Supplemental information

**Palmitate and oleate modify membrane fluidity and kinase activities of INS-1E β-cells alongside altered metabolism-secretion coupling**

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**Supplemental Figure S1.** Representative phase contrast images of INS-1E cells treated with glucose and free fatty acids (20x, scale bar: 40 µm)
Supplemental Figure S2. Integrity of human and mouse islets after 3 days of treatment with glucose (G5.5, G11.1, G25) and 0.4 mM BSA-complexed fatty acids. Quantification of cleaved-Caspase 3 and IPF-1/PDX-1 protein levels from treated human islets (A,C) and mice islets (B,D). Results are presented as means ± SEM of at least 4 independent islets cultures and expressed as ECL signal normalized to TUBULIN obtained from Western Blots shown in Figure 1.
Supplemental Figure S3. Contribution of mitochondrial β-oxidation to metabolic rate in INS-1E β-cells chronically exposed to glucose (G5.5, G11.1, G25) and 0.4 mM BSA-complexed fatty acids. Metabolic rates contributed by β-oxidation and non β-oxidation redox activities were calculated from Figure 2C by the subtraction of raw MTT values, as determined with the mitochondrial β-oxidation blocker etomoxir, to the raw MTT values obtained without etomoxir. Results are presented as percentage of the metabolic fitness of treated INS-1E cells measured in Figure 2.
Supplemental Figure S4. Mitochondrial function in INS-1E β-cells after 3 days of exposure to glucose (G5.5, G11.1, G25) and 0.4 mM BSA-complexed fatty acids. OCR traces from INS-1E β-cells treated with low G5.5 (A), control G11.1 (B) and high G25 (C) glucose ± fatty acids. After the 3-day treatments, cells were acutely stimulated with 15 mM glucose (Glc) before blockade of mitochondrial respiration (Oligo), then induction of uncoupled maximal respiration (FCCP), and finally inhibition of the electron transport chain (R/A). Results are presented as means ± SEM of 3 independent experiments, using triplicates in each individual independent experiment, and expressed as pmol of O₂ consumed per min. Mitochondrial membrane potential (ΔΨₘ) monitoring in INS-1E β-cells treated with low G5.5 (D), control G11.1 (E) and high G25 (F) glucose ± fatty acids. After 3 days of treatment, cells were acutely stimulated with 15 mM glucose (Glc) followed by 1 μM FCCP for total mitochondrial membrane depolarization. Results are presented as means ± SEM of 4 independent experiments, using triplicates in each individual experiment, and expressed as percentage of basal Rhodamin-123 fluorescence signal.
Supplemental Figure S5. Glucose-induced cytosolic calcium changes in INS-1E β-cells after 3 days of exposure to glucose (G5.5, G11.1, G25) and 0.4 mM BSA-complexed fatty acids. Cytosolic calcium kinetics (n=3 ± SD from 1 representative experiment out of at least 3 experiments) in INS-1E β-cells treated with low G5.5 (A), control G11.1 (B) and high G25 (C) glucose ± fatty acids expressed as 340/380 nm fluorescence ratio normalized to baseline. Cells were acutely challenged with 15 mM glucose (Glc) before addition of 30 mM KCl.
Supplemental Figure S6. Glucose-stimulated secretion in INS-1E β-cells after 3 days of exposure to glucose (G5.5, G11.1, G25) and 0.4 mM BSA-complexed fatty acids. Secretion kinetics (n=3 ± SD from 1 representative experiment out of 5 experiments) of INS-1E β-cells treated with low G5.5 (A), control G11.1 (B) and high G25 (C) glucose ± fatty acids expressed as relative luminescence units (RLU) normalized to baseline (prior to injection). Cells were acutely challenged with 15 mM glucose (Glc) before addition of 30 mM KCl. (D) Secretion measured in the supernatant of control G11.1 INS-1E cells for the very same wells by both luciferase-based assay (left) and insulin-targeted radioimmunoassay (right). Results are presented as AUC of the luminescent signal monitored over the 30 min period following glucose stimulation (left) and as immuno-reactive insulin present in the supernatant at the end of the same period (left; n=5 ± SD). (E) Secretion rates upon Glc stimulation presented as means ± SEM of 5 independent experiments, using triplicates in each individual independent experiment, and expressed as AUC from traces in Figure 5 of the luminescence signal per min; *p<0.05, **p<0.01, ****p<0.0001.