Supplementary Figure Legends

Supplementary Figure 1. Scheme of *Rosa26* locus targeting. The Southern blot shows the wild type and targeted locus upon EcoR1 digestion of genomic DNA and probed with probe A as shown in the schematic.

Supplementary Figure 2. Expansion of Marginal Zone B cells in *P110*/+; CD21-cre; B1-8f/J_HT* compound mutant mice.

Supplementary Figure 3. IKK2CA expression in mature B cells. Representative FACS analysis of splenic B cells of *C57Bl/6* (B6) and *IKK2CA/+; CD21-cre* mice. The left column shows expression of surface IgM on mature B cells and the right column shows GFP expression, indicative of transgene expression on mature B cells.

Supplementary Figure 4. QRT-PCR analysis of transcript levels of NF-κB genes and targets in sorted IgM^{pos} and IgM^{neg} mature splenic B cells of *CD21-cre; B1-8f/J_HT* and *P110*/+; CD21-cre; B1-8f/J_HT* mice. Each bar is an average of values obtained from two mice and the dots represent individual mice.

Supplementary Figure 5. Expression of MEK1DD and RacDA fails to rescue BCR^{neg} B cells. (A) FACS analysis of lymphocytes from spleens of *CD21-cre; B1-8f/J_HT* (left column), *MEK1DD/+; CD21-cre; B1-8f/J_HT* (middle panel) and *RacDA/+; CD21-cre; B1-8f/J_HT* mice. (B) Western blot analysis showing increase in phospho-Erk levels in splenic B cells isolated from *MEK1DD/+; CD21-cre* mice. (C) Rac activation assay showing efficient binding of RacDA to PAK1 in splenic B cells isolated from

RacDA/+; CD19-cre mice. The RacDA cDNA also contains 3X HA tag and can be detected using an anti-HA antibody.

Supplementary Figure 6. Comparison of cell size (FSC), and surface expression of the B cell markers B220, CD19, CD23 and CD21 between IgM^{pos} (grey) and IgM^{neg} (black) splenic follicular B cells of *CD21-cre; B1-8f/J_HT* (top row) and *PtenΔ/f; CD21-cre; B1-8f/J_HT* (bottom row) mice.

Supplementary Figure 7. Survival of resting B cells through the BCR is mediated by PI3K-FOXO pathway. FACS analysis of lymphocytes from bone marrow and peritoneal cavity (PEC) of *B1-8f/J_HT* (left column), *CD21-cre; B1-8f/J_HT* (middle column) and *Foxo1Δ/f; CD21-cre; B1-8f/J_HT* (right column) showing rescue of IgM^{neg} mature B cells by deletion of Foxo1.

Supplementary Figure 8. (A) FACS analysis of splenic B cells showing lack of MZ B cells (CD1d high, CD21 high) in the IgM^{neg} gate of *Foxo1Δ/f; CD21-cre; B1-8f/J_HT* (bottom row) mice. (B) Comparison of cell size (FSC, left column), and surface expression of CD23 (right column) between IgM^{pos} (grey) and IgM^{neg} (black) follicular B cells from spleen of *CD21-cre; B1-8f/J_HT* (top row) and *Foxo1Δ/f; CD21-cre; B1-8f/J_HT* (bottom row) mice.

Supplementary Figure 9. Limited Rescue of IgM^{neg} B cells by haplo-insufficiency of Foxo1. (A) Western blot analysis of sorted IgM^{pos} and IgM^{neg} splenic mature B cells from mice as indicated in the figure for Foxo1 (Top panel) and Actin (bottom panel). (B) FACS analysis of lymphocytes from spleen and lymph

nodes (LN) of *CD21-cre; B1-8f/J_HT* (left column) and *Foxo1Δ/+; CD21-cre; B1-8f/J_HT* (right column) showing significant rescue of IgM^{neg} B cells in the latter.

Supplementary Figure 10. Dependence of PI3K activity in mature B cells on BCR expression and moderate activation of PI3K by P110* ot Pten deletion. Immunoblot analysis for phospho-Akt and Akt (panel A), phospho- GSK3a/b and actin (panel B) and phospho-S6K and S6K (panel C) in sorted IgM+ and IgM- Mature B cells from mice of genotype as indicated in the figure. For activation experiments in lanes 1-4, panel C, mature B cells were sorted and incubated in B cell medium with 10% FCS at 37^{o}C for 1h before any further treatment. Subsequently cells were treated with PI3K inhibitor, LY294002 (10uM) or DMSO for 30min followed by anti-IgM (50ug/ml) stimulation for 15 min. Lanes 5-10 represent ex vivo isolated cells of the genotypes as indicated in the figure.

Supplementary Table1. Primers used for quantitative RT-PCR are listed

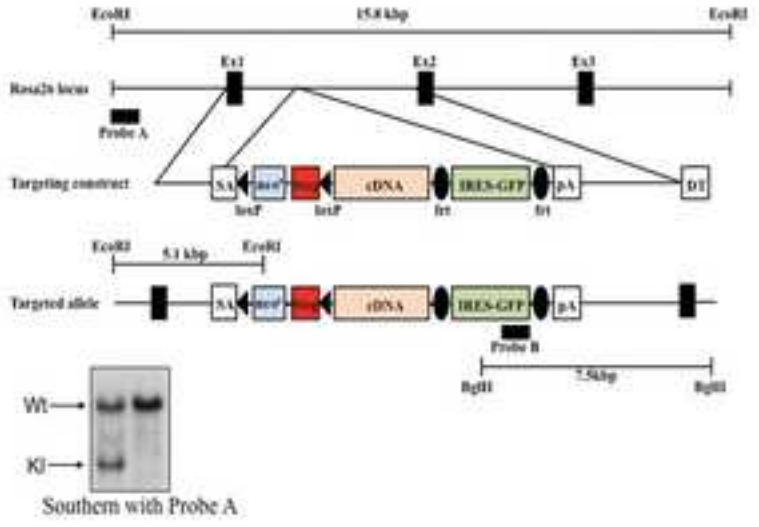
RAG1	Fw-TTGCTATCTCTGTGGCATCG Rev-AATTTCATCGGGTGCAGAAC
AICDA	Fw-GGACTTCGGCCACCTTC Rev-CATCTCAGAAACTCAGCCACG
BCL2L11	Fw-CTGCGCCCGGAGATACG Rev-CCATACCAGACGGAAGATAAA
P27/Kip1	Fw-GGCCCGGTCAATCATGAA Rev-CTTGCGCTGACTCGCTTCTT
IRF4	Fw-AGGTCTGCTGAAGCCTTGGC Rev-CTTCAGGGCTCGTCGTGGTC
NFkB1A	Fw-TTGGTCAGGTGAAGGGAGAC Rev-GCTTTCAGAAGTGCCTCAGC
cREL	Fw-GACAACAACCGGACATACCC Rev-CCATCTCTGCAATCTTTTCC
P100	Fw-TTTCCTTCGAGCTAGCGATG Rev-TTCGGGAGATCTTCAGGTTC
ID2	Fw-GACAGAACCAGGCGTCCAGG Rev-GGGAATTCAGATGCCTGCAAGGAC
BCLxL	Fw-GGTGAGTCGGATTGCAAGTT Rev-GCTGCATTGTTCCCGTAGAG
BCL3	Fw-TCCAGAATAACATAGCCGCTGT Rev- CATGCCAGGTGAATTGCAGTC

Supplementary methods

Rac Activation assay: This was done on B cell lysates using the rac activation assay kit from Upstate Biotechnology according to the manufacturers instructions.

Immunoblot analysis: To prepare whole cell lysates, sorted mature B cells (B220+, AA4.1-) were lysed for 10 min on ice with RIPA buffer containing complete protease inhibitor cocktail (Roche Diagnostics), 1 mM sodium orthovanadate, 5 mM sodium fluoride and 0.5mM phenylmethylsulfonyl fluoride. Antibodies directed against Pten (clone C38G6), Foxo1 (clone C38G6), Phospho-Akt (ser473, Cat No. 9271), Phospho-Gsk3α/β (clone 37F11), Phospho-P70S6K (clone 108D2), Phospho-Erk (Cat No. 9101), Akt (clone C73H10), P70S6K (clone 49D7) were from Cell Signalling Technologies. β-actin-specific monoclonal antibody (clone AC-74) was from Sigma-Aldrich. For activation experiments mature B cells were sorted and incubated in B cell medium (containing 10% FCS) at 37^{o}C for 1h before any treatment. Subsequently cells were treated with PI3K inhibitor, LY294002 (10uM) or DMSO for 30min followed, if applicable, by anti- IgM (50ug/ml) stimulation for 15 min.

[G] Supplemental Text and Figures



Supplementary Figure 1

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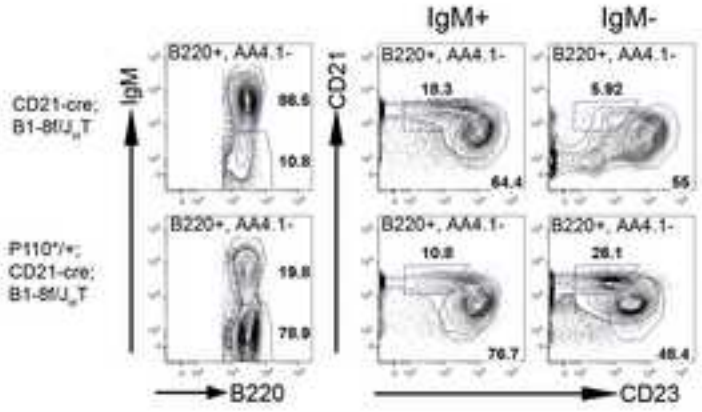

Supplementary Figure 2

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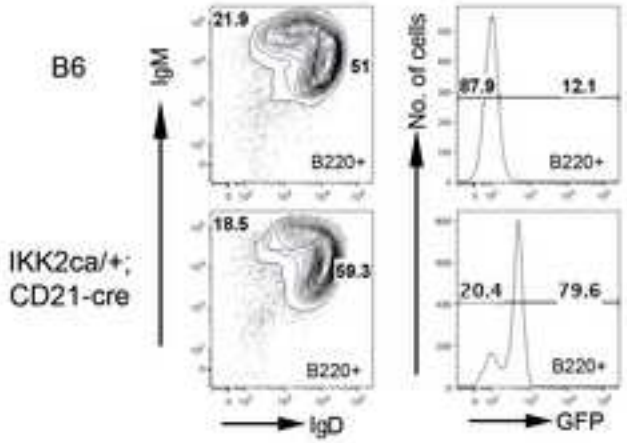

Supplementary Figure 3

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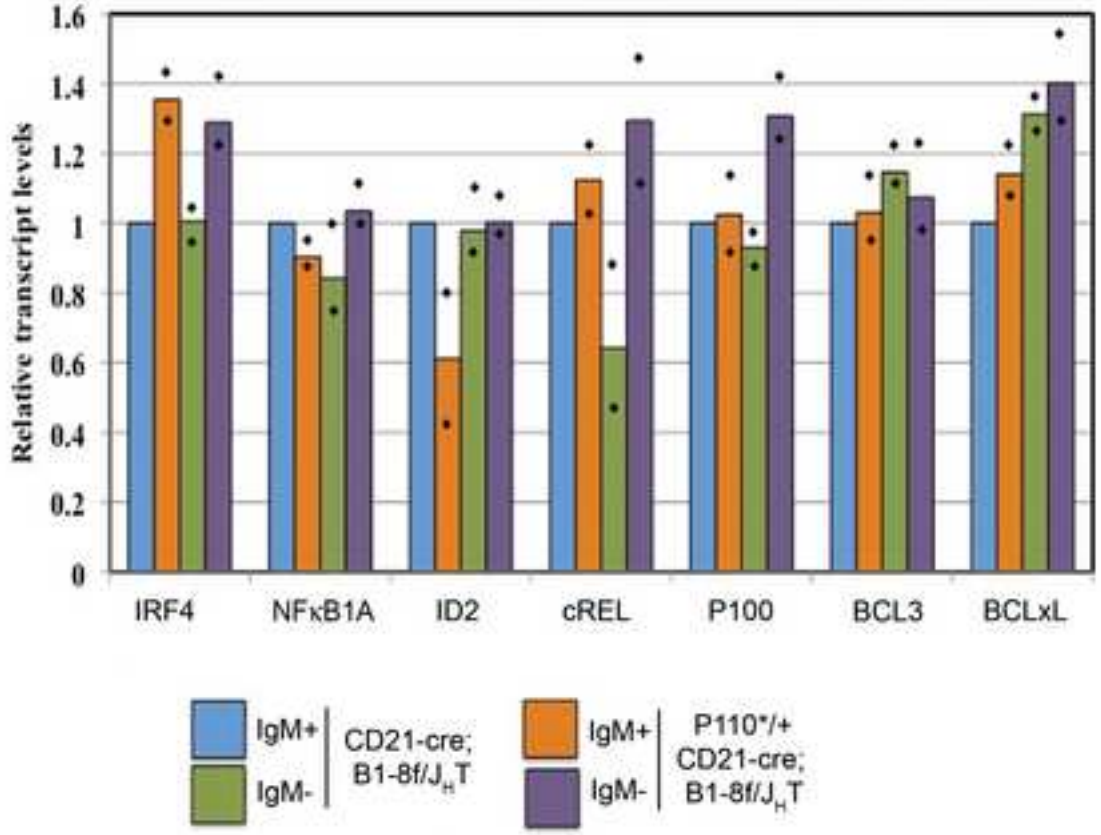

Supplementary Figure 4

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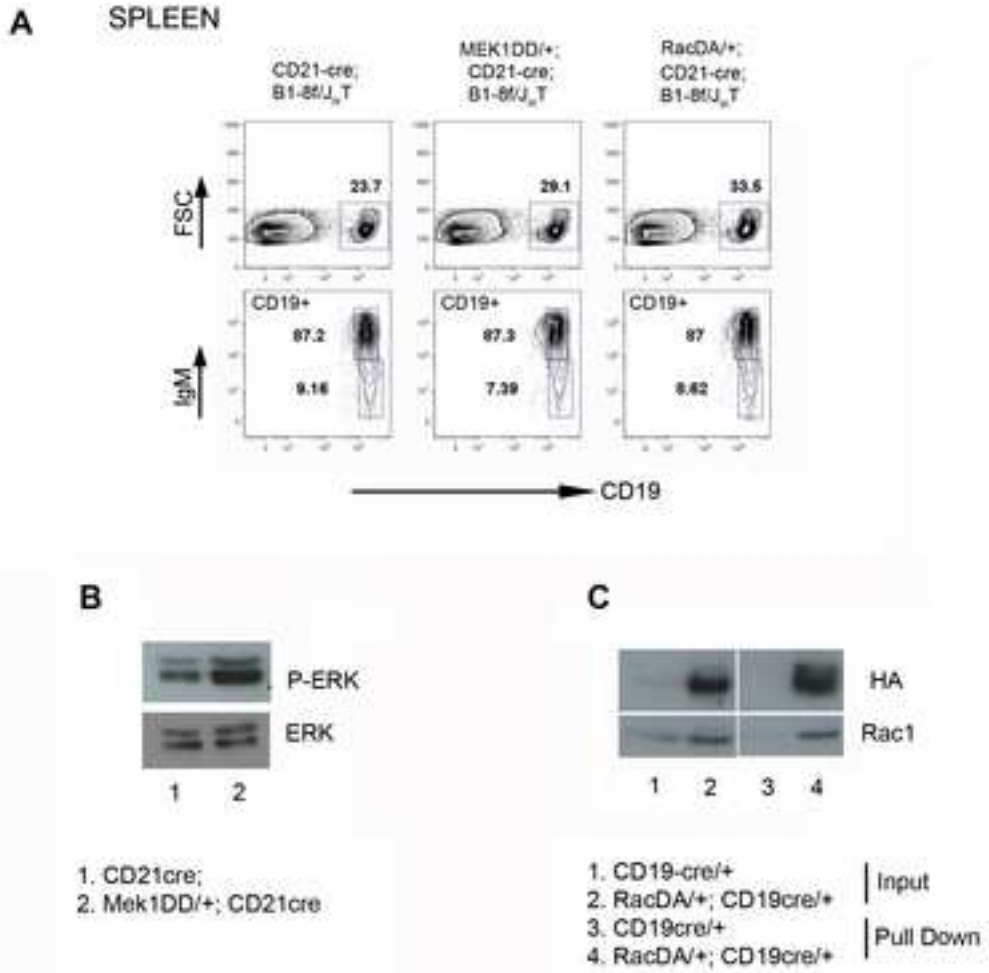

Supplementary Figure 5

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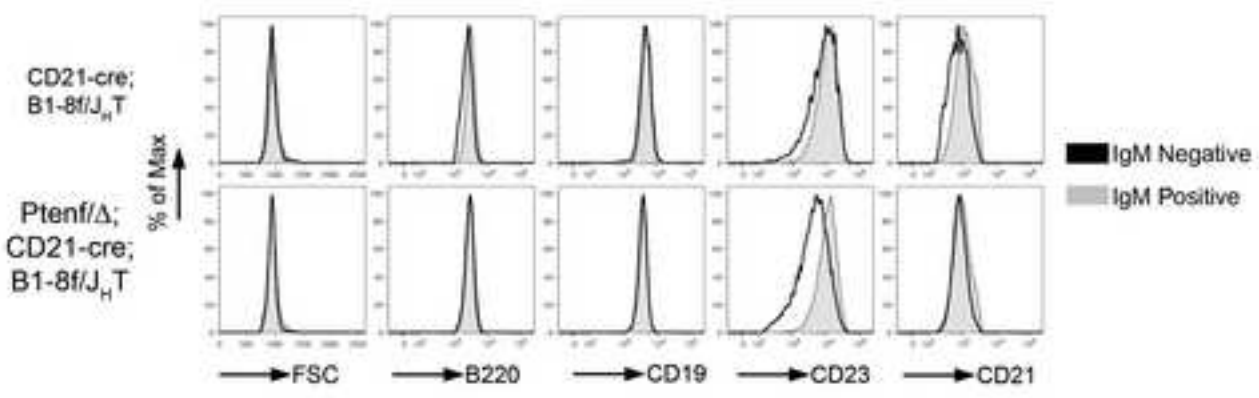

Supplementary Figure 6

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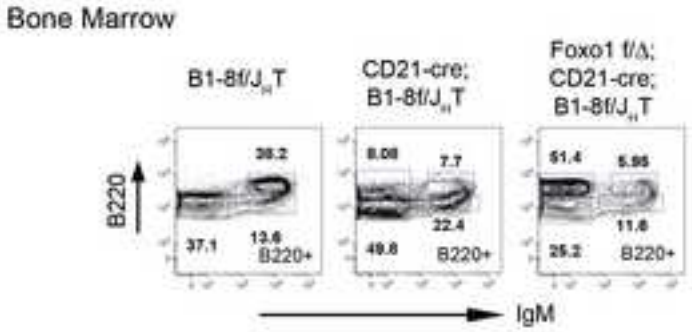

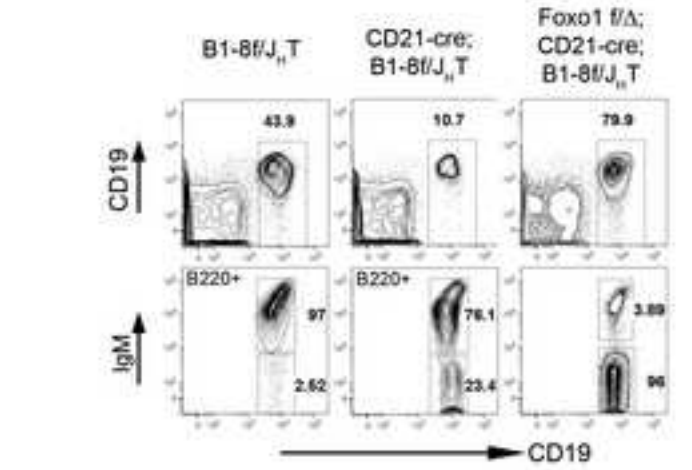

Supplementary Figure 7

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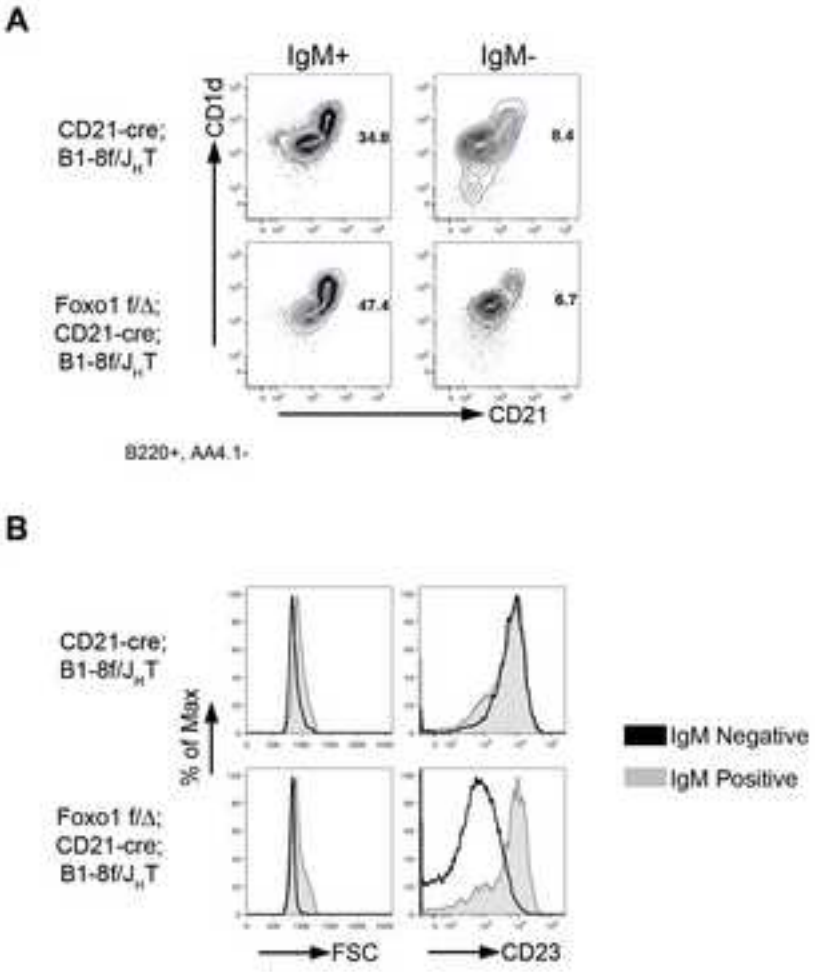
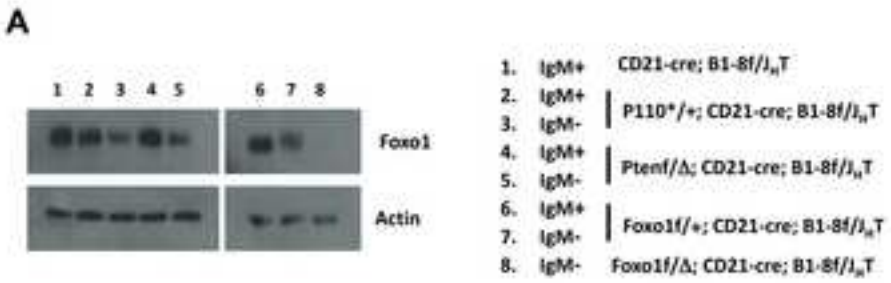
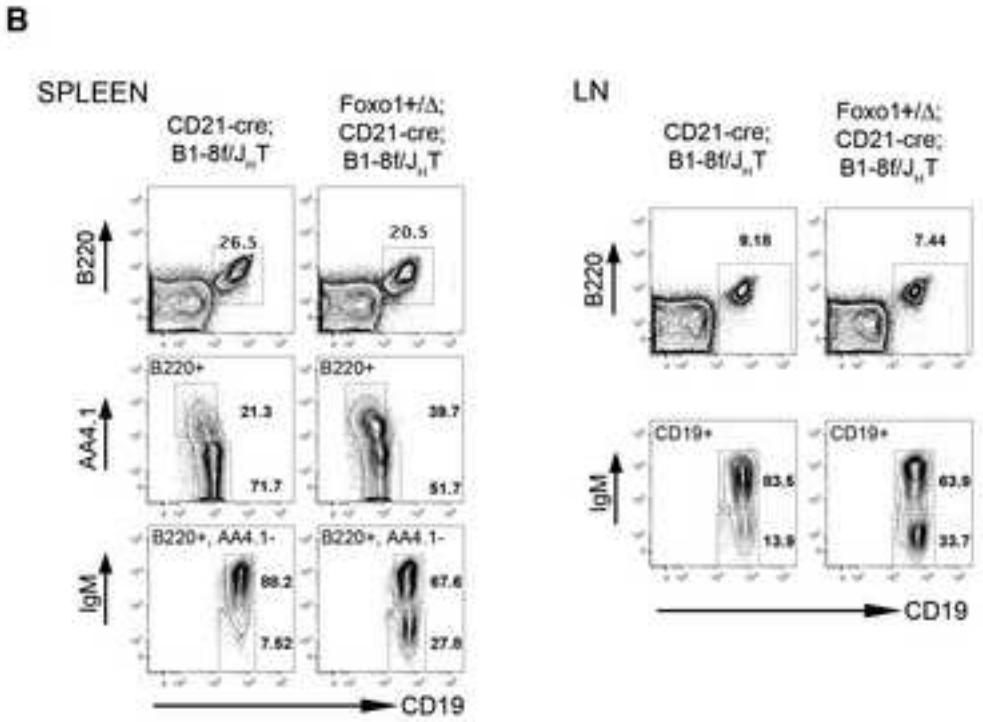
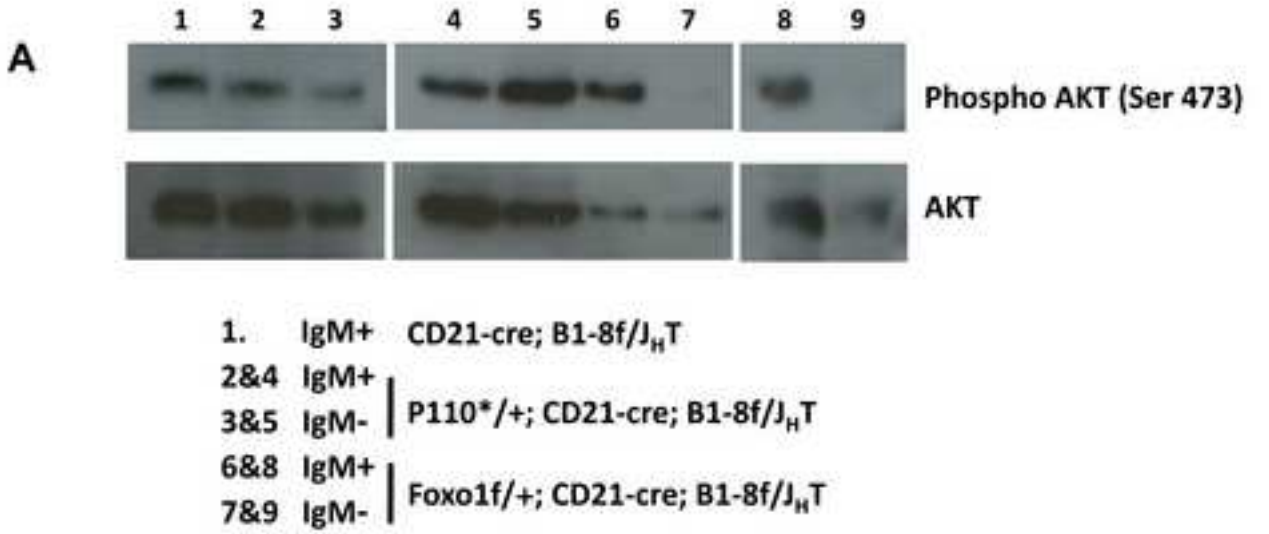


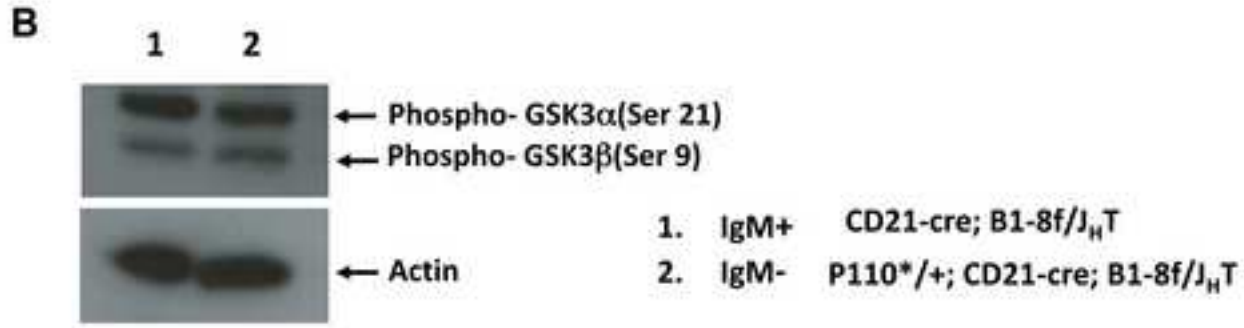
Supplementary Figure 8

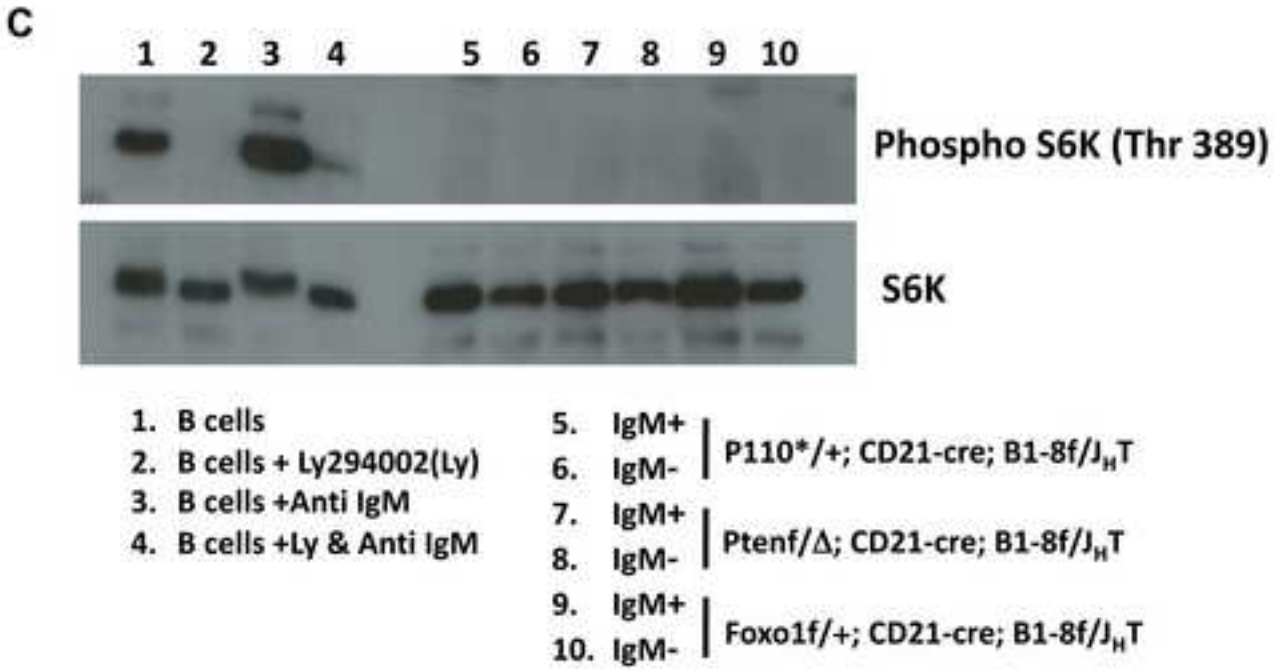




Supplementary Figure 9







Supplementary Figure 10