Supplementary information

CPT1C deficiency in SF1 neurons impairs early metabolic adaptation leading to obesity

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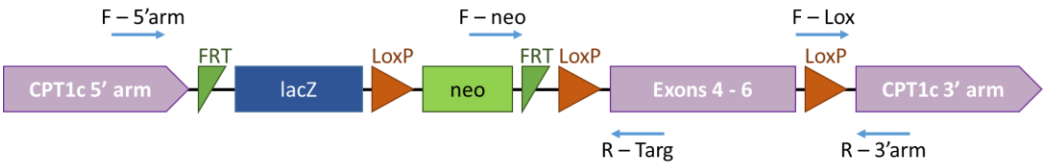
GENOTYPE	PRIMER	SEQUENCE	AMPLICON SIZE	VOLUME	AGAROSE GEL	ANNEALING
CPT1c – KO	F9	5'- GAGTCAGCCATGACCCGACTGTT -3'	200pb	0.9 µL	0.8%	65ºC – 30''
	R1	5'- CCGGTAGAATTGACCTGCAGGGGC -3'		0.6 µL		
	R9	5'- CGCTAAAGCCCAGACAGAACACAC -3'		1.6 µL		
KO FIRST 1	F – 5'arm	5'- ATGGACCCATAGTCTTGAAACCTGG-3'	625 pb and 432pb	2 µL	2%	63.5ºC – 30''
	F - Neo	5'- GGGATCTCATGCTGGAGTTCTTCG -3'				
	R - Targ	5'- GTGTTTGCCTAGCATGCGAACG -3'				
KO FIRST 2	F - Lox	5'- GAGATGGCGCAACGCAATTAATG -3'	292bp	2 µL	2%	63.5ºC – 30''
	R – 3'arm	5'- ATTCTTGATTCGTGTGACTCATTCCG -3'				
CONDITIONAL KO/FLOXED	F – 5'arm	5'- ATGGACCCATAGTCTTGAAACCTGG-3'	517pb	1.5 µL	2%	63.5ºC – 30''
	R - Targ	5'- GTGTTTGCCTAGCATGCGAACG -3'				
FLPO	F – FlpO	5'- CTATCGAATTCCACCATGGCTCCTAAGAAGAA -3'	1300pb	1 µL	0.8%	58ºC – 1'
	R - FlpO	5'- CAATGCGATGAATTCTCAGATCCGCCTCTTGATGTA -3'				
SF1-CRE	F – Cre	5'- CTGAGCTGCAGCGCAGGGACAT -3'	250pb	1 µL	2%	68ºC – 1'
	R – Cre	5'-TGCGAACCTCATCACTCGTTGCAT -3'				

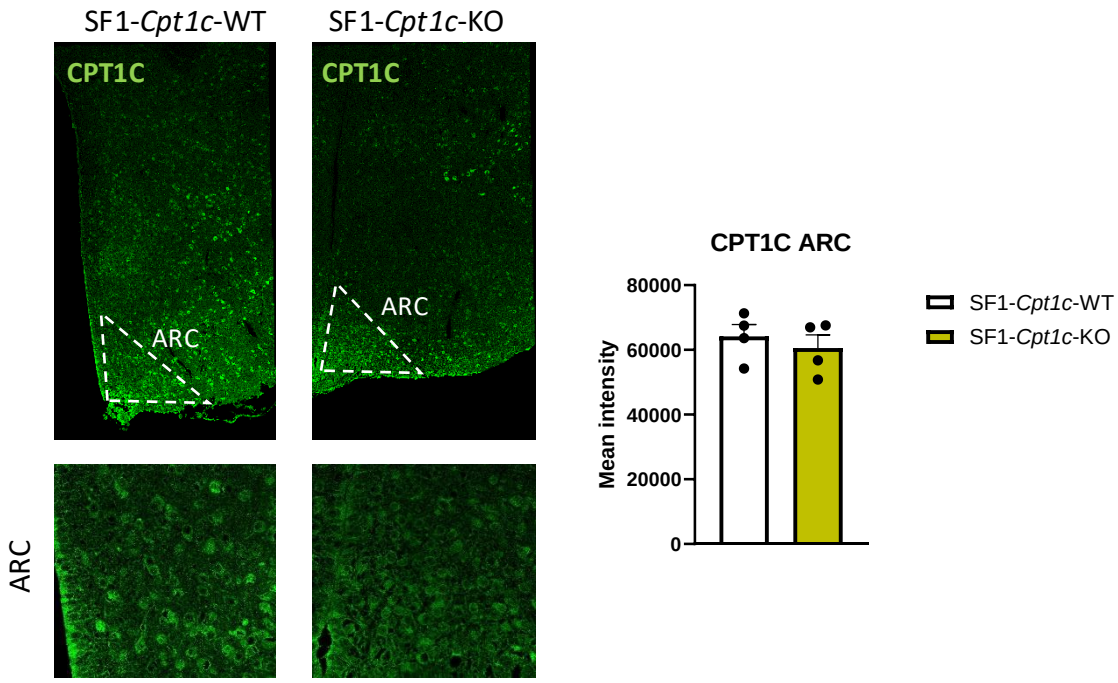
Supplemental Table 1. Genotyping primers. Oligonucleotides enhancers used for genotyping mice. Sequence and experimental details are also indicated.

Gene (protein)	Forward sequence	Reverse sequence
Acaca (ACCα)	5'-ATGGGCGGAATGGTCTCTTTC	5'-TGGGGACCTTGTCTTCATCAT
Agrp	5'-TTTGTCCTCTGAAGCTGTATGC	5'-GCATGAGGTGCCTCCCTA
Atgl	5'-AACAACAGCATCCAGTTCAA	5'-GGTTCAGTAGGTCATTCCTC
Cd36	5'-TGGCCAAGCTATTGCGACAT	5'-ACACAGCGTAGATAGACCTGC
Cpt1a	5'-GACTCCGCTCGCTCATTC	5'-AAGGCCACAGCTTGGTGA
Cpt1b	5'-TGCCTTTACATCGTCTCCAA	5'-GGCTCCAGGGTTCAGAAAGT
Dagla	5'-TATCTTCCTCTTCCTGCT	5'-CCATTTCGGCAATCATAC
Faah	5'-CAGCTACAAGGGCCATGCT	5'-TTCCACGGGTTCATGGTCTG
Fabp4	5'-GGATGGAAAGTCGACCACAA	5'-TGGAAGTCACGCCTTTCATA
Fasn	5'-CAGATGATGACAGGAGATGGAA	5'-CACTCACACCCACCCAGA
Gapdh	5'-TCCACTTTGCCACTGCA	5'-GAGACGGCCGCATCTTCTT
Hsl	5'-TCACGCTACATAAAGGCTGCT	5'-CCACCCGTAAAGAGGGAACT
Leptin	5'-CAGGATCAATGACATTTCACACA	5'-GCTGGTGAGGACCTGTTGAT
Lpl	5'-GGGAGTTTGGCTCCAGAGTTT	5'-TGTGTCTTCAGGGGTCCTTAG
Mgl1	5'-CCCAGTGGCACACCCAAG	5'-TAACGGCCACAGTGTTCCC
Nape-pld	5'-AAAACATCTCCATCCCGAA	5'-CGTCCATTTCCACCATCA
Npy	5'-TCCGCTCTGCGACACTACAT	5'-TGCTTTCCTTCATTAAGAGGT
Pgc1a	5'-GAAAGGGCCAAACAGAGAGA	5'-GTAAATCACACGGCGCTCTT
Pomc	5'-TGAACATCTTTGTCCCCAGAG	5'-TGCAGAGGCAAACAAGATTGG
Ppard	5'-AGGAGAAAGAGGAAGTGGCC	5'-GGGAGGAATTCTGGGAGAGG
Pparg	5'-CCGAAGAACCATCCGATTGAA	5'-GCCCAAACCTGATGGCATT
Prdm6	5'-CCTAAGGTGTGCCCAGCA	5'-CACCTTCCGCTTTTCTACCC
Scd1	5'-TTCCCTCCTGCAAGCTCTAC	5'-CAGAGCGCTGGTCATGTAGT
Adrb3 (β3AR)	5'-TCGACATGTTCCTCCACAAA	5'-GATGGTCCAAGATGGTGCTT

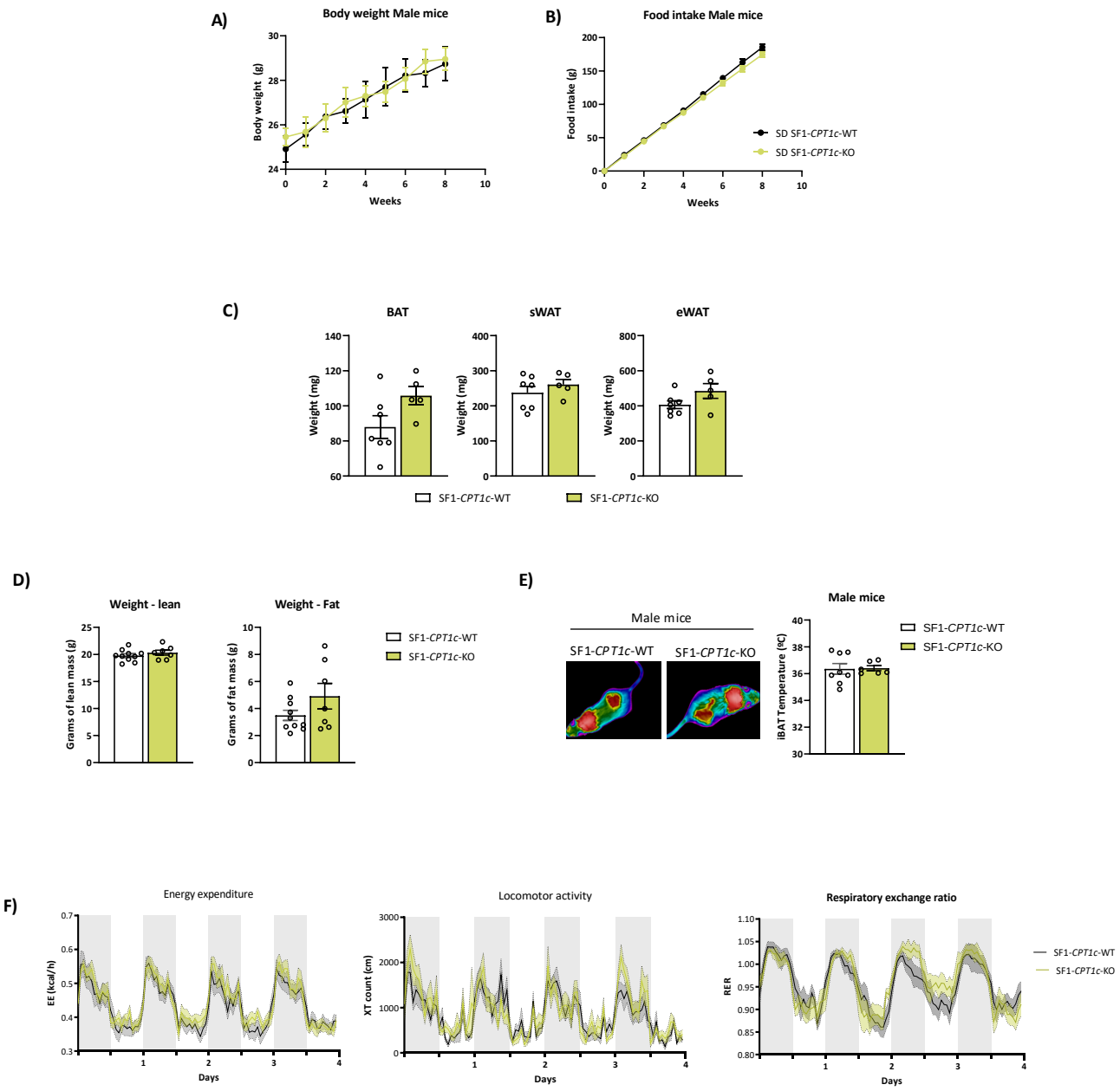
Supplemental Table 2. Oligonucleotides used for quantitative RT-PCR



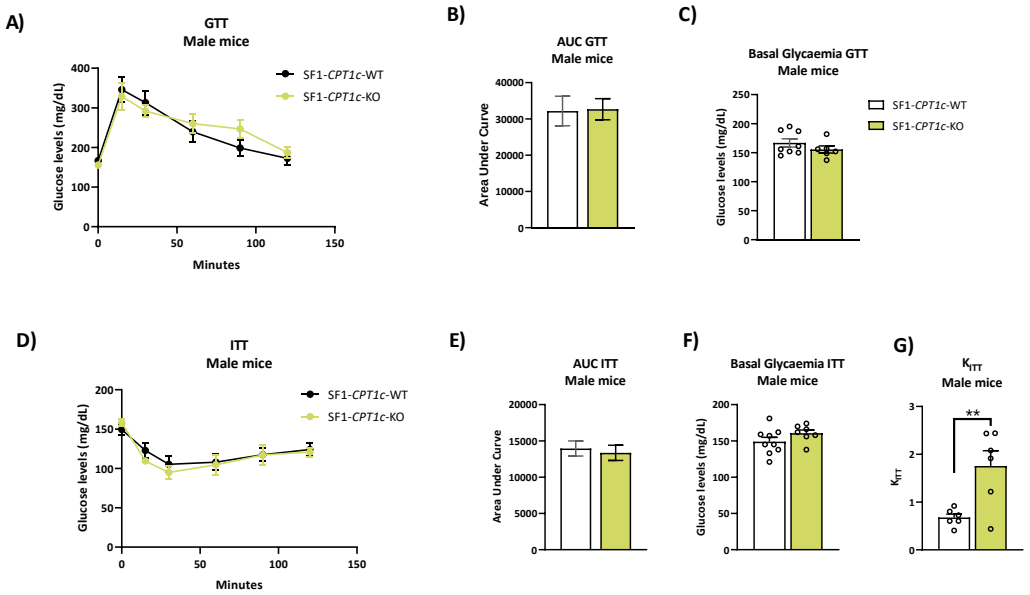
Supplemental Figure 1. Schematic representation of modified *Cpt1c* gene. All oligonucleotide enhancers used for genotyping are also represented.



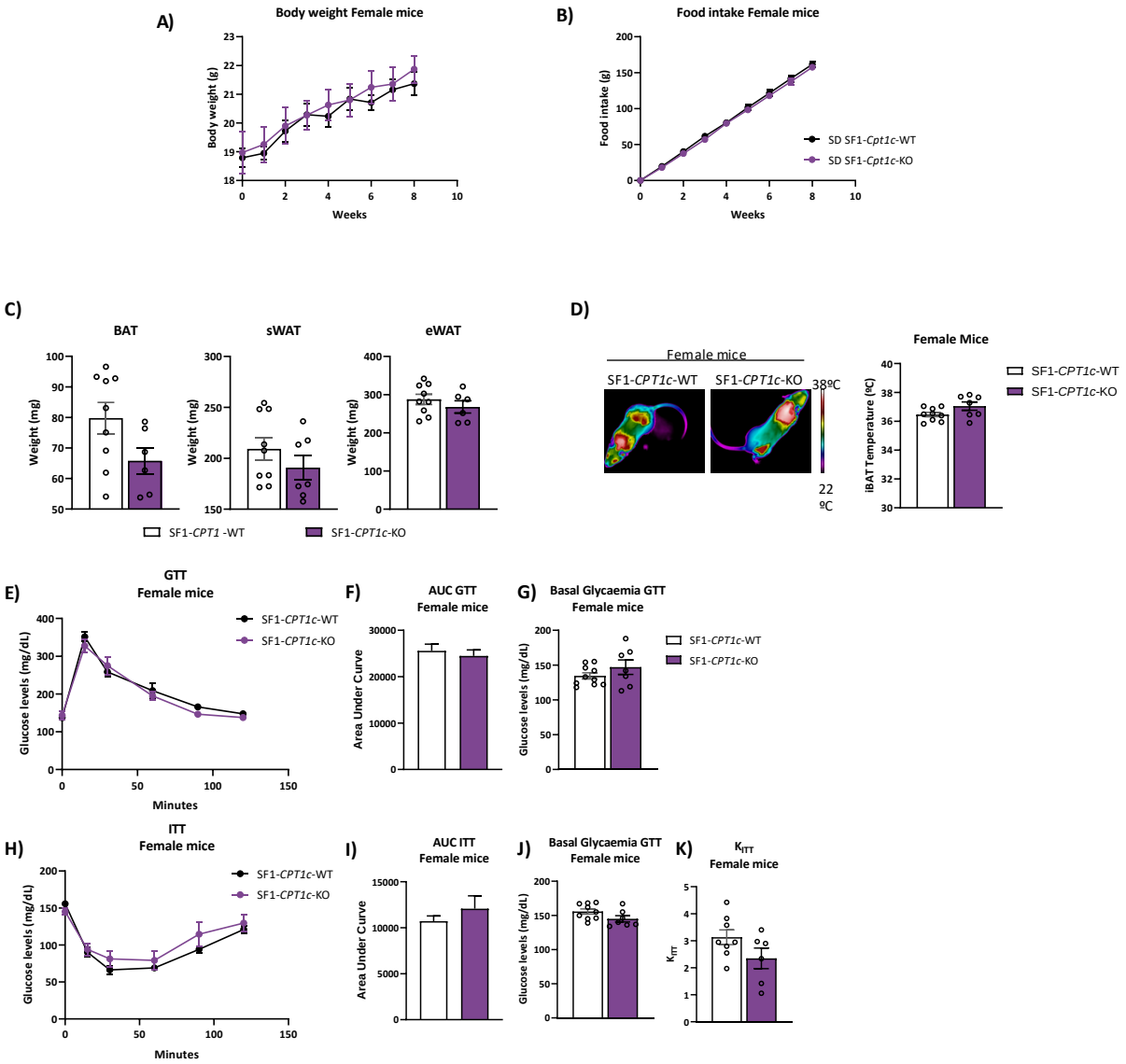
Supplemental Figure 2. Quantification of CPT1C immunostaining in the ARC. Representative images of selected areas for CPT1C quantification. Data were represented as mean ± SEM, male mice, between 8 and 12-week-old, n= 4/ group. Statistical significance was determined by t-student test.



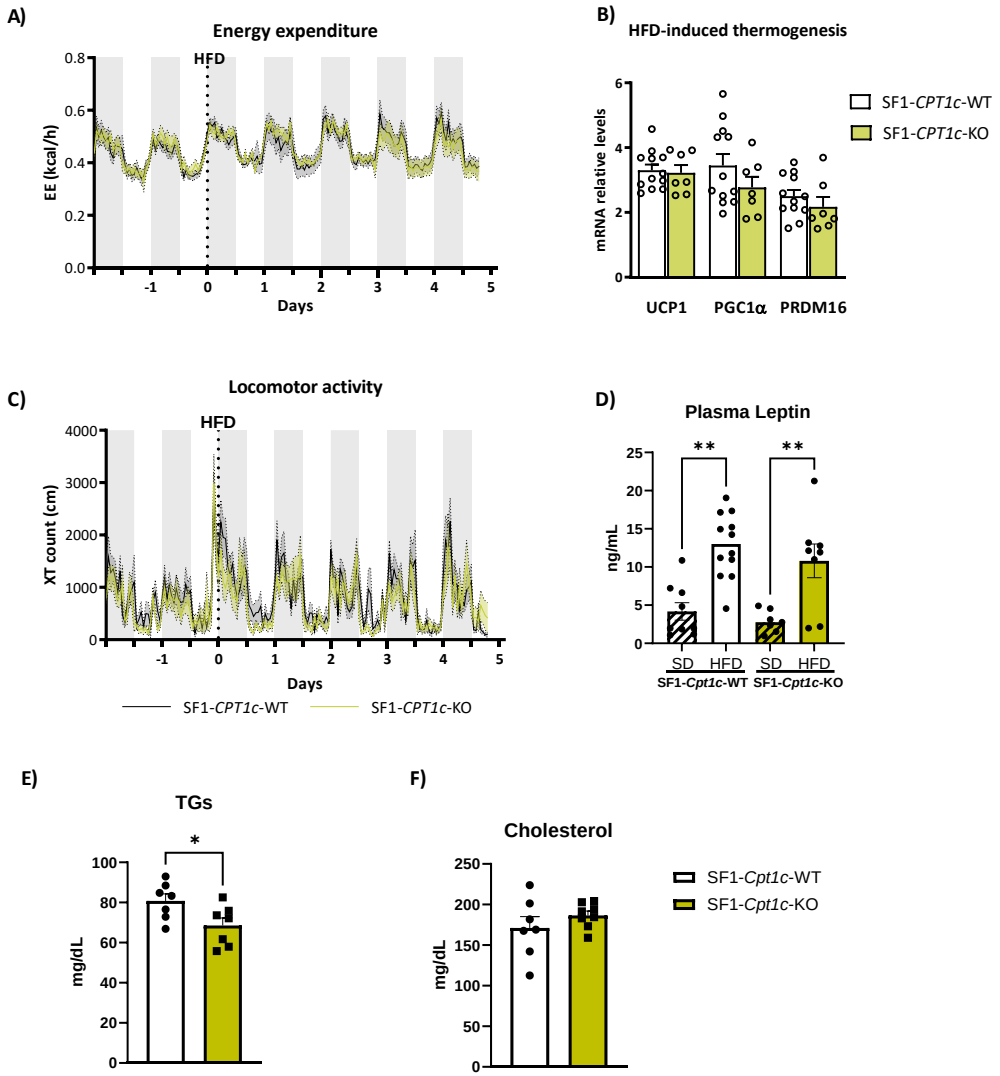
Supplemental Figure 3. Metabolic phenotype of SF1-*Cpt1c*-KO male mice under chow diet. **A)** Body weight. B) Food intake. C) Weight of BAT, sWAT and eWAT collected after 8 weeks on chow diet. D) MRI analysis of lean and fat mass. E) Representative IR pictures and iBAT temperature normalized to the temperature of back's mice. F) Energy expenditure, locomotor activity on the horizontal axis of the cage (XT) and Respiratory exchange ratio monitored for 4 days. Data were represented as mean ± SEM, male mice, between 8 and 12-week-old, n= 7-10/ group. Statistical significance was determined by t-student test and by ANOVA test with post-hoc Bonferroni.



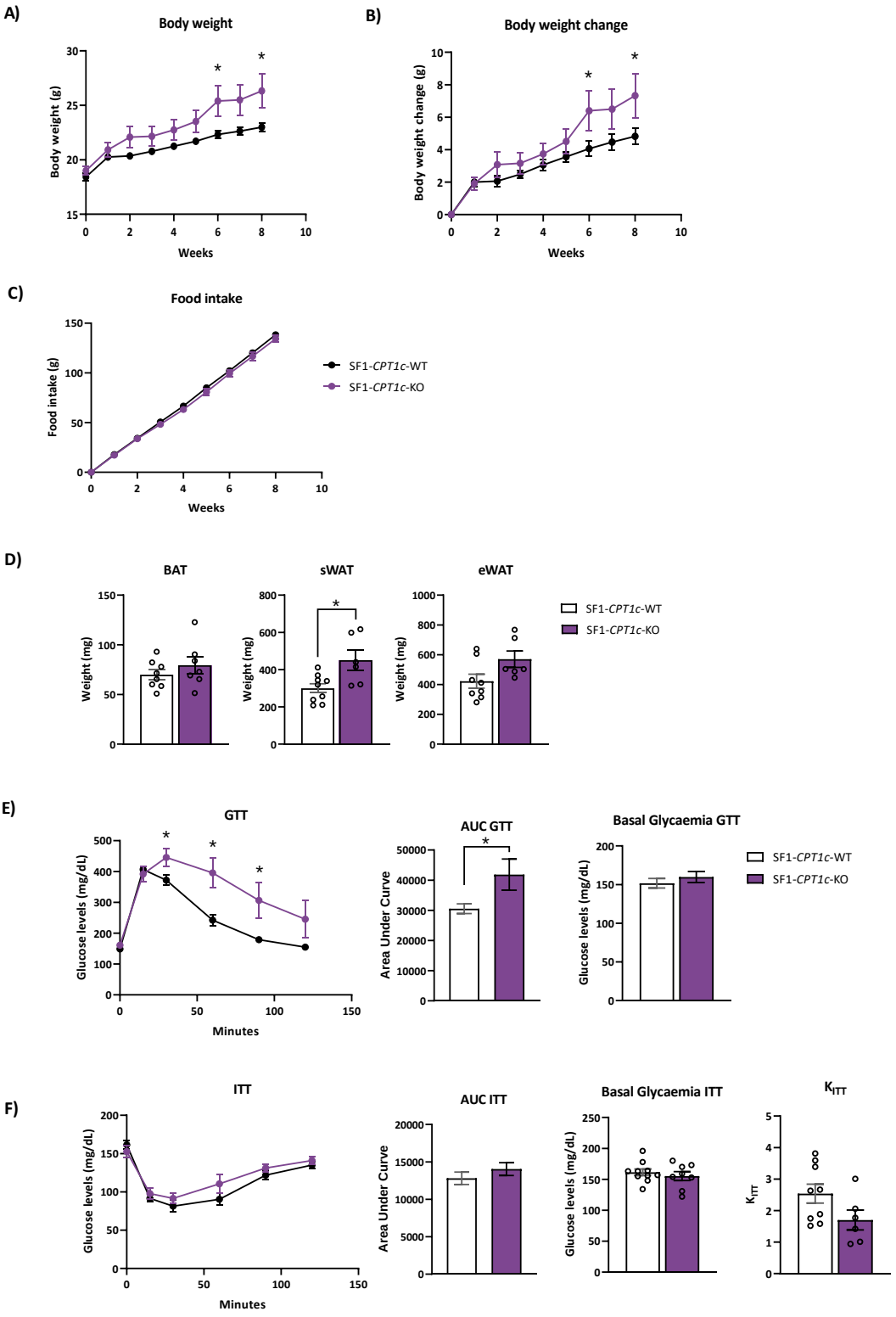
Supplemental Figure 4. Gucose and insulin sensitivity of male mice in chow diet. A) GTT. B) Area under curve of GTT. C) Basal glycaemia of GTT. D) ITT. E) Area under curve of ITT. F) Basal glycaemia of ITT. G) KITT calculated on the first 30 min of ITT. Data were represented as mean ± SEM, male mice, 8-weekold, n= 7-9/ group. Statistical significance was determined by ANOVA test and t-student test.



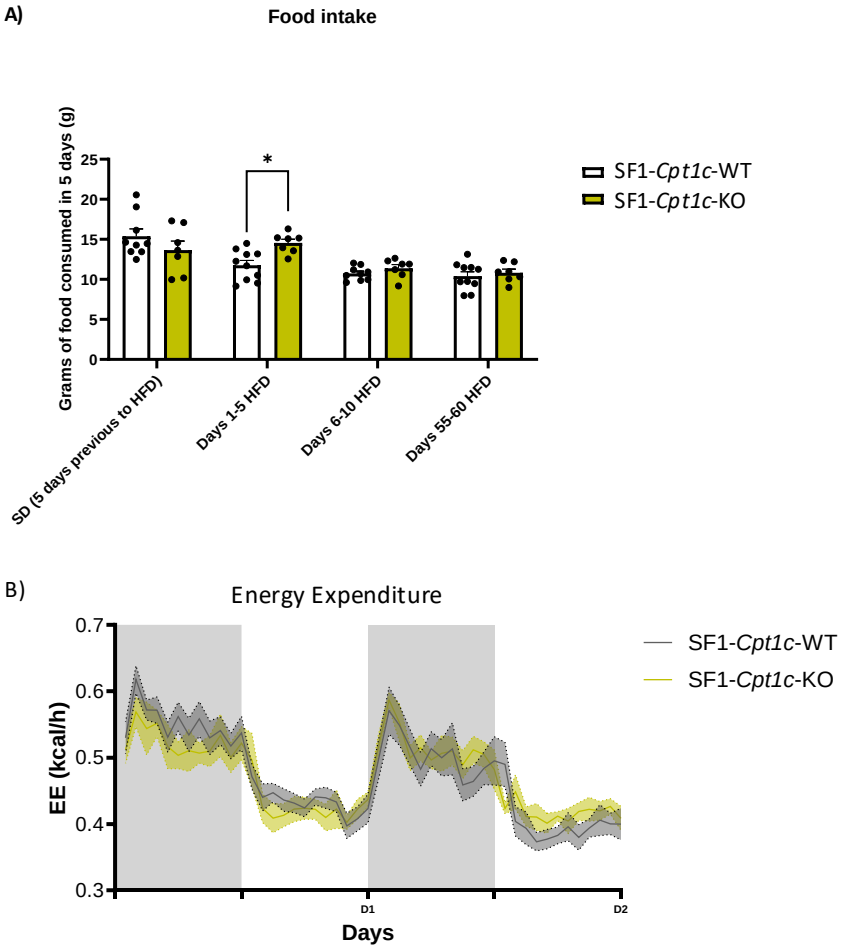
Supplemental Figure 5. Metabolic phenotype of SF1-*Cpt1c*-KO female mice under chow diet. A) Body weight. B) Food intake. C) Weight of BAT, sWAT and eWAT collected after 8 weeks on chow diet. D) Representative IR pictures and iBAT temperature normalized to the temperature of back's mice. E) GTT. F) Area under curve of GTT. G) Basal glycaemia of GTT.H) ITT. I) Area under curve of ITT. J) Basal glycaemia of ITT. K) KITT calculated on the first 30 min of ITT. Data were represented as mean ± SEM, male mice, 8-weeks-old, n= 7-9/ group. Statistical significance was determined by ANOVA test and t-student test.



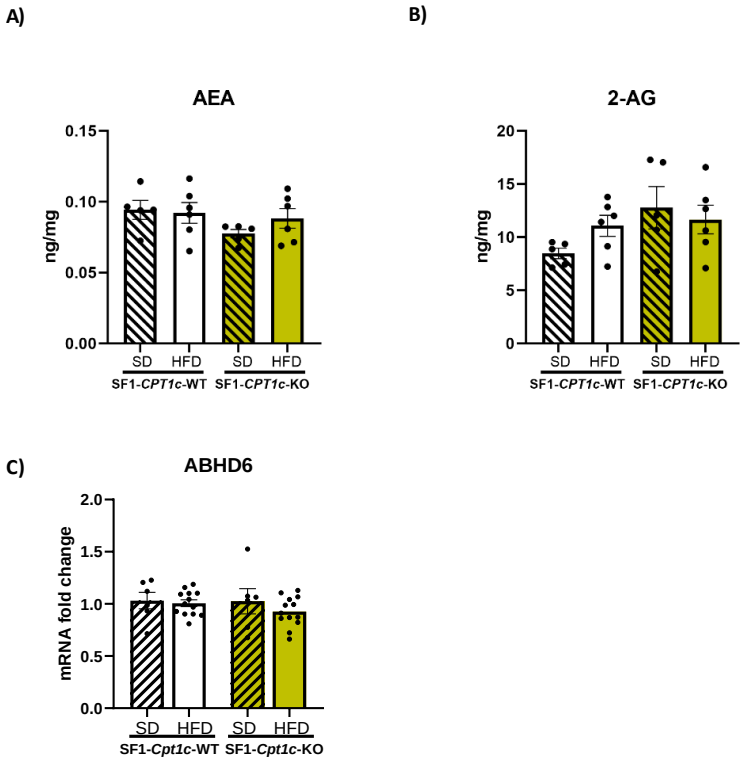
Supplemental Figure 6. Short-term high fat diet (HFD) in male mice. A) Energy expenditure. B) UCP1, PGC1α and PRDM16 mRNA thermogenic markers. C) Locomotor activity on the horizontal axis of the cage (XT). D) Plasma Leptin. E) Plasma triglycerides. F) Plasma cholesterol. Data were represented as mean ± SEM, male mice, between 16-week-old, n= 7-10/ group. Statistical significance was determined by ANOVA test with post-hoc Bonferroni; or t-student.



Supplemental Figure 7. Metabolic phenotype of SF1-*Cpt1c*-KO female mice under 8 weeks of HFD exposure. A) Body weight. B) Body weight change. C) Food intake. D) Weight of BAT, sWAT and eWAT collected after 8 weeks on high-fat diet. E) GTT. F) Area under curve of GTT. G) Basal glycaemia of GTT.H) ITT. I) Area under curve of ITT. J) Basal glycaemia of ITT. K) KITT calculated on the first 30 min of ITT. All tests performed after 8 weeks of high-fat diet exposure. Data were represented as mean ± SEM, male mice, 8-weeks-old, n= 7-9/ group. Statistical significance was determined by ANOVA test and t-student test.



Supplemental Figure 8. Long-term high fat diet (HFD) in male mice. (A) Cumulative food intake during a 5-day standard diet (SD) period prior to HFD administration, as well as during three separate 5-day intervals (days 1–5, 6–10, and 55–60) of HFD within an 8-week period. B) Energy Expenditure measured over two consecutive days. Data are presented as mean ± SEM for male mice aged approximately 16 weeks (n = 7–10 per group). Statistical significance was determined using ANOVA with post-hoc Bonferroni correction.



Supplemental Figure 9: eCB levels in the hippocampus of SF1-*Cpt1c*-KO and -WT mice under standard diet (SD) or HFD. A) Anandamide (AEA), B) 2-arachidonoylglycerol (2-AG). C) Hypothalamic ABHD6 mRNA levels. Data are represented as mean ± SEM, male mice, 8-12 week-old, n= 5-6/group. No statistical significance is observed. Statistical significance was determined by ANOVA test.