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

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7th Postgraduate Lymphoma Conference

Rome
March 16-17, 2023

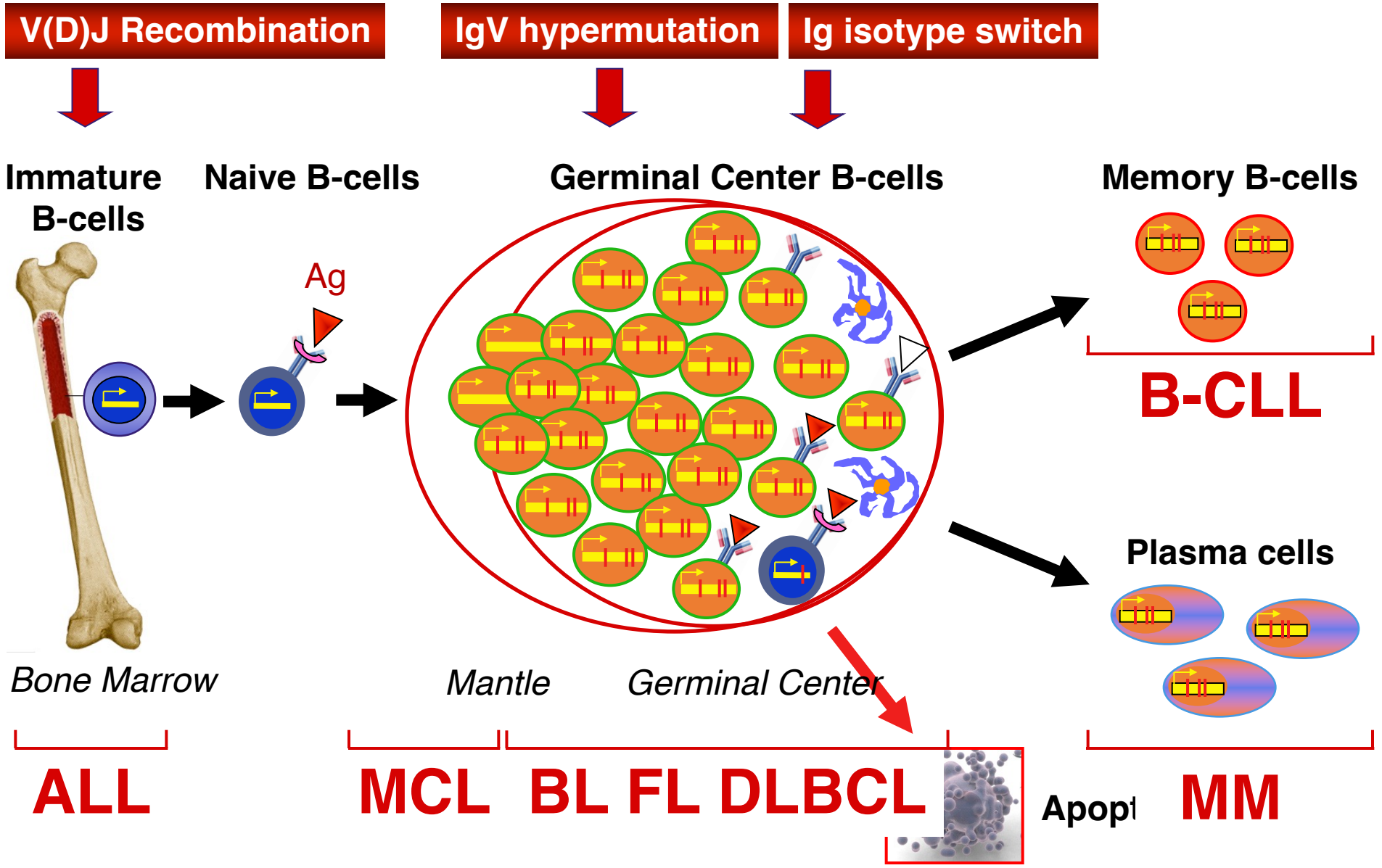
I have the following financial relationships to disclose:

Consultant for: NeoGenomics, Astra Zeneca, DiaTech

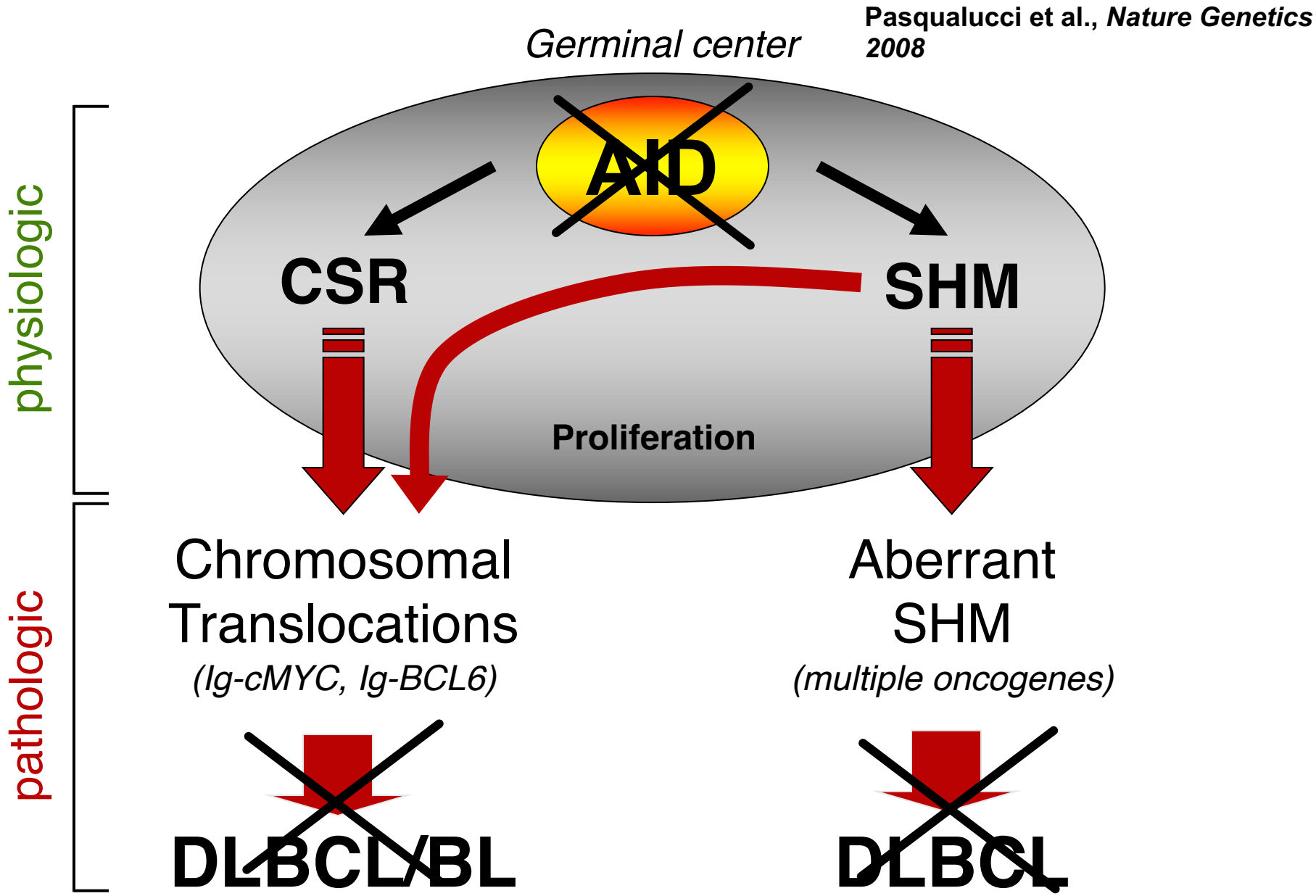
Research support: Astra Zeneca

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

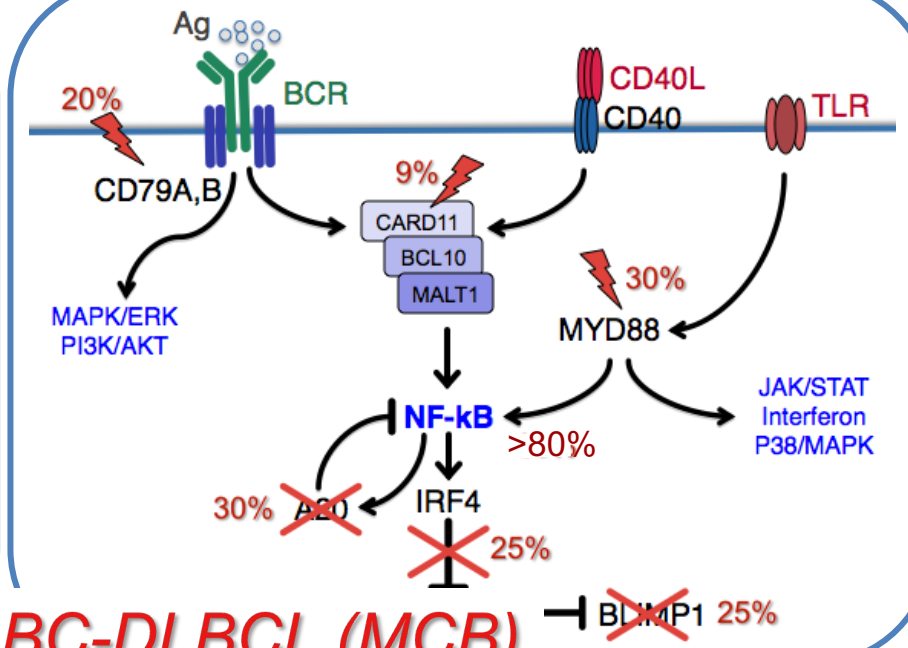
Escape from immune surveillance (CTL + NK)

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%

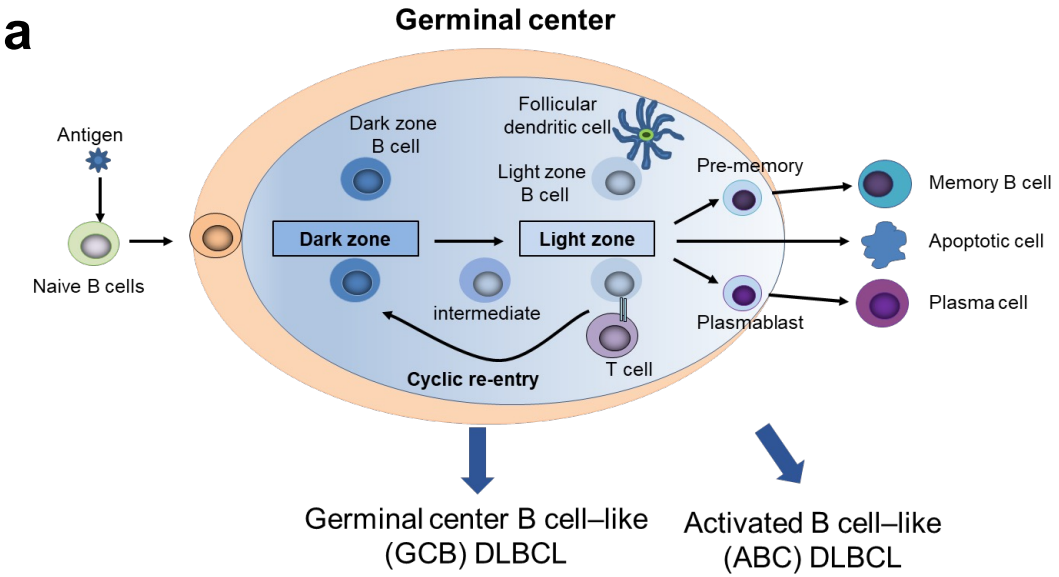
GCB-DLBCL (EZB)



ABC-DLBCL (MCB)

Genomic classification of DLBCL

a



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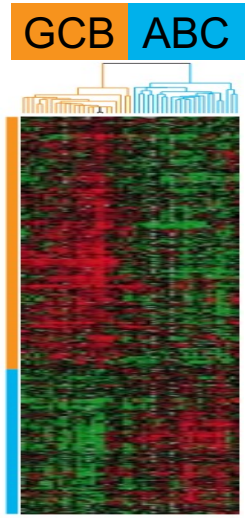
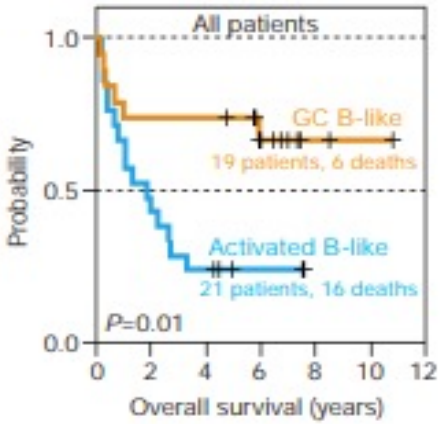


Alizadeh, *Nature, 2000*



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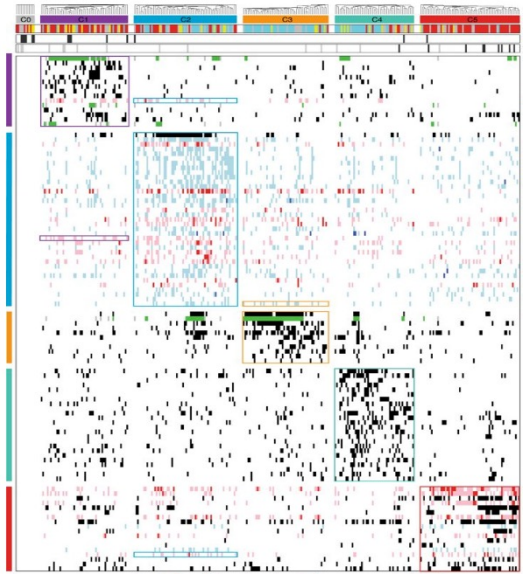
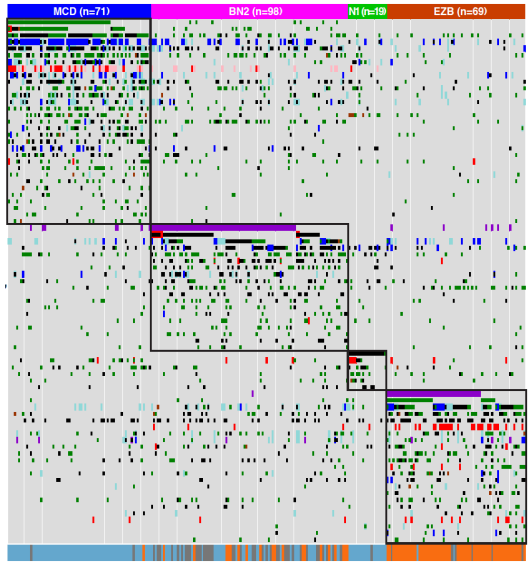
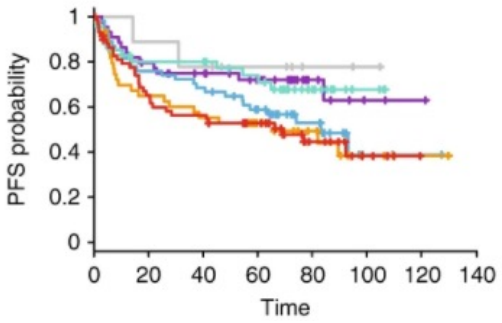


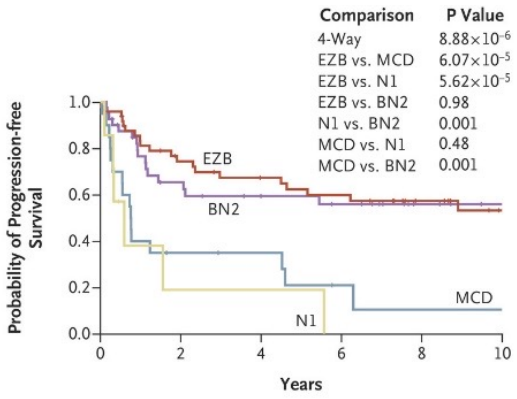
Chapuy et al, *Nature Med 2018*



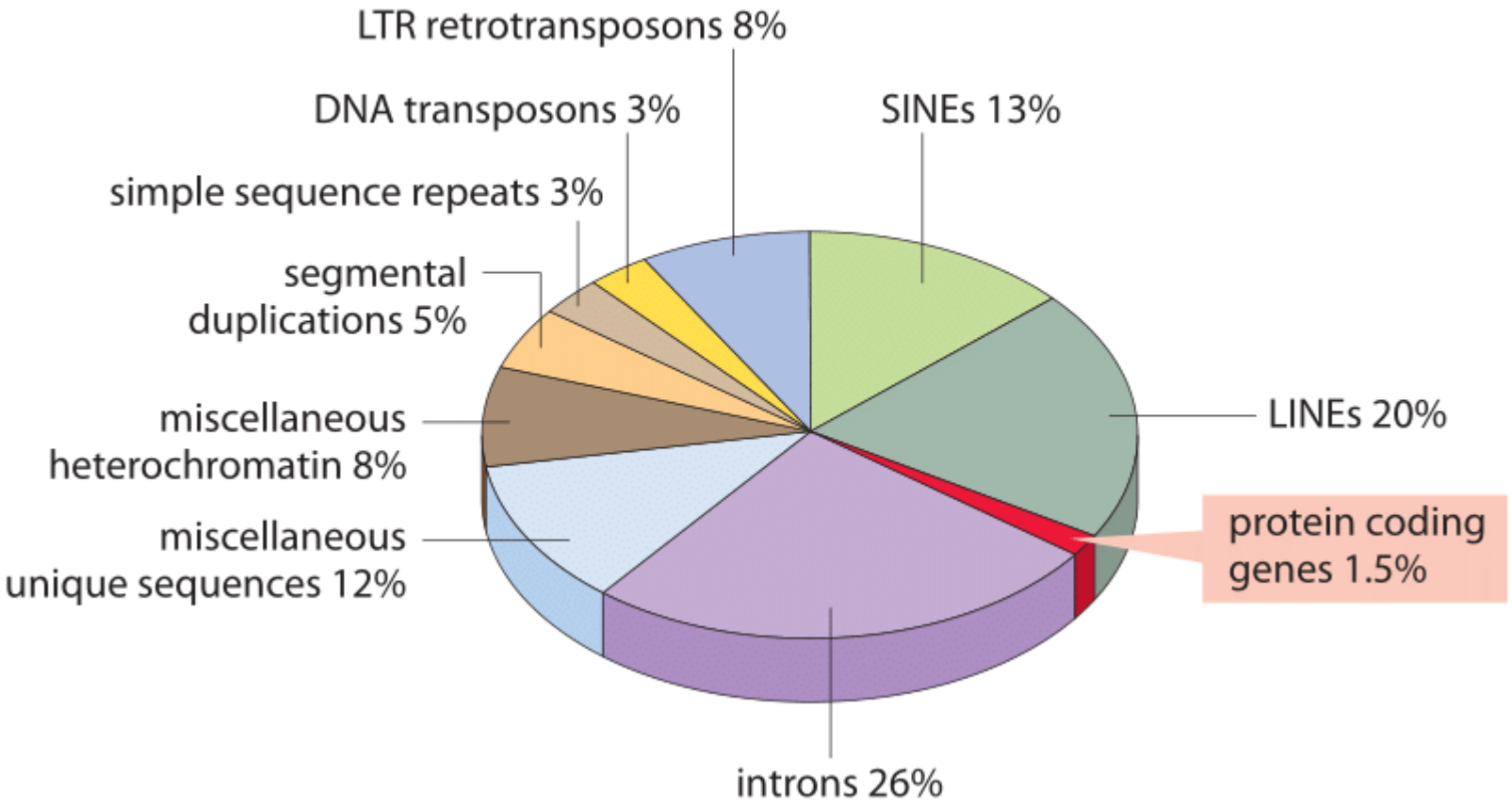
Schmitz et al, *NEJM 2018*

d



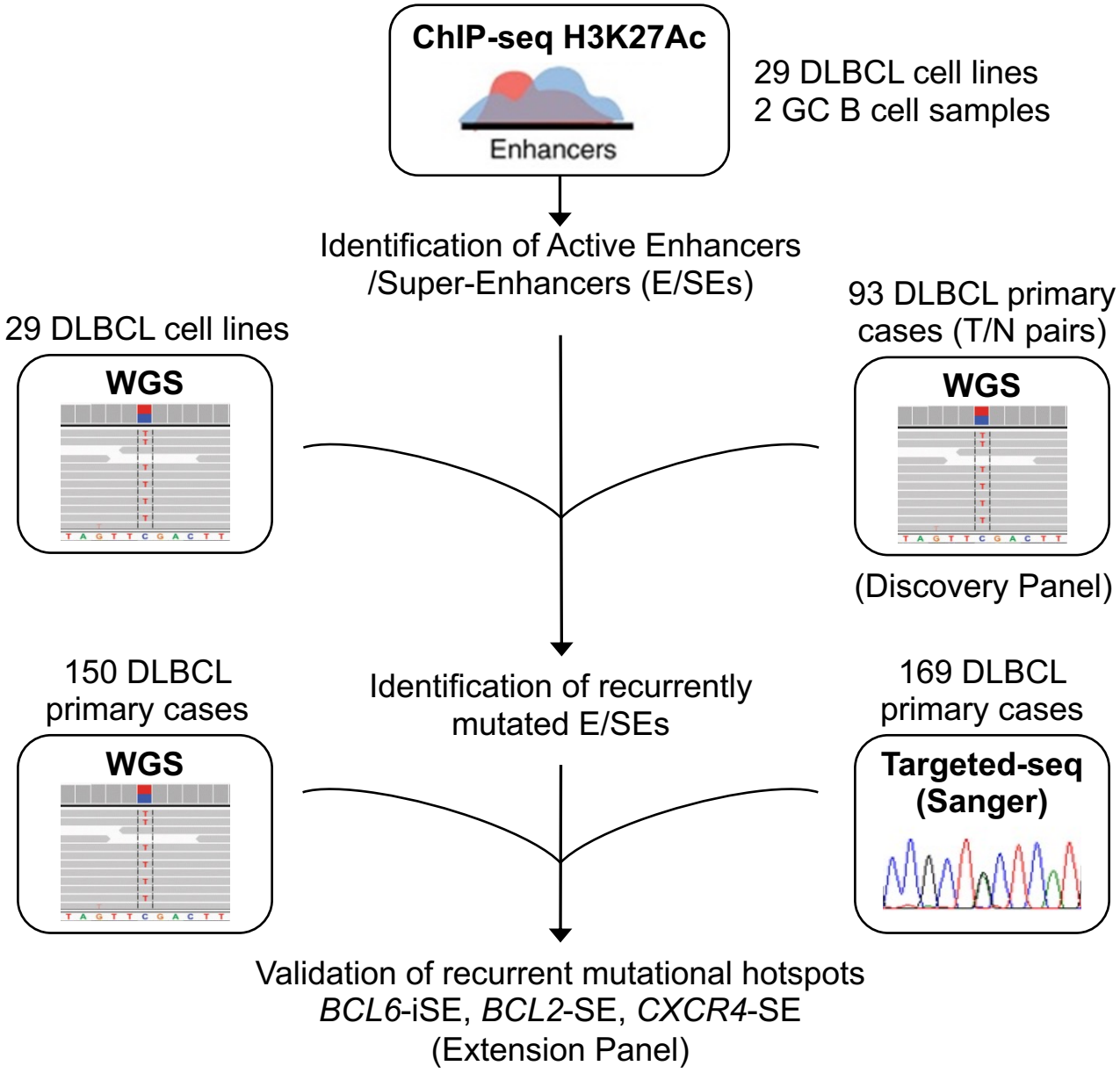


Protein coding genes represent <2% of the human genome: what about the non-coding genome?



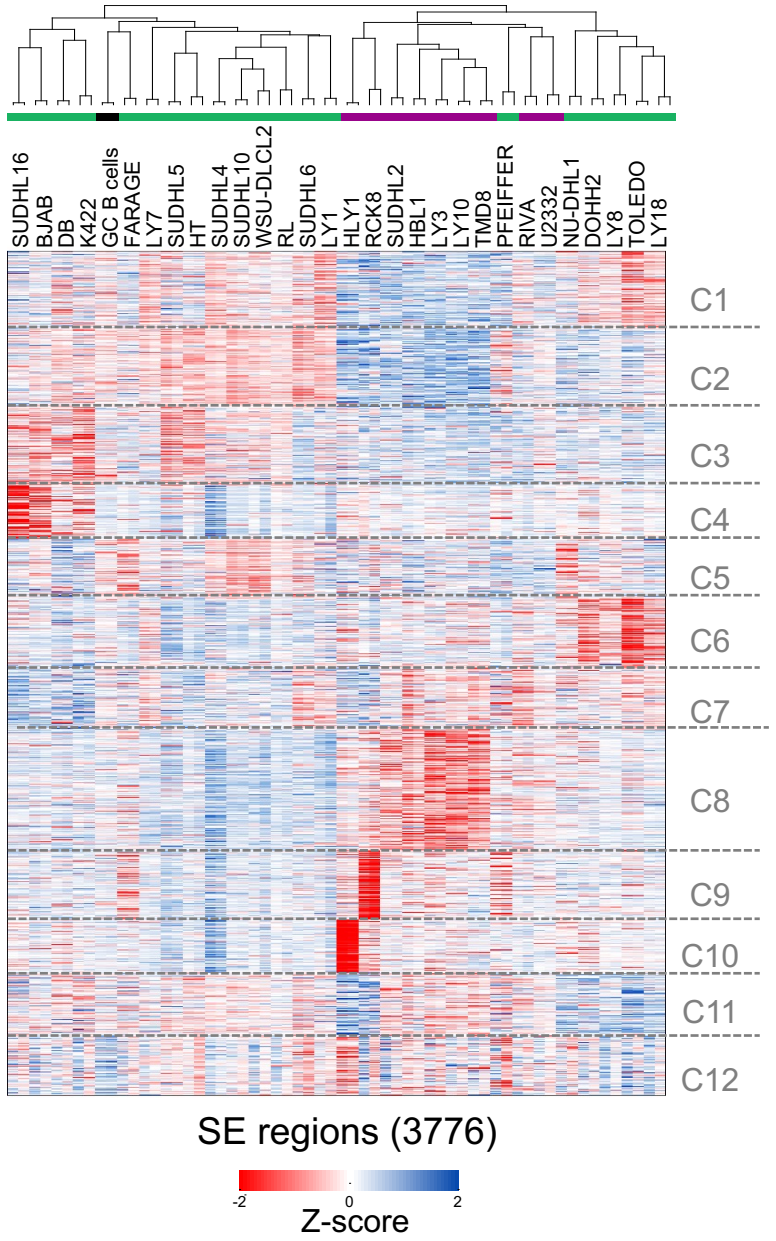
Genome-wide analysis of non-coding regulatory mutations in Diffuse Large B-cell Lymphoma

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



Bal et al., *Nature*, 2022

Shared and subtype-specific SEs in DLBCL

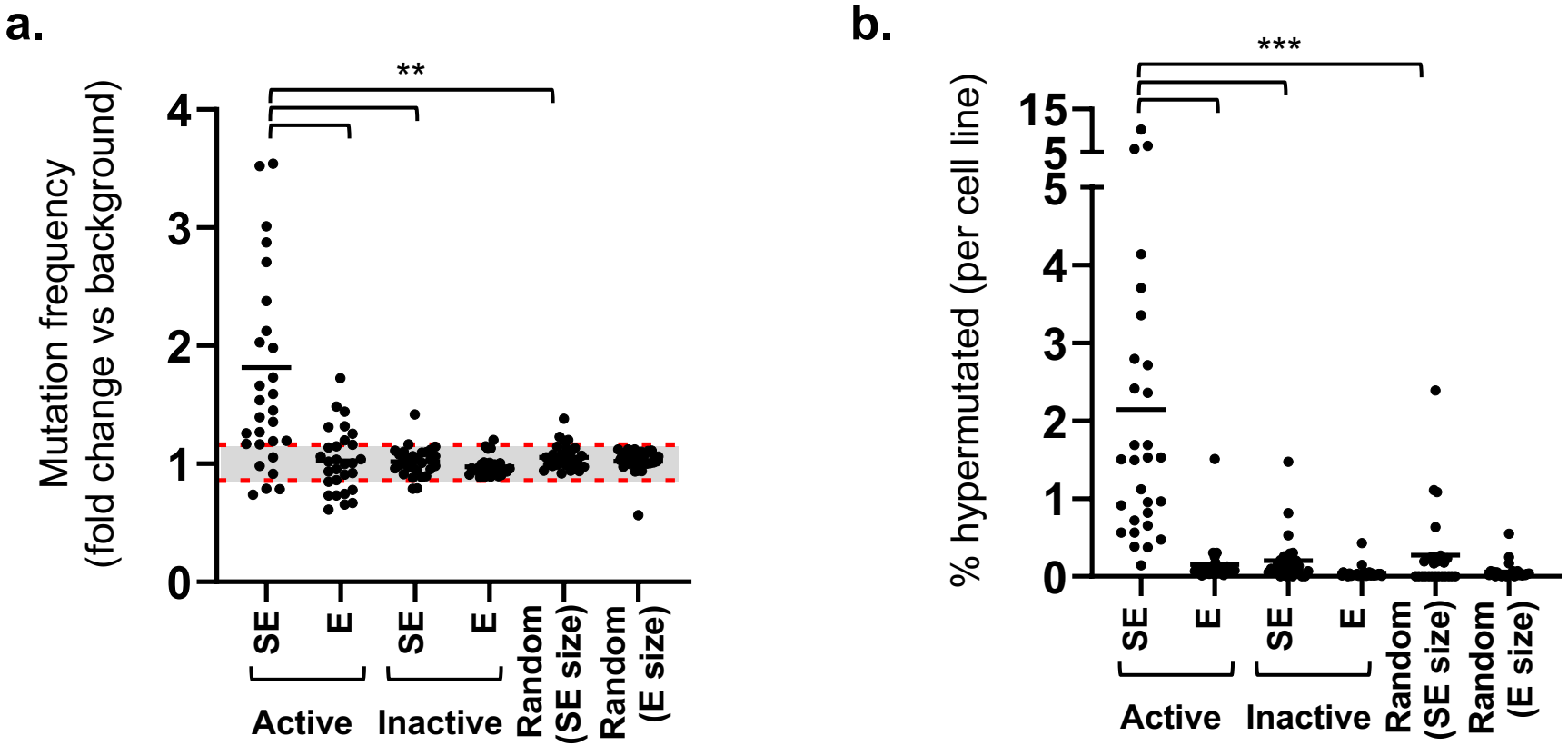


GCB-DLBCL cell lines

ABC-DLBCL cell lines

Normal GC B cells

SE regions are hypermutated in DLBCL cell lines

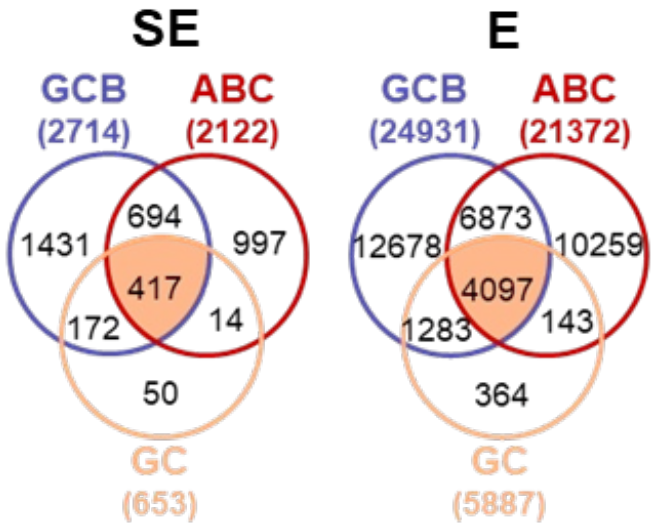


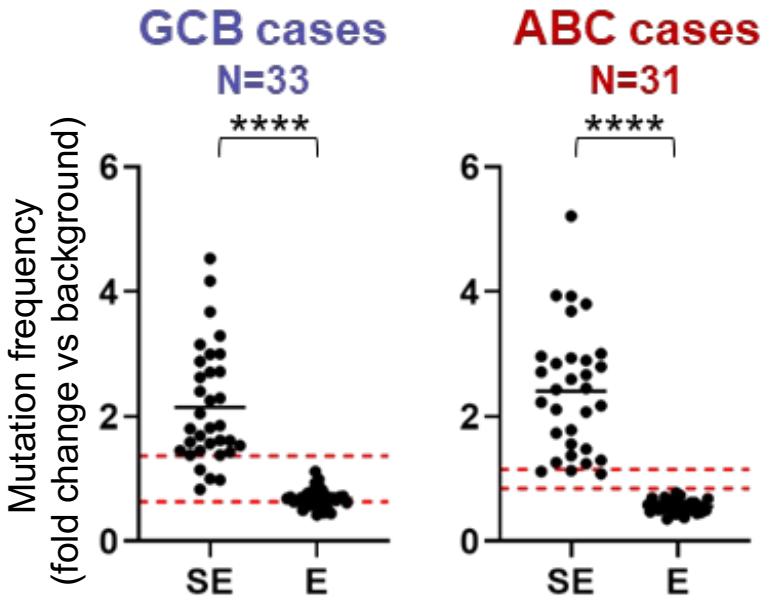
Hypermutated E/SEs:

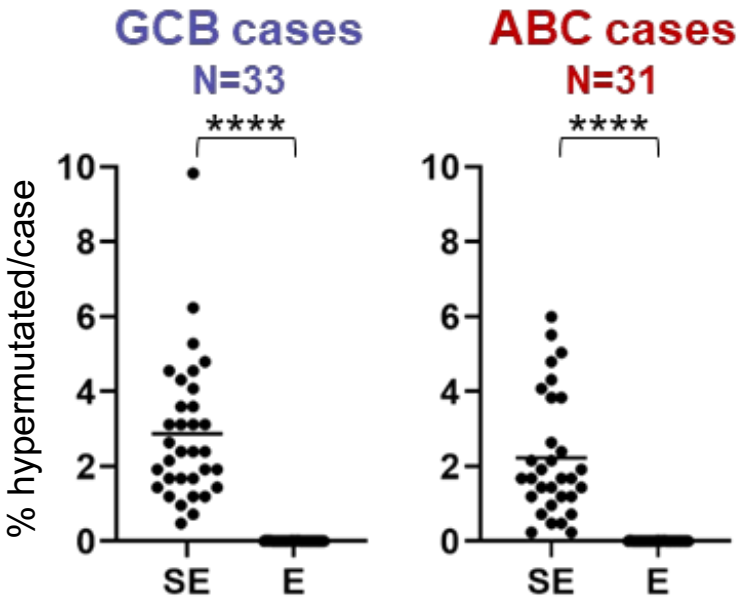
- >=3 mut with intermutation distance <=1kb
- Mutation frequency significantly higher with respect to background (mutations in the rest of the genome)

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

SEs are hypermutated in DLBCL primary cases

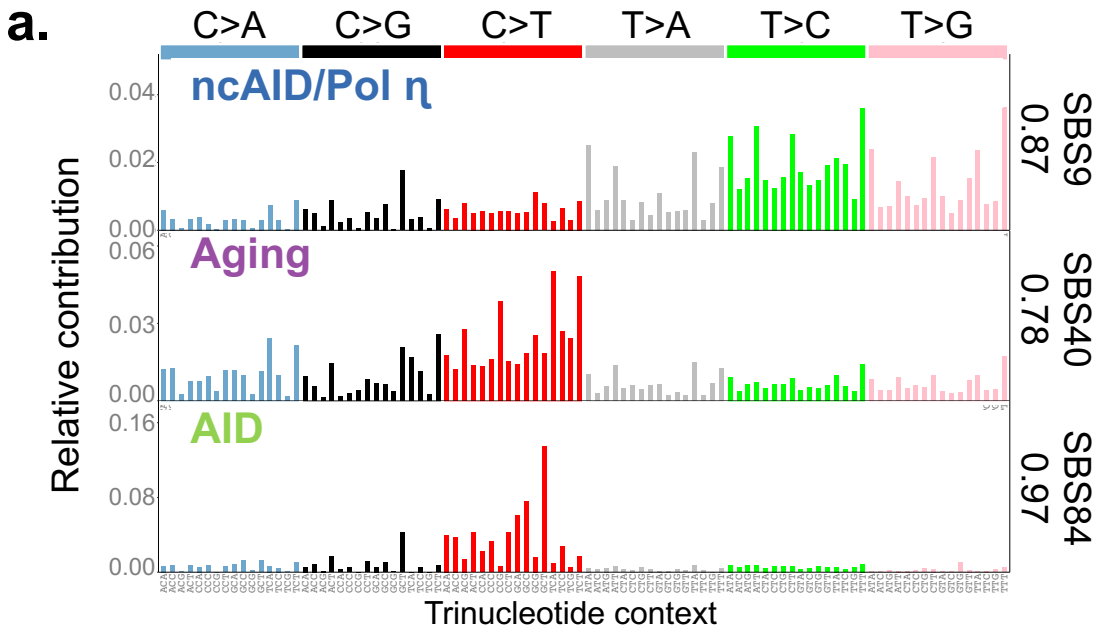


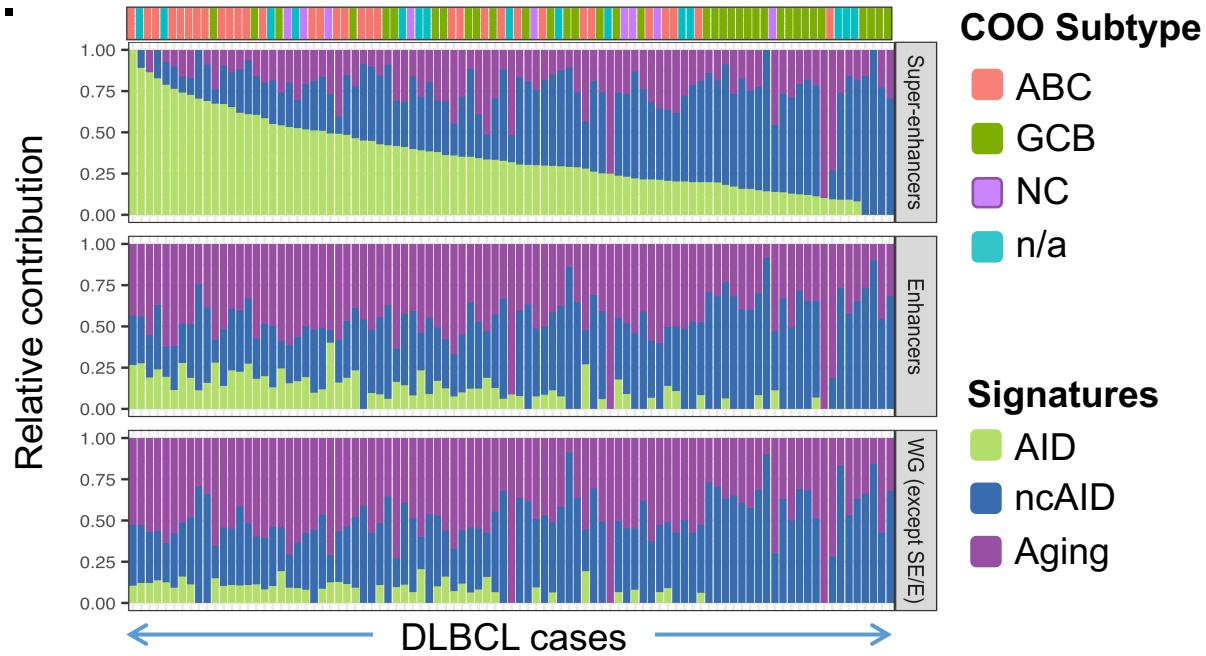


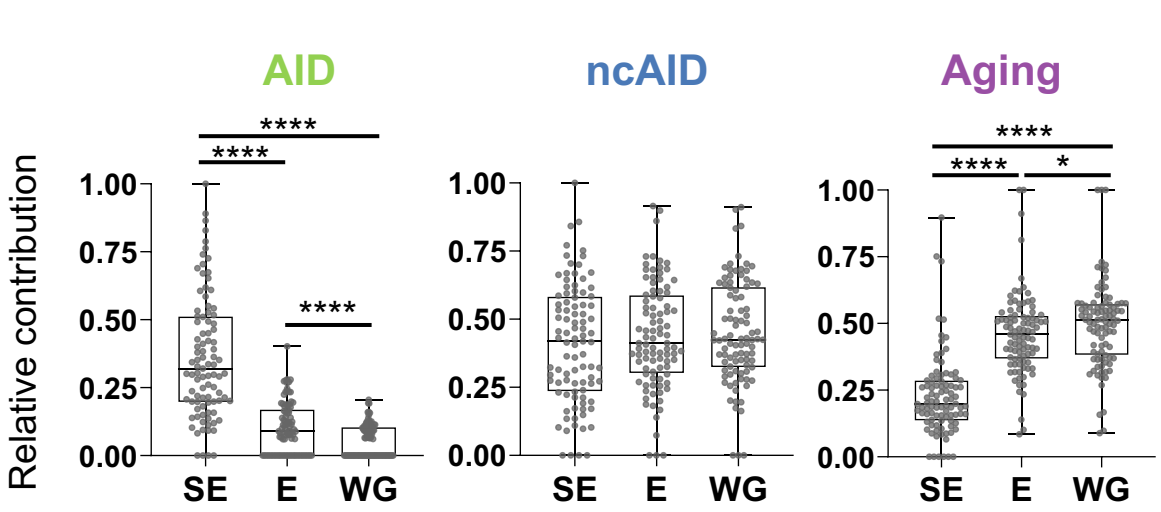


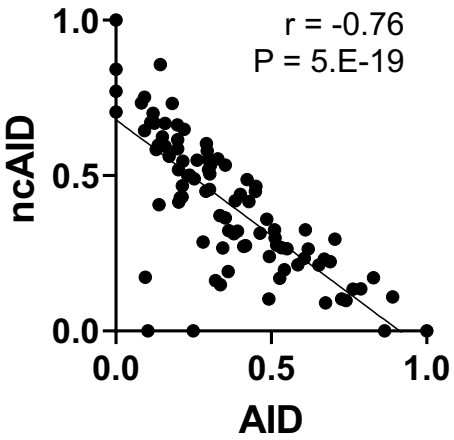
**** p<0.0001,paired t-test

Mutational signatures in SEs display AID hallmarks



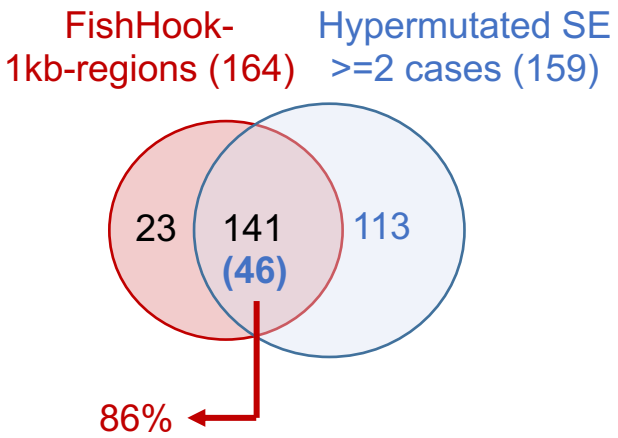




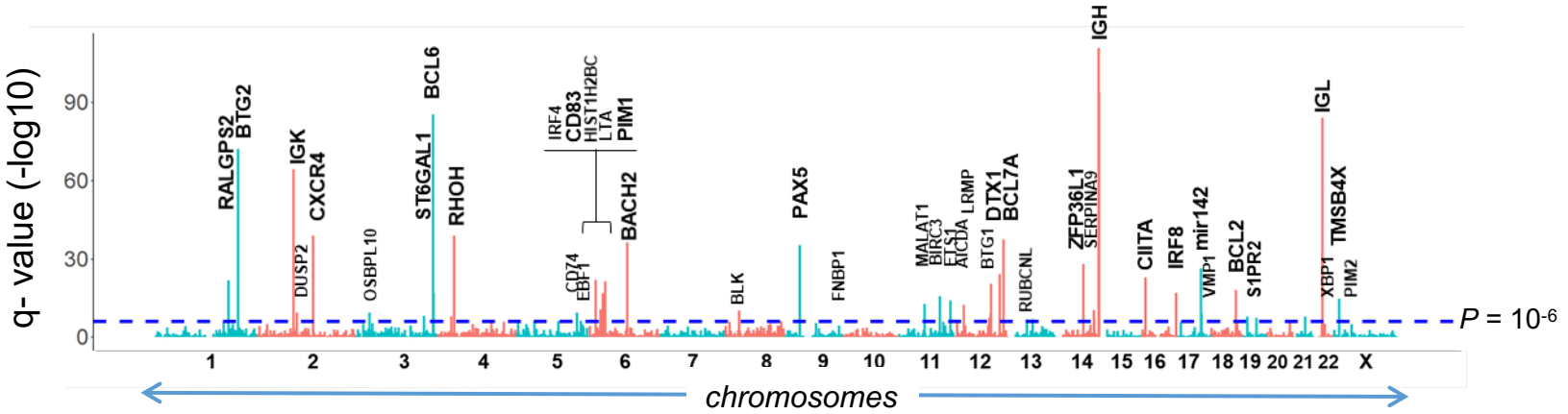


Candidate genes linked to recurrently mutated SEs are enriched in lymphoma oncogenes

a.



b.



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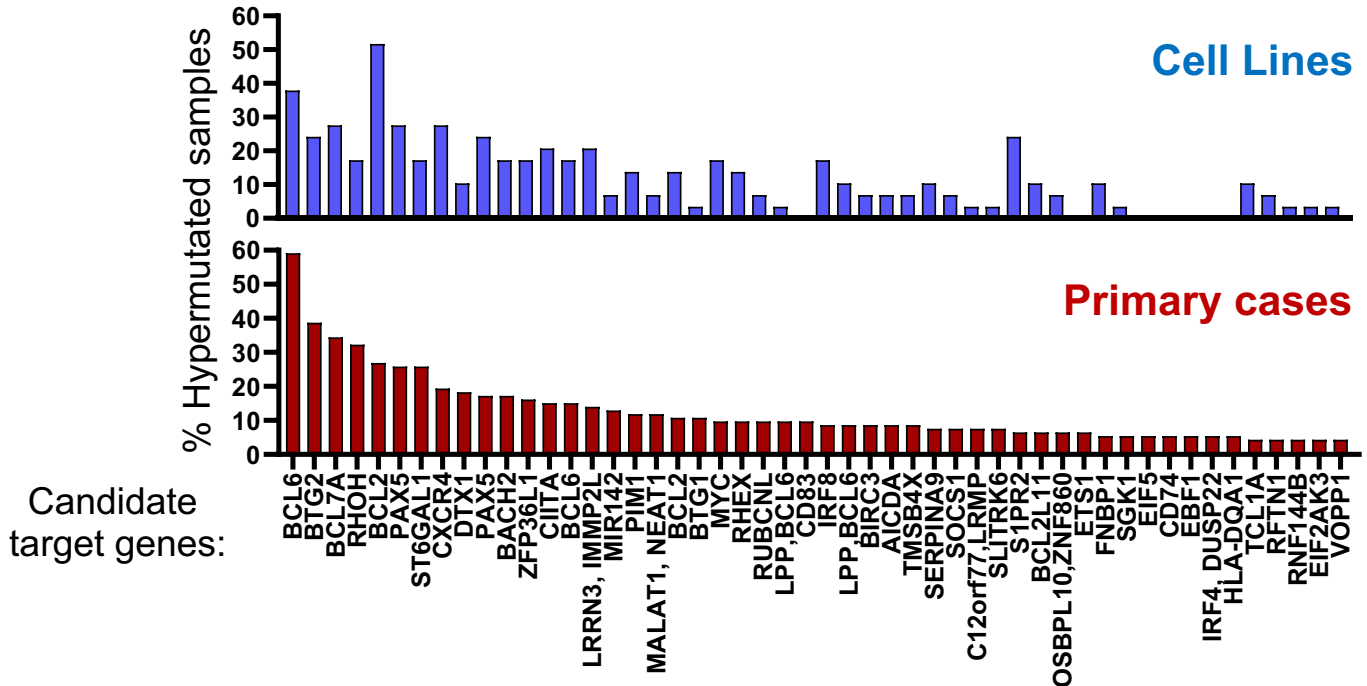
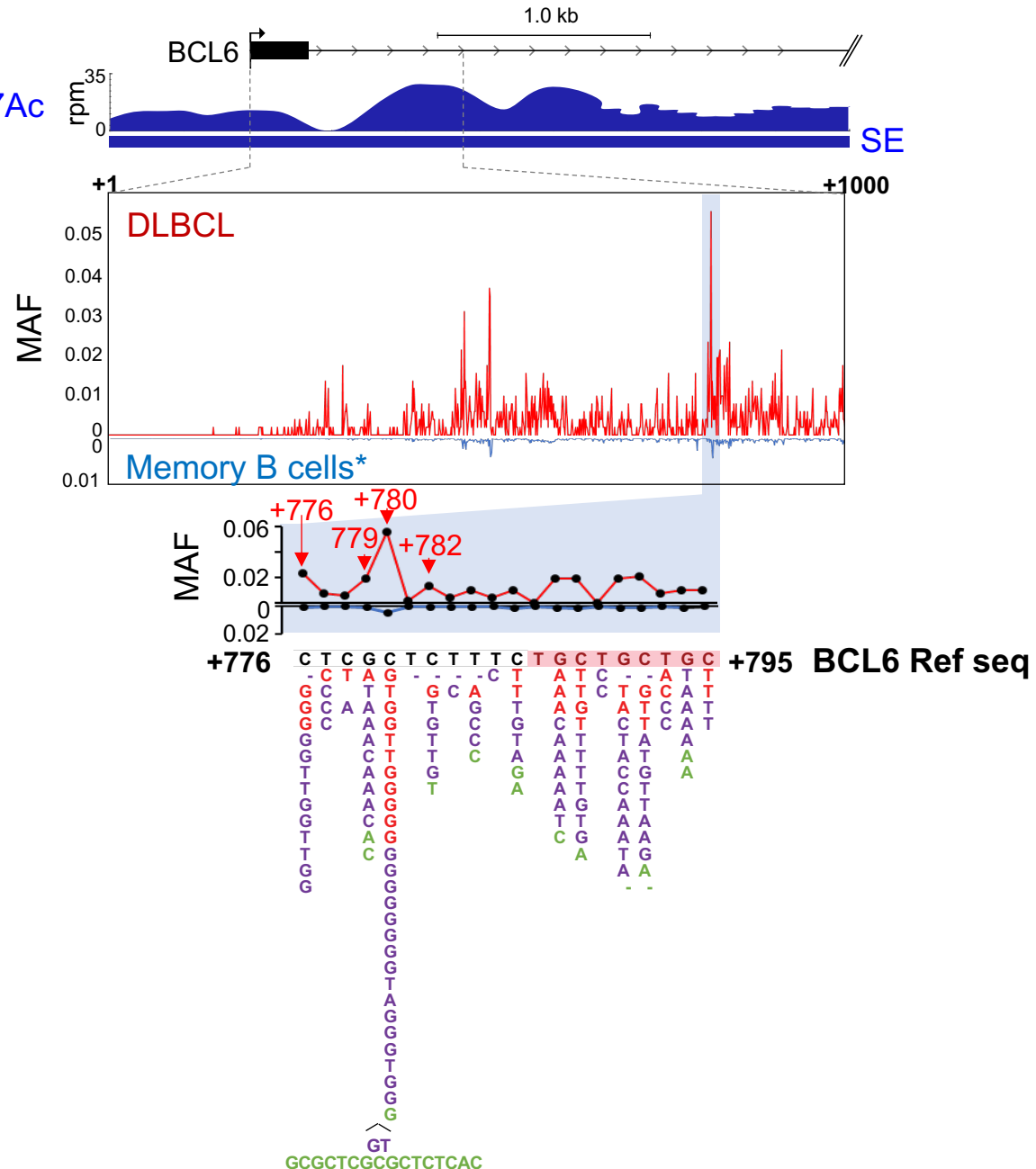


FishHook
(Imielinski et al, Cell 2017)

A highly recurrent mutational hotspot in the BCL6 intragenic SE



AID motif

Cases tested:

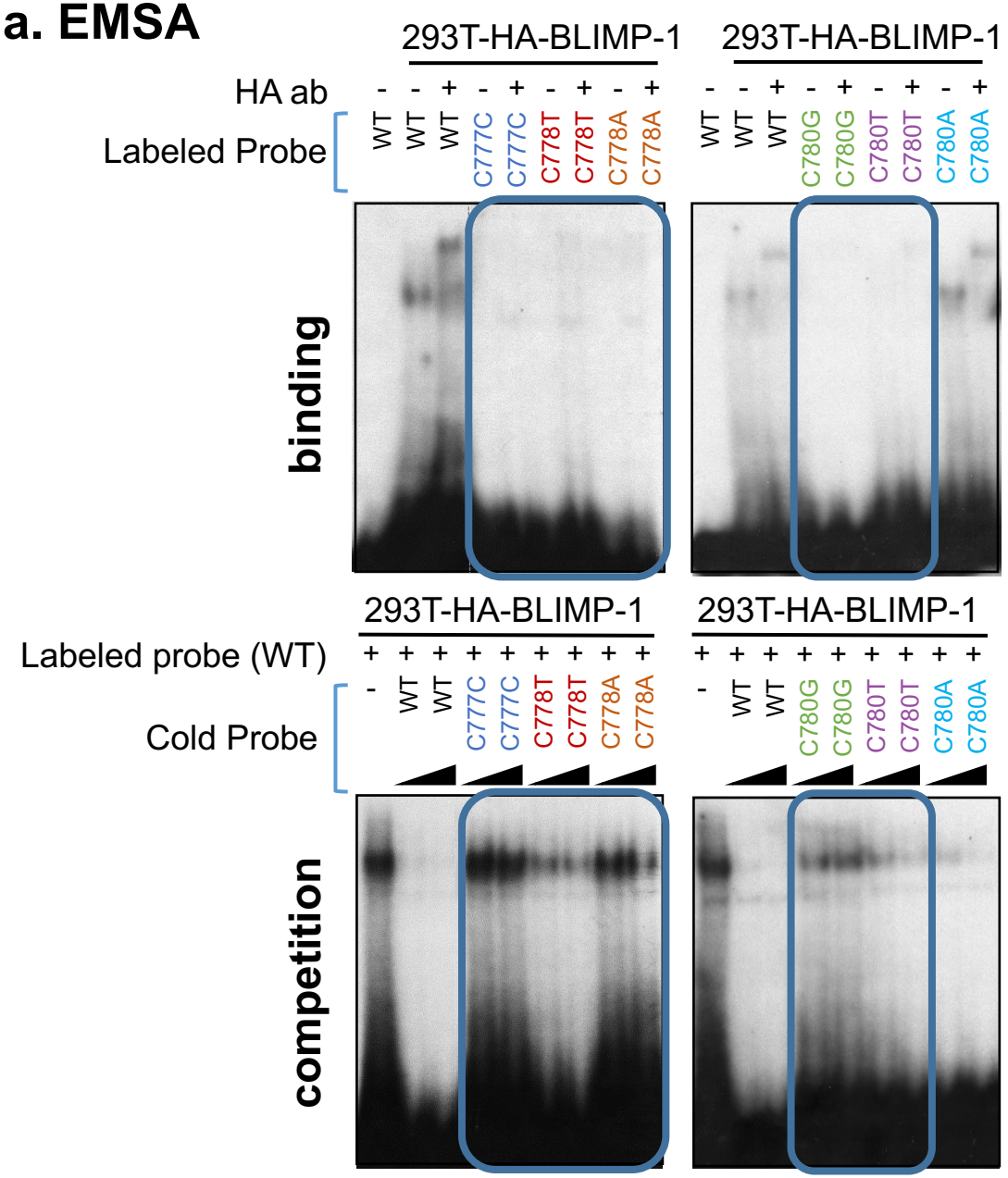
- primary cases (WGS, n= 75)
- primary cases (Sanger, n=176)
- cell lines (WGS, n=23)

(BCL6 Tx excluded)

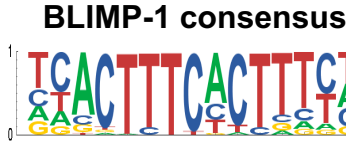
*Shen JC at al., PNAS, 2019

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

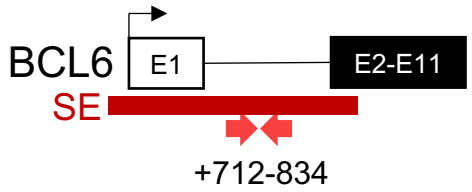
a. EMSA

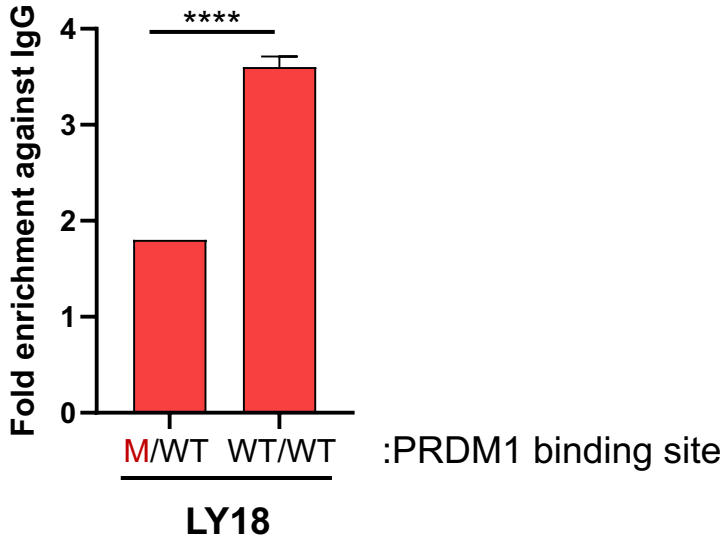


b. ChIP-qPCR (CD40 stimulated cells)

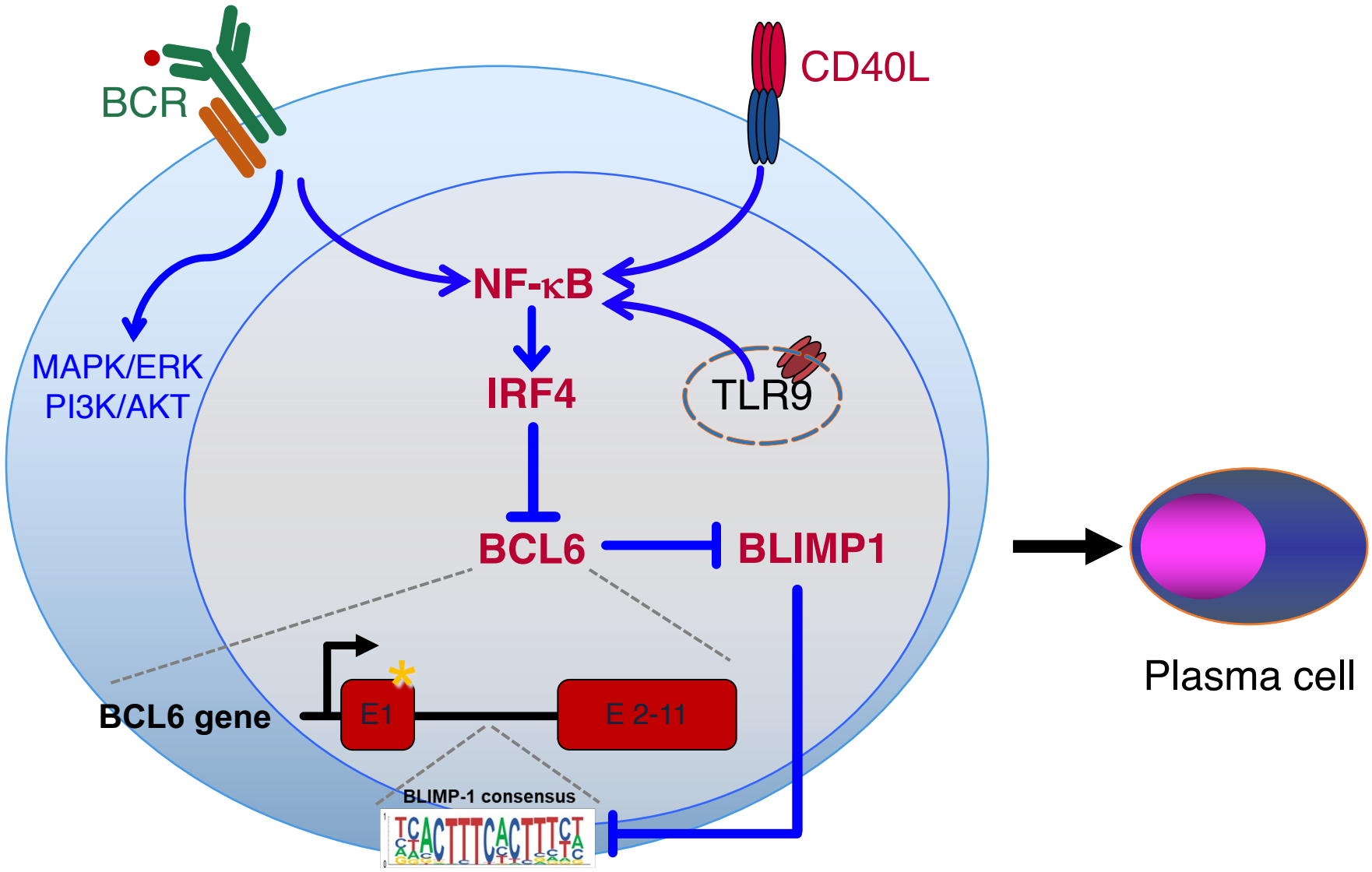



```
WT     +769 TCGCGCTCTCGCTCTTTCTGC +789
LY18A  +769 TCGCGCTCTCGGTCTTTCTGC +789
LY18B  +769 TCGCGCTCTCGCTCTTTCTGC +789
```



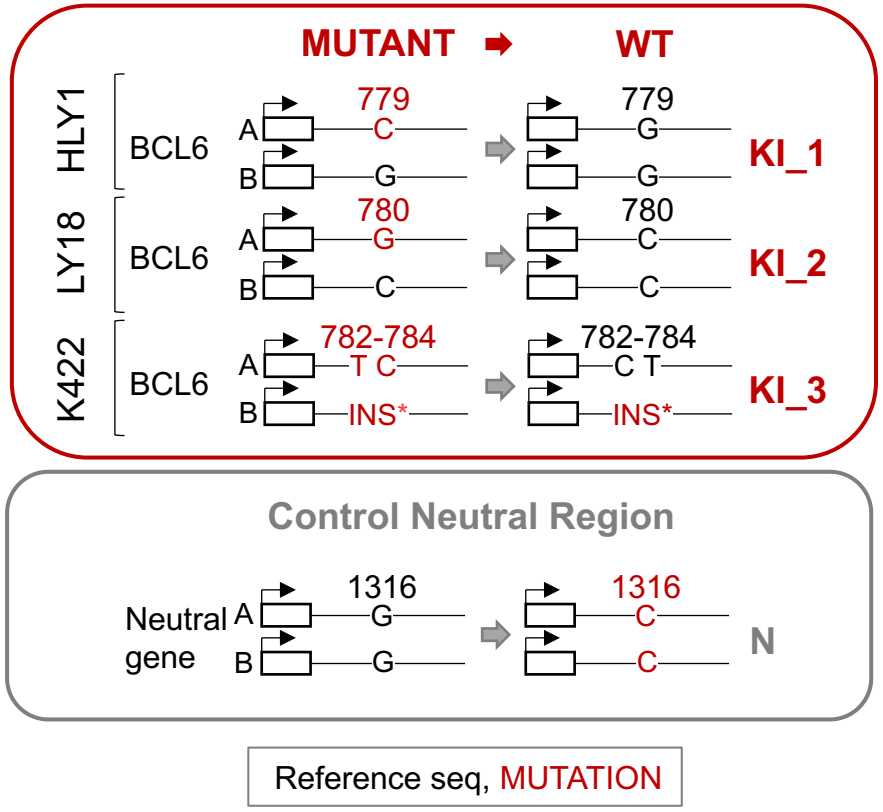


Downregulation of BCL6 expression is required for the initiation of post-GC differentiation

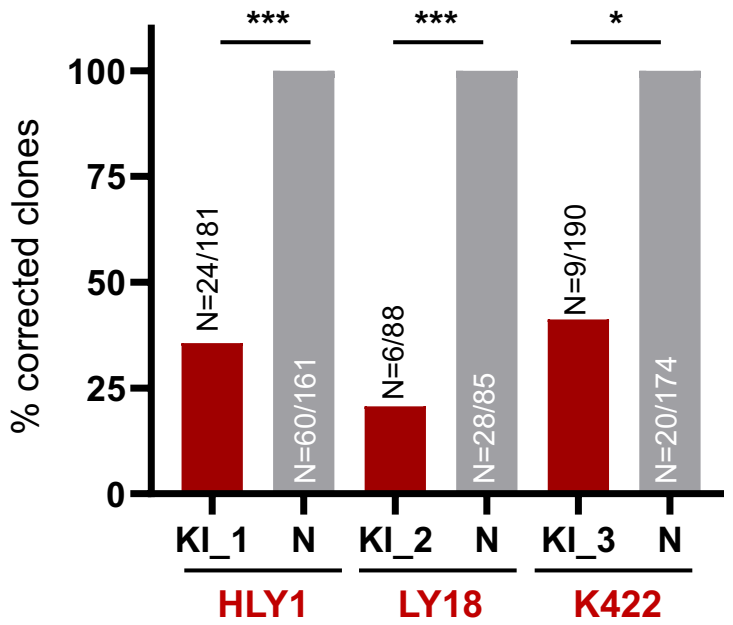


Correction of mutations in BCL6 SE leads to counter-selection

a. CRISPR/Cas9-editing of endogenous site

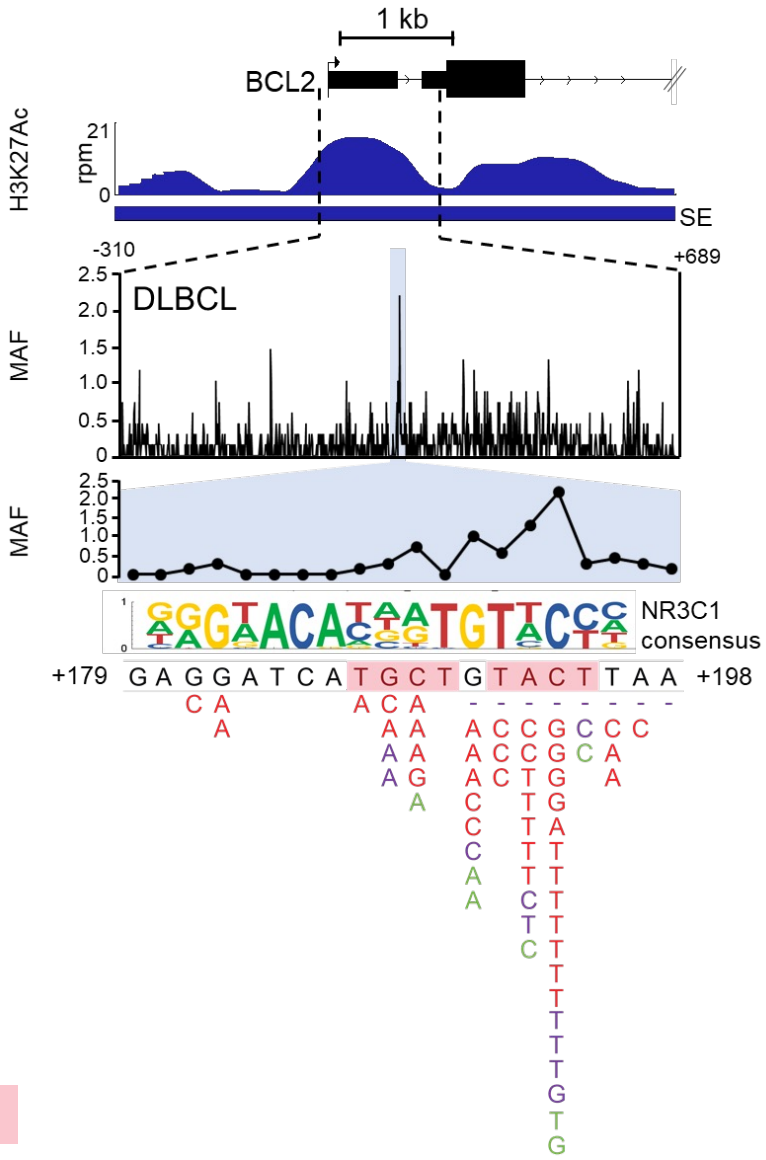


b. Clones Recovery



* Ins 16bp 780_781

Recurrent mutations in BCL2 intragenic SE target NR3C1 binding site

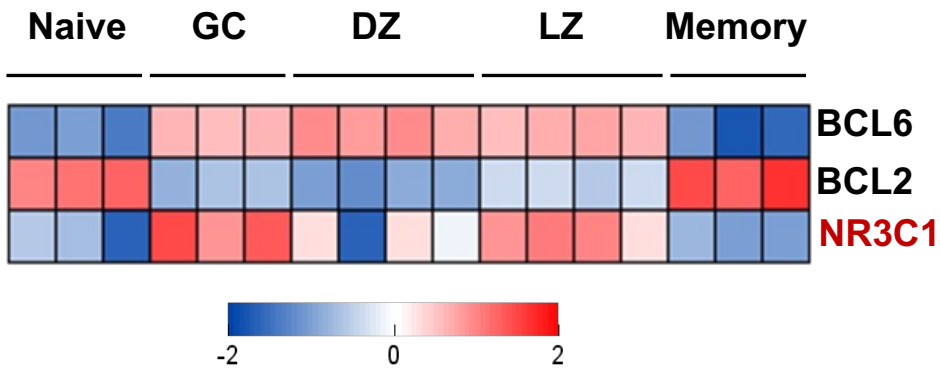


AID motif

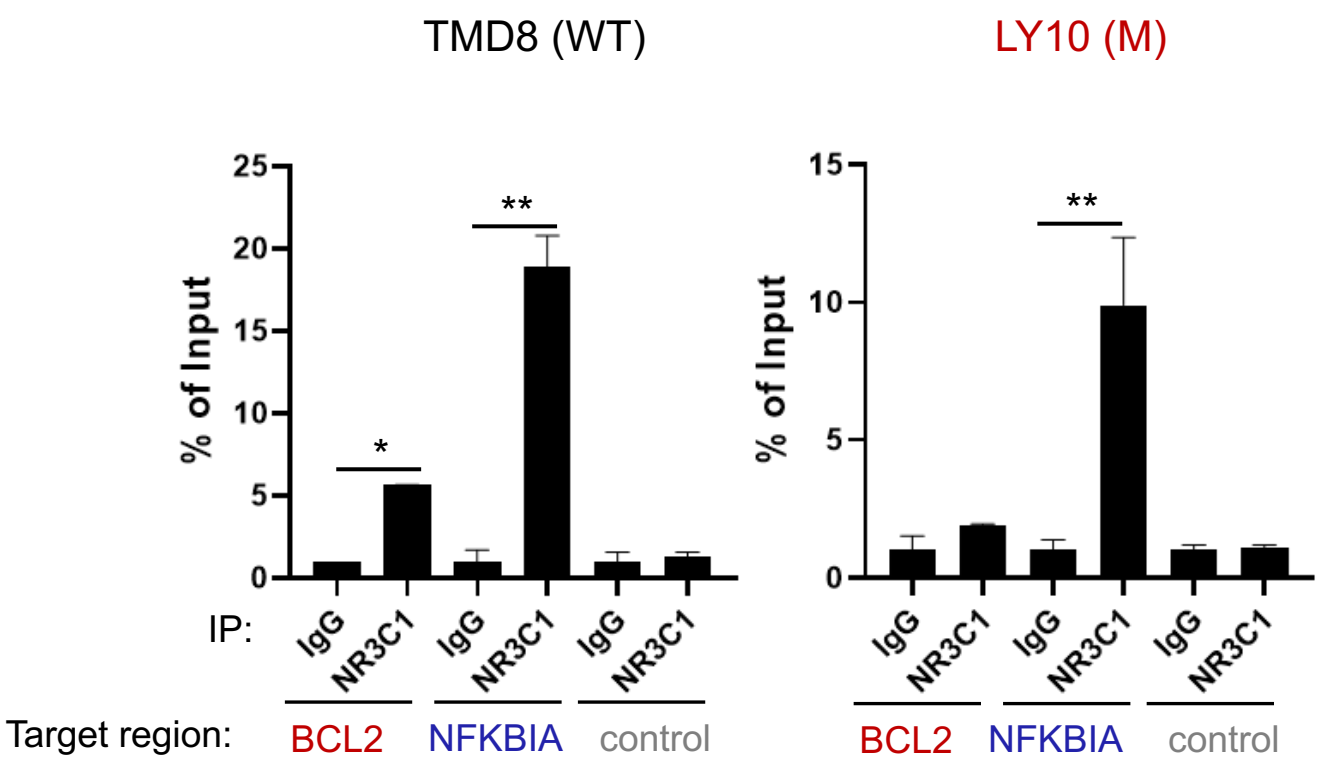
(BCL2Tx cases excluded)

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

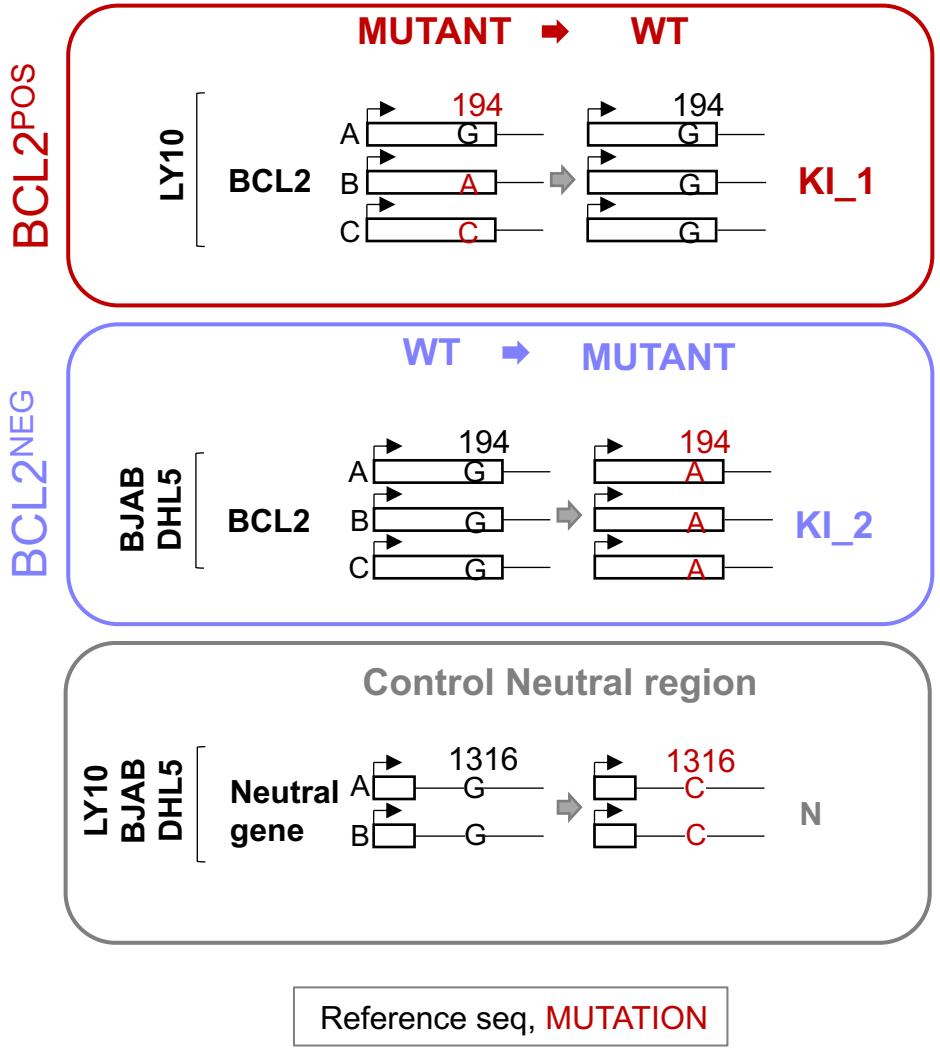


Mutations in BCL2 intragenic SE abrogate NR3C1 binding in DLBCL cells

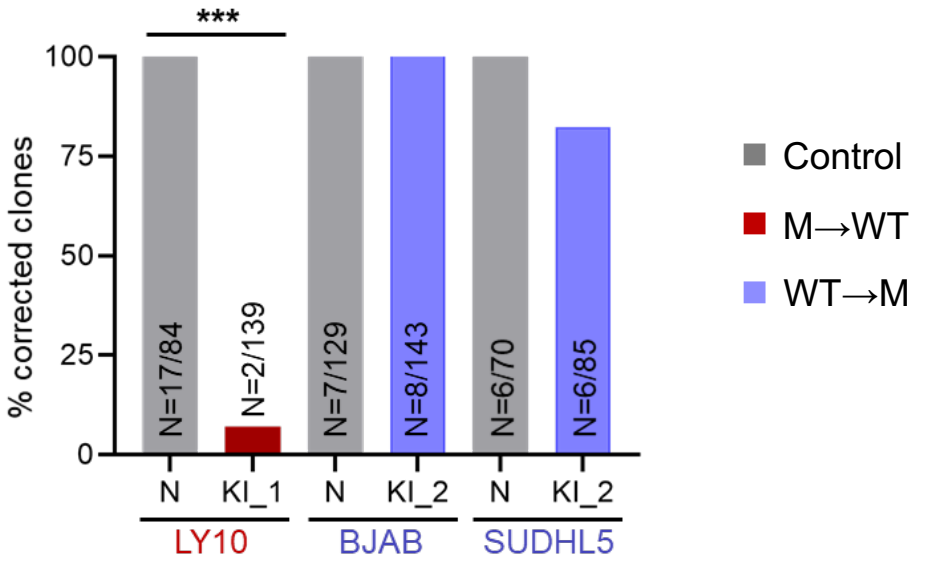


Correction of mutations in BCL2 iSE leads to counter-selection

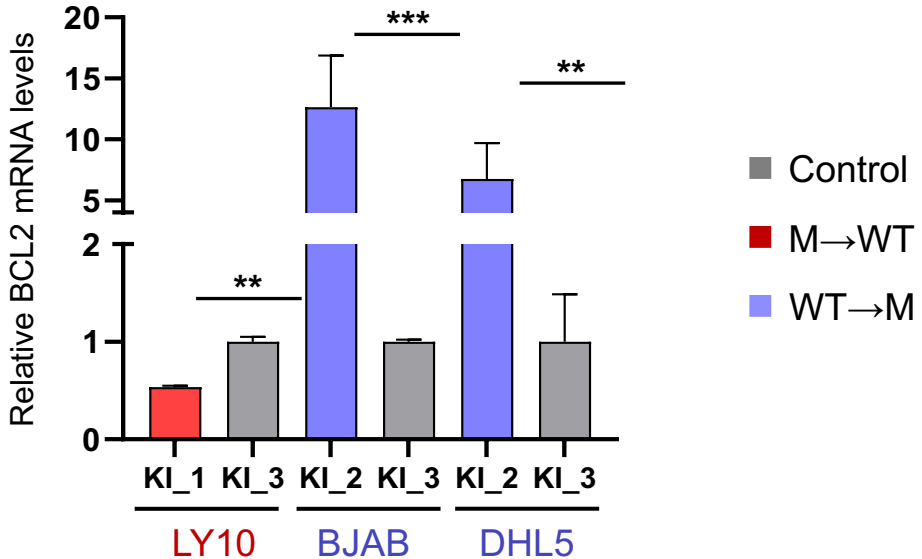
a. CRISPR/Cas9-editing of endogenous site



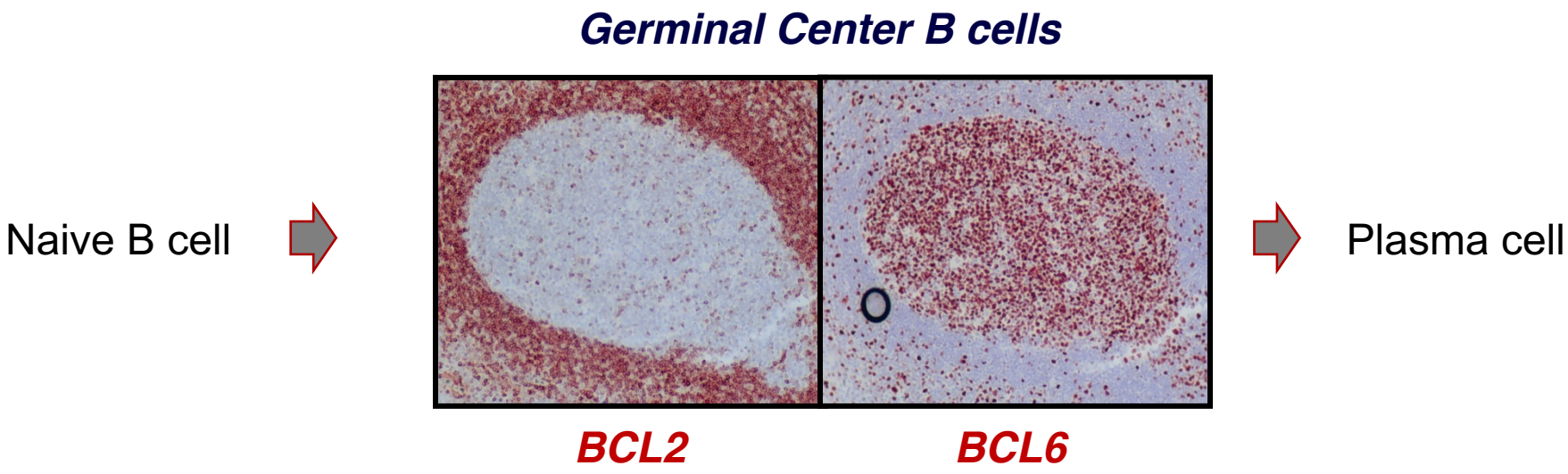
b. Clones Recovery

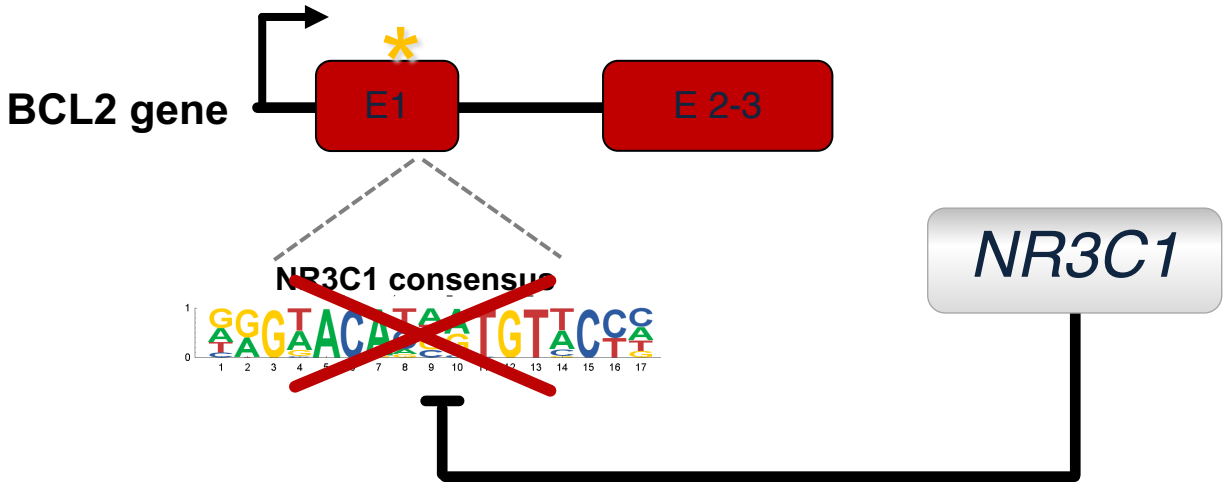


c. BCL2 mRNA expression

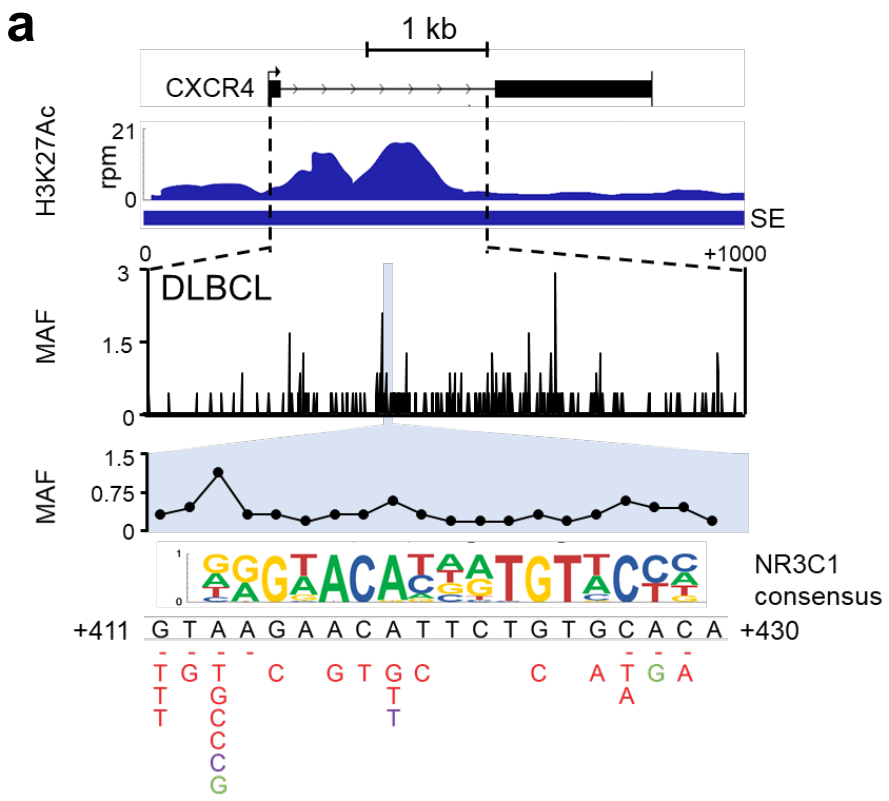


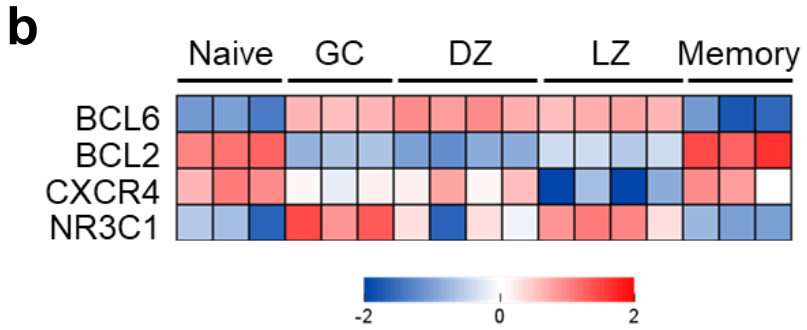
Multiple genetic lesions contribute to BCL2 deregulation in lymphoma





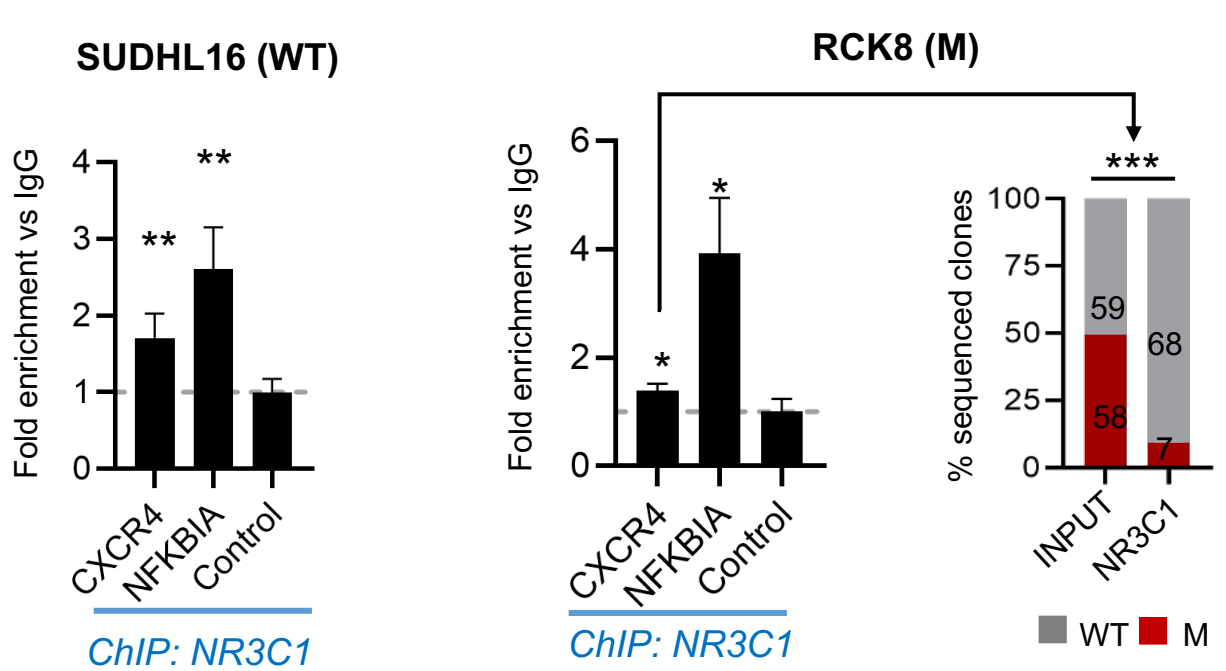
A mutational hotspot in the CXCR4 SE abrogates DNA-binding and transcriptional activation by the glucocorticoid receptor (NR3C1)





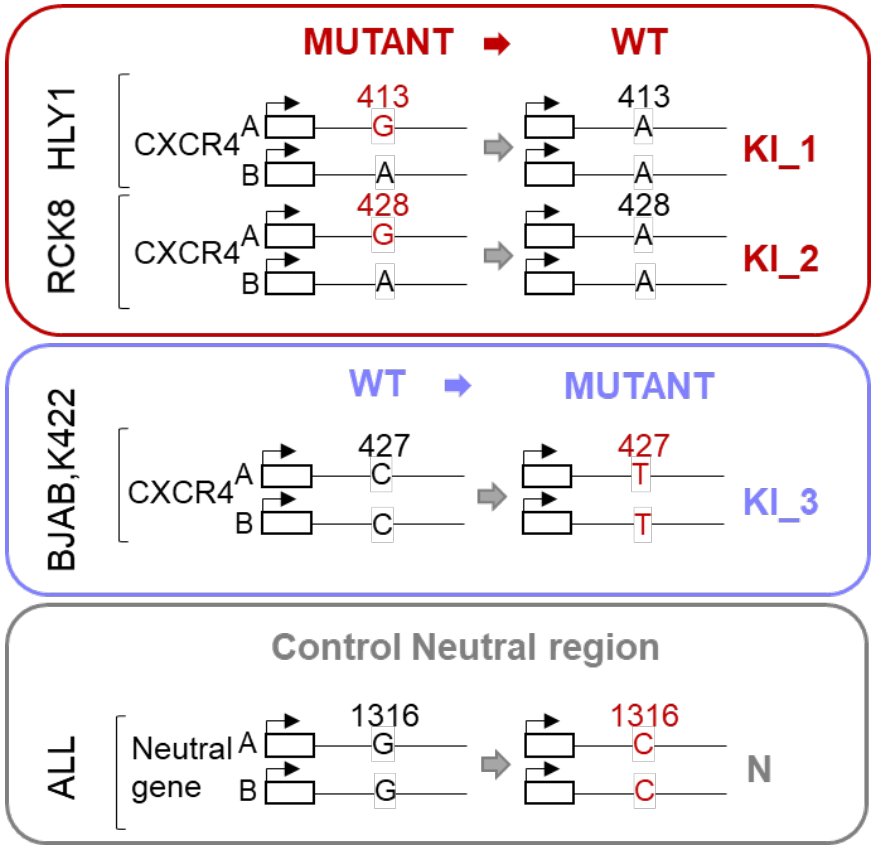
CXCR4

- 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



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

a



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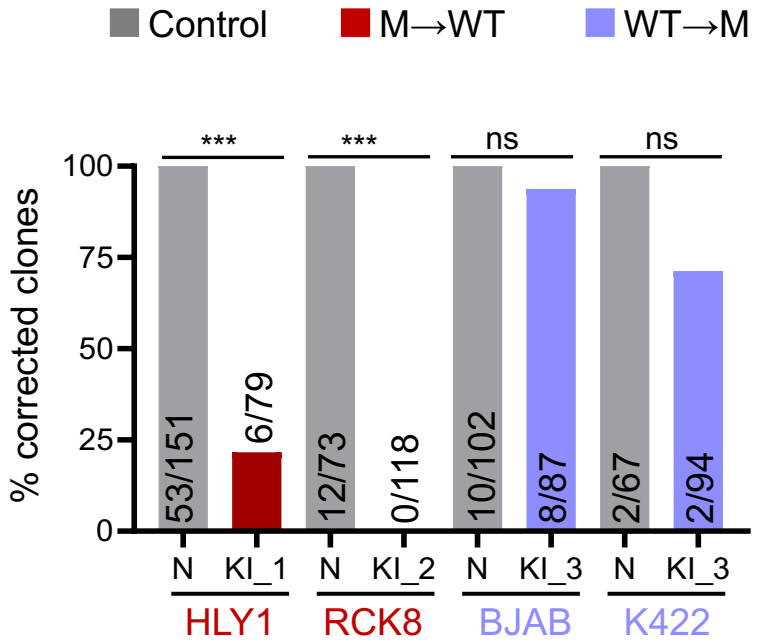

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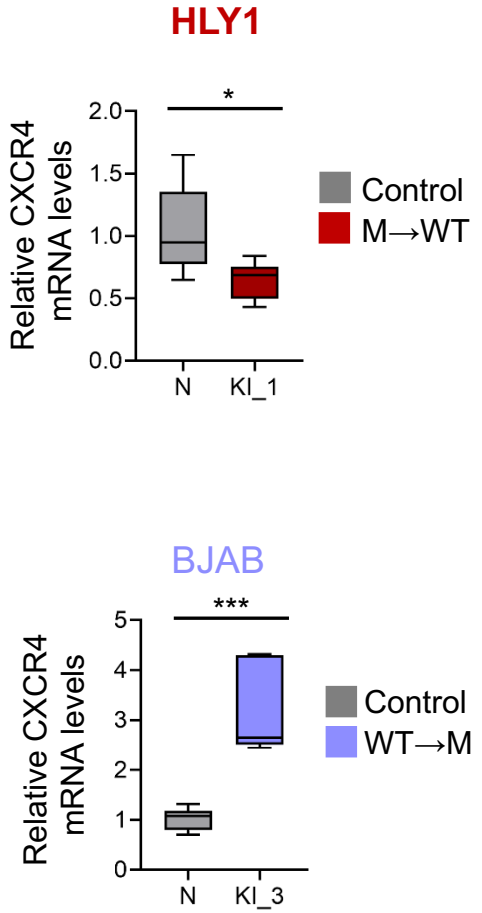
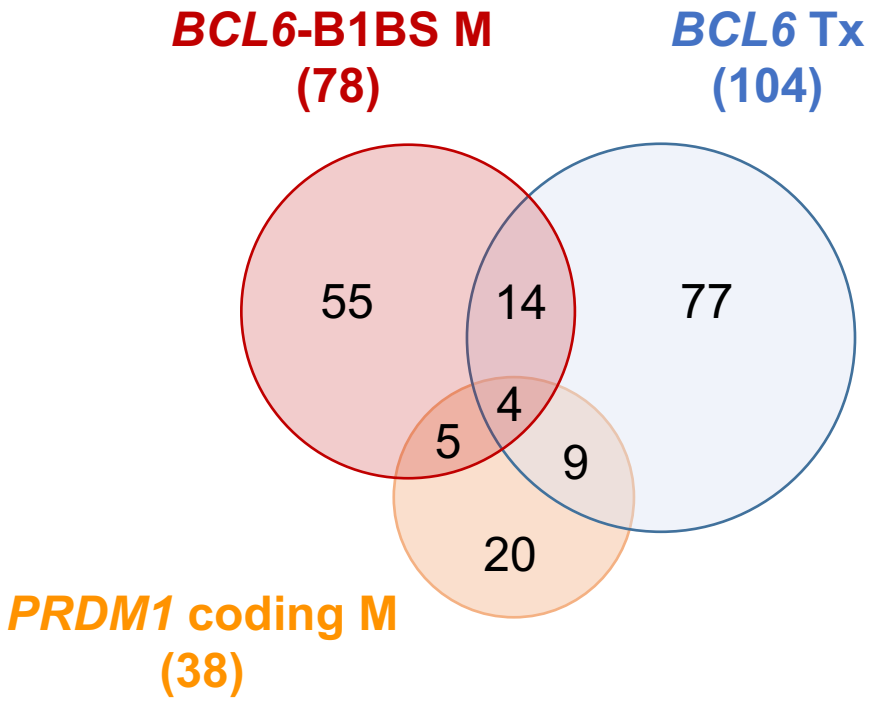
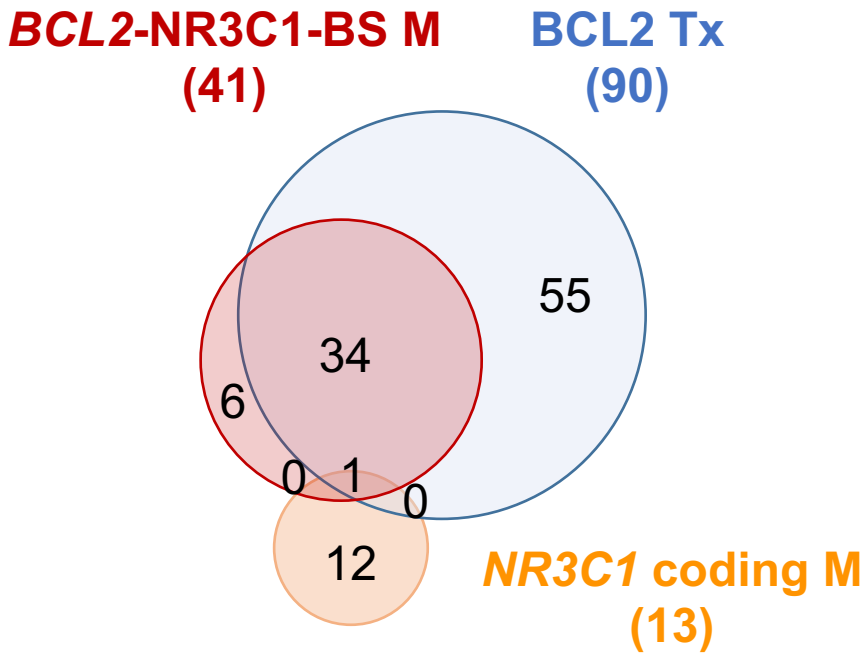
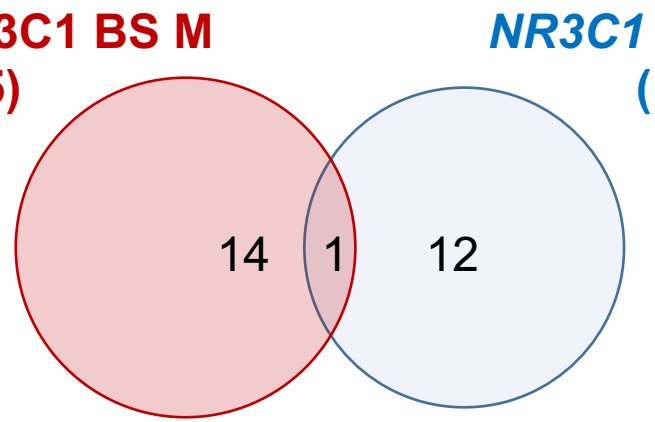


SE-mutations identify complementary mechanisms deregulating target gene expression







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

Acknowledgements

Institute for Cancer Genetics
Columbia University

Elodie Bal
Rahul Kumar
Marco Fangazio
Chuanijang Yu
Qiong Shen
Bowen Cai
Tongwei Mo
Hongyan Tang
Clarissa Corinaldesi
Claudio Scuoppo

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Mara Holloman
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Raul Rabadan

Former Members (2008-2018)

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Marta Crespo
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N. Compagno

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AID-mediated aberrant somatc hypermutation in DLBCL

nature
International journal of science

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

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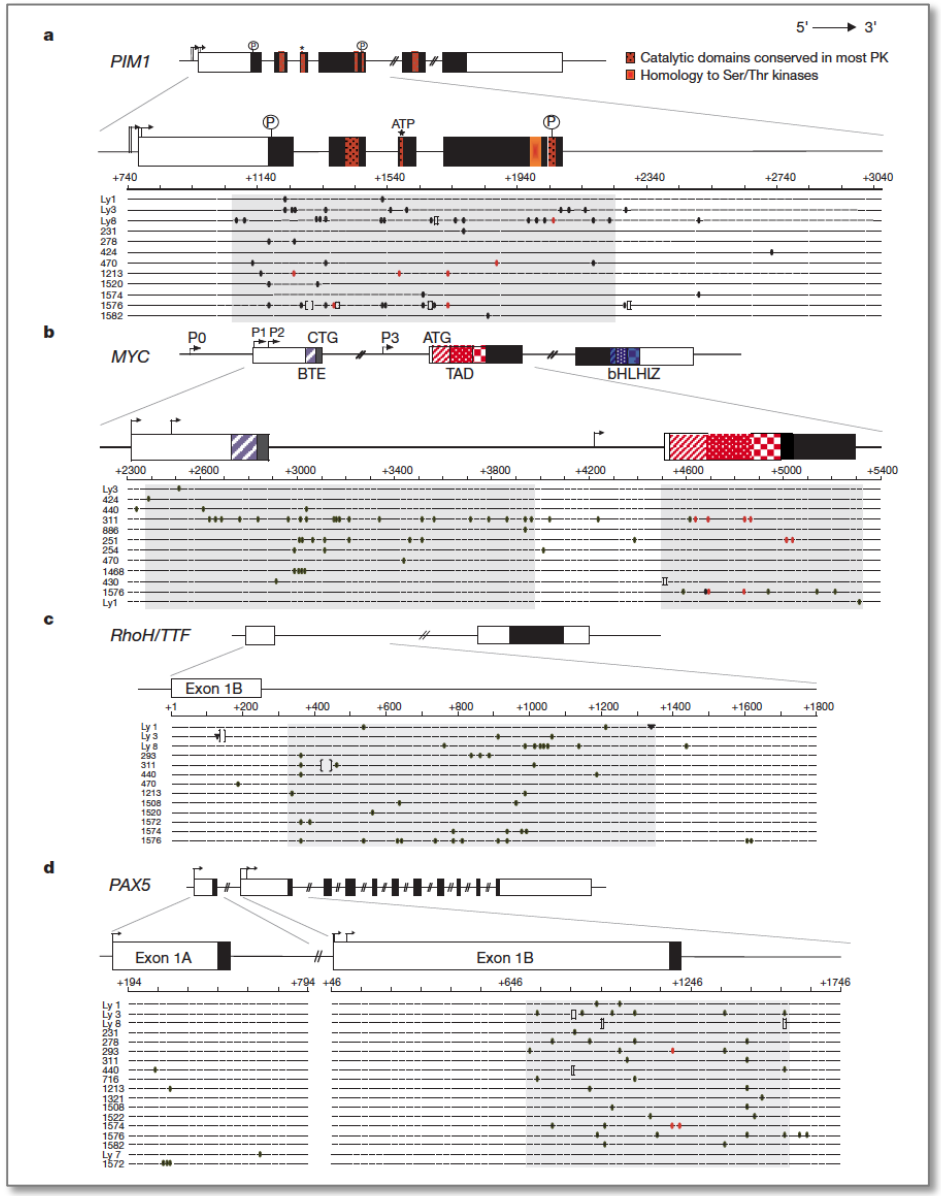
Nature, 2001



- 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

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

Pasqualucci et al., *Nature Genetics 2008*