Data S1. Supplementary Materials and methods.

Animals. Male albino Wistar rats (Velaz, s. r. o.; n=3; weighing 282-295 g) were housed at the Charles University animal care facility (23±1˚C, 55±10% humidity, air exchange 12-14 times/h, 12-h light-dark cycle). The animals had free access to tap water and diet (standard pelleted diet, Velaz, s. r. o.; 10% of energy from fat, 30% of energy from proteins, and 60% from carbohydrates). The animals received care according to the guidelines set out by the Animal-welfare Body of the Charles University (Hradec Kralove, Czech Republic) and the International Guiding Principles for Biomedical Research Involving Animals. The isolation of primary rat hepatocytes (PRH) was approved by the Animal-welfare Body of the Charles University (Hradec Kralove, Czech Republic) and the Ministry of Education, Youth and Sports (approval no. MSMT-11265/2022-3). Rats were euthanized with an isoflurane overdose (5%) followed by exsanguination following liver removal, and death was confirmed by the cessation of the heartbeat and respiration.

Hepatocyte isolation, culture and treatment. Hepatocytes were isolated by two-step collagenase perfusion from rat livers according to the protocol by Berry and Friend (1). The viability of the rat hepatocytes used in the experiments was set >90% (confirmed by the Trypan blue exclusion test). Isolated hepatocytes were suspended in William's E medium (Lonza Group, Ltd) with supplements and plated on collagen-coated wells. The seeding density was 10,000 cells per well. PRH were allowed to attach to collagen and establish a monolayer for the first 2 h in a humidified incubator containing 95% air and 5% CO_2 at 37˚C. The previous medium was then replaced with William's medium (containing supplements and no fetal bovine serum), and the cells were allowed to attach for 22 h. Following a 24-h attachment, the cells were treated with 1 mM oleate/palmitate (2/1) and 2 mM oleate/palmitate (1/1) (Merck & Co., Inc.) for another 24 h as described in the main manuscript. Information on the culture and treatment of HepaRG and HepG2 cell lines is available in the Materials and methods section of the main manuscript. After 24 h of treatment with free fatty acids, the Mito-stress test was performed in PRH, HepaRG and HepG2 cells

Analysis of cellular metabolism in non-permeabilized cells. The Mito-stress test was performed according to the protocol described in the main manuscript.

Analysis of glutamate-driven respiration in permeabilized cells. The analysis of glutamate-driven respiration was carried out according to the protocol described in the main manuscript.

References

1. Berry MN and Friend DS: High-yield preparation of isolated rat liver parenchymal cells: A biochemical and fine structural study. J Cell Biol 43: 506-520, 1969.

Figure S1. Calculated parameters of mitochondrial respiration in non-permeabilized PRH, HepaRG and HepG2 cells following exposure to free fatty acids. (A) Basal respiration, (B) maximal respiration, (C) spare respiratory capacity, (D) proton leak, (E) ATP production-linked respiration. Data are expressed as the mean ± SD. Statistical analyses were carried out using two- and one-way ANOVA followed by Tukey's and Dunnett's post hoc tests, respectively. *P<0.05, **P<0.01 and ***P<0.001. ns, not significant (n=16). PRH, primary rat hepatocytes; OCR, oxygen consumption rate; OA, oleate; PA, palmitate; BSA, bovine serum albumin.

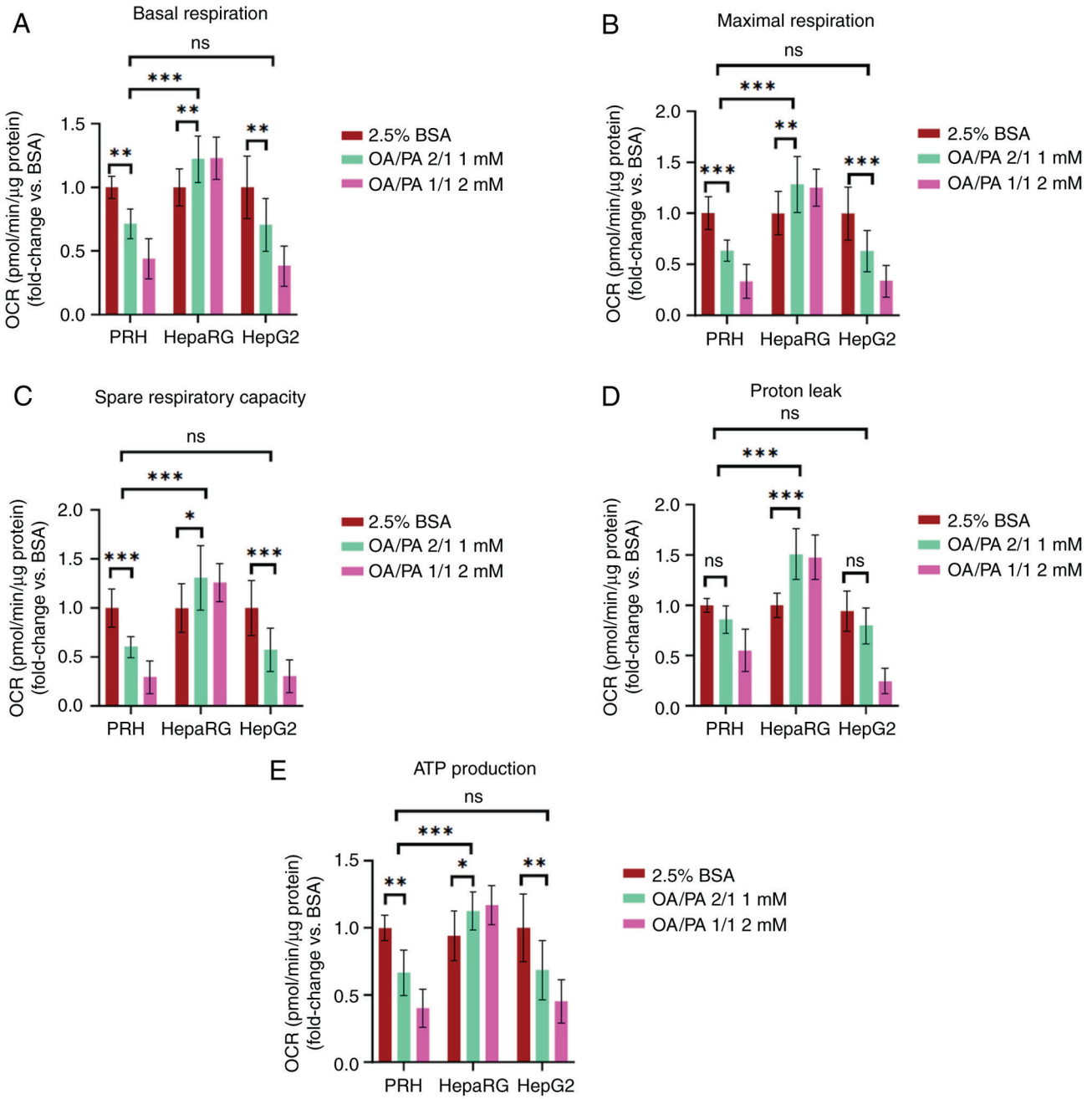

Figure S2. Calculated parameters of glutamate-driven respiration in permeabilized HepaRG and HepG2 cells following exposure to free fatty acids. Added substrates were glutamate and malate. OXPHOS (state 3), MRC (state 3u), LEAK respiration (state 4o). Data are expressed as the mean ± SD. Statistical analyses were carried out using one- or two-way ANOVA followed by Dunnett's and Tukey's post hoc tests, respectively. *P<0.05, **P<0.01 and ***P<0.001. ns, not significant (n=8). OXPHOS, oxidative phosphorylation; MRC, maximal respiratory capacity; OCR, oxygen consumption rate; OA, oleate; PA, palmitate; BSA, bovine serum albumin.