**Figure Legends**

**Figure 1: IL21-expressing CD4 T cells are the major population of activated CD4 T cells that arise in young naïve mice.**

**(A)** Percentagesof VFP+ of splenic CD4 T cells from 2 and 4 wk old naïve IL21-VFP mice and reporter-negative control mice (left). Each symbol is data from individual mice (right), with mean +/-SEM indicated. **(B)** Percentagesof VFP+ CD4T cells in blood and spleens at the indicated ages. Increase over time is significant by P ≤0.02 (Kruskal-Wallis). **(C)** Expressionpatterns of CD44, ICOS, CXCR5 and PD1 in VFP+ splenic CD4T cellsof 4 wk old naïve IL21-VFP mice. **(D)** Representative FACS plots comparing reporter expression of CD4 T cells fromsplenocytes offour to six week oldIL21-VFP (n=7), IL17-GFP (n=7), IL10-GFP (n=7), IFNγ-YFP (n=7) and FoxP3-GFP (n=7) reporter mice on a B6 background (left). Results summarized on graph (right) are percentages of reporter-positive CD4T cells (circle) and those co-expressing CD44 (triangle). Data are representative of at least three independent experiments. \*\*\* P ≤0.0001.

**Figure 2: Early IL21-VFP+ CD4T cells do not require B cells or CXCR5, but IL21, IL6, IFN1 and IL10 signaling influence their development.**

**(A)** Comparisons of frequencies of VFP+CD4T cells in PBL from *Ighm-/-* IL21-VFP and IL21-VFP WT controls and co-expression of CD44, ICOS, CXCR5 and PD1 (top) with combined results (bottom). **(B)** Comparisons of frequencies of VFP+CD4T cells in PBL from *Cxcr5-/-* IL21-VFP and WT control mice. Comparisons of CD4+ IL21-VFP+ CXCR5- PD1- cellsin:(**C**) 4 wk old *Il12b-/-* IL21-VFP and *Il12b+/-* mice; 6 wk old *Il10-/-* IL21-VFP and WT mice; 5 wk old *Ifnar-/-* IL21-VFP and WT mice; and **(D)** 4 to 6 wk old *Il6*-/- IL21-VFP, *Il21r*-/- IL21-VFP, *Il6*-/- *Il21r*-/- IL21-VFP, CD4-cre *Bcl6fl/fl* and IL21-VFP mice. \* P ≤0.05, \*\* P ≤0.01, \*\*\* P≤0.001; n.s, not significantly different. Data are representative of four to eight independent experiments.

**Figure 3: RNAseq-based transcriptomic analysis of natural CD4 T cell populations.**

**(A)** Comparisonsbetween microarray-based profiling datasets from Yusuf, *et al.* of 8 day Naïve CD4 T cells, TH1 cells, TFH cells, and GC TFH cells (top), and RNAseq-based profiling datasets from Choi, *et al.* of 3 day TFH (IL-2Rα−Blimp1-CXCR5+PD1lo) and 3 day TH1(IL-2Rα+Blimp1-CXCR5-) (bottom) to RNAseq profiles of N, ACT, and ACT IL21 populations. **(B)** Heat map of 471 signature genes for each sample group with representative KEGG and GO BP functional enrichments (P< 1 x 106 and 1X10-8, respectively. See **Table S1** for full signature gene lists and expression data and **Table S2** for enrichment data. **(C)** Scatterplot of the Log2 difference between each ACT sample and the N sample with the N, ACT, and ACT IL21 signature genes colored and other genes in grey. Reference genes are indicated. **(D)** Comparison of gene expression between the N, ACT and ACT IL21 populations for selected genes of interest for ACT IL21. Expression of each transcript is shown as percentages of total transcripts per million (TPM) for the indicated gene within each sample. **(E)** Genes of interest for TH1, TH2, NKT and TREG.See **Table S3** genes of interest expression data.

**Figure 4: nTFH display a diverse TCR repertoire but restricting its specificity does not alter nTFH development.**

**(A)** Analysis of *Trav* and *Trbv* gene utilization of N, ACT and ACT IL21 (nTFH). Mean TPM for each *Tcrav* and *Tcrbv* gene is shown. Pearson correlation coefficients: N vs. nTFH, *r* = 0.866 (\*\*); N vs. ACT, *r* = -0.00361; ACT vs. nTFH, 0.0760. **(B)** FACS analyses of CD4T cells from 4 and 14 wk old IL21-VFP *Tcrα*-/- OT2 Tg and non-transgenic IL21-VFP WT mice. Representative FACS plots of VFP and ICOS staining (left) and combined results (right) are shown. \* P ≤0.05, \*\* P ≤0.01. Results are representative of at least three independent experiments.

**Figure 5: nTFH cells persist after transfer and differentiate into full TFH after immunization.**

**(A)** Representative FACS plots (left) summary data (right) for B6.*Tcrα* -/- mice (CD45.1) recipient mice that received nTFH cells (VFP+ CXCR5- PD1-) sorted from B6.IL21-VFP (CD45.2) mice. About 500,000 nTFH cells were injected. Recipients were bled at 2 wks, 4 wks and 6 wks post transfer. *Tcrα-/-* mice that received no transferred cells were used as a negative control. **(B)** Distribution of TFH differentiation stage among the transferred VFP+ CD4 T cells. The majority of the cells were nTFH. **(C)** Comparison of unimmunized and immunized *Tcrα-/-* mice recipients of nTFH. Mice were immunized with DNP-KLH and analyzed 10 days later. *Tcrα-/-* mice that received no transferred cells served as negative control. **(D)** Comparison of the stages of TFH development in the mice from **(c)**. Results show appearance of TFH after immunization. Representative FACS plots (left) summary data (right). Data are representative of 3 independent experiments. \* P ≤0.05, \*\* P ≤0.01.

**Figure 6: nTFH are present in the thymus and abide by similar rules as those found in the periphery.**

**(A)** Thymic and spleen VFP expression in naïve IL21-VFP mice at 2 d, 2 and 4 wks of age. Representative FACS data from thymus (top) and spleen (bottom). **(B)** Summary thymus data from (**A**). **(C)** Four to 6 wk old reporter mice were analyzed for thymic levels of IFNy YFP (n=7), IL10 GFP (n=7), IL17 GFP (n=7), IL21 VFP (n=7) and FoxP3 GFP (n=3). Results are given as total percentage of reporter positive CD4+ CD8- T cells and of those that are also CD44+. **(D)** Spleens and thymi of 6 wk old IL21-VFP mice were analyzed by FACS for expression of. FACS plots comparing spleen and thymic expression of ICOS, CD44, CD5 and CD3 on VFP+ CD4 T cells (left) summary data (right) of 6wk old VFP-IL21 mice. **(E)** Comparison of thymic levels of VFP in standard IL21-VFP mice compared to mice lacking *Il6*, *Il21r* or both *Il6* and *Il21r.* (**F**) Comparison of nTFH present in the thymi of mice lacking one or two copies of *Aire* compared to WT controls. Each symbol represents data from a single mouse. Representative of at least three independent experiments. \* P ≤0.05, \*\* P ≤ 0.001.

**Figure 7: AIRE is critical for positive selection of nTFH in the thymus and FoxP3+ TREG regulates nTFH expansion in the periphery.**

**(A)** Comparison of nTFH in spleens of mice lacking one or two copies of *Aire* compared to WT controls (same mice from 6f) shows significant peripheral increase of IL21-VFP+cells. **(B)** Comparison of TFH differentiation stages from mice shown in 7a. **(C)** Left, FACS plots of expression of VFP and co-expression of CXCR5 and PD1 of the VFP+ gated splenic CD4T cells from 4 wk old IL21-VFP *Foxp3-/-* and IL21-VFP WT mice; Right, frequencies of VFP+CD4+ T cells in 2 and 4 wk old cohorts. Each symbol represents a single mouse. **(D)** Frequencies from **c** of VFP+CD4+ T cells further distinguished as nTFH (ICOShiCXCR5-/loPD1-), pre-TFH (ICOS-/loCXCR5hiPD1-) or full TFH (ICOShiCXCR5hiPD1+) **(E)** Examples and group comparisons of VFP expression by CD4+ single positive thymocytes from 2 wk old IL21-VFP *Foxp3-/-* and IL21-VFP WT mice. \* P ≤0.05, \*\* P ≤0.01, \*\*\* P ≤0.001, \*\*\*\* P≤0.0001. Results are representative of at least three independent experiments.