SUPPLEMENTAL MATERIAL

Fu et al., https://doi.org/10.1084/jem.20170901

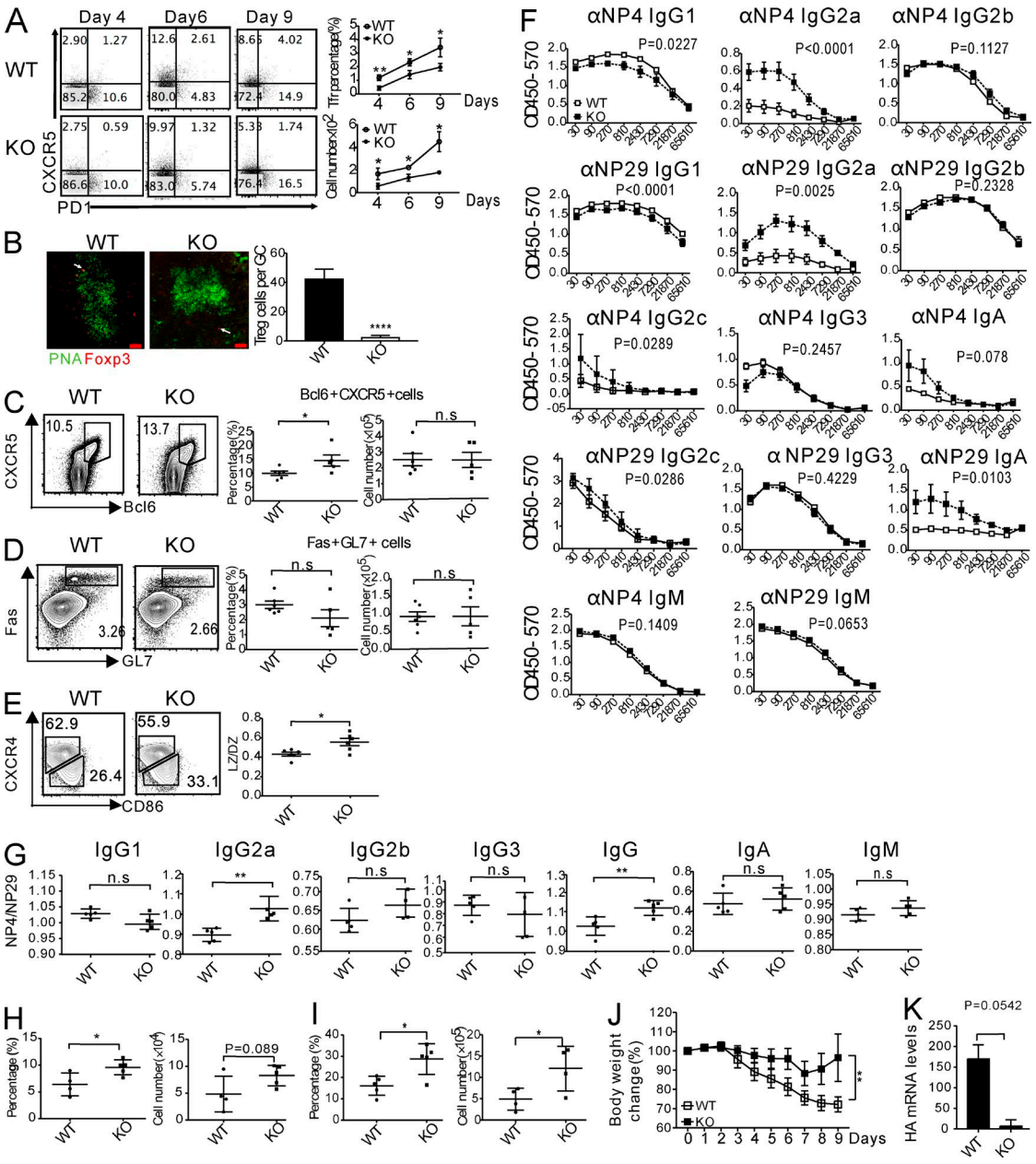

Figure S1. **Defects in Tfr cells do not greatly affect Tfh and GC B cell populations in primary immune response. (A)** Both WT and KO mice were immunized s.c. with KLH in CFA. FACS staining (left), frequency quantification (right, top row), and cell number (right, bottom row) analysis of CXCR5$^+$PD1$^+$ Tfr cells in CD4$^+$Foxp3$^+$ cells was performed by flow cytometry at various time points after immunization. *n* = 3 per group. **(B)** Cryosections of dLNs from the 9-d KLH-immunized mice were stained with PNA and anti-Foxp3 to identify Foxp3$^+$ cells in the GC. Total number of Foxp3$^+$ T reg cells in the GC was analyzed by confocal microscopy. Bars, 50 µm. *n* = 3 per group. **(C–F)** Control and KO mice were immunized with NP-KLH by s.c. injection. FACS staining (left), frequency quantification (middle), and cell number (right) analysis were performed on CXCR5$^+$Bcl6$^+$ Tfh cells (C) in CD4$^+$Foxp3$^-$ and GL7$^+$Fas$^+$ GC B cells (D) in B220$^+$ cells from dLNs obtained 9 d after s.c. immunization with NP-KLH. *n* = 5–6 per group. **(E)** GC (B220$^+$GL7$^+$Fas$^+$), LZ (CXCR4$^-$CD86$^+$), and DZ (CXCR4$^+$CD86$^-$) staining (left) and ratio analysis (right). *n* = 6. **(F)** Serum antigen-specific antibody was detected. *n* = 5 or 7 per group. 30 d after immunization, mice were given boosters with NP-KLH and IFA, and antibody affinity maturation (G) was analyzed on day 3 after challenge. **(H and I)** Frequency quantification (left) and cell number (right) analysis of GL7$^-$Fas$^-$IgD$^-$ CD38$^+$ memory B cells in CD19$^+$ B cells were done before (H) and on day 3 after (I) the secondary challenge. *n* = 4–5 per group. *Bcl6$^{fl/fl}$Foxp3Cre/Cre* were KO, and *Bcl6$^{fl/fl}$Foxp3WT/WT* mice from the *Bcl6$^{fl/fl}$Foxp3Cre/WT*x*Bcl6$^{fl/fl}$Foxp3WT* breeder were used as control. All data are a representative of two independent experiments. **(J and K)** Repeated results of influenza infection model using littermate *Bcl6$^{fl/wt}$ Foxp3Cre/Cre* as control. (J) Body weights of control and Tfr KO mice were monitored daily after influenza virus infection. (K) Mice were sacrificed at day 9 after infection, and viral titers in the lungs were assessed by quantitative RT-PCR measurement of active HA gene expression. *n* = 4 or 5 per group. Data shown are mean ± SEM; two-tailed *t* test; p-values in F and J were analyzed by two-way ANOVA; *, P < 0.05; **, P < 0.01; ***, P < 0.001; n.s., no significance.

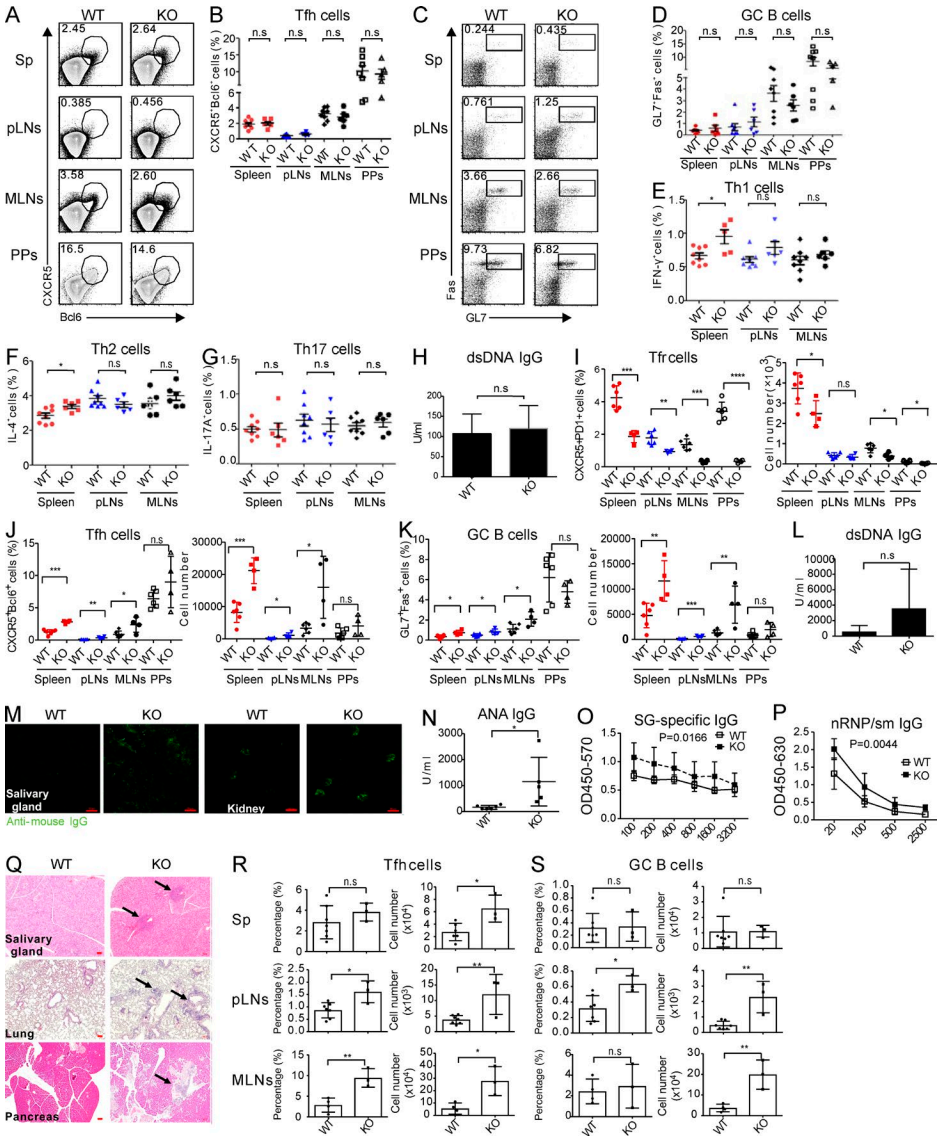

Figure S2. **Young *Bcl6*$^{fl/fl}$*Foxp3Cre/Cre* mice did not show autoimmunity. (A)** FACS staining of CXCR5$^+$Bcl6$^+$ Tfh cells among CD4$^+$Foxp3$^-$ cells in different organs of WT and KO mice. **(B)** Frequency quantification of Tfh cells in different organs of WT and KO mice. **(C)** FACS staining of GL7$^+$Fas$^+$ GC B cells among B220$^+$ cells in different organs of WT and KO mice. **(D)** Frequency quantification of GC B cells in different organs of WT and KO mice. **(E–H)** After restimulation with PMA and ionomycin for 5 h, IFN-γ (E), IL-4 (F), and IL-17A (G) expression in CD4$^+$ T cells from different organs was measured by flow cytometric analysis (H). Anti-dsDNA autoantibodies in the serum of WT and KO mice were measured by ELISA. All data are representative of two independent experiments. The mice used in A–H were 6–8-wk-old unmanipulated mice. *n* = 6–8 per group. **(I)** Flow cytometry quantification (top) and cell number (bottom) analysis of Bcl6$^+$CXCR5$^+$ Tfr cells in CD4$^+$Foxp3$^+$T cells from different organs of WT and KO mice. **(J)** Flow cytometry quantification (left) and cell number (right) analysis of Bcl6$^+$CXCR5$^+$ Tfh cells in CD4$^+$Foxp3$^-$ T cells from different organs of WT and KO mice. **(K)** Frequency quantification (left) and cell number (right) analysis of GL7$^+$Fas$^+$GC B cells in B220$^+$cells from different organs of WT and KO mice. *n* = 4 or 6 per group. **(L)** Anti-dsDNA autoantibodies in the sera of WT and KO mice were measured by ELISA. *n* = 4 or 6 per group. **(M)** Anti-IgG immunofluorescent staining of SG and kidney. Bars, 50 µm. *n* = 4 or 6 per group. The mice used in I–M were 12-wk-old unmanipulated mice. **(N)** Anti-ANA autoantibodies in the sera of WT and KO mice at 30 wk of age were measured by ELISA. *n* = 5 or 6 per group. **(O)** IgG autoantibodies against SG antigens in the sera of steady-state WT and KO mice were measured by ELISA. *n* = 5 or 11 per group. **(P)** Anti-nRNP/Sm autoantibodies in the sera of WT and KO mice were measured by ELISA. *n* = 6 per group. *Bcl6*$^{fl/fl}$*Foxp3Cre/Cre* were KO, and *Bcl6*$^{fl/fl}$*Foxp3WT/WT* mice from the *Bcl6*$^{fl/fl}$*Foxp3Cre/WTxBcl6*$^{fl/fl}$*Foxp3WT* breeder were used as control. **(Q–S)** Repeated data of the mice at 30 wk of age using littermate *Bcl6*$^{fl/fl}$*Foxp3Cre/WT* as control. (Q) Histopathology analysis of SG, lung, and pancreas from WT and KO mice at 30 wk of age. Black arrows indicate the immune cell infiltrates in KO mice. Bars, 100 µm. *n* = 3–7 per group. Flow cytometry quantitation (left) and cell number (right) analysis of CXCR5$^+$Bcl6$^+$ Tfh cells in CD4$^+$Foxp3$^-$ cells (R) and GL-7$^+$Fas$^+$ GC B cells in B220$^+$ cells (S) in different organs from the old steady-state control and KO mice. *n* = 3–7 per group. Sp, spleen; PPs, Peyer's patches. All experimental data were verified in at least two independent experiments. Data shown are mean ± SEM; two-tailed *t* test; *, P < 0.05; **, P < 0.01; ***, P < 0.001; n.s., no significance.

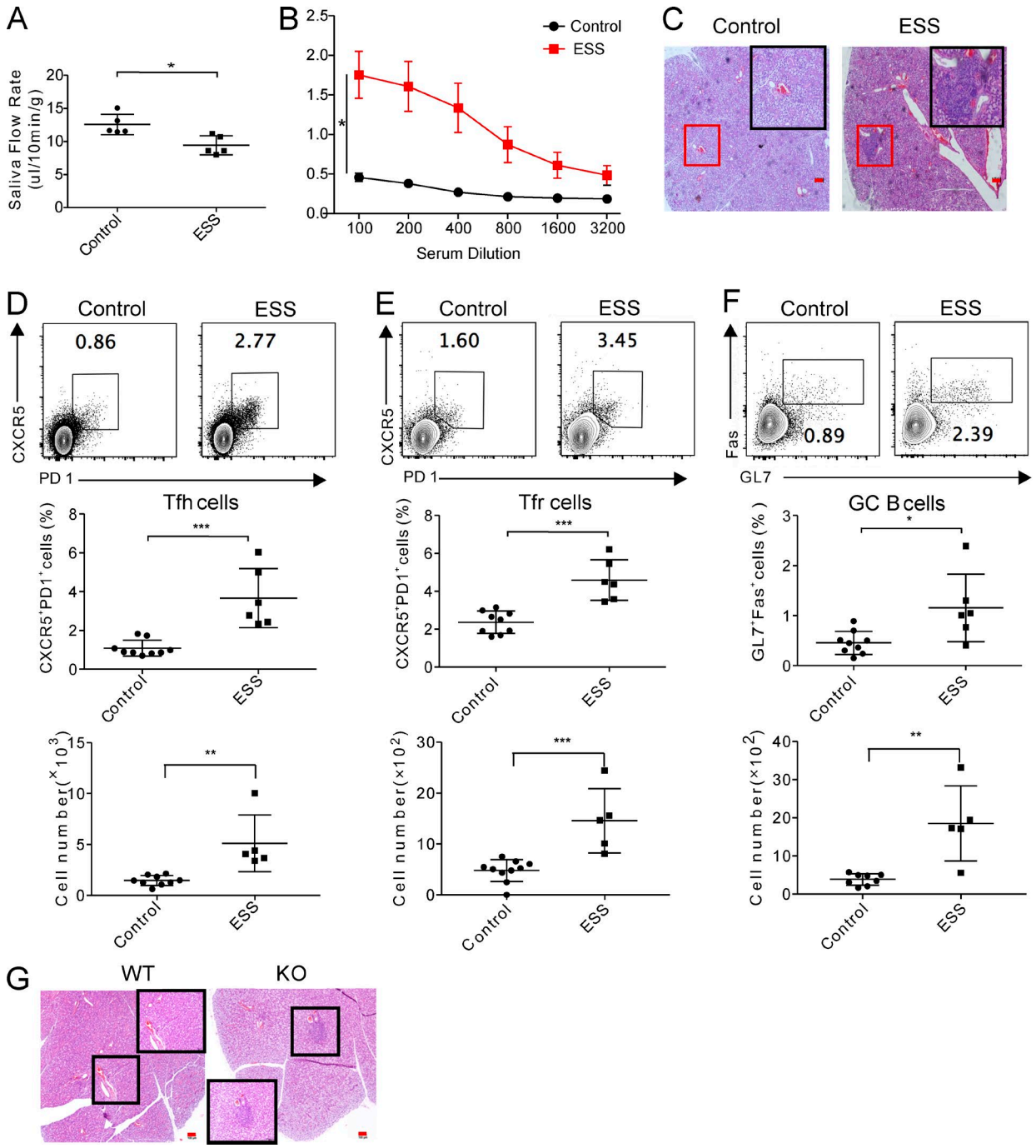

Figure S3. **Tfh, Tfr, and GC B cells were all elevated in the ESS model. (A)** Saliva flow rates were measured in SG protein-immunized mice for ESS induction (ESS) and control mice (control) immunized with adjuvant. Significant reduction in salivary secretion was detected in ESS mice on day 10 after immunization. $n = 5$ per group. **(B)** Autoantibodies against SG antigens were detected in the serum samples from ESS mice and controls 35 d after first immunization by ELISA. $n = 5$ per group. **(C)** Histological evaluation of glandular destruction in mice immunized with SG proteins for ESS induction and controls was performed on tissue sections of submandibular glands with H&E staining 15 wk after immunization. Bars, 100 µm. **(D–F)** FACS staining (top row), frequency quantitation (middle row), and cell number (bottom row) analysis of $CXCR5^+PD1^+$ Tfh cells among $CD4^+Foxp3^-$ cells (D), $CXCR5^+PD1^+$ Tfr cells among $CD4^+Foxp3^+$ cells (E), and Fas^+GL7^+GC B cells among $B220^+$ cells (F) in CLN at 15 wk after first immunization. $n = 6$ or 9 per group in C–F. All data are representative of two independent experiments; WT *Bcl6*$^{fl/fl}$*Foxp3WT/WT* mice from the *Bcl6*$^{fl/fl}$*Foxp3Cre/WTxBcl6*$^{fl/fl}$*Foxp3WT* breeder were used. **(G)** Repeated histological evaluation of glandular destruction in WT and KO mice 5 wk after ESS induction with H&E staining using littermate *Bcl6*$^{fl/fl}$*Foxp3Cre/WT* as control. Bars, 100 µm. WT, $n = 5$; KO, $n = 6$. Data shown are mean ± SEM; two-tailed *t* test; p-values in B were analyzed by two-way ANOVA; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., no significance.