APPENDIX

Table of Contents:

Appendix Supplementary Methods

Ex vivo lipolysis measurement

Isolated fat bodies from 20 larvae were homogenized on ice. TAG hydrolase activity was measured in the tissue extracts using a method based on ^{3}H-TO degradation, according to procedures described previously (Schweiger *et al*, 2014).

Cell culture and siRNA-mediated knockdown

For RNAi knockdown of *PKM2* in HepG2 cells, HepG2 cells were cultured at 37°C in high glucose DMEM (HyClone) supplemented with 10% fetal bovine serum (HyClone), 100 U/ml penicillin and 100 μg/ml streptomycin (HyClone) in a 5% CO_2 incubator. A total of 100 pmole siRNA oligonucleotides and dsRED expression plasmid (1 μg) were transfected into HepG2 cells plated in 6-well dishes using Lipofectamine 2000 (Invitrogen). The sequences of siRNA oligonucleotides (Shanghai GenePharma Co., Ltd) are as follows: for human *PKM2 #1*, sense 5'-GUGGUGAUCUAGGCAUUGATT -3' and antisense 5'-UCAAUGCCUAGAUCACCACTT -3'; for human *PKM2 #2*, sense 5'-CAUGCUGUCUGGAGAAACATT -3' and antisense 5'-UGUUUCUCCAGACAGCAUGTT -3'. Control oligonucleotides with comparable GC content were also obtained from Shanghai GenePharma Co., Ltd. Twenty-four hours after RNAi treatment, oleic acid (OA) was added into the medium at a final concentration of 100 μM. Twenty-four hours after OA treatment, cells were stained with BODIPY and imaged with a confocal microscope.

For S2 cell assays, *Drosophila* S2 cells were maintained in Sf-900 II serum-free

medium (Gibco) at 25°C. The *pUAST*-expression plasmids were co-transfected with *actin-GAL4* plasmid by Cellfectin II (Invitrogen) and PLUS reagent (Invitrogen). Forty-eight hours after transfection, cells were fixed in 4% paraformaldehyde in PBS for 20 min, permeabilized in PBST (0.1% Triton X-100) for 5 min, blocked with 10% BSA in PBST for 1 hour and incubated with mouse monoclonal anti-ATP5A antibody (1:500; Abcam, ab14748) overnight at 4 ℃. Alexa Fluor 555-conjugated goat anti-mouse (1:500; Invitrogen) were used as secondary antibodies. Following immunostaining, the cells were stained by DAPI for 5 min, washed three times in 1xPBS, mounted and immediately imaged with a confocal microscope.

Molecular biology

For qPCR, reactions were performed with Trans Start Green qPCR superMix (TransGen, Co., Ltd, Beijing, China) on an Agilent Technologies MX3000P system. Primers used were as follows (5'-3'):

TBP-F GGAGAGTTCTGGGATTGTAC

TBP-R CTTATCCTCATGATTACCGCAG

PKM2-F ATTATTTGAGGAACTCCGCCGCCT

PKM2-R ATTCCGGGTCACAGCAATGATGG

PKM1-F CGAGCCTCAAGTCACTCCAC

PKM1-R GTGAGCAGACCTGCCAGACT

PKL-F ATGGAAGGGCCAGCGGGGTATC

PKL-R GGCCCGATGGTGGCAATGAT

PKR-F CAGCTTCGGTCATGGGTCTCTAA

PKR-R AGCTGCTGCTGCTGGAAGAA

For construction of *pUAST-CG18660-EGFP*, the coding region of *CG18660* without the stop codon was fused with *EGFP* and inserted into the *pUAST-attB* vector (NotI and KpnI).

Gene set enrichment analysis

For RNA sequencing, total RNA was isolated from 3rd instar larval fat bodies using an RNeasy® Lipid Tissue Kit (QIAGEN). A total amount of 3 μg RNA per sample was used as input material for the RNA sample preparations. Sequencing libraries were

generated using the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer's recommendations and index codes were added to attribute sequences to each sample. Samples were sequenced on an Illumina HiSeq 2500 platform at Novogene Bioinformatics Technology Co., Ltd. The raw sequencing data of RNA-seq have been submitted to the Genome Sequence Archive (GSA) database with the accession number PRJCA000907.

Bam files were resampled and 75% of the total sequences were taken since the depth of the sequencing data was enough for quantitative analysis of the gene expression. The sequencing files were divided to create three repeats for the *dSeipin* and WT groups. As the GSEA software was designed for microarray data analysis, 74.1% the gene IDs were converted to microarray data IDs using the ID conversion tools from BioMart (version 0.9) (http://www.biomart.org/). The differentially expressed genes (DEGs) were quantified using DEseq with the selection criteria: p-value<0.05 and $\log_2$(Fold Change)>1. The gene set files were downloaded from the GSEA website (http://software.broadinstitute.org/gsea/index.jsp). The enrichment scores (ES) were calculated for each gene set in the *dSeipin* and WT groups and only the gene sets which contained 5~1,000 genes was used for calculation.

Stable isotope carbon metabolic tracing

Composition of medium (modified Schneider's Insect Solution):

Ingredients	mg/L
Disodium hydrogen phosphate	700
Magnesium sulphate heptahydrate	3700
Potassium chloride	1600
Potassium dihydrogen phosphate	450
Sodium chloride	2100
Glycine	250
L-Arginine hydrochloride	483
L-Aspartic acid	400
L-Cysteine hydrochloride	78
L-Cystine dihydrochloride	100

L-Glutamic acid	800
L-Glutamine	1800
L-Histidine hydrochloride monohydrate	540
L-Isoleucine	150
L-Leucine	150
L-Lysine hydrochloride	1650
L-Methionine	800
L-Phenylalanine	150
L-Proline	1700
L-Serine	250
L-Threonine	350
L-Tryptophan	100
L-Tyrosine disodium salt	620
L-Valine	300
ß-Alanine	500
Alpha-Ketoglutaric acid	200
D (+) Glucose (unlabeled)/ U-$^{13}C_6$- Glucose (labeled)	4000
Fumaric acid	100
L-Malic acid	100
Succinic acid	100
Yeast extract	2000

The pH was adjusted to 6.7 ± 0.2 and the solution was sterilized immediately by filtration using a membrane with porosity of 0.22 microns.

Glucose and DCA dietary treatment

Extra glucose was added to ordinary fly medium at the indicated mass-volume concentration. DCA (Adamas Reagent Co., Ltd. Cat#37358A) was added to ordinary fly medium at the specified molar concentration. Adult flies were transferred from normal medium to the vial containing glucose food or DCA-supplemented food. The 3rd instar larvae of the next generation were analyzed.

Appendix Supplemental Figure Legends

Appendix Figure S1. Lipolysis activities of *dSeipin* mutants are not significantly different from the wild-type control

^{3}H-labeled fatty acid released from ^{3}H-labeled TO was measured to determine the rate of lipolysis (*n*=3, 20 independent fat bodies for each repeat). Error bars represent ±SEM and statistical analyses were performed with Mann-Whitney test. ns: p>0.05.

Appendix Figure S2. Depletion of human PKM2 reduces fat storage in HepG2 cells

(A) Expression levels of various pyruvate kinases in HepG2 cells, as determined by qRT-PCR (*n*=3). The *TBP* gene, which encodes TATA-binding protein, was used as the internal control. Error bars represent ±SEM.

(B) HepG2 cells transfected with *PKM2* siRNAs. dsRED is a cotransfection maker. BODIPY staining labels lipid droplets. DAPI staining labels cell nuclei. White arrows indicate the positively transfected cells. Scale bar represents 20 μm.

Appendix Figure S3. Various genetic manipulations rescue the lipodystrophy caused by *SERCA* RNAi

Overexpressing *PyK* or *ERR*, or knockdown of *CG18660*, rescues the lipid storage defects in *SERCA* RNAi animals, while *MCU* overexpression fails to do so. Lipid droplets are stained by Nile red and nuclei are stained by DAPI.

UAS-eGFP-Moesin-actin binding domain (*GMA*) is used as the *UAS* control. Scale bar represents 50 μm.

Appendix Figure S4. Glycolytic gene transcripts are not reduced in *dSeipin* mutant fat bodies

(A) Relative mRNA levels of genes involved in glycolysis and the TCA cycle in *dSeipin* mutant fat bodies compared to wild type (WT). Data are from RNA-seq.

(B-D) Gene set enrichment analyses of RNA-seq data. The glycolysis-gluconeogenesis gene set (B) and the pyruvate metabolism gene set (C) are

not enriched, while the calcium signaling gene set (D) scores highly in this analysis. NES denotes normalized enrichment score. FDR denotes false discovery rate.

Appendix Figure S5. External glucose cannot rescue the lipodystrophy of *dSeipin* mutants

BODIPY staining labels lipid droplets and DAPI labels the nuclei of 3rd instar larval fat body. 3% or 10% glucose supplement does not rescue the fat body lipid storage phenotype of *dSeipin* mutants. Scale bar represents 50 μm.

Appendix Figure S6. ERMES is important for lipid storage

(A) Rhod-2 staining indicates the mitochondrial calcium levels in fat bodies from 3rd instar larvae with RNAi knockdown of different ERMES factors and MCU.

(B) Mean red fluorescence intensity per unit area measured by ImageJ software. Related to (A). *n*=6, error bars represent ±SEM and statistical analyses were performed with one-way ANOVA with a post-Dunnett's multiple comparison test. Each RNAi column is compared to the control column. ***P<0.001.

(C) BODIPY staining labels lipid droplets and DAPI labels the nuclei. Fat tissue-specific knockdowns of several ERMES factors and MCU result in decreased lipid storage.

Scale bar represents 50 μm (A and C).

Appendix Figure S7. Subcellular localization analysis of CG18660

(A) EGFP-fused CG18660 partially colocalizes with a mitochondrial marker in S2 cells. Mitochondria are labeled by anti-ATP5A antibody. DAPI staining labels the nuclei. The box in each top right corner is the zoomed picture of the region enclosed by the white frame. Scale bar represents 5 μm.

(B) Colocalization analysis calculated by Pearson's correlation coefficient (R). Related to (A). *n*=3, error bars represent ±SEM.

Appendix Figure S8. DCA rescues the lipid storage defect of *dSeipin* mutant

(A) BODIPY staining labels lipid droplets and DAPI labels the nuclei of 3rd instar larval fat bodies. Two different doses of DCA were used, and both can rescue the lipid storage phenotype of *dSeipin* mutants. Scale bar represents 50 μm.

(B) Determination of TAG levels in 3rd instar larval fat bodies of wild type and *dSeipin* mutants reared on different foods, related to (A) (n=6, ⩾10 independent fat bodies for each repeat).

Error bars represent ±SEM and statistical analyses were performed with one-way ANOVA with a post-Tamhane's T2 test. ***P<0.05.

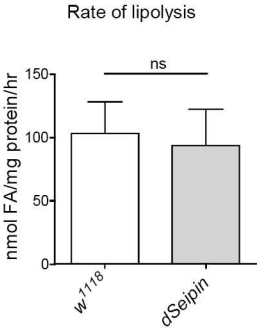
Appendix Table S1. Metabolic profiling of wild type versus *dSeipin* mutant fat bodies

Metabolomic profiling reveals changes in amino acids and TCA cycle and glycolysis metabolites in *dSeipin* mutant fat bodies compared to w^{1118}. Control and *dSeipin* mutant 3rd instar larval fat tissue extracts were subjected in quintuplicate to analysis on GC/MS platforms to compare the relative levels of metabolites. The fold change and t-test p value are shown for each metabolite.

Reference

Schweiger M, Eichmann TO, Taschler U, Zimmermann R, Zechner R, Lass A (2014) Measurement of lipolysis. *Methods Enzymol* 538: 171-193

Appendix Figure S1



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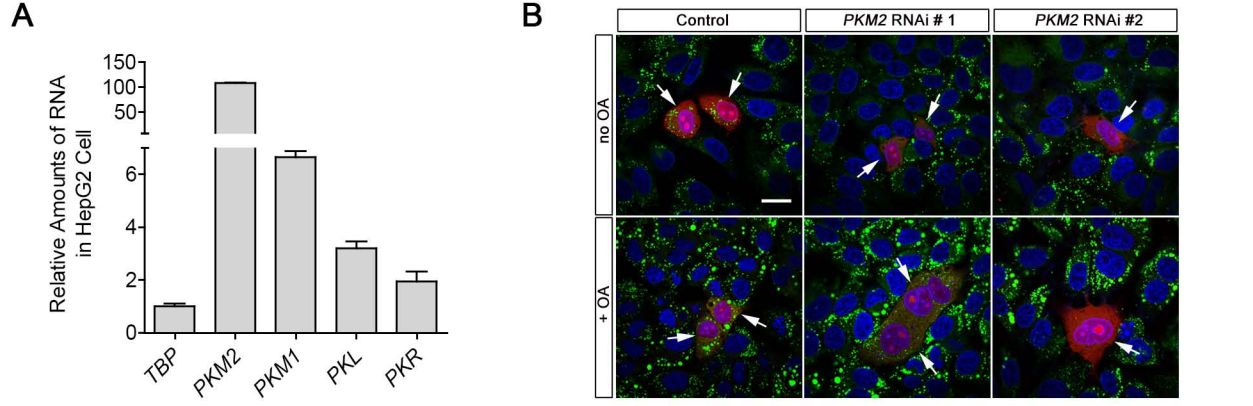

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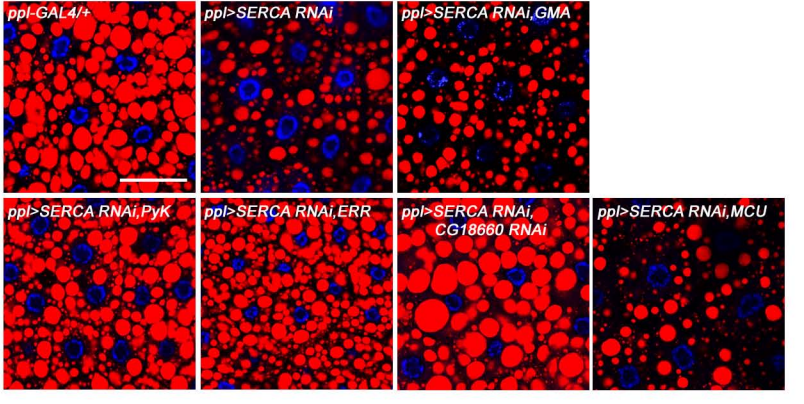

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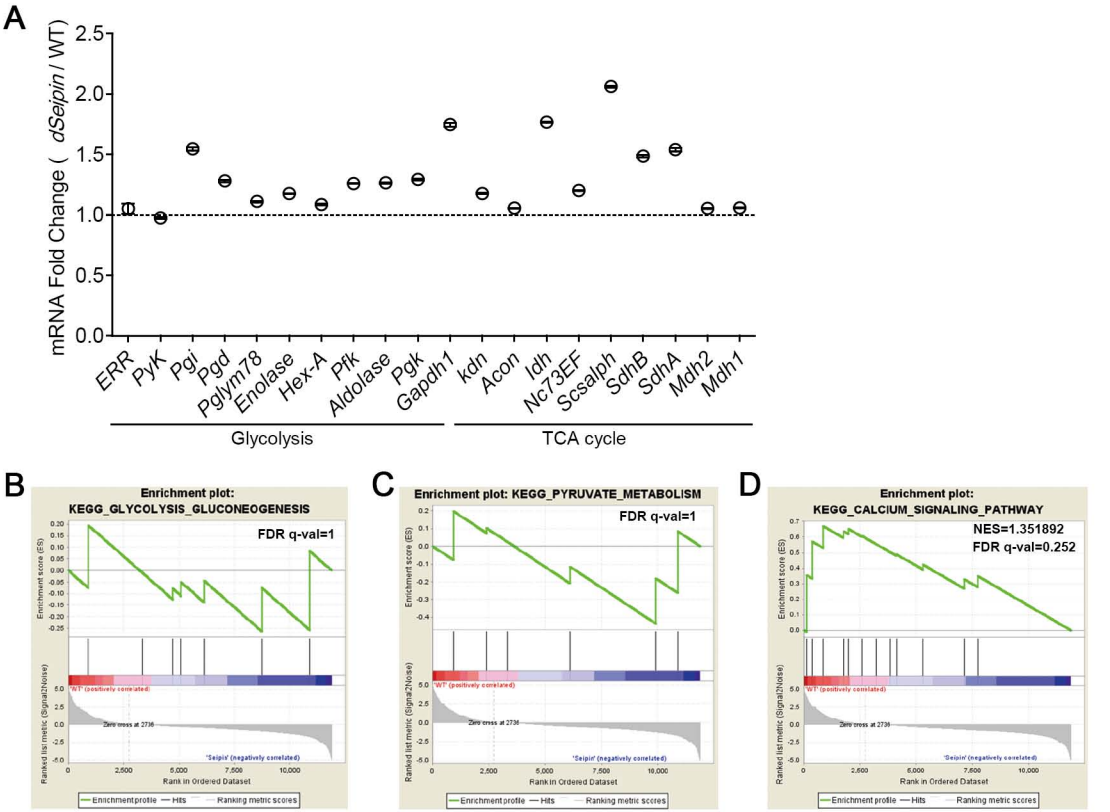

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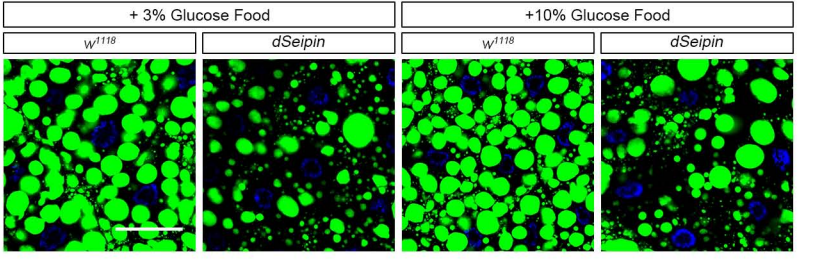

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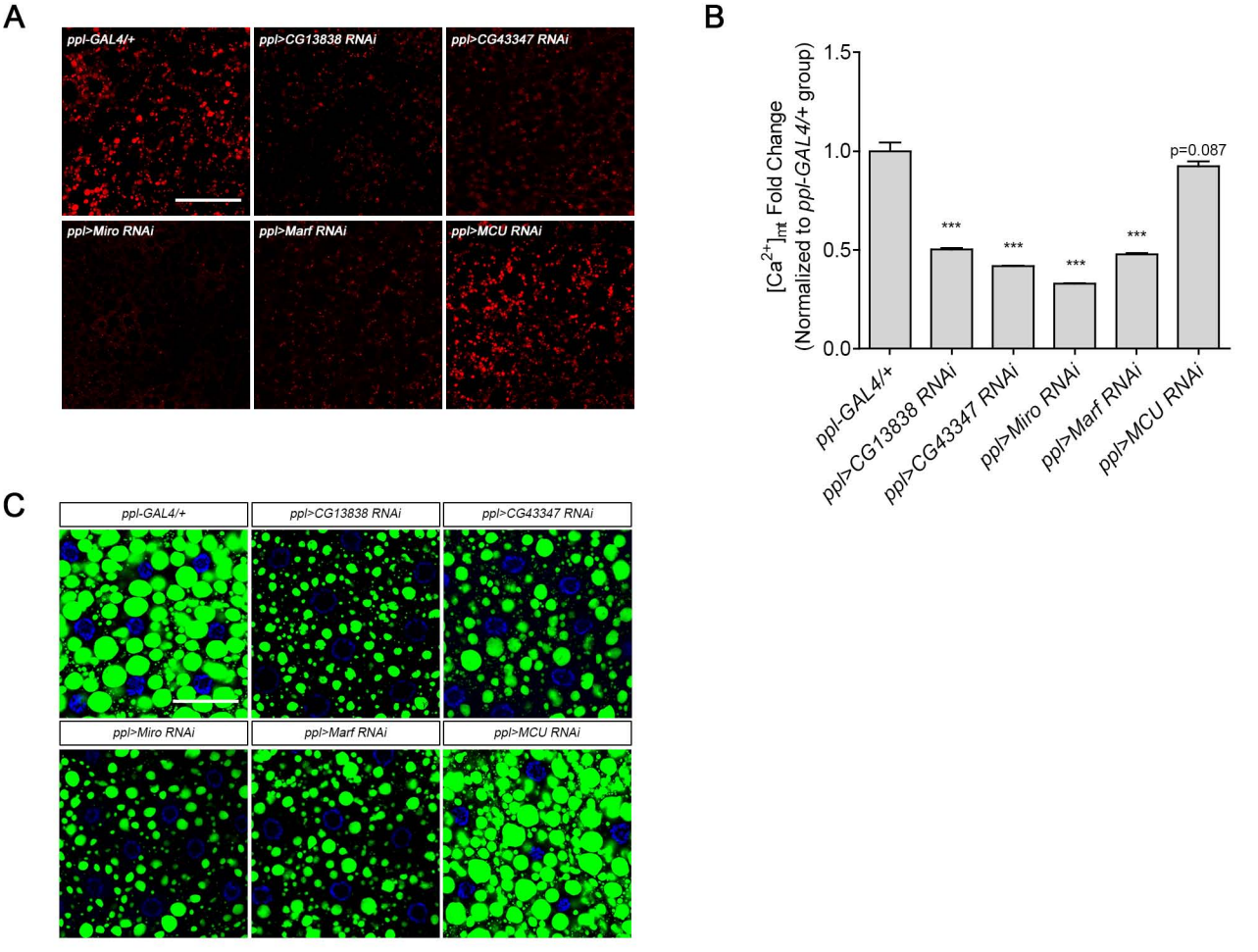
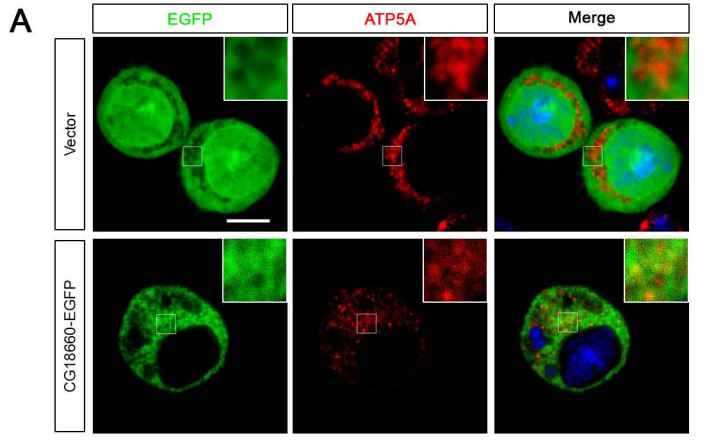
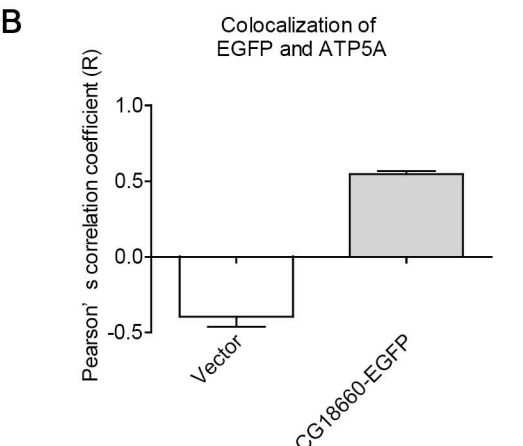
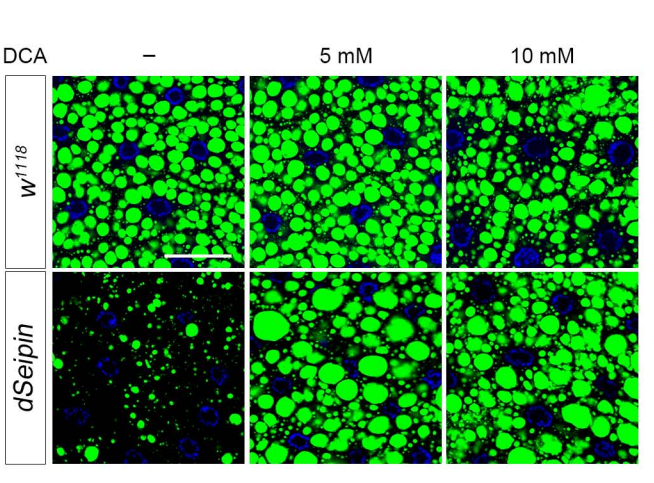


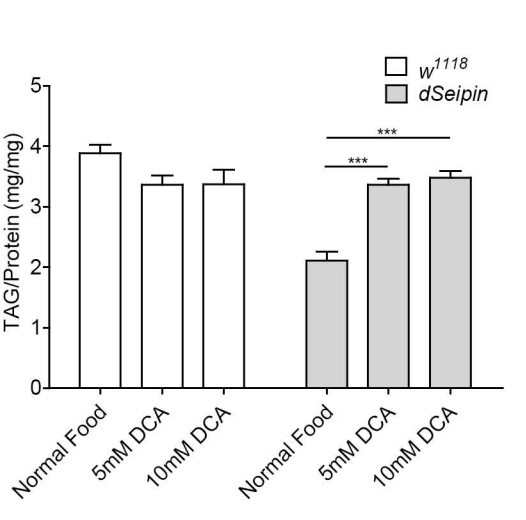
Appendix Figure S7





Appendix Figure S8





Appendix Table S1

	Metabolite	Fold change (*dSeipin*/WT)	p value			Metabolite	Fold change (*dSeipin*/WT)	p value
AMINO ACID					**ORGANIC ACID and OTHER**			
	L-valine	2.2057	0.0063			D-ribose-5-phosphate	1.4556	0.0370
	L-alanine	2.4390	0.0030			glycolic acid	2.7505	0.0320
	L-leucine	2.1749	0.0090			oxalic acid	1.2202	0.2778
	L-proline	3.0253	0.0016			urea	1.2484	0.2770
	L-serine	2.0724	0.0018			phosphoric acid	1.8566	0.0006
	DL-isoleucine	2.2919	0.0060			glycerol	1.3939	0.1468
	glycine	1.4796	0.0519			Putrescine	1.7391	0.0075
	L-threonine	3.0583	0.0208			glycerol 1-phosphate	1.0999	0.5445
	Homocysteine	3.4269	0.1058			O-phosphocolamine	0.9982	0.9905
	Beta- alanine	2.0397	0.0126			6-phosphogluconate	1.6243	0.0485
	aspartic acid	3.0365	0.0060			citrulline	2.1477	0.0008
	Pyroglutamic acid	1.8201	0.0013			Pantothenic acid	2.2779	0.0124
	L-glutamic acid	0.9571	0.8197			gluconic acid	1.6428	0.0456
	Phenylalanine1	2.3643	0.0010			cytindine-5-monosphate	2.4394	0.0030
	α-Hydroxyglutaric acid	1.5336	0.0489			palmitoleic acid	1.2468	0.2498
	Ornithine	2.2778	0.0051			L-DOPA	4.8216	0.0003
	L-asparagine	1.4097	0.1703			uric acid	2.7281	0.0052
	L-glutamine	2.1908	0.0005			6-phosphogluconate	1.6243	0.0485
	tyrosine	0.9158	0.9059			inosine	2.1271	0.0031
	L-histidine	7.8939	0.0010			Adenosine-5-monophaphate	3.2141	0.0484
	L-lysine	4.1460	0.0007			sorbose	0.4316	0.0212
	L-tryptophan	2.1837	0.0116			D-(+) trehalose	1.5942	0.0234
TCA CYCLE						myo-inositol	3.1305	0.0002
	citric acid+isocitric acid	0.0767	0.0295			talose	4.6295	0.0016
	fumaric acid	0.1002	0.0019			maltose	2.1611	0.0042
	D-malic acid	0.1386	0.0136			isomaltose	2.6297	0.0101
	alpha ketoglutaric acid	1.6197	0.2346			mannose	4.3824	0.0001
	succinic acid	1.7848	0.0209					
GLYCOLYSIS								
	pyruvate	3.1178	0.0006					
	L-(+) lactic acid	2.5358	0.0106					
	gluctose-6-phophate	1.9990	0.0329					
	3-phosphoglyceric acid	4.6250	0.0020					