Supplementary Methods

Cell lines

DLBCL cell lines (SU-DHL9, SU-DHL4, DOHH2, and PFEIFFER), the Mantle lymphoma cell line (Rec1), and Burkitt lymphoma cell lines (Daudi, Ramos, BJAB, and Raji) were maintained in 90% RPMI and 10% FCS (Hyclone, Logan, USA) supplemented with penicillin G and streptomycin. The BJAB cell line was kindly provided by Myung Soo Kang (Samsung Medical Center, Sungkyunkwan University, Seoul). Other cell lines were obtained from the American Type Culture Collection. Monthly tests were conducted for mycoplasma sp. and other contaminants.

Antibodies

The following antibodies were used: an anti-BCL6 (LN22) antibody (Novocastra, Newcastle, UK); anti-Blimp-1 (C14A4), anti-BCL6, anti-TAK1, anti-MyD88 (D80F5), anti-IκBαa (L35A5), anti-p105, anti-p100, anti-Rel B, anti-p65, anti-p52, and anti-PI3K antibodies (Cell Signaling Technologies, MA, USA); anti-PELI1 (F-7), anti-MUM1/IRF4 (M-17), anti-BCL2 (C21), anti-TRAF6 (H-274), anti-p38β (C-16), anti-ERK1/2, and anti-CyclinD1 antibodies (Santa Cruz Biotechnology, CA, USA); an anti-RIP1 antibody (BD Biosciences, CA, USA); an anti-HA (3F10) antibody (Roche, Manheim, Germany); an anti-Actin antibody (Sigma-Aldrich, MO, USA); an anti-Myc antibody (Bethyl Laboratories, TX, USA); anti-Lamin B1, anti-Ki67, anti-CD10 and anti-BCL2 (Abcam, Cambridge, UK) antibodies; anti-Foxp1 (Cell Marque, CA, USA); an APC-labeled anti-CD45R (B220) or anti-CD86 antibody, a PE-labeled anti-CD3e antibody, an FITC-labeled anti-MHC class II or anti-BCL2 antibody, a PE-labeled anti-BCL6 antibody, an APC-labeled anti-IgD (11-26C) or anti-B220 (RA3-6B2) antibody and anti-CD20, anti-CD23, and anti-CD43 (eBioR2/60) antibodies, an FITC-labeled anti-B220 antibody, and a PerCP-eF710-labeled anti-IgM (II/41) antibody (eBioscience, CA, USA); and an anti-IgM (μ-chain specific) antibody (Jackson Immunoresearch, PA, USA).

Plasmid construction, cell culture, transfection, and drug treatment

The full length cDNA sequence of the human PELI1 protein was PCR amplified using oligo-dT primers. Peli1ΔC included N-terminal 280 amino acids and lacked the C-terminal RING domain (1). PELI1 and PELI1 ΔC were subcloned into Myc- , GST-, or His_6-tagged fusion plasmids. pMSCV-BCL6-IRES-GFP (Addgene, MA, USA) was subcloned into HA- or GST-

tagged fusion plasmids. For shRNA synthesis, the following gene-specific sequences were generated using the pSuper vector (Oligoengine, WA, USA): the PELI1 shRNA [targeting open reading frame (ORF)] 5'-GGGTTCAACACACTAGCAT-3', 3'untranslated region (UTR) PELI1 shRNA 5'-GCTCCTTTGGATATGCAATTT-3', and the Luciferase shRNA 5'-CTACGCGGAATACTTCGA-3'. B-cell lines were grown in the RPMI1640 medium. HeLa cells and HEK-293 T cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% FBS (Hyclone) and pen/strep. For transient transfection, cells were electroporated using a microporator (Digital Biotechnology, Seoul, Korea) according to the manufacturer's instructions. The following drugs were used: 1 μg/ml LPS (Sigma-Aldrich), 25 μM MG132, and 100 μg/ml cycloheximide (A.G Scientific, CA, USA).

Flow cytometry and B-cell stimulation

Samples were obtained from blood, bone marrow, the spleen, and lymph nodes. Erythrocytes were lysed, and single-cell suspensions were prepared in phosphate buffered saline (PBS) and stained with an anti-B220 APC or anti-CD3 PE antibody. Splenocytes were stimulated with an anti-IgM or anti-CD40 antibody and were stained with an anti-CD86 APC or anti-MHC class II FITC antibody. Data were obtained using the FACS Canto II flow cytometer (BD Biosciences).

Cell fractionation

$B220^+$ B cells were isolated from splenocytes of wild-type or PELI1 Tg mice by using magnetic beads (Miltenyi Biotec, CA, USA) and cultured in the presence of LPS (1 μg/ml) for indicated time periods. To separate the cytoplasm and nuclei, cells were harvested and suspended in cold hypotonic buffer (HB; 10 mM Hepes, pH7.9, 10 mM KCl, 0.2 mM EDTA, 1 mM DTT, 1x protease inhibitor mixture, 0.25 mM PMSF). After incubation on ice for 15 min, NP-40 was added to a final concentration of 0.5%. Nuclei were recovered by centrifugation at 13,000 rpm for 30 s. Supernatants were collected as cytoplasmic extracts. The pellet was washed once with buffer HB, and proteins were extracted by adding high-salt (HS) buffer (20 mM Hepes, pH7.9, 420 mM NaCl, 2 mM EDTA, 1 mM DTT, 1x protease inhibitor mixture, 0.25 mM PMSF), followed by incubation at 4^oC for 30 min. The mixture was spun at 13,000 rpm for 15 min, and the supernatant was collected as a nuclear extract.

Retrovirus-mediated gene transfer and bone-marrow transplantation

To generate retroviruses expressing GFP or GFP-fused PELI1, 293T cells were transfected with the replication-incompetent helper vector pCL-Eco and pMSCV-HA-IRES-GFP or pMSCV-HA Peli1-IRES-GFP expression plasmids. Supernatants were collected, passed through 0.45 μm filters, and frozen in aliquots at -80^{o}C. Retroviral titers were determined by measuring the percentage of GFP-positive 3T3 cells. Bone marrow cells were obtained by flushing the tibia and femur with RPMI 1640 supplemented with 10% fetal bovine serum (FBS). Purified bone marrow cells were pre-incubated overnight in the 20% FBS/RPMI1640 medium supplemented with 5 ng/ml murine IL-3 (R & D Systems, MN, USA), 10 ng/ml murine IL-6 (R & D Systems), 50 ng/ml murine SCF (R & D Systems), and 50 ng/ml murine Flt3L (eBioscience). HSCs (1 x 10^6 cells) isolated from bone marrow were mixed with the retroviral supernatant and 8 μg/ml polybrene (Sigma-Aldrich). After triplicate spin infection, retroviral supernatants were removed and replaced with the 20% FBS/RPMI 1640 medium supplemented with 5 ng/ml murine IL-3, 10 ng/ml murine IL-6, 50 ng/ml murine SCF, and 50 ng/ml murine Flt3L. C57BL/6J mice were irradiated using fractionated doses (4.5 Gy x 2 = 9 Gy; 3 h intervals) and then transplanted with infected HSC cells (4 x 10^6) through a tail vein injection.

Histopathology

Tissue samples were fixed in 10% neutral buffered formalin, embedded in paraffin, cut at various depths in serial sections 3-6 μm thick, and stained with hematoxylin and eosin. Immunohistochemistry was performed using the Vectastain Elite ABC kit (Vector Laboratories, MN, USA) according to the manufacturer's instructions. The following antibodies were used: anti-CD45R, anti-CD3, anti-Ki67, anti-BCL2, anti-BCL6, anti-CD20, and anti-CD23 antibodies (primary) and biotinylated anti-rat IgG and anti-rabbit IgG antibodies (secondary). Sections were then incubated with an avidin-biotin-horseradish peroxidase complex (Vectastain Elite ABC kit; Vector Laboratories), and peroxidase activity was visualized using a 3,3′-diaminobenzidine substrate kit (Vector Laboratories). Finally, tissue sections were counterstained with Harris Hematoxylin (BBC Biochemical, WA, USA), and microscopic images were captured using the AxioCam digital microscope camera and the AxioVision image processing software package (Carl Zeiss, Oberkochen, Germany). DLBCL patient samples were histologically examined using microsections stained with hematoxylin and eosin. Representative core samples 2 mm in diameter were taken from formalin-fixed, paraffin-embedded (FFPE) blocks to construct a tissue microarray. Immunohistochemical

staining for PELI1 was performed using the Leica BOND-MAX automated immunostainer (Leica Microsystems, Newcastle, UK), and that for BCL6 was performed using the BenchMark XT Slide Preparation System (Ventana Medical Systems, Tucson, AZ, USA). Microscopic images were captured using the DP72 digital camera mounted on the BX51 microscope (Olympus Corp, Tokyo, Japan).

Fluorescence in situ hybridization (FISH)

A FISH analysis was conducted using serial sections 3 μm thick from each TMA block. Here the Vysis LSI BCL6 dual-color break-apart probe (Abbott-Vysis, Wiesbaden-Delkenheim, Germany) was used. After deparaffinization and dehydration, slides were immersed in 0.2N HCl, boiled in a microwave in citrate buffer (pH 6.0), incubated in 1M NaSCN for 35 min at 80^{o}C, immersed in a pepsin solution, and then fixed in 10% neutral-buffered formalin. The DNA probe set was applied to the slide and then incubated in a humidified chamber at 73^{o}C for 5 min to co-denature the target DNA and probe and subsequently at 37^{o}C for 19 h to achieve hybridization. After the post-hybridization washing, the slide was counterstained with 4', 6-diamidino-2-phenylindole (DAPI) and an anti-fade compound (*p*-phenylenediamine). At least 100 intact, non-overlapping nuclei were assessed under the Olympus BX51TRF microscope (Olympus) equipped with DAPI, green, orange, and triple-pass (DAPI／Green／Orange) filters (Abbott-Vysis). Here the positive predictive cutoff value was 10% of suspected tumor cells.

ELISA

For the detection of various serum cytokines, plasma samples were collected from mice and immediately stored at -80^{o}C in a freezer until the day of the assay. Novex®ELISA kits (for IL-6, IL-10, IL-12p40, and TNF-α) were purchased from Invitrogen. All reagents were provided in the ELISA kit, and all procedures were performed according to the manufacturer's instructions. Plates were read at 450 nm by using the Spectra Max Plus^{384} Microplate Reader (Molecular Devices, CA, USA).

In vitro binding and immunoprecipitation assays

For the GST pull-down assay, fusion proteins were adsorbed onto glutathione-protein A/G-Sepharose beads (Amersham Biosciences, NJ, USA) and incubated with whole-cell extracts

from LPS- treated RL7 cells. Bound proteins were separated by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by immunoblotting with an anti-BCL6 or anti-c-Rel antibody. For immunoprecipitation, transfected cells were treated with or without 2 μg/ml LPS for 24 h, resuspended in immunoprecipitation buffer (150 mM NaCl, 20 mM HEPES, 5 mM EDTA, 0.5% Nonidet P-40, 1 mM phenylmethanesulfonyl fluoride, 10 mM NaF, 1 mM Na_3Vo_4, 1 mM dithiothreitol supplemented with a mixture of protease inhibitors), and incubated at 4°C for 30 min. Then the cells were lysed by passing cell pellets five times through a 27-gauge needle. Lysates were centrifuged at 13,000 rpm for 30 min, and the insoluble debris was discarded. The lysates were then incubated with an anti-PELI1 antibody or normal IgG (control) and then with protein A/G agarose beads, which were later pelleted, washed three times in immunoprecipitation buffer, and analyzed by immunoblotting.

In vivo and in vitro ubiquitination assays

RL7 cells were transfected with an expression plasmid encoding Myc or Myc-tagged PELI1 and HA-tagged ubiquitin K63 (HA-Ub K63, kindly provided by Dr. Hong Tae Kim) in combination, as shown in Figure 5. Cells from each plate were collected into two aliquots. One aliquot (10%) was used for conventional immunoblotting, and the remaining cells (90%) were used for the immunoprecipitation of the BCL6 protein complex. Immunoprecipitates were washed three times with TNN buffer, and bound proteins were immunblotted with indicated antibodies. Purified GST or GST-BCL6 (1 μg) was incubated with purified His-PELI1 or His-PELI1 ΔC (100 ng) in conjunction with E1 (50 ng UBE1; Boston Biochem, MA, USA), E2 (400 ng UncH13/Uev1a; Boston Biochem), and HA-tagged ubiquitin K63 (2 μg HA-Ub K63; Boston Biochem) in ubiquitin reaction buffer composed of 5 mM Tris-HCl (pH 7.5), 2 mM $MgCl_2$, 2 mM ATP, and 100 mM NaCl. Reaction mixtures were incubated for 2 h at 37°C and analyzed by immunoblotting with an anti-BCL6 or anti-HA antibody and an anti-PELI1 antibody.

Quantitative real-time PCR

Total RNA was isolated using an RNeasy mini kit (QIAGEN, CA, USA) and cDNAs were synthesized using the QuantiTect Reverse Transcription kit (QIAGEN). qRT-PCR was performed with Rotor-Gene SYBR Green PCR kit (QIAGEN) using the Rotor-Gene Q 5plex PCR (QIAGEN) according to manufacturer instructions. Samples were assayed in duplicate,

and normalized to dual housekeeping gene (GAPDH; QT01658692 and Rn18s; QT02448075). All Primers (murine BCL6; QT01057196, Mme; QT00162589, Mybl1; QT01057637, LMO2; QT00115073, Entpd1; QT00099001, Ccnd2; QT00170618, IRF4; QT00109984, Pim1; QT00170541, Foxp1; QT01062929, Lrmp; QT00101101, Cxcr4; QT00249305, IL16; QT00116550) were purchased from QIAGEN. The heat-map was generated using the heatmap.2 package in R (http://www.R-project.org).

Statistical analysis

Data were analyzed using the GraphPad Prism 4.5 software package (GraphPad Software, CA, USA) and presented as the mean ±s.e.m. Spearman's correlation analysis was conducted to measure the strength of the linear relationship between PELI1 and BCL6 expression scores. PELI1 expression and clinicopathologic variables were compared through the χ^2testorFisher'sexacttest.Overall survival (OS) was defined as the period from the start of treatment to the date of the final follow-up or death from any cause. The OS rate of patients was estimated using the Kaplan-Meier method, and a log-rank test was conducted to compare differences. The p-value < 0.05 was considered significant, and a statistical analysis was conducted using SPSS 12.0 and the IBM-SPSS Statistics software package (version 19.0; IBM Corporation).

Reference

1. Lee YS, Kim JH, Kim ST, Kwon JY, Hong S, Kim SJ, and Park SH. Smad7 and Smad6 bind to discrete regions of Pellino-1 via their MH2 domains to mediate TGF-beta1-induced negative regulation of IL-1R/TLR signaling. *Biochem Biophys Res Commun.* 2010;393(4):836-843

Supplementary Figure Legends

Supplementary Figure 1. Generation of *PELI1* transgenic mice

(A) The vector construct used to generate conventional PELI1-transgenic (Tg) mice: CMV enh, human early cytomegalovirus enhancer; pA, β-globin gene polyadenylation sequence; Myc, Myc epitope. The vector was linearized, purified, and injected into the pronuclei of fertilized C57BL/6J mice. Among 14 pups derived from the quadruple embryo transfer, three pups (founder lines 2, 3, and 9) were positive based on the reverse-transcription-polymerase chain reaction (RT-PCR). **(B)** The transgene expression of PELI1 transgenic founder (line #9) was determined through RT-PCR (upper) and an immunoblot analysis using anti-Myc and anti-Actin antibodies (bottom). The amplification of the ribosomal L32 mRNA by RT-PCR verified the presence of RNA in all samples. The PCR primers specific to the human *PELI1* gene were 5'-GCAATAAGCAACAAAG-3'(forward) and 5'-ATGAGTCAAATCCTGCAG-3'(reverse). **(C)** The transgene expression of PELI1 transgenic founder (line #2) was determined through an immunoblot analysis using anti-PELI1 and anti-Actin antibodies.

Supplementary Figure 2. PELI1 expression led to impaired immune responses

(A) A peripheral blood CBC analysis of the white blood cell, lymphocyte, and monocyte content of non-Tg (n = 9) and PELI1-Tg (n = 12) mice at 12-14 months (error bars represent the mean ±s.e.m). **(B)** ELISA for serum cytokine concentrations in blood obtained from non-Tg and PELI1-Tg mice. Data are representative of two experiments with three mice per experiment (error bars represent the mean ±s.e.m). Asterisks indicate significant differences based on a one-way ANOVA (*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$); ns: not significant.

Supplementary Figure 3. The tumor incidence of mice overexpressing PELI1 by bone marrow transplantation

Total tumor incidences based on a macroscopic analysis of control GFP-transduced (n = 4) and GFP-PELI1-transduced (n = 5) mice (error bars represent the mean ±s.e.m.). Data are representative of three independent experiments. Figure 3 provides detailed information on the bone marrow transplantation of hematopoietic stem cells expressing PELI1.

Supplementary Figure 4. An examination of the molecular mechanism of PELI1-induced B-cell lymphomagenesis

(A) Splenic $B220^+$ cells were isolated from non-Tg littermates and PELI1-Tg mice and maintained in the absence (-) or presence (+) of lipopolysaccharide (LPS). At 24 h after

treatment, splenic $B220^+$ cells were harvested, lysed, and subjected to immunoblotting for indicated proteins. **(B)** Splenic $B220^+$ cells were isolated from TetO-PELI1-rtTA mice, a doxycycline-inducible PELI1-Tg mice, maintained in the absence (-) or presence (+) of doxycycline, and further treated with LPS as indicated. Treated splenic $B220^+$ cells were lysed, separated into cytoplasmic (CE) and nuclear (NE) fractions, and immunoblotted with anti-IkBα, anti-p52, anti-p65, anti-100, anti-p105, anti-Rel B, anti-PELI1, anti-Lamin B (a marker for nuclear fractions), and anti-Actin (a marker for cytoplasmic fractions) antibodies as indicated.

Supplementary Figure 5. The polyubiquitinations of BCL6 by the PELI1 E3 ubiquitin ligase

To compare the K63-mediated polyubiquitination of BCL6 by PELI1 through K48-mediated polyubiquitination, HeLa cells were transfected with Myc-PELI1, HA-BCL6, and HA-Ub wild-type K48 or K63 mutant expression plasmids. At 24 h after transfection, cells were treated with LPS for 18 h and harvested for immunoprecipitation with an anti-BCL6 antibody. The BCL6 protein complex was subjected to immunoblotting with anti-Ubiquitin, anti-BCL6, and anti-PELI1 antibodies. The overexpression of HA-Ub K63 with Myc-PELI1 clearly induced the appearance of high-molecular-mass species of BCL6 polypeptides, whereas the overexpression of HA-Ub K48 weakly polyubiquitinated BCL6.

Supplementary Figure 6. A comparison of B-cell development between young and old PELI1-Tg mice

Populations of $B220^+$ B cells (B220) and $CD3^+$ T cells (CD3) were measured by flow cytometry using cells isolated from the spleen and lymph nodes of young (6 weeks of age) and old (14 months of age) non-Tg and PELI1-Tg mice measured by flow cytometry. Data are representative of three independent experiments with three mice per experiment (mean ±s.e.m). ns: not significant.

Supplementary Figure 7. The detailed gene expression analysis on PELI1-induced lymphoid tumors.

qRT-PCR analysis of gene expression in the spleen of non-Tg (n = 9) and PELI1-Tg mice (n = 9). A group of genes characteristic of GCB and post-GCB (or ABC-like) subtypes are selected for analysis as indicated (2, 3). Relative mRNA expression in non-Tg mice is set to

be 1 after normalization with GAPDH and Rn18S levels. The relative fold changes ratio of GCB and post-GCB transcripts in PELI1-Tg sample as compared to non-Tg sample is $\log_2$ transformed and displayed in a heat-map. A filter was applied to categorize genes with fold changes, greater (dark blue) or lower (light blue) than zero.

Supplementary Figure 8. The frequency of the 5'noncoding-region mutation of the BCL6 gene in DLBCL patients.

(**A**) Representative images of the DNA sequencing of the 5'noncoding region of the BCL6 gene based on genomic DNA isolated from paraffin sections of 69 DLBCL specimens. The BCL6 5'noncoding region spanning the first exon is shown in the upper diagram. The direct sequencing of the PCR product obtained from genomic DNA is shown in the lower diagrams. Arrows indicate the position of the mutation. F indicates forward sequencing, and R, reverse sequencing. For a mutational analysis of the BCL6 promoter region at the DNA level, the genomic fragment spanning 5'noncoding region was amplified using primers (+39 forward 5'-ACGCTCTGCTTATGAGGA -3'and reverse +300 5'-CGGCAGCAACAGCAATAA -3') (4). The PCR product was resolved on 1.2% agarose gels and visualized by ethidium bromide staining. The same amplicons were subsequently gel purified and sequenced. (**B**) The frequency of the 5'noncoding-region mutation of the BCL6 gene in 66 DLBCL specimens.

Supplementary Figure 9. The overall survival rate according to PELI1 expression in the R-CHOP group with DLBCLs

A Kaplan-Meier curve showing the overall survival (OS) rate for a cohort of 71 DLBCL patients treated with R-CHOP according to their PELI1 expression status. High-PELI1 cases were defined based on the intensity (2-3), extent (> 10%), and location (3) of PELI1 expression in tumor cells. Differences in the OS rate were analyzed through Kaplan-Meier method with the log-rank test. The sharp drop in the OS rate from 67% to 50% in the low-PELI1 group was likely due to poor survival responses of three patients after 85 months of treatment.

Supplementary Figure 10. An analysis of IgH locus rearrangements in lymphoma-bearing PELI1 transgenic samples

The rearrangement of the variable region of mouse IgH was analyzed (5). Genomic DNA was prepared from the spleen of PELI1-Tg or non-Tg mice by using a genomic DNA isolation kit

(QIAGEN). An analysis of the rearranged status of the IgH locus was conducted by PCR using the primers V_H588 5'-CGAGCTCTCCAACACAGCCTACATGCAACTCAAC-3'and J_H3 5'-GTCTAG ATTCTCACAAGAGTCCGATAGACCCTGG-3'. SM: standard DNA marker; N: non-transgenic spleen used as the control.

References

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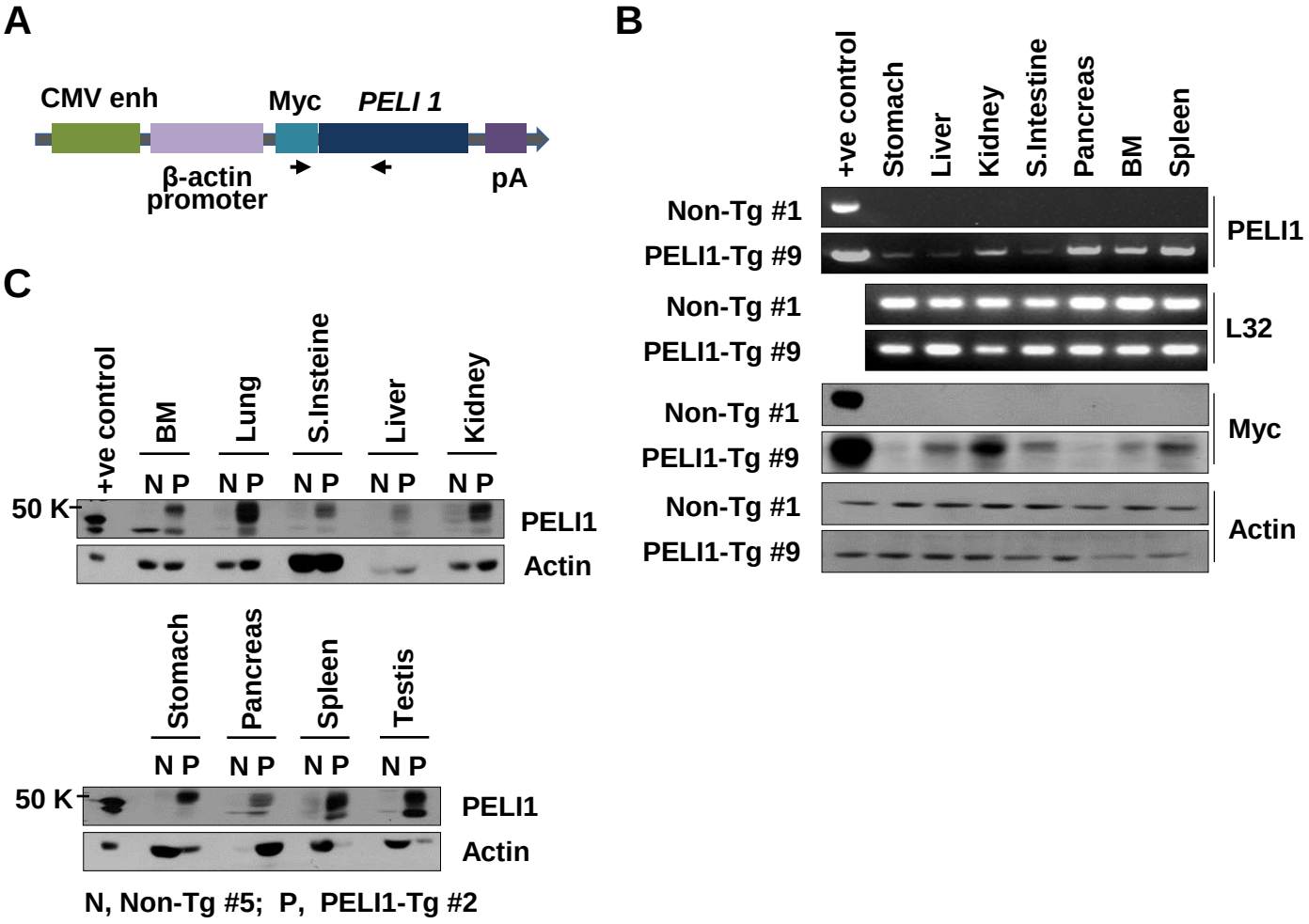
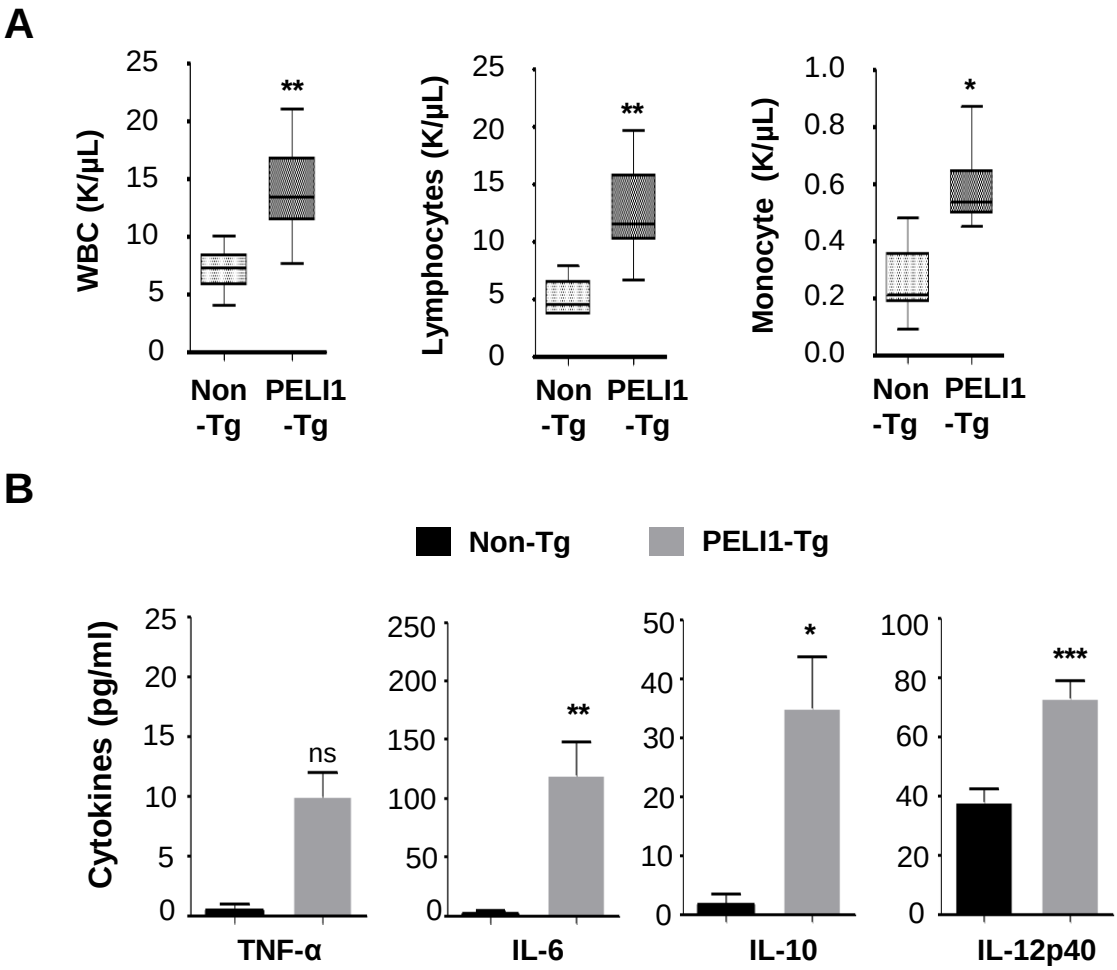
Supplementary Figure 1 (Park *et al*)



Supplementary Figure 2 (Park *et al*)



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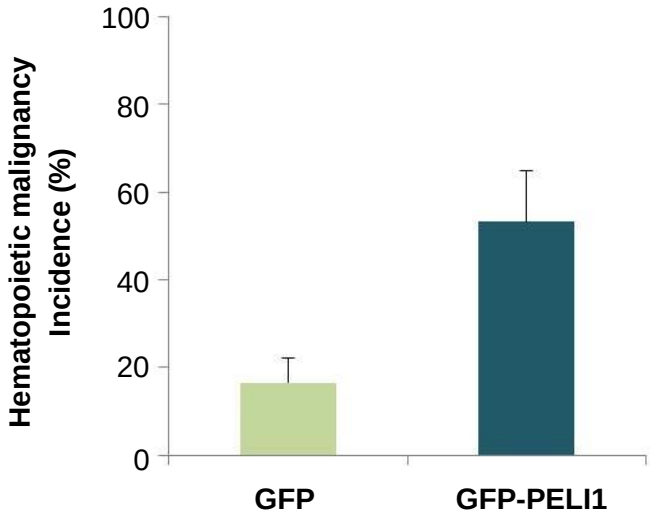

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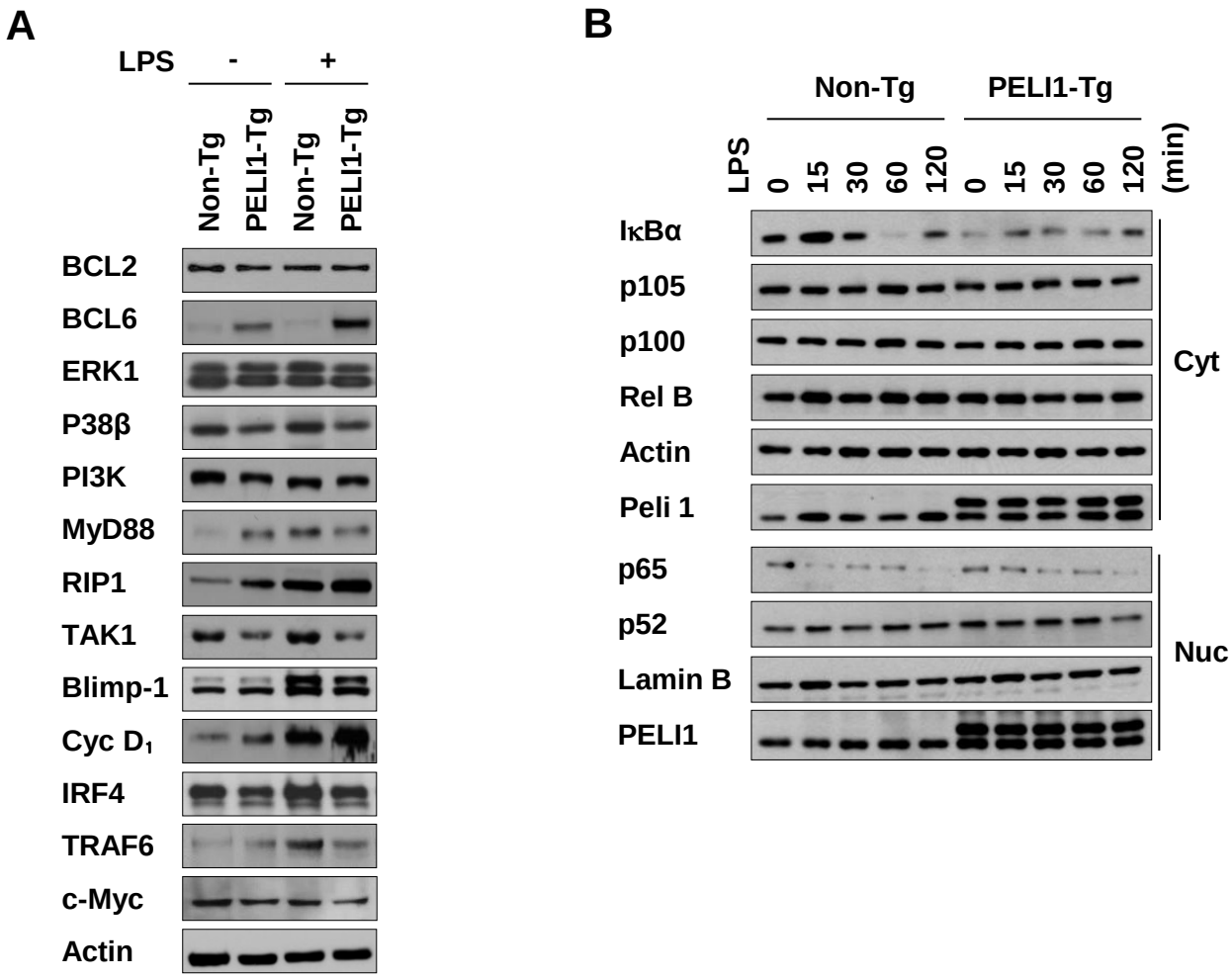

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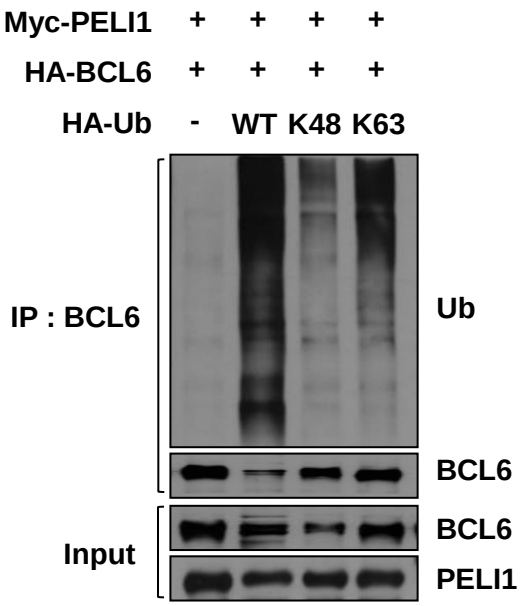

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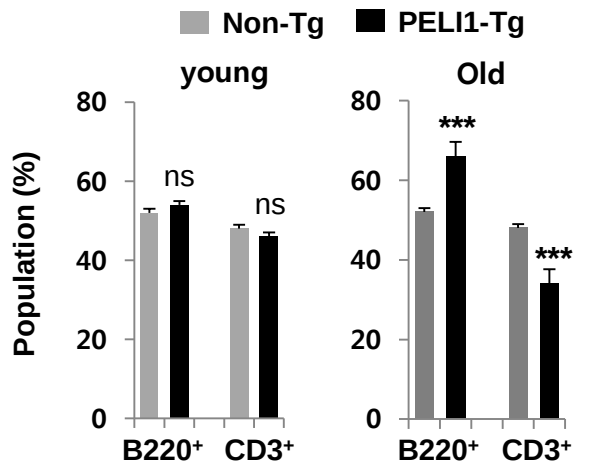
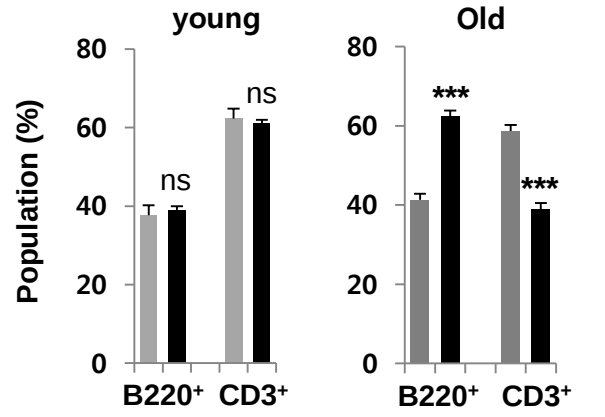
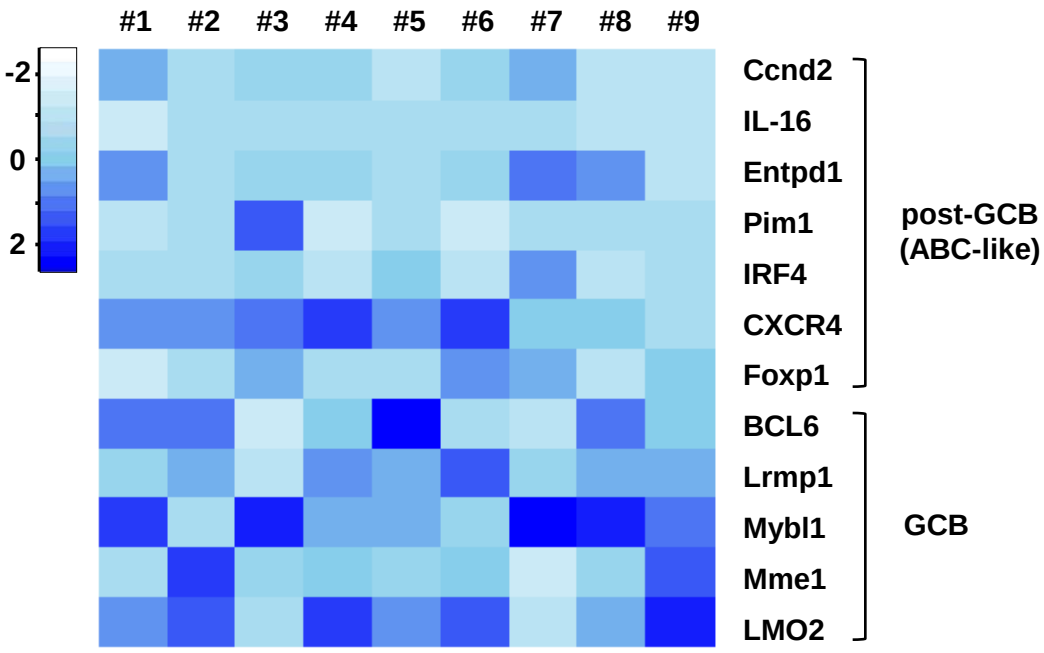


age	6 weeks	14 months
tumors in spleen	-	+



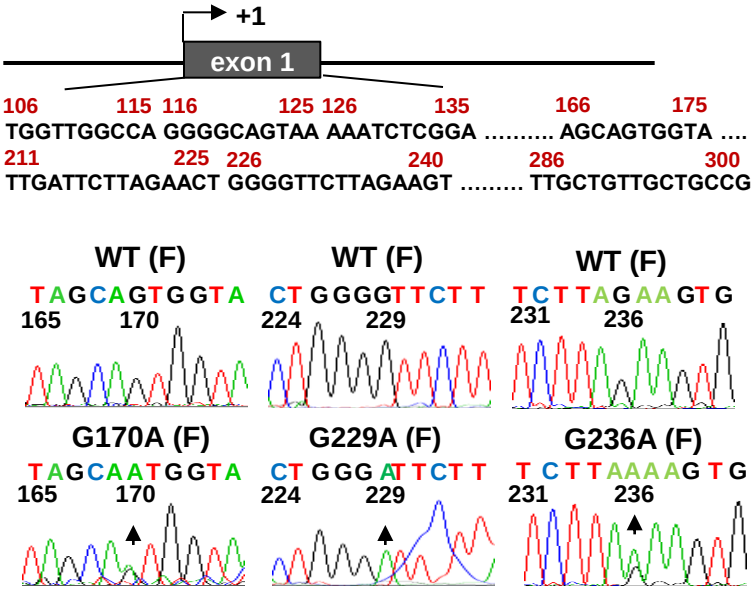
age	6 weeks	14 months
tumors in LNs	-	+

Supplementary Figure 7 (Park *et al*)



Supplementary Figure 8 (Park *et al*)

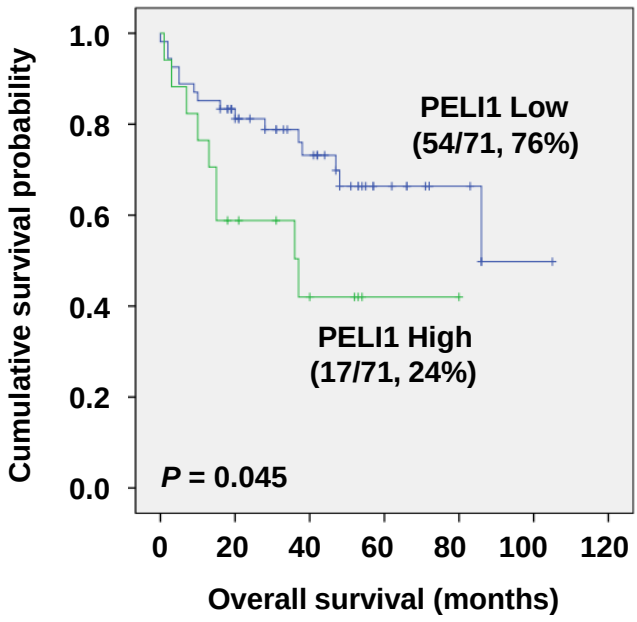
A



B

Sequence	Total (n)	Total (%)
Mutation G170A	1/66	4.5
Mutation G229A	1/66	
Mutation C236A	1/66	
No mutation	63/66	95.5

Supplementary Figure 9 (Park *et al*)



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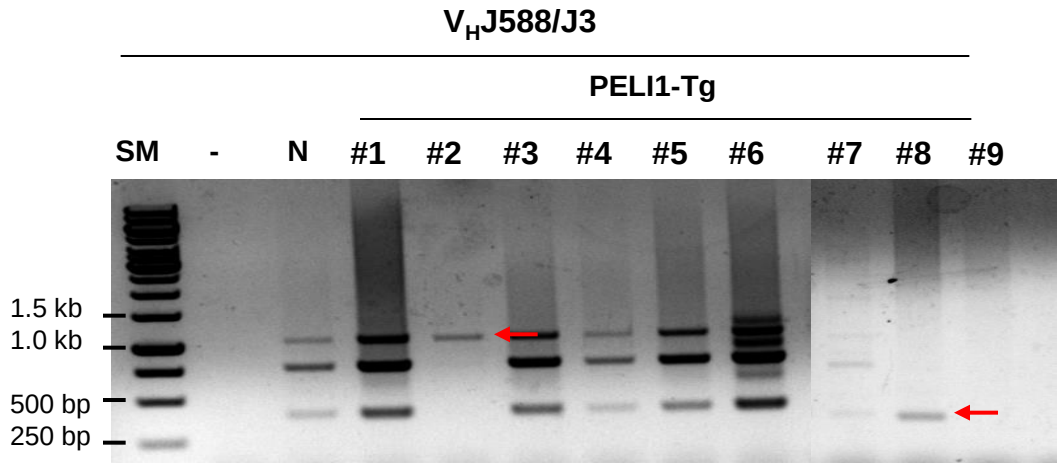

Supplementary Table 1. The identification of tumor cell types arose in the non-lymphoid organs of PELI1-Tg mice

Total tumor incidence (n = 49)	Tumor cell types	
	Carcinomas and adenomas	Lymphomas
Liver 19 (39%)	4 (22%)	15 (78%)
Lung 8 (16%)	2 (25%) + 1 (12%)*	5 (63%)
Intestine 7 (14%)	1 (14%) + 2 (29%)#	4 (57%)
Pancreas 7 (14%)	2 (29%)	5 (71%)
Prostate 1 (2%)	1 (100%)	0 (0%)

* Adenomas, # Tubular adenomas

Supplementary Table 2. Correlations of PELI1 expression with clinicopathologic features of DLBCL patients

		All	PELI1 expression		p-value
			Low	High	
		n = 113	n = 85	n = 28	
		n (%)	n (%)	n (%)	
Age	Mean (range)	57.7 (8-86)	57.3 (8-86)	58.9 (12-82)	0.626
	< 60	44 (51.8)	10 (35.7)	54 (47.8)	0.140
	≥ 60	41 (48.2)	18 (64.3)	59 (52.2)	
Sex	Male	46 (54.1)	16 (57.1)	62 (54.9)	0.78
	Female	39 (45.9)	12 (42.9)	51 (45.1)	
Primary site	Nodal	20 (23.5)	9 (32.1)	29 (25.7)	0.365
	Extranodal	65 (76.5)	19 (67.9)	84 (74.3)	
Stage	1. 2	50 (61)	13 (46.4)	63 (57.3)	0.179
	3, 4	32 (39)	15 (53.6)	47 (42.7)	
B symptom	Absent	65 (79.3)	22 (78.6)	87 (79.1)	0.938
	Present	17 (20.7)	6 (21.4)	23 (20.9)	
Bulky disease	Absent	70 (85.4)	23 (82.1)	93 (84.5)	0.684
	Present	12 (14.6)	5 (17.9)	17 (15.5)	
ECOG performance status	0, 1	72 (87.8)	16 (59.3)	88 (80.7)	0.001
	≥ 2	10 (12.2)	11 (40.7)	21 (19.3)	
LDH	Normal	37 (51.4)	7 (28)	44 (45.4)	0.043
	Elevated	35 (48.6)	18 (72)	53 (54.6)	
No. of extranodal sites	0, 1	64 (78)	22 (81.5)	86 (78.9)	0.705
	≥ 2	18 (22)	5 (18.5)	23 (21.1)	
BM involvement	Absent	70 (89.7)	21 (80.8)	91 (87.5)	0.231
	Present	8 (10.3)	5 (19.2)	13 (12.5)	
IPI group	Low to intermediated (0-2)	50 (68.5)	15 (57.7)	65 (65.7)	0.319
	High (3-5)	23 (31.5)	11 (42.3)	34 (34.3)	
BLC6 translocation	Absent	87 (77)	62 (72.9)	25 (89.3)	0.118*
	Present	26 (23)	23 (27.1)	3 (10.7)	
Choi classifier	GCB	22 (29.7)	10 (38.5)	32 (32.0)	0.412
	ABC	52 (70.3)	16 (61.5)	68 (68.0)	
EBV ISH	Negative	71 (94.7)	26 (96.3)	97 (95.1)	0.737
	Positive	4 (5.3)	1 (3.7)	5 (4.9)	

*Fisher exact test; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; BM, bone marrow; IPI, international prognostic index; GCB, germinal center B-cell-like; ABC, activated B cell-like; EBV ISH, Epstein-Barr virus In situ hybridization

Supplementary Table 3. Pearson correlation coefficients between PELI1 and BCL6 expression in GCB and non-GCB patients

GCB patients 35/102 (34%)		**BCL6 Intensity X Extent**	**PELI1 Intensity X Extent X Location**
BCL6 Intensity X Extent	Pearson correlation	1	0.341*
	Sig. (2-tailed)		0.045
	N	35	35
PELI1 Intensity X Extent X Location	Pearson correlation	0.341*	1
	Sig. (2-tailed)	0.045	
	N	35	35

*Correlation is significant at the 0.05 level (2-tailed).

non-GCB patients 67/102 (66%)		**BCL6 Intensity X Extent**	**PELI1 Intensity X Extent X Location**
BCL6 Intensity X Extent	Pearson correlation	1	0.542**
	Sig. (2-tailed)		< 0.001
	N	67	67
PELI1 Intensity X Extent X Location	Pearson correlation	0.542**	1
	Sig. (2-tailed)	< 0.001	
	N	67	67

**Correlation is significant at the 0.01 level (2-tailed). Sig, significant

Supplementary Table 4. Multivariate overall survival analysis in all patients with DLBCL

Variable		Category	Univariate analysis			Multivariate analysis		
			HR	95% CI	P-value	HR	95% CI	p-value
Comparison with risk factors								
PELI1		Low vs. High	2.576	1.338-4.957	0.005	3.745	1.674-8.376	0.001
Age, years		< 60 vs. ≥ 60	2.671	1.324-5.390	0.006	-	-	-
Sex		Male vs. Female	0.780	0.409-1.486	0.450	-	-	-
Primary site		Nodal vs. Extranodal	0.586	0.299-1.146	0.118	-	-	-
Stage		1, 2 vs. 3, 4	3.233	1.665-6.279	0.001	6.055	2.392-15.33	<0.001
B symptom		Absent vs. Present	2.299	1.157-4.568	0.017	-	-	-
Bulky disease		Absent vs. Present	1.034	0.432-2.475	0.941	-	-	-
Performance status		0, 1 vs. ≥ 2	3.097	1.553-6.175	0.001	-	-	-
LDH		Normal vs. Elevated	4.006	1.812-8.857	0.001	-	-	-
No. of extranodal sites		0, 1 vs. ≥ 2	0.988	0.434-2.250	0.977	-	-	-
Bone marrow involvement		Absent vs. Present	1.598	0.663-3.853	0.297	-	-	-
BCL6 translocation		Absent vs. Present	0.344	0.122-0.971	0.044	-	-	-
EBV ISH		Absent vs. Present	1.763	0.421-7.371	0.438	-	-	-
Comparison with IPI group								-
All patients*	Peli 1	Low vs. High				2.443	1.243-4.803	0.010
	IPI score	0 - 2 vs. 3 - 6	4.309	2.179-8.521	<0.001	4.029	2.033-7.988	<0.001
R-CHOP group**	Peli 1	Low vs. High				2.165	0.930-5.041	0.073
	IPI score	0 - 2 vs. 3 - 6				2.818	1.224-6.488	0.015

HR, hazard ratio; CI, confidence interval; LDH, lactate dehydrogenase; IPI, international prognostic index; EBV ISH, Epstein-Barr virus In situ hybridization. *Multivariate overall survival analysis in all patients with DLBCL. **Multivariate overall survival analysis in R-CHOP group with DLBCL.