SUPPLEMENTAL MATERIAL

Kaji et al., http://www.jem.org/cgi/content/full/jem.20120127/DC1

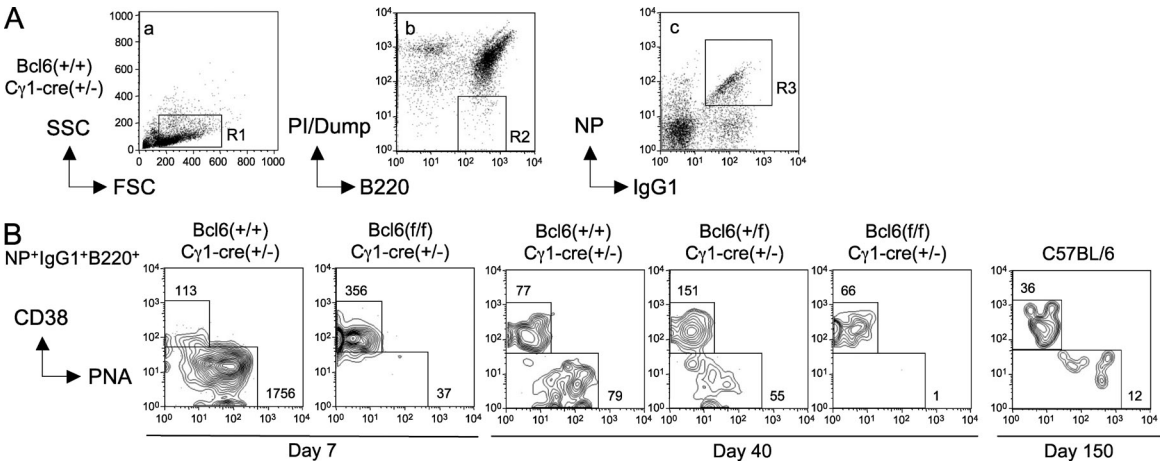

Figure S1. The strategy for analysis of NP-specific B cell subpopulations. (A and B) Splenocytes were recovered from Bcl6$^{+/+}$, Bcl6$^{+/f}$, and Bcl6$^{f/f}$ mice heterozygous for Cγ1-cre at day 7 or 40 after immunization with NP-CG/alum and stained with NIP-BSA^{PE}, anti-CD38^{AlexaFluor647}, anti-B220$^{PE\text{-}Cy7}$, anti-IgG1$^{Pacific\text{-}Blue}$, PNA^{FITC}, and streptavidin$^{PE\text{-}TexasRed}$ as described in Materials and methods. (A) A representative FACS profile for NP-specific/IgG1$^+$ B cells in Bcl6$^{+/+}$ mice heterozygous for Cγ1-cre at day 7 after immunization. Splenocytes were selected under a lymphocyte gate on forward with side light scatter (a, R1). Thereafter, B220$^+$ cells, which were negative for the biotinylated mAbs (Dump) and propidium iodide (PI), were selected by FACS gating (b, R2). (PI/Dump)$^-$/B220$^+$ cells were separated into NP-specific/IgG1$^+$ B cells (c, R3). (B) Representative FACS profiles for CD38$^+$/PNA^{lo} memory and CD-38^{dull} GC B cells in NP-specific/B220$^+$/IgG1$^+$ B cells in conditional Bcl6-deficient mice (Bcl6$^{f/f}$ × Cγ1-cre) and controls (Bcl6$^{+/f}$ × Cγ1-cre and Bcl6$^{+/+}$ x Cγ1-cre) at days 7 and 40 after immunization. A FACS profile from WT mice at day 150 after immunization is also shown. The number of gated cells per 10^6 cells in the lymphocyte gate is shown in each figure.

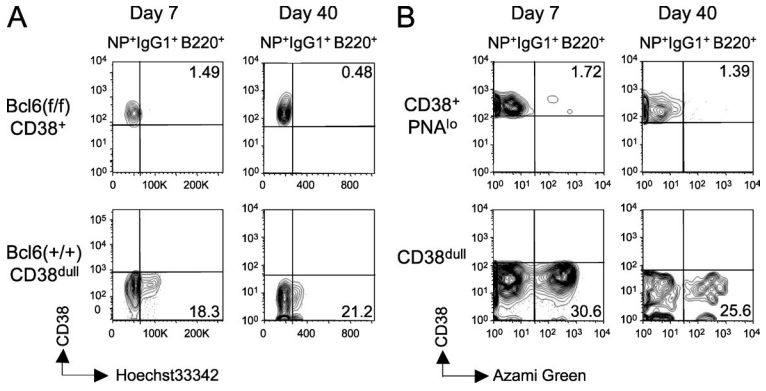

Figure S2. Cell cycle analysis of memory B cells. (A) A representative FACS profile of Hoechst 33342 staining in NP-specific/IgG1$^+$ memory B cells (CD38$^+$) in Bcl6 cKO mice and GC B cells (CD38^{dull}) in control mice at days 7 and 40 after immunization with NP-CG in alum. Splenocytes were incubated with Hoechst 33342 solution, followed by staining as described in Materials and methods. Numbers in plots indicate the percentage of positive cells. (B) A representative FACS profiles of Azami Green$^+$ cells in NP-specific memory (top) and GC B cells (bottom) in the spleen of FUCCI transgenic mice 7 and 40 d after immunization. B cells were enriched by MACS from individual spleens of immunized mice and stained as described in Materials and methods. Numbers in plots indicate percentage of Azami Green$^+$ cells.

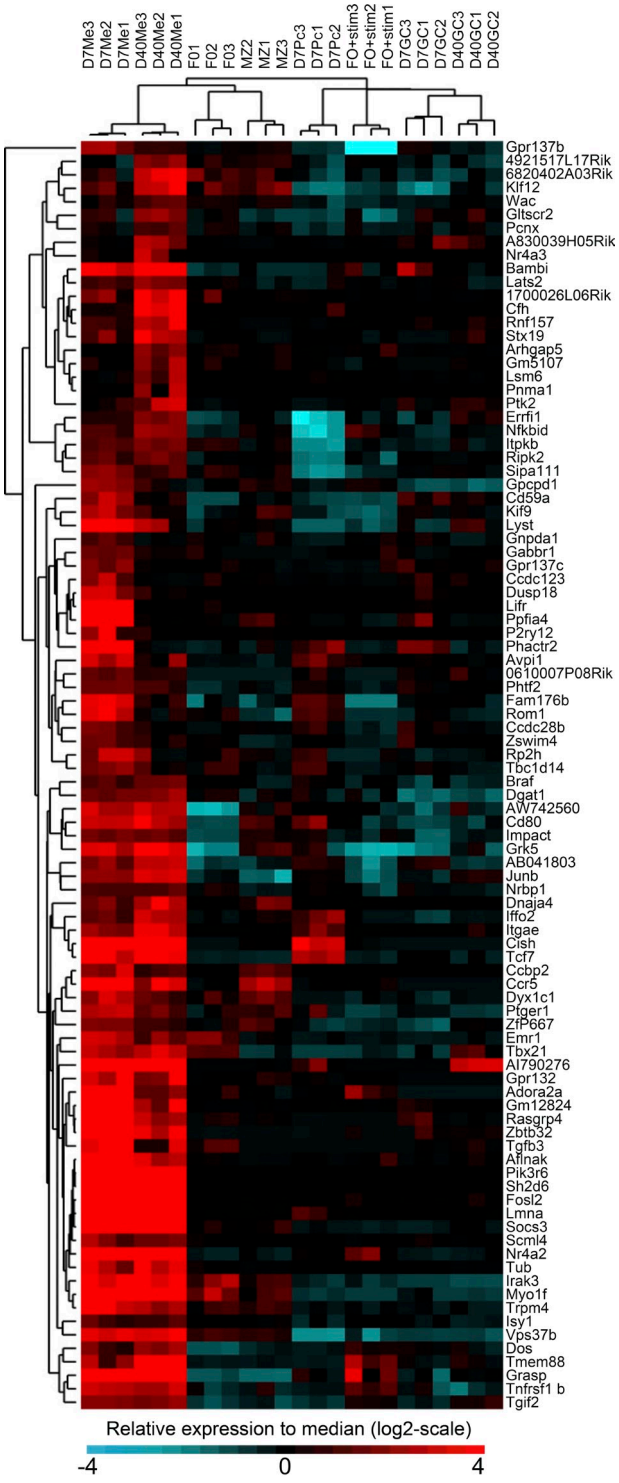

Figure S3. Microarray analysis of memory B cells. Cell-intrinsic differences in three replicates of day 7 and day 40 memory B cells (D7Me and D40Me, respectively), FO B cells (FO), MZ B cells (MZ), day 7 plasma cells (D7Pc), FO B cells stimulated in vitro with anti-IgM $(Fab')_2$ + anti-CD40 mAb (FO+stim), and day 7 and day 40 GC B cells (D7GC and D40GC, respectively). Hierarchical clustering was performed on each gene or individual experiment with Pearson correlation as a metric of similarity across genes or samples with similar expression patterns. Branch lengths represent the degree of similarity between sets. Gene expression profiles that were similar across the experimental samples were clustered together. 94 genes were identified as the memory B cell–specific genes with two distinct statistical methods, Tukey's multiple comparison test, and ROKU (Entropy + AIC). The color in the map depicts the relative expression value to median (log2-scale) for each gene: red and cyan represent high and low expression level, respectively. The heat map was generated using Java TreeView software.

Table S1. Primers for qRT-PCR

Gene	Direction	Sequence (5′–3′)
β-Actin	Forward	ACTATTGGCAACGAGCGGTTC
β-Actin	Reverse	GGATGCCACAGGATTCCATAC
Cfh	Forward	TCCCAGCCCCCTACAATAGAA
Cfh	Reverse	CAAGGAAGTCCAACACAGCGA
Fra-2	Forward	CAATCCCTATCCACGCTCACAT
Fra-2	Reverse	CCGATGGTCTTGATCACTCCAG
Sh2d6	Forward	TGCCCCATTCACTTTCTCTCC
Sh2d6	Reverse	TGCCTCTCAGTTTGAACCTGC
Nr4a3	Forward	GCCTGGCAAATAAAAACTGCC
Nr4a3	Reverse	AACCATCCCGACACTGAGACA
Grasp	Forward	TCATCGTAACAGCCTGTGCAA
Grasp	Reverse	TGAACCGACCAGAAAGACCAG
Pik3r6	Forward	CCCACCCATCCACAACATTT
Pik3r6	Reverse	AATTCCCAGGCATTCTCTGGC
Lmna	Forward	GCGGTAGAGGAAGTCGATGAA
Lmna	Reverse	ACCATTCTGACGCCTGATCTG
Socs3	Forward	TTTTCTTTGCCACCCACGG
Socs3	Reverse	TTCTCGCCCCCAGAATAGATG
Bambi	Forward	ACGGACACCATTCCAAGAAGG
Bambi	Reverse	CCGCATTTTGTCACAGGTCAG
Junb	Forward	TGGAGGACAAGGTGAAGACACT
Junb	Reverse	CATGACCTTCTGCTTGAGCTG
Vps37b	Forward	CGCAGACGGTTCAGCTTAACA
Vps37b	Reverse	TCAGGCGAGCTTTCTGAGCAT
Gpr132	Forward	AAGAACAAGGTGAAGCGCTCC
Gpr132	Reverse	GGAAAAGCTGGCAGCTTTGAC
Cish	Forward	TGTCAGTCAAAACCACCCGTG
Cish	Reverse	AAGGCCAGGATTCGAGGTCTT
Tmem88	Forward	TGATGGAACAGCTGAATGTGG
Tmem88	Reverse	CAGCCCAAACGTTCAGGAAT
Lifr	Forward	TCGCCTCATTTCTCCGGTT
Lifr	Reverse	GCGAGCACCACTTTGTCTTGA
Adora2a	Forward	CCTGCAGAACGTCACCAACTT
Adora2a	Reverse	GAAGCCAGTGCTGATGGTGAT
Nr4a1	Forward	TGTTGATGTTCCCGCCTTTG
Nr4a1	Reverse	ATGCGATTCTGCAGCTCTTCC
Nr4a2	Forward	AAGATCCCTGGCTTTGCTGAC
Nr4a2	Reverse	TTGGACCTGTATGCTAAGCGC
Tnfrsf1b	Forward	GCGCCTTGAAAACCCATTCT
Tnfrsf1b	Reverse	GGCCTTGCATAGCACATTTCC
Rog	Forward	TCCCATAGTACCCCCATCACTG
Rog	Reverse	GAAGCCAGCTGATTCTGACTCC
Nidogen1	Forward	TCACATGTCAGTGCAAGCCTG
Nidogen1	Reverse	CAAGGATGTGCTCTCGTTCCA
Gm12824	Forward	TTCTCCCAGGTGTTGTTTGCC
Gm12824	Reverse	CCCCGCAGTTGTAGTGTTTGA
Nfkbid	Forward	TCCAAGGATGCAGAATTCCC
Nfkbid	Reverse	GCTTGTATGACTGGCACCCAT
AW742560	Forward	CAGGATTGTCCCCACTTTGTGT
AW742560	Reverse	CATTATTAAAGCGAGCCCCTCC
M17	Forward	GATGACTTCAGCTCCCGTTCA
M17	Reverse	TTCACTAATGGCCTTCCCCTG

Table S1. Primers for qRT-PCR *(Continued)*

Gene	Direction	Sequence (5′–3′)
Blimp1	Forward	TTGAGCACCATGAACAACATCA
Blimp1	Reverse	GACGGGATACAACCTAGGGAAGA