Expanded View Figures

Figure EV1. Validation of the experimental system, flow cytometer gating strategy to identify and compare different bone marrow B lineage subsets and *ex vivo* and *in situ* analysis of cell viability in WT and KO mice.

A Schematic view of WT and KO (Δ exon 2) cDNA of *Dot1L* showing UTRs, exons, physiological translational stop codon for WT, multiple translational stop codons (position not to scale) generated after frameshifting induced in KO and location of primers used in RT–PCR to amplify the region flanking exon 2. Chromatograph generated by sanger sequencing showing *Cre*-mediated deletion of exon 2 leading to joining of exon 1 to exon 3 in KO. Agarose gel picture showing the deletion of 44 bp corresponding to exon 2 specifically in KO-B cells. Results represent the data from one experiment, and numbers represent biological replicates for each group (WT; $n = 1$, KO; $n = 2$).

B Representative flow cytometry plots showing gating strategy to identify and compare relative frequency of different B lineage precursor subsets in the bone marrow from WT and KO mice.

C Representative flow cytometry plots showing gating strategy to identify and compare relative frequency of live, early apoptotic, late apoptotic, and $DAPI^+$ cells in bone marrow.

D–H Statistical analysis of the relative frequency of live, early apoptotic, late apoptotic, and $DAPI^+$ cells from the total B lineage and its subsets in the bone marrow from WT and KO mice. Results represent the data from one experiment, and numbers represent biological replicates for each group (WT; $n = 3$, KO; $n = 3$).

I Statistical analysis of the relative frequency of live, early apoptotic, late apoptotic and $DAPI^+$ cells from mature spleen B cells from WT and KO mice. Results represent the data from one experiment, and numbers represent biological replicates for each group (WT; $n = 3$, KO; $n = 3$).

J Immunohistochemistry staining for CD3, Ki67 and cleaved caspase-3 on spleen sections from WT and KO mice. Results represent the data from at one experiment, and numbers represent biological replicates for each group (WT; $n = 4$, KO; $n = 4$).

Data information: Statistical analysis were performed using Student's two-tailed unpaired *t*-test. Statistical significance was determined by calculating *P*-value. *P*-value less than 0.05 was considered as significant. Bars and error bars indicate mean ± SD.

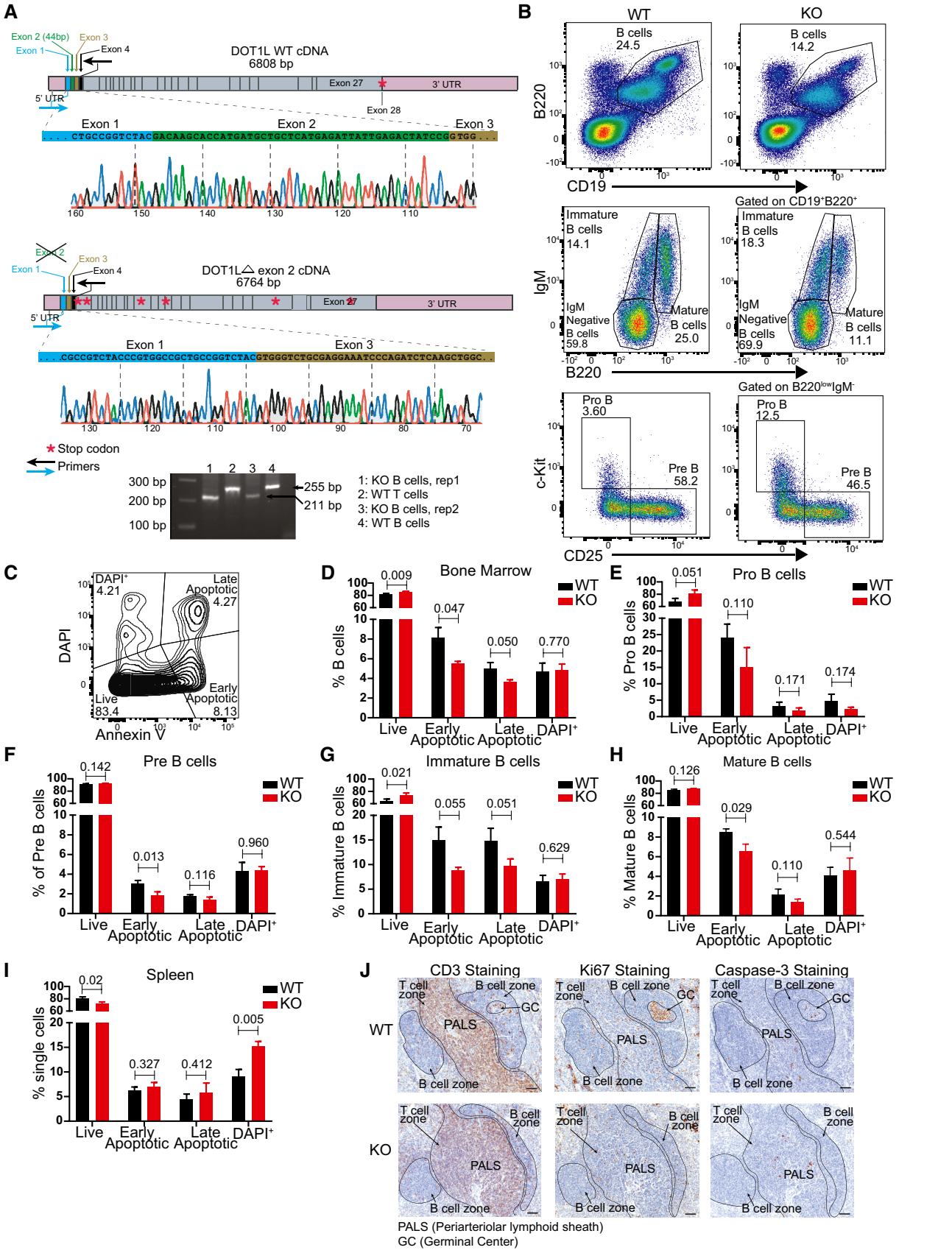
A
Exon 2 (44bp)
Exon 3
Exon 1
Exon 4
DOT1L WT cDNA
6808 bp
Exon 27
3' UTR
5' UTR
Exon 28
Exon 1
Exon 2
Exon 3
. . . CTGCCGGTCTACGACAAGCACCATGATGCTGCTCATGAGATTATTGAGACTATCCGGTGG . . .
160 150 140 130 120 110
Exon 2
Exon 3
Exon 1
Exon 4
DOT1L△ exon 2 cDNA
6764 bp
Exon 27
3' UTR
5' UTR
Exon 1
Exon 3
. . . CGCCGTCTACCCGTGGCCGCTGCCGGTCTACGTGGGTCTGCGAGGAAATCCCAGATCTCAAGCTGGC . . .
130 120 110 100 90 80 70
Stop codon
Primers
1 2 3 4
300 bp
200 bp
100 bp
255 bp
211 bp
1: KO B cells, rep1
2: WT T cells
3: KO B cells, rep2
4: WT B cells
B
WT
KO
B cells
24.5
B cells
14.2
B220
CD19
Gated on CD19+B220+
Immature
B cells
14.1
Immature
B cells
18.3
IgM
IgM
Negative
B cells
59.8
Mature
B cells
25.0
IgM
Negative
B cells
69.9
Mature
B cells
11.1
B220
Gated on B220lowIgM-
Pro B
3.60
Pro B
12.5
c-Kit
Pre B
58.2
Pre B
46.5
CD25
C
DAPI+
4.21
Late
Apoptotic
4.27
DAPI
Early
Apoptotic
8.13
Live
83.4
Annexin V
D
Bone Marrow
WT
KO
% B cells
0.009
0.047
0.050
0.770
Live
Early
Apoptotic
Late
Apoptotic
DAPI+
E
Pro B cells
% Pro B cells
0.051
0.110
0.171
0.174
F
Pre B cells
% of Pre B cells
0.142
0.013
0.116
0.960
G
Immature B cells
% Immature B cells
0.021
0.055
0.051
0.629
H
Mature B cells
% Mature B cells
0.126
0.029
0.110
0.544
I
Spleen
% single cells
0.02
0.327
0.412
0.005
J
CD3 Staining
Ki67 Staining
Caspase-3 Staining
WT
KO
T cell
zone
B cell zone
GC
PALS
B cell zone
PALS (Periarteriolar lymphoid sheath)
GC (Germinal Center)

Figure EV1.

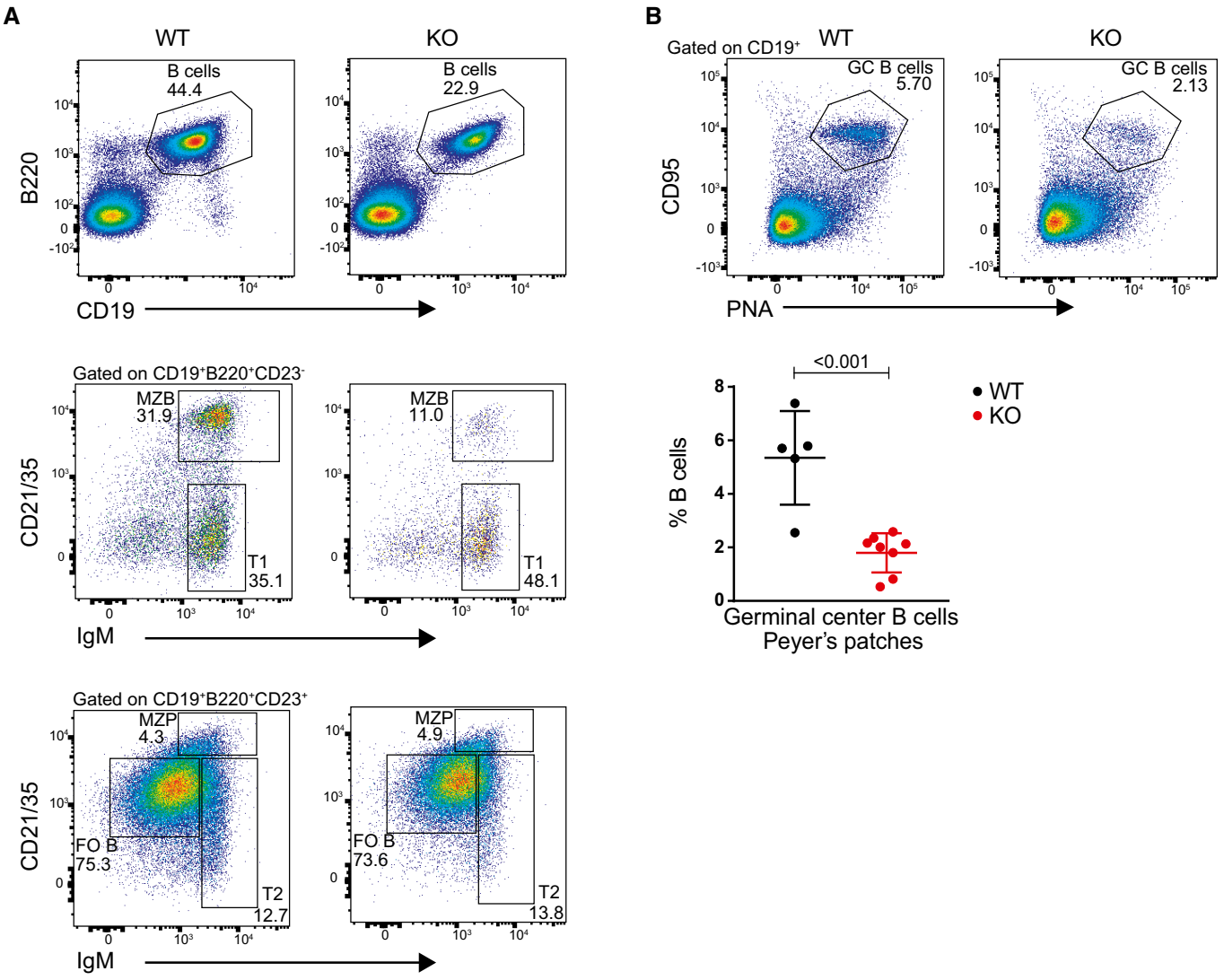

Figure EV2. Gating strategy to identify and compare the spleen B cells, different B-cell subsets, and germinal center B cells from Peyer's patches of WT and KO conditions.

A Representative flow cytometry plots showing gating strategy to identify and compare relative frequency of mature B cells and different B-cell subsets from the spleen of WT and KO mice.

B Representative flow cytometry plots showing gating strategy to identify and compare germinal center B cells from Peyer's patches from WT and KO mice (upper panel). Statistical analysis of the percentage of germinal center B cells of total B from Peyer's patches (lower panel). Results represent the data from three independent experiments, and numbers represent biological replicates for each group (WT; $n = 5$, KO; $n = 8$).

Data information: Statistical analyses were performed using the Student's two-tailed unpaired *t*-test. Statistical significance was determined by calculating the *P*-value. *P*-value less than 0.05 was considered as significant. Bars and error bars indicate mean ± SD.

Figure EV3. Dot1L activity in B cells controls proliferation in response to *in vitro* T cell-dependent stimuli, class switch recombination in response to *in vitro* T-independent stimuli, and viability in response to both the stimuli.

A Representative flow cytometry plots showing gating strategy to identify and compare relative frequency of IgG1 switched cells from WT and KO mice after 4 days of activation with anti CD40 + IL-4.

B, C Number of cell divisions traced by CTV dilution of B cells after 4 days of stimulation with LPS (B) or LPS + IL-4 (C). Data represent three biological replicates for each genotype.

D, E Statistical analysis of the percentage of IgG1 switched cells per generation of proliferating B cells after 4 days of stimulation with LPS (D) or LPS + IL-4 (E). Results represent the data from one experiment and numbers represent biological replicates for each group (WT; $n = 3$, KO; $n = 3$).

F, G Representative flow cytometry plots showing gating strategy to identify and compare relative frequency of IgG1 switched cells from WT and KO mice after 4 days of activation with LPS (F) or LPS + IL-4 (G).

H Representative flow cytometry plot from single cells showing gating strategy to identify live (Zombie NIR^-) and dead cells (Zombie NIR^+) after 4 days of stimulation with LPS + IL-4.

I–K Statistical analysis of dead cells indicated as the percentage of total single cells after 4 days of stimulation with LPS (I), LPS + IL-4 (J) or anti-CD40 + IL-4 (K). Results represent the data from one experiment and numbers represent biological replicates for each group (I: (WT) $n = 3$, (KO) $n = 3$; J: (WT) $n = 4$, (KO) $n = 4$; K: (WT) $n = 4$, (KO) $n = 4$).

L Representative histograms indicating the levels of cleaved caspase-3 in live (Zombie NIR^-) after 4 days of stimulation with LPS + IL-4. Cleaved caspase-3 levels on dead (Zombie NIR^+) population shown, as positive control.

M Intracellular flow cytometry staining for H3K79me2 in activated B-cell after 4 days of stimulation with LPS either in the presence of DOT1L inhibitor, Pinometostat or DMSO as a control. Data represent three biological replicates for each treatment.

N Statistical analysis of Median Fluorescence Intensity (MFI) of H3K79me after 4 days of stimulation with LPS either in the presence of DOT1L inhibitor, Pinometostat or DMSO as a control is shown. Results represent the data from one experiment, and numbers represent biological replicates for each treatment ($n = 4$).

O, P Representative flow cytometry plots showing gating strategy to identify and compare relative frequency of switched cells after 4 days of stimulation of naive B cells with LPS (O) or LPS + IL-4 (P) either in the presence of DOT1L inhibitor, Pinometostat, or DMSO as a control.

Q, R Statistical analysis of dead cells indicated as the percentage of total single cells after 4 days of stimulation with LPS (Q) or LPS + IL-4 (R) either in the presence of DOT1L inhibitor, Pinometostat, or DMSO as a control. Results represent the data from one experiment, and numbers represent biological replicates for each treatment ($n = 4$).

S Number of cell divisions traced by CTV dilution of B cells after 4 days of stimulation with LPS either in the presence of the DOT1L inhibitor Pinometostat or DMSO as a control. Data represent four biological replicates for each treatment.

T Statistical analysis of the percentage of IgG3 switched cells per generation of proliferating B cells after 4 days of stimulation with LPS either in the presence of the DOT1L inhibitor Pinometostat or DMSO as a control. Results represent the data from one experiment, and numbers represent biological replicates for each treatment ($n = 4$).

Data information: Statistical analyses were performed using Student's two-tailed unpaired *t*-test. Statistical significance was determined by calculating *P*-value. *P*-value less than 0.05 was considered as significant. Bars and error bars indicate mean ± SD.

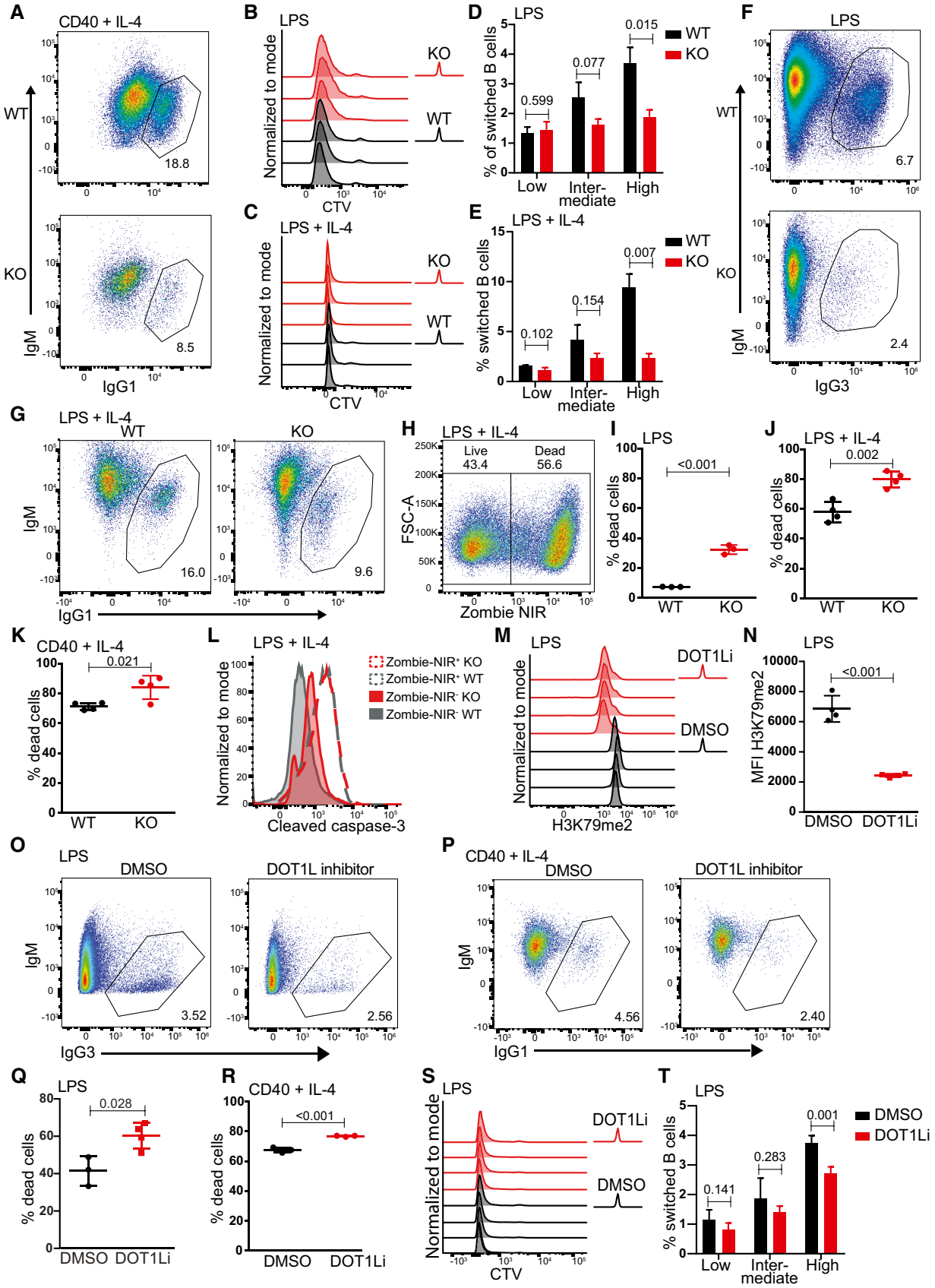

Figure EV3.

Figure EV4. *Dot1L*-deficient B cells show reduced serum Ig titers and aberrant plasma cells formation.

A–E Statistical analysis of resting serum titers of IgM (A), IgA (B), IgG1 (C), IgG2b (D), and IgG3 (E) from WT and KO mice. *P*-value for IgA (B) was calculated by Mann–Whitney test. Results represent the data from two independent experiments and numbers represent biological replicates for each group (WT; $n = 8$, KO; $n = 8$).

F Representative flow cytometry plots showing gating strategy to identify and compare plasma cells (Sca-I$^+$CD138$^+$) between WT and KO cells after 4 days of stimulation with LPS + IL-4 (left panel) and statistical analyses of their relative numbers (right panel). Results represent the data from three independent experiments, and numbers represent biological replicates for each group (WT; $n = 10$, KO; $n = 10$).

G Representative flow cytometry plots showing gating strategy to identify and compare activated B cells (CD138$^+$ Blimp1$^+$), Pre-PB cells (CD138$^-$ Blimp1$^+$) and plasma blast (PB) cells between WT and KO cells after 4 days of stimulation with LPS + IL-4 (left panel) and statistical analyses of their relative numbers (right panel). Results represent the data from one experiment, and numbers represent biological replicates for each group (WT; $n = 4$, KO; $n = 4$).

H Representative flow cytometry plots showing the relative surface density for B220 (CD45R) of WT and KO cells after 4 days of stimulation with LPS + IL-4 (left panel) and statistical analysis of MFI of B220 for each group (right panel). Results represent the data from three independent experiments, and numbers represent biological replicates for each group (WT; $n = 10$, KO; $n = 10$).

I Representative histograms showing the relative surface density for CD19 on total live WT and KO cells after 4 days of stimulation with LPS + IL-4 (left panel) and statistical analysis of MFI of CD19 for each group (right panel). Results represent the data from one experiment, and numbers represent biological replicates for each group (WT; $n = 4$, KO; $n = 4$).

Data information: Statistical analyses were performed using the Student's two-tailed unpaired *t*-test. Statistical significance was determined by calculating the *P*-value. A *P*-value less than 0.05 was considered as significant. Bars and error bars indicate mean ± SD.

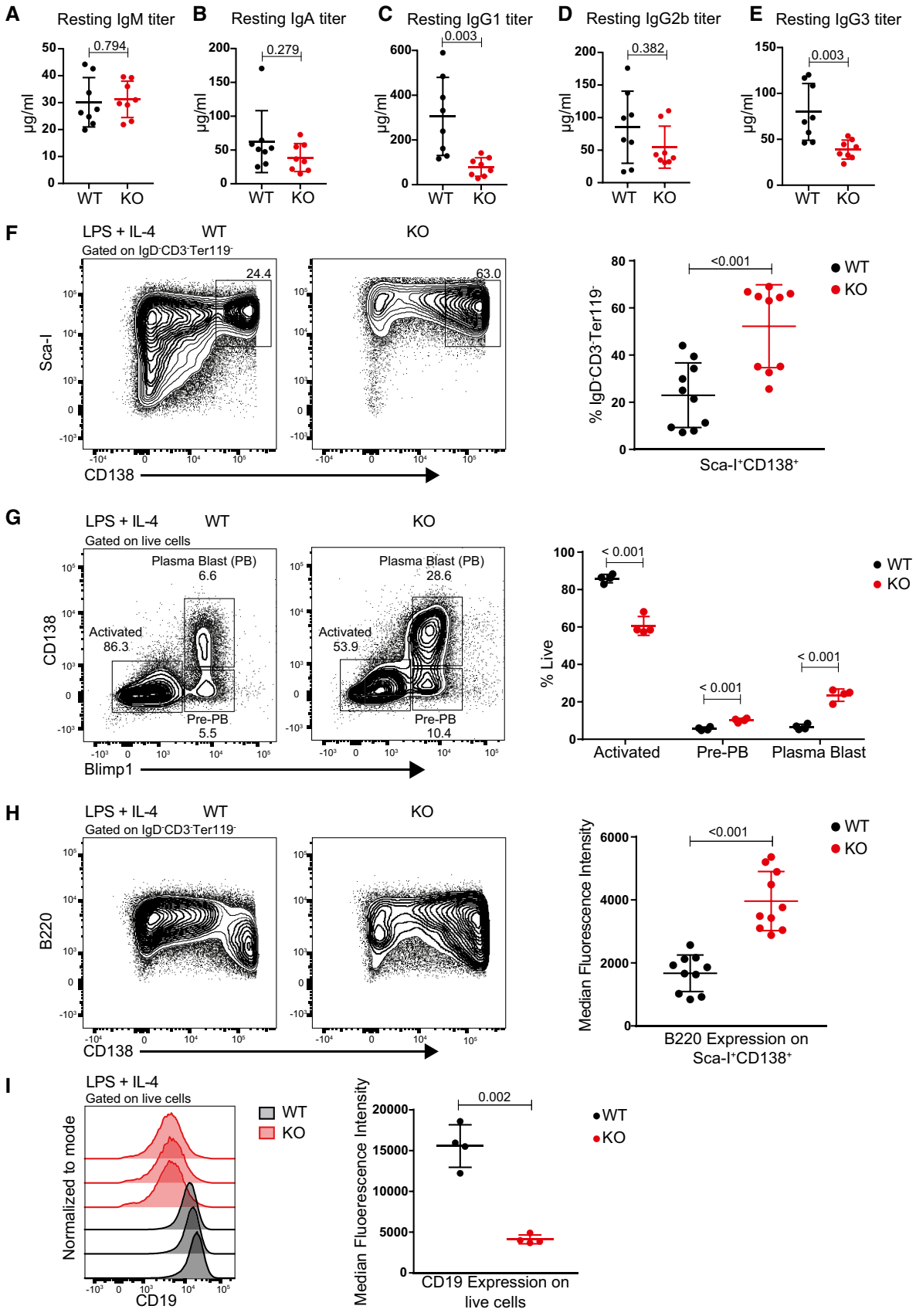

Figure EV4.

Figure EV5. *Dot1L* ablation in B cells results in transcriptional biasness, premature acquisition of plasma cell markers in naïve B cells, and represents transcriptional resemblance with *Ezh2*-KO plasma cells.

A–C H3K79me2 methylation at the *Myc* (A), *Bach2* (B), and *Prdm1* (C) loci from WT activated and naïve B cells, as determined by ChIP-seq reads per genomic content (RPGC). Data represent three independent biological replicates for each condition.

D Box plot showing the distribution of mean WT H3K79me2 level for *Bach2*, *Ezh2*, *Myc*, *Irf4*, and *Prdm1* among different indicated gene sets from activated and naïve B cells. Boxes in Box plot indicate Inter quartile range (IQR) and whiskers show 1.5 IQR of highest and lowest quartile. Central horizontal line within the bars represent median of the TMM normalized H3K79me2 counts + 1 values of the respective genes for each condition. Results represent the data generated from three biological replicates for each group.

E Relative distribution of plasma cell signature genes between WT and *Dot1L*-KO naïve B cells depicted by BARCODE plot. The *P*-values calculated via FRY test show the statistical significance of enrichment of each gene set. *P*-value less than 0.05 was considered as significant.

F Differential (FDR < 0.05) expression of *Irf4* transcripts as indicated by counts per million after TMM normalization from naïve and activated B cells from WT and KO mice. Data were generated from three independent biological replicates for each genotype. Bars and error bars indicate mean ± SD. Statistical significance is indicated by FDR after the Benjamini–Hochberg multiple testing correction performed by edgeR package using R language.

G MA-Plot of normalized RNA-Seq data generated from three independent biological replicates for each genotype showing relative distribution of Unfold protein response (UPR) genes between WT and *Dot1L*-KO Activated B cells.

H Lack of significant enrichment of UPR gene set between WT and *Dot1L*-KO activated B cells shown by Fast gene set enrichment analysis.

I Number of differentially (FDR < 0.05) expressed genes from naïve and activated WT and *Dot1L*-KO B cells. Results represent the data generated from three biological replicates for each group and condition.

J MA-Plot of normalized RNA-Seq data generated from three independent biological replicates for each genotype showing differentially (FDR < 0.05) expressed genes between *Ezh2*-KO and -WT plasma cells, using data GSE103126 (Guo *et al*, 2018).

K Relative distribution of up- (dark-red) and downregulated (green) genes from *Ezh2*-KO plasma cells between WT and *Dot1L*-KO naïve and activated B cells depicted by BARCODE plot. The *P*-values calculated via FRY test show the statistical significance of enrichment of each gene set. A *P*-value of less than 0.05 was considered as significant.

Source data are available online for this figure.

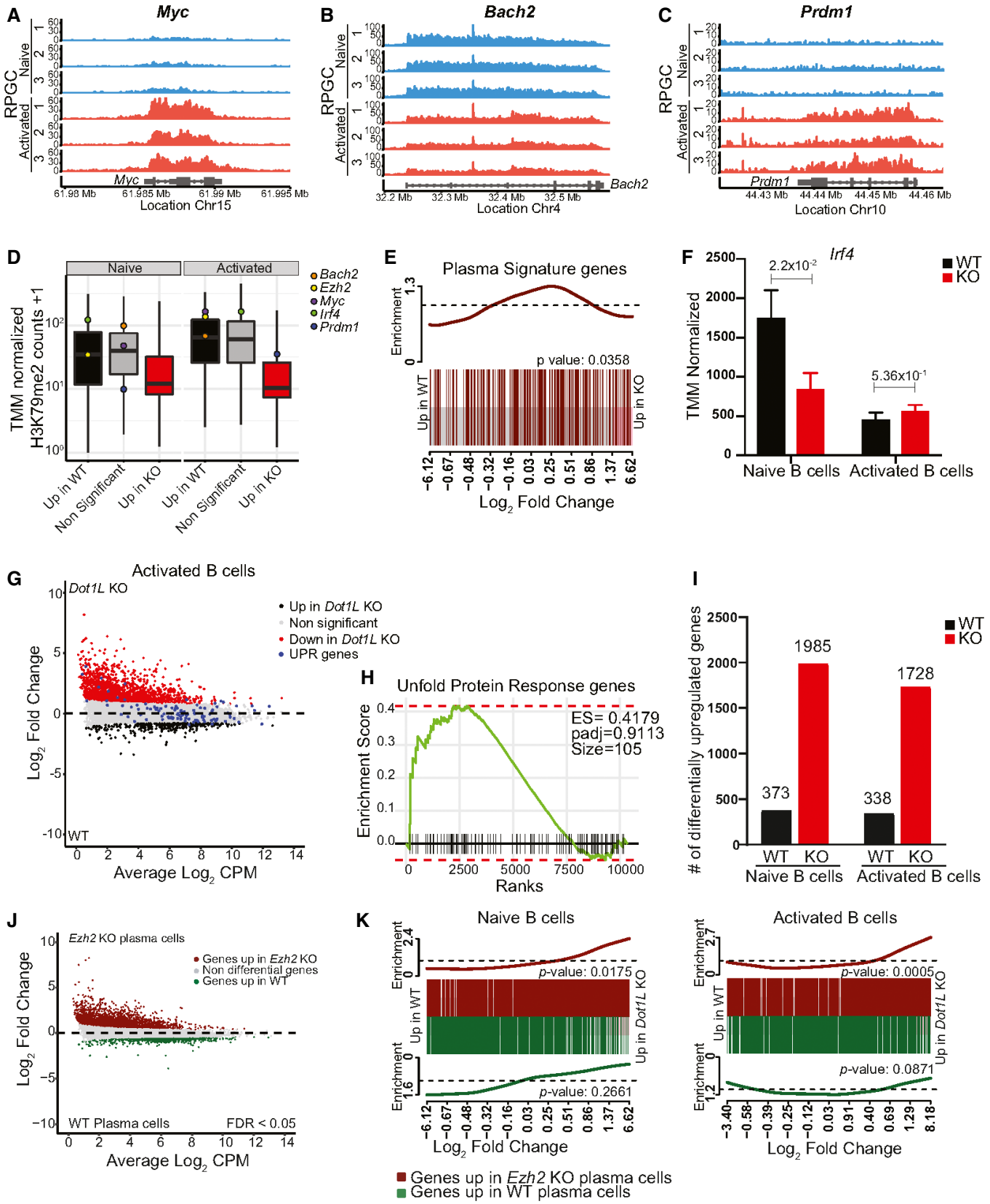

Figure EV5.