Supplementary Material

Suppl. Table 1. Annotation markers used for single cell RNA sequencing analysis.

Liver

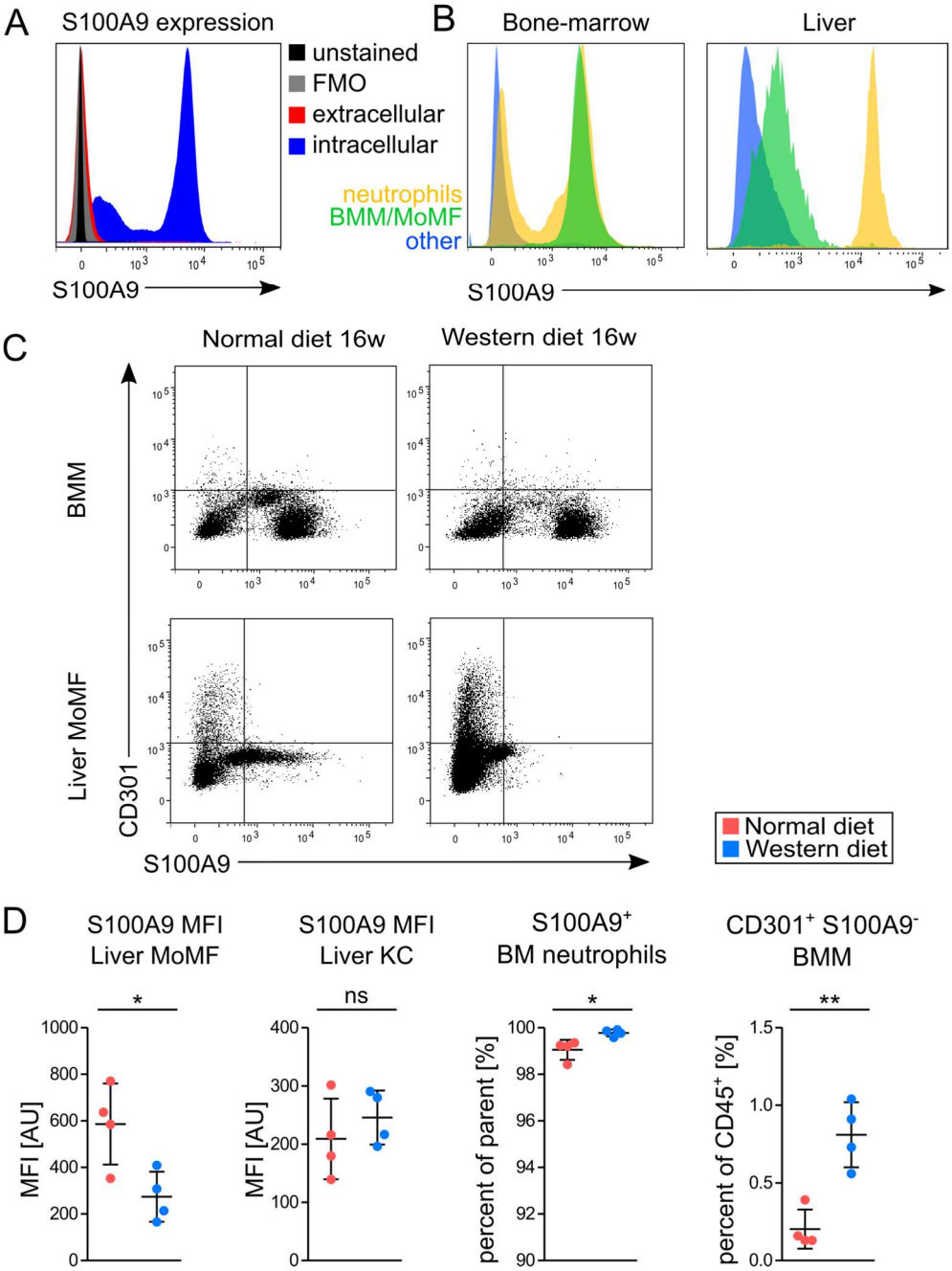
LY6C^{high} MoMF	LY6C^{low} MoMF	Kupffer cells	cDC1	cDC2	pDC	Ccr7 DC
Ccr2	*Lyz2*	*Adgre4*	*Id2*	*Cd209a*	*Cd209a*	*Ccr7*
F13a1	*Ccr2*	*Cd74*	*Irf8*	*Klrd1*	*Bst2*	
Fn1	*Nr4a1*	*Cd68*	*Xcr1*		*Siglech*	
Ly6c2		*Tlr4*			*Ly6c2*	
Lyz2		*Cd5l*				

Bone marrow

HSC	BMM	CMoP	preDC
Cd34	*Lyz2*	*Birc5*	*Birc5*
	Ccr2	*Ube2c*	*Irf8*
	Ly6c2	*S100a10*	*Bst2*

MoMF, monocyte-derived macrophages; cDC, conventional dendritic cells; pDC, plasmacytoid dendritic cells; HSC, hematopoietic stem cells; BMM, bone marrow monocytes; CMoP, common monocyte precursors

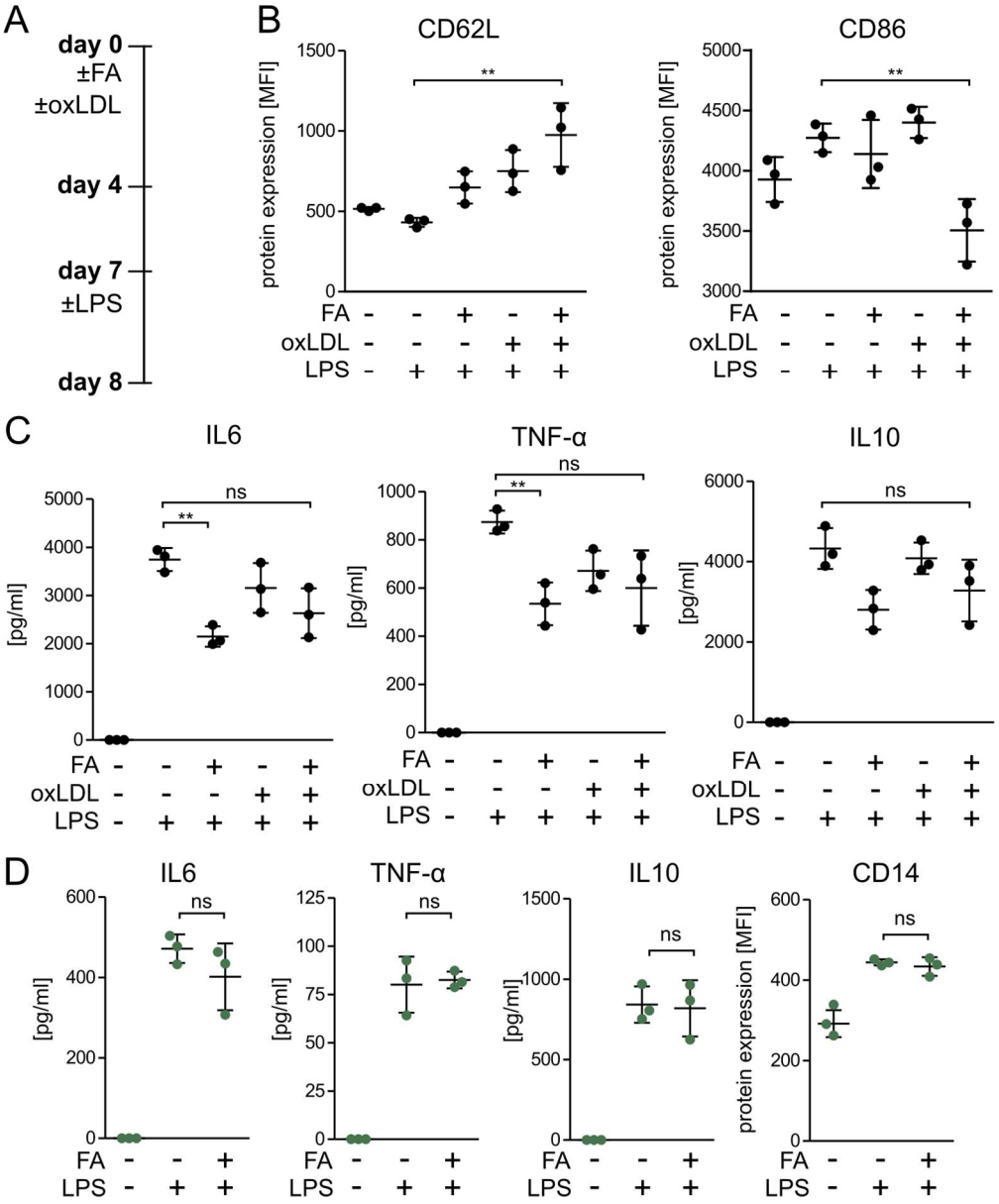
Supplementary Figure 1



Suppl. Figure 1. Changes in S100A9 protein expression in bone marrow monocytes and liver monocyte-derived macrophages (MoMF) during Western diet feeding. (A) Extracellular and intracellular staining for S100A9 in bone marrow leukocytes of ND control mice. (B) Intracellular staining for S100A9 of bone marrow and liver neutrophils, bone marrow monocytes (BMM) and liver monocyte-derived macrophages (MoMF) as well as other cells. (C) Representative dot plots showing CD301 versus S100A9 expression of BMM and liver MoMF of ND and WD fed mice. (D) Intracellular staining for S100A9 in liver MoMF and mean fluorescence intensity (MFI) of liver MoMF and Kupffer cells (KC) as determined by flow cytometry. All data

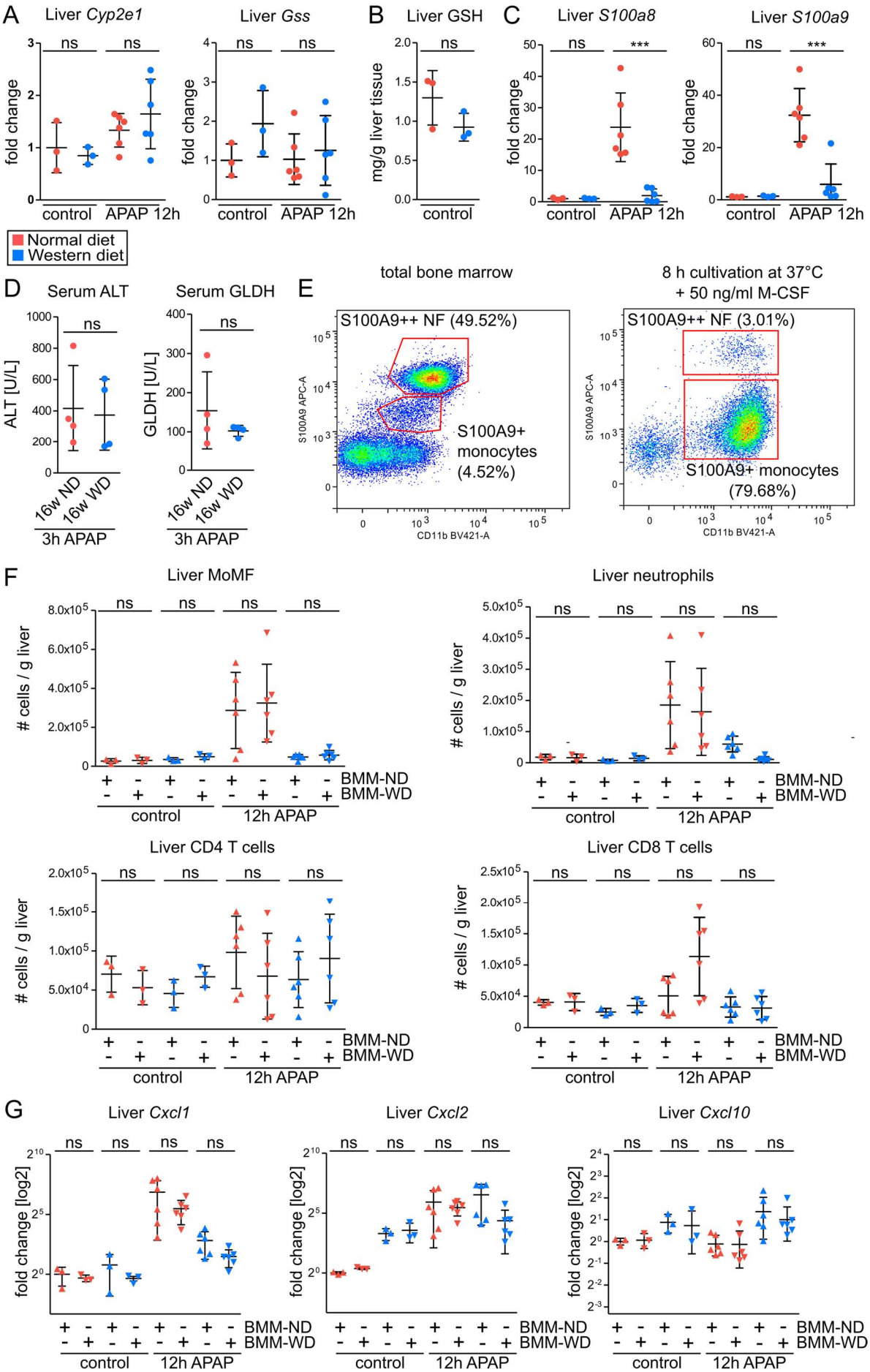
are presented as mean ± SD, n≥4 for all groups. *p<0.05, **p<0.01 (unpaired student t-test).

Supplementary Figure 2



Suppl. Figure 2. Interference of oxidized LDL with the FA induced polarization of bone marrow derived macrophages (BMDM). (A) Experimental setup (scheme). Cells were treated for the first 4 days with either fatty acids (FA), oxidized LDL (oxLDL) or both. On day 7, BMDM were stimulated with 100 ng/ml LPS for 24 h. (B) Mean fluorescence intensity (MFI) as quantified by fluorescence flow cytometry. (C) Quantification of the cytokine concentration in the supernatant of BMDM after LPS stimulation for 24 h. (D) Use of BMDM from *Tlr4*$^{-/-}$ mice (indicated by green circles). Quantification of the cytokine concentration in the supernatant and CD14 MFI after LPS stimulation for 24 h in BMDM generated from *Tlr4*$^{-/-}$ mice. All data are presented as mean ± SD, n=3 for all groups. **p<0.01 (ANOVA with multiple comparisons correction).

Supplementary Figure 3



Suppl. Figure 3. Acetaminophen (APAP) induced liver injury following the induction of NAFLD by Western diet feeding. (A) Normalized gene expression analysis of *Cyp2e1* and *Gss*, the two main APAP metabolizing enzymes, in liver tissue from mice that received either ND or WD for 16 weeks as well as from mice that received APAP for 12 h. (B) Hepatic glutathione (GSH) levels, the main substrate for APAP detoxification, shown as mg per g liver tissue. (C) Normalized gene expression analysis of *S100a8* and *S100a9* from mice that received either ND or WD for 16 weeks as well as from mice that received APAP for 12 h. (D) Serum ALT and GLDH activity from mice that had been fed by either ND or WD for 16 weeks, 3h after intravenous APAP injection. (E) Pseudo color blots showing the expression of S100A9 and CD11b on neutrophils (NF) and monocytes when freshly isolated from the bone marrow as well as following 8 h cultivation at 37°C prior to the adoptive transfer. (F) Absolute numbers of MoMF, neutrophils, CD4 and CD8 T cells per g liver tissue as determined by fluorescence flow cytometry (red: ND-fed recipients, blue: WD-fed recipients; donor conditions of the transferred cells are indicated below the graph). (G) Normalized gene expression analysis of *Cxcl1*, *Cxcl2* and *Cxcl10* from mice that received either ND or WD for 16 weeks and were adoptively transferred with BMM from ND or WD fed donors. All data are presented as mean ± SD, n≥3 for all groups. ***p<0.001 (ANOVA with multiple comparisons correction).

Supplementary Methods

Serum biomarker measurement

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) activities were measured (UV test at 37°C) in serum (Roche Modular pre-analytics system, Rotkreuz, Switzerland). MCP-1 (CCL2) levels were quantified in serum from mice by enzyme-linked immunosorbent assay according to the manufacturer's instructions (eBioscience, Germany).

Bone marrow derived macrophage polarization with fatty acids and oxidized LDL

BMDM were generated as described under material and methods of the main manuscript. For additional oxLDL prestimulation 20 ng/ml of human copper oxidized (Hoelzel Diagnostics, Germany) was added to the supernatant. After 4 days the medium was removed and cells were washed once with PBS to remove remaining FA or oxLDL containing medium. Afterwards the BMDM were cultivated for another 3 days with fresh medium until the final stimulation with 100 ng/ml LPS for 24 h.

Generation of BMM for adoptive transfer

Total bone marrow was isolated and cultivated in RPMI with 50 ng/ml murine M-CSF, 10% FCS and 1 % P/S for 8 h at 37°C. Afterwards cells were carefully washed three times with cold PBS to remove non adherend cells. BMM were then gently scraped by using a rubber cell scraper. Prior to injection, cells were washed once with cold PBS, centrifuged at 570 rcf and 4°C for 5 minutes. Afterwards cells were resuspended in sterile NaCl for injection and counted. 2 h after APAP, $3x10^6$ cells were adoptively transferred by an IV injection.

Gene expression analysis by qPCR

Total RNA was isolated from snap-frozen liver tissue by PeqGold™ Tri-Fast (VWR, Germany) according to the manufacturer's instructions. Next, 5 µg of isolated RNA were reverse transcribed into cDNA by using the First Strand cDNA Synthesis Kit (ThermoFisher Scientific, Germany) according to the manufacturer's instructions. For gene expression analysis PowerUP™ SYBR™ green master mix (ThermoFisher Scientific, Germany) was used with the Applied Biosystems™ QuantStudio™ 6 Flex Real-Time PCR System (ThermoFisher Scientific, Germany).

Analysis of shared genes and gene ontology pathways

Differentially expressed genes between the conditions ND and WD were determined with Seurat v2.3 for R (threshold = 0.25, minimum pct = 0.25). Odds ratio of overlapping genes between every single cluster and condition was calculated by GeneOverlap package[1] for R and the log2-scaled data were displayed as a heatmap.

Next, significantly expressed genes that were expressed by at least two clusters in one treatment condition were compared with all other clusters and treatments between both liver and bone marrow myeloid leukocytes. The number of shared genes was counted and displayed in a Venn diagram. Additionally, also most significantly shared genes were displayed for both ND and WD condition between liver and bone marrow cells.

In the final step, gene set enrichment analysis of significantly overrepresented gene ontology (GO) terms of biological process (BP) (adjusted p-value < 0.001) were calculated by using goseq.[2] The number of shared GO terms between clusters and conditions was visualized in a Venn diagram.

References

1 Shen L, Sinai M. GeneOverlap: Test and visualize gene overlaps. 2013.

2 Young M, Wakefield M, Smyth G*, et al.* Gene ontology analysis for RNA-seq: accounting for selection bias. Genome Biology 2010;**11**:R14.