RAGE deficiency does not affect non-alcoholic steatohepatitis and atherosclerosis in Western type diet-fed Ldlr$^{-/-}$ mice

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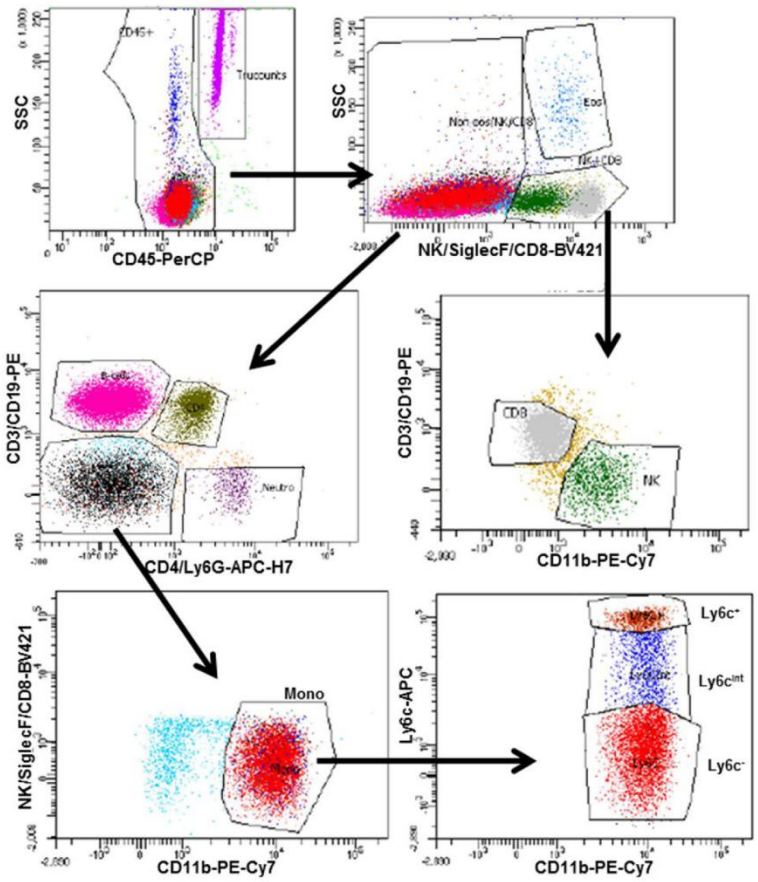
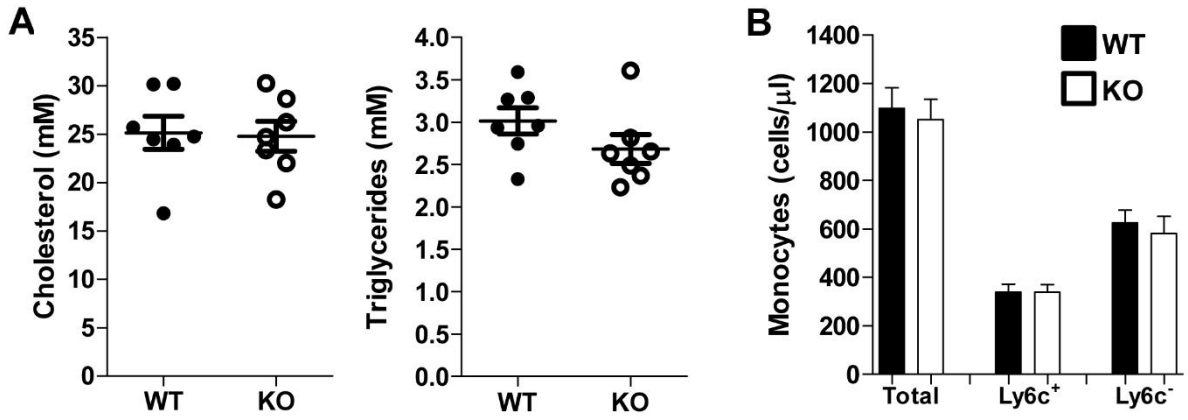


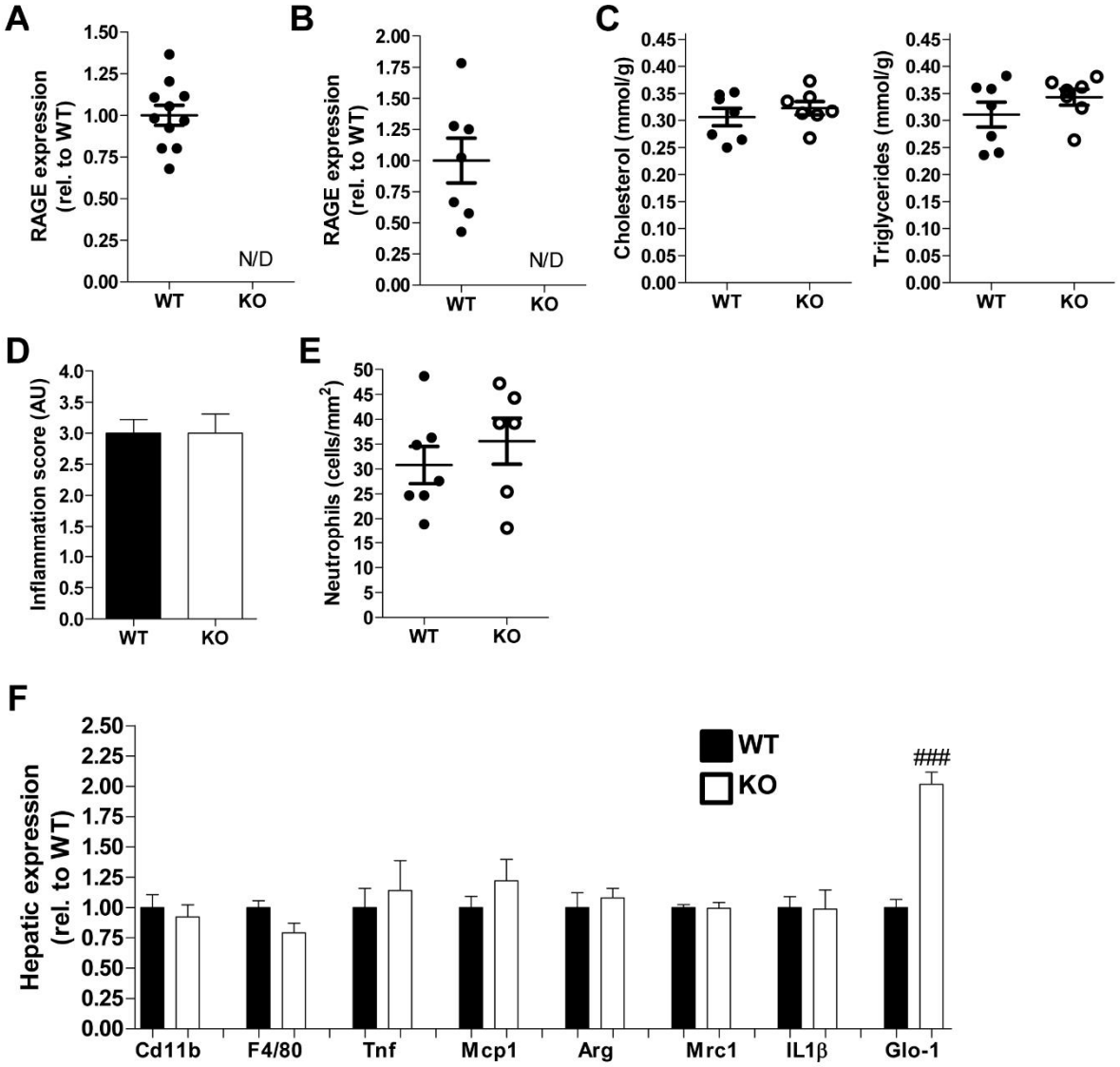
Suppl. Fig. 1. Gating strategy used for flow cytometry

FACS plots showing gating strategy used for circulating cells starting from CD45$^+$ cells. First, eosinophils (SSC$^+$, SiglecF$^+$), NK (SSC$^-$, NK1.1$^+$) and CD8$^+$ T-cells (SSC$^-$, CD8$^+$) were separated and further divided into CD8$^+$ T-cells (CD3$^+$) and NK-cells (CD11b$^+$). All other cells were divided into B-cells (CD19$^+$, CD4$^-$, Ly6G$^-$), CD4$^+$ cells (CD3$^+$, CD4$^+$), neutrophils (CD3$^-$, Ly6G$^+$) and remaining cells. Monocytes were separated from these remaining cells using CD11b before splitting all monocytes into Ly6c$^-$ (patrolling monocytes), Ly6c^{int} and Ly6c$^+$ (proinflammatory) monocytes based on their Ly6c expression. SSC, sidescatter.



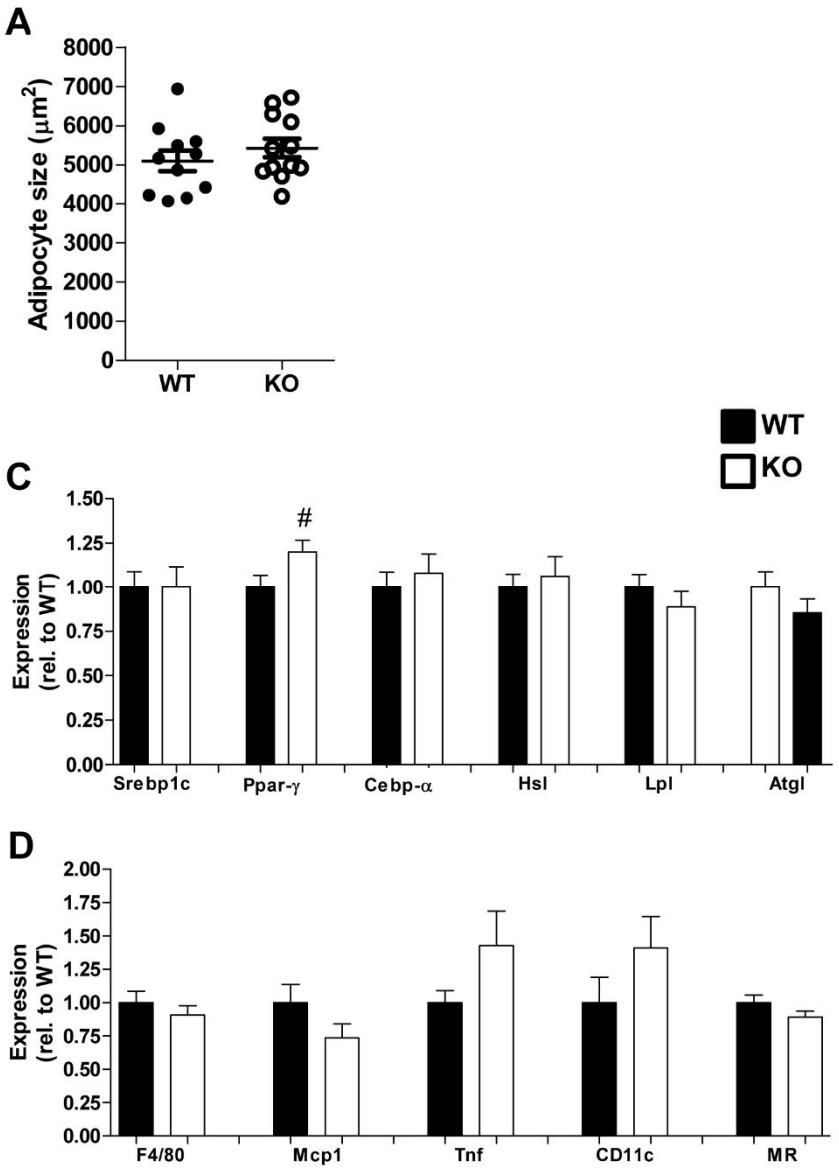
Suppl. Fig. 2. RAGE does not affect plasma lipid levels or monocytosis

(A) Plasma cholesterol and triglyceride levels after 3 weeks of WTD feeding. (B) Total circulating monocyte levels and subdivision in $Ly6C^+$ and $Ly6C^-$ monocytes measured by flow cytometry and presented as cells/µl after 3 weeks of WTD feeding. All data are means ± SEM. n = 7.



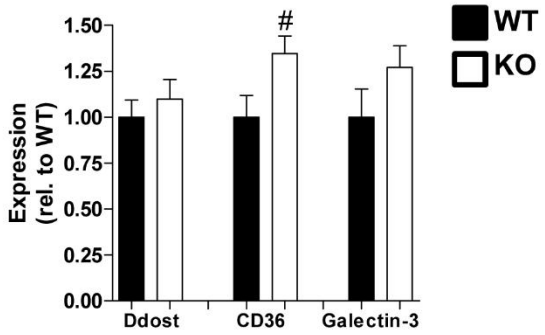
Suppl. Fig. 3. Hepatic steatosis, neutrophil infiltration and macrophage accumulation were unaffected by RAGE

(A, B) Hepatic RAGE expression levels after 12 (A; n = 11-12) and 3 weeks (B; n = 7) of WTD feeding. (C) Hepatic cholesterol and triglycerides levels after 3 weeks of WTD feeding. (D) Hepatic inflammation score (1, 2, 3 or 4 based on lobular inflammation and immune cell numbers) after 3 weeks of WTD feeding. (E) Quantification of neutrophils in the liver after 3 weeks of WTD. (F) Hepatic gene expression levels of inflammatory and immune cell specific markers after 3 weeks of WTD feeding. N/D = not detectable. All data are means ± SEM. ###P < 0.001 vs WT. n = 7.



Suppl. Fig. 4. RAGE deficiency does not influence adipocyte size, lipid metabolism or adipose tissue inflammation

(A) Adipocyte cell size after 12 weeks of WTD feeding. (B) Lipolysis and adipogenesis related gene expression in the vAT. (C) Gene expression levels of inflammatory and immune cell specific markers in vAT after 12 weeks of WTD feeding. All data are means ± SEM. n = 11-12.



Suppl. Fig. 5. The absence of RAGE stimulates expression of CD36, but not of other AGE-receptors

Gene expression levels of the AGE-receptors DDOST, CD36 and Galectin-3 in the liver after 12 weeks of WTD feeding. All data are means ± SEM. n = 11-12.

Suppl. Table 1. Antibodies used for flow cytometry of circulating cells

Flow cytometry antibodies with fluorescent label and manufacturer.

Antibody	Label	Manufacturer
Blood mix		
CD16/32	-	eBioscience
CD45	PerCP	Biolegend
CD3	PE	eBioscience
NK1.1	BV421	Biolegend
LY6G	APC-H7	BD
CD11B	PE-Cy7	BD
LY6C	APC	Miltenyi
CD4	APC-H7	BD
CD8	eFluor450	eBioscience
CD19	PE	eBioscience
SIGLECF	BV421	BD

Suppl. Table 2. Primer sequences

Forward and reverse qPCR primer sequences of all measured genes.

Gene	FW primer	RV primer
Mcp1	TTAAAAACCTGGATCGGAACCAA	GCATTAGCTTCAGATTTACGGGT
Tnf	CATCTTCTCAAAATTCGAGTGACAA	TGGGAGTAGACAAGGTACAACCC
F4/80	GGATGTACAGATGGGGGATG	TCTGTGGTGTCAGTGCAGGT
IL1β	CTGCAGCTGGAGAGTGTGGAT	CACTTTGCTCTTGACTTCTATCTTGTTG
Cd11b	ATGGACGCTGATGGCAATACC	TCCCCATTCACGTCTCCCA
Mrc1	TGCCAAAAATTATTGATCCTGTAACT	CGCCGGCACCTATCACA
Arg1	CATGGGCAACCTGTGTCCTT	CGATGTCTTTGGCAGATATGCA
Glo1	ATGACGAGACTCAGAGTTACCACAA	TAGACATCAGGAACGGCAAATCC
RAGE	GCTCGAATCCTCCCCAATG	TCCCCTCATCGACAATTCCA
Cd11c	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTCA
IL-6	TTCAACCAAGAGGTAAAAGATTTACATAA	CACTCCTTCTGTGACTCCAGCTT
iNOS	GCCCCTGGAAGTTTCTCTTC	TTCTGTGCTGTCCCAGTGAG
Col1a1	TGTTCAGCTTTGTGGACCTC	TCAAGCATACCTCGGGTTTC
Srebp1c	ACTTTTCCTTAACGTGGGCCT	ACTTTTCCTTAACGTGGGCCT
Ppar-γ	ATGATGGGAGAAGATAAAATCAAGTTC	CGGCTTCTACGGATCGAAAC
Cebp-α	GCGGGAACGCAACAACATC	GTCACTGGTCAACTCCAGCAC
Hsl	GAGGCCTTTGAGATGCCACT	AGATGAGCCTGGCTAGCACAG
Lpl	TGGCGTAGCAGGAAGTCTGA	TGCCTCCATTGGGATAAATGTC
Atgl	AGCATCTGCCAGTATCTGGTGAT	CACCTGCTCAGACAGTCTGGA
Ddost	CCCGGACAATCCCTTGGTTT	TGAGAATACCTCTGCGCACC
Cd36	GGAGCCATCTTTGAGCCTTCA	GAACCAAACTGAGGAATGGATCT
Galectin-3	CCCGCATGCTGATCACAATC	GGGGTTAAAGTGGAAGGCAA
Cyclophilin	TTCCTCCTTTCACAGAATTATTCCA	CCGCCAGTGCCATTATGG
B2-Micro	CTTTCTGGTGCTTGTCTCACTGA	GTATGTTCGGCTTCCCATTCTC

Suppl. Table 3. RAGE deficiency does not affect circulating immune cells

Levels of circulating T-cells, B-cells, neutrophils, NK-cells and eosinophils measured by flow cytometry in cells/µl after 12 weeks of WTD feeding. n = 11-12.

Cell type	WT (Mean ± SEM)	KO (Mean ± SEM)
All immune cells (cells/µl)	6268 ± 499	7309 ± 824
CD4 T-cells (cells/µl)	590 ± 53	731 ± 86
CD8 T-cells (cells/µl)	591 ± 37	625 ± 72
B-cells (cells/µl)	2744 ± 303	3741 ± 471
Neutrophils (cells/µl)	911 ± 289	1075 ± 245
NK-cells (cells/µl)	425 ± 77	296 ± 26
Eosinophils (cells/µl)	63,8 ± 11,2	109,3 ± 27,3

Suppl. Table 4. Circulating immune cells are not affected by RAGE

Levels of circulating T-cells, B-cells, neutrophils, NK-cells and eosinophils measured by flow cytometry in cells/µl after 3 weeks of WTD feeding. #P < 0.05 vs WT. n = 7.

Cell type	WT (Mean ± SEM)	KO (Mean ± SEM)
All immune cells (cells/µl)	10969 ± 393	12570 ± 723
CD4 T-cells (cells/µl)	1656 ± 124	1832 ± 139
CD8 T-cells (cells/µl)	1204 ± 79	1221 ± 76
B-cells (cells/µl)	5448 ± 213	6929 ± 456$^{\#}$
Neutrophils (cells/µl)	657 ± 70	684 ± 97
NK-cells (cells/µl)	257 ± 21	230 ± 18
Eosinophils (cells/µl)	138 ± 16	177 ± 20

Suppl. Table 5. Free AGE levels in the vAT are not affected by RAGE-deficiency

The CML, CEL and MG-H1 levels in the vAT after 12 weeks of WTD. n = 11-12. N/D = not detectable.

vAT AGE levels	WT (Mean ± SEM)	KO (Mean ± SEM)
CML (nmol/g)	78,7 ± 15,1	86,0 ± 15,2
CEL (nmol/g)	N/D	N/D
MG-H1 (nmol/g)	4,08 ± 0,64	3,84 ± 0,71