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**Supplemental Information**

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**Natural IgM prevents autoimmunity by enforcing B cell central tolerance induction**

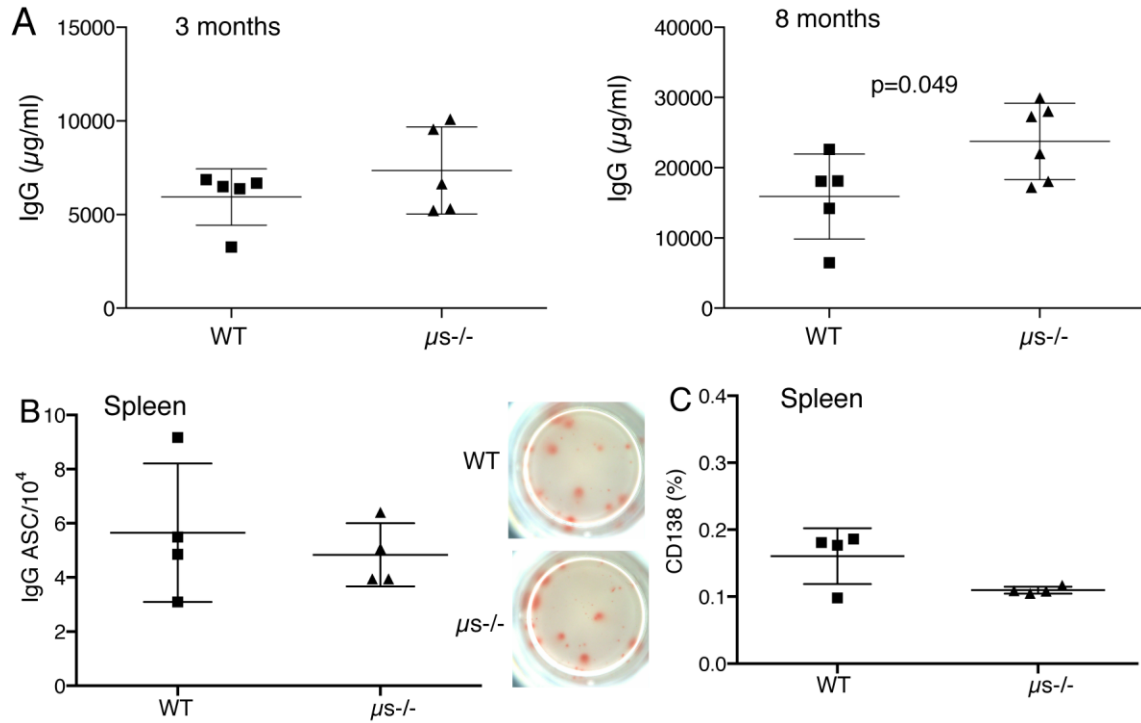
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1 **Supplemental Figure 1**



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3 **Supplemental Figure 1.  $\mu s^{-/-}$  mice have normal IgG levels.** (A) Frequencies  $\pm$  SD serum

4 levels total IgG in WT and  $\mu s^{-/-}$  mice at 3 and 8 months of life, as measured by ELISA. Serum

5 levels were similar at 3 months. By 8 months the  $\mu s^{-/-}$  mice showed subtle but statistically-

6 significant increases in total serum IgG concentrations (B) Frequencies  $\pm$  SD IgG antibody

7 secreting cells (ASC) in WT and  $\mu s^{-/-}$  spleens at 3 months of age, as measured by ELISPOT.

8 Images to the right depict representative wells (C) Frequencies  $\pm$  SD plasma cells (CD138+) in

9 WT and  $\mu s^{-/-}$  spleens at 3 months of age as measured by flow cytometry after gating on live

10 CD19+ lymphocytes. Each symbol represents values for a single mouse; horizontal line indicates

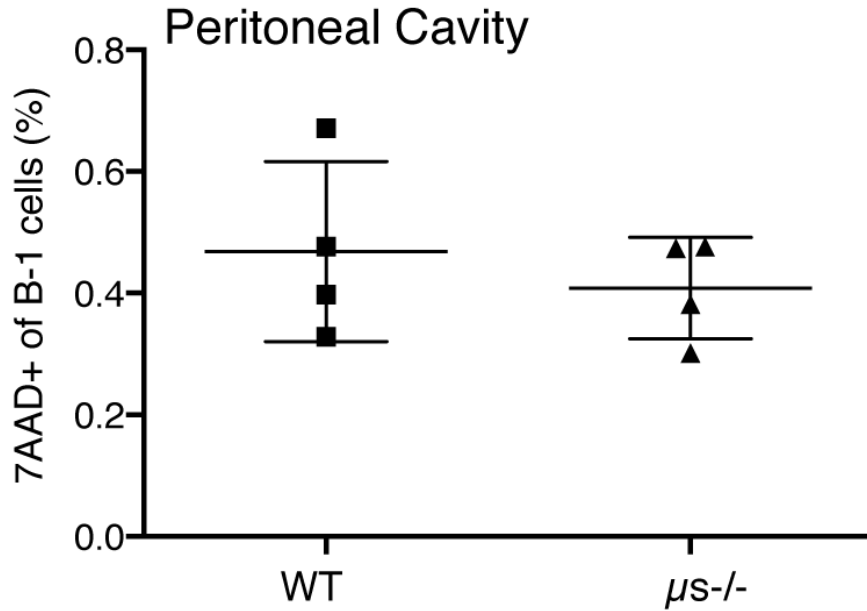
11 the mean for each group (n = 4 - 6 per group)

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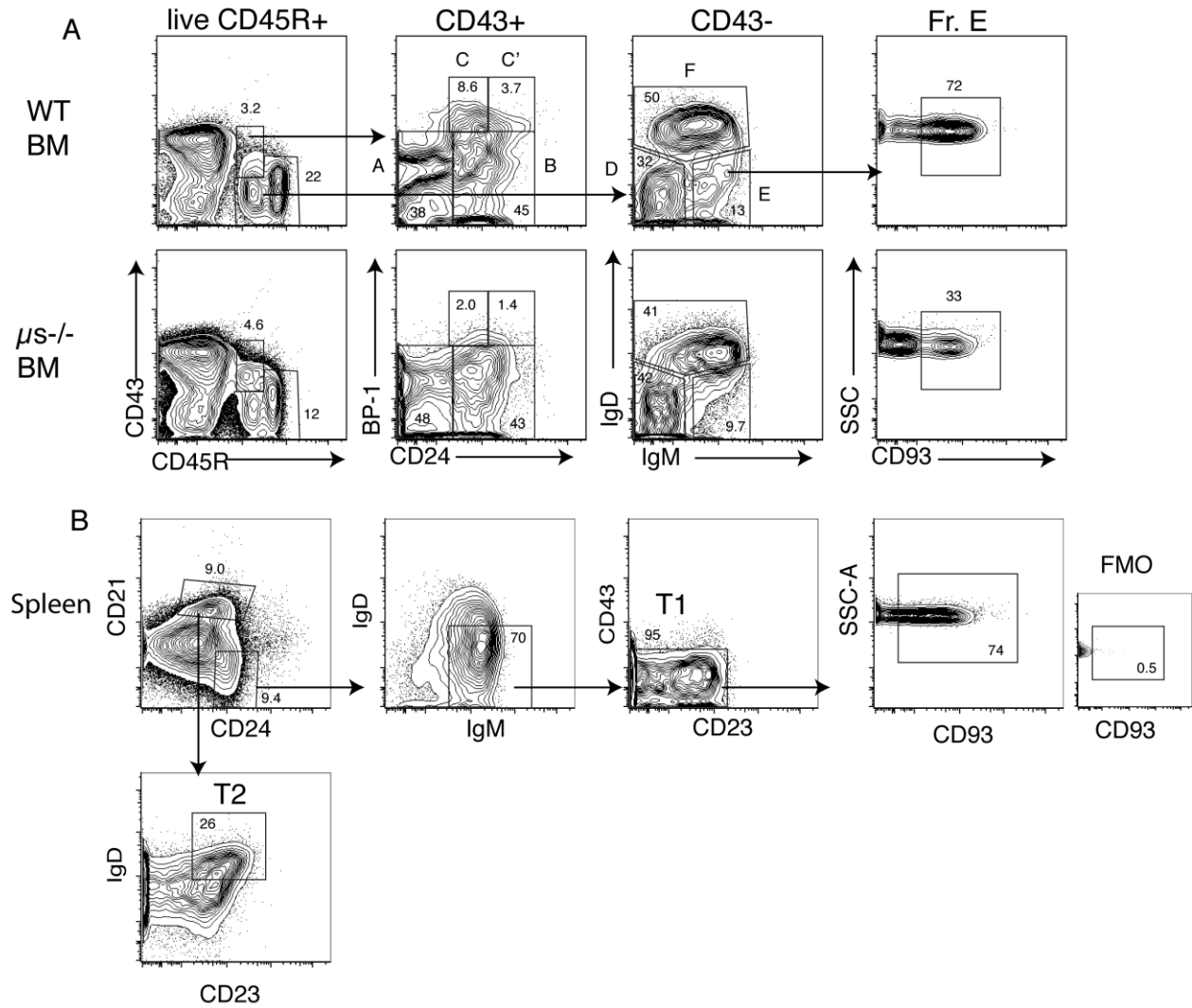
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**Supplemental Figure 2. Cell death among peritoneal cavity B-1 cells.** Shown are mean frequencies  $\pm$  SD of dead/dying cells among B-1 cells in peritoneal cavities of wildtype (WT) and sIgM-deficient ( $\mu S^{-/-}$ ) mice, as determined by flow cytometry staining dead/dying cells with 7-AAD staining and B-1 cells were identified by gating on  $IgM^{hi} IgD^{lo} CD23^{-} CD43^{+}$  (n=4 per group). Each symbol represents values for a single mouse; horizontal line indicates the mean for the group. No significant differences were noted between the groups.

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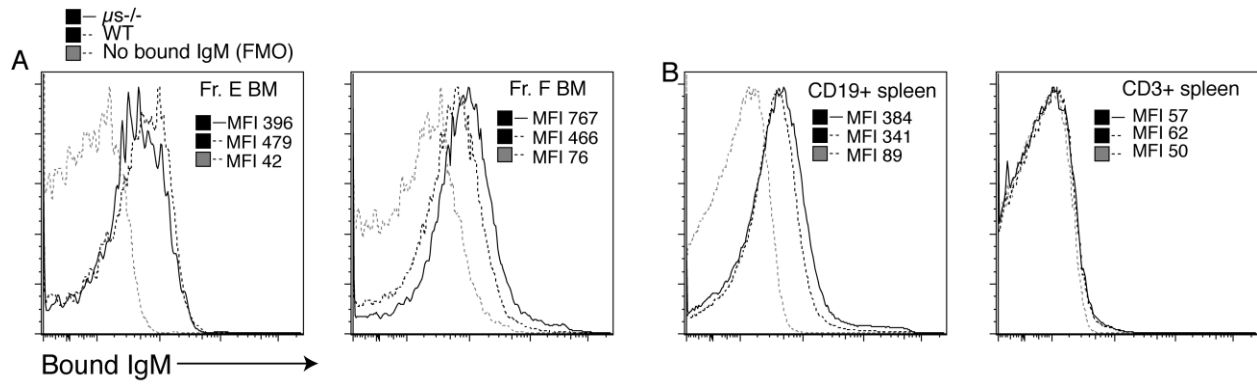
### Supplemental Figure 3



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### Supplemental Figure 3. Gating Strategy to identify B cell precursors in bone marrow and spleen.

(A) Shown are representative 5% contour plots with outliers of bone marrow samples, identifying B cell precursors according to Hardy: A, pre-pro; B, pro C; late pro; C' early pre; D late pre; E, immature; F, mature B cells. (B) Shown are representative 5% contour plots with outliers of spleen samples, identifying transitional B cells.



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3 **Supplemental Figure 4. Secreted IgM binds to bone marrow and spleen cells in vitro. (A)**

4 Shown are overlay histograms of cells from  $\mu s^{-/-}$  (dark grey stippled line) and wildtype (solid  
5 line) mice before (light grey line) and after incubation of cells with purified IgM for 1h in vitro.

6 (A) Bone marrow Hardy Fraction E and Fraction F B cells from WT and  $\mu s^{-/-}$  mice and (B)

7 spleen B cells (CD19+) and T cells (CD3+). Data are representative of two independent

8 experiments. MFI, mean fluorescent intensity.

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