Supplemental Material to:

Whole-body knock-out of the aryl hydrocarbon receptor (AhR) ameliorates atherosclerosis due to altered lipid metabolism

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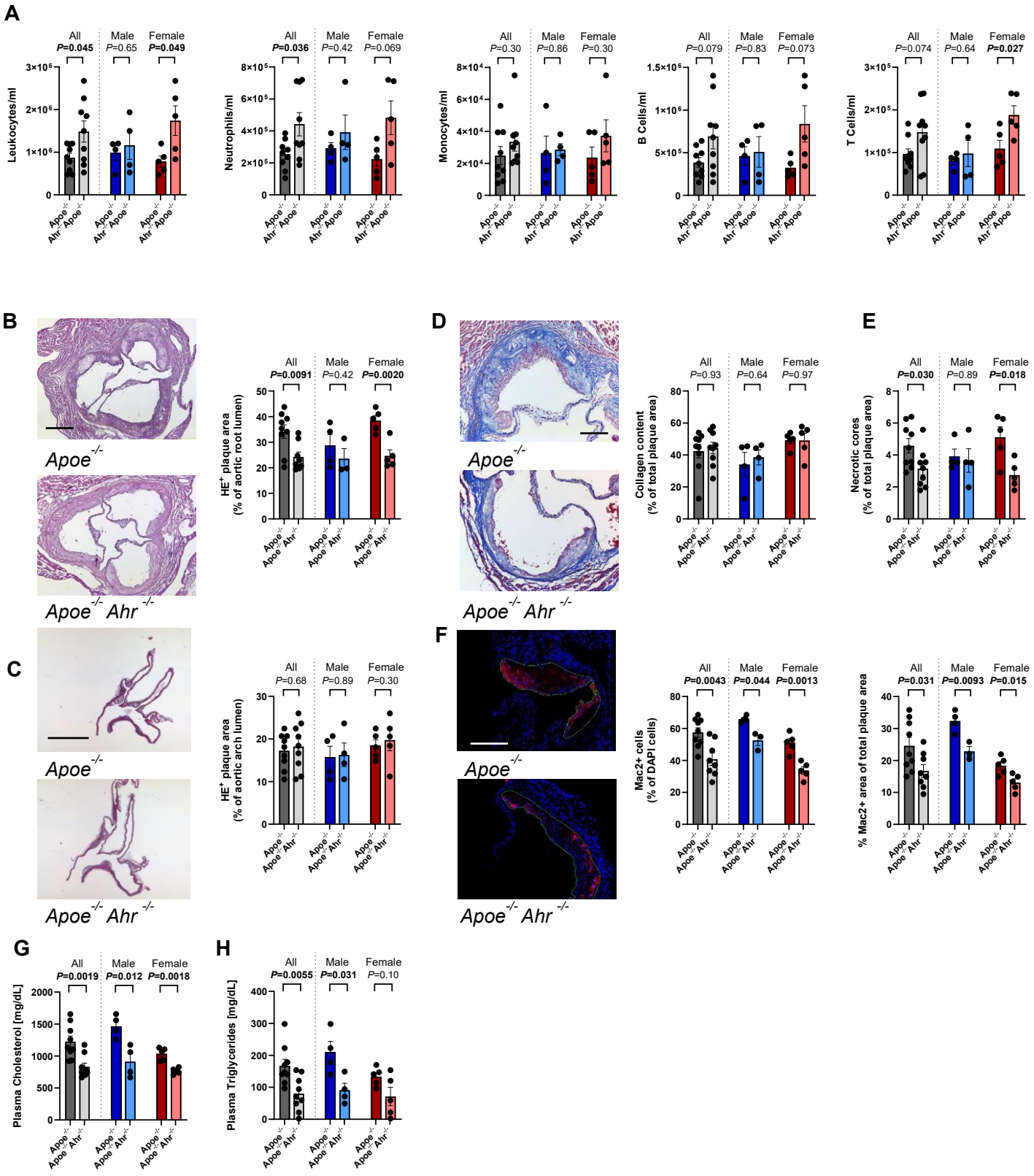

25 **Figure S1. Leukocytosis, though, reduced plaque development in mice lacking *Ahr*.**

26 All results are obtained from *Apoe*$^{-/-}$ and *Apoe*$^{-/-}$ *Ahr*$^{-/-}$ mice after 12 weeks of HFD, with combined
27 sexes, and separated by males and females, from a second independent experiment. **(A)** Blood
28 total leukocyte and leukocyte subset counts. **(B)** Representative pictures of HE-stained aortic roots
29 (scale = 200µm) with quantification of plaque lesion size. **(C)** Representative pictures and

quantification of plaque lesion size in aortic arches (scale = 2mm). **(D)** Representative pictures and quantification of collagen content in aortic roots, and **(E)** quantification of necrotic cores content. **(F)** Representative pictures and quantification of Mac2+ cells. **(G)** Plasma cholesterol levels, and **(H)** plasma triglyceride levels. Combined n=9, male n=4, female n=5, graphs represent mean ± SEM.

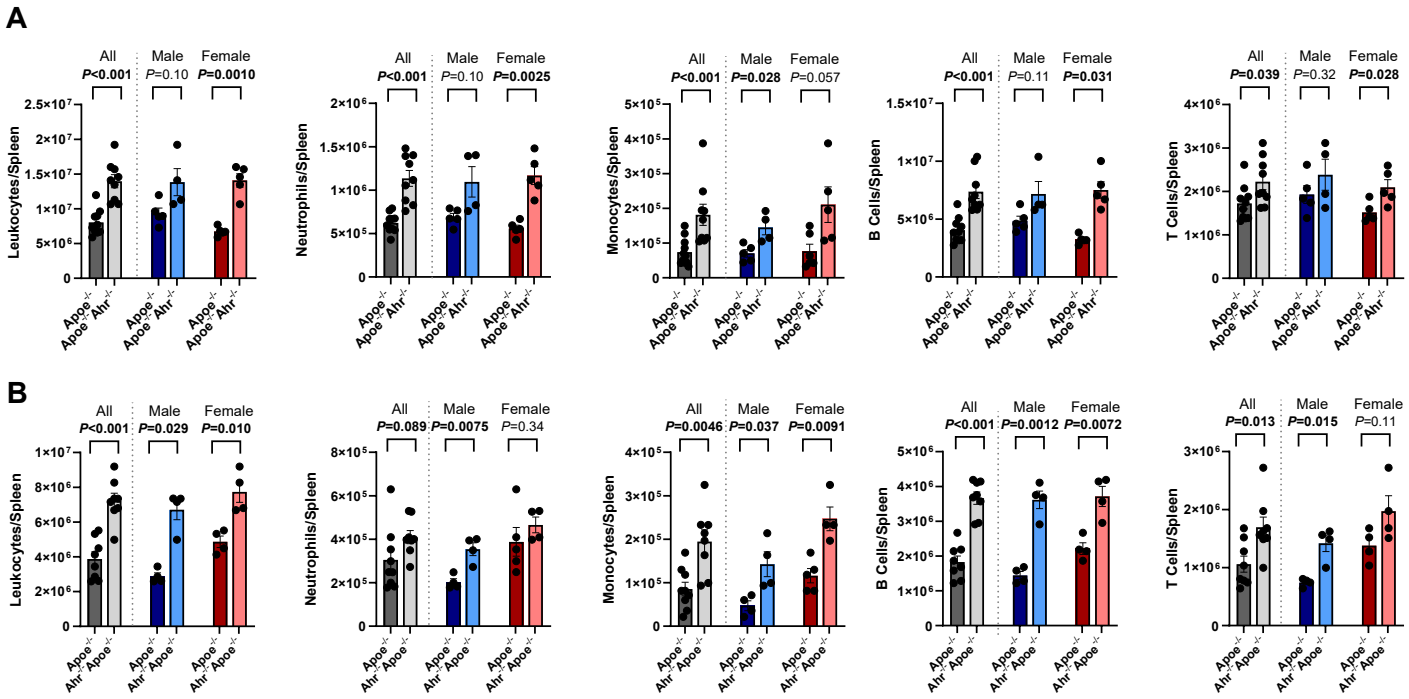

Figure S2. Increased splenic leukocyte counts in mice lacking *Ahr*.

All results are obtained from *Apoe*$^{-/-}$ and *Apoe*$^{-/-}$ *Ahr*$^{-/-}$ mice after 12 weeks of HFD, with combined sexes, and separated by males and females. **(A)** Spleen total leukocyte and leukocyte subset counts, related to Figure 1. **(B)** Spleen total leukocyte and leukocyte subset counts, related to Figure S1. Combined n=9-11, male n=4-6, female n=4-6, graphs represent mean ± SEM.

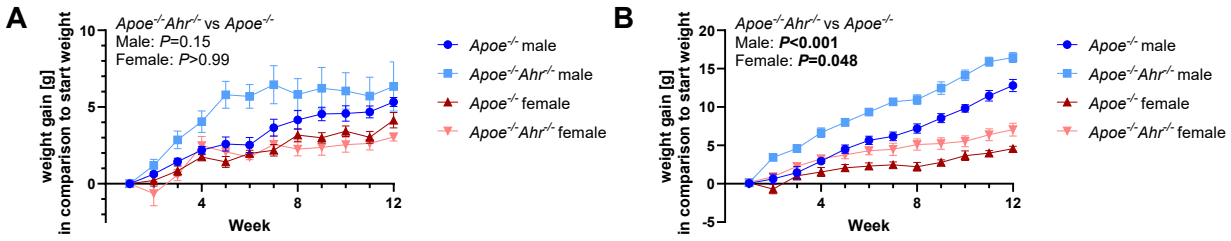

Figure S3. Body weight changes over time.

(A) Weight curve of *Apoe*$^{-/-}$ and *Apoe*$^{-/-}$ *Ahr*$^{-/-}$ mice along the 12-week HFD, male and female separated, related to Figure 1. **(B)** Weight curve of *Apoe*$^{-/-}$ and *Apoe*$^{-/-}$ *Ahr*$^{-/-}$ mice along the 12-week HFD, male and female separated, related to Figure S1. Combined n=9-11, male n=4-6, female n=4-6, graphs represent mean ± SEM.

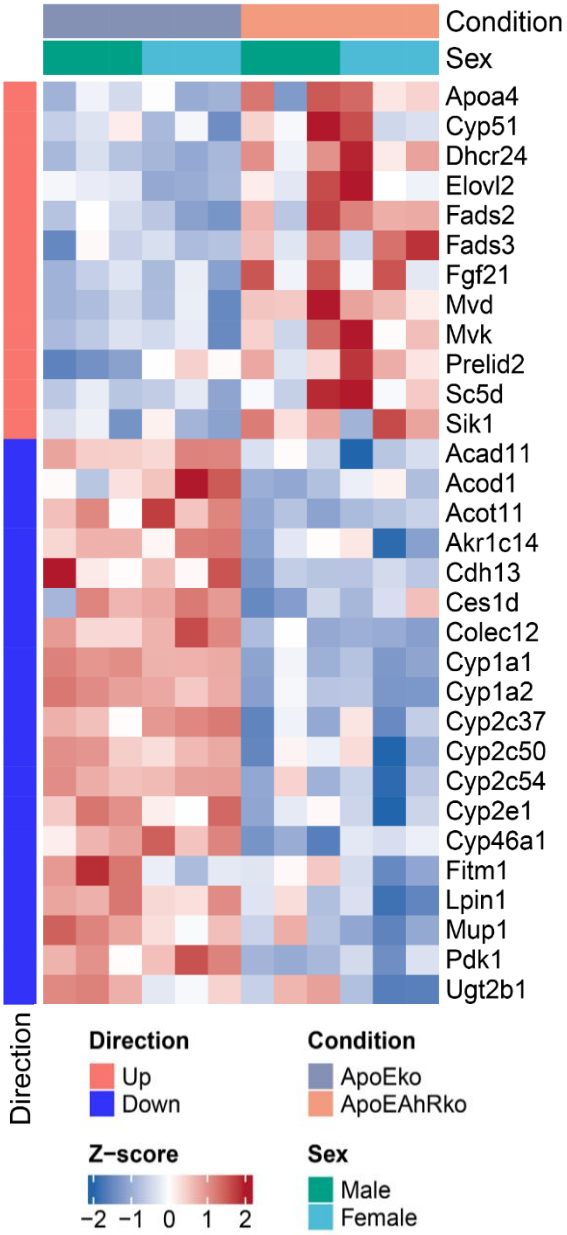

Figure S4. Heatmap of DEGs.

Heatmap of DEGs from the pathways identified in Figure 2F.

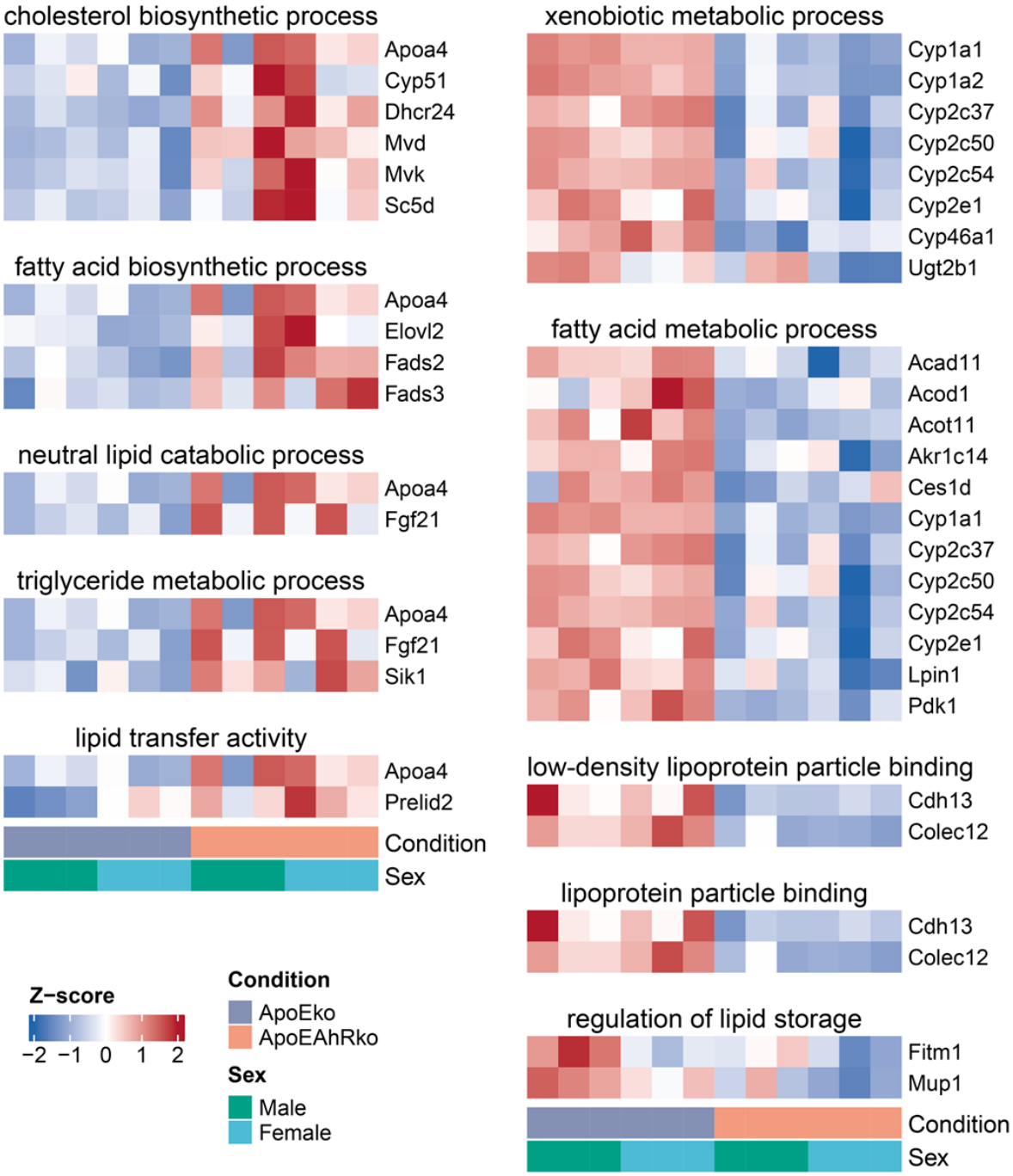

Figure S5. Heatmap of DEGs, sorted by pathway.

Heatmap of DEGs from the pathways identified in Figure 2F, sorted and visualized per individual pathway.

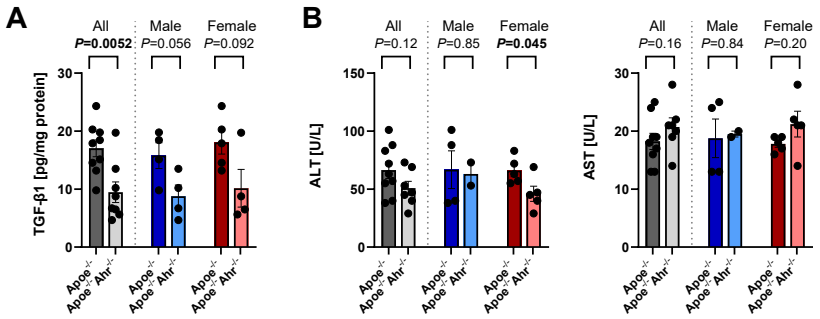

Figure S6. Effects of *Ahr* deficiency on liver damage and fibrosis parameters.

All results are obtained from *Apoe*$^{-/-}$ and *Apoe*$^{-/-}$ *Ahr*$^{-/-}$ mice after 12 weeks of HFD, with combined sexes, and separated by males and females. **(A)** TGF-β1 levels in the liver. **(B)** Plasma ALT and AST levels. Combined n=7-9, male n=2-4, female n=5, graphs represent mean ± SEM.

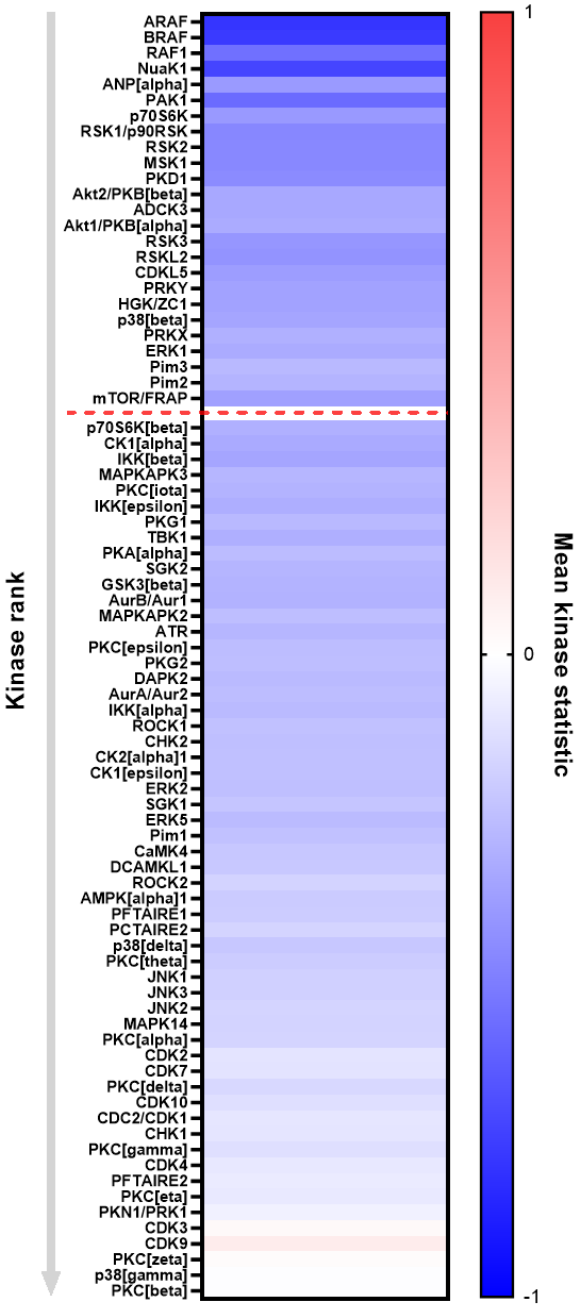

Figure S7. Complete list of liver kinomics results.

Heatmap of all measured STKs in the liver. Blue indicates decreased kinase activity in liver tissues of *Apoe*$^{-/-}$ *Ahr*$^{-/-}$ vs *Apoe*$^{-/-}$ mice. Red indicates increased kinase activity in liver tissues of *Apoe*$^{-/-}$ *Ahr*$^{-/-}$ vs *Apoe*$^{-/-}$ mice. Kinases above the red dotted line are significantly altered, while kinases below this dotted line are not significantly changed based on a mean final score cut-off of 1.2.

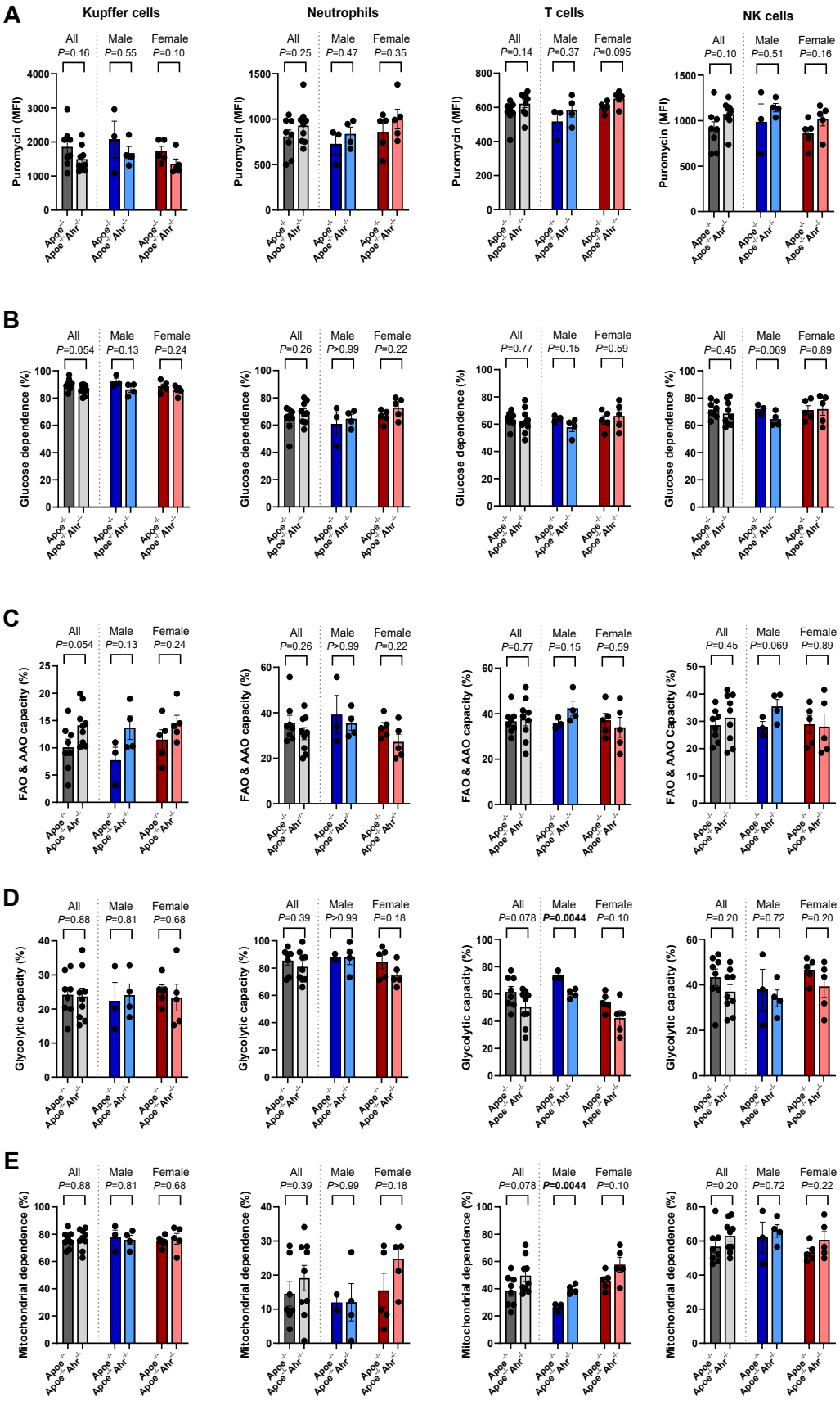

Figure S8. Effects of *Ahr* deficiency on liver immune cell metabolism.

All results are obtained from *Apoe*$^{-/-}$ and *Apoe*$^{-/-}$ *Ahr*$^{-/-}$ mice after 12 weeks of HFD, with combined sexes, and separated by males and females. **(A-E)** Results from SCENITH assay on liver immune

cells, showing **(A)** mean fluorescent intensity (MFI) of puromycin, **(B)** glucose dependency, **(C)** , glycolytic capacity, **(D)** fatty acid oxidation (FAO) & amino acid oxidation (AAO) capacity, and **(E)** mitochondrial dependency. Combined n=7-9, male n=2-4, female n=5, graphs represent mean ± SEM.

Table S1. Selection of signaling pathways focusing on inflammation, lipid metabolism, and glucose metabolism.

Description	ID	Adjusted *P* value
Inflammation		
IL-5 signaling pathway	WP127	4.00E-03
Interleukin-11 signaling pathway	WP2332	5.90E-03
Chemokine signaling pathway	WP3929	9.44E-03
TNF signaling pathway	hsa04668	8.67E-02
IL-2 signaling pathway	WP49	1.86E-01
IL-4 signaling pathway	WP395	4.64E-01
IL6 signaling pathway	WP364	4.90E-04
IL-3 signaling pathway	WP286	7.42E-04
IL-7 signaling pathway	WP205	3.91E-03
IL-17 signaling pathway	WP2112	6.02E-03
IL-1 signaling pathway	WP195	1.59E-02
Lipid Metabolism		
Long-chain fatty acid import into cell	GO:0044539	2.70E-04
Lipid and atherosclerosis	hsa05417	1.59E-03
Positive regulation of lipid metabolic process	GO:0045834	2.08E-03
LDL- influence on CD14 and TLR4	WP5272	3.67E-03
Lipid metabolism pathway	WP3965	5.03E-03
Regulation of fatty acid beta-oxidation	GO:0031998	5.54E-03
Non-alcoholic fatty liver disease	hsa04932	5.99E-03
Negative regulation of lipid transport	GO:0032369	9.01E-03
Glucose Metabolism		

Insulin signaling	WP481	6.59E-09
Response to insulin	GO:0032868	5.54E-03
Insulin secretion involved in cellular response to glucose stimulus	GO:0035773	2.02E-02
Regulation of glucose transmembrane transport	GO:0010827	4.01E-04
Glucose homeostasis	GO:0042593	2.52E-02
Insulin resistance	hsa04931	1.10E-04

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Table S2. Association between rs4410790 and lipid phenotypes in a meta-analysis of the GWAS studies in the Common Metabolic Disease and Knowledge Portal.

Lipid trait	*P* value	beta	std Err	N
Triglycerides	$2.55x10^{-28}$	0.0121	0.0008	3016430
HDL cholesterol	$4.13x10^{-18}$	-0.0087	0.0008	3035390
Triglyceride levels in individuals without type 2 diabetes	$6.24x10^{-18}$	0.0197	0.0023	402944
Triglyceride-to-HDL ratio	$1.55x10^{-14}$	0.0241	0.0025	418488
Non-HDL cholesterol	$5.54x10^{-14}$	0.0116	0.0015	1073110
LDL cholesterol	$1.96x10^{-10}$	0.0076	0.0007	3269200
Serum ApoB	$6.94x10^{-10}$	0.0116	0.0019	436068
Total cholesterol	$9.56x10^{-08}$	0.0055	0.0009	2804400
Serum ApoA	$4.08x10^{-5}$	-0.0078	0.0019	398828
Lauric acid	0.0073	-0.1856	0.069	490
Leptin	0.0304	0.0101	0.0036	136426
Capric acid	0.0655	0.1298	0.0703	475
Alpha-linolenic acid (ALA)	0.1199	0.1100	0.0706	488
Eicosapentaenoic acid (EPA)	0.1820	0.0909	0.068	485
Leptin adj BMI	0.2203	-0.0060	0.004	131812
Docosahexaenoic acid	0.3073	0.0691	0.0676	485
Stearic acid	0.3524	-0.0660	0.0709	492
Arachidonic acid (AA)	0.3696	0.0625	0.0696	487
Docosapentaenoic acid (DPA)	0.4269	0.0536	0.0674	485
Elaidic and trans-vaccenic acids	0.5720	0.0407	0.0721	434
Arachidic acid	0.5939	-0.0367	0.0689	487
Dihomo-gamma-linolenic acid (DGLA)	0.6299	0.0337	0.0698	488

Palmitic acid	0.6815	-0.0286	0.0696	492
Adrenic acid	0.7113	-0.0265	0.0716	485
Myristic acid	0.7370	-0.0239	0.0713	491
Oleic acid	0.7451	0.0226	0.0694	492
Calculated LDL cholesterol	0.8100	-0.0074	0.0309	2917
Linoleic acid	0.8145	0.0165	0.0704	491
Vaccenic acid	0.8499	-0.0135	0.0715	443
Triglyceride levels in individuals with type 2 diabetes	0.8581	0.0009	0.005	21176
Dyslipidemia	0.8724	0.0021	0.0132	56375
Lipoprotein(a)	0.9165	-0.0002	0.0018	349077
Palmitoleic acid	0.9827	-0.0015	0.0669	491

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Major Resources Table

Mouse Models (in vivo studies)

Mouse Model	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Ahr^{tm1Bra}/J (*Ahr*$^{-/-}$)	The Jackson Laboratory	C57BL/6J	Male& Female	Jax strain number 002831
Apoe^{tm1Unc}/J (*Apoe*$^{-/-}$)	The Jackson Laboratory	C57BL/6J	Male& Female	Jax strain number 002052

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration
Mac2	Cedarlane	CL8942AP	2.5µg/mL
Goat anti-rat DL488	Thermo Scientific	SA5-10018	0.66µg/mL
CD45-APC	Invitrogen	47-0451-82	1µg/mL
Cd115-FITC	Invitrogen	53-1152-82	2.5µg/mL
Gr1-V500	Biolegend	108437	1µg/mL
Cd11b-PeCy7	Invitrogen	25-0112-82	2.5µg/mL
B220-eF450	Invitrogen	48-0452-82	1µg/mL
CD3-PerCP	Invitrogen	45-0031-82	1µg/mL
CD4-APC	eBioScience	17-0041-82	1µg/mL
CD8-PE	eBioScience	12-0081-82	1µg/mL
CD45-APC-Cy7	Biolegend	103115	1µg/mL
Cd11b-V500	BD Bioscience	562127	1µg/mL
F4/80-PerCP	Biolegend	157318	1µg/mL
NK-1.1-PB	Biolegend	108721	2.5µg/mL
CD3-PE	Biolegend	100205	1µg/mL

Ly-6G-PE-Cy7	Biolegend	127617	1µg/mL
Anti-Purmycin- AF488- Clone 12D10	Sigma Aldrich	MABE343- AF647	0.25µg/mL

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98 **Other**

Description	Source / Repository
Triglyceride Cobas C Systems	Roche 04657594 190
Cholesterol Generation2 Cobas C systems	Roche 03039773190
TNF Elisa	Thermo Fisher Scientific (88-7324-88)
CCL2 Elisa	Thermo Fisher Scientific (88-7391-88)
Western type diet	SNIFF (TD88137)

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ARRIVE GUIDELINES

The ARRIVE guidelines (https://arriveguidelines.org/) are a checklist of recommendations to improve the reporting of research involving animals. Key elements of the study design should be included below to better enable readers to scrutinize the research adequately, evaluate its methodological rigor, and reproduce the methods or findings.

Study Design

Groups	Sex	Age	Number (prior to experiment)	Number (after termination)	Littermates (Yes/No)	Other description
Group 1 (Figure 1) *Apoe*^{tm1Unc}/J (*Apoe*$^{-/-}$)	M / F	8 weeks at start of experiment	5 M / 6 F	5 M / 6 F	No	-
Group 2 (Figure 1) Ahr^{tm1Bra}/J (*Ahr*$^{-/-}$)	M / F	8 weeks at start of experiment	5 M / 5 F	4 M / 5 F	No	-
Group 3 (Supplemental Figure 1) *Apoe*^{tm1Unc}/J (*Apoe*$^{-/-}$)	M / F	8 weeks at start of experiment	4 M / 5 F	4 M / 5 F	No	-
Group 4 (Supplemental Figure 1) Ahr^{tm1Bra}/J (*Ahr*$^{-/-}$)	M / F	8 weeks at start of experiment	4 M / 5 F	4 M / 5 F	No	-

Sample Size: Based on previous atherosclerosis studies conducted by our working group and other international working groups, the biologically relevant difference (effect size) can be set at a minimum of 45% compared to the control group. To obtain meaningful data sets, a group size of n1=n2=n3=14 animals is required for three groups to be compared in a one-factor variance analysis without repeated measurements with df=2 degrees of freedom, an effect size (contrast) of 45% and a standard deviation of 35%. However, due to limitations with the animal breeding, the experimental groups remained smaller (9-11 animals). Due to the fact that significant results could already be obtained, these smaller groups were sufficient in this case.

Inclusion Criteria: Besides the desired genotype and general well-being, no specific inclusion criteria were used for the animal experiments.

Exclusion Criteria: No specific exclusion criteria were defined. Only statistical outliers were removed from the data-set as described in the method section.

Randomization: No randomization was used, but group 1 and 2 as well as group 3 and 4 were maintained in identical animal/cage conditions to minimize potential confounders.

Blinding: Only the project leader was aware of the group allocation. All analysis were performed blindly.