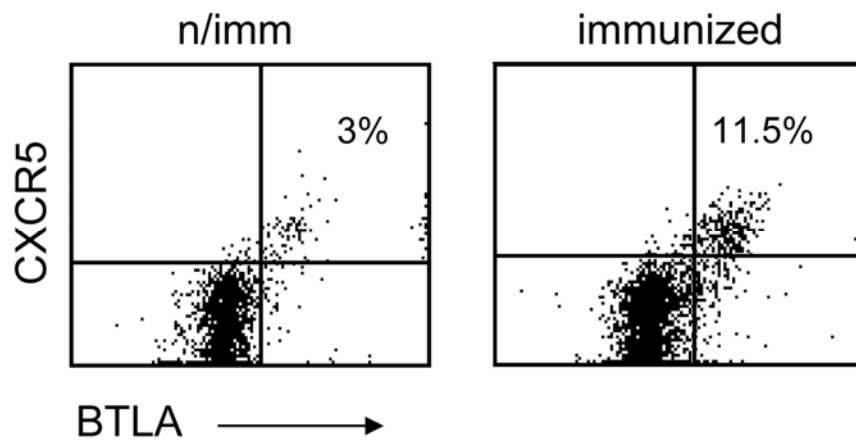# Generation of T Follicular Helper Cells Is

Mediated by Interleukin-21 but Independent

# of T Helper 1, 2, or 17 Cell Lineages

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Figure S1. T follicular helper cells co-express CXCR5 and BTLA. C57BL/6 mice were immunized with KLH in CFA. Seven days after the immunization, experimental mice were sacrificed and the Tfh cell induction was determined by staining CD4 cells with PE-labeled anti-BTLA mAb and biotinylated CXCR5 mAb, followed by APC-labeled streptavidin.
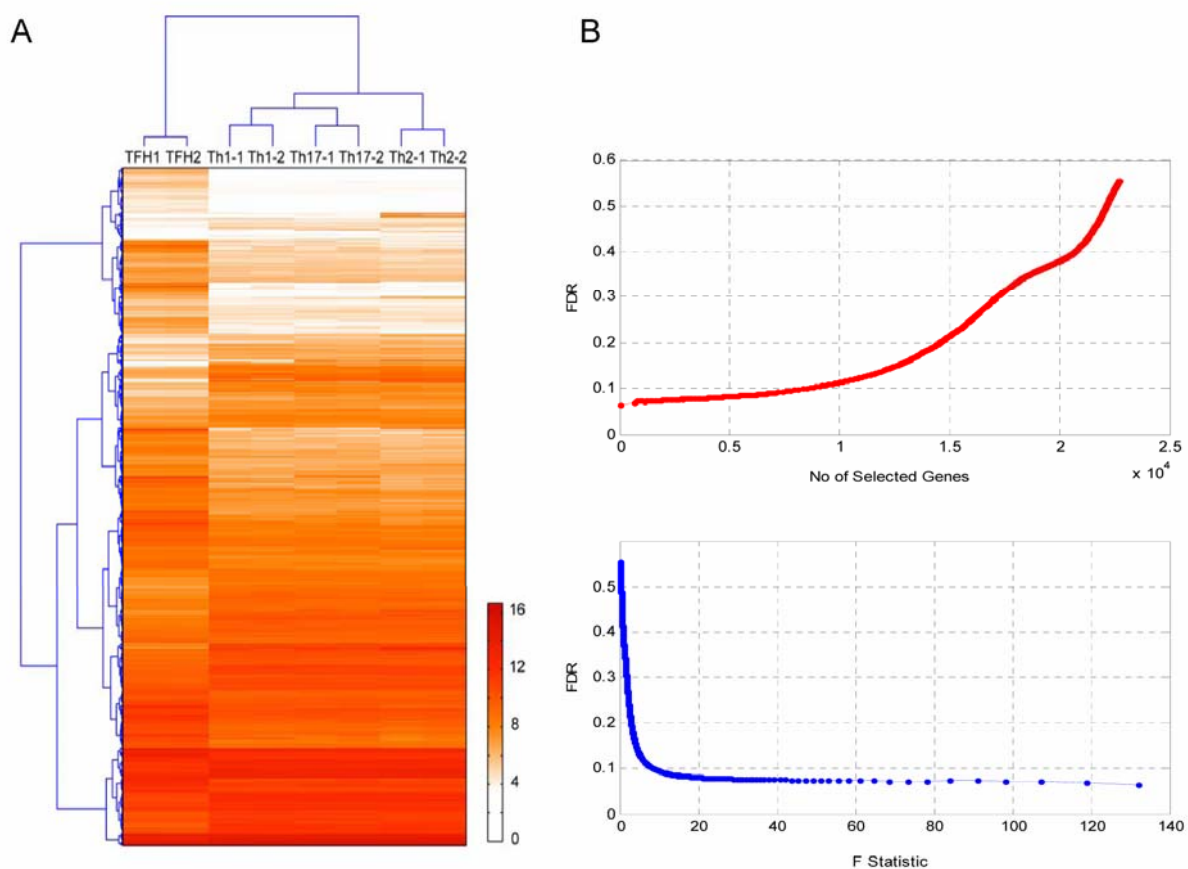
Figure S2. Gene expression profile of Tfh cells. (A). C57BL/6 mice were immunized with KLH in CFA. 7 days later CD4^{+}CD44^{hi}CXCR5^{+} BTLA^{+} (Tfh) cells were sorted and restimulated with anti-CD3 for 4 hours for gene profiling analysis. (B). FDR estimated from ANOVA tests using resampled samples.
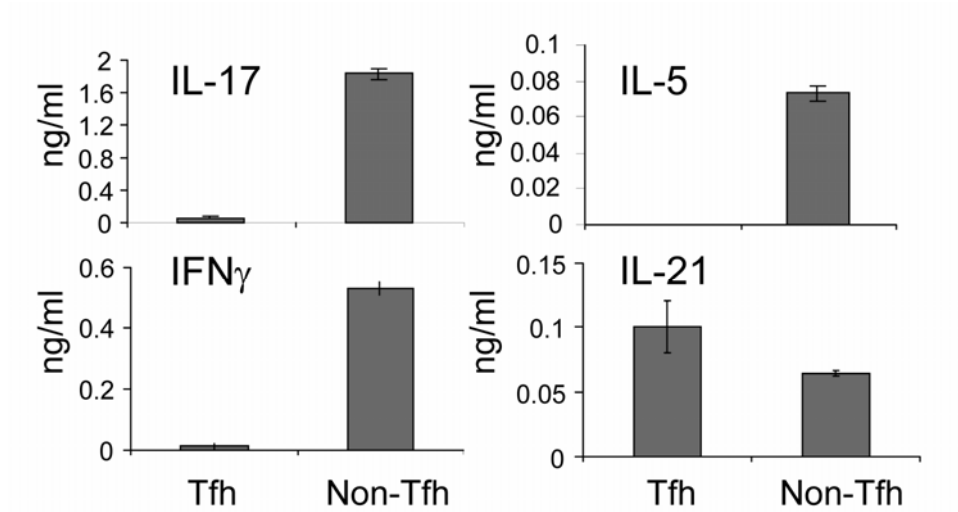
Figure S3. Antigen-specific cytokine production by Tfh cells. C57BL/6 mice were immunized with KLH in CFA. 7 days later CD4$^+$CD44^{hi}CXCR5$^+$ (Tfh) and CD4$^+$CD44hiCXCR5$^-$ (non-Tfh) cells were sorted and restimulated with KLH and irradiated APC for 48 hours for cytokine measurement by ELISA. The graph shows means ± standard deviation (SD).
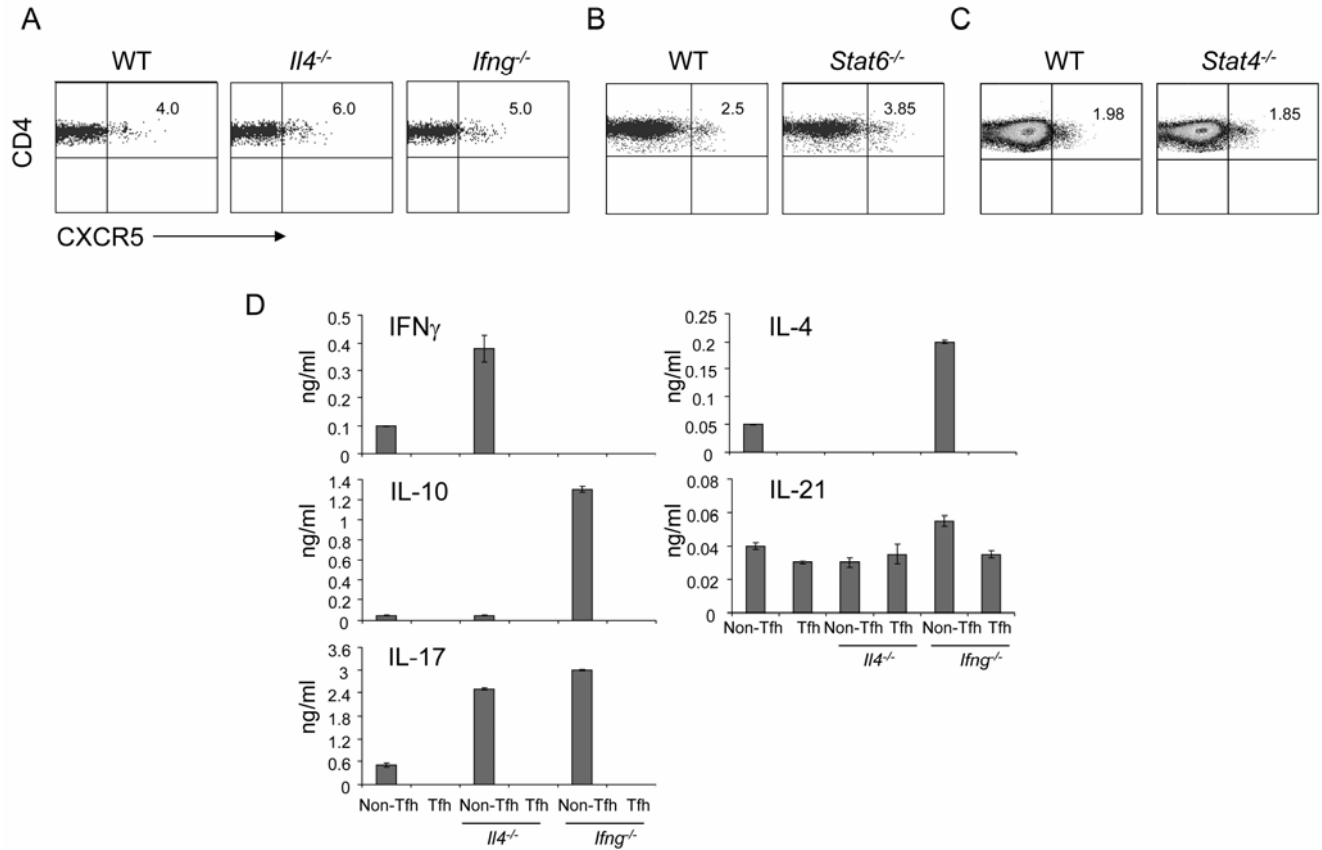
Figure S4. Tfh cell generation is independent of Th1 and Th2 cells. (A-C). Basal levels of CXCR5 expression in non-immunized *Il4*^{-/-}, *Ifng*^{-/-}, *Stat6*^{-/-} and *Stat4*^{-/-} mice were analyzed by staining with PerCP-labeled CD4 mAb and biotinylated CXCR5 mAb, followed by APC-labeled streptavidin. Numbers in dot plot quadrants represent the percentages. (D). *Il4*^{-/-}, *Ifng*^{-/-} and their appropriate controls (WT, 3 mice per group) were immunized with KLH emulsified in CFA. Seven days after the immunization, CD4$^+$CD44^{hi}CXCR5$^+$ (Tfh) and CD4$^+$CD44hiCXCR5$^-$ (non-Tfh) cells were sorted and restimulated with anti-CD3 for 4 hours for real-time RT-PCR analysis of Th subset-specific genes. The graph shows means ± standard deviation (SD).
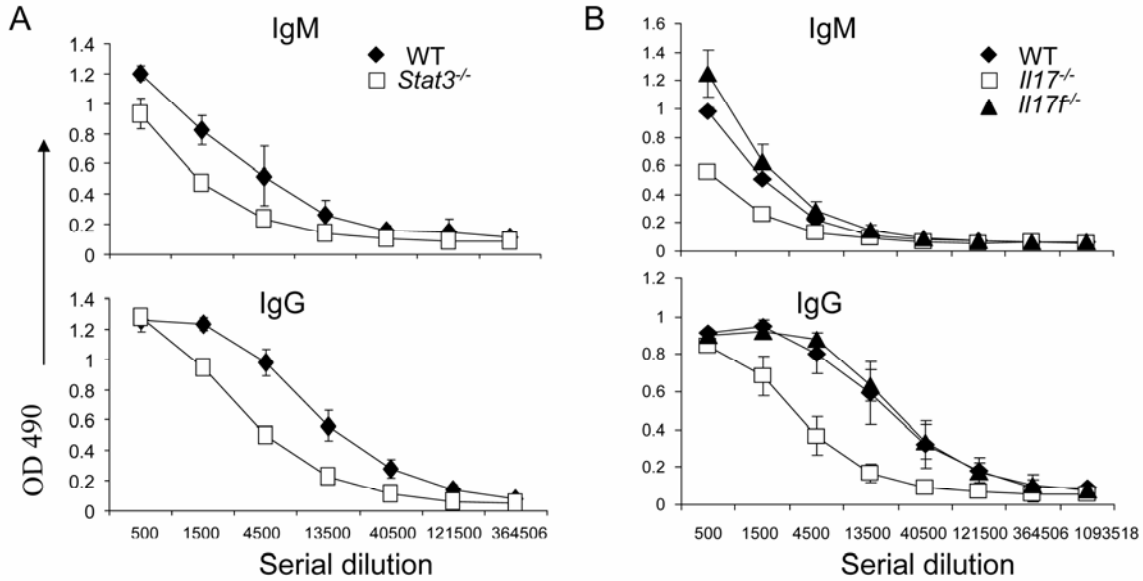
Figure S5.   Regulation of antibody responses by STAT3. T cell-specific *Stat3*^{-/-} (A), *Il17*^{-/-}, *Il7f*^{-/-} (B) and their controls (WT, 3 mice per group) were immunized with KLH in CFA. Seven days after the immunization, KLH-specific antibodies (IgM and IgG) were measured in the sera by ELISA. The graph shows means ± standard deviation (SD).
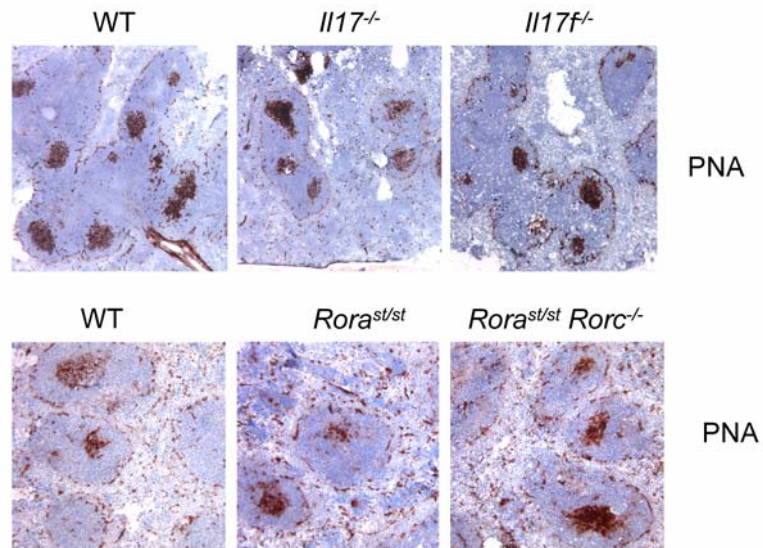
Figure S6. Normal germinal center reaction in IL-17, IL-17F and RORα/γ deficient mice.
Il17^{-/-}, *Il7f*^{-/-} , *Rora*[st/st], *Rora*[st/st]*Rorc*^{-/-} mice and their controls (WT, 3 mice per group) were immunized with KLH in CFA. Seven days after the immunization, germinal centers in the spleens of KLH-immunized mice were identified by PNA staining (brown).
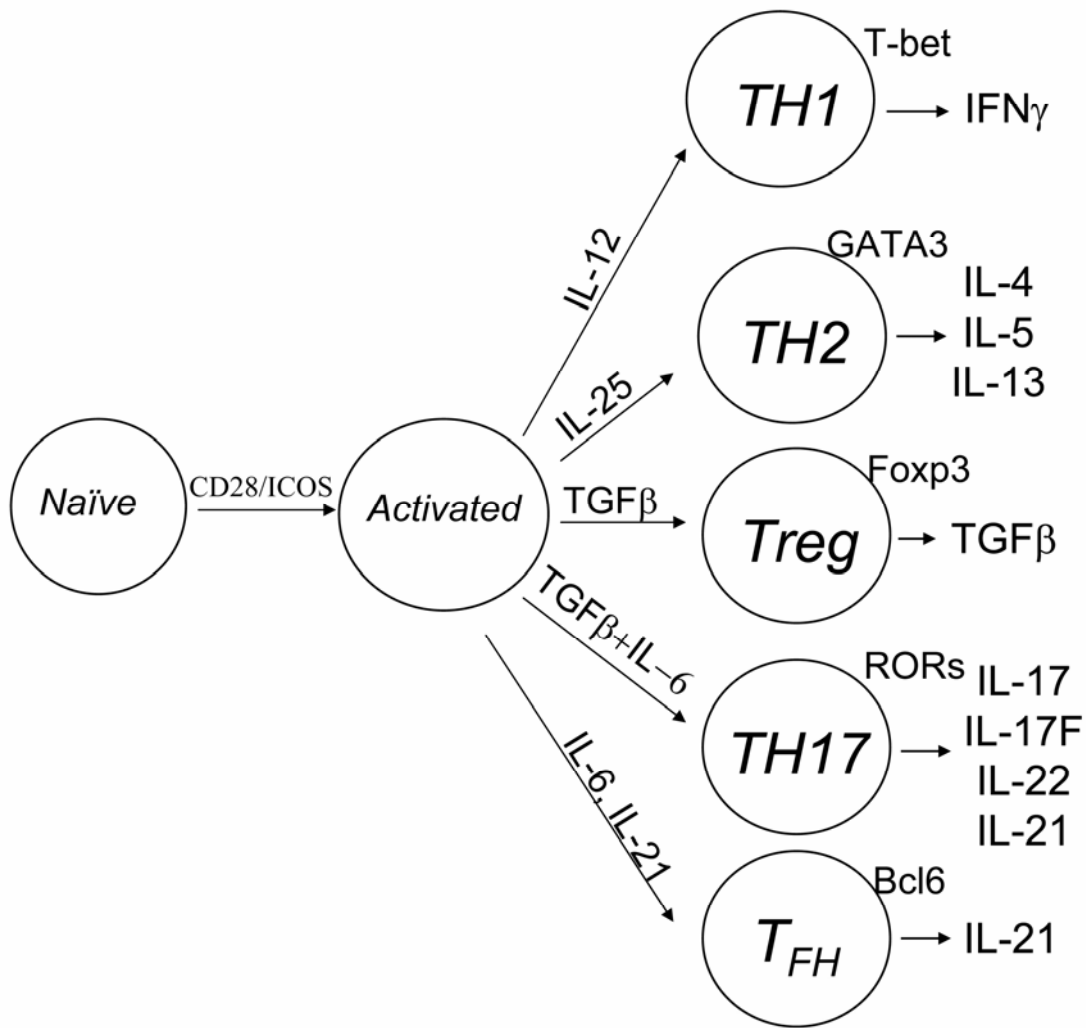
Figure S7. Schematic demonstration that development of Tfh cells, independent of Th1, Th2 or Th17 lineages, is regulated by IL-6 and IL-21.