

Supplemental Information

**A Macrophage Colony-Stimulating-
Factor-Producing $\gamma\delta$ T Cell Subset
Prevents Malarial Parasitemic Recurrence**

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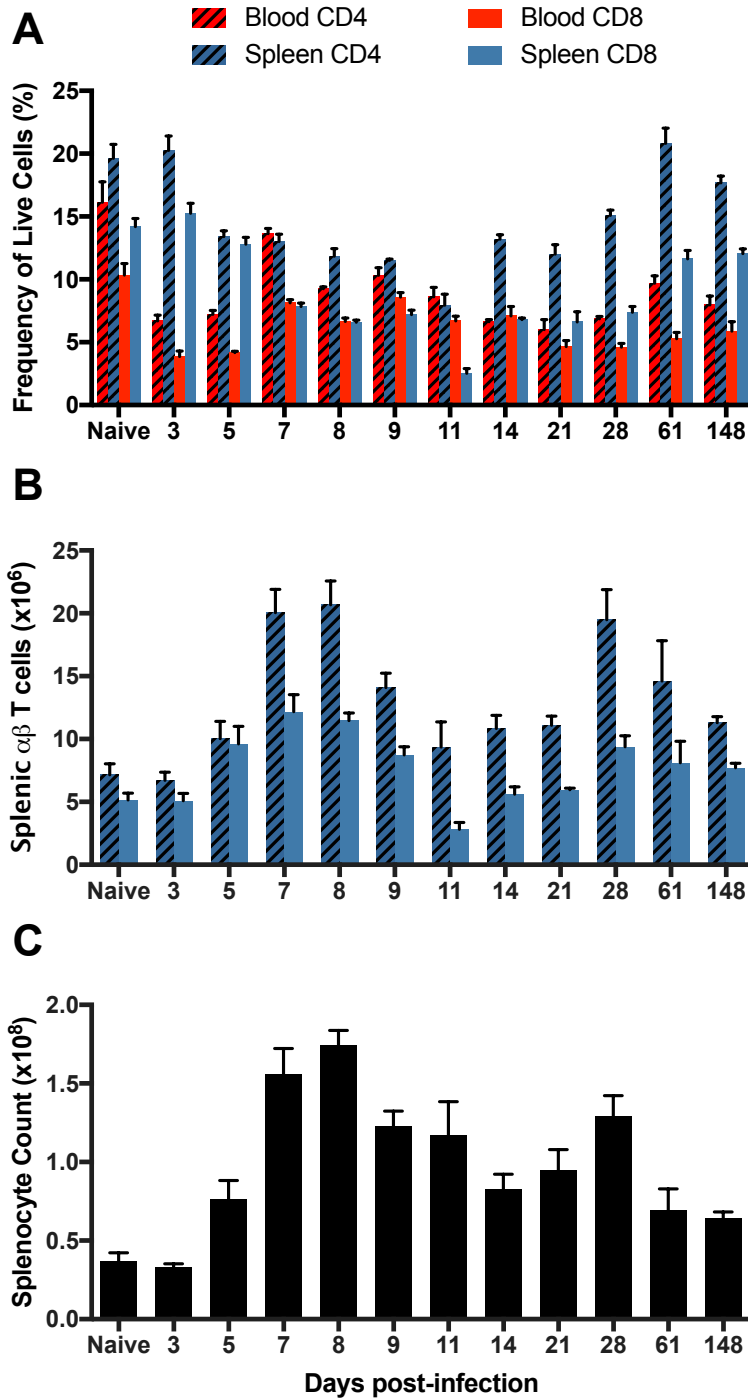


Figure S1. Related to Figure 1. Changing amounts of $\alpha\beta$ T cells and splenocytes during *P. chabaudi* infection.

(A and B) The frequency in the blood and spleen (A) and the absolute number in the spleen (B) of CD4⁺ and CD8⁺ $\alpha\beta$ T cells taken from C57BL/6 mice infected with 10^5 Pcc AJ-iRBCs.

(C) The number of splenocytes in C57BL/6 mice infected with 10^5 Pcc AJ-iRBCs.

(A-C) n=4 per time-point. Data shown as mean \pm SEM from one experiment.

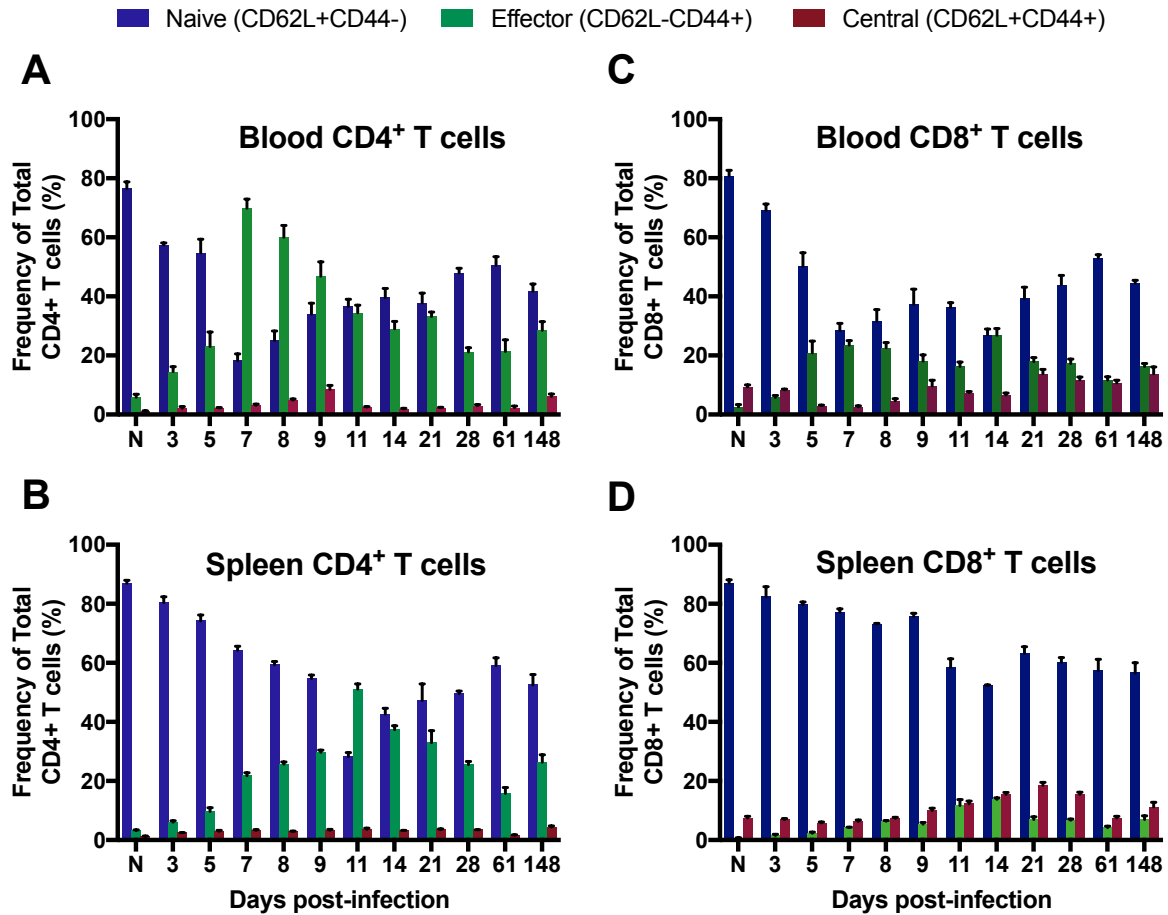


Figure S2. Related to Figure 1. Activated phenotypes of $\alpha\beta$ T cells during *P. chabaudi* infection.

(A-D) The frequencies of naïve (CD62L⁺CD44⁻), effector (CD62L⁻CD44⁺), and central memory (CD62L⁺CD44⁺) blood-borne CD4⁺ $\alpha\beta$ T cells (A), splenic CD4⁺ $\alpha\beta$ T cells (B), blood-borne CD8⁺ $\alpha\beta$ T cells (C), and splenic CD8⁺ $\alpha\beta$ T cells (D) in naïve (N) and infected C57BL/6 mice. Data shown as mean \pm SEM from one experiment; n=4 per time-point.

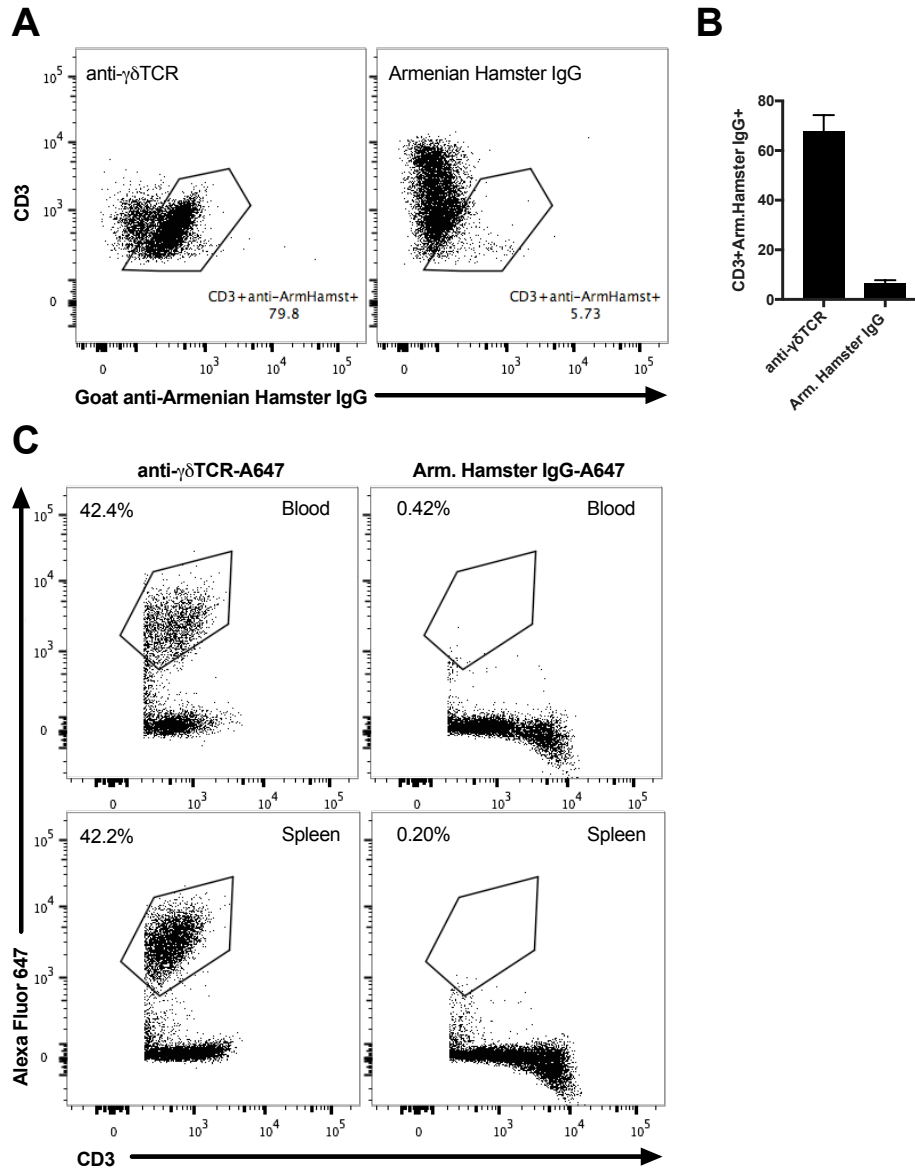


Figure S3. Related to Figure 3. $\gamma\delta$ T cells persist after anti- $\gamma\delta$ TCR antibody administration. (A and B) Representative plots (A) and quantification (B) of CD3⁺Armenian Hamster IgG⁺ cells among live CD3⁺CD4⁻CD8⁻ splenocytes obtained at 14 d.p.i. from infected C57BL/6 mice (n=4 per group). The animals were administered at 12 d.p.i. i.p. 200 μ g of anti- $\gamma\delta$ TCR antibody (clone GL3, Armenian Hamster IgG isotype) or irrelevant Armenian Hamster IgG isotype control (clone HTK888; anti-trinitrophenol). After fixation and permeabilization, the cells were stained with goat anti-Armenian Hamster IgG secondary antibody. Data are representative of two independent experiments.

(C) Representative plots of CD3⁺Alexa Fluor 647⁺ cells among live CD3⁺CD4⁻CD8⁻ cells obtained at 14 d.p.i. from infected C57BL/6 mice (n=3 per group). The animals were injected at 12 d.p.i. i.p. 200 μ g of Alexa Fluor 647-conjugated anti- $\gamma\delta$ TCR (clone GL3, Armenian Hamster IgG isotype) or irrelevant Armenian Hamster IgG isotype control (clone HTK888; anti-trinitrophenol). None of the antibodies used in the staining panel were conjugated to Alexa Fluor 647 or equivalent dyes. Data shown are from one experiment.

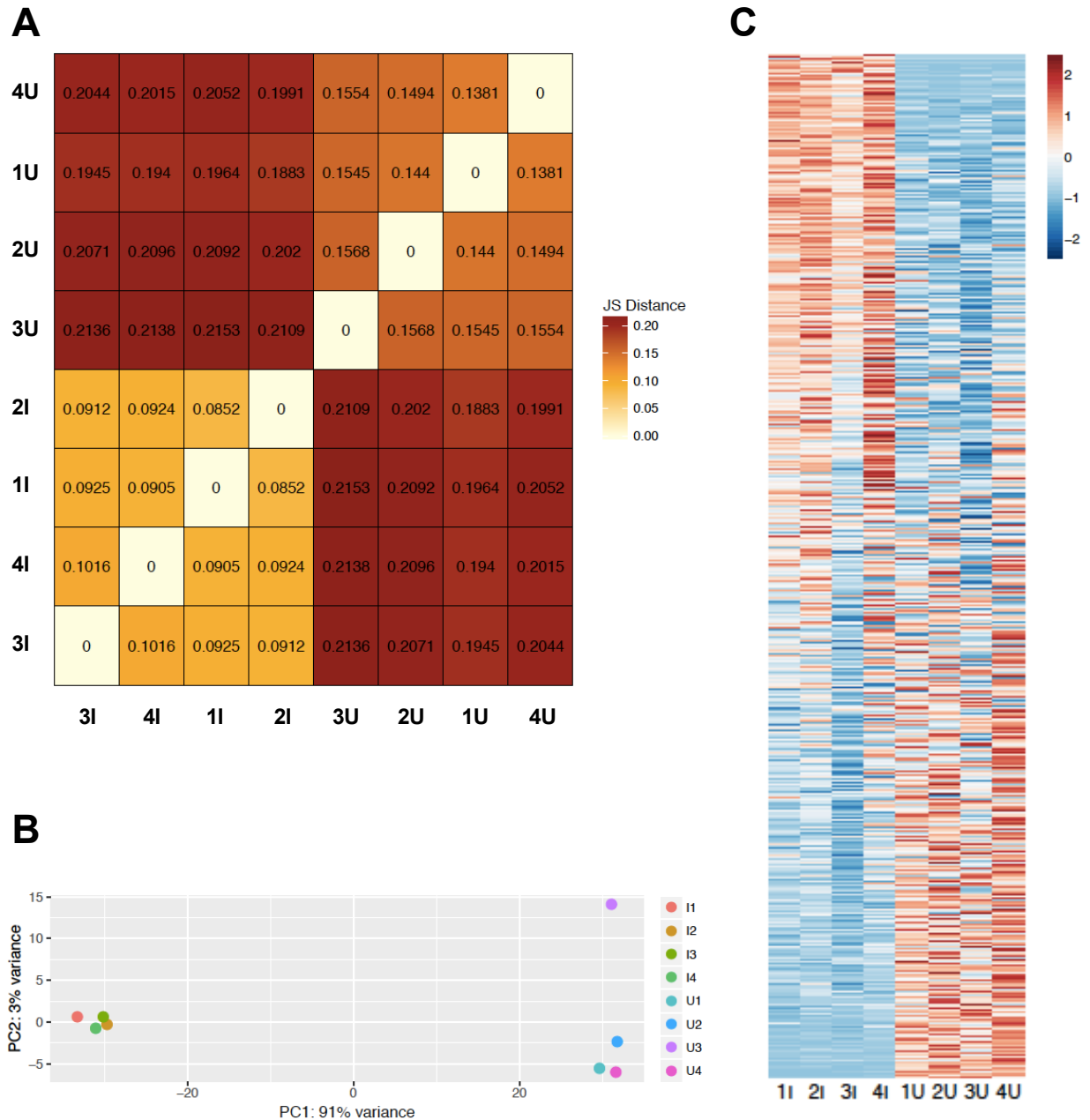


Figure S4. Related to Figure 5. Global comparison of $\gamma\delta$ T cells from infected and uninfected animals.

(A) Pairwise comparisons of the global transcriptomes of splenic $\gamma\delta$ T cells from infected (1I-4I) and uninfected (1U-4U) mice as measured by Jensen-Shannon (JS) distance scores. Samples were collected at 19 d.p.i..

(B) Principle component (PC) analysis transformation of global transcription by $\gamma\delta$ T cells from infected and uninfected animals. Percentage of total variance accounted for by PC1 and PC2 shown.

(C) Normalized global transcription. Using gene expression measurements, the heat map shows Z-scores normalized within each gene of the entire identified transcriptome (9892 genes). Each row shows a separate gene.

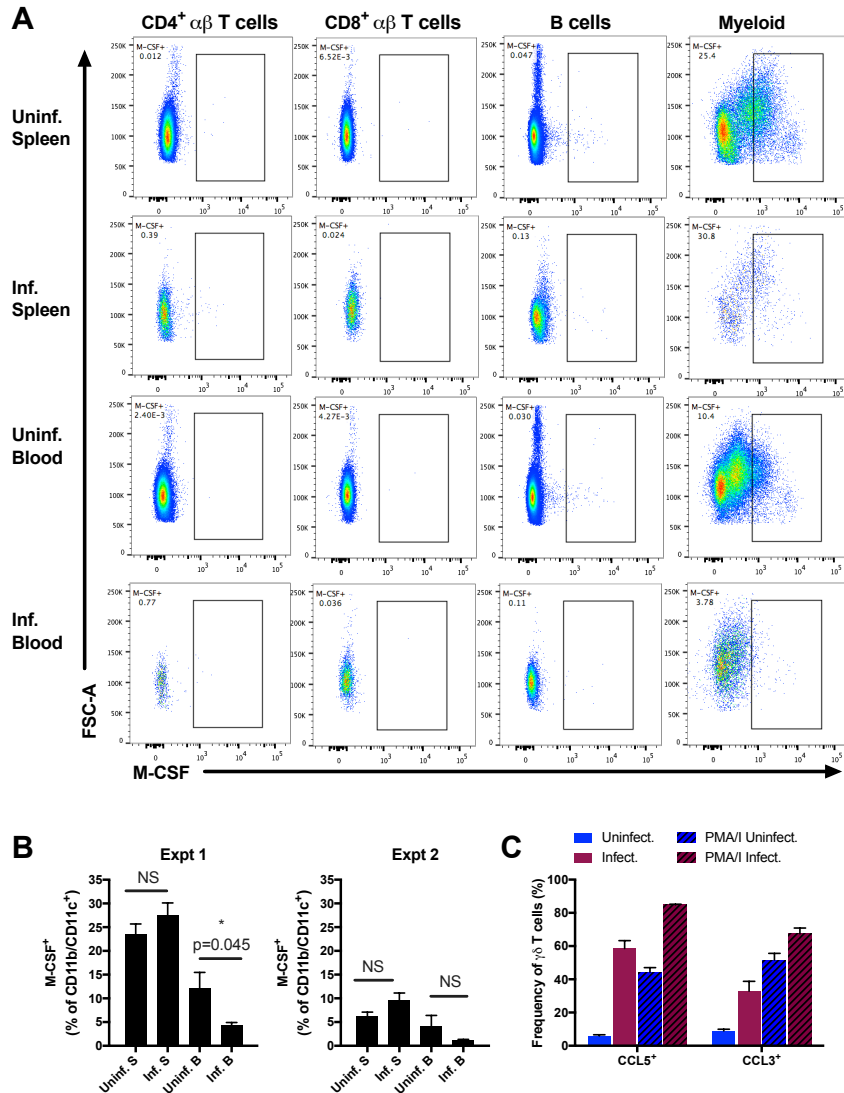


Figure S5. Related to Figure 5. M-CSF staining across leukocytes.

(A) Representative FACS pseudocolor plots of intracellular M-CSF staining in splenic and blood-borne CD4⁺ $\alpha\beta$ T cells (TCR β ⁺CD4⁺CD8⁻ CD11b/CD11c⁻TCR $\gamma\delta$ ⁻), CD8⁺ $\alpha\beta$ T cells (TCR β ⁺CD8⁺CD4⁻ CD11b/CD11c⁻TCR $\gamma\delta$ ⁻), B cells (CD19⁺CD4⁻CD8⁻CD11b/CD11c⁻TCR $\gamma\delta$ ⁻), and myeloid cells (CD11b⁺ and/or CD11c⁺, CD3⁻TCR β ⁻TCR $\gamma\delta$ ⁻CD19⁻) from infected and uninfected vehicle control animals at 19 d.p.i. are shown. Data are representative of two independent experiments.

(B) Quantified M-CSF staining in splenic (S) and blood-borne (B) myeloid cells obtained from infected and uninfected vehicle control animals at 19 d.p.i. from two independent experiments.

(C) Frequency of blood-borne $\gamma\delta$ T cells at 19 d.p.i. that are CCL5⁺ and CCL3⁺ with or without stimulation. Cells were cultured for 6 hours in the presence of protein trafficking inhibitors and in the absence or presence of PMA and ionomycin before staining. Data are representative of three independent experiments.

(A and B) n=5 per group, (C) n=4-5 per group. (B and C) Data shown as mean \pm SEM. Two-tailed, unpaired Student's *t*-test.

Table S1. Related to Figure 5. Clusters of Upregulated Genes

| Gene | Infected (FPKM±SEM) | Uninfected (FPKM±SEM) | Fold Change |
|-------------------------------------|---------------------|-----------------------|-------------|
| Killer Cell Like Receptors | | | |
| <i>Klra3</i> | 106.32 ± 23.71 | 2.59 ± 0.40 | 41.04 |
| <i>Klrb1b</i> | 229.14 ± 55.49 | 8.44 ± 2.50 | 27.14 |
| <i>Klre1</i> | 377.83 ± 52.65 | 24.57 ± 3.64 | 15.38 |
| <i>Klri1</i> | 462.52 ± 62.82 | 34.55 ± 5.44 | 13.39 |
| <i>Klrb1f</i> | 56.38 ± 4.46 | 4.82 ± 0.66 | 11.70 |
| <i>Klri2</i> | 99.38 ± 15.86 | 8.85 ± 2.24 | 11.22 |
| <i>Cd244</i> (NKR2B4) | 84.14 ± 5.38 | 8.23 ± 1.12 | 10.22 |
| <i>Klra9</i> | 17.05 ± 6.39 | 1.38 ± 0.20 | 12.33 |
| Cell Recruitment/Activation | | | |
| <i>Spp1</i> | 123.36 ± 11.87 | 0.35 ± 0.04 | 348.34 |
| <i>Csf1</i> (M-CSF) | 456.29 ± 26.90 | 1.82 ± 0.26 | 251.23 |
| <i>Ccl3</i> | 229.87 ± 11.20 | 1.20 ± 0.49 | 191.47 |
| <i>Ccl4</i> | 70.09 ± 12.07 | 3.13 ± 2.07 | 22.42 |
| <i>Ccl6</i> | 16.54 ± 2.29 | 0.83 ± 0.40 | 20.02 |
| <i>Ccl5^a</i> | 4085.63 ± 286.8 | 503.97 ± 62.26 | 8.11 |
| <i>Ccr12</i> | 62.56 ± 6.45 | 4.49 ± 1.24 | 13.94 |
| <i>Metrn1</i> | 36.40 ± 2.18 | 2.34 ± 0.07 | 15.56 |
| <i>S100a9</i> | 53.27 ± 12.54 | 2.48 ± 1.81 | 21.46 |
| Cytotoxicity Associated | | | |
| <i>Fcrl6</i> | 70.22 ± 19.23 | 0.76 ± 0.28 | 92.67 |
| <i>Gzmb</i> | 218.61 ± 26.32 | 6.59 ± 0.74 | 33.18 |
| <i>Ncf2</i> | 17.07 ± 1.22 | 0.69 ± 0.26 | 24.66 |
| Attenuated Growth/Exhaustion | | | |
| <i>Havcr2</i> (Tim3) | 27.01 ± 2.57 | 0.42 ± 0.25 | 64.34 |
| <i>Prdm1</i> (Blimp1) | 51.16 ± 3.58 | 1.86 ± 0.21 | 27.51 |
| <i>Tigit</i> | 72.69 ± 4.19 | 5.21 ± 1.60 | 13.96 |
| <i>Entpd1</i> | 30.97 ± 2.34 | 2.25 ± 0.27 | 13.75 |
| Apoptosis Regulation | | | |
| <i>Bcl2a1d</i> | 77.94 ± 8.19 | 3.33 ± 0.92 | 23.39 |
| <i>Bcl2a1b</i> | 154.06 ± 17.63 | 10.68 ± 1.31 | 14.43 |
| <i>Htra3</i> | 31.35 ± 5.10 | 0.69 ± 0.17 | 45.64 |
| <i>Casp7</i> | 65.23 ± 3.00 | 5.36 ± 0.86 | 12.17 |
| <i>Entpd1</i> | 30.97 ± 2.34 | 2.25 ± 0.27 | 13.75 |
| <i>Birc5</i> | 20.52 ± 0.93 | 1.58 ± 0.52 | 12.95 |
| <i>Plekhf1</i> | 17.66 ± 0.94 | 1.44 ± 0.34 | 12.28 |
| TCR Signaling | | | |
| <i>Plcg2</i> | 63.72 ± 1.30 | 3.97 ± 0.70 | 16.05 |
| <i>Pik3ap1</i> | 115.42 ± 6.47 | 8.39 ± 0.98 | 13.76 |
| <i>Lat2</i> | 98.50 ± 1.92 | 7.16 ± 0.76 | 13.75 |
| Cell Trafficking | | | |
| <i>Gcnt1</i> | 14.11 ± 1.18 | 1.41 ± 0.55 | 10.00 |
| <i>Hpse</i> | 15.13 ± 0.67 | 0.52 ± 0.04 | 28.91 |
| <i>Itgad</i> (CD11d) | 189.68 ± 7.68 | 4.20 ± 0.45 | 45.11 |
| <i>S1pr5</i> | 203.95 ± 13.95 | 4.55 ± 0.34 | 44.78 |
| <i>Adam8</i> | 21.64 ± 0.90 | 1.52 ± 0.48 | 14.26 |
| <i>Tiam2</i> | 11.09 ± 0.85 | 0.44 ± 0.37 | 24.94 |
| <i>Itgax</i> (CD11c) | 60.31 ± 3.11 | 3.22 ± 0.34 | 18.70 |

Mean expression values (FPKM±SEM) in $\gamma\delta$ T cells from infected mice (n=4) at 19 d.p.i. and uninfected control animals (n=4). Fold change calculated as (mean of infected)/(mean of uninfected). *Gene name* (Protein name). ^aNot in the top 50 upregulated immune-related genes.