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1 **Macrophage fumarate hydratase restrains mtRNA-mediated interferon**
2 **production**

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24

25 **Summary**

26 Metabolic rewiring underlies macrophage effector functions¹⁻³, but the mechanisms
27 involved remain incompletely defined. Here, using unbiased metabolomics and stable
28 isotope-assisted tracing, we show induction of an inflammatory aspartate-
29 argininosuccinate shunt following LPS stimulation. The shunt, supported by increased
30 ASS1 expression, also leads to increased cytosolic fumarate levels and fumarate-
31 mediated protein succination. Pharmacologic inhibition and genetic ablation of the
32 TCA cycle enzyme FH further elevates intracellular fumarate levels, suppresses

33 mitochondrial respiration, and increases mitochondrial membrane potential. RNA
34 sequencing and proteomic analysis demonstrates profound inflammatory effects
35 resulting from FH inhibition. Of note, acute FH inhibition suppresses IL-10 expression
36 leading to increased TNF- α secretion, an effect recapitulated by fumarate esters.
37 Unexpectedly, FH inhibition, but not fumarate esters, also increases IFN- β production
38 through mechanisms that are driven by mitochondrial RNA (mtRNA) release and
39 activation of the RNA sensors TLR7 and RIG-I/MDA5. This effect is recapitulated
40 endogenously when FH is suppressed following prolonged LPS stimulation.
41 Furthermore, cells from SLE patients also exhibit FH suppression, indicating a
42 potential pathogenic role for this process in human disease. We therefore identify a
43 protective role for FH in maintaining appropriate macrophage cytokine and interferon
44 responses.

45

46 **Keywords**

47 Macrophage, LPS, cytokines, type I interferon, mitochondria, metabolism,
48 mitochondrial retrograde signalling, fumarate hydratase, aspartate-argininosuccinate
49 shunt, fumarate, TNF- α , IL-10, SLE, mtRNA, TLR7, RIG-I, MDA5, antiviral signalling

50

51

52 **Main Text**

53 Stimulation of macrophages with the TLR4 ligand lipopolysaccharide (LPS) induces
54 metabolic reprogramming involving rewiring of the TCA cycle and mitochondrial
55 respiration, facilitating cytokine production. Changes in macrophage metabolism have
56 emerged as a major regulator of inflammation^{2,4-6}. While metabolic reprogramming is
57 crucial for macrophage activation⁷, the players involved and how they regulate
58 cytokine production remain incompletely characterised.

59

60 **Accumulation of fumarate in macrophages**

61 To evaluate metabolic alterations that occur during LPS stimulation, we employed an
62 unbiased liquid chromatography-mass spectrometry (LC-MS)-based metabolomics
63 approach to characterise the metabolome of inflammatory bone marrow-derived
64 macrophages (BMDMs). The TCA cycle metabolite fumarate stood out as one of the
65 most significantly upregulated metabolites upon exposure to acute LPS stimulation,
66 joining previously identified metabolites such as itaconate² (Fig. 1a). We also observed

67 a significant increase in fumarate-mediated protein succination⁸⁻¹⁰, resulting in the
68 formation of the fumarate-cysteine adduct, (S)-2-succinocysteine (2SC) (Extended
69 Data Fig. 1a-c).

70

71 As acute LPS stimulation failed to impair respiration (Fig. 1b, c), TCA cycle disruption
72 is unlikely to be sufficient for fumarate accumulation. Increased flux through the
73 aspartate-argininosuccinate shunt has been reported to support nitric oxide (NO)
74 production⁵. As fumarate is a by-product of argininosuccinate cleavage by
75 argininosuccinate lyase (ASL) in the cytosol, we hypothesised that argininosuccinate
76 may be a source of fumarate. Supporting this, we observed decreased aspartate, the
77 substrate for argininosuccinate, and increased argininosuccinate, fumarate, and
78 malate levels (Fig. 1d), consistent with increased flux through the shunt. This rewiring
79 also occurred during prolonged LPS stimulation (Extended Data Fig. 1d).

80

81 Argininosuccinate synthase (*Ass1*) and fumarate hydratase (*Fh1*) expression
82 increased and decreased respectively in LPS-stimulated BMDMs, as determined by
83 RT-qPCR (Fig.1e). Using available quantitative proteomics data^{2,11}, we found
84 argininosuccinate synthase (ASS1) to be upregulated, whereas levels of glutamic-
85 oxaloacetic transaminase 2 (GOT2), ASL and FH were not significantly altered (Fig.
86 1f). FH protein levels were suppressed only at later time points of LPS (Fig. 1g),
87 indicating that ASS1 induction is vital to the acute accumulation of fumarate.

88

89 Inhibition of the aspartate-argininosuccinate shunt with the GOT2 inhibitor
90 aminooxyacetic acid (AOAA)⁵ reduced aspartate, asparagine, argininosuccinate and
91 fumarate levels following LPS stimulation (Fig. 1h and Extended Data Fig. 1e).
92 Knockdown of *Asl* also prevented fumarate accumulation (Extended Data Fig. 1f, g)
93 indicating its dependency on the aspartate-argininosuccinate shunt, which would
94 increase cytosolic fumarate (Fig. 1i). With stable isotope-assisted tracing, we show
95 that glutamine-dependent anaplerosis is in part responsible for fumarate accumulation
96 and drives the aspartate-argininosuccinate shunt. U-¹³C-glutamine tracing
97 demonstrated glutaminolysis as a carbon source for the TCA cycle, aspartate-
98 argininosuccinate shunt metabolites, including fumarate, and glutathione (Extended
99 Data Fig. 2). ¹⁵N₂-glutamine tracing also demonstrated that glutamine nitrogen is a
100 source for glutathione synthesis and aspartate-argininosuccinate shunt metabolites

101 (Extended Data Fig. 3). Importantly, AOAA completely prevented the contribution of
102 glutamine nitrogen to aspartate, asparagine, arginine and citrulline, confirming its
103 inhibition of GOT2. Metabolomics on cytosolic fractions of resting and LPS-stimulated
104 macrophages showed that metabolites such as itaconate and succinate accumulate
105 in the cytosol following LPS stimulation (Extended Data Fig. 4a). Importantly, we also
106 found increased cytosolic argininosuccinate, fumarate and 2SC (Extended Data Fig.
107 4b).

108

109 We hypothesised that *Irg1*^{-/-} BMDMs (which are unable to synthesise itaconate) would
110 relieve inhibition of succinate dehydrogenase (SDH)^{4,12} and exhibit greater
111 accumulation of aspartate-argininosuccinate shunt metabolites. Metabolomics in *Irg1*^{-/-}
112 BMDMs revealed the expected decrease in itaconate and succinate, and increased
113 aspartate-argininosuccinate shunt metabolites, including fumarate and NO (Extended
114 Data Fig. 4c, d), providing further evidence linking mitochondrial TCA cycle activity to
115 an aspartate-argininosuccinate shunt (Extended Data Fig. 4e).

116

117 **FH inhibition causes metabolic rewiring**

118 FH catalyses the hydration of fumarate to malate in the mitochondrion and cytosol¹³,
119 the inhibition of which elevates cytosolic fumarate accumulation, perturbs urea cycle
120 metabolism and leads to renal cyst development¹⁴. Given protein levels of FH remain
121 stable during early LPS stimulation (Fig. 1g), we used a well-established
122 pharmacological inhibitor of FH (FHIN1)¹⁵ and a recently developed tamoxifen-
123 inducible CRE-ERT2-*Fh1*^{-/-} model to probe the role of FH activity and fumarate
124 accumulation in macrophages. However, since FH inhibition may lead to effects
125 independent of fumarate accumulation through mitochondrial and redox stress¹⁶, we
126 also used low concentrations of cell-permeable dimethyl fumarate (DMF) to deliver a
127 cysteine-reactive fumarate ester which does not inhibit respiration¹⁷⁻¹⁹. This approach
128 would uncouple the role of impaired mitochondrial bioenergetics following TCA cycle
129 disruption and fumarate-mediated electrophilic modification of cysteine residues.

130

131 Previous reports show that immunometabolites and their derivatives affect
132 macrophage function through regulation of metabolic pathways^{9,20,21}. We therefore
133 aimed to assess how FH inhibition and DMF may regulate macrophage metabolism.
134 First, comparing the effects of FHIN1 and DMF on mitochondrial bioenergetics, we

135 found that FHIN1 reduced ratios of ATP/ADP, ATP/AMP, and P-creatine/creatine while
136 DMF had no effect, demonstrating that FH sustains mitochondrial bioenergetics (Fig.
137 2a, Extended Data Fig. 5a). This was confirmed by respirometry, showing FHIN1
138 impaired basal respiration, ATP production and maximal respiration as measured by
139 OCR, while DMF had no effect (Fig. 2b). FHIN1 led to a distinct metabolic signature
140 characterised by alterations in TCA cycle metabolites including citrate, aconitate,
141 itaconate and succinate, indicating TCA cycle rewiring, as well as enhanced fumarate
142 and 2SC accumulation, supporting this approach in studying the roles of FH in
143 macrophages (Fig. 2c, e, Extended Data Fig. 5b). Principal component analysis (PCA)
144 showed a significant divergence of FHIN1 treatment to the other conditions (Fig. 2d).

145

146 Tamoxifen-inducible knockout of *Fh1* in macrophages (Extended Data Fig. 5c, d)
147 induced similar bioenergetic changes to FHIN1, demonstrated by reduced ATP/AMP
148 and P-creatine/creatine ratios, although the ATP/ADP ratio was unchanged (Extended
149 Data Fig. 5e). TCA cycle rewiring was also observed in *Fh1*^{-/-} macrophages, although
150 to a lesser extent than with FHIN1 (Extended Data Fig. 5f). Compensatory remodelling
151 during initial genetic inactivation of FH may buffer some of the acute changes
152 observed with FHIN1²². Importantly however, fumarate and 2SC levels were increased
153 in *Fh1*^{-/-} macrophages (Fig. 2f, Extended Data Fig. 5g), supporting our parallel use of
154 FHIN1 and *Fh1*^{-/-} macrophages.

155

156 Confirming previous reports⁹, DMF, and to a lesser extent FHIN1, suppressed
157 glycolysis (Extended Data Fig. 5h). GAPDH is reportedly inhibited by fumarate-
158 mediated succination^{9,23}. Consistently, FHIN1 increased the glyceraldehyde 3-
159 phosphate (G3P)/2/3-phosphoglycerate (2/3-PG) ratio (Extended Data Fig. 5i),
160 suggesting that endogenous fumarate accumulation may impair GAPDH activity. This
161 provides further evidence that FH impairment leads to modulation of cytosolic
162 processes.

163

164 As FHIN1 impaired respiration, we examined further mitochondrial parameters. We
165 first observed increased reactive oxygen species (ROS) production in cells treated
166 with FHIN1 but not DMF (Fig. 2g). FHIN1 treatment also increased staining intensity
167 of the mitochondrial membrane potential (MMP)-dependent dye mitotracker RED
168 (mtRED) (Extended Data Fig. 5j, k). Tetramethylrhodamine methyl ester (TMRM)

169 staining confirmed this result, as FHIN1 significantly increased staining while DMF had
170 no effect (Fig. 2h). Similarly, *Fh1*^{-/-} macrophages had increased MMP, as previously
171 reported in kidney epithelial cells²⁴ (Fig. 2h). We also observed a decreased
172 aconitate/citrate ratio in FHIN1-treated macrophages, indicative of impairment in the
173 fumarate- and redox-sensitive TCA cycle enzyme aconitase²⁵ (Fig. 2i). Although the
174 GSSG/GSH ratio was unchanged, FHIN1 led to a depletion of total glutathione (Fig.
175 2j), consistent with fumarate-mediated glutathione depletion^{26,27}. These data suggest
176 that FH inhibition induces profound redox stress responses.

177

178 **FH maintains appropriate cytokine responses**

179 To determine whether FH regulates macrophage activation and effector responses,
180 we performed RNA sequencing and proteomics to assess changes in the
181 transcriptome and proteome of FHIN1-treated BMDMs. Geneset enrichment analysis
182 (GSEA) identified an expected suppression in genes associated with metabolism, but
183 FHIN1 also decreased expression of inflammatory pathways, including IL-1 and IL-10
184 signalling (Fig. 3a). Increased expression of the heme-regulated inhibitor (HRI) stress
185 response, amino acid metabolism and tRNA aminoacylation was also observed (Fig.
186 3a), consistent with previous reports¹⁶. Further overrepresentation analysis (ORA) of
187 RNAseq data revealed TNF- α signalling to be the most highly upregulated pathway in
188 our analysis (Fig. 3b).

189

190 Comparing FHIN1 with DMF on cytokine readouts allowed us to determine the role of
191 protein succination following FH inhibition. Validating our transcriptomic analysis,
192 FHIN1 and DMF decreased IL-10 release and expression, while TNF- α release and
193 expression were increased (Fig. 3c, Extended Data Fig. 6a). Both compounds also
194 reduced IL-1 β expression and IL-6 release (Extended Data Fig. 6b), consistent with
195 previous reports^{10,28}, demonstrating widespread regulation of cytokine expression.

196

197 The less electrophilic fumarate ester, monomethyl fumarate (MMF), exhibited the
198 same effects on *Ii10* and *Tnfa* expression (Fig. 3d), supporting a role for their
199 regulation by fumarate. Shared transcriptomic changes of FHIN1 and DMF
200 demonstrated strong downregulation of the ERK1/2 cascade and PI3K signalling (Fig.
201 3e). A similar transcriptional fingerprint has been observed in FH-deficient
202 leiomyomas²⁹. We also observed increased amino acid metabolism and transport, and

203 autophagy transcripts (Extended Data Fig. 6c). Upon LPS stimulation, IL-10 is
204 regulated by ERK1/2 and PI3K-induced AP-1 activation³⁰, suggesting that
205 downregulation of this signalling axis by FHIN1 and DMF may repress IL-10. However,
206 we did not observe changes in the upstream kinases (AKT, JNK, ERK and p38) which
207 converge on AP-1 activation, (Extended Data Fig. 6d). Although we did observe
208 reduced *Jun* expression in our transcriptomics dataset (Extended Data Fig. 6e), this
209 could indicate reduced autoregulation by AP-1³¹. In this dataset, *Fos* was not reduced
210 (Extended Data Fig. 6f).

211

212 Interestingly, the thiol precursor N-acetyl cysteine (NAC) abrogated the suppression
213 of *IL10* by FHIN1 and DMF (Fig. 3f). The free thiols of NAC and its products would react
214 with and sequester fumarate, thereby reducing the modification of protein thiols and
215 suggesting that suppression of IL-10 results from a redox-dependent succination
216 event. The electrophile sulforaphane has been shown to reduce AP-1 activation via
217 modification of Cys-154 on c-Fos³². We therefore investigated if FHIN1 or DMF may
218 affect c-Fos activation, despite upstream regulators remaining unaffected. Using a c-
219 Fos transcription factor assay, we found that FHIN1 and DMF strongly impaired c-Fos
220 activation (Fig. 3g), providing evidence of direct regulation of c-Fos, potentially through
221 S-alkylation.

222

223 IL-10 signalling has been shown to repress TNF- α expression³³. We confirmed this
224 using an IL-10 receptor (CD210) blocking antibody targeting IL-10-mediated STAT3
225 phosphorylation, leading to augmented LPS-induced TNF- α release (Fig. 3h,
226 Extended Data Fig. 6g). We then examined whether recombinant IL-10
227 supplementation could rescue the increase in TNF- α . Indeed, with IL-10, FHIN1 failed
228 to impair STAT3 phosphorylation or augment TNF- α production (Fig. 3i, j), indicating
229 that the FHIN1- and DMF-driven induction of TNF- α is dependent on the suppression
230 of IL-10.

231

232 Confirming the role of FH in regulating this axis, inducible deletion of *Fh1* in
233 macrophages from heterozygous *Fh1*^{+/-} or homozygous *Fh1*^{-/-} mice (Extended Data
234 Fig. 5c, d, Extended Data Fig. 6h) resulted in decreased IL-10 expression and release
235 (Fig. 3k) and increased TNF- α release (Fig. 3l). Furthermore, FHIN1 also suppressed
236 *IL10* expression and increased *TNFA* expression in LPS-stimulated human peripheral

237 blood mononuclear cells (PBMCs) (Fig. 3m) and macrophages (Fig. 3n), indicating
238 that the FH-regulated IL-10/TNF- α axis is also active in human cells. Establishing the
239 role of LPS-driven fumarate accumulation on release of these cytokines, AOAA, which
240 reduces fumarate accumulation (Fig. 1h), modestly increased and reduced IL-10 and
241 TNF- α release respectively (Extended Data Fig. 6i), indicating that an increase in
242 ASS1, which results in fumarate accumulation, mildly regulates IL-10 and TNF- α
243 production. These effects are accentuated by pharmacological or genetic inhibition of
244 FH, leading to increased fumarate accumulation (Extended Data Fig. 6j). Therefore,
245 sustained expression and activity of FH may be viewed as protective against
246 excessive fumarate accumulation and dysregulated production of IL-10 and TNF- α .

247

248 FH inhibition also resulted in the activation of an NRF2 and ATF4 stress response in
249 macrophages (Extended Data Fig. 7a), in line with previous observations in epithelial
250 cells¹⁶. Proteomic analysis revealed that the inflammation-associated hormone
251 GDF15³⁴⁻³⁶ is one of the most significantly increased proteins with FHIN1 and DMF,
252 while FHIN1 also increased the recently identified mitochondrial glutathione importer,
253 SLC25A39³⁷, reinforcing the mitochondrial redox perturbation (Extended Data Fig. 7b,
254 c). Validating our proteomics data, FH inhibition drove GDF15 release from
255 macrophages (Extended Data Fig. 7d). Both ATF4 and NRF2 have been reported to
256 regulate GDF15 in different contexts^{35,38}, and silencing of each revealed that FHIN1-
257 driven GDF15 release was partly NRF2- but not ATF4-dependent (Extended Data Fig.
258 7e, f). This work defines two previously unappreciated signalling axes linked to FH
259 inhibition, uncovering its role in the regulation of IL-10/TNF- α and GDF15. The recent
260 developments identifying GDF15 as a mediator of immune tolerance, and the anti-
261 inflammatory properties of colchicine and NSAIDs^{38,39}, suggest that protective effects
262 of DMF in models of inflammation could be via GDF15. Additionally, increased TNF-
263 α levels potentially explain adverse events reported with fumarate esters⁴⁰.
264 Mechanistically, suppression of IL-10 may also explain why fumarate esters promote
265 enhanced TNF- α production during trained immunity, in addition to reported epigenetic
266 changes⁴¹.

267

268 **FH restrains mtRNA-driven IFN- β release**

269 RNAseq analysis of type I interferon (IFN) response genes revealed divergent effects
270 on IFN expression and signalling with FH inhibition, including an upregulation in *Ifnb1*
271 (IFN- β) expression and several interferon-stimulated genes (ISGs), such as *Irf1*, *Ifih1*,
272 *Rsad2* and *Ifit2* (Fig. 4a). However, other ISGs, such as *Lcn2*, were suppressed by
273 FHIN1 and DMF treatment (Fig. 4a & Extended Data Fig. 8a). Examination of specific
274 type I IFN signalling components downstream of the interferon- α/β receptor (IFNAR)
275 revealed that both FHIN1 and DMF treatment limited IFN- β -induced signal transducer
276 and activator of transcription 1 (STAT1) and Janus kinase 1 (JAK1) phosphorylation
277 (Extended Data Fig. 8b), indicating modest suppression of JAK/STAT signalling.
278 Activation of NRF2 by fumarate and derivatives (Extended Data Fig. 7) may be
279 responsible⁴². Indeed, *Ifnb1* expression was increased with FHIN1 and DMF following
280 *Nrf2* silencing (Extended Data Fig. 8c, d), suggesting that Nrf2 restrains interferon
281 transcription.

282

283 Strikingly, FHIN1, but not DMF or MMF, was found to increase IFN- β release from
284 LPS-stimulated macrophages (Fig. 4b, c). This was independent of NAC-sensitive
285 redox stress (Extended Data Fig. 8e), and was not due to augmented TLR4 signalling,
286 as LPS-induced TRAF3 levels and IL-1 β expression were not increased by FHIN1
287 (Extended Data Fig. 8f, g). FHIN1 and DMF did modestly augment LPS-induced p65
288 phosphorylation (Extended Data Fig. 8h), which may contribute to increased TNF- α
289 release⁴³. Given FH inhibition causes mitochondrial stress (Fig. 2) which is associated
290 with the release of immunostimulatory mitochondrial nucleic acids⁴⁴⁻⁴⁶, we
291 hypothesised that the IFN response was driven by cytosolic nucleic acid sensors, such
292 as cGAS. To support this, FH deficient-hereditary leiomyomatosis and renal cell
293 cancer (HLRCC) tumours exhibit changes in mitochondrial DNA (mtDNA)²². We first
294 used ethidium bromide (EtBr) to deplete mtDNA⁴⁷ (Extended Data Fig. 8i) before
295 treating cells with FHIN1 and LPS. We found that FHIN1 no longer boosted LPS-
296 induced IFN- β release in the presence of EtBr (Fig. 4d), indicating that increased IFN-
297 β release with FHIN1 may be mtDNA-dependent. We subsequently found that FHIN1
298 caused an increase in both mtDNA and mtRNA in cytosolic extracts (Fig. 4e, Extended
299 Data Fig. 8j). Given the established role of mtDNA in driving IFN responses^{44,45}, we
300 examined whether the cGAS-STING or TLR9 DNA-sensing pathways were required
301 for the increase in IFN- β . However, neither use of the STING inhibitor C-178⁴⁸ nor

302 silencing of *Cgas* (cGAS) or *Tmem173* (STING) had any effect on FHIN1-driven IFN-
303 β induction (Extended Data Fig. 8k-n). Targeting TLR9 using the competitive inhibitor
304 ODN 2088⁴⁹ or using siRNA also had no effect on this response (Extended Data Fig.
305 8k-n). Suppression of *Tmem173* expression by FHIN1 and DMF (Extended Data Fig.
306 8o) may explain why cGAS-STING signalling is redundant in our model, even in the
307 presence of cytosolic mtDNA. ETC inhibition, as we observe with FHIN1 treatment,
308 has also been shown to inhibit STING activation⁵⁰.

309

310 Since cytosolic mtRNA was also increased by FHIN1 (Fig. 4e), we performed
311 immunofluorescence staining with an antibody specific for double-stranded RNA
312 (dsRNA). Mitochondrial RNA has previously been shown to drive an IFN response in
313 human cells^{51,52}, and is known to be particularly immunostimulatory⁵³. FHIN1
314 treatment led to an accumulation of dsRNA relative to DMSO control (Fig. 4f). We
315 subsequently co-treated cells with FHIN1 and IMT1, the mitochondrial RNA
316 polymerase (POLRMT) inhibitor. The increase in mtRNA with FHIN1 was observed in
317 the cytosolic fraction but not in the whole cell fraction and was inhibited in both by co-
318 treatment with IMT1 (Extended Data Fig. 8p, q). Importantly, IMT1 also abrogated the
319 FHIN1-mediated boost in IFN- β release (Extended Data Fig. 8r), implicating the role
320 of mtRNA in driving this response. Mitochondrial ssRNA, resulting from a decline in
321 mitochondrial integrity, has also been implicated in driving TLR7-dependent IFN
322 signalling^{54,55}. We subsequently silenced *Tlr7* or the dsRNA sensors *Ddx58* (RIG-I)
323 and *Iffih1* (MDA5) (Extended Data Fig. 9a, b), all of which abrogated the boost in IFN-
324 β release observed with FH inhibition (Fig. 4g, h), confirming a non-redundant
325 requirement of these sensors and mtRNA, rather than mtDNA, for the FHIN1-driven
326 IFN response. Knockdown of the cell surface dsRNA sensor *Tlr3* did not affect the
327 augmentation in IFN- β release (Extended Data Fig. 9c). RIG-I and MDA5, although
328 predominantly described as dsRNA sensors, can also bind ssRNA⁵⁶, indicating that
329 the IFN response following FH inhibition is likely driven by a mixture of dsRNA and
330 ssRNA species. It is notable that FHIN1 also reduced *Ddx58* but not *Iffih1* expression,
331 which may warrant further investigation (Extended Data Fig. 9b). The signalling events
332 downstream of RIG-I/MDA5 activation include mitochondrial antiviral signalling protein
333 (MAVS) oligomerisation, followed by recruitment and phosphorylation of TANK-
334 binding kinase 1 (TBK1). We observed MAVS oligomerisation and increased TBK1

335 phosphorylation with FHIN1 treatment (Fig. 4i, Extended Data Fig. 9d). Intriguingly,
336 MAVS knockout did not impair the induction of IFN- β by FHIN1 (Extended Data Fig.
337 9e), perhaps indicating that compensatory TLR7 signalling is sufficient to drive type I
338 IFN following FH inhibition with chronic MAVS deficiency.

339

340 Previously, we demonstrated that FH inhibition causes mitochondrial stress (Fig. 2).
341 Changes in MMP have previously been correlated with increased type I IFN release⁵⁷,
342 thus we hypothesised that disturbances in MMP may be linked to mtRNA release and
343 IFN- β induction following FH inhibition. To support this, we induced changes in MMP
344 by using the ATP synthase inhibitor oligomycin A, which boosted MMP, the K⁺
345 ionophore valinomycin A, which non-significantly reduced MMP, or the uncoupler
346 CCCP, which significantly dissipated MMP (Extended Data Fig. 9f, h). All treatments
347 boosted LPS-driven IFN- β release, akin to FHIN1 (Extended Data Fig. 9g, h). MMF,
348 which does not increase LPS-induced IFN- β expression (Fig. 4c), did not affect MMP
349 (Extended Data Fig. 9i). Oligomycin treatment led to an accumulation of dsRNA to a
350 similar extent to that observed in cells treated with FHIN1 or transfected with dsRNA
351 (poly (I:C)), and increased mtRNA release into the cytosol (Extended Data Fig. 9j-l).
352 Valinomycin treatment similarly drove dsRNA accumulation (Extended Data Fig. 9m,
353 n), indicating that MMP-altering compounds induce an accumulation of mtRNA. As we
354 also observed an increase in cytosolic mtDNA levels following oligomycin treatment
355 (Extended Data Fig. 9l), it is still possible that IFN responses following
356 oligomycin/valinomycin/CCCP treatment are not exclusively driven by mtRNA. mtRNA
357 release from chondrocytes has recently been implicated in activating the immune
358 response and promoting osteoarthritis⁵⁸. As such, mitochondrial damage and nucleic
359 acid release are emerging as key pathogenic processes that may underlie many
360 immune-mediated diseases.

361

362 Tamoxifen-inducible *Fh1*^{-/-} BMDMs released more IFN- β upon LPS stimulation than
363 their *Fh1*^{+/+} counterparts (Fig. 4j). We also detected increased dsRNA accumulation in
364 *Fh1*^{-/-} BMDMs (Fig 4k, Extended Data Fig. 9o) which, coupled with the fact that
365 deletion of *Fh1* also drives mitochondrial membrane hyperpolarisation (Fig. 2h),
366 demonstrate that both genetic and pharmacological targeting of FH drive similar
367 mitochondrial retrograde type I IFN stress responses.

368

369 We next considered whether this response could be applied to an endogenous model
370 of LPS activation in the absence of pharmacological or genetic inactivation of FH.
371 Given LPS-induced FH suppression occurs predominantly during late-phase LPS
372 stimulation (24-48 h) (Fig. 1g), FH suppression at this time point may drive membrane
373 hyperpolarisation and the release of mtRNA. MMP was significantly increased
374 following 48 h LPS stimulation, but not following 4 h or 24 h stimulation (Extended
375 Data Fig. 10a). Although dsRNA did not accumulate following acute (4 h) LPS
376 stimulation (Extended Data Fig. 9j, k), we did observe increased dsRNA staining
377 following 24 h and 48 h LPS stimulation (Extended Data Fig. 10b, c). *Ddx58* and *Ifih1*
378 expression is LPS-inducible (Extended Data Fig. 10d), which may suggest that RIG-
379 I/MDA5 signalling is required during LPS stimulation. Indeed, silencing of *Ddx58* and
380 *Ifih1* reduced both 24 h and 48 h LPS-induced *Ifnb1* expression (Fig. 4l), indicating
381 that *Ifnb1* transcription during late-phase LPS stimulation is maintained by mtRNA
382 release. These results demonstrate that the mitochondrial retrograde type I IFN
383 response, which we initially unmasked by pharmacologically or genetically targeting
384 FH during early LPS signalling, is active endogenously during late-phase LPS
385 activation with potential implications for chronic inflammation, for example during
386 ageing⁵⁹.

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388 To determine whether FH inhibition leads to similar effects *in vivo*, we injected mice
389 with FHIN1 or DMF prior to administration of LPS, and measured IFN- β release into
390 the serum. FHIN1 increased LPS-induced IFN- β release, while DMF had no effect
391 (Fig. 4m), indicating that FH inhibition leads to a similar IFN response *in vivo* which
392 may have effects on bystander cells. We also treated human PBMCs with FHIN1 or
393 DMF prior to LPS stimulation and observed similar effects, as FHIN1 boosted, while
394 DMF suppressed LPS-induced IFN- β release (Fig. 4n).

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396 We hereby describe a mitochondrial retrograde signalling pathway leading from FH
397 inhibition to mitochondrial membrane hyperpolarisation and mtRNA release
398 (Supplementary Fig. 1). Mitochondrial stress may be an underlying mechanism that
399 contributes to type I IFN release in interferonopathies such as systemic lupus
400 erythematosus (SLE). It has previously been demonstrated that PBMCs from SLE
401 patients have impaired mitochondrial function and altered MMP^{60,61}. We therefore
402 examined *FH* expression in the whole blood of SLE patients and found significant

403 suppression of *FH* compared to healthy control samples (Fig. 4o). Autoantibodies to
404 dsRNA, as well as dsDNA, have been detected in SLE patients^{62,63}. However, it is
405 unclear whether *FH* suppression is a cause or consequence of increased IFN
406 signalling, as *Fh1* can also be inhibited by IFN- β stimulation in BMDMs (Extended
407 Data Fig. 10e). A negative feedback loop may exist whereby suppression of *FH* leads
408 to type I IFN release, which feeds back to further suppress *FH*. *FH* suppression has
409 previously also been linked to multiple sclerosis progression⁶⁴ and, in parallel to our
410 work, has been shown to promote a type I IFN response in kidney epithelial cells and
411 HLRCC tumours (Zecchini, Paupe *et al.*, under revision). This study and ours implicate
412 roles for *FH* in nucleic acid release, which may contribute to inflammation-driven
413 tumorigenesis and as a potential host defence mechanism in the context of viral
414 infection. Finally, the recent demonstration of aberrant dsRNA editing due to ADAR1
415 deficiency leading to MDA5 activation as a mechanism of common inflammatory
416 diseases also points to the clinical relevance of endogenously produced dsRNA,
417 suggesting that targeting this pathway may yield novel anti-inflammatory strategies⁶⁵.

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635 **Figure Legends**

636

637 **Figure 1 – LPS stimulation drives fumarate accumulation via glutamine**
638 **anaplerosis and an aspartate-argininosuccinate shunt**

639 Metabolite abundance (**a,d**) and bioenergetic ratios (**b**) in non-stimulated (NS) versus
640 LPS-stimulated BMDMs ($n = 3$; LPS 4 h; argininosuccinate ($P=0.000044$), fumarate
641 ($P=0.000141$), malate ($P=0.000219$)). **c**, Respirometry as measured by oxygen
642 consumption rate (OCR) of NS and LPS-stimulated BMDMs ($n = 6$ (NS); $n = 8$ (LPS);
643 LPS 4 h). n = technical replicates from 1 experiment performed with 3 pooled biological
644 replicates. Data are mean \pm s.d. **e**, *Ass1* and *Fh1* gene expression with LPS time-
645 course ($n = 9$; 24 h ($P=0.000729$), 48 h ($P=0.000001$)). **f**, Quantitative proteomics of
646 aspartate-argininosuccinate shunt enzymes in NS and LPS-stimulated BMDMs ($n = 4$,
647 LPS 24 h; ASS1 ($P=0.000156$)). **g**, FH protein levels with LPS time-course ($n = 1$). **h**,
648 Fumarate levels following LPS stimulation with or without aminooxyacetic acid (AOAA)
649 pre-treatment (1 h) ($n = 6$, LPS 4 h). **i**, Schematic of metabolic changes occurring
650 during early-phase TCA cycle rewiring. Created with BioRender.com. **b,d-f,h**, Data
651 are mean \pm s.e.m. n = biological replicates unless stated otherwise. P values
652 calculated using two-tailed Student's t -test for paired comparisons or one-way analysis
653 of variance (ANOVA) for multiple comparisons.

654

655 **Figure 2 – FH inhibition increases bioenergetic stress, fumarate levels and**
656 **mitochondrial membrane potential**

657 Bioenergetic ratios (**a**) and heatmap of top 50 differentially abundant metabolites (**c**)
658 in BMDMs pre-treated with vehicle (DMSO), FH inhibitor (FHIN1) or dimethyl fumarate
659 (DMF) ($n = 3$; LPS 4 h; ATP/ADP ($P=0.000004$), phosphocreatine/creatine
660 ($P=0.00000001$)). **b**, Respirometry of BMDMs pre-treated with DMSO, FHIN1 or DMF
661 ($n = 8$; LPS 4 h). n = technical replicates from 1 experiment performed with 3 pooled
662 biological replicates. Data are mean \pm s.d. **d**, PCA plot of metabolomics in BMDMs
663 pre-treated with DMSO, FHIN1 or DMF or ($n = 3$; LPS 4 h). **e**, Fumarate levels in
664 BMDMs pre-treated with DMSO or FHIN1 ($n = 9$; LPS 4 h). **f**, Fumarate and 2SC levels
665 in *Fh1^{+/+}* and *Fh1^{-/-}* BMDMs ($n = 3$; 96 h EtOH/TAM; LPS 4 h). **g**, Mean fluorescence
666 intensity (MFI) of CellROX staining in BMDMs pre-treated with DMSO, FHIN1 or DMF
667 ($n = 3$ (CellROX); $n = 4$ (TMRM); LPS 4 h). **h**, MFI of TMRM staining in BMDMs pre-
668 treated with DMSO, FHIN1 or DMF or *Fh1^{+/+}* and *Fh1^{-/-}* BMDMs ($n = 4$
669 (DMSO/FHIN1/DMF); $n = 3$ (*Fh1^{+/+}* and *Fh1^{-/-}*); 72 h EtOH/TAM; LPS 4 h). **i**,
670 Aconitate/citrate ratio following LPS stimulation with or without FHIN1 or DMF pre-
671 treatment ($n = 3$; LPS 4 h). **j**, GSH and GSSG levels following LPS stimulation with or
672 without FHIN1 or DMF pre-treatment ($n = 3$; LPS 4 h). **a,e-j** Data are mean \pm s.e.m. n
673 = biological replicates unless stated otherwise. P values calculated using two-tailed
674 Student's t -test for paired comparisons or one-way or ANOVA for multiple
675 comparisons.

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680 **Figure 3 – FH activity is required to maintain appropriate cytokine responses**

681 GSEA (a) and overrepresentation analysis (ORA) (b) of RNAseq in BMDMs pre-
682 treated with FHIN1 or DMSO ($n = 3$; LPS 4 h). c, IL-10 and TNF- α release from DMSO-
683 FHIN1- or DMF-pre-treated BMDMs ($n = 6$; LPS 4 h; FHIN1/IL-10 ($P=0.0000024$),
684 DMF/IL-10 ($P=0.0000018$), FHIN1/TNF ($P=0.000001$)). d, *Il10* and *Tnfa* expression in
685 DMSO- or MMF-pre-treated BMDMs ($n = 3$; LPS 4 h). e, Enrichment map plot of
686 shared significantly decreased genes in FHIN1- and DMF-pre-treated BMDMs ($n = 3$;
687 LPS 4 h). f, *Il10* expression in DMSO- FHIN1- or DMF-pre-treated BMDMs in the
688 presence of NAC ($n = 3$; LPS 4 h). g, c-Fos activity in DMSO- FHIN1- or DMF-pre-
689 treated BMDMs ($n = 3$; LPS 4 h; DMF ($P=0.0000298$)). h, TNF- α release from BMDMs
690 pre-treated with anti-CD210 antibody (1 h) ($n = 4$; LPS 4 h). Western blot for STAT3
691 and phospho-STAT3 (i) and TNF- α release (j) from DMSO- FHIN1- or DMF-pre-
692 treated BMDMs and co-treated with IL-10 ($n = 3$, LPS 4 h; DMF ($P=0.000163$)). k, *Il10*
693 expression and IL-10 release in *Fh1*^{+/+} and *Fh1*^{-/-} ($n = 5$ or 2)/*Fh1*^{+/-} ($n = 2$) BMDMs
694 (EtOH/TAM 72 h; LPS 4 h; *Il10* ($P= 0.000055$)). l, TNF- α release from *Fh1*^{+/+} and *Fh1*^{-/-}
695 ($n = 5$)/*Fh1*^{+/-} ($n = 2$) BMDMs (EtOH/TAM 72 h; LPS 4 h). m, *IL10* and *TNFA*
696 expression in DMSO- or FHIN1-pre-treated human PBMCs ($n = 8$, LPS 4 h; FHIN1
697 ($P=0.00000008$)). n, *IL10* and *TNFA* expression in DMSO- or FHIN1- pre-treated
698 human macrophages ($n = 3$, LPS 4 h; FHIN1 ($P=0.000028$)). c,d,f-h,j-n Data are mean
699 \pm s.e.m. i, 1 representative blot of 3 shown. $n =$ biological replicates. P values
700 calculated using two-tailed Student's t-test for paired comparisons or one-way ANOVA
701 for multiple comparisons.

702

703 **Figure 4 – FH impairment triggers IFN- β release via a mtRNA-driven retrograde**
704 **response**

705 a, Volcano plot of DMSO- or FHIN1-pre-treated BMDMs ($n = 3$; LPS 4 h). b, IFN- β
706 release from DMSO-, FHIN1- or DMF-pre-treated BMDMs ($n = 6$; LPS 4 h; FHIN1
707 ($P=0.000004$)). c, *Ifnb1* in DMSO- or MMF-pre-treated BMDMs ($n = 3$; LPS 4 h). d,
708 IFN- β release from BMDMs treated with EtBr (6 days), before pre-treatment with
709 DMSO or FHIN1 ($n = 6$; LPS 4 h). e, Cytosolic *D-loop* in DNA and RNA in DMSO- or
710 FHIN1- pre-treated BMDMs ($n = 4$ for mtDNA, $n = 5$ for mtRNA; LPS 4 h). f, dsRNA in
711 DMSO- or FHIN1-pre-treated BMDMs ($n = 3$; LPS 4 h). Scale bar = 20 μ m. g, *Ifnb1*
712 with *Tlr7* silencing in DMSO- or FHIN1-pre-treated BMDMs ($n = 3$; LPS 4 h). h, IFN- β
713 with *Ddx58* or *Ifih1* silencing in DMSO- or FHIN1-pre-treated BMDMs ($n = 7$; LPS 4 h).
714 i, MAVS in DMSO- or FHIN1-pre-treated BMDMs ($n = 3$; LPS 4 h). j, IFN- β in *Fh1*^{+/+}
715 and *Fh1*^{-/-} BMDMs ($n = 3$, EtOH/TAM 72 h; LPS 4 h). k, dsRNA in *Fh1*^{+/+} and *Fh1*^{-/-}
716 BMDMs ($n = 3$; EtOH/TAM 72 h; LPS 4 h). Scale bar = 20 μ m. l, *Ifnb1* with *Ddx58* or
717 *Ifih1* silencing ($n = 3$). m, Serum IFN- β of FHIN1- or DMF-treated mice prior to PBS or
718 LPS injection ($n = 5$ (PBS); $n = 10$ (FHIN1/LPS); $n = 11$ (Vehicle/LPS); $n = 12$
719 (DMF/LPS)). n, IFN- β release from DMSO-, FHIN1- or DMF-pre-treated human
720 PBMCs ($n = 3$; LPS 4 h). o, *FH* in whole blood from healthy controls and SLE patients
721 ($n = 30$; $P=0.0000005$). b-e,g,h,j,i-o, Data are mean \pm s.e.m. f,i,k, 1 representative
722 blot or image of 3 experiments shown. $n =$ biological replicates. P values calculated
723 using two-tailed Student's t-test for paired comparisons, one-way ANOVA for multiple
724 comparisons.

725

726

727 **Materials and Methods**

728

729 **Animal Details**

730 All mice were on a C57BL/6J0laHsd background unless stated below. Wild-type
731 (WT) mice were bred in-house. The inducible *Fh1^{+fl}* and *Fh1^{fl/fl}* mice were generated
732 on the C57BL/6 genetic background and their hind legs were generously donated by
733 Dr. Christian Frezza (University of Cambridge, UK). Vehicle (ethanol) treated *Fh1^{+/+}*
734 and *Fh1^{-/-}* were used as controls. Upon treatment with 4-hydroxy tamoxifen, Cre-
735 mediated chromatin excision results in the loss of either one (*Fh1^{+/-}*) or both (*Fh1^{-/-}*)
736 copies of *Fh1*, thus generating either heterozygous or null animals. Hind legs from
737 WT and *Mavs^{-/-}* mice were generously donated by Dr Cecilia Johansson (Imperial
738 College London, UK). These strains, originally obtained from S. Akira (World Premier
739 International Immunology Frontier Research Center, Osaka University, Osaka,
740 Japan), were *Ifna6^{flp/+}* but since *Ifna6* expression was not a primary readout the mice
741 are designated as WT and *Mavs^{-/-}*. *In vitro* experiments were performed with BMDMs
742 isolated from 6-18-week-old female and male mice. Although we did not use
743 statistical methods to calculate sample size, we decided to use a minimum of 3
744 biological replicates per experiment to account for biological variability, considering
745 the 3 Rs principle and the fact that most experiments were performed in primary
746 murine macrophages from inbred mice. All *in vitro* treatment groups were randomly
747 assigned. *In vitro* and *in vivo* experiments were not blinded due to lack of available
748 experimenters with required expertise. *In vivo* models were performed with 6-week-
749 old male mice and littermates were randomly assigned to experimental groups.
750 Animals were maintained under specific pathogen-free conditions in line with Irish
751 and European Union regulations. All animal procedures were ethically approved by
752 the Trinity College Dublin Animal Research Ethics Committee prior to
753 experimentation and conformed with the Directive 2010/63/EU of the European
754 Parliament.

755

756 **Generation of Murine BMDMs**

757 6-18-week-old mice were euthanised in a CO₂ chamber, and death was confirmed by
758 cervical dislocation. Bone marrow was subsequently harvested from the tibia, femur
759 and ilium and cells were differentiated in DMEM containing L929 supernatant (20%),
760 foetal calf serum (FCS) (10%), and penicillin/streptomycin (1%) for 6 days, after which

761 cells were counted and plated at 0.5×10^6 cells/ml unless otherwise stated. BMDMs
762 were plated in 12-well cell culture plates and left overnight to adhere.

763

764 **Isolation of Human PBMCs**

765 Human blood samples from healthy donors were collected and processed at the
766 School of Biochemistry and Immunology in TBSI (TCD). Blood samples were obtained
767 anonymously and written informed consent for the use of blood for research purposes
768 has been obtained from the donors. All the procedures involving experiments on
769 human samples have been approved by the School of Biochemistry and Immunology
770 Research Ethics Committee (TCD). Experiments were conducted according to the
771 TCD guide on good research practice, which follows the guidelines detailed in the
772 National Institutes of Health Belmont Report (1978) and the Declaration of Helsinki.
773 30 ml whole blood was layered on 20 ml Lymphoprep (Axis-Shield), followed by
774 centrifugation for 20 mins at $400 \times g$ with the brake off, after which the upper plasma
775 layer was removed and discarded. The layer of mononuclear cells at the plasma-
776 density gradient medium interface was retained, and 20 ml PBS was added. Cells
777 were centrifuged for 8 mins at $300 \times g$ and the resulting supernatant was removed and
778 discarded. The remaining pellet of mononuclear cells was resuspended, counted, and
779 plated at 1×10^6 cells/ml in RPMI supplemented with FCS (10%) and
780 penicillin/streptomycin (1%).

781

782 **Generation of Human Macrophages**

783 PBMCs were taken, and CD14⁺ monocytes were isolated using MagniSort Human
784 CD14 Positive Selection Kit (Thermo Fisher) according to the manufacturer's protocol.
785 CD14 Monocytes were then differentiated in T-175 flasks in RPMI containing FCS
786 (10%), penicillin/streptomycin (1%) and recombinant human M-CSF (1:1000). After 6
787 days, the supernatant was discarded, cells were scraped and counted, and human
788 monocyte-derived macrophages (hMDMs) were plated in 12-well plates at 1×10^6
789 cells/mL RPMI containing FCS (10%) and penicillin/streptomycin (1%).

790

791 **Whole Blood Isolation from SLE Patients**

792 All SLE patients (as per ACR diagnostic criteria) were recruited from Cedars-Sinai
793 Medical Center, CA, USA. Age- and sex-matched healthy donors who had no history
794 of autoimmune diseases or treatment with immunosuppressive agents were included.

795 All participants provided informed written consent and the study received prior
796 approval from the institutional ethics review board (IRB protocol. 19627). Blood was
797 collected into PAXgene RNA tubes (2.5 mL blood + 6.9 mL buffer) and stored at -
798 80°C. Before isolation of RNA, the tubes were thawed at room temperature for 16 h.
799 Total RNA was isolated using the PAXgene Blood RNA Kit according to
800 manufacturer's recommendations (PreAnalytiX GmbH, 08/2005, REF: 762174).

801

802 **Reagents**

803 LPS from *Escherichia coli*, serotype EH100 (ALX-581-010-L001), was purchased from
804 Enzo Life Sciences. High molecular weight poly (I:C) (tlrl-pic) and 2'-3'-cGAMP (tlrl-
805 nacga23) were purchased from Invivogen. Recombinant mouse IFN- β 1 (581302) and
806 recombinant mouse IL-10 (417-ML-005/CF) were purchased from Biolegend. ATP
807 disodium salt (A2383), dimethyl sulfoxide (DMSO) (D8418), aminooxyacetic acid
808 (AOAA) (C13408), valinomycin (V3639), 4-hydroxytamoxifen (H6278) and N-acetyl
809 cysteine (NAC) (A7250) were purchased from Sigma Aldrich. Oligomycin A from
810 *Streptomyces diastatochromogenes* (M02220) was purchased from Fluorochem.
811 Fumarate hydratase-IN-1 (FHIN1) (HY-100004), dimethyl fumarate (DMF) (HY-
812 17363), monomethyl fumarate (MMF) (HY-103252), IMT1 (HY-134539) and C-178
813 (HY-123963) were purchased from MedChemExpress. CPG ODN 1826 (130-100-
814 274) and ODN 2088 (130-105-815) were purchased from Miltenyi Biotec. CCCP
815 (M20036) was purchased from Thermo Fisher.

816

817 **Compound Treatments**

818 All compounds used DMSO as a vehicle except for 4-hydroxy tamoxifen (EtOH), NAC
819 (PBS), and AOAA for tracing experiments (Media). LPS was used at a concentration
820 of 100 ng/mL for indicated timepoints (2, 3, 4, 6, 8, 24, 48 h). FHIN1 (10 or 20 μ M),
821 MMF (50 or 100 μ M), DMF (25 μ M), AOAA (5 mM), oligomycin (10 μ M), CCCP (50
822 μ M) NAC (1 mM), and IMT1 (10 μ M) pre-treatments were performed for 3 h prior the
823 addition of LPS. Cells were treated with valinomycin (10 nM) 15 mins before LPS
824 stimulation. Anti-CD210 or IgG control (10 μ g/ml) antibodies were added to cells 1 h
825 prior to LPS stimulation. Recombinant mouse IL-10 protein (100 ng/ml) was added to
826 cells at the same time as LPS. Cells were treated with IFN- β 1 (220 ng/ml) for 3 h. Cells
827 were treated with C-178 (1 μ M) 1 h prior to LPS stimulation or transfection with 2'-3'-
828 cGAMP (1.5 μ g/ml) for 4 hrs to achieve cGAS-STING activation. Cells were treated

829 with ODN 2088 (1 μ M) for 1 hr prior to LPS stimulation or transfection with CPG ODN
830 1826 (1.5 μ g/ml) to achieve TLR9 activation. 3 different timepoints of 4-hydroxy
831 tamoxifen (TAM) (600 nM or 2 μ M) or EtOH treatment were performed- these are
832 specified in the individual figure legends. For '48 h' treatments EtOH/TAM was added
833 on day 5 of 6 during the BMDM differentiation protocol. On day 6 they were plated with
834 EtOH/TAM (left overnight) and treated the following day. For '72 h' treatments
835 EtOH/TAM was added on day 4 of 6 during the BMDM differentiation protocol. On day
836 6 they were plated with EtOH/TAM (left overnight) and treated the following day. For
837 '96 h' treatments EtOH/TAM was added on day 4 of 6 during the BMDM differentiation
838 protocol. On day 6 they were plated with EtOH/TAM and treated 2 days later.

839

840

841 **Antibodies**

842 Working dilutions of antibodies were 1/1000 unless otherwise stated. Anti-mouse
843 Lamin B1 (12586), STAT1 (9172), p-STAT1 (9167), JAK1 (3344), p-JAK1 (3331),
844 TBK1 (3504), p-TBK1 (5483), STAT3 (30835), p-STAT3 (9145), FH (4567), ASS1
845 (70720), α -tubulin (2144), α -tubulin (3873), MAVS (4983), ATF4 (11815), p-AKT
846 (13038), AKT (2920), p-JNK (9255), JNK (9252), p-ERK1/2 (9101), ERK1/2 (4695), p-
847 p38 (4511), p-38 (9212), TRAF3 (4729), p-p65 (3033) and GAPDH (2118) antibodies
848 were purchased from Cell Signaling. Anti-goat IL-1 β (AF-401-NA) was purchased from
849 R&D. Anti-2SC antibody was kindly provided by Dr. Norma Frizzell (University of South
850 Carolina, US). Anti-mouse β -actin antibody (1/5000) (A5316) was purchased from
851 Sigma Aldrich. Horseradish peroxidase (HRP)-conjugated anti-mouse (115-035-003),
852 anti-goat (705-035-003) and anti-rabbit (111-035-003) immunoglobulin G (IgG)
853 antibodies (all 1/2000) were purchased from Jackson ImmunoResearch. Anti-mouse
854 CD210 (112710) and anti-mouse IgG (406601) antibodies (both 10 μ g/ml) were
855 purchased from Biolegend. Anti-dsRNA antibody (clone rJ2, 1/60) was purchased from
856 Merck (MABE-1134). Alexa Fluor 488 goat anti-mouse IgG1 antibody (A21121) was
857 purchased from Invitrogen. Details of antibody validation are given in Table S1.

858

859 **RT-qPCR**

860 RNA extraction from cells was carried out using a PurelinkTM RNA kit (Invitrogen)
861 according to the manufacturer's instructions. BMDMs were treated as required, and

862 following treatment were instantly lysed in 350 μ l RNA lysis buffer. Isolated RNA was
863 quantified using a NanoDrop 2000 spectrophotometer, and RNA concentration was
864 normalised to the lowest concentration across all samples with RNase-free water. If
865 necessary, samples were DNase-treated after quantification using DNase I (Thermo
866 Fisher) according to the manufacturer's instructions. Isolated RNA samples were
867 normalised and converted into cDNA using the High-Capacity cDNA Reverse
868 Transcription Kit (Thermo Fisher) according to manufacturer's instructions. 10 μ l of
869 RNA (at a maximum concentration of 100 ng/ μ l) was added to 10 μ l of reverse
870 transcription master mix to complete the reaction mixture. Real-time quantitative PCR
871 was performed on the cDNA generated in the previous step, using primers designed
872 in-house and ordered from Eurofins Genomics, as detailed in Table S2. The reaction
873 was performed in a 96-well qPCR plate by a 7500 Fast Real-Time PCR machine
874 (Thermo Fisher). Relative expression ($2^{-\Delta\Delta CT}$) was calculated from the C_T values for
875 each sample and gene of interest.

876

877 **RNA Interference (RNAi)**

878 Pre-designed silencer select siRNAs for *Cgas* (s103166), *Tmem173* (s91058), *Tlr3*
879 (s100579), *Tlr9* (s96268), *Asl* (s99640), *Tlr7* (s100720), *Ddx58* (s106376), *Ifih1*
880 (s89787), *Nrf2* (s70522), *Aff4* (s62689) and negative control (4390843) were ordered
881 from Thermo Fisher. siRNA sequences are given in Table S2. Cells were transfected
882 with 50 nM siRNA using 5 μ l lipofectamine RNAiMAX according to manufacturer's
883 instructions (Thermo Fisher). Cells were transfected in medium without serum and
884 antibiotics which was replaced with complete medium 8 hours later. Cells were
885 subsequently left for at least a further 12 hours prior to treatment.

886

887 **Immunofluorescence**

888 Cells were plated on 20 mm cover slips in 12-well plates. Cells were treated as
889 required and Mitotracker Red CMXRos (100 nM, Thermo Fisher), was added to
890 medium 30 mins prior to end of cell treatments. After 30 min incubation, cells were
891 washed three times with warm PBS. Cells were subsequently fixed for 10 mins with
892 4% paraformaldehyde/PBS at 37°C. Cells were washed three times with PBS and
893 permeabilized for 1 hour in block solution (1% BSA, 22.52 mg/ml glycine, 0.1% tween
894 20 in PBS). Anti-dsRNA antibody (Merck) was diluted 1/60 in block solution and
895 incubated with cells overnight at room temperature. Cells were washed three times

896 with PBS for 5 mins/wash. A mix containing AF488-conjugated goat anti-mouse IgG1
897 antibody (1/1000) and DAPI (1/1000, Thermo Fisher) was subsequently added to cells
898 for 90 mins at room temperature in the dark. Cells were subsequently washed three
899 times with PBS for 5 mins/wash. Cover slips were mounted onto microscope slides
900 using 10-20 μ l ProLong Gold antifade reagent (Thermo Fisher). Slides were imaged
901 using a Leica SP8 scanning confocal microscope using 20.0 \times objective. Images were
902 analysed using the LAS X Life Science Microscope Software Platform (Leica). The
903 same microscope instrument settings were used for all samples and all images were
904 analysed using the same settings. Scale bars = 20 μ m. Quantification of dsRNA or
905 Mitotracker Red CMXRos signal intensity was performed using the measure function
906 in ImageJ 1.53t (NIH). Mean signal intensity was calculated for individual cells in single
907 colour images and displayed relative to signal intensity of control cells.

908

909 **Flow Cytometry**

910 Cells were plated in 12-well plates and treated as desired. CellROX Green (5 μ M,
911 Thermo Fisher) or TMRM (20 nM, Thermo Fisher) was added to cells 30 mins prior to
912 end of cell treatments. Cells were washed once in PBS and scraped into 200 μ l FACS
913 buffer (2 mM EDTA, 0.5% FCS in PBS). Acquisition of samples was performed on a
914 BD Accuri C6 flow cytometer. The gating strategy used for all flow cytometry
915 experiments consisted of debris exclusion by FSC-A vs SSC-A analysis and
916 subsequent doublet exclusion by FSC-A vs FSC-H analysis. A sample gating strategy
917 is provided in Supplementary Fig. 2. 10,000 cells was acquired per condition. Mean
918 fluorescence intensity (MFI) was calculated for all cells in each condition using FlowJo
919 v10.

920

921 **Liquid-Chromatography-Mass Spectrometry (LC-MS)**

922 **Steady-State Metabolomics**

923 BMDMs (3 independent mice) were plated at 0.5×10^6 cells/well in 12-well plates in
924 technical triplicate per condition, treated as indicated, snap frozen and stored at -80°C .
925 For metabolomics on cytosolic fraction, BMDMs were plated at 10×10^6 cells/10 cm
926 dish and rapid fractionation was performed as previously reported¹⁶. Metabolite
927 extraction solution (MES) (methanol/acetonitrile/water, 50:30:20 v/v/v) was added (0.5
928 mL per 1×10^6 cells) and samples were incubated for 15 min on dry ice. The resulting

929 suspension was transferred to ice-cold microcentrifuge tubes. Samples were agitated
930 for 20 min at 4°C in a thermomixer and then incubated at -20°C for 1 h. Samples were
931 centrifuged at maximum speed for 10 min at 4°C. The supernatant was transferred
932 into a new tube and centrifuged again at maximum speed for 10 min at 4°C. The
933 supernatant was transferred to autosampler vials and stored at -80°C prior to analysis
934 by LC-MS.

935

936 HILIC chromatographic separation of metabolites was achieved using a Millipore
937 Sequant ZIC-pHILIC analytical column (5 µm, 2.1 × 150 mm) equipped with a 2.1 ×
938 20 mm guard column (both 5 mm particle size) with a binary solvent system. Solvent
939 A was 20 mM ammonium carbonate, 0.05% ammonium hydroxide; Solvent B was
940 acetonitrile. The column oven and autosampler tray were held at 40°C and 4°C,
941 respectively. The chromatographic gradient was run at a flow rate of 0.200 mL/min as
942 follows: 0–2 min: 80% B; 2-17 min: linear gradient from 80% B to 20% B; 17-17.1 min:
943 linear gradient from 20% B to 80% B; 17.1-22.5 min: hold at 80% B. Samples were
944 randomized and analysed with LC–MS in a blinded manner and the injection volume
945 was 5 µl. Pooled samples were generated from an equal mixture of all individual
946 samples and analysed interspersed at regular intervals within sample sequence as a
947 quality control. Metabolites were measured with a Thermo Scientific Q Exactive Hybrid
948 Quadrupole-Orbitrap Mass spectrometer (HRMS) coupled to a Dionex Ultimate 3000
949 UHPLC or with Vanquish Horizon UHPLC coupled to an Orbitrap Exploris 240 mass
950 spectrometer (both Thermo Fisher Scientific) via a heated electrospray ionization
951 source.

952

953 For Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap Mass spectrometer
954 (HRMS) coupled to a Dionex Ultimate 3000 UHPLC, the mass spectrometer was
955 operated in full-scan, polarity-switching mode, with the spray voltage set to +4.5 kV/-
956 3.5 kV, the heated capillary held at 280°C and the heated electrospray ionization probe
957 held at 320°C. The sheath gas flow was set to 40 units, the auxiliary gas flow was set
958 to 15 units, and the sweep gas flow was set to 0 unit. HRMS data acquisition was
959 performed in a range of $m/z = 70-900$, with the resolution set at 70,000, the AGC
960 target at 1×10^6 , and the maximum injection time (Max IT) at 120 ms. Metabolite
961 identities were confirmed using two parameters: (1) precursor ion m/z was matched
962 within 5 ppm of theoretical mass predicted by the chemical formula; (2) the retention

963 time of metabolites was within 5% of the retention time of a purified standard run with
964 the same chromatographic method. Chromatogram review and peak area integration
965 were performed using the Thermo Fisher software XCalibur Qual Browser, XCalibur
966 Quan Browser software and Tracefinder 5.0 and the peak area for each detected
967 metabolite was normalized against the total ion count (TIC) of that sample to correct
968 any variations introduced from sample handling through instrument analysis. Absolute
969 quantification of 2SC was performed by interpolation of the corresponding standard
970 curve obtained from serial dilutions of commercially available standards (Sigma
971 Aldrich) running with the same batch of samples.

972

973 For the Orbitrap Exploris 240 mass spectrometer, MS1 scans, mass range was set to
974 $m/z=70-900$, AGC target set to standard and maximum injection time (IT) set to auto.
975 Data acquisition for experimental samples used full scan mode with polarity switching
976 at an Orbitrap resolution of 120000. Data acquisition for untargeted metabolite
977 identification was performed using the AcquireX Deep Scan workflow, an iterative
978 data-dependent acquisition (DDA) strategy using multiple injections of the pooled
979 sample. In brief, sample was first injected in full scan-only mode in single polarity to
980 create an automated inclusion list. MS2 acquisition was then carried out in triplicate,
981 where ions on the inclusion list were prioritized for fragmentation in each run, after
982 which both the exclusion and inclusion lists were updated in a manner where
983 fragmented ions from the inclusion list were moved to exclusion list for the next run.
984 DDA full scan-ddMS2 method for AcquireX workflow used the following parameters:
985 full scan resolution was set to 60000, fragmentation resolution to 30000, fragmentation
986 intensity threshold to $5.0e3$. Dynamic exclusion was enabled after 1 time and exclusion
987 duration was 10s. Mass tolerance was set to 5ppm. Isolation window was set to 1.2
988 m/z . Normalized HCD collision energies were set to stepped mode with values at 30,
989 50, 150. Fragmentation scan range was set to auto, AGC target at standard and max
990 IT at auto. Xcalibur AcquireX method modification was on. Mild trapping was enabled.

991

992 Metabolite identification was performed in the Compound Discoverer software (v 3.2,
993 Thermo Fisher Scientific). Metabolites were annotated at the MS2 level using both an
994 in-house mzVault spectral database curated from 1051 authentic compound
995 standards and the online spectral library mzCloud. The precursor mass tolerance was
996 set to 5 ppm and fragment mass tolerance set to 10 ppm. Only metabolites with

997 mzVault or mzCloud best match score above 50% and 75%, respectively, and RT
998 tolerance within 0.5 min to that of a purified standard run with the same
999 chromatographic method were exported to generate a list including compound names,
1000 molecular formula and RT. The curated list was then used for further processing in the
1001 Tracefinder software (v 5.0, Thermo Fisher Scientific), where extracted ion
1002 chromatographs for all compounds were examined and manually integrated if
1003 necessary. False positive, noise or chromatographically unresolved compounds were
1004 removed. The peak area for each detected metabolite was then normalized against
1005 the total ion count (TIC) of that sample to correct any variations introduced from
1006 sample handling through instrument analysis. The normalized areas were used as
1007 variables for further statistical data analysis. Statistical analysis was performed using
1008 MetaboAnalyst 5.0⁶⁶.

1009

1010 **Stable isotope-assisted tracing**

1011 BMDMs (3 independent mice) were plated at 0.5×10^6 cells/well in 12-well plates in
1012 technical triplicate per condition, treated as indicated in glutamine-free DMEM
1013 supplemented with U-¹³C-glutamine or ¹⁵N₂-glutamine, respectively. For ¹³C- and ¹⁵N-
1014 tracing analysis, the theoretical masses of ¹³C and ¹⁵N isotopes were calculated and
1015 added to a library of predicted isotopes in Tracefinder 5.0. These masses were then
1016 searched with a 5-ppm tolerance and integrated only if the peak apex showed less
1017 than 1% deviation in retention time from the [U-¹²C or ¹⁴N] monoisotopic mass in the
1018 same chromatogram. The raw data obtained for each isotopologue were corrected for
1019 natural isotope abundances using the AccuCor algorithm
1020 (<https://github.com/lparsons/accucor>) before further statistical analysis.

1021

1022 **EtBr Treatment**

1023 BMDMs were plated in the presence or absence of ultrapure ethidium bromide (100
1024 ng/ml) and incubated for a further 6 days prior to treatment. Depletion of mtDNA was
1025 determined by genomic DNA isolation followed by qPCR using primers specific for
1026 areas of mitochondrial DNA (D-loop) and areas of mtDNA that are not inserted into
1027 nuclear DNA (Non-NUMT).

1028

1029 **c-Fos Activity Assay**

1030 BMDMs from 3 mice were plated in 10 cm dishes at 0.5×10^6 cells/mL and left
1031 overnight. Cells were pre-treated with FHIN1 or DMF (3 h) prior to LPS stimulation (4
1032 h). Upon harvesting, nuclear extracts were isolated using a Nuclear Extraction Kit
1033 (ab113474) purchased from Abcam. Nuclear extracts were quantified via BCA assay
1034 and standardised. c-Fos relative activity was then quantified using the AP1
1035 transcription factor assay purchased from Abcam (Ab207196) according to the
1036 manufacturers protocol.

1037

1038 **Fumarate Assay**

1039 Analysis of fumarate levels were assessed using a fumarate colorimetric assay kit
1040 (Sigma MAK060) that uses an enzyme assay, which results in a colorimetric (450 nm)
1041 product proportional to the fumarate present, as per manufacturer's instructions.

1042

1043 **Nitrite Measurement**

1044 The Griess Reagent System (Promega G2930) was used according to manufacturer's
1045 instructions.

1046

1047 **RNA Sequencing**

1048 BMDMs (3 independent mice) were treated as indicated and RNA was extracted as
1049 previously detailed. mRNA was extracted from total RNA using poly-T-oligo-attached
1050 magnetic beads. After fragmentation, the first strand cDNA was synthesized using
1051 random hexamer primers, followed by the second strand cDNA synthesis. The library
1052 was checked with Qubit and real-time PCR for quantification and bioanalyzer for size
1053 distribution detection. Quantified libraries were pooled and sequenced on the
1054 NovaSeq 6000 S4 (Illumina). Differential expression analysis of two conditions/groups
1055 was performed using counted reads and the DESeq2 R package⁶⁷. Pathway
1056 enrichment analyses were performed as indicated in quantification and statistical
1057 analysis.

1058

1059 **Proteomic Analysis**

1060 **Sample Preparation**

1061 BMDMs (from 5 independent mice) were plated onto 10-cm dishes and treated as
1062 indicated. At the experimental endpoint, cells were washed with PBS on ice and
1063 centrifuged at 1500 rpm for 5 mins at 4°C and frozen at -80°C. Cell pellets were lysed,

1064 reduced and alkylated in 50 μ l of 6M Gu-HCl, 200 mM Tris-HCl pH 8.5, 10 mM TCEP,
1065 15 mM Chloroacetamide by probe sonication and heating to 95°C for 5 min. Protein
1066 concentration was measured by a Bradford assay and initially digested with LysC
1067 (Wako) with an enzyme to substrate ratio of 1/200 for 4 h at 37 °C. Subsequently, the
1068 samples were diluted tenfold with water and digested with porcine trypsin (Promega)
1069 at 37°C overnight. Samples were acidified to 1% TFA, cleared by centrifugation
1070 (16,000 g at RT) and approximately 20 μ g of the sample was desalted using a Stage-
1071 tip. Eluted peptides were lyophilized, resuspended in 0.1% TFA/water and the peptide
1072 concentration was measured by A280 on a nanodrop instrument (Thermo). The
1073 sample was diluted to 2 μ g/ 5 μ l for subsequent analysis.

1074

1075 **MS Analysis**

1076 The tryptic peptides were analysed on a Fusion Lumos mass spectrometer connected
1077 to an Ultimate Ultra3000 chromatography system (both Thermo Scientific)
1078 incorporating an autosampler. 2 μ g of de-salted peptides were loaded onto a 50 cm
1079 emitter packed with 1.9 μ m ReproSil-Pur 200 C18-AQ (Dr Maisch, Germany) using a
1080 RSLC-nano uHPLC systems connected to a Fusion Lumos mass spectrometer (both
1081 Thermo, UK). Peptides were separated by a 140 min linear gradient from 5% to 30%
1082 acetonitrile, 0.5% acetic acid. The mass spectrometer was operated in DIA mode,
1083 acquiring a MS 350-1650 Da at 120k resolution followed by MS/MS on 45 windows
1084 with 0.5 Da overlap (200-2000 Da) at 30k with a NCE setting of 27.

1085

1086 **Data Analysis**

1087 Raw files were analysed and quantified by searching against the Uniprot *Mus*
1088 *Musculus* database using DIA-NN 1.8 (<https://github.com/vdemichev/DiaNN>). Library-
1089 free search was selected, and the precursor ion spectra were generated from the
1090 FASTA file using the deep learning option. Default settings were used throughout apart
1091 from using “Robust LC (high precision)”. In brief, Carbamidomethylation was specified
1092 as fixed modification while acetylation of protein N-termini was specified as variable.
1093 Peptide length was set to minimum 7 amino acids, precursor FDR was set to 1%.
1094 Subsequently, missing values were replaced by a normal distribution (1.8 π shifted
1095 with a distribution of 0.3 π) to allow the following statistical analysis. Protein-wise linear
1096 models combined with empirical Bayes statistics are used for the differential

1097 expression analyses. We use the Bioconductor package limma to carry out the
1098 analysis using the information provided in the experimental design table.

1099

1100 **Digitonin Fractionation**

1101 BMDMs were plated at 0.5×10^6 cells/well and treated as desired. After treatment,
1102 cells were washed once with room temperature PBS, before being scraped on ice into
1103 ice cold PBS and pelleted at $500 \times g$ for 5 mins at 4°C . Supernatant was removed and
1104 discarded, and the pellet was resuspended in $400 \mu\text{l}$ extraction buffer (150 mM NaCl,
1105 50 mM HEPES pH 7.4, 25 $\mu\text{g/ml}$ digitonin). Samples were then placed in a rotating
1106 mixer at 4°C for 10 mins before centrifugation at $2000 \times g$ at 4°C for 5 mins. The
1107 resulting supernatant constituted the cytosolic fraction, from which RNA and DNA were
1108 isolated using an AllPrep DNA/RNA Mini Kit (Qiagen). Alternatively, the cytosolic
1109 fraction was concentrated using Strataclean Resin (Agilent) and analysed by western
1110 blot. The pellet constituted a fraction containing membrane-bound organelles which
1111 was lysed in RNA lysis buffer for RNA isolation or lysed in western blot lysis buffer for
1112 analysis by western blot. To determine the presence of mtRNA and mtDNA in the
1113 cytosol, qPCR was performed using primers specific for mitochondrial D-loop on cDNA
1114 which had been reverse-transcribed from RNA isolated from the cytosolic fraction
1115 (mtRNA) and on DNA isolated from the cytosolic fraction (mtDNA). In both cases,
1116 values were normalised using a housekeeping control gene (β -actin) amplified in
1117 cDNA which had been reverse-transcribed from RNA isolated from the membrane-
1118 bound fraction.

1119

1120 **MAVS Oligomerisation**

1121 BMDMs were plated at 1×10^6 cells/well in technical triplicate and treated as desired.
1122 After treatment, cells were washed twice with $200 \mu\text{l}$ cold PBS before being lysed in
1123 crosslinking lysis buffer (50 mM HEPES, 0.5% triton X-100, 1X protease inhibitor
1124 cocktail). Samples were placed on ice for 15 mins. Lysates were centrifuged for 15
1125 mins at $6000 \times g$ at 4°C and the supernatant was removed and frozen down as the
1126 'soluble fraction.' $20 \mu\text{l}$ of the soluble fraction was mixed with $5 \mu\text{l}$ of sample lysis buffer
1127 (0.125 M Tris pH 6.8, 10% glycerol, 0.02% SDS, 5% DTT) and run on a 10% gel. The
1128 insoluble pellet was resuspended in HEPES (50 mM) and washed 3 times by
1129 centrifuging at $6000 \times g$ at 4°C and removing the supernatant each time. After the final
1130 wash, the pellet was resuspended in $500 \mu\text{l}$ crosslinking buffer (50 mM HEPES, 150

1131 mM NaCl) and disuccinimidyl suberate (DSS, Thermo Fisher, made up in anhydrous
1132 DMSO) was added to the final concentration of 2 mM. Immediately following the
1133 addition of DSS, the sample was inverted several times and incubated for 45 mins at
1134 37°C. The sample was then centrifuged for 15 mins at 6000 x g at 4°C, before the
1135 supernatant was removed and the pellet was resuspended in 30 µl sample lysis buffer.
1136 The resuspended 'insoluble fraction' was subsequently boiled for 5 mins at 95°C
1137 before being run on a gel.

1138

1139 **Seahorse XF Glycolysis Stress Test**

1140 Cells were plated at 100,000 cells/well in 100 µl and were left overnight to adhere.
1141 Protocol was carried out according to manufacturer's instructions (Agilent). In brief,
1142 cells were treated as required, after which medium was replaced with Seahorse
1143 medium containing glutamine (2 mM). Cells were then placed in a CO₂-free incubator
1144 for 1 hour. Glycolysis stress test was subsequently performed using a Seahorse
1145 XFe96 Analyzer (Agilent) with the following injections:

1146 A- Glucose (10 mM)

1147 B- Oligomycin (1 µM)

1148 C- 2-DG (50 mM)

1149 Analysis was performed using Seahorse Wave Software (Agilent). Data shown are
1150 representative experiments containing at least 3 pooled biological replicates.

1151

1152 **Seahorse XF Mito Stress Test**

1153 Cells were plated at 100,000 cells/well in 100 µl and were left overnight to adhere.
1154 Protocol was carried out according to manufacturer's instructions (Agilent). In brief,
1155 cells were treated as required, after which medium was replaced with Seahorse
1156 medium containing glutamine (2 mM), glucose (10 mM) and pyruvate (1 mM). Cells
1157 were then placed in a CO₂-free incubator for 1 hour. Mito stress test was subsequently
1158 performed using a Seahorse XFe96 Analyzer (Agilent) with the following injections:

1159 A- Oligomycin (1 µM)

1160 B- FCCP (1 µM)

1161 C- Rotenone (500 nM)

1162 Analysis was performed using Seahorse Wave Software (Agilent). Data shown are
1163 representative experiments containing at least 3 pooled biological replicates.

1164

1165 **LPS-induced Inflammation Model**

1166 6-week-old male mice were used, and littermates were randomly assigned to
1167 experimental groups. Compounds were resuspended in 10% DMSO followed by 90%
1168 cyclodextrin/PBS (20% w/v). Mice were injected intraperitoneally with vehicle, FHIN1
1169 or DMF (both 50 mg/kg) at a volume of 200 µl per injection. 1 hour later, mice were
1170 injected intraperitoneally with PBS or LPS from *E.coli* (2.5 mg/kg, Sigma) at a volume
1171 of 100 µl per injection. 2 hours later, mice were euthanised and blood was harvested
1172 retro-orbitally. Blood was allowed to clot for 30 mins at room temperature before it was
1173 centrifuged at 5000 x g for 10 mins at 4°C. The serum was removed and IFN-β
1174 concentration was determined by ELISA.

1175

1176 **Western Blotting**

1177 Supernatant was removed from cells following stimulation and lysates were harvested
1178 in 30-50 µl lysis buffer (0.125 M Tris pH 6.8, 10% glycerol, 0.02% SDS, 5% DTT)
1179 Lysates were subsequently heated to 95°C for 5 mins to denature proteins. SDS-
1180 PAGE was used to resolve proteins by molecular weight. Samples were boiled at 95°C
1181 for 5 mins prior to loading into a 5% stacking gel. The percentage resolving gel
1182 depended on the molecular weight of the given protein. The Bio-Rad gel running
1183 system was used to resolve proteins and the Bio-Rad wet transfer system was used
1184 for the electrophoretic transfer of proteins onto PVDF membrane. Following transfer,
1185 the membrane was incubated in milk powder (5% in TBST) for 1 hr and subsequently
1186 incubated in primary antibody rolling overnight at 4°C. Primary antibodies targeting
1187 phospho-proteins were diluted in BSA (5% in TBST) as opposed to milk. The
1188 membrane was incubated for 1 hr with secondary antibody (diluted in 5% milk powder)
1189 at room temperature. Prior to visualisation, the membrane was immersed in
1190 WesternBright ECL Spray (Advansta). Protein visualisation took place on a ChemiDoc
1191 MPTM Imaging System (Bio-Rad), and both chemiluminescent and white light images
1192 were taken. Images were analysed using Image Lab 6.0.1 (Bio-Rad).

1193

1194 **ELISA**

1195 DuoSet ELISA kits for IL-1β, TNFα, IL-6, IL-10, and GDF15 were purchased from R&D
1196 Systems and were carried out according to the manufacturer's instructions with
1197 appropriately diluted cell supernatants added to each plate in duplicate or triplicate.
1198 IFN-β was determined using DuoSet ELISA kit from R&D Systems or Abcam

1199 (ab252363). Quantikine ELISA kit for IFN- β (R&D Systems) was used for
1200 determination of IFN- β concentration in serum samples and from human cells, and
1201 these were also carried out according to the manufacturer's instructions. Absorbance
1202 at 450 nm was quantified using a FLUOstar Optima plate reader. Corrected
1203 absorbance values were calculated by subtracting the background absorbance, and
1204 cytokine concentrations were subsequently obtained by extrapolation from a standard
1205 curve plotted on GraphPad Prism 9.2.0.

1206

1207 **Quantification and Statistical Analyses**

1208 Details of all statistical analyses performed can be found in the figure legends. Data
1209 were expressed as mean \pm standard error of the mean (SEM) unless stated otherwise.
1210 Representative western blots are shown. For metabolomics data, MetaboAnalyst 5.0⁶⁶
1211 was used to analyse, perform statistics, and visualise the results. Autoscaling of
1212 features (metabolites) was used for heatmap generation. One-way ANOVA corrected
1213 for multiple comparisons by the Tukey statistical test was used and a p.adjusted < 0.05
1214 was set as the cut-off. For proteomics data, protein signal intensity was converted to
1215 a log₂ scale and biological replicates were grouped by experimental condition. Protein-
1216 wise linear models combined with empirical Bayes statistics were used for the
1217 differential expression analyses. The Bioconductor package limma was used to carry
1218 out the analysis using an R based online tool⁶⁸. Data were visualised using a heatmap
1219 with autoscaled features (genes) and a Volcano plot, which shows the log₂ fold change
1220 on the x axis and the -log₁₀ adjusted p value on the y axis. The proteomics cut-offs for
1221 analysis were a log₂FC of 0.5 and a false discovery rate (FDR) < 0.05, determined
1222 using t statistics. RNA seq cut-offs were set to log₂FC of 1 and an FDR < 0.05.
1223 Overrepresentation analysis (ORA) of significant changes were assessed using
1224 Enrichr⁴ and the Bioconductor package clusterProfiler 4.0 in R (version 3.6.1). Further
1225 information on this visualisation method is available⁶⁹. Emapplots were generated
1226 using enrichplot package in R (version 3.6.1). GSEA analysis of RNAseq was
1227 performed using the Broad Institutes GSEA 4.1.0⁷⁰. Graphpad Prism 9.2.0 was used
1228 to calculate statistics in bar plots using appropriate statistical tests depending on the
1229 data including one-way ANOVA, two-tailed unpaired t test and multiple t tests.
1230 Adjusted p values were assessed using appropriate correction methods, such as
1231 Tukey, Kruskal-Wallis, and Holm-Sidak tests. Sample sizes were determined based
1232 on previous experiments using similar methodologies. All depicted data points are

1233 biological replicates taken from distinct samples, unless stated otherwise. Each figure
1234 consists of a minimum of 3 independent experiments from multiple biological
1235 replicates, unless stated otherwise. For *in vivo* studies, mice were randomly assigned
1236 to treatment groups. For metabolomics, proteomics and RNA sequencing analyses,
1237 samples were processed in random order and experimenters were blinded to
1238 experimental conditions.

1239

1240 **Data Availability**

1241 Proteomics data from Fig. 1d were previously deposited to the ProteomeXchange
1242 Consortium via the PRIDE partner repository with the dataset identifier PXD029155¹¹.
1243 All other proteomics, RNA sequencing data and metabolomics data have been
1244 deposited to Dryad (doi:10.5061/dryad.6wwpzgn28). All original gel images are
1245 provided in the source data file. All other source data are available from the
1246 corresponding author(s) upon request.

1247

1248 **References (methods)**

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1286

1287 **Author contributions**

1288 A.H., C.G.P., D.G.R. and L.A.J.O'N. conceptualised the project; A.H., C.G.P. and
1289 D.G.R. were lead experimentalists, provided intellectual input, designed all
1290 experiments, analysed and visualised the data and co-wrote the paper with input from
1291 all authors. E.A.D performed *in vivo* experiments. E.N.M., L.H., G.D.L.S., M.I., D.J.W.,
1292 S.V. and C.Je. generated data from SLE patients. J.E.T.K. assisted with
1293 immunofluorescence experiments. C.F., M.Ya., A.S.H.C. and E.P. assisted with
1294 metabolomics. A.B.C. and A.V.K. assisted with proteomics. A.F.M., M.Yi., T.A.J.R.,
1295 A.M.C. and H.A.P. performed *in vitro* experiments. C.F. and V.Z. provided inducible
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1297 provided macrophage lysates. C.Jo. provided *Mavs^{-/-}* mouse tissue. M.P.M. and C.F.
1298 provided intellectual input and oversaw a portion of the research programme.
1299 L.A.J.O'N. obtained funding and oversaw the research programme.

1300

1301 **Competing interests**

1302 The authors declare no competing interests.

1303

1304 **Materials & Correspondence**

1305 Correspondence and materials request should be addressed to A.H, D.G.R or
1306 L.A.J.O'N.

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1337 **Extended Data Figure 1 – LPS stimulation drives fumarate accumulation and**
1338 **protein succination**

1339 **a-c**, Fumarate-mediated protein succination with LPS ($n = 3$) and 2SC abundance in
1340 NS and LPS-stimulated BMDMs ($n = 5$; LPS 4 h). **d**, Heatmap of metabolites linked to
1341 aspartate-argininosuccinate shunt in NS and LPS-stimulated BMDMs ($n = 5$; LPS 24
1342 h) **e**, Metabolite abundance of aspartate-argininosuccinate shunt metabolites in LPS-
1343 stimulated BMDMs pre-treated with DMSO or AOAA ($n = 3$; LPS 4 h; aspartate
1344 ($P=0.0000005$)). **f**, *AsI* expression with silencing of *AsI* following LPS stimulation ($n =$
1345 3 ; LPS 24 h). **g**, Fumarate levels with silencing of *AsI* following LPS stimulation ($n = 3$;
1346 LPS 24 h). **c,e-h**, Data are mean \pm s.e.m. **a**, 1 representative blot of 3 shown. $n =$
1347 biological replicates. P values calculated using two-tailed Student's t -test for paired
1348 comparisons or one-way ANOVA for multiple comparisons.

1349

1350 **Extended Data Figure 2 – LPS stimulation drives fumarate accumulation via**
1351 **glutamine anaplerosis and an aspartate-argininosuccinate shunt**

1352 **a**, Schematic diagram indicating U-¹³C-glutamine tracing into distinct metabolic
1353 modules. **b**, U-¹³C-glutamine tracing into glutamate, α -KG and succinate in LPS-
1354 treated BMDMs (m+4 and m+5 labelling intensity and total isotopologue fraction
1355 distribution) ($n = 3$; LPS 4 h). **c**, U-¹³C-glutamine tracing into γ -glutamylcysteine, GSH
1356 and GSSG in LPS-treated BMDMs (m+5 labelling intensity and total isotopologue
1357 fraction distribution) ($n = 3$; LPS 4 h). **d**, U-¹³C-glutamine tracing into aspartate,
1358 argininosuccinate, fumarate and malate in LPS-treated BMDMs (m+4 labelling
1359 intensity and total isotopologue fraction distribution) ($n = 3$; LPS 4 h). Data are mean
1360 \pm s.e.m. $n =$ biological replicates. P values calculated using two-tailed Student's t -test
1361 for paired comparisons.

1362

1363 **Extended Data Figure 3 – LPS stimulation drives fumarate accumulation via**
1364 **glutamine anaplerosis and an aspartate-argininosuccinate shunt**

1365 **a**, Schematic diagram indicating ¹⁵N₂-glutamine tracing into distinct metabolic
1366 modules. **b**, ¹⁵N₂-glutamine tracing into glutamate and asparagine in LPS-treated
1367 BMDMs (m+1 and m+2 labelling intensity and total isotopologue fraction distribution)
1368 ($n = 3$; LPS 4 h). **c**, ¹⁵N₂-glutamine tracing into GSH and GSSG in LPS-treated BMDMs
1369 (m+1 and m+2 labelling intensity and total isotopologue fraction distribution) ($n = 3$;
1370 LPS 4 h). **d**, ¹⁵N₂-glutamine tracing into aspartate, arginine and citrulline in LPS-treated
1371 BMDMs (m+1 labelling intensity and total isotopologue fraction distribution) ($n = 3$;
1372 LPS 4 h; aspartate ($P=0.000001$)). Data are mean \pm s.e.m. $n =$ biological replicates. P
1373 values calculated using one-way ANOVA for multiple comparisons.

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1379 **Extended Data Figure 4 – Increase in aspartate-argininosuccinate shunt**
1380 **metabolites in cytosol and *Irg1*^{-/-} macrophages**

1381 Heatmap (min-max) of metabolites linked to mitochondrial bioenergetics and redox
1382 signalling (**a**) and the aspartate-argininosuccinate shunt (**b**) in NS and BMDMs ($n = 3$;
1383 LPS 24 h). **c**, Metabolite abundance of TCA cycle and aspartate-argininosuccinate
1384 shunt metabolites in WT and *Irg1*^{-/-} BMDMs ($n = 3$; LPS 24 h; itaconate
1385 ($P=0.000000000000002$, succinate ($P=0.000000003$), fumarate ($P=0.000018$)). **d**, Nitrite
1386 levels in WT and *Irg1*^{-/-} BMDMs ($n = 3$; LPS 24 h). **e**, Schematic of metabolic changes
1387 occurring during mid-phase TCA cycle rewiring in WT and *Irg1*^{-/-} BMDMs. Created with
1388 BioRender.com. Data are mean \pm s.e.m. $n =$ biological replicates. P values calculated
1389 using two-tailed Student's t-test for paired comparisons or one-way ANOVA for
1390 multiple comparisons.

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1392 **Extended Data Figure 5 – FH deletion increases bioenergetic stress, fumarate,**
1393 **and mitochondrial membrane potential**

1394 **a**, Bioenergetic ratios in BMDMs treated with DMSO or FHIN1 ($n = 3$). **b**, Fumarate
1395 and 2SC levels in BMDMs treated with DMSO or FHIN1 ($n = 3$). qPCR ($n = 5$) (**c**) and
1396 western blot ($n = 2$) (**d**) analysis of *Fh1* expression in *Fh1*^{+/+} and *Fh1*^{-/-} BMDMs
1397 (EtOH/TAM 72 h; LPS 4 h; *Fh1*^{+/+} NS vs *Fh1*^{+/+} LPS ($P=0.00000002$), *Fh1*^{+/+} NS vs *Fh1*^{-/-}
1398 ^{-/-} NS ($P=0.000000000000002$), *Fh1*^{-/-} NS vs *Fh1*^{-/-} LPS ($P=0.00000000000014$)). **e**,
1399 Bioenergetic ratios in *Fh1*^{+/+} and *Fh1*^{-/-} BMDMs ($n = 3$; EtOH/TAM 48 h). **f**, Heatmap
1400 of top 50 significantly abundant metabolites in *Fh1*^{+/+} and *Fh1*^{-/-} BMDMs ($n = 3$; LPS 4
1401 h). **g**, Fumarate and 2SC levels in *Fh1*^{+/+} and *Fh1*^{-/-} BMDMs ($n = 3$; EtOH/TAM 72 h).
1402 **h**, Glycolysis as measured by ECAR in BMDMs pre-treated with DMSO, FHIN1 or
1403 DMF ($n = 8$ (DMSO/FHIN1); $n = 6$ (DMF); LPS 4 h). $n =$ technical replicates from 1
1404 experiment performed with 3 pooled biological replicates. Data are mean \pm s.d. **i**,
1405 Glyceraldehyde 3- phosphate (G3P) and 2,3-phosphoglycerate (2/3-PG) levels and
1406 ratio in BMDMs pre-treated with DMSO or FHIN1 ($n = 3$; LPS 4 h; G3P ($P=0.00004$)).
1407 Immunofluorescence (**k**) and quantification (**j**) of Mitotracker red staining in BMDMs
1408 pre-treated with DMSO or FHIN1 ($n = 8$ (DMSO); $n = 19$ (FHIN1); LPS 4 h). $n =$
1409 technical replicates from representative experiment. Scale bar = 20 μ m. Data are
1410 mean \pm s.d. **a-c,e,g,i** Data are mean \pm s.e.m. Representative blots or images of 2 (**d**)
1411 or 1 experiment(s) (**j**) shown. $n =$ biological replicates unless stated otherwise. P
1412 values calculated using two-tailed Student's t-test for paired comparisons or one-way
1413 ANOVA for multiple comparisons.

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1420 **Extended Data Figure 6 – FH inhibition remodels inflammatory gene expression**

1421 **a**, *Il10* and *Tnfa* expression in BMDMs pre-treated with DMSO, FHIN1 or DMF ($n = 5$
1422 (*Il10*); $n = 6$ (*Tnfa*); LPS 4 h; FHIN1/*Il10* $P=0.000002$, DMF/*Il10* $P=0.0000004$). **b**, *Il1b*
1423 expression and IL-6 release in BMDMs pre-treated with DMSO, FHIN1 or DMF ($n = 6$;
1424 4 h LPS; DMF/*Il1b* ($P=0.000046$), DMF/IL-6 ($P=0.00000002$)). **c**, Enrichment map plot
1425 of shared significantly increased genes in BMDMs pre-treated with DMF or FHIN1
1426 compared to DMSO control ($n = 3$; LPS 4 h). **d**, Western blot of total and phospho-
1427 AKT, JNK, ERK and p38 levels in BMDMs pre-treated with DMSO, FHIN1 or DMF (n
1428 $= 2$). **e**, *Jun* expression in RNA seq from BMDMs pre-treated with DMF or FHIN1
1429 compared to DMSO control ($n = 3$; LPS 4 h). **f**, *Fos* expression in RNA seq from
1430 BMDMs pre-treated with DMF or FHIN1 compared to DMSO control ($n = 3$; LPS 4 h).
1431 **g**, Western blot of total and phospho-STAT3 levels in BMDMs pre-treated with anti-
1432 CD210 antibody (1 h) ($n = 4$; LPS 4 h). **h**, FH protein and gene expression levels in
1433 *Fh1^{+/+}* and *Fh1^{+/-}* BMDMs ($n = 2$; EtOH/TAM 72 h). **i**, ELISA of IL-10 and TNF- α release
1434 in BMDMs pre-treated with DMSO or AOAA ($n = 3$; LPS 4 h; IL-10 ($P=0.000483$)). **j**,
1435 Schematic depicting mild suppression of IL-10 expression during typical LPS signalling
1436 (right), and increased suppression of IL-10 following FH inhibition, leading to
1437 dysregulated TNF- α release (right). Created with BioRender.com. **a,b,e,f,h,i** Data are
1438 mean \pm s.e.m. 1 representative blot of 2 (**d, h**) or 4 (**g**) shown. $n =$ biological replicates.
1439 P values calculated using two-tailed Student's t-test for paired comparisons or one-
1440 way ANOVA for multiple comparisons.

1441

1442 **Extended Data Figure 7 – FH inhibition triggers the NRF2 and ATF4 stress**
1443 **response and promotes GDF15 release**

1444 **a**, Heatmap of significantly differentially expressed RNA seq data in BMDMs pre-
1445 treated with FHIN1 compared to DMSO control ($n = 3$; LPS 4 h). Volcano plots of
1446 proteomics in BMDMs pre-treated with DMSO, FHIN1 (**b**) or DMF (**c**) ($n = 5$; LPS 4 h).
1447 **d**, ELISA of GDF15 in BMDMs pre-treated with DMSO or FHIN1 ($n = 3$; LPS 4 h). **e**,
1448 *Nrf2* expression or ATF4 protein levels after silencing of *Nrf2* or *Atf4*, respectively, in
1449 BMDMs pre-treated with DMSO or FHIN1 ($n = 6$; LPS 4 h). **f**, *Gdf15* expression after
1450 silencing of *Nrf2* or *Atf4* respectively in BMDMs pre-treated with DMSO or FHIN1 ($n =$
1451 3, LPS 4 h; FHIN1/*Nrf2* RNAi ($P=0.000048$)). **d-f**, Data are mean \pm s.e.m. **e**, 1
1452 representative blot of 6 shown. $n =$ biological replicates unless stated otherwise. P
1453 values calculated using one-way ANOVA for multiple comparisons.

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1461 **Extended Data Figure 8 – IFN-β release following FH inhibition is independent**
1462 **of cGAS-STING**

1463 **a**, Heatmap (min-max) of significantly differentially expressed RNA seq data in
1464 BMDMs pre-treated with DMSO or DMF ($n = 3$; LPS 4 h). **b**, Phospho-STAT1, STAT1,
1465 phospho-JAK1 and JAK1 levels in BMDMs pre-treated with DMSO, FHIN1 or DMF (n
1466 $= 3$; LPS 4 h). **c**, *Ifnb1* expression after silencing of *Nrf2* in BMDMs pre-treated with
1467 DMSO, FHIN1 or DMF ($n = 3$, LPS 4 h). **d**, *Nrf2* expression after silencing of *Nrf2* in
1468 BMDMs pre-treated with DMSO, FHIN1 or DMF ($n = 3$, LPS 4 h; FHIN1
1469 ($P=0.0000008$), DMF ($P=0.0000012$)). **e**, *Ifnb1* expression in BMDMs pre-treated with
1470 DMSO or FHIN1 in the presence of NAC ($n = 3$; LPS 4 h). **f**, TRAF3 levels in BMDMs
1471 pre-treated with DMSO or FHIN1 ($n = 3$; LPS 4 h). **g**, IL-1β levels in BMDMs pre-
1472 treated with DMSO, FHIN1 or DMF ($n = 3$). **h**, p-p65 levels in BMDMs pre-treated with
1473 DMSO, FHIN1 or DMF ($n = 3$). **i**, *D-loop* and *Non-NUMT* DNA fold expression in EtBr-
1474 treated BMDMs ($n = 5$; *D-loop* ($P=0.000000000031$), *Non-NUMT* ($P=0.0000000012$)). **j**,
1475 Lamin B1 and α-tubulin in cytosolic and membrane-bound organelle fractions following
1476 digitonin fractionation ($n = 3$). **k**, IFN-β release from 2',3' cGAMP- or CpG-transfected
1477 BMDMs pre-treated (1 h) with C-178 or ODN2088 ($n = 3$ (cGAMP); $n = 4$ (CpG); 3 h).
1478 **l**, *Ifnb1* expression in BMDMs pre-treated with DMSO or FHIN1 in conjunction with C-
1479 178 or ODN2088 (1 h) respectively ($n = 3$; LPS 4 h). **m**, *Cgas*, *Tmem173* and *Tlr9*
1480 expression with silencing of *Cgas*, *Tmem173* and *Tlr9* respectively in BMDMs pre-
1481 treated with DMSO or FHIN1 ($n = 3$; LPS 4 h). **n**, IFN-β release with silencing of *Cgas*,
1482 *Tmem173* and *Tlr9* respectively from BMDMs pre-treated with DMSO or FHIN1 ($n =$
1483 3 ; LPS 4 h). **o**, *Tmem173* expression in BMDMs pre-treated with DMSO, FHIN1 or
1484 DMF ($n = 3$, LPS 4 h). **p**, *ND4*, *ND5* and *ND6* RNA levels in whole cell extracts of
1485 BMDMs pre-treated with DMSO or FHIN1 in the presence of IMT1 ($n = 5$; LPS 4 h;
1486 *ND5* ($P=0.000052$)). **q**, *ND4*, *ND5* and *ND6* RNA levels in cytosolic extracts of BMDMs
1487 pre-treated with DMSO or FHIN1 in the presence or absence of IMT1 ($n = 5$; LPS 4
1488 h). **r**, IFN-β release in BMDMs pre-treated with DMSO or FHIN1 in the presence of
1489 IMT1 ($n = 3$; LPS 4 h). **c-e,i,k-r**, Data are mean ± s.e.m. **b,f-h,j**, 1 representative of 3
1490 shown. $n =$ biological replicates. P values calculated using two-tailed Student's t-test
1491 for paired comparisons or one-way ANOVA for multiple comparisons.
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1504 **Extended Data Figure 9 – Mitochondrial membrane potential modifiers increase**
1505 **mtRNA and trigger IFN-β release**

1506 **a**, *Tlr7* expression with silencing of *Tlr7* in BMDMs pre-treated with DMSO or FHIN1
1507 ($n = 3$; LPS 4 h). **b**, *Ddx58* and *Ifih1* expression with silencing of *Ddx58* and *Ifih1*
1508 respectively in BMDMs pre-treated with DMSO or FHIN1 ($n = 5$; LPS 4 h;
1509 DMSO/*Ddx58* ($P=0.000000000002$), FHIN1/*Ddx58* ($P=0.000000813792$),
1510 DMSO/*Ifih1* ($P=0.000000009$), FHIN1/*Ifih1* ($P=0.00000014$)). **c**, *Tlr3* expression and
1511 IFN-β release with silencing of *Tlr3* in BMDMs pre-treated with DMSO or FHIN1 ($n =$
1512 3 ; LPS 4 h; DMSO/*Tlr3* ($P=0.000000007$), FHIN1/*Tlr3* ($P=0.000013487$)). **d**, TBK1 and
1513 p-TBK1 in BMDMs pre-treated with DMSO or FHIN1 ($n = 3$; LPS 4 h). **e**, *Ifnb1*
1514 expression in WT and *Mavs*^{-/-} BMDMs pre-treated with DMSO or FHIN1 ($n = 3$; LPS 4
1515 h). **f**, MFI of TMRM staining in BMDMs pre-treated with DMSO, FHIN1, oligomycin or
1516 valinomycin ($n = 3$, LPS 4 h). **g**, IFN-β release from BMDMs pre-treated with DMSO,
1517 FHIN1, oligomycin or valinomycin ($n = 4$; LPS 4 h; oligomycin ($P=0.0000003$)). **h**, MFI
1518 of TMRM staining and IFN-β release from BMDMs pre-treated with DMSO or CCCP
1519 ($n = 4$ (TMRM), $n = 3$ (IFN-β); LPS 4 h; CCCP/IFN-β ($P=0.00000008$)). **i**, MFI of TMRM
1520 staining in BMDMs pre-treated with DMSO or MMF ($n = 3$, LPS 4 h).
1521 Immunofluorescence (**j**) and quantification (**k**) of dsRNA in BMDMs pre-treated with
1522 DMSO, FHIN1 or oligomycin or transfected with poly (I:C) ($n = 8$; LPS 4 h). $n =$
1523 technical replicates from representative experiment. Data are mean \pm s.d. Scale bar =
1524 20 μ m. **l**, *D-loop* fold expression in DNA and RNA isolated from cytosolic fractions of
1525 digitonin-fractionated BMDMs pre-treated with DMSO or oligomycin ($n = 4$ for mtDNA,
1526 $n = 5$ for mtRNA). Immunofluorescence (**m**) and quantification (**n**) of dsRNA in BMDMs
1527 pre-treated with DMSO or valinomycin ($n = 9$ (DMSO); $n = 6$ (Valinomycin); LPS 4 h).
1528 $n =$ technical replicates from representative experiment. Data are mean \pm s.d. Scale
1529 bar = 20 μ m. **o**, Quantification of dsRNA immunofluorescence in *Fh1*^{+/+} and *Fh1*^{-/-}
1530 BMDMs ($n = 7$ (*Fh1*^{+/+} Control); $n = 6$ (*Fh1*^{+/+} LPS); $n = 12$ (*Fh1*^{-/-} Control); $n = 10$ (*Fh1*^{-/-}
1531 ^{-/-} LPS); EtOH/TAM 72 h; LPS 4 h). $n =$ technical replicates from representative
1532 experiment. Data are mean \pm s.d. **a-c,e-i,l** Data are mean \pm s.e.m. **d,j,m**, 1
1533 representative blot or image of 3 experiments shown. $n =$ biological replicates unless
1534 stated otherwise. P values calculated using two-tailed Student's t-test for paired
1535 comparisons, one-way ANOVA for multiple comparisons.

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1537 **Extended Data Figure 10- Prolonged LPS stimulation increases mitochondrial**
1538 **membrane potential and dsRNA**

1539 **a**, MFI of TMRM staining in BMDMs ($n = 3$). Immunofluorescence (**b**) and
1540 quantification (**c**) of dsRNA in BMDMs ($n = 8$ (0/48 h); $n = 9$ (24 h)). $n =$ technical
1541 replicates from representative experiment. Data are mean \pm s.d. Scale bar = 20 μ m.
1542 **d**, *Ddx58* and *Ifih1* expression in BMDMs ($n = 4$; LPS 4 h; *Ddx58* ($P=0.0000000010$),
1543 *Ifih1* ($P=0.00000012$)). **e**, *Fh1* expression in IFN-β-stimulated BMDMs ($n = 3$). **a,d,e**,
1544 Data are mean \pm s.e.m. **b**, 1 representative image of 3 experiments shown. $n =$
1545 biological replicates unless stated otherwise. P values calculated using two-tailed
1546 Student's t-test for paired comparisons, one-way ANOVA for multiple comparisons.

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