Full Methods

Mice. 6-12 week old C57BL/6 and were purchased from either the National Cancer Institute or Jackson Laboratories. B6(C)-H2-Ab1^{bm12}/KhEgJ (bm12) mice and B6.Cg-Igh^{a}Thy1^{a}GPi1^{a}/J (IgMa) mice were from Jackson labs. MD4 mice [27] and CXCL13-deficient mice [22] were from an internal colony. Bone marrow chimeras were generated as described [11] and analyzed after 6-12 weeks. NP-CGG immunizations were performed using 50 μg of NP-CGG (Solid Phase Sciences, USA) in Alum (Accurate Chemical & Scientific Corp., USA). Treatment with LTβR-Fc (kindly provided by Jeff Browning, Biogen Idec) was as described [20] using 100μg once a week for three weeks. Animals were housed in specific pathogen-free environment in the Laboratory Animal Research Center at UCSF and all experiments conformed to ethical principles and guidelines approved by the UCSF Institutional Animal Care and Use Committee.

Ebi2 gene targeting and retroviral constructs. A 5' homology arm (5.2kb) and 3' homology arm (3.7kb) were generated from mouse genomic DNA by PCR and cloned using BD In-Fusion Dry-Down PCR cloning kit into vector EGFP-polyA-loxP-Neo-loxP-DTA-PL452 (kindly provided by Nigel Killeen) to flank the EGFP-polyA-loxP-Neo-loxP insert. E14 (129) ES cells were transfected by standard techniques and 350 colonies were screened by long PCR (Roche Long Template PCR system) yielding 22 positive clones. Homologous recombination was confirmed by Southern blotting and three clones were used for microinjection into B6 blastocysts. Chimeras were bred to B6 mice and germline transmission was confirmed by allele-specific PCR and flow cytometric detection of GFP expression. *Ebi2*$^{GFP/+}$ mice were intercrossed with actin-Cre transgenic mice and deletion of the loxP flanked Neomycin resistance cassette was confirmed by PCR. *Ebi2*$^{GFP/+}$ (neo-) mice used in this study were backcrossed to B6 for at least 6 generations. The retroviral construct was made by inserting the mouse Ebi2 open reading frame, with a preprolactin-FLAG leader sequence [28] in place of the ATG, into the MSCV2.2 retroviral vector containing cytoplasmic-domain truncated human CD4 as an expression marker downstream of the internal

ribosomal entry site [8]. The control vector contained cytoplasmic domain truncated human Nerve Growth Factor receptor as an irrelevant insert.

Cell isolation, CFSE labeling, retroviral transduction and adoptive transfers. B cells were isolated and in some cases labeled with 2.5 μM of 5(and 6)-carboxy-fluorescein diacetate succinimidyl ester (CFSE, Molecular Probes) as described [29]. For in vivo analysis of Ebi2 expression, 5-10x10^6 purified B cells as in [11], were transferred into bm12 experiments. In vitro analysis of Ebi2 expression was performed by culturing 10^5 splenocytes from *Ebi2*$^{GFP/+}$ mice with 10-13μg/ml of anti-IgM (F(ab')2 goat anti-mouse IgM, Jaskson Immunoresearch), 10μg/ml anti-CD40 (clone FGK4.5, UCSF Hybridoma Core), or both, for 24 and 48h. Retroviral supernatant was generated using Phoenix packaging cells. Retroviral transduction of activated B cells was performed as described [8] using MD4 Ig-transgenic B cells. Transduced cells were adoptively transferred one day after spin-infection for transfers to non-immunized hosts or immediately after spin-infection for transfers to immunized hosts. For GC experiments, B6 mice received 10^5 MD4 B cells and 10^5 OTII CD4$^+$ T cells at day -1, were intraperitoneally immunized with 50μg HEL-OVA in RIBI adjuvant system (Sigma) at day 0 and received approximately 10^6 Ebi2 or control vector transduced cells at day 1. Mice were analyzed on day 5.

Flow cytometry. BM B cell subsets were analysed as in [30]. Spleen and lymph node cells were isolated and stained as described [11]. For analysis of GC B cell differentiation, cells were stained with PE-Cy5.5-conjugated anti-B220 (RA3-6B2; BD Biosciences), Pacific blue-conjugated anti-CD45.1, FITC-conjugated anti-IgD (11-26c.2a; BD Biosciences) and PE-Cy7-conjugated anti-Fas (Jo2; BD Biosciences). For analysis of Ebi2 expression in T cells, spleens and lymph nodes were stained with APC-conjugated anti-TCRβ (H57-597, eBioscience), PECy5.5-conjugated anti-CD4 (RM4-5, Invitrogen), with PE-conjugated anti-CD8 (CT-CD8a, Invitrogen), and with biotin-conjugated anti-NK1.1 (PK136, BD Biosciences)

ELISA. IgG1 anti-NP ELISA was performed by coating 96-well plates (Immunolon) with 10μg/mL of NP(30)BSA (Solid Phase Sciences) in phosphate buffered saline for at least 2h at 37ºC, and blocked with 5% (w/v) bovine serum albumin (Calbiochem) for 2h at ~25ºC. Serum samples were serially diluted (1:2) starting at 1:500 in PBS 0.01%Tween, incubated for 2h at ~25ºC, and NP-binding IgG1 was detected using biotin-conjugated anti-IgG1 (A85, BD Biosciences), followed by horseradish peroxidase-conjugated streptavidin (Jackson Immunoreserach). Color development was done using ABTS substrate (Southern Biotech) in 55mM citrate buffer containing 0.03% H_2O_2. OD measured at 405nm in a VERSAmax microplate reader using SoftMax pro 5.2 (Molecular Devices). The NP-specific IgG1 concentration was calculated by determining the dilution required to achieve an O.D. of 0.5, 1.0 and 1.5 (across these values, the correlation coefficient was >0.99 in all serum samples), averaged, and displayed as relative units to a standard serum sample.

Immunohistochemistry and Immunofluorescent microscopy. 5-7 μm cryosections were fixed and stained immunohistochemically as described [8] with combinations of the following antibodies: anti-IgD (11-26c.2a, BD Biosciences), anti-IgMa (DS-1, BD Biosciences), hCD4 (RPA-T4, BD Biosciences) and B220 (RA3-6B2, BD Biosciences). For immunofluorescence, staining with biotin-conjugated anti-IgD^a (AMS9.1, BD Biosciences) was detected with Alexa Fluor488®-conjugated streptavidin (Invitrogen), PE-conjugated anti-IgD^b (217-170, BD Biosciences), and Alexa 647-conjugated anti-CD4, and anti-CD8 (UCSF Hybridoma Core). The FDC network was stained using purified anti-CD35 (8C12, BD Biosciences) detected with APC-conjugated anti-rat IgG (Jackson Immunosearch). Marginal zone macrophages were stained with an anti-Ser4 antibody (P. Crocker, University of Glasgow) conjugated to Alexa Fluor647®. Analysis of T cell distribution was performed with biotin-conjugated anti-CD90.1 (Thy-1.1, clone HIS51) detected with Alexa Fluor488®-conjugated), and with PE-conjugated anti-CD90.2 (Thy-1.2, clone 30-H12). Sections were then blocked

with 5% normal rat serum before staining with additional antibodies. FITC-conjugated anti T- and B-cell activation antigen (GL7; BD Biosciences) was used to detect GCs. For detection of GFP, tissues were fixed in 4% paraformaldehyde and prepared as described [31]. Images were obtained with a Zeiss AxioObserver Z1 inverted microscope or a Zeiss AxioImager M1 upright microscope.

30. Pereira, J. P. *et al.* Cannabinoid receptor 2 mediates the retention of immature B cells in bone marrow sinusoids. *Nat. Immunol.* 10, 403 (2009)
31. Pappu, R. *et al.* Promotion of Lymphocyte Egress into Blood and Lymph by Distinct Sources of Sphingosine-1-Phosphate. *Science* 316, 295 (2007)