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Supplemental Information

Aryl Hydrocarbon Receptor Interacting Protein Maintains Germinal Center B Cells through Suppression of BCL6 Degradation

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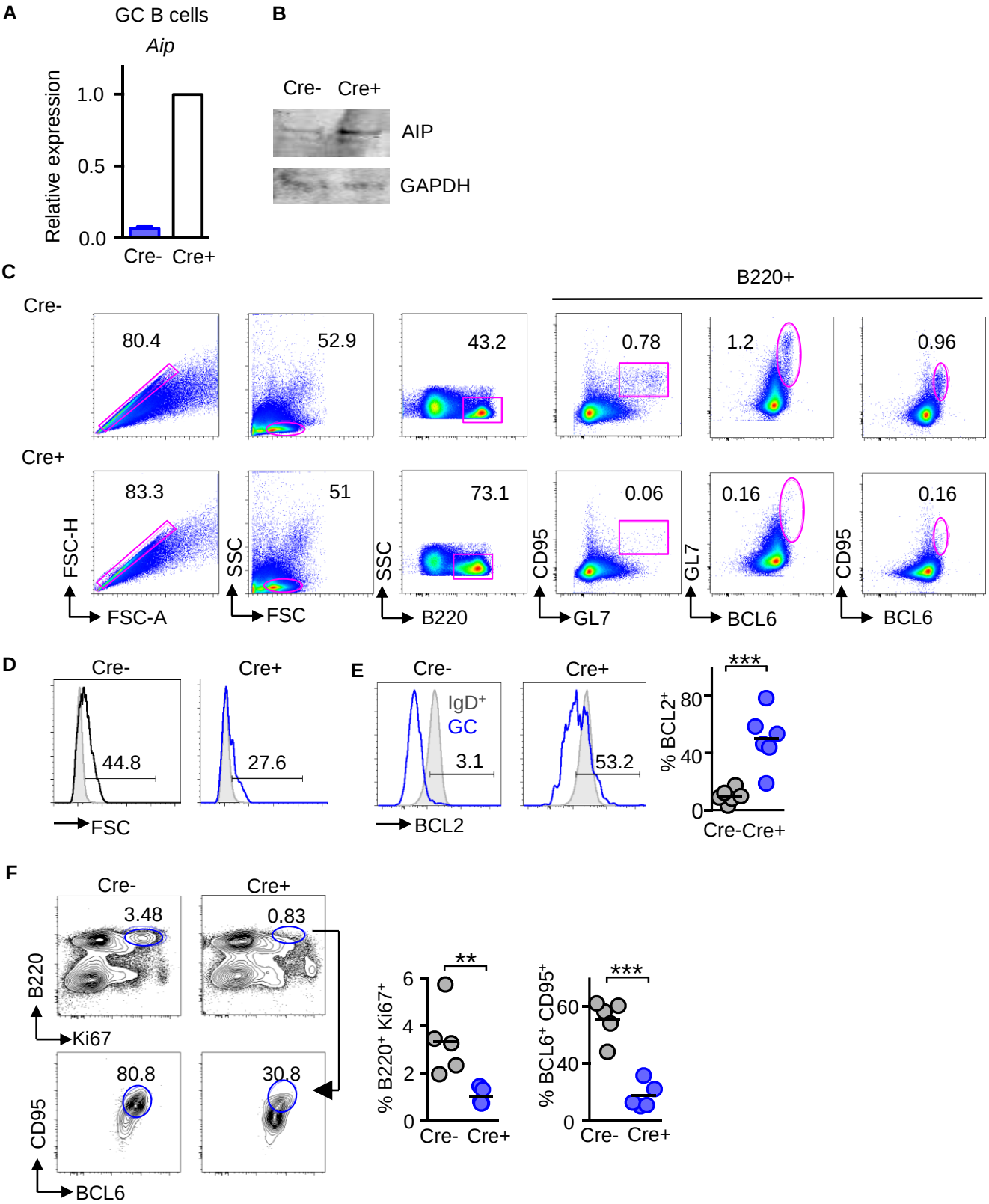

Figure S1. RAG1 Cre deletion of Aip and phenotype of GC B cells; related to Figure 1

(**A**) qPCR and (**B**) Western blot analysis of Aip from GC B cells from *Aip*$^{fl/fl}$ Cre^{+} and *Aip*$^{fl/fl}$ Cre^{-} mice. Data are plotted as mean ± SEM. (**C**) gating strategy to identify and analyze GC B cells. (**D**) size (FSC), (**E**) BCL2 expression, (**F**) proliferation of GC B cells from Cre^{-} and Cre^{+} mice.

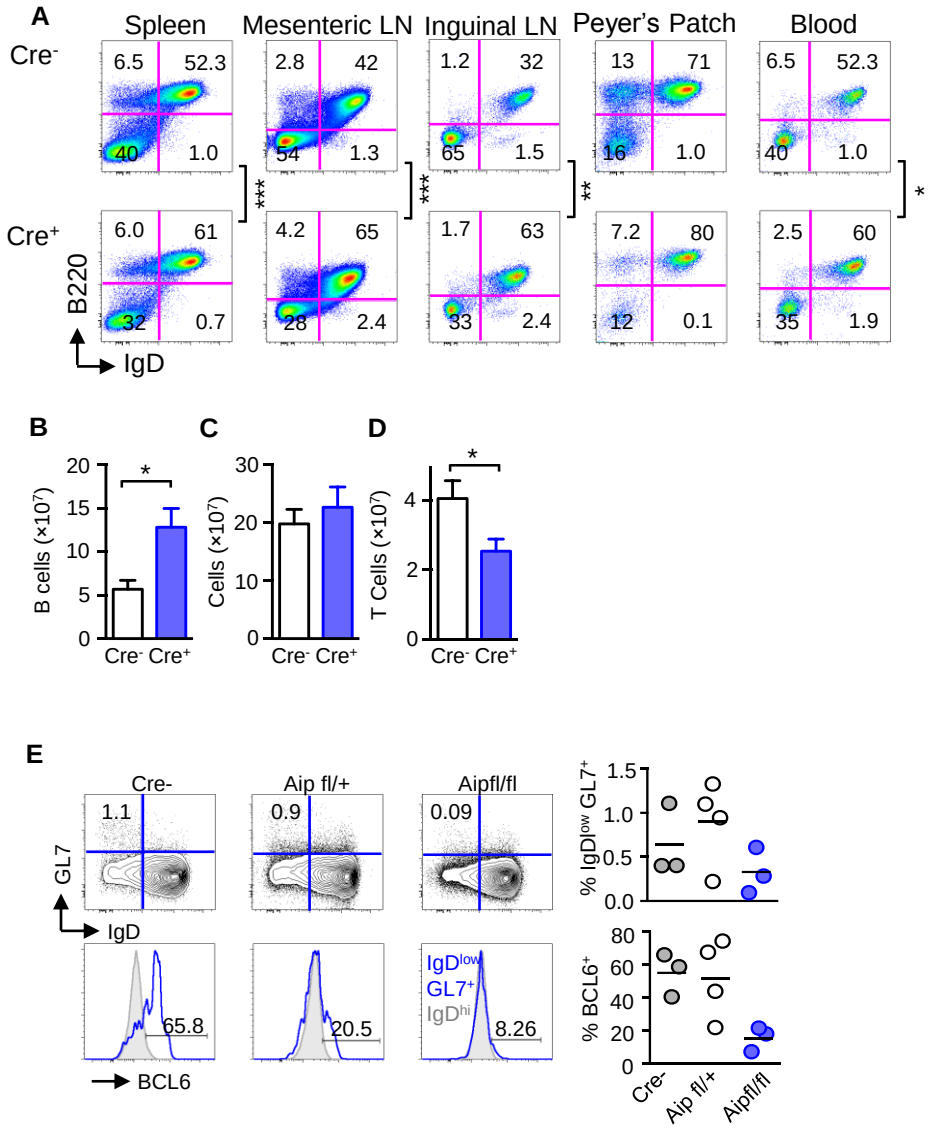

Figure S2. Increased B cells in the periphery of *Aip*$^{fl/fl}$ Cre^{+} mice; related to Figure 2

(**A**) Percentage of B cells in spleen, lymph nodes and circulating blood of B220^{+} IgD^{+} B cells from *Aip*$^{fl/fl}$ Cre^{+} mice compared to WT mice. Data shown as percentage ± SEM. Total number of B220^{+} IgD^{+} B cells (**B**) and total number of splenocytes (**C**). Total number of CD3^{+} T cells (**D**). Results are from 4-5 independent experiments, 2-3 mice per experimental group.

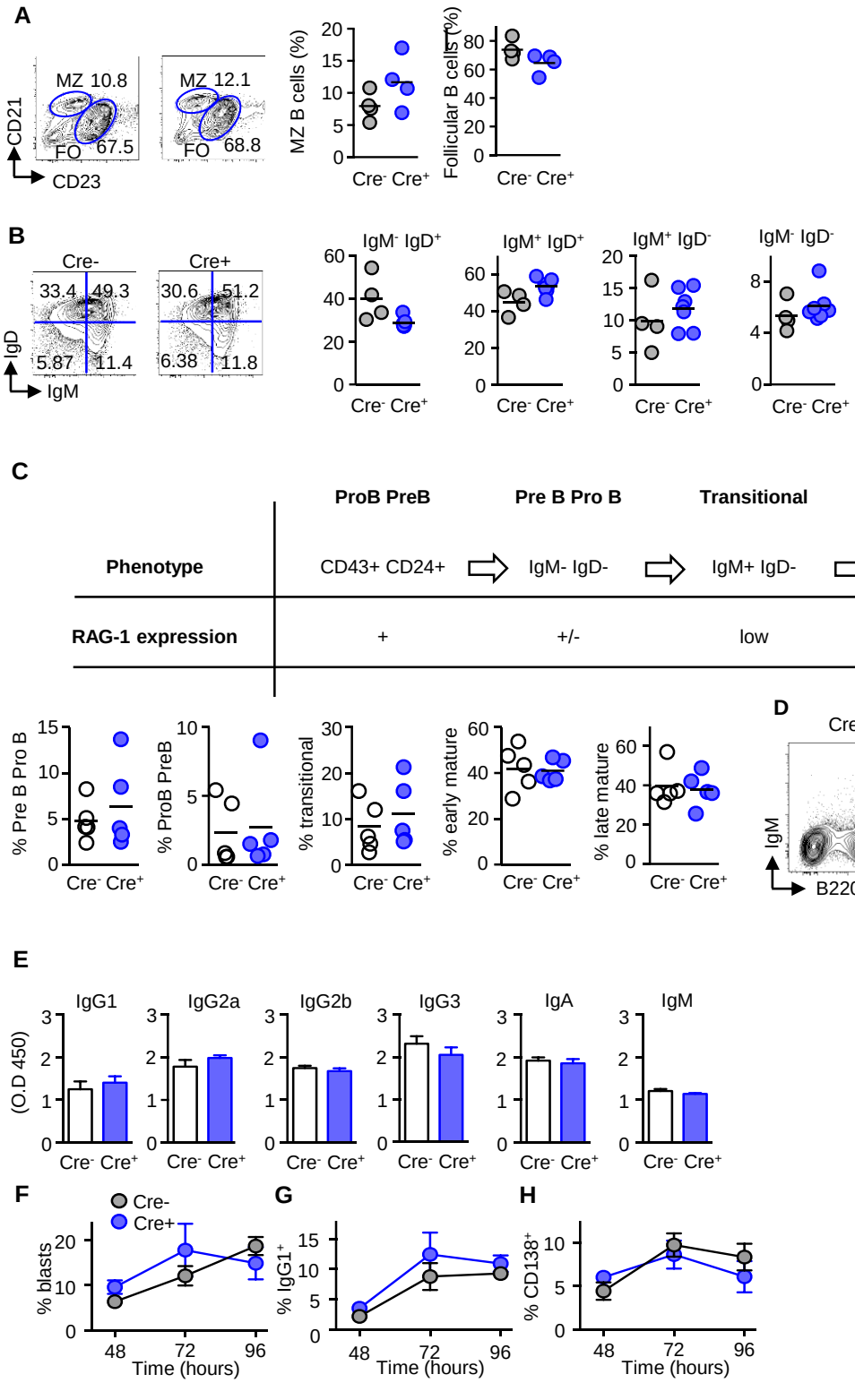

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Figure S3. No difference in Marginal Zone or Follicular B cells or B cell development in *Aip*$^{fl/fl}$ Cre^{+} mice; related to Figure 2

(**A**) Marginal zone (MZ) (CD21^{+} CD23^{-}) and Follicular (FO) CD21^{-} CD23^{+}) B cells were analysed by flow cytometry. (**B**) Expression of IgM or IgD on B cells from WT and *Aip*$^{fl/fl}$ Cre^{+} mice. (**C**) Bone marrow from the femurs of Aip$^{fl/fl}$ Cre^{+} and Cre^{-} mice and were analyzed for different stages of B cell development by flow cytometry. B220^{+} cells were stained with CD43 and CD24. The CD24^{+} cells were divided into different sub-populations depending upon their expression of IgM and IgD. (**D**) Bone marrow from the femurs of *Aip*$^{fl/fl}$ Cre^{+} and Cre^{-} mice and were analyzed for B220^{hi} IgM$^{+/-}$ B cells by flow cytometry. There was a significant reduction in the percentage of B220^{hi} IgM^{+} B cells from *Aip*$^{fl/fl}$ Cre^{+} mice compared to Cre^{-} mice. (**E**) serum from Cre- and Cre+ mice were examined by ELISA for immunoglobulin isotypes. B cells from Cre- and Cre+ were isolated and stimulated in vitro in the presence of anti-IgM, anti-CD40 and IL-4 and the percentage of proliferating cells (blasts) (**F**), IgG1 expression (**G**) and CD138 (**H**) examined at 48, 72 and 96 hours post stimulation by flow cytometry. 2-3 independent experiments, Results are from 2-3 mice per experimental group.

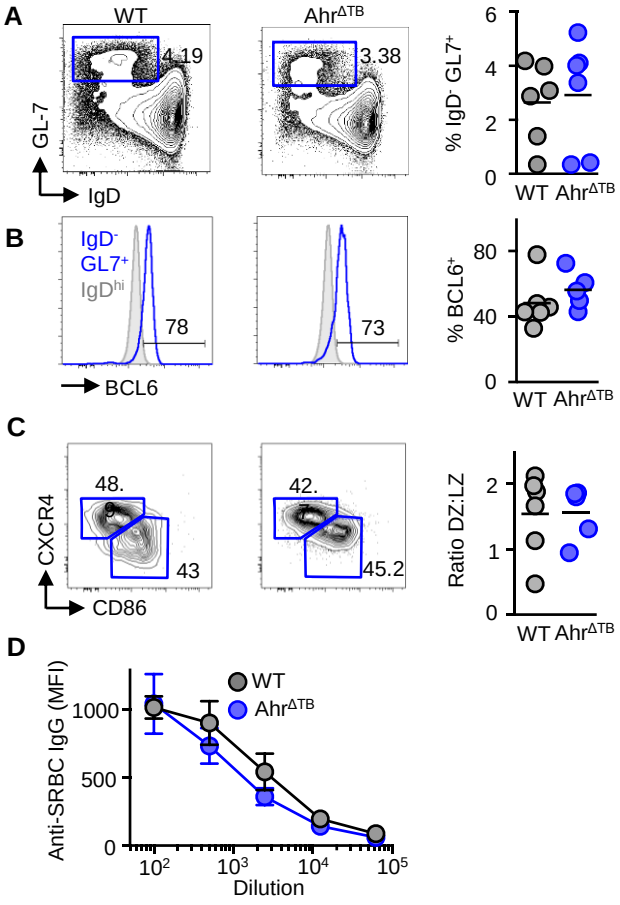

Figure S4. *AHR$^{\Delta TB}$* mice have normal GC B cells and immune responses towards SRBCs; related to Figure 2

(**A**) *AHR$^{\Delta TB}$* and Cre$^-$ mice were immunized with SRBCs and the percentage of GC B cells (B220$^+$ IgD$^-$ GL-7$^+$) examined. (**B**) BCL6 expression determined from *AHR$^{-/-}$* and *AHR$^{+/+}$* IgD$^-$ B cells. (**C**) DZ (CXCR4$^+$) and LZ (CD86$^+$) GC B cells from *AHR$^{\Delta TB}$* and Cre$^-$ B cells and the DZ/LZ ratio. (**D**) Serum from *AHR$^{\Delta TB}$* and Cre$^-$ mice immunized with SRBCs were serially diluted and incubated with SRBCs and the amount of IgG bound determined by flow cytometry. Results are from two independent experiments, 2-3 mice per experimental group. Data are plotted as mean ± SEM.

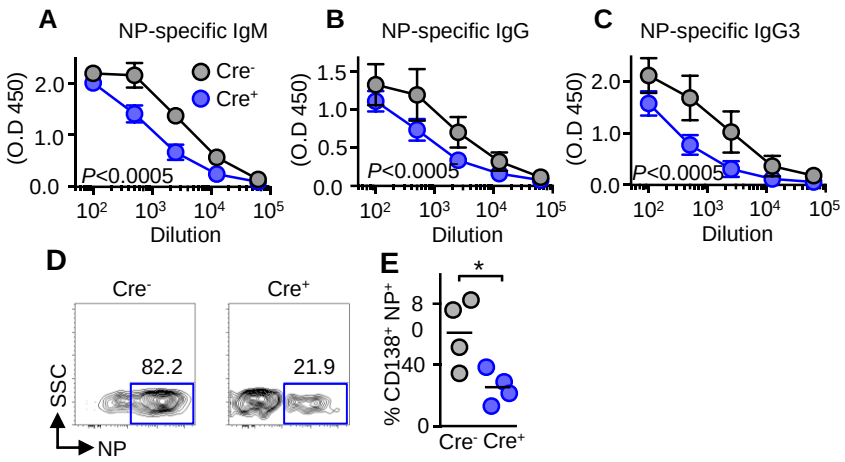

Figure S5. *Aip*$^{fl/fl}$ Cre^{+} mice decreased extra-follicular immune responses; related to Figure 1

Aip$^{fl/fl}$ Cre^{+} and Cre^{-} mice were immunized with NP-Ficoll and analyzed 14 days later NP-specific serum (**A**) IgM, (**B**) IgG and (**C**) IgG3 analyzed by ELISA. Data are plotted as mean ± SEM. (**D-E**) Percentage of NP-specific plasmablasts (B220$^{+/-}$ CD138^{+}) analyzed by flow cytometry. Representative FACS plots from at least 2 independent experiments, 2-3 mice per experimental group.

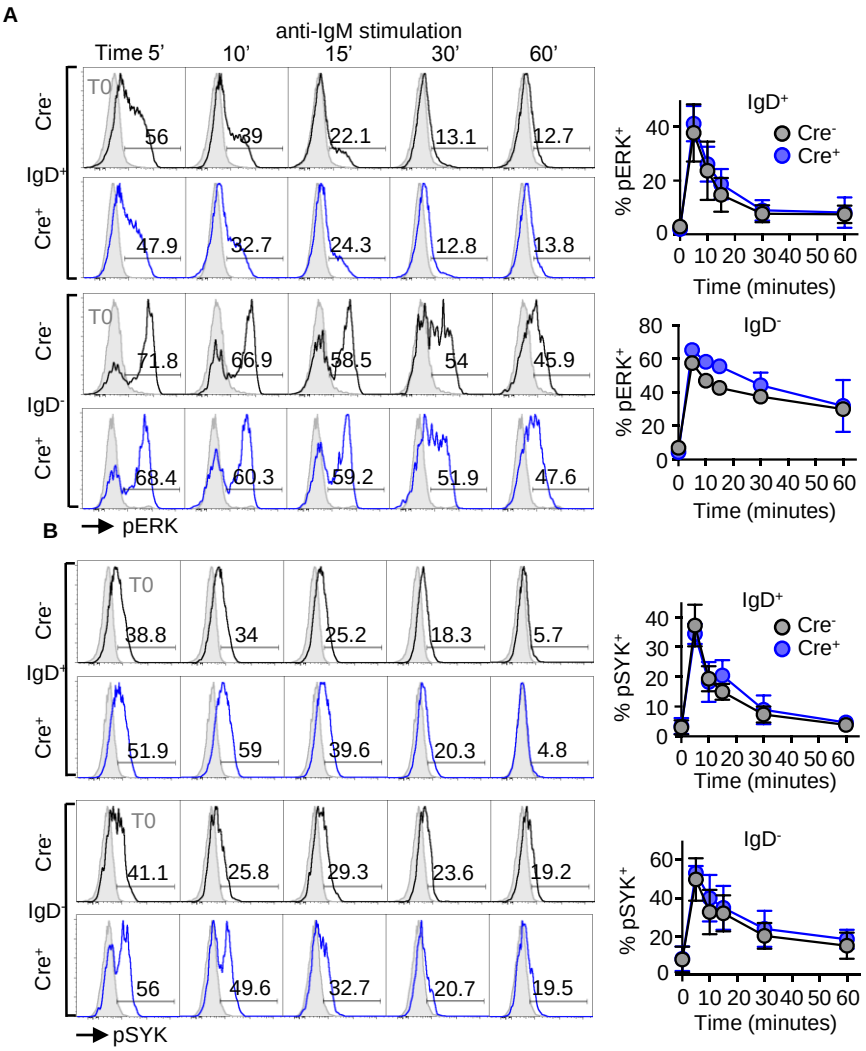

Figure S6. *Aip*$^{fl/fl}$ Cre^{+} mice have no differences in ERK or SYK phosphorylation following BCR stimulation; related to Figure 3

IgD^{-} B cells from *Aip*$^{fl/fl}$ Cre^{+} and Cre^{-} mice were stimulated with anti-IgM (10µg/ml) and examined for the expression of phosphorylated (**A**) ERK and (**B**) SYK from 5 to 60 minutes post-stimulation. Grey histograms show expression at time 0 (T0). Data expressed as percentage from time 0. Results are from two independent experiments with 1-2 mice per experimental group.