

Supplementary Materials for

ERKs Induce Expression of the Transcriptional Repressor Blimp-1 and Subsequent Plasma Cell Differentiation

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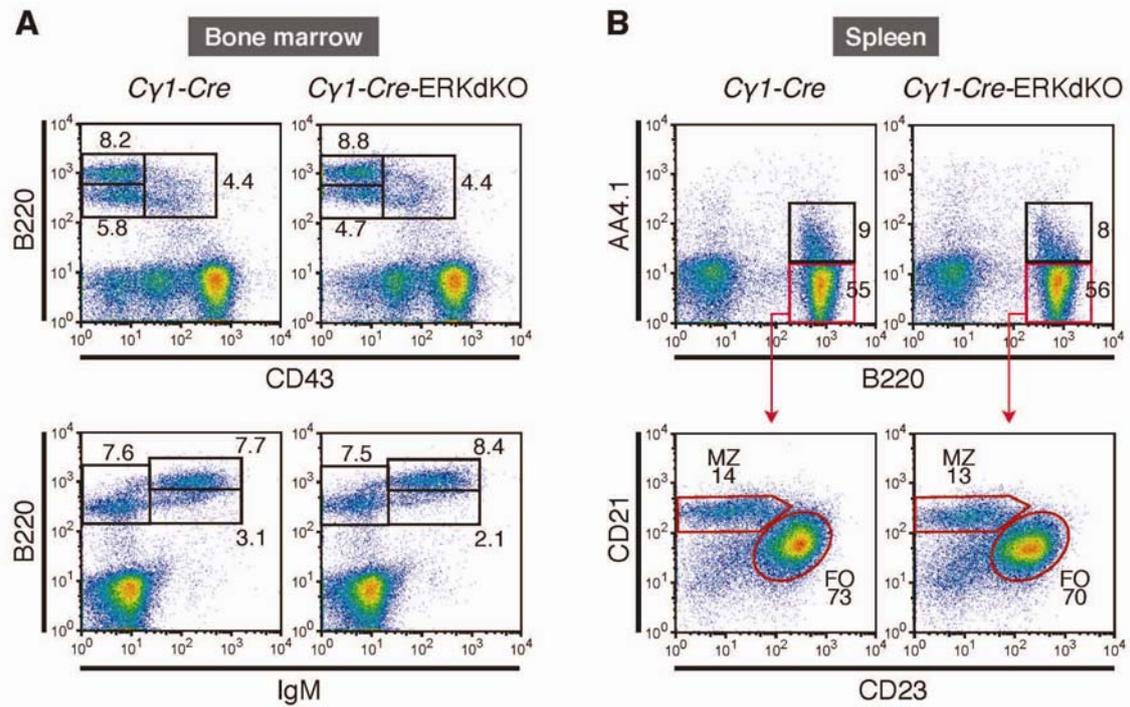


Fig. S1. Normal B cell development in *Cy1-Cre-ERKdKO* mice. **(A)** Representative flow cytometric analysis of bone marrow cells from *Cy1-Cre Mapk1^{+/+} Mapk3^{+/+}* mice (*Cy1-Cre*) or *Cy1-Cre Mapk1^{fl/fl} Mapk3^{-/-}* (*Cy1-Cre-ERKdKO*) mice. **(B)** Representative flow cytometric analysis of splenocytes from *Cy1-Cre* or *Cy1-Cre-ERKdKO* mice. Numbers in the plots represent the percentages of cells within each gate. Representative data from more than three mice are shown.

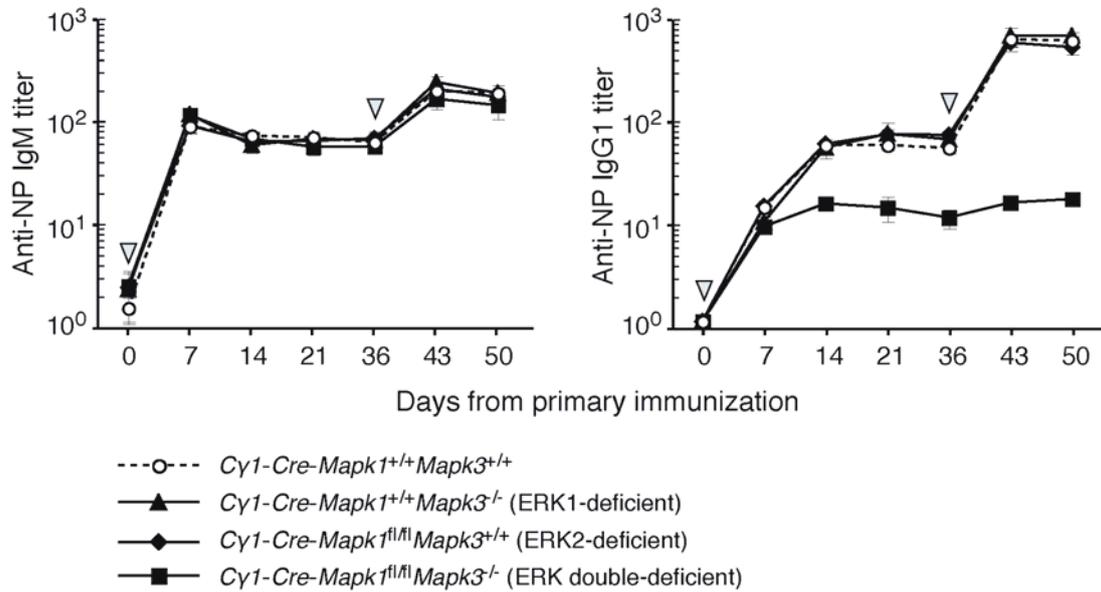


Fig. S2. Antibody responses to the immunization of ERK1-deficient, ERK2-deficient, and ERK double-deficient mice with NP-CGG and alum. The indicated mice were immunized with NP-CGG and alum on day zero. The time (in days) after primary immunization is indicated on the x-axis. Mice were subjected to secondary immunization at day 36 (boost) with NP-CGG alone (arrow heads). The amounts of NP-specific IgM and IgG1 antibodies in the sera of the indicated mice were measured by ELISA. Results represent the mean \pm SEM of four mice per group.

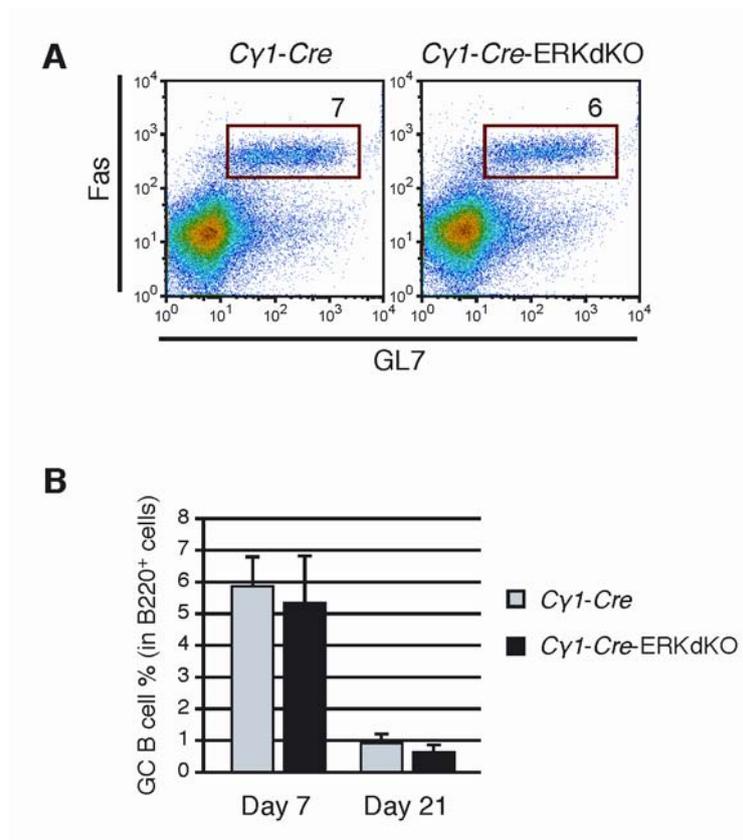


Fig. S3. Generation of GC B cells. (A) Flow cytometric analysis of splenocytes from *Cy1-Cre* and *Cy1-Cre-ERKdKO* mice after immunization with NP-CGG. The Fas^{high}GL7⁺ GC B cell population is boxed within the B220⁺ gate of live cells analyzed 7 days after immunization. (B) The percentages of GC B cells in the gate of B220⁺ live cells at days 7 and 21 are shown. The data are the mean ± SD from four individual mice.

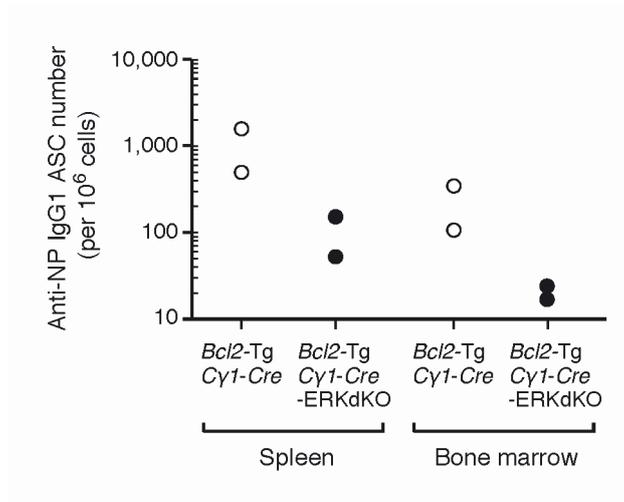


Fig. S4. The NP-specific IgG1 response of *Cγ1-Cre* or *Cγ1-Cre-ERKdKO* mice harboring the *Bcl2* transgene. On day 20 after immunization of mice with NP-CGG and alum, we determined the number of NP-specific IgG1 ASCs in the spleen and bone marrow by ELISPOT. Two mice from each genotype were counted.

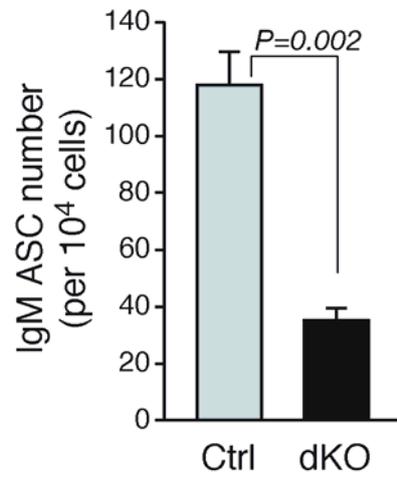


Fig. S5. Effect of ERK deficiency on the numbers of IgM ASCs. Splenic B cells from *Cγ1-Cre Mapk1^{+/+} Mapk3^{+/+}* (Ctrl) or *Cγ1-Cre Mapk1^{fl/fl} Mapk3^{-/-}* (dKO) mice were stimulated for 4 days with anti-CD40 antibody and IL-4. The numbers of IgM ASCs were determined by ELISPOT. Data are the mean \pm SD from three experiments.

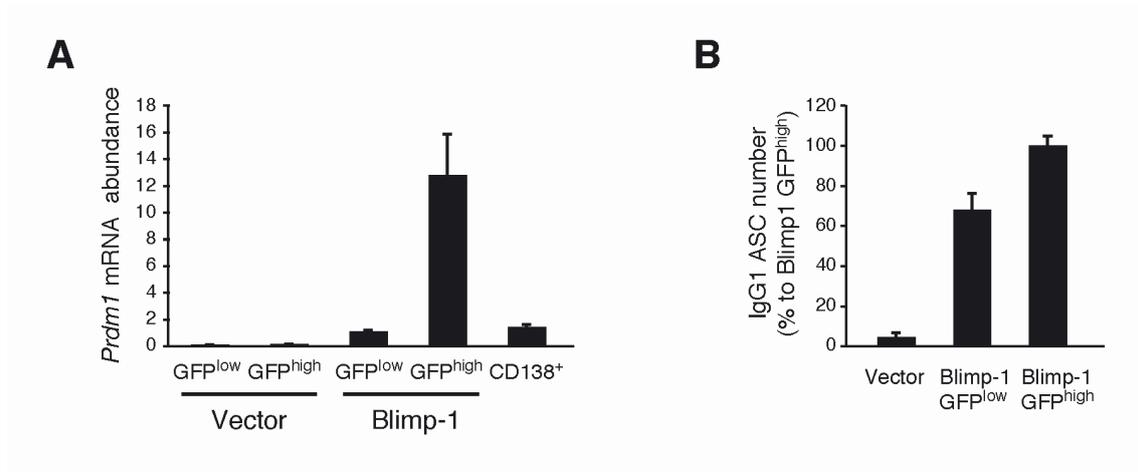


Fig. S6. Normal *Prdm1* expression in ERK double-deficient cells still restores the defect in plasma cell differentiation. Splenic B cells from *Cy1-Cre-ERKdKO* mice were infected with retrovirus expressing the indicated genes. After culturing for 3 days in the presence of anti-CD40 antibody and IL-4, we sorted GFP⁺ cells and cultured them for an additional day. **(A)** The abundance of *Prdm1* mRNA in GFP^{low} and GFP^{high} cells was measured by real-time RT-PCR. CD138⁺ plasma cells were sorted from the wild-type B cell culture. Data are normalized to the abundance of *Gapdh* mRNA and are shown as the mean \pm SD of triplicate samples. **(B)** ELISPOT assay to measure the numbers of IgG1 ASCs. For Blimp-1-expressing cells, GFP^{low} and GFP^{high} cells were separately sorted. Data are the relative cell number to Blimp-1 GFP^{high} cells and are shown as the mean \pm SD of triplicate samples.

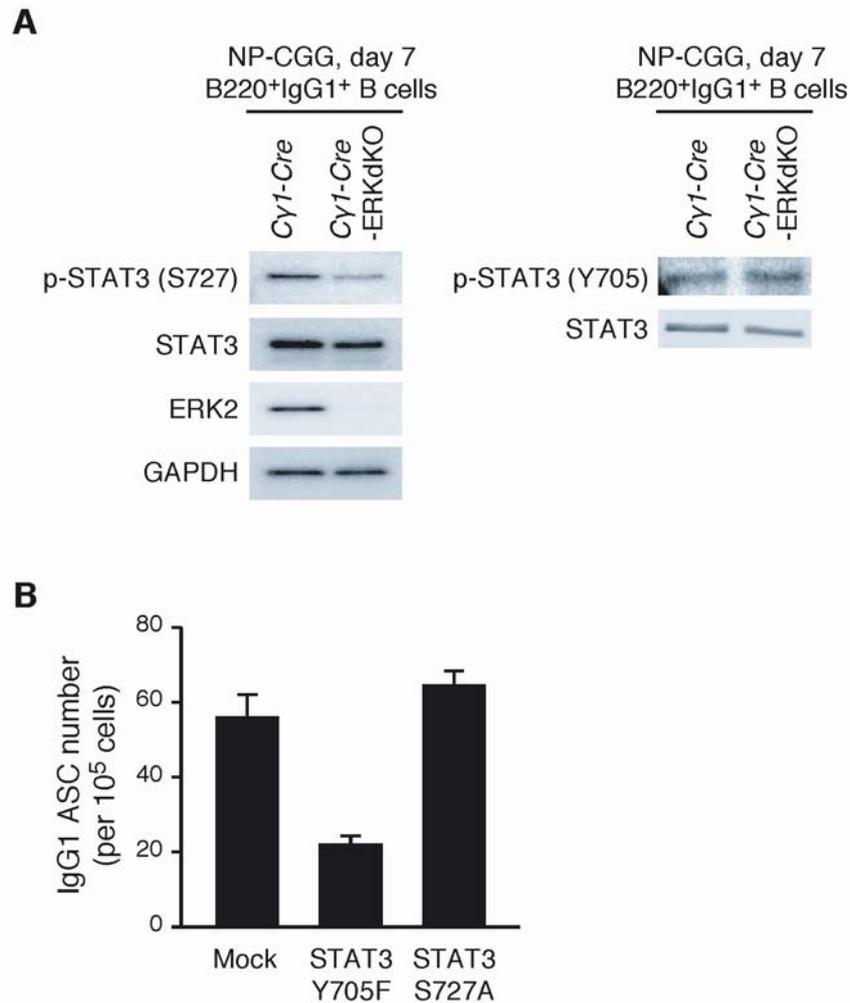


Fig. S7. Phosphorylation of STAT3 on Ser⁷²⁷ depends on ERKs, but is not required for plasma cell differentiation. **(A)** Western blotting analysis of the abundances of pSTAT3 (Tyr⁷⁰⁵ or Ser⁷²⁷), STAT3, ERK2, and GAPDH. IgG1⁺ splenic B cells were isolated at 7 days after immunization of *Cy1-Cre* or *Cy1-Cre-ERKdKO* mice with NP-CGG. **(B)** Naïve splenic B cells were infected with mock retrovirus or with retroviruses expressing STAT3 Y705F or STAT3 S727A, and GFP⁺ cells were purified. After culturing with anti-CD40 antibody and IL-4 for 4 days, we determined the numbers of IgG1 ASCs by ELISPOT. The data are given as the mean \pm SD.

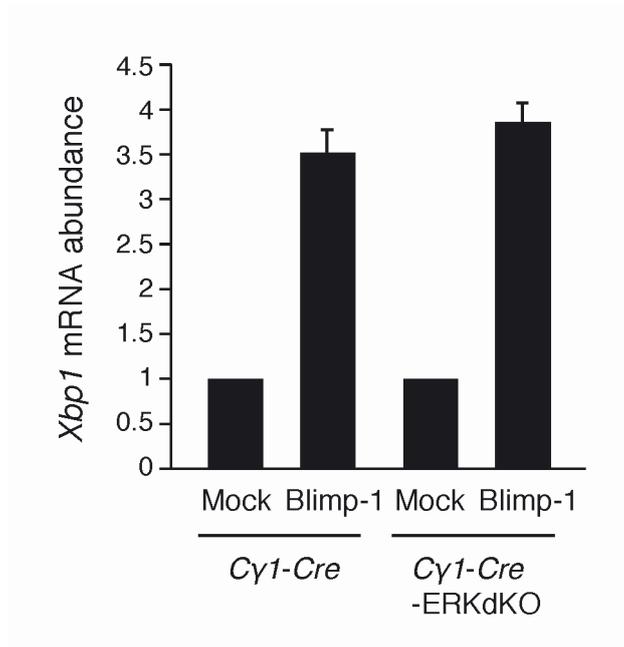


Fig. S8. Overexpression of Blimp-1 induces the expression of *Xbp1*. Splenic B cells treated with anti-CD40 antibody and IL-4 were infected with a retrovirus expressing GFP only (Mock) or with a retrovirus expressing Blimp-1 and GFP (Blimp-1). After culture for 3 days in the presence of anti-CD40 antibody and IL-4, we sorted GFP⁺ cells and determined the abundance of *Xbp1* mRNA by real-time RT-PCR. Data were normalized to the abundance of *Gapdh* mRNA and expressed as the fold-induction compared to cell infected with mock virus.