

Figure S1 (Related to Figure 1). Pcc infection promotes Th1-biased polyclonal CD4⁺ T-cell activation when compared with PbA infection.

B6 mice were adoptively transferred with PbT-II cells and uninfected (Uninf) or infected with PbA or Pcc on the following day, and spleen cells were analyzed as shown in Fig. 1. (A) Gating strategy for the analysis of PbT-II and host CD4⁺ T cells. The results for PbT-II cells are shown in Fig. 1, and those for

host CD4⁺ T cells are shown here. (B) Representative plots (upper) and summary graphs (lower panel) of surface staining in host CD4⁺ cells. (C) Representative plots (upper panel) and summary graphs (lower panel) of intracellular staining in host CD4⁺ cells. The numbers in each graph are the proportions (%) of the indicated subpopulations. Two-tailed Student's t-test was used to compare PbA- vs. Pcc-infected mice. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.001; ns p > 0.05. The data are shown as the mean \pm SD (A, B). (D) The proportions of host CD4⁺ cell subpopulations (upper) and Ly6C^{hi} cells in host Tbet^{hi}Tcf1^{lo} and Tbet^{lo}Tcf1^{hi}CD4⁺ cells (lower) are plotted in relation to parasitemia levels in each mouse. Each dot represents an individual mouse. Pearson's correlation (two-tailed) coefficient (r) and p values were determined (C). The data were obtained from the same mice as in Fig. 1.



Figure S2 (Related to Figure 1). Tfh-like cells from both PbA- and Pcc-infected mice express

CXCR3. B6 mice were adoptively transferred with PbT-II cells and infected with PbA or Pcc on the following day, and spleen cells were stained for CD4, CD45.1, CXCR3, CXCR5, fixed and permeabilized, and stained intracellularly for T-bet and Bcl-6. Representative flow cytometry profiles of T-bet/Bcl-6 on PbT-II (CD4⁺CD45.1⁺) (A) and host CD4⁺ T (CD4⁺CD45⁻) cells are shown (left). Th1 (T-bet^{hi}Bcl-6^{lo}, blue), Tfh-like (T-bet^{lo}Bcl-6^{hi}, red) and naïve like (T-bet^{lo}Bcl-6^{lo}, gray) populations were gated, and their CXCR5/CXCR3 profiles are shown in different colors. Geometric mean fluorescence intensity (gMFI) of CXCR3 is shown for Th1 (blue), Tfh-like (red) and naïve like (gray) populations in PbT-II cell (A) and host CD4⁺ T cells (B) in PbA and Pcc infected mice. Two-way ANOVA followed by Bonferroni test was used for the analysis. **p* <0.05; ***p* <0.01; *** *p* <0.001; **** *p* <0.0001;

ns *p* >0.05.



Figure S3 (Related to Figure 3). Sorting of PbT-II subpopulations from PbA-infected mice for transcriptome analysis.

B6 mice were adoptively transferred with PbT-II cells and infected with PbA on the following day. Seven days after infection, spleen cells were stained with APC-anti-CD45.1 mAb and PbT-II cells (CD45.1⁺) were enriched using Auto-MACS. The enriched PbT-II cells were stained for CD4, CD11a, and CD49d, and CD11a^{hi}CD49d^{hi} and CD11a^{hi}CD49d^{lo} PbT-II cells were sorted using BD FACSAria.



Figure S4 (Related to Figure 4). Inhibition of type I IFN signaling modulates CD4⁺ T-cell responses during infection with Pcc.

B6 mice were adoptively transferred with PbT-II cells, infected with Pcc on the next day, and treated with anti-IFNAR1 blocking mAb (blue) or IgG (orange) as shown in Figure 4. Parasitemia was monitored (A). Spleen cells were prepared 7 days after infection, stained with mAbs, and analyzed using flow cytometry. (B, C) Representative plots and summary graphs for CD45.2/CD45.1 in CD4⁺ cells (B) and for CD11a/CD49d and Ly6C/CD49d in PbT-II and host CD4⁺ T cells (C). The total number of PbT-II cells was calculated by multiplying the number of spleen cells with the ratio of PbT-II cells in each spleen. (D) Representative plots and summary graphs of the intracellular staining for T-bet /Tcf1 and Ly6C in T-bet^{hi}Tcf1^{lo} and Tbet^{lo}Tcf1^{hi} PbT-II and host CD4⁺ T cells. The numbers in each graph are the proportions (%) of the indicated subpopulations. A two-tailed Student's *t*-test was used for comparison. **p* <0.05; ***p* <0.01; *****p* <0.0001; ns *p* >0.05.