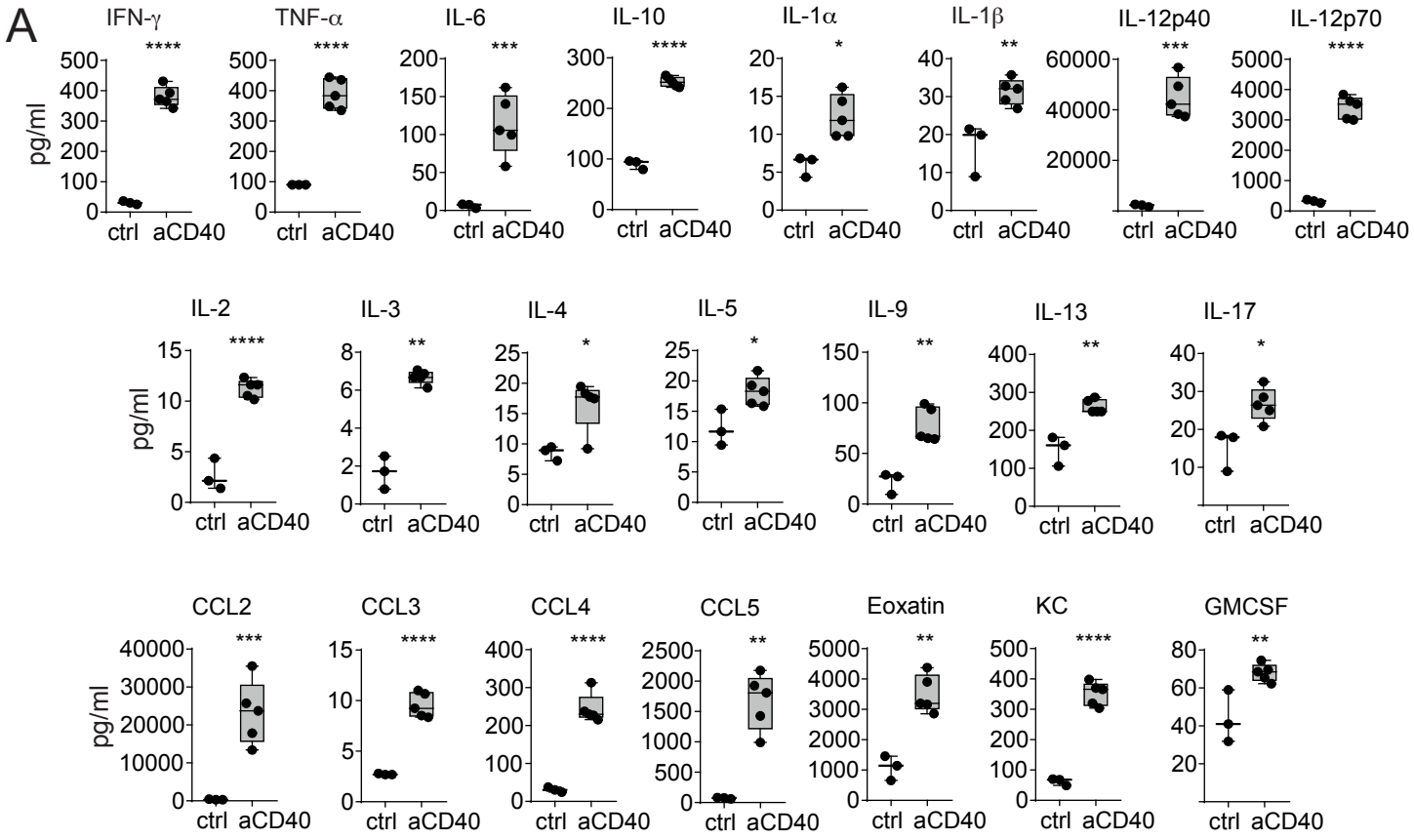
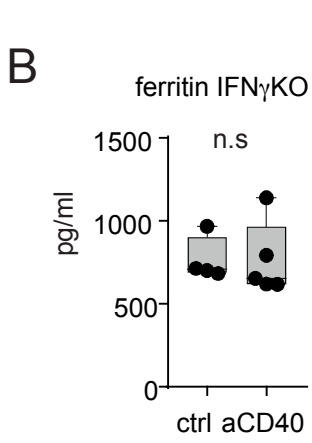
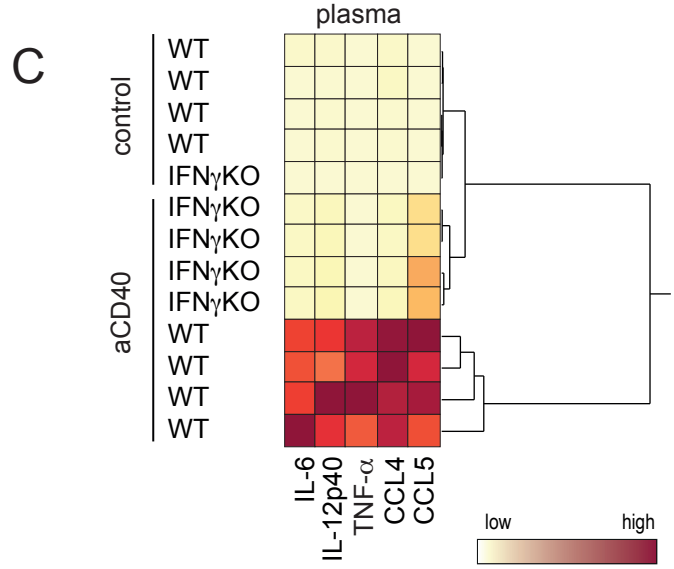
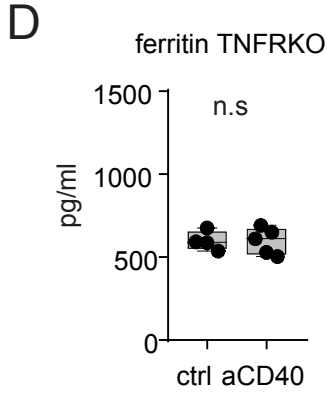
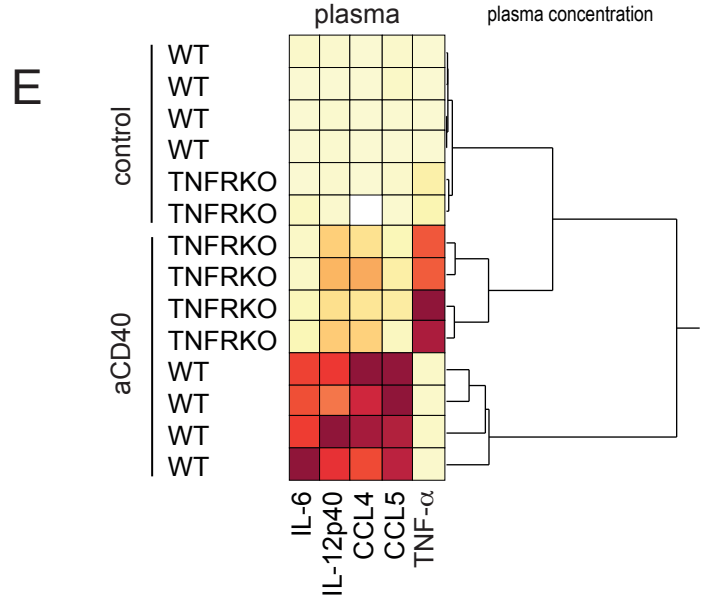

A
IFN-γ
TNF-α
IL-6
IL-10
IL-1α
IL-1β
IL-12p40
IL-12p70
IL-2
IL-3
IL-4
IL-5
IL-9
IL-13
IL-17
CCL2
CCL3
CCL4
CCL5
Eoxatin
KC
GMCSF
pg/ml
ctrl
aCD40


B
ferritin IFNγKO
n.s
pg/ml
ctrl
aCD40


C
plasma
control
aCD40
WT
IFNγKO
IL-6
IL-12p40
TNF-α
CCL4
CCL5
low
high
plasma concentration


D
ferritin TNFRKO
n.s
pg/ml
ctrl
aCD40


E
plasma
control
aCD40
WT
TNFRKO
IL-6
IL-12p40
CCL4
CCL5
TNF-α

SUPPLEMENTARY FIGURE 1

A. Plasma concentration of cytokines and chemokines in saline (n=3) or anti-CD40-treated (n=5) wild-type mice, 12 h post-injection.

B. Plasma ferritin concentrations in saline (n=4) and anti-CD40-treated (n=5) IFN-gamma knockout mice.

C. Hierarchical clustering analysis of plasma concentrations of cytokines and chemokines in saline and anti-CD40-treated wild-type (WT) and IFN-gamma knockout mice (yellow=low concentration, red=high concentration).

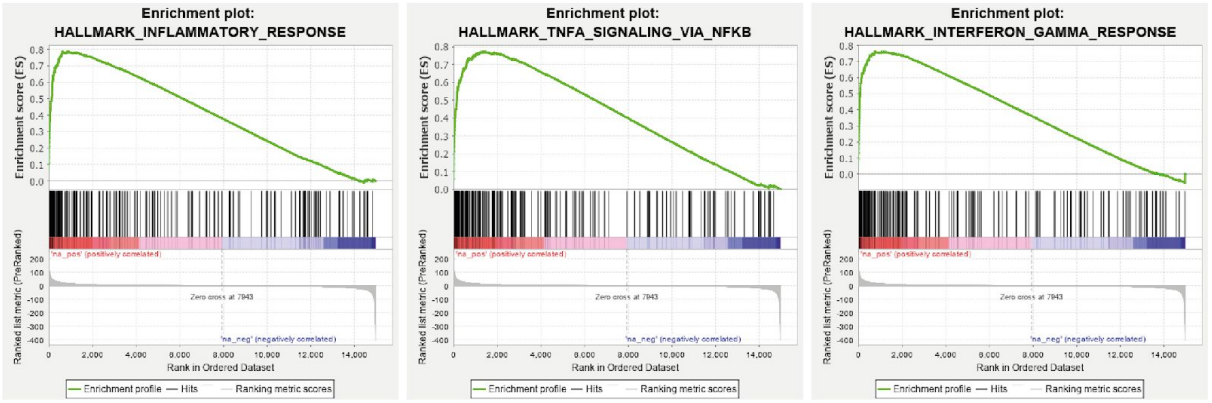
D. Plasma ferritin concentrations in saline (n=4) and anti-CD40-treated (n=5) TNFR1/2 knockout mice.

E. Hierarchical clustering analysis of plasma concentrations of cytokines and chemokines in saline and anti-CD40-treated wild-type (WT) and TNFR1/2 knockout mice (yellow=low concentration, red=high concentration).

Individual symbols represent one mouse; **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$, * $p\leq0.05$, n.s. $p>0.05$, for all panels.

anti-CD40 versus saline (bulk RNA-sequencing)

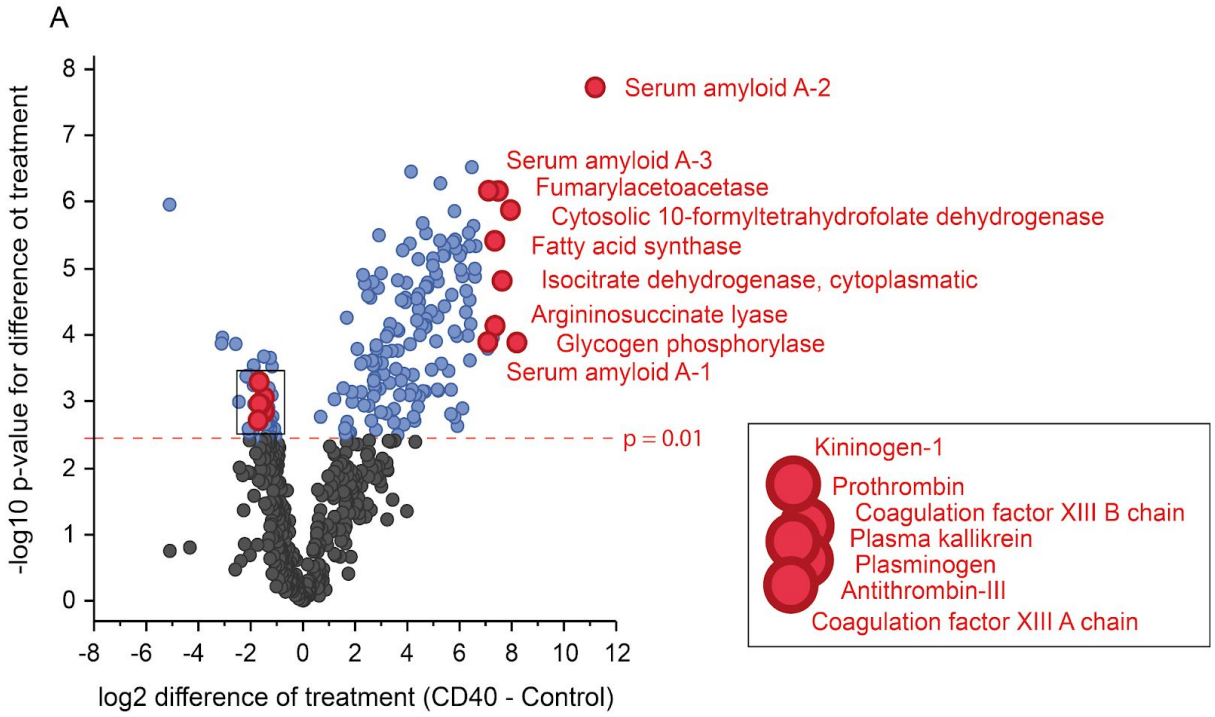
A



positively enriched gene sets

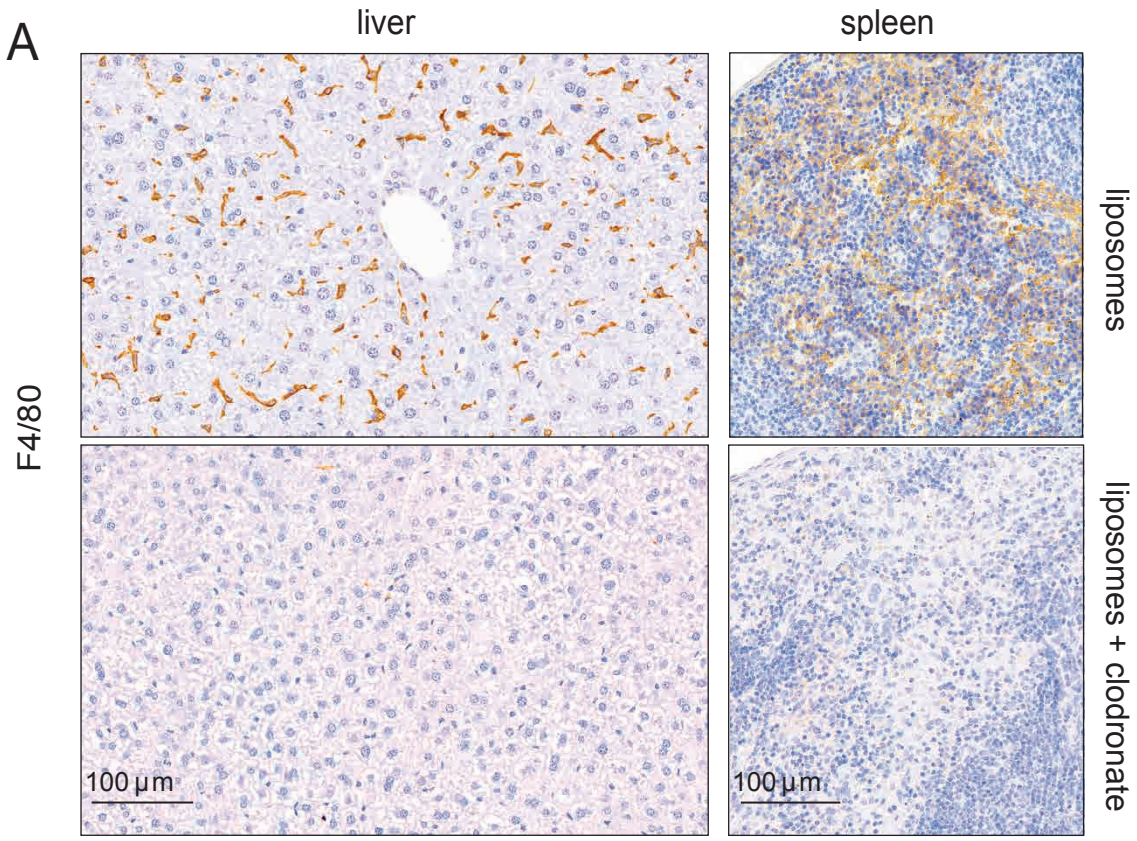
SUPPLEMENTARY FIGURE 2

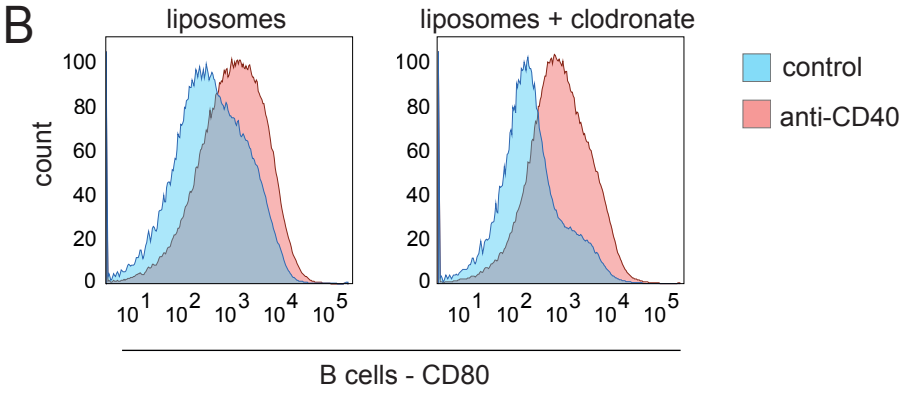
A. Differentially expressed genes in liver macrophages from anti-CD40 versus saline-treated mice (bulk RNA-seq data, Figure 1F) were analyzed by GSEA software using the hallmark gene set from the Molecular Signatures Database. Enrichment plots of the top 3 positively enriched hallmark gene sets are shown. Plots display running enrichment score and position of gene set members on the rank ordered gene list.

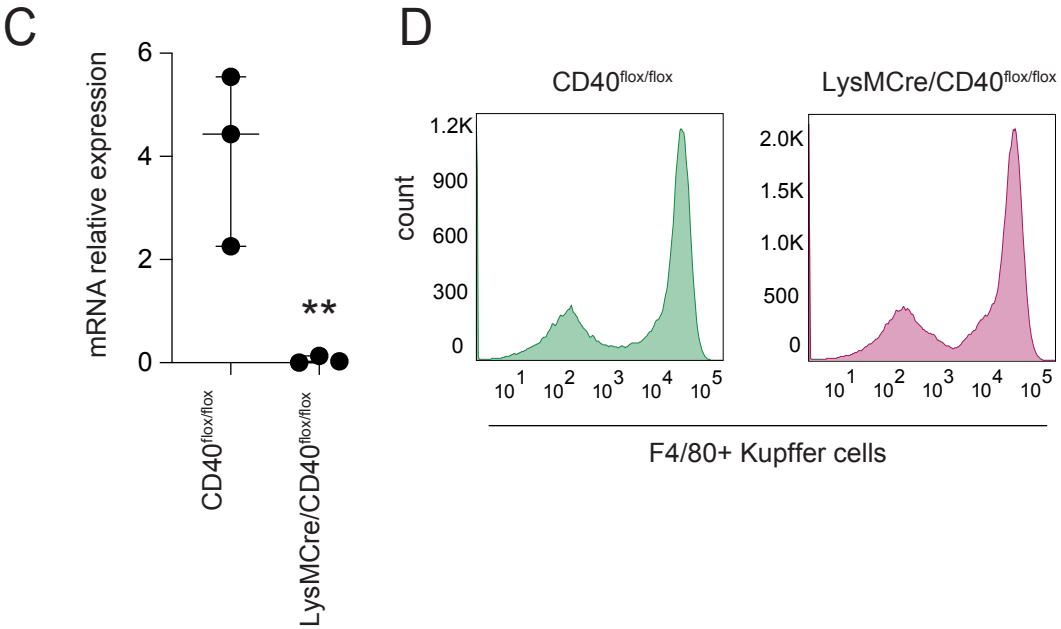


SUPPLEMENTARY FIGURE 3

A) Volcano plot of log-transformed and mean normalized ion intensity ratios of differentially changed proteins in plasma of anti-CD40-treated and control mice. Significantly changed proteins are highlighted in blue ($p<0.01$). The analysis was performed by quantitative liquid chromatography tandem mass spectrometry (LC-MSMS).


A
liver
spleen
F4/80
liposomes
liposomes + clodronate
100 µm
100 µm


B
liposomes
liposomes + clodronate
count
control
anti-CD40
B cells - CD80


C
mRNA relative expression
**
CD40flox/flox
LysMCre/CD40flox/flox
D
CD40flox/flox
LysMCre/CD40flox/flox
count
F4/80+ Kupffer cells

SUPPLEMENTARY FIGURE 4

A) F4/80 immunohistochemistry of representative liver and spleen sections of mice treated with control-liposomes or liposomes + clodronate demonstrating the effective macrophages depletion.

B) Representative flow-cytometric histograms of CD80 expression of $CD19^{high}$ $B220^{high}$ splenic B cells in saline (blue filled histogram) or anti-CD40 (red filled histogram)-treated mice. Mice were pretreated with control-liposomes (left histograms) or liposomes + clodronate (right histograms).

C) Cd40 mRNA expression levels measured by RT-PCR in F4/80+ purified liver macrophages from $CD40^{flox/flox}$ mice (wild-type) and LysMCre $CD40^{flox/flox}$ mice (n=3).

D) Representative flow-cytometric histograms of F4/80 expression in $CD40^{flox/flox}$ mice (wild-type) and LysMCre $CD40^{flox/flox}$ mice. The displayed cells were gated from live $CD45^{high}$ leukocytes.

Individual symbols represent one mouse; ** $p<0.01$ for all panels.

Supplementary Methods

Flow cytometry and FACS sorting

Non-parenchymal liver or spleen single cell suspensions were preincubated with LIVE/DEAD Fixable Near-IR cell stain kit (Invitrogen, L34976) and with Mouse BD Fc Block™ (≤ 1 µg/million cells in 100 µL, BD Biosciences 553141) at 4 °C for 5 min. The cell suspensions were then filtered through a 70-µm cell strainer (Sigma Aldrich, cat. n. CLS431751) and centrifuged at 300 x g for 5 min. **Antibodies:** Macrophages: Pacific Blue anti-CD45 (5 µg/mL, BioLegend 109820), FITC anti-CD11b (5 µg/mL, BD Biosciences 557396) and APC anti-F4/80 (5 µg/mL, BioLegend 123116) antibodies. Endothelial cells: Pacific Blue anti-CD45 (5 µg/ml, BioLegend 109820), FITC anti-CD102 (1 µg/ml, BD Biosciences 557396) and APC anti-CD31 (1 µg/ml, BioLegend 123116) antibodies. Dendritic cells: Pacific Blue anti-CD45 (5 µg/mL, BioLegend 109820, PE anti-CD11c (0.2 mg/mL, BD Biosciences, 553802) and Alexa Fluor anti-MHC II (I-A/I-E) (0.2 mg/mL, BD Biosciences, 562367). B cells: Pacific Blue anti-CD45R/B220 (5 µg/ml, BioLegend 103227), FITC anti-CD19 (5 µg/ml, BD Biosciences 553785) and PE anti-CD80 (2 µg/ml, BD Biosciences 553769) antibodies or Alexa Fluor anti-MHC II (I-A/I-E) (2 µg/mL, BD Biosciences, 562367). T cells: Pacific Blue anti-CD3 (2 µg/ml, BD Biosciences 558214), FITC anti-CD8 (5 µg/ml, BD Biosciences 553031) and APC anti-CD69 (2 µg/ml, BioLegend 104514) antibodies. NK cells: Pacific Blue anti-CD45R/B220 (5 µg/ml, BioLegend 103227), BUV395 anti-CD3 (0.2 mg/ml, BD Biosciences 740268), FITC anti-NK1.1 (0.5 mg/ml, BD Biosciences 561082), APC anti-CD69 (2 µg/ml, BioLegend 104514). Phagocytosed red blood cells: After cell fixation with 2% formaldehyde and membrane permeabilization with permeabilization buffer (eBioscience, 00-8333-56), ingested erythrocytes were stained intracellularly with PE anti-TER119 (2 µg/mL, Stemcell 60033) antibody.

Instruments: LSRFortessa (BD), FACSymphony (BD), ImageStream X Mk II Imaging Flow Cytometer (Amnis). BD FACSAria Flow Cytometer (cell sorting). Data was analyzed using FlowJo and IDEAS software.

Gene expression analysis

Bulk RNA-sequencing

RNA extraction: RNA was extracted from cells using the RNeasy microkit (Qiagen Hombrechtikon, Switzerland) according to the manufacturer's protocol. Concentration and purity was determined with a spectrophotometer (NanoDrop, Thermo Fischer) while an Agilent Technologies 2100 Bioanalyzer s was used to assess quality. Only samples with a RIN >9 were kept. .

Library preparation: cDNA libraries were generated from the RNA samples using the Illumina TruSeq RNA stranded kit following the manufacturer's instructions (total of 15 PCR cycles for amplification). Library quality was determined using an Agilent Fragment Analyzer with DNA High-Sensitivity Chips.

Sequencing: The libraries were pooled in equimolar amounts and sequenced in an Illumina NovaSeq 6000 sequencer (single-end 100 bp) with a depth of approximately 20 mio reads per sample.

Analysis: Reads were aligned to the reference genome Ensembl GRCm38.p5 Release 91 using STAR (v2.7.0e)[49]. The quality of alignment was evaluated using Samtools (v1.9). Counts were obtained using the featureCounts function of the Rsubread package (v1.22.2). Differential expression analysis was performed with the DEseq2 R package (v1.26.0), which included count normalization. *Gene set enrichment analysis (GSEA)* was performed for the differentially expressed genes (DEGs) using the GSEA-preranked tool of the GSEA desktop software version 4.0.2. DEGs were ranked based on *-log (pvalue)* times the sign of the fold change. The tested pathways were part of the hallmark gene sets of the Molecular Signatures Database (MSigDB database v7.0)[49,50].

Real-time PCR

Reverse transcription was performed with TaqMan reverse transcription reagents (Life Technologies, Basel, Switzerland). Real-time PCR was performed using Fast SYBR™ Green Master Mix (Applied Biosystems, 4385612) to determine the expression level of the target using the primers listed in the table below. Relative mRNA levels were calculated by the

7500 Fast System Sequence Detection Software Version 1.4 (Applied Biosystems) after normalization of each experimental sample to *Hprt* levels.

TARGET GENES	FW primer	REV primer
Il-6	gctaccaaactggatataatcagga	ccaggtagctatggtactccagaa
Tnf	tcttctcattcctgcttgtgg	gaggccatttgggaacttct
Il-12b	aaggaacagtgggtgtccag	gttagcttctgaggacacatcttg
Cxcl9	cttttcctcttgggcatcat	gcatcgtgcattccttatca
Cxcl10	gctgccgtcattttctgc	tctcactggcccgtcatc
Ccl2	catccacgtgttggctca	gatcatcttgctggtgaatgagt
Hprt	cctcctcagaccgctttttt	aacctggttcatcatcgctaa
Nos2	gcatcccaagtacgagtggt	ccatgatggtcacattctgc
Vcam1	tcttacctgtgcgctgtgac	actggatcttcagggaatgagt
Ccl5	tgcagaggactctgagacagc	gagtggtgtccgagccata
Cd40	aaggaacgagtcagactaatgtca	agaaacaccccgaaaatggt

Proteomics study

Sample preparation

Samples were further processed using a commercial iST Kit (PreOmics, Germany), according to the manufacturer's instructions. The obtained peptides were finally washed, eluted, dried, and re-solubilized in 20 µL of buffer (3% acetonitrile, 0.1% formic acid) and diluted 20 times for LC-MS-Analysis.

LC-MS/MS analysis

Mass spectrometry analysis was performed on a Q Exactive HF-X mass spectrometer (Thermo Scientific) equipped with a Digital PicoView source (New Objective) and coupled to a M-Class UPLC (Waters). Solvent A was 0.1% formic acid and solvent B was 0.1% formic acid, 99.9% acetonitrile. Separation was performed on a commercial MZ Symmetry C18 Trap Column (100Å, 5 µm, 180 µm x 20 mm, Waters) followed by nanoEase MZ C18 HSS T3 Column (100Å, 1.8 µm, 75 µm x 250 mm, Waters) with a flow rate of 300 nL/min and a gradient from 8 to 27% B in 85 min, 35% B in 5 min and 80% B in 1 min. The mass spectrometer was operated in data-dependent mode (DDA), acquiring a full-scan MS spectra (350−1'400 m/z) at a resolution of 120'000 at 200 m/z after accumulation to a target value of 3'000'000. The twenty most intense signals per cycle were fragmented by HCD (higher-energy collision dissociation), acquired at a resolution of 15'000 with a normalized collision energy of 25. The automatic gain and a maximum injection time control (AGC) was set to 100'000 ions. Precursors above an intensity of 110'000 were selected for MS/MS. The mass spectrometry proteomics data were processed using the local laboratory information management system (LIMS).

Protein identification and label free protein quantification

The obtained raw MS data were processed by MaxQuant (version 1.6.2.3), and protein identification was done by the integrated Andromeda search engine[48]. Spectra were searched against a Uniprot Mus musculus (Mouse) reference proteome (taxonomy 10090, version from 2016-09-02), concatenated to its reversed decoyed fasta database and common protein contaminants. Carbamidomethylation of cysteine was set as fixed and methionine oxidation and N-terminal protein acetylation were set as variable modifications. Trypsin/P was used for enzyme specificity allowing a minimal 7 amino acids for peptides length and a maximum of two missed-cleavages. MaxQuant Orbitrap default search settings were used. The maximum false discovery rate (FDR) was set to 0.01 for peptides and 0.05 for proteins. Label free quantification was enabled and 2 minutes window for match between runs was applied. Protein fold changes were computed based on Intensity values reported in the proteinGroups.txt file. The filter process for proteins with 2 or more peptides and a maximum of 4 missing values and normalization of the data with a modified robust z-score transformation to compute p-values using the t-test with pooled variance was done by a set of inbuild functions in the R package SRMService (W. Wolski, J.

Grossmann, C. Panse. 2018. SRMService - R-Package to Report Quantitative Mass Spectrometry Data. http://github.com/protViz/SRMService). If one of the conditions was missing all measurements of a protein, the average mean of the missing group was substituted by a pseudo fold change computed by the mean of 10% smallest protein intensities in that condition.