SUPPLEMENTAL MATERIAL

Nakano-Yokomizo et al., http://www.jem.org/cgi/content/full/jem.20101623/DC1

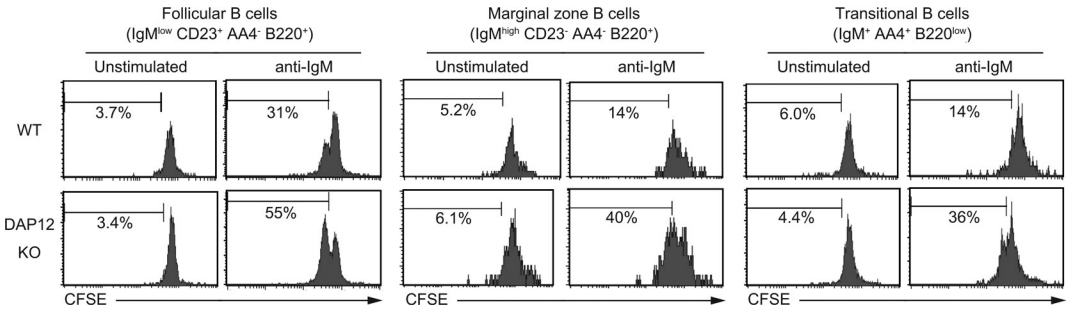

Figure S1. Enhanced proliferation of DAP12-deficient B cell subsets. Each subset of B cells was purified from the spleen of WT or DAP12-deficient mice by flow cytometry. The purified B cells were labeled with CSFE and stimulated with 10 µg/ml anti-IgM. Proliferation was analyzed by flow cytometry. Data are representative of two independent experiments.

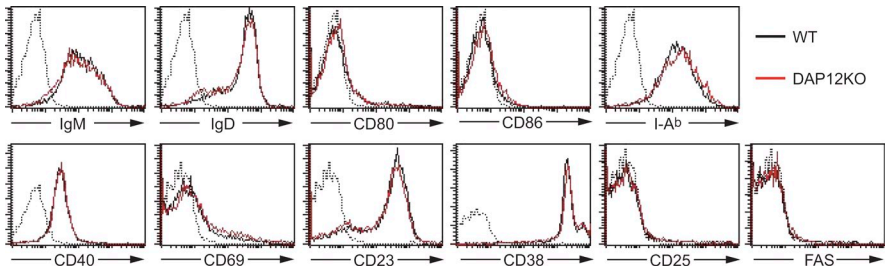

Figure S2. Maturation and activation markers of B cells derived from WT and DAP12-deficient (KO) mice are comparable. B cells were purified from the spleen of WT or DAP12-deficient mice by positive selection using magnetic beads with anti-B220 mAb and simultaneously stained with the FITC-conjugated or PE-conjugated mAb indicated and APC-conjugated B220 mAb. The expression of maturation or activation markers on B220$^+$ gated cells was assessed by flow cytometry. Data are representative of three independent experiments.

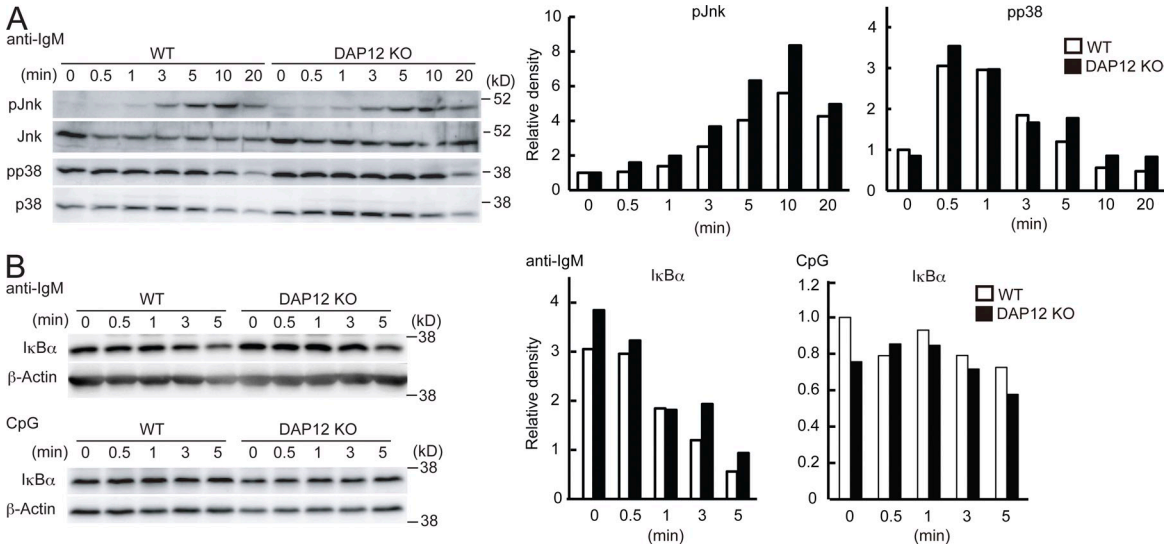

Figure S3. Comparable IKBα degradation in WT and DAP12-deficient B cells after stimulation with anti-IgM or CpG. Purified B cells from the spleens of WT and DAP12-deficient mice were stimulated with 5 µg/ml of $F(ab')_2$ fragments of anti-mouse IgM (A) or 0.06 µM CpG (A and B). The stimulated cells were lysed and then analyzed by immunoblotting with anti-phospho-Jnk, anti-Jnk, anti-phopho-p38, anti-p38, anti-IKBα, or anti-β-actin. The relative amount of each protein, as determined by densitometry, before and after stimulation is also shown.

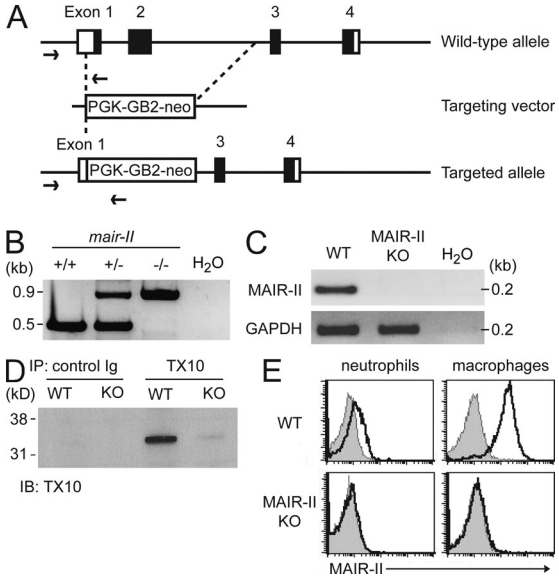

Figure S4. Generation of MAIR-II–deficient mice. (A) A targeting vector was designed to disrupt the MAIR-II (*Cd300d*) gene by homologous recombination. The partial fragment of the first exon (1) containing the start codon and the second exon (2) were replaced by a gene for neomycin resistance (pGK-GB2-Neo). (B) Genomic PCR. +/+, +/−, and −/− represents C57BL/6J mice that are WT, chimeric, or homozygous negative for *Cd300d*, respectively. (C) mRNA from the spleen cells of WT and MAIR-II–deficient mice were subjected to RT-PCR. (D) The spleen cells of WT and MAIR-II–deficient mice were lysed, immunoprecipitated with anti–MAIR-II mAb TX10 or control Ig, and then immunoblotted with anti–MAIR-II mAb TX10. (E) Spleen cells from WT and MAIR-II–deficient mice were simultaneously stained with Alexa Fluor 647–conjugated anti–MAIR-II (TX52) and either FITC-conjugated Gr-1 or FITC-conjugated F4/80. MAIR-II expression was analyzed on neutrophils (Gr-1$^+$ gate) and macrophages (F4/80$^+$ gate) by flow cytometry.

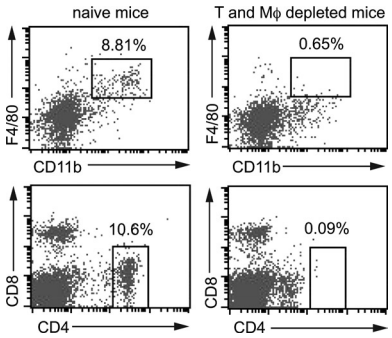

Figure S5. Depletion of CD4+ T cells and macrophages. WT and DAP12-deficient mice were injected i.p. with 100 µg anti-CD4 mAb (GK1.5) and with 500 µl of a suspension of Cl_2MBP-liposomes 2 and 1 d, respectively, before immunization with NP-Ficoll. Spleen cells from these mice or untreated PE-conjugated F4/80 or FITC-conjugated CD4 and PE-conjugated CD8 and then analyzed by flow cytometry.