

Supplementary Material

1. Supplementary tables

Supplementary table S1. A composition of diets used.

Diet	Western Diet (WD)		Control Diet (CD)	
%	g	kcal	g	kcal
Protein	12.4	11	10.3	11
Carbohydrate	54.9	48	76.0	79
Fat	21.3	42	4.3	10
Total		100		100
kcal/gm	4.61		3.84	
Ingredient	gm	kcal	gm	kcal
Casein, 30 Mesh	106	424	106	424
L-Cystine	1.6	6.4	1.6	6.4
Sucrose	150	600	150	600

Maltodextrin 10	100	400	150	600
Corn Starch	216	864	481	1924
Cellulose, BW200	50	0	50	0
Soybean Oil	0	0	25	225
Palm Oil	185	1665	20	180
Mineral Mix S10026	10	0	10	0
DiCalcium Phosphate	13	0	13	0
Calcium Carbonate	5.5	0	5.5	0
Potassium Citrate, 1 H ₂ O	16.5	0	16.5	0
Vitamin Mix V10001	10	40	10	40
Choline Bitartrate	2	0	2	0
Cholesterol, NF	1.8	0	0	0
Total	867.45	3999	1040.65	3999

Supplementary table S2. Sequences for primers used in RT-PCR.

Gene	Gene ID	Forward primer	Reverse primer
GAPDH	14433	TGCACCACCAACTGCTTAG	GGATGCAGGGATGATGTTC
ACTB	11461	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
B2m	12010	QuantiTect Primer Assays Cat. No. QT01149547 (Qiagen, Netherlands)	
IRA	16337	GGTTTTGTCCCCAGGCCAT	GTGCTCCTCCTGACTTGTGG
IRB	16337	CAATGGTGCCGAGGACAGTA	GTGCTCCTCCTGACTTGTGG
ACSL1	14081	ATCTGGTGGAACGAGGCAAG	TCCTTTGGGGTTGCCTGTAG
ENPP	18605	AGTGCTCCGCCTTAGAGTCC	GTCAGCAACTCCTCGACCCA
PTPN1	19246	GGCGCCAAGTTCATCATGGG	AGCTCCTTGCACTTCCCGTT
PTEN	19211	AGGCACAAGAGGCCCTAGAT	CTGACTGGGAATTGTGACTCC
CD36	12491	TCTCATGCCAGTCGGAGACA	CTGTACACAGTGGTGCCTGTT

Supplementary table S3. Bins (where VIP score > 1.5) and metabolite assignments (see the main text).

WT/CD vs. WT/WD			WT/CD vs. KO/CD			KO/CD vs. KO/WD			WT/WD vs. KO/WD		
Bin	VIP score	Metabolite assignment	Bin	VIP score	Metabolite assignment	Bin	VIP score	Metabolite assignment	Bin	VIP score	Metabolite assignment
1.32-- 1.34 ppm	7.638	lactate	1.32-- 1.34 ppm	5.738	lactate	1.32-- 1.34 ppm	4.958	lactate	0.86-- 0.88 ppm	4.036	lipid (-CH3)
0.86-- 0.88 ppm	2.892	lipid (-CH3)	3.22-- 3.24 ppm	3.766	β - glucose/lipid	0.86-- 0.88 ppm	4.026	lipid (-CH3)	3.22-- 3.24 ppm	3.126	β -glucose/ lipid
4.10-- 4.12 ppm	2.595	lactate	0.86-- 0.88 ppm	3.192	lipid (-CH3)	0.88-- 0.90 ppm	3.018	lipid (-CH3)	5.29-- 5.31 ppm	3.092	unsaturated lipid
3.22-- 3.24 ppm	2.202	β - glucose/lipid	3.26-- 3.28 ppm	2.338	β -glucose	1.28-- 1.30 ppm	2.817	lipid (-CH2-)n	0.88-- 0.90 ppm	2.933	lipid (-CH3)
0.84-- 0.86 ppm	2.131	lipid (-CH3)	1.26-- 1.28 ppm	2.261	lipid (-CH2-)n	0.84-- 0.86 ppm	2.811	lipid (-CH3)	1.28-- 1.30 ppm	2.687	lipid (-CH2-)n
1.46-- 1.48 ppm	2.010	alanine	1.46-- 1.48 ppm	2.222	alanine	3.22-- 3.24 ppm	2.647	β -glucose/ lipid	3.40-- 3.42 ppm	2.643	glucose
3.50-- 3.52 ppm	1.865	glucose	3.42-- 3.44 ppm	2.067	glucose	1.30-- 1.32 ppm	2.522	lipid (-CH2)n	1.32-- 1.34 ppm	2.577	lactate
3.72-- 3.74 ppm	1.862	glucose	0.84-- 0.86 ppm	1.874	lipid (-CH3)	5.29-- 5.31 ppm	2.521	unsaturated lipid	5.31-- 5.33 ppm	2.566	unsaturated lipid

3.90-- 3.92 ppm	1.794	glucose	1.48-- 1.50 ppm	1.867	alanine	2.76-- 2.78 ppm	2.159	lipid (=CH- CH2-CH=)	2.76-- 2.78 ppm	2.505	lipid (=CH- CH2-CH=)
3.84-- 3.86 ppm	1.647	glucose	1.24-- 1.26 ppm	1.866	lipid (-CH2-)n	4.10-- 4.12 ppm	2.098	lactate	2.02-- 2.04 ppm	2.229	lipid (=CH- CH2-CH-)
3.46-- 3.48 ppm	1.644	glucose	4.10-- 4.12 ppm	1.841	lactate	5.31-- 5.33 ppm	1.967	unsaturated lipid	1.26-- 1.28 ppm	2.225	lipid (-CH2-)n
0.92-- 0.94 ppm	1.594	BCAA isoleucine	3.02-- 3.04 ppm	1.662	creatine/creat inine	1.26-- 1.28 ppm	1.901	lipid (-CH2-)n	2.78-- 2.80 ppm	2.098	lipid (=CH- CH2-CH=)
3.24-- 3.26 ppm	1.539	β - glucose/lipid	1.04-- 1.06 ppm	1.521	BCAA valine	5.27-- 5.29 ppm	1.901	unsaturated lipid	0.84-- 0.86 ppm	2.039	lipid (-CH3)
						2.02-- 2.04 ppm	1.849	lipid (=CH- CH2-CH-)	1.30-- 1.32 ppm	2.001	lipid (-CH2-)n
						3.56-- 3.58 ppm	1.832	glucose	3.26-- 3.28 ppm	1.871	β -glucose
						3.64-- 3.66 ppm	1.776	glucose	5.27-- 5.29 ppm	1.841	unsaturated lipid
						3.76-- 3.78 ppm	1.667	glucose	3.76-- 3.78 ppm	1.803	glucose
						3.54-- 3.56 ppm	1.589	glucose	3.42-- 3.44 ppm	1.768	glucose
						1.46-- 1.48 ppm	1.580	alanine	3.72-- 3.74 ppm	1.661	glucose

						3.78-- 3.80 ppm	1.576	glucose	3.46-- 3.48 ppm	1.607	glucose
									1.46-- 1.48 ppm	1.594	alanine
									1.48-- 1.50 ppm	1.592	alanine
									3.82-- 3.84 ppm	1.568	glucose

Supplementary table S4. Summary of statistical analysis of the main effects and interactions of physiological and metabolic changes, behavioral changes in the open field, brain expression of IR subtypes and transcription factors, and metabolomic parameters in wild types and Sert knockout mice fed with control diet or Western diet (see Figs. 2, 4-6, Supp. Fig. S3). One-way ANOVA. Significant group differences are in bold; ns: not significant.

Parameter	Main effects		
	Genotype effect	Diet effect	Interaction
Body weight week 1	F=12.36, p=0.0019	F=0.1285, p=0.7232, ns	F=0.03424, p=0.8548, ns
Body weight week 2	F=17.29, p=0.0004	F=2.729, p=0.1121, ns	F=0.0004869, p=0.9826, ns
Body weight week 3	F=14.80, p=0.0009	F=7.261, p=0.0132	F=0.0002291, p=0.9881, ns

Glucose tolerance AUC	F=0.7898, p=0.3882, ns	F=10.91, p=0.0048	F=0.1614, p=0.6935, ns
Leptin blood level	F=10.08, p=0.0088	F=19.50, p=0.0010	F=2.656, p=0.1315, ns
Mean velocity	F=3.230, p=0.0844, ns	F=0.1343, p=0.7171, ns	F=4.240, p=0.05, ns
Number of transitions	F=7.153, p=0.0130	F=0.574, p=0.4557, ns	F=2.06, p=0.1636, ns
Duration of stretched posture	F=2.27, p=0.1444, ns	F=1.386, p=0.2502, ns	F=1.478, p=0.2355, ns
Duration of grooming	F=3.718, p=0.0653, ns	F=0.8463, p=0.3664, ns	F=4.517, p=0.0436
<i>IrA</i> expression in hippocampus	F=6.831, p=0.0150	F=3.689, p=0.0662, ns	F=12.11, p=0.0019
<i>IrA</i> expression in dorsal raphe	F=5.008, p=0.0357	F=7.286, p=0.0131	F=9.840, p=0.0048
<i>IrA</i> expression in prefrontal cortex	F=12.18, p=0.0019	F=5.229, p=0.0313	F=0.9133, p=0.3488, ns
<i>IrA</i> expression in hypothalamus	F=8.912, p=0.0063	F=4.636, p=0.0412	F=6.025, p=0.0214

<i>IrB</i> expression in hippocampus	F=1.171, p=0.2895, ns	F=1.243, p=0.2754, ns	F=6.729, p=0.0156
<i>IrB</i> expression in dorsal raphe	F=15.99, p=0.0007	F=1.176, p=0.2904, ns	F=2.747, p=0.1123, ns
<i>IrB</i> expression in prefrontal cortex	F=12.63, p=0.0016	F=0.8655, p=0.3615, ns	F=1.776, p=0.1952, ns
<i>IrB</i> expression in hypothalamus	F=2.118, p=0.158, ns	F=6.359, p=0.0184	F=15.4, p=0.0006
<i>ACSL1</i> expression in hippocampus	F=2.894, p=0.1052, ns	F=0.4987, p=0.4887, ns	F=7.709, p=0.012
<i>ACSL1</i> expression in dorsal raphe	F=3.839, p=0.0649, ns	F=4.978, p=0.0379	F=0.5091, p=0.4842, ns
<i>ACSL1</i> expression in prefrontal cortex	F=2.88, p=0.106, ns	F=0.09974, p=0.758, ns	F=0.3635, p=0.5537, ns
<i>ACSL1</i> expression in hypothalamus	F=0.1008, p=0.7543, ns	F=0.698, p=0.4138, ns	F=0.3041, p=0.5878, ns
<i>ENPP</i> expression in hippocampus	F=13.42, p=0.0017	F=2.153, p=0.1587, ns	F=4.066, p=0.0581, ns
<i>ENPP</i> expression in	F=0.8958, p=0.3558, ns	F=2.338, p=0.1428, ns	F=3.936, p=0.0619, ns

dorsal raphe			
<i>ENPP</i> expression in prefrontal cortex	F=0.7062, p=0.4111, ns	F=0.1466, p=0.7061, ns	F=0.8035, p=0.3813, ns
<i>ENPP</i> expression in hypothalamus	F=0.2164, p=0.6471, ns	F=0.019, p=0.8918, ns	F=0.05743, p=0.8132, ns
<i>PTPN1</i> expression in hippocampus	F=3.308, p=0.0847, ns	F=1.993, p=0.1742, ns	F=11.88, p=0.0027
<i>PTPN1</i> expression in dorsal raphe	F=9.489, p=0.0062	F=3.044, p=0.0972, ns	F=0.002973, p=0.9571, ns
<i>PTPN1</i> expression in prefrontal cortex	F=0.1376, p=0.7147, ns	F=3.059, p=0.0964, ns	F=0.01529, p=0.9029, ns
<i>PTPN1</i> expression in hypothalamus	F=0.1825, p=0.6740, ns	F=5.837, p=0.0259	F=0.1285, p=0.7239, ns
<i>PTEN</i> expression in hippocampus	F=1.762, p=0.2001, ns	F=8.608, p=0.0085	F=0.2820, p=0.6016, ns
<i>PTEN</i> expression in dorsal raphe	F=4.693, p=0.0432	F=0.5955, p=0.4498, ns	F=0.4606, p=0.5055, ns
<i>PTEN</i> expression in prefrontal cortex	F=7.579, p=0.0127	F=4.377, p=0.0501, ns	F=0.1866, p=0.6707, ns

<i>PTEN</i> expression in hypothalamus	F=0.9680, p=0.3375, ns	F=0.03909, p=0.8454, ns	F=0.002877, p=0.9578, ns
<i>CD36</i> expression in hippocampus	F=0.8532, p=0.3672, ns	F=1.194, p=0.2881, ns	F=0.002275, p=0.9625, ns
<i>CD36</i> expression in dorsal raphe	F=0.2069, p=0.6543, ns	F=0.1694, p=0.6852, ns	F=0.006875, p=0.9348, ns
<i>CD36</i> expression in prefrontal cortex	F=1.202, p=0.2867, ns	F=0.1550, p=0.6981, ns	F=0.5186, p=0.4802, ns
<i>CD36</i> expression in hypothalamus	F=2.994, p=0.0998, ns	F=0.7086, p=0.4104, ns	F=2.234, p=0.1514, ns
Lipid	F=13.61, p=0.0014	F=0.6235, p=0.4386, ns	F=3.071, p=0.0943, ns
Unsaturated lipid	F=3.014, p=0.0965, ns	F=2.758, p=0.1109, ns	F=4.339, p=0.0491
HDL	F=0.135, p=0.7168, ns	F=3.249, p=0.0852, ns	F=1.248, p=0.276, ns
VLDL	F=4.906, p=0.0379	F=8.572, p=0.008	F=0.7989, p=0.3815, ns
Glucose	F=16.38, p=0.0006	F=0.02511, p=0.8756, ns	F=9.263, p=0.0062
Lactate	F=3.433, p=0.0774, ns	F=16.05, p=0.0006	F=0.04025, p=0.8428, ns

Alanine	F=25.95, p<0.0001	F=24.63, p<0.0001	F=0.02009, p=0.8886, ns
Isoleucine	F=8.356, p=0.0085	F=27.56, p<0.0001	F=0.5327, p=0.4732, ns
Valine	F=20.69, p=0.0002	F=17.96, p=0.0004	F=0.1263, p=0.7258, ns

Supplementary table S5. Summary of statistical values of multiple comparisons for parameters with significant genotype x diet interaction: behavioral changes in the open field, brain expression of IR subtypes and transcription factors, and metabolomic parameters in wild types and Sert knockout mice fed with control diet or Western diet (Figs. 4-6, Supp. Fig. S3). *Post-hoc* Tukey's test. Significant differences are in bold; ns: not significant. WT - wild type, KO - knockouts, CD - control diet, WD - Western diet.

Parameter	post hoc Tukey's test			
	WT/CD WT/WD vs.	WT/CD KO/CD vs.	KO/CD KO/WD vs.	WT/WD KO/WD vs.
Duration of grooming	p=0.1982, ns	p=0.0442	p=0.804, ns	p=0.9989, ns
<i>IrA</i> expression in hippocampus	p=0.7231, ns	p=0.0015	p=0.0024	p=0.9277, ns

<i>IrA</i> expression in dorsal raphe	p=0.9914, ns	p=0.0071	p=0.001	p=0.9098, ns
<i>IrA</i> expression in hypothalamus	p=0.997, ns	p=0.0047	p=0.0102	p=0.9804, ns
<i>IrB</i> expression in hippocampus	p=0.754, ns	p=0.0769, ns	p=0.0475	p=0.6972, ns
<i>IrB</i> expression in hypothalamus	p=0.7824, ns	p=0.0052	p=0.0003	p=0.3044, ns
<i>ACSL1</i> expression in hippocampus	p=0.1098, ns	p=0.8785, ns	p=0.4569, ns	p=0.0294
<i>PTPN1</i> expression in hippocampus	p=0.016	p=0.6791, ns	p=0.4711, ns	p=0.0059
Unsaturated lipids	p=0.9905, ns	p=0.9952, ns	p=0.0653, ns	p=0.0466
Glucose	p=0.1875, ns	p=0.9074, ns	p=0.1535, ns	p=0.0001

Supplementary table S6. Summary of t-test and one-sample t-test statistical values for of physiological and metabolic changes, behavioral changes in the open field, brain expression of IR subtypes and transcription factors, and metabolomic parameters in wild types and Sert knockout mice fed with control diet or Western diet (Figs. 2, 4-6, Supp. Fig. S3). Significant differences are in bold; ns: not significant, WT - wild type, KO – knockouts.

Parameter	Paired comparison	Comparison with 100%	
		WT	KO
Body weight week 1	p=0.7617, ns	p=0.6068, ns	p=0.8536, ns
Body weight week 2	p=0.8218, ns	p=0.1812, ns	p=0.0833, ns
Body weight week 3	p=0.7939, ns	p=0.0745, ns	p=0.0075
Glucose tolerance AUC	p=0.8193, ns	p=0.0279	p=0.0569, ns
Leptin blood level	p=0.0013	p=0.0449	p=0.0087
Mean velocity	p=0.0038	p=0.0359	p=0.0264
Number of	p=0.1034, ns	p=0.4470, ns	p=0.1336, ns

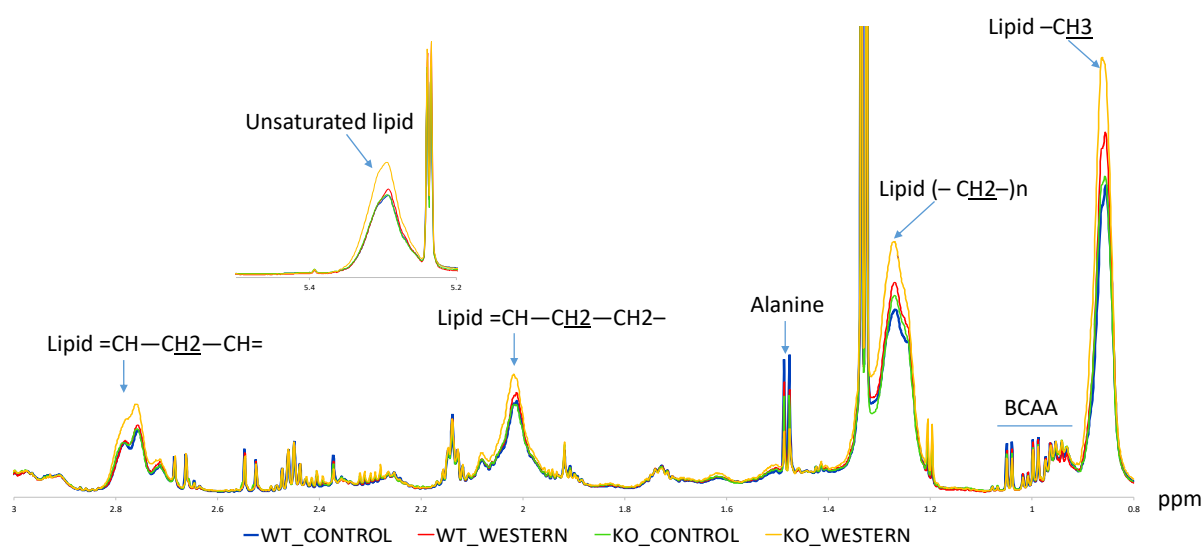
transitions			
Duration of stretched posture	p=0.1846, ns	p<0.0001	p=0.2202, ns
Duration of grooming	p=0.0034	p=0.0213	p=0.1148, ns
<i>IrA</i> expression in hippocampus	p=0.0006	p=0.1460, ns	p=0.0004
<i>IrA</i> expression in dorsal raphe	p=0.0098	p=0.7961, ns	p<0.0001
<i>IrA</i> expression in prefrontal cortex	p=0.6401, ns	p=0.0030	p=0.0334
<i>IrA</i> expression in hypothalamus	p=0.0041	p=0.6540, ns	p=0.0021
<i>IrB</i> expression in hippocampus	p=0.0022	p=0.2004, ns	p=0.0005
<i>IrB</i> expression in dorsal raphe	p=0.0446	p=0.4758, ns	p=0.0429
<i>IrB</i> expression in prefrontal cortex	p=0.0605, ns	p=0.6350, ns	p=0.0497

<i>IrB</i> expression in hypothalamus	p=0.0012	p=0.3514, ns	p<0.0001
<i>ACSL1</i> expression in the hippocampus	p=0.0031	p=0.0293	p=0.0578, ns
<i>ACSL1</i> expression in dorsal raphe	p=0.1602, ns	p=0.1027, ns	p=0.0278
<i>ACSL1</i> expression in prefrontal cortex	p=0.3577, ns	p=0.8114, ns	p=0.1973, ns
<i>ACSL1</i> expression in hypothalamus	p=0.4692, ns	p=0.4075, ns	p=0.3804, ns
<i>ENPP</i> expression in hippocampus	p=0.1650, ns	p=0.0064	p=0.7674, ns
<i>ENPP</i> expression in dorsal raphe	p=0.0190	p=0.6250, ns	p=0.0345
<i>ENPP</i> expression in prefrontal cortex	p=0.2582, ns	p=0.7106, ns	p=0.1322, ns
<i>ENPP</i> expression in hypothalamus	p=0.7256, ns	p=0.4389, ns	p=0.9393, ns

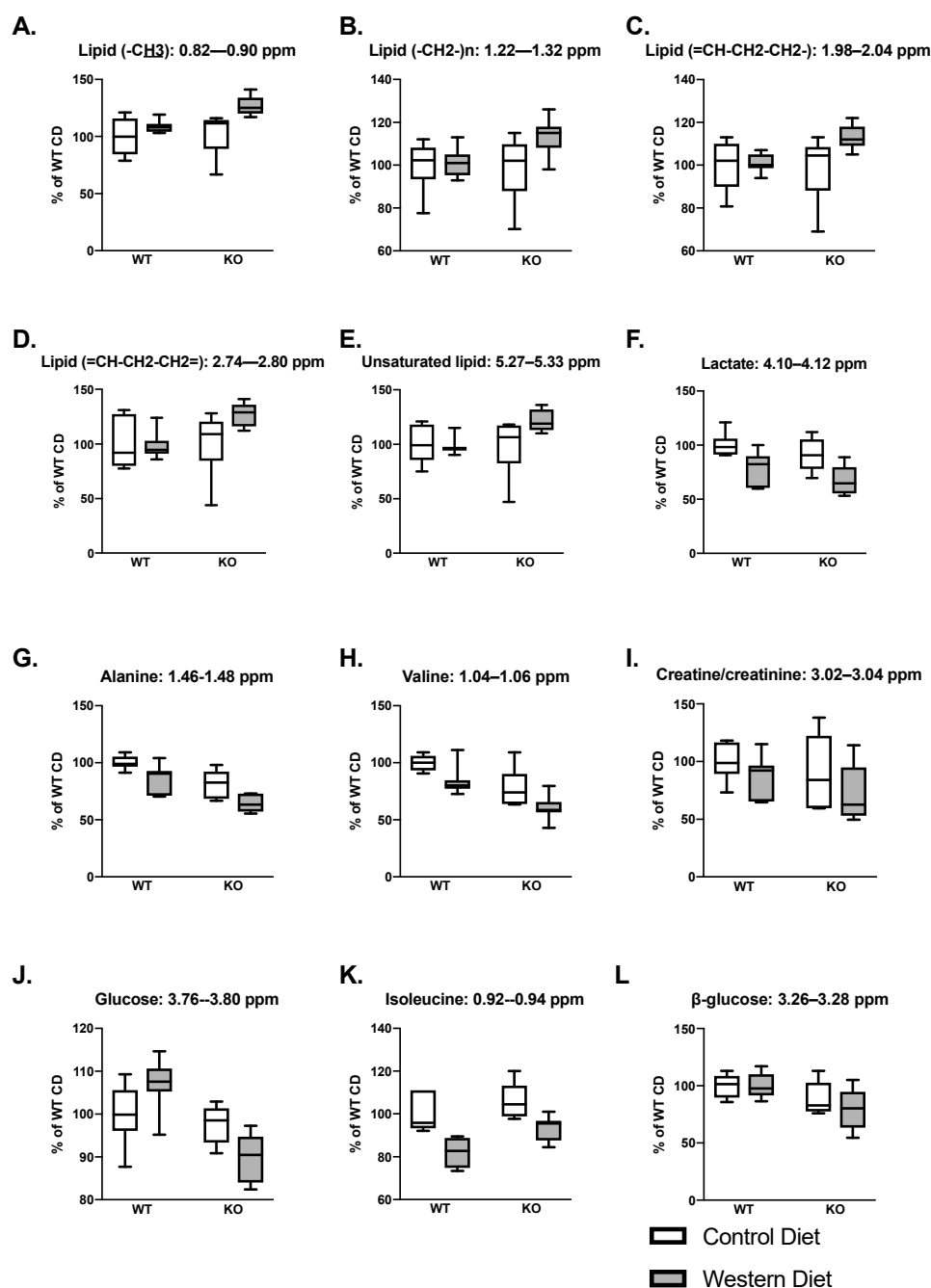
<i>PTPN1</i> expression in hippocampus	p=0.0006	p=0.0074	p=0.0971, ns
<i>PTPN1</i> expression in dorsal raphe	p=0.8898, ns	p=0.1054, ns	p=0.2781, ns
<i>PTPN1</i> expression in prefrontal cortex	p=0.8972, ns	p=0.1914, ns	p=0.1465, ns
<i>PTPN1</i> expression in hypothalamus	p=0.6591, ns	p=0.0481	p=0.0759, ns
<i>PTEN</i> expression in hippocampus	p=0.7976, ns	p=0.0190	p=0.0581, ns
<i>PTEN</i> expression in dorsal raphe	p=0.4624, ns	p=0.8873, ns	p=0.3937, ns
<i>PTEN</i> expression in prefrontal cortex	p=0.9828, ns	p=0.0111	p=0.0236
<i>PTEN</i> expression in hypothalamus	p=0.9641, ns	p=0.6591, ns	p=0.8567, ns
<i>CD36</i> expression in hippocampus	p=0.2858, ns	p=0.0293	p=0.1889
<i>CD36</i> expression in	p=0.9414, ns	p=0.7535, ns	p=0.6780, ns

dorsal raphe			
<i>CD36</i> expression in prefrontal cortex	p=0.2348, ns	p=0.7491, ns	p=0.1657, ns
<i>CD36</i> expression in hypothalamus	p=0.1722, ns	p=0.0690, ns	p=0.6611, ns

2. Supplementary figures

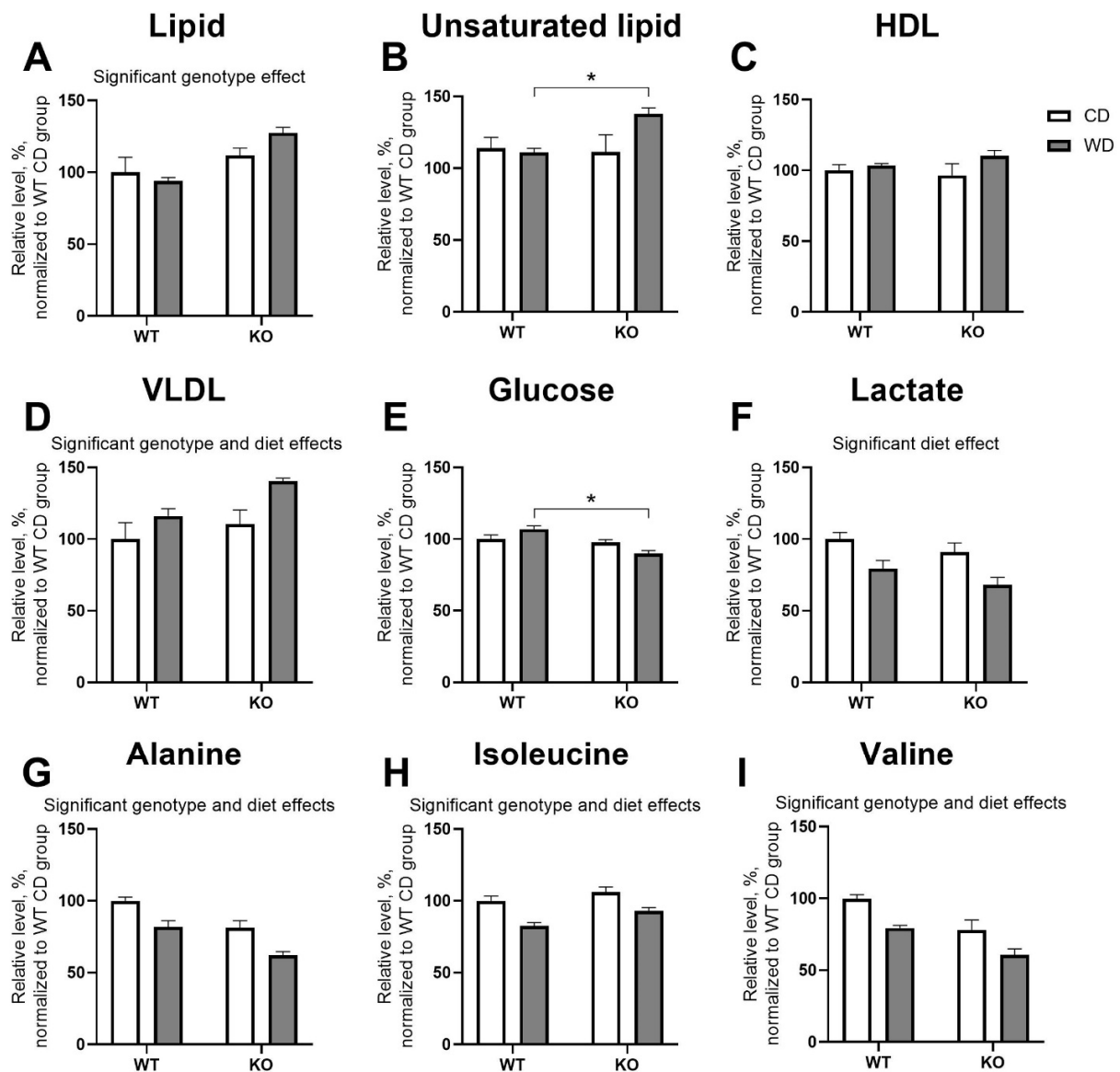


Supplementary Figure S1. Average ^1H CPMG spectra of WT/CD (blue), WT/WD (red), KO/CD (green), and KO/WD (yellow). Lipid signals as well as BCAAs and alanine are shown. Hydrogens that are responsible for the NMR signal are underlined.



Supplementary Figure S2. Boxplots showing average percent change of each metabolite signal relative to the WT/CD group are shown. (A) Average percent change of lipid (-CH₃) was higher in WD-fed groups compared to the WT/CD group. Average percent change of (B) lipid (-CH₂), (C) lipid (=CH-CH₂-CH₂-), (D) lipid (=CH-CH₂-CH₂=), and (E) unsaturated lipid was higher in KO/WD group compared to the WT/CD group. (F)

Average percent change of lactate was lower in WD-fed groups compared to the WT/CD group. Average percent change of (G) alanine, (H) valine, and (I) creatine/creatinine was lower in both WD-fed groups and KO/CD group compared to the WT/CD group. (J) Average percent change of glucose was higher in WT/WD group, but lower in KO/WD group compared to the WT/CD group. (K) Average percent change of isoleucine was lower in WD-fed groups compared to the WT/CD group. (L) Average percent change of β -glucose was lower in KO groups compared to the WT/CD group. The regions of the spectrum used to calculate the integrals are indicated in ppm.



Supplementary Figure S3. Metabolome changes between SERT-KO and WT mice housed on WD. (A) Significant genotype effect was found in lipid level. **(B)** Unsaturated lipid level was significantly higher in KO/WD mice compared to WT/WD group. **(C)** No significant group differences were found in HDL level. **(D)** Significant genotype and diet effects were shown for VLDL level. **(E)** Glucose concentration was significantly lower in KO/WD mice compared to WT/WD group. **(F)** Significant diet effect was revealed in lactate level. **(G)** There was a significant genotype and diet effects on alanine level, as well as on **(H)** isoleucine content and **(I)** valine concentration. Two-way ANOVA and Tukey's post hoc test (* $p < 0.05$). All data are mean \pm SEM.