SUPPLEMENTAL FIGURE 1

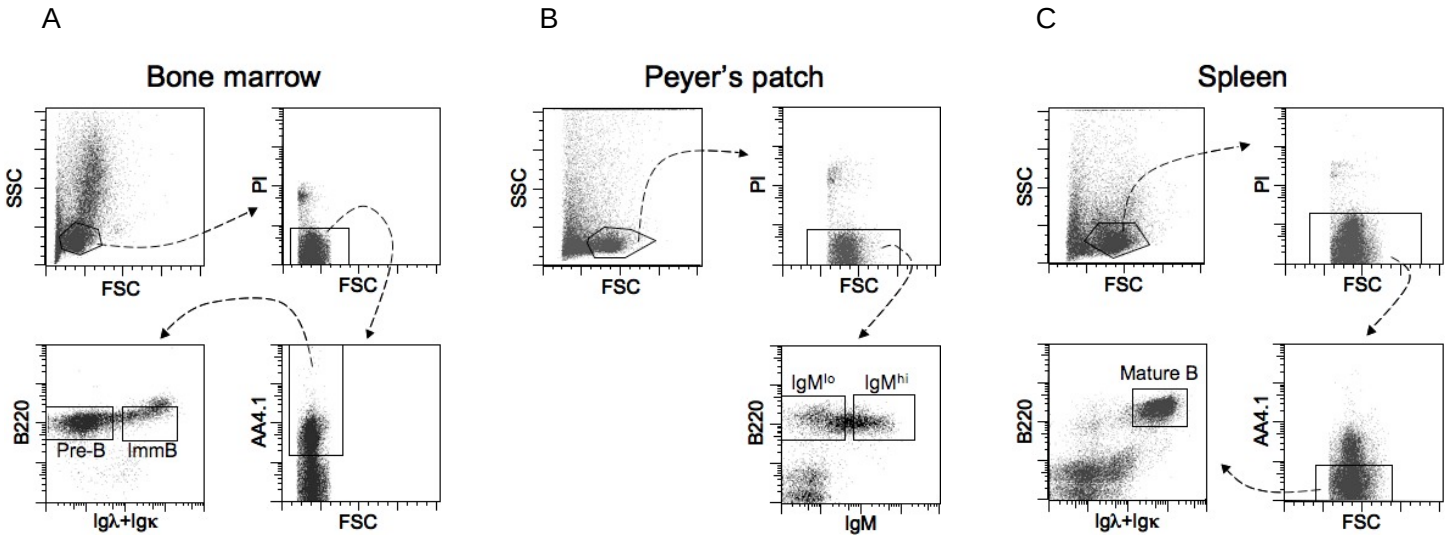

Figure S1. Cell Sorting Strategies

(A) Developing B cells were sorted from BM. Lymphocytes were gated according to their Forward Side Scatter (FSC) and Side Scatter (SSC) profile and living lymphocytes were gated by propidium iodide exclusion. Cells were triple-stained for B220, AA4.1 and light chains (λ and κ). Pre-B and immature B cells were gated on AA4.1-positive cells and sorted as $B220^{+}\lambda^{-}\kappa^{-}$ and $B220^{+}\lambda^{+}\kappa^{+}$, respectively. (B) PP-derived mature B cells were stained with anti-B220- and -IgM antibodies. Two distinct populations were sorted based on the level of IgM expression: $B220^{+}IgM^{lo}$ and $B220^{+}IgM^{hi}$. (C) Splenic cells were triple-stained with antibodies to B220, AA4.1 and light chains (λ and κ). Living lymphocytes were gated, and mature B lymphocytes were sorted as $B220^{+}\lambda^{+}\kappa^{+}AA4.1^{-}$.

SUPPLEMENTAL FIGURE 2

A

BM-IgA-10 **V_H-D-J_H1-Cα**

Nucleotide Sequence

```
CAG CTG CAG GAG TCT GGA CCT GAG CTG GTG AAG CCT GGC GCT TCA GTG GAG ATT TCC TGC AAG GCT
TCT GGT TAC TCA TTC ACT GGC TAC AAC ATG AAC TGG GTG AAG CGG AGC AAT GGA AAG AGC CTT GAG
TGG ATT GGA GTG ATT AAG CCT AAC TAT GGT CTT ACT AGC TAC AAT CAG AAA TTC ACG GTC AAG GCC
ACA TTG ACT GTA GAC CAA TCT TCC AGC ACA GCC TAC ATG CAG CTC AAC AGC CTG ACA TCT GAG GAC
TCT GCA GTC TAT TAC TGT GCA AGA TCC GAT GGT CAG GGG TTC TTC GAT GTC TGG GGC ACA GGG ACC
GCG GTC ACC GTC TCC TCA GAG TCT GCG AGA AAT CCC ACC ATC TAC CCA CTG ACA CTC CCA CCA GCT C
```

Amino Acid Coding

```
Q L Q E S G P E L V K P G A S V E I S C K A S G Y S F T G Y N M N W V K R S N G K S L E W
I G V I K P N Y G L T S Y N Q K F T V K A T L T V D Q S S S T A Y M Q L N S L T S E D S A
V Y Y C A R S D G Q G F F D V W G T G T A V T V S S E S A R N P T I Y P L T L P P A
```

BM-IgA-3 **V_H-D-J_H2-Cα**

Nucleotide Sequence

```
CAG CTG CAG GAG TCT GGA CCT GAG CTG GTG AAG CCT GGG GCT TCA GTG AAG TTG TCC TGC AAG GCT
TCT GGC TAC ACC TTC ACA AGC TAC GAT ATA AAC TGG GTG AAG CAG AGG CCT GGA CAG GGA CTT GAG
TGG ATT GGA TGG ATT TAT CCT AGA GAT GGT AGT ACT AAG TAC AAT GAG AAG TTC AAG GAC AAG GCC
ACA TTG ACT GTA GAC ACA TCC TCC AGC ACA GCG TAC ATG GAG CTC CAC AGC CTG ACA TCT GAG GAC
TCT GCA GTC TAT TTC TGT GCA AGA TTC TAC CCT AAC TTT GAC TAC TGG GGC CAA GGC ACC ACT CTC
ACA GTC TCC TCA GAG TCT GCG AGA AAT CCC ACC ATC TAC CCA CTG ACA CTC CCA CCA GCT C
```

Amino Acid Coding

```
Q L Q E S G P E L V K P G A S V K L S C K A S G Y T F T S Y D I N W V K Q R P G Q G L E W
I G W I Y P R D G S T K Y N E K F K D K A T L T V D T S S S T A Y M E L H S L T S E D S A
V Y F C A R F Y P N F D Y W G Q G T T L T V S S E S A R N P T I Y P L T L P P A
```

BM-IgA-27 **V_H-D-J_H3-Cα**

Nucleotide Sequence

```
CAG CTG CAG GAG TCT GGA GCT GAG CTG GCG AGG CCT GGG GCT TCA GTG AAG CTG TCC TGC AAG GCT
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TGG ATT GGA GAG ATT TAT CCT AGA AGT GTT AAT ACT TAC TAC AAT GAG AAG TTC AAG GAC AAG GCC
ACA CTG ACT GCA GAC AAA TCC TCC AGC ACA GCG TAC ATG GAG CTC CGC AGC CTG ACA TCT GAG GAC
TCT GCG GTC TAT TTC TGT GCA AGA TCA CCC TGG AGG TTT GCT TAC TGG GGC CAA GGG ACT CTG GTC
ACT GTC TCT GCA GAG TCT GCG AGA AAT CCC ACC ATC TAC CCA CTG ACA CTC CCA CCA GCT C
```

Amino Acid Coding

```
Q L Q E S G A E L A R P G A S V K L S C K A S G Y T F T S Y G I S W V K Q R T G Q G L E W
I G E I Y P R S V N T Y Y N E K F K D K A T L T A D K S S S T A Y M E L R S L T S E D S A
V Y F C A R S P W R F A Y W G Q G T L V T V S A E S A R N P T I Y P L T L P P A
```

BM-IgA-22 **V_H-D-J_H4-Cα**

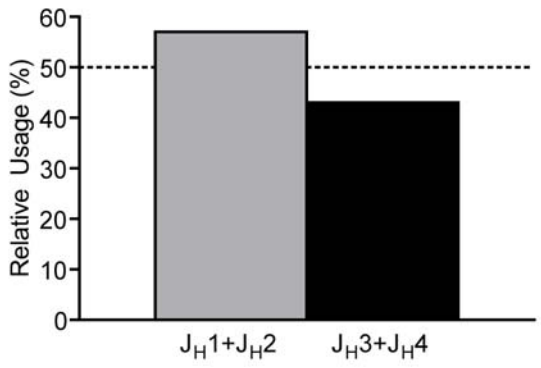
Nucleotide Sequence

```
CAG CTG CAG GAG TCT GGA CCT GGC CTC GTG AAA CCT TCT CAG TCT CTG TCT CTC ACC TGC TCT GTC
ACT GGC TAC TCC ATC ACC AGT GGT TAT TAC TGG AAC TGG ATC CGG CAG TTT CCA GGA AAC AAA CTG
GAA TGG ATG GGC TAC ATA AGC TAC GAT GGT AGC AAT AAC TAC AAC CCA TCT CTC AAA AAT CGA ATC
TCC ATC ACT CGT GAC ACA TCT AAG AAC CAG TTT TTC CTG CAG TTG AGT TCT GTG ACT TCT GAG GAC
ACA GCC ACA TAT TAC TGT GCA AGA GAG GGG ATA CTG GGA CGG GCT ATG GAC TAC TGG GGT CAA GGA
ACC TCA GTC ACC GTC TCC TCA GAG TCT GCG AGA AAT CCC ACC ATC TAC CCA CTG ACA CTC CCA CCA
GCT C
```

Amino Acid Coding

```
Q L Q E S G P G L V K P S Q S L S L T C S V T G Y S I T S G Y Y W N W I R Q F P G N K L E
W M G Y I S Y D G S N N Y N P S L K N R I S I T R D T S K N Q F F L Q L S S V T S E D T A
T Y Y C A R E G I L G R A M D Y W G Q G T S V T V S S E S A R N P T I Y P L T L P P A
```

B



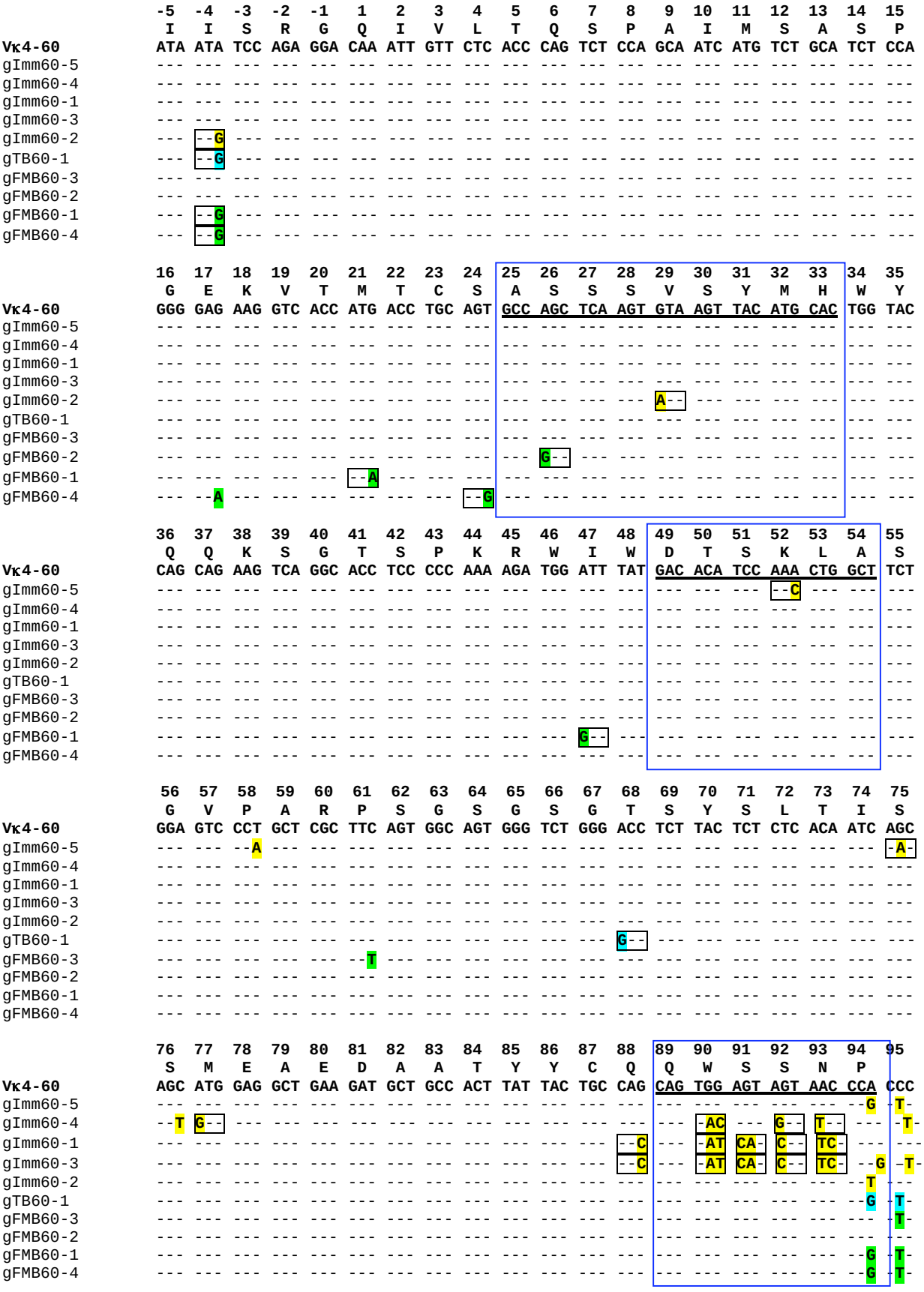
SUPPLEMENTAL FIGURE 2

Figure S2. Functional VDJ-C Recombination from *Aicda*$^{+/-}$ BM Immature IgA-Expressing Cells (B220^{+}AA4.1^{+}IgA^{+}).

Rearranged α-transcripts were amplified by universal V_H (MSHV) and CαR primers (see details in Supplemental Methods below). (A) Total 28 clones were cloned and characterized by DNA sequencing analysis. All of them were in-frame sequences and carried the Cα segment. Some of the representative clones are shown here. Nucleotide sequences and their amino acid sequences are presented. The areas highlighted in grey, blue, green and yellow are the V, D, J, Cα segments, respectively. It is noteworthy that the tested *Aicda*$^{+/-}$ mice were not backcrossed to C57BL/6 enough number of generations, therefore, we presume that some of V_H genes we identified were inherited from the CBA genetic background (from the original ES cell line) and perhaps the germline V_H DNA database is not complete. (B) The J_H segment usage in the 28 clones is depicted. More than half of the sequenced α-transcripts had J_H1 or J_H2 segments, suggesting that many of those clones are yet prior to extensive heavy chain secondary rearrangement. This result is similar to Jκ light chain usage where BM immature B cells carry more Jκ1 or Jκ2 than Jκ4 or Jκ5 in contrast to splenic mature B cells shown in Figure S5C. Individual sequences can be found from Genbank (Accession numbers: EF492991-EF493014 and EF513151-EF513154)

SUPPLEMENTAL FIGURE 3

CLUSTAL W (1.83) multiple sequence alignment of Vκ4-60 from genomic DNA



CLUSTAL W (1.83) multiple sequence alignment of Vκ4-68 from genomic DNA

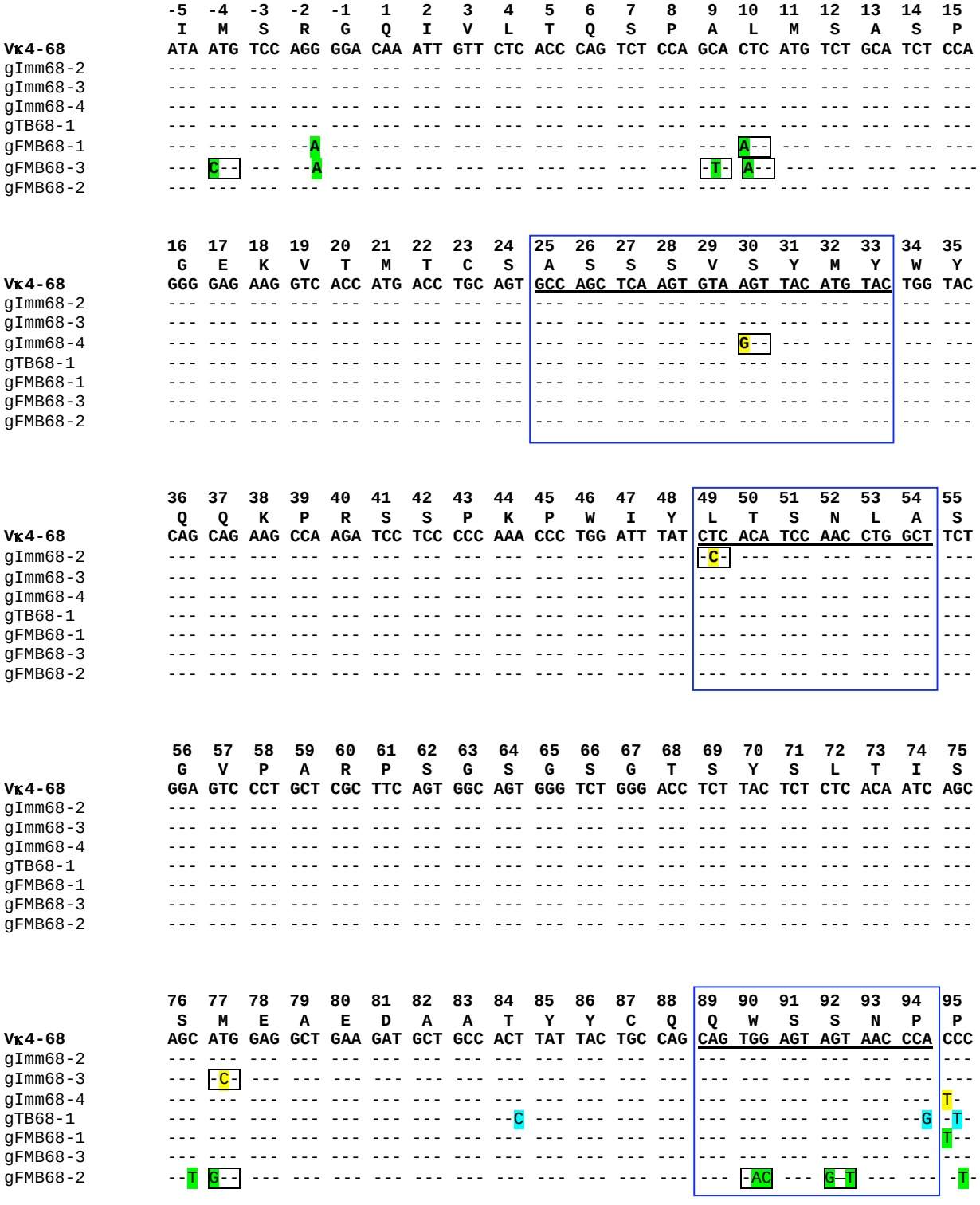


Figure S3. Clustal Alignment of Major Vκ4 Genomic DNA Sequences. *IgVκ4* light chain genes were amplified from rearranged genomic DNA using a specific primer set. DNA sequences of *IgVκ4-60* and *-68* were projected to ClustalW (http://www.ebi.ac.uk/clustalw). No identical two sequences were aligned above although some common nucleotide changes have been found, suggesting divergent sequential mutations from one original clone. The boxes represent the sequences of CDR1, 2 and 3, and highlighted letters indicates nucleotide changes. Germline sequence of *IgVκ4-60* or *-68* is shown at top of each alignment. Codons are numbered by homology with the sequences as in Figure S3. Clone name gImm, gTB, or gFMB stands for DNA sequences amplified from genomic DNA of immature B cells, transitional B cells, or follicuilar B cells, respectively. Nucleotide changes from immature B cells were highlighted with yellow, the ones from transitional B cells were with skyblue, and green highlights represent the nucleotide changes from follicular B cells. Small back boxes surrounding some nucleotide triple codes with a mutation represent amino acid replacement by the nucleotide chage. Large blue boxes with underlines in them are CDR1, CDR2, and CDR3, respectively, in order. DNA sequences isolated from rearranged Vκ4 genomic DNA are available from Genbank (Accession numbers: EF543864-EF544021).

SUPPLEMENTAL FIGURE 4

A

B

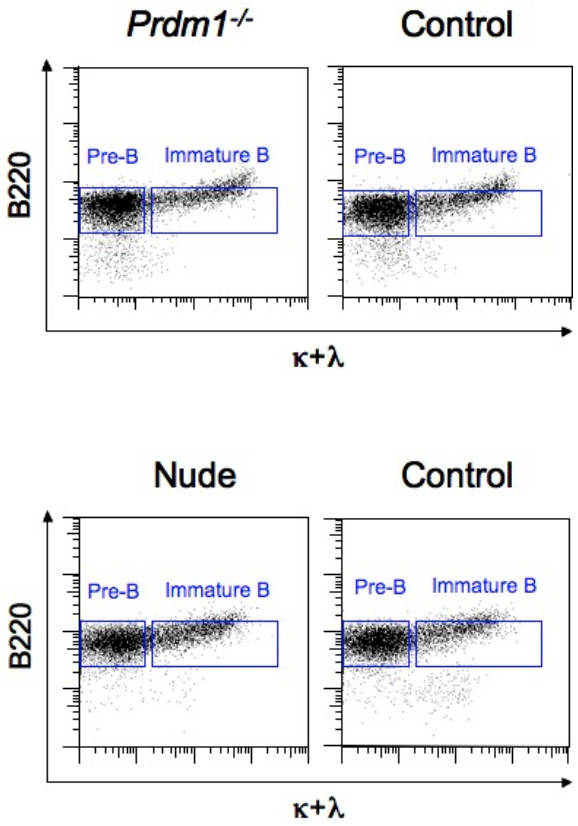


Figure S4. Normal Bone Marrow B cell Staining Profile of *Prdm1*$^{-/-}$ or Nude Mice.

Bone marrow single cell suspension from (A) *Prdm1*$^{-/-}$ mice or from (B) Nude mice was stained with FITC-conjugated anti-mouse κ and λ antibodies. They were subsequently stained with PE-conjugated anti-mouse AA4.1 antibody, and Alexa647-conjugated anti-mouse B220 antibody along with their wild-type littermate controls. PI was also used to exclude dead cells. Viable immature cells were gated on AA4.1+ lymphocytes and analyzed for B220 and κ/λ. As shown in the figures A and B, there is no difference in developing B cell staining profiles between the mutant and wild-type mice.

SUPPLEMENTAL METHODS

Flow Cytometry Staining Procedure

In order to stain pre-B, immature B (both from BM) and mature B cells (from spleen), goat anti-mouse λ and κ polyclonal antibodies labeled with FITC (SouthernBiotech), were used. Subsequently PE-labeled rat anti-mouse AA4.1- and Alexa647-labeled rat anti-mouse B220 monoclonal antibodies (both from Pharmingen) were used to stain the bone marrow and spleen cells. For Peyer's patch cells, Alexa647-labeled rat anti-mouse B220 and FITC-labeled goat anti-mouse IgM antibodies were used. Viable cells were gated by PI-exclusion. In Figure 2D, to stain surface IgA on developing B cells, FITC-labeled goat anti-mouse IgA antibody (IgG) or FITC-labeled goat IgG (as isotype control) (both from SouthernBiotech) were incubated first with single cell suspension. After washing out unbound antibodies, the cells were stained with rat anti-mouse B220-Alexa647- and rat anti-mouse AA4.1-PE monoclonal antibody (both from Pharmingen).

PCR Reaction

AID, RAG-2, PST, and GAPDH amplification was done at 94°C, 3 min; 40 cycles at 94°C, 30s; 55°C, 30s; and 72°C, 1min, followed by a 15 min final extension at 72°C. PCR for CT detection was done at 94°C, 3 min; 40 cycles at 94°C, 30s; 58°C, 1 min; and 72°C, 1 min, followed by a 15 min final extension at 72°C. Primers for CT and PST were previously described (Kinoshita et al., 2001; Muramatsu et al., 2000). Primer sequences for AID, RAG-2, and GAPDH were also described previously (Mao et al., 2004).

SUPPLEMENTAL METHODS

Cell Culture and *In Vitro* Stimulation for Positive Controls for CTs and PSTs

For positive controls of CTs and PSTs, splenic B cells were purified from C57BL/6 mice using StemSep B Cell Enrichment kit (StemCell Technologies). To induce each CSR *in vitro*, purified splenic B cells ($5x10^5$ cells/ml) were stimulated in 10%FCS/RPMI1640 as previously described (Peng et al., 2002). In brief, for CSR to IgG1, 25 μg/ml LPS (Sigma) + 10 ng/ml murine IL-4 (PeproTech); for CSR to IgG2a, 25 μg/ml LPS (Sigma) + 100 units/ml murine IFN-γ (PeproTech); for CSR to IgG2b and IgA, 25 μg/ml LPS (Sigma) + 1 ng/ml human TGF-β1 (PeproTech); for CSR to IgG3, 25 μg/ml LPS (Sigma). After 3 days of incubation, RNAs were prepared and first-strand cDNAs were generated.

Cloning and Sequencing

To amplify *Vκ4* genes, Platinum *Taq* DNA polymerase High Fidelity (Invitrogen) was used at 94°C, 3 min; 35 cycles at 94°C, 30s; 55°C, 30s; and 68°C, 1 min, followed by a 15 min final extension at 68°C. All PCR reactions were carried out according to manufacturer's instructions. Amplified Vκ4 from genomic DNA was cloned into pCRII-TOPO vector using TOPO Cloning Kit (Invitrogen) by following the manufacturer's protocol. Ligation products were transformed into TOP10 competent cell (Invitrogen). Randomly picked colonies were cultured in Terrific Broth (Invitrogen) overnight and plasmid DNAs were purified using QIAprep Spin Miniprep Kit (Qiagen). Plasmid DNAs containing PCR products with the right size were screened by *Eco*RI digestion and subsequently sequenced at Tufts University Core Facility (Tufts University School of Medicine, Boston, MA) or Genewiz Inc. (Plainfield, NJ).

SUPPLEMENTAL METHODS

PCR primers

For amplification of *IgVκ4* genes from cDNA (for the analysis of SHM in Blimp-1-deficient or nude immature B cells):

Vκ4F, 5'-CAAGTGCAGATTTTTCAGCTTCCT-3'

CκR, 5'-CACGACTGAGGCACCTCCAGA-3'

For amplification of rearranged *IgVκ4* genes from genomic DNA:

Vκ4-68F, 5'-GATTTTCAGCTTCCTGCTAATGAGTGCC-3'

Jκ5int3-R, 5'-TGATAATGAGCCCTCTCCAT-3'

For amplification of α-transcripts in cDNA from BM immature $AA4.1^{+}B220^{+}IgA^{+}$ sorted cells (Figure 2F):

MSHV, 5'-CGAGGTGCAGCTGCAGGAGTCTGG-3'

CαR primer sequence is described in a previous report (Kinoshita et al., 2001)

ELISA

To screen B cell-specific Blimp-1-deficient mice, serum IgM level was measured by ELISA. ELISA plates were coated with 5μg/well unlabeled goat polyclonal anti-mouse IgM antibody (SouthernBiotech). Bound serum antibody was detected with 1μg/ml Alkaline Phosphatase (AP)-conjugated goat anti-mouse IgM (μ heavy chain specific) antibody (SouthernBiotech). AP-conjugated antibody was detected with 4-nitrophenyl phosphate disodium salt hexahydrate (pNPP; Sigma-Aldrich) and optical density at 405 nm (OD_{450}) was determined in a Spectra Max 340 ELISA plate reader (Molecular Devices).

REFERENCES FOR SUPPLEMENTAL DATA

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Mao, C., Jiang, L., Melo-Jorge, M., Puthenveetil, M., Zhang, X., Carroll, M. C., and Imanishi-Kari, T. (2004). T cell-independent somatic hypermutation in murine B cells with an immature phenotype. Immunity *20*, 133-144.

Muramatsu, M., Kinoshita, K., Fagarasan, S., Yamada, S., Shinkai, Y., and Honjo, T. (2000). Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell *102*, 553-563.