

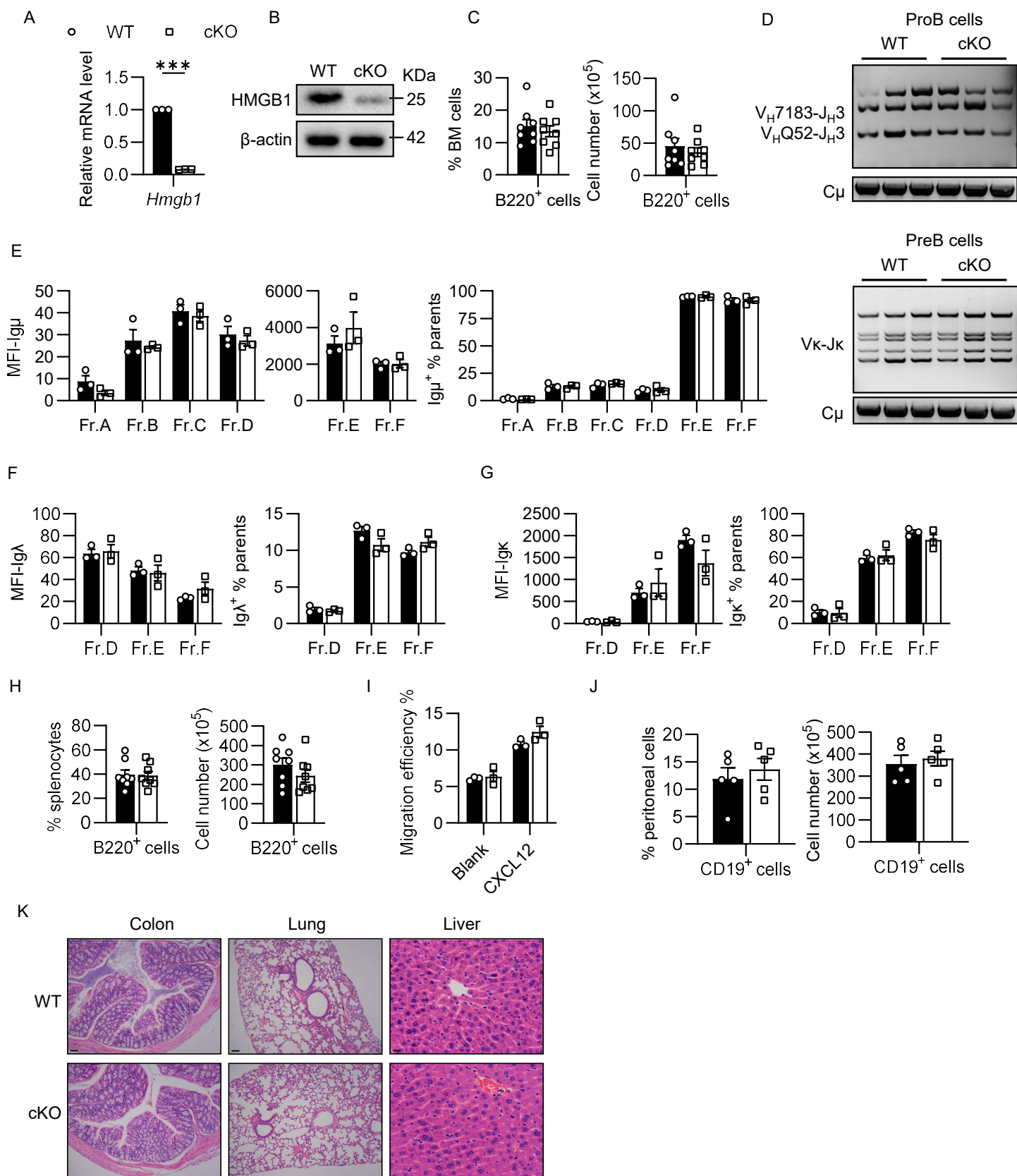


Fig. S1. HMGB1 is dispensable for maintaining total B cell numbers in the bone marrow, spleen, and peritoneal cavity, as well as for V(D)J recombination in B cells. (A) Quantitative PCR analysis of *Hmgb1* mRNA levels in purified splenic B cells from WT and cKO mice (n = 3). (B) Western blot analysis of HMGB1 protein expression in splenic B cells. (C, H and J) Mean proportions and absolute numbers of B220⁺ cells in bone marrow, spleen, and CD19⁺ cells in the peritoneal cavity. (D) Semi-quantitative PCR analysis of V_H and V_k chain rearrangements by degenerate primers in sorted bone marrow pro-B (B220⁺IgM⁻CD43⁺) and pre-B (B220⁺IgM⁻CD43⁻) cells (n = 3). (E-G). Flow cytometry analysis of Igμ, Igκ and Igλ MFI, as well as Igμ⁺, Igκ⁺ and Igλ⁺ populations, in bone marrow B cell subsets (n = 3) (I) Migration efficiency of splenic B cells toward CXCL12, assessed by Transwell assay (n = 3). (K) Representative H&E-stained sections of colon, lung, and liver (colon/lung: 10× objective, scale bar = 200 μm; liver: 40× objective, scale bar = 50 μm). Shown are representative images of H&E staining from one of three independent experiments. Data are representative of three independent experiments. Two-tailed unpaired Student's t-tests (A, C, H-J) and multiple t-tests (E-G) were used for statistical analysis. Data shown as mean ± SEM. *** p < 0.001.

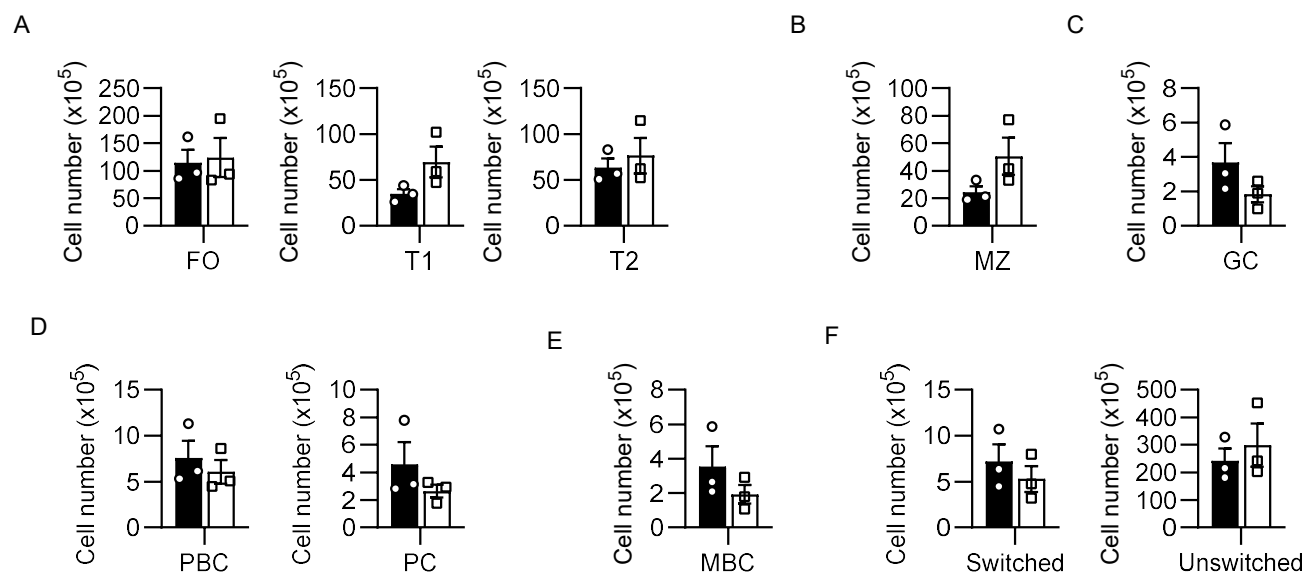


Fig. S2. The numbers of splenic cell subsets in cKO mice remains unchanged after being immunized by NP-Ficoll. (A-F) Absolute numbers of splenic B cell subpopulations in WT (n = 3) and cKO (n = 3) mice immunized with NP-Ficoll, analyzed by flow cytometry. Data are representative of three independent experiments. Two-tailed unpaired Student's t-tests were used for statistical analysis. Data shown as mean $\pm$ SEM. * p<0.05, ** p<0.01, *** p<0.001.

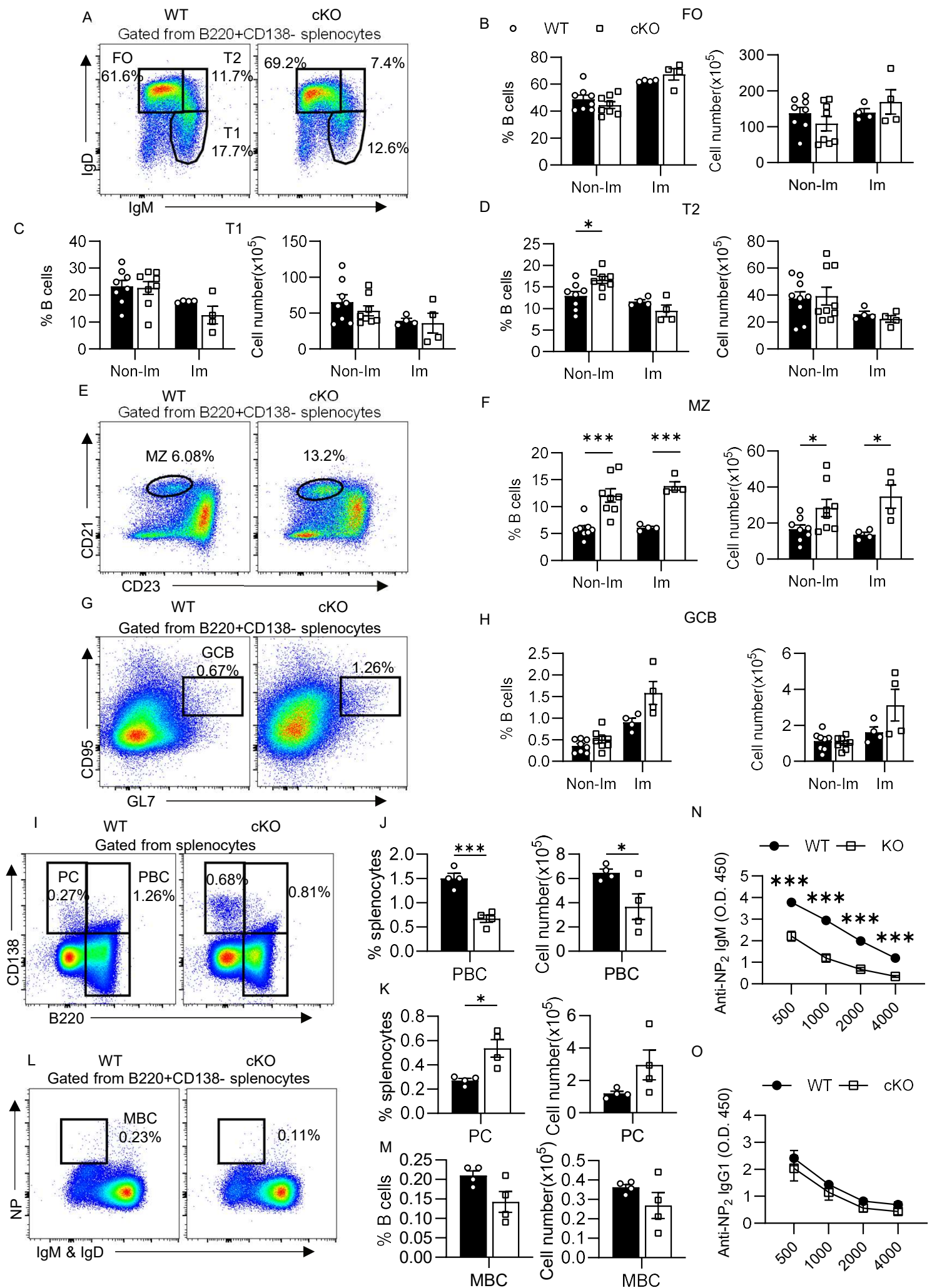


Fig. S3. HMGB1 participates in the primary TD-immune response. (A-M) Flow cytometry analysis of splenic cell subpopulations in WT (n = 4) and cKO (n = 4) mice 14 days after primary NP-KLH immunization. Mean proportions and absolute numbers are shown. (N-O) ELISA quantification of serum NP-IgM and NP-IgG1 titers (OD values) in primarily NP-KLH immunized mice. Data are representative of three experiments. Multiple t-tests (B-D, F, H, N-O) and two-tailed unpaired Student's t-tests (J-K, M) were used for statistical analysis. Data shown as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

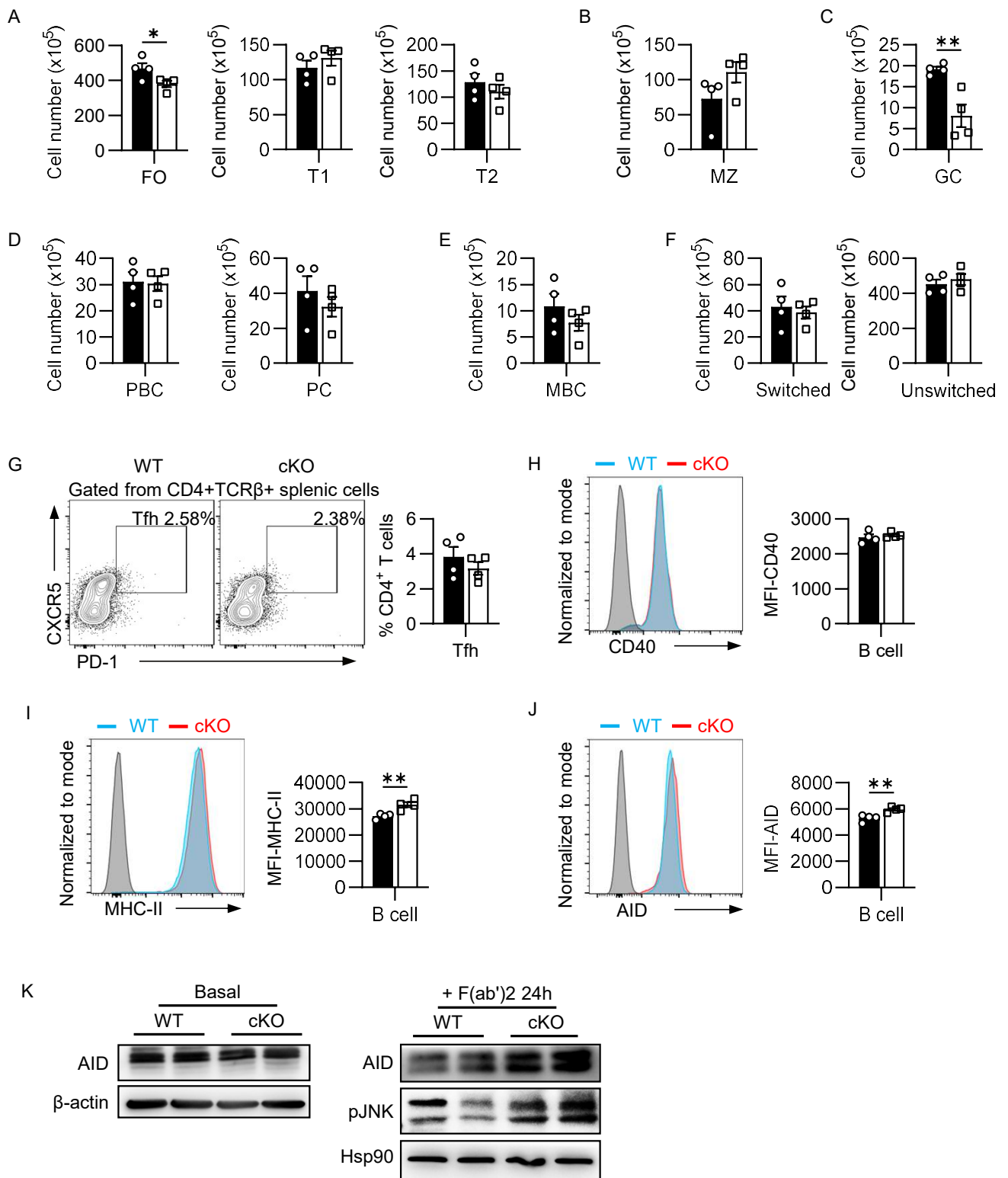


Fig S4. Secondary TD-immune response and signaling were altered in *Hmgbl* cKO mice. (A-F) Flow cytometry analysis of splenic subpopulation numbers in secondarily NP-KLH-immunized mice ($n = 4$). (G) Flow cytometry analysis for the proportion of Tfh cells. (H) Flow cytometry analysis for MFI of CD40, MHC II, and AID in B cells from secondarily NP-KLH-immunized mice. (K) Western blot analysis of AID expression in resting B cells, AID and pJNK1/2 expressions in B cells stimulated by anti-BCR for 24h. Data are representative of three experiments. Two-tailed unpaired Student's t -tests were used for statistical analysis. Data shown as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$.

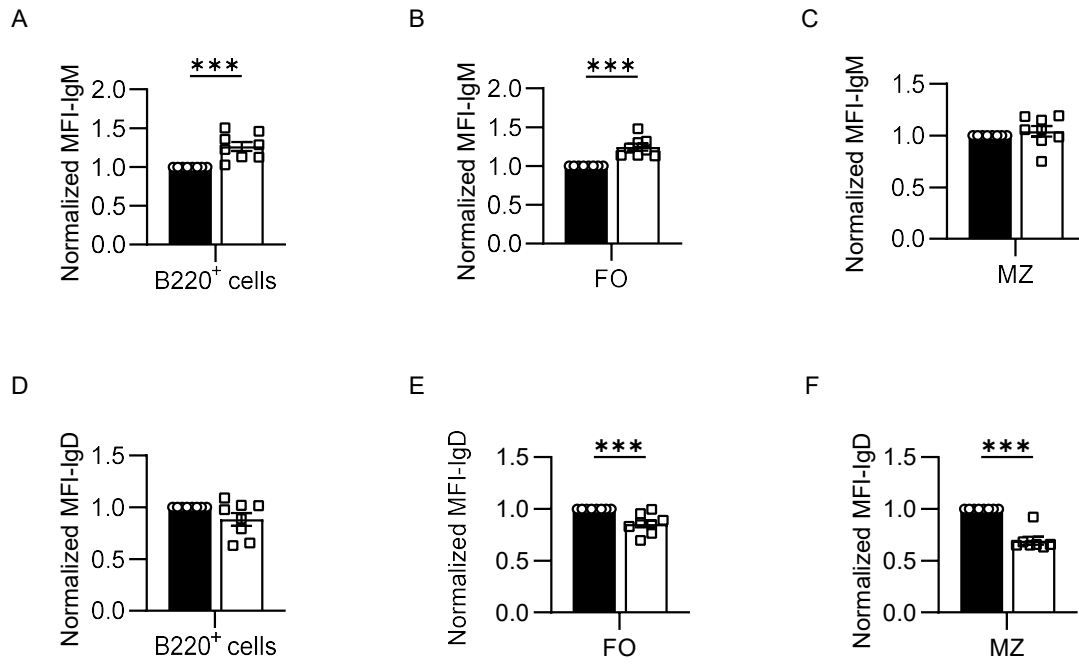


Fig. S5. Abnormal expressions of surface IgM and IgD in cKO B cell subsets. (A-C) Flow cytometry analysis of IgM MFI (n = 8) in B220⁺, FO, and MZ B cells. (D-F) IgD MFI in B220⁺ (n = 9), FO (n = 8), and MZ B cells (n = 7). Data are representative of three independent experiments. Two-tailed unpaired Student's t-tests were used for statistical analysis. Data shown as mean ± SEM. *** p<0.001.

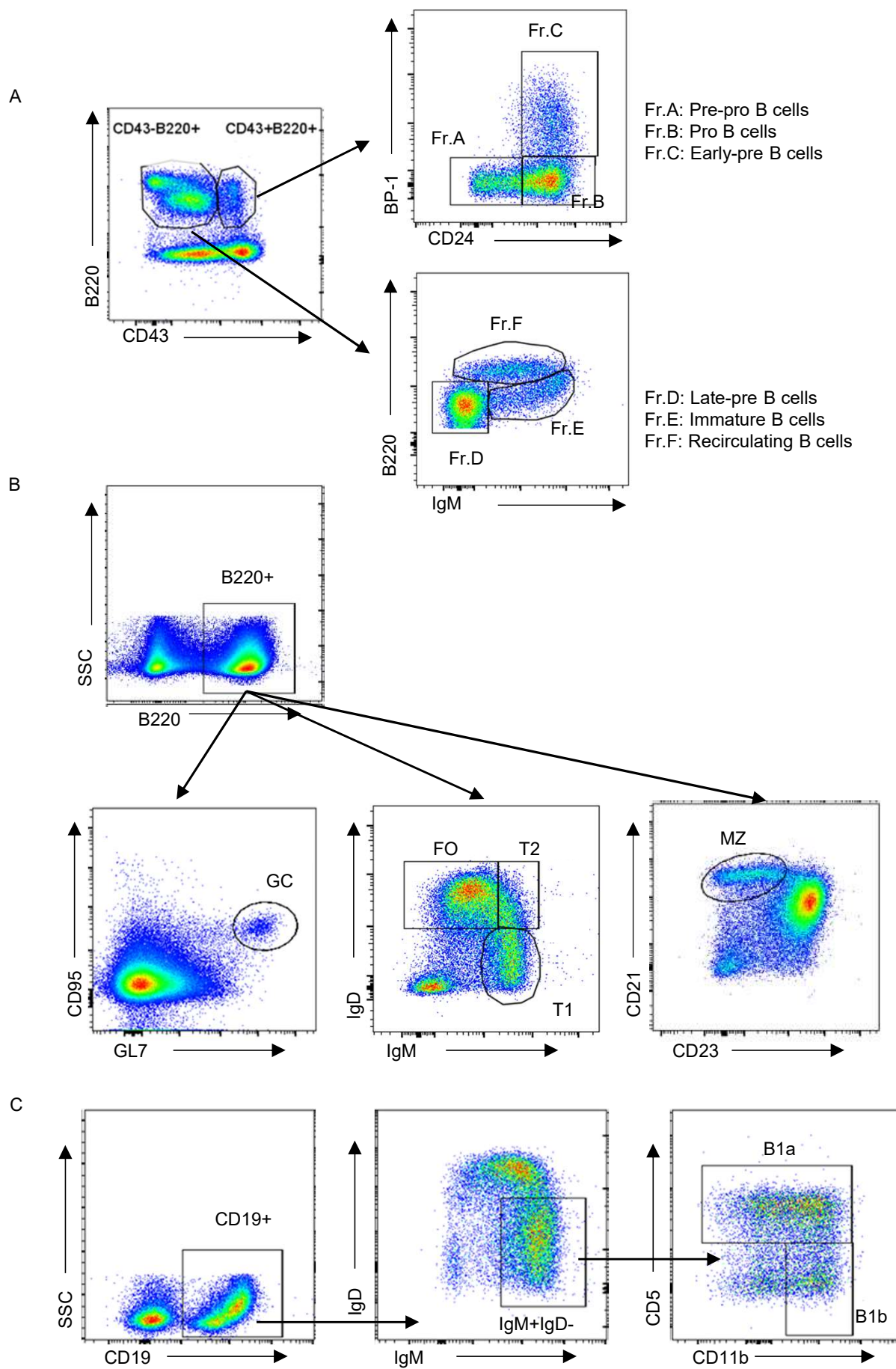


Fig. S6. Flow cytometry gating strategies. Gating schemes for: (A) bone marrow cells, (B) splenic lymphocytes, and (C) peritoneal cavity cells.