Figure S1

Administration of αGalCer did not accelerate anti-Gal Ab and anti-NeuGc Ab production in *GalT*$^{-/-}$ and *CMAH*$^{-/-}$ mice, respectively. To determine whether iNKT cells enhance Ab production against Gal and NeuGc Ags, we immunized *GalT*$^{-/-}$ and *CMAH*$^{-/-}$ mice with specific Ags together with the administration of αGalCer (4 μg/mouse) or PBS as a control. (A-B) To elicit anti-Gal Ab production, *GalT*$^{-/-}$ mice were immunized with Gal-bearing thymocytes obtained from F344 rats together with or without αGalCer. After the immunization, blood samples were obtained and subjected to ELISA for the detection of anti-Gal IgM and IgG. The average values ± SEM for the individual groups are shown. There were 4 animals in each group. (C-D) To elicit anti-NeuGc Ab production, CMAH$^{-/-}$ mice were immunized with NeuGc-expressing thymocytes obtained from F344 rats with or without αGalCer. After the immunization, blood samples were obtained and subjected to FCM for the detection of anti-NeuGc IgM and IgG. MFI values were used to follow Ab levels. The average values ± SEM for the individual groups are shown. There were 4 animals in each group.

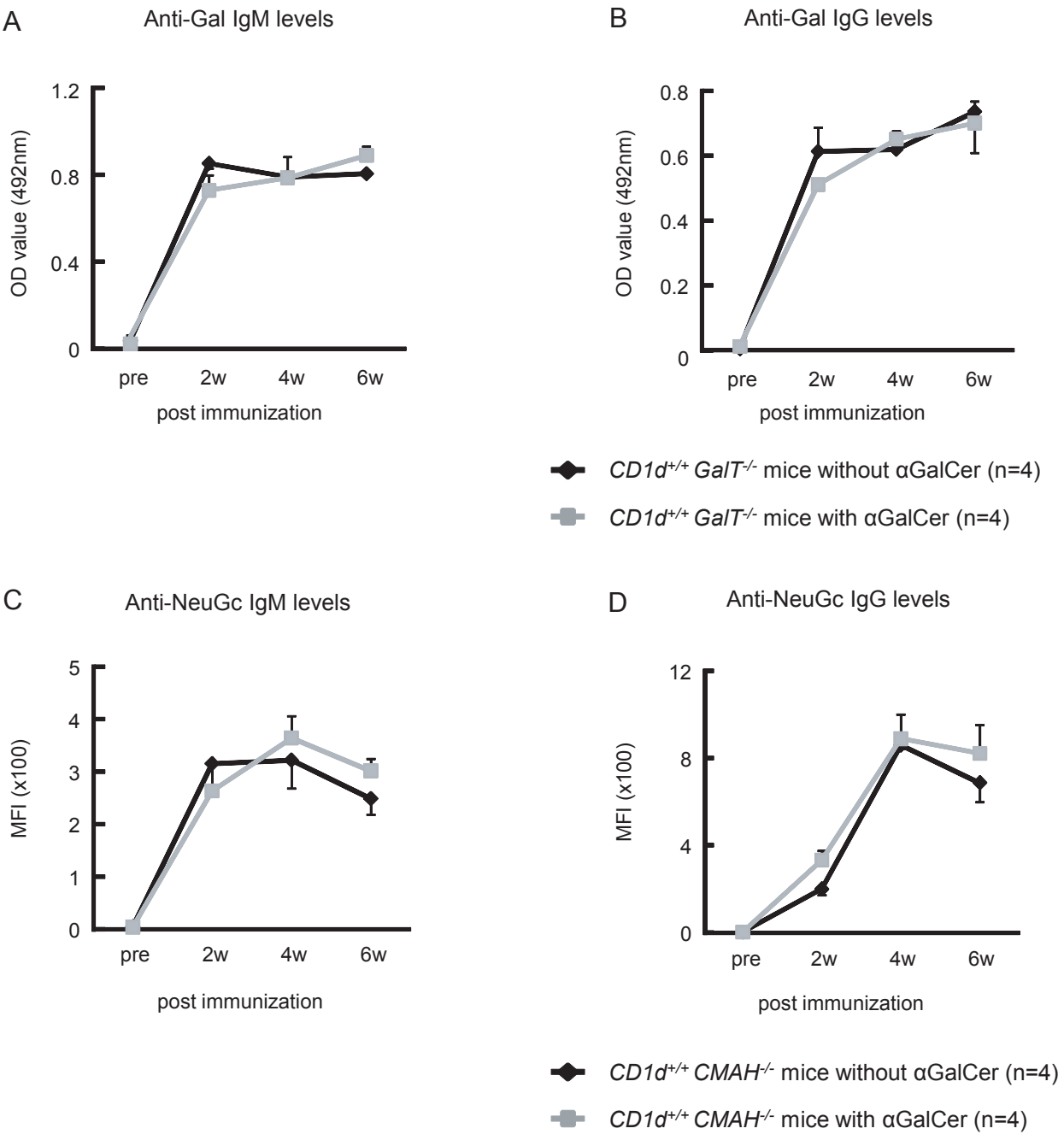

Figure S2

Ab production against allopeptide determinants was independent of iNKT cells. To elicit anti-allopeptide Ab production, Balb/c *CD1d*$^{-/-}$ mice (n = 6) and Balb/c WT (*CD1d*$^{+/+}$) mice (n = 6) were immunized 2 times with a 1-week interval, with thymocytes obtained from B6 mice (20 × 10^6 cells/mouse at each immunization). Blood samples were obtained at 3 and 4 weeks after the last immunization to detect anti-allopeptide IgM and IgG subclasses (IgG1, IgG2, and IgG3) (**A**, **B**, **C,** and **D**, respectively). Anti-allopeptide Abs were detected by indirect immunofluorescence staining of B6 wild-type (WT) mice thymocytes using FCM. A total of 10^6 thymocytes were incubated with 100 µL of serially diluted Balb/c mouse serum, washed, and then incubated with biotin-conjugated rat anti-mouse IgM, IgG1 (A85-1), IgG2 (R2-40), or IgG3 (R40-82) mAbs (BD PharMingen). The biotinylated mAbs were visualized using allophycocyanin-streptavidin. MFI values were used to follow Ab levels. The average values ± SEM for the individual groups are shown.

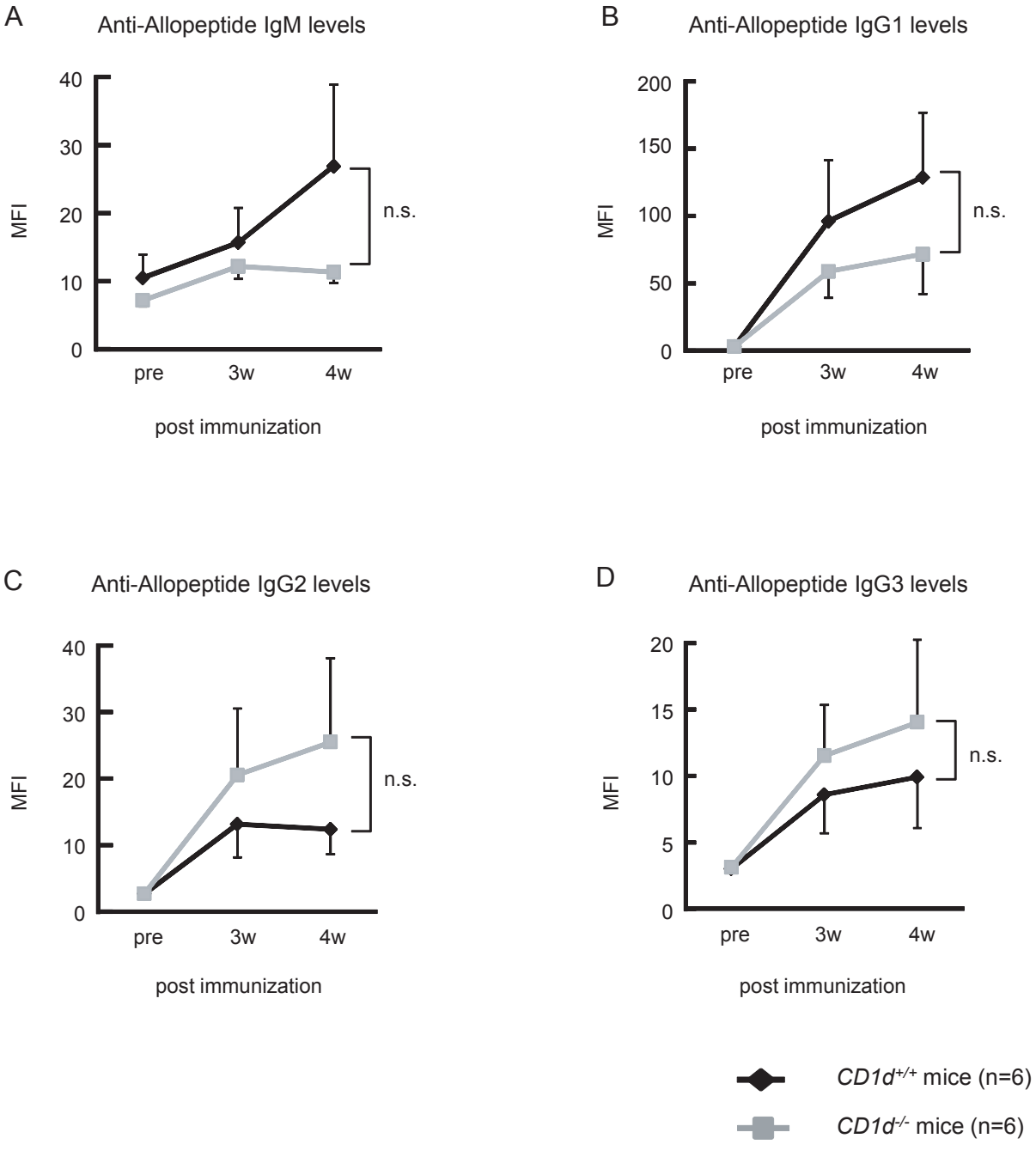

Figure S3

***CD1d*$^{-/-}$ mice display a slightly reduced proportion of B-1a cells.** B cell subclasses in various anatomical sites were classified by four-color FCM analysis. Peritoneal cavity (PerC), liver and spleen (Spl) mononuclear cells were stained with biotin-conjugated anti-mouse IgM (R6-60.2: BD PharMingen), fluorescein isothiocyanate (FITC)-conjugated anti-mouse CD11b (M1/70: BD PharMingen), and phycoerythrin (PE)-conjugated anti-mouse CD5 (53-7.3: BD PharMingen) mAb. The biotinylated mAb was visualized using allophycocyanin-streptavidin. Non-specific Fcγ receptor binding of labeled Abs was blocked by anti-mouse CD16/32 (2.4G2: BD PharMingen). An isotype-matched irrelevant mAb was used as a control. Dead cells were excluded from the analysis based on light scatter and staining with propidium iodide. B cell subclasses in the PerC, liver and Spl of untreated Balb/c *CD1d*$^{-/-}$ and WT *CD1d*$^{+/+}$ mice (n = 4 in each group) were analyzed. (A) Representative FCM results of B cell subclass analyses in the PerC, liver and Spl. We analyzed 50,000 cells per contour plot. B-1 cells were identified as IgM^{+}CD11b^{+} cells, while B-2 cells were identified as IgM^{+}CD11b^{-} cells. (B) IgM^{+}CD11b^{+} B cells were selected by gating and analyzed for the expression of CD5 to distinguish B-1a and B-1b cells. Thin lines represent negative control staining with isotype-matched Ab. Representative histograms were shown. (C) The average values ± SEM of the proportion of each B cell subclass (IgM^{+}CD11b^{+}CD5^{+} B-1a cells, IgM^{+}CD11b^{+}CD5^{-} B-1b cells, IgM^{+}CD11b^{-}CD5^{-} B-2 cells) among the total lymphocytes in the PerC, liver and Spl of *CD1d*$^{-/-}$ mice and WT *CD1d*$^{+/+}$ mice are shown. (D) The average values ± SEM of the absolute number of each B cell subclass isolated from the PerC, liver and Spl of *CD1d*$^{-/-}$ mice and WT *CD1d*$^{+/+}$ mice are shown. **P* < 0.05, ***P* < 0.01 compared to the data from *CD1d*$^{-/-}$ mice with those from *CD1d*$^{+/+}$ mice.