PELI2 regulates early B-cell progenitor differentiation and related leukemia via the IL-7R expression

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Supplementary Files:

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Supplemental Information

1. Supplementary Materials and Methods

Patient samples

Bone marrow (BM) samples from BCP-ALL patients and healthy donors without any malignant bone marrow disorder were obtained at the Qilu Hospital of Shandong University, China. Informed consent was obtained in accordance with the Declaration of Helsinki. Acquisition of bone marrow samples was performed with the informed consent of the patients. The use of human tissues was approved by the Medical Institutional Ethics Committee of Qilu Hospital, Shandong University, China. Bone marrow mononuclear cells were isolated by density gradient centrifugation using lymphocyte separation medium (Haoyang, China).

Generation of *PELI2* conditional knockout mice

We made *PELI2* conditional knockout mice via CRISPR/Cas9-mediated genome engineering. Briefly, Cas9 mRNA, sgRNA and donor were co-injected into zygotes. sgRNAs (5'-TGGGATTACAGCTGACAATT-3', 5'-ATCGGCAGCTAAACTTCCCA-3') directed Cas9 endonuclease cleavage in intron 1-2 and intron 2-3 and created a double-strand break. LoxP sites were inserted into intron 1-2 and intron 2-3 respectively by homologous recombination. LoxP sites were verified by DNA sequencing. Chimeric mice were generated by standard methods.

PELI2 deletion was achieved by crossing with transgenic Vav-cre mice (B6.Cg-Commd10Tg(Vav1-icre)A2Kio/J) purchased from Jackson laboratory (Bar Harbor, USA), by which exon 2 floxed by loxP sites were deleted in hematopoietic cells. This led to the disruption of *PELI2* expression. *PELI2*$^{fl/fl}$ littermate mice were genotyped by PCR with primers *PELI2*-tF1 (5'-GTGCCAGCAAAGAGTCTTAGTC-3') and *PELI2*-tR1(5'-GTAAACAAGTCCTGAGGTAGACTGC-3'). Excision after Vav-Cre recombination was confirmed by PCR with primers to detect a portion that remains post excision (*PELI2*-null-TF1:5'-GTCAAGCCAAGGGTCTATTGGG-3' and *PELI2*-null-TR1: 5'-CCTTCTAATCCTCCCTAAGCAGTACC-3'). *PELI2*$^{flox/flox}$ Ubc-cre-ERT2 mice (*PELI2*^{IKO}) were crossed with Ubc-cre-ERT2 mice (B6.Cg-Ndor1^{Tg}(UBC-cre/ERT2)1Ejb/2J) purchased from Jackson laboratory (Bar Harbor, USA). Excision after Ubc-cre-ERT2 recombination was confirmed by PCR with primers Universal-Cre-F (5'-ATTTGCCTGCATTACCGGTCG-3') and Universal-Cre-F (5'-CAGCATTGCTGTCACTTGGTC-3').

For treatment of tamoxifen, mice were injected intraperitoneally with tamoxifen (Sigma, 1 mg per mouse) in corn oil daily for five consecutive days and analyzed 7 days after the last injection. All mice were maintained on the C57BL/6 genetic background. C57BL/6 recipient mice were purchased from Charles River Laboratories (Beijing, China). Experiments were performed on age- and sex-matched cohorts. All animal studies were performed in

accordance with the Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees at Shandong University.

Cell culture

HEK293T cells were purchased from the Cell Bank of the Shanghai Institute for Biological Sciences, Chinese Academy of Science. HEK293T cells were cultured in Dulbecco's modified eagle medium (Gibco), and Nalm-6 and 697 cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum (Biological Industries) and 1% penicillin/streptomycin, respectively.

Lineage negative (Lin^-) bone marrow cells were cultured in StemSpan medium (StemCell Technologies) supplemented with mouse IL-3 (10 ng/mL, Peprotech), mouse IL-6 (10 ng/mL, Peprotech), mouse stem cell factor (50 ng/mL, Peprotech), and human low-density lipoprotein (20 μg/mL, StemCell Technologies).

Human $CD34^+$ cells were incubated in IMDM medium supplemented with 10% FBS in the presence of human granulate-macrophage colony-stimulating factor (100 ng/mL, Peprotech), human stem cell factor (100 ng/mL, Peprotech), human IL-3 (20 ng/mL, Peprotech), and human IL-6 (20 ng/mL, Peprotech).

Plasmids construction and cell transduction

Retroviral shRNA oligonucleotides against *PELI2*, TCF3, E2F1, MYB, GATA3 were cloned into pLKO.1 vector. The gene overexpression plasmids were constructed by standard molecular cloning: MIG-*PELI2* encodes mouse *PELI2* with indicated domain (FL:1-419aa; F1:1-287aa; F2: 1-168aa; F3: 87-287aa; R: 287-419aa) with a HA tag, MIG-PU.1 encodes mouse PU.1 with indicated domain (FL:1-282aa; ETS+PEST:112-282aa; ETS: 170-282aa) with a Flag tag. PLV-*PELI2* encodes human *PELI2* with indicated domain (FL:1-421aa; F1:1-287aa; F2: 1-168aa; F3: 87-287aa; R: 287-421aa) with a HA tag, PLV-TCF3 encodes human TCF3 with indicated domain (FL: 1-654aa; T1: 1-369aa; T2: 153-485aa; T3: 369-654) with a Flag tag. For luciferase assays, the human or mouse *IL-7R* promotor was subcloned into the pGL3-basic plasmid (Promega).

Lentivirus supernatants were prepared by transient cotransfection of HEK293T cells with package plasmids and lentiviral constructs encoding indicated object plasmid. Viral supernatants were collected after 48 and 72 hours.

For transduction, BM Lin^- cells were cultured overnight in StemSpan medium showed above. Lentiviral supernatants were applied to culture dishes and centrifuged at 2,500 rpm for 90 minutes in the presence of polybrene (8 μg/mL) and then incubated at 37 °C for an additional 24 hours. And cells were then transfected again. Nalm-6 and 697 cells were transfected once with object lentivirus by spin infection at 37 °C (1800 rpm, 90 min).

Bone marrow transplantation

Noncompetitive BM transplantation and competitive BM transplantation were performed as previously described.[1] For secondary transplantation assays, 2×10^6 total BM cells from 6 months post BMT mice were transplanted into lethally irradiated mice ($CD45.1^+$).

Limiting dilution assays

Different doses (10,000, 20,000, 40,000, 80,000) of BM cells from $PELI2^{CKO}$ and WT mice ($CD45.2^+$) together with 200,000 competitor cells ($CD45.1^+$) were transplanted into lethally irradiated (8.5 Gy) recipient mice ($CD45.1^+$). The percentage of donor-derived ($CD45.2^+$) cells was analyzed 16-weeks following transplantation as described above. HSC frequency was calculated using L-Calc software (StemCell Technologies Inc.) and ELDA software (bioinf.wehi.edu.au/software/elda/).

Homing assays

A total of 2×10^6 $PELI2^{CKO}$ and WT BM cells ($CD45.2^+$) were injected into lethally irradiated recipient mice ($CD45.1^+$). BM cells were harvested 18 hours following injection and the frequency of donor-derived cells ($CD45.2^+$) was evaluated by flow cytometry. Homing efficiency was calculated as: absolute numbers of $CD45.2^+$ $lineage^-$ cells / absolute numbers of $CD45.2^+$ $lineage^-$ cells transplanted.

Human BCP-ALL xenograft

The 6-week-old male NSG mice (Charles River Laboratories, Beijing, China)

were irradiated at 1 Gy before tail vein injection of 5×10^6 Nalm-6 cells infected with object virus. Mice were killed when demonstrating characteristic disease symptoms or becoming moribund. Cells were then isolated from spleen and bone marrow and the human CD45 and CD19 surface markers were assessed by flow cytometry analysis of leukemia burden *in vivo*.

BCP-ALL Patient-Derived Xenografts

Bone marrow mononuclear cells from BCP-ALL patients were purified by density gradient centrifugation using lymphocyte separation medium (Haoyang, China), and then cultured in IMDM medium supplemented with 10% FBS in the presence of granulate-macrophage colony-stimulating factor (GM-CSF, 100 ng/mL), stem cell factor (SCF, 100 ng/mL), IL-3 (20 ng/mL), IL-6 (20 ng/mL) and IL-7 (10 ng/mL). After overnight pre-stimulation, these primary cells were transduced with lentivirus encoding *PELI2* or non-target shRNA, followed by the second transduction after 12 hours. The cells were then transplanted into irradiated (1 Gy) 8-week-old female NSG recipient via tail vein injection (2×10^6 cells/mouse). After two months, cells from leukemic NSG mice were harvested and stained. The percentage of leukemic cells ($hCD45^+hCD19^+$) in bone marrow and spleen were analyzed by flow cytometry.

Flow cytometric analysis and cell sorting

Single-cell suspensions of BM, spleen and thymus were prepared and stained with a ‘‘cocktail’’ of antibody conjugates for 20 min at room temperature in flow

cytometry buffer. Data were collected on a FACS and were analyzed with NovoExpress software.

For cell apoptosis assays, cells were stained with surface markers and Annexin V/ propidium iodide (PI) for 15 min at room temperature in the dark according to the manufacturer's instructions, and were analyzed by flow cytometry.

For BrdU labeling, mice were injected intraperitoneally once with 100 μL BrdU solution (10mg/mL) and analyzed 3 hours later. Surface markers, and BrdU incorporation were detected and analyzed using flow cytometry.

For cell cycle assays, cells were collected and fixed by slowly adding ice-cold 75% ethanol in a flow cytometry tube with vortex. Following incubation for at least 6 hours at -20 °C, PI was added to the cell suspension for 30min at 37 °C and then analyzed on flow cytometer. The DNA content of the cells was analyzed for the percentage of cells in G0G1, S and G2/M phase.

For Ki67 staining, BM cells were fixed using Foxp3 Fixation/Permeabilization working solution (Thermo Fisher) according to the manufacturer's protocol. Then the cells were stained with mouse anti-Ki67 (Biolegend) and resuspended in Hoechst-containing FACS buffer. For intracellular staining of phospho-Stat5, freshly sorted $B220^{+}$ cells were stimulated with IL-7 for 30 min at 37 °C, and then were fixed as above and stained with anti-phospho-Stat5-APC.

For CLPs cell sorting, cells from BM were first depleted of mature hematopoietic cells with a lineage cell depletion kit (BD) and isolated by FACS cell sorter

(Beckman Coulter). For B220$^+$ cells sorting, freshly isolated BM cells were stained with biotin-B220 (BD) and then purified by magnetic bead depletion following the manufacturer's instructions (STEMCELL Technologies). Purified cells were shown to be highly enriched (≥87%).

Colony forming assays

For pre-B colony forming assays from BM Lin$^-$ cells, single-cell suspensions from *PELI2*^{CKO} and WT mice were plated in 35-mm dishes (35 cells/dish) with MethoCult M3630 (StemCell Technologies) supplemented with IL-7 according to the manufacturer's instructions. Cells were cultured at 37 °C with 5% CO_2, and colonies were counted and morphology assessed 7 days of culture.

CD34$^+$ cells from BM mononuclear cells of BCP-ALL were obtained using human CD34$^+$ selection cocktail (STEMCELL). CD34$^+$ cells were infected with *PELI2* knockdown lentivirus twice for 24 hours, and then the cells were seeded at 1000 cells in 35-mm dishes with the stem cell medium (H4434, STEMCELL), and then continuously incubated at 37°C in 5% humidified CO2. After incubation for 14 days, the colonies were counted.

Luciferase reporter assays

HEK293T cells were plated and then transfected with promoter plasmid pGL3-*IL-7R* with indicated plasmids. After 24 hours, cells were lysed and subjected to luciferase reporter assays according to manufacturer's instructions (Promega). pRL-MCV plasmid (Rellina luciferase) (Promega) was included as

an internal control. The fold change of relative luciferase intensity was calculated based on the ratio of the Firefly and Rellina luciferase activities.

Immunoblotting

Cells were lysed in RIPA buffer (Beyotime) with protease and phosphatase inhibitor mixture (Beyotime) for 30 min, then centrifuged at 12000 rpm, 4°C. The protein concentration was quantified by bicinchoninic acid (BCA) protein kit (Beyotime). The protein expression levels were determined by staining with primary antibodies.

Co-immunoprecipitation

The cells were harvested and suspended in 1000 μL Pierce IP lysis buffer (Thermo Fisher Scientific) supplemented with a proteinase inhibitor cocktail and incubated on ice for 30 min followed by centrifugation at 13 000 rpm for 30 min at 4°C. The cleared cell lysates were immunoprecipitated with beads conjugated with indicated antibodies at 4°C for 12 hours. The beads were then washed with IP buffer five times and boiled at 95 °C in 2× NuPAGE LDS Sample Buffer (Thermo Fisher Scientific) for 10 min. Aliquots were analyzed by immunoblotting.

RNA-sequencing

Total RNA of B220$^+$ cells were extracted using the Trizol reagent according to the manufacturer’s instructions (Invitrogen). RNA quality and quantity were determined using a Nano Drop and Agilent 2100 bioanalyzer (Thermo Fisher

Scientific). RNA sequencing was conducted using the BGIseq500 platform (Shenzhen, China). Genes with a false discovery rate less than 0.05 with a fold change more than 1.5 or less than 1.5 were considered differentially expressed genes.

EdU cell proliferation assays

EdU analysis of Nalm-6 cells were performed using the Cell-Light EDU Apollo567 *In vitro* Kit (RIBBIO). 1 $\times 10^5$ cells were harvested and labeled with 50μM EdU in medium for 2 hours in 37°C incubator. Cells were washed once with 1× PBS and the pellets were resuspended in 100 μL 4% PFA for 30 minutes at room temperature. After fixation, cells were washed with 2 mg/mL glycine for 5 minutes and then permeabilization with PBS containing 0.5% Triton X-100. After the 10-minute incubation period, 100 μL Apollo Stain mix was added to each sample and incubated for 30 minutes at room temperature. Washed three times with PBS containing 0.5% Triton X-100, DNA staining, covered slip, and observed by fluorescence microscope.

mRNA extraction and quantitative RT-PCR

Total RNA was extracted using the Trizol reagent according to the manufacturer's instructions (Invitrogen). RNA quality and quantity were determined using a Nano Drop and Agilent 2100 bioanalyzer (Thermo Fisher Scientific). RNA was reversely transcribed into cDNA using RT reagent kit (TaKaRa, Japan). A SYBR Green PCR kit was used for quantitative real-time

PCR and results were quantified with an Applied Biosystem System (ABI) with appropriate primers. The human housekeeping gene 18S was used as the RNA-loading control. Gene expression was determined by the delta CT method ($2^{-\Delta\Delta Ct}$). ΔCt = (Ct target gene – Ct housekeeping).

Statistical analysis

All statistical analysis were performed using Prism8 (GraphPad Software). All data were presented as mean±SD except where indicated otherwise. All comparisons were tested using unpaired two-tailed Student's t-test except where indicated otherwise. A *P* value of less than 0.05 was considered statistically significant. Survival was presented with a Kaplan-Meier survival plot. Blinding was performed for *in vitro* experiments with data analysis by different operators.

References

1. Lu Z, Huang L, Li Y, et al. Fine-Tuning of Cholesterol Homeostasis Controls Erythroid Differentiation. Adv Sci (Weinh). 2022;9(2):e2102669.

2. Supplemental Tables

Supplemental Table 1. Definition of hematopoietic cell types.

Hematopoietic Population	**Markers**
Lineage$^-$ Sca-1$^+$ c-Kit$^+$ (LSKs)	Lineage$^-$ Sca-1$^+$ c-Kit$^+$
SLAM-LSKs	CD48$^-$ CD150$^+$ LSK
Lineage$^-$ Sca-1$^-$ c-Kit$^+$ (LKs)	Lineage$^-$ Sca-1$^-$ c-Kit$^+$
Common myeloid progenitors (CMPs)	CD34$^+$ CD16/32$^-$ LK
Granulocyte/monocyte progenitors (GMPs)	CD34$^+$ CD16/32$^+$ LK
Megakaryocyte/erythrocyte progenitor (MEPs)	CD34$^-$ CD16/32$^-$ LK
Common Lymphoid Progenitors (CLPs)	Lineage$^-$ IL-7R$^+$ Sca1^{low} c-Kit^{low}
B cell-biased lymphoid progenitor (BLP)	Ly6D$^+$ CLPs
all-lymphoid progenitor (ALP)	Ly6D$^-$ CLPs
Pre-pro-B cells	B220$^+$ IgM$^-$ IgD$^-$ CD43$^+$ CD24$^-$
Pro-B cells	B220$^+$ IgM$^-$ IgD$^-$ CD43$^+$ CD24$^+$
Pre-B cells	B220$^+$ IgM$^-$ IgD$^-$ CD43$^-$ CD24$^+$
Immature BM B cells	B220$^+$ IgM$^+$ IgD$^-$
Mature BM B cells	B220$^+$ IgM$^+$ IgD$^+$
Double-negative thymocytes (DN)	CD4$^-$ CD8$^-$
CD4+ single-positive thymocytes	CD4$^+$ CD8$^-$
CD8+ single-positive thymocytes	CD4$^-$ CD8$^+$
Double-positive thymocytes (DP)	CD4$^+$ CD8$^+$

Supplemental Table 2. Primer sequences used in this study.

Gene	Oligonucleotides
Human-*PELI2*-shRNA-3	Forward：5’-CCGGGAACCTTACACAGCACGGATACTCGAGTA TCCGTGCTGTGTAAGGTTCTTTTTG-3′
	Reverse：5’-AATTCAAAAAGAACCTTACACAGCACGGATACTC GAGTATCCGTGCTGTGTAAGGTTC-3′
Human-*PELI2*-shRNA-4	Forward：5’-CCGGGCCTCATGGAACTCATGCATTCTCGAGAAT GCATGAGTTCCATGAGGCTTTTTG-3′
	Reverse：5’-AATTCAAAAAGCCTCATGGAACTCATGCATTCTCG AGAATGCATGAGTTCCATGAGGC-3′
Human-TCF3-shRNA-1	Forward: 5’-CCGGCCCGGATCACTCAAGCAATAACTCGAGTTATT GCTTGAGTGATCCGGGTTTTTG-3′
	Reverse：5’-AATTCAAAAACCCGGATCACTCAAGCAATAACTCGAG TTATTGCTTGAGTGATCCGGG-3′
Human-TCF3-shRNA-2	Forward：5’-CCGGATTGTGCCTAAGCGAAATATTCTCGAGAATATTTC GCTTAGGCACAATTTTTTG-3′
	Reverse：5’-AATTCAAAAAATTGTGCCTAAGCGAAATATTCTCGAG AATATTTCGCTTAGGCACAAT-3′
Human-HA-*PELI2*-FL	Forward：5’- CAGATCTCGAGCTCAAGCTTCGAATTCATGTACCCATACGACGT CCCAGACTACGCTATGTTTTCCCCTGGCCAGGAG -3′
	Reverse：5’- CTCCCCTACCCGGTAGAATTATCTAGATCAGTCA ATTGGACCTTGGAA-3′
Human-HA -*PELI2*-F1	Forward：5’-CAGATCTCGAGCTCAAGCTTCGAATTCATGTACCCATACGACGT CCCAGACTACGCTATGTTTTCCCCTGGCCAGGAG-3′
	REVERSE：5’-CTCCCCTACCCGGTAGAATTATCTAGATCACCCC ACAGGACACTGAGG-3′
Human-HA -*PELI2*-F2	Forward：5’-CAGATCTCGAGCTCAAGCTTCGAATTCATGTACCCATACG ACGTCCCAGACTACGCTATGTTTTCCCCTGGCCAGGAG-3′
	Reverse：5’-CTCCCCTACCCGGTAGAATTATCTAGATCAAAG AAATATGTTTTTGGA-3′
Human-HA -*PELI2*-F3	Forward：5’-CAGATCTCGAGCTCAAGCTTCGAATTCATGTACCCATACGAC GTCCCAGACTACGCTATGACTGTGGTGGTGGAGTAC-3′
	Reverse：5’-CTCCCCTACCCGGTAGAATTATCTAGATCACCCC

	ACAGGACACTGAGG-3′
Human-HA -*PELI2*-R	Forward：5’-CAGATCTCGAGCTCAAGCTTCGAATTCATGTACCCATA CGACGTCCCAGACTACGCTATGCTCAACACCCTGGCCTTC-3′
	Reverse：5’-CTCCCCTACCCGGTAGAATTATCTAGATCAGTC AATTGGACCTTGGAA-3′
Human-Flag-TCF3-FL	Forward：5’-AGATCTCGAGCTCAAGCTTCGAATTCATGGATTACAAG GATGACGACGATAAGATGAACCAGCCGCAGAGGAT-3’
	Reverse：5’- CTCCCCTACCCGGTAGAATTATCTAGATCACATGTGC CCGGCGGGGTTGTG-3’
Human-Flag-TCF3-T1	Forward：5’-AGATCTCGAGCTCAAGCTTCGAATTCATGGATTACAA GGATGACGACGATAAATGAACCAGCCGCAGAGGAT-3’
	Reverse：5’- CTCCCCTACCCGGTAGAATTATCTAGATCACCAC TGTGACGTTCCTGCCAG-3’
Human-Flag-TCF3-T2	Forward：5’- CAGATCTCGAGCTCAAGCTTCGAATTCATGGATTACAAGGAT GACGACGATAAGATGTCCGGCAGCTCCCGGCGG-3’
	Reverse：5’- CTCCCCTACCCGGTAGAATTATCTAGATCACTTTTC CTCTTCTCGCCGTTT-3’
Human-Flag-TCF3-T3	Forward：5’- CAGATCTCGAGCTCAAGCTTCGAATTCATGGATTACAAG GATGACGACGATAAGATGCCTCGAGCAGGAGCCCCC-3’
	Reverse：5’- CTCCCCTACCCGGTAGAATTATCTAGATCACATGTGC CCGGCGGGGTTGTG-3’
Mouse-HA -*PELI2*-FL	Forward：5’-TAGATCTCTCGAGGTTAACGAATTCATGTACCC ATACGACGTCCCAGACTACGCTATGTTTTCCCCGGGCCAGGAG-3′
	Reverse：5’-TTAGGGGGGGGGGGCGGAATTCTCAGTCCA CTGGACCTTGGAA-3′
Mouse-HA -*PELI2*-F1	Forward：5’-TAGATCTCTCGAGGTTAACGAATTCATGTACCCATAC GACGTCCCAGACTACGCTATGTTTTCCCCGGGCCAGGAG-3′
	Reverse：5’-TTAGGGGGGGGGGGCGGAATTCTCAAAGGCCCA CGGGGCACT-3′
Mouse-HA -*PELI2*-F2	Forward：5’-TAGATCTCTCGAGGTTAACGAATTCATGTACCCATAC GACGTCCCAGACTACGCTATGTTTTCCCCGGGCCAGGAG-3′
	Reverse：5’-TTAGGGGGGGGGGGCGGAATTCTCATCCAAG AAAGATATTTTTGG-3′
Mouse-HA -*PELI2*-F3	Forward：5’-TAGATCTCTCGAGGTTAACGAATTCATGTACCCATAC

	GACGTCCCAGACTACGCTATGACGGTAGTGGTGGAGTACACA-3′ Reverse：5’-TTAGGGGGGGGGGGCGGAATTCTCAAAGGCC CACGGGGCACT-3′
Mouse-HA -*PELI2*-R	Forward：5’-TAGATCTCTCGAGGTTAACGAATTCATGTACCCATAC GACGTCCCAGACTACGCTATGAACACCCTGGCCTTCCC-3′ Reverse：5’-TTAGGGGGGGGGGGCGGAATTCTCAGTCCACTGGA CCTTGGAA-3′
Mouse-Flag-Pu.1-FL	Forward：5’-TAGATCTCTCGAGGTTAACGAATTCATGGATTACAAGG ATGACGACGATAAGATGTTACAGGCGTGCAAAATGG-3′ Reverse：5’-TTAGGGGGGGGGGGCGGAATTCTCAGTGGGGCGGGAGGCG-3′
Mouse-Flag -Pu.1-PEST+ETS	Forward：5’-TAGATCTCTCGAGGTTAACGAATTCATGGATTACAAGG ATGACGACGATAAGATGCCACCCCACACCGGCCTCA-3′ Reverse：5’- TTAGGGGGGGGGGGCGGAATTCTCAGTGGGGCG GGAGGCG -3′
Mouse-Flag -Pu.1-ETS	Forward：5’-TAGATCTCTCGAGGTTAACGAATTCATGGATTACAAGG ATGACGACGATAAGATGCTTCTGCACGGGGAGACA-3′ Reverse：5’-TTAGGGGGGGGGGGCGGAATTCTCAGTGGGGCGGGAGGCG-3′
Mouse-IL-7R promotor	Forward：5’- TCTATCGATAGGTACCCTGGTATTTGTCAACCTGCCAG -3′ Reverse：5’- GAGTCAGTGCCCGGGCTAGCCATTCTGAGAGAGAGAG AGAGAGAG -3′
Human-IL-7R promotor	Forward:5’- AGAACATTTCTCTATCGATAGGTACCTTCAAAACAAGA TTAGAAATGAGGGC-3’ Reverse：5’-CTTGATGAGTCAGTGCCCGGGCTAGCGACAGAGAAG CTTAGATCTAGGG-3’
Mouse IL-7R promoter regions	Forward：5’-CCACCTTGCTCAAAAGTAAGC-3’ Reverse：5’-GAAGCACGCTTGTATGTGC-3’
Human IL-7R promoter regions	Forward：5’-TCTCATCTACTTAGCTCTGCCATT-3’ Reverse：5’-TCTTACACACCACAGTAGGCATTC-3’
Human-Ki67 primer	Forward：5’-CTTTGGGTGCGACTTGACGA-3′ Reverse：5’-ACAACTCTTCCACTGGGACG-3′
Human-*PELI2* primer	Forward：5’-CCCCCAATAAGGAGCCAGTG-3′ Reverse：5’-GCTGATAGCCTTGGATGCCT-3′
Human-IL-7R primer	Forward：5’-TGTCGCTCTGTTGGTCATCT-3′ Reverse：5’-AATCTGGCAGTCCAGGAAACT-3′

Human-TCF3 primer	Forward：5’-AGAAGCCCCAGACCAAACTG-3’
	Reverse：5’-GGGAGCTGAAAGCACCATCT-3’
Human-MYB primer	Forward：5’-AGTCAATGTCCCTCAGCCAG-3’
	Reverse：5’-GCTGCATGTGTGGTTCTGTG-3’
Human-GATA3 primer	Forward：5’-GAAGGCAGGGAGTGTGTGAA-3’
	Reverse：5’-GTCTGACAGTTCGCACAGGA-3’
Human-E2F1 primer	Forward：5’-GCATCCAGCTCATTGCCAAG-3’
	Reverse：5’-TCAGTGTCCTCGGAGAGCAG-3’
Mouse-18S primer	Forward：5’-GCAATTATTCCCCATGAACG-3’
	Reverse：5’-GGCCTCACTAAACCATCCAA-3’
Mouse- E2AE47 primer	Forward：5’-GCCGAAGAGGACAAGAAGG-3′
	Reverse：5’-CTTCTCCTCCAGGGACAG-3′
Mouse- Ebf1 primer	Forward：5’-ATGAAGAGGTTGGATTCTG-3′
	Reverse：5’-GCAGTTATTGTTGATTCC-3′
Mouse- Pax5 primer	Forward：5’-CAGTATTCTTCCTACAATGAT-3′
	Reverse：5’-AGGGCTGTAATAGTATGG-3′
Mouse- Foxo1 primer	Forward：5’-CCTTGAGCAGCCTAATGT-3′
	Reverse：5’-CGATGGACGGAATGAGAG-3′
Mouse- Cd79a primer	Forward：5’-CAGGAGCAGAAAACCCAAGT-3′
	Reverse：5’-AGGAGGGTAGGCCCTATAA-3′
Mouse- Cd79b primer	Forward：5’-GCGGAATTCCTTGGCACGGAACTTCTAGT-3′
	Reverse：5’-GCGAAGCTTCCTGTCCGAAGAGTCACTATG-3′
Mouse- VpreB primer	Forward：5’-GCAGAGCCTAGAAGATGG-3′
	Reverse：5’-TACAATCCAGCCTCCAATG-3′
Mouse- *PELI2* primer	Forward：5’-GGTGTCAAACCCAGCACAAT-3′
	Reverse：5’-GTGACCACGAAGTCAATGGG-3′
Mouse- ccnd3 primer	Forward：5’-CGCTGCGAGGAGGATGTCTT-3’
	Reverse：5’-CAACTGCCATGGAGCCACAG-3’
Mouse- IL-7R primer	Forward：5’-GCAAGGGGTGAAAGCAACTG-3′
	Reverse：5’-GACTCCACTCGCTCCAGAAG-3′
Mouse- Cd33 primer	Forward：5’-ACCATCCAGCTCAATGTTACCC-3′
	Reverse：5’-GTGATCGCTTCCTGCCTCTTGA-3′
Mouse- Cybb primer	Forward：5’-GAGGTTGGTTCGGTTTTGGC-3’
	Reverse：5’-CAGGAGCAGAGGTCAGTGTG-3′

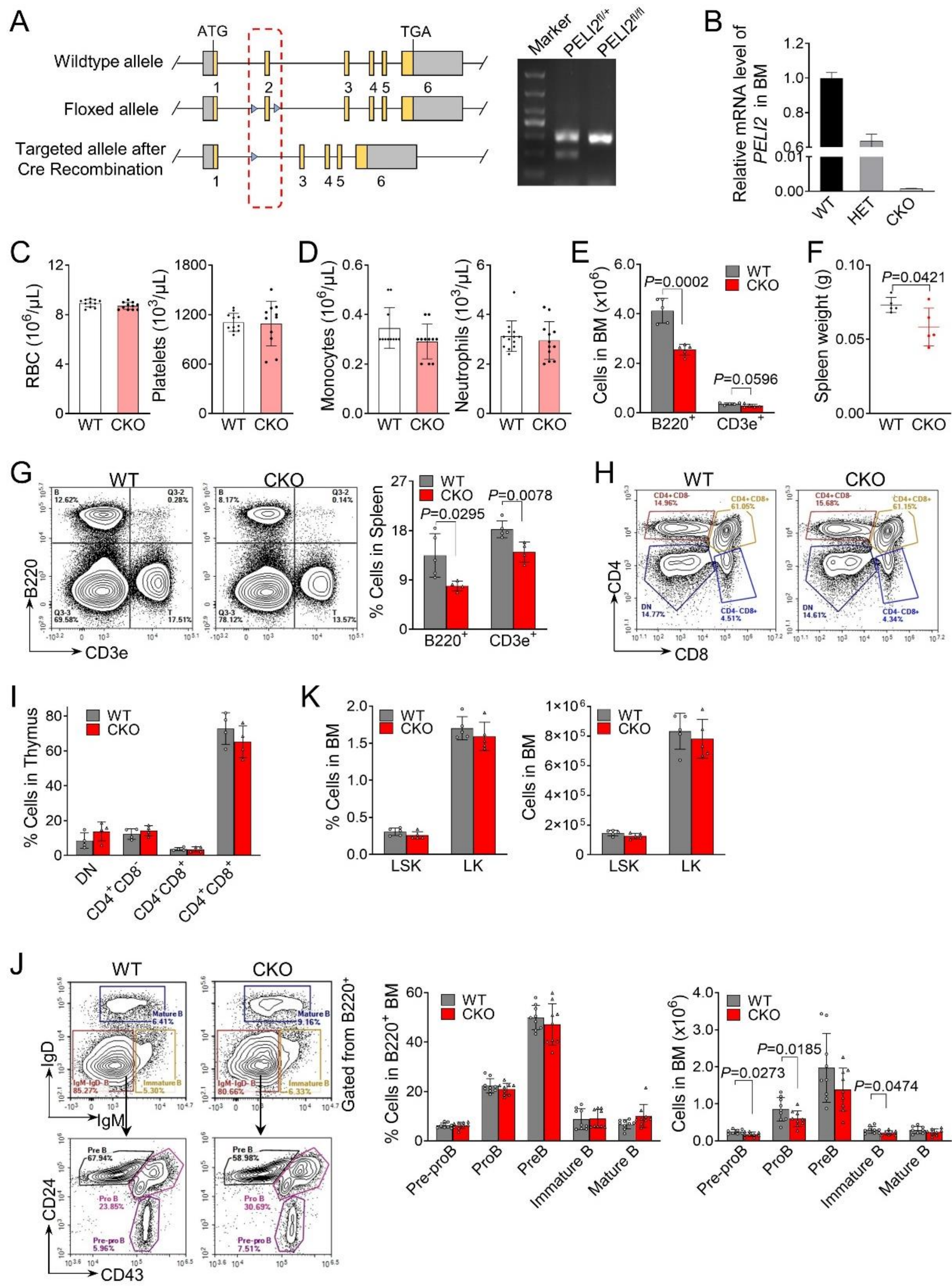
Mouse- Itgb2 primer	Forward：5’-AGATTCTCGGAGTGGAGGCT-3′
	Reverse：5’-CTTGGTGCATTCCTGGGACA-3′
Mouse- Ncf1 primer	Forward：5’-CGGCCTGATGACCTGAAACT-3′
	Reverse：5’-TGCCACGGTCATCTCTGTTC-3′
Mouse- Itgam primer	Forward：5’-AGCTTGGCTTTTTCAAGCGG-3′
	Reverse：5’-AAAGGCCGTTACTGAGGTGG-3′
Mouse- Hes1 primer	Forward：5’-ATAGCTCCCGGCATTCCAAG-3′
	Reverse：5’-GCGCGGTATTTCCCCAACA-3′
Mouse- Notch1 primer	Forward：5’-GGTATGCAAGGAGTCTGA AG-3′
	Reverse：5’-CACACTCATTGATGTCAACC-3′

Supplemental Table 3. Antibodies and commercial reagents used in this study.

Antibodies/Regents	Source	Catalog Number
Pellino2 antibody	Abcam	ab126982
IL-7R antibody	Affinity	DF6362
HSC70 antibody	Proteintech	10995-1-AP
AKT antibody	Affinity	AF6261
Phospho-AKT (Ser473) antibody	Affinity	AF0016
Pu.1 antibody	Affinity	DF13270
TCF3 antibody	Proteintech	21242-1-AP
GAPDH antibody	Abcam	ab9385
K48-linkage Specific Polyubiquitin	CST	D9D5
K63-linkage Specific Polyubiquitin	CST	D7A11
Phospho-ERK1/2 (Thr202/Tyr204) antibody	Affinity	AF1015
ERK antibody	Survicebio	GB11560
cMyc antibody	Proteintech	10828-1-AP
phospho-Histone H2A.X (Ser139) antibody	CST	9718T
FLAG-tag antibody	Affinity	T0053
HA-tag antibody	Affinity	T0050
Peroxidase-AffiniPure Goat Anti-Rabbit IgG	Jackson	111-035-003
Peroxidase-AffiniPure Goat Anti-Mouse IgG	Jackson	115-035-003
APC-anti-mouse-CD3ε	Biolegend	100312
PE-anti-mouse-CD11b	Biolegend	101207
FITC-anti-mouse-B220	Biolegend	103206
PB-anti-mouse-Ly-6G/Ly-6C	Biolegend	108430
APC-anti-mouse-CD4	Biolegend	100412
PE-anti-mouse-CD8a	eBioscience	50-1886
PE/CY7-anti-mouse-CD34	Biolegend	119326
APC/Cy7-anti-mouse-CD48	Biolegend	103432
PE-anti-mouse-CD16/32	Biolegend	101308
PE-anti-mouse-CD135	eBioscience	12-1351-83
PE-anti-mouse-CD150	Biolegend	115904

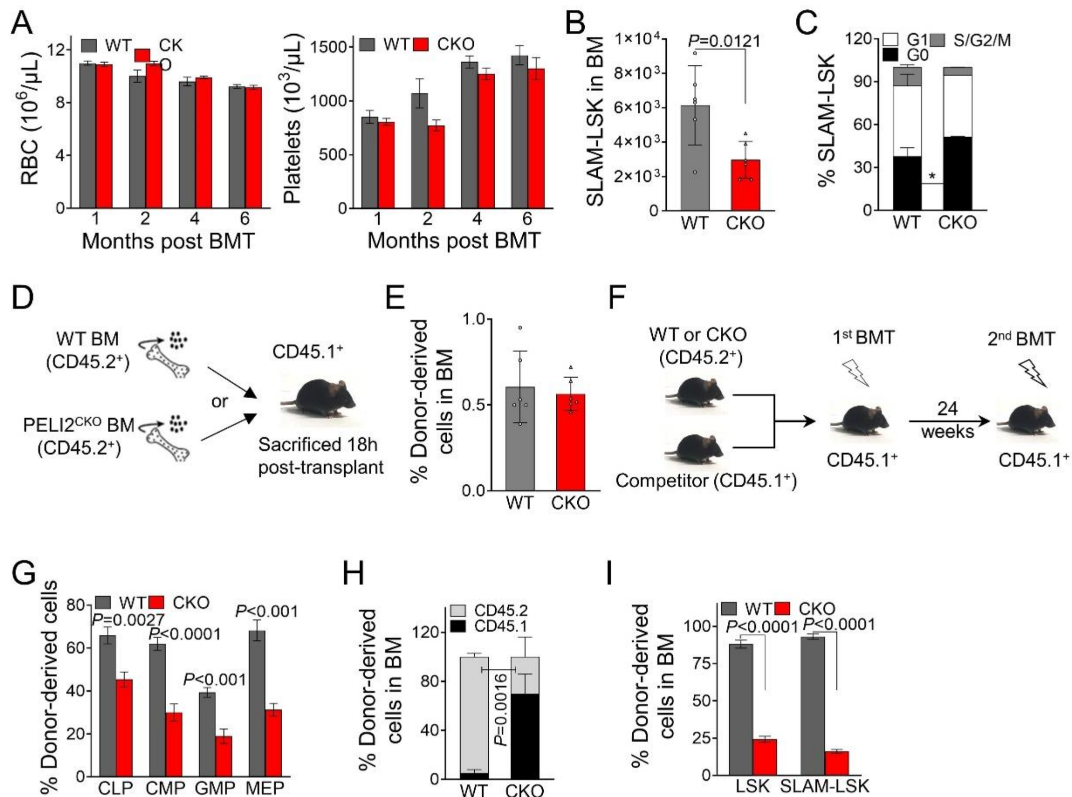
PB-anti-mouse-Lineage	Biolegend	133306
FITC-anti-mouse-Sca-1	eBioscience	11-5981-85
APC-anti-mouse-CD117	eBioscience	17-1171-83
PE-anti-mouse-CD127	Biolegend	121111
PB-anti-mouse-Ly6D	invitrogen	48-5974-80
APC/Cy7-anti-mouse-CD45.1	Biolegend	110715
PE-CY7-anti-mouse-CD45.2	eBioscience	25-0454-80
PE-anti-mouse-IgD	Biolegend	405705
APC-anti-mouse-IgM	Biolegend	406509
Pacific Blue™-anti-mouse-CD24	Biolegend	101819
PE/Cyanine7-anti-mouse-CD43	Biolegend	143209
Percp-anti-mouse-Ki67	Biolegend	652423
APC-anti-mouse-Annexin V	Biolegend	640920
APC-anti-mouse-BrdU	Biolegend	364113
APC-anti-human-CD45	Biolegend	304012
PE-anti-human-CD19	Biolegend	302207
Biotin Mouse Lineage Panel	BD Bioscience	559971
Biotin-B220	Biolegend	103204
Biotin-ckit	Biolegend	105804
Biotin-CD4	Biolegend	344610
Hexadimethrine bromide	Sigma	H9268
Dual-Luciferase® Reporter Assay System	Promega	REF.E1910
PrimerScript RT reagent	Takara	RR047A
SYBR Premix Ex Taq	Takara	RR420A
The SimpleChIP® Enzymatic Chromatin IP Kit	CST	9003
Cell-Light EDU Apollo567 In vitro Kit	RIBBIO	C10310-1
MG132	Selleck Chemicals	S2619

3. Supplemental Figure Legends



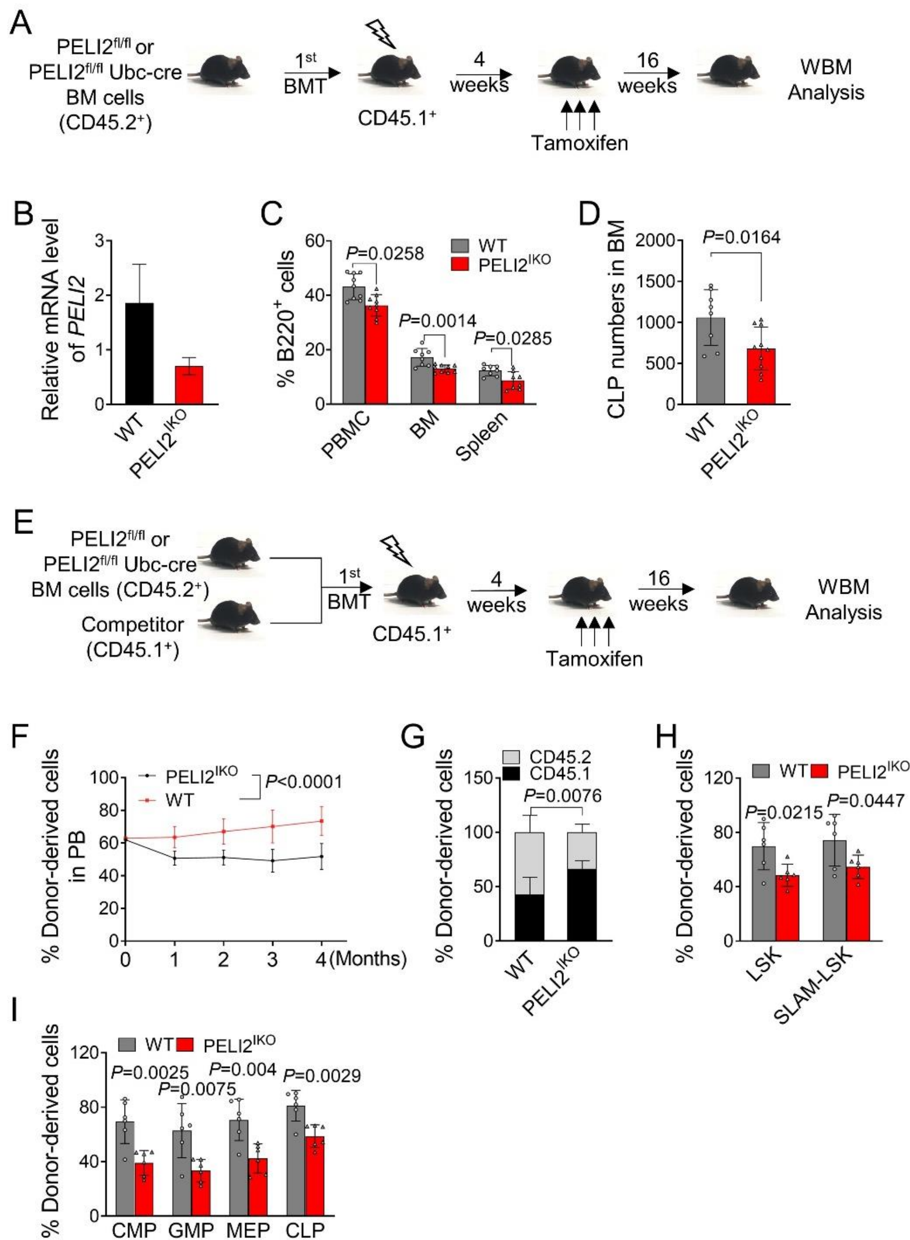
Supplementary Figure 1. Hematopoietic profile in *PELI2*$^{\text{CKO}}$ mice, related to Figure 1. (**A**) Schematic of *PELI2*$^{\text{fl/fl}}$ Vav-Cre gene-targeting strategy. (**B**) Quantification of mRNA expression of *PELI2* from bone marrow mononuclear

cell (MNCs) in WT and *PELI2*^{CKO} mice. Data were presented as mean±SD from three independent experiments. (**C-D**) Peripheral blood chimerism analysis of 8-week-old WT and *PELI2*^{CKO} mice. Each dot represents one mouse. Data were presented as mean±SD. (**E**) Quantification of the numbers of B cells and T cells in the bone marrow of 8-week-old WT and *PELI2*^{CKO} mice. Each dot represents one mouse. Data were presented as mean±SD. (**F**) Spleen weight of WT and *PELI2*^{CKO} mice at the age of 8 weeks. Each dot represents one mouse. Data were presented as mean±SD. (**G**) Representative flow cytometric analysis of B cells and T cells in the spleen of 8-week-old WT and *PELI2*^{CKO} mice (left). The percentage of B cells and T cells was quantified (right). Each dot represents one mouse. Data were presented as mean±SD. (**H**) Representative flow cytometric analyses of T cells in the thymus of 8-week-old WT and *PELI2*^{CKO} mice. (**I**) Statistical analyses on the percentage of different stages of T cells in H. Each dot represents one mouse. Data were presented as mean±SD. (**J**) Representative flow cytometric analysis of B cells in the bone marrow of 8-week-old WT and *PELI2*^{CKO} mice (left). The percentage and numbers of different stages of B cells were quantified (right). Each dot represents one mouse. Data were presented as mean±SD. (**K**) Quantification of the percentage and numbers of LSK and LK cells from 8-week-old WT and *PELI2*^{CKO} mice. Each dot represents one mouse. Data were presented as mean±SD.



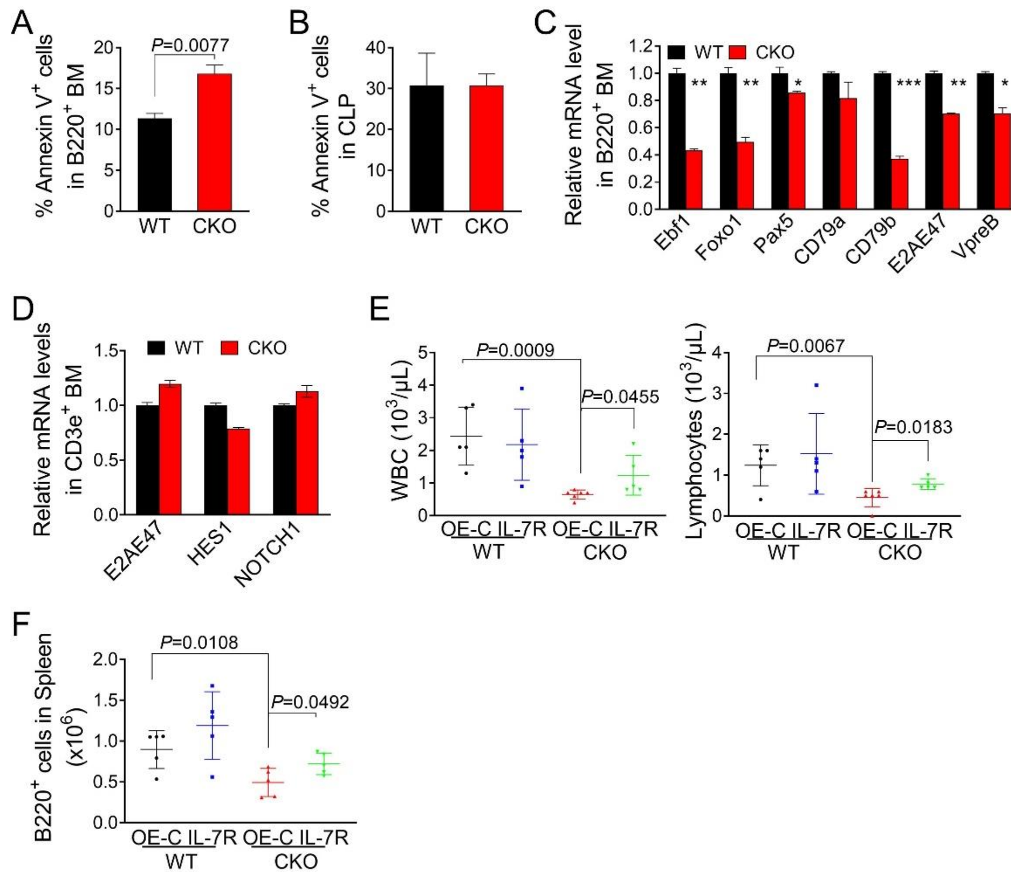
Supplementary Figure 2. Loss of *PELI2* impaired HSC repopulation capacity, related to Figure 2. (**A**) Peripheral blood chimerism analyses of WT and *PELI2*^{CKO} mice every month after 1st noncompetitive transplantation. Data were obtained from 8 mice in each group and presented as mean±SD. (**B**) The numbers of SLAM-LSK cells in the bone marrow of indicated mice 24 weeks after bone marrow transplantation. Each dot represents one mouse. Data were presented as mean±SD. (**C**) Cell cycle analysis by flow cytometry using Ki67 and Hoechst staining in SLMA-LSK cells from mice as in B. Data were presented as mean±SD from three independent experiments. * $P<0.05$. (**D**) Schematic illustration of HSC homing assays. **(E)** Quantification of homing efficiency in WT and *PELI2*^{CKO} HSCs. Each dot represents one mouse. Data were presented as mean±SD. (**F**) Schematic illustration of competitive transplantation with WT or *PELI2*^{CKO} bone marrow cells. (**G**) Quantification of

the percentage of donor derived CLP, CMP, GMP and MEP cells in the bone marrow of WT and *PELI2*^{CKO} mice 24 weeks after 1st competitive transplantation. Data were obtained from 8 mice in each group and presented as mean±SD. (**H**) Quantification of the percentage of donor derived total cells in the bone marrow of WT and *PELI2*^{CKO} mice 24 weeks after 2nd competitive transplantation. Data were obtained from 8 mice in each group and presented as mean±SD. (**I**) Quantification of the percentage of donor derived LSK and SLAM-LSK cells in the bone marrow of WT and *PELI2*^{CKO} mice as in H. Data were obtained from 8 mice in each group and presented as mean±SD. All *P* values were determined by unpaired two-tailed Student's t-test except where indicated otherwise.



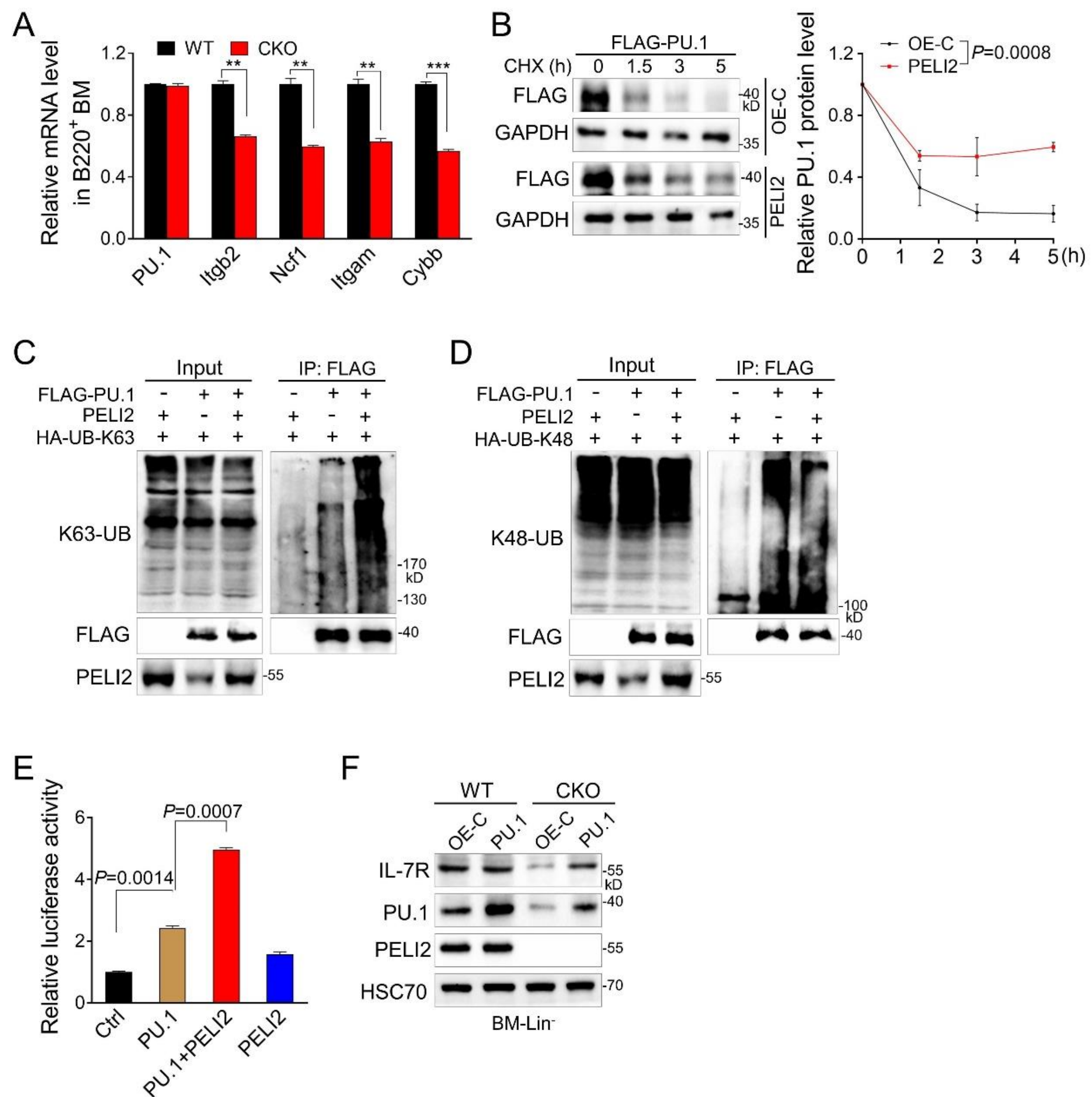
Supplementary Figure 3. *PELI2* played an intrinsic role in adult hematopoiesis, related to Figure 2. (**A**) Schematic illustration of noncompetitive transplantation with bone marrow cells of WT and *PELI2*^{IKO} mice. (**B**) Quantification of the mRNA expression of *PELI2* in the blood of WT and *PELI2*^{IKO} mice induced by tamoxifen after noncompetitive transplantation. Data were obtained from 10 mice in each group and presented as mean±SD. (**C**) Quantification of the percentage of B cells in the peripheral blood, bone

marrow (BM) and spleen of WT and *PELI2*^{IKO} mice 16 weeks after noncompetitive transplantation. Each dot represents one mouse. Data were presented as mean±SD. (**D**) Quantification of the numbers of CLP cells in bone marrow of WT and *PELI2*^{IKO} mice as in C. Each dot represents one mouse. Data were presented as mean±SD. (**E**) Schematic illustration of competitive transplantation with bone marrow cells of WT and *PELI2*^{IKO} mice. (**F**) Quantification of the percentage of donor-derived total cells in peripheral blood at the indicated time points in the 1st competitive transplantation assay. Data were obtained from 8 mice in each group and presented as mean±SD. (**G**) Quantification of the percentage of donor-derived bone marrow cells of WT and *PELI2*^{IKO} mice 16 weeks after 1st competitive transplantation. Data were obtained from 6 mice in each group and presented as mean±SD. (**H**) Quantification of the percentage of donor derived LSK and SLAM-LSK cells in the bone marrow of WT and *PELI2*^{IKO} mice as in G. Each dot represents one mouse. Data were presented as mean±SD. (**I**) Quantification of the percentage of donor derived CLP, CMP, GMP and MEP cells in the bone marrow of WT and *PELI2*^{IKO} mice as in G. Each dot represents one mouse. Data were presented as mean±SD. All *P* values were determined by unpaired two-tailed Student's t-test except where indicated otherwise.



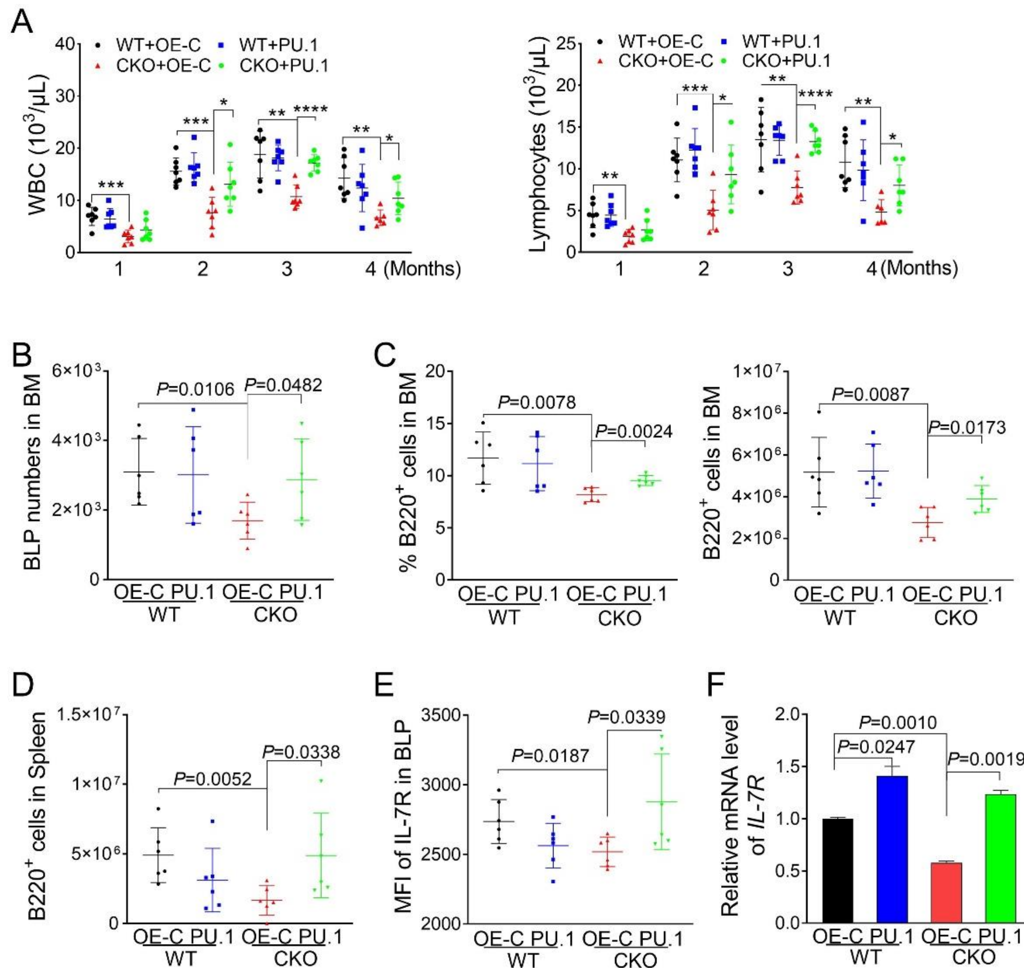
Supplementary Figure 4. Increased apoptosis and decreased transcription of B lymphoid genes in *PELI2*^{CKO} mice, related to Figure 3. (**A-B**) Quantification of the apoptosis of B cells and CLP cells in the bone marrow of WT and PELI^{CKO} mice based on Annexin V staining by flow cytometric analyses. Data were obtained from 3 mice in each group and presented as mean±SD. (**C**) Quantitative PCR analysis of genes related to B cell development in the bone marrow B220^{+} cells of WT and *PELI2*^{CKO} mice. Data were obtained from 3 mice in each group and presented as mean±SD. * P < 0.05; ** P < 0.01; *** P < 0.001. (**D**) Quantitative PCR analysis of genes related to T cell development in bone marrow CD3e^{+} cells of WT and *PELI2*^{CKO} mice. Data were obtained from 3 mice in each group and presented as mean±SD. **(E)** Peripheral blood analysis of recipient mice transplanted with WT and *PELI2*^{CKO} cKit^{+} BM cells transduced with lentivirus expressing IL-7R or

blank vector after 1-month transplantation. Each dot represents one mouse. Data were presented as mean±SD. **(F)** Quantification of $B220^{+}$cells in the spleen from mice as in E. Each dot represents one mouse. Data were presented as mean±SD. All *P* values were determined by unpaired two-tailed Student's t-test except where indicated otherwise.



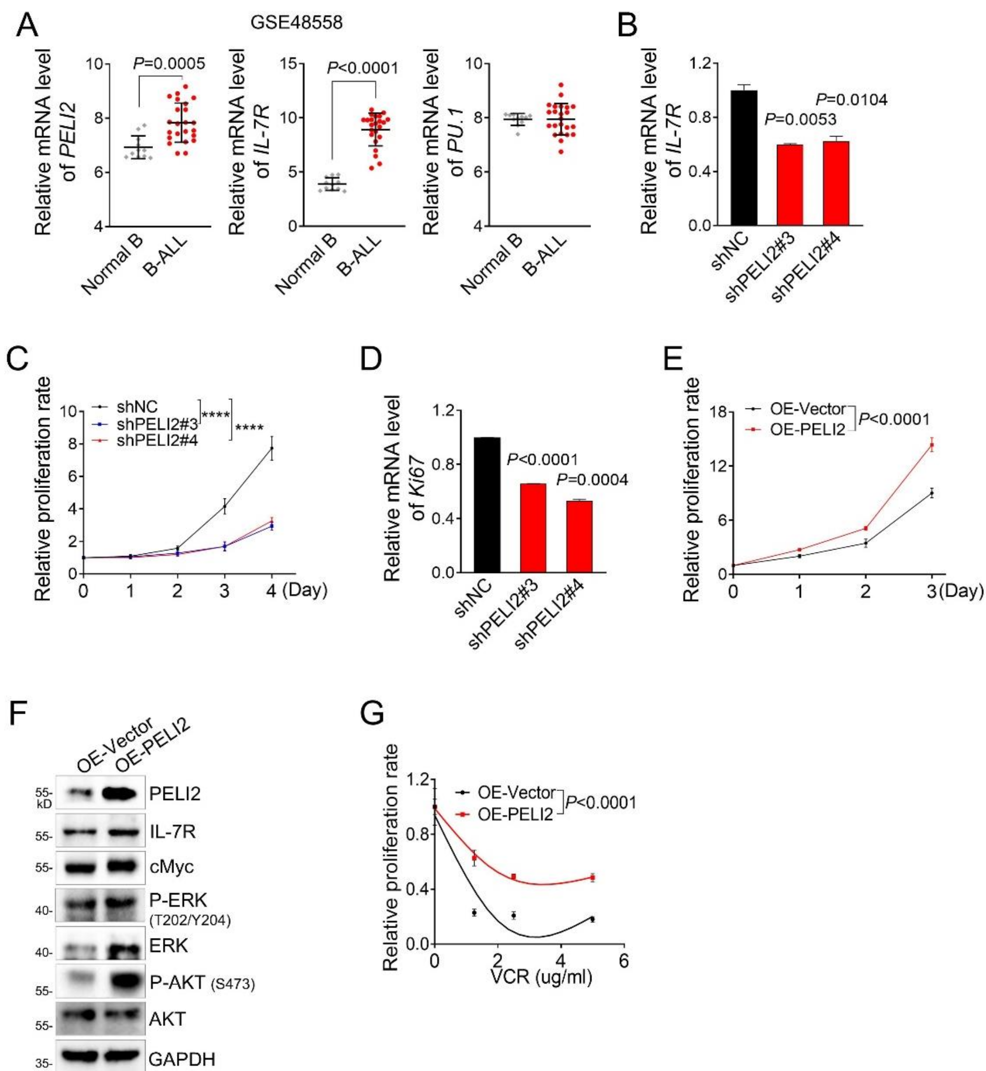
Supplementary Figure 5. ***PELI2*** **regulated PU.1 target genes and targeted PU.1 through ubiquitination, related to Figure 4. (A)** RT-qPCR analysis of *PU.1* target genes in the bone marrow B220+ cells of WT and *PELI2*^{CKO} mice. Data were obtained from 3 mice in each group and presented as mean±SD. ** *P* < 0.01; *** *P* < 0.001. (**B**) Immunoblotting analysis of PU.1 protein level in HEK293T cells with overexpressing Flag-PU.1 and *PELI2* or blank vector (OE-C) upon cycloheximide (CHX, 10 μM) treatment. PU.1 level was normalized to the change of GAPDH. Data were presented as mean±SD from three independent experiments. *P* value was determined by two-way ANOVA. (**C**)

Immunoblotting analysis of the K63-linked polyubiquitination of PU.1 immunoprecipitated from HEK293T cells co-overexpressing with PU.1, *PELI2* and K63-linked ubiquitin. (**D**) Immunoblotting analysis of the K48-linked polyubiquitination of PU.1 immunoprecipitated from HEK293T cells co-overexpressing with PU.1, *PELI2* and K48-linked ubiquitin. (**E**) Luciferase reporter assays of *IL-7R* promoter. HEK293T cells were cotransfected with luciferase reporter construct bearing the *IL-7R* promoter and PU.1, *PELI2*, both expressing plasmid or blank vector. Data were presented as mean±SD from three independent experiments. (**F**) Immunoblotting analysis of IL-7R and PU.1 in the bone marrow lineage^{-} cells from WT and *PELI2*^{CKO} mice transduced with lentivirus expressing PU.1 or blank vector (OE-C). HSC70 was used as a loading control.



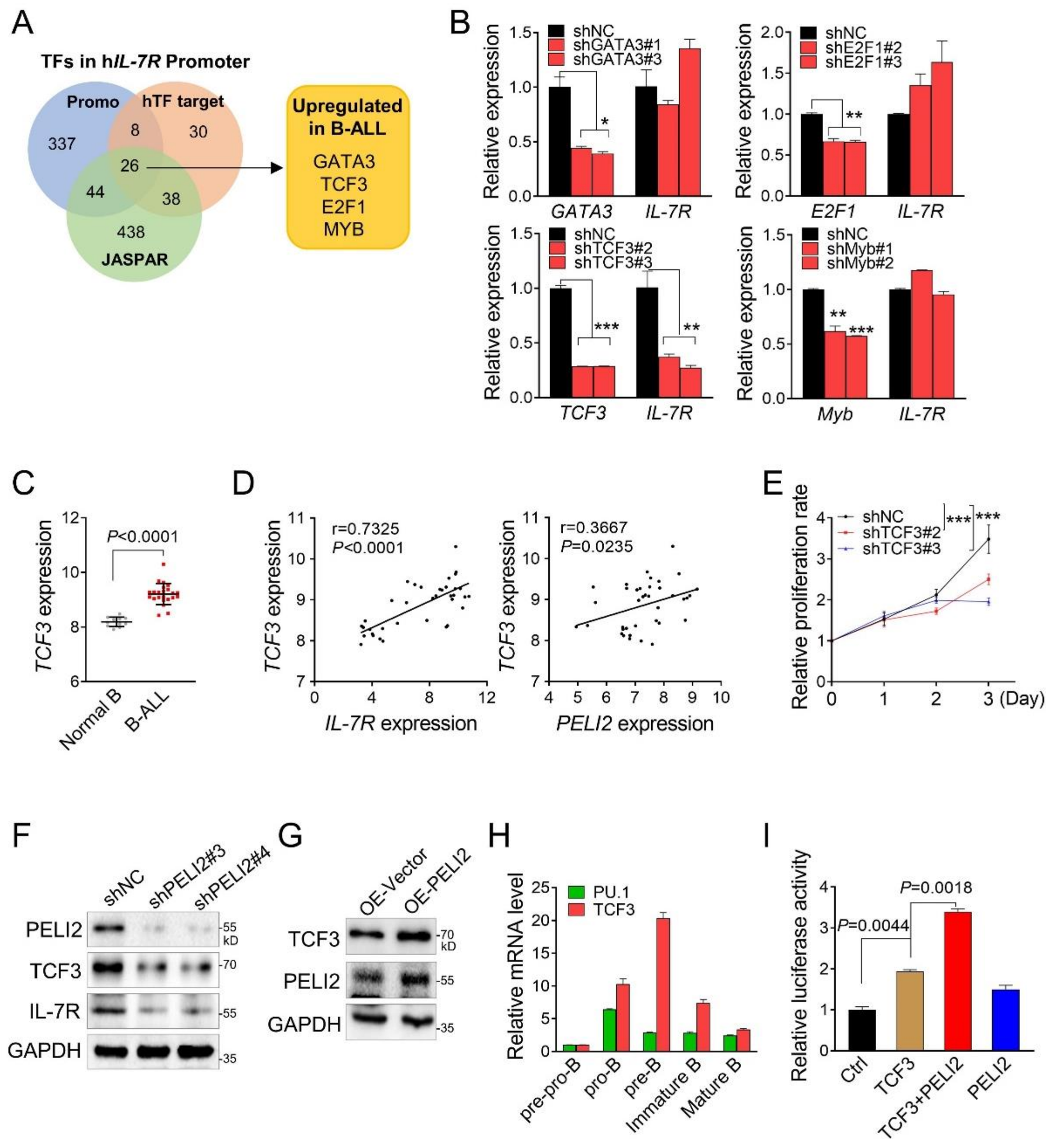
Supplementary Figure 6. Overexpression of PU.1 rescued reduced B cells in the *PELI2*^{CKO} mice, related to Figure 4. (A) Peripheral blood analysis of recipient mice transplanted with WT and *PELI2*^{CKO} cKit$^+$ BM cells transduced with lentivirus expressing PU.1 or blank vector. Each dot represents one mouse. Data were presented as mean±SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. **(B)** Quantification of BLP cells in the bone marrow from mice as in A 4-month after transplantation. Each dot represents one mouse. Data were presented as mean±SD. **(C-D)** Quantification of B cells (B220$^+$) in the bone marrow (C) and spleen (D) from mice as in A 4-month after transplantation. Each dot represents one mouse. Data were presented as mean±SD. **(E)** Flow

cytometric analysis of cell surface expression of IL-7R in the bone marrow BLP cells of indicated mice as in A 4-month after transplantation. Quantification of mean fluorescence intensity (MFI) of IL-7R was shown. Each dot represents one mouse. Data were presented as mean±SD. **(F)** Quantification of mRNA expression of *IL-7R* expression in the bone marrow B220$^+$ cells of indicated mice as in A 4-month after transplantation. Data were obtained from 3 mice in each group and presented as mean±SD. All *P* values were determined by unpaired two-tailed Student's t-test except where indicated otherwise.



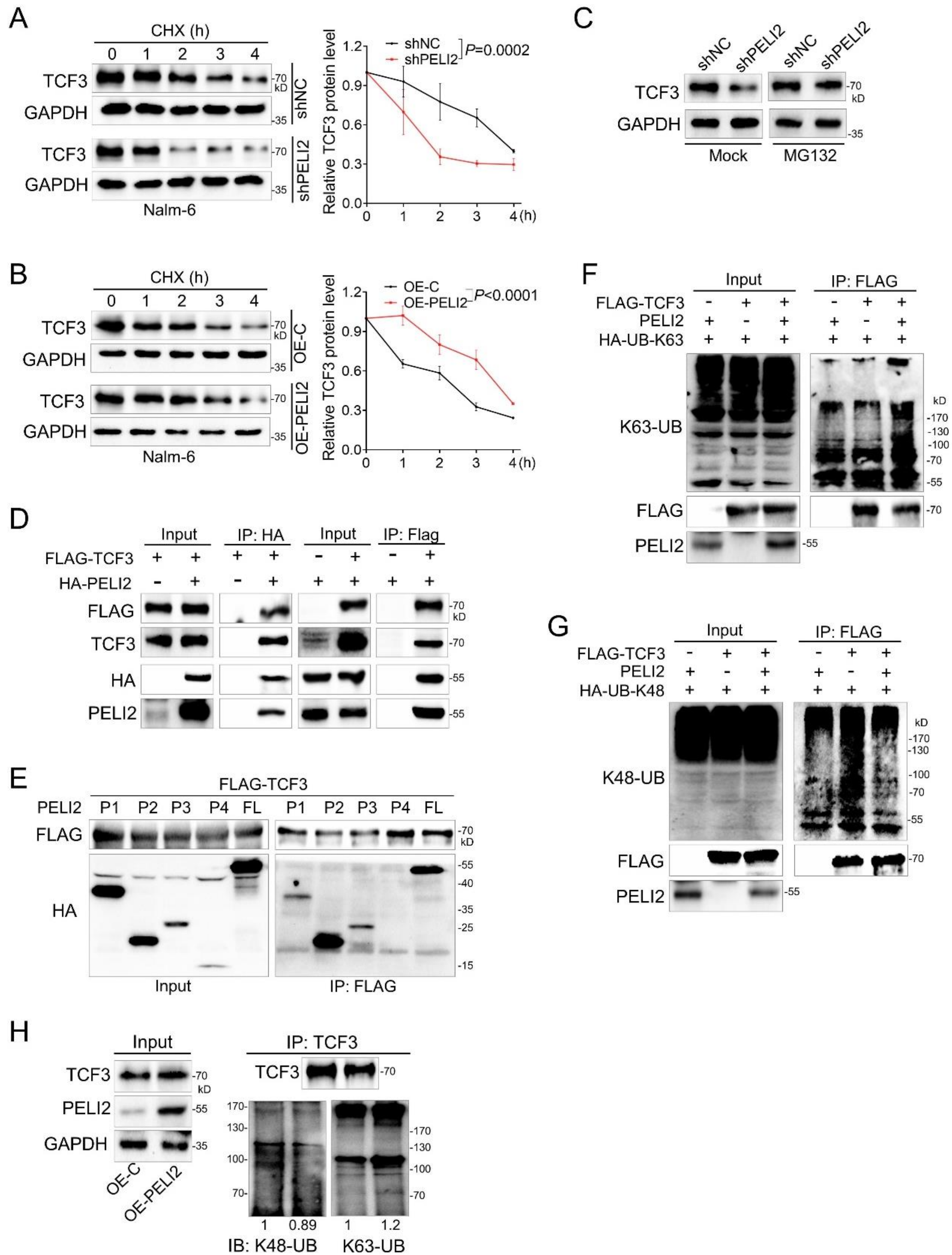
Supplementary Figure 7. Overexpress *PELI2* enhanced BCP-ALL cells proliferation through IL-7R signaling, related to Figure 5. (**A**) RNA-seq expression profiles of *PELI2*, *IL-7R* and *PU.1* in the BCP-ALL samples and normal B cell samples from public database (GSE48558). Each dot represents one human sample. Data were presented as mean±SD. (**B**) Quantification of mRNA expression of *IL-7R* in Nalm-6 cells transduced with retroviruses encoding indicated shRNAs. shNC represents a non-targeting shRNA. Data were presented as mean±SD from three independent experiments. (**C**) Statistical analysis of cell proliferation in 697 cells transduced with retroviruses

encoding indicated shRNAs. shNC represents a non-targeting shRNA. Data were obtained from three independent experiments and presented as mean±SD. *P* value was determined by two-way ANOVA. **** $P<0.0001$. (**D**) Statistical analysis of *Ki67* mRNA level in Nalm-6 cells as in B. Data were presented as mean±SD from three independent experiments. (**E**) Statistical analysis of cell proliferation in Nalm-6 cells with *PELI2* overexpression. Data were presented as mean±SD from three independent experiments. *P* value was determined by two-way ANOVA. (**F**) Immunoblotting analysis of indicated proteins in Nalm-6 cells as in E. GAPDH was used as a loading control. (**G**) *PELI2* promotes resistance to Vincristine (VCR). Nalm-6 cells with or without *PELI2* overexpression were cultured for 24 hours in complete medium and subsequently supplemented with VCR at 1.25, 2.5, or 5 ug/mL as indicated for 24 hours. The cell viability was determined by flow cytometric. Data were presented as mean±SD from four independent experiments. All *P* values were determined by unpaired two-tailed Student's t-test except where indicated otherwise.



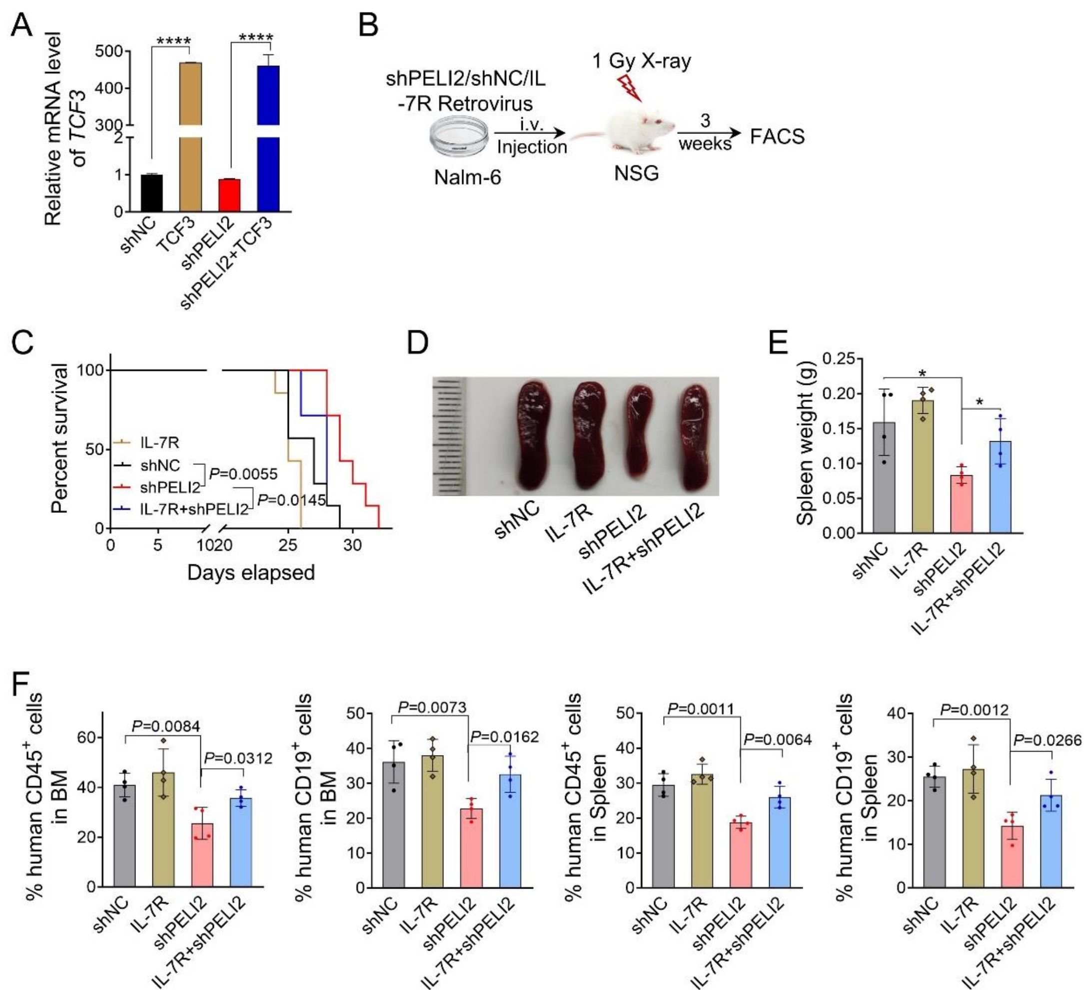
Supplementary Figure 8. *PELI2* regulated TCF3 expression, which was involved in IL-7R transcription, related to Figure 6. (**A**) Venn diagram indicated 26 common predicted transcription factors (TFs) in the human *IL-7R* promoter from three published databases and 4 genes among them are upregulated in BCP-ALL samples from GEO48558. (**B**) Quantification of mRNA expression of *IL-7R* in Nalm-6 cells transduced with retroviruses encoding indicated shRNAs. Data were presented as mean±SD from three independent experiments. shNC represents a non-targeting shRNA. * $P < 0.05$; ** $P < 0.01$;

*** *P* < 0.001. (**C**) RNA-seq expression profiles of *TCF3* in the BCP-ALL samples and normal B cell samples from public database (GSE48558). Each dot represents one human sample. Data were presented as mean±SD. (**D**) Correlation of *TCF3* expression with *IL-7R* or *PELI2* in primary BCP-ALL samples (GSE48558) with mRNA levels. Pearson's correlation coefficient (R), paired t-test, *P* values were shown. (**E**) Statistical analysis of cell proliferation in Nalm-6 cells transduced with retroviruses encoding indicated shRNAs. shNC represents a non-targeting shRNA. Data were obtained from three independent experiments. *P* value was determined by two-way ANOVA. *** *P*<0.001. (**F**) Immunoblotting analysis of TCF3 and IL-7R expression in Nalm-6 cells transduced with retroviruses encoding indicated sh*PELI2*. shNC represents a non-targeting shRNA. GAPDH was used as a loading control. (**G**) Immunoblotting analysis of TCF3 expression in Nalm-6 cells transduced with stable *PELI2* overexpress. GAPDH was used as a loading control. **(H)** Quantification of mRNA expression of *PU.1* and *TCF3* in B-lymphocyte from bone marrow. Data were presented as mean±SD from three independent experiments. (**I**) Luciferase reporter assays of *IL-7R* promoter. HEK293T cells were cotransfected with luciferase reporter construct bearing the *IL-7R* promoter and TCF3, *PELI2*, both expressing plasmid or blank vector. Data were presented as mean±SD from three independent experiments. All *P* values were determined by unpaired two-tailed Student's t-test except where indicated otherwise.



Supplementary Figure 9. *PELI2* interacted with TCF3 and stabilized TCF3 through ubiquitination, related to Figure 6. (**A**) Immunoblotting analysis of TCF3 protein level in Nalm-6 cells with stable *PELI2* knockdown upon cycloheximide (CHX, 10 μM) treatment. TCF3 level was normalized to the change of GAPDH. Data were presented as mean±SD from three independent

experiments. *P* value was determined by two-way ANOVA. (**B**) Immunoblotting analysis of TCF3 protein level in Nalm-6 cells with stable *PELI2* overexpression upon cycloheximide (CHX, 10 μM) treatment. TCF3 level was normalized to the change of GAPDH. Data were presented as mean±SD from three independent experiments. *P* value was determined by two-way ANOVA. (**C**) Nalm-6 cells with *PELI2* stably knockdown were treated with vehicle and MG132 (10 μM) for 4 hours together with the control cells. The proteins were then extracted and subjected to western blotting. GAPDH was used as a loading control. (**D**) Reciprocal co-IP of exogenous FLAG-TCF3 and HA-*PELI2* in HEK293T cells. The bands were obtained from independent membranes blotted with indicated antibodies. (**E**) Co-IP analysis of *PELI2* mutants binding to TCF3. HEK293T cells were co-transfected with *PELI2* truncations (HA tagged) and FLAG-TCF3, and IP analysis was performed with anti-FLAG antibody. (**F**) Immunoblotting analysis of the K63-linked polyubiquitination of TCF3 immunoprecipitated from HEK293T cells co-overexpressing with PU.1, *PELI2* and K63-linked ubiquitin. (**G**) Immunoblotting analysis of the K48-linked polyubiquitination of TCF3 immunoprecipitated from HEK293T cells co-overexpressing with TCF3, *PELI2* and K48-linked ubiquitin. (**H**) Immunoblotting analysis of ubiquitination of TCF3 immunoprecipitated from Nalm-6 cells transduced with indicated retroviruses with K63-linkage and K48-linkage specific polyubiquitin antibodies. OE-C represents overexpression of blank vector. The numbers indicated the relative band intensity of the ubiquitin blots to corresponding control. All *P* values were determined by unpaired two-tailed Student's t-test except where indicated otherwise.



Supplementary Figure 10. Replenishment of IL-7R reversed the suppression phenotypes of mice bearing *PELI2*-silencing Nalm-6, related to Figure 7. (**A**) Quantification of mRNA expression of *TCF3* in Nalm-6 cells transduced with indicated lentivirus. Data were presented as mean±SD from three independent experiments. ** $P < 0.01$; **** $P < 0.0001$. (**B**) Schematic representation of human BCP-ALL xenograft. Five million Nalm-6 cells, transduced with indicated retroviruses, were injected into irradiated NOD scid gamma (NSG) mice (1 Gy). shNC represents a non-targeting shRNA. (**C**) Kaplan-Meier survival curve of indicated Nalm-6-xenograft mice. Data were obtained from 7 mice in each group. *P* values were determined by Log-rank

(Mantel-Cox) test. (**D**) Representative spleen of 4 mice as in B was shown. (**E**) Statistical analysis of spleen weigh in D. Data were obtained from 4 mice in each group and presented as mean±SD. * $P < 0.05$. (**F**) Human CD45$^+$ and CD19$^+$ cells from bone marrow and spleen of indicated mice were analyzed by flow cytometry. Each dot represents one mouse. Data were presented as mean±SD. P values were determined by unpaired two-tailed Student's t-test except where indicated otherwise.