

Supplemental. *Optimal Germinal Center B cell Activation and T-dependent Antibody Responses Require the Expression of the Mouse Complement Receptor Cr1*

Supplemental Figure 1. Representative histograms of CD19 expression on bone marrow, peritoneal, and splenic B cells. *A* and *B*, Total bone marrow, peritoneal cavity, or splenic cells were isolated from naïve WT (filled histogram/black bars), *Cr1/2KO* (gray line histogram/gray bars), and *Cr1KO* (black line histogram/white bars) mice and surface CD19 expression was analyzed by FACS. *A*, Representative histograms of CD19 expression on pro-B, pre-B, immature, and mature B cells (top four plots); B1a and B1b cells from the peritoneal cavity (middle two plots); and total splenic B cells (bottom plot). *B*, Quantification of the geometric mean fluorescent intensity (gMFI) of CD19 staining by FACS analysis on B cell populations from the bone marrow, peritoneal cavity, and total splenic B cells. Bars represent the average gMFI for each genotype. CD19 gMFI was analyzed by ANOVA for each population and determined to be at least $p < 0.05$ where significance is shown pairwise (* = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$ by the student's t-test; $n = 3, 4$; sex matched 8-12 week old mice).

Supplemental Figure 2. RT-PCR quantification of splenic inflammatory gene expression. Intron spanning primer sets specific for arachidonate 5-lipoxygenase (*Alox5*), D site albumin promoter binding protein (*Dbp*), cathepsin G (*Ctsg*), coagulation factor V (*Fv*), lipocalin 2 (*Lcn2*), and killer cell lectin-like receptor, subfamily A, member 18 (*Klra18*) were used to quantify gene expression in mRNA obtained from total splenic extracts. All sample quantifications were normalized to 1000 β -actin transcripts. Bars represent the mean fold change from WT (center line) for *Cr1/2KO* (black) and *Cr1KO* (white). (WT $n = 3$, *Cr1KO* and *Cr1/2KO* $n = 4$; 12-16 week old female mice; BALB/C background; Error bars represent SEM; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ by student's t-test).

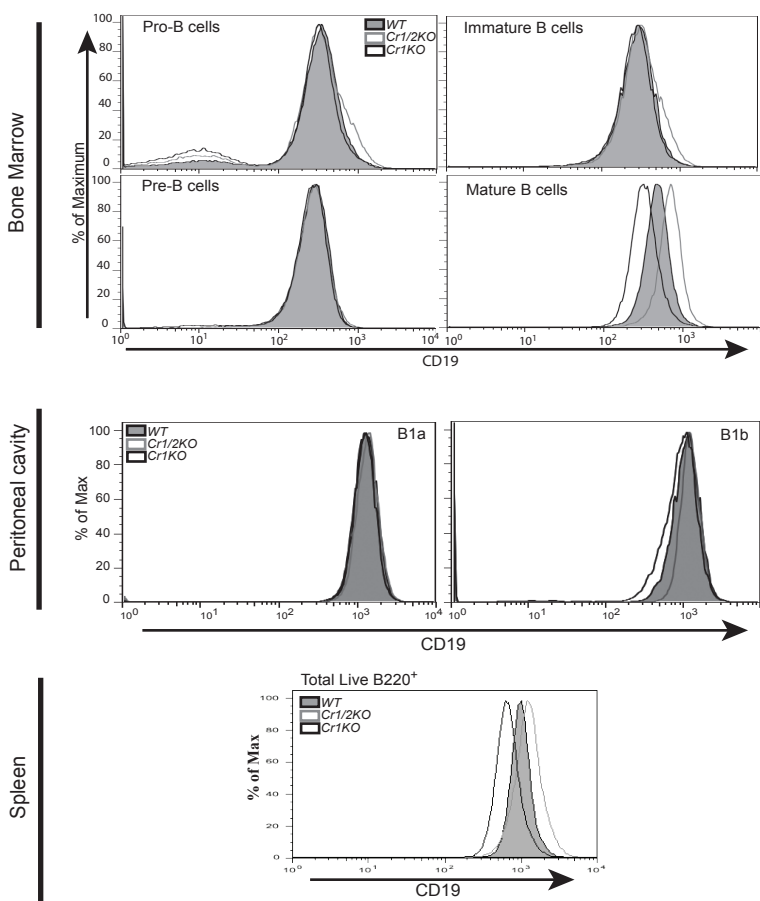
Supplemental Figure 3. TI-1, TI-2, and TD antigen specific immunoglobulin responses. Experiments were performed in which WT, *Cr1/2KO*, and *Cr1KO* mice

were immunized with the model antigens (A) TNP-LPS (TI-1) and (B) DNP-AECM-Ficoll (TI-2), as well as (C) TNP-KLH (TD) at both a low dose immunization (10 μ g) and high dose immunization (100 μ g). All mice that were immunized with TNP-KLH were administered a secondary immunization at 21 days. Serum samples were collected at seven day intervals post-immunization, and antigen specific immunoglobulin (IgM, IgG1, IgG2b, IgG2c, and IgG3) abundance was measured by ELISA. All data graphed here are included in the larger data set shown in **Fig.9**. (TI-1 n=4-7; TI-2 n=6; TD (low) n=4; TD (high) n=5; 8-12 week old sex matched mice; * = p<0.05; ** = p<0.01 significance between WT and *Cr1KO* by student's t-test).

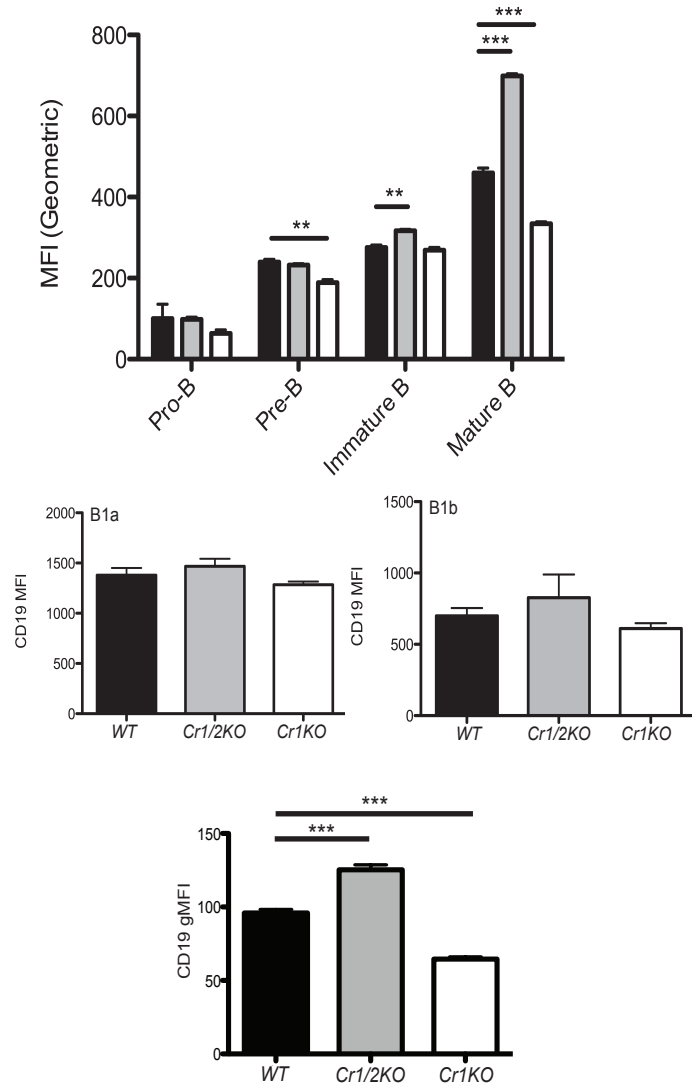
Supplemental Figure 4. FACS analysis of *C3KO* GC B cells following SRBC immunization. Percentage of live B220⁺ splenocytes that are IgD^{int} Fas⁺ in SRBC immunized WT and *C3KO* mice compared to mock PBS immunized mice and representative dot plots. (n=8 immunized, n=2 PBS; Error bars represent SEM; ns=not significant; *=p<0.05, **=p<0.01, ***=p<0.001 by student's t-test).

Supplemental Figure 1

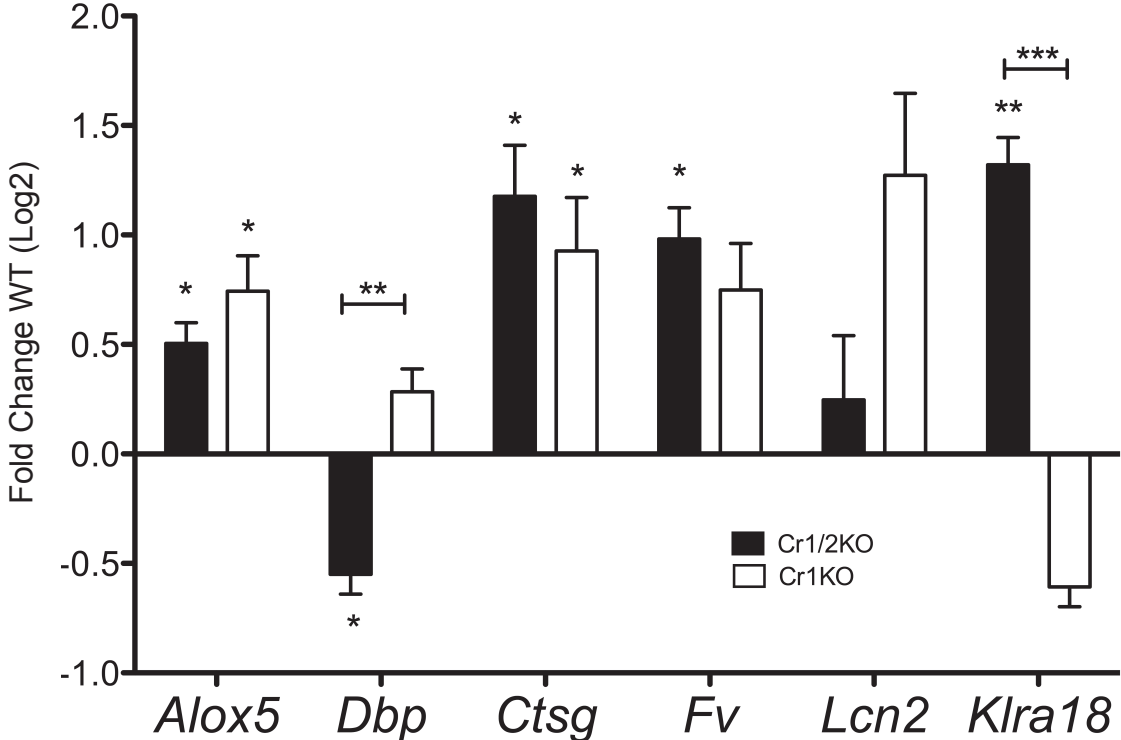
A



B

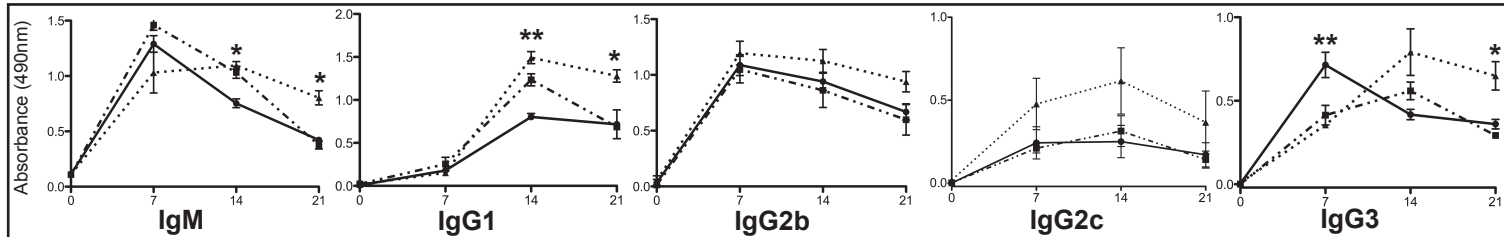


Supplemental Figure 2

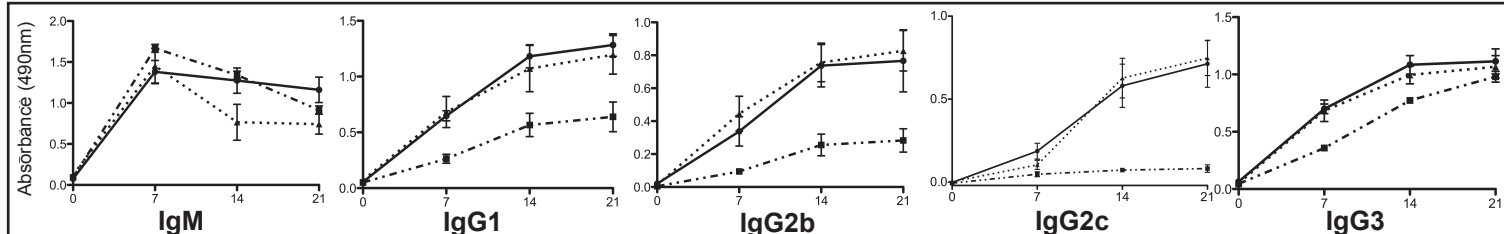


Supplemental Figure 3

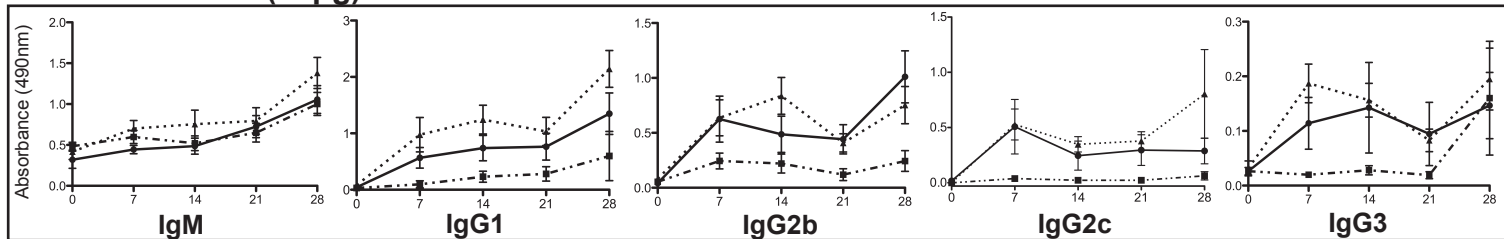
A TI-1 TNP-LPS



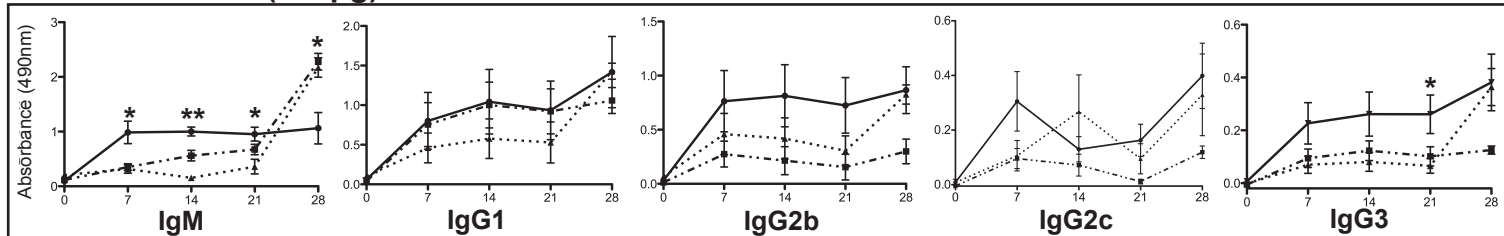
B TI-2 DNP-AECM-Ficoll



C TD TNP-KLH (10µg)



TD TNP-KLH (100µg)



WT ●—● Cr1/2KO ■—■ Cr1KO ▲—▲

Supplemental Figure 4

