

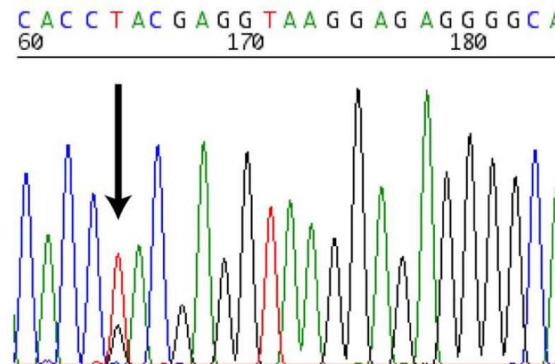
Therapy of Lymphoma Inspired by Functional and Structural Genomics

Interplay of Functional and Structural Genomics

Genome-wide
RNAi / CRISPR screens



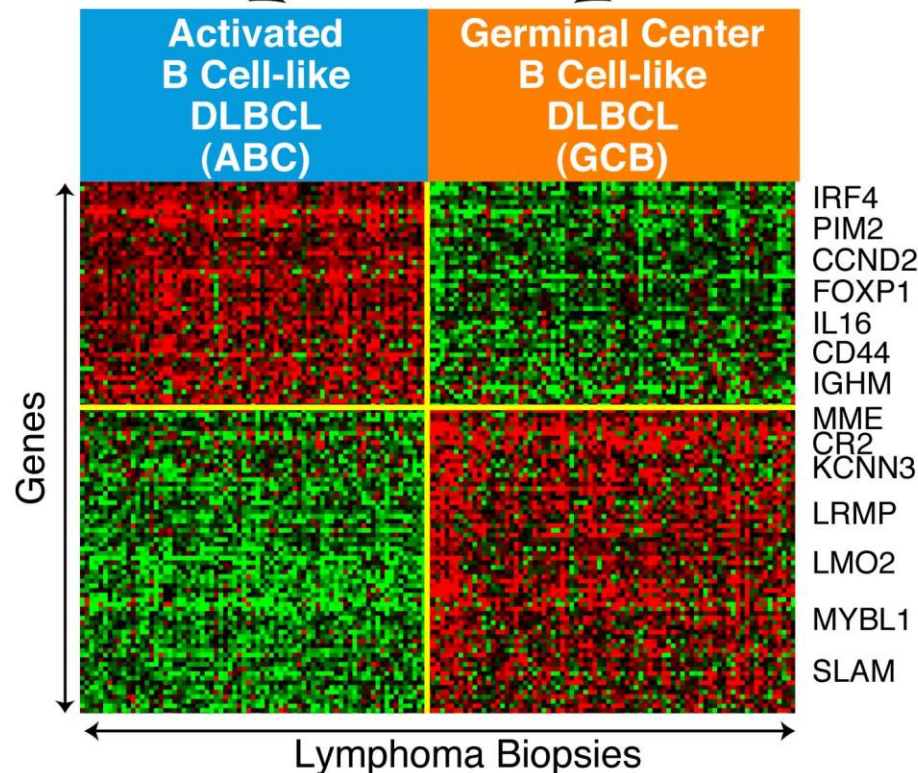
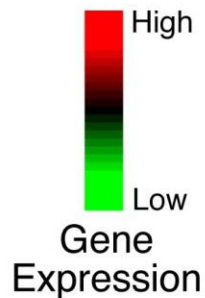
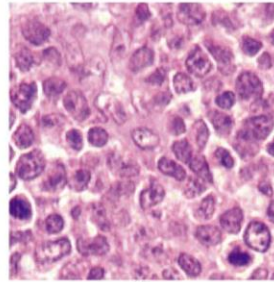
Oncogenic somatic mutation



Essential
cancer
pathways

Dissecting Cancer Into Molecularly and Clinically Distinct Subtypes by Gene Expression Profiling

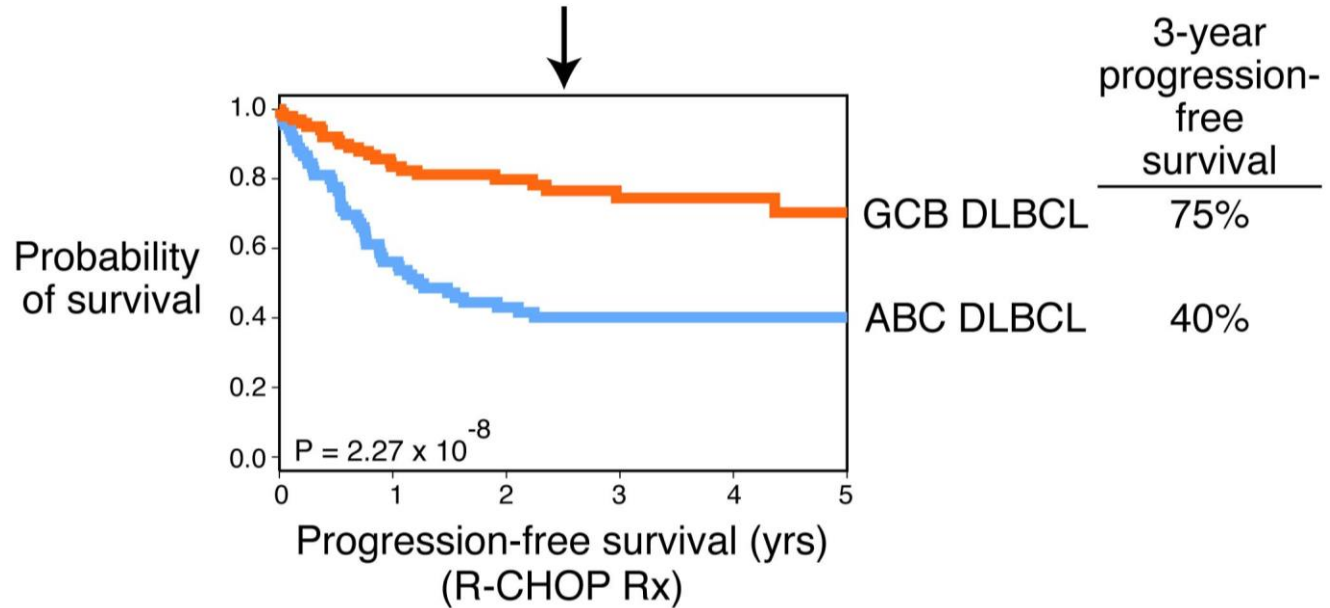
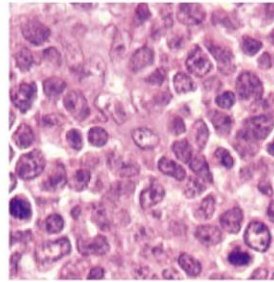
Diffuse Large B Cell Lymphoma (DLBCL)



Subtype-specific gene expression signatures

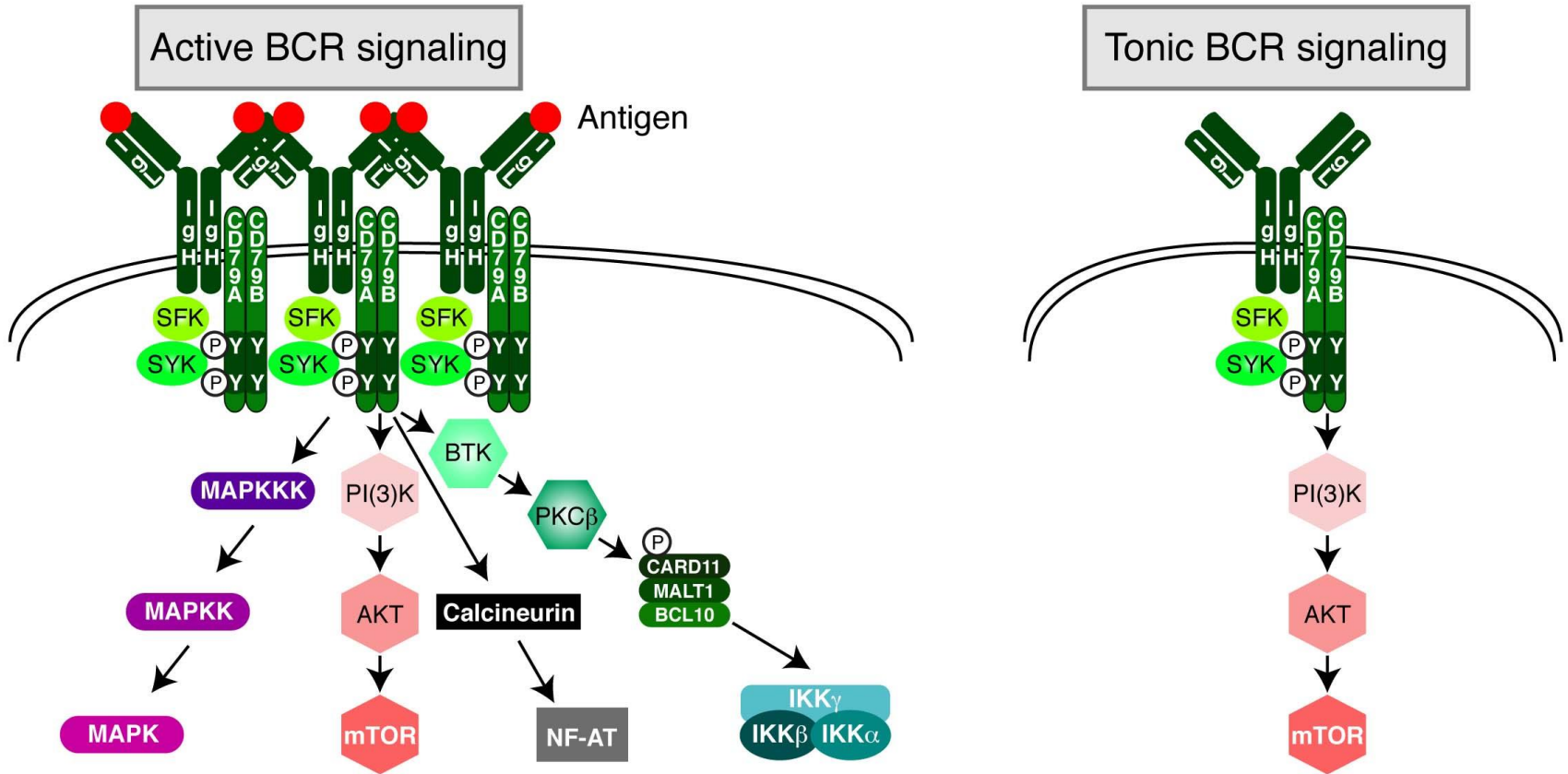
Dissecting Cancer Into Molecularly and Clinically Distinct Subtypes by Gene Expression Profiling

Diffuse Large B Cell
Lymphoma
(DLBCL)

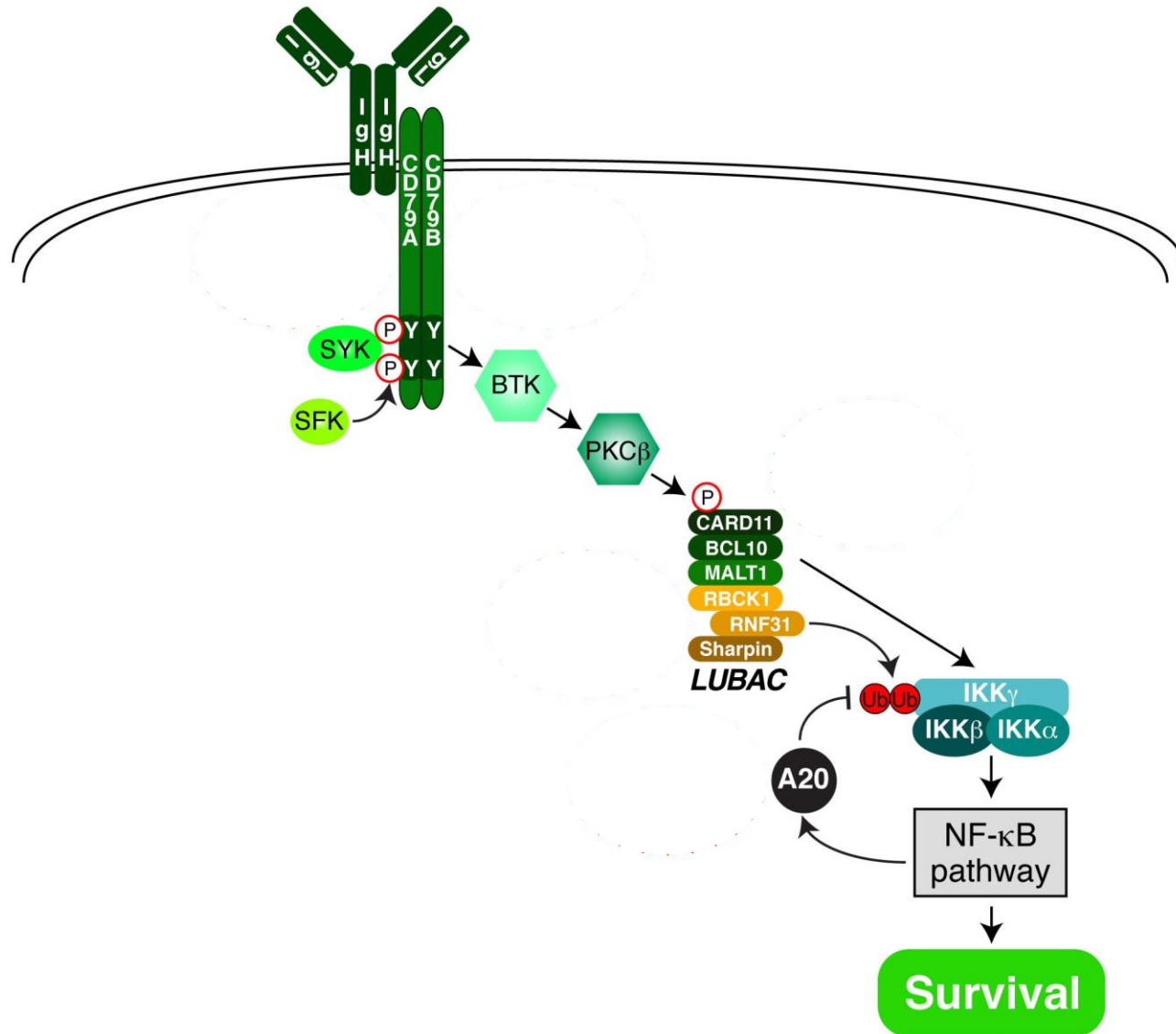


Subtype-specific response
To chemotherapy

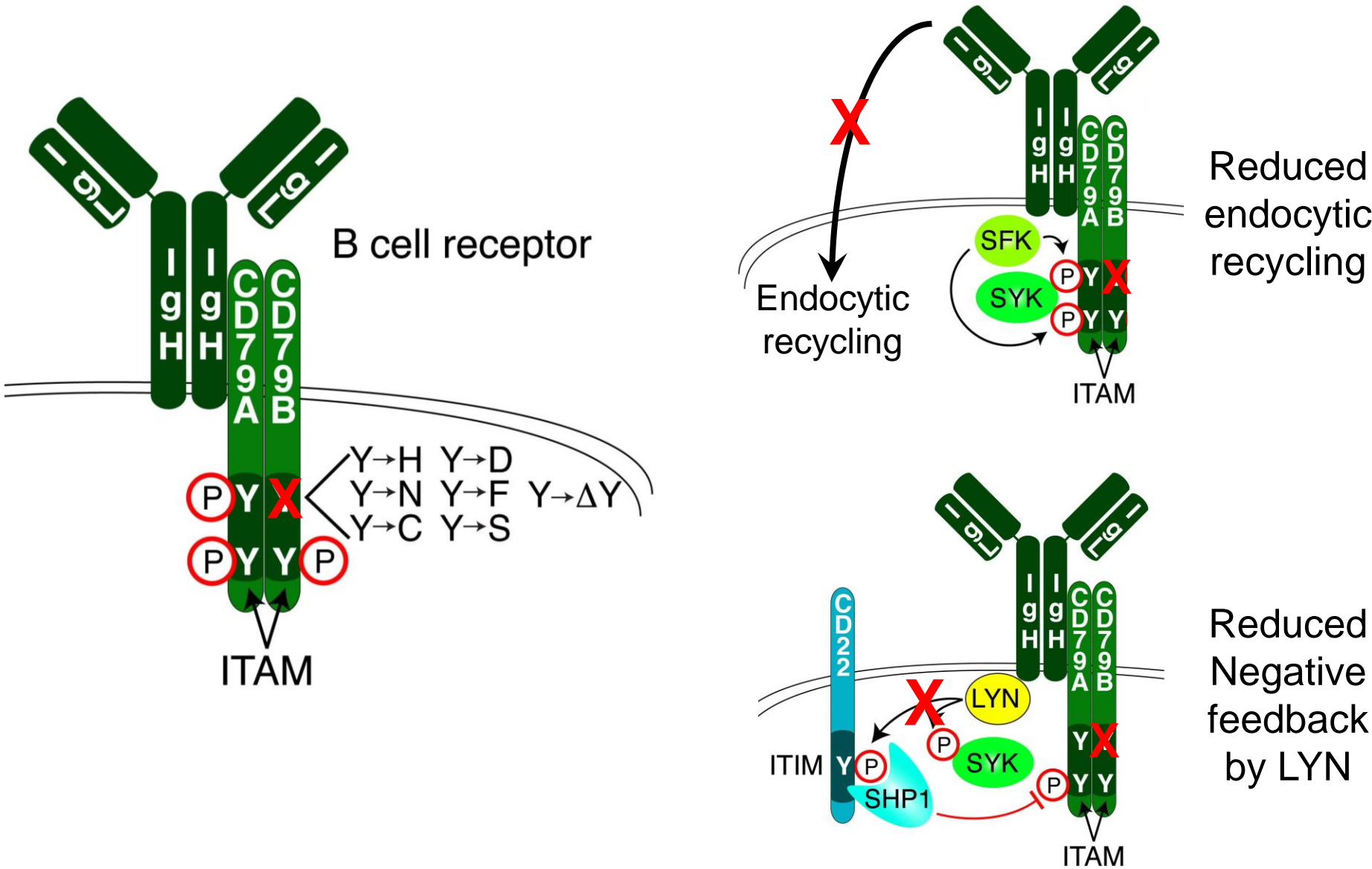
Active vs. Tonic BCR Signaling Differentiates Lymphoma Subtypes



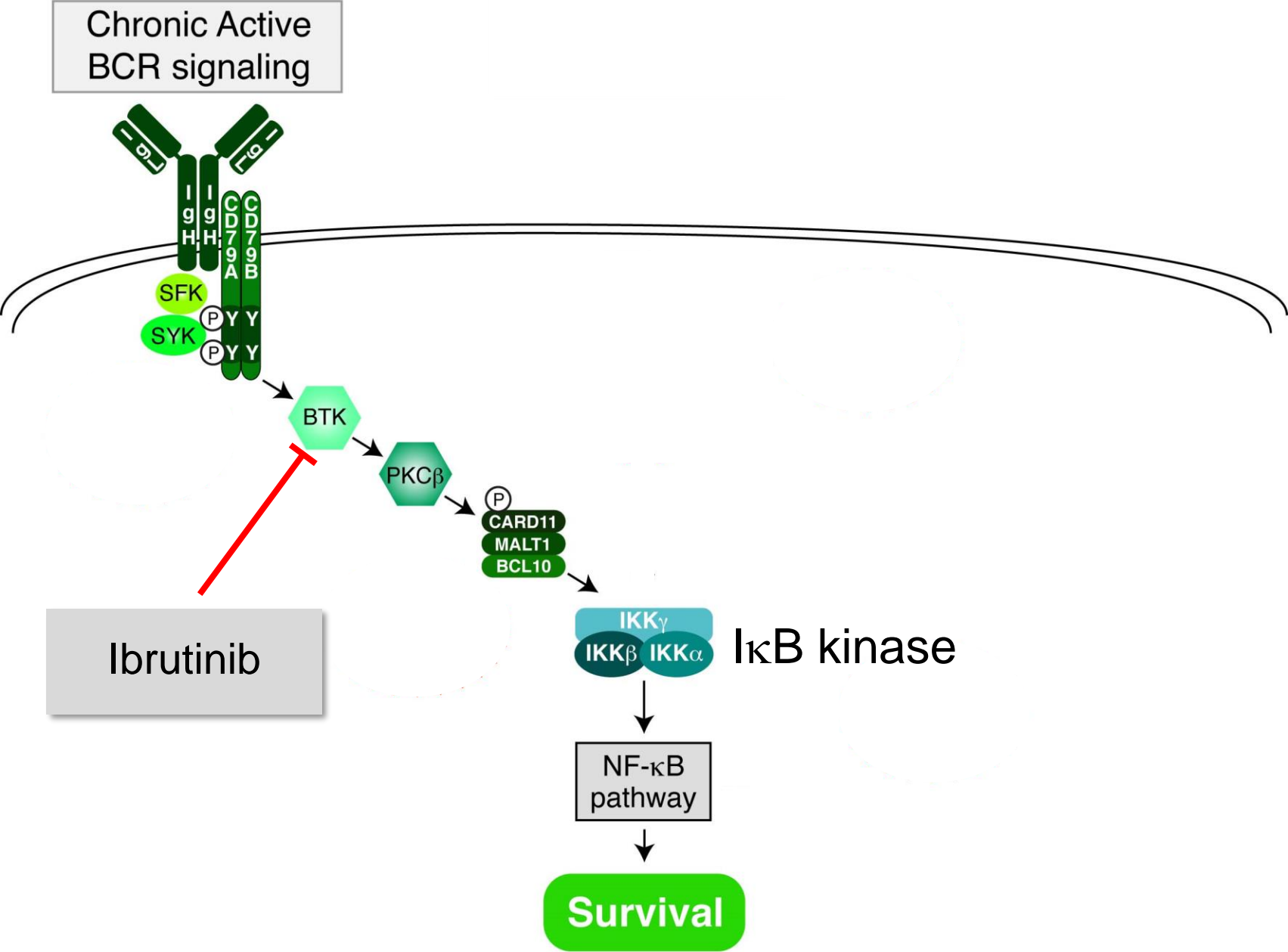
Multiple Oncogenic Mutations in ABC DLBCL Promote Chronic Active B Cell Receptor Signaling



CD79 ITAM Mutations Are Back Seat Drivers in ABC DLBCL



Blockade of BCR Signaling in ABC DLBCL with Ibrutinib

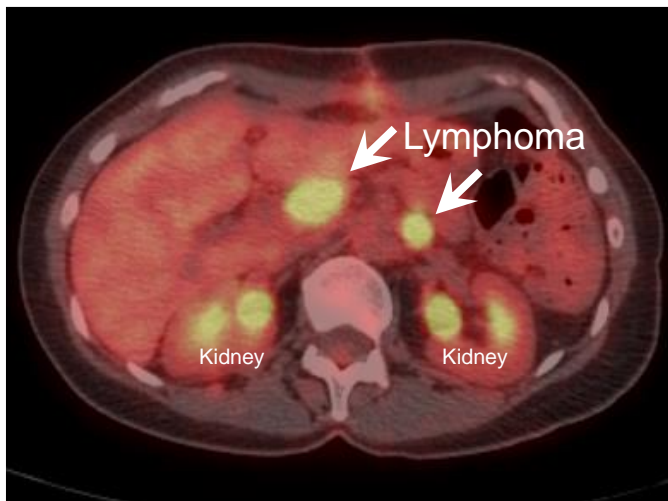
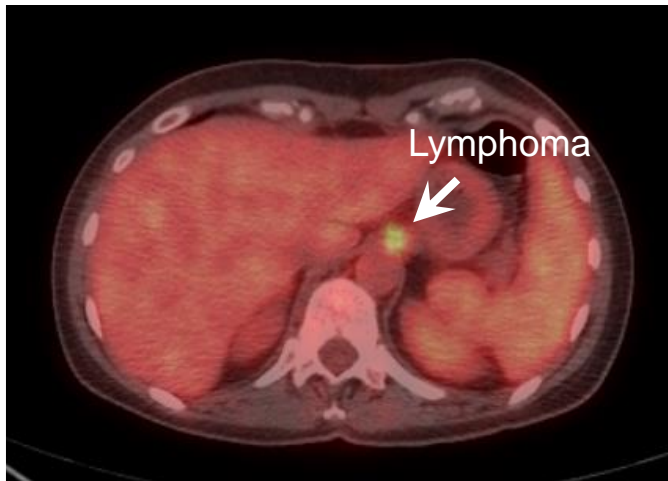


The Promise of Targeted Therapy in Cancer

- ❖ 52 year old female
ABC Diffuse Large B Cell Lymphoma
- ❖ Activating Mutation in B cell receptor subunit CD79B
- ❖ Relapse following 2 prior chemotherapies

DA-EPOCH-R + Campath	CR and relapse
DA-EPOCH-R	CR and relapse
- ❖ Treatment with Ibrutinib, a B cell receptor signaling inhibitor

PET/CT Scan Before and On Treatment With Ibrutinib



Before Rx

On Rx: week 8

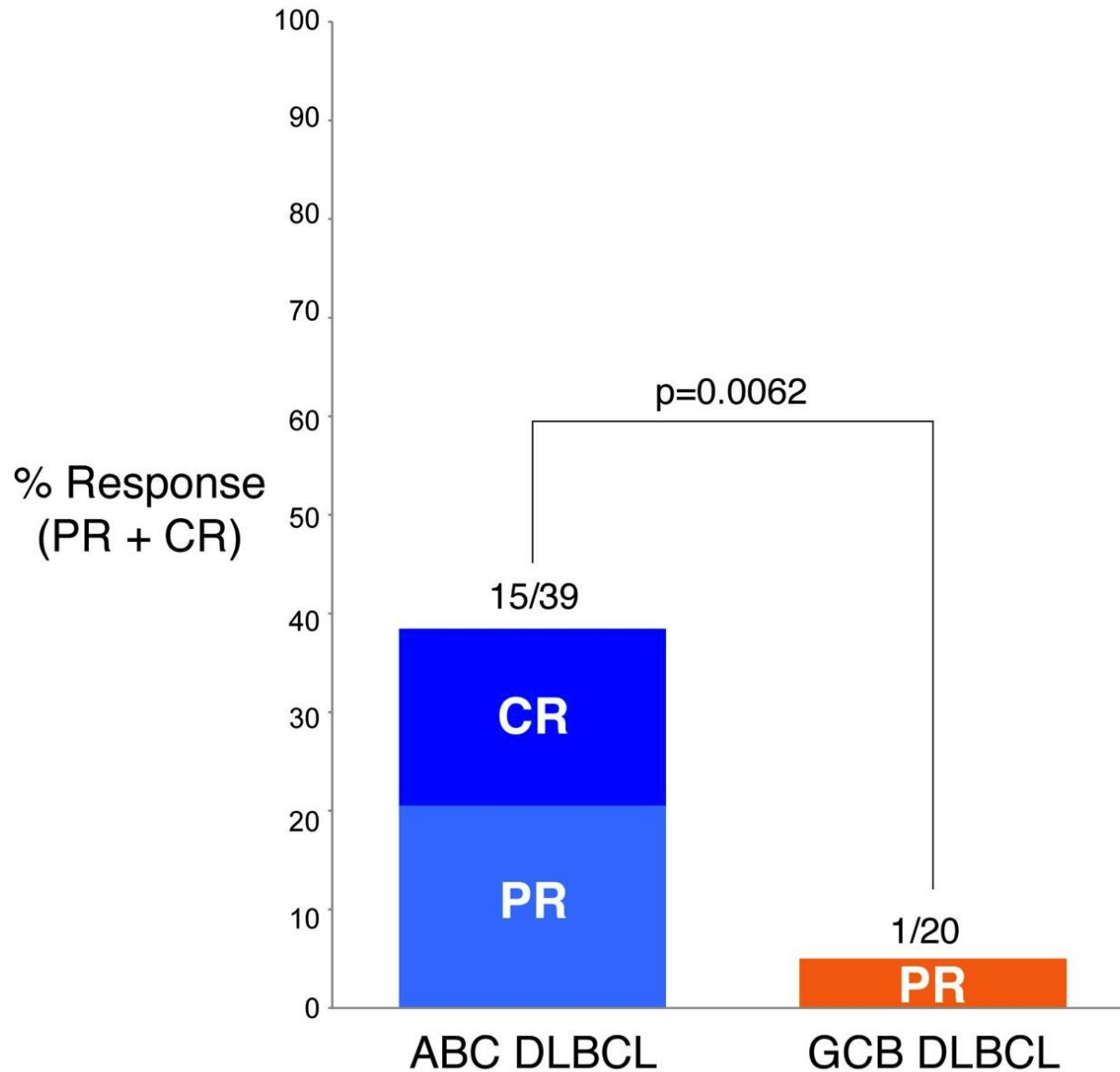
Ongoing Complete Response at > 6 years

Phase 2 Clinical Trial of Ibrutinib in Relapsed/refractory DLBCL

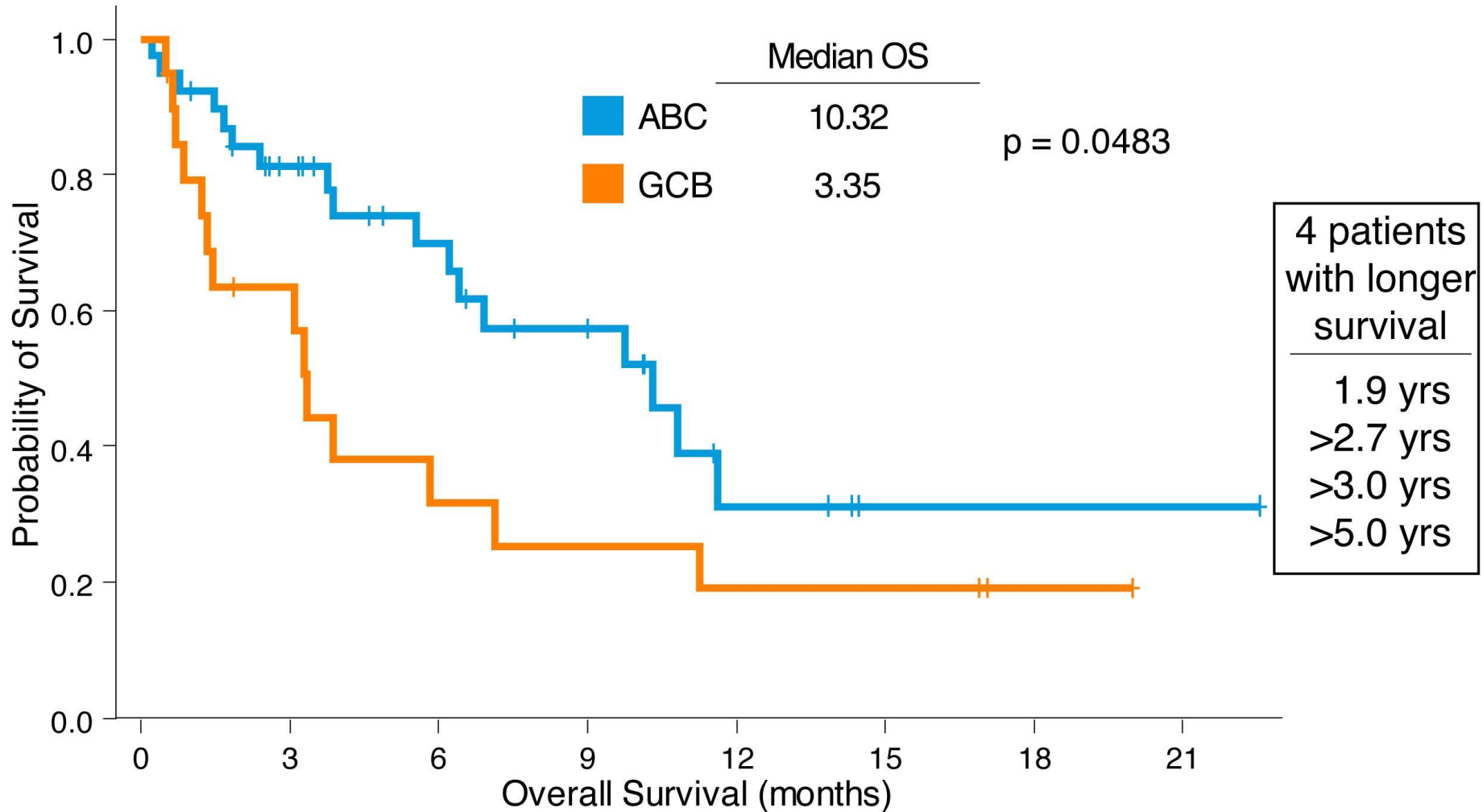
❖ Multicenter phase 2 trial

- Relapsed/refractory DLBCL (ABC and GCB subtypes)
- Subtype determined by immunohistochemistry and confirmed by gene expression profiling
- Ibrutinib 560 mg p.o. daily
- n=70

Ibrutinib is Preferentially Active in ABC DLBCL



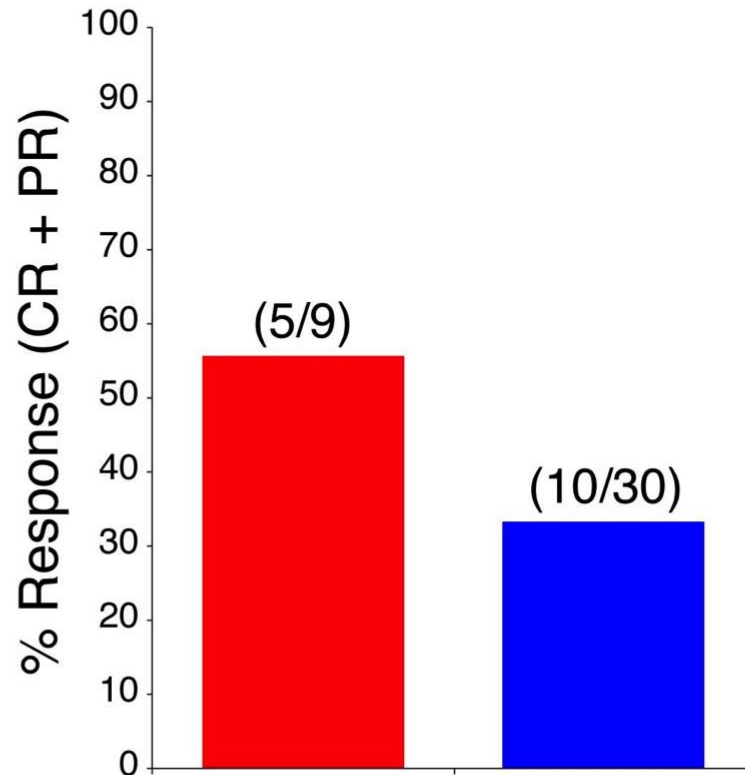
Ibrutinib Extends Overall Survival in Relapsed/Refractory ABC DLBCL



Can Analysis of Recurrent Genetic Lesions
Identify Ibrutinib Responders
Within ABC DLBCL?

Influence of B Cell Receptor Mutations on Ibrutinib Response in ABC DLBCL

CD79B ITAM motif



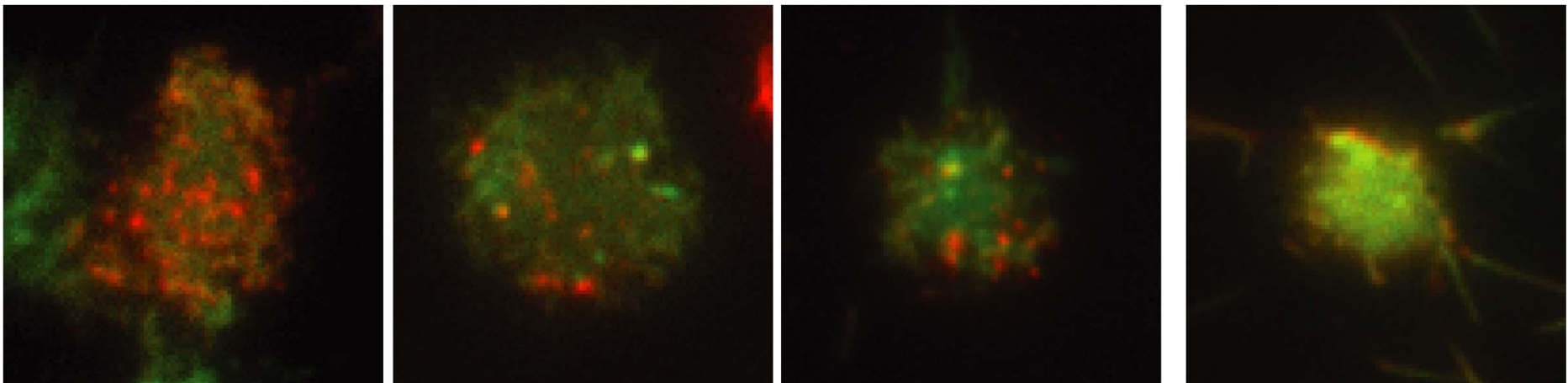
Cancers can be strongly addicted to
non-genetic signaling

Mutation \neq response

Clustering of B Cell Receptors on the ABC DLBCL Surface Suggests Antigen Engagement

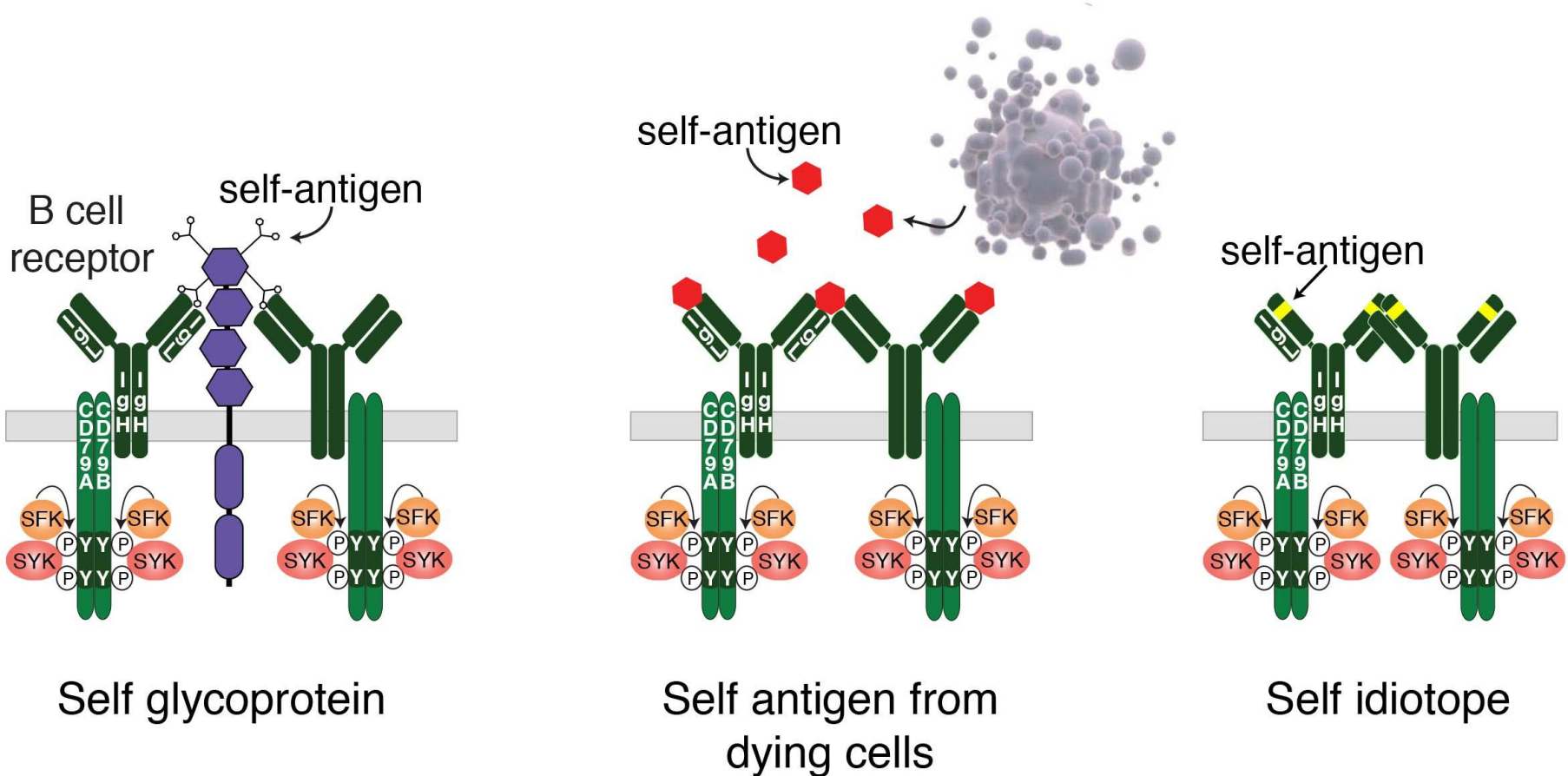
ABC DLBCL

GCB DLBCL



■ IgM
■ Membrane

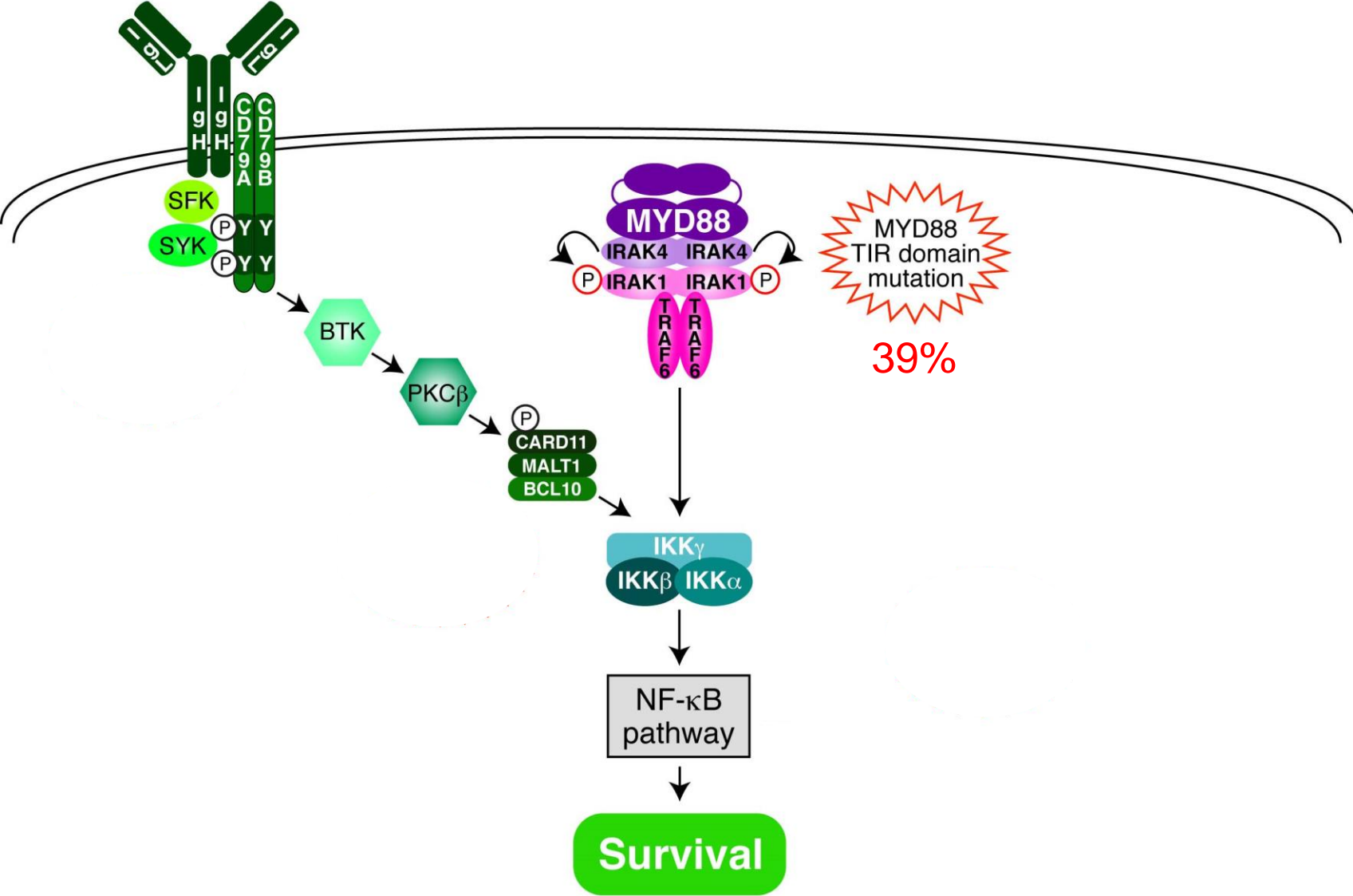
Self Antigens Drive B Cell Receptor Signaling in ABC DLBCL



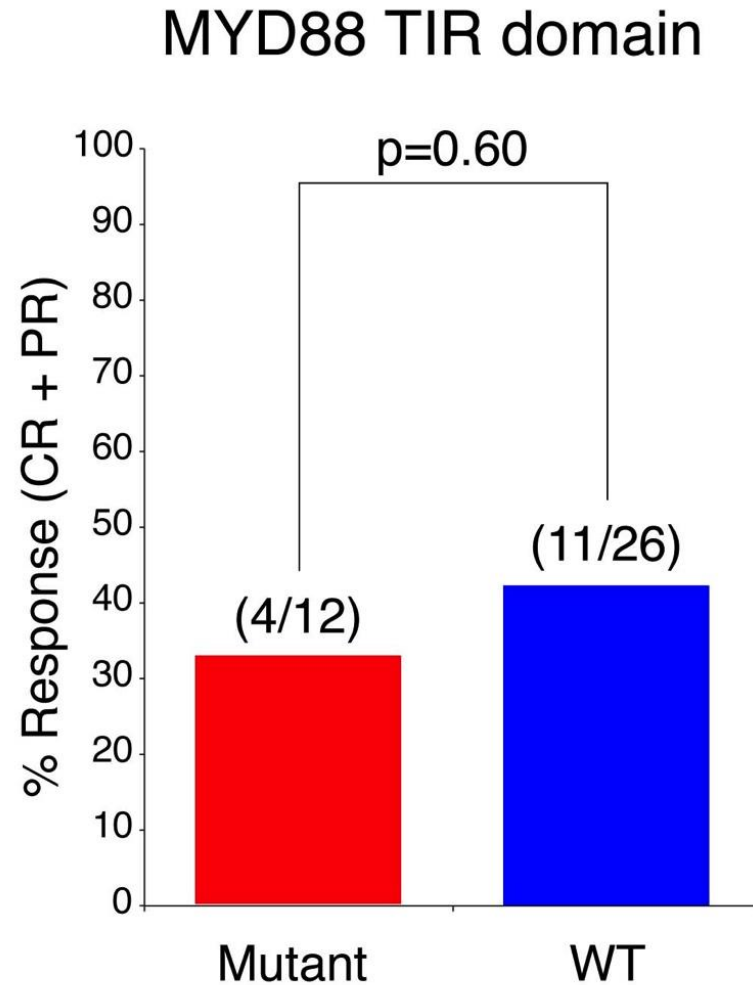
Constitutive MYD88 Signaling in ABC DLBCL

Chronic Active BCR signaling

Constitutive MYD88 signaling

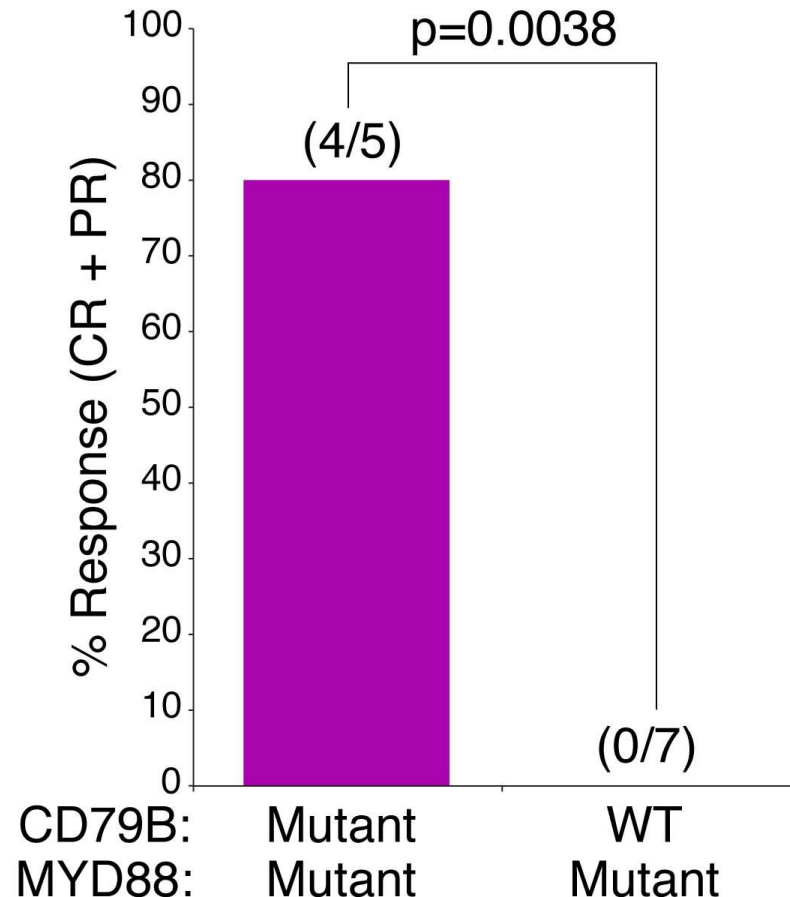


Influence of B Cell Receptor and MYD88 Pathway Mutations on Ibrutinib Response in ABC DLBCL

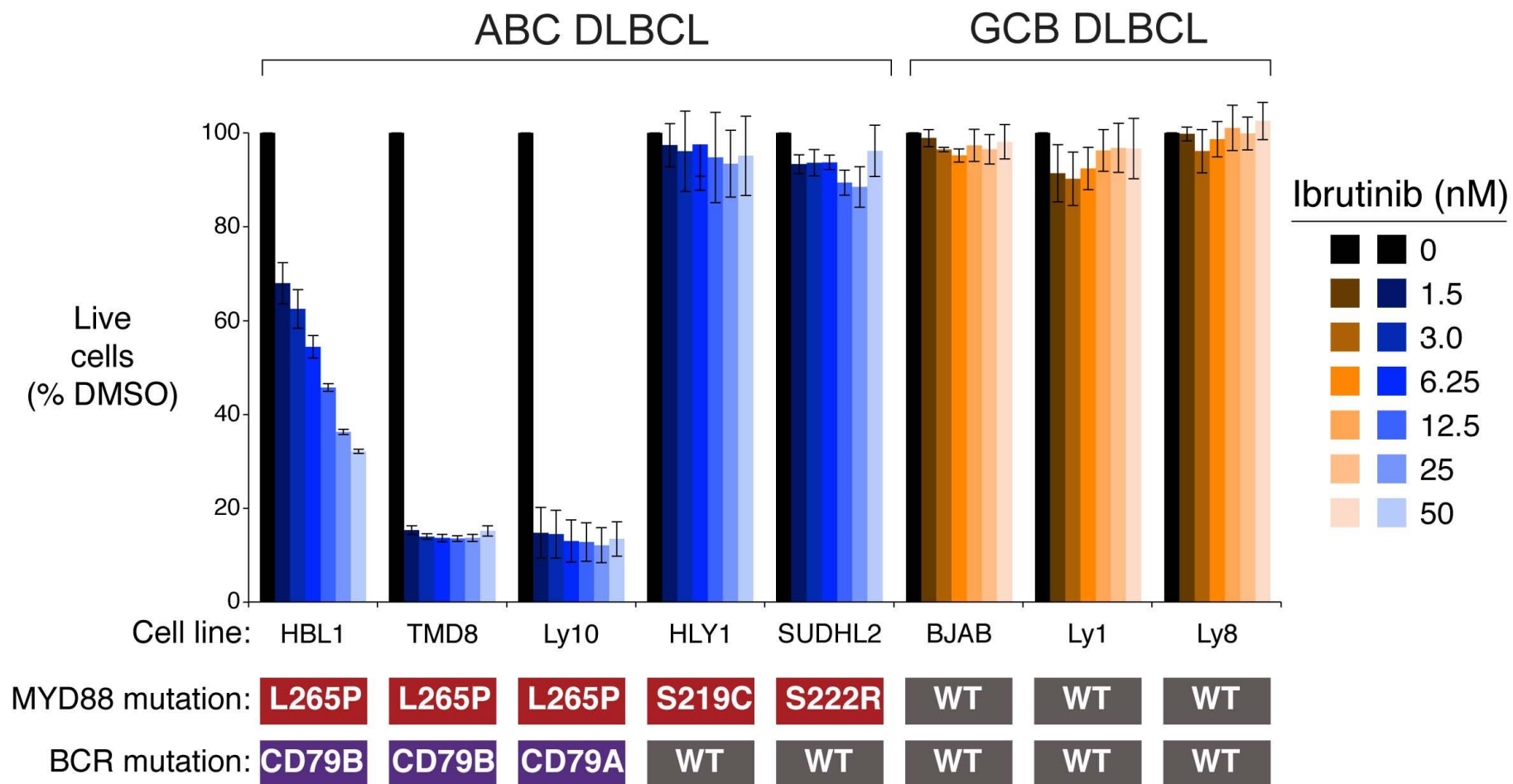


Influence of B Cell Receptor and MYD88 Pathway Mutations on Ibrutinib Response in ABC DLBCL

MYD88 TIR domain vs.
CD79A/B ITAM motif

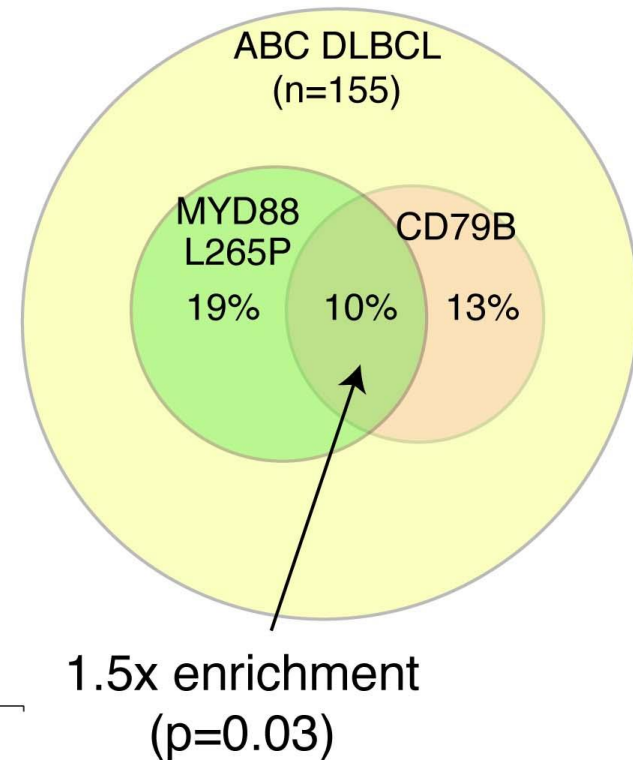
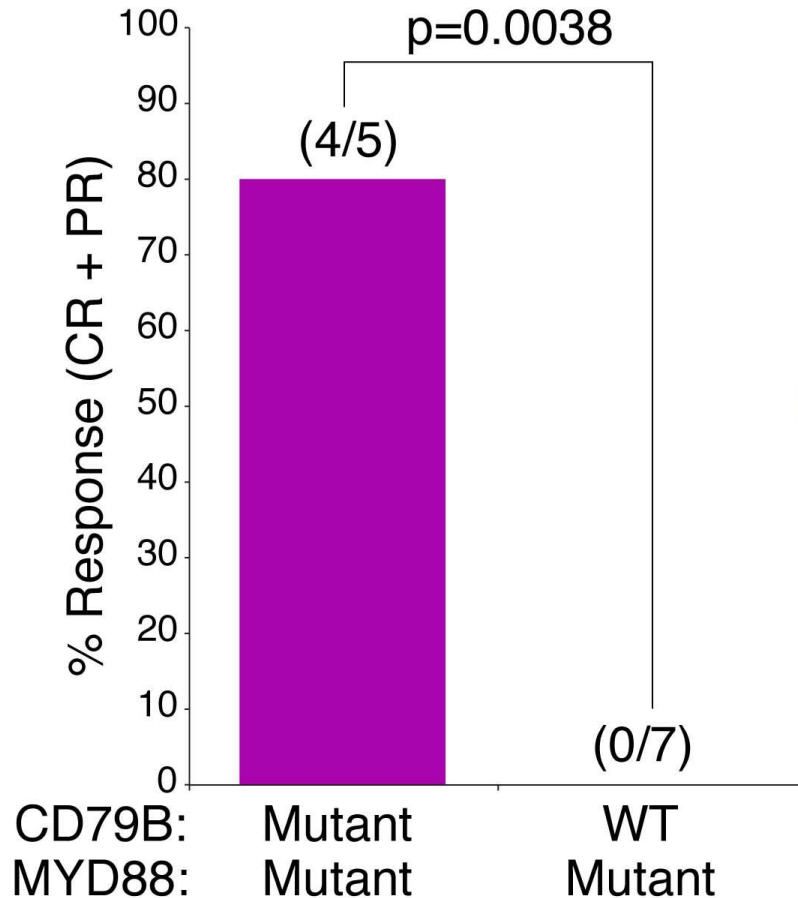


Ibrutinib-sensitive and -resistant Forms of ABC DLBCL

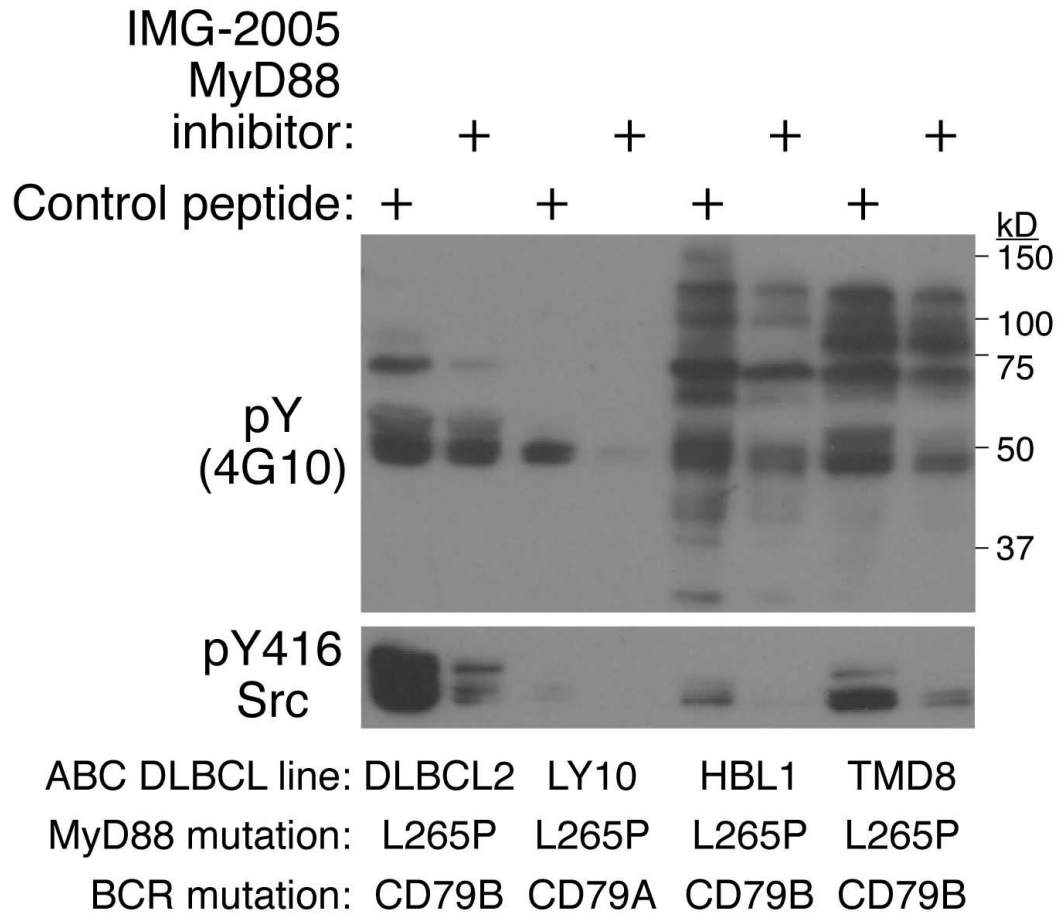


Influence of B Cell Receptor and MYD88 Pathway Mutations on Ibrutinib Response in ABC DLBCL

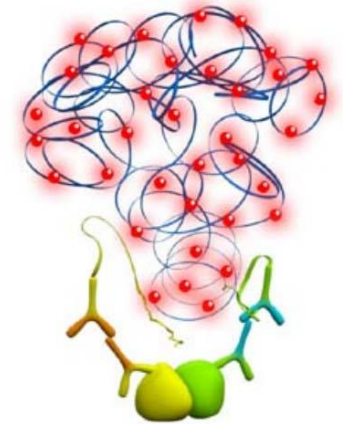
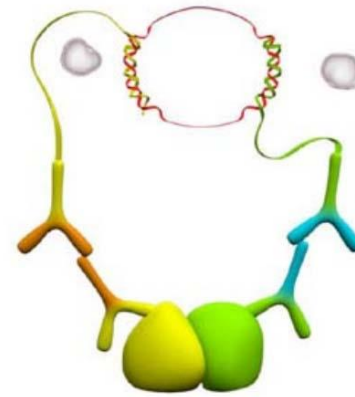
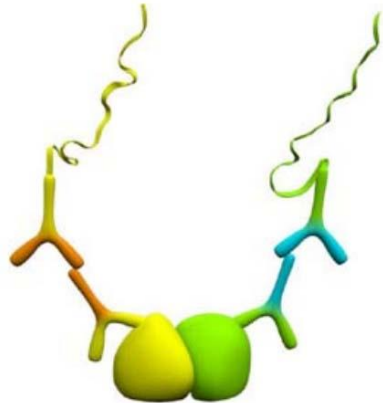
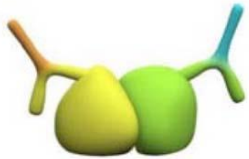
MYD88 TIR domain vs.
CD79A/B ITAM motif



MYD88 Inhibition Decreases Proximal B Cell Receptor Signaling in ABC DLBCL Cells



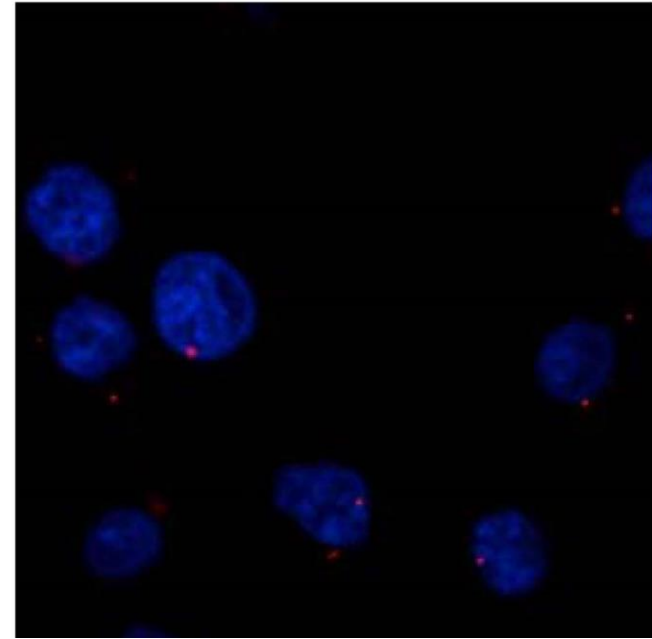
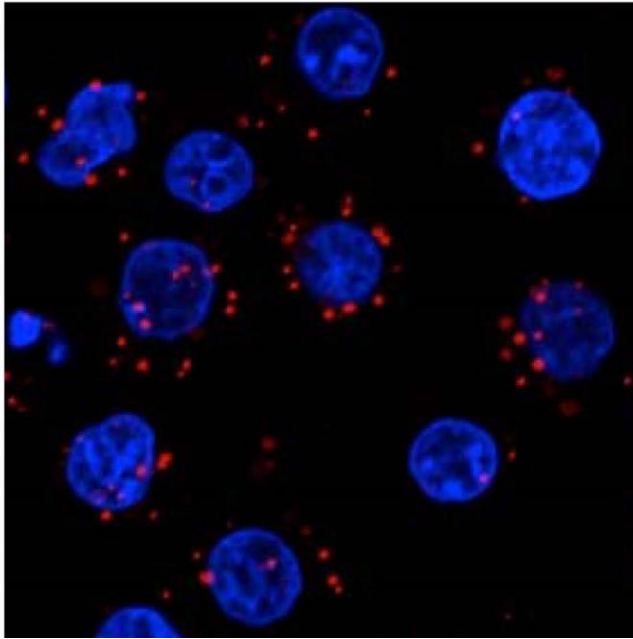
Seeing Protein Interactions in Cells: The Proximity Ligation Assay



Colocalization of Phosphorylated CD79A and MYD88 in the Cytoplasm of ABC DLBCL Cells

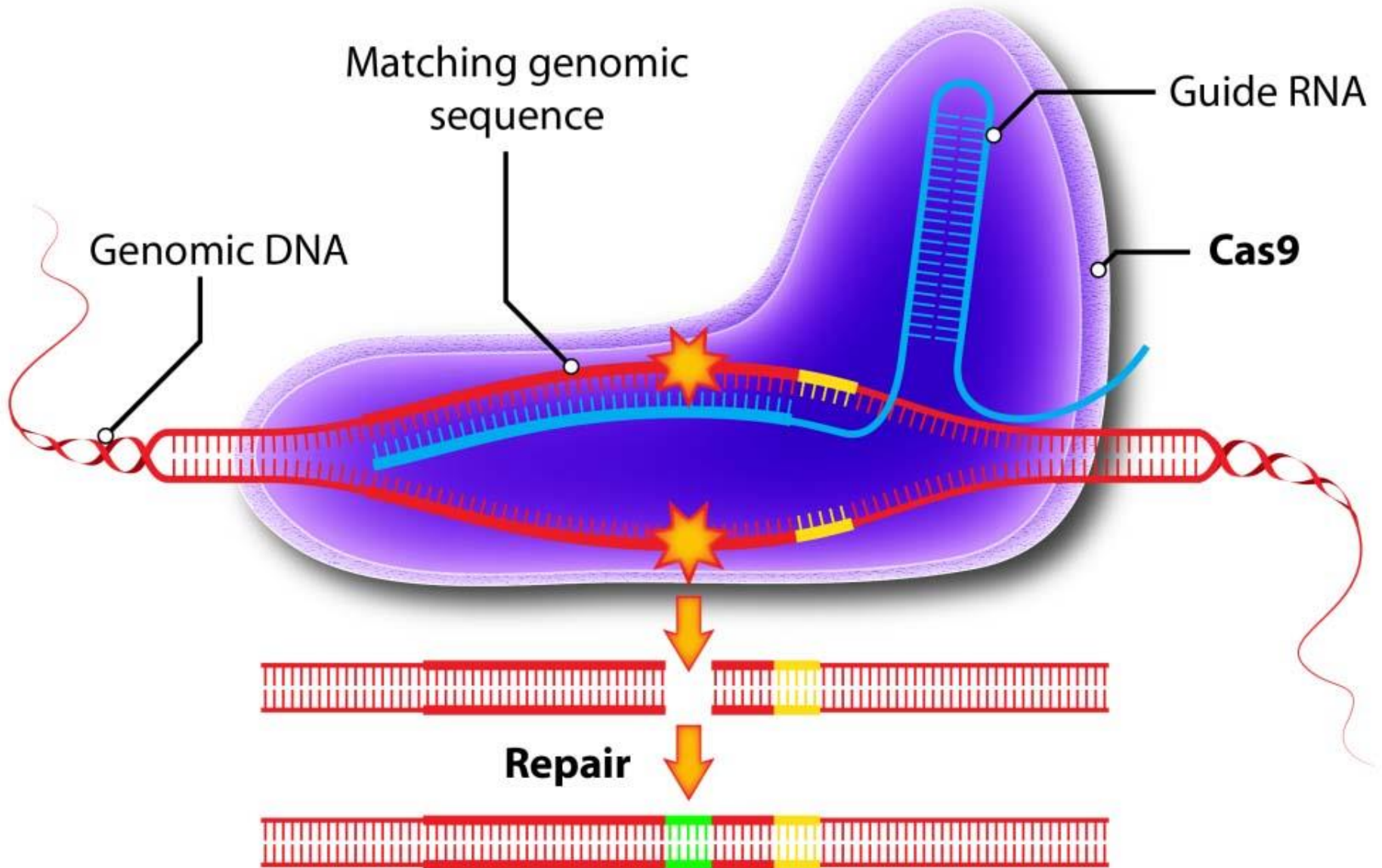
Control sgRNA

MYD88 sgRNA



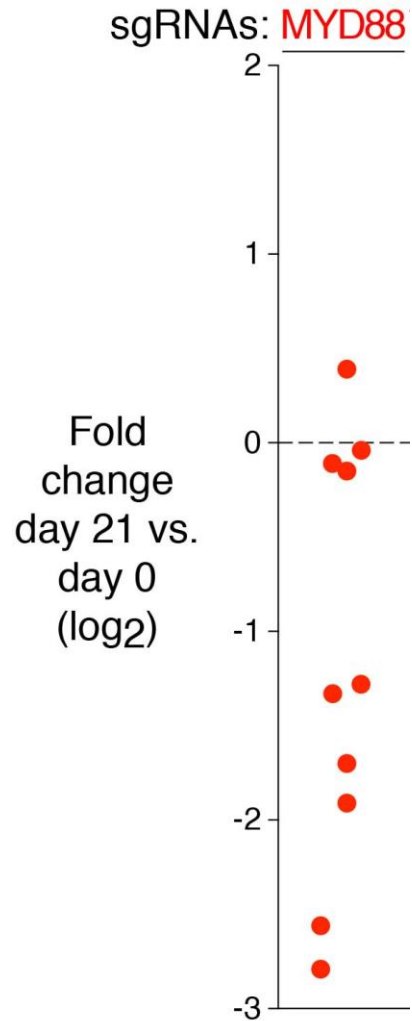
pY182 CD79A
x MYD88
Proximity
Ligation
Assay

The CRISPR Revolution

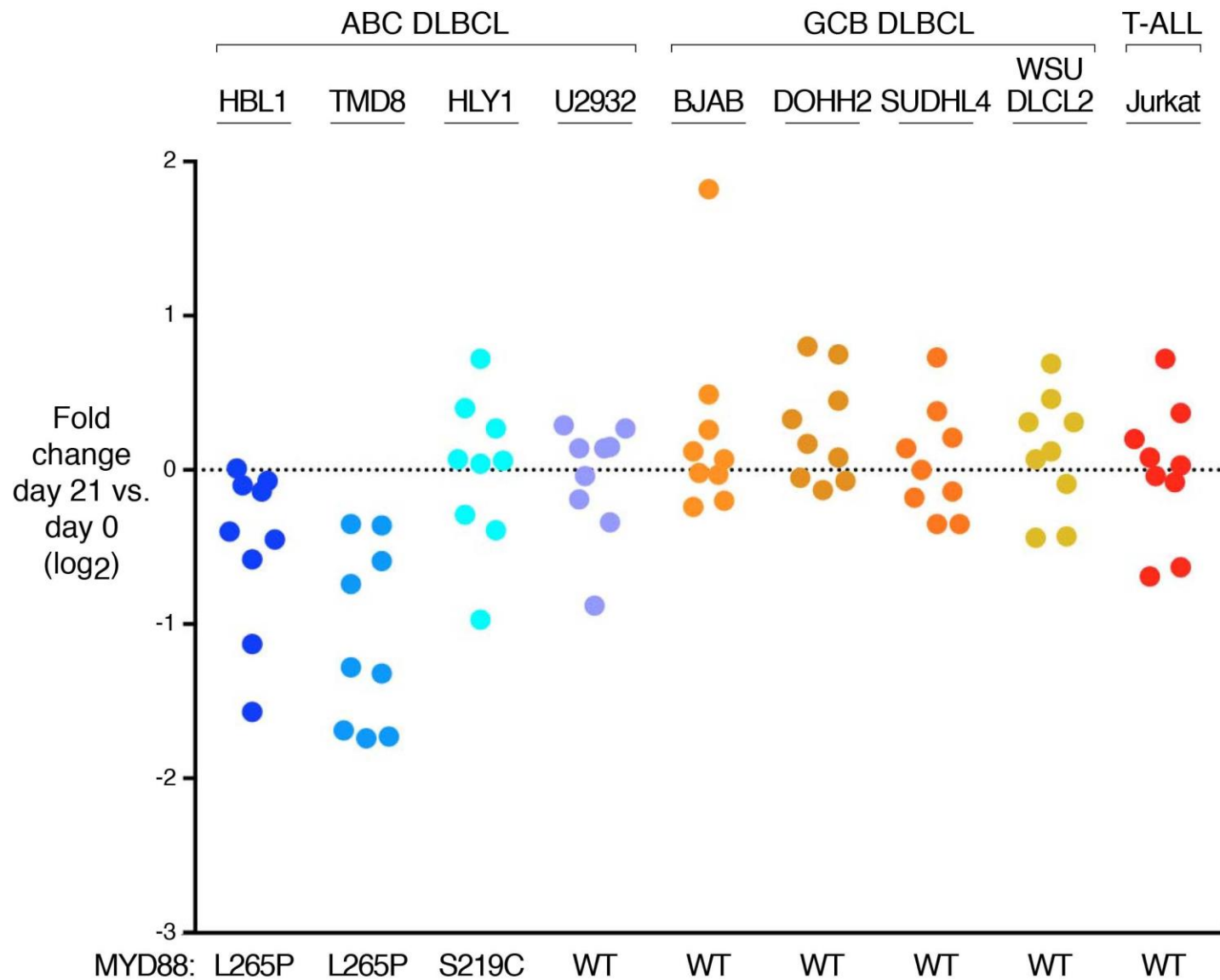


CRISPR-Cas9 Screening Identifies Essential Genes In ABC DLBCL Cell Lines

sgRNA toxicity in TMD8 ABC DLBCL cells

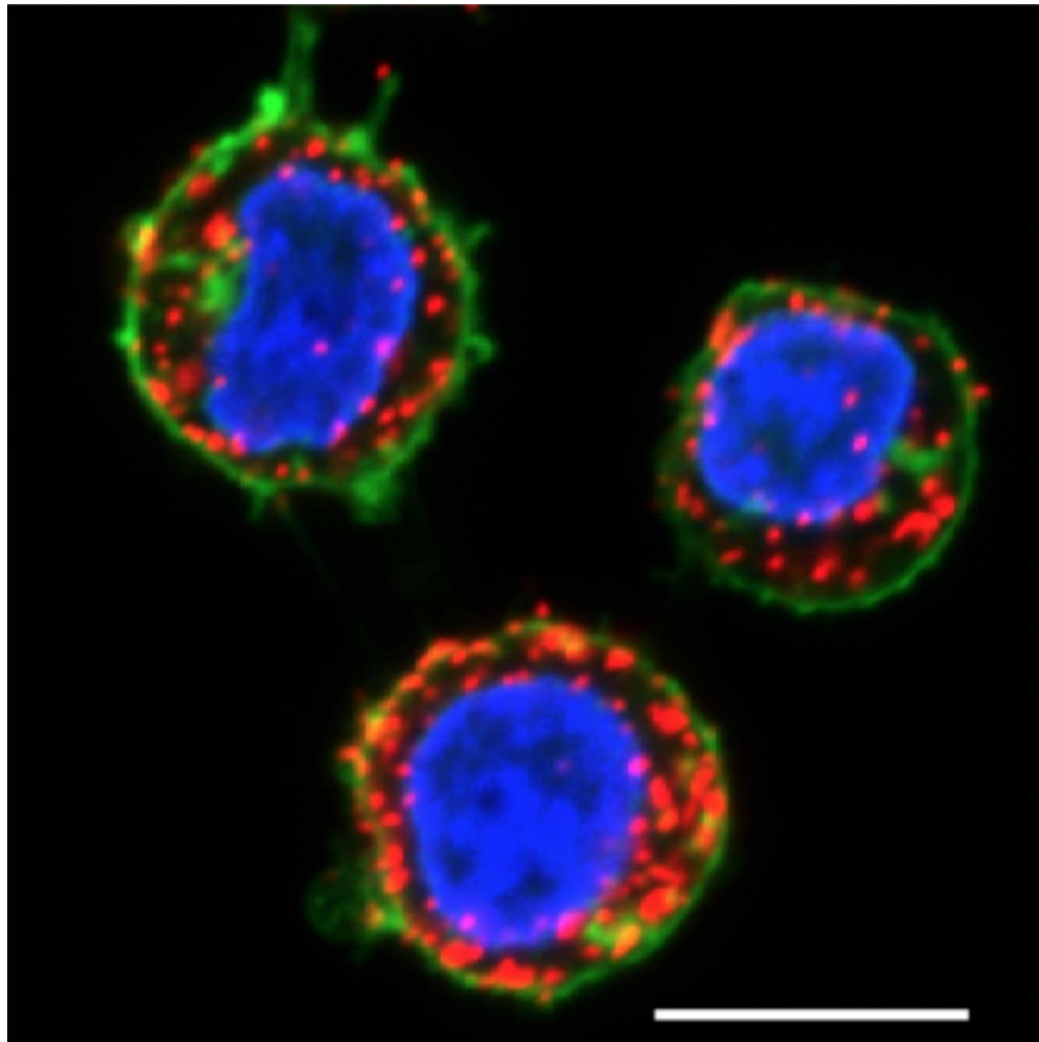


TLR9 is Required in ABC DLBCL Lines With MYD88 L265P



Colocalization of IgM and TLR9 in Cytoplasmic Vesicles in ABC DLBCL

IgM
X
TLR9
PLA

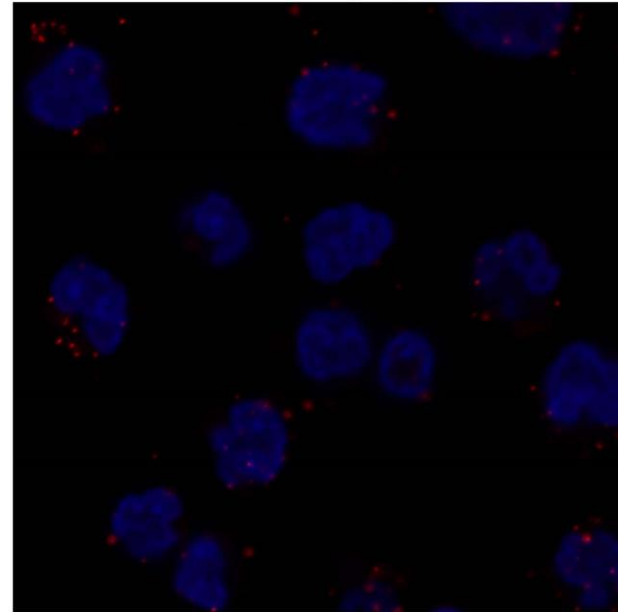
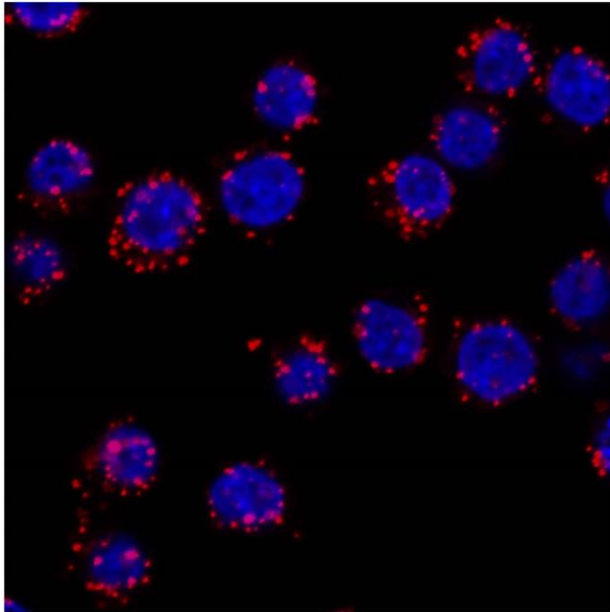


Colocalization of IgM and TLR9 in Cytoplasmic Vesicles in ABC but not GCB DLBCL

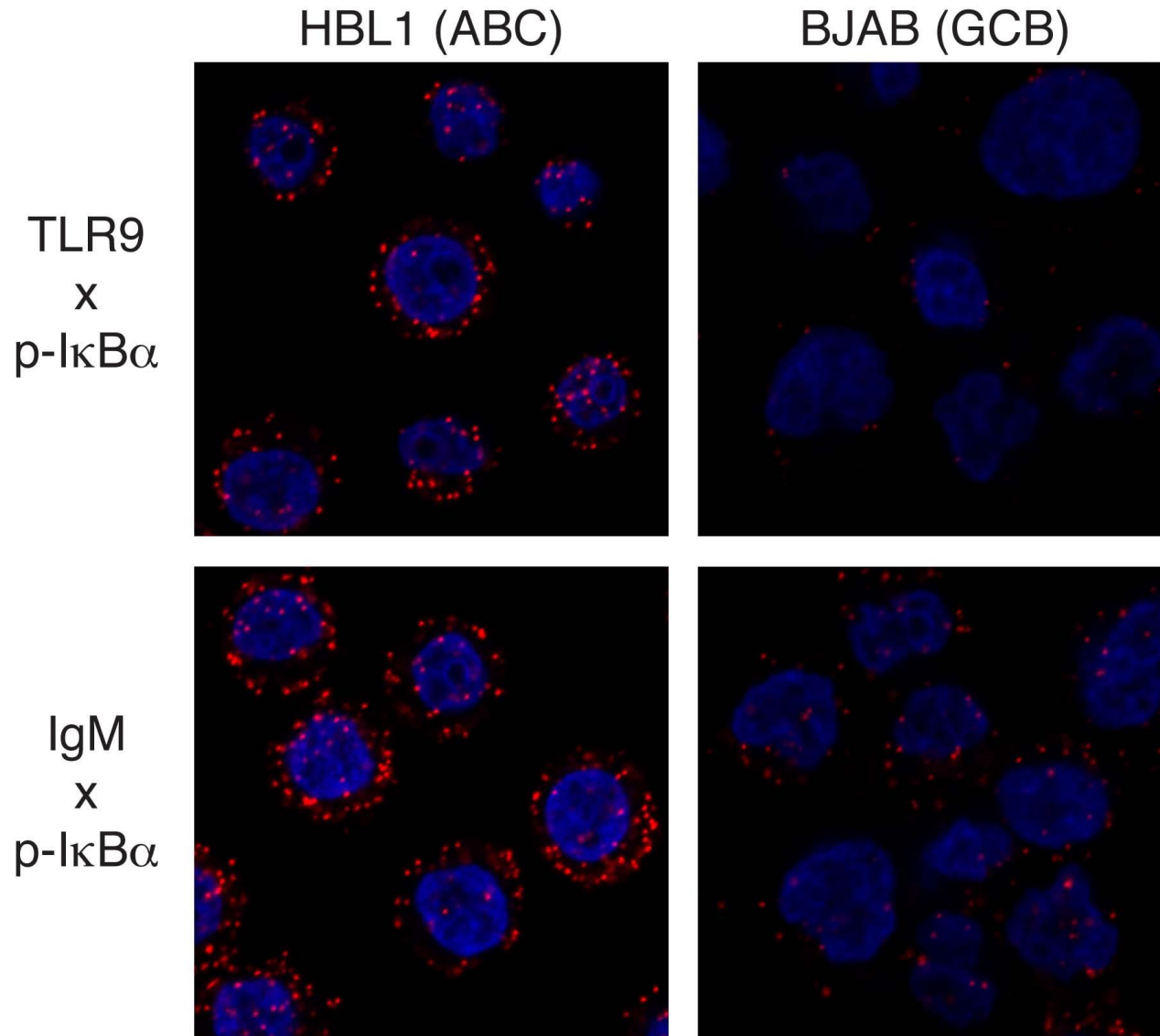
HBL1 (ABC DLBCL)

BJAB (GCB DLBCL)

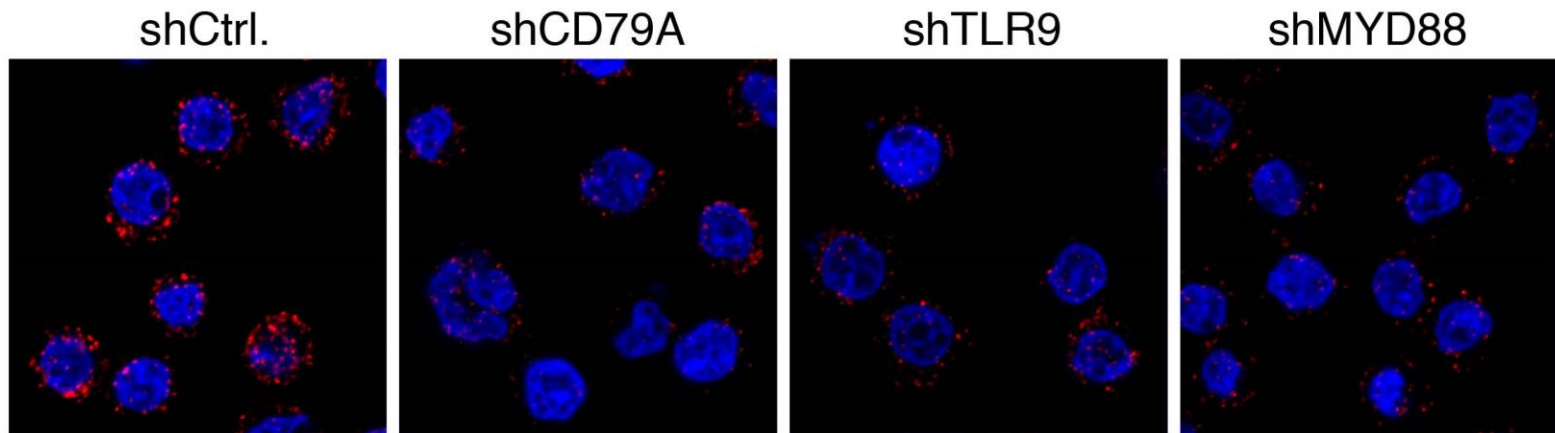
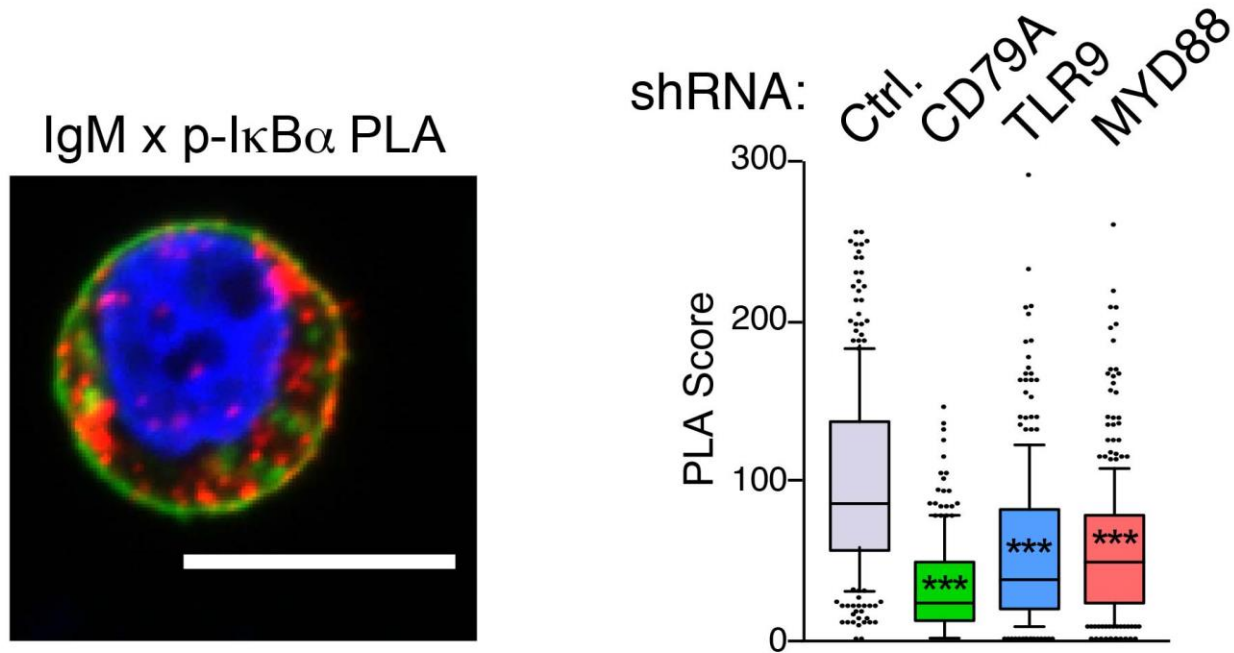
IgM x TLR9
Proximity
Ligation
Assay



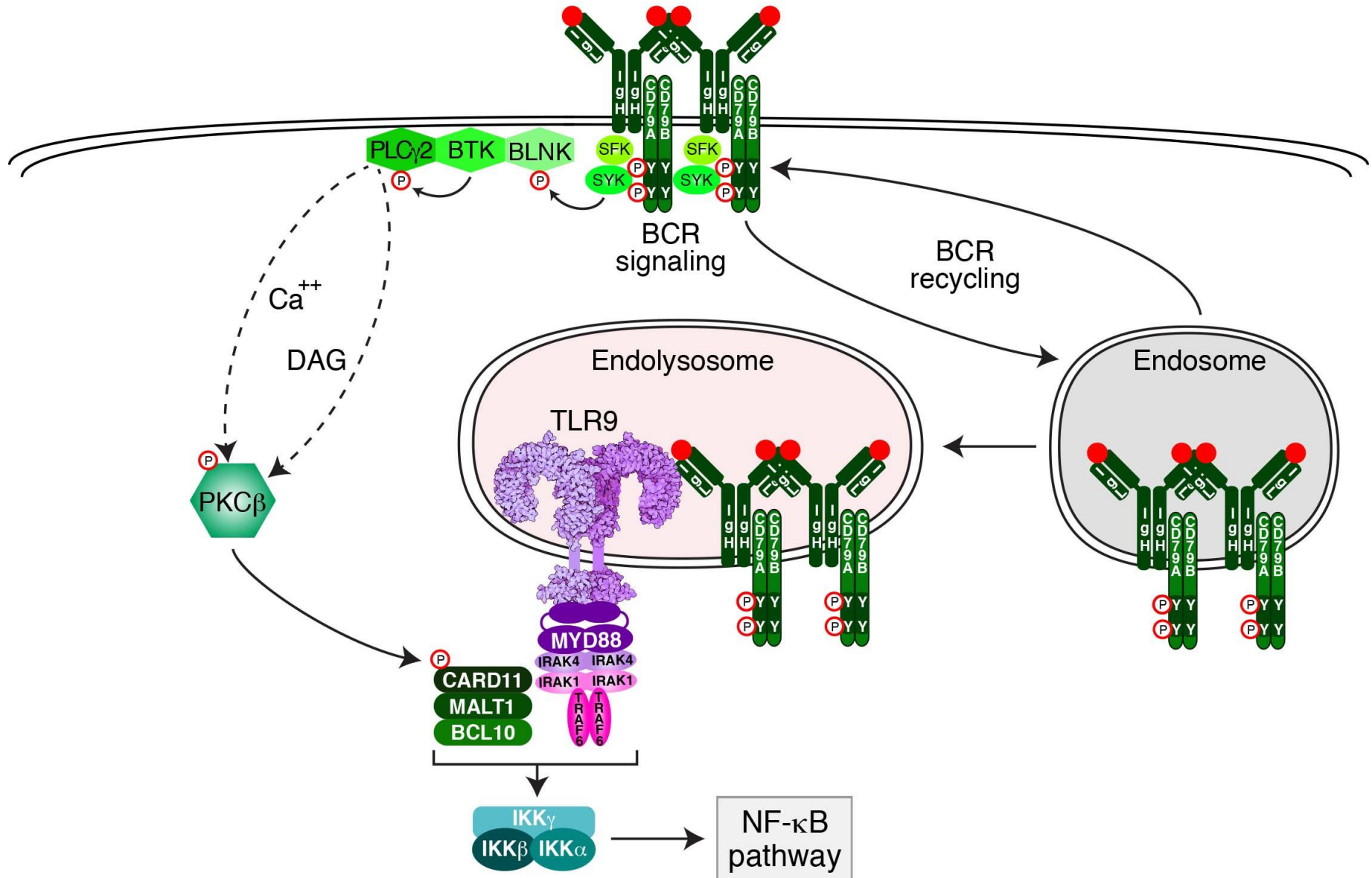
NF- κ B Pathway Activation in ABC DLBCL Occurs in the Lysosomal Compartment Containing TLR9 and IgM



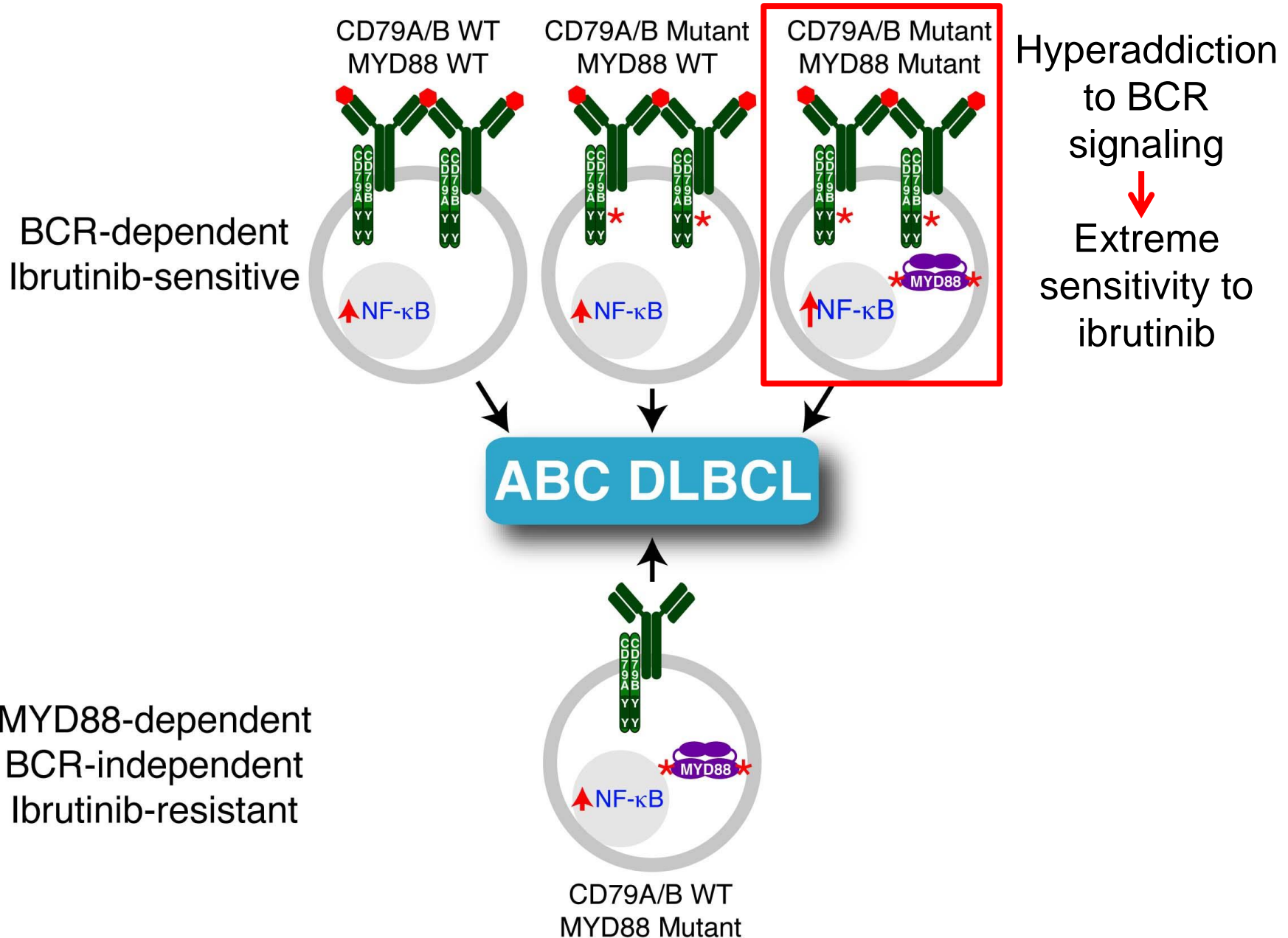
NF- κ B Activation by BCR and TLR/MYD88 Signaling is Colocalized in the Cytoplasm



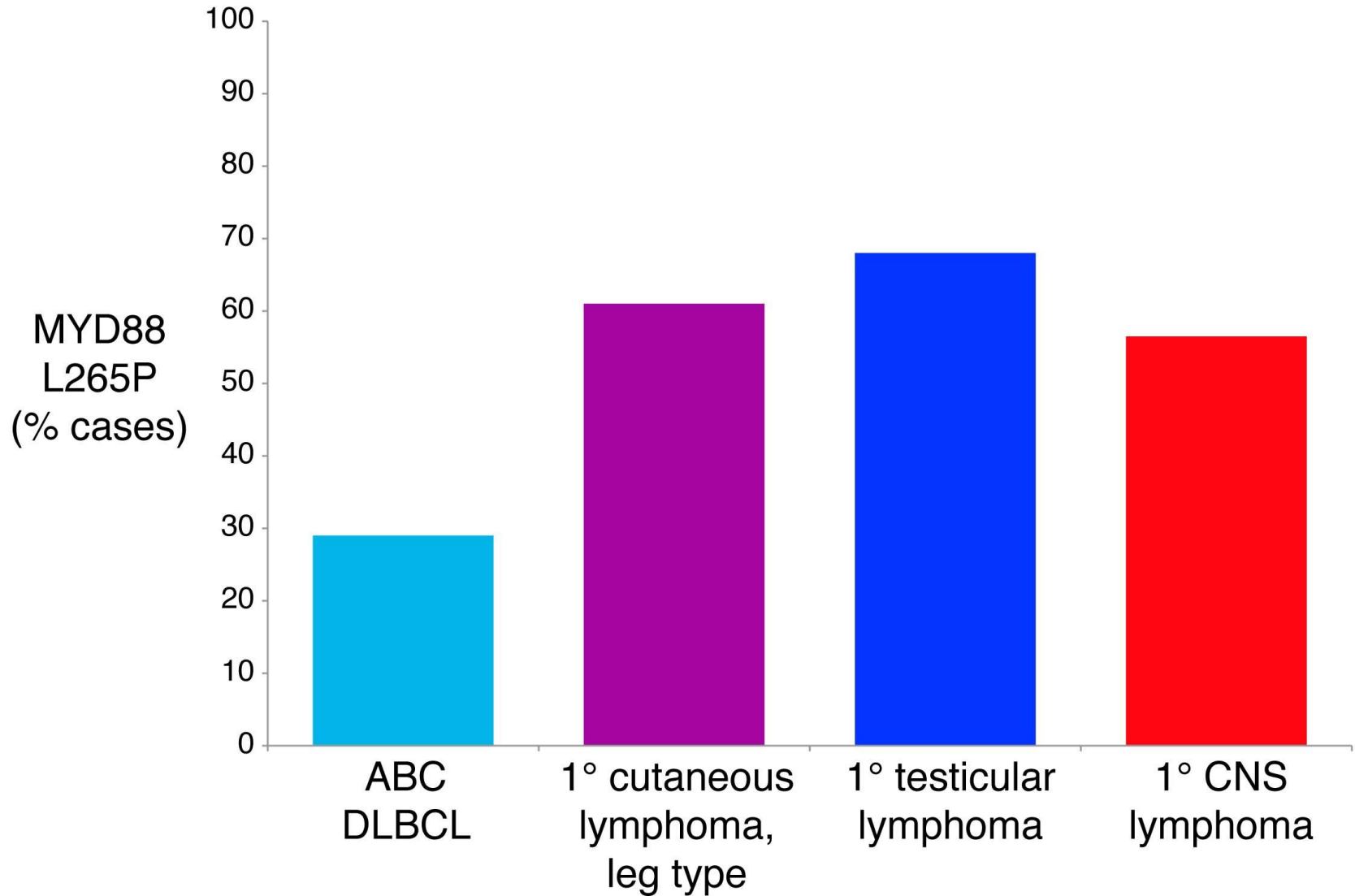
The BCR-MYD88 Superpathway in ABC DLBCL



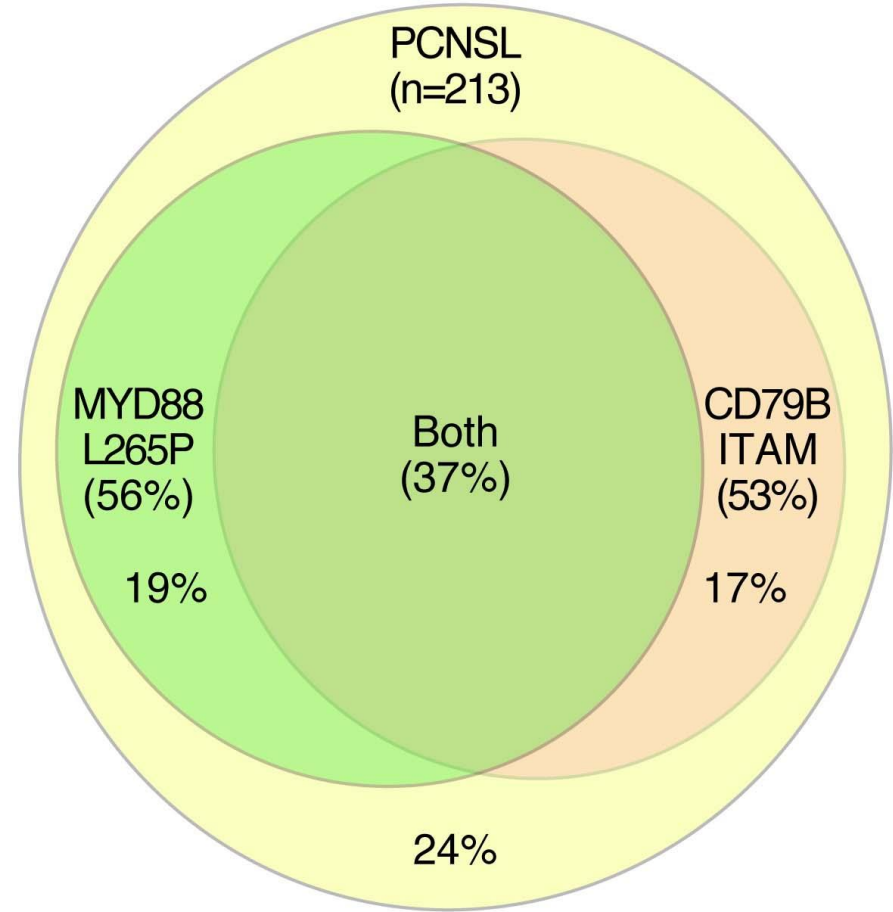
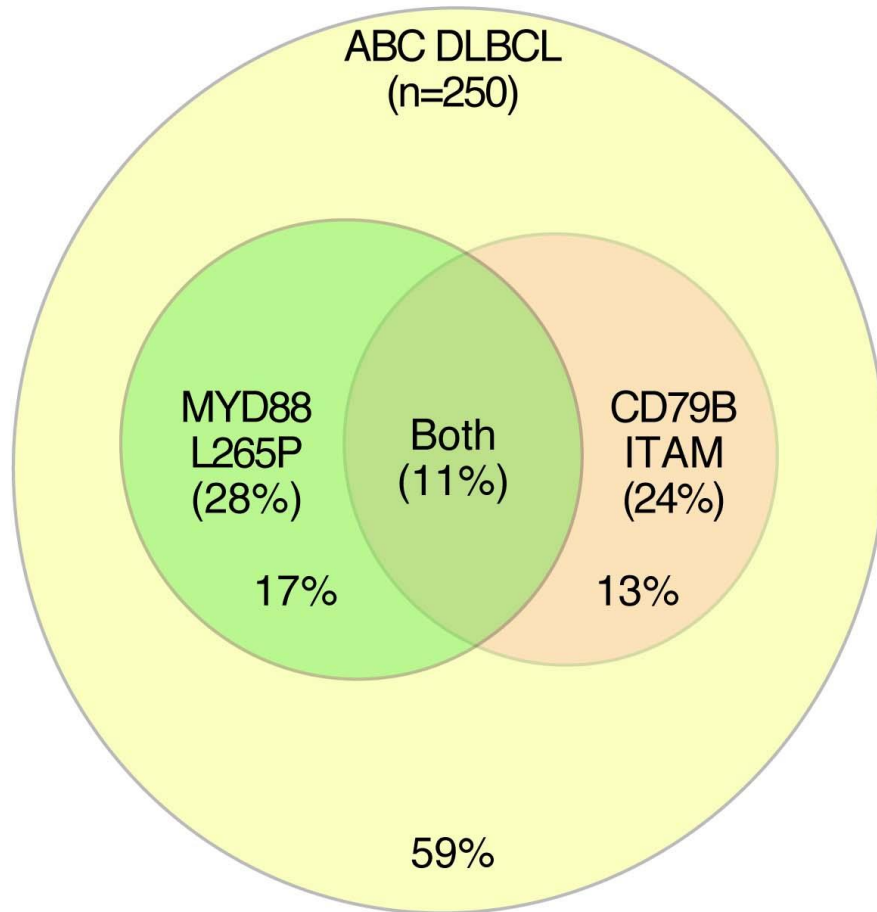
Two Pathogenetic Pathways to ABC DLBCL?



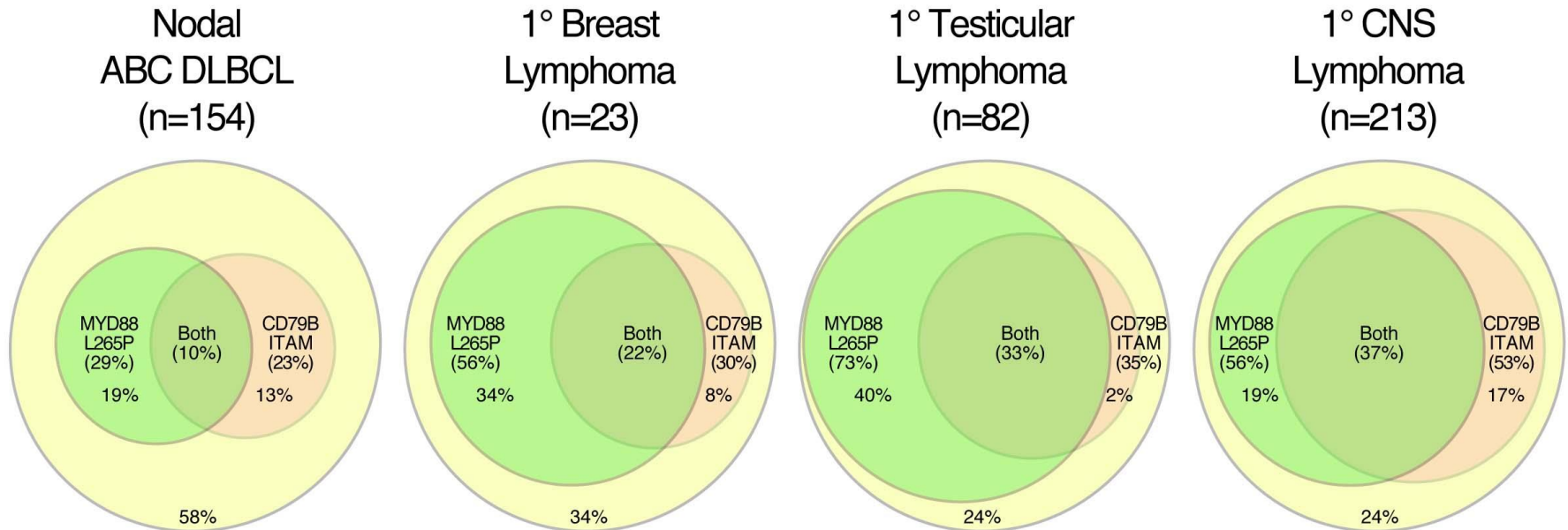
High Prevalence of MYD88 L265P Mutation in Extranodal Lymphomas with an ABC DLBCL Phenotype



Primary Central Nervous System Lymphoma is Enriched For Mutations in MYD88 and the BCR Subunit CD79B



Increased Coincidence of MYD88 L265P and CD79B Mutations in Extranodal DLBCL Tumors



Hypothesis:

Extranodal DLBCLs are hyper-addicted to BCR signaling

=> Will respond frequently to ibrutinib

Acknowledgements

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CRISPR Screens

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