Supporting Information

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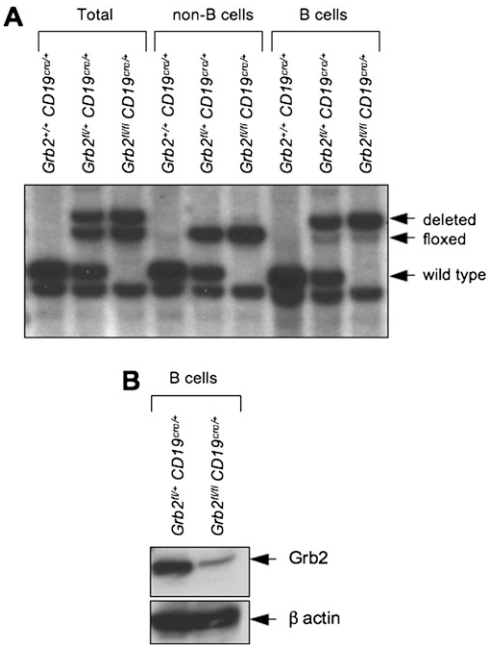

Fig. S1. Specific deletion of *grb2* in B cells. (*A*) Southern blot analysis of *grb2* alleles. Bands representing WT, floxed, and deleted *grb2* alleles are indicated by arrows. Genotype and cell types of the samples are indicated at the top of the blot. (*B*) Western blot analysis of *grb2* deletion in B cells.

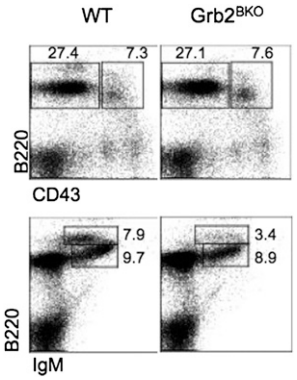

Fig. S2. Normal B-cell generation in the bone marrow (BM) of Grb2^{BKO} mice. Flow cytometry analysis of the BM B-cell subsets in Grb2^{BKO} mice. Shown are dot plots of BM cells stained for B220 and CD43 (*Upper*), B220 and IgM (*Lower*). Percentages of each B-cell subset are indicated in the plots.

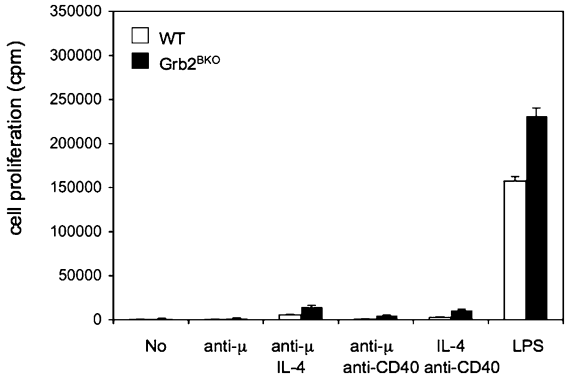

Fig. S3. In vitro proliferation of immature B cells. Immature T1 and T2 (AA4.1^{hi}CD24^{hi}) B cells were isolated by FACS sorting. Cells were cultured in 96-well plates (5×10^4 per well) in the presence of soluble anti-μ $F(ab)_2$, IL-4, anti-CD40, and LPS, either alone or in different combinations for 48 h. ^{3}H-thymidine (1 μCi per well) was added to the culture for 12 h. ^{3}H-thymidine incorporation was determined on a β-counter. Shown are representatives of more than three independent experiments.

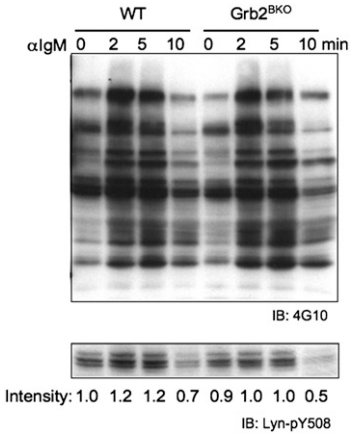

Fig. S4. B-cell receptor (BCR)-induced tyrosine phosphorylation of total cellular proteins and activation of Lyn kinase. Purified B cells were stimulated with anti-IgM for various periods. Tyrosine phosphorylation of total proteins and activation of Lyn were detected by an anti-phosphotyrosine antibody (4G10) and anti-active Lyn (Lyn-pY508).

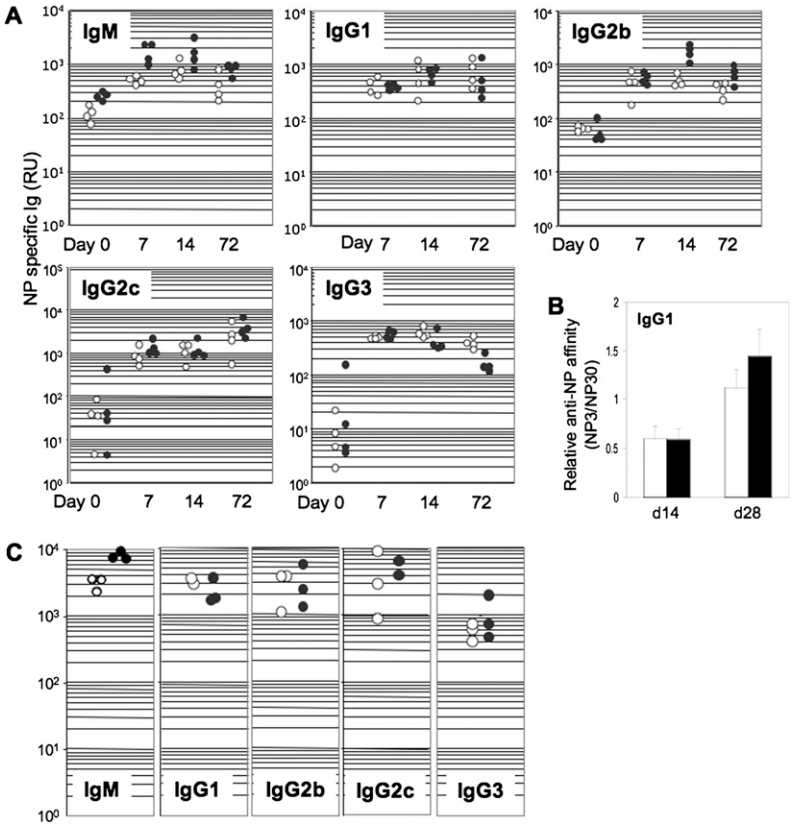

Fig. S5. *grb2*$^{-/-}$ B cells have undergone antibody affinity maturation and produce normal serum levels of antigen-specific IgM and IgG antibodies. (*A*) Serum levels of NP-specific Ig isotypes of WT (○) and Grb2^{BKO} (●) mice at indicated time points after NP-KLH immunization were measured by ELISA. Each symbol represents the value obtained from one mouse. (*B*) Relative affinities of IgG1 of WT (white bars) and Grb2^{BKO} (black bars) mice were determined at the indicated time points after antigenic challenge. Bars represent the mean ± SD (n = 5). (*C*) Serum levels of NP-specific Ig at day 7 after antigenic rechallenge. WT (○) and Grb2^{BKO} (●) mice.

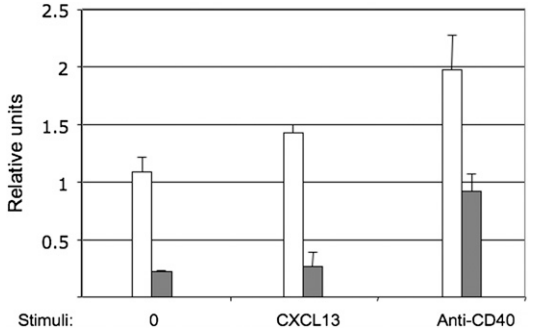

Fig. S6. In vitro up-regulation of lymphotoxin (LT)-β. Splenic B cells were purified from WT and Grb2^{BKO} mice using the mouse B-cell enrichment kit (StemCell Tech). B cells were rested in RPMI medium with 0.1% FCS at 4 °C overnight, and then stimulated with either 3 μg/mL of CXCL13 or 10 μg/mL of anti-CD40 at 37 °C for 3.5 h. Total RNA was prepared from cell lysates and LTβ transcript was quantified by qRT-PCR and are shown as relative units after normalized to the expression of 18sRNA in the corresponding samples. $P < 0.001$, n = 3.

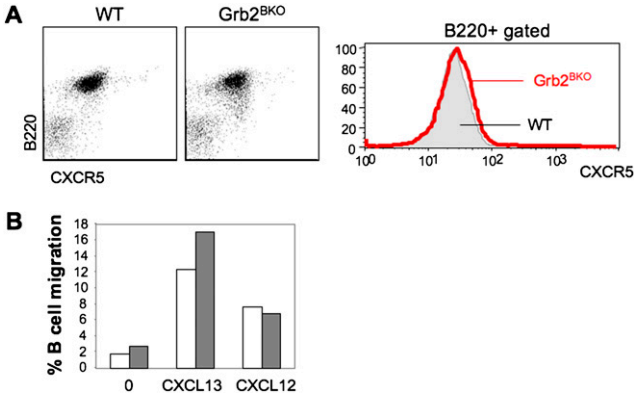

Fig. S7. *grb2*$^{-/-}$ B cells exhibit normal chemoattractant migration to CXCL12 and CXCL13. (*A*) Dot plots show splenocytes from WT and Grb2^{BKO} mice stained with antibodies against B220 and CXCR5. Histograms show the CXCR5 expression level on WT B cells (shaded) or on *grb2*$^{-/-}$ B cells (red). (*B*) Chemotaxis of WT (white bars) or *grb2*$^{-/-}$ (gray bars) B cells toward CXCL12 and CXCL13 were analyzed by a transwell assay. The input and migrated splenocytes were stained with anti–B220-Alexa 780, and the number of B cells in each population was determined by flow cytometry. The percentages of migration of splenic B cells toward CXCL13 and CXCL12 are shown.

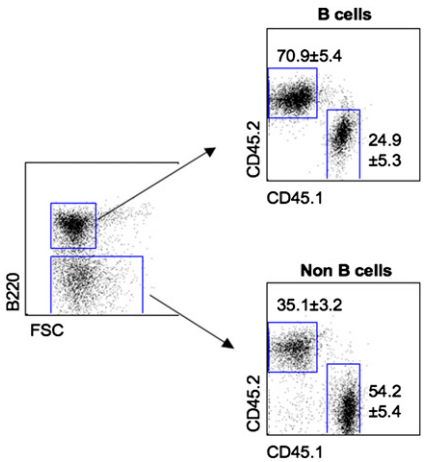

Fig. S8. Preferential expansion of *grb2*$^{-/-}$ B cells in BM chimeras. BM chimeras were generated by transferring equal numbers (10^6) of BM cells from CD45.1$^+$ WT and CD45.2$^+$ Grb2^{BKO} mice into irradiated CD45.1$^+$ WT recipients ($n = 4$). Flow cytometry analysis was carried out 2 mo after transplantation. Splenocytes were stained with anti–B220-Alexa 780, anti–CD45.1-Alexa 450, and anti–CD45.2-APC. B220$^+$ and B220$^-$ populations are gated, and the cells of Grb2^{BKO} or WT origin were displayed as CD45.2$^+$ and CD45.1$^+$ cells, respectively. The inversed chimerism between B cells and non-B cells indicates that *grb2*$^{-/-}$ B cells have undergone preferential expansion.

Table S1. Numbers of subset B cells in the bone marrow and spleen

	Grb2$^{+/+}$	Grb2^{BKO}
Bone marrow	(*N* = 6)	(*N* = 7)
Pre-B	5.9 ± 2.3	5.8 ± 2.2
Pro-B	1.6 ± 0.5	1.5 ± 0.2
Immature B	2.8 ± 1.3	0.9 ± 0.6
Recirculating B	2.3 ± 0.8	0.9 ± 0.6
Spleen	(*N* = 8)	(*N* = 8)
Immature B	4.0 ± 1.3	1.8 ± 0.8*
T1	1.7 ± 0.5	0.7 ± 0.4*
T2	2.0 ± 0.8	0.8 ± 0.3*
Mature B	23.0 ± 7.1	15.7 ± 5.7**
FO	16.4 ± 4.9	9.7 ± 4.2**
MZ	3.0 ± 1.3	2.4 ± 0.9
B1	1.9 ± 0.9	2.3 ± 1.1

Numbers shown here represent data from six to eight mice. Bone marrow pro-B, IgM-IgD-B220^{lo}CD43$^+$; Pre-B, IgM-IgD-B220^{lo}CD43$^-$; immature B, B220^{lo}IgM$^+$ IgD$^-$; recirculating B, B220^{hi}IgD$^+$. Cell surface markers for splenic B cell subsets are described in the legend to Fig. 1. Shown is mean ± SD. *$P < 0.01$; **$P < 0.05$.