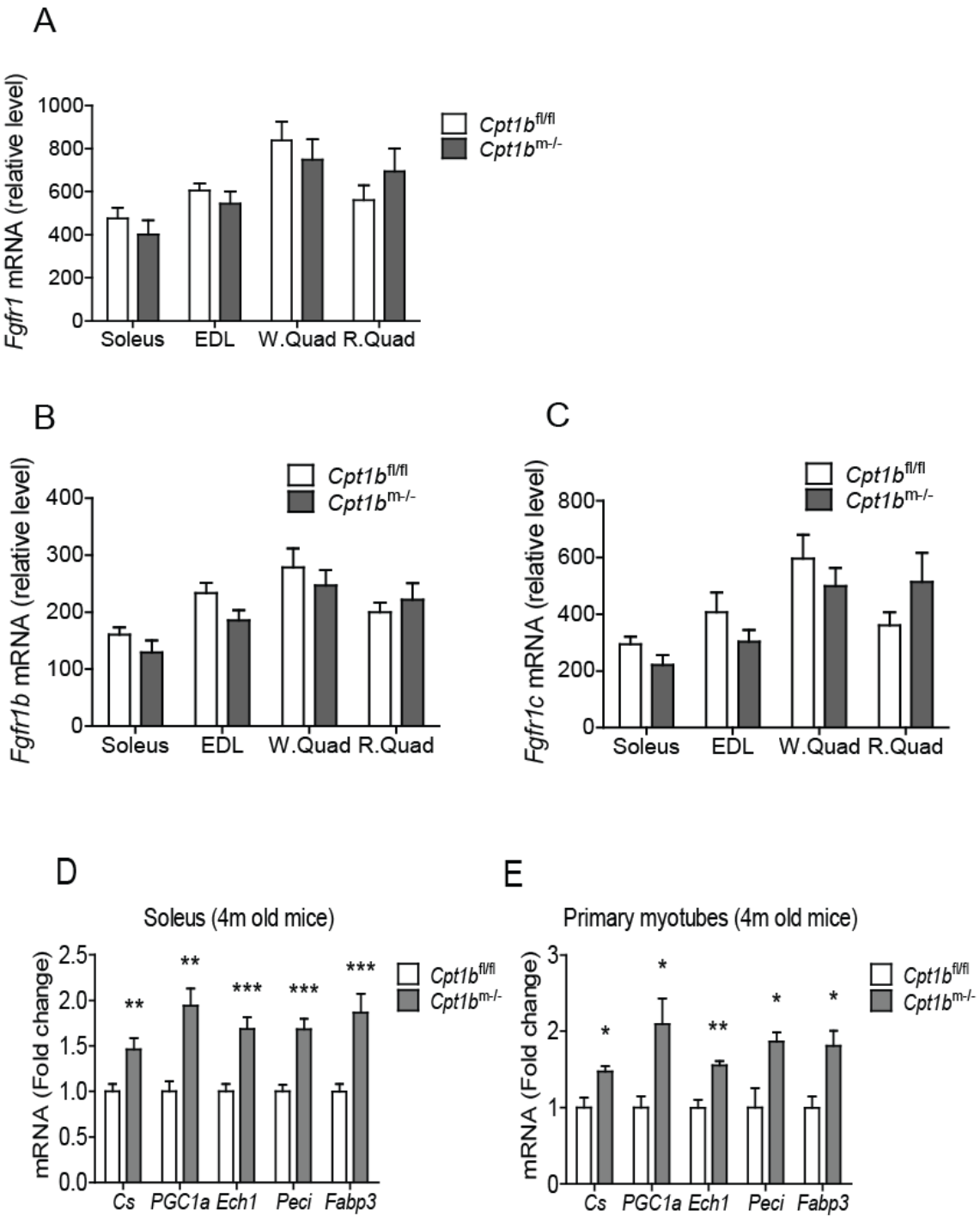


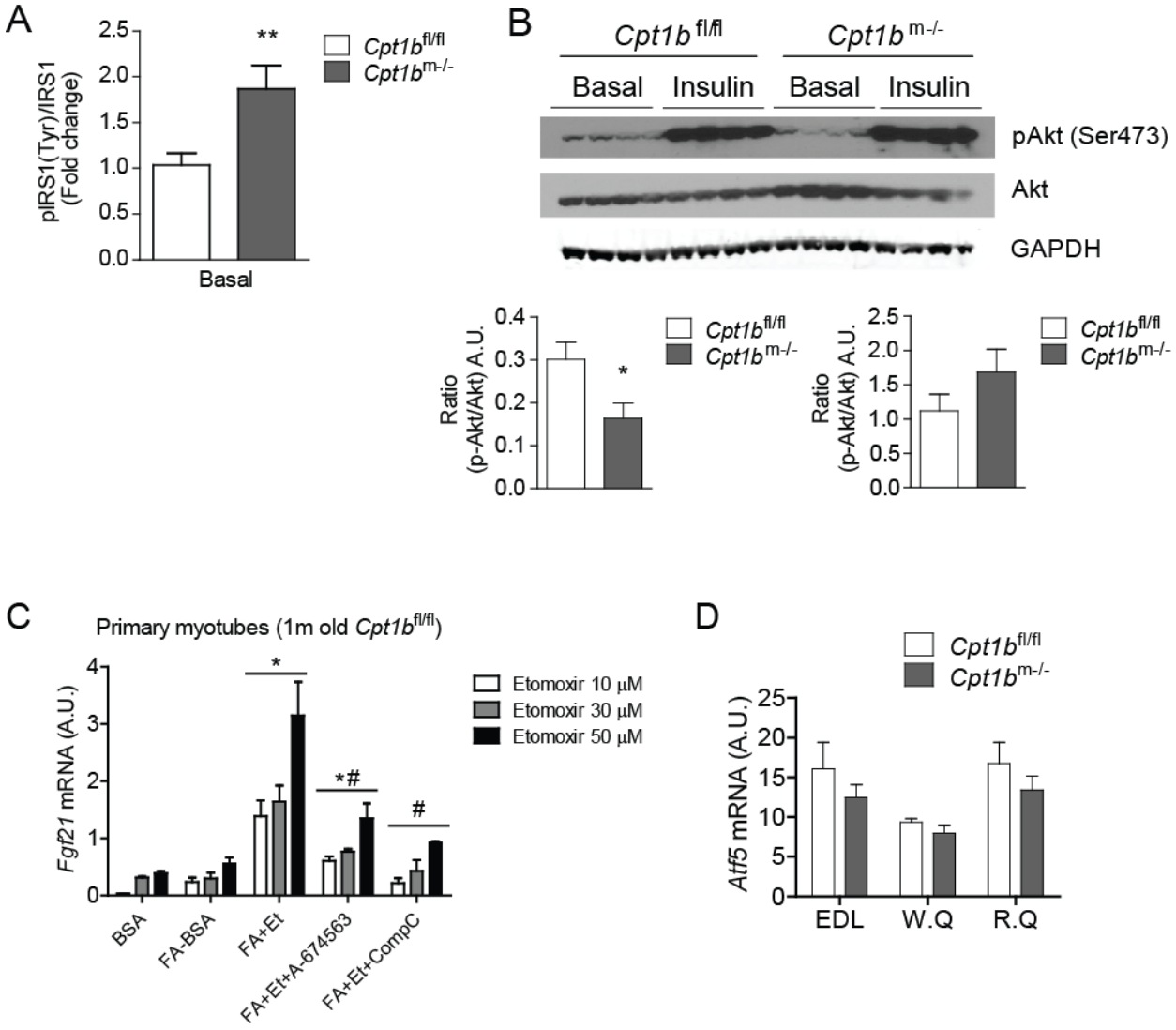
Supplementary Figure 1 is related to Figure 1.

A-C. Gene expression of *Fgfr1* (A), its isoforms *Fgfr1b* (B) and *Fgfr1c* (C), as examined by qRT-PCR in skeletal muscle from 4 month old *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ mice. Muscle tissue:

soleus, extensor digitorium longus (EDL), white and red quads (W.Quad and R.Quad). All data are presented as means ± s.e.m., *n=5-7* per group.

D-E. Expression of metabolic characteristic genes such as mitochondrial citric acid cycle (*Cs*), transcription factor that regulates energy metabolism (*PGC1α*), mitochondrial fatty acid oxidation (FAO) (*Ech1*), peroxisomal FAO (*Peci*), fatty acid (FA) storage (*Fabp3*) in soleus muscle (D) and primary muscle cells (E) from 4 month old *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ mice.

All data are presented as means ± s.e.m., **P* < 0.05, ***P* < 0.01, ****P* < 0.005 and higher significance between *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ mice.



Supplementary Figure 2 is related to Figure 2 and Figure 3 and Figure 4.

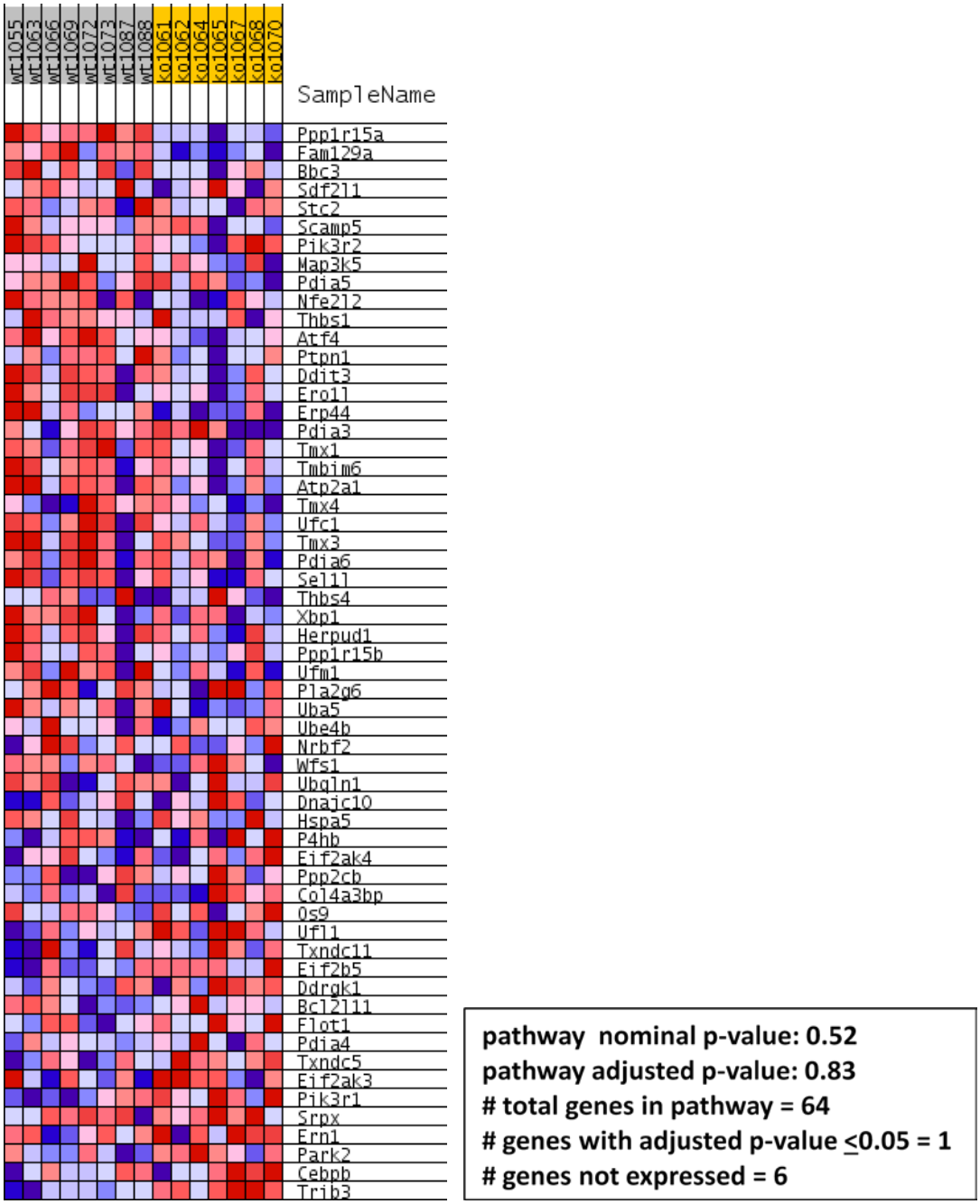
A-B is related to Figure 2. Activity of IRS1 at basal state, as examined by multiplex protein signaling assay for phosphorylation at Pan-Tyr in gastrocnemius muscle from 4 month old *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ mice (A). Immunoblot analysis of basal and insulin-stimulated Akt signaling by phosphorylation at Ser473 in red quad muscle from 4 month old *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ mice (B). GAPDH, a loading control. Image J software was used for densitometry quantification of the immunoblots. All data are presented as means ± s.e.m., *n=4* per group, **P* < 0.05, ***P* < 0.01.

C is related to Figure 3. Gene expression of *Fgf21*, as examined by qRT-PCR in primary myotubes established from 1 month old *Cpt1b*$^{fl/fl}$ mice. Primary muscle cells were differentiated into myotubes prior to treatment with BSA-conjugated FA (0.5 mM) and inhibitors such as Etomoxir (Et, 10μM, 30μM and 50μM), A-674563 (30μM) and Compound C (CompC, 30μM)

for 24h. All data are presented as means ± s.e.m., $^{*}P < 0.05$ and higher significance between treatments with BSA-conjugated FA (FA) versus inhibitors for the indicated Etomoxir dose group, and $^{\#}P < 0.05$ and higher significance between treatments with FA in presence of Etomoxir (FA+Et) versus other inhibitors for the indicated Etomoxir dose group.

D is related to Figure 4. Gene expression of *Atf5* as examined by qRT-PCR in skeletal muscle from 4 month old *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ mice. Muscle tissue: extensor digitorium longus (EDL), white and red quads (W.Quad and R.Quad). All data are presented as means ± s.e.m., *n=5-7* per group.

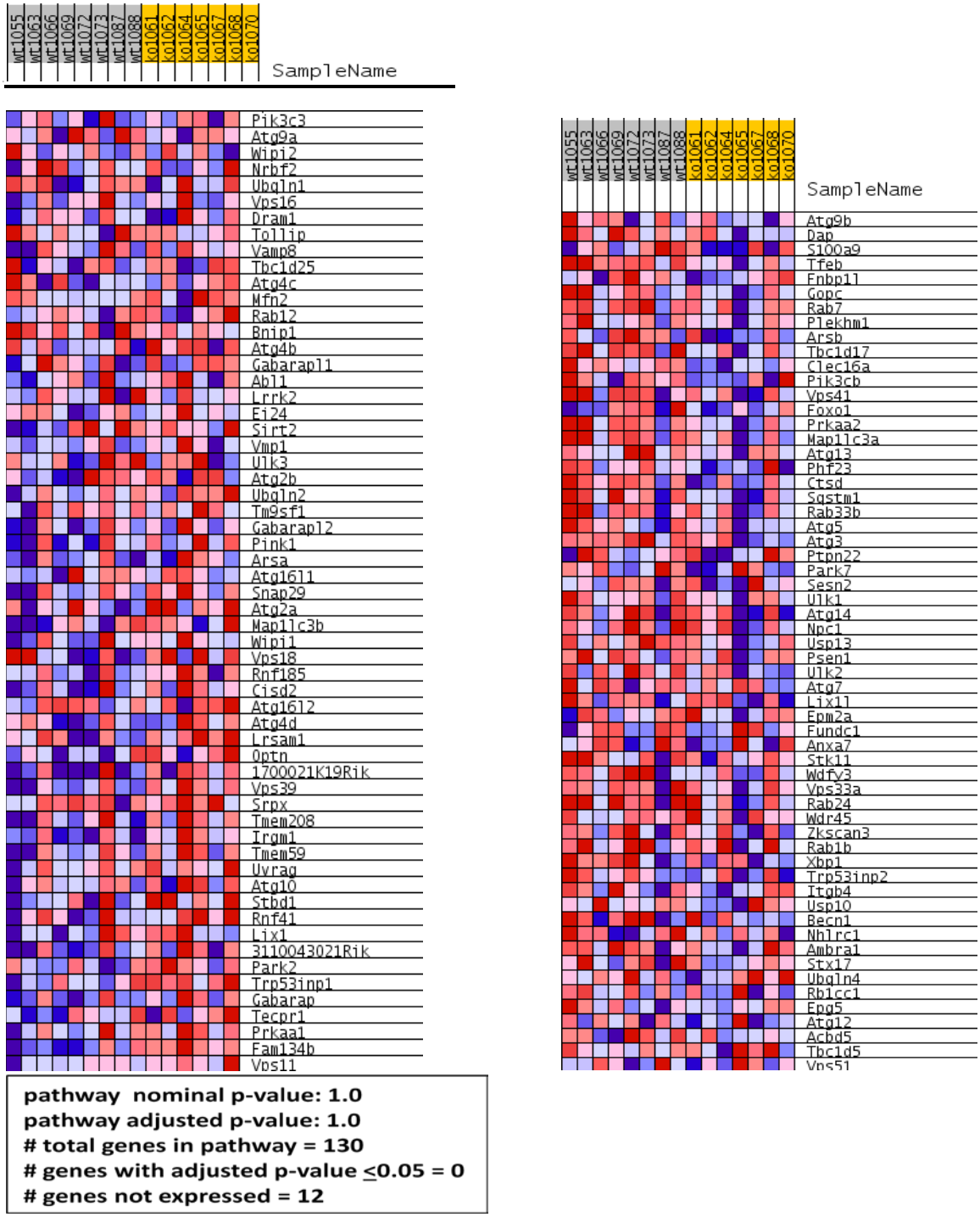
ER stress pathway



Supplementary Figure 3 is related to Figure 4.

ER stress pathway related gene expression pattern determined by Ingenuity Pathway Analysis (IPA) and Gene Set Enrichment Analysis (GSEA) in gastrocnemius muscle from *Cpt1b*$^{m-/-}$ (wt) and *Cpt1b*$^{fl/fl}$ (ko) mice, *n=7-8*.

Autophagy

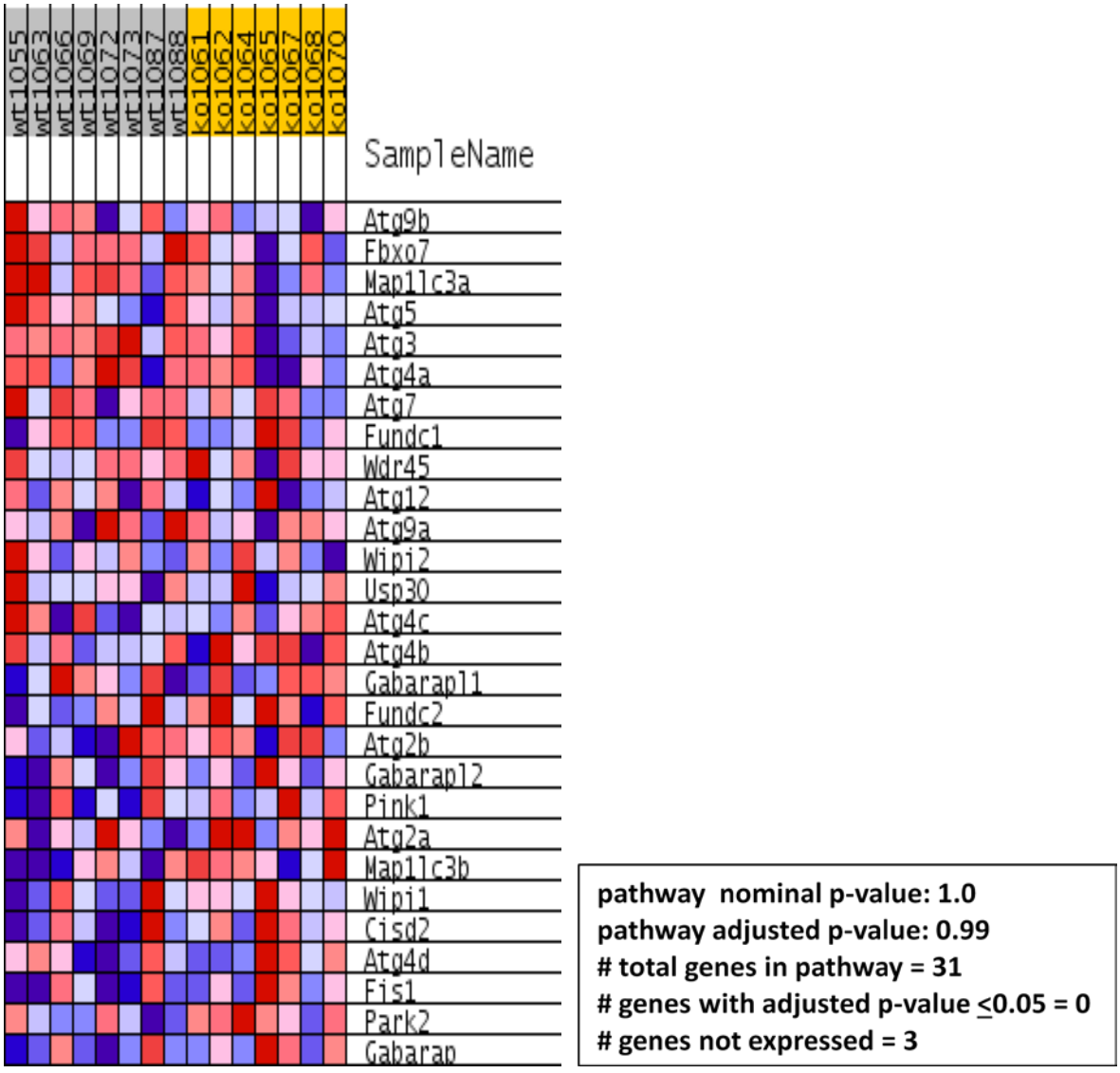


pathway nominal p-value: 1.0
pathway adjusted p-value: 1.0
 # total genes in pathway = 130
 # genes with adjusted p-value ≤0.05 = 0
 # genes not expressed = 12

Supplementary Figure 4 is related to Figure 4.

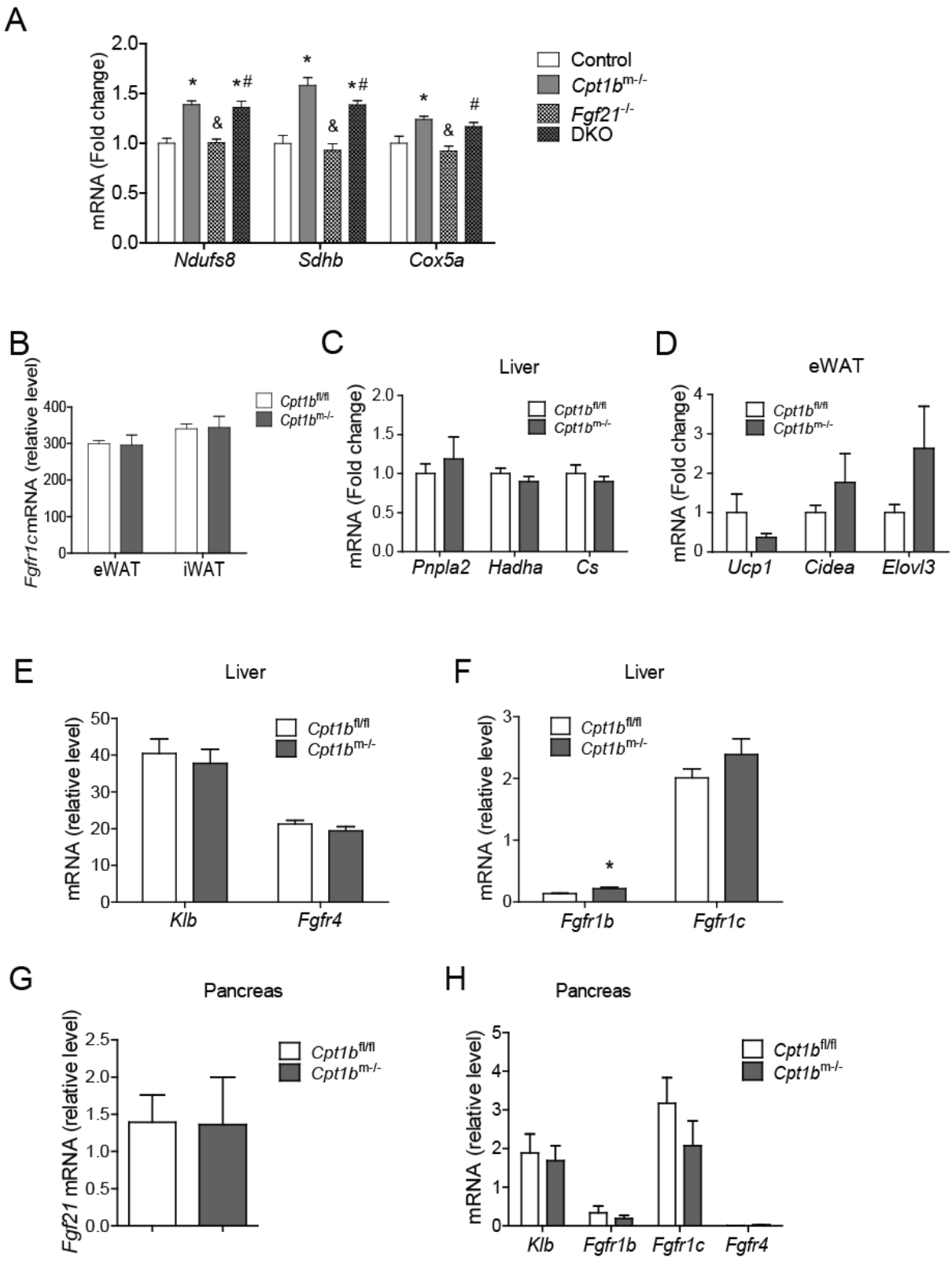
Authophagy related gene expression pattern determined by Ingenuity Pathway Analysis (IPA) and Gene Set Enrichment Analysis (GSEA) in gastrocnemius muscle from *Cpt1b*$^{m-/-}$ (wt) and *Cpt1b*$^{fl/fl}$ (ko) mice, *n=7-8.*

Mitophagy



Supplementary Figure 5 is related to Figure 4.

Mitophagy related gene expression pattern determined by Ingenuity Pathway Analysis (IPA) and Gene Set Enrichment Analysis (GSEA) in gastrocnemius muscle from *Cpt1b*$^{m-/-}$ (wt) and *Cpt1b*$^{fl/fl}$ (ko) mice, *n=7-8*.



Supplementary Figure 6 is related to Figure 5 and Figure 7.

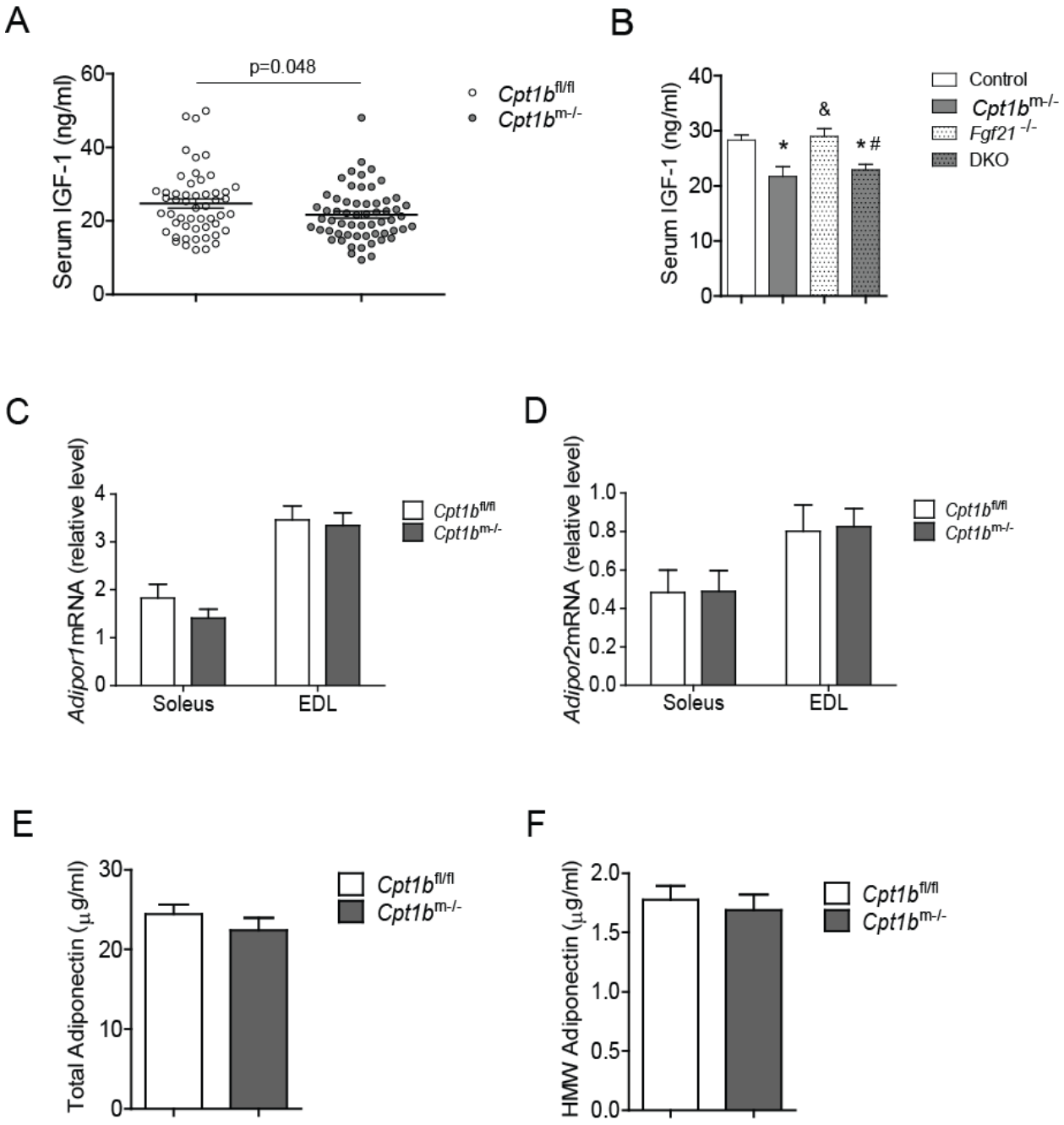
A is related to Figure 5. Expression of mitochondrial oxidative – phosphorylation genes in gastrocnemius muscle from 4 month old control (*Fgf21*$^{+/+}$ *Cpt1b*$^{fl/fl}$), *Cpt1b*$^{m-/-}$ (*Fgf21*$^{+/+}$ *Cpt1b*$^{-/-}$), *Fgf21*$^{-/-}$ (*Fgf21*$^{-/-}$ *Cpt1b*$^{fl/fl}$) and DKO (*Fgf21*$^{-/-}$ *Cpt1b*$^{m-/-}$) mice as examined by qRT-PCR (*n=4-8* per group). All data are presented as means ± s.e.m., **P* < 0.05 and higher significance compared to control mice, $^{\&}P$ < 0.05 and higher significance compared to *Cpt1b*$^{m-/-}$ mice, $^{\#}P$ < 0.05 and higher significance compared to *Fgf21*$^{-/-}$ mice.

B is related to Figure 7. Gene expression of *Fgfr1c* as determined by qRT-PCR in white adipose tissue (eWAT and iWAT) from 4 month old *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ mice. All data are presented as means ± s.e.m., *n=5-7* per group.

C-D is related to Figure 7. mRNA expression for lipolysis (*Pnpla2*) and mitochondrial FAO (*Hadha, Cs*) genes in liver from 4 month old *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ mice (C). mRNA expression of beige adipocyte marker genes in eWAT from 4 month old *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ mice (D). All data are presented as means ± s.e.m., *n=5-7* per group.

E-F. Gene expression of *Klb, Fgfr4* (E) and *Fgfr1b, Fgfr1c* (F) as determined by qRT-PCR in liver from 4 month old *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ mice. All data are presented as means ± s.e.m., *n=5-7* per group.

G-H. Gene expression of *Fgf21* (G) and *Klb* and Fgf-receptors (H) as determined by qRT-PCR in pancreas from 4 month old *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ mice. All data are presented as means ± s.e.m., *n=4* per group.



Supplementary Figure 7 is related to Figure 5.

A is related to Figure 5. Serum level of IGF-1 as determined by ELISA in fed *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ mice. All data are presented as means ± s.e.m., *n=52-59* per group.

B is related to Figure 5. Serum level of IGF-1 as determined by ELISA in fed 4 month old control (*Fgf21*$^{+/+}$ *Cpt1b*$^{fl/fl}$), *Cpt1b*$^{m-/-}$ (*Fgf21*$^{+/+}$ *Cpt1b*$^{m-/-}$), *Fgf21*$^{-/-}$ (*Fgf21*$^{-/-}$ *Cpt1b*$^{fl/fl}$) and DKO (*Fgf21*$^{-/-}$ *Cpt1b*$^{m-/-}$) mice, *n=7* per genotype. All data are presented as means ± s.e.m., *$P < 0.05$

significance compared to control mice, $^{\&}P < 0.05$ significance compared to *Cpt1b*$^{m-/-}$ mice and $^{\#}P < 0.05$ significance compared to *Fgf21*$^{-/-}$ mice.

C-D. Gene expression of adiponectin receptors *Adipor1* (C), *Adipor2* (D), as examined by qRT-PCR in soleus and extensor digitorium longus (EDL) muscle from 4 month old *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ mice. All data are presented as means ± s.e.m., *n=6* per group.

E-F. Serum levels of total adiponectin (E) and high molecular weight (HMW) adiponectin (F), as determined by ELISA in 5 month old, fed *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ mice. All data are presented as means ± s.e.m., *n=12* per group.

Supplemental Table 1: Primer Sequences for qRT-PCR

	Gene symbol	Ref.Seq ID	Forward primer	Reverse primer	Notes
1	*Fgf21*	NM_020013	TTCTTTGCCAACAGCCAGAT	GTCCTCCAGCAGCAGTTCTC	Mus musculus
2	*Klb*	NM_031180	ACACTGTGGGACACAACCTG	AGAGCCAACCCTTCTGATGA	
3	*Pnpla2*		CATGATGGTGCCCTATACTC	GTGAGAGGTTGTTTCGTACC	(Kim et al., 2013)
4	*Hadha*	NM_178878	TGACGCTGGTTATCTTGCTG	ATCAGGGCCTTCGATTCTTT	
5	*Cs*	NM_026444	CGGGAGGGCAGCAGTATCGG	ACCACCCTCATGGTCACTATGGATG	
6	*Ucp3*	NM_009464	AAAGGGACTTGGCCCAACA	CTTGATGATGTCGTAGGTCACCAT	
7	*Ech1*	NM_016772	TCGCTACTGCACTCAGGATG	AGCAGCCAAGCCCATATCTA	
8	*Peci*	NM_001110331	GGGGATAGAGGAGGCAGCTA	AACCAGAGGCTTGGGAAAGT	
9	*Fabp3*	NM_010174	GACGAGGTGACAGCAGATGA	CTGCACATGGATGAGTTTGC	
10	*PGC1α*	NM_008904	AGCCTCTTTGCCCAGATCTTC	CCATCTGTCAGTGCATCAAATGA	
11	*Adipor1*	NM_028320	GCATCTCTGCCATCATTGTG	ACACCACTCAAGCCAAGTCC	
12	*Adipor2*	NM_197985	TACACACAGAGACGGGCAAC	CCCAGGCACAGGAAGAATAC	
13	*Akt1*	NM_009652	GGATGTGGATCAGCGAGAGT	GCGGATGATAAAGGTGTTGG	
14	*Akt2*	NM_001110208	GCCCCTGACCAGACCTTAC	GTCGTGGCCTCTCAGTCTTC	
15	*Akt3*	NM_011785	GATGCGTCTACAACCCATCA	ACTTGCCTTCTCTCGAACCA	

16	*Nrf2*	NM_010902	GGCAGAGACATTCCCATTTG	GAGGGGCAGTGAAGACTGAA	
17	*Hif1α*	NM_010431	TGCTCATCAGTTGCCACTTC	CCATCTGTGCCTTCATCTCA	
18	*Xbp1u*	NM_001271730	CTGACGAGGTTCCAGAGGTG	CAGAGGTGCACATAGTCTGAGT	
19	*Xbp1s*	NM_001271730	CTGACGAGGTTCCAGAGGTG	TGCACCTGCTGCGGACTCA	
20	*Ddit3*	NM_007837	TATCTCATCCCCAGGAAACG	GGACGCAGGGTCAAGAGTAG	
21	*Atf4*		AGCAAAACAAGACAGCAGCC	ACTCTCTTCTTCCCCCTTGC	(Kim et al., 2013)
22	*Atf5*	NM_030693	GGCTGGCTCGTAGACTATGG	CGCTCAGTCATCCAATCAGA	
23	*Fgfr1b*	NM_001079909	GAGTAAGATCGGGCCAGACA	TCACATTGAACAGGGTCAGC	
24	*Fgfr1c*	NM_001079908	GACTCTGGCCTCTACGCTTG	TCGTCGTCGTCATCATCTTC	
25	*Fgfr1*	NM_001079908	ACCAAGAAGAGCGACTTCCA	AACCAGGAGAACCCCAGAGT	
26	*Fgfr4*	NM_008011	ACTCCATCGGCCTTTCCTAC	TGTTGTCCACGTGAGGTCTT	
27	*Ucp1*	NM_009463.2	ACTGCCACACCTCCAGTCATT	CTTTGCCTCACTCAGGATTGG	
28	*Cidea*	NM_007702.1	TGCTCTTCTGTATCGCCCAGT	GCCGTGTTAAGGAATCTGCTG	
30	*Elovl3*	NM_007703	TCCGCGTTCTCATGTAGGTCT	GGACCTGATGCAACCCTATGA	
31	*Ndufs8*	NM_144870	TTGCCTGCAAACTCTGTGAG	ATGTCATAGCGTGTCGTTCG	
32	*Sdhb*	NM_023374	GGAGGGCAAGCAACAGTATC	GCACACAGGATGCACTCGTA	
33	*Cox5a*	NM_007747	ACATTGATGCCTGGGAATTG	AATGATTTTGGGCTCAGGAA	

34	*Cyclophilin B (Ppib)*	NM_011149	TCCATCGTGTCATCAAGGACTT	CTCATCTGGGAAGCGCTCA	
35	*FGF21*	NM_019113	AGACATCCAGGTTCCTGTGC	AGACATCCAGGTTCCTGTGC	Homo sapiens
36	*Cyclophilin B (PPIB)*	NM_000942	ATGATCCAGGGCGGAGACT	CAGGCCCGTAGTGCTTCAG	

Kim, K.H., Jeong, Y.T., Oh, H., Kim, S.H., Cho, J.M., Kim, Y.N., Kim, S.S., Kim do, H., Hur, K.Y., Kim, H.K.*, et al.* (2013). Autophagy deficiency leads to protection from obesity and insulin resistance by inducing Fgf21 as a mitokine. Nat Med *19*, 83-92.

Quantitative RT-PCR. Total RNA from mouse tissue (snap frozen on liquid nitrogen at the collection) was isolated using RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and total RNA from mouse primary myotubes and HSMM was isolated using RNeasy Micro Kit (Qiagen). Pancreas RNA was prepared immediately upon tissue collection in Tri Reagent (Sigma, St. Louis, MO, USA) as described in (Fon Tacer et al., 2010). All samples were DNase digested to remove potential genomic DNA contamination. cDNA synthesized with the iScript cDNA synthesis kit was used for qRT-PCR with the SYBR Green system (Bio-Rad, Hercules, CA, USA).

Western blot analysis. Protein homogenates were prepared from muscle tissue in Cell Lysis Buffer (EMD Millipore, Danvers, MA, USA). Immunoblot analyses were performed using standard procedures followed by ECL or Odyssey Infrared detection. For detection of pAkt1,2 and Glut1, samples were heated at 900C for 2 minutes, and for Glut1 separation was at 75V for 3 hours. The antibodies used were: pAkt (Ser473), Akt, pAkt1 (Ser473), pAkt2 (Ser474), Akt1, pTSC2 (Ser1387), pTSC2 (Thr1462), TSC2, Atf4 all from Cell Signaling, Akt2, Glut1 from EMD Millipore, and GAPDH from Abcam, Active MAPK (pErk1/2) and Erk1/2 from Promega, and Klotho β from R&D Systems. Densitometric analysis was performed using ImageJ.

Histology. Inguinal white adipose tissue (iWAT) from mice were harvested and fixed with formalin. Then the tissue were embedded in paraffin and sectioned and stained with H&E at the Cell Biology and Bioimaging Core Facility.

Mouse primary muscle cell culture. Cultures were established from mixed hindlimb muscle from 1 month and 3-5 month old *Cpt1b*$^{m-/-}$ and *Cpt1b*$^{fl/fl}$ littermates (Rando and Blau, 1994). Collagenase digestion was used to isolate satellite cells (0.5% collagenase B, 1.2 U/ml Dispase II

(Roche) in Ham's F-10 media (Thermo Scientific)), and enrichment in non-collagen coated flasks before initiation of culture and between passages used to reduce fibroblast content. Cells were maintained in collagen-coated flasks in Rat Skeletal Muscle Cell Growth Medium (Cell Applications, Inc) and myoblasts at passage 3 were used for experiments. Briefly, the myoblasts at passage 2 were subcultured onto 24 well plates and grown to 80-90% confluence. Then cells were differentiated into fused multinucleated myotubes in Ham's F-10 media with 2% horse serum for 5-7 days. Myotubes were treated with either essentially fatty-acid free BSA or BSA-conjugated palmitate:oleate (1:1 ratio, total 0.5mM) plus 2.5mM carnitine in presence of vehicle (DMSO) or one or combination of the following inhibitors: Cpt1 inhibitor, Etomoxir (10, 30, 50, 100 μM); Akt1 inhibitor, A-674563 (30 μM); and AMPK inhibitor, Compound C (30 μM). Treatments were performed in serum-free MEM alpha media with nucleosides (Gibco) for 24h and cells were harvested for gene expression analysis. At least three independent cultures were performed for each gene expression analysis by qRT-PCR and multiple wells (at least three) were used per treatment.

Human skeletal muscle myoblast culture. Cryopreserved human skeletal muscle myoblasts (HSMM) from four Normal-Lean (BMI, 19.8±0.7), and four Diabetic-Obese (BMI, 32.6±4.2) subjects at passage 2 were purchased from Lonza (Walkersville, MD, USA). Undifferentiated myoblasts were maintained in human skeletal growth media (SkGM-2 Bullet Kit, Lonza, Walkersville, MD) and the media were changed every 2 days. Myoblasts at passage 4 were used for experiments (Vandanmagsar et al., 2014). Briefly, the myoblasts at passage 3 were subcultured onto 12-well (for protein assays) or 24-well (for gene expression analysis) culture plates and grown to 80-90% confluence. Then cells were differentiated into fused multinucleated myotubes for 3-5 days until myotubes were observed throughout the culture by switching to

fusion medium DMEM-F12 (Lonza) supplemented with 2% horse serum. For gene expression analysis, myotubes were treated with either essentially fatty-acid free BSA or BSA-conjugated palmitate:oleate (1:1 ratio, total 0.5mM) plus 2.5mM carnitine in presence of vehicle (DMSO, DDW) or one or combination of the following inhibitors: Cpt1 inhibitor, Etomoxir (100 μM); Akt1 inhibitor, A-674563 (50 μM); AMPK inhibitor, Compound C (30 μM), PPARα inhibitor, GW6471 (10 μM); PPARγ inhibitor, T0070907 (10 μM) and PPARδ inhibitor, GSK3787 (10 μM). Treatments were performed in serum-free MEM alpha media with nucleosides (Gibco) for 24h and cells were harvested for gene expression analysis. Essentially fatty-acid free BSA, sodium palmitate, sodium oleate, L-carnitine salt and etomoxir sodium salt hydrate were obtained from Sigma. A-674563, T0070907 and GSK3787 were obtained from Selleckchem and Compound C was obtained from Calbiochem. GW6471 was obtained from Santa Cruz. For protein assays, myotubes were treated with vehicle (PBS) or essentially fatty-acid free BSA or BSA-conjugated palmitate:oleate (1:1 ratio, total 0.5mM) or recombinant human FGF21 (200 ng/ml) for 12h and then cells were harvested for Multiplex Mapmate Cell signalling protein analysis. rhFGF21 was obtained from R&D Systems. At least two independent culture of HSMM per subject were performed for each gene expression analysis by qRT-PCR and multiple wells (at least three) were used per treatment.

Multiplex Mapmate signaling assay. Harvested muscle tissue from mouse was snap froze in liquid nitrogen. Then entire muscle tissue was powdered in liquid nitrogen and used for protein lysate preparation in Cell Signaling Lysis Buffer (Millipore). Differentiated and treated human myotubes were harvested in Cell Signaling Lysis Buffer (Millipore). Total protein lysates from mouse tissue and human cells were prepared according to the manufacturer instructions and used for Multiplex Map Cell signaling assays. The following mouse and human Map mates were

used: p-Akt (Ser473), p-IR (Tyr1162/1163), p-mTOR (Ser2448), p-Erk1/2 (Thr185/Tyr187) and total Akt, IR, mTOR, Erk1/2 (all from Millipore); and p-P70S6K (Thr421/Ser424), p-IRS-1 (Ser636/Ser639) (Bio-Rad); and total P70S6K, IRS-1 (Millipore). Human GAPDH (Millipore) map mate was used in all HSMM multiplex assays for normalization of protein data analysis. Milliplex Map Cell signaling buffer and Detection kit (Millipore) were used in all multiplex assays and Map mates were prepared and combined according to the manufacturer instructions. For mouse multiplex assays, 10 μg of tissue protein lysates was used in each assay and assays were run in duplicates. Phospho- and total Map mates were used in separate assays and mean fluorescence intensity (MFI) of phospho-protein was normalized to MFI of total protein. The values for muscle from *Cpt1b*$^{fl/fl}$ mice set to 1 and fold change was calculated for muscle from *Cpt1b*$^{m-/-}$ mice compared to *Cpt1b*$^{fl/fl}$ mice. For human multiplex assays, 20 ul of cell (protein) lysates was used in each assay and assays were run in duplicates. Phospho- and total Map mates were used in separate assays, but human GAPDH was used in each assay. First, mean fluorescence intensity (MFI) of phospho- and total map mates were normalized to MFI of GAPDH separately, than secondly ratios of phospho-protein to total protein were calculated to compare activation of the signaling pathways (Vandanmagsar et al., 2014). The values for control myotubes (PBS-treated) set to 1 and fold change was calculated as fold over control for each treatment.

Glucose Uptake. Glucose uptake was measured in mouse primary myotubes as described in (Noland et al., 2009). Briefly, primary myotubes were serum starved for 5h, and then incubated in a buffer containing 1mM pyruvate, 0.1% essentially fatty-acid free BSA and 5mM glucose. After incubation at 37C for 30 minutes, PBS or insulin (100 nM) was added. Following 15 min of incubation, pre-incubation buffer was removed and uptake buffer containing [H3]2-

deoxyglucose (1 μCi), 0.1% BSA and PBS or insulin (100 nM) was added. Following 45 min of incubation at 37C, cells were washed with ice cold PBS supplemented with 20 mM glucose. Then cell lysates were prepared in 0.1% SDS and radioactivity counts were obtained. For measurement of glucose uptake in presence of FGF21, mouse primary myotubes were pretreated with vehicle (PBS) or rmFGF21 (250 ng/ml) overnight (16h), and then PBS or rmFGF21 (250 ng/ml) was present during starvation and in all buffers through the glucose uptake assay. Insulin solution human was obtained from Sigma and recombinant mouse FGF21 was obtained from Prospec (Ness Ziona, Israel).