SUPPLEMENTAL MATERIAL

Duong et al., http://www.jem.org/cgi/content/full/jem.20091873/DC1

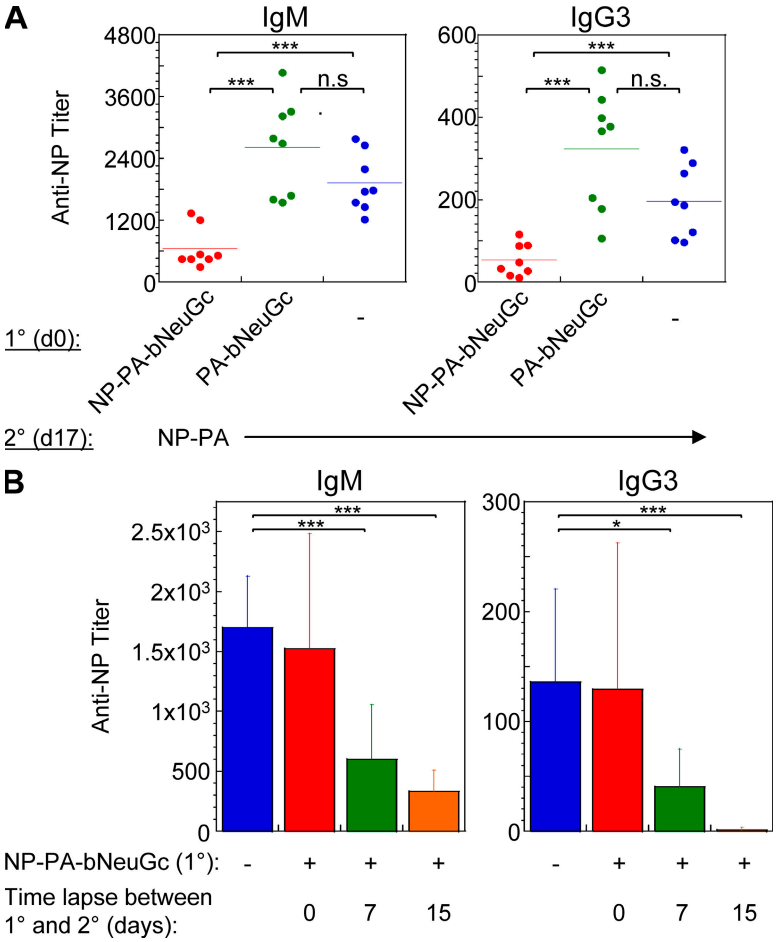

Figure S1. Antigen specificity and time course of tolerance induction. (A) Specificity analysis of tolerance induction. Mice were challenged as indicated with NP–PA–bNeuGc or PA–bNeuGc, or were untreated on day 0; all mice were then rechallenged on day 17 with the immunogenic compound NP–PA and tested for anti-NP serum antibodies on day 24. Horizontal bars represent means. (B) Tempo of tolerance induction. Mice tolerized with NP–PA–bNeuGc were rechallenged on days 0, 7, or 15 with NP–PA, and sera taken 7 d after rechallenge were assessed by ELISA for anti-NP IgM and IgG3 responses. For all experimental groups, eight mice were analyzed. Shown are means + SD. Antigen-specific tolerance was also observed in three other experiments with mice receiving NP-specific B cells from QM mice, including Fig. 6. Results in B were representative of at least three experiments. *, $P < 0.05$; and ***, $P < 0.005$ using the two-tailed Student's *t* test. n.s., not significant.

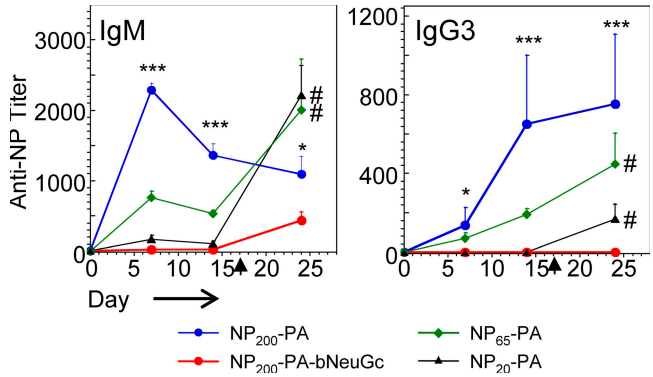

Figure S2. Effects of altering NP hapten density on responses to unsialylated compounds. B6 mice (n = 4/group) were challenged on day 0 with NP–PA conjugates containing densities of 200, 65, or 20 as indicated, or to NP–PA–bNeuGc with a density of 200 NP and 400 bNeuGc. Mice were then rechallenged on day 17 with NP–PA with a density of 200 NP (NP_{200}–PA). Shown are means + SD. Results are from a single experiment with four mice/group. *, P < 0.05; and ***, P < 0.005 using the two-tailed Student's t test. #, the response to the second challenge was not significantly different from the day 7 response to NP_{200}–PA.

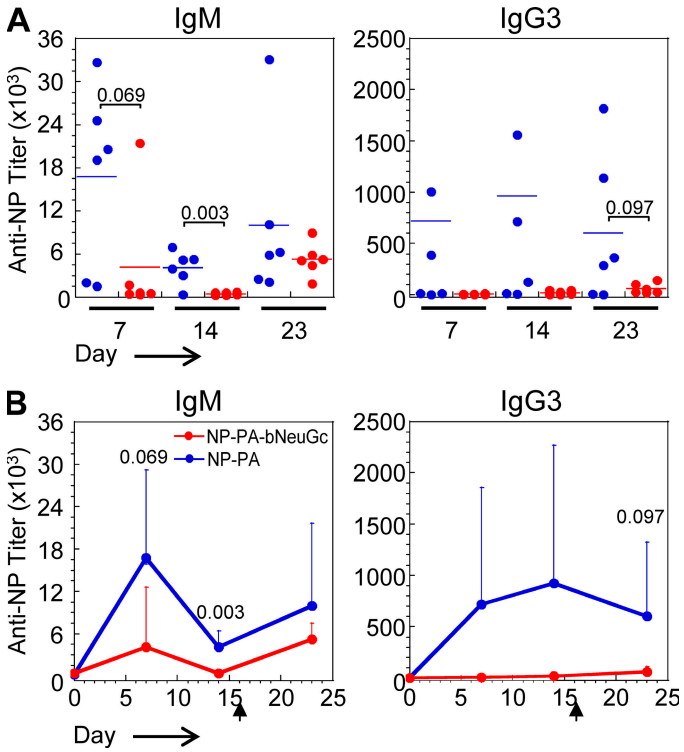

Figure S3. Analysis of *CD22*$^{-/-}$*;Siglecg*$^{-/-}$ mice for tolerance induction by NP–PA–bNeuGc and responses to NP–PA. (A and B) Serum IgM and IgG3 anti-NP responses of mice (n = 6/group) that were primed on day 0 with either NP–PA–bNeuGc (red) or NP–PA (blue) and boosted on day 16 with NP–PA (arrows). This experiment has been performed twice with similar results. (A) Individual data points indicate the serum titer of an individual mouse on a particular day after immunization. Horizontal bars represent means. (B) Data from A with SDs and p-values.

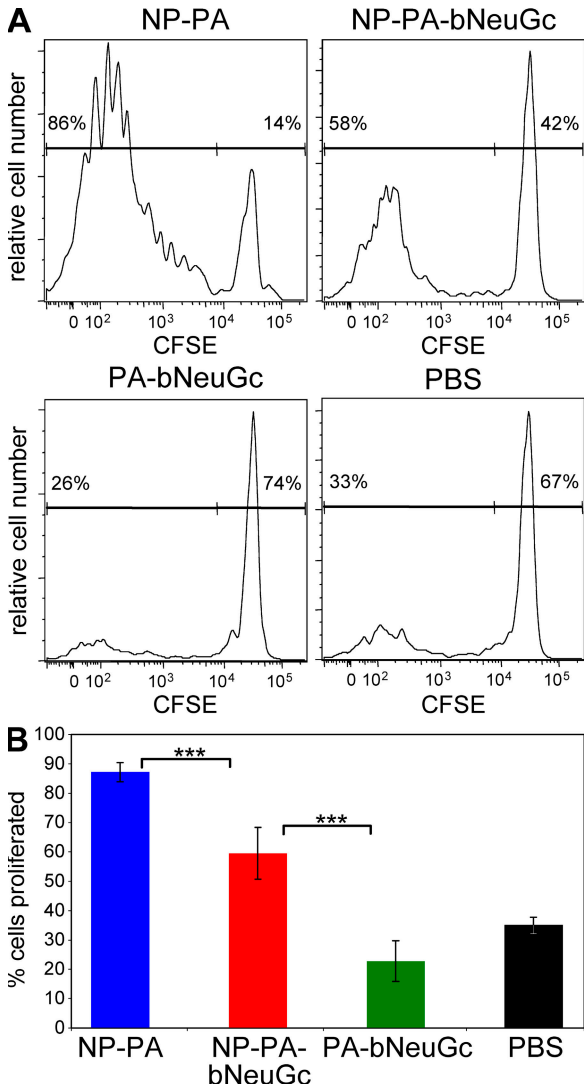

Figure S4. Analysis of QM B cell proliferation in vivo in cells labeled with CFSE. 10^7 NP-specific B cells/mouse isolated from QM transgenic mice were transferred to B6.Ly5a mice and immunized with NP–PA, NP–PA–bNeuGc, PA–bNeuGc, or PBS (n = 5, 4, 5, and 2 mice/group, respectively). CFSE fluorescence was measured in $Ly5b^+$ $B220^+$ gated B cells at day 7 after challenge. (A) Flow cytometry profiles of individual mice are shown. An analysis gate divides cells that have failed to undergo proliferation in vivo (right peaks) from those that underwent cell division. (B) Summary data from analyses as in A. Shown are means + SD. Data are representative of two experiments. ***, $P < 0.005$.

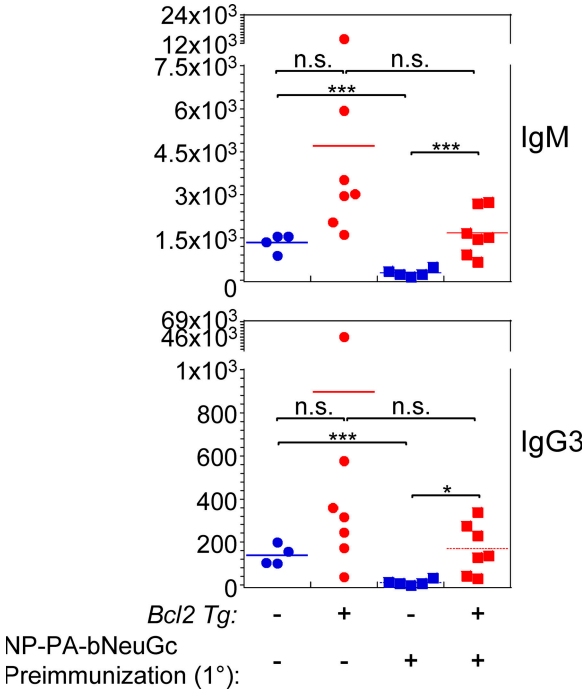

Figure S5. Alternative display of the data shown in Fig. 7 with a common scale and statistical comparisons. In vivo analysis of the effect of B cell–enforced Bcl2 expression on tolerance induction with NP–PA–bNeuGc. *Bcl2 Tg* or nontransgenic littermates were challenged with NP–PA–bNeuGc on day 0 followed by rechallenge with NP–PA on day 17. Shown are IgM and IgG3 antibody titers of sera obtained at day 7 after primary or secondary challenge. Note that one *Bcl2 Tg* mouse had an extremely high titer that is given on a different scale than the other data points. Horizontal bars represent means. *, $P < 0.05$; and ***, $P < 0.005$ using the two-tailed Student's *t* test. n.s., not significant.