SUPPLEMENTAL MATERIAL

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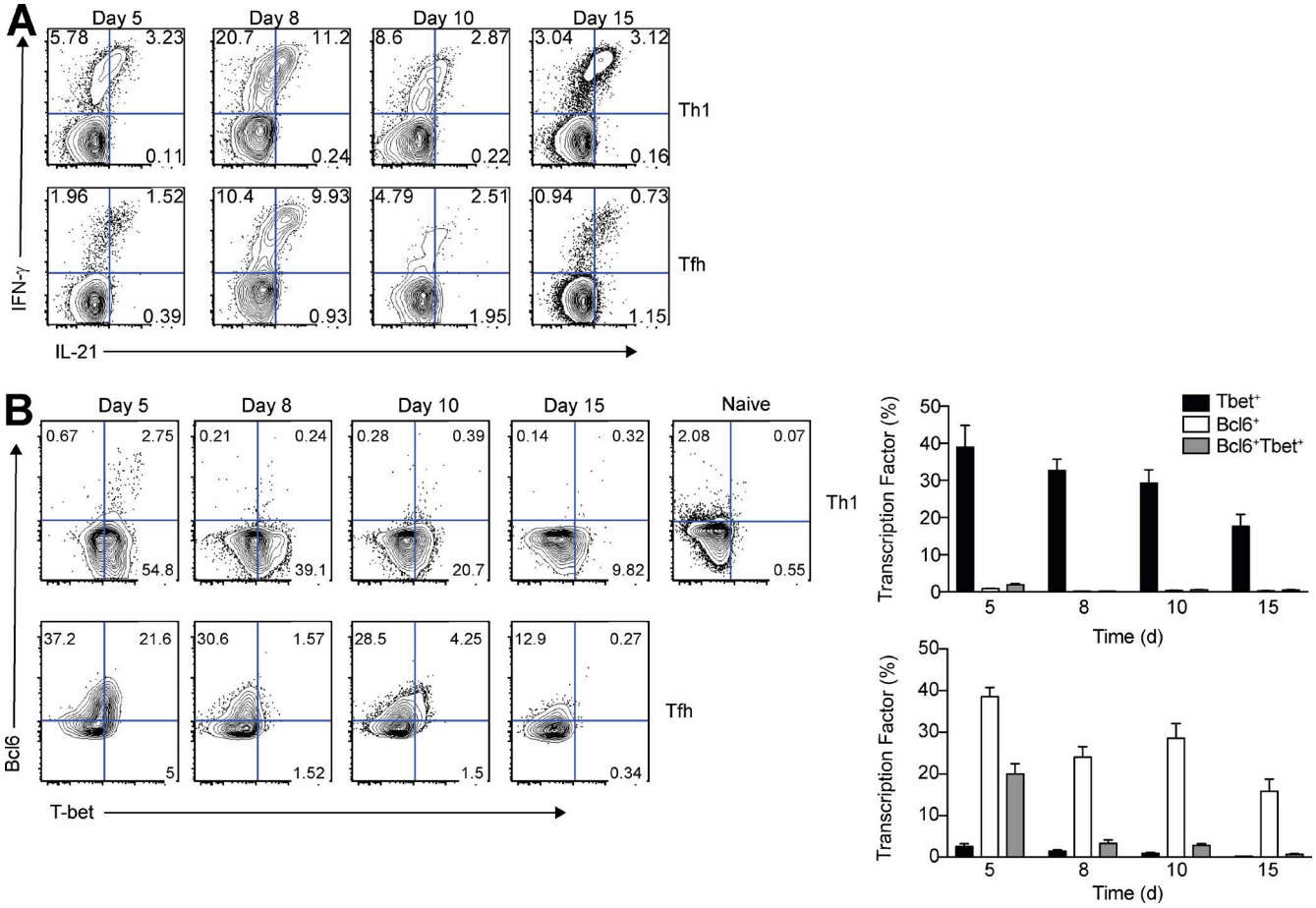

Figure S1. **Tfh cells express T-bet and coproduce IFN-γ and IL-21.** Thy1.2$^+$ B6 mice were infected with LCMV Armstrong. Splenic Thy1.2$^+$ PSGL-1^{hi}Ly6^{hi}CXCR5^{lo} Th1 and Thy1.2$^+$PSGL-1^{lo}Ly6^{lo}CXCR5^{hi}PD-1^{hi} Tfh cells in recipient spleens were analyzed at days 5, 8, 10, and 15 p.i. **(A)** Representative flow cytometry plots of intracellular IL-21 and IFN-γ staining in Th1 and Tfh cells. **(B)** Representative flow cytometry plots of intracellular staining for Bcl6 and T-bet in Th1 and Tfh cells with cell percentages of each transcription factor$^+$ population. Experiments were performed three times with $n \geq 5$ mice per group. Error bars represent SEM.

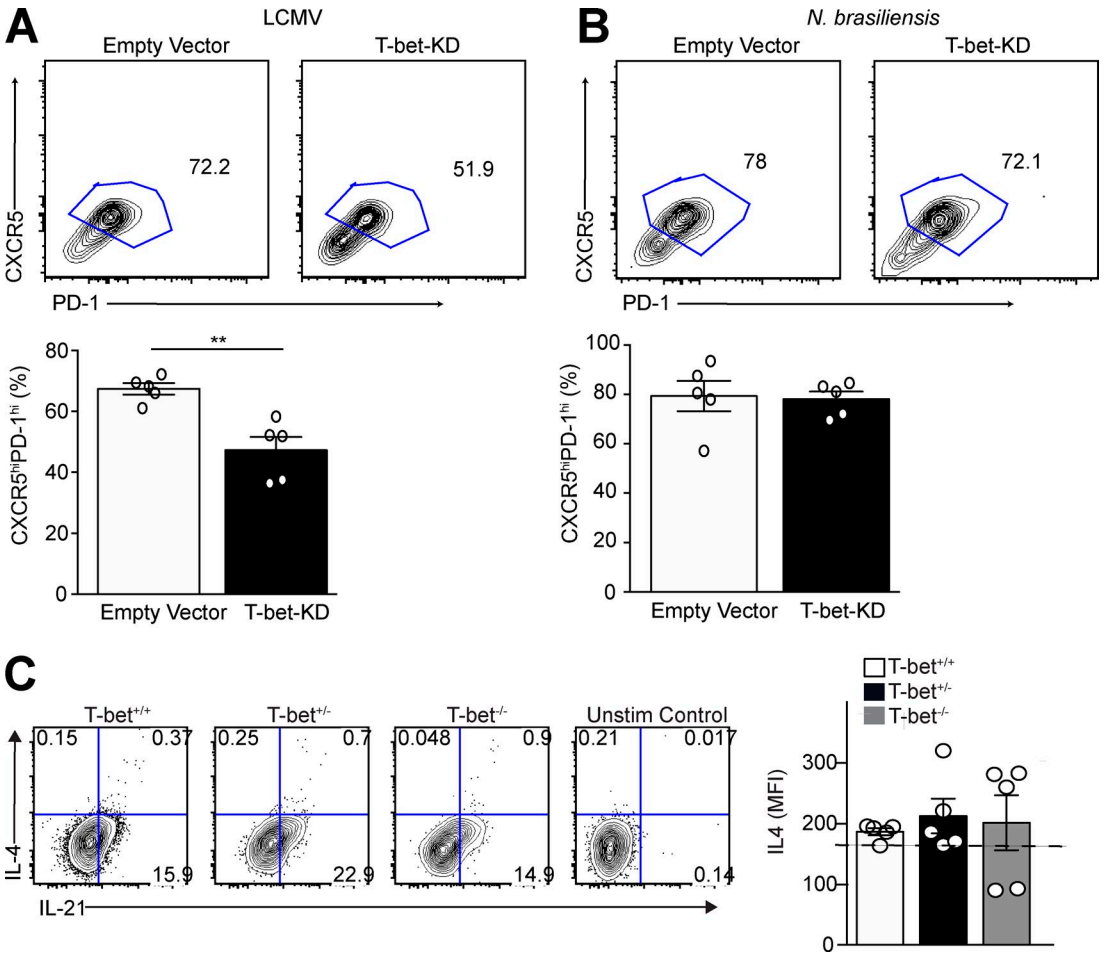

Figure S2. **T-bet is not required for Tfh cell differentiation in type 2 infection.** Thy1.1$^+$ Stg CD4$^+$ T cells or Thy1.1$^+$ OT-II (OVA-specific TCR) CD4$^+$ T cells were transduced with either a GFP-labeled retroviral T-bet knockdown or empty vector and transferred into Thy1.2$^+$ B6 mice followed by LCMV Armstrong or *N. brasiliensis* infection with OVA immunization (to activate TCR transgenic T cells), respectively. Transduced CD4$^+$Thy1.1$^+$GFP$^+$ PSGL-1^{lo}Ly6^{lo}CXCR5^{hi}PD-1^{hi} Tfh cells in recipient spleens were analyzed at 8 d p.i. **(A)** Representative flow cytometry plots of retrovirally transduced splenic Tfh cells after LCMV challenge with a bar graph that summarizes percentages of Tfh cells. **(B)** Representative flow cytometry plots of retrovirally transduced Tfh cells upon *N. brasiliensis* and OVA challenge with a bar graph that summarizes percentages of Tfh cells. **(C)** T-bet$^{+/+}$, T-bet$^{+/-}$, or T-bet$^{-/-}$ Thy1.1$^+$ Stg CD4$^+$ T cells were transferred to Thy1.2$^+$ B6 mice with LCMV Armstrong infection 24 h later. Spleens were harvested 8 d p.i. Representative flow cytometry plots of intracellular IL-21 and IL-4 staining in Tfh cells with a bar graph that summarizes MFI; dashed line represents mean MFI of unstimulated Tfh cells. Data are representative of two or three experiments with three to five recipients per group. **, $P < 0.01$ by Student's *t* test. Error bars represent SEM.

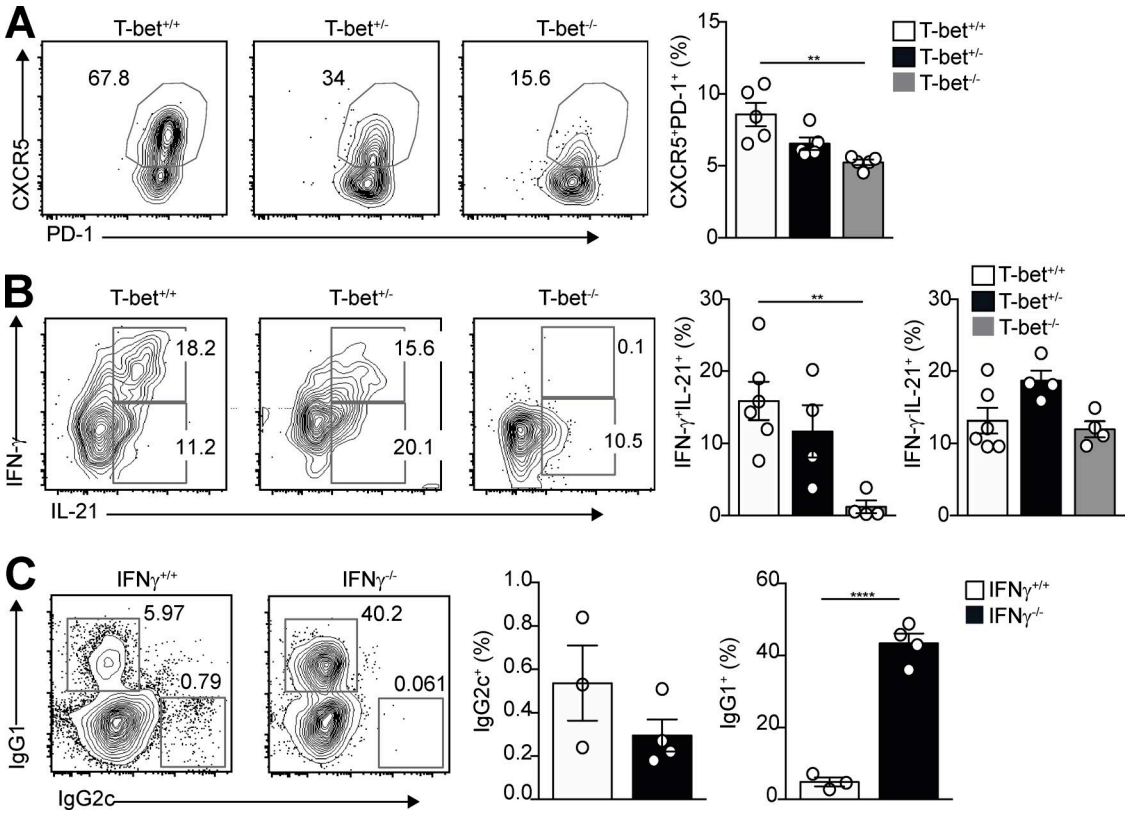

Figure S3. **T-bet expression in Tfh cells is necessary for proper GC development.** TCR-$\beta^{-/-}$ mice were infected with LCMV Armstrong a day after receiving in transfer T-bet$^{+/+}$, T-bet$^{+/-}$, or T-bet$^{-/-}$ Stg CD4^{+} cells. Splenic Thy1.1^{+}PSGL-1^{lo}Ly6^{lo}CXCR5^{hi}PD-1^{hi} Tfh cells in recipient spleens were analyzed at day 12 p.i. **(A)** Representative flow cytometry plots of CXCR5 and PD-1 staining of Tfh cells, with cell percentages and cell numbers. **(B)** Representative flow cytometry plots of intracellular IL-21 and IFN-γ staining in donor Tfh cells with summed percentages. **(C)** IFN-$\gamma^{+/+}$ and IFN-$\gamma^{-/-}$ mice were infected with LCMV Armstrong and sacrificed 12 d p.i. Representative flow cytometry plots of intracellular IgG1 and IgG2c staining of CD4^{-}B220^{+}IgD^{lo}CD95^{hi}GL-7^{hi} GC B cells with percentages of isotype^{+} cells. Data are representative of three experiments with three to five recipients per group. **, $P < 0.01$; ****, $P < 0.0001$ by Student's *t* test. Error bars represent SEM.

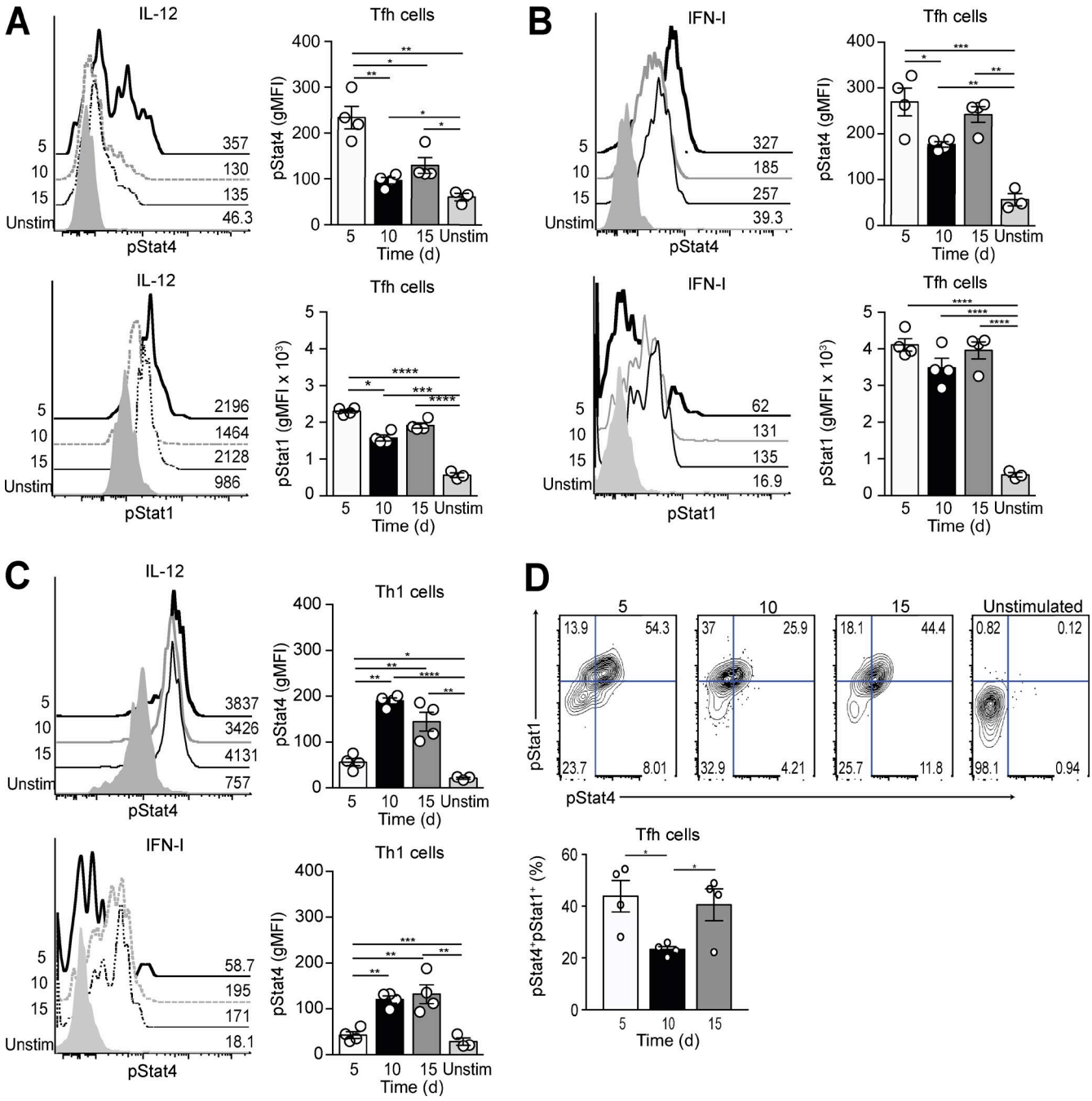

Figure S4. **Expression of pSTAT4 in Tfh cells.** Thy1.1$^+$ Stg TCR transgenic CD4$^+$ T cells were transferred into Thy1.2$^+$ B6 mice followed by infection with LCMV Armstrong 24 h later. Spleens were harvested at days 5, 10, and 15 p.i. **(A and B)** Total splenocytes were stimulated with either IL-12p40 (A) or IFN-β (B). Thy1.1$^+$PSGL-1^{lo}Ly6^{lo}CXCR5^{hi}PD-1^{hi} Tfh cells staining was followed by intracellular staining for pSTAT4 or pSTAT1, as quantified by gMFI. **(C)** Expression of pSTAT4 in PSGL-1^{hi}Ly6c^{hi} Th1 cells with quantified gMFI. **(D)** Representative flow cytometry plots of intracellular pSTAT1 and pSTAT4 staining of Tfh cells stimulated with IFN-β, with percentages of double-positive cells. Data are representative of three experiments with five recipients per group. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$ by Student's *t* test. Error bars represent SEM.

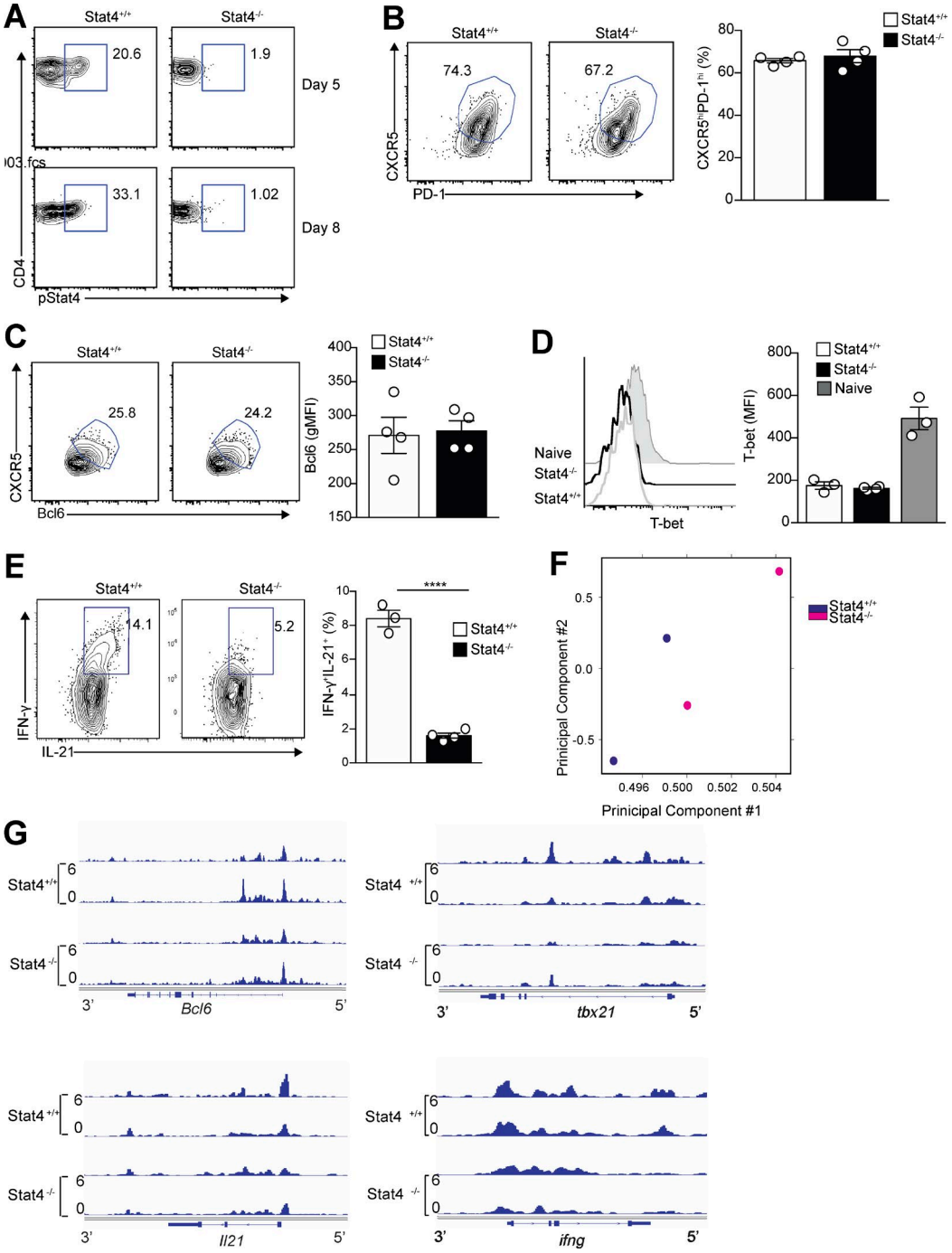

Figure S5. **STAT4 is required for cytokine production by Tfh cells but not their development.** Thy1.2 B6 mice were infected with LCMV a day after receiving naive Thy1.1$^+$STAT4$^{+/+}$ or Thy1.1$^+$STAT4$^{-/-}$ Stg CD4 T cells. **(A)** Total splenocytes from day 5 or day 8 p.i. were stimulated with IL-12p40, followed by intracellular staining for pSTAT4 in Thy1.1$^+$CD4$^+$ T cells, as quantified by gMFI. **(B)** Representative flow cytometry plots of day 8 p.i. splenic Thy1.1$^+$PS-GL-1^{lo}Ly6C^{lo}CXCR5^{hi}PD-1^{hi} Tfh cells with percentages summarized. **(C)** Staining for intracellular Bcl6 and for surface CXCR5 in CD4$^+$Thy1.1$^+$Ly6c^{lo}PSGL-1^{lo} STAT4$^{+/+}$ and STAT4$^{-/-}$ Tfh cells in recipients, with gMFI of Bcl6 staining. **(D)** Intracellular T-bet staining in STAT4$^{+/+}$ and STAT4$^{-/-}$ Tfh and in Th1 and naive CD4$^+$T cells with gMFI. **(E)** Representative flow cytometry plots of intracellular IL-21 and IFN-γ staining of STAT4$^{+/+}$ and STAT4$^{-/-}$ Tfh cells from recipient spleens, with cell percentages summarized. ATAC-seq performed on two replicates each of WT and STAT4-deficient cells to assess chromatin accessibility. **(F)** Principal component analysis performed on called ATAC peaks revealed that control and STAT4 knockout samples were not distinctly separated by group. **(G)** ATAC-seq at the *Bcl6*, *Tbx21*, *Ifng*, and *Il21* loci and flanking regions revealed no statistically significant differences in chromatin accessibility between control and STAT4-deficient cells. Sorted Tfh cells were pooled from five STAT4$^{+/+}$ or STAT4$^{-/-}$ Stg mice. Two independent sorts were analyzed for ATAC-seq. Data are representative of three experiments with five recipients per group. ****, $P < 0.0001$ by Student's *t* test. Error bars represent SEM.

Table S1. **Antibodies used for flow cytometry, ELISA, and microscopy**

Ag	Dilution	Clone	Fluorochrome	Source
CD4	1:200	RM4-5	Alexa Fluor 700, BV510	eBiosciences
CD44	1:200	IM7	e450	eBiosciences
PD-1	1:200	J43	PE-Cy7	eBiosciences
IgD	1:200	26-Nov	e450	eBiosciences
CD40L	1:50	MR1	APC	eBiosciences
B220	1:500	RA3-6B2	PE–Texas red	BD Biosciences
Thy1.1 (CD90.1)	1:200	OX-7	BV510	BD Biosciences
CXCR5		2G8	BV605	BD Biosciences
CD62L	1:200	MEL-14	APC	BD Biosciences
GL-7	1:200	GL-7	FITC	BD Biosciences
CD95	1:200	Jo2	PE-Cy7	BD Biosciences
IL-21R-FC chimera	1:40	Cat no. 596-MR		R&D Systems
Ly6C	1:200	AL-21	FITC	BD Biosciences
IgG2a	1:200	R19-15	PE	BD Biosciences
IFN-γ	1:500	XMG1.2	PeCy7	BD Biosciences
PSGL-1	1:1,000	2PH1	APC	Conjugated in house to BD Biosciences antibody
pSTAT4	1:10	SLEB11	APC	BD Biosciences
pSTAT1	1:10	4a	PE	BD Biosciences
Tbet	1:50	eBio4B10	APC	eBiosciences
Bcl6	1:50	K112-91	PE	BD Biosciences
IgG1	1:200	A85-1	FITC	BD Biosciences
CD138	1:400	281-2	APC	BD Biosciences
IgG1	1:1,000	Goat polyclonal	HRP, AP	Southern Biotech
CD4	1:200	RM4-5	FITC	eBiosciences
IgD	1:200	26-Nov	Alexa Fluor 647	eBiosciences
PNA	1:200		Biotin	Vector Labs
FITC	1:200	Rabbit polyclonal	Alexa Fluor 488	Invitrogen
Streptavidin	1:200		Alexa Fluor 555	Invitrogen
Anti-IgM AP	1:2,000	Goat polyclonal		Southern Biotech
Anti-IgG1 AP	1:2,000	Goat polyclonal		Southern Biotech
Anti-IgG2c AP	1:2,000	Goat polyclonal		Southern Biotech

Table S2. **Quantitative RT-PCR primers**

Gene name	Forward	Reverse
Bcl6	5'-CACACTCGAATTCACTCTG-3'	5'-TATTGCACCTTGGTGTTGG-3'
Tbx21	5'-CAACAACCCCTTTGCCAAAG-3'	5'-TCCCCCAAGCAGTTGACAGT-3'
Ifng	5'-GATGCATTCATGAGTATTGCCAAGT-3'	5'-GTGGACCACTCGGATGAGCTC-3'
Il21	5'-TGAAAGCCTGTGGAAGTGCAAACC-3'	5'-AGCAGATTCATCACAGGACACCCA-3'
Batf	5'-CACAGAAAGCCGACACCCTT-3'	5'-CACAGAAAGCCGACACCCTT-3'
Hprt	5'-ACCTCTCGAAGTGTTGGATACAG-3'	5'-CCTCGTATTTGCAGATTCAACTT-3'

Table S3 is a separate Excel document showing that the regulation of differential gene expression in STAT4$^{+/+}$ and STAT4$^{-/-}$ Tfh cells is not mediated at the level of chromatin accessibility.