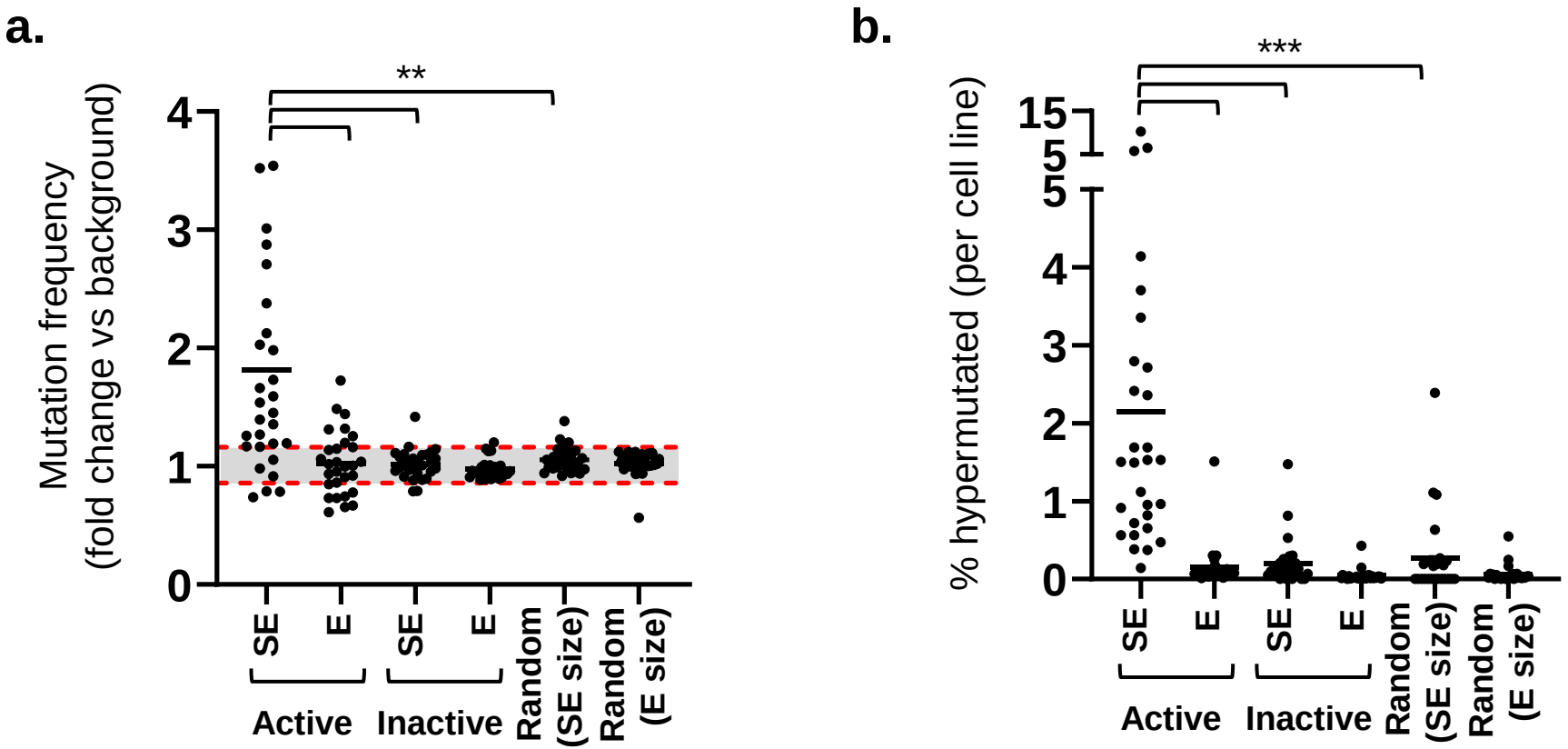


GCB-DLBCL cell lines

ABC-DLBCL cell lines

Normal GC B cells

SE regions are hypermutated in DLBCL cell lines

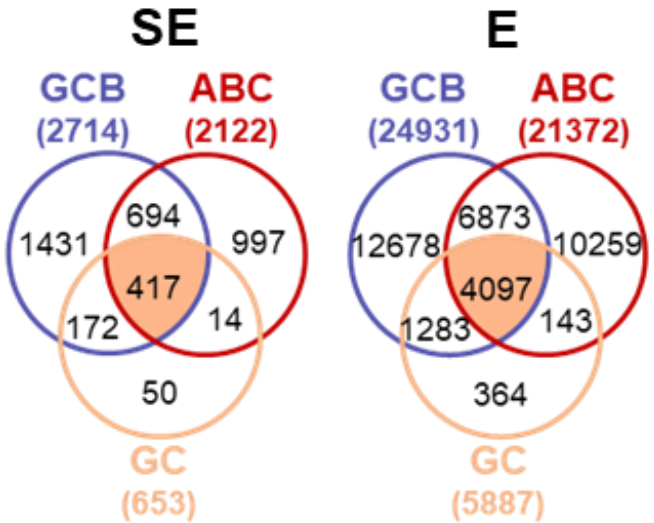


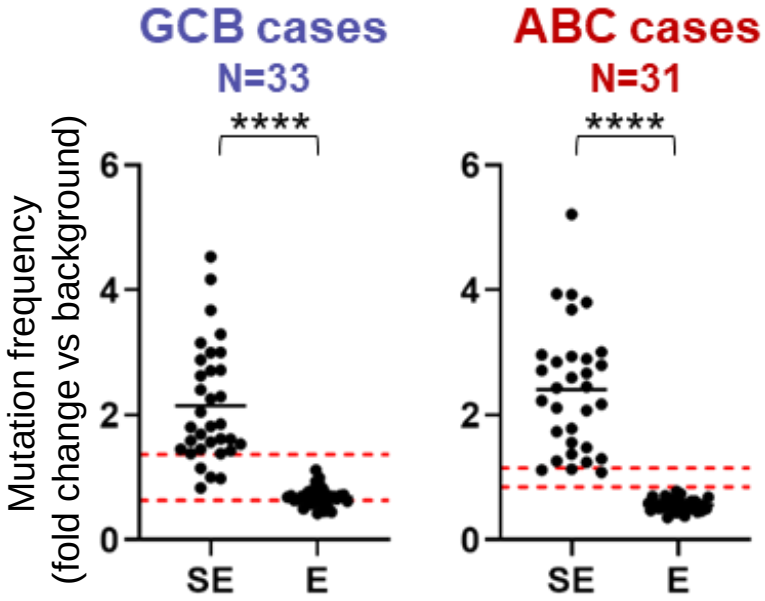
Hypermutated E/SEs:

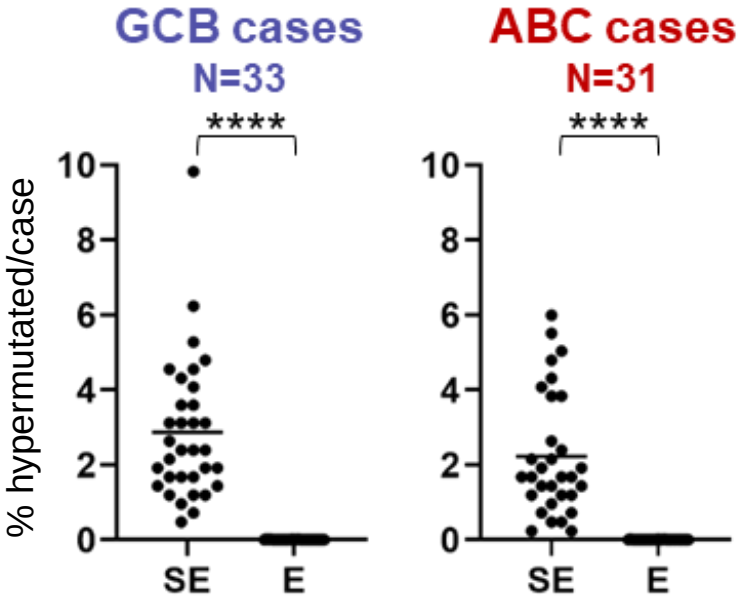
- >=3 mut with intermutation distance <=1kb
- Mutation frequency significantly higher with respect to background (mutations in the rest of the genome)

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

SEs are hypermutated in DLBCL primary cases



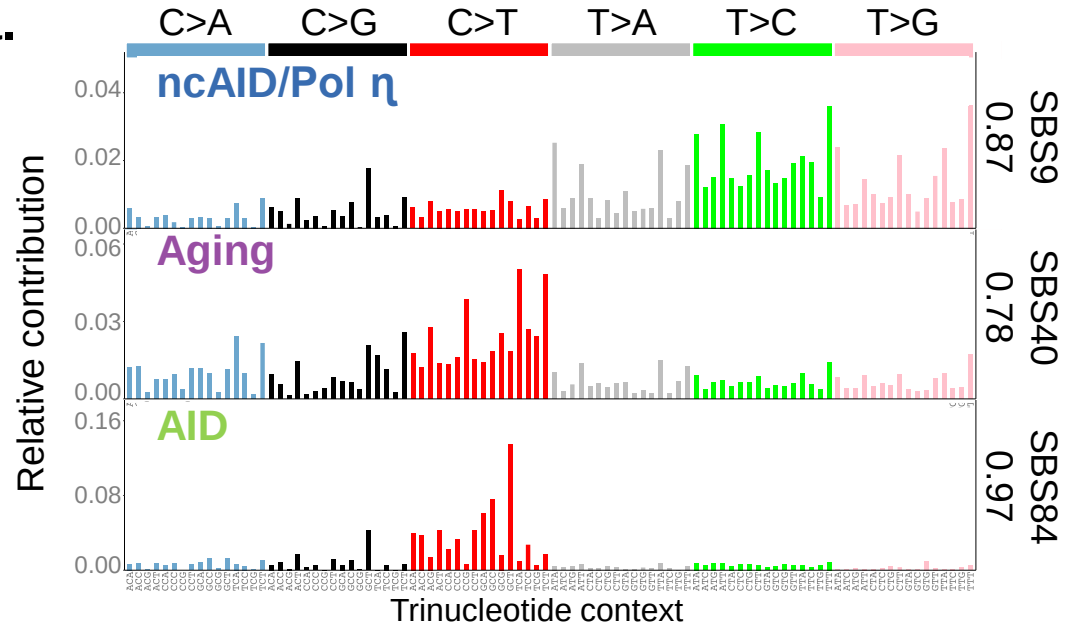




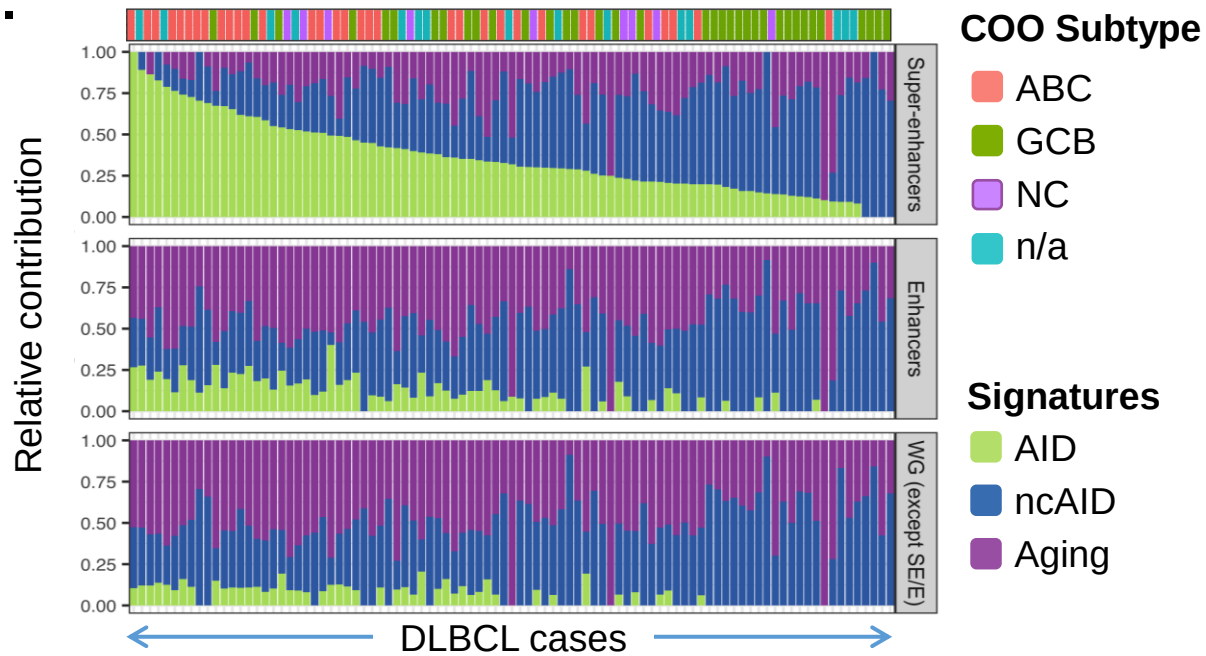
**** p<0.0001,paired t-test

Mutational signatures in SEs display AID hallmarks

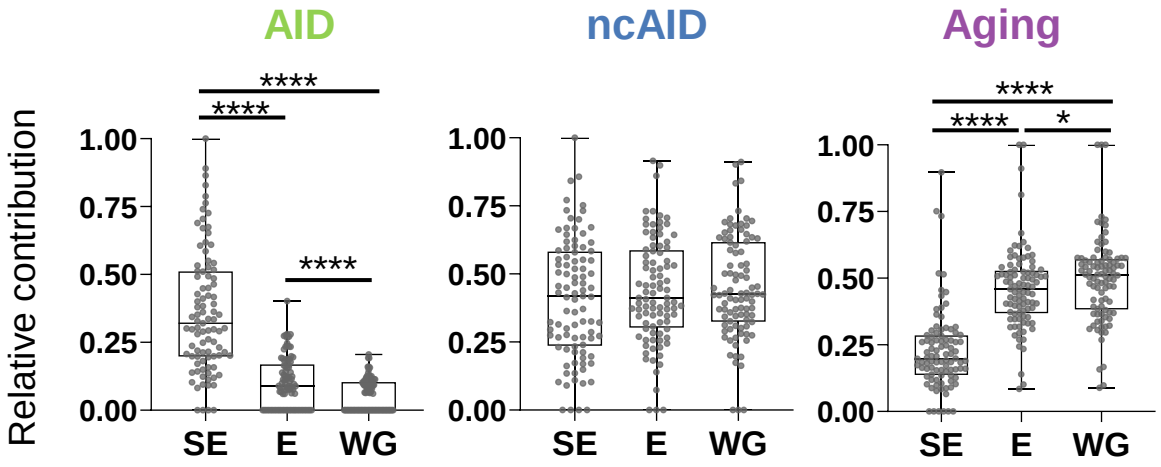
a.



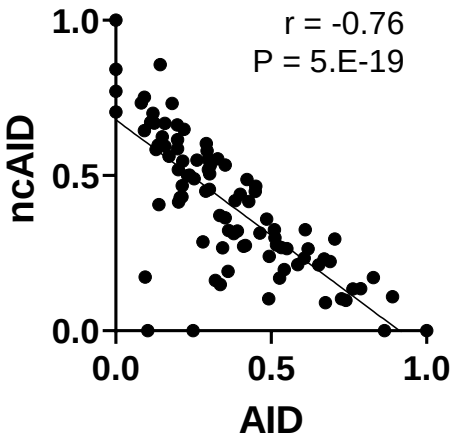
b.



c.

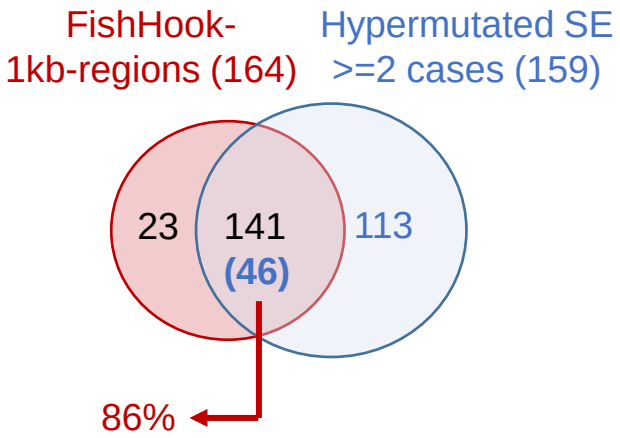


d. Inverse contribution of **ncAID** vs **AID** signature in SEs

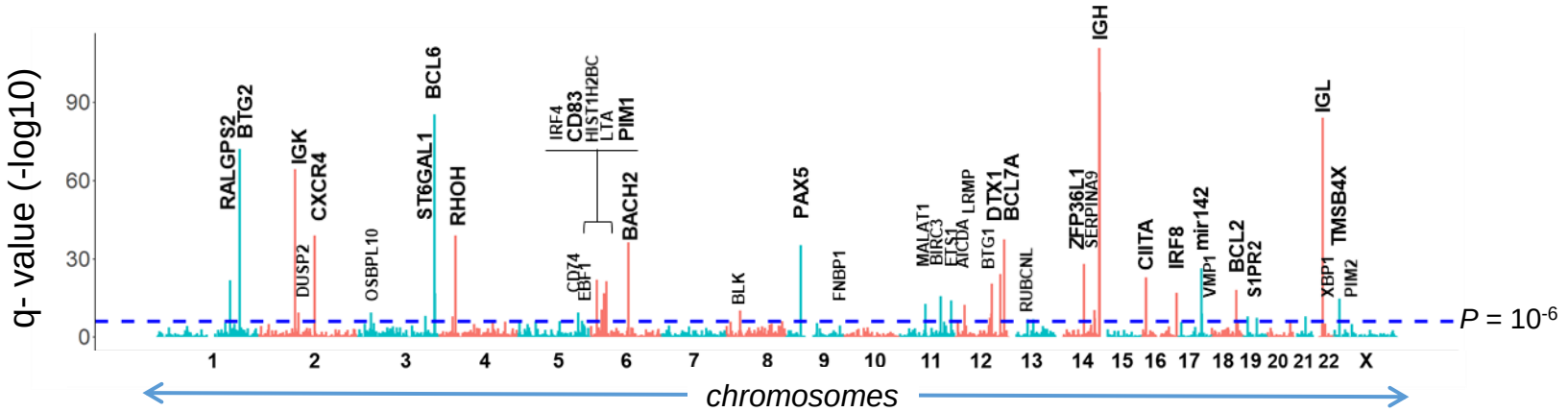


Candidate genes linked to recurrently mutated SEs are enriched in lymphoma oncogenes

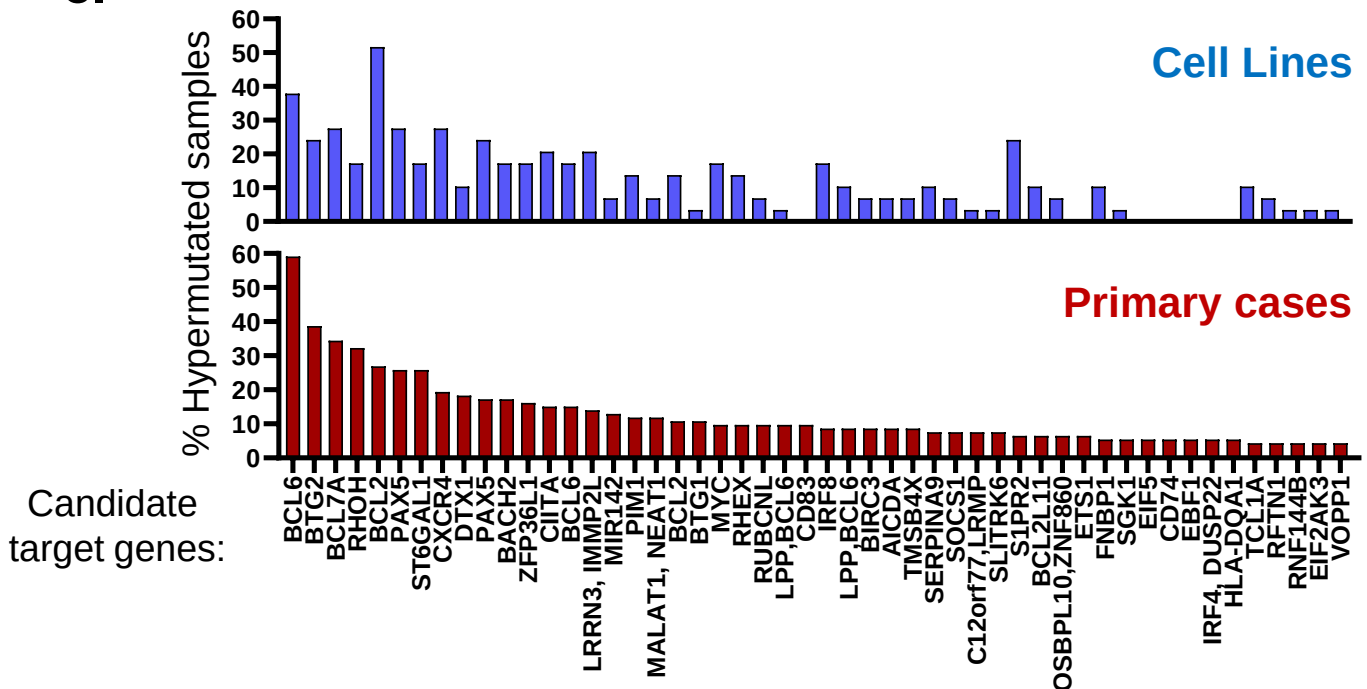
a.



b.

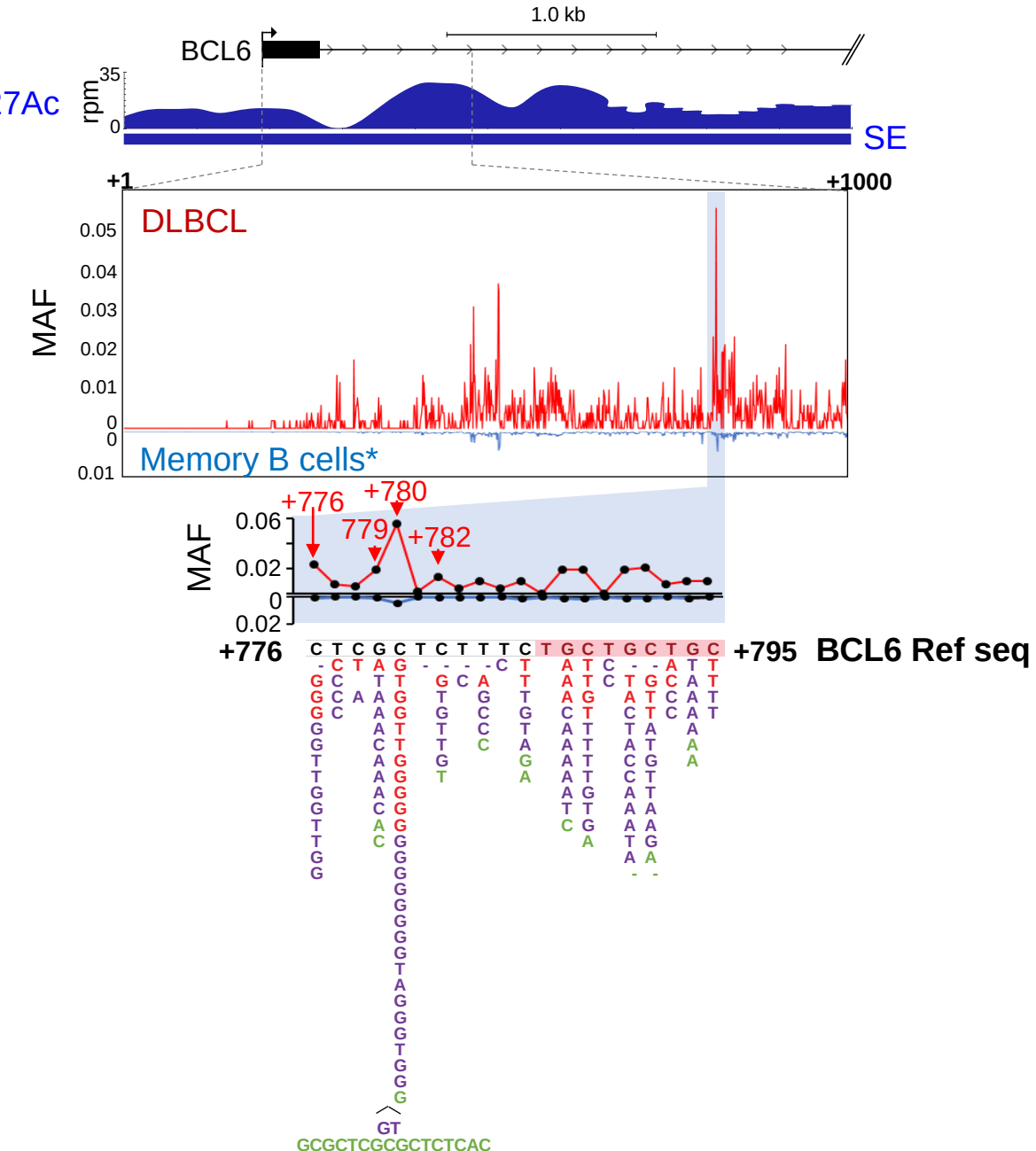


c.



FishHook
(Imielinski et al, Cell 2017)

A highly recurrent mutational hotspot in the BCL6 intragenic SE



AID motif

Cases tested:

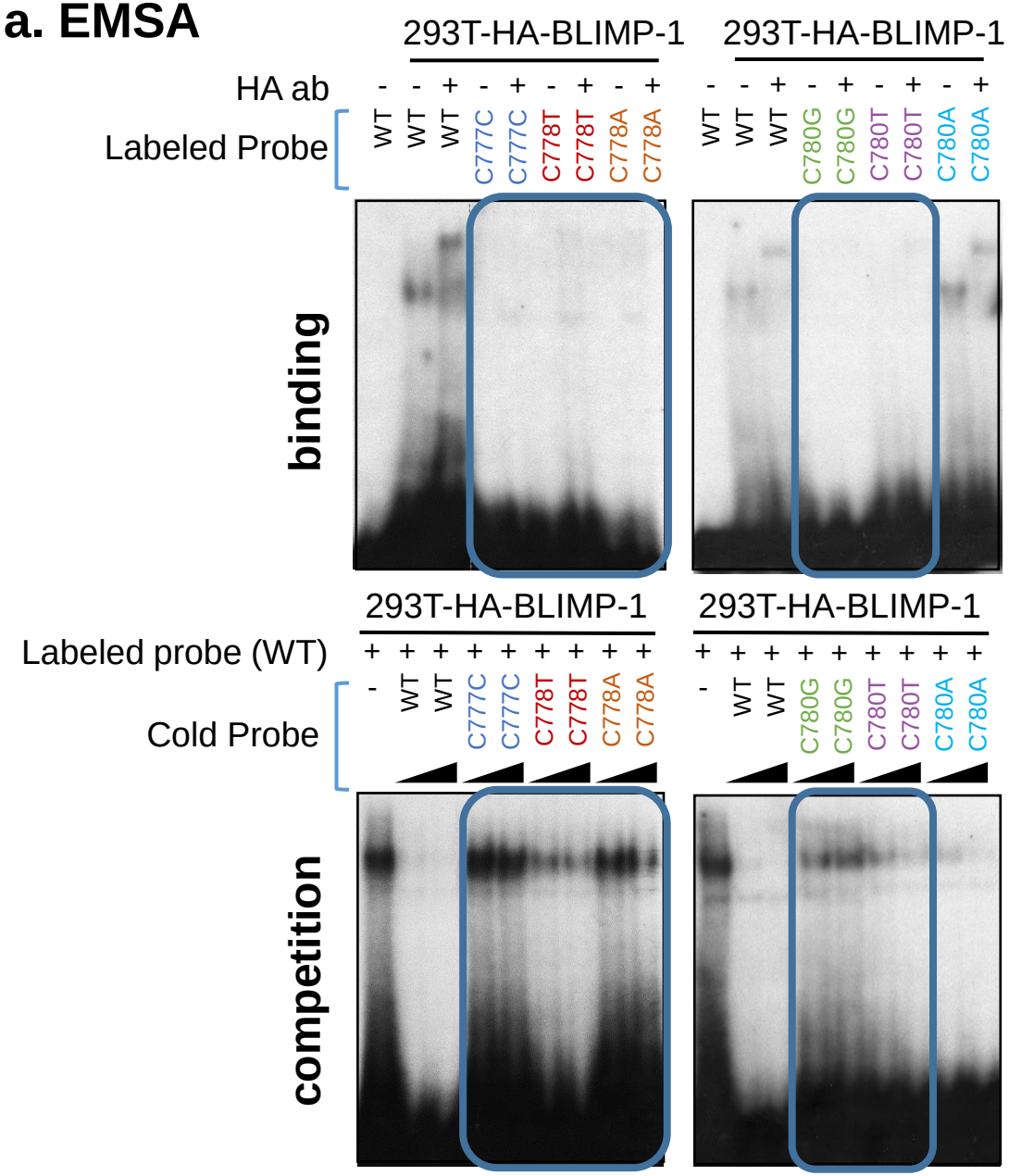
- primary cases (WGS, n= 75)
- primary cases (Sanger, n=176)
- cell lines (WGS, n=23)

(BCL6 Tx excluded)

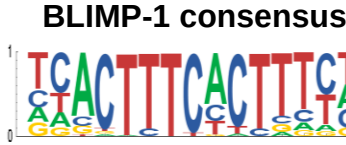
*Shen JC at al., PNAS, 2019

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

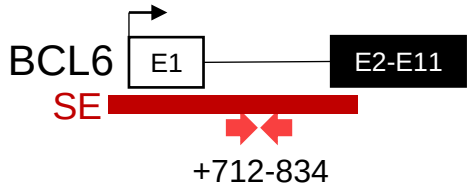
a. EMSA

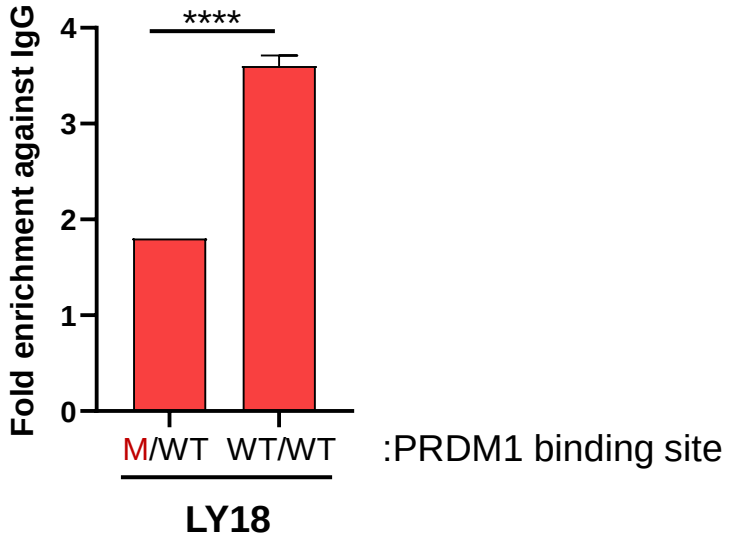


b. ChIP-qPCR (CD40 stimulated cells)

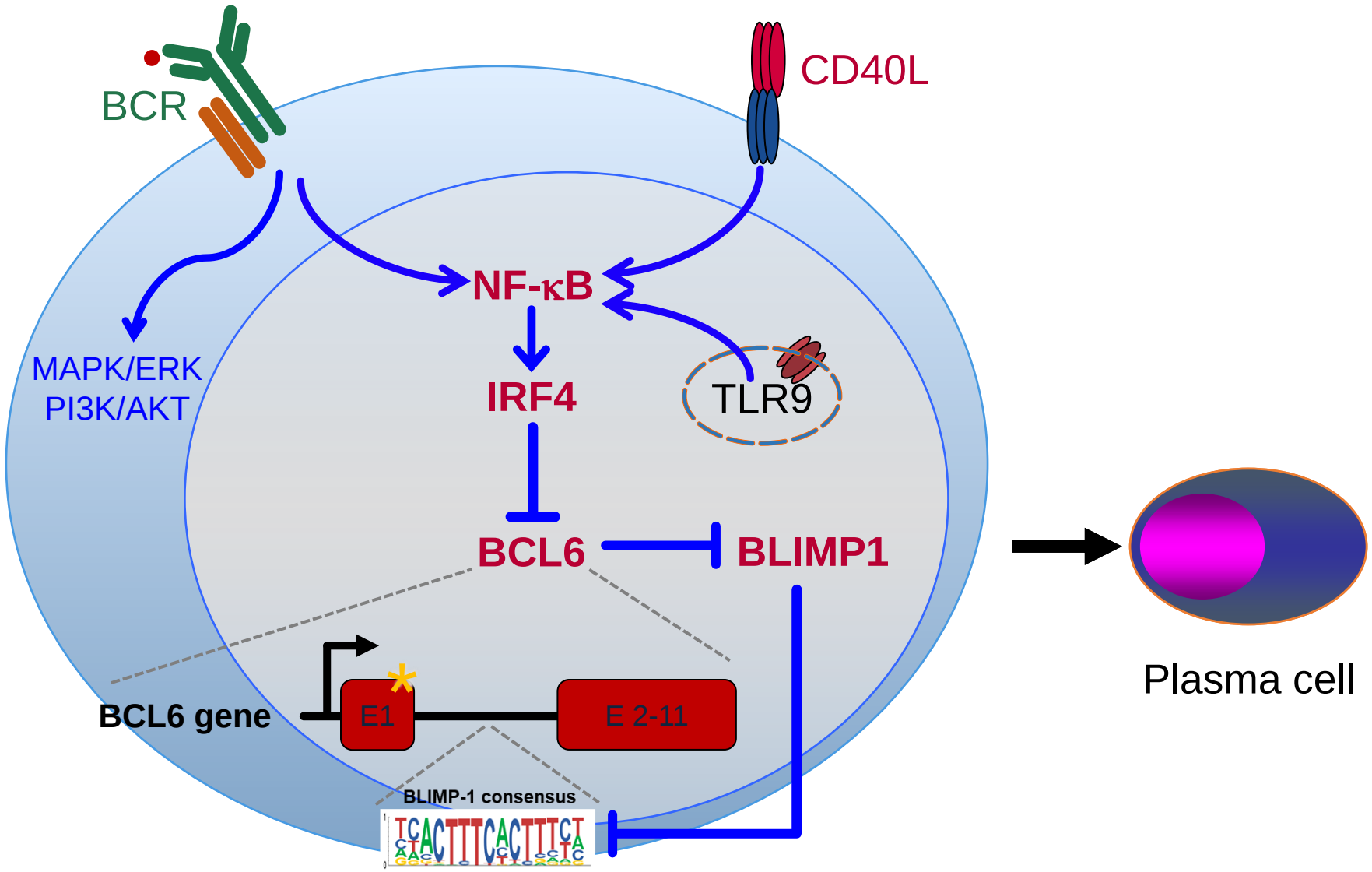



```
WT     +769 TCGCGCTCTCGCTCTTTCTGC +789
LY18A  +769 TCGCGCTCTCGGTCTTTCTGC +789
LY18B  +769 TCGCGCTCTCGCTCTTTCTGC +789
```



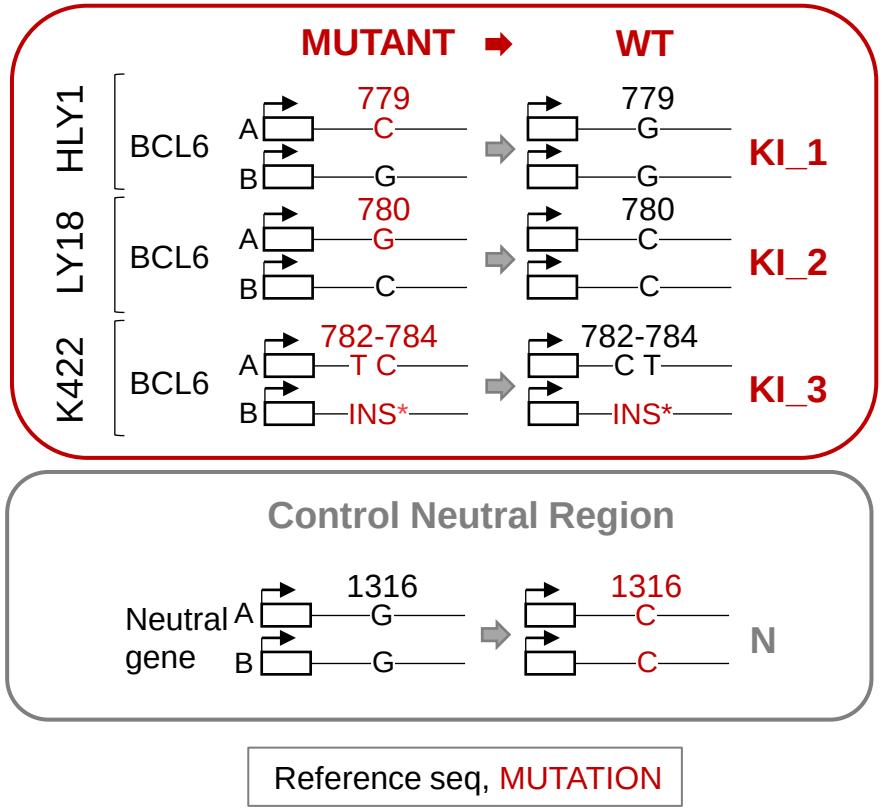


Downregulation of BCL6 expression is required for the initiation of post-GC differentiation

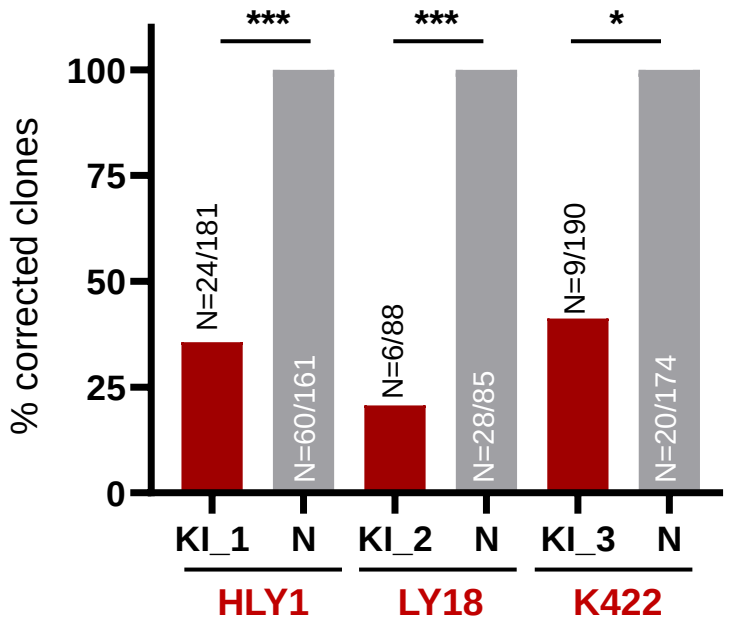


Correction of mutations in BCL6 SE leads to counter-selection

a. CRISPR/Cas9-editing of endogenous site

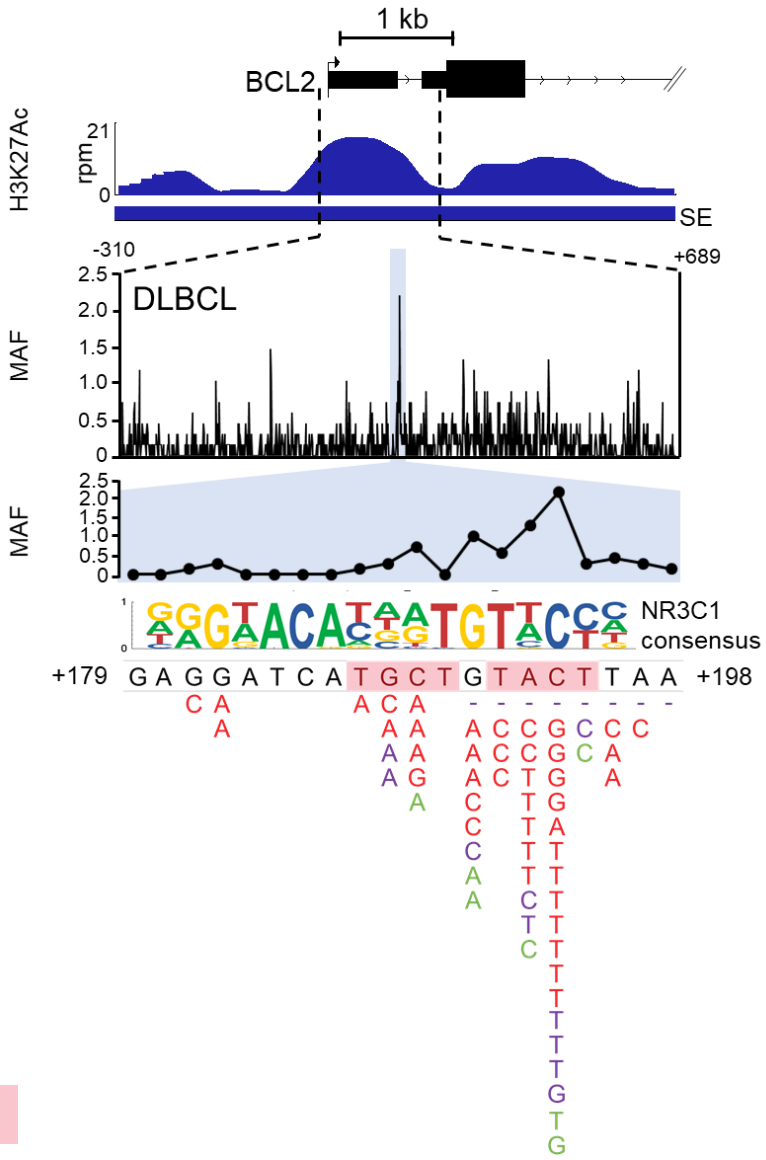


b. Clones Recovery



*** Ins 16bp 780_781**

Recurrent mutations in BCL2 intragenic SE target NR3C1 binding site

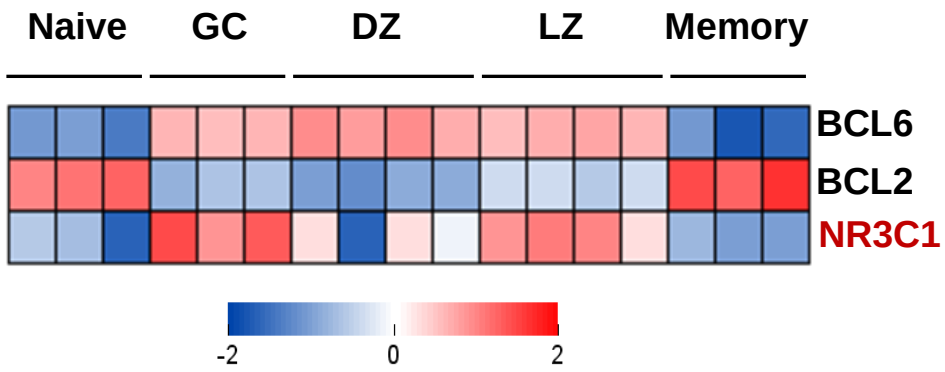


AID motif

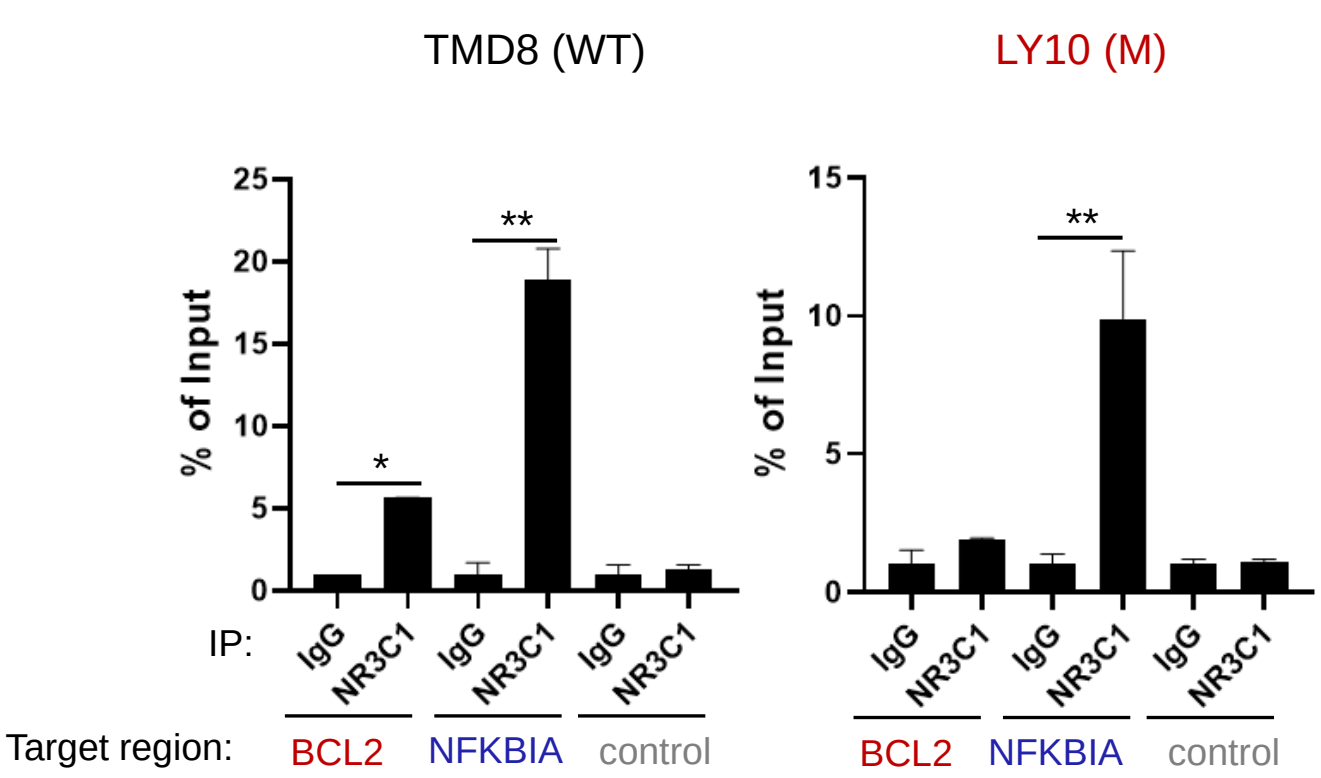
(BCL2Tx cases excluded)

NR3C1

- Glucocorticoid receptor and transcription factor
- Truncating mutations in 6% relapsed B-ALL
- Low expression linked to poor prognosis and tumor progression in B-ALL by upregulation of BCL2
- Inversely correlated with BCL2 expression in the GC

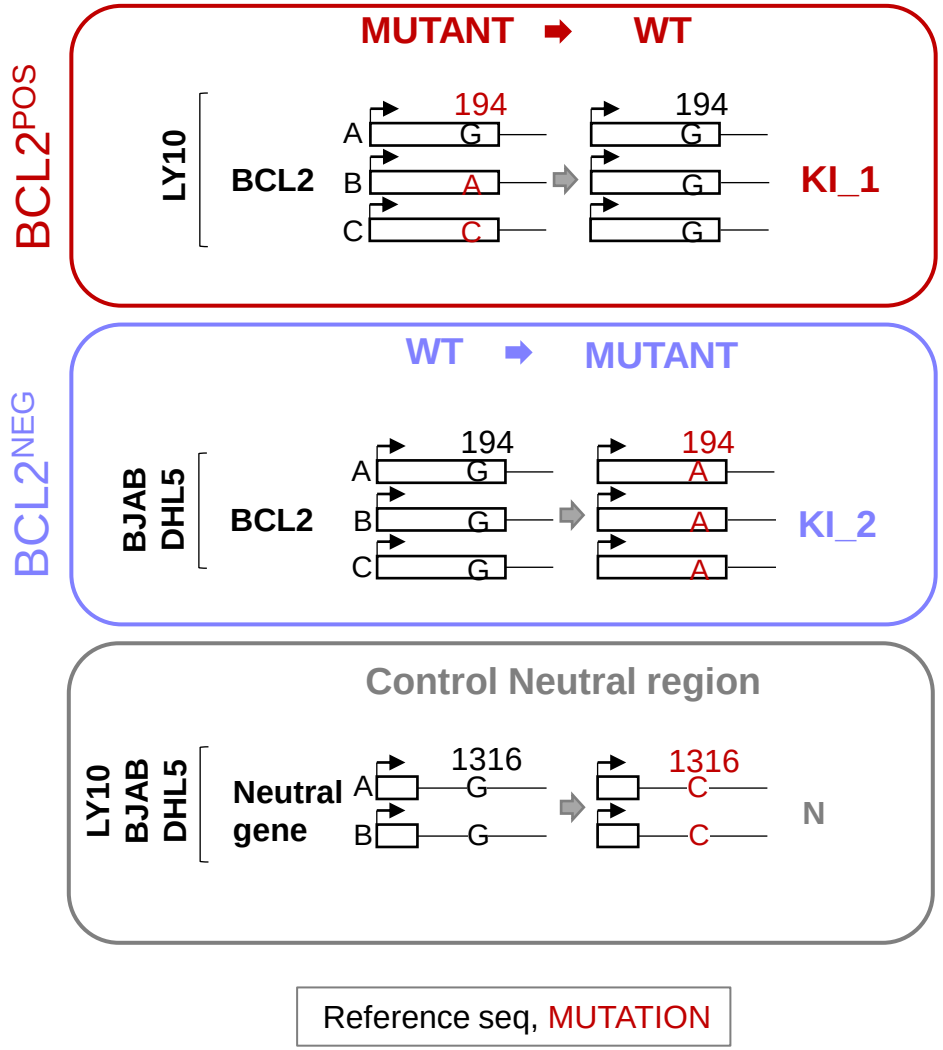


Mutations in BCL2 intragenic SE abrogate NR3C1 binding in DLBCL cells

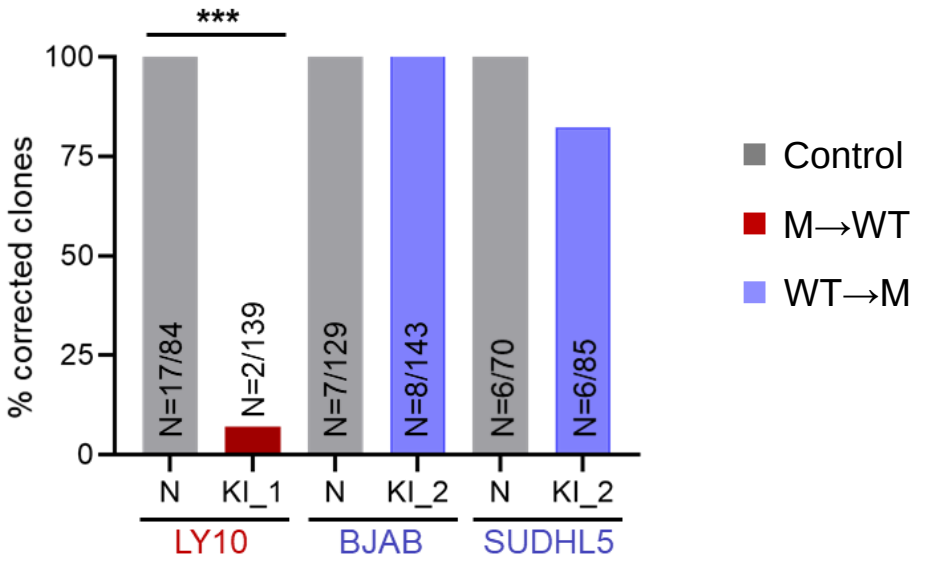


Correction of mutations in BCL2 iSE leads to counter-selection

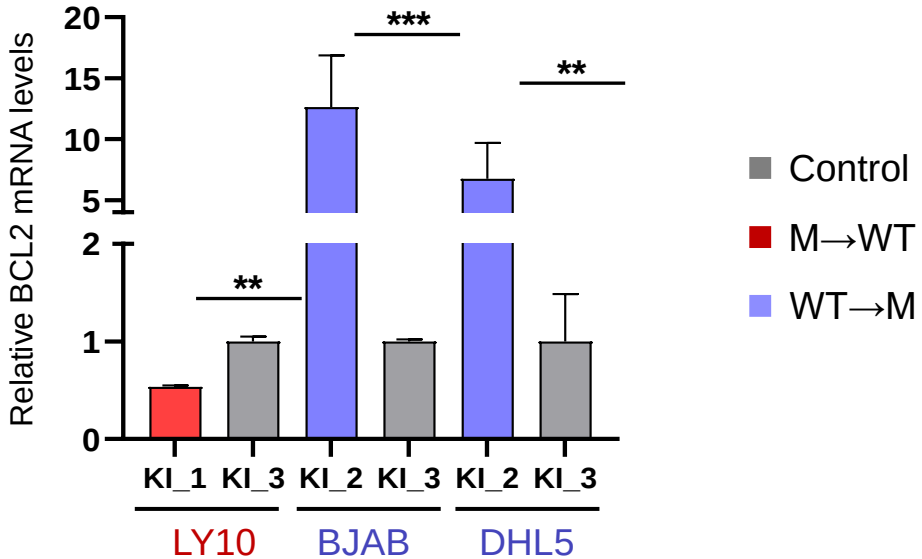
a. CRISPR/Cas9-editing of endogenous site



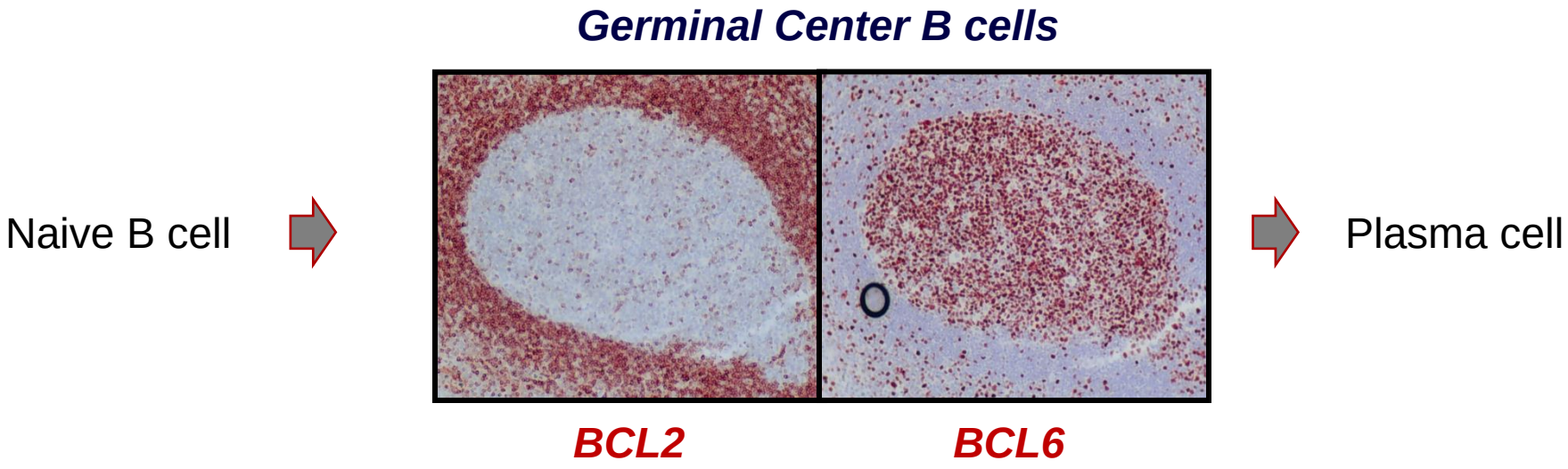
b. Clones Recovery

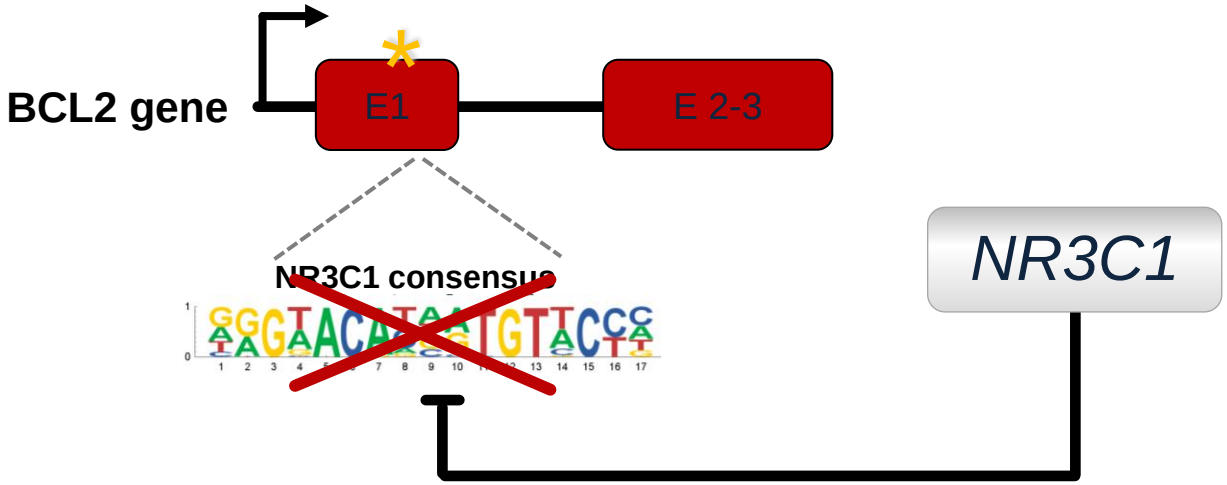


c. BCL2 mRNA expression



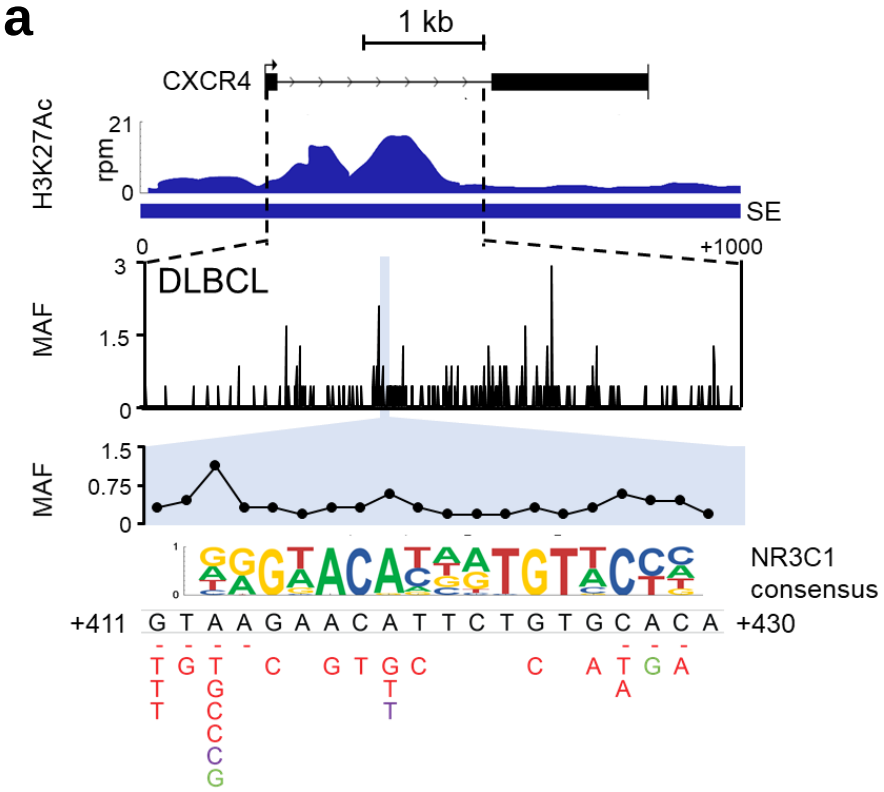
Multiple genetic lesions contribute to BCL2 deregulation in lymphoma





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

a



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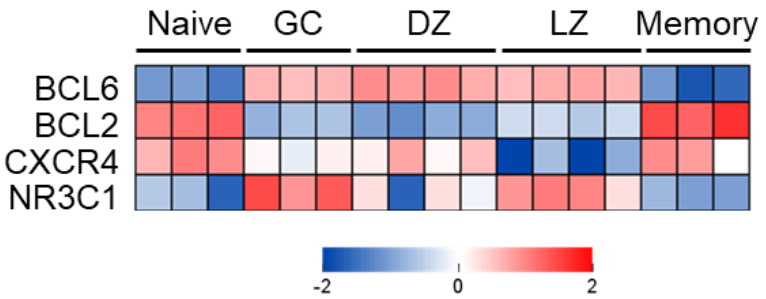
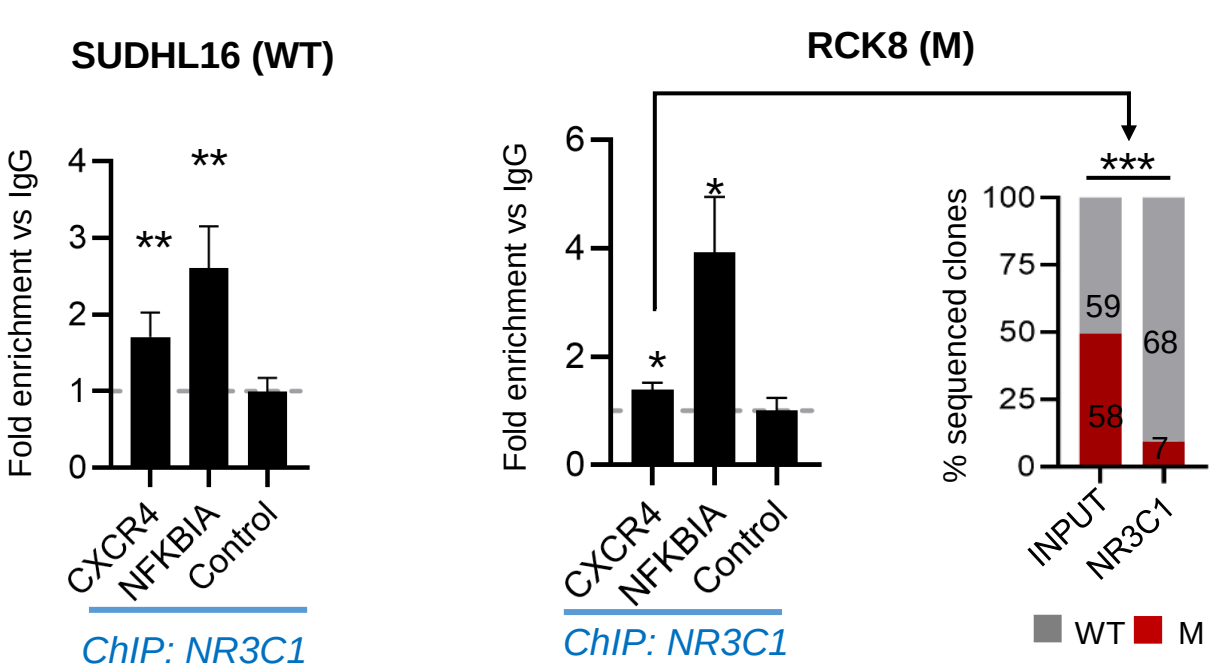


CXCR4

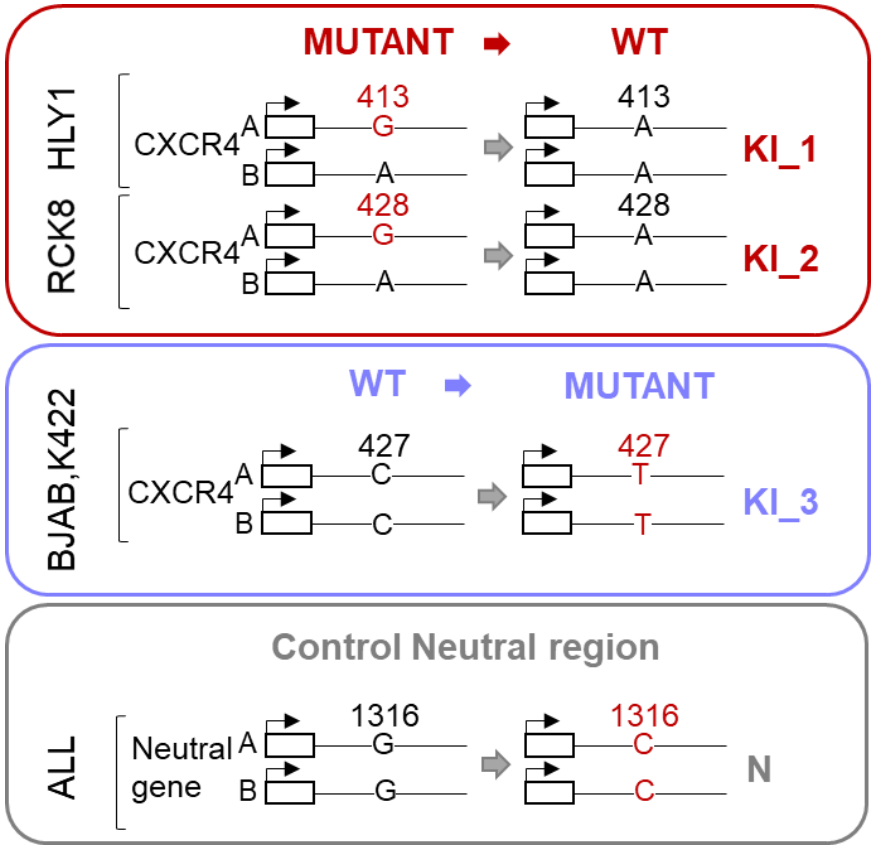
- Transmembrane receptor for the CXCL12/SDF1 chemokine
- Involved in MAPK activation and AKT signaling
- Essential role in cell migration and GC DZ/LZ organization
- Oncogenic truncating mutations in Waldenstroem Macroglobulinemia
- Coding mutations found in few DLBCL cases

c



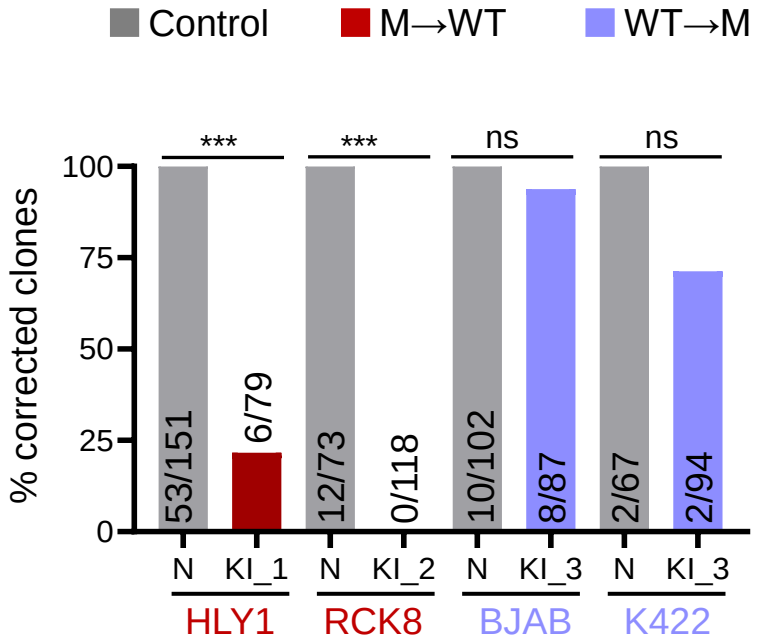
Correction of mutations in the CXCR4 SE induces counter-selection of lymphoma cells

a



WT, Mutation

b



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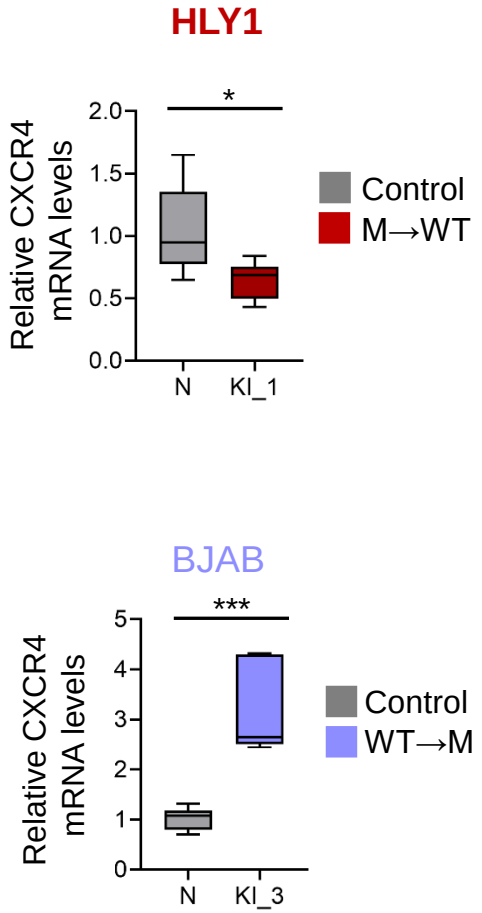
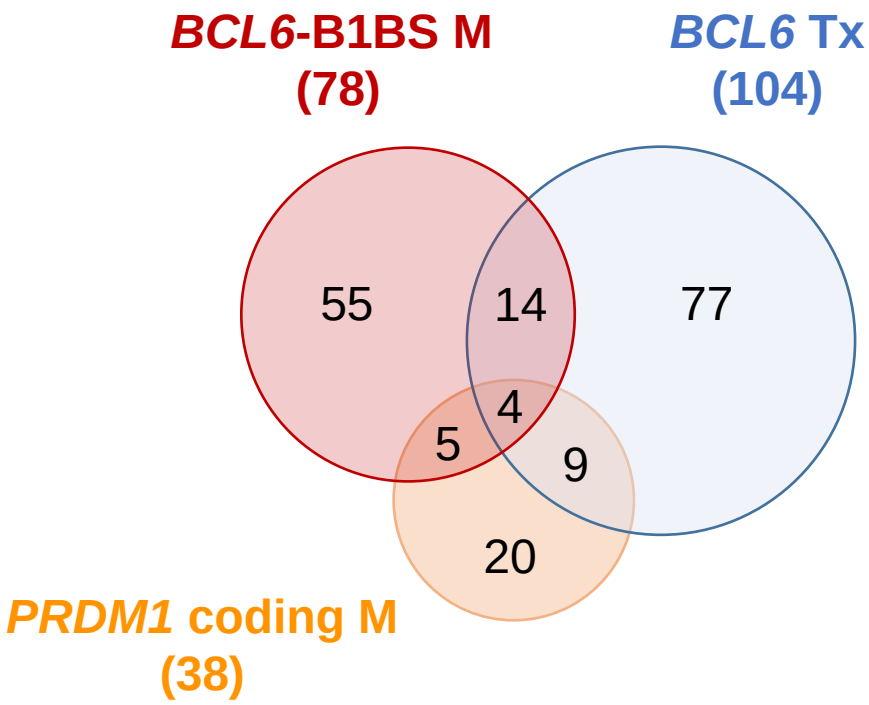
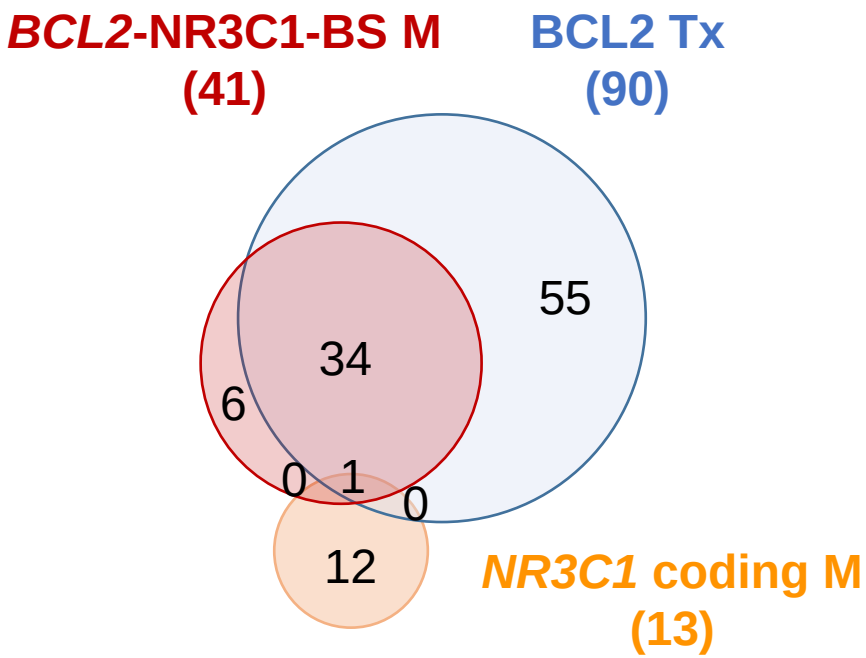
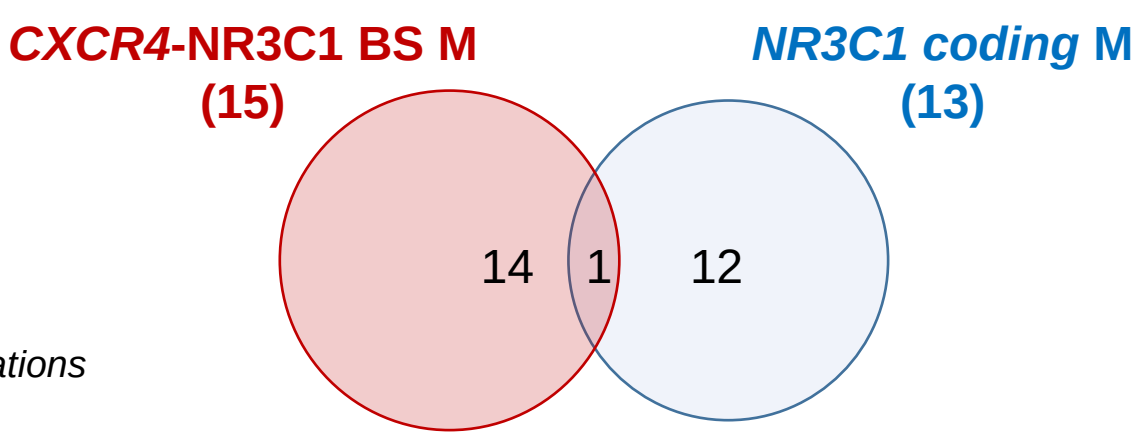


SE-mutations identify complementary mechanisms deregulating target gene expression







B1BS M = Blimp1 binding site mutations
NR3C1-BS M = NR3C1 binding site mutations
Tx, translocations

Summary

- 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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AID-mediated aberrant somatc hypermutation in DLBCL

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Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas

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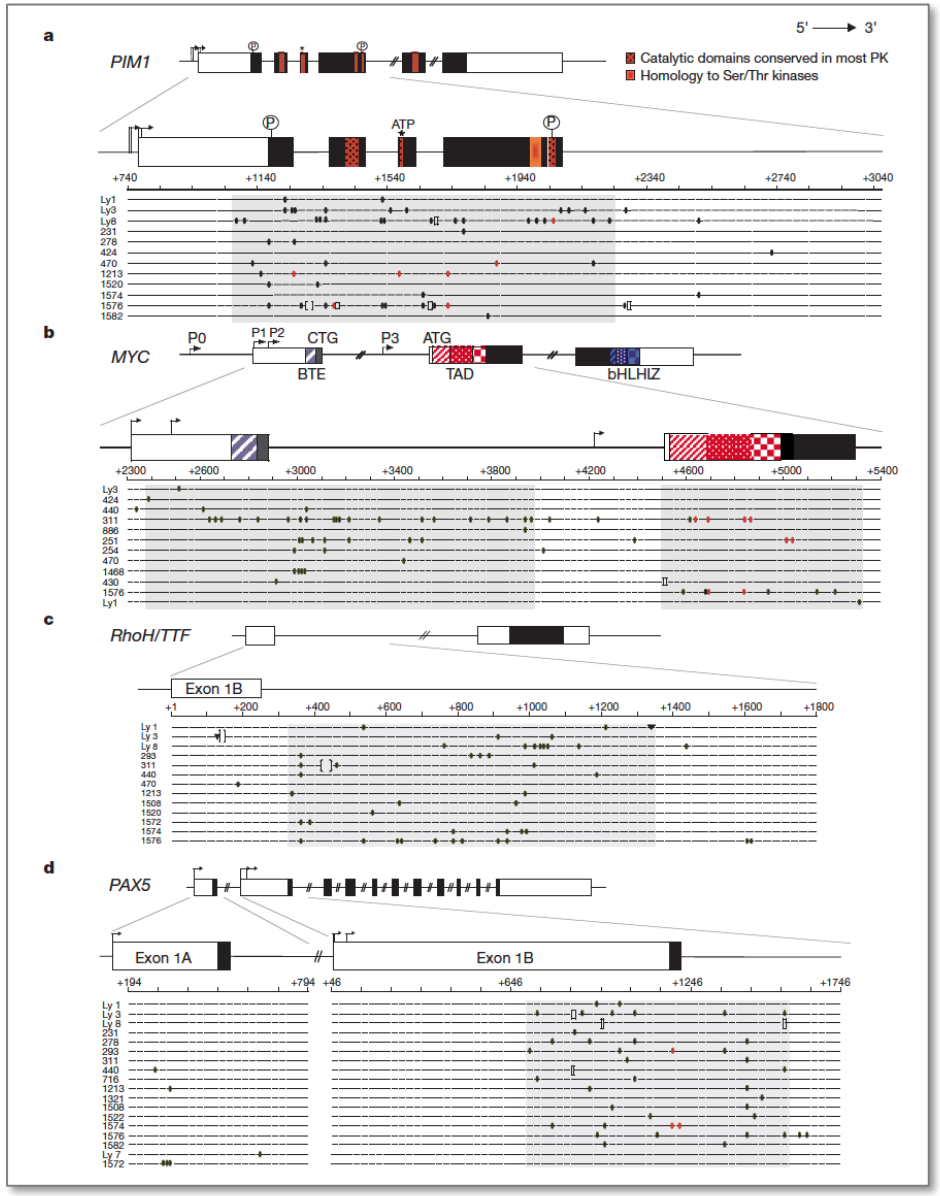
Nature, 2001



- 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

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Shen et al., *Science, 1998*
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Model for the generation of genetic lesions in B-NHL

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