FIGURE S1

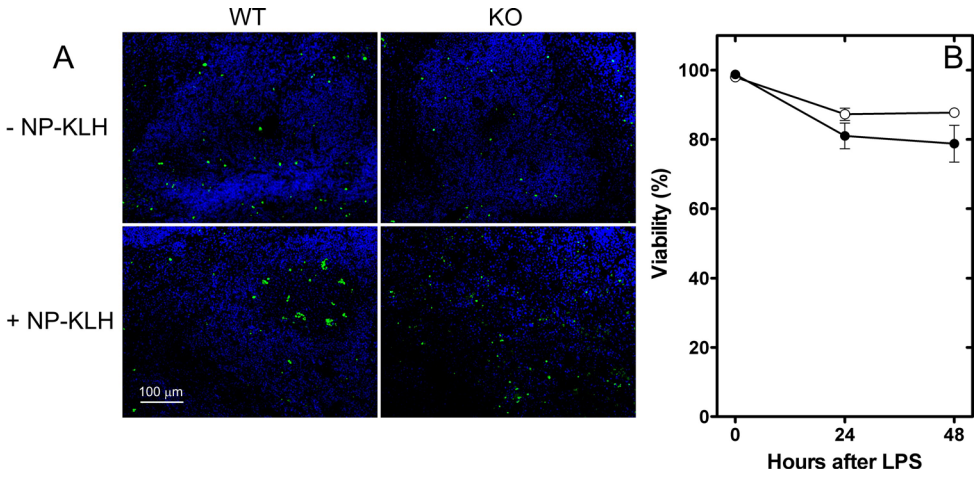

Fig. S1. Splenic apoptosis and B-cell viability. (*A*) WT or KO mice were injected with either saline or NP-KLH and spleens were isolated and frozen in OCT™, a cryoprotectant, seven days later. Cryosections (4 μm thickness) were stained using the TUNEL assay to indicate apoptosis (green) and with DAPI (blue) to indicate cell nuclei. Apoptotic clusters in the immunized WT spleens coincide with the germinal centers and reflect the selective degradation of B-cells expressing lower affinity IgGs. The data are representative of spleens isolated from 3 mice of each genotype. (*B*) Viability was determined using trypan blue exclusion in cultures of either WT (○) or KO (●) at indicated times after LPS stimulation. The data are the mean ± standard error from duplicate determinations in two independent experiments.

FIGURE S2

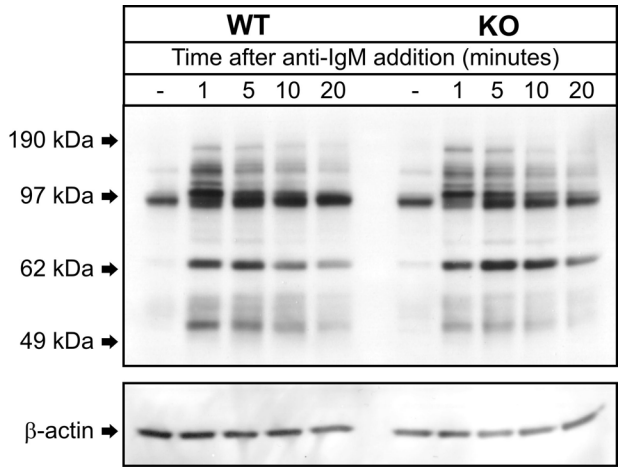

Fig. S2. Signaling in WT and CCT$\alpha^{-/-}$ B-cells in response to anti-IgM. WT and KO B-cells were incubated in vitro with anti-mouse IgM for the indicated times to trigger the signaling cascade mediated by the B cell receptor. Cell lysates were separated on SDS-PAGE and immunoblots were prepared using an anti-phosphotyrosine primary antibody (4G10, Millipore Corporation) followed by secondary antibody provided in the ECL detection kit (Amersham). Blots were stripped and re-probed with primary antibody against β-actin (Sigma-Aldrich). The blot is representative of 4 independent experiments with the same results.

FIGURE S3

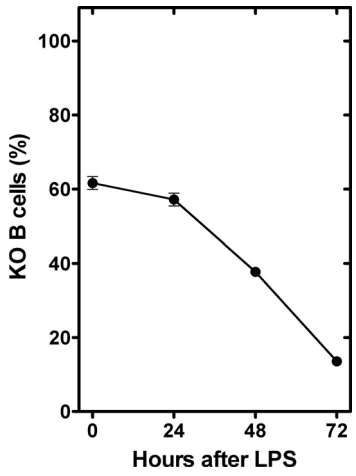

Fig. S3. CCTα transcript distribution in LPS-stimulated KO B-cell population. B-cells from KO spleens were cultured with LPS and the relative number of intact (WT) and deleted (KO) transcripts were quantified by PCR at times indicated. Data are the mean ± standard error from duplicate determinations in two independent experiments.

FIGURE S4

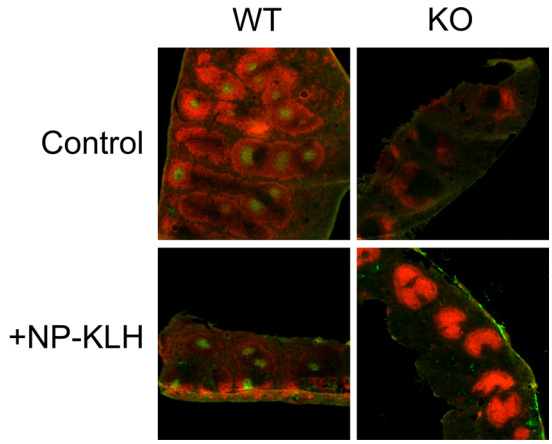

Fig. S4. Splenic germinal centers in naïve and immunized mice. Spleens from mice immunized with NP-KLH and control mice treated with adjuvant only were isolated and frozen in OCT™ cryoprotectant 7 days after immunization. Frozen splenic sections were stained to detect B-cells with fluorescently labeled anti-B220 (red) and with labeled peanut agglutinin (green) to detect germinal centers. The figure is representative of three individual mice per genotype per treatment.

FIGURE S5

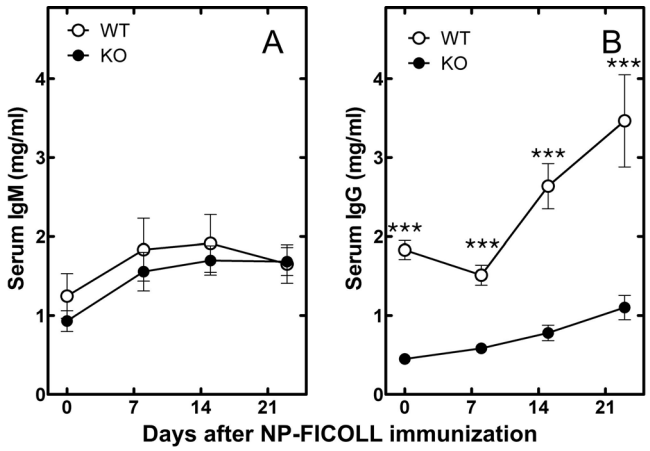

Fig. S5. Immunization with NP-FICOLL. WT (O) or KO (●) mice were immunized with NP-FICOLL to elicit a T cell-independent immune response from the B-cell population. At times indicated, serum IgM and IgG levels were determined using an ELISA assay. The data are the mean ± the standard error from 4 mice per group.

FIGURE S6

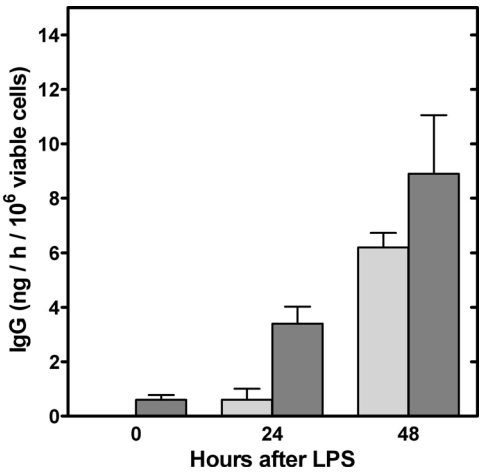

Fig. S6. Rate of IgG secretion in vitro. The amount of IgG secreted in vitro during a 1 hour period from KO (dark gray) or WT (light gray) B-cells was determined before and 24 or 48 hours after stimulation with LPS. The data are the mean ± standard error from at least three independent determinations in two independent experiments.

FIGURE S7

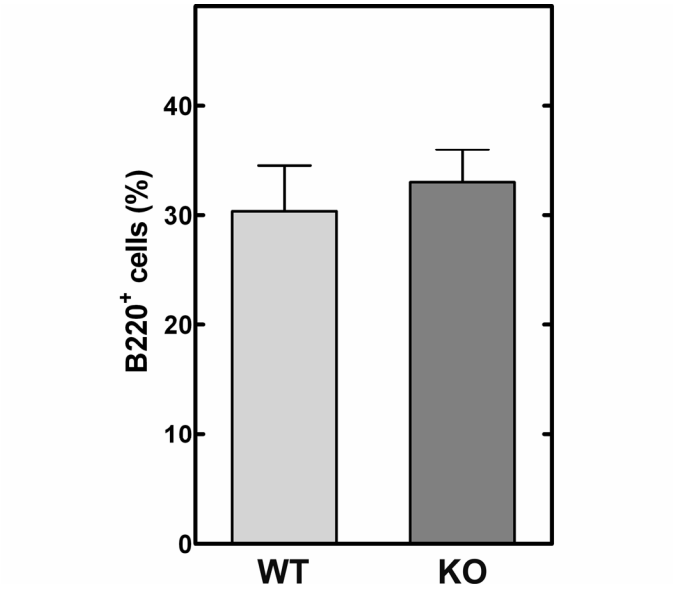

Fig. S7. Percentage of B-cells in bone marrow. The number of $B220^+$ cells in bone marrows isolated from adult (age 10-12 weeks) WT (light gray; n = 3) or KO (dark gray; n = 5) mice, was expressed as a percentage of the total number of cells. The data are the mean values $\pm$ standard error.