Supporting Information

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SI Methods

Flow Cytometry. Cells were stained in PBS containing 1% FBS, 2 mM EDTA, and 0.02% sodium azide. Cells were blocked with anti-Fcγ mAb and normal mouse serum and stained with the following Abs or chemical conjugates: NIP-PE, CD45.1-Alexa647, and CD4-Alexa633 (Memorial Sloan-Kettering Cancer Center mAb Core Facility); CD19-APC-Cy7, Fas-biotin, GL7-FITC, CD45.2-perCP-Cy5.5, CD4-FITC, Vα2-PE, Vβ6-FITC, PD-1-FITC, and CXCR5-PE (BD Pharmingen); and eFluor-450-streptavidin (eBioscience). Data were collected on a BD LSRII cytometer (BD Biosciences). DAPI was added to the samples before analysis to allow for dead cell exclusion. Cell doublets were also excluded from the analysis. Data were analyzed using FlowJo software (Tree Star).

T-Cell Assays. For generation of polyclonal NP-CGG specific CD4 T cells, B6 mice were immunized in the hocks and base of tail with 400 μg NP-CGG in 200 μL as an alum precipitate. Then, 7–10 d later, CGG-specific CD4 T cells were purified from draining lymph nodes by negative selection (Naïve CD4 T Cell Isolation Kit II; Miltenyi Biotech). For CGG presentation assays, resting B cells were purified from B1-8, B1-8.H2-O$^{-/-}$, and nontransgenic littermates by negative selection using CD43 MACS beads (Miltenyi Biotech) and activated overnight with 2 μg/mL anti-CD40 (mAb FGK45). Next, 2×10^4 NP$^+$ B cells or a matched number of total B cells from nontransgenic control mice were incubated for 30 min on ice with titrated amounts of NP-CGG (0.001–100 μg/mL). Cells were washed twice, and 1×10^5 CGG-specific polyclonal CD4 T cells were added and incubated at 37 °C for 72 h. For NP-OVA assays, B1-8 and B1-8.H2-O$^{-/-}$ mice were immunized i.p. with NP-OVA in alum (50 μg in 200 μL alum). Two days later, NP$^+$ splenic B cells were purified by staining cells with NP-PE, followed by the addition of anti-PE MACS beads (Miltenyi Biotech) and positive magnetic sorting. Then 2.5×10^4 MACS purified OT-II T cells (Naïve CD4 T Cell Isolation Kit II; Miltenyi Biotech) were added to titrated numbers of B cells ($0–1 \times 10^5$ cells/well) and incubated at 37 °C for 72 h. B cells were irradiated at 2000 rads before use as APCs. T-cell proliferation after 48 h was measured by the addition of 1μCi/well of [^{3}H]thymidine. Results are expressed as mean cpm (± SEM) of triplicate cultures. B- and T-cell purity was >85% as measured by flow cytometry.

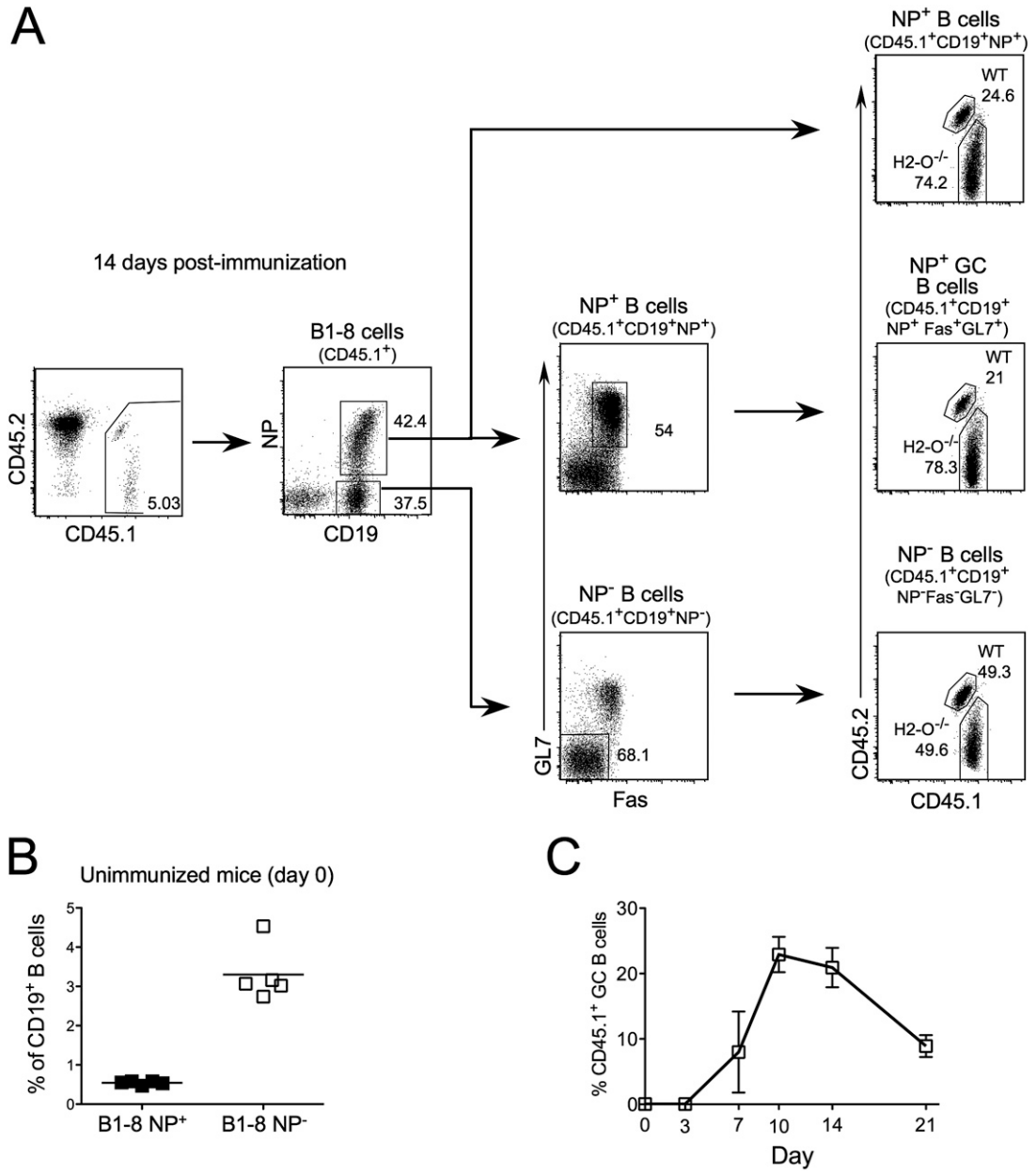

Fig. S1. Definition of B-cell subsets and characterization of overall GC response. (*A*) Splenocytes were gated on CD45.1 expression to define donor-derived B1-8 cells. B1-8 cells were gated on CD19 expression and NP specificity to define Ag-specific and nonspecific B cells. Ag-specific B cells were gated on CD45.1 and CD45.2 expression to determine WT and H2-O$^{-/-}$ contribution to total Ag-specific B-cell population, or were gated on Fas^{+} GL7^{+} cells to define GC subset. GC subset was gated for CD45.1 and CD45.2 expression to determine contribution of each donor. Nonspecific B cells (CD19^{+} NP^{-}) were gated on Fas^{-}GL7^{-} subset to exclude cells that participated in the GC reaction (most likely CGG-specific B cells), then gated on CD45.1 and CD45.2 expression to determine contribution of each donor. (*B*) Percentage of total B cells that are B1-8 NP^{+} or B1-8 NP^{-} B cells in unimmunized recipient mice at day 0. (*C*) Kinetics of GC response. Donor-derived GC B cells (CD45.1^{+} CD19^{+} Fas^{+} GL7^{+}) as percentage of donor-derived B cells (CD45.1^{+} CD19^{+}).

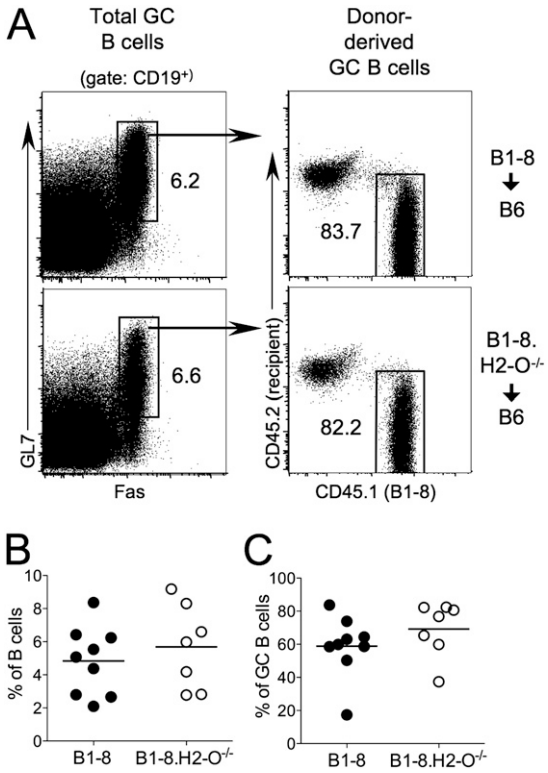

Fig. S2. In the absence of competition, NP$^+$ H2-O$^{-/-}$ B cells populate GCs similarly to WT B cells. Splenocytes containing 10^6 NP-specific B cells from B1-8.SJL or B1-8.H2-O$^{-/-}$.SJL donors were transferred into B6 mice. Recipients were immunized with NP-CGG 24 h later and analyzed 10 d later. (*A*) CD19$^+$ cells were gated for GC B cells (Fas$^+$ GL7$^+$), then gated on CD45.1$^+$ population to identify B1-8–derived cells. Numbers indicate percentage of each population. (*B*) Quantification GC as percentage of total CD19$^+$ cells. (*C*) Quantification of contribution of transferred B cells to GC population.

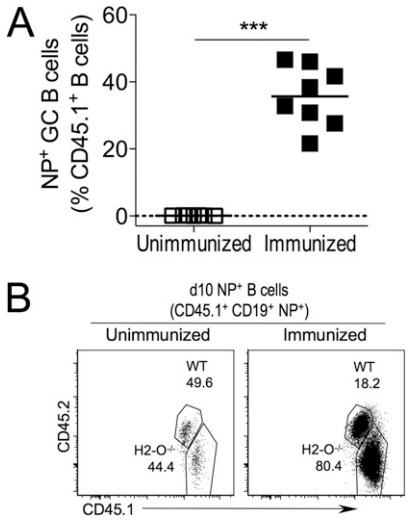

Fig. S3. Unimmunized B6 recipient mice lack NP-specific GC B-cell response. (*A*) B1–8 and B1–8.H2-O$^{-/-}$ B cells were transferred into B6 hosts, hosts were either immunized with NP-CGG in alum or were left unimmunized. Percentage of NP$^+$ GC B cells (CD19$^+$ Fas$^+$ GL7$^+$) B cells at 10 d postimmunization that were derived from transferred cells was measured by flow cytometry. Data are presented as percentage of total B1–8 and B1–8.H2-O$^{-/-}$ B cells in transferred recipients. (*B*) B1-8 and B1-8.H2-O$^{-/-}$ B cells were transferred into B6 hosts, immunized with NP-CGG, or left unimmunized and analyzed 10 d later. Contribution of B1–8. H2-O$^{-/-}$, and B1–8 B cells to Ag-specific B-cell subset. Numbers indicate size of each gated population as percentage of parent population.

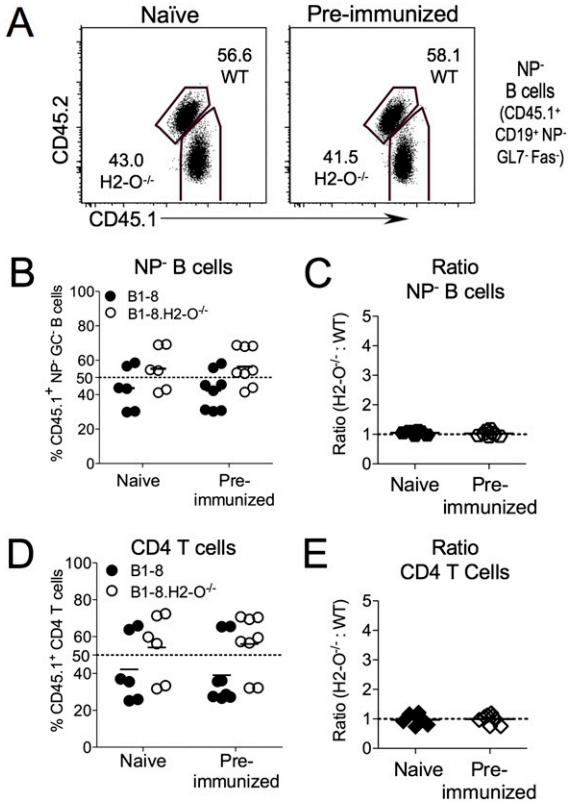

Fig. S4. Change in H2-O$^{-/-}$ and WT B-cell proportions is confined to Ag-specific B-cell subset in preimmunized mice. Contribution of H2-O$^{-/-}$ and WT cells to donor-derived NP^{-} non-GC B-cell population in naïve versus preimmunized hosts shown as (*A*) FACS plots, (*B*) percentages, and (*C*) normalized ratio of H2-O$^{-/-}$ to WT cells. (*D* and *E*) Quantification of contribution of H2-O$^{-/-}$ and WT cells to donor-derived CD4^{+} T-cell population as (*D*) percentages and (*E*) normalized ratio of H2-O$^{-/-}$ to WT cells.

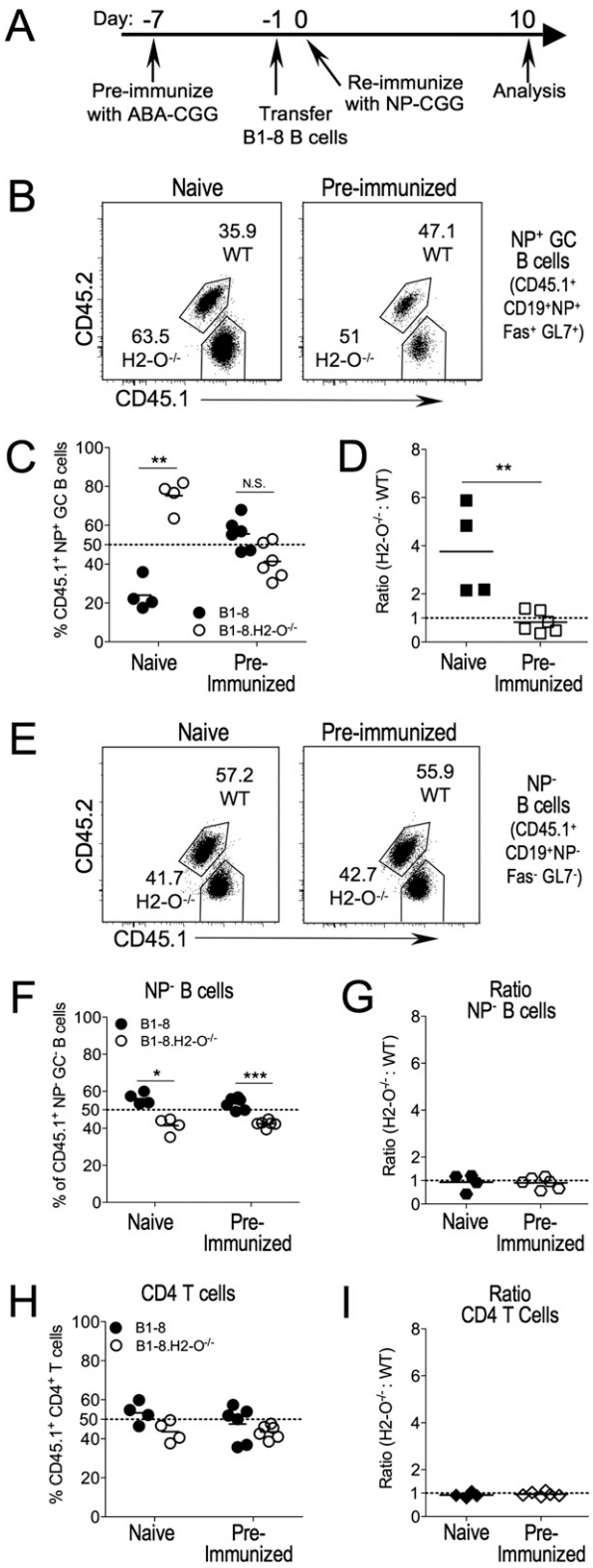

Fig. S5. Preimmunization with ABA-CGG to expand pool of CGG-specific T_{FH} cells before B-cell transfer results in similar numbers and percentages of B1-8 WT and H2-O$^{-/-}$ GC B cells after immunization with NP-CGG. (*A*) Schematic representation of experiment setup. Marked B1-8 and B1-8.H2-O$^{-/-}$ cells were mixed at a 1:1 ratio and transferred into either naïve hosts or hosts that had been immunized 7 d prior with ABA-CGG. The next day, ABA-CGG preimmunized mice were immunized with NP-CGG in alum (preimmunized). (*B*) Contribution of WT and H2-O$^{-/-}$ B1-8 cells to the NP^{+} GC B-cell pool upon preimmunization. (*C* and *D*) Quantification of population defined in B as percentage of donor-derived NP^{+} GC B cells (*C*) and as a ratio of H2-O$^{-/-}$ to WT populations, normalized to ratio in transferred mixture (*D*). (*E*–*G*) Contribution of H2-O$^{-/-}$ and WT cells to donor-derived NP^{-} non-GC B-cell population in naïve versus preimmunized hosts shown as (*E*) FACS plots, (*F*) percentages, and (*G*) normalized ratio of H2-O$^{-/-}$ to WT cells. (*H* and *I*) Quantification of the contribution of H2-O$^{-/-}$ and WT cells to donor-derived CD4^{+} T-cell population as (*H*) percentages and (*I*) normalized ratio of H2-O$^{-/-}$ to WT cells. Statistical significance was calculated using a two-tailed paired *t* test in *C, F,* and *H* and two-tailed unpaired *t* test in *D, G,* and *I*.

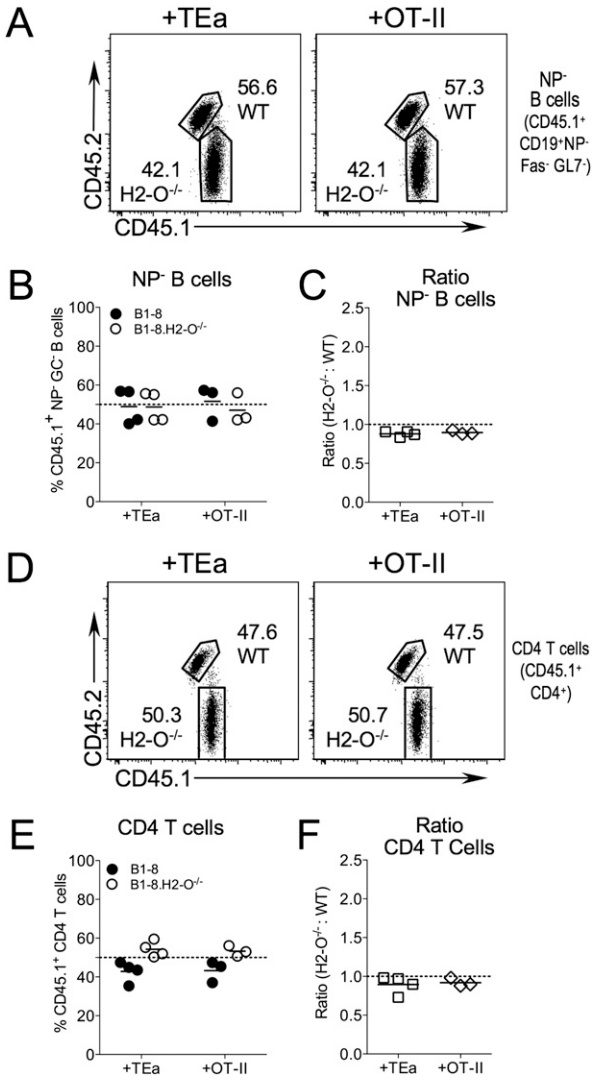

Fig. S6. Change in H2-O$^{-/-}$ and WT B-cell proportions is confined to Ag-specific B-cell subset upon the addition of OT-II T cells. (*A*–*C*) Contribution of H2-O$^{-/-}$ and WT cells to donor-derived non-GC NP^{-} B-cell population in hosts that received nonspecific (TEa) or Ag-specific (OT-II) T cells shown as (*A*) FACS plots (*B*) percentages, and (*C*) normalized ratio of H2-O$^{-/-}$ to WT cells. (*D*–*F*) Contribution of H2-O$^{-/-}$ and WT cells to donor-derived CD4^{+} T-cell population in hosts that received extra nonspecific or Ag-specific T cells shown as (*D*) FACS plots, (*E*) percentages, and (*F*) normalized ratio of H2-O$^{-/-}$ to WT cells.