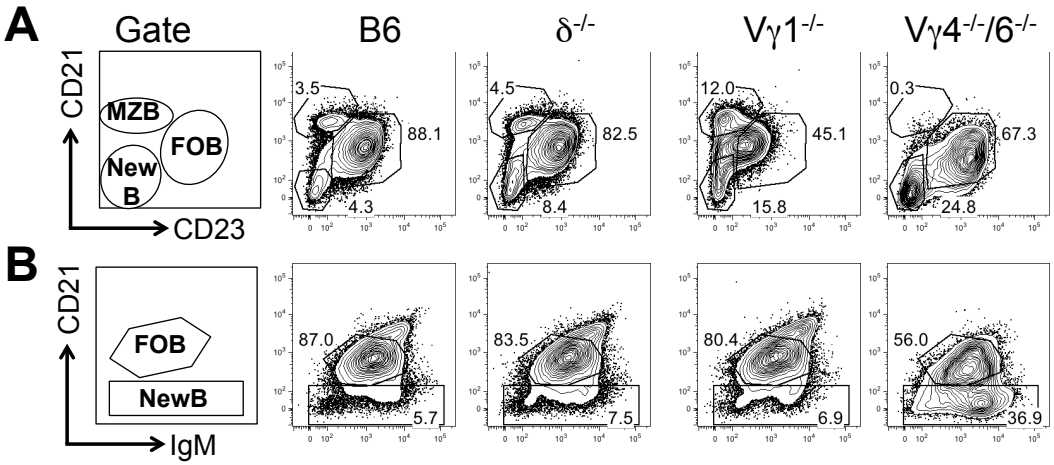
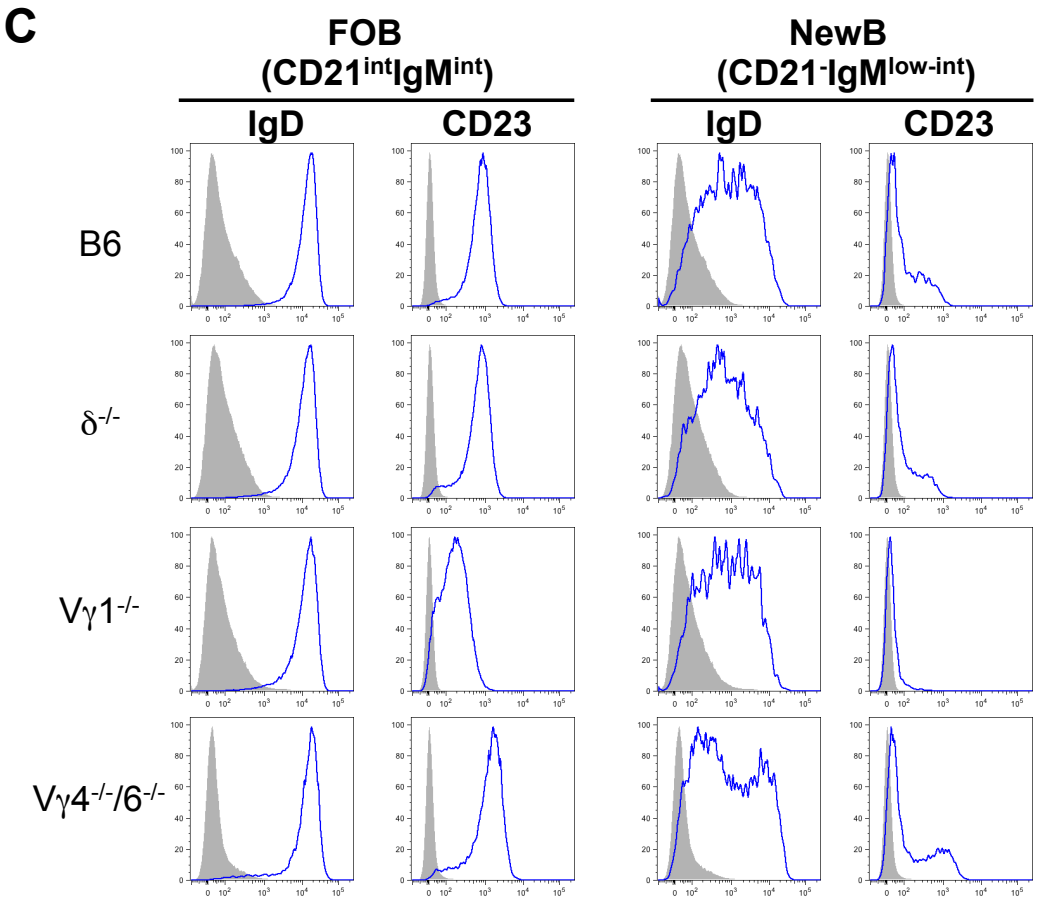
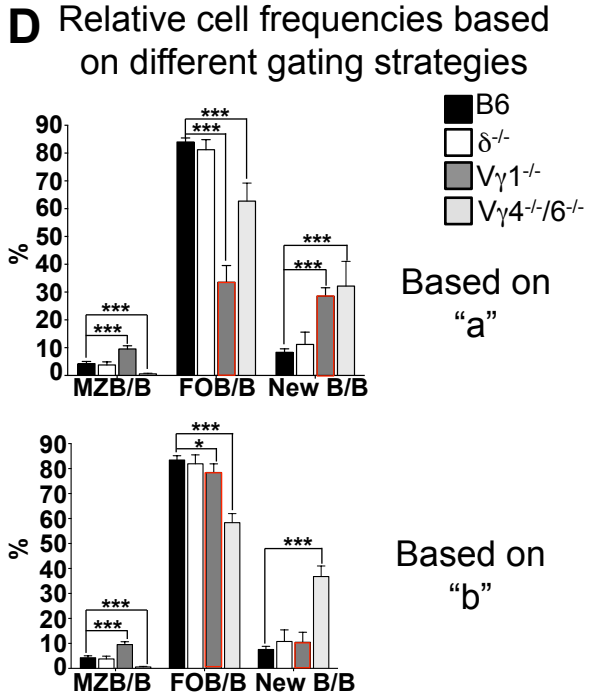
Supplemental Figure 1







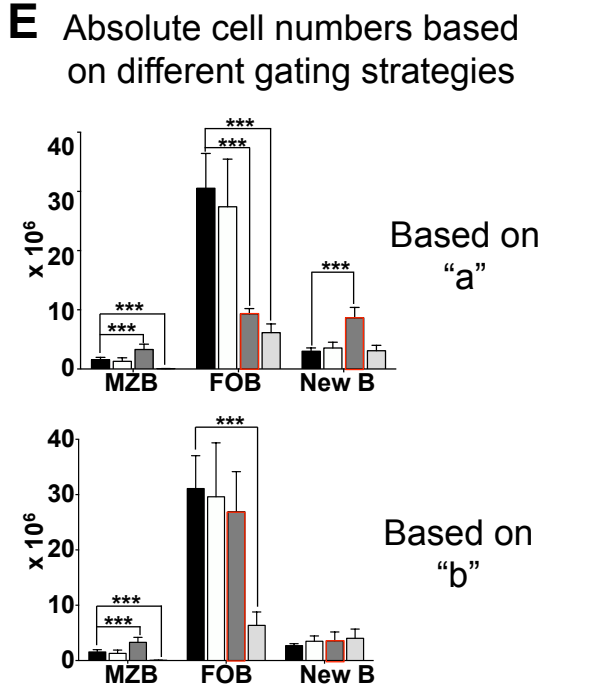


Figure S1 Effect of gating strategies on estimates of splenic B cell populations

(A) Mature splenic B cells in 8 wks old test panel mice as in Figure 3. (B) Mature splenic B cells in the same mice gated based on their expression of CD21 and IgM; the difference in relative frequencies of FOB cells and "new" B cells in B6.TCR-Vγ1$^{-/-}$ mice is related to the CD23-deficiency in this strain. (C) IgD and CD23 expression profiles in splenic FOB cells and "new" B cells in the test panel mice; both cell types express CD23 at much reduced levels in B6.TCR-Vγ1$^{-/-}$ mice, and at slightly increased levels in B6.TCR-Vγ4$^{-/-}$/Vγ6$^{-/-}$ mice. Minor changes in the expression of IgD are also seen. (D, E) Relative frequencies and absolute numbers of mature splenic B cells based on the gating strategies shown in (A) and (B). The largest differences between the two types of measurement are seen with FOB cells and "new" B cells in B6.TCR-Vγ1$^{-/-}$ mice (outlined in red). n=8 mice per group, *P<0.05, ***P<0.001

Supplemental Figure 2

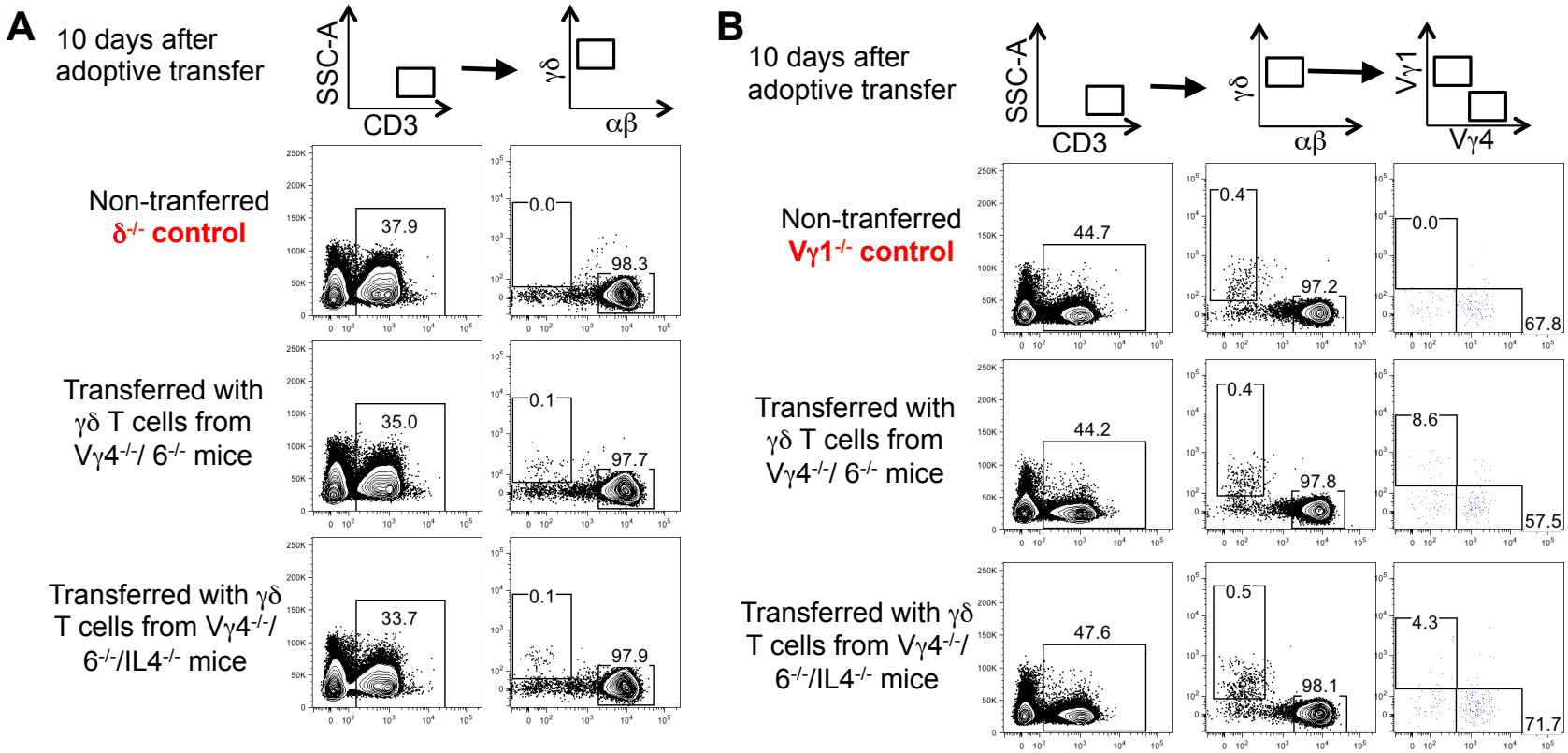


Figure S2 Reconstituting effect of transfer with γδ T cells on splenic γδ T cell populations, and effect of transferred residual γδ T cells from B6.TCR-Vγ4$^{-/-}$/6$^{-/-}$ mice on splenic B cells in B6.TCR-Vγ1$^{-/-}$ mice.

(A) 4 wks old female B6.TCR-δ$^{-/-}$ (δ$^{-/-}$) mice were transferred with 5x10^{6} purified splenic γδ T cells from B6.TCR-Vγ4$^{-/-}$/Vγ6$^{-/-}$ mice (Vγ4$^{-/-}$/6$^{-/-}$) or B6.TCR-Vγ4$^{-/-}$/Vγ6$^{-/-}$/IL-4$^{-/-}$ mice (Vγ4$^{-/-}$/6$^{-/-}$/IL-4$^{-/-}$), and examined 10 days later for the presence of γδ T cells in the spleen. Levels of reconstitution remained below 10% of normal γδ T cell numbers in the spleen. (B) 4 wks old female B6.TCR-Vγ1$^{-/-}$ (Vγ1$^{-/-}$) mice were transferred with purified splenic γδ T cells from B6.TCR-Vγ4$^{-/-}$/Vγ6$^{-/-}$ mice (Vγ4$^{-/-}$/6$^{-/-}$) or B6.TCR-Vγ4$^{-/-}$/Vγ6$^{-/-}$/IL-4$^{-/-}$ mice (Vγ4$^{-/-}$/6$^{-/-}$/IL-4$^{-/-}$), and examined 10 days later for the presence of Vγ1^{pos} γδ T cells in the spleen. Levels of reconstitution also remained well below normal γδ T cell numbers in the spleen. (C) B6.TCR-Vγ1$^{-/-}$ mice were transferred with purified splenic γδ T cells from B6.TCR-Vγ4$^{-/-}$/Vγ6$^{-/-}$ (Vγ4$^{-/-}$/6$^{-/-}$) or B6.TCR-Vγ4$^{-/-}$/Vγ6$^{-/-}$/IL-4$^{-/-}$ (Vγ4$^{-/-}$/6$^{-/-}$/IL-4$^{-/-}$) mice. 10 days after the transfer, B cell populations in the spleen were compared as detailed for Figure 3, using the indicated markers. Data of one representative experiment are shown. (D) Effect of transferred residual γδ T cells on absolute numbers of MZB cells in the spleen of B6.TCR-Vγ1$^{-/-}$ mice. n= 4 mice per group, *P<0.05

Supplemental Figure 3

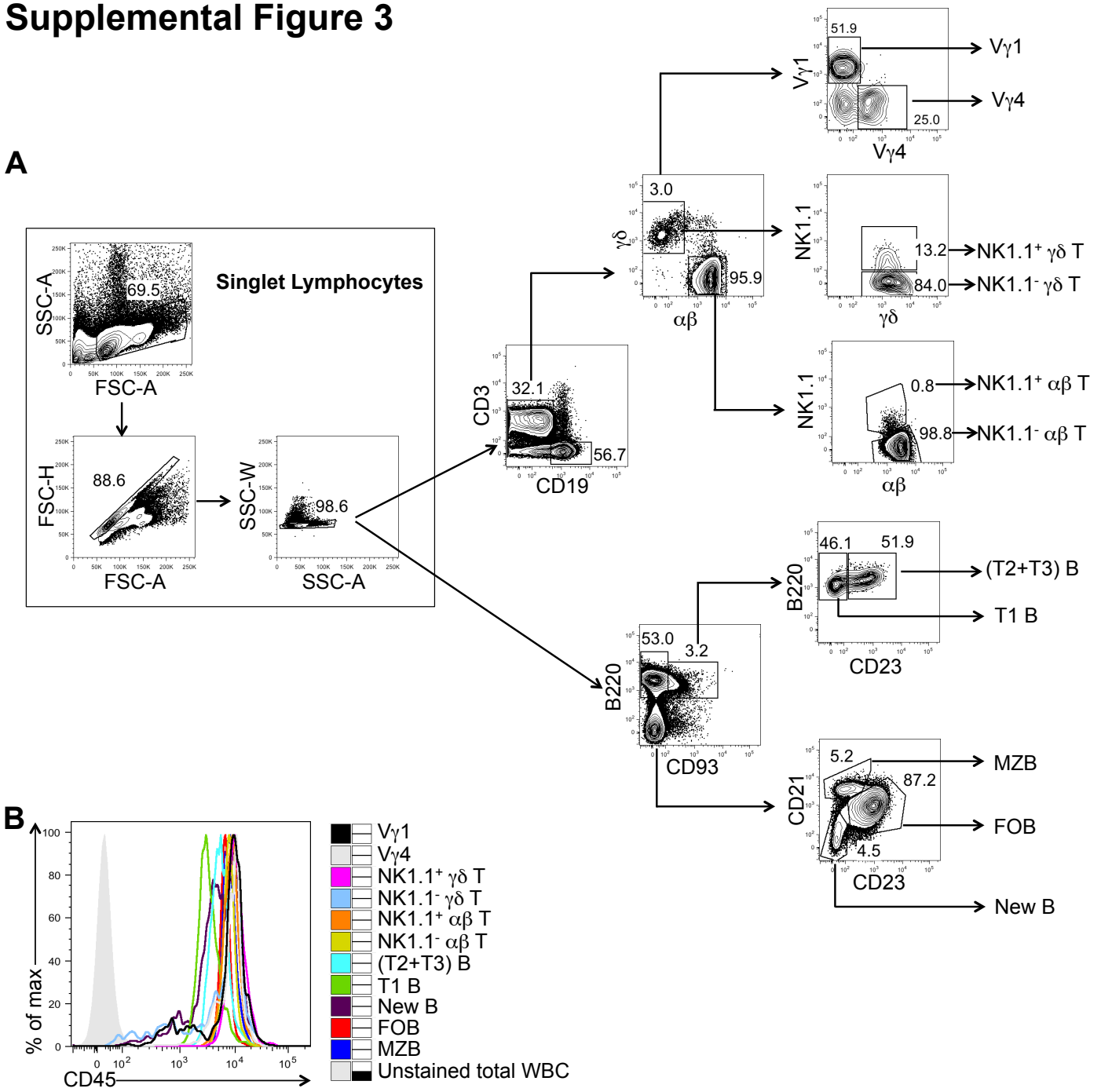


Figure S3

(A) Gating strategy for the *in vivo* stained C57BL/6 splenic lymphocyte populations shown in Figure 7.

(B) CD45 expression of the splenic lymphocyte populations shown in Figure 7a. C57BL/6 splenocytes were stained *in vitro* with specific mAbs detecting the indicated lymphocyte subsets, and compared for CD45 cell surface expression using cytofluorimetry.

Supplemental Figure 4

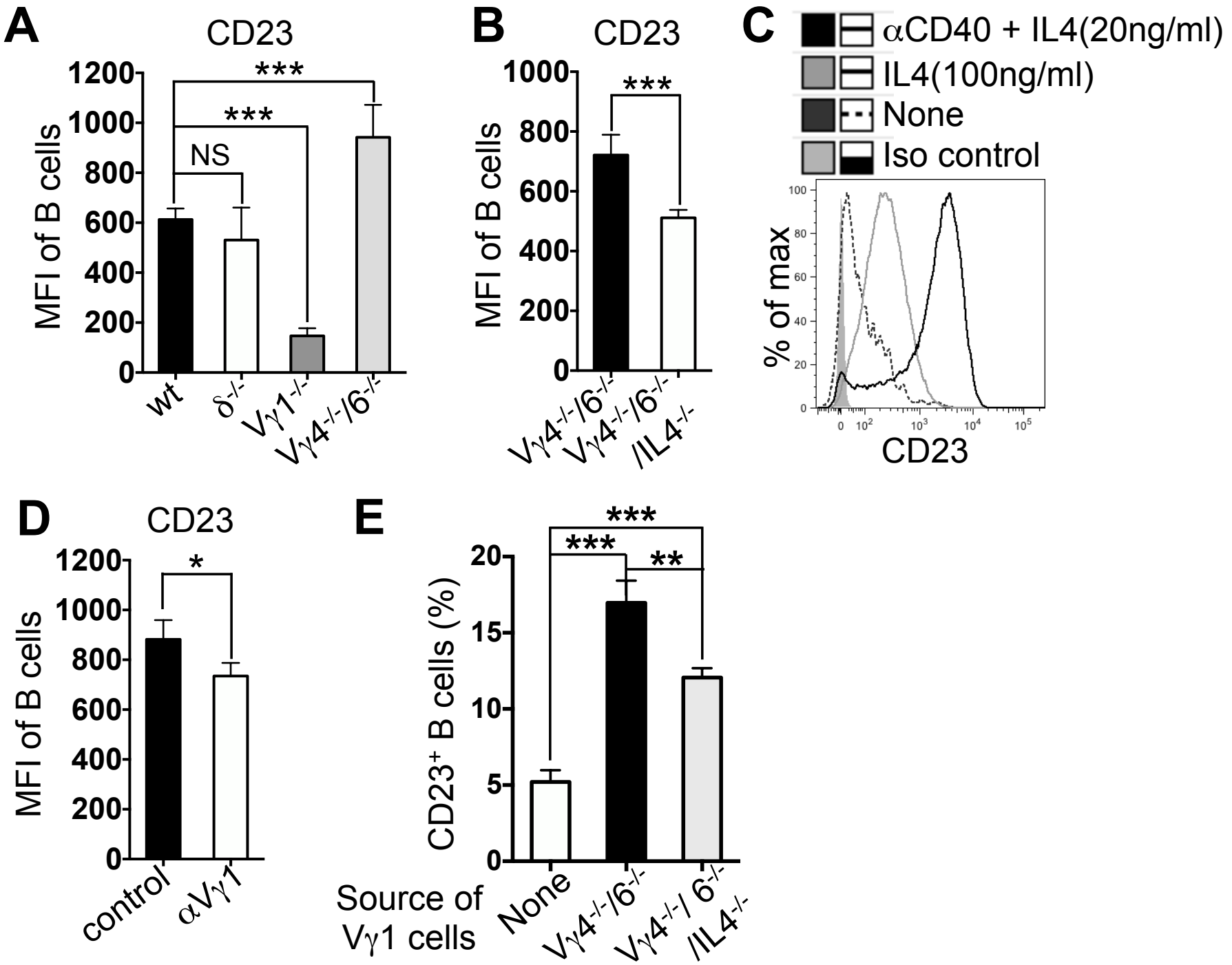


Figure S4 Effect of IL-4 on CD23 expression by B cells of B6.TCR-V$\gamma1^{-/-}$ mice.

(A) Comparison of CD23 expression (MFI) among total splenic B cells from C57BL/6 (wt), B6.TCR-$\delta^{-/-}$ ($\delta^{-/-}$), B6.TCR-V$\gamma1^{-/-}$ (V$\gamma1^{-/-}$) and B6.TCR-V$\gamma4^{-/-}$/$6^{-/-}$ (V$\gamma4^{-/-}$/$6^{-/-}$) mice.
(B) Comparison of CD23 expression (MFI) among total splenic B cells from B6.TCR-V$\gamma4^{-/-}$/$6^{-/-}$ (V$\gamma4^{-/-}$/$6^{-/-}$) and B6.TCR-V$\gamma4^{-/-}$/$6^{-/-}$/IL-$4^{-/-}$ (V$\gamma4^{-/-}$/$6^{-/-}$/IL-$4^{-/-}$) mice.
Panels A and B: n = 5 mice per group, ***P<0.001
(C) IL-4 alone or in combination with anti CD40 antibody restores CD23 expression on splenic B cells derived from B6.TCR-V$\gamma1^{-/-}$ (V$\gamma1^{-/-}$) mice. CD43-negative MZ B cell-rich splenic B cells from B6.TCR-V$\gamma1^{-/-}$ (V$\gamma1^{-/-}$) mice were cultured for 60 hrs alone or in the presence of IL-4 or IL-4 plus anti CD40 mAb. B cells were subsequently stained for CD23. Prior to the treatment, B cells of these mice expressed CD23 at lower levels than wt B cells. Data shown are representative of several similar experiments.