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A mouse model of human mitofusin 2-related lipodystrophy exhibits adipose-specific mitochondrial stress and reduced leptin secretion

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Abstract

Mitochondrial dysfunction has been reported in obesity and insulin resistance, but primary genetic mitochondrial dysfunction is generally not associated with these, arguing against a straightforward causal relationship. A rare exception, recently identified in humans, is a syndrome of lower body adipose loss, leptin-deficient severe upper body adipose overgrowth, and insulin resistance caused by the p.Arg707Trp mutation in *MFN2*, encoding mitofusin 2. How the resulting selective form of mitochondrial dysfunction leads to tissue- and adipose depot-specific growth abnormalities and systemic biochemical perturbation is unknown. To address this, $Mfn2^{R707W/R707W}$ knock-in mice were generated and phenotyped on chow and high fat diets. Electron microscopy revealed adipose-specific mitochondrial morphological abnormalities. Oxidative phosphorylation measured in isolated mitochondria was unperturbed, but the cellular integrated stress response was activated in adipose tissue. Fat mass and distribution, body weight, and systemic glucose and lipid metabolism were unchanged, however serum leptin and adiponectin concentrations, and their secretion from adipose explants were reduced. Pharmacological induction of the integrated stress response in wild-type adipocytes also reduced secretion of leptin and adiponectin, suggesting an explanation for the *in vivo* findings. These data suggest that the p.Arg707Trp MFN2 mutation selectively perturbs mitochondrial morphology and activates the integrated stress response in adipose tissue. In mice, this does not disrupt most adipocyte functions or systemic metabolism, whereas in humans it is associated with pathological adipose remodelling and metabolic disease. In both species, disproportionate effects on leptin secretion may relate to cell autonomous induction of the integrated stress response.

Introduction

Mitochondrial dysfunction has been implicated in the pathogenesis of a wide range of congenital and acquired conditions[1–3]. However, despite being central to cellular energy homeostasis, there has been little mechanistic evidence of a causal role for deranged mitochondrial function in human adiposity. Instead, most patients with inherited mitochondrial disorders have a neurological phenotype, though multisystem involvement is common[1,4]. This is true for disorders caused by many different mutations affecting mitochondrial proteins, whether encoded by the mitochondrial or nuclear genome. The mechanisms underlying such tissue-selective disease manifestations even in the face of constitutional mutations are unclear, but differing tissue requirements for oxidative phosphorylation, and interactions between the nuclear and mitochondrial genome may play a role[5–7].

We[8] and others[9–13] recently identified biallelic R707W mutations in the nuclear *MFN2* gene in patients with a remarkable adipose phenotype characterised by extreme upper body adiposity (lipomatosis) and lower body lipodystrophy. Affected patients also showed non-alcoholic fatty liver disease, dyslipidaemia and insulin resistant type 2 diabetes, likely secondary to the changes in adipose tissue. *MFN2* encodes mitofusin 2, a mitochondrial outer membrane protein that plays a key role in mitochiondrial fusion and tethering to other organelles[14]. Like patients with heterozygous complete loss-of-function mutations in *MFN2,* patients with the R707W mutation also often exhibit axonal peripheral neuropathy known as Charcot-Marie Tooth type 2A (CMT2A)[15–17], but the adipose and metabolic phenotype has uniquely been associated with the R707W allele to date. Evidence suggesting that the MFN2 R707W mutation does disrupt mitochondrial function in humans includes elevated serum lactate in affected patients, abnormal mitochondrial morphology seen on transmission electron microscopy of affected adipose tissue[8], and strong transcriptomic signatures of mitochondrial dysfunction and activation of the integrated stress response (ISR)[4] in the same tissue.

Despite normal or raised whole body adipose mass, and the relatively normal histological appearance of lipomatous upper body fat, plasma leptin concentrations were very low in the patients reported by Rocha *et al.*[8]. This observation was supported by Sawyer *et al.* who reported one patient with undetectable serum leptin (<0.6 ng/mL)[9] and Capel *et al.* who described five patients with serum leptin concentrations <1.6 ng/mL despite body mass indices (BMIs) within the normal range[10]. Leptin is a critical endocrine signal of adipose stores and yet what determines the rate of adipocyte leptin secretion remains poorly understood[18]. This surprising observation thus offered a rare opportunity to address this important issue. Circulating leptin concentration correlates with fat mass, and is usually

4

141 higher in women than men, with some adipose depots reported to release more than

142 others[19]. Leptin secretion is increased by insulin stimulation[20] but this effect is modest in the

143 context of serum leptin concentrations. Adipose depots that secrete higher leptin have

144 increased *LEP* mRNA and, at least *in vitro*, *LEP* mRNA increases in response to insulin

145 stimulation[21].

146 Most neuropathy-associated *MFN2* mutations are located within the protein's GTPase

147 domain[22–25], but to date, all patients with *MFN2*-associated multiple symmetric lipomatosis

148 (MSL) have had at least one R707W allele. Most cases have been homozygous for the

149 R707W mutation, with others compound heterozygous for R707W and a second, functionally

150 null mutation[8–13]. Arginine 707 lies in the highly conserved heptad-repeat (HR)2 region of

151 MFN2 but consensus on the precise orientation and/or function of this domain has not yet

152 been established[26]. The dominant model holds that the HR2 domains of Mitofusin 1 (MFN1)

153 and/or MFN2 face the cytosol and interact in *trans,* forming mitofusin homodimers or

154 heterodimers required for mitochondrial fusion and tethering to other organelles[27,28]. The

155 R707W mutation may disrupt this binding and subsequent MFN oligomerisation and

156 mitochondrial fusion/tethering.

157 Global knock-out of the core mitochondrial fusion-fission machinery proteins *Mfn1*, *Mfn2*,

158 *Opa1*, and *Drp1* in mice confers embryonic lethality in all cases[29–31]. Two homozygous loss-

159 of-function *Mfn2* knock-ins - H361Y and R94W - have also been reported. H361Y was also

160 embryonically lethal due to complete loss of detectable Mfn2 protein[32], while homozygous

161 R94W mice died at post-natal day 1[33]. Mfn2^{R94W} is expressed but is GTPase defective,

162 increasing mitochondrial fragmentation and preventing formation of Mfn2 homodimers[34].

163 Multiple tissue-specific knock-outs of *Mfn2* (and/or *Mfn1*) have been studied, including three

164 in adipose tissue[35–38]. Adipose-specific *Adipoq*::Cre *Mfn2* knock-out increased fat mass,

165 attributed to reduced energy expenditure, whether it occurred in embryonic life[36] or was

166 induced by tamoxifen in adult mice[38], with ultrastructural evidence of more rounded

167 mitochondria with fewer lipid droplet contacts[36]. Both Adipoq::Cre driven (adipose-specific)

168 and *Ucp1*::Cre driven (brown adipose-specific) *Mfn2* knock-out also caused cold intolerance

169 with "whitened" brown adipose tissue[37]. Plasma leptin concentrations were not reported in

170 Adipoq-Cre or *Ucp1*::Cre *Mfn2* knock-outs, but leptin concentration was higher and

171 adiponectin concentration lower in the tamoxifen-inducible adult Adipoq::Cre *Mfn2* knockout

172 model[38].

173 These studies suggest that *Mfn2* has a non redundant role in adipose tissues, but findings to

174 date are not readily reconcilable with the phenotype observed in humans with the MFN2^{R707W}

175 mutation. We thus generated and characterised mice homozygous for Mfn2^{R707W} to

176   determine the extent of tissue-specific manifestations of mitochondrial dysfunction and to

177   interrogate the effect of this mutant on adipose leptin secretion.

178

179 **Results**

180 *Generation of Mfn2$^{R707W/R707W}$ mice*

181 *Mfn2$^{R707W/R707W}$* mice were generated by CRISPR-Cas9 genomic engineering using an

182 ssODN (single-stranded oligo donor) template recoding Arginine to Tryptophan in codon 707

183 (**Figure 1A**). A single round of targeting yielded one founder (F0) mouse (**Figure 1B**) which

184 was used to expand a colony on a C57/BL6J background. An additional silent mutation

185 introducing an EcoRV restriction site was introduced to facilitate genotyping (**Figure 1C, D**).

186 We first compared expression of Mfn2 and its paralogue, Mfn1, in knock-in (KI) mice and

187 wild-type (WT) littermates in order to determine if the R707W change perturbed expression

188 of the mutant Mfn2 protein and/or resulted in a compensatory change in Mfn1. We observed

189 no consistent differences in Mfn1 or Mfn2 expression, relative to WT, in inguinal white

190 adipose tissue (WAT), liver, heart, or skeletal muscle in both chow and high fat diet (HFD)

191 fed mice (**Figure 1E**, **Figure 1-Figure supplements 1-4**). However, in brown adipose tissue

192 (BAT) expression of Mfn1 was lower in KI than in WT mice fed with chow diet for 6 months

193 (**Figure 1-Figure supplement 1D and 2D**). Similarly, expression of Mfn1 was lower in KI

194 than WT mice after only 4 weeks of chow diet **(Figure 1-Figure supplement 1G and 2G)**. In

195 HFD fed mice, both Mfn1 and Mfn2 expression was lower in BAT in KI compared to WT

196 mice (**Figure 1E**, **Figure 1-Figure supplement 3D and 4D**). In epididymal WAT, expression

197 of Mfn1 was also lower in KI than in WT mice fed with chow diet **(Figure 1-Figure**

198 **supplement 1F and 2F)**, but no differences were observed in HFD fed mice **(Figure 1-**

199 **Figure supplement 3F and 4F)**. We interpret these data as suggesting that the R707W

200 missense mutation does not directly reduce expression of Mfn2, nor does it result in a

201 compensatory change in Mfn1 in most tissues. However, in BAT the data suggests that the

202 cellular perturbation induced in brown adipocytes is associated with a very modest reduction

203 in expression of Mfn1 and 2.

204 *Mfn2$^{R707W/R707W}$ mice show adipose-selective alterations of mitochondrial structure*

205 *and function*

206 Next, we used transmission electron microscopy (TEM) to examine mitochondrial

207 ultrastructure **(Figure 2A)**. In BAT, mitochondria from KI mice had a tendency to exhibit a

208 decreased mitochondrial perimeter compared to WT mice (**Figure 2B**), but significantly

209 reduced size, assessed by the mitochondrial cross-sectional length/ width aspect ratio

210 analysis (**Figure 2C**), indicating that Mfn2^{R707W} leads to mitochondrial fragmentation in BAT.

211 Double membrane-bound structures representing autophagosomes consistent with

212 mitophagy were observed in lipomatous adipose tissue from human patients with the

213 MFN2^{R707W} mutation[8] , but these were not identified in the murine tissues examined.

214 Mitofusins may mediate contact between mitochondria and lipid droplets[36], and the extent of
215 these contacts was reduced in BAT from KI animals (**Figure 2D**). In addition, mitochondrial
216 cristae were disrupted in KI compared to WT animals (**Figure 2E**). In both inguinal (**Figure
217 2-figure supplement 1A-C)** and epididymal WAT (**Figure 2-figure supplement 1D-F**),
218 similar mitochondrial fragmentation and cristae defects were observed. In contrast, no
219 change in mitochondrial morphology, cristae number, or cristae structure was seen in the
220 heart, skeletal muscle or liver of KI mice (**Figure 2-figure supplement 2**).

221 Perturbed mitochondrial dynamics have been associated with decreased mitochondrial DNA
222 (mtDNA) content, as replication of mtDNA relies on balanced fusion and fission[39]. In
223 $Mfn2^{R707W/R707W}$ mice, mtDNA was reduced in BAT in both diet conditions, but not in any
224 other tissue analysed (WAT, heart, skeletal muscle, or liver) (**Figure 2F-G**). Immunoblotting
225 of electron transport chain components from chow fed animals showed no changes in liver
226 or heart (**Figure 2-Figure supplement 3A-B and 4A-B)**. However, mtCo1 (complex IV) and
227 Ndufb8 (complex I) were reduced in KI mice in brown and white adipose tissue (**Figure 2-
228 Figure supplement 3C-E and 4C-E**). In addition, Uqcrc2 (complex III) was also lower in
229 inguinal WAT.

230 To determine if these changes altered mitochondrial oxidative phosphorylation, we assessed
231 oxidative capacity in freshly isolated mitochondria from BAT and liver by high resolution
232 respirometry using Oroboros Oxygraphy. No significant differences were detected between
233 WT and KI mitochondria (**Figure 2-figure supplement 5A-B**). We further assessed
234 mitochondrial function in BAT *in vivo* by challenging mice with noradrenaline in cold (10
235 degrees) or thermoneutral (30 degrees) conditions to determine maximum thermogenic
236 capacity. Again, despite a trend towards reduced thermogenic capacity in KI animals, the
237 difference was not significant, and both groups manifested the expected increase in energy
238 expenditure at 10ºC (**Figure 2-figure supplement 5C-G**).

239 *Body composition and metabolic phenotype of Mfn2$^{R707W/R707W}$ mice*
240 We next assessed whether *Mfn2$^{R707W/R707W}$* mice phenocopy the severely abnormal adipose
241 topography and metabolic abnormalities of patients harbouring the same mutations. Male
242 mice fed with either chow or HFD for up to 6 months were assessed. Whole body mass and
243 composition, and masses of individual adipose depots and other organs were similar in KI
244 and WT mice throughout the study period (**Table 1**, **Figure 3A-C & Figure 3-figure
245 supplement 1A-D**). Moreover, no difference in hepatic steatosis nor lipid droplet size was
246 detected histologically in BAT or WAT (**Figure 3D-E & Figure 3-figure supplement 1F-G**).
247 In keeping with the normal body composition, fasting serum glucose, insulin, triglycerides,
248 cholesterol, lactate, and liver transaminase concentrations showed no difference between

249   WT and KI mice (**Figure 3F-I & Figure 3-figure supplement 1E, H**). There was also no

250   differences in hepatic expression of genes related to lipid metabolism or steatohepatitis

251   (**Figure 3-figure supplement 1I**). Dynamic testing of glucose and insulin tolerance was also

252   similar between genotypes (**Figure 3G-K**).

253

254   Similar results were observed in female mice harbouring the Mfn2^{R707W} mutation.

255   Specifically, there was no difference in body weight, fat mass, fasting glucose or insulin

256   between high fat fed WT and KI mice (**Figure 3-Figure supplement 2A-C**).

257   *Adipose-tissue specific activation of the integrated stress response in Mfn2^{R707W}*

258   *mice*

259   Mitochondrial dysfunction is sensed by cells, and triggers a series of adaptive responses to

260   maintain mitonuclear balance and cellular homeostasis[40]. Precise sensing and transducing

261   mechanisms vary among different forms of mitochondrial perturbation[41], and show some

262   redundancy[42]. Although details of the integration of these mechanisms in different tissue and

263   cellular contexts are not fully elucidated, it is clear that the transcription factor Atf4 plays a

264   crucial role[43]. Atf4 is translationally upregulated following phosphorylation of eIF2α, which is

265   a point of convergence of several cellular stress sensing pathways. The canonical eIF2α

266   kinase HRI is most closely implicated in linking mitochondrial dysfunction to eIF2α

267   phosphorylation[44,45], but mTORC1 appears to play a role in Atf4 upregulation independently

268   of eIF2α phosphorylation[46]. The response to sustained mitochondrial dysfunction overlaps

269   significantly with the response to many other cellular stressors including unfolded protein

270   induced stress (i.e. the unfolded protein response (UPR)), and is best characterised as a

271   cellular ISR[47].

272

273   Strong transcriptional evidence of ISR activation was found in adipose tissue of patients with

274   *MFN2^{R707W}*-related lipodystrophy[8]. We thus screened multiple tissues from the KI mice for

275   ISR activation. mRNA levels of *Atf4*, *Atf5*, and *Ddit3* (Chop)*,* all sentinel markers of the ISR,

276   were increased in BAT and epididymal WAT of KI mice (**Figure 4A-C**). *Ddit3* was also

277   higher in inguinal WAT from KI animals. mRNA levels of *Atf4*, *Atf5*, and *Ddit3* (Chop) were

278   unchanged in liver, heart, and skeletal muscle, except for a 1.4-fold rise in *Atf5* (p.adj = 0.03)

279   in skeletal muscle only. Phosphorylation of eIF2α and protein expression of Mthfd2, an Atf4-

280   upregulated enzyme playing a rate-limiting role in mitochondrial one carbon metabolism[48–50],

281   were also both strongly increased in BAT and in WAT in KI mice (**Figure 4D-F & Figure 4-**

282   **figure supplement 1E-G and 2**), whereas they were unchanged in the liver, skeletal muscle

283   and heart. mRNA expression of two important secreted mediators of the organismal

284   metabolic response to mitochondrial dysfunction, Gdf15 and Fgf21, trended towards an

285   increase in adipose tissue, but serum concentrations were unchanged in KI mice (**Figure 4-**
286   **figure supplement 1A-D**). This differs from observations in patients with MFN2[R707W]-related
287   lipodystrophy[50–52], and may relate to the fact that such patients manifest non-alcoholic fatty
288   liver disease, which was not seen in the KI animals.

289

290   Given the recent evidence implicating HRI kinase as a key mediator of eIF2α
291   phosphorylation induced by mitochondrial dysfunction[44,45], we next sought to determine if
292   this pathway was active in R707W KI mice. The pathway activating HRI kinase was reported
293   to be initiated by activation of the metalloendopeptidase Oma1 [53,54]. In addition to cleaving
294   Dele1[55], which activates HRI kinase, Oma1 also cleaves Opa1 (optic atrophy type 1)[56] and
295   itself upon activation. We thus immunoblotted tissue lysates for Opa1 and Oma1. No
296   evidence for Oma1 activity was found in liver, skeletal muscle or heart (**Figure 4-figure**
297   **supplement 3**). In BAT, we did detect a modest change in Oma1 expression and a slight
298   increase in the short / long Opa1 ratio, providing some evidence that the pathway may be
299   activated (**Figure 4-figure supplement 4**). However, the differences observed were small
300   precluding a confident conclusion at this stage. In WAT, clear bands for all five Opa1 forms
301   could not be visualised on repeated attempts and so we did not attempt to quantify the data.
302   Current WAT data thus do not suggest Oma1 activation (**Figure 4-figure supplement 4C-**
303   **D**). As far as we are aware, Oma1 activation has yet to be examined in Mfn2 tissue-specific
304   KO models so we cannot infer anything from those studies either. Thus we conclude that the
305   ISR is activated in selected tissues in the KI mice, but the question of how this is linked to
306   the Mfn2 R707W mutation remains open.

307   To obtain an unbiased view of the transcriptional consequences of the lipodystrophy-
308   associated Mfn2 mutation, we next applied bulk RNA sequencing (RNAseq) to BAT and
309   inguinal WAT from HFD fed mice. Inguinal, rather than epididymal, WAT was selected as
310   subcutaneous adipose tissue is predominantly affected in human MFN2-related and other
311   partial lipodystrophies[57]. Induction of the ISR was confirmed in both BAT and WAT (**Figure**
312   **4G-I & Figure 4-figure supplement 5**), with the "unfolded protein response" gene set, a
313   surrogate for the ISR, the top upregulated gene set in BAT and 6th in inguinal WAT. *Atf5*
314   and *Mthfd2* were confirmed among the most highly upregulated mRNAs in both tissues, and
315   a range of other well established ISR genes also showed increased expression. These
316   included *Ddit3* (Chop), *Trib3*, an Atf4-driven ISR component that exerts negative feedback
317   on the ISR, and *Gadd45a*, involved in ISR-induced G2/M checkpoint arrest[58].

318   The top upregulated gene set in inguinal WAT was "oxidative phosphorylation", driven solely
319   by increased expression of nuclear-encoded mitochondrial genes (**Figure 4-figure**
320   **supplement 1H**). In contrast, mitochondria-encoded genes were nearly universally

321   downregulated (**Figure 4-figure supplement 1I**), recapitulating the pattern seen in affected

322   human WAT[8]. In BAT, a similar but weaker pattern was seen on inspection of heatmaps, but

323   this was not sufficient to reach statistical significance.

324   Another finding common to mouse and human was the transcriptional evidence of mTORC1

325   activation. The "mTorc signalling" gene set was the second most upregulated group in BAT

326   and third most upregulated in WAT (**Figure 4-figure supplement 5B&E**). This activation is

327   consistent with the proposed role for mTORC1 in mediating the proximal ISR[46], and is of

328   interest given accumulating evidence that the mTORC1 inhibitor sirolimus may exert

329   beneficial effects in various mitochondrial diseases[59].

330   Although no increase in adipose tissue mass was seen in *Mfn2^{R707W}* mice the "adipogenesis"

331   gene set was upregulated in inguinal WAT under both diet conditions (**Figure 4H & Figure**

332   **4-figure supplement 5B**). However, closer inspection revealed a mixed profile of individual

333   gene expression. The most consistent finding was downregulation of the adipokine-encoding

334   mature adipocyte genes *Adipoq* and *Lep* in *Mfn2^{R707W}* mice (discussed below). *Adipoq* and

335   *Lep* were also downregulated in BAT (fold changes 0.64 (p.adj=6.3×10^{-4}) *and 0.31* (p.adj=0.03)

336   respectively), but the adipogenesis gene set was not enriched overall (**Figure 4-figure**

337   **supplement 5E**).

338   To assess for other potential drivers of adipose hyperplasia, we also examined

339   downregulated gene sets. The signature of epithelial-mesenchymal transition (EMT) was the

340   most strongly down-regulated set in mouse BAT and WAT (**Figure 4I & Figure 4-figure**

341   **supplement 5C, F**), and was also previously found to be downregulated in overgrown

342   human WAT in MFN2-associated multiple symmetric lipomatosis[8]. In bulk transcriptomic

343   data it is not possible to discern the cell type(s) responsible for this consistent signature.

344   However TGFβ family ligands are important mediators of EMT, some family members inhibit

345   adipogenesis[60], and they also play important roles in regulating mitochondrial function and in

346   responding to mitochondrial dysfunction[61].

347   We next sought to assess whether the increased demand for adipose expansion imposed by

348   HFD feeding exacerbates the transcriptional consequences of Mfn2 R707W homozygosity.

349   RNAseq was thus undertaken of inguinal WAT from mice maintained on HFD for 6 months.

350   Comparison to WT animals revealed strikingly concordant findings to those seen in chow-fed

351   mice (**Figure 4-figure supplement 5G**). No general differences were seen in the magnitude

352   of transcriptional changes induced by Mfn2 R707W homozygosity between conditions.

353   Oxidative phosphorylation, unfolded protein response and adipogenesis genes and Hallmark

354   gene sets were strongly upregulated, whereas genes in the EMT set were downregulated in

355   KI mice on both diets. An exception was the group of mRNAs related to cholesterol

356 homeostasis, for which diet strikingly modified the effect of genotype. They were the top
357 downregulated gene set in inguinal WAT in HFD-fed animals but were not significantly
358 altered on chow in the same depot and were upregulated in BAT (**Figure 4I & Figure 4-**
359 **figure supplement 5E**). Lower expression of key enzymes in cholesterol metabolism (e.g.
360 Hydroxymethylglutaryl-CoA synthase (*Hmgcs1*), mevalonate kinase (*Mvk*), and squalene
361 monooxygenase (*Sqle*)) in WAT on HFD is consistent with the response to inhibition of the
362 mitochondrial respiratory chain in primary human fibroblasts[62].

363 *Lower circulating leptin and adiponectin in mice homozygous for Mfn2^{R707W}*

364 One of the most striking aspects of the Mfn2^{R707W}-associated lipodystrophy phenotype is the
365 low or undetectable serum leptin concentration despite abundant whole body adiposity,
366 accounted for mostly by excess upper body adipose tissue of relatively normal histological
367 appearance[8]. Serum adiponectin concentrations are also low, however this is in keeping with
368 the "adiponectin paradox" widely seen in obesity with insulin resistance[63]. Mirroring these
369 human observations, KI mice showed low serum leptin and adiponectin concentrations on
370 both chow and HFD (**Figure 5A-C**), though unlike humans, the mice had normal fat mass
371 and insulin sensitivity. In both WT and KI mice serum leptin concentrations correlated
372 positively with whole body adiposity on chow and HFD, but a generalised linear model
373 revealed marked attenuation of the relationship between serum leptin concentration and
374 adipose mass in the KI mice (**Figure 5C & Figure 5 supplement 1A**). Immunoblotting of
375 WAT samples from KI mice fed a HFD confirmed that both leptin and adiponectin expression
376 was reduced (**Figure 5-Figure supplement 2**). Findings were similar in female mice, in
377 which serum adiponectin was significantly lower in KI mice (**Figure 3-Figure supplement**
378 **2F**), with a trend towards lower leptin (**Figure 3-Figure supplement 2G-I**).

379 RNAseq showed *Lep* mRNA in inguinal WAT to be lower in Mfn2^{R707W} than in WT mice under
380 both chow (fold change 0.35; p.adj = 3.2x10^{-4}, **Figure 4-Figure Supplement 5A**) and HFD
381 (fold change 0.66; p.adj = 5.3x10^{-4}, **Figure 4G**). *Adipoq* mRNA was also significantly lower in
382 chow (fold change 0.66; p.adj = 2.7x10^{-4}, **Figure 4-Figure Supplement 5A**) and HF fed mice
383 (fold change 0.57; p.adj = 3.3x10^{-15}, **Figure 4G**). RT-qPCR analysis confirmed lower *Lep*
384 mRNA in epididymal WAT from chow fed Mfn2^{R707W} animals (**Figure 5D**). RT-qPCR analysis
385 also found 50% lower *Adipoq* mRNA in both inguinal and epididymal WAT in chow fed
386 Mfn2^{R707W} mice and in inguinal WAT from HFD fed Mfn2^{R707W} mice (**Figure 5E**).

387 To assess leptin secretion directly, we studied production of adipokines from adipose
388 explants. Explants from KI mice fed on HFD for 4 weeks showed lower secretion of leptin
389 and adiponectin per gram of tissue (**Figure 5-figure supplement 1A-B**). KI explants also
390 exhibited minimal increase in leptin secretion after insulin and dexamethasone stimulation.

391   *Adipoq* mRNA was lower in KI than WT explants at baseline whereas *Lep* mRNA was no

392   different at baseline but failed to increase in KI explants following insulin and

393   dexamethasone stimulation (**Figure 5-figure supplement 1C**).

394   To assess whether induction of the ISR in adipose tissue may be responsible for the relative

395   leptin deficiency in both humans and mice homozygous for MFN2 R707W, we studied

396   adipocytes freshly isolated from mouse gonadal fat in floating culture, adapting a recently

397   described protocol[64]. We induced the ISR using each of two different well characterised

398   activators, namely thapsigargin (TG), an inhibitor of the endoplasmic reticulum (ER) Ca^{2+}

399   ATPase that depletes ER calcium[65,66], or tunicamycin (TN), which blocks protein

400   glycosylation. ISR induction was confirmed by increased *Atf4* and *Ddit3* mRNA and/or

401   protein expression, and by eIF2α phosphorylation (**Figure 5F-L**). *Lep* mRNA was modestly

402   reduced by TG but not by TN (**Figure 5H**), whereas both agents reduced intracellular leptin

403   protein expression (**Figure 5L**) and secretion (**Figure 5J**). Expression of adiponectin was

404   also reduced without a change in mRNA level whereas expression of Akt and the insulin

405   receptor beta subunit were not altered (**Figure 5I, K & L**). These data suggest that ISR

406   activation may have a bigger impact on secreted proteins than on intracellular proteins, in

407   keeping with the previous suggestion that the ISR tends to prevent a fall in intracellular

408   amino acid concentrations[66]. In seeking to validate this notion, we proceeded to measure the

409   serum concentration of adipsin, another 'adipokine' selectively secreted by adipocytes.

410   Serum adipsin concentrations were significantly lower in the KI mice than in WT controls in

411   both chow and HFD fed mice (**Figure 5M**), suggesting that low serum leptin and adiponectin

412   may be part of a wider pattern of reduced adipocyte-derived secreted proteins in *MFN2*[*R707W*]

413   KI mice.

414

415

Discussion

The recent discovery that humans homozygous for the MFN2 R707W mutation manifest striking adipose redistribution associated with serious metabolic disease is probably the clearest example to date of a causal link in humans between a mitochondrial perturbation and adipose dysregulation. MFN2-related lipodystrophy has some remarkable and currently poorly understood features. These include: a) a marked and often dramatic increase in upper body adiposity, contrasting with loss of lower limb adipose tissue; b) a severe reduction in plasma leptin concentration despite abundant, histologically near-normal upper body fat. These problems have to date been associated only with the R707W mutation. To investigate the molecular pathogenesis of MFN2 R707W-related lipodystrophy, and the role of MFN2 in leptin synthesis and secretion, we generated and characterised homozygous $Mfn2^{R707W/R707W}$ mice.

$Mfn2$ knock-out mice die in early embryogenesis[31] while mice homozygous for either of two human neuropathy-associated, GTPase null missense mutations (H361Y or R94W) die on day 0-1[33]. Mfn2 is nearly ubiquitously co-expressed with its closely related paralogue Mfn1, and this demonstrates that it has essential, non-redundant functions. Homozygous Mfn2 R707W mice, in contrast, were viable and bred normally, showing that Mfn2 R707W retains significant function. Mfn2 also has key metabolic functions in mature adipocytes: mice lacking Mfn2 in all adipocytes[36] or in brown adipocytes alone[37] did not show reduced adipose tissue, but they did exhibit lower energy expenditure, reduced expression of multiple oxidative phosphorylation subunits, and impaired cold tolerance. Paradoxically, however, both lines were protected from systemic insulin resistance. Mice in which Mfn2 was deleted in all adipocytes in adulthood showed increased obesity and elevated blood glucose[38]. In contrast, homozygous Mfn2 R707W mice showed no overt change in adipose mass, metabolic function, or thermogenic capacity even though the genetic alteration was constitutional. This confirms some retained Mfn2 function also in adipose lineages.

Primary anatomical and/or functional defects in humans with MFN2 R707W homozygosity have been observed only in adipose tissue and peripheral nerves, with some but not all people reported to have sensorimotor neuropathy. Such neuropathy is commonly observed in people with heterozygous loss of MFN2 function[15,67]. Although homozygous Mfn2 R707W KI mice had no overt anatomical adipose abnormality, and failed to show obvious neurological phenotypes, ultrastructural studies did reveal mitochondrial network disruption in adipose tissues, but not liver, skeletal muscle, or heart. The structural changes in mouse adipose mitochondria resembled those in adipose tissue from patients homozygous for the MFN2 R707W mutation[8], and in both species these were associated with robust activation of the ISR, which was also seen previously in tissue-specific $Mfn2$ knock-out mice[68]. The ISR

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452   was not activated in liver, muscle, and heart, strengthening evidence that Mfn2 R707W has
453   deleterious effects selectively in adipose tissue. We cannot conclusively exclude the
454   possibility that tissues other than brown and white adipose tissue are affected, as we have
455   not studied every tissue in the mice or patients, but if present, it is not associated with overt
456   phenotypes.

457   The reason for adipose-selectivity of abnormalities in Mfn2 R707W homozygous mice is not
458   established, but disruption of the function of Mfn2 in establishment or maintenance of
459   mitochondrial-lipid droplet contact sites, perhaps through interaction with an adipose-specific
460   protein, is plausible. We did observe reduced mitochondrial-lipid droplet contacts in brown
461   adipose tissue from Mfn2 R707W KI mice, as reported in adipocyte *Mfn2* knock-out animals,
462   but we were unable to replicate the direct mitofusin 2-perilipin 1 interaction previously
463   reported using co-immunoprecipitation[36]. This requires further characterisation in the context
464   of Mfn2[R707W].

465   Whether mitochondrial structural and functional perturbation mediates the overgrowth of
466   some adipose depots and loss of others in humans with MFN2 R707W homozygosity
467   remains to be proven. If it does, the mechanisms transducing dysfunction of a key organelle
468   into cellular hyperplasia in some adipose depots but loss of adipose tissue in others are also
469   unexplained. KI mice exhibit neither adipose loss nor hyperplasia, even when challenged by
470   a HFD. This failure to model the gross anatomical adipose abnormalities of humans, despite
471   evidence of mitochondrial dysfunction and attendant ISR, establishes that the cellular
472   abnormalities we describe are not sufficient to perturb adipose growth, but they may still be
473   necessary. Whether a permissive genetic background, or an undefined additional stressor,
474   are required as cofactors, remains to be determined.

475   Some of the transcriptomic changes observed that are common to mouse and human
476   adipose tissue do suggest both potential opportunities to intervene pharmacologically, and
477   mechanistic hypotheses relating to adipose hyperplasia that warrant further investigation.
478   For example, transcriptional evidence of strong mTORC1 activation, likely part of the
479   proximal ISR triggered by mitochondrial dysfunction, suggests that mTOR inhibitors such as
480   sirolimus are worthy of testing. It is possible that they may reduce the ISR, thereby
481   restraining compensatory adipose hyperplasia or even inducing synthetic lethality in cases of
482   MFN2 R707W homozygosity. Several previous studies have suggested that mTOR inhibition
483   may have beneficial effects in other primary mitochondrial disorders[59,69]. Downregulation of
484   TGFβ also merits further investigation as one candidate mechanism linking Mfn2 R707W
485   homozygosity to adipose hyperplasia. This is based on strong transcriptional evidence of
486   downregulated EMT in both mice and humans, on the important roles of TGFβ in EMT and

487 adipogenesis[70,71], and on the inter-relationship of TGFβ signalling with mitochondrial
488 dysfunction[72–75].

489 A further notable difference between the mice and humans with the homozygous MFN2
490 R707W mutation is that serum concentrations of GDF15 and FGF21 were increased in
491 people but not mice (**Table 2**). This likely reflects the fact that affected humans also have
492 fatty liver disease and diabetes, both strongly associated with elevated stress hormone
493 levels[76,77]. In keeping with this, we have shown in mice that the liver is the predominant
494 source of circulating FGF21 and GDF15, with little or no contribution from adipose tissue[78].

495 Although abnormal adipose growth and metabolic disease were not seen in KI mice, the low
496 plasma leptin concentration seen in human adipose overgrowth associated with MFN2
497 mutations was replicated. Leptin concentrations were not reported in previously described
498 adipocyte *Mfn2* knock-out mice[36,38], and although a different model of adipose-specific
499 mitochondrial dysfunction (*Tfam* knock-out) did show reduced serum leptin, fat mass was
500 also reduced compared to WT[79]. Lower adipose leptin secretion caused by Mfn2 R707W
501 does not appear to be predominantly transcriptionally mediated in mice, as in some
502 analyses, for example of adipose explants under basal conditions, leptin secretion was
503 reduced (**Figure 5-figure supplement 1A**) without alteration of leptin mRNA (**Figure 5-**
504 **figure supplement 1C**). Our findings suggest instead that the lower leptin secretion is a
505 consequence of ISR activation. Synthesis of adipokines is an amino acid-intensive process,
506 and activation of the UPR typically results in conservation of amino acids, in part through
507 reduction of protein secretion[66,80–82]. Stressing primary adipocytes with tunicamycin or
508 thapsigargin reduced leptin secretion without any effect on expression of non-secreted
509 proteins such as the insulin receptor. We also observed upregulation of pathways related to
510 amino acid metabolism (particularly in BAT) (**Figure 4 – Figure Supplement 4**), which
511 would be consistent with the known transcriptional effects of Atf4[83]. This suggests that the
512 low leptin may not be due to a mitofusin-specific mechanism, rather secondary to activation
513 of the ISR in adipose tissue as part of amino acid conservation. KI mouse data suggest that
514 other adipokines, including adiponectin and adipsin, are similarly affected.

515 This study has limitations. We characterised male homozygous KI mice only in detail, so
516 cannot extrapolate our results to females with confidence, though case series do not
517 suggest significant sexual dimorphism in the human disorder[8–10]. We also did not study
518 heterozygous animals, but as human MFN2 R707W-associated lipodystrophy shows
519 recessive inheritance, and as even homozygous mice do not exhibit lipodystrophy, a
520 phenotype in heterozygous animals seems unlikely. It is unclear to what extent the
521 phenotype observed in BAT is due to reduction in expression of both the mitofusins. Given
522 the concomitant reduction in expression of Oxphos components in BAT, the lower mitofusin

523 expression may be more reflective of general mitochondrial perturbation. Lastly, this study
524 has not directly assessed the ability of Mfn2^{R707W} mutants to mediate mitochondrial fusion.
525 However given the normal mitochondrial network morphology in non-adipose tissues in
526 homozygous KI mice and in dermal fibroblasts from humans homozygous for MFN2
527 R707W[8], any defect is likely mild and context dependent.

Conclusion

529 Mfn2^{R707W} KI mice show adipose-selective alteration of mitochondrial morphology and robust
530 activation of the integrated stress response, but no abnormal adipose growth or systemic
531 metabolic derangement. The KI mice do show suppressed leptin expression and plasma
532 leptin concentration, likely secondary to the adipose selective mitochondrial stress response.
533 The unique association of human lipodystrophy with the MFN2 R707W allele remains
534 unexplained, but transcriptomic analysis suggested that reduced TGFβ signalling warrants
535 further explanation as a potential cause of adipose hyperplasia, while mTOR inhibitors are
536 worthy of testing in models as a potential targeted therapy.

Methods

Key Resources Table				
Reagent type (species) or resource	**Designation**	**Source or reference**	**Identifiers**	**Additional information**
strain, strain background (*Mus musculus, C57BL/6J*)	Mfn2^{R707W}	This paper		See "Generation of the Mfn2$^{R707W/R707W}$ knock-in mouse"
antibody	Mouse monoclonal anti-Adiponectin	GeneTex	Cat# GTX80683	WB (1:1000)
antibody	Rabit polyclonal anti-Leptin	Abcam	Cat# ab9749	WB (1:1000)

antibody	Mouse monoclonal anti-Mfn1	Abcam	Cat# Ab126575	WB (1:250)
antibody	Rabbit monoclonal anti-Mfn2	Cell signalling	Cat# D2D10	WB (1:1000)
antibody	Rabit polyclonal anti-Mthfd2	Proteintech	Cat# 12270-1-AP	WB (1:1000)
antibody	Rabit polyclonal anti-Oma1	Proteintech	Cat# 17116-1-AP	WB (1:1000)
antibody	Mouse monoclonal anti-Opa1	BD Biosciences	Cat# 612606	WB (1:1000)
antibody	Mouse monoclonal OXPHOS cocktail	Abcam	Cat# Ab110413	WB (1:1000)
antibody	Rabbit monoclonal anti-Phos-Eif2a	Epitomics	Cat# 10901	WB (1:1000)

540   A full list of antibodies and primers are available in Supplementary Tables 1 and 2.

541

542   Generation of the $Mfn2^{R707W/R707W}$ knock-in mouse

543    $Mfn2^{R707W}$ mice were generated using CRISPR-Cas9 microinjection of fertilised oocytes at

544   The Wellcome Trust Centre for Human Genetics (Oxford, UK). Two single guide RNA

545   (sgRNA) sequences targetting exon 18 of the mouse $Mfn2$ gene (ENSMUSE00000184630)

546   were   used,   namely   5'-   CACCgTTCCTGCTCCAGATTATCTC-3'   and   5'-

547   AAACGAGATAATCTGGAGCAGGAAc-3'. The single-strand donor oligonucleotide (ssODN)

548   incorporated the desired R707W mutation by recoding codon 707 from CGA to TGG. (This

549   involved two point mutations in order to avoid a stop codon.) A silent mutation was added

550   upstream to generate an EcoRV restriction site (Figure 1).

551 Superovulated 3-week old C57BL/6J female mice were mated with C57BL/6J males.
552 Embryos were extracted on day 0.5 of pregnancy and cultivated until two pronuclei were
553 visible. One pronucleus was injected with purified sgRNA (20ng/µL), Cas9 protein (100
554 ng/µL), and the ssODN template (10ng/µL). Embryos were reimplanted into pseudopregnant
555 CD1 foster mothers at day 0.5 post-coitum. $Mfn2^{R707W}$ was confirmed by Sanger sequencing
556 of F0 founder males (with one additional upstream silent mutation ACC>ACA). Following
557 cryopreservation of embryos, the line was re-derived in a colony of C57BL/6J mice in
558 Cambridge, UK.

559 Genotyping utilised the upstream EcoRV restriction enzyme digestion site. For genotyping,
560 ear biopsies were digested in Chelix and proteinase K (0.1mg/mL) for 45 min at 50°C to
561 extract gDNA for use in PCR. gDNA was amplified using primers 5'-
562 AGTCCCTTCCTTGTCACTTAGT-3' and 5'-ATCTCACAAGAAAGCGAAATCC-3' and GoTaq
563 DNA Polymerase (Promega), then digested using EcoRV (New England Biosciences). Wild-
564 type (WT) *Mfn2* generates a PCR product of 523bp, homozygous knock-ins (KI) have two
565 bands at 327bp and 196bp, and heterozygotes have all three bands (Figure 1).

566 Mouse husbandry and phenotyping

567 All experiments were performed under UK Home Office-approved Project License 70/8955
568 except for thermogenic capacity assessments which were conducted under P0101ED1D.
569 Protocols were approved by the University of Cambridge Animal Welfare and Ethical Review
570 Board. Animals were co-housed in groups of 2-5 littermates of mixed genotype, on 12 hr
571 light/dark cycles. They had access to food and water *ad libitum* except when fasting prior to
572 experimental procedures.

573 Separate cohorts of male and female mice were studied. Unless otherwise stated (**Figure 3
574 – Figure Supplement 2**), data represents results from male mice. WT and KI male mice
575 aged 5 weeks were randomly allocated to HFD (45% kcal as fat, 4.7kcal/g, Research Diets
576 D12451i) or chow (Safe Diets R105-25) for up to 6 months. Investigators were blinded to
577 animal genotype at the point of data collection. Mice were weighed weekly. Tail blood
578 samples were collected four weekly into heparinised capillary tubes (Hawksley) and spun at
579 13,000g for 4 min for plasma analysis of leptin and adiponectin. 6 hr fasted blood samples
580 were obtained on weeks 16 and 24 of diet, and prior to sacrifice, for analysis of glucose,
581 insulin, and lactate, whilst other blood samples were from fed animals. For lactate, tail vein
582 blood was collected into fluoride oxalate tubes and centrifuged immediately before freezing
583 of plasma at -80°C.

584 An intraperitoneal glucose tolerance test (IPGTT) was performed on week 31 of chow and
585 HFD and an intraperitoneal insulin tolerance test (IPITT) was performed on week 32 of HFD
586 (only) after a 6 hr fast. 1g/kg of glucose and 0.75 units/kg of insulin were administered for

587   the IPGTT and IPITT, respectively. Blood glucose was measured at 0, 10, 20, 30, 60, 90,

588   and 120 min after the injections using a glucometer (Abbot Laboratories) and glucose test

589   strips (Abbot Laboratories). Insulin was measured at 0 min at the start of IPGTT from a tail

590   vein blood sample.

591

592   Body composition (lean and fat mass) was assessed prior to sacrifice by Time-Domain

593   Nuclear Magnetic Resonance (TD-NMR) using a Minispec Live Mouse Analyzer (Bruker).

594   Mice were sacrificed at 4- or 33- weeks on diet after a 6 hr fast. Tissues were weighed,

595   sections were removed for histological or electron microscopic analysis, and remaining

596   tissue was snap frozen in liquid nitrogen.

597

598   To estimate the number of mice required for experimental groups, We used data from the

599   *Adipoq*::Cre *Mfn2* knock-out mouse[36], aiming to determine a difference between fat mass in

600   Mfn2^{R707W} and wild-type. Mean fat mass in adipose-specific *Mfn2* knock-out = 3.8±0.43g.

601   Mean fat mass in wild-type = 2.9±0.14g. For 80% power at 0.05 significance, to detect 0.9g

602   difference, sample size: 8 animals per group.

603

604   Calorimetry studies

605   Eight-week-old chow-fed male mice were housed in either cold (10°C) or thermoneutrality

606   (30°C) for 4 weeks. Animals were anaesthetised using 90mg/kg of pentobarbital by

607   intraperitoneal injection and were placed in 2.7 l calorimetry chambers (Oxymax, Columbus

608   instruments, Ohio) attached to a Promethion calorimetry system (Sable Systems, Las

609   Vegas, NV, USA) pre-warmed to 30°C for 20 min for measurement of basal energy

610   expenditure. They were then given a subcutaneous injection of 2 µl/g of 0.5 mg/mL

611   noradrenaline bitartrate (NA) plus 1.66 µl/g of 18 µg/µl pentobarbital and NA-stimulated

612   energy expenditure was measured for 25 min. Animals were then sacrificed, and tissues

613   snap frozen as described above. Basal energy expenditure was calculated from the three

614   readings prior to NA-injection. Peak energy expenditure was calculated from the three

615   greatest readings 5-25 min after NA-injection. NA-induced energy expenditure was

616   calculated as the difference between peak and basal energy expenditure.

617   Transmission Electron Microscopy (TEM)

618   Chow-fed mice aged 8 weeks were sacrificed and white adipose tissue (inguinal and

619   epididymal), brown adipose tissue, skeletal muscle (quadriceps), heart, and liver were

620   removed, cut into <1 mm^3 cubes and fixed (2% glutaraldehyde/2% formaldehyde in 0.05 M

621   sodium cacodylate buffer pH 7.4 containing 2 mM calcium chloride) on a rocker at 4℃

622   overnight. Samples were then washed five times with 0.05 M sodium cacodylate buffer pH

623   7.4 and osmicated (1% osmium tetroxide, 1.5 % potassium ferricyanide, 0.05 M sodium

624   cacodylate buffer pH 7.4) for 3 days at 4°C.

625   Following initial osmication, samples were washed five times in DIW (deionised water) then

626   treated with 0.1 % (w/v) thiocarbohydrazide/DIW for 20 min at room temperature in the dark.

627   After washing five times in DIW, samples were osmicated a second time for 1 hr at room

628   temperature (2% osmium tetroxide/DIW). After washing five times in DIW, samples were

629   block stained with uranyl acetate (2% uranyl acetate in 0.05 M maleate buffer pH 5.5) for 3

630   days at 4°C. Samples were washed five times in DIW and then dehydrated in a graded

631   series of ethanol (50%/70%/95%/100%/100% dry) 100% dry acetone and 100% dry

632   acetonitrile, three times in each for at least 5min. Samples were infiltrated with a 50/50

633   mixture of 100% dry acetonitrile/Quetol resin (without benzyldimethylamine (BDMA))

634   overnight, followed by 3 days in 100% Quetol (without BDMA). Then, samples were

635   infiltrated for 5 days in 100% Quetol resin with BDMA, exchanging the resin each day. The

636   Quetol resin mixture is: 12 g Quetol 651, 15.7 g NSA, 5.7 g MNA and 0.5 g BDMA (all from

637   TAAB). Samples were placed in embedding moulds and cured at 60°C for 3 days.

638   Thin sections were cut using an ultramicrotome (Leica Ultracut E) and placed on bare 300

639   mesh copper TEM grids. Samples were imaged in a Tecnai G2 TEM (FEI/Thermo Fisher

640   Scientific) run at 200 keV using a 20 µm objective aperture to improve contrast. Images were

641   acquired using an ORCA HR high resolution CCD camera (Advanced Microscopy

642   Techniques Corp, Danvers USA).

643   Analysis was performed by manual measurement of individual mitochondrion from all

644   obtained images using Fiji[84]/ImageJ measurement tools by an investigator who was blinded

645   to the genotype of tissues/cells. In all tissues, mitochondrial perimeter and aspect ratio

646   (length/width) were determined. In addition, in brown adipose tissue, the number of cristae

647   per mitochondrion and mitochondrial-lipid droplet contacts were quantified. There were

648   insufficient mitochondrial-lipid droplet contact sites with high quality preservation to permit

649   this in white adipose tissue. In liver, mitochondrial-endoplasmic reticulum contact sites were

650   quantified. It was not possible to assess for mitochondria-ER contacts in other tissues due to

651   quality of preservation.

652   Isolation of mitochondria for Oroboros analysis

653   For *ex vivo* measurement of mitochondrial respiratory capacity, mitochondria were isolated

654   from BAT and liver of 12 week old chow-fed male mice using the protocols from McLaughlin

655   *et al.*[85] for BAT and Fernández-Vizarra *et al.*[86] for liver. In brief, tissues were isolated and

656   washed in buffer 'B' then homogenised using a drill-driven Teflon pestle and borosilicate

657   glass vessel. Homogenates were centrifuged at 800 x *g* for 10 min at 4°C to remove cellular

658   debris. Supernatant was removed, and then re-centrifuged at 10,000 x *g* for 10 min at 4°C

659   to enrich mitochondria. Supernatant was discarded and mitochondria were resuspended in

660   buffer 'A'. Mitochondria were quantified using a BioRad Protein Assay.

661   Fifty μg of protein were used per chamber for high-resolution respirometry, using Oroboros

662   (Innsbruck, Austria). There was sequential injection of: 20 μl of 1 M glutamate and 10 μl of 1

663   M malate (liver only), 20 μl of 0.5 M ADP, 20 μl of 1 M pyruvate, 20 μl of 2 M succinate, 1 μl

664   of 1 mM CCCP (liver only), 2 μl of 2 mM rotenone, and 2 μl of 2 mM antimycin A.

665   mtDNA content assay

666   Relative mtDNA content was assayed using real-time quantitative polymerase chain reaction

667   (RT-qPCR) quantification of mitochondrial *Rnr2* and nuclear *Hk2* DNA. DNA was extracted

668   from snap frozen murine tissue, using a DNeasy kit (Qiagen) as per the manufacturer's

669   instructions. DNA was quantified on Nanodrop and diluted to 4 ng/ul. RT-qPCR was

670   performed in triplicate for each sample using 8 ng DNA with primers for *Hk2* and *Rnr2*.

671   mt*Rnr2*/n*Hk2* was calculated using the standard curve method and expressed relative to

672   WT.

673   Protein extraction for WB

674   Fifty mg of frozen tissue was crushed using a pestle and mortar in liquid nitrogen. Powdered

675   tissue was dissolved in 800 μl of RIPA buffer (Sigma, R0278) containing protease (Sigma,

676   11836170001) and phosphatase inhibitors (Roche, 04906837001). Samples were sonicated

677   twice for 5 s at 30 Hz before centrifugation at 10,000 x g for 5 min at 4°C. Supernatant was

678   extracted and, for adipose samples, re-centrifuged to remove excess lipid.

679   Thirty-45 μg of protein lysates were mixed with NuPAGE 4x LDS buffer (ThermoFisher

680   Scientific), containing 0.05% 2-mercaptoethanol, and denatured for 5 min at 95°C. Samples

681   were run on 4-12% Bis-Tris gels (Invitrogen) and transferred onto a nitrocellulose membrane

682   using iBlot-2 (ThermoFisher Scientific). For Opa1 immunobloting, protein lysates were

683   prepared using 4x BOLT^{TM} LDS sample buffer and resolved using BOLT^{TM} 8% Bis-Tris with

684   BOLT^{TM} MOPS SDS running buffer (ThermoFisher Scientific). Membranes were washed in

685   Tris-buffered saline with 0.1% (vol/vol) Tween 20 (TBST, Sigma) before blocking in 5%

686   (wt/vol) skimmed milk powder dissolved in TBST. Membranes were incubated with primary

687   antibodies (**Supplementary Table 1**) at 4°C for 16 hr, washed with TBST five times for 5

688   min, followed by incubation with horseradish peroxidase (HRP)-conjugated secondary

689   antibodies for 1 hr at room temperature. Blots were developed using Immobilon Western

690   Chemiluminescent HRP Substrate (Millipore) with images acquired on BioRad ChemiDoc

691   Imaging system or ImageQuant LAS 4000 (GE Healthcare).

692   For Opa1 quantification, Oma1-cleaved S-Opa1 bands were calculated as described by

693   Shammas *et al.*[87], with the intensity of each of the five Opa1 bands on the blot measured.,

694   The intensity of c and e bands were summed and divided by the sum of the five bands (a–e)
695   to obtain the percentage of S-Opa1 generated by Oma1 from total Opa1.

696

697   RNA isolation and qPCR analysis

698   At the end of the study, tissues were harvested and immediately snap frozen in liquid
699   nitrogen and stored at -80°C. For RNA isolation, 30-50 mg of tissue was placed in Lysing
700   Matrix D tubes and homogenized in 800 µl TRI Reagent (T9424, Sigma) using the Fastprep-
701   24 Homogenizer for 30 s at 4-6 m/s (MP Biomedical). Homogenate was transferred to an
702   RNase free tube and 200 µl chloroform (Sigma) added. The samples were vortexed and
703   centrifuged at 13,000 rpm for 15 min at 4°C. The upper phase was then transferred to an
704   RNase free tube and mixed with an equal volume of 70% ethanol before loading onto RNA
705   isolation spin columns. RNA was extracted using a RNeasy Mini Kit (74106, Qiagen)
706   isolation kit following the manufacturer's instructions.
707   Total RNA of 600 ng was quantified using Nanodrop and converted to cDNA using MMLV
708   Reverse Transcriptase with random primers and RNase inhibitor (Promega). RT-qPCR was
709   performed using SYBR Green or TaqMan Universal PCR MasterMixes (Applied Biosystems)
710   on QuantStudio 7 Flex Real time PCR system (Applied Biosystems). Primers are listed in
711   **Supplementary Table 2**. Reactions were performed in triplicate and RNA expression was
712   normalised to *36b4*, *Hprt*, and *B2m* expression using the standard curve method.

713   Immunoassays

714   Mouse sera and plasma were analysed by the Cambridge Biochemical Assay Laboratory,
715   University of Cambridge. Leptin was measured using a 2-plex Mouse Metabolic
716   immunoassay kit from Meso Scale Discovery Kit (Rockville, MD, USA) according to the
717   manufacturers' instructions and with supplied calibrants. GDF15 was measured using a
718   modified Mouse GDF15 DuoSet ELISA (R&D Systems) as an electrochemiluminescence
719   assay on the Meso Scale Discovery platform. Adiponectin (K152BYC-2, MSD) was analysed
720   individually using the Meso Scale Discovery Kit (Rockville, MD, USA). NEFA were analysed
721   using the Free Fatty Acid Kit (half-micro test) (11383175001, Roche) and TG was measured
722   using an enzymatic assay (DF69A, Siemens Healthcare). Alanine aminotransferase (ALT,
723   product code DF143), aspartate aminotransferase (AST, product code DF41A), and total
724   cholesterol (product code DF27) were measured using automated enzymatic assays on the
725   Siemens Dimension EXL analyzer. Mouse Fgf21 was measured using an enzyme-linked
726   immunosorbent assay kit (R&D/ Biochne, cat no. MF2100).

Histological processing

Fresh tissue was fixed in 10% formalin for 24 hr at room temperature immediately following sacrifice. Tissue was then embedded in paraffin and 4 μm sections were cut then baked overnight at 50°C. For haematoxylin & eosin (H&E) staining, slides were dewaxed in xylene for 5 min twice, then dehydrated in 100% ethanol for 2 min twice. Following a 3 min water wash, slides were stained with filtered Mayer's haematoxylin (Pioneer Research Chemicals) for 7 min and blued in water for 4 min. Slides were then stained with 1% aqueous eosin (Pioneer Research Chemicals) for 4 min and briefly washed in water before dehydrating in 100% ethanol (1 min, twice) and cleared in xylene (2 min, twice) and mounting with Pertex. Slides were imaged using a Axio Scan Z1 slide scanner (Zeiss). Lipid droplet area and hepatic steatosis was quantified automatically using Halo software (Indica Labs).

Transcriptomic profiling in white and brown adipose tissue

RNA was isolated from three tissues/dietary conditions for RNA sequencing: (1) inguinal WAT from chow fed animals (n=7 WT, n=6 KI, single technical replicates); (2) inguinal WAT from HFD-fed animals (n=8 WT, n=8 KI, two technical replicates per animal); and (3) BAT from HFD-fed animals (n=6 WT, n=6 KI, single technical replicate). RNA was quantified using Agilent 2100 Bioanalyzer (Agilent Technologies Inc) and only samples with RNA Integrity Number ≥8 were used for library preparation. cDNA libraries were made using Illumina TruSeq RNA sample kits and sequencing was performed on Illumina NovaSeq 6000 with paired-end 150 bp reads (Novogene, Cambridge, UK). Raw reads all passed quality control for Q_{score}, error rate distribution, and AT/GC distribution.

Adapter sequences were removed from raw FASTQ files using cutadapt[88] and aligned to *Mus musculus* reference genome (GRCm38) using STAR[89]. Binary alignment/map (BAM) files were sorted using samtools[90] and counts were performed using featureCounts[91]. Differential gene expression (DGE) between WT and KI was performed using DESeq2[92], where significance was considered as a Benjamini-Hochberg false-discovery rate (FDR) corrected p-value <.01. Pathway analysis was performed with the EnrichR package for R[93–95] using significantly differentially expressed genes to determine enriched Hallmark[96] and Kyoto Encyclopaedia of Genes and Genomes (KEGG)[97] gene sets. Gene sets with FDR-corrected p-value <.05 were considered enriched. Figures were generated in R 4.0.2[98] using packages pheatmap, ggplot2, and dplyr.

Adipose explant experiments

Inguinal (subcutaneous) and epididymal (visceral) adipose tissue was harvested from 12-week-old male C57BL/6J mice fed either chow or HFD for 4 weeks (i.e. 8-12 weeks of age). Tissue was placed in Hanks' Balanced Salt Solution (HBSS, H9269, Sigma) and kept on ice

762   before cutting into 1-2 mm fragments. Approximately 100 mg fragments were incubated in a

763   12-well plate with M199 media ± 7 nM insulin (Actrapid, Novo Nordisk) and 25 nM

764   dexamethasone (D4902, Sigma). After 24 hr incubation, media were collected, spun down at

765   5,000 x g and stored at -80°C until leptin and adiponectin assay as above. Explant tissues

766   were weighed and snap frozen for RNA analysis.

767   Primary adipocyte experiments

768   Mature white adipocytes were isolated from 12-20-week-old chow-fed male or female

769   C57BL/6J or C57BL/6N mice as previously described[64,99] with modifications. Briefly, after

770   dissection, gonadal adipose tissue was washed, minced finely, and dissociated to a single-

771   cell suspension in Hanks' Balanced Salt Solution containing 2.25% (w/v) BSA (Sigma, Cat.

772   H9269) and 1 mg/mL collagenase (Sigma, C6885-1G) for approximately 15 min at 37°C in a

773   shaking incubator. Digested material was diluted with 6x volume of high glucose DMEM

774   media (Sigma, Cat. D6546) and connective tissue or undigested pieces were removed by

775   passing through a 100 µm nylon mesh (Fisherbrand, 11517532). Floating adipocytes were

776   washed twice with 6x volume of high glucose DMEM media before being used for

777   downstream experiments.

778   Mature adipocytes with a packed volume of 60 µL were then cultured in 500 µL of high

779   glucose DMEM media (supplemented with 10% FBS (Gibco Cat. 10270-106), 2 mM L-

780   glutamine (Sigma Cat. G7513) and 1 x Penn/Strep (Sigma Cat. P0781)) per well in the

781   presence of 100 nM insulin in a 24-well plate. On the third day of in vitro culture, 100 µL of

782   media was sampled followed by media replenishment. Cells were treated with indicated

783   concentration of Thapsigargin or Tunicamycin for 6 hr. Media were sampled at the end of

784   treatment and cells collected for subsequent RT-qPCR or western blotting. Adipokine

785   secretion from adipocytes during the 6 hr window was calculated as X=B-A*400/500 (A/B =

786   medium adipokine concentration before/after treatment).

787   Statistical analysis

788   Continuous data were expressed as mean ± standard error (SE). Normally distributed data

789   were analysed by t-test (for two group pairwise comparison) and one-way ANOVA (for three

790   or more groups) with post-hoc Bonferroni multiple comparisons test. FDR-corrected p-value

791   <.05 was considered significant.

792   Statistical tests of TEM data (pairwise comparisons of mutant to WT) were based on the

793   mean of independent biological replicates (i.e. the number of different samples, not the

794   number of mitochondria) and FDR adjustment for the number of tests[100].

795

796   All experiments were conducted at least three times using, where possible, randomisation of

797   sample order and blinding of experimenters handling samples. No data were excluded from

798   analysis. Data were analysed using R 4.0.2[98] and GraphPad Prism version 9 (GraphPad,

799   San Diego). Figures were made using BioRender.

800

801   Materials availability

802   All reagents used are publicly available. Primer sequences and antibodies are detailed in

803   Supplementary Tables 1 and 2. Code used in analysis is available from:

804   https://doi.org/10.5281/zenodo.5770057. Raw counts from transcriptomic analysis are

805   available from GEO under accession ID: GSE210771.

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1039 **Figure 1. Generation of a Mfn2^{R707W} knock-in mouse.** (A) Wild-type (WT) nucleotide and amino
1040 acid sequence around the Arg 707 codon. The CRISPR/Cas9 nuclease target is indicated in *green*.
1041 Below is part of the ssODN template with mutated nucleotides in *blue*, including the upstream silent
1042 mutation (at codon 704-705) to generate an EcoRV restriction site. (B) Sanger sequencing
1043 confirmation of the knock-in (KI) with restriction site in a founder (F0). (C) Illustration of the genotyping
1044 strategy: mutant alleles will digest into 327 bp and 196 bp fragments in response to EcoRV digestion.
1045 (D) Ear biopsies were digested using chelix and *Mfn2* amplified by PCR, then digested using EcoRV.
1046 Representative SYBR Safe DNA gel demonstrating genotyping for two WT, heterozygous, and
1047 homozygous KI mice. Image is representative of other genotyping gels. (E) Western blot from inguinal
1048 and epididymal white adipose tissue (WAT), brown adipose tissue (BAT), liver and skeletal muscle for
1049 expression of Mfn1 and Mfn2. Tissues are from WT and homozygous Mfn2^{R707W} KI mice fed a 45%
1050 kcal high fat diet (HFD) for 6 months. Due to variability across tissues, both *Gapdh* and Beta-tubulin
1051 (*Tubb*) are given as loading controls. The image is representative of at least three biological
1052 replicates.

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1055 **Figure 1-figure supplement 1. Expression of mitofusins on chow diet.** Western blots showing
1056 expression of Mfn2 and Mfn1 in liver (A), heart (B), skeletal muscle (C), BAT (D), inguinal WAT (E),
1057 and epididymal WAT (F) in WT and homozygous Mfn2^{R707W} KI mice fed chow diet for 6 months; and
1058 (G) expression of Mfn2 and Mfn1 in BAT in mice fed chow for 4 weeks. Each lane contains samples
1059 from a separate animal and blots are representative of at least three replicates. *Canx* (calnexin),
1060 *Gapdh*, and *Tubb* (β-tubulin) are loading controls.

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1063 **Figure 1-figure supplement 2. Quantification of western blots for mitofusins in tissues of chow**
1064 **diet-fed mice**. Densitometric quantification of Mfn1 and Mfn2 bands from liver (A), heart (B), skeletal
1065 muscle (C), BAT (D), inguinal WAT (E), and epididymal WAT (F) in male mice fed chow diet for 6
1066 months; and (G) BAT in mice fed chow diet for 4 weeks. Data are from n=3-9 animals. Each point
1067 represents data from a separate animal. Asterisks indicate p-values from pairwise comparisons (T-
1068 tests) that are false-discovery rate (FDR)-adjusted for multiple tests (* p-FDR <.05). WT in *blue*,
1069 homozygous Mfn2^{R707W} KI in *green*.

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1072 **Figure 1-figure supplement 3. Expression of mitofusins on high fat diet.** Western blots showing
1073 expression of Mfn2 and Mfn1 in liver (A), heart (B), skeletal muscle (C), BAT (D), inguinal WAT (E),
1074 and epididymal WAT (F) from WT and homozygous Mfn2^{R707W} KI mice fed a 45% kcal HFD for 6

1075   months. Each lane contains samples from a separate animal and blots are representative of at least

1076   three replicates. *Canx* (calnexin), *Gapdh*, and *Tubb* (β-tubulin) are given as loading controls.

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1078   **Figure 1-figure supplement 4.** Quantification of western blots for mitofusins in tissues of high fat

1079   diet-fed mice. Densitometic quantification of bands for Mfn1 and Mfn2 from male mice fed HFD for 6

1080   months is shown. Data are from liver (A), heart (B), skeletal muscle (C), BAT (D), inguinal WAT (E),

1081   and epididymal WAT (F). Data are from n=3-10 animals. Each point represents a separate animal.

1082   Asterisks indicate p-values from pairwise comparisons (T-tests) that are FDR-adjusted for multiple

1083   tests (* p-FDR <.05, ** p-FDR <.01). WT in *blue*, homozygous Mfn2^{R707W} KI in *green*.

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1086   **Figure 2. Effect of Mfn2^{R707W} on mitochondrial structure and function.** (A) Representative

1087   transmission electron microscopy (TEM) images of BAT with zoomed-in images of mitochondria

1088   (highlighted in *yellow*) bordering lipid droplets (outlined in *red*). (B) Quantification of mitochondrial

1089   perimeter from TEM on BAT. Each dot represents data from an individual mitochondrial cross section

1090   with each diamond showing the separate 6 biological replicates. p.adj gives the FDR-adjusted p-value

1091   from across all TEM analyses. (C) Quantification of mitochondrial aspect ratio (length/width) from

1092   TEM of BAT. (D) Quantification of mitochondrial-lipid droplet contact from TEM, expressed as

1093   proportion (%) of lipid droplet in contact with mitochondrial membrane on BAT. (E) Number of cristae

1094   per mitochondrion from TEM of BAT. (F-G) Mitochondrial DNA content in tissues from mice fed chow

1095   diet (F) or HFD (G) for 6 months. Each data point represents a separate animal. p-values are from

1096   pairwise comparisons (T-tests) between WT and Mfn2^{R707W} KI that are FDR-adjusted for multiple tests

1097   (* p-FDR <.05). WT in *blue*, homozygous Mfn2^{R707W} KI in *green*. AU, arbitrary units.

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1100   **Figure 2-figure supplement 1. Effect of Mfn2^{R707W} on mitochondrial morphology in white**

1101   **adipose tissue.** (A) Representative TEM images from inguinal WAT with zoomed-in images of

1102   mitochondria (highlighted in *yellow*) bordering lipid droplets (outlined in *red*). Quantification of

1103   mitochondrial perimeter (B) and aspect ratio (length/width) (C) from TEM in inguinal WAT. Each dot

1104   represents an individual mitochondrion with diamonds showing biological replicates. p.adj gives the

1105   FDR-adjusted p-value from across all TEM analyses. (D) TEM images from epididymal WAT with

1106   zoomed-in images of mitochondria (highlighted in *yellow*) bordering lipid droplets (outlined in *red*).

1107   Quantification of mitochondrial perimeter (E) and aspect ratio (length/width) (F) from TEM on

1108   epididymal WAT. FDR-adjusted p-values are from pairwise comparisons (T-tests) between WT and

1109   Mfn2^{R707W} KI. WT in *blue*, homozygous Mfn2^{R707W} KI in *green*.

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1111

1112   **Figure 2-figure supplement 2. Effect of Mfn2^{R707W} on mitochondrial morphology in liver, heart,**

1113   **and muscle.** (A) Representative TEM images from the heart with zoomed-in images of mitochondria

1114   (highlighted in *yellow*). (B) Quantification of mitochondrial perimeter from TEM on heart. Each dot

1115 represents an individual mitochondrion with diamonds showing biological replicates. p.adj gives the
1116 FDR-adjusted p-value from across all TEM analyses. (C) Quantification of mitochondrial aspect ratio
1117 (length/width) from TEM on heart. (D) TEM images from skeletal muscle with zoomed-in images of
1118 mitochondria (highlighted in *yellow*). Quantification of mitochondrial perimeter (E) and aspect ratio
1119 (length/width) (F) from TEM on skeletal muscle. (G) TEM images from liver with zoomed-in images of
1120 mitochondria (highlighted in *yellow*). Quantification of mitochondrial perimeter (H) and aspect ratio
1121 (length/width) (I) from TEM on liver. FDR-adjusted p-values are from pairwise comparisons (T-tests)
1122 between WT and Mfn2^{R707W} KI. WT in *blue*, homozygous Mfn2^{R707W} KI in *green*.
1123
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1125 **Figure 2-figure supplement 3. Altered expression of Oxphos protein subunits in adipose**
1126 **tissue.** Western blots showing expression of Oxphos protein subunit expression in mice fed chow diet
1127 for 6 months. Expression is shown from liver (A), heart (B), inguinal WAT (C), epididymal WAT (D),
1128 and BAT (E). Each lane contains samples from a separate animal and blots are representative of at
1129 least three replicates. *Canx* (calnexin) is used as total cellular control and *Tomm20* (or citrate
1130 synthetase (*Cs*)) is used as mitochondrial mass loading control.
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1133 **Figure 2-figure supplement 4. Quantification of western blots for Oxphos subunits from**
1134 **tissues of chow diet-fed mice.** Densitometric quantification of bands for mitochondrial Oxphos
1135 subunits from male mice fed chow diet for 6 months. Data are from liver (A), heart (B), inguinal WAT
1136 (C), epididymal WAT (D), and BAT (E). Data are from n=3-8 animals per genotype. Each point
1137 represents a separate animal. Asterisks indicate p-values from pairwise comparisons (T-tests) that
1138 are FDR-adjusted for multiple tests (* p-FDR <.05, ** p-FDR <.01). WT in *blue*, homozygous
1139 Mfn2^{R707W} KI in *green*.
1140
1141

1142 **Figure 2-figure supplement 5. Mfn2^{R707W} does not impair brown adipose tissue thermogenic**
1143 **capacity.** *Ex vivo* mitochondrial respirometry in liver (A, n=4) and BAT (B, n=9). Each data point
1144 represents data from a separate animal. Eight-week-old chow diet fed mice were exposed to cold
1145 (10°C, n=16-18) or thermoneutrality (30°C, n=13-14) for 4 weeks and then maximum thermogenic
1146 capacity was tested using noradrenaline (NA) stimulation under anaesthesia. (C) Trend in energy
1147 expenditure (kcal/hr) before and after noradrenaline stimulation for cold (solid line) and thermoneutral
1148 (dashed line) housed animals. (D) Quantification of maximum (peak) energy expenditure. P-values
1149 represent the difference between groups comparing 10°C and 30°C. (E) Area under the curve
1150 analysis using the minimum value at the point of noradrenaline (NA) injection at the base of the curve.
1151 (F) Basal energy expenditure prior to NA injection. (G) Difference between peak and baseline energy
1152 expenditure under both conditions. WT in *blue*, homozygous Mfn2^{R707W} KI in *green*. Each data point
1153 represents results from an individual animal. FDR-adjusted p-values are from pairwise comparisons
1154 (T-tests) between groups as indicated.

1157   **Figure 3. No difference in fat mass or glucose homeostasis in Mfn2^{R707W} mice compared to WT**

1158   **mice on chow or high fat diet.** Seven-week-old mice were fed chow (n=8-12) or 45% kcal HFD

1159   (n=13-14) for 6 months. (A) Absolute body mass for mice fed chow (solid line) and HFD (dashed line)

1160   over 6 months. (B) Time-domain nuclear magnetic resonance (TD-NMR) measurement of fat and lean

1161   mass of mice fed HFD. (C) The ratio of tissue weights versus body mass for multiple tissues,

1162   including four WAT depots, from mice fed HFD. (D) Quantification of lipid droplet area from

1163   histological specimens of adipose tissue from mice fed HFD for 6 months. (E) Representative

1164   histological images from WAT, liver, and BAT from mice fed HFD. Analyses of mouse plasma lactate

1165   (F), plasma glucose (G), and insulin (H), homeostatic model of assessment of insulin resistance

1166   (HOMA-IR, I) after a 6 hr fast. Change in plasma glucose during intraperitoneal glucose tolerance test

1167   (J) and intraperitoneal insulin tolerance test (K). ns, p>.05 on unpaired T-tests, adjusted for multiple

1168   comparisons. WT in *blue*, homozygous Mfn2^{R707W} KI in *green*. Each data point represents an

1169   individual animal.

1172   **Figure 3-figure supplement 1. No evidence of altered fat mass or glucose homeostasis in**

1173   **Mfn2^{R707W} compared to WT mice on chow or high fat diet for 6 months.** Seven-week-old mice

1174   were fed chow diet (n=8-12) or 45% kcal HFD (n=13-14) for 6 months. (A) Relative (%) change of

1175   body mass for mice fed chow diet (solid line) and HFD (dashed line) over 6 months. (B) TD-NMR

1176   measurement of fat and lean mass in mice fed a chow diet for 6 months. (C) Analysis of mouse serum

1177   biochemistry after 6 months of diet and a 6 hr fast for total cholesterol. (D) The ratio of tissue weights

1178   versus body mass for multiple tissues, including four WAT depots, from mice fed chow diet for 6

1179   months. (E) Serum triglycerides. (F) Quantification of lipid droplet area from histological specimens of

1180   adipose tissue from mice fed chow diet for 6 months. (G) Quantification of relative hepatic steatosis

1181   from histological images of liver. (H) Serum aspartate aminotransferase. (I) qPCR of genes implicated

1182   in the integrated stress response, lipid metabolism or inflammation in liver from animals fed HFD for 6

1183   months. Raw expression values for target genes were normalised to three housekeeping genes

1184   (*36b4, B2m,* and *Hprt*) and expressed relative to WT liver. ns, p>.05 on unpaired T-tests, adjusted for

1185   multiple comparisons. WT in *blue*, homozygous Mfn2^{R707W} in *green*. Each data point represents

1186   results from an individual animal.

1189   **Figure 3-figure supplement 2. Similar phenotype in female Mfn2^{R707W} knock-in mice fed high fat**

1190   **diet for 6 months.** Seven-week-old female mice were fed 45% kcal HFD (n=5-11) for 6 months. (A)

1191   Absolute body mass for over 6 months. (B) Body mass after 12 weeks on HFD. (C) TD-NMR

1192   measurement of fat and lean mass of female mice after 12 weeks on HFD. (D-F) Analysis of mouse

1193   serum biochemistry after 12 weeks of diet and a 6 hr fast for serum glucose (D), serum insulin (E),

1194   and serum adiponectin (F). Analysis of mouse serum leptin at 4 weeks (G), 12 weeks (H), and 24

1195   weeks (I) on HFD after a 6 hr fast. WT in *blue*, homozygous Mfn2[R707W] KI in *green*. Each data point

1196   represents an individual animal. Asterisks indicate p-values from pairwise comparisons (T-tests)

1197   between WT and Mfn2[R707W] KI that are FDR-adjusted for multiple tests (*** p-FDR <.001).

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1200   **Figure 4. Mfn2[R707W] causes an adipose tissue-specific induction of the integrated stress**

1201   **response.** qPCR of genes involved in the integrated stress response (ISR) for six tissues from

1202   animals fed chow diet for 6 months: *Atf4* (A), *Ddit3* (Chop, B), and *Atf5* (C). Each data point

1203   represents one animal. Target gene CT values were normalised to three housekeeping genes (*36b4,*

1204   *B2m,* and *Hprt*) and expressed relative to WT liver for each gene. WT in *blue*, homozygous Mfn2[R707W]

1205   in *green*. p-values are FDR-adjusted for multiple tests. Western blots from BAT (D), epididymal WAT

1206   (E), and liver (F) illustrating Ser51-phosphorylation of eIF2α and expression of Mthfd2 with calnexin

1207   (*Canx*) as loading control. Western blots are representative of at least three biological and technical

1208   replicates. (G) Volcano plot from bulk RNA sequencing (n=8 per genotype) of inguinal WAT from mice

1209   on HFD. Significantly differentially expressed genes (Log_2 fold change >1.5 and p-FDR <.001) are

1210   highlighted in orange. Pathway analysis using significantly differentially expressed genes for

1211   upregulated (H) and downregulated (I) Hallmark gene sets. The X-axis depicts a relative gene set

1212   enrichment score. All illustrated gene sets are enriched with p-FDR <.05. Asterisks indicate p-values

1213   from pairwise comparisons (T-tests) between WT and Mfn2[R707W] KI that are FDR-adjusted for multiple

1214   tests (* p-FDR <.05, ** p-FDR <.01, *** p-FDR <.001, **** p-FDR <.0001).

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1217   **Figure 4-figure supplement 1. Mfn2[R707W] causes adipose tissue-specific induction of the**

1218   **integrated stress response with perturbation of mitochondrial gene expression.** qPCR of genes

1219   involved in the ISR for six tissues from animals fed a chow diet for 6 months: *Gdf15* (A) and *Fgf21* (B).

1220   Each data point represents data from a separate animal. Target gene CT values were normalised

1221   against three housekeeping genes (*36b4, B2m,* and *Hprt*) and expressed relative to WT liver for each

1222   gene. P-values are FDR-adjusted for multiple tests. Serum FGF-21 (C) and GDF-15 (D) after 6

1223   months of chow diet or HFD. Western blots from inguinal WAT (E), skeletal muscle (F), and heart (G)

1224   illustrating Ser51-phosphorylation of eIF2α and expression of Mthfd2 with calnexin (*Canx*) as loading

1225   control. Western blots are representative of at least three biological replicates. Mthfd2 in heart (G) is

1226   the upper band within the box, as indicated by a horizontal line. (H) Heatmap of all genes from the

1227   citrate acid (tricarboxylic acid (TCA)) cycle KEGG pathway. Colour illustrates the Log_2 fold change

1228   normalised per gene. Those annotated with orange in the leftmost column had significant differential

1229   gene expression (DGE) with p-FDR <.001. (I) Heatmaps comparing mRNA expression of

1230   mitochondrial- and nuclear-encoded mitochondrial genes from inguinal WAT from HFD-fed animals.

1231   Asterisks indicate p-values from pairwise comparisons (T-tests) between WT and Mfn2[R707W] KI that

1232   are FDR-adjusted for multiple tests (*** p-FDR <.001).

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1234

1235 **Figure 4-figure supplement 2. Quantification of western blots for components of the integrated**
1236 **stress response in high fat diet-fed mice.** Densitometric quantification of bands for Mthfd2 and
1237 phosphorylated Ser-51 eIF2α from male mice fed HFD for 6 months. (A) BAT, (B) epididymal WAT,
1238 (C) liver, (D) inguinal WAT, (E) skeletal muscle, and (F) heart. Data are from 3-9 animals. Each point
1239 represents a separate animal. Asterisks indicate p-values from pairwise comparisons (T-tests)
1240 between WT and Mfn2^{R707W} KI that are FDR-adjusted for multiple tests (* p-FDR <.05, ** p-FDR <.01,
1241 *** p-FDR <.001). WT in *blue*, homozygous Mfn2^{R707W} KI in *green*.

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1244 **Figure 4-figure supplement 3. Mfn2^{R707W} does not affect Oma1 or Opa1 processing in heart,**
1245 **liver, and muscle.** Western blots of heart (A), liver (B), and skeletal muscle (C) for Opa1 and Oma1
1246 are shown. Tissues are from male mice fed chow diet for 6 months. Each lane contains samples from
1247 a separate animal, with band intensity quantified densitometrically. Opa1 is quantified as percentage
1248 of the short form of Opa1 (bands c and e of S-Opa1) to total Opa1 (long and short forms, bands a-e).
1249 ns, p>.05 on unpaired T-tests, adjusted for multiple comparisons. WT in *blue*, homozygous Mfn2^{R707W}
1250 KI in *green*.

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1253 **Figure 4-figure supplement 4. Evidence of modest activation of the Oma1-Opa1 pathway in**
1254 **brown adipose tissue from Mfn2^{R707W} mice.** Western blots of BAT for Opa1 and Oma1 are shown.
1255 Tissues are from male mice fed chow diet. (A) BAT (chow diet for 4 weeks), (B) BAT (chow diet for 6
1256 months), (C) Epididymal WAT (chow diet for 6 months), and inguindal WAT (chow diet for 6 months).
1257 Each lane contains samples from a separate animal with band intensity quantified densitometrically.
1258 Opa1 is quantified as percentage of the short form of Opa1 (bands c and e of S-Opa1) to total Opa1
1259 (long and short forms, bands a-e). ns, p>.05 on unpaired T-tests, adjusted for multiple comparisons.
1260 WT in *blue*, homozygous Mfn2^{R707W} KI in *green*. * p-FDR <.05.

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1263 **Figure 4-figure supplement 5. Transcriptional evidence of upregulation of the unfolded protein**
1264 **response and mTorc1 pathways in adipose tissue.** (A) Volcano plot from bulk RNA sequencing
1265 (n=7 WT and n=6 KI) of inguinal WAT from mice on chow diet, where significantly differentially
1266 expressed genes (Log$_2$ fold change >1.5 and p-FDR <.001) are highlighted in *orange*. Pathway
1267 analysis using significantly differentially expressed genes for upregulated (B) and downregulated (C)
1268 Hallmark gene sets. X-axis depicts a relative gene set enrichment score. (D) Volcano plot from bulk
1269 RNA sequencing (n=6 per genotype) of BAT from mice on HFD, where significantly differentially
1270 expressed genes (Log$_2$ fold change >1.5 and p-FDR <.001) are highlighted in *green*. Pathway
1271 analysis using significantly differentially expressed genes for upregulated (E) and downregulated (F)
1272 Hallmark gene sets. X-axis depicts a relative gene set enrichment score. All illustrated gene sets are
1273 enriched with p-FDR <.05. (G) Scatter plot of the top 100 up-/down-regulated genes from bulk RNA
1274 sequencing from inguinal WAT. For each gene, the fold change observed under HFD is plotted on the

1275   x-axis and fold change under chow diet is shown on the y-axis. P-values for each dietary condition are

1276   shown using size (HFD) and colour (chow diet).

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1279   **Figure 5. Mfn2^R707W^ decreases adipose secretion of leptin and adiponectin.** Fasting serum leptin

1280   (A) and adiponectin (B) from mice after 6 months on chow diet or HFD. Asterisks indicate t-tests

1281   comparing WT and Mfn2^R707W^ KI with p-values adjusted for multiple testing. (C) Relationship between

1282   leptin and body weight for animals fed HFD. Each data point represents one measurement of leptin,

1283   with multiple measurements per animal. Shaded area represents the 95% confidence interval. Data

1284   are from n=13-14 animals. qPCR of *Lep* (D) and *Adipoq* (E) from WAT depots. Each data point

1285   represents data from a separate animal. Target gene CT values were normalised against three

1286   housekeeping genes (*36b4, B2m,* and *Hprt*) and expressed relative to WT for each condition. (F-I)

1287   mRNA expression of *Atf4*, *Chop*, *Lep, and Adipoq* in primary adipocytes treated with either DMSO

1288   control or Thapsigargin (TG, 150 nM) or Tunicamycin (TN, 5 µg/mL) for 6 hr. Each data point

1289   represents an individual well from a separate biological experiment (n=5). Asterisks indicate

1290   significance on one-way ANOVA with correction for multiple comparisons. (J) Leptin and (K)

1291   Adiponectin secretion from primary adipocytes treated with either DMSO control or Thapsigargin (TG,

1292   150 nM) or Tunicamycin (TN, 5 µg/mL) over 6 hr. Asterisks indicate significance on one-way ANOVA

1293   with correction for multiple comparisons. (L) Representative western blots of primary adipocytes

1294   treated with either DMSO control or Thapsigargin (TG, 150 nM) or Tunicamycin (TN, 5 µg/mL) for 6

1295   hr. (M) Fasting serum adipsin (complement factor D) from mice after 6 months on chow diet or HFD.

1296   Asterisks indicate p-values from pairwise comparisons (T-tests) between indicated groups that are

1297   FDR-adjusted for multiple tests (* p-FDR <.05, ** p-FDR <.01, *** p-FDR <.001, **** p-FDR <.0001).

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1300   **Figure 5-figure supplement 1. Mfn2^R707W^ decreases adipose leptin and adiponectin secretion**

1301   **from multiple adipose depots and in different dietary conditions.** (A) Leptin secretion from

1302   explants of epididymal WAT in basal conditions and after insulin-dexamethasone stimulation. (B)

1303   Adiponectin secretion from epididymal WAT explants. (C) qPCR of *Lep* and *Adipoq* from epididymal

1304   explants from animals fed a HFD, where each data point represents an explant from a separate

1305   animal (n=11-13). (D) Relationship between leptin and body weight for animals on chow diet. Each

1306   data point represents one measurement of leptin, with multiple measurements per animal. Shaded

1307   area represents the 95% confidence interval. Data are from n=8-12 animals. Asterisks indicate p-

1308   values from pairwise comparisons (T-tests) between indicated groups that are FDR-adjusted for

1309   multiple tests (* p-FDR <.05, ** p-FDR <.01, **** p-FDR <.0001).

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1312   **Figure 5-figure supplement 2. Mfn2^R707W^ decreases leptin and adiponectin protein expression**

1313   **in white adipose tissue.** Western blots showing expression of leptin and adiponectin in male mice

1314   fed 45% kcal HFD for 6 months from epididymal (A) and inguinal (B) WAT. Expression is shown from

1315   epididymal and inguinal WAT with *Gapdh* used as a loading control. Each lane contains samples from

1316   a separate animal with band intensity quantified densitometrically. Asterisks indicate p-values from

1317   pairwise comparisons (T-tests) between WT and Mfn2^{R707W} KI that are FDR-adjusted for multiple tests

1318   (** p-FDR <.01, **** p-FDR <.0001). WT in *blue*, homozygous Mfn2^{R707W} KI in *green*.

1319

Tables

	Chow diet			High fat diet		
	WT	**Knock-in**	**p-FDR**	**WT**	**Knock-in**	**p-FDR**
Weight (g)	39.9 (1.2)	40.9 (1.4)	1	48.4 (1.7)	45.7 (1.5)	0.51
Lean mass (g)	19.97 (0.44)	20.53 (0.53)	0.85	16.50 (0.39)	15.51 (0.42)	0.2
Fat mass (g)	12.41 (0.97)	12.67 (1.29)	1	22.39 (1.63)	24.09 (1.10)	0.79
Leptin (ug/L)						
4 weeks	3.6 (0.9)	1.3 (0.2)	0.17	14.3 (2.6)	4.8 (0.5)	0.01
8 weeks	4.5 (0.8)	1.6 (0.3)	0.056	20.8 (3.3)	8.6 (0.9)	0.007
12 weeks	7.6 (0.4)	2.3 (0.5)	0.045	19.3 (4.0)	11.4 (1.5)	0.31
24 weeks (fasting)	15.4 (3.4)	6.0 (1.3)	0.047	57.0 (8.9)	15.6 (1.5)	0.003
Adiponectin (mg/L)						
4 weeks	24.8 (1.0)	16.4 (2.3)	0.068	21.0 (0.4)	11.0 (3.3)	4.20E-10
8 weeks	20.0 (0.7)	11.0 (0.7)	1.50E-05	29.4 (1.4)	16.0 (0.7)	5.30E-07
12 weeks	29.0 (1.4)	14.7 (0.7)	4.50E-05	33.0 (0.8)	18.2 (0.9)	7.20E-09
24 weeks (fasting)	29.0 (2.3)	15.4 (2.3)	0	27.9 (0.9)	15.5 (0.6)	4.30E-09
Fgf21 (ng/L)						
20 weeks	238 (26)	292 (22)	0.50	2,655 (203)	2,475 (268)	1
28 weeks	444 (51)	638 (96)	0.24	3,355 (911)	2,919 (289)	1
Gdf15 (ng/L) (28 weeks)	132 (6)	172 (14)	0.067	250 (36)	217 (23)	1
Glucose (mmol/L)	10.0 (0.5)	10.4 (0.4)	1	11.7 (0.5)	11.4 (0.5)	1
Insulin (µg/L) (33 weeks)	1.8 (0.3)	2.0 (0.3)	1	1.7 (0.2)	1.2 (0.1)	0.17
HOMA-IR	141.3 (29.0)	159.2 (23.3)	1	152.2 (22.9)	104.8 (12.1)	0.26
Insulin (µg/L) (GTT baseline)	1.8 (0.4)	2.3 (0.7)	1	2.3 (0.4)	1.4 (0.1)	0.1
Lactate (mmol/L)	4.2 (0.3)	4.4 (0.2)	1	4.5 (0.4)	4.8 (0.2)	1
NEFA (µmol/L)	1,688 (78)	1,564 (46)	0.39	922 (87)	1030 (90)	0.90
TG (mmol/L)	1.1 (0.1)	1.0 (0.1)	1	0.8 (0.1)	0.7 (0.0)	0.76
Total cholesterol (mmol/L)	3.0 (0.1)	3.1 (0.1)	1	6.3 (0.3)	5.2 (0.2)	0.042
ALT (IU/L)	64 (6)	68 (5)	1	144 (33)	45 (9)	0.076
AST (IU/L)	114 (20)	118 (16)	1	207 (32)	162 (22)	0.65

Table 1: **Weights and serum biochemistry for mice on chow or high fat diet for 6 months.** Seven-week-old mice were fed chow (n=8-12) or 45% kcal HFD (n=13-14) for 6 months. Blood was taken 4 weekly with 6 hr fasting blood taken on week 28. p-FDR are false-discovery rate adjusted p-values derived from unpaired T-tests. Values in brackets referred to standard error of the mean. ALT, alanine aminotransferase; AST, aspartate aminotransferase; FGF21, fibroblast growth factor 21; Gdf15, Growth and differentiation factor 15; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; NEFA, non-esterified fatty acids.

1331

Human	Phenocopy?	Mouse
Upper body adipose overgrowth	No	No difference in weight of any adipose depots
Lower limb lipoatrophy	No	
Insulin resistance	No	No difference on GTT or fasting insulin
Lower serum leptin concentration	Yes	Seen on chow or HFD; lower secretion seen from adipose explants
Severely reduced adipose leptin mRNA expression	Partial	Modest decrease only; only significant in some analyses
Lower serum adiponectin concentration	Yes	Seen on chow or HFD; lower secretion seen from adipose explants
Severely reduced adipose adiponectin mRNA expression	Yes	Seen on chow or HFD and in adipose explants
No change in WAT MFN2 protein expression (only over-grown WAT studied)	Yes	No difference in any tissue studied, though variable in WAT and BAT.
No difference in adipocyte size	Yes	True on both chow and HFD
Disorganised, fragmented WAT mitochondria on TEM	Partial	More circular mitochondria with trend towards reduced cristae
Upregulation of nuclear and down regulation of mitochondrial Oxphos transcriptional pathway	Yes	True in inguinal and epididymal WAT, and BAT
Lower Oxphos complex II and III but preserved complex I and IV protein	Partial	Lower complex I and IV protein in WAT
Lower WAT mtDNA	Partial	Lower mtDNA in BAT but not WAT
Transcriptional activation of ISR	Yes	Seen in inguinal and epididymal WAT, and BAT

1332 **Table 2**: **Comparison of human *MFN2^{R707W}*-associated lipodystrophy with phenotype of**
1333 ***Mfn2^{R707W/R7007W}* mice.** BAT, brown adipose tissue; GTT, glucose tolerance test; HFD, high fat diet;
1334 ISR, integrated stress response; MFN2, mitofusin 2; mRNA, messenger ribose nucleic acid; mtDNA,
1335 mitochondrial DNA; PLIN1, perilipin 1; TEM, transmission electron microscope; WAT, white adipose
1336 tissue.
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1338 **Supplementary Table Legends**

1339

1340 **Supplementary Table 1**: Antibodies used in this study.

1341

1342 **Supplementary Table 2**: Primer sequences used in this study. Fwd, forward primer; Rv, reverse

1343 primer.

1344

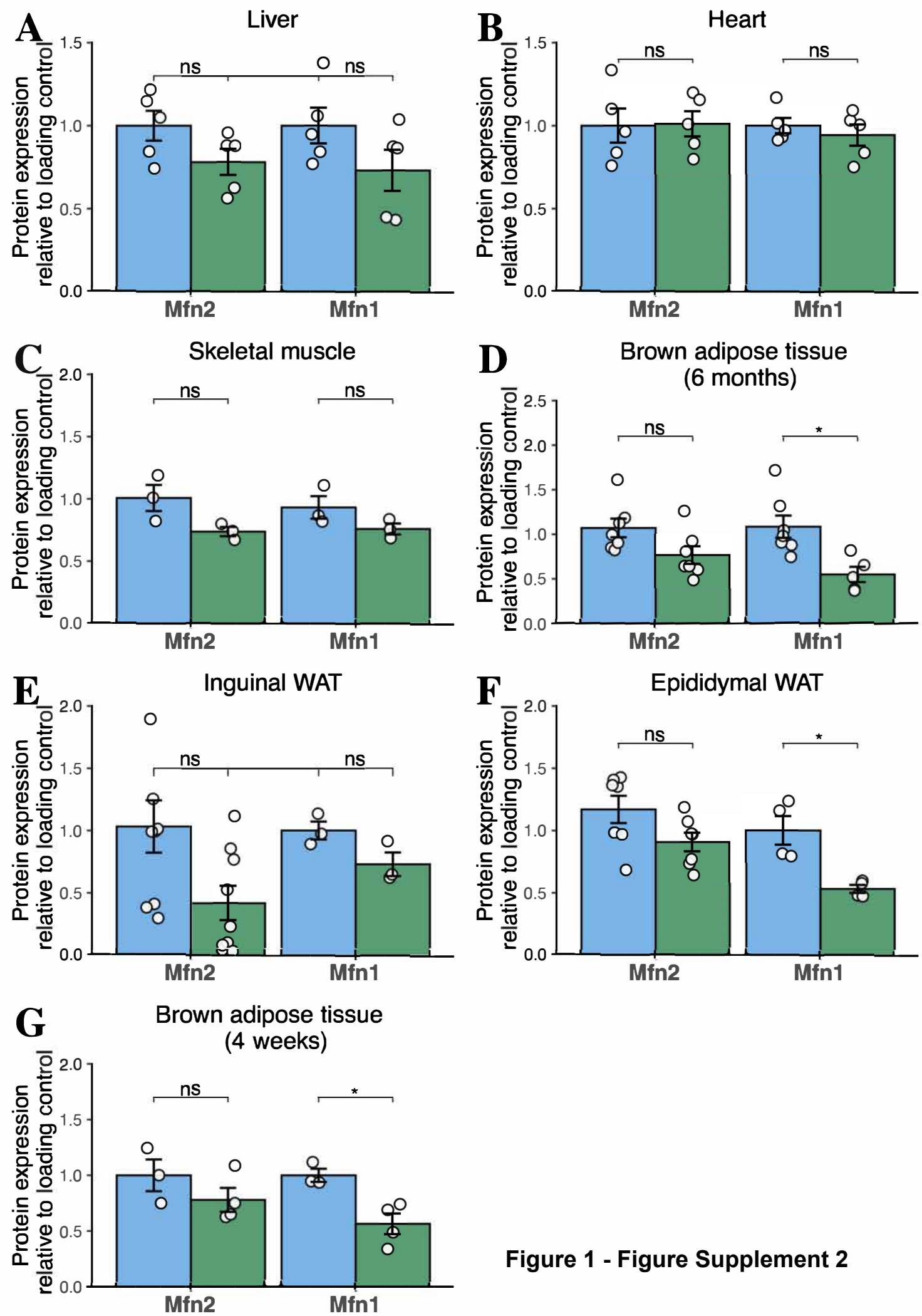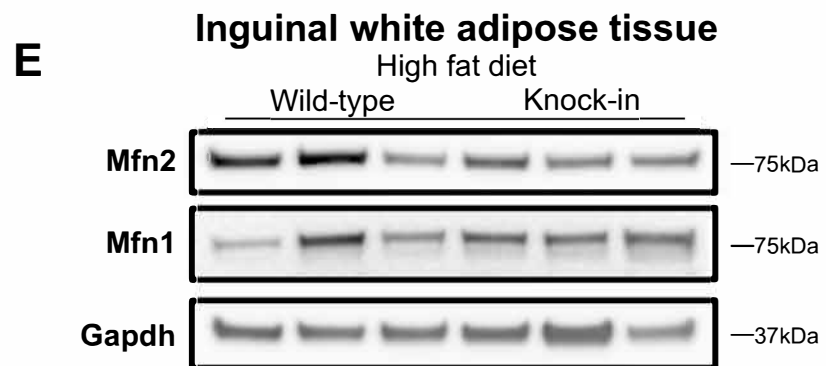**Source data titles**
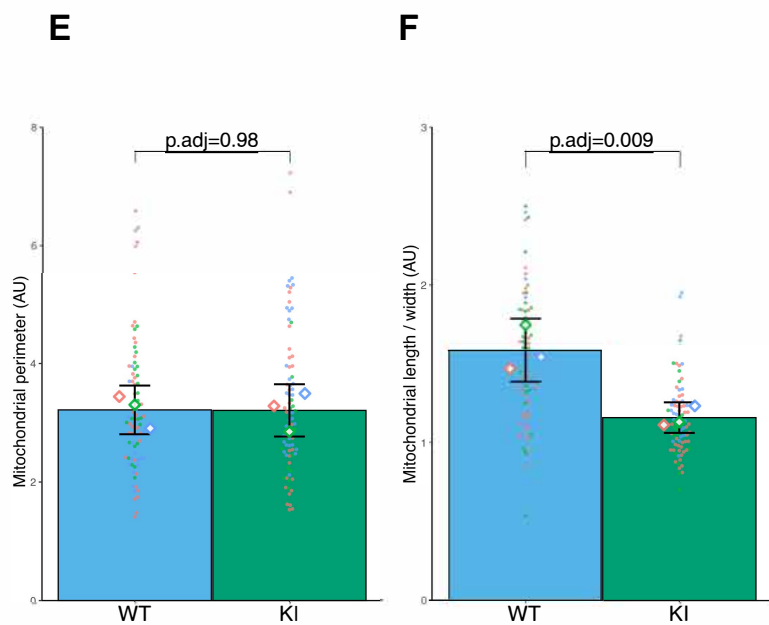
1346

1347   **Figure 1-Source data**. Raw & annotated immunoblots from Figure 1 E.

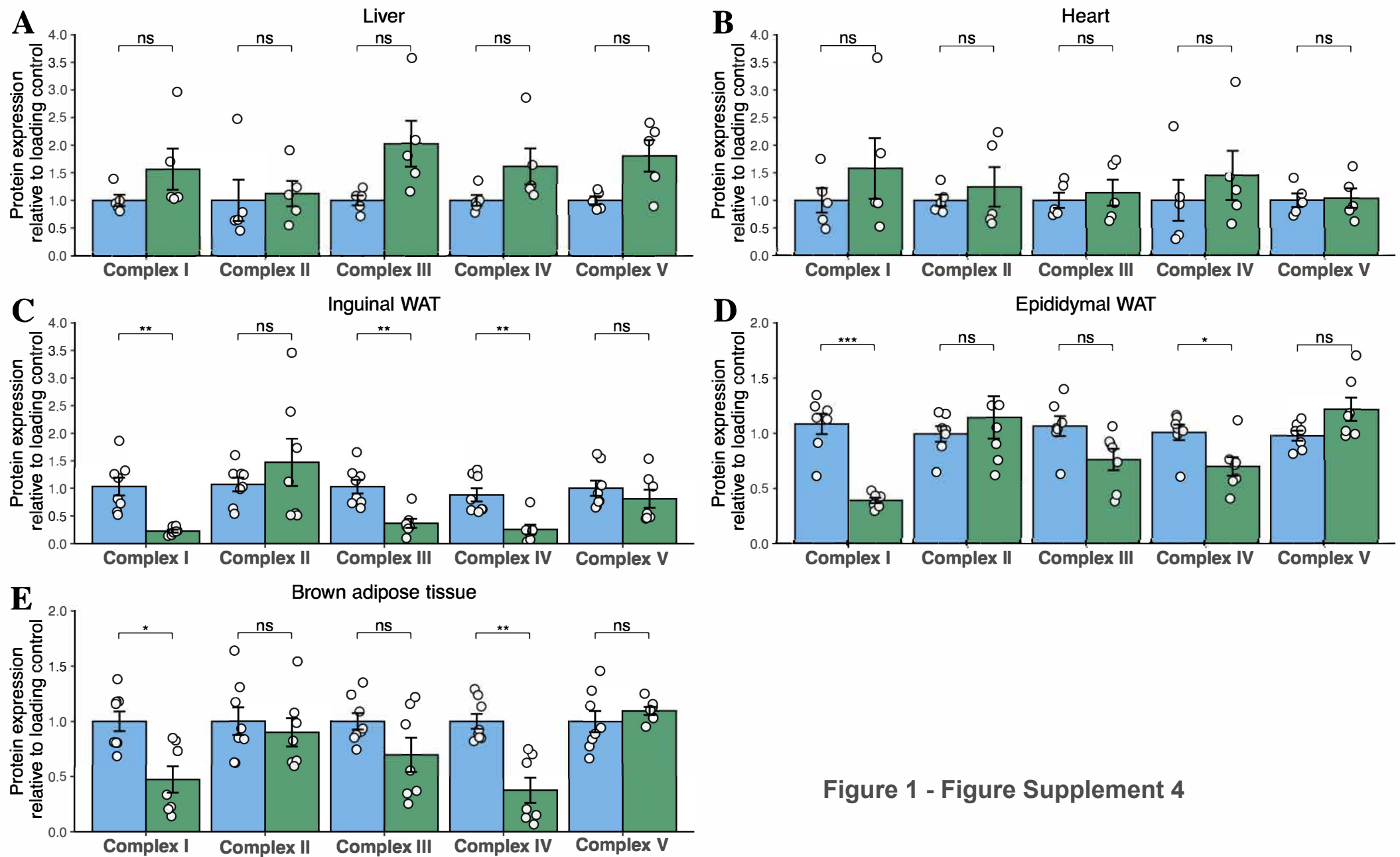1348   **Figure 1-Figure Supplement 1-Source data**. Raw & annotated immunoblots from Figure 1-

1349   Figure Supplement 1.

1350   **Figure 1-Figure Supplement 3-Source data**. Raw & annotated immunoblots from Figure 1-

1351   Figure Supplement 3.

1352   **Figure 2-Figure Supplement 3-Source data**. Raw & annotated immunoblots from Figure 2-

1353   Figure Supplement 3.

1354   **Figure 4-Source data**. Raw & annotated immunoblots from Figure 4 D-F.

1355   **Figure 4- Figure Supplement 1-Source data**. Raw & annotated immunoblots from Figure

1356   4-Figure Supplement 1 E-G.

1357   **Figure 4- Figure Supplement 3-Source data**. Raw & annotated immunoblots from Figure

1358   4-Figure Supplement 3.

1359   **Figure 4- Figure Supplement 4-Source data**. Raw & annotated immunoblots from Figure

1360   4-Figure Supplement 4.

1361   **Figure 5-Source data**. Raw & annotated immunoblots from Figure 5 L.

Figure 1

Liver
Chow diet

Wild-type   Knock-in

A

Mfn2   —75kDa

Mfn1   —75kDa

Gapdh   —37kDa

Heart
Chow diet

Wild-type   Knock-in

B

Mfn2   —75kDa

Mfn1   —75kDa

Canx   —75kDa

Skeletal muscle
Chow diet

Wild-type   Knock-in

C

Mfn2   —75kDa

Mfn1   —75kDa

Tubb   —50kDa

Brown adipose tissue
Chow diet (6 months)

Wild-type   Knock-in

D

Mfn2   —75kDa

Mfn1   —75kDa

Tubb   —50kDa

Inguinal white adipose tissue
Chow diet

Wild-type   Knock-in

E

Mfn2   —75kDa

Mfn1   —75kDa

Gapdh   —37kDa

Epididymal white adipose tissue
Chow diet

Wild-type   Knock-in

F

Mfn2   —75kDa

Mfn1   —75kDa

Gapdh   —37kDa

Brown adipose tissue
Chow diet (4 weeks)

Wild-type   Knock-in

G

Mfn2   —75kDa

Mfn1   —75kDa

Gapdh   —37kDa

Figure 1 - Figure Supplement 1

A Liver

B Heart

C Skeletal muscle

D Brown adipose tissue (6 months)

E Inguinal WAT

F Epididymal WAT

G Brown adipose tissue (4 weeks)

Figure 1 - Figure Supplement 2

Liver

A

High fat diet

Wild-type Knock-in

Mfn2 —75kDa

Mfn1 —75kDa

Tubb —50kDa

Heart

B

High fat diet

Wild-type Knock-in

Mfn2 —75kDa

Mfn1 —75kDa

Tubb —50kDa

Skeletal muscle

C

High fat diet

Wild-type Knock-in

Mfn2 —75kDa

Mfn1 —75kDa

Tubb —50kDa

Brown adipose tissue

D

High fat diet

Wild-type Knock-in

Mfn2 —75kDa

Mfn1 —75kDa

Canx —75kDa

Inguinal white adipose tissue

E

High fat diet

Wild-type Knock-in

Mfn2 —75kDa

Mfn1 —75kDa

Gapdh —37kDa

Epididymal white adipose tissue

F

High fat diet

Wild-type Knock-in

Mfn2 —75kDa

Mfn1 —75kDa

Gapdh —37kDa

Figure 1 - Figure Supplement 3

Fig 1 - Supp 4

Figure 2

Inguinal WAT

Wild-type

Mfn2^{R707W}

B

p.adj=0.85

Mitochondrial perimeter (AU)

WT | KI

C

p.adj=0.04

Mitochondrial length / width (AU)

WT | KI

Epididymal WAT

Wild-type

Mfn2^{R707W}

E

p.adj=0.98

Mitochondrial perimeter (AU)

WT | KI
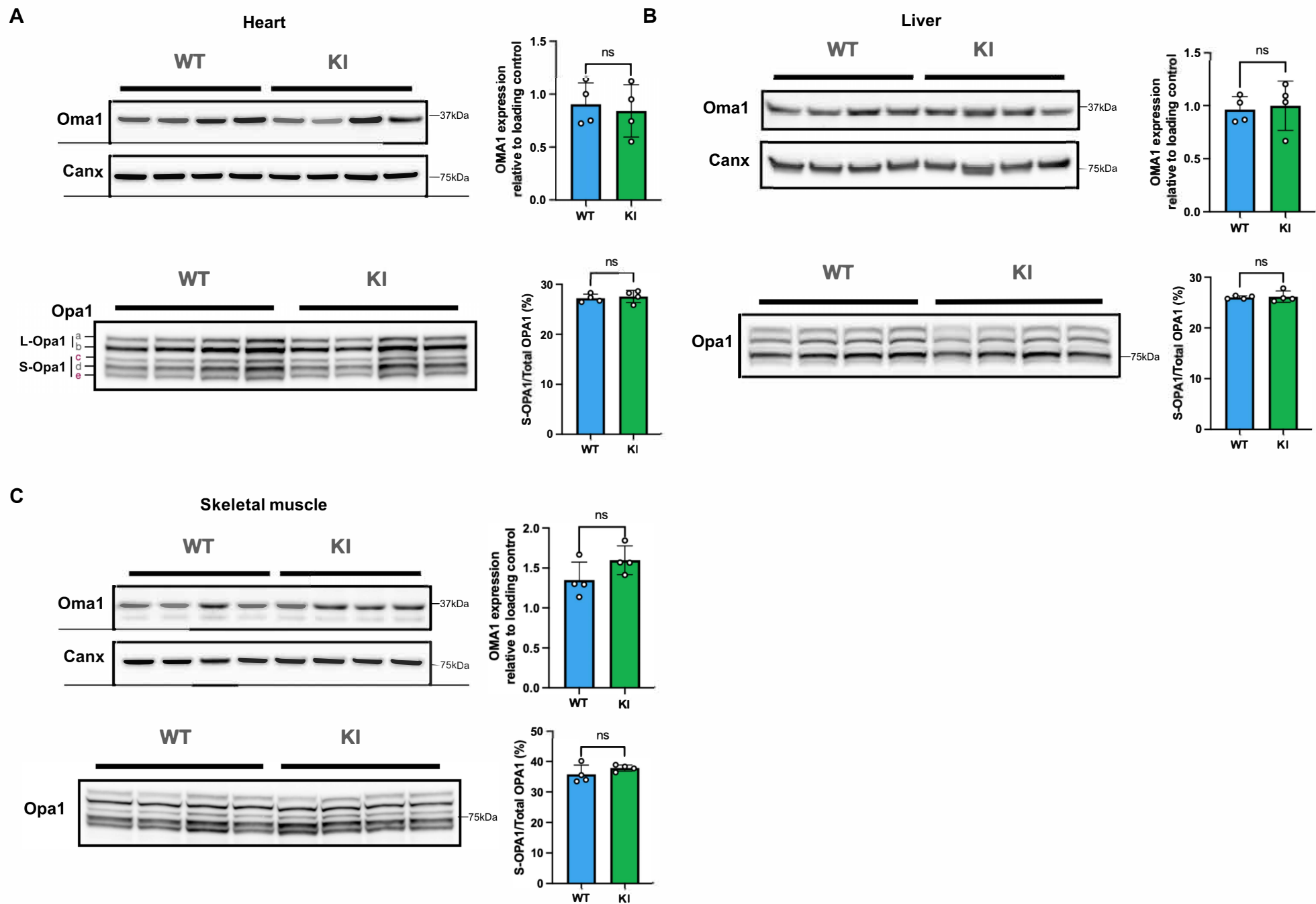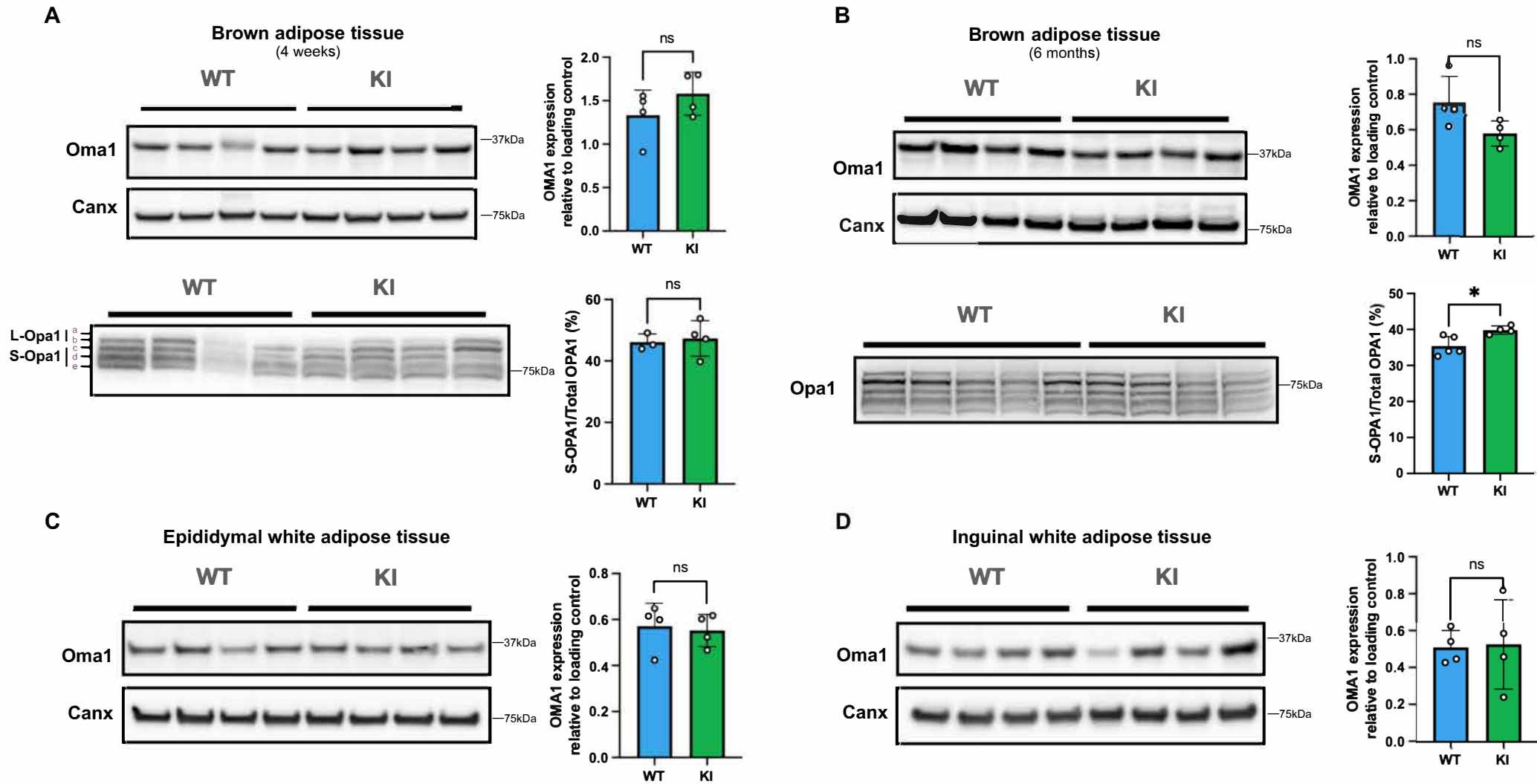
F

p.adj=0.009

Mitochondrial length / width (AU)

WT | KI

Figure 2 - Figure Supplement 1

Figure 2 - Figure Supplement 2

A Liver

B Heart

C Inguinal white adipose tissue

D Epididymal white adipose tissue

E Brown adipose tissue
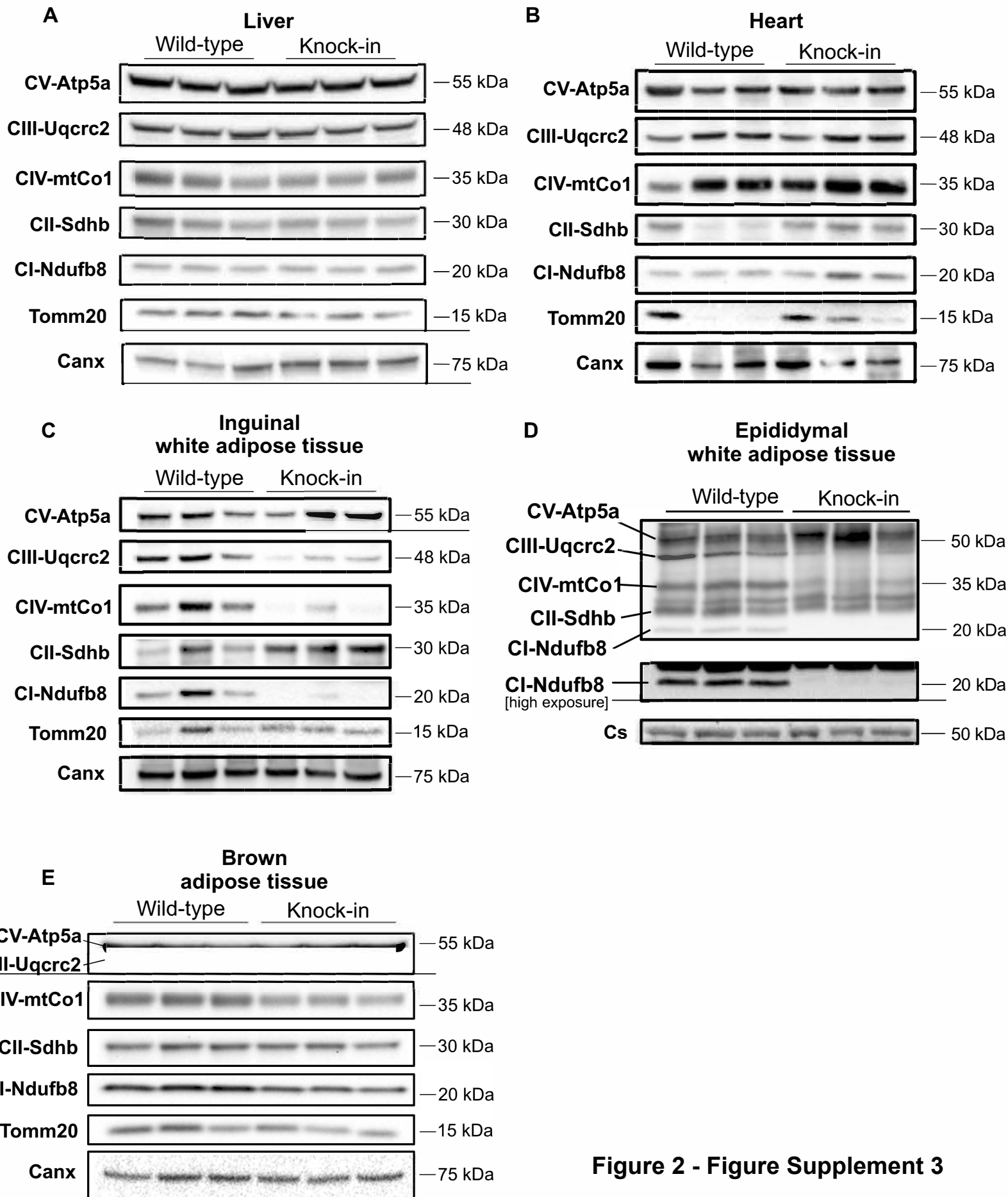
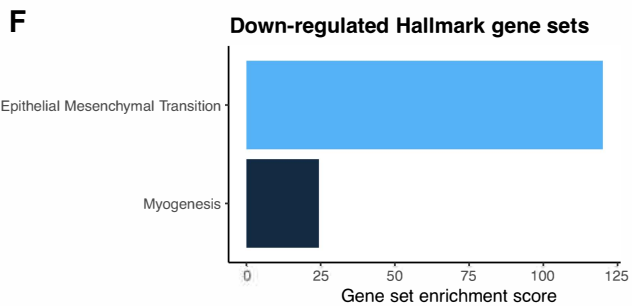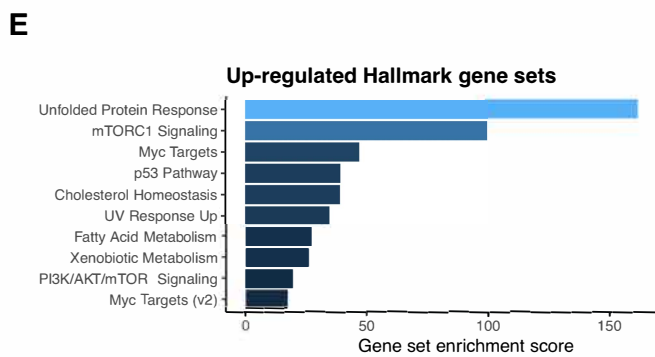Figure 2 - Figure Supplement 3

Figure 1 - Figure Supplement 4

A Liver

B BAT

C Maximum thermogenic capacity

Wild-type 10°C (n=18)
Knock-in 10°C (n=16)
Wild-type 30°C (n=14)
Knock-in 30°C (n=13)

D Maximum energy expenditure

p.adj =.0001
p.adj <.0001

E Area under the curve

p.adj = .008
p.adj = .0002
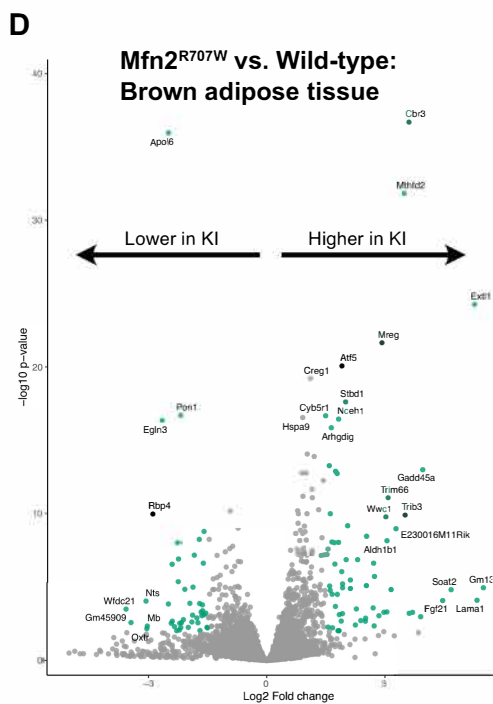
F Basal
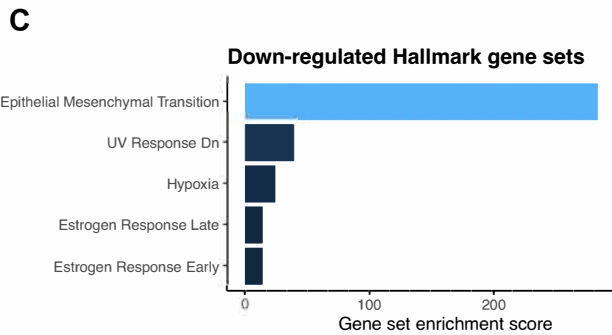
p.adj=.01
p.adj=.02

G NA-stimulated energy expenditure

p.adj =.0001
p.adj <.0001
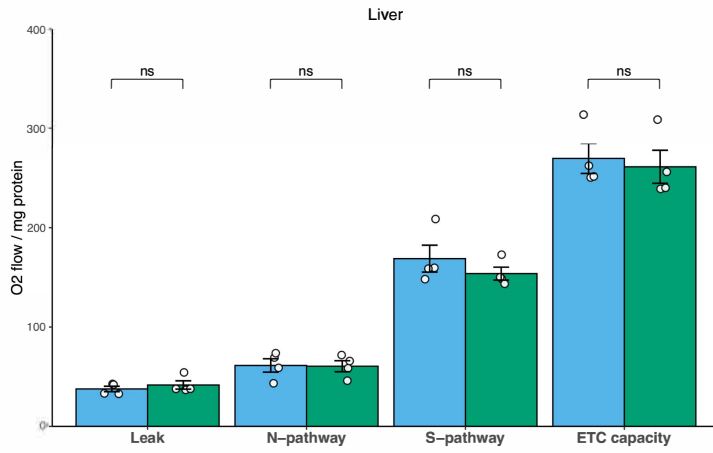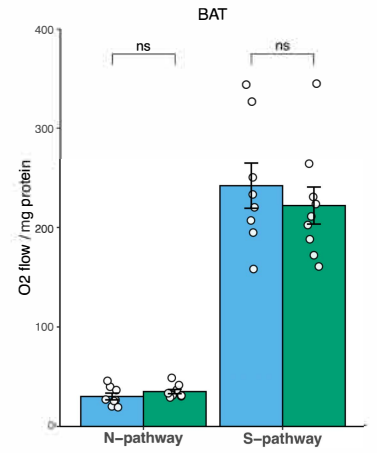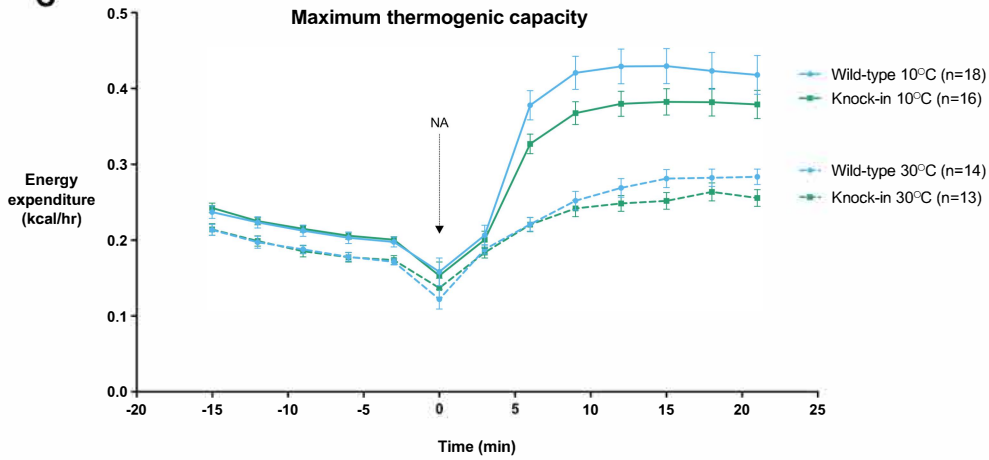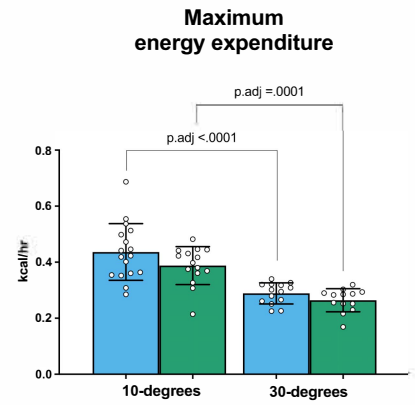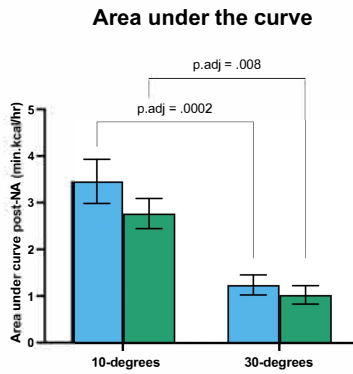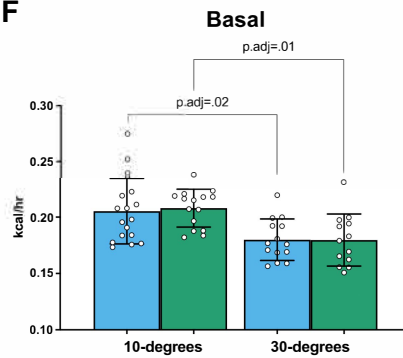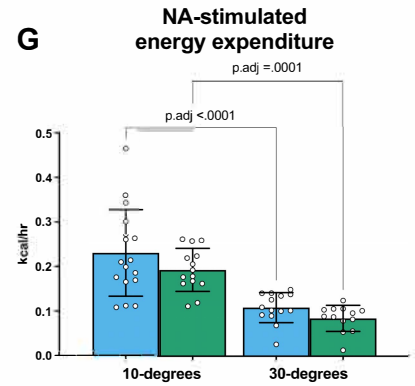
Figure 1 - Figure Supplement 5
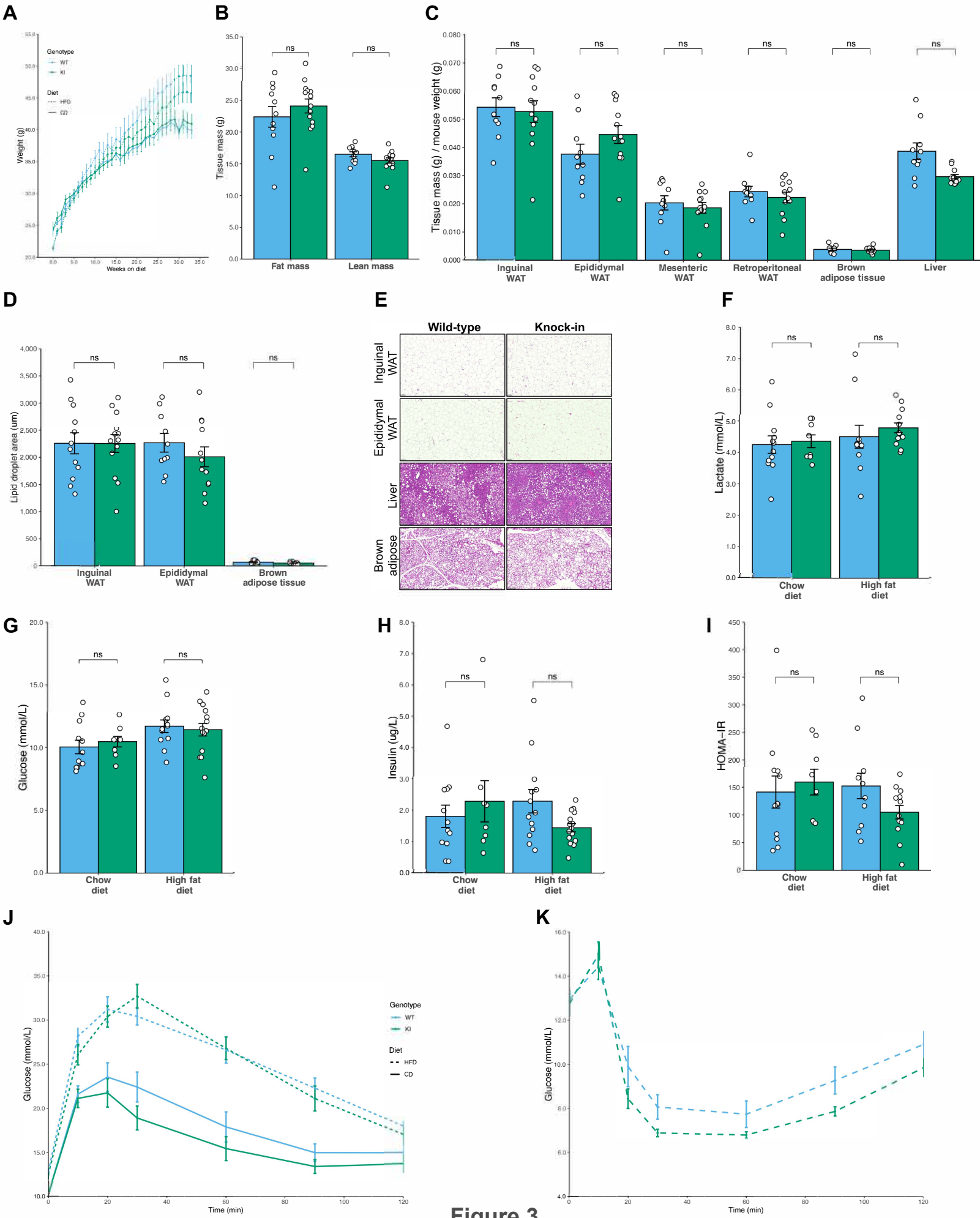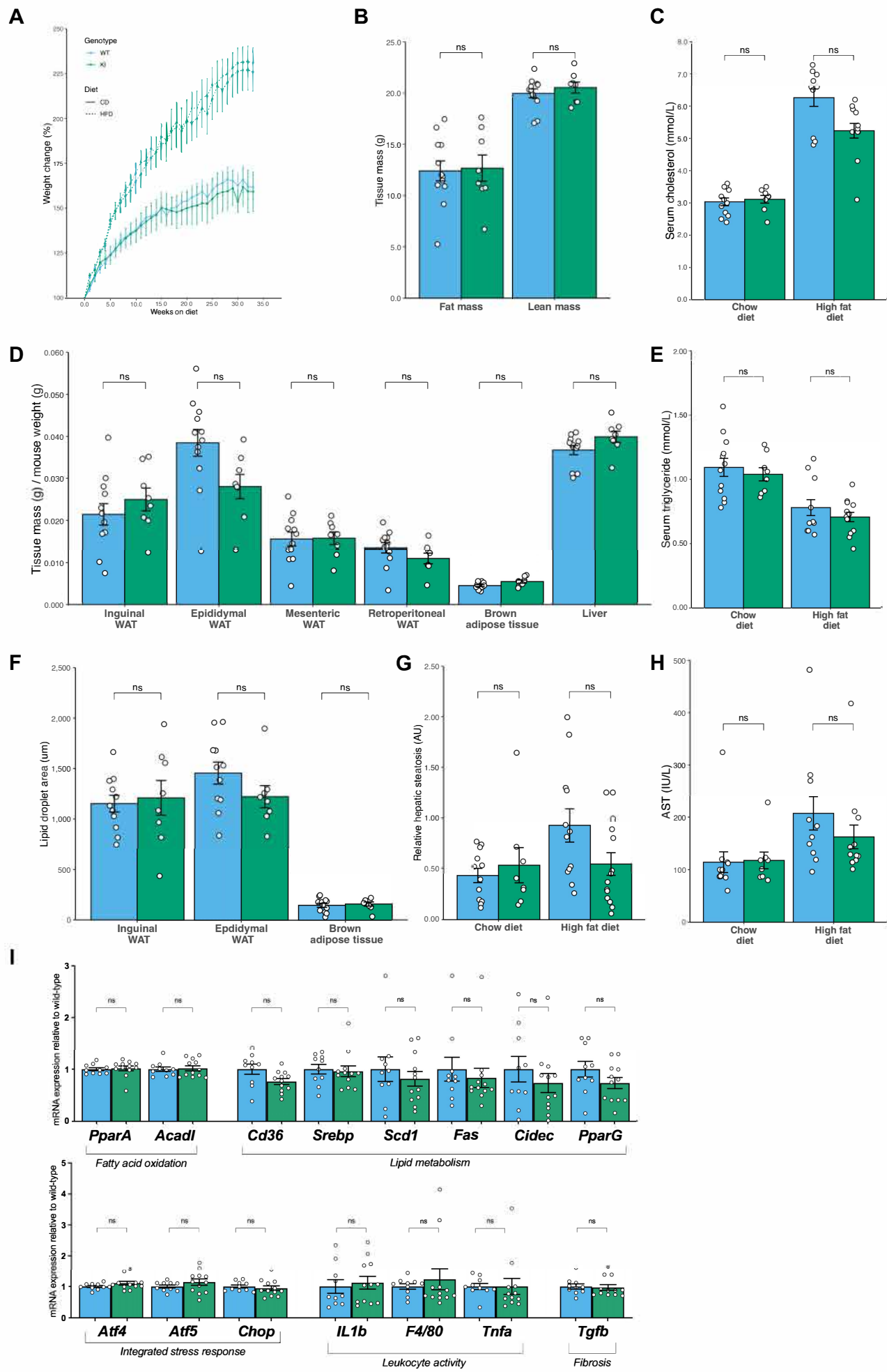
Figure 3

Figure 3 - Figure Supplement 1

Figure 3 - Figure Supplement 2

Figure 4

A *Gdf15*

B *Fgf21*

C

D

E Inguinal white adipose tissue

Phos-Eif2a (Ser51) —37 kDa
Total Eif2a —37 kDa
Mthfd2 —37 kDa
Canx —75 kDa

F Skeletal muscle

Phos-Eif2a (Ser51) —37 kDa
Total Eif2a —37 kDa
Mthfd2 —37 kDa
Canx —75 kDa

G Heart

Phos-Eif2a (Ser51) —37 kDa
Total Eif2a —37 kDa
Mthfd2 —37 kDa
Canx —75 kDa

H KEGG: TCA Cycle

I Nuclear-encoded mitochondrial genes   Mitochondrial-encoded genes

Log$_2$ fold change

Significant DGE
p-FDR <.001
n.s.

Figure 4 - Supp 3

Figure 4 - Figure Supplement 2

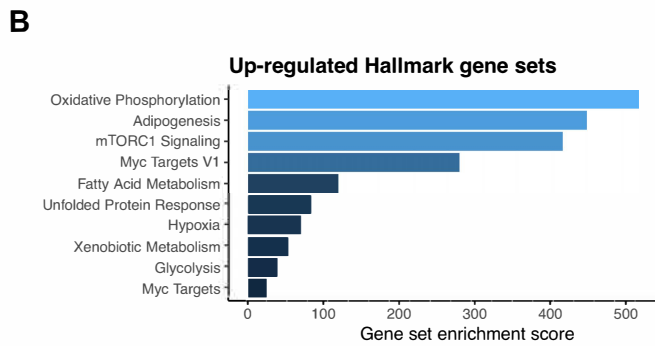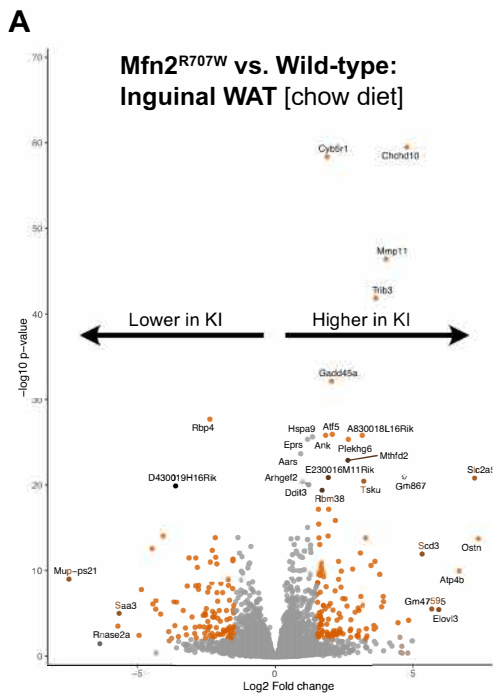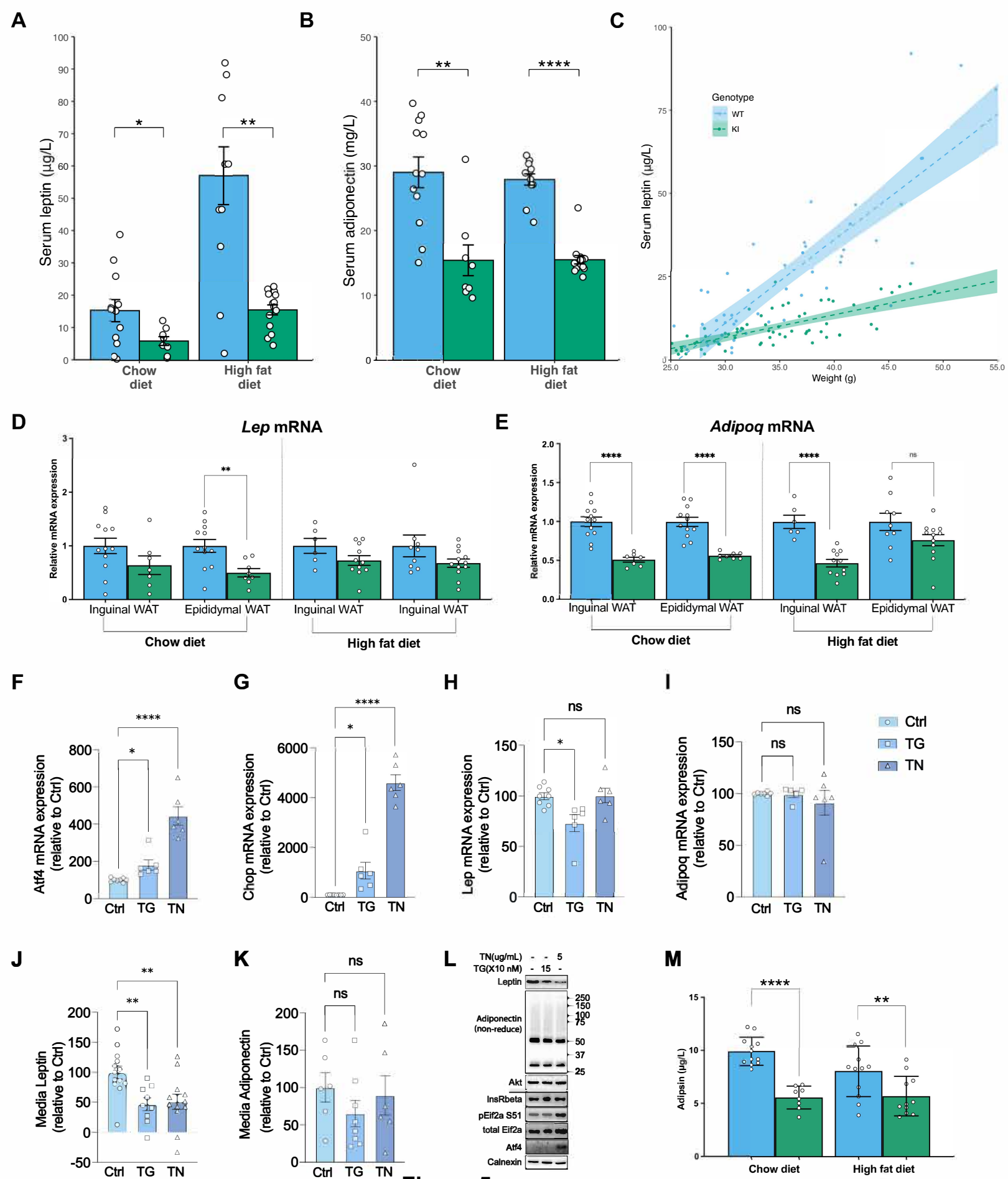**Figure 4 - Figure Supplement 3**
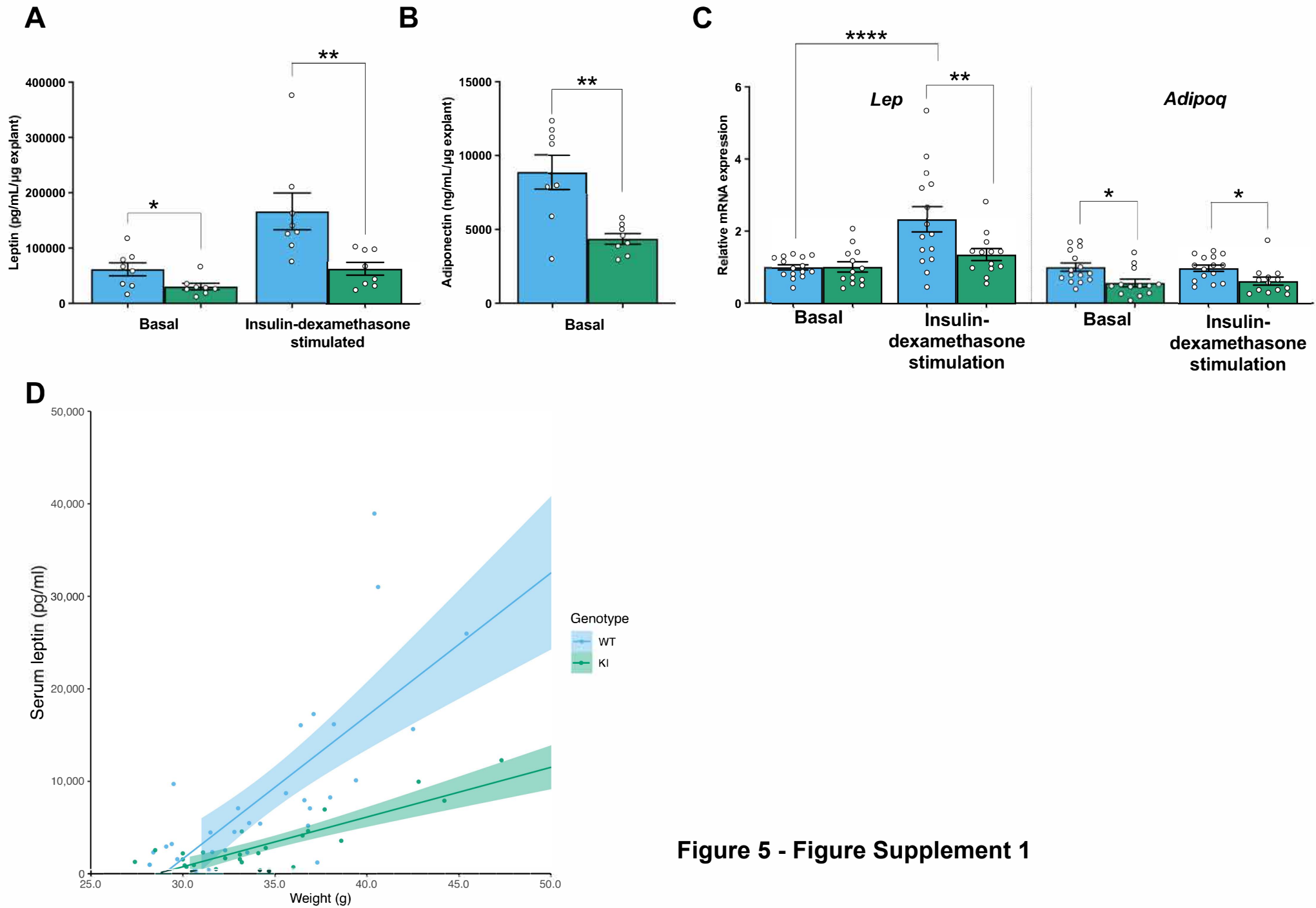
Figure 4 - Figure Supplement 4

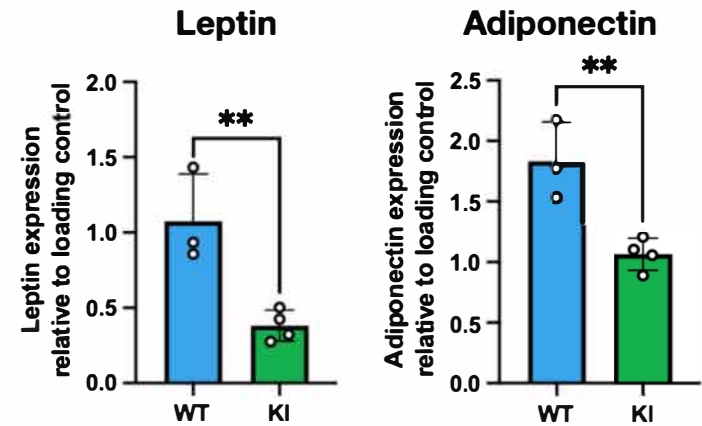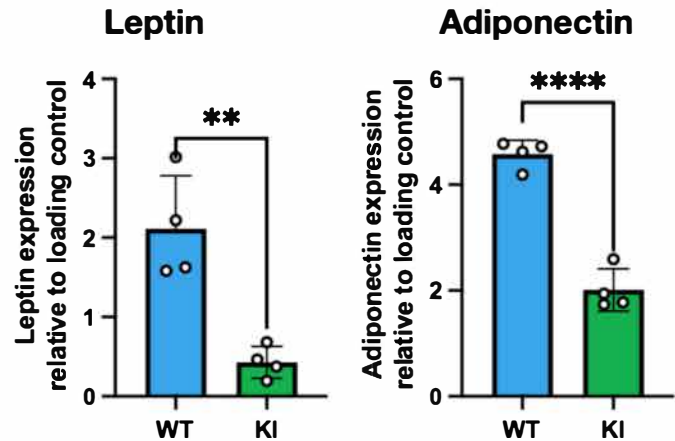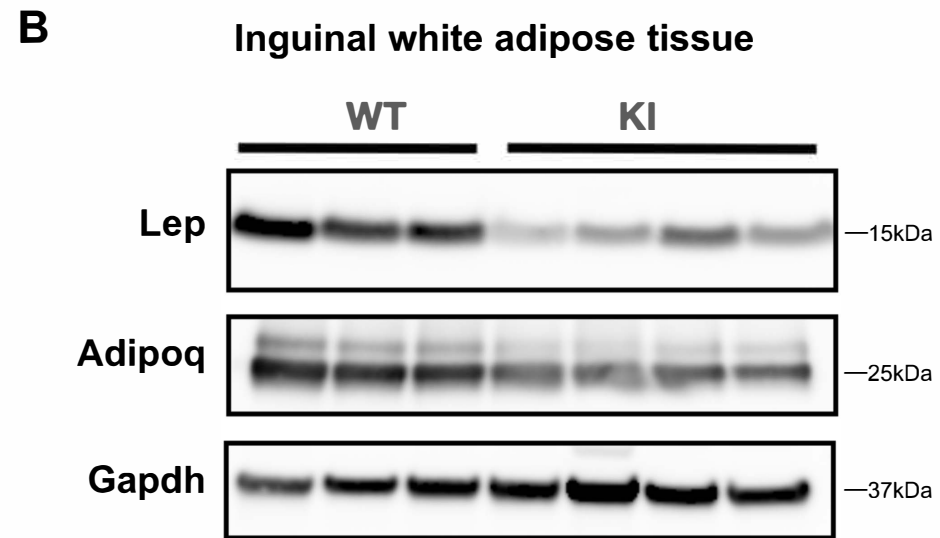Figure 4 - Figure Supplement 5

Figure 5

Figure 5 - Figure Supplement 1

A Epididymal white adipose tissue

WT   KI

Lep —15kDa

Adipoq —25kDa

Gapdh —37kDa

Leptin

Adiponectin

B Inguinal white adipose tissue

WT   KI

Lep —15kDa

Adipoq —25kDa

Gapdh —37kDa

Leptin
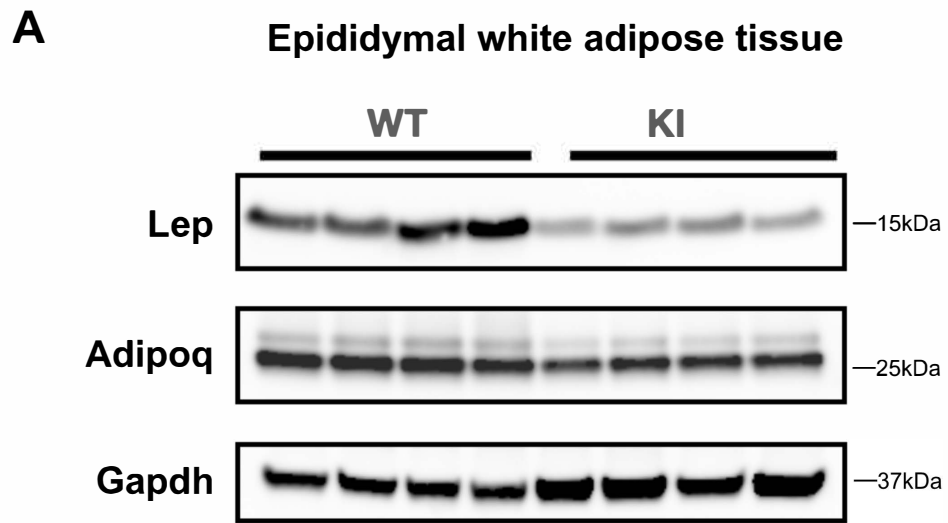
Adiponectin

Figure 5 - Figure Supplement 2

Primary	Supplier	CatID	Concentration	Diluent	2[ary]
Adiponectin	GeneTex	GTX80683	1 in 1000	5% milk	Mouse
AKT	Cell signalling	2920S	1 in 1000	5% BSA	Mouse
Anti-mouse HRP	Cell signalling	70765	1 in 5000	5% milk	N/A
Anti-rabbit HRP	Cell signalling	70751	1 in 5000	5% milk	N/A
Atf4	Cell signalling	11815S	1 in 1000	5% BSA	Rabbit
Beta-tubulin	Abcam	ab6046	1 in 1000	5% milk	Rabbit
Calnexin	Abcam	ab22595	1 in 5000	5% BSA	Rabbit
CitSynth	Abcam	Ab129095	1 in 1000	5% milk	Rabbit
Gapdh	GeneTex	GTX100118	1 in 5000	5% milk	Rabbit
Gapdh (for Opa1 blots)	Proteintech	10494-1-AP	1 in 1000	5% milk	Rabbit
InsR beta	Santa cruz	SC-57342	1 in 1000	5% BSA	Mouse
Leptin	Abcam	ab9749	1 in 1000	5% BSA	Rabbit
Mfn1	Abcam	Ab126575	1 in 250	5% milk	Mouse
Mfn2	Cell signalling	D2D10	1 in 1000	5% milk	Rabbit
Mthfd2	Proteintech	12270-1-AP	1 in 1000	5% milk	Rabbit
Oma1	Proteintech	17116-1-AP	1 in 1000	5% milk	Rabbit
Opa1	BD Biosciences	612606	1 in 1000	5% milk	Mouse
OXPHOS cocktail	Abcam	Ab110413	1 in 1000	5% milk	Mouse
Phos-Eif2a	Epitomics	10901	1 in 1000	5% BSA	Rabbit
Tom20	Abcam	Ab56783	1 in 500	5% milk	Mouse
Total Eif2a	D. Ron Lab	N/A	1 in 3000	5% milk	Rabbit

Supplementary File 1: Antibodies used in this study.

Primer	Sequence/TaqMan
36b4 Fwd	AGATGCAGCAGATCCGCAT
36b4 Rv	GTTCTTGCCCATCAGCACC
AdipoQ Fwd	GTTGCAAGCTCTCCTGTTCC
AdipoQ Rv	ATCCAACCTGCACAAGTTCC
Atf4 Fwd	GGGTTCTGTCTTCCACTCCA
Atf4 Rv	AAGCAGCAGAGTCAGGCTTTC
Atf5 TaqMan	Cat# Mm04179654_m1 (ThermoFisher Scientific)
B2m Fwd	ACTGATACATACGCCTGCAGAGTT
B2m Rv	TCACATGTCTCGATCCCAGTAGA
Ddit3 (Chop) Fwd	CCACCACACCTGAAAGCAGAA
Ddit3 (Chop) Rv	AGGTGAAAGGCAGGGACTCA
Fgf21 Fwd	CTGGGGGTCTACCAAGCATA
Fgf21 Rv	CACCCAGGATTTGAATGACC
Gdf15 TaqMan	Cat# Mm00442228_m1 (ThermoFisher Scientific)
gDNA_HK2_Fwd	GCCAGCCTCTCCTGATTTTAGTGT
gDNA_HK2_Rev	GGGAACACAAAAGACCTCTTCTGG
gDNA_Mt-Rnr2_Fwd	AACTCGGCAAACAAGAACCC
gDNA_Mt-Rnr2_Rev	CCCTCGTTTAGCCGTTCATG
Hprt Fwd	AGCCTAAGATGAGCGCAAGT
Hprt Rv	GGCCACAGGACTAGAACACC
Lep3 Fwd	CCAGGAtgacaccaaaaccct
Lep3 Rv	GATACCGACTGCGTGTGTGA
Mfn2 genotyping Fwd	AGTCCCTTCCTTGTCACTTAGT
Mfn2 genotyping Rv	ATCTCACAAGAAAGCGAAATCC

2   **Supplementary File 2**: Primer sequences used in this study. Fwd, forward primer; Rv, reverse primer.

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