

Supplementary Figure Legend

Supplementary Figure S1.

Age-related changes in the percent of MZ-P B cells and IFN α levels in BXD2 mice. Single cell suspensions were prepared from the spleens of BXD2 mice at the indicated age. **A**, Flow cytometry analysis of the percent of MZ-P B cells in the indicated mouse strain. Means \pm SEM (n=4-6 per age group); * $P < 0.05$ compared with 1-, 3-, and 8-mo-old BXD2 mice. **B**, CpG was intravenously injected into BXD2 mice in the indicated age groups and whose serum levels of IFN α was measured 4 hours later. Means \pm SEM (n=6 per age group); * $P < 0.05$ compared with 1-, 3-, and 8-mo-old BXD2 mice. **C**, Quantitative bar graph shows the percentages of pDCs that are CD80 and CD86-positive in each age group for BXD2 mice. Negative isotype controls were employed to determine the cut-off for percentage of cells that are positive for either CD80 or CD86. Means \pm SEM (n=4-6 per age group); * $P < 0.05$ compared with 1- and 3-mo-old BXD2 mice.

Supplementary Figure S2.

MZ-P B cells from BXD2 mice exhibited highest proliferation. **A, B**, Single-cell suspensions prepared from the spleens of 8 to 12-wk-old mice were sorted into FO, MZ, and MZ-P B cells based on the expression of CD21/35, CD23, IgM, and IgD. Sorted cells were cultured in triplicate wells (5×10^5 cells/well). Stimulators included (**A**) 10 $\mu\text{g/ml}$ anti-CD40 (HM40-3; Biolegend) plus 4 $\mu\text{g/ml}$ rabbit-anti-mouse Ig (clone 187.1, BD-PharMingen), and (**B**) 10 $\mu\text{g/ml}$ goat anti-mouse IgM (F(ab')₂ (Jackson ImmunoResearch Laboratories, West Grove, PA). The proliferative response was measured by a standard [³H]-thymidine incorporation assay at the 72 h time point. Cells were collected 12 h later. For each experimental group of two independent experiments, cells of the same B cell subset from at least three mice were pooled. **C**, QRT-PCR analysis of *Cdca3*, *Cdca5*, and *Cdca8* transcripts in FACS sorted FO, MZ and MZ-P B cells from the spleens of the indicated strain of mice. Means \pm SEM; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$, compared with MZ-P B cell population isolated from BXD2 mice (n=4 per group).