Supplementary Experimental Procedures

Mouse TRAF3 conditional gene targeting construct

We previously cloned ~23 kb of the mouse TRAF3 gene using PCR-based chromosomal walking techniques (Xie et al., 2004). Based upon this sequence information, we made a mouse TRAF3 conditional gene targeting construct that was designed to insert two loxP sites, recognition sequences of the bacterial phage P1 DNA recombinase Cre (Kuhn and Schwenk, 1997; Rajewsky et al., 1996), into two introns flanking the first two coding exons of the TRAF3 gene (Supplementary Fig. 1A). A 10.1 kb fragment of the mouse TRAF3 gene encompassing the first three encoding exons and their surrounding as well as intervening introns were used as homologous arms to make the targeting construct. The first loxP sequence together with a SpeI restriction enzyme site was ligated into the intron upstream of the translation initiation codon "ATG" at a Hpa I restriction enzyme site (about 230 bp upstream of "ATG"), while the second loxP sequence together with a neomycin resistance (neo^r) cassette was incorporated into the intron downstream of Exon 2 at an EcoRV restriction enzyme site (about 250 bp downstream of exon 2). A thymidine kinase (TK) cassette was ligated outside of the TRAF3 arms to serve as a negative selection marker against random insertion events. Due to concerns that the active transcription of the positive selection marker neo^r might interfere with TRAF3 expression, a pair of FRT sites, recognition sites of the yeast DNA recombinase FLP (Dymecki, 1996), were incorporated to flank the neo^r gene and thus enabled its removal by FLP-mediated recombination.

Generation of $TRAF3^{+/flox}$ mice

The conditional TRAF3 gene targeting construct was linearized with Not I, then transfected into W4 ES cells of the 129/SvJ mouse strain by electroporation. Transfected cells were selected with geneticin and gancyclovir as previously described (McDonald et al., 1999). Five of 280 double-resistant ES clones were identified to contain homologous recombination by genomic PCR screening using 2 sets of primers (pBYneoA + G4, and pBYneoA + G6)(Supplementary Fig. 2A and 2B). Three of the 5 ES clones were found to have both loxP sites inserted and to contain a single copy of the targeting construct as revealed by further genomic PCR analysis using primers FA3 and FT4 followed by SpeI digestion of the PCR products, as well as by Southern Blot analysis of SpeI-digested genomic DNA using probes A and Neo as described (McDonald et al., 1999)(Supplementary Fig. 2C-2E). Each of the three $TRAF3^{+/flox\text{-}FRT}$ ES clones was injected into wild type C57BL/6 blastocysts, then transferred into pseudo-pregnant ICR female recipient mice to generate chimeric mice as described (McDonald et al., 1999). The male chimeric mice were bred with wild type C57BL/6 female mice, and germ-line transmitted $TRAF3^{+/flox\text{-}FRT}$ F1 mice were obtained from each of the three ES clones as confirmed by both genomic PCR and Southern blot analyses (Supplementary Fig. 1B and data not shown).

The $TRAF3^{+/flox\text{-}FRT}$ mice were crossed with FLPe transgenic mice (Dymecki, 1996)(Jackson Laboratory, Bar Harbor, ME) to excise the neo^r gene (Supplementary Fig. 1A). $TRAF3^{+/flox}$ mice with the neo^r gene excised were identified by genomic PCR screening of mouse tail DNA using primers FC3 and BT6 (Supplementary Fig. 2F), and were bred with wild type C57BL/6 mice for one more generation to ensure germ-line transmission of the $TRAF3^{flox}$ allele (with the neo^r gene removed).

Primers

Primers used for PCR screening include: pBYneoA (5'- AGA GCA GCC GAT TGT CTG TT -3'); G4 (5'- GGT AAA CAC AGC ACT ACG GT -3'); G6 (5'- ATG GCG TTT CAG GGT AGG GAT -3'); FA5 (5'- CTT GGA CAG TAA AGG AAG TTG T -3'); FT4 (5'- GCT GGA GGT CTT GGA AGA AG -3'); FC3 (5'- GCC AAG AAA GCA TCA TCA AAG ACA -3'); BT6 (5'- GTC CTG GAT CTT GCT ATG AG -3'); U7 (5'- GTT ACA ATG AAG TTC TGG CAC -3'); Cre-F (5'- ATG CAA CGA GTG ATG AGG TT -3'); Cre-R (5'- ATC ATC AGC TAC ACC AGA GA -3'). Positions of the primers for the TRAF3 gene are depicted in Supplementary Fig. 1A. The pBYneoA primer sequence resides in the neo^r cassette of the targeting construct, whereas primers G4 and G6 are complementary to the genomic sequence downstream of, but not within, the 3' arm of the targeting vector. Thus, the primer set pBYneoA + G4 (or pBYneoA + G6) is predicted to amplify a 4.2 kb (or 4.3 kb) PCR product from ES clones with the $TRAF3^{flox\text{-}FRT}$ allele, but not to amplify any PCR products from the $TRAF3^{+}$ allele or from the targeting construct. The FA5 primer is complementary to the genomic sequence upstream of, but not within, the 5' arm of the targeting vector, while the FT4 primer is downstream of the first encoding exon. Therefore, the primer set FA5 + FT4 is predicted to amplify a 5.6 kb PCR product from the $TRAF3^{flox\text{-}FRT}$ allele or $TRAF3^{+}$ allele, but not to amplify any PCR products from the targeting construct. Primers FA3 and BT6 are flanking the second loxP insertion site, and are predicted to amplify a 533 bp PCR product from the $TRAF3^{+}$ allele, a 615 bp PCR product from the $TRAF3^{flox}$ allele (with the neo^r cassette removed by FLP-mediated recombination), and a 2.4 kb PCR product from the $TRAF3^{flox\text{-}FRT}$ allele (containing the neo^r cassette). In contrast, primers U7 and

BT6 are flanking both loxP insertion sites, and are thus predicted to amplify a 2.54 kb PCR product from TRAF3^{+} allele, a 2.69 kb PCR product from the TRAF3^{flox} allele, and a 645 bp PCR product from the TRAF3$^{\Delta}$ allele (with the first two encoding exons of the TRAF3 gene deleted through Cre-mediated recombination).

Antibodies (Ab) and reagents

Polyclonal rabbit Abs to TRAF1 (N19), TRAF3 (H122), TRAF6 (H274), Rel B (C19) and PKCδ (C17), and mouse anti-YY1 Ab (H-10) were from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal rabbit Ab to TRAF2 was from Medical and Biological Laboratories (Nagoya, Japan). Fluorescein isothiocyanate (FITC), phycoerythrin (PE), or Cy5 labeled monoclonal Abs against mouse CD45R (B220), CD3, CD19, CD23, CD5, IgM, IgG, IgD, AA4.1, c-Kit, CD1d, CD38, CD43, CD4, CD8, CD11b, Gr-1, FcR (2.4G2), MHC class II (I-A/I-E), CD40 and Fas, as well as an agonistic hamster anti-mouse CD40 (HM40-3) were purchased from eBioscience (San Diego, CA). Polyclonal goat Abs against mouse BAFF-R, TACI, or BCMA were from R&D Systems (Minneapolis, MN). Polyclonal rabbit Abs against total or phosphorylated IκBα, JNK, p38 or ERK, and polyclonal rabbit Abs against p100/p52 and NIK were from Cell Signaling Technology (Beverly, MA). Anti-actin Ab was from Chemicon (Temecula, CA). HRP-labeled secondary Abs and FITC-labeled rabbit anti-goat IgG were from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA). FITC, or biotin labeled monoclonal Abs against mouse CD21/35, CD25 or CD9, PerCP-B220, anti-mouse CD95 (Jo-2, hamster IgG) and hamster IgG isotype control were purchased from BD PharMingen (San Diego, CA). FITC-anti-mouse MOMA-1 and FITC-anti-mouse C3 were from Cedarlane laboratories (Ontario, Canada). Alkaline phosphatase (AP)-

conjugated polyclonal goat Abs specific for mouse IgM, IgG1, IgG2a, IgG2b, IgG3, IgA and IgE, and PE-conjugated goat anti-mouse IgG(H+L) were from Southern Biotechnology Associates (Birmingham, AL). A hybridoma producing anti-mouse IgE Ab (clone EM95, rat IgG2a) was provided by Dr. Thomas Waldschmidt of the University of Iowa (Iowa City, IA), and Ab purified from hybridoma supernatants by saturated $AmS0_4$ precipitation. Alexa fluor 350-labeled goat anti-mouse IgM polyclonal Ab was from Molecular Probes (Eugene, OR). Anti-mouse CD43 magnetic beads were from Miltenyi Biotec Inc. (Auburn, CA). Geneticin, gancyclovir, Elongase DNA polymerase, and tissue culture supplements including stock solutions of sodium pyruvate, L-glutamine, non-essential amino acids, and Hepes (pH 7.55) were from Invitrogen (Carlsbad, CA). DNA oligonucleotide primers were obtained from Integrated DNA Technologies (Coralville, IA). SRBCs were purchased from Elmira Biologicals (Iowa City, IA). Tissue-Tec OCT compound was from Sakura (Torrance, CA). FITC-labeled peanut agglutinin (PNA) and VectorShield mounting media were obtained from Vector Laboratories (Burlingame, CA). Solvent 100 mounting media was from IMEB Inc. (San Marcos, CA). Percoll, AP substrates and propidium iodide (PI) were purchased from Sigma-Aldrich Corp. (St. Louis, MO).

FACS staining of BAFF receptors

For detection of BAFF receptors, purified resting splenic B cells were stained with polyclonal goat anti-mouse BAFF-R, TACI, or BCMA Abs, followed by FITC-labeled rabbit anti-goat IgG Abs.

Fas-mediated apoptosis

Purified resting splenic B cells were first stimulated with 2 μg/ml of anti-mouse CD40 Ab (HM40-3) at 37^{o}C for 24 h to upregulate Fas, then washed and treated with 100 ng/ml of anti-Fas Ab (Jo-2) or hamster IgG isotype control Ab at 37^{o}C for 16 h, and subsequently fixed with 70% ethanol for cell cycle analysis.

Transwell co-culutre analysis

Purified resting splenic B cells from LMC and B-TRAF3$^{-/-}$ mice (0.3X10^{6} of each) were plated in the lower and upper chambers respectively, or conversely, of each well in a 24-well Costar transwell plates (pore size 0.4 μm, Corning Inc., Acton, MA) following the manufacturer's instructions. At each time point, cells in the lower chamber were fixed with ice-cold 70% ethanol, and apoptotic cells were analyzed by PI staining.

References

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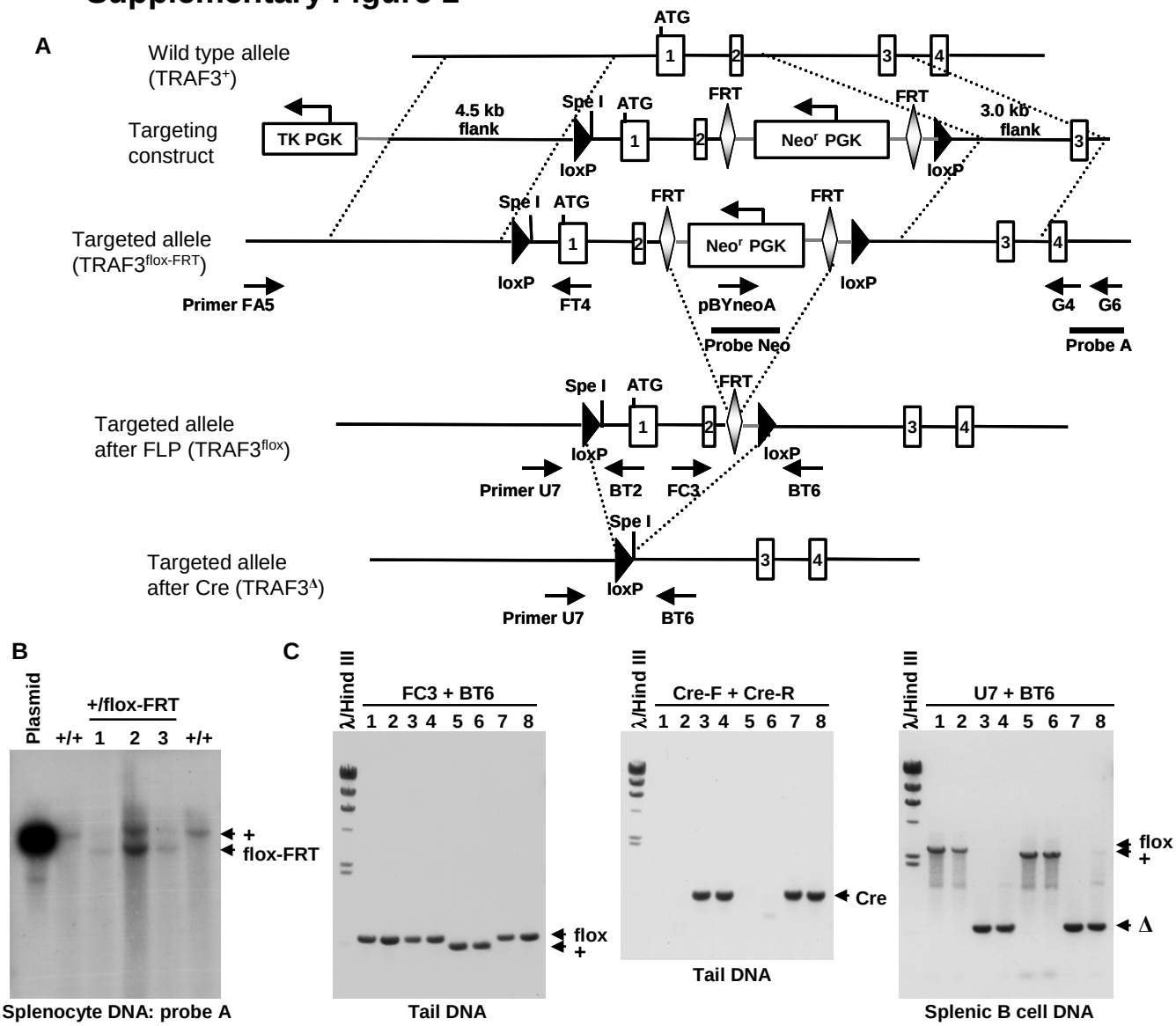
Supplementary Table 1

		B-LMC	B-TRAF3-/-	Fold
Spleen (x 10^6 cells)		91.25 ± 7.58	208.25 ± 25.47	2.28
Total B	B220+	54.41 ± 6.98	164.30 ± 16.83	3.02
Mature B	B220+AA4.1-	46.54 ± 2.92	149.71 ± 18.29	3.22
T1 transitional B	B220+AA4.1+IgM^{hi}CD23-	2.37 ± 0.39	3.35 ± 0.52	1.41
	B220+AA4.1+IgM^{hi}IgD-	2.40 ± 0.40	3.34 ± 0.20	1.39
T2 transitional B	B220+AA4.1+IgM+CD23+	4.11 ± 1.00	9.34 ± 1.26	2.27
	B220+AA4.1+IgM+IgD+	3.86 ± 0.97	8.99 ± 0.99	2.33
Newly formed or activated B	B220+IgM+CD21^{lo}CD23^{lo}	7.16 ± 1.93	17.36 ± 2.63	2.43
Marginal Zone B	B220+IgM+CD21^{hi}CD23^{int}	6.24 ± 0.83	37.23 ± 7.15	5.96
	B220+IgM+CD1d+CD9+	5.81 ± 1.16	35.89 ± 1.37	6.18
Follicular B	B220+IgM+CD21^{int}CD23^{hi}	39.87 ± 5.48	109.95 ± 10.34	2.76
Total T	CD3+	29.51 ± 2.09	40.65 ± 10.62	1.38
CD4+ T	CD4+	16.33 ± 2.74	23.34 ± 5.53	1.43
CD8+ T	CD8+	9.92 ± 0.76	11.80 ± 2.20	1.19
Lymph node (x 10^6 cells)		14.36 ± 3.65	83.75 ± 28.95	5.83
Total B	B220+	4.75 ± 1.10	63.05 ± 24.78	13.29
Mature B	B220+AA4.1-	4.42 ± 1.27	59.14 ± 23.11	13.38
Immature B	B220+AA4.1+	0.38 ± 0.06	2.88 ± 0.88	7.51
Newly formed or activated B	B220+IgM+CD21^{lo}CD23^{lo}	0.43 ± 0.12	4.46 ± 1.61	10.31
Follicular B	B220+IgM+CD21^{int}CD23^{hi}	4.27 ± 1.04	57.72 ± 23.95	13.52
Total T	CD3+	8.27 ± 2.10	20.99 ± 4.83	2.54
CD4+ T	CD4+	4.78 ± 1.20	12.59 ± 2.93	2.63
CD8+ T	CD8+	3.05 ± 0.95	7.17 ± 1.70	2.35
Bone marrow (x 10^6 cells)		26.41 ± 2.35	27.13 ± 2.51	1.03
Total B cells	B220+	9.95 ± 0.86	10.07 ± 2.05	1.01
Pro-B/Pre-B	B220+IgM-	4.91 ± 0.61	4.58 ± 1.35	0.93
Immature B	B220+AA4.1+IgM+IgD-	2.11 ± 0.39	1.90 ± 0.57	0.90
Recirculating mature B	B220+AA4.1-IgM+IgD+	1.53 ± 0.22	1.73 ± 0.43	1.13

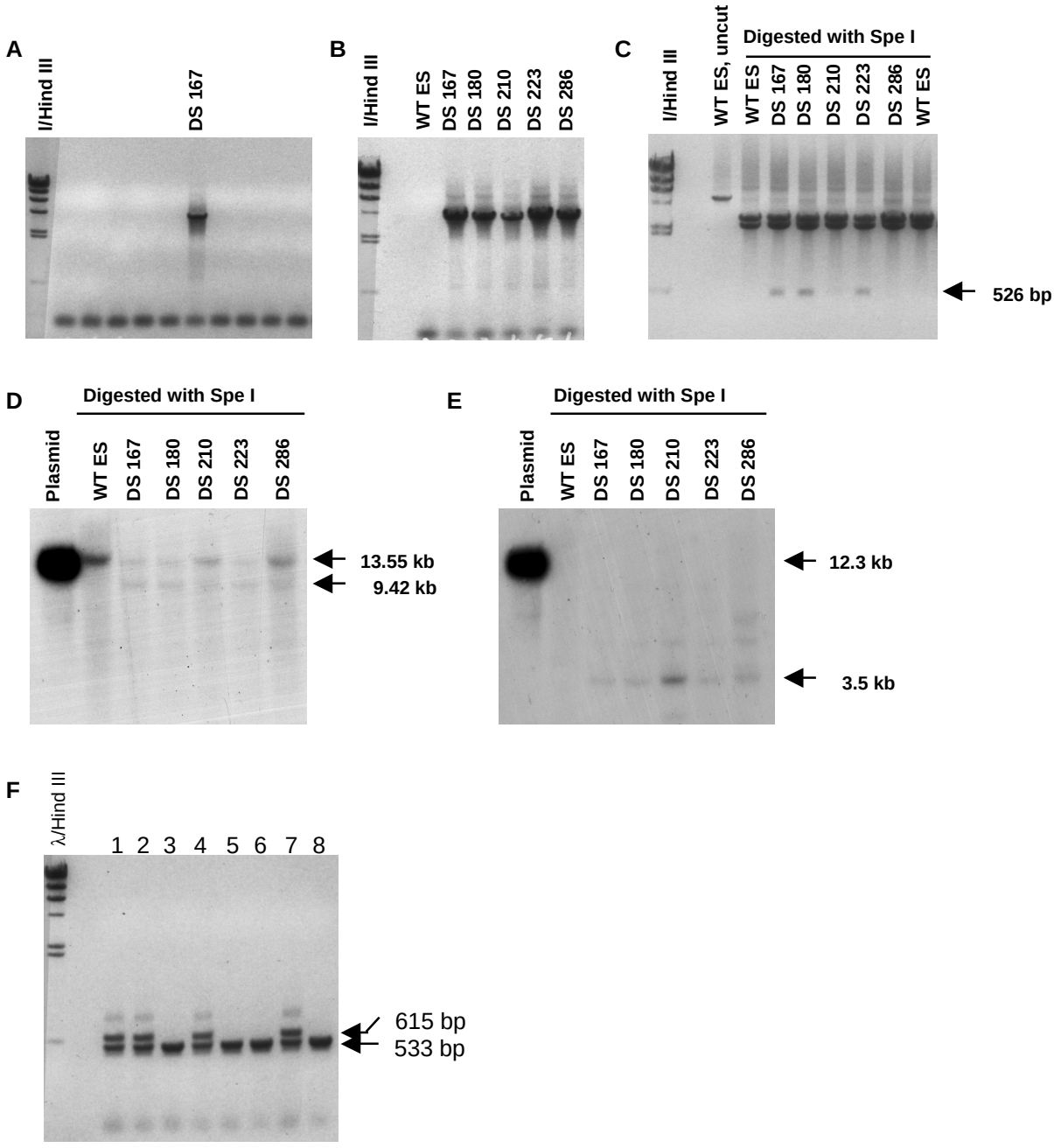
Supplementary Table 1. Summary of FACS analysis of B cell and T cell populations in spleen, lymph nodes and bone marrow. Single cell suspensions were prepared from spleens and lymph nodes of LMC and B-TRAF3$^{-/-}$ mice, and bone marrow cells were extracted from the two femurs of each mouse. B and T cells were analyzed by immunofluorescence staining and flow cytometry using cell surface markers described in the second column of the table. FACS data were analyzed using a FSC/SSC gating for single lymphocytes. Mice analyzed were 8 to 12 weeks old. Data shown are the results of four independent experiments (mean ± STDEV).

Supplementary Figure 1



Supplementary Figure 1. Conditional targeting of the TRAF3 gene in mice. (**A**) Targeting strategy. Schematic diagrams of the TRAF3 conditional targeting construct, the initial targeted locus, and the targeted locus after FLP- or Cre- mediated recombination are shown. Numbered boxes represent exons, and "ATG" indicates the start codon. Length of homologous sequence, the neo^r and TK cassettes, as well as loxP and FRT sites are indicated. PCR primers used to screen ES cell clones with homologous recombination, or to detect TRAF3^{flox} or TRAF3$^{\Delta}$ alleles in mice are indicated with arrows. Positions of probes used in Southern blot analysis are shown. (**B**) Southern blot analysis. Genomic DNA was isolated from splenocytes of LMC mice (+/+) and the representative germline-transmitted F1 mice (1, 2, 3) derived from each of the three ES clones (DS167, DS180, DS223), respectively. Southern blot analysis was performed on SpeI-digested DNA using probe A. A linearized plasmid DNA of 12.3 kb, which contains probe A, was used as the positive control (Plasmid) for Southern blot hybridization. (**C**) Genomic PCR analysis. The primer set FC3 + BT6 was used to detect the insertion of the second loxP site (TRAF3^{flox} allele) in tail genomic DNA, while the primer set U7 + BT6 was used to detect excision of exons 1 and 2 of the TRAF3 gene (TRAF3$^{\Delta}$ allele) in splenic B cell genomic DNA.

Supplementary Figure 2



Supplementary Figure 2. Representative results of PCR screening and Southern blot analysis. (**A**) Genomic DNA was isolated from G418-resistant W4 ES clones following transfection with the TRAF3 conditional gene targeting construct, then analyzed by PCR using primers pBYneoA and G4 (shown in Supplementary Fig. 1). A 4.2 kb PCR product was amplified from the targeted TRAF3 allele (clone DS167), but no product was amplified from WT allele or the targeting construct. (**B**) Clones with homologous recombination of the TRAF3 gene identified as in A, including DS167, DS180, DS210, DS223 and DS286, were further confirmed by PCR using another set of primers: pBYneoA and G6 (shown in Supplementary Fig. 1A). (**C**) The 5.6 kb PCR products amplified by using primers FA5 and FT4 (shown in Supplementary Fig. 1A) from wild type or targeted clones were gel purified, and then digested with the restriction enzyme SpeI to determine the insertion of the first loxP site, which would incorporate an additional SpeI site. Gel purified DNA (5.6 kb) before digestion with SpeI is labeled "uncut". The predicted 526 bp band of the correctly targeted TRAF3 allele with both loxP sites inserted was detected in DS167, DS180 and DS223 clones. (**D** and **E**) Southern blot analysis. Genomic DNA was isolated from WT W4 ES cells and clones with homologous recombination of the TRAF3 gene identified by PCR. DNA was digested with the restriction enzyme SpeI, and analyzed by Southern blot using probes A (D) and Neo (E) (positions shown in Supplementary Fig. 1A). A linearized plasmid DNA of 12.3 kb, which contains probes A and Neo, was used as the positive control (Plasmid) for Southern blot hybridization. Arrows indicate the 13.55 kb band of the $TRAF3^{+}$ allele, the 9.42 kb band of the $TRAF3^{flox\text{-}FRT}$ allele, the 12.3 kb band of the positive control DNA, and the 3.5 kb band of the neo^{r} of the TRAF3 targeting construct. (**F**) Tail genomic DNA was isolated from one litter of mice generated by breeding a $TRAF3^{+/flox\text{-}FRT}$ mouse with an FLP transgenic mouse. Genomic PCR was performed using primers FC3 and BT6 (shown in Supplementary Fig. 1A). As predicted, a 533 bp PCR product was amplified from the $TRAF3^{+}$ allele, while a 615 bp PCR product was amplified from the $TRAF3^{flox}$ allele (with the neo^{r} gene excised through FLP-mediated recombination, but still keeping the insertion of one FRT and two loxP sites). The $TRAF3^{flox\text{-}FRT\ allele}$ (containing the neo^{r} gene) is predicted to give a PCR product of 2.4 kb, which was not detected.

[F] Supplemental Fig. 3
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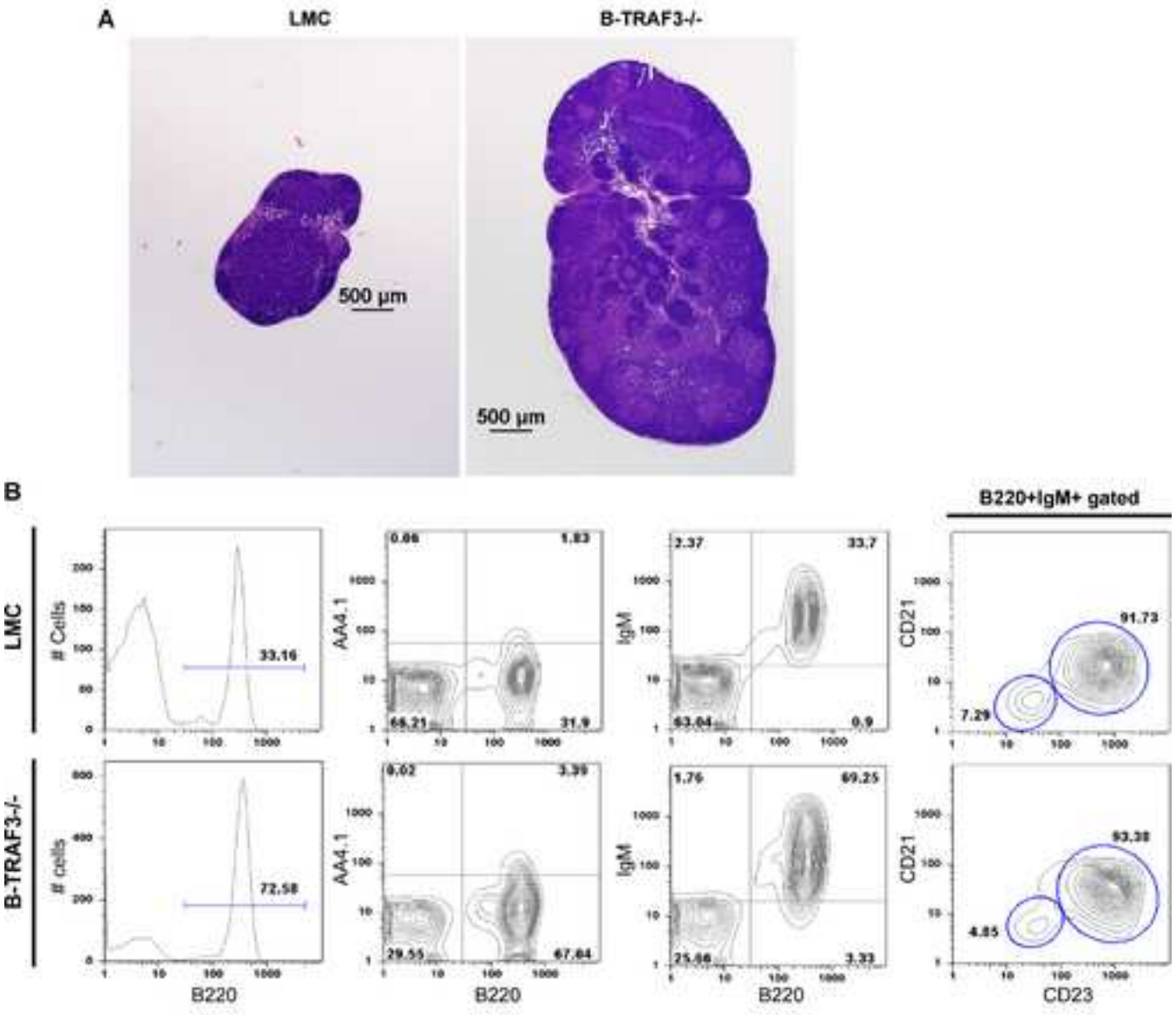
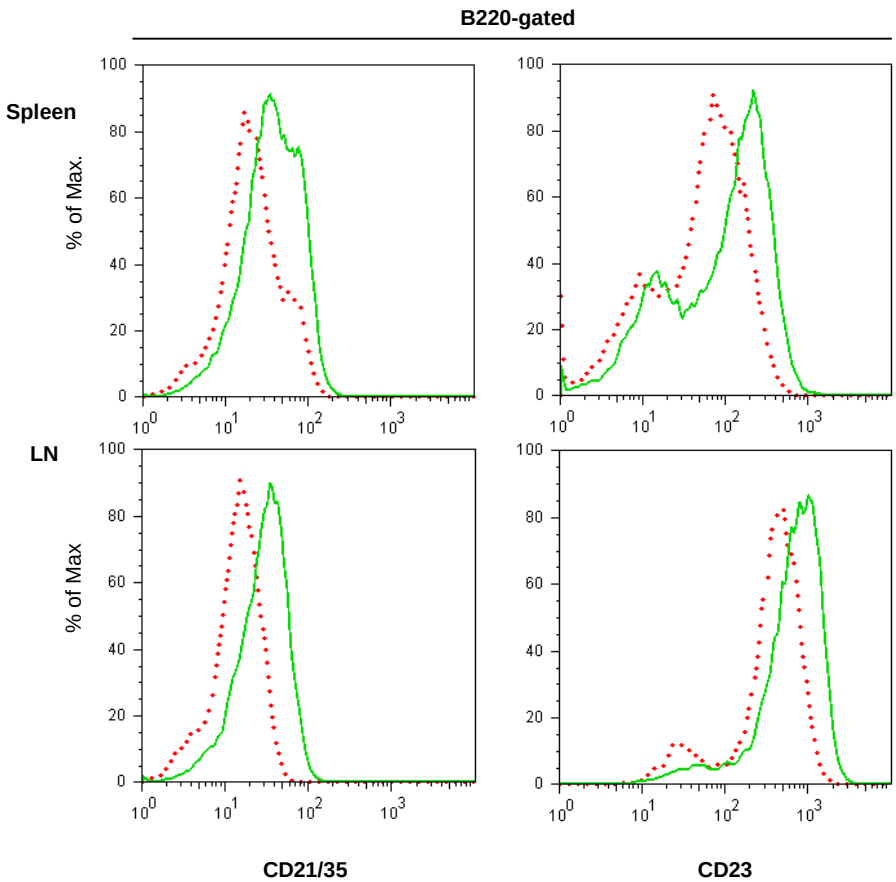
Supplementary Figure 3



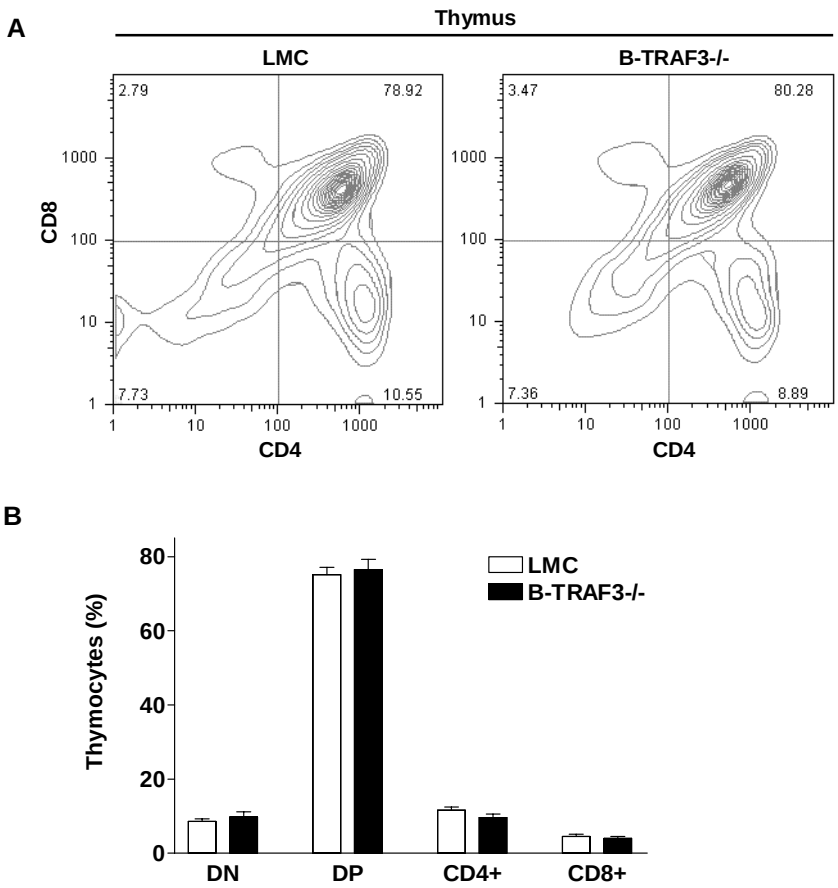
Supplementary Figure 3. Expanded B cell compartments in lymph nodes of B-TRAF3$^{-/-}$ mice. (A) Representative micrographs of cervical lymph nodes of LMC and B-TRAF3$^{-/-}$ mice stained with hematoxylin and eosin. (B) Representative FACS histograms or contour plots of lymph nodes of LMC and B-TRAF3$^{-/-}$ mice. Histograms in the left panels were scatter-gated on single lymphocytes and showed increased frequency of B cells (B220+) in B-TRAF3$^{-/-}$ mice as compared to LMC mice. Similar results were observed in three additional experiments. Mice analyzed were 8 to 12 weeks old.

Supplementary Figure 4



Supplementary Figure 4. Increased expression of CD21/35 and CD23 on splenic and lymph node B cells of B-TRAF3$^{-/-}$ mice. Expression of CD21/35 and CD23 on splenocytes and lymph node (LN) cells was determined by FACS analysis. Overlaid FACS histograms gated on B220+ lymphocytes of spleens and lymph nodes of LMC (dashed profile) and B-TRAF3$^{-/-}$ (solid profile) mice are shown. Mice analyzed were 8 to 12 weeks old. Results are representative of four independent experiments.

Supplementary Figure 5



Supplementary Figure 5. Normal thymocyte development in B-TRAF3$^{-/-}$ mice. CD4 and CD8 expression on thymocytes of LMC and B-TRAF3$^{-/-}$ mice were examined by FACS analysis. (**A**) Representative contour plots scatter-gated on lymphocytes. (**B**) Percentage of CD4^{-}CD8^{-} (DN), CD4^{+}CD8^{+} (DP), CD4^{+}, and CD8^{+} cells in the thymus of LMC and B-TRAF3$^{-/-}$ mice. Data shown are results of four independent experiments (mean ± SEM). Mice analyzed were 8 to 12 weeks old.

[F] Supplemental Fig. 6
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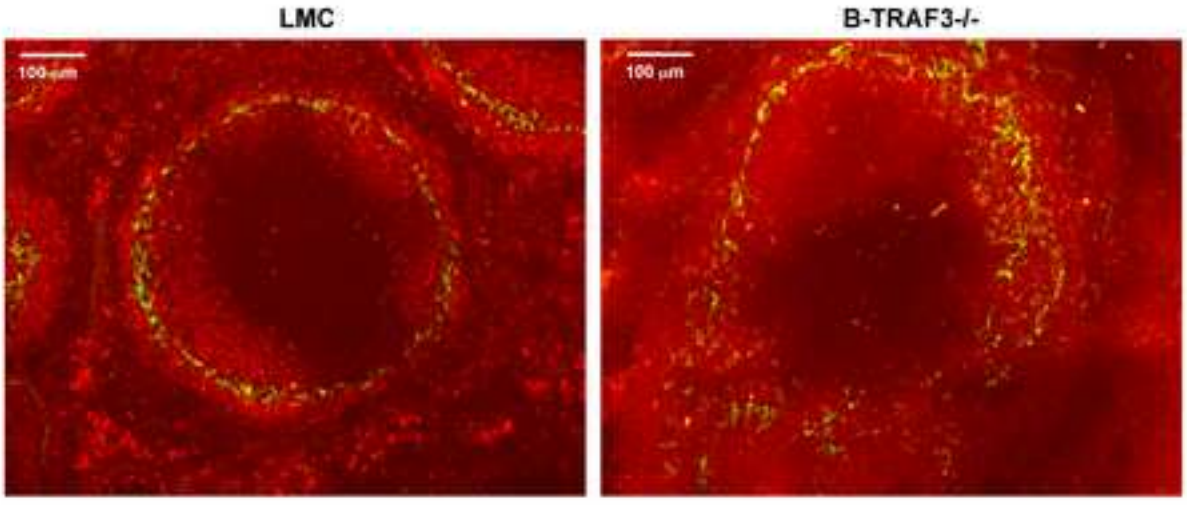
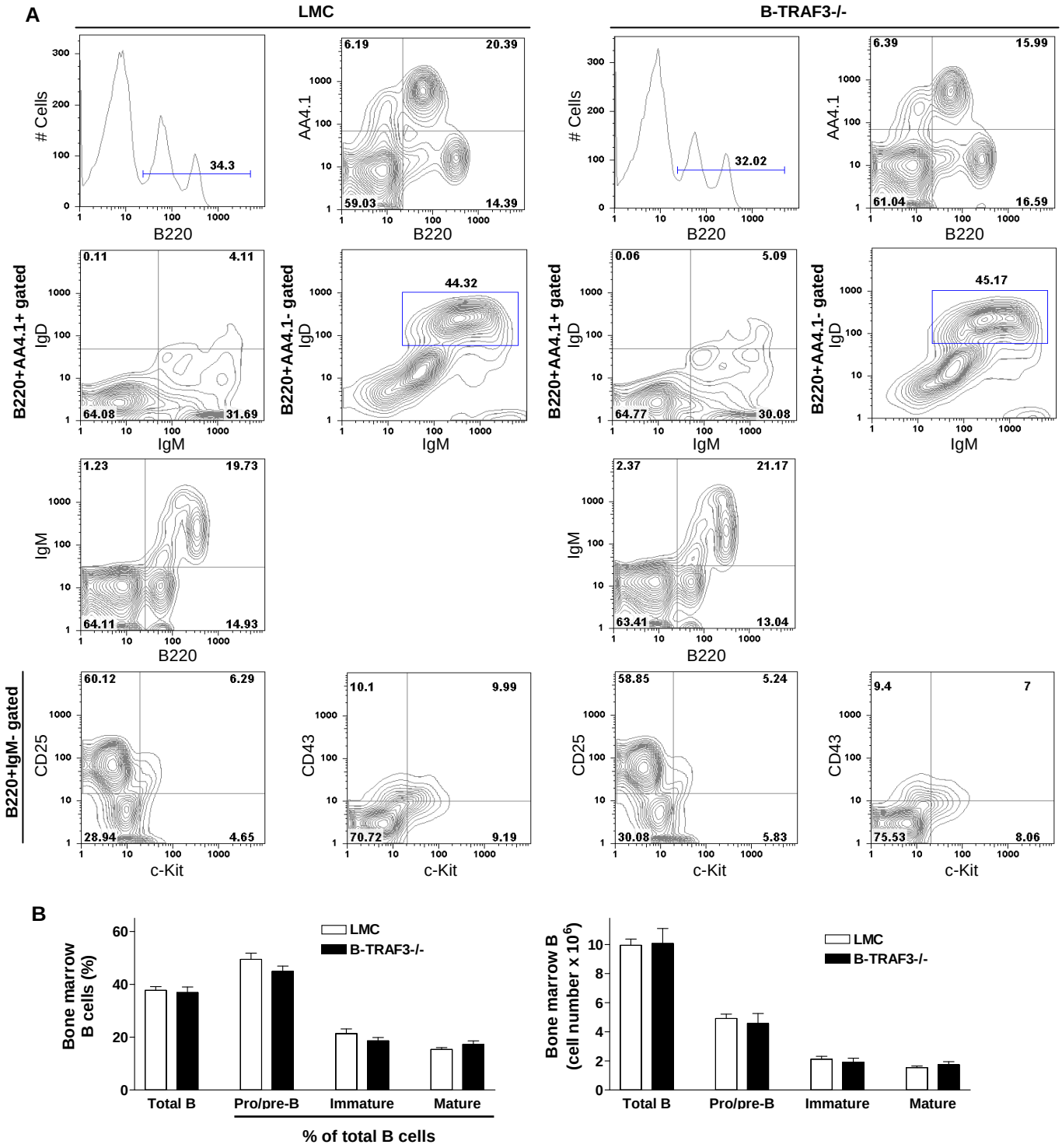
Supplementary Figure 6



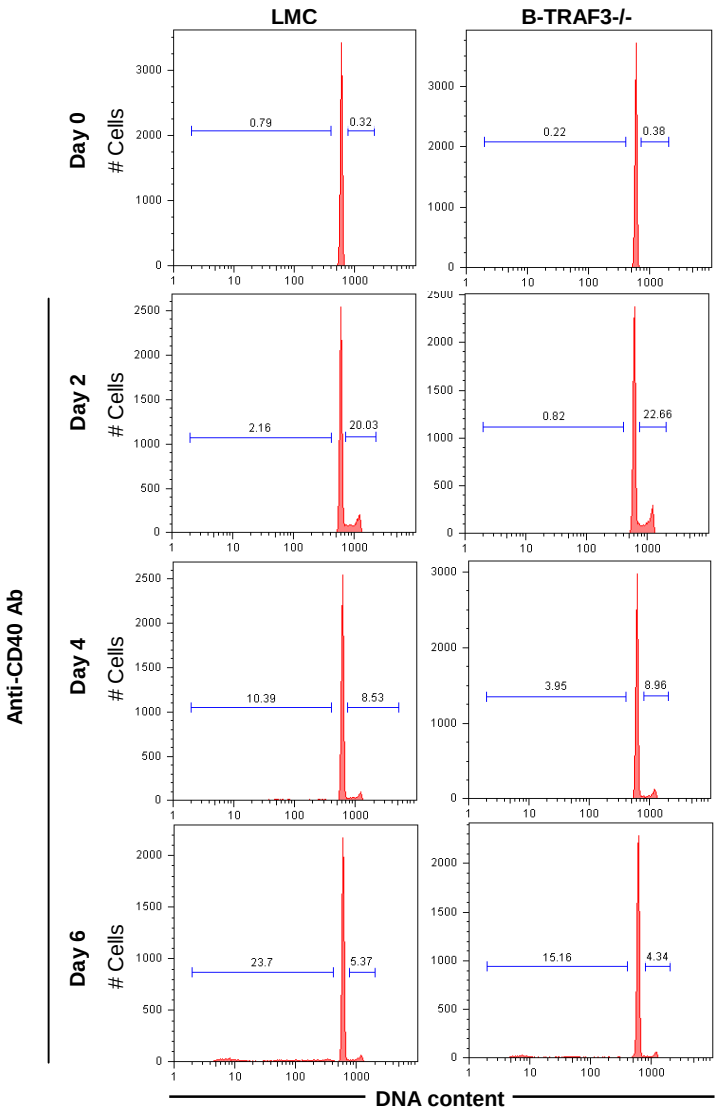
Supplementary Figure 6. Expanded marginal zone B cells in the spleen of B-TRAF3$^{-/-}$ mice. Frozen sections were prepared from spleens of naïve mice. Sections were fixed and then stained with FITC-anti-MOMA-1 (green) and PE-B220 (red). Results are representative of two independent experiments.

Supplementary Figure 7



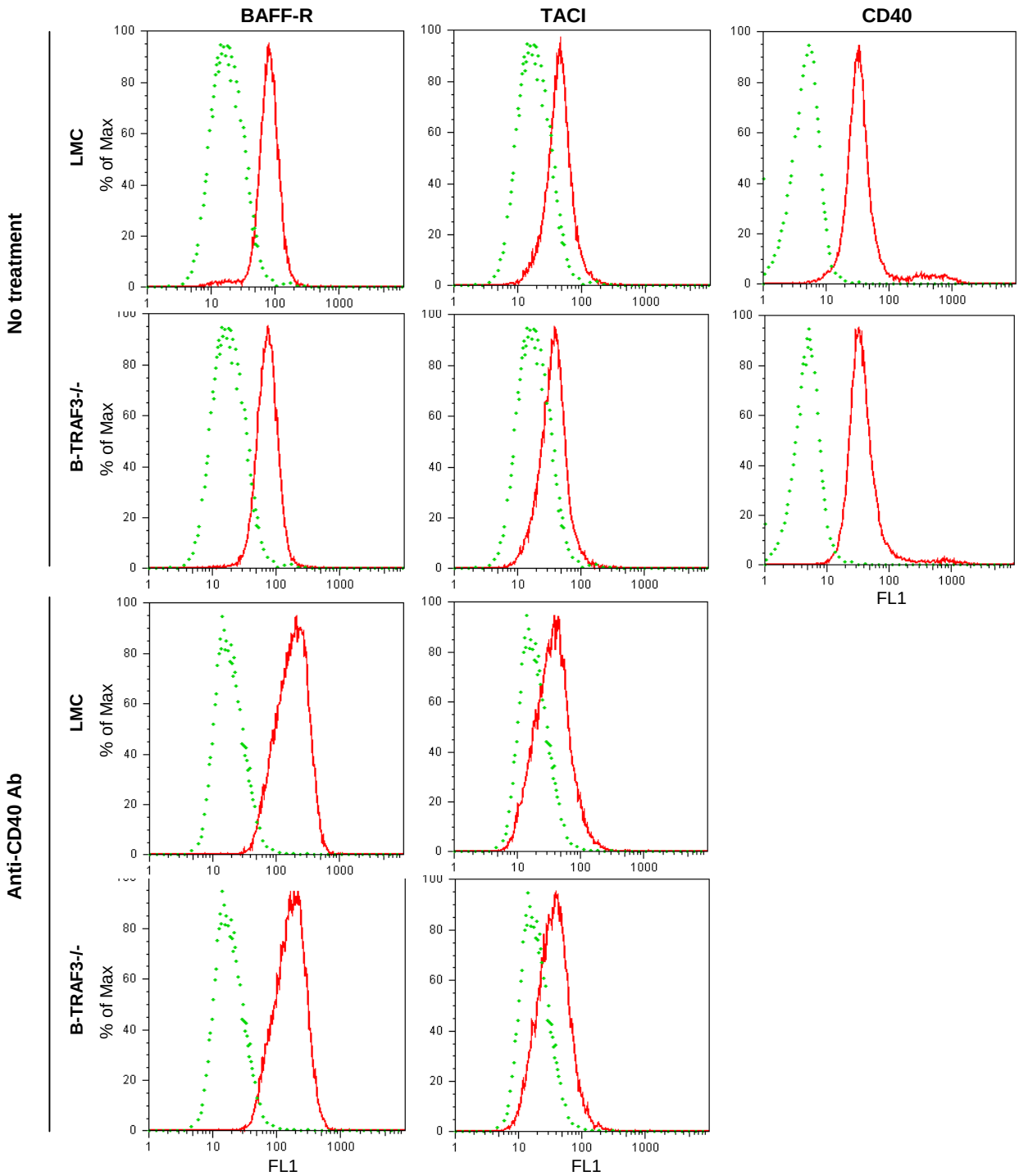
Supplementary Figure 7. Normal B cell populations in the BM of B-TRAF3$^{-/-}$ mice. Bone marrow cells were extracted from the 2 femurs of each mouse. B cell subpopulations were determined by FACS analysis. (**A**) Representative FACS histograms or contour plots of the BM of LMC and B-TRAF3$^{-/-}$ mice. Histograms in the top panels were scatter-gated on single nucleated cells and show unaltered frequency of B cells (B220+) in the BM of B-TRAF3$^{-/-}$ mice as compared to LMC mice. Similar results were observed in three additional experiments. (**B**) Percentage and numbers of B cell subpopulations in the BM of LMC and B-TRAF3$^{-/-}$ mice. Data shown are results of four independent experiments (mean ± SEM). Mice analyzed were 8 to 12 weeks old.

Supplementary Figure 8



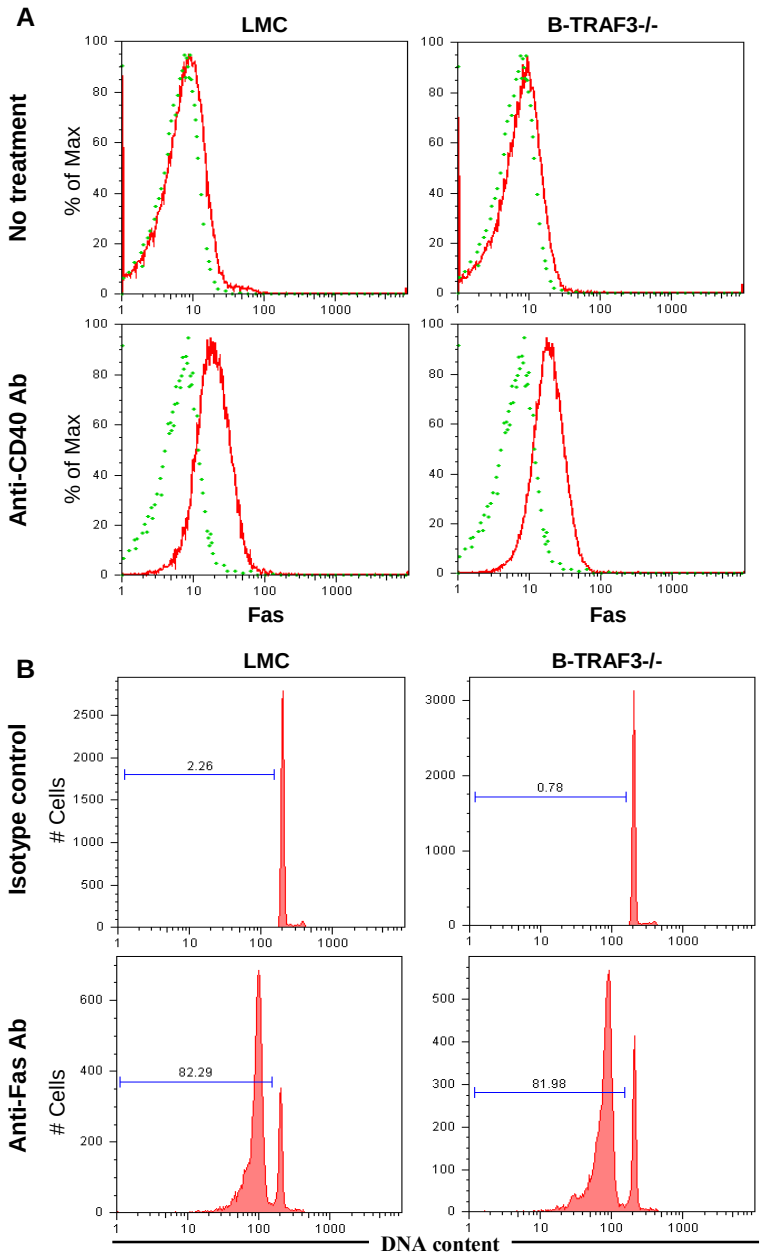
Supplementary Figure 8. Unaltered proleferation of TRAF3$^{-/-}$ splenic B cells. Resting splenic B cells were purified from 10- to 12- week-old LMC and B-TRAF3$^{-/-}$ mice, and cultured in the presence of 2 μg/ml anti-CD40 Abs. Cell cycle analysis was performed by PI staining and FACS. Representative histograms of PI staining are shown, and percentage of apoptotic cells (DNA content < 2n) and proliferating cells (2n < DNA content ≤ 4n) are indicated. Similar results were observed in two additional experiments.

Supplementary Figure 9



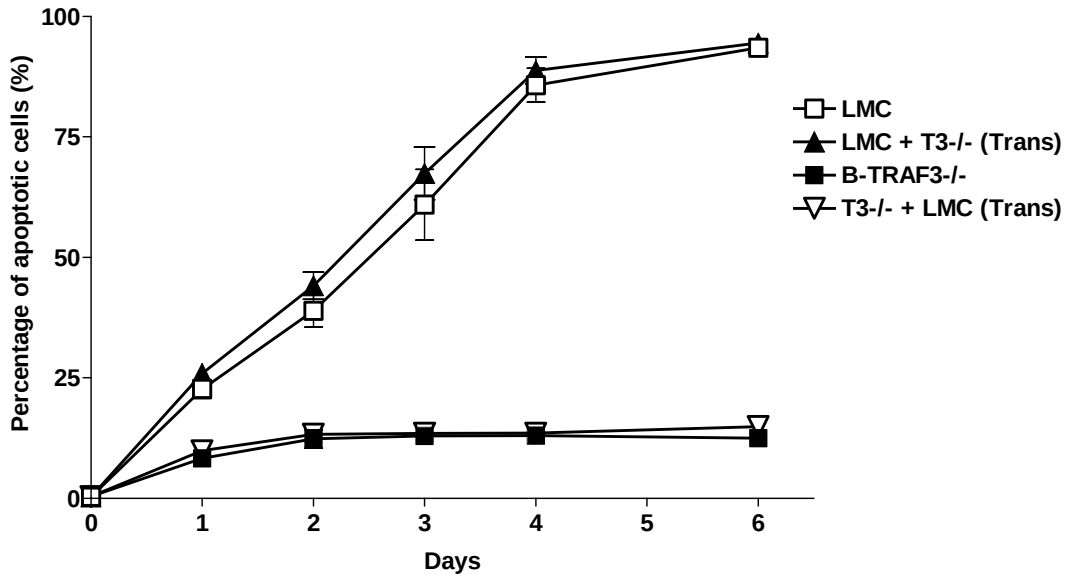
Supplementary Figure 9. Normal expression of BAFF-R, TACI and CD40 on TRAF3$^{-/-}$ resting splenic B cells. Resting splenic B cells were purified from 10- to 12- week-old LMC and B-TRAF3$^{-/-}$ mice, and cultured in the absence or presence of 2 μg/ml anti-CD40 Abs for 24 h. Expression of BAFF-R, TACI and CD40 was determined by FACS analysis. Overlaid FACS histograms of isotype control (dashed profile) and anti-BAFF-R, anti-TACI or anti-CD40 (solid profile) staining are shown. Results are representative of two independent experiments.

Supplementary Figure 10



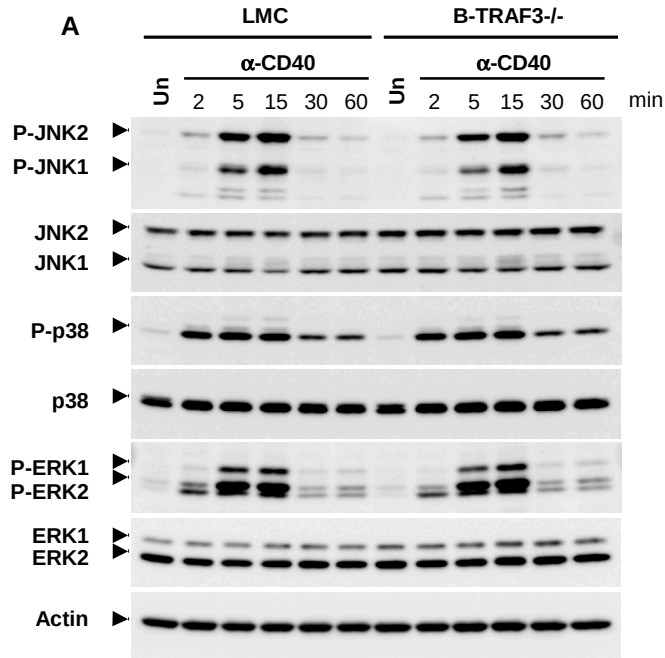
Supplementary Figure 10. Normal expression of Fas and Fas-mediated killing in TRAF3$^{-/-}$ splenic B cells. (**A**) Fas expression. Resting splenic B cells were purified from 10- to 12- week-old LMC and B-TRAF3$^{-/-}$ mice, and cultured in the absence or presence of 2 μg/ml anti-CD40 Abs for 24 h. Cells were stained with FITC-labeled isotype control or anti-Fas Abs and analyzed by FACS. Overlaid FACS histograms of isotype control (dashed profile) and anti-Fas (solid profile) staining are shown. (**B**) Fas-mediated killing. Cells as in (A) were cultured in the presence of 2 μg/ml anti-CD40 Abs for 24 h, and then incubated with 100 ng/ml of an isotype control or anti-Fas Abs for 16 h. Cell apoptosis was determined by PI staining and FACS analysis. Representative histograms of PI staining are shown, and percentages of apoptotic cells (DNA content < 2n) are indicated. Results are representative of two independent experiments.

Supplementary Figure 11



Supplementary Figure 11. Lack of involvement of soluble factors in extended lifespan of TRAF3$^{-/-}$ B cells. Resting splenic B cells were purified from 10- to 12-week-old LMC and B-TRAF3$^{-/-}$ mice and cultured in transwell co-culture plates. Cell apoptosis was examined by PI staining and FACS analysis at indicated time. Data shown are results of three independent experiments (mean ± SEM).

Supplementary Figure 12



Supplementary Figure 12. Normal activation of JNK, p38 and ERK in TRAF3$^{-/-}$ splenic B cells. Resting splenic B cells were purified from 10- to 12- week-old LMC and B-TRAF3$^{-/-}$ mice, and stimulated with 2 μg/ml anti-CD40 Abs for indicated times. Total cellular lysates were immunoblotted for phosphorylated (P-) or total JNK, p38 and ERK, followed by actin. Similar results were observed in two additional experiments.

[F] Supplemental Fig. 13
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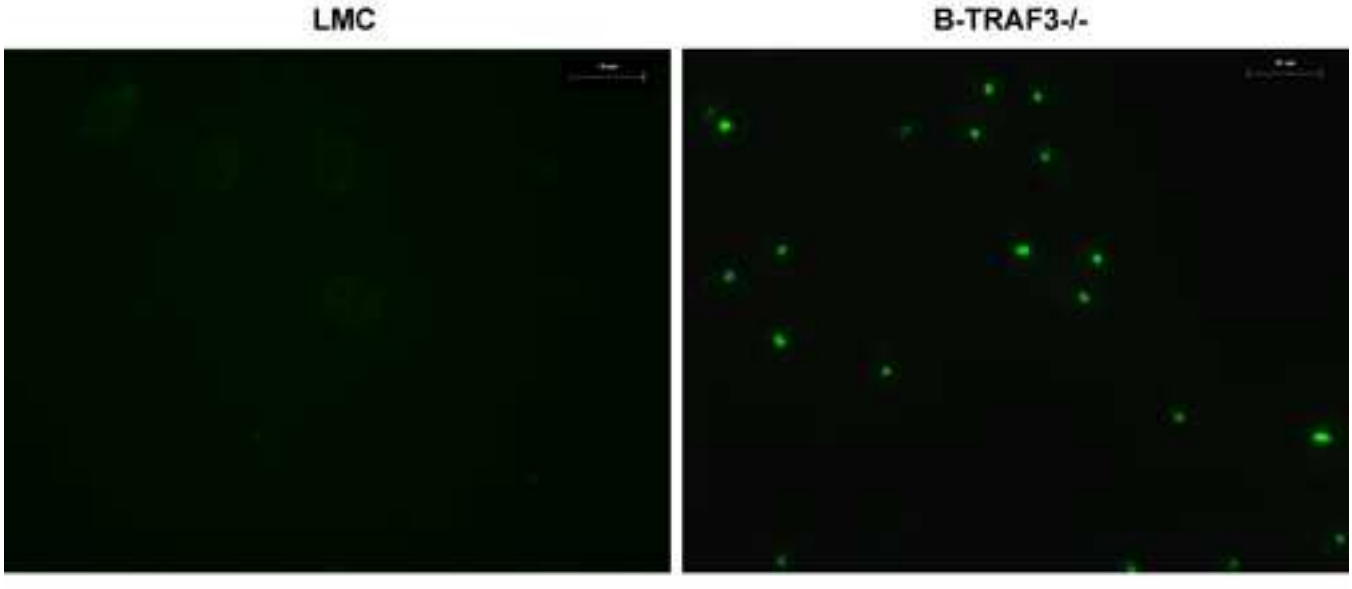
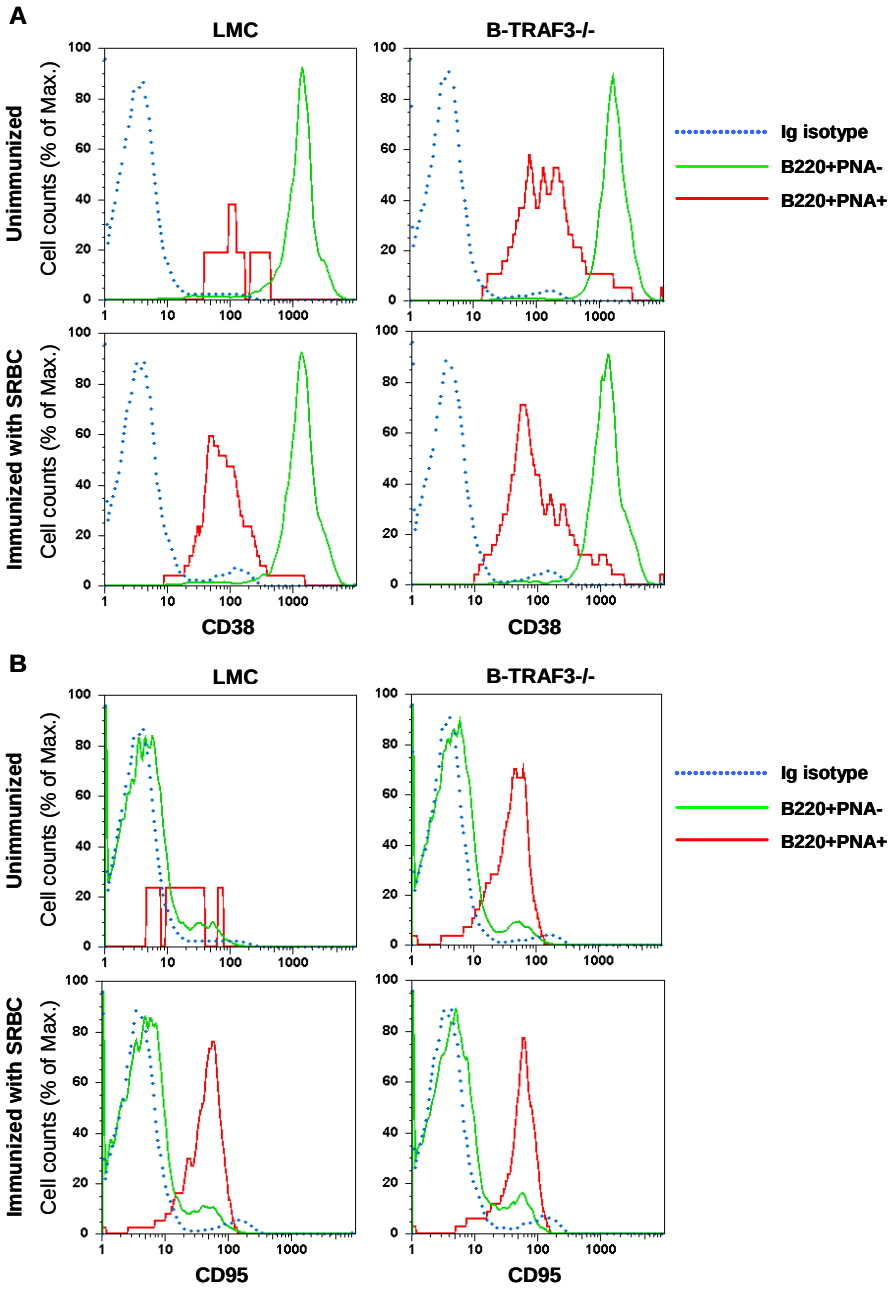
Supplementary Figure 13



Supplementary Figure 13. Representative results of anti-dsDNA autoantibodies detection by a Crithidia luciliae dsDNA kit. Sera from naïve LMC and B-TRAF3$^{-/-}$ mice were diluted 1:100 or 1:500 folds and tested for anti-dsDNA autoantibodies by a Crithidia Luciliae dsDNA kit. Mice analyzed were 10 to 12 weeks old.

Supplementary Figure 14



Supplementary Figure 14. Lower levels of CD38 and higher levels of CD95 (Fas) on GC B (B220+PNA+) cells as compared to non-GC B (B220+PNA-) cells. Expression of CD38 and CD95 on splenocytes was determined by FACS analysis. Littermate control (LMC) and B-TRAF3-/- mice with or without SRBC immunization were examined. Overlaid FACS histograms of CD38 (A) and CD95 (B) are shown. Dashed profile depicts Isotype control staining of splenocytes, green profile depicts CD38 (A) or CD95 (B) staining of B220+PNA- cells, and red profile depicts CD38 (A) or CD95 (B) staining of B220+PNA+ cells. Results are representative of three independent experiments.