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Antibody-induced erythrophagocyte reprogramming of Kupffer cells prevents anti-CD40 cancer immunotherapy-associated liver toxicity

Marc Pfefferlé[1], Irina L Dubach[1], Raphael M Buzzi[1], Elena Dürst[1], Nadja Schulthess-Lutz[1], Livio Baselgia[1], Kerstin Hansen[1], Larissa Imhof[1], Sandra Koernig[2], Didier Le Roy[3], Thierry Roger[3], Rok Humar[1], Dominik J Schaer[1] and Florence Vallelian[1]

[1]Division of Internal Medicine, University Hospital and University of Zurich, Zurich, Switzerland
[2]CSL Ltd., Research, Bio21 Institute, Parkville, Vic, Australia
[3]Infectious Diseases Service, Lausanne University Hospital and University of Lausanne,

Supplementary Figures

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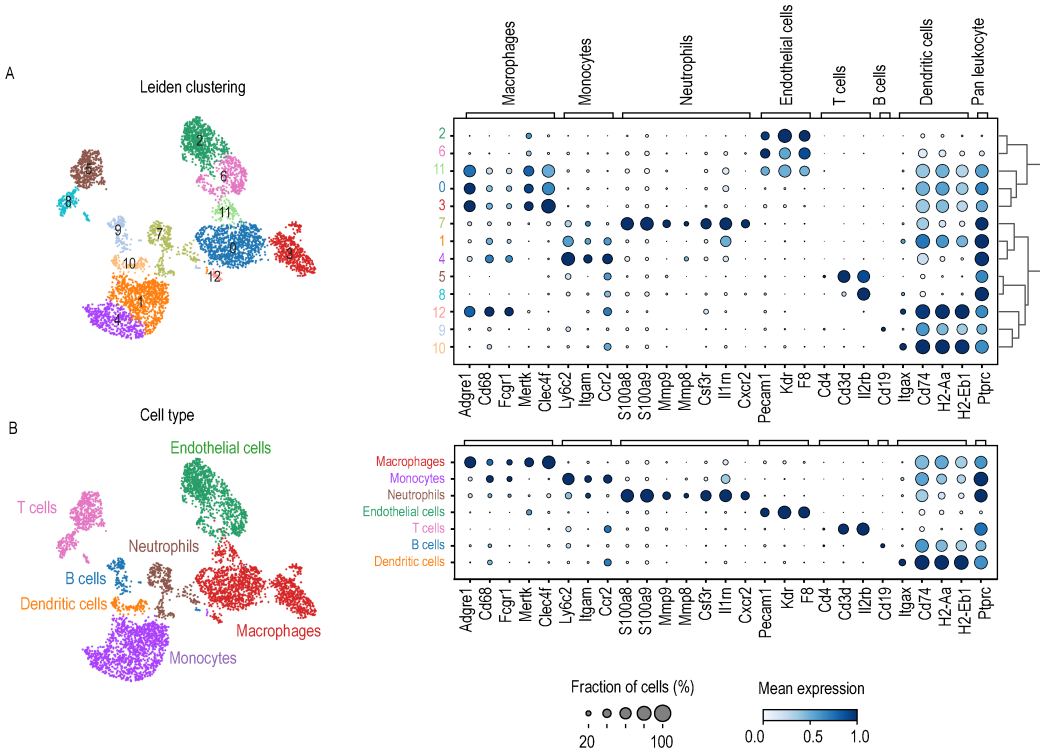
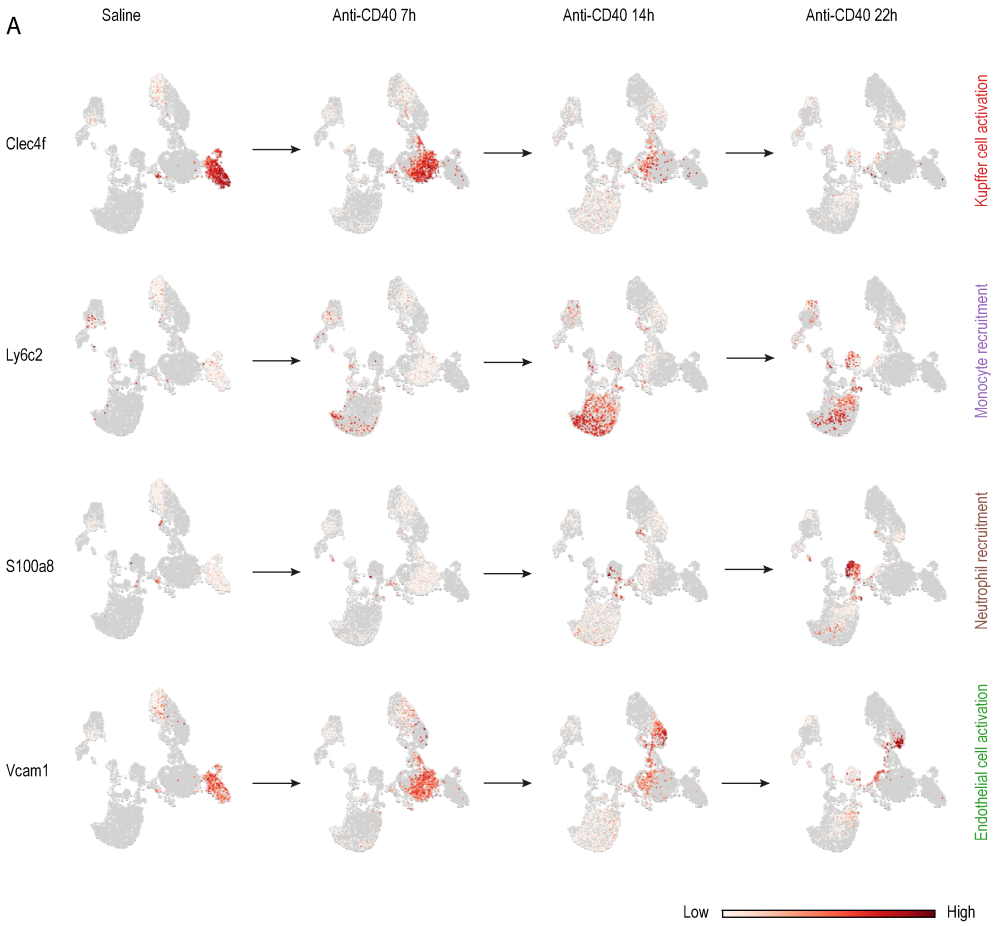


Supplementary Figure 1

Multiplexed sc-RNA-seq experiment performed with wild-type mice that were injected i.v. with an anti-CD40 mAb or saline (0 hours). After 7, 14 and 22 hours, livers were digested into single-cell suspensions and enriched for leukocytes using CD45-coated magnetic beads.

A. Left: UMAP plot with cells colored by clusters as determined by the Leiden unsupervised clustering algorithm. Right: Dot plot displaying the scaled expression of canonical marker genes for each cluster and the proportion of cells expressing the genes.
B. Left: UMAP plot with cells colored by cell identity as determined by the expression of canonical marker genes by each cluster. Right: Dot plot displaying the scaled expression of canonical marker genes for each cell type and proportion of cells in each cell type expressing the genes.



Supplementary Figure 2

Multiplexed sc-RNA-seq experiment performed with wild-type mice that were injected i.v. with an anti-CD40 mAb (20 mg/kg) or saline (0 hours). After 7, 14 and 22 hours, livers were digested into single-cell suspensions and enriched for leukocytes using CD45-coated magnetic beads.

A. UMAP plots with cells colored based on Clec4f, Ly6c2, S100a8, or Vcam1 expression levels separated by treatment condition (saline or anti-CD40 mAb 7, 14 and 22 hours post injection). The cells are superposed on the gray outline of cells from all conditions.

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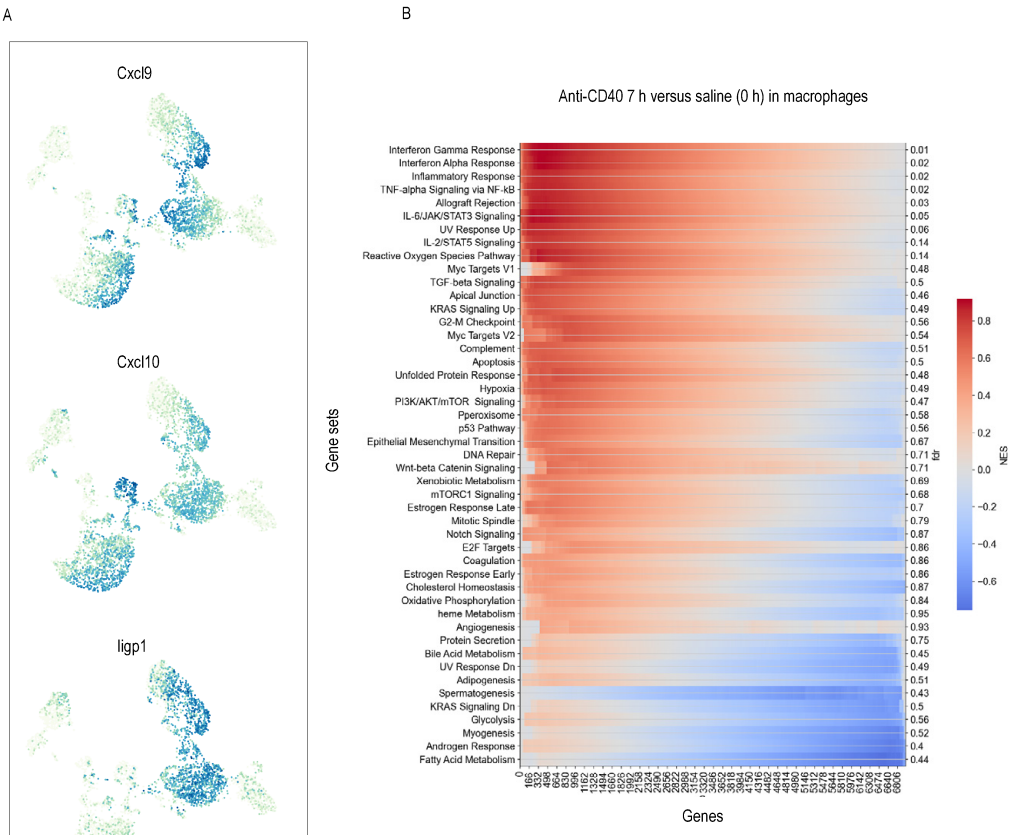

Supplementary Figure 3

Multiplexed sc-RNA-seq experiment performed with wild-type mice that were injected i.v. with an anti-CD40 mAb or saline (0 hours). After 7, 14 and 22 hours, livers were digested into single-cell suspensions and enriched for leukocytes using CD45-coated magnetic beads.

A. UMAP plots with cells from all conditions colored based on Cxcl9, Cxcl10, or Iigp1 expression levels.
B. Heatmap displaying the results of GSEA of genes differentially expressed between liver macrophages from anti-CD40 mAb-treated mice at 7 h post-treatment and those from saline-treated mice (0 h). The analysis was performed using hallmark gene sets from the Molecular Signature Database (MSigDB). Rows represent individual gene sets and are ordered by normalized enrichment score (NES). The heatmap displays the magnitude of the running enrichment score per gene set category (red = positive enrichment, blue = negative enrichment).

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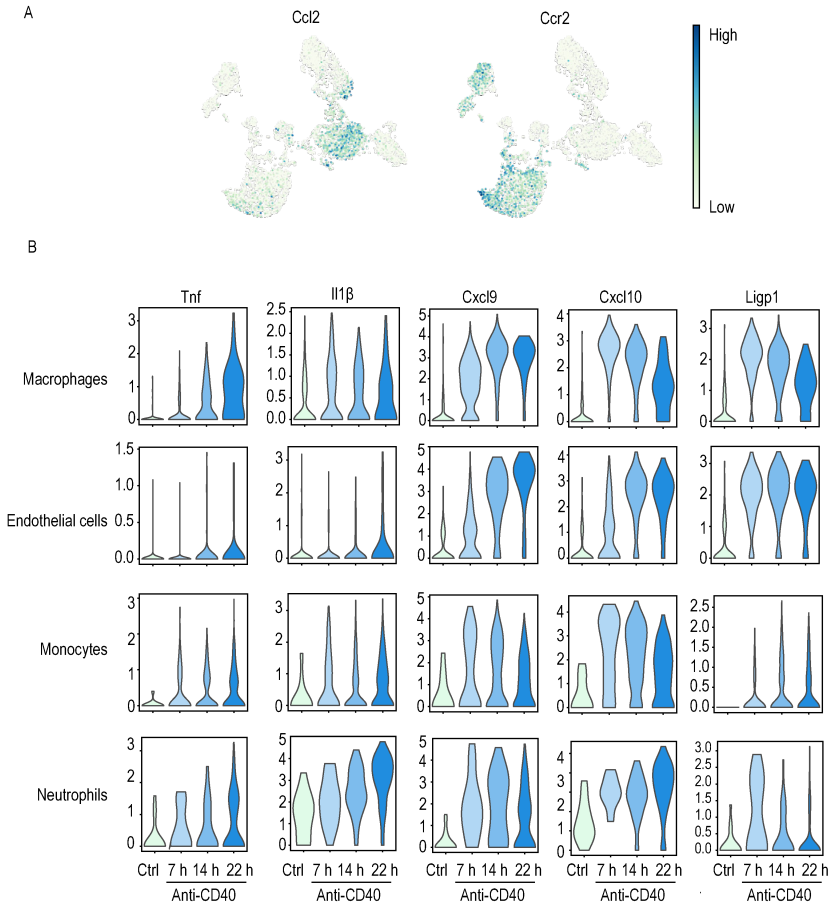
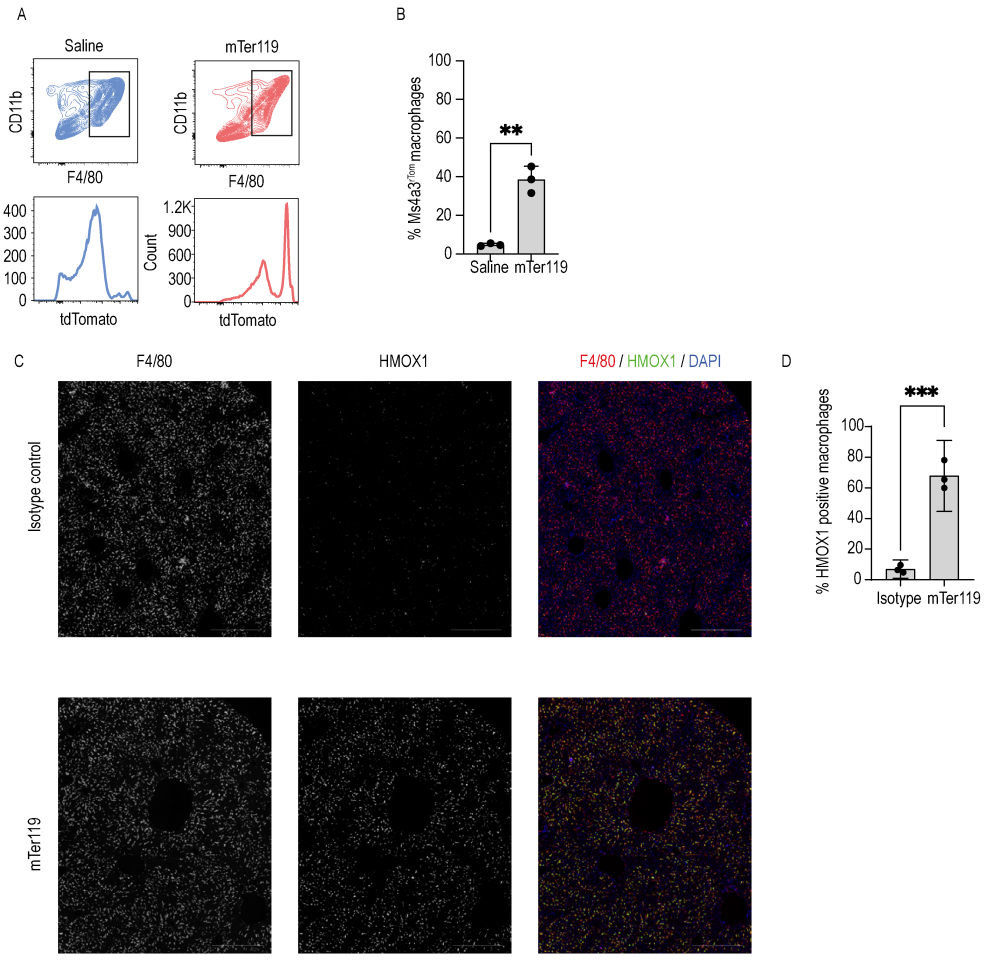


Supplementary Figure 4

Multiplexed sc-RNA-seq experiment performed with wild-type mice that were injected i.v. with an anti-CD40 mAb or saline (0 hours). After 7, 14 and 22 hours, livers were digested into single-cell suspensions and enriched for leukocytes using CD45-coated magnetic beads.

A. UMAP plots with cells from all conditions colored based on Ccl2 (left) or Ccr2 (right) expression levels.
B. Violin plots displaying the expression levels of Tnf, Il1b, Cxcl9, Cxcl10, and Iigp1 in macrophages, endothelial cells, monocytes, and neutrophils from mice treated with anti-CD40 (7, 14 or 22 h post-anti-CD40 treatment) or saline (Ctrl).



Supplementary Figure 5

A. Top: Flow cytometry contour plots of liver cell suspensions from mTer119-treated (red) or saline-treated (blue) Ms4a3^{Cre}-Rosa^{TdT} mice. The cells were stained for F4/80 and CD11b and gated from live CD45^{+} cells. Bottom: histogram displaying the intensity of the tdTomato channel (Ms4a3) in F4/80^{+} cells gated as shown in the contour plots above. The cells were run on a spectral flow cytometer.
B. Cumulative data for the percentage of Ms4a3^{+} cells in CD45^{+}F4/80^{+} cells from the liver cell suspensions of mice treated as described in A (each treatment n= 3).
C. Representative multispectral immunofluorescence images of paraffin-embedded liver sections from mTer119- and isotype control-treated C57BL/6 mice. Liver sections were stained with F4/80 (red) and HMOX1 (green) antibodies. Nuclei were counterstained with DAPI (blue). Images were acquired using a Phenolmager HT (Akoya). Scale bar 500 µm.
D. Percentage of HMOX1 and F4/80 double positive cells quantified from stained whole-liver sections of C57BL/6 mice subjected to mTer119 (n = 3) or isotype control treatment (n = 3). Slides were stained as described in C.

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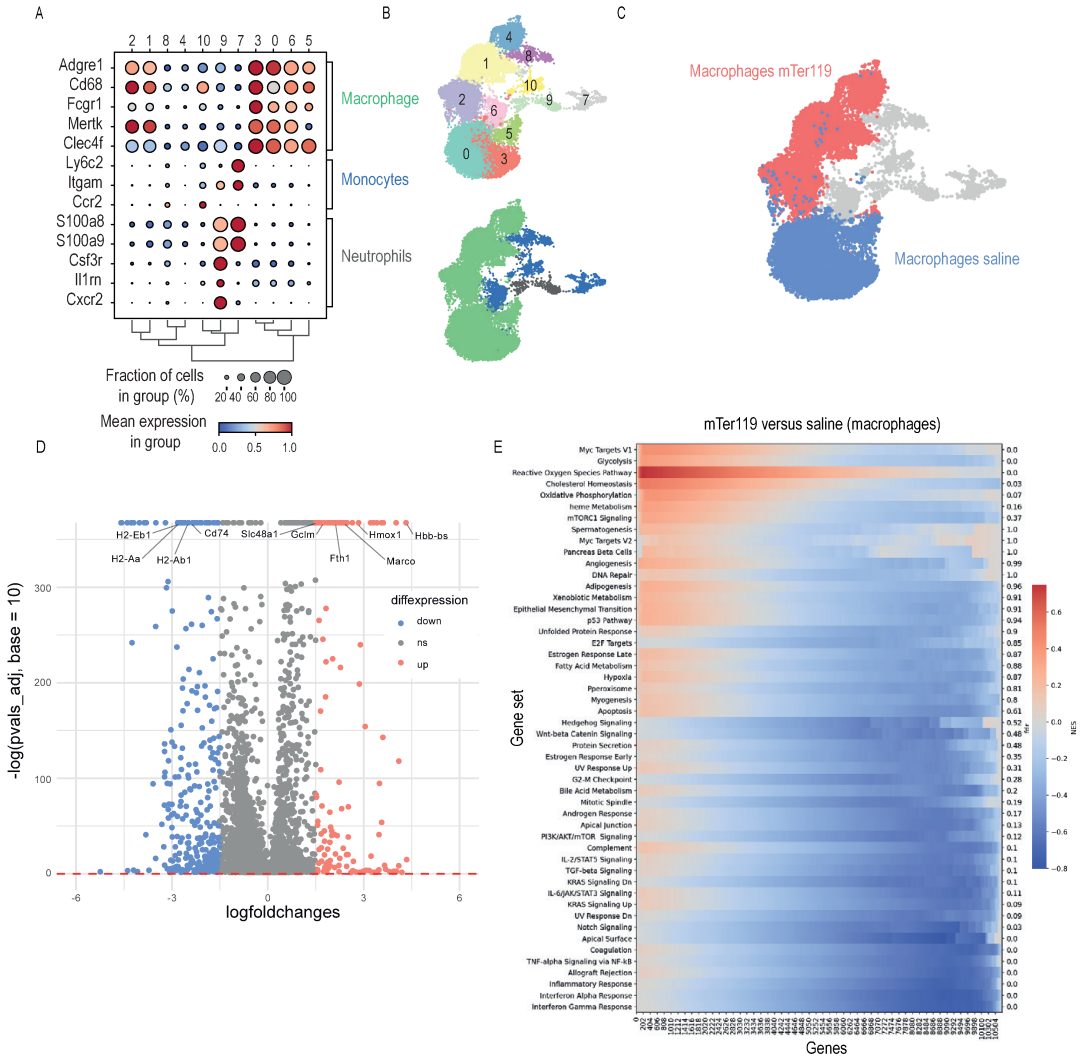

Supplementary Figure 6

Pooled sc-RNA-seq data from two sequencing experiments performed with liver cell suspensions from mTer119- and saline-treated mice. Before sequencing, the liver cells were enriched for macrophages using anti-F4/80- and anti-CD11b-coated magnetic beads. The dataset was subsetted to contain only phagocytes as outlined in Figure 3.

A. Dot plot displaying the scaled expression of canonical marker genes for each cell type and the proportion of cells in each cluster expressing those genes.
B. Top: UMAP plot displaying cells colored by clusters. Bottom: UMAP plot displaying cells colored by cell type. The colors indicate the marker gene-derived cell-type attribution delineated in A.
C. UMAP plot displaying cells with only macrophages highlighted, differentially colored depending on treatment (mTer119 or saline).
D. Volcano plot displaying the results of the differential gene expression analysis between liver macrophages from mTer119- versus saline-treated mice. Key genes related to the erythrophagocyte phenotype are highlighted.

E. Heatmap presenting the results of gene set enrichment analysis (GSEA) of differentially expressed genes between liver macrophages from mTer119- and saline-treated mice. The analysis was performed using hallmark gene sets from the Molecular Signature Database (MSigDB). Rows represent individual gene sets and are ordered by normalized enrichment score (NES). The heatmap displays the magnitude of the running enrichment score per gene set category (red = positive enrichment, blue = negative enrichment).

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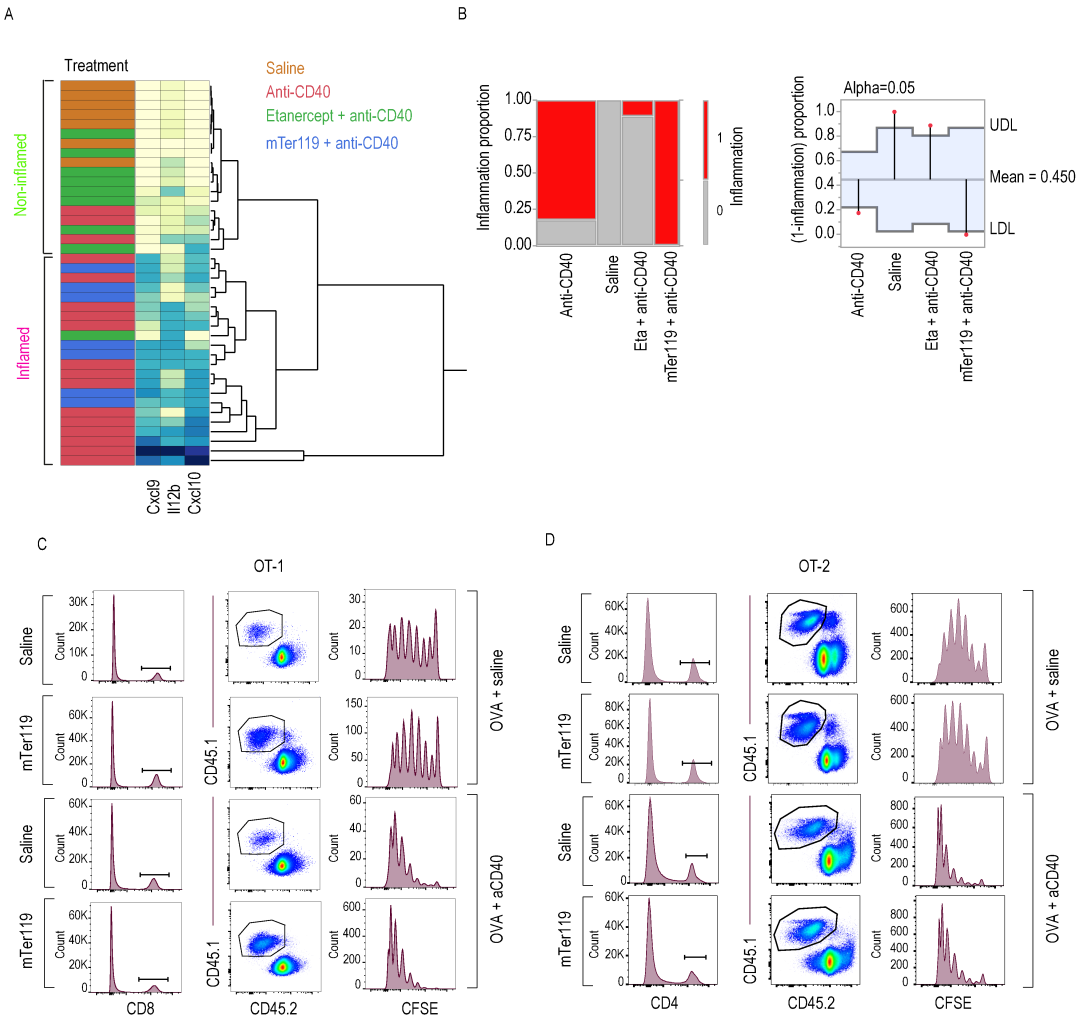

Supplementary Figure 7

A. Heatmap showing unsupervised hierarchical clustering analysis of Cxcl9, Cxcl10 and Il12b gene expression in peritoneal macrophages from C57BL/6 mice subjected to mTer119 (blue), etanercept (green), or saline (red) and challenged with and anti-CD40 mAb (20mg/kg i.p.) or saline (orange). Peritoneal cells were harvested after 16 h and enriched for macrophages with anti-F4/80-coated magnetic beads, and gene expression was measured by RT–qPCR (yellow = low expression, blue = high expression). The clustering analysis classified animals into the inflamed and noninflamed groups.

B. Left: Stacked bar plot displaying the proportion of mice with inflamed peritoneal macrophages from each condition described in A. Red indicates inflamed, gray indicates not inflamed. Right: Proportion data were analyzed by a chi^2 test ($p<0.001$); displayed is the analysis of means (ANOM) of the four groups (LDL= lower decision line, UDL= upper decision line; defining the confidence intervals).

C. Representative CFSE dilution profiles of adoptively transferred CD8$^+$ T cells in mTer119- or saline-treated mice injected with OVA or OVA+anti-CD40 as described in Figure 5C.

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Representative data for cells gated based on positive CD8 and CD45.1 expression are shown.

D. Representative CFSE dilution profiles of adoptively transferred $CD4^+$ T cells in mTer119- or saline-treated mice injected with OVA or OVA+anti-CD40 as described in Figure 5C. Representative data for cells gated based on positive CD4 and CD45.1 expression are shown.

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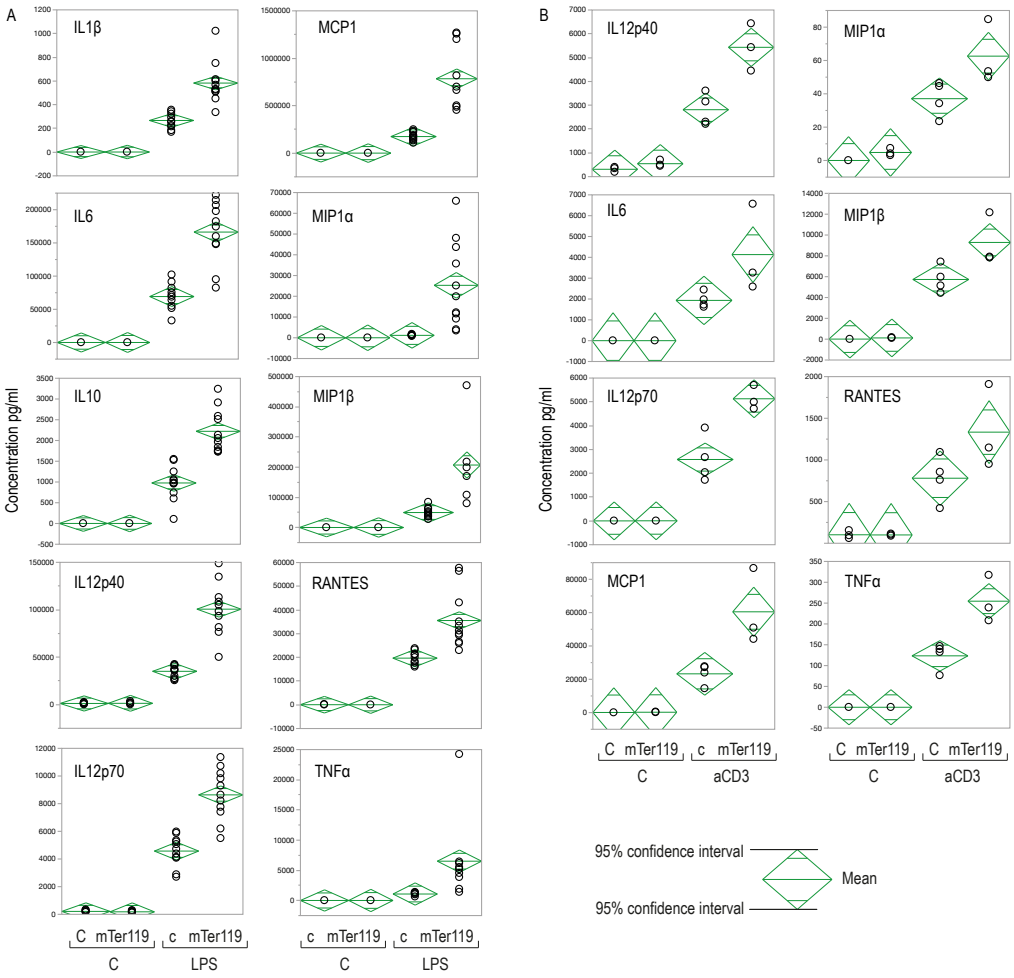

Supplementary Figure 8

A. Diamonds displaying the same data as in Figure 5A of the main manuscript. C57BL/6 mice were treated with mTer119 or saline and injected i.p. with LPS (6 mg/kg). Blood was sampled before (0 hours) and 4 hours after the LPS injection to measure cytokine levels. Every point represents a mouse (n = 11-12 for LPS).
B. Diamonds displaying the same data as in Figure 5B of the main manuscript. C57BL/6 mice were treated with mTer119 or saline and injected i.v. with an anti-CD3 antibody (100 µg). Four hours later, the animals were sacrificed, and blood was collected for proinflammatory cytokine measurement (n = 3-4)

Each dot represents one mouse. The green diamonds represent mean and 95% confidence intervals as indicated in the figure.