Syk tyrosine kinase is critical for B cell antibody responses and memory B cell survival

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Supplemental Figures
Supplemental Figure 1. Defective T-dependent antibody response in the absence of Syk in B cells. (A) Irradiated Rag1-deficient mice reconstituted with a mixture of μMT bone marrow and Syk-expressing (Syk\textsuperscript{fl/+}RMCM) or Syk-deficient (Syk\textsuperscript{fl/-}RMCM) bone
marrow were treated with tamoxifen, immunized 21d later with NP-CGG in Alum or Alum alone and analyzed 10d after that, as shown in the time-line. Dot plots show binding of antigen (NIP) and expression of IgG1 on the surface of splenic B cells or germinal center (GC) B cells. Gates indicate antigen-specific switched cells (NIP⁺IgG1⁺). Numbers indicate % of cells falling into gate and were used to determine data shown in Fig 3D. (B) SWHEL Syk⁺⁺⁺ or SWHEL Syk⁺⁺⁺RMCM mice were treated with tamoxifen and 21d later B cells were transferred into B6.SJL mice, recipient mice were immunized with SRBC or HEL-SRBC and analyzed 5d later, as shown in the time-line. Dot plots in top row show expression of B220 and CD138 on donor (CD45.2⁺) splenocytes and gates indicate B cells (B220⁺CD138⁻) and plasma cells (B220⁻CD138⁺). Dot plots in lower row indicate surface binding of PNA and expression of CD38 on donor-derived splenic B cells (CD45.2⁺B220⁺CD138⁻). Gates indicate non-germinal center (PNA²⁺CD38⁺) and germinal center (PNA⁺CD38⁻) cells. Numbers indicate % of cells falling into gate and were used to determine data shown in Fig 3E.
Supplemental Figure 2. Defective antibody recall response in the absence of Syk in B cells. Irradiated Rag1-deficient mice reconstituted with a mixture of μMT bone marrow and Syk-expressing (Syk^{fl/+}RMCM) or Syk-deficient (Syk^{fl/-}RMCM) bone marrow were immunized twice with NP-CGG in Alum. 85d after the second immunization the mice were treated with tamoxifen, and a further 21d later immunized with NP-CGG in PBS or PBS alone and analyzed 5d after that, as shown in the time-line. Dot plots show surface binding of antigen (NIP) and expression of IgD on splenic non-T cells (CD4^−CD8^−), which are mainly B cells, and on plasma cells (CD138^+). Gates show switched antigen-specific cells (NIP^+IgD^-) and were used to determine data shown in Fig 4A.