Supplementary Figures

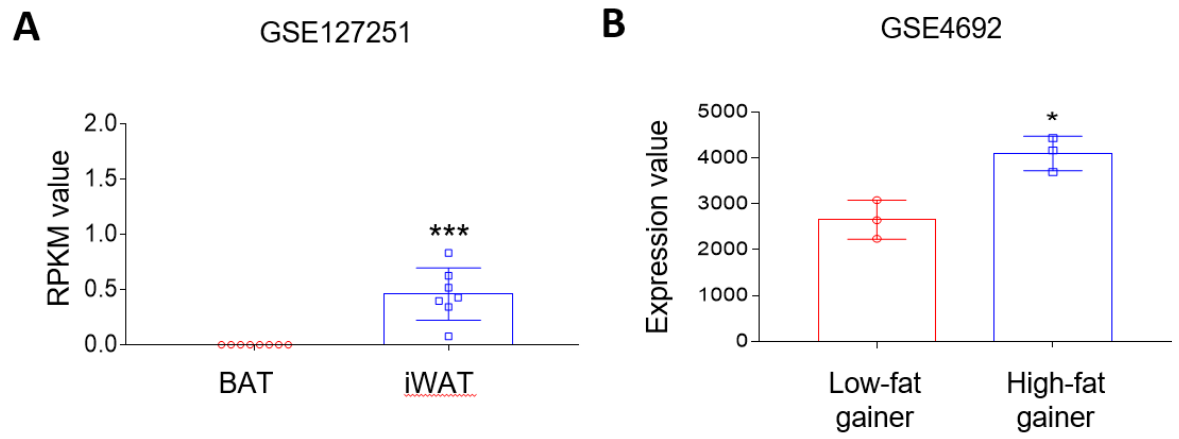


Figure S1. Tm4sf5 gene expression in adipose tissue.

A: *Tm4sf5* expression in brown adipose tissue (BAT) and inguinal white adipose tissue (iWAT) of mice, determined by publicly available transcriptomic analyses (n = 7-8 per group, Gene Expression Omnibus (GEO) repository, accession number GSE127251).

B: *Tm4sf5* expression in inguinal fat of mice characterized as low fat gainer or high fat gainer, determined by publicly available transcriptomic analyses (n = 3 per group, GEO accession number GSE4692).

Data represent the mean ± SEM (unpaired, two-tailed t-test; *P < 0.05, ***P < 0.001).

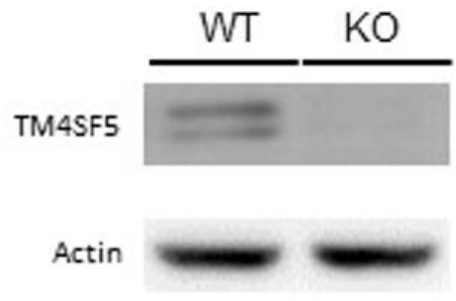


Figure S2. Immunoblot analysis of TM4SF5 in iWAT of wild-type (WT) and TM4SF5 KO mice.

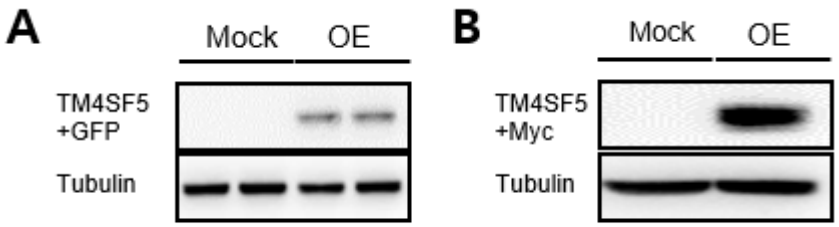


Figure S3. TM4SF5 overexpression in C3H10T1/2 adipocytes.

A: Immunoblot analysis of GFP in C3H10T1/2 adipocytes overexpressing GFP-fused TM4SF5 and mock-control.

B: Immunoblot analysis of Myc in C3H10T1/2 adipocytes overexpressing Myc-fused TM4SF5 and mock-control.

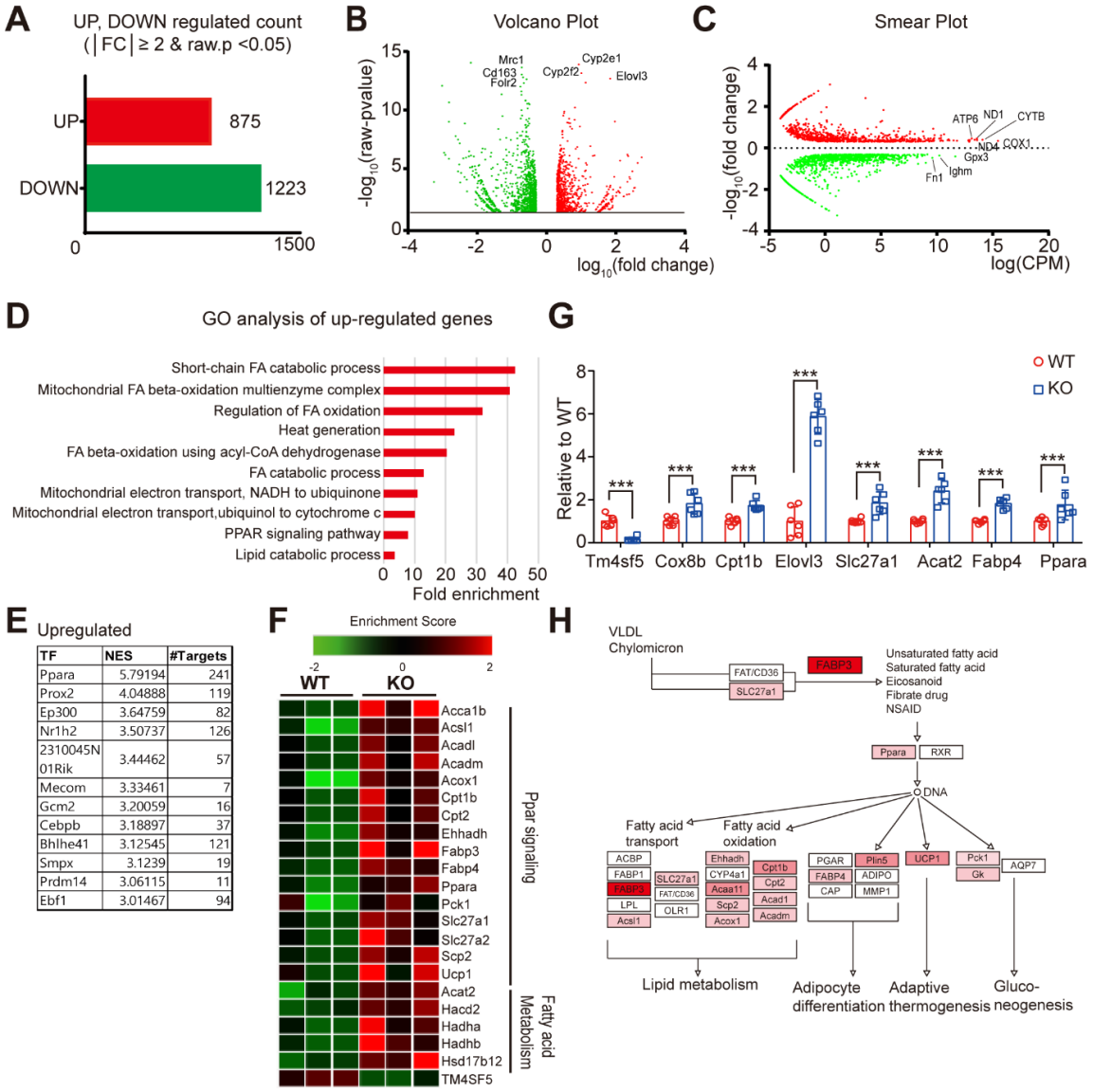


Figure S4. Transcriptomic characterization of iWAT of WT and TM4SF5 KO mice.

Total RNA in iWAT of WT and TM4SF5 mice (n = 3 per group) was analyzed.

A: The total number of differentially expressed genes in iWAT of TM4SF5 KO and WT mice identified by RNA sequencing (RNAseq) analysis (fold change (≥ 2) and p-value (< 0.05)).

B-C: Volcano plot (B) and smear plot (C) of differentially expressed genes in iWAT of TM4SF5 KO mice (fold change (≥ 2) and p-value (< 0.05)).

D: GO analysis of upregulated genes in iWAT of TM4SF5 KO mice.

E: Transcription factor analysis of differentially regulated genes in iWAT of TM4SF5 KO mice from RNAseq data.

F: Heatmap of the upregulated genes involved in PPARα signaling and fatty acid metabolism in iWAT of TM4SF5 KO mice.

G: qPCR analysis in iWAT of WT and TM4SF5 KO mice (n = 6 per group, mean ± SEM, ***P < 0.001; unpaired, two-tailed, t-test).

H: KEGG pathway analysis of genes significantly enriched in PPAR signaling pathways.

Upregulated DEGs from RNAseq are marked in red.

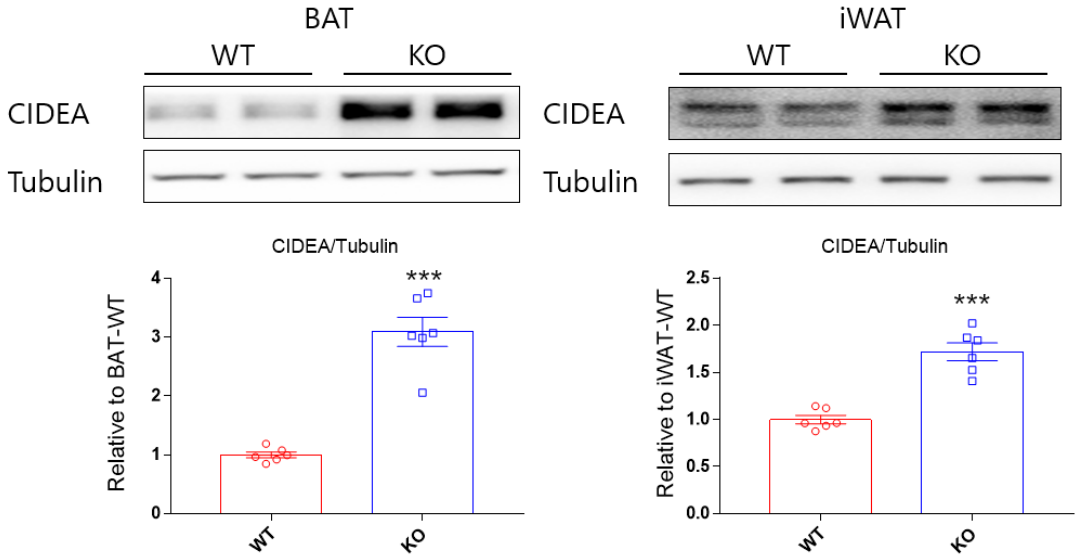


Figure S5. Immunoblot analysis of CIDEA in BAT and iWAT of wild-type (WT) and TM4SF5 KO mice.

Data represent the mean ± SEM of 6 mice per group (unpaired, two-tailed t-test: ***P < 0.001).

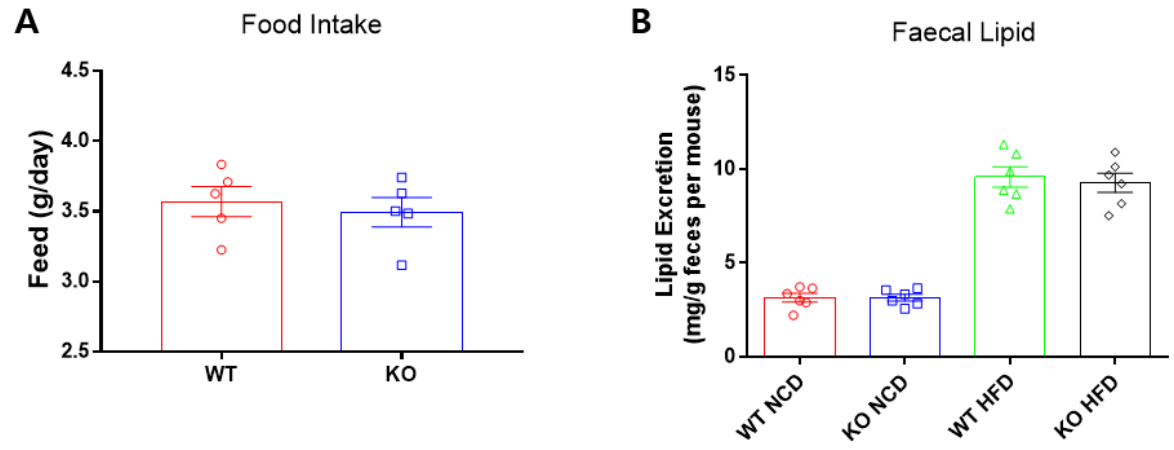


Figure S6. TM4SF5 KO does not affect food intake and faecal lipid contents.

A: Food intake of wild-type (WT) and TM4SF5 KO mice.

B: Lipid content in feces of WT and TM4SF5 KO mice after feeding NCD and HFD.

Data represent the mean ± SEM of 5-6 mice per group.

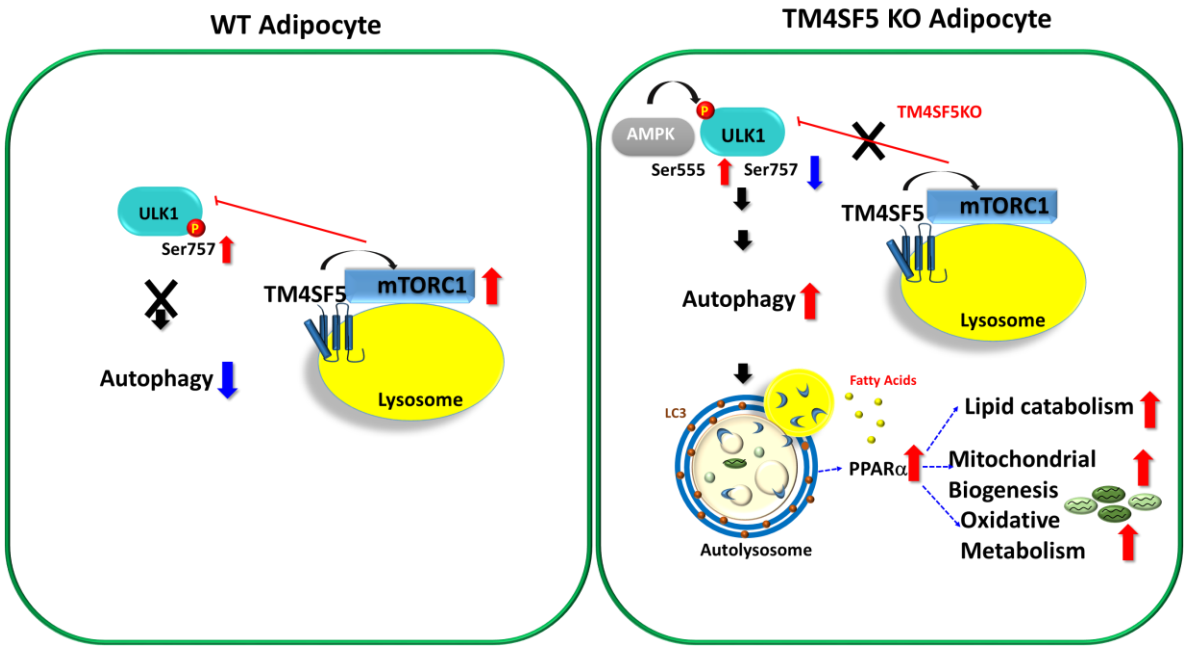


Figure S7. Graphical abstract: effects of TM4SF5 KO on adipocyte metabolism.

TM4SF5 activates mTORC1 signaling in WT adipocytes. Genetic deletion of TM4SF5 (TM4SF5 KO) suppresses mTORC1 activity and initiates autophagy. TM4SF5 KO improves lipid metabolism, mitochondrial biogenesis, and mitochondrial oxidative metabolism partly through PPARα signaling pathway.

Supplementary Tables

Table S1. Primers used for genotyping

Amplicon size	Forward (5′→3′)	Reverse (5′→3′)
WT: 279 bp KO: 250bp	CCAAGCCTCCCACCTGTTTA	GCTCCAGCATTCTCACCATC

Table S2. Antibodies used for western blots (WB) and immunofluorescence (IF)

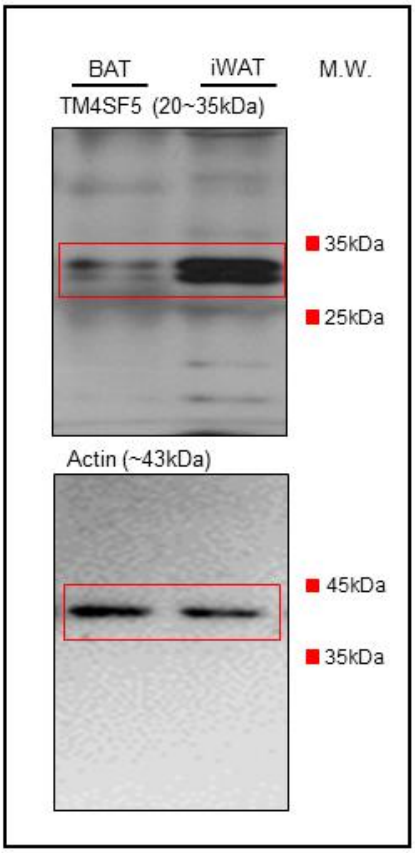
Antibody	Host	Company	Catalog #	Dilution
Actin	Mouse	Santa Cruz	sc-47778	1:1000 (WB)
LC3	Rabbit	Cell signaling	12741	1:1000 (WB) 1:200 (IF)
PLIN1	Goat	Santa Cruz	sc-47319	1:100 (IF)
MCAD	Mouse	Santa Cruz	sc-365108	1:100 (IF)
p62/SQSTM1	Rabbit	Cell signaling	5114	1:1000 (WB)
HSL	Rabbit	Cell signaling	4107	1:1000 (WB)
Phospho-HSL (Ser660)	Rabbit	Cell signaling	4126	1:1000 (WB)
Total oxphos rodent WB antibody cocktail	Mouse	Abcam	110413	1:1000 (WB)
UCP1	Rabbit	Alpha diagnostics	UCP11-A	1:2000 (WB)
Tubulin	Rabbit	Cell signaling	2148	1:1000 (WB)
F4/80	Rabbit	Cell signaling	30325	1:1000 (WB)
Phospho-mTOR (Ser2481)	Rabbit	Cell signaling	2974	1:1000 (WB)
mTOR	Rabbit	Cell signaling	2983	1:1000 (WB)
Phospho-ULK1 (Ser555)	Rabbit	Cell signaling	5869	1:1000 (WB)
Phospho-ULK1 (Ser757)	Rabbit	Cell signaling	14202	1:1000 (WB)
COXIV	Rabbit	Cell signaling	4850	1:1000 (WB)
ATGL	Rabbit	Cell signaling	2138	1:1000 (WB)
GFP	Goat	GeneTex	GTX26673	1:1000 (WB)
Phospho-AMPK (Thr172)	Rabbit	Cell signaling	2531	1:1000 (WB)
AMPK	Rabbit	Cell signaling	2532	1:1000 (WB)
Phospho-IRS1 (Ser612)	Rabbit	Cell signaling	2386	1:1000 (WB)
IRS1	Rabbit	Cell signaling	2382	1:1000 (WB)
Phospho-IRS2 (Ser388)	Rabbit	Sigma-Aldrich	07-1517	1:1000 (WB)
IRS2	Rabbit	Cell signaling	3089	1:1000 (WB)
Phospho-AKT (Ser473)	Mouse	Santa Cruz	sc-514032	1:1000 (WB)
AKT	Mouse	Santa Cruz	sc-81434	1:1000 (WB)
PPARα	Mouse	Santa Cruz	sc-398394	1:1000 (WB)
CIDEA	Rabbit	Novus Biologicals	NBP1-76950	1:1000 (WB)
TM4SF5 (Customized)	Rabbit	GeneScript	6440	1:10000 (WB)
Goat anti-rabbit IgG, HRP	Goat	Invitrogen	31460	1:3000 (WB)
Goat anti-mouse IgG, HRP	Goat	Jackson	115-035-174	1:1000 (WB)
Mouse anti-goat IgG, HRP	Mouse	Santa Cruz	sc-2354	1:1000 (WB)
Donkey anti-Rabbit IgG, Alexa Fluor 488	Donkey	Invitrogen	A-21206	1:500 (IF)
Donkey anti-Goat IgG, Alexa Fluor 594	Donkey	Invitrogen	A-11058	1:500 (IF)

Table S3. Primers used for qPCR

Genes	Forward (5′→3′)	Reverse (5′→3′)
Tm4sf5	TGTGTACTGGAAAGGTGCG	CCAAACTTGCAAGCTGAGGT
Ppia	GTGGTCTTTGGGAAGGTGAA	TTACAGGACATTGCGAGCAG
Cox8b	TGCGAAGTTCACAGTGGTTC	TCAGGGATGTGCAACTTCA
Cpt1b	ATGCTCCGAGGCATTTGTCA	GGTCAGCTGGCCATGGTATT
Elovl3	ACCTACATGAGAACGCGGAA	GTAGATGGCAAAGCACACGG
Slc27a1	TGCCACAGATCGGCGAGTTCTA	AGTGGCTCCATCGTGTCCTCA
Acat2	GAGATTGTGCCAGTGCTGGTGT	GTGACAGTTCCTGTCCCATCAG
Fabp4	TGGGGATTTGGTCACCATCCGGT	GGGCCCCGCCATCTAGGGTT
Ppara	GGAGGCAGCCGCTTACG	AACTTCAACTTGGCTGAGGCT

Full-length blots for figures

Figure 1B.



M.W. (Molecular weight)

Figure 2A. BAT

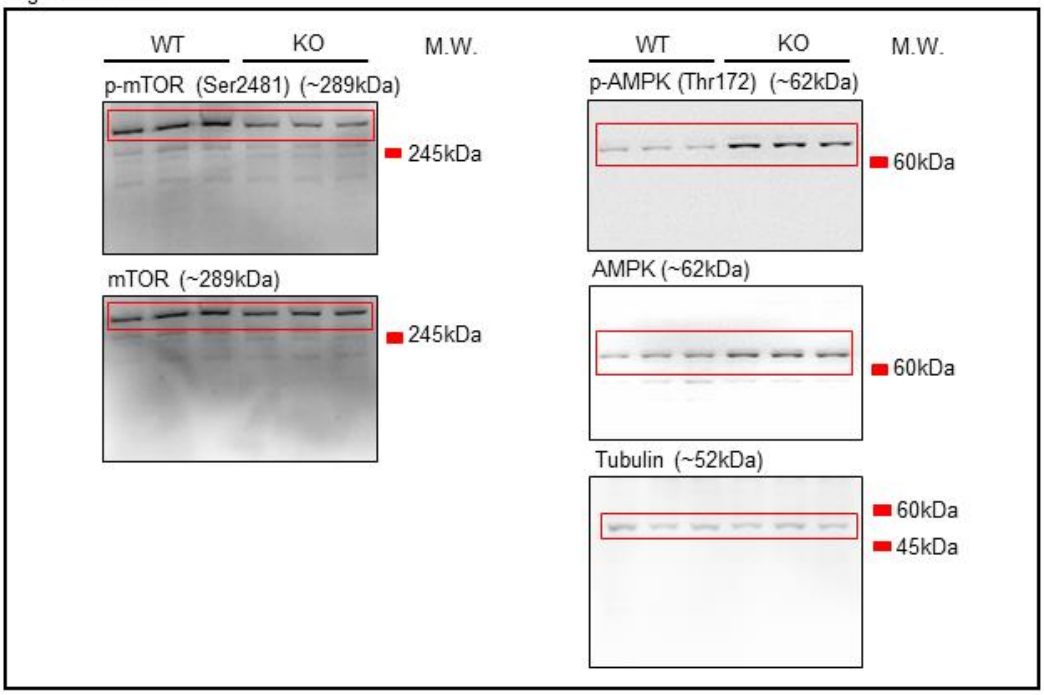
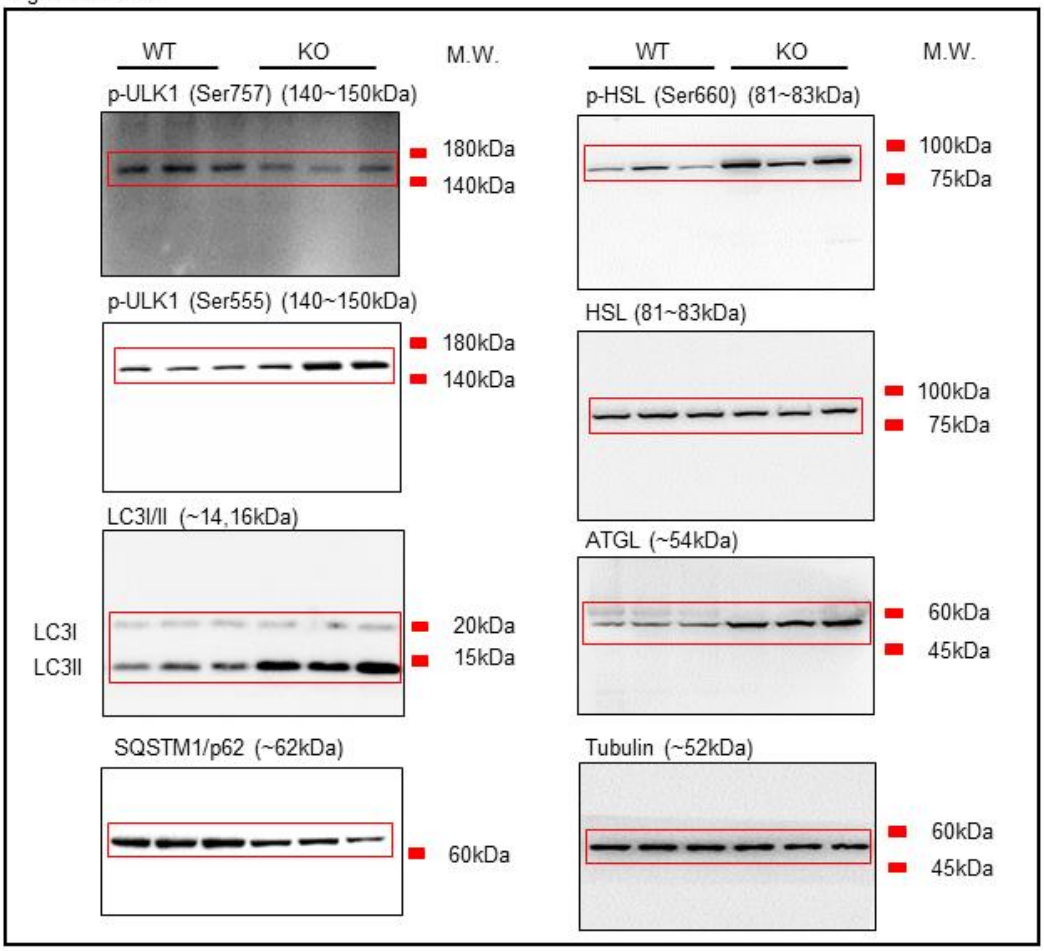


Figure 2B. BAT



M.W. (Molecular weight)

Figure 2A.

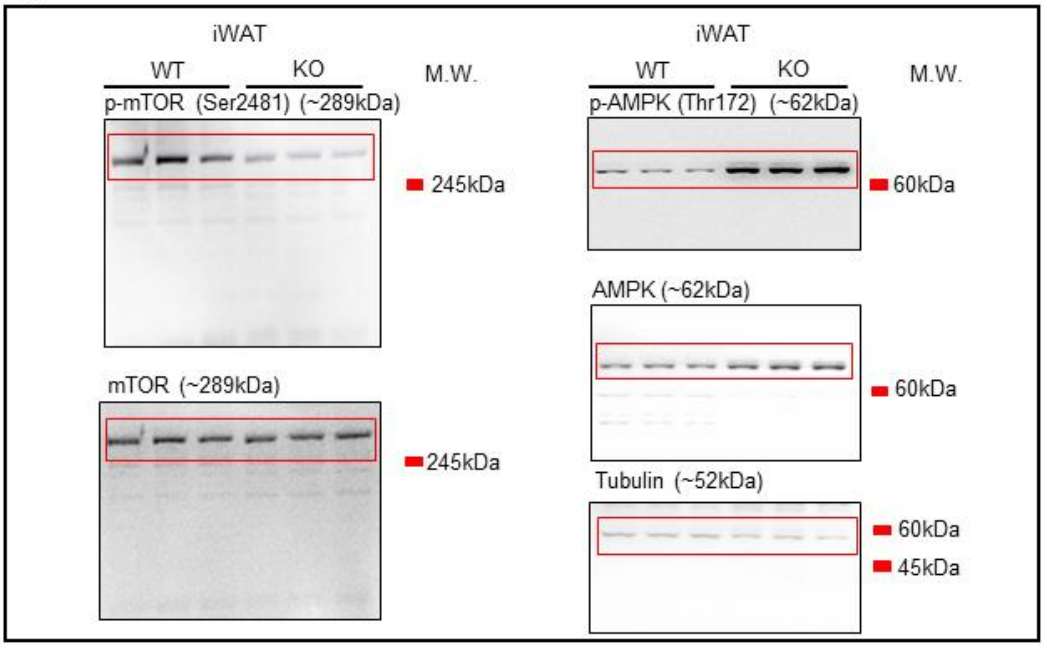
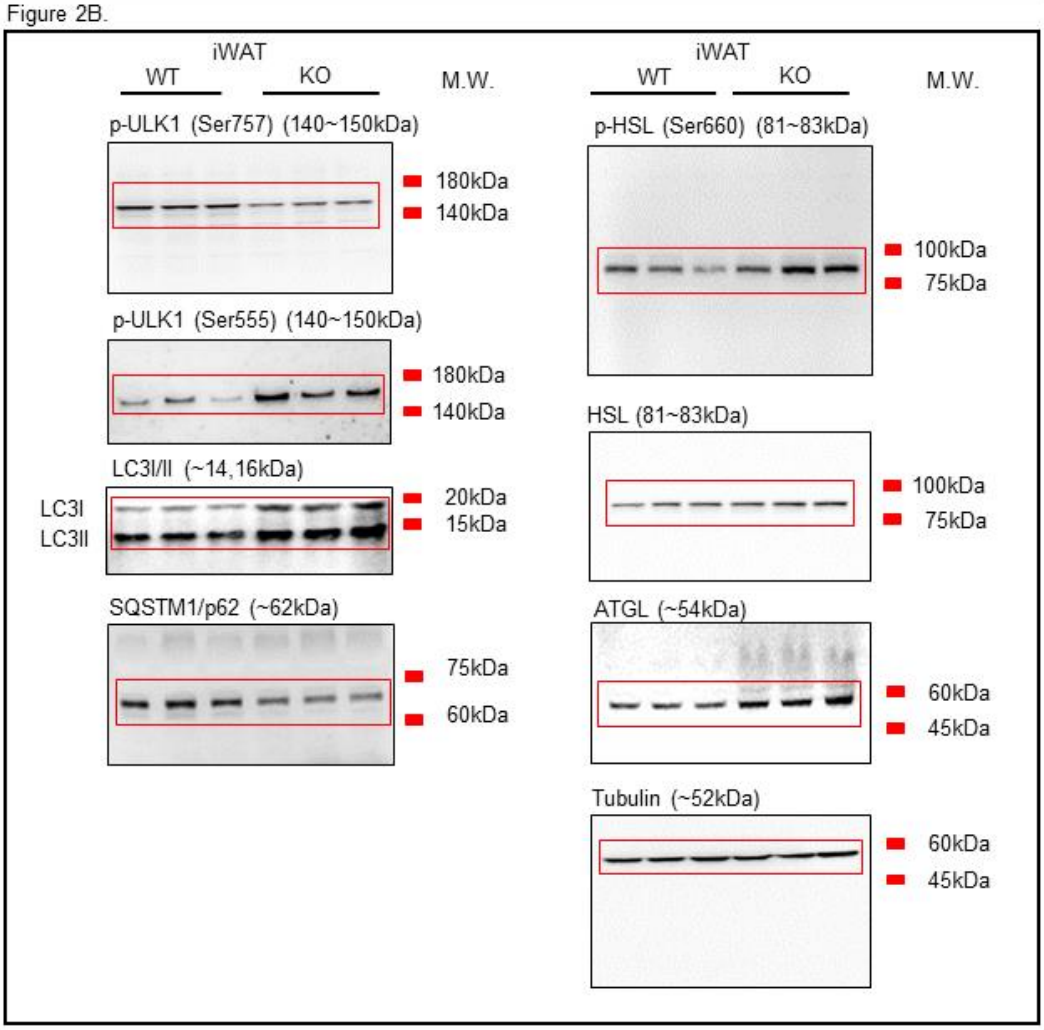


Figure 2B.



M.W. (Molecular weight)

Figure 3C.

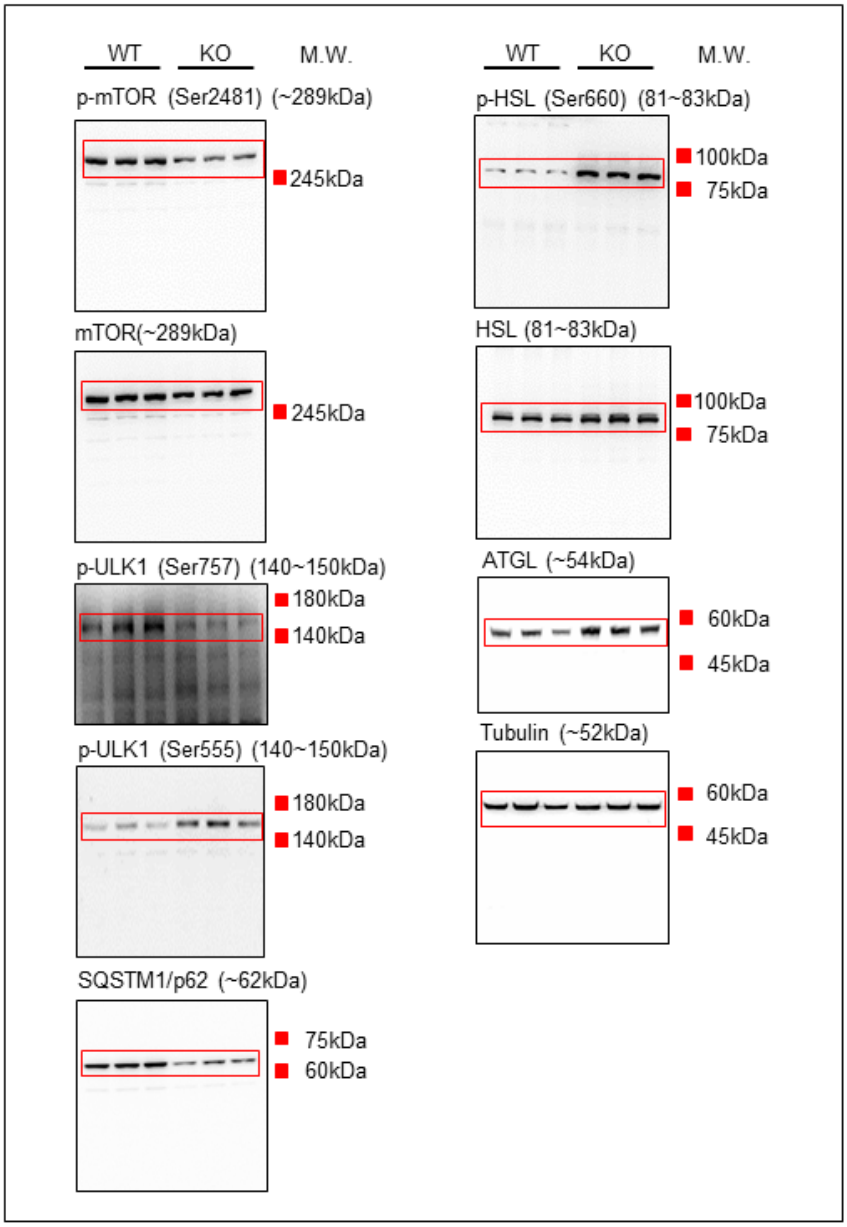
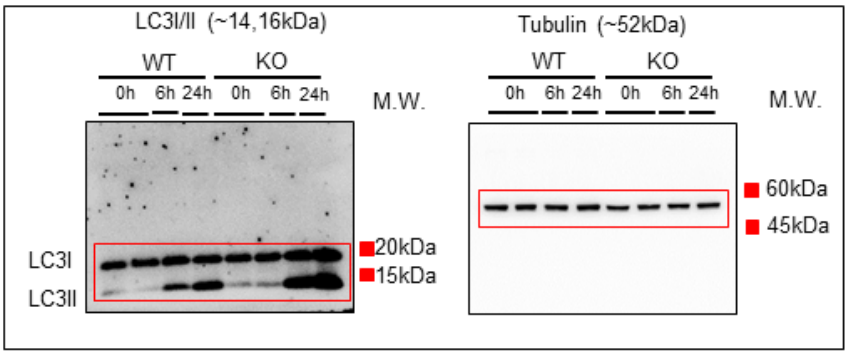
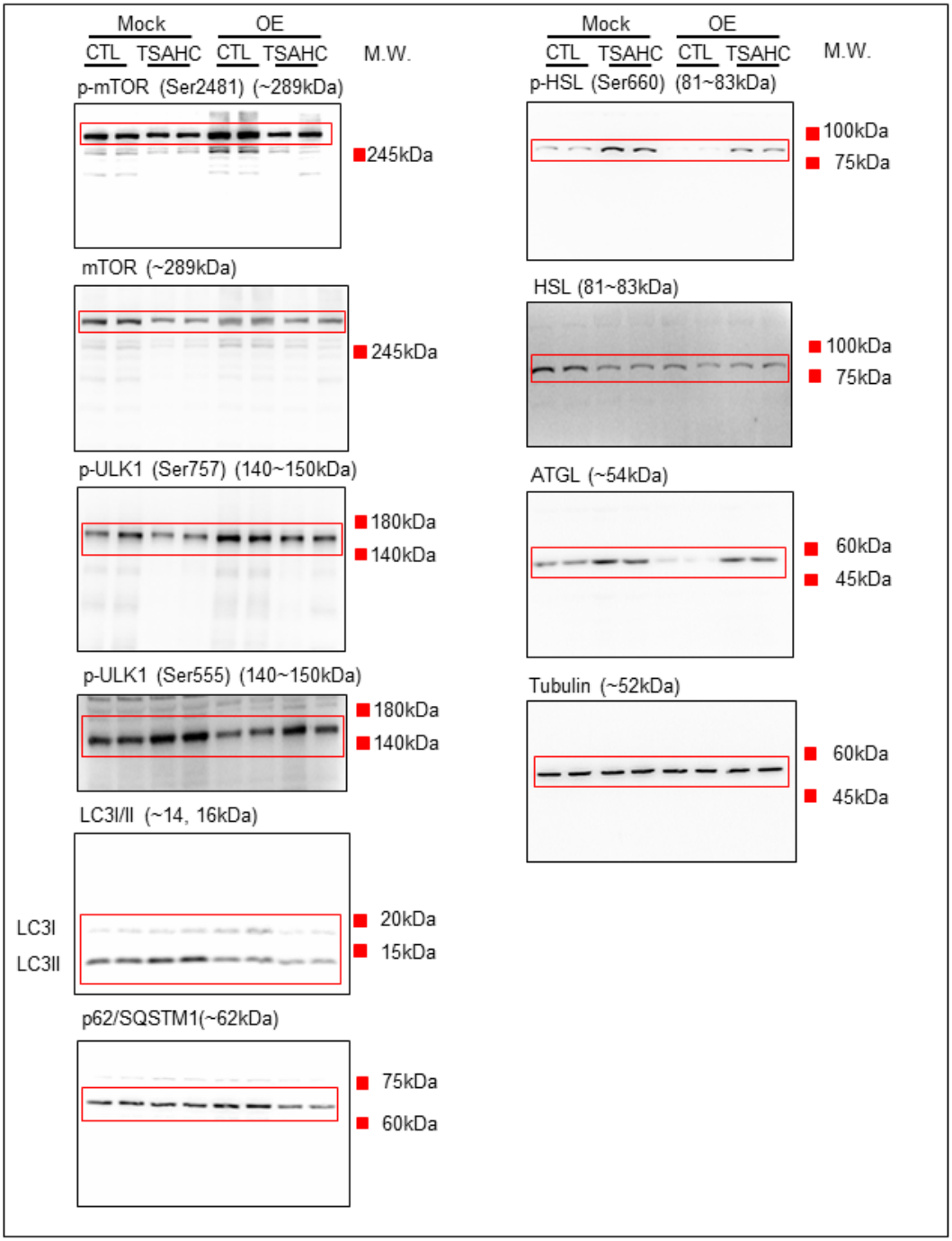


Figure 3D.



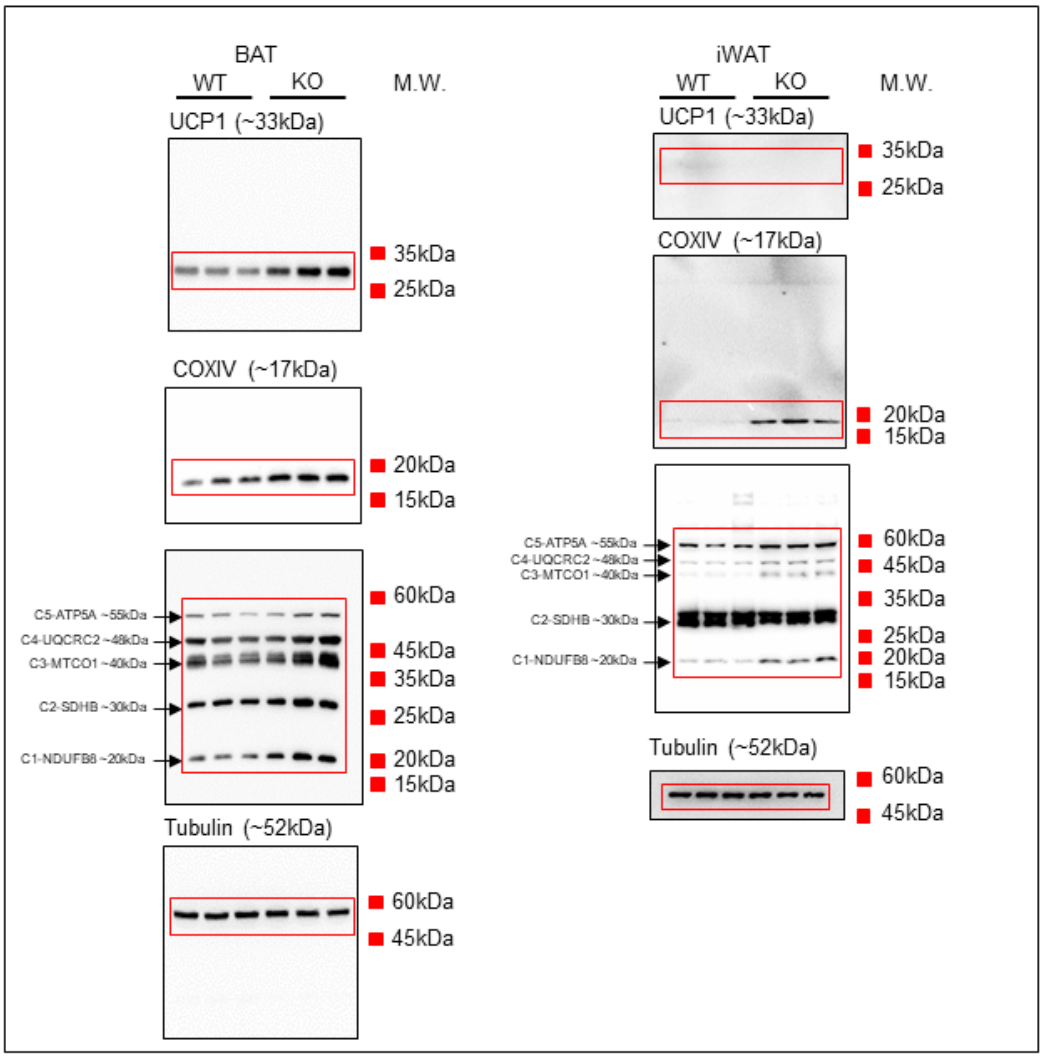
M.W. (Molecular weight)

Figure 4C.



M.W. (Molecular weight)

Figure 5A.



M.W. (Molecular weight)

Figure 6A.

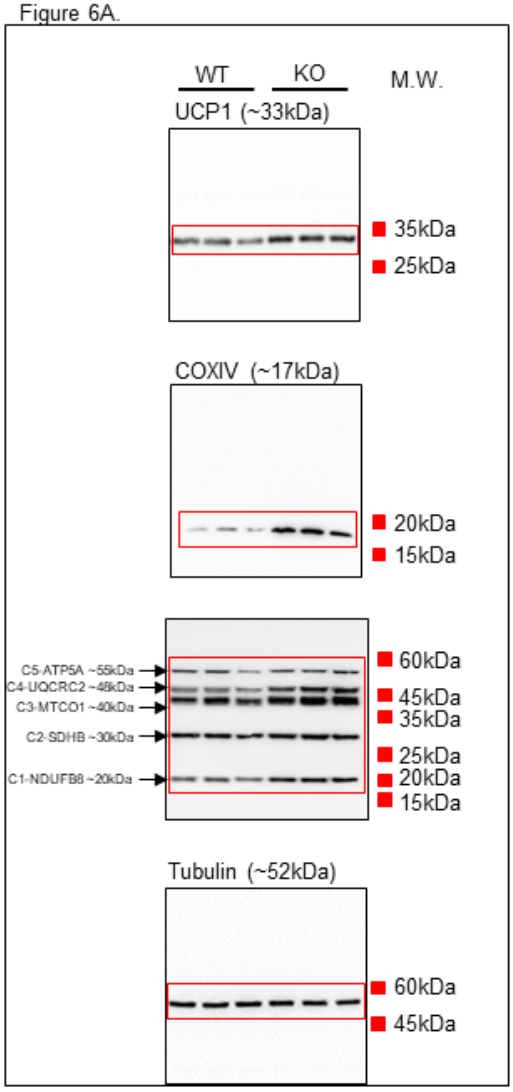
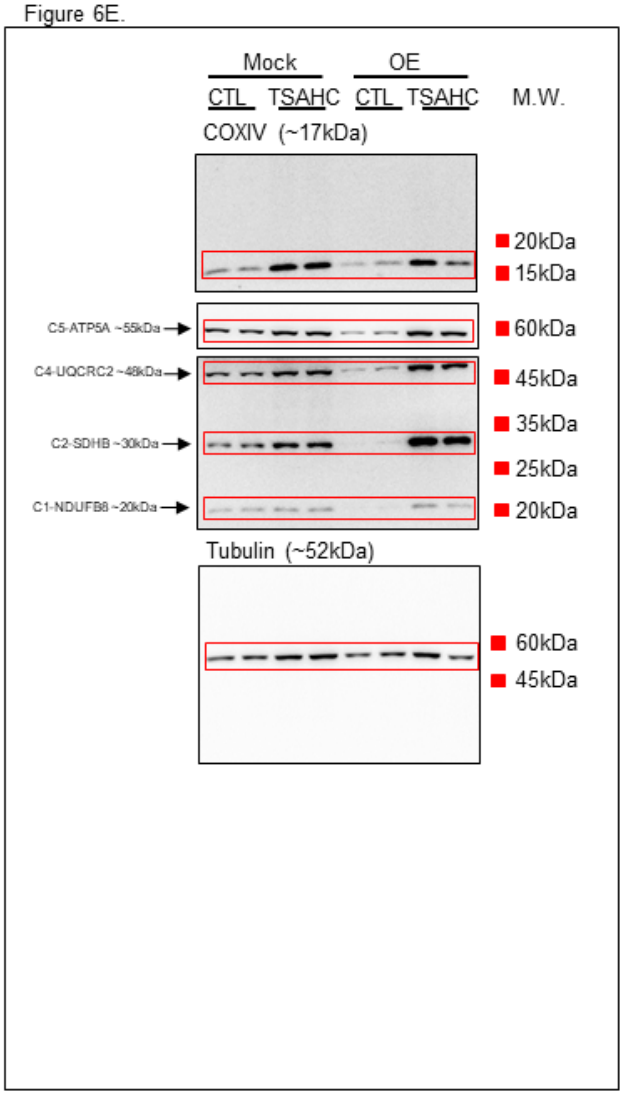


Figure 6E.



M.W. (Molecular weight)

Figure 7B.

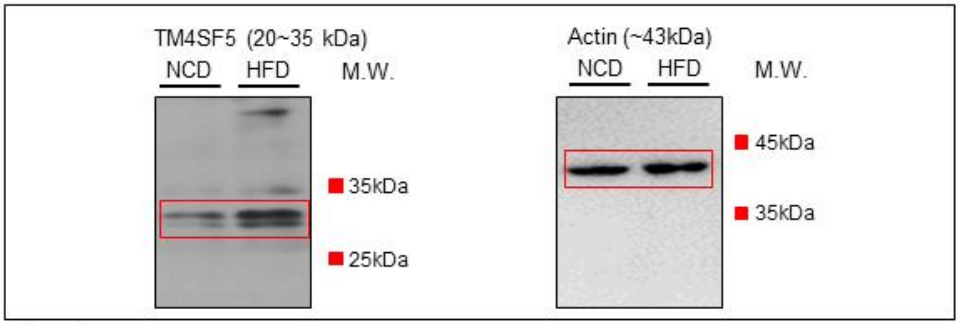
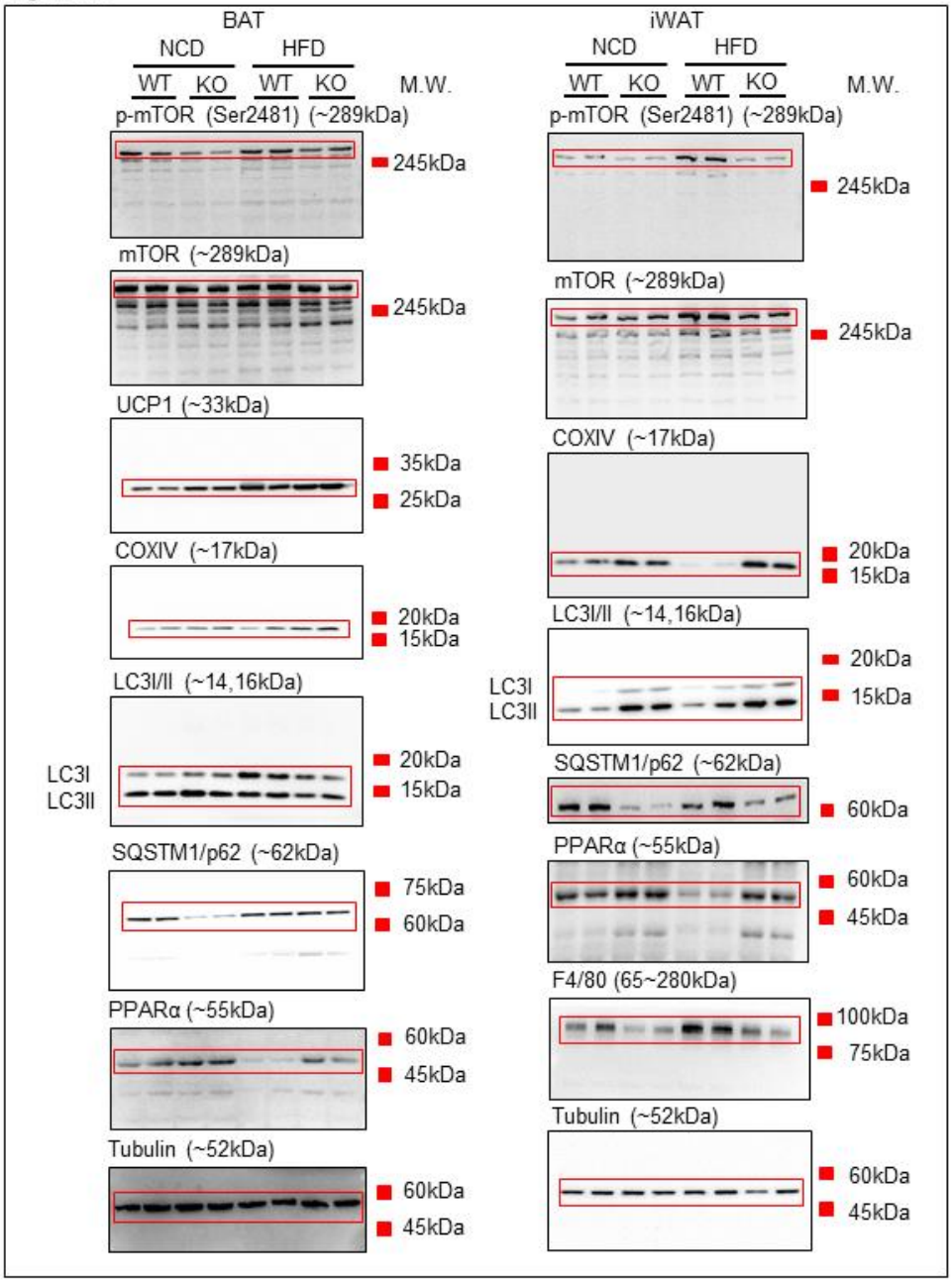
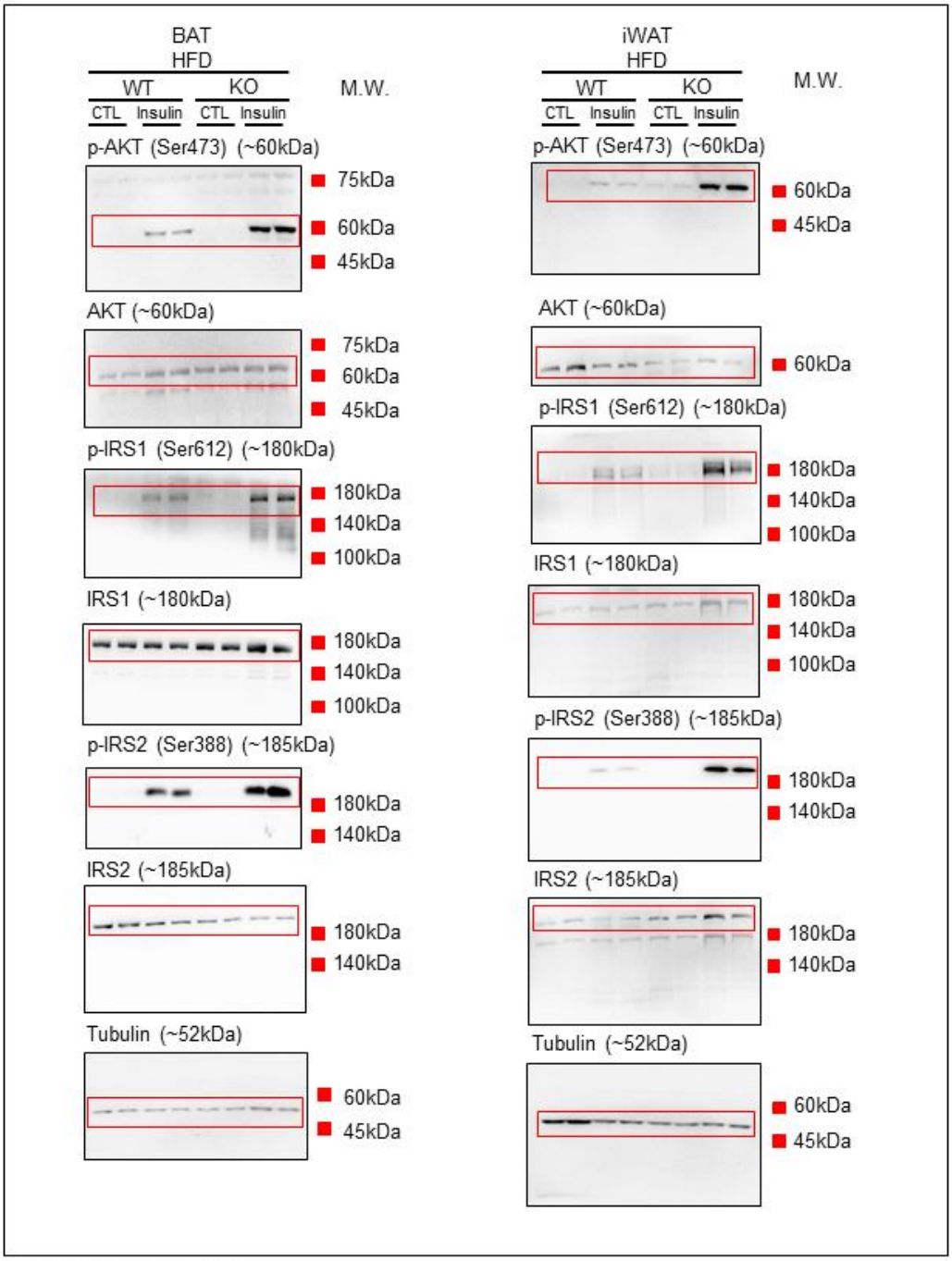


Figure 7F.



M.W. (Molecular weight)

Figure 8E.



M.W. (Molecular weight)