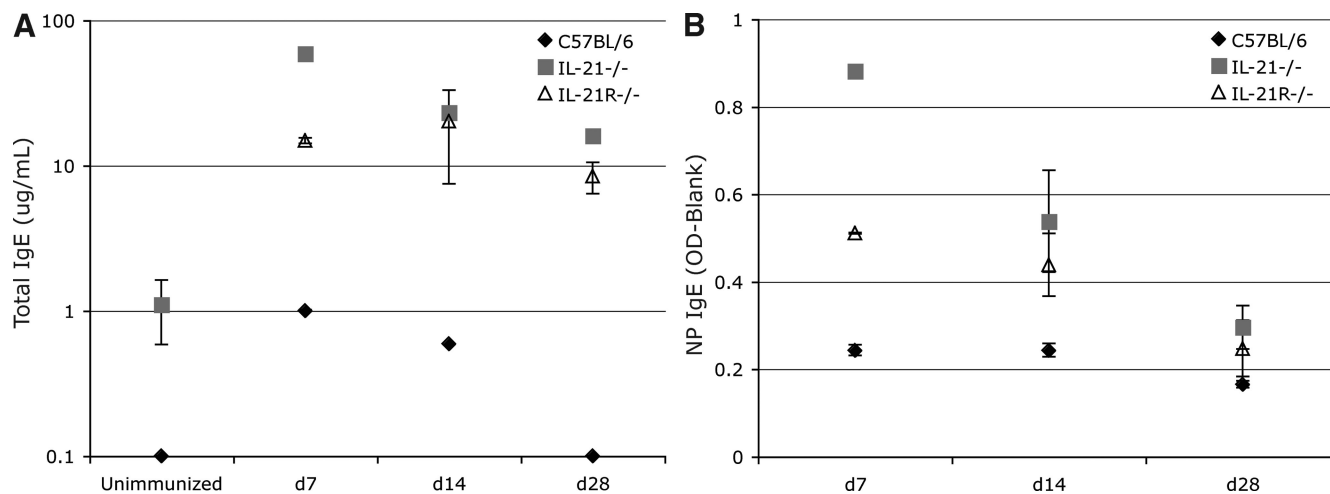
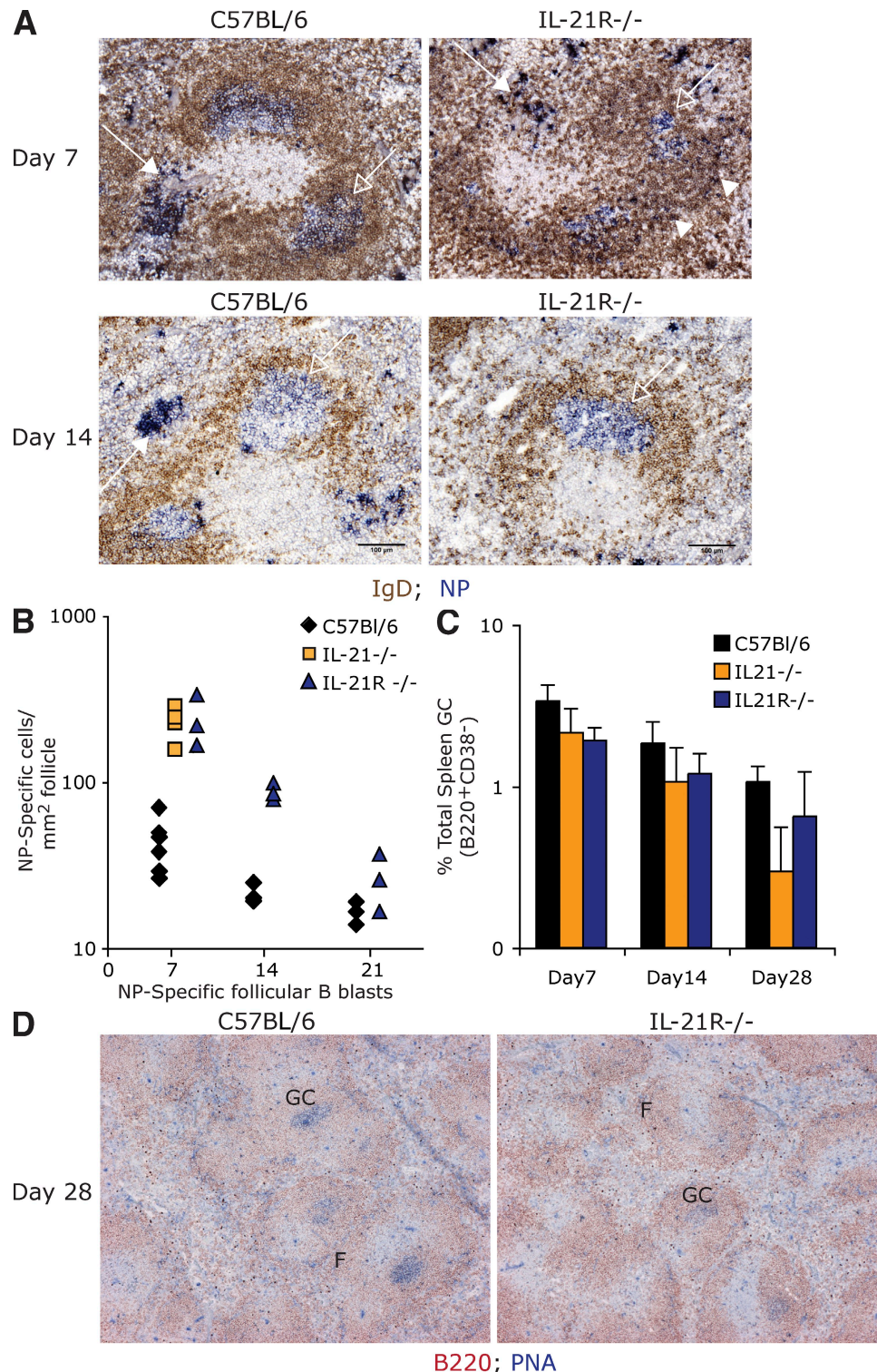


SUPPLEMENTAL MATERIAL

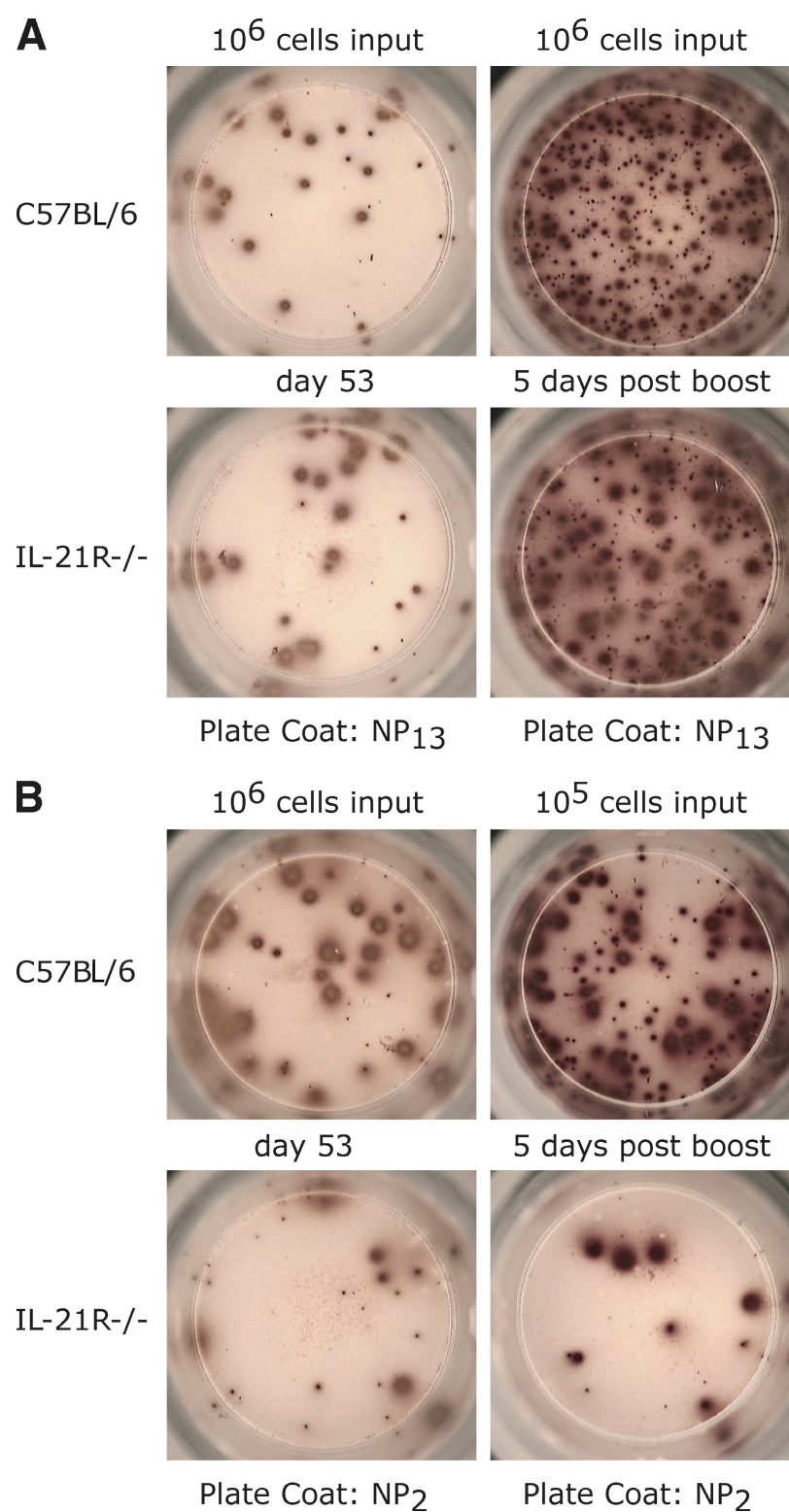
Zotos et al., <http://www.jem.org/cgi/content/full/jem.20091777/DC1>



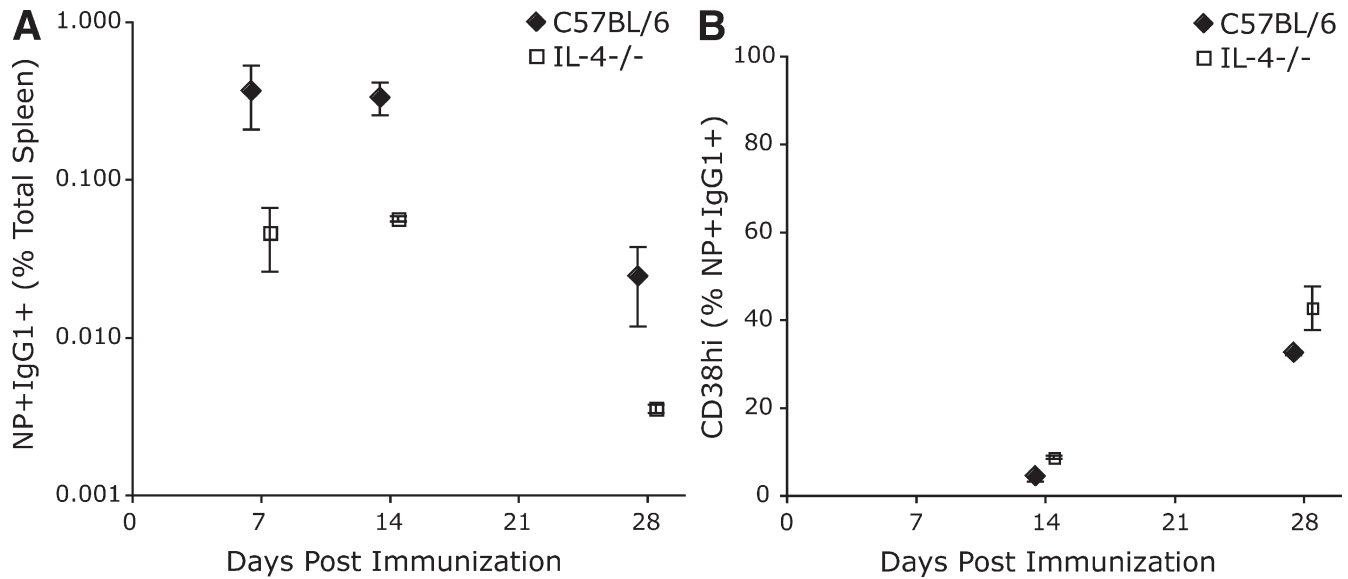
**Figure S1. Hyper IgE in absence of IL-21 signaling.** (A) Total serum IgE levels in C57BL/6, IL-21<sup>-/-</sup>, and IL-21R<sup>-/-</sup> mice, determined by ELISA, at times indicated before and after immunization with NP-KLH in alum. Error bars show mean ± SE. (B) Antigen-specific serum IgE in C57BL/6, IL-21<sup>-/-</sup>, and IL-21R<sup>-/-</sup> mice at the indicated times, showing mean ± SE. In the absence of an IgE standard, measurements were in optical density for the identical serum dilution.



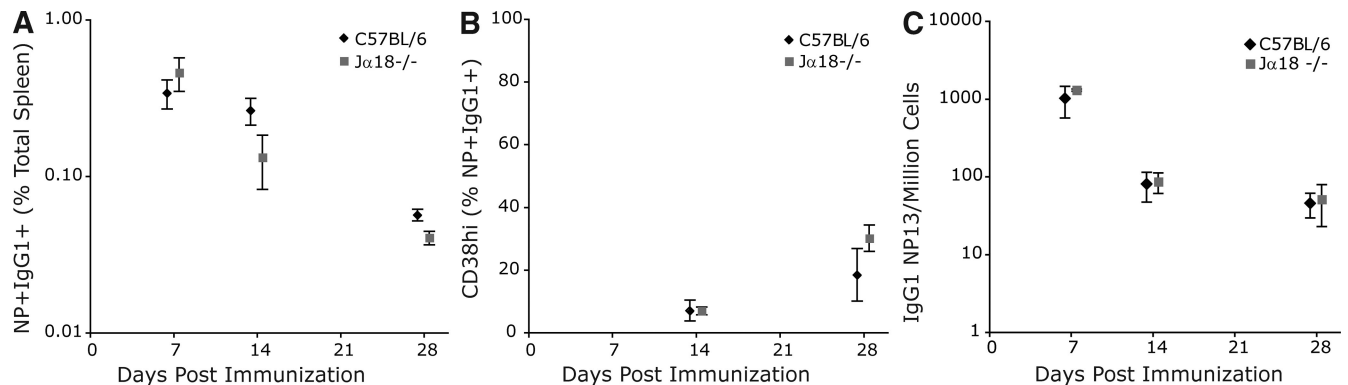
**Figure S2. Loss of IL-21 or IL-21R affects NP<sup>+</sup> B cell localization.** (A) Representative histology staining of spleen sections from C57BL/6 mice (left) and IL-21R<sup>-/-</sup> mice (right), 7 and 14 d after immunization, revealing NP-specific cells (blue) and B cells follicles with IgD (brown). Open arrows indicate GCs, solid arrows show plasma cell foci, and arrowheads show NP<sup>+</sup> cells in B cell follicles (only present in IL-21R<sup>-/-</sup> mice). (B) Time course of distribution of NP-specific B blasts in follicles. Each point is from an individual mouse and is derived from two experiments. (C) Time course of appearance of GC phenotype B cells in the spleens of control and IL-21-deficient mice after immunization. GC B cells were identified as B220<sup>+</sup>CD38<sup>-</sup>. Values are derived from at least two experiments and are the mean of between three and six mice (±SD) at each time. (D) GC staining of spleens from C57BL/6 and IL-21R<sup>-/-</sup> mice 28 d after immunization. GCs were revealed with PNA (blue). F, follicle revealed with B220 (red). Bars, 100 µm.



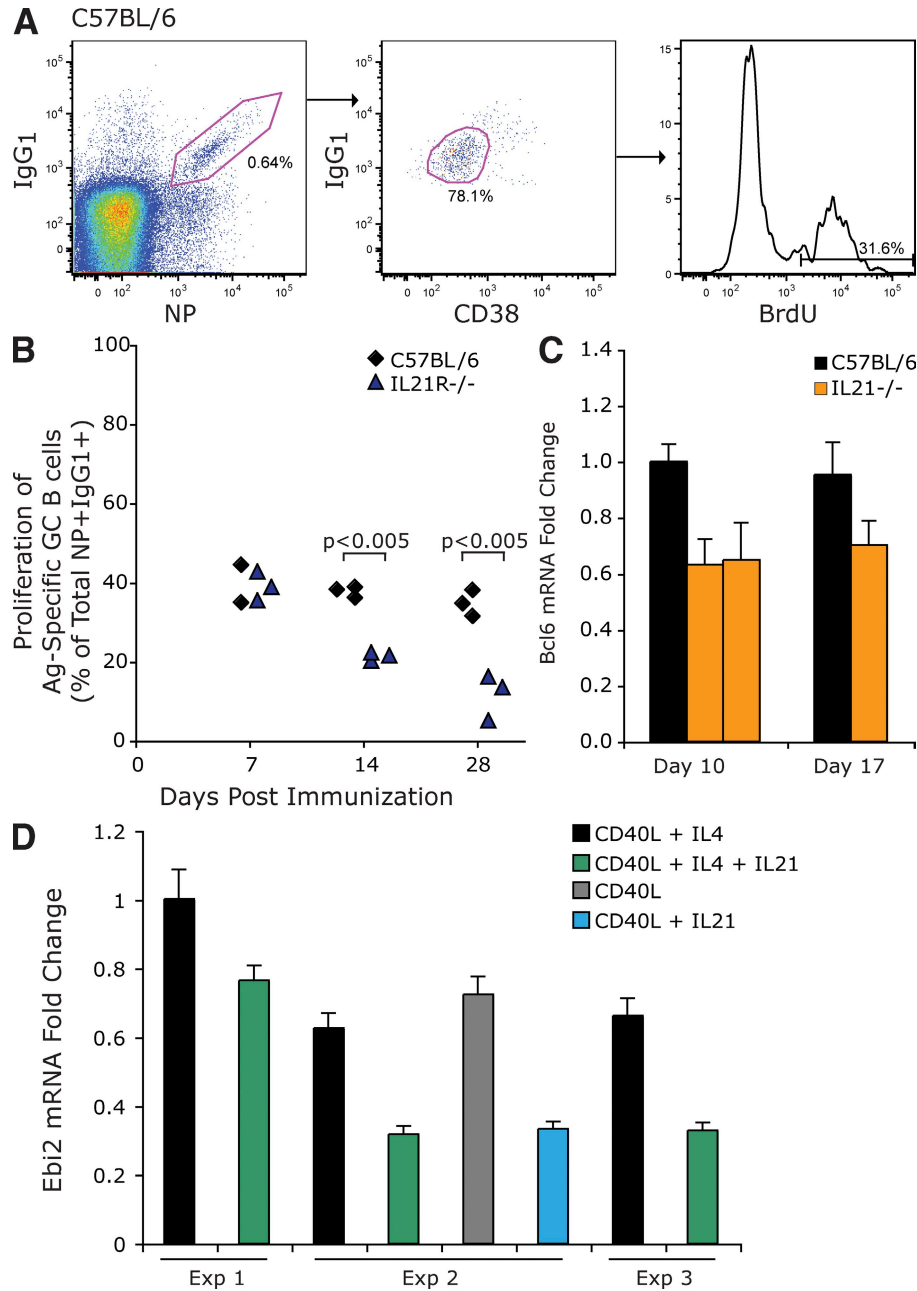
**Figure S3. Memory B cells in IL-21R<sup>-/-</sup> mice are functional.** IL-21R<sup>-/-</sup> and C57BL/6 mice were boosted with NP-KLH in PBS 53 d after primary immunization. The frequency of total (A) and high-affinity (B) NP-specific IgG1 ASC in spleen was measured by ELISpot 53 d after immunization (left) and then 5 d after boosting (right). Although boosting increased the frequency of NP-specific IgG1 ASC in IL-21R<sup>-/-</sup> mice, a lower fraction of these cells were secreting high-affinity antibody compared with controls. Note the lower input cell number in the post-boost high-affinity wells.



**Figure S4. Absence of IL-4 suppresses the immune response without distortion.** (A) IL-4<sup>-/-</sup> and C57BL/6 mice, immunized with NP-KLH in alum, were assessed for the frequency of NP-specific IgG1<sup>+</sup> B cells at the indicated time points. Symbols depict the mean ( $\pm$ SEM) of three mice per group at each time. (B) The proportion of NP-specific IgG1<sup>+</sup> B cells expressing high levels of CD38, and therefore of memory phenotype, was determined at each time point in both IL-4<sup>-/-</sup> and C57BL/6 mice and found not to differ significantly between groups. Error bars indicate  $\pm$ SD.



**Figure S5. Absence of NKT cells fails to replicate the effects of IL-21 deficiency on ASC or GC.** (A) NP+IgG1<sup>+</sup> B cells as a fraction of the spleen of C57BL/6 and TCR J $\alpha$ 18<sup>-/-</sup> mice at the indicated times after immunization. Values are mean  $\pm$  SEM of at least three mice per time point. (B) Proportion of NP-specific IgG1<sup>+</sup> B cells in the memory compartments at the indicated days after immunization for C57BL/6 and TCR J $\alpha$ 18<sup>-/-</sup> mice. Values are mean  $\pm$  SD of at least three mice per time point. (C) Frequency of NP-specific IgG1 ASC in the spleens of C57BL/6 and TCR J $\alpha$ 18<sup>-/-</sup> mice at the indicated times after immunization. Values are mean  $\pm$  SD of at least three mice per time point.



**Figure S6. Loss of IL-21 receptor reduces B cell proliferation in GCs.** Quantifying proliferation among NP-specific GC B cells in control and IL-21R<sup>-/-</sup> mice after immunization using BrdU. At the indicated days after immunization with NP-KLH in alum, mice were injected i.p. with a bolus of BrdU (2.5 mg/mouse in PBS) and sacrificed after a 5-h pulse. (A) GC B cells were identified by flow cytometry using cell surface markers (IgM<sup>-</sup>IgD<sup>-</sup>B220<sup>+</sup>NP<sup>+</sup>IgG1<sup>lo</sup>CD38<sup>lo</sup>) and BrdU incorporation by specific antibody staining after fixation and permeabilization. (B) Plot shows the proportion of NP<sup>+</sup>IgG1<sup>lo</sup> GC B cells that were BrdU<sup>+</sup> at the indicated times after immunization. Values of individual mice are shown, with two to three mice per time point per genotype in one experiment. (C) Amount of Bcl6 mRNA as determined by quantitative PCR in purified GC B cells from control and IL-21-deficient mice immunized 10 or 17 d previously with chicken gamma globulin i.p. in alum. 55,000 to 600,000 GC B cells were sorted and cells resuspended in lysis buffer and RNA was purified using RNeasy Micro kit according to the manufacturer's protocol (QIAGEN). Data are derived from at least two mice per time point per genotype from two experiments. (D) Effect of IL-21 on Ebi2 expression in CD40L-stimulated B cells. One million splenic B cells from C57BL/6 mice were stimulated overnight with a mitogenic dose of CD40L with or without IL-4, followed by the addition of IL-21 at 100 ng/ml final concentration. After a further 24-h culture, B cells were recovered and RNA purified using RNeasy Mini kit according to the manufacturer's protocol (QIAGEN). For both C and D, Superscript II (Invitrogen) and Oligo dT Primers (Bioline) were used to reverse transcribe cDNA from RNA. Gene expression was determined using the ABI Prism 7900 Sequence Detection System (Applied Biosystems) along with SYBR Green/Rox PCR master mix (Super Array Bioscience) and Bcl6- or Ebi2-specific primers (QIAGEN). Expression is normalized to the housekeeping gene HMBS in C and to HPRT in D. Data are derived from at least two mice per time point per genotype from two experiments in C and are representative of three independent experiments in D. Error bars indicate  $\pm$ SD.