Supporting Information

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SI Materials and Methods

PCR Protocols and Primers for Vβ5 and Bcl-6 Genotyping. Vβ5 primers were as follows: forward 5′-ACG TGT ATT CCC ATC TCT GG; reverse 5′-CTG TTC ATA ATT GGC CCG A. B-cell leukemia/lymphoma 6 (Bcl-6) primers were described previously (1). The Vβ5 PCR consisted of 15 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 61 °C, and 30 s at 72 °C, followed by 10 min at 72 °C. The Bcl-6 PCR consisted of 5 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 63 °C, and 30 s at 72 °C, followed by 10 min at 72 °C.

Quantitative PCR Protocol and Primers. PCRs were performed in 96-well Optical Reaction Plates (Applied Biosystems) and consisted of 2 min at 50 °C, and a 10-min 95 °C denaturation step, followed by 40 cycles of 15 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C, and then a dissociation stage (15 s at 95 °C, 30 s at 60 °C, and 15 s at 95 °C). The following primers were used: Bcl-6, forward 5′-GGA AGT TCA TCA AGG CCA GT; reverse 5′-GAC CTC GGT AGG CCA TGA, for a 114-nt product; blimp-1, forward 5′-AAA CTC CAT GAC CTC GCT ATG AC; reverse 5′-CAC CCT CAC CTC TGC ACT GA, for a 90-nt product; and hypoxanthine guanine phosphoribosyltransferase (HPRT), forward 5′-GTT GTT GGA TAT GCC CTT GAC; reverse 5′-CAA CTT GCG CTC ATC TTA GGC, for a 111-nt product. IL-4 primers were described previously (2). Reactions were run in triplicate, and results for Bcl-6, B lymphocyte-induced maturation protein-1 (Blimp-1), and IL-4 were normalized to the internal HPRT control. Bcl-6, IL-4, and HPRT primers are intron-spanning; the Blimp-1 primers are not.

Semiquantitative PCR Protocol and Primers. cDNA samples were quantified on a NanoDrop 1000 (Thermo Scientific). Mtv-8 primers amplify the Mtv-8 superAg and were as follows: forward 5′-TCT AAA CAA TTC GGA GAA CTC; reverse 5′-AAG ACT TGG ATA AGT TCC A, for an 890-nt product. The Mtv-8 PCR consisted of 15 min at 95 °C, 31 cycles of 1 min at 94 °C, 1 min at 40 °C, and 1 min 30 s at 72 °C, followed by 10 min at 72 °C. PCR using the HPRT primers described for quantitative PCR (qPCR) was performed as a control for quantitation. The HPRT PCR consisted of 5 min at 94 °C, 31 cycles of 30 s at 94 °C, 30 s at 60 °C, and 30 s at 72 °C, followed by 10 min at 72 °C. Samples were run on a 2% agarose gel stained with SYBR Safe (Life Technologies). Bands were quantified relative to the lowest dilution of Mtv-8^{+} blast cDNA on a ChemiDoc XRS+ (Bio-Rad) using Image Lab 4.1 software.

1. Dent AL, Hu-Li J, Paul WE, Staudt LM (1998) T helper type 2 inflammatory disease in the absence of interleukin 4 and transcription factor STAT6. *Proc Natl Acad Sci USA* 95 (23):13823–13828.
2. Newton C, et al. (2000) Induction of interleukin-4 (IL-4) by *Legionella pneumophila* infection in BALB/c mice and regulation of tumor necrosis factor alpha, IL-6, and IL-1β. *Infect Immun* 68(9):5234–5240.

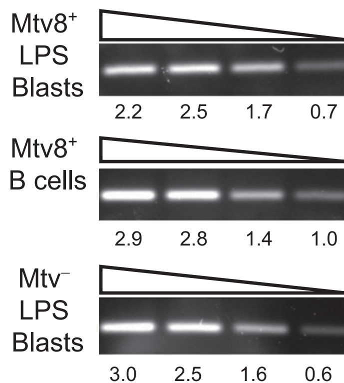

Fig. S1. HPRT control shows consistent cDNA quantification in semiquantitative PCR. Semiquantitative PCR was performed on serial threefold dilutions of Mtv-8^{+} B-cell and blast and Mtv^{-} blast cDNA for expression of HPRT, starting from 10 ng of cDNA. Numbers below gel images represent quantification of band intensity.

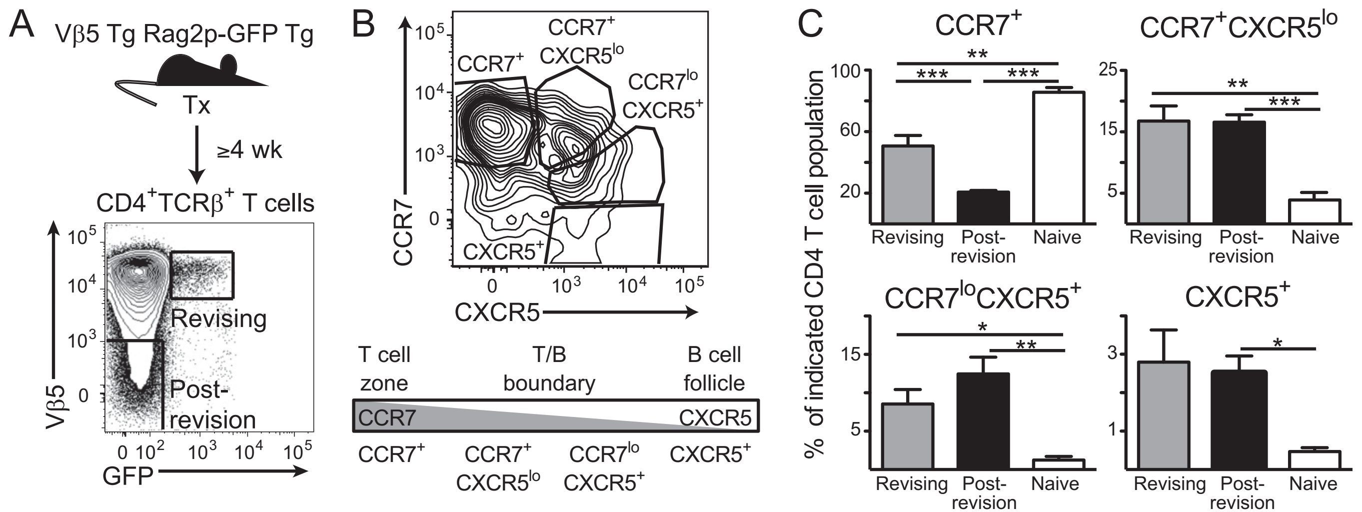

Fig. S2. Revising T cells tend to localize to the T cell–B cell boundary. (*A*) Peripheral CD4$^+$TCRβ$^+$ cells from Rag2p-GFP transgenic (Tg) Vβ5 Tg mice thymectomized (Tx) 4–6 wk previously were identified as revising or postrevision T cells. (*B*) Representative flow-cytometric plot shows CCR7 and CXCR5 expression by revising T cells. Scheme for localizing lymphocytes within secondary lymphoid organs based on their chemokine receptor expression pattern. (*C*) Shown are the % of revising (gray), postrevision (black), or naive (white, defined as in Fig. 4) CD4 T cells expressing the indicated levels of chemokine receptors. Bars indicate mean ± SEM from four pools of two to three mice per group in three independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$.

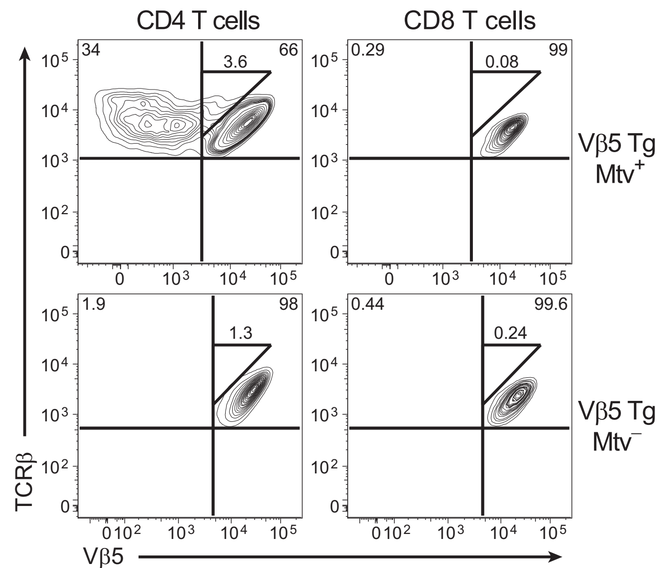

Fig. S3. Revising and postrevision CD4 T cells can be identified by flow cytometry. mLN cells from 28-wk-old Vβ5 Tg Mtv$^+$ and Mtv$^-$ mice were stained in separate experiments for CD4, CD8, Vβ5, and TCRβ. Revising T cells were identified as CD4$^+$TCRβ$^+$Vβ5^{lo} cells (triangular gate), and postrevision T cells were identified as CD4$^+$TCRβ$^+$Vβ5$^-$ cells (upper left quadrant gate). Numbers in upper corners represent the percent of cells in that quadrant; numbers above triangular gate represent the percent of cells in that gate.

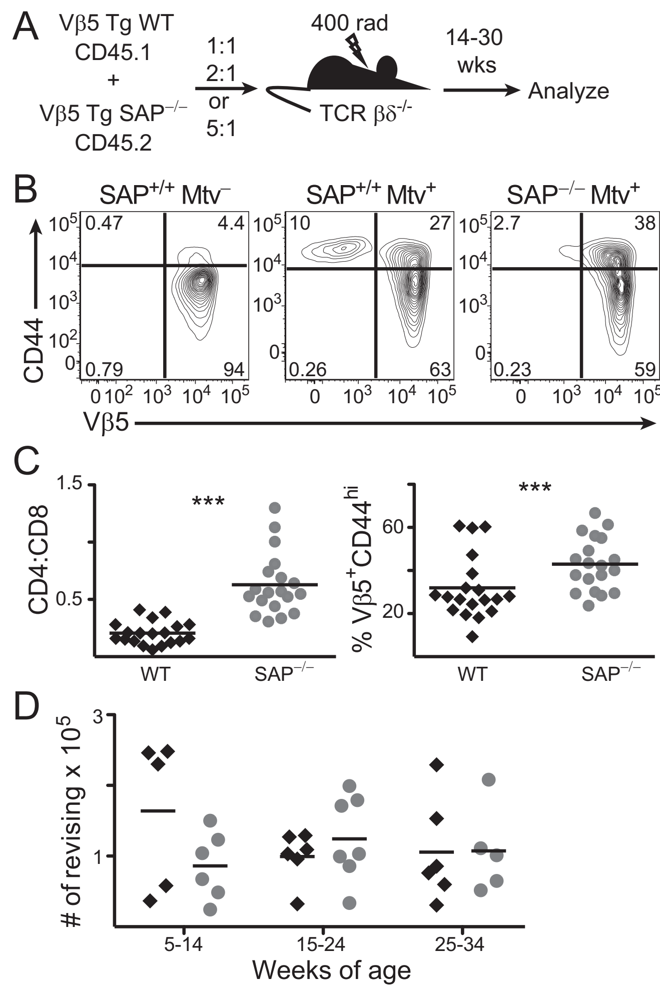

Fig. S4. SAP is not required for the initiation of revision. (*A*) Vβ5 Tg WT (black diamonds) and SAP$^{-/-}$ (gray circles) CD4 T cells were analyzed from the mLN of mixed bone-marrow chimeras 14–30 wk postreconstitution. (*B*) Representative flow-cytometric plots of mLN CD4 T cells from a 14-wk-old Vβ5 Tg Mtv^{-} mouse and a recipient of Vβ5 Tg WT and SAP$^{-/-}$ bone marrow 14 wk postreconstitution. Numbers in corners represent the percent of cells in that quadrant. (*C*) Ratio of CD4:CD8 T cells and % Vβ5^{+}CD44^{hi} of CD4 T cells were analyzed in mLN of mixed bone-marrow chimeras. Data are from four independent groups of chimeras totaling 19 mice in seven independent experiments. (*D*) mLN CD4 T cells from 5- to 34-wk-old Vβ5 Tg WT and SAP$^{-/-}$ mice were analyzed for total number of revising T cells. Data are from three to seven mice per group in five to nine independent experiments. ***$P < 0.005$.

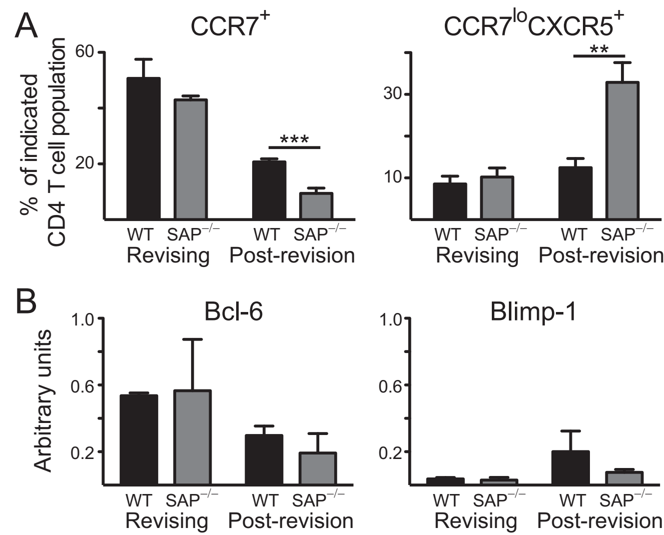

Fig. S5. Revising T cells are slightly less T follicular helper cell (Tfh)-like in the absence of SAP. Vβ5 Tg WT (black) and $SAP^{-/-}$ (gray) revising and postrevision T cells were analyzed for (*A*) % expressing the indicated chemokine receptors and (*B*) levels of RNA specific for Bcl-6 and Blimp-1. Bars indicate mean ± SEM from three to seven mice per group in two to three independent experiments. $^{**}P < 0.01$, $^{***}P < 0.005$.

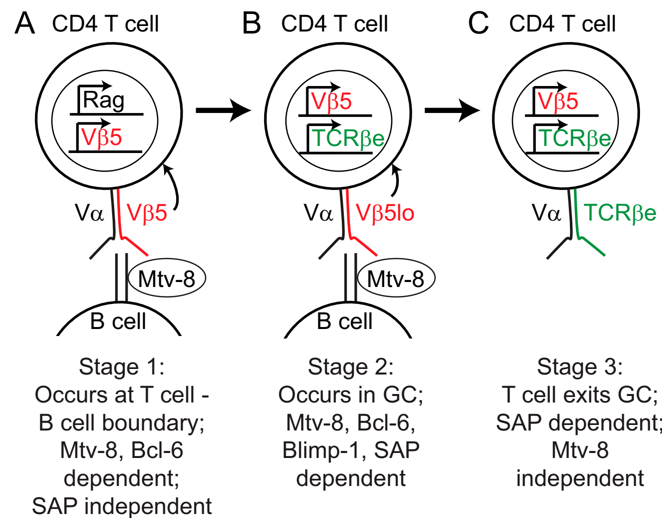

Fig. S6. Model of the multistep pathway of TCR revision. Revision occurs in three distinctly localized steps: (*A*) initiation at the T cell–B cell boundary, (*B*) completion in the germinal center (GC), and (*C*) exit from the GC.