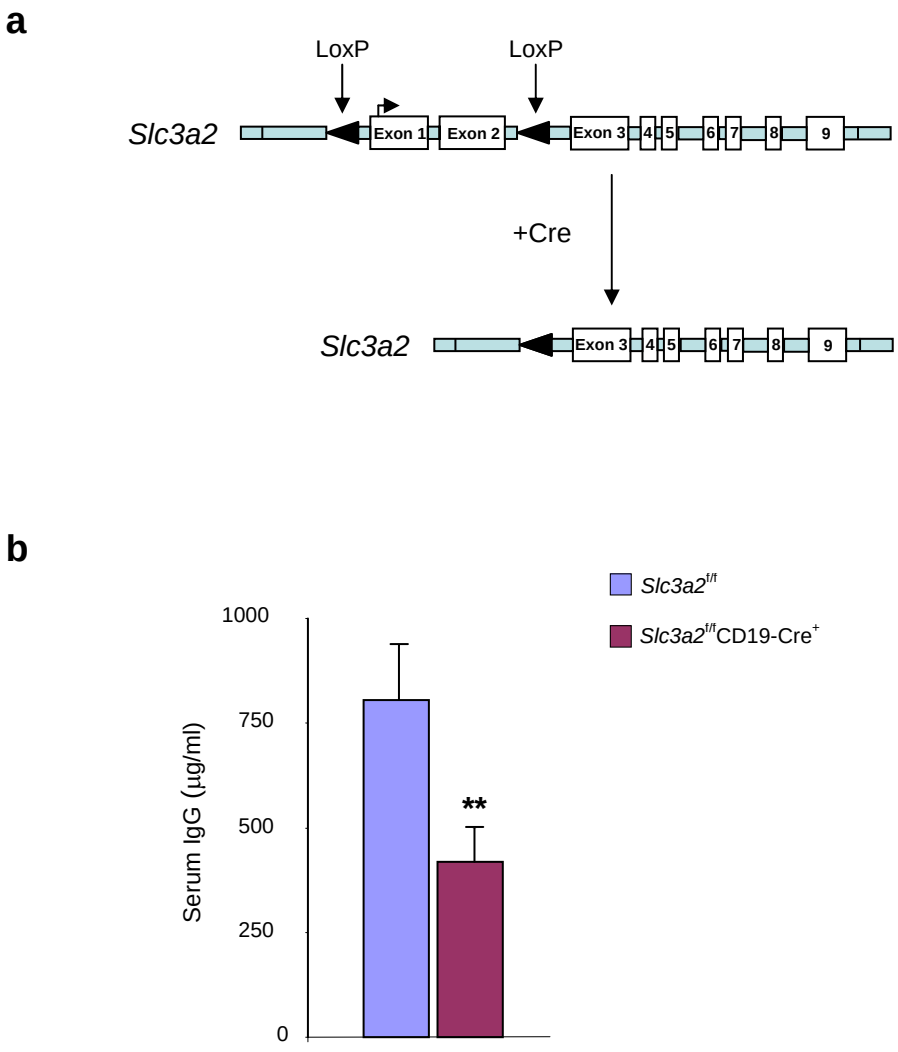
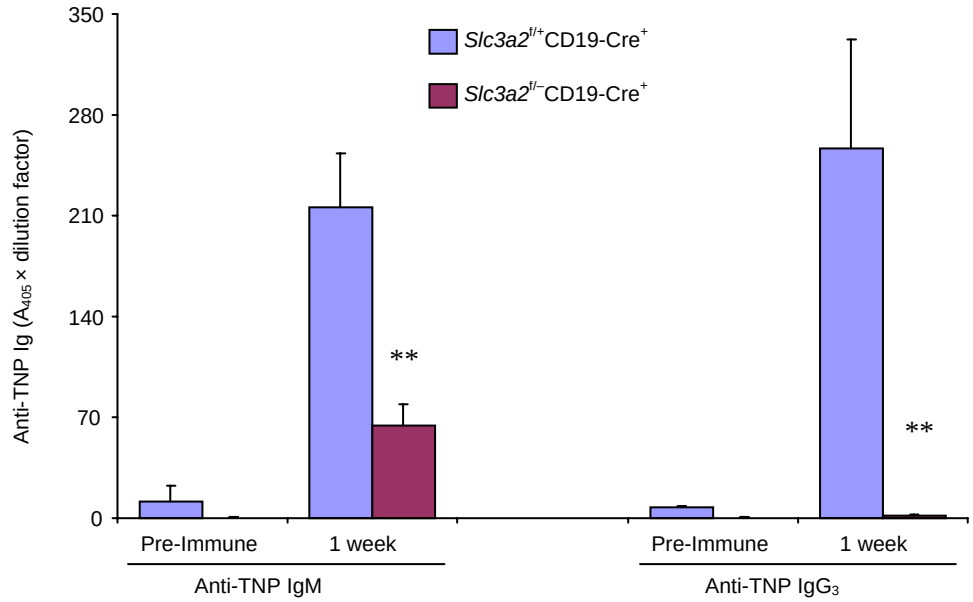
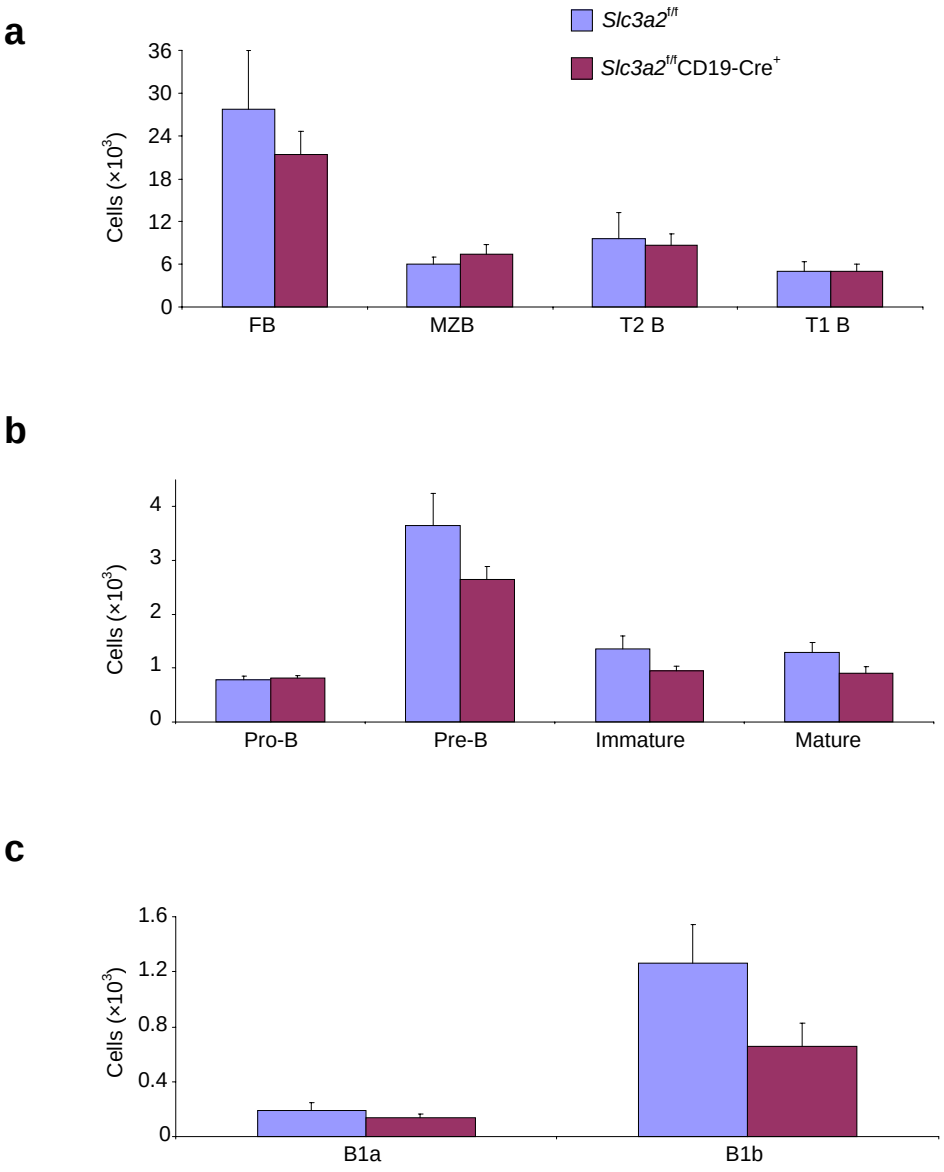
Supplementary Data:



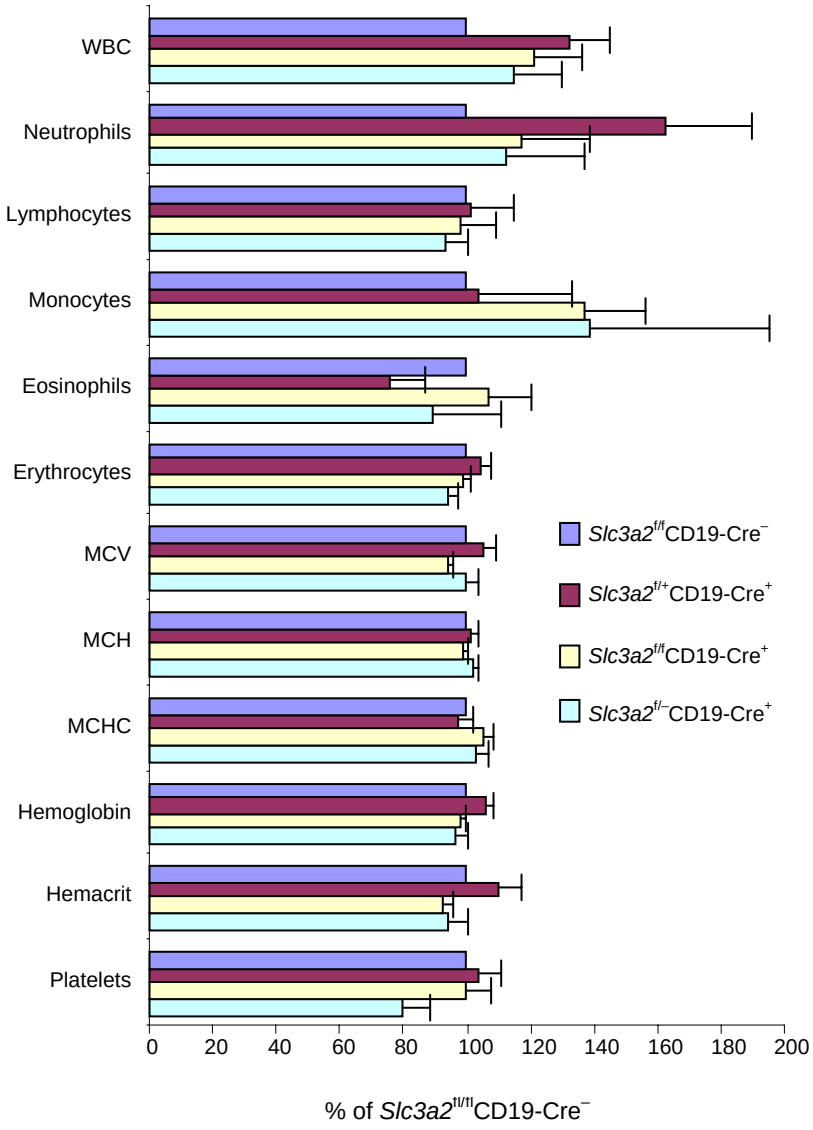
Supplementary Figure 1. Conditional genetic targeting of Slc3a2 and Circulating IgG in *Slc3a2*$^{f/f}$CD19-Cre^{+} mice. **(a)** Conditional genetic targeting of Slc3a2. Using homologous recombination in ES cells, a targeting vector encoding portion of the *Slc3a2* gene locus containing the start codon and loxP sites flanking exons 1 and 2 replaced the endogenous gene segment. Exposure to Cre recombinase excised the start codon and exons 1 and 2, which encode the cytoplasmic and transmembrane portions of CD98hc, resulting in loss of CD98hc expression. **(b)** Circulating IgG in *Slc3a2*$^{f/f}$CD19-Cre^{+} mice. Naïve adult (8-12 wk-old) *Slc3a2*$^{f/f}$CD19-Cre^{+} and littermate control mice were bled and serum analyzed by sandwich ELISA for total Ig. Error bars represent s.e.m. from 30 mice per group (**P = 0.017)**.**



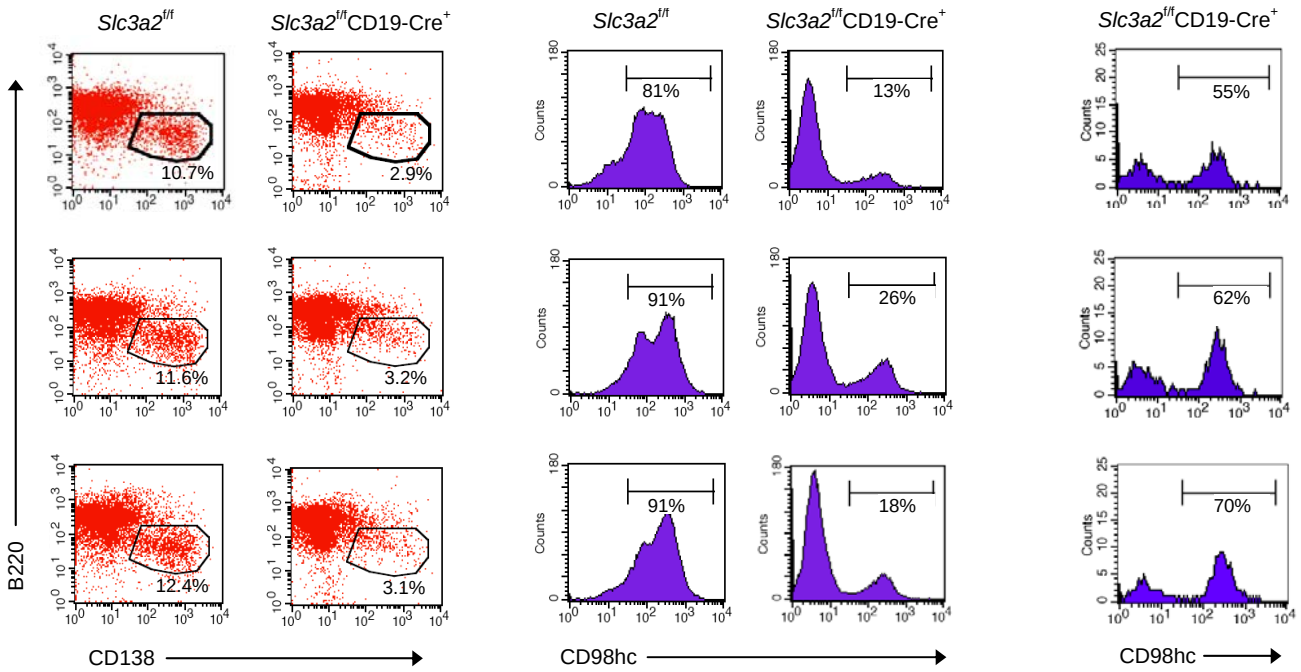
Supplementary Figure 2. Humoral responses in *Slc3a2*$^{f/+}$CD19-Cre^{+} vs. *Slc3a2*$^{f/-}$CD19-Cre^{+} mice. **(a)** Antibody response. To control for potential effects of one versus two endogenous *Cd19* alleles, and for Cre-mediated toxicity, adult (8-12 wk old) *Slc3a2*$^{f/+}$CD19-Cre^{+} and *Slc3a2*$^{f/-}$CD19-Cre^{+} littermate mice were immunized with 50 μg of a T cell-independent antigen, TNP-LPS, in PBS. Mice were bled before immunization (pre-immune) and at one wk after immunization to obtain serum, which was analyzed for anti-TNP IgM or anti-TNP IgG3 by direct ELISA. Error bars represent s.e.m. from 3 mice for each group (**P = 0.009). This experiment was performed once.



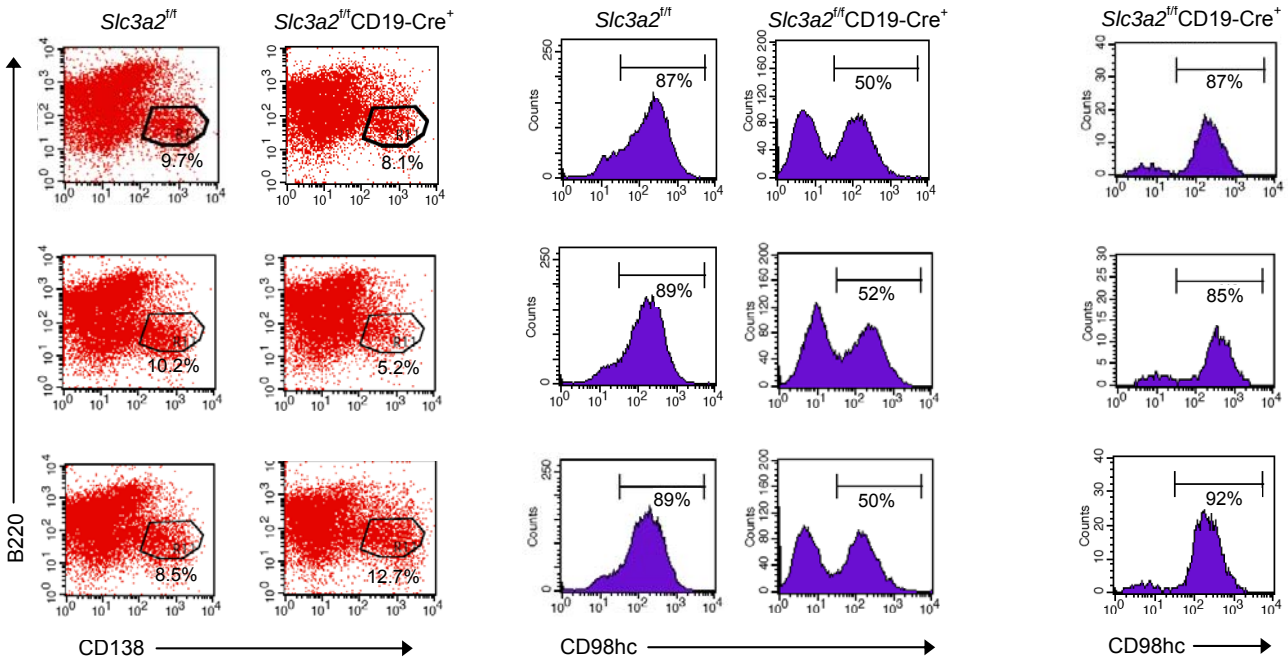
Supplementary Figure 3. Absolute numbers of B cells in various subsets in *Slc3a2*$^{f/f}$CD19-Cre^{+} mice. Indicated subsets in the spleen (**a**), BM (**b**), and peritoneal lavage (**c**) from adult (8-12 wk old) *Slc3a2*$^{f/f}$CD19-Cre^{+} or control mice were analyzed by flow cytometry. Frequency and total tissue cell number were used to derive total numbers for each subset. Error bars show s.e.m. from 4 mice per group.; experiment was repeated. Small differences in means for each subset are insignificant ($P > 0.05$). FB, follicular B; MZB, marginal zone B; T2, Transitional 2 B; T1, Transitional 1 B.



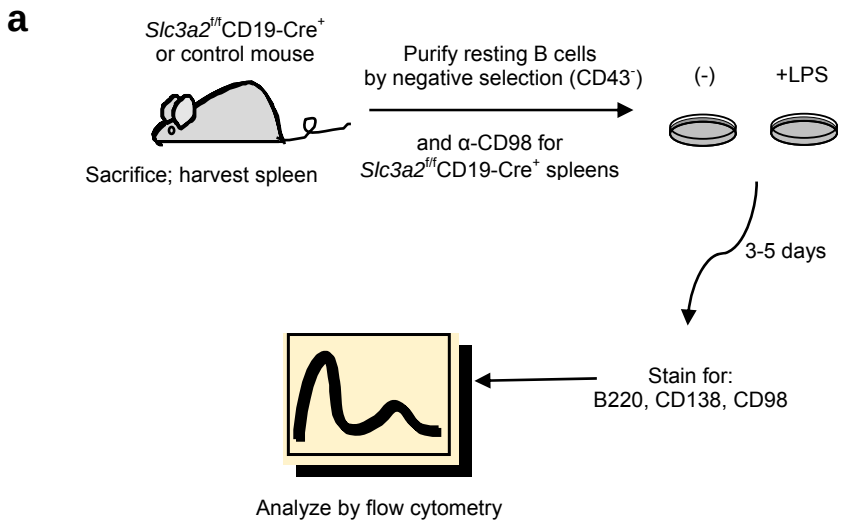
Supplementary Figure 4. Blood analysis of *Slc3a2*$^{f/f}$CD19-Cre^{+} mice. Adult (8-16 wk old) mice of the indicated genotypes were bled and analyzed for a variety of white blood cell populations as well as standard blood parameters such as hematocrit, hemoglobin, and platelets. No statistically significant differences were apparent with *n*=5-6 mice per group.

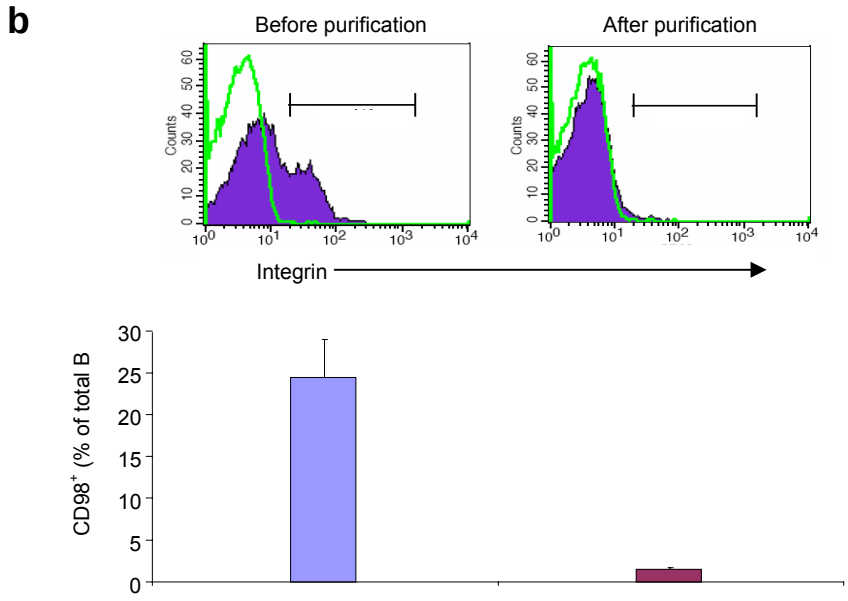


Supplementary Figure 5. Differentiation to plasma cells *in vitro* using CD98hc-depletion. Resting splenic B cells (CD43^{-}CD98hc^{-}) were purified using CD98hc depletion from *Slc3a2*$^{f/f}$CD19-Cre^{+} or littermate control mice, cultured with LPS for 5 days, stained for B220, CD98hc, or CD138 (Syndecan-1, a plasma cell marker), and analyzed by flow cytometry. Each dot plot depicts cells from one mouse after 5 days stimulation with LPS. The middle set of histograms is CD98hc staining on stimulated total B cells, while the right histograms shows % of CD98^{+} cells within the *Slc3a2*$^{f/f}$CD19-Cre^{+} plasma cell population. Experiment was repeated once.

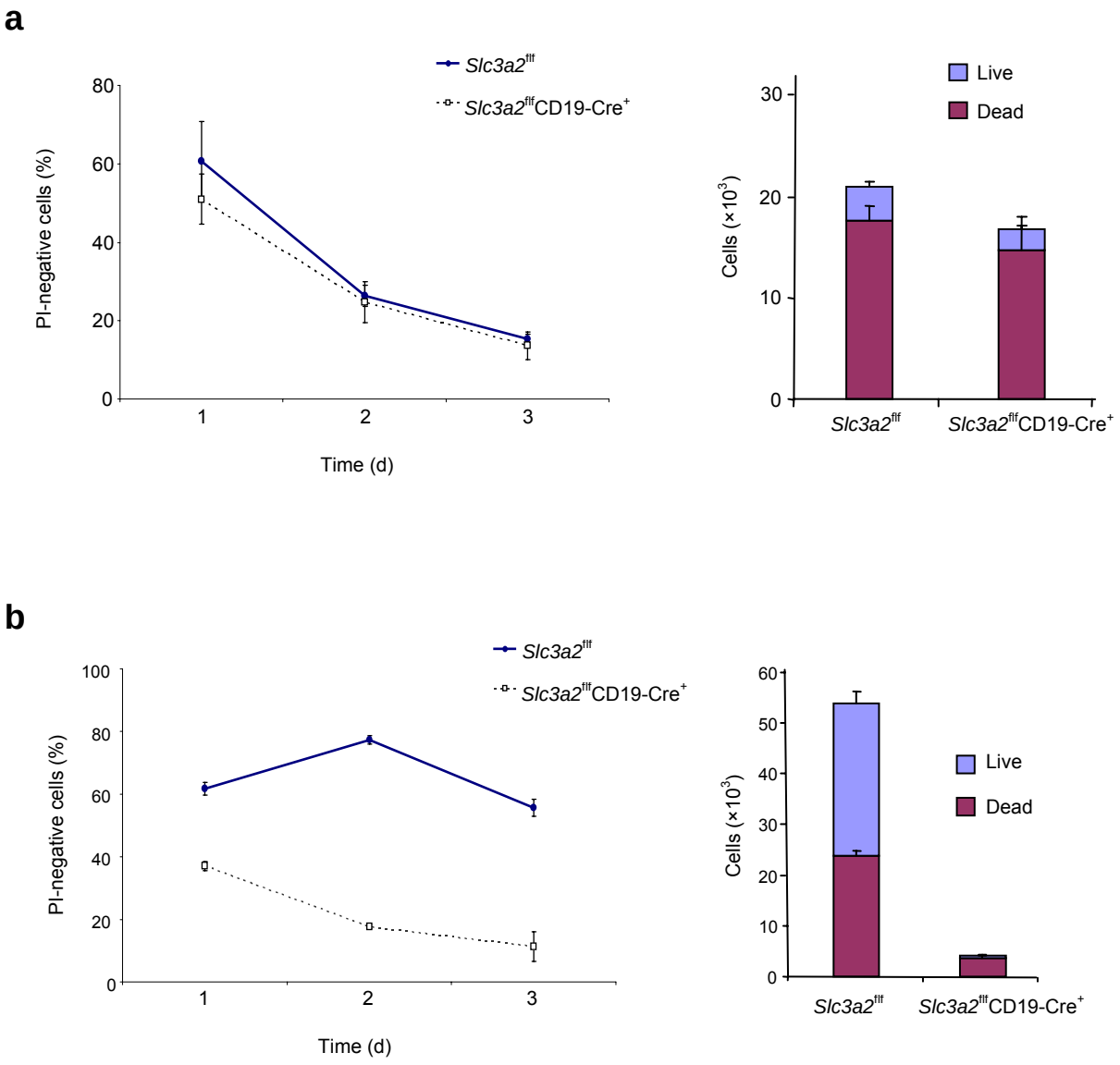


Supplementary Figure 6. Differentiation to plasma cells *in vitro* without CD98hc-depletion. Resting splenic B cells (CD43^{-}) were purified from *Slc3a2*$^{f/f}$CD19-Cre^{+} or littermate control mice, cultured with LPS for 5 days, stained for B220, CD98hc, or CD138 (Syndecan-1, a plasma cell marker), and analyzed by flow cytometry. Each dot plot depicts cells from one mouse after 5 days stimulation with LPS. The middle set of histograms is CD98hc staining on stimulated total B cells, while the right histograms shows % of CD98^{+} cells within the *Slc3a2*$^{f/f}$CD19-Cre^{+} plasma cell population.

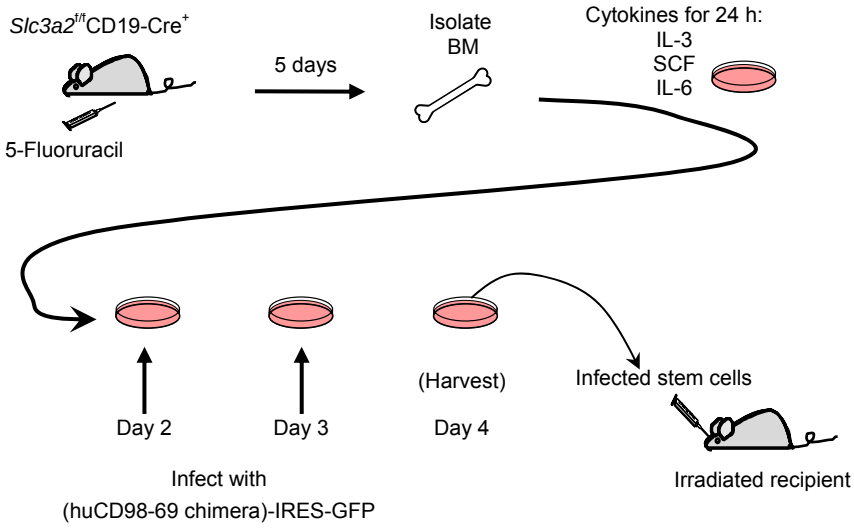




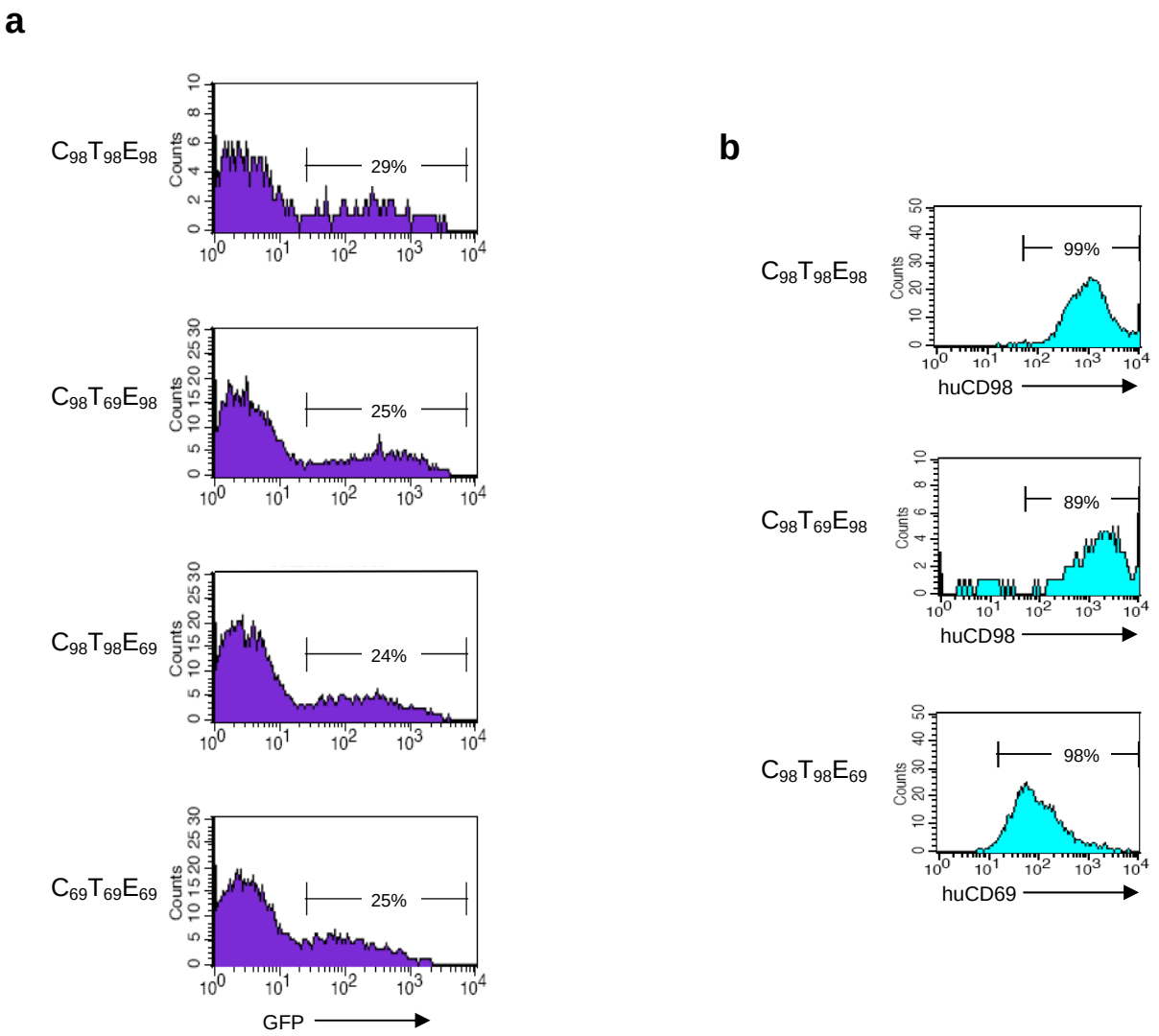
Supplementary Figure 7. Purification of CD98hc-deficient B cells. **(a)** Purification and depletion protocol. Resting B cells ($CD43^-$) were purified from splenocytes of 8-16 wk-old *Slc3a2*$^{f/f}$CD19-Cre$^+$ and littermate control mice by negative depletion of $CD43^+$ cells using magnetic beads. After purification, B cells were incubated, with or without LPS, for 3-5 days *in vitro* before staining for plasma cell formation or CFSE dilution analysis by flow cytometry. **(b)** Depletion efficiency. For *Slc3a2*$^{f/f}$CD19-Cre$^+$ (depicted in this figure), anti-CD98hc was included in the purification to deplete the approximately 25% of B cells that expressed CD98hc; <2% $CD98hc^+$ cells remained after depletion. Representative histograms are gated on $B220^+$ cells before and after depletion, and the bar graph below summarizes data from 3 mice from each group. Experiment was repeated three times.



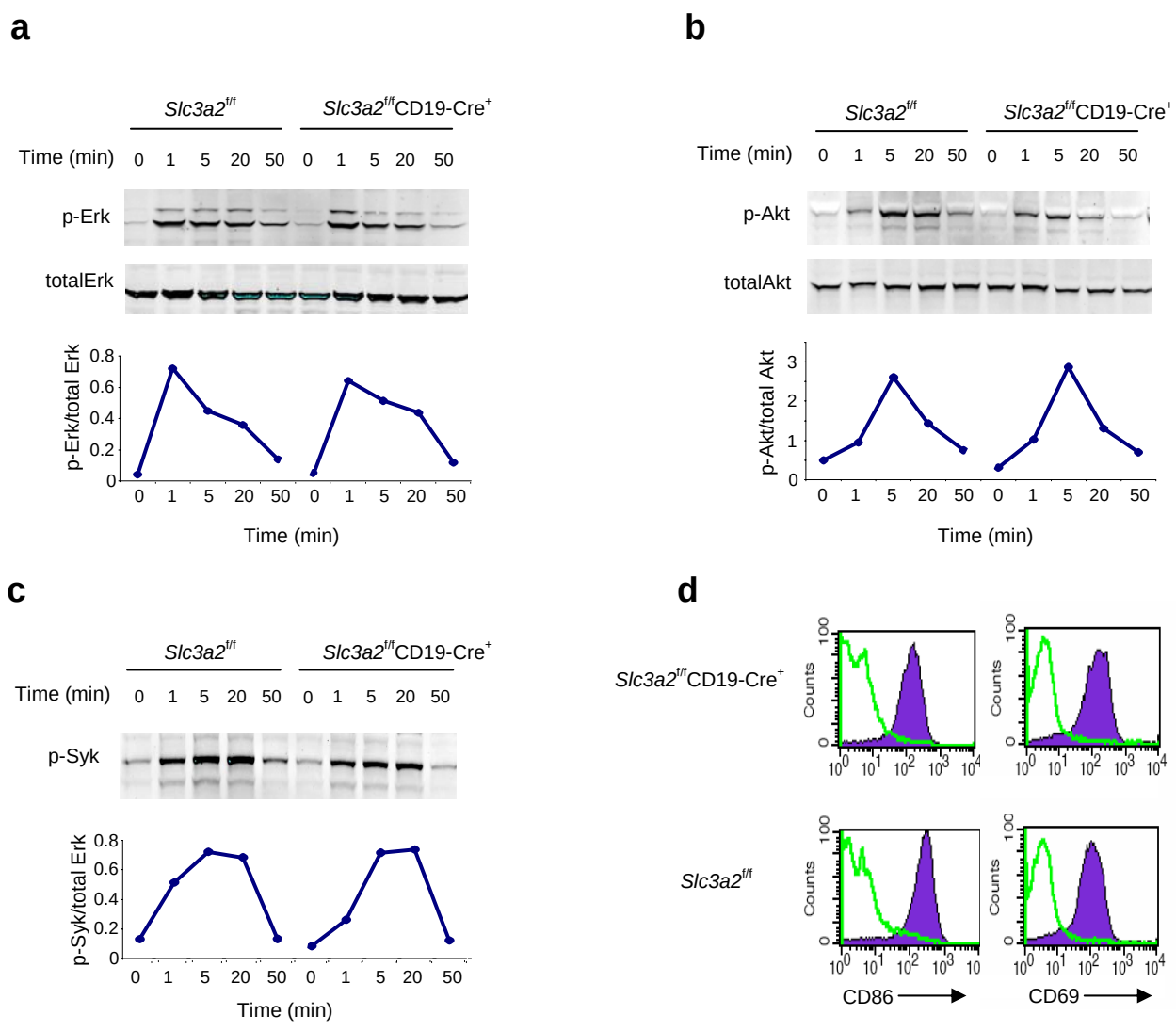
Supplementary Figure 8. Survival and proliferation of splenic B cells from *Slc3a2*$^{f/f}$CD19-Cre^{+} mice. Resting B cells (CD43^{-}) were purified from splenic single-cell suspensions from 8-12 wk-old *Slc3a2*$^{f/f}$CD19-Cre^{+} or littermate control mice. 400,000 B cells were cultured per well in a 48-well plate with anti-IgM (30 μg/ml) and IL-4 (50 ng/ml) (**b**) or were left unstimulated (**a**). Cells were harvested at 1, 2, or 3 days and stained by propidium iodide to mark dead cells. At day 3, total cell number and number of dead and live cells was also determined (bar graphs); please note different scales for unstimulated vs. stimulated cells at 3 days.. Error bars indicate s.e.m. from 3 mice for each group. Experiment was repeated once.



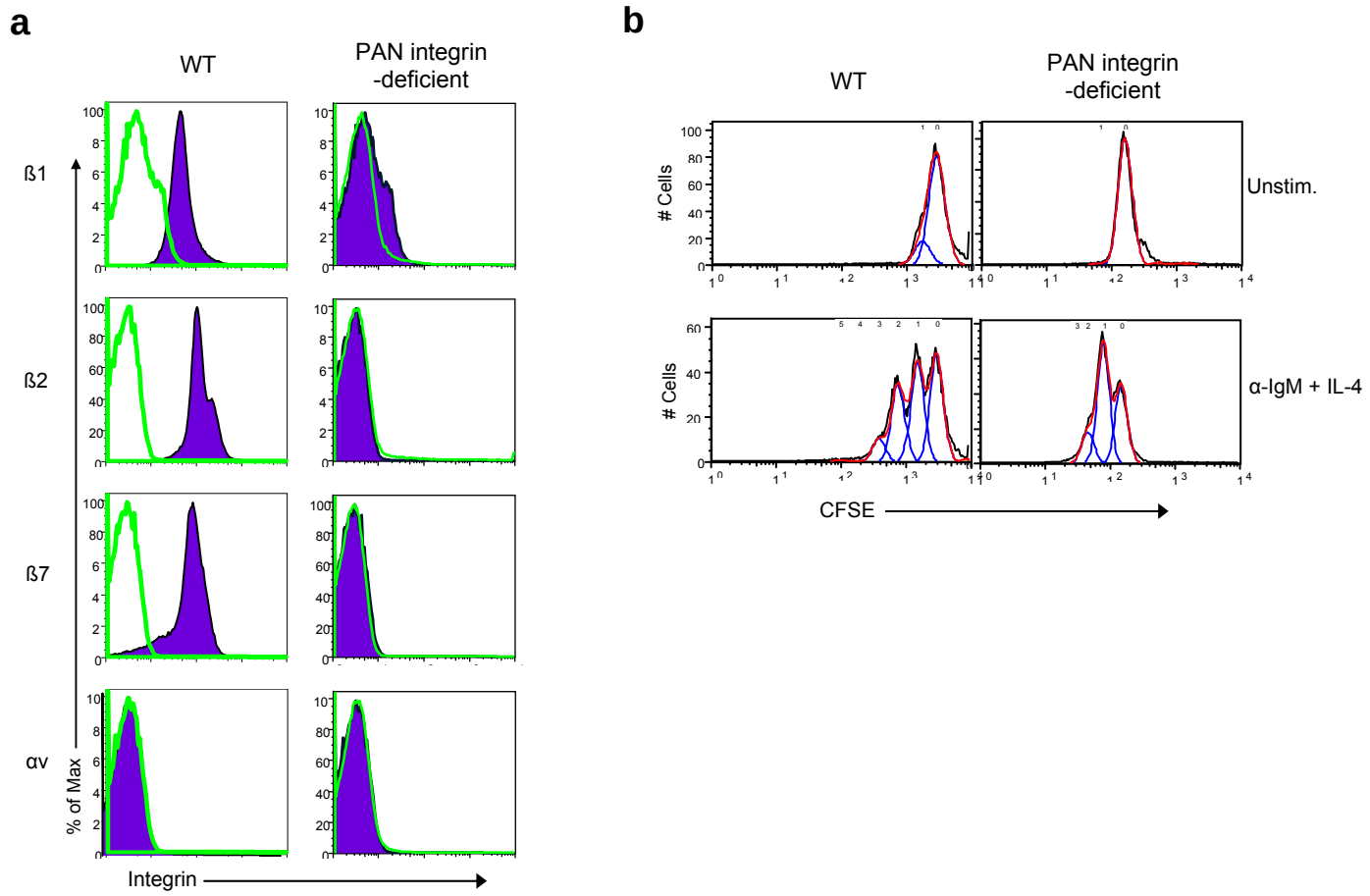
Supplementary Figure 9. Protocol for generating primary B cells lacking one or both functions of CD98. Stem cell-enriched BM isolated from adult *Slc3a2*$^{f/f}$CD19-Cre^{+} mice pretreated with 5-Fluorouracil was repeatedly infected with retrovirus in the presence of IL-3, IL-6 and SCF. On day 4 after BM isolation, cells were harvested % of stem cells infected with retrovirus was measured (24-29% for all for constructs). BM cells were injected into lethally irradiated recipient mice i.v. (*n*=3-5 per group).



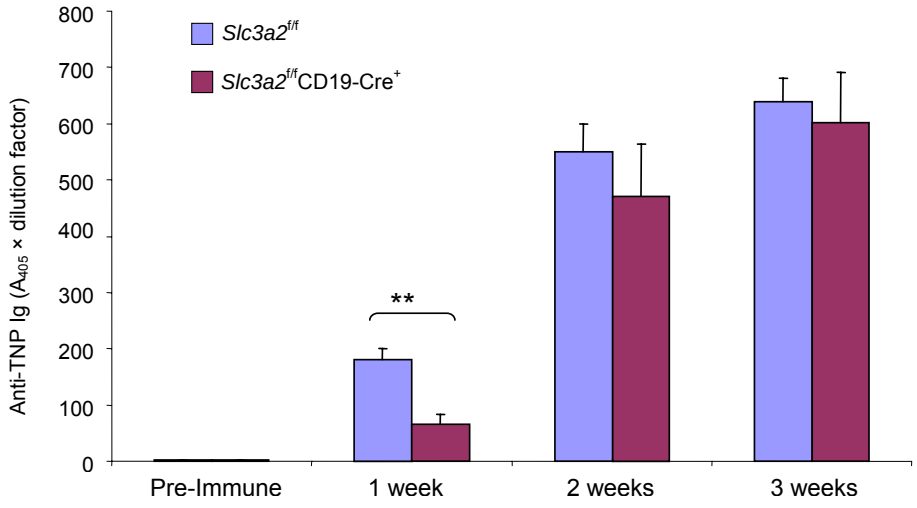
Supplementary Figure 10. Expression of human CD98-69 chimeras. **(a)** Efficiency of retroviral infection of stem cells. Before injection into irradiated recipient mice, BM cells infected on two successive days with indicated CD98-69chimera-IRES-GFP retroviruses were stained for the stem cell marker Sca-1, and analyzed for GFP expression by flow cytometry. Histograms are gated on stem cells (Sca-1^{+}); percentages are % GFP+ **(b)** Chimera expression. Purified (CD43^{-}mCD98^{-}) B cells from irradiated mice that had been reconstitued with CD98-69chimera-IRES-GFP retrovirus-infected BM were stained for human CD98hc or CD69 to assess chimera expression by GFP^{+} cells. Histograms are gated on GFP^{+} cells; numbers indicate % of GFP% cells that express chimera. Experiment was repeated twice.



Supplementary Figure 11. Early BCR signaling and activation in B cells lacking CD98hc. **(a-c)** BCR signaling. Resting B cells (CD43^{-}CD98^{-}) were purified from splenocytes of 8-12 wk-old *Slc3a2*$^{f/f}$CD19-Cre^{+} or littermate control mice and were stimulated with anti-IgM (10 ug/ml) and IL-4 (15 ng/ml) for 0-50 min. Cells were then immediatedly washed with ice-cold buffer, lysed, and phosphorylation of Erk1/2 (**a**), Akt (**b**), and Syk (**c**) were measured by immunoblotting. Plots below each lane show quantified staining density normalized to total Erk, Akt or Syk over time. This experiment was repeated 2 additional times with similar results. **(d)** B cell activation. Purified B cells from *Slc3a2*$^{f/f}$CD19-Cre^{+} or littermate control mice were stimulated for 24 h with anti-IgM (30 ug/ml) and expression of the early activation markers CD86 and CD69 was measured by flow cytometry. Filled histograms show anti-IgM-stimulated cells, open histograms show unstimulated cells. Experiments in (**a-d**) were repeated once.



Supplementary Figure 12. Pan integrin-deficient B cell proliferation. **(a)** Integrin expression *ex vivo*. Resting B cells ($CD43^-$) were purified from splenocytes of adult pan integrin-deficient mice (*Itgb1*^{flf}, *Itgav*^{flf}, *Itbg2*$^{-/-}$, *Itgb7*$^{-/-}$, mx1-CRE^+, poly IC treated) or littermate control mice and were depleted of residual integrin-$\beta1^+$ B cells in the case of the pan integrin-deficient B cells. Integrin expression on purified mature B cells was measured by flow cytometry after staining with specific antibodies. **(b)** B cell proliferation. Purified B cells from pan integrin-deficient mice or littermate control mice were labeled with CFSE and stimulated for 3 days with anti-IgM and IL-4. Cell division as measured by dilution of CFSE was assessed by flow cytometry. Histograms have been arranged to allow comparison with unstimulated cells, as CFSE labelling varied between samples. Data represents two experiments.



Supplementary Figure 13. Antibody response of *Slc3a2*$^{f/f}$CD19-Cre^{+} mice to T cell-dependent antigen over time. Adult (8-12 wk old) *Slc3a2*$^{f/f}$CD19-Cre^{+} or control mice were immunized with 100 μg of a T cell-dependent antigen, TNP-KLH, in Complete Freund's Adjuvant (CFA). Mice were bled before immunization (pre-immune) and at one, two, or three weeks after immunization to obtain serum, which was analyzed for anti-TNP IgG by direct ELISA. Error bars represent s.e.m. from 5 mice for each group (**P < 0.025). Experiment was repeated once.

Supplementary Methods

Hematology

Blood from adult (10-20 wk-old) *Slc3a2*$^{f/f}$CD19-Cre^{+} and control littermate mice was collected by tail bleed into tubes containing EDTA. Cell counts were obtained using an MS9 automated cell counter with veterinary reagents and parameters by the University of California San Diego Animal Care Program Diagnostic Laboratory who also manually performed differential counts on Wright-Giemsa–stained smears.

ELISPOT

Serial dilutions of spleen cells from *Slc3a2*$^{f/f}$CD19-Cre^{+} and littermate control mice (7 days after immunization with TNP-KLH in CFA) were incubated for 2-3 hours at 37°C in a 96-well PVDF plate (Millipore) coated overnight with TNP-OVA (Biosearch) and blocked with Casein (Pierce) for 2 hours. After vigorous washing to remove cells and incubation with HRP-conjugated anti-mouse IgG or anti-mouse IgM (Jackson Immunoresearch), plates were washed again and spots developed using an amino-ethyl-carbazole (Sigma) substrate for 5 min. Enumeration of spots was performed using an automated ELISPOT reader. Data with samples (triplicates) from two separate experiments was combined by normalizing to control responses.

Spreading assay

We used a modified version of the B cell spreading assay previously described [34]. Resting B cells (CD43^{-}) were purified from spleen cell suspensions of *Slc3a2*$^{f/f}$CD19-Cre^{+} or control mice by negative depletion and cultured at 1-2 × 10^{6} per well in a 24-well plate with 4 μg/ml anti-CD40 (eBioscience, clone 1C10) and 20 ng/ml IL-4 (Peprotech). After 24 hours, cells were harvested and cultured overnight in a 48-well plate that had been coated with anti-LFA-1 (eBioscience, clone 17/4) at 1 μg/2.5 cm^{2} and blocked with 2% BSA. Digital pictures were taken at identical magnification of control and *Slc3a2*$^{f/f}$CD19-Cre^{+} cells, which were then analyzed for cell area and perimeter by manual tracing of at least 30 cells per sample and quantitation using ImageJ software.

Signaling

Resting B cells from *Slc3a2*$^{f/f}$CD19-Cre^{+} and littermate control mice were purified as described above, using anti-CD98 depletion for *Slc3a2*$^{f/f}$CD19-Cre^{+} samples. Upregulation of activation markers was measured by staining purified B cells stimulated overnight with LPS (20μg/ml) using antibodies to CD86 and CD69 (BD Biosciences), followed by flow cytometry. For early signaling, 5 × 10^{6} purified B cells were incubated 37°at

C in PBS without serum for 15 min to equilibrate, and then stimulated with10 μg/ml $F(Ab')^2$ goat anti-mouse IgM (Jackson Immunoresearch) and 25 ng/ml IL-4 (Peprotech) for 0-50 min at 37°C in PBS without serum. Cells were immediately washed for 2 min at 5000 × *g* in cold PBS with the phosphatase inhibitors sodium fluoride (10 mM), sodium vanadate (1 mM), and β-glycerol phosphate (20 mM). B cells were lysed for 30 min on ice in 30 μl RIPA lysis buffer (50mM Tris, pH 7.4, 150mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 10 mM NaF, 1 mM Na_3VO_4, 20 mM β-glycerol phosphate) and EDTA-free protease inhibitor cocktail (Roche). Cellular debris was pelleted and removed by centrifugation at 12,000 x *g* for 20 min. Remaining lysate was combined with 6× SDS sample buffer containing β-ME and boiled for 5 min, resolved on a 4-20% Tris-gylcine polyacrylamide gel, and transferred to nitrocellulose membrane overnight. Membranes were blocked with 5% milk and stained with antibodies against total Erk2 (Santa Cruz, clone D-2), p27 (SantaCruz, clone M-197), total Akt (Cell Signaling, clone 40D4), p-Syk (Cell Signaling, clone C87C1), p-Akt (Cell Signaling, clone 193H12), and p-ERK1/2 (Cell Signaling, polyclonal). After secondary antibody incubation, detection was performed in the infrared range using a LiCor infrared scanner. For sustained (late) ERK signaling, purified B cells were stimulated as in the proliferation assay with anti-IgM and IL-4, but harvested, washed with cold PBS and phosphatase inhibitors, and lysed and blotted as for early signaling assays.

Statistical Analysis

All error bars represent standard error of the mean from the number of mice indicated in the figure legends. *P*-values listed in figure legends are from analysis using two-tailed Student's *t*-test. (** < 0.025, * <0.05, unless otherwise indicated)