SUPPLEMENTAL INFORMATION

CD22 regulates adaptive and innate immune responses of B cells.

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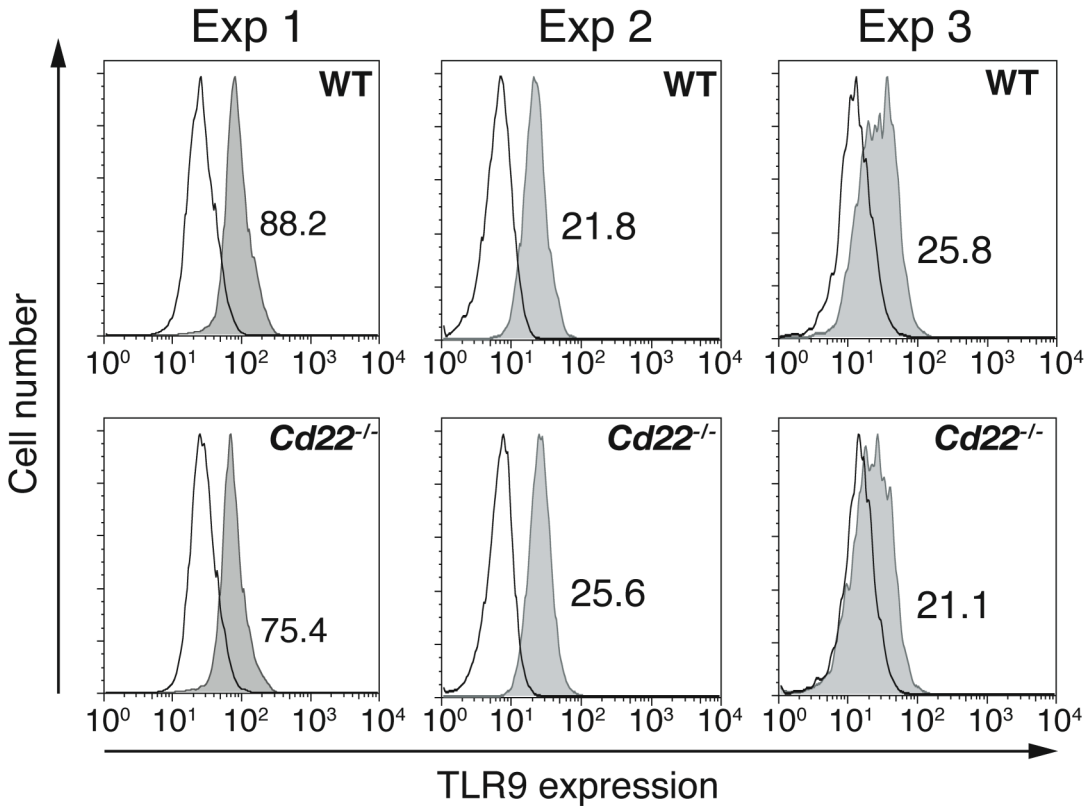
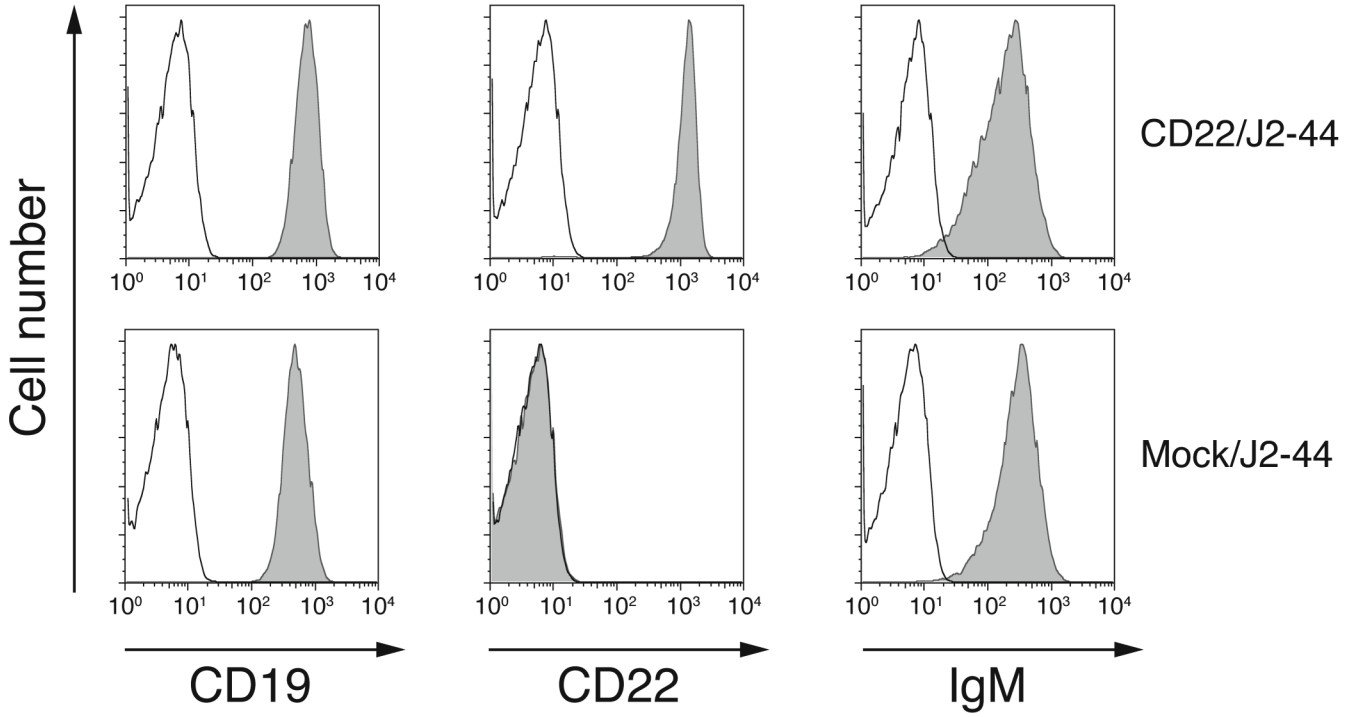
Supplementary text and figure

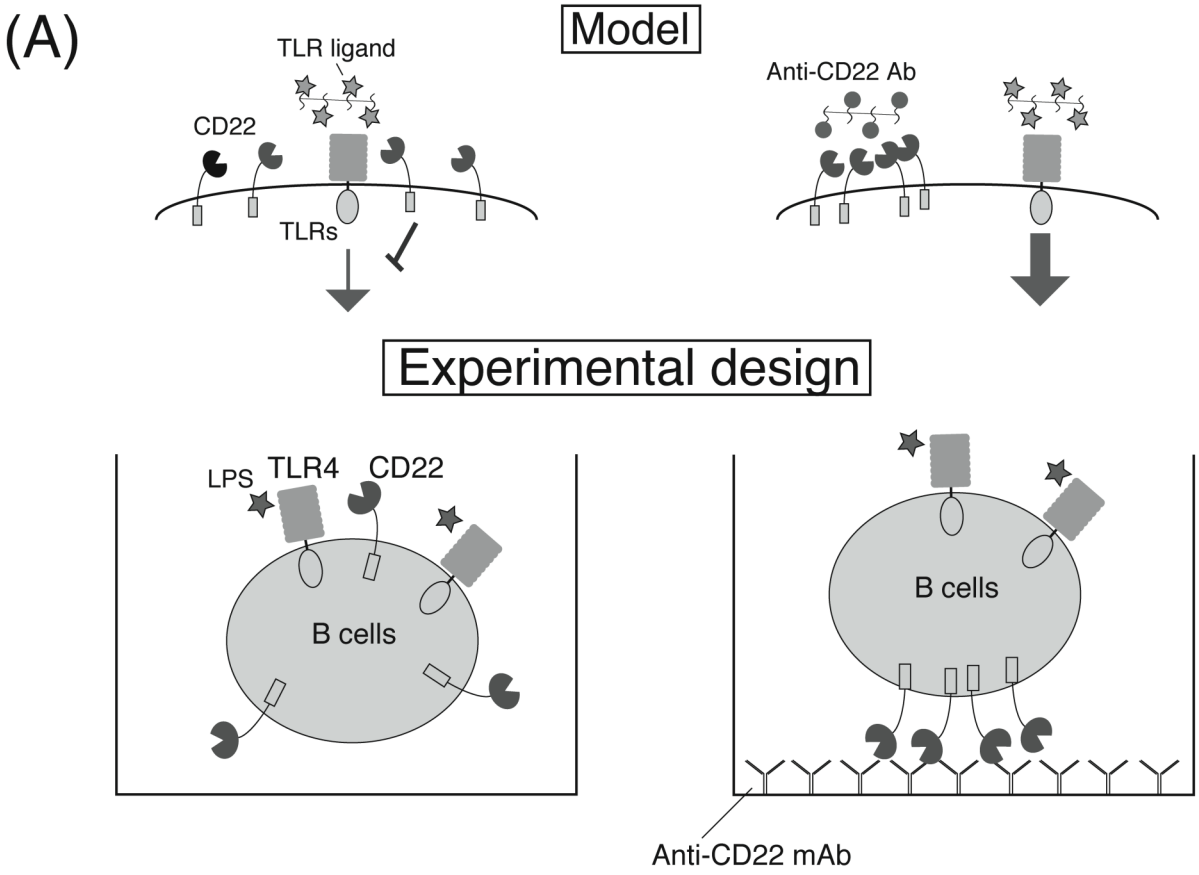
Supplementary Figure 1. TLR9 expression in B cells. Histograms of TLR9 staining of B cells from three independent experiments are shown. Black indicates isotype control Ab staining and gray histograms indicates anti-TLR9 staining. The MFI of *Cd22-/-* B cells relative to that of WT B cells is plotted in Figure 3.

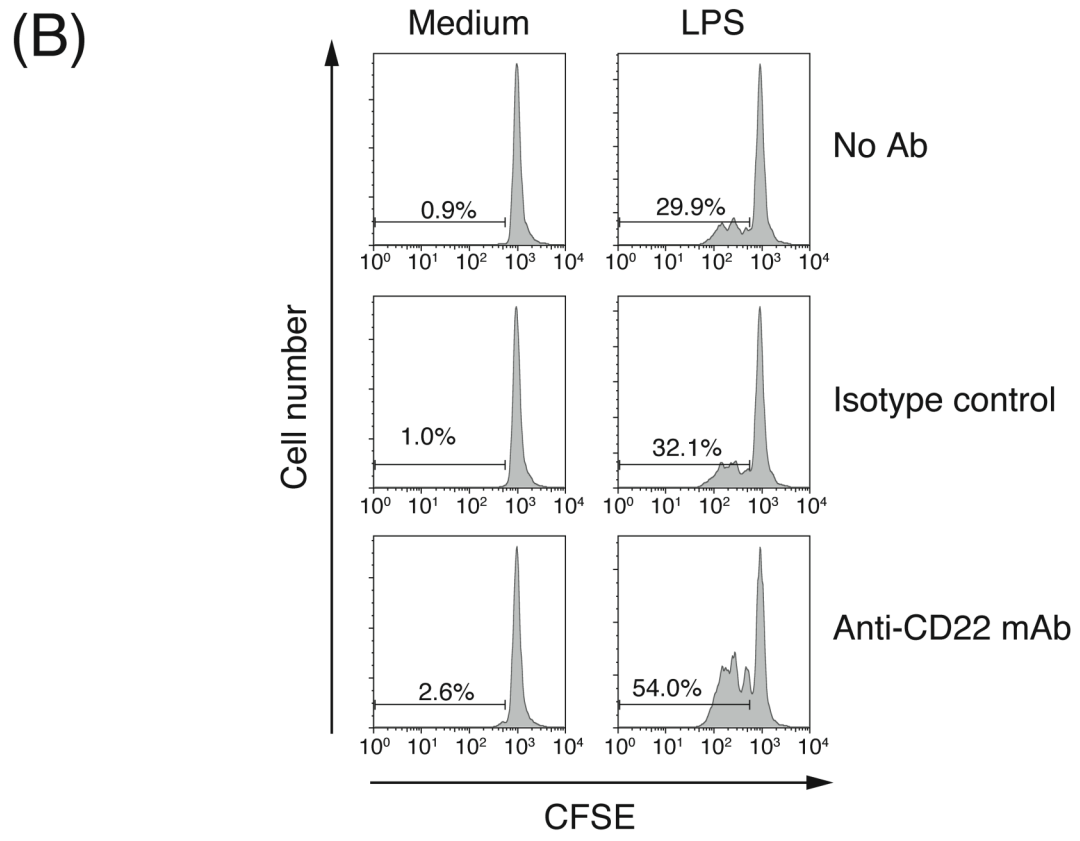


Supplementary Figure 2. Establishment of CD22 expressing J2-44 cells. CD22 expressing J2-44 cells and the mock transductants were stained with anti-CD19 (clone 1D3, BD biosciences), anti-CD22 (clone Cy34.1, BD biosciences), or anti-IgM$^{a/b}$ (kindly provided by Dr. David A. Nemazee in The Scripps Research Institute). The cells were analyzed by flow cytometry.

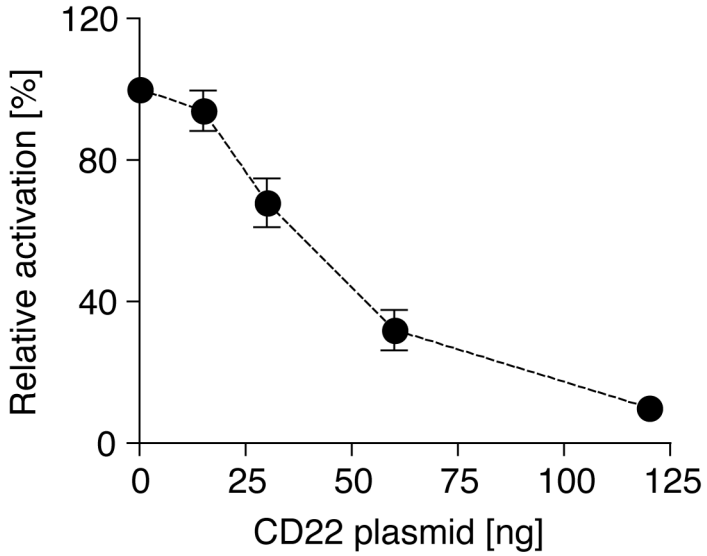


Supplementary Figure 3. Antibody-mediated sequestration of CD22 from TLR4 augments LPS-induced proliferation of WT B cells. (A) Model of this experiment is shown. We hypothesized that co-localization of CD22 with TLR may be important for its inhibitory effect, based on the observation by Doody GM, Justement LB, Delibrias CC, Matthews RJ, Lin J, Thomas ML, Fearon DT: A role in B cell activation for CD22 and the protein tyrosine phosphatase SHP. Science 1995;269:242-244. Therefore we expected that immobilized anti-CD22 Ab reduced the contact of CD22 with TLR4, thereby augmented the activation. (B) CFSE-labeled WT B cells were stimulated with or without soluble 3.0 μg/mL of LPS in the presence of immobilized anti-CD22 mAb or isotype-matched control Ab. After 2 days, cells were analyzed by flow cytometry. One of the results in Fig. 2B is shown.


(A)
Model
TLR ligand
CD22
TLRs
Anti-CD22 Ab
Experimental design
LPS
TLR4
CD22
B cells
B cells
Anti-CD22 mAb


(B)
Medium
LPS
No Ab
Isotype control
Anti-CD22 mAb
0.9%
29.9%
1.0%
32.1%
2.6%
54.0%
Cell number
CFSE

Supplementary Figure 4. Ectopic expression of CD22 inhibits NF-κB activation in a TLR4 reporter cell line. mTLR4/HEK293 cells were transfected with NF-κB inducible AP and indicated amount of mCD22 vector and stimulated with 10 μg/mL of LPS. The culture supernatant was incubated with the AP substrate at 37˚C. Then the enzymatic activity was measured by the absorbance of 405 nm. The activity relative to that in the absence of mCD22 plasmid was shown.



Supplementary Figure 5. IκBα, p38, JNK, and IRAK-1 analysis in WT and *Cd22*$^{-/-}$ B cells. Purified B cells were stimulated with 10 μg/mL of anti-mouse IgM or 1 μM CpG for indicated time period. Cells were lysed and subjected to western blot analysis. IκBα and IRAK-1 degradation and phosphorylation of p38 and JNK in *Cd22*$^{-/-}$ B cells upon CpG stimulation were indistinguishable from that in WT B cells.