Data Supplement

Figure S1

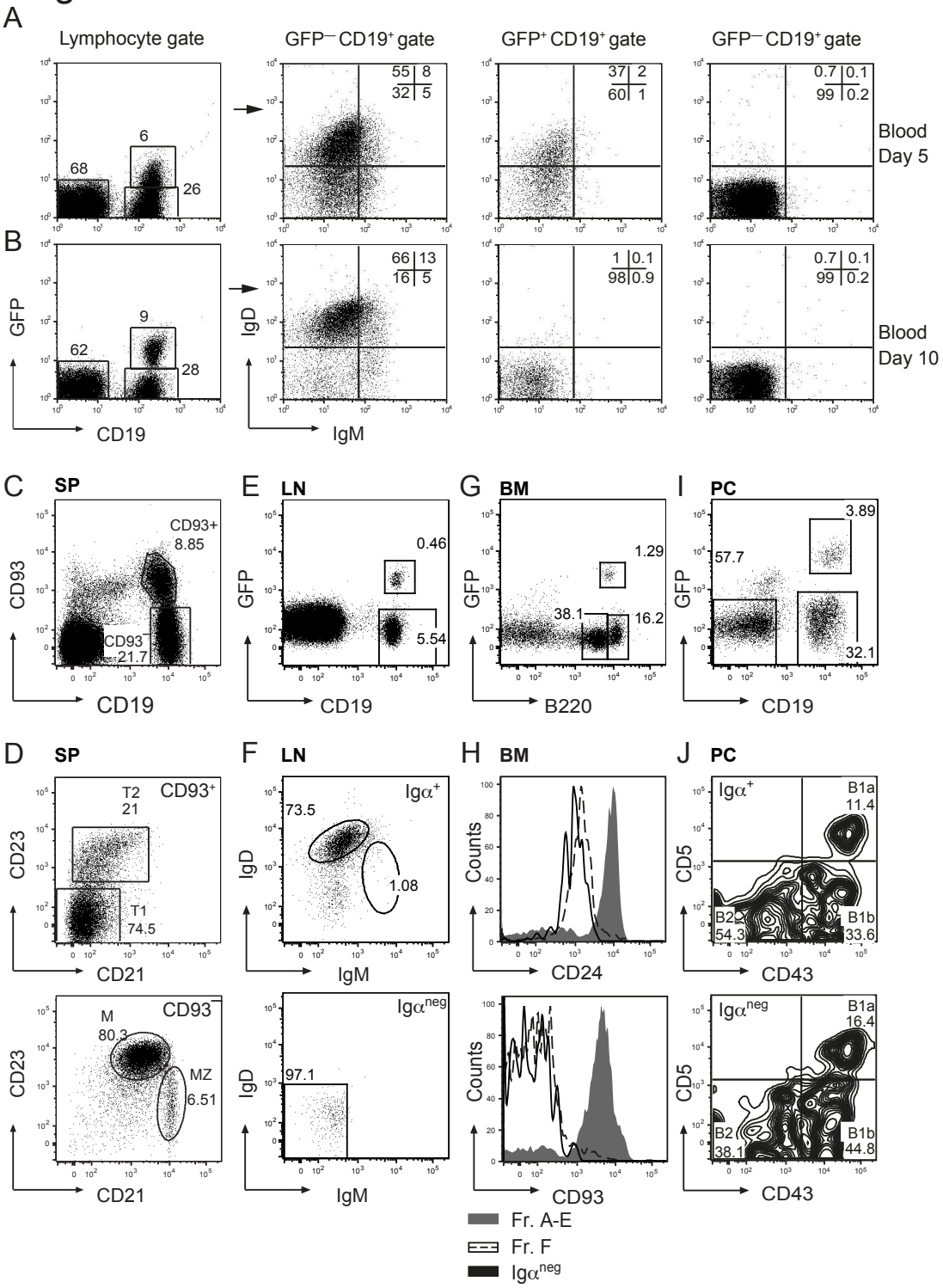

Figure S1. Generation and phenotypic analysis of Igα^{neg} B cells.

(A) Flow cytometric analysis of peripheral blood derived lymphocytes from a *cmb1* mouse at day 5 and (B) day 10 after tamoxifen treatment. Shown are CD19 versus GFP as well IgM versus IgD dot plots. Numbers in the corners of upper right quadrants represent the percentage of cells falling into each quadrant. The plots are representative of more than three mice. (C) Flow cytometric analysis of maturation markers expressed on the surface of *cmb1*-derived splenic B cells. A CD93 versus CD19 dot plot distinguishes the $CD19^{+}CD93^{+}$ and $CD19^{+}CD93^{-}$ B cells. (D) The $CD19^{+}CD93^{+}$ fraction is further subdivided according to the CD23 versus CD21 expression into $CD23^{-}CD21^{-}$ T1 or $CD23^{+}CD21^{lo}$ T2 B cells (lower left panel). The $CD19^{+}CD93^{-}$ fraction is subdivided into $CD23^{+}CD21^{+}$ FO B cells and $CD23^{lo}CD21^{hi}$ MZ B cells. (E) Flow cytometric analysis of LN-derived B cells. A representative CD19 versus GFP dot plot is shown with gates set on $CD19^{+}GFP^{-}$ and $CD19^{-}GFP^{+}$ cells. (F) IgM versus IgD dot plot of $Ig\alpha^{+}$ and $Ig\alpha^{neg}$ B cells in the LN. (G) Flow cytometric analysis of BM-derived $Ig\alpha^{+}$ and $Ig\alpha^{neg}$ B cells. A B220 versus GFP dot plot is shown with gates set on $B220^{hi}GFP^{+}$ $Ig\alpha^{neg}$ B cells, $B220^{hi}GFP^{-}$ B cells (corresponding to Fr. F) and $B220^{lo}$ B cells (Fr. A–E). (H) Flow cytometric analysis of CD24 (upper panel) and CD93 (lower panel) in BM-derived fractions. Histograms are shown. Fr. A–E: grey curves, Fr. F.: dashed lines, $Ig\alpha^{neg}$: solid black lines. (I) Flow cytometric analysis of PC-derived lymphocytes. Shown is a representative CD19 versus GFP dot plot with gates set on $Ig\alpha^{+}$ and $Ig\alpha^{neg}$ B cells. (J) Cytometric analysis of PC-resident fractions in $Ig\alpha^{+}$ (upper panel) and $Ig\alpha^{neg}$ (lower panel) populations. CD43 versus CD5 density plots are shown to distinguish B2 ($CD43^{-}CD5^{-}$), B1a ($CD43^{+}CD5^{+}$) and B1b ($CD43^{+}CD5^{-}$) B cells.

Figure S2

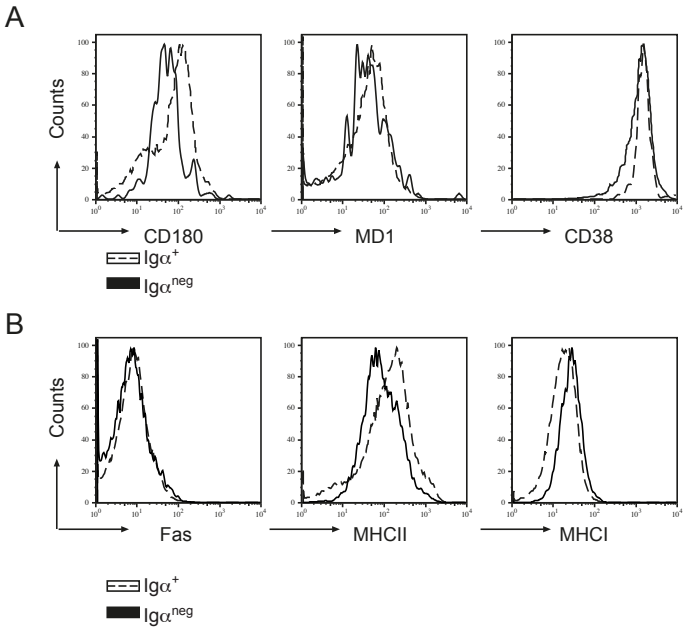

Figure S2. Characteristics of Igα^{neg} B cells

(A) Flow cytometric analysis of the amount of B cell surface protein CD180, MD-1, and CD38 on Igα^{+} (dashed line) and Igα^{neg} B cells (solid line). (B) Flow cytometric analysis of Fas, MHCII, and MHCI on Igα^{+} (dashed line) and Igα^{neg} B cells (solid line).

Figure S3

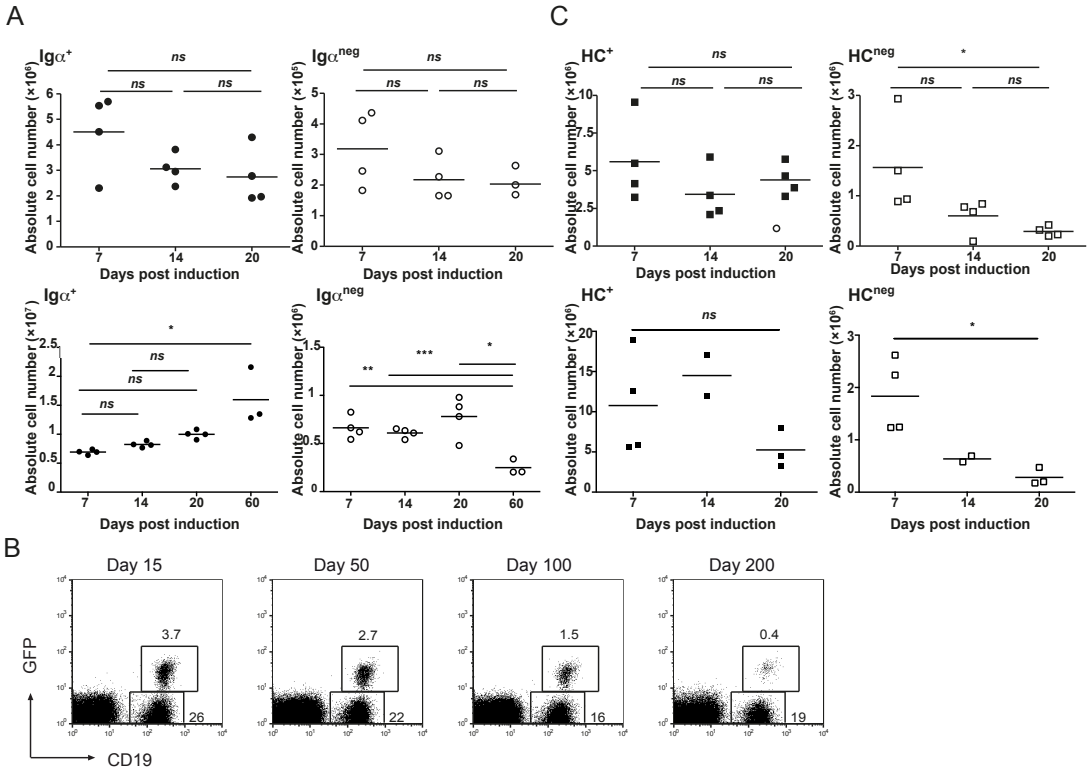

Figure S3. HC^{neg}B cells but not Igα^{neg} B cells disappear from the mature B cell pool within 20 days.

(A) Absolute cell numbers of *cmb1*-derived Igα^{+} B cells and Igα^{neg} calculated at days 7, 14, 20, and 60 (lower panel) after tamoxifen treatment. Asterisks (*, ** and ***) indicate statistically significant differences with P<0.05, P<0.01 and P<0.001, respectively. ns, not significant. P-Values were obtained using two-tailed non-parametric Mann-Whitney t-test. Shown are data from two separate experiments with two—four mice per group. (B) Flow cytometric analysis of blood-derived B cells from *cmb1* mice. Shown are CD19 versus GFP dot plots. Blood was taken from one to three mice per time point. (C) Absolute cell numbers of mb1-CreER^{T2}; B1-8 $^{fl/\Delta}$-derived HC^{+} and HC^{neg} B cells calculated at days 7, 14, and 20 after tamoxifen treatment. Asterisks (*, ** and ***) indicate statistically significant differences with

P<0.05, P<0.01 and P<0.001, respectively. ns, not significant. P-Values were obtained using two-tailed non-parametric Mann-Whitney t-test. Shown are data from two separate experiments with two—four mice per group.

Figure S4

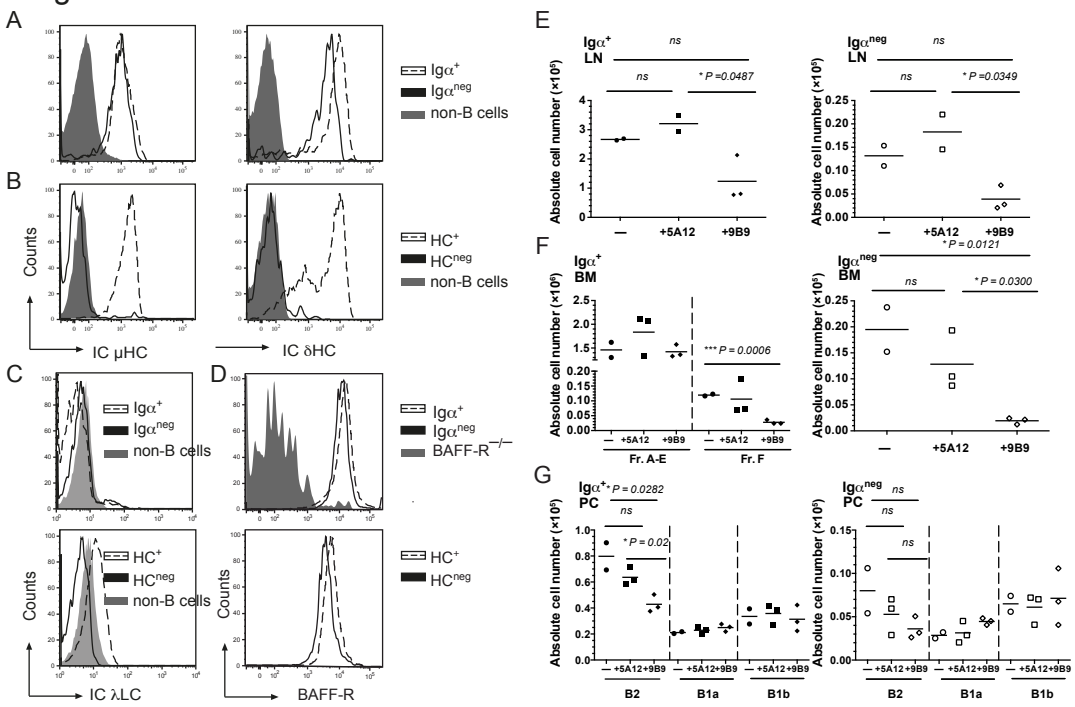

Figure S4. Intracellular expression of BCR components, expression of BAFF-R and the effect of BAFF-R blocking Abs on B cell numbers in LN, BM and PC

(A) Intracellular flow cytometric analysis of μ and δ HCs in Igα^{neg} and (B) HC^{neg} B cells. (C) Intracellular expression of λLC in HC^{neg} (left) and Igα^{neg}(right) B cells and their respective controls. Grey filled curves represent the non-B cell controls. Curves with solid lines represent the Igα^{neg} or HC^{neg} B cells and curves with dashed lines represent the respective control B cells. (D) Flow cytometric analysis of the amount of BAFF-R on the surface of Igα^{+} and Igα^{neg} (left panel), and HC^{+} and HC^{neg} (right panel). Igα^{+} or HC^{+} B cells: dashed lines, Igα^{neg} or HC^{neg} B cells: solid lines. BAFF-R^{-} B cells served as a negative control for the staining (grey filled curve in the left

panel). (E) Statistical analysis of Igα^+ and Igα^{neg} B cells from the LN of *cmb1* mice treated with anti-BAFF-R blocking Ab. *P*-Values were computed using a two-tailed Student's *t*-test and are indicated in the figure. The results of two to three mice per group are shown. Each symbol represents an individual animal. (F) Statistical analysis of BM-derived Igα^+ and Igα^{neg} B cells. Each symbol represents an individual animal. The analysis was performed as in (Fig. 5G). In the Igα^+ population, Fr. A–D and Fr. F were analyzed. (G) Analysis of PC-derived Igα^+ and Igα^{neg} B cells separated into B2, B1a, and B1b populations. *P*-Values were computed using a two-tailed Student's *t*-test and are indicated in the figure. The results of two to three mice per group are depicted. Each symbol represents an individual animal.