SUPPLEMENTARY DATA

Supplementary Figure S1. IP3R1, GRP75 and VDAC1 form a complex and interact with PDK4 in MAM interface, Related to Figure 1.

(A) Immunoblot of subcellular fractions isolated from C2C12 myotubes.

(B) Validation of interaction between MAM proteins IP3R1, GRP75 and VDAC1 by *in situ* PLA in C2C12 myoblasts (Scale Bars, 20μm).

(C) Co-IP with GRP75 antibody in C2C12 myotubes.

(D) & (F) Validation of IP3R1 and GRP75 knockdown in C2C12 myoblast.

(E) & (G) Evaluation of IP3R1-GRP75-VDAC1 interaction after knocking down IP3R1 or GRP75 by *in situ* PLA in C2C12 myoblast (Scale Bars, 20μm). Following bar graph represents the quantification plot of *in situ* PLA blobs of each respective panel. (Mean ±SD of n=3 of more than 30 cells per experiment; *P<0.05, **P<0.01; Student's t test)

(H) Evaluation of interaction site of PDK4 with MAM proteins by aligning the in situ PLA blobs with mito-GFP using confocal microscope (White Scale Bars, 10μm; Inset-Yellow Scale Bars, 2.5μm).
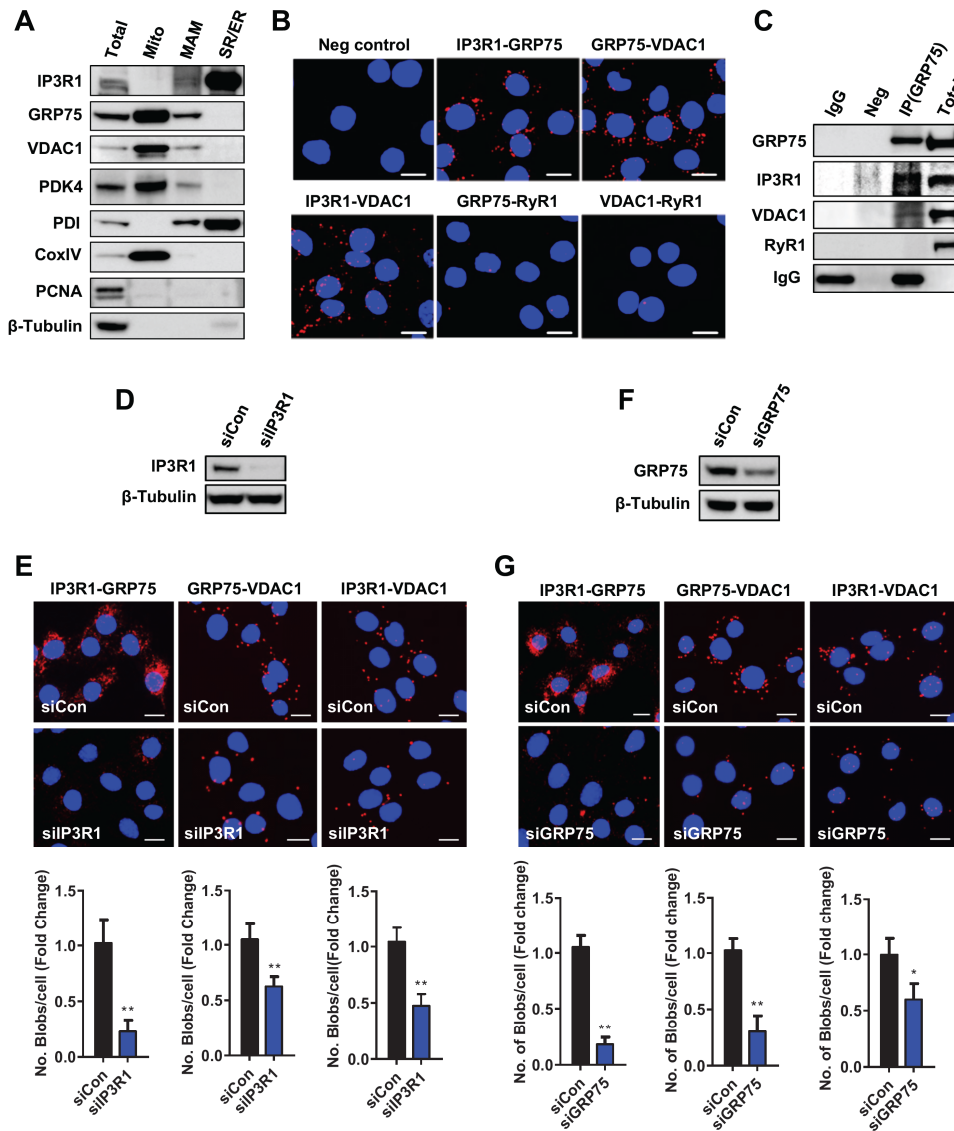
SUPPLEMENTARY DATA

H
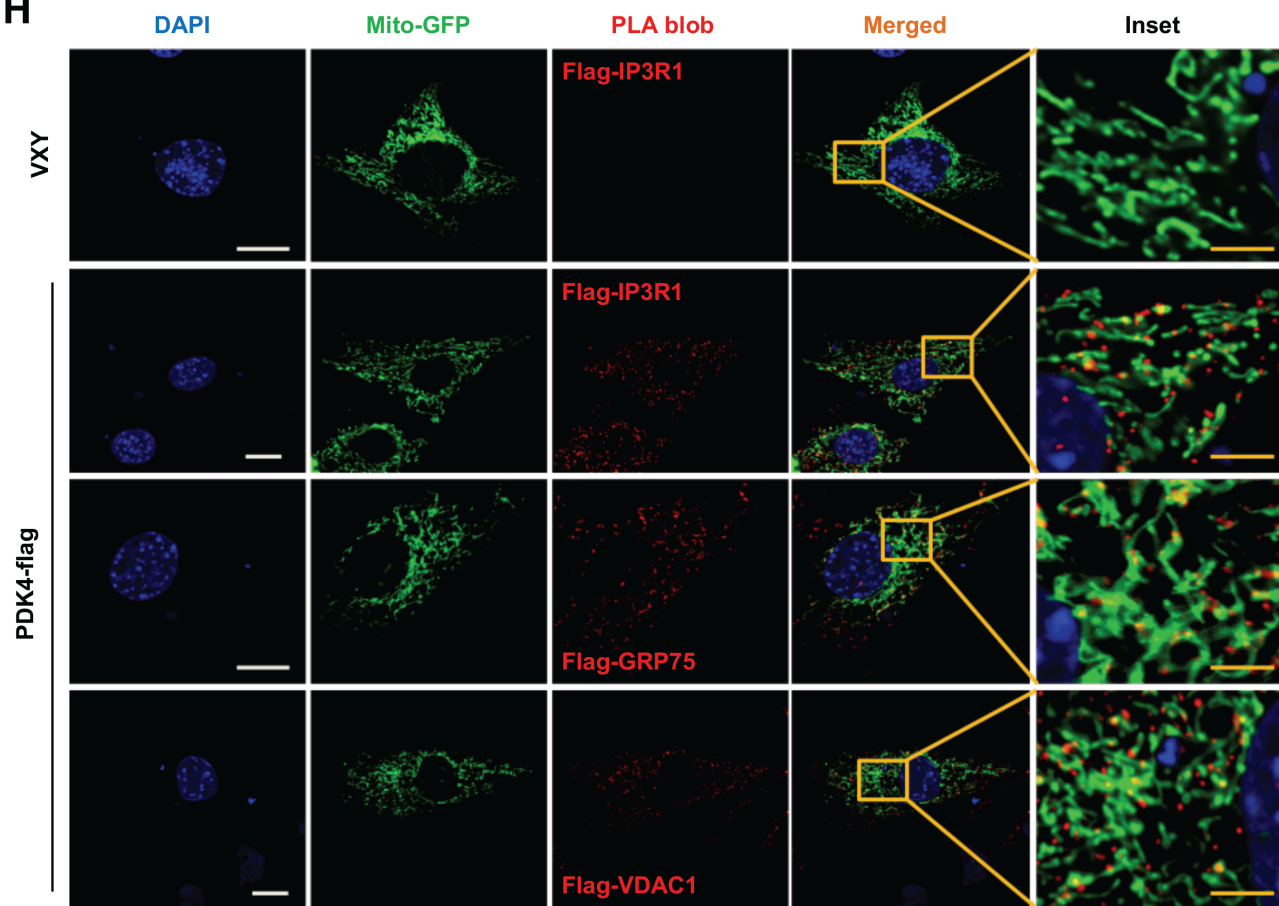
SUPPLEMENTARY DATA

Supplementary Figure S2. Regulation of MAM integrity by PDK4 is PDH independent, Related to Figure 2.

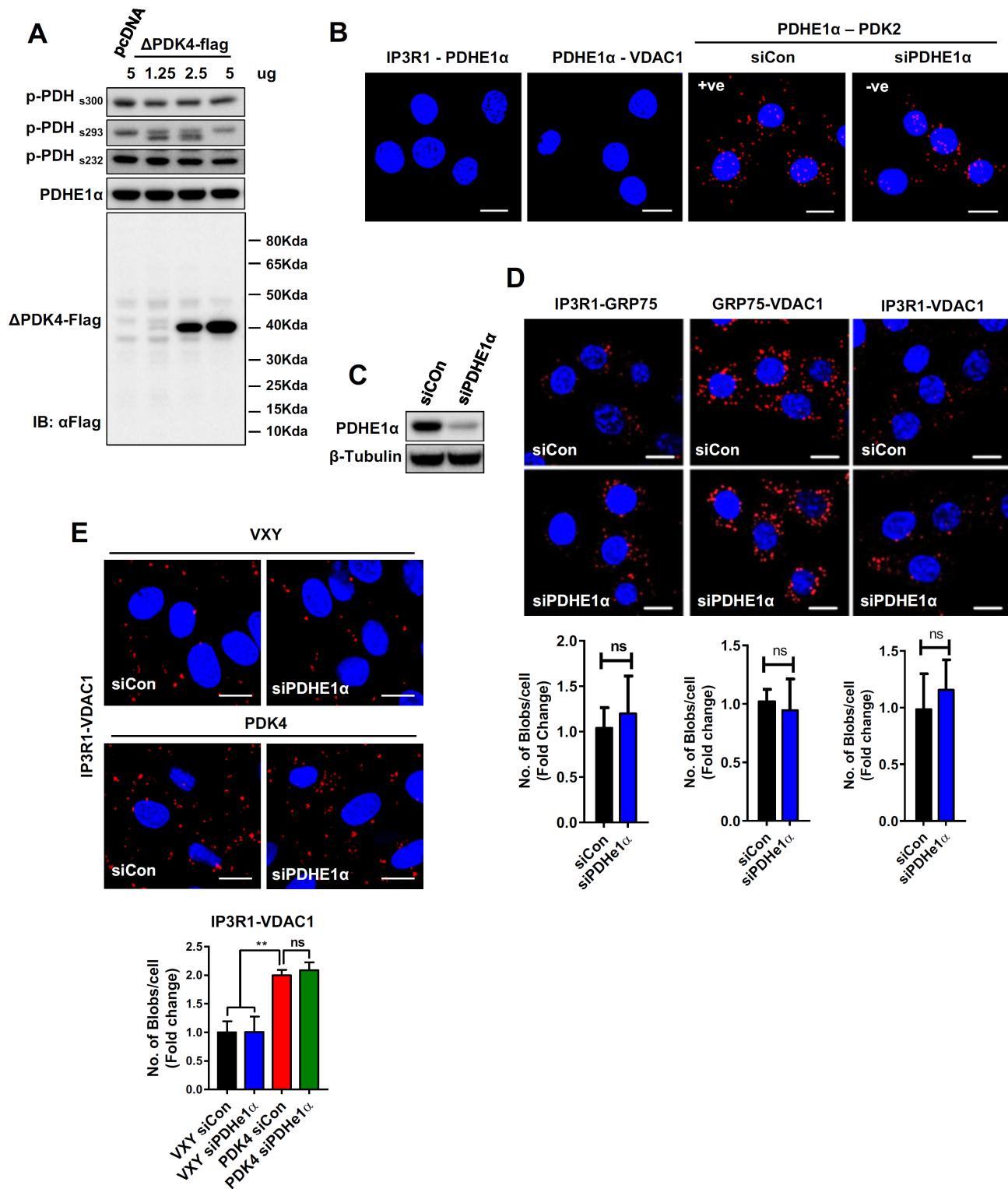(A) Dose dependent overexpression ΔPDK4-flag and evaluation of PDH phosphorylation in C2C12 myoblast by immunoblot.

(B) Evaluation of interaction between PDHE1α and IP3R1/VDAC1 by *in situ* PLA in C2C12 myoblast. Interaction between PDHE1α and PDK2 in absence or presence of siPDHE1α was used as negative (-ve) and positive (+ve) control respectively (Scale Bars, 20μm).

(C) Validation of PDHE1α knockdown by immunoblot.

(D) Evaluation of IP3R1-GRP75-VDAC1 interaction in PDHE1α knockdown C2C12 myoblast by *in situ* PLA (Scale Bars, 20μm). Following bar graph represents the quantification plot of *in situ* PLA blobs of each respective panel. (Mean ±SD of over 30 cells for each condition; ns –no significance; Student's t test).

(E) Evaluation of IP3R1 and VDAC1 interaction in VXY or PDK4-flag expressing C2C12 myoyubes in absence or presence of siPDHE1α by *in situ* PLA (Scale Bars, 20μm). (Below) Quantification (Mean ±SD of over 30 cells for each condition; **$P<0.01$; ns – no significance; one-way ANOVA)

A

p-PDH $_{s300}$
p-PDH $_{s293}$
p-PDH $_{s232}$
PDHE1α

ΔPDK4-Flag

IB: αFlag

pcDNA ΔPDK4-flag
5 1.25 2.5 5 ug

— 80Kda
— 65Kda
— 50Kda
— 40Kda
— 30Kda
— 25Kda
— 15Kda
— 10Kda

B

PDHE1α – PDK2

IP3R1 - PDHE1α   PDHE1α - VDAC1   siCon   siPDHE1α

+ve   -ve

C

siCon siPDHE1α

PDHE1α
β-Tubulin

D

IP3R1-GRP75   GRP75-VDAC1   IP3R1-VDAC1

siCon   siCon   siCon

siPDHE1α   siPDHE1α   siPDHE1α

E

VXY

IP3R1-VDAC1

siCon   siPDHE1α

PDK4

siCon   siPDHE1α

IP3R1-VDAC1

SUPPLEMENTARY DATA

Supplementary Figure S3. Saturated fatty acid palmitate enhanced MAM formation in skeletal muscle cells, Related to Figure 3.

(A) PDKs protein expression level after 0.4mM Pal/BSA treatment for 16 h in C2C12 myotubes.

(B) Quantification of figure A (Mean ±SD of n=3; *P<0.05*; Student's t test)

(C) Immunoblot of subcellular fractions isolated from C2C12 myotubes treated with 0.4mM Pal/BSA for 16 h. (Right) Quantification of MAM fractions (Mean ±SD of n=3; *P<0.05*, **P<0.01*; Student's *t* test)

(D) *in situ* PLA of IP3R1-VDAC1 interaction in C2C12 myotubes treated with 0.4mM Pal/BSA at various time points (hour – h) (Scale Bars, 20μm).

(E) Quantification of *in situ* PLA blobs of figure D. (Mean ±SD of over 30 cells for each condition; *P<0.05*, **P< 0.01*; Student's t test)

(F) IP3R1 and VDAC1 interaction was monitored by in situ PLA after treatment of increasing dose of Pal for 16 h (as indicated in the figure) with low glucose (5.5mM) or high glucose (25mM) media (as indicated in the figure) in C2C12 myotubes (Scale Bars, 20μm). (G) Quantification of in situ PLA blobs of figure F. (Mean ±SD of over 30 cells for each condition; *P<0.05*, **P<0.01*, ***P<0.001*; one-way ANOVA)

(H) Transcription factors of PDK4 was evaluated by Immunoblot after incubating with 0.4mM Pal/BSA at various time points in C2C12 myotubes.

(I) Immunoblot of gastrocnemius muscle homogenate isolated from 16 weeks CD and HFD fed mice. (Right) Quantification (Mean ±SD of n=2/group; *P<0.05*; Student's t test)

(J) Examination of PDK4 and PDK2 protein expression level after knocking down FOXO1 by immunoblot.
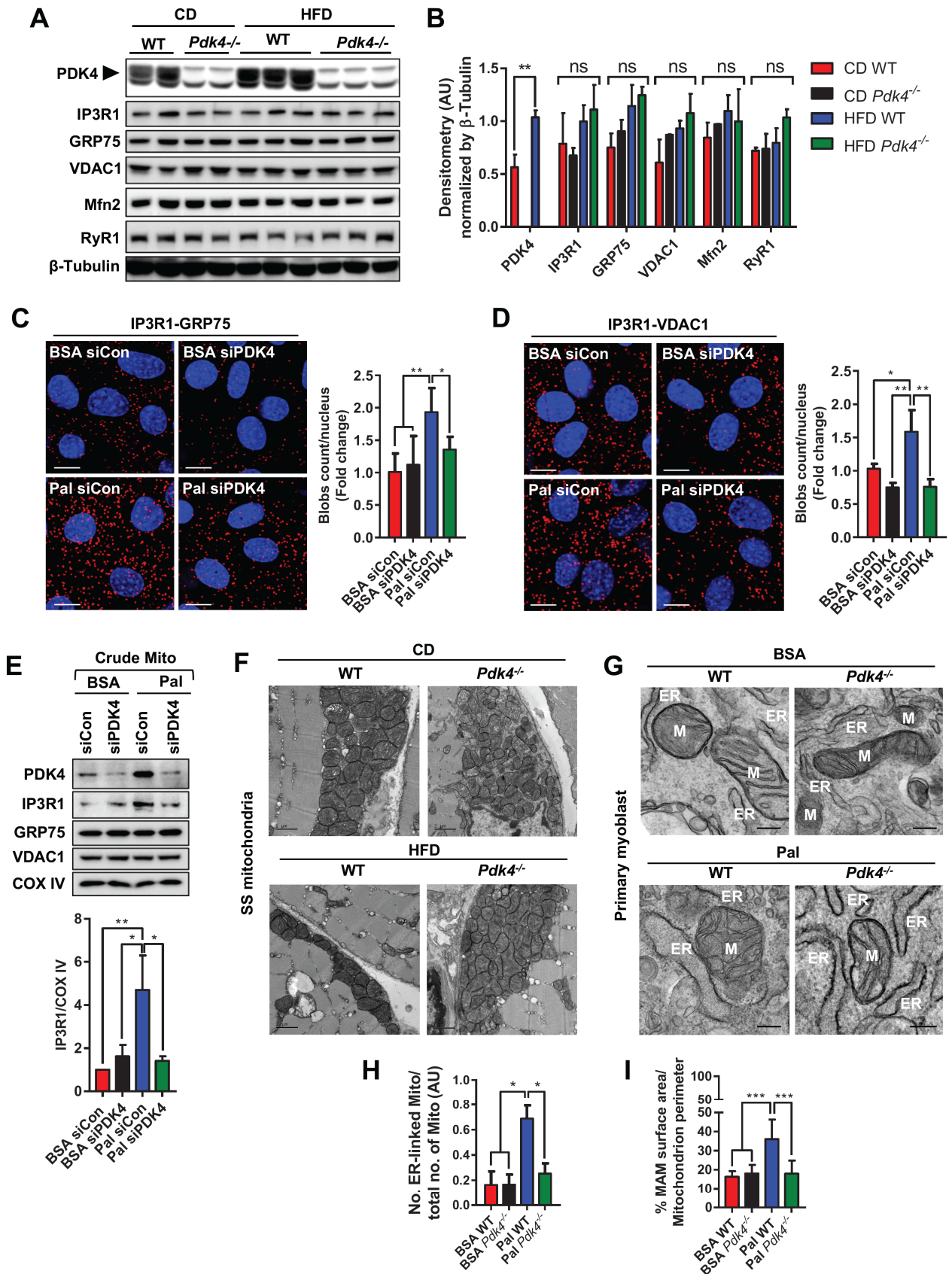
SUPPLEMENTARY DATA
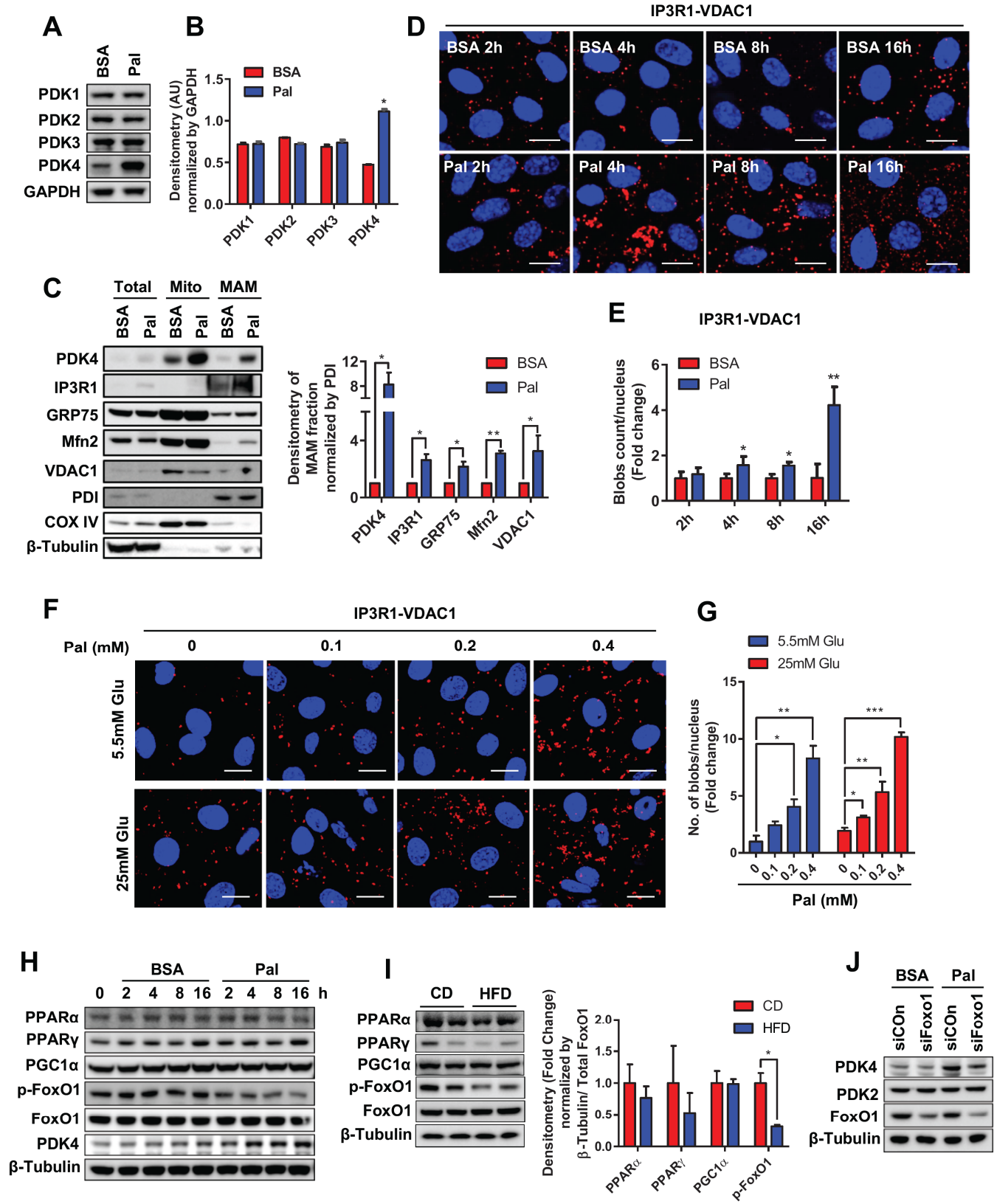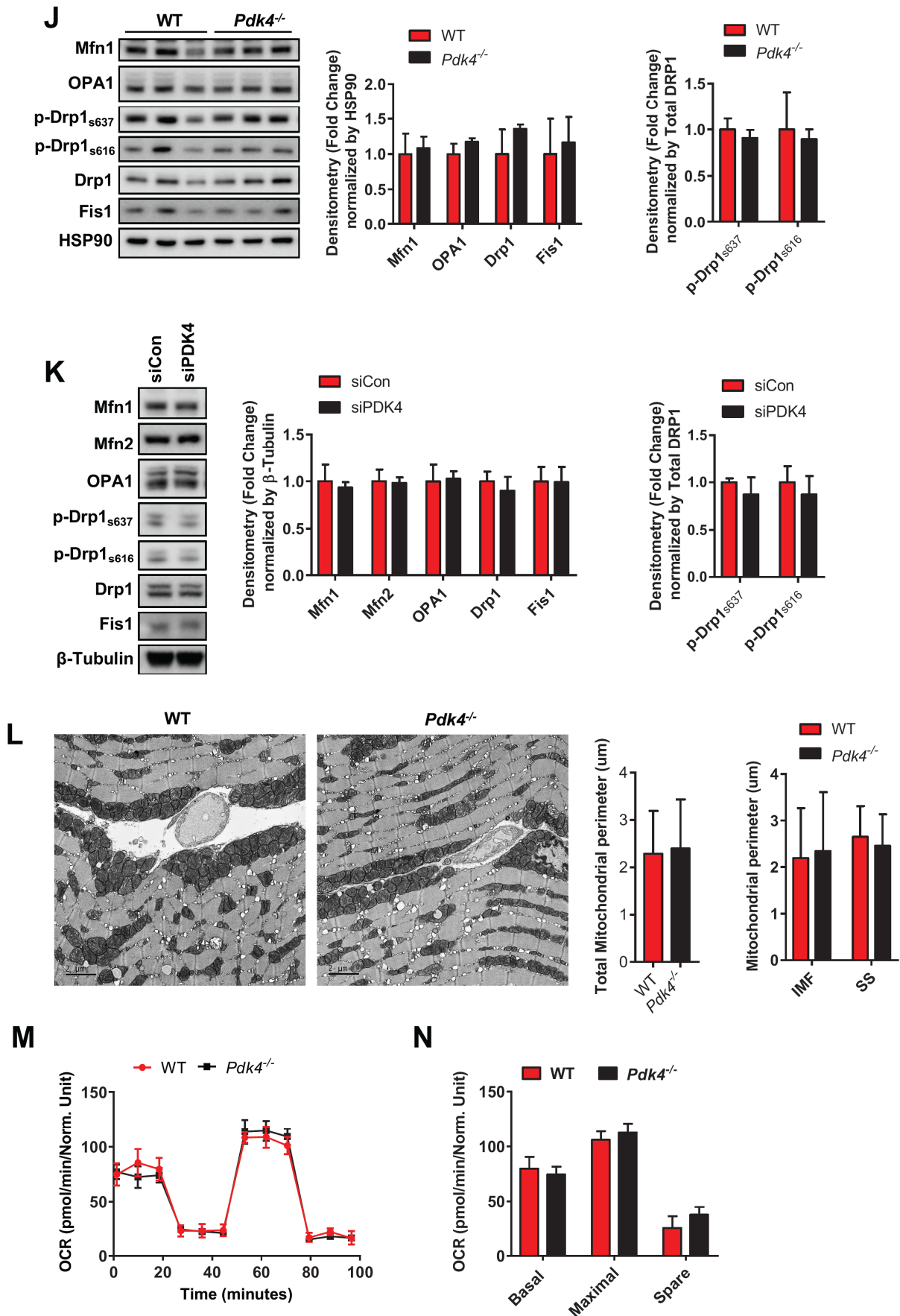
SUPPLEMENTARY DATA

Supplementary Figure S4. PDK4 deficiency reduced high fat diet or palmitate-induced MAM formation without affecting the expression levels of MAM and mitochondrial dynamics related proteins, Related to Figure 4.

(A) Evaluation of total protein expression levels of indicated MAM resident proteins in gastrocnemius muscle isolated from CD or HFD fed WT or *Pdk4*^{-/-} mice by immunoblot.

(B) Quantification of figure A (Mean ±SD of n=3; **$P<0.01$, ns − no significance; one-way ANOVA)

(C) & (D) Evaluation of interaction between IP3R1 and GRP75/VDAC1 by *in situ* PLA in siCon/siPDK4 transfected C2C12 myotubes after incubating with 0.4mM Pal/BSA for 16 h. (Right) Quantification of *in situ* PLA blobs of each respective panel (Scale Bars, 10μm) (Mean ±SD of n=3 of over 30 cells per experiment; *$P<0.05$, **$P<0.01$; one-way ANOVA).

(E) Examination of IP3R1 enrichment in crude mitochondria isolated from siCon/siPDK4 transfected C2C12 myotubes after incubating with 0.4mM Pal/BSA for 16 h. (Below) Quantification (Mean ±SD of n=3; *$P<0.05$, **$P<0.01$; one-way ANOVA).

(F) Representative TEM images of subsarcolemmal (SS) mitochondria in gastrocnemius muscle isolated from CD or HFD-fed WT and *Pdk4*^{-/-} mice.

(G) Representative TEM images of ER-mitochondria contacts in primary myoblast isolated from WT or *Pdk4*^{-/-} mice after stimulating 0.4mM Pal/BSA for 16 h. (Scale Bars, 500nm).

(H) Number of mitochondria linked with ER in total number of mitochondria in each microscopic field (n=10/group; Mean ±SD, *$P<0.05$, **$P< 0.01$; one-way ANOVA).

I) Percentage of MAM surface area per mitochondrion perimeter in each microscopic field n=20/group; Mean ±SD, ***$P< 0.001$; one-way ANOVA).

J) Evaluation of mitochondrial dynamics-related proteins in WT and *Pdk4*^{-/-} mice. (Right) Quantification (Mean ±SD of n=3/group; Student's t test).

K) Evaluation of mitochondrial dynamics related proteins in C2C12 myotubes transfected with siCon/siPDK4. (Right) Quantification (Mean ±SD of n=3; Student's t test).

L) TEM images showing the mitochondria morphology in gastrocnemius muscle isolated from WT and *Pdk4*^{-/-} mice (Scale Bars, 2um). (Right) Total mitochondria perimeter (um) and mitochondrial perimeter of SS and IMF (um) (n=74~123).

M) OCR level was measured in primary myotubes isolated from WT/ *Pdk4*^{-/-} mice gastrocnemius.

N) Quantification of Figure M.

SUPPLEMENTARY DATA

Supplementary Figure S5. Attenuation of MAM formation prevented palmitate-induced mitochondrial dysfunction, ER stress and insulin resistance, Related to Figure 5.

A) Mitochondrial ROS generation measurement in siCon/siIP3R1si/GRP75 transfected C2C12 myotubes after incubating with 0.4mM Pal/BSA for 16 h using CM-H2XRos normalized by MitoTracker green FM (Mean ±SD of n=2, *$P<0.05$, **$P< 0.01$; one-way ANOVA).

B) Cellular ATP was measured in siCon/siIP3R1/siGRP75 transfected C2C12 myotubes after incubating with 0.4mM Pal/BSA for 16 h by ATPlite luminescence assay. (n=3; Mean ±SD, *$P<0.05$, **$P<0.01$; one-way ANOVA).

C) Immunoblot of mitochondrial total OXPHOS-complex proteins in gastrocnemius muscle isolated from 16weeks CD or HFD-fed WT and *Pdk4$^{-/-}$* mice.

(D) Quantification of figure C (Mean ±SD of n=6/group; ns – no significance; one-way ANOVA)

(E-F) Palmitate induced ER stress was analyzed by immunoblot in IP3R1 or GRP75 knockdown C2C12 myotubes. (Right) Quantifications (Mean ±SD of n=3, *$P<0.05$, **$P<0.01$; one-way ANOVA).

(G) siCon/siPDK4 transfected C2C12 myotubes were incubated with 0.25µM Tg (thapsigargin) at different time points (hour – h) and examined the ER stress markers by immunoblot.
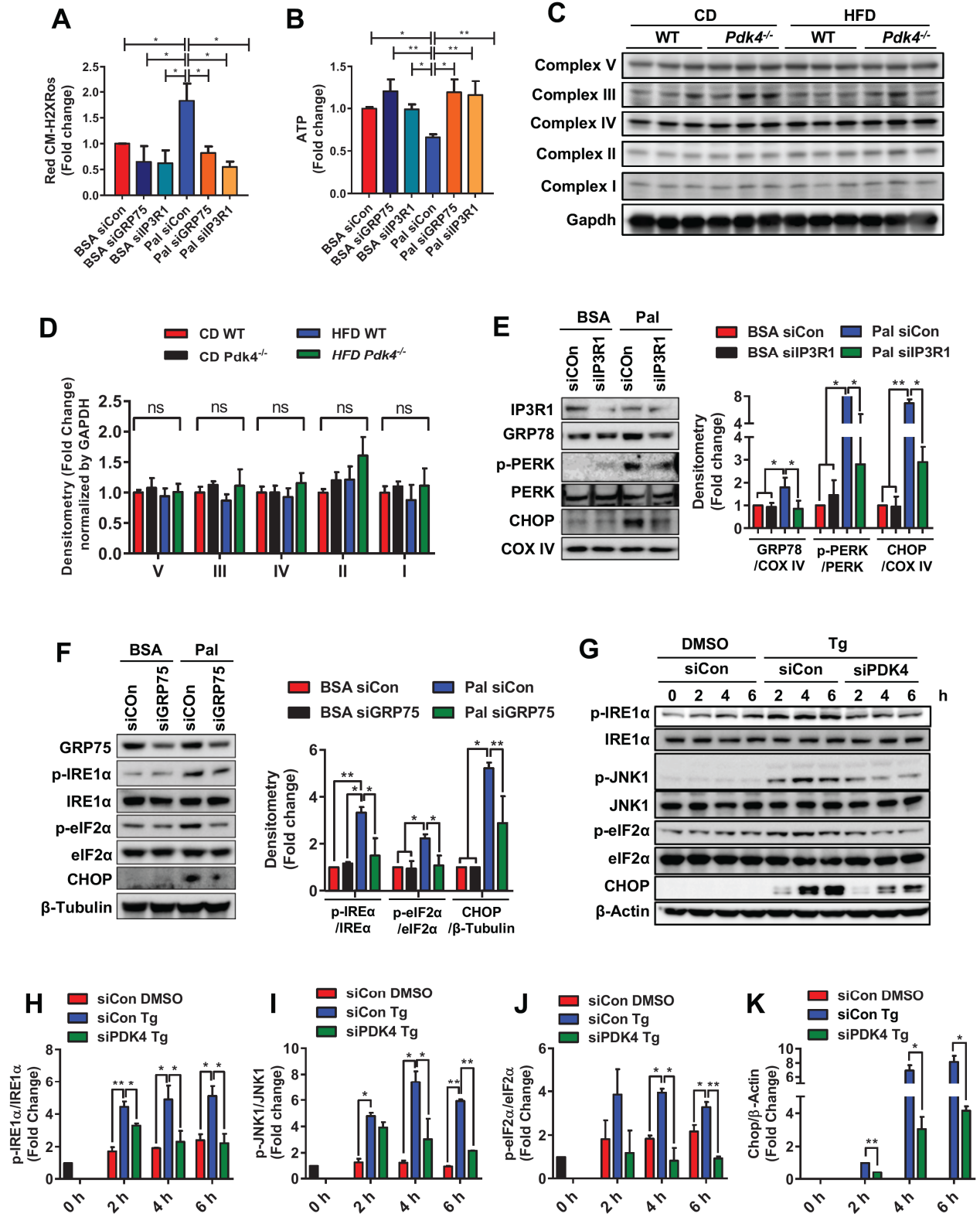
(H-K) Quantification of figure G (Mean ±SD of n=2, *$P<0.05$, **$P<0.01$; one-way ANOVA).

(L) IP3R1 and VDAC1 interaction were examined by *in situ* PLA in siCon/siPDK4 transfected C2C12 myotubes after incubating with 0.25µM Tg for 6 h (Scale Bars, 20µm). (Right) *in situ* PLA blobs quantification (Mean ±SD of more than 30 cells per condition; **$P<0.01$; one-way ANOVA).
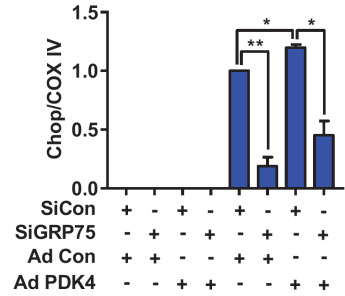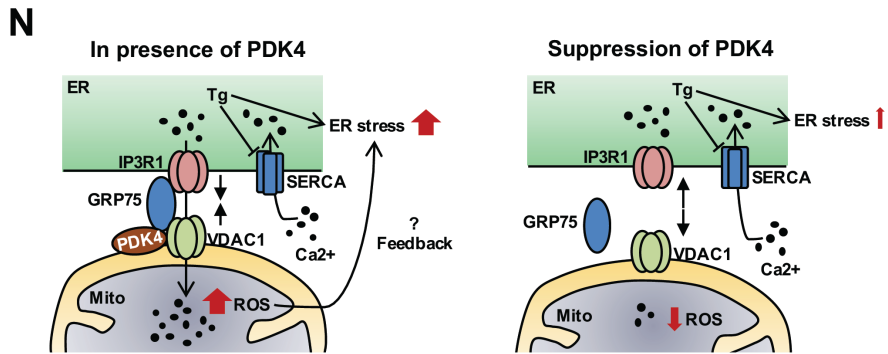
(M) C2C12 myotubes were transfected or transduced with siGPR75 or adeno virus (Ad) PDK4 or both siGRP75 and adPDK4 followed by treatment of 0.25µM Tg for 6 h. ER stress marker, CHOP expression was evaluated by immunoblot. (Below) Quantification (Mean ±SD of n=2, *$P<0.05$, **$P<0.01$; one-way ANOVA).

(N) Schematic representation, showing that PDK4 enhances Tg-induce ER stress through induction of MAM formation, possibly via a feedback mechanism induced by enhanced generation of mitochondria ROS. Whereas, suppression of PDK4 reduces Tg-induced ER stress by attenuating MAM formation

L

IP3R1-VDAC1

siCon | siCon Tg | siPDK4 Tg

M

	DMSO				Tg			
Ad PDK4	-	-	+	+	-	-	+	+
Ad Con	+	+	-	-	+	+	-	-
siGRP75	-	+	-	+	-	+	-	+
siCon	+	-	+	-	+	-	+	-

GRP75
PDK4
CHOP
COX IV

N

In presence of PDK4

Suppression of PDK4
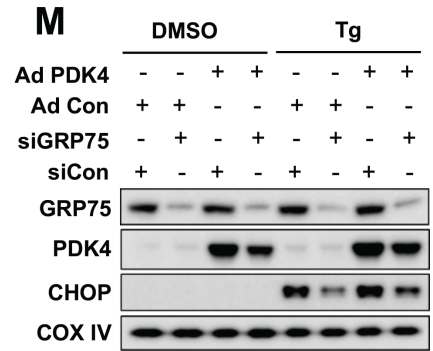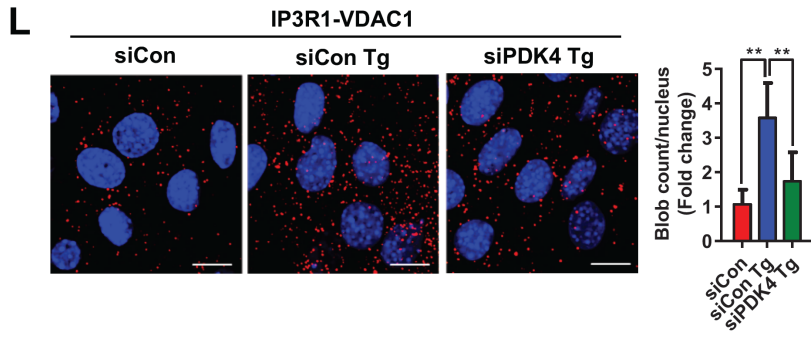
SUPPLEMENTARY DATA

Supplementary Figure S6. MAM suppression protects from palmitate-induced insulin resistance, Related to Figure 6.

(A) & (C) JNK phosphorylation was analyzed in siCon/siIP3R1/siGRP75 transfected C2C12 myotubes after incubating with 0.4mM BSA/Pal for 16 h by immunoblot. (Below) Quantification of each respective figures (Mean ±SD of n=3; *P<0.05, **P<0.01; one-way ANOVA)

(B) & (D) Insulin-stimulated AKT phosphorylation was evaluated in siIP3R1/siGRP75 transfected C2C12 myotubes after incubating with 0.4mM BSA/Pal for 16 h (Below) Quantification of each respective figures (Mean ±SD of n=3; *P<0.05, **P<0.01; one-way ANOVA)

(E) Mock or Linker-RH was transduced in C2C12 myotubes and JNK phosphorylation was examine by immunoblot. (Below) Quantification (Mean ±SD of n=3; **P<0.01; Student's t test)

(F) AKT phosphorylation was evaluated by immunoblot in mock or Linker-RH overexpressing C2C12 myotubes. (Below) Quantification (Mean ±SD of n=3; **P<0.01; Student's t test)

(G) Immunoblot analysis after mock or Linker-RH transduction in control or PDK4 knockdown C2C12 myotubes. (Right) Quantification (Mean ±SD of n=3; *P<0.05; one-way ANOVA)

(H) Insulin-stimulated AKT phosphorylation was evaluated after mock or Linker-RH transduction in control or PDK4 knockdown C2C12 myotubes. (Right) Quantification (Mean ±SD of n=3; **P<0.01; one-way ANOVA)
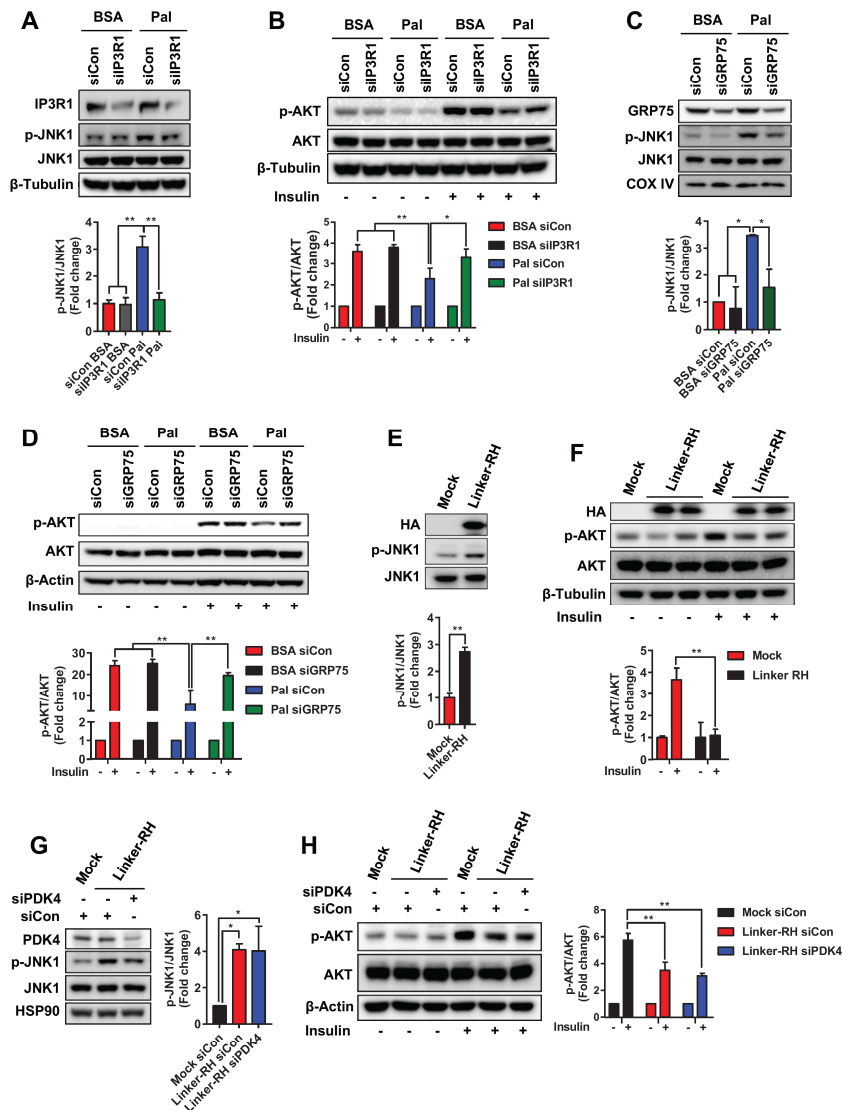
SUPPLEMENTARY DATA

Supplementary Table S1. Antibody information

Antibody Name	Provider	Catalog No.	Application	Dilution
IP3R1	Santa Cruz	sc-28614	IB/in situ PLA	1:1000/1:50
GRP75	Santa Cruz	sc-133137	IB/in situ PLA/IP	1:1000/1:100/1:100
PERK	Santa Cruz	sc-13073	IB	1:1000
PDK2	Santa Cruz	sc-100534	IB/in situ PLA	1:1000/1:50
PGC1α	Santa Cruz	sc-5816	IB	1:1000
GRP78	Santa Cruz	sc-1050	IB	1:1000
eIF2α	Santa Cruz	sc-11386	IB	1:1000
Fis1	Santa Cruz	sc-98900	IB	1:1000
VDAC1	Santa Cruz	sc-390996	IB/in situ PLA	1:1000/1:100
Drp1	Santa Cruz	sc-271583	IB	1:1000
VDAC1	Abcam	ab14734	IB/in situ PLA	1:1000/1:100
COX IV	Abcam	ab16056	IB	1:1000
IP3R1	Abcam	ab166871	IB/in situ PLA	1:1000/1:100
PPARα	Abcam	ab2779	IB	1:1000
p-IRE1α	Abcam	ab4817	IB	1:1000
Mfn1	Abcam	ab57602	IB	1:1000
Total Oxphos-Complex	Abcam	ab110413	IB	1:1000
PDI	Abcam	ab2792	IB	1:1000
Ryr1	Cell signaling	#8153	IB	1:1000
PDK1	Cell signaling	#3820	IB	1:1000
Flag	Cell signaling	#2368	IB/in situ PLA	1:1000/1:100
PDHE1α	Cell signaling	#3205	IB/in situ PLA	1:1000/1:100
Mfn2	Cell signaling	#9482	IB	1:1000
p-Drp1 s616	Cell signaling	#3455	IB	1:1000
p-Drp1 s637	Cell signaling	#4867	IB	1:1000
PPARγ	Cell signaling	#2435	IB	1:1000
p-Foxo1	Cell signaling	#9461	IB	1:1000
Foxo1	Cell signaling	#2880	IB	1:1000
p-PERK	Cell signaling	#3179	IB	1:1000
IRE1α	Cell signaling	#3294	IB	1:1000
p-eIF2α	Cell signaling	#9721	IB	1:1000
p-JNK	Cell signaling	#9251	IB	1:1000
Chop	Cell signaling	#2895	IB	1:1000
JNK	Cell signaling	#9252	IB	1:1000
p-AKT	Cell signaling	#9271	IB	1:1000
AKT	Cell signaling	#2920	IB	1:1000
HSP90	Cell signaling	#4874	IB	1:1000
GAPDH	Cell signaling	#2118	IB	1:1000
HA	Cell signaling	#2367	IB	1:1000
p-PDH s232	Calbiochem	AP1063	IB	1:10000
p-PDH s293	Calbiochem	AP1062	IB	1:10000
p-PDH s300	Calbiochem	AP1064	IB	1:10000
β-tubulin	ABM	AMB-G098	IB	1:1000
β-Actin	Sigma	A5441	IB	1:2000
Flag	Sigma	F1804	IB/in situ PLA	1:10000/1:500
p-IRS-1	Upstate	07-247	IB	1:1000
IRS-1	Upstate	06-248	IB	1:1000
PCNA	BD biosciences	#610664	IB	1:1000
OPA1	BD biosciences	#612606	IB	1:1000
PDK3	Anti-serum	NA	IB	1:1000
PDK4	Anti-serum	NA	IB	1:1000