Figure S1. *Grp75* expression is unchanged in muscle tissues of insulin resistance mice.

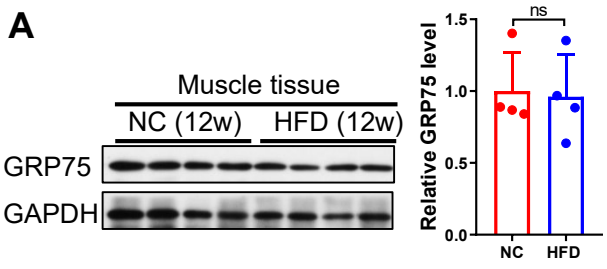


Figure S2. Transfection efficiency validation in 3T3-L1 and AML12 cells

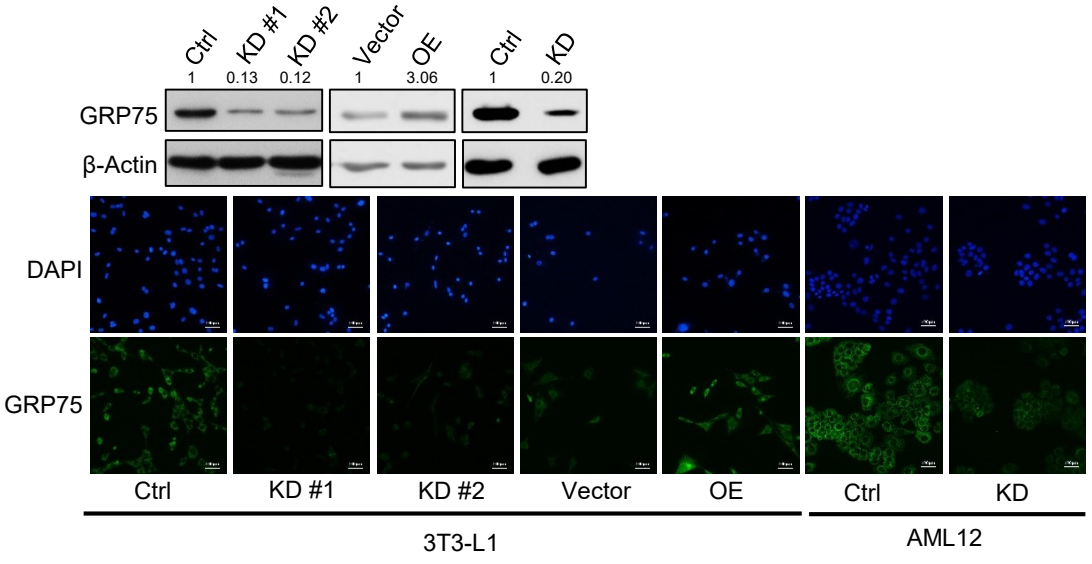


Figure S3. *Grp75* did not affect the differentiation of 3T3-L1 cells.

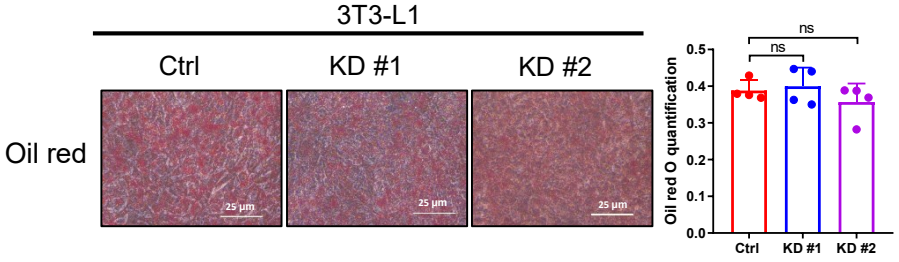
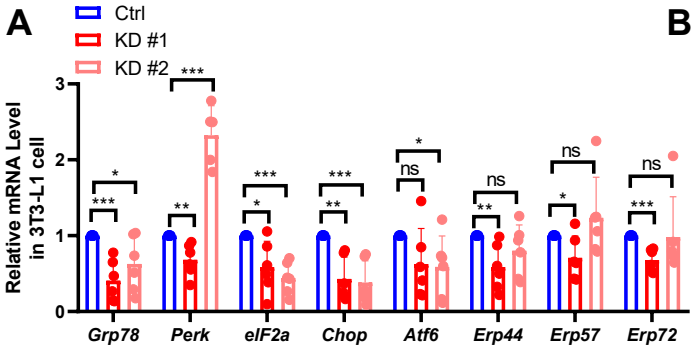
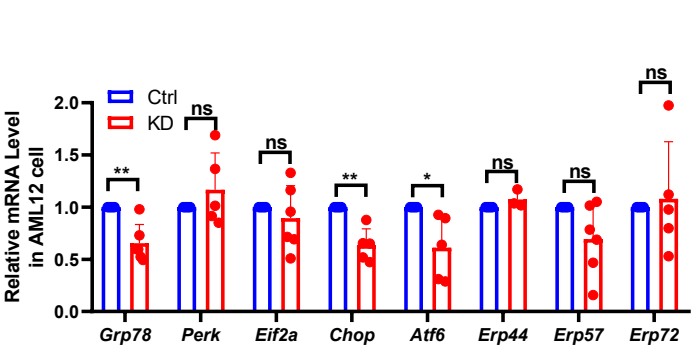


Figure S4. Related to Figure 2.





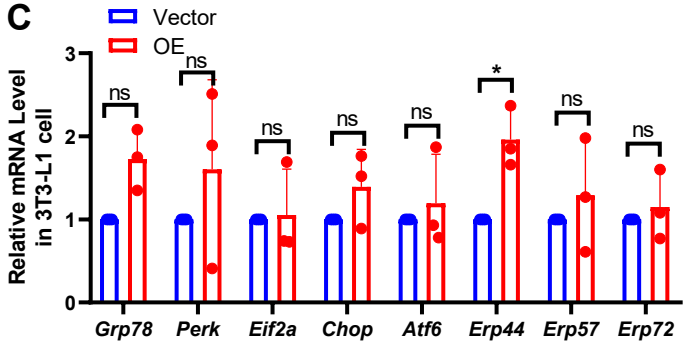


Figure S5.

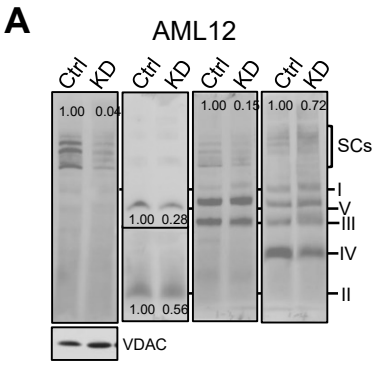

A
AML12
Ctrl
KD
Ctrl
KD
Ctrl
KD
Ctrl
KD
1.00
0.04
1.00
0.28
1.00
0.56
1.00
0.15
1.00
0.72
SCs
I
V
III
IV
II
VDAC

Figure S6.

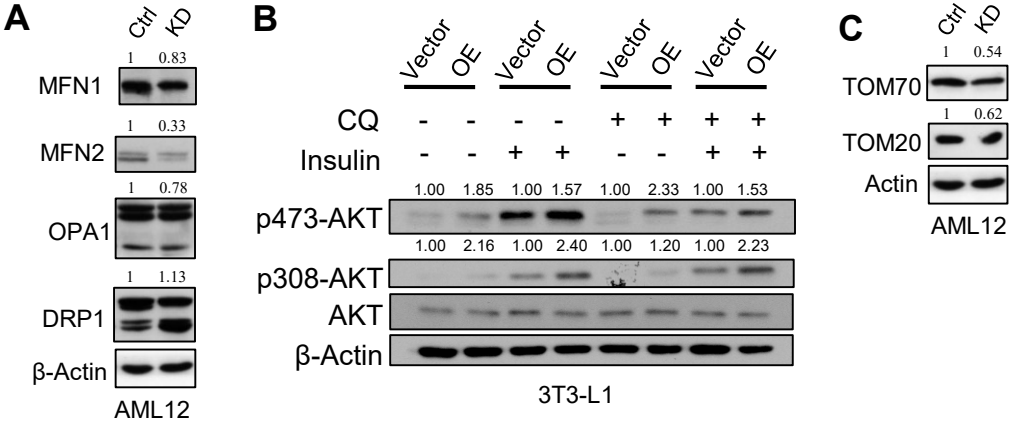

A
Ctrl
KD
1
0.83
MFN1
1
0.33
MFN2
1
0.78
OPA1
1
1.13
DRP1
β-Actin
AML12
B
Vector
OE
Vector
OE
Vector
OE
Vector
OE
CQ
Insulin
1.00 1.85 1.00 1.57 1.00 2.33 1.00 1.53
p473-AKT
1.00 2.16 1.00 2.40 1.00 1.20 1.00 2.23
p308-AKT
AKT
β-Actin
3T3-L1
C
Ctrl
KD
1
0.54
TOM70
1
0.62
TOM20
Actin
AML12

Figure S7

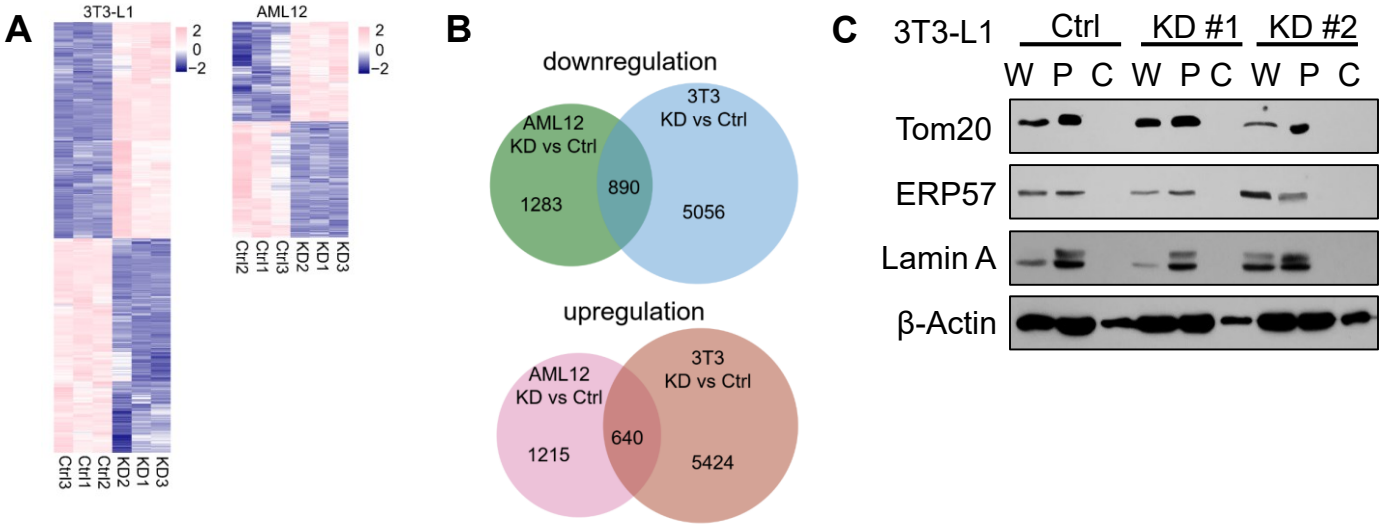

A
3T3-L1
AML12
2
0
−2
Ctrl3
Ctrl1
Ctrl2
KD2
KD1
KD3
B
downregulation
AML12
KD vs Ctrl
1283
890
3T3
KD vs Ctrl
5056
upregulation
AML12
KD vs Ctrl
1215
640
3T3
KD vs Ctrl
5424
C
3T3-L1
Ctrl
KD #1
KD #2
W P C W P C W P C
Tom20
ERP57
Lamin A
β-Actin

Figure S8

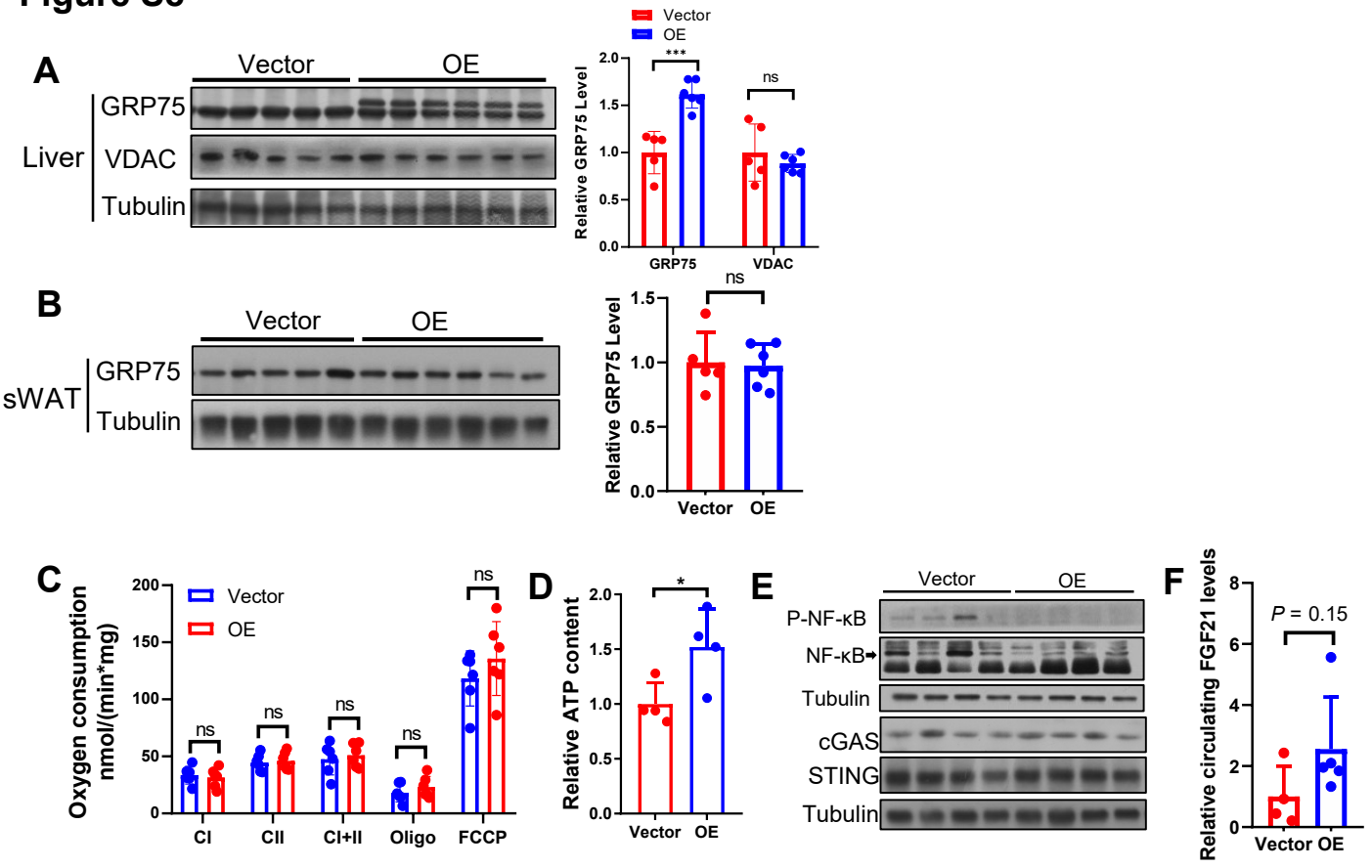

A
Vector
OE
Liver
GRP75
VDAC
Tubulin
Relative GRP75 Level
***
ns
GRP75
VDAC
B
sWAT
GRP75
Tubulin
ns
Vector
OE
C
Oxygen consumption nmol/(min*mg)
CI
CII
CI+II
Oligo
FCCP
D
Relative ATP content
*
E
P-NF-κB
NF-κB
Tubulin
cGAS
STING
F
Relative circulating FGF21 levels
P = 0.15

Figure S1. GRP75 expression is unchanged in muscle tissues of insulin resistance mice.
Representative western blotting of GRP75 in muscle from C57BL/6J mice fed NC (n = 4) or HFD (n = 4) for 12 weeks.

Figure S2. Transfection efficiency validation in 3T3-L1 and AML12 cells.
Western blotting and fluorescence microscopy were used to verify the transfection efficiency in *Grp75*-KD 3T3-L1 or AML12 cells and *Grp75*-OE 3T3-L1 cells.

Figure S3. GRP75 did not affect the differentiation of 3T3-L1 cells.
Oil red O staining in differentiated *Grp75*-KD and control 3T3-L1 cell.

Figure S4. Measurement of the ER stress markers in 3T3-L1 and AML12 cells.
(A-C) Relative mRNA levels of the ER stress markers in *Grp75*-KD 3T3-L1 or AML12 cells and *Grp75*-OE 3T3-L1 cells.

Figure S5. Mitochondrial complex content in *Grp75*-KD and control AML12 cells
BN-PAGE/immunoblotting of digitonin-solubilized cell lysates from *Grp*75-KD and control AML12 cells. Complexes I–V were probed with antibodies against Grim19, SDHA, UQCRC2, COXI, and ATP5a, respectively. VDAC was the internal control.

Figure S6. Related to Figure 4
(A) Representative western blotting showing relative amounts of DRP1, OPA1, MFN1/2 in *Grp75*-KD and control AML12 whole-cell extracts. Actin was the internal control.
(B) Representative western blotting showing relative amounts of phospho-Akt Ser473/Thr308 in *Grp75*-OE 3T3-L1 cell treated with or without 5 μM chloroquine for 24h. Actin was the internal control.
(C) Representative western blotting showing relative amounts of Tomm20 and Tomm70 in *Grp75*-KD and control AML12 whole-cell extracts.

Figure S7. Related to Figure 6
(A) Heatmap showed differentially expression genes in *Grp75*-KD and control 3T3-L1 and AML12 cells.
(B) Venn diagram showed the common downregulated and upregulated genes in *Grp75*-KD and control 3T3-L1 and AML12 cells.
(C) Western blots to detect Tom20, ERP57, Lamin A, and Actin proteins using specific antibodies for each protein. Differential centrifugation was used to isolate the precipitate fraction (P), and cytosolic supernatant fraction (C); the whole cell lysate (W) was used for comparison.

Figure S8. Related to Figure 7
(A) Representative western blotting and quantity results of GRP75 and VADC in the liver tissue of Vector (n=5) and *Grp75* overexpressed (n=6) mice. Tubulin was the

internal control.
(B) Representative western blotting and quantity results of GRP75 in the sWAT of Vector (n=5) and *Grp75* overexpressed (n=6) mice. Tubulin was the internal control.
(C) Rates of oxygen fluxes in the liver of Vector (n=6) and *Grp75* overexpressed (n=6) mice. CI: respiration related to complex I activity, measured in the presence of glutamate and malate; CII: respiration related to complex II activity, measured in the presence of succinate; CI + II: respiration related to combined complex I and II activity, measured in the presence of glutamate, malate, and succinate; Oligo: uncoupled mitochondrial respiration, measured after adding oligomycin; FCCP: maximum oxygen consumption, measured after adding FCCP.
(D) Relative ATP content in Vector (n=6) and *Grp75* overexpressed (n=7) mice.
(E) Representative western blotting showing relative amounts of phospho-NF-κB (phospho-NF-κB/NF-κB), cGAS and STING in Vector (n=4) and *Grp75* overexpressed (n=4) mice. Tubulin was the internal control.
(F) Relative serum FGF21 levels in Vector (n=4) and *Grp75* overexpressed (n=4) mice.

Regulation	Description	P value	P.adjust	Count
up	Cell cycle	2.47E-11	6.39E-09	22
up	Progesterone-mediated oocyte maturation	2.23E-05	0.002888	12
up	IL-17 signaling pathway	0.000122	0.010533	11
up	TNF signaling pathway	0.000212	0.013713	12
up	Colorectal cancer	0.000412	0.021329	10
up	Mismatch repair	0.0005	0.021584	5
up	Small cell lung cancer	0.000589	0.021801	10
up	T cell receptor signaling pathway	0.001425	0.040646	10
up	p53 signaling pathway	0.001637	0.040646	8
up	Hepatitis B	0.001731	0.040646	13
up	Human T-cell leukemia virus 1 infection	0.001886	0.040646	17
up	Cellular senescence	0.002033	0.040646	14
up	JAK-STAT signaling pathway	0.00204	0.040646	13
down	Ribosome	1.57E-12	4.46E-10	32
down	Biosynthesis of amino acids	3.93E-06	0.000559	14
down	Carbon metabolism	4.90E-05	0.004636	16
down	Aminoacyl-tRNA biosynthesis	9.89E-05	0.007021	11
down	Autophagy - other	0.000333	0.018891	7
down	Fructose and mannose metabolism	0.000595	0.026653	7
down	Proteasome	0.000657	0.026653	8
down	Inositol phosphate metabolism	0.001027	0.036468	10