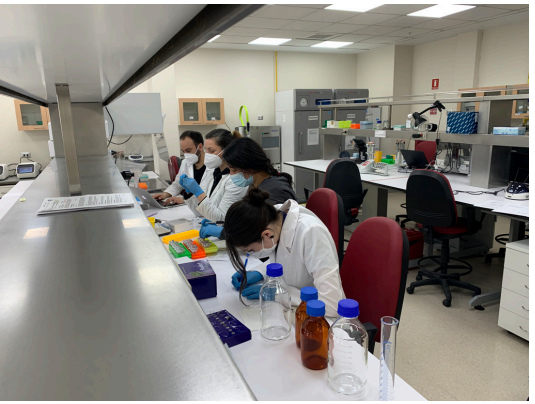

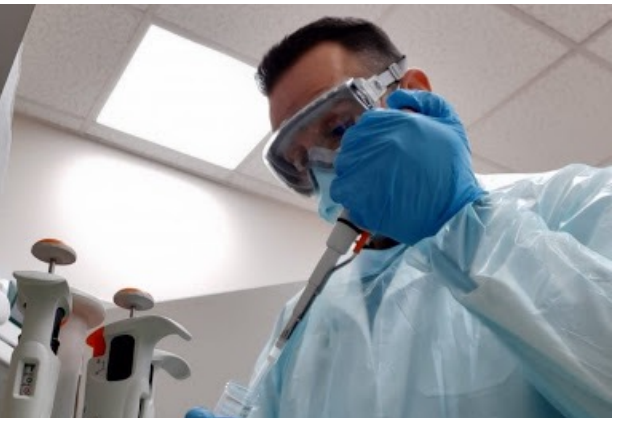

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cATG
CENTRO AUSTRAL DE TECNOLOGÍA GENÓMICA

Avances en Inmunopatogenesis de Neoplasias Linfoides B Indolentes

Puerto Varas, Abril 2024

UNIVERSIDAD DE MAGALLANES
CHILE

Centro Asistencial Docente y de Investigacion
CADI - UMAG

Public State Research Infrastructure at University of Magallanes

- 7.000 sqmt
- State-of-the-art equipment
- Basic and traslational research groups
  - Modulo Asistencial (clinical training and clinical research)
  - Laboratory of Molecular Medicine (diagnostics and traslational research)
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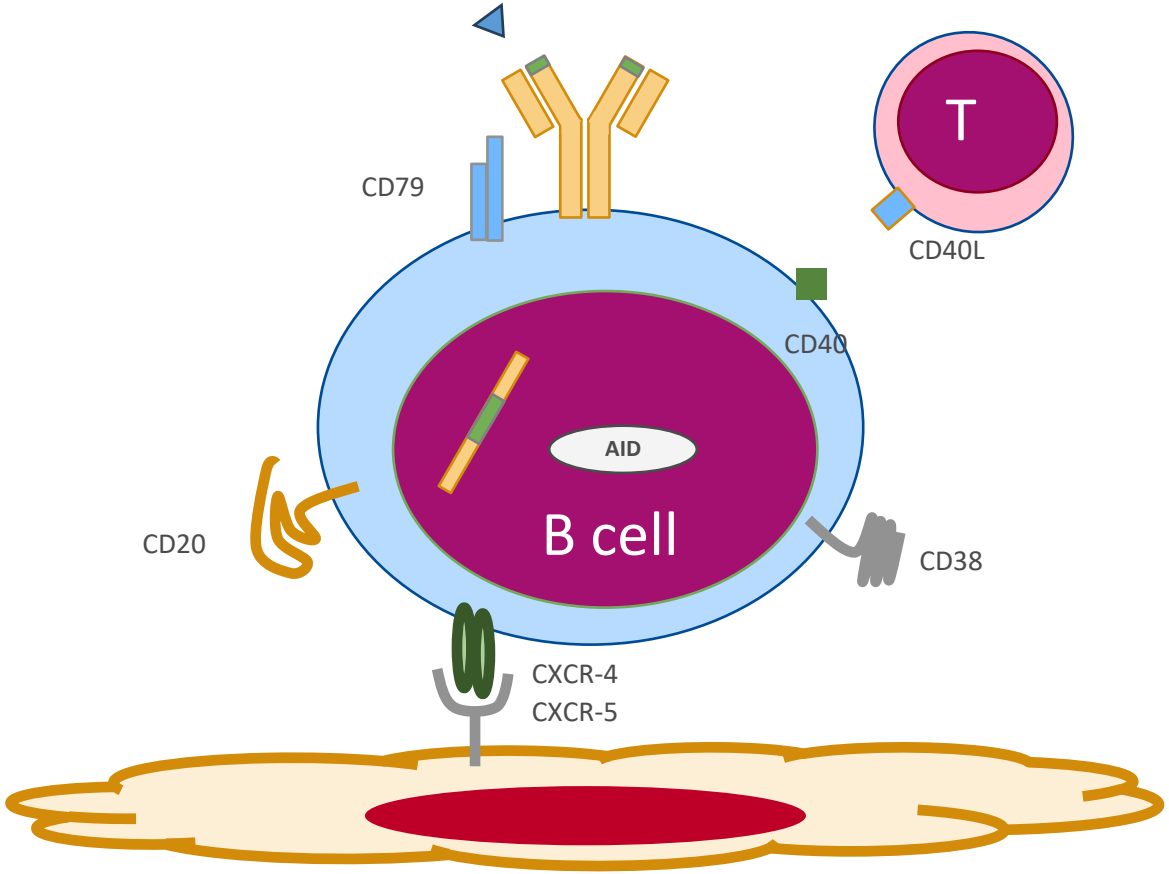
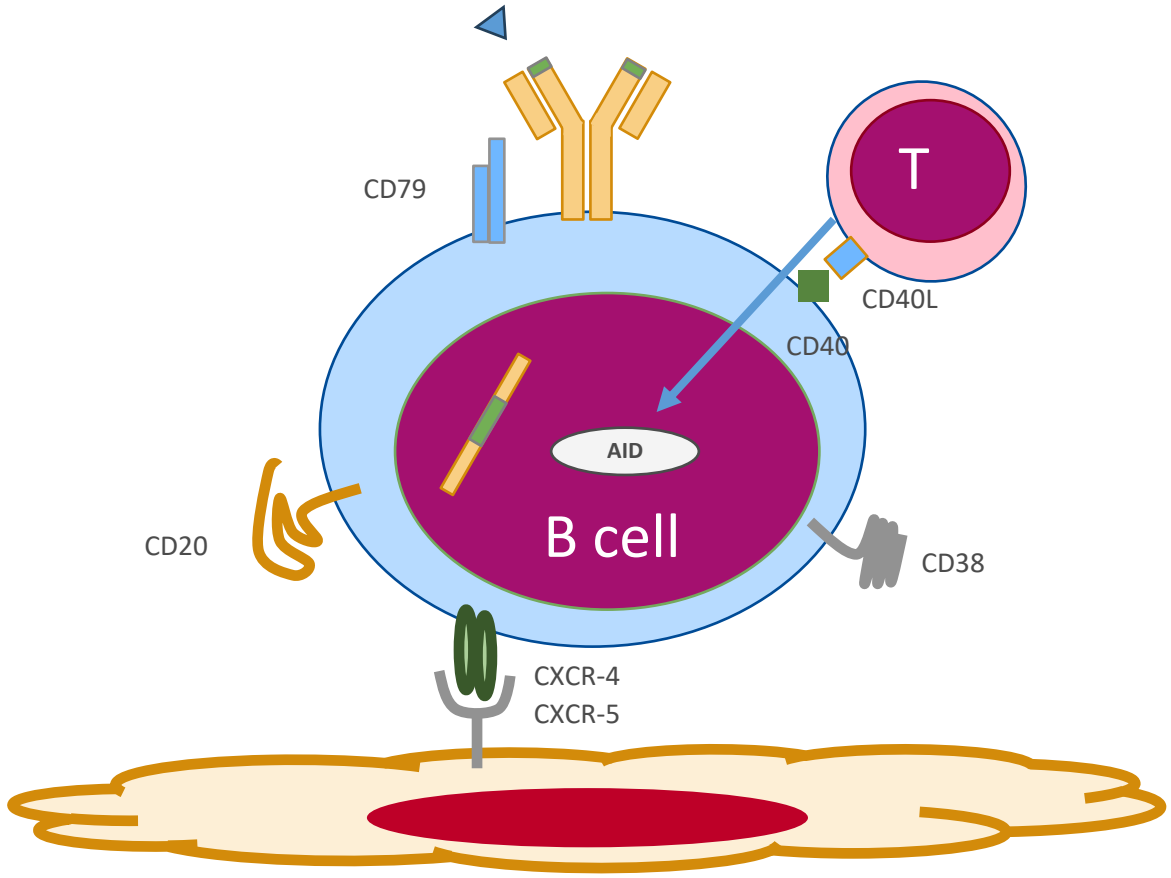
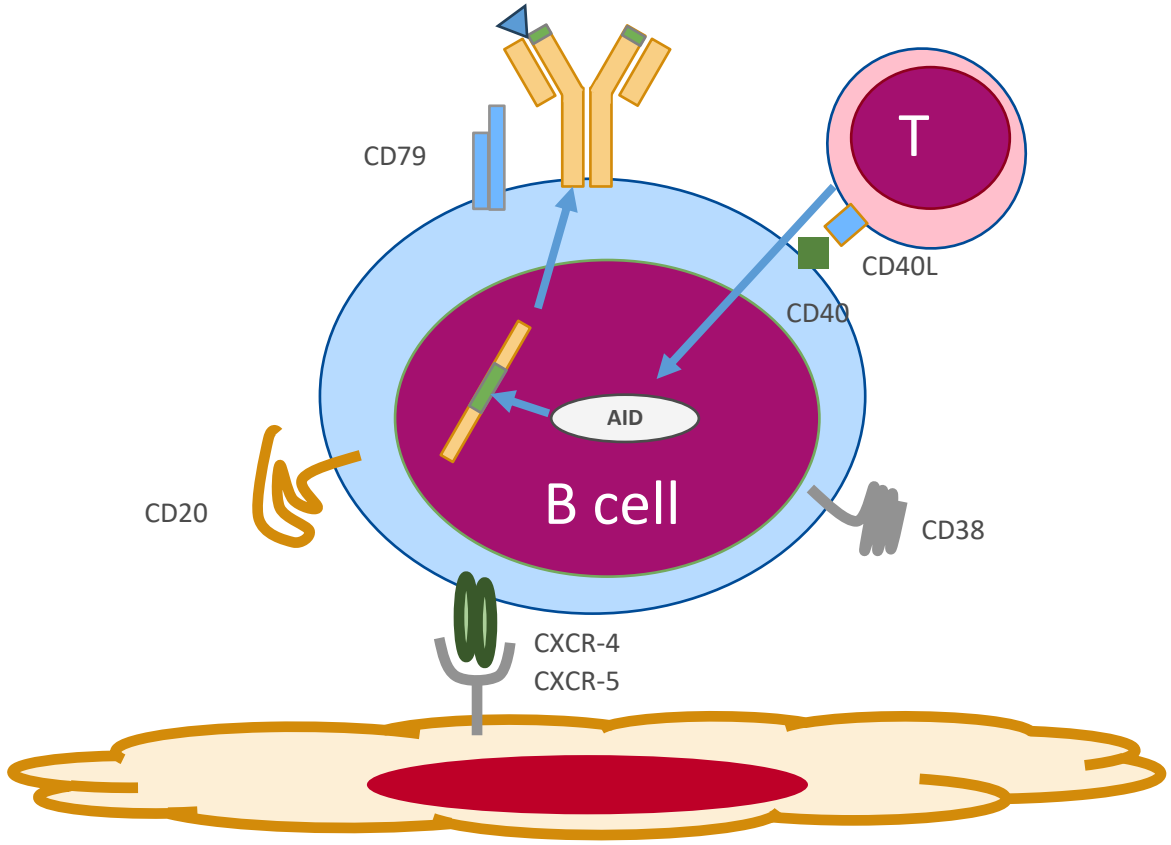
The B-cell



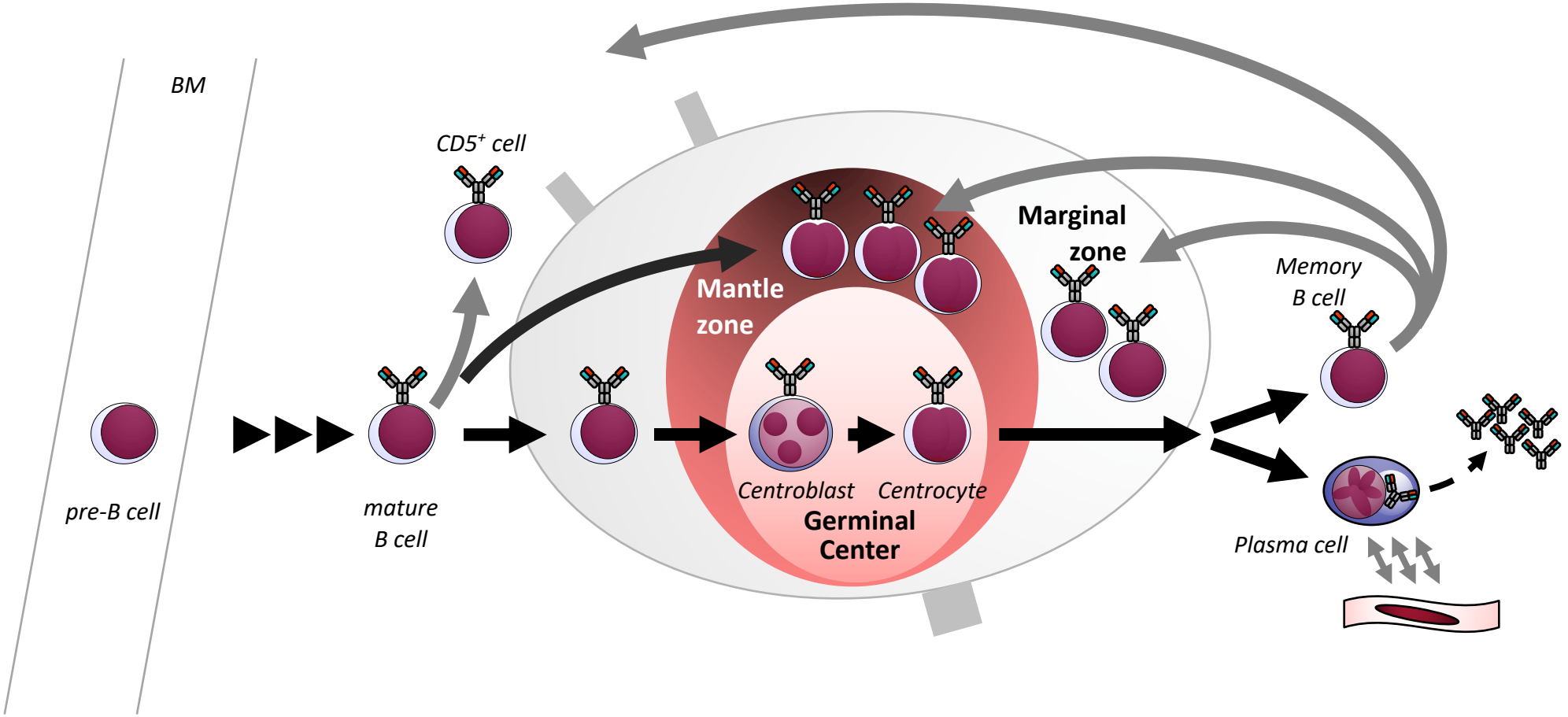
B-cell physiological interactions



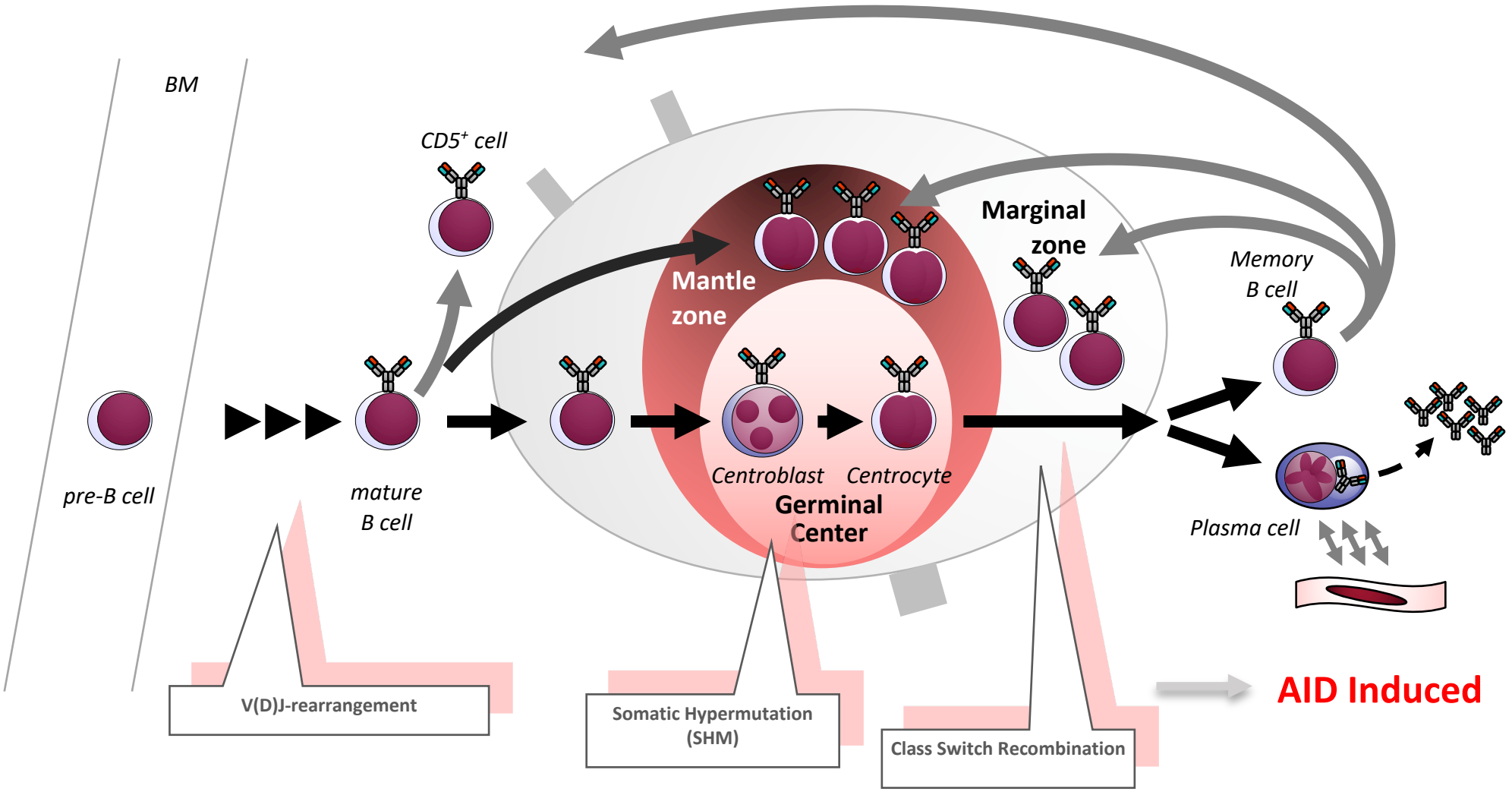
B-cell physiological interactions



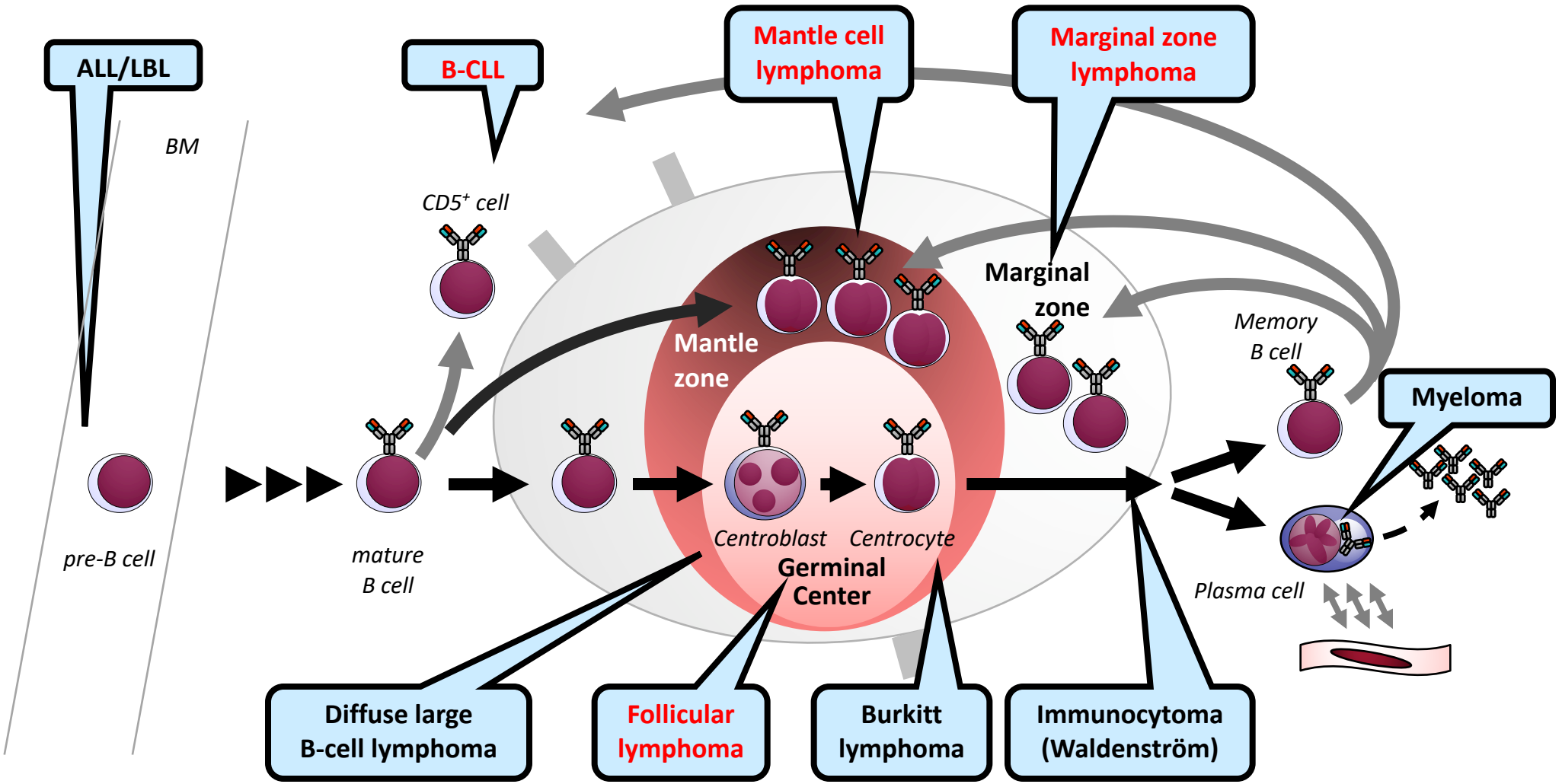
B-cell Biology



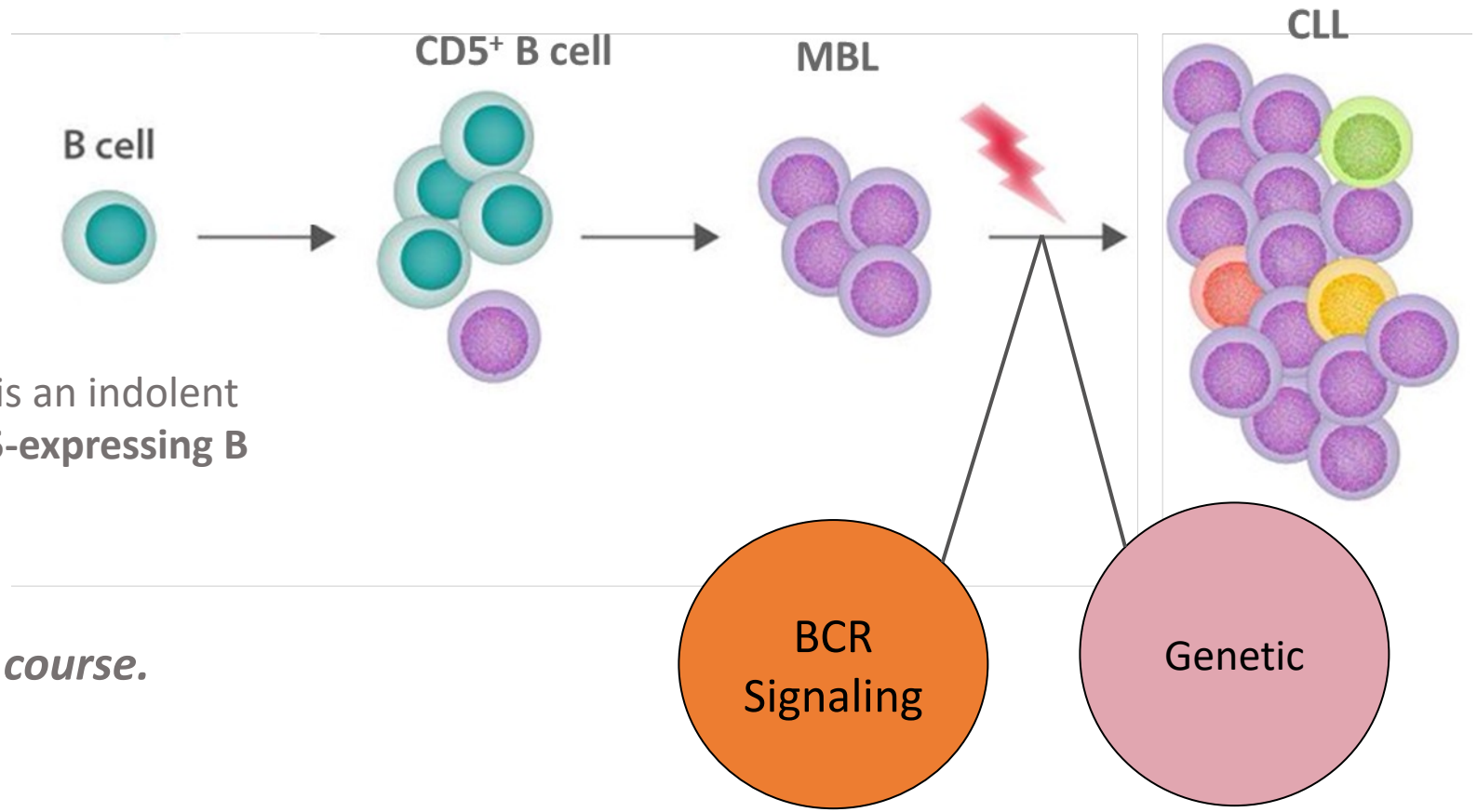
B-cell Biology



B-cell Lymphomas



Stepwise Immunopathogenesis



Chronic lymphocytic leukemia (CLL) is an indolent monoclonal expansion of mature **CD5-expressing B cells.**

CLL follows a
highly heterogenous clinical course.

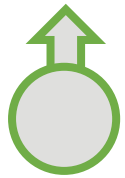
Sepulveda et al. ASH 2022

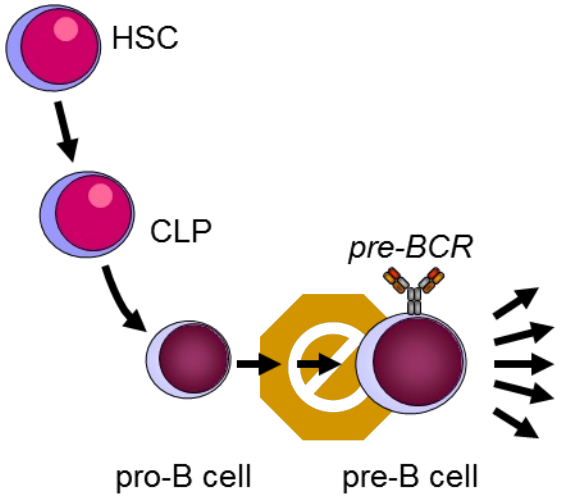
Lymphomagenesis in indolent B-cell Neoplasms

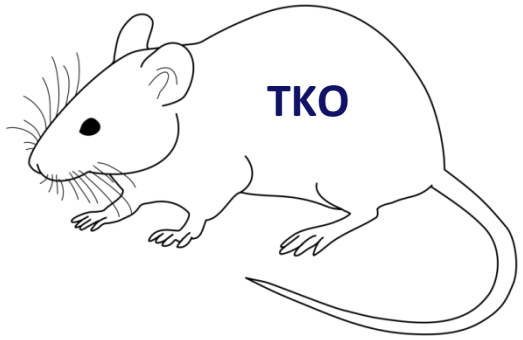
- Unique and distinct mechanisms in oncogenesis
- Physiological immune system mechanisms to promote and support tumor origin and progression
  - B-cell Receptor **Signaling**
  - Endogenous **mutagenesis**

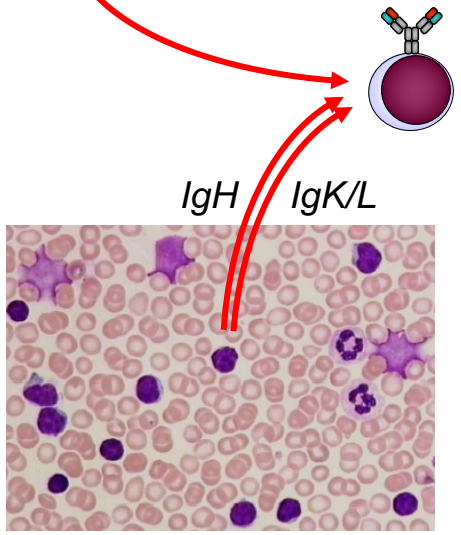
Ca^{2+} flux analysis

Functional BCR characterization









- rag2$^{-/-}$: No V(D)J recombination
- lambda5$^{-/-}$: No pre-BCR expression
- slp65$^{-/-}$: Interrupted BCR signalling
- Tg 4-OHT-dependent slp65-/-

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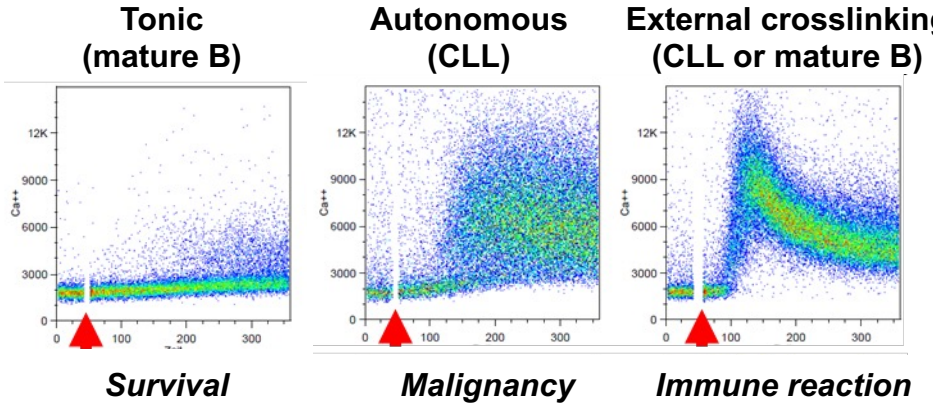
nature > letters > article

Letter | Published: 12 August 2012

Chronic lymphocytic leukaemia is driven by antigen-independent cell-autonomous signalling

Marcus Dühren-von Minden, Rudolf Übelhart, Dunja Schneider, Thomas Wossning, Martina P. Bach, Maike Buchner, Daniel Hofmann, Elena Surova, Marie Follo, Fabian Köhler, Hedda Wardemann, Katja Zirlik, Hendrik Veelken & Hassan Jumaa

Nature **489**, 309–312 (2012) | Cite this article

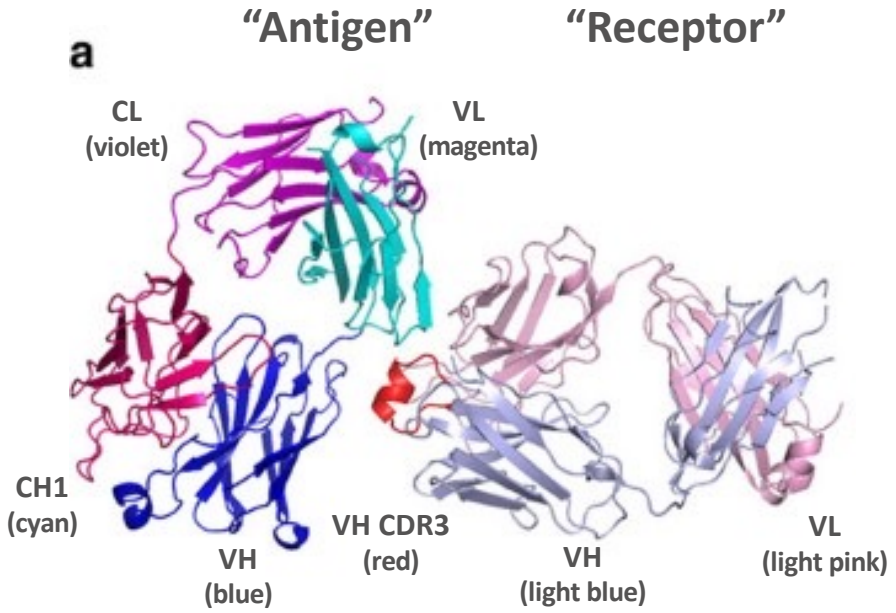


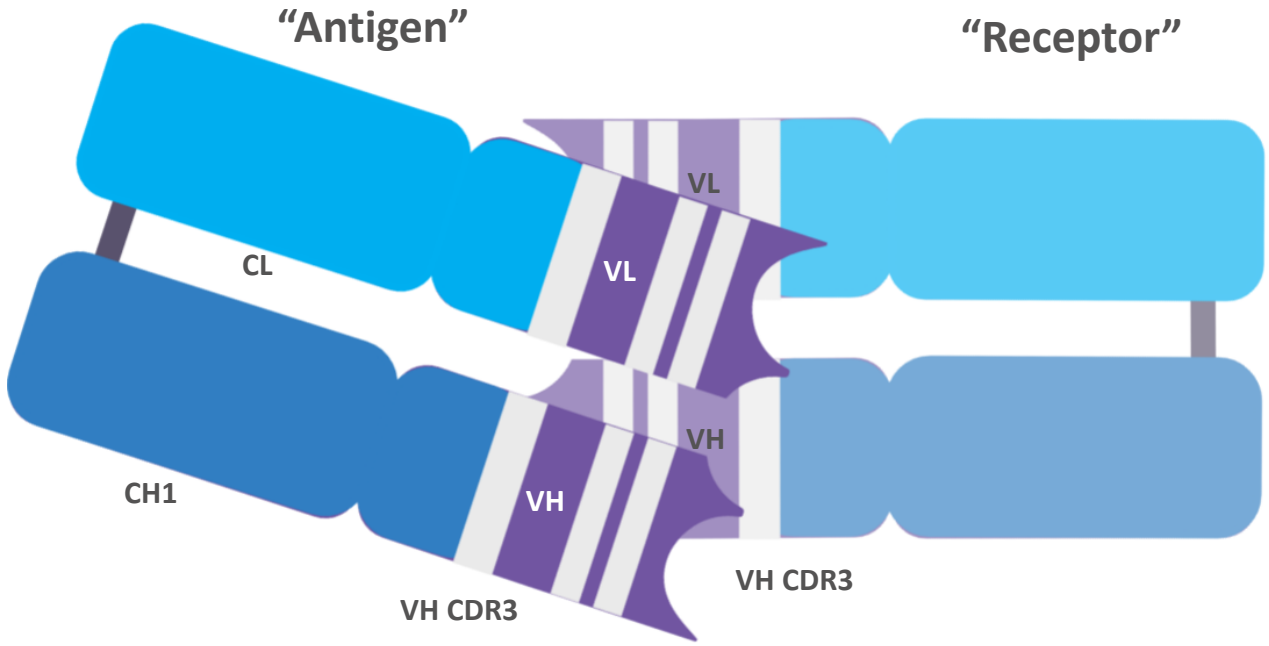
von Mindel et al. Nature 2012

Autonomous BCR signaling

BCR Signaling

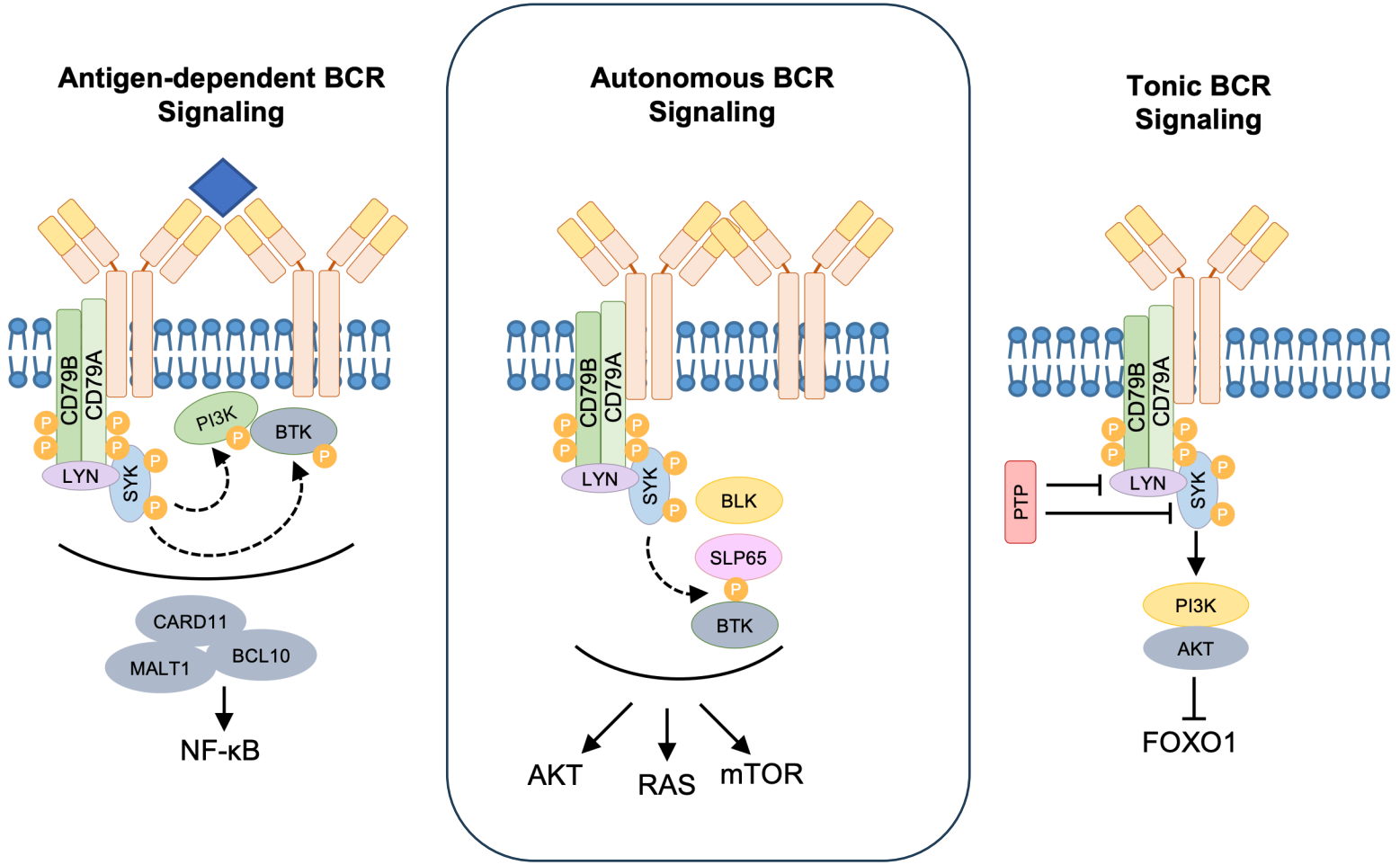
Autonomous BCR signaling without engagement of external antigen is an **indispensable oncogenic signal in CLL**.





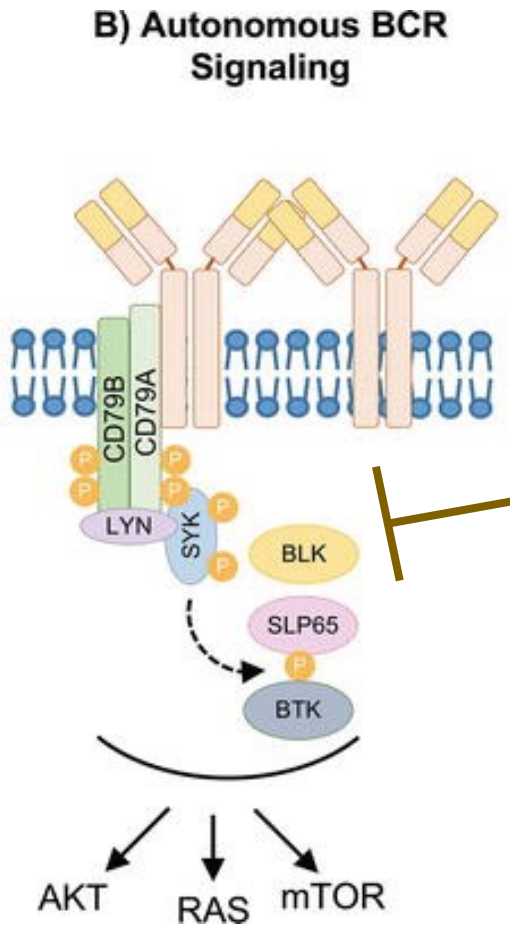
Minici *et al.*, 2017. Nature Communications

The Lymphomagenic B-cell Receptor



Sepulveda et al. Advances in Hematology 2018

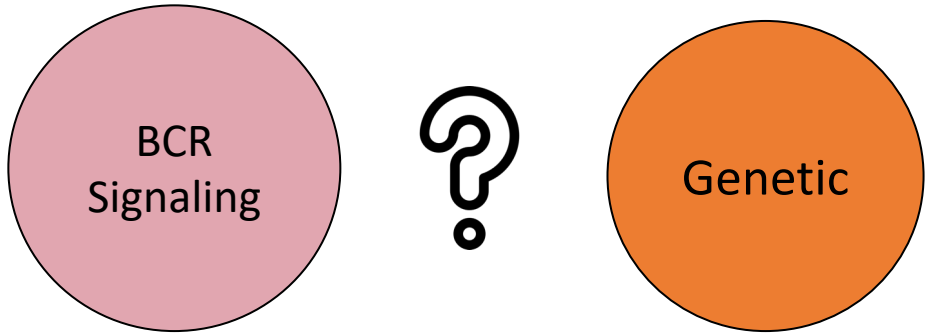
Targeting BCR Signalling

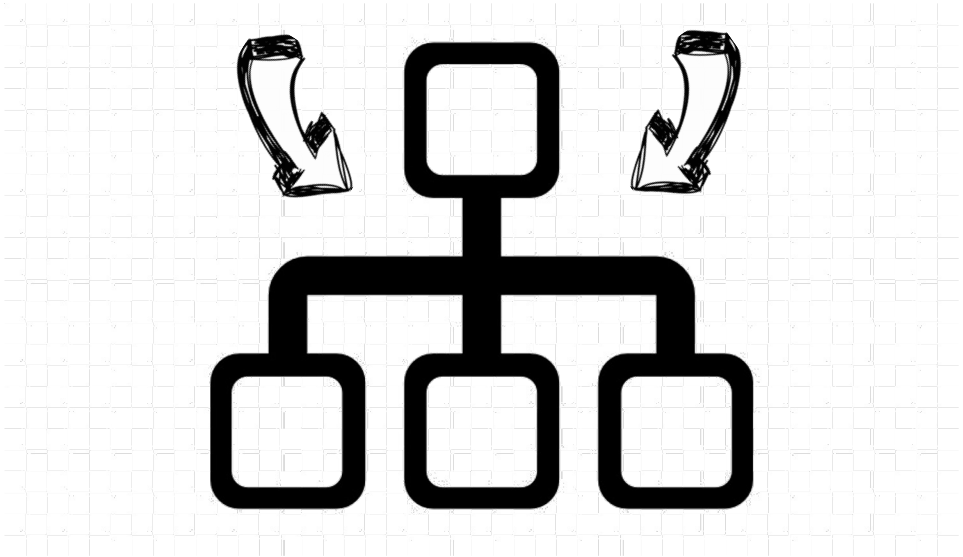


Irreversible BTK inhibitors:
- Ibrutinib
- Acalabrutinib
- Zanubrutinib
- Tirabrutinib
- Orelabrutinib

Reversible BTK inhibitors:
-ARQ-351
- LOXO-305
- GDC-0853
- Vecabrutinib

What is the order of events?





The **hierarchy and sequence of mechanisms**

- leading to benign, and longitudinally stable MBL
- and the events causing eventual progression to overt CLL

are unknown.

In order to investigate the **early stages in CLL ontogeny,** we sought to clarify whether **MBL cells express BCR with autonomous signaling capacity** and to compare the prevalence of **inherited risk loci and acquired genetic aberrations** in MBL-CLL sibling pairs.

julieta.sepulveda@umag.cl

Study Cohort

160

Patients with **CLL**

191

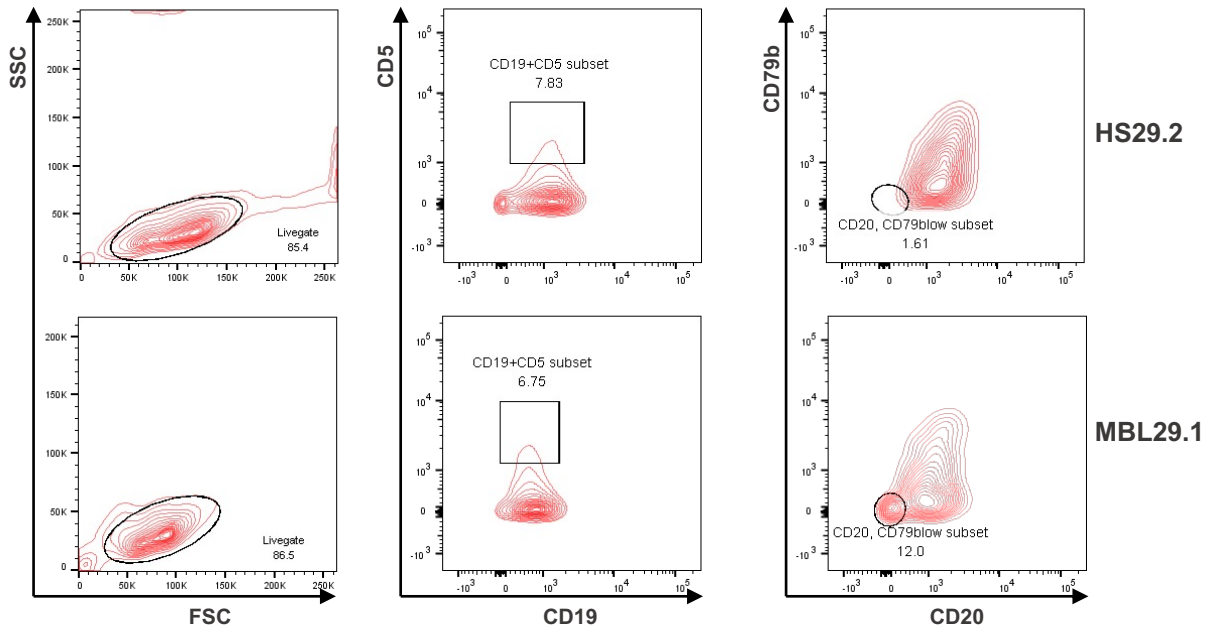
siblings of CLL patients

26

Patients with **CLL**

34

siblings with ***CLL-phenotype*** population



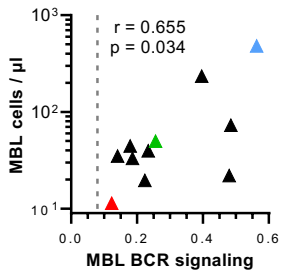
CLL-phenotype

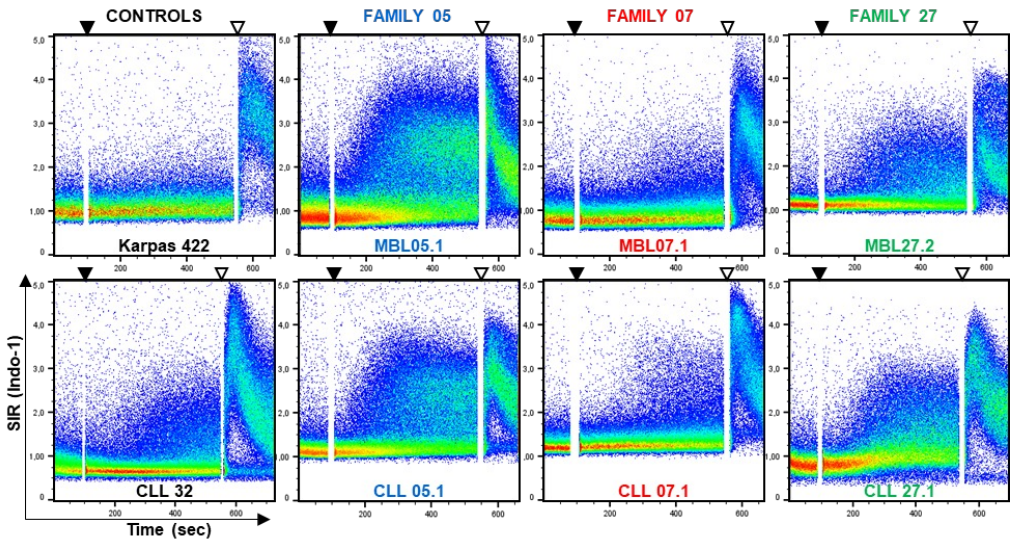
Mature B cells with the characteristic

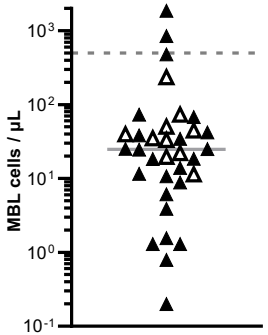
$CD5^{+}CD20^{low}CD79^{low}$

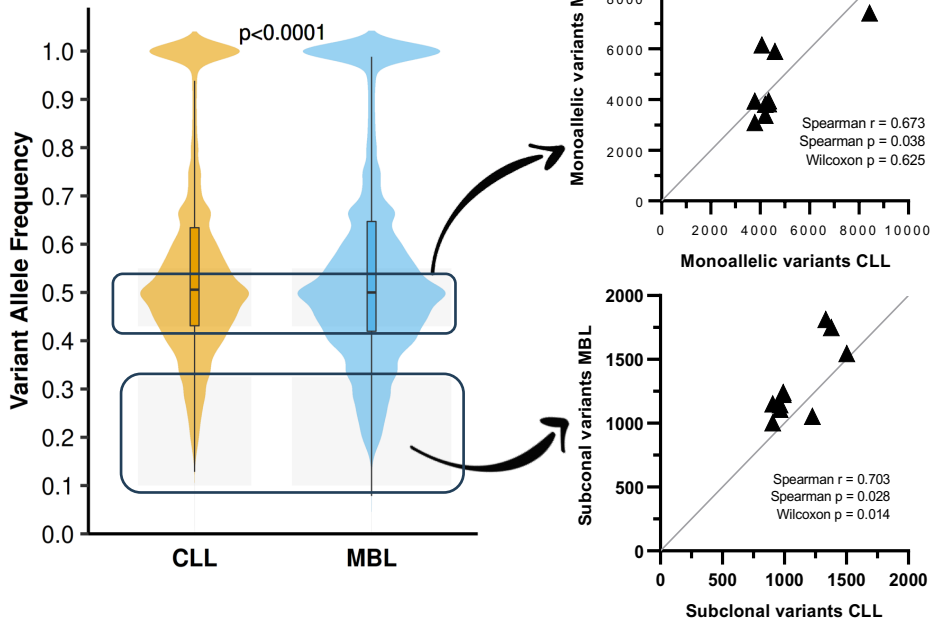
Comparison of BCR signaling and genome-wide variants in MBL-CLL siblings

BCR **signaling** increases as a function of the **cell count**









Subclonal variants defined by a VAF of 0.1-0.33 were **more prevalent in MBL** compared to CLL siblings

Sepulveda et al. Haematologica 2023

What about other B-cell Neoplasms?

What about other B-cell Neoplasms?

JEM Journal of Experimental Medicine

Brief Definitive Report | March 21 2024

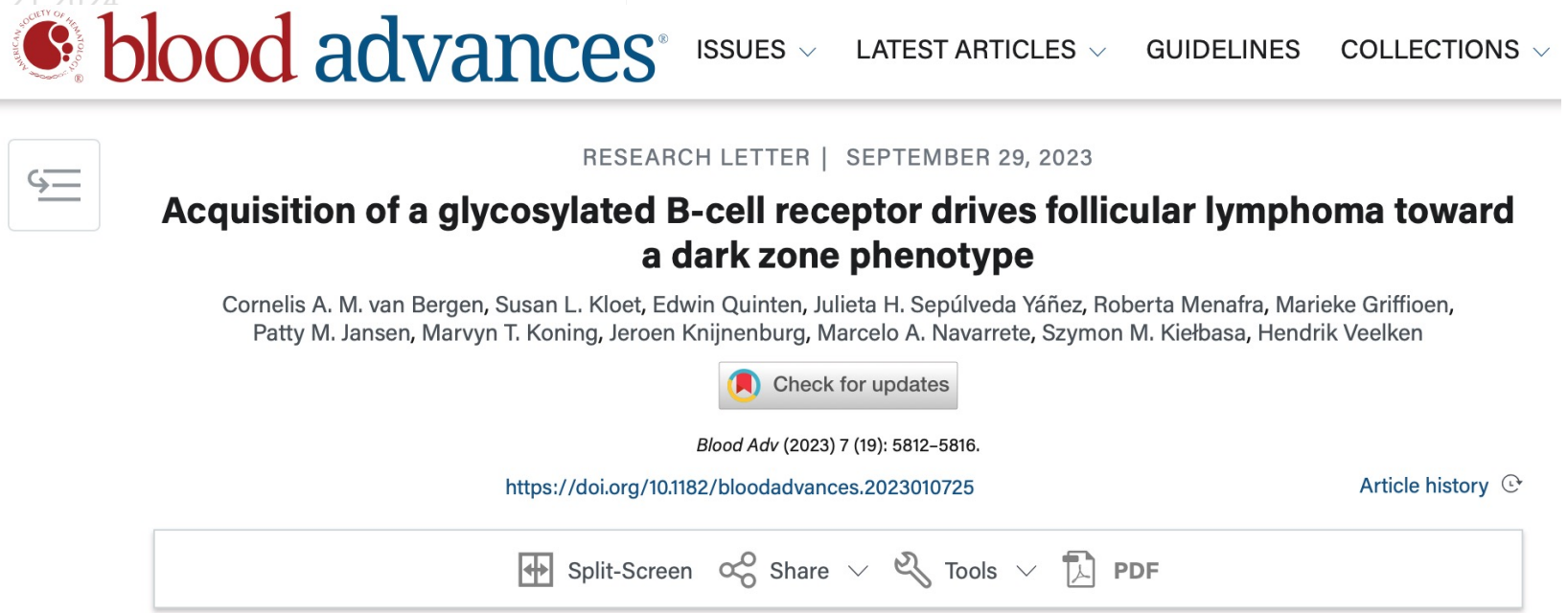
Antigen-independent, autonomous B cell receptor signaling drives activated B cell DLBCL

Janneke A. Eken, Marvyn T. Koning, Kristyna Kupcova, Julieta H. Sepúlveda Yáñez, Ruben A.L. de Groen, Edwin Quinten, Jurriaan Janssen, Cornelis A.M. van Bergen, Joost S.P. Vermaat, Arjen Cleven, Marcelo A. Navarrete, Bauke Ylstra, Daphne de Jong, Ondrej Havranek, Hassan Jumaa, Hendrik Veelken

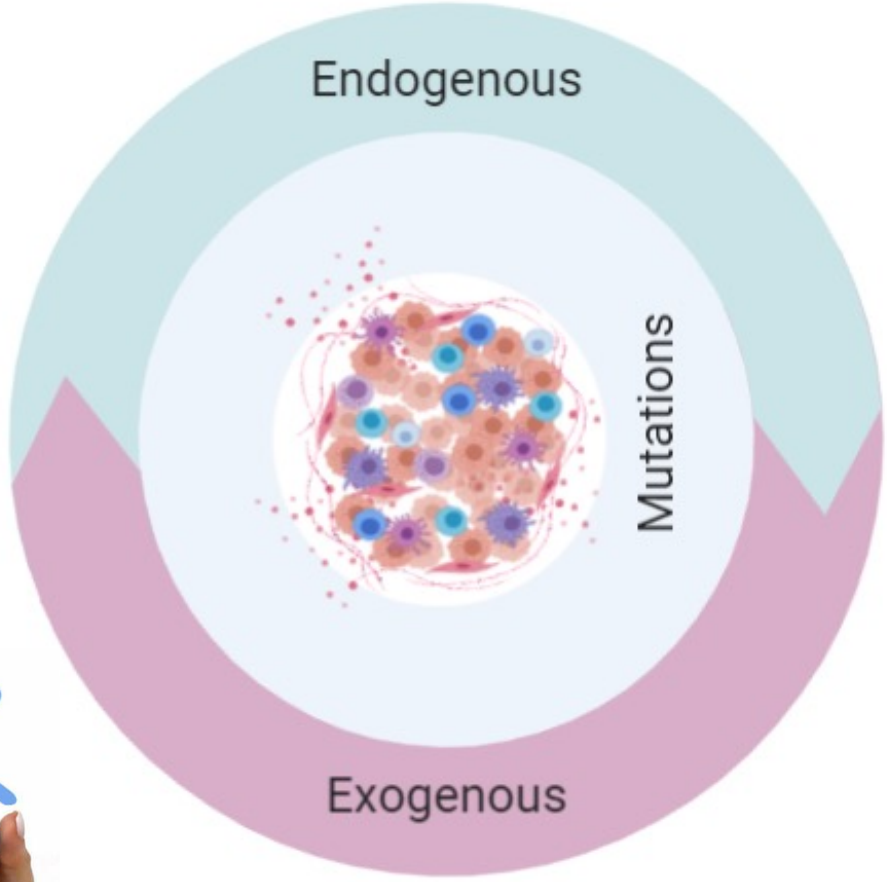
+ Author and Article Information

J Exp Med (2024) 221 (5): e20230941.
https://doi.org/10.1084/jem.20230941 | **Article history**

What about other B-cell Neoplasms?

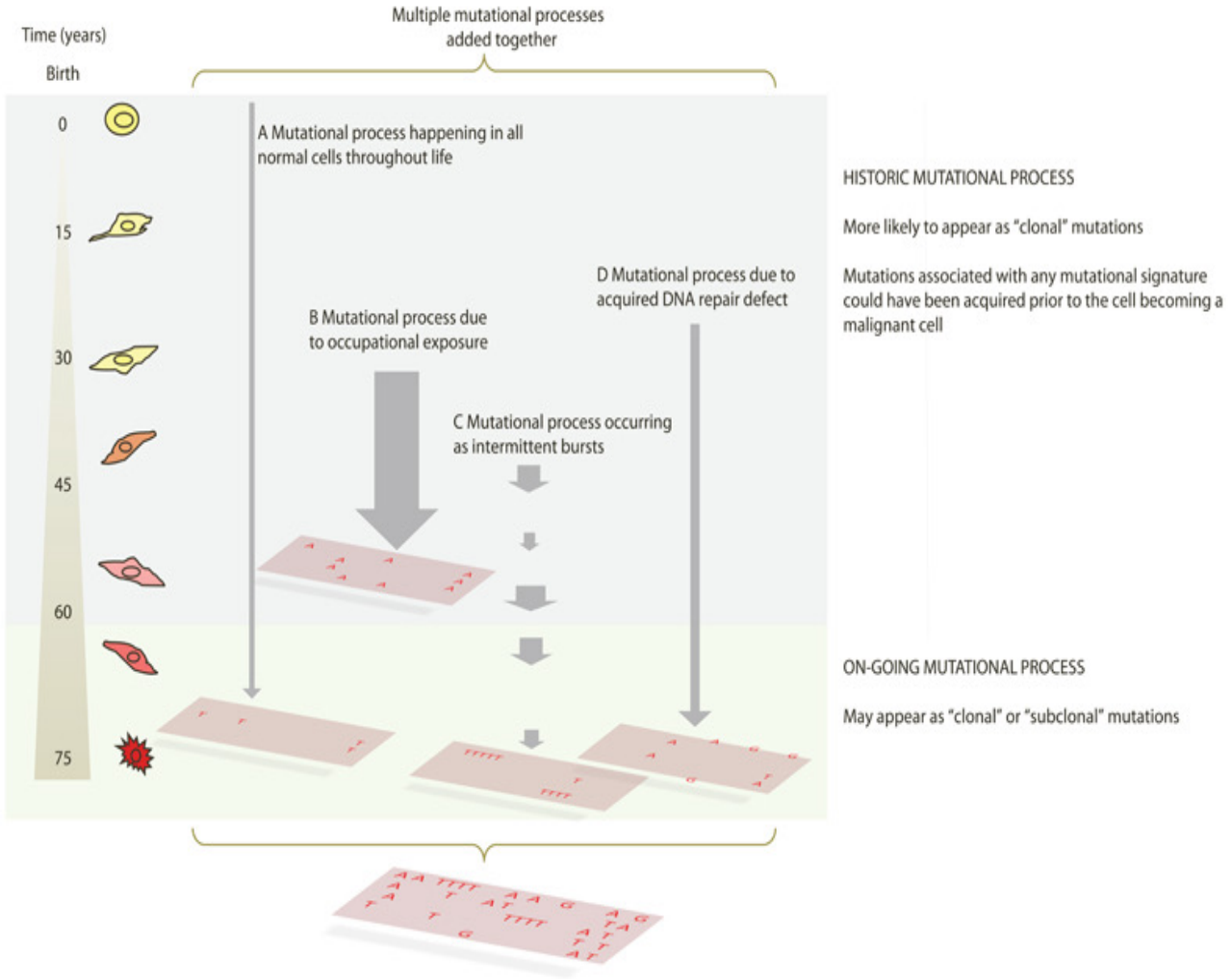


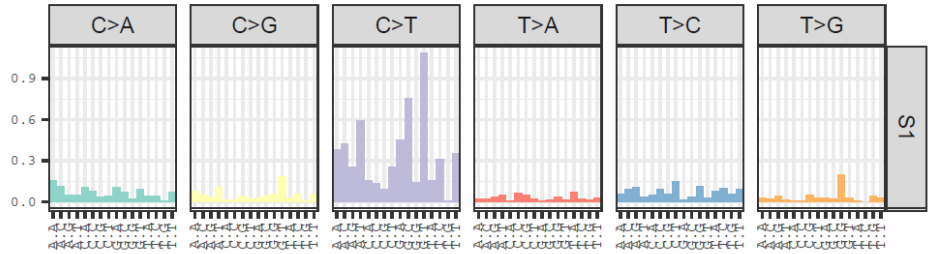
Hunting down the cause of cancer



Mutational Signatures

Somatic Pattern





Somatic pattern is a composite of all the mutational processes that have been active over the **lifetime** of the cancer patient.

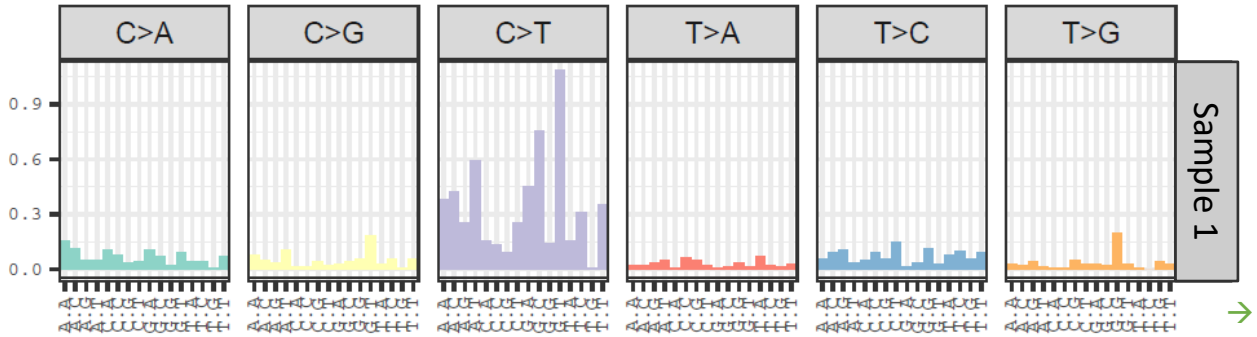
Somatic Patterns

C > A | C > G | C > T | T > A | T > C | T > G

16 possible combination each (4 types of 5’ base x 4 types of 3’)

C > A	C > G	C > T	T > A	T > C	T > G
A . A	A . A	A . A	A . A	A . A	A . A
A . C	A . C	A . C	A . C	A . C	A . C
A . G	A . G	A . G	A . G	A . G	A . G
C . A	C . A	C . A	C . A	C . A	C . A
C . G	C . G	C . G	C . G	C . G	C . G
C . T	C . T	C . T	C . T	C . T	C . T
G . A	G . A	G . A	G . A	G . A	G . A
G . C	G . C	G . C	G . C	G . C	G . C
G . T	G . T	G . T	G . T	G . T	G . T
G . G	G . G	G . G	G . G	G . G	G . G
T . A	T . A	T . A	T . A	T . A	T . A
T . C	T . C	T . C	T . C	T . C	T . C
T . G	T . G	T . G	T . G	T . G	T . G
T . T	T . T	T . T	T . T	T . T	T . T

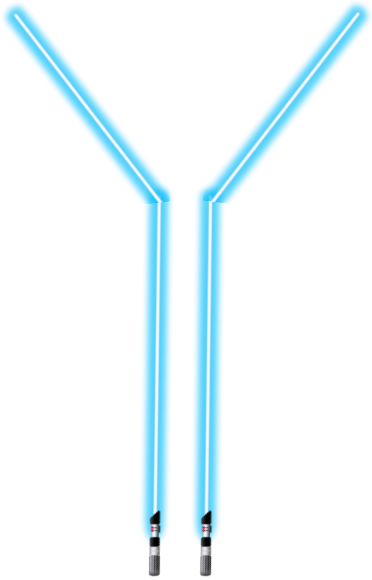
16 possible combination x 6 substitutions = 96 possible mutation types



→ 96 possible mutation types

AID is responsible for the normal adaptive immune response

(Arakawa et al, Science 2002)



AID is responsible for the normal adaptive immune response

(Arakawa et al, Science 2002)

Expression of AID has a **dark side**

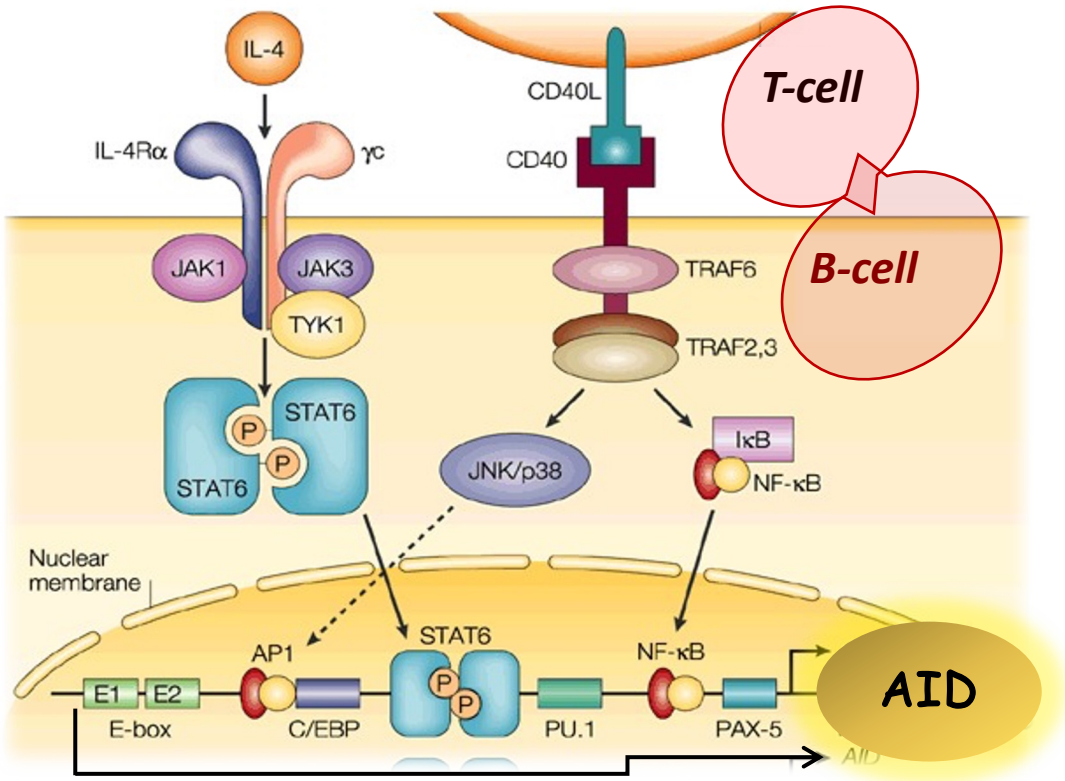
AID an oncogenic enzyme

(Okasaki et al., JEM, 2003)

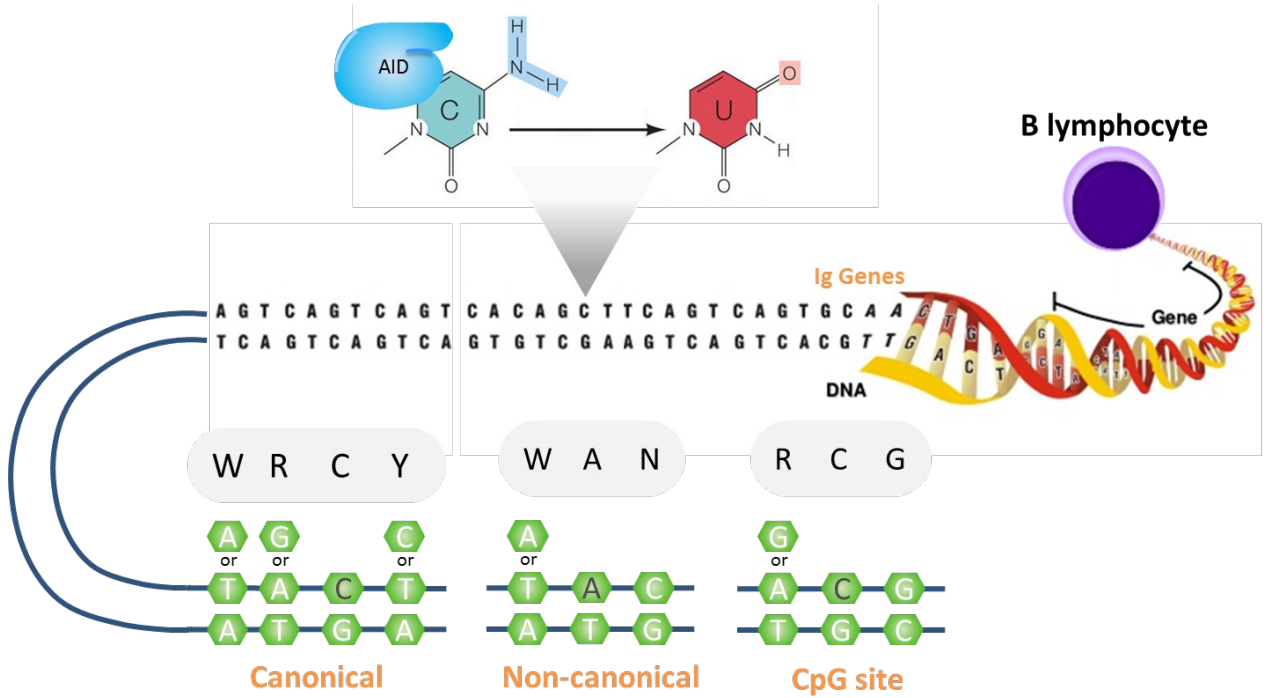
AID “off targets” mutations in lymphomas

(Robbiani et al., Mol. Cell, 2009)

Activation Induced Deaminase AID

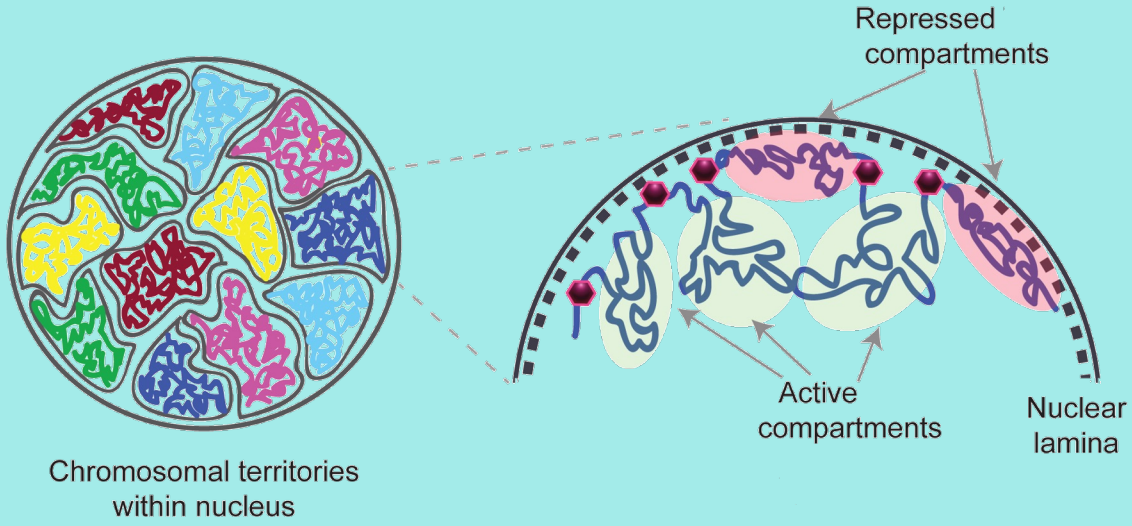


Modified from: Geha RS, et al., Nat Rev Immunol 3:721-732



Sepulveda-Yanez et al. Frontiers Immunology 2022

Mutational signatures and 3D structure



Two compartments:

The **active compartment (compartment A)** includes genomic regions characterized by transcription or epigenetics marks associated with open chromatin (H3K36me3), high density of genes, and DNase I hypersensitivity.

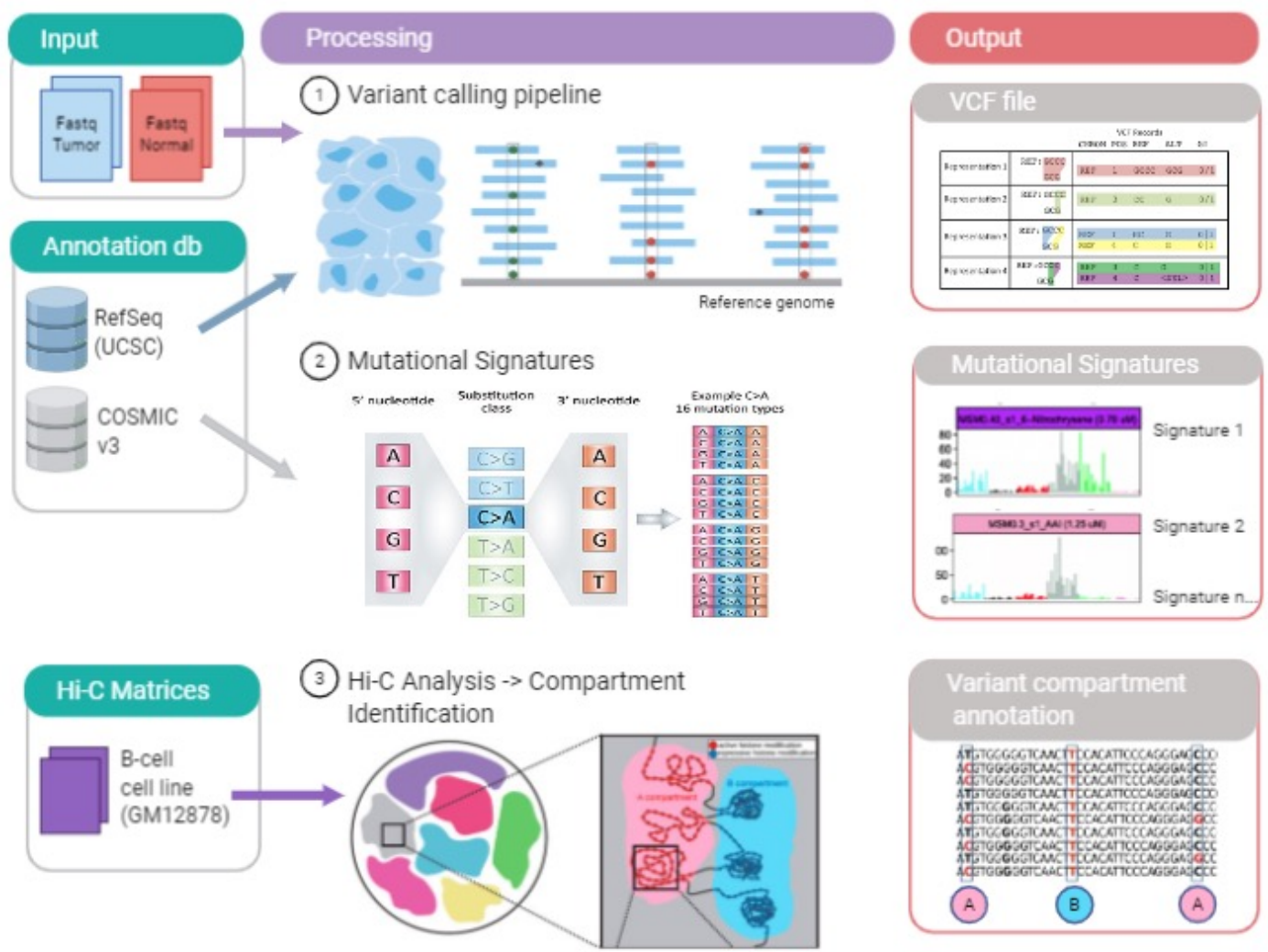
The **inactive compartment (compartment B)** represents the condensed DNA regions.

In the genome-wide context, the **three-dimensional (3D) chromatin structure** could play an important role in the activity of the different mutational mechanisms.

High-throughput Chromosome Conformation Capture (Hi-C) allows the identification of different states of the genome structure at sub-chromosomal scale.

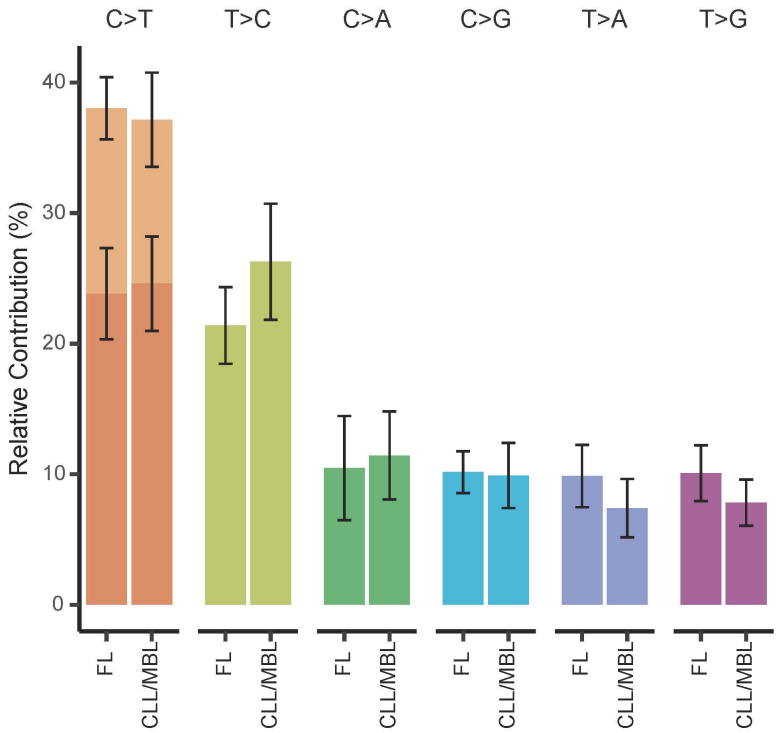
Hi-C maps from pro-B cells revealed that up to **96% of canonical AID target regions can be assigned to compartment A in the mouse genome**.

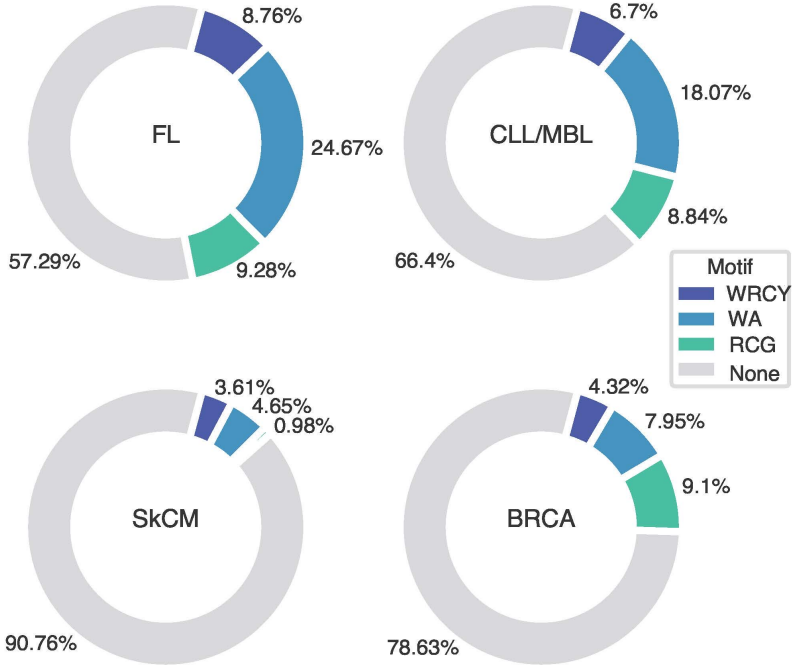
Overview

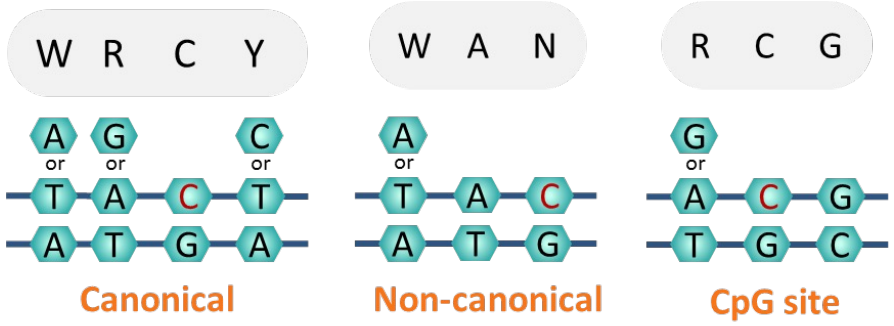


Sepulveda et al. IJMS 2022

Mutations AID-related and Pattern of





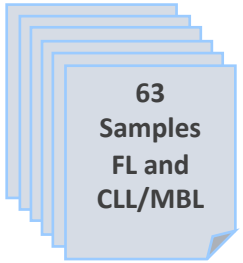


- Transition/transversion
- C>T: 35,6% (13,4)
- T>C: 23,7%

- FL: 42% related with AID
- CLL/MBL: 34% related with AID
- Skin Cutaneous Melanoma: 9%
- Breast cancer (BRCA) 21%

Sepulveda et al. IJMS 2022

Mutational signatures in B-cell lymphomas are related with AID-activity

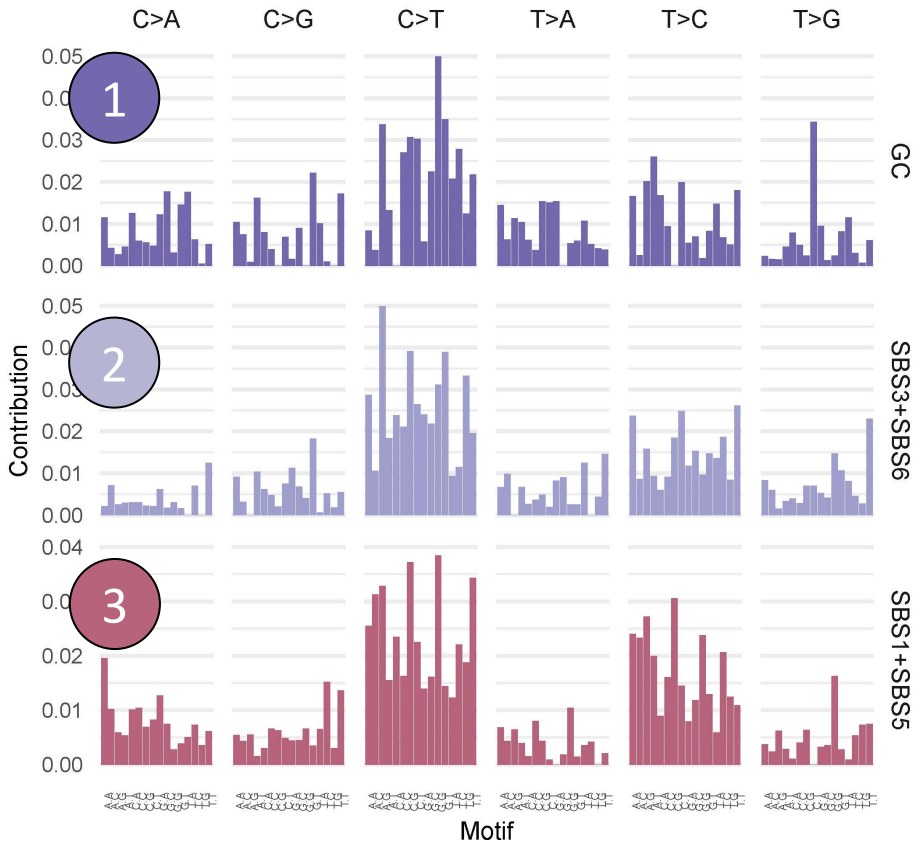


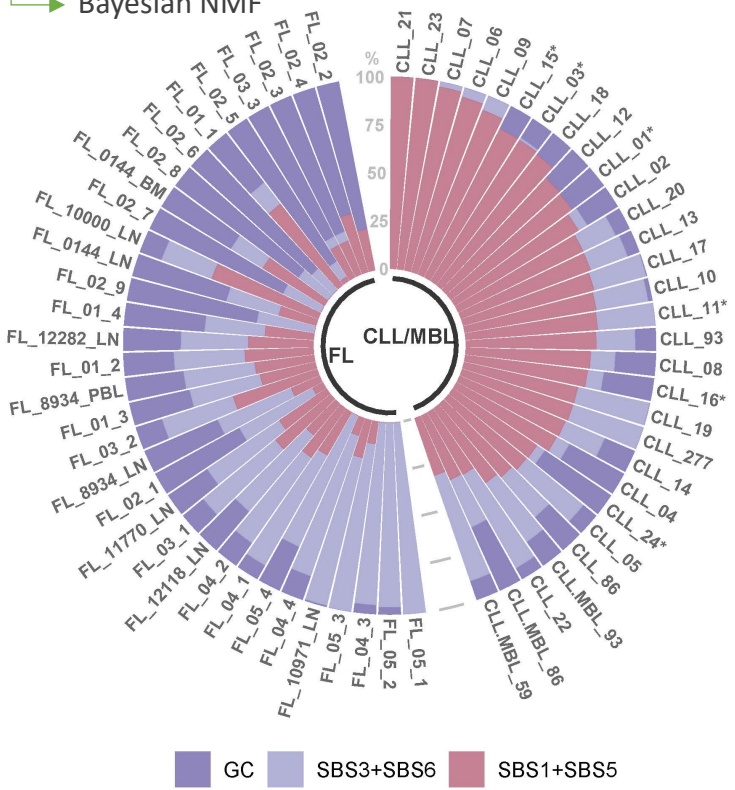
De novo signature extraction

- Multiplicative NMF
- Bayesian NMF

Similarity

Fitting approach





In this unsupervised analysis **84.5%** of the mutational spectrum variance was explained by 3 signatures

We analyzed whether the **extracted signatures** correspond to know mutational signatures described in the **COSMIC catalog** (v3-May 2019)

S1: unique composition **(novel signature)**
S2: DNA repair deficiency
S3: Ubiquitous process

GC and SBS3+SBS6 dominated the mutational landscape in **FL**
SBS1 and SBS6was more prominent in **CLL/MBL**

Sepulveda et al. ASH 2021

Conclusions

We defined the **mutational processes** that shape the mutational landscape of **FL and CLL** (global and localized) and integrated these signatures with **sub-chromosomal conformation data**

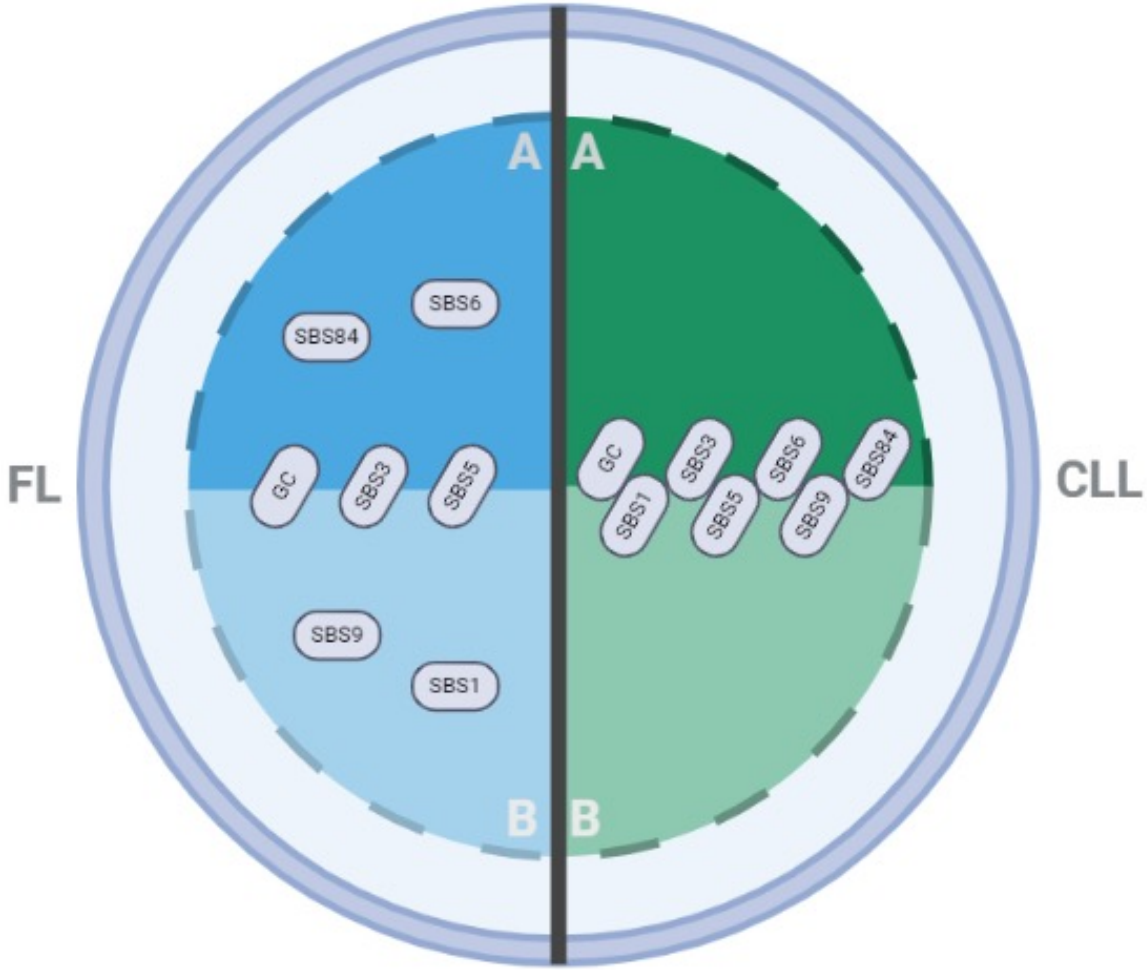
In **CLL**, mutational signatures are

evenly distributed across chromatin compartments.

In contrast, mutagenesis related to canonical AID activity and failures in DNA repair pathways in **FL** were **more frequently found in the active chromatin compartment.**

Since certain mutators such as **ongoing endogenous deamination** are more prone to occur in restricted areas of the tridimensional structure, **integration of genomic conformational data into signature analysis** could help to

better understand the biological relevance of deconvoluted mutational processes.



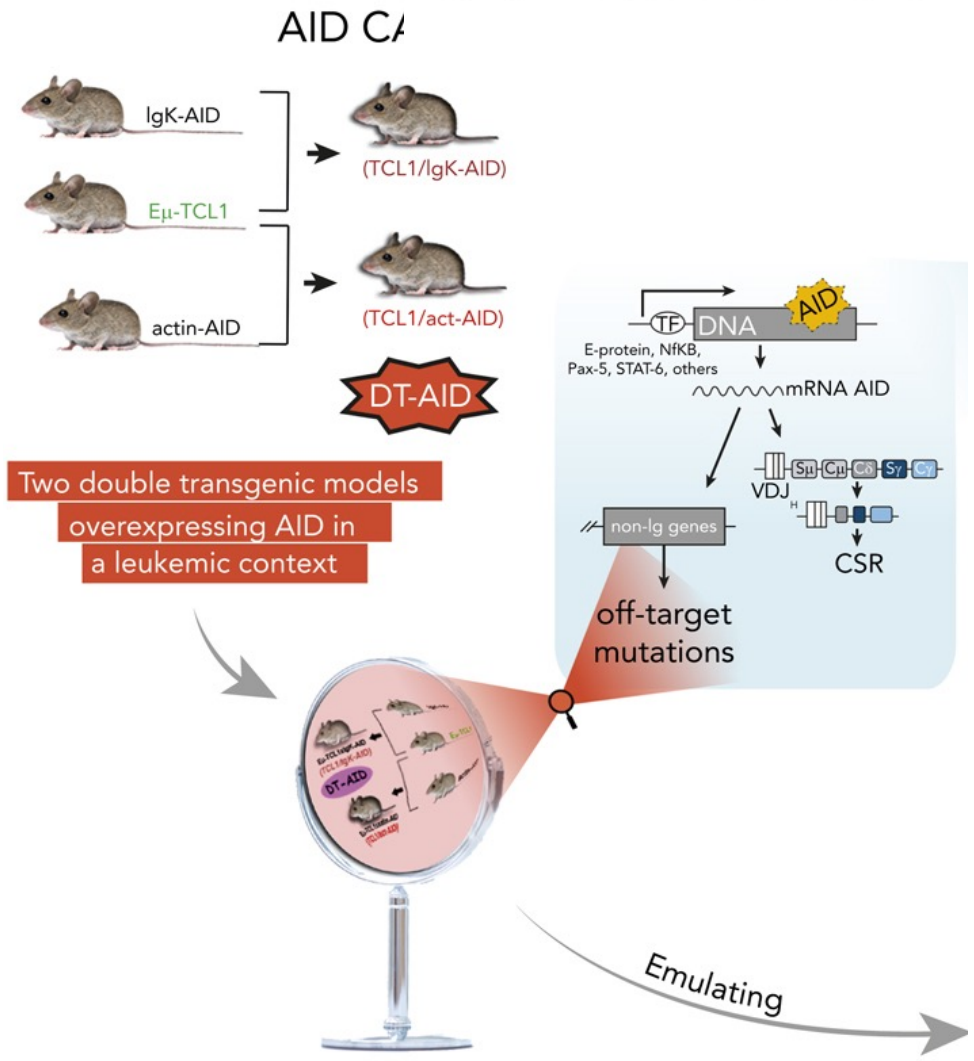
Conclussions

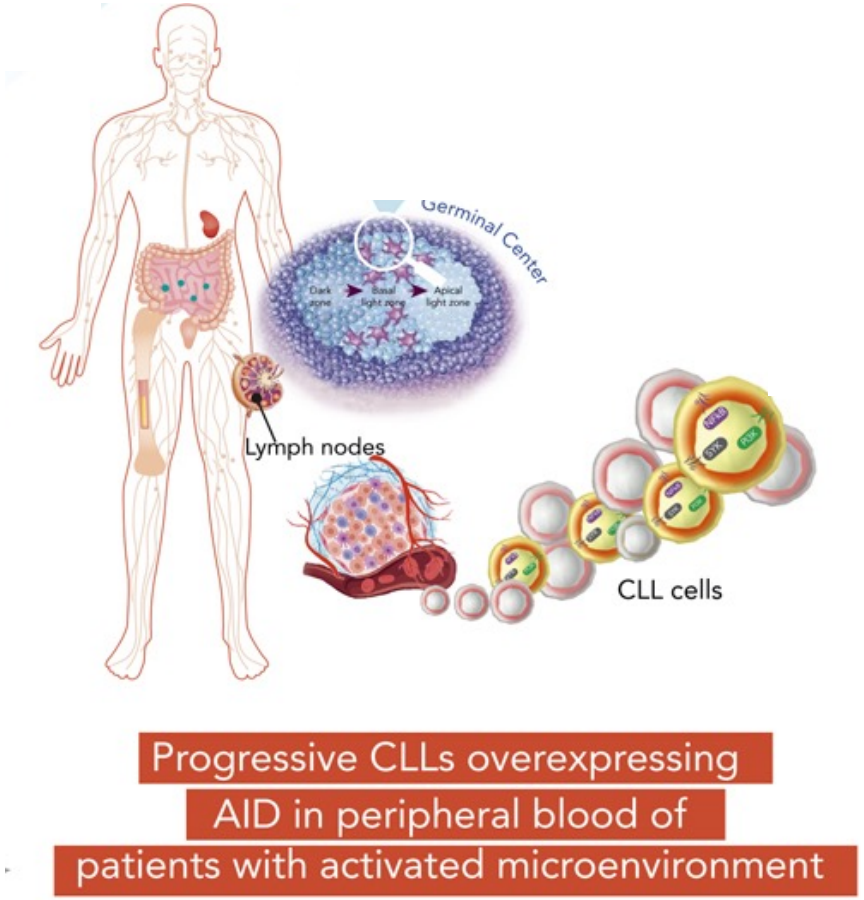
- **AID activity** shapes a sizable portion of the **genomic landscape** of indolent B-cell neoplasms (MBL, FL, and CLL)
- Differential **AID** activity in **nuclear compartments**

Based on statistical association of patterns in human samples … but it is the actual effect of AID?

Sepulveda et al. IJMS 2022, and Sepulveda et al. Frontiers Immunology 2022

AID effect *in Vivo*

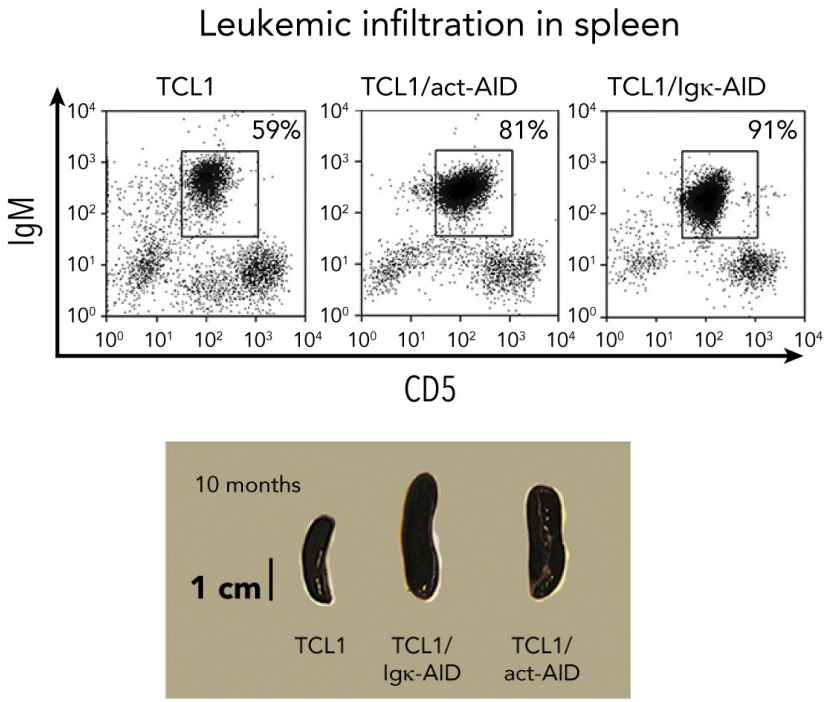


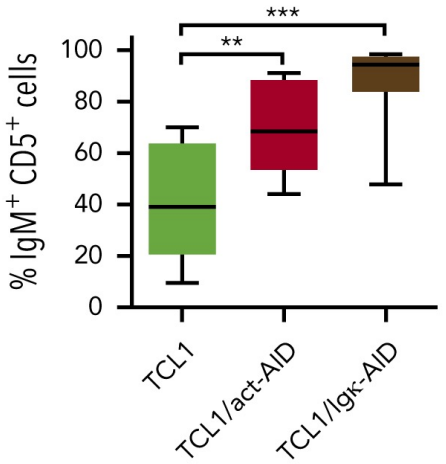


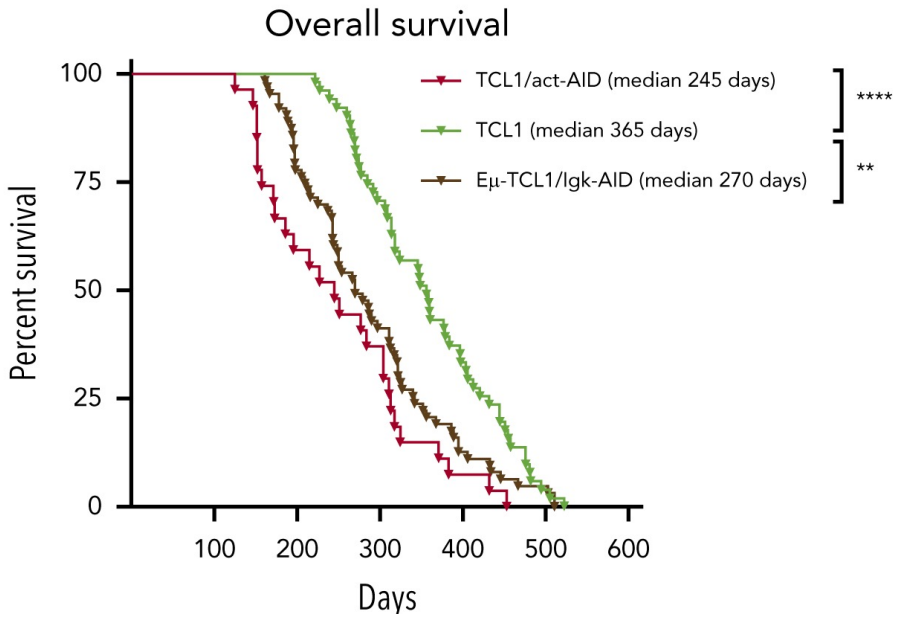
Progressive CLLs overexpressing
AID in peripheral blood of
patients with activated microenvironment

Morande et al. Blood 2021

AID overexpressing Mice Develop a More Aggressive B-cell Neoplasm

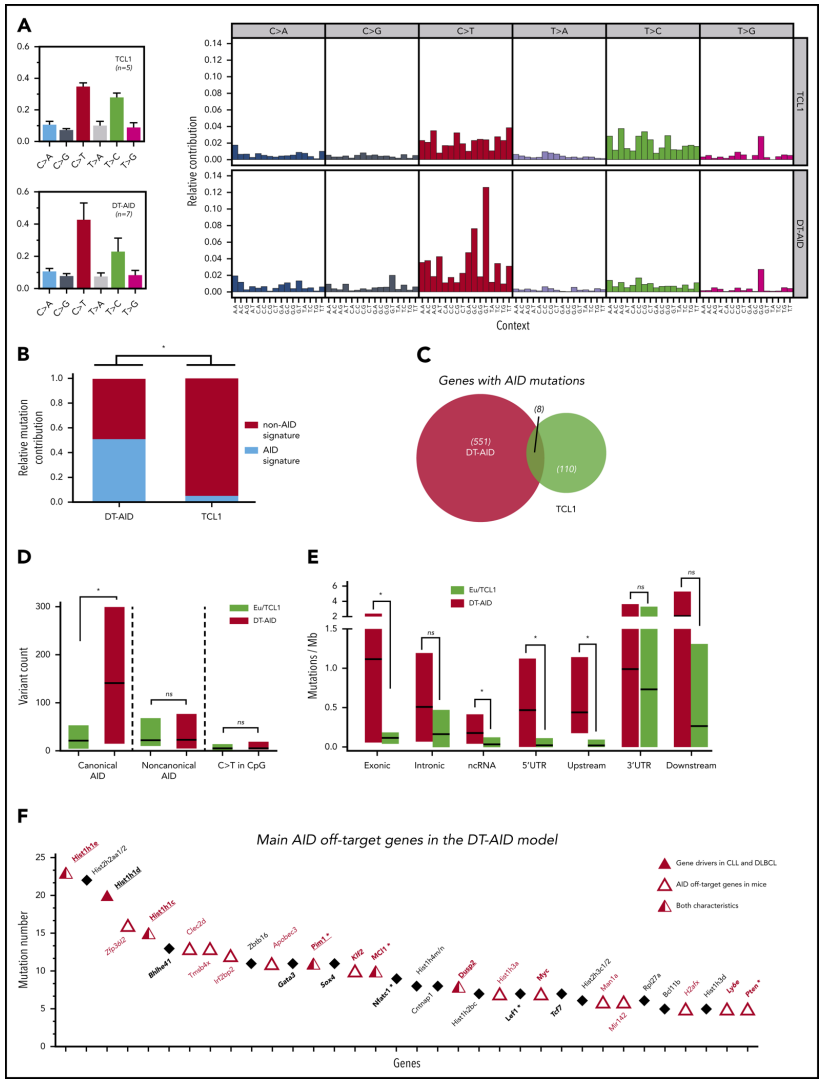


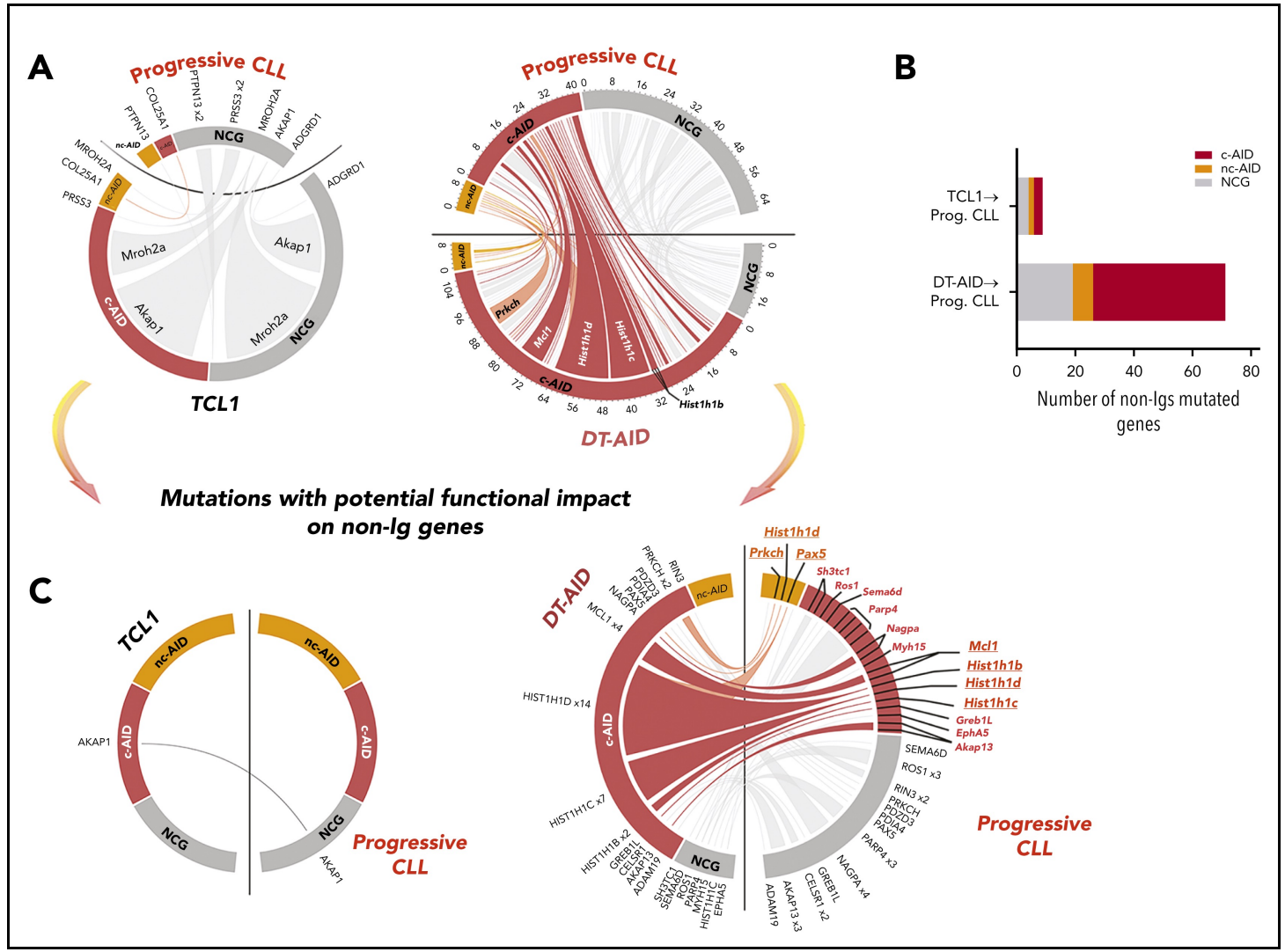




Morande et al. Blood 2021

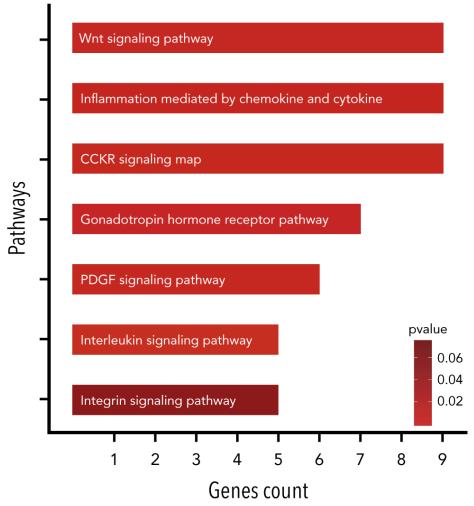
AID-induced Mutagenesis in Lymphomagenic Driver Genes

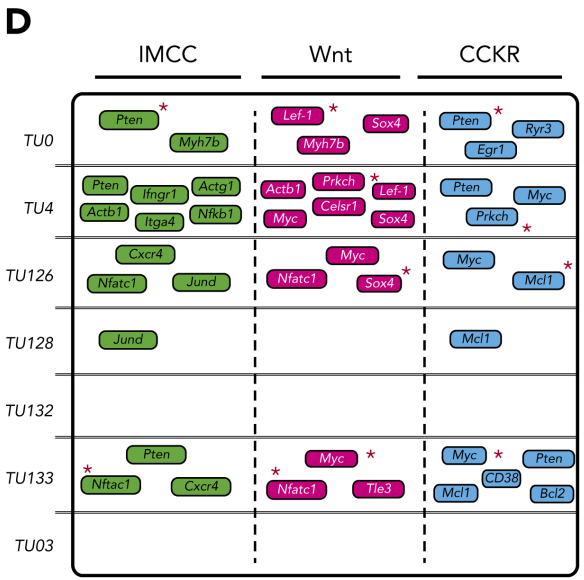


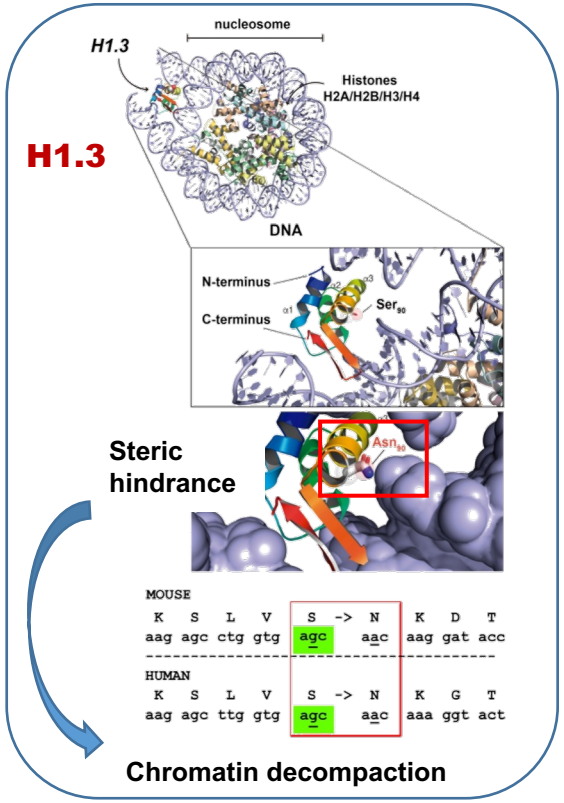


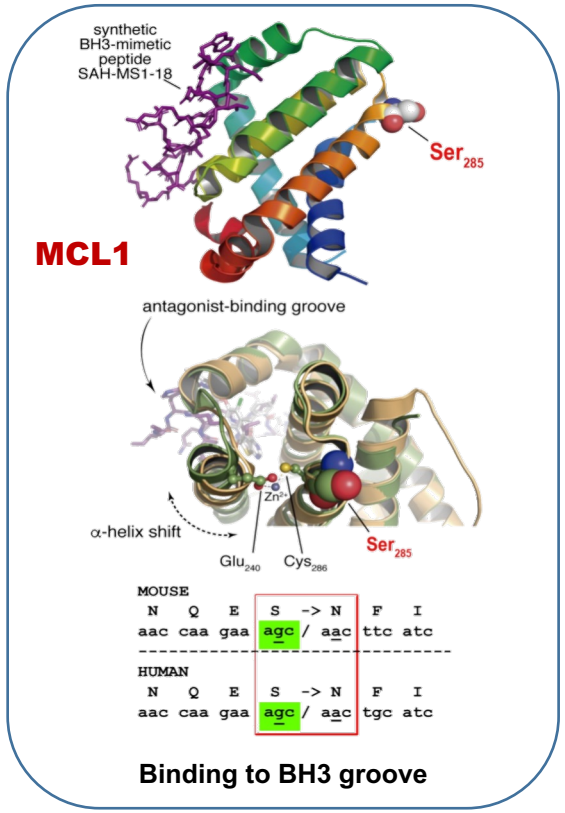
Morande et al. Blood 2021

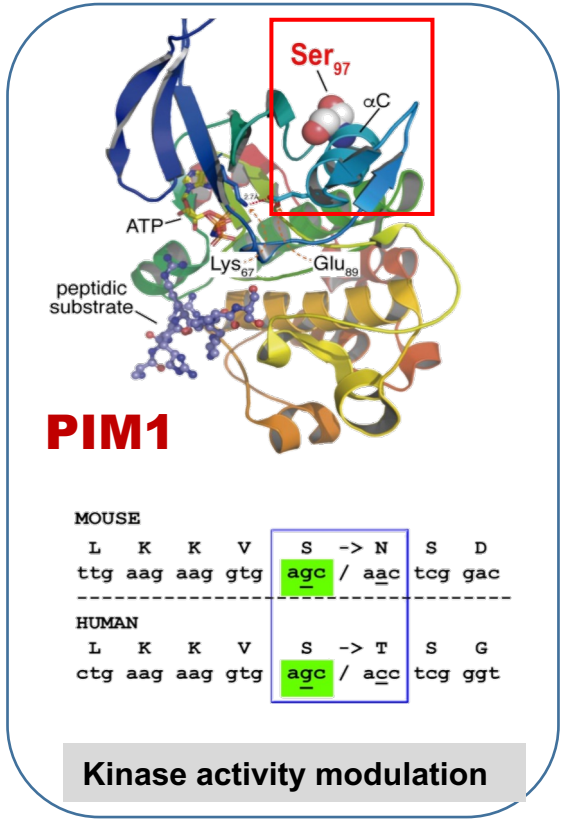
AID-induced Mutagenesis in Mice Mirrors Human Lymphomas











Morande et al. Blood 2021

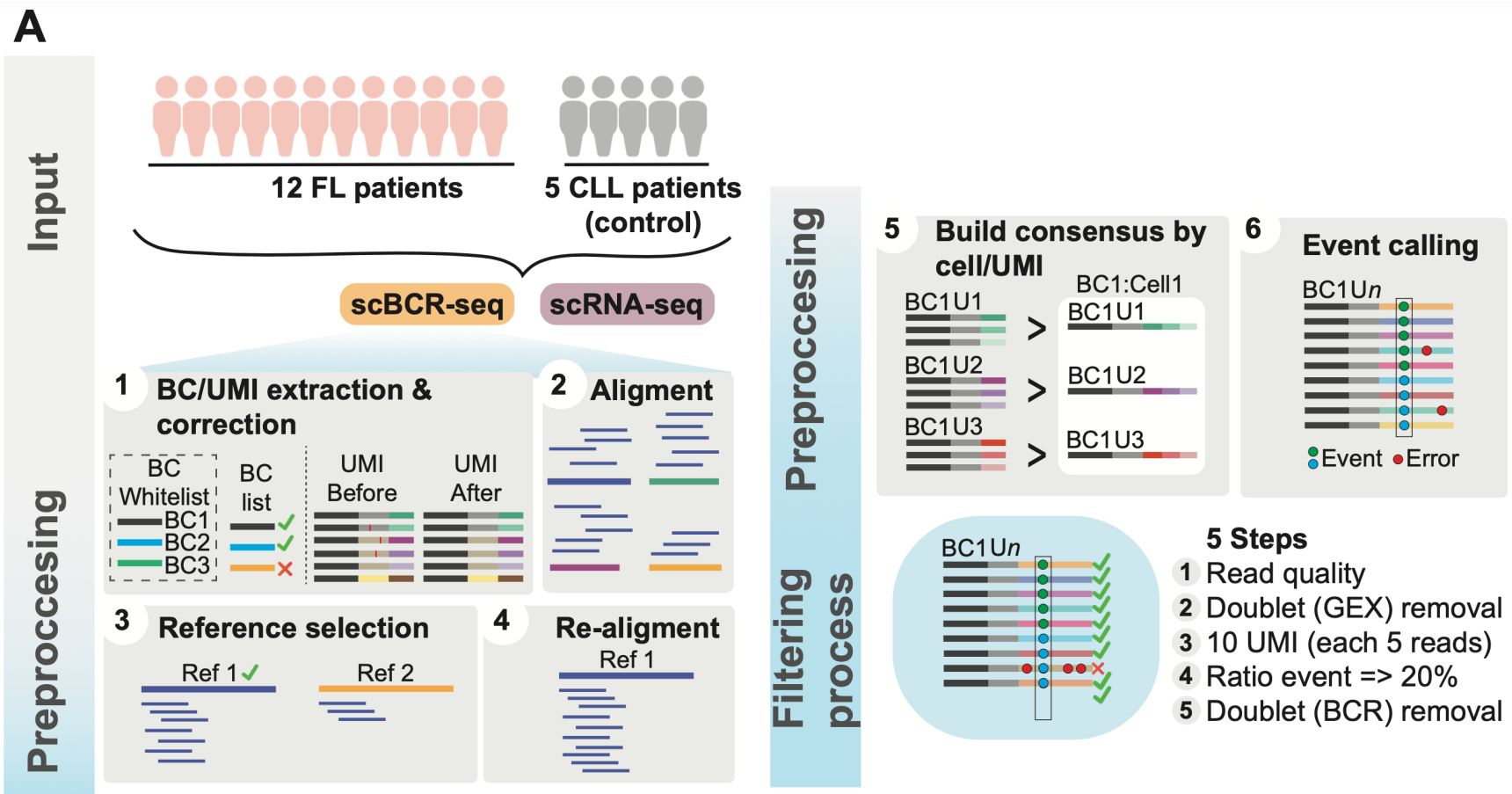
Conclussions

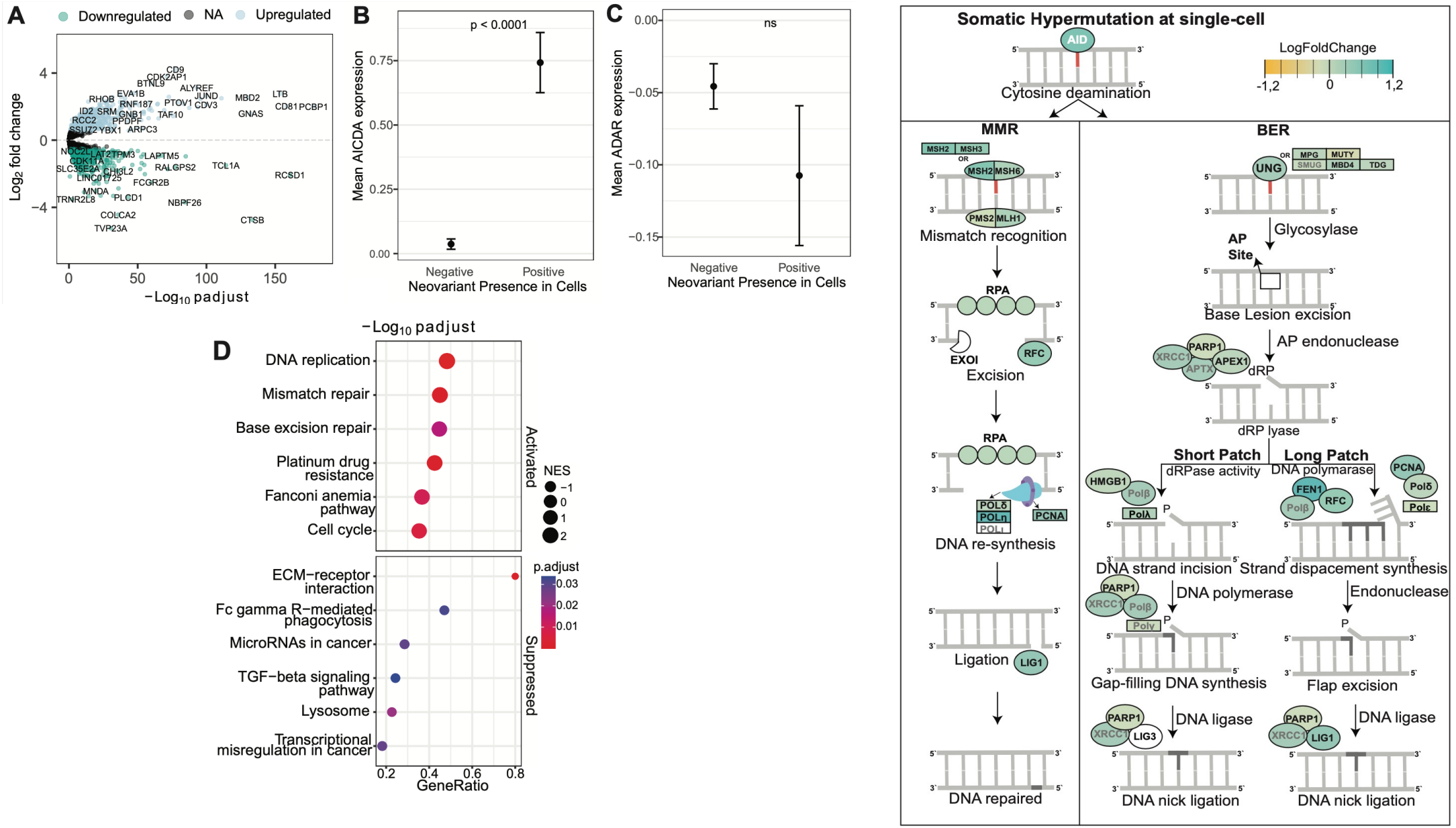
- **AID activity** shapes a sizable portion of the **genomic landscape** of indolent B-cell neoplasms
- Differential **AID** activity in **nuclear compartments**
- ***in vivo*** induction of **AID** mirrors events in human neoplasms

Based on statistical association of patterns and samples, and in vivo data in mice … but there is an ongoing effect of AID in humans?

Sepulveda et al. IJMS 2022, and Sepulveda et al. Frontiers Immunology 2022

Single Cell Transcriptomics of B-cell neoplasms





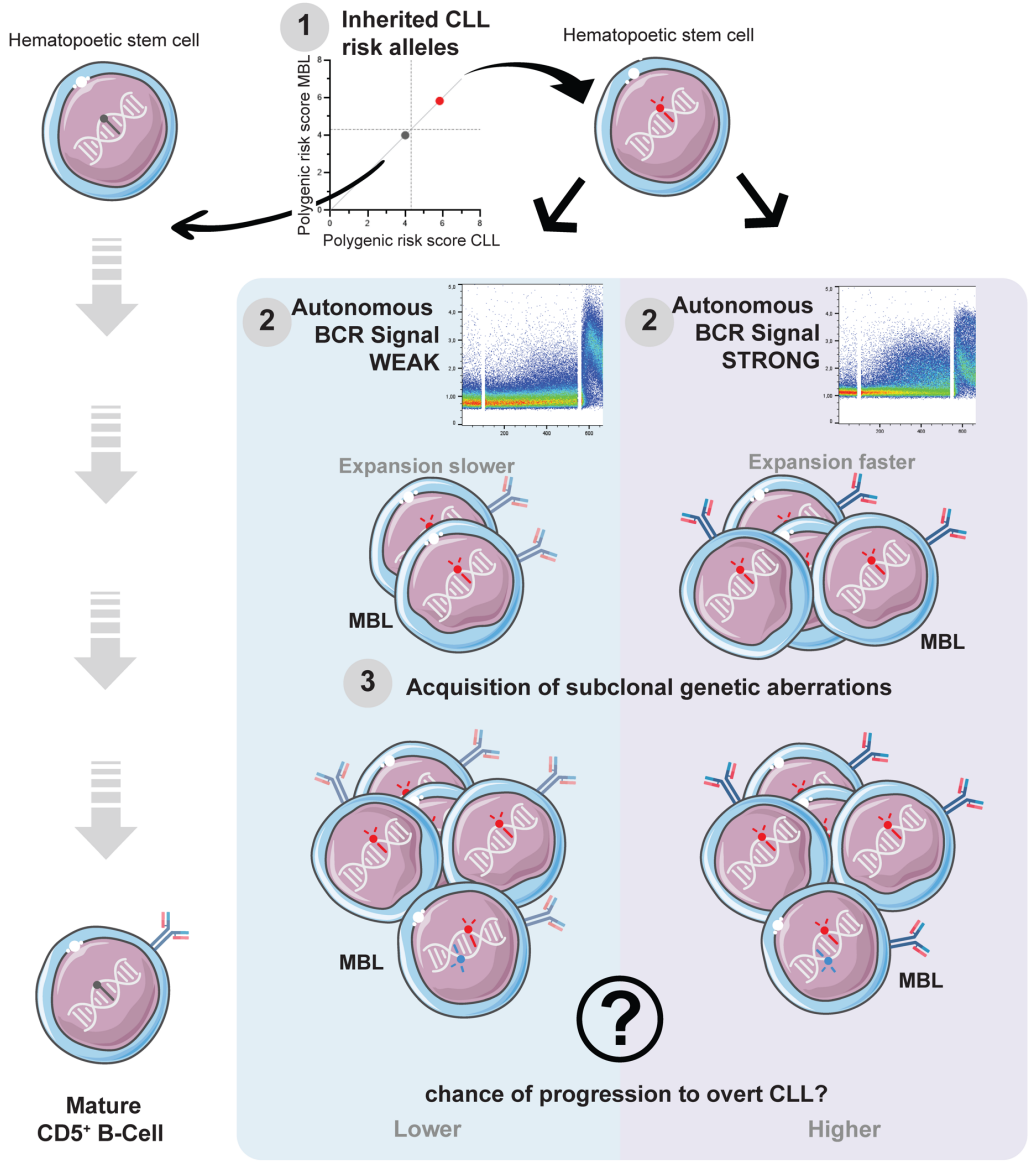
Sepulveda et al. ASH 2023 and 2024 Submitted

Conclussions

- **AID activity** shapes a sizable portion of the **genomic landscape** of indolent B-cell neoplasms
- Differential **AID** activity in **nuclear compartments**
- ***in vivo*** induction of **AID** mirrors events in human neoplasms
- Individual Lymphoma cells expressing **AID** transcripts display ongoing somatic **hypermutation** and activation of **DNA repair** pathways

Morande et al. Blood 2021, Sepulveda et al. IJMS 2022, and Sepulveda et al. Frontiers Immunology 2022, Sepulveda et al. ASH 2023

Stepwise CLL pathogenetic model



1 **Risk alleles** are found with **high and similar prevalence in CLL patients and MBL siblings**, suggesting that CLL risk loci predispose to clonal expansion of CLL phenotype cells in both low-count MBL and CLL

2 **Autonomous BCR signaling operates early in lymphomagenesis** as predicted by BCR stereotype, albeit at lesser strength in MBL.

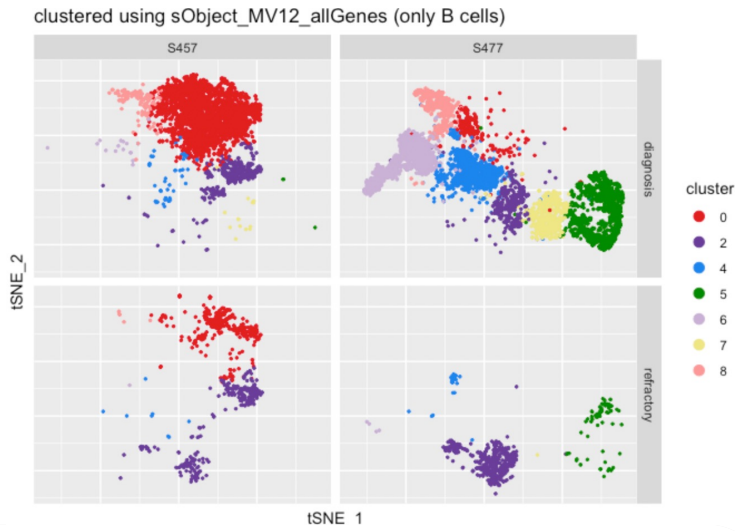
3 **AID-induced genetic changes**, drives tumor **progression**

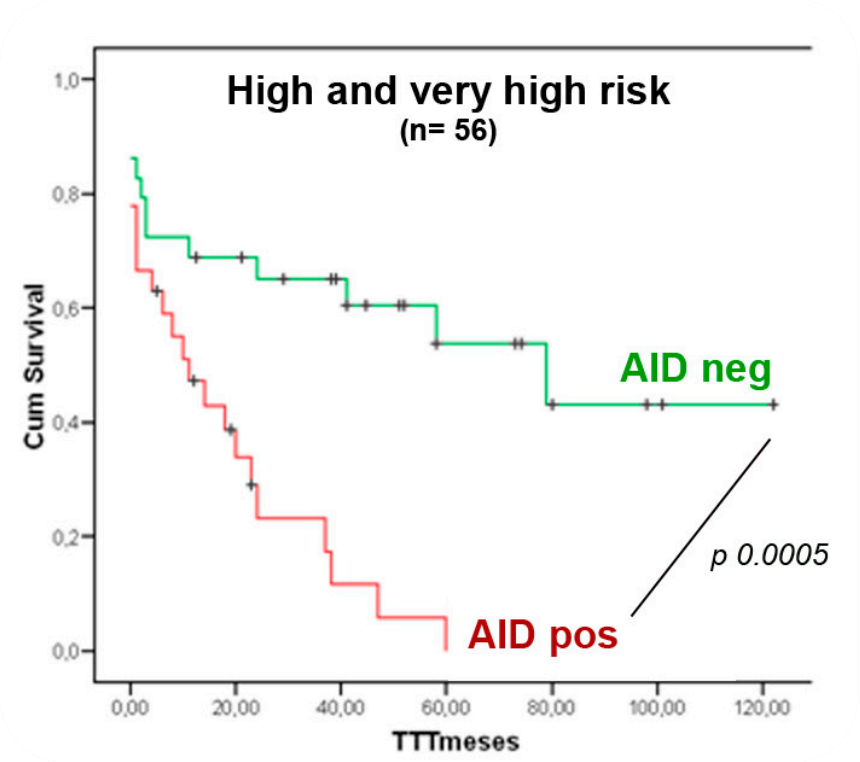

Muchas Gracias !
cATG
CENTRO AUSTRAL DE
TECNOLOGÍA GENÓMICA
Universidad de
Magallanes

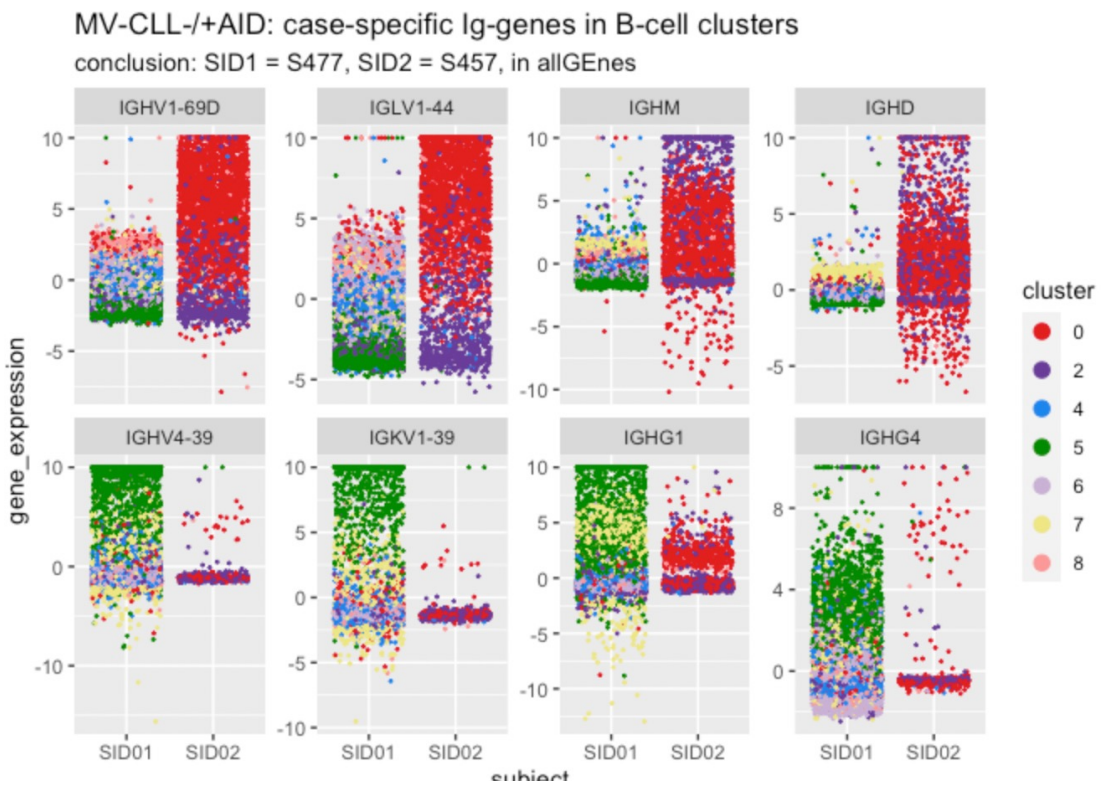
CLL study cohort

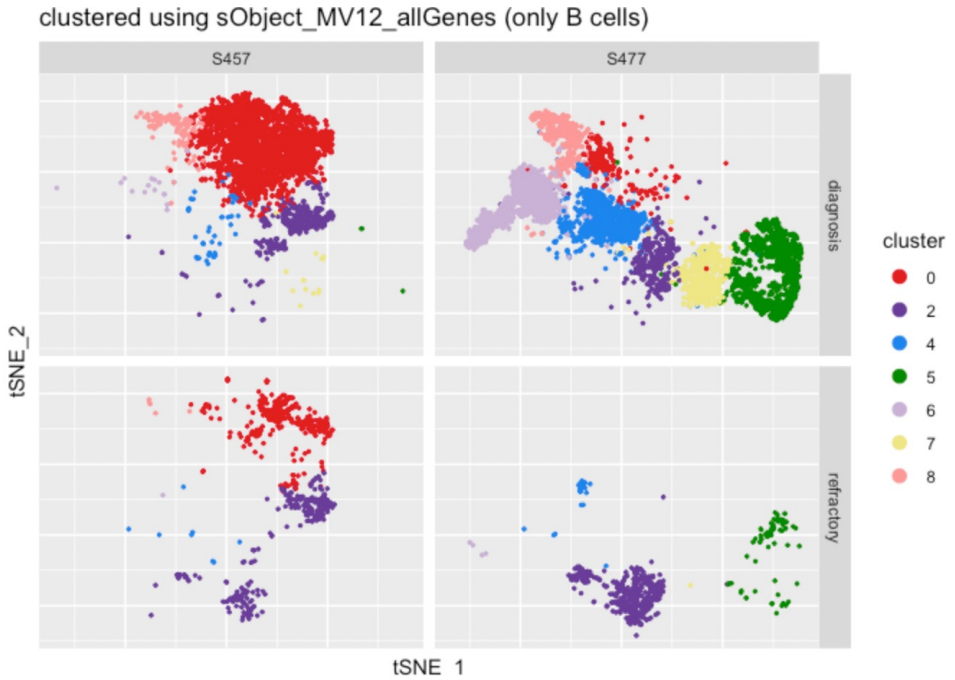
Low risk	•Watch and wait
Intermediate risk	•Not be treated unless disease presents symptoms
High risk	**•Start treatment except asymptomatic**
Very high risk	**•Treatment •Incorporation of novel drugs •Avoid chemoimmunotherapy**

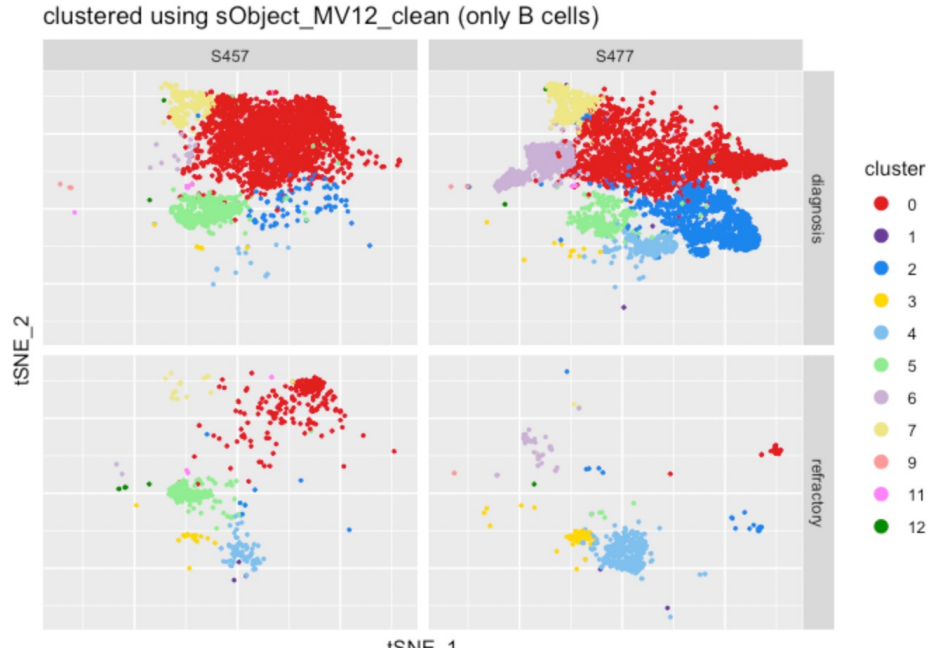
	CLL-IPI risk score	Patients	Median overall survival (months [95% CI])
Training dataset		1214	
Low	0–1	341 (28%)	NR
Intermediate	2–3	474 (39%)	105 (96–119)
High	4–6	337 (28%)	75 (68–82)
Very high	7–10	62 (5%)	29 (18–40)






MV-CLL-/+AID: case-specific Ig-genes in B-cell clusters
conclusion: SID1 = S477, SID2 = S457, in allGEnes
IGHV1-69D
IGLV1-44
IGHM
IGHD
IGHV4-39
IGKV1-39
IGHG1
IGHG4
gene_expression
SID01
SID02
subject
cluster
0
2
4
5
6
7
8


clustered using sObject_MV12_allGenes (only B cells)
S457
S477
diagnosis
refractory
tSNE_2
tSNE_1
cluster
0
2
4
5
6
7
8


clustered using sObject_MV12_clean (only B cells)
S457
S477
diagnosis
refractory
tSNE_2
tSNE_1
cluster
0
1
2
3
4
5
6
7
9
11
12

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