

SUPPLEMENTARY DATA

Perilipin 5 deletion unmasks an endoplasmic reticulum stress – fibroblast growth factor 21 axis in skeletal muscle

Running title: ER stress and FGF21 in Plin5 MKO mice

Magdalene K. Montgomery¹, Ruzaidi Mokhtar^{1,2}, Jacqueline Bayliss¹, Helena C Parkington¹, Victor M Suturin¹, Clinton R. Bruce³, Matthew J. Watt^{1*}

¹ *Monash Biomedicine Discovery Institute, Metabolic Disease and Obesity Program, and the Department of Physiology, Monash University, Clayton, Victoria, 3800, Australia;* ²*Biotechnology Research Institute, Universiti Malaysia Sabah, Jalan UMS 88400 Kota Kinabalu, Sabah, Malaysia;* ³*Institute for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Burwood, Australia.*

*Address correspondence to:

Matthew J. Watt, Ph.D.

Monash Biomedicine Discovery Institute, Metabolic Disease and Obesity Program
and the Department of Physiology,

Monash University, Clayton, Victoria, 3800, Australia

Phone: 613 9905 2584

Fax: 613 9905 2547.

Email: matthew.watt@monash.edu

SUPPLEMENTARY DATA

Supplementary Table S1. Antibodies

Protein	Company	Product Code
ACC (Ser212)	Cell Signalling Technology	3661
ACC	Cell Signalling Technology	3662
AMPK (Thr172)	Cell Signalling Technology	2535S
AMPK	Cell Signalling Technology	2532S
ATF6	Enzo Life Sciences	ALX-804-381-C100
CGI58	Abcam	ab73551
CHOP	Santa Cruz Biotechnology	sc575
Eif2 α (Ser51)	Cell Signalling Technology	3398
Eif2 α	Cell Signalling Technology	9722
IRE1 (Ser724)	Abcam	ab48187
JNK (Thr183/Tyr185)	Cell Signalling Technology	4668
JNK	Cell Signalling Technology	9252
MitoProfile® Total OXPHOS	MitoSciences	MS604
PGC1 α	Merck Millipore	AB3242
PLIN2/ADFP	ProteinTech	15284-1-AP
PLIN5/OXPAT	Progen Biotechnik	GP31
VDAC	Cell Signalling Technology	4661
XBP1	Santa Cruz Biotechnology	sc7160

SUPPLEMENTARY DATA

Supplementary Table S2. Primer Sequences

Gene	Forward Primer	Reverse Primer
ACC2	GCATGAAGGACATGTATGAG	AGGGATGTAGATGAGAATGG
ATF4	CAAGGAGGATGCCTTTTC	GTCATCCATTTCGAAACAGAG
CHOP	TTCACTACTCTTGACCCTGCGTC	CACTGACCACTCTGTTTCCGTTTC
CideA	TGACATTCATGGGATTGCAGAC	CATGGTTTGAAACTCGAAAAGGG
CPT1a	TGGCATCATCACTGGTGTGTT	GTCTAGGGTCCGATTGATCTTTG
EDEM	TGATGTTCTCTTCTACCCTC	CGTAGCATATCCACATTTGAC
FGF21	CAGTCCAGAAAGTCTCCTG	AGAAACCTAGAGGCTTTGAC
GRP78	AGGACAAGAAGGAGGATGTGGG	ACCGAAGGGTTCATTCCAAGTG
GRP94	AAACGGCAACACTTCGGTCAG	GCATCCATCTCTTCTCCCTCATC
HEDJ	GGTCACAAGGTCCATATTTTC	TGTTCTTTTGGAAAGTCCAC
HPRT	TCCTCCTCAGACCGCTTTT	CCTGGTTCATCATCGTAATC
IL6	CAAGAGACTTCCATCCAGTTGCC	CATTTCCACGATTTCCCAGAGAA
Kctd20	TGACAGTGACAGGTTATTGCG	AGGCATAGTCAAGTGAGAGGTC
MCP1	CACTCACCTGCTGCTACTCATTC	TCTGGACCCATTCTTCTTGG
Nrf2	CATTCCCGAATTACAGTGTC	GGAGATCGATGAGTAAAAATGG
P58IPK	TTCTCTAAGGATGAGAAGCC	GCTTCATCGTACATTTCTC
Plin5	GTGACTACCTGTGCCCTGGAC	TGATGGCTGCTGCAGGAAG
Sk. actin	TATTCCTTCGTGACCACAGCTGAACGT	CGCGAACGCAGACGCGAGTGCGC
Spata 13	TCAGCCCATCGAAGCTGGTA	AAGGACGACTGAGCGAGGT
TNF α	CCCCTTTACTCTGACCCCTTTATTG	AACCTGACCACTCTCCCTTTGC
UCP1	GTGAACCCGACAACTTCCGAA	TGCCAGGCAAGCTGAAACTC
XBP1s	CTGAGTCCGCAGCAGGT	TGTCAGAGTCCATGGGAAGA

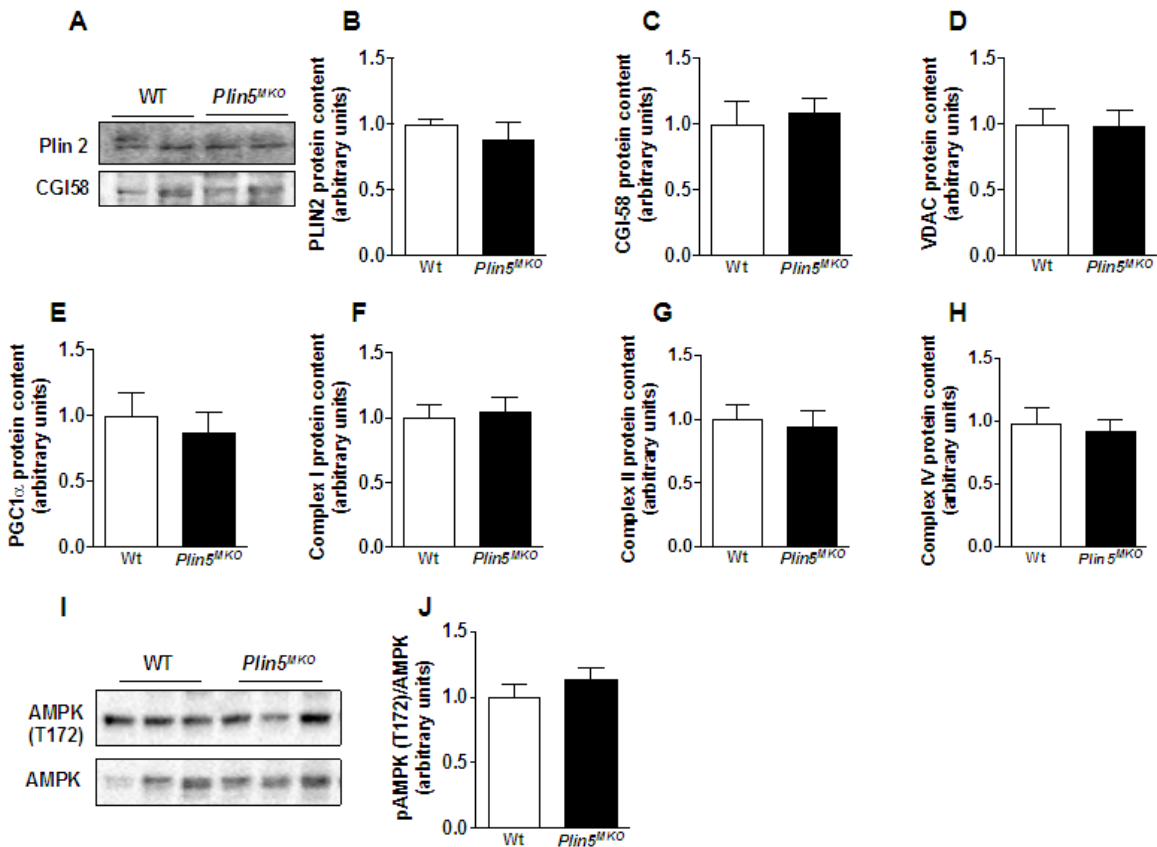
Supplementary Table S3. Tissue weights in WT and *Plin5*^{MKO} mice fed a high-fat diet

Tissue	WT	<i>Plin5</i> ^{MKO}
Liver (g)	1.38 \pm 0.09	1.26 \pm 0.04
Epididymal WAT (g)	2.51 \pm 0.09	2.56 \pm 0.13
Inguinal WAT (g)	2.05 \pm 0.20	1.72 \pm 0.16
Heart (g)	0.15 \pm 0.01	0.17 \pm 0.01
Quadriceps muscle (g)	0.36 \pm 0.01	0.34 \pm 0.01

WAT, white adipose tissue. Vales are means \pm SEM (n=8)

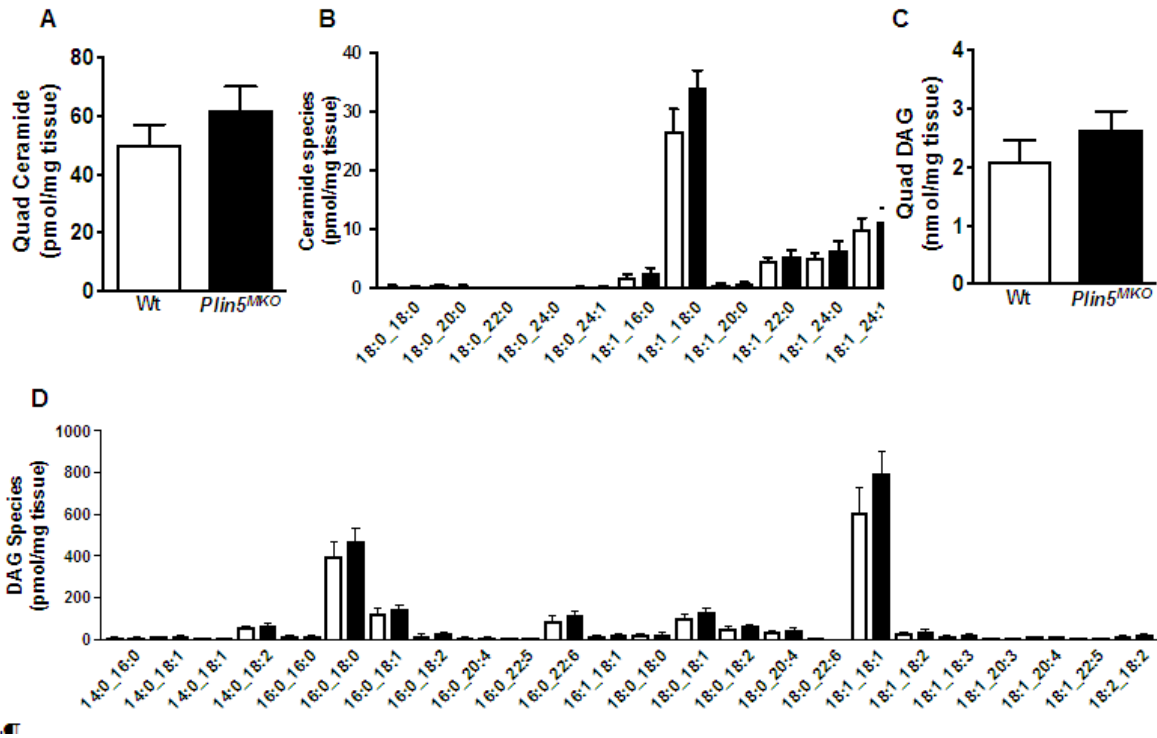
SUPPLEMENTARY DATA

Supplementary Figure S1. Metabolic profiling in WT (white) and *Plin5^{MKO}* mice (black) fed a high-fat diet. (A) Representative immunoblotting, as well as quantification of (B) Plin2 and (C) CGI58 protein content. Immunoblotting quantification of (D) VDAC, (E) PGC1 α , (F) NDUFB8 Complex 1 subunit, (G) CII-30 Complex II subunit, and (H) CIV-I Complex IV subunit. (I) Representative immunoblotting for total protein content and phosphorylation of AMP-activated kinase (AMPK, T172), and quantification of (J) changes in AMPK phosphorylation and (K) total AMPK protein content. Shown are means \pm SEM, with n=6-8 per group.



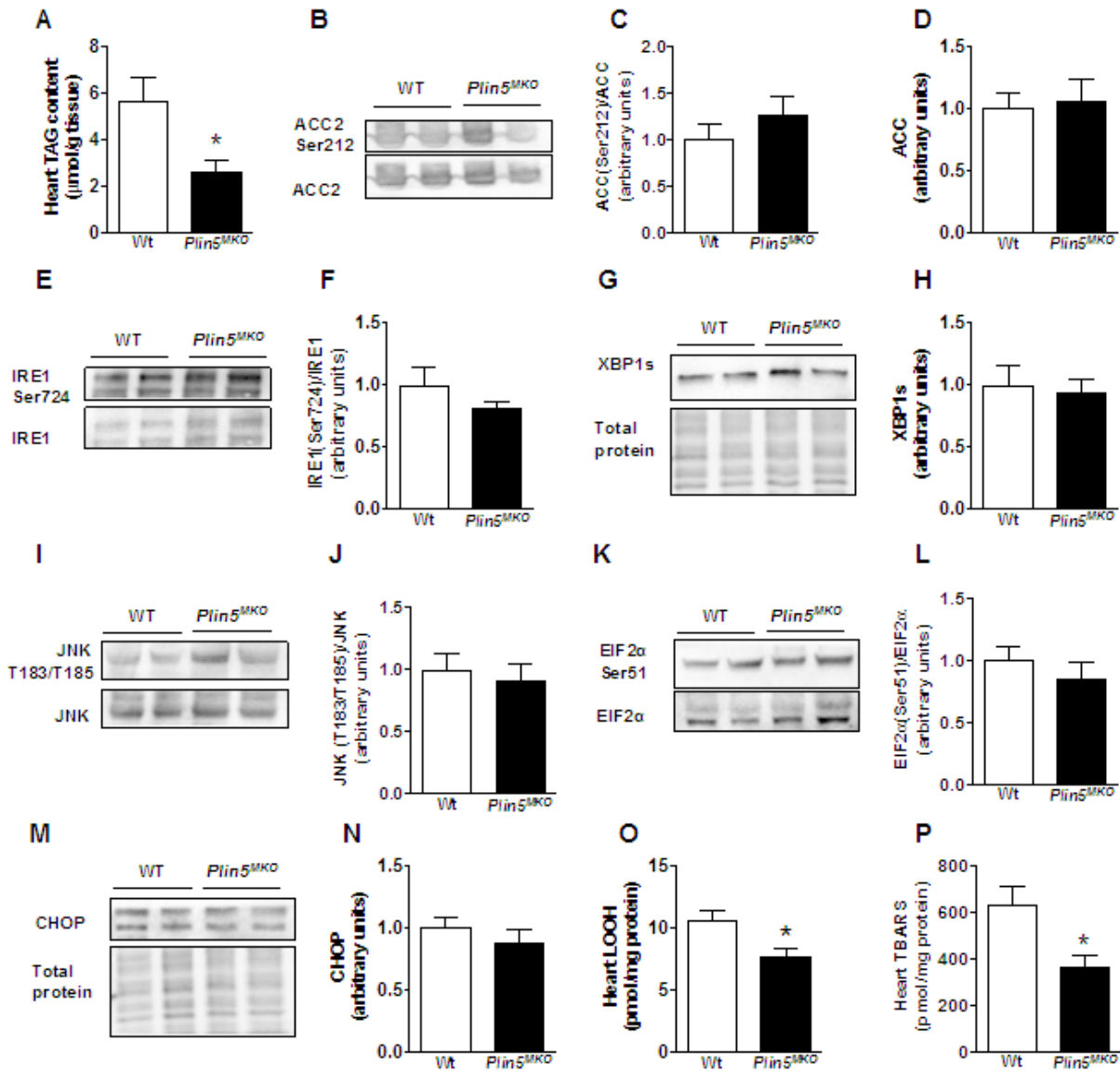
SUPPLEMENTARY DATA

Supplementary Figure S2. Quadriceps muscle lipidomics analysis. (A) total ceramide content, and (B) ceramide species. (C) total diacylglycerol content and (D) diacylglycerol species. Shown are means \pm SEM, with n=6 for WT and n=7 for *Plin5^{MKO}* mice.



SUPPLEMENTARY DATA

Supplementary Figure S3. Metabolic profiling in heart of WT (white) and *Plin5^{MKO}* mice (black) fed a high-fat diet. (A) Total heart triglyceride content (WT n=6, *Plin5^{MKO}* n=7). (B) Representative immunoblotting for total protein content and phosphorylation (Ser212) of Acetyl-CoA carboxylase (ACC2), as well as immunoblotting quantification of changes in (C) ACC2 phosphorylation and (D) total ACC2 protein content (WT n=8, *Plin5^{MKO}* n=8). (E-F). Representative immunoblotting and quantification of IRE1 phosphorylation (Ser724), (G-H) the spliced isoform of XBP1, (I-J) JNK phosphorylation (T183/T185), (K-L) eIF2 α phosphorylation (Ser51), and (M-N) CHOP (WT n=8, *Plin5^{MKO}* n=8). (O) Lipid Hydroperoxides (LOOH), (P) TBARS (WT n=8, *Plin5^{MKO}* n=8). *P<0.05 vs WT.



SUPPLEMENTARY DATA

Supplementary Figure S4. FGF21 mRNA expression in WT (white) and *Plin5*^{MKO} mice (black). FGF21 mRNA in (A) quadriceps muscle (WT n=4, *Plin5*^{MKO} n=4) and (B) liver (WT n=5, *Plin5*^{MKO} n=5) of chow-fed mice, as well as (C) in heart (WT n=8, *Plin5*^{MKO} n=8) and (D) inguinal adipose tissue (WT n=5, *Plin5*^{MKO} n=6) of HFD-fed mice.

