Supplementary Material

The Transcription Factor T-bet is Required for Optimal Type I Follicular Helper T Cell Maintenance during Acute Viral Infection

Pengcheng Wang1,2,#,Youping Wang1,#, Luoyingzi Xie1,#, Minglu Xiao1, Jialin Wu1, LifanXu1, QiangBai1, YaxingHao1, Qizhao Huang4, Xiangyu Chen1, Ran He1, Baohua Li1, Sen Yang3, Yaokai Chen3,\*,Yuzhang Wu1,\*and Lilin Ye1,\*

1Institute of Immunology, PLA,Third Military Medical University, Chongqing, China

2National Clinical Research Center of Kidney Diseases, Jinling Hospital, Nanjing, China

3Chongqing Public Health Medical Center, Chongqing, China

4Cancer Center, The General Hospital of Western Theater Command, Chengdu, China

#These authors contributed equally to this work

**\*Correspondence:**

Yaokai Chen  
yaokaichen@hotmail.com

Yuzhang Wu  
wuyuzhang@tmmu.edu.cn

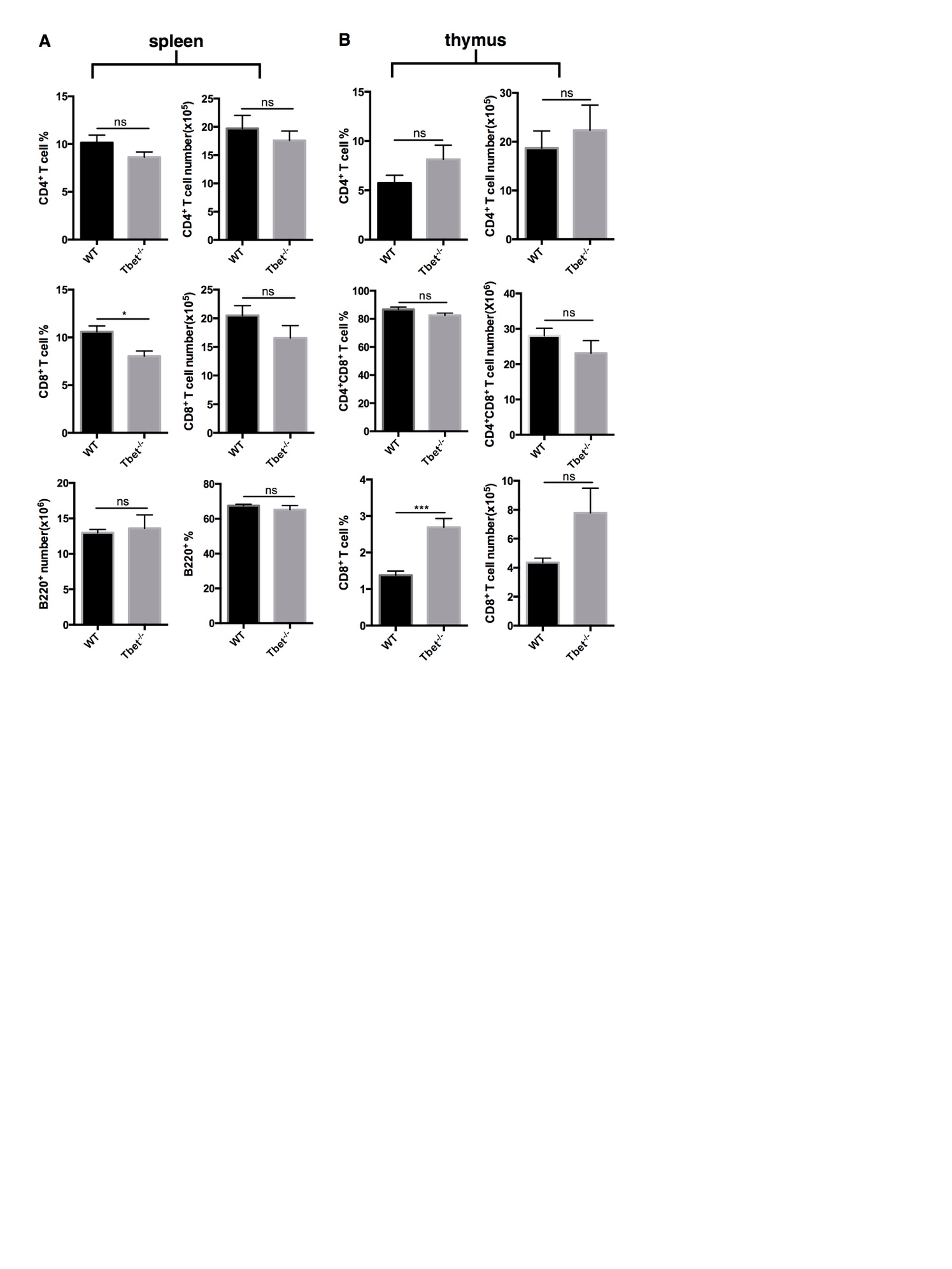
Lilin Ye  
yelilinlcmv@tmmu.edu.cn

**Supplementary Table**

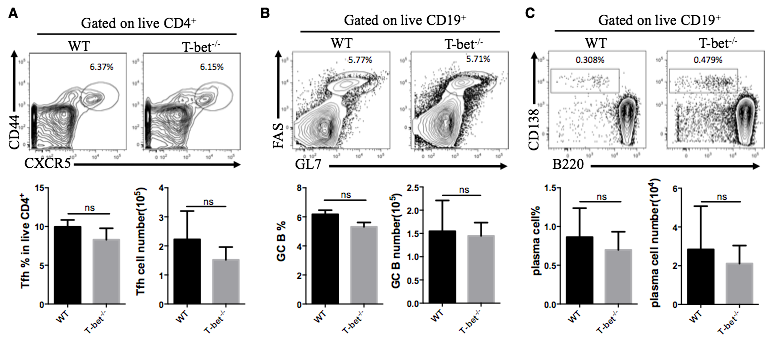
**Supplementary Table 1 | Antibodies and Reagents**

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| --- | --- | --- |
| Antibody/Reagent | Clone/Cat.No. | Company |
| CD4 | RM4-5 | Biolegend |
| CD8 | 53-6.7 | BD Biosciences |
| B220 | RA3-6B2 | eBioscience |
| CD19 | 6D5 | Biolegend |
| CD44 | IM7 | eBioscience |
| CD69 | H1.2F3 | Biolegend |
| CD25 | PC61.5 | Biolegend |
| T-bet | 4B10 | Biolegend |
| Foxp3 | FJK-16s | eBioscience |
| CXCR5 | 2G8 | BD Bioscience |
| TNFa | MP6-XT22 | Biolegend |
| IL2 | JES6-5H4 | Biolegend |
| IFNγ | XMG1.2 | BD Bioscience |
| SLAM | TC15-12F12.2 | Biolegend |
| Bcl6 | K112-91 | BD Bioscience |
| TCF1 | C46C7 | CST |
| PNA | FL-1071 | Vector Labs |
| FAS | JO2 | BD Bioscience |
| CD138 | 281-2 | BD Bioscience |
| Ki67 | B56 | BD Bioscience |
| Bcl2 | 51-15025X | BD Bioscience |
| Caspase3 | 51-68654X | BD Bioscience |
| CD45.1 | A20 | Biolegend |
| CD45.2 | 104 | Biolegend |
| Va2 | B20.1 | Biolegend |
| CD127 | A7R34 | Biolegend |
| CD62L | MEL-14 | eBioscience |
| PD-1 | RMP1-30 | eBioscience |
| GITR | DTA-1 | eBioscience |
| CD71 | R17217 | eBioscience |
| CD98 | RL388 | eBioscience |
| IgG2c | 130-097-950 | MiltenyiBiotec |
| IgD | 11-26c.2a | BD Bioscience |
| Tim3 | RMT3-23 | Biolegend |
| Annexin V | 51-65875X | BD Bioscience |
| Brdu | 51-23619U | BD Bioscience |
| FC-blocker | NB309 | Innovex Biosciences |
| KLRG1 | 2F1 | BD Bioscience |
| GL-7 | GL7 | BD Bioscience |

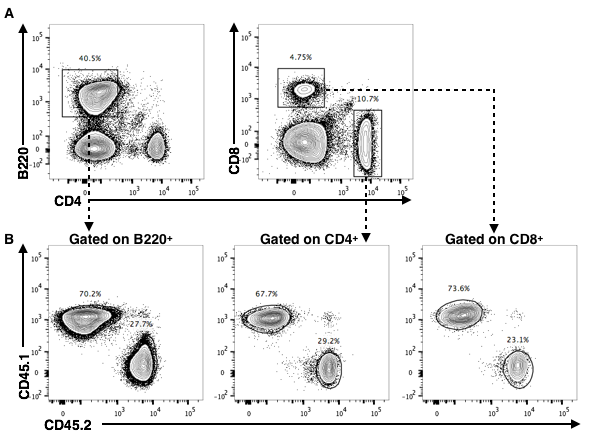
## Supplementary Figures



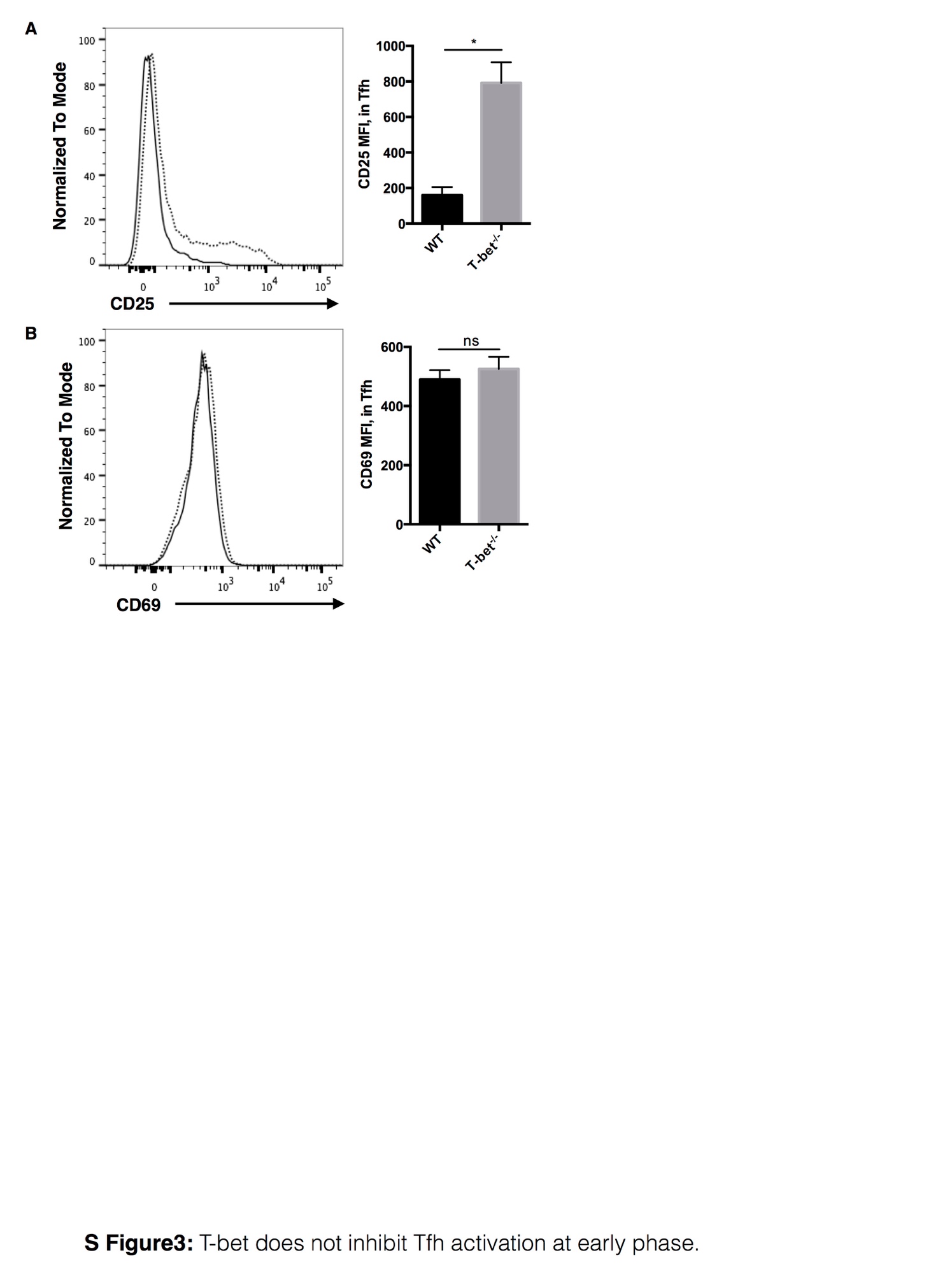
**S FIGURE 1 | Naive state of CD4/CD8+T cells in Tbx21-/- mice (associated with Figure 2).** Spleen as well as thymus were harvested from Naïve WT and Tbx21-/- mice and analyzed for naïve state of lymphocytes. **(A)** Summary the percentages and numbers of CD4+ T cells, CD8+ T cells and B220+ B cells in spleen. **(B)** Summary the percentages and numbers of CD4+ T cells, CD8+ T cells and CD4+CD8+ T cells in thymus. ns, not significant; \**P* < 0.05, \*\*\**P* < 0.001 (unpaired two-tailed *t*-test). Data are representative of two independent experiments with 3-5 mice per group (error bars, SEM).

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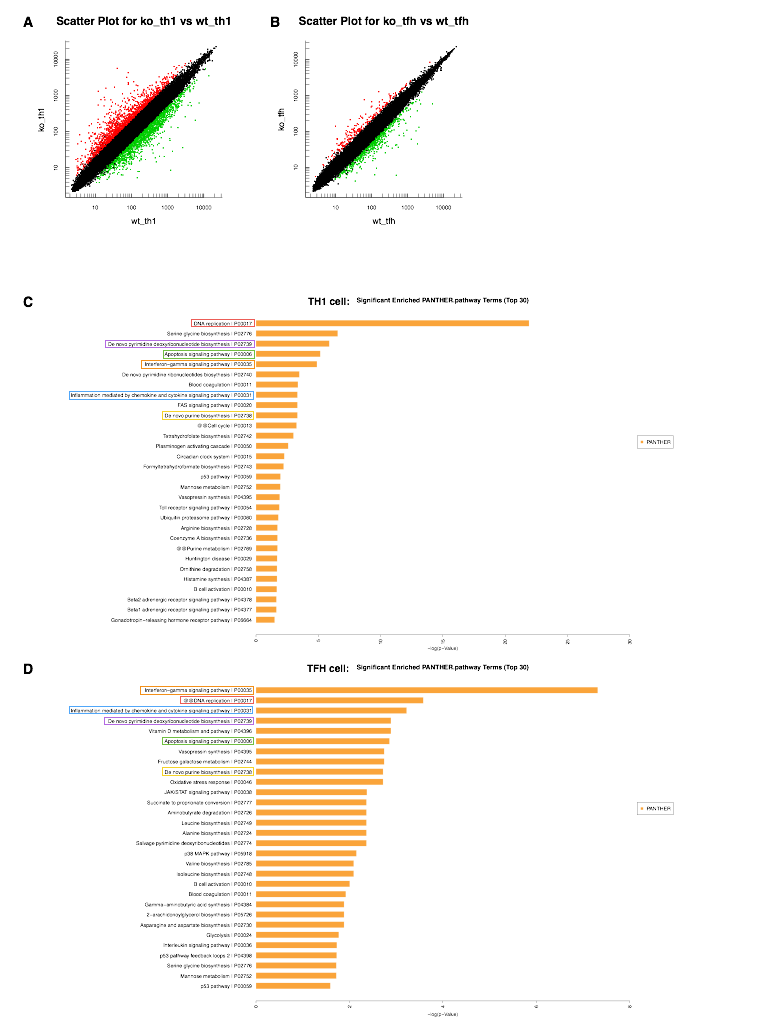
**S FIGURE 2 | T-bet is not required for Type II TFH cell response.** **(A-C)** WT and Tbx21-/- mice were immunized with NP-KLH. Draining lymph nodes were harvested at day8 post infection. Representative flow cytometry of TFH cells (CD44+CXCR5+) **(A)**, GC B cells (FAS+PNA+) **(B)** and plasma cells (CD138+B220low) **(C)** (up) with its percentages and numbers (below) in WT and Tbx21-/- mice. Numbers adjacent to outlined areas indicate percent of each cell subset in parent subset. ns, not significant; \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001 (unpaired two-tailed *t*-test). Data are representative of two independent experiments with 3-5 mice per group (error bars, SEM).



**S FIGURE 3 | successful reconstitution of bone marrow chimera mice (associated with Figure 4).** Bone marrow cells collected from CD45.2+ Tbx21-/- mice and CD45.1+ WT mice were mixed at a ratio of 3:7 and transferred intravenously into lethally irradiated (5.5 Gy, twice) naïve WT CD45.1+ mice (5 x 106 cells/mouse). 8 weeks later, peripheral blood of recipients was tested. **(A)** Flow cytometry of B220+ B cells, CD4+ T cells and CD8+ T cells in chimera mice at day60 post cell transfer. **(B)** Flow cytometry of WT (CD45.1+) and Tbx21-/- cells (CD45.2+) in B220+ B cells, CD4+ T cells and CD8+ T cells of chimera mice. Numbers adjacent to outlined areas in **(A,B)** indicate percent of each cell subset in parent subset.



**S FIGURE 4 | T-bet does not inhibit Tfh activation at early phase (associated with Figure 6). (A)** Flow cytometry of CD25 expression, with the summary of CD25 expression (showed as MFI) in SMARTA TFH cells at day2 post infection. **(B)** Flow cytometry of CD69 expression, with the summary of CD69 expression (showed as MFI) in SMARTA TFH cells at day2 post infection. ns, not significant; \**P* < 0.05 (unpaired two-tailed *t*-test). Data are representative of two independent experiments with 3-5 mice per group (error bars, SEM).



**S FIGURE 5 | Scatter plot of differentially expressed genes and Pathway enrichment of the TFH and TH1 cell transcriptomes (****associated with Figure 7). (A,B)** Scatter plot showing the differentially expressed genes in TH1 **(A)** and TFH **(B)** cells of Tbx21-/- mice relative to WT mice. The red plots represent upregulated genes, the green plots represent downregulated genes, and the black plots represent genes without clear differences in expression between Tbx21-/- and WT cells (fold change≥ 2). **(C)** PANTHER pathway enrichment analysis of TH1 cells (WT TH1*vs*Tbx21-/- TH1 cells). **(D)** PANTHER pathway enrichment analysis of TFH cells (WT TFH*vs*Tbx21-/- TFH cells).