Supplementary Data

Methods

Quantitative RT-PCR
Splenic mature B cells and CD4\(^+\) T cells were purified using a MACS system with biotin-anti-CD43, biotin-anti-AA4.1 (clone AA4.1, eBioscience) and streptavidin-microbeads or CD4\(^+\) T Cell Isolation Kit (Miltenyi Biotec). Total RNA was isolated with GenElute Mammalian Total RNA Miniprep Kit (Sigma) and cDNA was synthesized with Superscript II reverse transcriptase (Invitrogen) according to the manufacture’s instructions respectively. Quantitative PCR was performed with Platinum SYBR Green qPCR SuperMix-UDG with ROX (Invitrogen) and 7900HT PCR System (Applied Biosystems). PCR condition was 50˚C for 2 min, 95˚C for 2 min, and 45 cycles of 95˚C for 15 sec, 60˚C for 1 min and primer specificities were confirmed by dissociation curve. All data were analyzed on SDS software (Applied Biosystems) and gene expression was determined as an average of triplicate samples after drawing standard curve and normalized by an expression of cyclophilin B (Ppib) gene. Primer sequences were as follows: Tslpr forward, 5’-TGACGTCACGGGCTGATGTC-3’, and reverse, 5’-GAGGATGCAACCGGAAGTGA-3’; Il7r forward, 5’-GGATGGAGACCTAGAAGATG-3’, and reverse, 5’-GAGTTAGGCATTTCACCTCGT-3’; Ppib forward, 5’-GCTACAGGAGAAAGGATTTGAC-3’, and reverse, 5’-CGGCTGTCTGTCTTGGTGTGCTCTC-3’.
Supplementary Figure Legends

Supplementary Fig. 1.

Expression of TSLP receptor complex, TSLP receptor (TSLPR) and IL-7Rα, on splenic B cell subsets. (A) Flow cytometry of splenic B cell subsets from NLC or K5-TSLP mice treated with 1 mg/ml of dox for 3 weeks, stained for TSLPR and IL-7Rα. B cell subsets were identified by staining B220, CD21/CD35, and CD23. Filled histogram, isotype control; thick lines, anti-TSLP or anti-IL-7Rα. Data represent one of two independent experiments. (B) Quantitative PCR analysis of the expression of *Tslpr* and *Il7r* transcript in isolated splenic B cells from NLC or K5-TSLP mice received 1 mg/ml of dox in the drinking water for 3 weeks.
Supplementary Fig. 2.

Polyclonal B cell activation in K5-TSLP spleen depends on αβ T cells. (A) Immature B cell subsets in bone marrow from NLC/Tcrb<sup>+/−</sup>, NLC/Tcrb<sup>−/−</sup>, K5-TSLP/Tcrb<sup>+/−</sup>, and K5-TSLP/Tcrb<sup>−/−</sup> mice treated for 3 weeks with 1 mg/ml of dox. Cells were gated on B220<sup>+</sup>CD43<sup>+</sup> live lymphocyte, and immature B cell subsets were identified by staining for HSA/CD24 and BP-1. Numbers near boxed area indicate percent of gated subset. (B) Analysis of FO B cells from spleen of NLC/Tcrb<sup>+/−</sup>, NLC/Tcrb<sup>−/−</sup>, K5-TSLP/Tcrb<sup>+/−</sup>, and K5-TSLP/Tcrb<sup>−/−</sup> mice treated for 3 weeks with 1 mg/ml of dox. Splenocytes were stained for B220, CD21/35, CD23, and MHC class II and analyzed with flow cytometry. Cells were gated on FO B cells (B220<sup>+</sup>CD21/CD35<sup>low</sup>CD23<sup>+</sup>) and forward scatter (FSC), expression of CD21/CD35, CD23, and MHC class II were shown. Dotted line indicates the peak of each histogram of NLC/Tcrb<sup>+−</sup> B cells. Numbers indicate mean of FSC or fluorescent intensity in each histogram. Both results represent one of four independent experiments.
Supplementary Fig. 3.

IL-4 concentration in serum samples from (A) NLC, K5-TSLP/Tcrb<sup>+</sup>/<sup>-</sup>, or K5-TSLP/Tcrb<sup>-</sup><sup>-</sup> mice; (B) NLC, K5-TSLP mice received anti-CD4 or control antibodies; (C) K5-TSLP/Il4<sup>+</sup>/± or K5-TSLP/Il4<sup>-</sup>/± mice received 1 mg/ml of dox in the drinking water for 3 weeks. Samples are diluted and analyzed by ELISA for IL-4 * , $P < 0.02$; ** , $P < 0.01$; *** , $P < 0.05$. 
Supplementary Fig. 4.

(A) Flow cytometry of peripheral blood or splenic mature B cells from NLC or K5-TSLP mice with or without 1 mg/ml of dox treatment for 3 weeks, stained for IL-4Rα. Mature B cells were identified by B220 and CD23 expression. Data represent one of two independent experiments. (B) Mean fluorescence intensities of NLC or K5-TSLP mature B cells stained with IL-4Rα. *, $P < 0.01$; **, $P < 0.02$. 
Supplementary Fig. 5.

Autoimmune hemolytic anemia in K5-TSLP mice depends on αβ T cells. (A) Hematocrit in the blood of NLC/Tcrb+/−, NLC/Tcrb−/−, K5-TSLP/Tcrb+/−, and K5-TSLP/Tcrb−/− mice after 1 mg/ml, 3 weeks dox treatment. Average were indicated by horizontal bars. (B) Detection of anti-RBC antibodies by flow cytometry. RBCs form dox-treated NLC/Tcrb+/−, NLC/Tcrb−/−, K5-TSLP/Tcrb+/−, and K5-TSLP/Tcrb−/− mice were stained with anti-mouse IgM and anti-mouse IgG and analyzed. Data represent one of two independent experiments.