ARTICLE - Hematopoiesis

Adipocytes control hematopoiesis and inflammation through CD40 signaling

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Supplementary methods

Mice

Breeding and housing of all animals was done at the animal facility of the Amsterdam University Medical Center, location AMC, and the Leiden University Medical Center. All mice were backcrossed >10 times before inclusion in experiments. All the experimental procedures were approved by the Ethical Committee for Animal Welfare of Amsterdam University Medical Center, location AMC (AVD1180020171666) and Leiden University Medical Center (AVD1160020173305). After the selected dietary period mice were euthanized using a cocktail of ketamine (150 mg/kg) and xylazine (10mg/kg). Blood was obtained by cardiac puncture with an EDTA-coated syringe. Subsequently, after PBS perfusion, epididymal adipose tissue (EpAT), subcutaneous AT (ScAT), cardiac AT (CAT), liver, spleen, bone marrow (BM), thymus, and lymph nodes (LN) were removed and used for subsequent analyses. For atherosclerosis analysis, the heart and arterial tree were excised, fixed in 1% paraformaldehyde o/n, embedded in paraffin, and sectioned in 4 µm sections.

Glucose and insulin tolerance tests

In the DIO studies, one week prior to start and 12 weeks after the HFD and SFD, glucose tolerance tests (GTT) and insulin tolerance tests (ITT) were performed. For the GTT, 4h fasted mice were injected intraperitoneal with glucose (1mg/g body weight, Sigma-Aldrich). For the ITT, 4h fasted mice were injected intraperitoneal with insulin (1,1mU/g body weight, Sigma-Aldrich). Utilizing the tail vain, glucose levels were measured in whole blood using a glucometer (Bayercontour, Basel, Switzerland) at times indicated in the figures.

Analysis of atherosclerosis: morphometry and immunohistochemistry

Per mouse, 4 µm sections were cut over the length of 48 µm and stained using haematoxylin and eosin to visualize plaques in the aortic root. Two sections, 56 µm apart were stained with Picro-Sirius Red to visualize plaque collagen content. For other stainings, selected sections were rehydrated in xylene and a graded series of ethanol before pre-treatment with 0.3% H_2O_2 in methanol for 30 min, and sodium citrate antigen retrieval buffer (pH 6) at 100°C for 10 min (or, in the case of staining for CD3, TE antigen retrieval buffer, pH 9). Sections were then incubated with antibodies against Mac3 (1:100, clone M3/84, BD Biosciences), α-SMA (1:3000, clone 1A4, Sigma-Aldrich), ER-MP58 (1:200, Bio-Rad Laboratories B.V., Veenendaal, The Netherlands), or CD3 (1:100, clone 145-2C11, BD Biosciences). The primary antibody binding was detected by incubation with appropriate biotin-conjugated secondary antibodies (CD3/Mac3; E0468, Dako, Santa Clara, USA) and (α-SMA; 200-062-037, Jackson ImmunoResearch, Cambridgeshire, UK) followed by the ABC kit according to the manufacturer's instructions (Vector Labs, Burlingame, USA). Immunoreactivity was visualized with 3,3'diaminobenzidine (DAB; Dako/Agilent, Santa Clara, USA). Tissue sections were counterstained with Mayer's haematoxylin and mounted with Entellan (Merck, Darmstadt, Germany). Immunohistochemical staining omitting the primary antibodies served as negative controls. For assay of apoptotic cells, a HRP-DAB TUNEL kit (Abcam, Cambridge, UK) was used in accordance to the protocol. Images were recorded with a Leica DM6000 microscope with Las 4.1

software (Leica Microsystems, Wetzlar, Germany), and analysis was performed using Adobe Photoshop CS6, ImageJ, and Fiji.

Flow cytometry analysis

For flow cytometric analysis, EpAT, ScAT, CAT, spleen, BM, thymus, and LN were removed, rinsed in PBS and minced into small pieces. Adipose tissue was digested with Liberase (0.25 mg/mL, Roche, Woerden, Netherlands) for 45 min at 37°C. The digested samples were passed through a 70-μm nylon mesh (BD Biosciences, Breda, Netherlands). The SVF was obtained from the resulting pellet and resuspended in FACS buffer (0.5% BSA, 0.01% NaN_3, and 2mM EDTA in PBS). Erythrocytes in blood, spleen, and BM were removed by incubation with hypotonic lysis buffer (8.4 g of NH_4Cl and 0.84 g of $NaHCO_3$ per litre of distilled water). Cell suspensions were incubated with CD16/32 antibody (ThermoFisher) in FACS buffer to prevent non-specific binding of antibodies to the Fc receptor. Tissues were further stained with fluorophore-conjugated antibodies using CD4, CD138, Sca-1, CD27, CD38 (1:100, BD bioscience), CD16/32, Ly6G, CD8, CD3, CD19, CD135, CD48, CD45 (1:200, Biolegend), IgM (1:1600), Lin, CD150, CD127, CXCR4, CD34, CD11b, CD11c, F4/80, CD62L, CD44, FoxP3, IL-2, IFNγ, TNFα, B220, CD21, CD23, CD25, CD93 and Ly6C (1:100, eBioscience/ThermoFisher). A fixation/permeabilization kit (ThermoFisher) was used to perform intracellular staining according to manufacturer's instructions. Flow cytometry was performed using the BD LSRFortessa (BD Biosciences) and analyzed using FlowJo v10.5.3 software (FlowJo LLC, Ashland, USA).

T cell activation assay

Splenocytes were isolated and $CD4^+$ and $CD8^+$ T cells were separated using magnetic cell separation strategies (MACS, Miltenyi Biotec, Leiden, The Netherlands) positive selection. Cells were cultured 1*10^5 in a 9-well dish and stained with a 5 μM staining solution CFSE (ThermoFisher), along with 2,5 μl CD3/CD28 Dynabeads (Invitrogen) per well. T cells were cultured in RPMI medium + 10% FCS + 1% P/S (ThermoFisher), with 20 μl/ml IL-2 and 50 μM β-mercaptoethanol for 48-72 hours. After stimulation cells were analyzed by flow cytometry using the BD LSRFortessa (BD Biosciences) and FlowJo v10.5.3 software (FlowJo LLC, USA).

Monocyte migration assay

For analysis of migration capacity Boyden chambers (Corning® 5 μm polycarbonate membrane, Sigma Aldrich) were used. All monocytes Ly6Clo-hi were isolated from erylysed blood using the Becton Dickinson FacsAria SORP Cell Sorter (BD Biosciences). 70K monocytes were plated on the transwells and medium with 1 μg/ml CCL2 or control (NS) was added to the bottom of the wells. After 4h the cells from the top and bottom of the well were analyzed using the BD LSRFortessa (BD Biosciences) and FlowJo v10.5.3 software (FlowJo LLC, USA).

Plasma measurements

Blood cholesterol and triglycerides were assessed by enzymatic colorimetric kits according to the vendor's instructions (Roche Diagnostics, Indianapolis, USA). Mouse fasting plasma insulin levels were measured in samples from 4h fasted mice using an insulin ELISA kit according to the vendor's instructions (Mercodia, Uppsala, Sweden). Plasma leptin and corticosteroid levels (obtained at ~10

AM at sacrifice) were measured with a mouse leptin ELISA kit (ChrystalChem, Zaandam, Netherlands) and a mouse corticosterone competitive ELISA kit (ThermoFisher, Bleiswijk, Netherlands). Plasma cytokine levels were measured on a Luminex 200 system using a multiplex mouse Procartaplex panel (ThermoFisher) containing markers for IFN-γ, TNF-α, IL1β, IL2, IL4, IL5, IL6, IL10, IL12p70, IL17A, IL17F, IL23, G-CSF, IP-10, GM-CSF, KCGRO, MCP-1, MCP-3, MIP-1α, MIP-1β, and MIP-2.
Total IgM and IgG concentrations of plasma were detected using IgG and IgM mouse uncoated ELISA Kits (ThermoFisher).

Histology

Adipose tissues was collected, fixed in 4% paraformaldehyde and embedded in paraffin. Immunohistochemistry on EpAT was performed for CD45 (1:1000, BD bioscience) and MAC3 (1:100, BD bioscience), and adipocyte size was measured on EpAT H&E-stained sections. Femurs were collected, fixed in 4% paraformaldehyde, decalcified in EDTA solution for 3 weeks, and embedded in paraffin. Immunohistochemistry on femurs was performed for perilipin-1 (1:200, Abcam). Livers were embedded in OCT (Sakura, Alphen aan den Rijn, Netherlands) and frozen at -80 ^{o}C. To measure tissue lipid content, 5 µm thick cryosections of the liver and 8 µm thick cryosections of the adrenal gland were stained with Oil red O (Sigma-Aldrich, Zwijndrecht, Netherlands).

RNA isolation and gene expression analysis

Total RNA from adipose tissue, BM, and liver was extracted using TRIzol (Invitrogen, Carlsbad, USA). RNA was reverse transcribed using an iScript cDNA synthesis kit (Bio-Rad, Veenendaal, Netherlands), and quantitative PCR was performed with the SYBR Green PCR kit (Applied Biosystems, Leusden, Netherlands) on a ViiA7 real time PCR system (Applied Biosystems). Expression levels of transcripts obtained with real-time PCR were normalized to the mean expression levels of the house keeping genes *Gapdh* and *B2m*.

Adipocyte culture

Adipocytes from AdiCD40^{KO} and WT mice were cultured by differentiating pre-adipocytes on a collagen hydrogel (Corning®, Osdorp, Netherlands). Adipose tissue isolated from mice was minced and digested with Dispase II (10mg/ml, Sigma-Aldrich) and Collagenase D (200mg/ml, Roche) for 1h at 37^{o}C. The digested mixture was strained through a 70 µm filter and spun for isolation of pre-adipocytes. Pre-adipocytes were cultured for 5 days on rat tail collagen type 1 (100µg/ml, Corning®) pre-coated plates. Adherent pre-adipocytes were trypsinized (ThermoFisher) and diluted at $6*10^5$ cells/24 well. Cells were added to the hydrogel and cultured for 2 days with cell differentiation medium (DMEM/F12 + GlutaMAX (ThermoFisher) + 10% FCS + 1% P/S, Dexamethasone (5uM, Sigma), insulin (2,5ug/mL), isobutylmethylxanthine (0,5mM, ThermoFisher), and rosiglitazone (3uM, CaymanChem, Ann Arbor, USA). Additionally, differentiated adipocytes were cultured for 7 days with DMEM/F12 + GlutaMAX + 10% FCS + 1% P/S + insulin (2,5ug/mL) before experiments or analysis.

Statistical analysis

Data are expressed as mean±SD. Differences between groups were analyzed using an unpaired t test or Mann-Whitney U test if not conforming to Gaussian distribution as assessed by D'Agostino-Pearson omnibus normality test. A P value of <0.05 was considered significant. Outliers were identified using Grubbs' test (alpha=0.05). Statistical analysis was performed using Graphpad Prism 9.1.2.

Supplemental Figures

Supplemental Central Figure, haematopoietic stem cell flow cytometric markers and gating strategies for bone marrow and thymus.

Supplemental Figure 1A Relative mRNA CD40 expression in adipocytes derived from EpAT in AdiCD40^{KO} mice and WT littermates. B Weights of Adipocyte CD40-deficient and WT littermates at sacrifice of indicated diets. n=5 52-week old chow fed (Chow52), n=8 22-week old chow fed (Chow22), n=7 standard fat diet (SFD), n=8 high fat diet (HFD), n=8 SFD that underwent the metabolic cage study (SFDmc).

Supp. Fig. 2A Common lymphoid progenitor (CLP) as a percentage of Lin- cells in BM of AdiCD40^{KO} mice and WT littermates. B Flow cytometric analysis of CD19^{+} B-cells of CD45^{+} cells in BM. C Amount of naïve, effector memory, and central memory CD4 T-cells in BM. D Histological quantification of adipocytes and megakaryocytes per area in BM. E Total amount of thymocytes per gram thymus. F Early thymocyte (cKit^{+}) development of double negative (DN, CD25^{lo-hi} CD44^{lo-hi}) cells. G Selection of double positive thymocytes (cKit^{-}) into single positive CD4^{+} and CD8^{+} T-cells. H Plasma corticosterone levels in pg/ml. I Diameter of adrenal gland in n=5 AdiCD40^{KO} mice and n=5 WT littermates. I Percentage Oil-Red-O area in adrenal gland cross-section (n=5x5). Data are shown as mean±SD, n=8 AdiCD40^{KO} mice and n=8 WT littermates. *P<0.05, **P<0.01, ***P<0.001.

Supp. Fig. 3A Flow cytometric analysis of lymphoid populations in the inguinal LN. B Flow cytometric analysis of splenocytes isolated from WT and AdiCD40KO mice, showing naïve (CD62L+CD44-), central memory (CD62L^{+}CD44^{+}), and effector memory (CD62L^{-}CD44^{+}) CD4^{+} T-cells (n=4x3). C Intracellular cytokine expression of CD4^{+} T-cells at day 3 after stimulation with CD3/CD28 beads (n=4x3). D Intracellular cytokine expression of CD8^{+} T-cells at day 3 after stimulation with CD3/CD28 beads (n=4x3). E Flow cytometric analysis of splenocytes. F Flow cytometric analysis of the Lin-cKit+Sca- population presenting the myeloid lineage precursors. Data are shown as mean±SD, n=8 AdiCD40^{KO} mice and n=8 WT littermates. *P<0.05, **P<0.01, ***P<0.001.

Supp. Fig. 4A Common lymphoid progenitor (CLP) as a percentage of Lin- cells in BM of aged 52-week old AdiCD40^{KO} mice and WT littermates. B Flow cytometric analysis of the Lin-cKit+Sca-population presenting the myeloid lineage precursors. C 10% of Lin^{+}CD45^{+} cells in BM. D Activation status of CD4^{+} T-cells in BM. E CXCR4 expression on splenic CD4^{+} and CD8^{+} T-cells. F Activation status of CD4^{+} T-cells in spleen. G Transitional (T1/T2) B-cells and follicular B-cells in spleen. H Flow cytometric analysis of B-cell maturation in BM, indicating ProB, PreB, and immature B-cells. I Interstitial BM IgG in pg/ml. Data are shown as mean±SD, aged n=5 AdiCD40^{KO} mice and n=5 WT littermates. *P<0.05, **P<0.01, ***P<0.001.

Supp. Fig. 5A Weights (g) of CD40^{KO} mice and ApoE$^{-/-}$ littermates after 11 weeks of high cholesterol diet. **B** Plasma total cholesterol (tCHOL), VLDL, LDL, and HDL subtypes, along with **C** plasma tryglycerides (TRIG), free fatty acids (free glycerol) and VLDL, LDL, HDL subtypes in CD40^{KO} mice and ApoE$^{-/-}$ littermates. Data are shown as mean±SD, n=20 CD40^{AKO} mice and n=19 ApoE$^{-/-}$ littermates. *P<0.05

Supp. Fig. 6A Flow cytometric analysis of BM monocyte precursors, macrophage and dendritic cell progenitor (MDP), common monocyte precursor (cMoP), transitional pre-monocytes (tpmo), classical monocyte (CM), intermediate monocyte (IntM), and non-classical monocyte (NCM) (n=6x6). **B** Early CLP, CLP, and Late CLP in BM (n=15x15). **C** Flow cytometric analysis of activation status of CD4^{+} T-cells, naïve (CD62L^{+}CD44^{-}), effector memory (EM; CD62L^{-}CD44^{+}), and central memory (CM; CD62L^{+}CD44^{+}) in BM. **D** Activation status of CD4^{+} T-cells in blood. **E** Activation status of CD4^{+} T-cells in spleen. **F** Activation status of CD4^{+} T-cells in EpAT. **G** Expression of activation markers on CD4^{+} T-cells at day 3 after stimulation with CD3/CD28 beads (n=4x3). **H** Plasma cytokines in CD40^{KO} mice and ApoE$^{-/-}$ littermates. **I** Flow cytometric analysis of cKit^{+} thymocytes in thymus. **J** Selection of double positive thymocytes (cKit^{-}) into single positive CD4^{+} and CD8^{+} T-cells (n=8x8). Data are shown as mean±SD, n=20 CD40^{AKO} mice and n=19 ApoE$^{-/-}$ littermates. *P<0.05, **P<0.01, ***P<0.001.

Supp. Fig. 7A Flow cytometric analysis of CD45^{+} lymphocytes in blood and spleen of CD40^{KO} mice and ApoE$^{-/-}$ littermates. **B** Flow cytometric analysis of Ly6C monocytes in blood. **C** Expression of CCR2 receptor on all CD40AKO and ApoE$^{-/-}$ monocytes in trans-well (n=3x3). **D** Flow cytometric analysis of CD3^{+} T-cells, CD19^{+} B-cells, and CD11b^{+} innate immune cells in blood. Data are shown as mean±SD, n=20 CD40^{AKO} mice and n=19 ApoE$^{-/-}$ littermates. *P<0.05, **P<0.01

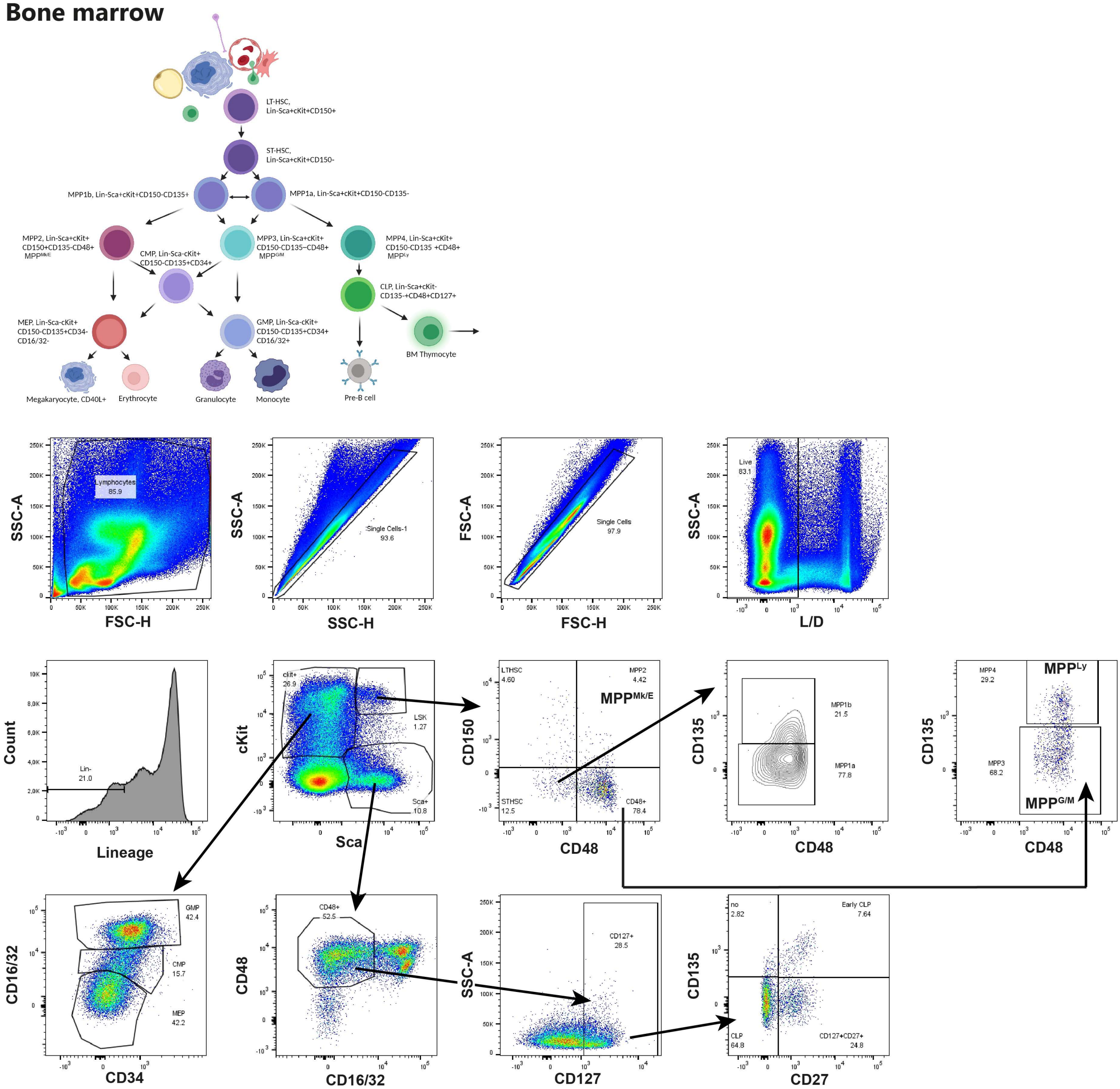
Supp. Fig. 8A Virmani score staging of atherosclerotic lesions, ix= initial xanthoma, pit= pathologic intimal thickening, fca= fibrous cap atheroma (thin and thick), in CD40^{KO} mice and ApoE$^{-/-}$ littermates. **B** Apoptotic cells in atherosclerotic roots were detected by the TUNEL (TdT-mediated dUTP nick end labeling) and area TUNEL+ macrophages in the atherosclerotic roots of ApoE-/- and CD40AKO mice. **C** Percentage collagen stained by sirius red (SR) in lesions. **D** Percentage smooth muscle cell stained by ASMA^{+} in lesions. **E** Flow cytometric analysis of CD45^{+} cells in aortic root and aortic arch (n=6x6). **F** Flow cytometric analysis of CD19^{+} B-cells, CD4^{+} and CD8^{+} T-cells, and F4/80^{+} macrophages in aortic roots and aortic arches (n=6x6). **G** Ly6C monocytes of CD11b^{+} innate cells in aortic root and aortic arch (n=6x6). **H** Histological quantification of ER-MP58 early monocyte staining in aortic root lesions. **I** Flow cytometric analysis of macrophages (F4/80^{+}) and Ly6C monocytes in cardiac/perivascular adipose tissue (CAT) (n=6x6). Data are shown as mean±SD, n=20 CD40^{AKO} mice and n=19 ApoE$^{-/-}$ littermates. *P<0.05

Supp. Fig. 9A Weights (g) of liver and epididymal adipose tissue (EpAT) of SFD fed AdiCD40^{KO} mice and WT littermates, along with HFD fed AdiCD40^{KO} and WT mice. **B** Area under the curve analysis of glucose tolerance test. **C** Insulin tolerance test glucose level (mmol/L) over time for SFD and HFD AdiCD40^{KO} mice and WT littermates. **D** Plasma insulin levels after 4h fasting in SFD and HFD fed AdiCD40^{KO} mice and WT littermates. **E** Triglycerides (mmol/L) levels in plasma of SFD and HFD fed AdiCD40^{KO} mice and WT littermates. **F** Cholesterol (mmol/L) levels in plasma of SFD and HFD fed AdiCD40^{KO} mice and WT littermates. **G** Leptin (ng/mL) levels in plasma of SFD and HFD fed AdiCD40^{KO} mice and WT littermates. Data are shown as mean±SD, n=7 SFD and n=8 HFD AdiCD40^{KO} and n=7 SFD and n=8 HFD WT littermates. *P<0.05, **P<0.01

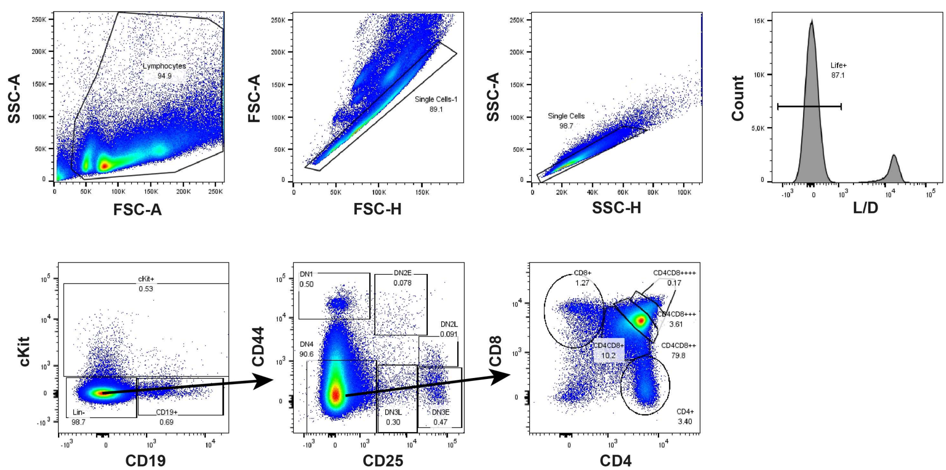
Supp. Fig. 10A Average Crown-like-structures determined through MAC3$^+$ staining in EpAT of HFD AdiCD40^{KO} mice and WT littermates. **B** Flow cytometric analysis of CD45$^+$ cells in 0,5 gram EpAT of HFD fed AdiCD40^{KO} mice and WT littermates. **C** Flow cytometric analysis of activation status of CD4$^+$ and CD8$^+$ T-cells in blood. **D** Plasma cytokines in HFD fed AdiCD40^{KO} mice and WT littermates. **E** Thymocyte (cKit$^-$) development of double negative (DN, CD25^{lo-hi} CD44^{lo-hi}) cells in thymus of HFD mice. **F** Flow cytometric analysis of CLP in BM of HFD fed mice. **G** Activation status of CD4$^+$ and CD8$^+$ T-cells in BM. Data are shown as mean±SD, n=8 HFD AdiCD40^{KO} and n=8 HFD WT littermates. *P<0.05, **P<0.01

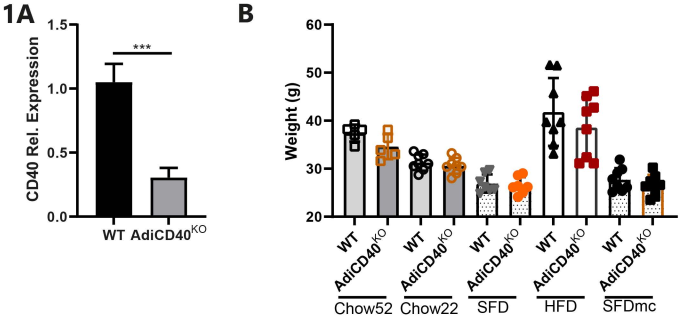
Supp. Fig. 11A Cumulative food intake (g) over time as observed in metabolic cage for n=8 SFD fed AdiCD40^{KO} and WT mice. **B** Indirect calorimetry measurement determined energy expenditure (kcal/h) during the light and dark period. **C** Volumetric oxygen intake (VO_2) along with **D** volumetric carbon dioxide release (VCO_2) release over time determined in metabolic cage in SFD fed AdiCD40^{KO} and WT mice. **E** Respiration quotient as determined in AdiCD40^{KO} and WT mice. **F** Relative glycerol tri[^{3}H]oleate uptake in different organs determined as dose/gram tissue of SFD fed AdiCD40^{KO} and WT mice. **G** Clearance of injected [^{14}C]deoxyglucose over time as determined in blood. **H** Relative [^{14}C]deoxyglucose uptake in different organs determined as dose/gram tissue of SFD fed AdiCD40^{KO} and WT mice. **I** STRING interaction network result for metabolic genes in adipose tissue and liver. The interactome can be observed, as well as relative fold-change in expression (low fold-change is light, high fold-change is blue), along with the grouping by the STRING software of clustered genes (https://string-db.org/, figure obtained 22-09-2022). Data are shown as mean±SD, n=8 SFD AdiCD40KO and n=8 SFD WT littermates. *P<0.05

Gating strategies
Bone marrow

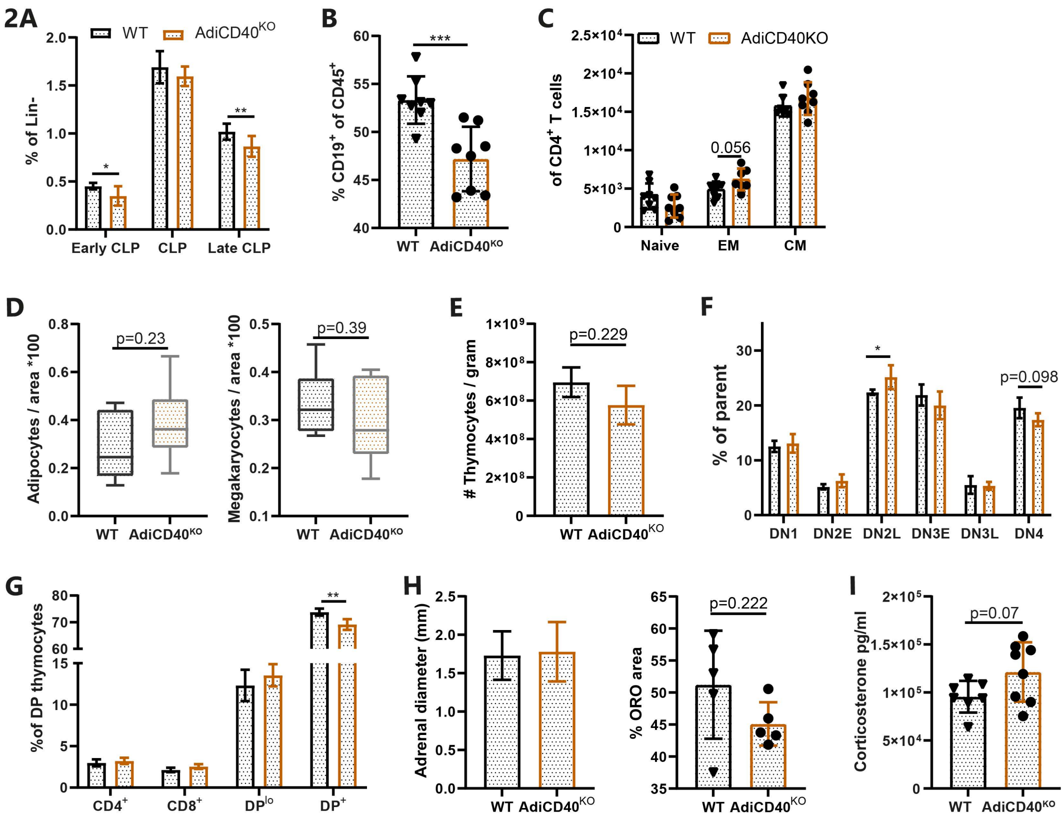


Thymus

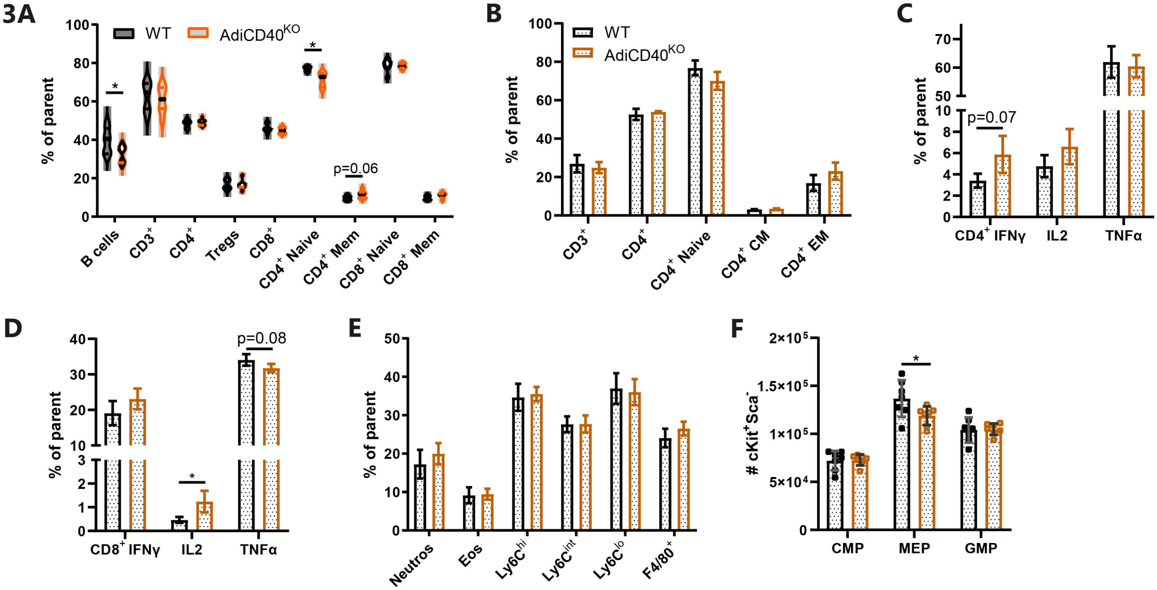




1A
CD40 Rel. Expression
1.5
1.0
0.5
0.0
***
WT
AdiCD40KO
B
Weight (g)
60
50
40
30
20
WT
AdiCD40KO
Chow52
Chow22
SFD
HFD
SFDmc



2A
WT
AdiCD40KO
% of Lin-
2.0
1.5
1.0
0.5
0.0
*
**
Early CLP
CLP
Late CLP
B
% CD19+ of CD45+
60
55
50
45
40
***
WT
AdiCD40KO
C
of CD4+ T cells
2.5×10^4
2×10^4
1.5×10^4
1×10^4
5×10^3
0
0.056
Naive
EM
CM
D
Adipocytes / area *100
0.8
0.6
0.4
0.2
0.0
p=0.23
Megakaryocytes / area *100
0.5
0.4
0.3
0.2
0.1
p=0.39
E
Thymocytes / gram
1×10^9
8×10^8
6×10^8
4×10^8
2×10^8
0
p=0.229
F
% of parent
30
20
10
0
*
p=0.098
DN1
DN2E
DN2L
DN3E
DN3L
DN4
G
%of DP thymocytes
80
70
60
15
10
5
0
**
CD4+
CD8+
DPlo
DP+
H
Adrenal diameter (mm)
2.5
2.0
1.5
1.0
0.5
0.0
% ORO area
65
60
55
50
45
40
35
p=0.222
I
Corticosterone pg/ml
2×10^5
1.5×10^5
1×10^5
5×10^4
0
p=0.07


3A
WT
AdiCD40KO
% of parent
100
80
60
40
20
0
*
*
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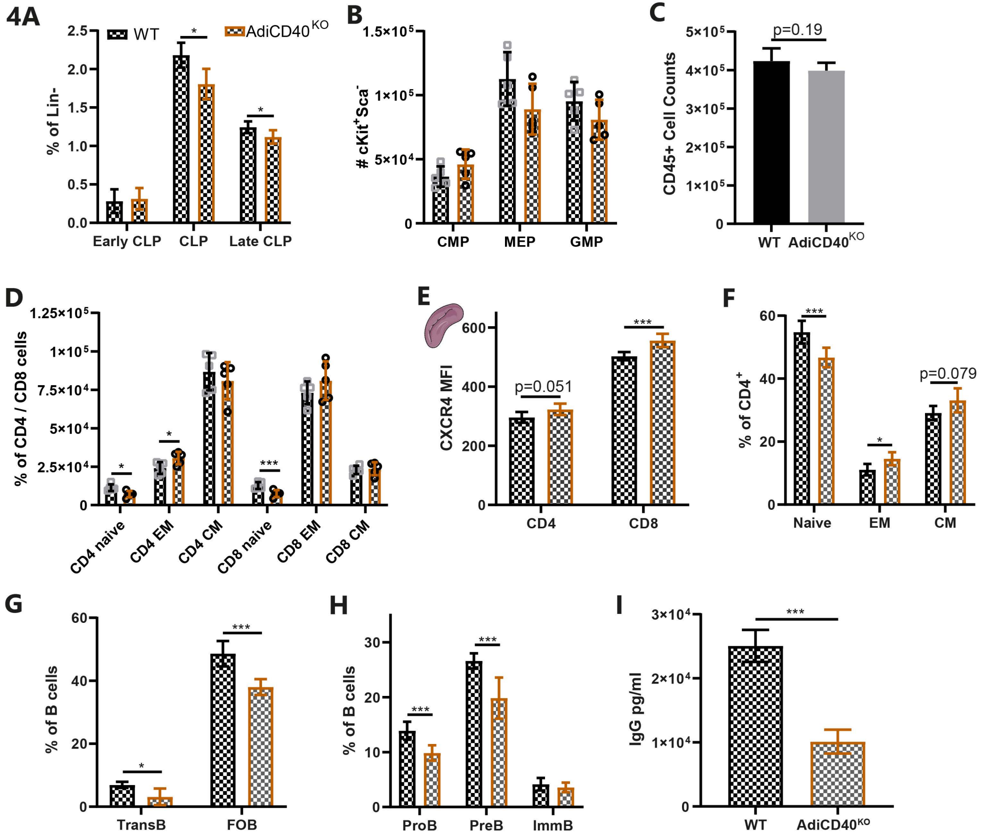
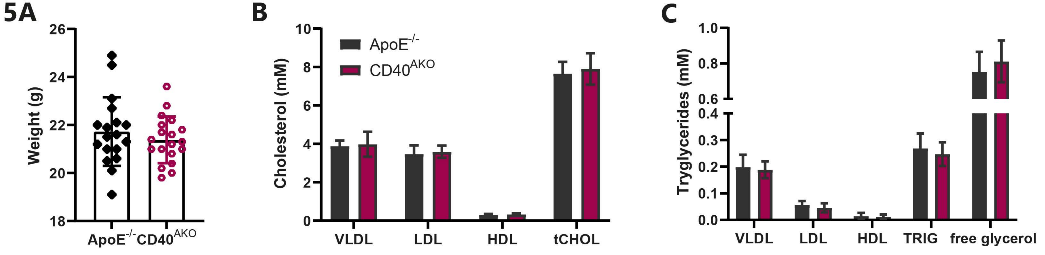
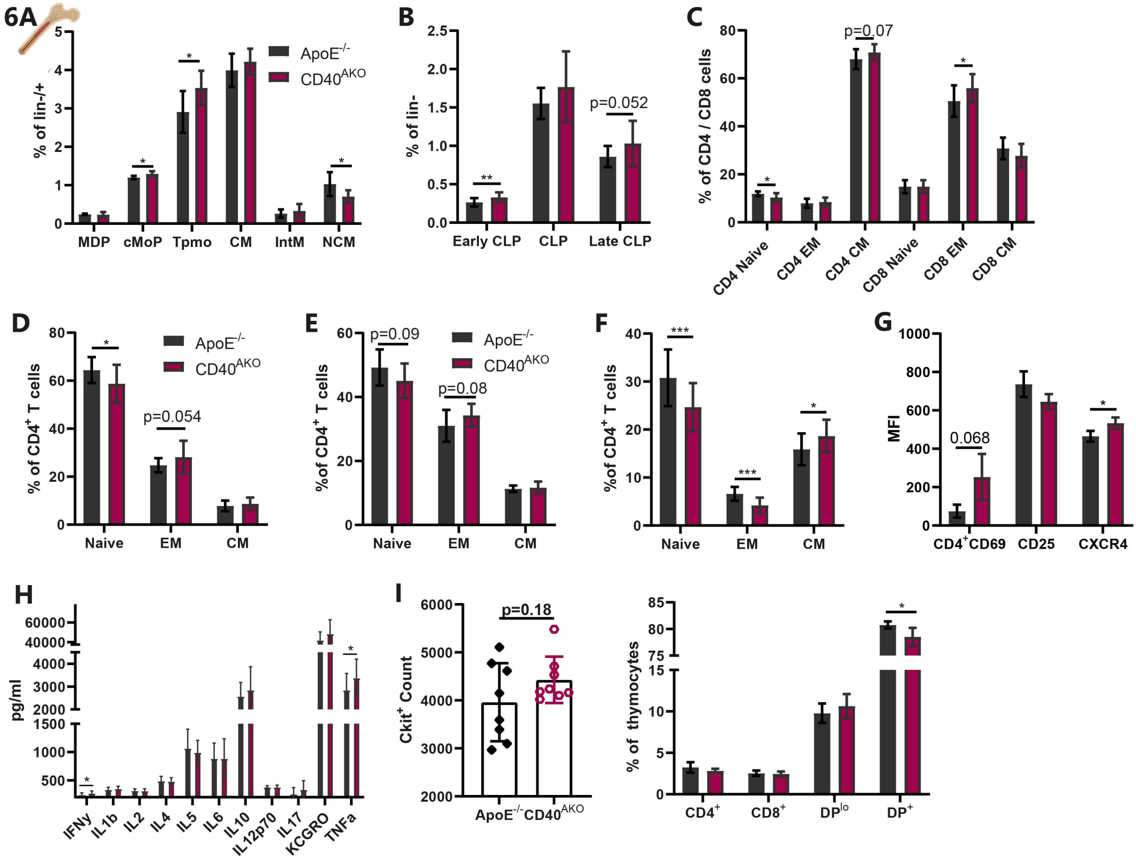
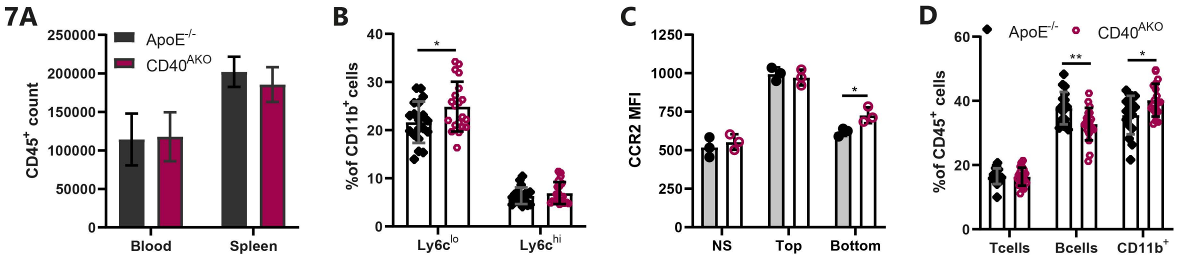
B cells
CD3+
CD4+
Tregs
CD8+
CD4+ Naive
CD4+ Mem
CD8+ Naive
CD8+ Mem
B
WT
AdiCD40KO
% of parent
CD3+
CD4+
CD4+ Naive
CD4+ CM
CD4+ EM
C
% of parent
70
60
50
10
8
6
4
2
0
p=0.07
CD4+ IFNγ
IL2
TNFα
D
% of parent
40
30
20
10
3
2
1
0
p=0.08
*
CD8+ IFNγ
IL2
TNFα
E
% of parent
50
40
30
20
10
0
Neutros
Eos
Ly6Chi
Ly6Cint
Ly6Clo
F4/80+
F
cKit+Sca-
2×10⁵
1.5×10⁵
1×10⁵
5×10⁴
0
*
CMP
MEP
GMP



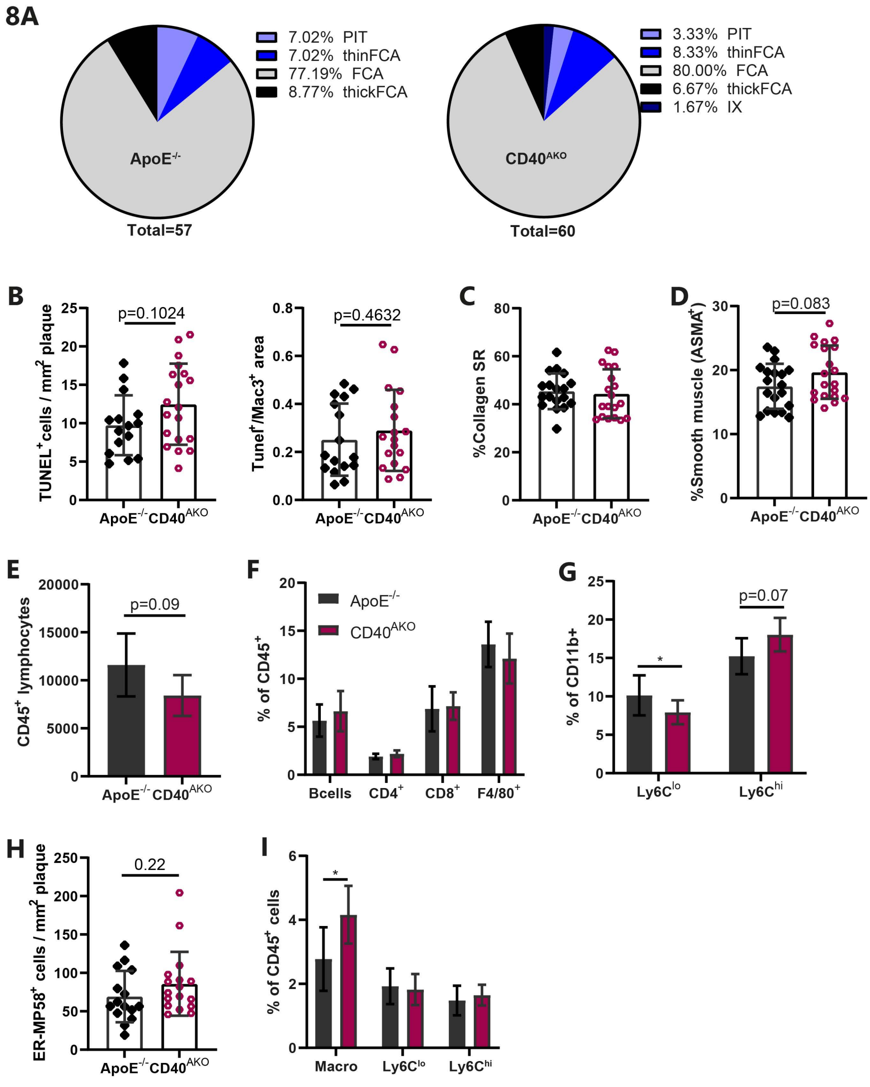
4A
WT
AdiCD40KO
% of Lin-
Early CLP
CLP
Late CLP
B
cKit+Sca-
CMP
MEP
GMP
C
CD45+ Cell Counts
p=0.19
WT
AdiCD40KO
D
% of CD4 / CD8 cells
CD4 naive
CD4 EM
CD4 CM
CD8 naive
CD8 EM
CD8 CM
E
CXCR4 MFI
p=0.051
CD4
CD8
F
% of CD4+
p=0.079
Naive
EM
CM
G
% of B cells
TransB
FOB
H
% of B cells
ProB
PreB
ImmB
I
IgG pg/ml
WT
AdiCD40KO

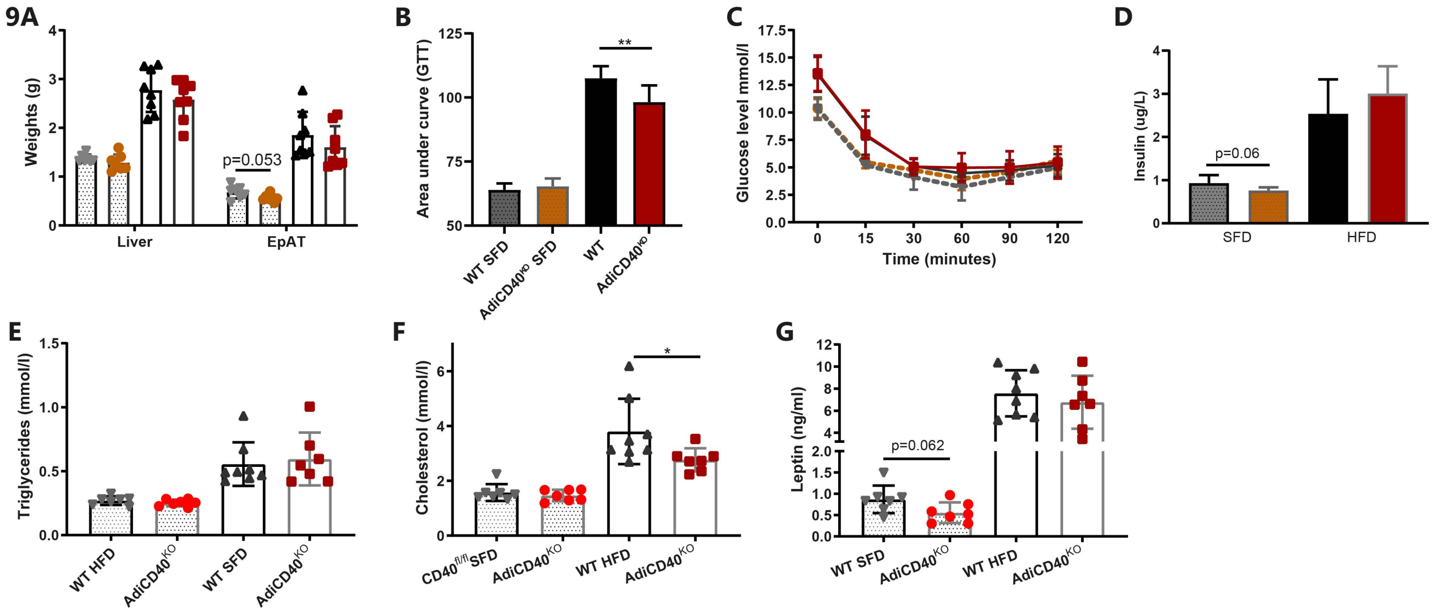

5A
Weight (g)
26
24
22
20
18
ApoE-/-
CD40AKO
B
Cholesterol (mM)
10
8
6
4
2
0
ApoE-/-
CD40AKO
VLDL
LDL
HDL
tCHOL
C
Tryglycerides (mM)
1.0
0.8
0.6
0.4
0.3
0.2
0.1
0.0
VLDL
LDL
HDL
TRIG
free glycerol

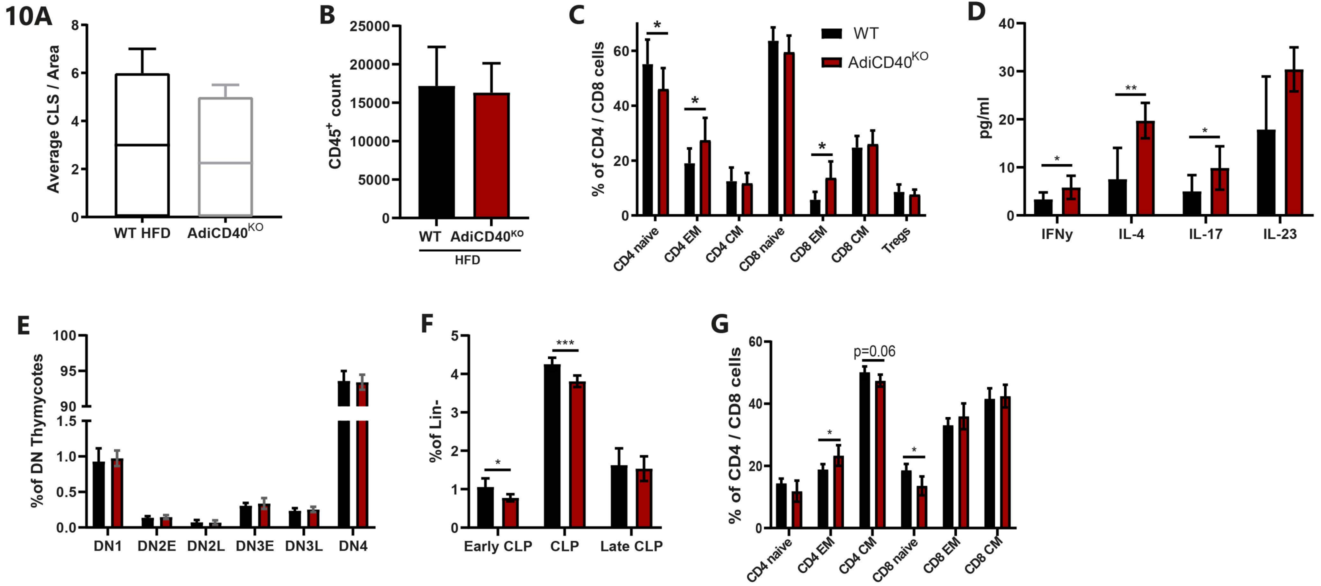

6A
% of lin-/+
ApoE-/-
CD40AKO
MDP
cMoP
Tpmo
CM
IntM
NCM
B
% of lin-
p=0.052
Early CLP
CLP
Late CLP
C
% of CD4 / CD8 cells
p=0.07
CD4 Naive
CD4 EM
CD4 CM
CD8 Naive
CD8 EM
CD8 CM
D
% of CD4+ T cells
ApoE-/-
CD40AKO
p=0.054
Naive
EM
CM
E
%of CD4+ T cells
p=0.09
p=0.08
ApoE-/-
CD40AKO
Naive
EM
CM
F
%of CD4+ T cells
Naive
EM
CM
G
MFI
0.068
CD4+CD69
CD25
CXCR4
H
pg/ml
IFNy
IL1b
IL2
IL4
IL5
IL6
IL10
IL12p70
IL17
KCGRO
TNFa
I
Ckit+ Count
p=0.18
ApoE-/-
CD40AKO
% of thymocytes
CD4+
CD8+
DPlo
DP+

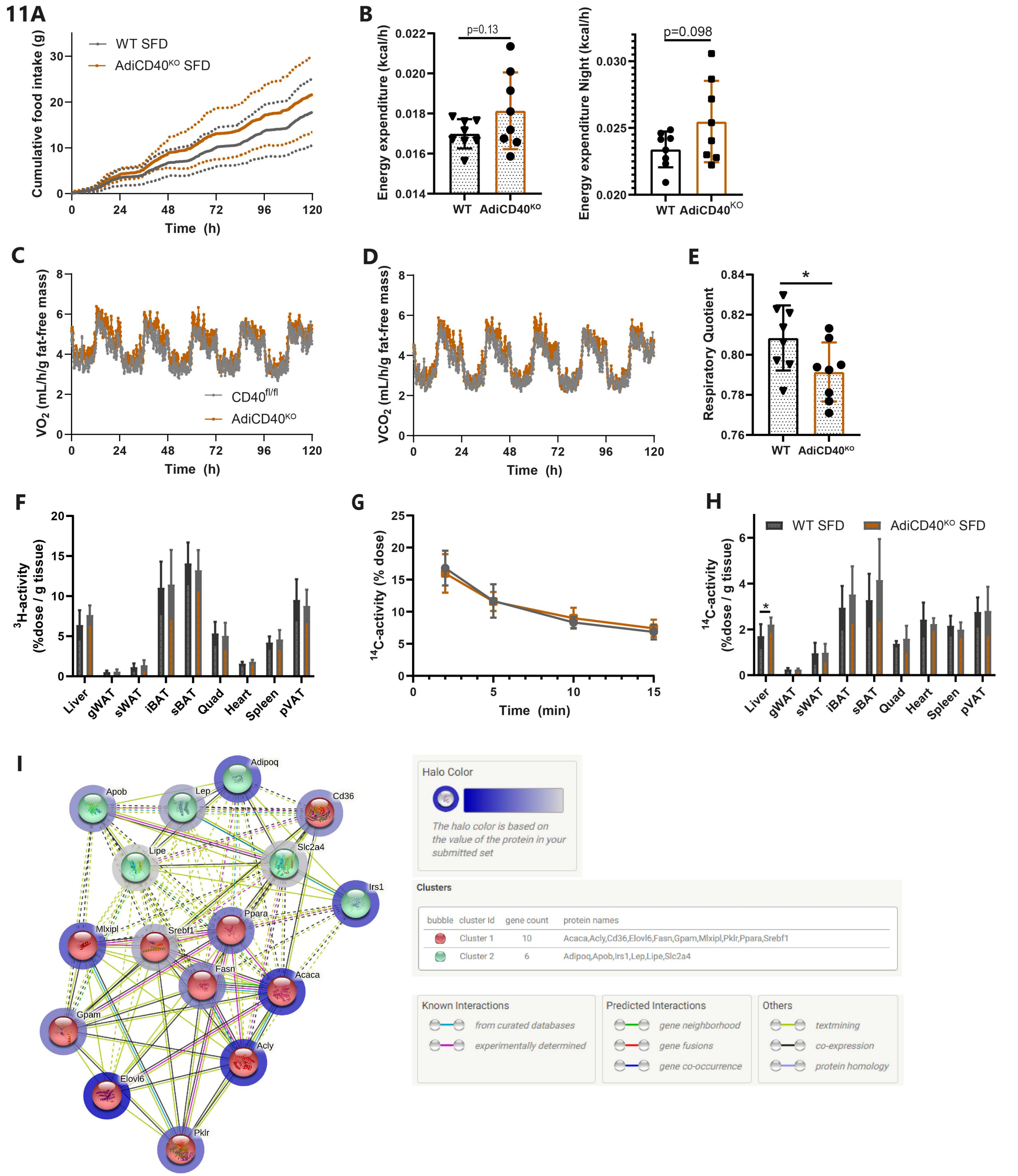


7A
ApoE-/-
CD40AKO
CD45+ count
250000
200000
150000
100000
50000
0
Blood
Spleen
B
%of CD11b+ cells
40
30
20
10
0
*
Ly6clo
Ly6chi
C
CCR2 MFI
1250
1000
750
500
250
0
*
NS
Top
Bottom
D
ApoE-/-
CD40AKO
%of CD45+ cells
60
40
20
0
**
*
Tcells
Bcells
CD11b+


8A
7.02% PIT
7.02% thinFCA
77.19% FCA
8.77% thickFCA
ApoE-/-
Total=57
3.33% PIT
8.33% thinFCA
80.00% FCA
6.67% thickFCA
1.67% IX
CD40AKO
Total=60
B
TUNEL+cells / mm2 plaque
p=0.1024
Tunel+/Mac3+ area
p=0.4632
C
%Collagen SR
D
%Smooth muscle (ASMA+)
p=0.083
ApoE-/- CD40AKO
E
CD45+ lymphocytes
p=0.09
F
% of CD45+
ApoE-/-
CD40AKO
Bcells
CD4+
CD8+
F4/80+
G
% of CD11b+
p=0.07
*
Ly6Clo
Ly6Chi
H
ER-MP58+ cells / mm2 plaque
0.22
I
% of CD45+ cells
*
Macro
Ly6Clo
Ly6Chi


9A
Weights (g)
Liver
EpAT
p=0.053
B
Area under curve (GTT)
**
WT SFD
AdiCD40KO SFD
WT
AdiCD40KO
C
Glucose level mmol/l
Time (minutes)
D
Insulin (ug/L)
p=0.06
SFD
HFD
E
Triglycerides (mmol/l)
WT HFD
AdiCD40KO
WT SFD
AdiCD40KO
F
Cholesterol (mmol/l)
*
CD40fl/flSFD
AdiCD40KO
WT HFD
AdiCD40KO
G
Leptin (ng/ml)
p=0.062
WT SFD
AdiCD40KO
WT HFD
AdiCD40KO


10A
Average CLS / Area
0
2
4
6
8
WT HFD
AdiCD40KO
B
CD45+ count
0
5000
10000
15000
20000
25000
WT
AdiCD40KO
HFD
C
% of CD4 / CD8 cells
0
20
40
60
WT
AdiCD40KO
*
*
*
CD4 naive
CD4 EM
CD4 CM
CD8 naive
CD8 EM
CD8 CM
Tregs
D
pg/ml
0
10
20
30
40
*
**
*
IFNy
IL-4
IL-17
IL-23
E
%of DN Thymycotes
0.0
0.5
1.0
1.5
90
95
100
DN1
DN2E
DN2L
DN3E
DN3L
DN4
F
%of Lin-
0
1
2
3
4
5
*
***
Early CLP
CLP
Late CLP
G
% of CD4 / CD8 cells
0
20
40
60
p=0.06
*
*
CD4 naive
CD4 EM
CD4 CM
CD8 naive
CD8 EM
CD8 CM


11A
WT SFD
AdiCD40KO SFD
Cumulative food intake (g)
Time (h)
B
p=0.13
Energy expenditure (kcal/h)
WT AdiCD40KO
p=0.098
Energy expenditure Night (kcal/h)
C
VO2 (mL/h/g fat-free mass)
CD40fl/fl
AdiCD40KO
D
VCO2 (mL/h/g fat-free mass)
E
Respiratory Quotient
F
3H-activity (%dose / g tissue)
Liver gWAT sWAT iBAT sBAT Quad Heart Spleen pVAT
G
14C-activity (% dose)
Time (min)
H
14C-activity (%dose / g tissue)
I
Adipoq Apob Lep Cd36 Lipe Slc2a4 Irs1 Ppara Mlxipl Srebf1 Fasn Acaca Gpam Acly Elovl6 Pklr
Halo Color
The halo color is based on the value of the protein in your submitted set
Clusters
bubble cluster Id gene count protein names
Cluster 1 10 Acaca,Acly,Cd36,Elovl6,Fasn,Gpam,Mlxipl,Pklr,Ppara,Srebf1
Cluster 2 6 Adipoq,Apob,Irs1,Lep,Lipe,Slc2a4
Known Interactions
from curated databases
experimentally determined
Predicted Interactions
gene neighborhood
gene fusions
gene co-occurrence
Others
textmining
co-expression
protein homology