**Supplementary Figure Legends**

**Figure S1 – B cell-specific TLR9 deficiency protected NOD mice from developing insulitis.** Pancreata from 20-wk-old female *TLR9*$^{fl/fl}$/CD19-Cre- and *TLR9*$^{fl/fl}$/CD19-Cre-+ NOD mice (n=6-7 mice/group, 105-116 islet/group) were assessed for immune cell infiltration. The insulitis was scored by the percentage of infiltration in each observed islet. Chi-square test was used for statistical analysis.

**Figure S2 – Representative gating strategy of flow cytometry.** **A**: Immune cells from splenocytes were first gated according to the SSA and FSC. The gated immune cells were further gated on the live cells using Zombie Dye (BioLegend). Gated live cells (Zombie Dye negative) were then gated using isotype control antibody (rat IgG2b) plot. Isotype control antibody negative but B200$^{+}$ cells were selected for further B cell subset analysis. **B:** Representative flow cytometry plots of gating CD21 and CD23 after gating on B cells (B220$^{+}$).

**Figure S3- The absolute cell number of different B cell subsets in different lymphoid tissues.** Immune cells were isolated from different lymphoid tissues of *TLR9*$^{fl/fl}$/CD19-Cre- and *TLR9*$^{fl/fl}$/CD19-Cre-+ NOD mice (females, 6-8 weeks). Absolute cell numbers of different B cell subsets were calculated as percentage of each subset in flow cytometric analysis ($10^5$) multiplied by the total cell numbers harvested from each tissue. Graphical summaries of the different B cell subsets are shown (A-E). Data were pooled from 2 independent experiments and assessed for significance using an unpaired, two-tailed Student's t Test.

**Figure S4 – B cell-specific TLR9 deficiency altered the frequency of B cell subsets in different lymphoid tissues at different age**

**A** and **B** show graphical summaries of B cells expressing CD21$^{high}$CD23$^{low}$ and CD21$^{high}$CD23$^{high}$ in 6-8-week-old female mice (*n*=7/group). **C** and **D** show graphical summaries of B cells expressing CD21$^{high}$CD23$^{low}$ and CD21$^{high}$CD23$^{high}$ in 30-32-week-old non-diabetic female mice (*n*=4/group). Data were pooled from 2 independent experiments and assessed for significance using a Student's t test.

**Figure S5- The differences in $IgM^+$ and $IgD^+$ single and double positive B cell subsets in different lymphoid tissues.**

**A-B** demonstrate flow cytometric profiles from 6-8-week-old female mice (*n*=6/group); **A** shows a graphical summary of flow cytometric analysis of B cells expressing $IgM^+IgD^-$, while **B**, shows graphical summaries of the frequency of B cells expressing $IgM^+IgD^+$. Data were pooled from 2 independent experiments and assessed for significance using a Student's t test.

**Figure S6- B cell-specific TLR9 deficiency decreased circulating IgG1.**

Circulating immunoglobulins (IgM, IgA, IgG and IgG1) were assessed from the serum samples of 10-12-week-old female mice (n=4-8/group) by ELISA. The concentration of each isotype was calculated based on the standard curve, respectively. Two-tailed unpaired Student's t test was used for the statistical analysis.

**Figure S7- B cell-specific TLR9 deficiency decreased $Blimp1^-$ plasmacytes.** Immune cells from different lymphoid tissues were isolated from 6-8-week-old female mice (n=5/group) followed by staining with markers for B cells and plasmacytes and analysis by flow cytometry. **A**, proportion of B220+CD138-Blimp1+ plasmacytes; **B**, B220+CD138+Blimp1+ plasmacytes; **C**, B220+CD138+Blimp1- plasmacytes. Two-tailed unpaired Student's t test was used for the statistical analysis.

**Figure S8- B cell-specific TLR9 deficiency did not affect CD8 T cells.** Immune cells from different lymphoid tissues were isolated from 6-8-week-old female mice (n=5/group) followed by staining with the markers for different CD8+ T cells and analysis by flow cytometry. **A**, proportion of naïve CD8+ T cells (TCRβ+CD8+CD44-CD62L+); **B**, proportion of memory CD8+ T cells (TCRβ+CD8+CD44+CD62L-); **C**, proportion of IGRP-tetramer+ CD8 T cells (TCRVβ8+CD8+IGRP tetramer+). Two-tailed unpaired Student's t test was used for the statistical analysis.

**Figure S89- B cell-specific TLR9 deficiency increased Treg cells.** Immune cells from different lymphoid tissues were isolated from 6-8-week-old female mice (n=4-5/group) followed by staining with the Treg markers (TCRβ, CD4, CD25 and Foxp3). Two-tailed unpaired Student's t test was used for the statistical analysis.