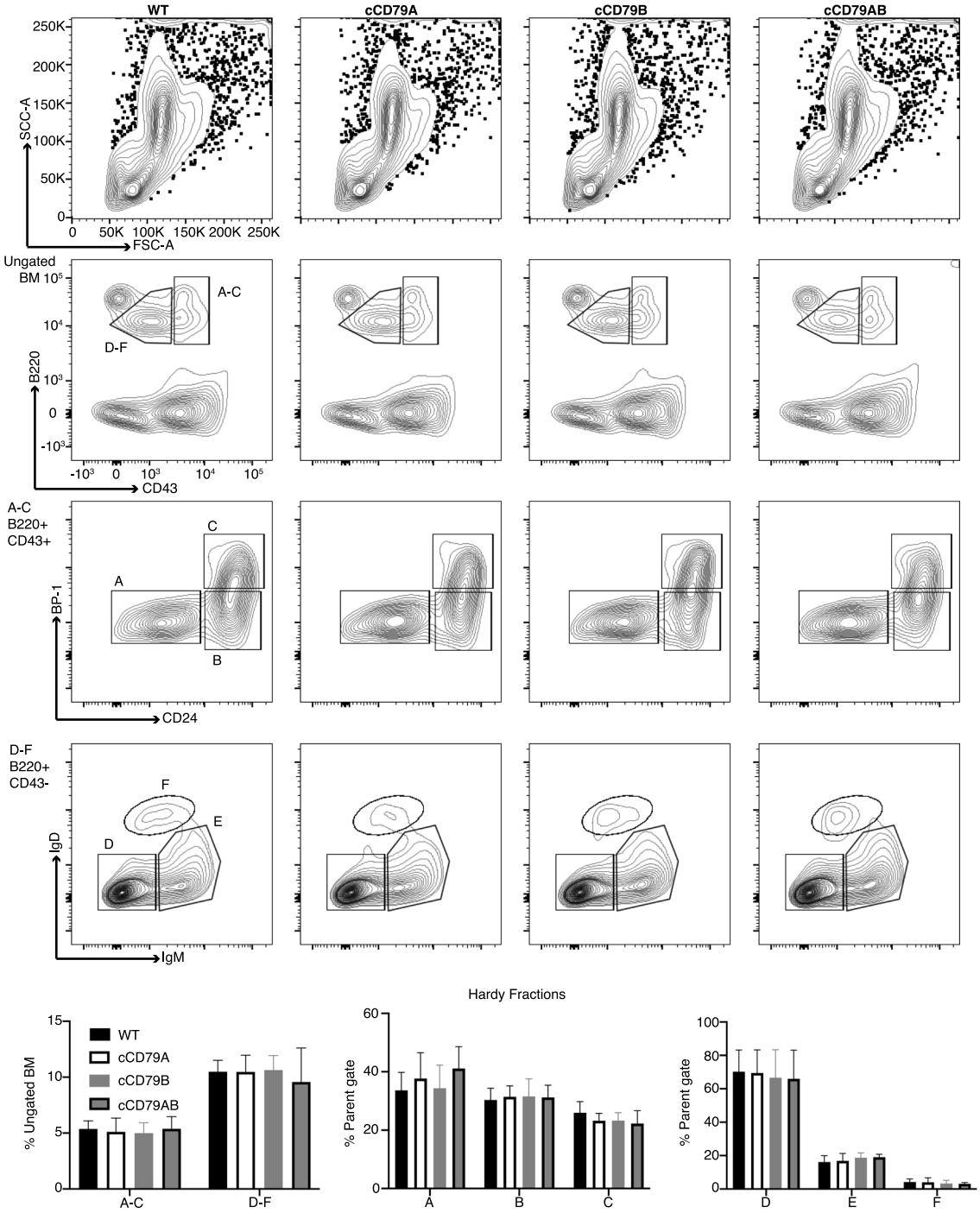
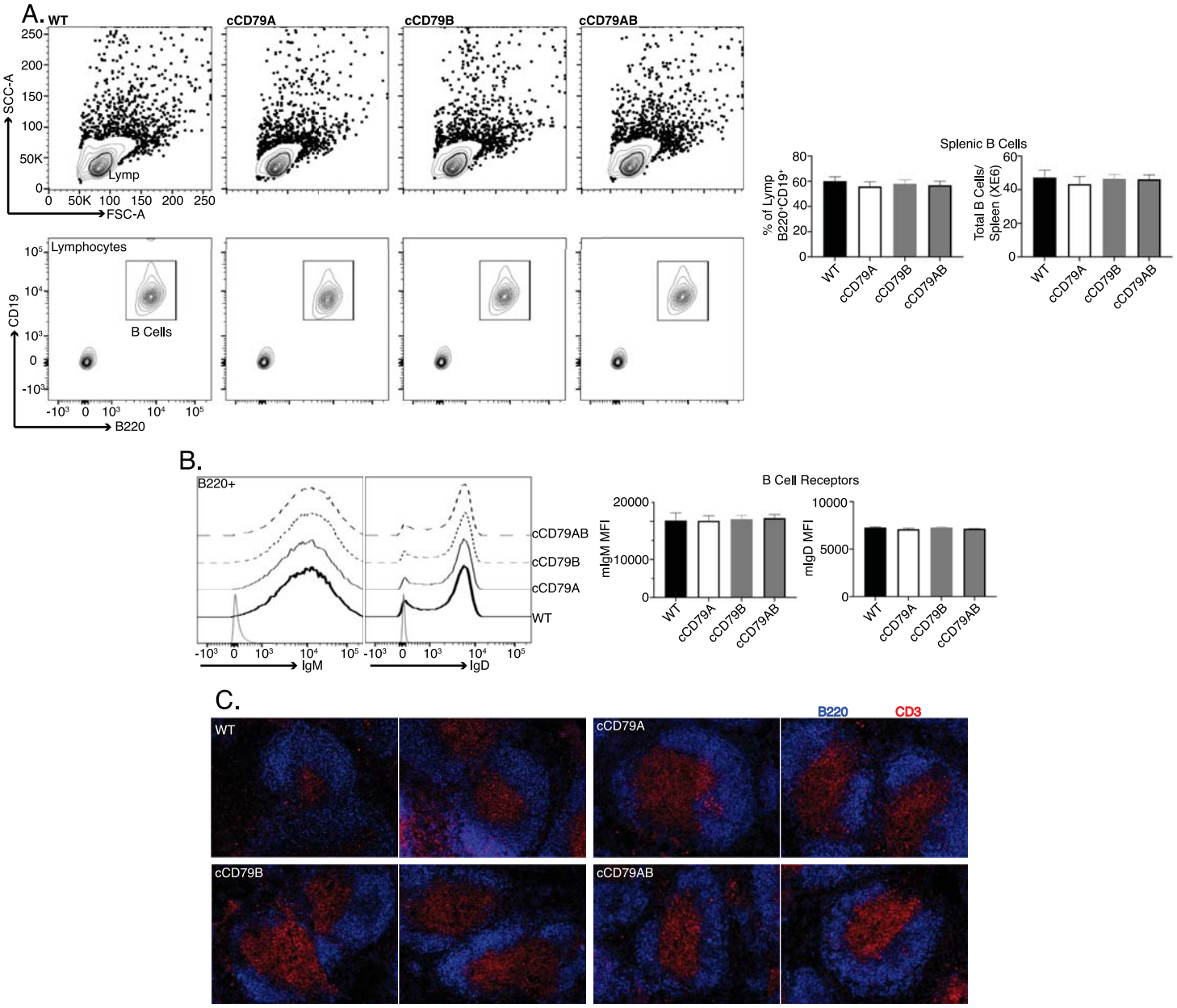
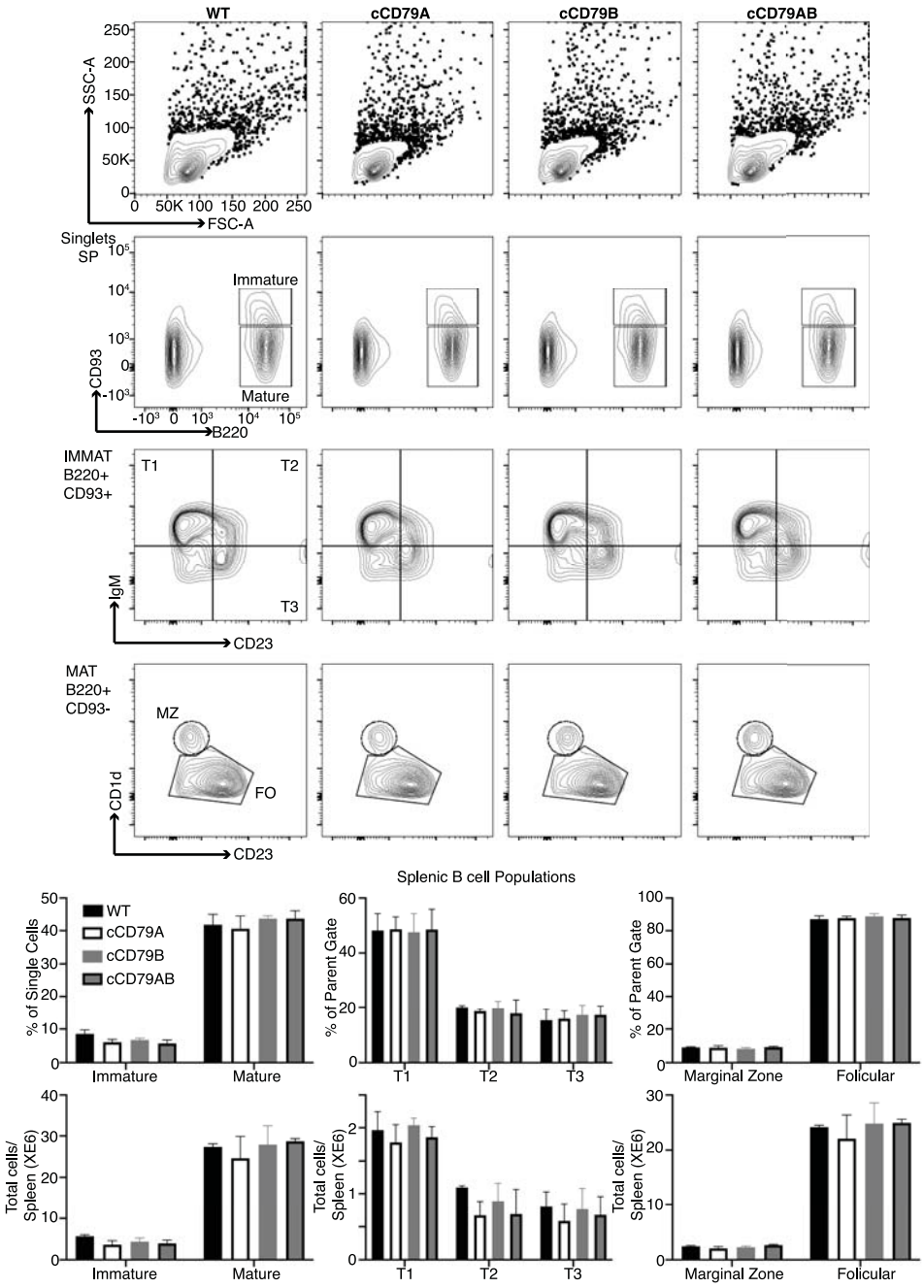


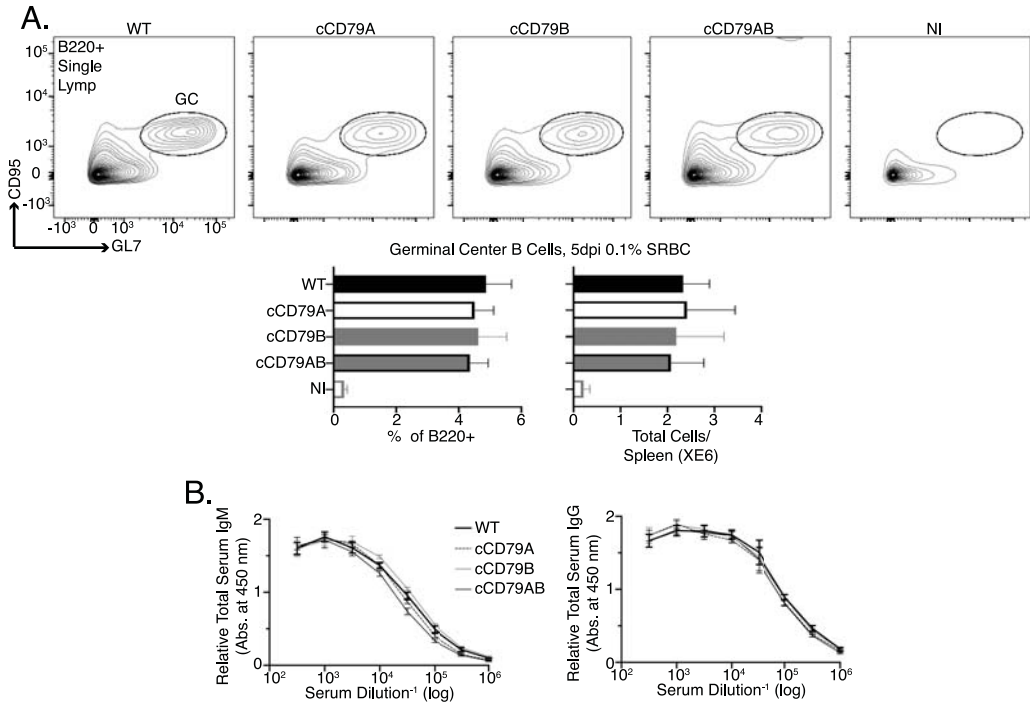
Supplemental Fig 1. B cell developmental populations appear normal in the bone marrow of cCD79 mice. Pre- and mature B cell antigen receptor expression and function are critical for proper development and function of the entire B cell compartment. To explore potential disruption of this process by cCD79, we characterized B cell development, BCR signaling and, B cell function in these mice (Supplamental Figs 1-4). To determine whether cCD79 manipulations impacted B cell development we analyzed B cell subpopulations in bone marrow of cCD79AB, cCD79A, cCD79B and wild-type mice using 6-color flow cytometric analysis to delineate developmental populations, i.e. Hardy fractions, spanning germline-arranged pro B cells to immature follicular-like B cells. Early B cell progenitors express B220 and CD43 and include both germline-arranged (Fraction A) and D-to-J rearranged (Fraction B/C) populations. Bone marrow B cells that are $B220^{+}$ and $CD43^{-}$ include pre-B cells expressing cytoplasmic IgM (Fraction D), immature B cells expressing surface IgM only (Fraction E) and, recirculating mature B cells expressing both IgM and IgD on the cell surface (Fraction F). As shown in Supplamental Fig 1, based on comparison with wild type mice, homozygous expression of chimeric CD79 supported normal B cell development in the bone marrow; all phenotypic populations are present at frequencies consistent with those observed in unmanipulated mice. There was no apparent difference in the size of the bone marrow B cell compartment in these mice. Representative cytograms and gating of RBC-depleted bone marrow cells from chimeric and control mice are shown here. Fractions A-C ($B220^{+}CD43^{+}$): A ($BP\text{-}1^{-}CD24^{-}$) Pre-pro B cells; B ($BP\text{-}1^{-}CD24^{+}$) Pro B cells; C ($BP\text{-}1^{+}CD24^{+}$) Large Pre-B cells. Fractions D-F ($B220^{+}CD43^{-}$): D ($IgM^{-}IgD^{-}$) Small Pre-B cells; E ($IgM^{+}IgD^{-}$) Immature transitional B cells; F ($IgM^{+}IgD^{+}$) Mature B cells. Proportions of Hardy fractions A-F depicted below. n= 5 male and 5 female mice per group. Error bars represent SEM. All data represents at least 3 independent experiments, representative data shown.



Supplemental Fig 2. Peripheral B cell populations appear normal in the spleens of cCD79 mice. We performed similar characterization of peripheral B cells from spleens of wild-type and homozygous cCD79 knock-in mice. Both frequencies and absolute numbers of B220+ CD19+ B cells were observed to be unaffected by the presence of the chimeric BCR components (Supplemental Fig 2A). Similarly, surface expression of neither IgM nor IgD were altered in cCD79 mice (Supplemental Fig 1, fraction F and Supplemental Fig 2B). This is important because CD79 is required for surface expression of immunoglobulin on B cells. In order to further assess B cell populations within the spleen, we utilized immunofluorescence microscopy. Accurate positioning of B cells within the spleen is vital to function when mounting an immune response. Thus, we wished to confirm immune cell orientation within the splenic architecture of the cCD79 mice. Supplemental Fig 2C shows no abnormality in splenic organization in the cCD79 mice, i.e., separation of B and T cells within the follicular and T cell zones. (A.) Representative cytograms and gating of RBC-depleted spleen cells from chimeric and control mice. Frequency and abundance of $CD19^{+}$ $B220^{+}$ B cells depicted below. n=3 male and 3 female mice per group. (B.) Flow analysis of IgM and IgD surface expression on B cells ($B220^{+}$). Gray lines show $B220^{-}$. n=3 male and 3 female mice per group. (C.) Immunofluorescence analysis of splenic architecture. B cells (B220) Blue; T cells (CD3) Red. 10X magnification. All data represents at least 3 independent experiments, representative data shown.



Supplemental Fig 3. Sub-populations of splenic B cells are unaffected by expression of cCD79 in mice. By flow cytometry, splenic B cells are further separated into populations of maturing transitional B cells and fully mature marginal zone and follicular cells. The immature B cell population (B220^{+} CD93^{+}) can be further subdivided into T1, T2 and, T3 as a function of IgM and CD23 expression. Mature B cells (CD93^{-} B220^{+}) can be distinguished as either marginal zone or follicular based on CD1d and CD23. In terms of surface phenotype, frequency and absolute numbers, splenic B cell populations in the cCD79 mice do not differ from those found in wild-type mice. Representative cytograms and gating of RBC-lysed spleen cells. Immature B cells (B220^{+} CD93^{+}): T1 (IgM^{+} CD23^{-}); T2 (IgM^{+} CD23^{+}); T3 (IgM^{low} CD23^{+}). Mature B cells (B220^{+} CD93^{-}): MZ (CD1d^{+} CD23^{-}); FO (CD1d^{+} CD23^{+}). Frequency and abundance of splenic B cell populations depicted below. n= 5 male and 5 female mice per group. All data represents at least 3 independent experiments, representative data shown.



Supplemental Fig 4. In vivo B cell activation and steady-state immunoglobulin concentrations are normal in cCD79 mice. We wished to approximate induction of germinal centers by immunizing WT and cCD79 knock-in mice with sheep red blood cells and analyzing by flow 5 days later. Here we see no differences in the frequency and number of $B220^{+}$ $GL7^{+}$ Fas^{+} B cells between knock-in and control animals. Also indicative of B cell function are levels of circulating immunoglobulins produced by their differentiated daughters. Serum IgM and IgG were found to be within the normal ranges in peripheral blood of cCD79 mice. In conclusion, B cell development, population of peripheral lymphoid organs and immunoglobulin levels appears to be unaffected by the expression of chimeric CD79. (A.) B cell activation in vivo. Approximation of germinal center ($B220^{+}CD95^{+}GL7^{+}$) formation 5 days post immunization with sheep red blood cells (0.1%, I.P.). n=4 male and 4 female mice per group. (B.) Relative, steady-state serum concentrations of IgM and IgG. n= 5 male and 5 female mice per group. All data represents at least 3 independent experiments, representative data shown.