Supplemental material

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Tables S1 and S2 are included as separate Excel files. Table S1 shows differentially expressed genes between CD4$^+$CD44^{high}CD25$^-$ tdTomato$^+$ZsGreen$^+$ and CD4$^+$CD44^{high}CD25$^-$tdTomato$^+$ZsGreen$^-$ cell populations. Table S2 show differentially expressed genes in CD4$^+$CD44^{high}CD25$^-$Tetramer$^+$-CXCR5^{high}PD-1^{high}tdTomato$^+$, CXCR5^{high}PD-1^{high}tdTomato$^-$, CXCR5^{low}PD-1^{low}tdTomato$^+$, and CXCR5^{low}PD-1^{low}tdTomato$^-$ antigen-specific cell populations.

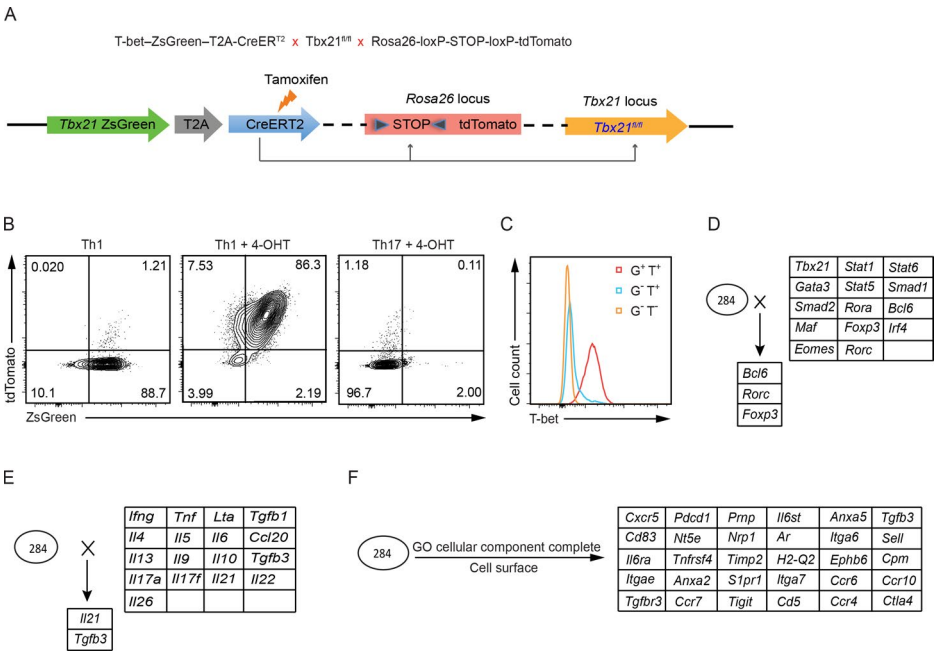

Figure S1. **Generation and characterization of an inducible T-bet fate–mapping mouse strain, and identification of Tfh-related gene expression in the ex–T-bet population. (A)** Genetic modifications of the inducible T-bet fate–mapping or inducible T-bet–deficient mice. TMX-induced Cre whose expression is driven by the *Tbx21* locus can fate-map the T-bet–expressing cells with tdTomato expression or conditionally delete the *Tbx21* gene. **(B)** Naive CD4 T cells sorted from ZTCE-tdTomato mice were cultured under Th1 or Th17 conditions with or without 4-OHT for 4 d. ZsGreen and tdTomato expression in CD4$^+$CD44^{high} cells were assessed by flow cytometry. Data are representative of two independent experiments. **(C)** ZTCE-tdTomato mice were treated (i.p.) with TMX on days 0, 2, and 5. After 10 d, CD4$^+$CD44^{high}CD25$^-$-ZsGreen$^+$tdTomato$^+$(G$^+$T$^+$), ZsGreen$^-$tdTomato$^+$(G$^-$T$^+$) and ZsGreen$^-$tdTomato$^-$(G$^-$T$^-$) populations were sorted. T-bet protein level was measured by intracellular staining. Data are representative of two independent experiments. **(D–F)** Comparing to T-bet–expressing (G$^+$T$^+$) population, 284 genes were up-regulated in ex–T-bet (G$^-$T$^+$) cells. Overlap of these genes with the transcription factor (D) and cytokine (E) genes that play important roles in T cell differentiation and functions. The cell surface proteins among 284 genes were identified through GO cellular component analysis (F).

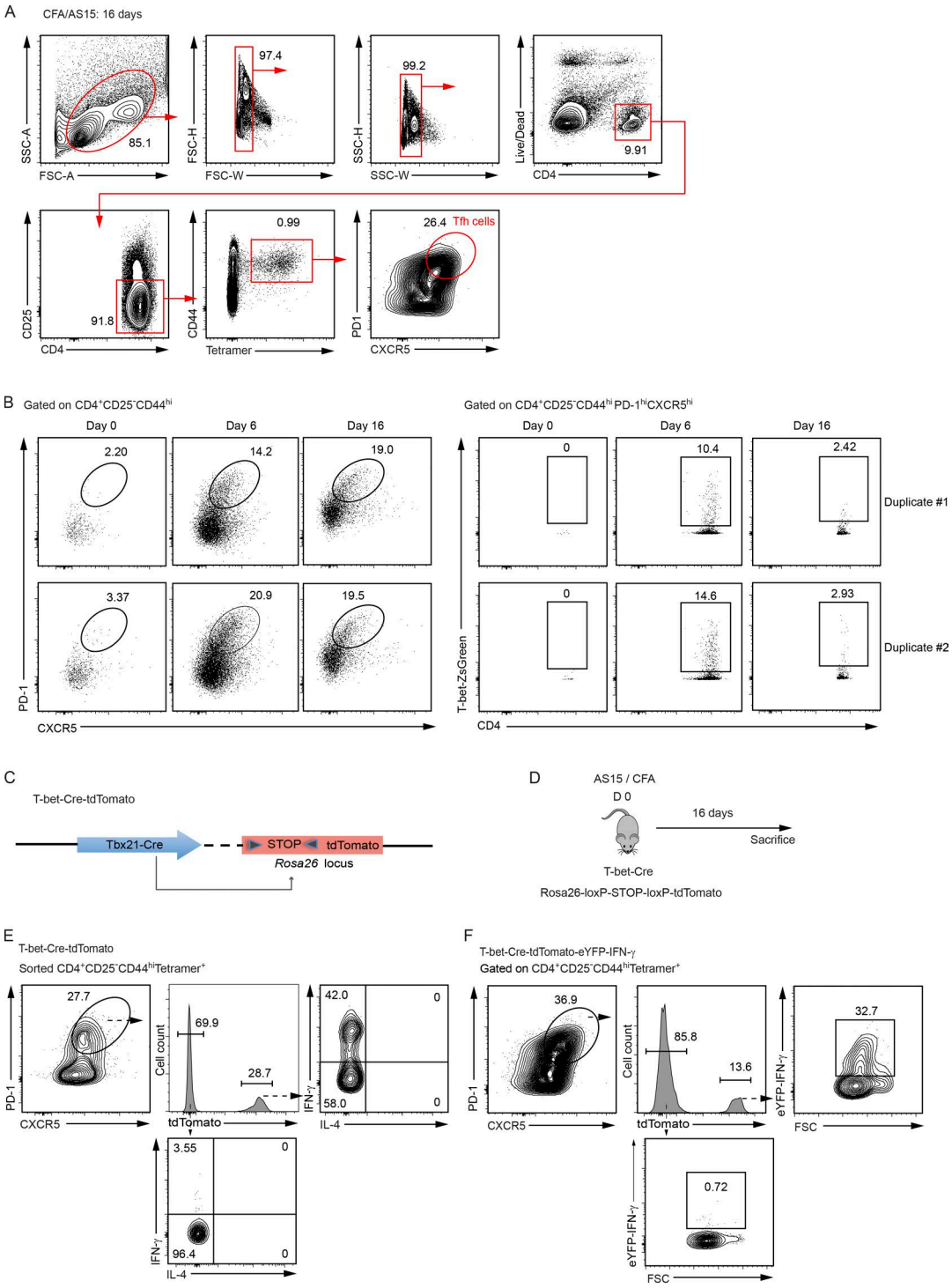

Figure S2. **Tfh cell gating strategy and immunization of constitutive T-bet fate mapping mice. (A)** Gating strategy for identifying antigen-specific Tfh cells after AS15/CFA immunization. **(B)** T-bet-ZsGreen mice were immunized with AS15/CFA for 6 or 16 d. dLN cells were analyzed by flow cytometry to measure the T-bet-ZsGreen expression among the $CD4^+CD44^{hi}CD25^-PD\text{-}1^{hi}CXCR5^{hi}$ Tfh population at different time points. Data are representative of two independent experiments. **(C)** Genetic modifications of the constitutive T-bet fate–mapping mice. Expression of a constitutively active Cre driven by the *Tbx21* locus can fate-map the T-bet–expressing cells with tdTomato expression. **(D)** Experimental procedure of immunizing T-bet-Cre-tdTomato mice with AS15/CFA. **(E)** $CD4^+CD44^{high}CD25^-Tetramer^+$ cells from AS15/CFA-immunized T-bet-Cre-tdTomato mice were purified and cultured in IL-2 supplemented media overnight. Cytokine production was assessed by intracellular staining 4 h after stimulation with PMA and ionomycin. Data are representative of two independent experiments. **(F)** Cells from dLN of immunized IFN-γ-eYFP-T-bet-Cre-tdTomato mice were stained with cell surface proteins. $eYFP^+$ cells among the $CD4^+CD44^{high}CD25^-Tetramer^+$-$tdTomato^+$ or -$tdTomato^-$ population were assessed by flow cytometry. Data are representative of three independent experiments.