# Title Page

**LYMPHOID NEOPLASIA**

**LUBAC accelerates B-cell lymphomagenesis by conferring B cells resistance to genotoxic stress**

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## Key Points

1. LUBAC accelerates B-cell lymphomagenesis through protection of DNA damage-induced apoptosis, thereby promoting AID-mediated mutations.

2. Inhibition of LUBAC by small molecules is a promising therapeutic strategy for B-cell lymphomas with NF-κB activation.

## Abstract

Linear ubiquitin chain assembly complex (LUBAC) is a key regulator of NF-κB signaling. Activating single-nucleotide polymorphisms of HOIP, the catalytic subunit of LUBAC, are enriched in patients with activated B cell–like diffuse large B-cell lymphoma (ABC-DLBCL), and expression of HOIP which parallels LUBAC activity is elevated in ABC-DLBCL samples. Thus, to clarify the precise roles of LUBAC in lymphomagenesis, we generated a mouse model with augmented expression of HOIP in B cells. Interestingly, augmented HOIP expression facilitated DLBCL-like B-cell lymphomagenesis driven by MYD88-activating mutation. The developed lymphoma cells partly shared somatic gene mutations with human DLBCLs, with increased frequency of a typical AID mutation pattern. *In vitro* analysis revealed that HOIP overexpression protected B cells from DNA damage-induced cell death through NF-κB activation, and the analysis of human DLBCL database showed that expression of HOIP positively correlated with gene signatures representing regulation of apoptosis signaling, as well as NF-κB signaling. These results indicate that HOIP facilitates lymphomagenesis by preventing cell death and augmenting NF-κB signaling, leading to accumulation of AID-mediated mutations. Furthermore, a natural compound that specifically inhibits LUBAC was shown to suppress the tumor growth in a mouse transplantation model. Collectively, our data indicates that LUBAC is crucially involved in B-cell lymphomagenesis through protection against DNA damage-induced cell death, and is a suitable therapeutic target for B-cell lymphomas.



**Keywords**: LUBAC, B-cell lymphoma, ABC-DLBCL, NF-κB, AID, cell death

## Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most frequent lymphoma subtype in adults,[1,2] and it is classified into two major categories, germinal center B cell–like (GCB)-DLBCL and activated B cell–like (ABC)-DLBCL, based on the gene expression profiling.[3-6] Since ABC-DLBCL has been shown to have a worse prognosis compared to GCB-DLBCL, new therapeutic strategies against ABC-DLBCL are warranted.[7-9]

ABC-DLBCL is characterized by constitutive NF-κB activation mediated by the B-cell receptor (BCR) and Toll-like receptor (TLR) signaling pathways, and many oncogenic mutations within these pathways have been identified. Among them, activating mutations of MYD88, a signaling molecule in the TLR pathway, including L265P, are present in around 30% of ABC-DLBCL cases,[10] and constitute the most frequent genetic abnormalities leading to aberrant NF-κB activation.

Protein ubiquitination is involved in multiple steps of the NF-κB pathway.[11] The linear ubiquitin chain assembly complex (LUBAC), which consists of the catalytic subunit HOIP (RNF31) and two accessory subunits, HOIL-1L and SHARPIN, promotes NF-κB activation and protects against cell death by synthesizing unique N-terminally linked linear polyubiquitin chains.[12-19] We previously reported that rare germline single-nucleotide polymorphisms (SNPs) in *HOIP* that increase LUBAC ligase activity are significantly enriched in ABC-DLBCL patients, suggesting that augmentation of LUBAC activity contributes to ABC-DLBCL pathogenesis.[20] The majority of ABC-DLBCLs in patients with these HOIP SNPs also harbor the MYD88 L265P mutation. Given that LUBAC plays a pivotal role in the NF-κB activation by linearly polyubiquitinating substrates, including the key NF-κB regulator NEMO,[18,19,21-24] it is speculated that LUBAC collaborates with MYD88 signaling in B-cell lymphomagenesis by further amplifying NF-κB activation.

By analyzing published clinical RNA sequencing (RNA-seq) gene expression data,[25] we found that expression of *HOIP* is elevated in human ABC-DLBCL (Figure 1A). As we previously reported that enforced expression of the catalytic subunit HOIP augments LUBAC functions,[17] we assumed that LUBAC activation is frequently involved in the pathogenesis of ABC-DLBCL, independent of SNPs in *HOIP*. To clarify the roles of LUBAC played in the pathogenesis of B-cell lymphoma, we established a gene-engineered mouse with enforced expression of HOIP in B cells. We found that increased expression of HOIP enhanced LUBAC activity, and it facilitated generation of MYD88-mediated DLBCL, whereas it could not lead to B-cell lymphoma development per se. Elevated expression of LUBAC was suggested to accelerate B-cell lymphomagenesis, not only by activating NF-κB in concert with MYD88-mediated signals, but also by protecting cells from DNA damage–induced apoptosis. Importantly, the mutations in B-cell lymphomas that arose in mice expressing an oncogenic MYD88 mutant and high levels of HOIP partially overlap with those reported in human DLBCLs, indicating the biological similarity between these tumors. Finally, by using a mouse lymphoma model with secondary transplantation of a newly established lymphoma cell line, we demonstrated that LUBAC inhibition represents a novel and promising therapeutic strategy against B-cell lymphomas.

# Methods

## Mice

Tissue-specific HOIP transgenic mice (ROSA26-STOP-Hoip-ires-eGFP-pA) and MYD88 L252P transgenic mice (ROSA26-STOP-Myd88_L252P-ires-eGFP-pA) (Accession No. CDB 1320K: http://www2.clst.riken.jp/arg/mutant%20mice%20list.html) were established as described in supplemental Methods. ROSA26-STOP-Hoip-ires-eGFP-pA or ROSA26-STOP-Myd88_L252P-ires-eGFP-pA transgenic mice were crossed with CD19-cre mice to express transgenic HOIP or MYD88 protein specifically in B cells from the pre–B cell stage.[26] All mice were maintained under specific pathogen–free conditions. All animal protocols were approved by Kyoto University and RIKEN Center for Biosystems Dynamics Research.

## Analysis of European Genome–phenome Archive and The Cancer Genome Atlas datasets

Clinical and RNA sequencing (RNA-seq) gene expression data derived from the core set of 624 human DLBCL samples were obtained from the European Genome–phenome Archive (EGA) (dataset identifier [ID]: EGAD00001003600),[25] and the Cancer Genome Atlas (TCGA) whole exome sequencing and RNA-seq data of 48 DLBCL samples (project ID: TCGA-DLBC) were obtained from the Broad Institute Firehose (http://gdac.broadinstitute.org/),[27,28] and were analyzed as described in supplemental Methods and supplemental Tables 1–4.

## Whole-exome sequencing

Lymphoma tissues obtained from the transgenic mouse model were analyzed by whole-exome sequencing using the SureSelect XT Mouse All Exon V2 kit (Agilent). Mouse tail DNA was used as a germline control. Sequence alignment to GRCm38/mm10 and mutation calling were performed using the Genomon pipeline (https://github.com/Genomon-Project) as previously described[29] with minor modifications. Candidate mutations with (i) $p < 0.01$ (Fisher's exact test), (ii) > 4 variant reads in tumor samples, and (iii) variant allele frequency (VAF) in tumor samples > 0.05 or > 0.2 were selected and manually reviewed. Human orthologues of mouse genes were assigned with the Ensembl 92 database. For each sample, the number of mutations, SNVs at C:G base pairs, transitions, and SNVs within the WRCY/RGYW motifs were calculated and compared using the Brunner–Munzel test. Enrichment of SNVs at C/G within the WRCY/RGYW motifs in genes were performed by binomial test. Gene enrichment analyses were performed with Fisher's exact test using the gene sets derived from supplemental Tables 5–8.[30,31]

**AlphaScreen binding assay for LUBAC inhibitors**

To search for inhibitors of linear polyubiquitination, an AlphaScreen-based HTS system was established using N-terminally FLAG-His–tagged ubiquitin (FLAG-Ub), C-terminally glutathione S-transferase (GST)-tagged ubiquitin (Ub-GST), ubiquitin-activating enzyme E1, UbcH7 as the E2 ubiquitin–conjugating enzyme, and Petit-LUBAC or Petit-SHARPIN as the E3 ubiquitin ligase, as described in supplemental Methods.

**Generation of a preclinical model for validation of LUBAC inhibitor**

The cell line HM876 was established as described in supplemental Methods. Transplantation of HM876 tumor cells was performed by subcutaneously injecting 5 ×

179 $10^6$ cells into 6-week-old C57BL/6 females, that were sublethally irradiated (4.5 Gy) 6
180 hours before transplantation. The animals were divided into three groups: the control
181 group (n = 7) received intraperitoneal injection of DMSO diluted in 5% glucose; the
182 other two groups were injected intraperitoneally with thiolutin diluted in 5% glucose at
183 2.5 or 5.0 mg/kg/day (n = 7). Thiolutin was administered from days 2 to 6 and days 9 to
184 13. On day 14, the animals were euthanized, and tumor weight was assessed.
185

## Results

### Augmented HOIP expression accelerates MYD88-mediated B-cell lymphomagenesis in mice

Based on the analysis of a publicly available database of gene expression in human B cells,[32] *HOIP* is physiologically expressed throughout B-cell development (supplemental Figure 1A). However, we previously reported that two rare SNPs of *HOIP* that augment LUBAC activity were enriched specifically in patients with ABC-DLBCL.[20] Because the protein expression level of HOIP determines the amount of other LUBAC subunits and activity of LUBAC,[17] we hypothesized that *HOIP* plays a key role in the activation of NF-κB pathway in ABC-DLBCL,[21,33,34] irrespective of the SNP status. We examined expression of *HOIP* in RNA sequencing (RNA-seq) data from 624 DLBCL samples in the European Genome–phenome Archive (EGA; dataset identifier [ID]: EGAD00001003600)[25] and found that its expression level was significantly higher in ABC-DLBCL than in GCB-DLBCL, as well as *HOIL-1L* and *SHARPIN*, that encode other subunits of LUBAC, although statistical significance was not observed in *SHARPIN* (Figure 1A; supplemental Figure 1B; supplemental Table 1). On the other hand, expression level of *OTULIN*, encoding a linear ubiquitin-specific deubiquitinase that negatively regulates LUBAC signaling,[35,36] was lower in ABC-DLBCL (supplemental Figure 1B; supplemental Table 1). These results are compatible to the increased LUBAC activity in ABC-DLBCL.

Therefore, to investigate the role of LUBAC played in B-cell lymphomagenesis, we generated mice expressing high levels of HOIP specifically in B cells from the pre–B cell stage (CD19-cre-HOIP) (Figure 1B). Bicistronic expression of eGFP allowed us to confirm that transgenic HOIP was specifically expressed in $CD19^+$ B cells (supplemental Figure 1C). In CD19-cre-HOIP mice, elevated expression of

HOIP increased expression of the other LUBAC subunits, thereby increasing the amount of trimeric LUBAC in B cells (supplemental Figure 1D). As expected, high levels of LUBAC increased expression of NF-κB target genes in splenic B cells despite mildly (Figure 1C; supplemental Figure 1E). Although some of the CD19-cre-HOIP mice aged over 14 months showed splenomegaly, no lymphoma development was observed (supplemental Figure 1F).

The majority of ABC-DLBCLs with the LUBAC-activating *HOIP* SNPs also carry the oncogenic MYD88 L265P mutation.[20] Consistent with this, we found that B cells with enforced HOIP expression proliferated more efficiently by TLR stimulation (CpG-DNA and Pam3CSK4) (Figure 1D), which suggested the synergistic effect of LUBAC and MYD88 signaling. To evaluate the combinatorial effect of LUBAC and MYD88 L265P, we generated mice in which *Myd88 L252P*, the equivalent to human *L265P*, was expressed specifically in B cells from the pre–B cell stage (CD19-cre-MYD88LP) (supplemental Figure 1G–I). MYD88 L252P increased proliferation and NF-κB activity of splenic B cells (supplemental Figure 1J–K). Hence, we assessed the synergistic effects of HOIP and MYD88 L252P on B-cell tumorigenesis in these mice. We evaluated the linear ubiquitin chains in B cells by using linear ubiquitin-specific tandem ubiquitin binding entity (M1-specific TUBE) (supplemental Figure 1L),[37,38] and found that the amount of linear ubiquitin chains was higher in splenic B cells of CD19-cre-HOIP/MYD88LP mice than in those of CD19-cre-MYD88LP mice (Figure 1E). As reported previously, B cell–specific expression of MYD88 L252P led to decreased survival.[39] We found that introduction of a HOIP transgenic allele significantly shortened the survival of CD19-cre-MYD88LP mice (Figure 1F).

Next, we examined pathological changes in CD19-cre-HOIP/MYD88LP and CD19-cre-MYD88LP mice. Mice with both genotypes developed marked

lymphosplenomegaly, and histological examination of spleens and lymph nodes revealed infiltrates of lymphoid cells in these organs (Figure 1G–H). In addition, human DLBCL-like eGFP and CD19-positive large abnormal B cells diffusely infiltrated into the affected organs in mice with both genotypes (Figure 1H; supplemental Figure 2A). Assessment of V(D)J recombination of immunoglobulin heavy chain loci using a PCR-based method confirmed the presence of monoclonal B-cell populations in all involved tissues derived from 14 mice (4 CD19-cre-MYD88LP and 10 CD19-cre-HOIP/MYD88LP) (Figure 1I; supplemental Figure 2B; Table 1). Lymphomas developed in 4 CD19-cre-MYD88LP mice and those in 8 of the 10 CD19-cre-HOIP/MYD88LP mice were positive for CD19, B220, and IgM and negative for CD138 by flow cytometric analysis (DLBCL-like lymphomas). These tumors were Irf4 positive and Bcl6 negative by immunohistochemical staining (Figure 1H). Moreover, sequence analysis of the variable regions of the clonally rearranged IgH gene revealed the presence of somatic hypermutations in most of the DLBCL-like lymphomas (supplemental Table 9). These results suggested that these tumors are mostly derived from post-germinal center B cells and are compatible with human ABC-DLBCL.[40] Tumor cells of the remaining two CD19-cre-HOIP/MYD88LP mice exhibited a plasma cell–like phenotype of CD19 and B220-negative and CD138-positive expression (Table 1). These results indicated that elevated expression of LUBAC potentially has a function to facilitate MYD88-mediated B-cell tumorigenesis.

**High LUBAC expression is associated with increased accumulation of AID-mediated somatic mutations**

We did not find any significant macroscopic, histological, and immunophenotypic differences between DLBCL-like lymphomas developed in CD19-cre-MYD88LP and CD19-cre-HOIP/MYD88LP mice (Figure 1G–H; Table 1). To understand the biological

background of the accelerated MYD88-mediated lymphomagenesis in the condition of augmented LUBAC activity, we performed whole-exome sequencing analyses of genomic DNA isolated from 12 lymphomas derived from 12 different mice (eight from CD19-cre-HOIP/MYD88LP and four from CD19-cre-MYD88LP mice). Significantly more mutations were detected in the whole exons of lymphoma cells derived from CD19-cre-HOIP/MYD88LP mice than in those from CD19-cre-MYD88LP mice (Figure 2A; supplemental Figure 3A), indicating that elevated LUBAC expression increased the number of somatic mutations. Twenty-six genes were found to be recurrently mutated non-synonymously in two or more samples among 12 mice. Twenty-three of them were recurrently mutated among those from eight CD19-cre-HOIP/MYD88LP mice. Moreover, 6 of them, including *Irf2bp2* and *Pim1*, were reported to be frequently mutated in human DLBCLs, especially in ABC-DLBCL (Figure 2B–C; supplemental Table 5).[30] These results suggested that B-cell lymphomas generated in mice expressing HOIP with MYD88 mutant transgene share some genome mutations with human DLBCLs.[25,30]

Notably, a significant proportion of recurrently mutated genes in lymphomas from CD19-cre-HOIP/MYD88LP mice were identified as known or predicted targets of aberrant somatic hypermutation induced by activation-induced cytidine deaminase (AID) (Figures 2C and 3A; supplemental Table 6).[25,30,31,41,42] AID plays essential roles in class-switch recombination and somatic hypermutation of the immunoglobulin genes during physiological B-cell maturation,[43] and is also involved in the pathogenesis of human DLBCL by introducing aberrant somatic hypermutations in non-immunoglobulin genes.[34,44-46] We found that somatic single-nucleotide variations (SNVs) within WRCY/RGYW motifs, or at C:G sites and transition mutations accumulated at higher levels in tumors derived from CD19-cre-HOIP/MYD88LP mice than in those from CD19-cre-MYD88LP mice (Figure 3B; supplemental Figure 3B–D;

supplemental Tables 7–8). Additionally, most of these mutations were located within 2 kb downstream of the transcription start site (TSS) of each target gene (Figure 3C; supplemental Figure 3E). Since all of these characteristics are known as hallmarks of AID-mediated somatic mutations, it is indicated that AID-mediated mutagenesis is involved in B-cell lymphoma development in CD19-cre-HOIP/MYD88LP mice.[47-49] Meanwhile, in the analyses of the whole-exome sequencing and RNA-seq data from 48 human DLBCL samples (Project ID: TCGA-DLBC),[27,28] we found that the frequency of AID-induced mutations positively correlated with the expression level of *HOIP* (Figure 3D–E; supplemental Table 4). Taken together, these results indicated that elevated expression of HOIP is associated with increased accumulation of somatic mutations of AID pattern, and augmented LUBAC activity is suspected to explain the facilitation of MYD88-mediated B-cell lymphomagenesis.

**Augmented LUBAC activity overcomes cell death induced by DNA damage thereby accelerating accumulation of somatic mutations**

Although AID has a strong preference for immunoglobulin genes, it produces off-target DNA damages as well, resulting in aberrant somatic mutations.[34,44,46,50] As shown above, AID-mediated mutations accumulated more prominently in CD19-cre-HOIP/MYD88LP mice compared to CD19-cre-MYD88LP mice. However, the expression levels of AID and the percentages of germinal center B cells in mesenteric lymph nodes were comparable between CD19-cre-HOIP/MYD88LP and CD19-cre-MYD88LP mice (Figure 4A). In addition, no correlation was found in the expression level of AID and HOIP in human DLBCLs (data not shown). Therefore, the altered expression level of AID did not seem to be the main reason for increased somatic mutations in lymphomas derived from CD19-cre-HOIP/MYD88LP mice.

Previous studies showed that LUBAC has functions in protecting cells from

316 genotoxic damage–induced apoptosis, as well as mediating NF-κB activation via
317 plasma membrane receptors.[51,52] Therefore, we examined the cell protective effect of
318 LUBAC against genotoxic stress. Enforced expression of HOIP protected HBL1, a
319 human ABC-DLBCL–derived cell line,[7] and murine splenic B cells from
320 cisplatin-induced cell death (Figure 4B–D; supplemental Figure 4A–B). We also found
321 that enforced expression of LUBAC protected Jurkat cells from cisplatin-induced cell
322 death by suppressing apoptosis (Figure 4E–H). It has been indicated that
323 LUBAC-mediated linear ubiquitination of NEMO is involved in genotoxic NF-κB
324 activation and protects cells from DNA damage–induced cell death.[52-55] Indeed,
325 expression of NF-κB target genes, including anti-apoptotic genes, was modestly but
326 significantly higher in splenic B cells of the CD19-cre-HOIP/MYD88LP mice than in
327 those of the CD19-cre-MYD88LP mice (supplemental Figure 5). Elevated levels of
328 HOIP not only augmented activation of NF-κB and expression of several anti-apoptotic
329 genes, but also enhanced linear ubiquitination of NEMO induced by cisplatin (Figure
330 4I–K; supplemental Figure 4C). In accordance with these results, RNA-seq analyses of
331 human DLBCLs revealed that expression of *HOIP* positively correlated with expression
332 of the genes involved in negative regulation of intrinsic apoptotic signaling, as well as
333 those involved in the NF-κB pathway (Figure 4L). These results suggested that
334 enhanced HOIP expression increases LUBAC activity and confers tumor cells
335 resistance to cisplatin-induced DNA damage by modulating expression of genes
336 associated with cell death.

337 On the other hand, AID-induced DNA alterations are repaired by the DNA
338 double-strand break repair machinery, which also functions in repairing
339 cisplatin-induced DNA damage.[41,56] Based on the observation that somatic mutations of
340 AID signature are increased in mouse lymphoma cells, it can be speculated that
341 increased LUBAC activity would also promote the accumulation of oncogenic somatic

342 mutations caused by AID, and in turn, facilitate the MYD88-mediated B-cell lymphoma
343 development.
344
345 **LUBAC is an effective target for the treatment of DLBCL**
346 Analysis of publicly available RNA-seq gene expression data[25,30] suggested that the
347 prognosis of primary refractory or relapsed DLBCL patients with high *HOIP* expression
348 is worse than those with low *HOIP* expression (supplemental Figure 6A). Indeed, we
349 showed that LUBAC is involved in B-cell lymphomagenesis by protecting cells from
350 DNA damage–induced apoptosis (Figure 4D,G; supplemental Figure 4B), which may
351 lead to resistance to cytotoxic chemotherapies.[51] We have previously described that
352 LUBAC represents a novel therapeutic target against this cancer because reduction of
353 LUBAC suppresses NF-κB activation and proliferation of ABC-DLBCL cells *in vitro*
354 cell culture.[20,57] We then tried to establish a preclinical model for B-cell lymphomas
355 using CD19-cre-HOIP/MYD88LP mice to evaluate whether LUBAC is a promising
356 drug target for B-cell lymphomas *in vivo*.
357 A cell line HM876, derived from a B-cell lymphoma with plasma cell–like
358 surface phenotype in a CD19-cre-HOIP/MYD88LP mouse, exhibited elevated
359 expression of trimeric LUBAC and constitutive activation of NF-κB, manifested by
360 phosphorylation and reduced expression of IκBα (Figure 5A; supplemental Figure 6B;
361 Table 1). Using HM876 cells, we established a mouse lymphoma model by secondary
362 transplantation of HM876 cells for *in vivo* drug evaluation (Figure 5B).
363 We next sought for small compounds that can inhibit the activity of LUBAC.
364 High-throughput screening (HTS) of 41,760 compounds in total using an
365 AlphaScreen-based method (supplemental Figure 6C) identified aureothricin as a
366 candidate LUBAC inhibitor (Figure 5C–D; supplemental Figure 6D–E). Because
367 thiolutin is a molecular derivative of aureothricin, we examined both compounds in

subsequent experiments (Figure 5C–E; supplemental Figure 6E).

Ubiquitin ligases are classified into three groups, RING, HECT, and RING-IBR-RING (RBR). LUBAC is an RBR ligase,[58] and thiolutin inhibited catalytic RBR domain of HOIP in LUBAC (Figure 5F). Thiolutin did not noticeably inhibit the activities of a HECT ligase (Nedd4) or a RING ligase (cIAP2) *in vitro*, and only slightly inhibited another RBR ligase (Parkin), when used in higher concentrations (supplemental Figure 6F), suggesting that its inhibitory function is specific for LUBAC. Thiolutin effectively suppressed CD40 ligand-mediated NF-κB activation, and decreased the protein expression levels of LUBAC subunits at concentration as low as 0.07 μM in ABC-DLBCL-derived DLBCL2 cells and HM876 cells (Figure 5G–H). Thiolutin did not decrease the amount of LUBAC components in the *in vitro* ubiquitination assay, nor obviously altered the gene expression of LUBAC subunits in DLBCL cells (supplemental Figure 6G–H). Thiolutin decreased the amount of linear ubiquitin chains without affecting the amount of K48- or K63-ubiquitin chains in cells (Figure 5I; supplemental Figure 6I), and appeared to decrease survival of ABC-DLBCL–derived cell lines more effectively than GCB-DLBCL-derived cell lines (Figure 5J). Likewise, thiolutin suppressed NF-κB activation and exerted significant cytotoxicity in HM876 cells *in vitro* (Figure 5A,K). These results suggested that the cytotoxic effect of thiolutin is mainly caused by the inhibition of LUBAC and the blockade of NF-κB signaling. To validate whether LUBAC is an effective therapeutic target for B-cell lymphomas, we intraperitoneally administered thiolutin to mice inoculated with HM876 cells, and found that thiolutin significantly decreased the tumor burden (Figure 5L–M), indicating that inhibition of LUBAC represents an effective treatment for B-cell lymphomas with NF-κB activation. Moreover, our results demonstrate that our mouse model provides a valuable preclinical platform for the development of novel therapeutic approaches for B-cell lymphomas.

## Discussion

Constitutive activation of NF-κB signaling is required for survival and proliferation of B cells and plays a crucial role in pathogenesis of ABC-DLBCL. Previously, we reported that rare germline SNPs in the gene encoding HOIP, which activates LUBAC ligase activity are accumulated in individuals with ABC-DLBCL.[20] We also found that expression of HOIP is elevated in ABC-DLBCL compared to GCB-DLBCL (Figure 1A). According to these observations, it is suspected that HOIP plays important roles broadly in ABC-DLBCL, whereas the precise contribution of HOIP and its functional protein complex LUBAC to lymphomagenesis has been poorly understood. Hence, we established mice models that allow enhanced expression of HOIP in B cells and assessed the roles of LUBAC in the pathogenesis of ABC-DLBCL. Because the LUBAC activating SNPs of HOIP accumulate in patients with ABC-DLBCL with oncogenic MYD88 L265P mutation,[20] we investigated the functional synergism of LUBAC and MYD88 in B-cell lymphomagenesis in mice. The results revealed that elevated expression of LUBAC accelerates MYD88-driven lymphomagenesis.

Our data showed that overexpression of HOIP increased NF-κB activation and enhanced proliferation of B cells upon MYD88 dependent signal activation (Figure 1C–D; supplemental Figure 1E), although it could not induce lymphomas in mice by itself (Figure 1F). However, enforced HOIP expression with oncogenic MYD88 L252P signaling facilitates tumor formation in mice, of which phenotype is DLBCL-like. More importantly, whole-exome sequence analysis of lymphomas developed in mice revealed higher somatic mutations in lymphomas with co-expression of HOIP, many of which are of AID signature and partially resemble those often seen in DLBCL patient samples (Figures 2B–C and 3A–C; supplemental Figure 3A–E). This suggests that the mouse model expressing HOIP and MYD88 L252P shares biological features with human

DLBCL.

NF-κB is known to be activated by genotoxic damages, including those triggered by AID, and it helps cell survival by inducing a variety of anti-apoptotic genes.[53] We previously reported that LUBAC-mediated linear ubiquitination of NEMO plays a key role in transducing nuclear genotoxic signals to the cytoplasm, and in turn inducing genotoxic stress-induced NF-κB activation.[52] As no significant difference in the expression levels of AID could be observed in B cells between CD19-cre-HOIP/MYD88LP and CD19-cre-MYD88LP mice (Figure 4A), we assume that increased mutation burden in the tumors of CD19-cre-HOIP/MYD88LP mice is rather a result of higher tolerability to genotoxic stress in the condition of higher catalytic activity of LUBAC (Figure 4D,G,J; supplemental Figure 4B). Therefore, elevated expression of LUBAC is considered to facilitate MYD88-mediated B-cell lymphomagenesis by conferring B cells resistance to genotoxic stress and, in turn, augmenting the accumulation of oncogenic mutations. Our findings are compatible with the previous report that apoptotic pathway countered MYD88-driven B-cell proliferation[59] and aberrant expression of anti-apoptotic protein Bcl-2 facilitated generation of oncogenic MYD88-driven DLBCL.[39,60,61]

In accordance with our mouse experiment, the expression level of HOIP appears to positively correlate with the number of somatic mutations of AID signature in human DLBCL (Figure 3E). Since no correlation was found in the expression level of AID and HOIP in human DLBCLs (data not shown), augmented protection of DNA damage-induced cell death by enhanced LUBAC expression might rather be a main cause for high mutation rates in human DLBCLs with high HOIP expression. Gene mutations recurrently found in human DLBCLs could barely be detected in lymphomas generated in mice with oncogenic MYD88 transgene alone. This could be simply due to the limited number of tumors that could be analyzed in CD19-cre-MYD88LP mice, or

446 the potential differences in B-cell developmental stage in which oncogenic MYD88
447 transgene are acquired between our model and human DLBCL.

448 We observed a population of mice with tumors of a more differentiated
449 phenotype with CD138 and IgM expression (Table 1) and the presence of serum M
450 proteins (data not shown). These tumors may possibly be the equivalent of
451 lymphoplasmacytic lymphoma (LPL) in humans, another B-cell malignancy in which
452 MYD88 L265P is closely involved.[62,63] Considering that these tumors were observed
453 only in CD19-cre-HOIP/MYD88LP mice (Table 1), augmented LUBAC activity may
454 have played some roles in their development, whereas there is presently no data of the
455 involvement of LUBAC in the pathogenesis of LPL.

456 Finally, we established a preclinical tumor transplantation model for human
457 B-cell lymphomas using a cell line derived from a CD19-cre-HOIP/MYD88LP mouse.
458 In this model, we showed that thiolutin, a specific inhibitor of LUBAC, suppressed the
459 growth of lymphoma cells. Reduction or deletion of LUBAC counteracts resistance to
460 cytotoxic chemotherapy, possibly by decreasing the expression of anti-apoptotic genes
461 that are induced by NF-κB activation.[51]

462 In summary, our results suggest that LUBAC has a function to accelerate B-cell
463 lymphomagenesis by conferring B cells resistance to genotoxic stress. We have also
464 shown that, as a direct regulator of NF-κB pathway, LUBAC is an effective treatment
465 target for lymphoma. Considering that resistance to genotoxic cell death is the common
466 feature of chemorefractory cancers, the inhibition of LUBAC would represent a
467 promising strategy for the treatment of multiple types of cancers.
468

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## Authorship Contributions

T.J., M.N., Y.S., K.I., and A.T-K. conceived and designed the project. T.J. performed most of the experiments. S.M. provided essential experimental support. A.S. and H.K. supported generation of transgenic mice. Y.K., K.K., and S.O. performed whole-exome sequencing analyses of the lymphomas derived from transgenic mice. H.A. performed analyses of clinical RNA-seq data. N.K., T.O., K.S., M.Y. developed and performed the HTS for small-molecule inhibitors for LUBAC. T.N., and F.I. performed experiments on aureothricin and thiolutin. K.S. advised on experimental design. T.J., M.N., K.I., and A.T-K. wrote the manuscript with contributions from all other authors.

## Disclosure of Conflicts of Interest

The authors declare that they have no conflict of interest.

## References


1. Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th edition). Lyon: International Agency for Research on Cancer (IARC). 2017.
2. Lenz G, Staudt LM. Aggressive lymphomas. *N Engl J Med*. 2010;362(15):1417-1429.
3. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000;403(6769):503-511.
4. Monti S, Savage KJ, Kutok JL, et al. Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response. *Blood*. 2005;105(5):1851-1861.
5. Shaffer AL, 3rd, Young RM, Staudt LM. Pathogenesis of human B cell lymphomas. *Annu Rev Immunol*. 2012;30:565-610.
6. Wright G, Tan B, Rosenwald A, Hurt EH, Wiestner A, Staudt LM. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. *Proc Natl Acad Sci USA*. 2003;100(17):9991-9996.
7. Kelly PN, Romero DL, Yang Y, et al. Selective interleukin-1 receptor-associated kinase 4 inhibitors for the treatment of autoimmune disorders and lymphoid malignancy. *J Exp Med*. 2015;212(13):2189-2201.
8. Nowakowski GS, LaPlant B, Macon WR, et al. Lenalidomide combined with R-CHOP overcomes negative prognostic impact of non-germinal center B-cell phenotype in newly diagnosed diffuse large B-Cell lymphoma: a phase II study. *J Clin Oncol*. 2015;33(3):251-257.
9. Tilly H, Gomes da Silva M, Vitolo U, et al. Diffuse large B-cell lymphoma (DLBCL): ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2015;26 Suppl 5:v116-125.
10. Ngo VN, Young RM, Schmitz R, et al. Oncogenically active MYD88 mutations in human lymphoma. *Nature*. 2011;470(7332):115-119.
11. Chen ZJ. Ubiquitination in signaling to and activation of IKK. *Immunol Rev*. 2012;246(1):95-106.
12. Fujita H, Rahighi S, Akita M, et al. Mechanism underlying IκB kinase activation mediated by the linear ubiquitin chain assembly complex. *Mol Cell Biol*. 2014;34(7):1322-1335.
13. Ikeda F, Deribe YL, Skanland SS, et al. SHARPIN forms a linear ubiquitin ligase complex regulating NF-κB activity and apoptosis. *Nature*. 2011;471(7340):637-641.
14. Kensche T, Tokunaga F, Ikeda F, Goto E, Iwai K, Dikic I. Analysis of nuclear factor-κB (NF-κB) essential modulator (NEMO) binding to linear and lysine-linked ubiquitin chains and its role in the activation of NF-κB. *J Biol Chem*.

2012;287(28):23626-23634.
15. Kirisako T, Kamei K, Murata S, et al. A ubiquitin ligase complex assembles linear polyubiquitin chains. *EMBO J*. 2006;25(20):4877-4887.
16. Kumari S, Redouane Y, Lopez-Mosqueda J, et al. Sharpin prevents skin inflammation by inhibiting TNFR1-induced keratinocyte apoptosis. *Elife*. 2014;3:e03422.
17. Tokunaga F, Nakagawa T, Nakahara M, et al. SHARPIN is a component of the NF-κB-activating linear ubiquitin chain assembly complex. *Nature*. 2011;471(7340):633-636.
18. Tokunaga F, Sakata S, Saeki Y, et al. Involvement of linear polyubiquitylation of NEMO in NF-κB activation. *Nat Cell Biol*. 2009;11(2):123-132.
19. Gerlach B, Cordier SM, Schmukle AC, et al. Linear ubiquitination prevents inflammation and regulates immune signalling. *Nature*. 2011;471(7340):591-596.
20. Yang Y, Schmitz R, Mitala J, et al. Essential role of the linear ubiquitin chain assembly complex in lymphoma revealed by rare germline polymorphisms. *Cancer Discov*. 2014;4(4):480-493.
21. Sasaki Y, Sano S, Nakahara M, et al. Defective immune responses in mice lacking LUBAC-mediated linear ubiquitination in B cells. *EMBO J*. 2013;32(18):2463-2476.
22. Satpathy S, Wagner SA, Beli P, et al. Systems-wide analysis of BCR signalosomes and downstream phosphorylation and ubiquitylation. *Mol Syst Biol*. 2015;11(6):810.
23. Yang Y, Kelly P, Shaffer AL, 3rd, et al. Targeting Non-proteolytic Protein Ubiquitination for the Treatment of Diffuse Large B Cell Lymphoma. *Cancer Cell*. 2016;29(4):494-507.
24. Yang YK, Yang C, Chan W, Wang Z, Deibel KE, Pomerantz JL. Molecular Determinants of Scaffold-induced Linear Ubiquitinylation of B Cell Lymphoma/Leukemia 10 (Bcl10) during T Cell Receptor and Oncogenic Caspase Recruitment Domain-containing Protein 11 (CARD11) Signaling. *J Biol Chem*. 2016;291(50):25921-25936.
25. Reddy A, Zhang J, Davis NS, et al. Genetic and Functional Drivers of Diffuse Large B Cell Lymphoma. *Cell*. 2017;171(2):481-494 e415.
26. Rickert RC, Roes J, Rajewsky K. B lymphocyte-specific, Cre-mediated mutagenesis in mice. *Nucleic Acids Res*. 1997;25(6):1317-1318.
27. Grossman RL, Heath AP, Ferretti V, et al. Toward a Shared Vision for Cancer Genomic Data. *N Engl J Med*. 2016;375(12):1109-1112.
28. Lohr JG, Stojanov P, Lawrence MS, et al. Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. *Proc Natl Acad Sci USA*. 2012;109(10):3879-3884.

29. Kataoka K, Nagata Y, Kitanaka A, et al. Integrated molecular analysis of adult T cell leukemia/lymphoma. *Nat Genet*. 2015;47(11):1304-1315.
30. Schmitz R, Wright GW, Huang DW, et al. Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma. *N Engl J Med*. 2018;378(15):1396-1407.
31. Alvarez-Prado AF, Perez-Duran P, Perez-Garcia A, et al. A broad atlas of somatic hypermutation allows prediction of activation-induced deaminase targets. *J Exp Med*. 2018;215(3):761-771.
32. Petri A, Dybkaer K, Bogsted M, et al. Long Noncoding RNA Expression during Human B-Cell Development. *PLoS One*. 2015;10(9):e0138236.
33. Dubois SM, Alexia C, Wu Y, et al. A catalytic-independent role for the LUBAC in NF-κB activation upon antigen receptor engagement and in lymphoma cells. *Blood*. 2014;123(14):2199-2203.
34. Pasqualucci L, Dalla-Favera R. Genetics of diffuse large B-cell lymphoma. *Blood*. 2018;131(21):2307-2319.
35. Keusekotten K, Elliott PR, Glockner L, et al. OTULIN antagonizes LUBAC signaling by specifically hydrolyzing Met1-linked polyubiquitin. *Cell*. 2013;153(6):1312-1326.
36. Rivkin E, Almeida SM, Ceccarelli DF, et al. The linear ubiquitin-specific deubiquitinase gumby regulates angiogenesis. *Nature*. 2013;498(7454):318-324.
37. Hjerpe R, Aillet F, Lopitz-Otsoa F, Lang V, England P, Rodriguez MS. Efficient protection and isolation of ubiquitylated proteins using tandem ubiquitin-binding entities. *EMBO Rep*. 2009;10(11):1250-1258.
38. van Wijk SJ, Fiskin E, Dikic I. Selective monitoring of ubiquitin signals with genetically encoded ubiquitin chain-specific sensors. *Nat Protoc*. 2013;8(7):1449-1458.
39. Knittel G, Liedgens P, Korovkina D, et al. B-cell-specific conditional expression of Myd88p.L252P leads to the development of diffuse large B-cell lymphoma in mice. *Blood*. 2016;127(22):2732-2741.
40. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*. 2004;103(1):275-282.
41. Chapuy B, Stewart C, Dunford AJ, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nat Med*. 2018;24(5):679-690.
42. Liu M, Duke JL, Richter DJ, et al. Two levels of protection for the B cell genome during somatic hypermutation. *Nature*. 2008;451(7180):841-845.
43. Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell*. 2000;102(5):553-563.
44. Lossos IS, Levy R, Alizadeh AA. AID is expressed in germinal center

614 B-cell-like and activated B-cell-like diffuse large-cell lymphomas and is not correlated
615 with intraclonal heterogeneity. *Leukemia*. 2004;18(11):1775-1779.
616 45. Mlynarczyk C, Fontan L, Melnick A. Germinal center-derived lymphomas: The
617 darkest side of humoral immunity. *Immunol Rev*. 2019;288(1):214-239.
618 46. Pasqualucci L, Neumeister P, Goossens T, et al. Hypermutation of multiple
619 proto-oncogenes in B-cell diffuse large-cell lymphomas. *Nature*.
620 2001;412(6844):341-346.
621 47. Khodabakhshi AH, Morin RD, Fejes AP, et al. Recurrent targets of aberrant
622 somatic hypermutation in lymphoma. *Oncotarget*. 2012;3(11):1308-1319.
623 48. Rada C, Milstein C. The intrinsic hypermutability of antibody heavy and light
624 chain genes decays exponentially. *EMBO J*. 2001;20(16):4570-4576.
625 49. Storb U, Peters A, Klotz E, et al. Cis-acting sequences that affect somatic
626 hypermutation of Ig genes. *Immunol Rev*. 1998;162:153-160.
627 50. Bahjat M, Guikema JEJ. The Complex Interplay between DNA Injury and
628 Repair in Enzymatically Induced Mutagenesis and DNA Damage in B Lymphocytes. *Int*
629 *J Mol Sci*. 2017;18(9).
630 51. MacKay C, Carroll E, Ibrahim AFM, et al. E3 ubiquitin ligase HOIP attenuates
631 apoptotic cell death induced by cisplatin. *Cancer Res*. 2014;74(8):2246-2257.
632 52. Niu J, Shi Y, Iwai K, Wu ZH. LUBAC regulates NF-κB activation upon
633 genotoxic stress by promoting linear ubiquitination of NEMO. *EMBO J*.
634 2011;30(18):3741-3753.
635 53. McCool KW, Miyamoto S. DNA damage-dependent NF-κB activation: NEMO
636 turns nuclear signaling inside out. *Immunol Rev*. 2012;246(1):311-326.
637 54. Wang CY, Mayo MW, Baldwin AS, Jr. TNF- and cancer therapy-induced
638 apoptosis: potentiation by inhibition of NF-κB. *Science*. 1996;274(5288):784-787.
639 55. Wu ZH, Miyamoto S. Induction of a pro-apoptotic ATM-NF-κB pathway and
640 its repression by ATR in response to replication stress. *EMBO J*.
641 2008;27(14):1963-1973.
642 56. Di Noia JM, Neuberger MS. Molecular mechanisms of antibody somatic
643 hypermutation. *Annu Rev Biochem*. 2007;76:1-22.
644 57. Fujita H, Tokunaga A, Shimizu S, et al. Cooperative Domain Formation by
645 Homologous Motifs in HOIL-1L and SHARPIN Plays A Crucial Role in LUBAC
646 Stabilization. *Cell Rep*. 2018;23(4):1192-1204.
647 58. Smit JJ, Monteferrario D, Noordermeer SM, van Dijk WJ, van der Reijden BA,
648 Sixma TK. The E3 ligase HOIP specifies linear ubiquitin chain assembly through its
649 RING-IBR-RING domain and the unique LDD extension. *EMBO J*.
650 2012;31(19):3833-3844.
651 59. Wang JQ, Jeelall YS, Beutler B, Horikawa K, Goodnow CC. Consequences of
652 the recurrent MYD88(L265P) somatic mutation for B cell tolerance. *J Exp Med*.

2014;211(3):413-426.
60. Lenz G, Wright GW, Emre NC, et al. Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. *Proc Natl Acad Sci USA*. 2008;105(36):13520-13525.
61. Monni O, Joensuu H, Franssila K, Klefstrom J, Alitalo K, Knuutila S. BCL2 overexpression associated with chromosomal amplification in diffuse large B-cell lymphoma. *Blood*. 1997;90(3):1168-1174.
62. Treon SP, Xu L, Yang G, et al. MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. *N Engl J Med*. 2012;367(9):826-833.
63. Kapoor P, Paludo J, Vallumsetla N, Greipp PR. Waldenström macroglobulinemia: What a hematologist needs to know. *Blood Rev*. 2015;29(5):301-319.

Table 1. Surface phenotypes of lymphomas in transgenic mice

| Tumor ID | Genotype | Surface phenotypes | | | | | | | | | | | Major site of involvement |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CD19 | B220 | IgM | IgD | CD5 | CD21 | CD23 | CD38 | CD138 | Igκ | Igλ | |
| 786 | CD19-cre-HOIP/MYD88LP | + | + | + | - | - | - | - | + | - | + | - | Spleen |
| 950 | CD19-cre-HOIP/MYD88LP | + | + | + | - | - | - | - | + | - | + | - | Spleen |
| 1032 | CD19-cre-HOIP/MYD88LP | + | + | + | - | - | - | - | + | - | + | - | Extranodal (subcutaneous) |
| 1074 | CD19-cre-HOIP/MYD88LP | + | + | + | - | - | - | - | + | - | + | - | Mesenteric lymph nodes |
| 1078 | CD19-cre-HOIP/MYD88LP | + | + | + | - | - | - | - | + | - | - | + | Peripheral lymph nodes |
| 1083 | CD19-cre-HOIP/MYD88LP | + | + | + | - | - | - | - | + | - | + | - | Peripheral lymph nodes |
| 1084 | CD19-cre-HOIP/MYD88LP | + | + | + | - | - | - | - | + | - | + | - | Peripheral lymph nodes |
| 1182 | CD19-cre-HOIP/MYD88LP | + | + | + | - | - | NA | NA | NA | - | + | - | Mesenteric lymph nodes |
| 1236 | CD19-cre-MYD88LP | + | + | + | - | - | - | - | + | - | + | - | Mesenteric lymph nodes |
| 1237 | CD19-cre-MYD88LP | + | + | + | - | - | - | - | + | - | + | - | Extranodal (subcutaneous) |
| 1289 | CD19-cre-MYD88LP | + | + | + | - | - | - | - | + | - | + | - | Peritoneal |
| 1385 | CD19-cre-MYD88LP | + | + | + | - | - | - | - | + | - | + | - | Peripheral lymph nodes |
| 876 | CD19-cre-HOIP/MYD88LP | - | - | + | - | - | - | - | + | + | + | - | Peritoneal |
| 1027 | CD19-cre-HOIP/MYD88LP | - | - | + | - | - | - | - | - | + | + | - | Peritoneal |

# Figure Legends

**Figure 1. Augmented LUBAC expression accelerates oncogenic MYD88-mediated B-cell lymphomagenesis in mice.** (A) Association of *HOIP* (*RNF31*) expression with cell-of-origin in human DLBCL. Boxes represent the median and the first and third quartiles, and whiskers represent the minimum and maximum of all data points. (B) Schematic representation of conditional expression of HOIP in mice. (C) Transcript levels of NF-κB target genes in unstimulated splenic B cells from mice (10 weeks old), normalized against *Actb* mRNA; n = 3 per genotype. Data are means ± SD. (D) Cell Trace Violet–labeled splenic B cells were cultured with or without stimuli. (E) Cell lysates of splenic B cells derived from CD19-cre, CD19-cre-MYD88LP, and CD19-cre-HOIP/MYD88LP mice were subjected to Halo-tagged linear ubiquitin-specific tandem ubiquitin binding entity (M1-specific TUBE) binding and Halo Tag based purification, and analyzed by immunoblotting. (F) Kaplan–Meier plots of survival of transgenic mice (n = 18, CD19-cre; n = 36, CD19-cre-HOIP; n = 26, CD19-cre-MYD88LP; and n = 33, CD19-cre-HOIP/MYD88LP). (G–I) Representative tumor involvement of lymphoid organs isolated from 9-month-old CD19-cre-HOIP/MYD88LP mice. (G) Macroscopic appearance of spleens (left) and lymph nodes (right). (H) Representative H&E and immunohistochemical staining for Irf4 and Bcl6 of spleens (CD19-cre mice) or tumors (CD19 cre-MYD88LP and CD19-cre-HOIP/MYD88LP mice). Scale bars: 200 μm; inset 20 μm. (I) Representative analyses of clonality. Tumor 1084–specific primers specifically amplified tumor 1084–specific V(D)J, but did not amplify V(D)J from genomic DNA of normal splenic B cells or tumor 786. (C and F) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (A and C), two-tailed unpaired Student's *t*-test; (F) log–rank test.

See also supplemental Figures 1–2 and supplemental Table 1.

693 **Figure 2. LUBAC facilitates somatic mutations in genes frequently mutated in**
694 **human DLBCL.** (A-C) Mutations with variant allele frequency (VAF) > 0.05 in tumor
695 samples were selected and analyzed. (A) Numbers of total mutations including both
696 synonymous and non-synonymous mutations in each tumor sample. Boxes represent the
697 median and the first and third quartiles, and whiskers represent the minimum and
698 maximum of all data points. (B) Venn diagram depicting the overlap between genes
699 recurrently mutated non-synonymously in lymphoma cells derived from
700 CD19-cre-HOIP/MYD88LP mice and those frequently detected in human DLBCL.[30]
701 (C) Mutational heatmap showing recurrently mutated genes across sequenced samples,
702 color-coded according to five types of genetic alteration. Above the mutational heatmap,
703 the bar graph indicates the number of non-synonymous mutations in each sample. To
704 the right of the mutational heatmap, the stacked bar graph indicates the percentage of
705 tumors that have each mutations, using the same five-color scheme. Target genes of
706 aberrant somatic hypermutation induced by AID in human DLBCL are labeled in red.
707 Black daggers indicates murine homologous genes frequently mutated in human
708 DLBCL.[30] Red and blue daggers indicate murine homologue of previously reported
709 altered genes significantly enriched in human ABC-DLBCL and GCB-DLBCL,
710 respectively.[30]
711 (A) *** $p < 0.001$. (A) Brunner–Munzel test; (B) Fisher's exact test.
712 See also supplemental Figure 3 and supplemental Table 5.
713
714 **Figure 3. LUBAC facilitates aberrant somatic hypermutations mediated by AID.**
715 (A-C) Mutations with VAF > 0.05 in tumor samples were selected and analyzed. (A)
716 Venn diagram depicting the overlap between genes recurrently mutated in lymphoma
717 cells derived from CD19-cre-HOIP/MYD88LP mice and murine homologue of known
718 or predicted targets of aberrant somatic hypermutation mediated by AID.[30] (B) Numbers

719 of SNVs at C/G within the WRCY/RGYW motifs (left), and numbers of C:G (center)
720 and transition mutations (right) in each tumor sample. (C) Mutation distribution in
721 targeted genes observed in lymphoma cells derived from eight
722 CD19-cre-HOIP/MYD88LP mice. Shadows indicate the 2 kb region downstream of the
723 transcription start site (TSS). (D) Numbers of nonsynonymous mutations in each human
724 DLBCL sample. (E) Numbers of SNVs at C/G within the WRCY/RGYW motifs (left),
725 and numbers of C:G (center) and transition mutations (right) in each human DLBCL
726 sample. (D and E) Average fold change of FPKM（high vs. low）= 1.36. Boxes
727 represent the median and the first and third quartiles, and whiskers represent the
728 minimum and maximum of all data points.
729 (B, D, and E), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (A) Fisher's exact test; (B)
730 Brunner–Munzel test; (D and E) two-tailed unpaired Student's *t*-test.
731 See also supplemental Figure 3 and supplemental Tables 6–8.
732
733 **Figure 4. Augmented LUBAC activity overcomes cell death induced by DNA**
734 **damage thereby accelerating accumulation of somatic mutations.** (A) Transcript
735 levels of *Aicda,* normalized to *Actb* (left panel) and percentages of germinal center B
736 cells (right panel) in mesenteric lymph nodes from 10-week-old mice; n = 3 per
737 genotype. Data are means ± SD. (B) HOIP-overexpressing HBL1 cells were established,
738 and immunoblot analyses were performed using lysates from WT, mock-transfected, or
739 HOIP-overexpressing HBL1 cells. (C) Live cells were analyzed by FACS using
740 TO-PRO-3 staining. HBL1 cells were treated with or without 10 μg/mL cisplatin for 0–
741 24 hours. (D) Percentage of live cells (± SD); n = 6 per group in three independent
742 experiments. (E) Immunoblot analyses were performed using lysates from WT,
743 HOIP-knockout, or HOIP-overexpressing Jurkat cells. (F) Live cells were analyzed by
744 FACS using FSC and TO-PRO-3 staining. Jurkat cells were treated with or without 0.5

μg/mL cisplatin for 0–72 hours. (G) Percentage of live cells (± SD) in three independent experiments. (H–J) Jurkat cells were treated with 3 μg/mL cisplatin for the indicated periods, followed by immunoblotting (H–I) or quantitative RT-PCR, normalized against *Actb* mRNA (J). (K) Jurkat cells were treated with 5 μg/mL cisplatin for the indicated periods. Whole-cell lysates were analyzed by anti-NEMO immunoprecipitation, followed by immunoblotting using antibodies against linear polyubiquitin and NEMO. (L) Correlation of expression of *HOIP* (*RNF31*) and negative regulation of intrinsic apoptotic signaling signature (left), and NF-κB signaling signature (right).

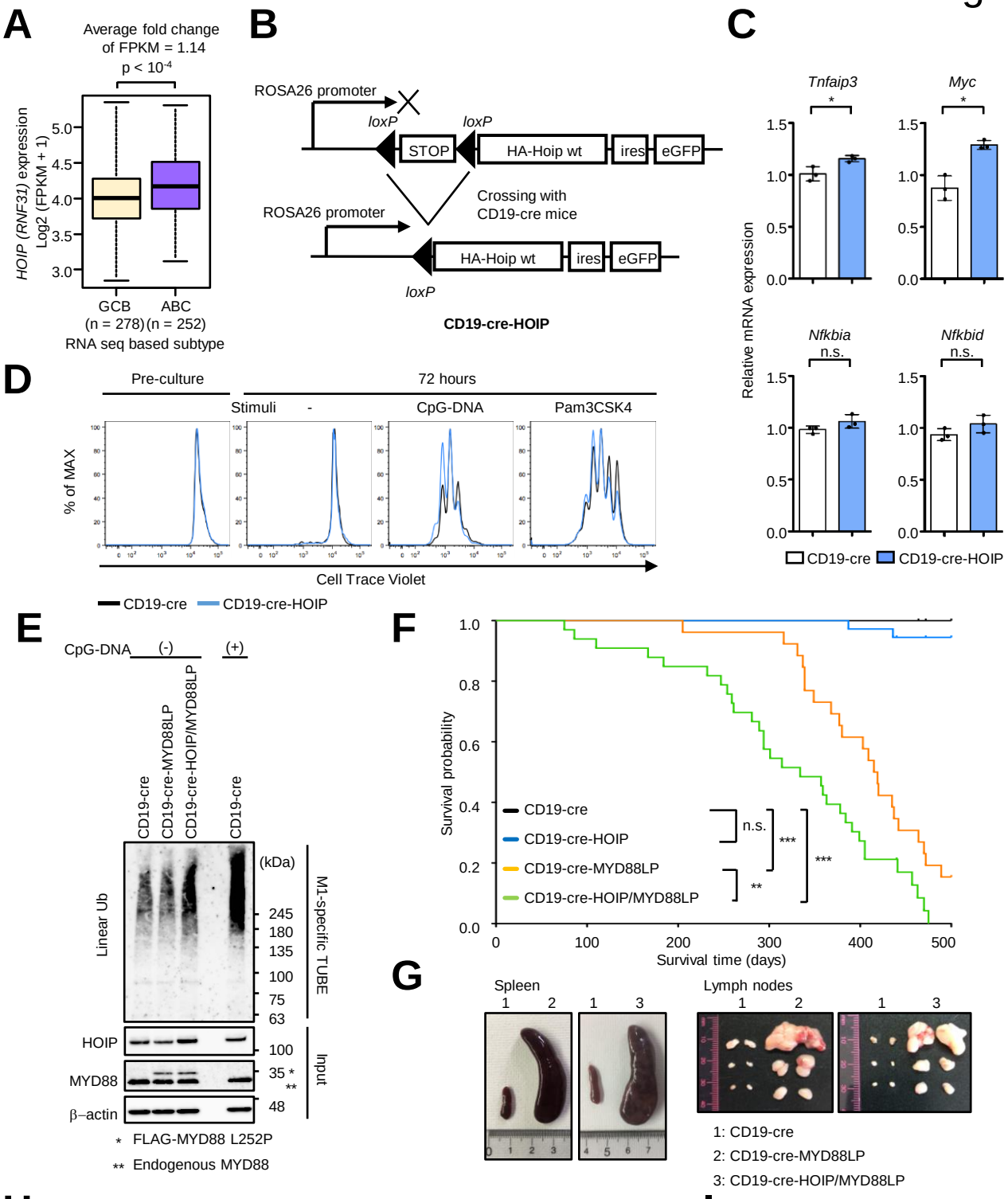
(A, D, G and J), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (A) one-way ANOVA with Turkey's post hoc test; (D, G and J) two-way ANOVA with Bonferroni post hoc test; (L) Pearson's correlation.

See also supplemental Figures 4 and 5, and supplemental Tables 1–4.

**Figure 5. LUBAC is an effective target for the treatment of DLBCL.** (A) Elevated phosphorylation and degradation of IκBα in unstimulated HM876 cells were suppressed by thiolutin. (B) Diagram of allogeneic transplantation model. (C) Chemical structure of aureothricin. (D) Inhibition of LUBAC ligase activity by aureothricin and thiolutin *in vitro*. (E) Chemical structure of thiolutin. (F) Thiolutin inhibited linear polyubiquitination mediated by HOIP (aa 699–1072). (G) Upon stimulation of DLBCL2 cells with CD40 ligand, thiolutin suppressed phosphorylation and degradation of IκBα in a dose-dependent manner. DLBCL2 cells were exposed to thiolutin or DMSO for 2 hours, and then stimulated with CD40 ligand (30 ng/mL) for the indicated times. (H) Levels of LUBAC components in DLBCL2 (upper panel) and HM876 (lower panel) cells treated with thiolutin were reduced in a dose-dependent manner. (I) Cell lysates of DLBCL2 (left panel) and HM876 (right panel) cells treated with or without thiolutin (0.1 μM) for two hours were analyzed by immunoblotting. Samples were probed with

771 anti-linear ubiquitin specific antibody (LUB9). (J) Viability of DLBCL lines after 48
772 hours treatment with the indicated concentrations of thiolutin, normalized against that of
773 control (DMSO-treated) cells. Data are means ± SD from three experiments. (K)
774 Viability of HM876 cells after 48 hours treatment with the indicated concentrations of
775 thiolutin, normalized against that of control (DMSO-treated) cells. Data are means ± SD
776 from three experiments. (L and M) Thiolutin suppressed growth of lymphomas *in vivo*.
777 (L) Gross appearance of engrafted tumors. (M) Tumor weight. Data are means ± SD.
778 (J, K and M) * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. (J and K) two-tailed unpaired
779 Student's *t*-test; (M) one-way ANOVA with Turkey's post hoc tests.
780 See also supplemental Figure 6.

Figure 1


A
Average fold change of FPKM = 1.14
p < 10-4
HOIP (RNF31) expression Log2 (FPKM + 1)
GCB (n = 278)
ABC (n = 252)
RNA seq based subtype
B
ROSA26 promoter
loxP
STOP
HA-Hoip wt
ires
eGFP
Crossing with CD19-cre mice
CD19-cre-HOIP
C
Tnfaip3
Myc
Nfkbia
Nfkbid
n.s.
Relative mRNA expression
CD19-cre
CD19-cre-HOIP
D
Pre-culture
72 hours
Stimuli
CpG-DNA
Pam3CSK4
% of MAX
Cell Trace Violet
CD19-cre
CD19-cre-HOIP
E
CpG-DNA
CD19-cre
CD19-cre-MYD88LP
CD19-cre-HOIP/MYD88LP
(kDa)
Linear Ub
M1-specific TUBE
HOIP
MYD88
β–actin
Input
* FLAG-MYD88 L252P
** Endogenous MYD88
F
Survival probability
Survival time (days)
CD19-cre
CD19-cre-HOIP
CD19-cre-MYD88LP
CD19-cre-HOIP/MYD88LP
n.s.
***
**
G
Spleen
Lymph nodes
1: CD19-cre
2: CD19-cre-MYD88LP
3: CD19-cre-HOIP/MYD88LP
H
CD19-cre
CD19-cre-MYD88LP
CD19-cre-HOIP/MYD88LP
H&E
Irf4
Bcl6
I
M
Normal Spl B
Tumor
1084
786
Cd19
T1084 specific VDJ

# Figure 2

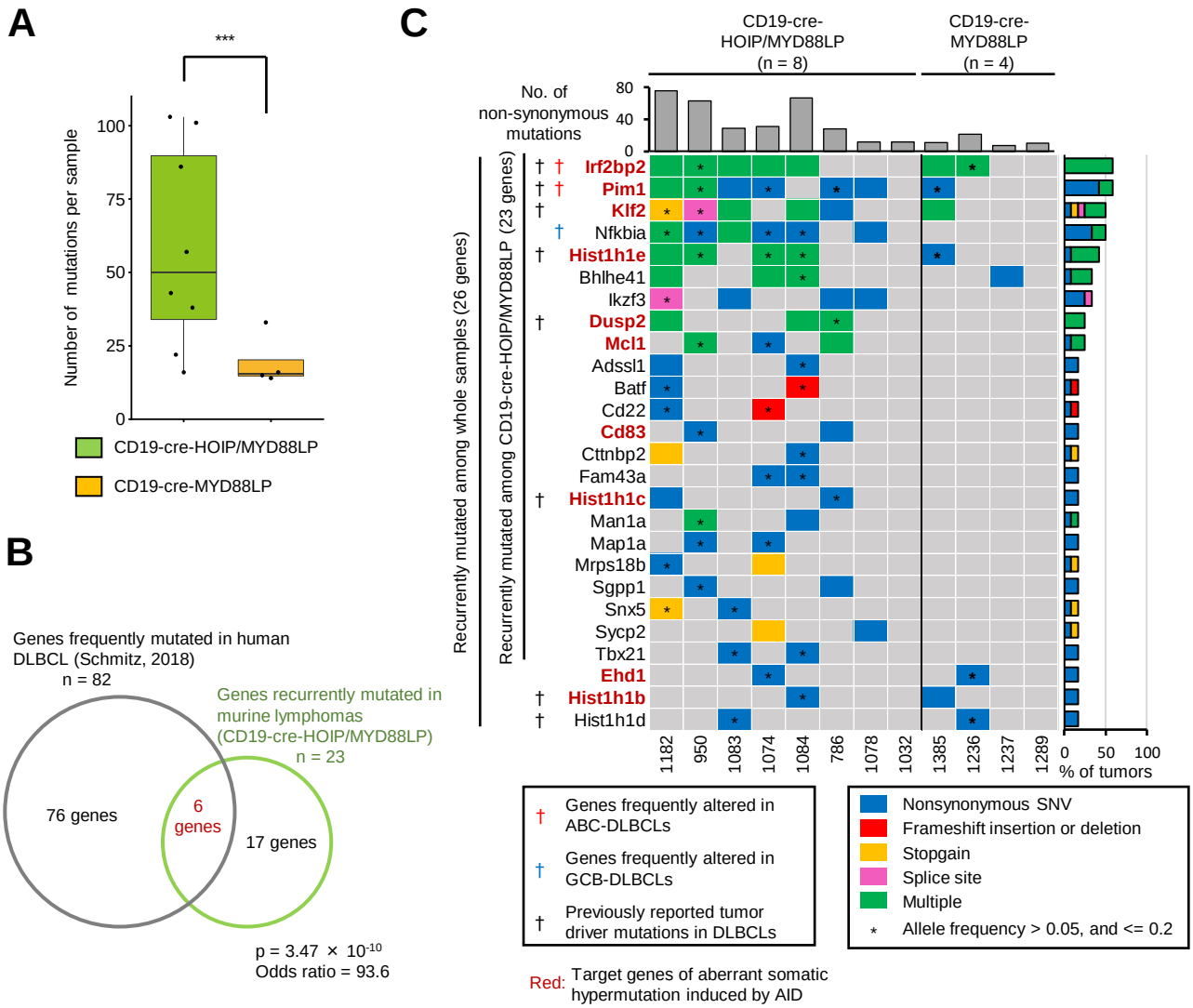

A
***
Number of mutations per sample
100
75
50
25
0
CD19-cre-HOIP/MYD88LP
CD19-cre-MYD88LP
B
Genes frequently mutated in human DLBCL (Schmitz, 2018)
n = 82
Genes recurrently mutated in murine lymphomas (CD19-cre-HOIP/MYD88LP)
n = 23
76 genes
6 genes
17 genes
p = 3.47 × 10-10
Odds ratio = 93.6
C
CD19-cre-HOIP/MYD88LP (n = 8)
CD19-cre-MYD88LP (n = 4)
No. of non-synonymous mutations
80
40
0
Recurrently mutated among whole samples (26 genes)
Recurrently mutated among CD19-cre-HOIP/MYD88LP (23 genes)
† † Irf2bp2
† † Pim1
† Klf2
† Nfkbia
† Hist1h1e
Bhlhe41
Ikzf3
† Dusp2
Mcl1
Adssl1
Batf
Cd22
Cd83
Cttnbp2
Fam43a
† Hist1h1c
Man1a
Map1a
Mrps18b
Sgpp1
Snx5
Sycp2
Tbx21
Ehd1
† Hist1h1b
† Hist1h1d
1182
950
1083
1074
1084
786
1078
1032
1385
1236
1237
1289
0
50
100
% of tumors
† Genes frequently altered in ABC-DLBCLs
† Genes frequently altered in GCB-DLBCLs
† Previously reported tumor driver mutations in DLBCLs
Red: Target genes of aberrant somatic hypermutation induced by AID
Nonsynonymous SNV
Frameshift insertion or deletion
Stopgain
Splice site
Multiple
* Allele frequency > 0.05, and <= 0.2

Figure 3

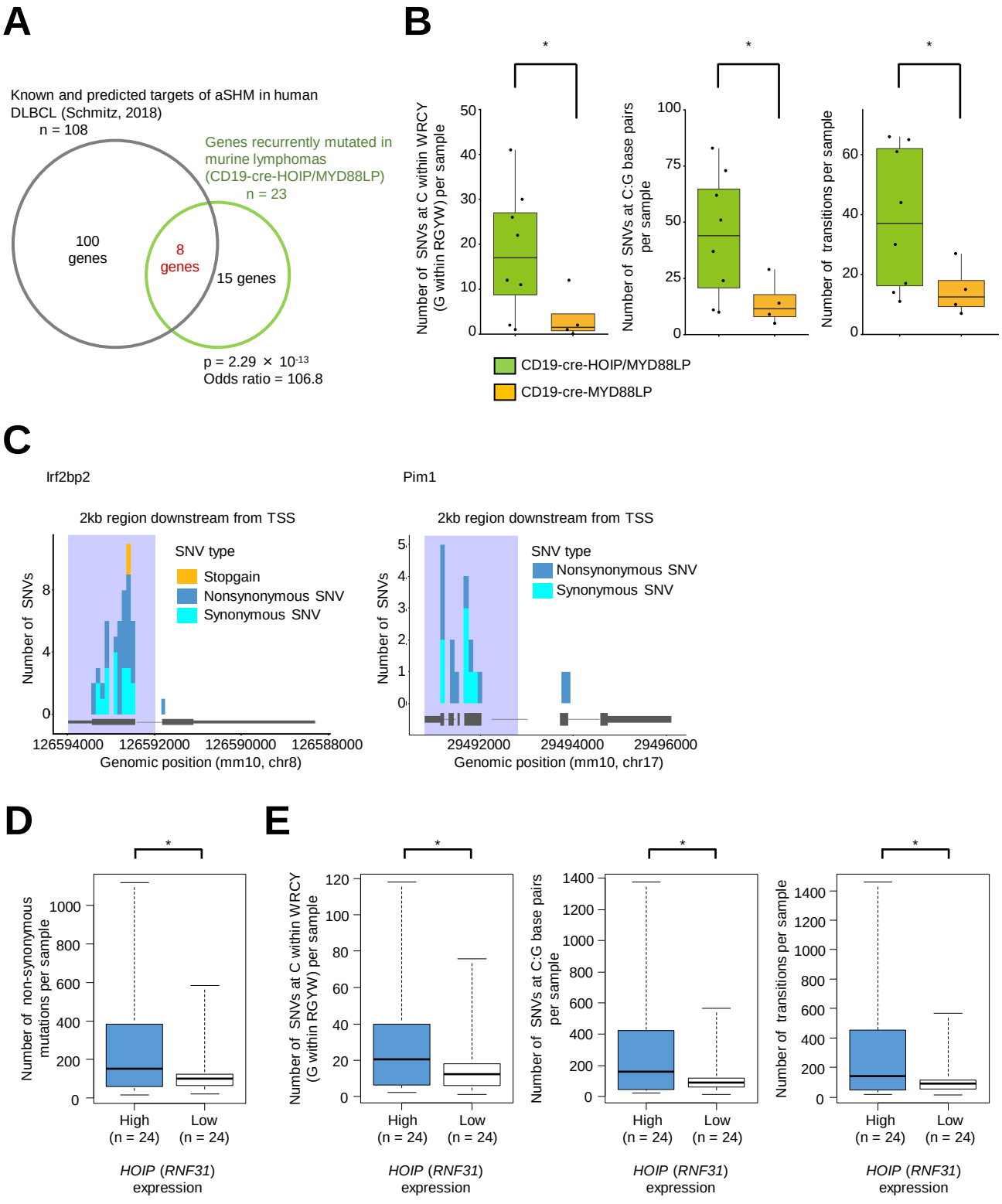
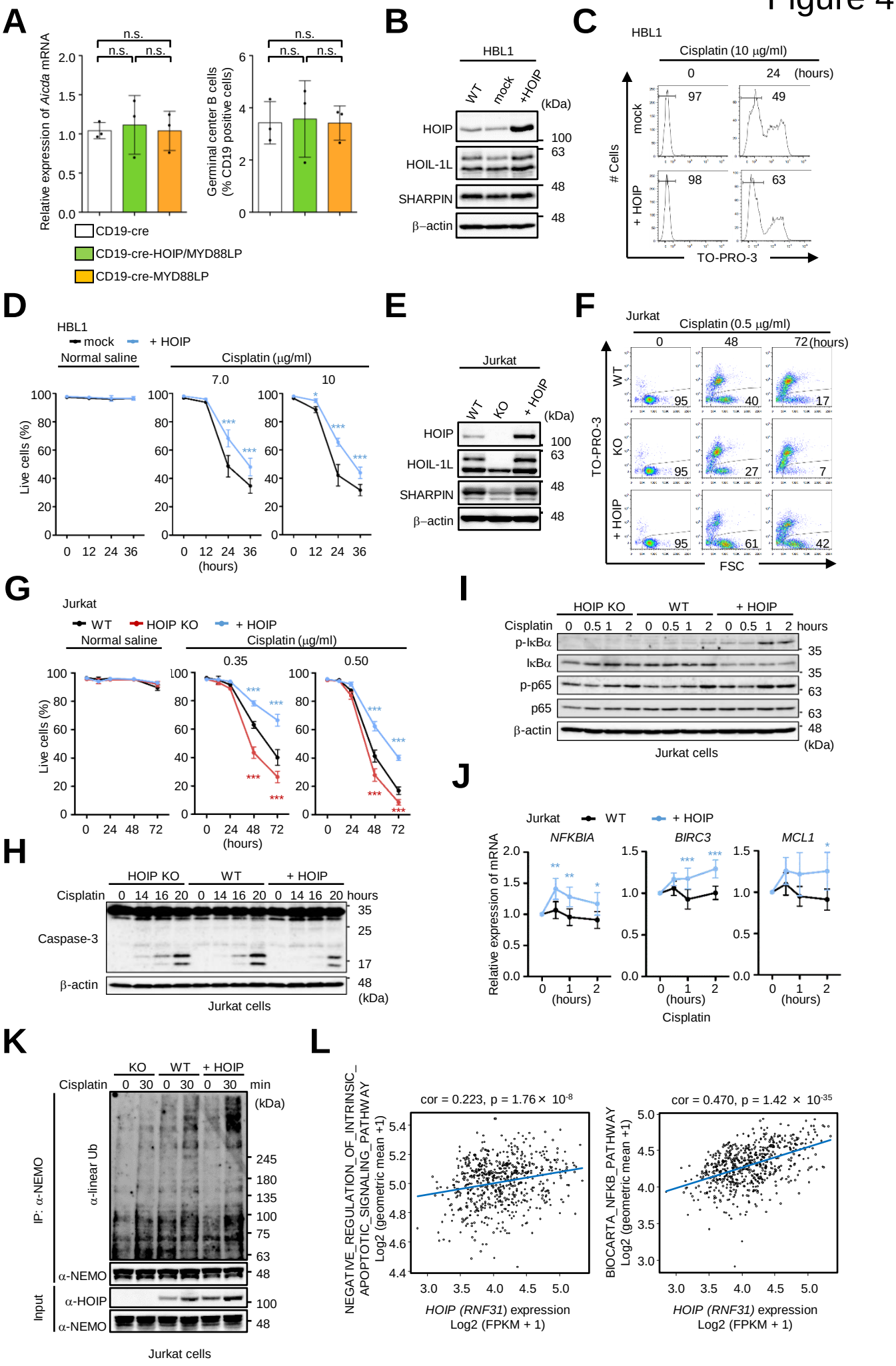
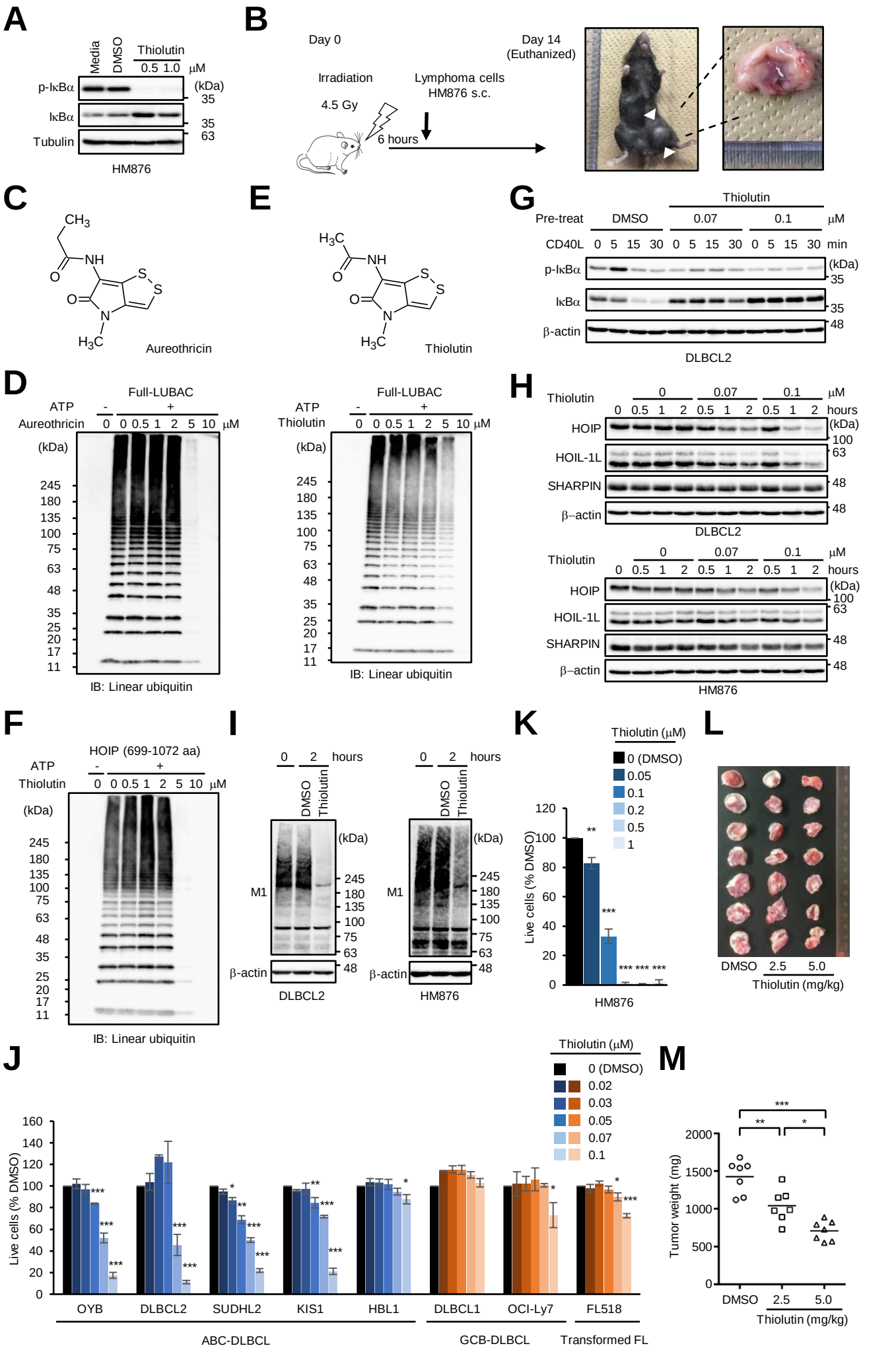

A
Known and predicted targets of aSHM in human DLBCL (Schmitz, 2018)
n = 108
Genes recurrently mutated in murine lymphomas (CD19-cre-HOIP/MYD88LP)
n = 23
100 genes
8 genes
15 genes
p = 2.29 × 10-13
Odds ratio = 106.8
B
Number of SNVs at C within WRCY (G within RGYW) per sample
Number of SNVs at C:G base pairs per sample
Number of transitions per sample
CD19-cre-HOIP/MYD88LP
CD19-cre-MYD88LP
C
Irf2bp2
Pim1
2kb region downstream from TSS
SNV type
Stopgain
Nonsynonymous SNV
Synonymous SNV
Number of SNVs
Genomic position (mm10, chr8)
Genomic position (mm10, chr17)
D
Number of non-synonymous mutations per sample
High (n = 24)
Low (n = 24)
HOIP (RNF31) expression
E
Number of SNVs at C within WRCY (G within RGYW) per sample
Number of SNVs at C:G base pairs per sample
Number of transitions per sample

Figure 4


A
Relative expression of Aicda mRNA
Germinal center B cells (% CD19 positive cells)
n.s.
CD19-cre
CD19-cre-HOIP/MYD88LP
CD19-cre-MYD88LP
B
HBL1
WT
mock
+HOIP
(kDa)
HOIP
HOIL-1L
SHARPIN
β–actin
C
HBL1
Cisplatin (10 μg/ml)
(hours)
mock
+ HOIP
# Cells
TO-PRO-3
D
HBL1
mock
+ HOIP
Normal saline
Cisplatin (μg/ml)
Live cells (%)
(hours)
E
Jurkat
WT
KO
+ HOIP
F
Jurkat
Cisplatin (0.5 μg/ml)
TO-PRO-3
FSC
G
Jurkat
WT
HOIP KO
+ HOIP
Normal saline
Cisplatin (μg/ml)
I
HOIP KO
WT
+ HOIP
Cisplatin
hours
p-IκBα
IκBα
p-p65
p65
β-actin
Jurkat cells
H
HOIP KO
WT
+ HOIP
Cisplatin
hours
Caspase-3
β-actin
Jurkat cells
J
Jurkat
WT
+ HOIP
NFKBIA
BIRC3
MCL1
Relative expression of mRNA
(hours)
Cisplatin
K
KO
WT
+ HOIP
Cisplatin
min
(kDa)
IP: α-NEMO
α-linear Ub
α-NEMO
Input
α-HOIP
α-NEMO
Jurkat cells
L
NEGATIVE_REGULATION_OF_INTRINSIC_APOPTOTIC_SIGNALING_PATHWAY
Log2 (geometric mean +1)
cor = 0.223, p = 1.76 × 10-8
HOIP (RNF31) expression
Log2 (FPKM + 1)
BIOCARTA_NFKB_PATHWAY
Log2 (geometric mean +1)
cor = 0.470, p = 1.42 × 10-35
HOIP (RNF31) expression
Log2 (FPKM + 1)

# Figure 5


A
Media
DMSO
Thiolutin
0.5 1.0 μM
p-IκBα
IκBα
Tubulin
(kDa)
35
35
63
HM876
B
Day 0
Irradiation
4.5 Gy
Lymphoma cells
HM876 s.c.
6 hours
Day 14
(Euthanized)
C
Aureothricin
E
Thiolutin
G
Thiolutin
Pre-treat
DMSO
0.07
0.1
μM
CD40L
0 5 15 30 0 5 15 30 0 5 15 30 min
p-IκBα
IκBα
β-actin
(kDa)
35
35
48
DLBCL2
D
Full-LUBAC
ATP
Aureothricin
0 0 0.5 1 2 5 10 μM
(kDa)
245
180
135
100
75
63
48
35
25
20
17
11
IB: Linear ubiquitin
Full-LUBAC
ATP
Thiolutin
0 0 0.5 1 2 5 10 μM
(kDa)
245
180
135
100
75
63
48
35
25
20
17
11
IB: Linear ubiquitin
H
Thiolutin
0
0.07
0.1
μM
0 0.5 1 2 0.5 1 2 0.5 1 2 hours
HOIP
HOIL-1L
SHARPIN
β–actin
(kDa)
100
63
48
48
DLBCL2
Thiolutin
0
0.07
0.1
μM
0 0.5 1 2 0.5 1 2 0.5 1 2 hours
HOIP
HOIL-1L
SHARPIN
β–actin
(kDa)
100
63
48
48
HM876
F
HOIP (699-1072 aa)
ATP
Thiolutin
0 0 0.5 1 2 5 10 μM
(kDa)
245
180
135
100
75
63
48
35
25
20
17
11
IB: Linear ubiquitin
I
0 2 hours
DMSO
Thiolutin
(kDa)
M1
245
180
135
100
75
63
β-actin
48
DLBCL2
0 2 hours
DMSO
Thiolutin
(kDa)
M1
245
180
135
100
75
63
β-actin
48
HM876
K
Thiolutin (μM)
0 (DMSO)
0.05
0.1
0.2
0.5
1
Live cells (% DMSO)
120
100
80
60
40
20
0
**
***
*** *** ***
HM876
L
DMSO
2.5
5.0
Thiolutin (mg/kg)
J
Thiolutin (μM)
0 (DMSO)
0.02
0.03
0.05
0.07
0.1
Live cells (% DMSO)
160
140
120
100
80
60
40
20
0
OYB
DLBCL2
SUDHL2
KIS1
HBL1
DLBCL1
OCI-Ly7
FL518
ABC-DLBCL
GCB-DLBCL
Transformed FL
M
Tumor weight (mg)
2000
1500
1000
500
0
***
**
*
DMSO
2.5
5.0
Thiolutin (mg/kg)

**Supplemental Information**

# LUBAC accelerates B-cell lymphomagenesis by conferring B cells resistance to genotoxic stress

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## Supplemental Methods

### Generation of tissue specific HOIP transgenic and MYD88 L252P transgenic mice

Tissue-specific HOIP transgenic mice (ROSA26-STOP-Hoip-ires-eGFP-pA) and MYD88 L252P transgenic mice (ROSA26-STOP-Myd88_L252P-ires-eGFP-pA) (Accession No. CDB 1320K: http://www2.clst.riken.jp/arg/mutant%20mice%20list.html) were established as follows: The cDNA encoding N-terminally HA-tagged murine HOIP or FLAG-tagged murine MYD88 L252P was subcloned into vector STOP-eGFP-ROSA26TV[1]. Bruce-4 ES cells derived from C57BL/6 embryos (for ROSA26-STOP-Hoip-ires-eGFP-pA) or TT2 ES cells derived from C57BL/6 × CBA F1 embryos (for ROSA26-STOP-Myd88_L252P-ires-eGFP-pA) transfected with the targeting vector were screened for homologous recombination. Homologous recombination at the 5' and 3' ends and single integration were confirmed by Southern blot analysis. The recombinant ES cells were injected into eight cell–stage Crl:ICR embryos to generate germline chimeras, and subsequent

chimeric breeding yielded ROSA26-STOP-Hoip-ires-eGFP-pA or ROSA26-STOP-Myd88_L252P-ires-eGFP-pA transgenic mice, which were then crossed with CD19-Cre mice.[2] Offspring were routinely genotyped by PCR with primers 5'-ACT GGA CCC AGC TAC CTT GTA TG-3' and 5'-GCA ATA TGG TGG AAA ATA AC-3' for the ROSA26-STOP-Hoip-ires-eGFP-pA allele, yielding a 367 bp product, and with primers 5'-GAC TAT ACC AAC CCT TGC AC-3' and 5'-CCT TGC TCA CCA TGG TTG TG-3' for the ROSA26-STOP-Myd88_L252P-ires-eGFP-pA allele, yielding a 783 bp product. Littermates were used in all subsequent experiments.

***In vitro* B-cell culture**

Splenic B cells were positively selected using anti-CD19 microbeads and a MACS Separation Column (Miltenyi Biotec); purity was > 90%. Purified splenic B cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 50 μM 2-ME, 10 mM HEPES-KOH (pH 7.4), and penicillin/streptomycin, and then stimulated with CpG-DNA (100 nM) (cat. no. tlrl-1826; InvivoGen) or Pam3CSK4 (1 μg/mL) (cat. no. tlrl-pms; InvivoGen) for the indicated times. For *in vitro* labeling, cells were incubated at 37°C for 10 minutes in RPMI1640 medium containing 5 μM Cell Trace Violet (Life Technologies), and then washed with RPMI medium containing 10% FBS. Labeled cells were cultured and exposed to various stimuli. After 72 hours, cell proliferation was measured by flow cytometry.

**Establishment of the HM876 cell line**

Lymphoma cells derived from the peritoneal cavity (T876) were seeded on 10 cm dishes in DMEM supplemented with 10% FBS, 50 μM 2-ME, 10 mM HEPES-KOH (pH 7.4),

and penicillin/streptomycin, and then incubated at 37°C in 5% $CO_2$. The medium was changed every 3–4 days. Continuously growing cell cultures were further passaged; frozen samples were prepared regularly from low passages.

**Cell lines**

HOIP KO Jurkat cells were established in our laboratory.[3] HBL1, OYB, DLBCL2, SUDHL2, KIS1,[4-6] DLBCL1, FL518, and Jurkat cells were cultured at 37°C in humidified air containing 5% $CO_2$ in RPMI medium (SIGMA) containing 10% FBS, 100 U/mL penicillin G, and 100 μg/mL streptomycin. OCI-Ly7[7] cells were cultured at 37°C in humidified air containing 5% CO2 in Iscove's modified Dulbecco's medium (SIGMA) containing 10% FBS, 100 U/mL penicillin G, and 100 μg/mL streptomycin.

**Lentiviral transduction of HBL1 and Jurkat cells**

Lentiviral transduction of HBL1 and Jurkat cells were performed as described previously.[8] Wild-type human *HOIP* cDNA was ligated into pCSII-EF-MCS-IRES2-Venus. The resultant plasmid was transfected into HEK293T cells along with pCMV-VSV-G-RSV-Rev and pMDLg/pRRE. After 12 hours, the culture medium was replaced with fresh DMEM containing 10 μM forskolin and incubated for 72 hours. Lentivirus in the culture supernatant was concentrated using a Lenti-X concentrator (Takara), and lentiviral titer was determined by measuring Venus expression. HBL1 or Jurkat cells were infected with lentivirus (multiplicity of infection = 10) in the presence of Polybrene (10 μg/mL). Infected Venus+ HBL1 or Jurkat cells were enriched using a FACSAria III cell sorter (BD Biosciences).

**Flow cytometry analysis**

Single-cell suspensions prepared from various lymphoid organs were stained with fluorochrome-conjugated antibodies. Flow cytometry data were acquired on a FACSCanto II (BD Biosciences), and the results were analyzed using the FlowJo software (Tree Star, Inc.). Antibodies used for analysis are listed below.

**BrdU proliferation assay**

BrdU was administered intraperitoneally to animals at 50 mg/kg, 1.5 hours prior to euthanasia. Splenocytes were stained with FxCycle Violet (Thermo Fisher) and fluorochrome-conjugated antibodies against BrdU (clone 3DE, cat. no. 364104; BioLegend), and then analyzed by FACSCanto II.

**Antibodies**

The following antibodies were used for flow cytometry analysis:

Biotinylated-anti-IgM (cat. no. 115-067-020; Jackson ImmunoResearch Laboratories), biotinylated-anti-CD21 (clone 7E9, cat. no. 123405; BioLegend), biotinylated-anti-Igκ (clone RMK-12, cat. no. 407203; BioLegend), streptavidin-APC (cat. no. 17-4317-82; eBioscience), streptavidin-PerCP (cat. no. 405213; BioLegend), PE-Cy7-anti-CD19 (clone 6D5, cat. no. 115520; BioLegend), PerCP-anti-B220 (clone RA3-6B2, cat. no. 103234 or 103224; BioLegend), PE-anti-IgD (clone 11-26, cat. no. 12-5993-81; eBioscience), APC-anti-Igλ (clone RML-42, cat. no. 407306; BioLegend), BV421-anti-CD5 (clone 53-7.3, cat. no. 562739; BD Biosciences), PE-anti-CD23 (clone B3B4, cat. no. 12-0232-82; eBioscience), PE-Cy7-anti-CD38 (clone 90, cat. no. 102718; BioLegend), and PE-anti-CD138 (clone 281-2, cat. no. 553714; BD Biosciences).

The following antibodies were used for immunoblot analysis: anti–mouse HOIP,[9] anti–human HOIP,[10] anti–HOIL-1L (clone 2E2, cat. no. MABC576; Merck Millipore), anti-SHARPIN (cat. no. ABF128; Merck Millipore), anti-MYD88 (cat. no. 4283; Cell Signaling Technology), anti–phospho-IκBα (cat. no. 9246; Cell Signaling Technology), anti-IκBα (cat. no. 4812; Cell Signaling Technology), anti–caspase-3 (cat. no. 9662; Cell Signaling Technology), anti-ubiquitin (clone P4D1, cat. no. sc-8017; Santa Cruz Biotechnology), anti–K48 polyubiquitin (clone Apu2, cat. no. 05-1307; Merck Millipore), anti–K63 polyubiquitin (clone Apu3, cat. no. 05-1308; Merck Millipore), anti–linear polyubiquitin (LUB9),[11] anti–linear polyubiquitin (clone 1E3, cat. no. MABS199; Merck Millipore), anti–phospho-NF-κB p65 (cat. no. 3033; Cell Signaling Technology), anti–NF-κB p65 (cat. no. sc-109; Santa Cruz Biotechnology), anti–β-actin (cat. no. A5316; Sigma-Aldrich), anti-NEMO (cat. no. KO159-3; MBL), anti–β-tubulin (cat. no. CLT9002; CEDARLANE), and anti-HA (clone Tana2, cat. no. M180-3; MBL).

The following antibodies were used for immunohistochemical staining: Anti-Irf4 (cat. no. 11247-2-AP; Proteintech), and anti-Bcl6 (clone D-8, cat. no. sc-7388; Santa Cruz Biotechnology).

**Histology**

Tissues were fixed with 4% paraformaldehyde, followed by paraffin embedding. Sections were prepared and stained with hematoxylin and eosin (H&E) and antibodies against Irf4, and Bcl6, as described previously.[12]

**Molecular analysis of tumor clonality**

Clonally rearranged V(D)J sequences of immunoglobulin heavy chains were amplified

from genomic DNA extracted from tumor tissue by nested PCR, as previously described.[13] A total of 100 ng of genomic DNA was used as the starting template for the first round of nested PCR. Initial reactions consisted of 2.5 µl of 10× *Taq* buffer (Qiagen), 2 mM $MgCl_2$, 100 nM dNTP mix, 0.5 nM VH-specific primer 1, 0.5 nM JH universal primer[13] (supplementary Table 10), 1.25 U of *Taq* DNA polymerase (Qiagen), 5 µl of 5× Q-solution (Qiagen), and $H_2O$ to a total volume of 25 µl. Thermal cycler conditions were as follows: 5 minutes at 96°C; 30 cycles of 96°C for 30 sec, 60°C for 30 sec, and 72°C for 1 minute; 5 minutes at 72°C; and 4°C hold. One microliter of the initial reaction was used as the template for the second round of PCR, which was conducted as described[13] except that VH-specific primer 1 was replaced by VH-specific primer 2, and one of four JH-specific primers (supplementary Table 10) was used instead of the JH universal primer. Thus, four independent second-round PCR amplifications, using each JH-specific primer individually, were conducted to isolate V(D)J rearrangements. PCR products were purified by gel purification and sequenced using the Big Dye Terminator Cycle sequencing kit (Applied Biosystems). Sequence alignment was performed using IgBLAST (http://www.ncbi.nlm.nib.gov/igblast). Primers were designed to anneal to tumor-specific complement-determining region 3 (CDR3). If specific bands were detected after PCR with 30 cycles, the V(D)J rearrangement was considered clonal.

**IgH somatic mutation analysis**

IgH-V gene rearrangements were PCR amplified using the PrimeSTAR Max DNA Polymerase (Takara) and forward and reverse primers designed in the process of analysis of tumor clonality described above. PCR products were purified by gel purification and sequenced by the Big Dye Terminator Cycle sequencing kit (Applied Biosystems).

Sequence alignment was performed using IgBLAST (http://www.ncbi.nlm.nib.gov/igblast) to determine $V_HD_HJ_H$ usage. The sequences were then aligned to their germline counterparts.

**Cell death and viability assays**

HBL1 cells and Jurkat cells were seeded at $5 \times 10^4$ cells per well in 24-well plates. Cells were pre-incubated for a minimum of 8 hours, and then exposed to the indicated concentration of cisplatin for 12, 24, or 36 hours (HBL1), or 12, 24, 48, or 72 hours (Jurkat). Purified splenic B cells were seeded at $2 \times 10^5$ cells per well in 96-well plates, and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 50 μM 2-ME, 10 mM HEPES-KOH (pH 7.4), and penicillin/streptomycin, stimulated with an anti-CD40 antibody (10 μg/ml) (HM40-3) (eBioscience) for 24 hours, and then exposed to the indicated concentration of cisplatin for 24 hours. Dead cells were labeled with TO-PRO-3 (Thermo Fisher), and the proportion of live cells was calculated from the percentage of TO-PRO-3–negative cells, as determined on a FACSCanto II.

**Quantitative RT-PCR (QPCR) analysis**

Total RNA was isolated using the RNeasy Micro or Mini Kit (Qiagen). DNase-treated RNA (20–200 ng) was reverse-transcribed using the high-capacity RNA-to-cDNA Kit (Applied Biosystems). Real-time PCR was performed using Power SYBR Green PCR master mix (Applied Biosystems) on an ABI 7900 Real-time PCR system (Applied Biosystems). All gene expression levels were normalized against the corresponding levels of β-actin mRNA. qPCR primer sequences were shown in supplemental Table 8.

## Immunoblotting

Cells were lysed in lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 2 mM phenylmethylsulfonyl fluoride (PMSF), protease inhibitor cocktail (Sigma-Aldrich), and phosphatase inhibitor cocktail (Nacalai Tesque). Lysates were centrifuged at 15,000 rpm for 20 minutes at 4°C, and the supernatant was used in subsequent steps. Samples were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes. After blocking in Tris-buffered saline containing 0.1% Tween 20 and 5% (w/v) nonfat dry milk, the membranes were immunoblotted with the appropriate primary antibodies, followed by the corresponding secondary antibodies. The membranes were visualized by enhanced chemiluminescence and analyzed on LAS3000 or LAS4000mini instrument (GE Healthcare). The following antibodies were used in immunoblotting assays:

## Tandem Ubiquitin Binding Entity (TUBE) assay

Halo-tagged linear ubiquitin chain specific Tandem Ubiquitin Binding Entity (M1-specific TUBE) was purified as described previously.[14,15] To measure linear ubiquitination in splenic B cells or Jurkat cells, 200 μg of cell lysates were subjected to incubation with 2 μg of M1-specific TUBE coupled with 20 μl of equilibrated Magne Halo Tag beads (Promega) at 4°C for 4 hours in 300 μl of buffer 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100. The precipitates were washed three times with the same buffer, boiled in sodium dodecyl sulfate sample buffer, and analyzed by immunoblotting.

**Immunoprecipitation of NEMO**

Jurkat cells were treated with cisplatin (5 μg/mL) at 37°C for the periods indicated in Figure 3J and lysed on ice for 20 minutes in lysis buffer containing 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1% Triton X-100, 2 mM PMSF, protease inhibitor cocktail (Sigma-Aldrich), phosphatase inhibitor cocktail (Nacalai Tesque), and 10 mM *N*-ethylmaleimide. Cysteine was added to a final concentration of 15 mM to neutralize the *N*-ethylmaleimide. The lysates were centrifuged at 15,000 rpm for 20 minutes at 4°C, and the supernatant was used in subsequent steps. Lysates were incubated with anti-NEMO antibody (Santa Cruz Biotechnology, sc-8330) for 60 minutes at 4°C, and then immobilized on rmp-Protein A–Sepharose beads (GE Healthcare). The beads were washed five times with lysis buffer. NEMO was eluted with sample buffer and analyzed by immunoblotting.

**Analysis of European Genome–phenome Archive and The Cancer Genome Atlas datasets**

Clinical and RNA sequencing (RNA-seq) gene expression data derived from the core set of 624 human DLBCL samples were obtained from EGA (dataset ID: EGAD00001003600).[16] Gene expression was measured in terms of fragments per kilobase of exon per million fragments mapped (FPKM) and normalized using the Cufflinks package (version 2.2.1). Quantile normalization was performed, and the data were $\log_2$ normalized. The Cancer Genome Atlas (TCGA) whole exome sequencing and RNA-seq data of 48 DLBCL samples (project ID: TCGA-DLBC) were obtained from the Broad Institute Firehose (http://gdac.broadinstitute.org/).[17,18] Mutation consequences were annotated using Oncotator (version 1.8.0), and single nucleotide polymorphisms (SNPs) that had minor allele frequency of 0.01 in the 1000 Genomes Phase 3 data were

removed.

**Defining correlated gene signatures**

Gene signatures were defined using a collection of widely used annotated gene set databases (BioCarta, Gene Ontology). To obtain better signal-to-noise estimates, genes whose expression data included FPKM value < 0.01 in any samples were excluded. Subsequently, BIOCARTA_NFKB_PATHWAY signature, and NEGATIVE_REGULATION_OF_INTRINSIC_APOPTOTIC_SIGNALING_PATHWAY signature were calculated as the geometric mean (log-average) of the expression of 22, and 66, as shown in supplemental Tables 2–4. Classification of DLBCL into ABC and GCB subtypes by RNA-seq were based on a previous report.[16]

**Survival analysis**

To assess the effects of *HOIP* expression on the survival of patients with DLBCL, we analyzed data from two independent cohorts of DLBCL patients with available clinical information and RNA-seq data on *HOIP* expression; Reddy, 2017 (n = 604),[16] and Schmitz, 2018 (n = 234).[19] If two Kaplan-Meier curves crossed early (≦18 months), differences between survival functions were examined by the log-rank test based on observations after the crossing point. For the cohort of Reddy, 2017, we analyzed overall survival following diagnosis of n = 102 patients who did not achieve complete response to the initial chemotherapy, and for the cohort of Schmitz, 2018, we analyzed survival after disease progression or relapse of n = 62 patients.

**B cell gene expression profiling**

Gene expression profiling data of flow-sorted B-cell subsets in human bone marrow was obtained at the Gene Expression Omnibus at the National Center for Biotechnology Information, Bethesda, USA (https://www.ncbi.nlm.nih.gov, GEO profiles/DATA sets) (dataset ID: GSE68878),[20] and analyzed using the integrated GEO2R tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/).

**Preparation of purified recombinant proteins**

Because it was difficult to obtain a sufficient quantity of LUBAC for high-throughput screening (HTS), two truncated subcomplexes of LUBAC were generated: Petit-LUBAC, which consists of residues 1–191 of human HOIL-1L and residues 474–1072 of human HOIP; and Petit-SHARPIN, which consists of residues 474–1072 of human HOIP (aa) and residues 172–346 of human SHARPIN.[3,10,21] Both Petit-LUBAC and Petit-SHARPIN can be expressed in a bacterial expression system and purified, and both proteins exhibit potent linear polyubiquitination activity. Recombinant Petit-LUBAC, Petit-SHARPIN, full-LUBAC (consisting of HOIP, HOIL-1L, SHARPIN, and HOIP [aa 699–1072]), E1, UbcH5c, UbcH7, and ubiquitin were prepared as described previously.[3,10,21] Briefly, pT7-7 was used to purify recombinant N-terminally FLAG-His–tagged ubiquitin, C-terminally glutathione S-transferase (GST)-tagged ubiquitin, UbcH5c, and UbcH7. pET Duet1 was used to purify Petit-LUBAC and Petit-SHARPIN. pVL1393 was used to purify full-LUBAC. FAST Bac vector was used to purify recombinant E1.

**Compound libraries**

The following libraries were screened: NPDepo (19,449 compounds, RIKEN, Japan); the MyriaScreen chemical library (10,000 compounds, Sigma-Aldrich, St. Louis, MO, USA);

LOPAC library (713 compounds, Sigma-Aldrich), the SPECS synthetic compound library (10,000 compounds, Specs, Netherlands), the NAMIKI synthetic compound library (318 compounds, NAMIKI, Japan), and the Isolated Natural Compound Library (1280 compounds, AIST, Japan).

**AlphaScreen binding assay for LUBAC inhibitors**

To search for inhibitors of linear polyubiquitination, an AlphaScreen-based HTS system was established using N-terminally FLAG-His–tagged ubiquitin (FLAG-Ub), C-terminally glutathione S-transferase (GST)-tagged ubiquitin (Ub-GST), ubiquitin-activating enzyme E1, UbcH7 as the E2 ubiquitin–conjugating enzyme, and Petit-LUBAC or Petit-SHARPIN as the E3 ubiquitin ligase. The candidate compounds were transferred to an AlphaPlate-384 (Perkin Elmer) with the following final concentrations: 10 μg/mL for NPDepo, 20 μM for MyriaScreen and LOPAC, 50 μM for SPECS, and 20 μg/mL for NAMIKI and the isolated natural compound library.

Ubiquitination reactions contained 100 ng of E1, 200 ng of E2/UbcH7, 1 μg of E3 (Petit-LUBAC or Petit-SHARPIN), 1 μg of FLAG-Ub, 250 ng of Ub-GST, and 2 mM ATP in 15 μl of buffer containing 20 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 1 mM DTT, and 0.01% (v/v) Tween20. ATP was added last to minimize autocatalytic ubiquitination by the ubiquitination enzymes. Reactions were allowed to proceed at 30°C for 0.5 hours (Petit-LUBAC) or 2–6 hours (Petit-SHARPIN).

After the ubiquitination reactions, AlphaScreen Glutathione Donor Beads (Perkin Elmer) and FLAG Detection Kit (Perkin Elmer) (both at a final concentration of 16 μg/mL for petit-LUBAC and 20 μg/mL for petit-SHARPIN) in 10 μl of 1× PBS buffer containing 5 mM EDTA-4Na were added. Plates were incubated at 23°C for 1 hour, laser

excitations were carried out at 680 nm, and readings were performed at 520–620 nm using the EnSpire Alpha plate reader (Perkin Elmer). Primary screening data from the AlphaScreen HTS assays were processed as follows: (1) Z', signal/background ratio (S/B), and coefficient of variation (CV) were calculated and compared with the minimum pass criteria (Z' > 0.5, S/B ratio > 2, CV < 20%); (2) the primary hits for the AlphaScreen assays were classified as compounds that led to a decrease in the normalized assay signal: > 60% (for Petit-LUBAC) or > 70% (for Petit-SHARPIN) for compounds from the NPDepo library; > 50% (for Petit-LUBAC) or > 60% (for Petit-SHARPIN) for compounds from the MyriaScreen library, and > 80% (for Petit-LUBAC) or > 80% (for Petit-SHARPIN) for compounds from other libraries. Based on these criteria, 568 hit compounds (NPDepo: 291; MyriaScreen: 223; LOPAC: 1; SPECS: 34; NAMIKI: 0; isolated natural compounds: 19) were identified. These compounds were tested for false-positive hits using the AlphaScreen TruHits kit (Perkin Elmer). Compounds with $IC_{50}$ > 20 μM were excluded. Finally, compounds that were immediately available were tested for inhibition of linear ubiquitination using *in vitro* ubiquitination assay.

**_In vitro_ ubiquitination assay**

E1 (5 μg/mL), E2/UbcH5 (10 μg/mL), each E3 (5 μg/mL for full-LUBAC; 50 μg/mL for Parkin, Nedd4, and cIAP2; and 60 μg/mL for HOIP [aa 699–1072]), and 250 μg/mL ubiquitin (SIGMA) were incubated at 37°C for 1 hour (full-LUBAC, HOIP [aa 699–1072], and cIAP2) or 3 hours (Parkin and Nedd4) in buffer containing Tris-HCl (pH 7.5) (50 mM for full-LUBAC, Parkin, Nedd4, and cIAP1/2; and 20 mM for HOIP [aa 699–1072]), 5 mM $MgCl_2$, 1 mM DTT, and 2 mM ATP. Ubiquitination reaction products were probed with anti-ubiquitin antibody or anti–linear polyubiquitin monoclonal antibody.

## Cell viability assay

Cells were seeded in 96-well flat-bottom plates at $5 \times 10^4$ cells/mL and treated with serial dilutions of the LUBAC inhibitor thiolutin or DMSO (as a solvent control). After incubation for 48 hours, cell viability was determined using the Cell Counting Kit-8 (Dojindo Laboratories). Cell treatment and viability analyses were performed in triplicate.

## Statistical analysis

Statistical analyses were performed using Prism 5 and R (https://www.r-project.org). Statistical tests included two-tailed unpaired Student's *t*-test, one-way ANOVA with Turkey's post hoc test, two-way ANOVA with Bonferroni post hoc test, the log–rank test, Fisher's exact test, binomial test, and the Brunner–Munzel test. Specific tests are identified in the respective figures. Differences are indicated as n.s. (not significant; $p > 0.05$), * $p < 0.05$, ** $p < 0.01$, or *** $p < 0.001$, unless indicated otherwise in the figures.

## References


1. Sasaki Y, Derudder E, Hobeika E, et al. Canonical NF-κB activity, dispensable for B cell development, replaces BAFF-receptor signals and promotes B cell proliferation upon activation. *Immunity*. 2006;24(6):729-739.

2. Rickert RC, Roes J, Rajewsky K. B lymphocyte-specific, Cre-mediated mutagenesis in mice. *Nucleic Acids Res*. 1997;25(6):1317-1318.

3. Sakamoto H, Egashira S, Saito N, et al. Gliotoxin suppresses NF-κB activation by selectively inhibiting linear ubiquitin chain assembly complex (LUBAC). *ACS Chem Biol*. 2015;10(3):675-681.

4. Kamesaki H, Miwa H, Ohno Y, et al. A novel B cell line established from Ki-1-positive diffuse large cell lymphoma. *Jpn J Cancer Res*. 1988;79(11):1193-1200.

5. Ohno H, Furukawa T, Fukuhara S, et al. Molecular analysis of a chromosomal translocation, t(9;14)(p13;q32), in a diffuse large-cell lymphoma cell line expressing the Ki-1 antigen. *Proc Natl Acad Sci USA*. 1990;87(2):628-632.

6. Ohno H, Nakagawa M, Kishimori C, et al. Diffuse large B-cell lymphoma carrying t(9;14)(p13;q32)/PAX5-immunoglobulin heavy chain gene is characterized by nuclear positivity of MUM1 and PAX5 by immunohistochemistry. *Hematol Oncol*. 2020.

7. Mehra S, Messner H, Minden M, Chaganti RS. Molecular cytogenetic characterization of non-Hodgkin lymphoma cell lines. *Genes Chromosomes Cancer*. 2002;33(3):225-234.

8. Sasaki K, Himeno A, Nakagawa T, Sasaki Y, Kiyonari H, Iwai K. Modulation of autoimmune pathogenesis by T cell-triggered inflammatory cell death. *Nat Commun*. 2019;10(1):3878.

9. Tokunaga F, Sakata S, Saeki Y, et al. Involvement of linear polyubiquitylation of

353 NEMO in NF-κB activation. *Nat Cell Biol*. 2009;11(2):123-132.
354 10. Kirisako T, Kamei K, Murata S, et al. A ubiquitin ligase complex assembles
355 linear polyubiquitin chains. *EMBO J*. 2006;25(20):4877-4887.
356 11. Sasaki Y, Sano S, Nakahara M, et al. Defective immune responses in mice
357 lacking LUBAC-mediated linear ubiquitination in B cells. *EMBO J*. 2013;32(18):2463-
358 2476.
359 12. Toda Y, Kono K, Abiru H, et al. Application of tyramide signal amplification
360 system to immunohistochemistry: a potent method to localize antigens that are not
361 detectable by ordinary method. *Pathol Int*. 1999;49(5):479-483.
362 13. Kline GH, Hayden TA, Riegert P. The initiation of B cell clonal expansion occurs
363 independently of pre-B cell receptor formation. *J Immunol*. 2001;167(9):5136-5142.
364 14. Hjerpe R, Aillet F, Lopitz-Otsoa F, Lang V, England P, Rodriguez MS. Efficient
365 protection and isolation of ubiquitylated proteins using tandem ubiquitin-binding entities.
366 *EMBO Rep*. 2009;10(11):1250-1258.
367 15. van Wijk SJ, Fiskin E, Dikic I. Selective monitoring of ubiquitin signals with
368 genetically encoded ubiquitin chain-specific sensors. *Nat Protoc*. 2013;8(7):1449-1458.
369 16. Reddy A, Zhang J, Davis NS, et al. Genetic and Functional Drivers of Diffuse
370 Large B Cell Lymphoma. *Cell*. 2017;171(2):481-494 e415.
371 17. Grossman RL, Heath AP, Ferretti V, et al. Toward a Shared Vision for Cancer
372 Genomic Data. *N Engl J Med*. 2016;375(12):1109-1112.
373 18. Lohr JG, Stojanov P, Lawrence MS, et al. Discovery and prioritization of somatic
374 mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. *Proc
375 Natl Acad Sci USA*. 2012;109(10):3879-3884.
376 19. Schmitz R, Wright GW, Huang DW, et al. Genetics and Pathogenesis of Diffuse

Large B-Cell Lymphoma. *N Engl J Med*. 2018;378(15):1396-1407.

20. Petri A, Dybkaer K, Bogsted M, et al. Long Noncoding RNA Expression during Human B-Cell Development. *PLoS One*. 2015;10(9):e0138236.

21. Tokunaga F, Nakagawa T, Nakahara M, et al. SHARPIN is a component of the NF-κB-activating linear ubiquitin chain assembly complex. *Nature*. 2011;471(7340):633-636.

**Supplemental Tables**

**Supplemental Table 1.** RNA-seq data derived from EGAD00001003600

**Supplemental Table 2.** Source of gene sets

**Supplemental Table 3.** Gene of BIOCARTA_NFKB_PATHWAY and NEGATIVE_REGULATION_OF_INTRINSIC_APOPTOTIC_SIGNALING_PATHWAY

**Supplemental Table 4.** Whole-exome sequencing data and RNA-seq data derived from TCGA-DLBC

**Supplemental Table 5.** Murine homologue of previously reported driver genes in human DLBCL

**Supplemental Table 6.** Murine homologue of previously known and predicted hypermutated genes in human DLBCL

**Supplemental Table 7.** Previously reported AID target genes in mice

**Supplemental Table 8.** Enrichment of WRCY mutations in genes mutated in lymphomas derived from transgenic mice

**Supplemental Table 9.** Analysis of somatic mutations in clonal IgH rearrangements of DLBCL-like lymphomas derived from transgenic mice

**Supplemental Table 10.** Primers for testing clonality of IgH V(D)J

**Supplemental Table 11.** Primer sequences used for real-time PCR

**Supplemental Table 1**

**LUBAC accelerates B-cell lymphomagenesis by conferring B cells resistance to genotoxic stress**

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**Supplemental Table 1. RNA-seq data derived from EGAD00001003600**

For each DLBCL patient sequenced, clinical features are shown. The **"Signatures"** section shows the geometric mean of the expression of genes involved in each pathway (see also supplemental Tables 2 and 3). Blank fields indicate that a measurement was not taken or was irrelevant to the patient.

| | | | | | | Signatures | | |
|---|---|---|---|---|---|---|---|---|
| Sample ID | Cell Of Origin by RNA-Seq | HOIP (RNF31) expression (log2[fpkm+1]) | HOIL1L (RBCK1) expression (log2[fpkm+1]) | SHARPIN expression (log2[fpkm+1]) | OTULIN expression (log2[fpkm+1]) | BIOCARTA_NFKB_PATHWAY | NEGATIVE_REGULATION_OF_INTRINSIC_APOPTOTIC_SIGNALING_PATHWAY | COO |
| 648 | Unclassified | 4.195717649 | 4.31519581 | 3.277641923 | 4.956514297 | 4.614161432 | 5.087244089 | 2 |
| 658 | GCB | 4.092387126 | 3.898355631 | 3.100934845 | 5.342089921 | 4.443884255 | 5.147378636 | 0 |
| 683 | ABC | 5.004024657 | 4.921808994 | 3.46800145 | 4.57766278 | 4.395643052 | 5.073229173 | 1 |
| 684 | GCB | 3.763522925 | 3.707060358 | 2.787447028 | 5.519449818 | 4.318039406 | 5.16707185 | 0 |
| 689 | ABC | 4.384255027 | 4.252816353 | 2.735319439 | 5.513209754 | 4.4557751 | 5.002923748 | 1 |
| 690 | Unclassified | 4.005425217 | 3.898355631 | 2.92274059 | 5.883301336 | 4.070451909 | 5.05442557 | 2 |
| 695 | GCB | 4.739987864 | 5.08722852 | 2.997202359 | 5.222936525 | 4.516688257 | 4.897774993 | 0 |
| 702 | GCB | 3.469948533 | 3.690909115 | 2.867809935 | 4.968035071 | 4.06139149 | 5.010415253 | 0 |
| 704 | ABC | 3.704276839 | 3.770049876 | 3.109557367 | 5.136238378 | 4.368531662 | 5.041074611 | 1 |
| 705 | GCB | 3.676195688 | 4.118120389 | 3.250321036 | 6.266936066 | 3.922399202 | 4.630682268 | 0 |
| 759 | ABC | 4.724882154 | 4.992352289 | 3.818200628 | 3.724416657 | 4.539771007 | 5.193364216 | 1 |
| 787 | ABC | 3.858287194 | 3.932834051 | 3.327465928 | 4.854269929 | 4.111559511 | 5.070042274 | 1 |
| 793 | ABC | 3.201302555 | 3.601592665 | 3.012642894 | 4.731683197 | 4.291487947 | 4.976847885 | 1 |
| 799 | Unclassified | 3.361162098 | 2.922018394 | 3.304065558 | 4.943040856 | 3.688718846 | 4.856810953 | 2 |
| 800 | ABC | 3.791105901 | 3.886273142 | 2.817437442 | 5.04368148 | 4.408336728 | 4.884653479 | 1 |
| 813 | ABC | 3.909467611 | 4.386568773 | 2.829095917 | 4.412480107 | 4.561161571 | 5.02626163 | 1 |
| 816 | ABC | 5.206608081 | 4.944309794 | 4.599632141 | 4.226338935 | 4.630389193 | 5.142343577 | 1 |
| 823 | ABC | 4.803982209 | 4.572335379 | 4.267855087 | 5.271658475 | 4.593382271 | 5.219421893 | 1 |
| 829 | ABC | 4.424910334 | 4.515693734 | 3.562500405 | 5.612903283 | 3.825286912 | 4.871292938 | 1 |
| 830 | ABC | 4.077833144 | 4.61063381 | 3.308286428 | 4.735105773 | 4.534273467 | 5.071999236 | 1 |
| 831 | GCB | 3.564386475 | 3.580399279 | 3.043617231 | 4.744531962 | 4.269243128 | 5.040704604 | 0 |
| 1008 | GCB | 3.400851219 | 3.475116413 | 2.837774914 | 6.061943906 | 4.116286614 | 4.989077553 | 0 |
| 1016 | GCB | 3.451793152 | 3.835169616 | 2.802980803 | 5.14881689 | 4.370900222 | 5.170748226 | 0 |
| 2043 | GCB | 5.052482388 | 4.788924404 | 4.212810172 | 5.79812658 | 4.276528485 | 4.872723623 | 0 |
| 2044 | ABC | 5.01380854 | 4.955677489 | 4.586447377 | 5.857875643 | 4.068806649 | 5.053505628 | 1 |
| 2045 | GCB | 4.224045569 | 4.820167832 | 3.496950094 | 5.203682858 | 4.310011749 | 4.853711449 | 0 |
| 2046 | Unclassified | 4.420214608 | 4.676746017 | 3.790473173 | 5.487265113 | 4.414159889 | 5.065319985 | 2 |
| 2047 | GCB | 4.288595033 | 4.546087557 | 3.941239784 | 5.984761383 | 4.373861161 | 4.801339811 | 0 |
| 2048 | GCB | 4.873401354 | 4.797385513 | 3.786090824 | 5.9770724 | 4.434605644 | 5.067935478 | 0 |
| 2057 | GCB | 4.781318677 | 5.064831481 | 3.808528441 | 5.074649601 | 4.535576642 | 5.352719476 | 0 |
| 2060 | GCB | 4.872152713 | 4.960800222 | 3.778063863 | 6.778105628 | 4.221656273 | 5.107111867 | 0 |
| 2072 | Unclassified | 4.840459425 | 5.808458312 | 3.838297801 | 5.972307807 | 4.57493274 | 4.936889033 | 2 |
| 2074 | ABC | 4.270693637 | 4.10831842 | 3.200650522 | 6.618631543 | 4.117870754 | 4.986115237 | 1 |
| 2075 | GCB | 4.682662876 | 5.482388672 | 5.146528312 | 4.330507022 | 4.562921307 | 4.921871084 | 0 |
| 2076 | GCB | 4.827167061 | 5.592195874 | 4.367302062 | 5.92430143 | 4.648262495 | 5.162684013 | 0 |
| 2078 | Unclassified | 4.095183016 | 4.786635557 | 3.855179987 | 4.137964507 | 4.234166871 | 4.947964946 | 2 |
| 2079 | GCB | 4.061624264 | 4.197258663 | 3.004287375 | 5.246849507 | 4.535740571 | 5.20118803 | 0 |
| 2080 | GCB | 4.360340965 | 4.708384743 | 3.772532367 | 4.49726199 | 4.348586048 | 5.067459855 | 0 |
| 2081 | ABC | 3.492202499 | 3.881289201 | 3.302094252 | 5.363927327 | 3.85914941 | 4.917344835 | 1 |
| 2084 | GCB | 3.984409398 | 3.445409 | 2.737599776 | 6.368712923 | 4.500009492 | 5.046654019 | 0 |
| 2085 | ABC | 3.2411119 | 3.991879203 | 2.282110364 | 5.979924179 | 3.878403093 | 4.616291747 | 1 |
| 2087 | ABC | 4.305376149 | 5.165925437 | 3.806987067 | 5.020316576 | 4.108682704 | 4.921288973 | 1 |
| 2088 | ABC | 3.166365158 | 4.263565685 | 3.317701231 | 4.934042802 | 3.995994794 | 4.664630489 | 1 |
| 2089 | Unclassified | 4.882975353 | 4.70978644 | 3.65477259 | 6.094017109 | 4.51460595 | 5.112486785 | 2 |
| 2091 | GCB | 4.078604791 | 4.041997226 | 3.368602846 | 5.957217133 | 4.318144805 | 5.043265305 | 0 |
| 2092 | GCB | 3.73300487 | 3.94991838 | 3.052886148 | 5.720174737 | 4.11430249 | 5.087951525 | 0 |
| 2093 | ABC | 3.648250318 | 4.241074585 | 2.894748459 | 5.189012409 | 4.395900744 | 4.954370233 | 1 |
| 2095 | Unclassified | 3.630010348 | 3.759158788 | 2.905950836 | 5.442321234 | 4.052617699 | 5.122827141 | 2 |
| 2097 | GCB | 3.71051805 | 4.104241445 | 2.918107457 | 5.981880481 | 4.245796801 | 5.080369296 | 0 |
| 2110 | GCB | 4.526718447 | 4.648788793 | 3.737727173 | 6.274733118 | 4.601314054 | 5.082345587 | 0 |
| 2112 | ABC | 4.746083244 | 4.322697373 | 3.573756472 | 5.258534024 | 4.590030272 | 5.220053807 | 1 |
| 2115 | ABC | 4.511175399 | 4.191219895 | 3.724416657 | 6.226341193 | 4.074984502 | 4.730955257 | 1 |
| 2122 | ABC | 3.428095667 | 3.978185584 | 3.034967696 | 6.290208883 | 4.191567621 | 4.936708015 | 1 |
| 2125 | GCB | 4.459943869 | 4.579059532 | 3.663858652 | 5.630217963 | 4.25523799 | 4.779373821 | 0 |
| 2126 | ABC | 4.27162497 | 4.56147475 | 4.331152529 | 5.201259568 | 4.318184549 | 5.029297736 | 1 |
| 2127 | Unclassified | 4.281446986 | 4.413146788 | 4.185881167 | 6.061943906 | 4.54772804 | 5.060628585 | 2 |
| 2128 | GCB | 5.134316748 | 4.787413284 | 4.367617312 | 5.137212562 | 4.241101193 | 4.914477494 | 0 |
| 2130 | GCB | 3.639180094 | 3.541263026 | 2.781471543 | 6.333516965 | 4.337611261 | 5.141039346 | 0 |
| 2141 | Unclassified | 4.42624329 | 4.759696639 | 3.09888476 | 4.622377455 | 4.648903636 | 4.939897509 | 2 |
| 2142 | GCB | 3.592034285 | 3.887869226 | 3.086599656 | 5.540666836 | 4.386661944 | 5.118110493 | 0 |
| 2147 | GCB | 4.333059477 | 4.849936944 | 3.626283426 | 5.601994529 | 4.728293014 | 5.071908877 | 0 |
| 2148 | ABC | 3.915418308 | 4.286353339 | 3.478321834 | 4.911183902 | 4.524358298 | 5.193710006 | 1 |
| 2149 | ABC | 3.866406497 | 4.588539878 | 3.275347265 | 4.670793182 | 4.299921218 | 5.164643059 | 1 |
| 2150 | ABC | 4.732067115 | 4.523976149 | 3.408235623 | 4.853463978 | 4.514378867 | 4.947765012 | 1 |
| 2153 | ABC | 4.26618951 | 3.943379427 | 3.184268699 | 5.252376895 | 4.114658524 | 5.046788368 | 1 |
| 2155 | ABC | 3.680181959 | 3.458236209 | 2.776588845 | 5.193444197 | 4.182282928 | 4.968581078 | 1 |
| 2156 | ABC | 3.497912317 | 3.388017347 | 2.415514368 | 6.708262699 | 3.997463784 | 4.832996513 | 1 |
| 2160 | ABC | 4.591017582 | 4.830633415 | 3.689025851 | 4.948062995 | 4.107513537 | 5.033980494 | 1 |
| 2191 | Unclassified | 4.546087557 | 4.710144652 | 4.087308038 | 5.119069315 | 4.54913414 | 5.135472264 | 2 |
| 2192 | ABC | 5.090344067 | 5.060883367 | 4.217271139 | 4.524663199 | 4.838161184 | 5.074063813 | 1 |
| 2193 | GCB | 4.276841004 | 4.831415658 | 3.255988684 | 6.086820979 | 4.309796314 | 4.872515965 | 0 |
| 2194 | ABC | 4.825186019 | 5.178328473 | 3.894621858 | 4.277168269 | 4.678047718 | 4.95406674 | 1 |
| 2195 | Unclassified | 4.966788376 | 5.612220989 | 4.512494324 | 4.519804673 | 4.596622837 | 5.208835238 | 2 |
| 2196 | GCB | 4.228917949 | 4.439976544 | 3.632240913 | 5.962846013 | 4.396469309 | 5.157322913 | 0 |
| 2199 | GCB | 4.300540073 | 4.054740766 | 3.244079307 | 4.995409873 | 3.827119194 | 4.941544193 | 0 |
| 2204 | ABC | 4.388904968 | 4.909957799 | 4.34088847 | 4.444041841 | 4.500530066 | 5.071401301 | 1 |
| 2205 | GCB | 4.214074615 | 4.628765016 | 3.380986739 | 5.619738687 | 4.472319262 | 5.134225566 | 0 |
| 2206 | ABC | 4.543990739 | 4.921808994 | 3.674934739 | 4.848380505 | 4.688335684 | 5.14345233 | 1 |
| 2209 | GCB | 4.307667577 | 2.96078718 | 3.876574227 | 6.537494635 | 4.104039011 | 5.025009939 | 0 |
| 2210 | ABC | 4.844028747 | 4.966346952 | 3.967291964 | 4.600660539 | 4.650999512 | 5.091176828 | 1 |
| 2211 | GCB | 4.351793714 | 4.434324282 | 4.15356604 | 5.034785816 | 4.182227022 | 5.080235675 | 0 |
| 2212 | ABC | 3.601592665 | 4.140106628 | 3.074100116 | 4.430290828 | 4.431782405 | 5.171829416 | 1 |
| 2213 | GCB | 4.730560109 | 5.176324895 | 4.783652516 | 5.580772162 | 4.529873697 | 5.090032966 | 0 |
| 2214 | ABC | 5.011641946 | 5.60399668 | 4.688916293 | 4.112097007 | 4.718743335 | 5.368719608 | 1 |
| 2215 | ABC | 4.740381169 | 4.970979726 | 4.162665783 | 5.589501341 | 4.361314164 | 4.919285936 | 1 |
| 2217 | GCB | 3.926314422 | 4.773763753 | 2.79634776 | 6.399766232 | 4.708544342 | 5.220224458 | 0 |
| 2219 | Unclassified | 4.481883564 | 4.630148904 | 3.719457032 | 6.534006812 | 4.61748658 | 5.074449136 | 2 |
| 2221 | ABC | 5.020744672 | 4.781318677 | 3.511659678 | 6.858805548 | 4.545684875 | 5.038220942 | 1 |
| 2224 | GCB | 4.568564369 | 4.54225418 | 3.745430063 | 6.303125109 | 4.62258162 | 5.072073488 | 0 |
| 2225 | ABC | 4.217590661 | 3.878793581 | 3.214294992 | 6.14734604 | 3.958093189 | 5.043934754 | 1 |
| 2226 | ABC | 4.495212983 | 4.472020492 | 3.59840924 | 4.336094867 | 4.452091235 | 5.12342685 | 1 |
| 2228 | Unclassified | 4.432299145 | 4.508803493 | 4.031296235 | 4.707645174 | 4.555330821 | 5.217066281 | 2 |
| 2229 | GCB | 4.065733257 | 4.963352138 | 4.945532397 | 4.908744762 | 3.658995093 | 4.800840102 | 0 |
| 2230 | ABC | 4.353560909 | 3.91006968 | 2.986373447 | 6.166566865 | 4.266278155 | 5.039940962 | 1 |
| 2231 | GCB | 4.097077255 | 3.99467065 | 3.143789183 | 6.177877073 | 4.699551597 | 5.133924567 | 0 |
| 2232 | ABC | 5.071191366 | 5.591520285 | 4.596417541 | 4.338954149 | 4.534439021 | 5.087880451 | 1 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2233 | GCB | 4.797749456 | 4.805490394 | 4.891732918 | 5.29376686 | 4.32054909 | 4.917211356 | 0 |
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| 2622 | ABC | 3.826528842 | 3.940301717 | 3.18629839 | 4.919756559 | 3.993889557 | 5.055249624 | 1 |
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| 2634 | ABC | 4.360676556 | 4.255403565 | 3.584477801 | 6.46353184 | 4.297428818 | 5.171726484 | 1 |
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| 2643 | ABC | 4.442021426 | 4.383607434 | 3.980375842 | 5.262083814 | 4.660321346 | 5.326293448 | 1 |
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| 2646 | ABC | 4.562161254 | 4.28078678 | 3.59297822 | 3.721638814 | 4.275547143 | 4.891313956 | 1 |
| 2647 | ABC | 4.324348185 | 4.429950454 | 3.451175954 | 5.288644512 | 4.311376912 | 5.258179522 | 1 |
| 2649 | ABC | 4.03658651 | 4.150407845 | 3.275347265 | 5.520067712 | 4.080777066 | 4.95622579 | 1 |
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| 2651 | ABC | 4.127585537 | 4.115231431 | 2.992981109 | 4.542950891 | 4.472628028 | 4.876462286 | 1 |
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| 2698 | ABC | 3.916340598 | 3.673371506 | 3.469645234 | 5.677705897 | 4.254411499 | 5.074174635 | 1 |

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| 2700 | GCB | 4.173030209 | 4.60426055 | 3.390298664 | 5.236814179 | 4.123270135 | 5.015453063 | 0 |
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| 2767 | ABC | 3.89002531 | 3.916340598 | 2.965039591 | 5.067101503 | 4.431322582 | 5.136161444 | 1 |
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| 2781 | GCB | 3.789198933 | 3.885335511 | 3.070646145 | 5.621825046 | 4.474948134 | 5.140632061 | 0 |
| 2783 | GCB | 3.607241114 | 3.663858652 | 2.852808201 | 5.442321234 | 4.276140677 | 5.070352332 | 0 |
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| 2809 | GCB | 4.237498759 | 4.580081477 | 3.647035161 | 5.1793391 | 4.408875332 | 4.926069629 | 0 |
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| 2812 | ABC | 4.26685871 | 4.667470936 | 3.91666244 | 4.289852242 | 4.436054021 | 5.275443334 | 1 |
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| 3883 | ABC | 3.880971315 | 3.756642178 | 3.176856281 | 5.097062197 | 4.396210639 | 5.152325757 | 1 |
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| 3894 | ABC | 4.465270782 | 4.751331283 | 3.794558604 | 4.509845323 | 4.550225367 | 5.109131401 | 1 |
| 3895 | GCB | 4.277805579 | 4.25839043 | 3.542498084 | 5.352395247 | 4.296457556 | 5.022739132 | 0 |
| 3896 | GCB | 4.099859078 | 3.952417921 | 4.009500529 | 4.523976149 | 3.805321193 | 4.885963204 | 0 |
| 3898 | GCB | 4.059777871 | 4.337079141 | 3.356920532 | 4.905104579 | 4.474140021 | 5.193104887 | 0 |
| 3900 | Unclassified | 3.536912539 | 4.671162195 | 4.170489057 | 2.751559553 | 4.306757487 | 5.034724775 | 2 |
| 3902 | GCB | 3.908854762 | 4.093627491 | 2.982555503 | 5.987520088 | 4.443200131 | 5.200063421 | 0 |
| 3903 | ABC | 3.658797203 | 4.329866538 | 3.33492745 | 6.758935343 | 4.15542868 | 5.051866226 | 1 |
| 3904 | GCB | 3.315451029 | 3.903282364 | 2.750416237 | 7.292089549 | 4.157729951 | 4.893404472 | 0 |
| 3905 | ABC | 3.950240011 | 3.961154307 | 2.778447715 | 5.849456869 | 4.214776541 | 5.140723073 | 1 |
| 3908 | GCB | 3.556842356 | 4.146562463 | 2.928862007 | 7.202122613 | 4.141914459 | 5.068475169 | 0 |
| 3910 | ABC | 3.78108047 | 4.595688028 | 3.216563999 | 4.554114605 | 4.258791372 | 5.053333258 | 1 |
| 3911 | GCB | 4.06513671 | 4.458912573 | 2.91991401 | 6.096109599 | 4.563656935 | 5.191885329 | 0 |
| 3914 | ABC | 3.437480941 | 3.835169616 | 2.263627849 | 5.641296762 | 4.270508597 | 4.906165237 | 1 |
| 3915 | Unclassified | 3.666913298 | 3.595806893 | 2.542909953 | 6.346964331 | 4.111363203 | 4.895130382 | 2 |
| 3918 | ABC | 4.516368635 | 4.200124283 | 2.992285909 | 5.033416951 | 4.229688625 | 5.040564425 | 1 |
| 3919 | ABC | 4.028209386 | 4.245016848 | 2.71887082 | 5.62254902 | 4.274800672 | 4.843853425 | 1 |
| 3922 | ABC | 4.242079247 | 4.106752731 | 3.318970755 | 4.026649105 | 3.944657774 | 4.780905677 | 1 |
| 3925 | GCB | 4.112716031 | 4.21952447 | 2.110658977 | 7.254644157 | 4.333888794 | 4.978752237 | 0 |
| 3928 | ABC | 4.274833051 | 4.366635325 | 3.374914956 | 5.034785816 | 4.500821824 | 5.230066397 | 1 |
| 3929 | GCB | 4.086380168 | 4.020415447 | 2.542909953 | 6.827025381 | 4.239298761 | 4.907587306 | 0 |
| 3930 | GCB | 4.199481319 | 3.697776704 | 3.272426428 | 6.675750511 | 4.107429735 | 4.895509317 | 0 |
| 3932 | GCB | 4.593895862 | 4.460956141 | 3.602243072 | 5.946127729 | 4.293542508 | 4.97242303 | 0 |
| 3933 | ABC | 3.450163381 | 3.641387913 | 3.18629839 | 5.949717129 | 4.2026754 | 5.054824304 | 1 |
| 3936 | GCB | 4.35309559 | 5.011232632 | 3.271452579 | 5.751850538 | 4.537377176 | 4.953949754 | 0 |
| 3937 | Unclassified | 3.888170844 | 3.89803618 | 2.612813311 | 5.105733581 | 4.357897662 | 5.172007691 | 2 |
| 3940 | GCB | 3.372551852 | 2.879464552 | 2.702235769 | 6.922084478 | 3.93714801 | 4.745024139 | 0 |
| 3944 | ABC | 4.512858235 | 4.148810961 | 3.504137376 | 5.135764408 | 4.018941862 | 5.0709639 | 1 |
| 3945 | ABC | 4.568735284 | 5.330444398 | 3.375877304 | 5.326578681 | 4.53848076 | 5.086550018 | 1 |
| 3948 | GCB | 3.709855176 | 4.284392679 | 3.12559996 | 4.849161554 | 4.343346561 | 5.055502919 | 0 |
| 3949 | GCB | 3.738779943 | 3.898668777 | 2.725605337 | 5.881546187 | 4.242403765 | 5.100059052 | 0 |
| 3950 | ABC | 3.738633057 | 4.075004712 | 2.837427849 | 5.455982766 | 4.303992475 | 4.817845913 | 1 |
| 3951 | GCB | 3.667516603 | 3.862682275 | 2.567263431 | 6.688892279 | 4.294598916 | 5.054145882 | 0 |
| 3952 | Unclassified | 3.791741968 | 4.579733613 | 2.92237668 | 5.212040703 | 4.393124526 | 5.092325959 | 2 |
| 3954 | Unclassified | 4.093627491 | 4.92879602 | 3.063764179 | 5.176848007 | 4.702077895 | 5.07038631 | 2 |
| 3956 | GCB | 3.860812799 | 4.575203088 | 3.728095949 | 7.43360276 | 4.240417895 | 4.939168885 | 0 |
| 3957 | ABC | 4.113057101 | 3.612205112 | 3.321598011 | 5.397878199 | 4.199483787 | 5.101009878 | 1 |
| 3958 | ABC | 3.831757463 | 3.983443907 | 2.610150469 | 5.566920152 | 4.06644462 | 4.834416078 | 1 |
| 3959 | ABC | 3.842711173 | 4.270043866 | 3.537235661 | 4.727124222 | 4.143295868 | 5.018581997 | 1 |
| 3960 | GCB | 3.263258232 | 3.482086247 | 2.885096234 | 6.12984053 | 3.819023791 | 4.785977533 | 0 |
| 3963 | GCB | 3.717960159 | 4.437963907 | 2.928862007 | 7.465573521 | 4.091541033 | 4.610146435 | 0 |
| 3964 | ABC | 4.668223081 | 4.553066036 | 3.34984433 | 5.045470204 | 4.113270365 | 5.056098591 | 1 |
| 3965 | ABC | 3.612854989 | 3.851713898 | 2.835981242 | 5.809296151 | 4.112157959 | 4.993313395 | 1 |
| 3966 | GCB | 3.779864356 | 4.03376207 | 2.821440182 | 5.648980304 | 4.208717472 | 5.08954604 | 0 |
| 3967 | Unclassified | 3.886607349 | 4.302177405 | 3.075477759 | 6.624010061 | 4.171285415 | 5.061776604 | 2 |
| 3968 | Unclassified | 3.935050703 | 4.357393798 | 3.332357622 | 6.732311968 | 4.182738418 | 5.064611533 | 2 |
| 3969 | GCB | 3.222573676 | 3.18629839 | 2.274090493 | 5.753354715 | 3.911227382 | 4.737652063 | 0 |
| 3971 | GCB | 3.455340999 | 3.795179674 | 2.426883273 | 8.095517141 | 4.226232803 | 4.9156315 | 0 |
| 3972 | GCB | 4.049770921 | 4.270693637 | 2.898711802 | 7.757309548 | 4.302683356 | 4.92014803 | 0 |
| 3974 | GCB | 3.78950792 | 4.348815968 | 3.361162098 | 5.856249304 | 4.140378804 | 5.060379396 | 0 |
| 3975 | GCB | 3.971034898 | 4.109929049 | 3.291363091 | 6.716032087 | 4.052584549 | 4.986225535 | 0 |
| 3976 | ABC | 4.198533996 | 4.702491733 | 2.913161045 | 5.83626283 | 4.44865637 | 4.986589592 | 1 |
| 3977 | GCB | 4.376004437 | 4.783652516 | 3.576659141 | 5.885004778 | 4.516840335 | 5.139289829 | 0 |
| 3980 | GCB | 3.678059856 | 4.163898839 | 2.799282764 | 5.071191366 | 4.221734552 | 5.08815788 | 0 |
| 3981 | ABC | 4.888509492 | 5.620439858 | 4.043548879 | 4.889730899 | 4.757577537 | 5.089092934 | 1 |
| 3983 | GCB | 3.904508978 | 4.348815968 | 3.120791188 | 5.677705897 | 4.058043055 | 4.715617974 | 0 |
| 3985 | ABC | 3.963294739 | 4.233356898 | 3.120791188 | 5.008304761 | 4.329455903 | 5.086926611 | 1 |
| 3987 | Unclassified | 3.811626951 | 3.638537584 | 2.564456763 | 6.692764837 | 4.486289232 | 5.22528798 | 2 |
| 3989 | GCB | 3.874459581 | 3.45209844 | 3.029790241 | 5.289661332 | 3.964155135 | 4.876499274 | 0 |
| 3990 | ABC | 4.117802001 | 4.34813777 | 2.87981132 | 4.917716995 | 4.365672399 | 5.077681001 | 1 |
| 3992 | ABC | 3.342691019 | 3.895532441 | 2.908110017 | 5.462563582 | 4.016654471 | 4.84903993 | 1 |
| 3997 | Unclassified | 3.759760351 | 3.947424166 | 2.466152853 | 5.489039813 | 4.156284189 | 4.962608688 | 2 |

Supplemental Table 2. Source of gene sets

| Signature | Pathway Collection | Original Pathway Source | Accession |
|---|---|---|---|
| BIOCARTA_NFKB_PATHWAY | Biocarta | http://cgap.nci.nih.gov/Pathways/BioCarta/h_nfkbPathway | N/A |
| NEGATIVE_REGULATION_OF_INTRINSIC_APOPTOTIC_SIGNALING_PATHWAY | Gene Ontology | http://www.geneontology.org/ | GO:2001243 |

# Supplemental Table 3. Gene of BIOCARTA_NFKB_PATHWAY and NEGATIVE_REGULATION_OF_INTRINSIC_APOPTOTIC_SIGNALING_PATHWAY

| BIOCARTA_NFKB_PATHWAY | NEGATIVE_REGULATION_OF_INTRINSIC_APOPTOTIC_SIGNALING_PATHWAY |
|---|---|
| CHUK | AKT1 |
| FADD | ARHGEF2 |
| IKBKB | BAG5 |
| IKBKG | BCL2 |
| IL1R1 | BCL2L1 |
| IRAK1 | BCL2L12 |
| MAP3K1 | BCL2L2 |
| MAP3K14 | CD44 |
| MAP3K7 | CD74 |
| MYD88 | CLU |
| NFKB1 | CREB3 |
| NFKBIA | CXCL12 |
| RELA | DDX3X |
| RIPK1 | ELL3 |
| TAB1 | FIGNL1 |
| TLR4 | GPX1 |
| TNF | GRINA |
| TNFAIP3 | HERPUD1 |
| TNFRSF1A | HIF1A |
| TNFRSF1B | HSPA1A |
| TRADD | HSPB1 |
| TRAF6 | HSPH1 |
| | HTRA2 |
| | HYOU1 |
| | ING2 |
| | IVNS1ABP |
| | KDM1A |
| | LRRK2 |
| | MAPK7 |
| | MCL1 |
| | MIF |
| | MMP9 |
| | NDUFA13 |
| | NDUFS3 |
| | NFE2L2 |
| | NOC2L |
| | NONO |
| | OPA1 |
| | PARK7 |
| | PARL |
| | PGAP2 |
| | PINK1 |
| | PPIF |
| | PTPN1 |
| | PTTG1IP |
| | RRM2B |
| | RRN3 |
| | SIRT1 |
| | SNAI2 |
| | SOD2 |
| | SRC |
| | SYVN1 |
| | TAF9 |
| | TAF9B |
| | TMBIM6 |
| | TMEM161A |
| | TPT1 |
| | TRAP1 |
| | TRIAP1 |
| | TRIM32 |
| | TXNDC12 |
| | USP47 |
| | VDAC2 |
| | WFS1 |
| | XBP1 |
| | ZNF385A |

# Supplemental Table 4. Whole-exome sequencing data and RNA-seq data derived from TCGA-DLBC

| Sample ID | RNF31 expression (log2[fpkm+1]) | RNF31 level | Number of non-synonymous mutations | Mutations related to AID | | |
|---|---|---|---|---|---|---|
| | | | | Number of SNVs at C/G within WRCY/ RGYW motifs | Number of SNVs at C:G pairs | Number of transition SNVs |
| TCGA_FA_8693 | 3.433192779 | low | 139 | 16 | 89 | 117 |
| TCGA_FA_A4BB | 3.378465047 | low | 60 | 7 | 63 | 62 |
| TCGA_FA_A4XK | 3.605882128 | low | 57 | 13 | 56 | 53 |
| TCGA_FA_A6HN | 3.923326525 | high | 147 | 38 | 156 | 135 |
| TCGA_FA_A6HO | 4.088918093 | high | 38 | 4 | 39 | 36 |
| TCGA_FA_A7DS | 3.749852318 | low | 66 | 17 | 72 | 63 |
| TCGA_FA_A7Q1 | 3.407832607 | low | 115 | 17 | 122 | 108 |
| TCGA_FA_A82F | 3.525318585 | low | 126 | 19 | 124 | 104 |
| TCGA_FA_A86F | 4.025166318 | high | 63 | 9 | 56 | 49 |
| TCGA_FF_8041 | 3.96908928 | high | 158 | 20 | 164 | 159 |
| TCGA_FF_8042 | 4.335474524 | high | 222 | 20 | 191 | 172 |
| TCGA_FF_8043 | 3.73604922 | low | 92 | 10 | 90 | 92 |
| TCGA_FF_8046 | 3.734145124 | low | 67 | 11 | 60 | 57 |
| TCGA_FF_8047 | 4.015725016 | high | 101 | 11 | 107 | 115 |
| TCGA_FF_8061 | 3.260872087 | low | 138 | 15 | 112 | 125 |
| TCGA_FF_8062 | 3.615960329 | low | 120 | 21 | 110 | 103 |
| TCGA_FF_A7CQ | 4.015156781 | high | 101 | 21 | 100 | 94 |
| TCGA_FF_A7CR | 3.828972878 | high | 143 | 32 | 140 | 128 |
| TCGA_FF_A7CW | 3.902906089 | high | 36 | 4 | 37 | 33 |
| TCGA_FF_A7CX | 3.824814506 | high | 98 | 11 | 99 | 91 |
| TCGA_FM_8000 | 3.724454855 | low | 113 | 6 | 104 | 111 |
| TCGA_G8_6324 | 4.024117965 | high | 1116 | 118 | 1373 | 1456 |
| TCGA_G8_6325 | 3.793983219 | high | 436 | 47 | 509 | 515 |
| TCGA_G8_6326 | 4.151311915 | high | 373 | 38 | 405 | 449 |
| TCGA_G8_6906 | 3.925795544 | high | 469 | 52 | 534 | 521 |
| TCGA_G8_6907 | 3.78420038 | high | 389 | 42 | 440 | 456 |
| TCGA_G8_6909 | 3.788774811 | high | 677 | 84 | 667 | 651 |
| TCGA_G8_6914 | 3.71859885 | low | 400 | 45 | 465 | 486 |
| TCGA_GR_7351 | 3.738807949 | low | 583 | 76 | 567 | 569 |
| TCGA_GR_7353 | 4.538902117 | high | 414 | 47 | 441 | 480 |
| TCGA_GR_A4D4 | 3.755117882 | low | 76 | 5 | 55 | 44 |
| TCGA_GR_A4D5 | 3.52981948 | low | 49 | 6 | 59 | 51 |
| TCGA_GR_A4D6 | 4.039498559 | high | 28 | 2 | 31 | 31 |
| TCGA_GR_A4D9 | 3.510295565 | low | 62 | 6 | 52 | 40 |
| TCGA_GS_A9TQ | 3.582656442 | low | 67 | 5 | 60 | 54 |
| TCGA_GS_A9TT | 3.478160411 | low | 131 | 21 | 136 | 118 |
| TCGA_GS_A9TU | 3.797060331 | high | 41 | 3 | 39 | 40 |
| TCGA_GS_A9TV | 4.358108537 | high | 55 | 4 | 33 | 43 |
| TCGA_GS_A9TW | 3.835903844 | high | 180 | 21 | 174 | 144 |
| TCGA_GS_A9TX | 3.727934043 | low | 20 | 1 | 14 | 16 |
| TCGA_GS_A9TY | 3.442669245 | low | 109 | 8 | 106 | 89 |
| TCGA_GS_A9TZ | 3.812335231 | high | 294 | 36 | 304 | 259 |
| TCGA_GS_A9U3 | 3.739806669 | low | 62 | 5 | 61 | 60 |
| TCGA_GS_A9U4 | 3.923842062 | high | 16 | 2 | 23 | 17 |
| TCGA_RQ_A68N | 4.078441915 | high | 155 | 19 | 161 | 145 |
| TCGA_RQ_A6JB | 3.577042044 | low | 58 | 3 | 46 | 44 |
| TCGA_RQ_AAAT | 3.691684788 | low | 111 | 19 | 108 | 99 |
| TCGA_VB_A8QN | 3.488698312 | low | 117 | 15 | 139 | 129 |

For each DLBCL patient sequenced, clinical features are shown.[1,2] The "Mutations related to AID" section shows number of single nucleotide variations (SNVs) at C/G within WRCY/RGYW motifs, and at C:G pairs and number of transition mutations. Blank fields indicate that a measurement was not taken or was irrelevant to the patient.


1. Grossman RL, Heath AP, Ferretti V, et al. Toward a Shared Vision for Cancer Genomic Data. *N Engl J Med*. 2016;375(12):1109-1112.
2. Lohr JG, Stojanov P, Lawrence MS, et al. Discovery and prioritization of somatic mutations in diffuse large B-cell lymphoma (DLBCL) by whole-exome sequencing. *Proc Natl Acad Sci USA*. 2012;109(10):3879-3884.

Supplemental Table 5. Murine homologue of previously reported driver genes in human DLBCL

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Actb | Bcor | Dazap1 | Gna13 | Itpkb | Nfkbie | Setd1b | Tnfaip3 | Zeb2 |
| Actg1 | Btg1 | Dtx1 | Gnai2 | **Klf2** | Notch2 | Sgk1 | Tnfrsf14 | Zfp36l1 |
| Ankrd11 | Btg2 | **Dusp2** | Grhpr | Klhl14 | Osbpl10 | Socs1 | Tox | |
| Arid1a | Card11 | Ebf1 | Hist1h1b | Klhl6 | Pax5 | Spen | Triobp | |
| Arid1b | Ccnd3 | Ep300 | **Hist1h1c** | Kmt2d | Pde4dip | Stat3 | Trp53 | |
| B2m | Cd70 | Ets1 | Hist1h1d | Ltb | **Pim1** | Syne2 | Ttn | |
| Bcl10 | Cd79b | Etv6 | **Hist1h1e** | Mef2b | Pim2 | Tbl1xr1 | Ube2a | |
| Bcl2 | Cdkn2a | Ezh2 | **Irf2bp2** | Mpeg1 | Plec | Tet2 | Vmp1 | |
| Bcl6 | Ciita | Fas | Irf4 | Myc | Pou2f2 | Tmem30a | Wdfy4 | |
| Bcl7a | Crebbp | Foxo1 | Irf8 | Myd88 | Prdm1 | Tmsb4x | Zc3h12a | |

Among 90 genes mutated with frequency greater than 5% in human DLBCL,[1] eight genes lacking a murine homologue were excluded. Genes non-synonymously mutated in lymphomas derived from CD19-cre-HOIP/MYD88LP mice are bold.

1. Schmitz R, Wright GW, Huang DW, et al. Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma. *N Engl J Med*. 2018;378(15):1396-1407.

Supplemental Table 6. Murine homologue of previously known and predicted hypermutated genes in human DLBCL

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Actb | Cd74 | Ets1 | Hist1h2ab | Hist2h2ab | Itpkb | Ms4a1 | Prrt2 | Tcl1 |
| Actg1 | **Cd83** | Etv6 | Hist1h2ao | Hist2h2ac | **Klf2** | Myc | Rcc1 | Tmsb4x |
| Arid5b | Cdkn1b | Fam102a | Hist1h2bc | Hist2h2bb | Klhl14 | Ncoa3 | Rftn1 | Tnf |
| Atxn2 | Ciita | Fcgr4 | Hist1h2be | Hist2h2be | Klhl21 | Nol9 | Rhoh | Tnfrsf14 |
| Bach2 | Cxcr4 | Foxc1 | Hist1h2bg | Hist2h3b | Limd2 | Osbpl10 | Rnf144b | Ube2j1 |
| Bcl2 | Dmd | Foxo1 | Hist1h2bk | Id3 | Lrmp | Pax5 | S1pr2 | Ube3c |
| Bcl6 | Dtx1 | Gadd45b | Hist1h2bm | Igll1 | Lst1 | **Pim1** | Serpina9 | Vmp1 |
| Bcl7a | Dtx4 | Grhpr | Hist1h3b | Il10ra | Lta | Pim2 | Sgk1 | Wee1 |
| Birc3 | **Dusp2** | H2afj | Hist1h3c | Il16 | Ltb | Pou2af1 | Socs1 | Zfp36l1 |
| Btg1 | Egr1 | Hist1h1b | Hist1h3h | **Irf2bp2** | Map3k3 | Ppp1r9b | Spred2 | Zfp36l2 |
| Btg2 | Ehd1 | **Hist1h1c** | Hist1h4d | Irf4 | **Mcl1** | Pramef25 | St6gal1 | Zfp608 |
| Cd44 | Eif4a2 | **Hist1h1e** | Hist2h2aa2 | Irf8 | Mpeg1 | Pramef8 | Tas1r1 | Zfp804a |

Among the previously reported 126 genes,[1] 18 genes lacking a murine homologue were excluded.

Genes non-synonymously mutated in lymphomas derived from CD19-cre-HOIP/MYD88LP mice are bold.

1. Schmitz R, Wright GW, Huang DW, et al. Genetics and Pathogenesis of Diffuse Large B-Cell Lymphoma. *N Engl J Med*. 2018;378(15):1396-1407.

## Supplemental Table 7. Previously reported AID target genes in mice

| | | | | | | |
|---|---|---|---|---|---|---|
| 1810065E05Rik | Cd79a | Fas | Jchain | Pik3ap1 | Rpl3 | Tcf4 |
| Aatk | Cd79b | Fchsd2 | Junb | **Pim1** | Rpl32 | Terc |
| Abl2 | **Cd83** | Fen1 | Kpna2 | Plac8 | Rpl35 | Tet2 |
| Ablim1 | Cdk11b | Fli1 | Kpnb1 | Plcb2 | Rpl4 | Tex14 |
| Acot7 | Cdk4 | Fnbp1 | Lmo1 | Pms2 | Rpl41 | Tfap4 |
| Actb | Chd2 | Foxo4 | Lrmp | Pola1 | Rplp0 | Tfdp1 |
| Ada | Chek1 | Fth1 | Lsp1 | Pold1 | Rps12 | Tmsb4x |
| Adar | Ciita | Ftl1 | Ltb | Pold4 | Rps14 | Tnf |
| Agk | Clk1 | Gadd45b | Ly6e | Pou2af1 | Rps5 | Tnfaip3 |
| Aicda | Cnbp | Gadd45g | Lyn | Pou2f1 | Rps6 | Top1 |
| Akap8 | Cox4i1 | Galnt1 | **Man1a** | Pou2f2 | Rps9 | Top2a |
| Apex1 | Cox6a1 | Gas5 | Mcm2 | Ppard | Rrm1 | Topors |
| Apobec1 | Cox8a | Gdi2 | Mcm6 | Ppia | Rrm2 | Tra2b |
| Apoe | Cradd | Gna13 | Mcm7 | Ppp1r15b | Runx1t1 | Ttf1 |
| Atf5 | Csk | Grap | Mdfi | Ppp1r16b | Safb | U2af2 |
| Atp5b | Csnk1d | Gsta3 | Mdh2 | Prdx1 | Vimp | Uba3 |
| Atp5e | Cyth1 | H2afx | Mef2b | Psmc3 | Serinc3 | Ubac2 |
| Atp5o | Daxx | H3f3b | Mir142b | Ptbp2 | Sf3b1 | Ubb |
| B2m | Ddb2 | Hdac1 | Mlh3 | Pten | Sh3bp5 | Ube2b |
| Bad | Ddx20 | Hdac9 | Mlst8 | Ptma | Sipa1 | Ube2n |
| Bcl11a | Ddx5 | Hdgf | Mrpl51 | Ptprc | Sirt6 | Ube4b |
| Bcl6 | Dnmt1 | Hells | Ms4a1 | Rac1 | Slbp | Ubox5 |
| Bid | Dok1 | Hist1h1a | Msh2 | Rad23a | Slc30a6 | Ubtf |
| Blk | Dsg4 | Hist1h1b | Msh5 | Rad51 | **Snx5** | Uhmk1 |
| Bmp2k | Dusp6 | Hnrnpa2b1 | Msh6 | Rad9a | Sod1 | Vav1 |
| Brca1 | Dyrk3 | Hnrnpf | Mtor | Ranbp1 | Sp3 | Vav2 |
| Btg1 | E2f1 | Hsf4 | Mtss1 | Rbm15 | Spib | Vcl |
| Btg2 | E2f2 | Hvcn1 | Mybbp1a | Rbm19 | Sra1 | Vdac1 |
| C2cd3 | E4f1 | Id3 | Myc | Rbm39 | Srsf10 | Xbp1 |
| Cacng4 | Ebf1 | Ikzf1 | Mycbp2 | Recql4 | St6gal1 | Zc3h15 |
| Calm1 | Eef1a1 | Il21r | Ncl | Rel | Stau1 | Zcchc7 |
| Cat | Eif2a | Il4ra | Nfkb2 | Rev3l | Stx3 | |
| Ccne2 | Eif3a | Ildr2 | Ntan1 | Rfc2 | Sv2b | |
| Cd19 | Eif3d | Ipmk | Parp1 | Rgs13 | Swap70 | |
| **Cd22** | Eif4a2 | Ipo7 | Pax5 | Rhoh | Syk | |
| Cd24a | Eif5a | Irf4 | Pcna | Rims1 | Taf4 | |
| Cd37 | Ell | Irf8 | Pex13 | Rnf2 | Taf9 | |
| Cd48 | Erh | Itga4 | Phip | Rpa1 | Tapbp | |
| Cd53 | Eri1 | Itgal | Pias1 | Rpia | Tcea1 | |
| Cd74 | Ets1 | Itgb2 | Pick1 | Rpl29 | Tcf3 | |

Among the previously reported 291 genes,[1] 20 genes duplicated or with no correspondence in the current database were excluded. Genes non-synonymously mutated in lymphomas derived from CD19-cre-HOIP/MYD88LP mice are bold.

1. Alvarez-Prado AF, Perez-Duran P, Perez-Garcia A, et al. A broad atlas of somatic hypermutation allows prediction of activation-induced deaminase targets. *J Exp Med*. 2018;215(3):761-771.

Supplemental Table 8. Enrichment of WRCY mutations in genes mutated in lymphomas derived from transgenic mice

| Mouse genotype group | Gene | No. of SNVs at C in WRCY in the indicated gene | No. of WRCY motifs in the indicated gene | No. of all WRCY motifs in the whole exome | No. of samples in the indicated genotype group | No. of SNVs at C in total WRCY among the indicated genotype group | Rate of SNVs in WRCY motifs | p value (Binomial test) | Gene sets | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Alvarez-Prado, 2018 | Schmitz, 2018 |
| CD19-cre-HOIP/MYD88LP | Irf2bp2 | 23 | 109 | 2254406 | 8 | 145 | 8.04E-06 | 1.52E-94 | FALSE | **TRUE** |
| CD19-cre-HOIP/MYD88LP | Pim1 | 11 | 56 | 2254406 | 8 | 145 | 8.04E-06 | 1.35E-45 | **TRUE** | **TRUE** |
| CD19-cre-HOIP/MYD88LP | Hist1h1e | 8 | 54 | 2254406 | 8 | 145 | 8.04E-06 | 1.82E-32 | FALSE | **TRUE** |
| CD19-cre-HOIP/MYD88LP | Klf2 | 7 | 55 | 2254406 | 8 | 145 | 8.04E-06 | 4.40E-28 | FALSE | **TRUE** |
| CD19-cre-HOIP/MYD88LP | Bhlhe41 | 7 | 84 | 2254406 | 8 | 145 | 8.04E-06 | 9.83E-27 | FALSE | FALSE |
| CD19-cre-HOIP/MYD88LP | Nfkbia | 6 | 70 | 2254406 | 8 | 145 | 8.04E-06 | 3.54E-23 | FALSE | FALSE |
| CD19-cre-HOIP/MYD88LP | H2-Ab1 | 5 | 47 | 2254406 | 8 | 145 | 8.04E-06 | 5.15E-20 | FALSE | FALSE |
| CD19-cre-HOIP/MYD88LP | Fgl2 | 3 | 102 | 2254406 | 8 | 145 | 8.04E-06 | 8.92E-11 | FALSE | FALSE |
| CD19-cre-HOIP/MYD88LP | Man1a | 3 | 127 | 2254406 | 8 | 145 | 8.04E-06 | 1.73E-10 | **TRUE** | FALSE |
| CD19-cre-HOIP/MYD88LP | Tap1 | 3 | 167 | 2254406 | 8 | 145 | 8.04E-06 | 3.96E-10 | FALSE | FALSE |
| CD19-cre-HOIP/MYD88LP | Cd83 | 2 | 47 | 2254406 | 8 | 145 | 8.04E-06 | 6.99E-08 | **TRUE** | **TRUE** |
| CD19-cre-HOIP/MYD88LP | Hes5 | 2 | 49 | 2254406 | 8 | 145 | 8.04E-06 | 7.60E-08 | FALSE | FALSE |
| CD19-cre-HOIP/MYD88LP | Mcl1 | 2 | 51 | 2254406 | 8 | 145 | 8.04E-06 | 8.24E-08 | FALSE | **TRUE** |
| CD19-cre-HOIP/MYD88LP | Socs3 | 2 | 53 | 2254406 | 8 | 145 | 8.04E-06 | 8.90E-08 | FALSE | FALSE |
| CD19-cre-HOIP/MYD88LP | Hist1h1b | 2 | 59 | 2254406 | 8 | 145 | 8.04E-06 | 1.11E-07 | **TRUE** | **TRUE** |
| CD19-cre-HOIP/MYD88LP | Eef1a1 | 2 | 75 | 2254406 | 8 | 145 | 8.04E-06 | 1.79E-07 | **TRUE** | FALSE |
| CD19-cre-HOIP/MYD88LP | Dusp2 | 2 | 86 | 2254406 | 8 | 145 | 8.04E-06 | 2.36E-07 | FALSE | **TRUE** |
| CD19-cre-HOIP/MYD88LP | Gm21948 | 2 | 87 | 2254406 | 8 | 145 | 8.04E-06 | 2.42E-07 | FALSE | FALSE |
| CD19-cre-HOIP/MYD88LP | Myc | 2 | 89 | 2254406 | 8 | 145 | 8.04E-06 | 2.53E-07 | **TRUE** | **TRUE** |
| CD19-cre-HOIP/MYD88LP | Sgpp1 | 2 | 93 | 2254406 | 8 | 145 | 8.04E-06 | 2.76E-07 | FALSE | FALSE |
| CD19-cre-HOIP/MYD88LP | Ehd1 | 2 | 116 | 2254406 | 8 | 145 | 8.04E-06 | 4.31E-07 | FALSE | **TRUE** |
| CD19-cre-HOIP/MYD88LP | Sik1 | 2 | 176 | 2254406 | 8 | 145 | 8.04E-06 | 9.95E-07 | FALSE | FALSE |
| CD19-cre-HOIP/MYD88LP | Tlr9 | 2 | 254 | 2254406 | 8 | 145 | 8.04E-06 | 2.07E-06 | FALSE | FALSE |
| CD19-cre-MYD88LP | Irf2bp2 | 5 | 109 | 2254406 | 4 | 15 | 1.66E-06 | 1.49E-21 | FALSE | **TRUE** |

The **"No. of SNVs at C in WRCY in the indicated gene"** shows total number of SNVs at C/G within WRCY/RGYW motifs observed in the indicated genes among all the lymphomas derived from the indicated genotype group. The **"No. of SNVs at C in WRCY in the indicated gene"** shows the total number of WRCY/RGYW motifs in the indicated gene. The "**No. of all WRCY motifs in the whole exome**" shows total number of WRCY/RGYW motifs in the whole exome. The **"No. of samples in the indicated genotype group"** shows the number of samples in the indicated genotype (8 CD19-cre-HOIP/MYD88LP, 4 CD9-cre-MYD88LP). The **"No. of SNVs at C in total WRCY among the indicated genotype group"** shows the total number of SNVs at C/G within WRCY/RGYW motifs observed in lymphomas derived from the indicated genotype (145 CD19-cre-HOIP/MYD88LP, 15 CD9-cre-MYD88LP). The **"Rate of SNVs in WRCY motifs"** were calculated as follows: Rate of SNVs in WRCY motifs = [No. of SNVs at C in total WRCY among the indicated genotype group/ (No. of all WRCY motifs in the whole exome × No. of samples in the indicated genotype group)]. The **"Gene set"** section shows whether the indicated gene is included in each gene set (yes = "TRUE", no = "FALSE").

Supplemental Table 9. Analysis of somatic mutations in clonal IgH rearrangements of DLBCL-like lymphomas derived from transgenic mice

| Tumor ID | Genotype | IGHV usage | No. of nucleotides analyzed | No. of mutations | Frequency of mutations (%) |
|---|---|---|---|---|---|
| 786 | CD19-cre-HOIP/MYD88LP | IGHV1-39*01 | 267 | 8 | 3.00 |
| 950 | CD19-cre-HOIP/MYD88LP | IGHV1-72*01 | 192 | 23 | 11.98 |
| 1032 | CD19-cre-HOIP/MYD88LP | IGHV1-81*01 | 233 | 1 | 0.43 |
| 1074 | CD19-cre-HOIP/MYD88LP | IGHV1-55*01 | 195 | 3 | 1.54 |
| 1083 | CD19-cre-HOIP/MYD88LP | IGHV1-63*01 | 248 | 1 | 0.40 |
| 1084 | CD19-cre-HOIP/MYD88LP | IGHV1-80*01 | 195 | 3 | 1.54 |
| 1182 | CD19-cre-HOIP/MYD88LP | IGHV1-87*01 | 184 | 40 | 21.74 |
| 1236 | CD19-cre-MYD88LP | IGHV2-2*01 | 221 | 0 | 0.00 |
| 1237 | CD19-cre-MYD88LP | IGHV1-59*01 | 196 | 0 | 0.00 |
| 1289 | CD19-cre-MYD88LP | IGHV1-87*01 | 205 | 9 | 4.39 |
| 1385 | CD19-cre-MYD88LP | IGHV1-69*02 | 198 | 2 | 1.01 |

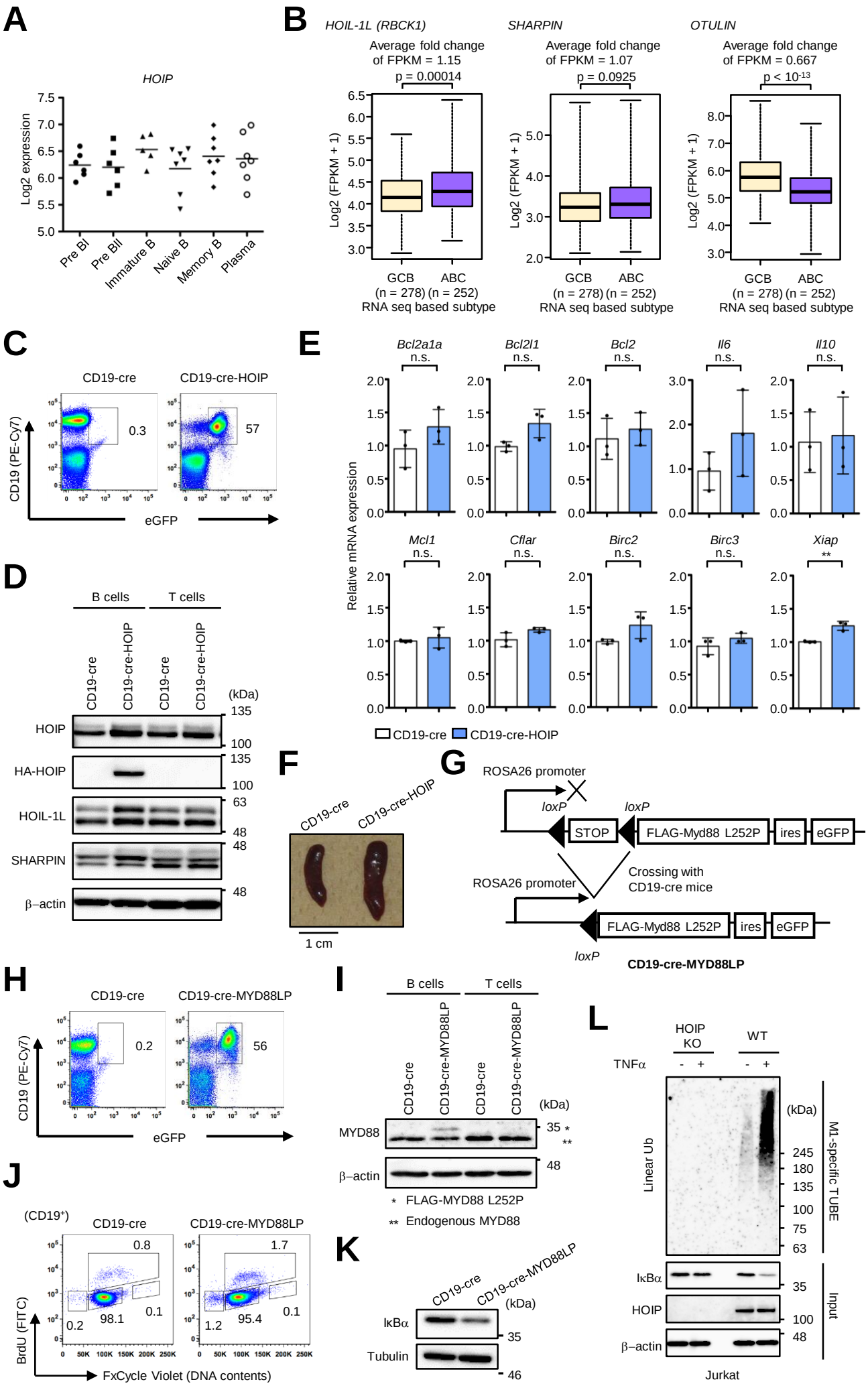
Supplemental Table 10. Primers for testing clonality of IgH V(D)J

| Primer | Sequence (5'–3') |
|---|---|
| VH-specific primers 1 | |
| 36-60 | CCTGGCCTCGTGAAACCTTCTCAG |
| 81X | GGAGGCTTAGTGCAGCCTAGAGAG |
| J558 | CTTCAGTGAAGATATCCTGCAAGG |
| Q52 | CCCAGGTGCAGCTGAAGCAGTCAG |
| VH-specific primers 2 | |
| 36-60 | GTCTCTCAGGCGCGCCGTCACTGG |
| 81X | TCCCTGAGGCGCGCCTGTGAATCC |
| J558 | GGCTTCTGGCGCGCCATTTACTGG |
| Q52 | AGCCTGTCCATCACCTGCACAGTC |
| JH universal primer | GAAAACTCCATAACAAAGG |
| JH-specific primers | |
| JH1 | AGCTTCTGCAGCATGCAGAGTGTG |
| JH2 | GGCCAGGATCCCTATAAATCTCTG |
| JH3 | ACAAAGGGGTTGAATCTTGATTCC |
| JH4 | AAAATAAAGACCTGGAGAGGC |

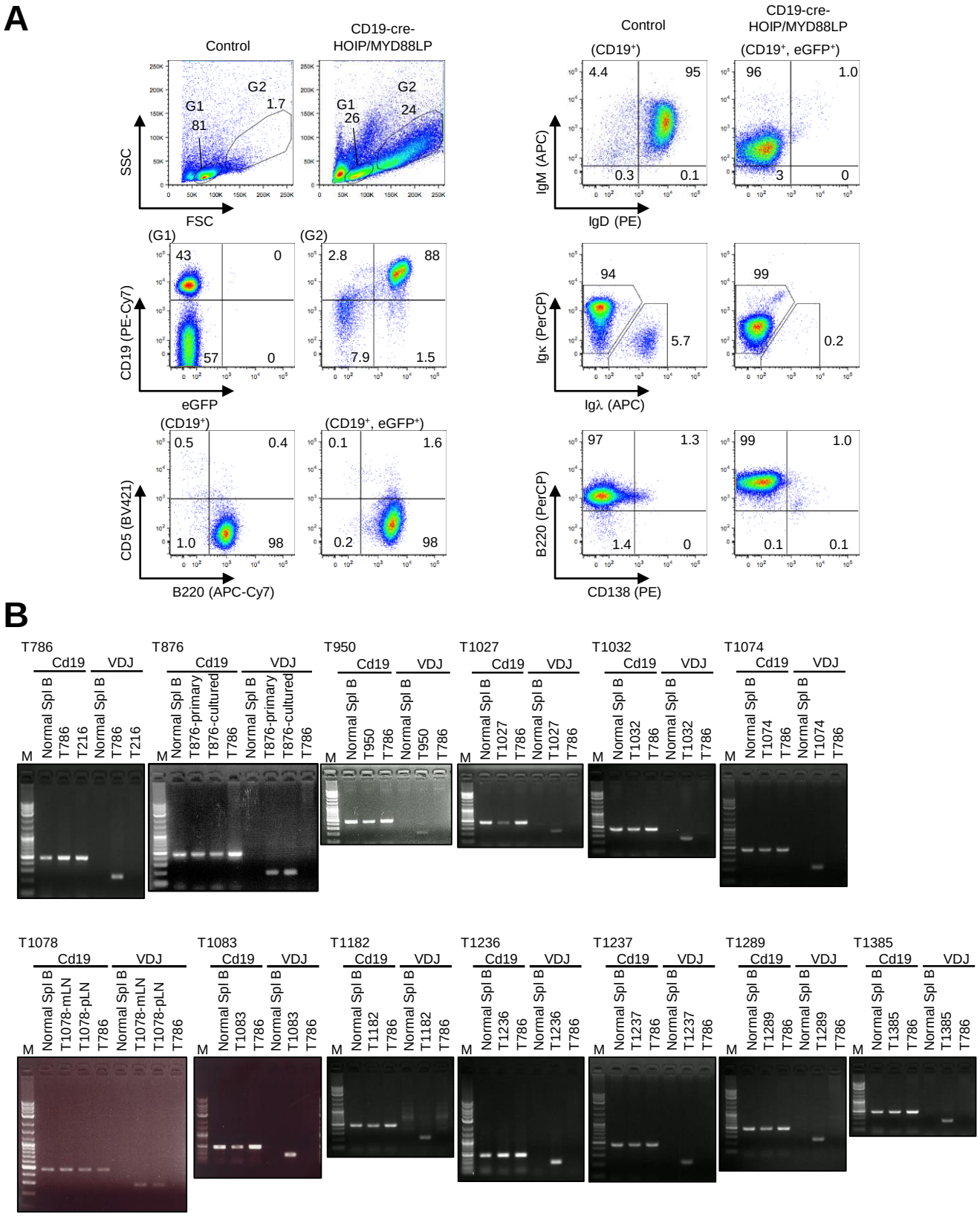
Supplemental Table 11. Primer sequences used for real-time PCR

| | Forward primer sequence (5' – 3') | Reverse primer sequence (5' – 3') |
|---|---|---|
| Mouse | | |
| *Aicda* | TCTGCTACGTGGTGAAGAGGAG | CCAGTCTGAGATGTAGCGTAGG |
| *Bcl2* | CCTGTGGATGACTGAGTACCTG | AGCCAGGAGAAATCAAACAGAGG |
| *Bcl2l1* | GCCACCTATCTGAATGACCACC | AGGAACCAGCGGTTGAAGCGC |
| *Bcl2a1a* | TCCACAAGAGCAGATTGCCCTG | GCCAGCCAGATTTGGGTTCAAAC |
| *Birc2* | GATACGGATGAAGGGTCAGGAG | GGGTCAGCATTTTCTTCTCCTGG |
| *Birc3* | GGACATTAGGAGTCTTCCCACAG | GAACACGATGGATACCTCTCGG |
| *Cflar* | GCTCTACAGAGTGAGGCGGTTT | CACCAATCTCCATCAGCAGGAC |
| *Il6* | TACCACTTCACAAGTCGGAGGC | CTGCAAGTGCATCATCGTTGTTC |
| *Il10* | CGGGAAGACAATAACTGCACCC | CGGTTAGCAGTATGTTGTCCAGC |
| *Mcl1* | AGCTTCATCGAACCATTAGCAGAA | CCTTCTAGGTCCTGTACGTGGA |
| *Myc* | TCGCTGCTGTCCTCCGAGTCC | GGTTTGCCTCTTCTCCACAGAC |
| *Nfkbia* | GCCAGGAATTGCTGAGGCACTT | GTCTGCGTCAAGACTGCTACAC |
| *Nfkbid* | GTGGAGGATCTGTTGAACCTGG | TCTCTGGCTTCCAGGTCAACCT |
| *Tnfaip3* | AGCAAGTGCAGGAAAGCTGGCT | GCTTTCGCAGAGGCAGTAACAG |
| *Xiap* | GGCAGAATATGAAGCACGGATCG | CACTTGGCTTCCAATCCGTGAG |
| *β-Actin* | CATTGCTGACAGGATGCAGAAGG | TGCTGGAAGGTGGACAGTGAGG |
| Human | | |
| *BCL2* | ATCGCCCTGTGGATGACTGAGT | GCCAGGAGAAATCAAACAGAGGC |
| *BCL2L1* | GCCACTTACCTGAATGACCACC | AACCAGCGGTTGAAGCGTTCCT |
| *BIRC2* | CAGACACATGCAGCTCGAATGAG | CACCTCAAGCCACCATCACAAC |
| *BIRC3* | GCTTTTGCTGTGATGGTGGACTC | CTTGACGGATGAACTCCTGTCC |
| *HOIP* | CTGGATCGTCATGGCAACCTTG | ACATCACCTCCGTGCTGGAACA |
| *HOIL1L* | TGACAACACCTACTCGTGCTCG | CACTGCGGTTTTCAGCAATGGAG |
| *MCL1* | CCAAGAAAGCTGCATCGAACCAT | CAGCACATTCCTGATGCCACCT |
| *NFKBIA* | TCCACTCCATCCTGAAGGCTAC | CAAGGACACCAAAAGCTCCACG |
| *SHARPIN* | TGGCTGTGAGATGTGTAGCACC | CCTGGAGATGTCGGACTTGTGA |
| *XIAP* | TGGCAGATTATGAAGCACGGATC | AGTTAGCCCTCCTCCACAGTGA |
| *β-ACTIN* | CACCATTGGCAATGAGCGGTTC | AGGTCTTTGCGGATGTCCACGT |

# Supplemental Figure 1

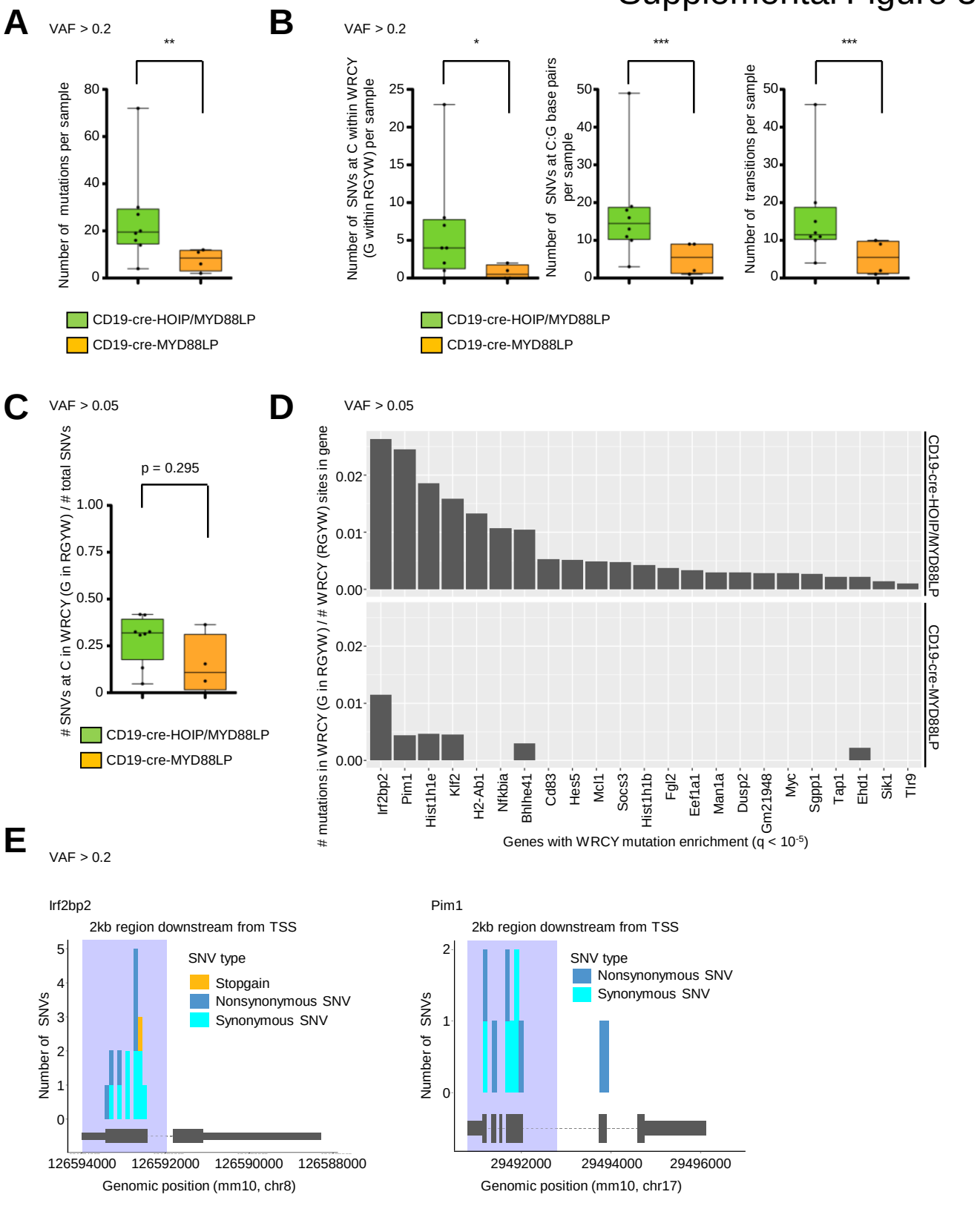


**Supplemental Figure 1. Generation of a mouse strain expressing HOIP or MYD88 L252P specifically in B cells.** (A) Expression profiles of *HOIP* in B-cell subsets in human bone marrow. (B) Association of *HOIL-1L* (*RBCK1*), *SHARPIN*, and *OTULIN* expression with cell-of-origin in human DLBCL. Boxes represent the median and the first and third quartiles, and whiskers represent the minimum and maximum of all data points. (C) B cell–specific expression of eGFP reporters in splenocytes from CD19-cre-HOIP mice. (D) B cell–specific expression of HOIP was confirmed by immunoblot analysis using MACS-purified splenic B or T cells. Expression of HOIL-1L and SHARPIN was also specifically elevated in B cells. β-actin was used as a loading control. (E) Transcript levels of NF-κB target genes in unstimulated splenic B cells from mice (10 weeks old), normalized against *Actb* mRNA; n = 3 per genotype. Data are means ± SD. (F) Macroscopic appearance of spleens in 14-month-old CD19-cre-HOIP mice. (G) Schematic representation of conditional expression of MYD88 L252P (the mouse equivalent of the human L265P mutation) in a Cre recombinase–dependent manner. (H) B cell–specific expression of eGFP reporters in splenocytes from CD19-cre-MYD88LP mice. (I) B cell–specific expression of transgenic MYD88 was confirmed by immunoblot analysis using MACS-purified splenic B or T cells. β-actin was used as a loading control. (J) *In vivo* BrdU analyses of splenic B cells. BrdU was administered by bolus intraperitoneal injection (50 mg/kg) 1.5 hours before dissection. (K) Immunoblots of MACS-purified splenic B cells from control and CD19-cre-MYD88LP transgenic mice (10 weeks old). (L) Cell lysates of HOIP KO or wild type Jurkat cells with or without TNFα stimulation were subjected to Halo-tagged linear ubiquitin-specific tandem ubiquitin binding entity (M1-specific TUBE) binding and Halo Tag based purification, and analyzed by immunoblotting. (E) ** $p < 0.01$. (B and E), two-tailed unpaired Student's *t*-test.

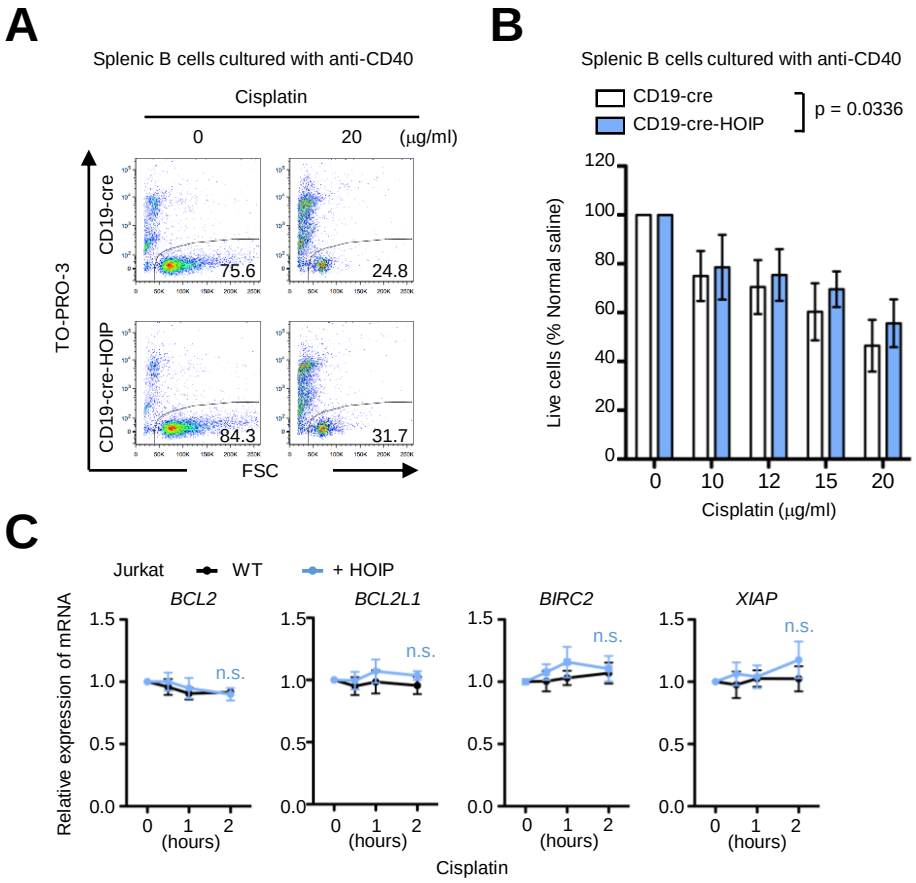



**Supplemental Figure 2. Surface immunophenotypes of lymphomas derived from transgenic mice and clonality analysis of the tumors.** (A) Representative data of FACS analysis for surface immunophenotypes of lymphoma cells. Large cells (G2) in tumor-affected organs were positive for eGFP, and eGFP-positive cells were analyzed for surface antigens. (B) Analyses for clonality of 13 cases (except for T1084, which is shown in Figure 1I).

# Supplemental Figure 3

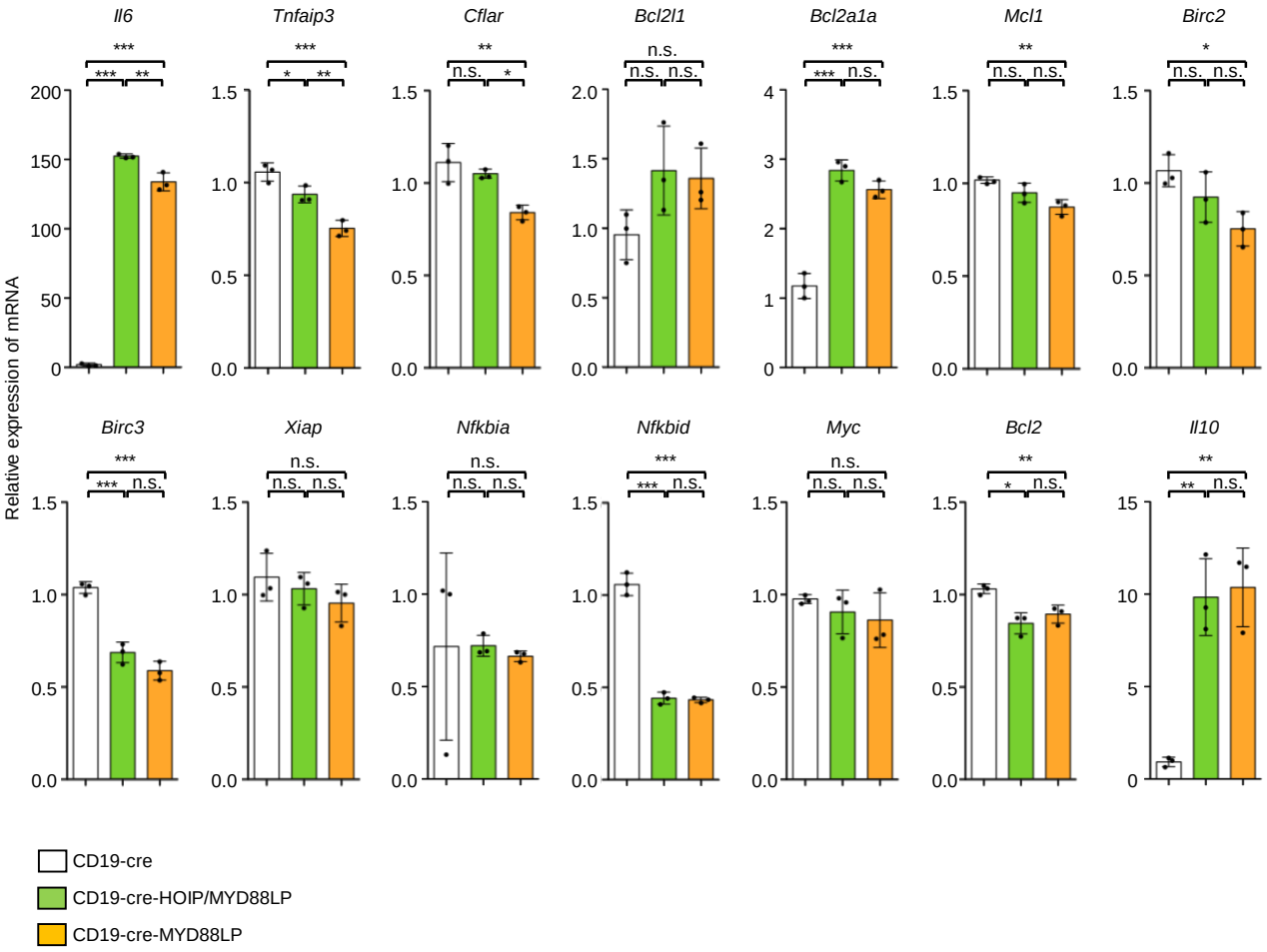


**Supplemental Figure 3. LUBAC facilitates aberrant somatic hypermutations mediated by AID.** (A, B, and E) Mutations with variant allele frequency (VAF) > 0.2 in tumor samples were selected and analyzed. (A) Numbers of mutations in each tumor sample. (B) Numbers of SNVs at C/G within the WRCY/RGYW motifs (left), and numbers of C:G (center) and transition mutations (right) in each tumor sample. (C-D) Mutations with VAF > 0.05 in tumor samples were analyzed. (C) Proportion of SNVs at C/G within the WRCY/RGYW motifs to total SNVs in each tumor samples. (D) Ratio of the number of SNVs at C/G within the WRCY/RGYW motifs to the number of WRCY/RGYW motifs in indicated genes among tumors derived from CD19-cre-HOIP/MYD88LP mice (upper panel) and CD19-cre-MYD88LP mice (lower panel). (E) Mutation distribution in targeted genes observed in lymphoma cells derived from eight CD19-cre-HOIP/MYD88LP mice. Shadows indicate the 2 kb region downstream of the transcription start site (TSS). (A, B, and C) Boxes represent the median and the first and third quartiles, and whiskers represent the minimum and maximum of all data points. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Brunner–Munzel test.



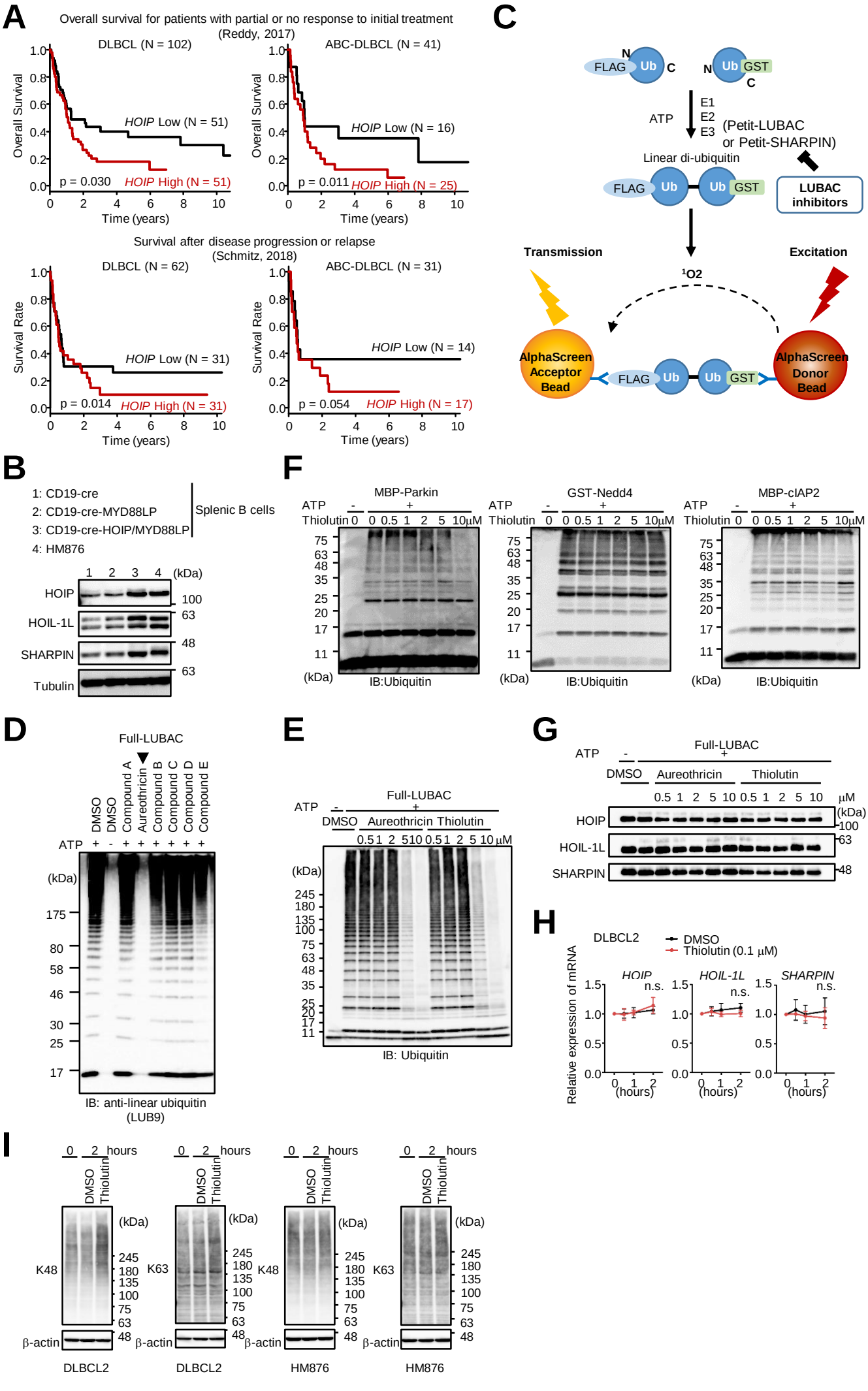

**Supplemental Figure 4. Augmented LUBAC activity overcomes cell death induced by DNA damage thereby accelerating accumulation of somatic mutations.** (A) Live cells were analyzed by FACS using TO-PRO-3 staining. Mouse splenic B cells derived from indicated genotypes were cultured with anti-CD40 antibody (10 μg/mL) for 24 hours and then were treated with or without cisplatin for 24 hours. (B) Percentage of live cells (± SD); n = 6 per group in three independent experiments. (C) Jurkat cells were treated with 3 μg/mL cisplatin for the indicated periods, followed by quantitative RT-PCR, normalized against *ACTB* mRNA; n = 3 per genotype. Data are means ± SD. (B and C) two-way ANOVA test.

# Supplemental Figure 5




**Supplemental Figure 5. Enforced LUBAC expression augments expression of NF-κB target genes.** Transcript levels of NF-κB target genes in MACS-purified unstimulated splenic B cells from CD19-cre, CD19-cre-HOIP/MYD88LP, and CD19-cre-MYD88LP mice (10 weeks old), normalized against *Actb* mRNA; n = 3 per genotype. Data are means ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (one-way ANOVA with Turkey's post hoc tests).

# Supplemental Figure 6



**Supplemental Figure 6. LUBAC is an effective target for the treatment of DLBCL.** (A) Overall survival of DLBCL cases following diagnosis who did not achieve complete response to initial therapy (Upper panels). Survival rate of DLBCL cases after disease progression or relapse (lower panels). If two Kaplan-Meier curves crossed early (≦18 months), differences between survival functions were examined by the log-rank test based on observations after the crossing point. (B) Immunoblot analyses were performed using lysates from splenic B cells of the indicated mouse genotypes and HM876 cells. (C) A schematic diagram of the AlphaScreen-based HTS system. Linear di-ubiquitin is formed in an enzymatic process that utilizes FLAG-Ub and Ub-GST, and Petit-LUBAC or Petit-SHARPIN as the E3 ubiquitin ligase enzyme, as well as E1, E2, and ATP. Anti-GST donor and anti-FLAG acceptor beads simultaneously capture linear di-ubiquitin. Proximity of acceptor and donor beads, induced by the production of linear di-ubiquitin, generates a luminescent signal upon irradiation at 680 nm. LUBAC inhibitors block formation of linear di-ubiquitin, thereby decreasing signal intensity. (D) *In vitro* ubiquitination assay confirmed that aureothricin (arrowhead) inhibited linear polyubiquitination mediated by LUBAC. Samples were probed with anti-linear ubiquitin antibody (LUB9). (E) *In vitro* Ubiquitination assay of LUBAC. Samples were probed with anti-ubiquitin antibody (P4D1). (F) *In vitro* ubiquitination assay of other types of E3 ligases, such as Parkin (left panel), Nedd4 (center panel), and cIAP2 (right panel). (G) *In vitro* Ubiquitination assay of LUBAC. Samples were probed with anti-HOIP, anti-HOIL-1L, and anti-SHARPIN antibody. (H) DLBCL2 cells were treated with or without thiolutin (0.1 μM) for the indicated periods, followed by quantitative RT-PCR, normalized against *ACTB* mRNA in three independent experiments. (I) Cell lysates of DLBCL2 and HM876 treated with or without thiolutin (0.1 μM) for 2 hours were analyzed by immunoblotting. Samples were probed with anti-K48 specific antibody (leftmost panel for DLBCL2, and middle right panel for HM876) or K63 specific antibody (middle left panel for DLBCL2, and rightmost panel for HM876). (A), log–rank test; (H) two-way ANOVA test.