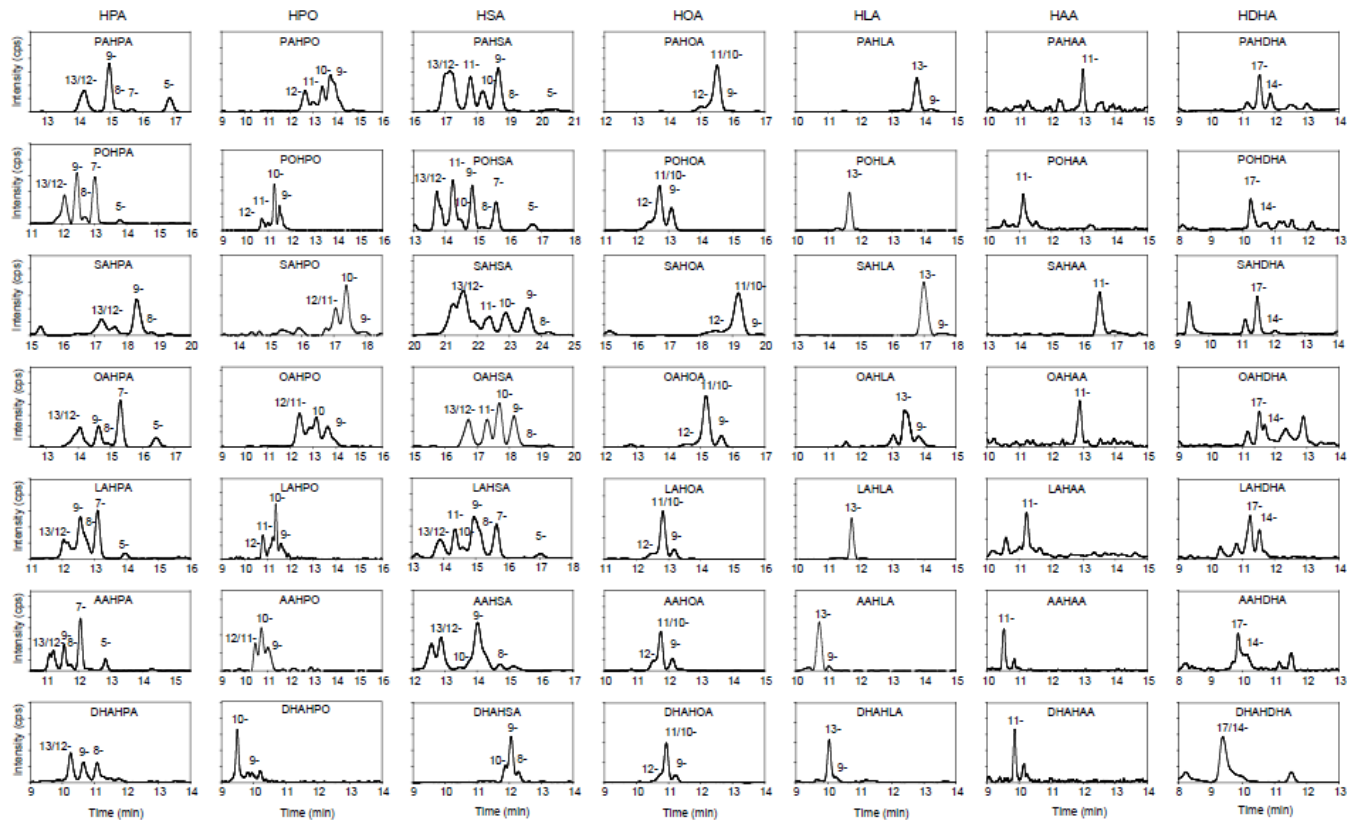


SUPPLEMENTARY DATA

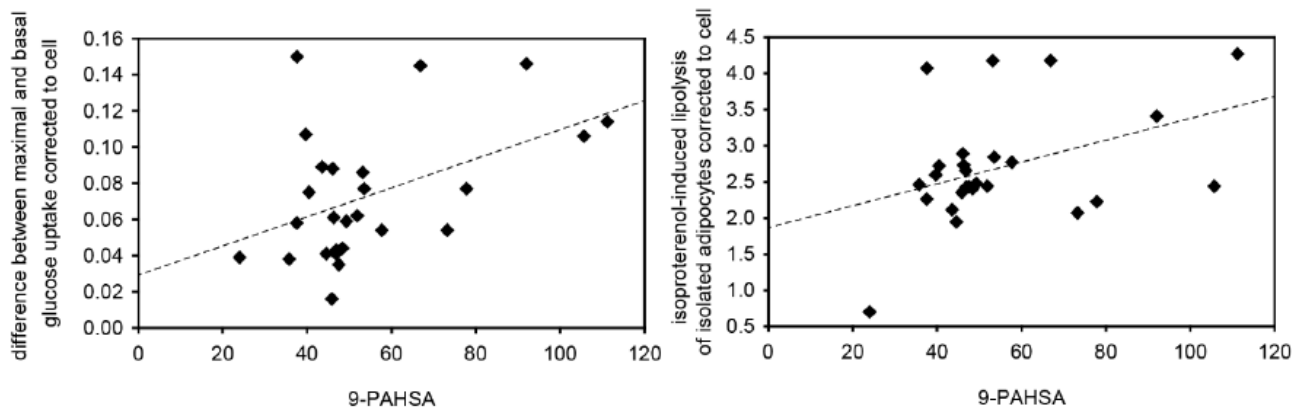
**Supplementary Figure S1.** Chromatographic profiles of FAHFAs. Epididymal adipose tissue samples were extracted and separated on YMC-Triart C18 ExRS 1.9  $\mu$ m 2.1x150 mm column. Illustrative profiles for HAA and HDHA regioisomers were acquired from mice fed omega-3 PUFA rich diet (1; 2). PA, palmitic acid; PO, palmitoleic acid; SA, stearic acid; OA, oleic acid; LA, linoleic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; H- prefix denotes hydroxy fatty acid.

In case of HPO and HOA, limited information on the position of double bond was acquired based on the MS/MS/MS spectra. Supposed configuration: 9-HPO and 9-HOA:  $\Delta^{10}$ ; 10-HPO and 10-HOA:  $\Delta^8$ ; 11-, 12-HPO and 11-, 12-HOA:  $\Delta^9$ ;



## SUPPLEMENTARY DATA

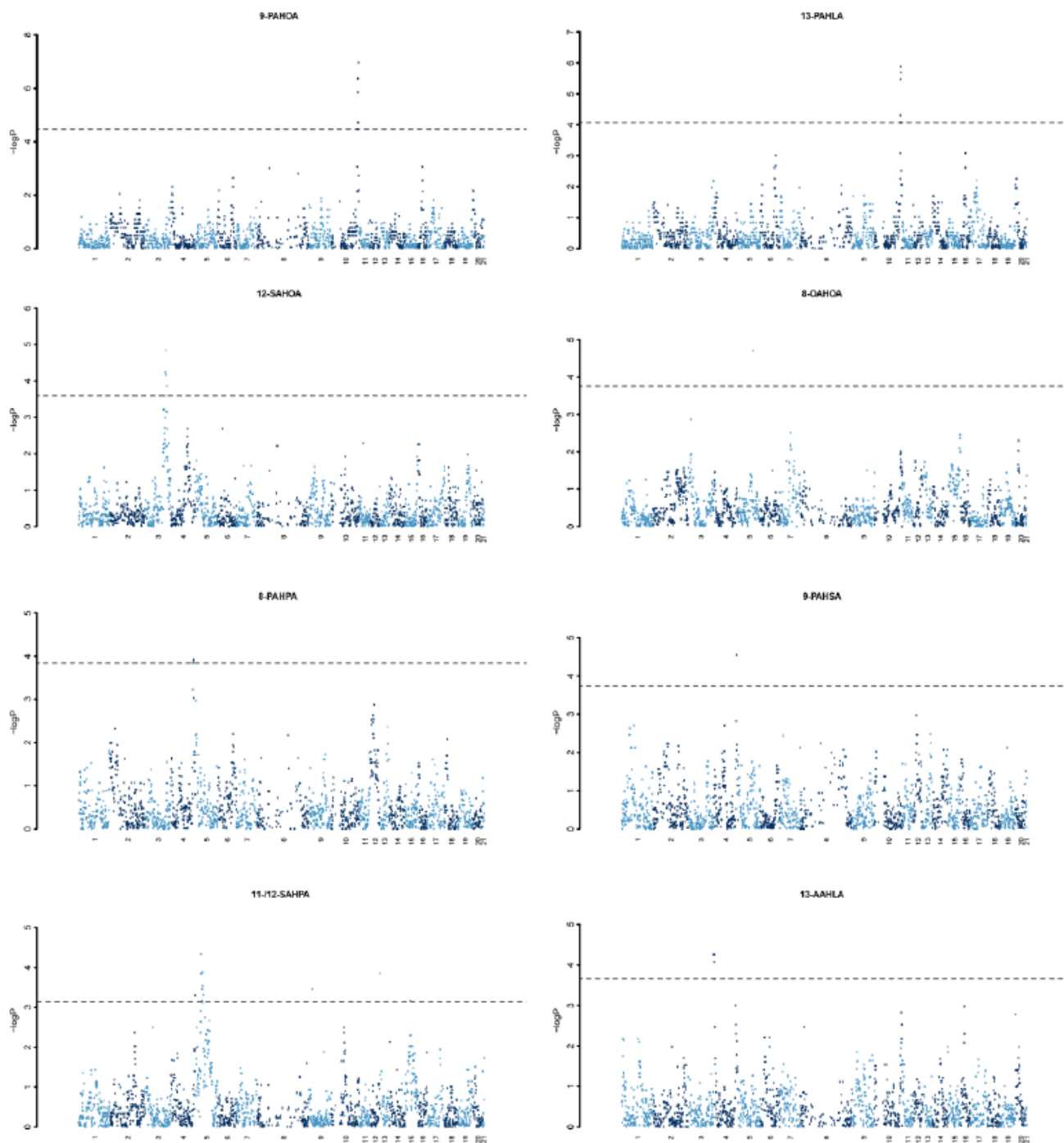
**Supplementary Figure S2.** Correlations between 9-PAHSA trait and physiological parameters of the BXH/HXB RI strains. Difference between maximal and basal glucose uptake into collagenase-liberated adipocytes and isoproterenol-induced lipolysis of isolated adipocytes plotted versus 9-PAHSA trait levels. Physiological data acquired from GeneNetwork (3).



# SUPPLEMENTARY DATA

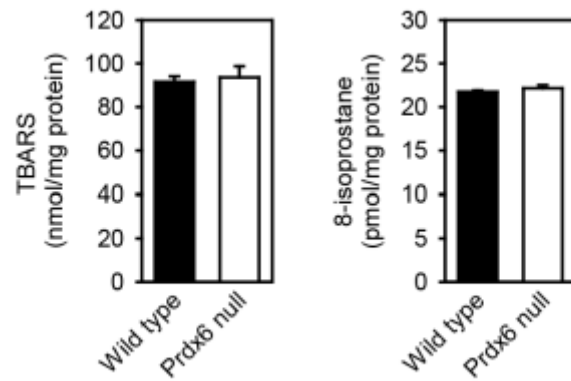
## Supplementary Figure S3. Manhattan plots of FAHFA traits

Dash line defines FDR threshold.



## SUPPLEMENTARY DATA

**Supplementary Figure S4.** Lipid peroxidation in wild type and Prdx6 null WAT. Levels of thiobarbituric acid reactive substances (TBARS) and 8-isoprostanes in WAT dissected from wild type and Prdx6 null mice. Data are expressed as means  $\pm$  standard error, n=3.



**Supplementary Table S1.** Optimized MS parameters for FAHFA measurement

Q1, precursor ion; Q3 product ion; DP, declustering potential; CE, collision energy.

ID	Q1 [M-H] <sup>-</sup>	Q3 FA	Q3 HFA	Q3 HFA-H <sub>2</sub> O	DP	CE
PAHPO	507.4	255.2	269.2	251.2	-130	-35
PAHPA	509.4	255.2	271.2	253.2	-130	-35
PAHLA	533.4	255.2	295.2	277.2	-130	-35
PAHOA	535.4	255.2	297.2	279.2	-130	-35
PAHSA	537.5	255.2	299.3	281.3	-130	-35
PAHAA	557.4	255.2	319.2	301.2	-130	-35
PAHDHA	581.4	255.2	343.2	325.2	-130	-35
POHPO	505.4	253.2	269.2	251.2	-130	-35
POHPA	507.4	253.2	271.2	253.2	-130	-35
POHLA	531.4	253.2	295.2	277.2	-130	-35
POHOA	533.4	253.2	297.2	279.2	-130	-35
POHSA	535.5	253.2	299.3	281.3	-130	-35
POHAA	555.4	253.2	319.2	301.2	-130	-35
POHDHA	579.4	253.2	343.2	325.2	-130	-35
LAHPO	531.4	279.2	269.2	251.2	-130	-35
LAHPA	533.4	279.2	271.2	253.2	-130	-35
LAHLA	557.4	279.2	295.2	277.2	-130	-35
LAHOA	559.4	279.2	297.2	279.2	-130	-35
LAHSA	561.5	279.2	299.3	281.3	-130	-35
LAHAA	581.4	279.2	319.2	301.2	-130	-35
LAHDHA	605.4	279.2	343.2	325.2	-130	-35
OAHPO	533.4	281.2	269.2	251.2	-130	-35
OAHPA	535.4	281.2	271.2	253.2	-130	-35
OAHLA	559.4	281.2	295.2	277.2	-130	-35
OAHOA	561.4	281.2	297.2	279.2	-130	-35
OAHSA	563.5	281.2	299.3	281.3	-130	-35
OAHAA	583.4	281.2	319.2	301.2	-130	-35
OAHDHA	607.4	281.2	343.2	325.2	-130	-35
SAHPO	535.5	283.3	269.2	251.2	130	35
SAHPA	537.5	283.3	271.2	253.2	-130	-35
SAHLA	561.5	283.3	295.2	277.2	-130	-35
SAHOA	563.5	283.3	297.2	279.2	-130	-35
SAHSA	565.6	283.3	299.3	281.3	-130	-35
SAHAA	585.5	283.3	319.2	301.2	-130	-35
SAHDHA	609.5	283.3	343.2	325.2	-130	-35
AAHPO	555.4	303.2	269.2	251.2	-130	-35
AAHPA	557.4	303.2	271.2	253.2	-130	-35
AAHLA	581.4	303.2	295.2	277.2	-130	-35

## SUPPLEMENTARY DATA

AAHOA	583.5	303.2	297.2	279.2	-130	-35
AAHSA	585.5	303.2	299.3	281.3	-130	-35
AAHAA	605.4	303.2	319.2	301.2	-130	-35
AAHDHA	629.4	303.2	343.2	325.2	-130	-35
DHAHPO	579.4	327.2	269.2	251.2	-130	-35
DHAHPA	581.4	327.2	271.2	253.2	-130	-35
DHAHLA	605.4	327.2	295.2	277.2	-130	-35
DHAHOA	607.5	327.2	297.2	279.2	-130	-35
DHAHSA	609.5	327.2	299.3	281.3	-130	-35
DHAHAA	629.4	327.2	319.2	301.2	-130	-35
DHAHDHA	653.4	327.2	343.2	325.2	-130	-35

SUPPLEMENTARY DATA

**Supplementary Table S2.** QTL associated with FAHFA traits

SDP	Trait	Genomic Range	p.value	FDR	beta	t.stat	beta_se	ci_upper	ci_lower
SDPG_04161793548	X9.PAHSA	4-161793548-161793548	2.79E-05	0.046932635	-0.183081616	-5.07118007	0.036102369	-0.112320972	-0.25384226
SDPG_13071646655	X12.13.PAHSA	13-71646655-72510384	7.86E-05	0.044160896	0.25459247	4.678066316	0.054422587	0.36126074	0.1479242
SDPG_13078909101	X12.13.PAHSA	13-78909101-78909101	2.27E-05	0.019103329	0.257097403	5.149244016	0.049929155	0.354958547	0.159236259
SDPG_13080093944	X12.13.PAHSA	13-80093944-81219060	2.27E-05	0.019103329	0.257097403	5.149244016	0.049929155	0.354958547	0.159236259
SDPG_13081943244	X12.13.PAHSA	13-81943244-83733914	1.16E-04	0.048985049	0.242172951	4.529814791	0.053461998	0.346958468	0.137387434
SDPG_13083744138	X12.13.PAHSA	13-83744138-85418436	1.72E-04	0.057951633	0.24095815	4.381403448	0.054995654	0.348749632	0.133166667
SDPG_04159937580	X8.PAHPA	4-159937580-160514422	1.44E-04	0.081157675	0.387286553	4.447458398	0.087080422	0.557964179	0.216608926
SDPG_04161793548	X8.PAHPA	4-161793548-161793548	1.28E-04	0.081157675	0.30195506	4.49200988	0.06722048	0.4337072	0.170202919
SDPG_04166069314	X8.PAHPA	4-166069314-167152491	1.21E-04	0.081157675	0.40117426	4.515841485	0.0888371	0.575294975	0.227053545
SDPG_10104672318	X9.PAHOA	10-104672318-104732023	3.46E-05	0.011661292	0.244878086	4.988902974	0.049084556	0.341083816	0.148672357
SDPG_10105504952	X9.PAHOA	10-105504952-105761650	1.42E-06	0.000797405	0.281542166	6.214339228	0.045305246	0.370340448	0.192743885
SDPG_10105769352	X9.PAHOA	10-105769352-105964319	4.28E-07	0.000360233	0.289369192	6.688007016	0.043266879	0.374172276	0.204566109
SDPG_10105969527	X9.PAHOA	10-105969527-106177758	1.92E-05	0.008071153	0.274516729	5.213220194	0.052657804	0.377726025	0.171307432
SDPG_10106181521	X9.PAHOA	10-106181521-107689156	1.09E-07	0.000184418	0.336331521	7.238509846	0.046464193	0.42740134	0.245261702
SDPG_10104672318	X13.PAHLA	10-104672318-104732023	4.93E-05	0.020777125	-0.227127602	-4.854631121	0.046785759	-0.135427514	-0.318827689
SDPG_10105504952	X13.PAHLA	10-105504952-105761650	3.35E-06	0.001880199	-0.258848927	-5.881068008	0.044013932	-0.17258162	-0.345116233
SDPG_10105769352	X13.PAHLA	10-105769352-105964319	1.35E-06	0.001752817	-0.264971881	-6.234736055	0.042499294	-0.181673265	-0.348270498
SDPG_10105969527	X13.PAHLA	10-105969527-106177758	8.42E-05	0.028374352	-0.243465606	-4.652136987	0.052334144	-0.140890684	-0.346040527
SDPG_10106181521	X13.PAHLA	10-106181521-107689156	2.08E-06	0.001752817	-0.296235071	-6.065372674	0.048840374	-0.200507937	-0.391962204
SDPG_05130556071	X8.OAHOA	5-130556071-130556071	2.01E-05	0.033818481	-0.35202829	-5.195582126	0.067755312	-0.219227878	-0.484828703
SDPG_04181531098	X11.12.SAHPA	4-181531098-181531098	4.95E-04	0.083348954	0.255805118	3.978345573	0.064299371	0.381831885	0.129778351
SDPG_05049742388	X11.12.SAHPA	5-49742388-50644238	1.42E-04	0.059882138	-0.324853563	-4.453654472	0.0729409	-0.1818894	-0.467817726
SDPG_05050656645	X11.12.SAHPA	5-50656645-52311164	4.62E-05	0.059882138	-0.345957452	-4.879582271	0.070898989	-0.206995433	-0.484919471
SDPG_05052356181	X11.12.SAHPA	5-52356181-54231285	1.42E-04	0.059882138	-0.324853563	-4.453654472	0.0729409	-0.1818894	-0.467817726
SDPG_05054244647	X11.12.SAHPA	5-54244647-54612807	6.90E-04	0.088995984	-0.294528395	-3.850330577	0.076494314	-0.14459954	-0.44445725
SDPG_05054633806	X11.12.SAHPA	5-54633806-54986141	3.42E-04	0.072021144	-0.307947307	-4.119700264	0.07474993	-0.161437444	-0.45445717
SDPG_05055867111	X11.12.SAHPA	5-55867111-57175062	3.42E-04	0.072021144	-0.307947307	-4.119700264	0.07474993	-0.161437444	-0.45445717
SDPG_05057202014	X11.12.SAHPA	5-57202014-60139253	6.90E-04	0.088995984	-0.294528395	-3.850330577	0.076494314	-0.14459954	-0.44445725
SDPG_05060214465	X11.12.SAHPA	5-60214465-60938429	3.42E-04	0.072021144	-0.307947307	-4.119700264	0.07474993	-0.161437444	-0.45445717
SDPG_05060988192	X11.12.SAHPA	5-60988192-62843226	7.39E-04	0.088995984	-0.29625819	-3.823565808	0.077482174	-0.144393129	-0.44812325

SUPPLEMENTARY DATA

SDPG_05063114762	X11.12.SAHPA	5-63114762-63162152	1.32E-04	0.059882138	-0.323527813	-4.482585576	0.072174375	-0.182066038	-0.464989589
SDPG_05063164285	X11.12.SAHPA	5-63164285-63706439	2.89E-04	0.072021144	-0.310029409	-4.183880452	0.074100924	-0.164791597	-0.455267221
SDPG_05065239187	X11.12.SAHPA	5-65239187-66480071	4.80E-04	0.083348954	-0.3019751	-3.990091407	0.075681249	-0.153639853	-0.450310347
SDPG_15025754773	X11.12.SAHPA	15-25754773-26250248	6.84E-04	0.088995984	0.315493383	3.853577938	0.081870248	0.475959069	0.155027696
SDPG_03136383584	X12.SAHOA	3-136383584-136578961	2.54E-04	0.085539159	-0.220889599	-4.233348851	0.052178454	-0.118619829	-0.323159369
SDPG_03137777747	X12.SAHOA	3-137777747-139087656	5.88E-05	0.037890575	-0.232240547	-4.788105941	0.048503636	-0.137173421	-0.327307674
SDPG_03139108660	X12.SAHOA	3-139108660-150071378	1.43E-05	0.024072631	-0.249758886	-5.324873544	0.046904191	-0.157826672	-0.341691101
SDPG_03150844988	X12.SAHOA	3-150844988-151580802	6.75E-05	0.037890575	-0.241448801	-4.73605112	0.050981038	-0.141525967	-0.341371636
SDPG_03154833237	X12.SAHOA	3-154833237-155286618	1.41E-04	0.059311448	-0.22758677	-4.457286849	0.051059485	-0.12751018	-0.32766336
SDPG_04000102881	X13.AAHLA	4-102881-7774756	5.59E-05	0.046549713	0.547352104	4.807520437	0.113853308	0.770504587	0.324199621
SDPG_04007780467	X13.AAHLA	4-7780467-8821996	8.29E-05	0.046549713	0.524520465	4.658116847	0.112603544	0.745223411	0.303817519
SDPG_04008906412	X13.AAHLA	4-8906412-8940940	5.59E-05	0.046549713	0.547352104	4.807520437	0.113853308	0.770504587	0.324199621
SDPG_04013806614	X13.AAHLA	4-13806614-14635595	2.17E-04	0.091502059	0.50084549	4.292626971	0.116675754	0.729529969	0.272161012

FDR < 0.1

Upper Confidence Interval 95% = beta + 1.96 \* beta\_standard\_error

Lower Confidence interval 95% = beta - 1.96 \* beta\_standard\_error



## SUPPLEMENTARY DATA

**Supplementary Table S3.** Gene names, symbols, abbreviations and qPCR primer sequences.

<i>Rat</i>			
Gene Name	Gene Symbol	Forward primer	Reverse primer
Peroxisredoxin 6	Prdx6	GCTGGCGCAGCAGTTGCAG	CAGTTGACTGGAAGAAGGGAGAGAG
Microsomal Glutathione S-Transferase 1	Mgst 1	GCCAATGAAACCCAGGCTTCACAC	CCATCCACAGGCTCTACCCCTTTGT
Microsomal glutathione S-transferase 3	Mgst 3	TCCCGTTCCTCAGTGGCAGGA	GGCTACTACACGGGAGACCCTAGC
Solute carrier family 2 member 4	Glut 4	GGGTTTCCAGTATGTTGCGGATGC	CCGGCCTCTGGTTTCAGGCA
MLX interacting protein-like	ChREBP total	CTTGAAACCTTCACCAGG	TACTGTTCCCTGCCTGCTC
	ChREBP alpha	AGGCTCAAGCATTCTGAAGAG	TGCATCGATCACAGGTCATT
	ChREBP beta	CTTGTCGCCGCATAGCAAC	TCTGCAGATCGCGCGGAG
ATP citrate lyase	Acly	CCAGGCCAGCCATAACGG	GGAGTATGGGCTTCATCGGGCA
Acetyl-CoA carboxylase alpha	Acc1	TGCCAGTCGCTCAGCCAAG	CGGGAAAGCAGGGGATCCGT
Fatty acid synthase	Fasn	TCCCATCACACCTGGGACA	CTGGACCGCCGTGACCTGAG
Peptidylprolyl isomerase A (cyclophilin A)	Ppia	AGCATACAGTCTGGCAT	TCACCTTCCCAAAGACCAC
<i>Murine</i>			
Gene Name	Gene Symbol	Forward primer	Reverse primer
Peroxisredoxin 6	Prdx6	TCGGAGAGGGTGGGAACCTACC	CAGTTGACTGGAAGAAGGGAGAGAG
Microsomal Glutathione S-Transferase 1	Mgst 1	CCTGTTTGCTGAGGAAGGGGA	TGCGCAGAGCCCACCTGAATGA
Microsomal glutathione S-transferase 3	Mgst 3	ACACGGTGGTGCCCATCAGG	GGCTACTACACAGGAGACCCTAGCA
Nuclear factor, erythroid derived 2, like 2	Nfe2l2	AAGGTCCATCTCCCGAACC	CTGAAAAGCGGCTCAGCACC
MLX interacting protein-like	Mlxip1	ATCTTGGTCTTAGGGTCTTCAGG	CACTCAGGGAATACAGCGCTAC
Actin, beta	Actb	GAACCCTAAGGCCAACCCTGAAAAGAT	ACCGCTCGTTGCCAATAGTGATG

## Methods

## References:

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2. Brezinova M, Kuda O, Hansikova J, Rombaldova M, Balas L, Bardova K, Durand T, Rossmeisl M, Cerna M, Stranak Z, Kopecky J: Levels of palmitic acid ester of hydroxystearic acid (PAHSA) are reduced in the breast milk of obese mothers. *Biochim Biophys Acta* 2017;1863:126-131
3. Aitman TJ, Gotoda T, Evans AL, Imrie H, Heath KE, Trembling PM, Truman H, Wallace CA, Rahman A, Dore C, Flint J, Kren V, Zidek V, Kurtz TW, Pravenec M, Scott J: Quantitative trait loci for cellular defects in glucose and fatty acid metabolism in hypertensive rats. *Nat Genet* 1997;16:197-201