Abstract #33 | Poster

An *In Vivo PiggyBac* Insertional Mutagenesis Screen Reveals Oncogenic Lesions Cooperating With *Myd88*^{L265P}

S. Höfmann[1], R. Flümann[2, 3], J. Hansen[2, 5], S. Klein[1], J. Meinel[7], P. Pfeiffer[2], H. Goldfarb-Wittkopf[2, 5], A. Lütz[2, 5], J. Wirtz[2, 5], M. Möllmann[1], T. Zhou[1], A. Tabatabai[1], T. Lohmann[2], F. Beleggia[2, 4], B. W. Pelzer[6], F. Ullrich[1], A. Arora[1], T. Persigehl[8], R. Büttner[7], B. v. Tresckow[1], R. D. Jachimowicz[2, 3], G. Knittel[1], H.C. Reinhardt[1]

[1] University Hospital Essen, Dept. of Hematology and Stem Cell Transplantation, West German Cancer Center, German Cancer Consortium Partner Site Essen, Center for Molecular Biotechnology, Essen, Germany
[2] University of Cologne, Faculty of Medicine and University Hospital Cologne, Department I of Internal Medicine, Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf, Cologne, Germany
[3] University of Cologne, Center for Molecular Medicine, Cologne, Germany
[4] Mildred Scheel School of Oncology Aachen Bonn Cologne Düsseldorf (MSSO ABCD), Faculty of Medicine and University Hospital of Cologne, Cologne, Germany
[5] Max Planck Institute for Biology of Ageing, Cologne, Germany
[6] Cornell University, Division of Hematology/Oncology, Department of Medicine, Weill Cornell Medicine, New York, United States of America
[7] University of Cologne, Faculty of Medicine and University Hospital Cologne, Institute of Pathology, Cologne, Germany
[8] University of Cologne, Faculty of Medicine and University Hospital Cologne, Department of Radiology and Interventional Radiology, Cologne, Germany

Diffuse large B cell lymphoma (DLBCL) is the most common type of Non-Hodgkin lymphoma originating from transformed germinal center-experienced B cells. DLBCL has been divided into two transcriptionally defined subtypes, activated B cell or germinal center B cell DLBCL (ABC and GCB DLBCL, Alizadeh et al., 2000). More recently, DLBCL cases have been classified on the basis of their genetic features and several clusters with different mutational profiles have been identified (Chapuy et al., 2018, Schmitz et al., 2018, Wright et al., 2020). The MCD/C5 cluster is defined by recurrent mutations in *MYD88*, *PRDM1* and frequent *BCL2* gain, amongst others. We introduced a conditional allele leading to a B cell-specific *Myd88*^{L252P} mutation (orthologous position of the human p.L265P mutation) in mice that develop B cell proliferation and occasional transformation into DLBCL (Knittel et al., 2016). Combination with *BCL2* overexpression and a genetically engineered block in plasmacytic differentiation by loss of *Prdm1* or overexpression of *Spib* increased lymphomagenesis (Flümann et al., 2021).

We performed an *in vivo piggyBac* insertional mutagenesis screen to identify genes and pathways that cooperate with *Myd88*^{L252P} in lymphomagenesis. These mice harbor a transposon and a conditionally expressed transposase on a *Myd88*^{L252P} background. The transposase mobilizes transposable elements, which can then reintegrate into the genome. Depending on the exact integration site and orientation of the transposon cassette, it can either silence or drive the expression of genes (Rad et al., 2015). In comparison to mice harboring only the *Myd88* mutation or just the *piggyBac* system, *Myd88*/*piggyBac* mice lived significantly shorter (**Fig. 1A**) and developed B220^{+}/CD138^{-} lymphomas (**Fig. 1B-C**). The isolated DNA from these lesions allowed the detection of common transposon insertion sites and the identification of

genes significantly enriched for integrations. We observed known genetic drivers of human MCD/C5 DLBCL, such as *PIM1* and *ETV6* (**Fig. 1D**). Moreover, *TBL1XR1* and *SPIB* were identified as common insertion sites, as well as *BCL2, BIM1* and *BCL-XL* (**Fig. 1D**), further validating our approach of engineering a plasma cell differentiation block as well as anti-apoptotic *BCL2* overexpression on a *Myd88* mutant background to model MCD DLBCL. The hits identified in this screen were significantly distinct from hits identified to drive *Myc*-driven B cell lymphomagenesis (Weber et al., 2019). Additionally, we identified several candidate genes that are not reported to be frequently genetically altered in DLBCL, however involved in processes relevant to B cell biology, particularly to B cell receptor signaling (**Fig. 1D**).

Since several screen hits were associated with ‘B cell receptor signaling’ and *CD79B* ITAM mutations are an additional hallmark of MCD/C5 DLBCL (Chapuy et al., 2018, Schmitz et al., 2018, Wright et al., 2020), we introduced a conditional *Cd79b* p.Y195H allele to our $Cd19^{Cre/wt}$;$Myd88^{cond.p.L252P/wt}$;$Rosa26^{LSL.BCL2\text{-}IRES\text{-}GFP/wt}$;$Prdm1^{fl7fl}$ MCD/C5 DLBCL model (**Fig. 2A**). *Cd79b* WT and mutant lymphomas did not show significant differences in their immunohistochemical and transcriptional phenotype (**Fig. 2B-C**). We observed increased B cell receptor (BCR) signaling activity for *Cd79b* p.Y195H tumors, indicated by higher levels of phosphorylated SYK and PLCg2 (**Fig. 2D**). Additionally, an increased formation of cytoplasmic signaling complexes comprised of MYD88 and several components of the BCR pathway, including MALT1 and BTK, were detected in *Cd79b* mutant lymphomas (**Fig. 2E**). Treatment with the BTK inhibitor ibrutinib reduced the complex formation to levels observed in *Cd79b* WT lymphomas, demonstrating the BTK dependence of these complexes (**Fig. 2E**). Consequently, we investigated the effects of ibrutinib treatment in *Cd79b* mutant and wildtype MCD DLBCL mouse models and found *Cd79b* mutant lymphomas to be significantly more sensitive to ibrutinib treatment than their *Cd79b* WT counterparts.

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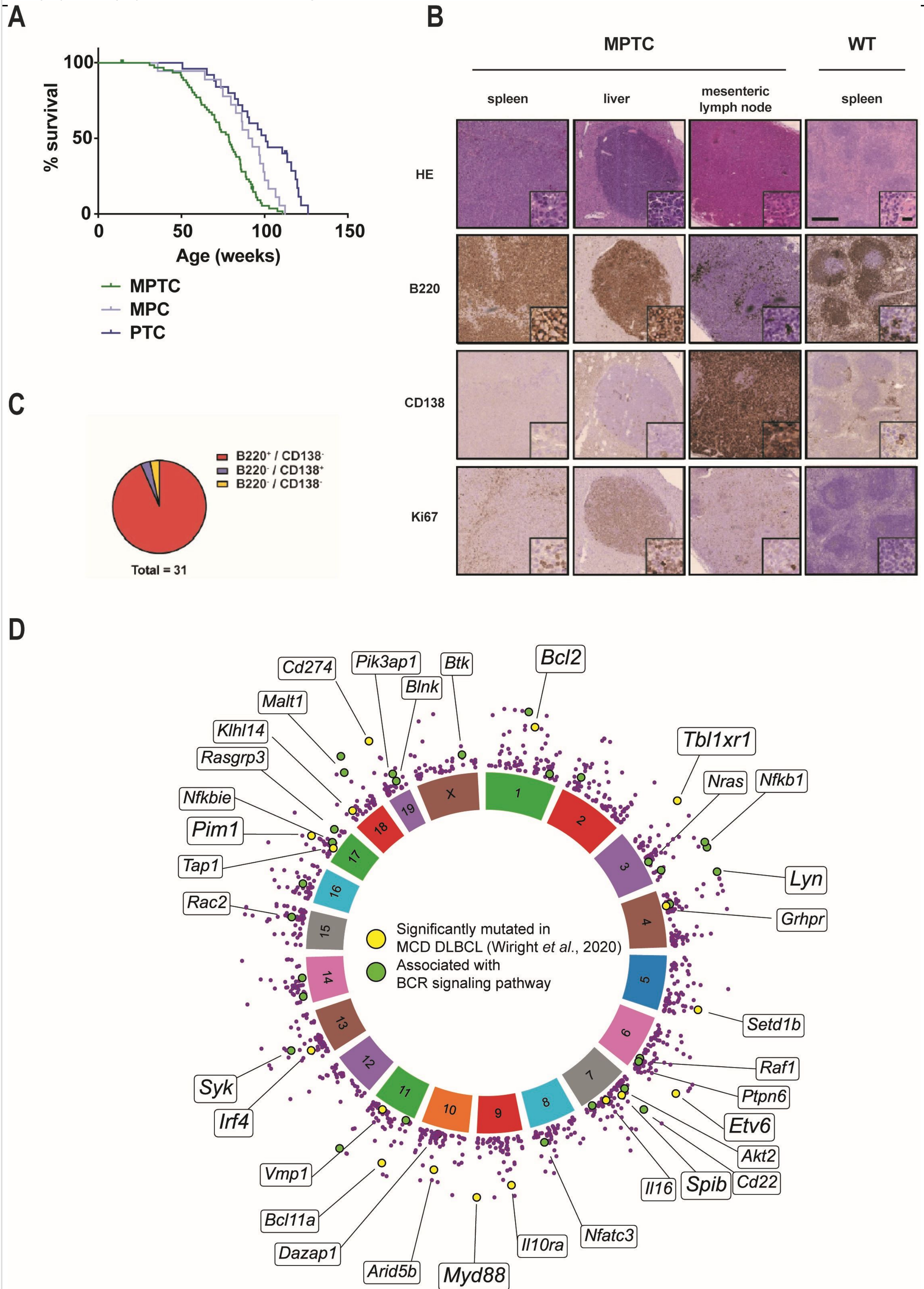

Fig. 1

A) Survival was determined for *Myd88*$^{p.L252P}$/*piggyBac* (MPTC) mice and mice harboring only *Myd88*$^{p.L252P}$ (MPC) or just the

piggyBac system (PTC). **B)** Representative immunohistochemical staining of lymphoma tissue isolated from MPTC and WT mice. **C)** Quantification of frequency of B220⁺/Cd138⁻ and B220⁻/Cd138⁺ tumors. **D)** Significant *piggyBac* hits that are found to be significantly mutated in MCD DLBCL (Wright et al., 2020) (yellow) or associated with the BCR signaling pathway (green).

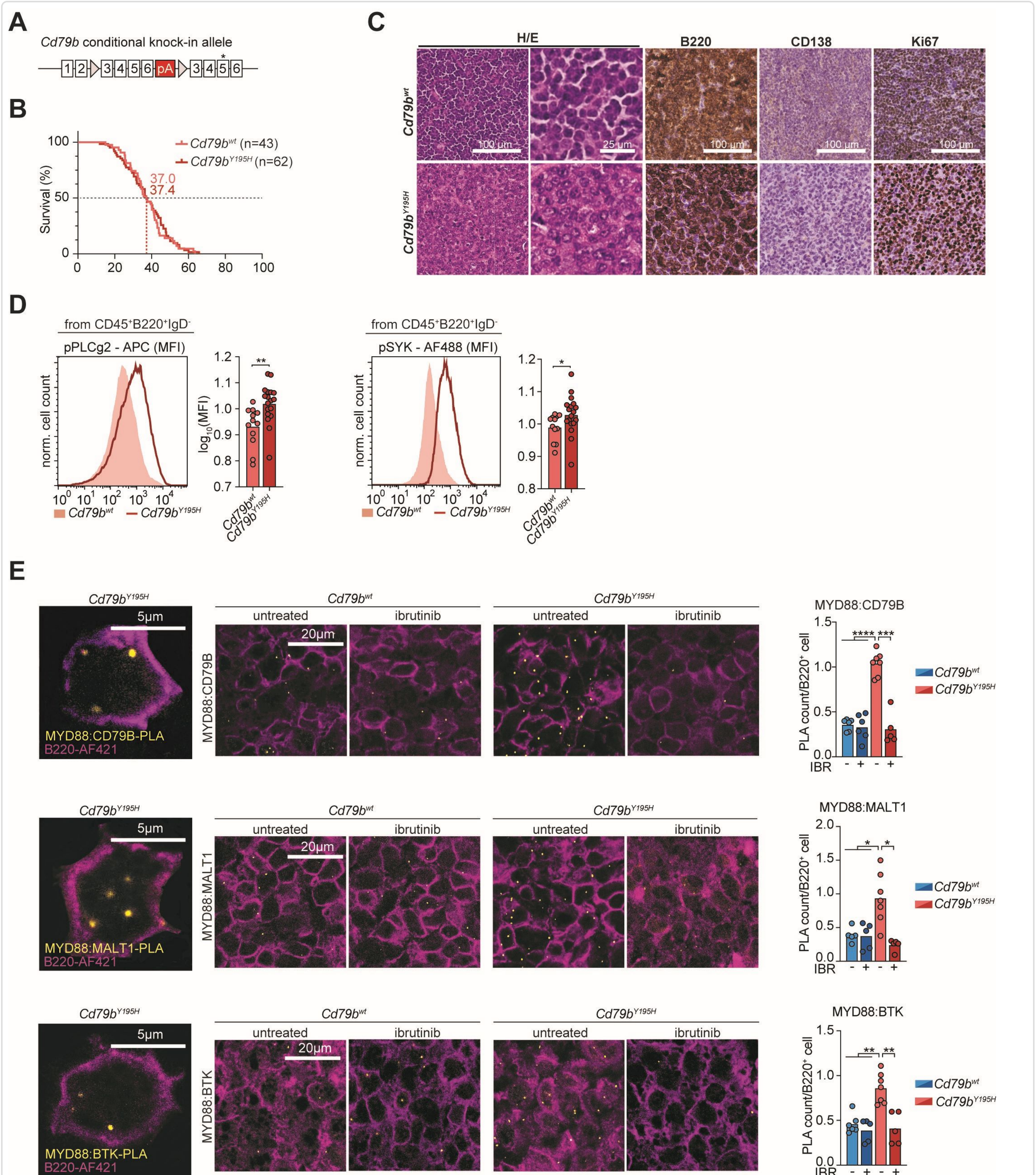

Fig. 2:

A) Schematic visualizations of the *Cd79b*$^{p.Y195H}$ allele. Triangular shapes represent loxP sites. **B)** Survival was determined for *Cd79b*$^{p.Y195H}$ and Cd79b^{wt} mice harboring additional *Cd19*$^{Cre/wt}$;*Myd88*$^{cond.p.L252P/wt}$;*Rosa26*$^{LSL.BCL2-IRES-GFP/wt}$;*Prdm1*^{fl7fl} alleles. **C)** Lymphoma cells of *Cd79b*^{wt} and *Cd79b*$^{p.Y195H}$ lymphomas were analyzed for levels of phosphorylated PLCg2 and SYK by

flow cytometry. **D)** Proximity ligation assays (PLAs) to detect the proximity of MYD88 and CD79B were performed on FFPE samples of *Cd79b*^{wt} and *Cd79b*$^{p.Y195H}$ lymphomas.