Supporting Information Appendix

The SWI/SNF chromatin remodeling complex regulates germinal center formation by repressing Blimp-1 expression

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SI Materials and Methods

Cell culture and transfection. HEK293T, SW-13 and phoenix-eco cells were maintained in DMEM (WelGENE) medium containing 10% fetal bovine serum (FBS), 100U/ml streptomycin and penicillin. BAL17 and Raji cells were cultured in RPMI-1640 medium containing 10% FBS and 50μM 2-mercaptoethanol. For transient transfection to HEK293T, calcium phosphate ($CaPO_4$) method was used and BAL17 and Raji cells were transfected using a Microporator Neon system (Life technologies). For SW-13 cells, FuGENE HD (Roche) transfection reagent was used.

RNA extraction and quantitative RT-PCR. Total RNA was isolated using TRI-reagent (Molecular Research Center) according the manufacturer's instructions. Equivalent quantities of total RNA were reverse-transcribed with SuperScript III (Life technologies) or QuantiTect (Qiagen) and diluted cDNA was analyzed by real-time PCR (StepOnePlus; Applied Biosystem). The primers using for amplification of the cDNA are described in SI Appendix, Table S2.

V(D)J Rearrangement analysis and Southern blot analysis. $B220^{+}IgM^{-}$ B cells of $Srg3^{fl/fl}$ or $Srg3^{fl/fl}CD19Cre^{+}$ mice were digested with proteinase K, and genomic DNA was isolated by phenol-chloroform extraction methods. PCR analysis of immunoglobulin genes was performed with primers as previously described (1). HSS3 was used as loading control. DNA product amplified by PCR were separated on 1% agarose gels by electrophoresis and transferred to hybond-N membranes (Amersham). Southern blotting was analyzed with ^{32}P-labeled oligonucleotide probes.

Splenic B cell isolation and culture. FO B cells ($B220^{+}CD21^{-}CD23^{+}$) were purified from spleen by FACSAria II (BD bioscience, NCIRF) and cultured at a density of $1X10^{6}$ cells per ml in the presence of IL-4 (10ng/ml; Peprotech) and LPS (25ug/ml; Sigma-Aldrich) for 3 days.

T cell isolation and culture. Naïve $CD4^{+}$ T cells ($CD4^{+}CD25^{-}CD44^{-}$) were purified from spleen and lymph node using FACSAria II (BD Bioscience). For some experiments, naïve $CD4^{+}$ T cells were obtained by $CD4^{+}$ T cell isolation kit (Miltenyi biotech). To activate T cells for retroviral transduction, $CD4^{+}$ T cells were cultured with plate-bound anti-CD3 (10μg/ml; 2C11; BD Bioscience) and soluble anti-CD28 (2μg/ml; 37.51; BD Bioscience) in the presence of IL-2 (20U/ml; R&D System). For T_{FH}-like differentiation, cultures were supplemented with anti-IFN-γ (10μg/ml; XMG1.2; e-bioscience), anti-IL-4 (10μg/ml; 11B11; e-bioscience), anti-TGF-β (20μg/ml; 1D11; R&D System) and IL-6 (20ng/ml; Peprotech) during 24hr.

Retroviral infection. To generate retroviruses for infection into cells, the packaging phoenix-eco cells were transfected with vectors expressing GFP alone (Empty-RV), GFP plus Bcl-6 (Bcl-6-RV), Myc-tagged Srg3 (Srg3-RV) or Cre-recombinase (Cre-RV) and viral supernatants were harvested 2 days later. $CD4^{+}$ T and B cells stimulated as described previously were infected with the viral supernatants in the presence of polybrene (8μg/ml) by spin infection for 90min at 2400rpm. The DNA vector expressing Bcl-6 was a gift from Hida ye (Albert Einstein College of Medicine).

Immunoprecipitation and western blotting. For western blotting, cells were lysed with RIPA buffer (10mM Tris-Cl (pH 8.0), 1mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 140mM NaCl) supplemented with protease inhibitors (Roche). Whole cell extracts were separated by SDS-PAGE gels and transferred to Immobilon-P membrane (Millipore). For detection of protein-protein interactions, nuclear extracts were prepared from Raji or BAL17 cells. About 500μg of nuclear extracts were diluted in IP buffer (50mM Tris-Cl (pH. 7.05), 250mM NaCl, 0.05% NP-40, 1% Triton X1-00, 1mM EDTA, 1mM NaF) supplemented with protease inhibitors and were immunoprecipitated with rabbit polyclonal IgG (negative control; Millipore), with 2μg of antibody for Bcl-6 or with antiserum against Srg3. Immune complexes were immobilized on Protein A Sepharose (GE Healthcare) and washed at least 3 times with cold IP buffer. The washed pellets were eluted with loading buffer and the eluates were analyzed by western blotting using the antibodies as described above.

Luciferase reporter assay. A reporter construct containing the Blimp-1 basal promoter and BRE1 in intron 5 as previously described (2) was transfected into Brg1-deficient SWI-13 cells using FuGENE HD (roche). The luciferase activity in the whole cell lysates was measured with luciferase assay kit (Promega) according to the manufacturer's instruction. A luciferase activity was normalized for transfection efficiency by β-galactosidase activity expressed from a co-transfected LacZ plasmid.

Adoptive transfer. Transduced B cells using retroviral infection were mixed with naïve $CD4^+$ T cells obtained from WT C57BL/6 mice at 1:1 ratio, and then cells were transferred into *Rag2*$^{-/-}$ host mice by intravenous injection via tail vein. Reconstituted mice were rested for 3 days before immunization with NP-KLH precipitated in Alum. After 7 days, flow cytometric analysis was done. For transfers of transduced $CD4^+$ T cells into *TCRβ*$^{-/-}$ mice, 5×10^4~1×10^5 cells were transferred.

Chromatin immunoprecipitation assay (ChIP). $B220^+GL\text{-}7^+Fas^+$ GC B cells and $B220^{lo}CD138^+$ PCs were purified from C57BL/7 mice immunized with NP-KLH 7 day after immunization using FACSAria II (BD bioscience). 1×10^6 cells (for GC B cells and plasma B cells; splenocytes of 3 mice per experiment were pooled and harvested for purification of plasma B cells) and 1×10^7 cells (for BAL17 cells) were used for ChIP assays. A solution of 37% (vol/vol) formaldehyde (Merck) was added to the culture medium to a final concentration of 1% and cells were incubated for 10 min with mild rocking at RT, followed by washing twice with cold PBS. The cells were lysed for 10 min in ice with SDS lysis buffer (1% SDS, 10mM EDTA, 50mM Tris-Cl (pH 8.1)). Lysates were sonicated to an average length of 100-500 base pairs and then, were diluted 10-folds in dilution buffer (1% Triton X-100, 2 mM EDTA, 150mM NaCl, 20mM Tris-HCl (pH 8.1)). After that, to reduce nonspecific background phenomena, lysates were precleared for 1hr with salmon-sperm DNA/Protein-A agarose (50% slurry), followed by incubation overnight at 4°C with isotype-control anti-rabbit or mouse IgG (Millipore), anti-Pol II (Santa Cruz Biotechnology), anti-Bcl-6, anti-Srg3, anti-$H3K4^{me3}$ (Millipore) or anti-$H3K27^{me3}$ (Millipore). The samples were washed sequentially once with a low salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.1), 150 mM NaCl), a high salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.1), 500 mM NaCl), LiCl washing buffer (0.25 M LiCl, 1% Nonidet P-40, 1 %

deoxycholate, 1 mM EDTA, 10 mM Tris-HCl (pH 8.1)) and twice with TE buffer. Crosslinked chromatin-histone complexes were eluted with elution buffer (1% SDS, 0.1 M $NaHCO_3$). After that, crosslinking was reversed by heating at 65°C for 4 hr, followed by proteinase K treatment for an additional 1 hr. DNA was purified with a QIAquick Spin kit (Qiagen) and eluted DNA was analyzed by PCR with indicated primers.

CFSE Staining. $B220^+CD43^-$ B cells were sorted from spleen of $Srg3^{fl/fl}$ or $Srg3^{fl/fl}CD19Cre^+$ mice and were labeled for 10min at 37°C with 1μM CFSE (Carboxyfluorescein diacetate, succinimidyl ester; Molecular Probe, Life technologies) in PBS/0.1% BSA. After staining, cells were cultured with IgM (5μg/ml; Jackson Immunoresearch) only or IgM plus IL-4 (10ng/ml) for 3 days. For analysis of proliferation of $CD4^+$ T cells, naïve $CD4^+$ T cells isolated from spleen of $Srg3^{fl/fl}$ or $Srg3^{fl/fl}CD4Cre^+$ mice were labeled with CSFE in same procedure of B cells and were cultured with plate-bound anti-CD3 (10μg/ml; 2C11; BD Bioscience) and soluble anti-CD28 (2μg/ml; 37.51; BD Bioscience) in the presence of IL-2 (20U/ml) for 3 days.

Flow cytometry. $1\sim3\times10^6$ cells were used for antibody staining. Biotinylated antibodies were revealed by PerCP-Cy 5.5-, PE, or APC-conjugated streptavidin (BD Bioscience). The antibodies used for generation of GC B cells are as follows: Phycoerythrin (PE)-conjugated NP (4-hydroxy-3-nitroophenyl) was purchased from Bioresearch Technologies. FITC-conjugated peanut agglutinin (PNA) was purchased from Sigma-Aldrich. The following antibody conjugates were purchased from BD Bioscience: Anti-B220 (RA3-62), anti-CD19 (1D3), anti-Fas (15A7), anti-GL7 (GL7), anti-CD4 (GK1.5), anti-CD8 (53-6.7), anti-CD138/Syndecan-1 (281-2), anti-IgM (R6-60.2), anti-IgG1 (A85-1), anti-CD279/PD1 (J43), anti-CD25 (7D4), anti-CD44 (IM7). Anti-IgD (11-26C.2a) was purchased from BioLegend. For analysis of T_{FH} cells, CXCR5 staining was done as a three-step staining as previously described (3). In brief, CXCR5 staining was done using purified anti-CXCR5 (2G8; BD Bioscience) for 1hr on ice, and secondary staining was done using biotinylated anti-rat IgG (Jackson Immunoresearch) for 30min, followed by PerCP-Cy5.5-conjugated strepavidin (BD Bioscience). For flow cytometric analysis of SRBC-mediated T_{FH} cells in Figure 5A, CXCR5 staining was done using APC-conjugated anti-CXCR5 (BD Bioscience).

Restriction endonuclease accessibility assay. 1×10^6 cells of $B220^+CD23^+CD21^-$ FO B cells and Brg1/Srg3-depleted BAL17 cells were used for restriction endonuclease accessibility assay. Germinal center ($B220^+GL\text{-}7^+Fas^+$) and plasma B cells ($B220^{lo}CD138^+$) were obtained from C57BL/6 mice immunized with NP-KLH 7 days after immunization using FACSAria (2×10^5 cells were used per experiment). Cells were washed in cold PBS and cell pellets were lysed with NP-40 lysis buffer (10mM Tris (pH7.4), 10mM NaCl, 3mM $MgCl_2$, 0.5% NP-40, 0.15mM spermine (Sigma), and 0.5mM spermidine (Sigma)) and incubated on ice for 5 min. Nuclei were pelleted at 3000rpm for 5min followed by washing in RE buffer (10mM Tris-Cl (pH 7.4), 50mM NaCl, 10mM $MgCl_2$, 0.2mM EDTA, 0.2mM EGTA, 1mM β-Mercaptoethaol, 0.15mM spermine, and 0.5mM spermidine). Nuclei were suspended in appropriate digestion buffer for indicated restriction enzyme with incubation at 37°C for 10 min. Reactions were terminated by proteinase K buffer (100mM Tric-Cl (pH 7.5), 200mM

NaCl, 2mM EDTA, and 1% SDS) and incubated at 55°C for 1hr. Samples were incubated with proteinase K (25mg/ml) overnight at 37°C and DNA was purified with Tissue DNA purification kit (LaboPass). About one hundred nanograms of genomic DNA were used to perform quantitative real-time PCR. DNA samples recovered from 'Cut' (treated with indicated restriction enzyme; +RE) or 'Uncut' (-RE) were used to perform quantitative real-time PCR analysis with indicated primers (Table S2). Digestion efficiency of restriction enzyme was normalized to GAPDH intron lacking recognition sites for both SacII and PstI restriction enzymes and chromatin accessibility was calculated as the ratio of 'uncut/cut'.

Histology. Mouse spleen were fixed in 10% formalin, dehydrated in 100% ethanol, and embedded in paraffin wax at 58 °C. For H&E staining, sections (4 μM) were rehydrated and stained with hematoxylin and eosin. Imaging was performed using a fluorescence microscopy (Axio Observer, Zeiss) and a confocal laser scanning microscopy (LSM710, Zeiss).

References

1. Fuxa M*, et al.* (2004) Pax5 induces V-to-DJ rearrangements and locus contraction of the immunoglobulin heavy-chain gene. *Genes & development* 18(4):411-422.
2. Tunyaplin C*, et al.* (2004) Direct repression of prdm1 by Bcl-6 inhibits plasmacytic differentiation. *J Immunol* 173(2):1158-1165.
3. Johnston RJ*, et al.* (2009) Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science* 325(5943):1006-1010.

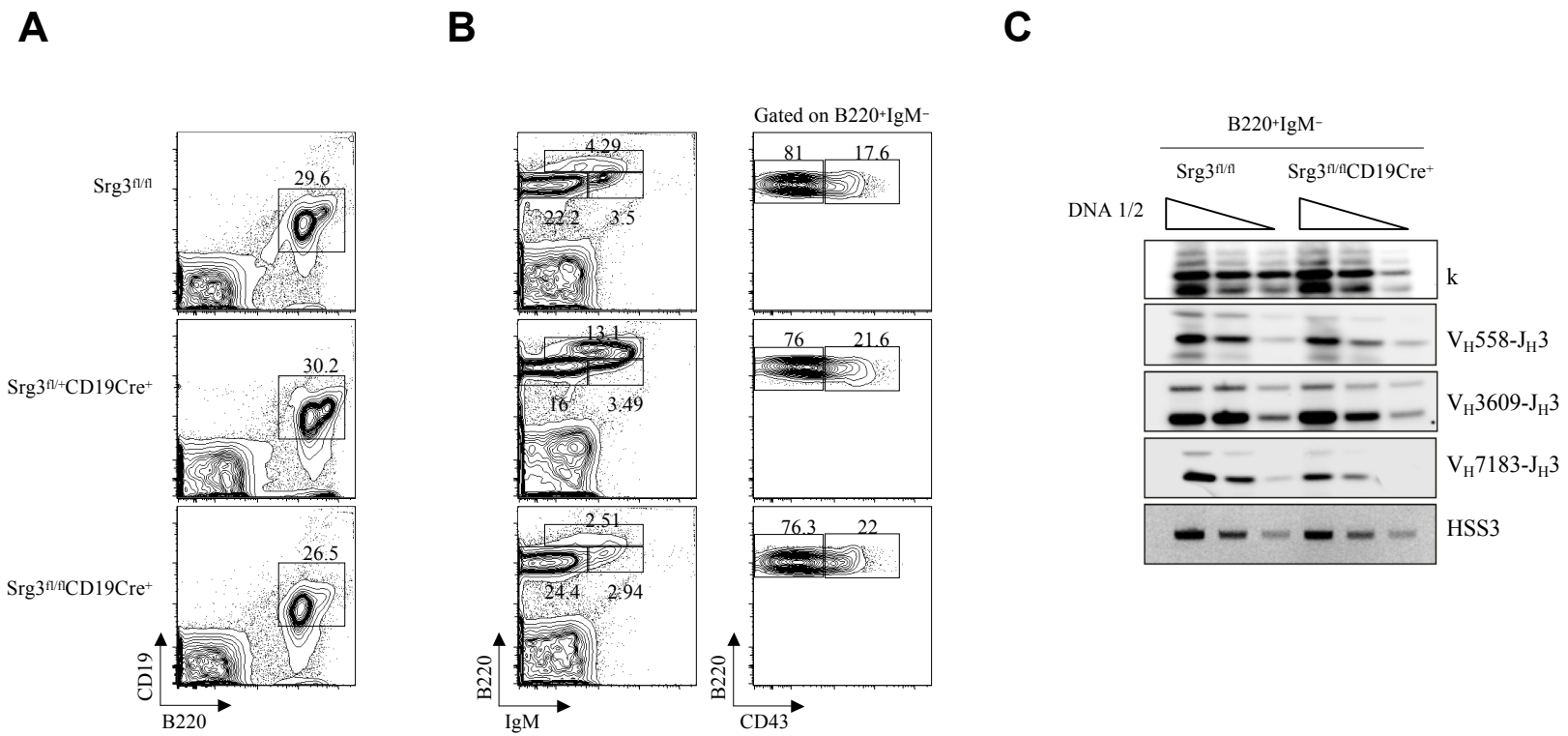

Figure S1. Normal B ell development in $Srg3^{fl/fl}CD19cre^{+}$ mice. (A) Flow cytometry of B cells ($B220^{+}CD19^{+}$) in the bone marrow of $Srg3^{fl/fl}$, $Srg3^{fl/+}CD19Cre^{+}$ and $Srg3^{fl/fl}CD19Cre^{+}$ mice. Numbers in plots indicate the frequency of populations. (B) Flow cytometry of B cell subsets of pro-B ($IgM^{-}B220^{+}CD43^{+}$), pre-B ($IgM^{-}B220^{+}CD43^{-}$), immature B ($IgM^{+}B220^{+}$) and mature recirculating B ($IgM^{-}B220^{high}$) cells. Numbers adjacent to the outlined areas indicate the frequency of B cell subsets. Data are representative of more than four independent experiments. (C) Southern blot analysis of genomic DNA from $B220^{+}IgM^{-}$ B cells for V_H7183-, V_HJ558-, V_H3609-DJ_H3 rearrangement at IgH locus and V_k-J_k rearrangement at Ig kappa locus. Three-fold serial dilutions were analyzed and the PCR product of IL-4 locus hypersensitivity site 3 (HSS3) was used as loading control of input DNA.

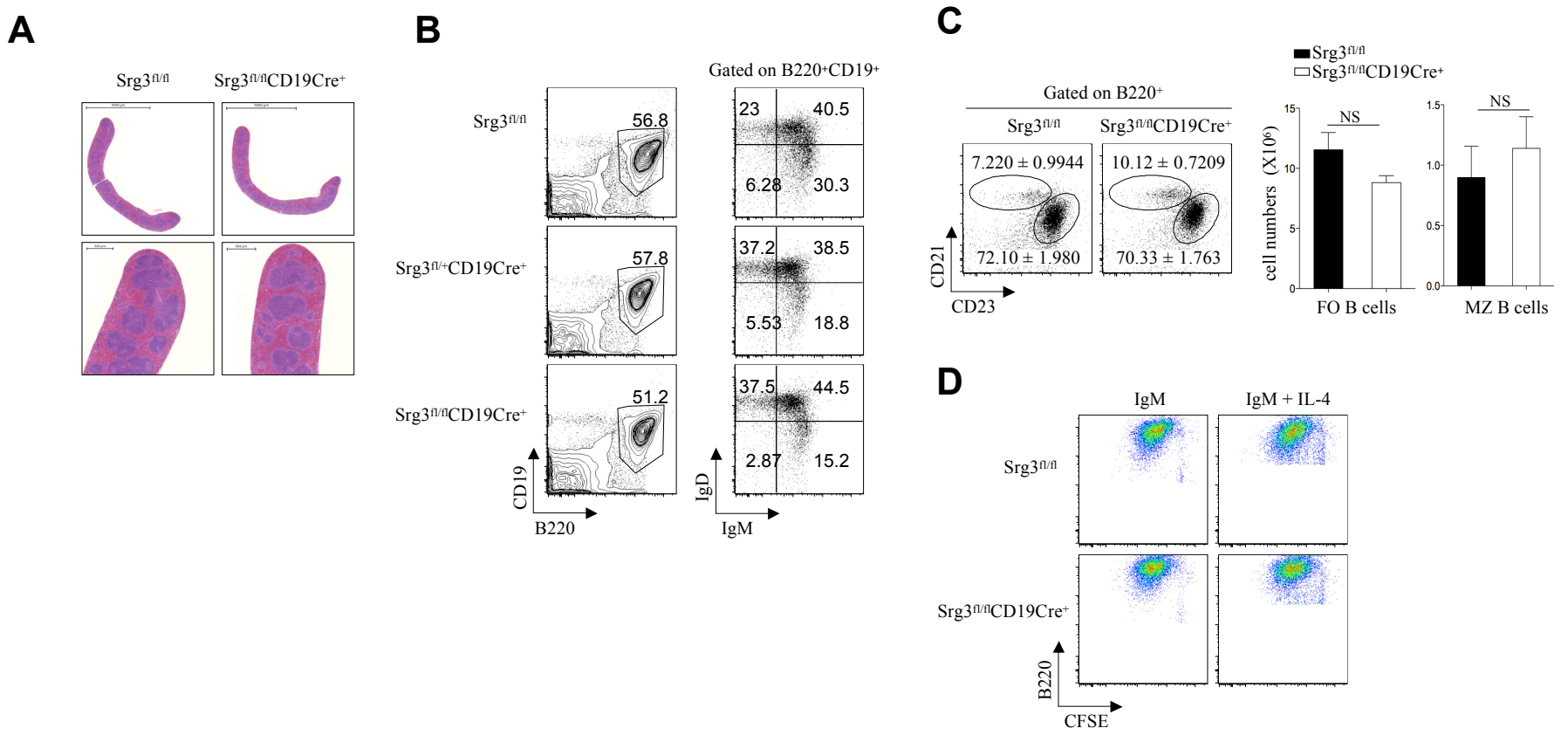

Figure S2. B cell development in the periphery is not altered by Srg3 deletion. (A) Paraffin-embedded splenic sections from $Srg3^{fl/fl}$ and $Srg3^{fl/fl}CD19Cre^{+}$ mice were stained with H&E and visualized by light microscopy. Data are representative of 2 experiments. (B) Representative flow cytometry of B cells ($B220^{+}CD19^{+}$) in the spleen of $Srg3^{fl/fl}$, $Srg3^{fl/+}CD19Cre^{+}$ or $Srg3^{fl/fl}CD19Cre^{+}$ mice. $B220^{+}CD19^{+}$ cells were further analyzed for the expression of surface molecules, IgM and IgD according to the maturation. T1 ($IgM^{+}IgD^{-}$), T2 ($IgM^{+}IgD^{+}$) and mature B cells ($IgM^{-}IgD^{+}$). (C) Representative flow cytometric analysis of follicular (FO) ($CD21^{-}CD23^{+}$) and marginal zone (MZ) ($CD21^{+}CD23^{-}$) B cells. Numbers in plots indicate the percentages of indicated populations. Frequency and cell numbers of MZ and FO B cells were calculated (n=4). (D) CFSE-labeled FO B cells isolated from $Srg3^{fl/fl}$ or $Srg3^{fl/fl}CD19Cre^{+}$ mice were activated with IgM (5μg/ml) or IgM plus IL-4 (10ng/ml). After 3 days, cells were harvested and stained with anti-B220 for flow cytometric analysis.

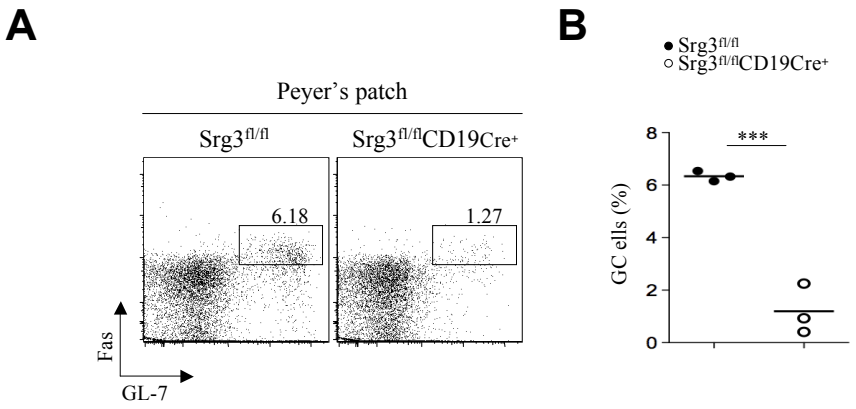

Figure S3. Spontaneous generation of GC B cells in Peyer's patch is impaired in $Srg3^{fl/fl}CD19Cre^{+}$ mice. (A) Flow cytometry of GC B cells in Peyer's patch from unimmunized mice. Cells gated on $B220^{+}$ were analyzed for the expression of surface molecules, Fas and GL-7. (B) Frequency of $Fas^{+}GL\text{-}7^{+}$ GC B cells obtained from $Srg3^{fl/fl}$ and $Srg3^{fl/fl}CD19Cre^{+}$ mice. Each individual dot represents one mouse; the lines show the mean percentage. ***$P < 0.001$.

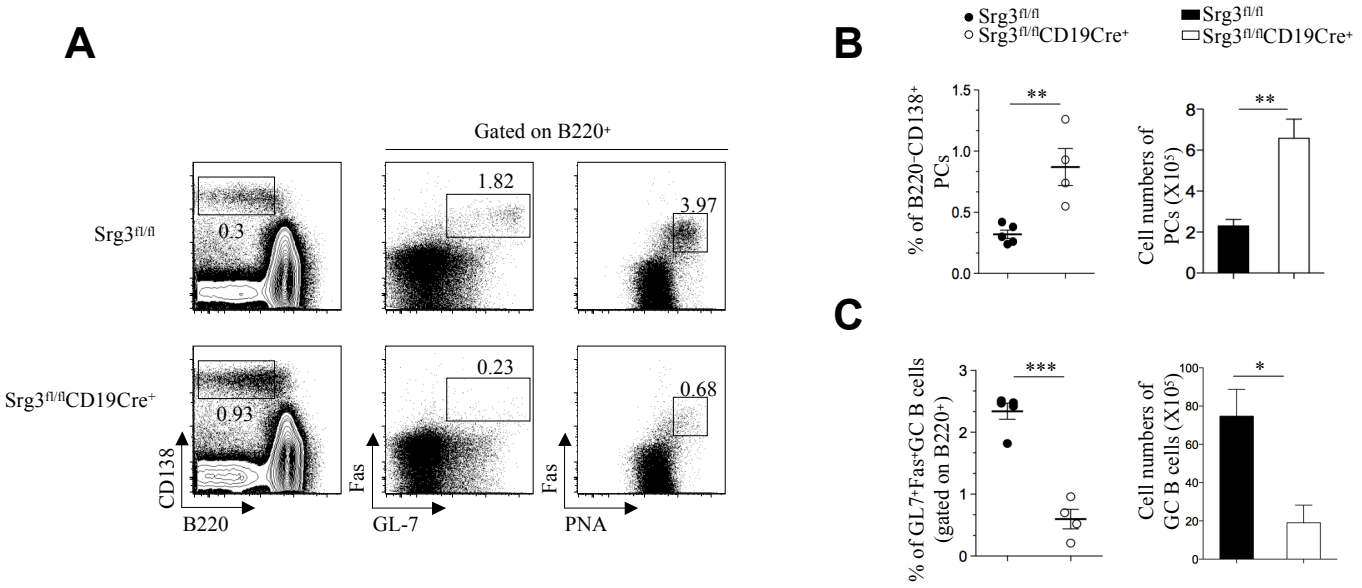

Figure S4. The formation of GC B cells is impaired in $Srg3^{fl/fl}CD19Cre^{+}$ mice after immunization with NP-KLH. (A) Flow cytometry of GC B cells obtained from the spleen of mice immunized with NP-KLH on day7. Cells gated on $B220^{+}$ were analyzed for the expression of surface molecules, CD138, Fas, GL-7 and PNA. (B) Frequency and cell numbers of $CD138^{+}B220^{lo}$ PCs. (C) Frequency and cell numbers of $CD138^{-}B220^{+}$ $Fas^{+}GL\text{-}7^{+}$ GC B cells. *P < 0.05, **P < 0.01, ***P < 0.001.

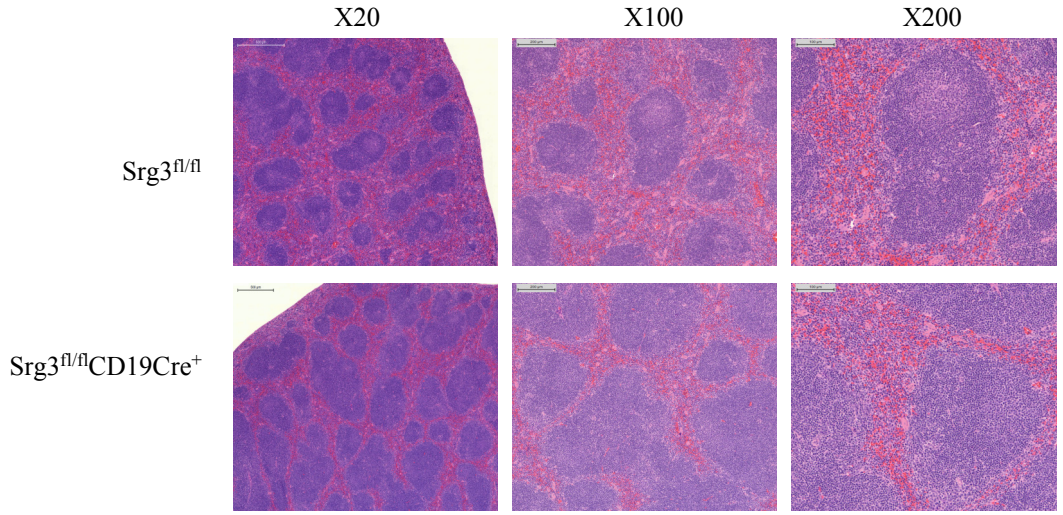

Figure S5. Reduced GC B cell formation in the spleen of immunized $Srg3^{fl/fl}CD19Cre^{+}$ mice. Paraffin-embedded splenic sections of $Srg3^{fl/fl}$ and $Srg3^{fl/fl}CD19Cre^{+}$ mice were prepared 7 days after immunization with NP-KLH. These samples were stained with H&E and visualized by light microscopy to analyze the splenic architecture, that is the size and distribution of red and white pulp. Data are representative of two independent experiments. Original magnification is indicated in the figures.

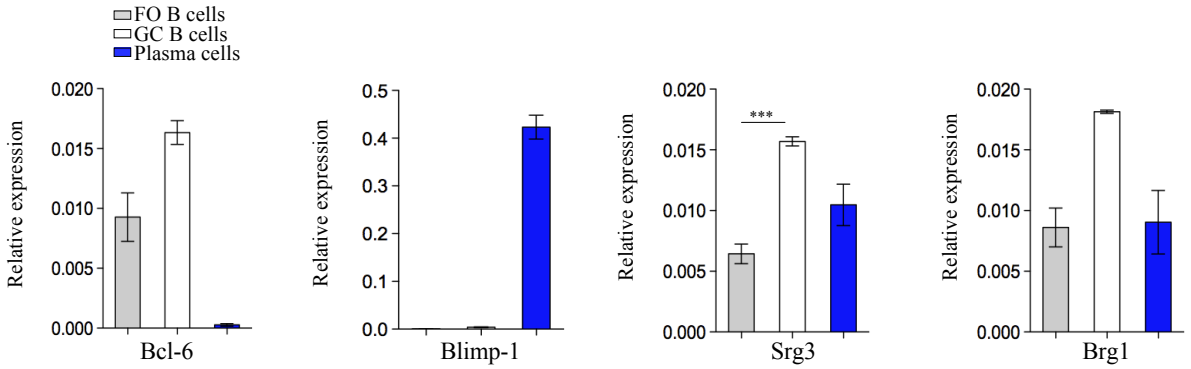

Figure S6. Differential expression of Srg3 in FO, GC B cells and PCs. Quantitative RT-PCR analysis of gene expression in FO, GC B cells and PCs (n=3-4). C57BL/6 WT mice were immunized with NP-KLH and cell subsets– FO B cells ($CD138^{-}PNA^{-}B220^{+}CD21^{-}CD23^{+}$), GC B cells ($CD138^{-}PNA^{+}B220^{+}$) and PCs ($CD138^{+}PNA^{-}B220^{lo/-}$)– were purified at day 7 after immunization. ***$P < 0.001$.

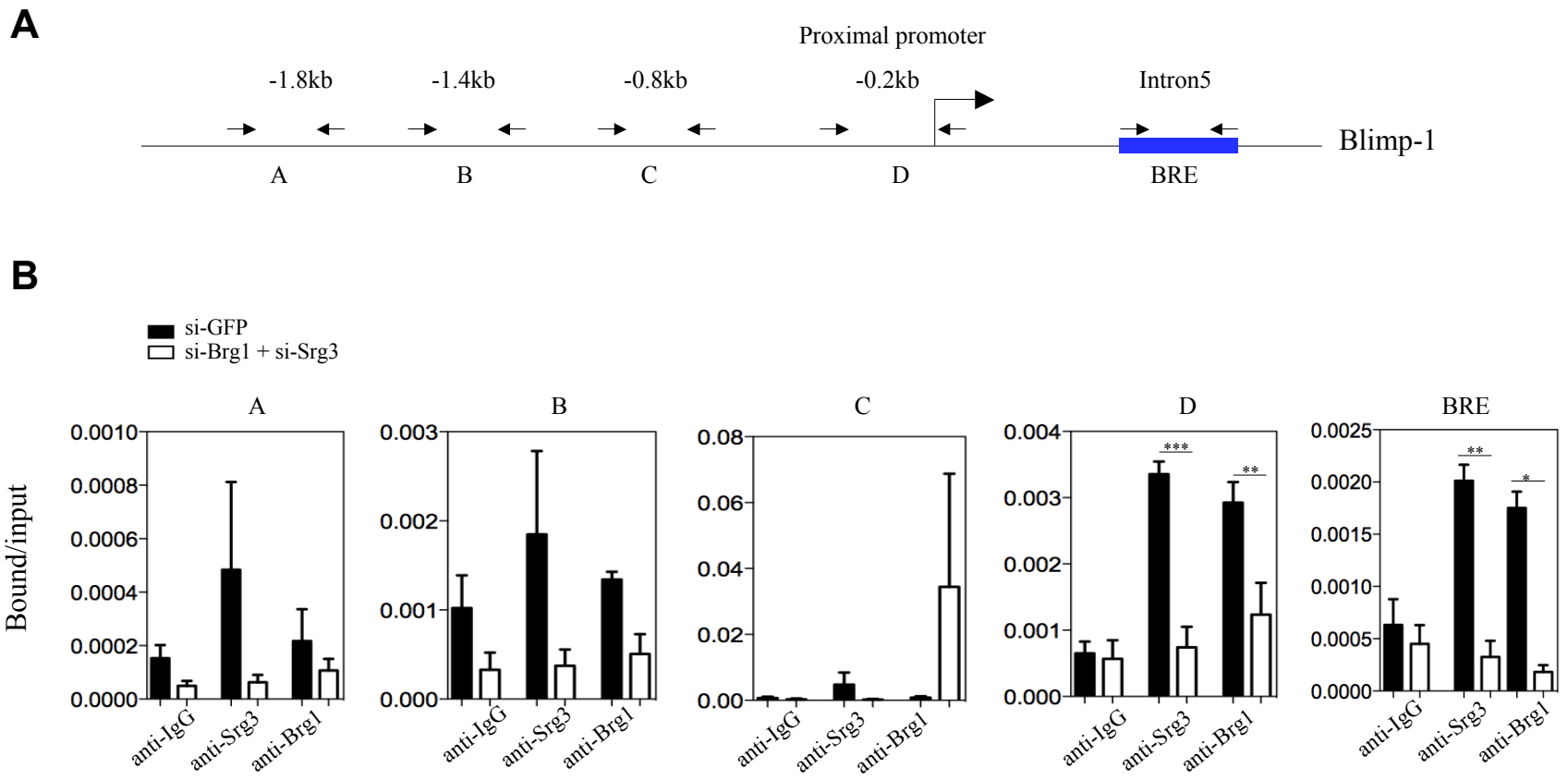

Figure S7. The SWI/SNF complex binds to promoter region and BRE at Blimp-1 locus. (A) Schematic representation of Blimp-1 locus. PCR primer pairs for ChIP analysis were indicated. (B) ChIP analysis of the binding of Srg3 or Brg1 to the Blimp-1locus on crosslinked chromatin from intact or Brg1/Srg3-depleted BAL17 cells; immunoprecipitated (IP) with anti-srg3, anti-brg1 or isotype control antibody (anti-IgG1), followed by PCR amplification of input DNA or immunoprecipitated DNA with indicated primers. *P < 0.05, **P < 0.01, ***P < 0.001.

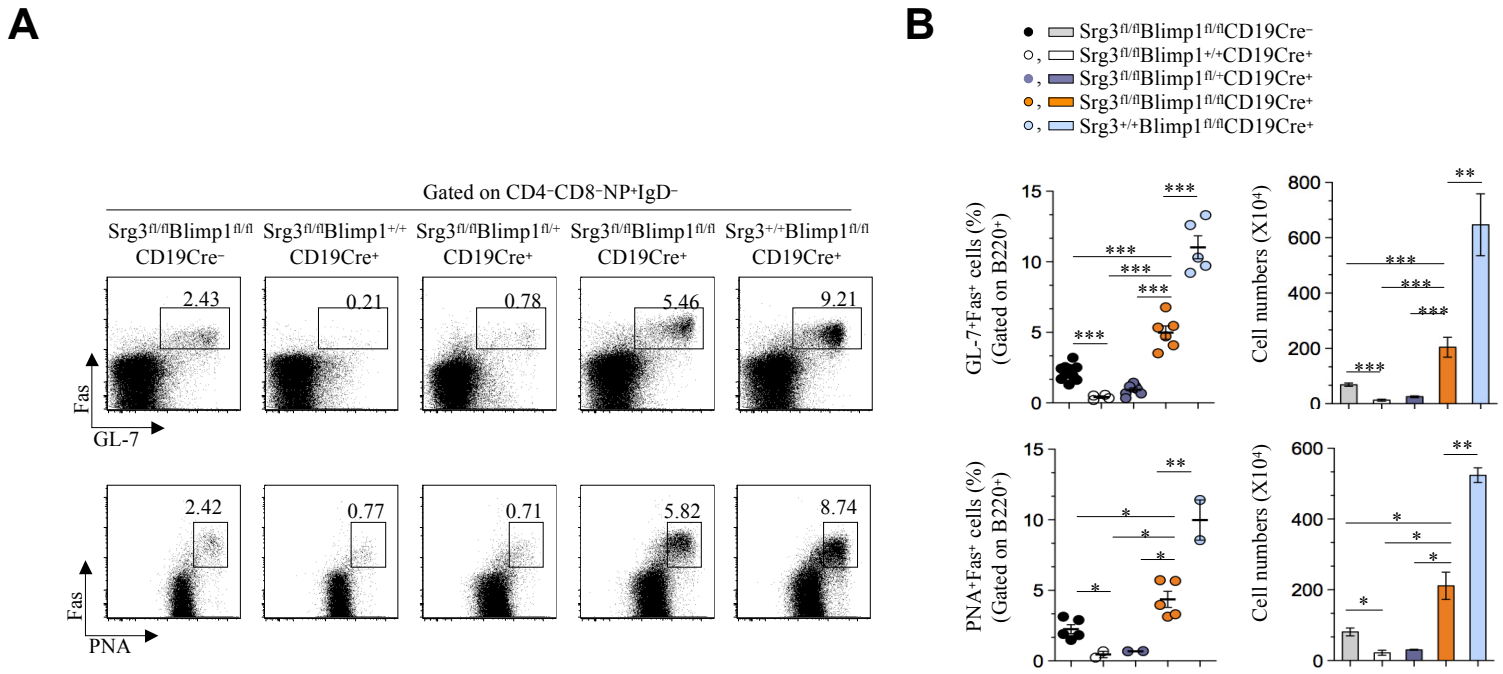

Figure S8. Impaired GC B cell generation is restored by Blimp-1 deletion in Srg3$^{fl/fl}$CD19Cre^{+} mice. (A) Flow cytometry of GC B cells obtained from the spleen of NP-KLH-immunized mice on day7. Cells gated on B220^{+} were analyzed for the expression of Fas with GL-7 (upper panels) or PNA (lower panels). Numbers adjacent to outlined area indicate the percentage of populations. (B) Frequency and cell numbers of Fas^{+}GL-7^{+} (upper panels) or Fas^{+}PNA^{+} (lower panels) GC B cells. Each individual dot represents one mouse; the lines show the mean percentage. Error bars represent a SEM; *P < 0.05, **P < 0.01, ***P < 0.001.

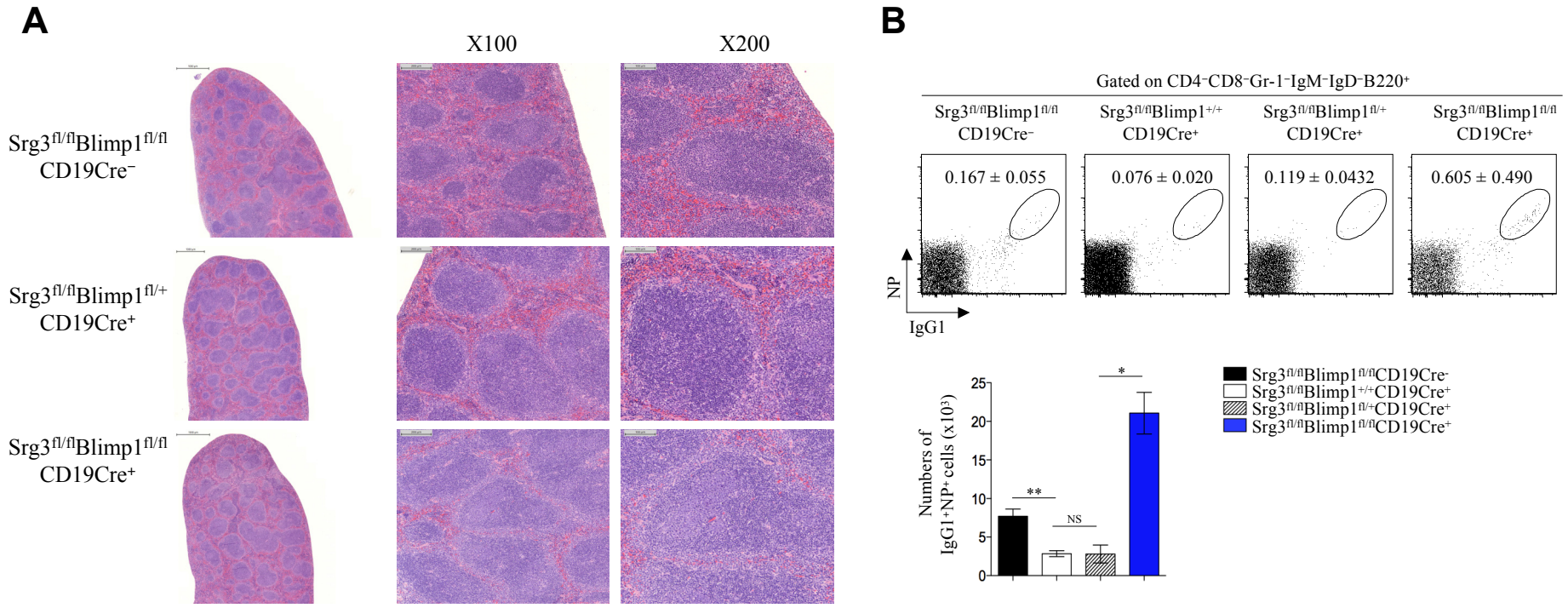

Figure S9. Impaired GC B cell generation is restored by Blimp-1 deletion in Srg3$^{fl/fl}$CD19Cre^{+} mice. (A) Paraffin-embedded splenic sections of Srg3$^{fl/fl}$ and Srg3$^{fl/fl}$CD19Cre^{+} mice were prepared 7 days after immunization with NP-KLH. These samples were stained with H&E and visualized by light microscopy to analyze the splenic architecture, that is the size and distribution of red and white pulp. Data are representative of two independent experiments. Original magnification is indicated in the figures.
(B) Flow cytometry of NP^{+}IgG1^{+} cells gated on CD4^{-}CD8^{-}IgM^{-}IgD^{-}Gr-1^{-}B220^{+} isotype-switched B cells. Numbers adjacent to outlined area indicate the average percentage of populations and SEM (n=3). Cell numbers (right panels) of NP^{+}IgG1^{+} B cells. *P < 0.05, **P < 0.01, NS, not significant.

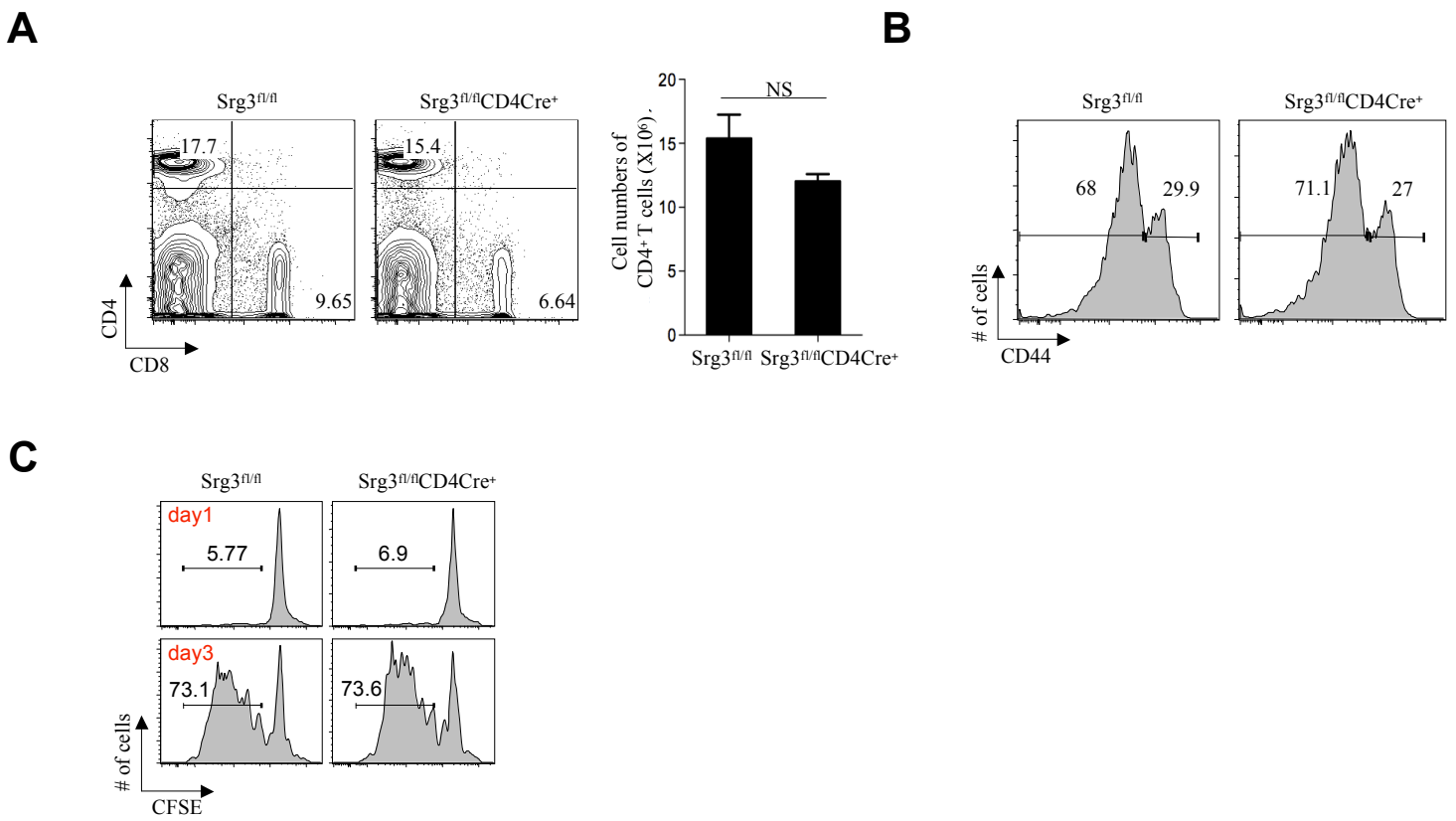

Figure S10. T cell development in the periphery is normal in $Srg3^{fl/fl}CD14Cre^{+}$ mice. (A) Flow cytometry of splenic $CD4^{+}$ and $CD8^{+}$ T cells in $Srg3^{fl/fl}$ and $Srg3^{fl/fl}CD4Cre^{+}$ mice. Numbers in quadrants indicate frequency of cells. Cell numbers of $CD4^{+}$ T cells were calculated (right panels, n=4). NS, not significant. (B) Surface expression of activation marker, CD44, on splenic $CD4^{+}$ T cells. This is representative of at least 3 independent experiments. (C) Proliferation of CFSE-labeled $CD4^{+}$ T cells of $Srg3^{fl/fl}$ and $Srg3^{fl/fl}CD4Cre^{+}$ mice in response to stimulation with anti-CD3 (10μg/ml) and anti-CD28 (2μg/ml) in the presence of IL-2 (20U/ml) at day1 (upper panels) and day3 (lower panels).

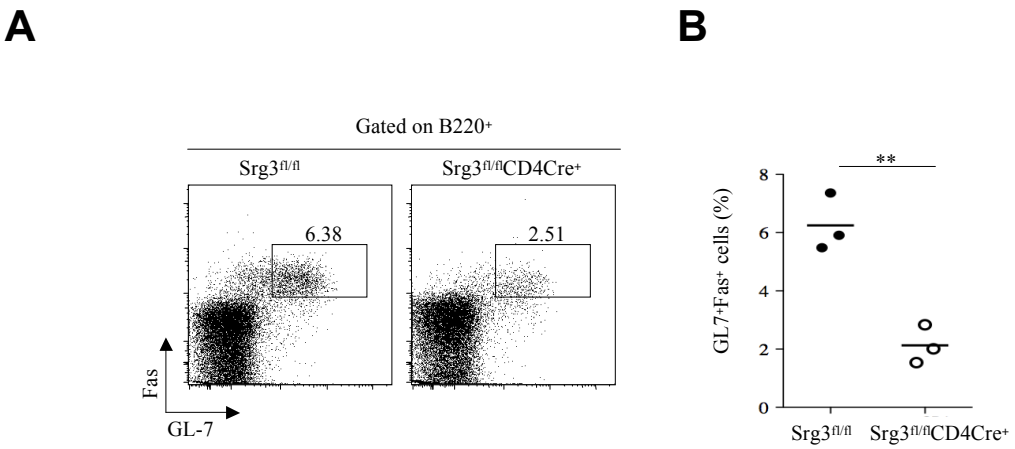

Figure S11. The development of GC B cells in Peyer's patch is impaired in $Srg3^{fl/fl}CD4Cre^{+}$ mice. (A) Flow cytometry of GC B cells in Peyer's patch from unimmunized $Srg3^{fl/fl}$ and $Srg3^{fl/fl}CD4Cre^{+}$ mice. Cells gated on $B220^{+}$ were analyzed for the expression of surface molecules, Fas and GL-7. (B) Frequency of $Fas^{+}GL\text{-}7^{+}$ GC B cells in Peyer's patch. Each individual dot represents one mouse; the lines show the mean frequency. $^{**}P < 0.005$.

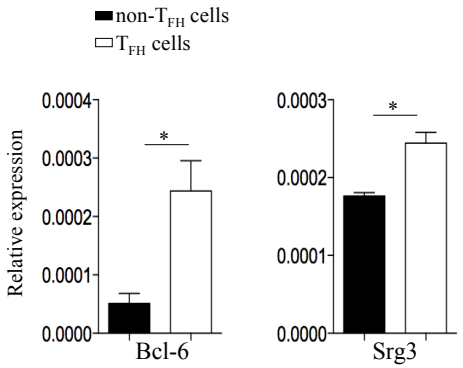

Figure S12. The expression of Srg3 in non-T_{FH} and T_{FH} cells was detected by quantitative RT-PCR analysis (n=3). C57BL/6 WT mice were immunized with NP-KLH and cell subsets– non-T_{FH} cells ($CD4^+CD19^-PD\text{-}1^-CXCR5^-$) and T_{FH} cells ($CD4^+CD19^-PD\text{-}1^{hi}CXCR5^{hi}$)– were purified at day 7 after immunization. *$P < 0.05$.

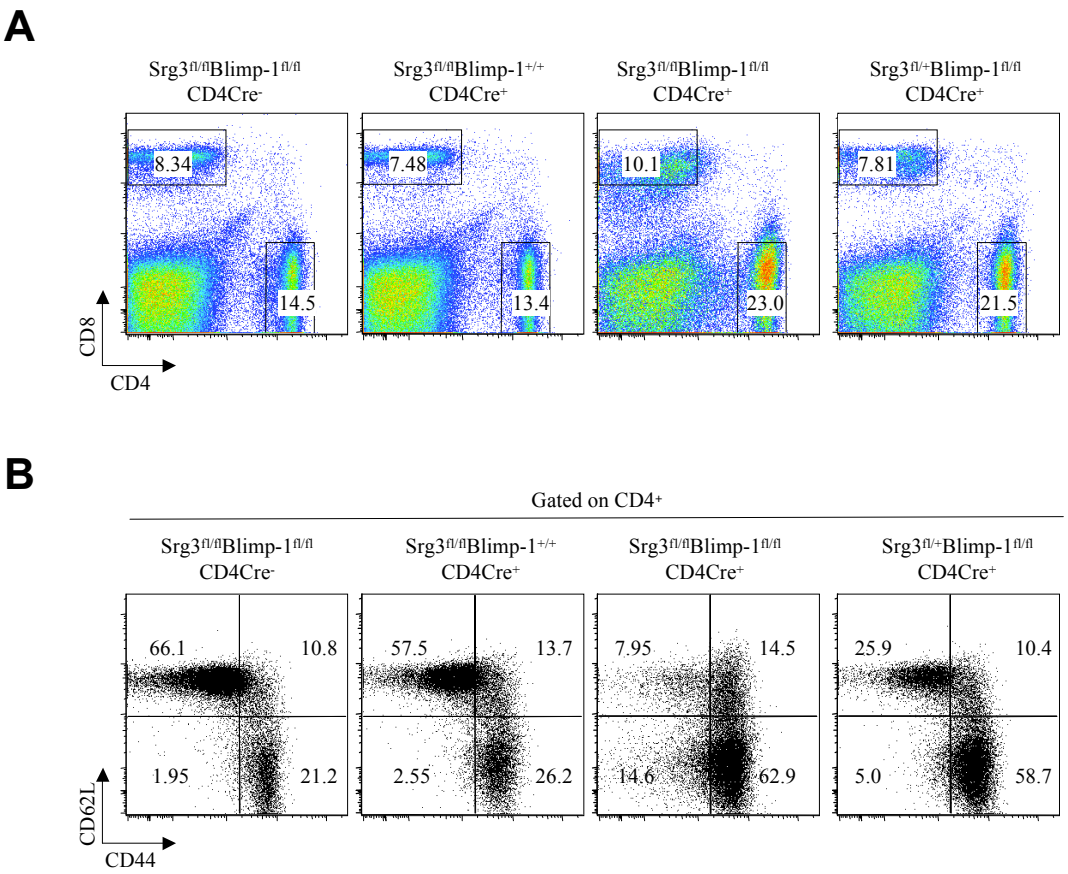

Figure S13. Hyperactivation of peripheral CD4⁺ T cells in Srg3$^{fl/fl}$Blimp-1$^{fl/fl}$CD4Cre^{+} mice. (A) Flow cytometry of peripheral CD4⁺ and CD8⁺ T cells in the spleen of indicated mice. (B) Surface expression of activation markers of CD62L and CD44 on splenic CD4⁺ T cells. Data are representative of two independent experiments. Data are representative of at least three mice.

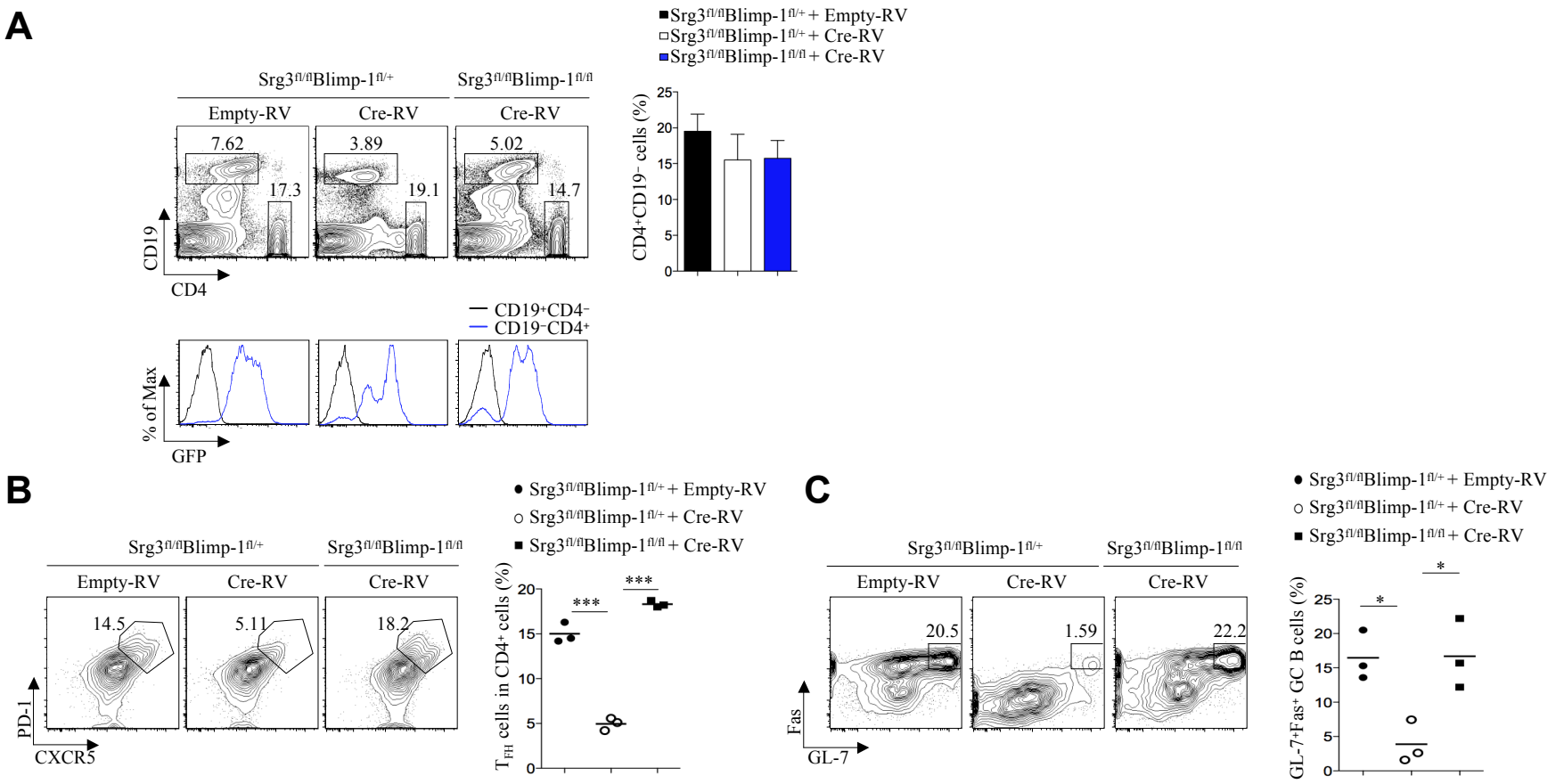

Figure S14. The deletion of Blimp-1 rescues the perturbed differentiations of T_{FH} and GC B cells in Srg3-deficient CD4$^+$ T cells. CD4$^+$ T cells isolated from Srg3$^{fl/fl}$Blimp-1$^{fl/+}$ or Srg3$^{fl/fl}$Blimp-1$^{fl/fl}$ mice were transduced with Empty-RV or Cre-RV and were transferred into *Rag2*$^{-/-}$ mice with WT B cells subsequently immunized with NP-KLH. (A) Expansion of the transduced CD4$^+$ T cells after immunization was analyzed with flow cytometry (upper left). Transduction efficiency of CD4$^+$ T cells was confirmed by GFP expression (lower left). Bar graphs show frequency of CD4$^+$ T cells in the spleen (upper right). (B) The differentiation of T_{FH} cells was analyzed by flow cytometry at day 7 after immunization (left) and the frequency of T_{FH} cells in CD4$^+$ T cells was calculated (right). Each individual dot represents one mouse. ***$P < 0.001$. (C) Flow cytometry of GC B cells gated on CD4$^-$CD19$^+$ in mice described in (B) (left). Frequency of GC B cells in CD4$^-$B220$^+$ B cells was statistically analyzed (right). Each individual dot represents one mouse. *$P < 0.05$.

Table S1. Primers for ChIP analysis and restriction endonuclease accessibility assay

Blimp-1 locus	Forward primer	Reverse primer
-1.8kb	5’-ACTGCTGTGCAGTGGAGACA-3’	5’-TTCTCAGAAAGGTCCAAGTGA-3’
-1.4kb	5’-GAACAAGCCTGTCTTTATGATTGA-3’	5’-ATCCAGATCCACACCCACAT-3’
-0.8kb	5’-TGTTTTGTAATGAGAAGCAGGAA-3’	5’-ATTAGTGGGGCTCGTTCTGA -3’
-0.2kb	5’-GCGGCTGGTAGGAGTGAAT-3’	5’-ACTGGAGGGCCGAGTGTC-3’
BRE	5’-TGCTTTCTCGGTTTCAGTTG-3’	5’-GAGTGAGCTGCTTTGGAAGG-3’
exon7	5’-CACCTGAGAGTGCACAGTGG-3’	5’-GGCACTCATGTGGCTTCTCT -3’

GAPDH intron	Forward primer	Reverse primer
	5’-TTACTTTCGCGCCCTGAG-3’	5’-GCGGTTCATTCATTTCCTTC -3’

Table S2. Primers for real-time quantitative RT-PCR

Genes	Forward primer	Reverse primer
Brg1	5’-CATCGCGCTCATCACATACC-3’	5’-CAAATTCATACGCCCAGTTT-3’
Srg3	5’-AGCTGTATTGCTGGCACAGG-3’	5’-CTGGCTTGACATCCTCACTG-3’
Icosl	5’-TGACCCACCTCCTTTTCAAG-3’	5’-TTAGGGTCATGCACACTGGA-3’
CD40	5’-GTGAGGAGATGAGAAGGCAA -3’	5’-CACTGTAGGACGGATGCTGC-3’
Slam	5’-AAAAGTGTCCGCATCCTCGTC-3’	5’-ATTGAAAGTGGTAGCCATCCTCC-3’
Irf4	5’-GCTGCATATCTGCCTGTATTACCG-3’	5’-GTGGTAACGTGTTCAGGTAACTCGTAG-3’
PDL1	5’-ACAAGCGAATCACGCTGAAAG-3’	5’-GGCCTGACATATTAGTTCATGCT-3’
Ox40L	5’-AGGACCCTCCAATCCAAAGAC-3’	5’-GAGCTGATGAATAGTTGCCCAT-3’
Blimp-1	5’-TCTGTTCAAGCCGAGGCATCC-3’	5’-TCTTGGGAACTGTGTCATTAG-3’
XBP-1	5’-TCCGCAGCACTCAGACTATG-3’	5’-ACAGGGTCCAACTTGTCCAG-3’
Bcl-6	5’-TAGAGCCCATAAGACAGTGCT-3’	5’-CACCGCCATGATATTGCCTTC-3’
Pax5	5’-GGGCTCCTCATACTCCATCA-3’	5’-TGCTGTGAACAGGTCTCC-3’
Bach2	5’-CGCTGTCGAAAGAGGAAGCTG-3’	5’-CCTGGATCTGCTCTGGACTCTG-3’
Icos	5’-TGACCCACCTCCTTTTCAAG-3’	5’-TTAGGGTCATGCACACTGGA-3’
c-Maf	5’-GTGCAGCAGAGACACGTCCT-3’	5’-CAACTAGCAAGCCCACTC-3’
actb	5’-GTGGCATCCATGAAACTACA -3’	5’-CTCATCGTACTCCTGCTTGC-3’
gapdh	5’-CATGGCCTTCCGTGTTCCTA-3’	5’-CCTGCTTCACCACCTTCTTGAT-3’
Btla	5’-TTCTGACTTGGCAGGAAGG-3’	5’-ATGGCAGTGCTTGGTGATTT-3’
CXCR5	5’-CGACATCAGACAGTGACCAGCC-3’	5’-GTCCTGTAGGGGAATCTCCGTG-3’
PD-1	5’-GCTCACTTCAGGTTTACCACAAGC-3’	5’-GCCCAACAGTAGGATTCAGGAGAC-3’
IL-21	5’-GCCAAACTCAAGCCATCAAACC-3’	5’-TTCTCATACGAATCACAGGAAGGG-3’