Online Methods

Mice.

WT 129S6/SvEv, C57BL/6, BALB/c, B6.SJL,and *Rag2*$^{-/-}$ (H-2^d) mice were from Taconic. *Batf*$^{-/-}$ mice on 129S6/SvEv, C57BL/6, and BALB/c background were used. *Batf*$^{-/-}$ mice on a 129S6/SvEv background were previously generated[2]. *Batf*$^{-/-}$ mice were backcrossed five generation to the C57BL/6 background and eight generation to the BALB/c background. *Aicda*$^{-/-}$ mice were obtained from T. Honjo (Kyoto University, Kyoto, Japan). The Animal Studies Committee at Washington University School of Medicine had reviewed and approved all of the protocols used in the current study.

Flow cytometry analysis.

Data collected by FACS Calibur or FACS Canto (BD Biosciences) was analyzed with FlowJo software (Tree Star). Biotin-conjugated anti-CD40 ligand (MR1), anti-OX40 (OX86), and anti-ICOS (7E.17FG9) were from eBioscience. Biotin-conjugated anti-CXCR5 (2G8), anti-IgG1 (A85-1), anti-IgG2a (R19-15), anti-IgG2b (R12-3), and IgG3 (R40-82), fluorescein isothiocyanate (FITC)-conjugated anti-GL7 (GL7) , allophycocyanin (APC)-conjugated anti-B220 (RA3-6B2), anti-IgM (II/41), anti-CD4 (RM4-5), anti-CD138 (281-2) and streptavidin, phycoerythrin-conjugated anti-CD25 (7D4) and streptavidin, Phycoerythrin-indotricarbocyanine-conjugated anti-Fas (Jo2), and Pacific Blue-conjugated anti-mouse CD4 (RM4-5) were from BD Biosciences. FITC-conjugated anti-CD4 (RM4-5), anti-CD62L (MEL14), and APC-conjugated anti-human CD4 (S3.5) were from Invitrogen. FITC-conjugated goat anti-mouse IgA was purchased from Southern Biotech.

T cell isolation and culture.

Naïve $CD4^+CD62L^+CD25^-$ T cells were purified from spleens on a MoFlo cytometer (DakoCytomation). Some experiments used $CD4^+$ T cells isolated with CD4 microbeads (Miltenyi Biotech). $CD4^+$ T cells were cultured at 1×10^6 cells/ml with plate-bound anti-CD3 (145-2C11; BioXcell) and soluble anti-CD28 (37.5; BioXcell). For gene expression analysis, cultures were supplemented with IL-6 (20 ng/ml, Peprotech), anti-IL-4 (11B11, 10 μg/ml; BioXcell), anti-IFN-γ (XMG1.2, 10 μg/ml; BioXcell), and anti-TGF-β (1D11, 20 μg/ml; R&D systems).

B cell isolation and culture.

Splenic B cells were enriched by negative selection of CD43-experssing cells with CD43 microbeads (Miltenyi Biotech) and cultured at 5×10^5 cells/ml either with LPS (10 μg/ml; Sigma-Aldrich) alone (for IgG3), LPS plus recombinant mouse IL-4 (2.5 ng/ml; Peprotech) (for IgG), LPS plus recombinant mouse IFN-γ (20 ng/ml; Peprotech) (for IgG2a), LPS plus recombinant human TGF-β1 (2 ng/ml; R& D systems) and BAFF (100 ng/ml; eBioscience) (for IgG2b), or LPS plus TGF-β1 (1 ng/ml), recombinant IL-5 (5 ng/ml; R&D systems), APRIL (180 ng/ml; R&D systems), and retinoic acid (10 nM; Sigma-Aldrich) to induce CSR to IgA. Cells were harvested on day 1, 2, or 4 for gene expression analysis, on day 4 for the analysis of surface immunoglobulins, and day 7 for ELISA to detect secreted immunoglobulin isotypes. Some experiments used enriched B cells were labeled with 1 μM carboxyfluorescein diacetate succinimidyl diester (CFSE) (Sigma-Aldrich) for 8 min at 25°C with 20×10^6 cells/ml in PBS. Cells were incubated for 1 min with an equal volume of FCS and were washed twice with media containing 10% FCS.

Microarray analysis.

Total RNA was isolated from cells using RNeasy Kit (Quiagen). Biotinylated antisense cRNA was generated using two cycle target preparation kit (Affymetrix). After fragmentation, cRNA

was hybridized to Affymetrix GeneChip Mouse Genome 430 2.0 Arrays. Data were normalized and expression values were modeled using DNA-Chip analyzer (dChip) software (www.dChip.org).

Retroviral infections.

GFP-RV[40], BATF-GFP-RV[2], and c-Maf GFP-RV[41] were previously described. Bcl-6-GFP RV and CD40L-GFP RV were from D. Bhattacharya (Washington University). AID-GFP-RV was generated by PCR-cloning of AID cDNA followed by subcloning to GFP-RV. AID cDNA was cloned from activated B cell RNA using primers AID-5'-BglII, 5'-TCAAGATCTTCACGCTGGAGACCGAT-3' and AID-3'-XhoI, 5'-TTCCTCGAGGTTGCTTTCAAAATCCC-3'. PCR product was inserted into Bgl II-Xho I site in GFP-RV. Bcl-6-hCD4-RV was generated by PCR cloning of Bcl-6 cDNA followed by subcloning to hCD4-GFP-RV. Bcl-6 cDNA was cloned from splenic B cell RNA using primers Bcl-6-5'-Bgl II, 5'- GCCGGACACCAGATCTAAAGCAAA-3' and Bcl-6-3'-Bgl II, 5'-GGCGAGGAAGATCTCATGCTTCAT-3'. PCR product was inserted into Bgl II site in hCD4-GFP-RV. Retroviral vectors were transfected into Phoenix E cells as described previously[42] and viral supernatants were collected 2 days later. T cells or B cells were stimulated as described for 24 h and were infected with the viral supernatants in the presence of polybrene (6 μg/ml for T cells and 8 μg/ml for B cells) by spin infection at 2500 rpm for 60 min.

Reporter constructs.

The retroviral reporter, human CD4 (hCD4)-pA-GFP-RV, in which a cytoplasmic truncated hCD4 marks viral infection and GFP is used to report promoter activity, has been described previously[43]. 390 bp Iα promoter was cloned by PCR using primers Iα-5'-Hind III, 5'-GGTCTCCCTTCTAAGCTTAATTG-3' and Iα-3'-HindII, 5'-ATGGTAGAAAGCTTAGGACTGG-3', and inserted into Hind III site in hCD4-pA-GFP RV[43].

996 bp Bcl-6 promoter was cloned by PCR using primers 5' Cla bcl6 pro, 5'-GACTACATCGATGTGTTGGAGGTTGGGGCTG-3' and 3' Bam bcl6 pro+ex1, 5'-CGGATCCAGCAATAATCACCTGGTGTCC-3', and inserted into Cla I-Hind III site. 5' conserved regions of Bcl-6 locus were cloned by PCR using primers as following; for ECR1 (1.5 kb), Bcl-6-ECR1-F, 5'- GACATCGATACAACAAAACCATACACCT-3' andBcl-6-ECR1-R, 5'-CCATCGATCCTTGGTACAATTTCCTTTT-3', for ECR2 (680 bp), Bcl-6-ECR2-F, 5'-CTATCGATGGACAGTCTAGTCTTTGC-3' and Bcl-6-ECR2-R, 5'-CCATCGATCAAGTCAAAGTGTGTATG-3', for ECR3 (700 bp), Bcl-6-ECR3-F, 5'-GTATCGATGAGCCAGGTCTGTGTTC-3' and Bcl-6-ECR3-R, 5'-CCATCGATATCTGCCCCTCTTTAGCAAA-3', for ECR4 (400 bp), Bcl-6-ECR4-F, 5'-CAATCGATGGAGTTAGACCAGGAT-3' and Bcl-6-ECR4-R, 5'-TGATCGATATACATTTCAGCTAAAGAT-3'. These cloned regions were inserted into ClaI site of hCD4-pA-GFP-Bcl-6-promoter.

Digestion-Circularization (DC)-PCR.

DC-PCR analysis was carried out as described previously[25]. Genomic DNA was isolated from B cells before or after stimulation with LPS plus IL-4 for 4 days and digested with EcoRI. Digested DNA was purified and self-ligated. Ligated DNA was purified again and served as a template for PCR using primers as reported previously[25].

ChIP assays.

Cells were fixed for 10 min at room temperature in 1% (wt/vol) formaldehyde. Crosslinking was terminated by the addition of 150 mM glycine. After being washed with ice-cold PBS, cells were lysed by sonication in SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris, pH 8.1). Debris was removed by centrifugation and immunoprecipitations were performed with rabbit anti-BATF polyclonal antibody or rabbit anti-acetyl-Histone H3 (Millipore) and Dynabeads® Protein G

(Invitrogen). Immunoprecipitated DNA released from cross-linked proteins was quantified by real-time PCR and was normalized to input DNA. Sequences of all PCR primers used are supplied in **Supplementary Table 1**.

Quantitative real-time PCR.

Total RNA and cDNA were prepared with the RNeasy Mini Kit (Qiagen) and Superscript III reverse transcriptase (Invitrogen). Real-time PCR analysis was performed using ABI SYBR Green master mix and Step One Plus™ Real-Time PCR system (Applied Biosystem). The PCR conditions were 10 min at 95°C followed by 40 two-step cycles consisting of 15s at 95°C and 1 min at 60°C. Primer sequences used to evaluate relative gene expression were supplied in **Supplementary Table 1**.

Immunization and enzyme-linked immunosorbent assay (ELISA).

Basal serum immunoglobulin titers were quantified by ELISA with the horseradish peroxidase-conjugated SBA Clonotyping System (SouthernBiotech). For evaluation of T cell-dependent or T cell-independent antibody responses, mice were immunized intraperitoneally with 50 μg nitrophenol (NP)-conjugated chicken γ-globulin or 25 μg TNP-Ficoll, respectively (Biosearch Technologies). Anti-NP and anti-TNP titers were measured by ELISA against plate-bound NP- or TNP-conjugated BSA (Biosearch Technologies) and isotype-specific horseradish peroxidase-conjugated secondary antibodies (SouthernBiotech). For evaluation of antibody responses in T cell and B cell-reconstituted *Rag2*$^{-/-}$ mice, *Rag2*$^{-/-}$ mice were immunized with 400 μl of 5% (vol/vol) sheep red blood cells (Sigma-Aldrich) in PBS. Anti-SRBC titers were measured by ELISA against plate-coated SRBC and isotype-specific alkaline phosphatase-conjugated secondary antibodies (SouthernBiotech). Titers for all ELISA experiments are presented as the greatest serum dilution that provided an average optical density exceeding 1.5-fold over the average background optical density at 405 nm. Germinal center formation and follicular helper

T-cell development were evaluated after intraperitoneal immunization of mice with sheep red blood cells as described.

Electrophoretic mobility shift assay.

Nuclear extracts were prepared from B cells activated with LPS plus IL-4 for 24 hours or T cells activated with αCD3 and αCD28 in the presence of IL-6 for 24 hours and with PMA and ionomycine for last 4 hours. For EMSA analysis, the AP-1 consensus probe[37] (top:AGCTTCGCTTGATGAGTC and bottom: GCCGACTGAGTAGTTCGC) or +17 kb of the AID locus (top: GGAATGTTCAAAGCATTAGTCACTAGGT and bottom: GTGACCTAGTGACTAATGCTTTGAACAT) were used after labeling with ^{32}P-dCTP. The probe (2.5×10^4 cpm per reaction) was used along with 3-5 μg of nuclear extracts and 1 μg polydIdC. For competitor-supershift assay, BATF binding to the AP-1 consensus probe was assessed by anti-BATF supershit. 100-fold excess unlabeled probes from c-Maf, Bcl-6, CD40L, or AID locus (**Supplementary Table 2**) were used to compete for BATF binding to the AP-1 consensus probe. Nuclear extracts were preincubated for 10 min at room temperature with anti-BATF or competitor oligos before ^{32}P-labeled probe was added. All EMSA gels were 10% bisacrylamide and subjected to electrophoresis in 0.4x TBE, pH7.5. Gels were dried and exposed for autoradiography.

Statistical Analysis.

A Student's unpaired two-tailed *t*-test was used to indicate statistically significant differences between indicated groups. Differences with a P value <0.05 were considered significant.

Accession codes: