SUPPLEMENTAL MATERIAL

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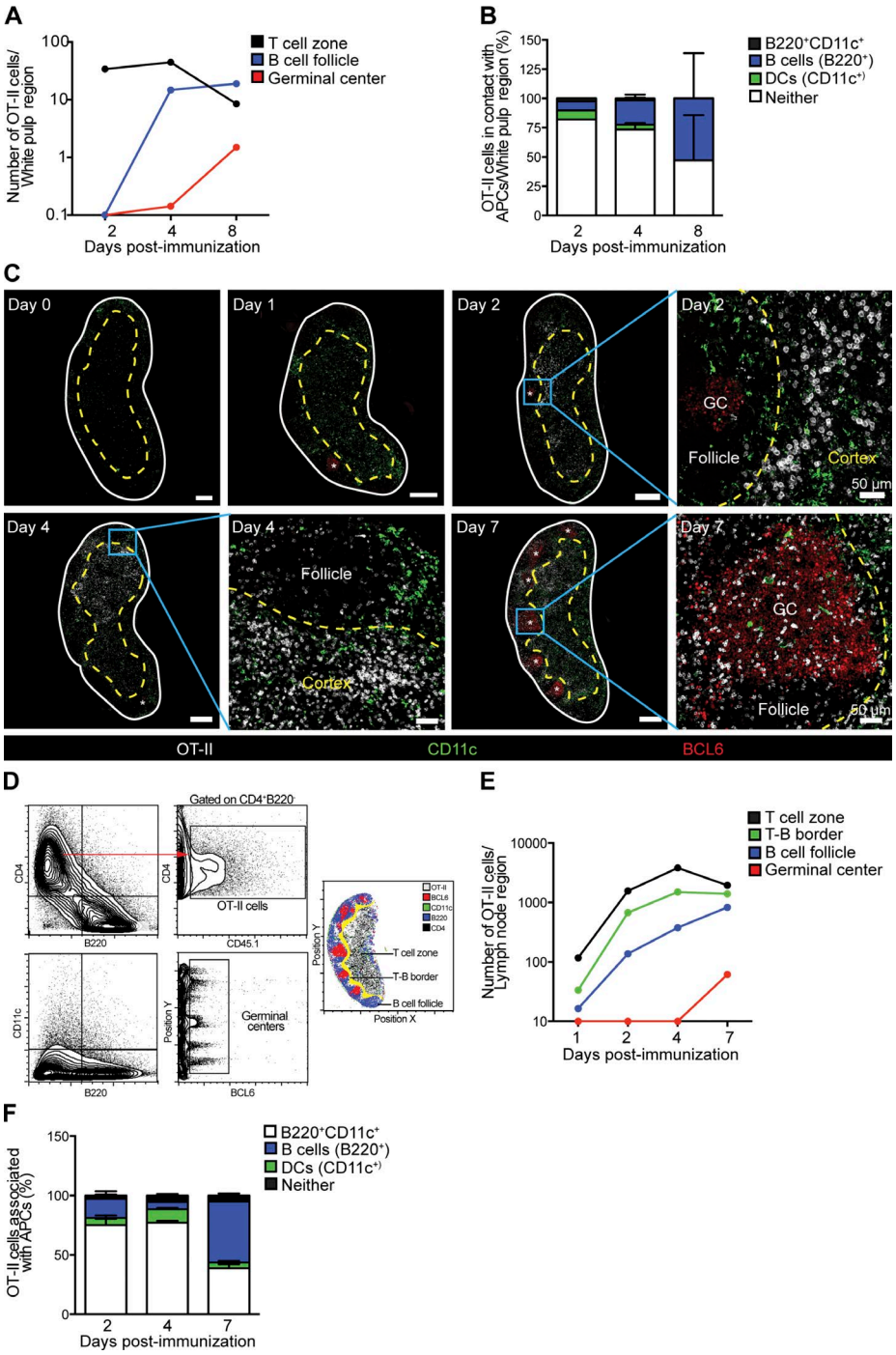

Figure S1. **Kinetics and localization of antigen-specific T cells during GC response.** (A–F) Naive CD45.1$^+$ OT-II cells were adoptively transferred to mice 1 d before i.p. or s.c. immunization with NP-OVA/Alum. Frozen sections were prepared from spleens (A and B) or draining LNs (dLNs; C–F) harvested 0–8 d after immunization and processed for confocal microscopy and histocytometry. (A) Number of OT-II cells in white pulp regions of the spleen. (B) Histocytometric analysis of OT-II cells in association with CD11c$^+$ or B220$^+$ APCs in the spleen. Data are from two to three images from two mice per time point. (C) Immunofluorescence images of dLN sections depicting the magnitude and location of the OT-II response at the indicated time points. White lines denote LN capsule, dashed yellow lines correspond to the boundary between the B cell follicles and T cell zone, and GCs are marked by asterisks. Bars: (main images) 300 µm; (magnifications) 50 µm. (D) Representative histocytometry contour plots and gating strategy used to identify the spatial distribution of B cells, T cells, DCs, OT-II cells, and GCs. Gate used to quantify OT-II cells in the T–B cell border is shown in yellow. (E) Number of OT-II cells per dLN region quantified by histocytometry. (F) Histocytometric analysis of OT-II cells in association with CD11c$^+$ or B220$^+$ APCs in the dLN. Mean ± SEM, n = 2–4 dLNs from two mice per time point.

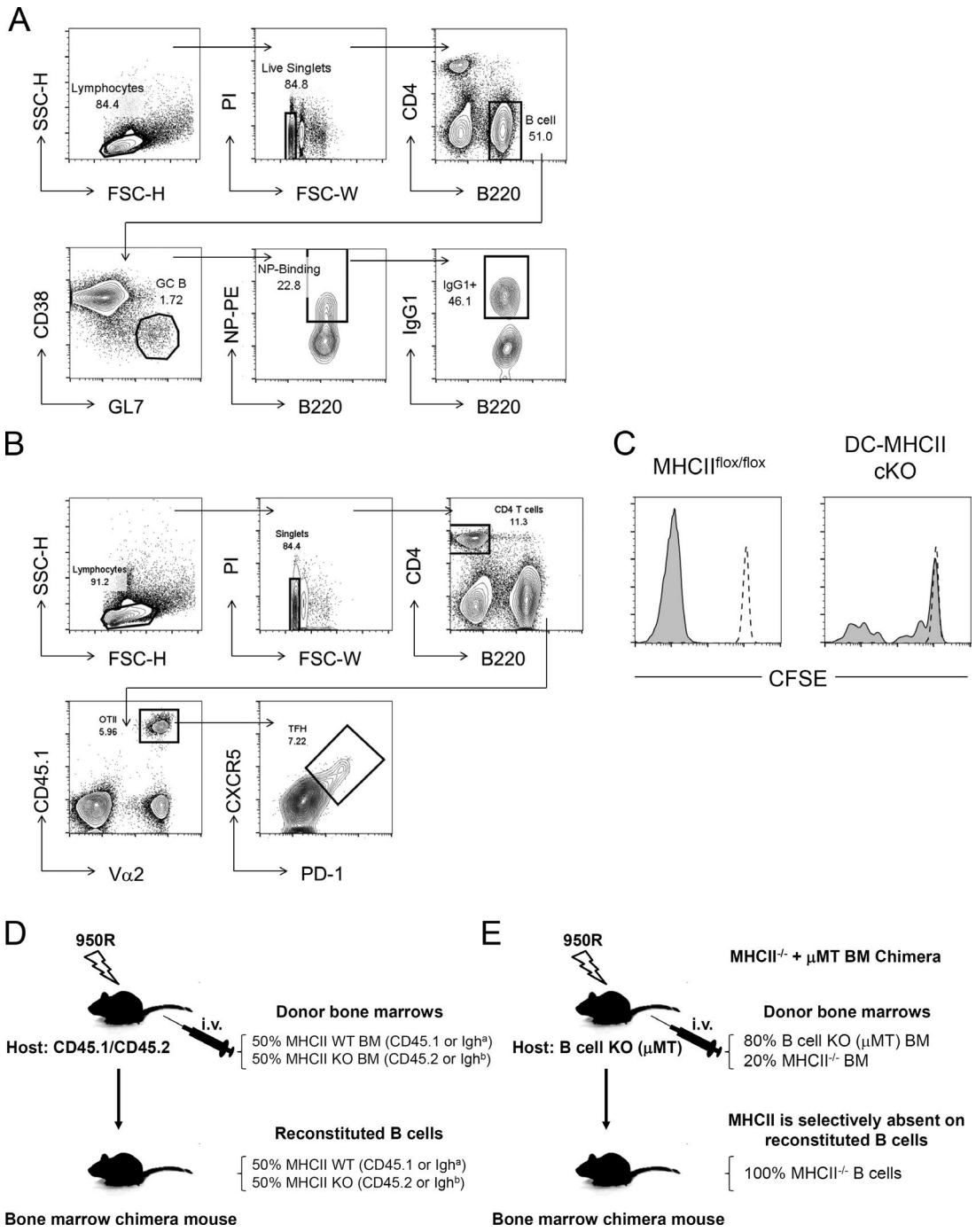

Figure S2. **Nonredundant role of MHCII on DCs and B cells for TD antigen–specific GC B cell development, IgG1 production, and Tfh development.** (A) Gating strategy for detecting NP-specific GC B cells. GC B cells were defined as $B220^+$ $GL7^+$ $CD38^{dull}$ or $B220^+$ $GL7^+$ Fas^+ cells. NP-specific GC B cells were identified as NP-PE binding. (B) Gating strategy for analyzing Tfh OT-II cells. Transferred OT-II T cells were identified as $CD4^+$ $B220^-$ $CD45.1^+$ $V\alpha2^+$. Tfh cells were defined as $CXCR5^{high}$ $PD\text{-}1^{high}$. Data shown are representative flow cytometry plots. (C) CFSE-labeled OT-II T cells (CD45.1) were transferred to the indicated recipient mice (CD45.2) followed by NP-OVA/Alum immunization, and transferred OT-II cells were analyzed at day 8. Cell division of OT-II cells was analyzed by CFSE dilution. Shaded histograms: OT-II cell in immunized mouse. Dashed histograms: OT-II cells in unimmunized mouse. Data shown are representative of two independent experiments. (D) Schematic procedure for generation of WT + MHCII KO mixed BM chimeras. (E) Schematic procedure for generation of B cell KO (μMT) + MHCII KO mixed BM chimeras ($MHCII^{-/-}$ + μMT) in which MHCII is selectively absent on B cells.

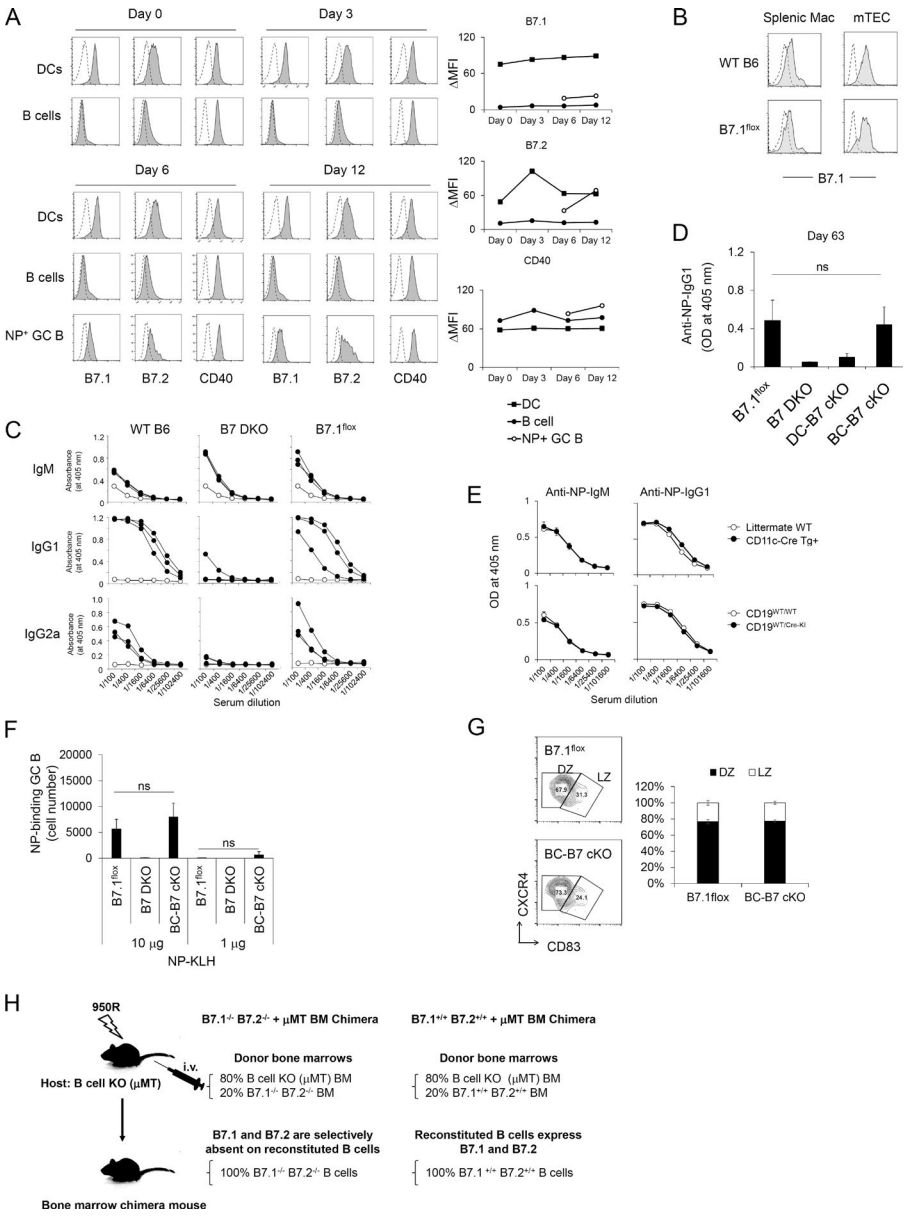

Figure S3. **Cellular requirement of B7–CD28 pathway for GC response.** (A) B7 and CD40 expression kinetics during the course of GC response. WT B6 were immunized with NP-KLH/Alum i.p. At the indicated time points, B7 and CD40 expression levels on splenic DC ($CD11c^+$ $B220^-$), B cells ($B220^+$ $CD11c^-$), and NP^+ GC B cells ($B220^+$ $CD38^{dull}$ $GL7^+$ NP^+) were analyzed by flow cytometry. Each time point, $n = 3$. Graphs show mean ± SEM of ΔMFI (specific antibody–isotype control antibody). Representative of two independent experiments. Shaded histograms are stainings of specific antibodies. Dashed-line histograms are stainings of isotype control antibodies. (B) B7.1 expression in $B7.1^{flox}$ BAC Tg mice on splenic macrophages ($CD11b^+$ $CD11c^-$ $B220^-$) and thymic medullary epithelial cells (mTEC; $MHCII^{high}$ $UEA1^+$ $Ly51^-$). Data are representative of at least three independent experiments. (C) Mice were immunized with TNP-KLH/Alum i.p. Serum titer of TNP-specific IgM, IgG1, and IgG2a antibodies were analyzed by ELISA at day 21. WT B6, $n = 3$; B7 DKO, $n = 3$; and $B7.1^{flox}$, $n = 3$. Closed circles represent individual immunized mice. Open circles represent unimmunized B6. (D) Mice were immunized with NP-KLH/Alum i.p. Serum titer of NP-specific IgG1 antibodies were analyzed by ELISA at day 63. Each strain, $n = 3$. Data are representative of at least three independent experiments. ns, not significant. (E) CD11c-Cre mice and CD19-Cre mice were immunized with NP-KLH/Alum i.p. Serum titer of NP-specific IgM and IgG1 was measured by ELISA at day 21. $CD11c\text{-}Cre^+$, $n = 3$; control $CD11c\text{-}Cre^-$, $n = 5$; $CD19\text{-}Cre^+$, $n = 4$; and control $CD19\text{-}Cre^-$, $n = 4$. Mean ± SEM. (F) Mice were immunized with low-dose antigen (10 and 1 mg NP-KLH/Alum), and NP-specific GC B cells were analyzed at day 7. Data presented are the combined result of three independent experiments. The total numbers of mice in the three combined experiments are 10 mg NP-KLH immunized group: $B7.1^{flox}$, $n = 6$; BC-B7 cKO, $n = 5$; and B7 DKO, $n = 4$. 1 mg NP-KLH immunized group, each strain $n = 3$. (G) Mice were immunized with NP-KLH/Alum, and NP-specific light zone (LZ; $CD83^{high}$ $CXCR4^{low}$/dark zone (DZ; $CD83^{low}$ $CXCR4^{high}$ GC B cells were analyzed by flow cytometry at day 8. Data presented are the combined result of two independent experiments. The total numbers of mice in the two combined experiments are $B7.1^{flox}$, $n = 5$; and BC-B7 cKO, $n = 4$. (H) Schematic procedure for generation of B cell KO + B7 DKO mixed BM chimeras ($B7.1^{-/-}$ $B7.2^{-/-}$ + μMT) in which B7 is selectively absent on B cells.

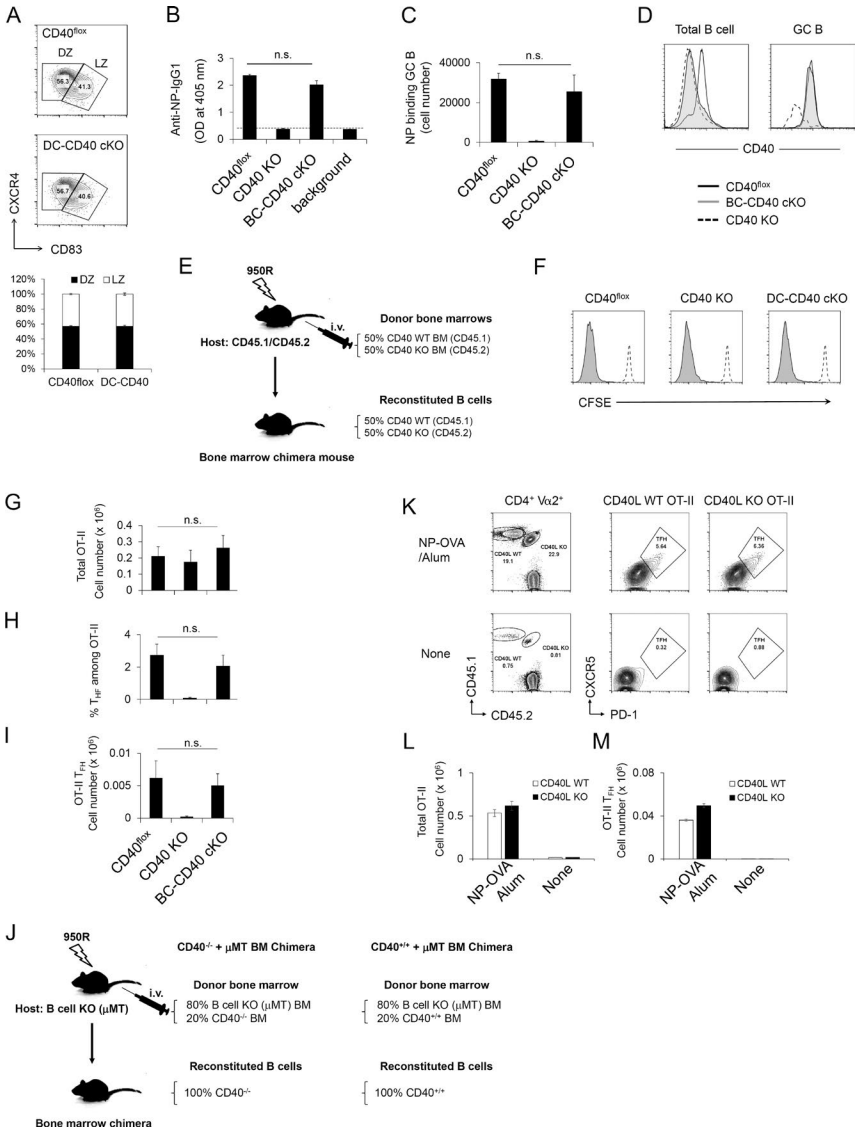

Figure S4. **Cellular requirement of CD40L–CD40 pathway for GC response.** (A) GC compartment of DC-CD40 cKO mice. Light zone (LZ) and dark zone (DZ) GC B cell composition. Mice were immunized with NP-KLH/Alum, and NP-specific LZ ($CD83^{high}$ $CXCR4^{low}$)/DZ ($CD83^{low}$ $CXCR4^{high}$) GC B cells were analyzed by flow cytometry at day 8. $n = 2$. Combined result of two independent experiments. (B) NP-specific IgG1 production in BC-CD40 cKO ($CD40^{flox}$ BAC Tg × CD19-Cre) mice. Serum titer of NP-specific IgG1 was measured at 3 wk after NP-KLH/Alum i.p. immunization. Pooled data of three independent experiments are shown. $CD40^{flox}$, $n = 6$; CD40 KO, $n = 6$; and BC-CD40 cKO, $n = 7$. (C) NP-specific GC B cells in BC-CD40 cKO mice. BC-CD40 cKO mice were immunized with NP-KLH/Alum, and the absolute number of NP-specific GC B cells ($B220^+$ $GL7^+$ Fas^+ NP-PE^+) in the spleen was analyzed by flow cytometry on day 8 after immunization. $CD40^{flox}$, $n = 3$; CD40 KO, $n = 4$; and BC-CD40 cKO, $n = 5$; unimmunized, $n = 3$. Graph represents pooled data of three independent experiments. (D) CD40 expression on GC B cells in BC-CD40 cKO mice. GC B cells and non-GC B cells were analyzed at day 8 after NP-KLH/Alum immunization. Data shown are representative of at least three independent experiments. (E) Schematic procedure for generation of WT + CD40 KO mixed BM chimeras. Statistical significance was evaluated by Student's *t* test. (F) OT-II expansion in DC-CD40 cKO mice. CFSE-labeled OT-II T cells (CD45.1) were transferred to the indicated mice, and mice were immunized with NP-OVA/Alum. At day 8 after immunization, OT-II cells were analyzed. Shaded histograms and dashed histograms indicate OT-II cells in immunized and unimmunized mice, respectively. Data shown are representative of two independent experiments. (G–I) OT-II T cell response in BC-CD40 cKO mice ($CD40^{flox}$ × CD19Cre). OT-II T cells (CD45.1) were transferred to the indicated mice strains and mice were immunized with NP-OVA/Alum. At day 8 after immunization, OT-II cells were analyzed. Recovered OT-II cell number (G), percentage of Tfh phenotype cells among OT-II (H), and total number of Tfh phenotype of OT-II cells (I). Graphs shown are pooled data of three independent experiments. Statistical significance was evaluated by Student's *t* test. n.s., not significant. (J) Schematic procedure for generation of B cell KO (μMT) + CD40 KO mixed BM chimeras ($CD40^{-/-}$ + μMT) in which CD40 is selectively absent on B cells. (K–M) CD40L WT (CD45.1) and CD40L KO (CD45.1/CD45.2) OT-II were cotransferred to B6 recipient mice (CD45.2) followed by NP-OVA/Alum immunization. OT-II cells were analyzed at day 8 after immunization. (K) Representative flow cytometry plots for OT-II Tfh analysis. (L) Total OT-II cell number. (M) Tfh OT-II cell number. Each group, $n = 3$. Data shown are representative of two independent experiments. Statistical significance was evaluated by Student's *t* test. All error bars represent means ± SEM.

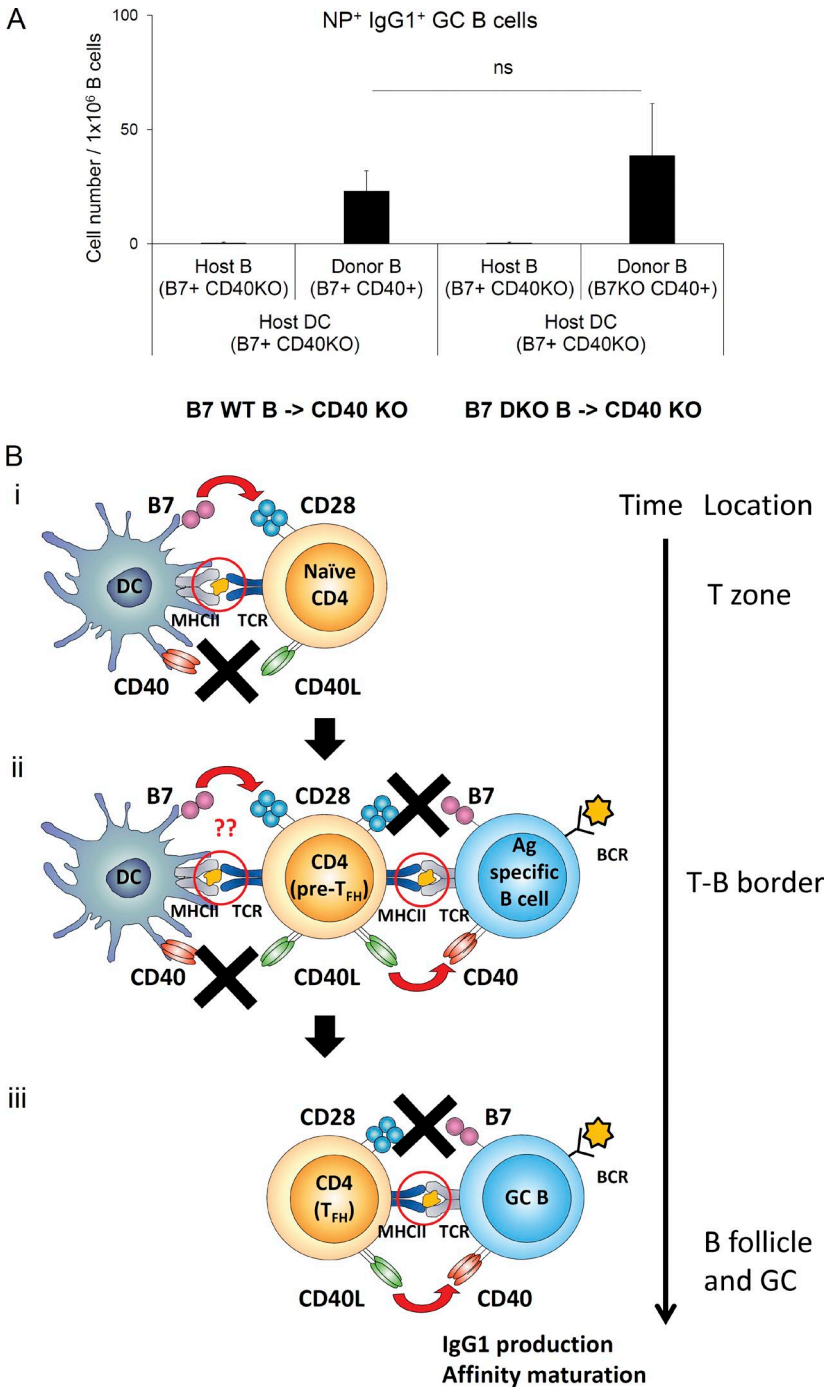

Figure S5. **GC response of WT and B7 DKO B cells in CD40 KO host mice.** (A) Purified WT B cells or B7 DKO B cells (3×10^7) were transferred to CD40 KO host mice, and mice were immunized the next day with NP-OVA/Alum i.p. 7 d later, spleen cells were analyzed for antigen-specific GC B cell populations ($B220^+$ $GL7^+$ $CD38^{dull}$ NP^+ $IgG1^+$). Data shown are normalized cell numbers of antigen-specific GC B cells per million B cells in each population (mean ± SEM, $n = 3$). Statistical significance was evaluated by Student's *t* test. ns, not significant. Similar results were obtained in two independent experiments. (B) A model of distinct roles of B7 and CD40 on DCs and B cells for GC response. (i) MHCII-dependent T–DC interaction in the T cell zone is required to initiate Tfh and GC responses. B7 co-stimulation is essential for T–DC interaction, whereas CD40 co-stimulation is dispensable. (ii) At a subsequent stage in the GC response, MHCII-dependent interaction between antigen-specific B cells and pre-Tfh cells occurs at the border of T and B cell zones. CD40 on B cells is essential, whereas B7 on B cells is dispensable. It is possible that B7 co-stimulation in T–DC interaction is still required in this stage, but that is yet to be determined. (iii) B7 on B cells is not required for GC responses in B cell follicles, including expansion of antigen-specific GC B cells, class switching to IgG1, and affinity maturation, but CD40 on B cells has an essential and cell-intrinsic role for these responses.