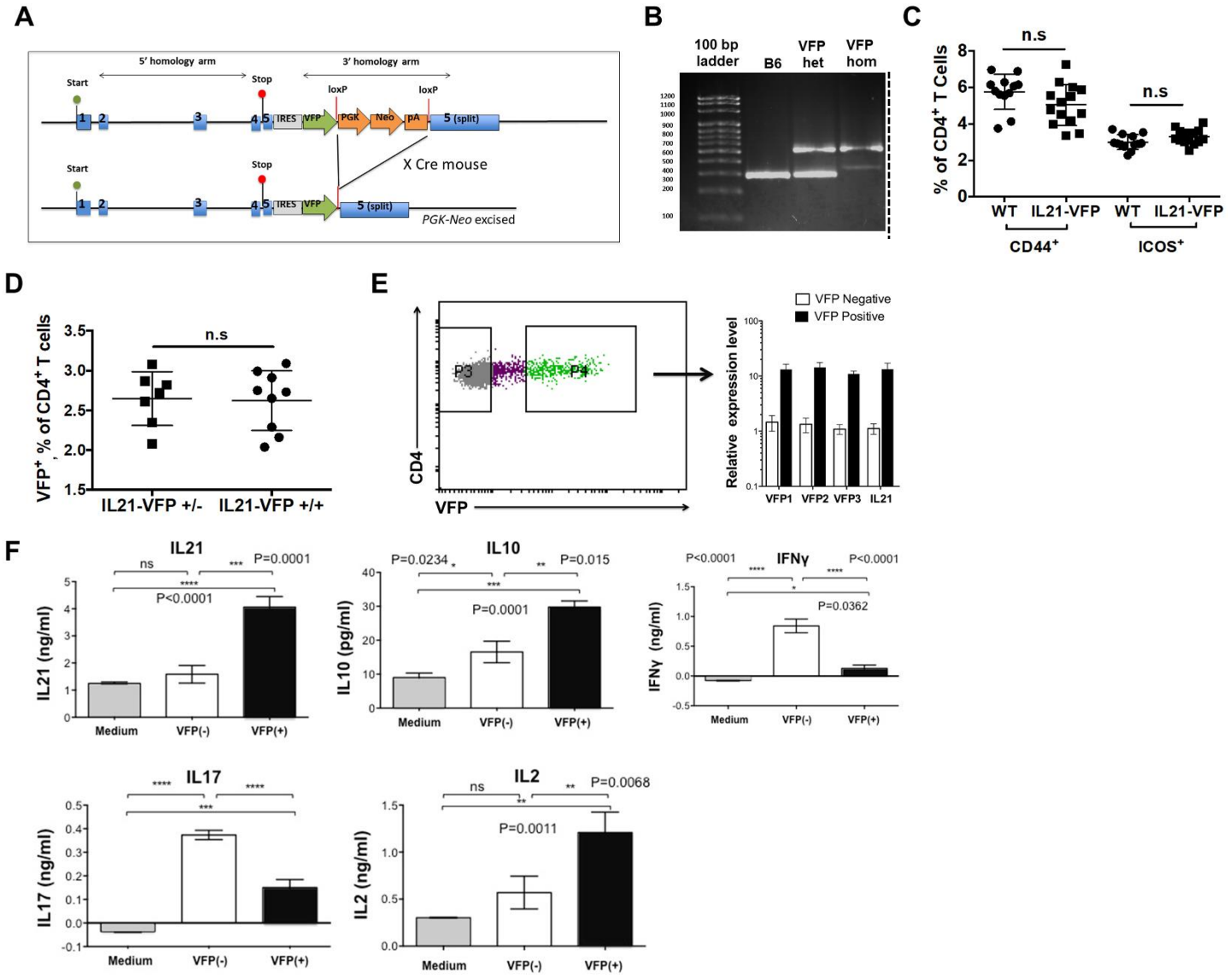


**Figure S1.** Development and characterization of IL21-VFP reporter mice. Related to **Figure 1**. (**A**) IL21-VFP reporter construct design. An IRES-VFP containing NeoR selection cassette flanked by loxP sites was inserted into non-coding exon 5 of the Il21 locus. The NeoR cassette was genetically excised by crossing to a germline expressed cre resulting in mice transmitting the bicistronic IL21-IRES-VFP reporter. (**B**) PCR confirmation of germline transmission (n=3) Note: lanes to the right of VFP homo were cropped out of the image shown because they were genotype reactions for non-related mice. (**C**) FACS comparisons of the frequencies of splenic CD44$^{hi}$ and ICOS$^{hi}$ CD4$^{+}$ T cells from B6.IL21-VFP homozygous (n=14) and WT B6 (n=12) mice. (**D**) Frequencies of VFP$^{+}$ CD4$^{+}$ T cells from B6.IL21-VFP heterozygous (n=7) and homozygous (n=9) mice. (Mann-Whitney). C, D are combined data from 2 experiments. (**E**) Splenic CD4$^{+}$ T cells from IL21-VFP heterozygous mice were FACS sorted after stimulation for 24 hrs in culture with CD3/CD28 antibodies. Data shown are the mean fold changes +/- S.D. of cells from B6.IL21-VFP mice after ΔΔCt normalization to 18s RNA. VFP 1, 2 & 3 are three different primer sets used for RT-qPCR (n=3). (**F**) Spleen cells from IL21-VFP mice were stimulated with antiCD3/CD28 for 36hrs after which VFP$^{+}$ and VFP$^{-}$ CD4$^{+}$ T cells were FACS-sorted and cultured independently for 24hrs (n=3). Serum was then collected and used for cytokine profiling by ELISA. *P ≤0.05, ** P ≤0.01, *** P ≤0.001, **** P ≤0.000, n.s = not significant. All error bars represent mean ± SD.

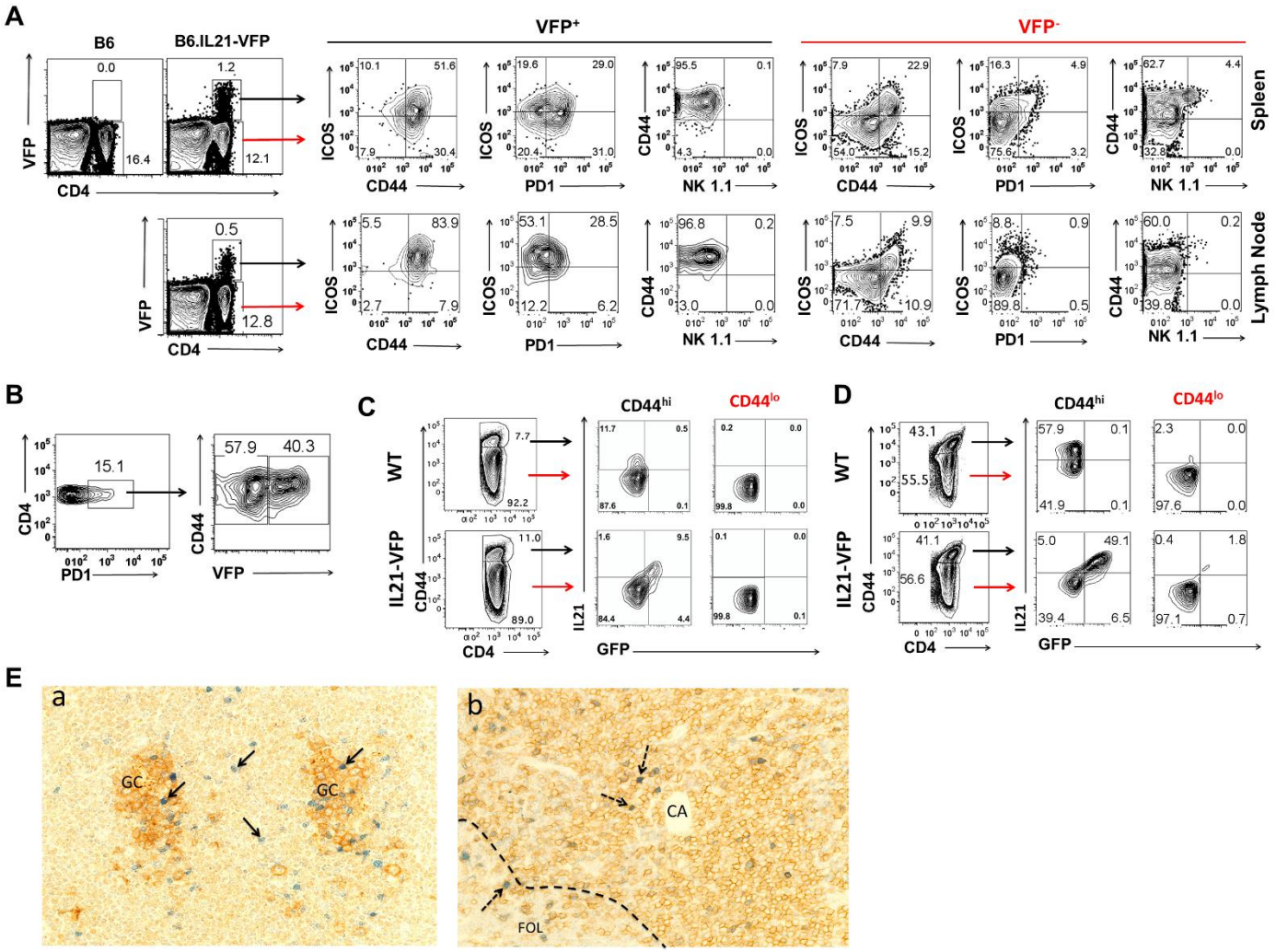


**Figure S2**. Further characterization of IL21-VFP reporter mice. Related **to Figure 1.** (**A**) FACS profiling of splenic and lymph node cells from IL21-VFP heterozygous mice 11 days after immunization with DNP-KLH in CFA (n=3). (**B**) Representative flow plot showing the frequencies of $PD1^+$ $CD4^+$ T cells from splenocytes of immunized IL21-VFP mice that are activated (CD44hi) and $VFP^+$ or $VFP^-$, with approximately 40-50% of $PD1^+$ cells being $VFP^+$. A-B, representative of two independent experiments (n=3). (**C**) Intracellular staining of IL21 and VFP (detected by anti-GFP) of splenic $CD44^{hi}$ and $CD44^{lo}$ $CD4^+$ T cells from 6 wk old B6.IL21-VFP heterozygous and B6 WT mice after a 3hr culture with PMA/Ionomycin (n=4). (**D**) Similar analysis of splenic $CD4^+$ T cells from 12 wk old BXSB.Yaa IL21-VFP homozygous and WT mice (n=4). (**E**) Immunohistochemical localization of $VFP^+$ T cells in the spleen of a 12 wk old BXSB.Yaa IL21-VFP homozygote (40X magnification). a, B cell follicle stained with anti-GFP (blue) and PNA (brown); b, T cell zone stained with anti-GFP (blue) and anti-CD4 (brown). GC, germinal center; CA, central arteriole; Dotted arrows, ETFH; solid arrows, FO TFH. Note, cells with highest intensity staining are in GCs (n=3).

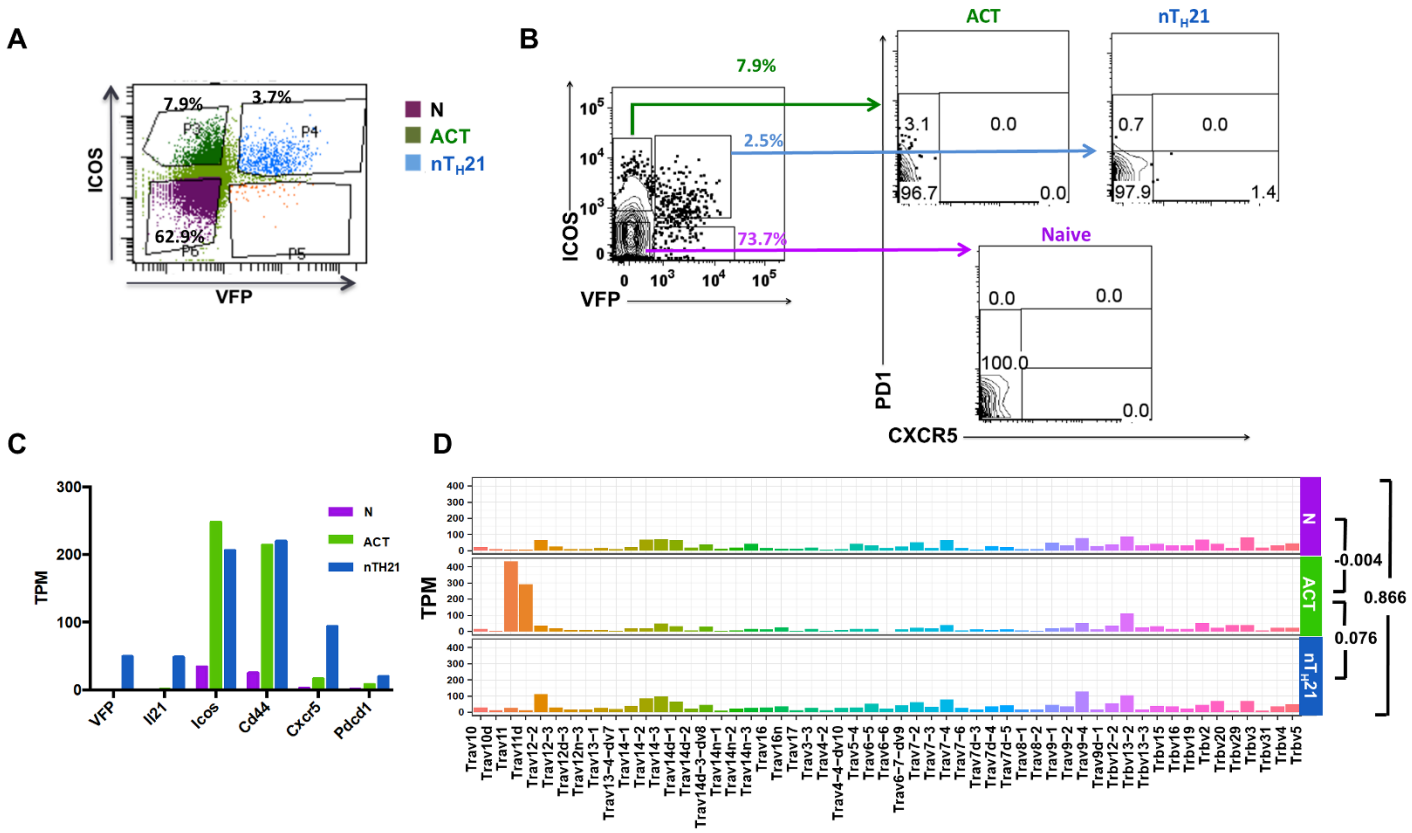


**Figure S3.** RNA sequencing sort parameters, validation and a/b TCR profiling. Related to **Figure 3**. (**A**) Splenocytes from naïve 4 wk old IL21-VFP mice were pooled into two biological replicates. Each pool was then enriched for $CD4^+$ T cells and FACS-sorted based on the following criteria: N, naïve ($VFP^-$ $ICOS^{lo}$); ACT, activated ($VFP^-$ $ICOS^+$); and $nT_H21$ ($VFP^+$ $ICOS^+$). (**B**) Representative pre-sort analysis of samples gated as in (A) evaluating PD1 and CXCR5 staining. (**C**) Gene expression confirmations showing equivalent expression of VFP and Il21 by the $nT_H21$ population and equivalently high levels of Cd44 expression by the ACT and $nT_H21$ populations. (**D**) Analysis of Trav and Trbv gene utilization of N, ACT and $nT_H21$. Mean TPM for each Tcrav and Tcrbv gene is shown. Pearson correlation coefficients are indicated.

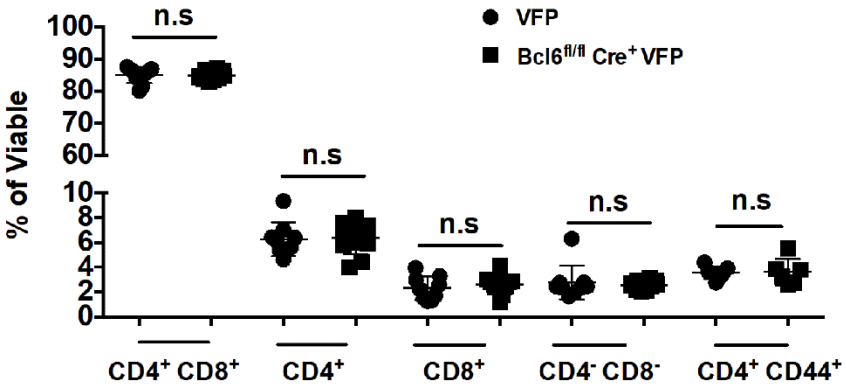


**Figure S4**. Further characterization of thymocytes comparing WT (n=9) and BCL6-floxed IL21-VFP mice (n=10). Related to **Figure 4F**. Relative frequencies of $CD4^+$ $CD8^+$, $CD4^-$ $CD8^-$, $CD4^+$ $CD8^-$, $CD4^-$ $CD8^+$ and activated $CD44^+$ $CD4^+$ $CD8^-$ thymocytes of the samples in Figure 4F are shown. All error bars represent mean ± SD.

# Supplemental Experimental Procedures

## Mouse stocks, genotyping and breeding

Genotypic confirmation of precise targeted transgenesis of the IL21-VFP allele was established by Southern blot and DNA sequence analyses at Ozgene. Genetic excision of the *loxP*-PGK-neo selection cassette-*loxP* was genetically excised by crossing to cre-transgenic "deletor" mice. Following genetic confirmations by DNA sequencing, mice carrying the knock-in allele were monitored using a three primer PCR assay with the following primers: *a,* F-AATGCATTTCTTTCACTTCCATGTT; *b,* R-TTAGTTAATGGGCGAAAGGATCTTA; *c*, F-AACGAGAAGCGCGATCACAT. The WT band (primers a and b) has an expected length of 293bp and the VFP band (primers c and b) is 501 bp. PCR was performed using 40 cycles of 95C for 30 seconds, 60C for 1 min and 70C for 1 min.

The following additional mouse strains were used: B6.Cg-*Il21*$^{tm1.1Hm}$/HmDcr; B6.129S2-*Ifnar1*$^{tm1Agt}$; B6.129(Cg)-*Il21r*$^{tm1Wjl}$; B6.129S2-*Il6*$^{tm1Kopf}$/J; B6.129S2-*Igh6*$^{tm1Cgn}$/J; B6.129P2-*Il10*$^{tm1Cgn}$/J; B6.Cg-Tg(TcraTcrb)425Cbn/J; B6.129S2-*Tcra*$^{tm1Mom}$/J; B6.129S1-*Il12b*$^{tm1Jm}$/J; B6.129S2(Cg)-*Cxcr5*$^{tm1Lipp}$/J; B6.Cg-*Foxp3*$^{sf}$/J; B6.Cg-*Foxp3*$^{tm2Tch}$/J, B6(Cg)-*Il10*$^{tm1Karp}$/J, B6.129S4-*Ifng*$^{tm3.1Lky}$/J, C57BL/6-*Il17a*$^{tm1Bcgen}$/J; B6.129S2-*Aire*$^{tm1.1Doi}$*/J*; B6.129S(FVB)-*Bcl6*$^{tm1.1Dent}$/J; B6.SJL-*Ptprc*$^{a}$ *Pep3*$^{b}$/BoyJ (Ly5.1), B6(Cg)-Tg(KRN)Doi/Dcr and Nur77 transgenic C57BL/6-Tg(Nr4a EGFP/cre)820Khog/J. BXSB.*Yaa Il21-VFP* and NOD-*Il21-VFP* mice were generated by backcrossing the *Il21-VFP* allele onto BXSB /MpJ or NOD/ShiLtJ respectively for at least 10 generations. PCR genotyping for knockouts and transgenes was performed using oligonucleotide primers recommended at the JAX MICE website. Conditional *Bcl6*$^{fl/fl}$ *IL21-VFP* mice were produced by crossing B6.129S(FVB)-*Bcl6*$^{tm1.1Dent}$/J mice with B6.Cg-Tg(CD4-cre)1Cwi/BfluJ and separately with B6.IL21-VFP reporter mice to generate *Bcl6*$^{fl/fl}$ *Cd4-cre*$^{+/?}$ and *Bcl6*$^{fl/fl}$ *IL21*$^{VFP/VFP}$ which were then intercrossed to produce *Bcl6*$^{fl/fl}$ *IL21*$^{VFP/+}$ *Cd4-cre*$^{+}$ and *cre*$^{-}$ for experiments. K/BXN *IL21-VFP* mice were generated by crossing B6.*KRN*$^{Tg/+}$ *IL21*$^{VFP/+}$ to NOD (*KRN*$^{-}$ *VFP*$^{-}$) mice or B6- *KRN*$^{Tg/Tg}$ to NOD-IL21$^{VFP/+}$ and genotyped for transmission of the KRN transgene and *Il21-VFP*.

B6.KRN mice were derived from B10.BR-KRN TCR transgenic mice kindly provided by Drs. Mathis and Benoist (Joslin Clinic, Boston MA).

**Antibodies used for flow cytometry and cell sorting:**

| Antigen | Clone | Fluorochrome | Company |
|---|---|---|---|
| B220 | RA3-6B2 | PB | Biolegend |
| B220 | RA3-6B2 | Biotin | BD Bioscience |
| CD11b | M1/70 | PE cy7 | Biolegend |
| CD11b | M1/70 | Biotin | BD Bioscience |
| CD11c | HL3 | Biotin | BD Bioscience |
| CD11c | HL3 | PE | BD Bioscience |
| CD4 | GK 1.5 | Pacific Orange | Made at JAX |
| CD4 | GK 1.5 | A650 | BD Bioscience |
| CD44 | IM7.8.1 | APC cy7 | BD Bioscience |
| CD62L | MEL-14 | PE cy7 | BD Bioscience |
| CD8 | 53-6.72 | A700 | BD Bioscience |
| CD8 | 53-6.72 | Biotin | BD Bioscience |
| CXCR5 | L138D7 | Biotin | Biolegend |
| GR1 | RB6-8C5 | APC cy7 | BD Bioscience |
| ICOS | C398.4A | A647 | Biolegend |
| NK 1.1 | PK136 | PE | BD Bioscience |
| PD1 | RMP1-30 | PE Cy7 | Biolegend |
| Streptavidin | | BV421 | Biolegend |
| Cd3e | 145-2C11 | PE-CF594 | BD Bioscience |
| CD5 | 53-7.3 | BV421 | BD Bioscience |
| GFP | FM264G | AF488 | BioLegend |

***Transcriptomic data analysis***

For comparison of our RNAseq data with antigen-induced $T_{FH}$ microarray and RNAseq datasets, gene expression files from 8-day naïve CD4 T cells, $T_H1$ cells, $T_{FH}$ cells, GC $T_{FH}$ cells (Yusuf et al., 2010) and 3-day $T_{FH}$ and $T_H1$ cells (Choi et al., 2015) were downloaded from gene expression omnibus (GSE21380 and GSE67334). The 8-day and 3-day data were measured by microarray and RNA-seq, respectively. For comparison of these with our N, ACT, and $nT_H21$ data: 1) gene expression levels by microarray and RNAseq were transformed into log2 intensity and log2 TPM scales; 2) platform and study differences were corrected by subtracting the first principle component of the combined samples for each transcript; and 3) hierarchical clustering

was performed on the combined samples based on the residual transcript levels. Identification of genes that best discriminate N, ACT, and $nT_H21$ populations were defined by three criteria: maximum mean expression in the signature population; mean TPM greater than 20 in the signature population; and differential expression in the signature population relative to the other two populations ($P < 0.05$, pairwise t-test). All analyses were done with R (www.r-project.org) except when specified otherwise. To estimate the expression of VFP relative to native mouse genes, the VFP sequence was added to the mouse transcriptome file.

TCR usages were determined by aligning all RNAseq transcripts to Ensembl. TR_V_genes and are reported as TPM. TCR_J and TCR_D segments were excluded because of difficulties in aligning short sequences. Transcripts with maximum expression <5 TPM were filtered out, resulting in a total of 68 *Tcrav* and *Tcrbv* genes.

**Gene expression by RT-qPCR**

Total RNA was extracted with the RNeasy Micro kit (Qiagen) followed by cDNA synthesis using the QuantiTect Reverse Transcription kit (Qiagen) according to the manufacturer's protocol. SuperScript III Platinum Two-Step qRT-PCR Kit with SYBR Green (Invitrogen) was used for quantification. Samples were run in technical triplicates on the ViiA Real Time PCR system (Life Technologies) and mean expression was normalized to 18sRNA using the ΔΔCt method. Primer sequences: *Il21* F-GAAGAT GGCAATGAAAGCCTGT, R-AGGATGTGGGAGAGGAGACTGA; *VFP1* F-AAG CTGACCCTGAAGTTCATCTGC, R-CTTGTAGTTGCCGTCGTCCTTGAA; *VFP2* F- CAACAGCCACAACGTCTATATCAT, R-CAACAGCCACAACGTCTA TAT CAT; *VFP3*
F-TGCTGCCCGACAA, R-TCACGAACTCCAGCA G.

**Serum cytokine detection**

For serum cytokine analysis, splenocytes from naïve B6.IL21-VFP mice were cultured for 36 hrs with 5 µg/ml of anti-CD3 and 1 µg/ml of anti-CD28 in 5% FBS-supplemented DMEM. Cells were then sorted for being $CD4^+VFP^-$ or $CD4^+VFP^+$. $VFP^-$ and $VFP^+$ cells were cultured independently in 5% FBS-supplemented DMEM for an additional 24 hrs. For ELISA, supernatants collected were added to plates coated with purified anti-mouse IL2 (BD; clone JES6-1A12), anti-mouse IL10 (BD; clone JES5-2A5), anti-mouse IL17 (BD; clone TC11-18H10), anti-mouse IFN gamma (BD; clone R4-6A2) and anti-mouse IL21 (Peprotech; polyclonal rabbit) (2ug/ml). Respective purified recombinant murine cytokines were used as standards. Cytokines were detected by biotinylated secondary antibodies and anti-mouse IL2 (BD; clone JES6-5H4), anti-mouse IL10 (BD; clone JES5-16H3), anti-mouse IL17 (BD; clone TC11-8H4), anti-mouse IFN gamma (BD; clone XMG1.2) or anti-mouse IL21 (Peprotech; polyclonal rabbit) (1ug/ml) as revealed by colorimetry using avidin-HRP (Sigma; 1:1000) and TMB (Invitrogen) after the reaction was stopped using 7% sulphuric acid (Sigma). Absorbance was measured at 450nm and concentrations were calculated using respective standard curves.

***Intracellular staining of IL21***

For intracellular staining of IL21 and VFP, 1-2 $x10^7$ RBC-lysed spleen cells were prepared and cultured at 37*º* for 3 hr in 2 ml of 10% FBS-supplemented DMEM containing 1 µl /ml of Cell Activation Cocktail (BioLegend #423303). Cells harvested were then washed and stained with Fixable Viability Dye eFluor780 (eBioscience, #65-0865-14) diluted 1:100 in 1% amine free PBS, 30 min at RT, washed 2X, and stained for surface markers: PE (clone IM7; BioLegend #103008); CD3e PE-CF594 (clone 145-2C11, BDBioscience, #582288); CD4 BV570 (clone RM4-5 BioLegend, #100542; CD8a AF700 (clone 53-6.72) BioLegend #100730 (all 1:40) for 30 min at RT in the dark and washed 2X. Samples were then fixed with 1ml Fixation Buffer (BioLegend #420801) for

20 min at RT, washed 2x3ml with 1x BD PhosflowPerm/Wash Buffer (BDBiosciences Cat. #557885), pelleted, Fc blocked with 10 ul CD16/32 (clone 24G.2 Tonbo Bioscience, #40-0161-M001), and stained with 1µgrmIL21R (596-MR, R&D Systems), and 1µg anti-GFP AF488, (clone FM264G; BioLegend). Data were acquired on an LSRII (BDBioscience) with FMO gating and analyzed using FlowJo v 9.4.8 software.

***Immunohistochemisty***

Spleens from IL21-VFP mice were fixed in 10% neutral buffered formalin and imbedded in paraffin. Sections were treated with citrate for antigen retrieval. For CD3 detection, sections were blocked with normal rabbit serum and biotin/avidin blockade reagent (Vector Laboratories SP-2001), stained with 1:50 rabbit rat anti-CD3 biotinylated IgG1 (Biorad MCA1477) for 60 min followed by rabbit anti-rat IgG using the ABC Elite Reagent (Vector Laboratories AK-5200) for detection. GFP on the same sections was revealed after blockade with normal goat anti-rabbit IgG using 1:2500 rabbit anti-GFP (Abcam ab6556) at 4º followed by goat anti-rabbit ABC-AP for detection. Peanut agglutinin (PNA) staining was revealed after biotin/avidin blockade by staining with 1:1000 PNA-biotin (Vector lab B-1075) at 4º followed by staining with APC. Histology images were viewed with an Olympus BX41 microscope (10-100X objectives) and photographed with an Olympus DP71 camera. DP controller software (Version 3.3.1.292) was used for image acquisition.