**Figure Legends**

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

**(a)** Representative FACS profiling of VFP expression by splenic CD4 T cells from 2 and 4 wk old naïve IL21-VFP mice (left). Each symbol indicates data from an individual mouse (right). Samples from B6 mice lacking the reporter are shown as controls. Results are percentagesof VFP+ CD4 T cells from PBL and splenocytes for mice analyzed at the indicated ages. **(b)** Percentagesof VFP+ CD4T cell PBL and splenocytes for groups of mice analyzed at the indicated ages. **(c)** Patterns of CD44, ICOS, CXCR5 and PD1 expression by VFP+ splenic CD4T cellsfrom 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) heterozygous reporter mice on a B6 background (left). Results summarized on graph (right) are reported as percentage of reporter-positive CD4T cells (black) and those co-expressing CD44 (white). Data are representative of at least three independent experiments. \*\*\* P ≤0.0001 (Mann Whitney). \*\*P ≤0.02 (Kruskal-Wallis).

**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)** RepresentativeFACS results comparing the frequencies of VFP+CD4T cells in PBL from *Ighm-/-* IL21-VFP and IL21-VFP wild-type (WT) controls and co-expression of CD44, ICOS, CXCR5 and PD1 (top) with combined results (bottom). **(b)** Similar analyses comparing the frequencies of VFP+CD4T cells in PBL from *Cxcr5-/-* IL21-VFP and wild-type controls. Flow cytometry results comparing the frequency 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 and IL21-VFP mice. \* P ≤0.05, \*\* P ≤0.01, \*\*\* P≤0.0003; n.s, not significantly different (Mann Whitney). Data are representative of four to eight independent experiments.

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

**(a)** **Comparisons** between microarray-based profiling datasets from Yusuf, *et al.* of 8 day Naïve CD4 T cells, TH1 cells, TFH cells, and GC TFH cells (up), 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 functional enrichments for GO and KEGG annotations with FDR < 0.01. See **Supplementary Dataset 1** for more details. **(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 TFH related genes. 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 **Supplementary Dataset 2** for data and statistical information.

**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 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 analysis of CD4T cells from 4 and 14 wk old IL21-VFP *Tcrα*-/- OT2 Tg and non-transgenic IL21-VFP WT mice. Representative profiles of VFP and ICOS staining (left) and combined results (right) are shown. \* P ≤0.05, \*\* P ≤0.01; n.s, not significantly different (Mann Whitney). 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 (right) summary data (left) for B6.*Tcrα* -/- mice (with 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 vast majority of the cells remained as nTFH. **(c)** Comparison of unimmunized and immunized *Tcrα-/-* mice that received transferred nTFH. Mice were immunized with DNP-KLH and analyzed 10 days later. *Tcrα-/-* mice that received no transferred cells served as a 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 (right) summary data (left). Data are representative of 3 independent experiments. \* P ≤0.05, \*\* P ≤0.01; n.s, not significantly different (Mann Whitney).

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

**(a)** Data comparing thymic and spleen VFP expression in naïve IL21-VFP mice at 2 days, 2 wks and 4 wks of age. Representative FACS data from thymus (top) and spleen (bottom). **(b)** Summary data of thymus from (**a**). **(c)** Four to six week 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 (white) and subset of those that are also CD44+ (black). **(d)** The spleens and thymi of 6 wk old IL21-VFP mice were analyzed by FACS for expression of ICOS, CD44, CD5 and CD3. Representative FACS plots comparing spleen and thymic expression of the indicated markers on VFP+ CD4 T cells (left) summary data (right). **(e)** FACS data comparing 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. Results show significant decrease in thymus. Data are representative of at least three independent experiments each containing at least 4 mice. \* P ≤0.05; n.s, not significantly different (Mann Whitney).

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

**(a)** Comparison of nTFH present in the spleen 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)** Right, FACS examples of expression of VFP and co-expression of CXCR5 and PD1 of the VFP+ gated splenic CD4+ T cells from 4 wk old IL21-VFP *Foxp3-/-* and IL21-VFP WT mice; left, frequencies of VFP+CD4+ T cells in 2 and 4 wk old cohorts. **(d)** Frequencies from **c** of VFP+CD4+ T cells further distinguished as nTFH (ICOShiCXCR5-/loPD1-), pre-TFH (ICOS-/loCXCR5hiPD1-), 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; n.s, not significantly different (Mann Whitney). Results are representative of at least three independent experiments.