

SUPPLEMENTARY DATA

**Supplementary Table 1.** Gene expression in VF isolated from WT males (n=5), WT females (n=4), *Aldh1a1*<sup>-/-</sup> males and females (both, n=4) on a HF diet (180d) was examined by TaqMan quantitative PCR using 18S as normalization control. Significance was examined between males and females in each genetic group (columns ‘WT’ and ‘KO’) as well as between WT males/KO males, and WT females/KO females (Column ‘P value’) by nonparametric Mann-Whitney U test and Kruskal-Wallis rank test (for *Ucp1*).

Gene	WT			KO			P value	
	Mean		P Value	Mean		P Value	WT/KO	
	M	F	M/F	M	F	M/F	M/M	F/F
<i>Pref1</i>	1.73E-08	3.03E-08	N.S.	1.07E-05	3.40E-08	N.S.	N.S.	N.S.
<i>PPAR<math>\gamma</math></i>	1.42E-05	1.40E-05	N.S.	1.82E-05	1.52E-05	N.S.	N.S.	N.S.
<i>Fabp4</i>	0.00203	0.00211	N.S.	0.00416	0.00147	N.S.	N.S.	N.S.
<i>RXR<math>\alpha</math></i>	1.93E-05	1.57E-05	N.S.	1.83E-05	1.28E-05	N.S.	N.S.	N.S.
<i>RXR<math>\beta</math></i>	2.97E-06	3.04E-06	N.S.	2.69E-06	2.03E-06	N.S.	N.S.	N.S.
<i>RXR<math>\gamma</math></i>	6.55E-08	1.24E-07	N.S.	1.41E-07	1.09E-07	N.S.	N.S.	N.S.
<i>Atgl</i>	7.10E-05	8.09E-05	N.S.	9.34E-05	5.79E-05	N.S.	N.S.	N.S.
<i>Leptin</i>	0.00061	0.00062	N.S.	0.00051	0.00031	N.S.	N.S.	0.0098
<i>Ucp1</i>	5.49E-06	3.89E-06	N.S.	1.04E-06	3.74E-05	0.001	N.S.	0.001
<i>Ucp2</i>	1.28E-04	1.03E-04	N.S.	8.73E-05	5.78E-05	N.S.	N.S.	N.S.
<i>Ucp3</i>	2.25E-06	1.80E-06	N.S.	4.95E-06	5.24E-06	N.S.	0.0050	0.0004
<i>Prdm16</i>	1.43E-06	9.86E-07	N.S.	1.13E-06	9.10E-07	N.S.	N.S.	N.S.
<i>Pgcl<math>\alpha</math></i>	5.87E-07	3.87E-07	N.S.	5.01E-07	6.89E-07	N.S.	N.S.	0.0055
<i>Dio2</i>	1.36E-07	1.35E-07	N.S.	6.95E-08	6.34E-07	N.S.	N.S.	N.S.
<i>Fndc5</i>								
<i>CD36</i>	2.31E-03	2.09E-03	N.S.	2.50E-03	2.22E-03	N.S.	N.S.	N.S.
FAS	7.01E-05	4.67E-05	N.S.	1.46E-03	2.89E-04	N.S.	0.0393	0.0522
ACC1	1.67E-05	1.40E-05	N.S.	2.75E-05	7.35E-05	N.S.	0.0019	0.0349
SCD1	1.11E-03	1.24E-03	N.S.	4.00E-03	4.58E-03	N.S.	0.0148	0.0086

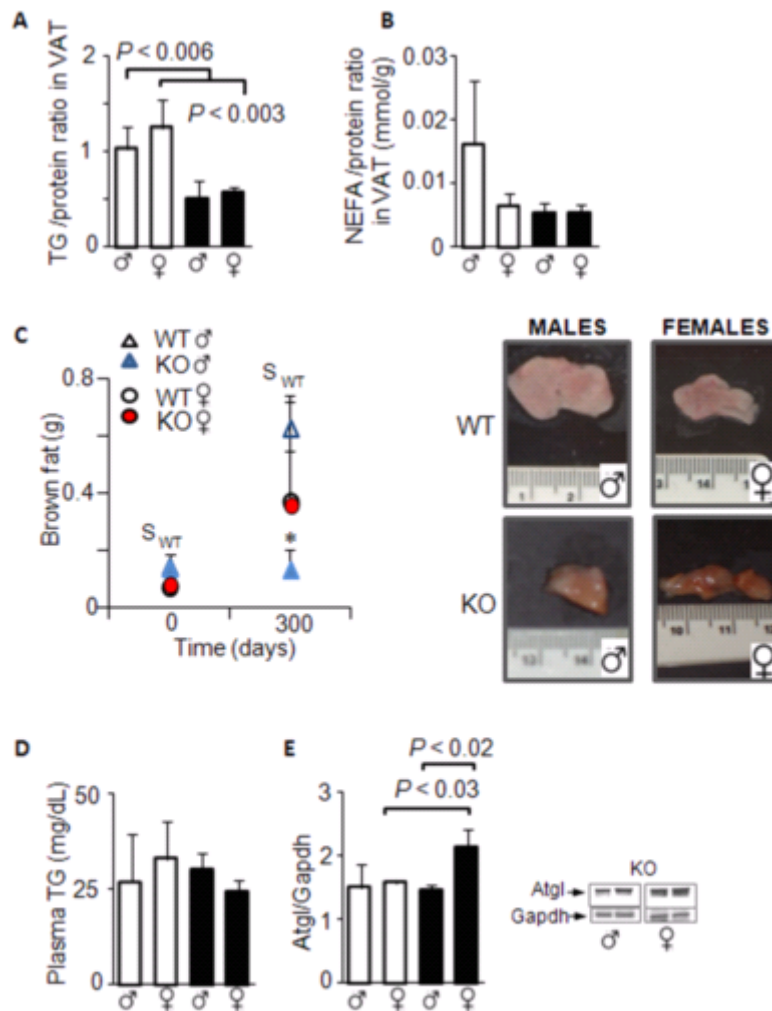
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**Supplementary Figure 1. (A&B)** Triglyceride (TG) and non-esterified fatty acids (NEFA) were measured from homogenates of VF in mice on a HF diet (300d) and normalized per protein content (n=4 per group). Throughout this legend: Data are shown as mean±SD. P, Mann-Whitney U test.

**(C)** Brown fat weight in WT and *Aldh1a1*<sup>-/-</sup> mice on a regular (n=5 per group) and HF diet (n=7 per WT sex group, n=4 KO males, n=6 KO females). \*, significant difference between brown fat mass in WT and KO males on a HF diet. S<sub>WT</sub> significant difference between WT males and females. Insert shows representative examples of brown fat from all groups on a HF diet. Our previous study indicated differences in *Ucp1* expression in this fat (Ziouzenkova et.al, Nat Med, 2007).

**(D)** TG levels in plasma of WT and *Aldh1a1*<sup>-/-</sup> mice on a HF diet (180d). n=3 per group. TG levels were not statistically different among groups.

**(E)** Western blot analysis of Atgl protein levels in VF isolated from WT (n=3 per sex group) and *Aldh1a1*<sup>-/-</sup> mice (n=4 per sex group) on a HF diet (300d). Insert shows a representative example of Atgl levels in two male and female *Aldh1a1*<sup>-/-</sup> mice.



SUPPLEMENTARY DATA

**Supplementary Figure 2.** Validation of responsiveness of RARE (Tg(RARE-Hspa1b/lacZ)12Jrt/J to nanomolar RA concentrations and  $\beta$ -galactosidase primer.

(A&B) Representative staining of  $\beta$ -galactosidase protein expression (brown staining) in hepatic and adipose tissues from RARE-lacZ mice injected with vehicle or RA (B), but no staining in C57BL/6J (WT) mice (A). WT or transgenic male mice (12-15 weeks old, regular chow) expressing  $\beta$ -galactosidase (lacZ) gene under the control of the RARE (Tg (RARE-Hspa1b/lacZ) 12Jrt/J were injected 3 times (every 48 hours) with 1mL PBS with or without RA (500nM). Immediately after third injection mice were harvested, their hepatic and visceral adipose tissue were embedded in paraffin and stained with antibody to  $\beta$ -galactosidase (Abcam) according to manufacturer instructions. Brown staining indicates 10x magnification.(C) mRNA  $\beta$ -galactosidase expression measured using TaqMan assay in livers isolated from WT and RARE-lacZ mice. (12-15 weeks old, regular chow, n=3). Throughout this legend: P was determined by 2-way ANOVA.

