

## SUPPLEMENTARY MATERIAL

**Supplementary Figure 1.** Loss of hepatic fatty acid oxidation does not alter body weight or adiposity during low fat feeding, related to Figure 1.

**Supplementary Figure 2.** Loss of hepatic fatty acid oxidation does not significantly alter serum TBARS or transcriptional programming of oxidative stress genes related to Figure 2.

**Supplementary Figure 3.** Loss of hepatic fatty acid oxidation did not affect bone quality related to Figure 3.

**Supplementary Figure 4.** Transcriptional programming of bile acid metabolism genes in the liver was not significantly altered in Cpt2<sup>L/-</sup> mice by HFD, related to Figure 4.

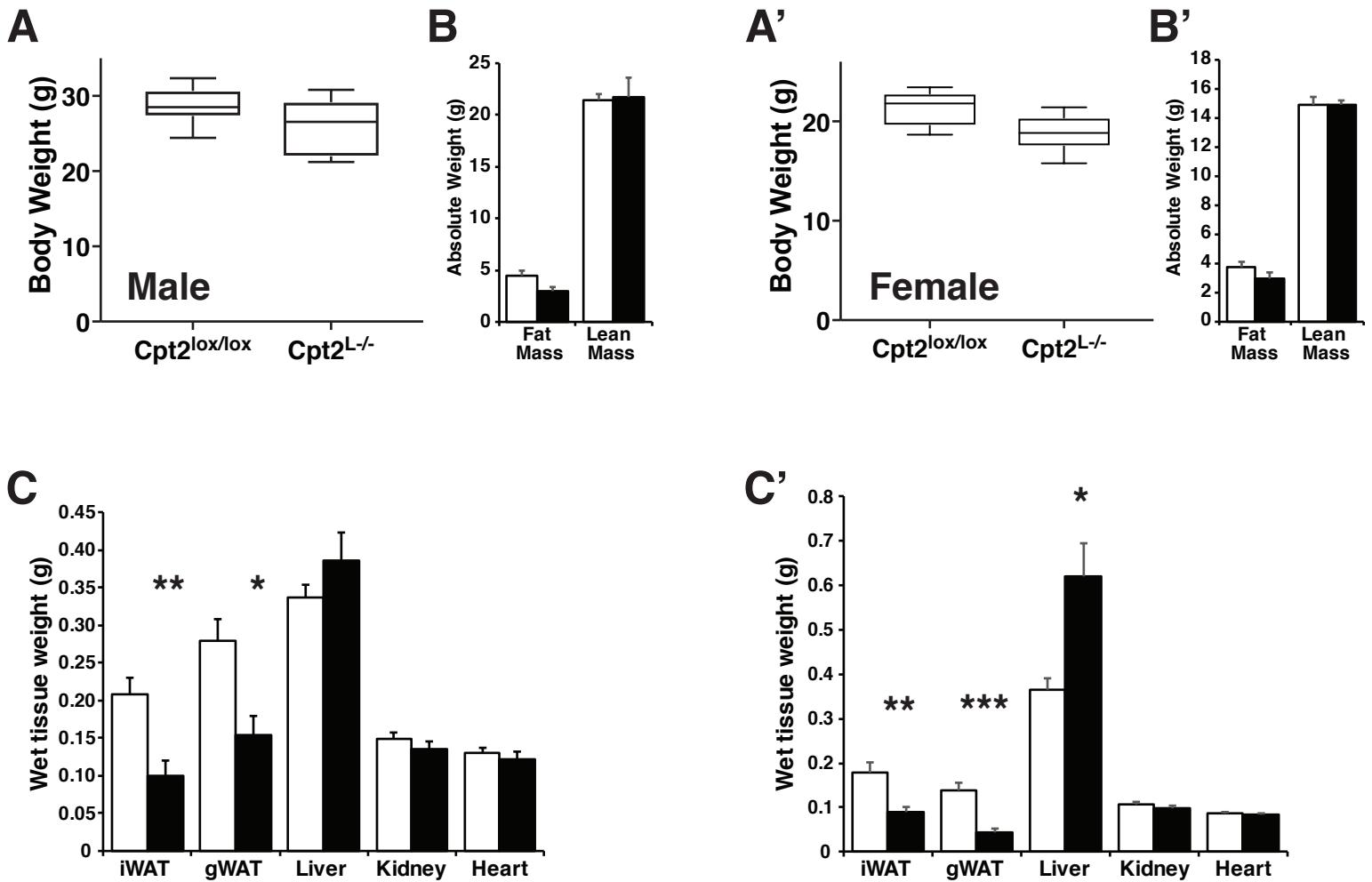
**Supplementary Figure 5.** 2-Deoxy-glucose uptake in skeletal muscle and adipose tissue is not different in Cpt2<sup>lox/lox</sup> and Cpt2<sup>L/-</sup> mice under hyperinsulinemic-euglycemic clamp, related to Figure 5.

**Supplementary Figure 6.** Liver-specific loss of Cpt2 did not induce browning in adipose tissue, related to Figure 6.

**Supplementary Figure 7.** Effects of feeding female Cpt2L/- mice a high fat diet at thermoneutrality, related to Figure 7.

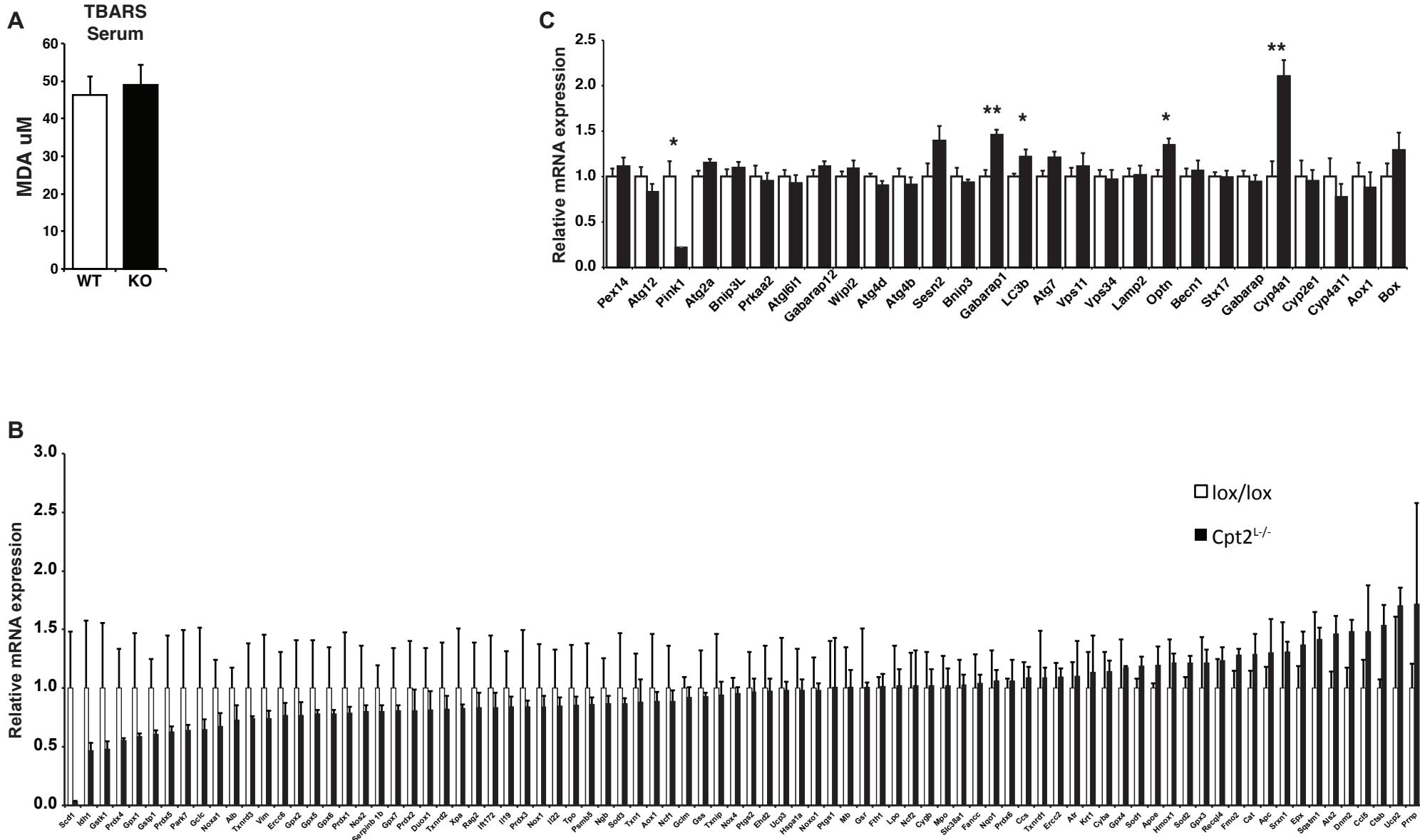
**Supplementary Table 1.** List of PCR primers, related to Experimental Procedures.

**Supplementary Table 2.** Fatty acid composition of Liver and iWAT of Cpt2<sup>lox/lox</sup> and Cpt2<sup>L/-</sup> mice fed a HFD for 16wks, related to Figure 2.



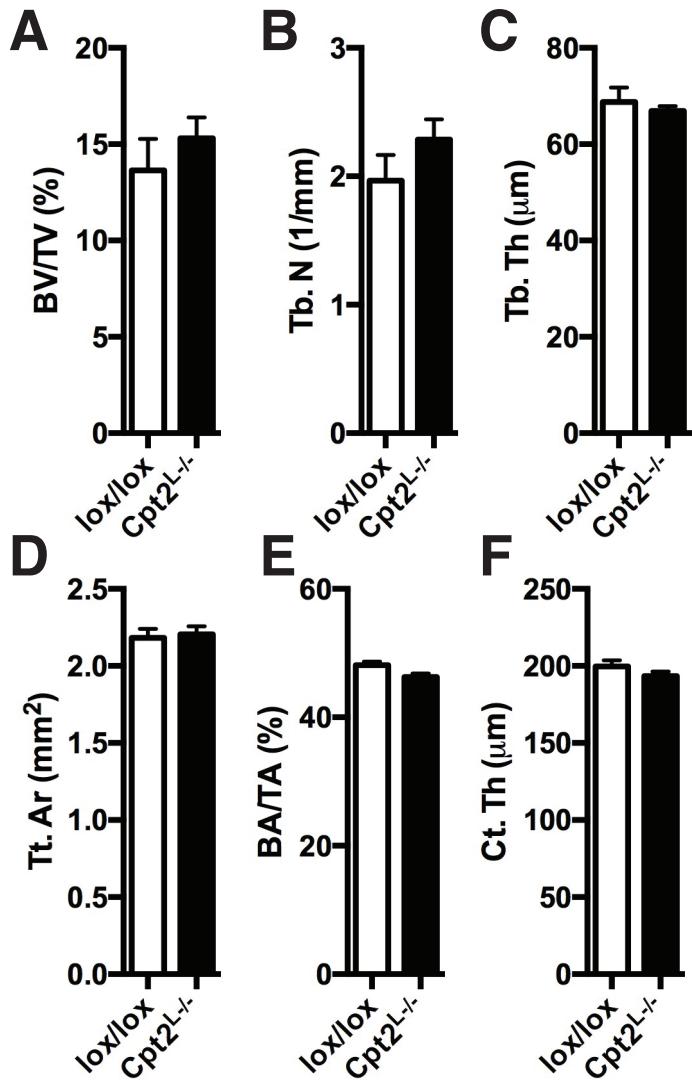
**Supplementary Figure 1. Loss of hepatic fatty acid oxidation does not alter body weight or adiposity during low fat feeding, related to Figure 1.**

- (A) Body weight of male Cpt2<sup>lox/lox</sup> and Cpt2<sup>L/-</sup> mice fed a LFD for 16wks (n=5-10).
  - (B) Body compositions of male Cpt2<sup>lox/lox</sup> and Cpt2<sup>L/-</sup> mice fed a LFD for 16wks measured by EchoMRI (n=5-10).
  - (C) Wet weights of iWAT and gWAT unilateral depots, liver and kidney for male Cpt2<sup>lox/lox</sup> and Cpt2<sup>L/-</sup> mice fed a LFD for 16wks (n=5-10).
- A', B' and C' indicate body weight, body composition and wet tissue weights, respectively, of female Cpt2<sup>lox/lox</sup> and Cpt2<sup>L/-</sup> mice fed a LFD for 16 weeks (n=5-8).
- Data are expressed as mean  $\pm$  SEM. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001



**Supplementary Figure 2. Loss of hepatic fatty acid oxidation does not significantly alter serum TBARS or transcriptional programming of oxidative stress genes, related to Figure 2.**

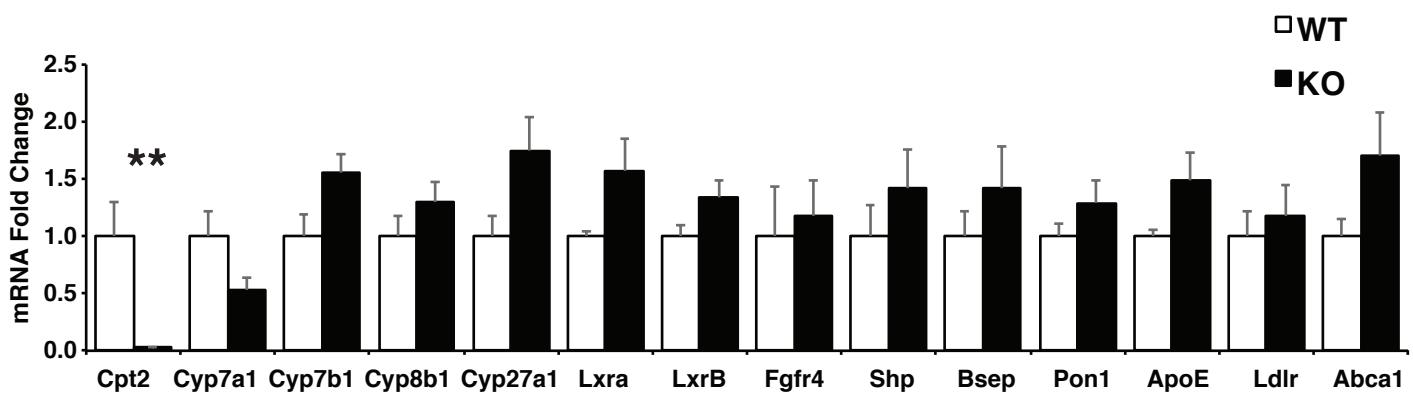
- (A) TBARS assay measuring lipid peroxidation in serum of male Cpt2<sup>lox/lox</sup> and Cpt2<sup>L-/-</sup> mice fed a HFD for 16wks (n=6).
  - (B) Gene expression profiling of genes involved in oxidative stress via oxistress array in livers of male Cpt2<sup>lox/lox</sup> and Cpt2<sup>L-/-</sup> mice fed a HFD for 16wks (n=3).
  - (C) Gene expression profiling of genes involved in peroxisome and autophagy genes in livers of male Cpt2<sup>lox/lox</sup> and Cpt2<sup>L-/-</sup> mice fed a HFD for 16wks (n=6).
- Data are expressed as mean  $\pm$  SEM. \*p<0.05; \*\*p<0.01; \*\*\*p<0.001



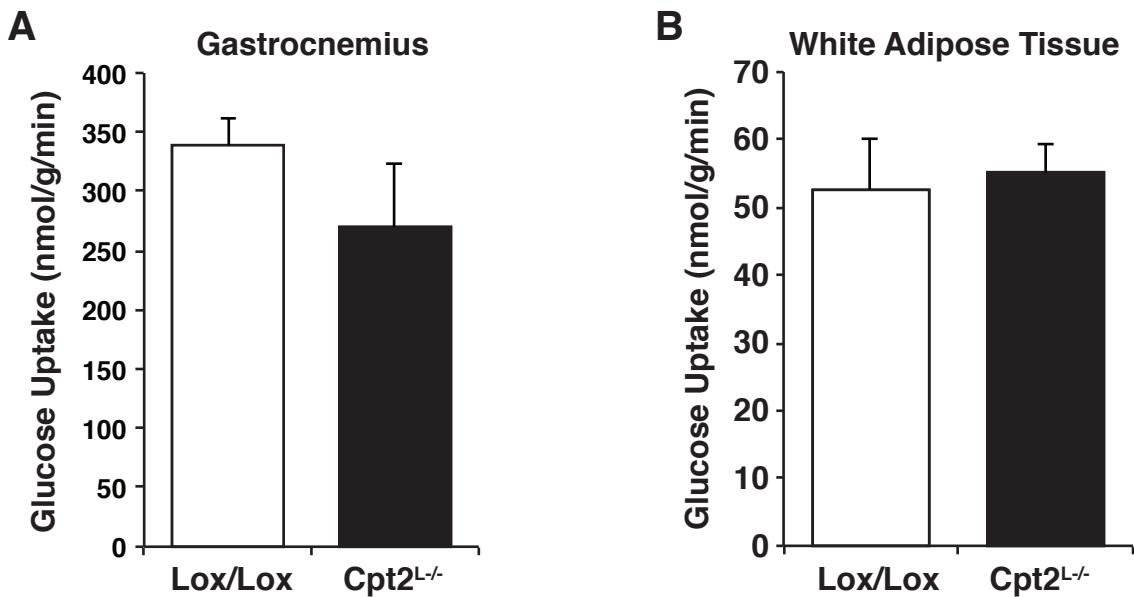
**Supplementary Figure 3. Loss of hepatic fatty acid oxidation did not affect bone structure, related to Figure 3.**

- (A) Trabecular bone volume/tissue volume (BV/TV) of Cpt2lox/lox and Cpt2L-/- mice fed a HFD for 16wks.
- (B) Trabecular number of Cpt2lox/lox and Cpt2L-/- mice fed a HFD for 16wks.
- (C) Trabecular thickness of Cpt2lox/lox and Cpt2L-/- mice fed a HFD for 16wks.
- (D) Cortical bone volume/tissue volume (BV/TV) of Cpt2lox/lox and Cpt2L-/- mice fed a HFD for 16wks.
- (E) Cortical number of Cpt2lox/lox and Cpt2L-/- mice fed a HFD for 16wks.
- (F) Cortical thickness of Cpt2lox/lox and Cpt2L-/- mice fed a HFD for 16wks.

Data are expressed as mean  $\pm$  SEM (n=8-12)



**Supplementary Figure 4.** Transcriptional programming of bile acid metabolism genes in the liver was not significantly altered in Cpt2L<sup>-/-</sup> mice by HFD, related to Figure 4. Data are expressed as mean ± SEM. \*\*p<0.01 (n=4).

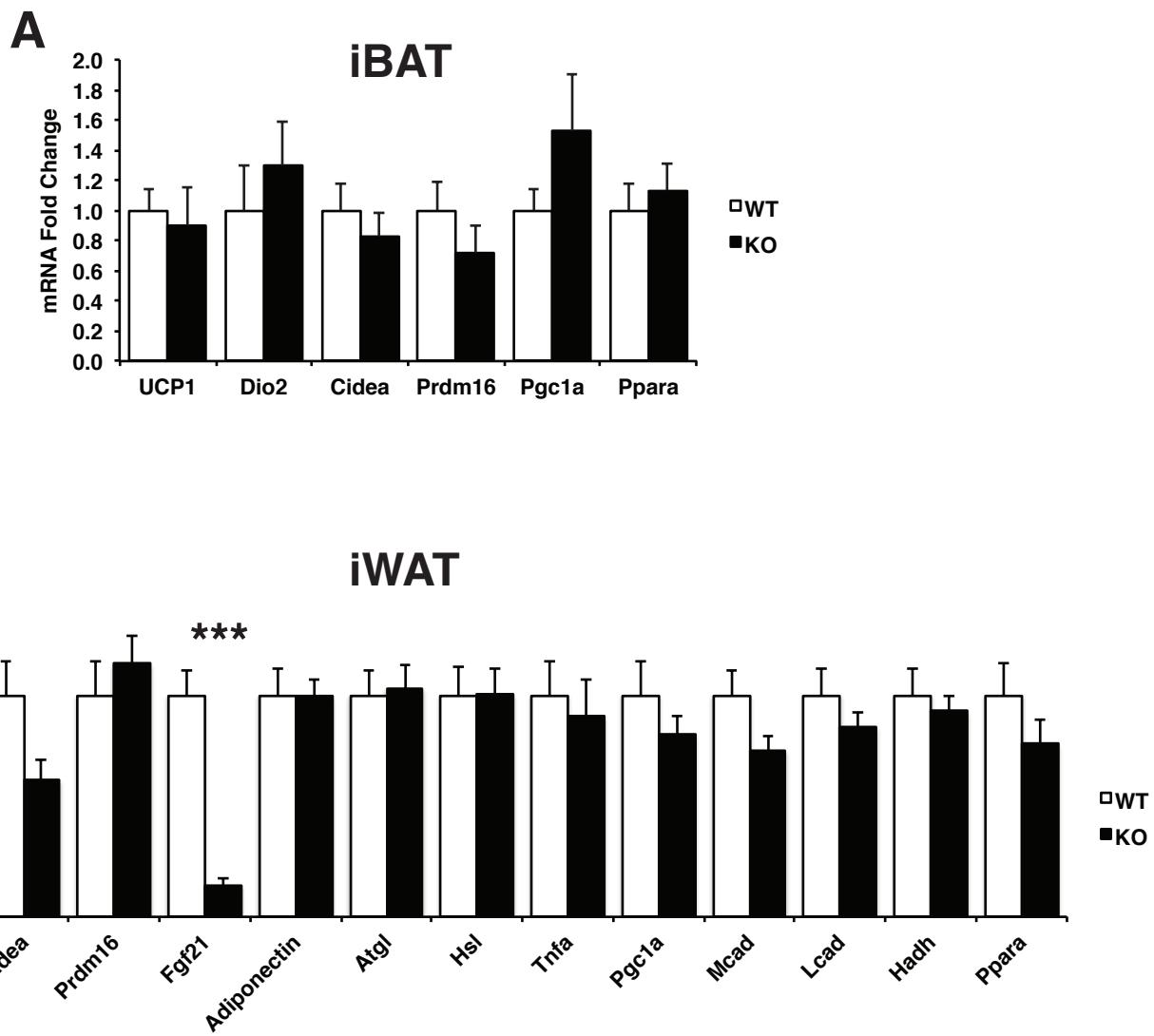


**Supplementary Figure 5.** 2-Deoxy-glucose uptake in skeletal muscle and adipose tissue is not different in  $\text{Cpt2}^{\text{lox}/\text{lox}}$  and  $\text{Cpt2}^{\text{L-/-}}$  mice under hyperinsulinemic-euglycemic clamp, related to Figure 5.

(A) 2-Deoxy-glucose uptake in Gastrocnemius muscle in  $\text{Cpt2}^{\text{lox}/\text{lox}}$  and  $\text{Cpt2}^{\text{L-/-}}$  mice ( $n=7-9$ ).

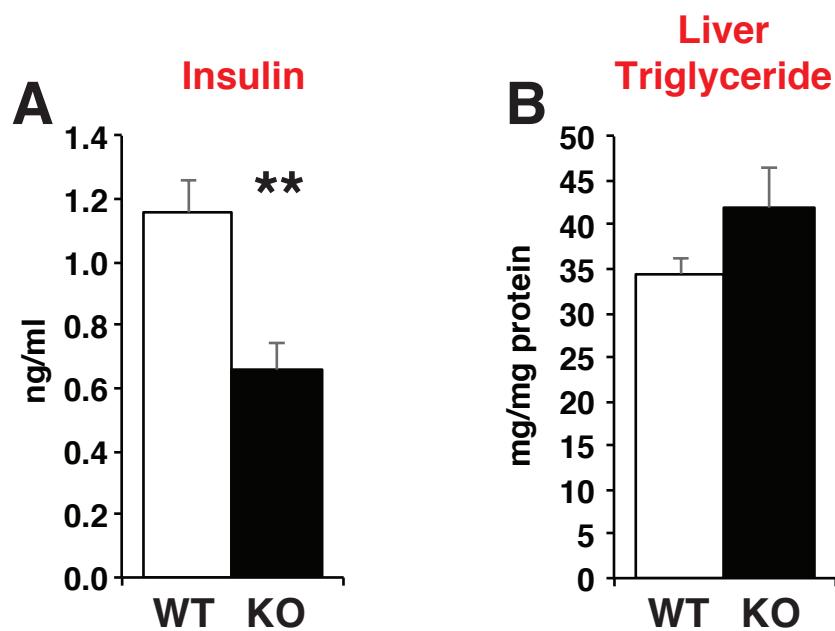
(B) 2-Deoxy-glucose uptake in gWAT in  $\text{Cpt2}^{\text{lox}/\text{lox}}$  and  $\text{Cpt2}^{\text{L-/-}}$  mice ( $n=7-9$ ).

Data are expressed as mean  $\pm$  SEM.



**Supplementary Figure 6. Liver-specific loss of Cpt2 did not induce browning in adipose tissue, related to Figure 6.**

- (A) BAT gene expression in Cpt2<sup>lox/lox</sup> and Cpt2<sup>L-/-</sup> mice fed a HFD for 16wks (n=6).
  - (B) iWAT gene expression in Cpt2<sup>lox/lox</sup> and Cpt2<sup>L-/-</sup> mice fed a HFD for 16wks (n=6).
- Data are expressed as mean  $\pm$  SEM. \*\*p<0.01; \*\*\*p<0.001



**Supplementary Figure 7. Effects of feeding female Cpt2L<sup>-/-</sup> mice a high fat diet at thermoneutrality, related to Figure 7.**

(A) Serum insulin concentration of female Cpt2lox/lox and Cpt2L<sup>-/-</sup> mice fed a HFD for 16wks at 30°C (n=6).  
 (B) Liver triglyceride concentration of female Cpt2lox/lox and Cpt2L<sup>-/-</sup> mice fed a HFD for 16wks at 30°C (n=6). Data are expressed as mean ± SEM. \*\*p<0.01

**Supplementary Table 1. List of PCR primers, related to Experimental Procedures.**

Gene	Forward	Reverse
<i>fgf15</i>	gaggaccaaaacgcgaaatt	acgtccgtatggcaatcg
<i>fyr</i>	gcttgatgtctacaaaagctg	cgttgtatggtaatgtcc
<i>ostα</i>	atgcatctggtaacagaa	gagtagggaggtgagcaagc
<i>ostb</i>	gaccacagtgcagagaagc	cttgtatgaccaccaggac
<i>ibabp</i>	caaggctaccgtgaagatgg	cccacgacccctcgaaatct
<i>diet1</i>	actgttcggttctggtctac	ctccagatgcacacgtggtg
<i>cyp4a1</i>	ctcatctgccctctcag	tccattttggacttcagc
<i>cyp2e1</i>	atgtctccctcgagatgt	gatgtccctccaggtaggtcc
<i>cyp4a11</i>	aggagctcaacaggaccag	cctgtatggctgaaggcacac
<i>Aox1</i>	agctccgtatgcggcagacat	ttcttgcggaaacagagccccagaatg
<i>box</i>	gggcattccacatccggttg	tggctctcgagcagatcagc
<i>pex14</i>	aggactgacagacgaagagatt	tgtatgatagccaaggcaccata
<i>atg12</i>	ggcctcgaaacagtgttta	cagcaccgaaatgtctgt
<i>pink1</i>	ttctccgcagtcggtag	ctgtttctctcgatcagcc
<i>atg2a</i>	ccacctctgcaaatcgca	ccagttgtctgtatccatcaca
<i>bnip3l</i>	atgctcaacttagtcgagccg	ctcatgtgtgcacccaggaa
<i>prkaa2</i>	caggccataaagtggcagtt	aaaagtctgtcgagtgctga
<i>atg16l1</i>	cgaatctggactgtggatga	agcaggaacttgcgcagagag
<i>gabarapl2</i>	tcgggctctcagattgtgac	atggccttctcgaggaggaa
<i>wipi2</i>	aggataaacacgtccctagct	tctctccacaatgcagacatct
<i>atg4d</i>	gtcaagtatgttggcagtt	tgtcacccctctccatcacaat
<i>atg4b</i>	catccatcagatagcgc当地	tgtttccatccatcaccaca
<i>sesn2</i>	tccgagtgccattccgagat	tccgggtgttagacccatcac
<i>bnip3</i>	tcctggtagaaactgcattc	gctggcattccaaacagtattt
<i>gabarapl1</i>	ggaccacccttcgagttac	cctcttatccagatcaggacc
<i>lc3b</i>	cccaccaagatcccaatgt	ccaggaacttgcgttgc
<i>atg7</i>	tgcctatgtatctgttc	caccaactttatcttgc
<i>vps11</i>	aaaagagagacggcgaatc	agccagtaacggatagtt
<i>vps34</i>	cctggacatcaacgtgcag	tgtctctggatagccagaaa
<i>lamp2</i>	gatgtgcctctccggta	attggactgaacggctcta
<i>optn</i>	atgtcccatcaacctctgagc	tcaaatgcgccttcatagcttg
<i>becn1</i>	ggaaaagaaccgcgaaatgt	aaactgtccgtgtccagatg
<i>stx17</i>	tcaaagtggcaggaatgtcag	aattttccacctgtgaaatgt
<i>gabarap</i>	aaggaggcatccgtcgaga	gctttggggctttccac
<i>Acsl1</i>	atctgggaaacggggcaag	tccctttgggtgcctgt
<i>Pgc1a</i>	caacatgtcaagccaaaccaaca	cgctcaatgttttgcataatgg
<i>Acot1</i>	gacaagaagacgttcatccgt	catcggatagaactgcgttcc
<i>Acot2</i>	agtcaacgcgc当地atgt	gctcttccaaatctgtgt
<i>Acox1</i>	acgcacccatctgtcttc	agattggtagaaatgtgt
<i>Cpt1a</i>	ccatctgtctgtacaagggtt	cctacttgcgttacagctgt
<i>Mcad</i>	aacacttactatgcctcgattca	ccatgcctccgaaaatgt
<i>Lcad</i>	tttcctcgaggcatgatttt	gccagttttccagac
<i>Hadha</i>	tgcatttgcgcagttac	gttggcccagattcgttca
<i>Ppara</i>	gcgtacggcaatgtgtt	gaacgcgttccctcagg
<i>Pdk4</i>	atctaaccatgcgc当地aaacc	ggaacgtacacaatgtggat
<i>Gpnmb</i>	cattcccatctcgaaatgt	aaatggcagatgtgtt
<i>Phospho1</i>	aagcacatcatccacatcc	tttgttccagatgtgt
<i>Fabp3</i>	acctggaaagctgtggacag	tgtatggtagatgtgtt
<i>Atf3</i>	cgaagactggagcaaaatgt	caggtagtgc当地aaatc
<i>Plin3</i>	tccaagctggactgtttag	gcccattgttccatgt
<i>Jun</i>	ccttctacgacgtccctc	gttcaagggtatgtgtt
<i>Agpat9</i>	ggccttcggattatccctgg	cttggggcttctgt
<i>Pex11a</i>	gacgccttcatccgagtcg	cggccttgcgttgc
<i>Ehhadh</i>	cagatgaaggactcaagctg	accttggcaatggctgt
<i>Myc</i>	atgccttcacgttgcact	cgc当地ataggatggagagca

**Supplementary Table 2. Fatty acid composition of Liver and iWAT of Cpt2lox/lox and Cpt2L/- mice fed a HFD for 16wks, related to Figure 2.**

	LIVER ug/mg tissue						LIVER %						ADIPOSE ug/mg tissue						ADIPOSE %						
	LOX/LOX	SE	Cpt2 A-/-	SE	ttest	LOX/LOX	SE	Cpt2 A-/-	SE	ttest	LOX/LOX	SE	Cpt2 A-/-	SE	ttest	LOX/LOX	SE	Cpt2 A-/-	SE	ttest	LOX/LOX	SE	Cpt2 A-/-	ttest	
C10:0 - Capric	0.04	0.02	0.19	0.02	0.000	0.00%	0.00156%	0.01%	0.00064%	0.001	0.00706377	0.06	0.0138287	0.551	0.01%	0.000348%	0.01%	0.001273%	0.115						
C12:0 - Lauric	0.22	0.12	1.13	0.11	0.000	0.02%	0.00937%	0.07%	0.00399%	0.001	0.04726923	0.46	0.08712445	0.224	0.04%	0.001450%	0.07%	0.005894%	0.001						
C14:0 - Myristic	6.93	1.58	19.28	1.33	0.000	0.61%	0.11349%	1.22%	0.02382%	0.001	5.43	0.75667368	6.86	1.28265766	0.343	0.71%	0.015598%	1.02%	0.076703%	0.002					
C15:0 - Pentadecanoic	1.31	0.25	3.59	0.27	0.000	0.12%	0.01780%	0.23%	0.00608%	0.000	0.72	0.1076194	0.76	0.12799941	0.809	0.09%	0.002555%	0.11%	0.005950%	0.020					
C16:0 - Palmitic	264.84	11.30	329.22	11.69	0.003	24.22%	0.69111%	21.04%	0.57787%	0.007	148.79	18.2648431	124.75	17.5636436	0.373	19.61%	0.308535%	18.87%	0.599516%	0.345					
C17:0 - Heptadecanoic	2.72	0.25	5.44	0.57	0.001	0.25%	0.01253%	0.34%	0.02072%	0.002	2.85	0.64300314	1.75	0.37411783	0.194	0.36%	0.037552%	0.26%	0.022852%	0.090					
C18:0 - Stearic	53.79	2.84	79.40	3.64	0.000	4.92%	0.18758%	5.06%	0.10182%	0.544	43.53	6.12948929	27.37	4.37418293	0.069	5.68%	0.197134%	4.11%	0.156936%	0.001					
C20:0 - Arachidic	2.76	0.48	0.69	0.08	0.004	0.26%	0.04739%	0.04%	0.00373%	0.003	0.66	0.11122809	0.41	0.07850584	0.111	0.09%	0.004051%	0.06%	0.004789%	0.015					
C22:0 - Behenic	1.44	0.24	0.61	0.02	0.012	0.14%	0.02710%	0.04%	0.00234%	0.010	0.17	0.01548838	0.14	0.02454148	0.290	0.02%	0.000887%	0.02%	0.001576%	0.296					
C23:0 - Tricosanoic	0.21	0.01	0.34	0.01	0.000	0.02%	0.00159%	0.02%	0.00114%	0.321	0.05	0.00481086	0.05	0.00812591	0.951	0.01%	0.00378%	0.01%	0.000566%	0.338					
C24:0 - Lignoceric	0.42	0.03	0.50	0.01	0.043	0.04%	0.00426%	0.03%	0.00120%	0.178	0.12	0.01328557	0.10	0.02532926	0.559	0.02%	0.001311%	0.02%	0.002085%	0.686					
C25:0 - Pentacosanoic	0.01	0.00	0.02	0.00	0.000	0.00%	0.00005%	0.00%	0.00004%	0.001	0.01	0.00075026	0.01	0.00100028	0.226	0.00%	0.000031%	0.00%	0.000080%	0.179					
C26:0 - Hexacosanoic	0.01	0.00	0.01	0.00	0.022	0.00%	0.00005%	0.00%	0.00002%	0.049	0.01	0.00107886	0.01	0.00110238	0.199	0.00%	0.000025%	0.00%	0.000071%	0.105					
C28:0 - Octacosanoic	0.00	0.00	0.00	0.00	0.306	0.00%	0.00002%	0.00%	0.00002%	0.602	0.00	0.00025447	0.00	0.00013062	0.149	0.00%	0.000020%	0.00%	0.000021%	0.249					
C30:0	0.00	0.00	0.00	0.00	0.333	0.00%	0.00003%	0.00%	0.00002%	0.700	0.00	0.00039531	0.00	0.00022175	0.520	0.00%	0.000034%	0.00%	0.000021%	0.822					
C10:1 - Caproic	0.02	0.01	0.10	0.01	0.001	0.00%	0.00105%	0.01%	0.00043%	0.002	0.00	0.00063755	0.01	0.00119983	0.313	0.00%	0.000047%	0.00%	0.000084%	0.032					
C12:1 - Dodecanoic	0.00	0.00	0.00	0.00	0.010	0.00%	0.00002%	0.00%	0.00003%	0.255	0.00	0.00053283	0.01	0.00269868	0.010	0.00%	0.000038%	0.00%	0.000286%	0.001					
C16:1(n-9) - Oleic	33.46	4.94	73.91	5.45	0.000	2.99%	0.31445%	4.68%	0.16089%	0.001	9.79	1.86703136	8.20	1.47194833	0.533	1.24%	0.08427%	1.22%	0.087244%	0.868					
C17:1 - Heptadecenoic	2.80	0.27	4.74	0.38	0.002	0.25%	0.01278%	0.30%	0.00922%	0.019	1.86	0.45630072	2.20	0.31572185	0.566	0.23%	0.049353%	0.33%	0.014491%	0.078					
C18:1(n-9) - Oleic	310.16	15.70	408.56	15.28	0.002	28.29%	0.75215%	26.09%	0.634551	0.057	284.32	25.540741	240.22	21.4074887	0.230	38.39%	0.562447%	37.42%	1.546898%	0.676					
C20:0(n-9) - Eicosenoic	9.48	1.56	3.80	0.41	0.010	0.18%	0.13629%	0.24%	0.01526%	0.002	6.21	1.04955933	4.46	0.76459089	0.226	0.80%	0.032586%	0.67%	0.037482%	0.110					
C20:3(n-9) - Mead	1.34	0.13	1.12	0.15	0.028	0.12%	0.01184%	0.07%	0.00678%	0.005	0.20	0.02650522	0.16	0.03130611	0.325	0.03%	0.000598%	0.02%	0.001884%	0.155					
C22:1(n-9) - Erucic	0.65	0.12	0.14	0.01	0.003	0.06%	0.01074%	0.01%	0.00043%	0.002	0.21	0.03740123	0.14	0.02728136	0.176	0.03%	0.001113%	0.02%	0.001657%	0.074					
C24:1(n-9) - Nervonic	0.72	0.05	0.78	0.04	0.362	0.07%	0.00644%	0.05%	0.00176%	0.042	0.16	0.01666818	0.15	0.03080184	0.770	0.02%	0.001346%	0.02%	0.002417%	0.846					
C26:1(n-9) - Nervonic	0.00	0.00	0.01	0.00	0.000	0.00%	0.00002%	0.00%	0.00001%	0.001	0.01	0.00118466	0.01	0.000980305	0.330	0.00%	0.000072%	0.00%	0.000072%	0.314					
C14:1 - Myristoleic	0.09	0.03	0.27	0.04	0.003	0.01%	0.00189%	0.02%	0.000156%	0.007	0.13	0.0184071	0.43	0.05957057	0.007	0.02%	0.000769%	0.08%	0.0009052%	0.000					
C16:1(n-7) - Palmitoleic	30.54	5.18	69.22	7.16	0.002	2.72%	0.34599%	4.36%	0.23860%	0.005	24.80	3.89312471	36.06	5.69897638	0.128	3.20%	0.127407%	5.42%	0.370022%	0.000					
C18:1(n-7) - Vaccenic	24.99	2.61	26.43	2.43	0.701	2.27%	0.19631%	1.67%	0.07384%	0.003	21.85	3.49138473	16.56	2.61152013	0.272	2.81%	0.101260%	2.48%	0.106578%	0.099					
C18:1(n-5)	0.31	0.06	0.22	0.04	0.248	0.03%	0.00494%	0.01%	0.00229%	0.037	0.07	0.00642565	0.10	0.02422916	0.317	0.01%	0.000324%	0.01%	0.002796%	0.139					
C20:3(n-7)	0.31	0.03	0.54	0.07	0.014	0.03%	0.00162%	0.03%	0.00349%	0.186	0.06	0.00787352	0.10	0.01921108	0.089	0.01%	0.000278%	0.01%	0.001467%	0.001					
C14:2 - Myristoleic	0.10	0.09	0.70	0.09	0.001	0.01%	0.00696%	0.04%	0.00371%	0.002	0.01	0.00138113	0.03	0.00693986	0.008	0.00%	0.000059%	0.01%	0.000530%	0.000					
C16:2 - Palmitoleic	1.45	0.71	6.53	0.61	0.000	0.12%	0.00508%	0.41%	0.02049%	0.001	0.64	0.11041326	0.67	0.1217345	0.845	0.08%	0.003801%	0.10%	0.006647%	0.057					
C18:2(n-6) - Linoleic	210.79	10.67	310.70	17.91	0.001	19.23%	0.34950%	19.73%	0.11901%	0.237	179.61	23.1676577	154.42	19.987566	0.442	23.57%	0.162580%	23.52%	0.401715%	0.938					
C18:2(n-6) - Conjugated linoleic	0.44	0.05	0.71	0.08	0.016	0.04%	0.00276%	0.04%	0.00380%	0.310	0.54	0.08622002	0.51	0.0826964	0.836	0.07%	0.034640%	0.08%	0.004396%	0.307					
C18:3(n-6) - Gamma Linolenic	12.14	7.22	63.56	7.92	0.001	1.03%	0.35788%	3.98%	0.37048%	0.003	4.00	0.06302432	1.85	0.41464242	0.004	0.05%	0.00797%	0.28%	0.036756%	0.000					
C20:2(n-6) - Eicosadienoic	4.74	0.32	4.61	0.48	0.824	0.43%	0.02739%	0.29%	0.01799%	0.002	3.68	0.54364564	4.25	0.90325473	0.586	0.48%	0.009387%	0.63%	0.059936%	0.031					
C20:4(n-6) - Arachidonic	45.37	2.05	54.96	4.34	0.063	4.20%	0.32560%	3.48%	0.13851%	0.092	2.28	0.2323422	3.72	0.77189244	0.083	0.31%	0.017444%	0.55%	0.057308%	0.002					
C22:2(n-6) - Docosadienoic	0.15	0.02	0.05	0.00	0.003	0.01%	0.00009%	0.00%	0.00009%	0.002	0.06	0.00928523	0.05	0.00944337	0.464	0.01%	0.002680%	0.01%	0.000592%	0.648					
C22:4(n-6) - Adrenic	4.76	0.49	3.89	0.55	0.266	0.44%	0.05369%	0.24%	0.02439%	0.012	0.43	0.01567565	0.51	0.12938186	0.580	0.06%	0.003318%	0.07%	0.009819%	0.115					
C22:5(n-6) - Docosapentenoic	1.61	0.19	1.62	0.26	0.982	0.15%	0.02051%	0.10%	0.01236%	0.088	0.09	0.01333967	0.08	0.02686837	0.802	0.01%	0.001291%	0.01%	0.002312%	0.925					
C24:2	0.07	0.00	0.11	0.01	0.000	0.01%	0.00034%	0.01%	0.00035%	0.466	0.04	0.00355734	0.04	0.00795877	0.911	0.01%	0.000333%	0.01%	0.000602%	0.673					
C26:2 - Hexacosadienoic	0.00	0.00	0.00	0.00	0.036	0.00%	0.00002%	0.00%	0.00002%	0.435	0.00	0.00023046	0.00	0.0003644											