Supplemental Figures

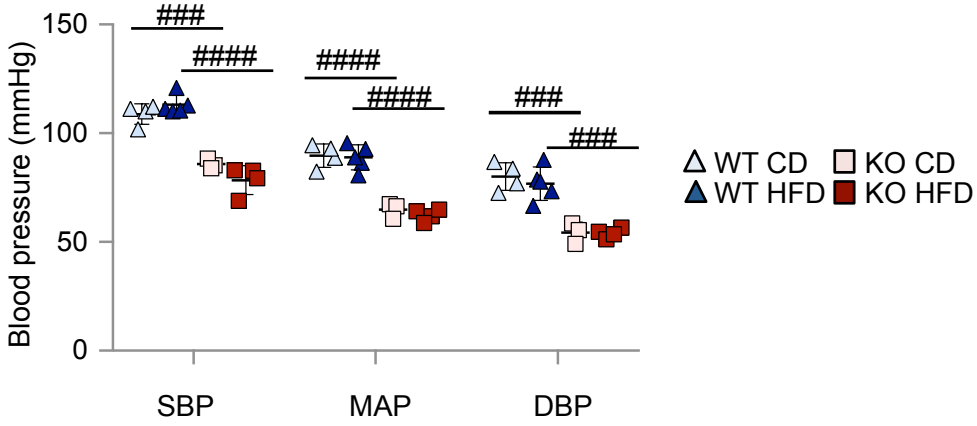

Figure S1, related to Figures 1 and 3. Effects of 10-week chow diet (CD) or high-fat diet (HFD) feeding on systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) of WT and ASKO mice. CD and HFD-fed ASKO mice had lower BP than diet-matched WT mice. HFD feeding did not increase BP in both genotypes. Data presented as mean±SD; n=3-5/group; $^{###}P < 0.001$, $^{####}P < 0.0001$ by one-way ANOVA followed by Tukey's post hoc test.

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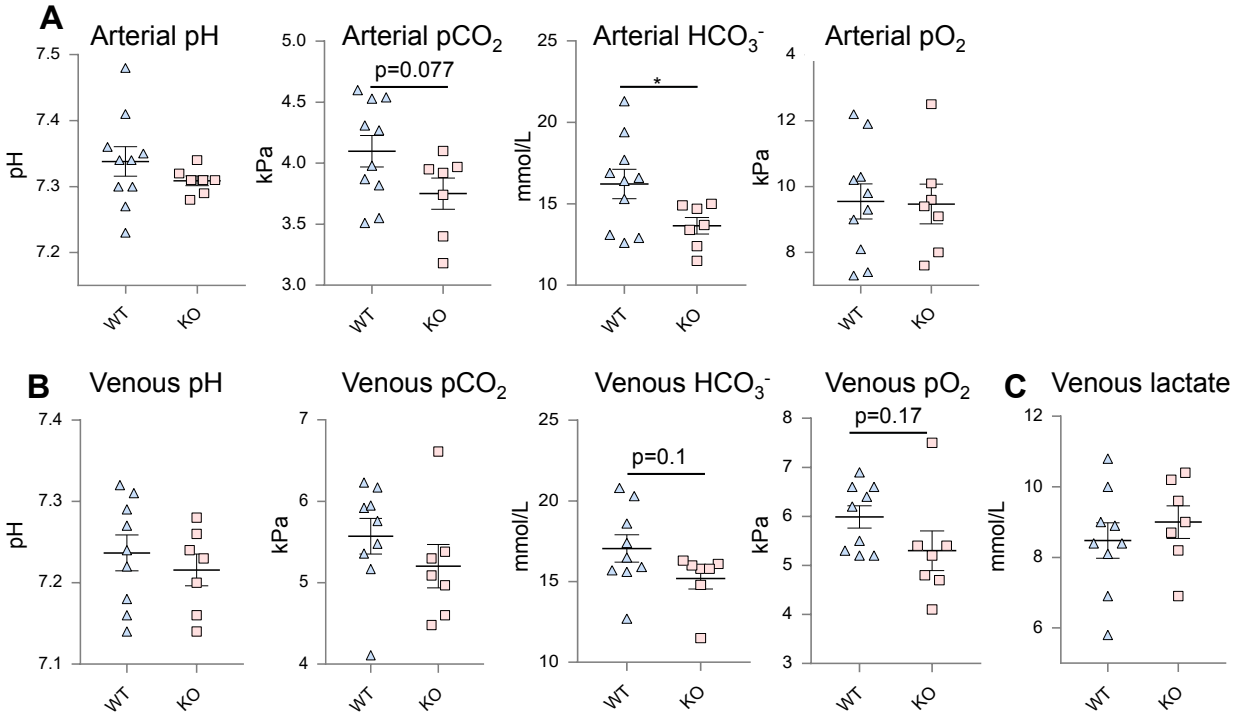


Figure S2, related to Figure 1. Blood gases of WT and ASKO mice at the age of 5-9 months maintained on chow diet (CD) at 22°C. Lower HCO_3^- and a trend towards lower pCO_2 and pH of ASKO mice than WT mice suggested that ASKO mice had metabolic acidosis. (**A**) Arterial blood was sampled from ventral tail artery. (**B and C**) Venous blood was sampled from superficial temporal vein. All data presented as mean±SEM; n=7-10/group; **P*<0.05 WT versus ASKO by Welch's unpaired t test.

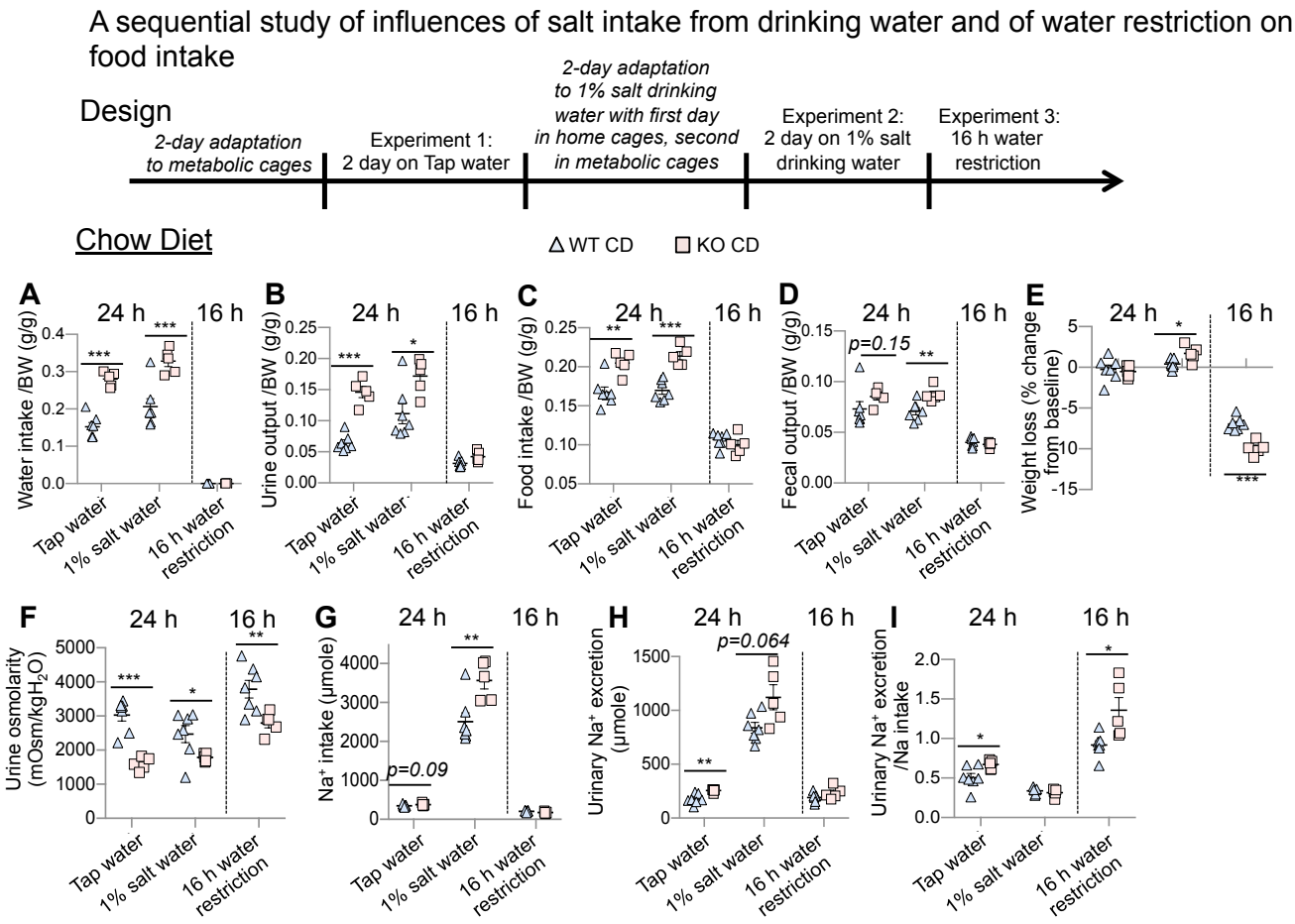
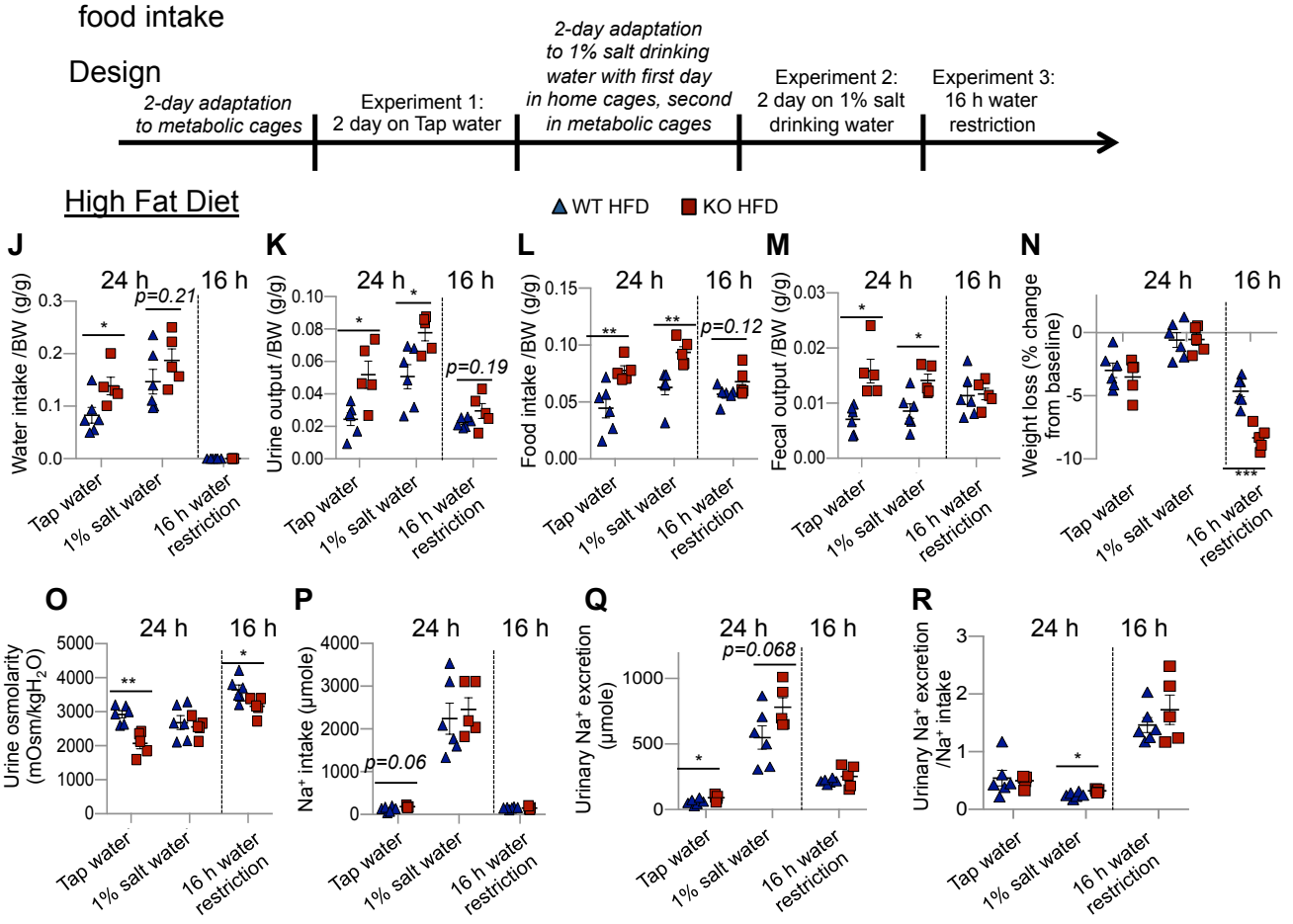


Figure S3, related to Figure 1. 1% NaCl in drinking water increased fluid and food intake in both genotypes with chow diet (CD) and high-fat diet (HFD) feeding, whereas 16-hour water restriction reduced food consumption in both genotypes and caused a greater weight loss in ASKO mice at 22°C. (**A-I**) In CD feeding condition, (**A**) water intake, (**B**) urine output, (**C**) food intake, (**D**) fecal output, (**E**) weight loss, (**F**) urine osmolarity, (**G**) Na^+ intake, (**H**) urinary Na^+ excretion, and (**I**) urinary Na^+ excretion/ Na^+ intake of CD-fed 2-3.5 month-old mice on tap water (in normal condition) or on 1% NaCl drinking water or challenged with 16-hour water restriction. These experiments were performed in the same CD-fed mice. n=5-7/group. mean±SEM. S3J-S3R in next page

A sequential study of influences of salt intake from drinking water and of water restriction on food intake



(**J-R**) In HFD feeding condition, (**J**) water intake, (**K**) urine output, (**L**) food intake, (**M**) fecal output, (**N**) weight loss, (**O**) urine osmolarity, (**P**) Na^+ intake, (**Q**) urinary Na^+ excretion, and (**R**) urinary Na^+ excretion/ Na^+ intake of 9-week HFD-fed mice on tap water (normal condition) or on 1% NaCl drinking water or challenged with 16-hour water restriction. These experiments were performed in the same HFD-fed mice. n=5-6/group. mean±SEM. In all data, $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$, WT versus ASKO on tap water or on 1%NaCl drinking water or challenged with 16-hour water restriction by Welch's unpaired t test. Continued S3

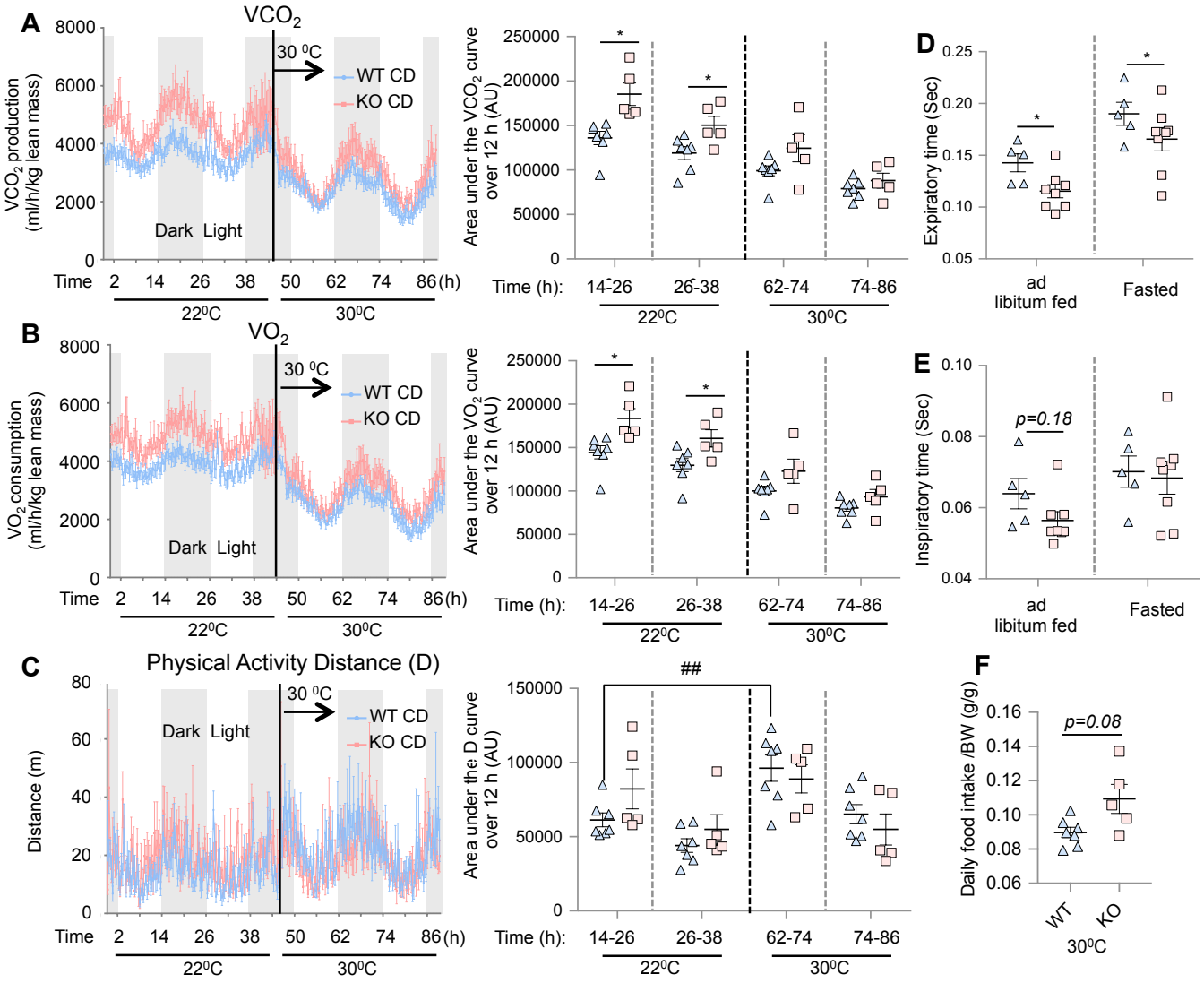


Figure S4, related to Figure 2. Respiratory parameters of mice fed chow diet (CD). ASKO mice had greater CO_2 production, O_2 consumption, and shorter expiratory time, but were not more active than WT mice at 22°C. 12-15 hours of fasting at 22°C eliminated and thermoneutral housing alleviated these changes. (**A-C**) Temporal changes of (**A**) CO_2 production (**B**) O_2 consumption (**C**) physical activity of 4-month-old mice at 22°C and 30°C. n=5-7/group. mean±SEM (**D-E**) (**D**) Expiratory time (**E**) inspiratory time per breath in 3 month-old ad libitum fed mice and after 12-15 hours of fasting at 22°C. n=5-8/group. mean±SEM. (**F**) Food intake of 4-month-old mice at 30°C. n=5-7/group. mean±SEM; In all data, * $p<0.05$ WT versus ASKO; $^{\#\#}$ $p< 0.01$, WT at 22°C versus WT at 30°C by Welch's unpaired t test.

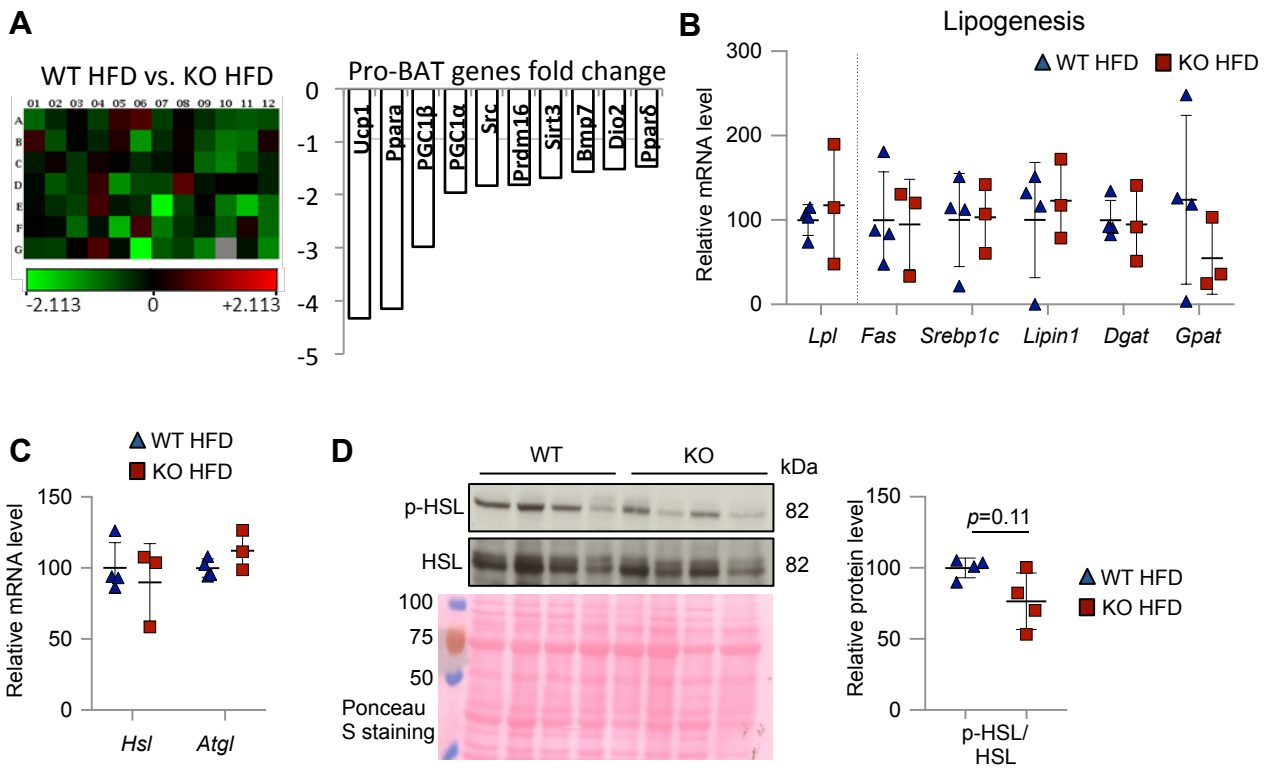
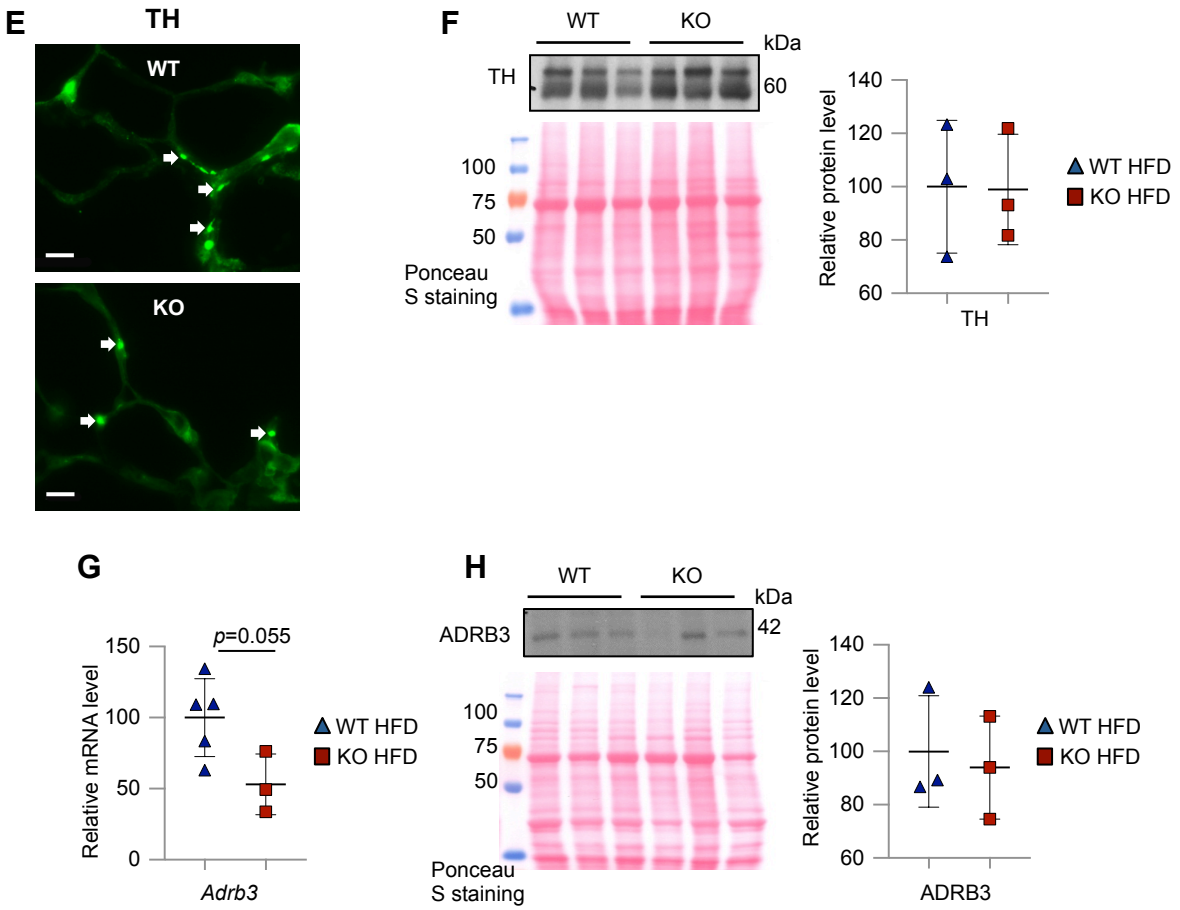


Figure S5, related to Figure 4. Perirenal fat of ASKO mice had reduced thermogenic reprogramming in response to 14-week high-fat diet (HFD) feeding, which was not related to a reduced sympathetic input. (**A**) Heat map of genes involved in adipocyte metabolism and genes differently expressed between the perirenal fat of HFD-fed WT and ASKO mice. (Pooled RNA from 3 mice/group). (**B and C**) mRNA levels of (**B**) lipogenesis genes (**C**) hormone-sensitive lipase (*Hsl*) and adipocyte triglyceride lipase (*Atgl*) in perirenal fat of HFD-fed mice. n=3-4/group. mean±SD. (**D**) Western blot autoradiographic images (left panel) and relative protein levels of p-HSL/HSL (right panel) in perirenal fat of HFD-fed WT and ASKO mice. Ponceau S stained blot is shown for loading controls. n=4/group. mean±SD. S5E-S5H in next page



(**E**) representative digital microphotograph of fluorochrome-labelled tyrosine hydroxylase (TH) protein in perirenal fat of HFD-fed WT and ASKO mice. Scale bar 10 μm. n=1/group. Though detectable, immunostaining showed TH protein in the collected perirenal fat specimen was sparsely present, so was it in perirenal fat of chow diet-fed WT mice, compared with that in brown adipose tissue of HFD-fed WT mice. This does not permit a reliable quantification analysis. (**F**) Western blot analysis of TH in mesenteric fat of HFD-fed WT and ASKO mice (left panel). Protein levels for TH were normalized to Ponceaus S staining (right panel). n=3/group. mean±SD. (**G**) mRNA levels of beta3-adrenergic receptor (*Adrb3*) in perirenal fat of HFD-fed WT and ASKO mice. n=3-5/group. mean±SD. (**H**) Western blot analysis of ADRB 3 in perirenal fat of HFD-fed WT and ASKO mice (left panel). Protein levels for ADRB3 were normalized to Ponceau S staining (right panel). n=3/group. mean±SD. All data analyzed by Welch's unpaired t test. Continued S5

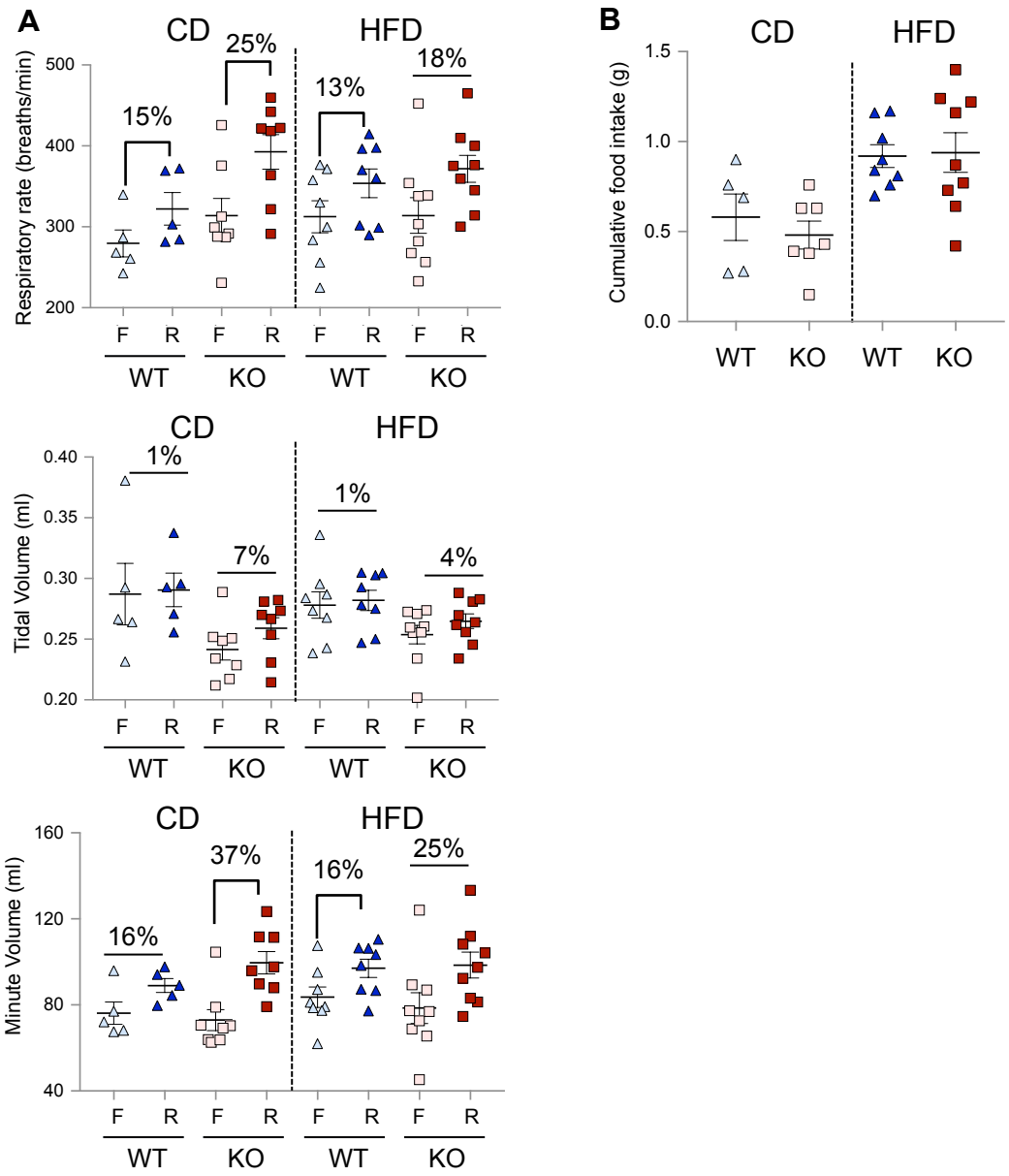


Figure S6, related to Figure 5. Respiratory burden of chow diet (CD)-fed ASKO mice decreased with the switch to high-fat diet (HFD) feeding. (**A**) Respiratory rate, tidal volume and minute volume of mice after 12-15 hours of fasting and in response to 1.5-hour refeeding of CD or HFD (n=5-9/group). F: fasting, R: refeeding. (**B**) 1.5-hour cumulative food intake during refeeding after 12-15 hours of fasting (n=5-9/group). Experiments performed in the same mice, first with CD feeding at the age of 2.5 months, and 1 month later with HFD feeding, but 3 more WT and 1 more 2.5-3 month old ASKO mice were recruited for the HFD experiment. The percentage changes of average mean of each respiratory parameter induced by refeeding are shown on the graphs. All data presented as mean±SEM. Data of 1.5-hour cumulative food intake after refeeding of CD are also shown in Figure 1F.

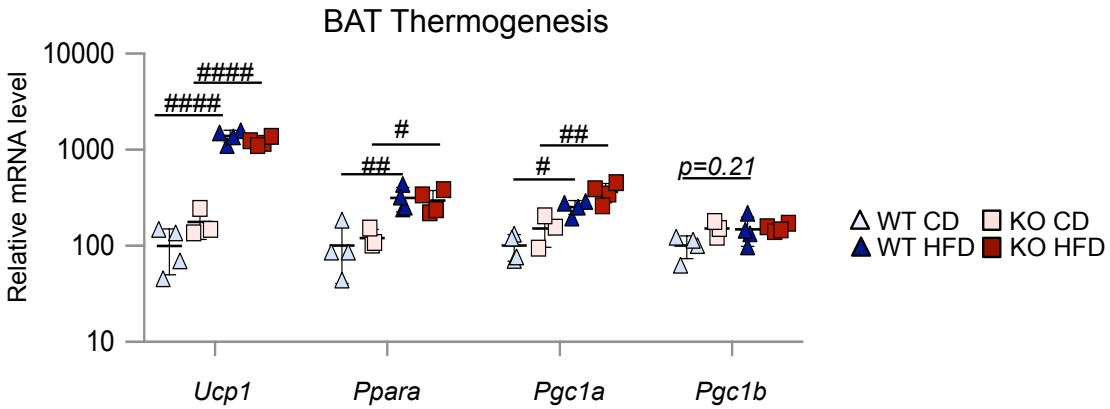


Figure S7, related to Figure 5. Chronic high-fat diet (HFD) feeding activated brown adipose tissue (BAT) thermogenic reprogramming in both WT and ASKO mice. Data presented as mean±SD; n=4 per group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#\#}P < 0.0001$ by one-way ANOVA followed by Tukey's post hoc test.

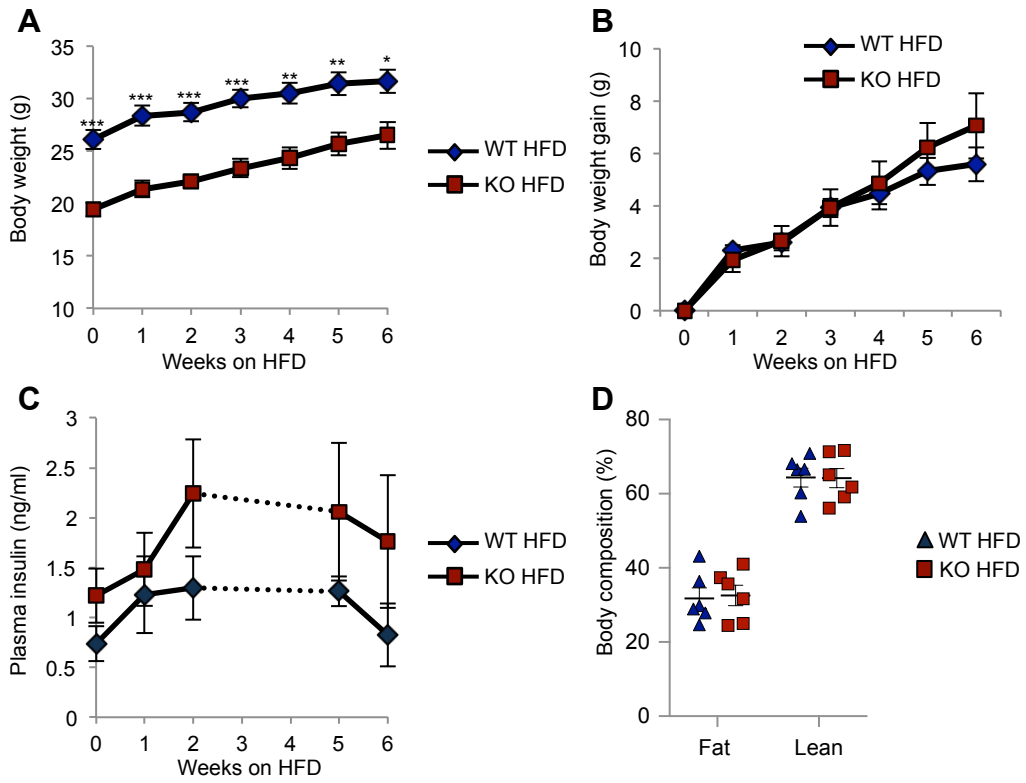


Figure S8, related to Figure 6. Differential hyperinsulinemic responses of WT and ASKO mice to high-fat diet (HFD). ASKO mice tended to have higher insulin levels than WT mice already after 1 week on HFD. (**A and B**) BW and BW gain tracked weekly from the beginning of HFD feeding in 9 week-old mice. (**C**) Corresponding plasma insulin levels. Blood was collected from tail during the dark phase. (**D**) Body composition in week 6 of HFD feeding. All data presented as mean±SEM; n=6/group; **P* < 0.05, ***P* < 0.01, ****P* < 0.001, HFD-fed WT versus HFD-fed ASKO by Welch's unpaired t test.

Supplemental Tables

Table S1. The composition of (A) the chow diet and of (B) the high fat diet used in the experiment. Information as provided by the producers.

(**A**) Chow diet (Kliba 3436 Extrudate)

Major nutrients			Vitamins	
Dry matter	(88.0%)		• Vitamin A	14000IU/kg
• Crude protein	18.5%		• Vitamin D3	1000IU/kg
• Crude fat	4.5%		• Vitamin E	110mg/kg
• Crude fiber	4.5%		• Vitamin K3	2mg/kg
• Crude ash	6.5%		• Vitamin B1	30mg/kg
• Nitrogen Free Extract	54.0%		• Vitamin B2	20mg/kg
			• Vitamin B6	14mg/kg
Amino acids			• Vitamin B12	0.05mg/kg
• Arginine	1.10%		• Nicotinic acid	70mg/kg
• Lysine	1.00%		• Pantothenic acid	33mg/kg
• Methionine	0.39%		• Folic acid	2mg/kg
• Methionine+cystine	0.76%		• Biotin	0.22mg/kg
• Tryptophan	0.20%		• Choline	2000mg/kg
• Threonine	0.65%		• Vitamin C	40mg/kg
Major mineral elements			Trace elements	
• Calcium	1.05%		• Iron	250mg/kg
• Phosphorus	0.80%		• Zinc	60mg/kg
• Magnesium	0.20%		• Copper	14mg/kg
• Sodium	0.20%		• Iodine	1mg/kg
• Potassium	0.78%		• Magnesium	60mg/kg
• Chloride	0.36%		• Selenium	0.3mg/kg
Energy content	***Calculated values***			
Protein, *% energy*	22.40			
Carbohydrate, *% energy*	65.35			
Fat, *% energy*	12.25			
Metabolizable energy	13.1 MJ/kg			
Gross energy	16.1MJ/kg			

Table S1B in next page

(**B**) High fat diet (Research diet D12492)

Ingredient	
Casein, 30 Mesh	200gm
L-Cystine	3gm
Corn Starch	0gm
Maltoextrin 10	125gm
Sucrose	68.8gm
Cellulose, BW200	50gm
Soybean Oil	25gm
Lard	245gm
Mineral Mix S10026	10gm
DiCalcium Phosphate	13gm
Calcium Carbonate	5.5gm
Potassium citrate, $1H_20$	16.5gm
Vitamin Mix V10001	10gm
Choline Bitartrate	2gm
FD and C Blue Dye #1	0.05gm
Energy content	***Calculated values***
Protein, *% energy*	20
Carbohydrate, *% energy*	20
Fat, *% energy*	60
Metabolizable energy	21.6 MJ/kg

continued Table S1

Table S2: Primer sequences used in quantitative RT-PCR experiments.

Genes	Forward	Reverse
Cebpα	GACAAGAACAGCAACGAGTA	AGCTGGCGGAAGATGC
Pparγ	TTCACAAGAGCTGACCCAAT	AAGCCTGATGCTTTATCCCC
Fas	AAGCGGTCTGGAAAGCTGAA	TCTGAACCACTCACACCCAC
Srebp1	AGCCACACTTCATCAAGGCA	GTGGATGGGCAGTTTGTCTG
Ucp1	TTCATCAACTCTCTGCCAGG	GTACAATCCACTGTCTGTCTG
Pparα	AGAGAATCCACGAAGCCTAC	GTGGAAGAATCGGACCTCTG
Pgc1α	CGAGGACACGAGGAAAGGAA	CACTGGCCTGAATCTGTGGA
Pgc1β	CACGGTTTTATCACCTTCCG	ATGGCTTCGTACTTGCTTTT
Mcad	AGCCAATGATGTGTGCTTAC	GGCTTTACTAGCGGGTACTT
Ecah	GTCAATGCCATCAGTCCAAC	GGCTTCTGGTATCGCTGTAT
Hcad	TTCGTGACCAGGCAATTCTT	CCCTTCTTGGATTTTGCCAG
Acaa	GGGAATATTTCTTCCCGCCT	GTTGTCACAGGCACAATCTC
Adrb3	GGTAATCATAGCCATCGCC	TAGCAGTTACACAGAGCACG
Atgl	CAACGCCACTCACATCTACG	CAGCAGGCAGGGTCTTTAGT
Hsl	ATGGCATCAACCACTGTGAG	GCCAGGCTGTTGAGTACCTT
Tfam	CCCCTCGTCTATCAGTCTTG	TTTGCATCTGGGTGTTTAGC
Nrf1	TGTAGCCACGTACACTGAGC	GAGGCCGTTTCCGTTTCTTC
Nrf2	GGAAGTGTCAAACAGAACGG	GGCTTTTTGATGACCAGGAC
Gapdh	CCATCACCATCTTCCAGGAG	TCCATGGTGGTGAAGACAC

Table S3: Antibodies origin and dilution used.

Primary Ab	Manufacturer catalog #	Host species	Dilution for IB	Dilution for IF
Hormone Sensitive Lipase (HSL)	Cell Signaling #4107	Rabbit	1/250	
phospho-Hormone Sensitive Lipase (pHSL)	Cell Signaling #4126	Rabbit	1/500	
Adipose triglyceride lipase (ATGL)	Cell Signaling #2138	Rabbit	1/500	
Tyrosine hydroxylase (TH)	Sigma-Aldrich #T1299	Mouse	1/1000	1/200
Adrenergic receptor β3 (ADRB3)	Novusbio #NBP1-00716	Rabbit	1/250	
β-Actin	Cell Signaling #3700	Mouse	1/2000	

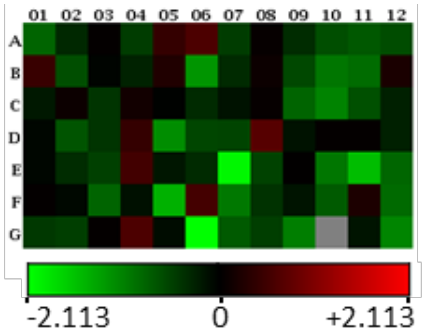
Secondary Ab	Manufacturer catalog #	Host species	Dilution for IB	Dilution for IF
Rabbit IgG, HRP	GE Healthcare Europe GmbH #NA934	Donkey	1/25000	
Mouse IgG, HRP	Jackson ImmunoResearch Laboratories #115-035-166	Goat	1/50000	
Mouse, AlexaFluor 488	Invitrogen #A-11029	Goat		1/300

IF: Immunofluorescence; HRP: Horseradish peroxidase

Table S4: Real-time PCR array of genes involved in adipogenesis and adipocyte function.

Well	Gene Symbol	Fold Regulation (2^(-ΔΔ Ct))
A01	Acacb	-1.78
A02	Adipoq	-1.26
A03	Adrb2	1.02
A04	Agt	-1.41
A05	Angpt2	1.35
A06	Axin1	1.54
A07	Bmp2	-1.41
A08	Bmp4	1.04
A09	Bmp7	-1.29
A10	Ccnd1	-1.56
A11	Cdk4	-1.66
A12	Cdkn1a	-1.54
B01	Cdkn1b	1.38
B02	Cebpa	-1.57
B03	Cebpb	-1.02
B04	Cebpd	-1.22
B05	Cfd	1.2
B06	Creb1	-2.37
B07	Ddit3	-1.28
B08	Dio2	1.07
B09	Dkk1	-1.51
B10	Dlk1	-1.96
B11	E2f1	-1.86
B12	Egr2	1.15
C01	Fabp4	-1.14
C02	Fasn	1.08
C03	Fgf1	-1.34
C04	Fgf10	1.12
C05	Fgf2	-1.02
C06	Fos	-1.28
C07	Foxc2	-1.1
C08	Gata2	1.03
C09	Gata3	-1.78
C10	Hes1	-2.13
C11	Insr	-1.58
C12	Irs1	-1.2
D01	Irs2	-1.04
D02	Jun	-1.62
D03	Klf15	-1.34
D04	Klf2	1.34
D05	Klf3	-2.25
D06	Klf4	-1.5
D07	Lep	-1.48
D08	Lipe	1.63
D09	Lmna	-1.11
D10	Lpl	1.03

D11	Lrp5	1.02
D12	Mapk14	-1.2



E01	Ncoa2	-1.03
E02	Ncor1	-1.29
E03	Ncor2	-1.45
E04	Nr0b2	1.46
E05	Nr1h3	-1.14
E06	Nrf1	-1.27
E07	Ppara	-4.15
E08	Ppard	-1.47
E09	Pparg	1.01
E10	Ppargc1a	-1.96
E11	Ppargc1b	-2.97
E12	Rb1	-1.81
F01	Retn	1.03
F02	Runx1t1	-1.05
F03	Rxra	-1.78
F04	Sfrp1	-1.1
F05	Sfrp5	-1.79
F06	Shh	1.47
F07	Sirt1	-1.99
F08	Sirt2	-1.33
F09	Sirt3	-1.12
F10	Slc2a4	-1.69
F11	Src	1.17
F12	Srebf1	-1.83
G01	Stat5a	-1.38
G02	Taz	-1.43
G03	Tcf7l2	1.03
G04	Tsc22d3	1.56
G05	Twist1	-1.07
G06	Ucp1	-4.33
G07	Vdr	-1.67
G08	Wnt1	-1.43
G09	Wnt10b	-1.96
G10	Wnt3a	-1.29
G11	Wnt5a	-1.13
G12	Wnt5b	-1.95
H01	Actb	-1.17
H02	B2m	1.1
H03	Hprt1	1.12
H04	Ldha	1.18
H05	Rplp1	-1.24