B Cell-Specific Loss of Lyn Kinase Leads to Autoimmunity

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[3] Abbreviations used in this paper: SFK, Src family kinase; [Ca^{2+}], intracellular Ca^{2+} concentration; T1, transitional stage 1; T2, transitional stage 2; T3, transitional stage 3; Fol., Follicular; MZ, marginal zone; GC, germinal center; Ig, immunoglobulins

Running title: B cell-specific Lyn deletion causes autoimmunity

Supplemental Materials

Figure S1: Generation of B cell-specific Lyn-deficient mice.

(A) Representative dot plots showing Lyn protein expression levels determined by intracellular flow cytometry analysis of peripheral blood leukocytes of the indicated lineages (2 month-old mice).

(B) Percentage of Lyn^+ cells in the indicated peripheral blood populations of 2 month-old mice. Data represent mean ± SEM of independent experiments from 5-8 mice per group. ** $P \leq 0.01$, *** $P \leq 0.001$ (One-way ANOVA).

Figure S2: Representative labeling and gating strategies used to analyze FACS data and define the different B cell subpopulations in the spleen.

Representative dot plots from spleen of 2 month-old WT mice. The numbers in gates represent the frequency of gated cells. Cells were first defined as $DAPI^-$ and $PerCP\text{-}Cy5.5^-$ population, PerCP-Cy5.5 being used to exclude $CD11b^+$ and $TCR\beta^+$ cells. Subsequent gating allowed the identification of the following strategies B cell populations.

(A) Plasma cells were identified as $B220^{lo/-}$ $CD138^{hi}$ cells.

(B) Gating on the $B220^+$ IgD^- population, followed by subsequent gating on Fas^+ $GL7^+$ cells allowed the identification of GC B cells.

(C) Gating strategies used to identify transitional T1, T2 and T3 cells, as well as mature follicular (I and II) and MZ B cells. The different subsets were defined as follow:

T1: $CD19^+$ IgD^{lo} IgM^{hi} $CD93^+$ $CD23^-$ $CD21^-$

T2: $CD19^+$ IgD^{lo} IgM^{hi} $CD93^+$ $CD23^+$ $CD21^-$

T3: $CD19^{+}$ IgD^{hi} IgM^{lo} $CD93^{+}$ $CD23^{+}$ $CD21^{lo}$

MZ: $CD19^{+}$ IgD^{lo} IgM^{hi} $CD93^{-}$ $CD23^{-}$ $CD21^{+}$

Fol. I: $CD19^{+}$ IgD^{hi} IgM^{lo} $CD93^{-}$ $CD21^{lo}$

Fol. II: $CD19^{+}$ IgD^{hi} IgM^{hi} $CD93^{lo/-}$ $CD21^{lo}$

Figure S3: *Lyn*$^{-/-}$ and B-*lyn*$^{-/-}$ display altered architecture of the lymphoid tissues

H&E staining of spleen (upper panel) and lymph node (lower panel) sections from 8 month-old control, *lyn*$^{-/-}$ and B-*lyn*$^{-/-}$ mice. Representative pictures are shown. Scale bars represent 200 μm.

Figure S1 - Lamagna C. *et al.*

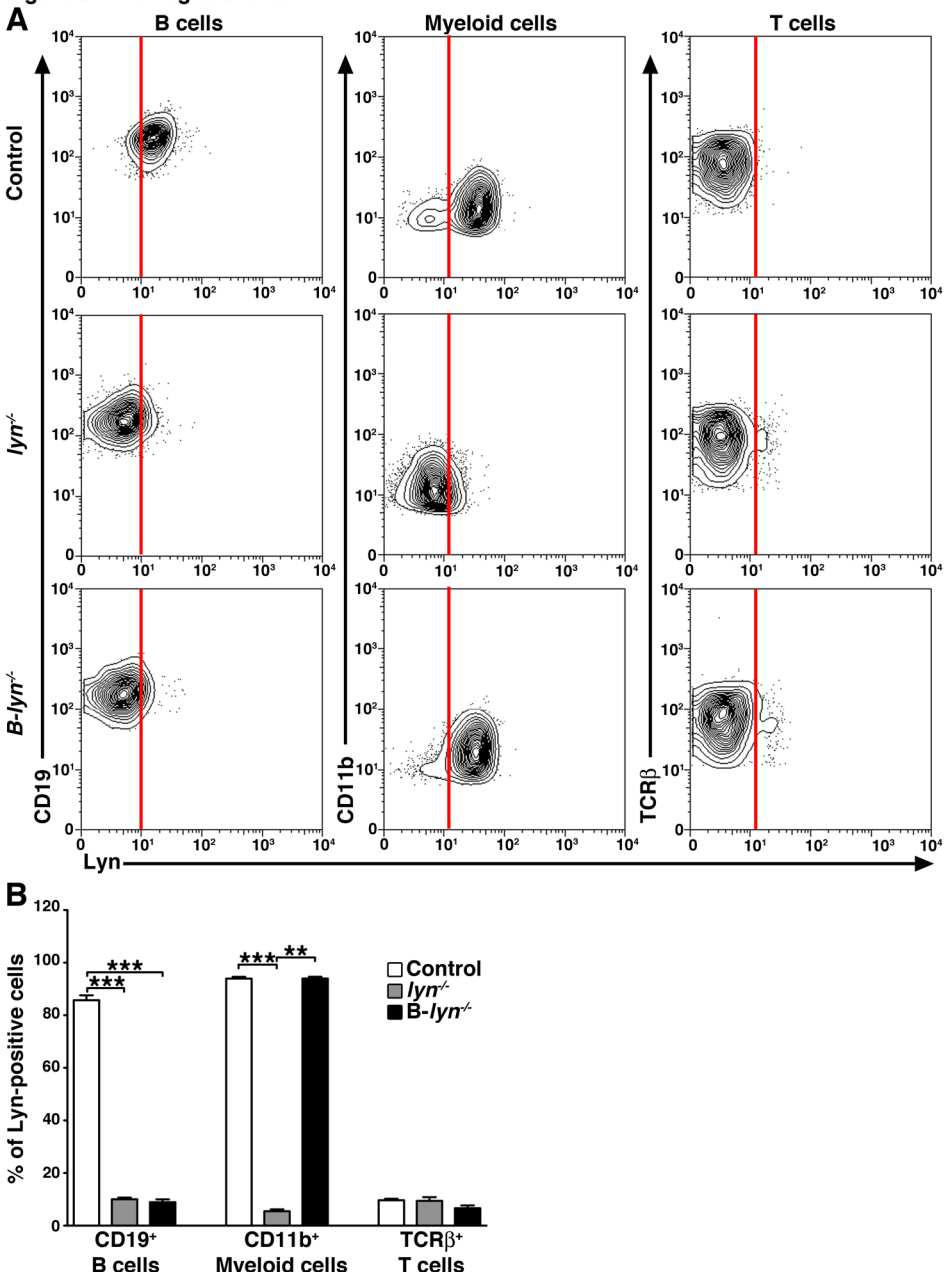

Figure S2 - Lamagna C. *et al.*

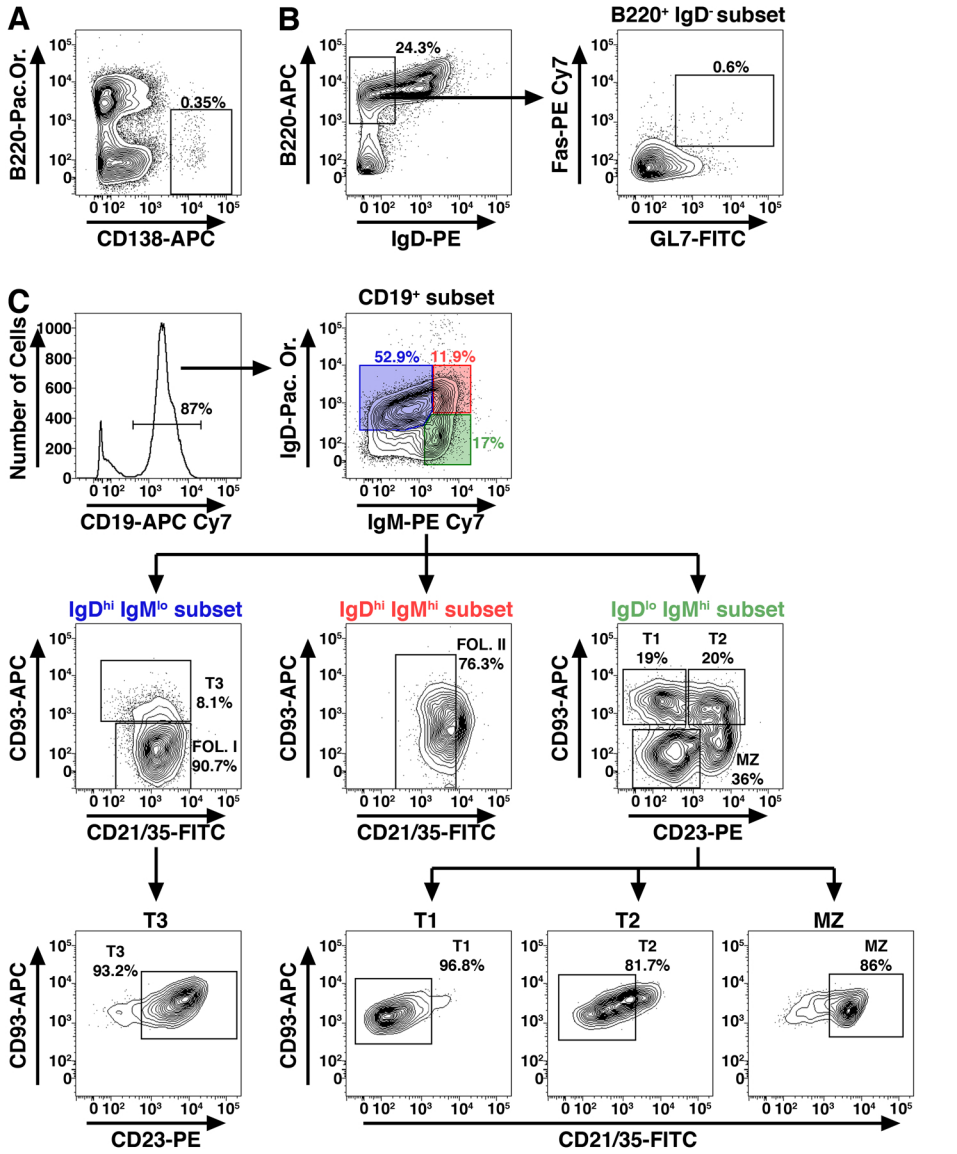

Figure S3 - Lamagna C. *et al.*