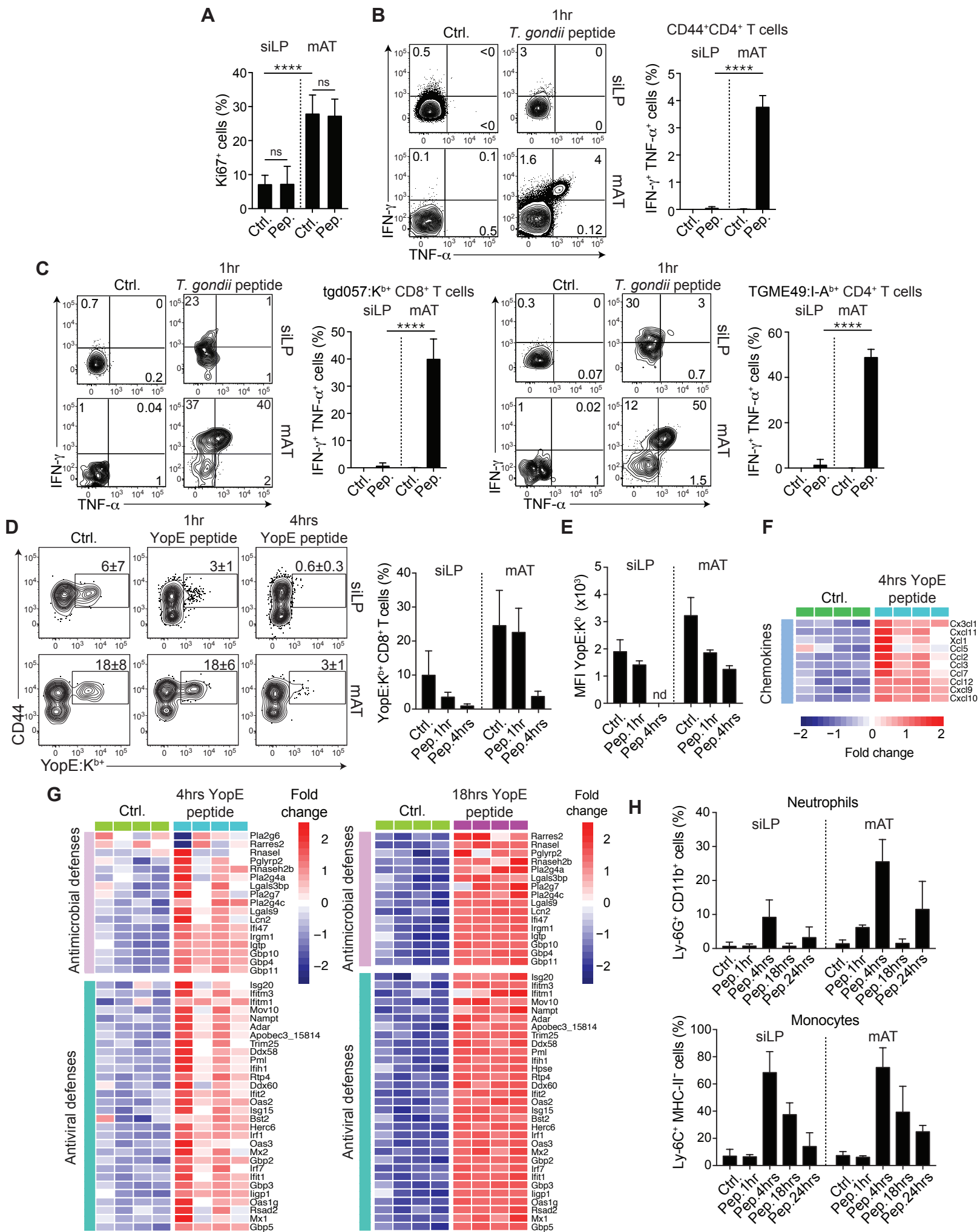
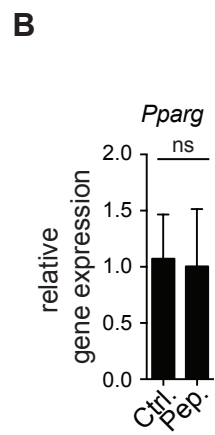
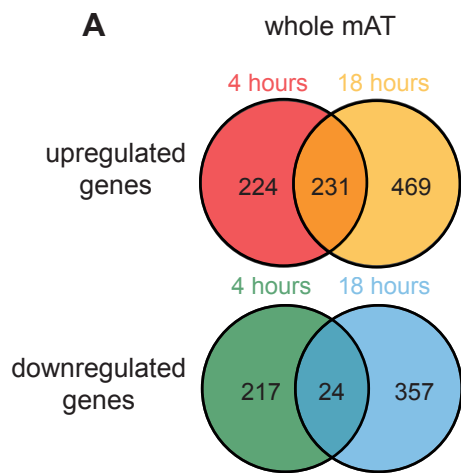


Han, Glatman Zaretsky *et al.* - Supplemental Figure 4





Han, Glatman Zaretsky *et al.* - Supplemental Figure 6

Supplementary Figure 1. Related to Figure 1.

(A) Naïve mice were injected *i.v.* with anti-CD45 AF-780 antibody. mAT was isolated from perfused (+) and non-perfused (-) mice. The frequency of CD4⁺ and CD8⁺ T cells labeled or not labeled by the *i.v.* injection of CD45 antibody is shown. (B) Representative contour plots showing total CD90.2⁺TCRβ⁺ T cells from gonadal adipose tissue (gAT), subcutaneous adipose tissue (scAT), and mesenteric adipose tissue (mAT) isolated from naïve C57BL/6 mice. (C-E) Adipose tissue was isolated from naïve mice and evaluated for T cell populations, transcription factor expression and cytokine production after PMA/ionomycin restimulation with BFA. (C) Representative plots show CD8⁺ and CD4⁺ T cells and Foxp3⁺Treg cells and IFN-γ, IL-17A, IL-5 and IL-13 production by CD4⁺ T cells in the mAT. (D) Numbers of CD8⁺ T cells, Foxp3⁺Treg cells and Foxp3⁺CD4⁺ T cells in mAT (left). Numbers of T-bet⁺CD8⁺ T cells, T-bet⁺CD4⁺ T cells and GATA-3⁺CD4⁺ T cells in mAT (right). (E) Frequency of IL-17A⁺CD4⁺ T cells and IL-5⁺IL-13⁺CD4⁺ T cells in the gAT, scAT and mAT. (F) Gating strategy for memory T cell populations. T_{EM}, T_{RM} and T_{CM} populations were identified by their expression of CD44, CD62L, CD127 and CD69. (G) Frequency of CD8⁺ T cells, CD4⁺ T cells and Treg cells derived from host (black bars) or donor (white bars) cells in the indicated organs of parabiotic animals from **Figure 1F**. (H-I) Surface expression of the indicated markers on cells from the siLP, mLN, and mAT of naïve mice was determined by flow cytometry analysis. (H) Surface expression of indicated markers on CD8⁺ and CD4⁺ T_{RM} (CD44⁺CD62L⁻CD69⁺) cells from the siLP and mAT and naïve CD8⁺ and CD4⁺ T cells (CD44⁻CD62L⁺) from the mLN. (I) Contour plots showing CD103 and CD69 expression on CD44⁺CD62L⁻CD8⁺ (left) and CD4⁺ (right) T cells in the indicated organs. (J) mAT was isolated from uninfected rhesus macaques and processed for flow cytometric analysis. Representative contour plots from concatenated samples show TNF-α and IFN-γ production by CD3⁺CD4⁺ naïve, T_{CM/SCM}, and T_{RM} cells. Numbers in all contour plots represent mean±SD. Error bars in all bar graphs represent standard deviation. Data are representative of at least 2 experiments with at least 4 mice, 3 pairs of parabiotic animals, or a total of 3 rhesus macaques. All cytokine production shown is after PMA/ionomycin stimulation.

Supplementary Figure 2. Related to Figure 2.

(A-C) Mice were infected with a fluorescent reporter strain of *T. gondii* by oral gavage and evaluated by flow cytometry. Numbers in representative contour plots indicate the mean±SD. (A) Representative contour plots of *T. gondii* fluorescence in the indicated organs of naïve, acutely (7 days post infection) or chronically (>6 weeks post infection) infected mice. (B) Representative contour plots showing IFN-γ production by tg057:K^{b+}CD8⁺ (top) and TGME49:I-A^{b+}CD4⁺ (bottom) T cells after PMA/ionomycin stimulation in the indicated organ >6 weeks post infection. (C) Bar graphs show the total numbers of IFN-γ⁺CD8⁺ and CD4⁺ T cells in the indicated organ >6 weeks after *T. gondii* infection. (Student's t test) (D) The number of CD44⁺YopE:K^{b+}CD8⁺ T cells in the mLN, siLP, or mAT was determined by flow cytometry at the indicated time points post-infection. (E) Representative images show the mLN of naïve or infected mice (>4 weeks post infection) as indicated. Top images show the mLN connected to the mAT and gastrointestinal tract. Bottom images show the isolated mLN at higher magnification. (F) Flow-cytometric analysis of CD127 and CD69 expression within YopE:K^{b+}CD8⁺ T cells in the indicated organs 4 weeks post-infection with *Yptb* WT or *Yptb* Δ*yopM*. (G) Mice were infected with *Yptb* Δ*yopM* and bacterial burden was assessed in the indicated organs of naïve mice and mice infected 7 days or >4 weeks previously. DNA was isolated from feces and quantified by RT-qPCR (left). The indicated organs were isolated from mice at the indicated time points post-infection and bacterial burden was assessed by CFU within the tissue (right). (H) Quantification of CD44⁺YopE:K^{b+}CD8⁺ T cells in the siLP, gAT and mAT >4 weeks post-infection with *Yptb* Δ*yopM*. (I) The quantification of CD44⁺tg057:K^{b+}CD8⁺ T cells (white) and TGME49:I-A^{b+}CD4⁺ T cells (black) in the siLP, gAT and mAT >6 weeks post-infection with *T. gondii*. Numbers in representative contour plots indicate

mean±SD. Error bars in all graphs represent standard deviation. Data are representative of at least 2 experiments with at least 4 mice per group. ns not significant, *p<0.05.

Supplementary Figure 3. Related to Figure 3.

(A-B) Mice were infected with *Yptb ΔyopM* by oral gavage. >4 weeks post-infection, effector (T_{EM} : CD44⁺CD62L⁻CD69⁻) and resident (T_{RM} : CD44⁺CD62L⁻CD69⁺) memory CD8⁺ T cells were sorted from the siLP, spleen, and mAT for gene expression analysis by RNA-Seq. **(A)** Pathway analysis was performed and gene pathways were organized into clusters based on shared genes, represented here as a clustergram, showing gene pathways differentially regulated between T_{EM} cells in the spleen and mAT. **(B-C)** Within each pathway cluster shown in **(A)**, the average value of the ratios of the positively or negatively regulated genes was calculated. Bar graphs show the log₂ ratio of the total number of up- or down-regulated genes in mAT T_{RM} and siLP T_{RM} (mAT/siLP)(B) or mAT T_{EM} and spleen T_{EM} (mAT/spleen)(C) for each differentially regulated cluster. **(D)** >4 weeks post-infection with *Yptb ΔyopM*, mice received BrdU i.p. daily for 12 days. The percentage of BrdU⁺YopE:K^{b+}CD44⁺CD69⁺ CD8⁺ T_{RM} in the indicated organ is quantified. Data are representative of at least 2 experiments with at least 3 mice per group. Error bars in bar graphs represent standard deviation. **p<0.01, ****p<0.0001

Supplementary Figure 4. Related to Figure 4.

(A) Pooled mAT, scAT and gAT isolated from either naïve donors or donors >4 weeks post-infection with *Yptb ΔyopM* were subcutaneously transplanted into *Rag1*^{-/-} mice. *Rag1*^{-/-} mice receiving adipose tissues from previously infected mice were either left untreated or injected with anti-CD4 and anti-CD8 depleting antibodies. 2 weeks post surgery, animals were challenged *i.v.* with 200 CFU of *Yptb* WT. A schematic of the experiment is shown.

Supplementary Figure 5. Related to Figure 5.

(A) >4 weeks post-infection with *Yptb ΔyopM*, mice injected *i.v.* with YopE₆₉₋₇₇ peptide or vehicle control (ctrl.) and evaluated by flow cytometry at the indicated time points post-injection. The percentage of YopE:K^{b+}CD8⁺ T cells expressing Ki67 1 hour after injection is quantified in the indicated organs. **(B-C)** Mice were infected orally with a fluorescent reporter strain of *T. gondii*. 6 weeks post-infection, mice were injected *i.v.* with MHC-I and MHC-II peptides or vehicle control. **(B)** Representative contour plots (left) of IFN-γ and TNF-α expression in CD44⁺CD4⁺ T cells in the siLP or mAT. The frequency IFN-γ⁺ TNF-α⁺ cells within the CD44⁺CD4⁺ T cells was quantified (right). **(C)** Representative contour plots show IFN-γ and TNF-α expression in tg057:K^{b+}CD8⁺ (left) and TGME49:I-A^{b+}CD4⁺ (right) T cells in the siLP or mAT. The frequency of IFN-γ⁺ TNF-α⁺ cells within the CD44⁺tg057:K^{b+}CD8⁺ (left) or TGME49:I-A^{b+}CD4⁺ (right) T cells was quantified. **(D-E)** >4 weeks post-infection with *Yptb ΔyopM*, mice injected *i.v.* with YopE₆₉₋₇₇ peptide or vehicle control (ctrl.) and evaluated by flow cytometry at the indicated time points post-injection. **(D)** Expression of YopE:K^{b+} on CD8⁺ T cells in the siLP or mAT 1 or 4 hours after injection is shown in representative contour plots (left) and quantified on the right. **(E)** MFI of YopE:K^{b+} staining on CD8⁺ T cells is quantified for the siLP and mAT after injection. **(F-G)** A microarray was performed on total mAT isolated from mice >4 weeks post-infection with *Yptb ΔyopM* at 4 or 18 hours post-injection with YopE₆₉₋₇₇ peptide or vehicle control. **(F)** Heatmap shows relative gene expression levels of chemokines 4 hours post-injection. **(G)** Heatmap represents differential antimicrobial and antiviral gene expression 4 hours (left) or 18 hours (right) post-injection. **(H)** Quantification of neutrophils (top) (gated on live, CD45⁺TCRβ⁻Siglec F⁻NK1.1⁻B220⁻ cells) or monocytes (bottom) (gated on live, CD45⁺TCRβ⁻Siglec F⁻NK1.1⁻B220⁻CD11b⁺Ly-6G⁻CD64⁺CCR2⁺ cells) at the indicated time points post-vehicle control or peptide injection in the siLP or mAT. Numbers in representative contour plots indicate mean±SD. Error bars in all graphs represent standard

deviation and statistics are calculated using one way Anova adjusted for multiple comparisons. Data are representative of at least 2 experiments with at least 3 mice per group. ns not significant, **** $p < 0.0001$.

Supplementary Figure 6. Related to Figure 6.

(A) >4 weeks post-infection with *Yptb ΔyopM*, mice were injected intravenously with YopE₆₉₋₇₇ peptide or vehicle control. At 4 or 18 hours post-injection, the total mAT was isolated for microarray analysis. The venn diagram indicates the numbers of up- (top) or down- (bottom) regulated genes at 4 and/or 18 hours post-peptide injection compared to control injected mAT. (B) Adipocytes were isolated from mAT 18 hours after vehicle control or peptide injection of mice >4 weeks post infection with *Yptb ΔyopM*. Expression of *Pparg* was determined by RT-qPCR. Error bars in all bar graphs represent standard deviation. Data are representative of at least 2 experiments with at least 2-6 mice per group. ns not significant.