

**Supplementary Figure 1: Analysis of Germinal Center reaction in mixed bone marrow chimera, generated from 50 % CD22<sup>-/-</sup> + 50 % WT (CD45.1) (A), 90 % CD22<sup>-/-</sup> + 10 % WT (CD45.1) (B, C) or 50–70 % CD22-Y2,5,6,F + 50–30 % WT (CD45.1) (D) bone marrow cells.**

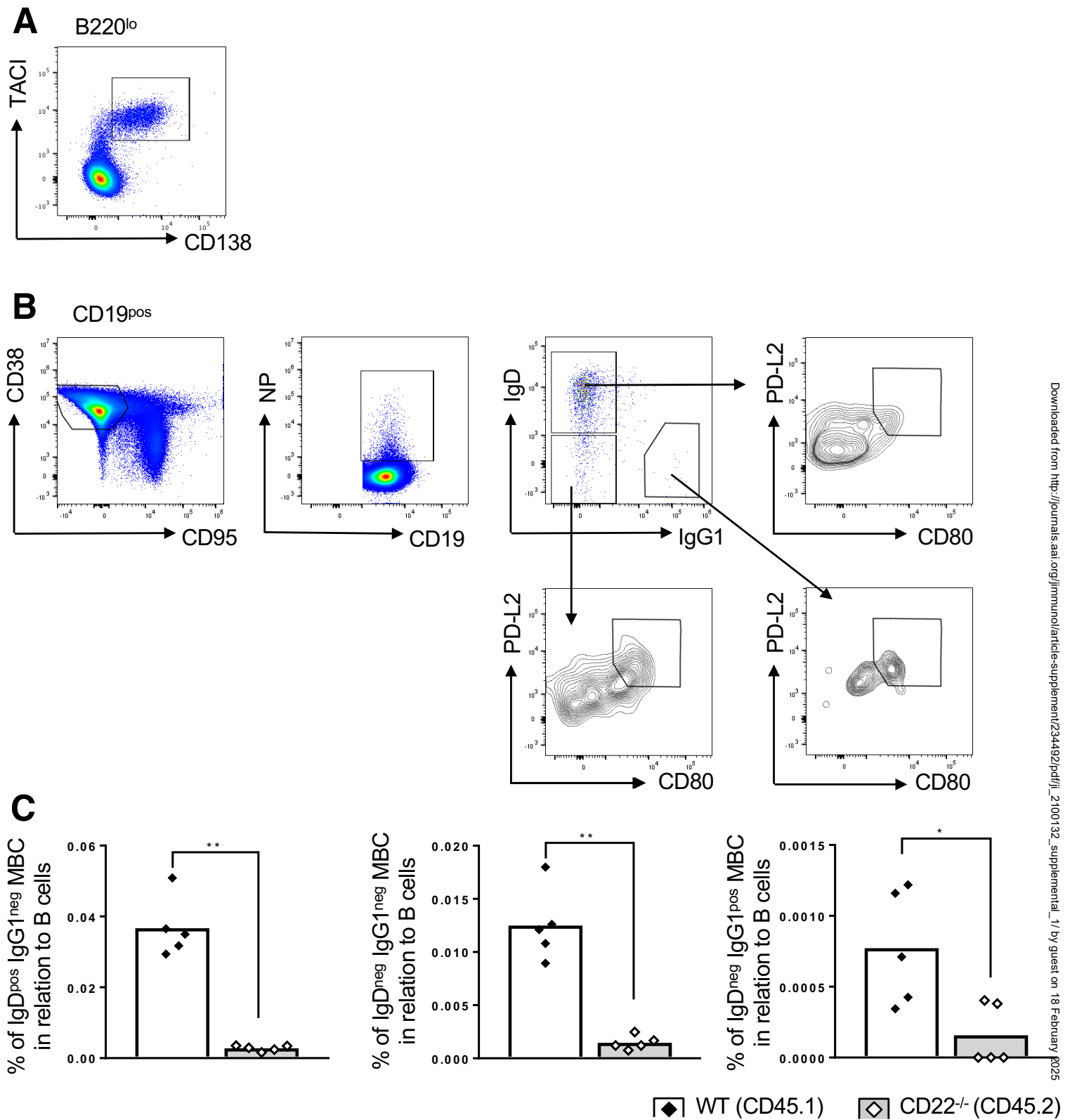
**A** Bone marrow single cell suspensions from mixed bone marrow chimera were stained with indicated antibodies and analyzed by flow cytometry. Dot plot shows gating strategy for indicated bone marrow B cell subsets. Bar Chart shows percentage of CD22<sup>-/-</sup> (CD45.2<sup>pos</sup>) and WT (CD45.1<sup>pos</sup>) Pro-B cells (B220<sup>pos</sup> IgM<sup>neg</sup>, c-kit<sup>pos</sup>, CD25<sup>neg</sup>) Pre-B cells (B220<sup>pos</sup> IgM<sup>neg</sup>, c-kit<sup>neg</sup>, CD25<sup>pos</sup>), immature B cells (B220<sup>pos</sup> IgM<sup>pos</sup>) and mature recirculating B cells (B220<sup>hi</sup> IgM<sup>pos</sup>). Pro = Pro-B cells, Pre = Pre-B cells, Imm. = Immature B cells, Mat. Rec. = Mature recirculating B cells

**B** Bar chart shows percentage of CD22<sup>-/-</sup> (CD45.2<sup>pos</sup>) and WT (CD45.1<sup>pos</sup>) naïve splenic B cells (left) or Germinal Center B cells (right) from mixed bone marrow chimera. Analyses were performed 8, 14 and 21 days after NP-KLH immunization. The red dotted line represents the percentage of originally injected CD22<sup>-/-</sup> (CD45.2<sup>pos</sup>) bone marrow cells at reconstituting of irradiated recipients to generate mixed bone marrow chimera. The blue dotted line shows the average percentage of CD22<sup>-/-</sup> (CD45.2<sup>pos</sup>) naïve B cells at day of analysis. BM = Bone marrow.

**C** Bar charts show percentage of CD22<sup>-/-</sup> (CD45.2<sup>pos</sup>) and WT (CD45.1<sup>pos</sup>) Germinal Center B cells of all B220<sup>pos</sup> cells in relation to the genotype specific (CD45.1<sup>pos</sup> or CD45.2<sup>pos</sup>) B220<sup>pos</sup> B cell population in mixed bone marrow chimera (based on data from B). Analyses were performed at day 14 after NP-KLH immunization.

**D** Bar charts show percentage of CD22-Y2,5,6F (CD45.2<sup>pos</sup>) and WT (CD45.1<sup>pos</sup>) Germinal Center B cells of all B220<sup>pos</sup> cells in relation to the genotype specific (CD45.1<sup>pos</sup> or CD45.2<sup>pos</sup>) B220<sup>pos</sup> B cell population in mixed bone marrow chimera. Analyses were performed at day 14 after NP-KLH immunization.

Data information: In (A–D), data are presented as mean. Each dot represents one mouse. Data in (A) were collected from 6, in (B,C) from 1 per time point and in (D) from 2 independent experiments. Graphs show data from (A) 20 mice, (B) 3–6 mice per time point, (C) 6 mice and (D) 14 mice. \**p* < 0.05, \*\*\**p* < 0.001 (Mann-Whitney test).



**Supplementary Figure 2: Analysis of plasma cells (A) and memory B cells (C,D) in NP-KLH immunized mixed bone marrow chimeras, generated from CD22<sup>-/-</sup> and WT (CD45.1) bone marrow cells.**

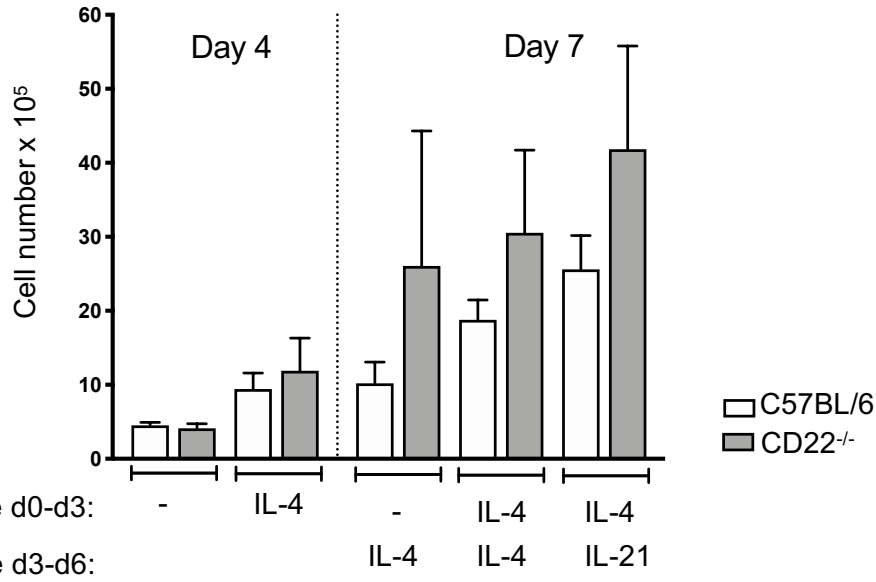
**A)** Splenic single cell suspensions from mixed bone marrow chimera were stained with indicated antibodies and analyzed by flow cytometry. Dot plot shows gating strategy for Plasma cells (B220<sup>lo</sup> CD138<sup>pos</sup> TACI<sup>pos</sup>).

**B)** Splenic single cell suspensions from mixed bone marrow chimera were stained with indicated antibodies and analyzed by flow cytometry. Dot plots show gating strategy for IgD<sup>pos</sup> IgG1<sup>neg</sup> NP-specific memory B cells (CD19<sup>pos</sup> CD38<sup>pos</sup> CD95<sup>neg</sup> NP<sup>pos</sup> IgD<sup>pos</sup> IgG1<sup>neg</sup> CD80<sup>pos</sup> PD-L2<sup>pos</sup>), IgD<sup>neg</sup> IgG1<sup>neg</sup> memory B cells (CD19<sup>pos</sup> CD38<sup>pos</sup> CD95<sup>neg</sup> NP<sup>pos</sup> IgD<sup>neg</sup> IgG1<sup>neg</sup> CD80<sup>pos</sup> PD-L2<sup>pos</sup>) and IgD<sup>neg</sup> IgG1<sup>pos</sup> memory B cells (CD19<sup>pos</sup> CD38<sup>pos</sup> CD95<sup>neg</sup> NP<sup>pos</sup> IgD<sup>neg</sup> IgG1<sup>pos</sup> CD80<sup>pos</sup> PD-L2<sup>pos</sup>).

**C)** Bar charts shows percentage of CD22<sup>-/-</sup> (CD45.2<sup>pos</sup>) and WT (CD45.1<sup>pos</sup>) IgD<sup>pos</sup> IgG1<sup>neg</sup> NP-specific memory B cells, IgD<sup>neg</sup> IgG1<sup>neg</sup> NP-specific memory B cells and IgD<sup>neg</sup> IgG1<sup>pos</sup> NP-specific memory B cells in relation to genotype specific (CD45.1<sup>pos</sup> or CD45.2<sup>pos</sup>) CD19<sup>pos</sup> B cells from mixed bone marrow chimera. Analyses were performed at day 14 after NP-KLH immunization. MBC = Memory B cells.

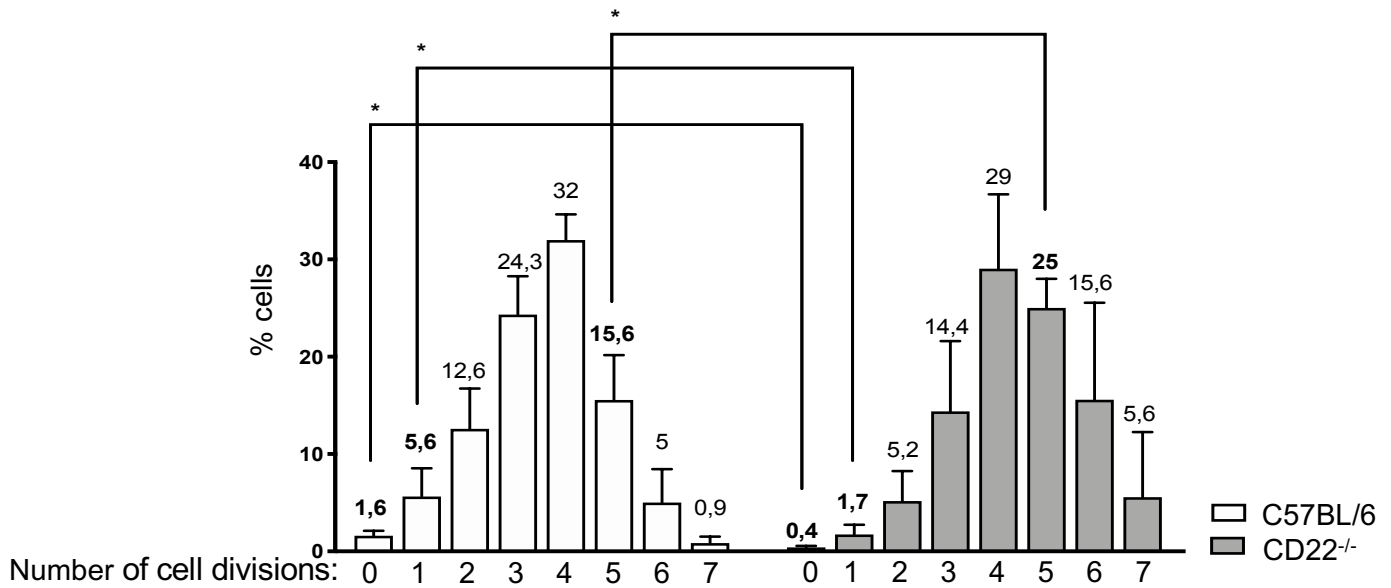
Data information: In (C), data are presented as mean. Each dot represents one mouse. Data were collected from 5 mice in 1 experiment.

\*p < 0.05, \*\*p < 0.01 (Mann-Whitney test).

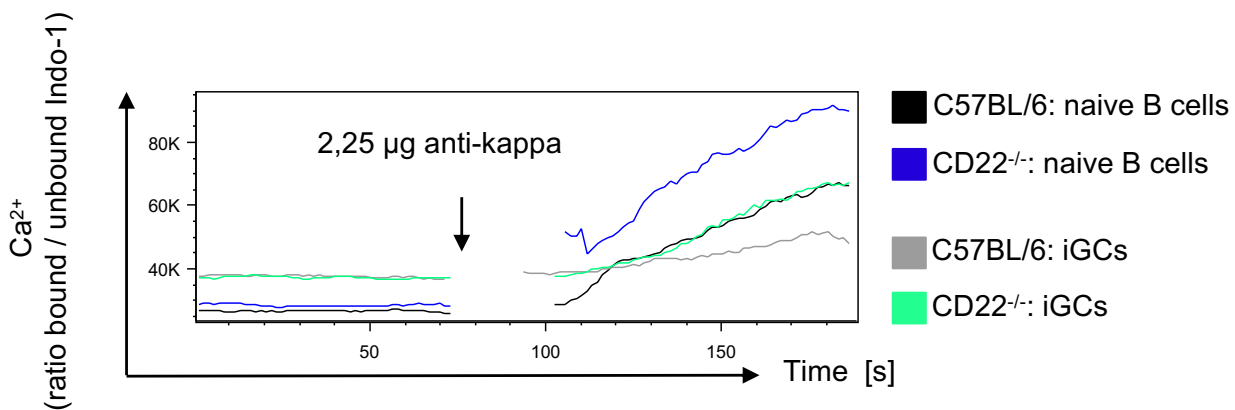
**A**

Cytokine added to culture d0-d3:

Cytokine added to culture d3-d6:

**B**

Number of cell divisions:

**C**

**Supplementary Figure 3: Proliferation and signaling analysis of *in vitro* generated Germinal Center B cells (iGCs) from C57BL/6 and CD22<sup>-/-</sup> mice.**

**A)** Germinal center cultures were generated *in vitro*. Therefore splenic naïve B cells were isolated from C57BL/6 and CD22<sup>-/-</sup> mice and cultivated on inactivated 40LB feeder cells in the presence of indicated cytokines (1 ng/ml IL-4 or 10 ng/ml IL-21) for 3-76 days. At day 3 and 6 cells were counted by hemocytometer and total cell number was calculated.

**B)** Proliferation analyses of C57BL/6 and CD22<sup>-/-</sup> iGCs were performed. Therefore splenic naïve B cells were isolated from C57BL/6 and CD22<sup>-/-</sup> mice, labeled with CTV and cultivated on inactivated 40LB feeder cells in the presence of IL-4 or for 3 days. Cells were analyzed via flow cytometry. Bar chart shows percentage of cells that underwent a certain number of cell divisions.

**C)** 2x10<sup>6</sup> splenic cells or iGCs of the indicated genotype were loaded with Indo-1. Ca<sup>2+</sup> baseline was measured under unstimulated conditions for 50 sec, afterwards BCR was stimulated. Ca<sup>2+</sup> mobilization was continued to 3 min. Curves show nBCs (B220<sup>pos</sup> GL7<sup>neg</sup> PNA<sup>neg</sup>) and iGCs (B220<sup>pos</sup> GL7<sup>pod</sup> PNA<sup>pos</sup>). Data shown are representative of 5 independent experiments. iGC BC = *In vitro* induced germinal center B cells.

Data information: In (A,B), data are presented as mean ± SD and show the results of 4 independent experiments. (C) shows a representative example of 5 independent experiments \*p < 0.01 (Mann-Whitney test).