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Supplementary Information for

PGC1A regulates the IRS1:IRS2 ratio during fasting to influence hepatic metabolism downstream of insulin

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SUPPLEMENTAL METHODS

Animals

Conditional floxed *Ppargc1a* KO mice (B6.129-*Ppargc1a*$^{tm2.1Brsp}$/J or *Ppargc1a*$^{f/f}$) were bred with Tg(Alb-cre)$^{21Mgn/J}$ (Alb-cre$^{+/0}$) mice expressing Cre recombinase under the hepatocyte-specific albumin promoter. We generated liver-specific heterozygote mice (LH; *Ppargc1a*$^{f/+}$Alb-cre$^{+/0}$) or liver-specific KO mice (LKO; *Ppargc1a*$^{f/f}$ Alb-cre$^{+/0}$), on a mixed background of C57BL/6J, C567BL/6N and 129/Sv. Mice carrying floxed *Ppargc1b* alleles, encoding PGC1B were bred with the LKO mice to generate liver-specific double knockout mice (LA/BKO) or controls (*Ppargc1a*$^{f/f}$-*Ppargc1b*$^{f/f}$). Mice were fed regular chow (18% kcal from fat content, Teklad Global diet 2018, Envigo). Animals were allowed *ad libitum* access to food and maintained at 22^{o}C (12h light, 12h dark cycle). All experiments were performed in accordance with IRCM animal facility institutional animal care and use committee regulations.

Primary Hepatocytes Isolation and Culture

Primary mouse hepatocytes from 10- to 12-week-old male and female mice were isolated by two-step liberase perfusion (Liberase TL, Roche) and 50% percoll gradient purification. Cells were plated in monolayers and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 0.2% BSA (Fatty acid free, Fisher Scientific), 5.5 mM glucose, 2 mM sodium pyruvate, 0.1 μM dexamethasone, 1% penicilin/streptomycin and 1 nM insulin for up to 48 hours. Primary hepatocytes were infected with adenoviruses with minimal multiplicity of infection ensuring 100%

infection of cells. Equal titers were used for all viruses. After overnight incubation with virus, media was exchanged for regular maintenance and cells were cultured for an additional 36 hours for over-expression and 60 hours for knock-down. To assess insulin or glucagon response, cells were incubated overnight in high glucose (25 mM) media lacking dexamethasone and insulin. Insulin and glucagon were added at indicated concentrations and times.

Immunoblotting

Proteins were isolated in radioimmunoprecipitation assay buffer containing protease and phosphatase inhibitors (Roche). Samples were resolved by SDS-PAGE, blotted, and probed with antibodies. Anti-Phospho-Ser473 AKT (#9271, 1/2000), total AKT (#9272, 1/2000), Phospho-Y1146 IRβ (#3021, 1/1000), IRS2 (#4502, 1/2000), and TCF4 (#2569, 1/2000) were purchased from Cell Signaling. Anti-Beta actin was from Millipore Sigma (#A5316, 1/10000), PGC1A from Millipore Sigma (#ST1202, clone 4C1.3, 1/1000) and IRS1 from Bethyl Laboratories (#A301-158A, 1/1000).

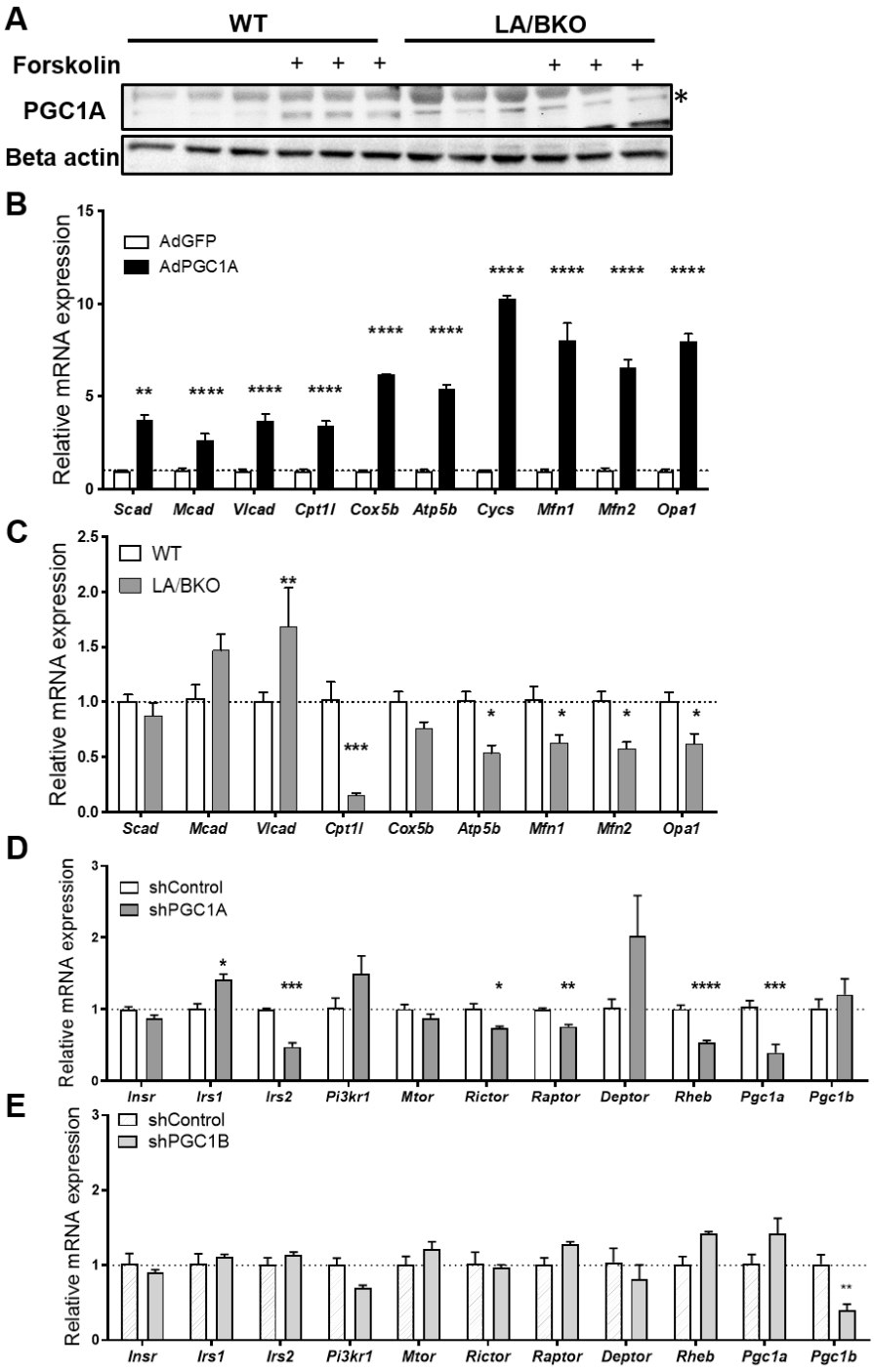

Fig. S1. PGC1 levels and effects on gene expression

Immunoblot of PGC1A in WT and LA/BKO primary hepatocytes treated or not with forskolin (known PGC1A inducer) * is a non specific band, non inducible protein. Gene expression measured by qPCR in primary hepatocytes infected with adenoviruses encoding for vector Control, B) shPGC1A, C) shPGC1B, D) PGC1A, or (E) primary hepatocytes isolated from wild type (WT) or liver specific PGC1A and PGC1B double knockout mice (LA/BKO). * $P < .05$, ** $P < .01$, ***$P < .001$, ****$P < .0001$ vs control. N=6±SEM

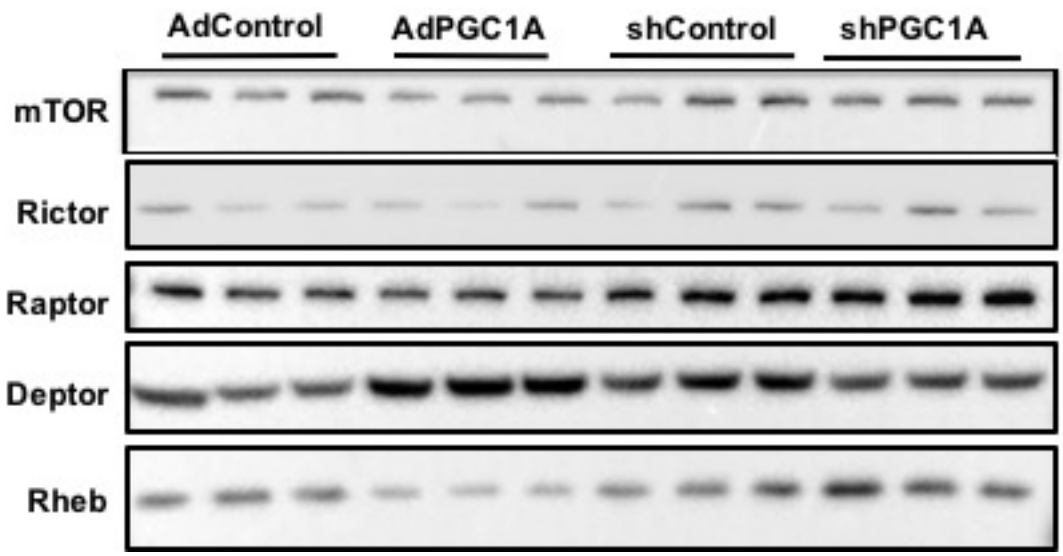

Fig. S2. Effect of PGC1A levels on Mtor pathway

Immunoblots of mTOR pathway members (MTOR, RICTOR, RAPTOR, DEPTOR, RHEB) in primary hepatocytes. Cells were infected with adenoviruses encoding vector control or PGC1A, or short hairpin control or against PGC1A.

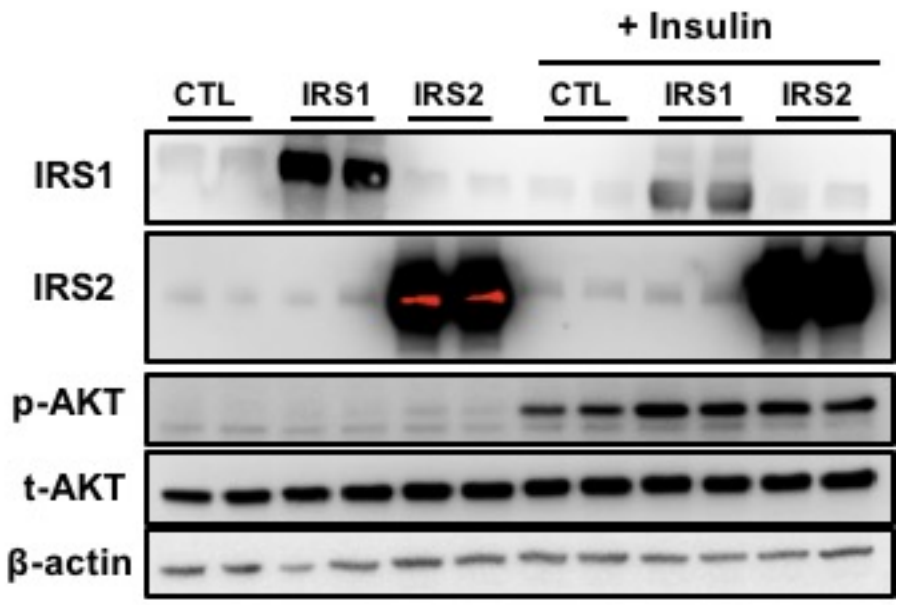

Fig. S3: Increased IRS1 or IRS2 potentiate activation of AKT in response to insulin.

Primary hepatocytes were infected with adenoviruses encoding a vector control (CTL), IRS1 or IRS2. A) Immunoblots of IRS1, IRS2, p-AKT, t-AKT and a loading control. Cells were starved overnight and treated with 100 nM insulin or vehicle for 15 min.

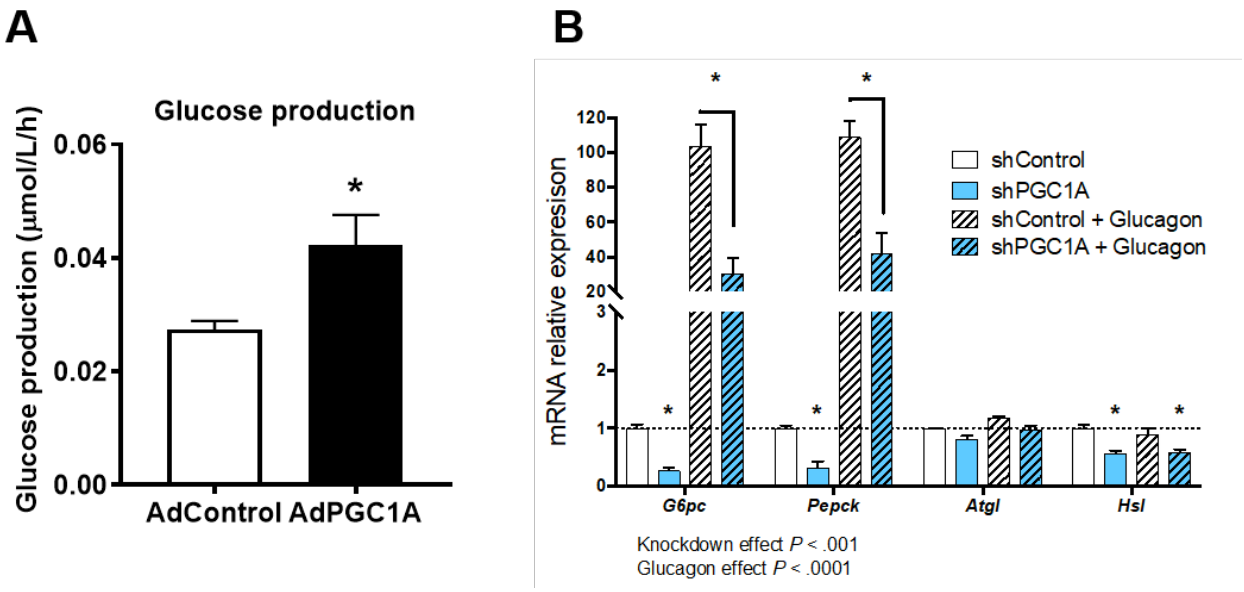

Fig. S4. Effect of PGC1A on glucose production and glucagon-induced gene expression

A) Glucose production in primary hepatocytes infected with a control vector or an adenovirus encoding for PGC1A. B) Gene expression in primary mouse hepatocytes following acute knockdown of PGC1A by shRNA, treated or not with glucagon (2h, 40 nM). N=4±SEM. * P < .05 vs control. Experiments were performed at least twice

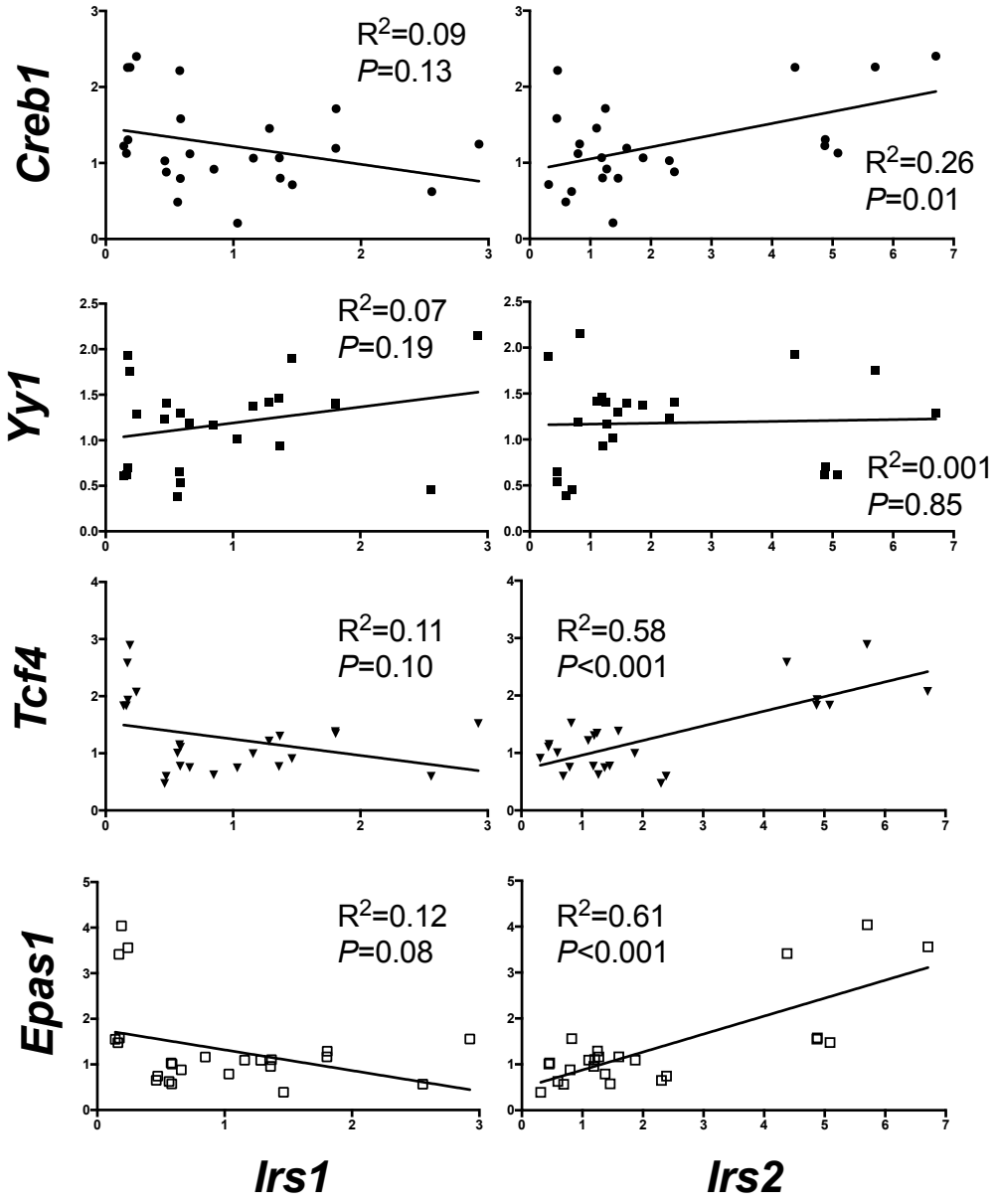

Fig. S5. Correlation of *Irs1* and *Irs2* gene expression with candidate transcription factors *Creb1, Yy1*, *Epas1*, *Tcf4* in primary hepatocytes expressing PGC1A to various degrees. After adjusting for multiple comparisons (Bonferroni), threshold for significance is $P < .005$

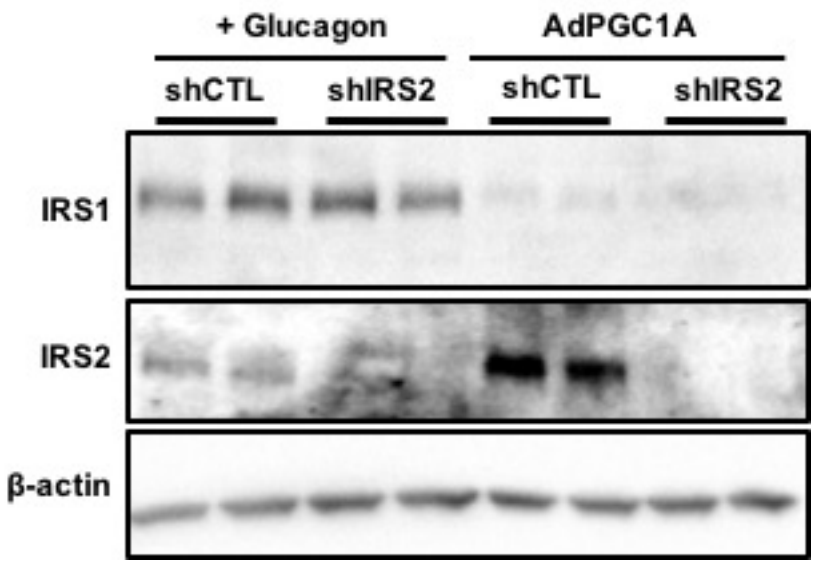

Fig. S6. Validation of IRS2 knockdown.

Immunoblots of IRS1, IRS2 and a loading control in primary hepatocytes. Cells were infected with shRNA with a control sequence not recognizing any known mouse RNA (shCTL) or sh against IRS2 (shIRS2) and treated with 40 nM glucagon for 4 hours or coinfected with adenovirus expressing PGC1A, as indicated.

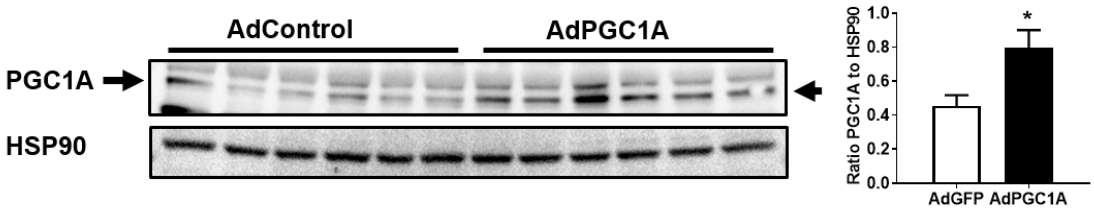

Fig. S7. Validation of PGC1A overexpression in liver.

Immunoblots of PGC1A and a loading control (HSP90) in liver. Mice were injected with $2x10^9$ infectious units intravenously with adenoviruses encoding for GFP (AdControl) or PGC1A (AdPGC1A). Mice were euthanized after a 6h fast and livers collected and immediately frozen in liquid nitrogen. Right panel is quantification by densitometry. N=6 + S.E.M. * $P < .05$

Table S1. Primers sequences

F: forward primer R: reverse primer

Hprt	F: GGC CAG ACT TTG TTG GAT TTG R: TGC GCT CAT CTT AGG CTT TGT
Insr	F: TGT GGC CTG TCG CAA CTT CTA TCT R: AGT GAA GGT CTT GGC AGA AGC TGA
Irs1	F: AGC GCG CCT GGA GTA TTA TGA GAA R: GTC AGC CCG CTT GTT GAT GTT GAA
Irs2 #1	F: AAA GTG GCC TAC AAC CCT TAC CCA R: TCA TCG CTC TTG CAG CTA TTG GG
Irs2 #2	F: AGC CAG GAG ACA AGA ACT CC R: AGT GAT GGG ACA GGA AGT CG
Pi3kr1	F: AAG GAG CTG GTG CTA CAT TAT C R: CGC CTC TGT TGT GCA TAT ACT
Deptor	F: GGT GGT TCT CAG GCA TTC TAT C R: TTT GAG ATG GTG CCG TCT ATC
Mtor	F: CGG GAC TAC AGA GAG AAG AAG A R: CAT CAA CGT CAG GTG GTC ATA G
Rictor	F: GCT CGT GGG CAG GTA TTA TT R: GAG CAG ACC TCG CCT TAT TT
Raptor	F: CCT GGA GTC ACA CTG GAT TTG R: GGT ATC TGT GAT GGC TGT GAA G
Rheb	F: GTC TGT GGG AAA GTC CTC ATT R: GTG TTC TCT ATG GTT GGA TCG T
PGC1A KO validation	F: AGC CGT GAC CAC TGA CAA CGA G R: GCT GCA TGG TTC TGA GTG CTA AG
Ppargc1a	F: GGA CAT GTG CAG CCA AGA CTC T R: CAC TTC AAT CCA CCC AGA AAG CT
Ppargc1b	F: AGT CAG CGG CCT TGT GTC AA R: ACT CTG GGA CAG GGC AGC A
Scad	F: ACC AAA GCT TGG ATC ACC AAC TCC R: AAC CAG GAA GGC ACT GAT ACC CTT
Mcad	F: AAC ACT TAC TAT GCC TCG ATT GCA R: CCA TAG CCT CCG AAA ATC TGA A
Vlcad	F: GGC CAA GCT GGT GAA ACA CAA GAA R: ACA GAA CCA CCA CCA TGG CAT AGA
Cpt1l	F: GAA CCC CAA CAT CCC CAA AC R: TCC TGG CAT TCT CCT GGA AT
Cox5b	F: ATG CTA CCT CCA AAG GCA GCT TC R: TGC AGC CCA CTA TTC TCT TGT TGC
Atp5b	F: CAC CAA GAA GGG ATC GAT CAC R: CAG GTC ATC AGC AGG CAC AT
Cycs	F: GCA AGC ATA AGA CTG GAC CAA A R: TGT TGG CAT CTG TGT AAG AGA ATC

Mfn1	F: CCT ACT GCT CCT TCT AAC CCA R: AGG GAC GCC AAT CCT GTG A
Mfn2	F: CCT ACT GCT CCT TCT AAC CCA R: AGG GAC GCC AAT CCT GTG A
G6pc	CAG GCA TTG CTG TGG CTG AAA CTT TAG CAG GTA GAA TCC AAG CGC GAA
Pck1	F: CAG GAT CGA AAG CAA GAC AGT R: AAG TCC TCT TCC GAC ATC CAG
Atgl	F: CAT GAT GGT GCC CTA TAC TCT G R: CTA CCC GTC TGC TCT TTC ATC
Hsl	F: CAT CAA CCA CTG TGA GGG TAA G R: AAG GGA GGT GAG ATG GTA ACT
Creb1	F: GCT CCC ACT GTA ACC TTA GTG R: GGA CTT GTG GAG ACT GGA TAA C
Yy1	F: GAA GAG CGG CAA GAA GAG TTA R: GCT TCT GCT CCC ACT TCT TAT
Tcf4	F: GCA TCC CTC ACC CGG CCA TC R: GCC ACC TGC GCC CGA GAA TC
Epas1	F: CAG CTT CCT TCG GAC ACA TAA R: CTC CAA GGC TTT CAG GTA CAA
Floxed PGC1A genotyping	F: TCC AGT AGG CAG AGA TTT ATG AC R: TGT CTG GTT TGA CAA TCT GCT AGG TC
Floxed PGC1B genotyping	F: TCT GAG CCT TCG GTT CTA CTT ACA R: ATC AAT CAA TCA ATC AAT CAA TCA ATCA