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Mtfp1 ablation enhances mitochondrial respiration and protects against hepatic steatosis

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30 **Abstract**

31 Hepatic steatosis is the result of an imbalance between nutrient delivery and metabolism in the

32 liver. It is the first hallmark of Non-alcoholic fatty liver disease (NAFLD) and is characterized by the

33 accumulation of excess lipids in the liver that can drive liver failure, inflammation, and cancer.

34 Mitochondria control the fate and function of cells and compelling evidence implicates these

35 multifunctional organelles in the appearance and progression of liver dysfunction, although it

36 remains to be elucidated which specific mitochondrial functions are actually causally linked to

37 NAFLD. Here, we identified Mitochondrial Fission Process 1 protein (MTFP1) as a key regulator of

38 mitochondrial and metabolic activity in the liver. Deletion of *Mtfp1* in hepatocytes is physiologically

39 benign in mice yet leads to the upregulation of oxidative phosphorylation (OXPHOS) activity and

40 mitochondrial respiration, independently of mitochondrial biogenesis. Consequently, hepatocyte-

41 specific knockout mice are protected against high fat diet-induced hepatic steatosis and metabolic

42 dysregulation.   Additionally, we find that deletion of *Mtfp1* in liver mitochondria inhibits

43 mitochondrial permeability transition pore opening in hepatocytes, conferring protection against

44 apoptotic liver damage in vivo and ex vivo. Our work uncovers novel functions of MTFP1 in the

45 liver, positioning this gene as an unexpected regulator of OXPHOS and a therapeutic candidate for

46 NAFLD.

47 **Introduction**

48   Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in

49 industrialized countries, whose incidence is rapidly expanding worldwide[1]. NAFLD is a frequent

50 comorbidity of type 2 diabetes and obesity with its prevalence calculated at ~30% in the general

51 population and 80% among obese people. NAFLD encompasses a spectrum of pathologies

52 ranging from simple steatosis characterized by triglyceride accumulation in hepatocytes, to non-

53 alcoholic steatohepatitis (NASH), whose hallmarks are inflammation and fibrogenesis, which can

54 further progress into cirrhosis and hepato-cellular carcinoma (HCC), the deadliest form of liver

55 cancer[2].

56   Considerable efforts have been made in recent decades to better understand the

57 mechanisms of NAFLD progression and therapeutic targets that might subsequently alleviate the

58 burden of this spectrum of pathologies. The progression of NAFLD is currently explained by a

59 "multiple parallel-hit" hypothesis, which implicates the synergistic and concerted action of multiple

60 events originating from various liver cell types[3]. In hepatocytes, oxidative stress and mitochondrial

61 dysfunction have been suggested to contribute to hepatocyte damage and death, tissue

62 inflammation and fibrosis[4]. This model highlights the complexity and heterogeneity of NAFLD

63 progression and underscores the central involvement of hepatocytes mitochondria in the

64 progression of NAFLD. Mitochondria are essential organelles that are deeply integrated in cellular

65 homeostasis. Most famous for their production of ATP via oxidative phosphorylation (OXPHOS),

66 mitochondria also regulate programmed cell death and inflammation through the sequestration and

67 release of pro-apoptotic factors and pro-inflammatory molecules[5,6]. Yet which of the multiple

68 functions of mitochondria are directly implicated in the onset of steatosis, inflammation, hepatocyte

69 death, and subsequent tissue remodeling has not been defined[7].

70   The relevance of mitochondria to liver function is highlighted by mitochondrial dysfunction

71 observed in inborn and acquired liver pathologies: mutations in mitochondrial genes that cause

72 genetic diseases manifest with liver dysfunction, while strong associations also exist between

73 mitochondrial dysfunction and acquired liver diseases such as NAFLD, viral hepatitis, and ischemic

74 liver injury[8,9]. Mitochondrial respiration has been reported to decline during the progression of liver

75 dysfunction in humans[10] and perturbation of mitochondrial structure has been reported in liver

76 biopsies from patients with NASH[11], lending support to the notion that the maintenance of

77 mitochondrial integrity is paramount to liver function. Promoting enhanced oxygen consumption

78 with targeted uncouplers in the liver reduces the deleterious accumulation of hepatic lipid storage

79 and steatosis in rodents and primates[12,13], yet broader system-wide mitochondrial uncoupling has

80 catastrophic effects on other organs and is incompatible with life[14]. We recently showed that

81 mitochondrial uncoupling in cardiac mitochondria is regulated by Mitochondrial Fission Process 1

82 (MTFP1), uncovering a novel role of this protein in bioenergetic regulation[15]. MTFP1 is a protein

83 localized at the inner membrane (IMM) whose namesake derives from a putative role in

84 mitochondrial fission in vitro[16,17] and has garnered interest as marker of liver dysfunction in

85 humans. Retrospective studies revealed a link between *MTFP1* expression in tumoral tissue and

86 patient survival in HCC[18], the most common type of liver cancer, whose development is heavily

87 influenced by NAFLD progression[2]. Indeed, compelling evidence implicates MTFP1 in metabolic

88 sensing and programmed cell death regulation in vitro[17,19,20], although its pertinence in the liver

89 function and metabolism has never been explored.

90   Here, we report the generation of a liver-specific *Mtfp1* knockout mouse model (LMKO) and

91 discover that, contrary to what we observed for the heart[15], MTFP1 is dispensable for organ

92 function. We report that deletion of *Mtfp1* in vivo in hepatocytes enhances hepatic OXPHOS

93 activity and confers protection against diet-induced liver steatosis, weight gain and systemic

94 glucose dysregulation when mice are fed a high fat diet (HFD). In sum, our data reveal liver-

95 specific effects of MTFP1 ablation in vivo that position this gene as a therapeutic candidate for

96 NAFLD.

97 **Results**

98 **Generation and characterization of liver specific *Mtfp1* KO mouse model**

99   To investigate the link between mitochondrial function and liver metabolism we generated a

100 liver-specific KO mouse model in which we specifically deleted *Mtfp1* in post-natal hepatocytes

101 (LMKO mice; Fig. 1A, S1A-B). Conditional mice (*Mtfp1^LoxP/LoxP*) previously generated on a

102 C57Bl6/N background[15] were crossed to Alb-Cre recombinase transgenic mice (*Alb-Cre^tg/+*) to

103 generate LMKO mice (*Alb-Cre^tg/+Mtfp1^LoxP/LoxP*). Genetic deletion of *Mtfp1* in LMKO mice was

104 specific to the liver (Fig. 1B) and caused a profound depletion of mRNA (Fig. 1C) and protein (Fig.

105 1D) expression in liver extracts. MTFP1 ablation in hepatocytes did not affect perinatal survival

106 (Fig. 1E) and histological liver analysis performed on LMKO mice were unremarkable and

107 indistinguishable from those of control littermates (Fig. 1F). Contrary to the ablation of MTFP1 in

108 the heart[15], LMKO mice did not manifest any overt defects during their lifetime under normal chow

109 diet (NCD). Comprehensive assessment of liver structure and function revealed no defects: liver

110 mass (Fig. 1G) was unchanged in LMKO mice and the levels of circulating biomarkers of liver

111 damage such as alanine aminotransaminase (ALAT) and aspartate aminotransaminase (ASAT)

112 were not increased relative to those of control littermates (Fig. 1H). Circulating levels of cholesterol

113 and triglycerides were normal (Fig. 1I) and metabolic cage performance was not impaired (Fig. 1J-

114 S1C). Similarly, we observed no evidence of pathological gene expression changes by RNAseq

115 analysis (Fig. S1D, Supplemental Dataset 1). In fact, control and LMKO livers revealed virtually no

116 gene dysregulation (only 27 out of >25,000 transcripts) including no upregulation of established

117 markers[21] of NAFLD or HCC defined in humans (Fig. S1D, Supplemental Dataset 1). Taken

118 together, our observations demonstrate that the deletion of *Mtfp1* in hepatocytes does not

119 negatively impact the liver under basal conditions.

120

121 ***Mtfp1* deletion increases OXPHOS activity and mitochondrial respiration**

122   Given our previous observations that MTFP1 ablation reduces bioenergetic efficiency of

123 cardiac mitochondria[15], we decided to directly assess the impact of *Mtfp1* deletion on hepatic

124 mitochondrial bioenergetics. To this end, we performed high resolution fluor-respirometry on

125 isolated hepatic mitochondria isolated from control and LMKO mice, simultaneously measuring

126　both oxygen consumption rates (JO_2) and mitochondrial membrane potential ($\Delta\Psi$) changes with

127　Rhodamine 123 (RH-123)[22]. JO_2 and RH-123 were recorded from mitochondria incubated with

128　respiratory substrates promoting the delivery of electrons to complex I (state 2; pyruvate,

129　glutamate, and malate (PGM) or complex II (state 2; succinate and rotenone), and also in the

130　presence of palmitoyl-carnitine plus malate then in the phosphorylating (state 3: ADP), non-

131　phosphorylating (state 4: oligomycin (OLGM) to inhibit ATP synthase) (Fig. 2A). Notably, and in

132　contrast to MTFP1-deficient cardiac mitochondria[15], respiration in LMKO liver mitochondria was

133　significantly increased in phosphorylating (state 3) conditions in the presence of any of the

134　respiratory substrates we tested. Pyruvate, glutamate, and malate led to a 49% increase in state 3

135　respiration and succinate and rotenone led to a 57% increase in respiration (Fig. 2B). Interestingly,

136　we observed a 200% increase in state 3 respiration in the presence of palmitoyl carnitine, a fatty

137　acid ester derivative, pointing to an increased efficiency of fatty-acid derived energy metabolism

138　caused by hepatocyte-specific deletion of *Mtfp1*.

139　　　Additionally, LMKO liver mitochondria showed a higher respiratory control ratio (RCR) in

140　the presence palmitoyl carnitine plus malate (Fig 2C). Despite a marked increase in state 3

141　respiration, we did not observe a genotype-specific difference in mitochondrial membrane potential

142　(Fig. 2D), which initially surprised us since increased oxygen consumption rates are typically

143　accompanied by reduction in membrane potential due to dissipation of the protonmotive force via

144　complex V (to synthesize ATP). The most parsimonious explanation for this result is that MTFP1

145　ablation promotes a commensurate increase in the activities of both cytochrome *c* oxidase

146　(complex IV) and the ATP synthase (complex V). Indeed, when we measured the specific activities

147　of complex IV (Fig. 2E) and complex V (Fig. 2F) in separate assays, we found a ~20% increase in

148　LMKO mitochondria relative to control littermate controls, suggesting a similar contribution of both

149　complexes to increase respiration while maintaining mitochondrial membrane potential. We further

150　confirmed our findings by measuring oxygen consumption rates in mitoplasts supplied with either

151　NADH, Cyt c, succinate and rotenone to drive electron transport via Complex II or NADH, Cyt c,

152　succinate and malonate to drive electron transport via Complex I (Fig. 2G). In both assays, oxygen

153　consumption was elevated in LMKO liver mitochondria demonstrating that Complex IV activity is

154　intrinsically augmented upon MTFP1 ablation independently of the protonmotive force. Together,

155　these observations implied either that MTFP1 ablation triggers a specific upregulation of

156　components of the OXPHOS machinery or the induction of a general mitochondrial biogenesis

157　response. To differentiate between these possibilities, we assessed mitochondrial mass using

158　multiple molecular and imaging-based methods. Quantification of mitochondrial mass in primary

159　hepatocytes isolated from control or LMKO mice expressing a genetically encoded matrix-targeted

160　YFP (mitoYFP) showed no differences in fluorescent signal intensity or surface area (Fig. S2A B),

161　indicating that mitochondrial mass is unaffected by MTFP1 ablation in primary hepatocytes.

162　Interestingly, we found a modest increase in mitochondrial elongation in LMKO mitochondria (Fig.

163　S2C) quantified by supervised machine learning[23], consistent with previous studies in cultured

164　cells[15,17]. Quantification of mtDNA content in liver tissue revealed no genotype-specific differences

165　in mice fed a NCD (Fig. S2D) and transmission electron microscopy (TEM) analyses of liver

166　sections showed no differences in mitochondrial area between control and LMKO mice (Fig. S2E).

167　RNAseq analyses showed no evidence of gene expression signatures typically associated with

168　increased mitochondrial biogenesis or integrated stress responses (Fig. S2E, Supplemental

169　Dataset 1) and shotgun liver proteomics performed on control and LMKO liver extracts revealed no

170　global upregulation of mitochondrial proteins (Fig. 1D, Supplemental Dataset 2). Taken together,

171　our data strongly argue that a specific and coordinated increase in both complex IV and V activity

172　in hepatocytes enhances respiration in liver mitochondria deleted of *Mtfp1,* enabling them to

173　consume nutrient-derived respiratory substrates at an elevated rate.

174

175　**MTFP1 interacts with OXPHOS-related proteins in the liver.**

176　To gain insights into the mechanisms responsible for the increased specific activities, we

177　assessed the relative complex abundance by grouping mitochondrial proteins quantified by

178　proteomics in the liver of NCD-fed LMKO mice according to the macromolecular complexes to

179　which they belong (Fig. 2H, Supplemental Dataset 2). These data revealed a significant increase

180　in Complex V subunits, which could be confirmed by quantitative SDS-PAGE (Fig. 2I) and Blue-

181　native polyacrylamide gel electrophoresis (BN-PAGE) analyses (Fig. 2J). BN-PAGE analysis of

182　the steady-state levels of OXPHOS complexes in LMKO liver mitochondria revealed an increase in

183　Complex V dimers (Fig. 2J) to an increase commensurate with the reported increase in ATPase

184 activity (Fig. 2F). Taken together, our data suggest that improved assembly and/or maintenance of

185 Complex V along with an increased activity of Complex IV is responsible for the enhanced

186 respiration observed in LMKO liver mitochondria.

187 To gain insights into the relationship between MTFP1 and macromolecular complex

188 assembly in the inner mitochondrial membrane (IMM), we sought to assess the interactome of

189 MTFP1 in the liver. We generated a hepatocyte-specific transgenic mouse model enabling the

190 expression of FLAG-MTFP1 from the *Rosa26* locus (Fig. 3A). We verified that the Hepatocyte[FLAG-

191 MTFP1] mice, expressed FLAG-MTFP1 correctly in the IMM by protease protection assay (Fig. 3B).

192 We then subjected liver mitochondria to co-immunoprecipitation (co-IP) and mass spectrometry

193 (MS/MS) to define interacting protein partners, which enabled the identification of 112 specific

194 interactors of MTFP1 with fold change (FC) >2 by MS/MS analysis (Figure 3C, D, Supplemental

195 Dataset 3) that could be ascribed to the mitochondrial proteome according to the Mitocarta 3.0

196 compendium[24]. We then decided to subdivide this list into binary and enriched interactors: binary

197 interactors were those proteins we could only identify in Hepatocyte[FLAG-MTFP1] but not control liver

198 mitochondria CoIP eluates while enriched interactors were those proteins for which peptide

199 abundance was greater in CoIP eluates from Hepatocyte[FLAG-MTFP1] mice than in control mice.

200 Classification of binary interacting proteins revealed a wide range of mitochondrial

201 functions, with a conspicuous abundance of factors involved in mitochondrial translation and

202 OXPHOS (Fig. 3D). On the other hand, enriched interactors did not reveal an enrichment of

203 proteins required for mitochondrial translation, but rather those involved in various metabolic

204 processes within mitochondria, including carrier proteins of the SLC25A family. 2D-BN-PAGE

205 analyses revealed the formation of an MTFP1 macromolecular complex in liver mitochondria,

206 which was absent in LMKO mice, and that co-migrated with SLC25A4 (ANT1) (Fig. 3E), as we

207 recently described in the heart[15]. However, we found little overlap with the cardiac interactome of

208 MTFP1[15] (7 out of 113 proteins), implying that there may be tissue-specific physical interactions

209 and complex assembly associated with MTFP1 beyond ANT1 that regulate respiration. Altogether,

210 these data indicate that post-transcriptional modulation of mitochondrial gene expression and IMM

211 complex abundance and activities are induced in a specific manner by the deletion of MTFP1 in

212 hepatocytes, leading to enhanced mitochondrial respiration.

213

Mtfp1 deletion protects against hepatic steatosis induced by high fat diet

215   Given enhanced stimulated respiratory activity of LMKO liver mitochondria, we next

216 decided to assess the response of LMKO mice to high fat diet (HFD) feeding, a classical metabolic

217 burden for the liver that causes nutrient overload, hepatic steatosis, and systemic metabolic

218 dysregulation that can be counteracted by accelerating oxygen consumption via mitochondrial

219 uncoupling[12,13]. We fed control and LMKO mice a HFD for a period of 16 weeks beginning at 8

220 weeks of age, which was previously shown to drive diet-induced obesity and NAFLD[25]. In control

221 mice, we observed that chronic HFD feeding triggered the hypertrophy of livers (Fig. 4A), which

222 were appreciably paler and heavier (Fig. 4B) than those of control mice on NCD. HFD feeding

223 also induced weight gain (Fig. 4C) and accumulation of hepatic triglycerides (TG), (Fig. 4D).

224 Histological analyses showed a massive accumulation of lipid droplets (in white), consistent with

225 both micro- and macro-steatosis (Fig. 4E). In contrast, HFD-fed LMKO mice were protected from

226 weight gain (Fig. 4C) and liver dysfunction, exhibiting a 26% of reduction in liver mass compared to

227 diet-matched controls (Fig. 4A, 4B). Concordantly, histological analyses of livers from HFD-fed

228 LMKO mice revealed a reduction in steatosis (Fig. 4E), a complete rescue of hepatic triglycerides

229 accumulation back to levels measured in NCD-treated mice (Fig. 4D), and a reduction in circulating

230 levels of ALAT by 64% and ASAT by 32% (Fig. 4F). Metabolic protection against HFD-feeding in

231 LMKO mice was reflected at the molecular level: liver RNAseq analyses showed that HFD feeding

232 induced significant transcriptional dysregulation in control livers with 854 upregulated genes and

233 758 downregulated genes (Fig. 4G, S3). Gene ontology analyses revealed an enrichment of

234 various metabolic pathways dysregulated by HFD-feeding in control mice including, lipid and

235 branched chain amino acid metabolism, PPAR signaling, and steroid biosynthesis, and fatty acid

236 metabolism. These alterations were absent in HFD-fed LMKO mice, in which only 214 upregulated

237 genes and 164 downregulated genes were detected, most of which were involved in pathways of

238 stress response and immune signaling (Fig. 4H). At the protein level, we observed that in HFD

239 feeding in control mice induced global reduction in complex IV by liver proteomic analyses (Fig.

240 S4A). This response was blunted in LMKO mice (Fig. S4B), leading to a relative increase in

241 complex IV proteins in HFD-fed LMKO mice compared to littermate, diet-matched controls (Fig.

242 S4C).

243 Given the protection against hepatocyte and liver damage observed in vivo and in vitro

244 upon the deletion of *Mtfp1*, we asked whether the protection against HFD-induced metabolic

245 dysregulation of the liver could be explained by differential sensitivity to cell death. However,

246 TUNEL assays performed on histological sections from HFD-fed control and LMKO mice revealed

247 an absence hepatocyte apoptosis (Fig. 4I), which is consistent with previous findings that HFD

248 causes limited liver cell death[25]. Altogether, our data demonstrate that *Mtfp1* deletion in

249 hepatocytes confers metabolic resistance to hepatic steatosis in vivo (Fig. 4J) in a manner that is

250 independent of apoptotic resistance.

251

252 **Mtfp1 deletion improves hepatocyte metabolism in a cell-autonomous.**

253 Having demonstrated that LMKO mice are protected against diet-induced steatosis, we

254 next sought to define the breadth of systemic protection by performing a battery of metabolic tests

255 on control and LMKO mice after HFD-feeding. Fat accumulation is known to affect systemic

256 glucose homeostasis. Consistent with improved liver metabolism promoted by the ablation of

257 MTFP1 in hepatocytes, metabolic cage analyses revealed improved systemic respiration in LMKO

258 mice fed an HFD. The lower respiratory exchange ratio (RER) observed in LMKO mice (Fig. 5A)

259 suggests that LMKO mice metabolize nutrient-derived lipids more efficiently than control

260 littermates, which can very likely be attributed to intrinsic differences in the livers of these mice.

261 Food and water intake were not altered between control and LMKO mice nor were distance

262 measurement on HFD (Fig. S5A-C), pointing to a specific effect of MTFP1 on energy expenditure

263 by the liver that is revealed under HFD feeding. Metabolic dysregulation caused by HFD feeding is

264 known to increase *de novo* glucose synthesis by the liver of rodents[27] and so we sought to assess

265 gluconeogenesis by performing intraperitoneal pyruvate tolerance tests (IP-PTT). We observed a

266 1.5-fold increase blood glucose levels in HFD-fed control mice relative to NCD littermates, which

267 was reduced by 14% in the HFD-fed LMKO group (Fig. 5B), indicating that *Mtfp1* deletion in

268 hepatocytes protects the liver against diet-induced dysregulation of gluconeogenesis. Consistent

269 with these findings, intraperitoneal glucose tolerance tests (IP-GTT) revealed LMKO mice to be

270   modestly protected from HFD-induced glucose intolerance. In control mice, HFD feeding led to a

271   1.7-fold increase of the area under curve (AUC) of IP-GTT relative to normal chow diet (NCD)

272   controls, which reduced by 17% in HFD-fed LMKO mice relative to diet-matched controls (Fig. 5C).

273   To exclude that improved glucose tolerance in HFD-fed LMKO mice was the consequence of

274   altered insulin resistance, rather than improved gluconeogenesis, we performed intraperitoneal

275   insulin tolerance tests (IP-ITT). We observed no genotype-specific differences in insulin sensitivity

276   (Fig. 5D) on either HFD or NCD, indicating that improved glucose tolerance in LMKO mice is not

277   caused by increased systemic insulin sensitivity. Concordantly, we did not observe differences in

278   basal insulin levels between control and LMKO mice fed a NCD (Fig S5E). While LMKO mice

279   gained less weight upon HFD feeding (Fig. 4C), we observed no genotype-specific differences in

280   body mass composition by NMR minispec analysis (Fig. 5E) nor in the circulating levels of

281   cholesterol and triglycerides (Fig. 4F). In line with these findings, LMKO mice were not protected

282   from extra-hepatic fat mass accumulation (Fig. 5G), white adipose tissue accumulation nor

283   adipocyte hypertrophy (Fig. S5E, F), all of which are common metabolic consequences of diet-

284   induced obesity[17,19,28]. Altogether, our data demonstrate that the protection against diet-induced

285   metabolic dysregulation conferred to mice by hepatocyte-specific deletion of *Mtfp1* is primarily

286   restricted to the liver.

287   To determine whether hepatocyte deletion of *Mtfp1* prevents steatosis in a cell-autonomous

288   manner, we established an assay in which we could mimic HFD-induced steatosis in primary

289   hepatocytes isolated from NCD-fed mice. Primary hepatocytes were plated and cultured in the

290   presence of Intralipid (IntLip): a complex lipid emulsion composed of linoleic, oleic, palmitic, and

291   stearic acids, which are the most abundant fatty acids found in HFD. We optimized our assay

292   conditions to ensure that limited damage, death, or differentiation under both treated and non-

293   treated (NT) conditions was occurring. Indeed, IntLip feeding of primary hepatocytes isolated from

294   NCD-fed control mice led to a 1.6-fold increase in intracellular lipid accumulation after 24 hours,

295   which could be visualized by live-cell imaging with BODIPY fluorescence (Fig. 5H) and quantified

296   by high-throughput confocal imaging (Figure 5I). Notably, deletion of *Mtfp1* in primary hepatocytes

297   rescued this phenotype: BODIPY staining intensities in NT and IntLip-treated *Mtfp1*^{-/-} cells were

298   indistinguishable to those of NT primary hepatocytes isolated from control littermates. Thus, we

299   conclude that the deletion of *Mtfp1* in hepatocytes confers direct protection to the liver against

300   HFD-induced metabolic dysregulation by improving hepatocyte metabolism in a cell-autonomous

301   manner.

302

303   **Mtfp1 deletion protects against hepatic cell death**

304   Given the previous implications of MTFP1 in cell death sensitivity reported in various

305   cultured cell lines[17,19,28] and the association of MTFP1 and liver tumor progression in humans[29], we

306   sought to directly test whether MTFP1 was involved in the regulation of cell survival in vivo. We

307   measured the response to liver apoptosis by treating 3 month old mice for 24 hours with FAS

308   antibodies, which specifically induces apoptotic liver damage via the activation of the FADD

309   signaling pathway in a manner than is regulated by IMM integrity[30]. Macroscopically, the livers of

310   WT mice treated with FAS showed hepatic damage characterized by the appearance of fibrotic

311   and necrotic foci (Fig. 6A). FAS-induced liver damage resulted in a loss of liver mass (Fig. S6A), a

312   dramatic increase of ALAT, ASAT and lactate dehydrogenase (LDH), a more general marker of

313   tissue damage, by 35, 85 and 45-fold respectively in control livers (Fig. 6B, S6B). Hematoxylin

314   and eosin (H&E) staining of control livers revealed tissue disruption, characterized by the presence

315   of apoptotic bodies and hyper-eosinophilic cytoplasm, conferring large areas of architecture

316   dysregulation (Fig. 6C), all of which features are consistent with apoptotic liver damage[15,23]. In

317   contrast, H&E analyses of LMKO livers revealed far less FAS-induced damage (Fig. 6C) and

318   plasma analyses revealed an 84% reduction of ALAT, an 86% reduction of ASAT, and a 97%

319   reduction of LDH compared to FAS-injected control mice (Fig. 6B, Fig. S6B). Finally, TUNEL

320   assays performed on histological liver sections demonstrated a profound reduction of cell death in

321   FAS-treated LMKO mice compared to control mice (Fig. 6D). Taken together, these studies

322   demonstrate that hepatocyte-specific *Mtfp1* depletion protects against apoptosis-induced liver

323   damage in vivo.

324   To understand whether MTFP1 ablation protects hepatocytes from cell death in a cell-

325   autonomous manner, we isolated primary hepatocytes from control and LMKO mice at 8-10 weeks

326   of age and performed quantitative cell death assays ex vivo. To induce cell death, primary

327   hepatocytes were treated with hydrogen peroxide (H_2O_2) [1mM] at 16 hours post plating and cell

328   death was by kinetically monitored by propidium iodide (PI) uptake using an automated live-cell

329   imaging approach powered by supervised machine learning previously developed for cultured cell

330   lines[31,32]. Consistent with our observations in vivo, $Mtfp1^{-/-}$ primary hepatocytes treated with H_2O_2

331   ex vivo showed a significant resistance against cell death (52% reduction at 4 hours) compared to

332   control primary hepatocytes (Fig. 6E, F), arguing that MTFP1 ablation confers protection to

333   autonomous hepatocyte death ex vivo. Programmed cell death in the liver, and more specifically

334   in hepatocytes, can be tightly regulated through the opening of the mitochondrial permeability

335   transition pore (mPTP)[32]. To test whether $Mtfp1$ deletion impacted mPTP opening, we isolated

336   hepatic mitochondria and simulated mPTP opening with calcium chloride overload (Fig. 6G). We

337   observed a reduced sensitivity of mPTP opening in hepatic mitochondria isolated from LMKO mice

338   compared to control littermates (Fig. 6G), which could be rescued by the pre-treatment with the

339   mPTP inhibitor Cyclosporin A (CsA)[19]. Taken together, we conclude that $Mtfp1$ deletion in

340   hepatocytes confers protection against mPTP opening and cell death induction.

341

342   **Discussion**

343   Pre-clinical transgenic animal models have been instrumental in dissecting the underlying

344   molecular remodeling of hepatocytes during the development of fatty liver disease and its

345   sequelae. These studies have demonstrated the unassailable pathophysiological relevance of

346   mitochondria, yet data from a large array knockout mice of mitochondrial genes have yielded a

347   more complex picture: some hepatocyte-specific knockouts confer resistance to NAFLD/NASH

348   while others inhibit normal liver function[33–37]. Pleotropic effects of the individual mitochondrial

349   genes that were targeted in previous studies combined with the potential for metabolic cross-talk

350   between tissues in vivo has made it challenging to pinpoint how modulating mitochondrial activity

351   may be used to combat liver disease. Here, we discovered that Mitochondrial Fission Process 1

352   (MTFP1) plays an important role metabolic role in the liver of mice that is critical for NAFLD but not

353   under basal conditions. MTFP1 was first identified as a metabolically-regulated inner mitochondrial

354   membrane IMM) protein[16–18,33] initially implicated in mitochondrial fission and cell death resilience

355   in a variety of cell lines[16–18,38]. In the liver, the discovery that MTFP1 protein expression is

356   predictive of hepatocellular carcinoma survival and recurrence risk in humans[15], prompted us to

357   directly investigate its role in the liver.

358   Here, we created Liver-specific *Mtfp1* knockout (LMKO) mice (Fig. 1A-E, S1A,B) by expressing a

359   hepatocyte-specific Cre-recombinase[39] in a conditional mouse model of *Mtfp1*[15]. We discovered

360   that LMKO mice are healthy and virtually indistinguishable from littermate control mice, showing no

361   defects in liver function and no indication of liver damage nor inflammatory responses and liver

362   RNAseq analyses only showed 0.1% of genes were dysregulated, none of which were involved in

363   pathogenic hepatic remodeling (Fig. 1, S1). While the livers of LMKO mice appeared unchanged,

364   characterization of hepatic mitochondria revealed two major functional improvements: increased

365   respiratory capacity and resistance to permeability transition pore opening. High resolution fluor-

366   respirometry measurements of MTFP1-deficient liver mitochondria showed increased ADP-

367   stimulated (state 3) oxygen consumption rates under all substrates we tested, which was

368   associated a commensurate increase in the specific activities of complex IV and complex V (Fig.

369   2B-F). Quantitative label-free proteomic studies revealed a ratio-metric increase in the levels of

370   Complex V subunits, which paralleled the increased levels of assembled Complex V detected by

371   BN-PAGE, pointing to an increased assembly and/or maintenance of the ATP synthase complex

372   (Fig. 2G-I). Enhanced respiration was not associated with proton leak (Fig. 2B,C) nor cristae

373   alterations (Fig. S2D) that we reported in MTFP1-deficient hearts[34], leaving us wondering: how

374   does MTFP1 ablation enhance OXPHOS activity and mitochondrial coupling and do so specifically

375   in the liver?

376   The spare respiratory capacity of hepatocytes has been reported to supersede that of

377   cardiomyocytes, indicating that under basal conditions cardiomyocyte mitochondria are working far

378   closer to their maximal capacity[40]. In contrast, morphometric studies of cardiac mitochondria from

379   rodents reveal cristae densities that approach the physical and functional limits exemplified by

380   tuna[41] and hummingbird hearts[42], consistent with the notion that there may be little additional IMM

381   real estate upon which to build additional OXPHOS complexes in the heart[43]. In the liver,

382   mitochondria have a comparatively reduced IMM density, OXPHOS protein content[44], and IMM

383   protein half-lives[45] and would therefore be capable of increasing OXPHOS complex assembly to

384   enhance mitochondrial coupling and respiration. In rodents, systemic thyroid hormone injections

385 can increase hepatocyte cristae density to increase the content and activity of the respiratory

386 chain[46,47]. On the other hand, genetic models of obesity manifest the oppositive effect, with altered

387 cristae organization in swollen mitochondria and reduced levels of cytochrome *c* oxidase[48], which

388 we also confirmed by proteomic studies of HFD-fed control mice (Fig. S4A, B). Yet to the best of

389 our knowledge, LMKO mice represent the first genetic mouse model that elevates respiration by

390 increasing the activity of Complex IV and V rather than by boosting total mitochondrial mass or by

391 promoting uncoupling.

392 MTFP1 ablation in the liver enhances state 3 respiration regardless of the substrate source

393 (Fig. 2B) yet this increase is most profound when the palmitoyl-carnitine is supplied in excess, as

394 evidenced by the increased respiratory control ration (RCR) in the presence of fatty acids but not

395 carbohydrate-derived substrates (Fig. 2B), revealing that MTFP1-deficient liver mitochondria are

396 capable of coupling voracious beta enhanced OXPHOS activity. A clear prediction of increased

397 beta oxidation capacity of hepatocyte mitochondria is an enhanced propensity to metabolize

398 excess lipids and therefore a resistance against diet-induced hepatic steatosis in vivo[1]. Indeed,

399 when we challenged LMKO mice with a long-term a high fat diet (HFD), they showed remarkable

400 resistance to fatty liver disease and metabolic dysregulation despite equal levels of food

401 consumption and in-cage activity. Metabolic protection appears to be restricted to the liver, as

402 LMKO mice were not protected against the increased extra-hepatic adiposity, dysregulated

403 systemic glucose metabolism, and aberrant glucose-stimulated insulin secretion by the pancreas

404 that caused by HFD feeding. Mimicking diet-induced steatosis in the tissue culture dish with

405 primary hepatocytes isolated from NCD-fed mice, we could show that excessive lipid droplet

406 accumulation was blunted in *Mtfp1*^{-/-} primary hepatocytes ex vivo that is consistent with enhanced

407 mitochondrial coupling in these cells (Fig. 5H).

408 The discovery that MTFP1 ablation in the liver is benign and even beneficial under stress

409 conditions is in stark contrast to our recent report that *Mtfp1* deletion in cardiomyocytes causes

410 heart failure[43]. However, defects in inner membrane integrity observed in *Mtfp1*^{-/-} cardiac

411 mitochondria were not observed in *Mtfp1*^{-/-} liver mitochondria: membrane potential was maintained

412 (Fig. 2C) and mPTP was increased rather than decreased (Fig. 6G). Moreover, the livers of LMKO

413 mice show increased mitochondrial respiratory capacity (Fig. 2B) and no signs of cell death or

414   inflammation (Fig. 4I, S3A), while MTFP1 deficiency in the heart triggered cardiomyocyte death,

415   fibrotic remodeling, and heart failure[15].   At first, we found these physiological discrepancies

416   perplexing, since the heart and liver are both rely heavily on mitochondria yet further exploration of

417   the literature provided conspicuous examples demonstrating that these two organs can in fact

418   respond differently to mitochondrial dysfunction. For example, ablation of Complex I activity in the

419   cardiomyocytes can cause a fatal hypertrophic cardiomyopathy in mice[49] while its ablation in

420   hepatocytes does not impact liver structure and function[37]. Similarly, interfering with the activity of

421   the mitochondrial fusion proteins OPA1[50,51] or Mitofusins[52] specifically in cardiomyocytes yields

422   disastrous outcomes for cardiac function, yet in doing so in the liver is either benign[33,34] or even

423   advantageous against NASH[35]. Similarly, cardiac deletion of the adenine nucleotide transporter

424   elicits cardiac hypertrophy but in the liver, its genetic or biochemical inhibition in the liver can be

425   beneficial, protecting against hepatic steatosis and metabolic dysregulation[36,53]. In mice, system-

426   wide disruption of mitochondrial genes such as *Chchd10*[54,55], *Dnm1l*[56], *Wars2*[57]*,* and *Cox1*[58] can

427   trigger multi-organ dysfunction in which the liver, but not the heart, is spared, further illustrating that

428   the liver may respond differently (or not at all) to genetic insults otherwise catastrophic to other

429   tissues.

430   But why does the ablation of MTFP1 impact liver mitochondria so differently? It is likely that

431   divergent cell type-specific effects reflect the different metabolic, structural, and functional

432   characteristics of mitochondria they harbor. Indeed, compelling differences between the heart and

433   liver have been documented at the level of mitochondrial network morphology, IMM and cristae

434   organization[59], mitochondrial proteome composition and protein half-lives[45], OXPHOS complex

435   organization and assembly[60], substrate utilization and enzymatic activities of the respiratory

436   chain[60,61], and even the molecular regulation of the mPTP[61–65]. Consistent with these observations,

437   we found little overlap between the differentially expressed mitochondrial genes and proteins of

438   knockout hearts and livers: no genes besides *Mtfp1* were commonly dysregulated between both

439   knockout tissues while only 13% (35 out of 264 proteins) shared common differential expression

440   patterns (Fig. S7). MTFP1 interactomes were also remarkably dissimilar, with only 7 out of 113

441   interacting partners of liver MTFP1 found to overlap with the cardiac MTFP1 interactome. MTFP1

442   is devoid of conserved motifs or catalytic domains and forms a complex of approximately 150kDa,

443   which is significantly smaller than the fully assembled mito-ribosome and ATP synthase complexes

444   with whose subunits it interacts and thus we posit that the impacts on mitochondrial function that

445   result from the ablation MTFP1 may reflect its role as a protein scaffold in the IMM. Indeed,

446   MTFP1 co-migrates with both adenine nucleotide translocase (ANT1) and Cyclophilin D (encoded

447   by *Ppif*), both of which have been previously physically and functionally connected to mitochondrial

448   ATP synthesis[62–66] and the mPTP. Our data also support a role for MTFP1 as a regulator of mPTP

449   activity in the liver, as inactivation of hepatocytes slows mPTP opening response in liver

450   mitochondria and protects against liver cell death in primary hepatocytes and in mice (Fig. 6). This

451   pro-survival role of MTFP1 in the liver appears to be independent from the metabolic protection its

452   deletion confers against HFD-induced hepatic steatosis, since HFD-feeding does not cause cell

453   death (Fig. 4I).

454   The metabolic protection conferred to MTFP1-deficient livers that are already hyper-

455   proficient in mitochondrial respiration and fatty acid oxidation is best appreciated under chronic

456   HFD feeding. Intriguingly, recombinant inbred mouse models that are susceptible to

457   NAFLD/NASH show a downregulation of *Mtfp1* expression in the liver while those that are resistant

458   do not[67]. While diet-induced hepatic steatosis predisposes humans to NASH and cirrhosis, HFD-

459   feeding alone does trigger NASH in laboratory mice so we have not yet learned whether LMKO

460   mice are protected from the downstream sequelae of hepatic steatosis. Nevertheless, LMKO

461   mice have availed themselves to be a unique tool to understand how enhancing mitochondrial

462   respiration can combat metabolic liver disease and will undoubtedly prove useful for the in vivo

463   exploration and development of therapeutic targets for other inherited and acquired liver diseases.

464

465   **Materials and Methods**

466   ***Animals***

467   Animals were handled according to the European legislation for animal welfare (Directive

468   2010/63/EU). All animal experiments were performed according to French legislation in

469   compliance with the European Communities Council Directives (2010/63/UE, French Law 2013-

470   118, February 6, 2013) and those of Institut Pasteur Animal Care Committees (CETEA is Comité

471   d'Ethique en Expérimentation Animale 89). The specific approved protocol number is #20687-

472   2019051617105572. Mice were housed within a specific pathogen free facility and maintained

473   under standard housing conditions of a 14-10h light-dark cycle, 50-70% humidity, 19-21°C with

474   free access to food and water in cages enriched with bedding material and gnawing sticks. Alb-

475   Cre recombinase[39] and mitoYFP[68] mouse lines are commercially available (MGI: 2176946 and

476   5292496). The *Mtfp1* conditional mouse model[68] was generated by PolyGene on a C57Bl/6N

477   genetic background using two LoxP sites and an FRT-flanked neomycin cassette. The first LoxP

478   site and neomycin resistance cassette is located in intron 1 and the second LoxP site is inserted in

479   intron 3. The FRT-flanked neomycin cassettes were removed *in vivo* by Flp-recombination by

480   crossing to Flp-deleter breeding step. Hepatocyte specific FLAG-MTFP1 Knock-In (KI) mice (*Alb-*

481   *Cre*[tg/+]*Mtfp1*^{+/+}, *CAG*[tg+/]) were generated by crossing an inducible mouse model for mCherry-P2A-

482   FLAG-MTFP1[69] generated by PolyGene AG (Switzerland) on a C57Bl6/N background expression

483   under the CAG promoter with mice expressing the Cre recombinase under the control of the

484   hepatocyte-specific albumin Cre promoter. Liver specific *Mtfp1* knockout mice (LMKO) were

485   generated by mating AlbCre+ mice with *Mtfp1*[LoxP/LoxP] mice, this led to the deletion of flanked region

486   containing exons 2 and 3 (925 bp in total), resulting in the loss of the majority of the coding region

487   of *Mtfp1* and a loss of *Mtfp1* expression. For fluoresce microscopy analysis of primary hepatocytes

488   the triple transgenic line AlbCre+; mitoYFP+; *Mtfp1*[LoxP/LoxP] was generated.

489   Mice were fed a normal chow diet (NCD, Teklad global protein diet; 20% protein, 75%

490   carbohydrate, 5% fat) or high fat diet (HFD) containing 22 kJ% carbohydrates 24 kJ% protein and

491   54 kJ% fat (Ssniff® EF acc. E15742-347) for 16 weeks starting from the age of 8 weeks. During

492   the treatment mice were monitored weekly for the body weight and every other week for the

493   glycaemia, which was measured using a glucometer (Accu-Chek Performa, Roche, 1486023). To

494   induce hepatic cell death, Fas antigen, CD95 (BD BioSciences, 554254) was intra-peritoneal

495   injected in 18-hour fasted mice at the concentration of 225μg/kg (BW). Mice were sacrificed 24

496   hours post injection.

497   Mouse genotype was determined at weaning. For the genotyping, genomic DNA was isolated from

498   mouse tail snip using lysis buffer Tris-HCl (EuroMedex, 26-128-3094-B) pH8.5 100mM;

499   Ethylenediaminetetraacetic acid (EDTA, 5mM; EuroMedex, EU0084-B); Sodium Dodecyl Sulfate

500 (SDS, 0.2%; EuroMedex, 1833); Sodium Chloride (NaCl, 200mM; EuroMedex, 1112-A);

501 Proteinase K 400μg/ml (Sigma-Aldrich, 03115879001); samples were incubated at 56°C until

502 digested and then centrifuged for 3 min at 16000g. Supernatants were transferred to fresh tubes,

503 and DNA was precipitated first with 1000 μl of isopropanol, centrifuged at 16000g for 10 minutes at

504 4°C, pellet were washed with 500 μl of 70% ethanol, centrifuged at 16000g for 10 minutes at 4°C,

505 and DNA was resuspended in H_2O. PCR was performed using the 5x Hot FIREpol (SOLIS

506 BIODYNE, 755015) at 94°C for 3 minutes, (94°C for 15 seconds, 55°C 30 seconds, 72°C for 1

507 minute) for 35 cycles, 72°C for 7 minutes and 4°C. Primers used for genotyping are listed in

508 Supplemental Dataset 4.

509 ***Metabolic studies***

510 Food and water intake, respiratory exchange ratio (RER), and physical activity were tested for 5

511 days using the PhenoMaster system (TSE Systems, Bad Homburg, Germany), which allowed

512 continuous undisturbed and controlled recording. during day and night periods. 5 days before the

513 recording, the animals were placed in the room and in cages similar to the PhenoMaster cages, for

514 acclimatization. The following parameters were recorded every 3 minutes and averaged hourly or

515 for the light and dark phase as indicated: food consumption (g), water consumption (ml), distance

516 covered (cm), and RER (ratio between the amount of carbon dioxide (CO_2) produced in

517 metabolism and oxygen (O_2) used). RER has a range from 0.7 to 1.0 according to the fuel source,

518 when close to 0.7 means that fats are the predominant source, closest to 1.0, carbohydrates are

519 predominantly consumed.

520 For Pyruvate Tolerance Test (IP-PTT): 18-hour fasted mice were administered intra-peritoneal

521 injections of sterile pyruvate (Sigma-Aldrich, P2256) at 1 g/kg (BW). Glucose and Insulin Tests for

522 Glucose Tolerance Test (IP-GTT): 18-hour fasted mice were administered intra-peritoneal

523 injections of sterile glucose (EuroMedex, UG3050) at 2 g/kg. For Insulin Tolerance Test (IP-ITT): 6-

524 hour fasted mice were administered intra-peritoneal injections of Insulin aspart (NovoRapid) at 1

525 g/kg (BW). For all the tests, blood glucose levels were measured before injection and 15, 30, and

526 60 post-injection using a glucometer (Accu-Chek Performa, Roche). For glucose-stimulated insulin

527 production, mice were fasted for 18 hours and were administered intra-peritoneal injections of

528 sterile glucose (EuroMedex, UG3050) at 2 g/kg. Insulin levels were analyzed by ELISA (Crystal

529 Chem, 90082).

530 Whole blood was collected from the submandibular vein of awake mice in tubes containing heparin

531 (Sigma-Aldrich, H4784) at the concentration of 50U/ml and centrifuged at 3000g for 10 minutes at

532 4°C to isolate plasma. Plasma analyses of transaminases (ASAT and ALAT), LDH, cholesterol and

533 triglycerides (TG) were performed by the 'Microdosages biochimiques' at the 'Institut de

534 Physiologie et Pathologie, Claude Bernard' core facility (Paris).

535 ***Analysis of body composition***

536 Fat and lean mass were determined in awake Control and LMKO mice by nuclear magnetic

537 resonance (NMR) using the Contactless Check Weighing (CCW) by MiniSpec MQ (Bruker

538 MiniSpec Plus) with a 0.15-T magnetic field and a 6.2-MHz frequency pulse. This device measures

539 the mass of fat, lean tissue, and fluid based on prior calibration. Mice were placed in an acrylic

540 cylinder (48-mm diameter) and was loosely restrained within the cylinder by pushing a plunger.

541 The cylinder was then positioned inside the bore of the magnet maintained at constant

542 temperature of 37°C. The measurements were recorded as % of fat, lean, and fluids.

543 ***Tissue analyses***

544 Histological and morphometric analysis: liver and white adipose tissue were fixed with phosphate-

545 buffered 10% formalin (Sigma-Aldrich, F5554) for 20 hours then washed in 70% ethanol, then

546 embedded in paraffin, sectioned by microtome (4 um) and stained with hematoxylin & eosin. Cell

547 death was assessed using the TUNEL assay kit – HRP – DAB (AbCam, ab206386). All slides were

548 scanned using the Slide scanner Axio Scan.Z1 (ZEISS). Representative pictures were obtained

549 using the CZI software (Zen 2.3 lite; ZEISS).

550 Triglyceride assay: snap frozen liver tissue was used to assess hepatic triglyceride content by

551 fluorometric assay (AbCam, ab178780).

552 ***Isolation of mitochondria***

553 Livers were gently homogenized on ice in Mitochondria Isolation Buffer (MIB: Sucrose 275mM

554 (EuroMedex, 200-301-b); ethylene glycol tetra acetic acid (EGTA-KOH pH7 1mM; EuroMedex,

555   E4378) Tris pH 7.4 20mM; Bovine Serum Albumin (free fatty acid BSA, 0.25 mg/ml; Sigma-Aldrich,

556   A6003) and protease inhibitors (Roche). Differential centrifugation at 1000g for 10 minutes at 4°C

557   yielded supernatant that was subsequently centrifuged at 3000g for 15 minutes at 4°C. The

558   resulting crude mitochondrial pellet fraction was resuspended in MIB and protein concentration

559   was quantified with Bradford assay (Sigma-Aldrich, B6916) using a spectrophotometer (Infinite 200

560   Pro, Tecan). Isolated mitochondria were used for Blue-Native Polyacrylamide Gel Electrophoresis

561   (BN-PAGE), SDS-PAGE or for High-Resolution Respirometry (HRR) assays.

562   ***High Resolution Respirometry***

563   Mitochondrial oxygen consumption was measured with an Oxygraph-2k (Oroboros Instruments,

564   Innsbruck, AT). 50 to 500 μg of mitochondria were diluted in 2.1 ml of mitochondrial respiration

565   buffer (Magnesium Chloride ($MgCl_2$, 3mM; Sigma-Aldrich, M3634); Lactobionic Acid 60mM

566   (Sigma-Aldrich, 153516); Taurine 20mM (Sigma-Aldrich, T0625); Potassium phosphate monobasic

567   (KH_2PO_4, 10mM; EuroMedex, 2018); 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-

568   Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES-KOH pH 7.4 20 mM; EuroMedex, 10-

569   110); Sucrose 110mM; EGTA-KOH pH7 0.5mM and free fatty acid BSA 1g/l). To ensure

570   mitochondrial quality and integrity, cytochrome *c* (CytC, 2μM;Sigma-Aldrich, C7752) was used.

571   The oxygen consumption (JO_2) rate was measured using either Pyruvate 10mM, Glutamate 5mM

572   (Sigma-Aldrich, G1626) and Malate 5mM (Sigma-Aldrich, M1000), or Palmitoyl-Carnitine (PC,

573   25uM; Sigma-Aldrich, P1645). To specifically assess Complex II activity, Rotenone 0.5 μM (Sigma-

574   Aldrich, R8875) and Succinate 10mM (Sigma-Aldrich, S2378) were used as substrates. Oxygen

575   consumption was assessed in the phosphorylating state with Adenosine 5′-diphosphate

576   monopotassium salt dihydrate (ADP, 0.2 mM; Sigma-Aldrich, A5285) or non-phosphorylating state

577   by adding oligomycin (OLGM, 5 μM, Sigma-Aldrich, O4876).

578   The cytochrome *c* oxidase (COX) activity was measured in the presence of Ascorbate-Na (2mM;

579   Sigma-Aldrich, A4034; pH6 with Ascorbic acid (Sigma-Aldrich, A7506)) and N,N,N′,N′-Tetramethyl-

580   p-phenylenediamine dihydrochloride (TMPD, 0.5mM; Sigma-Aldrich, 87890), with prior inhibition

581   with Antimycin A (AMA, 2,5μM; Sigma-Aldrich, A8674), finally KCN (2mM, Sigma-Aldrich, 60178)

582   was injected to evaluate the effective complex IV-dependent respiration.

583 The ATPase activity was assessed by colorimetric assay. Mitoplasts were incubated for 15

584 minutes in the following buffer: triethanolamine 75mM (Sigma-Aldrich, T58300), $MgCl_2$ 2mM pH8.9

585 followed by the addition of ATP 5mM (Sigma-Aldrich, A6419) or ATP 5mM and Oligomycin 5μM (to

586 determine the oligomycin-insensitive ATPase activity) or ADP 5mM (used as negative control).

587

588 ***Mitochondrial swelling assay***

589 Mitochondrial permeability transition pore opening was monitored by calcium-induced

590 mitochondrial swelling and change of light scattering (absorbance 540 nm). Freshly isolated

591 hepatic mitochondria from control and LMKO mice aged 8-10 week were resuspended in Ca^{2+}

592 uptake buffer (120mM KCl, 5mM MOPS, 5mM KH_2PO_4, 10mM Glutamate, 5mM Malate, pH 7.4 in

593 presence of cOmplete™ EDTA-free Protease Inhibitor Cocktail (Sigma 4693132001) at a

594 concentration of 500 μg/mL and stimulated by the addition of a single pulse of 125μM $CaCl_2$. The

595 absorbance at 540 nm was measured at intervals of 30 seconds for 20 minutes at 37°C using the

596 microplate reader (Tecan). Cyclosporin A (1μM) was used to inhibit the mPTP opening.

597 ***BN-PAGE of liver mitochondria***

598 1D BN-PAGE was performed as described previously[69] with some modifications. Briefly, liver

599 mitochondria (100 μg, mitochondrial protein concentration was determined with DC Protein Assay

600 BIO-RAD) were isolated from control and LMKO mice and incubated with digitonin extraction buffer

601 (HEPES (30mM), potassium Acetate (150 mM), Glycerol (12%), 6-Aminocaproic acid (2mM),

602 EDTA (2mM), high purity digitonin (4.5g/g), pH=7.2). Mitochondria were vortexed 1 hour at 4ºC to

603 solubilize membranes and after centrifugated at 21,000 g for 30 minutes. Supernatants were

604 transferred to a new tube and mixed with loading dye (0.0124% (w/v), Coomassie brilliant blue G-

605 250 (InVitroGen, BN2004)). Digitonin solubilized mitochondria were loaded 3-12% Bis-Tris

606 Invitrogen™ Novex™ NativePAGE™ 3-12% acrylamide gel (1mm) (Invitrogen, BN2011BX10)

607 using the anode running buffer (Invitrogen, BN2001) and Cathode Buffer Additive (0.5%) added to

608 the anode buffer (Invitrogen, BN2002). Samples were migrated at 80V 20mA for 45 minutes and at

609 150V 20 mA for 13 hours. Gel was incubated in Transfer buffer (Tris (0.304% w/v), Glycine (1.44%

610 w/v)) plus SDS (0.2% v/v) and β-mercaptoetanol (0.2% v/v)) for 30 minutes at room temperature

611 (RT) to denature proteins. After the incubation, gels were transferred on polyvinylidene difluoride

612 (PVDF) in transfer buffer (Tris (0.304% w/v), Glycine (1.44% w/v), Ethanol (10%v/v)) at 400mA

613 20V for 3 hours and 30 minutes. After transfer, membranes were washed with methanol to remove

614 Coomassie blue staining. For immunodetection, membranes were blocked with 5% milk in Tween-

615 Tris-buffered saline (TTBS) for 1 hour at RT and incubated overnight with the specific primary

616 antibody diluted in blocking solution. The membrane was washed three times in TTBS and

617 incubated for 2 hours at RT with the appropriate secondary antibody conjugated with horseradish

618 peroxidase (HRP). Finally, membranes were incubated in Tris-HCL (0.1 M pH 8.5) plus Luminol

619 and p-Coumaric acid for 3 minutes and luminescence was detected using a ChemiDoc TM

620 XRS+Imaging System. Band intensity was determined with Image Lab Software

621 For 2D BN-PAGE, first dimension native gels were incubated in MOPS1x SDS Running Buffer

622 (Fished Scientific) supplemented with β-mercaptoetanol (0.2% v/v) for 30 minutes at RT and then

623 run in a denaturing second dimension electrophoresis (2D-SDS PAGE). The gels were run in

624 MOPs buffer and then transferred to a nitrocellulose membrane. Immunodetection was performed

625 as previously described. Primary antibodies used for immunoblotting of BN-PAGE are listed in

626 Supplemental Dataset 4.

627

628 ***Protein extraction, immunoblotting and immunoprecipitation***

629 To prepare total cell lysates for immunoblot analysis, cells were washed twice with cold Dulbecco's

630 Phosphate-Buffered Saline (PBS; GIBCO, 14190169), scraped from the dishes and collected in

631 tubes, then centrifuged at 16000g for 10 minutes at 4°C. For liver samples, tissue was pulverized

632 or isolated mitochondria were spun at 3000g for 10 minutes at 4°C. Cell or mitochondria pellet or

633 tissue powder were resuspended in RIPA buffer (Tris pH 7.4 50mM; NaCl 150 mM; Triton X-100

634 (EuroMedex, 2000C) 1%; Sodium Deoxycholate 0.05% (Sigma-Aldrich, D6750); SDS 0.10%;

635 EDTA 1 mM; protease inhibitors and PhosphoStop Inhibitors (Roche), homogenized with a pestle

636 (Eppendorf, 033522) and incubated at 4°C for 30 minutes on a rotating wheel, then spun at

637 16000g for 10 minutes. Protein concentration was determined with Bradford assay using a plate

638 reader (TECAN). Protein extracts were prepared in Laemmli Buffer (Bio-Rad, 1610747) with 2-

639   Mercaptoethanol 355mM (Sigma-Aldrich, M3148) and boiled at 95°C for 5 minutes, then resolved

640   by SDS-PAGE (Mini Protean chamber and TGX gels, Bio-Rad) and transferred onto nitrocellulose

641   membrane (Trans-blot Turbo Transfer system and kit, Bio-Rad) followed by blocking with 3% milk

642   for 30 minutes. Membranes were then incubated with the primary antibodies (listed in Table 2) and

643   HRP-linked secondary antibodies (anti-mouse or anti-rabbit IgG; Thermo Fisher Scientific,

644   10368172 and 11034). Clarity Western ECL (Bio-Rad) was used for the detection with ChemiDoc

645   (Bio-Rad). Densitometric analysis of immunoblots was performed using Image Lab software (Bio-

646   Rad). The signal of the quantified bands was normalized to loading controls or Stain-free TGX

647   staining as indicated[70] and presented as fold difference over control treatment or control genotype

648   as the invariant control.

649

650   ***Proteomics***

651   Liver extract proteins were extracted and denatured in RIPA buffer. Samples were sonicated using

652   a Vibracell 75186 and a miniprobe 2 mm (Amp 80% // Pulse 10 off 0.8, 3 cycles) and further

653   centrifuged. Protein assay was performed on supernatant (Pierce 660 nm, according to

654   manufacturer instructions) and 100 µg of each extract was delipidated and cleaned using a

655   Chloroform / Methanol / Water precipitation method. Briefly, 4 volume of ice-cold methanol were

656   added to the sample and vortex, 2 volume of ice-cold chloroform were added and vortex and 3

657   volume of ice-cold water was added and vortex. Samples were centrifuged 5 min at 5000 g. The

658   upper layer was removed and proteins at the interface were kept. Tubes were filled with ice-cold

659   methanol and centrifuged at max speed for 5 min. Resulting protein pellet was air-dried and then

660   dissolved in 130 µl of 100mM NaOH before adding 170 µl of Tris 50 mM pH 8.0, tris (2-

661   carboxyethyl)phosphine (TCEP) 5 mM and chloroacetamide (CAA) 20 mM. The mixture was

662   heated 5 min at 95 °C and then cooled on ice. Endoprotease LysC (1µg) was use for an 8h

663   digestion step at (37° C) followed with a trypsin digestion (1 µg) at 37°C for 4 h. Digestion was

664   stopped adding 0.1% final of trifluoroacetic acid (TFA). Resulting peptides were desalted using a

665   C18 stage tips strategy (Elution at 80% Acetonitrile (ACN) on Empore C18 discs stacked in a P200

666   tips) and 30 µg of peptides were further fractionated in 4 fractions using

667   poly(styrenedivinylbenzene) reverse phase sulfonate (SDB-RPS) stage-tips method as previously

668   described[71,72]. Four serial elutions were applied as following: elution 1 (80 mM Ammonium formate

669   (AmF), 20% (v/v) ACN, 0.5% (v/v) formic acid (FA)), elution 2 (110 mM AmF, 35% (v/v) ACN, 0.5%

670   (v/v) FA), elution 3 (150 mM AmmF, 50% (v/v) ACN, 0.5% (v/v) FA) and elution 4 (80% (v/v) ACN,

671   5 % (v/v) ammonium hydroxide). All fractions were dried and resuspended in 0.1% FA before

672   injection.

673   NanoLC-MS/MS

674   LC-MS/MS analysis of digested peptides was performed on an Orbitrap Q Exactive Plus mass

675   spectrometer (Thermo Fisher Scientific, Bremen) coupled to an EASY-nLC 1200 (Thermo Fisher

676   Scientific). A home-made column was used for peptide separation (C_{18} 50 cm capillary column

677   picotip silica emitter tip (75 µm diameter filled with 1.9 µm Reprosil-Pur Basic C_{18}-HD resin, (Dr.

678   Maisch GmbH, Ammerbuch-Entringen, Germany)). It was equilibrated and peptide were loaded in

679   solvent A (0.1 % FA) at 900 bars. Peptides were separated at 250 nl.min^{-1}. Peptides were eluted

680   using a gradient of solvent B (ACN, 0.1 % FA) from 3% to 7% in 8 min, 7% to 23% in 95 min, 23%

681   to 45% in 45 min (total length of the chromatographic run was 170 min including high ACN level

682   step and column regeneration). Mass spectra were acquired in data-dependent acquisition mode

683   with the XCalibur 2.2 software (Thermo Fisher Scientific, Bremen) with automatic switching

684   between MS and MS/MS scans using a top 12 method. MS spectra were acquired at a resolution

685   of 35000 (at *m/z* 400) with a target value of 3×10^6 ions. The scan range was limited from 300 to

686   1700 *m/z*. Peptide fragmentation was performed using higher-energy collision dissociation (HCD)

687   with the energy set at 27 NCE. Intensity threshold for ions selection was set at 1×10^6 ions with

688   charge exclusion of z = 1 and z > 7. The MS/MS spectra were acquired at a resolution of 17500 (at

689   *m/z* 400). Isolation window was set at 1.6 Th. Dynamic exclusion was employed within 45 s.

690   Data Processing

691   Data were searched using MaxQuant (version 1.5.3.8) using the Andromeda search engine[73]

692   against a reference proteome of Mus musculus (53449 entries, downloaded from Uniprot the 24[th of]

693   July 2018). The following search parameters were applied: carbamidomethylation of cysteines was

694   set as a fixed modification, oxidation of methionine and protein N-terminal acetylation were set as

695   variable modifications. The mass tolerances in MS and MS/MS were set to 5 ppm and 20 ppm

696   respectively. Maximum peptide charge was set to 7 and 5 amino acids were required as minimum

697   peptide length. A false discovery rate of 1% was set up for both protein and peptide levels. All 4

698   fractions per sample were gathered and the iBAQ intensity was used to estimate the protein

699   abundance within a sample[74].

700   The label-free quantitative (LFQ) proteomics data was annotated in Perseus v.1.6.14.0[75] with

701   Mouse MitoCarta 3.0[77] for individual OXPHOS complexes, mitoribosome and whole mitochondria

702   using uniprot IDs. The Relative Complex Abundance (RCA) of the OXPHOS and mitoribosome

703   were plotted with an in-house R script (R v.4.0.3, R studio v.1.3.1093) correcting for differential

704   mitochondrial content using the ratio mean of LFQ values of mitochondria "+" annotations from

705   MitoCarta 3.0. The mean of normalized values and standard deviation were calculated for each

706   subunit of each complex comparing the LMKO and control mouse values from quantitative

707   proteomics data along with the 95% confidence interval based on the t-statistic for each complex.

708   A paired t-test calculated the significance between the LMKO and control mouse values for each

709   complex. P-value *** = $p \leq 0.001$, ns= non-significant.

710   Mass spectrometry data have been deposited at the ProteomeXchange Consortium

711   (http://www.proteomexchange.org) via the PRIDE partner repository[76,77] with the dataset identifier

712   PXD041197.

713

714   **Co-Immunoprecipitation assay**

715   500 µg of cardiac liver mitochondria were freshly isolated from heart tissue of cardiomyocyte

716   specific Flag-MTFP1 Knock-In (KI) mice (*Alb-Cre^tg/+Mtfp1^+/+, CAG^tg+/*) and Control mice (*Albe-*

717   *Cre^+/+Mtfp1^+/+, CAG^tg/+*) as described above. Mitochondria were lysed in IP buffer (20 mM HEPES-

718   KOH pH 7.5, 150 mM NaCl, 0.25% Triton X-100, protease inhibitor cocktail) on ice for 20 min and

719   then centrifugated at 10000 g, 4°C for 15 min. Supernatant obtained by centrifugation was then

720   incubated with 20 µL of anti-FLAG magnetic beads (Sigma M8823) for 2 hours at 4°C. The

721   immunocomplexes were then washed with IP buffer without Triton X-100 and eluted with Laemmli

722   Sample Buffer 2x at 95°C for 5 min. Protein were stacked in a 15 % SDS-PAGE gel with a 10 min

723   long migration at 80 V. Proteins were fixed in gel and migration was visualized using the Instant

724   Blue stain (Expedeon). Bands were excised for digestion. Gel bands were washed twice in

725   Ammonium bicarbonate (AmBi) 50 mM, once with AmBi 50 mM / ACN 50 % and once with 100%

726   ANC. Gel band were incubated for 30 min at 56°C in 5 mM dithiothreitol (DTT) solution for

727   reduction. Gel bands were washed in AmBi 50 mM and then in 100% ACN. Alkylation was

728   performed at room temp in the dark by incubation of the gel bands in Iodocateamide 55 mM

729   solution. Gel bands were washed twice in AmBi 50mM and in 100% ACN. 600 ng of trypsin were

730   added for 8h digestion at 37°C. Peptides were extracted by collecting 3 washes of the gel bands

731   using AmBi 50 mM / 50 % ACN and 5 % FA. Peptides clean up and desalting was done using

732   Stage tips (2 disc Empore C18 discs stacked in a P200 tip).

733   LC-MS/SM analysis of digested peptides was performed on an Orbitrap Q Exactive HF mass

734   spectrometer (Thermo Fisher Scientific, Bremen) coupled to an EASY-nLC 1200 (Thermo Fisher

735   Scientific). A home-made column was used for peptide separation (C_{18} 30 cm capillary column

736   picotip silica emitter tip (75 µm diameter filled with 1.9 µm Reprosil-Pur Basic C_{18}-HD resin, (Dr.

737   Maisch GmbH, Ammerbuch-Entringen, Germany)). It was equilibrated and peptide were loaded in

738   solvent A (0.1 % FA) at 900 bars. Peptides were separated at 250 nl.min^{-1}. Peptides were eluted

739   using a gradient of solvent B (ACN, 0.1 % FA) from 3% to 26% in 105 min, 26% to 48% in 20 min

740   (total length of the chromatographic run was 145 min including high ACN level step and column

741   regeneration). Mass spectra were acquired in data-dependent acquisition mode with the XCalibur

742   2.2 software (Thermo Fisher Scientific, Bremen) with automatic switching between MS and MS/MS

743   scans using a top 12 method. MS spectra were acquired at a resolution of 60000 (at *m/z* 400) with

744   a target value of 3×10^6 ions. The scan range was limited from 400 to 1700 *m/z*. Peptide

745   fragmentation was performed using HCD with the energy set at 26 NCE. Intensity threshold for

746   ions selection was set at 1×10^5 ions with charge exclusion of z = 1 and z > 7. The MS/MS spectra

747   were acquired at a resolution of 15000 (at *m/z* 400). Isolation window was set at 1.6 Th. Dynamic

748   exclusion was employed within 30 s. Data are available via ProteomeXchange with identifier

749   PXD041197.

750   <u>Data Processing</u>

751   Data were searched using MaxQuant (version 1.6.6.0) [1,2] using the Andromeda search engine

752   [3] against a reference proteome of Mus musculus (53449 entries, downloaded from Uniprot the

753   24[th of] July 2018). A modified sequence of the protein MTP18 with a Flag tag in its N-ter part was

754   also searched.

755   The following search parameters were applied: carbamidomethylation of cysteines was set as a

756   fixed modification, oxidation of methionine and protein N-terminal acetylation were set as variable

757   modifications. The mass tolerances in MS and MS/MS were set to 5 ppm and 20 ppm respectively.

758   Maximum peptide charge was set to 7 and 5 amino acids were required as minimum peptide

759   length. A false discovery rate of 1% was set up for both protein and peptide levels. The iBAQ

760   intensity was used to estimate the protein abundance within a sample.

761   Quantitative analysis was based on pairwise comparison of intensities. Values were log-

762   transformed (log2). Reverse hits and potential contaminant were removed from the analysis.

763   Proteins with at least 2 peptides (including one unique peptide) were kept for further statistics.

764   Intensities values were normalized by median centering within conditions (normalizeD function of

765   the R package DAPAR). Remaining proteins without any iBAQ value in one of both conditions

766   have been considered as proteins quantitatively present in a condition and absent in the other.

767   They have therefore been set aside and considered as differentially abundant proteins. Next,

768   missing values were imputed using the impute.MLE function of the R package imp4. Statistical

769   testing was conducted using a limma t-test thanks to the R package limma[113]. An adaptive

770   Benjamini-Hochberg procedure was applied on the resulting p-values thanks to the function

771   adjust.p of R package cp4p[114] using the robust method previously described[115] to estimate the

772   proportion of true null hypotheses among the set of statistical tests. The proteins associated to an

773   adjusted p-value inferior to a FDR level of 1% have been considered as significantly differentially

774   abundant proteins. Mass spectrometry data have been deposited at the ProteomeXchange

775   Consortium (http://www.proteomexchange.org) via the PRIDE partner repository[103,104] with dataset

776   identifier that is pending

777

778   **Liver RNA sequencing and RT-qPCR**

779   Total RNA was isolated from 50–100 mg of snap-frozen liver tissue by the NucleoSpin RNA kit

780   (Macherey-Nagel, 740955). Quality control was performed on an Agilent BioAnalyzer. Libraries

781   were built using a TruSeq Stranded mRNA library Preparation Kit (Illumina, USA) following the

782   manufacturer's protocol. Two runs of RNA sequencing were performed for each library on an

783   Illumina NextSeq 500 platform using paired-end 75bp. The RNA-seq analysis was performed with

784 Sequana. In particular, we used the RNA-seq pipeline (version 0.9.13)

785 (https://github.com/sequana/sequana_rnaseq) built on top of Snakemake 5.8.1[78]. Reads were

786 trimmed from adapters using Cutadapt 2.10 then mapped to the mouse reference genome

787 GRCm38 using STAR 2.7.3a[79]. FeatureCounts 2.0.0 was used to produce the count matrix,

788 assigning reads to features using annotation from Ensembl GRCm38_92 with strand-specificity

789 information[80]. Quality control statistics were summarized using MultiQC 1.8[81]. Statistical analysis

790 on the count matrix was performed to identify differentially regulated genes, comparing different

791 diets among same genotypes or different genotypes under same diet. Clustering of transcriptomic

792 profiles were assessed using a Principal Component Analysis (PCA). Differential expression

793 testing was conducted using DESeq2 library 1.24.0[82] scripts based on SARTools 1.7.0 indicating

794 the significance (Benjamini-Hochberg adjusted p-values, false discovery rate FDR < 0.05) and the

795 effect size (fold-change) for each comparison. Over-representation analysis (ORA) was performed

796 to determine if genes modulated by HFD in control or LMKO mice are more present in specific

797 pathways. ORA was performed on WebGestalt (https://www.webgestalt.org/). RNAseq data have

798 been deposited at ENA with the dataset identifier E-MTAB-12920.

799 For RT-qPCR, 1 μg of total RNA was converted into cDNA using the iScript Reverse Transcription

800 Supermix (Bio-Rad). RT-qPCR was performed using the CFX384 Touch Real-Time PCR Detection

801 System (Bio-Rad) and SYBR® Green Master Mix (Bio-Rad) using the primers listed in

802 Supplemental Dataset 4. Gapdh was amplified as internal standard. Data were analyzed

803 according to the $2-\Delta\Delta CT$ method[83].

Primary hepatocyte isolation and imaging

805 Primary hepatocytes were isolated from 6-8 week-old mice as previously described[84].

806 Briefly, a catheter (22G feeding needle) was connected to a pump and inserted into the vena cava.

807 Livers were perfused first with perfusion buffer (NaCl 0.15M; Potassium Chloride (KCl, 2.7mM;

808 EuroMedex; P017); Sodium phosphate dibasic (Na_2HPO_4, 0.2mM; Sigma-Aldrich 255793);

809 HEPES-KOH pH 7.4 10mM and EDTA 0.5mM, pH 7.65) at the speed of 3.5 ml/min for 10 minutes,

810 and then with the collagenase buffer (NaCl 0.15M, KCl 2.7mM, Na_2HPO_4 0.2mM, HEPES-KOH pH

811 7.4 10mM, Calcium Chloride ($CaCl_2$, 0.8mM; Sigma-Aldrich C3881) and Collagenase type I

812   500µg/ml (Thermo Fisher Scientific, 17018029). Liver lobes were collected in Washing Medium

813   (William's medium (Thermo Fisher Scientific) supplemented with 10% Fetal Bovine Serum (FBS;

814   GIBCO, 10270), a mix of penicillin (100U/ml) and streptomycin (100µg/ml) (Pen-Strep; Sigma-

815   Aldrich, P4333) and amphotericin B (Fungizone, 250ng/ml; GIBCO, 15290018)) and passed

816   through a cell strainer. Cells were centrifuged at 40g for 2 minutes, supernatant was removed and

817   resuspended again with Washing Medium. This procedure was repeated 3 times. Finally,

818   hepatocytes were counted with Trypan Blue using the Countless II FL Automated Cell counter

819   (Invitrogen) and plated at 1.2×10^5 cells/cm^2 in Culturing Medium (William's medium supplemented

820   with 20% FBS, a mix of Insulin (1,7µM), Transferrin (68,75nM) and Selenium (38,73nM) (ITS;

821   Sigma-Aldrich, I3146), dexamethasone (25nM; Sigma-Aldrich, D4902), penicillin (100U/ml) and

822   streptomycin (100µg/ml) and Fungizone (250ng/ml) on pre-coated plates (collagen I (40µg/ml,

823   Gibco) and glacial acetic acid 0.1% (Sigma-Aldrich, A6283); and incubated at 37°C then washed

824   with PBS.

825   For cell death assays, primary hepatocytes were seeded in Cell Carrier Ultra 96-well plates

826   (PerkinElmer, 6055302), treated with NucBlue Solution (Thermo Fisher)) (diluted 1 drop in 10 ml)

827   for 30 minutes, then, after 2 washes in PBS, cells were treated or not with hydrogen peroxide

828   (H_2O_2, 1mM; Sigma-Aldrich, 95294) in the presence of Propidium Iodide (PI; Thermo Fisher,

829   640922) (1:500).. Cells were immediately imaged with the Operetta CLS High-Content imaging

830   system (PerkinElmer) at 37°C and 5% CO_2. Images were taken every 2 hours for 10 hours with a

831   20x water-immersion objective (1.0 NA). PI and NucBlue were excited with the 530-560nm and

832   355-385nm LEDs respectively. Images were analyzed using Harmony 4.9 software (Perkin Elmer)

833   using the analysis sequence in Supplemental Dataset 4.

834   Quantification of mitochondrial morphology: primary hepatocytes were isolated from

835   mitoYFP+ mice and seeded in Cell Carrier Ultra 96-well plates, treated with 2µM NucBlue for 30

836   minutes, then, after 2 washes in PBS. Cells were immediately imaged with the Operetta CLS High

837   Content imaging system at 37°C and 5% CO_2. Images were taken 16 hours post-plating with a 63x

838   water-immersion objective (1.15 NA). MitoYFP and Hoechst were excited with the 460-490nm and

839   355-385nm LEDs respectively. Images were analyzed using the PhenoLOGIC supervised machine

840   learning pipeline in Harmony 4.9 software (Perkin Elmer) with the analysis sequence in Table 4.

841　The algorithm was used to identify the best properties able to segregate into "Short" or "Long"

842　populations according to their mitochondrial network. 50 cells of each population were manually

843　selected to for supervised machine learning training. Dead cells identified by propidium iodide were

844　excluded directly by the algorithm and not included in the quantification. To mimic HFD steatosis in

845　primary hepatocytes isolated, culture medium of the cells was supplemented with Intralipid (0.65%)

846　for 24 hours. Intralipid is a complex lipid emulsion composed linoleic, oleic, palmitic, and stearic

847　acids.　BODIPY™ 558/568 C12 (Invitrogen D3835)　was used to stain intracellular lipid

848　accumulation.

849

850　**Statistical Analyses**

851　Experiments were repeated at least three times and quantitative analyses were conducted blindly.

852　Randomization of groups (e.g., different genotypes) was performed when simultaneous, parallel

853　measurements were not performed (e.g., Oroboros, hepatocyte isolation). For high-throughput

854　measurements (e.g., mitochondrial morphology, cell death), all groups were measured in parallel

855　to reduce experimental bias. Statistical analyses were performed using GraphPad Prism v9

856　software. Data are presented as mean ± SD or SEM where indicated. The statistical tests used,

857　and value of experiment replicates are described in the figure legends. Comparisons between two

858　groups were performed by unpaired two-tailed T test. To compare more than two groups or groups

859　at multiple time points 1-way ANOVA or 2-way ANOVA was applied. Tests were considered

860　significant at p-value < 0.05 (*p< 0.05; **p< 0.01; ***p < 0.001; ****p< 0.0001).

861

862

863 **Figure legends**

864 **Figure 1. Hepatic deletion of *Mtfp1* in mice does not impair basal liver function.**

865 **A)** Generation of hepatocyte-specific *Mtfp1* knockout mice (LMKO). Conditional *Mtfp1$^{LoxP/LoxP}$*

866 mice were crossed to Alb-Cre recombinase mice to generate LMKO mice. *Mtfp1* exons 2 and

867 3 are flanked by LoxP sites (blue arrowheads) and a single FRT site (green arrowhead), which

868 are excised by Cre-recombinase to yield the deleted (Δ) *Mtfp1* allele.

869 **B)** PCR genotyping of Mtfp1 alleles from DNA isolated from indicated different organs of a

870 control and a LMKO mice allele-specific primers (Supplemental Dataset 4).

871 **C)** Normalized *Mtfp1* expression in liver in control and LMKO mice at 24-weeks measured by

872 RNAseq (Supplemental Dataset 1). n=4. Data are means ± SD, unpaired Student's t

873 test.****p<0.001.

874 **D)** MTFP1, VDAC1, TOMM40 and TIMM20 protein levels. Equal amounts of protein extracted

875 from livers of control (n=4) and LMKO (n=4) mice at 8 weeks of age separated by SDS-PAGE

876 and immunoblotted with indicated antibodies.

877 **E)** Genotype distribution from *Mtfp1$^{LoxP/LoxP}$ Alb-Cre$^{tg/+}$* x *Mtfp1$^{LoxP/LoxP}$* intercrosses were not

878 different from expected Mendelian distributions using two-tailed binomial tests of live female

879 (n=202; p>0.9999) and male (n=155; p=0.7481) mice.

880 **F)** (Top) Representative images of livers from control and LMKO mice fed at 24 weeks of

881 age. Scale bar=1cm. (Bottom) Representative H&E staining n=4-5. Scale bar=100μm.

882 **G)** Liver mass of 24-week old control and LMKO mice fed a normal chow (NCD) n=9. Data

883 are means ± SD. unpaired Student's t test, ns=not significant

884 **H)** Alanine (ALAT; left) and Aspartate transaminase (ASAT; right) levels in plasma of control

885 (n=3) and LMKO (n=3) mice. Data are means ± SD. unpaired Student's t test, ns=not

886 significant . *: p<0.05.

887 **I)** Cholesterol (left) and triglycerides (TG, right) levels in plasma from G). Data are means ±

888 SD. unpaired Student's t test, ns=not significant.

889 **J)** Metabolic cage analyses of NCD-fed control and LMKO mice at 24 weeks of age. Total

890 distance covered, food, and water during dark and light phases n=3. Data

891 are means ± SD. unpaired Student's t test, **: p<0.01. ns=not significant

892

Figure 2. *Mtfp1* deletion promotes mitochondrial respiration via OXPHOS upregulation

A) Substrates from fatty acid oxidation (mustard) and glycolysis (purple, blue) are metabolized in the TCA cycle which delivers fuels the electron transport chain (ETC) complexes by providing NADH and FADH to complexes I (purple) and II (blue), respectively. ETC creates an electrochemical gradient driving the phosphorylation of ADP by Complex V. Specific inhibitors of complex I (rotenone), complex V (oligomycin).

B) Oxygen flux (JO_2) of isolated liver mitochondria from 16-week old control and LMKO mice on NCD measured in the presence of pyruvate, glutamate, and malate (state 2) or succinate and rotenone (state 2) or palmitoyl carnitine and malate (state 2) followed by ADP (state 3). Non-phosphorylating respiration was measured in the presence of oligomycin (state 4). Respiratory Control Ratio (State3:4) data are represented as the means ± SD of 4 independent experiments. 2-tailed, unpaired Student's t test. *: $p<0.05$.

C) Respiratory Control Ratio (RCR) from 16 week old control and LMKO mice on NCD measured in the presence of pyruvate, glutamate and malate plus ADP (state 3) or palmitoyl carnitine and malate plus ADP(state 3) divided Non-phosphorylating respiration in the presence of oligomycin (state 4).

D) Mitochondrial membrane potential ($\Delta\Psi$) measured by quenching of Rhodamine 123 (RH123) fluorescence in liver mitochondria from B). $\Delta\Psi$ was measured in presence of pyruvate, malate, and glutamate (PGM) (state 2) followed by the addition of ADP (state 3) and Oligomycin (OLGM, state 4), CCCP and potassium cyanide (KCN). Data represent mean ± SD; Multiple t-test.

E) Complex IV activity assessed in isolated liver mitochondria from NCD-fed 16-week old control and LMKO mice by recording oxygen flux (JO_2) in the presence of Antimycin A (AMA), Ascorbate and N,N,N′,N′-Tetramethyl-p-phenylenediamine dihydrochloride (TMPD), previously incubated with of pyruvate, glutamate, and malate (PGM) and ADP. n=5. Data are means ± SD. 2-tailed, unpaired Student's t test. *: $p<0.05$.

F) Complex V (ATP synthase) activity assessed in isolated liver mitochondria from NCD-fed 16-week old control and LMKO mice in the reverse mode in the presence of ATP. Oligomycin

921   (OLGM) or ADP were used as negative controls. n=10. Data are means ± SD. 2-tailed,

922   unpaired Student's t test. *: p<0.05.

923   **G)** Mitoplast respiration assessment. Mitoplasts were supplied with either NADH, malate, and ADP

924   (complex I) or FADH, rotenone, and ADP (complex II) n=5. Data are means ± SD. 2-tailed,

925   unpaired Student's t test. *: p<0.05.

926   **H)** (Top) Volcano plot of liver proteome of LMKO mice analyzed by mass spectrometry.(Purple)

927   Mitochondrial proteins (MitoCarta 3.0), (Green) Non-mitochondrial proteins more abundant in

928   control liver. (Blue) Non-mitochondrial proteins significantly more abundant in LMKO liver.

929   (Bottom) Relative complex abundance (RCA) plot comparing the levels of the OXPHOS

930   complexes (CI-CV) and the mitoribosome (mtribo) between LMKO and control mice fed. NCD.

931   The graph represents the relative values of each complex ratio between LMKO and controls.

932   The dotted line represents the control mean value of each complex and error bars represent

933   95% confidence interval of the mean. Paired t-test. *** = p<0.001, ns=non-significant.

934   **I)** Proteins levels of different OXPHOS complexes subunits. Equal amounts of protein

935   extracted from hepatic mitochondria isolated from NCD-fed 16-week old control (n=4) and

936   LMKO (n=4) mice were separated by SDS-PAGE, immunoblotted with indicated antibodies

937   (left) and quantified by densitometry (right) relative to Timm22 loading control. Data are means

938   ± SD. 2-tailed, unpaired Student's t test. *: p<0.05.

939   **J)** 1D BN-PAGE (left) and densitometric quantification (right) of OXPHOS complexes in hepatic

940   mitochondria isolated from NCD-fed 16-weeks old control and LMKO. n=4. Data are means ± SD.

941   2-tailed, unpaired Student's t test. *: p<0.05.

942

943   **Figure 3. MTFP1 interacts with various IMM components in the liver.**

944   **A)** Generation of transgenic (Tg) Hepatocyte[FLAG-MTFP1] mice constitutively expressing FLAG-MTFP1

945   from the *Ros26* locus.

946   **B)** Protease protection assay of liver mitochondria isolated from control and transgenic (Tg)

947   Hepatocyte[FLAG-MTFP1] mice analyzed by immunoblot with indicated antibodies.

948   **C)** Liver mitochondria co-immunoprecipitation (co-IP) in WT and FALG_MTFP1 mitochondria.

949   Elution fraction (Elu).

950 **D)** Volcano plot of the FLAG-MTFP1 interactome analyzed by mass spectrometry (left). (Purple)

951 Mitochondrial proteins exclusively present in FLAG-MTFP1 eluates (binary interactors) or enriched

952 greater than two-fold listed in Supplementary Data 5. (Green) Non-mitochondrial proteins enriched

953 in Hepatocyte[FLAG-MTFP1] livers. (Blue) Non-mitochondrial proteins enriched in control liver. (Right)

954 Functional classification of 113 mitochondrial proteins identified in Co-IP eluates (Supplementary

955 Data 4).

956 **E)** Second dimension electrophoresis (2D BN-PAGE) analysis of hepatic mitochondria isolated

957 from control (top) and cMKO (bottom) mice fed a NCD immunoblotted using the indicated

958 antibodies.

959

960 **Figure 4. Hepatic deletion of *Mtfp1* protects mice from high fat diet-induced steatosis.**

961 **A)** Representative images of livers from control and LMKO mice fed a normal chow diet

962 (NCD, from Fig. 1D) or a high fat diet (HFD) for 16 weeks.

963 **B)** Liver weight of 24-week old control and LMKO mice fed a NCD or HFD for 16 weeks. n=7. Data

964 are means ± SD. 2-way ANOVA, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$

965 **C)** Body mass of control and LMKO mice fed a NCD or HFD for16 weeks. n=7. Data are

966 means ± SD. 2-way ANOVA, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

967 **D)** Triglycerides (TG) measurement in liver tissue from 24-week old control and LMKO mice

968 fed a NCD or HFD for 16 weeks. n=4-5. Data are means ± SD. 2-way ANOVA, **: $p < 0.01$,

969 **E)** H&E staining of control and LMKO livers from A). Scale bar=100μm.

970 **F)** Alanine (ALAT; left) and Aspartate transaminase (ASAT; right) levels in plasma of 24-

971 week old control and LMKO mice fed a NCD or HFD for 16 weeks. Data are means ± SD. 2-

972 way ANOVA, *: $p < 0.05$, ***: $p < 0.001$, ****: $p < 0.0001$

973 **G)** Differential modulation of liver gene expression by HFD. Upregulation (left) and

974 downregulation (right) of mitochondrial (pink) and non-mitochondrial genes (orange or teal) in

975 LMKO and control livers.

976 **H)** Pathway enrichment analysis of differentially expressed genes depicted in G).

977 **I)** TUNEL staining of control and LMKO livers from A). Scale bar=100μm

978

Figure 5. LMKO mice are protected against diet-induced metabolic dysregulation

A) Whole-body Respiratory Exchange ratios (RER; VCO_2/VO_2) in control and LMKO mice after 16 weeks of HFD. n=3. Data are means ± SD. 2-way ANOVA, **: $p<0.01$.

B) Pyruvate Tolerance Test performed after 18h fasting. Left panel: glycaemia measured at indicated time. Right panel: area under the curve (AUC). n=4 NCD; n=5 HFD groups. Data are means ± SD. 2-way ANOVA, *: $p<0.05$, **: $p<0.01$, ****: $p<0.0001$.

C) Glucose Tolerance Test performed after 18h fasting. Left panel: glycaemia measured at indicated time. Right panel: area under the curve (AUC). n=4-9. Data are means ± SD 2-way ANOVA, **: $p<0.01$.

D) Insulin Tolerance Test performed after 6 hours fasting. Left panel: glycaemia measured at indicated time after insulin injection. Right panel: area under the curve (AUC). n=4. Data are means ± SD. 2-way ANOVA, *: $p<0.05$, **: $p<0.01$, ***: $p<0.0001$, ****: $p<0.0001$.

E) Body composition analysis of control and LMKO mice fed NCD or HFD by Nuclear Magnetic Resonance (NMR). n=5. Left panel: fat mass; right panel: lean mass. Data are means ± SD. 2-way ANOVA, *: $p<0.05$, **: $p<0.01$, ***: $p<0.0001$, ns=not significant

F) Plasma cholesterol (left) and triglycerides (TG) in control and LMKO mice fed NCD or HFD. n=4-5. Data are means ± SD. 2-way ANOVA, *: $p<0.05$, **: $p<0.01$, ***: $p<0.0001$, ns=not significant

G) Epididymal white adipose tissue (eWAT) mass in control and LMKO mice fed NCD or HFD. n=5 NCD, n=7 HFD. Data are means ± SD. 2-way ANOVA, *: $p<0.05$, **: $p<0.01$.

H) Ex vivo steatosis measurement. Representative images of primary hepatocytes isolated from control and LMKO mice treated with IntraLipid (0.65%) for 24h and stained for nuclei (NucBlue, blue) and lipid droplets (BODIPY, orange).

I) BODIPY intensity quantification of H) Data are means of 3 independent experiments ± SD. unpaired Student's t test, **: $p<0.01$

Figure 6. Hepatic deletion of *Mtfp1* protects mice from FAS-induced liver damage.

A) Representative images of livers from control (n=2) and LMKO (n=4) mice treated with FAS or NaCl for 24 hours.

B) Alanine (ALAT; left) and Aspartate transaminase (ASAT; right) levels in plasma of mice

from A). Data are means ± SD. unpaired Student's t test, *: p<0.05, **: p<0.01.

C) H&E staining of control and LMKO livers from A). Scale bar=100μm.

D) TUNEL staining of control and LMKO livers from A). Scale bar=100μm.

E) Representative of high content imaging of primary hepatocytes from control and LMKO mice

treated with or without H_2O_2 at 4 hours (1mM) treatment. Blue: nuclei; Orange: propidium iodide

(PI).

F) Cell death quantification of PI uptake of 3 independent experiments from E). Data are means ±

SEM. 2-way ANOVA, *: p<0.05.

G) Mitochondrial permeability transition pore (mPTP) opening assay. Liver mitochondria

from control and LMKO mice were treated with $CaCl_2$ 120μM to induce the mPTP opening

by light scattering at 540nm. Cyclosporin A (CsA) 1 μM used to block mPTP opening. Data

are means of 3 independent experiments ± SEM. 2-way ANOVA, *: p<0.05.

Figure 7. Model for the regulation of liver resilience by MTFP1

Mtfp1 deletion in hepatocytes of liver-specific Mtfp1 knockout (LMKO) mice promotes liver

resilience in vivo by 1) increasing the resistance to mitochondrial permeability transition pore

(mPTP) opening and liver apoptosis and 2) enhancing oxidative phosphorylation (OXPHOS)

activity and resistance against fatty liver disease (hepatic steatosis) induced by chronic high fat

diet (HFD) feeding.

Supplemental Figures

Figure S1. Generation and characterization of *Mtfp1* hepatocyte-specific knockout mice

A) Targeting strategy for conditional inactivation of mouse *Mtfp1* in LMKO mice. To allow deletion

of *Mtfp1* exons 2 and 3 were flanked in both cases by LoxP sites (blue arrowheads). Flox denotes

NeoR cassette containing LoxP targeted locus. LoxP denotes NeoR cassette-deleted targeted

locus. Δ denotes deletion induced by Cre-recombinase. FRT sites (green) initially flank NeoR

cassette (yellow). Exons 2 and 3 were deleted by Cre-recombinase.

B) Genotyping PCR for the distal LoxP site in the conditional *Mftp1* locus from genomic DNA.

1037   The wild type (WT; 387 bp) and the conditional (LoxP; 469 bp) alleles are shown for *Mtfp*^{WT/WT},

1038   *Mtfp1*^{LoxP/WT}, and *Mtfp1*^{LoxP/LoxP} mice.

1039   **C)** Respiratory Exchange Ratio (RER) for control and LMKO mice on fed a normal chow diet (NCD).

1040   RER analyses for 24 hours are shown. Mean values for day and night periods. N=4. Data are means

1041   ± SD. 2-tailed, unpaired Student's t test. ns=not significant.

1042   **D)** Upregulated (orange) and downregulated (cyan) genes in LMKO mice on a normal chow diet

1043   corresponding to Supplemental Dataset 1.

1044

1045   **Figure S2. Impact of *Mtfp1* deletion on mitochondrial mass**

1046   **A)** Representative images of primary hepatocytes isolated from control and LMKO mice

1047   expressing mitoYFP (green). Nuclei in blue. Scale bar=100µm.

1048   **B)** Quantification of mitochondrial imaging in primary hepatocytes from control and LMKO

1049   expressing mitoYFP mice. Single cell mitoYFP intensity is normalized to hepatocyte area.

1050   **C)** Quantification of mitochondrial morphology in primary hepatocytes from control and LMKO mice

1051   performed by supervised machine learning.

1052   **D)** Mitochondrial DNA content measured by qPCR relative to nuclear DNA in liver biopsies from

1053   control and LMKO mice fed a normal chow (NCD) or high-fat (HFD) diets for 16 weeks. N=4. Data

1054   are means ± SD. 2-way ANOVA, *: p<0.05. **: p<0.01.

1055   **E)** Mitochondrial surface determined by transmission electron microscopy (TEM) of control

1056   and LMKO liver sections.   Median (yellow dotted line) and Quartiles (solid colored line). 2-

1057   tailed, unpaired Student's t test. ns=not significant.

1058

1059   **Figure S3. Diet-induced transcriptional remodeling in control and LMKO mice**

1060   **A)** Venn diagram overlap of upregulated genes in LMKO and control mice on normal chow diet

1061   (NCD) or high fat diet (HFD) corresponding to Supplemental Dataset 1.

1062   **A)** Venn diagram overlap of downregulated genes in LMKO and control mice on normal chow diet

1063   (NCD) or high fat diet (HFD) corresponding to Supplemental Dataset 1.

1064

1065   **Figure S4. Diet-induced proteomic remodeling in control and LMKO mice**

1066 **A)** (Top) Volcano plot of liver proteome of LMKO mice analyzed by mass spectrometry.(Purple)

1067 Mitochondrial proteins (MitoCarta 3.0), (Green) Enriched non-mitochondrial proteins more

1068 (Blue) Enriched non-mitochondrial proteins corresponding to Supplemental Dataset 3.

1069 **B)** Relative complex abundance (RCA) plot comparing the levels of the OXPHOS

1070 complexes (CI-CV) and the mitoribosome (mtribo). The graph represents the relative values

1071 of each complex ratio between two groups. The dotted line represents the control mean value

1072 of each complex and error bars represent 95% confidence interval of the mean. Paired t-test.

1073 *** = $p < 0.001$, ns= non-significant.

1074

1075 **Figure S5. LMKO mice are protected against diet-induced metabolic dysregulation**

1076 **A)** Total food consumption during dark and light phases in HFD-fed control and LMKO mice.

1077 n=3. Data are means ± SD. unpaired Student's t test, ns=not significant.

1078 **B)** Total water consumption during dark and light phases in HFD-fed control and

1079 LMKO mice n.=3. Data are means ± SD. unpaired Student's t test, ns=not significant.

1080 **C)** Total distance covered during dark and light phases in HFD-fed control and LMKO mice.

1081 n=3. Data are means ± SD. unpaired Student's t test, ns=not significant

1082 **D)** Plasma insulin levels measured in control and LMKO mice treated fed with NCD or

1083 HFD after 16 hours fasting (0 minute) or fasting followed by glucose injection (30 minutes).

1084 n=4-5. Data are means ± SD. 2-way ANOVA, ***: $p < 0.001$

1085 **E)** Representative images of extrahepatic white adipose tissue (eWAT) after H&E staining

1086 from control and LMKO mice fed with NCD or HFD for 16 weeks. n=4-5. Scale bar=100μm.

1087 **F)** Quantification of adipocyte area in G).Data are means ± SD. 2-way ANOVA, *: $p < 0.05$.

1088 **G)** Body weight of control and LMKO mice on NCD and HFD. Data are means ± SD

1089 **H)** Fasting glycaemia of control and LMKO mice on NCD and HFD. Data are means ± SD

1090

1091 **Figure S6. Hepatic deletion of *Mtfp1* protects mice from FAS-induced liver damage.**

1092 **A)** Lactate dehydrogenase (LDH) plasma levels of 16-week old control and LMKO after

1093 treatment with FAS (n=4) or NaCl (n=2) for 24 hours. Data are means ± SD. 2-tailed, unpaired

1094 Student's t test. *: $p < 0.05$

1095   **B)** Liver to body weight ratio of 16-week old control and LMKO before and after treatment with

1096   FAS (n=6) or NaCl (n=4) for 24 hours. Data are means ± SD. 2-way ANOVA, *: $p<0.05$ ns=not

1097   significant.

1098

1099   **Figure S7. Differential MTFP1 expression in animal models and human patients**

1100   **A)** Venn diagram comparison of differentially expressed genes in MTFP1-deficient livers and

1101   hearts from LMKO and cMKO mice. LMKO data are reported in Supplemental Dataset 1 and

1102   cMKO data were reported previously[15].

1103   **B)** Venn diagram comparison of differentially expressed proteins in MTFP1-deficient livers and

1104   hearts from LMKO and cMKO mice. LMKO data are reported in Supplemental Dataset 2 and

1105   cMKO data were reported previously[15].

1106   **C)** Violin plot of *MTFP1* expression (transcripts per kilobase (TPkB) in NASH human patients

1107   biopsies (GSE162694) previously analyzed[67]. F0-F4 scale refers to the severity of the severity of

1108   fibrosis as previously described[85]. Data are means ± SD. 1-way ANOVA, **: $p<0.01$ ****: $p<0.0001$.

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1301
1302   **Acknowledgments**

Figure 1

Figure 2

Oxygen Consumption

B Pyruvate Glutamate Malate / Succinate Rotenone / Palmitoylcarnitine Malate

C Respiratory Control Ratio

D Membrane potential

E Complex IV

F Complex V

G Mitoplast respiration
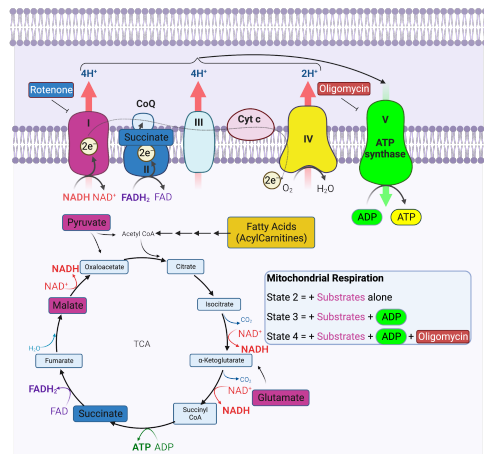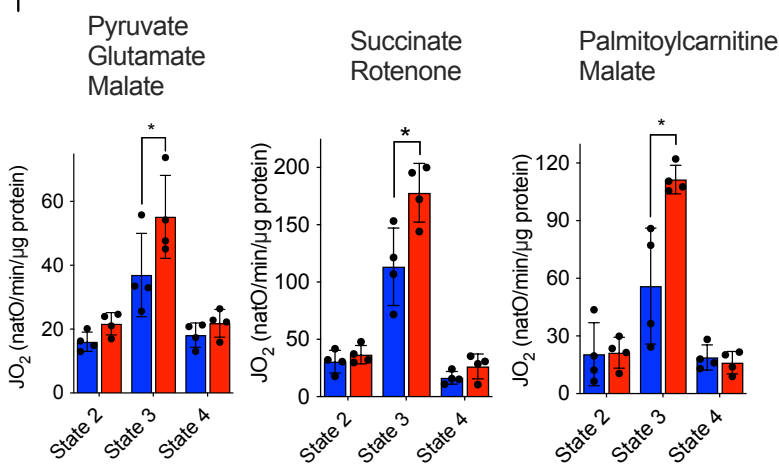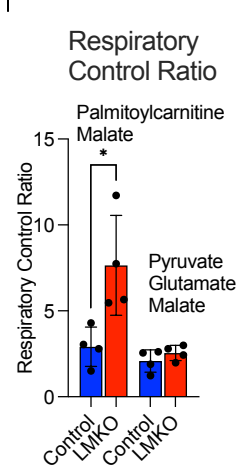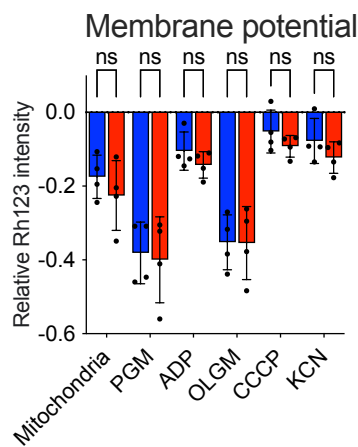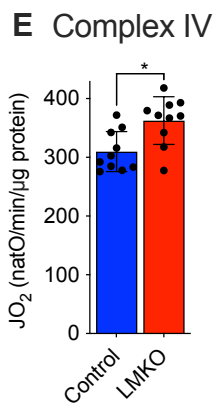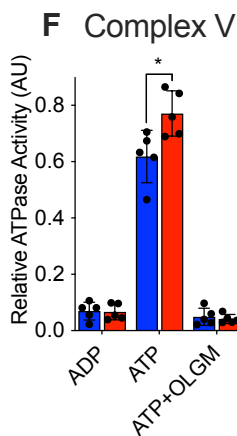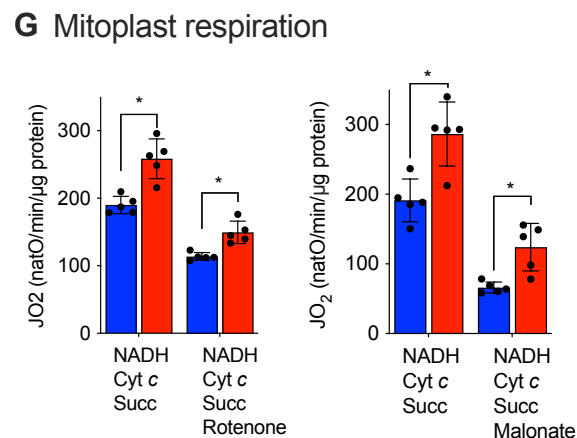
H Liver proteome / Relative Complex Abundance

I

J

Figure 3

A

Rosa ^{FLAG-MTFP1}   *Alb-Cre* ^{Tg/+}
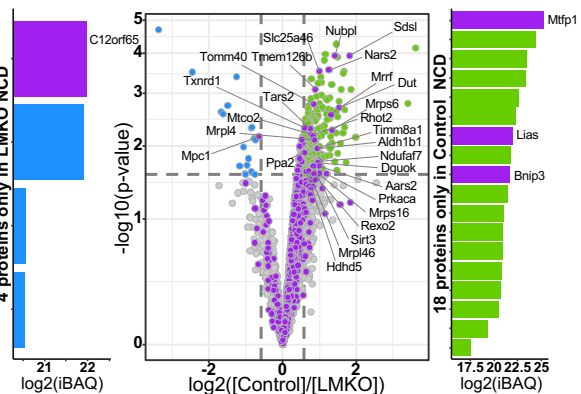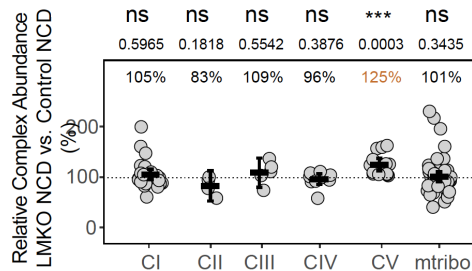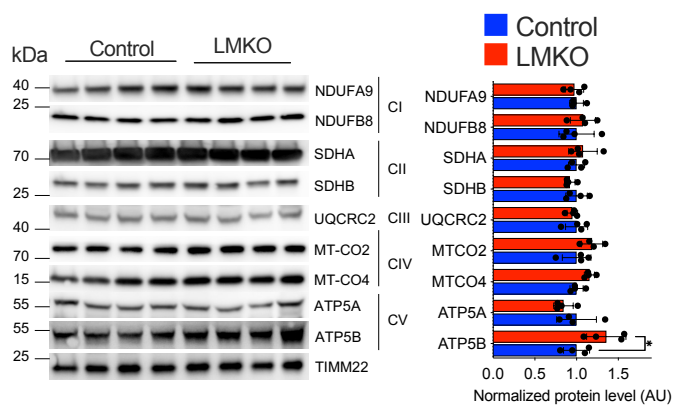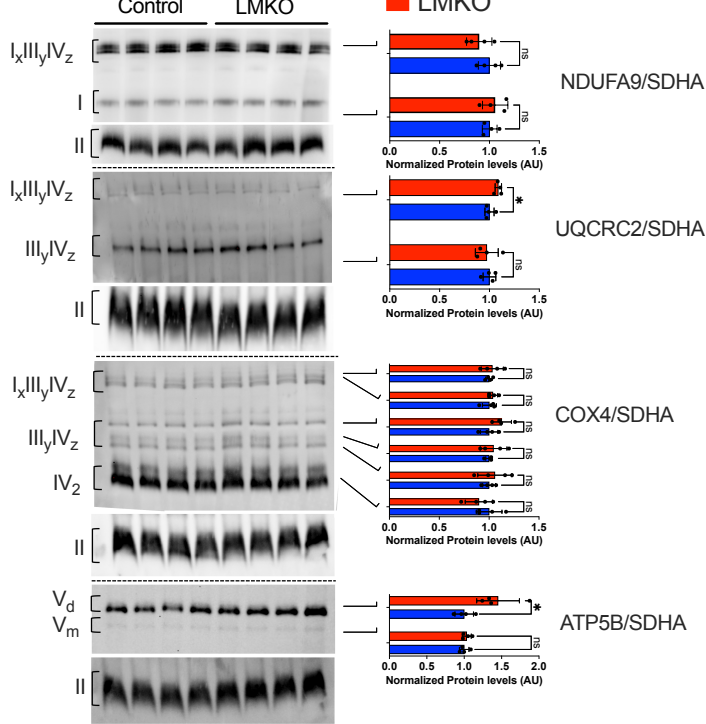
Hepatocyte ^{FLAG-MTFP1}

B

Control   Hepatocyte^{FLAG-MTFP1}

Proteinase
Swelling
Triton

70 — MFN2
35 — TOMM40
15 — FIS1
25 — ANT1
15 — CYTC
15 — MTFP1
25 — FLAG
     TIMM22
15 — NDUFB8

C

CoIP ^{FLAG}

Control   Hepatocyte^{FLAG-MTFP1}

IN FT Elu   IN FT Elu

MTFP1

Stain Free

D

3 mito proteins only in WT

Stoml2
Ndufc2
Bcl2l13
Dnajc11

log2(iBAQ)   18 20 22

-log10(p-value)

log2([FLAG-MTFP1]/[WT])

42 mito proteins only in FLAG-MTFP1

Mtfp1
Timm8a1
Chchd2
Cyb5b
C12orf65
Hdhd3
Mcee
Lactb2
Mrps34
Mrrf
Lyrm4
Ociad2
Mrpl41
Vdac3

log2(iBAQ)   16 20 24 28

Binary Interactors

- Detox
- FeS Cluster
- Folate and 1C
- Metabolism
- MICOS
- Mito Dynamics
- Mito Protease
- **Mito Translation**
- mtDNA
- **OXPHOS**
- RNA
- Translocase
- Unknown

Enriched Interactors

- Carrier
- Detox
- FeS
- Folate and 1C
- **Metabolism**
- Mito Dynamics
- Chaperone
- Mito Translation
- mtDNA
- **OXPHOS**
- Translocase
- Unknown

E

Liver mitochondria control
1D BN-PAGE 4-16%

SDS PAGE 4-20%

25/15 — MTFP1
25/15 — PPIF
35 — ANT1
55 — *ATP5A
180/130/100/70 — SDHA
15 — MRPS14

Liver mitochondria LMKO
1D BN-PAGE 4-16%

SDS PAGE 4-20%

25/15 — MTFP1
25/15 — PPIF
35 — ANT1
55 — *ATP5A
180/130/100/70 — SDHA
15 — MRPS14

Figure 4

A 16-week diet intervention

B Liver mass

C Body mass

D Liver lipids

E H&E Staining

F Liver dysfunction biomarkers

G Upregulated by HFD | Upregulated by HFD

H Pathways modulated in LMKO by HFD

Pathways modulated in Control by HFD

I Tunel Staining

Figure 5

Figure 6

A Control · LMKO (NaCl, FAS) — 1cm

B Liver dysfunction biomarkers

ALAT (U/l) · ** · Control · LMKO · NaCl · FAS

ASAT (U/l) · * · Control · LMKO · NaCl · FAS

C Control · LMKO (NaCl, FAS)

D Control · LMKO (NaCl, FAS)

E Cell death imaging of Primary Hepatocytes

0h · 4h · Control · LMKO · Untreated · H_2O_2 · DAPI · PI

F Control · Control + H_2O_2 · LMKO · LMKO + H_2O_2

Cell Death (%PI uptake) · Time after induction (h)

G mPTP opening in liver mitochondria

$CaCl_2$ · relative Abs_{540nm} · time (seconds)

Control · Control+CsA · LMKO · LMKO+CsA

Figure 7