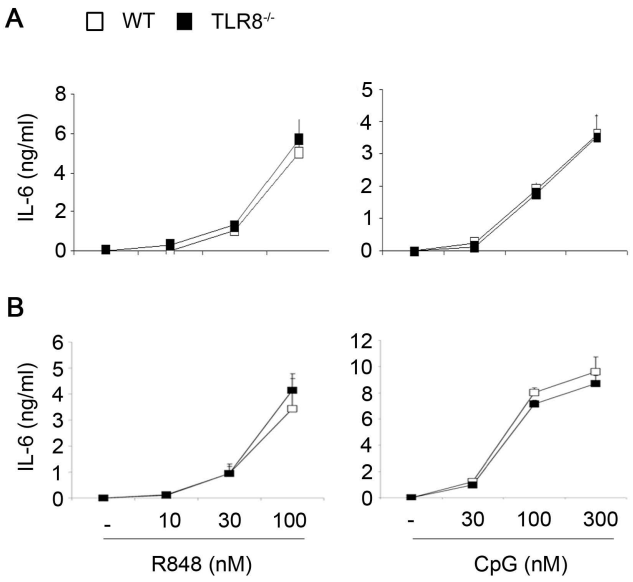
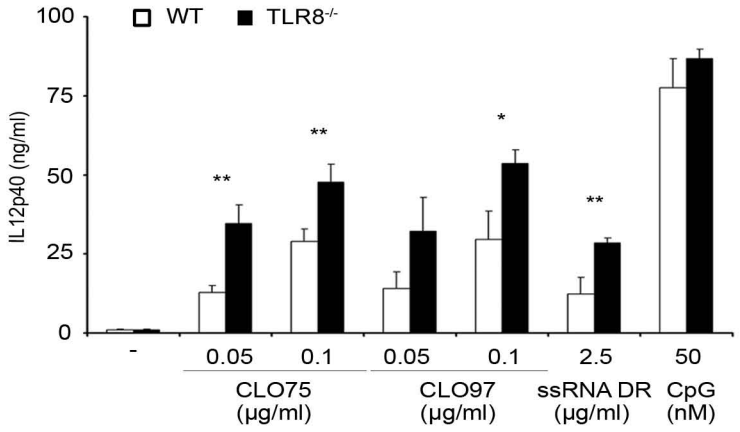
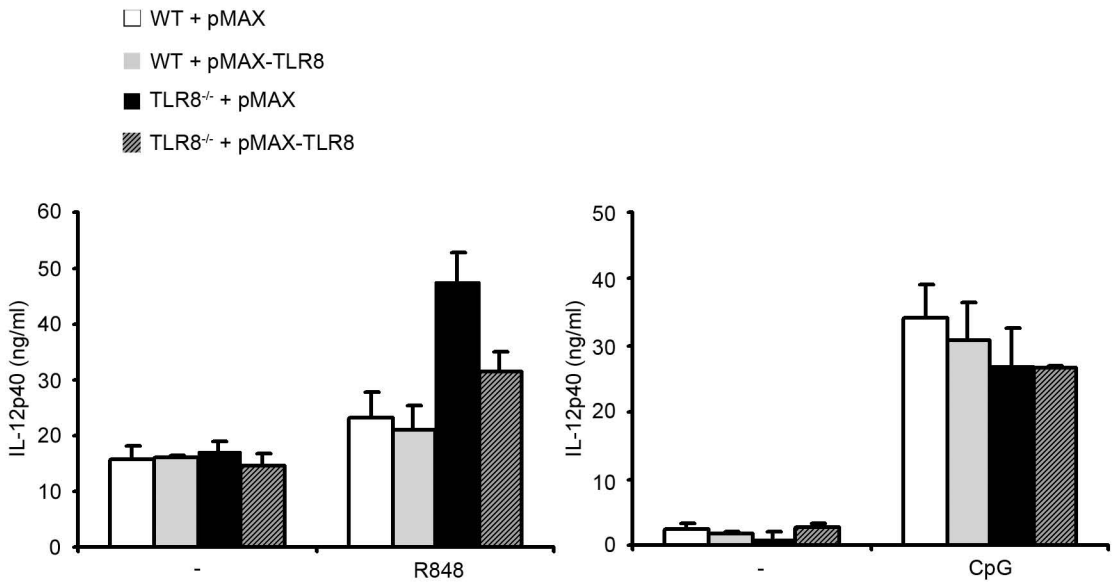


Supplemental Figure 1

Similar IL-6 production by wild-type and TLR8$^{-/-}$ macrophages and splenocytes. Wild-type or TLR8$^{-/-}$ (A) BMMs or (B) splenocytes were left untreated or stimulated with the indicated amounts of R848 or CpG for 20h. The levels of IL-6 in the culture supernatants were determined by ELISA. Data indicate the mean ± SD of four mice per group. Figures are representative of three independent experiments.

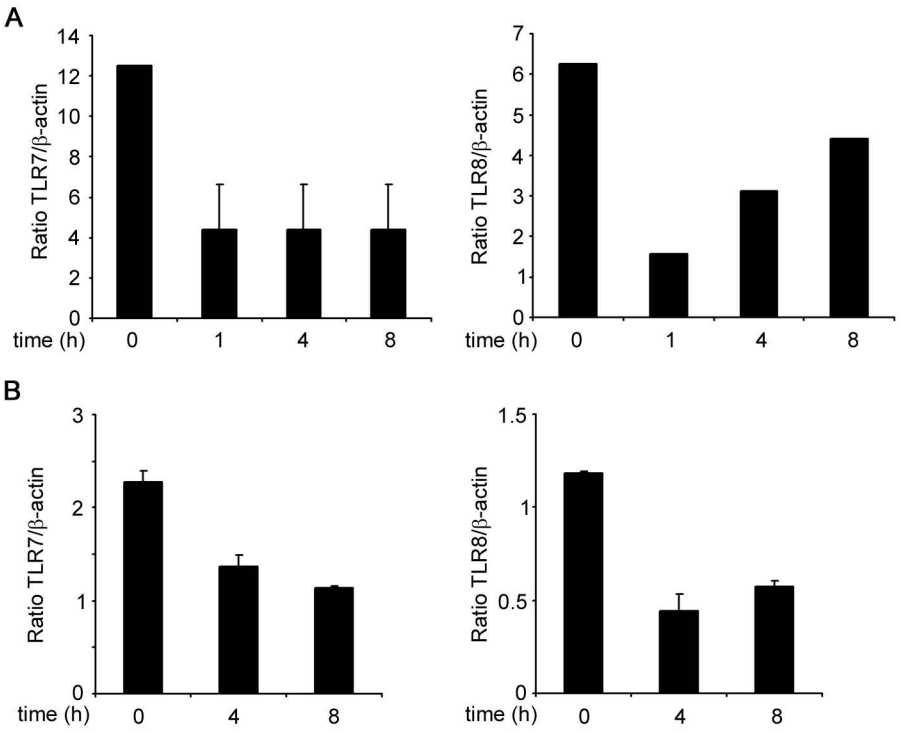


Supplemental Figure 2
Enhanced IL-12p40 production by TLR8$^{-/-}$ BMDCs in response to various TLR7 ligands. BMDCs from wild-type or TLR8$^{-/-}$ mice were stimulated with the indicated amounts of CL075 (TLR7/8 ligand), CL097 (TLR7/8 ligand), ssRNA-DR/LyoVec (TLR7/8 ligand) or CpG for 20 h. The concentration of IL-12p40 in the culture supernatants were assessed by ELISA. Data indicate the mean ± SD of three to four mice per group. *, $p<0.05$; **, $p<0.01$.

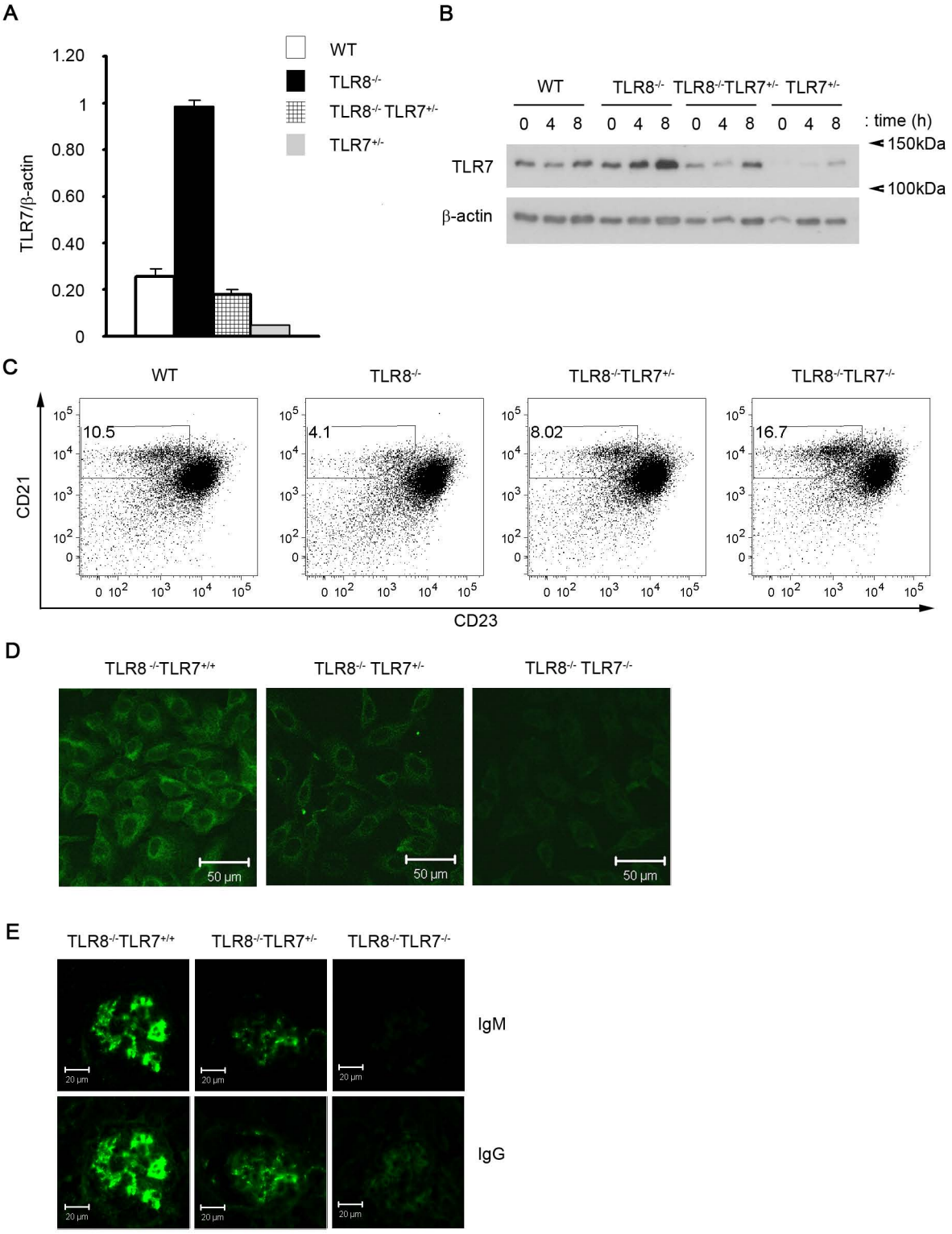


Supplemental Figure 3

Rescue of the phenotype TLR8$^{-/-}$ phenotype by the addition of a functional TLR8 gene. Wild-type or TLR8$^{-/-}$ BMDCs were transfected in triplicates with 1µg of TLR8 expression vector (pMAX-TLR8) or empty vector (pMAX) via the AMAXA kit (according to manufacturers instructions). Two hours later, cells were left untreated of stimulated with 50 nM R848 or 50 nM CpG. After 16 h, culture supernatants were collected and the concentration of IL-12p40 was assessed by ELISA. Data indicate the mean ± SD of triplicates.

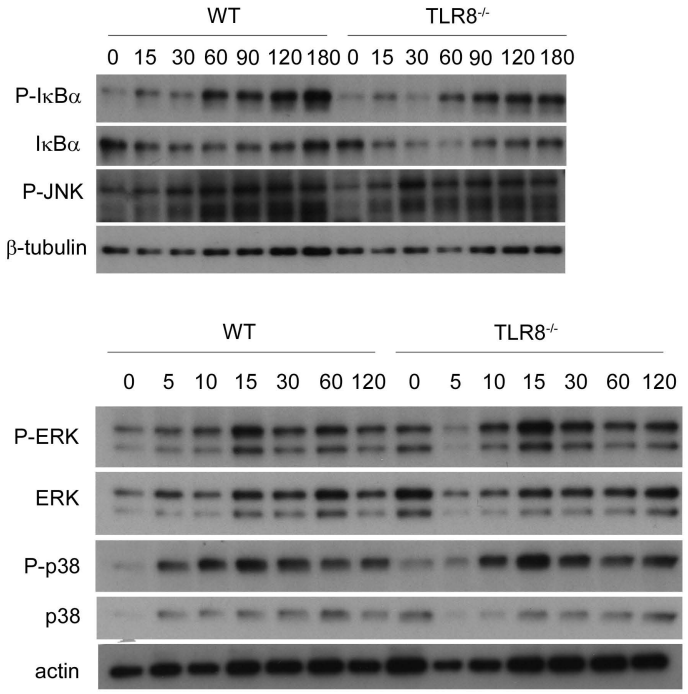


Supplemental Figure 4
TLR7 and TLR8 mRNA expression in IFN-γ or TNFα activated BMDCs. Wild-type BMDCs were left untreated or stimulated with (A) 2 ng/ml IFN-γ or (B) 10 ng/ml TNFα for the indicated time points. Total RNA was extracted from the cells and the expression of TLR7 or TLR8 was assessed by quantitative PCR. Data indicate the mean ± SD of duplicates.



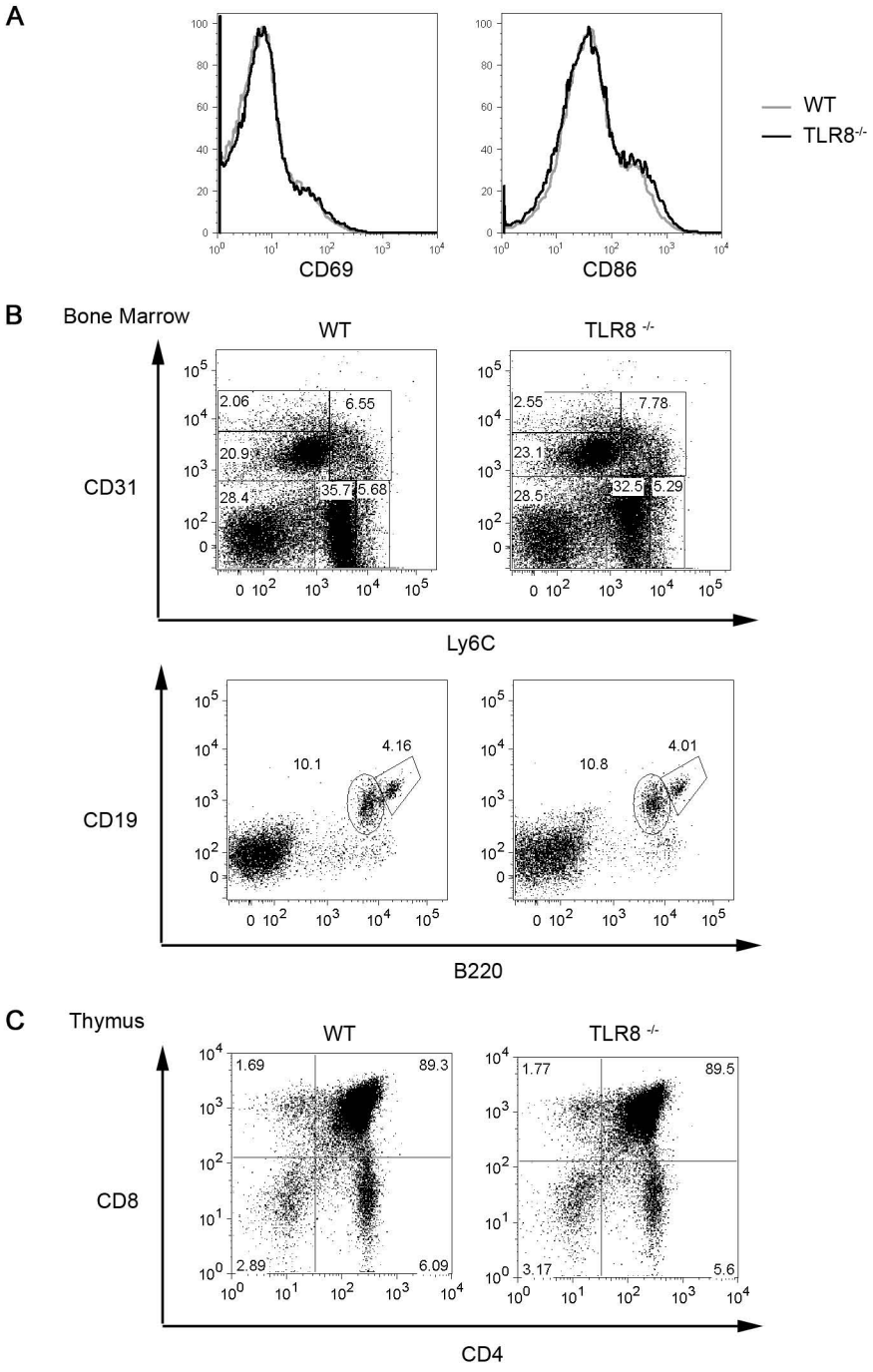
Supplemental Figure 5

Comparison of the responses and phenotype of TLR8$^{-/-}$, TLR8$^{-/-}$TLR7$^{+/-}$ and TLR8$^{-/-}$TLR7$^{-/-}$ BMDCs and mice. Wild-type, TLR8$^{-/-}$, TLR8$^{-/-}$TLR7$^{+/-}$ and TLR7$^{+/-}$ BMDCs were (A and B) left untreated or (B) stimulated with 100 nM R848 for the indicated time points. Total RNA or protein was extracted from the cells and the expression of TLR7 was assessed by (A) quantitative PCR or (B) Western blot and β-actin was used as control. (C) Erythrocyte-depleted splenocytes from age and sex matched wild-type, TLR8$^{-/-}$, TLR8$^{-/-}$TLR7$^{+/-}$ and TLR8$^{-/-}$TLR7$^{-/-}$ mice were analyzed by flow cytometry for the expression of CD19, CD21 and CD23. The numbers denote the percentage of MZ B cells (CD21^{high}CD23$^{low/neg}$) on CD19^{+} gated cells. (D) ANA staining patterns on Hep2 human epithelial cells for serum derived from TLR8$^{-/-}$TLR7$^{+/+}$, TLR8$^{-/-}$TLR7$^{+/-}$ and TLR8$^{-/-}$TLR7$^{-/-}$ mice at 1:160 dilution. (E) IgM and IgG immunofluorescence staining of kidney sections from TLR8$^{-/-}$TLR7$^{+/+}$ (9 weeks old), TLR8$^{-/-}$TLR7$^{+/-}$ (11 weeks old) and TLR8$^{-/-}$TLR7$^{-/-}$ (17 weeks old) mice. In panel A, data indicate the mean ± SD of duplicates. In panels C to E, data are representative of three mice per group.



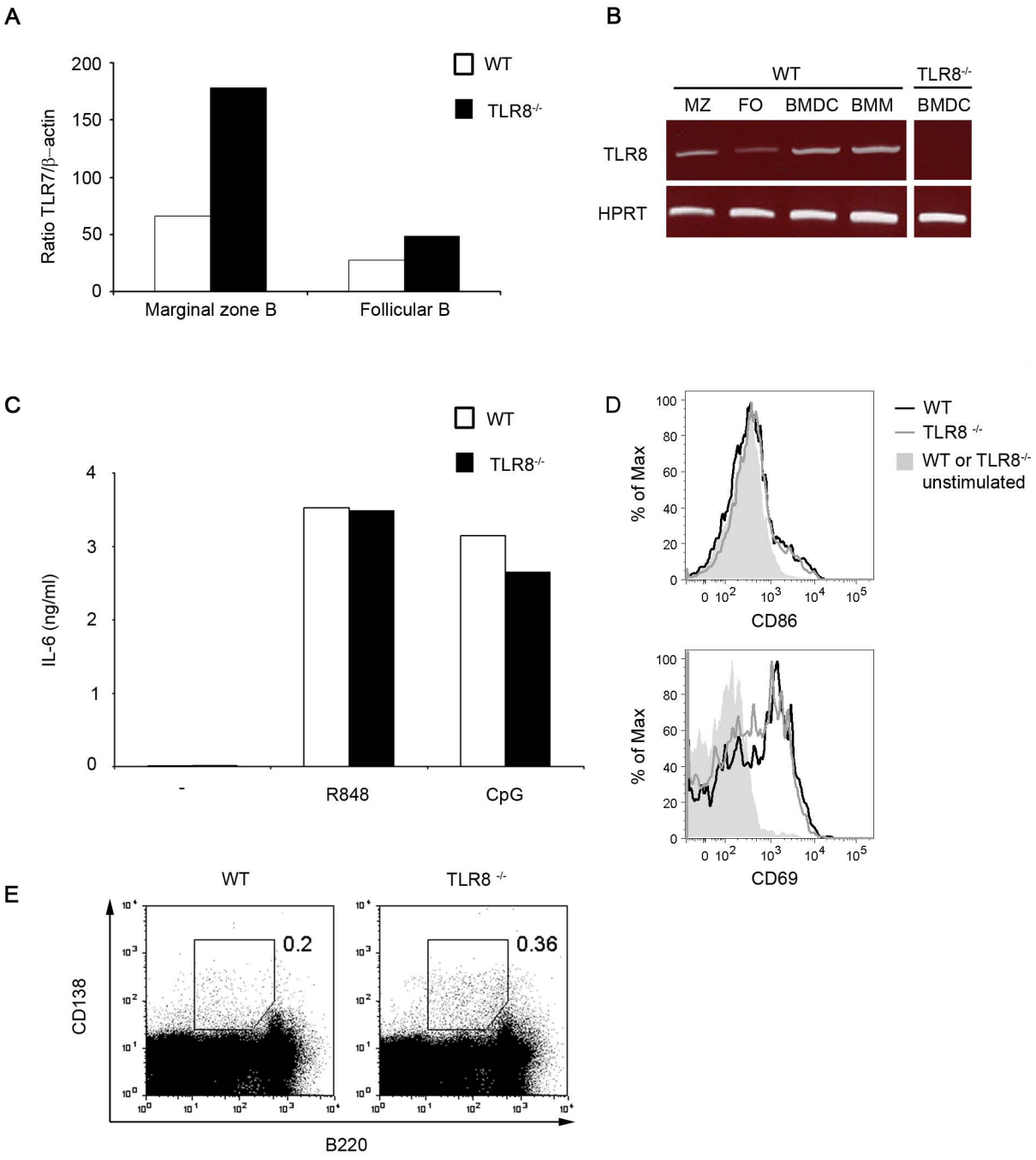
Supplemental Figure 6

Activation of Iκ-Bα and MAP kinases in wild-type and TLR8$^{-/-}$ BMDCs upon R848 stimulation. Wild-type or TLR8$^{-/-}$ BMDCs were stimulated with 100 nM R848 and at the indicated time points, cells were lysed and phosphorylation of Iκ-Bα, JNK, p38 and ERK and degradation of Iκ-Bα were determined by Western blot. Actin, β-tubulin and total ERK and p38 were used as loading controls. Figures are representative of three independent experiments.



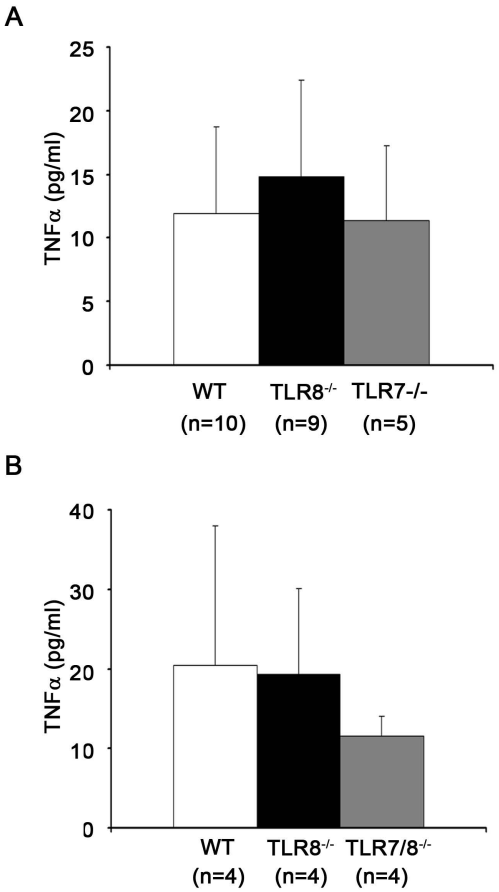
Supplemental Figure 7

No spontaneous activation on TLR8$^{-/-}$ B cells and normal myeloid progenitor fractions of the BM and thymic development in TLR8$^{-/-}$ mice. (A) The expression of activation markers CD69 and CD86 was assessed by flow cytometric analysis on B220^{+} gated cells from wild-type and TLR8$^{-/-}$ mice. (B) Bone marrow cells from 12 weeks old wild-type and TLR8$^{-/-}$ mice were analyzed by flow cytometric analysis using antibodies specific for CD31, Ly6C, CD19 and B220. With anti-CD31 and anti-Ly6C, the cell subsets were defined as follows: blast cells, CD31^{high}/Ly6C^{low}; lymphoid cells CD31^{med}/Ly6C^{low}; erythroid cells CD31^{low}/Ly6C^{low}; myeloid progenitors and plasmacytoid cells CD31^{high}/Ly6C^{high}; granulocytes CD31^{low}/Ly6C^{med}; and monocytes, CD31^{low}/Ly6C^{high}. With anti-CD19 and anti-B220, the cell subsets were defined as B220^{high}CD19^{+}, recirculating B cells and B220^{+}CD19^{+}, pro-, pre- and immature-B cells. (C) Thymic cells from wild-type and TLR8$^{-/-}$ mice stained with anti-CD4 and anti-CD8 gated on live cells and analyzed by flow cytometric analysis. The percentage of cells in each quadrant is indicated.



Supplemental Figure 8

Analysis of wild-type and TLR8$^{-/-}$ MZ B cells, follicular B cells and plasmocytes. MZ and follicular (FO) B cells were isolated from wild-type and TLR8$^{-/-}$ spleens by Flow cytometry. (A) Expression of TLR7 mRNA by quantitative PCR of wild-type and TLR8$^{-/-}$ MZ and FO B cells. (B) Expression of TLR8 mRNA by PCR on cDNA derived from wild-type MZ and FO B cells, BMDCs and BMMs. cDNA from TLR8$^{-/-}$ BMDCs was included as negative control. (C and D) Wild-type or TLR8$^{-/-}$ MZ B cells were left untreated or stimulated with 100 nM R848 or 100 nM CpG overnight. (C) The levels of IL-6 production in culture supernatants were assessed by ELISA. Data are representative of three independent experiments. (D) The expression of activation markers CD86 and CD69 was assessed by flow cytometric analysis (E) Flow cytometric analysis of splenocytes from 3 months old wild-type or TLR8$^{-/-}$ mice shows the B220^{lo}CD138^{+} population. Data are representative of three independent experiments.



Supplemental Figure 9

Similar spontaneous TNF production in wild-type, TLR8$^{-/-}$, TLR7$^{-/-}$ or TLR7/8$^{-/-}$ mouse sera. Sera were collected from wild-type, TLR8$^{-/-}$, TLR7$^{-/-}$ or TLR7/8$^{-/-}$ mice aged (A) 3 or (B) 6 months old, and the production of TNF in their sera was assessed by ELISA. Data indicate the mean ± SD of each mouse group.