SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Corticosterone, ER stress, body composition and whole body bioenergetics of $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ mice, related to Figure 2.

(A) Respiratory exchange ratio and energy expenditure in fed fast and refed $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ female mice (n=6).
(B) Total fat and lean mass of $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ female mice (n=6).
(C) Ambulatory activity and food intake during fed, fast and refed $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ female mice (n=6).
(D) Ambulatory activity and food intake during fed, fast and refed $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ male mice (n=5-7).
(E) Serum concentration of Corticosterone in fed, 24 hr fasted and ketogenic diet-fed $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ male mice (n=5).
(F) Gene expression of *Atf4*, *Chop*, *Xbp(s)* and *Xbp(t)* in the liver of fed, 24 hr fasted and ketogenic diet-fed $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ male mice (n=6).

Data are expressed as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001.

Figure S2. The loss of liver fatty acid oxidation alters fatty acid metabolism in the liver, related to Figure 3.

(A) Gene expression of *de novo* fatty acid biosynthesis genes of fed and 24 hr fasted $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ mice (n=6).
(B) Western blots of proteins involved in hepatic fatty acid metabolism with Hsc70 as loading control from the composite Figure 3B.

Data are expressed as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001.

Figure S3. Gluconeogenic gene expression, cardiac and kidney fatty acid metabolism, related to Figure 4.

(A) Gene expression of gluconeogenic genes *Pck1, Pck2, G6pase* and *Pcx* in liver of fed and 24 hr fasted $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ mice (n=6).
(B) Gene expression of gluconeogenic genes *Pck1, Pck2, G6pase* and *Pcx* in kidney of fed and 24 hr fasted $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ mice (n=6).
(C) Gene expression of fatty acid oxidation genes in the heart of fed and 24 hr fasted $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ mice (n=6).
(D) Western blots of proteins involved in kidney fatty acid metabolism with Hsc70 as loading control from the composite Figure 4B.

Data are expressed as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001.

Figure S4. A ketogenic diet results in compensation from kidney, gastrocnemius muscle and heart, related to Figure 5.

(A) Gene expression of gluconeogenic genes *Pck1, Pck2,* and *G6pase* in liver of $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ mice fed a ketogenic diet (n=6).

(B) Gene expression of gluconeogenic genes *Pck1, Pck2,* and *G6pase* in kidney of $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ mice fed a ketogenic diet (n=6).

(C) Serum concentrations of insulin in $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ mice fed a ketogenic diet (n=5-7).

(D) Gene expression of fatty acid oxidation genes in kidney of $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ mice fed a ketogenic diet (n=6).

(E) Gene expression of fatty acid oxidation genes in gastrocnemius muscle of $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ mice fed a ketogenic diet (n=6).

(F) Gene expression of fatty acid oxidation genes in heart of $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ mice fed a ketogenic diet (n=6).

Data are expressed as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001.

Table S1. Daily blood acylcarnitine profile of $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ mice fed a chow diet then a ketogenic diet for 4 days, related to Figure 6.

Table S2. Liver acylcarnitine profile of $Cpt2^{lox/lox}$ and $Cpt2^{L-/-}$ mice fed a chow diet or a ketogenic diet for 4 days, related to Figure 6.

Figure S1

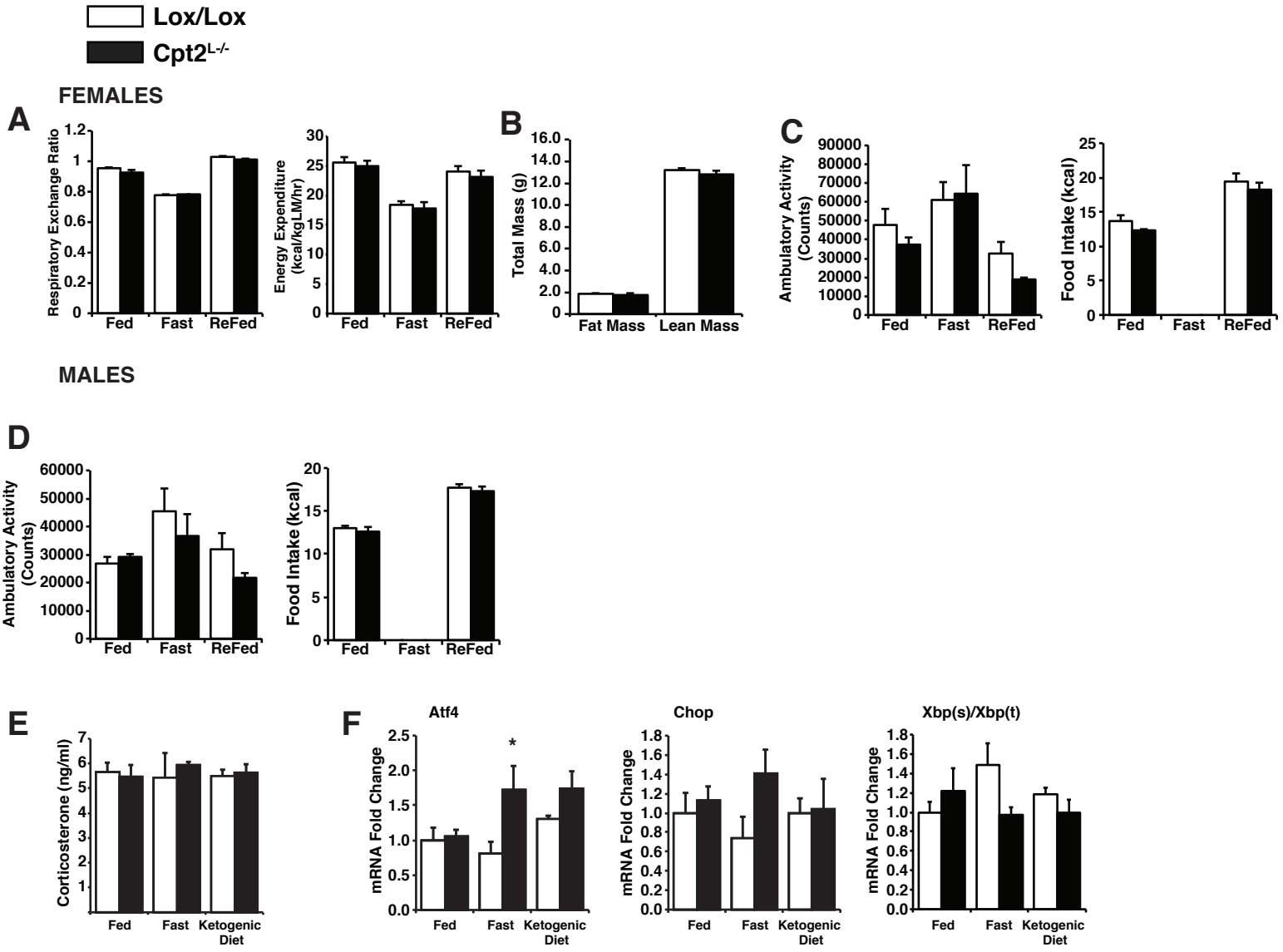
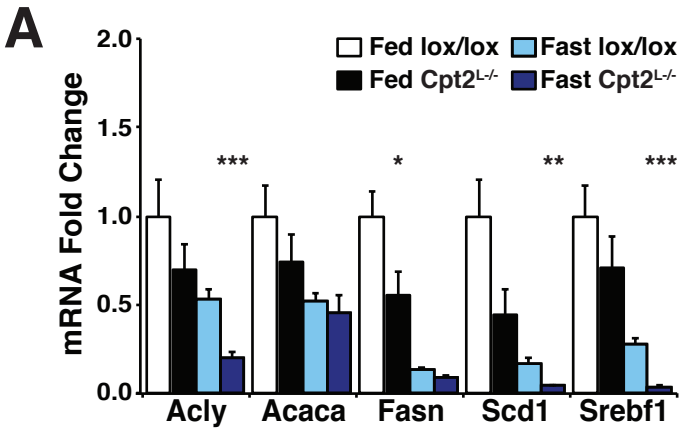

Lox/Lox
Cpt2L-/-
FEMALES
A
Respiratory Exchange Ratio
Energy Expenditure (kcal/kgLM/hr)
Fed
Fast
ReFed
B
Total Mass (g)
Fat Mass
Lean Mass
C
Ambulatory Activity (Counts)
Food Intake (kcal)
MALES
D
Ambulatory Activity (Counts)
Food Intake (kcal)
E
Corticosterone (ng/ml)
Ketogenic Diet
F
Atf4
Chop
Xbp(s)/Xbp(t)
mRNA Fold Change
*

Figure S2


A
mRNA Fold Change
2.0
1.5
1.0
0.5
0.0
Fed lox/lox
Fast lox/lox
Fed Cpt2L-/-
Fast Cpt2L-/-
***
*
**
***
Acly
Acaca
Fasn
Scd1
Srebf1

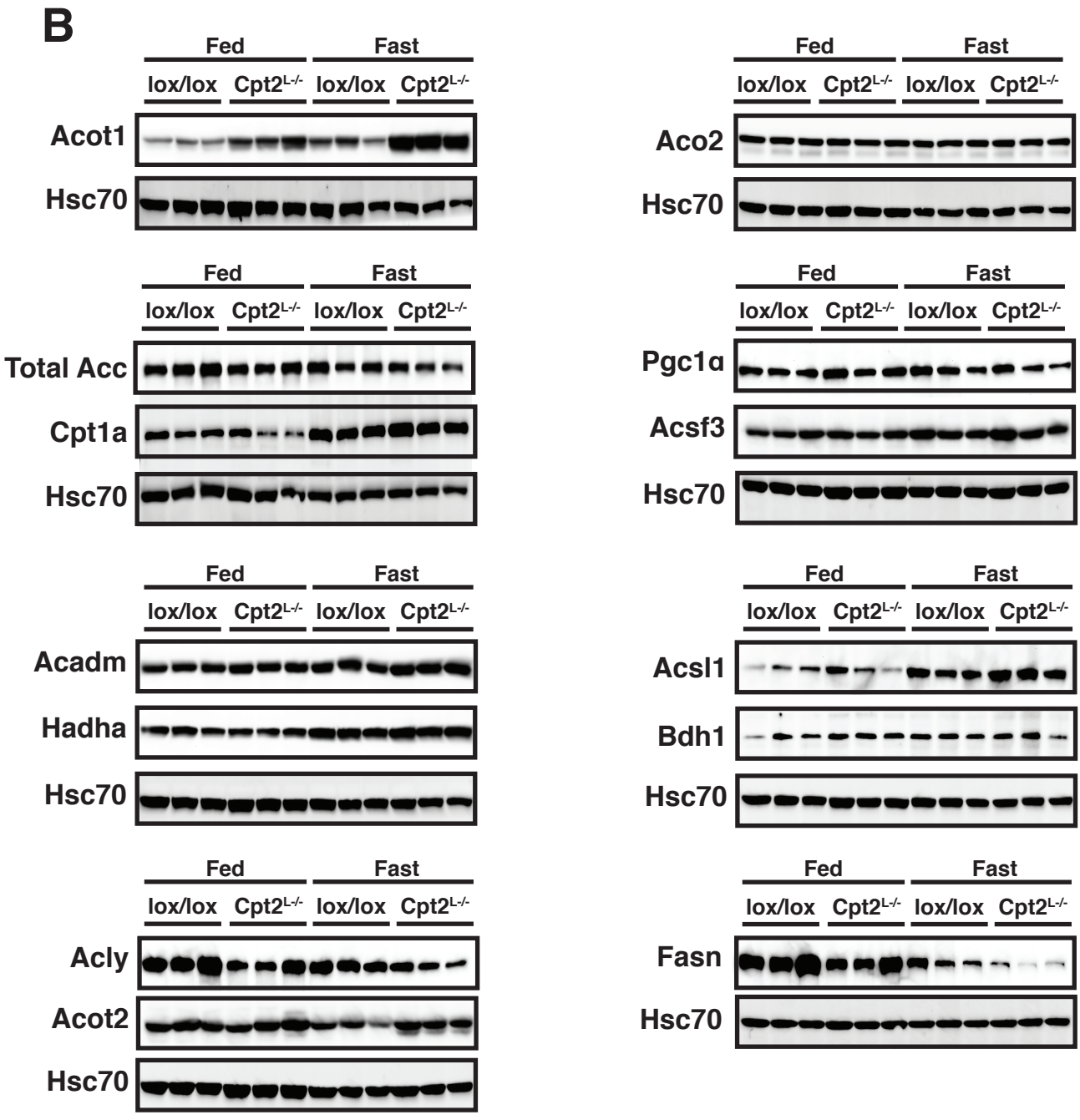
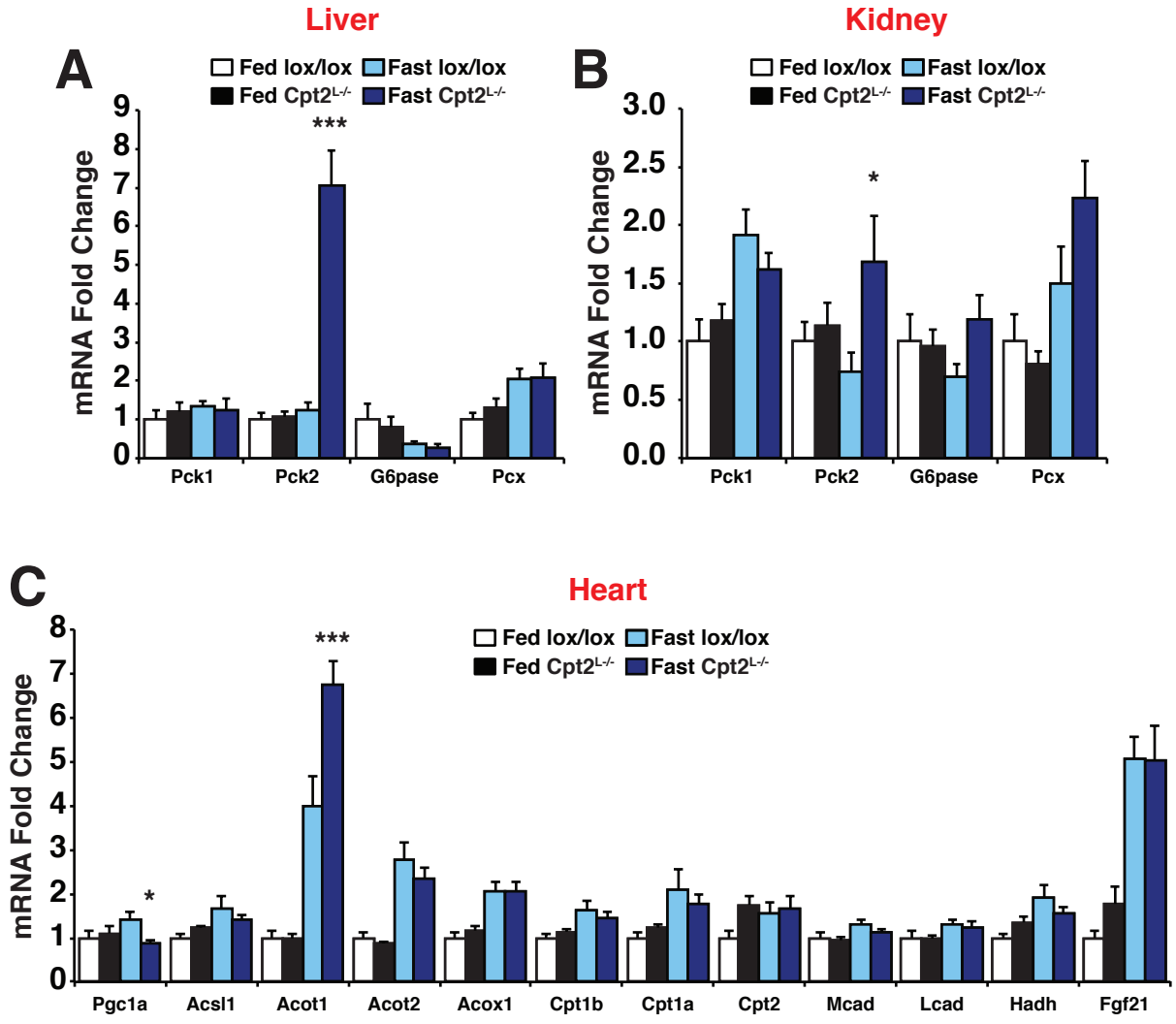

B
Fed
Fast
lox/lox
Cpt2L-/-
Acot1
Hsc70
Aco2
Total Acc
Cpt1a
Pgc1α
Acsf3
Acadm
Hadha
Acsl1
Bdh1
Acly
Acot2
Fasn

Figure S3


Liver
A
Fed lox/lox
Fast lox/lox
Fed Cpt2L-/-
Fast Cpt2L-/-
mRNA Fold Change
***
Pck1
Pck2
G6pase
Pcx
Kidney
B
*
Heart
C
Pgc1a
Acsl1
Acot1
Acot2
Acox1
Cpt1b
Cpt1a
Cpt2
Mcad
Lcad
Hadh
Fgf21

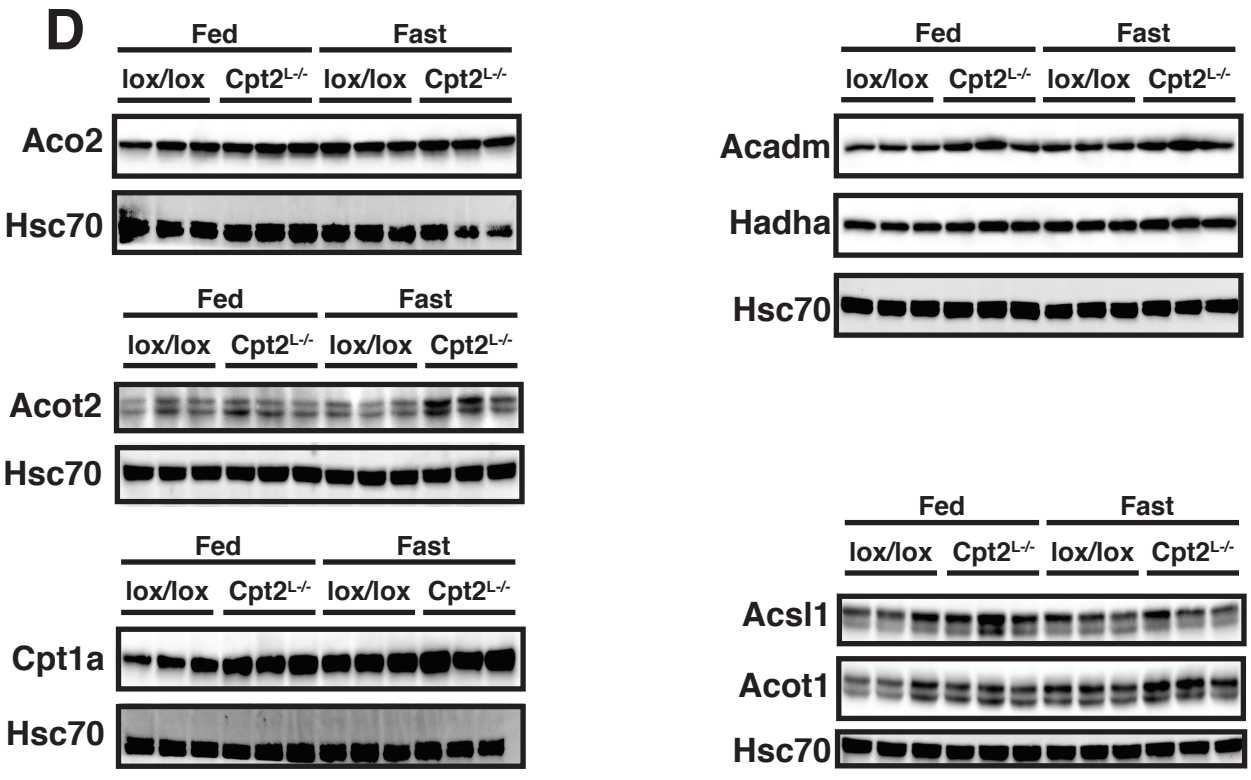
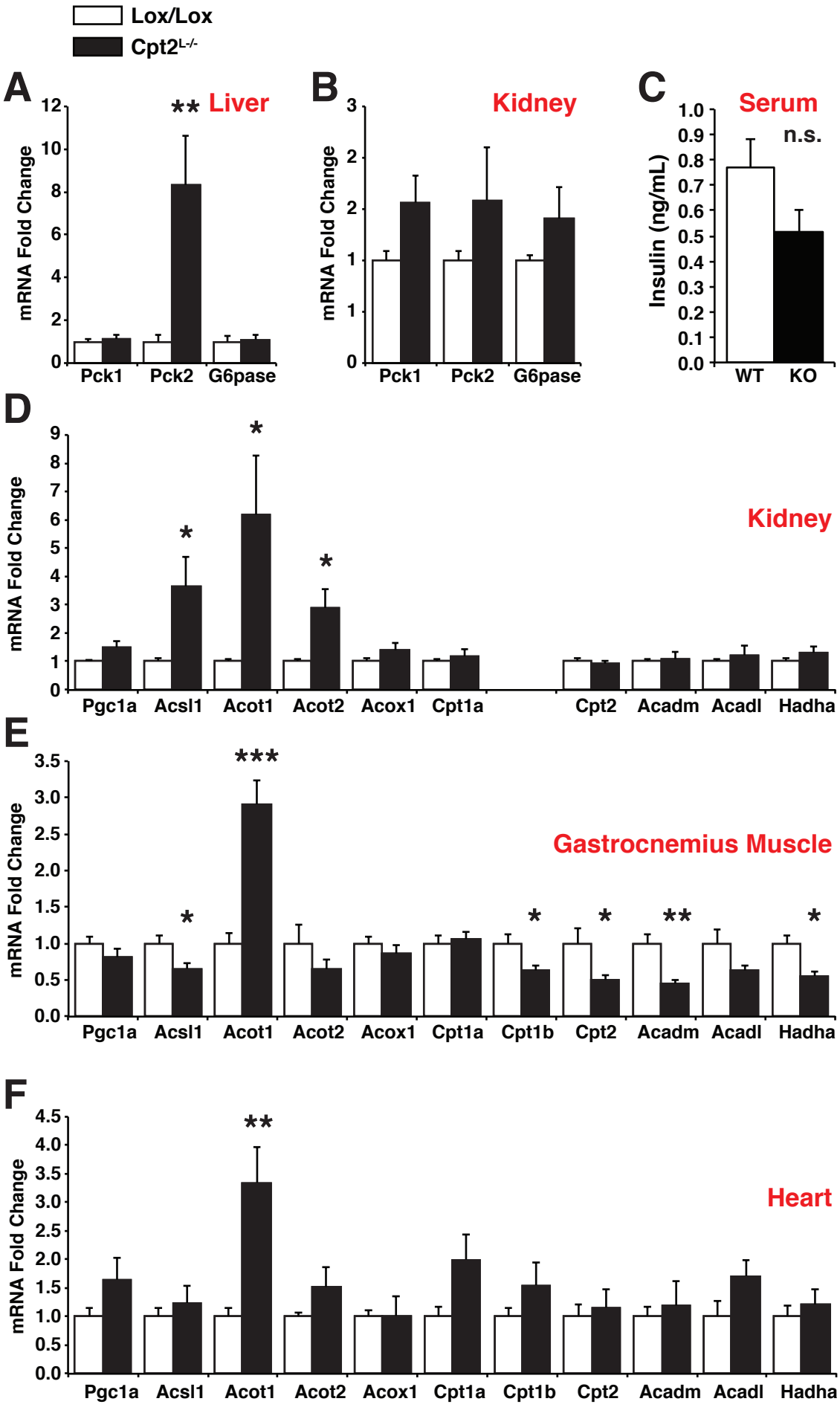

D
Fed
Fast
lox/lox
Cpt2L-/-
Aco2
Hsc70
Acadm
Hadha
Acot2
Cpt1a
Acsl1
Acot1

Figure S4


Lox/Lox
Cpt2L-/-
A
Liver
**
mRNA Fold Change
Pck1
Pck2
G6pase
B
Kidney
C
Serum
n.s.
Insulin (ng/mL)
WT
KO
D
Kidney
Pgc1a
Acsl1
Acot1
Acot2
Acox1
Cpt1a
Cpt2
Acadm
Acadl
Hadha
E
Gastrocnemius Muscle
***
Cpt1b
F
Heart

Supplemental Table 1. Daily blood acylcarnitine profile of Cpt2lox/lox and Cpt2L-/- mice fed a chow diet then a ketogenic diet for 4 days.

Acylcarnitines	Lox/Lox					Cpt2L-/-				
(nmol/ml)	Chow	1	2	3	4	Chow	1	2	3	4
Total	27.406 ± 2.206	26.094 ± 2.42	20.704 ± 1.351	21.222 ± 1.877§	23.928 ± 2.42§	7.15 ± 0.571####	8.366 ± 0.96####	9.27 ± 1.242####	9.53 ± 0.675####	10.616 ± 1.357####
C0	29.424 ± 3.309	17.332 ± 1.97§§§§	15.672 ± 2.025§§§§	16.672 ± 0.729§§§§	16.278 ± 1.634§§§§	4.478 ± 0.576####	3.56 ± 0.633####	4.005 ± 0.416####	3.748 ± 0.259####	3.803 ± 0.337####
C2	21.616 ± 2.423	19.296 ± 2.344	14.884 ± 0.989§§§	15.604 ± 1.234§§	17.858 ± 1.795	3.458 ± 0.3####	3.538 ± 0.579####	2.948 ± 0.151####	2.964 ± 0.391####	3.024 ± 0.344####
C3	0.644 ± 0.084	0.896 ± 0.141	0.684 ± 0.057	0.658 ± 0.078§	0.57 ± 0.099§	0.066 ± 0.005####	0.172 ± 0.021####	0.114 ± 0.007####	0.088 ± 0.006####	0.076 ± 0.015####
C3-DC	0.142 ± 0.031	0.118 ± 0.015	0.154 ± 0.013	0.11 ± 0.026	0.134 ± 0.015	0.06 ± 0.01##	0.052 ± 0.008#	0.082 ± 0.01#	0.05 ± 0.007	0.074 ± 0.01
C4	0.47 ± 0.106	0.332 ± 0.038	0.31 ± 0.039	0.328 ± 0.048	0.354 ± 0.038	0.166 ± 0.036###	0.206 ± 0.031	0.238 ± 0.025	0.254 ± 0.032	0.274 ± 0.057
C4-OH	0.23 ± 0.017	0.284 ± 0.014	0.328 ± 0.048	0.376 ± 0.079§§	0.44 ± 0.054§§	0.058 ± 0.015##	0.102 ± 0.018##	0.084 ± 0.017##	0.112 ± 0.017##	0.09 ± 0.006*,####
C4-DC	0.098 ± 0.011	0.098 ± 0.016	0.086 ± 0.005	0.112 ± 0.025	0.112 ± 0.032	0.048 ± 0.007	0.05 ± 0.013	0.05 ± 0.007	0.046 ± 0.005##	0.036 ± 0.004##
C5:1	0.014 ± 0.002	0.016 ± 0.002	0.02 ± 0.006	0.022 ± 0.006	0.018 ± 0.006	0.026 ± 0.008	0.012 ± 0.004	0.022 ± 0.006	0.012 ± 0.002	0.268 ± 0.241
C5	0.066 ± 0.006	0.062 ± 0.011	0.068 ± 0.024	0.096 ± 0.004	0.07 ± 0.005	0.024 ± 0.006#	0.026 ± 0.005	0.024 ± 0.004#	0.032 ± 0.006#	0.038 ± 0.007
C5-OH	0.088 ± 0.01	0.082 ± 0.008	0.062 ± 0.009	0.074 ± 0.01	0.098 ± 0.015	0.026 ± 0.005####	0.028 ± 0.006###	0.036 ± 0.005	0.034 ± 0.006##	0.028 ± 0.002####
C5-DC/C10-OH	0.026 ± 0.002	0.028 ± 0.006	0.032 ± 0.005	0.032 ± 0.006	0.034 ± 0.004	0.01 ± 0	0.022 ± 0.005	0.018 ± 0.004	0.028 ± 0.007	0.026 ± 0.008
C6	0.086 ± 0.012	0.098 ± 0.019	0.076 ± 0.005	0.084 ± 0.012	0.104 ± 0.018	0.052 ± 0.007	0.052 ± 0.008#	0.042 ± 0.002	0.062 ± 0.012	0.044 ± 0.007##
C8:1	0.042 ± 0.007	0.028 ± 0.004	0.032 ± 0.004	0.03 ± 0.004	0.036 ± 0.012	0.028 ± 0.002	0.024 ± 0.006	0.028 ± 0.006	0.026 ± 0.007	0.03 ± 0.005
C8	0.09 ± 0.023	0.084 ± 0.009	0.07 ± 0.008	0.092 ± 0.012	0.124 ± 0.035	0.038 ± 0.006	0.028 ± 0.004	0.022 ± 0.002	0.034 ± 0.007#	0.032 ± 0.004###
C10:1	0.122 ± 0.053	0.074 ± 0.011	0.058 ± 0.01	0.102 ± 0.03	0.114 ± 0.023	0.042 ± 0.004	0.052 ± 0.009	0.052 ± 0.013	0.044 ± 0.009	0.068 ± 0.012
C10	0.052 ± 0.009	0.054 ± 0.009§	0.06 ± 0.003	0.064 ± 0.008	0.09 ± 0.018*,§	0.026 ± 0.007	0.036 ± 0.008	0.028 ± 0.005	0.02 ± 0.003##	0.032 ± 0.005####
C12:1	0.032 ± 0.002	0.028 ± 0.004	0.03 ± 0.004	0.036 ± 0.006	0.034 ± 0.009	0.024 ± 0.006	0.028 ± 0.006	0.018 ± 0.004	0.024 ± 0.005	0.016 ± 0.004
C12	0.108 ± 0.02	0.098 ± 0.019	0.08 ± 0.006	0.122 ± 0.031	0.098 ± 0.014	0.056 ± 0.012	0.052 ± 0.008	0.048 ± 0.006	0.062 ± 0.006#	0.062 ± 0.007
C12:1-OH	0.04 ± 0.015	0.026 ± 0.002	0.024 ± 0.005	0.038 ± 0.021	0.022 ± 0.004	0.018 ± 0.004	0.018 ± 0.004	0.02 ± 0.004	0.022 ± 0.004	0.018 ± 0.004
C12-OH	0.03 ± 0.01	0.026 ± 0.002	0.034 ± 0.008	0.026 ± 0.006	0.03 ± 0.003	0.008 ± 0.002#	0.012 ± 0.002	0.016 ± 0.005	0.012 ± 0.002	0.014 ± 0.002
C14:2	0.05 ± 0.014	0.036 ± 0.009	0.028 ± 0.005	0.034 ± 0.007	0.036 ± 0.005	0.044 ± 0.007	0.024 ± 0.002	0.03 ± 0.007	0.036 ± 0.005	0.036 ± 0.004
C14:1	0.104 ± 0.025	0.108 ± 0.008	0.096 ± 0.012	0.122 ± 0.023	0.102 ± 0.014	0.084 ± 0.009	0.056 ± 0.009	0.062 ± 0.008	0.096 ± 0.017	0.062 ± 0.011
C14	0.172 ± 0.008*	0.304 ± 0.048	0.256 ± 0.025	0.264 ± 0.063	0.222 ± 0.033	0.14 ± 0.016	0.138 ± 0.014#	0.136 ± 0.012#	0.168 ± 0.024	0.158 ± 0.02
C14:1-OH	0.034 ± 0.005	0.05 ± 0.011	0.038 ± 0.008	0.052 ± 0.008	0.04 ± 0.007	0.024 ± 0.005	0.024 ± 0.005	0.024 ± 0.005	0.034 ± 0.005	0.024 ± 0.01
C14-OH	0.046 ± 0.015	0.048 ± 0.004	0.038 ± 0.002	0.06 ± 0.013	0.058 ± 0.006	0.018 ± 0.004#	0.016 ± 0.002#	0.014 ± 0.002	0.016 ± 0.002###	0.016 ± 0.004###
C16:1	0.122 ± 0.007	0.162 ± 0.032	0.11 ± 0.017	0.12 ± 0.027	0.126 ± 0.025	0.174 ± 0.017	0.14 ± 0.01	0.148 ± 0.026	0.198 ± 0.03	0.176 ± 0.025
C16	1.424 ± 0.041	1.392 ± 0.3	1.158 ± 0.139	0.996 ± 0.142	1.144 ± 0.136	0.942 ± 0.124	1.368 ± 0.129	1.726 ± 0.329	1.688 ± 0.211	2.012 ± 0.382*,#
C16:1-OH	0.062 ± 0.01	0.09 ± 0.014	0.076 ± 0.014	0.074 ± 0.019	0.092 ± 0.018	0.038 ± 0.009	0.064 ± 0.01§	0.108 ± 0.017*	0.114 ± 0.013*	0.148 ± 0.034***
C16-OH	0.054 ± 0.007*	0.07 ± 0.008	0.08 ± 0.008	0.074 ± 0.015	0.094 ± 0.024	0.018 ± 0.002	0.014 ± 0.004##	0.024 ± 0.007##	0.02 ± 0.003##	0.022 ± 0.004##
C18:2	0.254 ± 0.021	0.278 ± 0.05	0.216 ± 0.031	0.158 ± 0.023	0.226 ± 0.038	0.214 ± 0.027	0.214 ± 0.013	0.258 ± 0.046	0.274 ± 0.056	0.292 ± 0.058
C18:1	0.586 ± 0.012	1.01 ± 0.198	0.736 ± 0.081	0.552 ± 0.07	0.706 ± 0.113	0.714 ± 0.087	0.866 ± 0.065§	1.278 ± 0.316	1.336 ± 0.204#	1.66 ± 0.44*,§§,#
C18	0.378 ± 0.017	0.69 ± 0.1	0.674 ± 0.094	0.598 ± 0.052#	0.588 ± 0.098###	0.462 ± 0.04	0.876 ± 0.136§	1.5 ± 0.323**	1.554 ± 0.192**	1.918 ± 0.563****
C18:2-OH	0.032 ± 0.012	0.024 ± 0.004	0.016 ± 0.002	0.014 ± 0.005	0.024 ± 0.005	0.016 ± 0.004	0.014 ± 0.002	0.022 ± 0.006	0.014 ± 0.002	0.022 ± 0.002
C18:1-OH	0.044 ± 0.01	0.062 ± 0.008	0.054 ± 0.007	0.054 ± 0.014	0.072 ± 0.019	0.024 ± 0.002	0.022 ± 0.002	0.04 ± 0.01	0.046 ± 0.008	0.046 ± 0.018
C18-OH	0.034 ± 0.004	0.042 ± 0.008	0.042 ± 0.004	0.044 ± 0.005	0.06 ± 0.009*	0.01 ± 0##	0.01 ± 0###	0.014 ± 0.002##	0.01 ± 0.003####	0.016 ± 0.004####

Symbol	Significance
*	Chow vs. Keto; *p<0.05; **p<0.01; ***p<0.001; ****$p<0.0001$
#	Lox/Lox vs. Cpt2L-/-; #p<0.05; ##p<0.01; ###p<0.001; ####$p<0.0001$
§	Effect of time (day1-day5); §$p<0.05$; §§p<0.01; §§§p<0.001;§§§§p<0.0001

Supplemental Table 2. Liver acylcarnitine profile of Cpt2lox/lox and Cpt2L-/- mice fed a chow diet or a ketogenic diet for 4 days.

Acylcarnitines (pmol/mg)	Lox/Lox		$Cpt2^{L-/-}$	
	Chow	Keto	Chow	Keto
Total	43.41 ± 3.58	80.34 ± 12.9*,##	35.23 ± 2.3	30.65 ± 3.23
C0	179.69 ± 21.85	120.04 ± 24.04	218.07 ± 13.26	79.63 ± 3.25***
C2	22.65 ± 1.67	30.83 ± 8.03	11.75 ± 0.9	5.88 ± 1.38 #
C3	4.87 ± 1.02	2.11 ± 0.4*	2.17 ± 0.38#	1.06 ± 0.12
C3-DC	1.27 ± 0.27	0.49 ± 0.09*	0.28 ± 0.03	0.08 ± 0.02##
C4	2.36 ± 0.38	2.58 ± 0.46	0.3 ± 0.05##	0.39 ± 0.03##
C4-OH	2.55 ± 0.61	1.9 ± 0.31	0.11 ± 0.01##	0.05 ± 0.01#
C4-DC	0.19 ± 0.05	0.84 ± 0.18**	0.11 ± 0.06	0.35 ± 0.08
C5:1	0.01 ± 0	0.01 ± 0	0.01 ± 0	0 ± 0#
C5	0.31 ± 0.06	0.32 ± 0.07	0.12 ± 0.01	0.03 ± 0.01##
C5-OH	0.15 ± 0.02	0.21 ± 0.04	0.09 ± 0.01	0.01 ± 0###
C5-DC/C10-OH	1.75 ± 0.55	4.79 ± 0.62*	4.91 ± 0.6##	3.64 ± 0.56
C6	0.17 ± 0.04	0.47 ± 0.08**	0.1 ± 0.01	0.05 ± 0.01###
C8:1	0.05 ± 0.01	0.09 ± 0.03	0.03 ± 0.01	0.05 ± 0
C8	0.2 ± 0.05	3.74 ± 0.9***	0.09 ± 0.01	0.1 ± 0.02###
C10:1	0.04 ± 0	0.11 ± 0.01****	0.03 ± 0	0.06 ± 0.01##
C10	0.04 ± 0.01	0.28 ± 0.06***	0.03 ± 0.01	0.06 ± 0.01##
C12:1	0.03 ± 0	0.23 ± 0.05***	0.03 ± 0.01	0.06 ± 0.01##
C12	0.19 ± 0.03	1.76 ± 0.26****	0.18 ± 0.03	0.32 ± 0.03####
C12:1-OH	0.09 ± 0.01	0.44 ± 0.06****	0.04 ± 0.01	0.04 ± 0####
C12-OH	0.58 ± 0.09	0.98 ± 0.11*	0.32 ± 0.06	0.28 ± 0.04###
C14:2	0.03 ± 0	0.33 ± 0.06****	0.06 ± 0.01	0.13 ± 0.02##
C14:1	0.1 ± 0.01	1.15 ± 0.24***	0.15 ± 0.02	0.22 ± 0.03##
C14	0.28 ± 0.06	3.36 ± 0.61****	0.57 ± 0.08	1.22 ± 0.1##
C14:1-OH	0.04 ± 0.01	0.44 ± 0.08****	0.05 ± 0.01	0.16 ± 0.02##
C14-OH	0.14 ± 0.04	1.13 ± 0.28**	0.04 ± 0.01	0.04 ± 0##
C16:1	0.26 ± 0.07	1.28 ± 0.21**	1.37 ± 0.18###	0.39 ± 0.09**,##
C16	1.37 ± 0.27	6.16 ± 0.76****	4.56 ± 0.51##	5.16 ± 0.56
C16:1-OH	0.09 ± 0.01	0.47 ± 0.07****	0.09 ± 0.01	0.36 ± 0.04**
C16-OH	0.09 ± 0.02	0.31 ± 0.08**	0.05 ± 0.01	0.05 ± 0.01##
C18:2	0.64 ± 0.08	2.8 ± 0.43***	2.07 ± 0.19##	0.82 ± 0.09*, ###
C18:1	1.32 ± 0.27	6.82 ± 0.84****	3.84 ± 0.3#	4.17 ± 0.4#
C18	1.37 ± 0.41	3.34 ± 0.43*	1.58 ± 0.13****	5.28 ± 0.54#
C18:2-OH	0.05 ± 0.01	0.12 ± 0.02**	0.02 ± 0	0.03 ± 0##
C18:1-OH	0.09 ± 0.02	0.3 ± 0.08*	0.04 ± 0.01	0.07 ± 0.01#
C18-OH	0.06 ± 0.01	0.15 ± 0.02***	0.02 ± 0	0.02 ± 0####

Symbol	Significance
*	Chow vs. Keto; *p<0.05; **p<0.01; ***p<0.005; ****$p<0.001$
#	Lox/Lox vs. Cpt2L-/-; #p<0.05; ##p<0.01; ###p<0.005; ####$p<0.001$