

SUPPLEMENTAL MATERIAL

Swanson et al., <http://www.jem.org/cgi/content/full/jem.20092695/DC1>

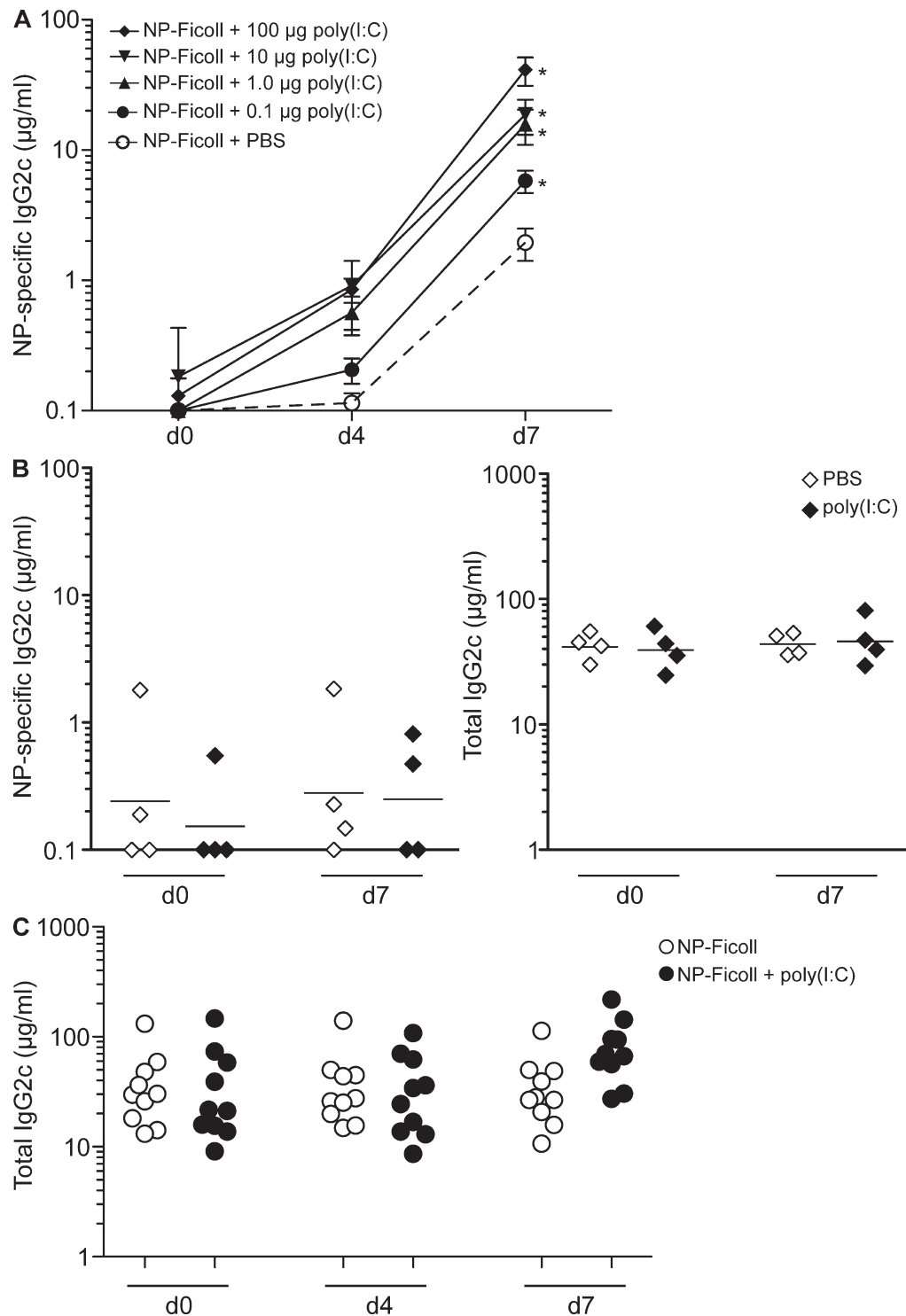


Figure S1. Poly(I:C) enhancement of the IgG2c antibody response to NP-Ficoll is antigen specific and dose dependent. (A) Wild-type C57BL/6 mice were immunized i.p. with 5 μg NP-Ficoll alone or 5 μg NP-Ficoll + poly(I:C) at the indicated doses. Serum NP-specific IgG2c antibodies were measured preimmune (day 0) and at days 4 and 7 after immunization. Data are represented as the geometric mean \pm SEM. Results were combined from two independent experiments with four to six mice per group. *, $P < 0.005$. (B) Wild-type C57BL/6 mice were immunized i.p. with PBS or 100 μg poly(I:C). Both serum NP-specific IgG2c and total antigen nonspecific IgG2c were measured preimmune (day 0) and 7 d after immunization. Data are from one experiment with four mice per group. Horizontal bars represent geometric means. (C) Wild-type C57BL/6 mice were immunized i.p. with 5 μg NP-Ficoll alone or 5 μg NP-Ficoll + 100 μg poly(I:C). Total serum IgG2c antibodies were measured preimmune (day 0) and at days 4 and 7 after immunization. Symbols represent individual mice. Data were combined from two independent experiments with five mice per group per experiment.

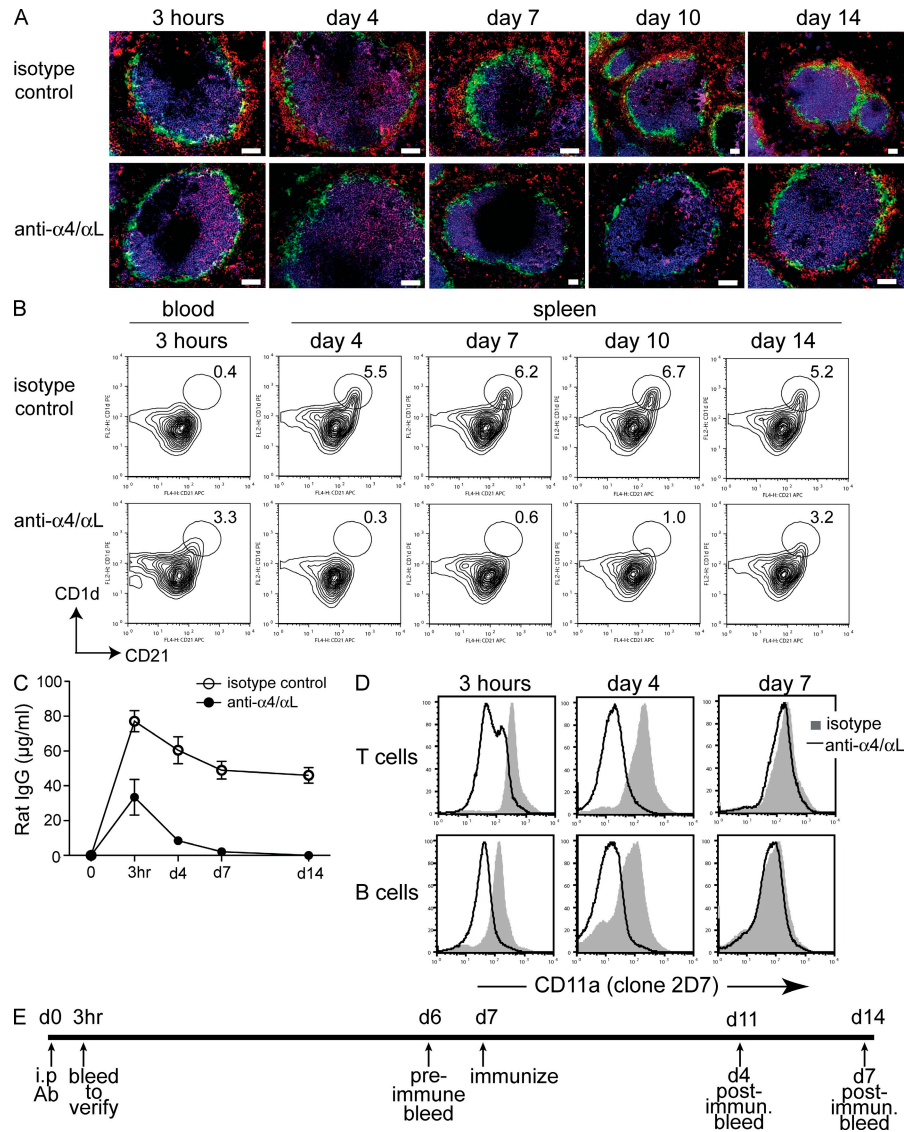


Figure S2. Injection of anti-α4/αL antibodies causes a rapid, but transient, depletion of MZ B cells from the spleen. (A–E) To characterize the kinetics and duration of MZ B cell depletion from the spleen with anti-α4/αL antibodies, wild-type C57BL/6 mice were injected i.p. with 100 μg each of anti-α4 + anti-αL antibodies or rat IgG isotype control antibodies. Samples were subsequently collected and analyzed as described at the indicated time points. (A) Immunofluorescence histology of mouse spleens frozen at the indicated times after injection of anti-α4/αL (bottom row) or isotype control (top row) antibodies. Sections were stained for MOMA-1 (green), IgM (red), and IgD (blue). MZ B cells (IgM^{high} IgD^{low}) are visualized under normal conditions as an IgM^{bright} (red) ring of cells located at the interface of the red and white pulp and situated adjacent to the MOMA-1⁺ (green) macrophages. B cell follicles comprised by FO B cells (IgM^{low} IgD^{high}) are within the white pulp and visualized as IgD^{bright} (blue) cells. Data are from a single experiment with 10 mice total analyzed and one mouse per treatment for each time point. (B) Flow cytometric plots of B lymphocytes from peripheral blood or spleens isolated at the indicated times after injection of anti-α4/αL or isotype control antibodies. The presence of MZ B cells was evaluated by phenotypic discrimination as a CD21^{high} CD1d^{high} population (shown gated) or similarly as a CD21^{high} CD23^{low} population (not depicted). Numbers indicate the percentage of MZ B cells comprising the total B cell population (gated on B220⁺ cells). Data are from a single experiment, 10 mice total analyzed with one mouse per treatment for each time point. (C) Serum clearance of rat IgG antibodies measured by ELISA at the indicated times after injection of anti-α4/αL or isotype control antibodies. Data are expressed as arithmetic mean ± SEM. Data are from one experiment with four mice total per treatment group. (D) Surface integrin αL (CD11a) expression analyzed by flow cytometry on splenic T cells (gated on TCR-β⁺ cells) and B cells (gated on B220⁺ cells) after injection of anti-α4/αL or isotype control antibodies. CD11a surface expression was evaluated with an anti-αL antibody (clone 2D7) that recognizes a different epitope than the anti-αL antibody (clone: M17/4) used for MZ B cell depletion injections. Data are from a single experiment with six mice total analyzed and one mouse per treatment for each time point. (E) Time line of MZ B cell depletion, immunization, and bleeds for data presented in Fig. 3 D.