4th POSTGRADUATE

CLL Conference

Bologna
November 13-14
2023

Royal Hotel Carlton

President:
Pier Luigi Zinzani

Genetic portrait of CLL: insights from mouse models

Dimitar Efremov, MD, PhD
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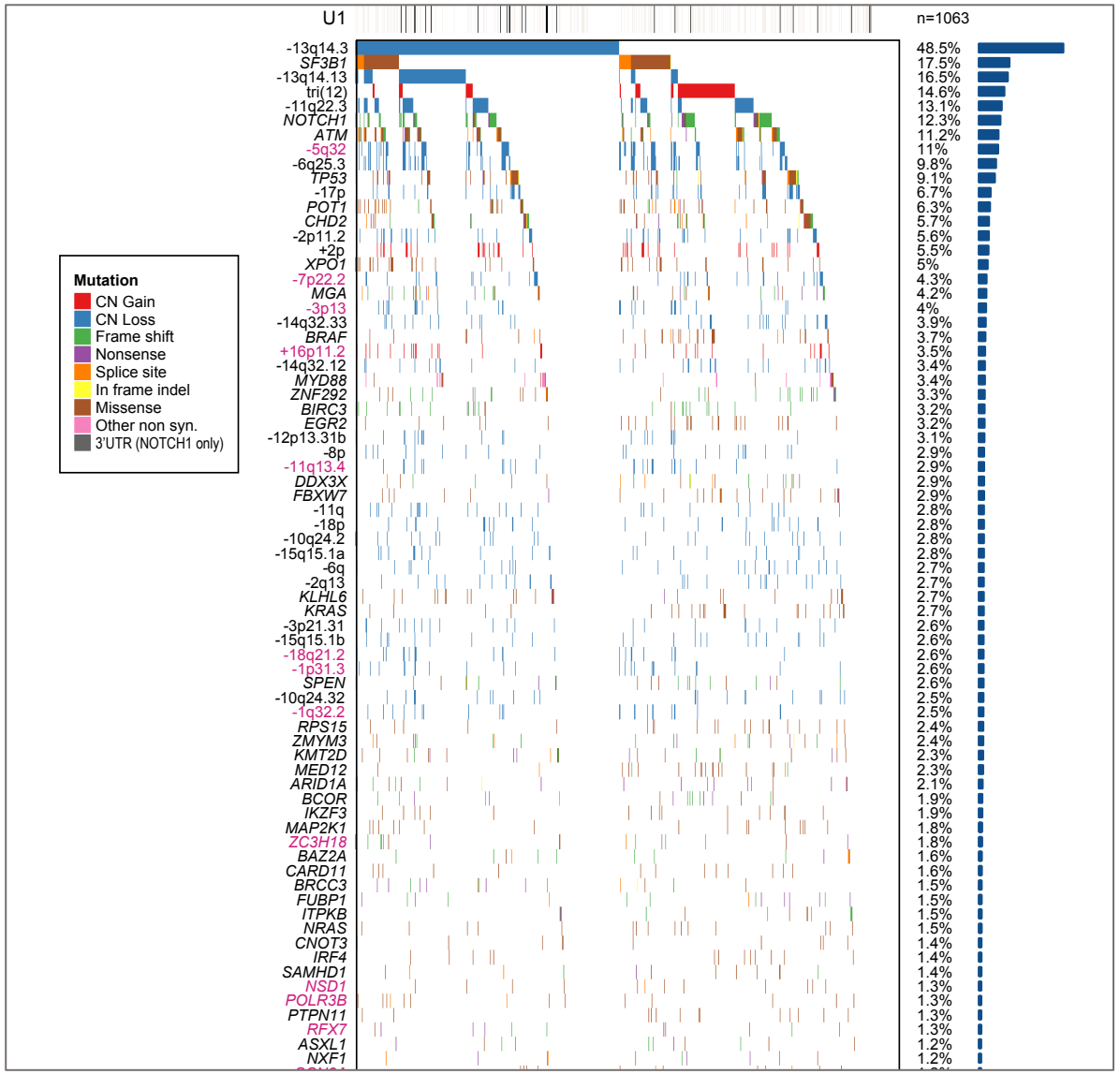
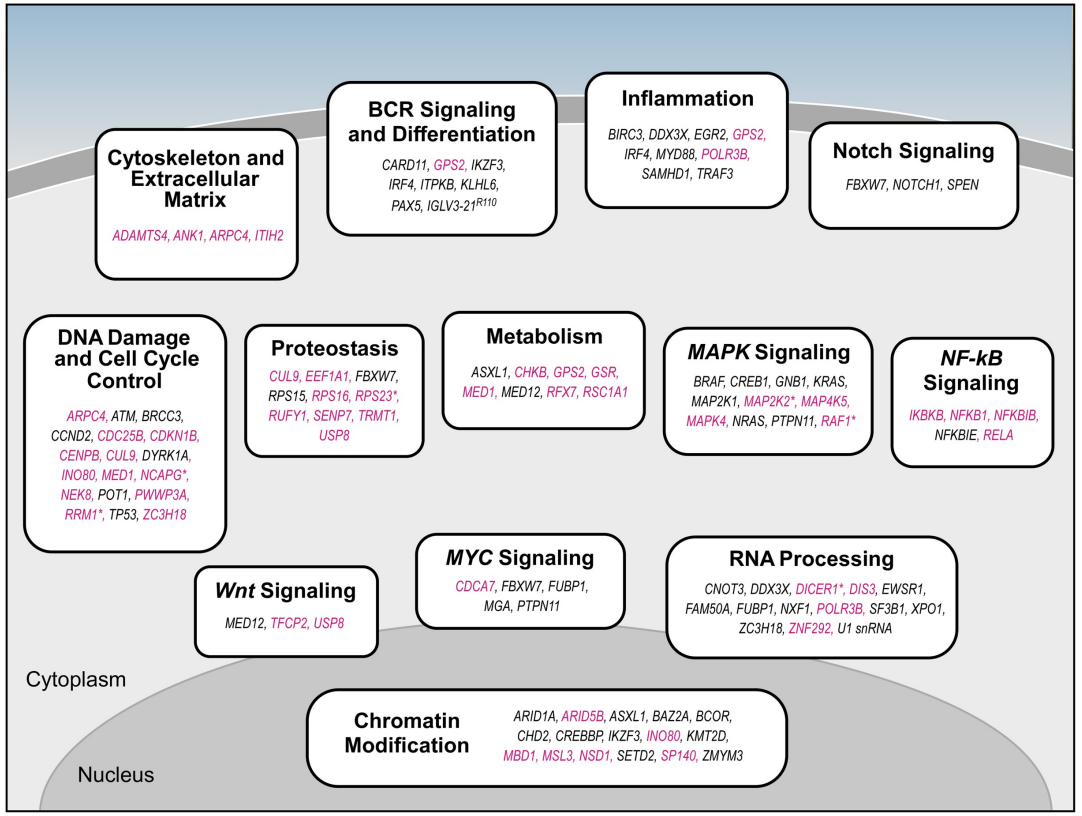
Chronic Lymphocytic Leukemia – Genetic lesions



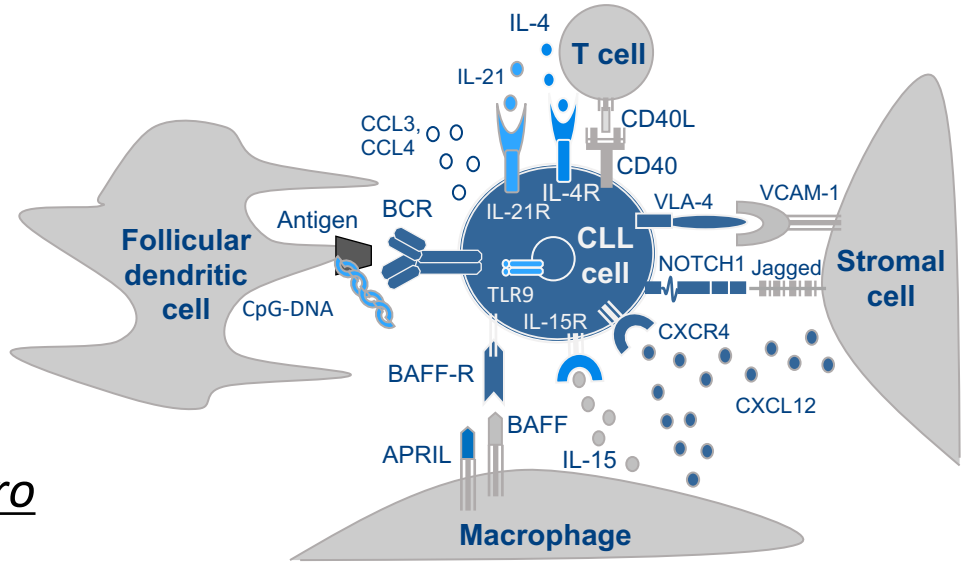
- ***sSNV/indel in 82 putative CLL driver genes*** *(59 mutated in <2% of patients)*
- ***sCNA in 130 genomic regions***



Knisbacher BA et al. *Molecular map of chronic lymphocytic leukemia and its impact on outcome.* Nat Genet. 2022

Chronic Lymphocytic Leukemia – Microenvironmental signals

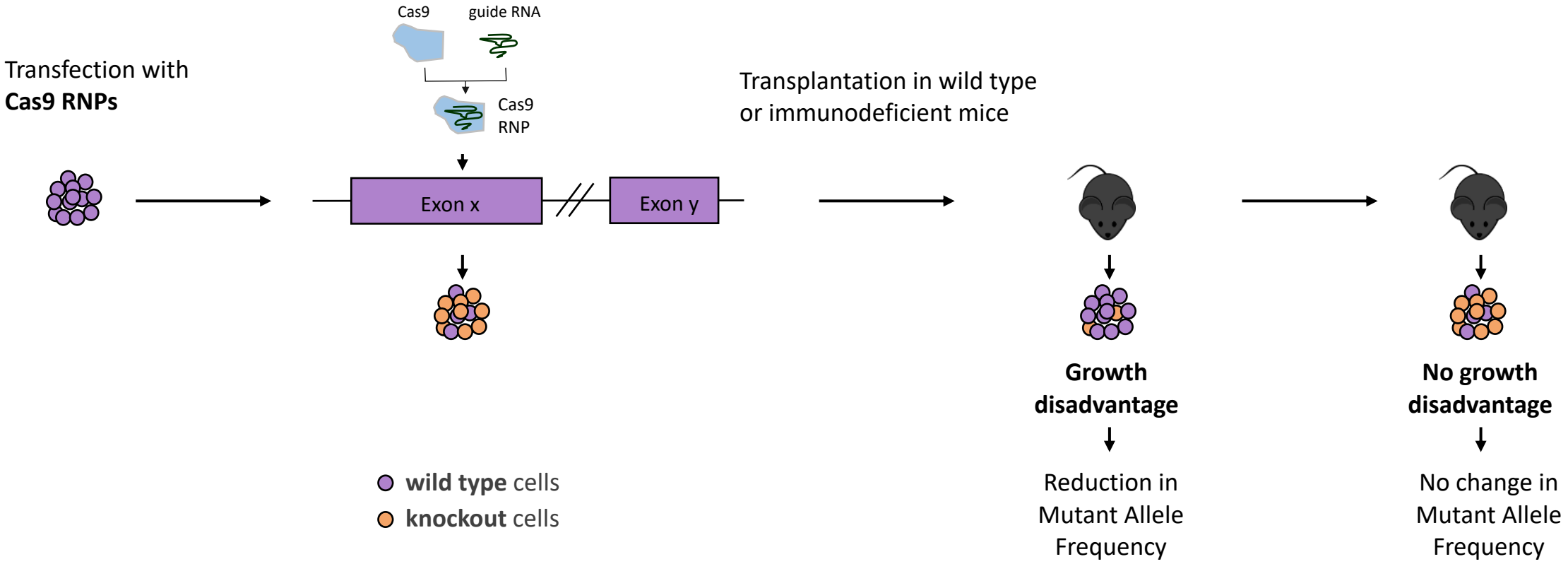
- Microenvironmental stimuli that increase CLL cell **survival** *in vitro*
  - T cells (CD40L, IL-4)
  - Mesenchymal stromal cells (Jagged, VCAM-1, CXCL12)
  - Macrophages (BAFF, APRIL, IL-15, CXCL12, Wnt5a)
  - B-cell receptor (BCR) ligands (apoptosis-associated autoantigens)
  - Toll-like receptor (TLR) ligands (CpG-unmethylated mitochondrial DNA)



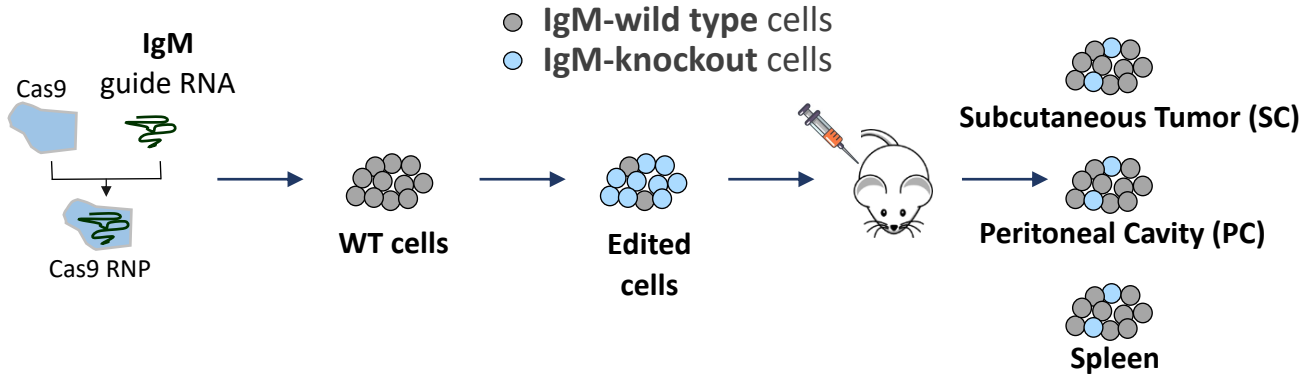
- Microenvironmental stimuli that induce CLL cell **proliferation** *in vitro*
  - T cell derived (CD40L + IL-4 + IL-21)
  - Toll-like receptor ligands (CpG-DNA) + IL-15/IL-2

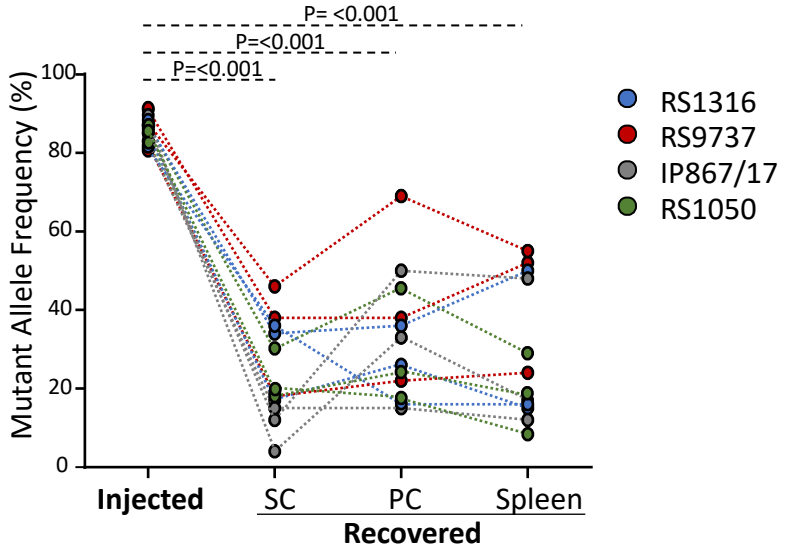
- Microenvironmental stimuli that induce **resistance** to the **novel drugs** *in vitro*
  - B cell receptor stimulation (venetoclax resistance)
  - Toll-like receptor ligands (CpG-DNA) (venetoclax resistance)
  - T cell coculture (CD40 stimulation) (venetoclax resistance)
  - Macrophage coculture (venetoclax resistance)
  - IL4 (ibrutinib resistance)
  - Integrin VLA-4 stimulation (ibrutinib resistance)

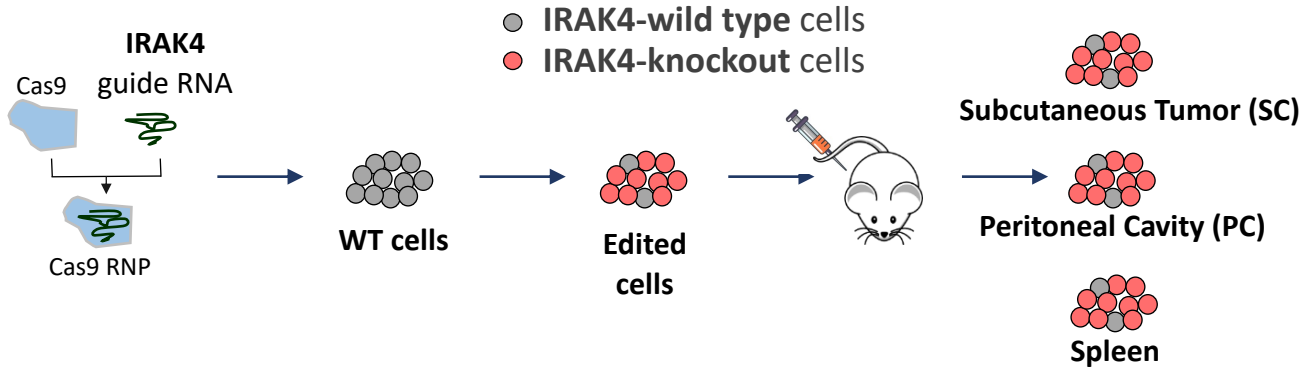
Genome editing of intracellular signaling pathways in primary murine CLL or patient-derived Richter Syndrome cells **to study *in vivo* the role of microenvironmental signals** in CLL cell growth, survival and treatment resistance

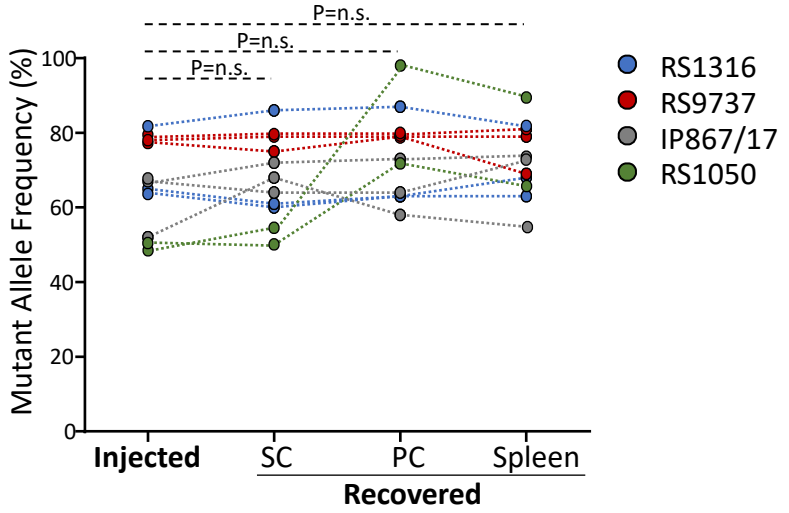


Genetic disruption of B cell receptor (BCR) but not Toll-like receptor (TLR) signaling suppresses the growth of xenografted human Richter syndrome cells *in vivo*





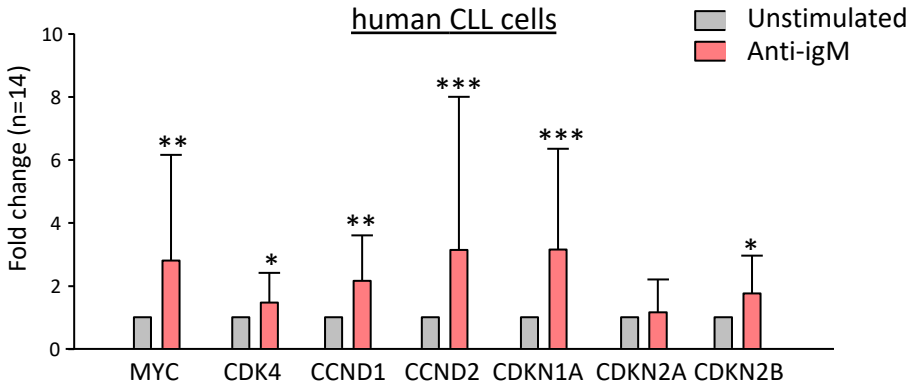


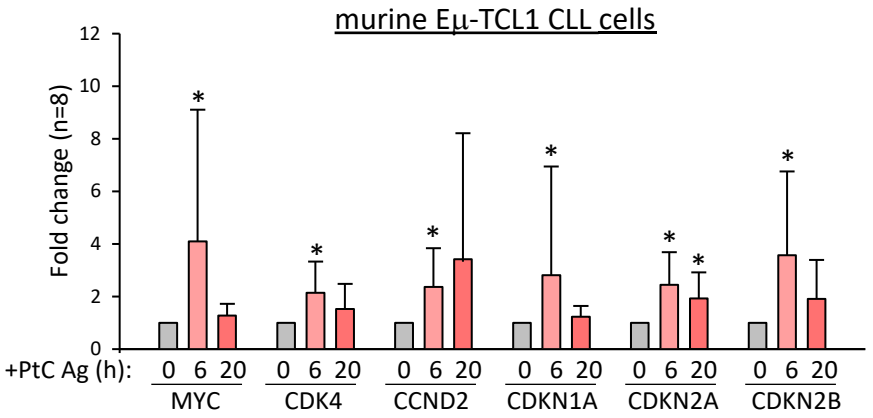


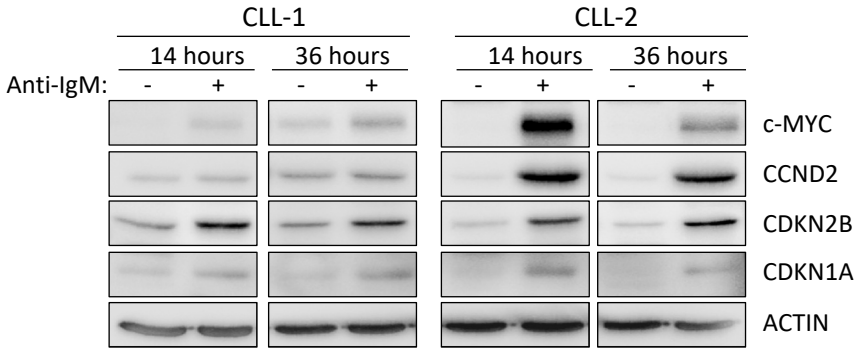
Martines C et al. *Macrophage- and BCR-derived but not TLR-derived signals support the growth of CLL and Richter syndrome murine models in vivo*. **Blood** 2022; 140:2335-2347

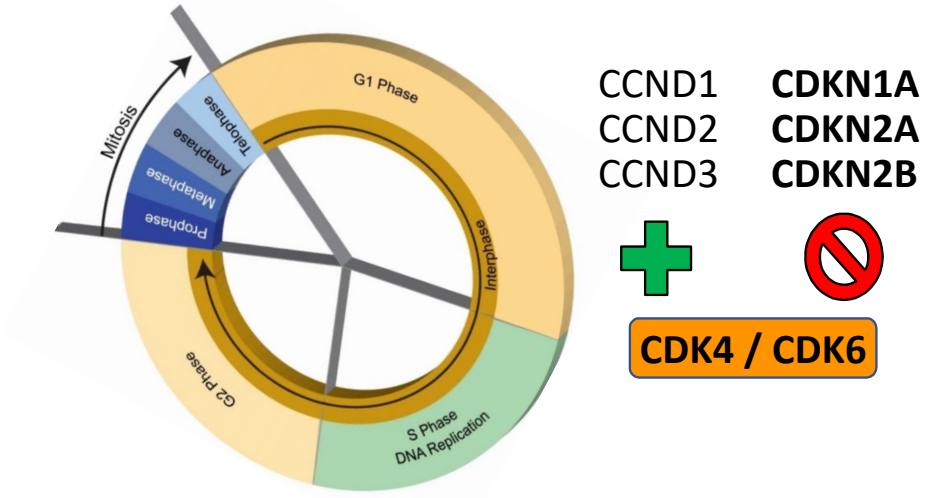
Cooperation between microenvironmental signals and genetic lesions during CLL transformation

BCR signaling induces both **positive** (CCND1, CCND2, CDK4, MYC) and **negative** (CDKN1A, CDKN2A, CDKN2B) **cell cycle regulators** in human and murine CLL B cells







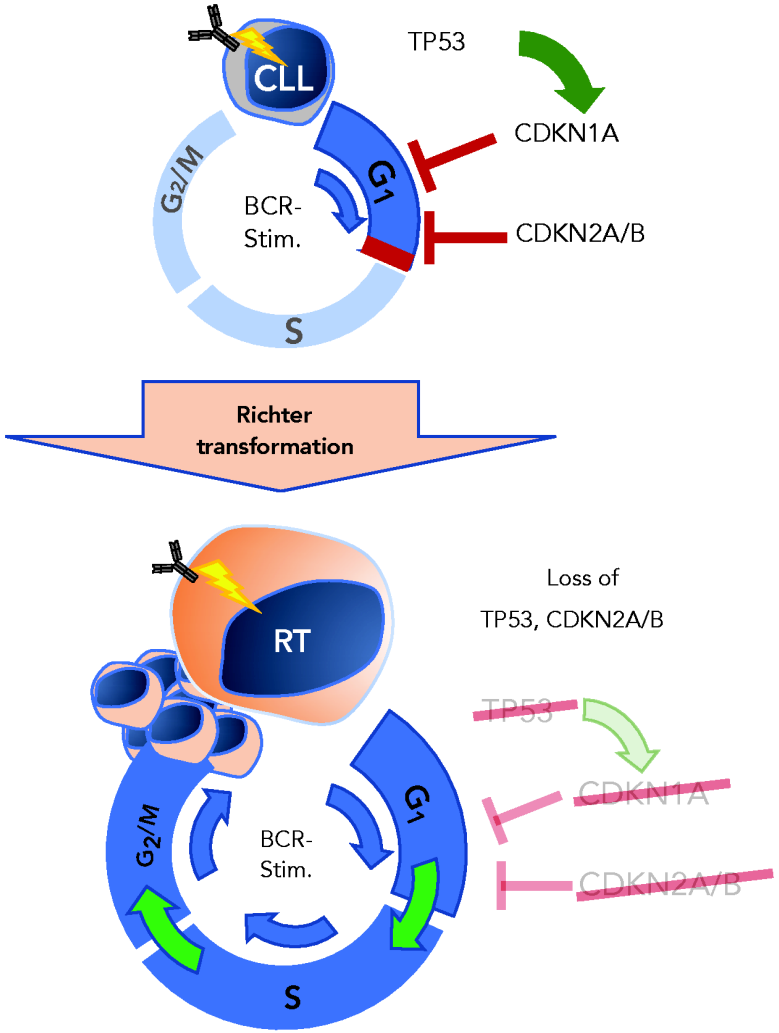


Chakraborty S et al. *B-cell receptor signaling and genetic lesions in TP53 and CDKN2A/CDKN2B cooperate in Richter transformation*. **Blood**. 2021; 138:1053-1066

Common genetic lesions in clonally-related Richter Transformation

Genetic lesion	Frequency in CLL	Frequency in RT	Cellular Pathway
TP53 mutation and/or deletion (del17p13)	10% -15%	**>80%**	DNA damage response and cell cycle (induces CDKN1A)
CDKN2A/CDKN2B deletion (del9p21)	1.5%	**40 - 50%**	Cell cycle
MYC abnormalities: t(8;14), amp(8q24)	5 -7%	15 - 30%	MYC signaling
MGA deletion (del15q)	4%	20%	MYC signaling
NOTCH1 mutation	10% -15%	30%	NOTCH1 signaling

Parry EM et al. Nat Med. 2023; 29(1):158-169
Nadeu F et al. Nat Med. 2022; 28(8):1662-1671
Klintman J et al. Blood. 2021;137(20):2800-2816.
Fabbri G. J Exp Med. 2013; 210(11):2273-2288
Chigrinova E et al. Blood. 2013; 122(15):2673-2682
Leeksma AC et al. Haematologica. 2021; 106(1):87-97.
Knisbacher BA et al. Nat Genet. 2022; 54(11):1664-1674

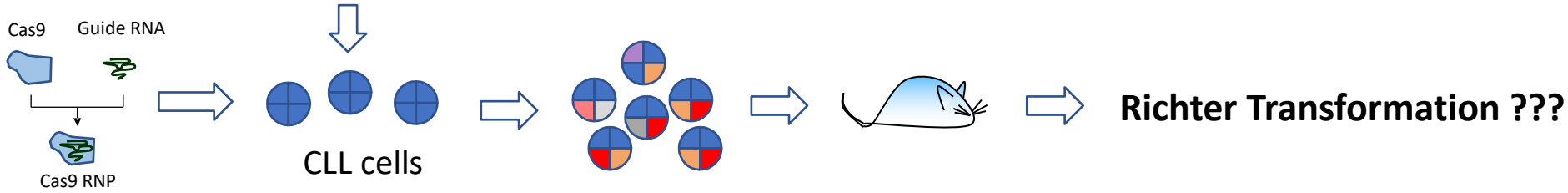


Adapted from Pallasch CP, Blood. 2021; 138:1005-1007

Can combined introduction of TP53, CDKN2A and CDKN2B loss-of-function genetic lesions in autoreactive murine Eμ-TCL1-derived CLL cells result in Richter Transformation?

CRISPR/Cas9 genome editing

Targeted genes:
TP53; CDKN2A; CDKN2B

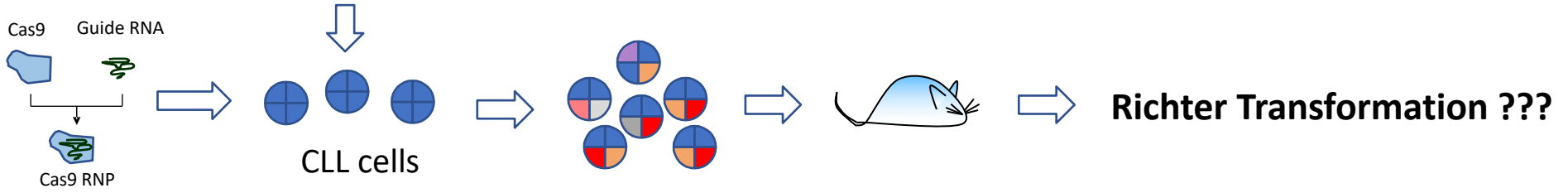


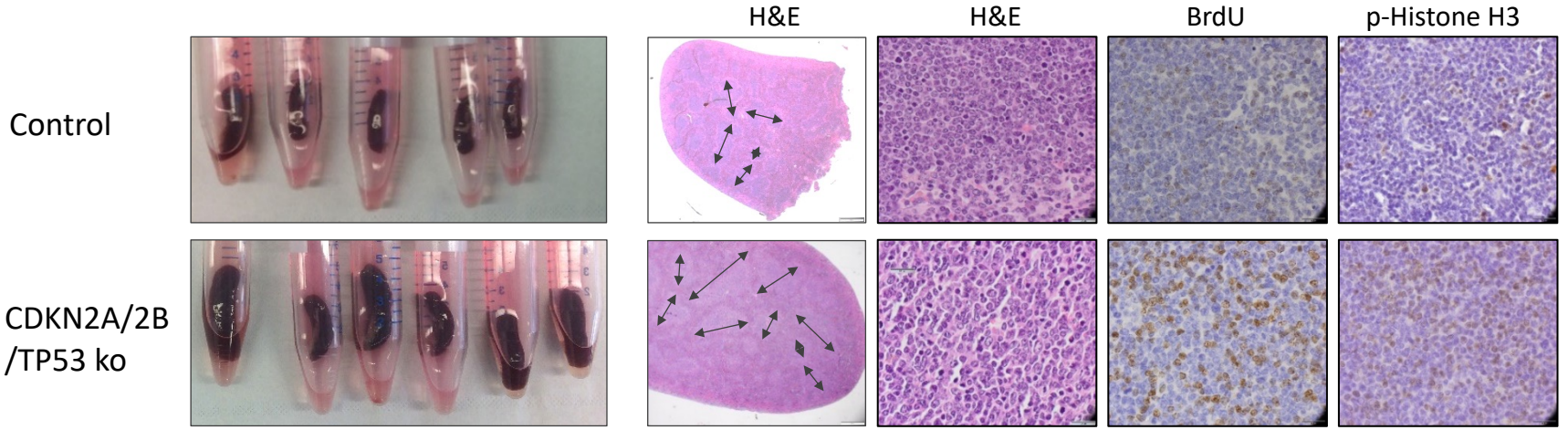
Chakraborty S et al. *B-cell receptor signaling and genetic lesions in TP53 and CDKN2A/CDKN2B cooperate in Richter transformation*. **Blood**. 2021; 138:1053-1066

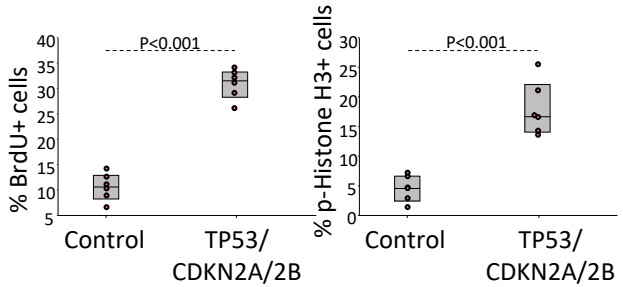
Combined introduction of TP53/CDKN2A/CDKN2B genetic lesions in murine Eμ-TCL1-derived CLL cells results in development of aggressive tumors with features of Richter's Transformation

CRISPR/Cas9 genome editing

Targeted genes: **TP53; CDKN2A; CDKN2B**

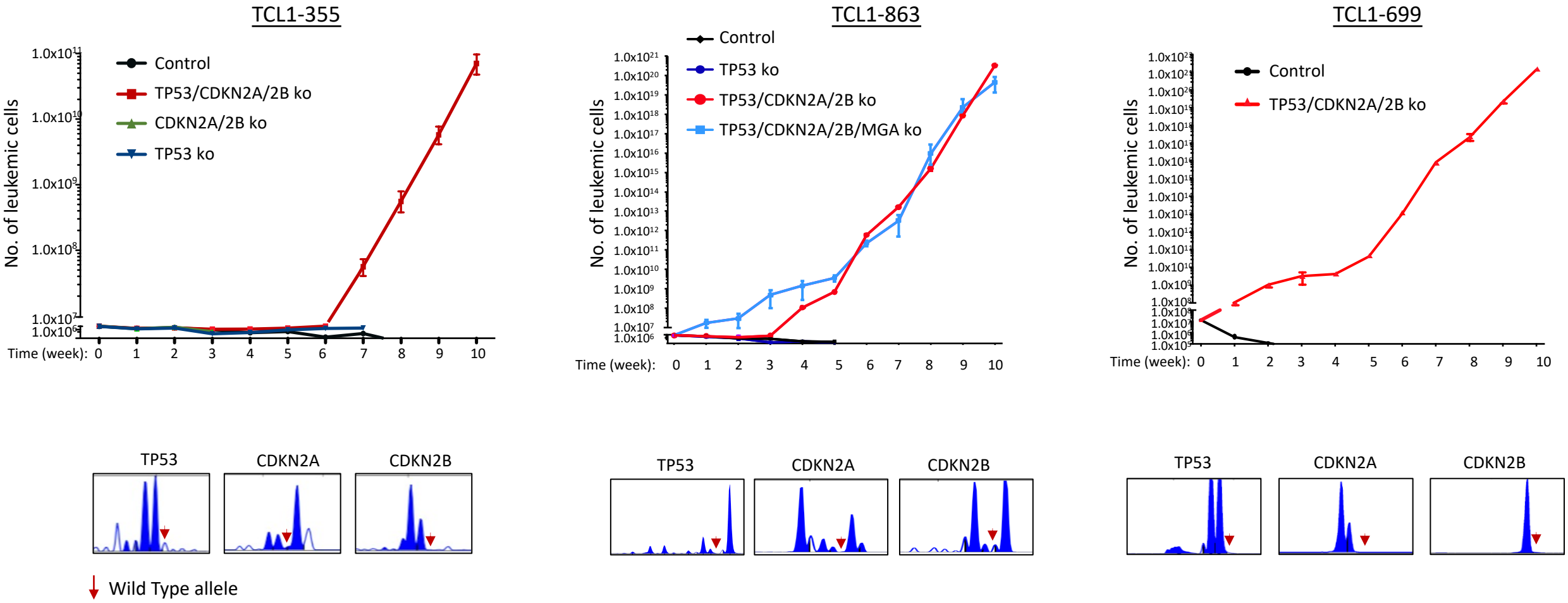






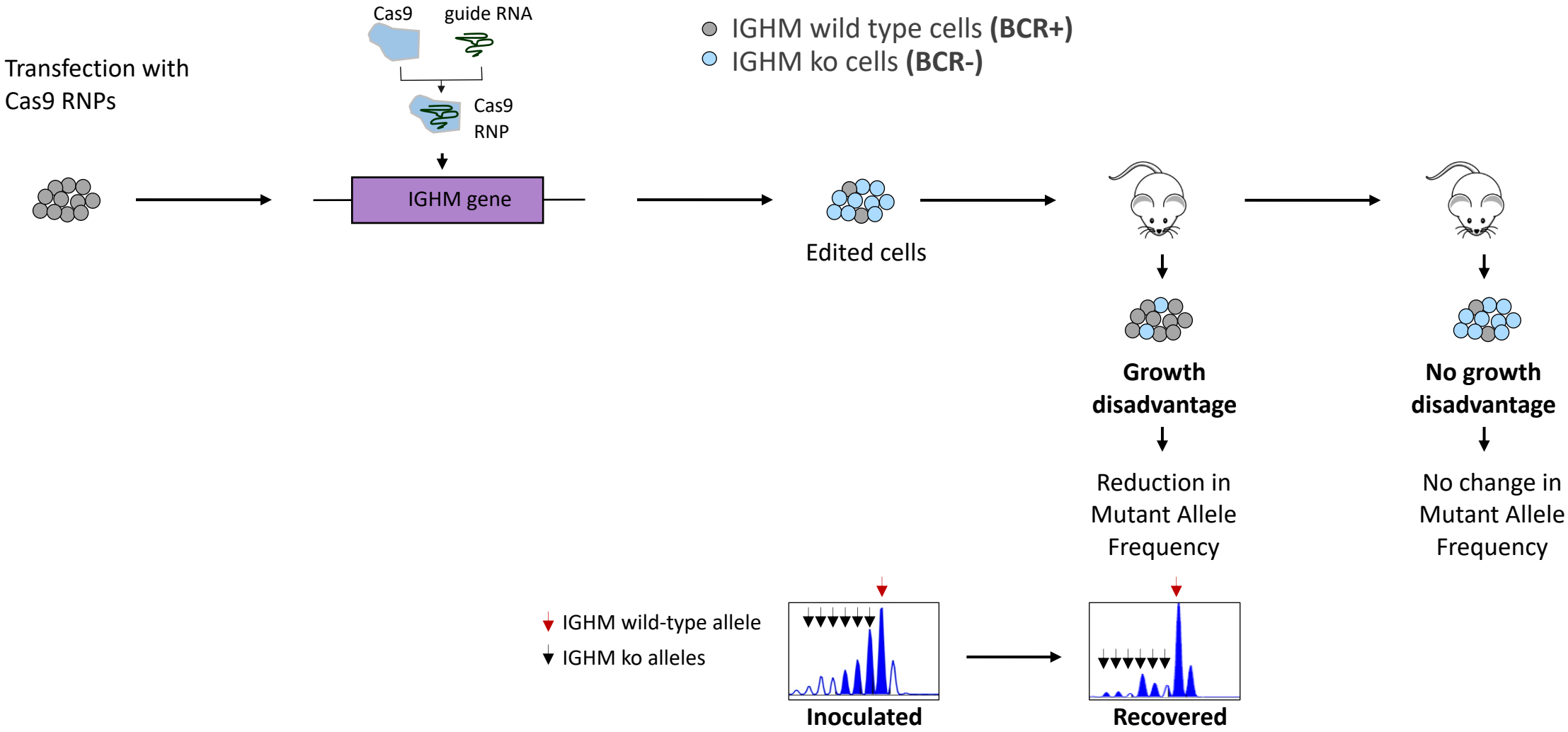
Chakraborty S et al. *B-cell receptor signaling and genetic lesions in TP53 and CDKN2A/CDKN2B cooperate in Richter transformation*. **Blood**. 2021; 138:1053-1066

Biallelic disruption of TP53, CDKN2A and CDKN2B in murine Eμ-TCL1-derived CLL cells results in spontaneous leukemia cell proliferation *in vitro*



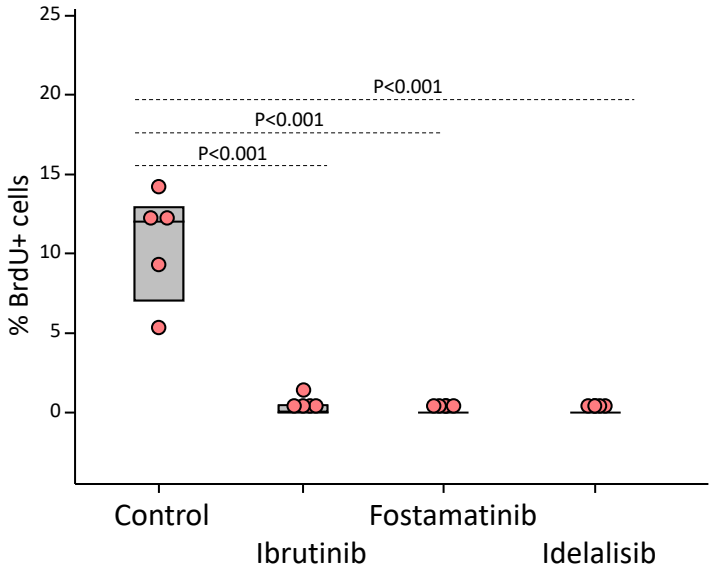
Chakraborty S et al. *B-cell receptor signaling and genetic lesions in TP53 and CDKN2A/CDKN2B cooperate in Richter transformation*. **Blood**. 2021; 138:1053-1066

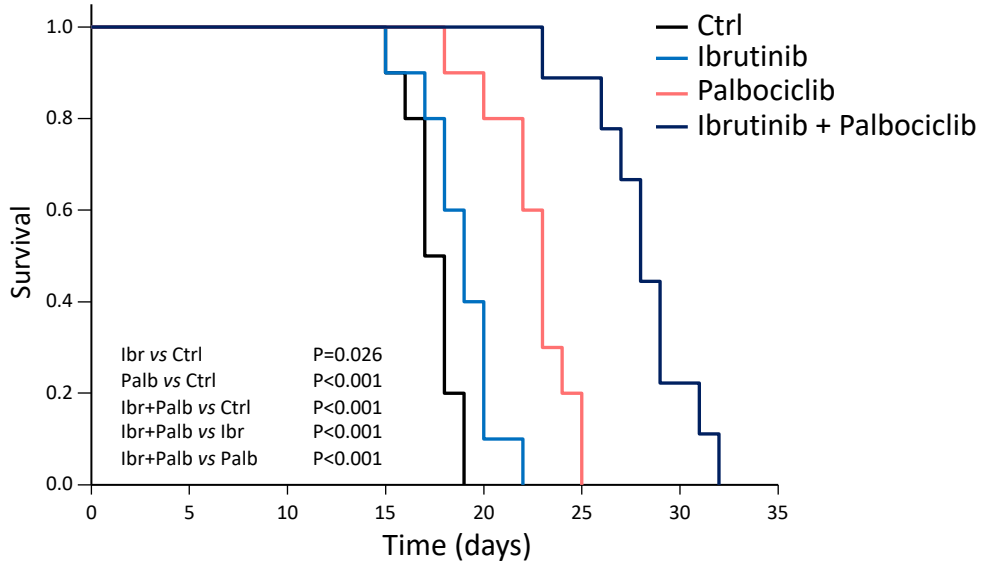
Murine Eμ-TCL1-derived RS cells with IGHM knockout are negatively selected in vivo

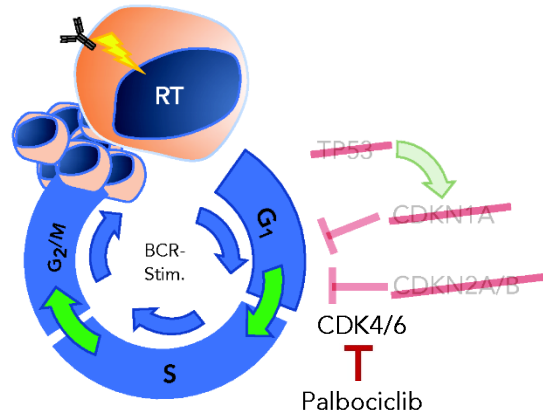


Chakraborty S et al. *B-cell receptor signaling and genetic lesions in TP53 and CDKN2A/CDKN2B cooperate in Richter transformation*. **Blood**. 2021; 138:1053-1066

Murine Eμ-TCL1-derived Richter Syndrome cells with TP53/CDKN2A/2B genetic lesions are sensitive to combination treatment with a BCR and CDK4/6 inhibitor



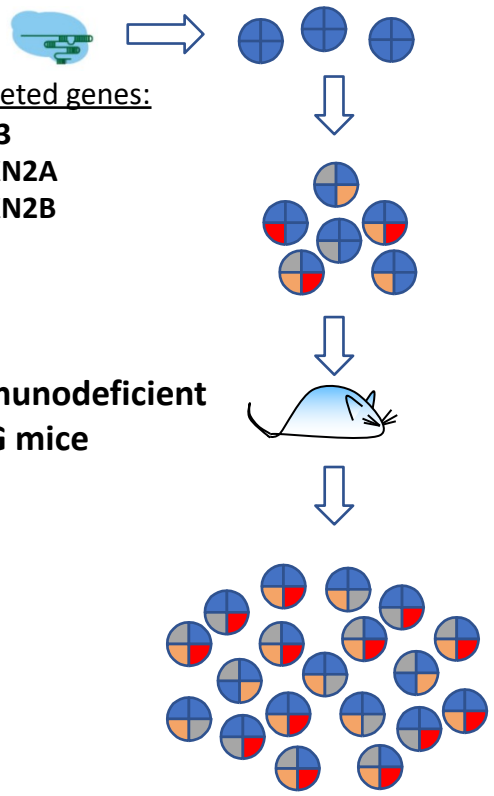


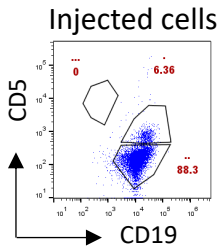


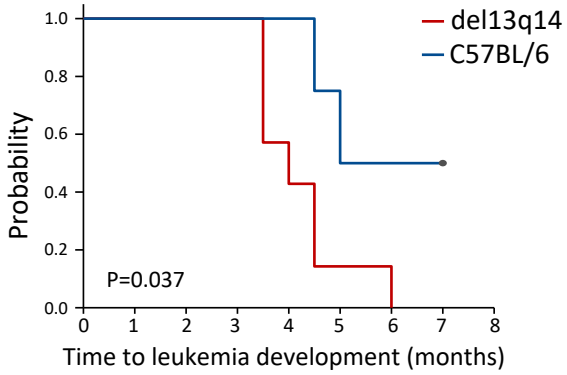
Chakraborty S et al. *B-cell receptor signaling and genetic lesions in TP53 and CDKN2A/CDKN2B cooperate in Richter transformation*. **Blood**. 2021; 138:1053-1066

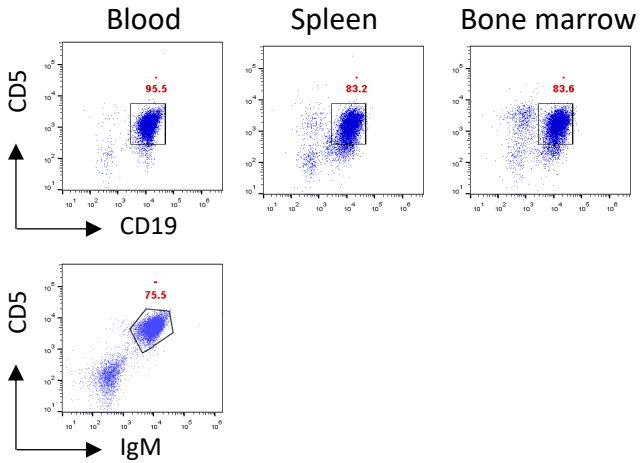
Biallelic disruption of TP53, CDKN2A and CDKN2B in non-leukemic B cells from del13q14 or C57BL/6 mice results in development of CD5+/IgM+ B cell tumors with features of Richter's Transformation

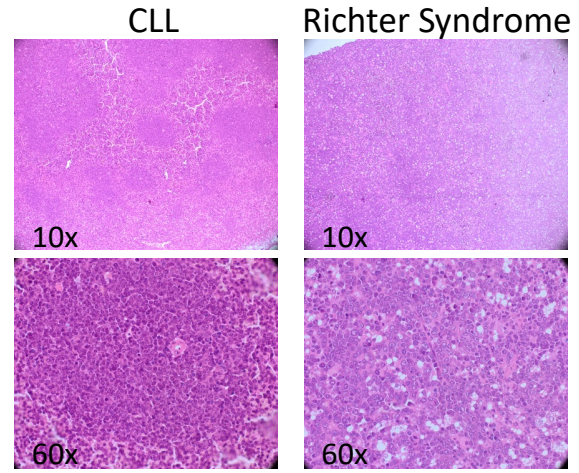
non-leukemic murine B cells
(del13q14 n=7, C57BL/6 n=4)

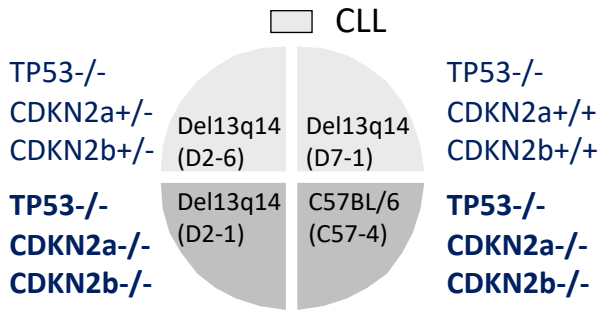


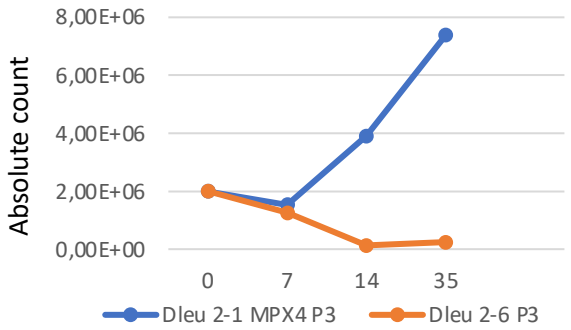






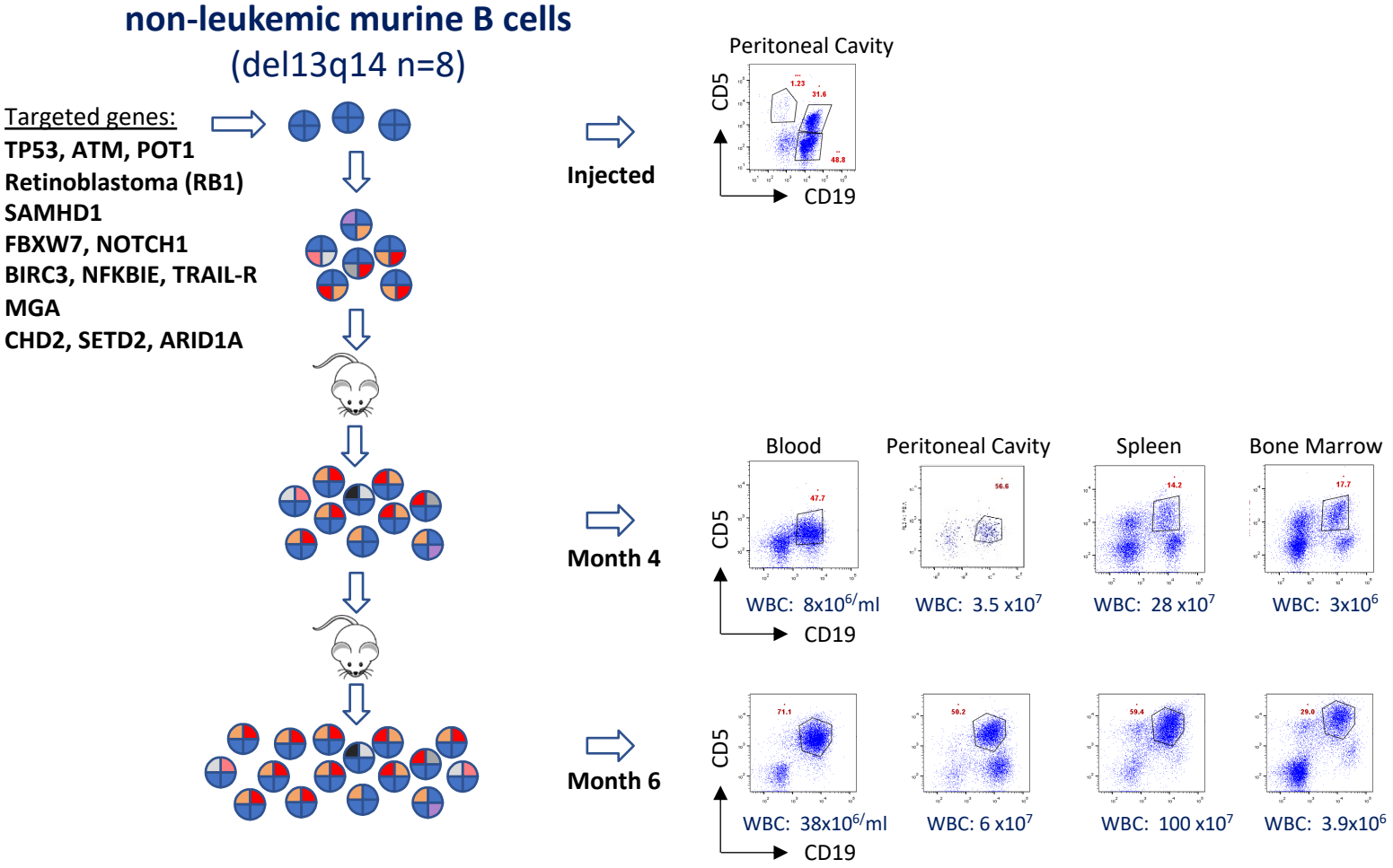


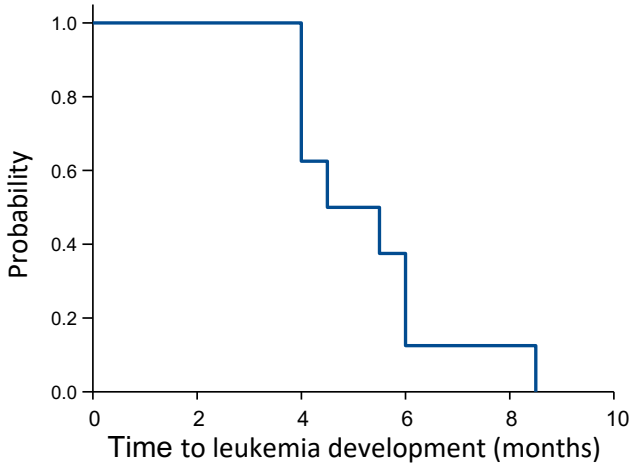


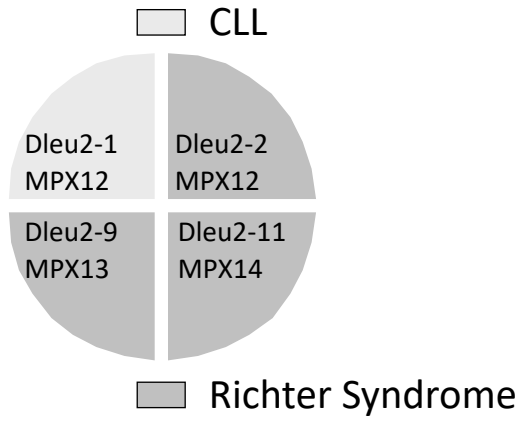


Chakraborty S et al, unpublished

Generation of murine CLL/RS models by multiplexed CRISPR/Cas9 editing of TP53 and 12-14 CLL driver genes other than CDKN2A/CDKN2B





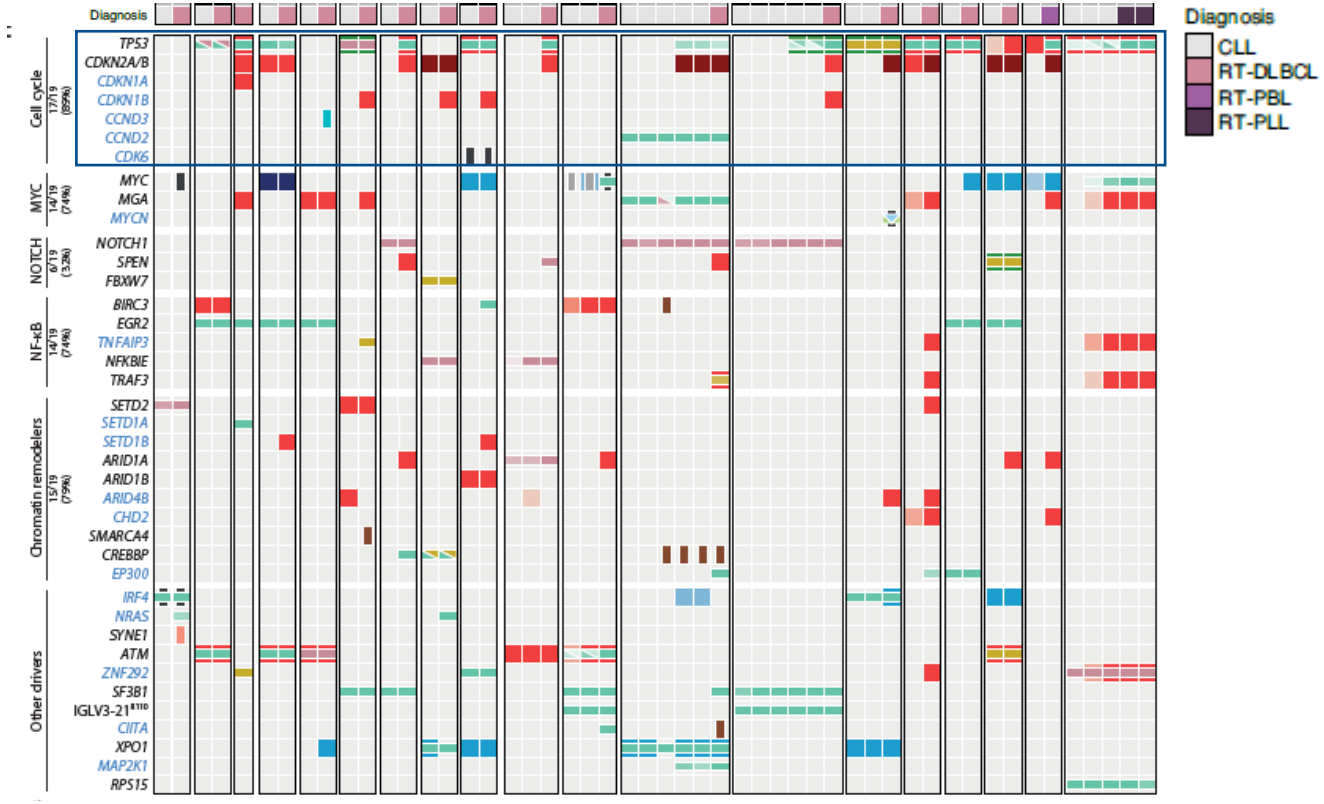


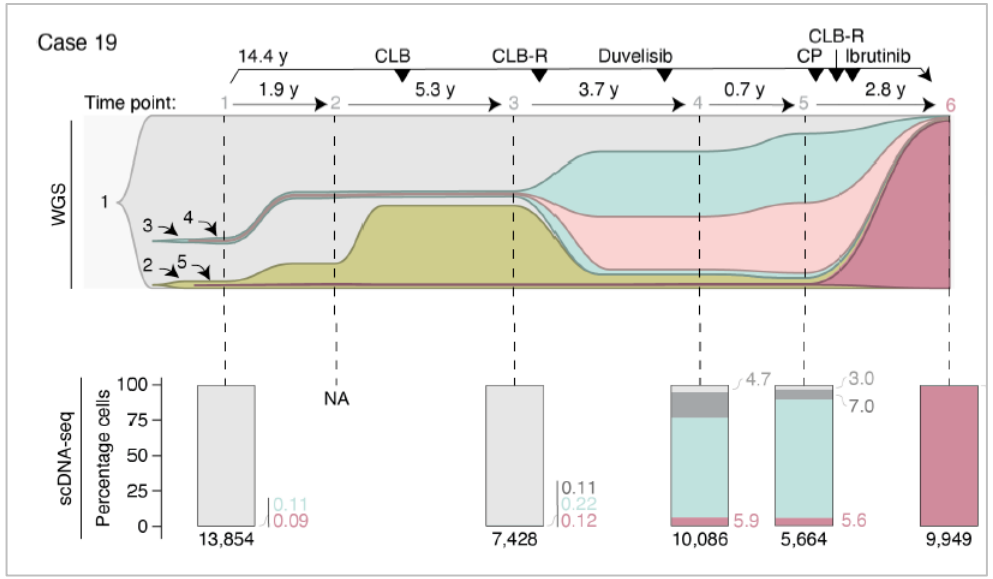
Chakraborty S et al, unpublished

Can events in the tumor microenvironment contribute to Richter Transformation?

Early seeding of Richter transformation: early acquisition of driver alterations

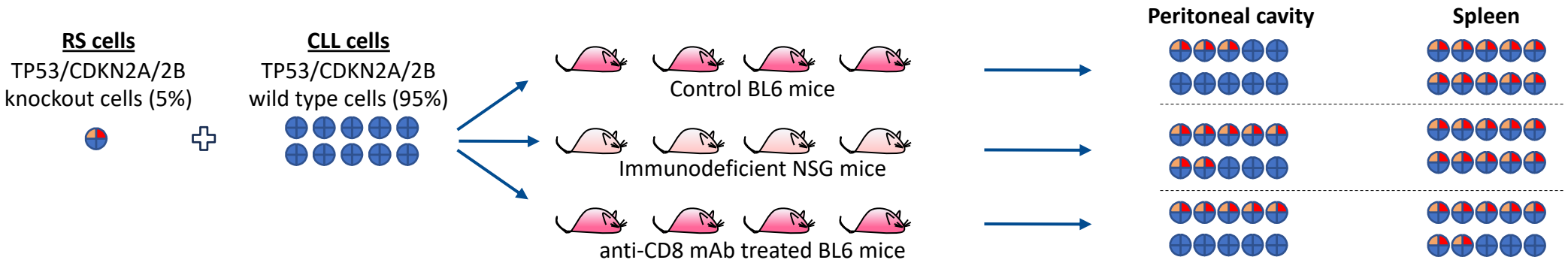
- Deletions or inactivating mutations in cell cycle inhibitors or activating mutations in positive cell cycle regulators present in >90 of Richter Syndrome cases
- Small subclones of cells with genomic features of RS may be present for many years prior to the appearance of the clinical manifestations of Richter Syndrome

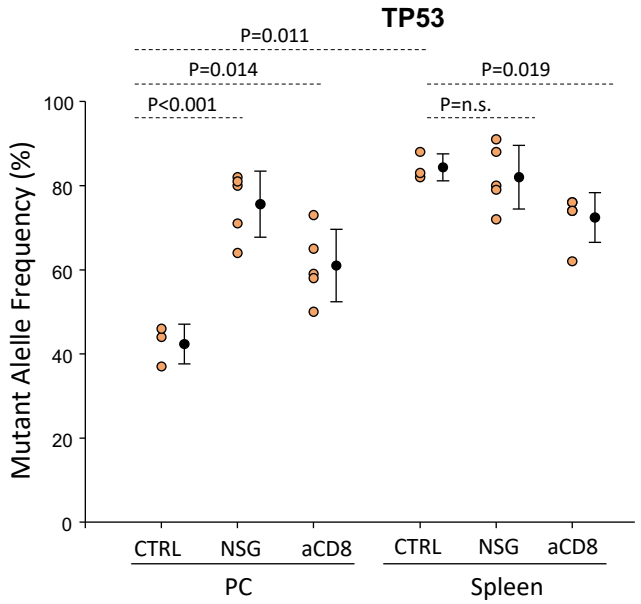


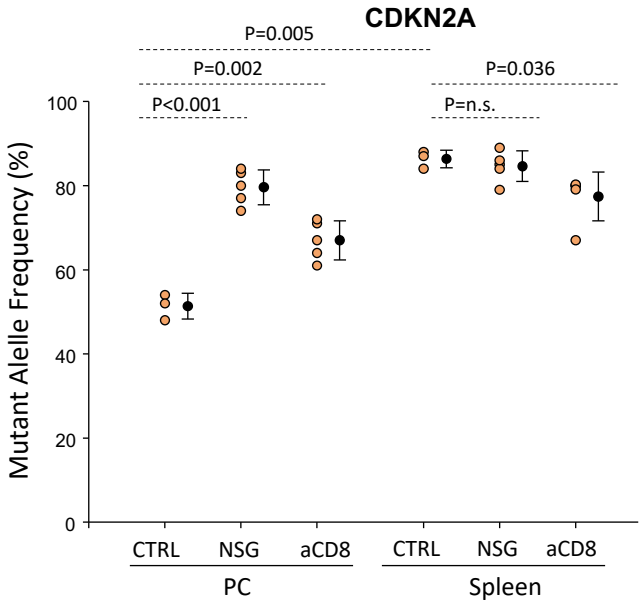


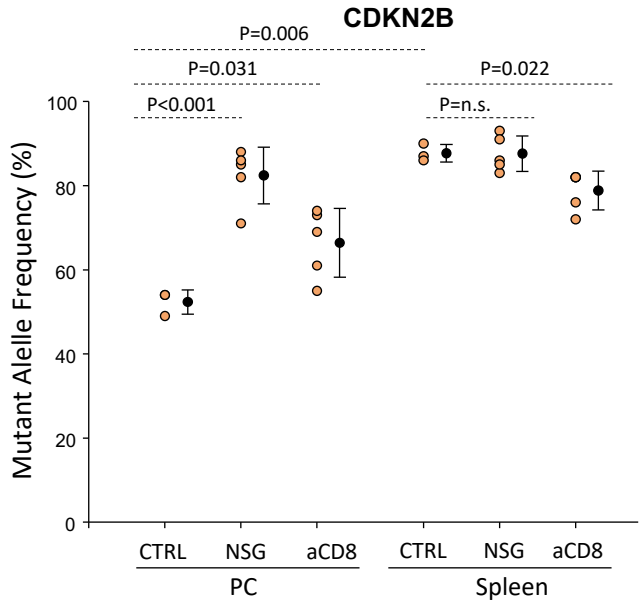
Nadeu F et al. *Detection of early seeding of Richter transformation in chronic lymphocytic leukemia*. **Nat Med.** 2022; 28:1662-1671

Murine RS cells with TP53/CDKN2A/2B lesions are differently selected in peritoneal cavity vs spleen and in immunodeficient vs immunocompetent mice







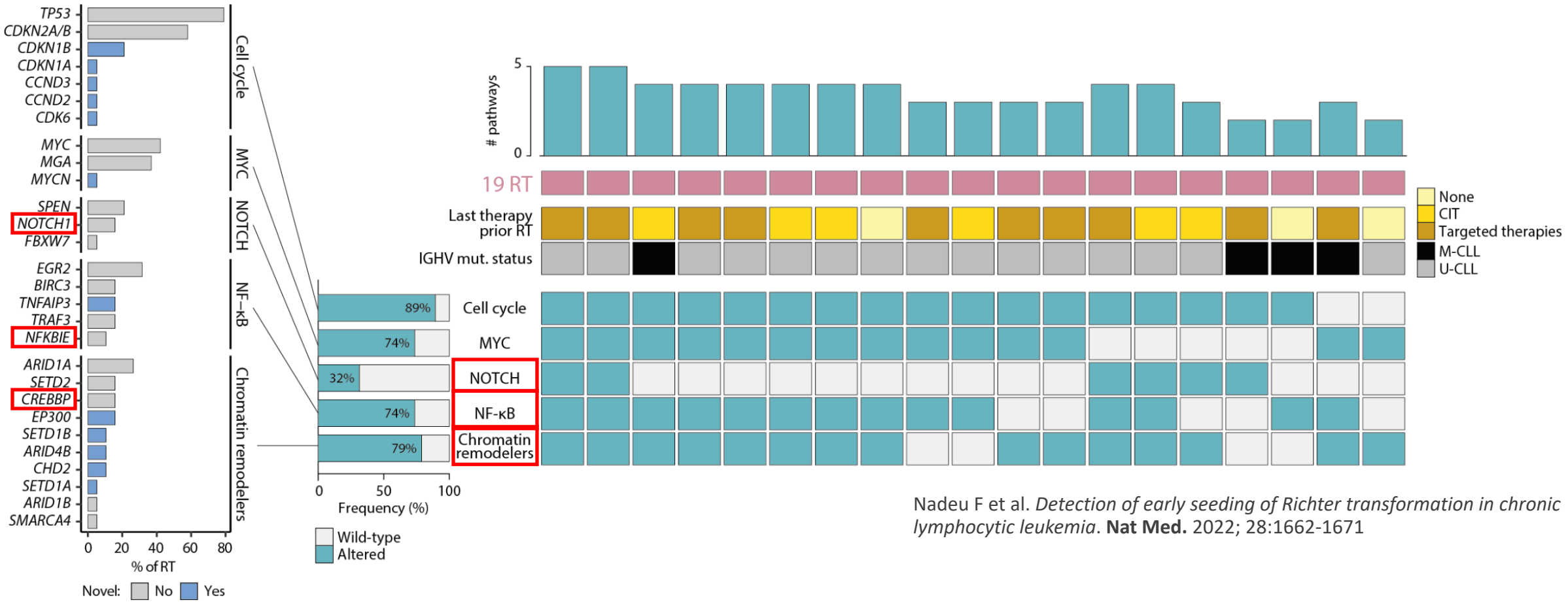


Martines C et al, unpublished

Potential mechanisms that can cause loss of immune control in Richter Syndrome

- Mutations in NOTCH1 and certain chromatin modifiers contribute to immune evasion by downregulating expression of MHC class II genes and upregulating PD-L1 expression on the malignant B cells

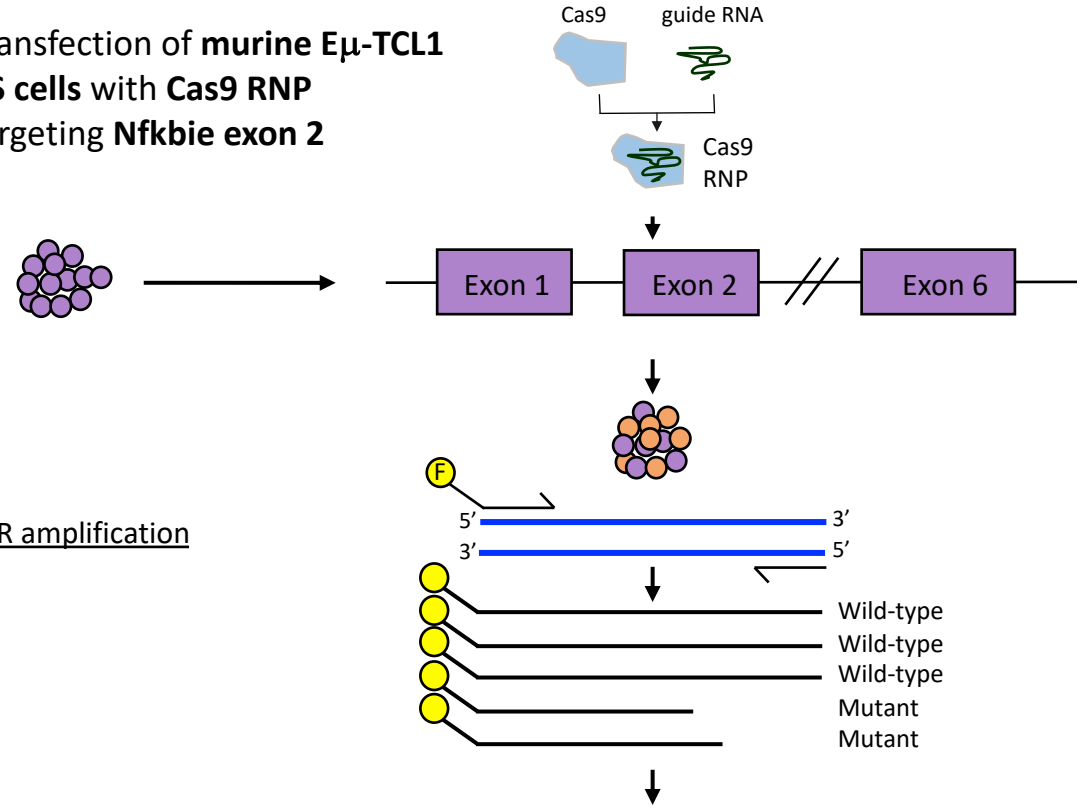
Mangolini M et al. Nat Commun. 2022; 13:6220
Fontes JD et al, Mol Cell Biol. 1999; 19(1):941-7
Green MR et al, Proc Natl Acad Sci U S A. 2015; 112(10):E1116-25

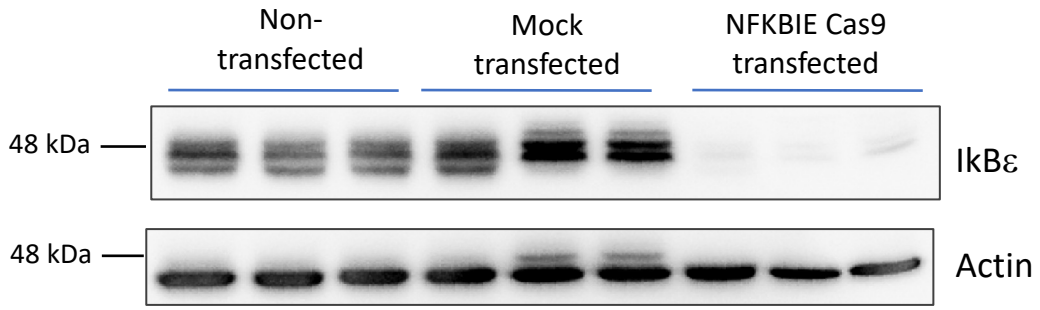


Nadeu F et al. *Detection of early seeding of Richter transformation in chronic lymphocytic leukemia*. **Nat Med.** 2022; 28:1662-1671

Introduction of loss-of-function NFKBIE mutations in TCL1-derived Richter Syndrome cells by CRISPR/Cas 9 editing

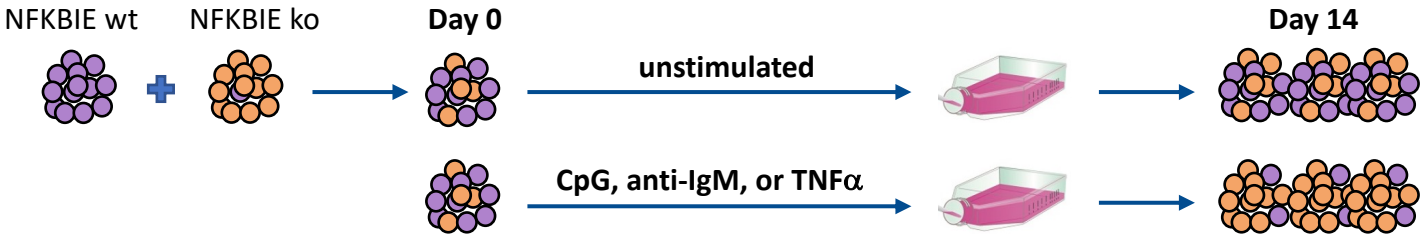
Transfection of **murine Eμ-TCL1 RS cells** with **Cas9 RNP** targeting **Nfkbie exon 2**

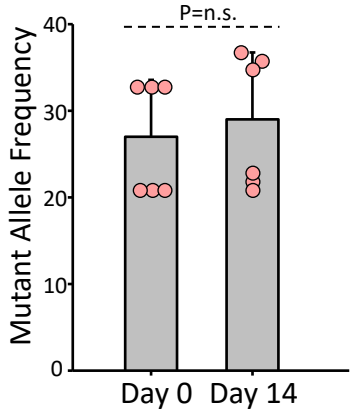


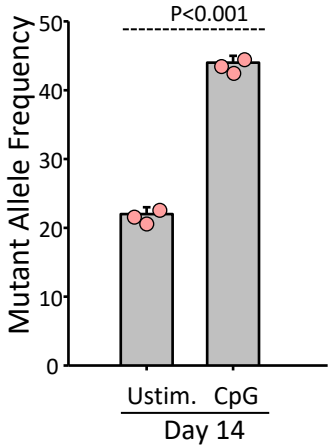


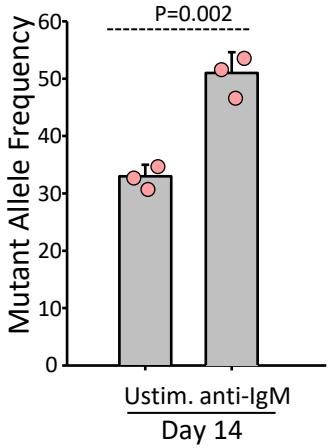
Bonato A et al, 2023 (manuscript submitted)

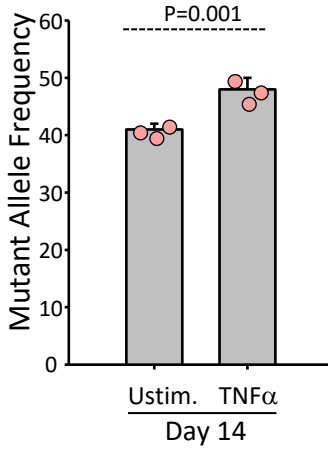
Murine RS cells with NFKBIE-mutations are positively selected by microenvironmental signals that activate the NF-kB pathway

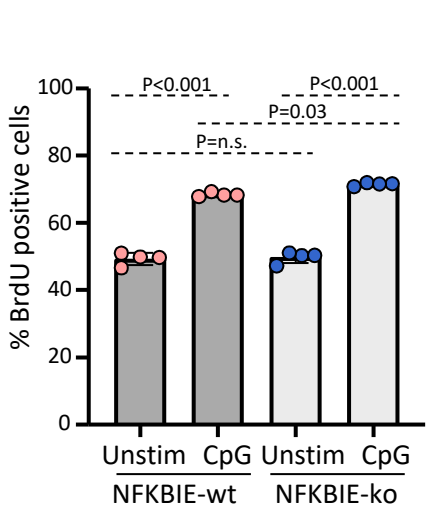


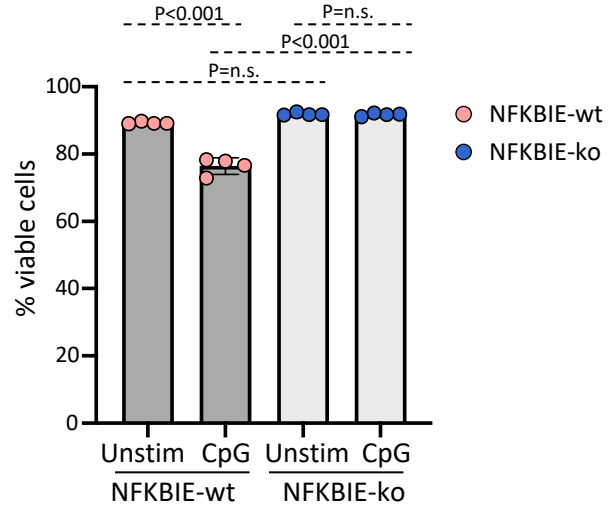






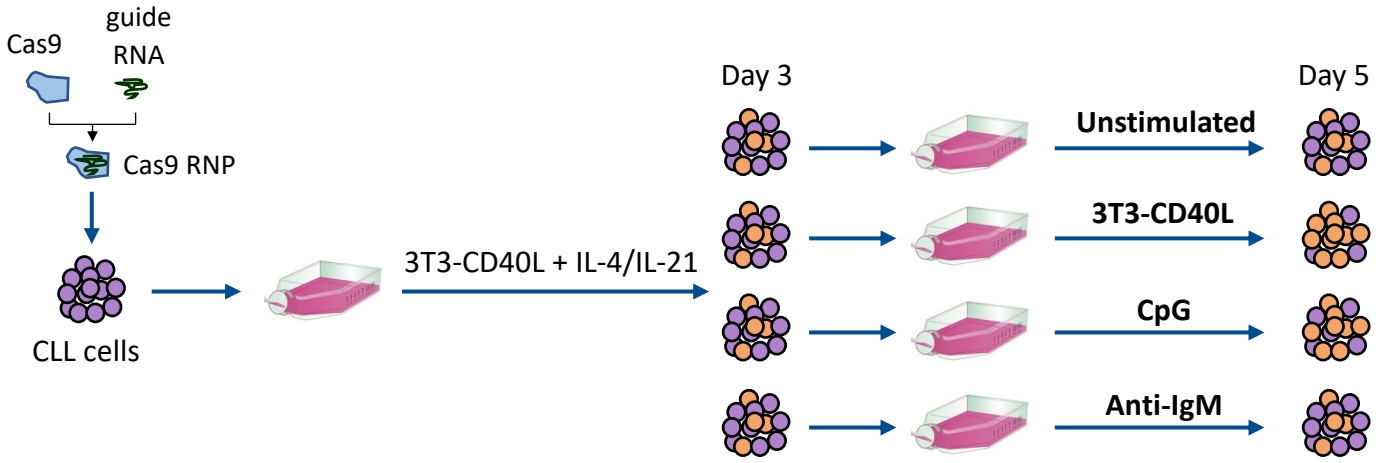


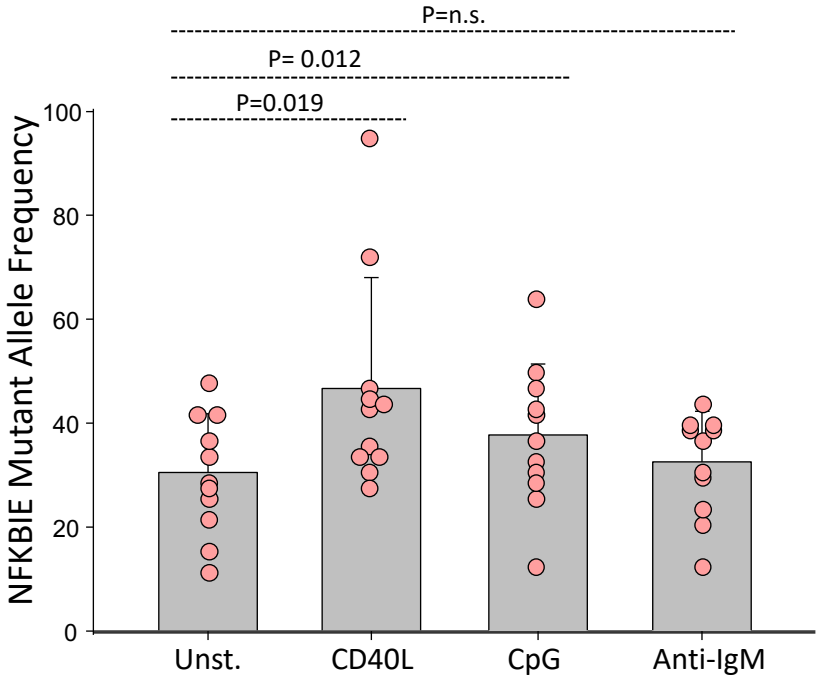




Bonato A et al, 2023 (manuscript submitted)

Human CLL cells with NFKBIE-mutations are positively selected by microenvironmental signals that activate the NF-kB pathway

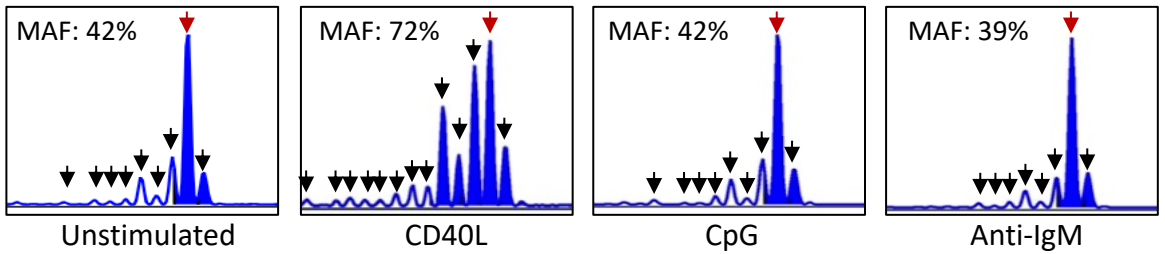




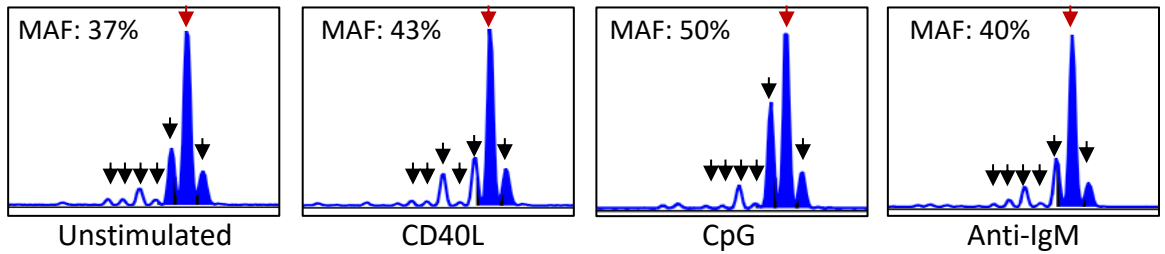
Wild-type allele

Mutant alleles

CLL G398

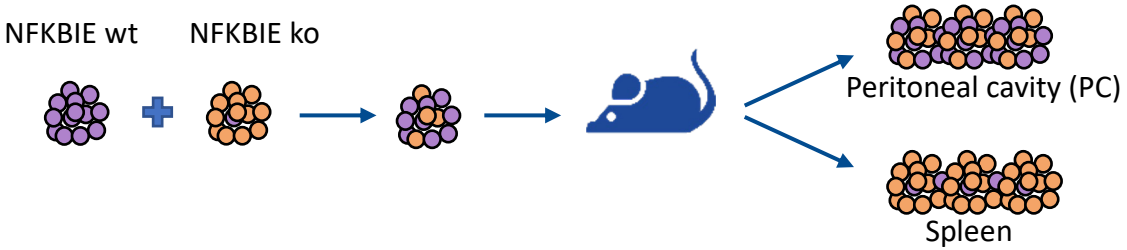


CLL G392

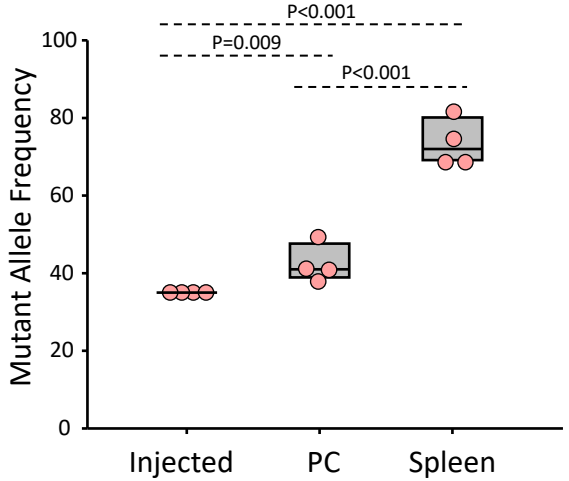


Bonato A et al, 2023 (manuscript submitted)

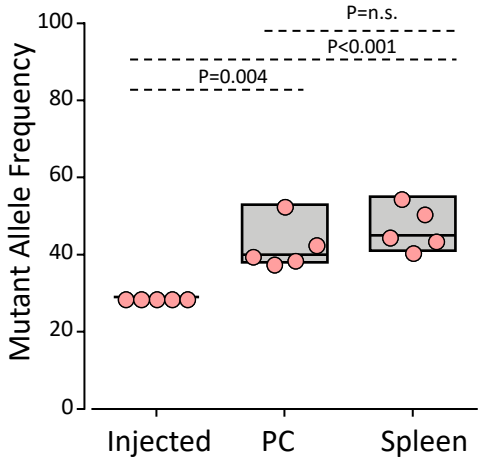
NFKBIE-mutated murine RS cells are differently selected in different anatomical compartments of immunocompetent and immunodeficient mice



TCL1-355 TKO cells transplanted in C57BL/6 **immunocompetent** mice

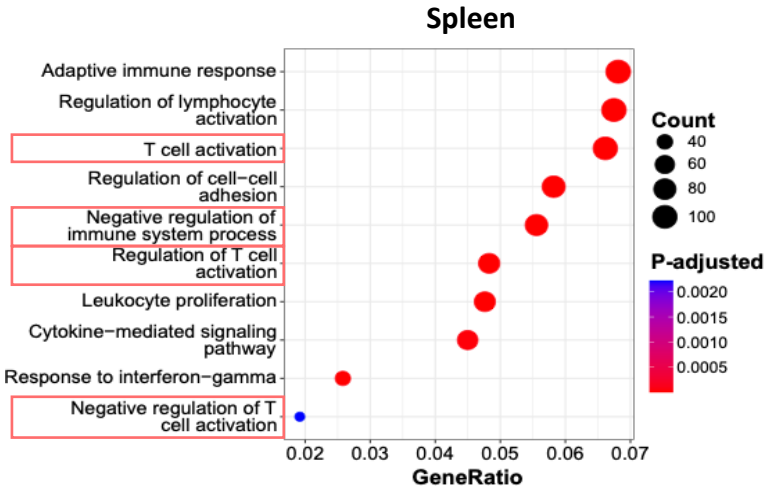


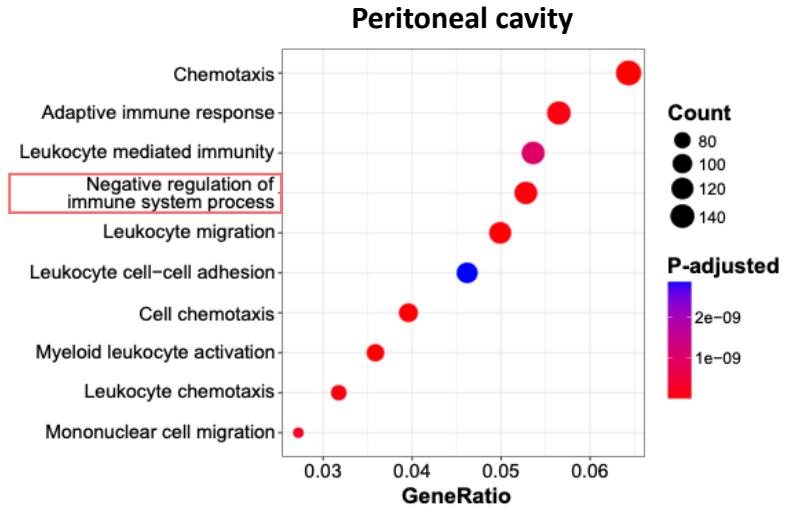
TCL1-355 TKO cells transplanted in NSG **immunodeficient** mice

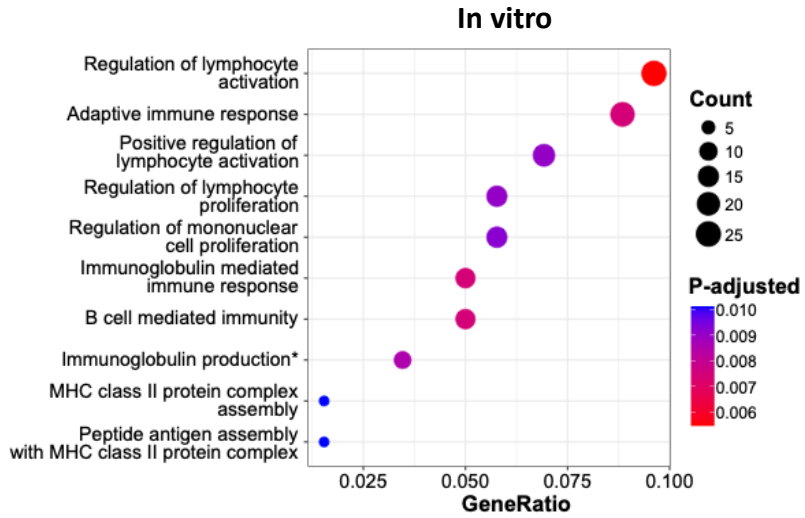


Bonato A et al, 2023 (manuscript submitted)

NFKBIE-mutated murine RS cells induce changes in the tumor immune microenvironment

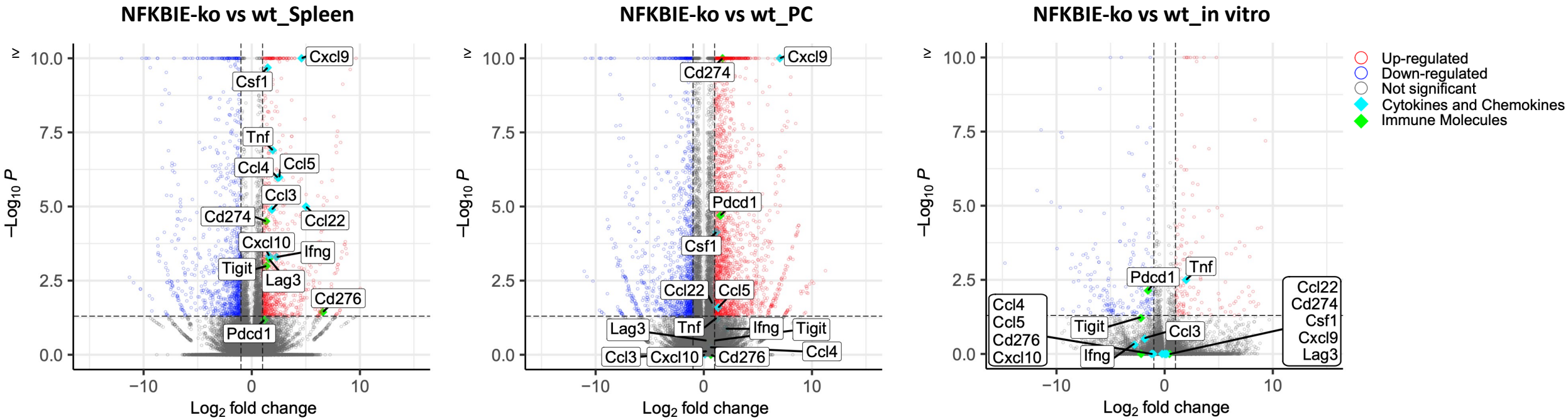






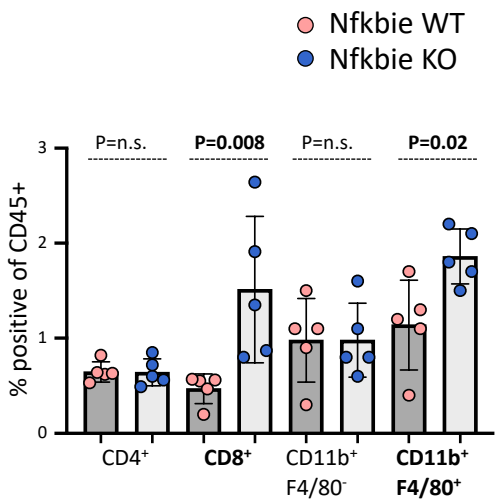
Bonato A et al, 2023 (manuscript submitted)

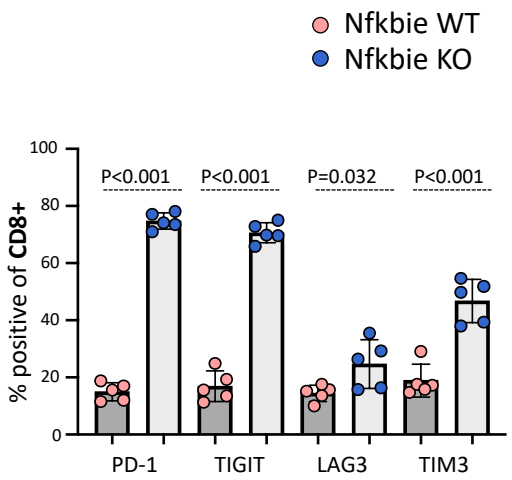
Cytokines and chemokines involved in recruitment of T cells and macrophages and inhibitory immune checkpoint molecules are enriched in spleens of mice receiving NFKBIE-mutated RS cells

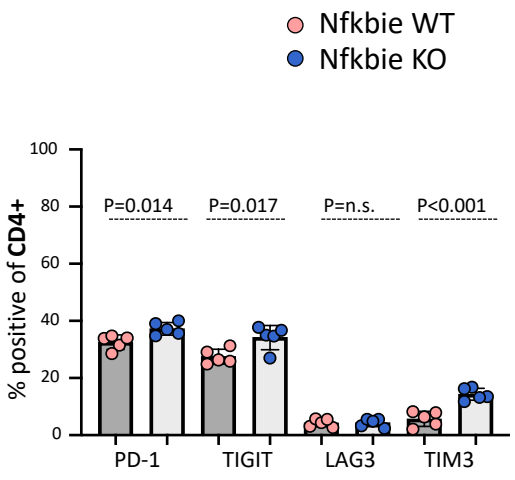


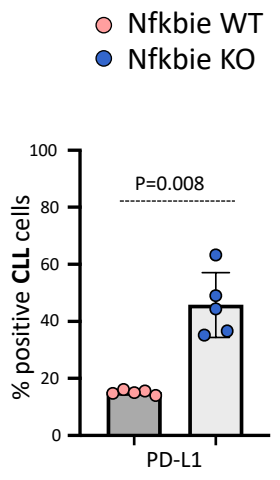
Bonato A et al, 2023 (manuscript submitted)

Spleens of mice receiving NFKBIE-mutated CLL cells show increased expression of inhibitory checkpoint molecules on CD4+ and CD8+ T cells



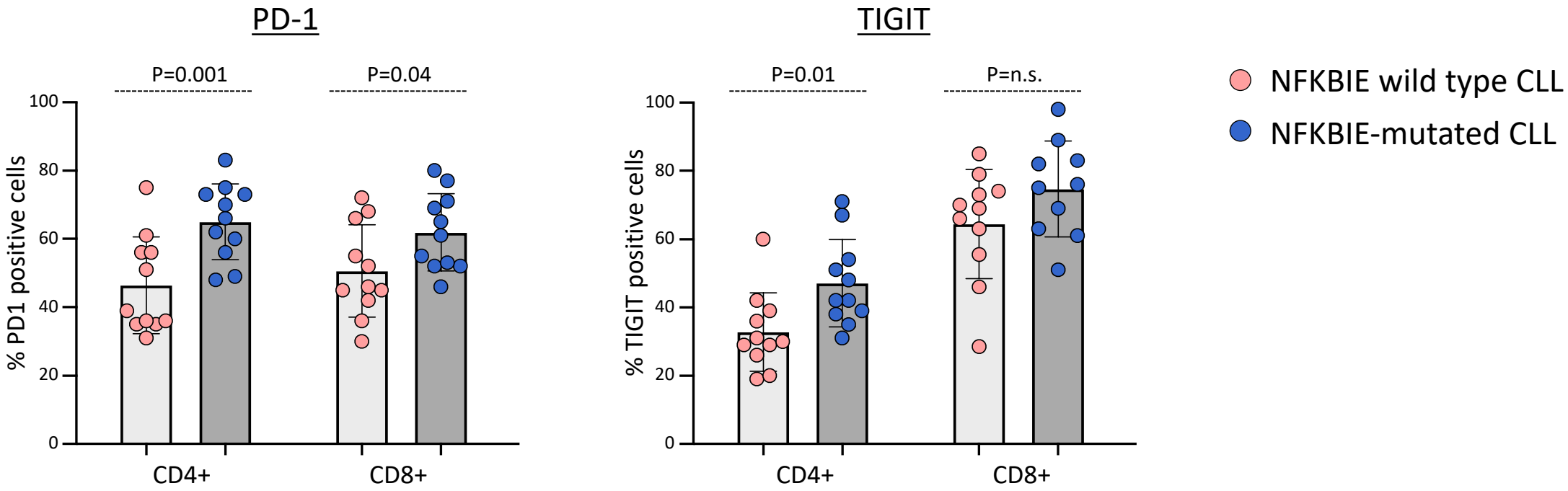






Bonato A et al, 2023 (manuscript submitted)

TIGIT and PD-1 expression by CD4+ and CD8+ T cells in NFKBIE-mutated CLL (VAF >0.2) vs NFKBIE-wild type CLL patients



Bonato A et al, 2023 (manuscript submitted)

Take-home messages

- *In vivo* murine CLL and RS models with patient-specific genetic lesions can be rapidly generated by CRISPR/Cas9 editing and can be used to study the mechanisms underyling CLL progression and Richter transformation

- Cell cycle deregulation caused by combined loss of TP53 and CDKN2A/2B represents one mechanism of Richter transformation

- Genetic lesions in some CLL driver genes may contribute to Richter transformation by facilitating immune escape of the malignant B cells

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Con la ricerca, contro il cancro.

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