Supporting Information

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SI Materials and Methods

Generation of Bone Marrow Chimeras. $Rag^{-/-}$ mice were sublethally irradiated (730 rads) and reconstituted by i.v. injection of d17 fetal liver cells (4×10^5 cells) or adult bone marrow cells (1×10^6 cells) from B6 CD45.1 mice that had both been depleted of $Gr1^+$ cells by AutoMACS. Chimeras were analyzed after 2 mo by gating on $CD45.1^+$ cells.

Parabiotic Mice. Parabiosis surgery were performed as described in ref. 1.

Antibodies and Flow Cytometry. Monoclonal antibodies for flow cytometry against mouse B220 (RA3-6B2), CD19 (ebio1D3), Igκ (187.1), MHC class II (M5/114.15.2), CD43 (S7), CD45.2 (104), CD45.1 (A20), CD69 (H1.2F3), CD86 (GL1), ICOSL (HK5.3), IgM (11/41), IgD (11-26), CD93 (AA4.1), CD21(ebio4E3), CD24/HSA (M1/69), CD23 (B3B4), CD5 (53-7.3), CD4 (RM4-5), CD8 (53.6.7), CD90.2 (53-2.1), CD11c (N418), and MHC II A^{g7} (10-2.16) were purchased from eBioscience, BD Biosciences, or Biolegend, or provided by the Fitch Monoclonal Antibody Facility (University of Chicago). Samples were collected on a FACS Canto (BD), and data were analyzed using FlowJo (Tree Star).

Generation of Clonotypic Antibodies for 121 Heavy and Light Chain. The 6.121 mouse IgG1 monoclonal antibody was purified from 6.121 hybridoma cells and was used as immunogen. Lewis rats were hyperimmunized intraperitoneally following the protocol of Hayakawa (2). Three days after the final boost, spleen cells were fused with SP2/mIL-6 cells. Clonotypic specificity was screened by comparing reactivity to 6.121 and control 6.149 IgG by ELISA, and by staining B cells of 121 knockin and control mice. Fine specificity was then examined by staining B cells of various mice as shown in Fig. S3.

Immunohistology of Thymus Sections. Sections from OCT-embedded thymi were stained according to standard immunofluorescence protocol (dehydration, acetone fixation, immunostaining), and imaged on an Axiovert 200M inverted wide-field fluorescence microscope (Zeiss). Image processing was performed using ImageJ (NIH).

1. Thomas SY, et al. (2011) PLZF induces an intravascular surveillance program mediated by long-lived LFA-1-ICAM-1 interactions. *J Exp Med* 208(6):1179–1188.

2. Hayakawa K, et al. (2003) Positive selection of anti-thy-1 autoreactive B-1 cells and natural serum autoantibody production independent from bone marrow B cell development. *J Exp Med* 197(1):87–99.

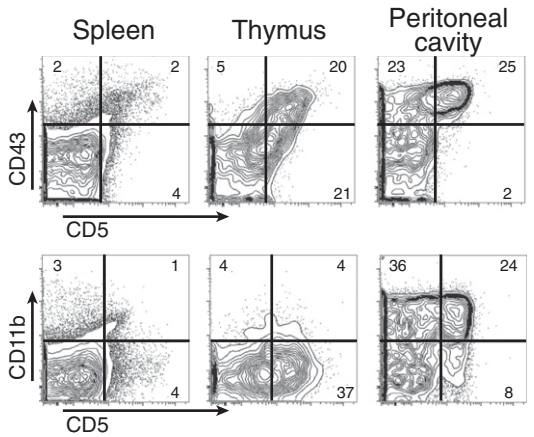

Fig. S1. Surface phenotype of thymic B cells vs. B1 B cells. Staining of splenic, thymic, and peritoneal cavity B cells for classical surface markers of the B1 B-cell lineage. Gated on $CD19^+B220^+$ B cells.

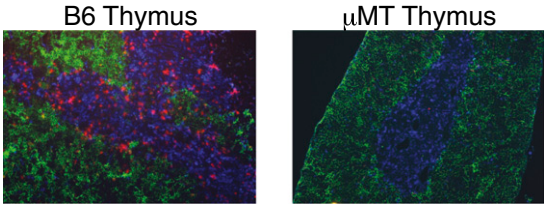

Fig. S2. Localization of thymic B cells. Representative thymic sections showing localization of thymic B cells in 6- to 8-wk-old B6 mice with $\mu MT^{-/-}$ mice as a negative control. Staining shows thymic cortex (CDR1-Alexa 488) in green, thymic medulla (CD11c-Dylight 649) in blue, and thymic B cells (CD19-PE) in red. 10× magnification.

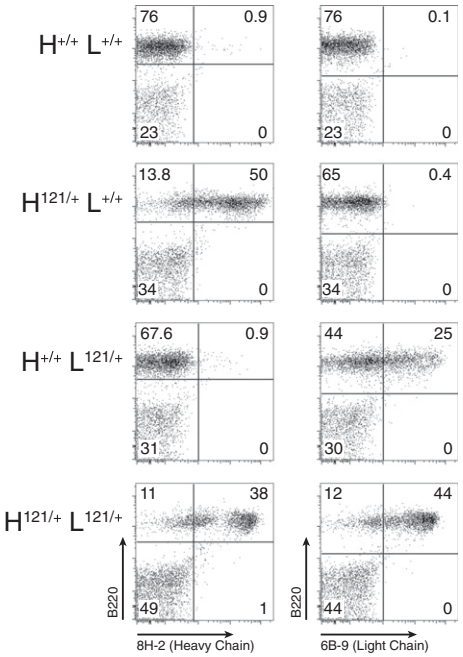

Fig. S3. Confirmation of clonotypic antibodies 8H2 and 6B9 for 121 heavy and light chains respectively. Splenocytes from WT ($H^{+/+}$, $L^{+/+}$), heavy chain only ($H^{121/+}$, $L^{+/+}$), light chain only ($H^{+/+}$, $L^{121/+}$), and heavy and light chain ($H^{121/+}$, $L^{121/+}$) knock-in mice were stained with the indicated 121-specific monoclonal antibody and B220.

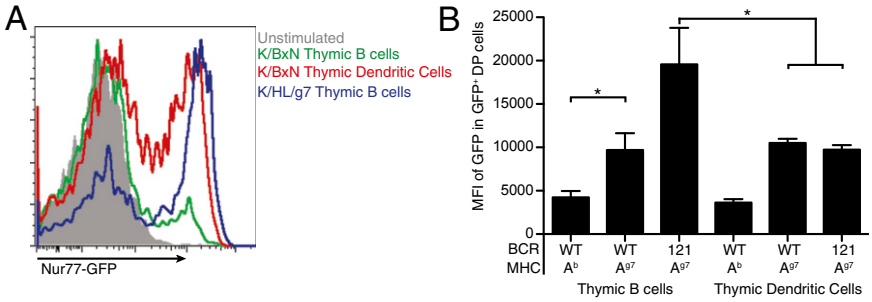

Fig. S4. Median fluorescence intensity (MFI) of Nur77-GFP expression in KRN double-positive (DP) thymocytes in antigen-presenting cell coculture assay. (*A*) Representative histogram showing induction of Nur77 GFP in KRN DP cells by K/BxN thymic B cells (green), K/BxN thymic dendritic cells (red), and K/HL/g7 thymic B cells (blue). (*B*) Quantification of the MFI of Nur77-GFP in GFP^+ DP cells. n = 3–6 mice per group from five independent experiments.