Supplemental Experimental Procedures

Fasting Studies; Plasma and Liver Metabolite Analysis

Age and sex matched mice were randomly divided into *ad libitum* chow fed or fasting groups. The fasting period was 24 h from 09:00 – 09:00. Blood glucose and lactate were measured at the beginning and end of the fasting period. Mice were subsequently sacrificed by asphyxiation with CO_2, and plasma was collected from the inferior vena cava, and stored on ice with EDTA to prevent clotting. Blood was then centrifuged at 8,000 x g for 8 min to separate cells and plasma. Livers were frozen in liquid nitrogen and stored at -80°C for later analysis.

Plasma triglyceride and cholesterol concentrations were measured by Infinity colorimetric assay kits (Thermo Fisher Scientific). Plasma non-esterified fatty acids and total ketone concentrations were measured using enzymatic assays (Wako Diagnostics). Plasma urea was assessed by a commercial assay kit (Abnova). Plasma insulin was measured by an immunoassay (Singulex) by the Core Laboratory for Clinical Studies at Washington University. Liver triglyceride was measured by solubilizing 100 mg liver tissue in saline and solubilizing lipid with 0.5% sodium deoxycholate. Liver TAG was then determined by colorimetric assay (Thermo Fisher Scientific). Liver glycogen was measured by colorimetric assay (Sigma).

Metabolic Tolerance Tests and Streptozotocin-Induced Insulin Deficiency

Pyruvate tolerance tests (PTT), alanine tolerance tests (ATT), and glucose tolerance tests (GTT) were performed after an overnight (16 h) fast and while housed on aspen chip bedding. Mice were injected i.p. with 2 g/kg body weight Na-pyruvate (PTT), 2 g/kg body weight L-alanine (ATT), or 1 g/kg body weight D-glucose (GTT) dissolved in sterile saline. Insulin tolerance tests were performed by injecting i.p. 0.75 U/kg body weight insulin (Humulin), after a short 2 h fast. For all tolerance tests, blood glucose and lactate were measured using a One-Touch Ultra glucometer (LifeScan) and a Lactate Plus lactate meter (Nova Biomedical), respectively with a single drop of tail blood at T= 0, 15, 30, 60, 90, and 120 minutes after challenge. GTT and ITT in db/db crossed mice were performed similarly, but after 4 h fast.

A subset of mice was injected with a single dose of 180 μg/g body weight streptozotocin (STZ; Santa Cruz Biotechnology) to render mice insulin-deficient. Blood glucose and lactate was measured daily pre-STZ injection and for six days following STZ injection at 15:00.

Pyruvate and Alanine Mitochondrial Transport Assays

Mitochondria were isolated from fresh mouse livers by differential centrifugation and resuspended in assay buffer containing 120 mM KCl, 1 mM EGTA, 5 mM KH_2PO_4, and 10 mM HEPES, pH 7.4. Measurements of pyruvate or alanine uptake of the isolated mitochondria were performed in 100 μl assay buffer containing 50-100 μg mitochondrial protein, 2 μM of ^{14}C-pyruvate or ^{3}H-alanine for 3 min at room temperature. The mitochondrial-bound ^{14}C -pyruvate and ^{3}H -alanine were separated from the reactants by filtration through a nitrocellulose membrane filter (0.2 μm) and repeated washing with ice cold assay buffer. ^{14}C-pyruvate and ^{3}H-alanine radioactivity in the nitrocellulose filters were determined by scintillation counting. Non-specific uptake of ^{14}C-pyruvate and ^{3}H-alanine were determined by pretreatment of a subset of mitochondria with either 20 μM UK-5099 to inhibit MPC activity or 500 μM unlabeled alanine to limit ^{3}H-alanine uptake. Specific uptake was expressed as DPM/mg/min.

Hepatocyte Glucose Production Assay

All experiments were performed 10-12 hours after the hepatocytes were plated. To determine rates of glucose production, hepatocytes were washed twice with PBS and then incubated for 2 h in Hank's balanced salt solution (HBSS) containing 127mM NaCl, 3.5 mM KCl, 0.44 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 0.33 mM Na_2HPO_4, 1 mM $CaCl_2$, 20 mM HEPES, pH 7.4. Media was removed and hepatocytes were washed with fresh HBSS, and incubated for 3 h in HBSS containing glucagon (100 ng/ml) alone or with 5 mM sodium pyruvate in the absence or presence of either 2.5 μM UK-5099, 15 μM MSDC-0602, or 500 μM amino-oxyacetate (AOA). At the end of this incubation period, the media was collected and assayed for glucose concentrations with a glucose oxidase-based glucose assay kit (Sigma). Rates of glucose production were expressed as μg glucose/mg cell protein/h.

Quantitative RT-PCR

Total RNA was isolated using the RNAzol method (RNA-Bee, Tel-Test). cDNA was made by use of a reverse transcription kit (Invitrogen), and real-time PCR was performed using an ABI PRISM 7500 sequence detection system (Applied Biosystems) and a SYBR green master mix. Arbitrary units of target mRNA were corrected by measuring the levels of *36B4* mRNA. The sequences of the oligonucleotides are available upon request.

Western Blotting

Whole-cell lysates from tissue or isolated primary hepatocytes were collected in HNET buffer containing protease and phosphatase inhibitors. Lysates were normalized to protein concentration, denatured, and run down Criterion precast PAGE gels (BioRad). Antibodies used were α-Tubulin (Sigma), and MPC1 and MPC2 (gifts from Michael Wolfgang).

Palmitate Oxidation Measurement

Palmitate oxidation rates were assessed in lysates from primary hepatocytes using [^{3}H]palmitate as previously described (Chen et al., 2008).

Mitochondrial Respiration and Membrane Potential

Isolated primary hepatocytes were permeabilized with digitonin, and high-resolution respirometry was conducted using an Oxygraph O2k (Oroboros Instruments). 0.5 x 10^6 cells was suspended in 2 mL respiration for each trace. Respiratory substrates utilized were 5 mM pyruvate/2 mM malate, 10 mM glutamate/2 mM malate, or 5 mM succinate with 0.5 μM rotenone. All substrates were used in the presence of 2 mM ADP.

Respiration was performed with the same substrate concentrations as above with 50 μg of mitochondria isolated by differential centrifugation. 15 μM MSDC-0602 and 10 μM UK-5099 were used to inhibit pyruvate-stimulated respiration.

Mitochondrial membrane potential was assessed by plating isolated hepatocytes onto Tissue-Tek chamber slides, and staining with 100 nM tetramethylrhodamine, ethyl ester, perchlorate (TMRE) in HBSS for 30 min. Hepatocytes were then washed twice with HBSS and imaged on an inverted fluorescence microscope (Nikon Instruments). Average TMRE fluorescence per cell was determined with ImageJ (NIH).

References Cited

Chen, Z., Gropler, M.C., Norris, J., Lawrence, J.C., Jr., Harris, T.E., and Finck, B.N. (2008). Alterations in hepatic metabolism in fld mice reveal a role for lipin 1 in regulating VLDL-triacylglyceride secretion. Arterioscler Thromb Vasc Biol *28*, 1738-1744.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1, related to Figure 1 and Figure 2, further characterization of LS-*Mpc2*$^{-/-}$ mice.

(A) Body weights of 13 week old mice. (B) Relative liver weights of 13 week old mice. (C) Indirect calorimetry, energy expenditure, spontaneous activity, and H_2O/food consumption as measured in Phenomaster metabolic cages. (D) Blood glucose concentrations during an i.p. glucose tolerance test (GTT) after an overnight fast. (E) Blood lactate concentrations during the GTT. (F) Blood glucose concentrations during an i.p. insulin tolerance test (ITT) after a 2 h fast. (G) Blood lactate concentrations during the ITT. Data expressed as mean ± S.E.M. *$p \leq 0.05$ for fl/fl vs. LS-*Mpc2*$^{-/-}$. $^{\dagger}p \leq 0.05$ for light vs. dark.

Supplemental Figure 2, related to Figure 3, body and tissue weights and blood lactates or LS-*Mpc2*$^{-/-}$ mice crossed into the db/db background.

(A) Weekly body weights of fl/fl and LS-*Mpc2*$^{-/-}$ mice crossed into the db/db background. (B) Gonadal white adipose tissue (WAT) and (C) subcutaneous WAT weights from mice crossed into the db/db background. (D) Relative liver weights and (E) liver triglyceride content from mice crossed into dbdb background. (F) Weekly blood lactate concentrations of fl/fl and LS-*Mpc2*$^{-/-}$ mice crossed into the db/db background. Data expressed as mean ± S.E.M. *$p \leq 0.05$ for fl/fl vs. LS-*Mpc2*$^{-/-}$. $^{\dagger}p \leq 0.05$ for dbWT vs. dbdb.

Table S1. Hepatocyte cellular organic and amino acid and extracellular glucose U-^{13}C-pyruvate isotopomers with MPC inhibition, related to experiments in Figure 2.

Metabolite	Genotype	Treatment	Isotopomer Distribution M0	M1	M2	M3	M4	M5	M6	Concentration (ng/mL)
Lactate	fl/fl	Vehicle	74.80 ± 0.77	2.88 ± 0.15	3.70 ± 0.14	19.13 ± 0.73				611.81 ± 20.67
		UK-5099	72.81 ± 0.37	2.33 ± 0.25	2.94 ± 0.15	21.92 ± 0.78				772.92 ± 206.24
		MSDC-0602	72.64 ± 0.35	2.04 ± 0.55	3.28 ± 0.37	22.05 ± 0.57				577.82 ± 2.63
	LS-*Mpc2*$^{-/-}$	Vehicle	74.74 ± 1.82	2.34 ± 0.22	3.35 ± 0.17	20.57 ± 0.42				622.09 ± 3.93
		UK-5099	73.57 ± 0.38	1.73 ± 0.40	2.59 ± 0.20	22.11 ± 0.23				839.12 ± 193.97
		MSDC-0602	74.26 ± 0.11	2.06 ± 0.14	3.09 ± 0.22	20.59 ± 0.48				639.94 ± 54.19
Pyruvate	fl/fl	Vehicle	66.65 ± 1.77	6.69 ± 0.22	11.05 ± 0.32	15.61 ± 2.31				137.31 ± 21.68
		UK-5099	58.07 ± 1.55†	5.18 ± 0.23†	6.55 ± 0.16†	30.19 ± 1.94†				188.02 ± 7.92
		MSDC-0602	54.18 ± 2.39†	7.70 ± 0.30	10.89 ± 0.23	27.24 ± 1.85†				179.05 ± 15.79
	LS-*Mpc2*$^{-/-}$	Vehicle	67.20 ± 1.92	4.25 ± 0.56*	6.07 ± 0.25*	22.48 ± 2.72*				189.71 ± 52.04
		UK-5099	57.29 ± 3.44	4.45 ± 0.23	6.12 ± 0.01	32.15 ± 3.21				273.07 ± 2.65*
		MSDC-0602	59.20 ± 1.59†	3.87 ± 0.50*	5.36 ± 0.36*	31.57 ± 2.45				270.58 ± 10.20*
Citrate	fl/fl	Vehicle	30.65 ± 2.42	7.61 ± 0.22	25.09 ± 1.07	19.32 ± 0.21	9.17 ± 0.80	7.21 ± 0.40	0.97 ± 0.14	1336.06 ± 82.39
		UK-5099	42.06 ± 1.47†	9.79 ± 0.23†	23.13 ± 0.67	15.93 ± 0.12†	5.45 ± 0.38†	3.41 ± 0.41†	0.24 ± 0.12†	575.06 ± 14.31†
		MSDC-0602	30.86 ± 1.70	8.21 ± 0.38	26.37 ± 0.69	18.03 ± 0.22†	9.22 ± 0.70	6.53 ± 0.76	0.79 ± 0.15	835.28 ± 3.75†
	LS-*Mpc2*$^{-/-}$	Vehicle	38.69 ± 0.13	9.38 ± 0.14*	23.83 ± 0.21	17.67 ± 0.63	6.10 ± 0.03	4.17 ± 0.53*	0.15 ± 0.07*	480.50 ± 11.25*
		UK-5099	37.42 ± 1.80	9.47 ± 1.30	24.91 ± 1.15	16.44 ± 0.37	7.39 ± 0.68	3.98 ± 0.90	0.40 ± 0.01†	365.16 ± 5.72*†
		MSDC-0602	42.89 ± 0.40*†	10.85 ± 0.94	24.07 ± 1.68	13.58 ± 0.66*†	5.53 ± 0.03*†	2.89 ± 0.22*	0.19 ± 0.06*	374.59 ± 33.06*
Succinate	fl/fl	Vehicle	90.60 ± 1.55	2.03 ± 0.25	4.90 ± 0.79	1.05 ± 0.21	1.42 ± 0.30			472.49 ± 8.63
		UK-5099	93.04 ± 1.27	2.42 ± 0.37	3.52 ± 0.66	0.48 ± 0.09†	0.54 ± 0.16†			445.14 ± 15.52
		MSDC-0602	92.43 ± 0.38	1.92 ± 0.10	3.78 ± 0.27	0.90 ± 0.09	0.97 ± 0.12			432.36 ± 8.44†
	LS-*Mpc2*$^{-/-}$	Vehicle	94.91 ± 0.01	1.84 ± 0.22	2.37 ± 0.13*	0.44 ± 0.03*	0.44 ± 0.04*			380.94 ± 2.92*
		UK-5099	92.40 ± 0.70	2.58 ± 0.26	3.52 ± 0.63	0.73 ± 0.11	0.76 ± 0.21			398.22 ± 6.06
		MSDC-0602	91.62 ± 0.20†	3.39 ± 0.19*†	3.80 ± 0.02†	0.56 ± 0.02†	0.62 ± 0.03†			412.14 ± 0.91†
Malate	fl/fl	Vehicle	42.07 ± 0.15	12.09 ± 0.07	21.36 ± 0.12	18.26 ± 0.21	6.22 ± 0.25			467.18 ± 51.53
		UK-5099	58.31 ± 0.89†	12.75 ± 0.14†	15.81 ± 0.44†	9.67 ± 0.32†	3.45 ± 0.28†			177.80 ± 35.31†
		MSDC-0602	48.21 ± 0.15†	12.18 ± 0.71	19.80 ± 0.40	14.09 ± 0.08†	5.72 ± 0.54			244.24 ± 24.55†
	LS-*Mpc2*$^{-/-}$	Vehicle	53.12 ± 0.39*	12.44 ± 0.69	17.11 ± 0.01*	12.57 ± 0.10*	4.76 ± 0.99			146.05 ± 10.61*
		UK-5099	55.06 ± 0.39†	12.74 ± 1.04	17.06 ± 0.42	10.58 ± 0.16†	4.56 ± 0.07			136.49 ± 8.43
		MSDC-0602	59.20 ± 1.93	12.79 ± 1.25	14.90 ± 0.28*†	8.97 ± 0.04*†	4.13 ± 0.36			135.98 ± 1.74*
αKG	fl/fl	Vehicle	56.13 ± 5.00	6.61 ± 2.03	17.79 ± 4.72	8.32 ± 2.60	8.19 ± 0.44	2.96 ± 1.10		19.15 ± 3.76
		UK-5099	66.42 ± 3.10†	5.43 ± 1.40	16.12 ± 0.38	6.74 ± 0.84	3.32 ± 0.39†	1.97 ± 0.08		11.06 ± 0.49
		MSDC-0602	53.51 ± 6.37	6.18 ± 1.40	21.66 ± 2.76	9.80 ± 0.79	5.74 ± 0.46†	3.12 ± 0.97		11.47 ± 0.12
	LS-*Mpc2*$^{-/-}$	Vehicle	69.65 ± 1.11	5.97 ± 2.29	13.28 ± 0.79	5.26 ± 2.18	4.52 ± 0.73*	1.32 ± 0.53		8.65 ± 0.70*
		UK-5099	64.62 ± 5.01	6.09 ± 0.73	15.84 ± 2.05	7.18 ± 1.64	4.23 ± 0.26	2.04 ± 0.34		10.20 ± 0.02
		MSDC-0602	73.08 ± 1.25	3.74 ± 1.72	13.67 ± 1.20	3.52 ± 0.27*	3.27 ± 0.67	2.71 ± 0.21		11.96 ± 1.56
Alanine	fl/fl	Vehicle	50.65 ± 0.21	4.54 ± 0.25	6.66 ± 0.03	39.67 ± 0.02				2.83 ± 0.01
		UK-5099	55.16 ± 0.59†	3.73 ± 0.27	4.39 ± 0.18†	38.21 ± 1.07				1.40 ± 0.03†
		MSDC-0602	51.63 ± 0.40	3.26 ± 0.62	5.04 ± 0.66	41.66 ± 1.67				3.16 ± 0.12
	LS-*Mpc2*$^{-/-}$	Vehicle	53.51 ± 0.09*	4.03 ± 0.15	5.19 ± 0.19*	38.73 ± 0.24				2.45 ± 0.25
		UK-5099	53.53 ± 1.06	3.24 ± 0.58	3.99 ± 0.51	40.76 ± 2.23				1.54 ± 0.06
		MSDC-0602	55.17 ± 0.33*	4.11 ± 0.37	4.74 ± 0.30*	37.54 ± 1.05				2.08 ± 0.12*
Aspartate	fl/fl	Vehicle	39.07 ± 0.52	11.22 ± 0.06	22.13 ± 0.21	20.86 ± 0.07	6.72 ± 0.19			7.01 ± 0.27
		UK-5099	55.06 ± 0.57†	11.95 ± 0.21	17.17 ± 0.31†	12.35 ± 0.42†	3.46 ± 0.05†			2.05 ± 0.06†
		MSDC-0602	44.31 ± 0.54†	11.99 ± 0.51	21.31 ± 1.04	16.52 ± 0.41†	5.87 ± 0.42			4.44 ± 0.59†
	LS-*Mpc2*$^{-/-}$	Vehicle	44.72 ± 0.37*	12.97 ± 0.29*	20.67 ± 0.16*	16.79 ± 0.16*	4.85 ± 0.08*			2.46 ± 0.09*
		UK-5099	49.51 ± 0.23*†	12.23 ± 0.84	19.62 ± 0.66	13.98 ± 0.08†	4.67 ± 0.33			1.43 ± 0.11*†
		MSDC-0602	52.39 ± 0.86*†	13.37 ± 0.92	18.28 ± 0.17†	12.17 ± 0.20*†	3.78 ± 0.03†			1.54 ± 0.03*†
Glucose (µM)	fl/fl	Vehicle	24.42 ± 0.09	11.93 ± 0.05	22.42 ± 0.01	21.21 ± 0.04	9.46 ± 0.01	7.90 ± 0.20	2.66 ± 0.00	170.04 ± 1.10
		UK-5099	33.63 ± 0.70†	12.92 ± 0.18†	20.55 ± 0.23†	18.53 ± 0.17†	7.22 ± 0.05†	5.38 ± 0.06†	1.79 ± 0.02†	114.47 ± 4.02†
		MSDC-0602	28.20 ± 0.30†	12.55 ± 0.13†	21.93 ± 0.01†	20.13 ± 0.08†	8.38 ± 0.09†	6.64 ± 0.03†	2.17 ± 0.04†	130.93 ± 4.85†
	LS-*Mpc2*$^{-/-}$	Vehicle	29.00 ± 0.04*	12.33 ± 0.09*	21.37 ± 0.05*	20.10 ± 0.02*	8.21 ± 0.03*	6.69 ± 0.00*	2.29 ± 0.05*	124.17 ± 3.45*
		UK-5099	34.49 ± 2.07	12.28 ± 0.42	19.79 ± 0.56	19.78 ± 0.13*	7.52 ± 0.25	5.58 ± 0.25	2.06 ± 0.03*†	107.59 ± 5.14
		MSDC-0602	35.04 ± 0.03*†	12.31 ± 0.35	19.82 ± 0.14*†	18.11 ± 0.19*†	7.10 ± 0.12*†	5.60 ± 0.04*†	2.02 ± 0.19*	119.86 ± 6.60

* $p < 0.05$ WT vs KO within treatment

† $p < 0.05$ Vehicle vs treatment within genotype

Table S2. Plasma and liver characteristics in vehicle or STZ-injected mice, related to Figure 3.

	Vehicle		Streptozotocin	
	fl/fl	LS-*Mpc2*$^{-/-}$	fl/fl	LS-*Mpc2*$^{-/-}$
Plasma Insulin (pg/mL)	573.0 ± 91.9	661.5 ± 66.1	146.9 ± 22.4†	140.9 ± 17.3†
Plasma Free Fatty Acids (mM)	0.62 ± 0.13	0.85 ± 0.15	1.59 ± 0.24†	1.10 ± 0.10
Plasma Total Ketones (mM)	0.14 ± 0.03	0.27 ± 0.07	0.67 ± 0.21†	0.52 ± 0.08†
Plasma TAG (mg/dL)	41.1 ± 5.23	32.8 ± 3.8	152.9 ± 34.0†	89.1 ± 17.5†
Plasma Cholesterol (mg/dL)	64.1 ± 4.4	55.2 ± 3.0	95.6 ± 14.4†	67.8 ± 1.3†

† $p < 0.05$ Saline vehicle vs Streptozotocin

Table S3. Plasma and liver characteristics and liver Glutaminolysis and TCA cycle gene expression in fed and fasted mice, related to Figure 4.

		ad-lib fed		24 h fast	
		fl/fl	LS-$Mpc2^{-/-}$	fl/fl	LS-$Mpc2^{-/-}$
	Plasma Insulin (pg/mL)	708.1 ± 83.2	631.3 ± 121.3	183.5 ± 32.7†	174.8 ± 44.6†
	Plasma Free Fatty Acids (mM)	0.51 ± 0.05	0.53 ± 0.12	1.15 ± 0.18†	1.36 ± 0.13†
	Plasma Total Ketones (mM)	0.12 ± 0.01	0.20 ± 0.06	1.36 ± 0.14†	1.55 ± 0.20†
	Plasma TAG (mg/dL)	62.6 ± 14.3	61.8 ± 7.1	53.8 ± 7.7	74.4 ± 5.9*
	Plasma Cholesterol (mg/dL)	66.8 ± 3.6	70.9 ± 2.7	74.7 ± 4.6	81.0 ± 3.1†
	Plasma Urea (mM)	14.26 ± 0.68	15.23 ± 0.90	18.13 ± 1.42†	17.40 ± 1.49
	Liver TAG (µg/mg wet wt)	3.34 ± 0.37	2.99 ± 0.39	25.69 ± 4.53†	32.05 ± 2.72†
Hepatic Expression of genes encoding enzymes in glutaminolysis or the TCA cycle	*Gls1*	1.0 ± 0.07	1.13 ± 0.12	1.30 ± 0.22	1.56 ± 0.16
	Gls2	1.0 ± 0.06	1.12 ± 0.11	1.30 ± 0.20	1.22 ± 0.16
	Glud1	1.0 ± 0.05	1.07 ± 0.05	1.01± 0.13	1.05 ± 0.09
	Ogdh	1.0 ± 0.12	0.98 ± 0.04	1.77 ± 0.06†	2.61 ± 0.36†
	Sdha	1.0 ± 0.08	1.05 ± 0.09	1.03 ± 0.16	1.55 ± 0.13*†
	Mdh2	1.0 ± 0.11	0.83 ± 0.09	1.69 ± 0.25†	1.54 ± 0.12†
	Cs	1.0 ± 0.07	0.93 ± 0.07	0.99 ± 0.15	1.06 ± 0.10
	Idh2	1.0 ± 0.09	1.06 ± 0.06	1.45 ± 0.15†	2.86 ± 0.46*†

* $p < 0.05$ fl/fl vs LS-$Mpc2^{-/-}$

† $p < 0.05$ fed vs fasted

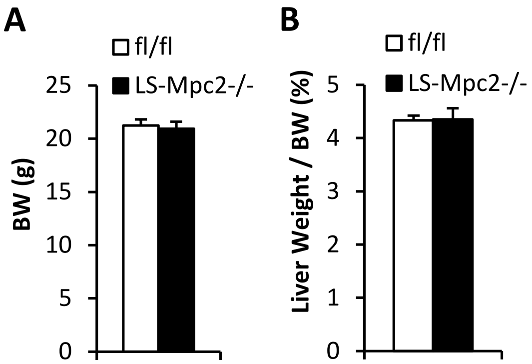
Table S5. Hepatocyte cellular organic and amino acids and extracellular glucose U-^{13}C-pyruvate isotopomers with ALT inhibition, related to experiment in Figure 7.

Metabolite	Genotype	Treatment	Isotopomer Distribution							Concentration
			M0	M1	M2	M3	M4	M5	M6	(ng/mL)
Lactate	fl/fl	Vehicle	84.28 ± 5.83	2.03 ± 0.62	2.63 ± 0.79	11.31 ± 4.66				597.35 ± 16.21
		AOA	82.23 ± 3.10	2.74 ± 0.28	3.26 ± 0.68	11.76 ± 2.26				529.87 ± 32.64
	LS-$Mpc2^{-/-}$	Vehicle	82.01 ± 4.28	1.87 ± 0.29	2.53 ± 0.49	16.34 ± 2.45				606.87 ± 8.94
		AOA	75.54 ± 1.88	1.09 ± 0.08*†	1.35 ± 0.15*†	22.02 ± 1.72†				721.70 ± 90.90
Pyruvate	fl/fl	Vehicle	60.01 ± 4.27	9.02 ± 1.44	14.06 ± 1.90	16.91 ± 1.29				138.86 ± 9.13
		AOA	50.84 ± 1.71	6.34 ± 1.70	9.12 ± 2.13	33.70 ± 2.14†				222.90 ± 30.68†
	LS-$Mpc2^{-/-}$	Vehicle	63.21 ± 2.49	6.16 ± 1.20	8.50 ± 1.52*	22.13 ± 1.88*				135.88 ± 37.67
		AOA	54.86 ± 0.11†	1.33 ± 0.08*†	1.72 ± 0.06*†	41.85 ± 0.38*†				301.76 ± 66.78†
Citrate	fl/fl	Vehicle	30.88 ± 1.07	8.23 ± 0.38	24.67 ± 0.52	20.05 ± 0.44	8.82 ± 0.39	6.56 ± 0.41	0.79 ± 0.12	1051.43 ± 168.45
		AOA	36.18 ± 5.85	8.18 ± 0.71	23.80 ± 2.20	16.50 ± 1.69	8.62 ± 0.94	6.02 ± 0.48	0.69 ± 0.08	917.03 ± 77.68
	LS-$Mpc2^{-/-}$	Vehicle	42.17 ± 2.03*	9.62 ± 0.16*	21.76 ± 1.20*	18.84 ± 0.73	4.57 ± 0.89*	3.00 ± 0.71*	0.04 ± 0.09*	425.84 ± 34.44*
		AOA	67.22 ± 1.71*†	11.25 ± 0.42*†	12.56 ± 1.1*†	6.26 ± 0.4*†	1.13 ± 0.18*†	1.37 ± 0.17*	0.20 ± 0.05*	243.66 ± 45.42*†
Succinate	fl/fl	Vehicle	89.55 ± 0.88	2.58 ± 0.34	5.30 ± 0.39	1.10 ± 0.09	1.47 ± 0.13			525.46 ± 30.79
		AOA	89.55 ± 0.81	2.86 ± 0.13	4.99 ± 0.40	1.17 ± 0.13	1.44 ± 0.15			496.20 ± 44.35
	LS-$Mpc2^{-/-}$	Vehicle	93.97 ± 0.55*	2.29 ± 0.29	2.94 ± 0.34*	0.38 ± 0.04*	0.42 ± 0.03*			467.05 ± 49.83
		AOA	97.19 ± 0.24*†	1.55 ± 0.10*†	1.09 ± 0.12*†	0.13 ± 0.01*†	0.04 ± 0.01*†			466.49 ± 44.65
Malate	fl/fl	Vehicle	42.00 ± 0.26	13.38 ± 0.75	21.59 ± 0.19	16.90 ± 0.81	6.13 ± 0.12			352.12 ± 69.71
		AOA	41.75 ± 1.61	14.34 ± 0.48	23.57 ± 0.69†	13.28 ± 0.76†	7.06 ± 0.63			292.55 ± 66.95
	LS-$Mpc2^{-/-}$	Vehicle	54.38 ± 0.87*	12.94 ± 0.41	16.86 ± 0.23*	12.30 ± 0.29*	3.52 ± 0.83*			162.13 ± 13.95*
		AOA	76.24 ± 1.36*†	10.33 ± 0.34*†	8.00 ± 0.62*†	4.39 ± 0.30*†	1.04 ± 0.42*†			95.61 ± 10.21*†
αKG	fl/fl	Vehicle	42.56 ± 5.88	9.15 ± 1.11	24.42 ± 2.89	11.80 ± 1.51	7.75 ± 0.22	4.32 ± 0.59		20.77 ± 1.80
		AOA	34.36 ± 1.18	9.97 ± 0.78	28.35 ± 0.53	13.34 ± 0.27	9.22 ± 0.13†	4.76 ± 0.13		44.72 ± 4.77†
	LS-$Mpc2^{-/-}$	Vehicle	61.57 ± 4.69*	8.29 ± 1.66	17.65 ± 2.54*	7.04 ± 1.41*	3.82 ± 0.50*	1.63 ± 0.30*		11.33 ± 1.57*
		AOA	69.42 ± 0.32*	8.17 ± 0.71	14.13 ± 0.57*	5.25 ± 0.05*	1.79 ± 0.23*†	1.24 ± 0.33*		22.48 ± 2.97*†
Alanine	fl/fl	Vehicle	50.39 ± 0.21	6.04 ± 0.88	7.97 ± 0.76	37.07 ± 1.52				2.49 ± 0.20
		AOA	57.64 ± 0.32†	2.03 ± 0.13†	3.86 ± 0.16†	37.85 ± 0.40				3.81 ± 0.16†
	LS-$Mpc2^{-/-}$	Vehicle	55.48 ± 1.19*	4.34 ± 0.20	5.64 ± 0.29*	35.92 ± 1.64				2.24 ± 0.16
		AOA	61.86 ± 1.06*†	0.74 ± 0.05*†	0.96 ± 0.04*†	37.87 ± 1.11				2.91 ± 1.09
Aspartate	fl/fl	Vehicle	39.34 ± 0.33	11.58 ± 0.23	21.73 ± 0.25	20.98 ± 0.14	6.37 ± 0.22			7.64 ± 0.40
		AOA	40.48 ± 1.54	12.77 ± 0.21†	24.06 ± 0.85†	15.66 ± 0.53†	7.03 ± 0.47			3.39 ± 0.26†
	LS-$Mpc2^{-/-}$	Vehicle	47.19 ± 1.46*	12.55 ± 0.27*	19.65 ± 0.60*	16.89 ± 0.19*	3.72 ± 0.65*			4.06 ± 0.93*
		AOA	64.19 ± 0.99*†	12.06 ± 0.15*	14.01 ± 0.39*†	8.00 ± 0.31*†	1.74 ± 0.19*†			1.15 ± 0.33*†
Glucose (μM)	fl/fl	Vehicle	24.61 ± 0.27	11.30 ± 0.36	22.04 ± 0.22	21.37 ± 0.11	9.52 ± 0.09	8.25 ± 0.23	2.91 ± 0.15	162.14 ± 4.59
		AOA	24.24 ± 0.78	11.14 ± 0.40	21.72 ± 0.33	21.53 ± 0.11	9.91 ± 0.15†	8.42 ± 0.09	3.04 ± 0.11	141.48 ± 4.40
	LS-$Mpc2^{-/-}$	Vehicle	29.74 ± 0.44*	12.08 ± 0.15*	21.26 ± 0.13*	20.05 ± 0.03*	7.97 ± 0.14*	6.58 ± 0.07*	2.32 ± 0.03*	131.40 ± 4.44*
		AOA	45.95 ± 0.94*†	12.53 ± 0.4*	16.93 ± 0.12*†	15.31 ± 0.2*†	4.53 ± 0.19*†	3.36 ± 0.03*†	1.39 ± 0.09*†	98.13 ± 11.41*†

* $p < 0.05$ WT vs KO within treatment

† $p < 0.05$ Vehicle vs AOA within genotype

Figure S1



C

	Light		Dark	
	fl/fl	LS-*Mpc2*$^{-/-}$	fl/fl	LS-*Mpc2*$^{-/-}$
VO_2 (ml/min/kg)	60.9 ± 2.4	59.4 ± 1.4	68.3 ± 2.8†	70.7 ± 1.9†
VCO_2 (ml/min/kg)	55.5 ± 2.8	53.5 ± 1.5	67.7 ± 3.1†	69.1 ± 2.6†
RER	0.88 ± 0.02	0.90 ± 0.01	0.99 ± 0.01†	0.97 ± 0.02†
Energy Exp. (kcal/h/kg)	18.1 ± 0.8	17.6 ± 0.4	20.6 ± 0.9†	21.3 ± 0.6†
Activity Counts	272 ± 30	307 ± 40	675 ± 102†	870 ± 164†
Average Speed (cm/s)	2.9 ± 0.3	3.2 ± 0.4	5.9 ± 0.8†	7.7 ± 1.3†

	fl/fl	LS-*Mpc2*$^{-/-}$
H_2O Intake (ml/24 h)	3.4 ± 0.4	3.2 ± 0.4
Food Intake (g/24 h)	3.3 ± 0.2	3.1 ± 0.2

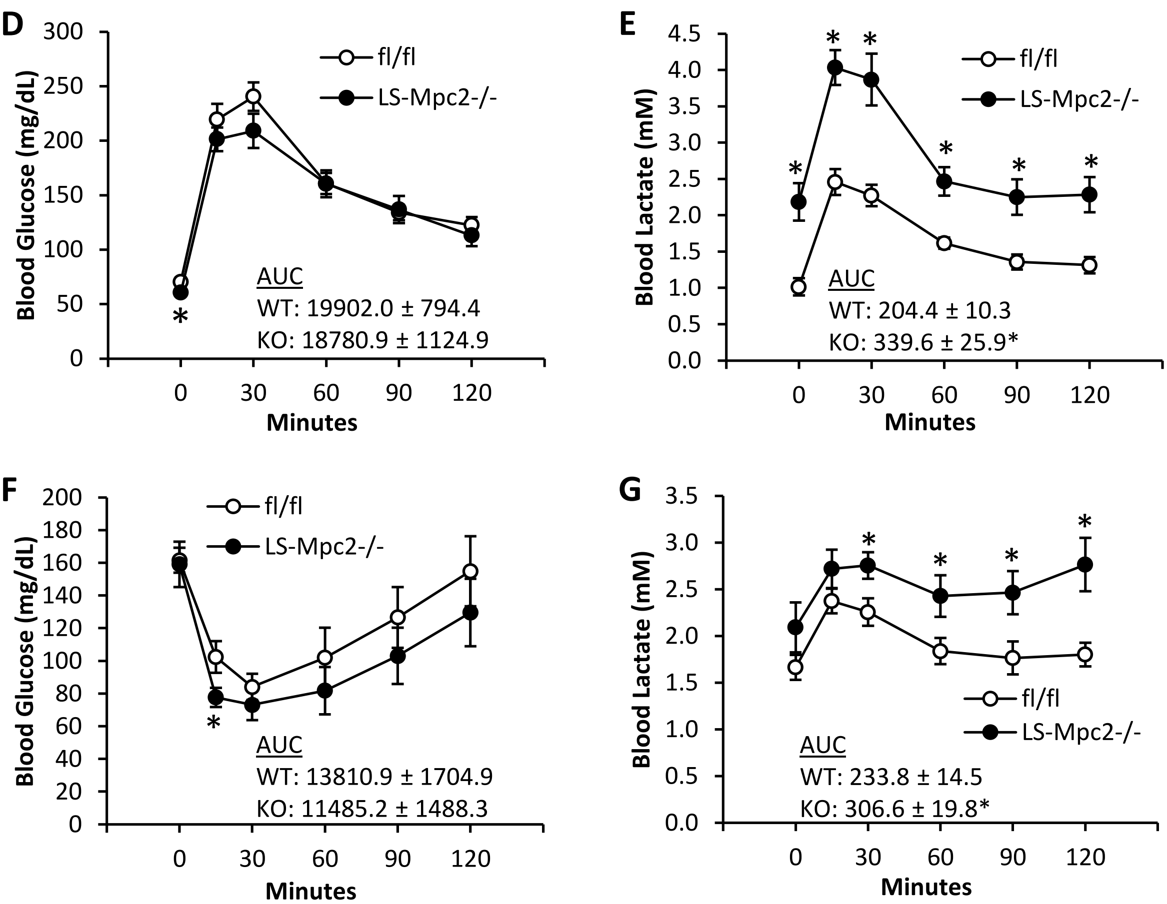
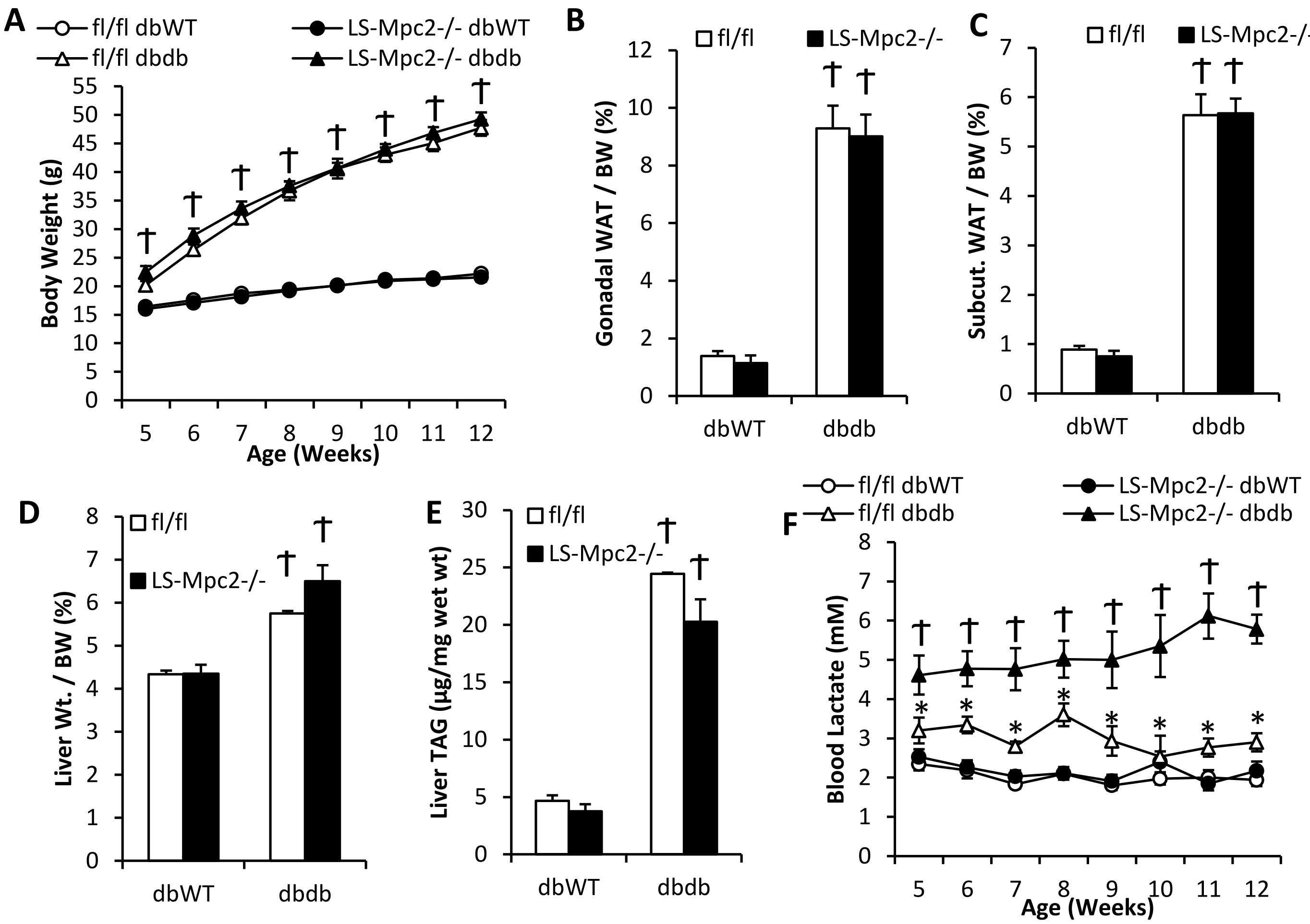


Figure S2



A
fl/fl dbWT
LS-Mpc2-/- dbWT
fl/fl dbdb
LS-Mpc2-/- dbdb
Body Weight (g)
Age (Weeks)
B
fl/fl
LS-Mpc2-/-
Gonadal WAT / BW (%)
dbWT
dbdb
C
fl/fl
LS-Mpc2-/-
Subcut. WAT / BW (%)
dbWT
dbdb
D
fl/fl
LS-Mpc2-/-
Liver Wt. / BW (%)
dbWT
dbdb
E
fl/fl
LS-Mpc2-/-
Liver TAG (µg/mg wet wt)
dbWT
dbdb
F
fl/fl dbWT
LS-Mpc2-/- dbWT
fl/fl dbdb
LS-Mpc2-/- dbdb
Blood Lactate (mM)
Age (Weeks)