Supplemental Material for

CD22 is a recycling receptor that can shuttle cargo between the cell surface and endosomal compartments of B cells

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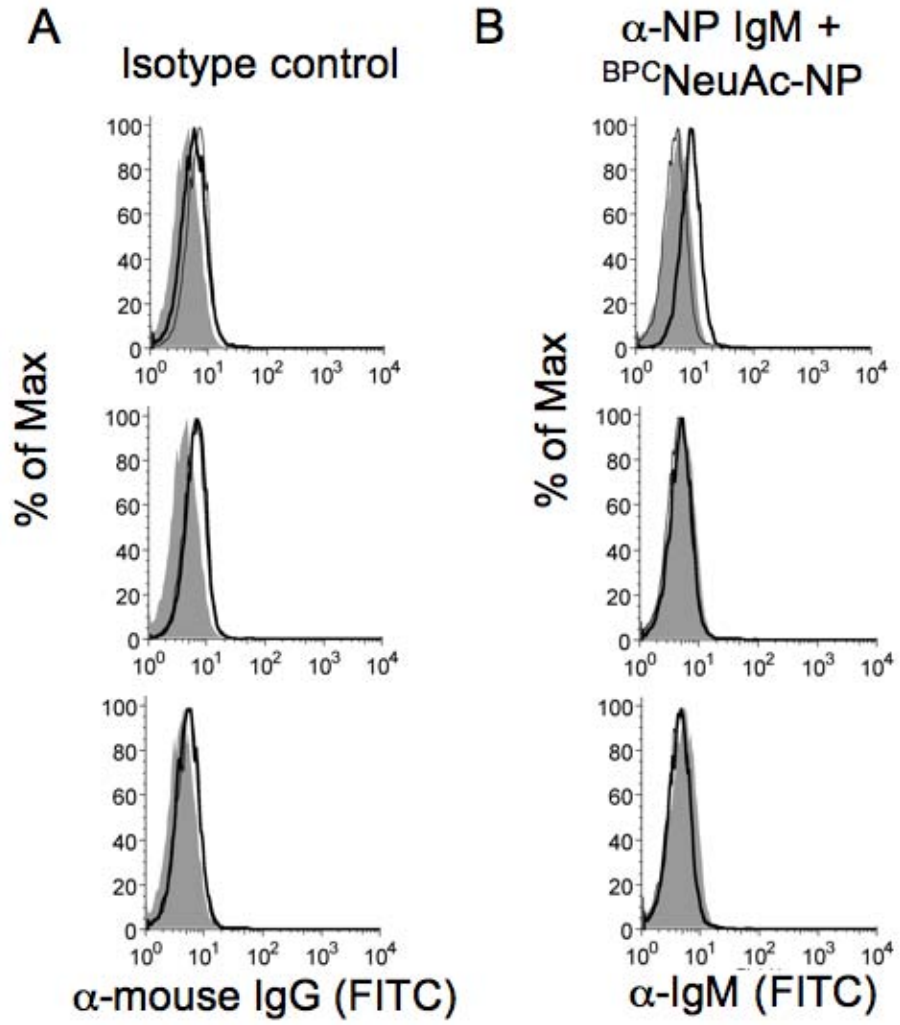
Materials and Methods

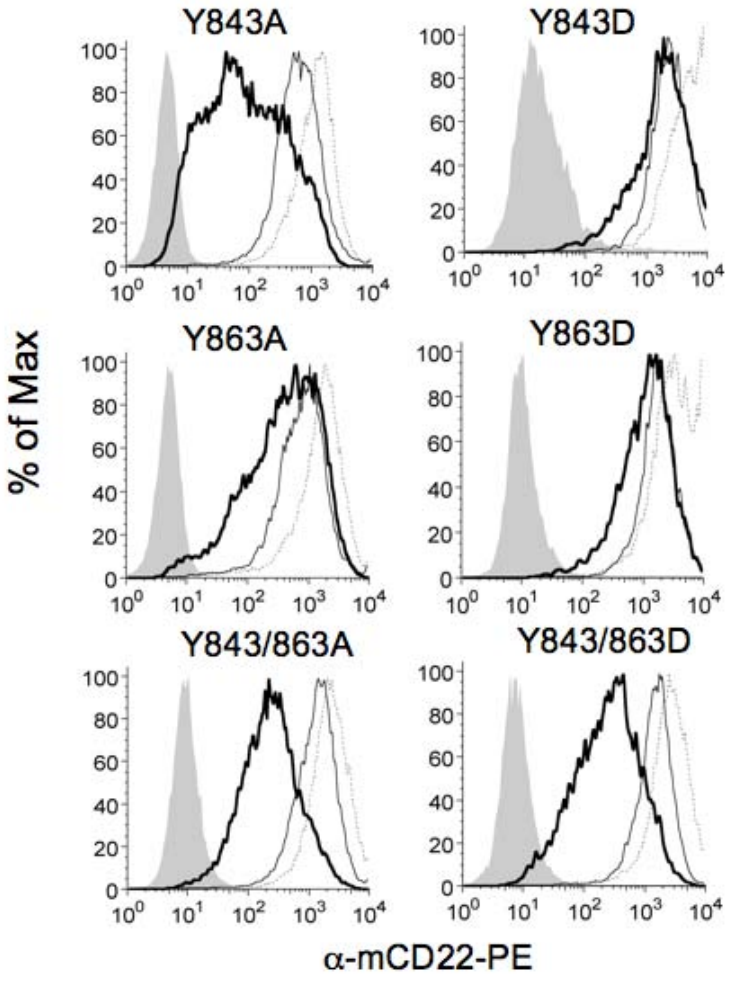
Stability of disulfide-linked biotin

BJAB cell surface proteins were biotinylated as described for the internalization and recycling experiments. After washing away unreacted biotinylation reagent with 25 mM lysine, cells were warmed to 37 ˚C in RPMI/10%FBS/50μM 2-ME for 90 minutes to mimic the conditions of the internalization/recycling experiments. Cells were then washed twice with cold DPBS, and lysed and immunoprecipitated with streptavidin-agarose resin as described in the main text.

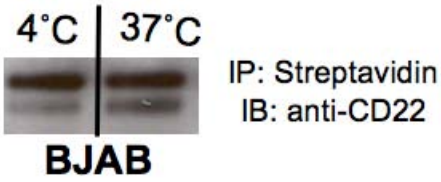
Supplemental Fig. 1. Evidence for recycling is not seen for the isotype control of αCD22 (Figure 2) or for αNP Anti-mouse IgG (a) or αNP IgM and ^{BPC}NeuAc-NP (b) were used in place of anti-CD22 as controls for the antibody recycling experiment Figure 2. Following the same protocol, neutral and acid-washed cells were stained with a labeled secondary antibody (a). Thick lines represent neutral-washed cells, thin lines represent acid-washed cells, and filled curves represent control in the absence of antibody. Acid-washed cells from the first step were warmed again to allow antibody to be recycled back to the cell surface. Following this 37˚ C incubation, neutral and acid-washed cells were stained with secondary antibody (b). As a control, acid-washed cells were incubated at 4˚ C instead of 37˚ C in the second step (c).



Supplemental Fig. 2. αCD22 uptake by alanine and aspartate mutants of Y843 and Y863. Murine B cell transfectants of single and double CD22 mutations from tyrosine 843 and 863 to alanine (a) or aspartate (b) were tested for internalization of fluorescently labeled αCD22 antibody. Cells were incubated at 4 ˚ C with antibody and then washed (thin line) or not washed (dotted line) prior to the 37 ˚C incubation. Cells that were not washed prior to 37˚ C incubation were also acid-washed following the warming step to reveal internalized antibody (thick line). Cells that were acid-washed following the 4 ˚C incubation were included as control (filled curve).



Supplemental Fig. 3. Disulfide-linked biotin is stable to endosomal conditions. Biotinylated BJAB cells were warmed to 37 ˚C for 90 minutes to mimic endocytosis and recycling experimental conditions in order to determine whether the biotin is lost to reduction upon internalization. Streptavidin pull-down and probing for CD22 by western blot reveals no loss in the biotin label from CD22.



Supporting Figure S4. αNP and ^{BPC}NeuAc-NP do not accelerate the constitutive rate of CD22 endocytosis. Steps A-C from Figure 3 were carried out to measure endocytosis of CD22 in BJAB cells. Samples from the last three lanes included 40 μg/mL αNP IgM and 2 μM ^{BPC}NeuAc-NP in the warming/internalization step.