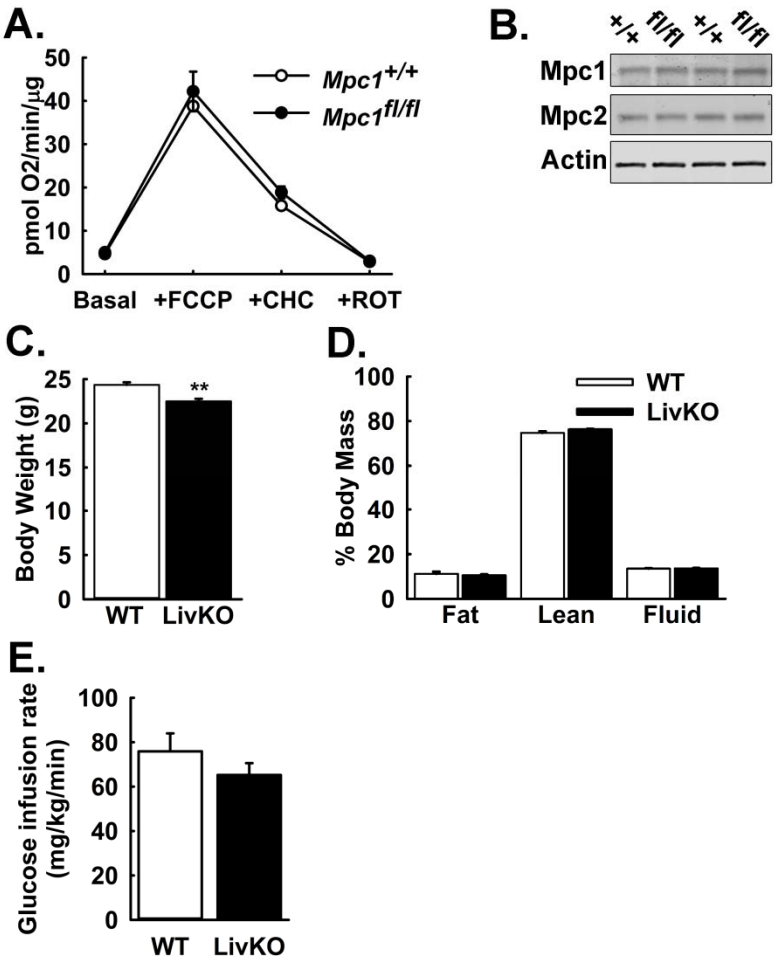
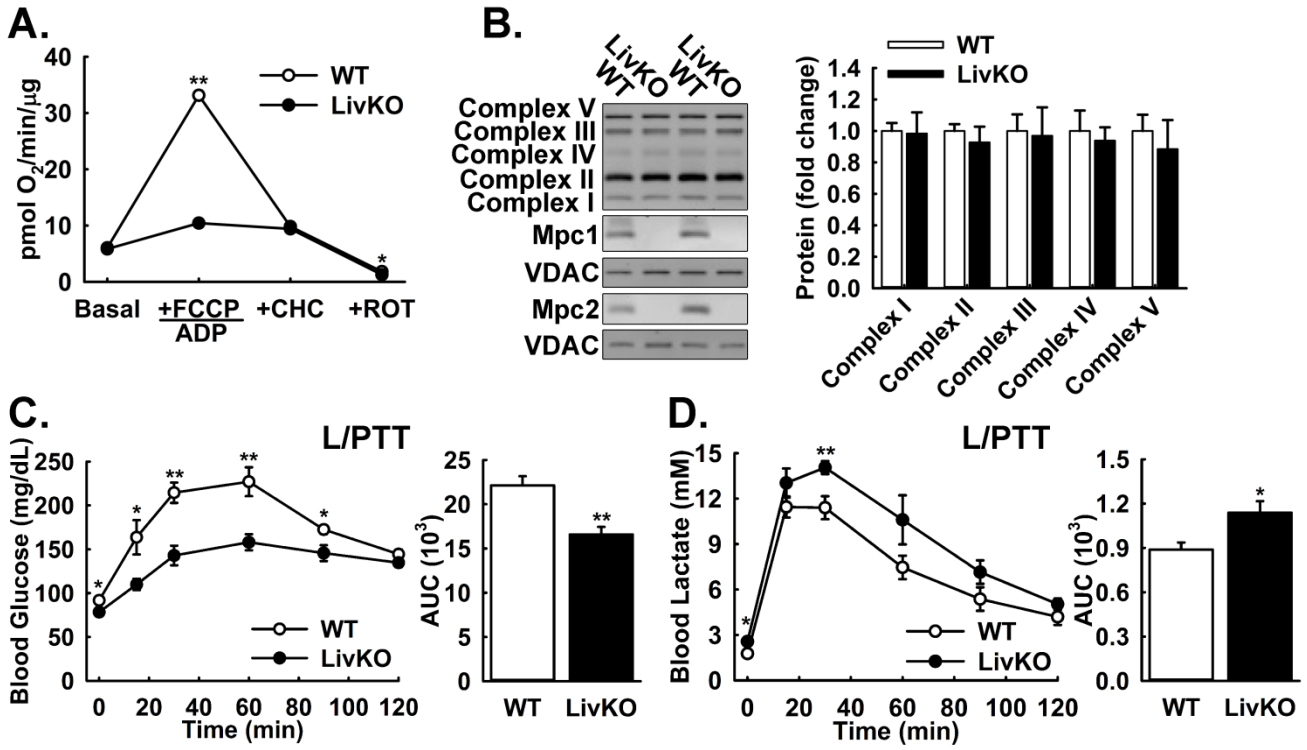
Supplemental Figure 1, related to Figure 1

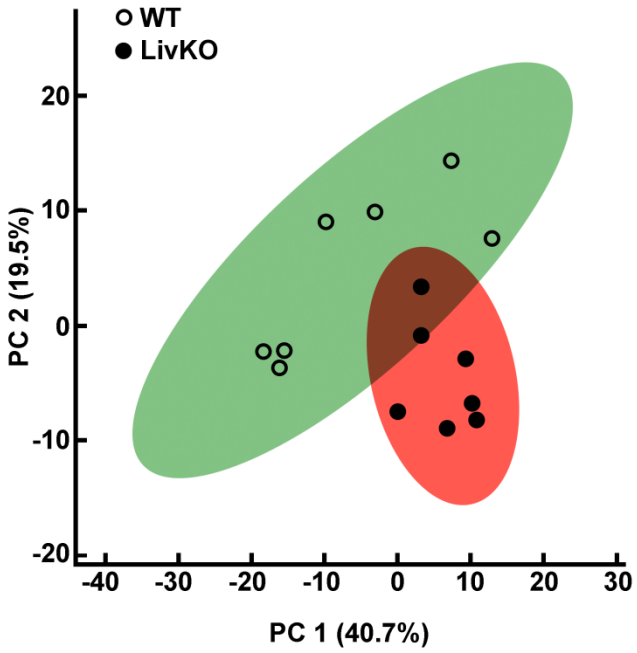


Supplemental Figure 2, related to Figure 2



Supplemental Figure 3, related to Figure 4

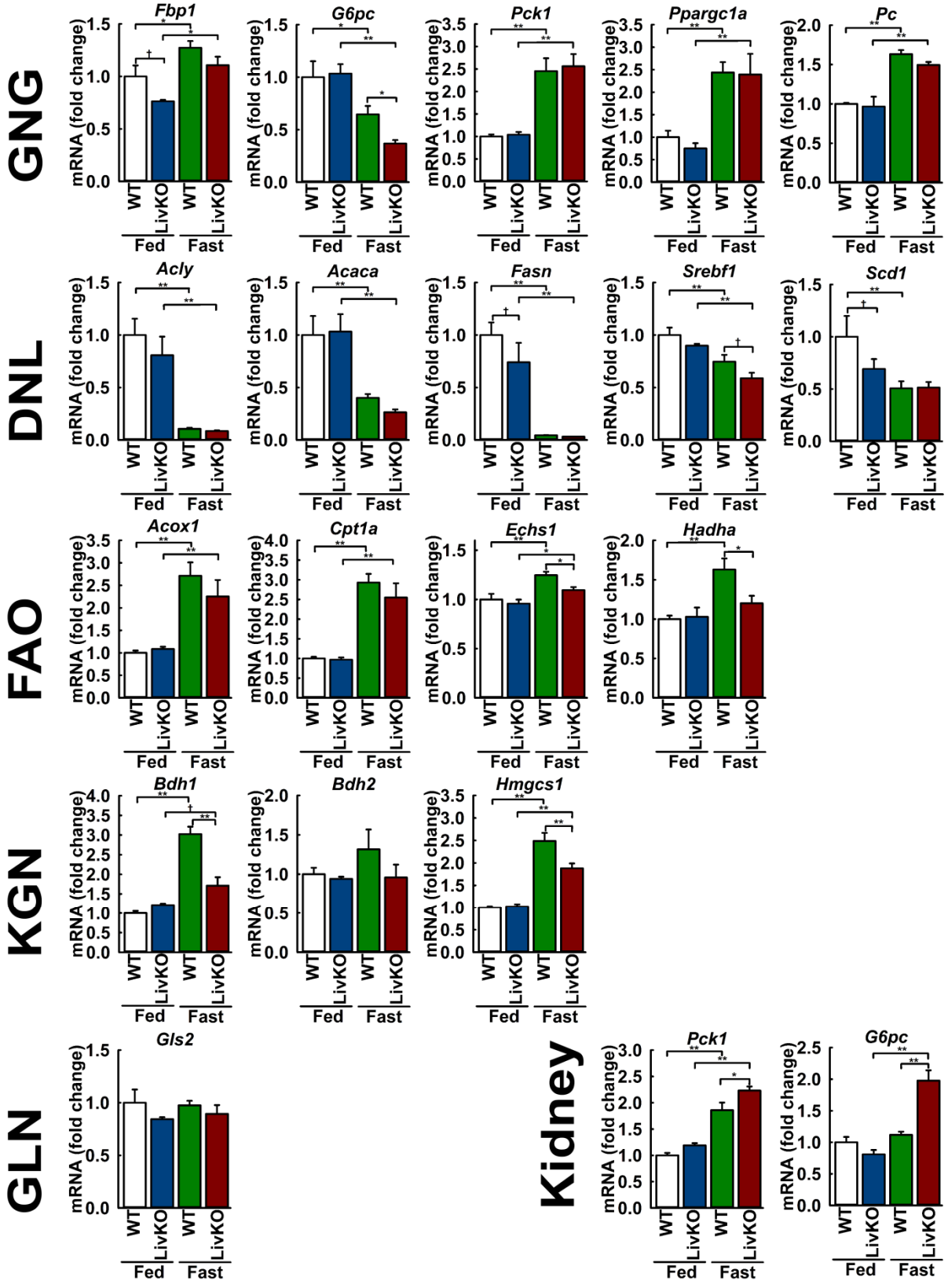
A.



B.

	Metabolite	LivKO/ WT
TCA	citric acid	1.14
	aconitate	1.33
	isocitric acid	0.77
	2-ketoglutaric acid	2.33**
	succinic acid	1.31
	fumaric acid	0.82
	malic acid	0.67
Glycolysis	3-phosphoglycerate	1.50
	2-phosphoglycerate	3.09*
	DHAP	1.33
	phosphoenolpyruvate	1.22
	1,3-bisphosphoglycerate	0.95
	fructose	1.24
	glucose	1.13
	fructose-6-phosphate	1.53*
	glucose-6-phosphate	1.55
Pentose Phosphate Pathway	ribose	2.21
	sedoheptulose	1.53*
	sedoheptulose-7-P	1.60
	erythrose	0.42
	erythrose-4-phosphate	1.06
	ribitol or xylitol	1.19
	ribose(xyl)-5-phosphate	1.37
Amino Acids	lysine	4.13**
	valine	1.60*
	leucine	1.41
	isoleucine	1.18
	threonine	1.43
	homoserine	1.74**
	glycine	1.14
	serine	1.36
	alanine	1.27
	glutamic acid	0.76
	glutamine	0.69*
	proline	1.72**
	aspartic acid	0.34***
	asparagine	2.10*
	methionine	1.24
	cysteine	1.32
	phenylalanine	1.13
	tyrosine	1.08
	tryptophan	1.44*

Supplemental Figure 4, related to Figure 4



Supplemental Figure 5, related to Figure 5

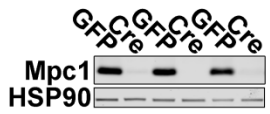
A.

Serum Parameters (HFD, WT vs Mpc1 LivKO)

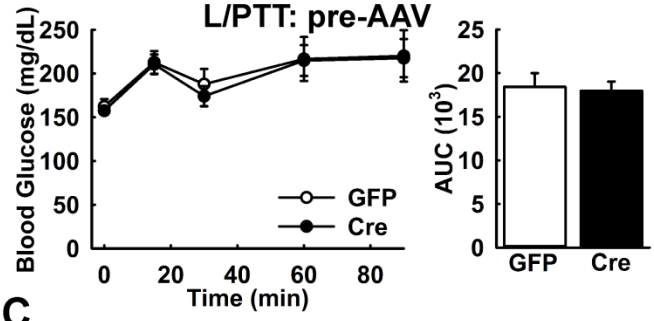
	4hr Fast		18hr Fast		
	WT	LivKO	WT	LivKO	
Insulin	1.66 ± 0.27	1.37 ± 0.19	0.45 ± 0.04	0.40 ± 0.02	ng/mL
Triglycerides	54.8 ± 3.7	65.2 ± 2.5*	79.2 ± 6.6	80.9 ± 7.8	mg/dL
NEFAs	0.32 ± 0.07	0.38 ± 0.07	0.60 ± 0.02	0.57 ± 0.04	mEq/L
Cholesterol	207.4 ± 6.6	207.3 ± 11.1	171.5 ± 7.1	170.4 ± 8.1	mg/dL
Ketones	189.9 ± 29.3	371.0 ± 26.2**	633.4 ± 71.4	683.7 ± 39.4	µmol/L
Lactate	2.02 ± 0.27	1.96 ± 0.11	1.57 ± 0.10	2.16 ± 0.13**	mmol/L
Glucose	190.0 ± 6.2	167.2 ± 5.0**	139.2 ± 5.0	136.0 ± 7.0	mg/dL
AST/ALT	1.55 ± 0.10	1.58 ± 0.14	1.52 ± 0.11	1.75 ± 0.07	

Data are mean ± SEM
*= p<0.05, **= p<0.01

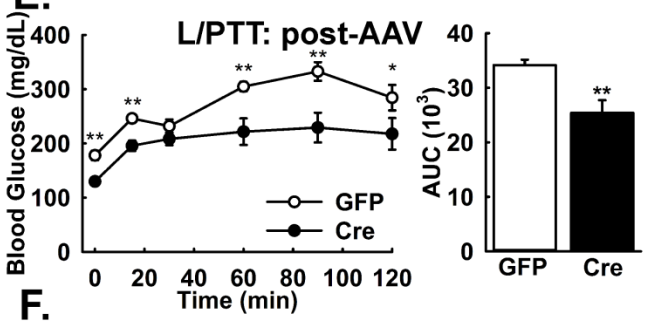
D.



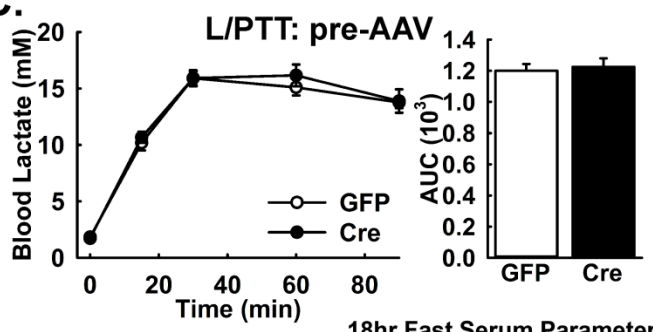
B.



E.



C.



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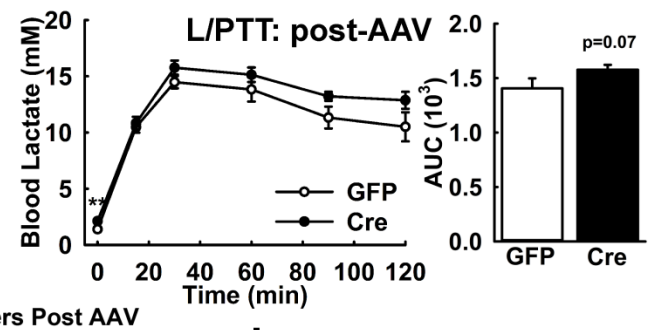
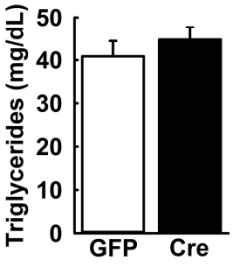


G.



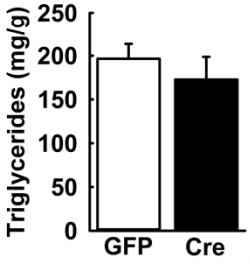
H.

18hr Fast Serum Parameters Post AAV

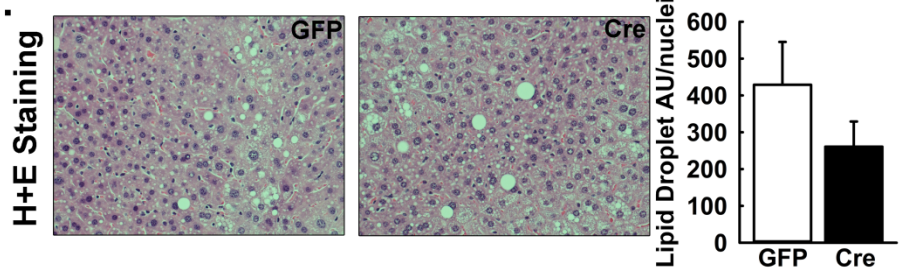
	AAV-GFP	AAV-Cre	
Insulin	1.84 ± 0.16	0.90 ± 0.09**	ng/mL
Triglycerides	64.4 ± 5.3	83.5 ± 5.6*	mg/dL
NEFAs	N.D.	N.D.	mEq/L
Ketones	609.7 ± 44.8	797.0 ± 95.2	µmol/L
Lactate	1.68 ± 0.31	2.08 ± 0.16	mmol/L
Glucose	178.8 ± 8.7	122.5 ± 7.8**	mg/dL
AST/ALT	1.16 ± 0.12	1.26 ± 0.12	

Data are mean ± SEM
*= p<0.05, **= p<0.01

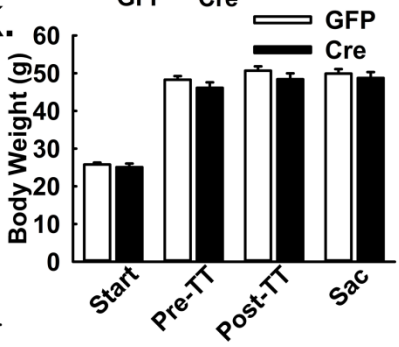
J.



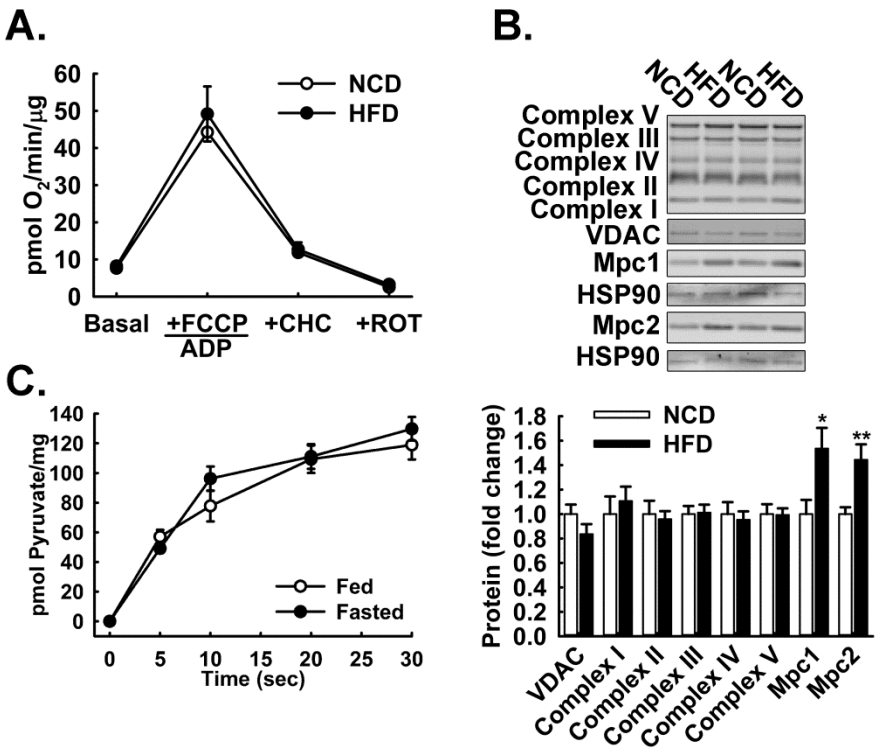
I.



K.



Supplemental Figure 6, related to Figure 6



Supplemental Figure and Table Legends

Supplemental Table 1, related to Figure 4:

Absolute concentration, isotopomer distribution, and isotopomer concentration of metabolites 30 minutes after $U^{13}C$-pyruvate/$U^{13}C$-lactate injection in Mpc1 LivKO versus WT livers.
Data are presented as mean ± SEM.

Supplemental Table 2, related to Figure 4:

Relative steady-state abundance of 118 metabolites in Mpc1 LivKO versus WT livers.
Data is presented as the fold change (LivKO/WT) and the associated p-value.

Supplemental Figure 1, related to Figure 1:

A: Pyruvate-driven (10 mM pyruvate/2 mM malate) respiration by liver mitochondria isolated from *Mpc1*$^{fl/fl}$ and *Mpc1*$^{+/+}$ mice. (n=4)
B: Western Blot analysis of liver lysates from *Mpc1*$^{fl/fl}$ and *Mpc1*$^{+/+}$ mice probed for Mpc1, Mpc2, or actin as indicated.
C: Body weight of WT and Mpc1 LivKO mice. (n=8).
D: Percentage body mass of fat, lean, and fluid of WT and Mpc1 LivKO mice. (n=8)
E: Glucose infusion rate required during terminal 20 minutes of the euglycemic hyperinsulinemic clamp. (n=5-6)
Data are presented as mean ± SEM (**p<0.01).

Supplemental Figure 2, related to Figure 2:

A: Pyruvate-driven (2.5 mM pyruvate/0.25 mM malate) respiration by liver mitochondria isolated from WT and Mpc1 LivKO mice. (n=4)
B: Western blot analysis and densitometric quantification of liver mitochondrial lysates from WT and Mpc1 LivKO mice. Membranes were probed for electron transport chain components

(Complex I-V) Mpc1, Mpc2, and VDAC. Densitometric quantification of ETC components are relative to VDAC. (n=8).

C: Blood glucose excursion in female WT and Mpc1 LivKO mice during a lactate/pyruvate tolerance test (L/PTT). Blood glucose was measured serially and an AUC calculated. (n=6)

D: Blood lactate clearance in female WT and Mpc1 LivKO mice during a lactate/pyruvate tolerance test (L/PTT). Blood lactate was measured serially and an AUC calculated. (n=6)

Data are presented as mean ± SEM (*p < 0.05, **p<0.01).

Supplemental Figure 3, related to Figure 4:

A: Principle component analysis (PCA) score plot of the first and second principle components (PCs) from 108 polar metabolites. PC 1 explains 40.7% of chemical variance while PC 2 explains 19.5%. From the PCA data two separate groups were observed corresponding to WT and Mpc1 LivKO mice. (n=7)

B: Relative steady-state abundance of select metabolites in Mpc1 LivKO versus WT livers (LivKO/WT) catergorized into TCA cycle, Glycolysis, Pentose Phosphate Pathway, and Amino Acids. With respect to the Mpc1 LivKO mice increased metabolites are highlighted in green and decreased metabolites are highlighted in red. (n=7)

Data are presented as mean ± SEM (*p<0.05, **p<0.01, ***p<0.001).

Supplemental Figure 4, related to Figure 4:

qPCR analysis of mRNA isolated from the liver or kidney of WT and Mpc1 LivKO mice fed ad lib or fasted for 18 hours. Pathways analyzed include: GNG, gluconeogenesis; DNL, de novo lipogenesis; FAO, fatty acid oxidation; KGN, ketogenesis; GLN, glutaminolysis; and Kidney, mRNA isolated from kidney. *G6pc* data shown generated using *G6pc* #1 primers. (Liver n=4-6 (*Srebf1*, *Fbp1* fed LivKO n=3), Kidney n=4-9)

Data are presented as mean ± SEM (2-way ANOVA; †p<0.1, *p<0.05, **p<0.01).

Supplemental Figure 5, related to Figure 5:

A: 4 and 18 hour fasted serum parameter of WT and Mpc1 LivKO mice after being fed a HFD for 12 weeks. (n=10)

B-C: HFD *Mpc1*$^{fl/fl}$ mice underwent a L/PTT prior to AAV-GFP (GFP) or AAV-Cre (Cre) injection. (B) Blood glucose was measured serially and an AUC was calculated. (C) Blood lactate was measured serially and an AUC was calculated. (n=10)

D: Western blot analysis of AAV-GFP and AAV-Cre injected mice at the time of sac. Membranes were probed for Mpc1 and HSP90.

E-F: Three weeks after AAV-GFP or AAV-Cre injection L/PTTs were repeated on HFD *Mpc1*$^{fl/fl}$ mice. (E) Blood glucose was measured serially and an AUC was calculated. (F) Blood lactate was measured serially and an AUC was calculated. (n=10)

G: Serum triglycerides of ad lib fed HFD *Mpc1*$^{fl/fl}$ mice after AAV-GFP or AAV-Cre injection. (n=8-10)

H: Serum parameters of AAV-GFP- and AAV-Cre-treated, HFD *Mpc1*$^{fl/fl}$ mice after an 18 hour fast. (n=8-10)

I: Representative H+E stained liver sections of AAV-GFP- and AAV-Cre-treated, HFD *Mpc1*$^{fl/fl}$ mice. Quantification of lipid droplet area relative to number of nuclei per field. (n=6)

J: Liver triglycerides of AAV-GFP- and AAV-Cre-treated, HFD *Mpc1*$^{fl/fl}$ mice after an 18 hour fast. (n=7)

K: Body weights of AAV-GFP- and AAV-Cre-treated HFD *Mpc1*$^{fl/fl}$ mice before, during and after injection. (n=10)

Data are presented as mean ± SEM (*$p < 0.05$, **$p<0.01$).

Supplemental Figure 6, related to Figure 6:

A: Pyruvate driven respiration (2.5 mM pyruvate/0.25 mM malate) by liver mitochondria isolated from NCD and HFD mice. (n=4)

B: Western blot analysis and densitometric quantification of liver lysates from NCD and HFD mice. Membranes were probed for electron transport chain components (Complex I-V) Mpc1, Mpc2, VDAC, and HSP90. Densitometric quantification of ETC components are relative to HSP90. (n=8)

D: ^{14}C-Pyruvate uptake in isolated liver mitochondria from 18hr fasted and ad lib fed WT mice. (n=8).

Data are presented as mean ± SEM (*$p < 0.05$, **$p<0.01$).

Table S1 - Isotopomer Distribution and Concentration

Metabolite Concentration (nmol/mg) x Isotopomer Distribution (%) = Isotopomer Concentration (nmol/mg)

	Concentration (nmol/mg)		m0	m+1	m+2	m+3	m+4	m+5	m+6	Total Enrichment
Glutamine (5 carbon)	0.92 ± 0.07	WT	81.78 ± 0.66 0.74 ± 0.06	5.52 ± 0.14 0.049 ± 0.004	9.55 ± 0.38 0.084 ± 0.007	3.01 ± 0.17 0.024 ± 0.002	0.080 ± 0.06 0.0006± 0.0005	0.067 ± 0.03 0.0005 ± 0.0002		0.15 ± 0.007
	1.04 ± 0.05	LivKO	85.37 ± 0.38 0.89 ± 0.045	4.39 ± 0.23 0.046 ± 0.003	7.86 ± 0.18 0.082 ± 0.002	2.19 ± 0.12 0.023 ± 0.0004	0.10 ± 0.06 0.0011 ± 0.0004	0.083 ± 0.01 0.0009 ± 0.0001		0.15 ± 0.005
	1.13	LivKO/WT	1.04 1.23	0.80 0.93	0.82 0.97	0.73 0.94	1.29 1.94	1.25 1.60		0.99
	0.13	p-value	0.00 0.10	0.00 0.71	0.01 0.56	0.01 0.38	0.80 0.41	0.65 0.13		0.86
Glutamate (5 carbon)	0.84 ± 0.07	WT	77.10 ± 0.81 0.62 ± 0.054	7.50 ± 0.17 0.060 ± 0.005	11.68 ± 0.46 0.094 ± 0.009	3.68 ± 0.19 0.030 ± 0.003	-0.02 ± 0.08 -0.0004 ± 0.0005	0.05 ± 0.02 0.0004 ± 0.0001		0.18 ± 0.016
	1.13 ± 0.32	LivKO	81.88 ± 0.56 0.93 ± 0.25	6.17 ± 0.18 0.069 ± 0.019	9.33 ± 0.27 0.11 ± 0.03	2.57 ± 0.17 0.030 ± 0.009	0.01 ± 0.02 -0.0001 ± 0.0004	0.04 ± 0.004 0.0004 ± 0.0001		0.15 ± 0.031
	1.13	LivKO/WT	1.06 1.49	0.82 1.15	0.80 1.12	0.70 1.00	-0.73 0.35	0.74 1.09		0.83
	0.36	p-value	0.00 0.30	0.00 0.66	0.00 0.72	0.00 0.99	0.71 0.73	0.57 0.81		0.36
Aspartate (4 carbon)	0.49 ± 0.03	WT	75.88 ± 1.29 0.37 ± 0.029	9.14 ± 0.28 0.045 ± 0.004	6.27 ± 0.35 0.031 ± 0.003	7.92 ± 0.60 0.039 ± 0.005	0.78 ± 0.11 0.004 ± 0.001			0.12 ± 0.012
	0.31 ± 0.07	LivKO	85.12 ± 2.29 0.26 ± 0.049	5.83 ± 0.87 0.020 ± 0.007	4.00 ± 0.41 0.013 ± 0.004	4.59 ± 0.95 0.017 ± 0.007	0.46 ± 0.09 0.002 ± 0.001			0.052 ± 0.018
	0.64	LivKO/WT	1.12 0.70	0.64 0.45	0.64 0.44	0.58 0.43	0.58 0.41			0.44
	0.04	p-value	0.01 0.09	0.01 0.01	0.00 0.01	0.02 0.03	0.04 0.03			0.02
Alanine (4 carbon)	0.85 ± 0.06	WT	76.04 ± 0.93 0.63 ± 0.044	2.06 ± 0.11 0.017 ± 0.002	2.63 ± 0.06 0.022 ± 0.002	19.98 ± 0.99 0.16 ± 0.014	-0.70 ± 0.03 -0.0057 ± 0.0004			0.20 ± 0.0156
	1.33 ± 0.49	LivKO	78.20 ± 1.20 1.02 ± 0.36	1.51 ± 0.09 0.021 ± 0.008	1.75 ± 0.09 0.025 ± 0.010	19.21 ± 1.18 0.27 ± 0.11	-0.67 ± 0.04 -0.0094 ± 0.004			0.31 ± 0.125
	1.55	LivKO/WT	1.03 1.63	0.73 1.20	0.66 1.12	0.96 1.66	0.96 1.65			1.56
	0.31	p-value	0.19 0.31	0.00 0.67	0.00 0.80	0.63 0.36	0.59 0.37			0.41
Glucose (6 carbon)	1.61 ± 0.16	WT	67.68 ± 3.24 1.05 ± 0.14	8.60 ± 0.705 0.15 ± 0.008	10.67 ± 1.09 0.18 ± 0.006	10.06 ± 1.08 0.173 ± 0.0059	1.31 ± 0.163 0.022 ± 0.0009	1.14 ± 0.153 0.019 ± 0.0008	0.543 ± 0.075 0.009 ± 0.0003	0.55 ± 0.018
	1.36 ± 0.11	LivKO	78.40 ± 2.02 1.05 ± 0.10	6.30 ± 0.53 0.10 ± 0.007	7.01 ± 0.65 0.10 ± 0.009	6.70 ± 0.65 0.10 ± 0.008	0.65 ± 0.09 0.0095 ± 0.001	0.56 ± 0.08 0.0083 ± 0.0009	0.384 ± 0.06 0.006 ± 0.0006	0.31 ± 0.026
	0.85	LivKO/WT	1.16 1.00	0.73 0.62	0.66 0.56	0.67 0.57	0.50 0.43	0.49 0.43	0.71 0.60	0.57
	0.25	p-value	0.02 0.98	0.03 0.00	0.02 0.00	0.02 0.00	0.01 0.00	0.01 0.00	0.13 0.00	0.00
Lactate (3 carbon)	436.94 ± 1.60	WT	70.25 ± 1.12 305.55 ± 29.08	1.88 ± 0.16 8.01 ± 0.62	2.34 ± 0.05 10.16 ± 0.95	25.53 ± 1.31 113.22 ± 15.13				131.39 ± 16.40
	698.36 ± 33.58	LivKO	71.86 ± 0.81 501.47 ± 23.01	1.55 ± 0.10 10.86 ± 0.89	1.78 ± 0.06 12.39 ± 0.68	24.81 ± 0.89 173.64 ± 11.83				196.89 ± 12.50
	1.60	LivKO/WT	1.02 1.64	0.83 1.36	0.76 1.22	0.97 1.53				1.50
	0.00	p-value	0.27 0.00	0.10 0.03	0.00 0.09	0.66 0.01				0.01
Pyruvate (3 carbon)	29.42 ± 2.65	WT	73.36 ± 1.16 21.59 ± 1.94	5.75 ± 0.41 1.67 ± 0.014	6.29 ± 0.29 1.82 ± 0.12	14.60 ± 1.54 4.35 ± 0.72				7.84 ± 0.82
	34.08 ± 2.84	LivKO	73.13 ± 0.89 24.85 ± 1.91	2.26 ± 0.22 0.76 ± 0.07	2.49 ± 0.25 0.83 ± 0.07	22.13 ± 1.19 7.64 ± 0.94				9.22 ± 0.98
	1.16	LivKO/WT	1.00 1.15	0.39 0.45	0.40 0.46	1.52 1.75				1.18
	0.26	p-value	0.88 0.26	0.00 0.00	0.00 0.00	0.00 0.02				0.30

Table S1 - Isotopomer Distribution and Concentration

Metabolite Concentration (nmol/mg) x Isotopomer Distribution (%) = Isotopomer Concentration (nmol/mg)

	Concentration (nmol/mg)		m0	m+1	m+2	m+3	m+4	m+5	m+6	Total Enrichment
Citrate (6 carbon)	89.91 ± 9.15	WT	69.45 ± 1.01 62.30 ± 6.16	10.95 ± 0.31 9.79 ± 0.96	8.11 ± 0.33 7.24 ± 0.70	10.23 ± 0.67 9.46 ± 1.42	1.10 ± 0.11 0.99 ± 0.14	0.11 ± 0.04 0.09 ± 0.04	0.051 ± 0.01 0.04 ± 0.01	27.61 ± 3.15
	37.80 ± 3.84	LivKO	74.86 ± 0.60 28.26 ± 2.83	9.78 ± 0.16 3.70 ± 0.39	8.45 ± 0.32 3.25 ± 0.46	5.95 ± 0.37 2.22 ± 0.22	0.83 ± 0.05 0.31 ± 0.03	0.081 ± 0.02 0.03 ± 0.01	0.040 ± 0.02 0.01 ± 0.01	9.53 ± 1.05
	0.42	LivKO/WT	1.08 0.45	0.89 0.38	1.04 0.45	0.58 0.23	0.76 0.32	0.74 0.36	0.79 0.32	0.35
	0.00	p-value	0.00 0.00	0.01 0.00	0.47 0.00	0.00 0.00	0.05 0.00	0.55 0.18	0.61 0.01	0.00
AKG (5 carbon)	3.27 ± 0.61	WT	78.20 ± 0.71 2.55 ± 0.46	7.40 ± 0.17 0.24 ± 0.046	10.76 ± 0.38 0.36 ± 0.075	3.42 ± 0.22 0.12 ± 0.026	0.15 ± 0.05 0.004 ± 0.001	0.063 ± 0.02 0.002 ± 0.001		0.72 ± 0.15
	4.54 ± 0.68	LivKO	81.91 ± 0.89 3.72 ± 0.57	6.58 ± 0.31 0.30 ± 0.05	8.97 ± 0.46 0.41 ± 0.06	2.35 ± 0.21 0.10 ± 0.011	0.16 ± 0.02 0.008 ± 0.002	0.036 ±0.01 0.002 ± 0.0004		0.82 ± 0.12
	1.39	LivKO/WT	1.05 1.46	0.89 1.24	0.83 1.13	0.69 0.88	1.08 2.15	0.57 0.80		1.13
	0.19	p-value	0.01 0.14	0.04 0.39	0.01 0.63	0.01 0.64	0.85 0.14	0.17 0.62		0.62
Succinate (4 carbon)	16.63 ± 0.37	WT	82.12 ± 0.951 13.65 ± 0.237	5.67 ± 0.178 0.95 ± 0.05	11.07 ± 0.66 1.84 ± 0.126	0.90 ± 0.21 0.15 ± 0.038	0.24 ± 0.05 0.041 ± 0.0085			2.98 ± 0.11
	11.73 ± 0.69	LivKO	89.45 ± 0.62 10.48 ± 0.595	3.55 ± 0.22 0.42 ± 0.042	6.64 ± 0.37 0.78 ± 0.073	0.27 ± 0.08 0.032 ± 0.010	0.085 ± 0.01 0.0099 ± 0.0014			1.25 ± 0.12
	0.71	LivKO/WT	1.09 0.77	0.63 0.44	0.60 0.42	0.30 0.21	0.35 0.24			0.42
	0.00	p-value	0.00 0.00	0.00 0.00	0.00 0.00	0.01 0.01	0.00 0.00			0.00
Fumarate (4 carbon)	11.02 ± 0.57	WT	70.98 ± 1.15 7.81 ± 0.40	10.99 ± 0.44 1.21 ± 0.072	7.00 ± 0.12 0.77 ± 0.04	9.77 ± 0.64 1.09 ± 0.108	1.26 ± 0.23 0.14 ± 0.024			3.20 ± 0.22
	6.30 ± 0.44	LivKO	74.49 ± 1.12 4.68 ± 0.28	11.31 ± 0.34 0.71 ± 0.045	5.49 ± 0.39 0.35 ± 0.05	7.34 ± 0.61 0.47 ± 0.068	1.36 ± 0.20 0.088 ± 0.016			1.62 ± 0.17
	0.57	LivKO/WT	1.05 0.60	1.03 0.59	0.79 0.46	0.75 0.43	1.08 0.64			0.51
	0.00	p-value	0.05 0.00	0.57 0.00	0.00 0.00	0.02 0.00	0.76 0.11			0.00
Malate (4 carbon)	61.01 ± 2.28	WT	70.23 ± 1.01 42.94 ± 2.11	11.09 ± 0.25 6.76 ± 0.25	7.23 ± 0.25 4.39 ± 0.10	10.43 ± 0.66 6.32 ± 0.323	1.02 ± 0.10 0.61 ± 0.044			18.07 ± 0.44
	38.45 ± 2.22	LivKO	75.32 ± 0.84 28.88 ± 1.40	9.65 ± 0.15 3.72 ± 0.27	6.04 ± 0.18 2.34 ± 0.21	8.23 ± 0.51 3.20 ± 0.356	0.75 ± 0.08 0.30 ± 0.046			9.54 ± 0.86
	0.63	LivKO/WT	1.07 0.67	0.87 0.55	0.84 0.53	0.79 0.51	0.74 0.49			0.53
	0.00	p-value	0.00 0.00	0.00 0.00	0.00 0.00	0.02 0.00	0.06 0.00			0.00
OAA (4 carbon)	0.73 ± 0.07	WT	72.30 ± 1.37 0.52 ± 0.05	10.89 ± 0.36 0.079 ± 0.008	7.00 ± 0.40 0.052 ± 0.007	8.87 ± 0.66 0.066 ± 0.011	0.94 ± 0.20 0.0069 ± 0.0016			0.20 ± 0.027
	0.55 ± 0.04	LivKO	77.52 ± 1.14 0.43 ± 0.036	9.83 ± 0.49 0.055 ± 0.006	5.17 ± 0.50 0.028 ± 0.002	6.53 ± 0.71 0.036 ± 0.004	0.94 ± 0.16 0.0054 ± 0.0013			0.12 ± 0.011
	0.76	LivKO/WT	1.07 0.82	0.90 0.69	0.74 0.54	0.74 0.54	1.01 0.77			0.61
	0.08	p-value	0.02 0.16	0.12 0.05	0.02 0.01	0.04 0.03	0.98 0.47			0.03

Table S2 - Steady State Metabolomic Fold Change

Metabolite	LivKO/WT	p-value
lactic acid	3.70	0.000083
pyruvic acid	1.59	0.0022
glycerol	1.40	0.10
glyceric acid	1.25	0.20
citric acid	1.14	0.28
aconitate	1.33	0.071
isocitric acid	0.77	0.34
2-ketoglutaric acid	2.33	0.019
succinic acid	1.31	0.087
fumaric acid	0.82	0.22
malic acid	0.67	0.048
2-hydroxyglutarate	1.08	0.24
2-aminoadipic acid	2.55	0.017
lysine	4.13	0.0048
valine	1.60	0.043
leucine	1.41	0.12
isoleucine	1.18	0.35
threonine	1.43	0.090
homoserine	1.74	0.0015
glycine	1.14	0.24
serine	1.36	0.21
alanine	1.27	0.21
glutamic acid	0.76	0.16
glutamine	0.69	0.024
proline	1.72	0.0071
aspartic acid	0.34	0.000084
asparagine	2.10	0.010
methionine	1.24	0.15
cysteine	1.32	0.098
homocysteine	2.42	0.027
phenylalanine	1.13	0.26
tyrosine	1.08	0.64
tryptophan	1.44	0.019
sarcosine	3.03	0.0066
4-hydroxyproline	1.16	0.28
ornithine	4.80	0.0031
N-acetylglutamate	2.15	0.014
N-acetylaspartate	1.38	0.16
N-acetylglycine	1.13	0.57
B-alanine	1.76	0.0050
phosphate	1.10	0.54
diphosphate	1.16	0.49
1-phosphoglycerol	1.11	0.67

Table S2 - Steady State Metabolomic Fold Change

Metabolite	LivKO/WT	p-value
2-phosphoglycerol	1.57	0.30
3-phosphoglycerate	1.50	0.20
2-phosphoglycerate	3.09	0.033
DHAP	1.33	0.47
phosphoenolpyruvate	1.22	0.54
1,3-bisphosphoglycerate	0.95	0.69
fructose	1.24	0.13
mannose or galactose	1.07	0.59
ribose	2.21	0.099
glucose	1.13	0.22
glucose-1-phosphate	1.59	0.11
fructose-6-phosphate	1.53	0.038
glucose-6-phosphate	1.55	0.12
mannitol	1.11	0.60
sorbitol	0.97	0.71
galacitol	1.60	0.41
gluconic acid	1.17	0.16
inositol	1.19	0.024
myo-inositol phosphate	0.85	0.44
sedoheptulose	1.53	0.0045
sedoheptulose-7-P	1.60	0.057
GalNAc	1.35	0.48
GlcNAc	0.86	0.63
erythrose	0.42	0.10
erythrose-4-phosphate	1.06	0.86
ribitol or xylitol	1.19	0.17
ribose(xyl)-5-phosphate	1.37	0.44
fucose	1.36	0.0039
lauric acid	1.14	0.28
myristic acid	1.45	0.014
palmitiladic acid	1.27	0.23
17:0 FA	1.28	0.30
palmitic acid	1.12	0.57
linoleic acid	1.26	0.30
oleic acid	1.38	0.14
elaidic acid	1.06	0.75
stearic acid	1.20	0.41
19:0 FA	1.30	0.32
arachidonic acid	1.15	0.58
20:1 FA	1.24	0.32
20:0 FA	0.84	0.24
22:0 FA	1.57	0.14
1-monolinoleoylglycerol	1.18	0.057

Table S2 - Steady State Metabolomic Fold Change

Metabolite	LivKO/WT	p-value
1-monooleoylglycerol	1.16	0.090
1-monostearylglycerol	1.28	0.16
1-monopalmitoylglycerol	1.13	0.49
cholesterol	1.19	0.31
pantothenic acid	1.31	0.011
phosphoethanolamine	1.33	0.026
xanthine	3.14	0.28
hypoxanthine	3.02	0.29
adenosine	1.29	0.41
inosine	1.27	0.25
adenine	1.33	0.20
uracil	1.61	0.29
adenosine-5'-monophosphate	1.59	0.42
urea	1.57	0.00029
creatinine	2.80	0.0065
3-hydroxybutyrate	0.79	0.0047
aminomalonic acid	0.93	0.52
uric acid	4.00	0.21
dopamine	2.40	0.082
B-hydroxy-B-methylglutaric ac	0.61	0.00032
hydroxyphenyllactic acid	2.39	0.0036
sn-glycero-3-phosphoethanolamine	0.80	0.123
2-hydroxybutyric acid	3.44	0.000022
nicotinamide	1.43	0.13
GSH reduced	0.94	0.72
ascorbate	1.03	0.90
2-hydroxypentanoic acid	1.62	0.0000067
2-aminobutyric acid	2.21	0.00039
N-methyl B-alanine	2.37	0.00053
benzoic acid	1.01	0.91
hexanedioic acid	1.21	0.12
3-hydroxyhexanedioic acid	0.83	0.25

Supplemental Experimental Procedures

Animals and Animal Care

Animal work was performed in accordance with the University of Iowa Animals Use and Care Committee (IACUC). Unless otherwise stated all experiments were performed with 8-12 week old male mice. Mice were maintained on Harlan Scientific 2920i diet (normal chow) or Research Diets Inc D12492i (high fat) feed. Wildtype C57Bl/6J mice were purchased directly from Jackson Laboratories (000664). Wildtype 275-299g Sprague Dawley rats were purchased directly from Harlan Laboratories.

Generation of *Mpc1*$^{fl/fl}$ and *Mpc1*$^{fl/fl}$ Alb-Cre mice

The generation of mice carrying the *Mpc1*^{fl} allele was performed with the assistance of the University of Utah Transgenic Gene-targeting mouse facility. *Mpc1*^{fl} mouse embryonic stem cells were obtained from EuCOMM (IKMC:51198) and utilized a knockout-first approach (Skarnes et al., 2011). The *Mpc1*^{fl} construct was composed of a promoter driven LacZ and NeoR cassettes flanked with FRT sequences upstream of exons 3-5 of the Mpc1 allele which, in turn, were flanked by LoxP sequences. Recombinant mouse ES cells from a C57Bl6/N genetic background were injected into blastocysts and implanted into pseudopregnant C57Bl6/J female mice. High percentage chimeric mice were interbred with WT C57Bl6/J to test for germline transmission of the *Mpc1*^{fl} construct (BC=1). The LacZ/NeoR cassette was removed by breeding heterozygous mice with C57Bl6/J + Flp mice (BC=2), which express the *S. cerevisiae* FLP1 FLPe variant driven by the ROSA promoter in a widespread manner which results in recombination at the FRT sites and removal of the intervening sequences (Figure 1B). Offspring were subsequently crossed with WT C57Bl6/J mice and genotyped to confirm Flp removal and NeoR excision (BC=3). Mice negative for both Flp and NeoR were used to establish our experimental mouse colonies. Mpc1 knockout mice were generated by interbreeding *Mpc1*$^{fl/fl}$ with mice hemizygous for Cre recombinase under the control of

the albumin promoter, which results in the recombination of the LoxP sites and removal of exons 3-5. Experimental cohorts were generated by crossing *Mpc1*$^{fl/fl}$ and *Mpc1*$^{fl/fl}$ Alb-Cre mice to generate *Mpc1*$^{fl/fl}$ Alb-Cre liver specific knockout mice (Mpc1 LivKO) and *Mpc1*$^{fl/fl}$ (WT) littermates, which were used as controls.

Acute Mpc1 Liver Specific Knockout

AAV8.TBG.PI.Cre.rBG (AV-8-PV1090, AAV-Cre) and AAV6.TBG.PI.eGFP.WPRE.bGH (AV-8-PV0146, AAV-GFP) were purchased from the UPenn Vector Core. *Mpc1*$^{fl/fl}$ mice were anesthetized with isofluorane and injected retro-orbitally with either AAV-Cre or AAV-GFP at a concentration of $2x10^{11}$ genome copies per mouse diluted in 100µL PBS. Mice were allowed to recover and monitored for any health concerns.

Tolerance Tests, CLAMS, and NMR

In general, prior to any tolerance test mice were placed into fresh cages and fasted for the indicated amount of time. Dosages, unless otherwise stated, were based on lean mass and prepared in sterile water. Injections were administered IP. Blood glucose and blood lactate were measured using a One Touch UltraMini glucometer and Nova Biomedical Lactate Plus lactate meter, respectively. Glucose tolerance test (GTT) – 6hr fast, 2.0g/kg. Combined Lactate Pyruvate tolerance test (L/PTT) – 18hr fast, 3.0g/kg. 10 parts lactate and 1 part pyruvate. Glutamine tolerance test (QTT) - 18hr fast, 2.0 g/kg. Insulin Tolerance Test (ITT) - 4hr fast, 1.5 U/kg Insulin (Humalin, Eli Lilly) in 0.9% saline. Comprehensive Lab Animal Monitoring System measurements (Columbus Instruments) and body composition measurements (NMR, Bruker MiniSpec) were performed under the direction of the University Of Iowa Fraternal Order Of Eagles Diabetes Research Center Metabolic Phenotyping Core.

Traced Hyperinsulinemic Euglycemic Clamps

A silicone catheter (0.012" ID, Dow-Corning 501-001) was introduced into the jugular vein of mice during isoflurane anesthesia. Mice recovered from catheter placement for 7-10 days. Clamps were performed in 18 hour fasted, unrestrained, conscious mice. Whole-body glucose flux was traced by infusion of 0.05μCi/min D-[3-^{3}H]-glucose coupled to a priming 5μCi bolus. After an 80 min basal sampling period, insulin (Novolin R, Novo Nordisk, Denmark) administration was initiated with a 40mU/kg bolus followed by 4mU/kg/min continuous infusion. Blood glucose measurements were taken every ten minutes via tail vein sampling. At 75 min, [1-^{14}C]-2-deoxy-D-glucose was infused in a single 10μCi bolus. Plasma samples for determination of tracer enrichment and hormone measurement were taken at -80, -20, -10, 0, 80, 90, 100, 110 and 120 minutes, where time zero was the initiation of insulin infusion. Tissue samples were collected at the end of the clamp from liver, heart, kidney, white adipose tissue, brown adipose tissue, gastrocnemius, soleus, and extensor digitorum longus for determination of tracer enrichment. Glucose appearance and disappearance rates were calculated using Steele's equations (Steele et al., 1956). Plasma insulin was measured by chemiluminescence ELISA (#80-INSMR-CH01, American Laboratory Products Company, NH).

Western Blots

Snap-frozen liver tissue was homogenized in a buffer containing 40mM HEPES, 120mM NaCl, 50mM NaF, 5mM Sodium Pyrophosphate decahydrate, 5mM b-glycerolphosphate, 1mM EDTA, 1mM EGTA, 10% Glycerol (v/v), 1% Igepal CA-630 (v/v), with 1X protease inhibitor (G Biosciences, 786-437) and DTT (1μM). Homogenates were incubated at 4^{o}C for 30 minutes and centrifugation at 21,000x*g* before the supernatants were collected. Proteins were separated by 10% Tricine-SDS-PAGE gel (Schagger, 2006), transferred to 0.22μM nitrocellulose membranes (GE Healthcare, 10600001), and blocked with TBST (50mM Tris, 150mM NaCl, and 0.05% Tween-20) supplemented with 5% nonfat dry milk, incubated with primary antibodies at 4^{o}C overnight and

fluorescent secondary antibodies for 1 hour (ThermoFischer Scientific), and visualized using the Li-Cor Odyssey CLx system.

Antibodies

Primary Antibodies: Mpc1 (1:1,000, Cell Signaling Technology #14462), Mpc2 (1:2000, Cell Signaling Technology #46141), VDAC (1:1,000, Cell Signaling Technology #4661), HSP90 (1:2,000, Cell Signaling Technology #4874), b-actin (1:10,000, Sigma A5441), Total OXPHOS (1:1000, Abcam ab110413). Secondary antibodies: Goat anti-Mouse DyLight 800 (1:10,000, ThermoFischer SA5-10176), Donkey anti-Rabbit DyLight 680 (1:5,000, ThermoFischer SA5-10042), Goat anti-Rabbit DyLight 800 (1:10,000, ThermoFischer 35571).

Pyruvate Uptake Assay

Mitochondria were isolated essentially as described previously (Rogers et al., 2011) in buffer containing 70mM Sucrose, 210mM d-mannitol, 1mM EGTA, 0.5% fatty acid free BSA, and 5mM HEPES pH 7.2. The pyruvate uptake protocol was based on previously published methodology (Aires et al., 2008; Halestrap, 1975). Mitochondria were resuspended to approximately 6.8 mg/mL in Uptake Buffer (120mM KCL, 5mM KH_2PO_4, 1mM EGTA, 5mM HEPES pH 7.2, 1μM Rotenone, and 1μM Antimycin A) and were divided into two equal aliquots. One aliquot was treated with 2mM α-Cyano-4-hydroxycinnamic acid (CHC, Sigma-Aldrich 476870) and the other was treated with a volume of uptake buffer equal to the volume of CHC added. 165μL of treated mitochondria were rapidly mixed with 55μL of 4X Pyruvate buffer (160mM HEPES pH 6.8, 1μM Rotenone, 1μM Antimycin A, 0.12mM ^{14}C-Pyruvate, and 0.28mM ^{12}C-Pyruvate (Perkin Elmer NEC256050UC) generating the pH gradient needed to initiate uptake. 50μL samples were rapidly mixed with 100μL Stop buffer (Uptake Buffer supplemented with 10mM CHC and 40mM HEPES pH 6.8) to halt uptake. Mitochondria were recovered by passing the solution through a 0.8μM cellulose filter (Millipore AAWP-142-50) and a 0.45μM nitrocellulose filter (Bio-Rad 162-0115). Filters were

washed twice with 140µL Wash buffer (Uptake Buffer supplemented with 2mM CHC, 40mM HEPES pH 6.8, and 5mM Pyruvate) and placed into scintillation vials for quantification. Mitochondria pre-treated with CHC were used as a negative control and counts were subtracted from non-pre-treated mitochondria.

Oxygen Consumption Rate (OCR) Measurements

Oxygen consumption rate experiments were performed essentially as described previously (Rogers et al., 2011) using a Seahorse Bioscience XF-96 extracellular flux analyzer. Briefly, 5µg of isolated liver mitochondria suspended in a buffer containing 70mM Sucrose, 220mM d-mannitol, 10mM KH_2PO_4, 5mM $MgCl_2$, 5mM HEPES pH 7.2, 1mM EGTA, and 0.2% fatty acid free BSA were attached to V3-PET seahorse plates by centrifugation at 2000x*g* for 20 minute. Substrate-containing buffer was added such that the final concentrations were 10mM Pyruvate/2mM Malate (high pyruvate concentration), 2.5mM Pyruvate/0.25mM Malate (low pyruvate concentration), or 10mM Glutamate/2mM Malate. A three injection protocol was utilized with three replicate measurements taken between each injection. Each replicate consisted of a 1 minute mix step, a 1 minute wait step, and a 3 minute measurement step. After basal measurements were acquired maximum oxygen consumption was stimulated by the addition of 4mM ADP (Sigma-Aldrich A2754) and 1µM FCCP (Sigma-Aldrich C2920), (Port A injection). MPC specific activity was inhibited by the addition by 1mM CHC (Port B injection). Complex I activity was inhibited by 5µM Rotenone (Port C injection). Oxygen consumption was normalized to protein loading.

Tissue Culture and Gluconeogenesis Assay

Primary Rat and Mouse hepatocytes were isolated according to previously published methods (Chen et al., 2007). Briefly, rats or mice were anesthetized and the portal was vein catheterized. ~30mL (Mice) or ~200mL (Rat) liver perfusion buffer (GIBCO 17701-038) at 8ml/min (Mice) or 10mL/min (Rat) was perfused. Following this wash livers were digested with Liver Digestion Media

(HBSS supplemented with 25mM HEPES pH 7.4, 0.5mg/mL Type II collagenase, 0.06mg/mL Trypsin Inhibitor, 2.0 mg/mL fatty acid free BSA, and 1x Pen/Strep). After digestion the liver was excised, the capsule removed, and hepatocytes shaken free in low-glucose DMEM. Isolated hepatocytes were filtered through 100μM cell strainer and centrifuged at 93x*g* for 3 minutes. Cells were resuspended in 30mL Wash media (low Glucose DMEM supplemented with 1x Pen/Strep, 5% FBS, and 10mM HEPES pH 7.4) and the wash repeated 3 times. After the final wash the cells were resuspended in Attachment Media (Williams' E Medium supplemented with 5% FBS, 1X pen/Strep, 10nM Insulin, and 10nM Dexamethasone). Cell viability was measured using Trypan Blue. All hepatocyte isolations had a cell viability of greater than 95%. Cells were plated on collagen coated plates at a density of $0.8x10^6$ cells/well in 2mL volume. After four hours the media above the cells was removed and replaced with Experimental Media (Low-glucose DMEM supplemented with 5% FBS, 1X Pen/Strep, 100nM Dexamethasone). Cells were maintained at 5% CO_2 in humidified air.

Hepatocytes were serum starved for 3 (mouse) or ~16 (rat) hours prior to the start of the experiment in low-glucose DMEM supplemented with 1x Pen/Strep. Hepatocytes were washed twice with PBS and pre-treated with glucose-free DMEM media containing desired substrates and 100nM glucagon for 2 hours. The pre-condition media was replaced with the corresponding media containing chemical inhibitors 5μM UK5099 (Sigma-Aldrich PZ0160), 250μM β-Chloro-L-alanine hydrochloride (Sigma-Aldrich C9033), individually or in combination; or vehicle. Samples of the media were collected after two and four hours. Glucose levels in the samples were measured with a Glucose Assay Kit (Sigma-Aldrich HK20) according to manufacturer's direction.

qPCR

Total RNA from liver tissue was extracted using TRIzol (Life Technologies 15596018) method. For quantitative real-time PCR (qPCR) analysis, an equal amount of RNA was reverse transcribed using High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems 4368814) followed by

qPCR reactions using SYBR Green (Life Technologies 11760-100). Relative abundance of mRNA was normalized to ribosomal protein 36B4 (Akamine et al., 2007). qPCR primers were designed using Primer-Blast (Ye et al., 2012) or previously published (Ai et al., 2012; Chu et al., 2010; Li et al., 2011; Lu et al., 2014; Seo et al., 2011; Wang et al., 2011; Youm et al., 2015).

Primers

	Forward	**Reverse**
36b4	5'-CGTCCTCGTTGGAGTGACA	5'-CGGTGCGTCAGGGATTG
Acac1	5'-GAGTGACTGCCGAAACATCTCTG	5'-GCCTCTTCCTGACAAACGAGT
Acly	5′-AGGAAGTGCCACCTCCAACAGT	5′-CGCTCATCACAGATGCTGGTCA
Acox1	5'-TGTTAAGAAGAGTGCCACCAT	5'-ATCCATCTCTTCATAACCAAATTT
Bdh1	5′-GCTTCCTTGTATTTGCTGGC	5'-TTCTCCACCTCTTCACTGTTG
Bdh2	5′-TCTCAATGAATCTCAACGTCCG	5′-ATCTGTTCTCCACCCCTTTG
Cpt1a	5'-ACTCCTGGAAGAAGAAGTTCA T	5'-AGTATCTTTGACAGCTGGGAC
Echs1	5'-CTTCACTGTAAGGGCAGGTG	5'- CTTGAGTTGGGAATCAGCAG
Fasn	5'-CGGTAGCTCTGGGTGTA	5'-TGCTCCCA GCTGCAGGC
Fbp1	5'-TGTGTTAGAACAGAACCA	5'-TCTGAGCATAGCAGGCTTCTT
Gls2	5'-AGCGTATCCCTATCCACAAGTTCA	5'-GCAGTCCAGTGGCCTTCAGAG
G6pc #1	5'-AGGAACGCCTTCTATGTCCTCTTTC	5'- GCGTTGTCCAAACAGAATCCACTTG
G6pc #2	5’-GTGGCAGTGGTCGGAGACT	5’-ACGGGCGTTGTCCAAAC

G6pc #3	5’-TTCAACCTCGTCTTCAAGTGGAT	5’-CACGGAGCTGTTGCTGTAGTAGTC
Hadha	5'-TGGATGTGGATGACATTGCT	5'-GGGGAAGAGTATCGGCTAGG
Hgmcs1	5′-CAGGTGAAGATCGTGGAAGTC	5′-TGGGAGAAACAAAGCTGGTG
Mpc1	5'-AACTACGAGATGAGTAAGCGGC	5'-GTGTTTTCCCTTCAGCACGAC
Mpc2	5'-CCGCTTTACAACCACCCGGCA	5'-CAGCACACACCAATCCCCATTTCA
Pc	5'-CAGGAGAACATCCGCATCAAT	5'-CTCACCACTCCGGAAAACCT
Pck1	5'-CGGAAGAGGACTTTGAGAAAGCATTC	5'-GCGAGTCTGTCAGTTCAATACCAATC
Ppargc1a	5'-AAACTTGCTAGCGGTCCTCA	5'-TGGCTGGTGCCAGTAAGAG
Scd1	5'-TGGGTTGGCTGCTTGTG	5'-GCGTGGGCAGGATGAAG
Srebf1	5'-GGAGCCATGGATTGCACATT	5'-CCTGTCTCACCCCCCAGCATA

Liver Triglyceride and Glycogen Measurements

Liver triglycerides were extracted essentially as previously described (Folch et al., 1957). Liver tissue (10-20mg) was homogenized in 4ml of chloroform:methanol (2:1) and incubated at room temperature for 30 minutes. Next, 1ml of 50mM NaCl was added, and the mixture was vigorously vortexed for 20 seconds forming an emulsion. This was then centrifuged at 1000x*g* for 10 minutes, and the organic phase, containing the extracted lipids, was collected. The extraction was then dried under a stream of N_2 and resuspended in 1ml chloroform. An aliquot of the resuspended lipids was mixed with 10µl of chloroform:triton-x 100 (1:1) and allowed to dry completely. 1ml of Infinity Triglyceride Reagent (Thermo Scientific TR22421) was added, vortexed, and allowed to

react for 15 minutes. This mixture was then analyzed spectrophotometrically according the manufacturer's direction.

Liver glycogen measurements were performed using the acid hydrolysis approach (Passonneau and Lauderdale, 1974). Briefly, ~10mg of liver tissue was placed into tubes containing 250µL 2M HCL. The samples were boiled for 2 hours with intermittent vortexing. Samples were returned to the original 250µL with water and neutralized with 250µL 2M NaOH and 10µL 1M Tris pH 7.4. Glucose concentrations were measured with the Glucose Assay Kit (Sigma-Aldrich HK20) according to manufacturer's direction.

Serum Analysis

Insulin measurements were performed using the Ultra-Sensitive Mouse Insulin ELISA Kit (Crystal Chem 90080). Serum cholesterol and triglycerides levels were determined using the Infinity Cholesterol Reagent (Thermo Scientific TR13421) and Infinity Triglyceride Reagent (Thermo Scientific TR22421) respectively. Serum ketone and NEFA levels were measured using the Total Ketone Bodies reagents (Wako Diagnostics 415-73301, 411-73401, and 412-73791) and NEFA-HR(2) Reagent (Wako Diagnostics 999-34691, 995-34791, 991-34891, 993-35191, and 276-76491). AST and ALT activities were determined using the Infinity AST (GOT) Reagent (Thermo Scientific TR70121) and the Infinity ALT (GPT) Reagent (Thermo Scientific TR71121), respectively. All reagents were used according to manufacturer's directions.

Tracer Metabolomics

WT and Mpc1 LivKO mice were fasted for 18 hours prior to being injected I.P. with a 10% solution of U-^{13}C-pyruvate/U-^{13}C-lactate (2:8) (Cambridge Isotopes CLM-1579 and CLM-2440) at 3.0mg/kg. 30 minutes after injection mice were anesthetized with isoflurane and a liver lobe was freeze-clamped. Samples were stored at -80C until they were prepared for GC-MS analysis of glucose, organic acids, and amino acids. Glucose concentrations and ^{13}C-Mass isotopomer enrichment

was determined by monitoring *m/z* 287 and 293 as previously described (Sunny and Bequette, 2010). Following conversion to *tert*-butyldimethylsilyl derivatives, organic acids were determined by monitoring their characteristic *m/z* as previously described (Des Rosiers et al., 1994). Similarly, amino acids were converted to trimethylsilyl/*tert*-butyldimethylsilyl derivatives and observed by their characteristic *m/z* (Molnár-Perl and Katona, 2000; Sobolevsky et al., 2003).

Steady State Metabolomics

WT and Mpc1 LivKO mice were fasted for 18 hours prior to the start of the experiment. Blood for serum analysis was collected before mice were anesthetized with isoflurane. Liver and kidney samples were freeze-clamped and stored at -80C. Steady metabolic analysis was performed as previously reported (A et al., 2005; Cox et al., 2009).

Data Analysis

Unless otherwise noted all data represent Mean ± SEM. The Grubbs test was used to identify outliers. SigmaPlot was used to prepare figures and to perform statistical analysis as indicated.

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