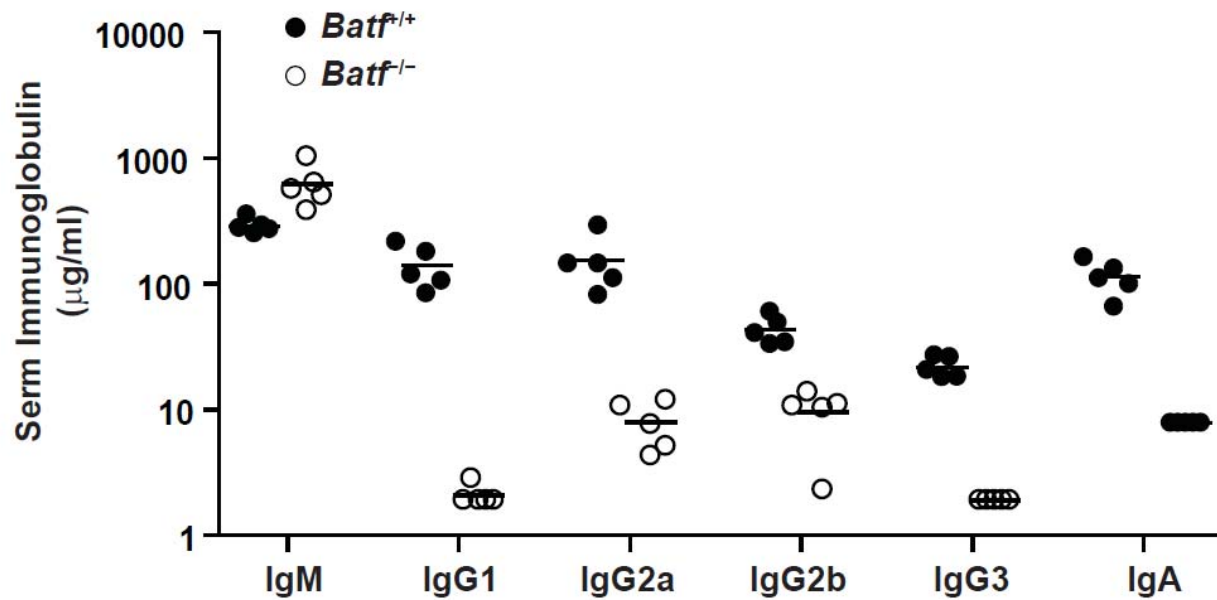


Supplementary Text and Figures

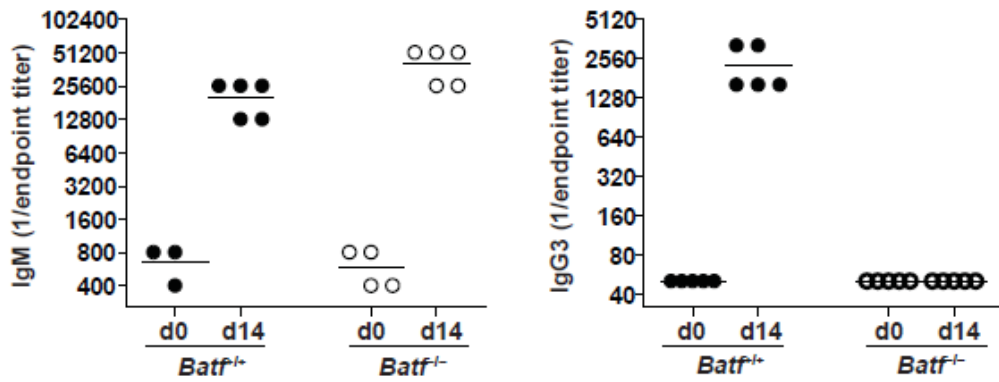
Supplementary Figure 1



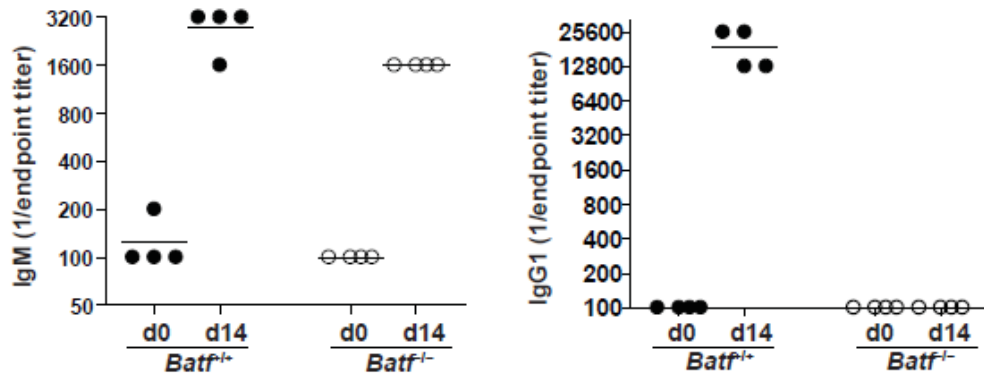
Supplementary Fig. 1. *Batf*^{-/-} mice have altered basal immunoglobulin titers. ELISA of basal serum immunoglobulin titers in unimmunized *Batf*^{+/+} or *Batf*^{-/-} mice.

Supplementary Figure 2

a



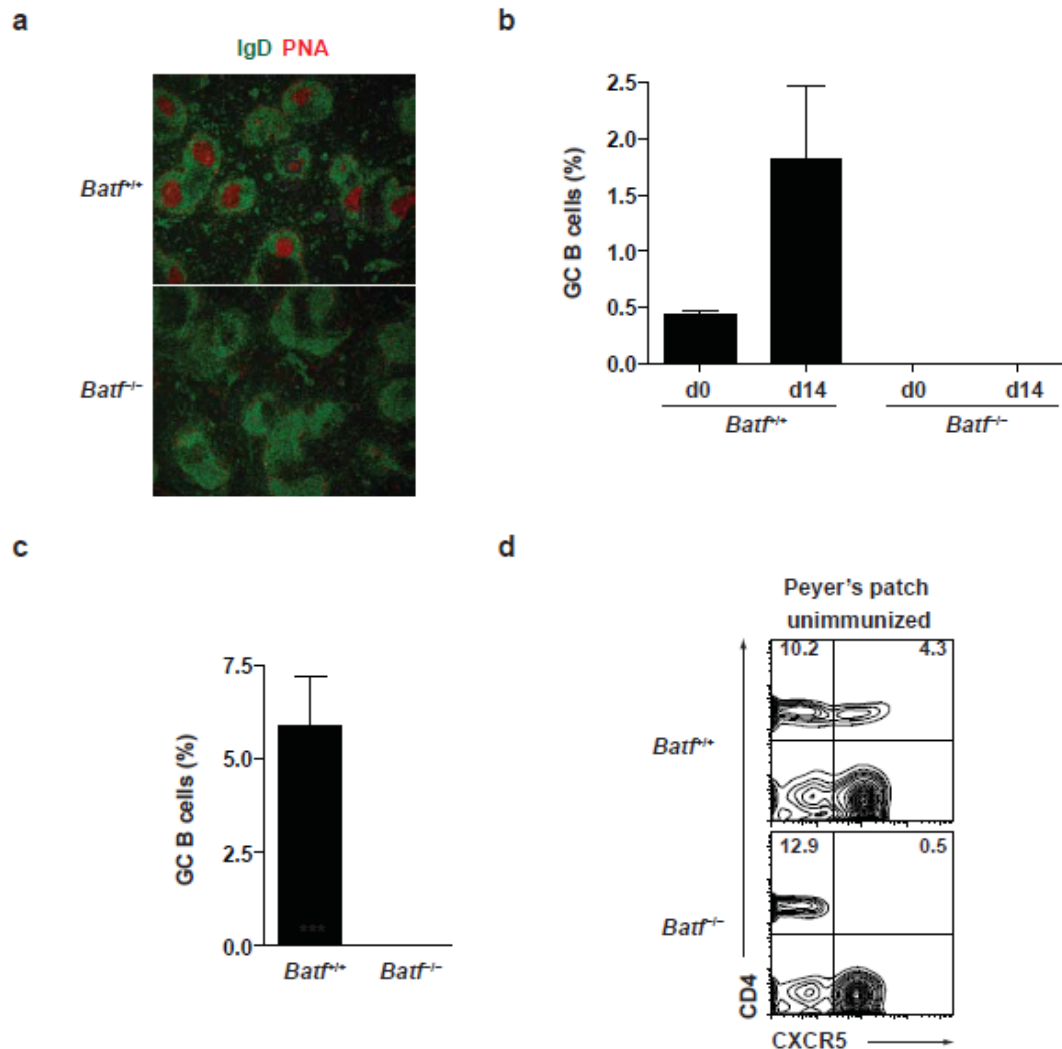
b



Supplementary Fig. 2. *Batf*^{-/-} mice have deficient antibody responses upon immunization. (a) TNP-specific antibody responses in *Batf*^{-/-} mice immunized with TNP-Ficoll. TNP-specific IgM and IgG3 titers in serum obtained before (day 0) and on day 14 after immunization were measured by ELISA against plate-bound TNP-conjugated BSA. The TNP-specific titer is defined as the greatest dilution at which average optical density at 405 nm exceeds that of background by 1.5-fold. Each symbol represents an individual mouse; small horizontal bars indicate mean values. Data are representative of two experiments. (b) NP-specific antibody responses of *Batf*^{-/-} mice immunized with NP-conjugated chicken γ -globulin in alum. NP-specific IgM and IgG1 titers in serum obtained before (day 0) and on

day 14 after immunization were measured by ELISA against plate-bound NP-conjugated BSA. Data are representative of two experiments.

Supplementary Figure 3

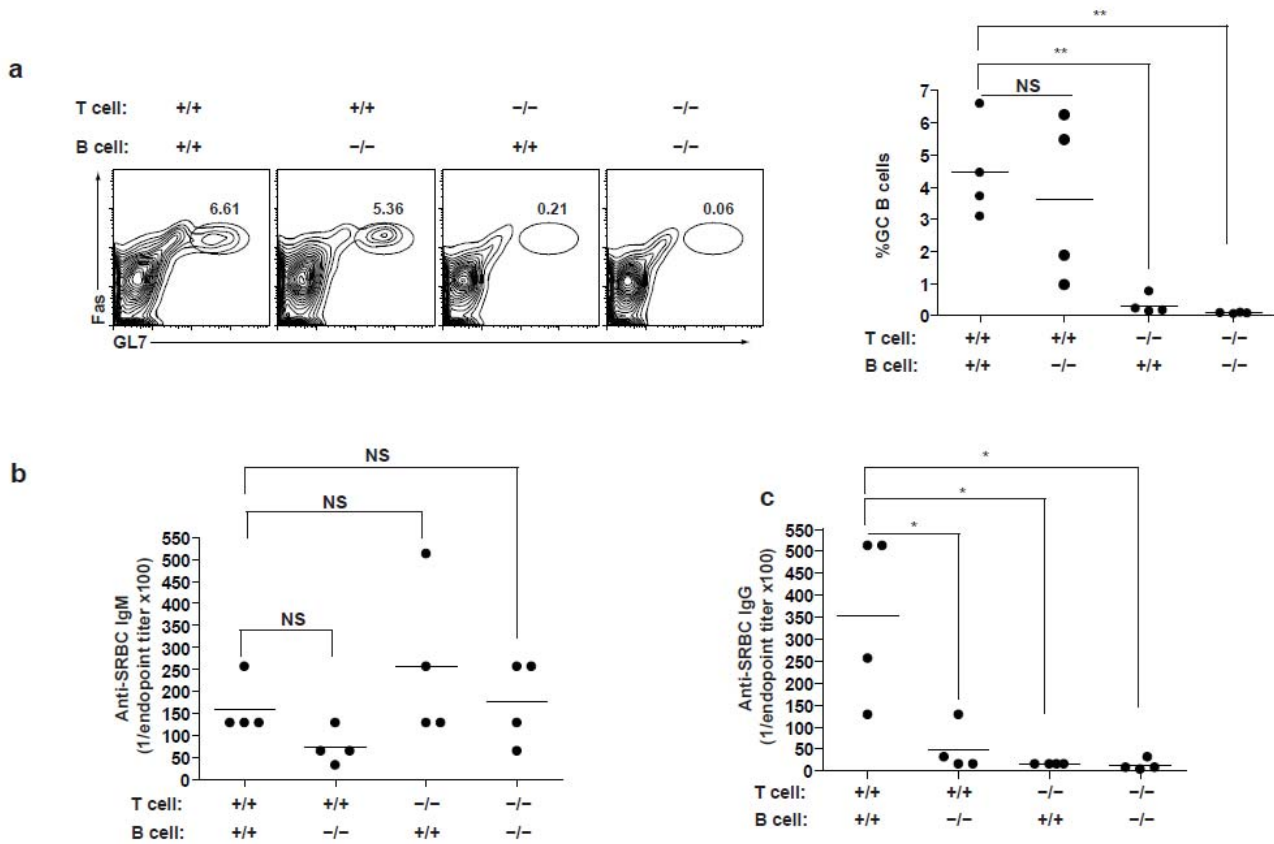


Supplementary Fig. 3. *Batf*^{-/-} mice have deficient germinal center B cell development. **(a)** Staining of germinal center in spleen sections prepared 10 d after immunization with sheep red blood cells (SRBC). The sections were stained with anti-IgD and peanut agglutinin (PNA). Original magnification, x100. Data are representative of two experiments. **(b)** Frequency of B220⁺Fas⁺GL7⁺ germinal center B cells in spleen before and 14 days after SRBC-immunization. Data are from 3 mice per group and are representative of two experiments. **(c)** Frequency of B220⁺Fas⁺GL7⁺ germinal center B cells in Peyer's patches from unimmunized mice. Data are from 3 mice per group and are representative of two

experiments. **(d)** FACS of CD4 and CXCR5 expression in Peyer's patches from unimmunized mice.

Data are representative of three experiments.

Supplementary Figure 4

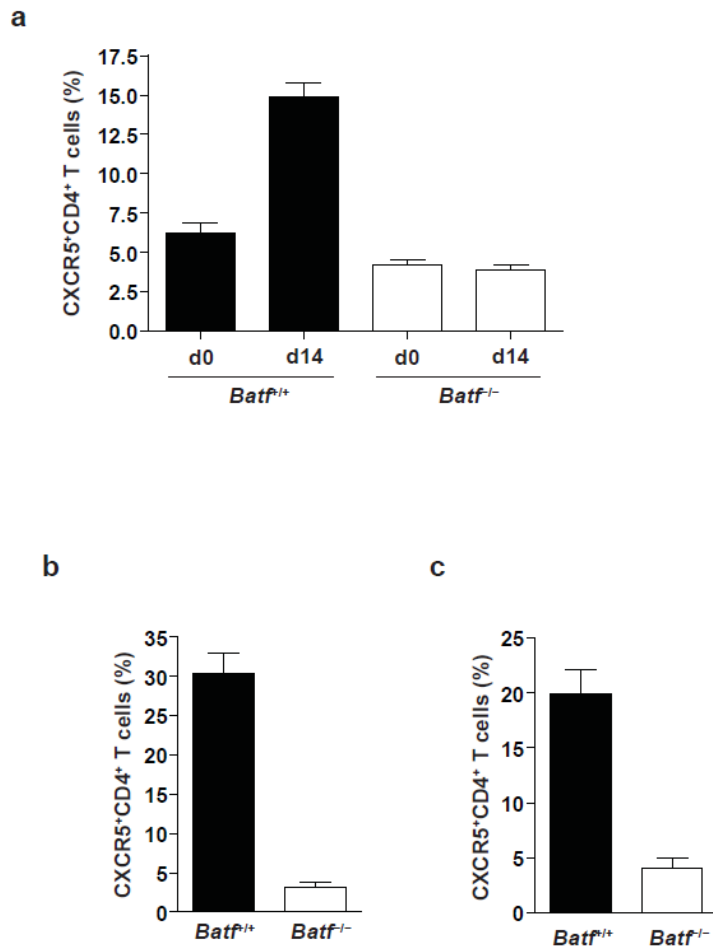


Supplementary Fig. 4. Batf controls both B- and T-cell function required for antibody responses.

CD4⁺ T cells and B-cells were purified from *Batf*^{+/+} or *Batf*^{-/-} mice, mixed, and transferred into *Rag2*^{-/-} mice as indicated. **(a)** Development of germinal center B cells in T- and B-cell reconstituted *Rag2*^{-/-} mice 10 days after immunization with SRBC. Left panel; the cells were stained with anti-B220, anti-GL7, and anti-Fas. The data are gated on B220⁺ cells. Right panel; The frequency of B220⁺Fas⁺GL7⁺ cells from 4 mice per group. ***P*<0.005 (unpaired student *t*-test). NS; not significant. **(b and c)** SRBC-specific IgM **(b)** and IgG1 **(c)** antibody responses in T-B-cell-reconstituted *Rag2*^{-/-} mice. The serum was collected 14 days after immunization with SRBC and anti-SRBC titer was determined by ELISA

as in Supplementary Figure 2. * $P < 0.05$ (unpaired student t -test). NS; not significant. Data are representative of two experiments.

Supplementary Figure 5



Supplementary Fig. 5. Development of follicular helper T cells is defective in *Batf*^{-/-} mice. **(a)**

Frequency of CD4⁺CXCR5⁺ T cells in spleen before or 14 days after immunization with SRBC. Data are from 3 mice per group and are representative of two experiments. **(b)** Frequency of CD4⁺CXCR5⁺ T cells in peyer's patches from unimmunized mice. Data are from 3 mice per group and are representative of two experiments. **(c)** Frequency of CD4⁺CXCR5⁺ T cells in CD45.2 cells in spleens of SJL mice. SJL mice were transferred with CD4⁺CD62L⁺ CD45.2⁺ cells from *Batf*^{+/+} or *Batf*^{-/-} mice

and immunized with SRBC 7 days before. Data are from 3 mice per group and representative of two experiments.

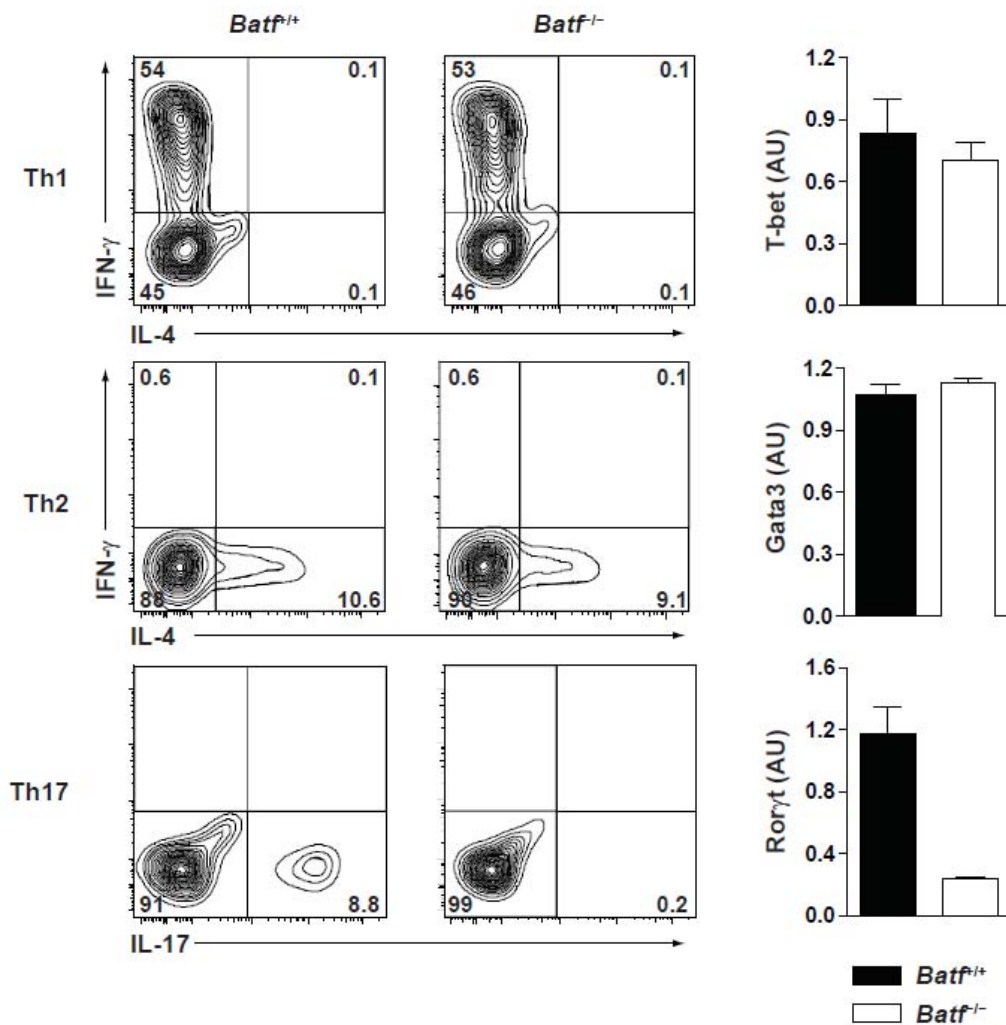
Supplementary Figure 6



Supplementary Fig. 6. Gene expression microarray analysis of activated *Batf*^{+/+} or *Batf*^{-/-} CD4⁺ T cells. Naïve CD4⁺ T cells were purified from *Batf*^{+/+} or *Batf*^{-/-} mice and cultured with anti-CD3/CD28 in the presence of IL-6, anti-IL-4, anti-IFN- γ , and anti-TGF- β . On day 1 or day3, cells were harvested,

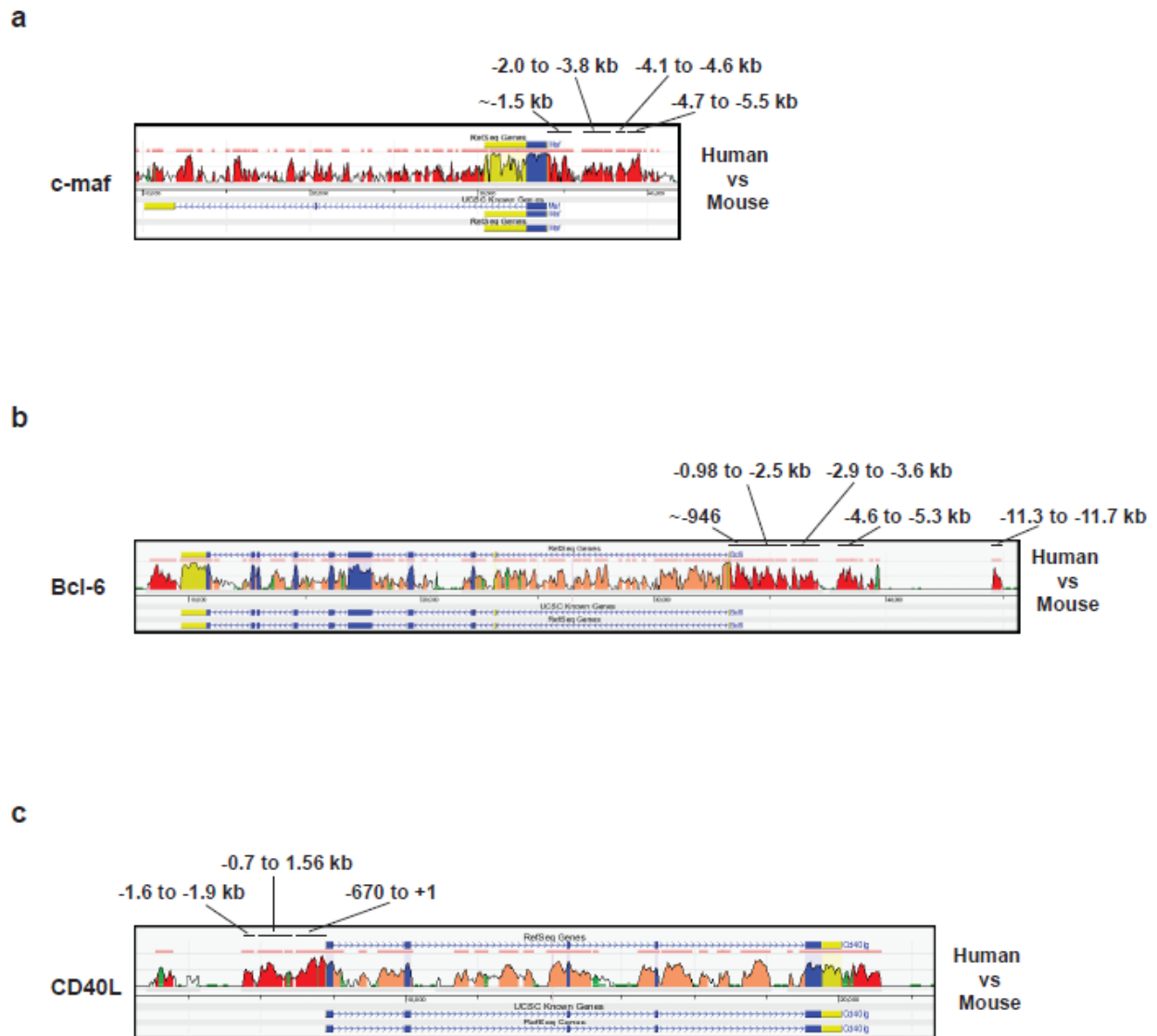
and RNA was prepared and subjected to gene expression microarray analysis. A representative heat map of genes differentially expressed at least 5-fold in *Batf*^{+/+} or *Batf*^{-/-} T cells is presented.

Supplementary Figure 7



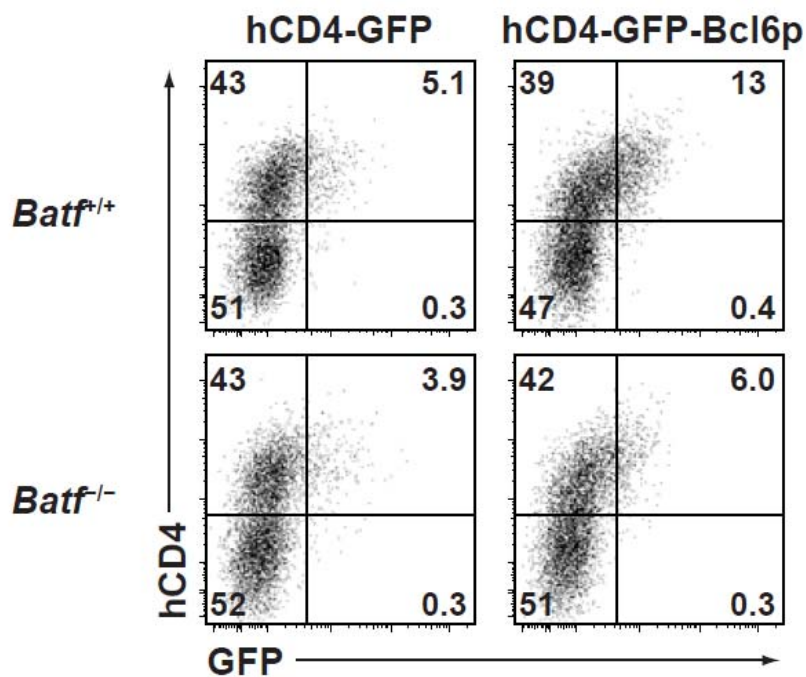
Supplementary Fig. 7. Th2 response is normal in *Batf*^{-/-} CD4⁺ T cells. Naïve CD4⁺ T cells were purified from *Batf*^{+/+} or *Batf*^{-/-} mice and stimulated with anti-CD3/CD28 in the presence of IL-12, IFN- γ and anti-IL-4 (Th1), IL-4, anti-IFN- γ (Th2), or IL-6, TGF- β , anti-IL-4, anti-IFN- γ (Th17). At day 5, cells were harvested and re-stimulated with PMA and ionomycin for 4 hours. Expression of intracellular IL-4, IFN- γ , and IL-17 was examined by FACS. Expression of T-bet, GATA-3, ROR γ t mRNA was measured by q-PCR.

Supplementary Figure 8



Supplementary Fig. 8. Conserved non-coding regions in Bcl-6 and c-Maf. Shown are vista alignment plots displaying the sequence similarity between human and mouse c-Maf (**a**), Bcl-6 (**b**), and CD40L (**c**) loci. The sequences within these indicated conserved regions were analyzed for identification of potential Batf binding sites.

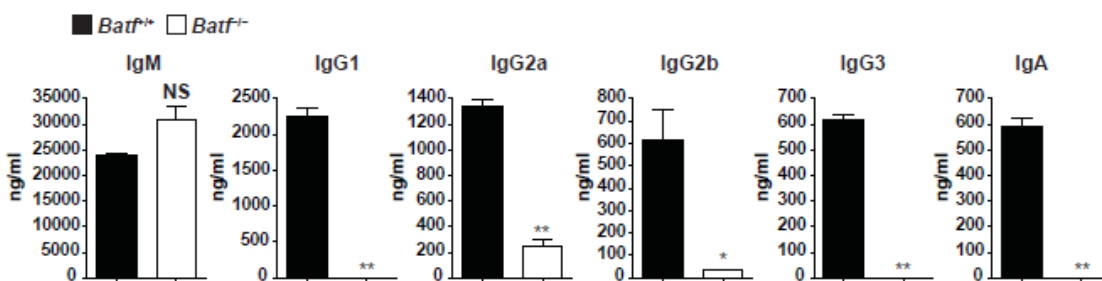
Supplementary Figure 9



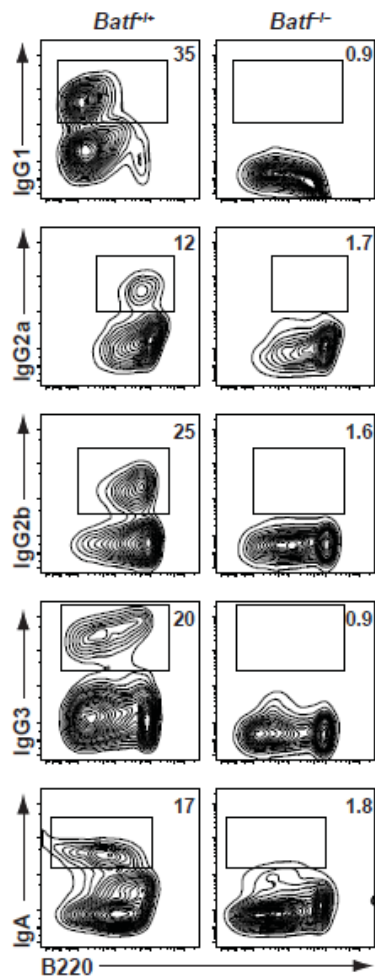
Supplementary Fig. 9. Bcl-6 promoter activity in activated CD4⁺ T cells. CD4⁺ T cells were cultured with anti-CD3 and anti-CD28 in the presence of IL-6, anti-IL-4, anti-IFN- γ , and anti-TGF- β and infected with hCD4-pA GFP-RV (left) or hCD4-pA GFP-Bcl-6-promoter-RV (right). hCD4 and GFP expression was examined on day 3 after stimulation. Data are from two independent experiments.

Supplementary Figure 10

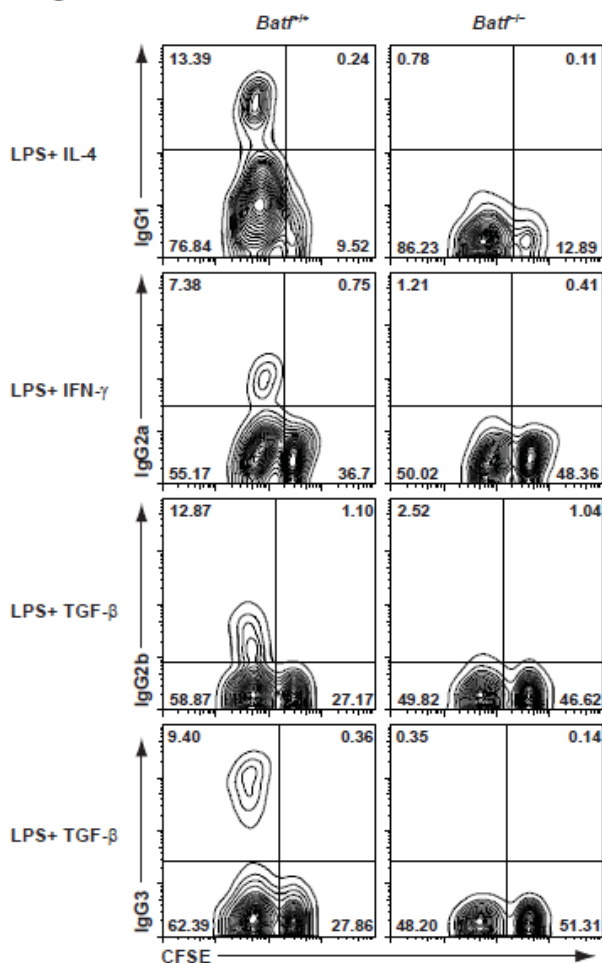
a



b

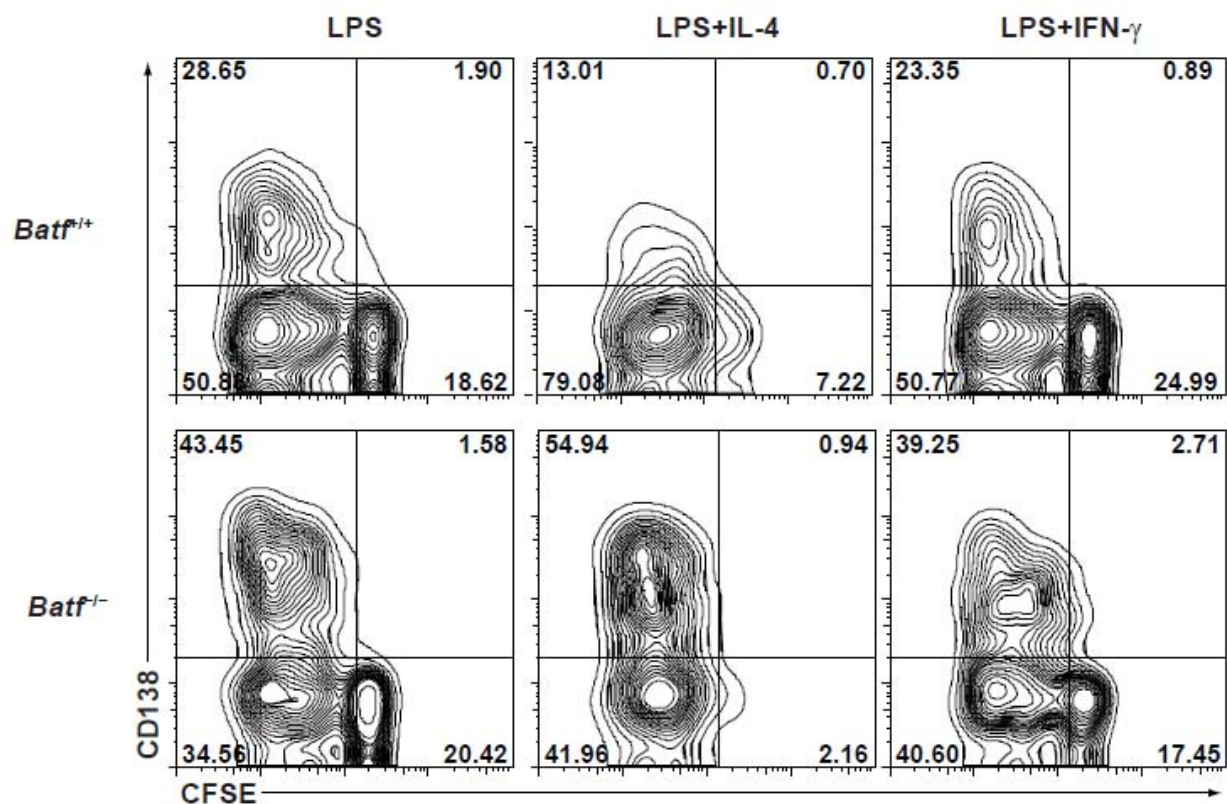


c



Supplementary Fig. 10. *Batf*^{-/-} B cells proliferate normally but do not express switched Immunoglobulin. **(a)** Immunoglobulin titers in supernatants of cultures of *Batf*^{+/+} or *Batf*^{-/-} B-cells stimulated for 7 days with LPS alone (IgM and IgG3), LPS plus IL-4 (IgG1), LPS plus IFN- γ (IgG2a), or LPS plus TGF- β (IgG2b and IgA). Data are representative of three experiments with three pairs of *Batf*^{+/+} or *Batf*^{-/-} B-cells. **P*<0.01 and ***P*<0.0001 (unpaired student *t*-test). NS; not significant. Data are from three independent experiments (mean and s.e.m.). **(b)** FACS of immunoglobulin expression by *Batf*^{+/+} or *Batf*^{-/-} B-cells stimulated for 4 days with LPS alone (IgG3), LPS plus IL-4 (IgG1), LPS plus IFN- γ (IgG2a), LPS plus TGF- β and BAFF (IgG2b), or LPS plus IL-5, TGF- β , APRIL, and Retinoic acid (IgA). Data are representative of three experiments. **(c)** Splenic B cells from *Batf*^{+/+} or *Batf*^{-/-} mice were labeled with CFSE and cultured for 4 days with LPS alone (IgG3), LPS plus IL-4 (IgG1), LPS plus IFN- γ (IgG2a), or LPS plus TGF- β (Ig2b). CFSE dilution and surface immunoglobulin expression were examined by FACS. Data are representative of three experiments.

Supplementary Figure 11



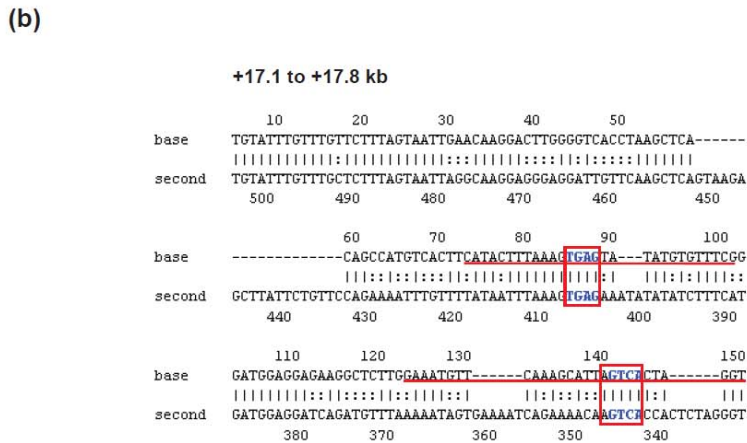
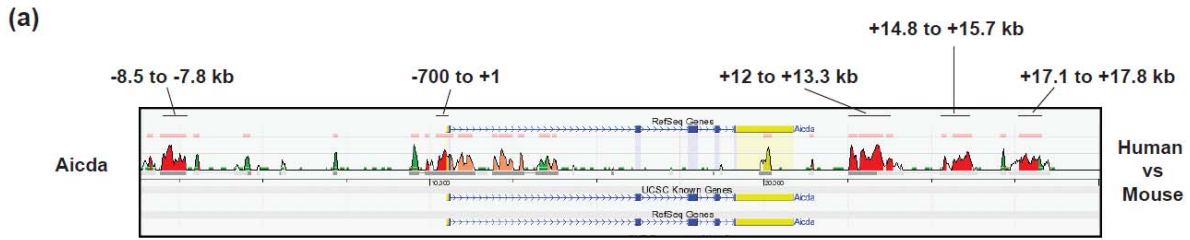
Supplementary Fig. 11. *Batf*^{-/-} B cells differentiate into CD138⁺ plasma cells. B-cells were purified from spleens of *Batf*^{+/+} or *Batf*^{-/-} mice, labeled with CFSE, and cultured for 4 days with LPS alone, LPS plus IL-4, or LPS plus IFN- γ . CFSE dilution and CD138 expression were examined by FACS. Data are representative of three experiments.

Supplementary Figure 12



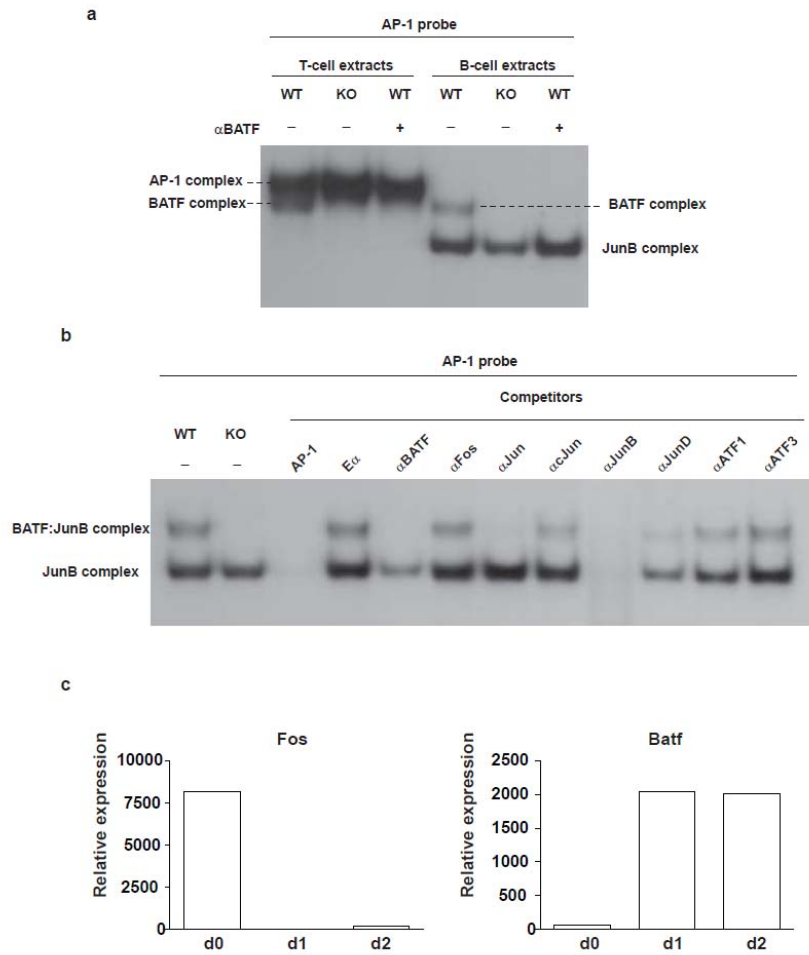
Supplementary Fig. 12. Gene expression microarray analysis of activated *Batf*^{+/+} or *Batf*^{-/-} B cells. B cells were purified from *Batf*^{+/+} or *Batf*^{-/-} mice and cultured with LPS. On day 1 or day2, cells were harvested and subjected to gene expression microarray analysis. A representative heat map of genes differentially expressed at least 5-fold in *Batf*^{+/+} or *Batf*^{-/-} B cells is presented.

Supplementary Figure13



Supplementary Fig. 13. Conserved non-coding regions in AID locus. **(a)** Vista alignment plot displaying the sequence similarity between human and mouse AID locus. The conserved regions that were used for identification of potential Batf binding sites are shown. **(b)** Sequence alignment of a region of +17.1 to +17.8 kb. The conserved TGAG/C sequences are shown in blue in red boxes and the probes that were used for this region are underlined with red lines.

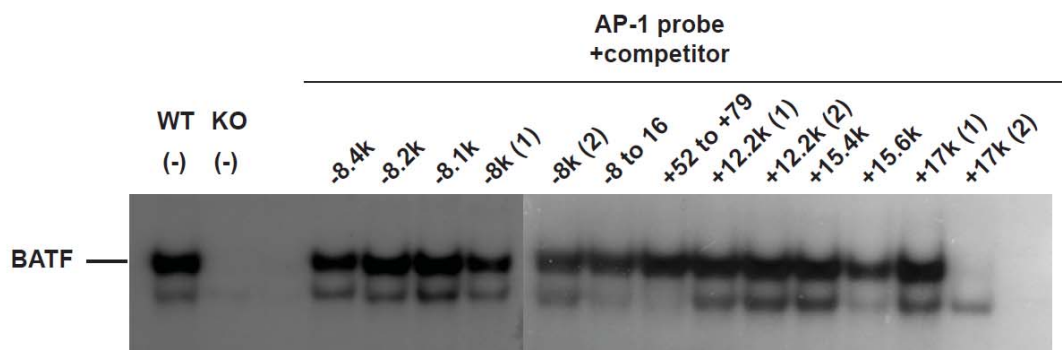
Supplementary Figure 14



Supplementary Fig. 14. EMSA assay for Batf binding in B cells. **(a)** Nuclear extracts were prepared from T cells that were activated with α CD3/ α CD28 for 1 day or B cells that were activated with LPS plus IL-4 for 1 day and were analyzed for DNA binding ability to a consensus AP-1 probe by EMSA. **(b)** B cell nuclear extracts were analyzed for DNA binding ability to a consensus AP-1 probe in the presence of AP-1 or E α oligos or the indicated antibodies. **(c)** Microarray data from B cells presented in Supplementary figure 12 of B cells activated with LPS and harvested on day 1 (D1) or day 2 (D2) after activation was normalized with microarray data of unstimulated B cells (D0) purified at the same

time. Shown are the normalized expression values exported from dCHIP analysis software for Fos and Batf on the indicated day.

Supplementary Figure 15



Supplementary Fig. 15. Identification of potential Batf binding sites AID locus. B cells purified from *Batf*^{+/+} or *Batf*^{-/-} mice were cultured with LPS plus IL-4 for 24 hours. Nuclear extracts were analyzed for DNA binding ability to a consensus AP-1 probe by EMSA. Annealed double-stranded oligonucleotides, between 29 and 32 bp in length, from the indicated regions of AID locus were used as competitors. Data are representative of two experiments.

Supplementary Table 1. Primers for real-time qRT-PCR and ChIP-qPCR

	Forward primer	Reverse primer
<u>Real-time qRT-PCR primers</u>		
c-maf	5'-AGCAGTTGGTGACCATGTGCG-3'	5'-TGGAGATCTCCTGCTTGAGG-3'
Bcl-6	5'-AGGCCTCCTTCCGCTACAAG-3'	5'-CAAATGTTACAGCGATAGGGTTTCT-3'
Blimp-1	5'-TCTGTTCAAGCCGAGGCATCC-3'	5'-TCTTGGGAAGTGTGTCATTAG-3'
CXCR5	5'-CGACATCAGACAGTGACCAGCC-3'	5'-GTCCTGTAGGGGAATCTCCGTG-3'
IL-21	5'-GCCAAACTCAAGCCATCAAACC-3'	5'-TTCTCATAACGAATCACAGGAAGGG-3'
IRF4	5'-GCCCAACAAGCTAGAAAAG-3'	5'-TCTCTGAGGGTCTGGAAACT-3'
AID	5'-TGCTACGTGGTGAAGAGGAG-3'	5'-TCCCAGTCTGAGATGTAGCG-3'
HPRT	5'-AGCCTAAGATGAGCGCAAGT-3'	5'-TTACTAGGCAGATGGCCACA-3'
Germline transcripts		
I μ -C μ	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5'-TCTGAACCTTCAAGGATGCTCTTG-3'
I γ 3-C γ 3	5'-AACTACTGCTACCACCACCACCAG-3'	5'-ACCAAGGGATAGACAGATGGGG-3'
I γ 1-C γ 1	5'-GGCCCTTCCAGATCTTTGAG-3'	5'-ATGGAGTTAGTTTGGGCAGCA-3'
I γ 2b-C γ 2b	5'-GATGGGGAGGAGTTGGCAGAT-3'	5'-CGGAGGAACCAGTTGTATC-3'
I γ 2a-C γ 2a	5'-GCTGATGTACCTACCGAGAGA-3'	5'-GCTGGGCCAGGTGCTCGAGGTT-3'
I α -C α	5'-CAAGAAGGAGAAGGTGATTGAG-3'	5'-GAGCTGGTGGGAGTGTGATCAGT-3'
Post switch transcripts		
I μ -C γ 3	5'-CTCGGTGGCTTTGAAGGAAC-3'	5'-ACCAAGGGATAGACAGATGGGG-3'
I μ -C γ 1	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5'-ATGGAGTTAGTTTGGGCAGCA-3'
I μ -C γ 2b	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5'-CGGAGGAACCAGTTGTATC-3'
I μ -C γ 2a	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5'-GCTGGGCCAGGTGCTCGAGGTT-3'
I μ -C α	5'-ACCTGGGAATGTATGGTTGTGGCTT-3'	5'-TAATCGTGAATCAGGCAG-3'
<u>ChIP-qPCR</u>		
I μ	5'-GCAGGTCCGCTGGACTAACT-3'	5'-CAACCTTGTTCCCTTAATTTTGCT-3'
I γ 3	5'-CTACTGCCTCTTCTCTTACCCTGAA-3'	5'-TCCTTGGGTGTAGAGACACTGTCA-3'
I γ 1	5'-CAGGTGCACAGAGAAACAGGAGTA-3'	5'-GTGAGGCGGTACCCTTGAGA-3'
I γ 2b	5'-TGGGGCAAGGACTGTGAATC-3'	5'-GGCTCTCTCCAGTCTCTCAGCT-3'
I γ 2a	5'-GATCCCTGCCAGCTTCTCT-3'	5'-TGCTCCGGCTTCTGACTGT-3'
I α	5'-ACACACCTGTCTCCACCACA-3'	5'-TGGCTGGAAGCTGTTTTTCT-3'
HS3A	5'-CACACACTGGGCTGCAACTG-3'	5'-TGTCTCCATTCTGTGCCTCTGA-3'
HS1,2	5'-CCAGTTATCACCCTGCTGGAA-3'	5'-GTGTTGAGCCACCCATCCTT-3'
HS3B	5'-CCTTTCTGATGAAGCCCTGTCT-3'	5'-CAGCTATTCTGGCAGAGGACTAGA-3'
HS4	5'-GCCAGGGCCTTCTAACCAA-3'	5'-GGTTCAGCTCATGGCTTCTCA-3'

Supplementary Table 2. Oligos for EMSA

	Top (sequence 5' to 3')	Bottom (sequence 5' to 3')
c-maf		
-47 to -70	GGGCAAGCTGGCTCACCCGCTGGCCA	GGGTGGCCAGCGGGTGAGCCAGCTTG
-951 to -980	GGCACGGGTGCTATAAACTCAGCAAT	GGAAATGCTGAGTTTTATAGCACCCGT
-1.0 k	GGCGGAGAGTGCCAGTCAATTTCCCATT	GGAAATGGGAAATTGACTGGCACTCTCC
-1.1 k (1)	GGGCTGTGATTGACAGCTCCGAAAAGTTT	AGGAAACTTTTCGGAGCTGTCAATCACAG
-1.1 k (2)	GGTGTCCCAGCTGACAGTCAGCTGATTGG	GGCCAATCAGCTGACTGTCAGCTGGGAC
-2.7 k	GGTGGCGCTGTTGAGGTAAAAACCAA	GAGTTGGTTTTTACCTCAACAGCGCC
-2.9 k	GGACAGGCACAGGTCTCTCAGCCAGAA	GGTATTCTGGCTGAGAGACCTGTGCCTG
-3.1 k	GGCGAAGTCAGGATTTTCTGAGAATTA	AGGTAATTCTCAGAAAATCCTGACTTC
-3.2 k	GGCTTAATATATGACTTAAGTGTCGA	GGTTCGGACACTTAAGTCATATATTA
-3.5 k	GGTCACCTCAGTATTGACATAGTCTT	GTGAAGACTATGTCAATACTGAGGTG
-4.3 k	GGTGCTAAATTAATAATGAGTGCTTGCA	GGTTGCAAGCACTCATTTTAATTTAGC
-4.5 k	GGGTTGAACTGGTCAATAATGACAAGCG	GTGCGCTTGTCAATTATTGACCAGTTCAA
-5.4 k	GGCAGAAATATATTCTCAGGCATAATG	GTGCATTATGCCTGAGAATATATTTCT
-5.5 k	GAGTAATTCATGAAGTGACTATTTATG	GAGCATAAATAGTCACTTCATGAATTA
Bcl-6		
-339 to -368	GGCAAATTAGTTCTCAGAATTCAGAG	GGCCTCTGGAATTCTGAGAACTAATTT
-300 to -331	GGTACAGCGAAGACGCCGACGTCACGGAG	GGGCTCCGTGACGTCGGCGTCTTCGCTGT
-276 to -304	GGCCCACGTGACGGCGGCGGAGCGGG	GGGCCCGCTCCGCCGCCGTCACGTGG
-2.3 kb	GGATCAATTTGACAAAGTCACCGTGT	GGACACGGTGACTTTGTCAAATTGA
-2.4 kb	GGAATACTCTAAAATGAGAGGGAAGGGG	GGTCCCCTTCCCTCTCATTTTAGAGTAT
-1.7 kb (1)	GAGTCTCACTCATCCAGGGATGAGTAGGAG	GGCCTCCTACTCATCCCTGGATGAGTGAGA
-1.2 kb	GGAATTTCTGAGAGTCAACAGTAACAGA	GGCTCTGTTACTGTTGACTCTCAGGAAAT
-1.8 kb	GGCCAGAACAGCCTGACCTTTGAAAGA	GGCTCTTTCAAAGGTCAGGCTGTTCTG
-1.7 kb (2)	GGATATTATTTAGCTCACACCCTTCTTG	GGGCAAGAAGGGTGTGAGCTAAATAATA
-2.9 kb	GGGTTTGAGGTATGAGAAGGGTAAATT	GGGAATTTACCCTTCTCATACCTCAA
-3.5 kb	GGAGTCCCCGATCCTGAGTATTAGCAA	TGGTTGCTAATACTCAGGATCGGGGAC
-4.9 kb	GGCACCTGAGTTTACAAATACTCAAGTCC	GGGAGGGACTTGAGTATTTGTAACTCAGG
-5.1 kb	GGTAGGCTTATGCAGAGTCAGGTGTACAG	GGTCTGTACACCTGACTCTGCATAAGCCT
-5.3 kb	GGGACGGTTTGATGACTTGAATTTGAG	GGCCTGAAATTCAAGTCATCAAACCGT
-5.3 kb (2)	GGCCTCTGCAGCCTGACCTCAGGGGA	GCGTCCCCTGAGGTCAGGCTGCAGAG
-11.7 kb	GGATTTTGATAACTCACTCATCACTTC	GGTGAAGTGATGAGTGAGTTATCAAAA
-11.6 kb	GGAAGAGTAAAGTTAGCTCAGAATAATTG	GAGCAATTATTCTGAGCTAACTTTACTCT

Supplementary Table 2 (cont.)

	Top (sequence 5' to 3')	Bottom (sequence 5' to 3')
<u>CD40L</u>		
-38 to -64	GGCCACTTTGACAGTCTTCTCATGCTG	AGGCAGCATGAGAAGACTGTCAAAGTG
-62 to -89	GCTCTTAACTAATCCTGAGTAAGGCGGC	GTGGCCGCCTTACTCAGGATTAGTTAAG
-367 to -392	GGTAGTGGACTCATTGTCACTTTCCTT	GGCAAGGAAAGTGACAAATGAGTCCACT
-507 to -532	GGATCAAAGTCCTGAGAAGCAATTAAGG	GGCCCTAATTGCTTCTCAGGACTTTGA
-541 to -565	GGTAGAAAGATTTTATTGAGCTTAGAG	GGTCTCTAAGCTCAATAAAAATCTTTCT
-564 to -588	GGCATGTAGTTTTGACTAGTAAAGCTA	GGCTAGCTTTACTAGTCAAAACTACAT
-1.0 kb	GGAATCATTTGGAAAACATTTTCATA	GGCTATGAAATGAGTTTTCCAAATGAT
-1.1 k	GCGAAAACAGTGTACAAACCTGAGGTG	GGCCACCTCAGGTTGTGACACTGTTTT
-1.4 k	GGAACCAATGCTTCTGACTTGACTGAT	GTGATCAGTCAAGTCAGAAGCATTGGT
<u>AID</u>		
-8.4 kb	GGGCCACAAAGTGAGAAAAACAAAAT	GCGATTTTTGTTTTTCTCACTTTGTGG
-8.2 kb	GTGACCCGAGGAGTCATGTCTGAGTGT	GGCACACTCAGACATGACTCCTCGGGT
-8.1 kb	GCGAAACCTCACCTGTGTGTCTCATAGG	GGCCCTATGAGACACACAGGTGAGGTTT
-8.0 kb (1)	GGCTCGATTTCCAAGTCACAGTATTTTT	GGCAAAAATACTGTGACTTGGAAATCGA
-8.0 kb (2)	GGCAATAGAAGTTAATGAGGGGAAAGAGA	GGTTCTCTTTCCCCTCATTA ACTTCTATT
-8 to -16	GGGCCAGTGCTCTGTACACAACAG	GTGCTGTTGTGTGACAGAGCACTGG
+52 to +79	GAGACTTTGAGGGAGTCAAGAAAGTCACG	GAGCGTGACTTTCTTGACTCCCTCAAAGT
+12.2 kb (1)	GGTAAGTCTACAAGAGGAGTCAGTTTAGA	GGGTCTAAACTGACTCCTCTTGTAGACTT
+12.2 kb (2)	GGTTAGACCCCTAGTGTGTCAGAGTGTG	GGTCACACTCTGACAACACTAGGGGTCTA
+15.4 kb	GAGGGAACCTTAGTGGAGTCACAGGCCA	GGATGGCCTGTGACTCCACTAAGGTTC
+15.6 kb	GGGGTTTTAAGACTGTCATGGTCCATGC	GGTGCATGGACCATGACAGTCTTAAAC
+17 kb (1)	GGCATACTTTAAAGTGAGTATATGTGTT	GGAAACACATATACTCACTTTAAAGTAT
+17 kb (2)	GGAATGTTCAAAGCATTAGTCACTAGGT	GTGACCTAGTGACTAATGCTTTGAACAT