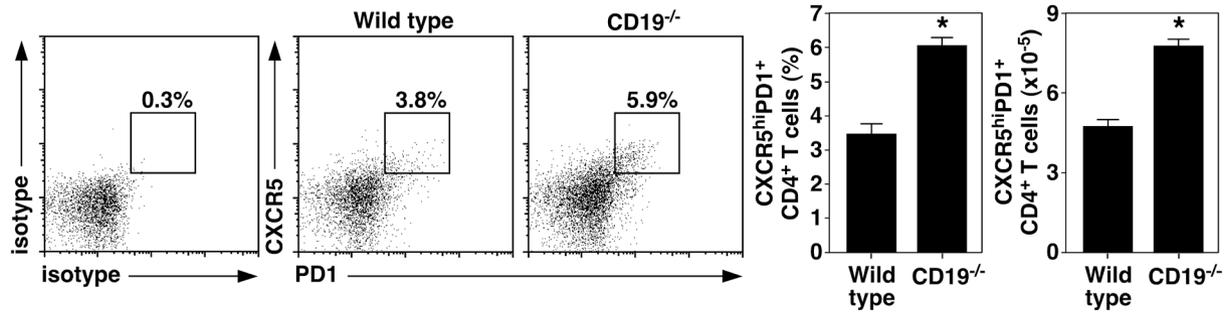


**Supplementary Figure 1.** IL-21 induces regulatory B10 cell function. **a**, IL-21 induces B10 cell IL-10 production and secretion. Purified spleen CD19<sup>+</sup> B cells from wild type mice were cultured with medium alone or containing the indicated cytokines for 48 or 72 h. To visualize IL-10-competent B cells, monensin was added to the cultures 5 h before the cells were stained for surface CD19 and cytoplasmic IL-10 expression and analyzed by flow cytometry. Bar graphs indicate mean ( $\pm$ s.e.m.) IL-10<sup>+</sup> B cell frequencies or numbers at 48 and 72 h from individual mice in three independent experiments. Significant differences between media versus cytokine sample means are indicated: \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ . **b-d**, IL-21R, CD40 and MHC-II expression are not required for B10 or B10pro cell development. Purified spleen B cells from wild type and **b**, IL-21R<sup>-/-</sup> **c**, CD40<sup>-/-</sup> or **d**, MHC-II<sup>-/-</sup> mice were cultured with monensin alone or L+PIM for 5 h to quantify B10 cell frequencies. Alternatively, B10+B10pro cell frequencies were determined after culturing the cells *ex vivo* with agonistic CD40 mAb for 48 h, with L+PIM added during the final 5 h of culture. Representative histograms and bar graphs indicate mean ( $\pm$ s.e.m.;  $\geq 3$  mice per group) percentages and numbers of IL-10<sup>+</sup> B cells in one of two experiments with equivalent results.



**Supplementary Figure 2.** T follicular helper cells are present in CD19<sup>-/-</sup> mice. Representative flow cytometry analysis of CXCR5<sup>hi</sup>PD1<sup>+</sup> cells among spleen CD4<sup>+</sup> T cells from wild type and CD19<sup>-/-</sup> mice. Bar values represent mean ( $\pm$ s.e.m.) CXCR5<sup>hi</sup>PD1<sup>+</sup> cell frequencies among CD4<sup>+</sup> T cells from three mice. Significant differences between sample means are indicated: \*,  $p < 0.05$ .