

Supplementary Materials for

**Drp1 controls complex II assembly and skeletal muscle metabolism by Sdhaf2
action on mitochondria**

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The PDF file includes:

Figs. S1 to S7
Tables S1 to S3
Legends for tables S4 and S5

Other Supplementary Material for this manuscript includes the following:

Tables S4 and S5

Fig. S1

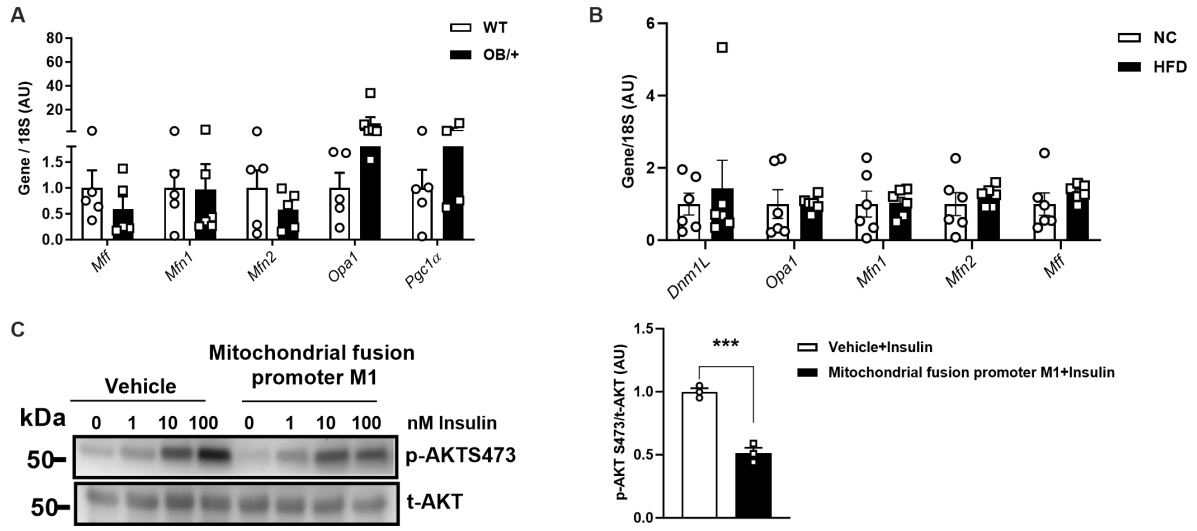


Fig. S2

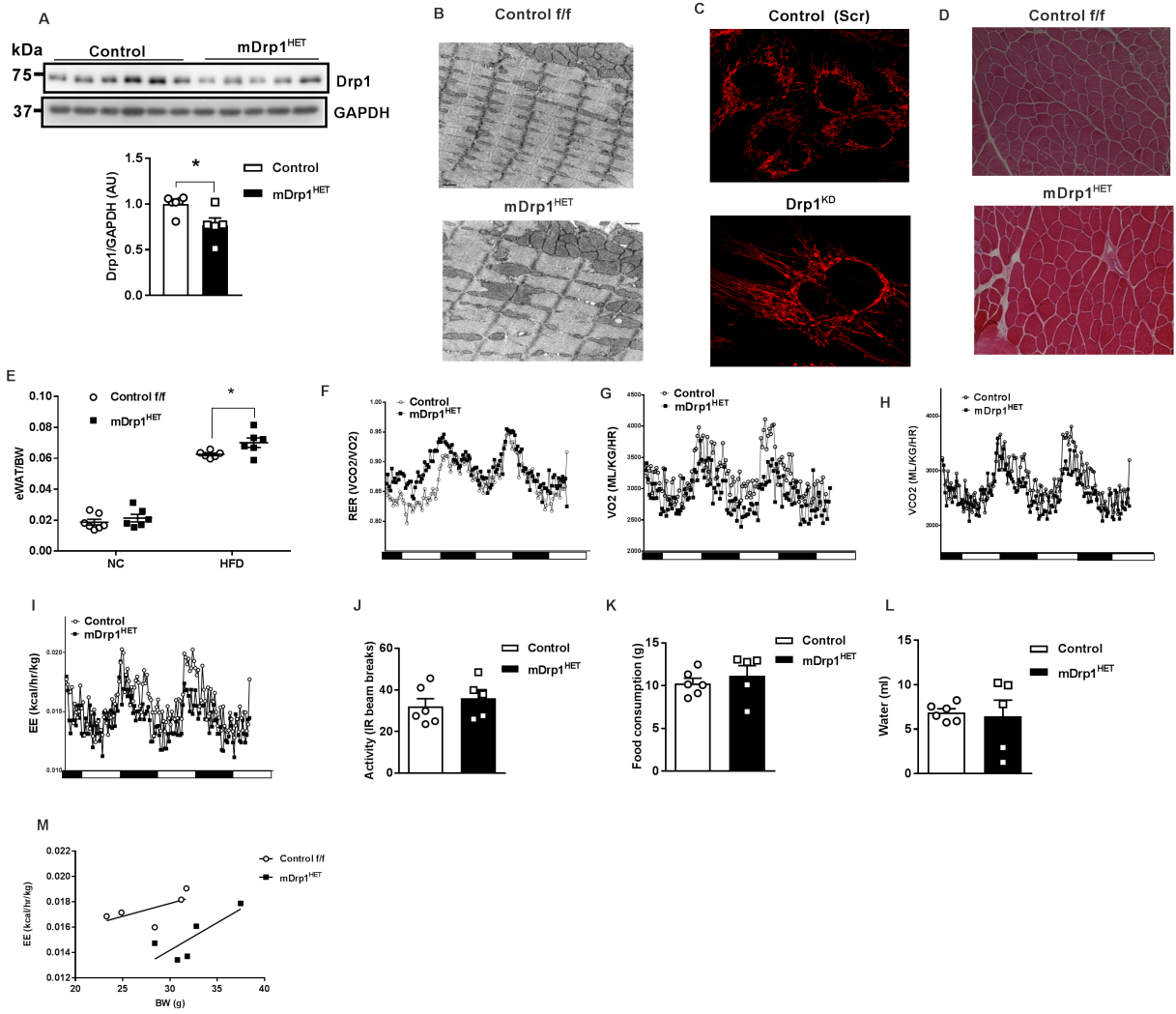


Fig. S3

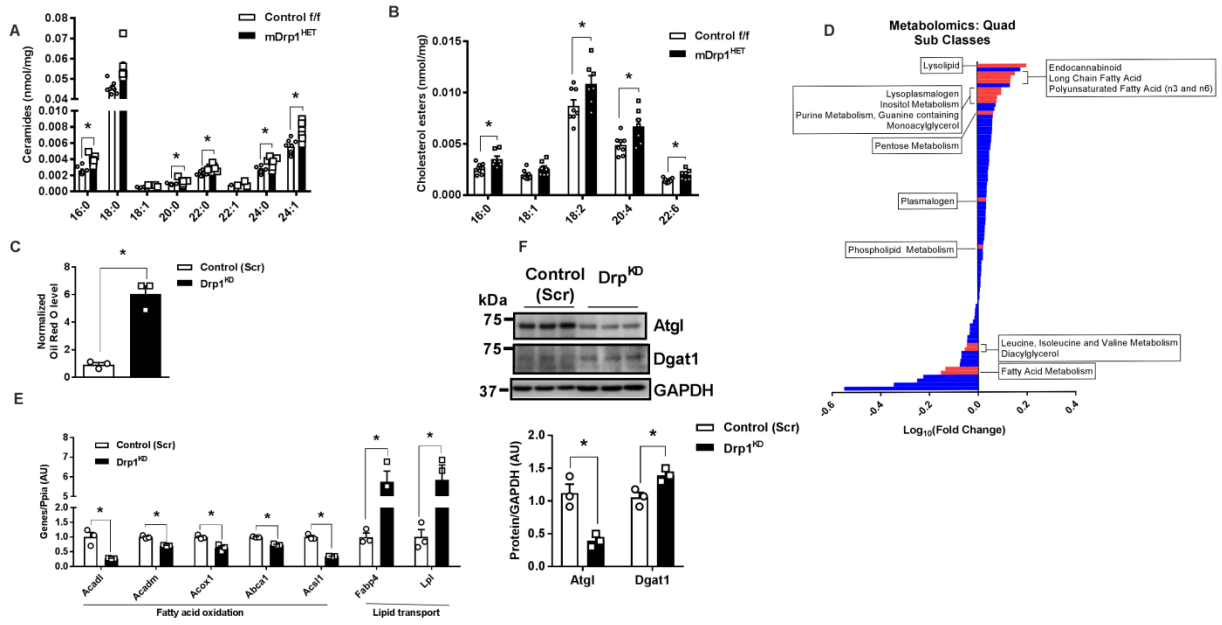


Fig. S4

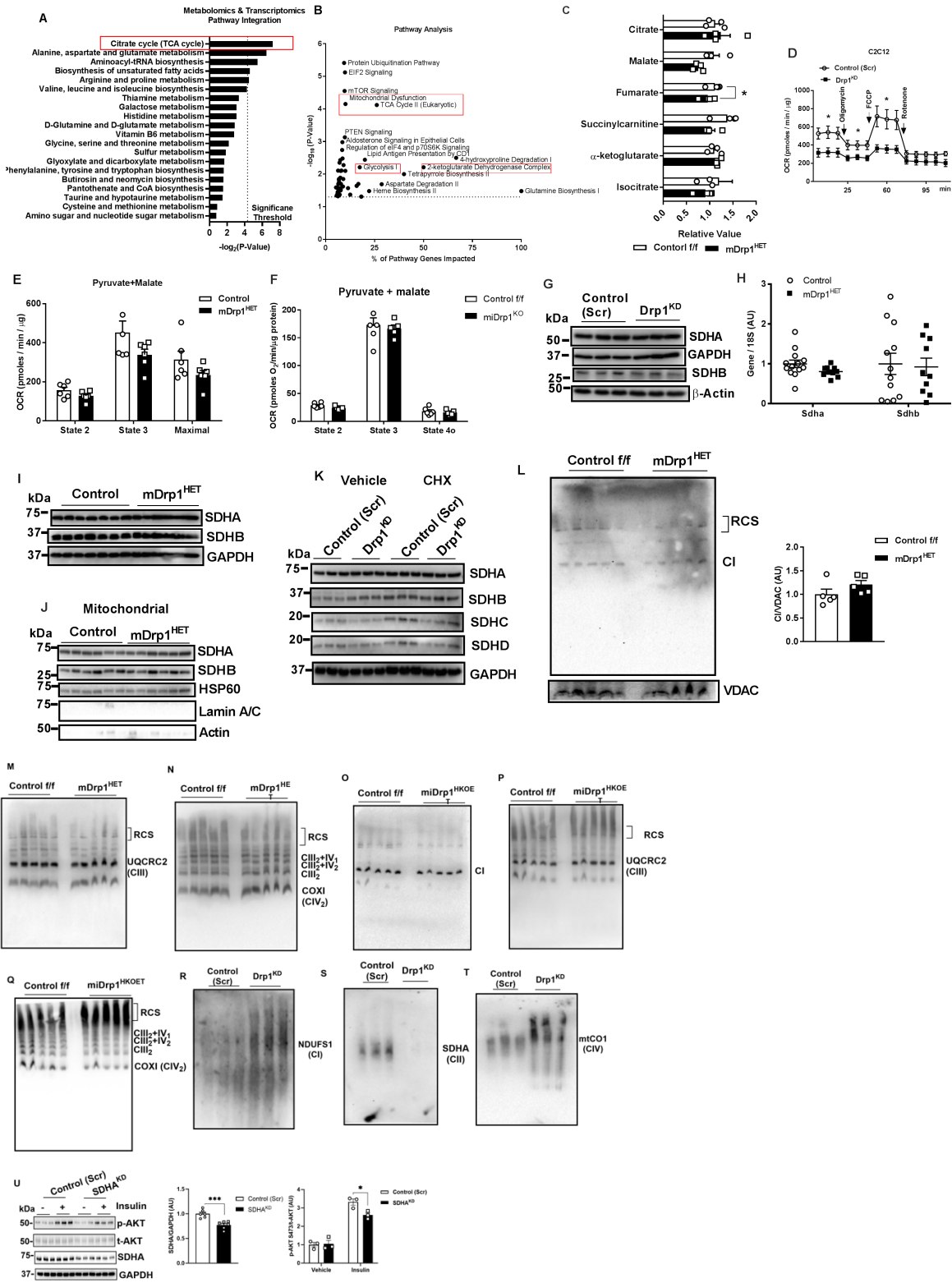


Fig. S5

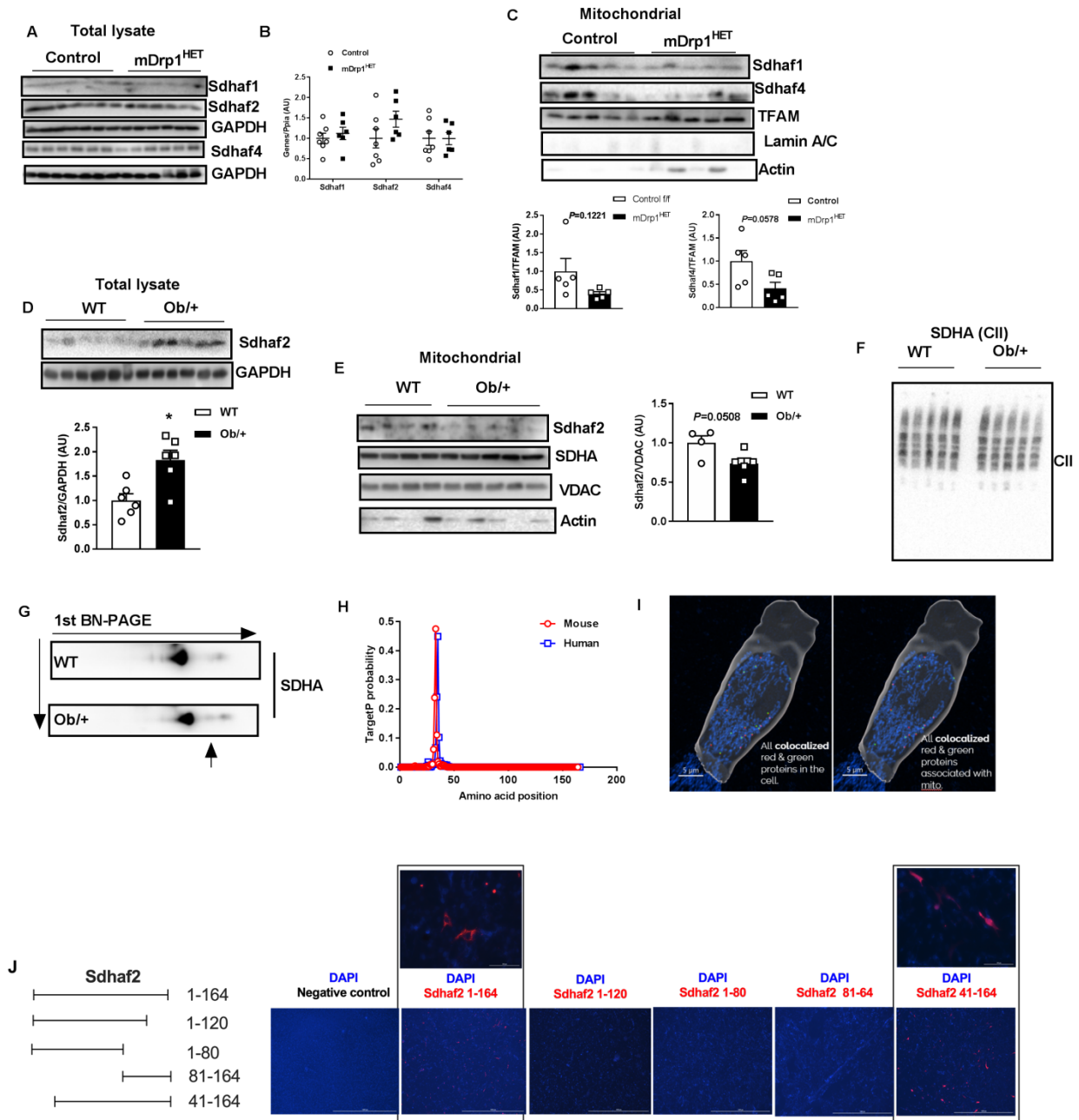


Fig. S6

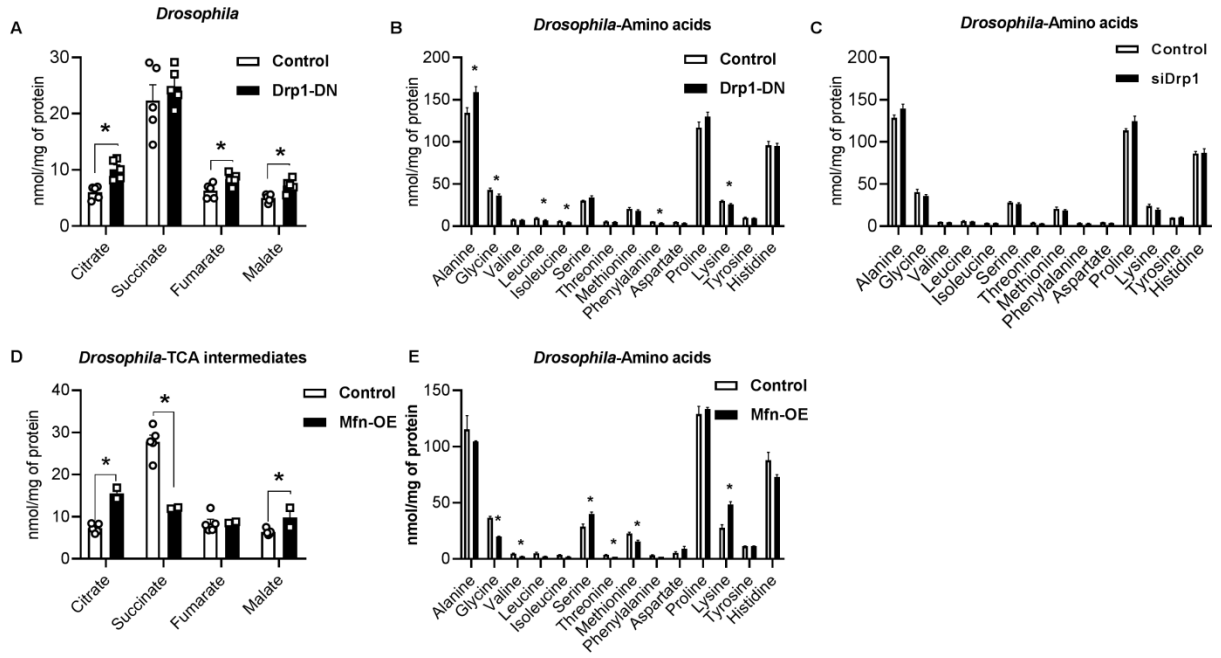


Fig. S7

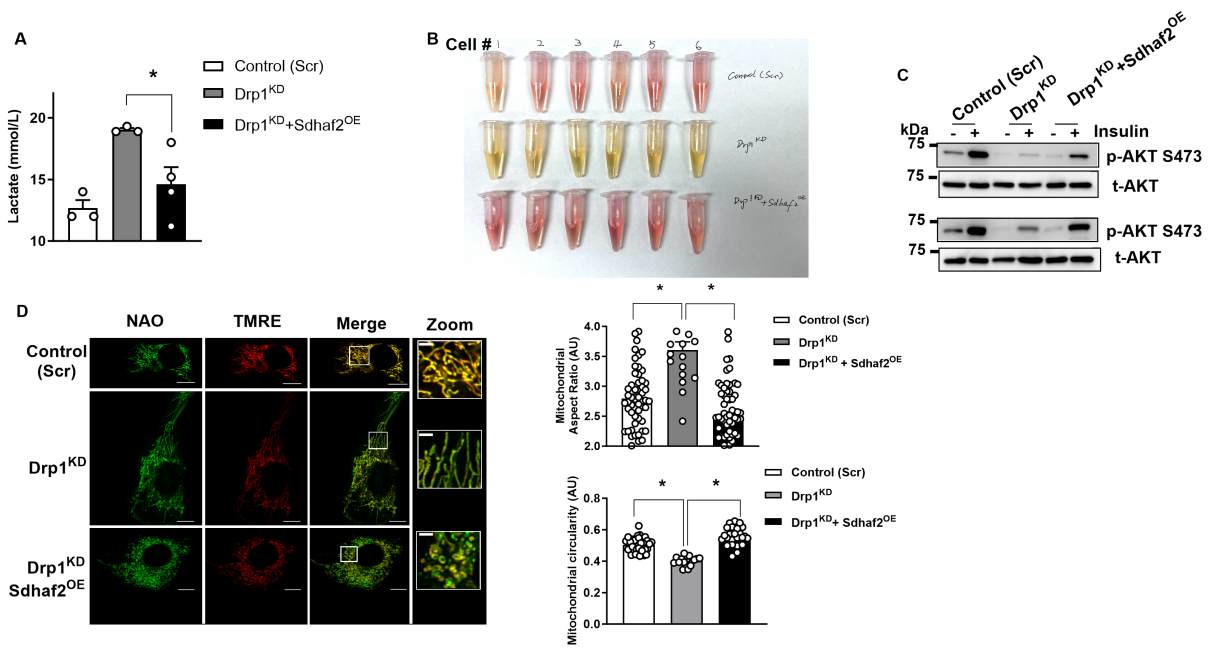


Table S1. Top 25 Genes Correlated with *DNM1L* in Human Muscle

Top 25 Genes Correlated with <i>DNM1L</i> in Human Muscle (P<0.01)	Bicor
HCCS	0.851
MRPS30	0.821
MTX2	0.821
HIGD1A	0.806
MRPL35	0.803
GHITM	0.798
TIMM17A	0.798
CYB5B	0.796
MRPL47	0.79
ERCC8	0.788
MRP510	0.787
VDAC2	0.786
ATP5B	0.783
FAM210A	0.782
SLC25A3	0.781
NCOA4	0.779
NDUFA5	0.778
PHB	0.777
IMMT	0.775
UBA5	0.775
GTPBP8	0.774

IMPA1	0.774
CYCS	0.773
ENOPH1	0.772
DLAT	0.771

Table S2. Antibody list.

Antibody	Company	Catalog Number
Drp1 S616	Cell Signaling	4494S
Drp1	abcam	ab56788
Drp1	Cell Signaling	8570S
Mfn2	Sigma	059K4767
Mfn2	abcam	ab56889
Opa1	BD Biosciences	612606
Gapdh	Cell Signaling	5174S
p-AMPK	Cell Signaling	2535S
t-AMPK	Cell Signaling	2532S
p-ACC (Ser79)	Cell Signaling	11818T
t-ACC	Cell Signaling	3676T
Atgl	Cell Signaling	2138S
Dgat1	Novus	NB100-57086
VDAC	Cell Signaling	4661S
SDHA	Cell Signaling	11998S
SDHA	NOVUS	NBP2-67130
t-AKT	Cell Signaling	9272
Cleaved caspase 3	Cell Signaling	9661S
Sdhaf2	Novus	NBP1-92259
TFAM	abcam	ab131607
Lamin A/C	Cell Signaling	4777T
Lamin A/C	abcam	ab133256
Actin	abcam	ab8227-50
GST	abcam	ab19256
p-AKT S473	Cell Signaling	9271S
SDHB	Santa Cruz Biotechnology	sc-271548
SDHB	proteintech	10620-I-AP
HSP60	Cell Signaling	12165T
NDUFS1 (CI)	Novus	NBP1-56520
UQCRC2 (CIII)	Santa Cruz Biotechnology	sc-390378
COXI (CIV)	Invitrogen	MA5-15078
Sdhaf1	invitrogen	PA5-34393
Sdhaf4	Invitrogen	PA5-56871
SDHC	Santa Cruz Biotechnology	sc-515102
SDHD	Santa Cruz Biotechnology	sc-293275
LC3B	Cell Signaling	3868P
LC3B	Cell Signaling	2775S
LC3B	NOVUS	NB100-2220

Table S3. Primer list.

Primer	Sequence-F	Sequence-R
18S	CGCCGCTAGAGGTGAAATTCT	CGAACCTCCGACTTTTCGTTCT
Dnm1l	CGTGGACTAGCTGCAGAATG	TGCCTCAGATCGTCGTAGTG
Ppia	AGCCAAATCCTTTCTCTCCAG	CACCGTGTTCTTCGACATCA
Acadl	GTAGCTTATGAATGTGTGCAACTC	GTCTTGCGATCAGCTCTTTCATTA
Acadm	GCAGCCAATGATGTGTGCTTAC	CACCCTTCTTCTCTGCTTTGGT
Acox1	GCCCAACTGTGACTTCCAT	GGCATGTAACCCGTAGCACT
Abca1	CTTCCCACATTTTTGCCTGG	AAGGTTCCGTCCTACCAAGTCC
Acs1l	ACCATCAGTGGTACCCGCTA	CTTCCAACCAACACCCTCAT
Fabp4	TTCGATGAAATCACCCGAGA	GGTCGACTTTCCATCCCCTT
Lpl	AGGACCCCTGAAGACAC	GGCACCCAACCTCTCATA
Sdha	TACTACAGCCCCAAGTCT	TGGACCCATCTTCTATGC
Sdhb	GTCTGTGCCCTCGACAG	TGACGTCAGGAGCCAAAAT
Sdhaf1	GAGTATCTGTATCGCCGGGG	CGCCCCTGTATCTTCACAAG
Sdhaf2	TGCTGTCTCCCTTGCCTAGT	ATCCCCCTCTTTCTGCTCTC
Sdhaf4	GTCTGGAGAGCAGCAAGACC	TGGGTCCACCTTTTTCTTTG

Table S4. Untargeted metabolomic analysis of muscle from mDrp1^{HET} vs. Control f/f mice.

Table S5. RNA-sequencing analysis of muscle from mDrp1^{HET} vs. Control f/f mice.

Fig. S1. Skeletal muscle Dnm1/Drp1 is associated with transcripts regulating mitochondrial inner membrane assembly and oxidative metabolism. (A) Mitochondrial fission and fusion gene expression in the quadriceps muscle of WT and Ob/+ mice (n=6 mice per genotype). (B) Insulin-stimulated AKT phosphorylation at Serine 473 in WT C2C12 myotubes administrated with vehicle vs. mitochondrial fusion promoter M1. The densitometry on the right represents the p-AKT/t-AKT ratio in cells subjected to 10 nM insulin treatment (n=3). All values are presented as mean±SEM; *= p<0.05 determined by Unpaired Student's t test two-tailed. AU=arbitrary units.

Fig. S2. Skeletal muscle Drp1 maintains glucose homeostasis and metabolic flexibility in male mice. (A) Drp1 protein levels in the quadriceps of Control f/f and mDrp1^{HET} mice (n=5-6 mice per genotype). (B) Enlarged and hyperfused mitochondria in soleus muscle of mDrp1^{HET} vs. Control f/f mice. (C) Elongated mitochondria in Drp1^{KD} vs. Control (Scr) myocytes stained with TMRM. (D) H&E staining of gastrocnemius muscles of Control f/f and mDrp1^{HET} mice. (E) Epididymal white adipose tissue (eWAT)/body weight (BW) ratio of NC and HFD-fed male Control f/f and mDrp1^{HET} mice (n=6-13 mice per genotype). Indirect calorimetry analysis of Control f/f and mDrp1^{HET} mice (n=6 mice per genotype), including (F) RER, (G) VO₂, (H) VCO₂, (I) EE, (J) activity, (K) food consumption, and (L) water. (M) Normalized EE with body weight as covariant of NC-fed male Control f/f and mDrp1^{HET} mice (n=5 mice per genotype per condition). All values are presented as mean±SEM; *= p<0.05 determined by unpaired Student's t test two-tailed. AU=arbitrary units.

Fig. S3. Skeletal muscle Drp1 deletion impairs fatty acid oxidation, leading to lipid accumulation. (A) Lipidomic analysis of ceramides and (B) cholesterol esters in Control f/f and mDrp1^{HET} mice (n=7-8 per genotype). (C) Oil Red O stained lipid in the differentiated Control (Scr) and Drp1^{KD} myotubes (n=4 biological replicates). (D) Metabolomic analysis of quadriceps muscles from mDrp1^{HET} vs. Control f/f mice (n=5 mice per genotype). (E) Fatty acid oxidation and lipid transport gene expression in Control (Scr) and Drp1^{KD} myocytes (n=3 biological replicates). (F) Immunoblot analysis of Atgl and Dgat1 in Control (Scr) and Drp1^{KD} myocytes (n=3 biological replicates). All values are presented as mean±SEM; *= p<0.05 determined by unpaired Student's t test two-tailed. AU=arbitrary units.

Fig. S4. Muscle Drp1 deletion reduces mitochondrial Complex II assembly and activity. (A) and (B) Integrated metabolomics (n=5 per genotype) and transcriptomics (n=3 per genotype) pathway analysis of Control f/f and mDrp1^{HET} mice. (C) TCA metabolite levels in skeletal muscle of NC-fed Control f/f and mDrp1^{HET} mice (n=4 per genotype, 5 months of age). (D) Oxygen consumption rate (OCR) of Control (Scr) and Drp1^{KD} myotubes (n=4-5 biological replicates). (E) Oxygen consumption rate (OCR) of mitochondria isolated from gastrocnemius muscles of NC-fed Control f/f and mDrp1^{HET} mice, (F) Control f/f and miDrp1^{KO} mice with the substrates of pyruvate+malate (n=5 per genotype). (G) Immunoblot of SDHA and SDHB in Control (Scr) and Drp1^{KD} myotubes (n=3 biological replicates). (H) mRNA expression and (I) protein levels of SDHA and SDHB in the quadriceps muscle Control f/f and mDrp1^{HET} mice (n=11-12 per genotype). (J) Immunoblot of SDHA and SDHB in the mitochondrial fractions of the gastrocnemius muscles of NC-fed male Control f/f and mDrp1^{HET} mice (n=6 per genotype, 5 months old). (K) Immunoblot of SDHA, SDHB, SDHC, and SDHD in Control (Scr) vs. Drp1^{KD} myocytes treated with vehicle or 100 mM CHX for 10 hours (n=3 biological replicates). (L) Representative Blue Native-PAGE gels showing Complex I subunit (NDUFS1), (M) Complex III (UQCRC2), and (N) Complex IV (mtCO1) in the isolated mitochondrial fraction of NC-fed male Control f/f and mDrp1^{HET} mice (n=5 per group). (O) Representative Blue Native-PAGE gels showing Complex I subunit (NDUFS1), (P) Complex III (UQCRC2), and (Q) Complex IV (mtCO1) in the isolated mitochondrial fraction of NC-fed male Control f/f and miDrp1^{KO} mice (n=5 per group). (R) Representative Blue Native-PAGE gels showing Complex I subunit NDUFS1 and (S) Complex III subunit UQCRC2, and (T) Complex IV subunit mtCO1 in the isolated mitochondrial fraction of Control (Scr) and Drp1^{KD} myotubes (n=3 biological replicates). (U) Immunoblot analysis of AKT phosphorylation at Serine 473, total AKT, SDHA in Control

(Scr) and SDHA^{KD} C2C12 myocytes ± 10 nM insulin for 15 minutes (n=3 biological replicates). All values are presented as mean±SEM; *= p<0.05 determined by unpaired Student's t test two-tailed. AU=arbitrary units.

Fig. S5. Drp1 interacts with Sdhaf2 and enhances its mitochondrial translocation. (A) Protein levels and (B) mRNA levels of Sdhaf1, Sdhaf2, and Sdhaf4 in the quadriceps muscles of Control f/f and mDrp1^{HET} mice (n=6 mice per genotype). (C) Immunoblot of Sdhaf1 and Sdhaf4 in the mitochondrial fraction of gastrocnemius muscles from Control f/f and mDrp1^{HET} mice (n=5 mice per genotype). Densitometric quantification analysis was performed by normalizing Sdhaf4 protein to TFAM. (D) Immunoblot of Sdhaf2 in total muscle lysate (n=6 mice per genotype) and (E) the mitochondrial fraction of quadriceps muscles of WT and Ob/+ mice (n=4-5 mice per genotype). (F) Blue Native-PAGE and (G) two-dimensional blue native/SDS gel electrophoresis analysis of Complex II assembly (blot SDHA) in the quadriceps muscles of WT and Ob/+ mice (n=5 mice per genotype). (H) Prediction of human and mouse Sdhaf2 mitochondrial localization signal by TargetP. (I) Confocal microscopic analysis of Drp1 and Sdhaf2 colocalization in WT C2C12 myoblasts by Imaris. (J) Proximity labeling assay of Drp1 with truncated Sdhaf2 in WT C2C12 myocytes. All values are presented as mean±SEM; *= p<0.05 determined by unpaired Student's t test two-tailed. AU=arbitrary units.

Fig. S6. Metabolic impacts of a dominant-negative Drp1 K38A in C2C12 myocytes and Drosophila. (A) TCA cycle intermediates and (B) amino acid levels in Control and Drp1-DN Drosophila (n=4-5). (C) Amino acid levels in Control and Drp1-RNAi Drosophila. (D) TCA cycle intermediates and (E) amino acid levels in Mfn overexpression (Mfn-OE) drosophila (n=4-5). All values are presented as mean±SEM; *= p<0.05 determined by unpaired Student's t test two-tailed. AU=arbitrary units.

Fig. S7. Sdhaf2 overexpression rescues impaired fatty acid oxidation and insulin action. (A) Lactate levels in the culture medium of Control (Scr), Drp1^{KD}, Drp1^{KD}+Sdhaf2^{OE} myocytes (n=3-4 biological replicates). (B) Picture of the culture medium of Control (Scr), Drp1^{KD}, Drp1^{KD}+Sdhaf2^{OE} myocytes (n=6 biological replicates). (C) Immunoblot of phospho-AKT S473 and total AKT in Control (Scr), Drp1^{KD}, Drp1^{KD}+Sdhaf2^{OE} myotubes with and without insulin treatment. (D) Mitochondrial morphology in Control (Scr), Drp1^{KD}, and Drp1^{KD}+Sdhaf2^{OE} myocytes. All values are presented as mean±SEM; *= p<0.05 determined by unpaired Student's t test two-tailed. AU=arbitrary units.