Supplemental data

Fasiglifam (TAK-875) has dual potentiating mechanisms via Gαq-GPR40/FFAR1 signaling branches on glucose-dependent insulin secretion

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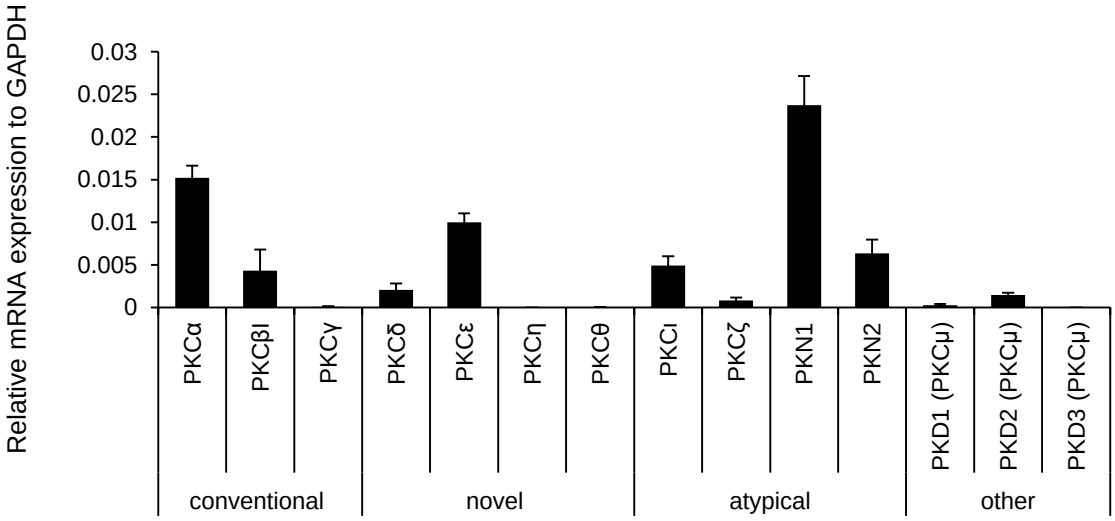

Supplementary Figure S1. mRNA levels of PKC and PKD family enzymes in MIN6 cells.

mRNA levels were measured by qRT-PCR and normalized to GAPDH using the comparative CT method ($(2^{-\Delta\Delta Ct})$). Data are shown as means ± SD of three experiments performed in duplicate.

Method

Quantitative real-time PCR (qRT-PCR) analyses for PKC and protein kinase D (PKD)

Total RNA was extracted from MIN6 cells using ISOGEN (Nippon Gene, Toyama, Japan) according to the manufacturer's instructions. cDNAs were synthesized from 1 μg of total RNA. The reverse transcription reactions were performed at 42°C using a random primer and SuperScriptII reverse transcriptase (Life Technologies). The mixture was dissolved in 40 μl of distilled water and the resulting cDNA was used as the template for qRT-PCR using a Prism 7900HT sequence detector (Applied Biosystems, Carlsbad, CA, USA). Thermal cycling parameters were 2 min at 50°C, 10 min at 95°C, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. mRNA levels were analyzed with the comparative Ct method ($2^{-\Delta\Delta Ct}$) using *GAPDH* as the housekeeping gene. The TaqMan Gene Expression assays (Applied Biosystems) used were: Mm00440858_m1 *(Prkca)*, Mm00435749_m1 *(Prkcb)*, Mm00440861_m1 *(Prkcg)*, Mm00440891_m1 *(Prkcd)*, Mm00440894_m1 *(Prkce)*, Mm00435756_m1 *(Prkch)*, Mm01340228_m1 *(Prkcq)*, Mm00435769_m1 *(Prkci)*, Mm00776345_g1 *(Prkcz)*, Mm00723995_m1 *(Pkn1)*, Mm00618304_m1 *(Pkn2)*, Mm00435790_m1 *(Prkd1)*, Mm00626821_m1 *(Prkd2)*, Mm01232233_m1 *(Prkd3)*. For measuring *GAPDH*, the following primers and probes were used: Forward primer: 5′-GTCATCATCTCCGCCCCTT-3′, Reverse primer: 5′-ATATTTCTCGTGGTTCACACCCA-3′, TaqMan probe: 5′FAM-

35 TGCCGATGCCCCCATGTTTGT-3′.