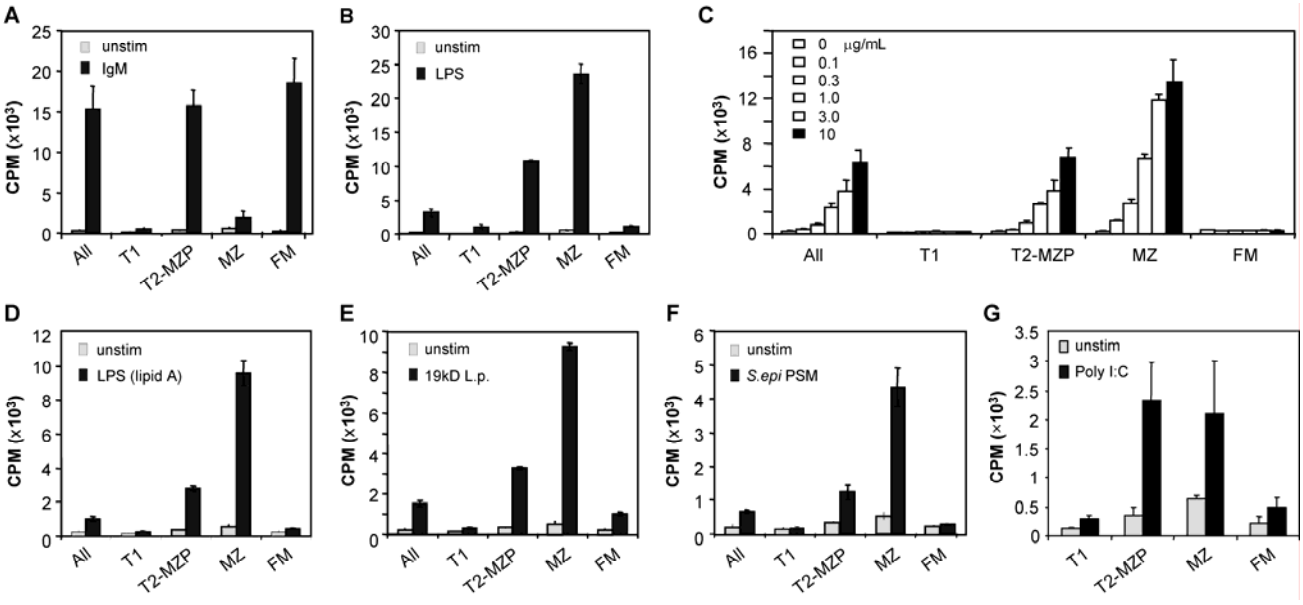
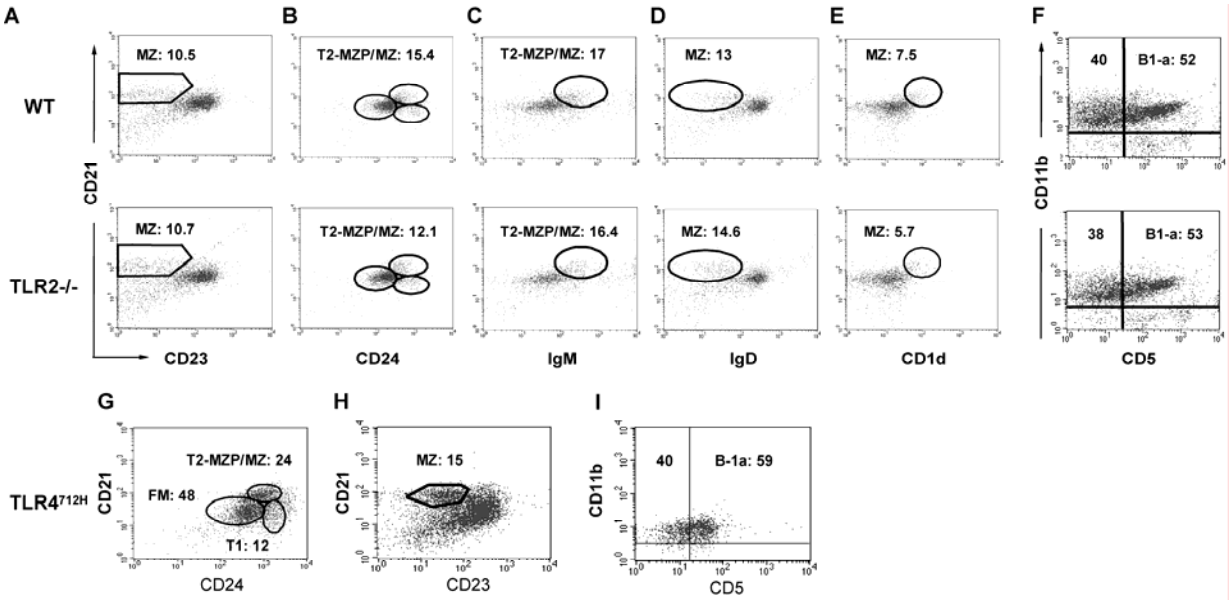
SUPPLEMENTAL DATA

Supplemental Figure 1



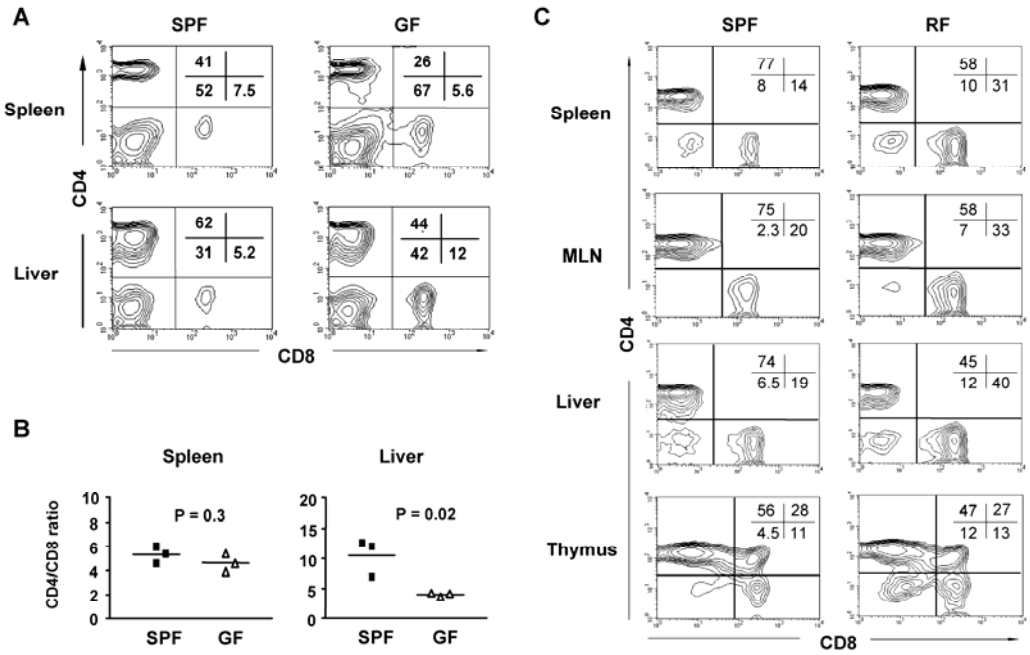
Supplemental Figure 1. Four splenic B cell populations exhibit distinct proliferative responses to adaptive and innate-like immune receptor engagement. Proliferative responses of FACS-sorted Balb/c splenic B cell sub-populations upon stimulation with (A) anti-IgM (10 μg/mL), (B-C) *S. minnesota* LPS, (D) phenol extracted *S. minnesota* LPS, (E) *M. tb* 19kD lipoprotein, (F**)** *S. epi.* PSM, all at 1 μg/mL unless otherwise indicated; and (G) 100 μg/mL poly (I:C). Essentially identical results as in (b) were obtained using *S. typhosa* LPS, and in analysis of additional wild-type murine stains including C57BL/6 and 129 mice. Representative results of one of more than 5 similar experiments are shown.

Supplemental Figure 2



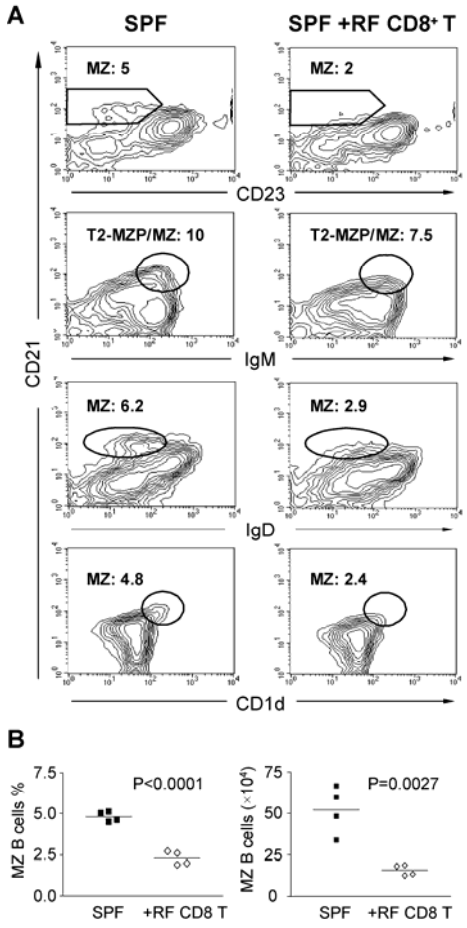
Supplemental Figure 2. <u>B cell subpopulations in TLR2-/- and TLR4 mutant mice</u>. Lymphocytes were prepared from TLR2-/- mice (TLR2-/-) and wildtype littermate controls (WT), and B220+ cells analyzed for B cell subsets in the spleen for CD21 and (A) CD23, (B) CD24, (C) IgM, (D) IgD, and (E) CD1d, or peritoneum for (F) CD11b/CD5. To analyze TLR4 mutant mice, splenic and peritoneal cells were prepared from C3H/HeJ mice ($TLR4^{712H}$), and B220+ cells were analyzed for T1, T2-MZP/MZ, MZ, B-1a, and B-1b B cells: (G) splenic CD21/HSA(CD24), (H) splenic CD21/CD23, and (I) peritoneal CD11b/CD5. Innate-like B cells are preserved in TLR2 and TLR4 mutant mice.

Supplemental Figure 3



Supplemental Figure 3. The percentage of $CD8^+$ T cells is profoundly increased in RF mice. Single cells were prepared from the different lymphoid compartments including spleen, liver of GF mice (A-B), and mesenteric lymph nodes (MLN) and thymus of RF and SPF control mice (C). Lymphocytes were stained for CD3, CD4, and $CD8^+$ T cells. (A) Percentages of CD4 vs. CD8 in SPF and GF mice are indicated in FACS plots. (B) Tabulated CD4/CD8 ratio in spleen and liver of SPF and GF mice showing the increase of $CD8^+$ T cells in the liver of GF mice. (C) $CD4^+$ vs. $CD8^+$ T cells in SPF and RF mice were examined in the different lymphoid compartments. The data represents the phenotypes observed in 24 GF mice and more than one hundred RF mice.

Supplemental Figure 4



Supplemental Figure 4. In vivo depletion of MZ B cells by RF $CD8^+$ T cells. Four weeks old SPF mice were transferred i.v. with 4×10^7 of splenic RF $CD8^+$ T cells and i.p. with 100 μg of microbial antigens from RF mice. On day three after cell transfer, splenocytes were collected and stained for CD21, CD23, CD1d, IgM, and IgD in different staining combinations. The MZ B cells were analyzed in the gate of CD19+ B cell population and indicated markers to determine the % MZ B cells in different staining patterns. (A) Percentage of MZ B cells from SPF mice with or without transfer of RF $CD8^+$ T cells; (B) Tabulated percentage and absolute number of MZ B cells in $CD19^+CD21^{hi}CD23^{lo}$ gate. The data represents at least three individual experiments.