

Supplementary Information

CFP1 promotes germinal center affinity maturation and restrains memory B cell differentiation through H3K4me3 modulation

Yanan Zhao^{1†}, Shuoxu Gong^{1†}, Yaling Yang², Jingning Bai¹, Yimiao Lu¹, Meiling Liu¹, Wanyu Bai^{1,2*}, Junchao Dong^{1,2*}

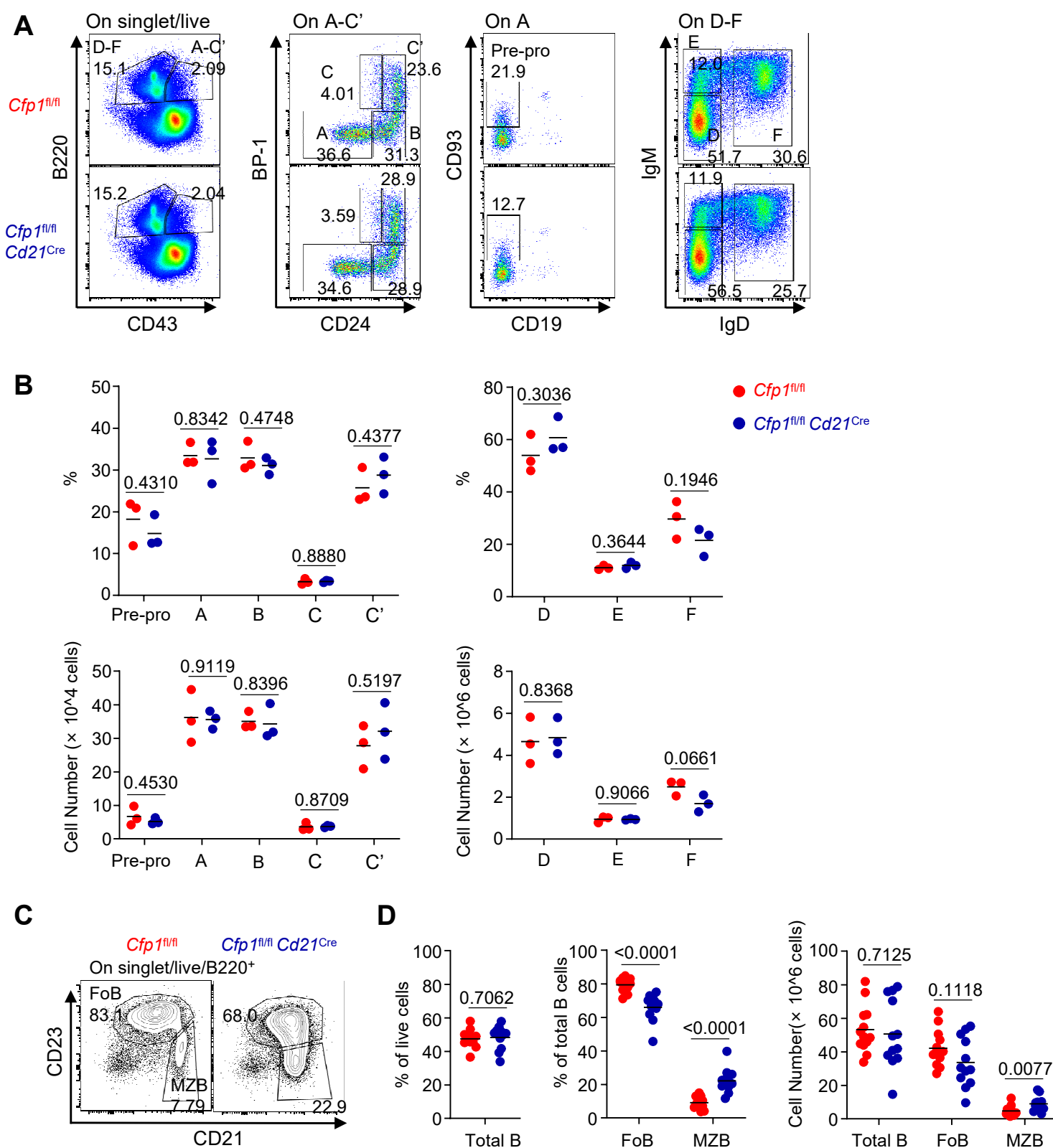
¹Department of Immunology and Microbiology, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, China

²Pediatric Hematology Laboratory, Division of Hematology/Oncology, Department of Pediatrics, The Seventh Affiliated Hospital of Sun Yat-Sen University, Shenzhen, China

[†]Equal contributions

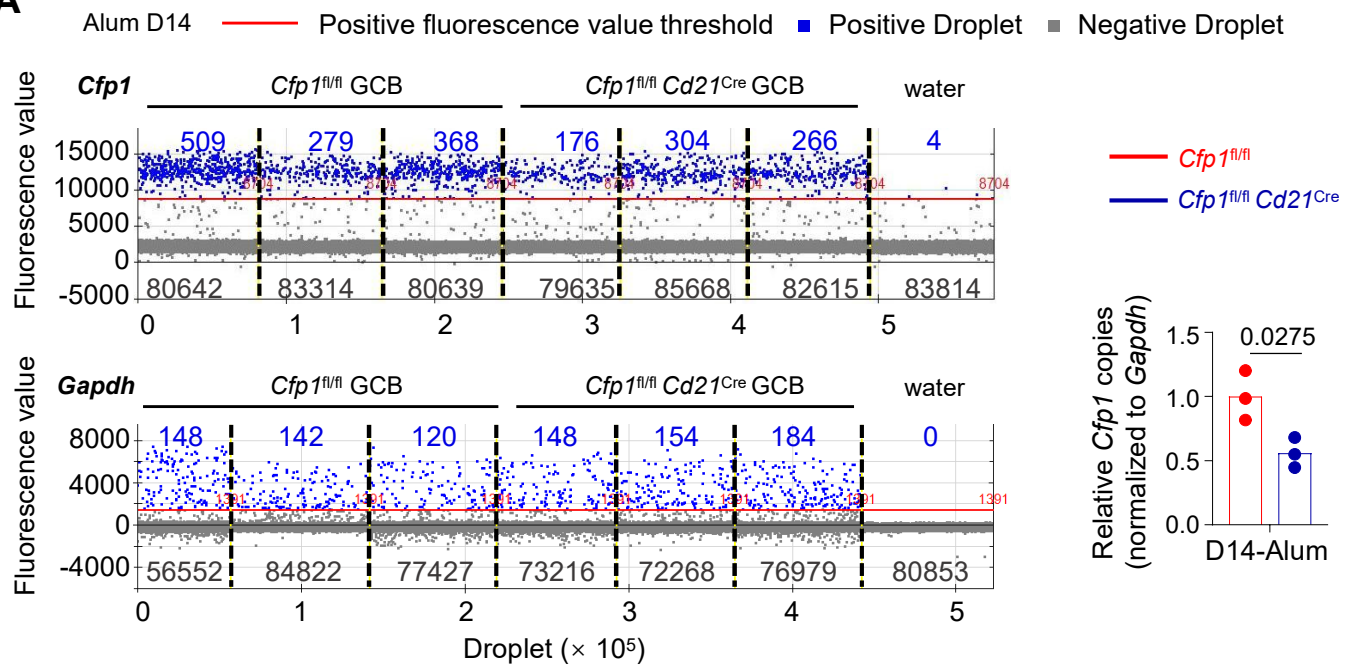
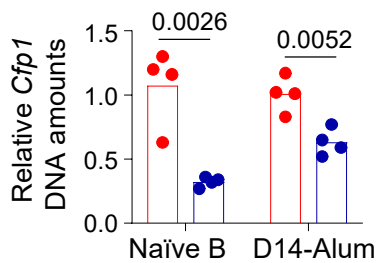
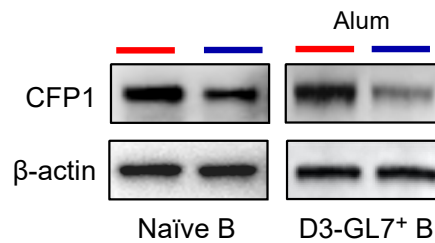
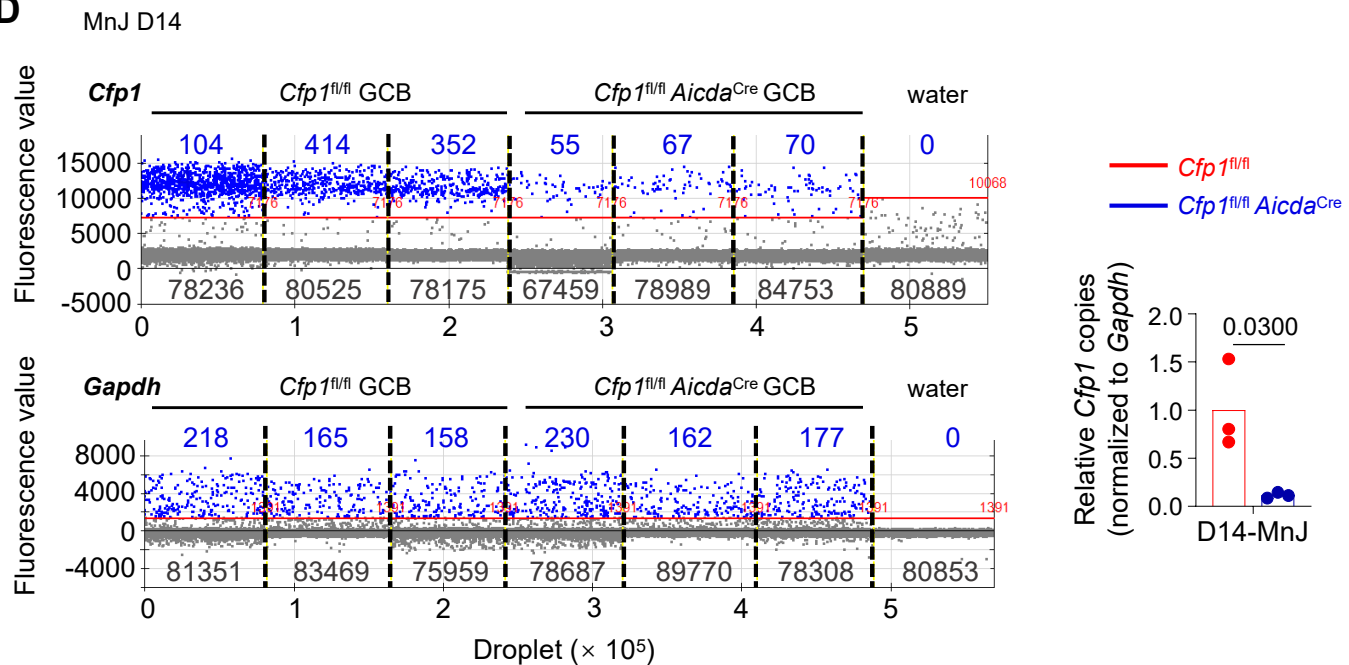
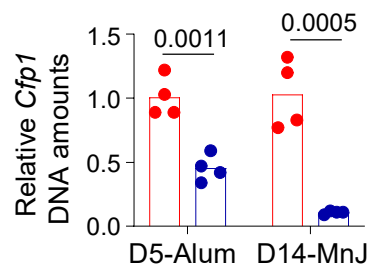
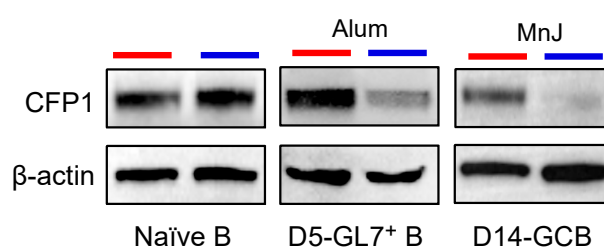
*Correspondence: baiwy6@163.com (W.B.), dongjch@mail.sysu.edu.cn (J.D.)

**This PDF file includes: Supplementary Figures 1 – 14
Supplementary Tables 1 – 3**



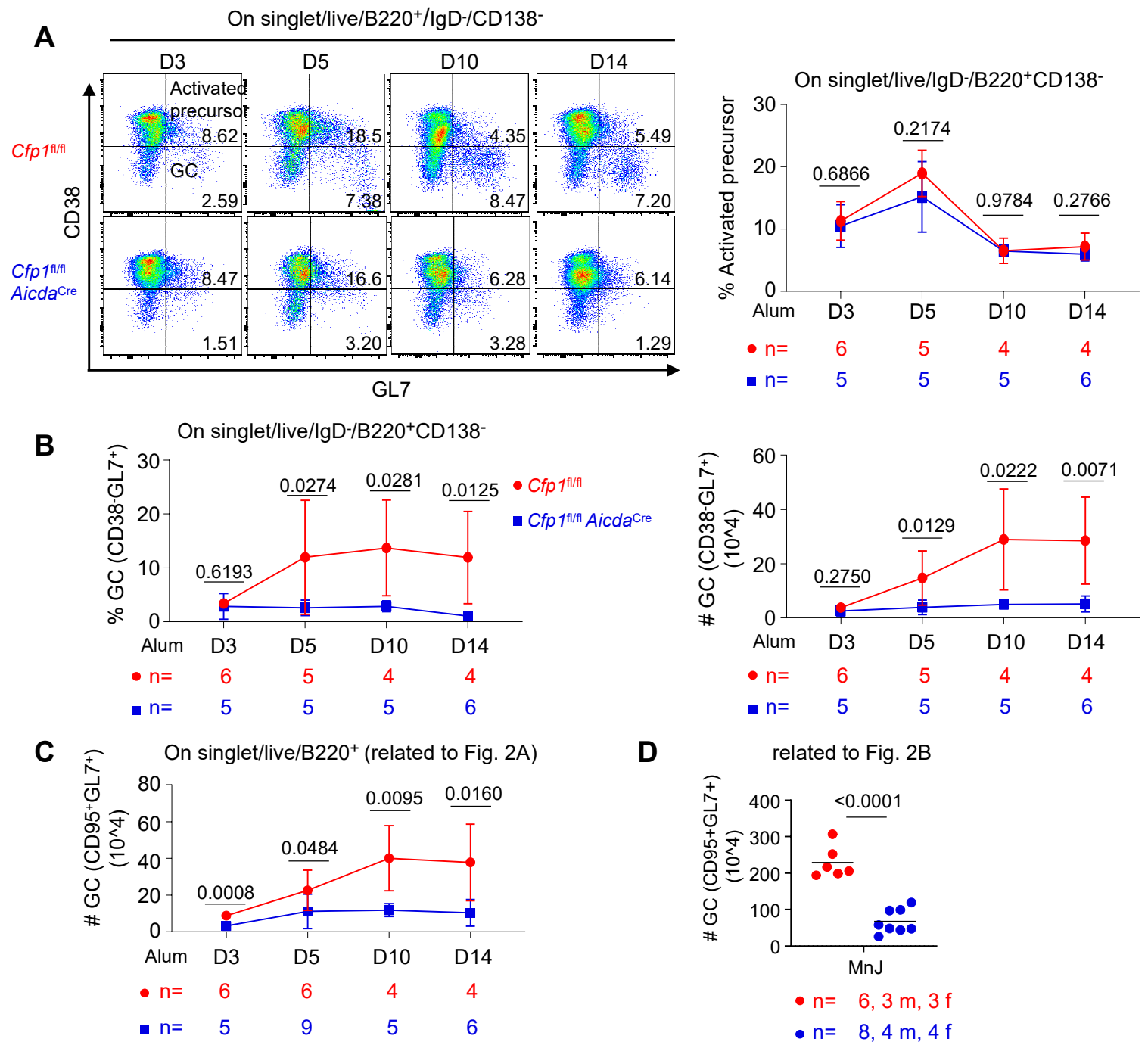
Supplementary Figure 1. B cell development is minimally affected by *Cfp1* deletion.

(A-B) representative FACS plots and quantitation of B cell subsets at different developmental stages in the bone marrow, according to Hardy's scheme. Data were pooled from two independent experiments. $n = 3$; 1 males (m), 2 females (f). (C-D) representative FACS plots and quantitation of splenic B cell subsets. Data were pooled from four independent experiments. *Cfp1*^{fl/fl} ($n = 13$; 6 m, 7 f); *Cfp1*^{fl/fl} *Cd21*^{Cre} ($n = 12$; 6 m, 6 f). (A-D) Each symbol indicates one mouse and lines denote means. P -values by unpaired t-test (two-tailed).

A**B****C****D****E****F**

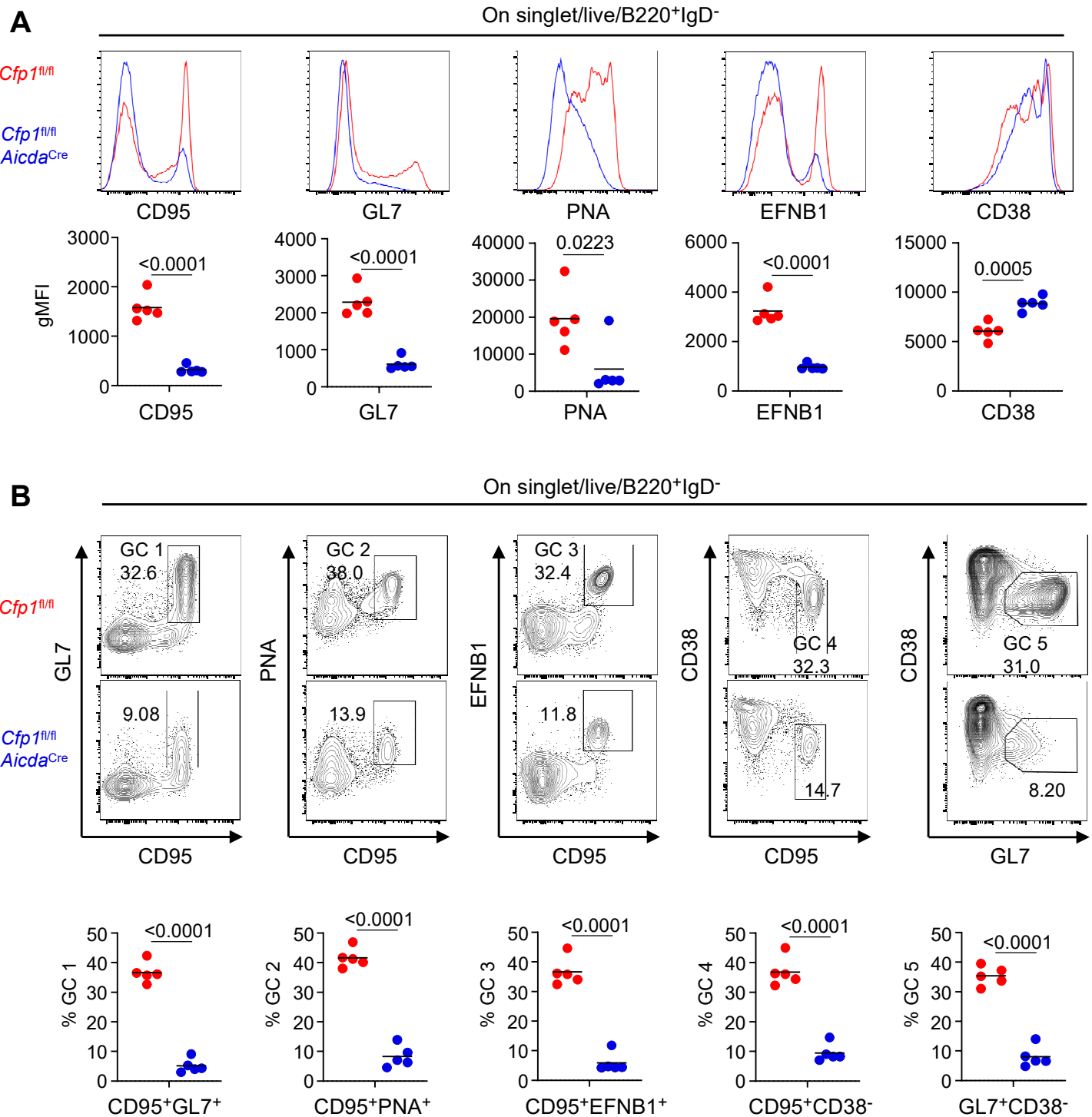
Supplementary Figure 2. Analyses of *Cfp1* deletion efficiency.

(A) Quantification of *Cfp1* expression by droplet digital PCR (ddPCR) in GC B cells sorted by FACS from *Cfp1^{fl/fl}* and *Cfp1^{fl/fl}Cd21^{Cre}* mice immunized with NP-KLH/Alum on day 14. One-Dimensional fluorescence amplitude plot (left) showing EvaGreen fluorescence intensity (Y-axis) across droplets (X-axis). Vertical dashed lines partition biological replicates. Each replicate shows: Negative droplets (gray), Positive droplets (blue), Negative/positive classification was determined by a No-Template Control (water)-derived cutoff (red solid line). The concentration (copies/ μ l) was calculated as described in the Methods section (see “Droplet digital PCR Analysis” for details). The *Cfp1* copies were calculated by normalizing *Cfp1* concentration to the reference gene *Gapdh* (2 copies/diploid cell) using the formula: $Cfp1 \text{ copies/cell} = (Cfp1 \text{ copies}/\mu\text{l}) / (Gapdh \text{ copies}/\mu\text{l}) \times 2$. The relative copies of *Cfp1* was normalized to the mean value of *Cfp1^{fl/fl}* mice. $n = 3$; 1 males (m), 2 females (f). **(B)** Naïve splenic B cells harvested from *Cfp1^{fl/fl}* and *Cfp1^{fl/fl}Cd21^{Cre}* mice or splenic GCB cells were examined for expression of *Cfp1* by qPCR. Data were pooled from two independent experiments. $n = 4$; 2 m, 2 f. **(C)** CFP1 expression in naïve B cells, day 3 GL7⁺ B cells from NP-KLH/Alum immunized *Cfp1^{fl/fl}* and *Cfp1^{fl/fl}Cd21^{Cre}* mice analyzed by immunoblotting. **(D)** *Cfp1^{fl/fl}* and *Cfp1^{fl/fl}Aicda^{Cre}* mice immunized with NP-KLH/MnJ, boosted on day 8 and GCB cells were sorted on day 14. Same ddPCR analysis as in (A), except for cutoff = 7176. $n = 3$; 1 m, 2 f. **(E)** GL7⁺ B cells harvested from *Cfp1^{fl/fl}* and *Cfp1^{fl/fl}Aicda^{Cre}* mice immunized with NP-KLH/Alum on day 5 or splenic GCB cells isolated as (D) were examined for expression of *Cfp1* by qPCR. Data were pooled from two independent experiments. $n = 4$; 2 m, 2 f. **(F)** CFP1 expression in naïve B cells, day 3 GL7⁺ B cells, day 5 GL7⁺ B cells and day 14 GCB cells from NP-KLH/Alum or NP-KLH/MnJ immunized *Cfp1^{fl/fl}* and *Cfp1^{fl/fl}Aicda^{Cre}* mice analyzed by immunoblotting. **(A-B and D-E)** Bars represent the mean. *P*-values by unpaired t-test (two-tailed).



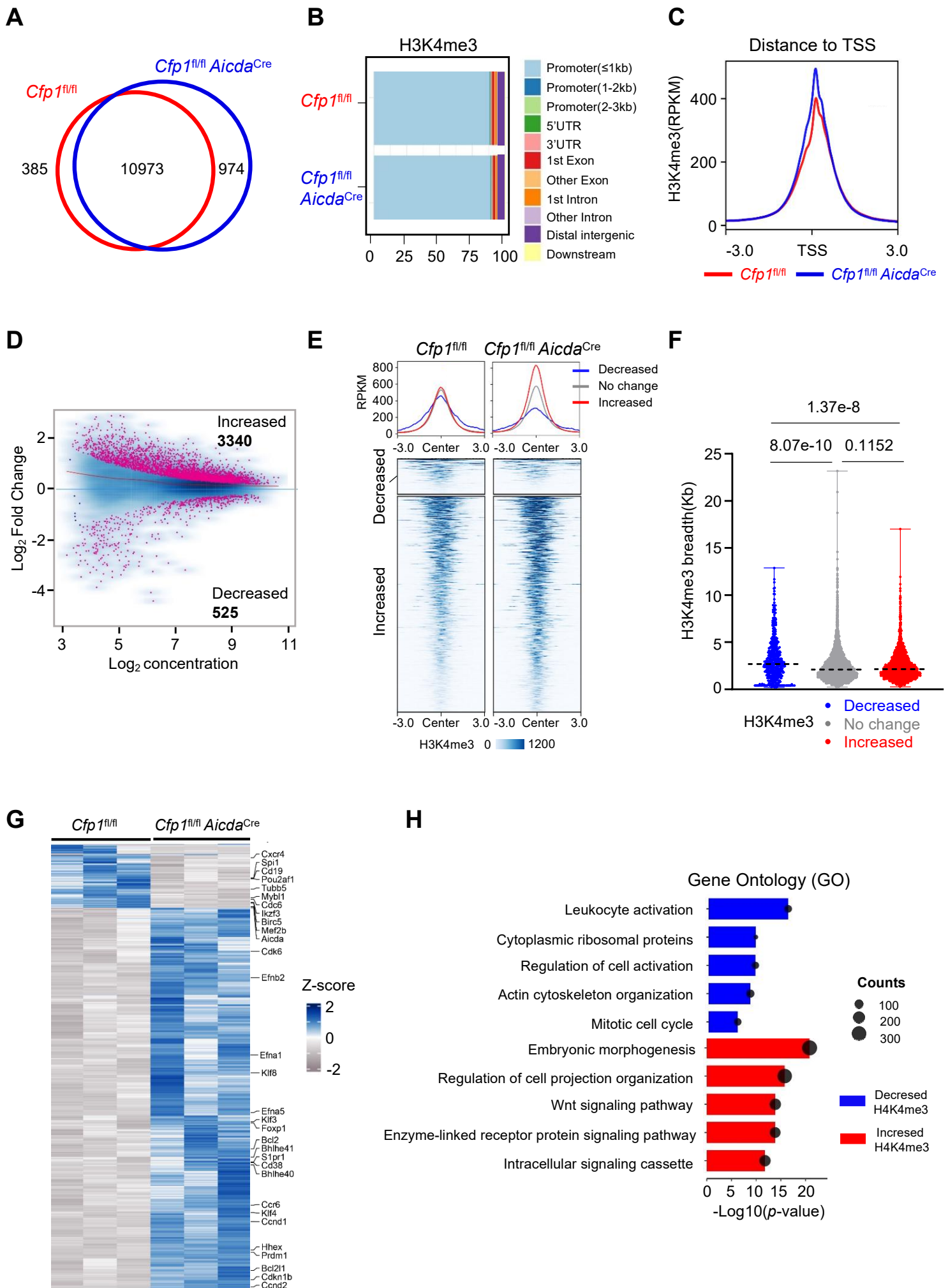
Supplementary Figure 3. *Cfp1^{fl/fl} Aicda^{Cre}* B cells can undergo activation in vivo.

(A) Mice were immunized with NP-KLH/Alum and analyzed at indicated time points. FACS plots (left) and ratio (right) of activated precursors were shown. Data were from three independent experiments; *Cfp1^{fl/fl}* (Day3, 3 males (m), 3 females (f); Day 5; 2 m, 3 f; Day10/14, 2 m, 2 f); *Cfp1^{fl/fl} Aicda^{Cre}* (Day3/5/10, 2 m, 3 f; Day14, 3 m, 3 f). **(B)** Ratio (left) and absolute number (right) of B220⁺IgD⁻CD138⁻CD38⁺GL7⁺ GCB cells from mice as in (A). **(C)** Absolute numbers of B220⁺CD95⁺GL7⁺ GCB cells from control and *Cfp1*-deficient mice immunized with NP-KLH/Alum for indicated time. Related to Fig. 2 A, *Cfp1^{fl/fl}* (Day3/5, 3 m, 3 f; Day 10/14, 3 m, 3 f); *Cfp1^{fl/fl} Aicda^{Cre}* (Day3, 2 m, 3 f; Day5, 5 m, 4 f; Day10, 2 m, 3 f; Day14, 3 m, 3 f). **(D)** Absolute numbers of B220⁺CD95⁺GL7⁺ GCB cells from control and *Cfp1*-deficient mice immunized with NP-KLH/MnJ for indicated time from three independent experiments. Related to Fig. 2 B. **(A-C)** Data points represent the mean $\pm$ SD. **(D)** Each symbol indicates one mouse and lines denote means. *P*-values by unpaired t-test (two-tail).



Supplementary Figure 4. GC entry of *Cfp1^{fl/fl} Aicda^{Cre}* B cells with additional surface marker sets.

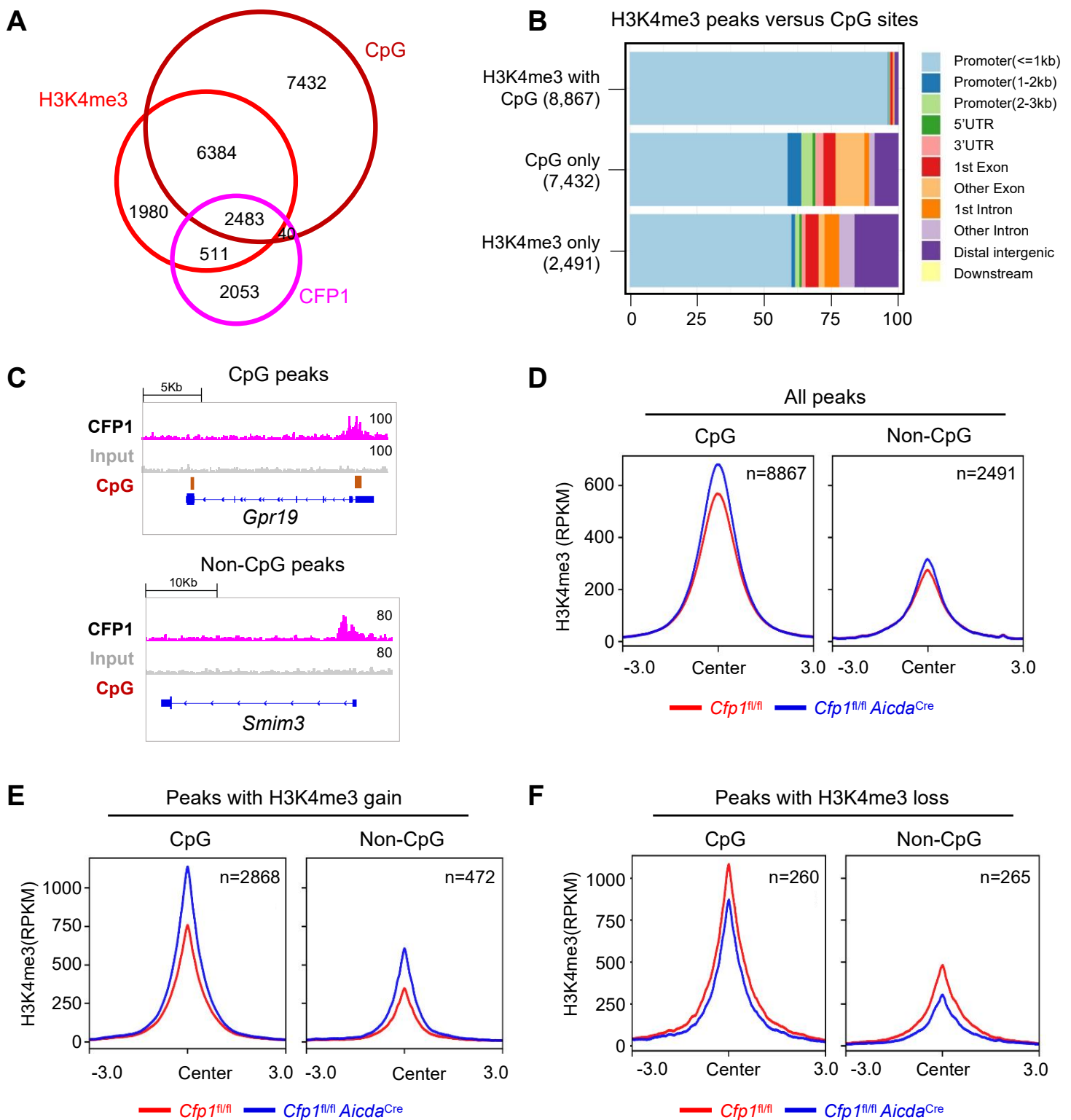
Mice were immunized with NP-KLH/MnJ, boosted on day 8 and analyzed on day 14. **(A)** gMFI analysis of CD95, GL7, PNA, EFNB1 and CD38 in splenic B220⁺IgD⁻ B cells from *Cfp1^{fl/fl}* and *Cfp1^{fl/fl} Aicda^{Cre}* mice. Data were pooled from two independent experiments; $n = 5$; 3 males (m), 2 females (f). **(B)** FACS plots (top) and quantitation (bottom) of GCB ratio using CD95 plus GL7/PNA/EFNB1/CD38 or GL7 plus CD38. Data were pooled from two independent experiments; $n = 5$; 3 m, 2 f. Each symbol indicates one mouse and lines denote means. P -values by unpaired t-test (two-tail).



Supplementary Figure 5

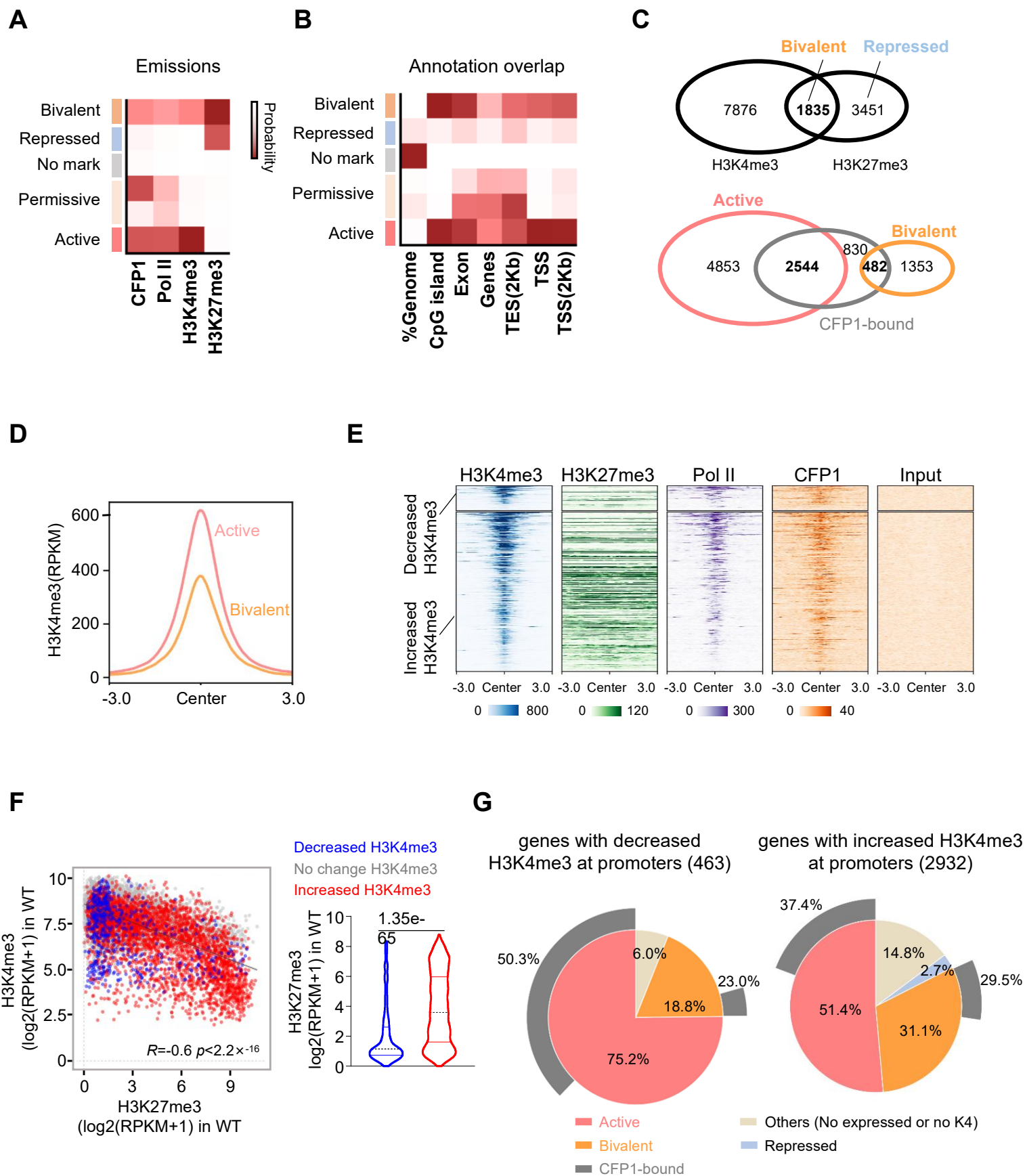
Supplementary Figure 5. *Cfp1* ablation impacts H3K4me3 domains linked to GC B cell functions.

Mice were immunized with NP-KLH/MnJ, boosted on day 8 and GCB cells were sorted on day 14 for CUT&Tag; $n = 3$; 1 males (m), 2 females (f). **(A)** Venn diagram showing H3K4me3 peaks in GC B cells from *Cfp1*^{fl/fl} and *Cfp1*^{fl/fl}*Aicda*^{Cre} mice. **(B)** Genome distribution of H3K4me3 peaks relative to gene features. **(C)** Mean read density (RPKM) of H3K4me3 around the transcription start site (TSS $\pm$ 3kb) in GC B cells from *Cfp1*^{fl/fl} and *Cfp1*^{fl/fl}*Aicda*^{Cre} mice. **(D)** MA plot showing intensity and differential H3K4me3 peaks (in magenta, Diffbind FDR<0.05) between GCB cells from control and *Cfp1*^{fl/fl}*Aicda*^{Cre} mice. **(E)** Illustration of the average density of H3K4me3 peaks with signal loss, gain or no change in control and *Cfp1*-KO GCB cells. **(F)** Scatter dot plot showing the baseline breadth of H3K4me3 peaks in control cells with increased, decreased, and unchanged signals upon *Cfp1* loss. *P*-values was calculated by unpaired Wilcoxon rank sum test (two-tailed) and the line denotes the median. **(G)** Heatmap of H3K4me3 marks with significant signal change by *Cfp1* deletion. **(H)** GO analysis for pathway enrichment of genes with significantly decreased and increased H3K4me3 signals upon *Cfp1* loss ranked by q-values.



Supplementary Figure 6. H3K4me3 peaks coincide with CpG Islands in GCB cells.

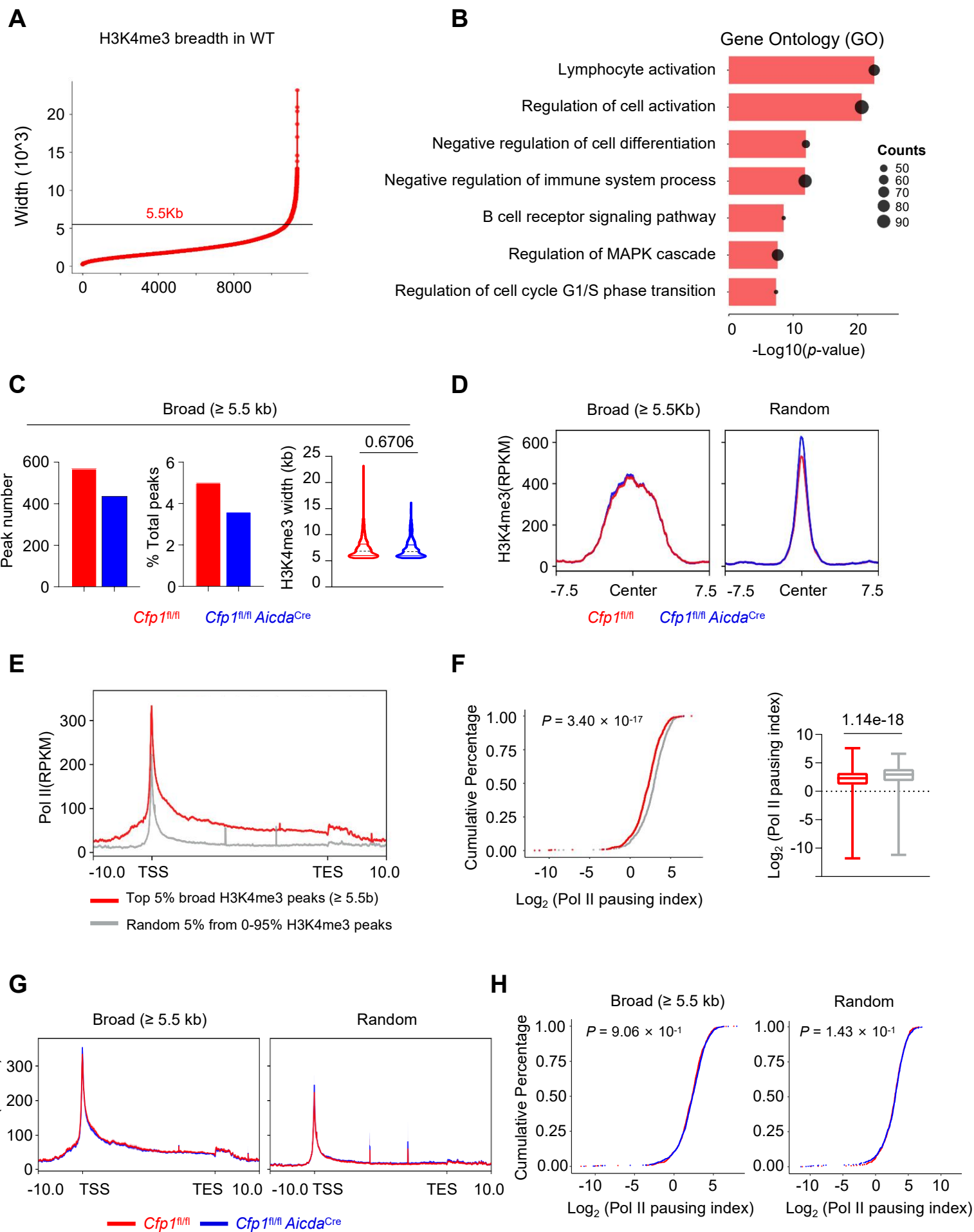
Cfp1^{fl/fl} control iGCB cells cocultured with 40LB feeders were used for CFP1 ChIP-seq ($n = 3$; 1 males (m), 2 females (f)). **(A)** Venn diagram illustrating the overlap between H3K4me3, CFP1 peaks and CpG sites in control cells. **(B)** Genomic distribution of H3K4me3 peaks in relation to CpGs. **(C)** Genomebrowser views of CFP1 binding at CpG gene *Gpr19* and non-CpG gene *Smim3*. **(D)** Mean signal density (RPKM) of H3K4me3 at CpG and non-CpG sites in the control and *Cfp1*^{fl/fl}*Aicda*^{Cre} GCB cells. **(E-F)** Mean signal density (RPKM) of H3K4me3 peaks with signal gain **(E)** or loss **(F)** upon *Cfp1* deletion at CpG and non-CpG sites in control and *Cfp1*^{fl/fl}*Aicda*^{Cre} GCB cells.



Supplementary Figure 7

Supplementary Figure 7. Cfp1 maintains H3K4me3 at active genes and restrains bivalent genes.

(A) ChromHMM emission plots depicting defined states in GCB cells from *Cfp1*^{fl/fl} control mice with H3K4me3, H3K27me3 and Pol II CUT&Tag and CFP1 ChIP-seq data. **(B)** Heatmap for state functional enrichments of each state in **(A)** at key genomic regions. **(C)** Genes identified in each subset as bivalent (upper) defined by H3K4me3 and H3K27me3 occurrence in regions ± 3 kb of all TSSs, repressed with only H3K27me3, and active with only H3K4me3 exhibited concurrent gene expression (mean normalized counts by Deseq2 >20). Bottom Venn diagram shows CFP1-bound active and bivalent genes. **(D)** Mean H3K4me3 signal around peak center (± 3 kb) on active and bivalent genes in control GCB cells. **(E)** The signal of H3K4me3, H3K27me3, Pol II, and CFP1 in control cells were plotted over the peak center (± 3 kb) of regions where H3K4me3 was decreased (mentioned in Fig. S5D) and increased. **(F)** Scatter plot showing correlation of H3K4me3 and H3K27me3 signals of control GCB cells on increased, decreased or no change H3K4me3 regions (left). The Violin plot showing the H3K27me3 average peak signals in increased and decreased H3K4me3 regions (right). **(G)** Pie chart illustrates the distribution of different modifications in decreased and increased H3K4me3 genes at promoters.



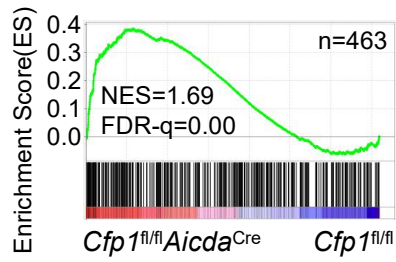
Supplementary Figure 8

Supplementary Figure 8. CFP1 deficiency does not affect broad H3K4me3 domains in GCB cells.

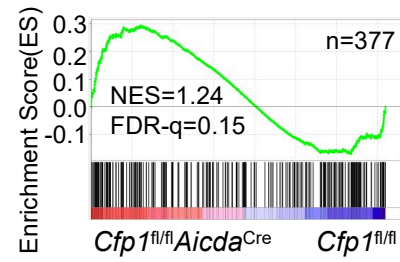
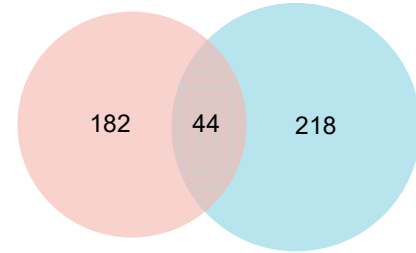
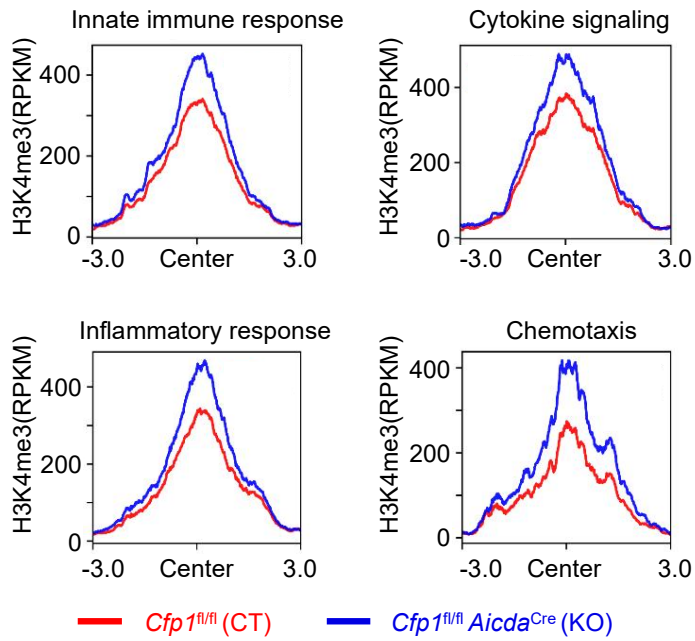
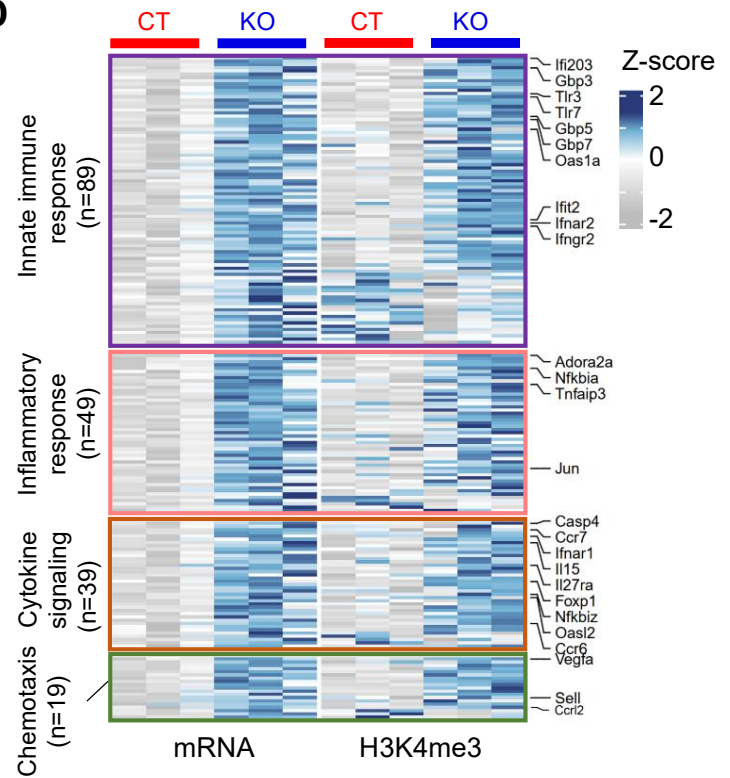
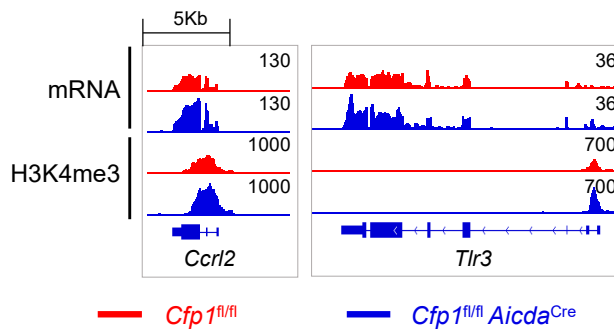
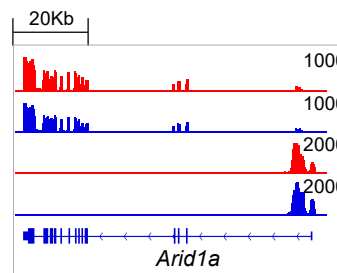
(A) A ranked plot of H3K4me3 breadth among GCB cells derived from *Cfp1^{fl/fl}* mice. Top 5% broadest H3K4me3 peaks ($\geq 5.5\text{Kb}$) was classified as broad peaks. **(B)** GO analysis for pathway enrichment of genes located at broad peaks. The top seven pathways ranked by *q*-values are shown. **(C)** Calculate the number of H3K4me3 peaks, the percentage of broad peaks relative to the total number of peaks, and the average width of broad peaks in both control and *Cfp1^{fl/fl}Aicda^{Cre}* GCB cells. **(D)** Mean H3K4me3 signal enrichment of broad and random H3K4me3 domains in control and *Cfp1^{fl/fl}Aicda^{Cre}* GCB cells. Random Forest models form non top 5% broadest H3K4me3 peaks in control cells as random group. **(E)** Mean Pol II signal enrichment of broad and random H3K4me3 domains in control GCB cells. TES: transcription termination site. **(F)** Accumulative plots of gene proportion against Pol II pausing index in control GCB cells with broad and random peaks (left). Turkey box showing the Pol II pausing index (right). **(G)** Mean Pol II signal enrichment of broad and random H3K4me3 domains in control and *Cfp1^{fl/fl}Aicda^{Cre}* GCB cells. **(H)** Accumulative plots of gene proportion against Pol II pausing index in control and *Cfp1^{fl/fl}Aicda^{Cre}* GCB cells with broad and random peaks. **(C right and F right)** *P*-values was calculated by unpaired Wilcoxon rank sum test (two-tailed) and the middle line denotes the median. **(F left and H)** *P*-values was calculated by KS test.

A

Regulation of Innate immune response

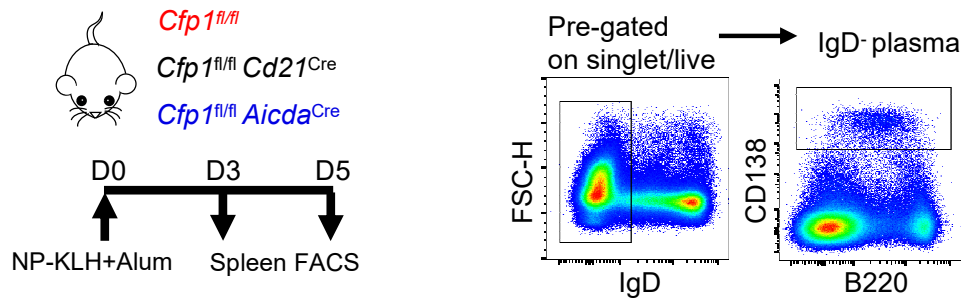
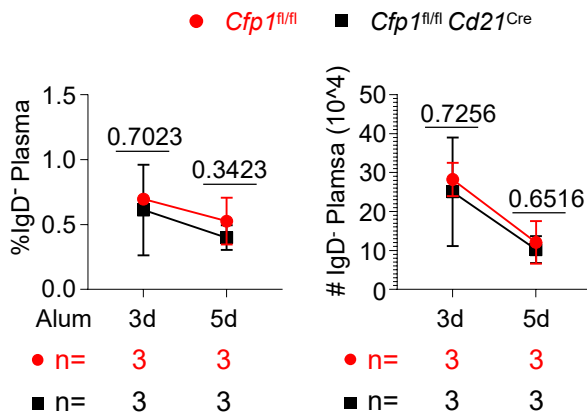
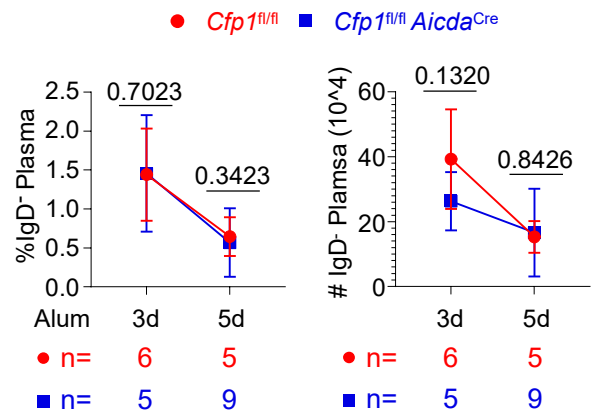
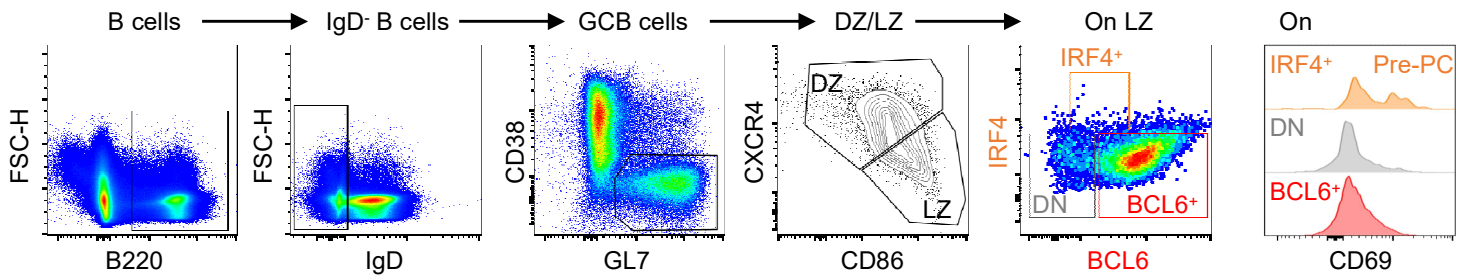


Regulation of Inflammatory response

**B**Cg1 Arid1a
KO > control*Cfp1^{fl/fl}Aicda^{Cre}* >
Cfp1^{fl/fl}**C****D****E****F**

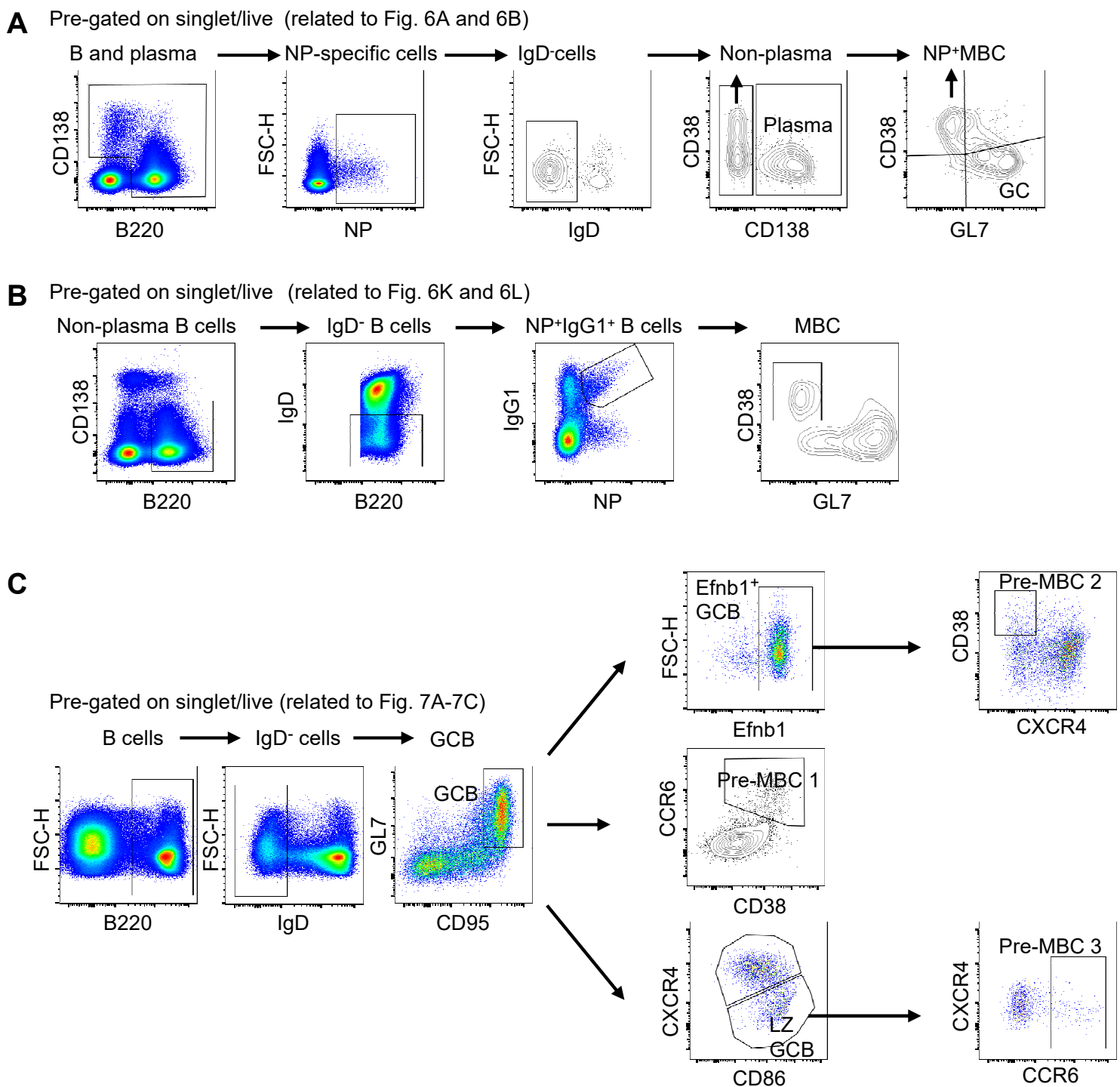
Supplementary Figure 9. *Cfp1*-deficient GCB cells upregulate inflammatory genes.

(A) GSEA analysis of inflammation-related signature genes with RNA-seq data in GC B cells from control and *Cfp1*^{fl/fl} *Aicda*^{Cre} mice. Gene sets for regulation of Innate immune response and regulation of Inflammatory response were analyzed with normalized enrichment scores (NES) and false discovery rate (FDR) q-values (q) shown. **(B)** Venn diagram showing the overlap between upregulated genes in the four inflammation-related pathways (Chemotaxis, Cytokine signaling, Inflammatory response and Innate response) in *Cg1-Arid1a* KO versus control GCB cells and *Aicda-Cfp1* KO versus control GCB cells. **(C)** Mean H3K4me3 signal profiles around peak center (± 3 kb) on promoters of the selected upregulated inflammation-related signature genes (Fig. S9 B) in *Cfp1*^{fl/fl} *Aicda*^{Cre} versus control GCB cells. **(D)** Heatmap for mRNA and H3K4me3 (z score-normalized FPKM/RPKM values) of gene sets from (Fig. S9 B). Duplicated genes were shown only once across different pathways. Up regulated genes: n=262; without H3K4me3 modifications: n=66. **(E)** Genomebrowser tracks for the mRNA and H3K4me3 signals of the *Ccr12* and *Tlr3* genes from control and *Cfp1*^{fl/fl} *Aicda*^{Cre} GCB cells. **(F)** Genomebrowser tracks for the unchanged mRNA and H3K4me3 signals of the *Arid1a* gene from control and *Cfp1*^{fl/fl} *Aicda*^{Cre} GCB cells.

A**B****C****D** Pre-gated on singlet/live (related to Fig. 5E)

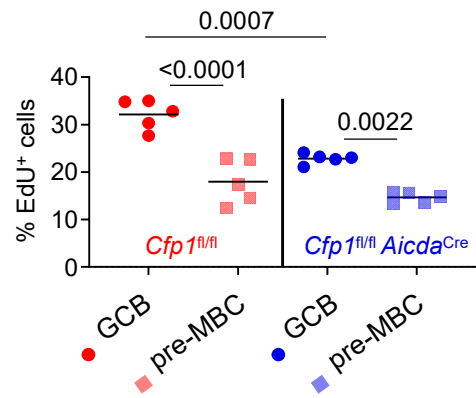
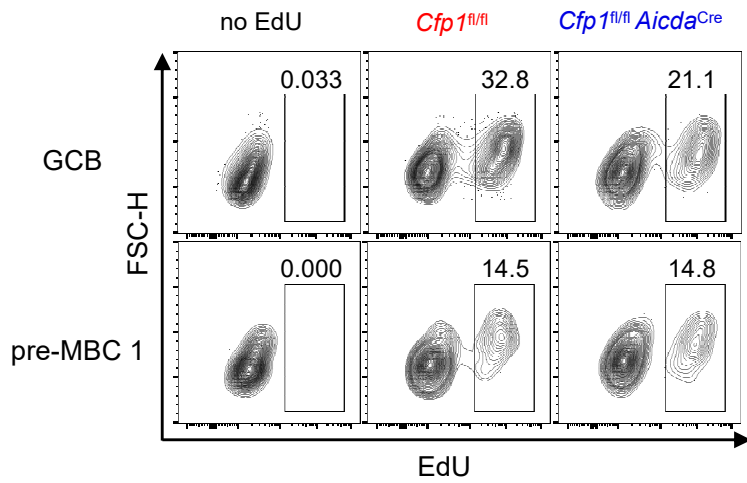
Supplementary Figure 10. *Cfp1* knockout does not affect early plasma generation via the EF pathway.

(A) Immunization strategy and gating strategy for extrafollicular plasma analysis. **(B)** Plasma cell frequency (% of IgD⁻) and absolute number at indicated time points in control and *Cfp1^{fl/fl} Cd21^{Cre}* mice. Data were pooled from two independent experiments; *n* = 3; 1 males (m), 2 females (f). **(C)** Plasma cell frequency (% of IgD⁻) and absolute number at indicated time points in control and *Cfp1^{fl/fl} Aicda^{Cre}* mice. Data were pooled from three independent experiments; *Cfp1^{fl/fl}* (Day3 *n* = 6; 3 m, 3 f; Day 5 *n* = 5; 2 m, 3 f); *Cfp1^{fl/fl} Aicda^{Cre}* (Day3 *n* = 5; 2 m, 3 f; Day5 *n* = 9; 5 m, 4 f). Data points represent the mean ± SD. P-values by unpaired t-test (two-tail). **(D)** Gating strategy to identify PC precursors (BCL6^{lo} IRF4⁺ CD69⁺) in LZ GCB (B220⁺ IgD⁻ GL7⁺ CD38⁻ CD86^{hi} CXCR4^{lo}).

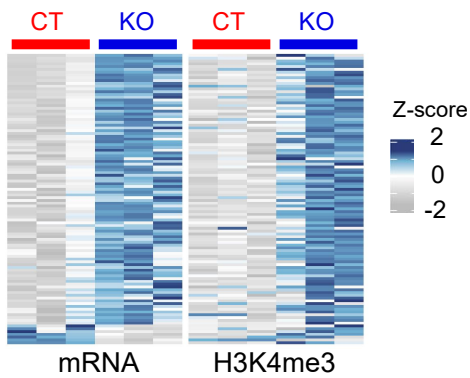


Supplementary Figure 11. Flow cytometry gating strategy for identification of memory B cells and memory precursors.

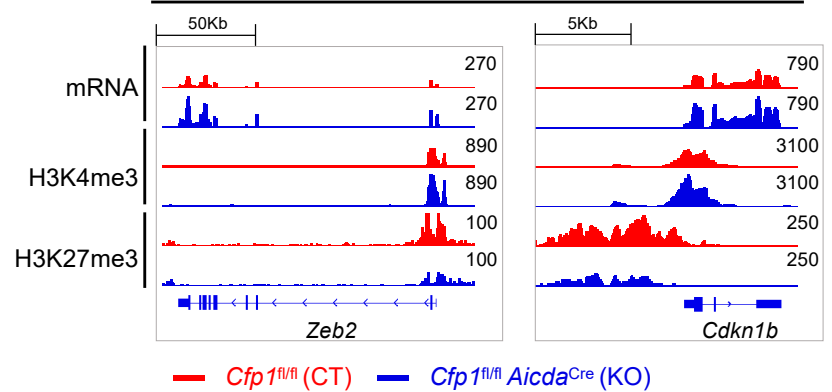
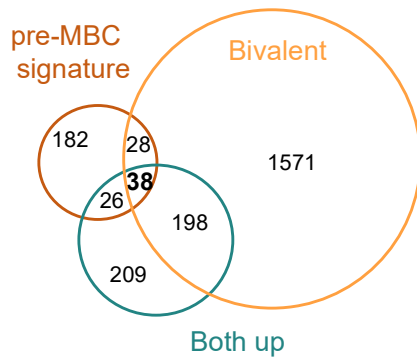
(A) Representative gating strategy for evaluating NP-specific memory B cells. Non-doublet lymphocytes were further gated on B220⁺NP⁺IgD⁻ cells. Memory B cells were defined as CD138⁻CD38⁺GL7⁻. **(B)** Representative gating strategy for evaluating NP+IgG1⁺ memory B cells. Non-doublet lymphocytes were further gated on B220⁺CD138⁻IgD⁻NP⁺IgG1⁺ cells. Memory B cells were defined as CD38⁺GL7⁻. **(C)** Representative different gating strategy for evaluating pre-MBC. Non-doublet lymphocytes were further gated on B220⁺IgD⁻CD95⁺GL7⁺ cells. Pre-MBC 1 were defined as CD38⁺CCR6⁺, Pre-MBC 2 were defined as Efnb1⁺CD38⁺CXCR4⁻, Pre-MBC3 further gated on CD86^{hi}CXCR4^{lo} (Light zone GCB cells) and were defined as CCR6⁺.

A**B**

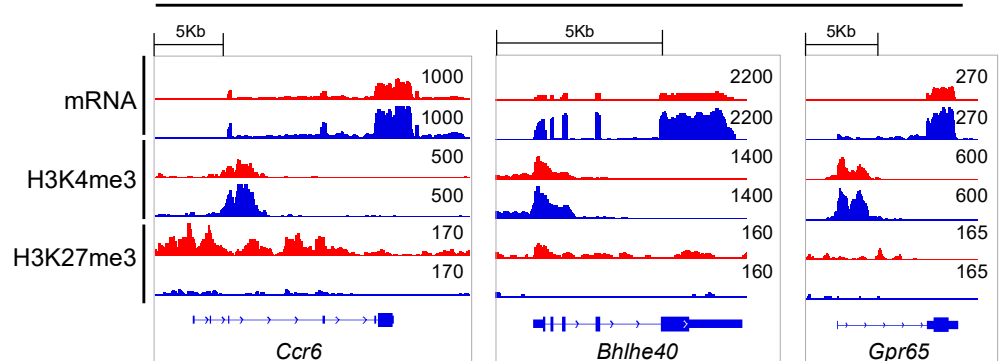
Pre-MBC signature with DEGs and H3K4me3 modification (n=104)

**D**

Pre-MBC signature/ Both up/ *Bcl6* targets (GCB cells)

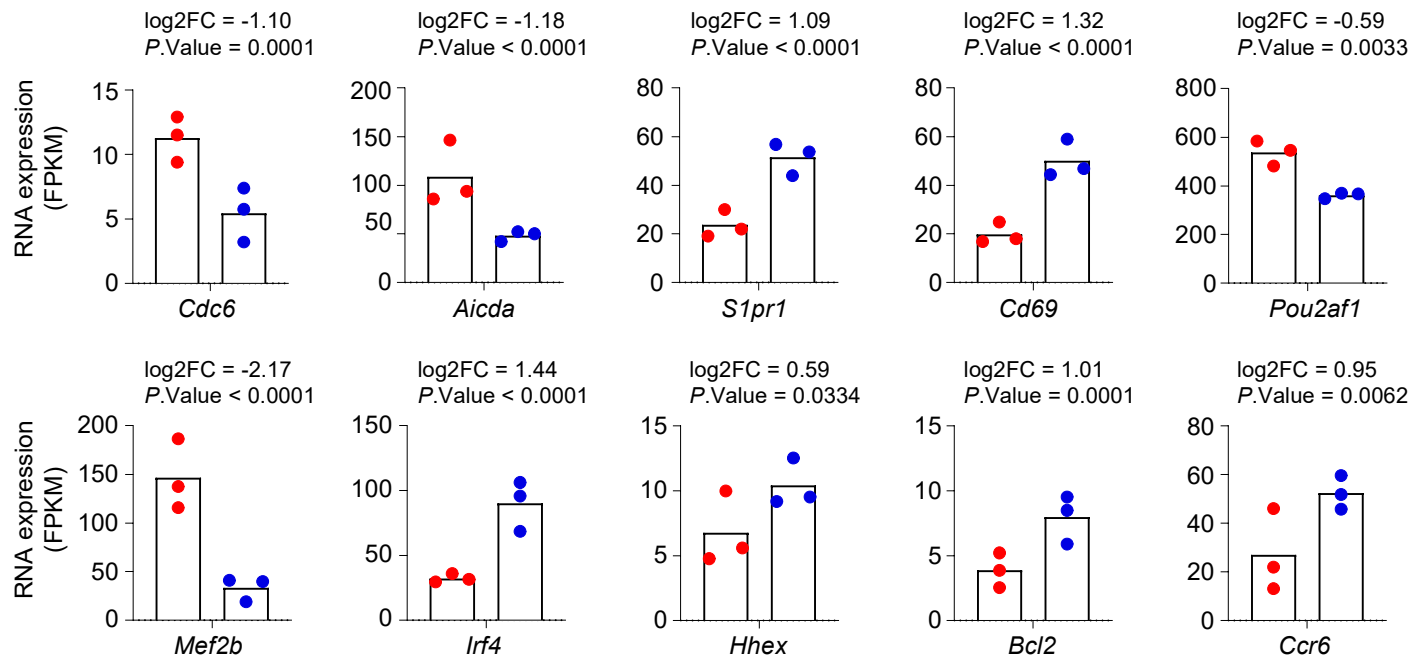
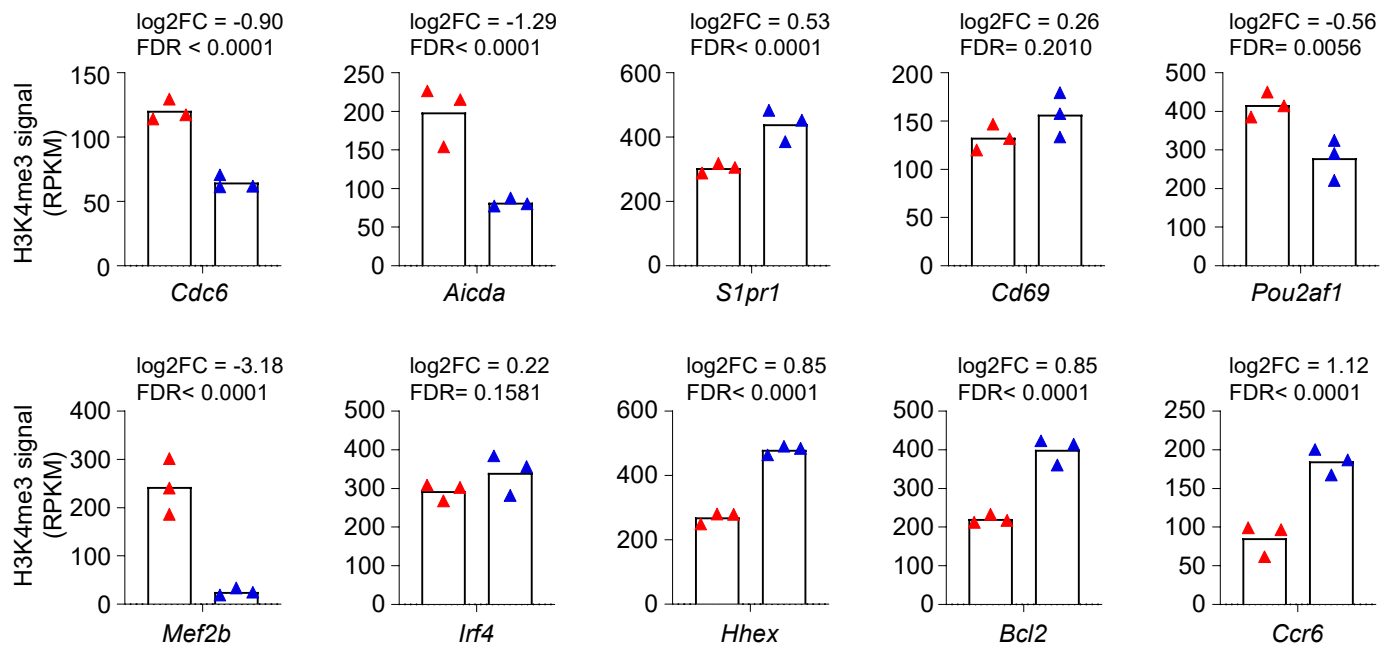
**C****E**

Pre-MBC signature/ Both up/ Non *Bcl6* targets (GCB cells)



Supplementary Figure 12. Both downregulation of BCL6 and upregulation of H3K4me3 contributed to the induction of the pre-MBC signature genes.

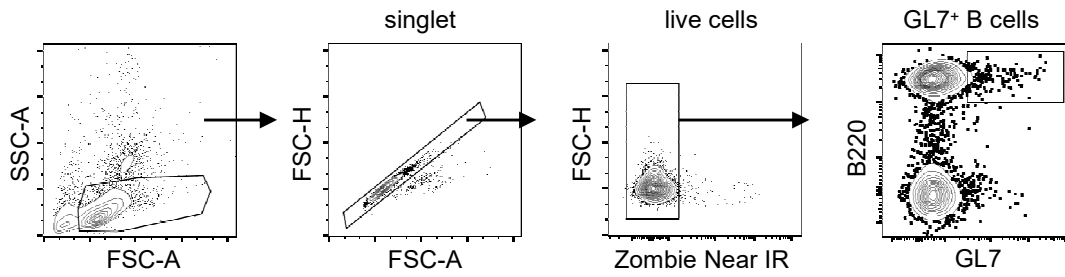
(A) Mice were immunized with NP-KLH/MnJ, boosted on day 8 and analyzed on day 14. Mice were injected with EdU intraperitoneally and then analyzed by flow cytometry 2.5 hours later. Flow cytometry plots (left) and ratio (right) of EdU-positive GCB cells (B220⁺IgD⁻CD95⁺GL7⁺EdU⁺) or pre-MBC (B220⁺IgD⁻CD95⁺GL7⁺CD38⁺CCR6⁺EdU⁺) of *Cfp1^{fl/fl}* and *Cfp1^{fl/fl}Aicda^{Cre}* mice. Data were pooled from two independent experiments; n = 5; 2 males (m), 3 females (f). Each symbol indicates one mouse and lines denote means. *P*-values by unpaired t-test (two-tail). **(B)** Heatmap for the mRNA and H3K4me3 marks for the differentially expressed genes (DEG) (Fold change >1.4-fold; p-values < 0.05) in Pre-MBC signature of *Cfp1^{fl/fl}* and *Cfp1^{fl/fl}Aicda^{Cre}* GCB cells. **(C)** Venn diagram showing the overlap between pre-MBC signature genes, bivalent genes and “Both up” genes. **(D)** Genome browser tracks of mRNA, H3K4me3 and H3K27me3 for *Zeb2* and *Cdkn1b*, which are BCL-6 targets and associated with the pre-memory signature related to Fig.7 G, in *Cfp1^{fl/fl}* and *Cfp1^{fl/fl}Aicda^{Cre}* GCB cells. **(E)** Genome browser tracks of mRNA, H3K4me3 and H3K27me3 for *Ccr6*, *Bhlhe40* and *Gpr65*, which are not BCL-6 targets but associated with the pre-MBC signature related to Fig. 7 G, in *Cfp1^{fl/fl}* and *Cfp1^{fl/fl}Aicda^{Cre}* GCB cells.

A**B**

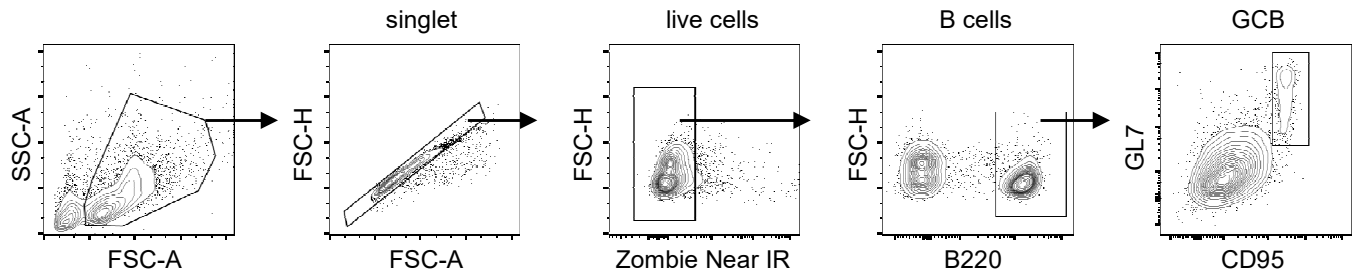
Supplementary Figure 13. Gene expression levels and H3K4me3 modification profiles.

(A) Expression levels of representative genes. Data from RNA-seq are presented as dot plots, $n = 3$; 3 females (f). Bars represent the mean values. Log2FC and P Values were determined by R package DESeq2. **(B)** H3K4me3 signal of representative genes. Data from H3K4me3 CUT&Tag are presented as triangle plots, $n = 3$; 1 males (m), 2 females (f). Bars represent the mean values. Log2FC and FDR were determined by R package DiffBind.

A (For Western Blot)



B (GCB For Western Blot, DD-PCR, qPCR, bulk-RNA-seq, CUT&Tag, 4C-HTGTS, 3C-qPCR)



Supplementary Figure 14. Gating strategies used for cell sorting.

(A) Gating strategy to sort GL7⁺ B cells (B220⁺GL7⁺) for Supplementary Figure 2 (Western Blot). **(B)** Gating strategy to sort GCB cells (B220⁺CD95⁺GL7⁺) for Supplementary Figure 2 (Western Blot, DD-PCR, qPCR); for bulk-RNA-seq, CUT&Tag, 4C-HTGTS, 3C-qPCR.

Supplementary Table1. Oligonucleotides

Primer	Sequence 5'->3'
Cut&Tag primers	
Universal i5	AATGATACGGCGACCAACCGAGATCTACACTCGTCGGCAGCGTCA GATGTGTAT
Barcoded i7	CAAGCAGAAGACGGCATAACGAGAT <u>NNNNNNNN</u> GTCTCGTGGGC TCGGAGATGTG
SHM of JH4 intron primers	
JH4_iEur	GACTAGTCCTCTCCAGTTTCGGCTGAATCC
JH4_J558	GGAATTCGCCTGACATCTGAGGACTCTGC
JH4Fi5	TTCCCTACACGACGCTCTTCCGATCT <u>NNNNNN</u> TAAGAATGGCCTC TCCAGGTC
JH4Ri7	AGTTCAGACGTGTGCTCTTCCGATCT <u>NNNNNN</u> ATGAGCCTCCAA AGTCCCT
JH4_P5	AATGATACGGCGACCAACCGAGATCTACAC <u>NNNNNNNN</u> ACACTCT TTCCCTACACGACGCTCTTCC
JH4_P7	CAAGCAGAAGACGGCATAACGAGAT <u>NNNNNNNN</u> GTGACTGGAGTT CAGACGTGTGCTCTTCCG
ddPCR and qPCR primers	
Cfp1 F	GTGAGTAGCTGCAGTGCTTG
Cfp1 R	CACTCACCACACATCCGAGC
GAPDH F	TACCTGATGAACCTAAGC
GAPDH R	GTGAAAGGGGCAGTGTCT
3C-qPCR primers	
Promoter 1	CACCTGACAGCTAGAATCAAT
Enhancer 1	TGAAGGTGGCCATTGTGAGG
Enhancer 2	CTCAGAGTCTCAGAGTCAAGATG
GAPDH F	TACCTGATGAACCTAAGC
GAPDH R	GTGAAAGGGGCAGTGTCT
4C-HTGTS primers	
Bio-Bcl6- promoter	GAGACGTGGGGCTGATCTTCGGCAT
I5-RED-Bcl6	CCCTACACGACGCTCTTCCGATCT <u>NNNNNN</u> GATACACATAGGAAA CTTGGAGCC
I7-Blue	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACTATAGGG CACGCGTGG
P5-I5	AATGATACGGCGACCAACCGAGATCTACAC <u>NNNNNNNN</u> ACACTCT TTCCCTACACGACGCTCTTCCGATCT
P7-I7	CAAGCAGAAGACGGCATAACGAGAT <u>NNNNNNNN</u> GTGACTGGAGTT CAGACGTGTGCTCTTC
NNNNNN represents barcode sequence	

Supplementary Table2. Antibodies

Host Species	Fluorescence	Anti	Clone	Company	Cat
Rat		CD16/CD32		BD Biosciences	553142
Rat	APC-Cy7	B220	RA3-6B2	BioLegend	103223
Rat	AF700	B220	RA3-6B2	eBioscience	56-0452-82
Rat	APC	B220	RA3-6B2	eBioscience	17-0452-82
Rat	eF450	B220	RA3-6B2	eBioscience	48-0452-82
Rat	FITC	GL7	GL7	BioLegend	144604
Rat	Pacific Blue	GL7	GL7	BioLegend	144613
Mouse	PE	CD95	SA367H8	BioLegend	152608
Mouse	APC	CD95	SA367H8	BioLegend	152604
Armenian Hamster	PE	CD95	Jo2(RUO)	BD Biosciences	561985
Rat	APC	CXCR4	L276F12	BioLegend	146507
Rat	BV605	CXCR4	L276F12	BioLegend	146519
Rat	eF450	CD86	GL1	eBioscience	48-0862-82
Rat	PE-Cy7	CD86	GL1	BioLegend	105014
Rat	BV510	IgD	11-26C. 2a	BioLegend	405723
Rat	biotin	IgD	11-26C. 2a	BioLegend	405734
Rat	eF450	IgD	11-26C (11-26)	eBioscience	48-5993-82
Rat	PC5.5	CD38	90/CD38	BD Biosciences	562770
Rat	biotin	CD38	90/CD38	BD Biosciences	553762
Rat	PE-Cy7	CD38	90	BioLegend	102717
Rat	biotin	CD38	90	BioLegend	102722
Rat	PE	CD138	281-2	BioLegend	142503
Rat	biotin	CD138	281-2	BD Biosciences	553713
Armenian Hamster	BV421	CCR6	29-2L17	BioLegend	129817
Rat	APC-Cy7	CD19	1D3	BD Biosciences	557855
Rat	APC	CD93	AA4.1	eBioscience	17-5892-82
Rat	BV605	CD24	M1/69	BioLegend	101827
Mouse	PE	BP-1	BP-1	BD Biosciences	553735
Rat	PC5.5	CD43	S11	BioLegend	143220
Rat	FITC	IgM	II/41	BD Biosciences	553437
Rat	APC-Cy7	IgM	II/41	eBioscience	47-5790-82
Rat	biotin	IgM	II/41	BD Biosciences	553436
Rat	PE	CD21	7.00E+09	BioLegend	123409
Rat	FITC	CD21	7.00E+09	BioLegend	123407
Rat	PE-Cy7	CD23	B3B4	BioLegend	101614
Rat	FITC	IgG1	A85-1	BD Biosciences	553443
Rat	PE	IgG1	A85-1	BD Biosciences	550083
Rat	BV421	IgG1	X56	BD Biosciences	742475
Rat	PE	IgG1	RMG1-1	BioLegend	406608
Rat	BV650	IgG1	RMG1-1	BioLegend	406629
Rat	APC	Ki67	16A8	BioLegend	652406
Mouse	APC	Brdu	3D4	BioLegend	364114
Mouse	AF647	BCL6	K112-91	BD Biosciences	561525
Rat	PE-Cy7	IRF4	IRF4. 3E4	BioLegend	646413
Armenian hamster	PE	CD69	H1.2F3	eBioscience	12-0691-81
Rat	BV421	PD-L2	TY25	BioLegend	107219
Armenian Hamster	BV605	CD80	16-10A1	BD Biosciences	563052

Supplementary Table2. Antibodies

Host Species	Fluorescence	Anti	Company	Cat
		Zombie NIR	BioLegend	423106
		LIVE/DEAD Blue	Invitrogen	L34961
	PE	Sreptavidin	BioLegend	405204
	PE-Cy7	Sreptavidin	BioLegend	405206
	FITC	Sreptavidin	Invitrogen	11-4317-87
	BV605	Sreptavidin	BioLegend	405229
	BV421	Sreptavidin	BioLegend	405225
	PE	NP	Biosearch Technologies	N-5070-1
	Fluorescein	Peanut Agglutinin (PNA)	Vector laboratories	FL-1071
Goat		Mouse Ephrin-B1 Biotinylated Antibody	R&D Systems	BAF473
Rabbit		Anti-CGBP (CFP1) antibody	Abcam	ab198977
Mouse		Beta Actin antibody	proteintech	66009-1-Ig
Goat		Goat Anti-Mouse IgG1-HRP	Southern Biotech	1071-05
Goat		Goat Anti-Mouse IgM-HRP	Southern Biotech	1021-05
Rabbit		Anti-trimethyl-Histone H3 (Lys4)	Sigma Aldrich	07-473
Goat		HRP-conjugated Goat Anti-Rabbit IgG(H+L)	proteintech	SA00001-2
Horse		Anti-mouse IgG, HRP-linked Antibody	Cell Signaling Technology	7076
Rabbit		Tri-Methyl-Histone H3 (Lys27) (C36B11) Rabbit mAb	Cell Signaling Technology	9733T
Mouse		Rpb1 CTD (4H8) Mouse mAb	Cell Signaling Technology	2629T
Rabbit		Rabbit Anti-Mouse IgG H&L	Abcam	ab46540
Guinea Pig		Guinea Pig anti-Rabbit IgG H&L	Antibodies Online	ABIN101961

Supplementary Table3. RNA and H3K4me3 replicates for Genomebrowser track related to Supplementary Figure 13

	RNA(FPKM)							
Symbol	WT1	WT2	WT3	CKO1	CKO2	CKO3	RNA log2FC	RNA p.value
Cdc6	11.51269265	12.91405498	9.392678435	7.380414815	5.743145481	3.212685128	-1.103733206	0.00013042
Aicda	146.4604688	93.69455568	85.92413825	41.99879133	50.19151089	52.21482387	-1.180483845	3.71E-06
S1pr1	22.04355431	19.16525018	30.06278993	53.7460107	56.81723821	43.95521462	1.093934948	1.26E-06
Bcl6	71.75543215	40.1243142	49.63549207	15.3243248	18.22457272	8.76900857	-1.964794941	9.68E-13
Cd69	17.95590624	16.85113888	24.89729881	44.41917278	46.82560672	58.94976719	1.328992598	1.29E-07
Pou2af1	584.3749	546.1529525	482.0574871	366.6464998	369.9441854	346.8341549	-0.596810396	0.003387685
Mef2b	186.4980484	137.4550643	115.7754254	39.92133468	41.12271101	19.05959897	-2.179984226	2.15E-14
Irf4	29.49608027	31.58088897	35.9742049	106.2732933	95.88036259	68.53198933	1.443946191	7.35E-11
Hhex	5.597836916	4.765359022	9.975361641	9.522375322	12.52097947	9.173939886	0.59957045	0.033440632
Bcl2	3.872103405	2.55006452	5.213650133	8.493628177	9.52513918	5.899837773	1.016504453	0.000157825
Ccr12	0.994028934	5.077216239	1.884483853	4.962372128	5.757518624	5.230165243	0.930924501	0.037199876
Tlr3	0.777457871	0.51349565	0.999949903	1.201441901	1.168920723	1.107777422	0.591440836	0.044585514
Arid1a	38.59328596	25.76589909	38.98041092	30.8955918	36.42567516	20.36693201	-0.266324193	0.272133472
Zeb2	1.936405028	1.455798166	3.272160285	5.988008413	5.691456639	4.678568808	1.280982185	4.38E-06
Cdkn1b	16.61357555	12.87023552	18.6997185	27.8949965	30.98081794	24.95166129	0.780265962	0.000202023
Ccr6	21.9920564	13.07433457	46.04690394	45.78604597	59.54899121	51.81483427	0.954364111	0.006223882
Bhlhe40	15.76179808	21.26010111	31.28321394	93.21782629	101.4224965	59.68080983	1.854746106	5.05E-11
Gpr65	3.373463052	3.833827849	5.321060569	9.602095113	9.297176739	14.75870696	1.428007697	2.41E-06
	H3K4me3(RPKM)							
Symbol	WT1	WT2	WT3	CKO1	CKO2	CKO3	H3K4me3 log2FC	H3K4me3 FDR
Cdc6	117.3472077	114.1155426	129.5451331	61.36442123	61.7996357	70.71573125	-0.907838902	9.813E-05
Aicda	154.0834117	215.0840209	226.4988077	87.73702147	77.32945081	80.37300257	-1.29052865	7.75033E-10
S1pr1	305.5603576	317.8432762	288.088235	385.7407793	483.2519366	451.9086997	0.535952442	2.83828E-05
Bcl6	437.7013588	452.9728123	458.2896746	368.3273097	357.7846077	369.6079935	-0.29203022	0.013023644
Cd69	131.6605362	146.6440195	119.8117269	133.4427207	179.2189186	157.6266655	0.267369387	0.201025809
Pou2af1	385.1905136	414.3852487	449.8837956	220.9393698	324.9297235	290.5004853	-0.569627031	0.005613682
Mef2b	186.0591653	240.3951104	301.5297485	33.7486949	24.48253198	18.81765035	-3.181789097	7.66E-21
Irf4	302.5096	308.7863	268.0151	281.8402	384.262	356.3161	0.226075536	0.158157237
Hhex	248.5673722	278.9193996	280.3552237	463.3683985	483.9732897	490.6622446	0.851676827	1.22854E-13
Bcl2	217.2962201	212.7874697	233.1758159	361.0576434	414.7130851	423.254395	0.851382691	2.43166E-11
Ccr12	130.0190113	158.3453053	134.1734638	238.5863637	210.3043538	222.5452673	0.686084121	5.09373E-05
Tlr3	31.3892042	35.64133129	42.41231428	77.16736679	50.97175436	73.77071285	0.933979575	0.006782652
Arid1a	748.4607072	720.5071843	792.0036003	831.822844	777.4111402	800.5684922	0.093588199	0.37256586
Zeb2	319.6514909	339.3200009	325.0998347	555.8476341	556.0509245	555.3370048	0.7711005337	6.78595E-14
Cdkn1b	560.6439192	587.2114499	545.503993	784.8739241	835.7704859	898.3472145	0.587032309	3.09064E-10
Ccr6	61.6573712	96.55355202	99.07175719	167.5416946	187.0390284	200.4057674	1.12518546	1.10179E-07
Bhlhe40	172.9749702	177.7583559	159.7469681	234.7025775	295.9990769	307.5327718	0.716101804	3.96406E-06
Gpr65	105.0957839	97.93033056	92.23485179	146.251305	182.1349827	161.5833881	0.748617584	0.000152893

RNA: log2FC and P. value were determined by R package DESeq2 (two-sided Wald test; Benjamini-Hochberg FDR correction for multiple comparisons).

H3K4me3: log2FC and FDR were determined by R package DiffBind (DESeq2-based; two-sided test; Benjamini-Hochberg correction for multiple comparisons).