Supplementary File 1

Supplementary Methods & References

Generation of *floxed-Pparα* mice

The *floxed-Pparα* mouse strain was generated at the Mouse Clinical Institute (Illkirch, France). High-fidelity PCR amplification of genomic DNA was used to generate a 4.5-kb 5′ long arm, a 0.7-kb targeting arm including exon 4 (FA: floxed fragment), and a 3.2-kb 3′ long arm including exon 5, which were assembled in a vector containing a neomycin resistance cassette and loxP and Flippase Recognition Target (FRP) sites. This targeting vector was electroporated into P1 ES cells (MCI-129Sv/Pas background). Homologous recombination was verified by PCR and Southern blot analysis using a Neoprobe, a 5′ external probe (5′-AATGTTAGACAGGAATGGCAATGCC-3′; 5′-CTCTGTGTACAGCTGTCTTTTGAAC-3′), a 3′ external probe (5′-CTACTGCCCTTGGTACCTTGAAATG-3′; 5′-CCTACCGTCTTTGTTACCTTCTTGC-3′), and three genomic DNA digestions (one with NsiI for the 5′ insertion and two with HindIII or NdeI for the 3′ insertion). To remove the neocassette, one positive ES cell clone was electroporated with a Flipase-expressing plasmid. The resultant recombination was screened by PCR.

The derived ES cell clones were injected into C57BL/6J blastocysts to produce chimeric mice expressing the *floxed-Pparα* locus. Mice carrying the floxed allele were genotyped by PCR using HotStar Taq DNA Polymerase (5 U/µL, Qiagen) and forward (Ef; 5′-CTGTACTTTGTAGACATCTGAGAGGCG-3′) and reverse primers (Er; 5′-TAGGTACCGTGGACTCAGAGCTAG-3′ (figure 1 A). The amplification conditions were as follows: 15 min at 95°C; then 25 cycles of 94°C for 1 min, 65°C for 1 min, and 72°C for 1 min; and 72°C for 10 min. The wild-type and floxed alleles amplified 279-bp and 380-bp fragments, respectively. The obtained conditional knockout mouse strain was backcrossed with C57BL/6J.

Generation of *Pparα* hepatocyte-specific knockout (*Pparα*$^{hep-/-}$) animals

Pparα$^{hep-/-}$ animals were created at INRA's rodent facility (Toulouse, France) by mating the *floxed-Pparα* mouse strain with C57BL/6J *albumin-Cre* transgenic mice (gifted from Prof. Didier Trono, EPFL, Lausanne, Switzerland) to obtain *albumin-Cre*$^{+/-}$*Pparα*$^{flox/flox}$ mice, i.e.

Ppar$\alpha^{hep-/-}$ mice. *Ppar*α deletion was confirmed by PCR using HotStar Taq DNA Polymerase (5 U/µL, Qiagen) and a forward (Lf; 5′-AAAGCAGCCAGCTCTGTGTTGAGC-3′ and reverse primer (Er; 5′-TAGGTACCGTGGACTCAGAGCTAG-3′) (figure 1A). Amplification conditions were as follows: 95°C for 15 min; followed by 35 cycles of 94°C for 1 min, 65°C for 1 min, and 72°C for 1 min; and 72°C for 10 min. This reaction produced 450-bp, 915-bp, and 1070-bp fragments with exon 4 deletion, the wild-type allele, and the floxed allele, respectively. The albumin-Cre allele was detected by PCR using the following primers pairs: CreU (5′-AGGTGTAGAGAAGGCACTTAG-3′ and CreD (5′-CTAATCGCCATCTTCCAGCAGG-3′), and G2lox7F (5′-CCAATCCCTTGGTTCATGGTTGC-3′) and G2lox7R (5′-CGTAAGGCCCAAGGAAGTCCTGC-3′). *Albumin-Cre*$^{-/-}$ *floxed-Ppar*α (*Ppar*$\alpha^{hep+/+}$) littermates and wild-type C57BL/6J mice were used as controls.

PPARα-deficient C57BL/6J mice (*Ppar*$\alpha^{-/-}$) were bred at INRA's transgenic rodent facility. Age-matched C57BL/6J mice (provided by Charles River) were acclimated to local animal facility conditions prior to experiments. Mouse housing was temperature-controlled (at 22-24°C), with a 12-hour light/12-hour dark cycle. All studied mice were male and were fed a standard rodent diet (Safe 04 U8220G10R). Mice were killed at Zeitgeber time (ZT) 14 unless stated otherwise, with ZT0 being when the lights are turned on and ZT12 when lights are turned off.

DNA preparation for genotyping

DNA was extracted from tail tissue and stored at −20°C. Samples were mixed with 75 µL 25 mM NaOH, and 0.2 mM NA_2EDTA (pH 12), then incubated for 10 min at 95°C. Samples were next cooled on ice for 10 min, mixed, and neutralized with 75 µL 40 mM Tris-HCL (pH 5.0). After centrifugation (6 min; 14 000 rpm), 2.5 µL of supernatant was used for PCR with HotStar Taq Polymerase (5 U/µL, Qiagen) following the manufacturer's instructions.

In vivo experiments

Fenofibrate treatment

Fourteen-week-old wild-type C57BL/6J (WT), floxed wild-type (*Ppar*$\alpha^{hep+/+}$), *Ppar*$\alpha^{hep-/-}$, and *Ppar*$\alpha^{-/-}$ mice received the PPARα agonist fenofibrate (Sigma) (100 mg/kg/day) or vehicle (aqueous 3% gum Arabic) by gavage for 10 days (n=6 animals/genotype/treatment).

Fasting and Fasting–refeeding experiment

Eight-week-old WT, (*Ppar*$\alpha^{hep+/+}$), *Ppar*$\alpha^{hep-/-}$, and *Ppar*$\alpha^{-/-}$ mice were fed *ad libitum*, fasted for 24 hours from ZT14, or fasted for 24 hours from ZT14 and then re-fed for the next 24 hours with glucose in water (200 g/L; Sigma). All mice were killed at ZT14 (n=6 mice/genotype/experimental condition). Wild-type (C57BL6/J) and *Fgf21*$^{-/-}$ mice (12 month-old) were sacrificed either at the fasted state (a 24hour fast) or at the fed state at ZT14, (n=5 mice/genotype/experimental condition).

Circadian experiment

Eleven-week-old C57BL/6J mice were fed *ad libitum* or fasted from ZT0–ZT24. At ZT0, ZT4, ZT8, ZT12, ZT14, ZT16, ZT20, and ZT24, six mice from each condition were killed by cervical dislocation.

CL316243 activation of β3-adrenergic receptor

Four-month-old (WT) and *Ppar*$\alpha^{hep-/-}$ mice were fasted at ZT0; given CL316243 (3 mg/mL/kg; Sigma C5976) or vehicle (0.5% carboxymethyl cellulose in sterilized water; Fluka, 21900) at ZT6; and killed at ZT14.

Nutritional challenge with a methionine- and choline-deficient (MCD) diet

Eighteen-week-old WT, *Ppar*$\alpha^{hep-/-}$, and *Ppar*$\alpha^{-/-}$ mice were fed for two weeks with a MCD (A02082002B) or control diet (A02082003B) obtained from Research Diet. Mice were killed at ZT8 (n=6 animals/genotype/group).

Nutritional challenge with a High Fat Diet

Eighteen-week-old WT, *Ppar*$\alpha^{hep-/-}$, and *Ppar*$\alpha^{-/-}$ mice were fed for two weeks with a HFD (D12492) or control diet (D12450J) obtained from Research Diet. Mice were killed at ZT8 (n=6 animals/genotype/group).

Aging experiment

WT, *Ppar*$\alpha^{hep-/-}$ and *Ppar*$\alpha^{-/-}$ mice (n=12 each) were weighed weekly for 51 weeks. Mice were then killed at ZT14.

Adenoviral FGF21 expression

FGF21 adenovirus or control (Genecust) was delivered to mice (WT, *Ppar*$\alpha^{hep-/-}$, and *Ppar*$\alpha^{-/-}$) through retro-orbital injection (5.10^9 [pfu]/mouse). Four days later, mice were fasted for 24h and sacrificed at ZT14.

Blood and tissue samples

Prior to sacrifice, blood was collected from the submandibular vein with a lancet into EDTA-coated tubes (BD Microtainer, K2E tubes). Plasma was prepared by centrifugation (1500*g*, 10 min, 4°C) and stored at −80°C. Following euthanasia by cervical dislocation, organs were removed, weighed, dissected when necessary, and prepared for histological analysis, or snap-frozen in liquid nitrogen and stored at −80°C.

Liver neutral lipids analysis

Tissue samples were homogenized in methanol/5 mM EGTA (2:1, v/v), and then lipids (corresponding to an equivalent of 2 mg tissue) were extracted following the Bligh–Dyer method using chloroform/methanol/water (2.5:2.5:2.1, v/v/v), in the presence of the internal standards glyceryl trinonadecanoate, stigmasterol, and cholesteryl heptadecanoate (Sigma). TGs, free cholesterol, and cholesterol esters were analysed by gas-liquid chromatography using a Focus Thermo Electron system with a Zebron-1 Phenomenex fused-silica capillary column (5 m, 0.32-mm i.d., 0.50-mm film thickness). Oven temperature was programmed to increase from 200 to 350°C at 5°C/min, and the carrier gas was hydrogen (0.5 bar). The injector and the detector temperatures were 315°C and 345°C, respectively.

Liver fatty acid analysis

To measure total hepatic fatty acid methyl ester (FAME) molecular species, lipids corresponding to an equivalent of 1 mg of liver were extracted in the presence of glyceryl triheptadecanoate (0.5 µg) as an internal standard. The lipid extract was transmethylated with 1 ml of BF3 in methanol (14% solution; Sigma-Aldrich) and 1 ml of hexane for 60 minutes at 100°C and evaporated to dryness, and the FAMEs were extracted with hexane/water (2:1). The organic phase was evaporated to dryness and dissolved in 50 µl ethyl acetate. A sample (1 µl) of total FAME was analyzed by gas-liquid chromatography (Clarus 600 Perkin Elmer system, with Famewax RESTEK fused silica capillary columns, 30-m×0.32-mm i.d., 0.25-µm film thickness). Oven temperature was programmed from 110°C to 220°C at a rate of 2°C per minute, and the carrier gas was hydrogen (7.25 psi). The injector and the detector were at 225°C and 245°C, respectively.

Transcriptomic analysis

A model was fitted using the limma lmFit function (1), and correction for multiple testing was applied using False Discovery Rate (Benjamini et al. 1995). Probes with an adjusted p value ≤0.05 were considered differentially expressed between conditions. Hierarchical clustering was applied to samples and differentially expressed probes using Pearson's correlation coefficient as distance and Ward's criterion for agglomeration. Gene Ontology (GO) Biological Process enrichment was evaluated using a conditional hypergeometric test (GOstats package,(3)). Functional annotation clustering of GO Biological Process were performed using DAVID Bioinformatics Resources 6.7 ((4,5)). Gene-gene interaction network were predicted using "Search Tool for the Retrieval of Interacting Genes" ((6) String V10).

Supplementary references

(1) Wettenhall JM, Smyth GK. limmaGUI: a graphical user interface for linear modeling of microarray data. Bioinformatics. 2004, 12;20(18):3705-6.

(2) Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A practical and powerful Approach to multiple testing. Journal of the royal Statistical Society. Series B (methodological), Vol.57, No.1 (1995), 289-300.

(3) Falcon S, Gentleman R. Using GOstats to test gene lists for GO term association. Bioinformatics. 2007 Jan 15;23(2):257-8.

(4) Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID Bioinformatics Resources. Nature Protoc. 2009;4(1):44-57.

(5) Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res. 2009;37(1):1-13.

(6) Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ, von Mering C. STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res. 2015 Jan;43(Database issue):D447-52. doi: 10.1093/nar/gku1003.

Supplementary File 2: Oligonucleotide sequences for real-time PCR

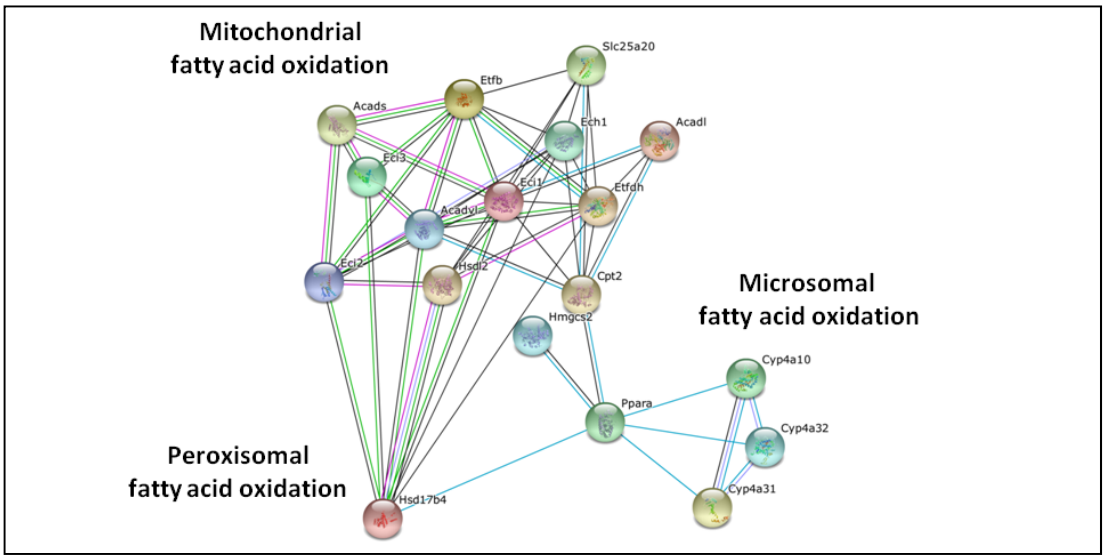
Gene	NCBI Refseq	Forward primer (5'-3')	Reverse primer (5'-3')
Acadl	NM_007381	AGAAGTTCATCCCCCAGATGAC	GGCGTTCGTTCTTACTCCTTGT
Acox1	NM_015729	CAGACCCTGAAGAAATCATGTGG	CAGGAACATGCCCAAGTGAAG
Cyp4a10	NM_010011	TCCAGCAGTTCCCATCACCT	TTGCTTCCCCAGAACCATCT
Cyp4a14	NM_007822	TCAGTCTATTTCTGGTGCTGTTC	GAGCTCCTTGTCCTTCAGATGGT
Fasn	NM_007988	AGTCAGCTATGAAGCAATTGTGGA	CACCCAGACGCCAGTGTTC
Fgf21	NM_020013	AAAGCCTCTAGGTTTCTTTGCCA	CCTCAGGATCAAAGTGAGGCG
Fsp27	NM_178373	AGGCCCTGTCGTGTTAGCAC	CATGATGCCTTTGCGAACCT
G6pd	NM_019468	GTGGGATCCTGAGGGAAGAGT	GATGGTGGGATAGATCTTCTTCTTG
Hmgcs2	NM_008256	TGCAGGAAACTTCGCTCACA	AAATAGACCTCCAGGGCAAGGA
Plin5	NM_025874	CGCTCCATGAGTCAAGCCA	CTCAGCTGCCAGGACTGCTA
Pparα	NM_011144	CCCTGTTTGTGGCTGCTATAATTT	GGGAAGAGGAAGGTGTCATCTG
Pparβ/δ	NM_011145	AAGTGGCCATGGGTGACG	TGGTCCAGCAGGGAGGAAG
Pparγ	NM_011146	CCACCAACTTCGGAATCAGCT	TTTGTGGATCCGGCAGTTAAGA
Scd1	NM_009127	CAGTGCCGCGCATCTCTAT	CAGCGGTACTCACTGGCAGA
Tbp	NM_013684	ACTTCGTGCAAGAAATGCTGAA	GCAGTTGTCCGTGGCTCTCT
Tnfα	NM_013693	TCCCCAAAGGGATGAGAAGTTC	GCGCTGGCTCAGCCACT
Vnn1	NM_011704	ATGAGGTTTATGCCTTTGGAGC	CCACAGGTGCGTAAATTGGTAG

Supplementary File 3

A - Functional annotation clustering GO (p-value < 0.01; DAVID Bioinformatics Resources 6.7) of the 99 Genes down-regulated in *Pparα*$^{hep-/-}$ mice compared to WT mice whatever the dietary status (fed, fasted, fasted-refed).

Functional categories	GO references	Number of genes
Mitochondrion	GO:0005739	24
Oxidation reduction	GO:0055114	13
Mitochondrial part	GO:0044429	11
Fatty acid metabolic process	GO:0006631	8
Endoplasmic reticulum	GO:0005783	8

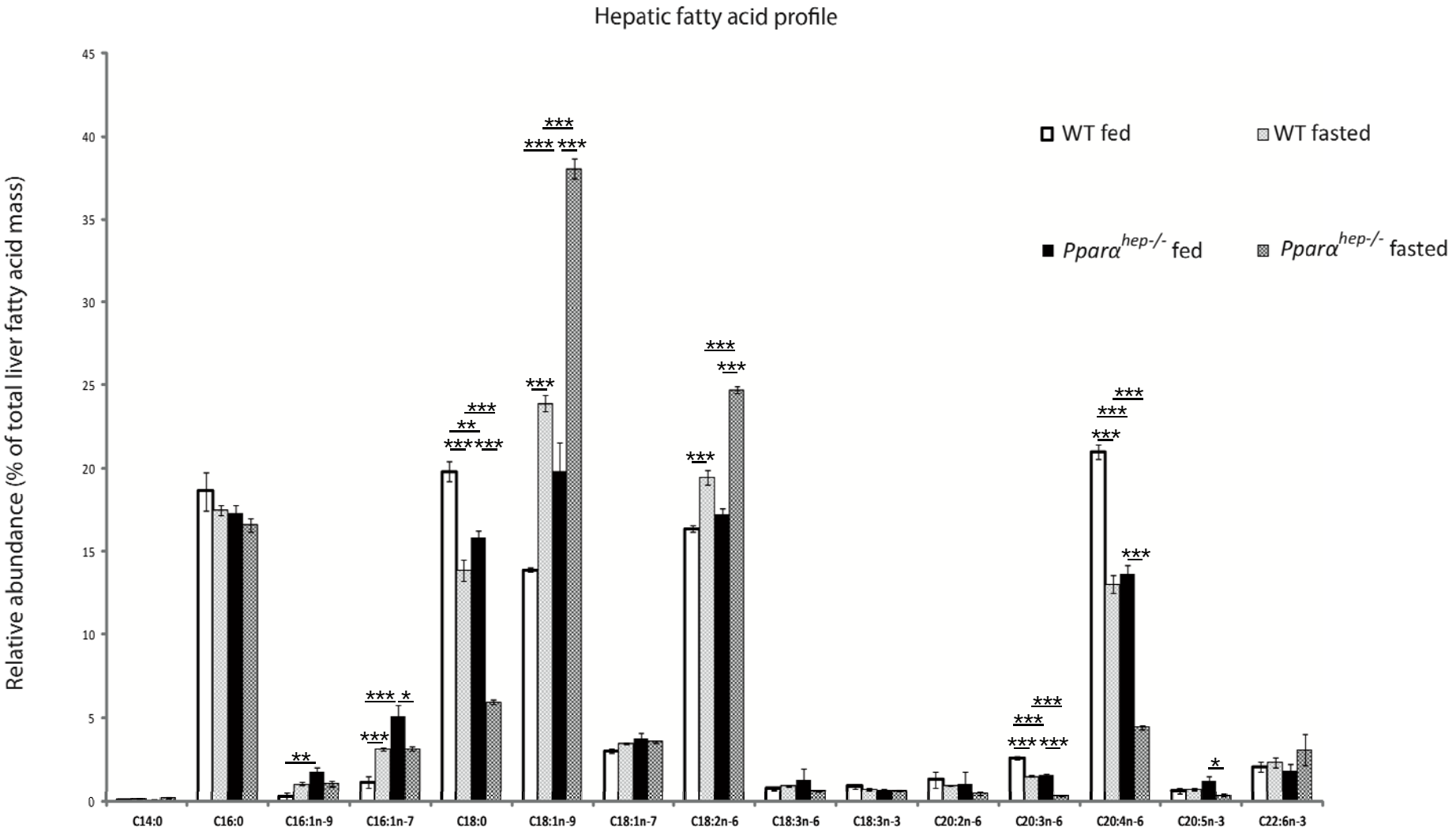
B - Predicted gene-gene interaction network (Search Tool for the Retrieval of Interacting Genes / String V10) amongst genes down-regulated in *Pparα*$^{hep-/-}$ mice compared to WT mice whatever the dietary status (fed, fasted, fasted-refed).



C - Functional annotation clustering GO (p-value < 0.01; DAVID Bioinformatics Resources 6.7) of the 27 Genes up-regulated in *Pparα*$^{hep-/-}$ mice compared to WT mice whatever the dietary status (fed, fasted, fasted-refed)

Functional categories	GO references	Number of genes
Endopeptidase activity	GO:0004175	4
Cytoskeleton organization	GO:0007010	3

Supplementary File 4



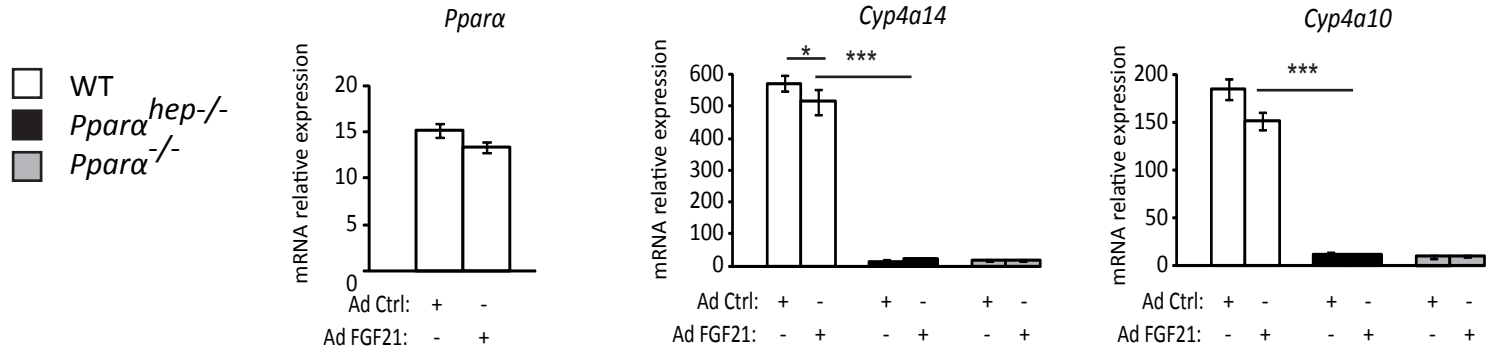
Hepatic fatty acid profile is modified by fasting and sensitive to hepatocyte *Pparα* deficiency. Relative abundance of hepatic fatty acids in WT and *Pparα*$^{hep-/-}$ mice fed or fasted for 24 hours was quantified by gas-liquid chromotography. Data are shown as mean ±SEM (n= 8 per group). *p≤0.05, **p≤0.01, ***p≤0.005.

(1) Gene Name	Regulation in fed mice over-expressing FGF21	(2)	Log FC $Ppar\alpha^{hep-/-}$ vs WT Fed	Adj.P. Val $Ppar\alpha^{hep-/-}$ vs WT Fed	Log FC $Ppar\alpha^{hep-/-}$ vs WT Fasted	Adj.P. Val $Ppar\alpha^{hep-/-}$ vs WT Fasted
Fmo	Up	*Fmo*	0,041	0,972	**1,909**	6,14E-06
Cyp2b9	Up	*Cyp2b9*	0,913	0,403	0,159	0,888
Mt1	Up	*Mt1*	**4,099**	3,34E-07	**1,110**	0,043
Igfal	Down	*Igfal*	0,076	0,942	-0,609	0,135
Ugt3a1	Down	*Ugt3a1*	**-2,070**	0,017	**-3,630**	1,08E-07
Osgin1	Down	*Osgin1*	-0,458	0,568	**1,162**	0,007
F11	Down	*F11*	0,229	0,520	**0,801**	5,52E-05
Scp2	Down	*Scp2*	0,085	0,632	-0,134	0,191
Aox3	Down	*Aox3*	**-1,032**	0,001	**-0,620**	0,014
Elovl3	Down	*Elovl3*	0,513	0,760	**-2,515**	0,001
Hes6	Down	*Hes6*	-0,087	0,860	-0,367	0,091
Selenbp2	Down	*Selenbp2*	ND	ND	ND	ND
Ela1	Down	*Ela1*	ND	ND	ND	ND
Ugt2b1	Down	*Ugt2b1*	-0,103	0,844	**0,881**	6,47E-05
Alas2	Down	*Alas2*	**0,586**	0,022	**0,418**	0,029
Nudt	Down	*Nudt*	-0,032	0,926	**-0,655**	1,18E-06
Gstp1	Down	*Gstp1*	-0,410	0,174	0,240	0,309
Cml4	Down	*Cml4*	ND	ND	ND	ND
Mcm10	Down	*Mcm10*	-0,842	0,149	-0,064	0,928
Sucnr1	Down	*Sucnr1*	**-2,181**	1,03E-05	**-1,792**	3,28E-06
C6	Down	*C6*	**0,254**	0,568	**0,927**	0,001
Cyp7b1	Down	*Cyp7b1*	0,418	0,515	-0,369	0,403
Cyp2d9	Down	*Cyp2d9*	-0,228	0,529	-0,373	0,077
Cyp4a12b	Down	*Cyp4a12b*	-0,461	0,466	**-1,277**	0,001
Mup4	Down	*Mup4*	0,095	0,627	-0,047	0,764
Serpina12	Down	*Serpina12*	**-1,947**	0,001	0,678	0,146
Serpina1e	Down	*Serpina1e*	**-0,565**	0,012	-0,149	0,490
Cyp4a12a	Down	*Cyp4a12a*	-0,663	0,248	**-1,303**	0,001
Hsd3b5	Down	*Hsd3b5*	-0,531	0,585	**1,377**	0,008

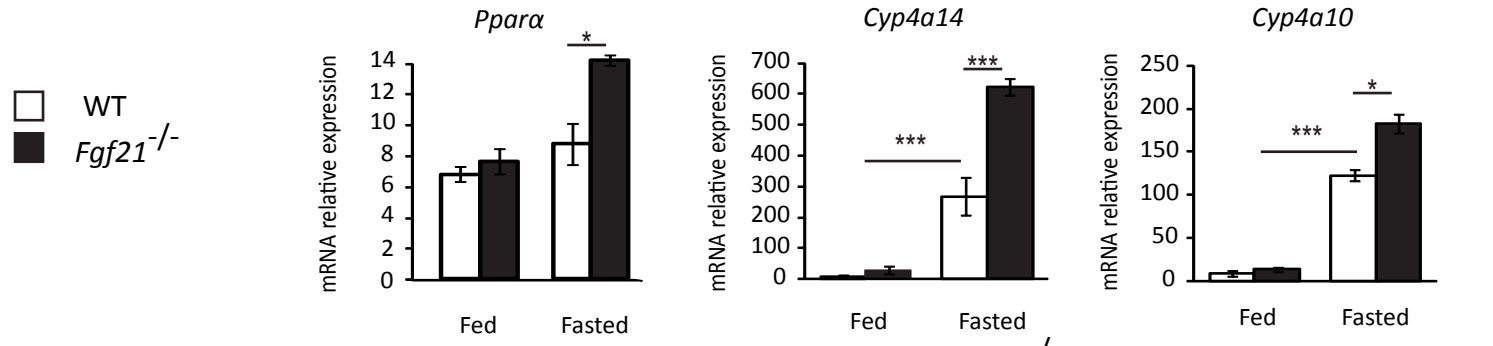
Up - regulated by FGF21 | Up - regulated by PPARα

Down - regulated by FGF21 | Down - regulated by PPARα

A: (1) Genes whose mRNA expression is significantly regulated in the liver of fed mice overexpressing FGF21 (data from GSE39313, Zhang *et al.*, 2012, Elife) and **(2)** their relative dependence on hepatocyte PPARα in our transcriptome analysis



B: Hepatic mRNA expression levels of *Pparα*, *Cyp4a14* and *Cyp4a10* in response to 24h fasting with (+) or without (-) adenoviral overexpression of FGF21 in WT, $Ppar\alpha^{hep-/-}$ and $Ppar\alpha^{-/-}$ mice. Data are shown as mean ± SEM. *p≤0.05, **p≤0.01, ***p≤0.005.



C: Hepatic mRNA expression levels of *Pparα*, *Cyp4a14* and *Cyp4a10* in WT and $Fgf21^{-/-}$ mice in response to 24h fasting. Data are shown as mean ± SEM. *p≤0.05, **p≤0.01, ***p≤0.005.

Supplementary File 6 **List of genes down-regulated by Fenofibrate (Log FC>1) and fasting in WT but not in PPARalpha hep-/-**

GeneName	SystematicName	logFC_LKO_Feno.LWT_Feno
Gm10804	NR_040533	4.338990155
Slco1a4	NM_030687	3.946688844
Gpr110	NM_133776	3.724069586
Blnk	NM_008528	3.594694282
Rgs16	NM_011267	3.328073207
Gck	NM_010292	2.704553909
Apoa4	NM_007468	2.698950403
Gm4477	NM_001253910	2.632084145
Ihh	NM_010544	2.628286186
Sdr9c7	NM_027301	2.600846197
Arhgef16	NM_001112744	2.600390221
Kcp	NM_001029985	2.587688912
Smpd3	NM_021491	2.502656144
Omd	NM_012050	2.446293066
Avpr1a	NM_016847	2.321034098
Clec2h	NM_053165	2.319711826
Cys1	NM_138686	2.251262731
Tuba8	NM_017379	2.21333475
Irf5	NM_012057	2.205714139
Il20	AK078698	2.188175561
Evc2	NM_145920	2.148281671
Il22ra1	NM_178257	2.14017001
Irx1	NM_010573	2.135892072
Plekhf1	NM_024413	2.071611419
Vasn	NM_139307	2.064549145

Cebpe	NM_207131	2.057939233
Cyp2c54	NM_206537	2.00580505
Fmn2	NM_019445	2.004180784
Ntf5	NM_198190	1.974077658
Espn	NM_207687	1.930187477
Usp18	NM_011909	1.929054274
Gldn	NM_177350	1.902430883
Snhg11	NM_175692	1.901885961
Gm10804	NR_040532	1.880303132
Mx2	NM_013606	1.833471447
Espn	NM_207687	1.752424815
Pkdcc	NM_134117	1.74787401
Dnajb11	NM_026400	1.693734168
Apol9a	NM_173786	1.682036041
Itpka	NM_146125	1.653329387
Evc	NM_021292	1.640295176
Cyp2c54	NM_206537	1.634821607
Ifi27l1	NM_026790	1.614340511
Nat8	NM_023455	1.611264421
Kalrn	ENSMUST00000023522	1.606828632
Sult1c2	NM_026935	1.597522687
Cyp2c50	NM_134144	1.530074931
Aqp4	NM_009700	1.520689629
Osgin1	NM_027950	1.51068762
Apol9b	NM_001168660	1.50682592
Apol9a	NM_173786	1.491777402
Cyp2c38	NM_010002	1.458490249
Plekhg5	NM_001004156	1.423607071

Rsad2	NM_021384	1.421197068
Irf7	NM_016850	1.419635985
Pde4b	NM_019840	1.416581869
Slco1a1	NM_013797	1.395577407
Slc17a1	NM_009198	1.37865757
Rtp4	NM_023386	1.36680329
Hist3h2a	NM_178218	1.361828009
Tnfrsf25	NM_033042	1.357712549
Cyp1a2	NM_009993	1.337789611
Synj2	NM_001113353	1.336116722
Kalrn	NM_177357	1.324905667
Slco2a1	NM_033314	1.323539871
Ifit1	NM_008331	1.320506833
Ear11	NM_053113	1.312705755
Crym	NM_016669	1.289927484
Nupr1	NM_019738	1.268707101
Tiam2	NM_011878	1.25324319
Gga2	NM_028758	1.248227354
Igsf8	NM_080419	1.245743645
Tmem161a	NM_145597	1.239198661
Pcp4l1	NM_025557	1.232257914
Hsd11b1	NM_008288	1.230227521
Samd1	NM_001081415	1.224727976
Dntt	NM_009345	1.21978365
Ppp4r4	NM_028980	1.213756156
Ugt2b1	NM_152811	1.209964061
Gstm2	NM_008183	1.205891998
C6	NM_016704	1.203122019

Gstm2	NM_008183	1.198638812
Pcbp4	NM_021567	1.189312741
Grm8	NM_008174	1.179630647
Gm2a	NM_010299	1.162405548
Pla1a	NM_134102	1.162394508
Ifit3	NM_010501	1.158512211
Gstm2	NM_008183	1.156968065
Oas1a	NM_145211	1.143199028
Oas1a	NM_145211	1.136708122
Lrp2	NM_001081088	1.123876743
Cdk20	NM_053180	1.115737794
Prodh	NM_011172	1.106241577
Mgat2	NM_146035	1.097829676
Cyp2c29	NM_007815	1.095120899
Mast4	NM_175171	1.091260632
Pcsk9	NM_153565	1.089930194
Adora1	NM_001008533	1.076713157
Gvin1	NM_029000	1.073977553
Pigf	NM_008838	1.069604813
Efhd2	NM_025994	1.062608113
Rtkn	NM_133641	1.061122385
Prss8	NM_133351	1.060518018
Armcx3	NM_027870	1.055236819
Oas1f	NM_145153	1.054884833
Slc37a1	NM_153062	1.05113534
Fam47e	NM_001033478	1.048260327
Wif1	NM_011915	1.047675224
Bhlhe40	NM_011498	1.041719532

Homer2	NM_011983	1.041124787
Mx1	NM_010846	1.039795857
Cmpk2	NM_020557	1.036328603
Agap2	NM_001033263	1.024070237
Prss8	NM_133351	1.020288658
Rnd2	NM_009708	1.017853772
Sqle	NM_009270	1.017528952
Neurl1a	NM_021360	1.009526359

Supplementary File 7: Functional annotation clustering GO (p-value < 0.01; DAVID Bioinformatics Resources 6.7) of the 698 Genes repressed by fenofibrate and fasting in WT and *Pparα hep+/+* but not in *Pparα hep-/-* mice (nor in *Pparα-/-* mice)

Functional categories	GO references	Number of genes
Endoplasmic reticulum	GO:0005783	61
Endoplasmic reticulum part	GO:0044432	24
Microsome	GO:0005792	13
Endosome	GO:0005768	21
Lysosome	GO:0005764	14
Regulation of Ras protein signal transduction	GO:0046578	13
Organic anion transmembrane transporter activity	GO:0008514	4

Supplementary File 8 **List of genes up-regulated by Fenofibrate (Log FC>2) and fasting in WT but not in PPARalpha hep-/-**

GeneName	SystematicName	logFC_LKO_Feno.LWT_Feno
Vnn1	NM_011704	-6.327507248
Cyp4a32	NM_001100181	-6.196767964
Cyp4a14	NM_007822	-5.960408744
Cyp4a31	NM_001252539	-5.634232845
Cyp4a10	NM_010011	-5.439267814
Krt23	NM_033373	-5.390005991
Cyp4a10	NM_010011	-5.344449564
Cyp4a10	NM_010011	-5.311799175
Rad51l1	NM_009014	-5.168389733
Gm15441	NR_040409	-5.022337513
Cyp4a31	NM_201640	-4.994452308
Agpat9	NM_172715	-4.981241516
Acot2	NM_134188	-4.774193064
Cyp4a31	NM_001252539	-4.739402899
Serinc2	NM_001253386	-4.694405198
Mfsd2a	NM_029662	-4.559186147
Rab30	NM_029494	-4.460954496
Serinc2	NM_172702	-4.442471841
Acot3	NM_134246	-4.39217577
Ehhadh	NM_023737	-3.943620325
Serinc2	NM_001253386	-3.720914131
Cidec	NM_178373	-3.699841322
Acot3	NM_134246	-3.695985114
Slc25a34	NM_001013780	-3.540701791

Tmem43	NM_028766	-3.519129781
Clstn3	NM_153508	-3.501309851
Dlg4	NM_007864	-3.459813265
Raet1e	NM_198193	-3.417569383
Acot5	NM_145444	-3.407106296
Rtn4	NM_194054	-3.319773259
Mtnr1a	NM_008639	-3.316641914
Gal3st1	NM_016922	-3.241994667
Mogat1	NM_026713	-3.208183376
Enc1	NM_007930	-3.202252717
Rufy4	NM_001034060	-3.201516745
Lgals4	NM_010706	-3.144456887
Spc25	NM_001199123	-3.115026503
Hsd17b11	NM_053262	-3.109470457
Lgals4	NM_010706	-3.092738086
Gm4952	NM_001013762	-3.043309935
Lgals4	NM_010706	-3.025184183
Fitm1	NM_026808	-3.02238903
Retsat	NM_026159	-2.986135359
Cda	NM_028176	-2.979215647
Qpct	NM_027455	-2.973192363
Gna15	NM_010304	-2.943823781
Cbfa2t3	NM_009824	-2.938950583
Fbf1	NM_172571	-2.901652108
Decr2	NM_011933	-2.836606019
Slc22a5	NM_011396	-2.815808723
Slc25a20	NM_020520	-2.793833675
G0s2	NM_008059	-2.791025291

Acaa1b	NM_146230	-2.786928971
Rab30	NM_029494	-2.77538891
Rarres1	NM_001164763	-2.7711581
Paqr7	NM_027995	-2.739895455
E2f8	NM_001013368	-2.739403931
Lgals6	NM_010707	-2.708925191
Tmtc2	NM_177368	-2.630701853
Slc35f2	NM_028060	-2.624275495
Ddhd2	NM_028102	-2.600504871
Cpt1b	NM_009948	-2.57618651
Nceh1	NM_178772	-2.551744536
Aldh3a2	NM_007437	-2.5462088
Abhd6	NM_025341	-2.541903604
Fitm2	ENSMUST00000109418	-2.541338387
Tmem98	NM_029537	-2.527726347
Plin5	NM_001077348	-2.522330477
Ech1	NM_016772	-2.510705785
Abhd6	NM_025341	-2.502624595
Paqr9	NM_198414	-2.492189808
Cox6b2	NM_183405	-2.488991428
Sema5b	NM_013661	-2.486591564
Chrna2	NM_144803	-2.443155692
Eci3	NM_026947	-2.432854016
Dnase1	NM_010061	-2.393569551
Sema5b	NM_013661	-2.389349368
Hr	NM_021877	-2.362630738
Etfdh	NM_025794	-2.361997894
Caln1	NM_021371	-2.338121081

Cerkl	NM_001048176	-2.325203282
Acsl1	NM_007981	-2.322008159
Tmed5	NM_028876	-2.320397015
Pex11a	NM_011068	-2.317353629
Acot8	NM_133240	-2.313631361
Eci2	NM_011868	-2.303602498
Slc6a16	XM_355900	-2.290913244
Slc22a21	NM_019723	-2.280482356
Unc5b	NM_029770	-2.270525456
Fitm2	NM_173397	-2.256975833
Cpt2	NM_009949	-2.250380721
Paqr9	NM_198414	-2.242478857
Cpt2	NM_009949	-2.23990941
Olfr15	NM_008762	-2.238433986
Raet1c	NM_009018	-2.222906132
Acot8	NM_133240	-2.218917378
Hsdl2	NM_024255	-2.216041134
Celf2	NM_010160	-2.210853201
Ctif	NM_201354	-2.180316331
Lamb3	NM_008484	-2.179424444
Mmd	ENSMUST00000004050	-2.168017347
Decr1	NM_026172	-2.167975542
Mmd	NM_026178	-2.16265343
Raet1b	NM_009017	-2.133017556
Celf2	NM_010160	-2.123555678
Crat	NM_007760	-2.116353216
Adam32	NM_153397	-2.08602317
Txnip	NM_001009935	-2.07753964

Pxmp4	NM_021534	-2.064830579
Slc16a11	NM_153081	-2.058554385
Slc16a13	NM_172371	-2.055575511
Mmd	ENSMUST00000134929	-2.022985304
Gm7969	XM_982175	-2.002981056

Supplementary File 9: Functional annotation clustering GO (p-value < 0.01; DAVID Bioinformatics Resources 6.7) of the 907 Genes induced by fenofibrate and fasting in WT and *Pparα hep+/+* but not in *Pparα hep-/-* mice (nor in *Pparα-/-* mice)

Functional categories	GO references	Number of genes
Mitochondrion	GO:0005739	219
Generation of precursor metabolites and energy	GO:0006091	50
Fatty acid metabolic process	GO:0006631	39
Peroxisome	GO:0005777	37
Mitochondrial matrix	GO:0005759	37
Cofactor binding	GO:0048037	37
Hydrogen ion transmembrane transporter activity	GO:0015078	17
Carboxylic acid catabolic process	GO:0046395	16
Cellular respiration	GO:0045333	16
O-acyltransferase activity	GO:0003988	11
Proteasome complex	GO:0000502	14
Nucleotide binding	GO:0000166	112
Iron ion binding	GO:0005506	26
Ligase activity, forming carbon-sulfur bonds,	GO:0016877	9
Oxidoreductase activity, acting on NADH or NADPH	GO:0016651	9
Acyl-CoA metabolic process	GO:0006637	7
Oxidative phosphorylation	GO:0006119	11
Cellular nitrogen compound biosynthetic process	GO:0044271	27
Protein homodimerization activity	GO:0042803	18
Vitamin metabolic process	GO:0006766	10
3-hydroxyacyl-CoA dehydrogenase activity	GO:0003857	5
Carboxylic acid binding	GO:0031406	12
Mitochondrial outer membrane	GO:0005741	10
Magnesium ion binding	GO:0000287	29
Oxidoreductase activity, acting on the CH-NH group of donors	GO:0016645	29
Mitochondrion organization	GO:0007005	13

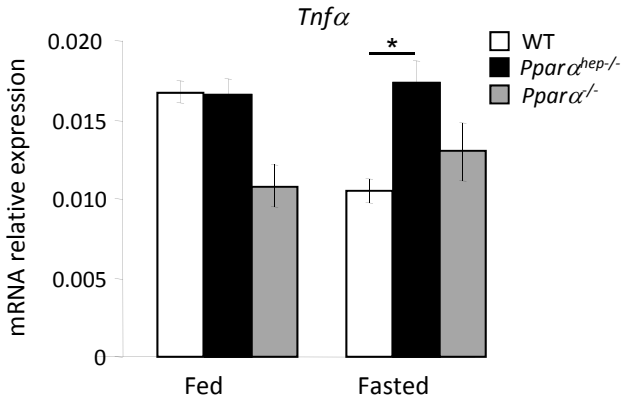
A

GeneName	logFC fasted vs fed	adj.P.Val
Cyp4a14	*8.90*	*4.43E-20*
Cyp4a31	*6.45*	*2.71E-20*
Cyp4a10	*6.43*	*7.32E-20*
Igfbp1	6.04	6.91E-11
Cyp4a31	*5.56*	*5.01E-19*
Cyp4a32	*5.06*	*3.81E-14*
Apoa4	4.73	8.80E-17
Ppp1r3g	4.72	2.06E-08
Fsp27	*4.34*	*3.51E-14*
Acot3	*3.87*	*3.72E-11*
Fgf21	*3.86*	*1.14E-06*

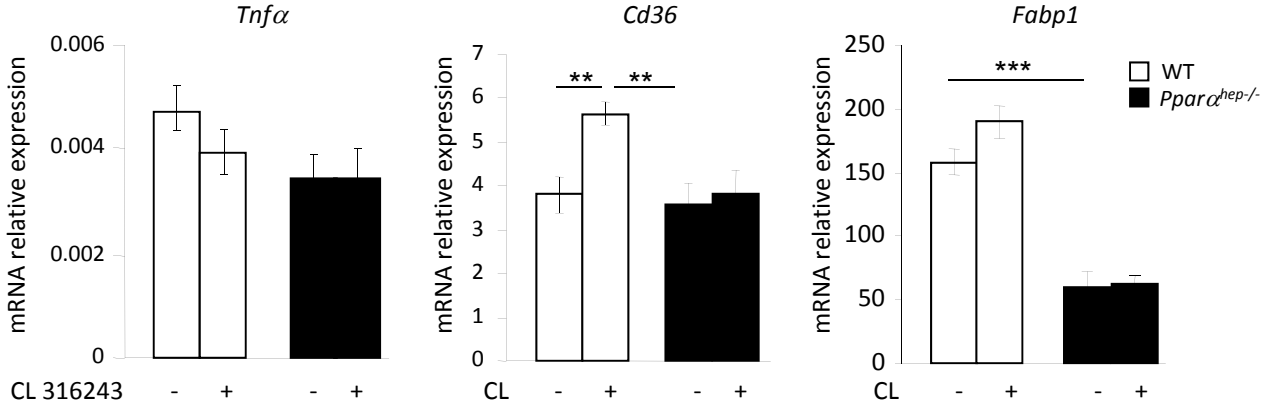
B

GO_id	Term	Genes	p-value
GO:0006629	lipid metabolic process	26	2.01E-9
GO:0044281	small molecule metabolic process	31	2.14E-9
GO:0044710	single-organism metabolic process	47	5.03E-8
GO:0006082	organic acid metabolic process	22	2.14E-7
GO:0044699	single-organism process	84	3.45E-7
GO:0044763	single-organism cellular process	79	8.02E-7
GO:0019752	carboxylic acid metabolic process	20	1.68E-6
GO:0006790	sulfur compound metabolic process	13	2.27E-6
GO:0032787	monocarboxylic acid metabolic process	16	2.37E-6
GO:0043436	oxoacid metabolic process	20	6.9E-6
GO:0006637	acyl-CoA metabolic process	8	7.61E-6
GO:0035383	thioester metabolic process	8	7.61E-6
GO:0044255	cellular lipid metabolic process	19	8.09E-6
GO:0006732	coenzyme metabolic process	12	9.41E-6
GO:0001676	long-chain fatty acid metabolic process	8	2.29E-5
GO:0051186	cofactor metabolic process	12	1.09E-4
GO:0006631	fatty acid metabolic process	11	1.29E-3
GO:0032789	unsaturated monocarboxylic acid metabolic process	3	1.97E-3
GO:0032788	saturated monocarboxylic acid metabolic process	3	1.97E-3
GO:0032869	cellular response to insulin stimulus	8	3.62E-3
GO:0006641	triglyceride metabolic process	6	4.41E-3
GO:0008202	steroid metabolic process	9	8.25E-3
GO:0019217	regulation of fatty acid metabolic process	6	8.54E-3
GO:0006639	acylglycerol metabolic process	6	1.16E-2
GO:0006638	neutral lipid metabolic process	6	1.27E-2
GO:0009987	cellular process	79	2.97E-2
GO:0071375	cellular response to peptide hormone stimulus	8	4.35E-2
GO:0032868	response to insulin	8	4.75E-2
GO:0010565	regulation of cellular ketone metabolic process	7	6.67E-2
GO:1901653	cellular response to peptide	8	6.96E-2
GO:0008152	metabolic process	63	9.02E-2

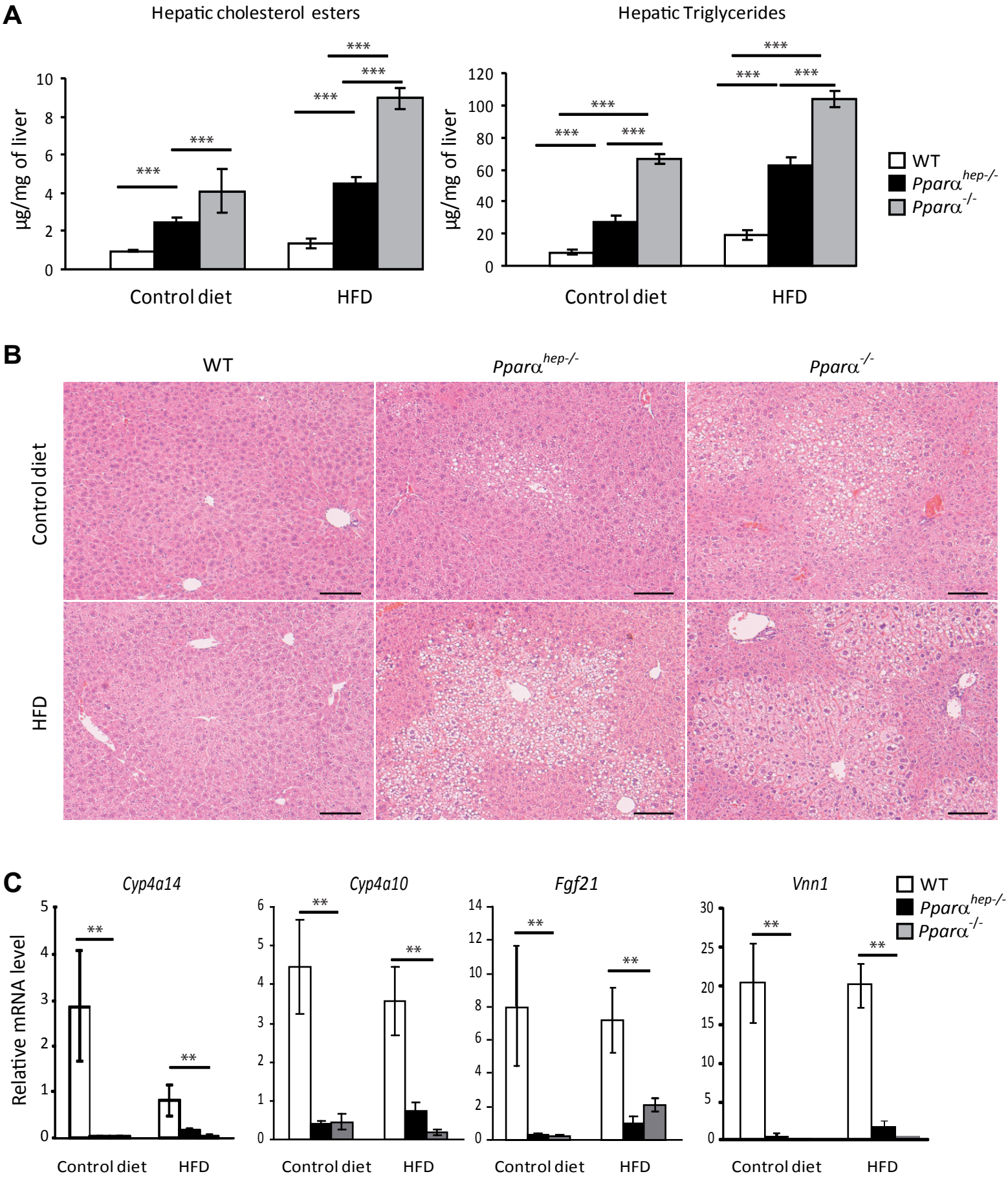
C



D



A. Top 11 genes induced by fasting in WT mice. In red genes regulated by fenofibrate and by fasting and dependent on hepatocyte PPARα activity. **B** Top GO biological process sensitive to fasting in WT mice (130 genes regulated with log FC>1.5). **C** Hepatic mRNA expression levels of *Tnfα* measured by qRT-PCR in liver samples of WT, *Pparα*$^{-/-}$, *Pparα*$^{hep-/-}$ 8 week-old male fed or fasted for 24 hours. **D**. Hepatic mRNA expression levels of *Tnfα*, *Cd36* and *Fabp1* measured by qRT-PCR in 4 month-old male WT and *Pparα*$^{hep-/-}$ mice treated with the β3-adrenergic receptor agonist CL316243 or vehicule at ZT6 and then killed at ZT14. Data are shown as mean ± SEM. *p≤0.05, **p≤0.01, ***p≤0.05.

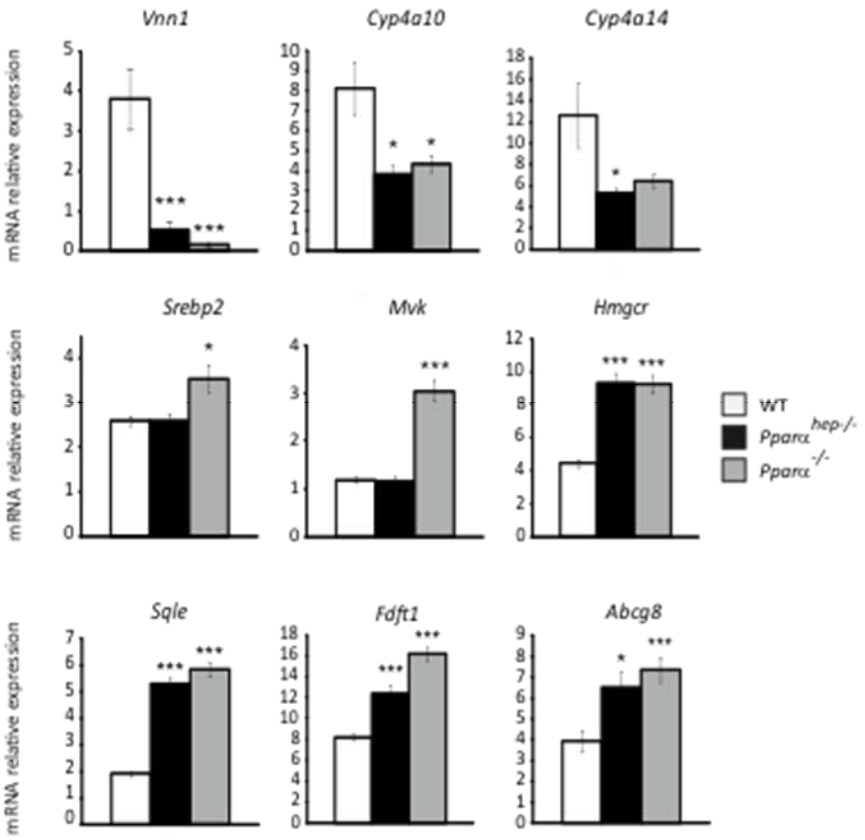


Liver PPARα deficiency aggravates steatosis in response to a High Fat Diet (HFD). Wild-type (WT), PPARα hepatocyte knockout (*Pparα*$^{hep-/-}$) and PPARα knockout (*Pparα*$^{-/-}$) mice were fed a HFD or a control diet for 2 weeks and were killed at ZT8. (A) Quantification of hepatic triglycerides and cholesterol esters. (B) Representative pictures of hematoxylin/eosin staining on liver sections. Scale bar, 100 µm. (C) Hepatic mRNA expression levels of *Cyp4a14, Cyp4a10, Fgf21* and *Vnn1*. Data are shown as mean +/- SEM. * p≤0.05, * p≤0.01, * p≤0.005.

A

Gene name	Ref Seq	Description	LogFC *Pparα* $^{hep-/-}$ vs WT	Adj.P.Val
Apon	NM_133996	Apolipoprotein N (Apon)	0.79	9.85E-05
Apof	NM_133997	apolipoprotein F (Apof)	0.37	0.016
Apol10a	NM_177744	Apolipoprotein L 10a	0.39	0.031
Apoa4	NM_007468	Apolipoprotein A-IV	1.97	6.03E-05
Abcg5	NM_031884	ATP-binding cassette, sub-family G (WHITE), member 5 (Abcg5)	-0.93	0.001
Abcg8	NM_026180	ATP-binding cassette, sub-family G (WHITE), member 8 (Abcg8)	-0.81	0.002
Abcb4	NM_008830	ATP-binding cassette, sub-family B (MDR/TAP), member 4 (Abcb4)	-0.53	0.009

B



***Pparα* deficiency impact hepatic cholesterol metabolism.** A. Table listing significant differentially expressed genes related to cholesterol metabolism in liver samples from *Pparα*$^{hep-/-}$ vs WT mice. Data are extracted from microarrays analusis performed on samples from 8 week-old male mice in the fed state. B.Hepatic mRNA expression levels of PPARα target genes (*Vnn1*, *Cyp4a10* and *Cyp4a14*) and cholesterol metabolism related genes (*Srebp2*, *Mvk*, *Hmgcr*, *Sqle*, *Fdft1* and *Abcg8*) measured by qRT-PCR in fed 52 week-old male mice from WT, *Pparα*$^{hep-/-}$ and *Pparα*$^{-/-}$ genotypes. Data are shown as mean ± SEM. *p≤0.05, ***p≤0.005.