SUPPLEMENTAL

Supplemental Figure Legends

Figure S1. Loss of CPT2 results in no gross anatomical differences. A) Volumes of brain regions based on MRI data using $CPT2^{lox/lox}$ (n = 3) and $CPT2^{B-/-}$ (n = 4) mice. 1. lateral ventricles 2. cortex 3. hippocampus, and 4. cerebellum. Data are expressed as mean ± S.E.M. B) Fractional anisotropy from a representative $CPT2^{lox/lox}$ and $CPT2^{B-/-}$ mouse. C) *Cpt2* mRNA abundance across tissues using $CPT2^{lox/lox}$ (n = 6) and $CPT2^{B-/-}$ mice (n = 6). D) wet tissue weight across tissues as a percentage of body weight using $CPT2^{lox/lox}$ (n = 5) and $CPT2^{B-/-}$ (n = 6). Represented data analyzed using student's two-tailed t-tests. *α = 0.05; **α = 0.01; ***α = 0.001; ns, not significant.

Figure S2. Nestin-Cre does not have any impact on anxiety. *3-Zone Open Field Test* using adult $CPT2^{lox/+}$ (n = 12) and Nestin-Cre;$CPT2^{lox/+}$ (n = 5) mice. A) Distances traveled in outer, middle, and center zones. B) Number of entries in outer, middle, and center zones. C) Total time spent in outer, middle, and center zones. D) Total distance traveled. E) Mean velocity. F) Total number of mobile episodes. G) Total number of immobile episodes. H) Total time spent mobile. I) Total time spent immobile. J) Grooming time. K) Time rearing. Represented data analyzed using student's two-tailed t-tests. Data are expressed as mean ± S.E.M *α = 0.05; **α = 0.01; ***α = 0.001; ****α = 0.0001; ns, not significant.

Figure S3. Loss of brain-specific long-chain fatty acid oxidation results in a mild increase in anxiety-like behavior and a potential minor cognitive deficit. *3-Zone Open Field Test* A) Total percentage of time spent in the inner, middle, and outer zones by adult female $CPT2^{lox/lox}$ and $CPT2^{B-/-}$ mice (n = 10). B) Average velocity of adult female $CPT2^{lox/lox}$ and $CPT2^{B-/-}$ mice

while in inner middle and outer zones (n = 10). Represented data analyzed using student's two-tailed t-tests. Data are expressed as mean ± S.E.M *α = 0.05; **α = 0.01; ***α = 0.001; ****α = 0.0001; ns, not significant.

Figure S4. Deletion of *Cpt2* in brain does not impact TCA or glycolytic metabolites. Fold changes and relative abundances of A) TCA and B) glycolytic metabolites in whole hippocampus from 24-hour fasted 9-week old $CPT2^{B-/-}$, $CPT2^{L-/-}$, and $PPAR\alpha^{-/-}$ in comparison and normalized to $CPT2^{lox/lox}$ (n = 6). Statistical significance of represented metabolites determined using two-stage false discovery rate (FDR) method of Benjamini, Krieger, and Yekutieli with a FDR (Q) of 10%. Fold changes in green boxes are significantly increased, fold changes in red boxes are significantly decreased, and fold changes in yellow boxes are not significantly affected by genotype. The same data are represented as mean of relative species abundance ± S.E.M. in adjacent graphs. *α = 0.05; **α = 0.01; ***α = 0.001; ****α = 0.0001; ns, not significant.

Figure S5. Diet does not significantly impact steady-state long-chain acylcarnitines in $CPT2^{B-/-}$ brain. A-D) Concentrations of steady-state acylcarnitines were determined using approximately 25mg of cortex from 9-week old fed and 24-hour fasted $CPT2^{lox/lox}$ and $CPT2^{B-/-}$ mice (n = 6). Additionally, steady-state acylcarnitines were determined using approximately 25mg of cortex from 18-week old $CPT2^{lox/lox}$ and $CPT2^{B-/-}$ mice after 15-weeks on low-fat or high-fat diet (n = 6). E) Select acylcarnitines from media collected from cultured P2 cortical astrocytes from $CPT2^{lox/lox}$ and $CPT2^{B-/-}$ mice. Astrocytes were incubated overnight in 100μM

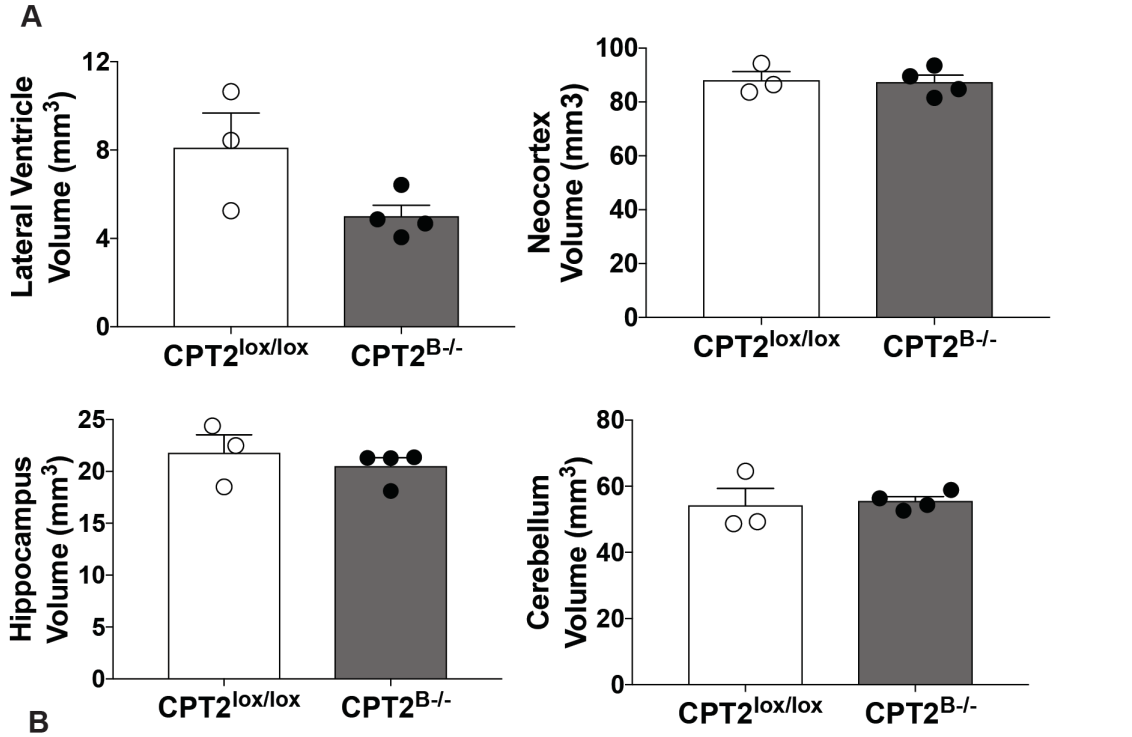
oleate media. Data are expressed as mean ± S.E.M. Represented data analyzed using ordinary two-way analysis of variance with Sidak's tests for multiple comparisons. *α = 0.05; **α = 0.01; ***α = 0.001; ****α = 0.0001; ns, not significant.

Table S1. qRT-PCR primers

Table S2 unbiased and targeted metabolomics data

All data from unbiased 24-hour fasted hippocampus metabolome (sheet 1), cortical acylcarnitines from mice under different dietary states (sheet 2), and arterial and venous acylcarnitines (sheet 3).

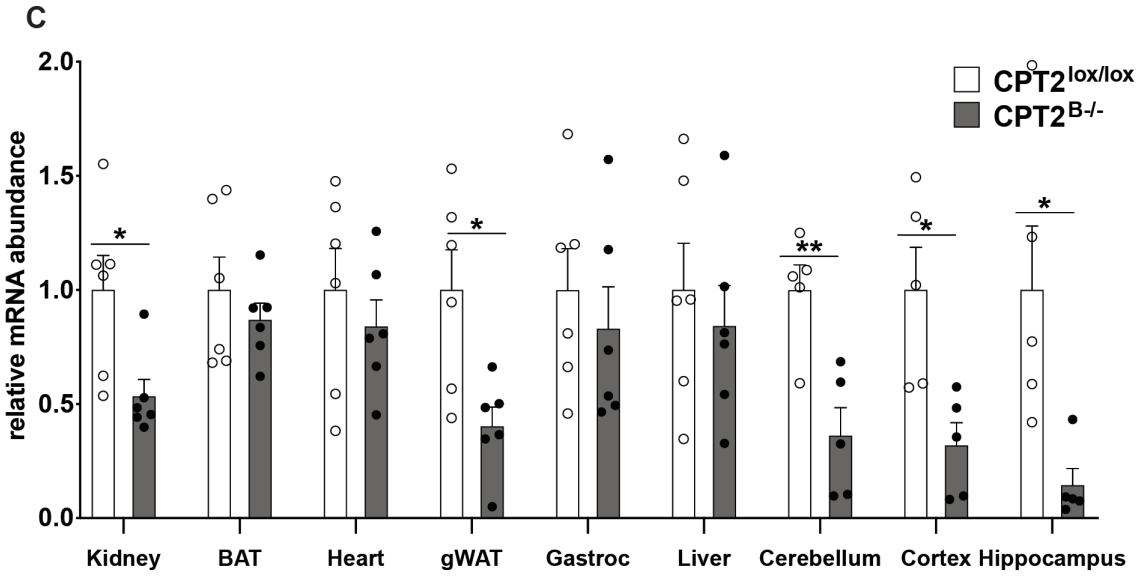
S1

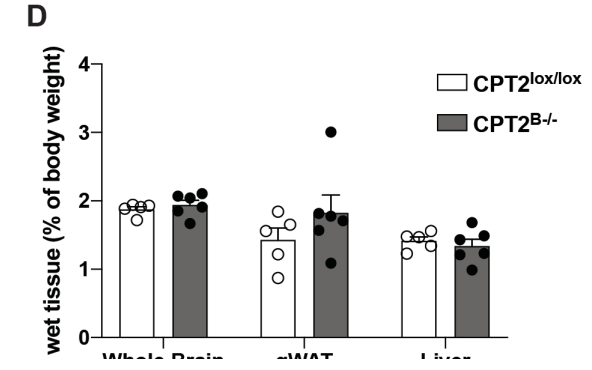


B

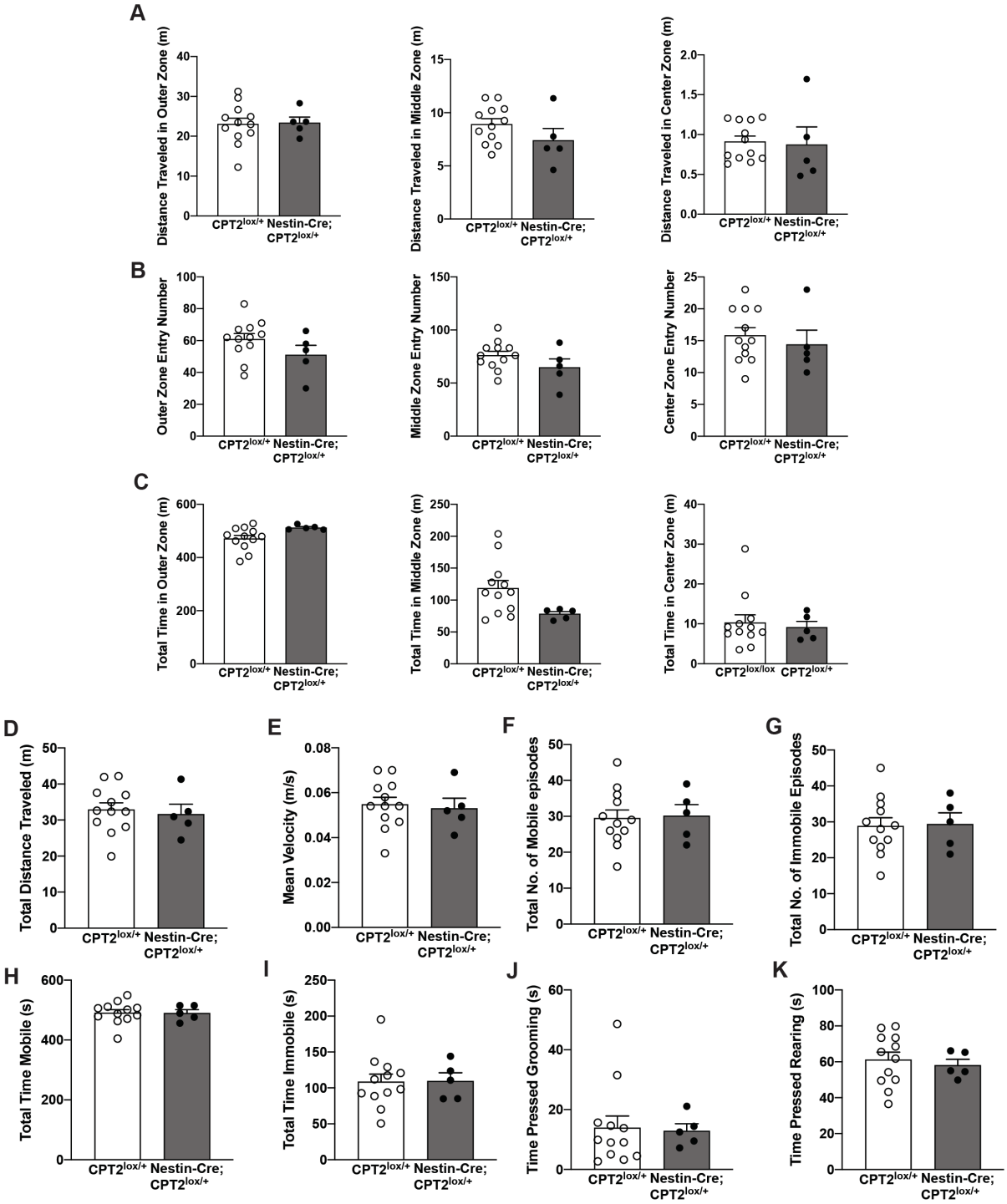
Fractional Anisotropy

CPT2lox/lox	CPT2B-/-
0.4365	0.4752

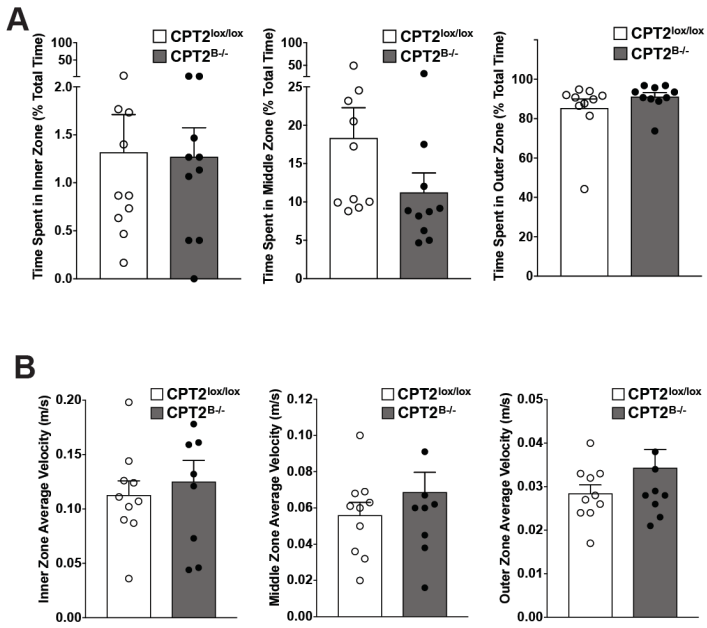




S2


A
Distance Traveled in Outer Zone (m)
Distance Traveled in Middle Zone (m)
Distance Traveled in Center Zone (m)
B
Outer Zone Entry Number
Middle Zone Entry Number
Center Zone Entry Number
C
Total Time in Outer Zone (m)
Total Time in Middle Zone (m)
Total Time in Center Zone (m)
CPT2lox/+
Nestin-Cre; CPT2lox/+
CPT2lox/lox
D
Total Distance Traveled (m)
E
Mean Velocity (m/s)
F
Total No. of Mobile episodes
G
Total No. of Immobile Episodes
H
Total Time Mobile (s)
I
Total Time Immobile (s)
J
Time Pressed Grooming (s)
K
Time Pressed Rearing (s)

S3

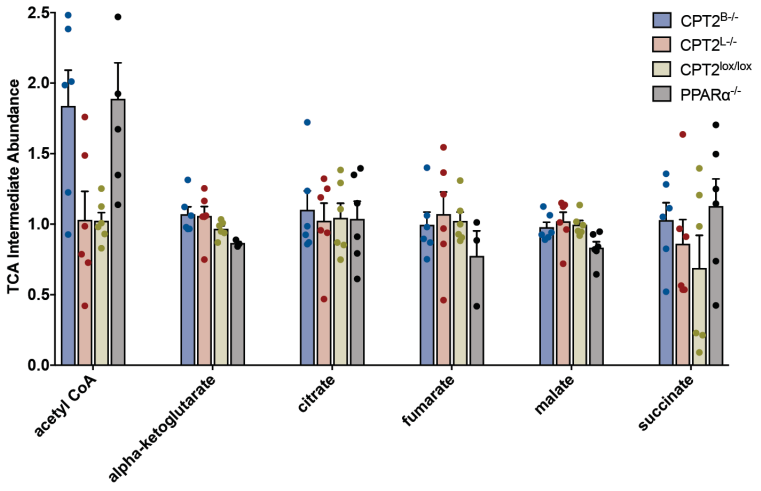

A
CPT2lox/lox
CPT2B-/-
Time Spent in Inner Zone (% Total Time)
Time Spent in Middle Zone (% Total Time)
Time Spent in Outer Zone (% Total Time)
B
Inner Zone Average Velocity (m/s)
Middle Zone Average Velocity (m/s)
Outer Zone Average Velocity (m/s)

S4

A

TCA Intermediates (Fold Change to $CPT2^{lox/lox}$)

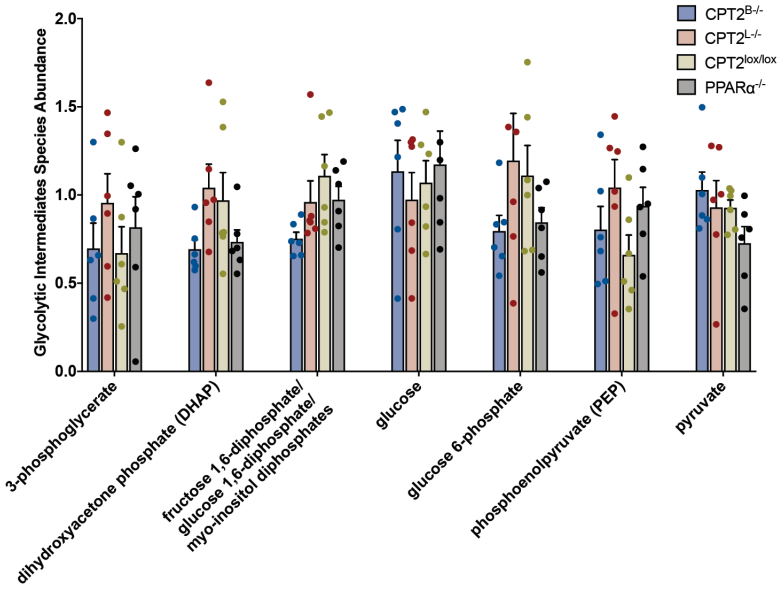
Species	$CPT2^{B-/-}$	$CPT2^{L-/-}$	$PPAR\alpha^{-/-}$
acetyl-CoA	1.798	1.007	1.847
alpha-ketoglutarate	1.107	1.094	0.958
citrate	1.055	0.980	0.993
fumarate	0.974	1.048	0.796
malate	0.982	1.024	0.837
succinate	1.494	1.250	1.640



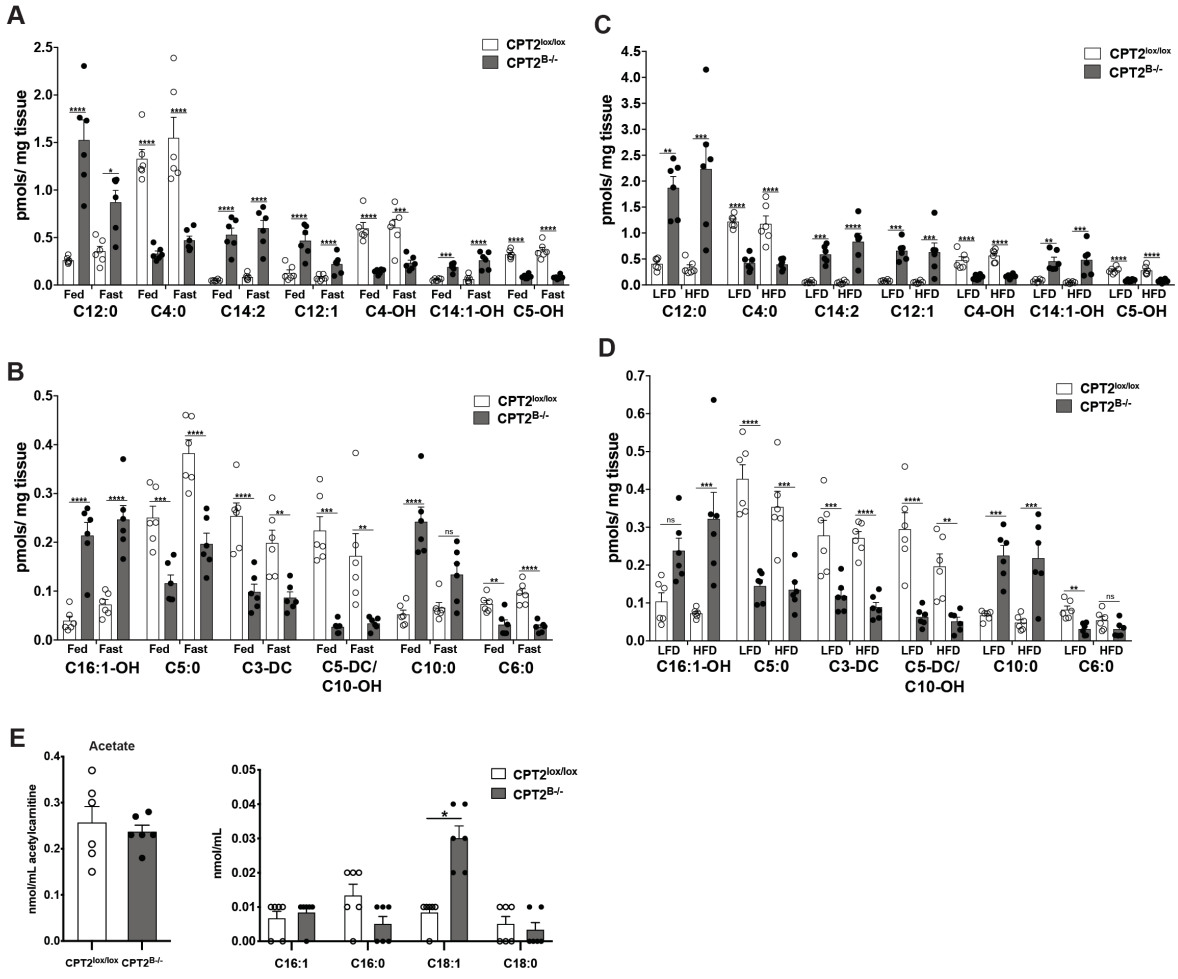
B

Glycolysis (Fold Change to $CPT2^{lox/lox}$)

Species	$CPT2^{B-/-}$	$CPT2^{L-/-}$	$PPAR\alpha^{-/-}$
3-phosphoglycerate	1.038	1.424	1.217
dihydroxyacetone phosphate	0.713	1.075	0.757
fructose 1,6 bisphosphate	0.678	0.865	0.877
glucose	1.061	0.910	1.097
glucose-6-phosphate	0.716	1.076	0.761
phosphoenolpyruvate	1.218	1.578	1.422
pyruvate	1.109	1.003	0.783



S5


A
CPT2lox/lox
CPT2B-/-
pmols/ mg tissue
Fed
Fast
C12:0
C4:0
C14:2
C12:1
C4-OH
C14:1-OH
C5-OH
C
LFD
HFD
B
C16:1-OH
C5:0
C3-DC
C5-DC/
C10-OH
C10:0
C6:0
D
E
Acetate
nmol/mL acetylcarnitine
nmol/mL
C16:1
C16:0
C18:1
C18:0