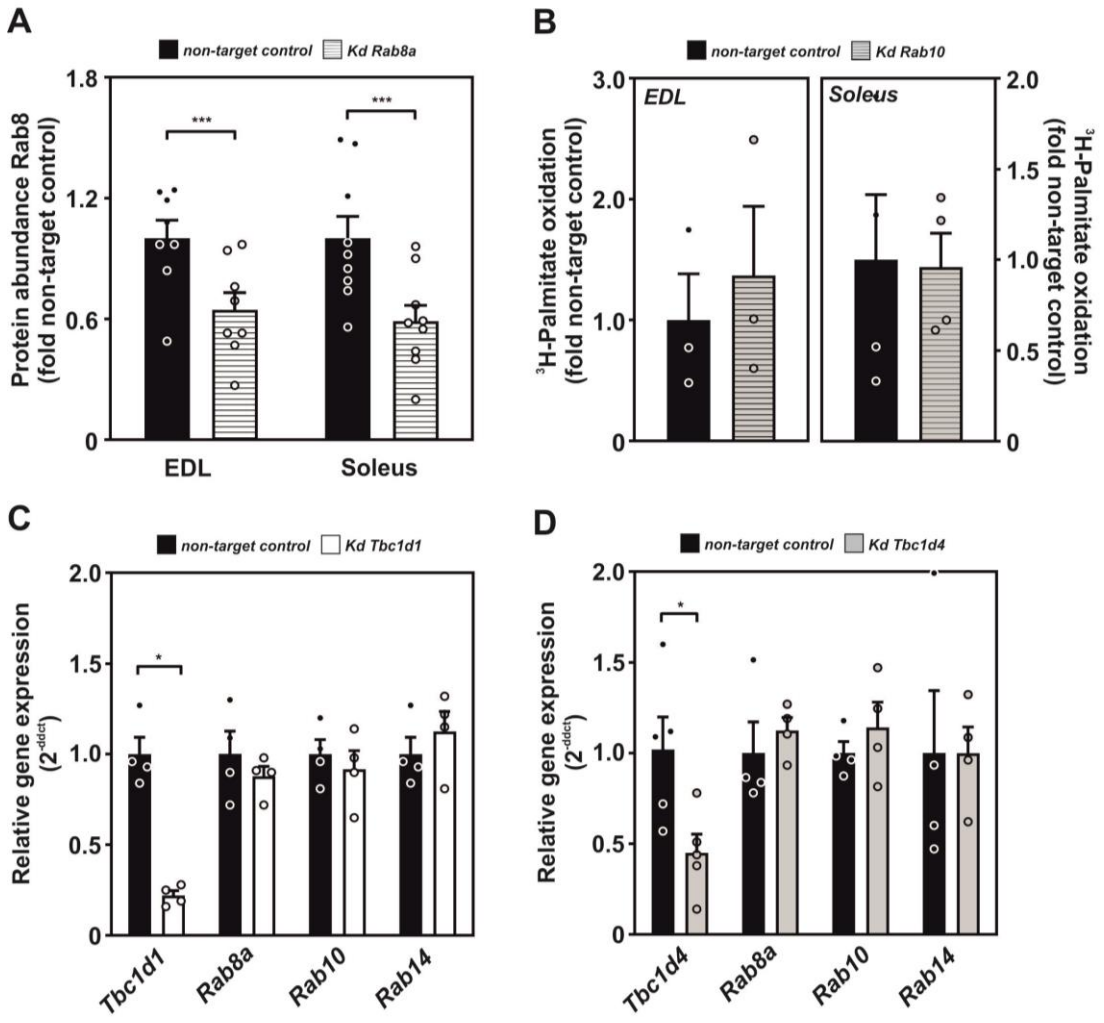
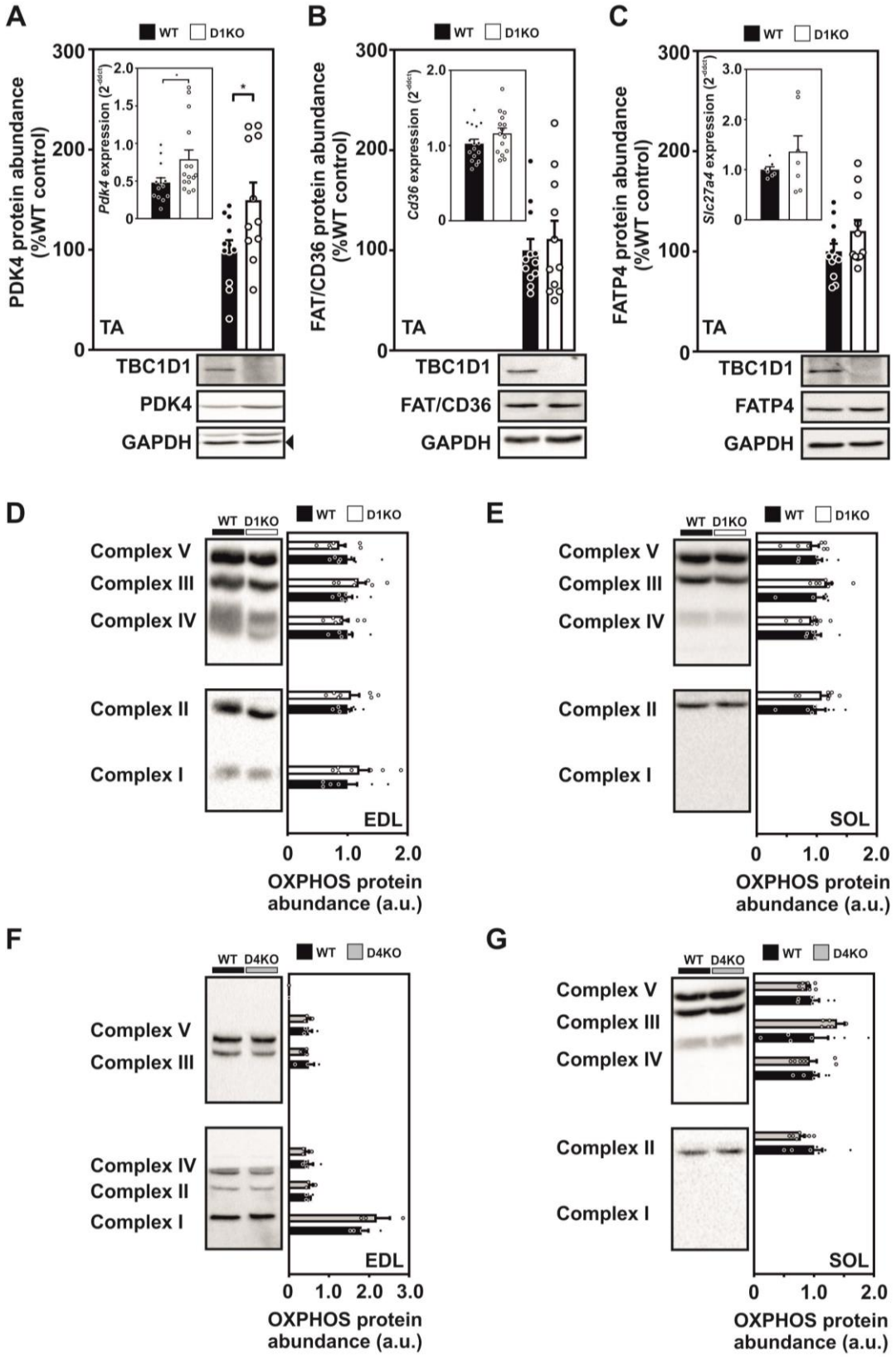
Supplemental material

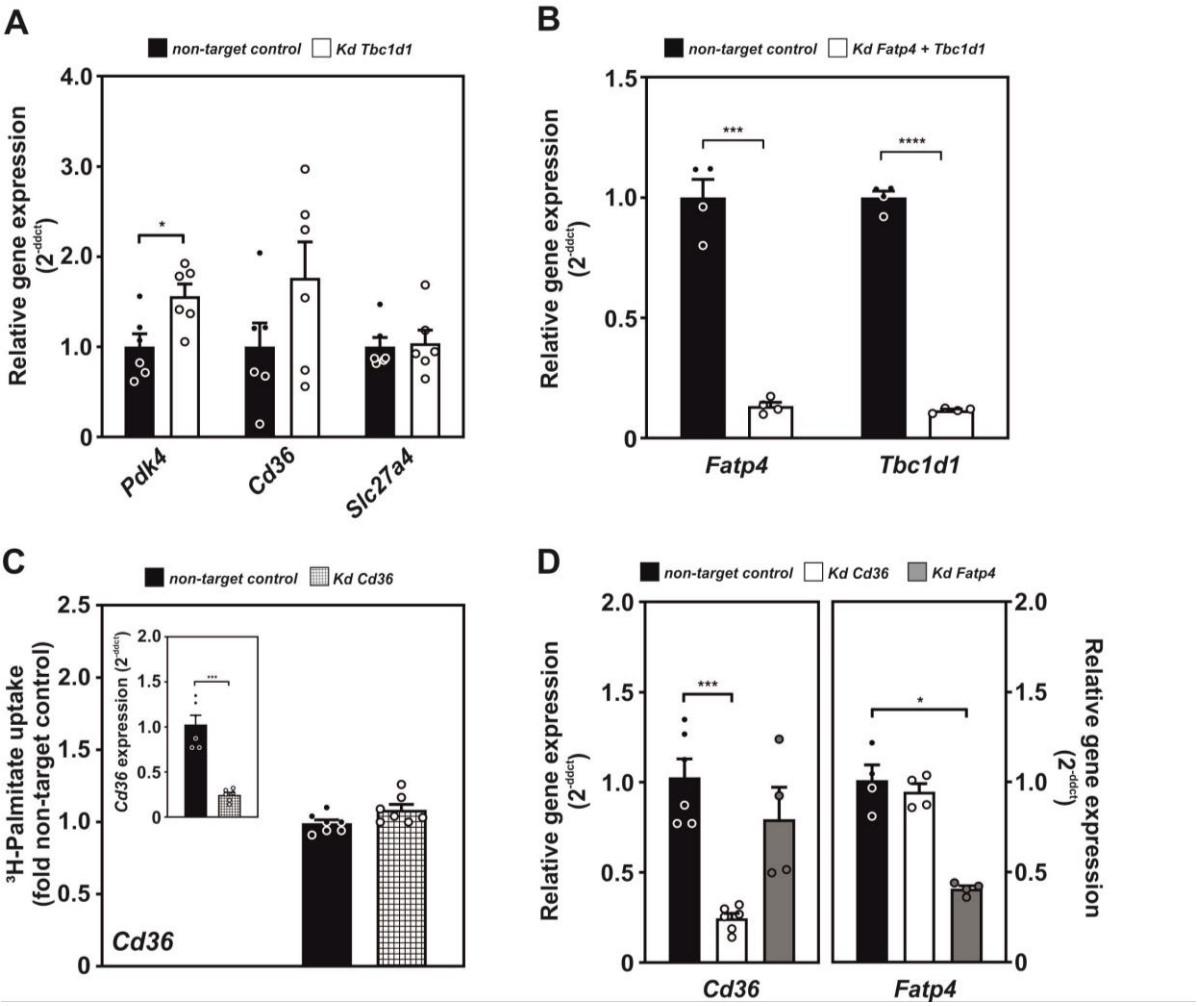


Supplemental Figure 1. (A) Protein abundance of Rab8a following siRNA-mediated knockdown of *Rab8* via IVE in EDL and Soleus muscle from C57BL/6J mice. Data are presented as mean ± SEM (n = 4). *p < 0.05, non-target control vs. *Rab8a* kd (paired two-tailed Student's t-test). (B) *Ex vivo* fatty acid oxidation in intact isolated Soleus skeletal muscle after *in vivo* electrotransfection-mediated knockdown of *Rab10*. Data are presented as mean ± SEM (n = 3). non-target control vs. Rab10 kd (paired two-tailed Student's t-test). (C) Relative mRNA expression of *Tbc1d1, Rab8a, Rab10* and *Rab14* in C2C12 myotubes following siRNA-mediated silencing of *Tbc1d1*. Data are presented as mean ± SEM (n = 4). *p < 0.05, non-target control vs. *Tbc1d1* kd, (paired two-tailed Student's t-test). (D) Relative mRNA expression of *Tbc1d4, Rab8a, Rab10* and *Rab14* in C2C12 myotubes following siRNA-mediated silencing of *Tbc1d4*. Data are presented as mean ± SEM (n = 4-5). *p < 0.05, non-target control vs. *Tbc1d1* kd (paired two-tailed Student's t-test).



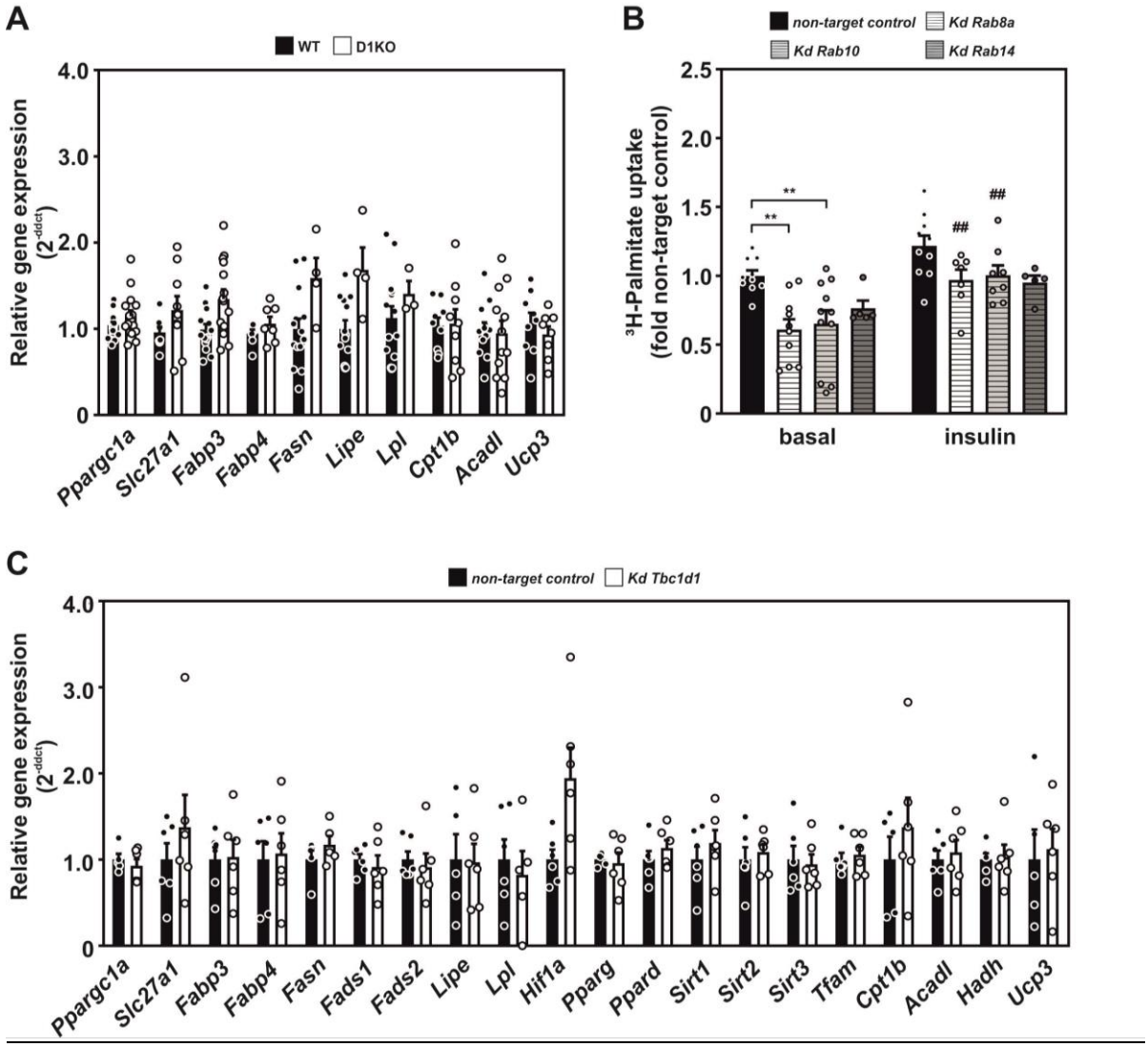
Supplemental Figure 2. Protein abundance of (A) PDK4, (B) FAT/CD36 and (C) SLC27A4/FATP4 in WT vs. D1KO mouse Tibialis anterior (TA) muscle samples. The corresponding gene expression analysis for *Pdk4*, *Cd36* and *Slc27a4/Fatp4*, respectively, can be seen in the interlaced graphs of either panel figure A, B and C. Data are presented as mean

± SEM (n = 7-16). *p < 0.05 (unpaired two-tailed Student`s t-test). Protein abundance of Complexes I – V of the OXPHOS complex in EDL muscle from WT vs. (D) D1KO or (F) D4KO mice and Soleus (SOL) muscle from WT vs. (E) D1KO or (G) D4KO mice. Data are presented as mean ± SEM (n = 3-7). *p < 0.05 (unpaired two-tailed Student`s t-test).



Supplemental Figure 3. (A) Relative mRNA expression of *Pdk4*, *Cd36* and *Slc27a4* (= FATP4) in C2C12 myotubes following siRNA-mediated silencing of *Tbc1d1*. Data are presented as mean ± SEM (n = 6), non-target control vs. *Tbc1d1* kd (paired two-tailed Student's t-test). (B) Validation of knockdown efficiency via quantitative Realtime-PCR in C2C12 myotubes following siRNA-mediated silencing of both, *Tbc1d1* and FATP4 (*Slc27a4*). Data are presented as mean ± SEM (n = 4), non-target control vs. *Tbc1d1/Fatp4* kd (paired two-tailed Student's t-test). (C) Uptake of ^{3}H-palmitate into C2C12 myotubes after siRNA-mediated knockdown of *Cd36*. Knockdown (Kd) efficiency for *Cd36* can be seen in the interlaced graph of the panel figure F. Data are presented as mean ± SEM (n = 6-7). ***p < 0.001, non-target control vs. *Cd36* kd (paired two-tailed Student's t-test). (D) Relative mRNA expression of *Cd36* and *Slc27a4* (= FATP4) in C2C12 myotubes following siRNA-mediated silencing of *Cd36* and

Slc27a4/Fatp4, respectively. Data are presented as mean ± SEM (n = 4-6), non-target control vs. *Cd36* or *Slc27a4/Fatp4* kd, respectively (one-way ANOVA with Dunnett *post hoc* test).



Supplemental Figure 4. (A) Relative gene expression of genes involved in the myocellular regulation of fatty acid metabolism in skeletal muscle from WT and D1KO mice, respectively. Data are presented as mean ± SEM (n = 4-14). *$p < 0.05$ (unpaired two-tailed t-test). (B) Uptake of ^{3}H-palmitate into C2C12 myotubes after siRNA-mediated knockdown of *Rab8a*, *Rab10* and *Rab14* under basal and insulin-stimulated conditions, respectively. Data are presented as mean ± SEM (n = 5-11). **$p < 0.01$ between knockdown conditions and $^{\#\#}p < 0.01$ for the stimulation with insulin within the respective genotype. (mixed model two-way ANOVA with repeated measures analysis for the Kd condition and Tukey *post hoc* test). (C) Relative gene expression of genes involved in the myocellular regulation of fatty acid metabolism in C2C12 myotubes following siRNA-mediated silencing of *Tbc1d1*. Data are presented as mean ± SEM (n = 6), non-target control vs. respective *Tbc1d1* kd (paired two-tailed Student's t-test).

Supplemental Table 1: Chemicals and buffer ingredients

Compound	Supplier
5-aminoimidazole-4-carboxyamide-1-β-D-ribofuranoside (AICAR)	Toronto Research Chemicals, North York ON, Canada
BSA Fraction V, fatty acid free	Carl Roth, Karlsruhe, Germany
Butyric Acid	Sigma Aldrich, Steinheim, Germany
Complete protease inhibitor cocktail	Roche Diagnostics, Mannheim, Germany
D(+)-Glucose, ≥99.5 %	Sigma Aldrich, Steinheim, Germany
Dulbecco´s modified eagle medium (DMEM), high glucose, L-Glutamine, sodium pyruvate, phenol red, w/o HEPES	Gibco at Thermo Fisher Scientific, Darmstadt, Germany
Fetal Bovine Serum (FBS)	Thermo Fisher Scientific, Darmstadt, Germany
Horse Serum (HS)	ATCC at LGC Standards, Wesel, Germany
Invivofectamine 2.0	Invitrogen, Carlsbad, California
Isoflurane	Piramal, Morpeth, UK
Oleic Acid	Sigma Aldrich, Steinheim, Germany
Palmitic Acid Sigma Ultra approx. 99 %	Sigma Aldrich, Steinheim, Germany
Pentadecanoic acid	Sigma Aldrich, Steinheim, Germany
PhosSTOP phosphatase inhibitor cocktail	Roche Diagnostics, Mannheim, Germany
Protease inhibitor cocktail	Roche Diagnostics, Mannheim, Germany

Supplemental Table 2: Buffers and cell culture media

Description	Ingredients
C2C12 culture medium	DMEM (4.5 g/L glucose), 10 vol % FCS, 1 vol % P/S
C2C12 differentiation medium	DMEM (4.5 g/L glucose), 2 vol % HS, 1 vol % P/S
C2C12 starvation medium	DMEM (4.5 g/L glucose), 1 vol % P/S
C2C12 transfection medium	DMEM (4.5 g/l glucose)
Ex vivo fatty acid oxidation/uptake HOT incubation buffer	KHB, 15 mM mannitol, 5 mM glucose, 0.6 mM cold palmitate, 20 % fatty acid-free BSA, 1.4 µM ^{3}H-palmitate
Ex vivo fatty acid oxidation/uptake pre-incubation buffer	KHB, 15 mM mannitol, 5 mM glucose, 3.5 mM fatty acid-free BSA
In vitro fatty acid oxidation HOT buffer	C2C12 differentiation medium, 11.8 µM ^{14}C-palmitate, 6.24 µM fatty acid-free BSA, 1 µM L-carnitine
In vitro fatty acid uptake HOT buffer	KRH, 8.5 nM ^{3}H-palmitate/-oleate/-butyrate, 2.5 µM fatty acid-free BSA, 5 µM unlabeled cold palmitate/oleate/butyrate
In vitro fatty acid uptake pre-HOT buffer	KRH, 40 µM fatty acid-free BSA
In vitro fatty acid uptake washing buffer	KRH, 0.1 % fatty acid-free BSA
Krebs-Henseleit buffer (KHB)	Stock I: 118.5 mM NaCl, 0.047 mM KCl, 0.012 mM KH_2PO_4, 0.25 mM $NaHCO_3$ Stock II: 0.025 mM $CaCl_2$, 0.012 mM $MgSO_4$, 0.05 mM HEPES

Description	Ingredients
Krebs-Ringer-HEPES (KRH) buffer (pH 7.4)	136 mM NaCl, 4.7 mM KCl, 1.25 mM $MgSO_4$, 1.25 mM $CaCl_2$, 10 mM HEPES
Lysis buffer (for tissue and cell protein isolation)	20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 % Triton X-100
Protein lysis buffer	20 mM Tris-HCL, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100
SDS-PAGE 1x electrophoresis buffer	25 mM Tris-HCl, 192 mM Glycine, 0.1 % (w/v) SDS
SDS-PAGE 1x separation gel buffer (pH 8.8)	500 mM Tris-HCl, 0.4 % SDS
SDS-PAGE 1x stacking gel buffer (pH 6.8)	0.5 M Tris, 0.4 % SDS
Transfer buffer (Tank blotting)	25 mM Tris-HCl, 192 mM Glycine, 20 % Methanol
Tris-buffered saline with Tween-20 (TBS-T)	10 mM Tris-HCl, 150 nM NaCl, 0.5 % Tween-20, pH 8.0

Supplemental Table 3: Genotyping primers

Primer	Sequence (5' → 3')
Tbc1d1-WT	Fwd: GGACAAGCAGCTTTCTTGTTT Rev: TCCTGGTCCAGAAGCGAG
Tbc1d1-KO	Fwd: CAACATTCTGAAGGCCTTCTG Rev: TCCCTGGCTACAAGCTGAGT
Tbc1d4 *Tbc1d4*-WT *Tbc1d4*-KO	Fwd: AGTAGACTCAGAGTGGTCTTGG Rev: GTCTTCCGACTCCATATTTGC Rev: GCAGCGCATCGCCTTCTATC
Cd36 *Cd36*-NEO *Cd36*	Fwd: AGCTCATACATTGCTGTTTATGCATG Fwd: GGTACAATCACAGTGTTTTCTACGTGG Rev: CCGCTTCCTCGTGCTTTACGGTATC

Supplemental Table 4: siRNA oligonucleotide sequences

All siRNA oligonucleotides utilized in this study were purchased from Dharmacon (now: Horizon). The sequence and ordering information are listed in the following table.

Designation	Sequence (5' → 3')	Ordering Number
siCd36	GAAAGGAUAACAUAAGCAAUU	D-062017-01
siFatp1	UGACGGUGGUACUGCGCAA	J-061607-09
siFatp4	AGACCAAGGUGCGACGGUA	J-063631-09
siNT	UAGCGACUAAACACAUCAAUU	D-001210-01
siRab10	GGGCAAAGACCUGCGUCCUU	J-040862-12
siRab14	GGUGUUGAAUUUGGUACAA	J-040866-09
siRab28	GAGCAUAUGCGAACAGUAA	J-040871-11
siRab8a	CAGGAGCGGUUUCGAACAA	J-040860-09
siRab8b	CGAACAAUUACGACAGCAU	J-055301-10
siTbc1d1	GAUCAGAGGUCAUAUUUAAUU	D-040360-01
siTbc1d4	AAGCUAUACACCAGCAAAU	J-040174-05

Supplemental Table 5: Radiochemicals

Compound	Concentration [mCi/ml]	Specific activity [Ci/mmol]	Supplier
[1-^{14}C]-Palmitic Acid	0.1	0.056	Hartmann Analytic, Braunschweig, Germany
[9,10-^{3}H(N)]-Oleic Acid	5	60	American Radiolabeled Chemicals, St. Louis MO, USA
n-[2.3-^{3}H]-Butyric Acid	1	120	American Radiolabeled Chemicals, St. Louis MO, USA
[9,10-^{3}H(N)]-Palmitate	1	50	Hartmann Analytic, Braunschweig, Germany

Supplemental Table 6: Antibodies

Name	Host Species	Supplier	Ordering #	Dilution used in TBS-T
FAT/CD36	Rabbit	Sigma-Aldrich	HPA002018	1:1000 + 5 % BSA
FATP4	Rabbit	Abcam	ab200353	1:1000 + 5 % milk powder
PDK4	Rabbit	Abcam	ab214938	1:1000 + 5 % milk powder
RAB10	Rabbit	Cell Signaling	#4262	1:1000 + 5 % milk powder
RAB8a	Mouse	BD Biosciences	610845	1:2000 + 5 % milk powder
TBC1D1	Rabbit	Cell Signaling	#4629	1:1000 + 5 % milk powder
GAPDH	Rabbit	Cell Signaling	#2118	1:1000 + 5 % BSA
Anti-Rabbit-HRP	Goat	Dianova	111-035-003	1:10,000; 1:20,000
Anti-Mouse-HRP	Rabbit	Dianova	315-035-008	

Supplemental Table 7: qRT-PCR primers

All qRT-PCR primers used throughout this study were designed using the NCBI primer blast online tool and purchased from Eurogentec.

Target gene	Primer sequences (5' → 3')	Product length
Acadl	Fwd: ATTGCTGAGTTGGCGATTTC Rev: GCTGCACCGTCTGTATGTGT	112 bp
Cd36	Fwd: CCTAGTAGGCGTGGGTCTGA Rev: ACGGGGTCTCAACCATTCATC	99 bp
Cpt1a	Fwd: CTCAGTGGGAGCGACTCTTCA Rev: GGCCTCTGTGGTACACGACAA	103 bp
Cpt1b	Fwd: CAGCGCTTTGGGAACCACAT Rev: CACTGCCTCAAGAGCTGTTCTC	105 bp

Target gene	Primer sequences (5' → 3')	Product length
Fabp3	Fwd: ACCTGGAAGCTAGTGGACAG Rev: TGATGGTAGTAGGCTTGGTCAT	106 bp
Fabp4	Fwd: TGAAATCACCGCAGACGACA Rev: ACACATTCCACCACCAGCTT	141 bp
Fads1	Fwd: CTCGTGATCGACCGGAAGG Rev: TGCCACAAAAGGATCCGTGG	114 bp
Fads2	Fwd: GCCCCTTGAGTATGGCAAGA Rev: TACATAGGGATGAGCAGCGG	99 bp
Fasn	Fwd: TTGCTGGCACTACAGAATGC Rev: AACAGCCTCAGAGCGACAAT	192 bp
Hadh	Fwd: GAGGCGATGCGTCTAAGGAA Rev: TCCATTTCATGCCACCCGTC	136 bp
Hif1a	Fwd: CAAGATCTCGGCGAAGCAA Rev: GGTGAGCCTCATAACAGAAGCTTT	113 bp
Lipe	Fwd: GGAGCTCCAGTCGGAAGAGG Rev: GTCTTCTGCGAGTGTCACCA	98 bp
Lpl	Fwd: CAGCTGGGCCTAACTTTGAG Rev: AATCACACGGATGGCTTCTC	206 bp
Pdk4	Fwd: CCTTTGGCTGGTTTTGGTTA Rev: CCTGCTTGGGATACACCAGT	225 bp
Ppard	Fwd: ACCGAGTTCGCCAAGAACAT Rev: AGCCCGTCTTTGTTGACGAT	128 bp
Pparg	Fwd: CGGGCTGAGAAGTCACGTT Rev: TGCGAGTGGTCTTCCATCAC	200 bp
Ppargc1a	Fwd: GAGTCTGAAAGGGCCAAACA Rev: TGCATTCCTCAATTTCACCA	148 bp
Rab10	Fwd: CGGACGATGCCTTCAATACC Rev: AGGAGGTTGTGATGGTGTGA	144 bp
Rab12	Fwd: CAGGTCATCATCATCGGCTC Rev: ATTTTAAAGTCAACACCCACGG	113 bp
Rab14	Fwd: GTTCAGAGCGGTTACACGGA Rev: TCCTTGCGTCTGTCAACCAG	116 bp
Rab28	Fwd: GCACAGGGAATCCTCTTGGT Rev: TAAAGCAACCAGGGGCTGAG	123 bp
Rab8a	Fwd: CCAGTGCAAAGGCCAACATC Rev: CTGGTCCTCTTCTGCTGCTC	151 bp
Rab8b	Fwd: AGGAAAATGAACGACAGCAAT Rev: CATCAAAGCAGAGAACAGCG	104 bp
Scl27a1	Fwd: TGCTTTGGTTTCTGGGACTT Rev: CCGAACACGAATCAGAACAG	149 bp
Scl27a4	Fwd: ACTGTTCTCCAAGCTAGTGCT Rev: GATGAAGACCCGGATGAAACG	106 bp
Sirt1	Fwd: GCTGACGACTTCGACGACG Rev: TCGGTCAACAGGAGGTTGTCT	101 bp
Sirt2	Fwd: GCCTGGGTTCCCAAAAGGAG Rev: GAGCGGAAGTCAGGGATACC	145 bp
Sirt3	Fwd: ATCCCGGACTTCAGATCCCC Rev: CAACATGAAAAAGGGCTTGGG	126 bp

Target gene	Primer sequences (5' → 3')	Product length
Tbc1d1	Fwd: ACAGTGTGGGAAAAGATGCT Rev: AGGTGGAACTGCTCAGCTAG	143 bp
Tbc1d4	Fwd: CCAACAGTCTTGCCTCAGAG Rev: GAATGTGTGAGCCCGTCTTC	146 bp
TBP	Fwd: GCGGCACTGCCCATTTATTT Rev: GGCGGAATGTATCTGGCACA	236 bp
Tfam	Fwd: GGGAATGTGGAGCGTGCTAA Rev: CAGACAAGACTGATAGACGAGGG	96 bp
Ucp3	Fwd: GTCTGCCTCATCAGGGTGTT Rev: CCTGGTCCTTACCATGCAGT	204 bp
β-Actin	Fwd: CCACCATGTACCCAGGCATT Rev: AGGGTGTAAAACGCAGCTCA	253 bp

Supplemental Table 8: Fatty acid reference standard for gas chromatography.

Trivial name	Carboxyl-reference	double bond reference (Δ^x)	ω-reference
Mystiric acid	C14:0		
Pentadecanoic acid	C15:0		
Palmitic acid	C16:0		
Palmitoleic acid	C16:1	cis-Δ^9	ω-7
Stearic acid	C18:0		
Oleic acid	C18:1	cis-Δ^9	ω-9
Linoleic acid	C18:2	cis,cis-$\Delta^{9, 12}$	ω-6
α-Linolenic acid	C18:3	cis,cis,cis-$\Delta^{9, 12, 15}$	ω-3
Arachidonic acid	C20:4	cis,cis,cis,cis-$\Delta^{5, 8, 11, 14}$	ω-6