SUPPLEMENTAL MATERIAL

Miletic et al., http://www.jem.org/cgi/content/full/jem.20091962/DC1

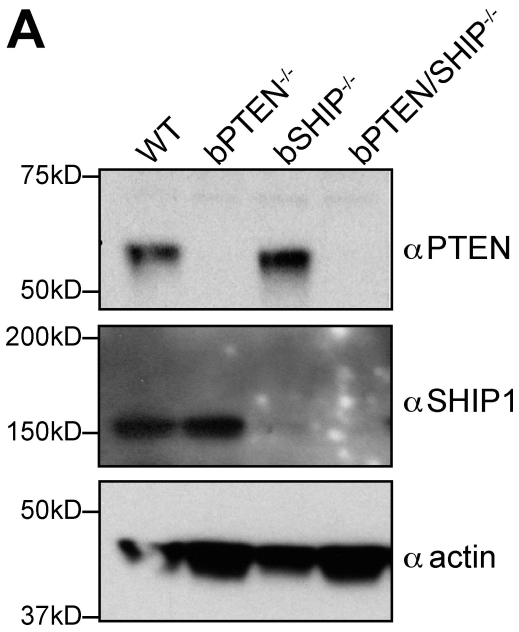

Figure S1. Deletion of PTEN and SHIP in B cells of bPTEN/SHIP$^{-/-}$ mice is complete. (A) Splenic B cells were purified from WT, bPTEN$^{-/-}$, bSHIP$^{-/-}$, and bPTEN/SHIP$^{-/-}$ mice. Lysates generated from cells were subjected to Western blot analysis with antibodies against PTEN or SHIP. Actin was used as a loading control. Data are representative of $n > 3$ experiments.

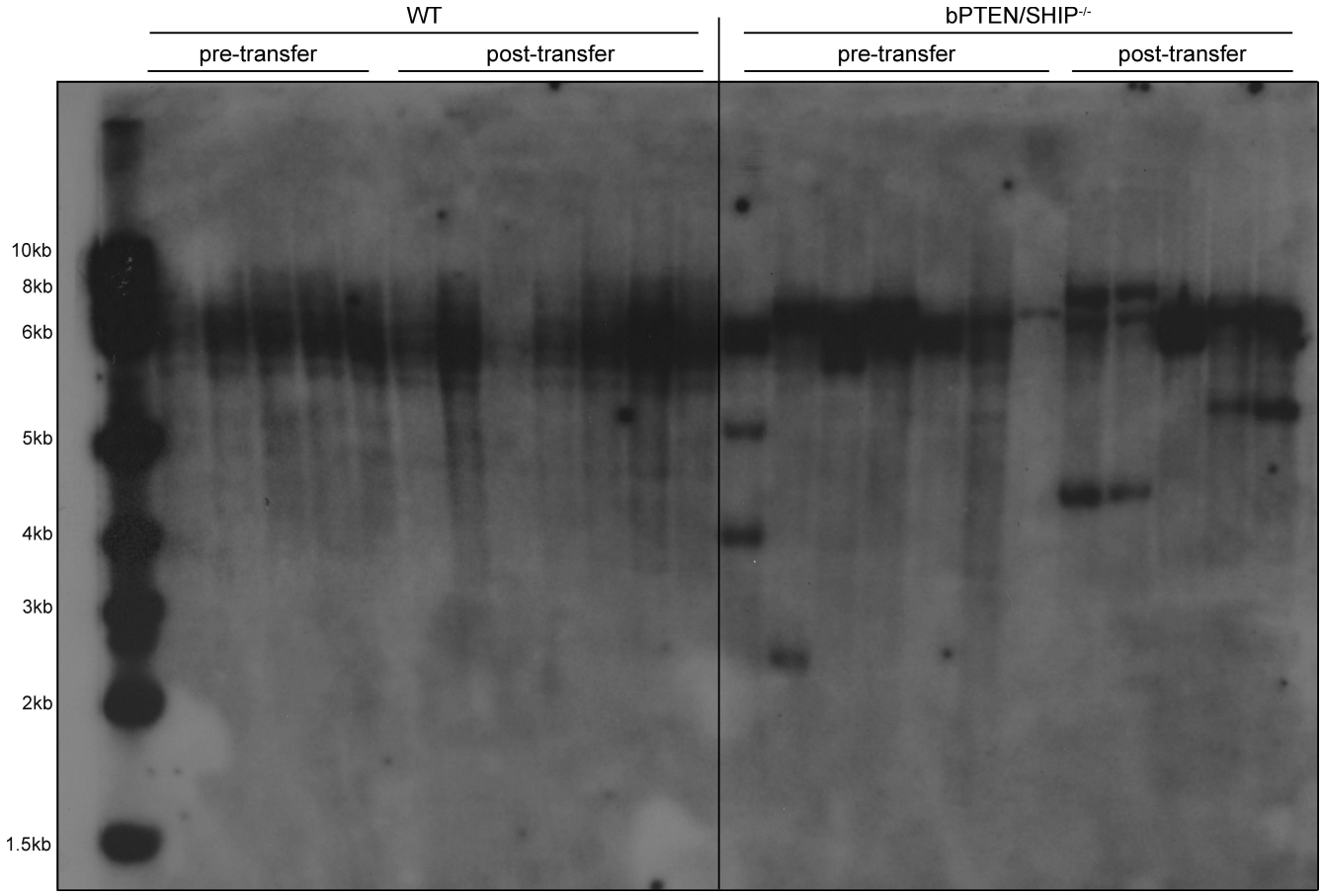

Figure S2. bPTEN/SHIP$^{-/-}$ B cells have clonal Ig repertoires after transfer into TCR-βδ$^{-/-}$ recipients. Rearrangements of the BCR heavy chain of WT and bPTEN/SHIP$^{-/-}$ B cells before transfer into TCR-βδ$^{-/-}$ mice (pre-transfer) or isolated from recipient animals (post-transfer) were detected using the *pJ11* probe, which preferentially detects rearrangements to J_H1, J_H2, and J_H3.

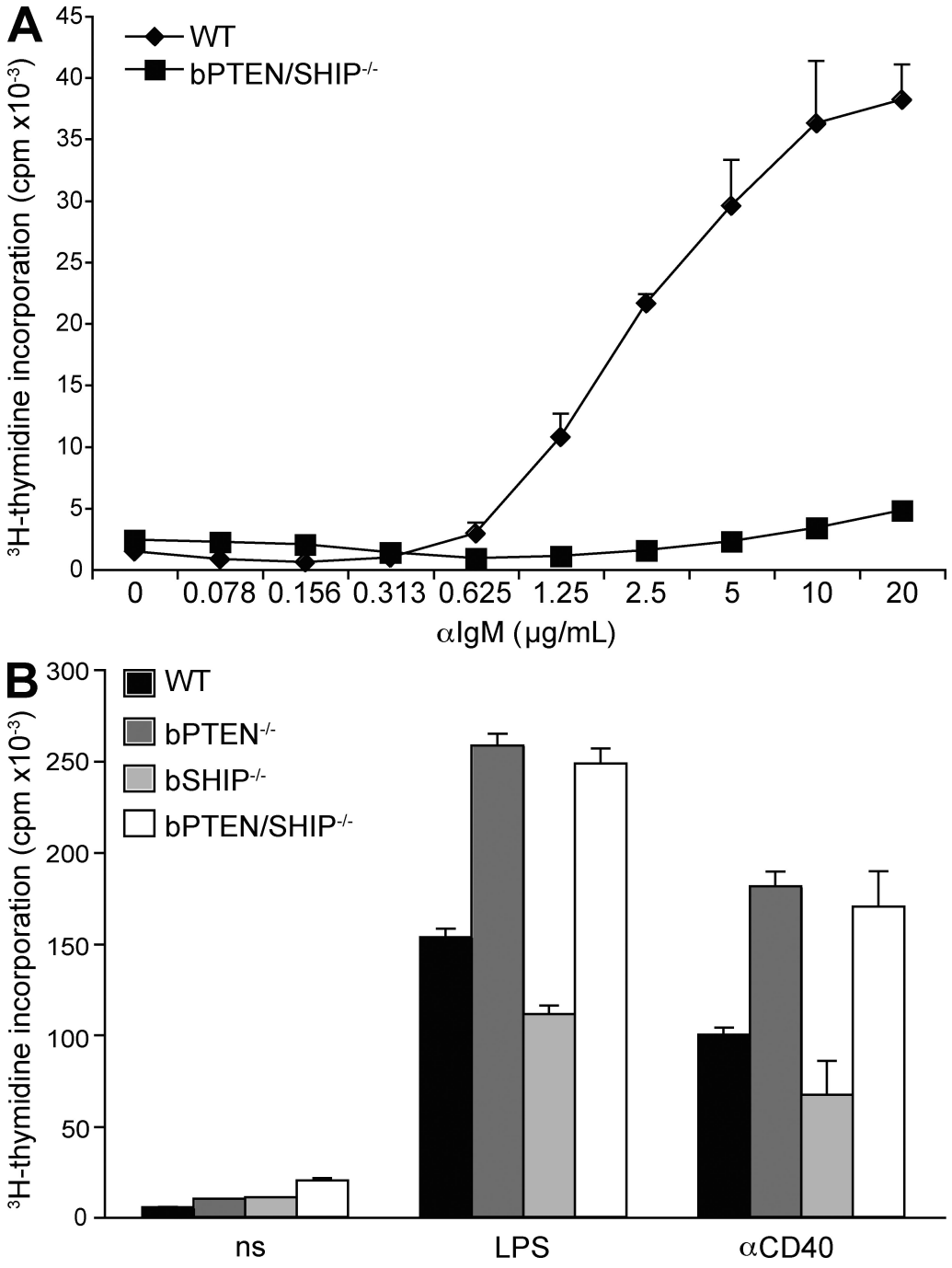

Figure S3. bPTEN/SHIP$^{-/-}$ B cells fail to proliferate in response to stimulation with anti-IgM but proliferate robustly to LPS or anti-CD40 treatment. (A) Purified splenic B cells from WT or bPTEN/SHIP$^{-/-}$ mice were stimulated with increasing concentrations of anti-IgM F(ab')$_2$ (α-IgM), as indicated. Proliferation was determined at 48 h by ^{3}H-thymidine incorporation. (B) Splenic B cells were purified from WT, bPTEN$^{-/-}$, bSHIP$^{-/-}$, and bPTEN/SHIP$^{-/-}$ mice and cultured in media alone (ns) or in the presence of LPS or anti-CD40 (α-CD40). Proliferation was determined at 48 h by ^{3}H-thymidine incorporation. In A and B, y-axis values shown are $\times 10^{-3}$ cpm. All assays were conducted in triplicates and SDs are shown as error bars. The results are representative of six experiments.

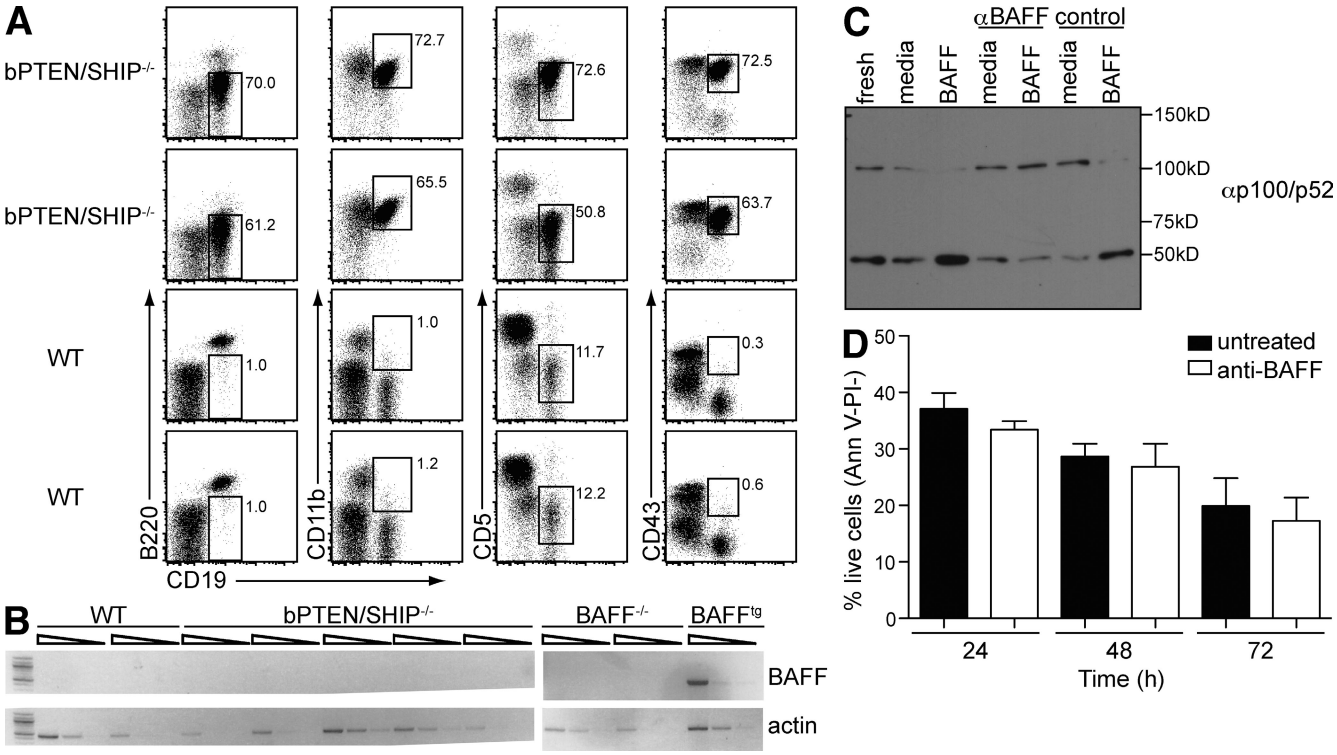

Figure S4. bPTEN/SHIP$^{-/-}$ B cells can survive and expand after transfer into BAFF$^{-/-}$ recipients but do not produce autocrine BAFF. (A) bPTEN/SHIP$^{-/-}$ or WT splenic B cells from age-matched mice were transferred intravenously into BAFF$^{-/-}$ recipients, and donor cell accumulation in recipient peripheral blood was assessed by flow cytometry. Shown are four representative animals 5.5 mo after transfer. Two received bPTEN/SHIP$^{-/-}$ B cells and two received WT B cells. (n = 8 WT recipients and 11 bPTEN/SHIP$^{-/-}$ recipients). Flow cytometry plots show PBLs from representative chimeras stained for CD19, B220, CD11b, CD5, and CD43. (B) Semiquantitative RT-PCR analysis of BAFF expression in WT and bPTEN/SHIP$^{-/-}$ B cells. BAFF$^{-/-}$ B cells or splenocytes from BAFF transgenic (BAFF^{tg}) animals overexpressing BAFF were used as controls. (C) Conversion of NF-κB p100 to p52 was used to determine effectiveness of BAFF-blocking Ig in WT B cells treated with BAFF. α-BAFF = BAFF-blocking Ig; control = isotype control Ig. Data are representative of two experiments. (D) bPTEN/SHIP$^{-/-}$ B cells were left untreated (media) or treated with BAFF-blocking Ig (anti-BAFF), and viability was assessed over time by annexin V (Ann V) and propidium iodide (PI) staining. The graph shows the percentage of live annexin V–propidium iodide^{-} B cells in culture at time points indicated. Data are representative of two experiments with two mice each. Error bars represent SD of the percentage of annexin V–propidium iodide^{-} B cells for WT and bPTEN/SHIP$^{-/-}$ mice at each time point shown.