SUPPLEMENTARY MATERIAL

Co-presentation of antigen and ligands of Siglec-G induces B cell tolerance independent of CD22

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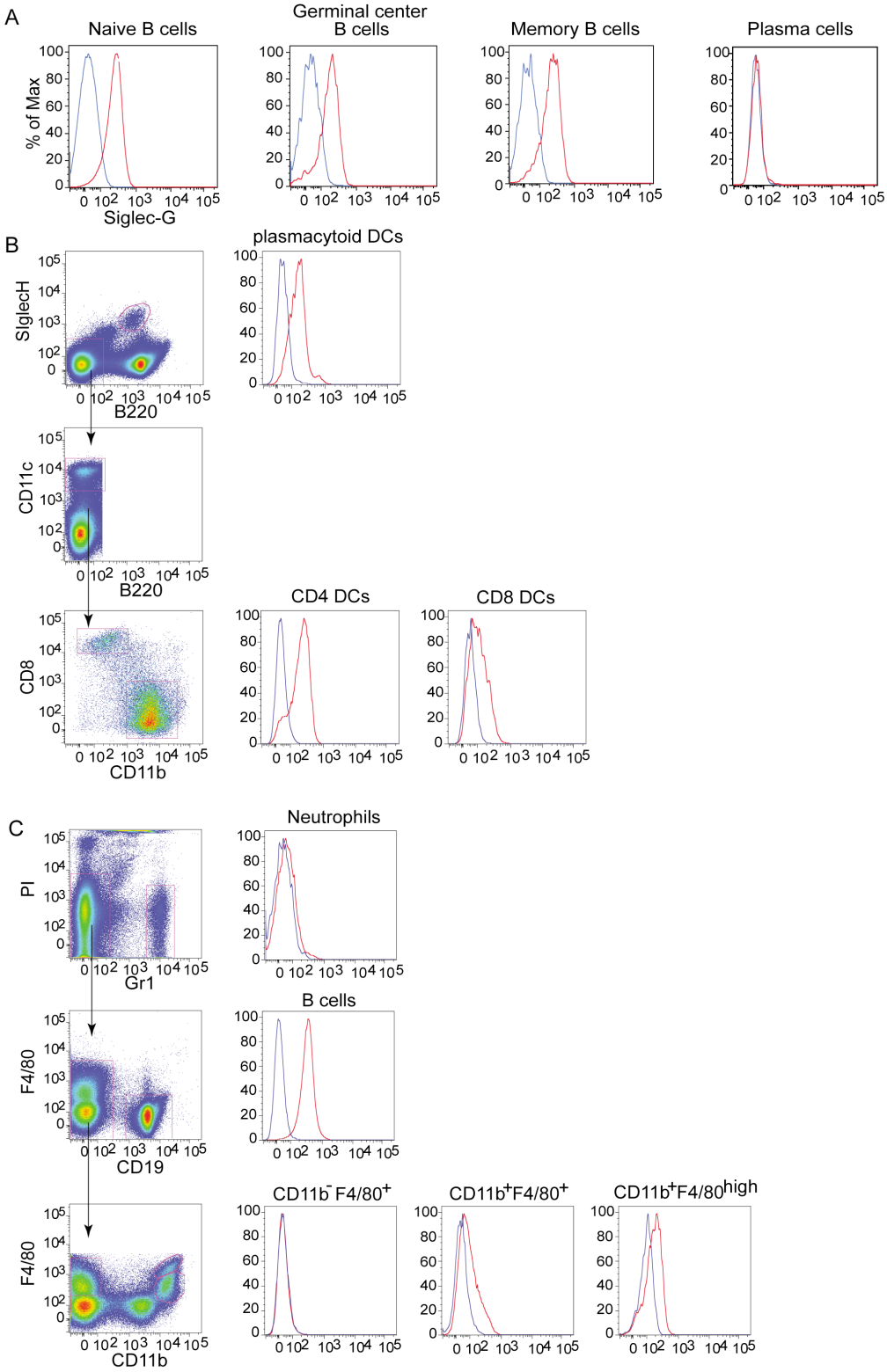

Figure SI1. Analysis of Siglec-G expression on activated B cell subsets, in dendritic cell subsets, and in different cell types in the liver. *A*, Siglec-G is expressed on naive B cells, germinal center B cells (gated for $CD19^+B220^+IgM^-IgD^-GL7^+CD38^-$), memory B cells (gated for $CD19^+B220^+IgM^-IgD^-CD80^+$), and plasma cells (gated for $B220^-IgM^-IgD^-Ter119^-Gr1^-CD11b^-NK1.1^-CD3^-CD138^+$). Cells were isolated from the spleen, stained with the appropriate antibodies for gating along with Alexa 647-conjugated anti-Siglec-G antibody, and analysed by FACS. *B*, Siglec-G is expressed on plasmacytoid DCs, $CD11b^+$ DCs and $CD8^+$ DCs with the highest amounts on $CD11b^+$ DCs. *C*, In the liver, Siglec-G is expressed on B cells and in low amounts on macrophages.

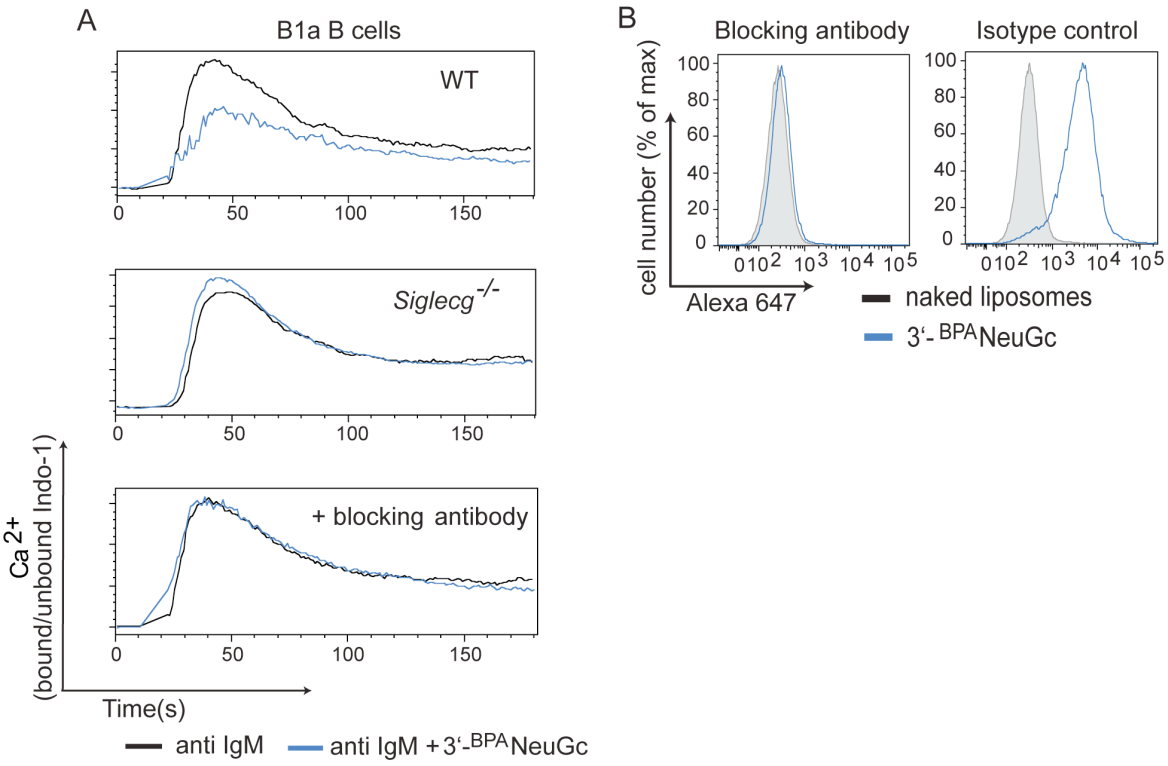

Figure SI2. The Siglec-G antibody blocks the ligand binding site of Siglec-G. *A*, The inhibition of Ca^{2+}-flux by liposomes displaying anti-IgM + 3'-^{BPA}NeuGc in B1a cells from the peritoneal cavity is abrogated in B1a cells from *Siglecg*$^{-/-}$ mice and B1a cells pretreated with unlabeled Siglec-G antibody. B1a cells from the peritoneal cavity ($B220^{low}CD5^{+}$) were preincubated with the Siglec-G antibody (15 min), stimulated after 10 seconds with liposomes displaying anti-IgM (black line) or anti-IgM + 3'-^{BPA}NeuGc (blue line), and the intracellular Ca^{2+}-mobilization was measured by FACS. *B*, The anti-Siglec-G antibody blocks binding of fluorescent liposomes displaying 3'-^{BPA}NeuGc. Splenic B cells were incubated with unlabeled Siglec-G antibody for 15 min, further incubated with Alexa 647-labeled naked liposomes (black line) or 3'-^{BPA}NeuGc liposomes (blue line), washed, and analyzed by FACS.

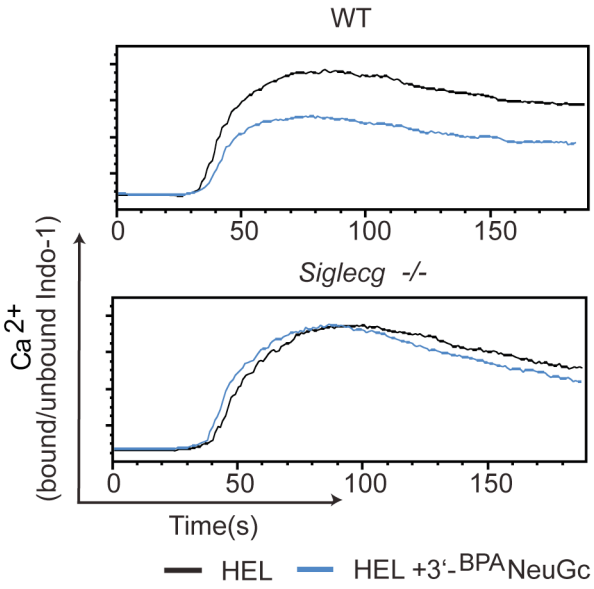

Figure SI3. STALs displaying Siglec-G ligand inhibit intracellular Ca^{2+}-mobilization in HEL-specific B cells. Ca^{2+}-flux in purified IgM^{HEL} B cells was inhibited after stimulation with liposomes displaying HEL + 3'-^{BPA}NeuGc compared to liposomes displaying HEL alone. Inhibition was Siglec-G dependent since no inhibition was observed in Siglec-deficient B cells.

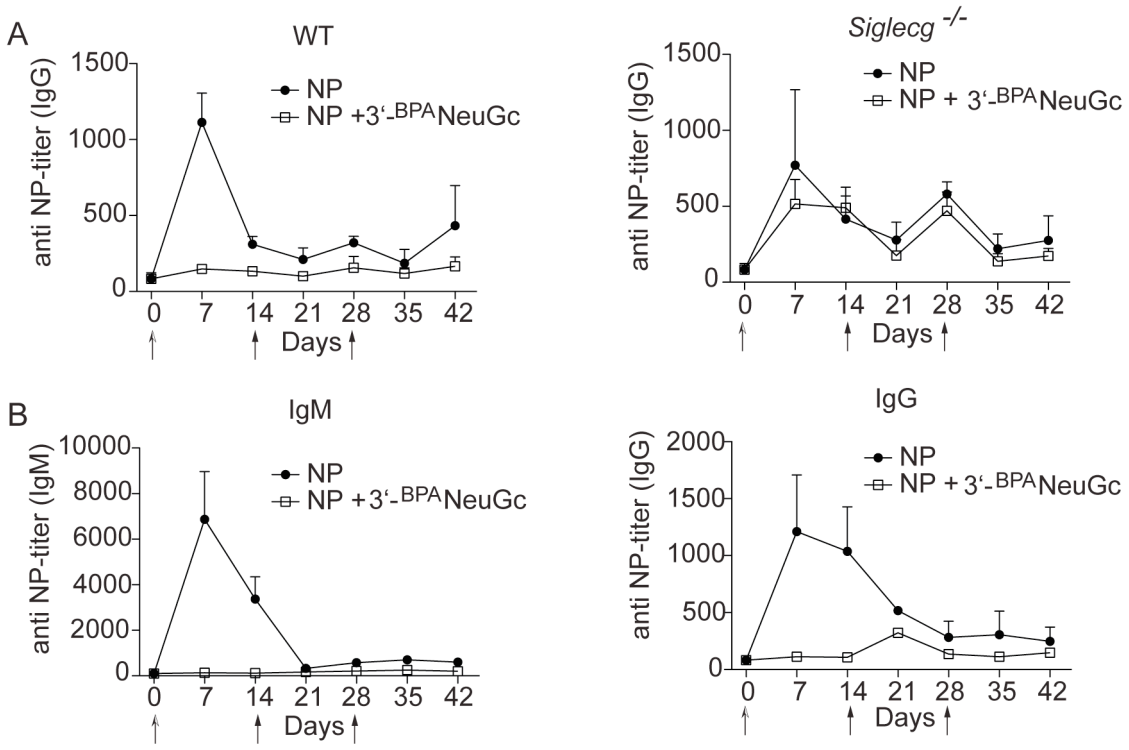

Figure SI4. Liposomes displaying NP and 3'-^{BPA}NeuGc induce tolerance to NP. *A*, Production of IgG in *WT* and *Siglecg*$^{-/-}$ mice injected intraperitoneally with the indicated liposomes (n=4). *B*, Production of IgM and IgG in mice injected intravenously with the indicated liposomes (n=4). Mice were injected at day 0 with antigenic liposomes displaying either antigen alone or antigen and Siglec-G ligand. On day 14 and 28 all mice received liposomes with antigen alone. Antibody titers were assessed by ELISA. Data represents mean +/- s.e.m.