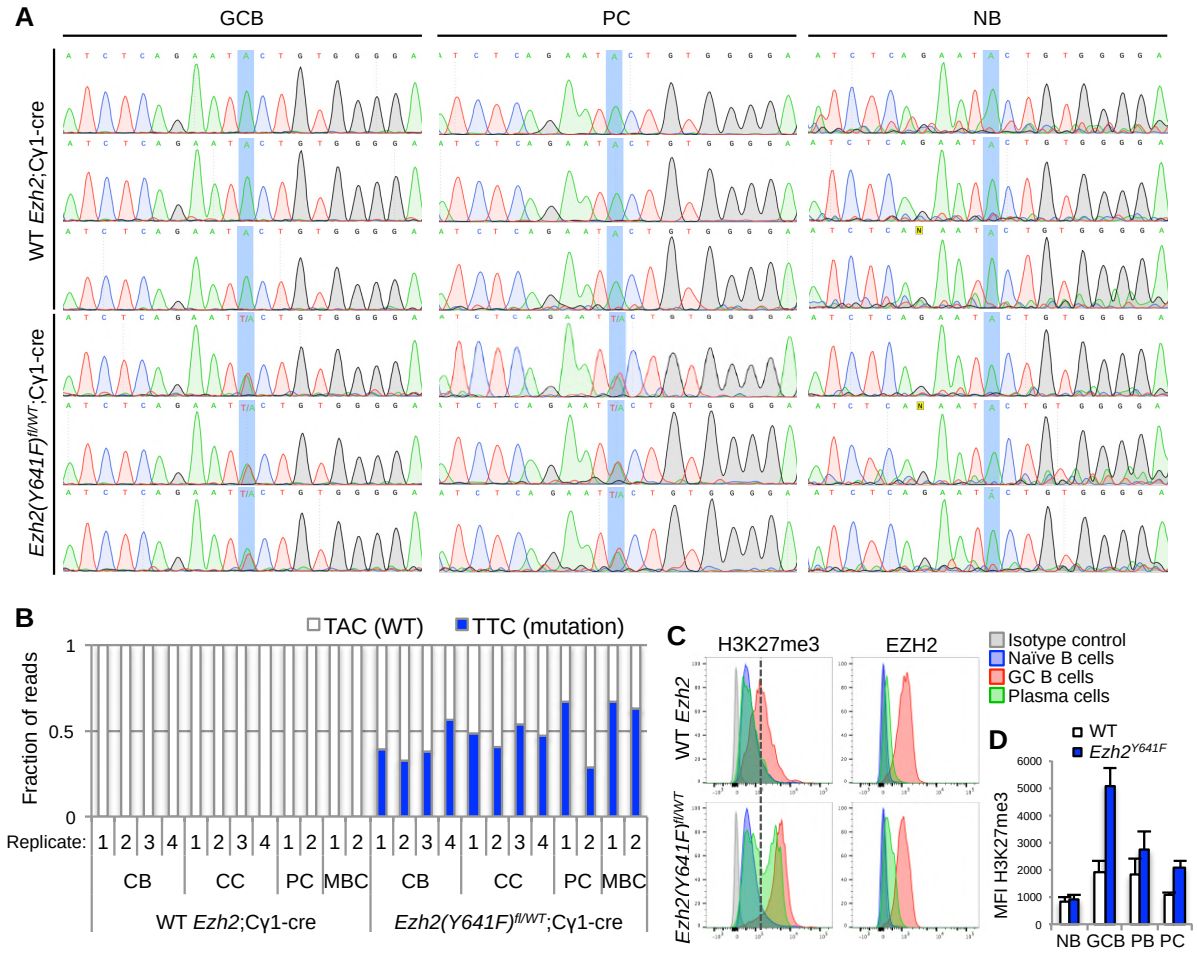
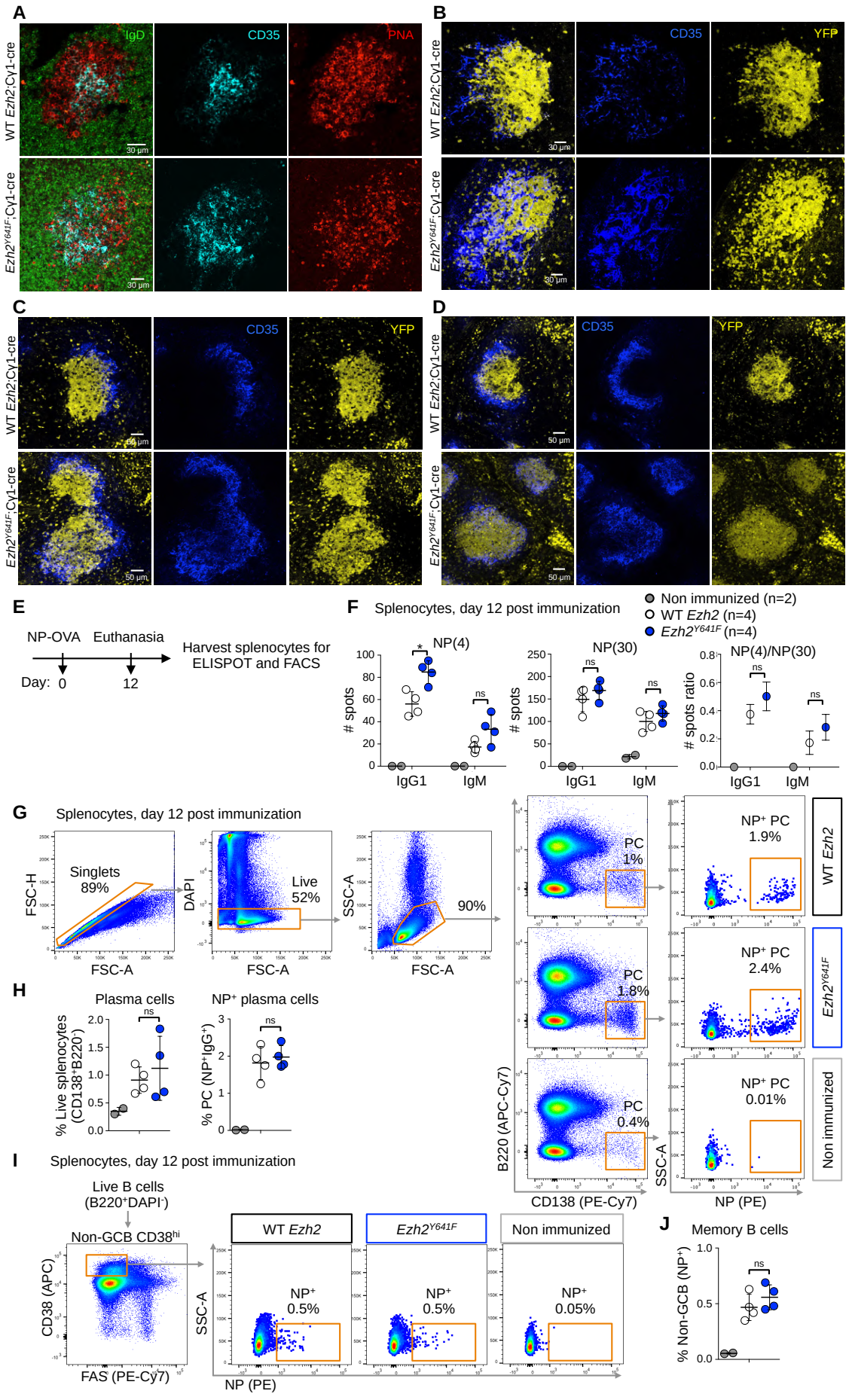
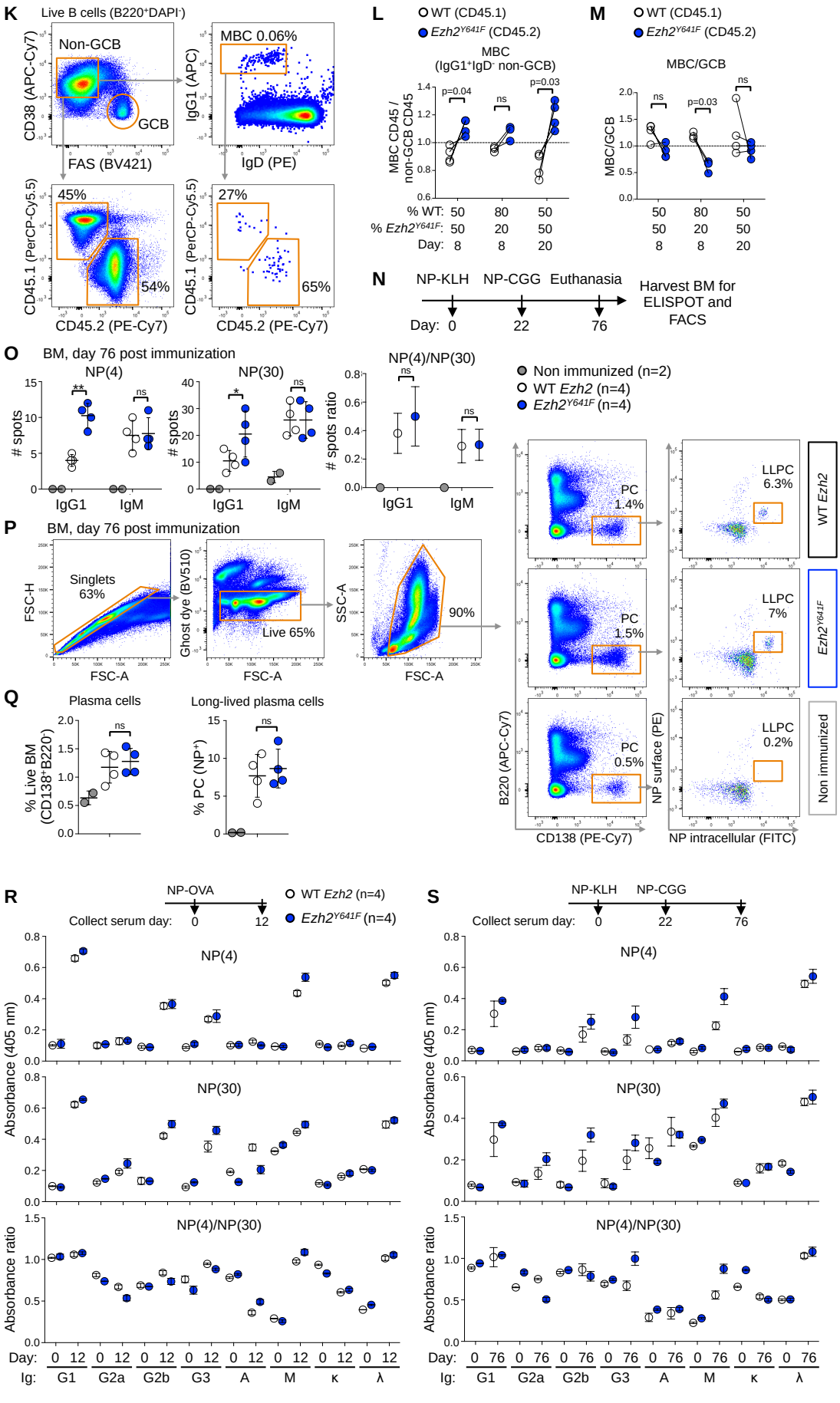


Figure S1. Related to Figure 1. Expression of mutant *EZH2*^{Y641F} upon Cre recombination.
A. Sanger sequencing of cDNA synthesized from RNA of sorted GC B-cells (GCB), plasma cells (PC) and naive B-cells (NB) of *Ezh2(Y641F)*$^{fl/WT}$*;Cγ1-cre* and WT *Ezh2;Cγ1-cre* mice reveals the expression of *Ezh2* Y641F point mutation (TAC: tyrosine -> TTC: phenylalanine) upon recombination in mice carrying the mutant allele. Note that NB sequences are poor quality due to the extremely low levels of expression of *Ezh2*.
B. Fraction of reads of RNA-seq displaying WT (TAC) or mutant (TTC) *Ezh2* allele in sorted centroblasts (CB), centrocytes (CC), plasma cells (PC) and memory B-cells (MBC).
C. Splenocytes from immunized *Ezh2(Y641F)*$^{fl/WT}$*;Cγ1-cre* and WT *Ezh2;Cγ1-cre* mice were stained to identify NB (FAS$^-$CD38$^+$IgD$^+$), GCB (FAS$^+$CD38$^-$) within B-cells (B220$^+$) and PC (B220$^-$CD138$^+$IgG1$^+$), cells were permeabilized and stained for H3K27me3 and EZH2 using fluorochrome-conjugated antibodies.
D. Mean fluorescence intensity of H3K27me3 (average of 5 control and 5 mutant *Ezh2* mice).


A
IgD
CD35
PNA
WT Ezh2;Cy1-cre
Ezh2Y641F;Cy1-cre
30 µm
B
CD35
YFP
C
50 µm
D
E
NP-OVA
Euthanasia
Day: 0
12
Harvest splenocytes for ELISPOT and FACS
F
Splenocytes, day 12 post immunization
Non immunized (n=2)
WT Ezh2 (n=4)
Ezh2Y641F (n=4)
NP(4)
NP(30)
NP(4)/NP(30)
# spots
# spots ratio
IgG1
IgM
ns
G
Splenocytes, day 12 post immunization
Singlets 89%
Live 52%
90%
FSC-H
FSC-A
DAPI
SSC-A
PC 1%
PC 1.8%
PC 0.4%
NP+ PC 1.9%
NP+ PC 2.4%
NP+ PC 0.01%
B220 (APC-Cy7)
CD138 (PE-Cy7)
NP (PE)
WT Ezh2
Ezh2Y641F
Non immunized
H
Plasma cells
NP+ plasma cells
% Live splenocytes (CD138+B220-)
% PC (NP+IgG+)
I
Splenocytes, day 12 post immunization
Live B cells (B220+DAPI-)
Non-GCB CD38hi
CD38 (APC)
FAS (PE-Cy7)
NP+ 0.5%
NP+ 0.5%
NP+ 0.05%
J
Memory B cells
% Non-GCB (NP+)


K
Live B cells (B220+DAPI-)
Non-GCB
GCB
CD38 (APC-Cy7)
FAS (BV421)
MBC 0.06%
IgG1 (APC)
IgD (PE)
45%
54%
27%
65%
CD45.1 (PerCP-Cy5.5)
CD45.2 (PE-Cy7)
L
WT (CD45.1)
Ezh2Y641F (CD45.2)
MBC
(IgG1+IgD- non-GCB)
MBC CD45 / non-GCB CD45
p=0.04
ns
p=0.03
% WT: 50 80 50
% Ezh2Y641F: 50 20 50
Day: 8 8 20
M
WT (CD45.1)
Ezh2Y641F (CD45.2)
MBC/GCB
ns
p=0.03
ns
50 80 50
50 20 50
8 8 20
N
NP-KLH
NP-CGG
Euthanasia
Harvest BM for ELISPOT and FACS
Day: 0 22 76
O
BM, day 76 post immunization
NP(4)
NP(30)
NP(4)/NP(30)
# spots
# spots ratio
**
*
ns
IgG1
IgM
Non immunized (n=2)
WT Ezh2 (n=4)
Ezh2Y641F (n=4)
P
BM, day 76 post immunization
Singlets 63%
FSC-H
FSC-A
Ghost dye (BV510)
Live 65%
SSC-A
90%
Q
Plasma cells
Long-lived plasma cells
% Live BM (CD138+B220-)
% PC (NP+)
PC 1.4%
LLPC 6.3%
PC 1.5%
LLPC 7%
PC 0.5%
LLPC 0.2%
WT Ezh2
Ezh2Y641F
Non immunized
B220 (APC-Cy7)
CD138 (PE-Cy7)
NP surface (PE)
NP intracellular (FITC)
R
NP-OVA
Collect serum day: 0 12
WT Ezh2 (n=4)
Ezh2Y641F (n=4)
NP(4)
NP(30)
NP(4)/NP(30)
Absorbance (405 nm)
Absorbance ratio
Day: 0 12 0 12 0 12 0 12 0 12 0 12 0 12 0 12
Ig: G1 G2a G2b G3 A M κ λ
S
NP-KLH
NP-CGG
Collect serum day: 0 22 76
NP(4)
NP(30)
NP(4)/NP(30)
Absorbance (405 nm)
Absorbance ratio
Day: 0 76 0 76 0 76 0 76 0 76 0 76 0 76 0 76
Ig: G1 G2a G2b G3 A M κ λ

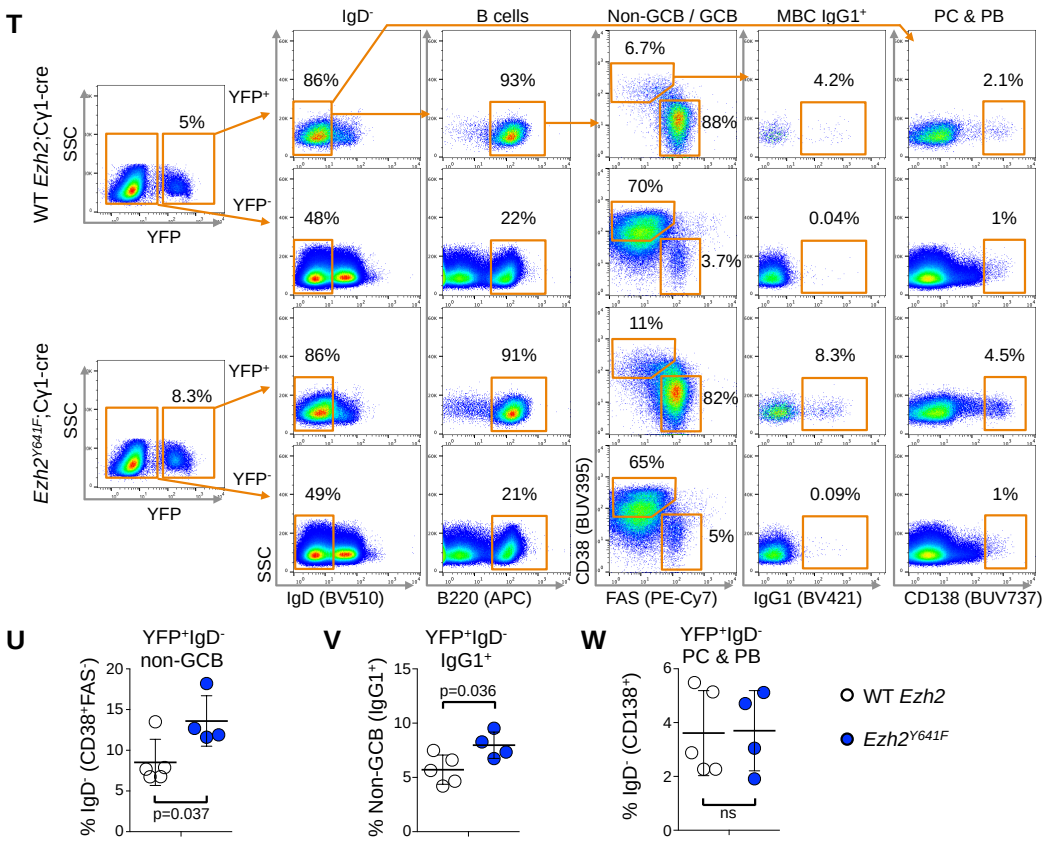


Figure S2. Related to Figure 2.

A. Immunofluorescence confocal microscopy images of *Cγ1-cre* GCs (top panels) and *Ezh2(Y641F)*$^{fl/WT}$;*Cγ1-cre* (bottom panels) in spleen sections stained with the indicated antibodies.

B-D. Immunofluorescence confocal microscopy images of GCs in spleen sections of *Rosa26-lox-stop-lox-YFP* reporter strain crossed with *Cγ1-cre* (top panels) or *Ezh2(Y641F)*$^{fl/WT}$;*Cγ1-cre* (bottom panels). Mice were injected with 2 µg anti CD35-BV421 16 hours before euthanasia. YFP cells represent GC B-cells and CD35 (blue) is a marker of FDC, showing the light zone.

E. Four *Ezh2*^{Y641F} and 4 WT mice were immunized with NP-OVA and euthanized 12 days later. Splenocytes were collected for ELISPOT and FACS experiments. Two non-immunized mice were included to evaluate basal levels.

F. For ELISPOT assays, one million splenocytes were incubated per well of 96-well plate with NP(4)-BSA or NP(30)-BSA. The number of spots per well were counted and plotted as average per spleen. Each dot represents a mouse and horizontal black lines are means ± SEM; unpaired t test, *p<0.05. The number of spots in plates coated with NP(4) were divided by the number of spots in plates with NP(30) to determine the production of high affinity antibodies. Each dot represents the mean value for the group of mice.

G, I, P. Gating strategy of splenocytes of one representative sample per group.

H, J, Q. Analysis of flow cytometry data of splenocytes gated as shown in **G**, **I** and **P**, respectively. Each dot represents a mouse. Horizontal black bars represent means ± SEM; paired t test.

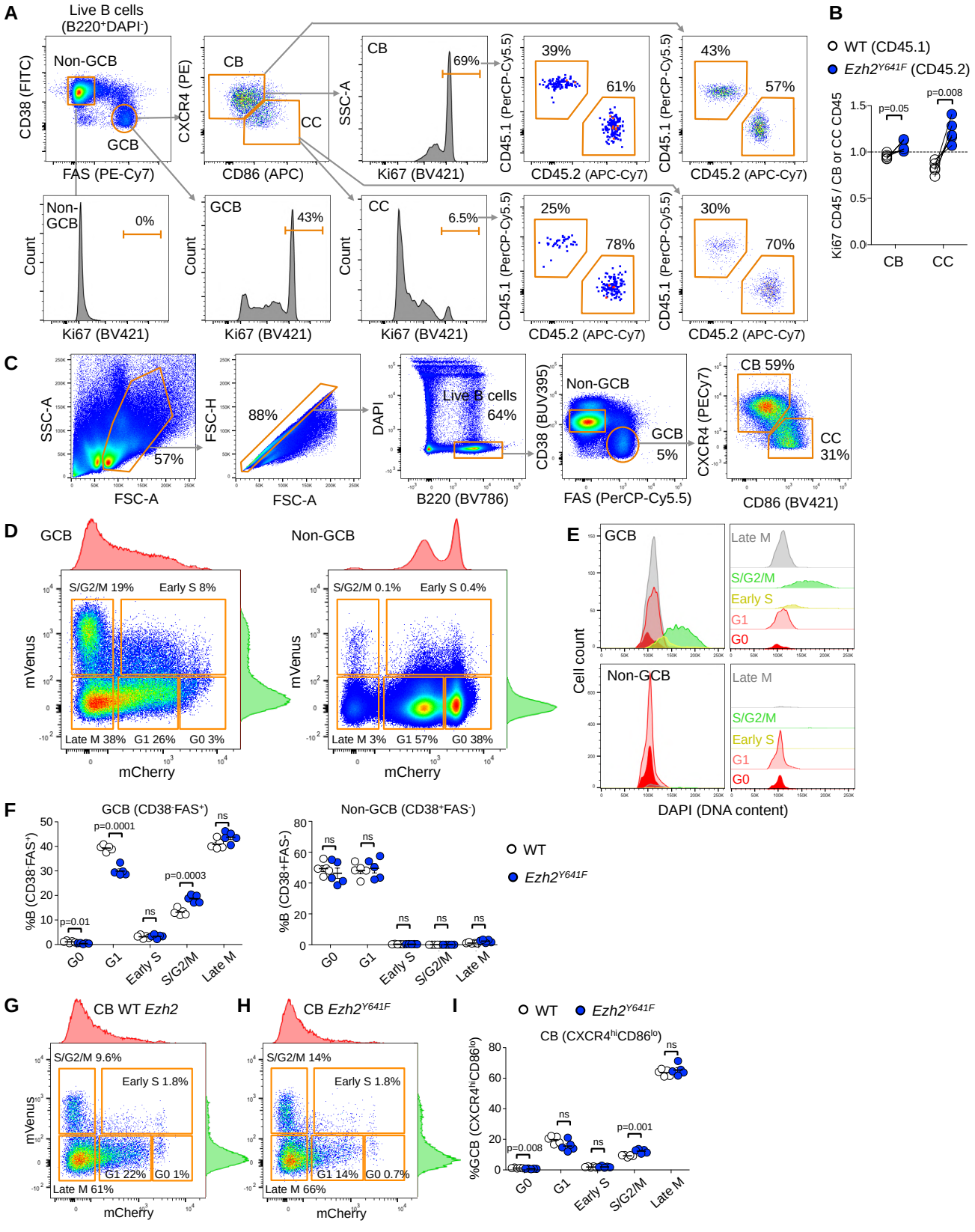
K-M. Mixed chimera experiments were performed as shown in **Figure 1A** and splenocytes were collected 8 or 20 days post immunization with SRBC for flow cytometry analysis. Gating strategy of splenocytes of one representative sample is shown in **K**. Data in **L** was analyzed by normalizing the percentage of CD45.1$^+$ memory B-cells (MBC, IgG1$^+$IgD$^-$) to their parental non-GC B-cells (CD38$^+$FAS$^-$), and equivalent normalization with CD45.2$^+$ populations (n=4 per group). MBC normalized to GC B-cells (**M**). Each pair of connected dots represents a mouse; p values paired t tests.

N. Four *Ezh2*^{Y641F} and 4 WT mice were immunized with NP-KLH, followed by a booster immunization 22 days later with NP-CGG, and then sacrificed 54 days later. Bone marrow was collected for ELISPOT and flow cytometry experiments. Two non-immunized mice were included to evaluate basal levels.

O. For ELISPOT assays, one million bone marrow cells were incubated per well of 96-well plate with NP(4)-BSA or NP(30)-BSA. Analysis was done as in **F**.

R-S. For ELISA assays, sera dilutions of 1:1000 were tested for the indicated antibodies. The lower panels show the ratio of absorbance of plates coated with NP(4)-BSA divided by the absorbance of plates coated with NP(30)-BSA. Each dot represents the mean value for the group of mice.

T-W. Four *R26-lox-stop-lox-YFP;Ezh2*^{Y641F} and 5 *R26-lox-stop-lox-YFP;Cγ1-cre* control mice were immunized with SRBC and euthanized 8 days later. Splenocytes were analyzed by flow cytometry, gating first on YFP$^+$ cells. Gating strategy of splenocytes of one representative sample per group is shown in **T**. As a reference, sub-gating in YFP negative cells is also shown. Each dot in **U-W** represents a mouse. Horizontal black bars represent means ± SD; p values unpaired t test. MBC: memory B-cell; PC: plasma cell; PB: plasmablast; LLPC: long-lived plasma cell.


A
Live B cells (B220+DAPI-)
CD38 (FITC)
FAS (PE-Cy7)
Non-GCB
GCB
CXCR4 (PE)
CD86 (APC)
CB
CC
SSC-A
Ki67 (BV421)
69%
CD45.1 (PerCP-Cy5.5)
CD45.2 (APC-Cy7)
39%
61%
43%
57%
Count
0%
43%
6.5%
25%
78%
30%
70%
B
WT (CD45.1)
Ezh2Y641F (CD45.2)
Ki67 CD45 / CB or CC CD45
p=0.05
p=0.008
C
SSC-A
FSC-A
57%
FSC-H
88%
DAPI
Live B cells
64%
B220 (BV786)
CD38 (BUV395)
FAS (PerCP-Cy5.5)
GCB
5%
CXCR4 (PECy7)
CB 59%
CC
31%
CD86 (BV421)
D
GCB
Non-GCB
mVenus
mCherry
S/G2/M 19%
Early S 8%
Late M 38%
G1 26%
G0 3%
S/G2/M 0.1%
Early S 0.4%
Late M 3%
G1 57%
G0 38%
E
Late M
S/G2/M
Early S
G1
G0
Cell count
DAPI (DNA content)
F
GCB (CD38-FAS+)
Non-GCB (CD38+FAS-)
%B (CD38-FAS+)
%B (CD38+FAS-)
p=0.0001
p=0.0003
p=0.01
ns
G0
G1
Early S
S/G2/M
Late M
WT
Ezh2Y641F
G
CB WT Ezh2
S/G2/M 9.6%
Early S 1.8%
G1 22%
G0 1%
Late M 61%
H
CB Ezh2Y641F
S/G2/M 14%
Early S 1.8%
G1 14%
G0 0.7%
Late M 66%
I
CB (CXCR4hiCD86lo)
%GCB (CXCR4hiCD86lo)
p=0.008
p=0.001

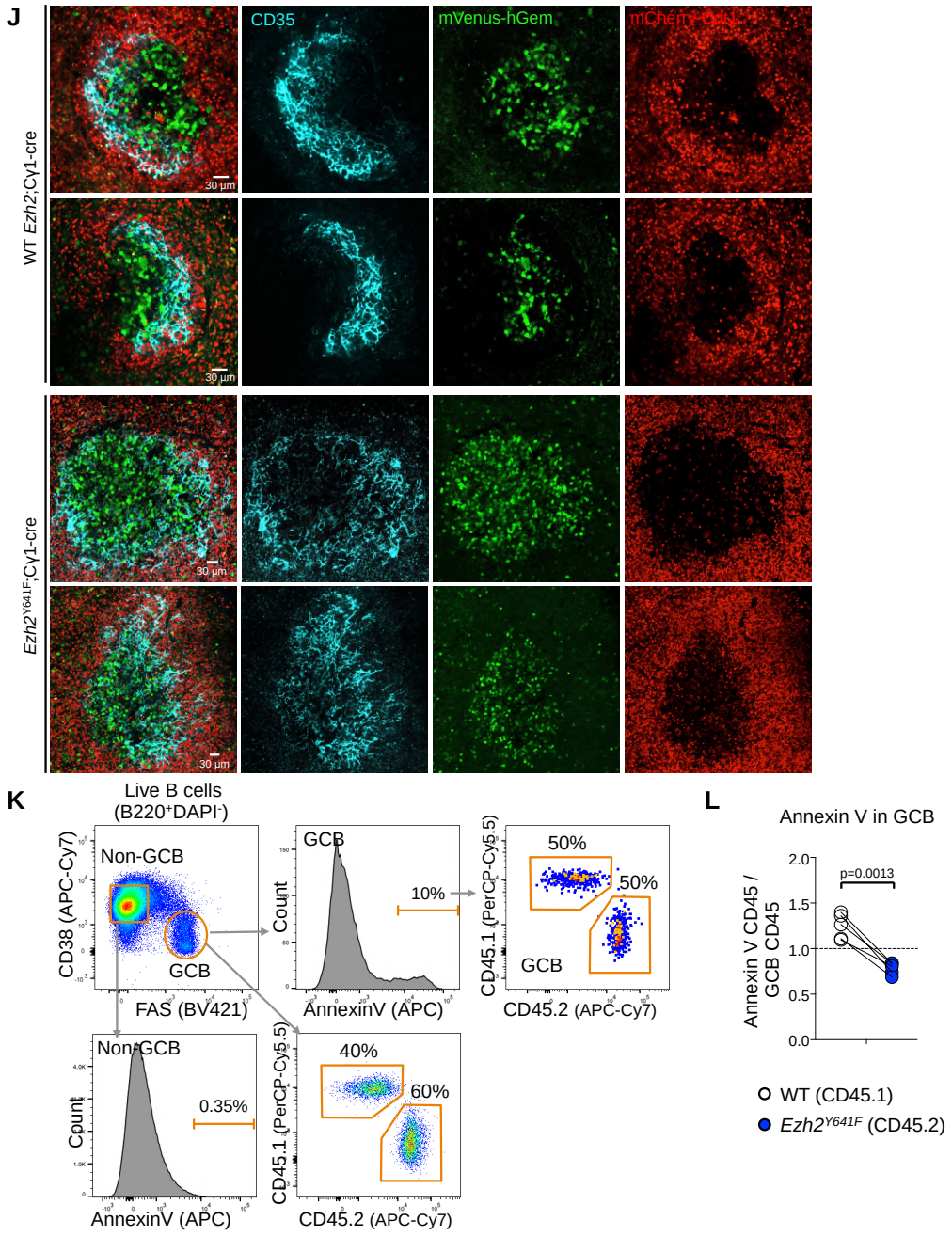


Figure S3. Related to Figure 3.

A-**B**. Gating strategy of lymph nodes of one representative sample. Flow cytometry data was analyzed by normalizing the percentage of CD45.1$^+$ Ki67$^+$ to their parental CD45.1$^+$ centroblasts (CB, CXCR4^{hi}CD86^{lo}) or centrocytes (CC, CXCR4^{lo}CD86^{hi}), and equivalent normalization with CD45.2$^+$ populations (n=4 per group). Each pair of connected dots represents a mouse; p values paired t tests.

C. Gating strategy of splenocytes of one representative *R26-Fucci2aR*;*Cγ1-cre* sample.

D. Flow cytometry analysis of Fucci2a splenocytes from mice immunized with SRBC for 8 days.

E. The gating strategy of Fucci2a was validated by evaluating DNA content using DAPI staining in fixed *R26-Fucci2aR* splenocytes.

F. Quantification of GC B-cells and non-GC B-cells according to the gating shown in **D**. Each dot represents a mouse and horizontal black lines are means ± SEM; p values unpaired t tests.

G-I. Gating strategy and quantification of Fucci2a centroblasts. Values in **I** shown as in **F**.

J. Immunofluorescence confocal microscopy images of GCs in spleen sections of *R26-Fucci2aR*;*Ezh2*^{Y641F} and *R26-Fucci2aR*;*Cγ1-cre* mice. Mice were injected with 2 µg anti CD35-BV421 16 hours before euthanasia.

K-**L**. Gating strategy of lymph nodes of one representative sample and quantification, analyzed as in **B**.

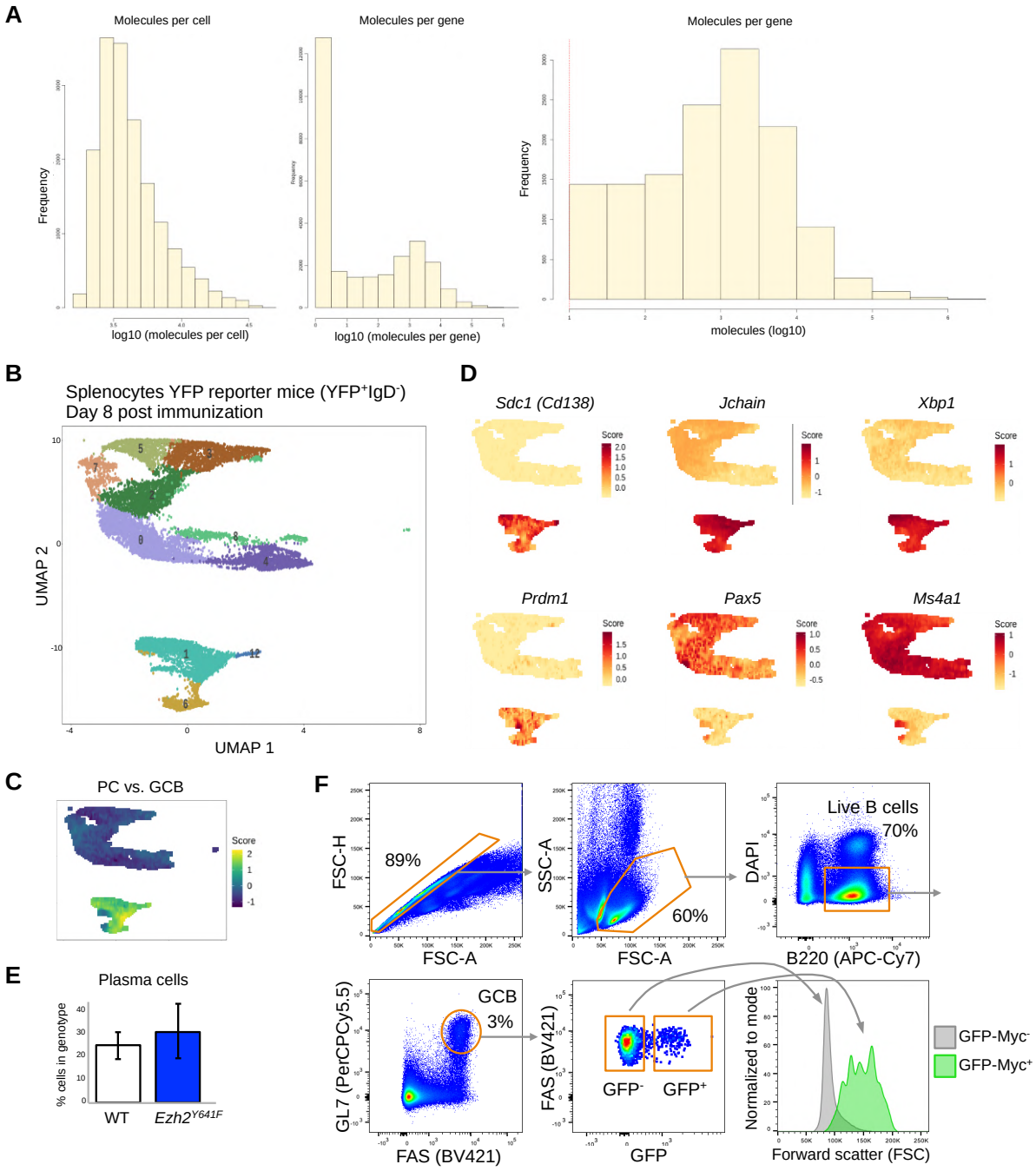
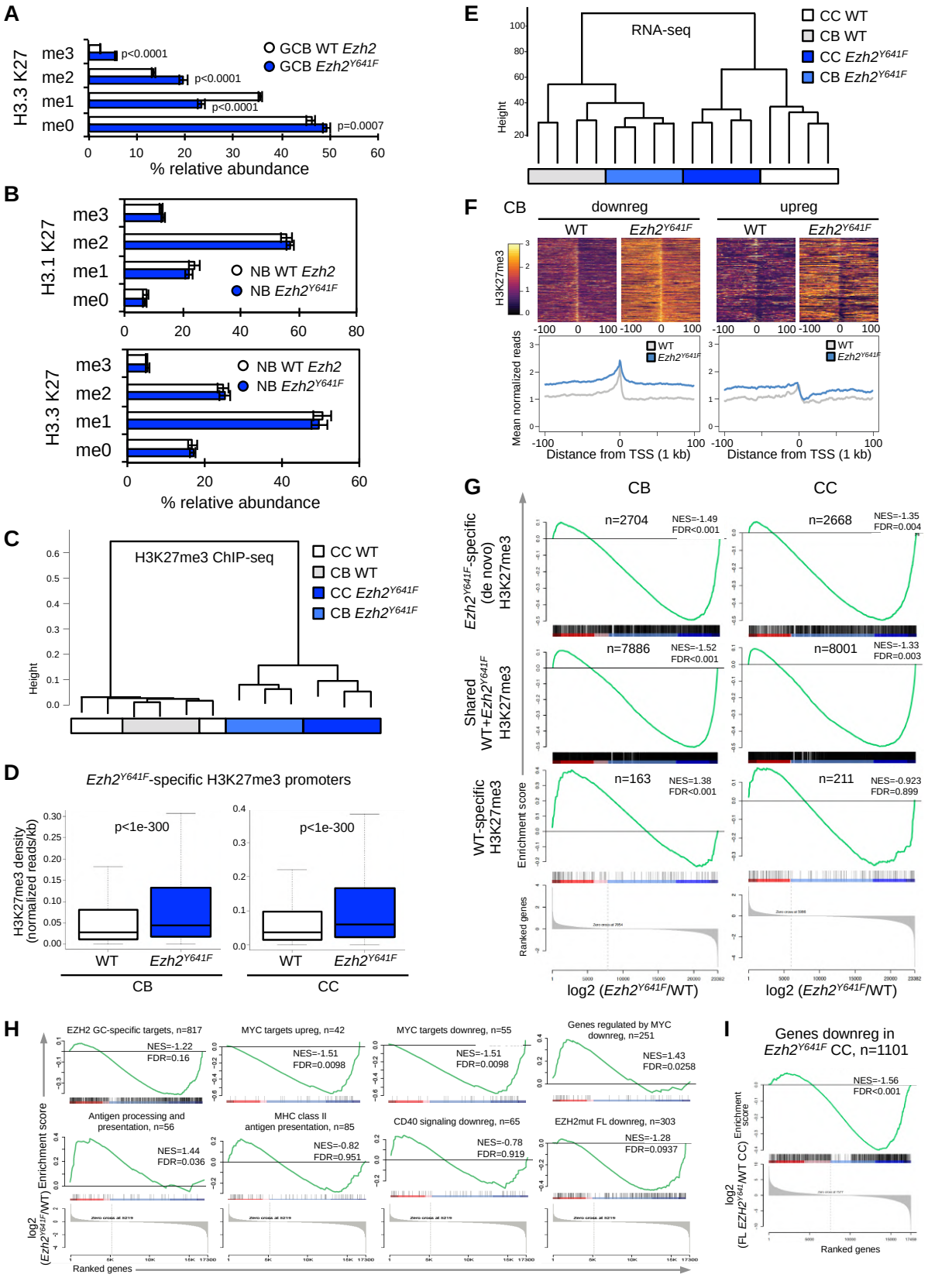
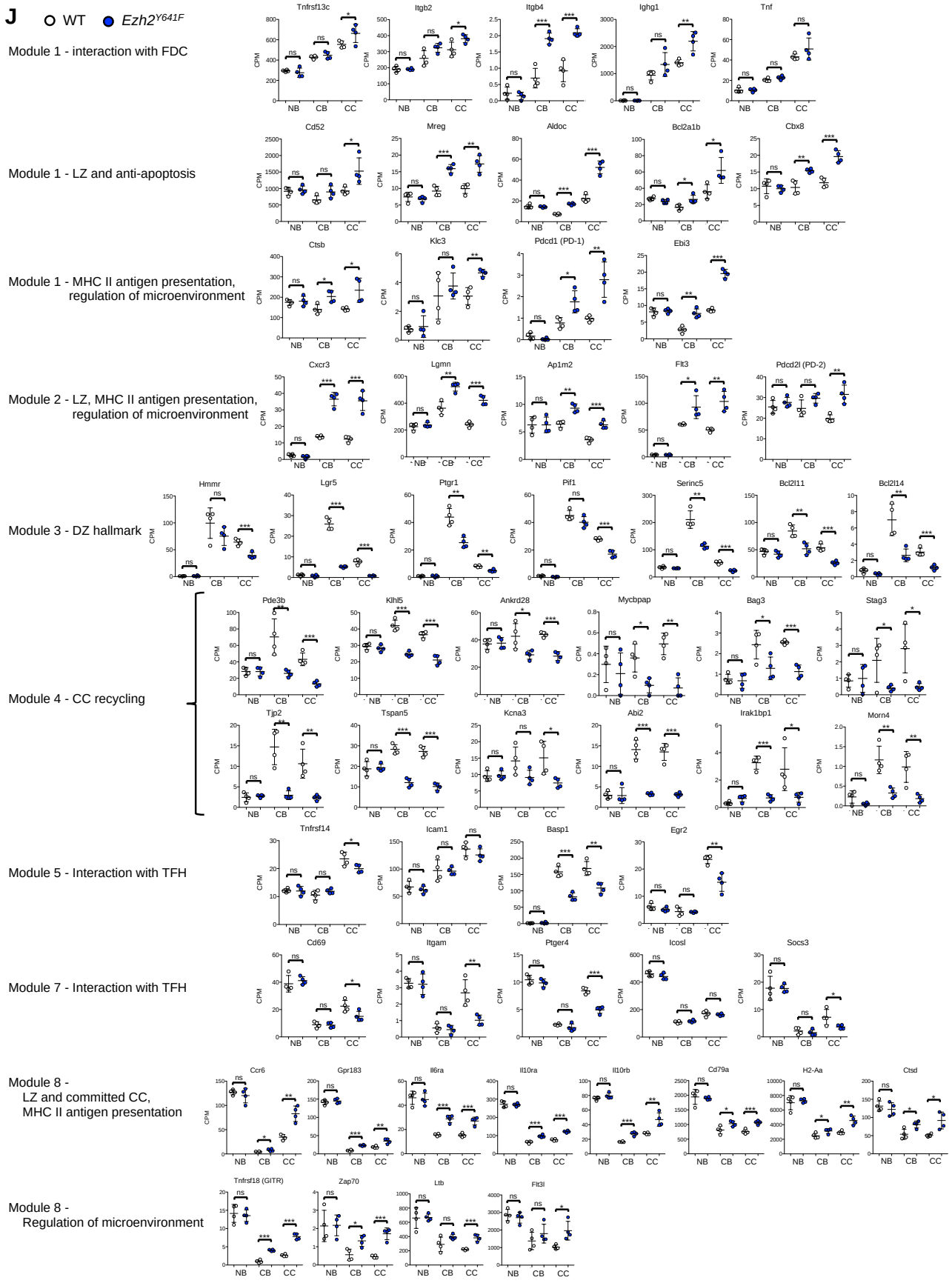


Figure S4. Related to Figure 4.
A-E. YFP^{+}IgD^{-} splenocytes were sorted from 3 *R26-lox-stop-lox-YFP;Ezh2*^{Y641F} and 3 *R26-lox-stop-lox-YFP;Cγ1-cre* mice 8 days after SRBC immunization and were subjected to single cell RNA-seq using 10X Genomics Chromium platform. Quality control metrics for sequencing is shown in **A**. Dimensionality reduction with UMAP on normalized gene expression values was performed using K nearest neighbor analysis and graph based clustering to assign cells to clusters with distinct expression profiles (**B**). Plasma cell signature (**C**) and specific gene expression (**D**) were projected on clusters. After normalization for total number of analyzed cells, the abundance of *Ezh2*^{Y641F} and WT in plasma cells was calculated (**E**). Data is shown as means ± SE for n=3 mice per group.
F. Gating strategy of splenocytes of one representative *GFP-Myc*;*Cγ1-cre* sample.
See also **Table S1**.


A
H3.3 K27
me3
me2
me1
me0
p<0.0001
p<0.0001
p<0.0001
p=0.0007
GCB WT Ezh2
GCB Ezh2Y641F
% relative abundance
B
H3.1 K27
NB WT Ezh2
NB Ezh2Y641F
H3.3 K27
% relative abundance
C
H3K27me3 ChIP-seq
CC WT
CB WT
CC Ezh2Y641F
CB Ezh2Y641F
Height
D
Ezh2Y641F-specific H3K27me3 promoters
H3K27me3 density (normalized reads/kb)
p<1e-300
p<1e-300
WT
Ezh2Y641F
CB
CC
E
RNA-seq
CC WT
CB WT
CC Ezh2Y641F
CB Ezh2Y641F
Height
F
CB
downreg
upreg
WT
Ezh2Y641F
H3K27me3
Mean normalized reads
Distance from TSS (1 kb)
G
CB
CC
Ezh2Y641F-specific (de novo) H3K27me3
n=2704
NES=-1.49
FDR<0.001
n=2668
NES=-1.35
FDR=0.004
Shared WT+Ezh2Y641F H3K27me3
n=7886
NES=-1.52
FDR<0.001
n=8001
NES=-1.33
FDR=0.003
WT-specific H3K27me3
n=163
NES=1.38
FDR<0.001
n=211
NES=-0.923
FDR=0.899
Enrichment score
Ranked genes
log2 (Ezh2Y641F/WT)
H
EZH2 GC-specific targets, n=817
NES=-1.22
FDR=0.16
MYC targets upreg, n=42
NES=-1.51
FDR=0.0098
MYC targets downreg, n=55
NES=-1.51
FDR=0.0098
Genes regulated by MYC downreg, n=251
NES=1.43
FDR=0.0258
Antigen processing and presentation, n=56
NES=1.44
FDR=0.036
MHC class II antigen presentation, n=85
NES=-0.82
FDR=0.951
CD40 signaling downreg, n=65
NES=-0.78
FDR=0.919
EZH2mut FL downreg, n=303
NES=-1.28
FDR=0.0937
log2 (Ezh2Y641F/WT)
Enrichment score
Ranked genes
I
Genes downreg in Ezh2Y641F CC, n=1101
NES=-1.56
FDR<0.001
log2 (FL EZH2Y641F/WT CC)
Enrichment score
Ranked genes


J
WT
Ezh2Y641F
Module 1 - interaction with FDC
Tnfrsf13c
Itgb2
Itgb4
Ighg1
Tnf
Module 1 - LZ and anti-apoptosis
Cd52
Mreg
Aldoc
Bcl2a1b
Cbx8
Module 1 - MHC II antigen presentation, regulation of microenvironment
Ctsb
Klc3
Pdcd1 (PD-1)
Ebi3
Module 2 - LZ, MHC II antigen presentation, regulation of microenvironment
Cxcr3
Lgmn
Ap1m2
Flt3
Pdcd2l (PD-2)
Module 3 - DZ hallmark
Hmmr
Lgr5
Ptgr1
Pif1
Serinc5
Bcl2l11
Bcl2l14
Module 4 - CC recycling
Pde3b
Klhl5
Ankrd28
Mycbpap
Bag3
Stag3
Tjp2
Tspan5
Kcna3
Abi2
Irak1bp1
Morn4
Module 5 - Interaction with TFH
Tnfrsf14
Icam1
Basp1
Egr2
Module 7 - Interaction with TFH
Cd69
Itgam
Ptger4
Icosl
Socs3
Module 8 - LZ and committed CC, MHC II antigen presentation
Ccr6
Gpr183
Il6ra
Il10ra
Il10rb
Cd79a
H2-Aa
Ctsd
Module 8 - Regulation of microenvironment
Tnfrsf18 (GITR)
Zap70
Ltb
Flt3l
CPM
NB
CB
CC
ns

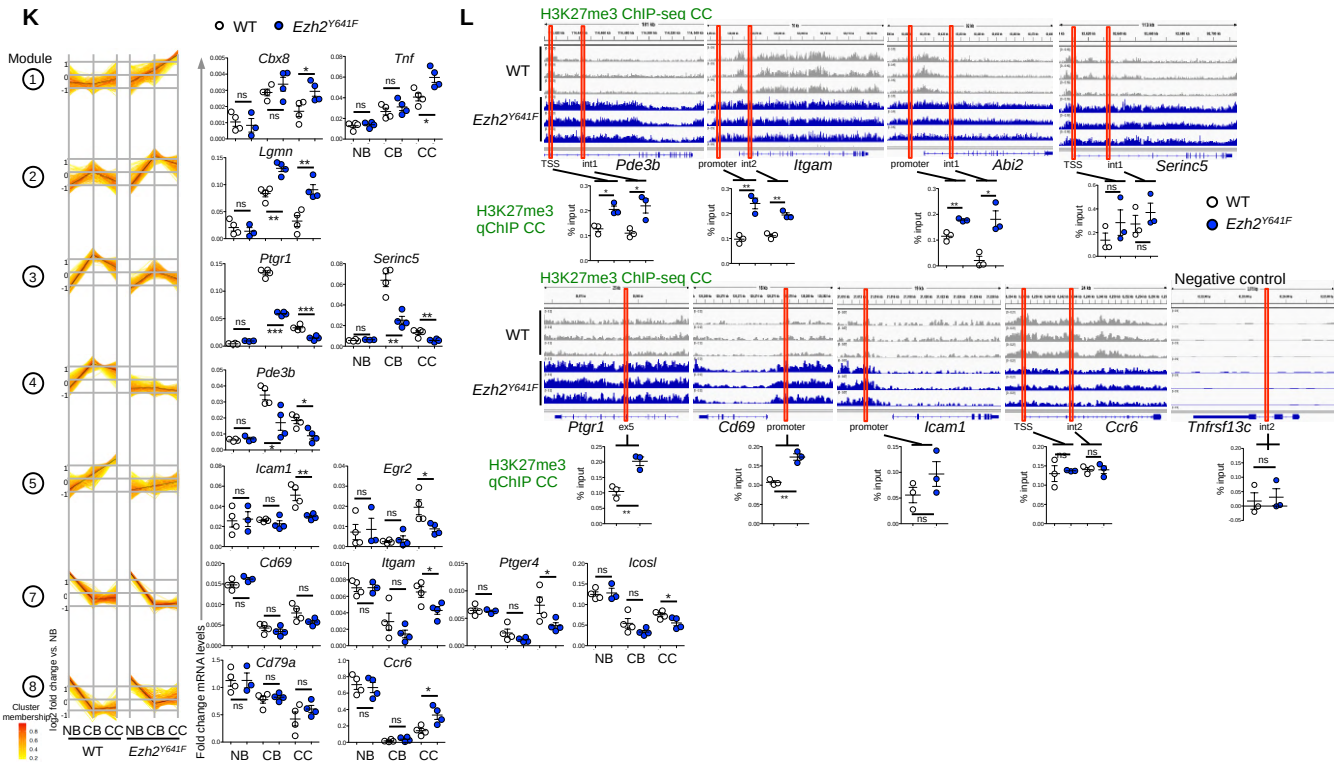


Figure S5. Related to Figure 5.

A-B. Relative abundance of H3K27 modifications was assessed by liquid chromatography separation and mass spectrometry of histone tryptic peptides from sorted GC B cells (**A**) and naive B cells (**B**) of 5 *Ezh2*^{Y641F} and 5 *Cγ1-cre* control mice immunized with SRBC and euthanized 8 days later. Data is shown as means ± SEM; p value unpaired t test.

C. Hierarchical clustering was performed with normalized H3K27me3 reads within promoters (TSS±5kb) of WT *Ezh2* and *Ezh2*^{Y641F} centroblasts and centrocytes. Clustering was performed using correlation distance (1-correlation coefficient) of 95th percentile variable promoters and Ward's method.

D. Boxplot showing H3K27me3 density between *Ezh2*^{Y641F} de novo H3K27me3 and nearest adjacent promoter.

E. Hierarchical clustering was performed using Ward's method with Euclidean distance on TPM (transcripts per million) values for the highly variable genes (variance in the top 10 percentile).

F. H3K27me3 normalized read density heat maps at promoters associated with differentially expressed genes in centroblasts (top). Bottom plot shows mean H3K27me3 profile across loci interval.

G. GSEA of *Ezh2*^{Y641F}-specific, WT-specific, and shared H3K27me3 marked promoters in *Ezh2*^{Y641F} vs. WT GC B-cells.

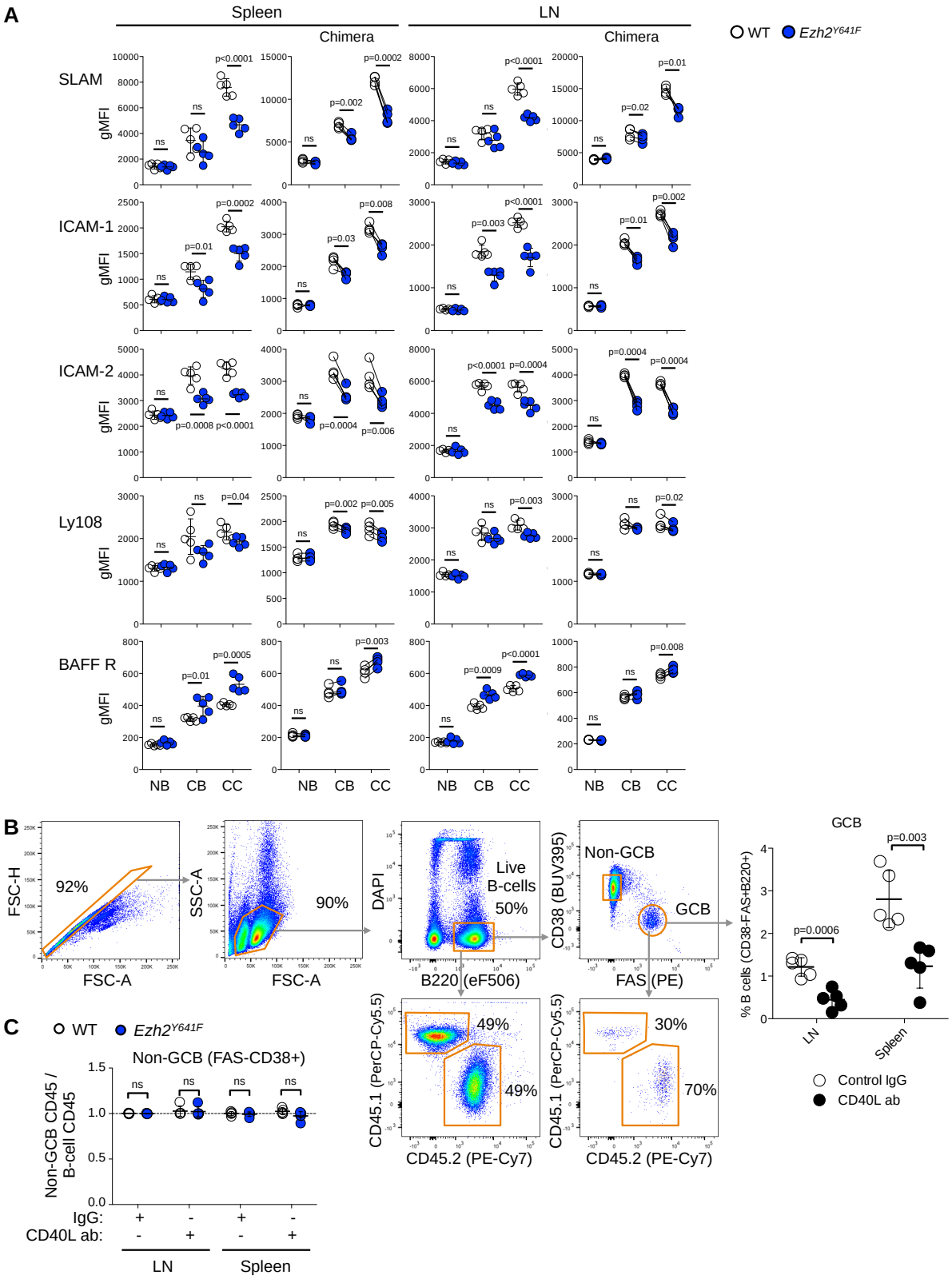
H. GSEA of PAGE gene signatures in *Ezh2*^{Y641F} centrocytes vs. WT. See also **Table S2.**

I. GSEA enrichment of genes down-regulated in murine *Ezh2*^{Y641F} centrocytes against the gene expression profile of *EZH2* mutant FL cases vs. WT human tonsilar centrocytes (Green et al., 2015).

J. mRNA expression levels of indicated transcripts as normalized counts per million (CPM) from naive B-cells, centroblasts and centrocytes RNA-seq.

K. Four *Ezh2*^{Y641F} and 4 *Cγ1-cre* mice were immunized with SRBC and euthanized 8 days later. Naive B-cells (NB), centroblasts (CB) and centrocytes (CC) were sorted and subjected to RT-qPCR using primers for the shown transcripts. Each dot represents a mouse and horizontal black lines represent mean fold change mRNA levels normalized to *Hprt1* (for *Ptgr1, Ccr6*), *Gapdh* (for *Cbx8*, *Lgmn*, *Serinc5*, *Cd79a*, *Itgam*, *Ptger4*) or *Rpl13* (for *Pde3b*, *Cd69*, *Egr2*, *Icosl*, *Icam1, Tnf*) ± SEM.

L. H3K27me3 ChIP-seq tracks and corresponding qChIP validation. Data collected for qChIP was an independent experiment performed with a different cohort of 3 mice per group. Each dot represents a mouse and horizontal black lines represent mean ± SEM.


A
Spleen
LN
Chimera
Chimera
WT
Ezh2Y641F
SLAM
ICAM-1
ICAM-2
Ly108
BAFF R
gMFI
NB
CB
CC
B
FSC-H
FSC-A
92%
SSC-A
FSC-A
90%
DAPI
Live
B-cells
50%
B220 (eF506)
CD38 (BUV395)
Non-GCB
GCB
FAS (PE)
CD45.1 (PerCP-Cy5.5)
49%
49%
CD45.2 (PE-Cy7)
30%
70%
CD45.2 (PE-Cy7)
GCB
% B cells (CD38-FAS+B220+)
p=0.0006
p=0.003
LN
Spleen
Control IgG
CD40L ab
C
WT
Ezh2Y641F
Non-GCB (FAS-CD38+)
Non-GCB CD45 /
B-cell CD45
IgG:
CD40L ab:
LN
Spleen

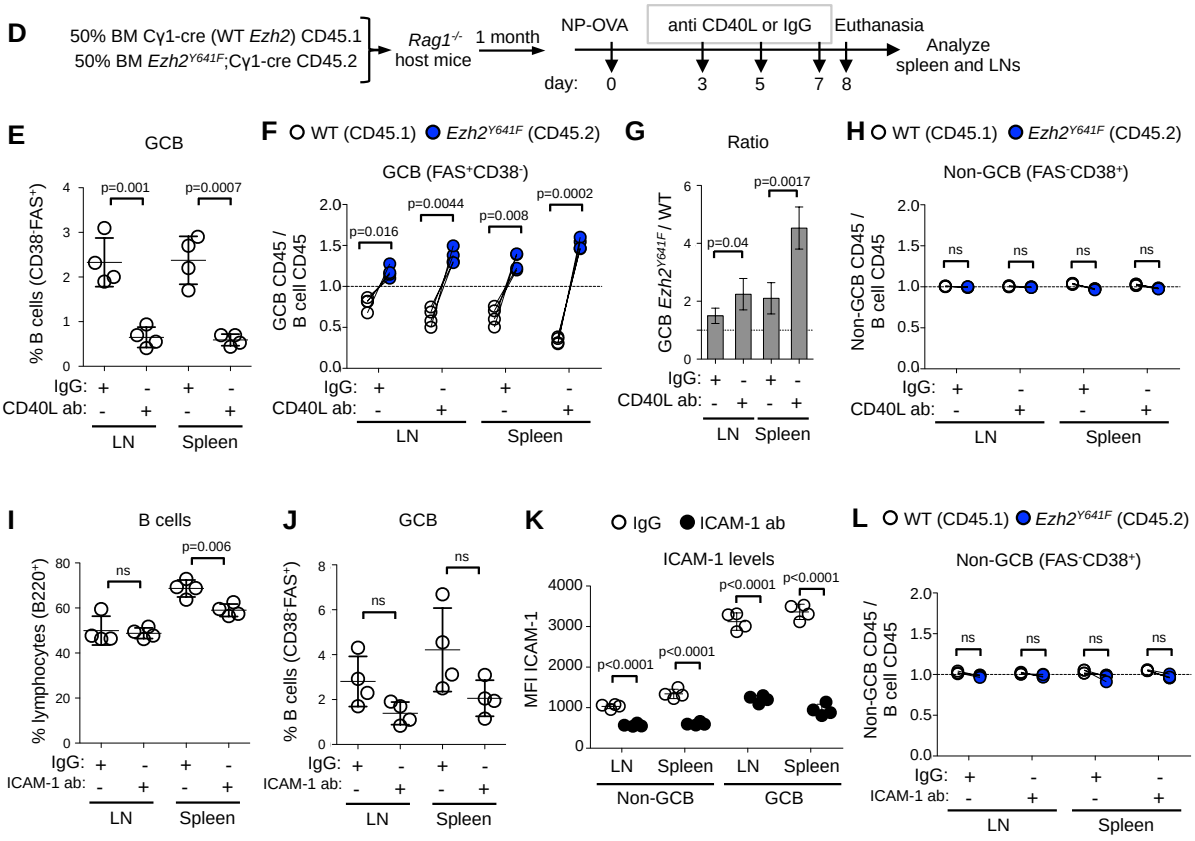


Figure S6. Related to Figure 6.

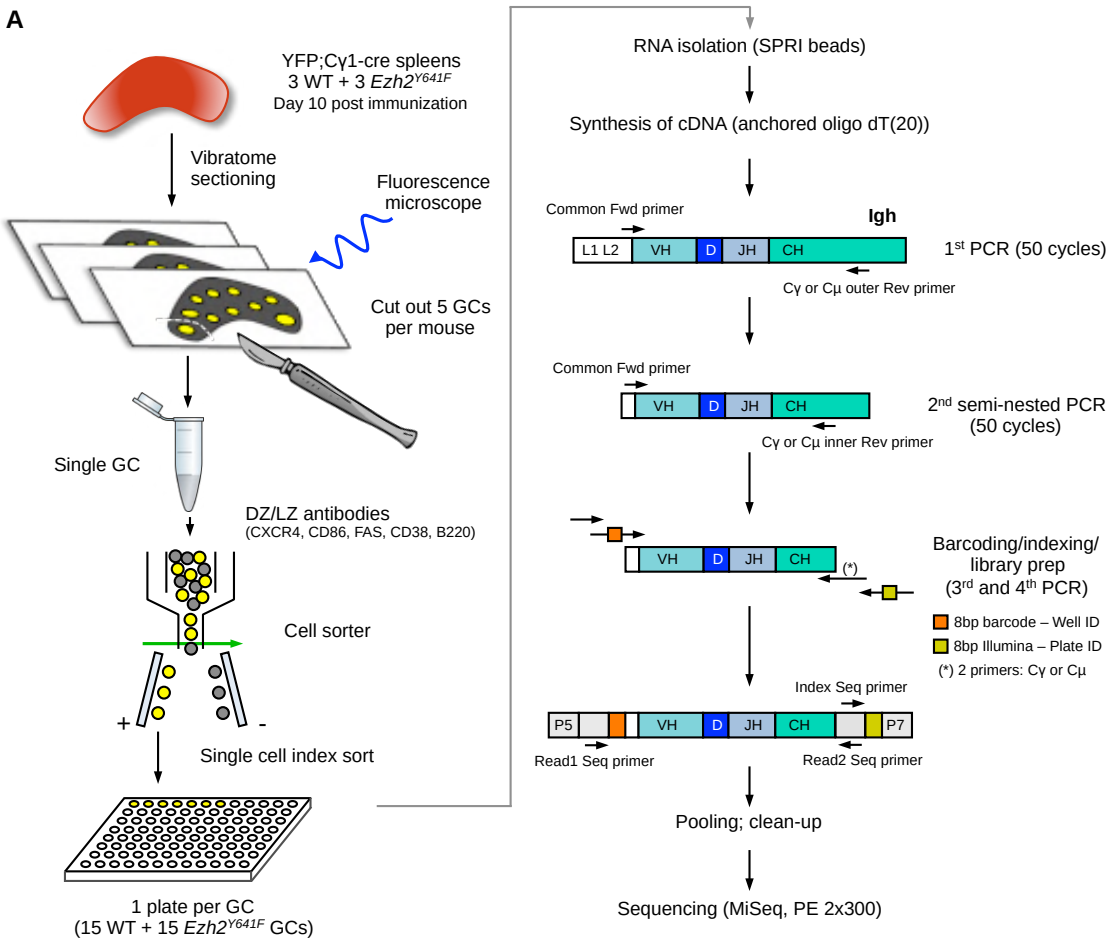
A. Five *R26-lox-stop-lox-YFP;Ezh2*^{Y641F}, 5 *R26-lox-stop-lox-YFP;Cγ1-cre* control, and 4 mixed chimera (50%WT+50%*Ezh2*^{Y641F}) were immunized with NP-OVA and euthanized 8 days later. Splenocytes and popliteal lymph node cells were collected for FACS. gMFI for each protein was assessed in naive B-cells (CD38^{hi}FAS^{-}IgD^{+} fraction of B cells), centroblasts (CXCR4^{hi}CD86^{low} fraction of YFP^{+} GC B-cells) and centrocytes (CXCR4^{low}CD86^{hi} fraction of YFP^{+} GC B-cells). Each dot represents a mouse. Horizontal black bars represent means ± SEM, p values unpaired (in non-chimera) and paired (in chimera) t tests.

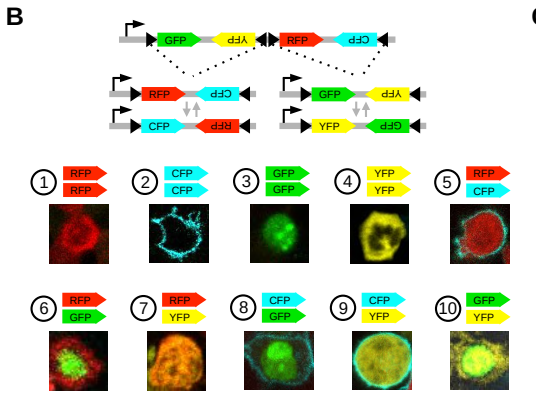
B. Gating strategy of lymph nodes of one representative sample and quantification of total GC B-cells in lymph nodes and spleen, to show the effect of anti CD40L antibody in GC formation. Each dot represents a mouse and horizontal black lines are means ± SEM; p values unpaired t tests.

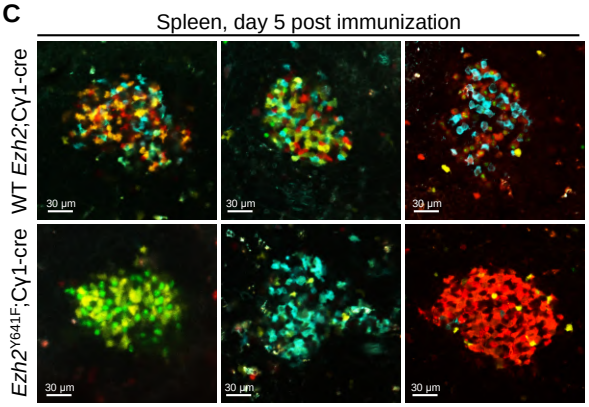
C. Flow cytometry data was analyzed by normalizing the percentage of CD45.1^{+} non-GC B-cells (CD38^{+}FAS^{-}) to their parental CD45.1^{+} B-cells (B220^{+}DAPI^{-}), and equivalent normalization with CD45.2^{+} populations (n=4 per group).

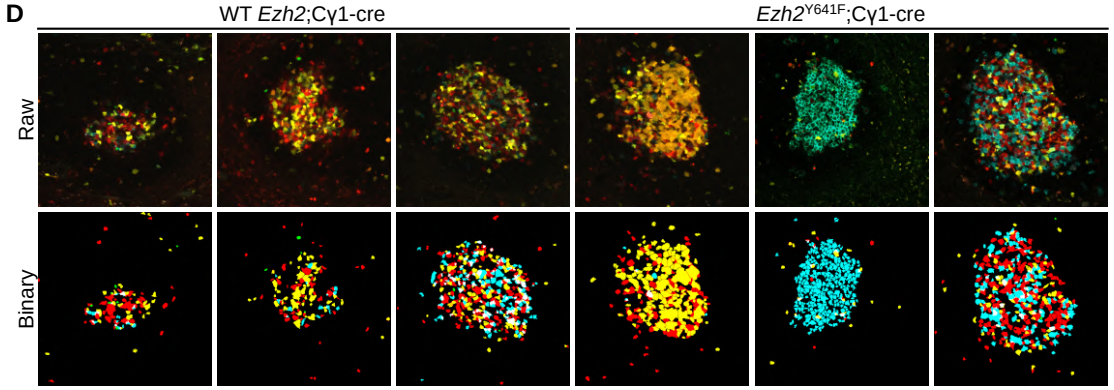
D-H. Four mixed chimera mice were immunized with NP-OVA and 3 days later they received 100 µg anti CD40L antibody or control IgG antibody i.v. Mice received a second dose of antibody on day 5 and a third dose on day 7, and were euthanized the next day (**D**). Percentage of GC B-cells in spleen and popliteal and inguinal lymph nodes was analyzed by flow cytometry (**E**). Data was analyzed by normalizing the percentage of CD45.1^{+} GC B-cells (CD38^{-}FAS^{+}) (**F**) and non GC B-cells (CD38^{+}FAS^{-}) (**H**) to their parental CD45.1^{+} B-cells (B220^{+}DAPI^{-}), and equivalent normalization with CD45.2^{+} populations (n=4 per group). Each pair of connected dots represents a mouse; p values from paired t tests. Proportions of *Ezh2*^{Y641F} GC B-cells were divided by WT GC B-cells (numbers shown in **F**) to calculate the ratio (**G**); p values from unpaired t tests.

I-L. Chimera mice were treated as described in **Figure 6J**. Percentages of B-cells (**I**) and GC B-cells (**J**) were analyzed by flow cytometry. Protein levels of ICAM-1 were evaluated by flow cytometry to confirm effectiveness of anti ICAM-1 antibody treatment (**K**). Flow cytometry data shown in **L** was analyzed as in **H**.


A
YFP;Cγ1-cre spleens
3 WT + 3 Ezh2Y641F
Day 10 post immunization
Vibratome sectioning
Fluorescence microscope
Cut out 5 GCs per mouse
Single GC
DZ/LZ antibodies
(CXCR4, CD86, FAS, CD38, B220)
Cell sorter
Single cell index sort
1 plate per GC
(15 WT + 15 Ezh2Y641F GCs)
RNA isolation (SPRI beads)
Synthesis of cDNA (anchored oligo dT(20))
Common Fwd primer
Igh
L1 L2
VH
D
JH
CH
1st PCR (50 cycles)
Cγ or Cμ outer Rev primer
Common Fwd primer
2nd semi-nested PCR (50 cycles)
Cγ or Cμ inner Rev primer
Barcoding/indexing/ library prep (3rd and 4th PCR)
8bp barcode – Well ID
8bp Illumina – Plate ID
(*) 2 primers: Cγ or Cμ
Index Seq primer
P5
P7
Read1 Seq primer
Read2 Seq primer
Pooling; clean-up
Sequencing (MiSeq, PE 2x300)


B
GFP
YFP
RFP
CFP


C
Spleen, day 5 post immunization
WT Ezh2;Cγ1-cre
Ezh2Y641F;Cγ1-cre
30 μm


D
WT Ezh2;Cγ1-cre
Ezh2Y641F;Cγ1-cre
Raw
Binary

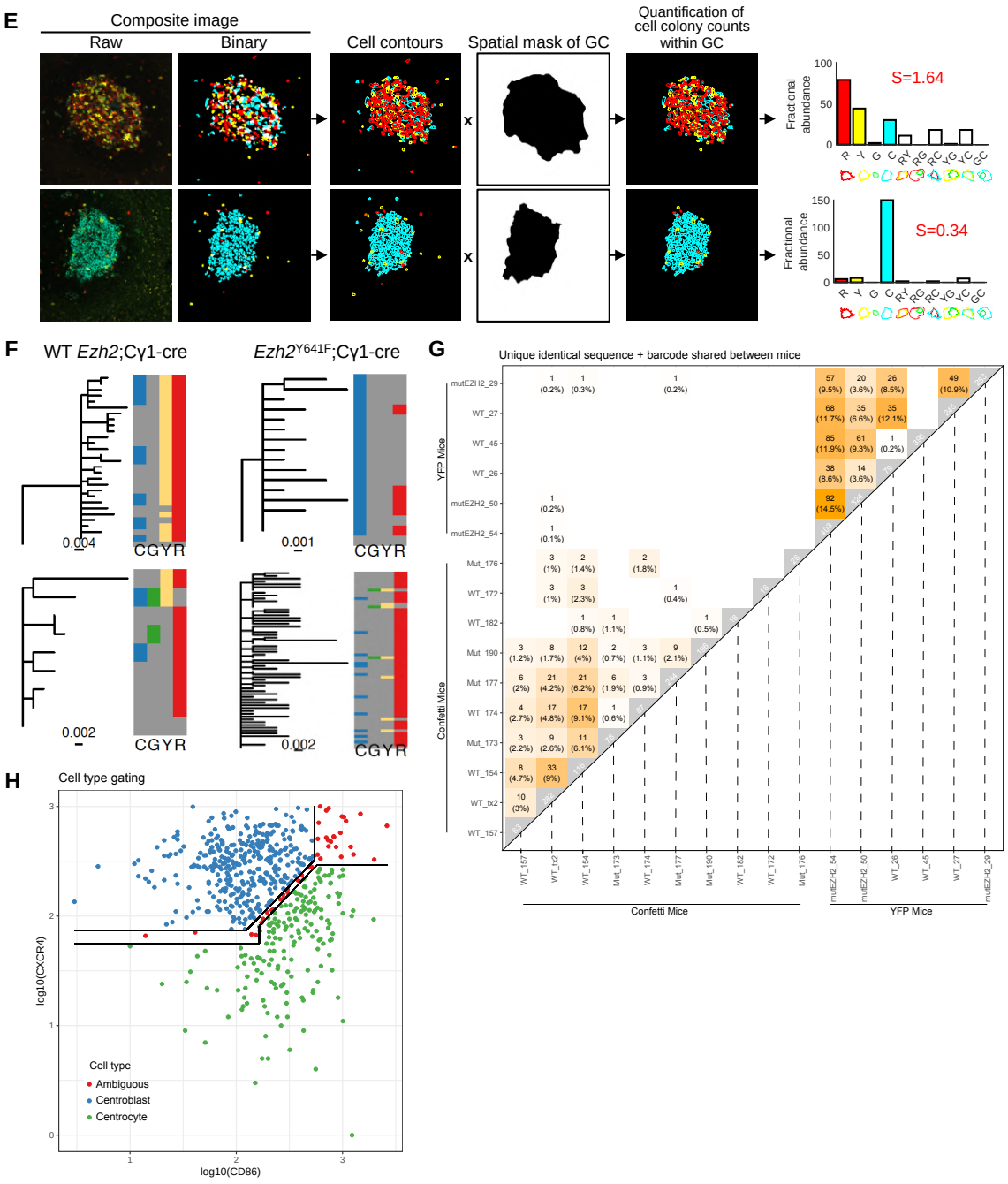


Figure S7. Related to Figure 7.

A. Flow chart of single cell genomic clonality assays. Three *R26-lox-stop-lox-YFP;Cγ1-cre* and 3 *R26-lox-stop-lox-YFP;Ezh2(Y641F)*$^{fl/WT}$*;Cγ1-cre* fresh spleens from mice immunized with NP-OVA for 10 days were vibratome cut. Individual YFP GCs were identified by fluorescence microscopy, 5 GCs per mouse were extracted, single cell suspension prepared, stained with CB/CC markers, and single cell index sorted. IgG1 and IgM loci were amplified and sequenced (Tiller et al., 2009).

B. Different possible outputs in *R26R-Confetti* in homozygosis.

C. Representative confocal image of GCs in spleens 5 days after immunization with SRBC.

D. Representative confocal images and their corresponding binary images after image processing for both WT *Ezh2;Cγ1-cre* and *Ezh2(Y641F)*$^{fl/WT}$*;Cγ1-cre* mice. Top row represent the raw images, and the bottom row illustrating the pseudo-colored binary images.

E. Sequence of image processing pipeline, raw images are binarized, boundaries are delineated and the abundance of each clone within the spatially defined GC is enumerated.

F. Example linage trees of Confetti clones.

G. Overlap of unique sequence and barcode combinations shared between different YFP and Confetti mice. Diagonal elements represent the total number of unique sequence + barcode combinations observed within a mouse, while off-diagonal elements represent the number and percentage (Jaccard index) of unique sequence + barcode combinations shared among two mice. Sequences shared between two mice (upper left triangle) were used to calibrate minimum sequence read cutoffs for YFP and Confetti mice, leading to the final set of sequences for analysis (lower right triangle).

H. Gating strategy for centroblast and centrocyte identity based on CXCR4 and CD86 fluorescence. Each dot represents a sequence (which had undergone all processing steps detailed in *Sequence processing*) from one of six YFP mice.

Table S1. Related to STAR Methods. Quality control of single cell RNA-seq using 10X Genomics Chromium.

Sample	**WT_1**	**WT_2**	**WT_3**	**EZH2_1**	**EZH2_2**	**EZH2_3**
Estimated number of cells	2,413	2,294	2,075	4,953	3,596	1,785
Total number of reads	243,772,949	284,179,979	239,709,808	231,817,568	246,711,689	250,412,306
Fraction reads in cells	54.40%	46%	46.90%	74.30%	53.50%	44.30%
Mean reads per cell	101,024	123,879	115,522	46,803	68,607	140,287
Median genes per cell	1,431	1,395	1,251	1,620	1,416	1,575
Total genes detected	14,355	14,554	14,492	14,909	14,962	14,477
Median UMI counts per cell	3,475	3,555	3,090	4,131	3,606	4,068

$YFP^{+}IgD^{-}$ splenocytes were sorted from 3 *R26-lox-stop-lox-YFP;Ezh2*^{Y641F} and 3 *R26-lox-stop-lox-YFP;Cγ1-cre* mice 8 days after SRBC immunization and were subjected to single cell RNA-seq using 10X Genomics Chromium platform. Seurat package was used to identify genes and cells suitable for inclusion in the analysis.

Table S3. Related to STAR Methods. Enriched pathways in upregulated and downregulated genes in WT vs *EZH2*^{Y641F} centrocytes.

Pathway name	Published pathway name	p value up	p value down	Overlapping gene orthologs in up	Overlapping gene orthologs in down
EZH2 GC-specific targets	GCB_deNovo_bivalent	0.363	4.24E-10	*PRKCZ,RPL22,CLSTN1,ID3,OMA1,ATP1A1,PROX1,DNMT3A,MAPKAPK3,BDH1,SCARB2,RPL37,HEXB,WDR41,RP9,CLN8,ASAH1,PDLIM2,SUSD1,RSU1,PDCD4,RNH1,CD44,GDPD5,CADM1,ST14,BLOC1S1,PLXNC1,LAMP1,FNTB,NPC2,IMP3,IFT140,RARA,SCPEP1,MBP,ATP5D,GAMT,BLVRB,LENG9,SLCO4A1,IL10RB,PDXK,POLR2F*	*C1orf159,ATG4C,BTBD8,GSTM4,KCNA3,ARHGEF11,POLR3GL,GLUL,CAPN2,EHBP1,ALMS1,KLF7,UBE2E3,ABI2,ACSL3,GPC1,FILIP1L,RYK,CEP70,GNB4,ATP11B,UCHL1,ANTXR2,TRIO,IL6ST,ADAMTS6,ELL2,CCDC112,RNF130,DUSP22,LCA5,TUBE1,TMEM170B,TNS3,FIGNL1,CLDN12,ABCB4,SRPK2,STAG3,UBN2,ZDHHC2,SGK3,FAM84B,NDRG1,MOB3B,GLIPR2,TMEM2,CDC14B,SLC44A1,WDR31,PBX3,RALGPS1,ABCA2,NMT2,VIM,EGR2,UBE2D1,SORBS1,FRAT1,FRAT2,CHST15,HSPA12A,USP47,AMPD3,CLCF1,NRGN,PTMS,BHLHE41,PPFIBP1,CHPT1,CHST11,SLC38A2,SCN8A,DRAM1,ATP11A,ZFHX2,ARHGAP5,GPHN,PPP1R13B,CEP152,SMAD3,CRTC3,MAZ,ZDHHC1,CAMKK1,CENPV,DYNLL2,WIPI1,CASKIN2,RFX2,ANO8,ATRN,C20orf194,RNF24,RTEL1,NRIP1,RSPH1,RPS6KA3*
MYC targets upreg	YU_MYC_TARGETS_UP	0.884	1.42E-08	*CLIC4*	*ASPM,DTL,BUB1,ECT2,CCNB1,KIF20A,ANLN,MKI67,E2F8,RACGAP1,BRCA2,TFDP1,KIAA0101,TOP2A,BIRC5*
MYC targets downreg	YU_MYC_TARGETS_DN	4.90E-08	0.672	*CD2,CD74,HLA-DMA,HLA-DQA1,HLA-DQB1,HLA-DRB1,LTB,BLK,UNC93B1,IL10RA,GNS,EVL,PLD4,CTSH,ACP5*	*GBP4,PYGM,SSPN*
Genes regulated by MYC downreg	DANG_REGULATED_BY_MYC_DN	0.000823	0.1	*ID3,CD48,ARPC4,VHL,LXN,GM2A,CTSB,IRF7,FTH1,THY1,ITM2B,LAMP1,NPC2,IFI35,RARA,SCPEP1,GAA,ACP5,MAN2B1,AKT2,FCGRT,FTL,CSTB,TSPO,TMSB4X*	*PEA15,KIF14,CKAP2L,KLF7,GPC1,GLT8D1,ACSL1,HNRNPH1,PIM1,AKAP12,MAGI2,NDRG1,GADD45G,ITGB1,FADS2,MDM2,RB1,DLEU2,NXN,PMAIP1,PLS3*
Antigen processing and presentation	KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	1.94E-03	0.974	*CTSS,CD74,HLA-DMA,HLA-DOA,HLA-DQA1,HLA-DQB1,HLA-DRB1,CTSB,LGMN*	*NFYA*
MHC class II antigen presentation	REACTOME_MHC_CLASS_II_ANTIGEN_PRESENTATION	3.56E-08	0.017	*CTSS,RAB7A,CD74,HLA-DMA,HLA-DOA,HLA-DQA1,HLA-DRB1,CTSB,DCTN6,DCTN3,CTSD,DCTN2,LGMN,CTSH,AP1M2,KLC3,AP2S1,CTSA,AP1B1*	*KIF2C,KIFAP3,KIF15,KIF2A,KIF20A,KIF11,RACGAP1,KIF23,DYNLL2,AP1S2,KIF4A*
CD40 signaling downreg	BASSO_CD40_SIGNALING_DN	0.00136	0.00643	*ID3,PTPN22,PTPN18,HSD17B8,LTB,CDH17,ISG20,CSF2RB,NCF4,TSPO*	*RERE,CRYZ,TRIB2,RAPGEF2,UBE2J1,HDAC9,PRKAR2B,GCNT1,BICD2,POU2AF1*
EZH2mut FL downreg	EZH2_mut_down_347_Ash_FL	0.0197	1.58E-07	*PGM1,CTSS,TXNIP,ADI1,KIDINS220,SUCLG1,ARHGAP15,SCARB2,ANKMY2,BLVRA,PTPRCAP,PRCP,MAML2,DERA,RNF41,UNC119B,ITM2B,TMOD3,ISG20,CD79B,MRPL34,TSPO,ZCCHC18,ARHGEF6*	*CDKN2C,SSX2IP,GCLM,KCNA3,ACP6,GLUL,NUCKS1,AKT3,SOCS5,NCK2,GTDC1,GCA,UBE2E3,HIBCH,ABI2,IFT57,PRKCI,TAPT1,RBPJ,HADH,PDLIM5,GPM6A,TRIO,MAST4,PIM1,BACH2,GLCCI1,TNS3,PTPN12,NAPEPLD,TJP2,VIM,DNAJC1,ARL3,PCGF5,SLK,CHST11,TM7SF3,USP44,MLXIP,MKL2,PCTP,FAM117A,FMR1*

Table S5. Related to STAR Methods. Primers used for qPCR.

Used for	Gene		Sequence (5'-3')
cDNA	*Tnfrsf13c*	Fwd	TCTGGTGAGAAACTGCGTGTC
		Rev	GTCAGCGCCAGTATCAGTCC
	Cbx8	Fwd	ATTCGCAAAGGACGCATGGAA
		Rev	CCTCGCTTTTTGGGGCCATA
	Lgmn	Fwd	TGGACGATCCCGAGGATGG
		Rev	GTGGATGATCTGGTAGGCGT
	Lgr5	Fwd	ACCTGTGGCTAGATGACAATGC
		Rev	TCCAAAGGCGTAGTCTGCTAT
	Serinc5	Fwd	GGCTGCTGCCCTAAGTTCC
		Rev	CCGGCTTGAGTCTTTTTACAGA
	Pde3b	Fwd	AAAGCGCAGCCGGTTACTAT
		Rev	CACCACTGCTTCAAGTCCCAG
	Tnfrsf14	Fwd	CAGGCCCCTACAGACAACAC
		Rev	ACTCGTCTCCCACAAGGAACT
	Icam1	Fwd	GTGATGCTCAGGTATCCATCCA
		Rev	CACAGTTCTCAAAGCACAGCG
	Itgam	Fwd	ATGGACGCTGATGGCAATACC
		Rev	TCCCCATTCACGTCTCCCA
	Ptger4	Fwd	ACCATTCCTAGATCGAACCGT
		Rev	CACCACCCCGAAGATGAACAT
	Ptgr1	Fwd	GAAGGCTTCCCTACGGACG
		Rev	GGCTGCAACTCTCATGTAAGGA
	Abi2	Fwd	GCTCTCTTCGACAGCTACACG
		Rev	ACACTTGCTAAGGATTGAGTGG
	Egr2	Fwd	GCCAAGGCCGTAGACAAAATC
		Rev	CCACTCCGTTCATCTGGTCA
	Cd69	Fwd	CCCTTGGGCTGTGTTAATAGTG
		Rev	AACTTCTCGTACAAGCCTGGG
	Icosl	Fwd	ACGCCATTTCAACTTGAGTGG
		Rev	TCCCTGGAGACTTGTAAGGCA
	Cd79a	Fwd	TCTTCTTGTCATACGCCTGTTTG
		Rev	GATGTTAGACTGAAGGCTGAACC
	Ccr6	Fwd	CCTGGGCAACATTATGGTGGT
		Rev	CAGAACGGTAGGGTGAGGACA
	Hprt1	Fwd	TATGCCGAGGATTTGGAAAA
		Rev	AATCCAGCAGGTCAGCAAAG
	Gapdh	Fwd	CTGCACCACCAACTGCTTAG
		Rev	GGATGCAGGGATGATGTTCT
	Rpl13	Fwd	ATCGTGAGGTGCCCTACAGT
		Rev	GACTCCGTGGACTTGTTTCG
	Ltb	Fwd	TGGCAGGAGCTACTTCCCT
		Rev	TCCAGTCTTTTCTGAGCCTGT
	Tnf	Fwd	CCCTCACACTCAGATCATCTTCT
		Rev	GCTACGACGTGGGCTACAG

Used for	Gene		Sequence (5'-3')
ChIP	*Icam1*_prom	Fwd	AGGGCAGGGGAGGAAATTAC
		Rev	GTCCTTCGCTGAGTGGAGAT
	*Cd69*_prom	Fwd	CATGCTGCTGTTCATCACCA
		Rev	GGCTGGTAGTCTTGGGTTCT
	*Ptgr1*_ex5	Fwd	GCCGCTACAATCGTTCCTTT
		Rev	ACCCCTGACTCCCATGAATG
	*Pde3b*_TSS	Fwd	GACCACTTCTTTCACGTCCG
		Rev	AACGACGGTCTCCTGAACAT
	*Pde3b*_int1	Fwd	TCCTCCTGTCTGCTTTGTCA
		Rev	GGCTAGGAAGATGGCTCAGT
	*Itgam*_prom	Fwd	TTGCAGGGTTCAAAGCTGAC
		Rev	CAAACCCCACCACAGACATG
	*Itgam*_int2	Fwd	TACAGTGTGGTTGTTGCAGC
		Rev	CCAGGGGTGTTCACAGAAGA
	*Abi2*_prom	Fwd	TGTACTTTCCCTGCCCAGTT
		Rev	TTGCACAGGGTAGAGGAGTC
	*Abi2*_int1	Fwd	GGTCTAGGGTTTGTGGGTCA
		Rev	AATCTCTGCCTGCCATGGTA
	*Ccr6*_TSS	Fwd	CCTTGCCTACCAGAAATCGC
		Rev	GCTACTGGAGGAAGGAAGTGT
	*Ccr6*_int2	Fwd	TGCAGCCAGTGAATTCCCTA
		Rev	TCATTCTTCGCTCTGCTCCA
	*Lgr5*_prom	Fwd	CATCTCCCCTGTCCCTGTTT
		Rev	CATATGCAGCAGAGGATGGC
	*Lgr5*_ex5	Fwd	TCACCAGCTCCATCTCAGAC
		Rev	CTTTGAACCCTAAGCGGCTG
	*Serinc5*_TSS	Fwd	GTTTCCCGAACGTGGATCTG
		Rev	GATCCGCTTGCCTTTGGTAG
	*Serinc5*_int1	Fwd	CGTGGGAGCTGGAATGTAGA
		Rev	ACCTCCCTATCTTCACCCCT
	*Tnfrsf14*_prom	Fwd	CCCCACAAGACCTCCACTAG
		Rev	TAACTCCAGCGGTGTCAAGT
	*Tnfrsf13c*_int2	Fwd	CCTCCGTCCCAAACATCTCT
		Rev	GACACAGGACTGAGGGAGAC

Table S7. Related to STAR Methods. Assessing reliability of Confetti color combinations in separating sequence-defined B cell clonal clusters.

Germinal Center	Sequences	Clones	p(same clone)	p(same clone | same color)
Mut_173_GC2.4	41	11	0.24	0.62
Mut_173_GC2.5	18	5	0.37	0.45
Mut_176_GC3.1	7	3	0.55	0.68
Mut_177_GC4.1	25	8	0.53	0.80
Mut_177_GC4.2	63	9	0.59	0.72
Mut_177_GC4.3	26	19	0.08	0.21
Mut_177_GC4.4	34	11	0.47	0.74
Mut_177_GC4.6	34	6	0.56	0.95
Mut_190_GC3i-S3	69	3	0.94	1.00
Mut_190_GC3i-S8-1	54	4	0.47	0.54
Mut_190_GC3i-S8-2	5	2	0.68	0.68
WT_154_GC3.3	14	8	0.16	0.62
WT_172_GC1.6	14	7	0.30	0.68
WT_174_GC1.2	27	15	0.13	0.32
WT_174_GC1.3	34	4	0.54	0.62
WT_182_GC2.2	1	1	1.00	1.00
WT_tx2_GC2.2	34	14	0.25	0.46
WT_tx2_GC2.3	6	4	0.33	1.00
WT_tx2_GC2.4	17	7	0.33	0.73
WT_tx2_GC2.5	31	9	0.40	0.86
Mean	29.11	7.84	0.42	0.67

Each row represents a germinal center (GC); p(same clone) is the probability that two random sequences (with replacement) within the GC are from the same clone; p(same clone | same color) is the probability that two random sequences of the same color combination are from the same clone. The row **Mean** contains mean values for all germinal centers excluding WT_182_GC2.2, which had only a single sequence.