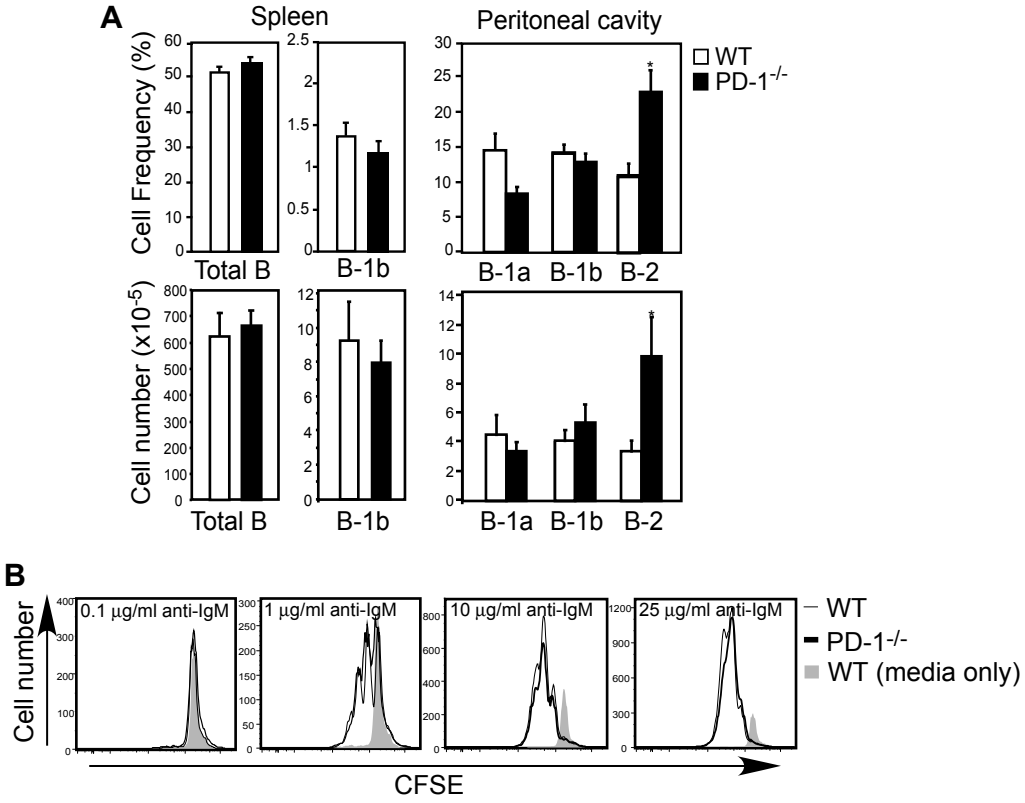
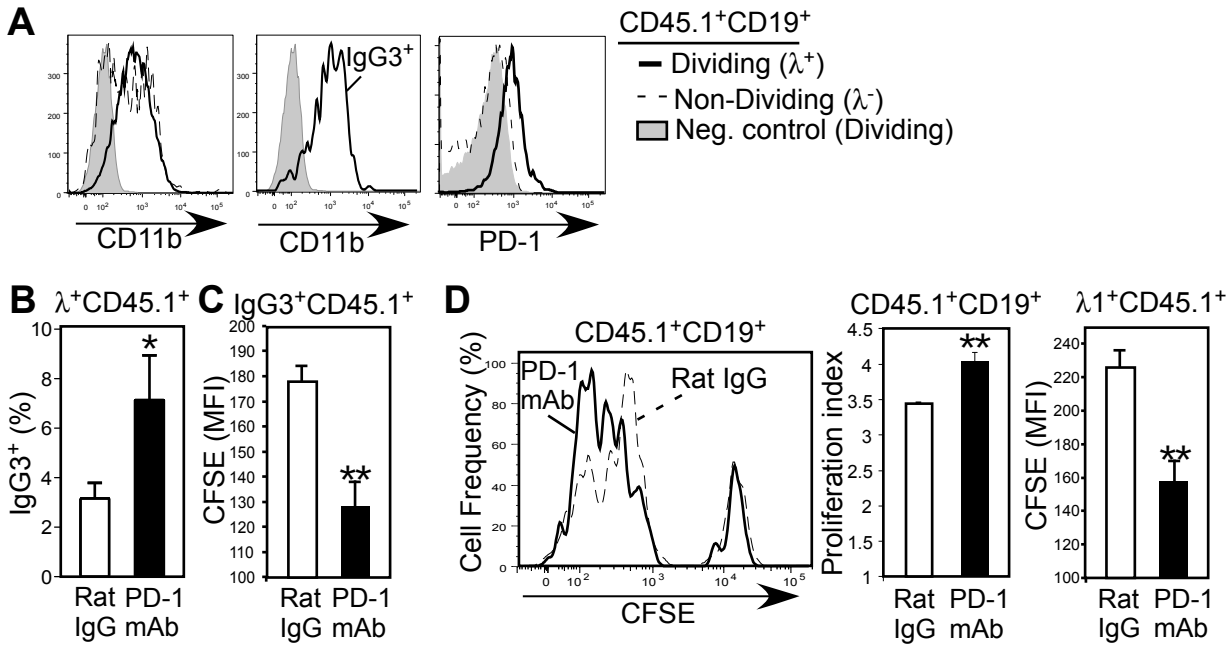


Supplemental Figure 1. B cell phenotypes in PD-1$^{-/-}$ mice. A) Mean (±SEM) peritoneal and spleen B cell frequencies and numbers in wild type and PD-1$^{-/-}$ mice (n=6 mice/group). B cells were defined as CD19^{+}, B-1a cells as CD19^{+}CD11b^{+}CD5^{+}, B-1b cells as CD19^{+}CD11b^{+}CD5^{-}, and B-2 cells as CD19^{+}CD11b^{-}CD5^{-}. Results are representative of four independent staining experiments obtained for 10-11 mice per genotype. B) Splenic B cell proliferation in response to BCR crosslinking. Purified splenic B cells were CFSE-labeled and stimulated 72 hours with F(ab‘)2 goat anti-mouse IgM (Jackson Immuno-research). Cells were stained with 7AAD and 50,000 total events were collected, with 7AAD^{neg} cells analyzed for CFSE loss. Results are representative of those obtained with 3 mice per genotype.

Supplemental Figure 1



Supplemental Figure 2. PD-1 regulates Ag-specific B-1b cell proliferation *in vivo* via B cell-intrinsic expression. Allotype-marked ($CD45.1^+$) peritoneal B-1b cells from B1-8^{hi} IgH knock-in mice were CFSE-labeled and transferred into the peritoneal cavities of $CD45.2^+$ $PD\text{-}1^{-/-}$ mice (5×10^5/mouse). The next day, mice (4-5/group) were immunized i.p. with 0.1 μg NP-Ficoll (d0). On d1, mice received 200 μg PD-1 blocking mAb (RMP1-14) or isotype control mAb i.p. NP-specific ($\lambda^+CD45.1^+$) $CD19^+$ B cells were analyzed by flow cytometry at d3 post-immunization. A) Expression of CD11b and PD-1 by dividing (λ^+) and non-dividing (λ^-) $CD45.1^+$ $CD19^+$ cells and $IgG3^+$ cells (for CD11b) recovered from the peritoneal cavities of recipient mice. Gray shaded histograms indicate isotype control staining for the dividing (λ^+) population. B-C) Increased $IgG3^+\lambda^+CD45.1^+$ peritoneal B-1b cell frequencies are found in mice treated with PD-1 blockade (B) and these $IgG3^+$ cells show increased CFSE loss relative to mice treated with control mAb (C). D) Division in the adoptively transferred $CD45.1^+$ B cell pool is increased in the spleens of mice treated with PD-1 blockade as evidenced by increased CFSE loss (left panel) and increased proliferation indices (middle panel). Significantly increased CFSE loss is also observed among high affinity $\lambda1^+$ NP-specific B cells (right panel).

Supplemental Figure 2