Supplemental Experimental Procedures

Transcriptomic study

Array hybridization- The quality of total RNA was evaluated by the Agilent 2100 Bioanalyzer system (Agilent Technologies). 150 ng of total RNA for each sample was processed using the Affymetrix GeneChip Whole Transcript Sense Target Labeling Assay (Affymetrix). The double-stranded cDNA was synthesized with random hexamers tagged with a T7 promoter sequence. The double-stranded was used as a template for amplification with T7 RNA Polymerase producing many copies of antisense cRNA. Next, random hexamers were used to reverse transcribe the cRNA to produce single-stranded DNA in the sense orientation. The single-stranded DNA was fragmented and labeled with terminal deoxynucleotidyl transferase (TdT). Hybridization cocktails containing ~2 μg of fragmented and labeled DNA target were prepared and applied to GeneChip Mouse Gene 1.0 ST arrays. Hybridization was performed in a 45°C hybridization oven (Affymetrix), at 60 rpm for 16 hours. The arrays (n = 5 for each genotype) were washed and stained with streptavidin phycoerythrin in a Affymetrix Fluidics Station 450, and then scanned using the Affymetrix 3000 7G scanner. Command Console Software (AGCC) was used to produce .CEL intensity files. The GeneChip Mouse Gene 1.0 ST array comprised 770 317 unique 25-mer oligonucleotide features constituting 28 853 gene-level probe sets.

Microarray analysis-Gene expression analysis was subjected to one filter of significance. This filter excluded all genes that had a mean expression value that was ≤ 30. This value was chosen based on the average background and the signal intensity for the GPR40 gene

in the KO islets. Both values were around 15 and the filter value was set at 2 times this background. Statistical analyses of gene expression was performed using Student *t*-test. $P < 0.05$ was considered significant.

Lipid profiling study

Preparation of packed capillary columns. Capillary liquid chromatography (LC) columns were slurry-packed with 5 μm Jupiter C18-RP particles (Phenomenex, Torrance, CA) as previously described (1). Briefly, the stationary phase was added to a stainless steel reservoir, to which an empty fused silica capillary (Polymicro Technologies, Phoenix, AZ) was connected. The opposite end of the capillary was connected to a stainless steel union (Valco, Houston, TX) containing a stainless steel screen (2 μm mesh, Valco) that served as a frit. Acetonitrile was used as the packing solvent and was delivered at constant pressure by syringe pump (ISCO, Lincoln, NE). Initially, a pressure of 100 psi was applied. The pressure was then increased stepwise to and held constant at 7,000 psi for 5-10 min under sonication.

LC-MS analysis of lipid extracts. Dried lipid extracts were reconstituted in 30 μL methanol, vortexed for 10 s, and centrifuged at 13400 x g for 5 min. The supernatants were then transferred to autosampler vials and maintained at 4°C until analysis. An automated LC system with two 150 μm x 65 cm capillary columns was used for reversed-phase separation of lipids, as previously described (2). Detailed construction of similar LC systems has been described elsewhere (3; 4). The mobile phases were (A) 10 mM ammonium acetate in 50:50 water/methanol and (B) 10 mM ammonium acetate in 50:50

methanol/acetonitrile. The LC system was equilibrated at 6,000 psi with mobile phase A prior to injecting 1 μL of sample. Exponential gradient elution was initiated 3 min after sample loading with an initial column flow of ~1 μL/min. After 90 min of gradient separation, the mobile phase mixer was purged with 5 mL of mobile phase B, followed by a 6 min column wash. Finally, the mobile phase mixer was purged with 10 mL of mobile phase A, which represented the end of one separation cycle. While gradient elution is performed on one column, the other column is equilibrated with mobile phase A. The capillary LC system was coupled to a hybrid linear ion-trap Fourier transform ion cyclotron resonance mass spectrometer (LTQ-FT, ThermoFisher, San Jose, CA) via a home-built electrospray ionization (ESI) interface. Briefly, the commercial ESI source was replaced with an electrodynamic ion funnel (5), and stainless steel unions were used to connect ESI emitters to the capillary separation columns without sheath gas or makeup liquid. The capillary temperature and ESI voltage were 200°C and 2.2 kV, respectively, and data were acquired in positive ion mode over the *m/z* range 100-1,000. The LTQ-FT duty cycle was ~1.6 sec and consisted of one high-mass resolution MS scan followed by 5 data-dependent low-mass resolution MS/MS scans.

Processing of quantitative LC-MS data. LC-MS datasets were processed using the PRISM Data Analysis system (6), a series of software tools (e.g. Decon2ls, VIPER (7); freely available at http://ncrr.pnl.gov/software/) developed in-house. The first step involved deisotoping of the high-resolution MS data to give the monoisotopic mass, charge state, and intensity of the major peaks in each mass spectrum. The data were next examined in a 2-D fashion to identify groups of mass spectral peaks that were observed in sequential spectra using an algorithm that computes a Euclidean distance in n-

dimensional space for combinations of peaks. Each group, generally ascribed to one detected species and referred to as a "feature", has a median monoisotopic mass, central normalized elution time (NET), and abundance estimate computed by summing the intensities of the MS peaks that comprise the entire feature.

The lipid identities of detected features in each LC-MS dataset were initially determined by comparing their measured monoisotopic masses and NETs to the calculated monoisotopic masses and observed NETs of each of the lipids in an accurate mass and time (AMT) tag database within search tolerances of ±3 ppm and ±0.03 NET for monoisotopic mass and elution time, respectively (2). Non-linear chromatographic alignment of LC-MS datasets was also performed with the LCMSWARP algorithm (8) during database matching by using the NETs of lipid AMT tags as retention time locks. All lipids initially identified by this approach were confirmed by manual inspection of raw data. Additional identifications were made by manual inspection of accurate mass, isotopic distribution, and MS/MS information (2) for those features that did not match the lipid AMT tag database but were found to be statistically significant (see below) between the various culture conditions.

After database matching and chromatographic alignment, intensity normalization was applied using the expectation maximization algorithm (2; 9). Briefly, this algorithm analyzes the histogram of log ratios of intensities of features common to two or more datasets and finds the peak apex of this distribution by assuming that the histogram is a mixture of a normal density corresponding to unchanged features and uniform density background corresponding to changed features. The expectation maximization algorithm

calculates the normal and uniform parts of the histogram, and the shift in intensity is applied to all features in the aligned dataset. It is important to note that all lipid features (*i.e.* both identified and unidentified) were considered during intensity normalization.

Statistical analysis of processed LC-MS data. Normalized lipid abundances were transformed to log 2 scale, and a data matrix was created to facilitate statistical analyses. Lipid features (both identified and unidentified) were included in statistical analyses if they were observed in a minimum of three out of five samples per sample type. It is important to note that, for most lipid features within a culture condition, more observations than the required minimum were present. Changes in the lipid profiles of islets isolated from GPR40 WT and KO mice, both untreated and treated with palmitate, were assessed using analysis of variance (ANOVA) to determine main effects. In addition, palmitate-induced (WTC versus WTP or KOC versus KOP) or genotype-induced (WTC versus KOC or WTP versus KOP) changes in the lipid profiles of isolated islets were examined using a *t*-test. Principal components analysis (PCA) (10) was also performed with the comparisons described above to identify lipid profiles characteristic of palmitate treatment in both islets isolated from GPR40 WT and KO mice. Missing lipid feature abundance values were replaced by the average lipid feature abundance for the sample type to aid visualization in PCA only.

Supplemental References

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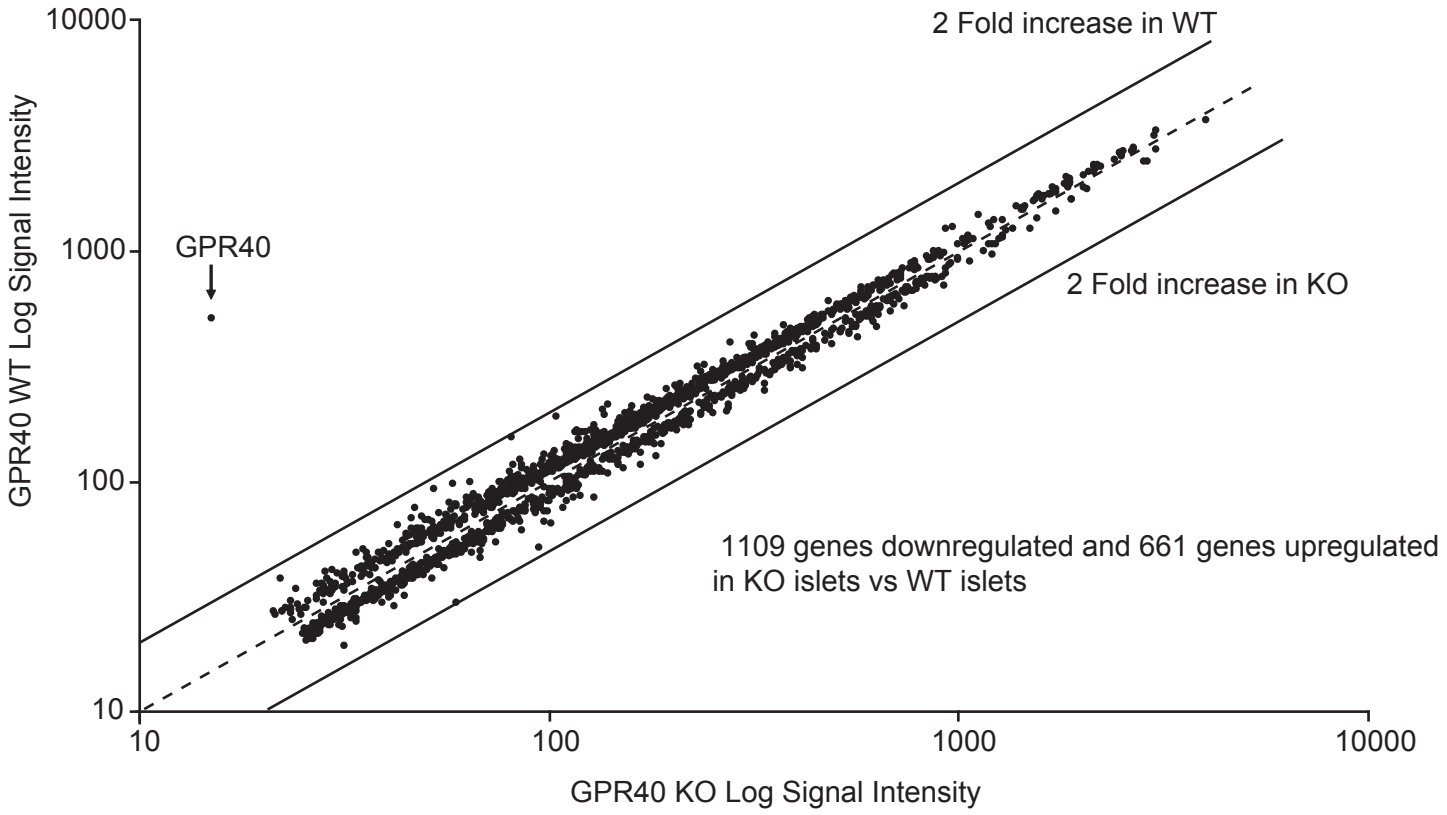
Supplemental Figure Legends

Fig. 1. Mean LOG intensities for the 1770 genes significantly modulated in GPR40 WT and KO islets. Microarray analyses were performed using RNA extracted from fed GPR40 WT and KO mice (13-15 weeks old, n = 5 arrays for each genotype) and the Affymetrix GeneChip Mouse Gene 1.0 ST array as described in Suppl. Methods. Statistical analyses of gene expression was performed using Student *t*-test. $P < 0.05$ was considered significant.

Fig. 2. Scores plot from a Principal Component Analysis of lipid profiles from islets samples. Score plot of GPR40 WT (A) and KO (B) lipid features that were significantly different between control and palmitate treated conditions in 5 independent experiments. Fifty-nine lipid features were significantly modulated by palmitate in WT islets and sixty-two lipid features were modulated by palmitate in KO islets.

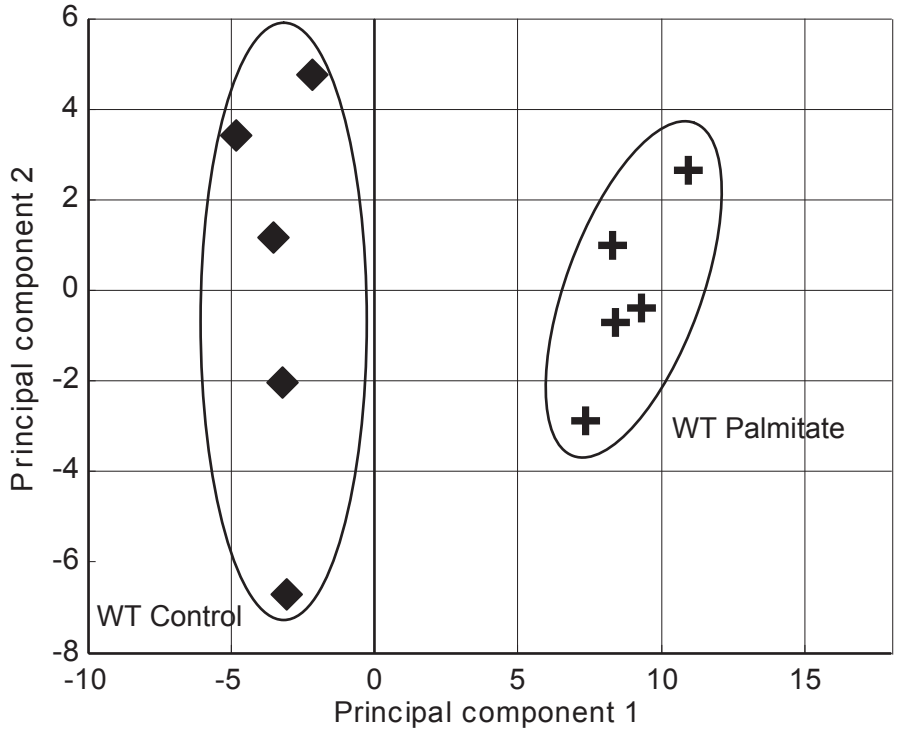
Fig. 3. Lipid profiles in islets isolated from GPR40 KO and WT mice. Log 2 relative abundances of phospholipids (A) and sphingomyelins (B) in isolated islets. Islets were isolated from fed mice and batches of 250 islets were incubated in complete RPMI at 16.7 mM glucose with or without 0.5 mM palmitate for 1 h. Lipid extracts were subjected to LC-MS as described in Suppl. Experimental Procedures. PE and PC, phosphatidyl ethanolamine and choline respectively. Data are expressed as mean ± SE of 5 independent experiments. *, ***, $p < 0.05$ and $p < 0.001$, respectively, versus WT Control. #, ###, $p < 0.05$ and $p < 0.001$ respectively, versus KO Control.

Supplemental FIGURE 1

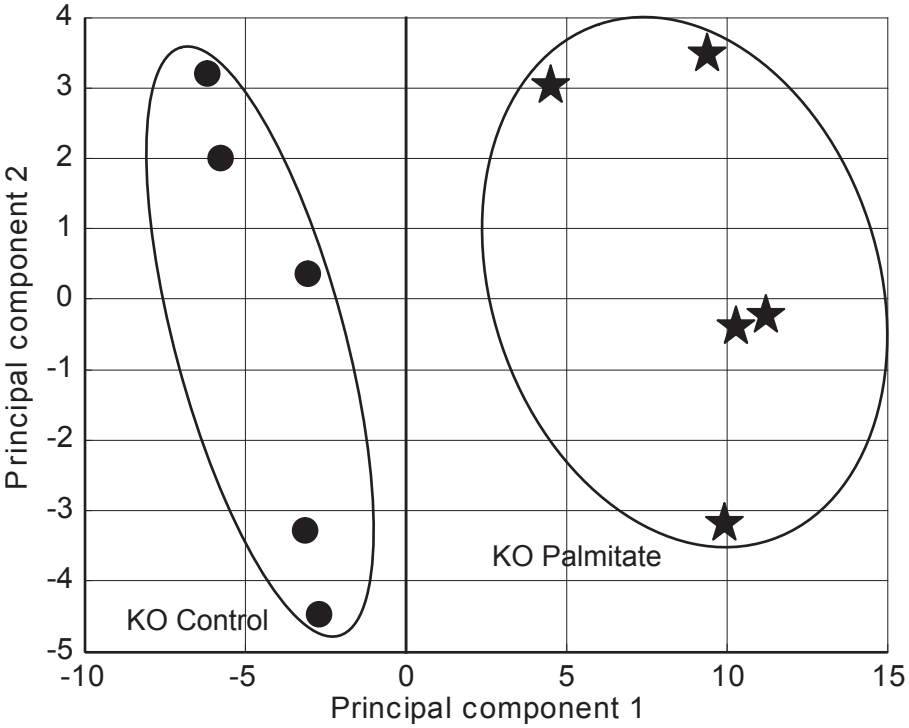


Supplemental FIGURE 2

A



B



Supplemental FIGURE 3