Supplementary Materials and methods

BAFF measurement

Mouse peripheral blood was drawn from retro orbital sinus using 0.5 ml insulin syringe with BD Ultra-Fine^{TM} 31G needle (BD) and collected into a 1.5 ml microcentrifuge tube (USA Scientific INC) with heparin (Sigma, 30 Units/ml). Plasma was collected by centrifugation at 600g for 5 min at 4 ˚C and stored in -80 ˚C until soluble BAFF was measured using an ELISA kit (R&D Systems).

Flow cytometric analyses of B cell subsets

Red blood cells (RBC) were lysed with ammonium-chloride-potassium (ACK) buffer (0.15 M NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA, purchased from Fisher Scientific) before cells were stained with anti-CD3-FITC (17A2), anti-CD19-Brilliant Violet 421^{TM} (6D5), anti-H-2K^{b}-PE (AF6-88.5), anti-H-2K^{d}-PE/CY7 (SF1-1.1), anti-CD45-APC (30-F11), anti-CD16/32 (TruStainFcX^{TM}) and 7-AAD for 30 min on ice. CountBright^{TM} absolute counting beads (Thermo Fisher Scientific) were used to enumerate donor T or B cells. Immature, activated B cell subsets and BAFF receptors were determined using Zombie Aqua^{TM} (Biolegend), anti-CD93-PE (AA4.1, BD Bioscience), anti-GL-7-PerCP/Cyanine5.5 (GL7), anti-CD19-Brilliant Violet 421^{TM}, anti-NOTCH2-APC (HMN2-35), anti-CD21-APC/CY7 (7E9), anti-CD23-FITC (B3B4), anti-BAFF-R-PE (7H22-E16), anti-BCMA-FITC (161616, R&D Systems) and anti-TACI-APC (ebio8F10-3, ThermoScientific). All antibodies were from Biolegend if not otherwise indicated. The stained cells were detected by Canto II flow cytometry and results were analyzed by flowjo software (BD Bioscience).

BAFF-R occupancy test

For excess volume culture BAFF dissociation assay (Sarantopoulos S. Blood. 2011; 117(7):2275-83.), the splenocytes, purified B cells or peripheral blood cells from BAFF-Tg mice were cultured in 4 ml RPMI with 10% FBS for 2.5 hrs. The cells were pipetted every 30 min. For acid elution assay, splenocytes, purified B cells from splenocytes or peripheral blood cells from BAFF-Tg mice

were pelleted and resuspended in citrate buffer (0.1M citric acid, 0.2M Na_2HPO_4 in 150mM PBS, pH2.4) for 1 min at RT (Smulski CR. Cell Rep. 2017;18(9):2189-2202). Citric acid and Na_2HPO_4 were purchased from Millipore Sigma. Acid treatment was stopped by PBS. Then excess volume cultured cells and the citrate buffer treated cells were stained with Zombie Aqua^{TM} viability kit first for 15 min at RT, then furtherly stained with anti-CD19-Brilliant Violet 421(6D5, Biolegend), anti-BAFF-R-PE (7H22-E16, Biolegend), for another 30min on ice. After washing, the cells were analyzed by flow cytometry. The results were similar if using purified spleen B cells, peripheral blood B cells or splenocytes B cells. Data from blood or splenocytes were shown in manuscript.

Phosphoflow and intracellular staining

Peripheral blood cells were incubated for 15 min at 37 °C water bath after lysis of RBC. Then the blood cells were stimulated with anti-IgM (10 μg/ml, $F(ab')_2$ fragment and μ chain specific, Jackson ImmunoResearch Laboratories) for 5 min at 37 °C. Stimulation was stopped with fixation/permeabilization solution (eBioscience^{TM} Foxp3/Transcription factor staining buffer set, Thermo Fisher Scientific) and the fixation of cells was incubated at RT for 20 min. After washing once with permeabilization buffer, anti-B220-Pacific Blue (RA3-6B2, Biolegend), anti-pSYK-FITC (I120-722, BD-Bioscience) and anti-pBLNK-APC (J117-1278, BD-Bioscience) were added and incubated for another 1 hour at RT before analyzing by flow cytometry. For total mouse SYK or BLNK protein expression, cells were stained with anti-B220-Pacific blue (RA3-6B2, Biolegend), anti-SYK-PE (5F5, Biolegend) and anti-BLNK-FITC (2B11, BD Bioscience). Proliferating B cells were also identified by anti-Ki-67-APC (SolA15, Thermo Fisher Scientific) intracellular staining using above method and flow cytometric analysis.

CFSE dilution assay

Spleens from disease mice, control mice or syngeneic BMT mice were isolated and splenocytes were prepared. After red blood cells were lysed with ACK buffer, the splenocytes were loaded

with 5 μM CFSE (Molecular Probes, ThermoFisher Scientific) in PBS containing 5% FBS for 5 min at RT. Then CFSE loaded cells were washed 3 times with 5% FBS-PBS. After washing, the CFSE loaded cells were stimulate with anti-IgM (10 μg/ml, Jackson ImmunoResearch Laboratories) for 5 days. The CFSE diluted cells were detected by flow cytometry.

Immunohistochemistry (IHC) staining

Lung and liver tissues from cGVHD and control mice at Day 83 post-BMT were harvested and fixed in 4% paraformaldehyde (Santa Cruz Biotechnology) in PBS overnight. Embedding tissues in paraffin, section preparation and immunohistochemistry staining were performed in Research Immunohistology lab at Duke Pathology. Briefly, the antigen retrieval was processed in 10 mM sodium citrate (pH 6.1) at 100 °C for 20 min. Anti-CD3 (RM-9107-S, ThermoScientific) or B220 antibody (RA3-6B2, Biolegend, San Diego, CA) were incubated with sections for 45 min at RT. After washing, the sections were incubated with biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) for another 30 min at RT. Then the sections were incubated with avidin-biotin complex with HRP enzyme (Vector Laboratories) for 30 min at RT after washing. Finally, the staining was visualized with DAB substrate (Vector Laboratories) after washing and developed for 5 min. The images of lung and liver IHC staining were captured by microscope (BX46, Olympus, Waltham, MA) with camera SPOT Idea (model 28.2, 5MP, SPOT IMAGING^{TM}, Sterling Heights, MI).

Quantitating the images by Image J

Open Image J (free download website: https://imagej.nih.gov/ij/download.html).In the menu Plugins, select IHC tool box tools to open IHC Tool Box window. IHC Tool box download website: https://imagej.nih.gov/ij/plugins/ihc-toolbox/index.html. Open image file in Image J. Select a most represented small area of positive staining, then Click Training in IHC Tool Box (This step tells ImageJ the positive staining color for IHC or fluorescence). Then new window of positive staining

image named Colour Filter and another window named Color Chooser box are opened. Adjust the Choose Threshold to let the image in Colour Filter to best represent the positive staining area. Select Adjust Color Threshold in menu of Image to open Threshold Color box. Adjust Brightness, using Red color to mark the positive staining area in Colour Filter image. Click Select in Threshold Color box to select the positive staining area. In the menu of Analyze, click Set Measurements. Select Area for IHC images and select Integrated density for fluorescence to measure the selected area. Click the Measure in the menu of Analyze to get the value of area or integrated density.

Stromal cell isolation and cell sorting

The spleen, 4 cervical, 2 inguinal, and 2 brachial lymph nodes were isolated from each mouse and digested with RPMI 1640 medium containing collagenase P (0.2mg/ml, Sigma), Dispase II (0.8mg/ml, Sigma) and DNase I (100u/ml, Qiagen) at 37 ℃ for 45 min (Fletcher AL. Front Immunol. 2011; 2: 35). Cells released into solution were collected every 15min and added into stop solution (PBS, 2% FBS, 5mM EDTA). After RBCs were lysed, leukocytes were removed with anti-CD45-biotin antibody (30-F11) and EasySep™ Strepavidin RapidSpheres™ beads (Stemcell Technologies). Cells were stained with Zombie Aqua™ for 15min at RT, and then stained with anti-CD45-APC (30-F11), anti-podoplanin (PDPN)-PE (8.1.1), anti-CD31-Pacific Blue (390), anti-H-2K^{d}-PE/CY7 (SF1-1.1) and anti-MadCam-FITC (MECA-367) (all from Biolegend) for another 30min on ice. The Zombie^{-}CD45^{-}H-2Kd^{+}anti-PDPN^{+}CD31^{-}MadCam^{-} fibroblastic reticular cells (FRC) were sorted and counted by flow cytometry (MoFlo Astrios EQ cell sorter, Beckman). Viably frozen peripheral blood mononuclear cells (PBMCs) from HCT patients were obtained with institutional review board protocols from Duke University, the National Cancer Institute (NIH), and the Dana-Farber Cancer Institute in accordance with the Declaration of Helsinki. Mouse CD4^{+} T cells and patient CD4^{+}CXCR5^{+} T_{FH} were sorted via flow cytometry (purity >95%) before RNA extraction.

Conjunctiva tissue cell preparation

Conjunctiva tissue was isolated and digested in collagenase P (0.2 mg/ml, Sigma) and DNase I (100u/ml, Qiagen) in RPMI without serum at 37 ℃ for 45 min. The digestion was stopped with PBS containing 2% FBS and 5mM EDTA (pH 7.4). The red blood cells were lysed with ACK buffer. The cell population was analyzed by surface staining and flow cytometry. Staining panels: Zombie Aqua™, anti-CD45-PE/CY7 (30-F11), anti-CD19-Brilliant Violet 421™ (6D5), anti-CD3-FITC (17A2), anti-GL7-PerCP/Cyanine5.5 (GL7) and anti-Ly6G (1A8). All antibodies and Zombie Aqua™ are from Biolegend.

qPCR BAFF transcript measurement

Total RNA were extracted using RNeasy Plus Mini kit (Qiagen). cDNA was synthesized using iScrip™ cDNA Synthesis Kit (Cat# 1708891, BIO-RAD). Bio-Rad iTaq Universal SYBR Green Supermix (Cat# 1725121) qPCR reagents and ThermoFisher-ABI StepOne Plus System were used. Delta-delta Ct method was used to calculate the relative BAFF expression level. Cycle threshold (Ct) value of GAPDH is subtracted from Ct value of BAFF to normalize the input amount of cDNA. The difference of Ct values is named ΔCt. Set the average of BM only group as the calibrator. Subtract the ΔCt of calibrator from the ΔCt of sample and name the new difference $\Delta\Delta$Ct. The fold change was calculated by the equation $2^{-\Delta\Delta Ct}$. Mouse BAFF expression was measured by qPCR using the following two primers (designed by primer-BLAST of NCBI): TTGCAAGCAGACCTGATGAAC (forward) and TGCCGGTGTCAGGAGTTTG (reverse). Normalization control mouse GAPDH: CGTCCCGTAGACAAAATGGT (forward) and TTGATGGCAACAATCTCCAC (reverse). Primers for human BAFF qPCR: ACCGCGGGACTGAAAATCT (forward) and CACGCTTATTTCTGCTGTTCTGA (reverse). Normalization control human Actin: GCTGTGCTACGTCGCCCT (forward) and AAGGTAGTTTCGTGGATGCC (reverse).

In vitro NOTCH2 stimulation assay

Peripheral blood was withdrew from recipients receiving BM only or BAFF-Tg BM only cells. After red blood cells were lysed by ACK buffer, the blood cells were stimulated with anti-IgM (1 μg/ml or 5 μg/ml, Jackson ImmunoResearch Laboratories) overnight for 18hrs. The cells were stained with7-AAD, anti-CD19-Brilliant blue 421 (6D5), anti-NOTCH2-APC (HMN2-35) (all from Biolegend) for 30 min on ice. The expression of NOTCH2 was analyzed by flow cytometry. The B cell population was gated on 7-AAD^{-}CD19^{+} live cells. To test the response to NOTCH ligand stimulation, OP9 and OP9-DL1 cell lines were used, which were provided by Dr. Yuan Zhuang in Duke Immunology. OP9 or OP9-DL1 cells were pre-seeded in 96-well-plate and blood cells from BM only or BAFF-Tg BM only were added after red blood cells were lysed. The cells were cultured with or without anti-IgM stimulation for 48 hrs. The cells were stained with 7-AAD and B220-Pacific blue for 30 min on ice, and then fixed after washing. The proliferation of B cells were checked by anti-Ki-67 intracellular staining and analyzed by flow cytometry.

Analyzing the stability and degradation of SYK

To test the degradation of SYK after BCR stimulation, splenocytes from BAFF-Tg or wild type control mice were stimulated with anti-IgM (10μg/ml, Jackson ImmunoResearch Laboratories) overnight (for 18 hrs). Then the B cells were purified using EasySep^{TM} mouse B cells isolation kit (Stemcell Technologies), The expression of SYK were visualized by Western blot with Anti-SYK antibody (D3Z1E) from Cell signaling. For cycloheximide chase assay, B cells were purified from spleen of BAFF Tg mice or wild type mice using EasySep^{TM} mouse B cell isolation kit. Purified B cells were treated with cycloheximide (10 μg/ml, Fisher Scientific) overnight and then stimulated with anti-IgM (10 μg/ml,) for 4, 6, 8, 10 and 12 hrs, respectively. The expression of SYK was visualized by Western blot.

Western blot

Cell lysates were prepared with Complete M lysis buffer (Roche, MilliporeSigma, St. Louis, MO). Protein concentration of cell lysates were measured by Pierce™ BCA protein assay kit (ThermoFisher Scientific, Rockford, IL). Proteins were separated on NuPAGE™ 4 to 12% Bis-Tris Mini Protein gel and then transferred to PVDF membrane using iBlot™ system (invitrogen™, ThermoFisher Scientific). PVDF membrane was block with 5% nonfat milk in TBS for 1 hour at RT and then probed with anti-SYK (Clone D3ZIE, Cell signaling Technology, Danvers, MA) overnight at 4°C. After washing with TBS (Corning) containg 0.02% Tween 20 (Bio-Rad) for three times at RT. The PVDF membrane was incubated with working concentration anti-rabbit IgG-Alexa Fluor 680 (A-21109, Molecular Probes, ThermoFisher, Eugene, OR) at RT for 1 hour. Loading control was checked with anti-actin (clone I-19, Santa Cruz Biotechnology, INC, Dallas, TX) for 2 hrs at RT. After washing, the membrane was incubated with IRDye 800CW Anti-goat IgG (926-32214, LI-CRO, Lincoln, NE) for one hour at RT. After washing, the target band were detected by Odyssey imaging system (LI-COR). The intensity of SYK and actin was analyzed by Image Studio Lite software (LI-COR).

Auto-antibodies detection

Salivary glands tissue from female RAG2 knockout mice on C56BL/6 background (008449, Jackson laboratory) or BALB/c background (601-F, Taconic) were isolated and cryo-sections were prepared. The cryo-sections were fixed in pre-cold acetone at -20 °C for 10 min and washed in PBS. The tissue sections were blocked with anti-mouse CD16/32 antibody (TrueStain FcX™, Biolegend) and goat plasma (Stemcell Technologies) for 1 hour at RT. Plasma from recipient mice at Day 63 post-BMT were incubated with cryo-sections at 4°C overnight (Wu T. J Immunol 2013; 191:488-499). After washing with PBS, the sections were incubated with goat anti-mouse IgG-Alexa Fluor 488 (Fc fragment specific, Jackson Immuno Research Laboratories, INC. West Grove, PA) for one hour at RT. After washing, the fluorescence were detected and images were taken by fluorescence microscope with ApoTome (Axiovert 200M, ZEISS) at X200 amplification lenses.

No obvious fluorescence signals were found on B6 salivary glands tissue sections (data not shown). The fluorescence intensity of image per field were quantitated by Image J software.

Statistics. Body weight changes after BMT were analyzed by repeated ANOVA method. Data of two groups were performed using unpaired and nonparametric test (Mann-Whitney test) or unpaired t test with Welch's correction, and multiple groups data were analyzed by ordinary one-way ANOVA with Tukey's multiple comparisons test, one-way ANOVA nonparametric test (Kruskal–Wallis test), ordinary two way ANOVA or multiple t test with few assumptions using Prism 8 software (GraphPad Prism version 8.4.3) with $p<0.05$ denoting a significant difference.

Supplementary Figure Legends

Supplementary Figure 1. Mouse model with manifestations of chronic GVHD. (A) Increased collagen deposition in lung and liver of cGVHD mice (Masson's Trichrome; 100x). (B) Increased B cell infiltration in lung and liver tissues from cGVHD mice (anti-B220 mAb; 100x). (C) Increased T cell infiltration in lung and liver tissues from cGVHD mice (anti-CD3 mAb; 100x). Images were captured by Olympus BX46 microscope with SPOT Idea 28.2 5 MP camera. The area of positive staining per lung bronchus and liver portal triad was quantified from ≥3 different images of each individual mouse sample by Image J software and the results were summarized in figures of right panels. The lung and liver tissues were collected at Day 83 after allo-BMT and serial sections were stained as described in A-C. N=10 (BM only) and n=9 (BM+Sp). Statistical analysis was performed by Mann-Whitney test using GraphPad Prism 8 software. *$P< .05$ and **$P< .01$.

Supplementary Figure 2. Soluble BAFF production is primarily recipient-derived after allogeneic allo-BMT. (A) B cell numbers at three time points (Day 19, 42, and 61) after allo-BMT in recipients receiving BAFF KO BM only versus BAFF KO BM+Sp. (B) Serial post-transplant soluble BAFF levels per blood B cell (so-called BAFF:B cell ratios) in recipients receiving BAFF KO that developed cGVHD manifestations (BAFF KO BM+Sp) compared to BAFF KO BM alone control mice (BAFF KO BM only) without disease manifestations (n=10 in each group). (C) BAFF transcript expression in donor cell populations. $CD8^+$, $CD19^+$, $Gr\text{-}1^+CD11b^+$ or $Gr\text{-}1^-F4/80^+$ cell populations were sorted through surface staining and flow cytometry from splenocytes at Day 50 (BM only and BM+Sp) or Day 54 (Syn) post-BMT. The purity of each population is > 95%. BAFF transcript levels were measured by qPCR and analyzed by ΔΔCT method. The data was normalized to a total BM cells sample from BM+Sp group mouse (set as 1).The fold change was calculated by the equation $2^{-\Delta\Delta Ct}$ (Fold change = $2^{-\Delta\Delta CT}$). No significant difference was found in (C) between BM only and BM+Sp. N=5 of each group. Some samples were below detectable range in the qPCR experiments. Statistical analysis was performed by Mann-Whitney test

(Supplementary Figure 2A and 2B) or Kruskal–Wallis test (Supplementary Figure 2C) using GraphPad Prism 8 software. $^{**}P < .01$, $^{***}P < .001$ and $^{****}P < .0001$.

Supplementary Figure 3. Consistent low B cell number in cGVHD mice. (A) B cell numbers of peripheral blood were calculated overtime by flow cytometry in cGVHD mice overtime post-BMT. The data shown is representative of three repeats. N=10 (BM+Sp and BM only) and n=5 (Syn). (A) Splenocyte number. The splenocytes from allo-BMT BALB/c recipients receiving wild type BM only, wild type BM+Sp, BAFF-Tg BM only or BAFF-Tg BM+Sp, as well as C57BL/6 recipients receiving BAFF-Tg BM only (Syngeneic BAFF-Tg) were isolated and counted at day 84 or day 92 post-BMT. (B) B cell number per spleen. B cell frequency was detected by flow cytometry and the cells were gated on Zombie^{-}CD19^{+} cells. B cell number was calculated using splenocyte number and B cell frequency. (C) Representative flow cytometric profiles of CD93^{+} transitional B cells that were pre-gated on CD19^{+} live cells (not shown) from blood samples at day 42 post-BMT. The numbers represent percentage of CD19^{+}CD93^{+} cells. Statistical analysis was performed by Kruskal–Wallis test (Supplementary Figure 3A) or ordinary one-way ANOVA with Tukey's multiple comparisons test (Supplementary Figure 3B and 3C) using GraphPad Prism 8 software. $^{*}P < .05$, $^{***}P < .001$ and $^{****}P < .0001$. NS, not significant ($P > .05$).

Supplementary Figure 4. The expression of TACI and BCMA. (A-B) Transmembrane activator and CAML interactor (TACI) and B-cell maturation antigen (BCMA) were detected by flow cytometry on blood B cells from mice with or without cGVHD on Day 34-36 post allo-BMT. B cells were gated on 7-AAD^{-}CD19^{+} cells and the median fluorescence intensity (MFI) of TACI (A) or BCMA (B) staining was determined. These data were pooled from two separate experiments. N=21 (BM only), n=15 (BM+Sp) and n=10 (Syn, Syn BM only = 5 and Syn BM+Sp = 5 combined). Statistical analysis was performed by Kruskal–Wallis test using GraphPad Prism 8 software. $^{*}P < .05$. NS, not significant ($P > .05$).

Supplementary Figure 5. BCR hyper-responsiveness and surface IgM expression levels on B cells from cGVHD mice. (A) Representative dot plots of intracellular phospho-flow staining of peripheral blood cells taken at Day 54 post-BMT and gated on B220$^+$ cells with anti-phosphorylated SYK (pSYK, pY348, left panels) and anti-phosphorylated BLNK (pBLNK, pY84, right panels) after stimulation with anti-IgM (10μg/ml) for 10min at 37°C. The stimulation was stopped by fix/perm buffer prior to surface and intracellular staining described above. (B) Comparison of the frequency of pBLNK$^+$ B cells from BM only versus BM+Sp mice. N=5 of each group. (C, D) The expression level of IgM on B cells as detected by flow cytometry. Peripheral blood cells were stained with anti-IgM, anti-CD19, anti-GL7 and Zombie Aqua^{TM} after red blood cells were lysed at Day 36 post-BMT. The MFI of surface IgM was assessed, with gating on Zombie$^-$CD19$^+$ total B cells (C) or on Zombie$^-$CD19$^+$GL7$^+$ B cells (D). N=10 (BM only), n=8 (BM+Sp) and n=5 (Syn). (E) BAFF together with BCR stimulation promotes GL7 expression. B cells from blood of naïve B6 mice were treated with 5 μg/ml anti-IgM or vehicle control overnight (18hrs) with or without additional BAFF (5 ng/ml). The cells were then harvested and the MFI of GL7 on CD19$^+$ B cells was measured by flow cytometry. N=5. Statistical analysis was performed by unpaired t test with Welch's correction (Supplementary Figure 6B) or ordinary one-way ANOVA with Tukey's multiple comparisons test (Supplementary Figure 6C-6E) using GraphPad Prism 8 software. *P< .05, **P< .01, ***P< .001 and ****P< .0001. NS, not significant (P>0.05).

Supplementary Figure 6. Circulating blood CD19$^+$GL7$^+$ B cells are not equivalent to germinal center B cells. (A) The expression of CD95 and GL7 on circulating blood B cells was analyzed by surface staining and flow cytometry at Day 84 post-BMT. The cells were pre-gated on Zombie$^-$CD19$^+$ live B cells. The numbers indicate percentage of cells within each quadrant. The representative flow cytometry dot plots are from 5 mice of each group tested and results are

similar. (B) CD95 was highly expressed on $CD19^-$ non-B cells as assessed on $Zombie^-CD19^+$ or $Zombie^-CD19^-$ lymphocytes from representative BM only recipient mouse. Dashed line represents isotype ctrl antibody staining. This representative flow cytometry histogram is used to indicate that anti-CD95 antibody is working in surface staining shown in Supplementary Figure 6A. Total 5 mice in each group were tested as shown in Supplementary Figure 6A and results are similar. (C) The frequency of $GL7^+$ B cells in spleen. The percentage of $CD19^+GL7^+$ B cells was assessed by surface staining and flow cytometry over time post-BMT. The cells were pre-gated on $Zombie^-CD19^+$ live B cells. Data represent the combined results from five separate experiments. Syngeneic BMT represents BALB/c recipient mice receiving BALB/c BM+Sp. N=10 (Day 8 BM only, Day 8 BM+Sp and Day 30/40 BM only), n= 9 (Day 30-40 BM+Sp), n=11 (Day 84 BM only), n=4 (Day 84 BM+Sp), n=5 (Day 8 Syn) and n=4 (Day 30 Syn). Day 84 Syn was not done. Statistical analysis was performed by Kruskal–Wallis test (Supplementary Figure 6C Day 8 and Day 30-40) or Mann-Whitney test (Supplementary Figure 6C Day 84) using GraphPad Prism 8 software. *$P< .05$ and **$P< .01$. NS, not significant ($P> .05$).

Supplementary Figure 7. Representative flow cytometric histograms showing NOTCH2 expression on B cells from recipients receiving BAFF Tg donor cells. (A) B cells from peripheral blood of BAFF-Tg BM only recipients or WT BM only recipients express similar level of IgM. Shown are representative flow cytometry profiles (five mice in each group were tested and the results are similar) from blood B cells at Day 42 post-BMT. (B) Peripheral blood cells were collected at Day 80 post-BMT and stimulated with anti-IgM (1 or 5 µg/ml) overnight (for 18 hrs) after red blood cells were lysed. The expression of surface NOTCH2 was assessed by flow cytometry. The cells were pre-gated on $7\text{-}AAD^-CD19^+$ live B cells. Results are representative flow cytometry histogram from each group (6 mice in WT BM only recipient mice group and 8 mice in BAFF-Tg BM only recipient mice group tested) producing similar results.

Supplementary Figure 8. Marginal zone B cells are not increased in cGVHD mice. (A) Representative flow cytometry profiles of follicular B cells ($CD23^{high}CD21^{low}$) and marginal zone B cells (MZ, $CD23^{low}CD21^{high}$) in peripheral blood (upper panels) and in splenocytes (bottom panels). The cells were pre-gated on $Zombie^{-}CD19^{+}$ cells. The peripheral blood cells were analyzed at Day 42 post-BMT. The splenocytes were analyzed at Day 84 or at Day 92 post-BMT. (B) MZ B cell numbers were calculated using B cell number as shown in Supplementary Figure 3C and frequency within the MZ B cell gate (Figure 5G). This data was combined from three individual experiments. N=10 (BM only), n=8 (BM+Sp), n=12 (BAFF-Tg BM only), n=9 (BAFF-Tg BM+Sp) and n=5 (Syn-BAFF-Tg). Statistical analysis was performed by ordinary one-way ANOVA with Tukey's multiple comparisons test using GraphPad Prism 8 software. *$P < .05$, ***$P < .001$ and ****$P < .0001$. NS, not significant ($P > .05$).

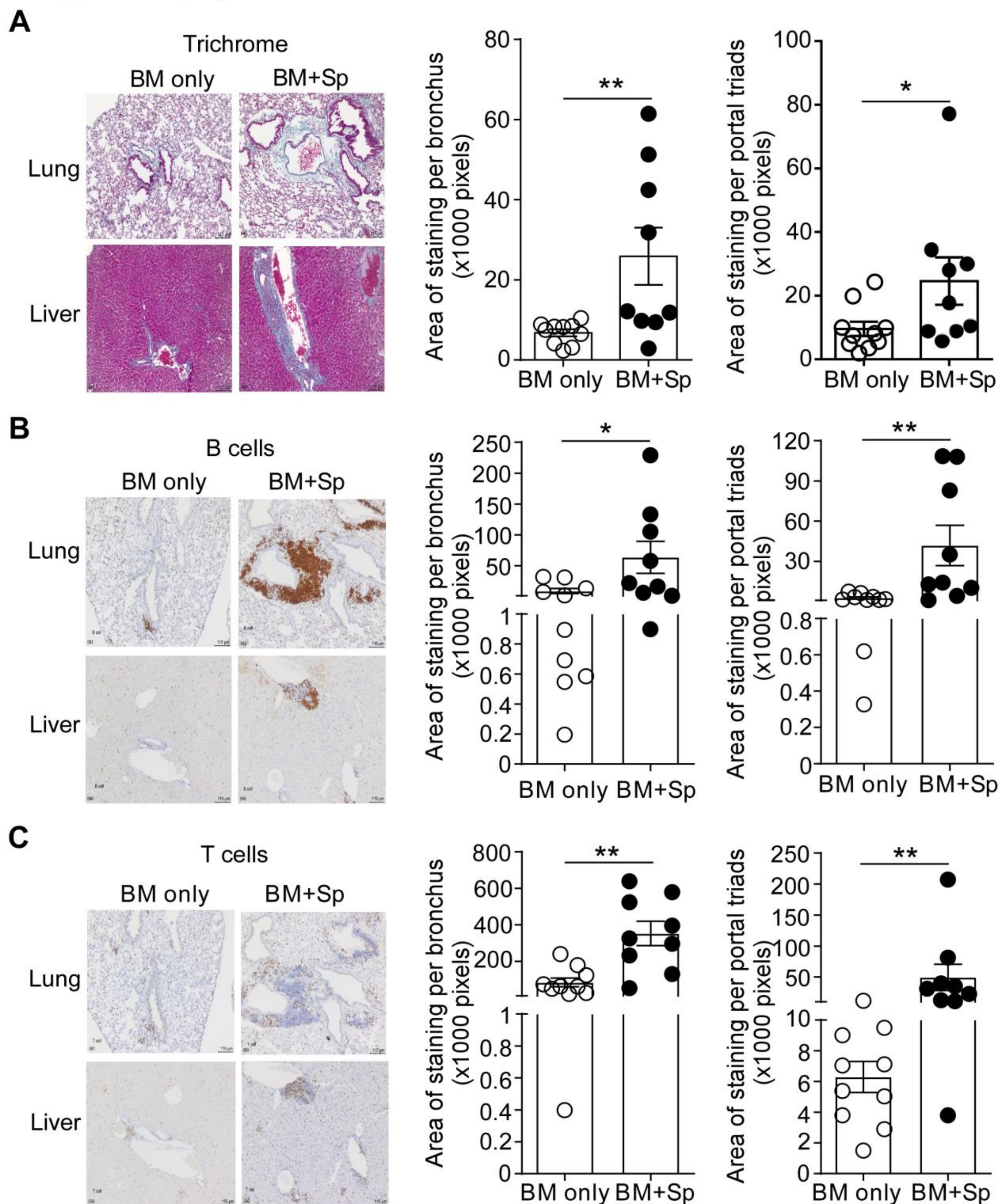
Supplementary Figure 9. The expression of BLNK in B cells from cGVHD mice. (A) BLNK protein expression level after BCR engagement. Blood B cells from WT or BAFF-Tg mice were stimulated with anti-IgM (10 μg/ml) overnight (18 hrs). The expression of BLNK was assessed by intracellular staining and flow cytometric analysis, with pre-gating on $Zombie^{-}CD19^{+}$ cells. N=10 of each group. (B-D) SYK and BLNK protein levels in blood B cells of BM only and BM+Sp mice were determined at Day 66 post-BMT by intracellular staining and flow cytometry. The B cells were pre-gated on $Zombie^{-}B220^{+}$ cells. The representative flow cytometry histograms were showed in (B) and (C). The MFI was used to represent the expression BLNK (D). The results in (D) are representative of three repeats. N=9 (BM only) and n=5 (BM+Sp). (E) Representative flow cytometry histogram of IgM expression on B cells from BAFF-Tg or wild type splenocytes. The cells were pre-gated on $Zombie^{-}CD19^{+}$ live cells. (F, G and H) SYK and BLNK protein levels in peripheral blood B cells from naïve mice or recipients of allo-BM only, syngeneic BAFF-Tg BM only versus BAFF-Tg BM only at Day 159 after allo-BMT. Cells were stained intracellularly and analyzed by flow cytometry, with pre-gating on $Zombie^{-}CD19^{+}$ cells. The representative flow

cytometry histograms were showed in (F) and (G). The MFI was used to represent the expression of SYK (F) and BLNK (H). (H) Statistical comparison of BLNK MFI in B cells from the indicated groups. N=9 (BAFF-Tg BM only), n=10 (wild type BM only or Syn-group) and n=4 (naïve B6 mice). Statistical analysis was performed by ordinary one-way ANOVA with Tukey's multiple comparisons test (Supplementary Figure 9A and 9H) or Mann-Whitney test (Supplementary Figure 9D) using GraphPad Prism 8 software.*P< .05, **P< .01, ***P< .001 and ****P< .0001. NS, not significant (P> .05).

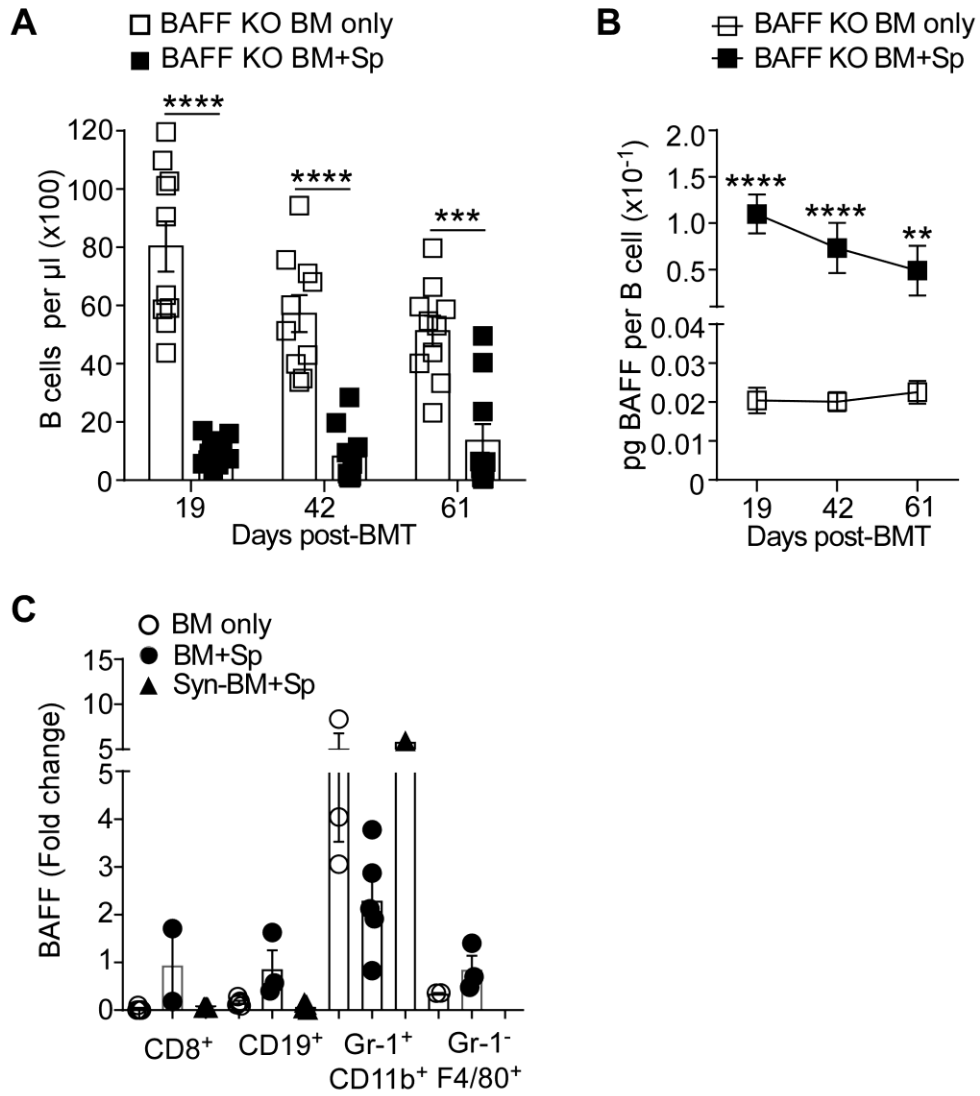
Supplementary Figure 10. B cells from high BAFF environment are sensitive to BCR stimulation-induced cell death. (A-B) Peripheral blood was withdrawn at different time points after allo-BMT. BAFF levels in plasma were assessed by ELISA (A). B cell numbers were calculated with anti-CD19 staining and flow cytometry analysis along with counting beads in the samples (B). N=9 (BAFF-Tg BM only), n=9 (BAFF-Tg BM+Sp at Day 20 and Day 42). n=5 (BAFF-Tg BM+Sp at Day 63) and n=5 (Syn-BAFF-Tg BM only). These results are representative of three repeat experiments. (C) Peripheral blood from mice receiving wild type or BAFF-Tg donor cells was withdrawn at Day 120 after allo-BMT. After the red blood cells were lysed, the cells were cultured overnight (18 hrs) in the presence or absence of anti-IgM (10 µg/ml). Cell death was assessed by 7-AAD staining and flow cytometry analysis, with gating on $CD19^+$ cells. N=8 (BAFF-Tg BM only), n=6 (BM only) and n=3 (naïve B6 mice). (D) The frequency of $CD19^+CD93^+$ transitional B cells in peripheral blood at Day 42 after BMT as assessed by flow cytometry. This data was combined from three individual experiments. N=22 (BM only), n=9 (BAFF-Tg BM only) n=9 (BAFF-Tg BM+Sp) and n=5 (Syn-BAFF-Tg). (E) The frequency of T3 B cells is increased in recipients receiving BAFF-Tg donor cells. T3 B cell frequency was assessed by flow cytometry at different time points after BMT. N=10 mice in each group. Statistical analysis was performed by ordinary one-way ANOVA with Tukey's multiple comparisons test (Supplementary Figure 10A,

10C-10D) or Kruskal–Wallis test (Supplementary Figure 10B and 10E) using GraphPad Prism 8 software.$^{*}P < .05$, $^{**}P < .01$ and $^{****}P < .0001$. NS, not significant ($P > .05$).

Supplementary Figure 1



Supplementary Figure 2



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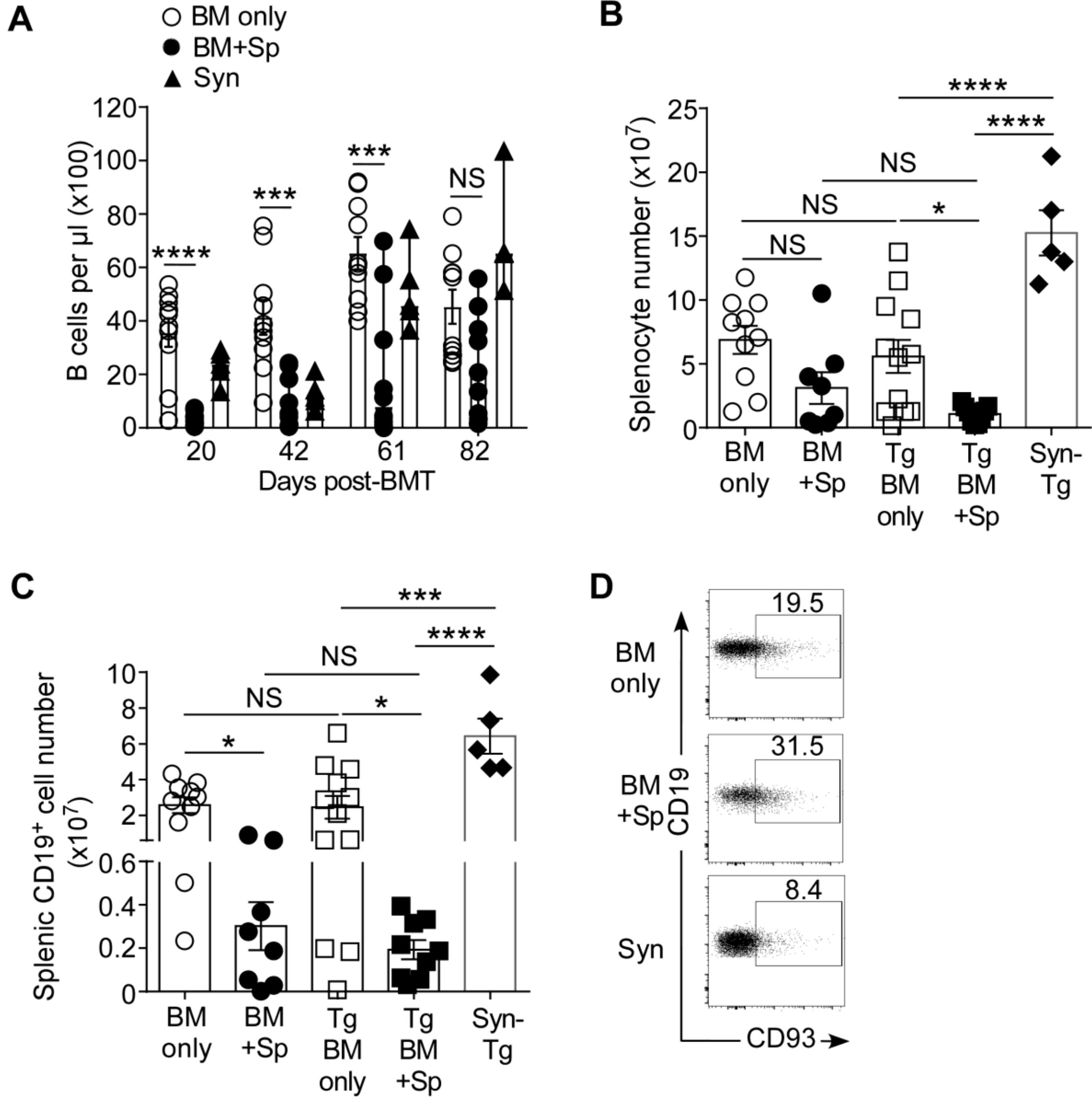

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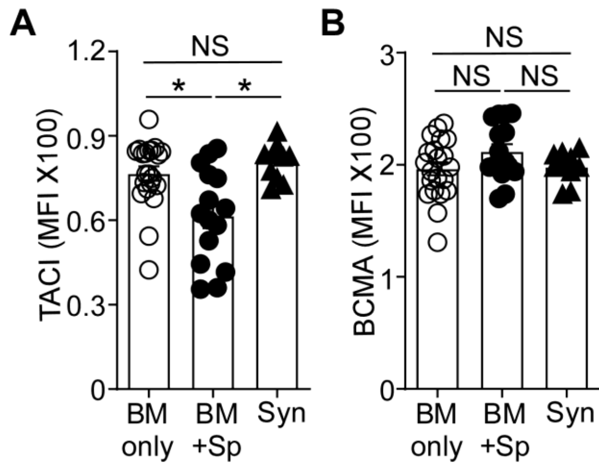

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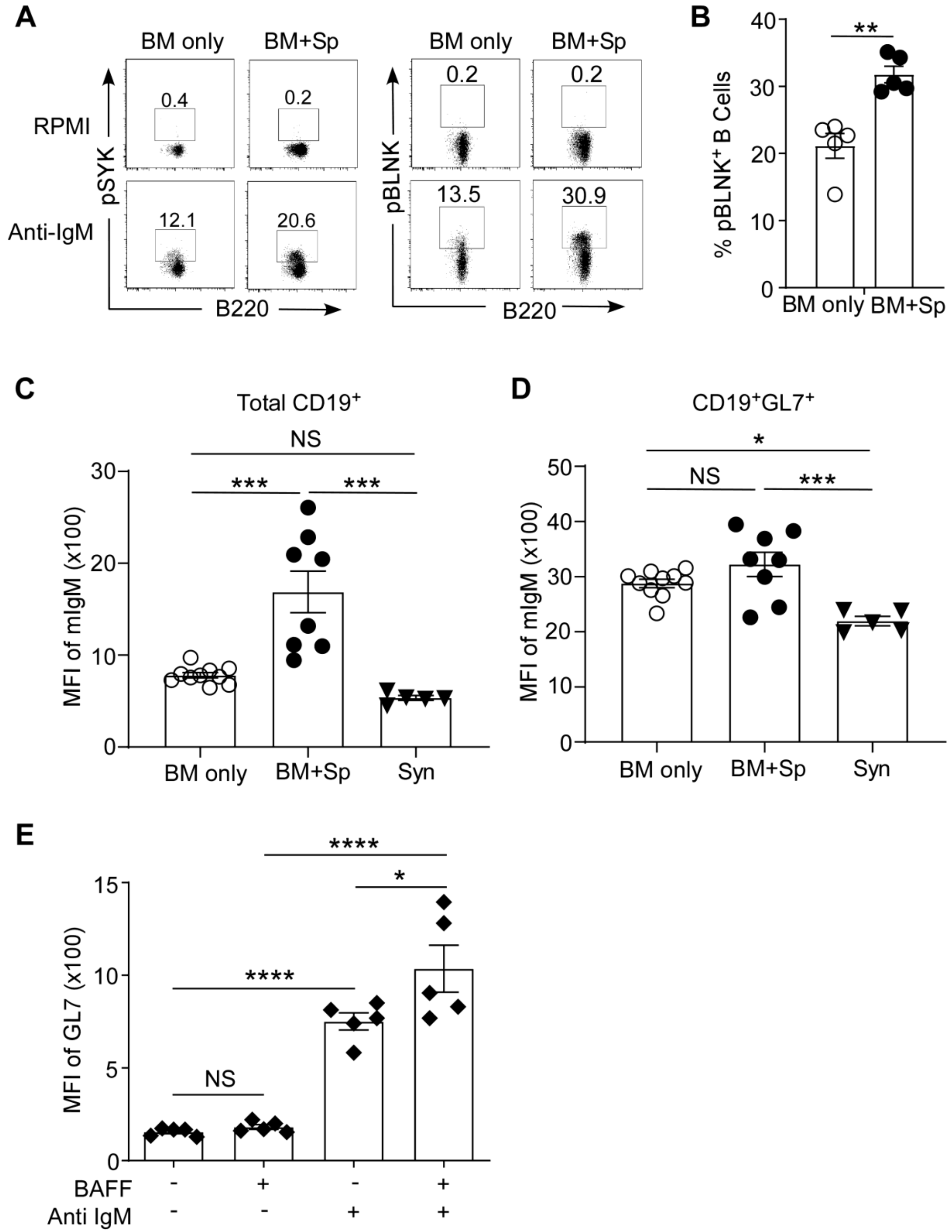
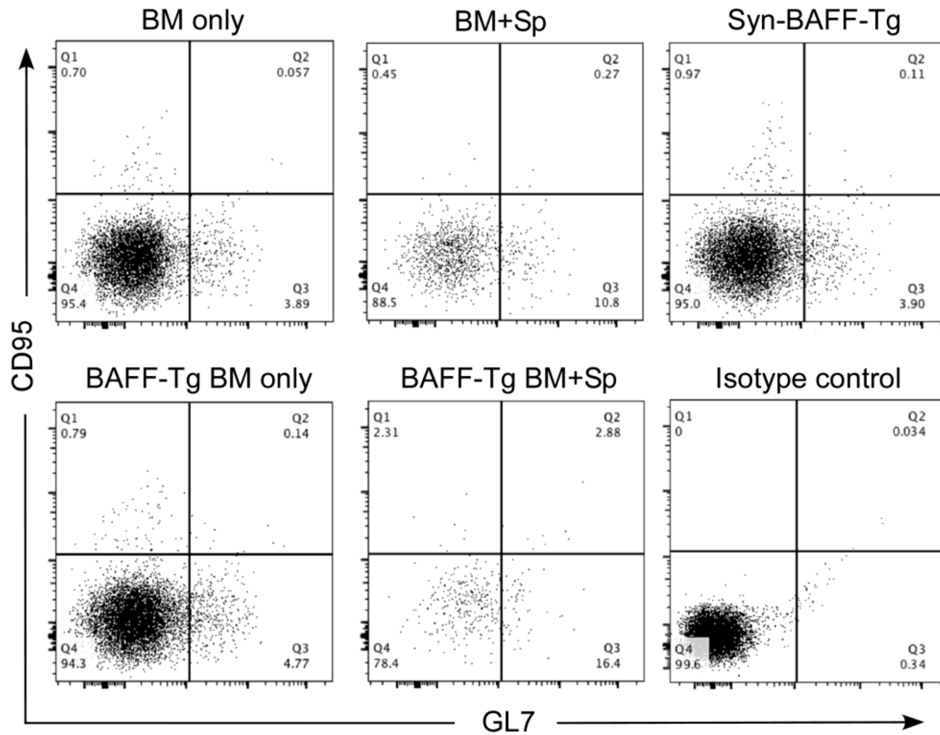
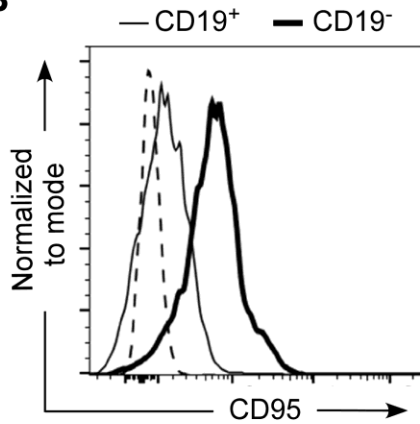
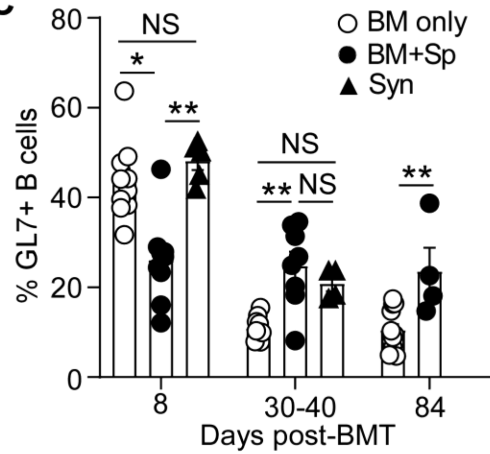


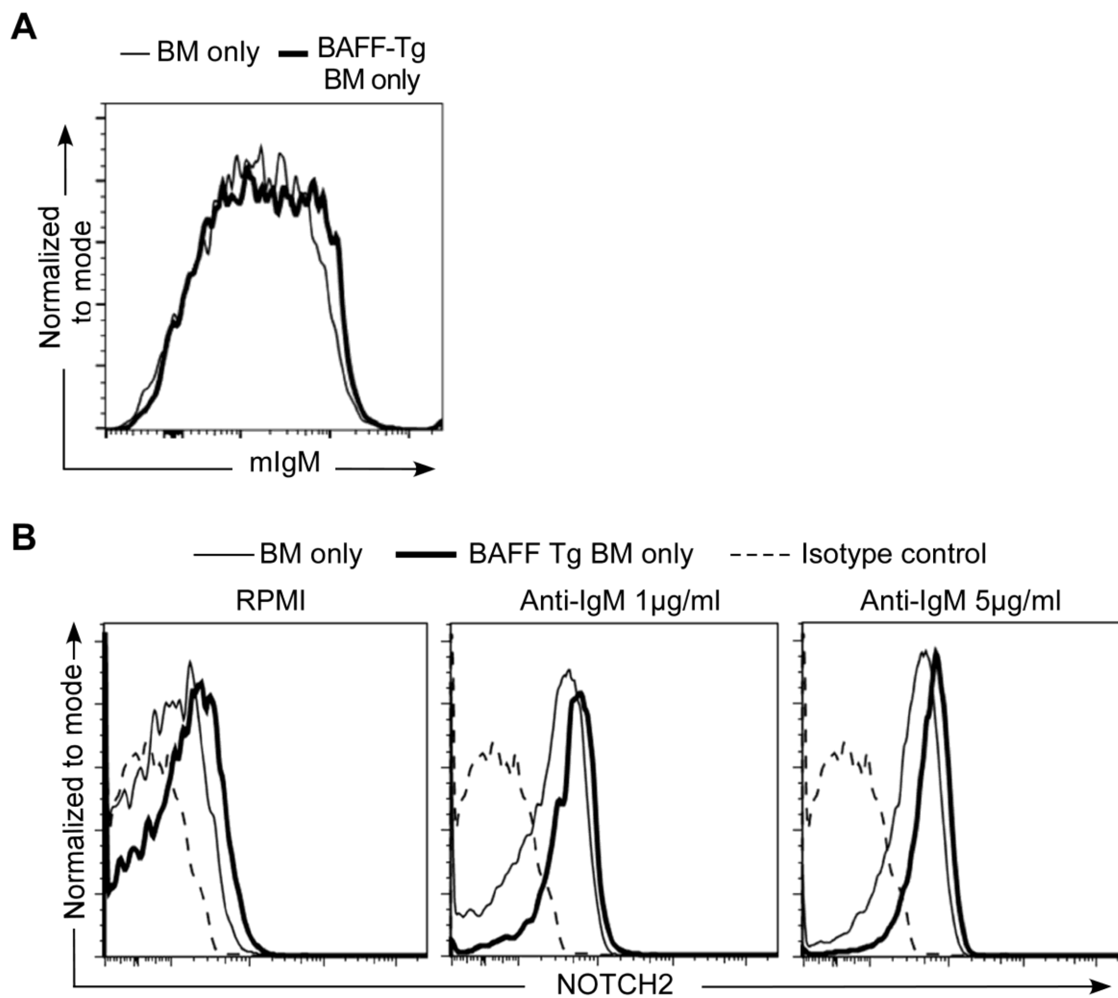
Supplementary Figure 6



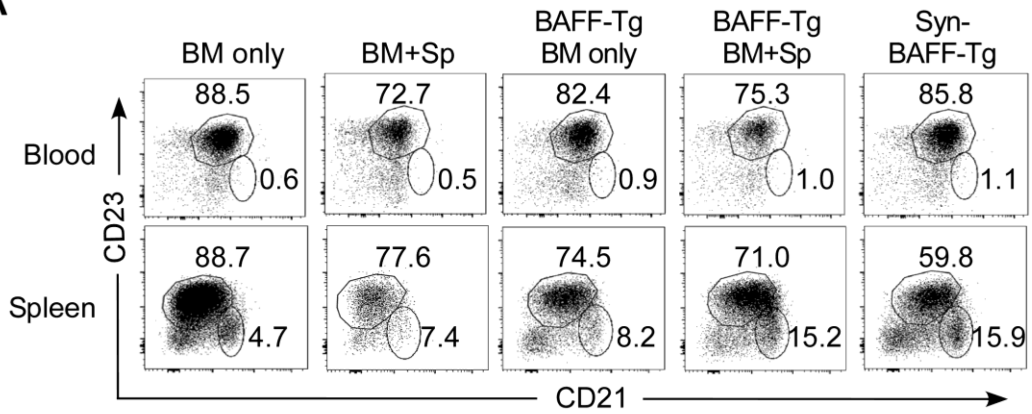


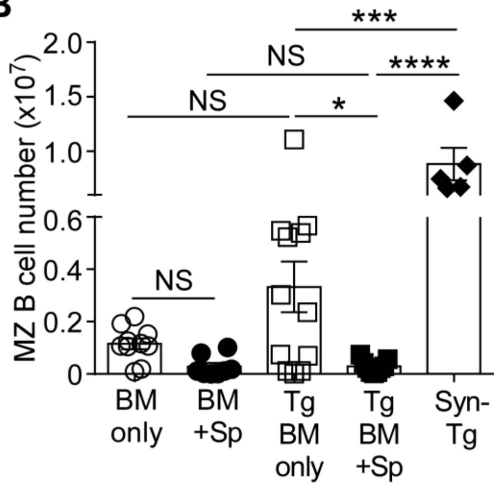


Supplementary Figure 7

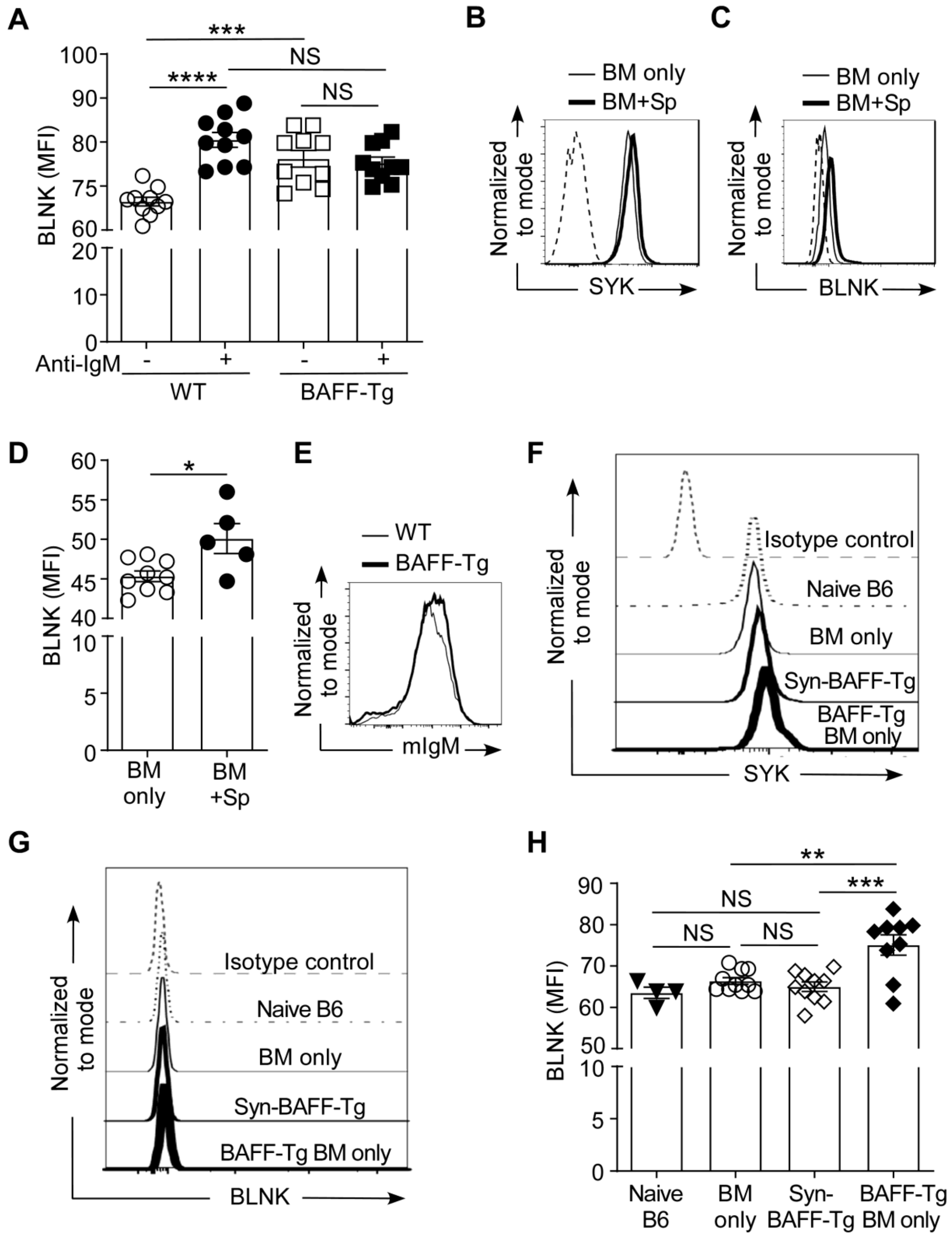


Supplementary Figure 8





Supplementary Figure 9



Supplementary Figure 10

A

□ BAFF-Tg BM only
■ BAFF-Tg BM+Sp
◇ Syn-BAFF-Tg

BAFF (ng/ml)
Days post-BMT
20 42 63
**** **** **** * **** NS

B

□ BAFF-Tg BM only
■ BAFF-Tg BM+Sp
◇ Syn-BAFF-Tg

B cells per µl (x100)
Days post-BMT
20 42 63
** * * * NS **

C

% Dead cells (7-AAD+)
Anti-IgM - + - + - +
BAFF-Tg BM only
BM only
Naive B6
* * NS NS NS

D

% CD19+CD93+ B cells
BAFF-Tg BM+Sp
BM only
BAFF-Tg BM only
Syn-BAFF-Tg
NS NS NS

E

○ BM only
□ BAFF-Tg BM only
◇ Syn-BAFF-Tg

% T3 B cells
Days post-BMT
33 61 80
* NS ** NS * * NS NS *