Supplemental Tables and Legends

Table s1. qRT-PCR primers. Abbreviations: ATP synthase (*Atp5o*), branched-chain aminotransferase 2 (*Bcat2*), branched-chain alpha-keto acid dehydrogenase (*Bckdha*), CCAAT/enhancer-binding protein alpha (*Cebpa*), cytochrome C oxidase Subunit 5A (*Cox5a*), glucose transporter 4 (*Slc2a4* or *Glut4*), hydroxyisobutyrate dehydrogenase (*Hibadh*), lactate dehydrogenase a (*Ldha*), lactate dehydrogenase b (*Ldhb*), carbohydrate response element binding protein as MLX interacting protein-like (*Mlxipl*),nuclear respiratory factor 1 (*Nrf1*), pyruvate dehydrogenase (*Pdh*), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*Ppargc1a*), peroxisome proliferator-activated receptor gamma (*Pparg*), sterol regulatory element-binding protein (*Srebp1*), Tata box binding protein (*Tbp*), and mitochondrial transcription factor A (*Tfam*).

Gene Abbreviation	Forward Sequence	Reverse Sequence
Atp5o	5'-AGGCCCTTTGCCAAGCTT-3'	5'-TTCTCCTTAGATGCAGCAGAGTACA-3'
Bcat2	5'-CGGACCCTTCATTCGTCAGA-3'	5'-CCATAGTTCCCCCCCAACTT-3'
Bckdha	5'-CCAGGGTTGGTGGGATGAG-3'	5'-GGCTTCCATGACCTTCTTTCG-3'
Cebp	5′-GTGTGCACGTCTATGCTAAACCA-3'	5′-GCCGTTAGTGAAGAGTCTCAGTTTG-3'
Cox5a	5'-GCTGCATCTGTGAAGAGGACAAC-3'	5'-CAGCTTGTAATGGGTTCCACAGT-3'
Slc2a4(Glut4)	5'-GATGAGAAACGGAAGTTGGAGAGA-3'	5'-GCACCACTGCGATGATCAGA-3'
Hibadh	5'- GCAGCGGTGTGTTCTAGGTC-3'	5'- ACACGTCATAGAGGATGAGTGG-3'
Ldha	5′-GGCTTGTGCCATCAGTATCT-3'	5′-CCCGCCTAAGGTTCTTCATTAT-3′
Ldhb	5'-AGTCTCCCGTGCATCCTCAA-3'	5'-AGGGTGTCCGCACTCTTCCT-3'
Nrf1	5'-ACCCTCAGTCTCACGACTAT-3'	5'-GAACACTCCTCAGACCCTTAAC-3'
Mlxipl	5′-CACTCAGGGAATACACGCCTAC-3′	5′-ATCTTGGTCTTAGGGTCTTCAGG-3′
Pdh	5'-GAAGGCCCTGCATTCAACTTC-3'	5'-ATAGGGACATCAGCACCAGTGA-3'
Ppargc1a	5'-GACAATCCCGAAGACACTACAG-3'	5'-AGAGAGGAGAGAGAGAGAGAGA-3'
Pparg	5′-TTCAGCTCTGGGATGACCTT-3'	5′-CGAAGTTGGTGGGCCAGAAT-3'
Srebp1	5′-ATCGCAAACAAGCTGACCTG-3'	5′-AGATCCAGGTTTGAGGTGGG-3'
Tbp	5'-GGGATTCAGGAAGACCACATA-3'	5'-CCTCACCAACTGTACCATCAG-3'
Tfam	5'-GAAGGGAATGGGAAAGGTAGAG-3'	5'-ACAGGACATGGAAAGCAGATTA-3'

Table s2 Primary antibodies used for western blot experiments. Abbreviations: branched-chain aminotransferase 2 (BCAT2), branched-chain alpha-keto acid dehydrogenase E1α (BCKDHE1α), carbohydrate response element binding protein (ChREBP), fatty acid synthase (FAS), goat polyclonal (GP), horse radish peroxidase conjugate (HRP), mouse monoclonal (MM), oxidative phosphorylation (OXPHOS), rabbit polyclonal (RP), and Sterol regulatory-element binding protein 1 c (SREBP1c). *Notes: Target molecular weight was based on product datasheet. Molecular weights for all targets were verified against sizes suggested by product brochures. * Indicates band is observed at 68 kd.† indicates this molecular weight corresponds to mature SREBP1.*

Antibody Target	***Type***	***Dilution***	***Company***	***Item***	***Approx. Mol Wt.***	***Product Link***
pAkt (Ser 473)	RP	1:1000	SC Biotechnology	sc-7985-R	62kd	p-Akt1/2/3 (Ser 473)
Akt	RP	1:1000	Cell Signaling	9272	62kd	Akt Antibody#9272
β-Actin	MM	1:500	SC Biotechnology	sc-47778	43kd	Datasheet
BCAT2	RP	1:1000	Bioss	BS-6589R	44kd	Datasheet
pBCKDHa(Ser293)	RP	1:1000	AbCam	ab200577	50kd	Phospho BCKDHA (S293)
BCKDHa	RP	1:1000	ProSci	31-325	50kd	BCKDHA Antibody
ChREBP	GP	1:1000	SC Biotechnology	sc-21189	62/78/91/93 kd	ChREBP (P-13): sc-21189
FAS	RP	1:1000	SC Biotechnology	sc-20140	270kd	Fatty Acid Synthase
HIBADH	MM	1:1000	SC Biotechnology	Sc-398288	35kd	Datasheets
PPARγ	RP	1:1000	SC Biotechnology	sc-7196	57kd*	PPARγ (H-100): sc-7196
SREBP1c	RP	1:1000	SC Biotechnology	sc-366	68kd†	SREBP-1 (C-20): sc-366
Total OXPHOS	MM	1:1000	AbCam	ab110413	Varied	Total OXPHOS
Goat Anti-Rabbit IgG	HRP	1:5000	AbCam	ab6721	NA	ab6721
Rabbit Anti-Mouse IgG	HRP	1:5000	AbCam	ab6728	NA	ab6728

Table s3 General chemicals and reagents used in experiments.

EXPERIMENT	DESCRIPTION	VENDOR	PRODUCT #
CELL CULTURE	D-Fructose	Sigma	F0127-100G
CELL CULTURE	Insulin	Sigma	I6634-50MG
CELL CULTURE	DMEM Cell Culture Media	Lonza	95042-512
CELL CULTURE	Avantor® Seradigm, Select Grade Fetal Bovine Serum (FBS)	Avantor	1300-500
CELL CULTURE	Donor Horse Serum	Corning	35-030-CV
CELL CULTURE	Penicillin : Streptomycin 100X	Avantor	SV30010
CELL CULTURE	Eiosin Y	Sigma	E4009-5G
SEAHORSE ASSAY	Seahorse XFe96/XF Pro FluxPak	Agilent	103792-100
SEAHORSE ASSAY	FCCP	Enzo Life Sciences	BML-CM120-0010
SEAHORSE ASSAY	Rotenone	Enzo Life Sciences	350-360-G001
SEAHORSE ASSAY	Oligomycin	Enzo Life Sciences	380-037-M010
SEAHORSE ASSAY	D-Glucose	Sigma	G8769-100ML
SEAHORSE ASSAY	Sodium Pyruvate	Sigma	S8636-100ML
SEAHORSE ASSAY	L(+)-Glutamine 200 mM 100X	VWR	VWRL0131-0100
FLUORESCENT STAINING	DAPI	Biotium	40009
FLUORESCENT STAINING	NONYL ACRIDINE ORANGE	Biotium	70012
FLUORESCENT STAINING	Nile Red	TCI America	N0659-500MG
QRT-PCR	iScript™ cDNA Synthesis Kit	Biorad	1708891
QRT-PCR	iQ™ SYBR® Green Supermix	Biorad	1708886
QRT-PCR	Microseal 'B' PCR Plate Sealing Film	Biorad	MSB1001
QRT-PCR	Hard-Shell® 96-Well PCR Plates,	Biorad	HSP9601
WESTERN BLOT	BUFFER RIPA LYSIS/EXTRACTION 100ML	G-Bioscience	786-489
WESTERN BLOT	Protease Inhibitor Cocktail	Avantor	M306-5ML
WESTERN BLOT	Precision Plus Protein Dual Color Standards	Biorad	1610374
WESTERN BLOT	Clarity Western ECL Substrate	Biorad	1705061

Supplemental Figure

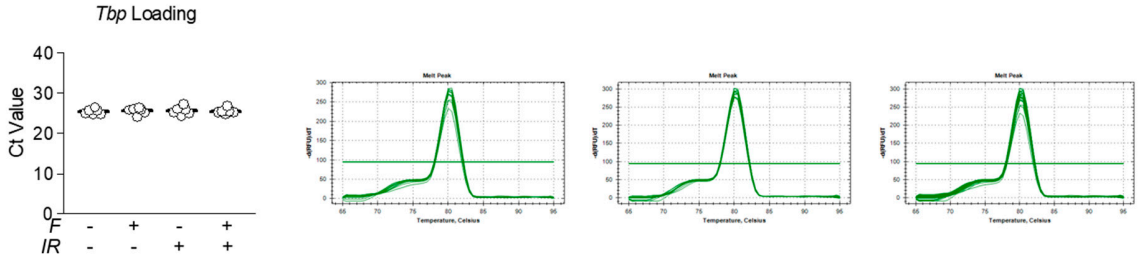


Figure s1 Effect of fructose with and without insulin resistance on myotube qRT-PCR loading control. Effect of fructose at 100µM for 24 hours with and without insulin resistance (IR) on mRNA expression of Tata binding protein (*Tbp*) with melt curves from two independent experiments (left and middle melt curves), and combined (right melt curve).

Notes: Data were analyzed using two-way ANOVA followed by one-way ANOVA with Bonferroni's correction for multiple comparisons used to assess differences in gene expression, protein expression, and lipid content. No statistical differences were observed. Gene expression was conducted using 3 replicates per group across 2 independent cell culture experiments with n=6 for the final analysis.

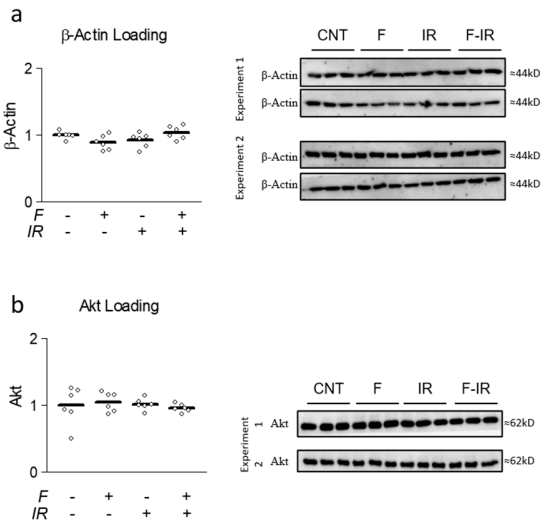


Figure s2 Effect of fructose with and without insulin resistance on myotube Western blot loading control. (a and b) Effect of fructose at 100µM for 24 hours with and without insulin resistance (IR) on protein expression of (a) β-actin or (b) Akt.

Notes: Data were analyzed using two-way ANOVA followed by one-way ANOVA with Bonferroni's correction for multiple comparisons used to assess differences in protein expression. No statistical differences were observed. Protein expression was conducted using 3 replicates per group across 2 independent cell culture experiments with n=6 for the final analysis.

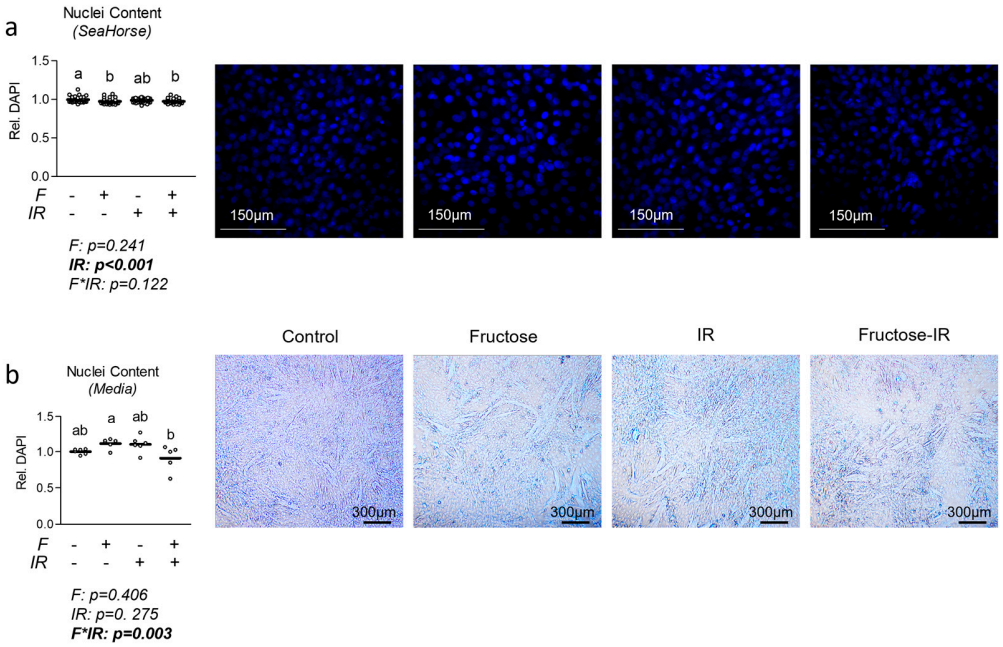


Figure s3 Effect of fructose with and without insulin resistance on myotube nuclei content. (a and b) Effect of fructose at 100µM for 24 hours with and without insulin resistance (IR) on myotube nuclei content from (a) Seahorse assays or (b) media collection experiments.

Notes: Data were analyzed using two-way ANOVA followed by one-way ANOVA with Bonferroni's correction for multiple comparisons used to assess differences in protein expression. Groups with dissimilar letters indicate $p<0.05$ between groups. Nuclei content measurements for Seahorse assays were performed using n=23 individual replicates per treatment condition and were repeated across 2 independent cell culture experiments with n=46 per group in the final analyses. Nuclei content measurements for media collection were performed using n=3 individual replicates per treatment condition and were repeated across 2 independent cell culture experiments with n=5-6 per group in the final analyses.