SUPPLEMENTAL MATERIAL

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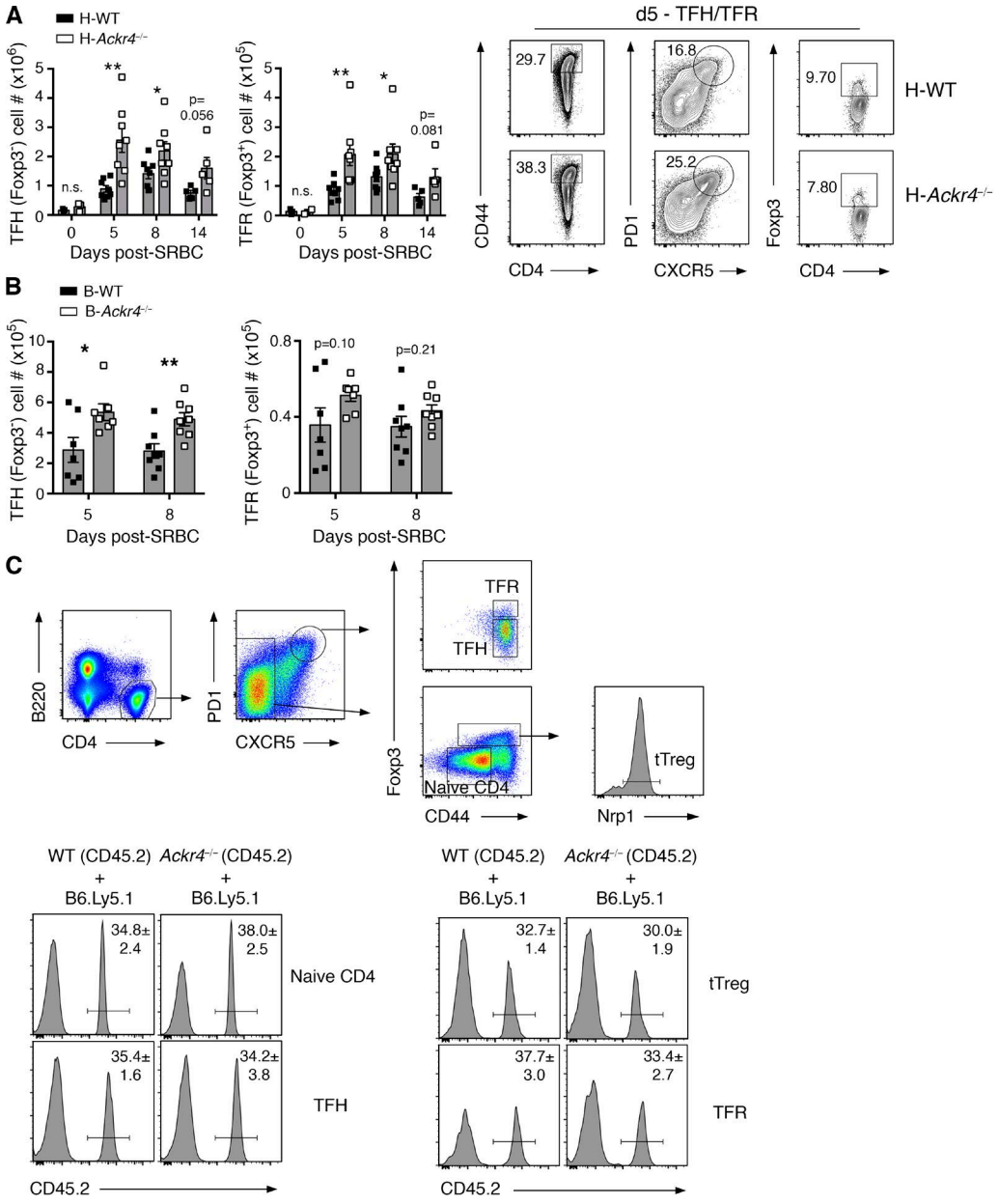

Figure S1. **Follicular T cell responses are enhanced in the absence of B cell ACKR4 (related to Figs. 1 and 3 A). (A)** TFH ($B220^{-}CD4^{+}CD44^{hi}PD1^{hi}CXCR5^{hi}Foxp3^{-}$) and TFR ($B220^{-}CD4^{+}CD44^{hi}PD1^{hi}CXCR5^{hi}Foxp3^{+}$) cell numbers in spleen of H-WT and H-*Ackr4*$^{-/-}$ mice after SRBC immunization as described in Fig. 1 (C and D; means ± SEM). Representative plots are from day 5 after SRBC immunization. Data were analyzed using a two-tailed, unpaired Student's *t* test or a two-tailed, nonparametric Mann-Whitney test. **(B)** TFH and TFR cell numbers in B-WT and B-*Ackr4*$^{-/-}$ mice described in Fig. 1 (E and F; means ± SEM). Data were analyzed using a two-tailed, unpaired Student's *t* test or a two-tailed, nonparametric Mann-Whitney test, as appropriate. **(C)** Representative flow-cytometric gating strategy identifying naive $CD4^{+}$ T cells, thymic-derived T regulatory (tTreg) cells, TFH, and TFR cells in mixed BM chimeras, as described in Fig. 3 A. Left: Representative histograms of $CD45.2^{+}$ cells among concurrent naive $CD4^{+}$ T cell and TFH compartments. Right: Representative histograms of $CD45.2^{+}$ cells among concurrent tTreg cell and TFR compartments. Numbers in plots indicate means ± SEM frequencies of $CD45.2^{+}$ cells among indicated cell populations (n = 6 mice/group). Data are representative of two independent experiments.

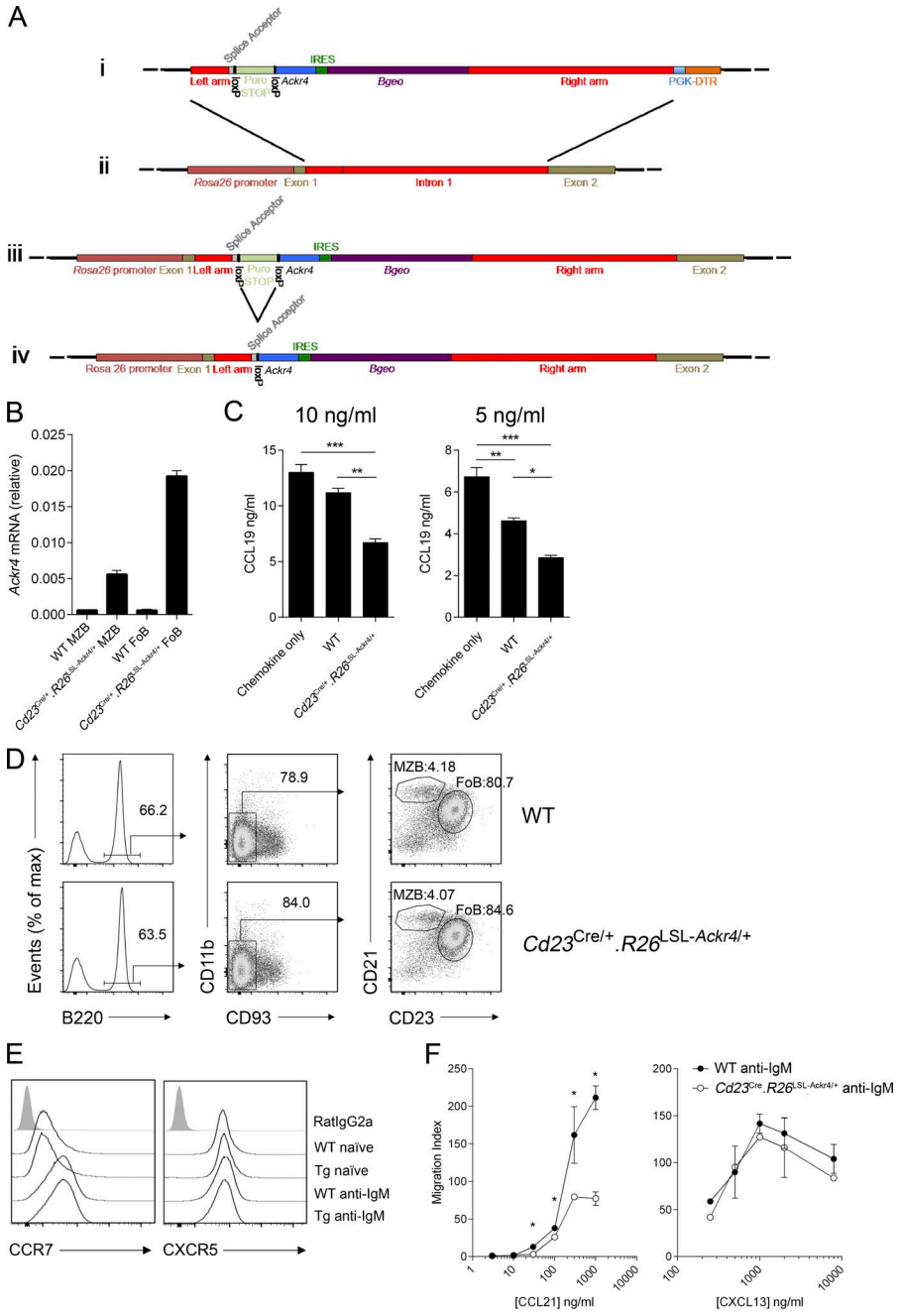

Figure S2. ***Cd23*$^{Cre/+}$*.Rosa26*$^{LSL\text{-}Ackr4/+}$ mice (related to Fig. 3 C). (A)** Rosa26 targeting strategy. (i) *Rosa26*$^{LSL\text{-}Ackr4}$ targeting construct; (ii) WT *Rosa26* locus; (iii) targeted *Rosa26* locus; and (iv) targeted *Rosa26* locus after *Cd23*-Cre–mediated recombination. loxP:loxP, targets for Cre-mediated recombination; Puro-STOP, puromycin resistance gene, followed by STOP cassette (transcriptional pause sequence with two polyA sites); IRES, internal ribosome entry site; *Bgeo*, fusion of β-gal and neomycin resistance cDNA; PGK-DTR, diphtheria toxin receptor control by the PGK promoter. **(B)** Quantitative PCR analysis of *Ackr4* transcript abundance in sorted marginal zone B cells and Fo B cells (n = 3 mice/genotype; gated as shown in D). Data are presented relative to the housekeeping gene *Rplp0* (means ± SD). **(C)** CCL19 scavenging assay. Sorted Fo B cells from WT (n = 3) or *Cd23*$^{Cre/+}$*.Rosa26*$^{LSL\text{-}Ackr4/+}$ (n = 3) mice were incubated with 10 or 5 ng/ml recombinant CCL19 for 3 h at 37°C. CCL19 concentration in cell-free supernatants was determined by ELISA. Samples with no addition of cells (chemokine only) were used to determine input CCL19 concentration. One-way ANOVA with the Bonferroni multiple comparisons test (means ± SEM). **(D)** Representative flow cytometric analysis of marginal zone B cell (B220$^+$CD11b$^-$CD93$^-$CD23$^{lo/-}$CD21$^+$) and Fo B cell (B220$^+$CD11b$^-$CD93$^-$CD23$^+$CD21$^{lo/-}$) frequencies in spleen of WT (n = 3) and *Cd23*$^{Cre/+}$*.Rosa26*$^{LSL\text{-}Ackr4/+}$ (n = 3) mice. max, maximum. **(E)** Representative flow cytometric analysis of CCR7 and CXCR5 expression on unstimulated (rested overnight) and anti-IgM-stimulated (5 µg/ml; 24 h) WT and *Cd23*$^{Cre/+}$*Rosa26*$^{LSL\text{-}Ackr4/+}$ B cells. **(F)** Anti-IgM–stimulated (24 h) WT and *Cd23*$^{Cre/+}$*.Rosa26*$^{LSL\text{-}Ackr4/+}$ B cell transwell chemotaxis to CCL21 and CXCL13 (means ± SEM). Unpaired, two-tailed Student's t test. (C and F) *, P < 0.05; **, P < 0.01; ***, P < 0.001.