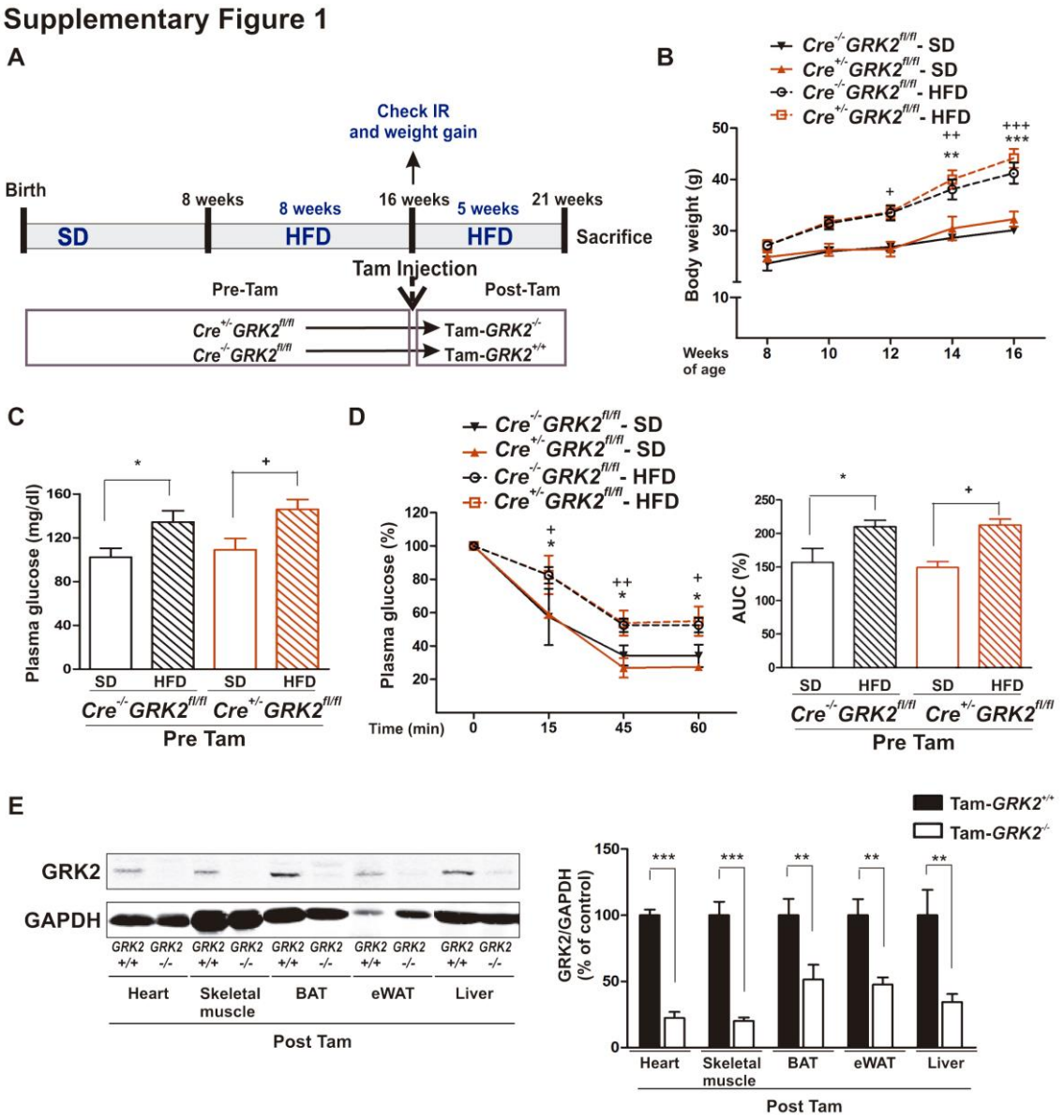
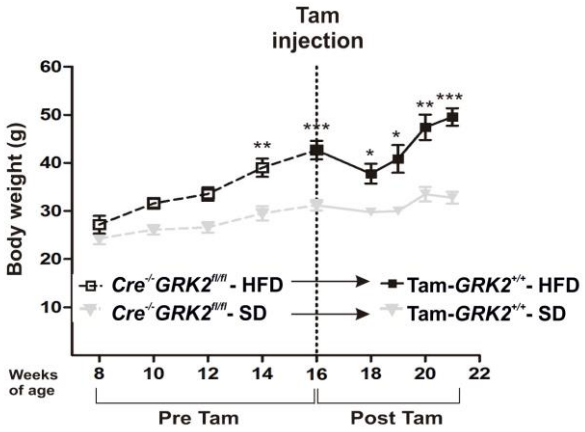


Supplementary Figure 1. Characterization of an obese and insulin-resistant phenotype after eight weeks of HFD, before tamoxifen treatment, and quantification of tamoxifen-induced GRK2 depletion. (A) Scheme of the experimental design: 8 weeks after birth, mice were initiated on a HFD and the establishment of insulin resistance and obesity was confirmed 8 weeks later; at this stage, mice were injected with tamoxifen, and maintained on HFD for 5 more weeks before sacrifice (totalling 13 weeks of HFD). SD, standard diet. Pre-Tam or Post-Tam, previous to or after tamoxifen injection respectively. (B) Body weight progression (g) during 8 weeks of standard (SD, solid lines) or high fat diet (HFD, dotted lines), before tamoxifen treatment in the indicated mouse

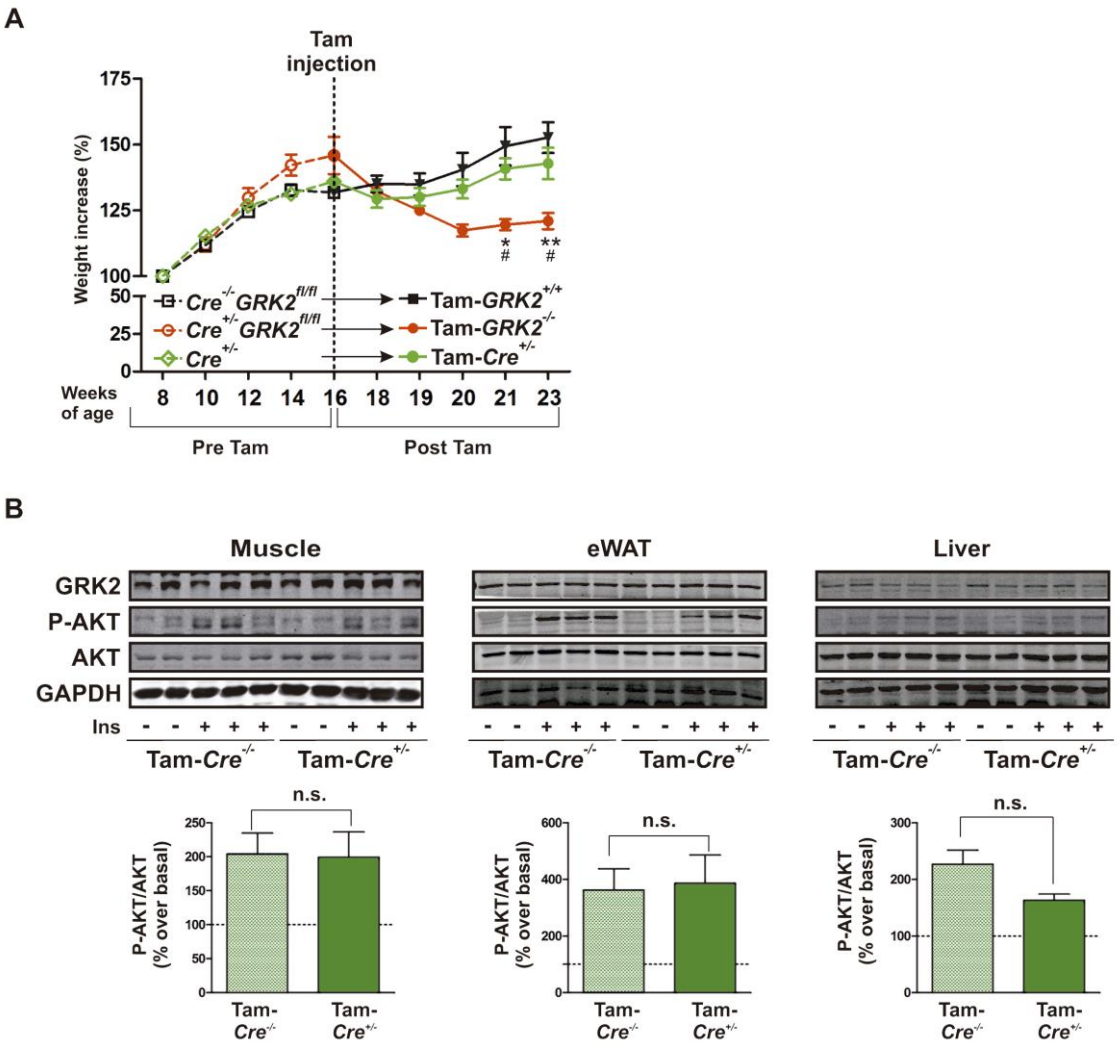
genotypes. (C-D) Circulating fasting glucose concentrations (C) and intraperitoneal ITTs (D) after 8 weeks of SD (solid lines) or HFD (dotted lines), before tamoxifen administration and histogram showing the ITT area under the curve (AUC). (E) Lysates of different tissues including heart, skeletal muscle, brown adipose tissue (BAT), epididymal white adipose tissue (eWAT) and liver were analyzed by Western Blot with antibodies against GRK2 and GAPDH Representative immunoblots and densitometric analysis are shown. Results are expressed as percent change from control ($Cre^{-/-}GRK2^{fl/fl}$) mice. For (B) to (E), results are means ± SEM of 6-7 mice per group. Statistical analysis was performed by two-way repeated measures ANOVA followed by Bonferroni's *post hoc* test (B) to (D) and unpaired two tailed t-test (E). *P < 0.05, **P < 0.01, ***P<0.005 compared to $Cre^{-/-}GRK2^{fl/fl}$ mice on a HFD. +P < 0.05, ++P < 0.01, +++P<0.005 compared to $Cre^{+/-}GRK2^{fl/fl}$ mice on a HFD. ++P < 0.01, +++P<0.005 compared to $Cre^{+/-}GRK2^{fl/fl}$ mice on a HFD.

Supplementary Figure 2



Supplementary Figure 2. HFD-induced weight gain in control mice compared with SD-fed mice. Body weight progression (g) during 8 weeks of standard (SD, grey lines) or high fat (HFD, black lines) diets, before (dotted lines) and after (solid lines) tamoxifen treatment in *Cre*$^{-/-}$*GRK2*$^{fl/fl}$ control mice. Results are means ± SEM of 6-7 mice per group. Statistical analysis was performed by two-way repeated measures ANOVA followed by Bonferroni's *post hoc* test. *P < 0.05; **P < 0.01; ***P<0.005

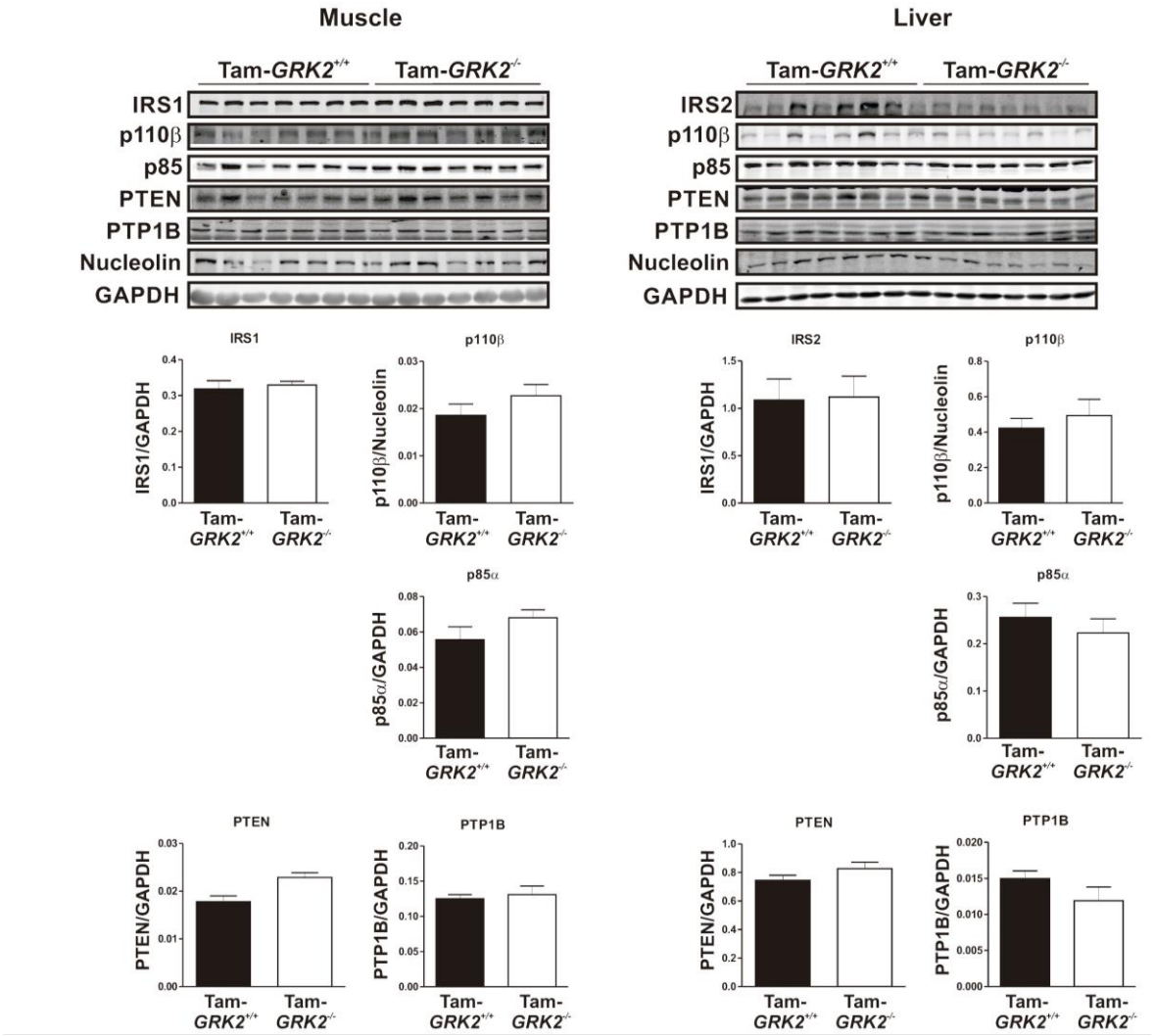
Supplementary Figure 3



Supplementary Figure 3. CreER expression or translocation to the nucleus does not affect body weight gain or insulin-induced phosphorylation of AKT in muscle, eWAT, or liver. (A) Body weight evolution (g) before (dotted lines) and after (solid lines) tamoxifen treatment in CreER-expressing *Cre*$^{+/-}$ mice, without floxed GRK2 alleles, compared to *Cre*$^{-/-}$*GRK2*$^{fl/fl}$ and *Cre*$^{+/-}$*GRK2*$^{fl/fl}$ mice pre- or post-tamoxifen treatment. Data are represented as means ± SEM of 5-7 mice per group. Statistical analysis was performed by two-way repeated measures ANOVA followed by Bonferroni's *post hoc* test. *P < 0.05, **P < 0.01 compared to Tam-*GRK2*$^{+/+}$; #P < 0.05 compared to Tam-*Cre*$^{+/-}$. (Open symbols, dotted lines = Pre-Tam; filled symbols, solid

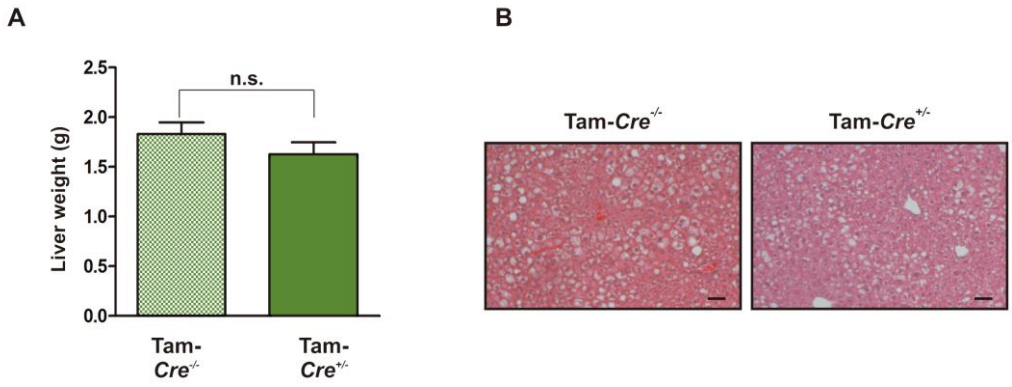
lines = Post-Tam; Black = $Cre^{-/-}GRK2^{fl/fl}$; Red = $Cre^{+/-}GRK2^{fl/fl}$; Green=$Cre^{+/-}$). (B) Mice that did or did not express Cre recombinase without floxed GRK2 alleles were treated or not with insulin for 10 min and tissue lysates from muscle, eWAT and liver were subjected to Western blot and probed with antibodies against GRK2, total and phosphorylated AKT (Ser^{473}), and GAPDH. Representative immunoblots and densitometric quantitations are shown. Results are expressed as % of stimulation over untreated mice (basal). Data are means ± SEM of 6 animals per genotype, 3 animals per treatment. Statistical significance was analyzed by unpaired two tailed t test. *$P < 0.05$

Supplementary Figure 4



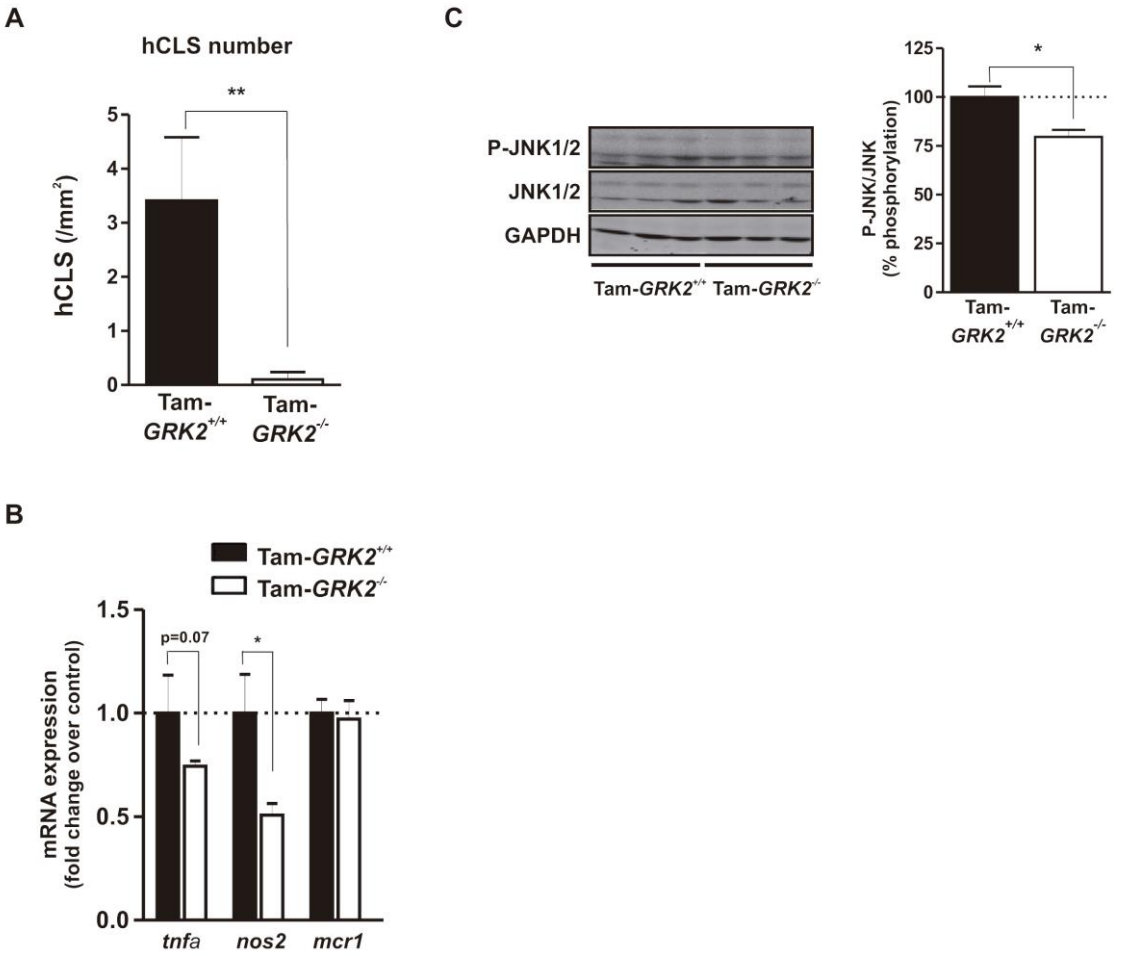
Supplementary Figure 4. The abundance of components in or inhibitors of the insulin signaling pathway is not changed upon GRK2 loss in muscle or liver. Tissue lysates from muscle and liver were subjected to Western blot and probed with antibodies against IRS1, IRS2, PI3K isoforms p110 and p85, PTEN, PTP1B, nucleolin, and GAPDH. Representative immunoblots and densitometric quantitations are shown. Results are expressed as arbitrary units and normalized by either nucleolin or GAPDH. Data are means ± SEM of 7-8 mice per group. Statistical significance was analyzed by unpaired two tailed t test or Mann-Whitney test, depending on the normality of the distribution of the data. *$P < 0.05$

Supplementary Figure 5



Supplementary Figure 5. CreER expression or translocation to the nucleus does not affect lipid accumulation in the liver. (A) Liver weight in mice expressing (Tam-*Cre*$^{+/-}$) or not (Tam-*Cre*$^{-/-}$) Cre recombinase. Statistical significance was analyzed by unpaired two tailed t test. (B) Liver sections from HFD-fed control (*Cre*$^{-/-}$) and Tam-*Cre*$^{+/-}$ mice were stained with hematoxylin and eosin (scale bar 50 μm). Images are representative of 5 mice per genotype. *P <0.05.

Supplementary Figure 6



Supplementary Figure 6 Decreased abundance of inflammation markers in the liver upon tamoxifen-induced GRK2 loss. Mice from both genotypes were fed a HFD for 8 weeks, injected with tamoxifen and maintained for 5 more weeks in the HFD. (**A**) Quantification of the characteristic hepatic crown-like structures within liver sections stained with F4/80 from control and Tam-*GRK2*$^{-/-}$ mice, indicated by arrows in Figure 5E. Results are means ± SEM of 4 mice per genotype. *P < 0.05; **P < 0.01. (**b**) qPCR was used to measure the hepatic expression of mRNAs for *tnfa*, *mcr1* and *nos2*. Results were normalized against *ywhaz* and *gapdh* mRNAs. Values are represented as fold change over control mice and are means ± SEM of 6 mice per genotype.; *P<0,05. (**c**) Representative immunoblots and densitometric analysis are shown for total and phosphorylated JNK1/2. Values are represented as fold change over control mice and are

means ± SEM of 3-4 mice per genotype. Statistical significance was analyzed by unpaired two-tailed t test or Mann-Whitney test, depending on the normality of the distribution of the data. *P <0.05.

Supplementary Table 1. Primers used for real time PCR analysis:

Emr1 (F4/80)	Forward Reverse	5′-AGTACGATGTGGGGCTTTTG-*3′* 5′-CCCCATCTGTACATCCCACT-3′
Tnf	Forward Reverse	5′-TCTTCTCATTCCTGCTTGTGG-3′ 5′-GGTCTGGGCCATAGAACTGA-3′
Mrc1(CD206)	Forward Reverse	5′-CCACAGCATTGAGGAGTTTG-3′ 5′-ACAGCTCATCATTTGGCTCA-3′
Nos2 (iNOS)	Forward Reverse	5′-TGAACTTGAGCGAGGAGCA-3′ 5′-TTCATGATAACGTTTCTGGCTCT-3′
Gapdh	Forward Reverse	5′-CTGCACCACCAACTGCTTAGC-3′ 5′-GGTCATGAGCCCTTCCACAAT-3′
Ywhaz	Forward Reverse	5′- TTACTTGGCCGAGGTTGCT-3 5′-TGCTGTGACTGGTCCACAAT-3′
Cpt1a	Forward Reverse	5′- GACTCCGCTCGCTCATTC -3′ 5′-TCTGCCATCTTGAGTGGTGA-3′
Cpt1b	Forward Reverse	5′-GAGTGACTGGTGGGAAGAATATG -3′ 5′-GCTGCTTGCACATTTGTGTT-3′
pparg	Forward Reverse	5′-TGCTGTTATGGGTGAAACTCTG -3′ 5′-TCTGTGTCAACCATGGTAATTTCT-3′
fasn	Forward Reverse	5′-GCTGCTGTTGGAAGTCAGC -3′ 5′-AGTGTTCGTTCCTCGGAGTG-3′
dio2	Forward Reverse	5′-AGCTTCCTCCTAGATGCCTACA -3′ 5′-CCGAGGCATAATTGTTACCTG-3′
Ucp1	Forward Reverse	5′-GGCCTCTACGACTCAGTCCA -3′ 5′-TAAGCCGGCTGAGATCTTGT-3′
ppargc1	Forward Reverse	5′-CCCTTCTTTGCCATTGAATC -3′ 5′-AATGTTAGGAAAGTTTAGCATCTGG-3′
lipe	Forward Reverse	5′- AGCGCTGGAGGAGTG-3′ 5′-CCGCTCTCCAGTTGAACC-3′