

Macrophage fumarate hydratase restrains mtRNA-mediated interferon production

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Metabolic rewiring underlies the effector functions of macrophages^{1–3}, but the mechanisms involved remain incompletely defined. Here, using unbiased metabolomics and stable isotope-assisted tracing, we show that an inflammatory aspartate–argininosuccinate shunt is induced following lipopolysaccharide stimulation. The shunt, supported by increased argininosuccinate synthase (ASS1) expression, also leads to increased cytosolic fumarate levels and fumarate-mediated protein succination. Pharmacological inhibition and genetic ablation of the tricarboxylic acid cycle enzyme fumarate hydratase (FH) further increases intracellular fumarate levels. Mitochondrial respiration is also suppressed and mitochondrial membrane potential increased. RNA sequencing and proteomics analyses demonstrate that there are strong inflammatory effects resulting from FH inhibition. Notably, acute FH inhibition suppresses interleukin-10 expression, which leads to increased tumour necrosis factor secretion, an effect recapitulated by fumarate esters. Moreover, FH inhibition, but not fumarate esters, increases interferon- β production through mechanisms that are driven by mitochondrial RNA (mtRNA) release and activation of the RNA sensors TLR7, RIG-I and MDA5. This effect is recapitulated endogenously when FH is suppressed following prolonged lipopolysaccharide stimulation. Furthermore, cells from patients with systemic lupus erythematosus also exhibit FH suppression, which indicates a potential pathogenic role for this process in human disease. We therefore identify a protective role for FH in maintaining appropriate macrophage cytokine and interferon responses.

Stimulation of macrophages with the TLR4 ligand lipopolysaccharide (LPS) leads to reprogramming of central metabolic pathways involved in bioenergetics, which can facilitate cytokine production. Changes in macrophage metabolism have emerged as a major regulator of inflammation^{1–7}. Although metabolic reprogramming is crucial for macrophage activation the players involved and how they regulate cytokine production remain incompletely characterized.

Accumulation of fumarate in macrophages

To evaluate metabolic alterations that occur during LPS stimulation, we used an unbiased metabolomics approach based on liquid

chromatography–mass spectrometry to characterize the metabolome of inflammatory bone-marrow-derived macrophages (BMDMs). The TCA cycle metabolite fumarate stood out as one of the most significantly upregulated metabolites following exposure to acute LPS stimulation, joining previously identified metabolites such as itaconate² (Fig. 1a). We also observed a significant increase in fumarate-mediated protein succination^{8–10}, which resulted in the formation of the fumarate-cysteine adduct (S)-2-succinocysteine (2SC) (Extended Data Fig. 1a–c).

As acute LPS stimulation failed to impair respiration (Fig. 1b,c), TCA cycle disruption is unlikely to be sufficient for fumarate accumulation. Increased flux through the aspartate–argininosuccinate shunt has been reported to support nitric oxide production⁵. As fumarate

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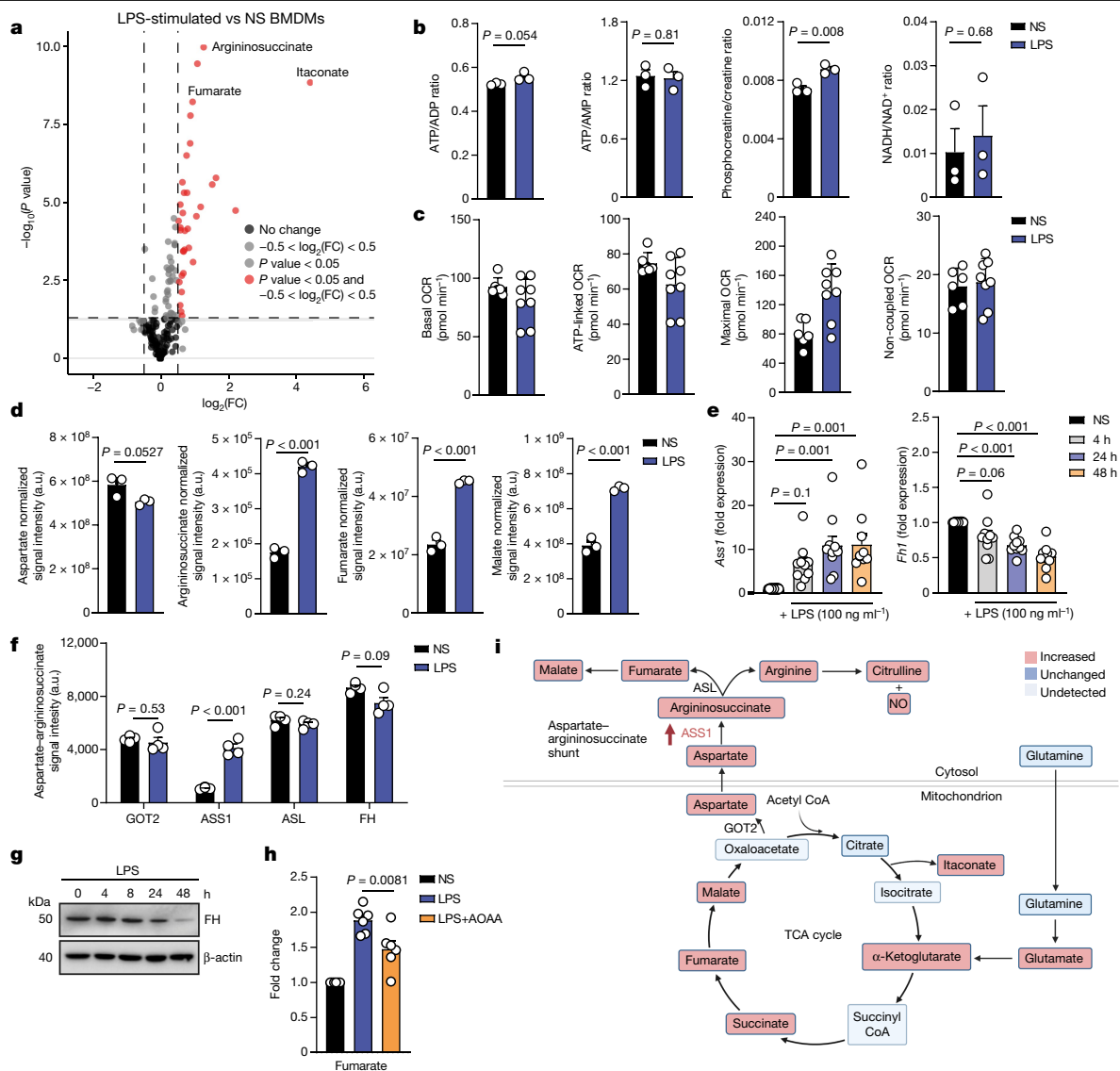


Fig. 1 | LPS stimulation drives fumarate accumulation through glutamine anaplerosis and an aspartate-argininosuccinate shunt. a, b, d, Metabolite abundance (**a, d**) and bioenergetic ratios (**b**) in non-stimulated (NS) and LPS-stimulated BMDMs ($n = 3$). LPS 4 h: argininosuccinate, $P = 0.000044$; fumarate, $P = 0.000141$; malate, $P = 0.000219$. a.u., arbitrary units; FC, fold change. **c,** Respirometry as measured by oxygen consumption rates (OCRs) of NS and LPS-stimulated BMDMs ($n = 6$ (NS) or 8 (LPS); LPS 4 h). $n =$ technical replicates from 1 experiment performed with 3 pooled biological replicates. Data are mean \pm s.d. **e,** *Ass1* and *Fh1* gene expression with LPS time course ($n = 9$). LPS 24 h, $P = 0.000729$; LPS 48 h, $P = 0.000001$. **f,** Quantitative proteomics

of aspartate-argininosuccinate shunt enzymes in NS and LPS-stimulated BMDMs ($n = 4$). LPS 24 h: *Ass1*, $P = 0.000156$. **g, FH** levels with LPS time course ($n = 1$). **h,** Fumarate levels following LPS stimulation with or without A/OAA pre-treatment (1 h) ($n = 6$, LPS 4 h). **i,** Schematic of metabolic changes occurring during early-phase TCA cycle rewiring (LPS 4 h). For **b, d, f, h,** data are mean \pm s.e.m. $n =$ biological replicates unless stated otherwise. P values calculated using two-tailed Student's *t*-test for paired comparisons or one-way analysis of variance (ANOVA) for multiple comparisons. Schematic in panel **i** was created using BioRender (<https://biorender.com>).

is a by-product of argininosuccinate cleavage by argininosuccinate lyase (ASL) in the cytosol, we proposed that argininosuccinate may be a source of fumarate. In support of this hypothesis, we observed decreased aspartate, the precursor to argininosuccinate, and increased argininosuccinate, fumarate and malate levels (Fig. 1d), a result consistent with increased flux through the shunt. This rewiring also occurred during prolonged LPS stimulation (Extended Data Fig. 1d).

Argininosuccinate synthase (*Ass1*) and FH (encoded by *Fh1* in mice and *FH* in humans) expression increased and decreased, respectively in LPS-stimulated BMDMs, as determined by quantitative PCR with reverse transcription (RT-qPCR) (Fig. 1e). Analyses of available quantitative proteomics data^{2,11} showed that *ASS1* was upregulated, whereas levels of glutamic-oxaloacetate transaminase 2 (GOT2), ASL and FH were not

significantly altered (Fig. 1f). FH levels were suppressed only at later time points of LPS treatment (Fig. 1g), which indicates that *ASS1* induction is vital to the acute accumulation of fumarate.

Inhibition of the aspartate-argininosuccinate shunt with the GOT2 inhibitor aminooxyacetic acid (AOAA)⁵ reduced aspartate, asparagine, argininosuccinate and fumarate levels following LPS stimulation (Fig. 1h and Extended Data Fig. 1e). Knockdown of *Asl* also prevented fumarate accumulation (Extended Data Fig. 1f, g), which indicated the dependency of fumarate production on the aspartate-argininosuccinate shunt, which would increase cytosolic fumarate levels (Fig. 1i). Stable isotope-assisted tracing showed that glutamine-dependent anaplerosis is in part responsible for fumarate accumulation and drives the aspartate-argininosuccinate shunt. U-¹³C-glutamine tracing

demonstrated glutaminolysis as a carbon source for the TCA cycle, aspartate–argininosuccinate shunt metabolites, including fumarate, and glutathione (Extended Data Fig. 2). $^{15}\text{N}_2$ -glutamine tracing also demonstrated that glutamine nitrogen is a source for glutathione synthesis and aspartate–argininosuccinate shunt metabolites (Extended Data Fig. 3). Notably, AOA prevented the contribution of glutamine nitrogen to aspartate, asparagine, arginine and citrulline, thereby confirming its inhibition of GOT2. Metabolomics analysis of cytosolic fractions of resting and LPS-stimulated macrophages showed that metabolites such as itaconate and succinate accumulated in the cytosol following LPS stimulation (Extended Data Fig. 4a). Increased levels of argininosuccinate, fumarate and 2SC were also present in the cytosol (Extended Data Fig. 4b).

We proposed that *Irg1*^{-/-} BMDMs (which are unable to synthesize itaconate) would relieve the inhibition of succinate dehydrogenase^{4,12} and exhibit greater accumulation of aspartate–argininosuccinate shunt metabolites. Metabolomics analysis of *Irg1*^{-/-} BMDMs revealed the expected decrease in itaconate and succinate levels, and increased aspartate–argininosuccinate shunt metabolites, including fumarate and nitric oxide (Extended Data Fig. 4c,d). This result provides further evidence linking mitochondrial TCA cycle activity to an aspartate–argininosuccinate shunt (Extended Data Fig. 4e).

FH inhibition causes metabolic rewiring

FH catalyses the hydration of fumarate to malate in mitochondria and the cytosol¹³. Inhibition of this process increases cytosolic fumarate accumulation, perturbs urea cycle metabolism and leads to renal cyst development¹⁴. FH levels remained stable during early LPS stimulation (Fig. 1g). Therefore, we used a well-established pharmacological inhibitor of FH (FHINI)¹⁵ and a recently developed tamoxifen-inducible model of *cre-ERT2*-expressing *Fh1*^{-/-} mice to analyse the role of FH activity and fumarate accumulation in macrophages. However, because FH inhibition may lead to effects independent of fumarate accumulation through mitochondrial and redox stress¹⁶, we also used low concentrations of cell-permeable dimethyl fumarate (DMF) to deliver a cysteine-reactive fumarate ester, which does not inhibit respiration^{17–19}. This approach uncouples the role of impaired mitochondrial bioenergetics following TCA cycle disruption and fumarate-mediated electrophilic modification of cysteine residues.

Previous reports have shown that immunometabolites and their derivatives affect macrophage function through the regulation of metabolic pathways^{4,9,20}. We therefore aimed to assess how FH inhibition and DMF may regulate macrophage metabolism. First, comparing the effects of FHINI and DMF on mitochondrial bioenergetics, we found that FHINI reduced ratios of ATP/ADP, ATP/AMP and P-creatine/creatine. By contrast, DMF had little effect, which demonstrates that FH sustains mitochondrial bioenergetics (Fig. 2a and Extended Data Fig. 5a). This result was confirmed through respirometry experiments, which showed that FHINI impaired basal respiration, ATP production and maximal respiration, as measured by oxygen consumption rates, whereas DMF had no effect (Fig. 2b). FHINI led to a distinct metabolic signature characterized by alterations in TCA cycle metabolites, including citrate, aconitate, itaconate and succinate. This result indicates that the TCA cycle is rewired and enhances fumarate and 2SC accumulation, and provides support for using this approach to study the roles of FH in macrophages (Fig. 2c,e and Extended Data Fig. 5b). Principal component analysis showed significant divergence of FHINI treatment to the other conditions (Fig. 2d).

Tamoxifen-inducible knockout of *Fh1* in macrophages (Extended Data Fig. 5c,d) induced similar bioenergetic changes as those induced by FHINI, such as reduced ATP/AMP and P-creatine/creatine ratios, although the ATP/ADP ratio was unchanged (Extended Data Fig. 5e). TCA cycle rewiring was also observed in *Fh1*^{-/-} macrophages, although to a lesser extent than with FHINI (Extended Data Fig. 5f). Compensatory remodelling during initial genetic inactivation of FH may buffer some of

the acute changes observed with FHINI (ref. ²¹). However, fumarate and 2SC levels were increased in *Fh1*^{-/-} macrophages (Fig. 2f and Extended Data Fig. 5g), which provides support for our parallel use of FHINI and *Fh1*^{-/-} macrophages.

Confirming previous reports⁹, DMF, and to a lesser extent FHINI, suppressed glycolysis (Extended Data Fig. 5h). GAPDH is reportedly inhibited by fumarate-mediated succination^{9,22}. Consistently, FHINI increased the ratio of glyceraldehyde 3-phosphate to 2/3-phosphoglycerate (G3P/2/3-PG) (Extended Data Fig. 5i), which suggests that endogenous fumarate accumulation may impair GAPDH activity. This result provides further evidence that FH impairment leads to the modulation of cytosolic processes.

As FHINI impaired respiration, we examined additional mitochondrial parameters. We first observed increased reactive oxygen species production in cells treated with FHINI but not DMF (Fig. 2g). FHINI treatment also increased the staining intensity of the mitochondrial membrane potential (MMP)-dependent dye Mitotracker Red (Extended Data Fig. 5j,k). Tetramethylrhodamine methyl ester (TMRM) staining confirmed this result, as FHINI significantly increased staining, whereas DMF had no effect (Fig. 2h). Similarly, *Fh1*^{-/-} macrophages had increased MMP values, as previously reported in kidney epithelial cells²³ (Fig. 2h). We also observed a decreased aconitate/citrate ratio in FHINI-treated macrophages, which was indicative of impairment in the fumarate-sensitive and redox-sensitive TCA cycle enzyme aconitase²⁴ (Fig. 2i). Although the GSSG/GSH ratio was unchanged, FHINI led to a depletion of total glutathione (Fig. 2j), which is consistent with fumarate-mediated glutathione depletion^{25,26}. These data suggest that FH inhibition induces substantial redox stress responses.

FH maintains appropriate cytokine responses

To determine whether FH regulates macrophage activation and effector responses, we performed RNA sequencing (RNA-seq) and proteomics to assess changes in the transcriptome and proteome of FHINI-treated BMDMs. Gene set enrichment analysis (GSEA) identified an expected suppression in genes associated with metabolism. However, FHINI also decreased the expression of pathways that affect inflammation, including interleukin-1 (IL-1) and IL-10 signalling (Fig. 3a). Increased expression of components of the haem-regulated inhibitor stress response, amino acid metabolism and tRNA aminoacylation was also observed (Fig. 3a), consistent with previous reports¹⁶. Overrepresentation analysis of RNA-seq data revealed that tumour necrosis factor (TNF) signalling was the most highly upregulated pathway in our analysis (Fig. 3b).

Comparing FHINI with DMF on cytokine readouts allowed us to determine the role of protein succination following FH inhibition. FHINI and DMF decreased IL-10 release and expression, whereas TNF release and expression were increased (Fig. 3c and Extended Data Fig. 6a). This result validated our transcriptomics analysis. Both compounds also reduced IL-1 β expression and IL-6 release (Extended Data Fig. 6b), consistent with previous reports^{10,27}, thereby demonstrating the widespread regulation of cytokine expression.

The less electrophilic fumarate ester monomethyl fumarate (MMF) exhibited the same effects on *Il10* and *Tnf* expression (Fig. 3d), which provides support for a role for fumarate in their regulation. Shared transcriptomic changes induced by FHINI and DMF demonstrated strong downregulation of the ERK1 and ERK2 cascade and PI3K signalling (Fig. 3e). A similar transcriptional fingerprint has been observed in FH-deficient leiomyomas²⁸. We also observed increased amino acid metabolism and transport and autophagy transcripts (Extended Data Fig. 6c). Following LPS stimulation, IL-10 is regulated by ERK1 and ERK2 and PI3K-induced AP-1 activation²⁹, which suggests that downregulation of this signalling axis by FHINI and DMF may repress IL-10. However, we did not observe changes in the upstream kinases AKT, JNK, ERK and p38, which converge on AP-1 activation (Extended Data Fig. 6d). Although we did observe reduced *Jun* expression in our transcriptomics dataset

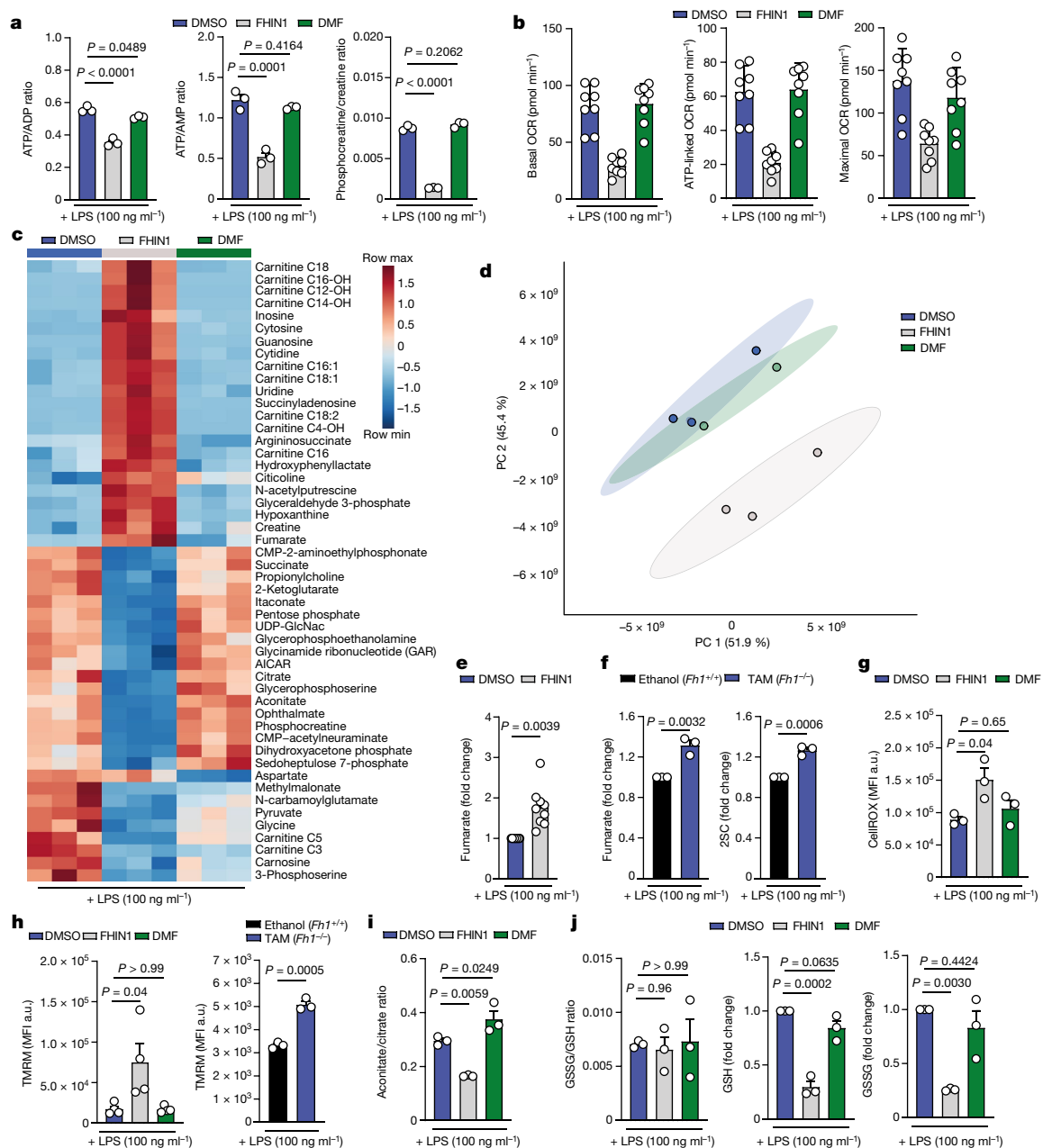


Fig. 2 | FH inhibition increases bioenergetic stress, fumarate levels and MMP. **a, c**, Bioenergetic ratios (**a**) and heatmap of top 50 differentially abundant metabolites (**c**) in BMDMs pre-treated with vehicle (DMSO), FHINI or DMF ($n = 3$; LPS 4 h). LPS 4 h: ATP/ADP, $P = 0.000004$; phosphocreatine/creatine, $P = 0.0000001$. **b**, Respirometry of BMDMs pre-treated with DMSO, FHINI or DMF ($n = 8$; LPS 4 h). $n =$ technical replicates from 1 experiment performed with 3 pooled biological replicates. Data are mean \pm s.d. **d**, Principal component analysis plot of metabolomics in BMDMs pre-treated with DMSO, FHINI or DMF ($n = 3$; LPS 4 h). **e**, Fumarate levels in BMDMs pre-treated with DMSO or FHINI ($n = 9$; LPS 4 h). **f**, Fumarate and 2SC levels in $Fh1^{+/+}$ and $Fh1^{-/-}$ BMDMs ($n = 3$; 96 h ethanol and 4-hydroxytamoxifen (TAM); LPS 4 h). **g**, Mean fluorescence

intensity (MFI) of CellROX staining in BMDMs pre-treated with DMSO, FHINI or DMF ($n = 3$; LPS 4 h). **h**, MFI of TMRM staining in BMDMs pre-treated with DMSO, FHINI or DMF or $Fh1^{+/+}$ and $Fh1^{-/-}$ BMDMs ($n = 4$ (DMSO, FHINI and DMF) or $n = 3$ ($Fh1^{+/+}$ and $Fh1^{-/-}$); 72 h ethanol and TAM; LPS 4 h). **i**, Aconitate/citrate ratio following LPS stimulation with or without FHINI or DMF pre-treatment ($n = 3$; LPS 4 h). **j**, GSH and GSSG levels following LPS stimulation with or without FHINI or DMF pre-treatment ($n = 3$; LPS 4 h). For **a, e–j**, data are mean \pm s.e.m. $n =$ biological replicates unless stated otherwise. P values calculated using two-tailed Student's t -test for paired comparisons or one-way or ANOVA for multiple comparisons.

(Extended Data Fig. 6e), this could indicate reduced autoregulation by AP-1 (ref. ³⁰). In this dataset, *Fos* was not reduced (Extended Data Fig. 6f).

Notably, the thiol precursor *N*-acetyl cysteine (NAC) abrogated the suppression of *Il10* by FHINI and DMF (Fig. 3f). The free thiols of NAC and its products would react with and sequester fumarate, thereby reducing the modification of protein thiols and suggesting that suppression of *Il10* results from a redox-dependent succination event. The electrophile sulforaphane has been shown to reduce AP-1 activation through the

modification of Cys154 on c-Fos³¹. We therefore investigated whether FHINI or DMF may affect c-Fos activation, despite upstream regulators remaining unaffected. c-Fos transcription factor assays showed that FHINI and DMF strongly impaired c-Fos activation (Fig. 3g), which provides evidence of direct regulation of c-Fos, potentially through S-alkylation.

Il10 signalling has been shown to repress *Tnf* expression³². We confirmed this effect using an *Il10* receptor (CD210) blocking antibody

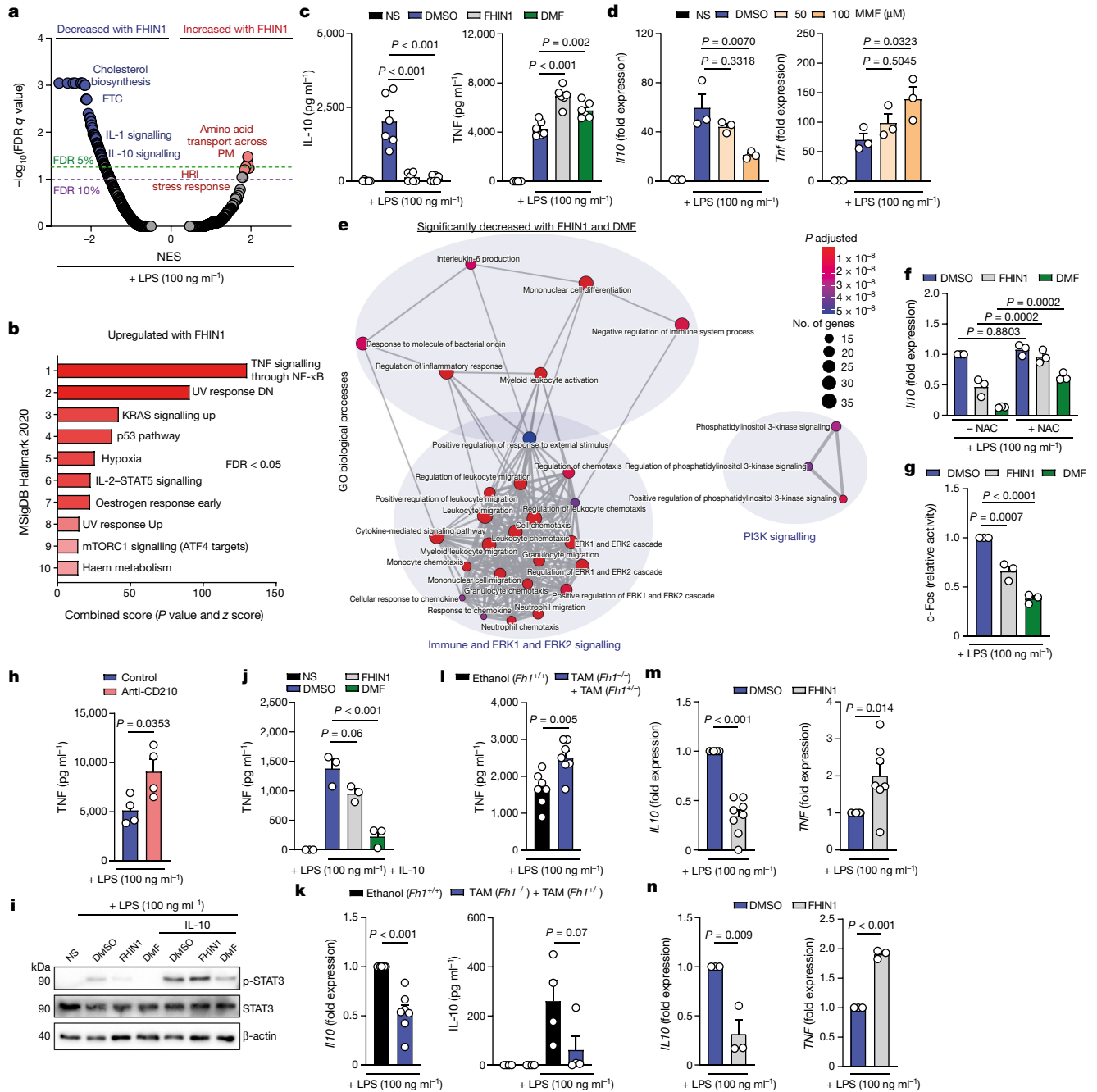


Fig. 3 | FH activity is required to maintain appropriate cytokine responses. **a, b**, GSEA (**a**) and overrepresentation analysis (**b**) of RNA-seq data of BMDMs pre-treated with FH1N1 or DMSO ($n = 3$; LPS 4 h). HRI, haem-regulated inhibitor. **c**, IL-10 and TNF release from BMDMs pre-treated with DMSO, FH1N1 or DMF ($n = 6$; LPS 4 h). FH1N1 and IL-10, $P = 0.000024$; DMF and IL-10, $P = 0.000018$; FH1N1 and TNF, $P = 0.000001$. **d**, *IL10* and *Tnf* expression in BMDMs pre-treated with DMSO or MMF ($n = 3$; LPS 4 h). **e**, Enrichment map plot of shared significantly decreased genes in BMDMs pre-treated with FH1N1 or DMF ($n = 3$; LPS 4 h). **f**, *IL10* expression in BMDMs pre-treated with DMSO, FH1N1 or DMF in the presence of NAC ($n = 3$; LPS 4 h). **g**, c-Fos activity in BMDMs pre-treated with DMSO, FH1N1 or DMF ($n = 3$; LPS 4 h). DMF, $P = 0.000298$. **h**, TNF release from BMDMs pre-treated with anti-CD210 antibody (**h**) ($n = 4$; LPS 4 h). **i, j**, Western blot for

STAT3 and phospho-STAT3 (pSTAT3; **i**) and TNF release (**j**) from BMDMs pre-treated with DMSO, FH1N1 or DMF and treated with IL-10 ($n = 3$, LPS 4 h). DMF, $P = 0.000163$. **k**, *IL10* expression and IL-10 release in *Fh1^{-/-}* and *Fh1^{+/+}* ($n = 5$ or 2) and *Fh1^{+/+}* ($n = 2$) BMDMs (ethanol and TAM 72 h; LPS 4 h). *IL10*, $P = 0.000055$. **l**, TNF release from *Fh1^{+/+}* and *Fh1^{-/-}* ($n = 5$) and *Fh1^{+/+}* ($n = 2$) BMDMs (ethanol and TAM 72 h; LPS 4 h). **m**, *IL10* and *Tnf* expression in human PBMCs pre-treated with DMSO or FH1N1 ($n = 8$, LPS 4 h). FH1N1, $P = 0.00000008$. **n**, *IL10* and *Tnf* expression in human macrophages pre-treated with DMSO or FH1N1 ($n = 3$, LPS 4 h) FH1N1, $P = 0.000028$. For **c, d, f, h, j, k, n**, data are mean \pm s.e.m. For **i**, blot is representative of three. $n =$ biological replicates. P values calculated using two-tailed Student's t -test for paired comparisons or one-way ANOVA for multiple comparisons.

that targets IL-10-mediated STAT3 phosphorylation. This blockade leads to augmented LPS-induced TNF release (Fig. 3h and Extended Data Fig. 6g). We then examined whether recombinant IL-10 supplementation could rescue the increase in TNF release. IL-10 with FH1N1 failed to impair STAT3 phosphorylation or augment TNF production

(Fig. 3i, j), which indicates that the induction of TNF driven by FH1N1 or DMF depends on the suppression of IL-10.

We sought to confirm the role of FH in regulating this axis. Inducible deletion of *Fh1* in macrophages from heterozygous *Fh1^{+/-}* or homozygous *Fh1^{-/-}* mice (Extended Data Figs. 5c, d and 6h) resulted in decreased

IL-10 expression and release (Fig. 3k) and increased TNF release (Fig. 3l). Furthermore, FHIN1 also suppressed *IL10* expression and increased *TNF* expression in LPS-stimulated human peripheral blood mononuclear cells (PBMCs) (Fig. 3m) and macrophages (Fig. 3n). This result indicates that the FH-regulated IL-10–TNF axis is also active in human cells. Establishing the role of LPS-driven fumarate accumulation in the release of these cytokines, AOA, which reduces fumarate accumulation (Fig. 1h), modestly increased and reduced IL-10 and TNF release, respectively (Extended Data Fig. 6i). This result indicates that an increase in ASS1, which results in fumarate accumulation, mildly regulates IL-10 and TNF production. These effects were accentuated by pharmacological or genetic inhibition of FH, which led to increased fumarate accumulation (Extended Data Fig. 6j). Therefore, sustained expression and activity of FH may be viewed as protective against excessive fumarate accumulation and dysregulated production of IL-10 and TNF.

FH inhibition also resulted in the activation of a NRF2 and ATF4 stress response in macrophages (Extended Data Fig. 7a), which is in line with previous observations in epithelial cells¹⁶. Proteomics analysis revealed that the inflammation-associated hormone GDF15 (refs. 33–35) was one of the most significantly increased proteins following FHIN1 and DMF treatment, whereas FHIN1 also increased the recently identified mitochondrial glutathione importer SLC25A39 (ref. 36). This result reinforces the fact that mitochondrial redox is perturbed (Extended Data Fig. 7b,c). In support of our proteomics data, FH inhibition drove GDF15 release from macrophages (Extended Data Fig. 7d). Both ATF4 and NRF2 have been reported to regulate GDF15 in different contexts^{34,37}, and silencing of each revealed that FHIN1-driven GDF15 release partly depended on NRF2 but not ATF4 (Extended Data Fig. 7e,f). This work defines two previously unappreciated signalling axes linked to FH inhibition, uncovering its role in the regulation of IL-10, TNF and GDF15. Recent developments that have identified GDF15 as a mediator of immune tolerance, and the anti-inflammatory properties of colchicine and nonsteroidal anti-inflammatory drugs^{37,38}, suggest that protective effects of DMF in models of inflammation could be mediated at least in part through GDF15. Moreover, increased TNF levels potentially explain adverse events reported with fumarate esters³⁹. Mechanistically, suppression of IL-10 may also explain why fumarate esters promote enhanced TNF production during trained immunity, in addition to reported epigenetic changes⁴⁰.

FH restrains mtRNA-driven IFN β release

RNA-seq analysis of type I interferon (IFN) response genes revealed divergent effects on IFN expression and signalling following FH inhibition, including an upregulation in *Irfb1* (which encodes IFN β) expression and several interferon-stimulated genes, such as *Irf1*, *Irfh1*, *Rsad2* and *Ift2* (Fig. 4a). However, other interferon-stimulated genes, such as *Lcn2*, were suppressed by FHIN1 and DMF treatment (Fig. 4a and Extended Data Fig. 8a). Examination of specific type I IFN signalling components downstream of the IFN α/β receptor (IFNAR) revealed that both FHIN1 and DMF treatment limited IFN β -induced STAT1 and JAK1 phosphorylation (Extended Data Fig. 8b), which indicated that there was modest suppression of JAK–STAT signalling. Activation of NRF2 by fumarate and derivatives (Extended Data Fig. 7) may be responsible⁴¹. Indeed, *Irfb1* expression was increased after FHIN1 and DMF treatment following *Nrf2* silencing (Extended Data Fig. 8c,d), which suggests that NRF2 restrains IFN transcription.

Notably, FHIN1, but not DMF or MMF, increased IFN β release from LPS-stimulated macrophages (Fig. 4b,c). This effect was independent of NAC-sensitive redox stress (Extended Data Fig. 8e) and was not due to augmented TLR4 signalling, as LPS-induced TRAF3 levels and IL-1 β expression were not increased by FHIN1 (Extended Data Fig. 8f,g). FHIN1 and DMF modestly augmented LPS-induced p65 phosphorylation (Extended Data Fig. 8h), which may contribute to increased TNF release⁴². Given that FH inhibition causes mitochondrial stress (Fig. 2),

which is associated with the release of immunostimulatory mitochondrial nucleic acids^{43–45}, we proposed that the IFN response is driven by cytosolic nucleic acid sensors, such as cGAS, RIG-I or MDA5. In support of this hypothesis, FH deficient-hereditary leiomyomatosis and renal cell cancer tumours exhibit changes in mitochondrial DNA (mtDNA)²¹. We first used ethidium bromide to deplete mtDNA⁴⁶ (Extended Data Fig. 8i) before treating cells with FHIN1 and LPS. FHIN1 no longer boosted LPS-induced IFN β release in the presence of ethidium bromide (Fig. 4d), which indicated that the increased IFN β release with FHIN1 may depend on mtDNA. We subsequently found that FHIN1 caused an increase in both mtDNA and mtRNA in cytosolic extracts (Fig. 4e and Extended Data Fig. 8j). Given the established role of mtDNA in driving IFN responses^{43,44}, we examined whether the cGAS–STING or TLR9 DNA-sensing pathways are required for the increase in IFN β . However, treatment with the STING inhibitor C-178 (ref. 47) or silencing of *Cgas* (which encodes cGAS) or *Tmem173* (which encodes STING) had no effect on FHIN1-driven IFN β induction (Extended Data Fig. 8k–n). Targeting TLR9 using the competitive inhibitor ODN 2088 (ref. 48) or using siRNA also had no effect on this response (Extended Data Fig. 8k–n). Suppression of *Tmem173* expression by FHIN1 or DMF (Extended Data Fig. 8o) may explain why cGAS–STING signalling is redundant in our model, even in the presence of cytosolic mtDNA. ETC inhibition, as observed with FHIN1 treatment, has also been shown to inhibit STING activation⁴⁹.

As cytosolic mtRNA was also increased by FHIN1 (Fig. 4e), we performed immunofluorescence staining with an antibody specific for double-stranded RNA (dsRNA). mtRNA has previously been shown to drive an IFN response in human cells^{50,51}, and is known to be particularly immunostimulatory⁵². FHIN1 treatment led to an accumulation of dsRNA relative to cells treated with dimethylsulfoxide (DMSO) as control (Fig. 4f). We subsequently treated cells with both FHIN1 and IMT1, an inhibitor of mitochondrial RNA polymerase (POLRMT). The increase in mtRNA following FHIN1 was observed in the cytosolic fraction but not in the whole cell fraction and was inhibited in both conditions with IMT1 treatment (Extended Data Fig. 8p,q). Notably, IMT1 also partly abrogated the FHIN1-mediated increase in IFN β release (Extended Data Fig. 8r), which implies that mtRNA has a role in driving this response. Mitochondrial single-stranded RNA (ssRNA), which results from a decline in mitochondrial integrity, has also been implicated in driving TLR7-dependent IFN signalling^{53,54}. We subsequently silenced *Tlr7* or the dsRNA sensors *Ddx58* (which encodes RIG-I) and *Irfh1* (which encodes MDA5) (Extended Data Fig. 9a,b), all of which abrogated the increase in IFN β release observed with FH inhibition (Fig. 4g,h). This result confirms that there is a non-redundant requirement of these sensors and mtRNA, rather than mtDNA, for the FHIN1-driven IFN response. Knockdown of the cell surface dsRNA sensor *Tlr3* did not affect the augmentation in IFN β release (Extended Data Fig. 9c). RIG-I and MDA5, although predominantly described as dsRNA sensors, can also bind ssRNA⁵⁵, which indicates that the IFN response following FH inhibition is probably driven by a mixture of dsRNA and ssRNA species. It is notable that FHIN1 also reduced *Ddx58* but not *Irfh1* expression, which may warrant further investigation (Extended Data Fig. 9b). The signalling events downstream of RIG-I–MDA5 activation include mitochondrial antiviral signalling protein (MAVS) oligomerization, followed by recruitment and phosphorylation of TANK-binding kinase 1 (TBK1). We observed MAVS oligomerization and increased TBK1 phosphorylation following FHIN1 treatment (Fig. 4i and Extended Data Fig. 9d). Notably, *Mavs* knockout did not impair the induction of IFN β by FHIN1 (Extended Data Fig. 9e), which may indicate that compensatory TLR7 signalling is sufficient to drive type I IFN responses following FH inhibition with chronic MAVS deficiency.

We previously demonstrated that FH inhibition causes mitochondrial stress (Fig. 2). Changes in MMP have previously been correlated with increased type I IFN release⁵⁶. Therefore we proposed that disturbances in MMP may be linked to mtRNA release and IFN β induction following FH inhibition. To support this hypothesis, we induced changes in MMP by using the ATP synthase inhibitor oligomycin A, which increased MMP,

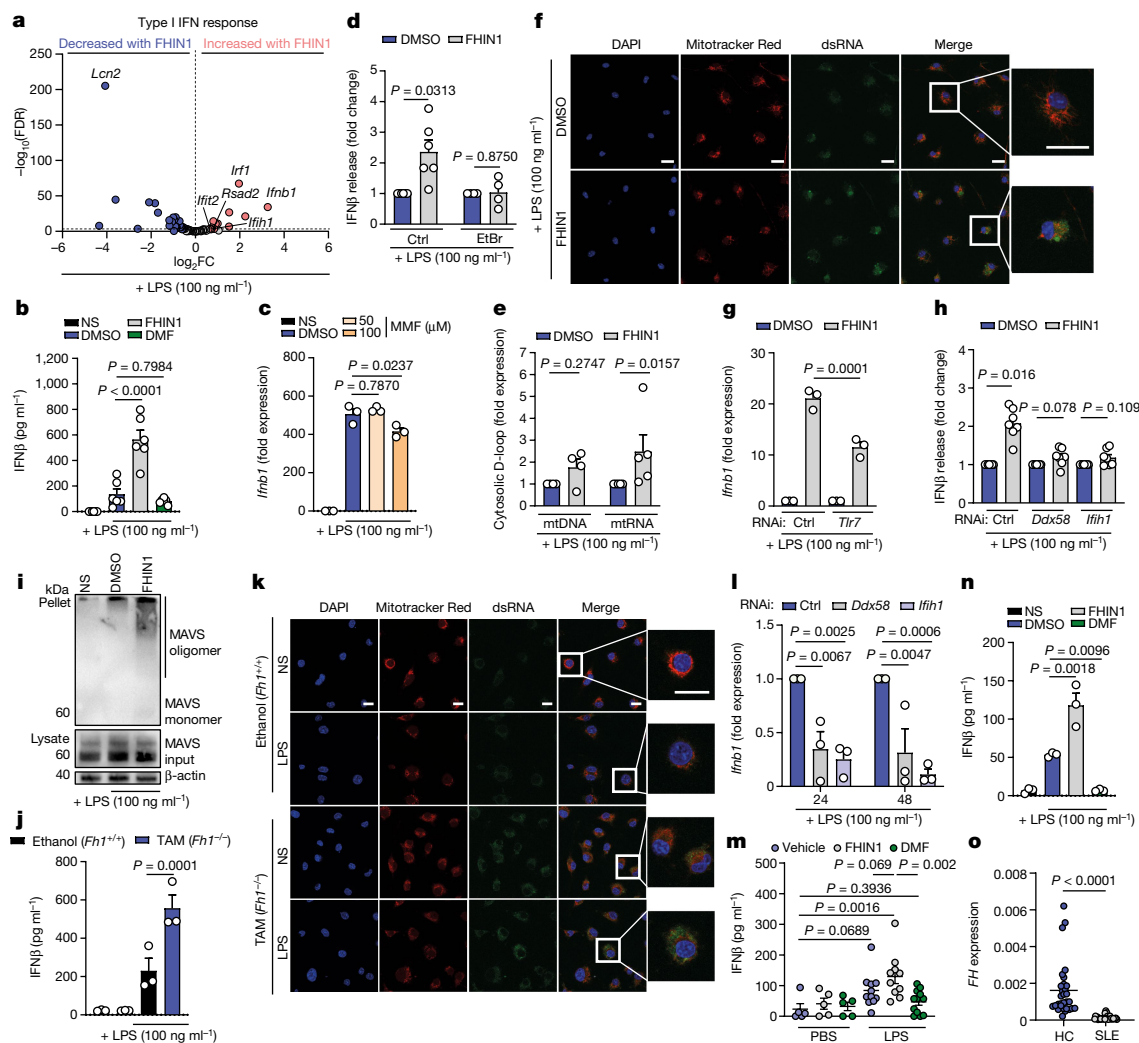


Fig. 4 | FH impairment triggers IFN β release through a mtRNA-driven retrograde response. **a**, Volcano plot of type I IFN response in BMDMs pre-treated with DMSO or FHINI ($n = 3$; LPS 4 h). **b**, IFN β release from BMDMs pre-treated with DMSO, FHINI or DMF ($n = 6$; LPS 4 h). FHINI, $P = 0.000004$. **c**, *Ifnb1* expression in BMDMs pre-treated with DMSO or MMF ($n = 3$; LPS 4 h). **d**, IFN β release from BMDMs treated with ethidium bromide (EtBr) (for 6 days) before pre-treatment with DMSO or FHINI ($n = 6$; LPS 4 h). Ctrl, control. **e**, Cytosolic D-loop expression in DNA and RNA in BMDMs pre-treated with DMSO or FHINI ($n = 4$ (mtDNA) or 5 (mtRNA); LPS 4 h). **f**, dsRNA immunofluorescence in BMDMs pre-treated with DMSO or FHINI ($n = 3$; LPS 4 h). **g**, *Ifnb1* with *Tlr7* silencing in BMDMs pre-treated with DMSO or FHINI ($n = 3$; LPS 4 h). **h**, IFN β with *Ddx58* or *Ifih1* silencing in BMDMs pre-treated with DMSO or FHINI ($n = 7$; LPS 4 h). **i**, MAVS

in BMDMs pre-treated with DMSO or FHINI ($n = 3$; LPS 4 h). **j**, IFN β levels in *Fh1*^{+/+} and *Fh1*^{-/-} BMDMs ($n = 3$; ethanol and TAM 72 h; LPS 4 h). **k**, dsRNA immunofluorescence in *Fh1*^{+/+} and *Fh1*^{-/-} BMDMs ($n = 3$; ethanol and TAM 72 h; LPS 4 h). **l**, *Ifnb1* with *Ddx58* or *Ifih1* silencing ($n = 3$). **m**, Serum IFN β of mice treated with FHINI or DMF before PBS or LPS injection ($n = 5$ (PBS), 10 (FHINI and LPS), 11 (vehicle and LPS) or 12 (DMF and LPS)). **n**, IFN β release from human PBMCs pre-treated with DMSO, FHINI or DMF ($n = 3$; LPS 4 h). **o**, *FH* expression in whole blood from healthy individuals (HC) and patients with SLE ($n = 30$; $P = 0.0000005$). For **b–e, g, h, j, l–o**, data are mean \pm s.e.m. For **f, i, k**, blot or image is representative of three experiments. $n =$ biological replicates. P values calculated using two-tailed Student's t -test for paired comparisons, one-way ANOVA for multiple comparisons. Scale bars, 20 μ m (**f, k**).

the K⁺ ionophore valinomycin A, which nonsignificantly reduced MMP, or the uncoupler CCCP, which significantly dissipated MMP (Extended Data Fig. 9f, h). All treatments boosted LPS-driven IFN β release, similar to effects with FHINI (Extended Data Fig. 9g, h). MMF, which does not increase LPS-induced IFN β expression (Fig. 4c), did not affect MMP (Extended Data Fig. 9i). Oligomycin treatment led to an accumulation of dsRNA to a similar extent to that observed in cells treated with FHINI or transfected with dsRNA (poly(I:C)), and increased mtRNA release into the cytosol (Extended Data Fig. 9j–l). Valinomycin treatment similarly drove dsRNA accumulation (Extended Data Fig. 9m, n), which indicated that compounds that alter MMP induce an accumulation of mtRNA. As we also observed an increase in cytosolic mtDNA levels following oligomycin treatment (Extended Data Fig. 9l), it is still possible that IFN responses following oligomycin, valinomycin and CCCP treatment are not exclusively driven by mtRNA. mtRNA release from

chondrocytes has recently been implicated in activating the immune response and promoting osteoarthritis⁵⁷. As such, mitochondrial damage and nucleic acid release are emerging as key pathogenic processes that may underlie many immune-mediated diseases.

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

We next considered whether this response could be applied to an endogenous model of LPS activation in the absence of pharmacological or genetic inactivation of FH. Given that LPS-induced FH suppression occurs predominantly during late-phase LPS stimulation (24–48 h)

(Fig. 1g), FH suppression at this time point may drive membrane hyperpolarization and the release of mtRNA. MMP was significantly increased following 48 h of LPS stimulation, but not following 4 h or 24 h of stimulation (Extended Data Fig. 10a). Although dsRNA did not accumulate following acute (4 h) LPS stimulation (Extended Data Fig. 9j,k), we did observe increased dsRNA staining following 24 h and 48 h of LPS stimulation (Extended Data Fig. 10b,c). *Ddx58* and *Ifih1* expression is induced by LPS (Extended Data Fig. 10d), which suggests that RIG-I-MDA5 signalling is required during LPS stimulation. Indeed, silencing of *Ddx58* and *Ifih1* reduced *Ifnb1* expression induced by 24 h and 48 h of LPS stimulation (Fig. 4l). This result indicates that *Ifnb1* transcription during late-phase LPS stimulation is maintained by mtRNA release. These results demonstrate that the mitochondrial retrograde type I IFN response, which we initially unmasked by pharmacologically or genetically targeting FH during early LPS signalling, is active endogenously during late-phase LPS activation. These data have potential implications for chronic inflammation, for example, during ageing⁵⁸.

To determine whether FH inhibition leads to similar effects *in vivo*, we injected mice with FHIN1 or DMF before administration of LPS, and measured IFN β release into the serum. FHIN1 increased LPS-induced IFN β release, whereas DMF had no effect (Fig. 4m), which indicates that FH inhibition leads to a similar IFN response *in vivo*, which may have effects on bystander cells. We also treated human PBMCs with FHIN1 or DMF before LPS stimulation and observed similar effects. That is, FHIN1 increased, whereas DMF suppressed, LPS-induced IFN β release (Fig. 4n).

In summary, we described a mitochondrial retrograde signalling pathway leading from FH inhibition to mitochondrial membrane hyperpolarization and mtRNA release (Supplementary Fig. 1). Mitochondrial stress may be an underlying mechanism that contributes to type I IFN release in interferonopathies such as systemic lupus erythematosus (SLE). It has previously been demonstrated that PBMCs from patients with SLE have impaired mitochondrial function and altered MMP^{59,60}. We therefore examined *FH* expression in the whole blood of patients with SLE and found significant suppression of *FH* compared with samples from healthy individuals (Fig. 4o). Autoantibodies to dsRNA, as well as dsDNA, have been detected in patients with SLE^{61,62}. However, it is unclear whether FH suppression is a cause or consequence of increased IFN signalling, as *Fh1* can also be inhibited by IFN β stimulation in BMDMs (Extended Data Fig. 10e). A negative feedback loop may exist whereby suppression of FH leads to type I IFN release, which feeds back to further suppress FH. FH suppression has also been previously linked to multiple sclerosis progression⁶³ and, in parallel to our work, has been shown to promote a type I IFN response in kidney epithelial cells and hereditary leiomyomatosis and renal cell cancer tumours (Zecchini et al.⁶⁴). That study and ours implicate roles for FH in nucleic acid release, which may contribute to inflammation-driven tumorigenesis and as a potential host defence mechanism in the context of viral infection. Finally, a recent study⁶⁵ reported that aberrant dsRNA editing due to ADARI deficiency leads to MDA5 activation as a mechanism of common inflammatory diseases. Together, these data point to the clinical relevance of endogenously produced dsRNA and suggest that targeting this pathway may lead to new anti-inflammatory strategies.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-023-05720-6>.

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Article

Methods

Animal details

All mice were on a C57BL/6JOLA^{Hsd} background unless stated below. Wild-type (WT) mice were bred in-house. The inducible *Fh1*^{fl/fl} and *Fh1*^{fl/f1} mice were generated on the C57BL/6 genetic background, and their hind legs were donated by C. Frezza (University of Cambridge, UK). *Fh1*^{+/+} and *Fh1*^{-/-} treated with vehicle (ethanol) were used as controls. Following treatment with 4-hydroxytamoxifen (TAM), Cre-mediated chromatin excision results in the loss of either one (*Fh1*^{+/-}) or both (*Fh1*^{-/-}) copies of *Fh1*, thus generating either heterozygous or null animals. Hind legs from WT and *Maus*^{-/-} mice were donated by C. Johansson (Imperial College London, UK). These strains, originally obtained from S. Akira (World Premier International Immunology Frontier Research Center, Osaka University, Osaka, Japan), were *Ifna6*^{6^{fl/+}}, but since *Ifna6* expression was not a primary readout, the mice are designated as WT and *Maus*^{-/-}. In vitro experiments were performed with BMDMs isolated from 6–18-week-old female and male mice. Although we did not use statistical methods to calculate sample sizes, we decided to use a minimum of three biological replicates per experiment to account for biological variability, considering the three Rs principle (replacement, reduction and refinement) and the fact that most experiments were performed in primary macrophages from in-bred mice. All in vitro treatment groups were randomly assigned. In vitro and in vivo experiments were not blinded owing to the lack of available experimenters with required expertise. In vivo models were performed using 6-week-old male mice, and littermates were randomly assigned to experimental groups. Animals were maintained under specific pathogen-free conditions in line with Irish and European Union regulations. All animal procedures were approved by the Trinity College Dublin Animal Research Ethics Committee before experimentation and conformed with the Directive 2010/63/EU of the European Parliament.

Generation of mouse BMDMs

Mice (6–18 weeks old) were euthanized in a CO₂ chamber, and death was confirmed by cervical dislocation. Bone marrow was subsequently collected from the tibia, femur and ilium and cells were differentiated in DMEM containing L929 supernatant (20%), FCS (10%) and penicillin–streptomycin (1%) for 6 days, after which cells were counted and plated at 0.5×10^6 cells per ml unless otherwise stated. BMDMs were plated in 12-well cell culture plates and left overnight to adhere.

Isolation of human PBMCs

Human blood samples from healthy donors were collected and processed at the School of Biochemistry and Immunology at the Trinity Biomedical Sciences Institute (TCD). Blood samples were obtained anonymously, and written informed consent for the use of blood for research purposes was obtained from the donors. All the procedures involving experiments on human samples were approved by the School of Biochemistry and Immunology Research Ethics Committee (TCD). Experiments were conducted according to the TCD guide on good research practice, which follows the guidelines detailed in the National Institutes of Health Belmont Report (1978) and the Declaration of Helsinki. Whole blood (30 ml) was layered on 20 ml Lymphoprep (Axis-Shield), followed by centrifugation for 20 min at 400g with the brake off, after which the upper plasma layer was removed and discarded. The layer of mononuclear cells at the plasma-density gradient medium interface was retained, and 20 ml PBS was added. Cells were centrifuged for 8 min at 300g and the resulting supernatant was removed and discarded. The remaining pellet of mononuclear cells was resuspended, counted and plated at 1×10^6 cells per ml in RPMI supplemented with FCS (10%) and penicillin–streptomycin (1%).

Generation of human macrophages

PBMCs were obtained, and CD14⁺ monocytes were isolated using a MagniSort Human CD14 Positive Selection kit (Thermo Fisher) according to

the manufacturer's protocol. CD14 monocytes were then differentiated in T-175 flasks in RPMI containing FCS (10%), penicillin–streptomycin (1%) and recombinant human M-CSF (1:1,000). After 6 days, the supernatant was discarded, cells were scraped and counted, and human monocyte-derived macrophages were plated in 12-well plates at 1×10^6 cells per ml in RPMI containing FCS (10%) and penicillin–streptomycin (1%).

Whole blood isolation from patients with SLE

All patients with SLE (as per the diagnostic criteria of the American College of Rheumatology) were recruited from the Cedars–Sinai Medical Center. Age- and sex-matched healthy donors who had no history of autoimmune diseases or treatment with immunosuppressive agents were included. All participants provided informed written consent, and the study received approval from the institutional ethics review board (IRB protocol number 19627). Blood was collected into PAXgene RNA tubes (2.5 ml blood plus 6.9 ml buffer) and stored at -80°C . Before isolation of RNA, the tubes were thawed at room temperature for 16 h. Total RNA was isolated using a PAXgene Blood RNA kit according to the manufacturer's recommendations (PreAnalytiX, 08/2005, 762174).

Reagents

LPS from *Escherichia coli*, serotype EH100 (ALX-581-010-L001), was purchased from Enzo Life Sciences. High molecular weight poly(I:C) (tlrl-pic) and 2'-3'-cGAMP (tlrl-nacga23) were purchased from InvivoGen. Recombinant mouse IFN β 1 (S81302) and recombinant mouse IL-10 (417-ML-005/CF) were purchased from BioLegend. ATP disodium salt (A2383), DMSO (D8418), AOAA (C13408), valinomycin (V3639), TAM (H6278) and NAC (A7250) were purchased from Sigma Aldrich. Oligomycin A from *Streptomyces diastatochromogenes* (M02220) was purchased from Fluorochem. FHINI (HY-100004), DMF (HY-17363), MMF (HY-103252), IMT1 (HY-134539) and C-178 (HY-123963) were purchased from MedChemExpress. CPG ODN 1826 (130-100-274) and ODN 2088 (130-105-815) were purchased from Miltenyi Biotec. CCCP (M20036) was purchased from Thermo Fisher.

Compound treatments

All compounds used DMSO as a vehicle except for TAM (ethanol), NAC (PBS) and AOAA for tracing experiments (culture medium). LPS was used at a concentration of 100 ng ml^{-1} for indicated time points (2, 3, 4, 6, 8, 24 and 48 h). FHINI (10 or 20 μM), MMF (50 or 100 μM), DMF (25 μM), AOAA (5 mM), oligomycin (10 μM), CCCP (50 μM), NAC (1 mM) and IMT1 (10 μM) pre-treatments were performed for 3 h before the addition of LPS. Cells were treated with valinomycin (10 nM) 15 min before LPS stimulation. Anti-CD210 or IgG control ($10 \mu\text{g ml}^{-1}$) antibodies were added to cells 1 h before LPS stimulation. Recombinant mouse IL-10 protein (100 ng ml^{-1}) was added to cells at the same time as LPS. Cells were treated with IFN β 1 (220 ng ml^{-1}) for 3 h. Cells were treated with C-178 (1 μM) 1 h before LPS stimulation or transfection with 2'-3'-cGAMP ($1.5 \mu\text{g ml}^{-1}$) for 4 h to achieve cGAS–STING activation. Cells were treated with ODN 2088 (1 μM) for 1 h before LPS stimulation or transfection with CPG ODN 1826 ($1.5 \mu\text{g ml}^{-1}$) to achieve TLR9 activation. Three different time points of TAM (600 nM or 2 μM) or ethanol treatment were performed (specified in the individual figure legends). For 48-h treatments, ethanol and TAM were added on day 5 of 6 during the BMDM differentiation protocol. On day 6, they were plated with ethanol and TAM (left overnight) and treated the following day. For 72-h treatments, ethanol and TAM were added on day 4 of 6 during the BMDM differentiation protocol. On day 6, they were plated with ethanol and TAM (left overnight) and treated the following day. For 96-h treatments, ethanol and TAM were added on day 4 of 6 during the BMDM differentiation protocol. On day 6, they were plated with ethanol and TAM and treated 2 days later.

Antibodies

Working dilutions of antibodies were 1:1,000 unless otherwise stated. Anti-mouse lamin B1 (12586), STAT1 (9172), p-STAT1 (9167), JAK1 (3344),

p-JAK1 (3331), TBK1 (3504), p-TBK1 (5483), STAT3 (30835), p-STAT3 (9145), FH (4567), ASS1 (70720), α -tubulin (2144), α -tubulin (3873), MAVS (4983), ATF4 (11815), p-AKT (13038), AKT (2920), p-JNK (9255), JNK (9252), p-ERK1/2 (9101), ERK1/2 (4695), p-p38 (4511), p-38 (9212), TRAF3 (4729), p-p65 (3033) and GAPDH (2118) antibodies were purchased from Cell Signaling. Anti-goat IL-1 β (AF-401-NA) was purchased from R&D. Anti-2SC antibody was provided by N. Frizzell (University of South Carolina, USA). Anti-mouse β -actin antibody (1:5,000) (A5316) was purchased from Sigma Aldrich. Horseradish peroxidase (HRP)-conjugated anti-mouse (115-035-003), anti-goat (705-035-003) and anti-rabbit (111-035-003) IgG antibodies (all 1:2,000) were purchased from Jackson ImmunoResearch. Anti-mouse CD210 (112710) and anti-mouse IgG (406601) antibodies (both 10 μ g ml⁻¹) were purchased from BioLegend. Anti-dsRNA antibody (clone rJ2, 1:60) was purchased from Merck (MABE-1134). Alexa Fluor 488 goat anti-mouse IgG1 antibody (A21121) was purchased from Invitrogen. Details of antibody validation are given in Supplementary Table 1.

RT-qPCR

RNA extraction from cells was carried out using a Purelink RNA kit (Invitrogen) according to the manufacturer's instructions. BMDMs were treated as required, and following treatment were instantly lysed in 350 μ l RNA lysis buffer. Isolated RNA was quantified using a NanoDrop 2000 spectrophotometer, and RNA concentration was normalized to the lowest concentration across all samples with RNase-free water. If necessary, samples were DNase-treated after quantification using DNase I (Thermo Fisher) according to the manufacturer's instructions. Isolated RNA samples were normalized and converted into cDNA using a High-Capacity cDNA Reverse Transcription kit (Thermo Fisher) according to the manufacturer's instructions. Next, 10 μ l of RNA (at a maximum concentration of 100 ng μ l⁻¹) was added to 10 μ l of reverse transcription master mix to complete the reaction mixture. Real-time qPCR was performed on the cDNA generated in the previous step, using primers designed in-house and ordered from Eurofins Genomics, as detailed in Supplementary Table 2. The reaction was performed in a 96-well qPCR plate using a 7500 Fast Real-Time PCR machine (Thermo Fisher). Relative expression ($2^{-\Delta\Delta CT}$) was calculated from the C_T values for each sample and gene of interest.

RNA interference

Pre-designed silencer select siRNAs for *Cgas* (s103166), *Tmem173* (s91058), *Tlr3* (s100579), *Tlr9* (s96268), *Asl* (s99640), *Tlr7* (s100720), *Ddx58* (s106376), *Irf1* (s89787), *Nrf2* (s70522), *Atf4* (s62689) and negative control (4390843) were ordered from Thermo Fisher. siRNA sequences are given in Supplementary Table 2. Cells were transfected with 50 nM siRNA using 5 μ l Lipofectamine RNAiMAX according to the manufacturer's instructions (Thermo Fisher). Cells were transfected in medium without serum and antibiotics, which was replaced with complete medium 8 h later. Cells were subsequently left for at least a further 12 h before treatment.

Immunofluorescence

Cells were plated on 20 mm cover slips in 12-well plates. Cells were treated as required and Mitotracker Red CMXRos (100 nM, Thermo Fisher) was added to medium 30 min before the end of cell treatments. After 30 min of incubation, cells were washed three times with warm PBS. Cells were subsequently fixed for 10 min with 4% paraformaldehyde in PBS at 37 °C. Cells were washed three times with PBS and permeabilized for 1 h in block solution (1% BSA, 22.52 mg ml⁻¹ glycine and 0.1% Tween 20 in PBS). Anti-dsRNA antibody (Merck) was diluted 1:60 in block solution and incubated with cells overnight at room temperature. Cells were washed three times with PBS for 5 min per wash. A mix containing AF488-conjugated goat anti-mouse IgG1 antibody (1:1,000) and DAPI (1:1,000, Thermo Fisher) was subsequently added to cells for 90 min at room temperature in the dark. Cells were subsequently

washed three times with PBS for 5 min per wash. Cover slips were mounted onto microscope slides using 10–20 μ l ProLong Gold antifade reagent (Thermo Fisher). Slides were imaged using a Leica SP8 scanning confocal microscope with a $\times 20.0$ objective. Images were analysed using LAS X Life Science Microscope Software Platform (Leica). The same microscope instrument settings were used for all samples, and all images were analysed using the same settings. Quantification of dsRNA or Mitotracker Red CMXRos signal intensity was performed using the measure function in ImageJ 1.53t (NIH). The mean signal intensity was calculated for individual cells in single colour images and displayed relative to signal intensity of control cells.

Flow cytometry

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

Liquid chromatography–mass spectrometry

Steady-state metabolomics. BMDMs (3 independent mice) were plated at 0.5×10^6 cells per well in 12-well plates in technical triplicate per condition, treated as indicated, snap frozen and stored at -80 °C. For metabolomics of the cytosolic fraction, BMDMs were plated at 10×10^6 cells per 10 cm dish, and rapid fractionation was performed as previously reported¹⁶. Metabolite extraction solution (methanol:acetonitrile:water, 50:30:20 v/v/v) was added (0.5 ml per 1×10^6 cells), and samples were incubated for 15 min on dry ice. The resulting suspension was transferred to ice-cold microcentrifuge tubes. Samples were agitated for 20 min at 4 °C in a thermomixer and then incubated at -20 °C for 1 h. Samples were centrifuged at maximum speed for 10 min at 4 °C. The supernatant was transferred into a new tube and centrifuged again at maximum speed for 10 min at 4 °C. The supernatant was transferred to autosampler vials and stored at -80 °C before analysis by liquid chromatography–mass spectrometry (LC–MS).

HILIC chromatographic separation of metabolites was achieved using a Millipore Sequant ZIC–pHILIC analytical column (5 μ m, 2.1×150 mm) equipped with a 2.1×20 mm guard column (both 5 mm particle size) with a binary solvent system. Solvent A was 20 mM ammonium carbonate and 0.05% ammonium hydroxide; solvent B was acetonitrile. The column oven and autosampler tray were held at 40 °C and 4 °C, respectively. The chromatographic gradient was run at a flow rate of 0.200 ml min⁻¹ as follows: 0–2 min: 80% solvent B; 2–17 min: linear gradient from 80% solvent B to 20% solvent B; 17–17.1 min: linear gradient from 20% solvent B to 80% solvent B; 17.1–22.5 min: hold at 80% solvent B. Samples were randomized and analysed by LC–MS in a blinded manner, and the injection volume was 5 μ l. Pooled samples were generated from an equal mixture of all individual samples and analysed interspersed at regular intervals within a sample sequence as a quality control. Metabolites were measured with a Thermo Scientific Q Exactive Hybrid Quadrupole–Orbitrap mass spectrometer coupled to a Dionex Ultimate 3000 UHPLC or with Vanquish Horizon UHPLC coupled to an Orbitrap Exploris 240 mass spectrometer (both Thermo Fisher Scientific) through a heated electrospray ionization source.

For the Thermo Scientific Q Exactive Hybrid Quadrupole–Orbitrap Mass spectrometer (HRMS) coupled to a Dionex Ultimate 3000 UHPLC, the mass spectrometer was operated in full-scan, polarity-switching mode, with the spray voltage set to +4.5 kV/–3.5 kV, the heated capillary held at 280 °C and the heated electrospray ionization probe held

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at 320 °C. The sheath gas flow was set to 40 units, the auxiliary gas flow was set to 15 units and the sweep gas flow was set to 0 unit. HRMS data acquisition was performed in a range of $m/z = 70-900$, with the resolution set at 70,000, the AGC target at 1×10^6 and the maximum injection time (max IT) at 120 ms. Metabolite identities were confirmed using two parameters: (1) precursor ion m/z was matched within 5 ppm of theoretical mass predicted by the chemical formula; (2) the retention time of metabolites was within 5% of the retention time of a purified standard run with the same chromatographic method. Chromatogram review and peak area integration were performed using the Thermo Fisher software XCalibur Qual Browser, XCalibur Quan Browser software and Tracefinder 5.0. The peak area for each detected metabolite was normalized against the total ion count of that sample to correct any variations introduced from sample handling through instrument analysis. Absolute quantification of 2SC was performed by interpolation of the corresponding standard curve obtained from serial dilutions of commercially available standards (Sigma Aldrich) running with the same batch of samples.

For the Orbitrap Exploris 240 mass spectrometer, MS1 scans, the mass range was set to $m/z = 70-900$, AGC target set to standard and maximum injection time (IT) set to auto. Data acquisition for experimental samples used full scan mode with polarity switching at an Orbitrap resolution of 120,000. Data acquisition for untargeted metabolite identification was performed using the AcquireX Deep Scan workflow, an iterative data-dependent acquisition strategy using multiple injections of the pooled sample. In brief, the sample was first injected in full scan-only mode in single polarity to create an automated inclusion list. MS2 acquisition was then carried out in triplicate, whereby ions on the inclusion list were prioritized for fragmentation in each run, after which both the exclusion and inclusion lists were updated in a manner such that fragmented ions from the inclusion list were moved to the exclusion list for the next run. Data-dependent acquisition full-scan ddMS2 method for AcquireX workflow used the following parameters: full scan resolution was set to 60,000, fragmentation resolution to 30,000 and fragmentation intensity threshold to 5.0×10^3 . Dynamic exclusion was enabled after 1 time and exclusion duration was 10 s. Mass tolerance was set to 5 ppm. The isolation window was set to 1.2 m/z . Normalized HCD collision energies were set to stepped mode with values at 30, 50 and 150. Fragmentation scan range was set to auto, AGC target at standard and max IT at auto. Xcalibur AcquireX method modification was on. Mild trapping was enabled.

Metabolite identification was performed using the Compound Discoverer software (v.3.2, Thermo Fisher Scientific). Metabolites were annotated at the MS2 level using both an in-house mzVault spectral database curated from 1,051 authentic compound standards and the online spectral library mzCloud. The precursor mass tolerance was set to 5 ppm and fragment mass tolerance set to 10 ppm. Only metabolites with mzVault or mzCloud best match score above 50% and 75%, respectively, and retention time tolerance within 0.5 min to that of a purified standard run with the same chromatographic method were exported to generate a list including compound names, molecular formula and retention time. The curated list was then used for further processing in the Tracefinder software (v.5.0, Thermo Fisher Scientific), in which extracted ion chromatographs for all compounds were examined and manually integrated if necessary. False positive, noise or chromatographically unresolved compounds were removed. The peak area for each detected metabolite was then normalized against the total ion count of that sample to correct any variations introduced from sample handling through instrument analysis. The normalized areas were used as variables for further statistical data analysis. Statistical analysis was performed using MetaboAnalyst (v.5.0)⁶⁶.

Stable isotope-assisted tracing. BMDMs (3 independent mice) were plated at 0.5×10^6 cells per well in 12-well plates in technical triplicate per condition and treated as indicated in glutamine-free DMEM

supplemented with U-¹³C-glutamine or ¹⁵N₂-glutamine, respectively. For ¹³C- and ¹⁵N-tracing analysis, the theoretical masses of ¹³C and ¹⁵N isotopes were calculated and added to a library of predicted isotopes in Tracefinder 5.0. These masses were then searched with a 5 ppm tolerance and integrated only if the peak apex showed less than 1% deviation in retention time from the [U-¹²C or ¹⁴N] monoisotopic mass in the same chromatogram. The raw data obtained for each isotopologue were corrected for natural isotope abundances using the AccuCor algorithm (<https://github.com/lparsons/accucor>) before further statistical analysis.

Ethidium bromide treatment

BMDMs were plated in the presence or absence of ultrapure ethidium bromide (100 ng ml⁻¹) and incubated for a further 6 days before treatment. Depletion of mtDNA was determined by genomic DNA isolation followed by qPCR using primers specific for areas of mitochondrial DNA (D-loop) and areas of mtDNA that are not inserted into nuclear DNA (non-NUMT).

c-Fos activity assay

BMDMs from 3 mice were plated in 10 cm dishes at 0.5×10^6 cells per ml and left overnight. Cells were pre-treated with FHN1 or DMF (3 h) before LPS stimulation (4 h). After collection, nuclear extracts were isolated using a Nuclear Extraction kit (ab113474) purchased from Abcam. Nuclear extracts were quantified using a BCA assay and standardized. c-Fos relative activity was then quantified using the AP-1 transcription factor assay purchased from Abcam (Ab207196) according to the manufacturer's protocol.

Fumarate assay

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

Nitrite measurement

The Griess reagent system (Promega G2930) was used according to the manufacturer's instructions.

RNA-seq

BMDMs (three independent mice) were treated as indicated and RNA was extracted as detailed above. mRNA was extracted from total RNA using poly-T-oligo-attached magnetic beads. After fragmentation, the first strand cDNA was synthesized using random hexamer primers, followed by the second strand cDNA synthesis. The library was checked using Qubit and real-time PCR for quantification and a bioanalyser for size distribution detection. Quantified libraries were pooled and sequenced on a NovaSeq 6000 S4 (Illumina). Differential expression analysis of two conditions per group was performed using counted reads and the DESeq2 R package⁶⁷. Pathway enrichment analyses were performed as indicated in quantification and statistical analysis section below.

Proteomic analysis

Sample preparation. BMDMs (from five independent mice) were plated onto 10 cm dishes and treated as indicated. At the experimental end point, cells were washed with PBS on ice and centrifuged at 1,500 r.p.m. for 5 min at 4 °C and frozen at -80 °C. Cell pellets were lysed, reduced and alkylated in 50 µl of 6 M Gu-HCl, 200 mM Tris-HCl pH 8.5, 10 mM TCEP, 15 mM chloroacetamide by probe sonication and heating to 95 °C for 5 min. Protein concentration was measured using a Bradford assay and initially digested with LysC (Wako) with an enzyme/substrate ratio of 1:200 for 4 h at 37 °C. Subsequently, the samples were diluted ten-fold with water and digested with porcine trypsin (Promega) at 37 °C overnight. Samples were acidified to 1% TFA, cleared by centrifugation

(16,000g at room temperature) and approximately 20 µg of the sample was desalted using a Stage-tip. Eluted peptides were lyophilized, resuspended in 0.1% TFA/water and the peptide concentration was measured by A280 on a nanodrop instrument (Thermo). The sample was diluted to 2 µg in 5 µl for subsequent analysis.

Mass spectrometry analysis. The tryptic peptides were analysed using a Fusion Lumos mass spectrometer connected to an Ultimate Ultra3000 chromatography system (both Thermo Scientific) incorporating an autosampler. In brief, 2 µg of de-salted peptides were loaded onto a 50 cm emitter packed with 1.9 µm ReproSil-Pur 200 C18-AQ (Dr Maisch) using a RSLC-nano uHPLC system connected to a Fusion Lumos mass spectrometer (both Thermo). Peptides were separated using a 140 min linear gradient from 5% to 30% acetonitrile, 0.5% acetic acid. The mass spectrometer was operated in DIA mode, acquiring a MS 350–1,650 Da at 120 k resolution followed by MS/MS on 45 windows with 0.5 Da overlap (200–2,000 Da) at 30 k with a NCE setting of 27.

Data analysis. Raw files were analysed and quantified by searching against the UniProt *Mus musculus* database using DIA-NN 1.8 (<https://github.com/vdemichev/DiaNN>). Library-free search was selected, and the precursor ion spectra were generated from the FASTA file using the deep-learning option. Default settings were used throughout apart from using 'Robust LC (high precision)'. In brief, carbamidomethylation was specified as the fixed modification whereas acetylation of protein amino termini was specified as the variable. Peptide length was set to a minimum of 7 amino acids, precursor false discovery rate (FDR) was set to 1%. Subsequently, missing values were replaced by a normal distribution (1.8π shifted with a distribution of 0.3π) to allow the following statistical analysis. Protein-wise linear models combined with empirical Bayes statistics were used for the differential expression analyses. We use the Bioconductor package *limma* to carry out the analysis using the information provided in the experimental design table.

Digitonin fractionation

BMDMs were plated at 0.5×10^6 cells per well and treated as desired. After treatment, cells were washed once with room temperature PBS before being scraped on ice into ice-cold PBS and pelleted at 500g for 5 min at 4 °C. Supernatant was removed and discarded, and the pellet was resuspended in 400 µl extraction buffer (150 mM NaCl, 50 mM HEPES pH 7.4, and 25 µg ml⁻¹ digitonin). Samples were then placed in a rotating mixer at 4 °C for 10 min before centrifugation at 2,000g at 4 °C for 5 min. The resulting supernatant constituted the cytosolic fraction, from which RNA and DNA were isolated using an AllPrep DNA/RNA Mini kit (Qiagen). Alternatively, the cytosolic fraction was concentrated using Strataclean resin (Agilent) and analysed by western blotting. The pellet constituted a fraction containing membrane-bound organelles, which was lysed in RNA lysis buffer for RNA isolation or lysed in western blot lysis buffer for analysis by western blotting. To determine the presence of mtRNA and mtDNA in the cytosol, qPCR was performed using primers specific for mitochondrial D-loop on cDNA, which had been reverse-transcribed from RNA isolated from the cytosolic fraction (mtRNA) and on DNA isolated from the cytosolic fraction (mtDNA). In both cases, values were normalized using a housekeeping control gene (*Actb*, which encodes β-actin) amplified in cDNA, which had been reverse-transcribed from RNA isolated from the membrane-bound fraction.

MAVS oligomerization

BMDMs were plated at 1×10^6 cells per well in technical triplicate and treated as desired. After treatment, cells were washed twice with 200 µl cold PBS before being lysed in crosslinking lysis buffer (50 mM HEPES, 0.5% Triton X-100 and 1× protease inhibitor cocktail). Samples were placed on ice for 15 min. Lysates were centrifuged for 15 min at 6,000g at 4 °C and the supernatant was removed and frozen down as the soluble

fraction. Next, 20 µl of the soluble fraction was mixed with 5 µl of sample lysis buffer (0.125 M Tris pH 6.8, 10% glycerol, 0.02% SDS and 5% DTT) and run on a 10% gel. The insoluble pellet was resuspended in HEPES (50 mM) and washed 3 times by centrifuging at 6,000g at 4 °C and removing the supernatant each time. After the final wash, the pellet was resuspended in 500 µl crosslinking buffer (50 mM HEPES and 150 mM NaCl) and disuccinimidyl suberate (Thermo Fisher, made up in anhydrous DMSO) was added to the final concentration of 2 mM. Immediately following the addition of disuccinimidyl suberate, the sample was inverted several times and incubated for 45 min at 37 °C. The sample was then centrifuged for 15 min at 6,000g at 4 °C, before the supernatant was removed and the pellet was resuspended in 30 µl sample lysis buffer. The resuspended insoluble fraction was subsequently boiled for 5 mins at 95 °C before being run on a gel.

Seahorse XF glycolysis stress test

Cells were plated at 100,000 cells per well in 100 µl and were left overnight to adhere. The protocol was carried out according to the manufacturer's instructions (Agilent). In brief, cells were treated as required, after which the medium was replaced with Seahorse medium containing glutamine (2 mM). Cells were then placed in a CO₂-free incubator for 1 h. The glycolysis stress test was subsequently performed using a Seahorse XFe96 analyzer (Agilent) with the following injections: glucose (10 mM); oligomycin (1 µM); and 2-DG (50 mM).

Analysis was performed using Seahorse Wave software (Agilent). Data shown are representative experiments containing at least three pooled biological replicates.

Seahorse XF mito stress test

Cells were plated at 100,000 cells per well in 100 µl and were left overnight to adhere. The protocol was carried out according to manufacturer's instructions (Agilent). In brief, cells were treated as required, after which the medium was replaced with Seahorse medium containing glutamine (2 mM), glucose (10 mM) and pyruvate (1 mM). Cells were then placed in a CO₂-free incubator for 1 h. The mito stress test was subsequently performed using a Seahorse XFe96 analyzer (Agilent) with the following injections: oligomycin (1 µM); FCCP (1 µM); and rotenone (500 nM)

Analysis was performed using Seahorse Wave software (Agilent). Data shown are representative experiments containing at least three pooled biological replicates.

LPS-induced inflammation model

Male mice (6 weeks old) were used, and littermates were randomly assigned to experimental groups. Compounds were resuspended in 10% DMSO followed by 90% cyclodextrin in PBS (20% w/v). Mice were intraperitoneally injected with vehicle, FHIN1 or DMF (both 50 mg kg⁻¹) at a volume of 200 µl per injection. After 1 h, mice were intraperitoneally injected with PBS or LPS from *E. coli* (2.5 mg kg⁻¹, Sigma) at a volume of 100 µl per injection. After 2 h, mice were euthanized and blood was collected retro-orbitally. Blood was allowed to clot for 30 min at room temperature before it was centrifuged at 5,000g for 10 min at 4 °C. The serum was removed and the IFNβ concentration was determined by ELISA.

Western blotting

Supernatant was removed from cells following stimulation, and lysates were collected in 30–50 µl lysis buffer (0.125 M Tris pH 6.8, 10% glycerol, 0.02% SDS and 5% DTT) Lysates were subsequently heated to 95 °C for 5 min to denature proteins. SDS-PAGE was used to resolve proteins by molecular weight. Samples were boiled at 95 °C for 5 min before loading into a 5% stacking gel. The percentage resolving gel depended on the molecular weight of the given protein. The Bio-Rad gel running system was used to resolve proteins, and the Bio-Rad wet transfer system was used for the electrophoretic transfer of proteins

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onto a PVDF membrane. Following transfer, the membrane was incubated in milk powder (5% in TBST) for 1 h and subsequently incubated in primary antibody rolling overnight at 4 °C. Primary antibodies targeting phospho-proteins were diluted in BSA (5% in TBST) as opposed to milk. The membrane was incubated for 1 h with secondary antibody (diluted in 5% milk powder) at room temperature. Before visualization, the membrane was immersed in WesternBright ECL Spray (Advansta). Protein visualization was performed using a ChemiDoc MPTM imaging system (Bio-Rad), and both chemiluminescent and white light images were taken. Images were analysed using Image Lab 6.0.1 (Bio-Rad).

ELISA. DuoSet ELISA kits for IL-1 β , TNF, IL-6, IL-10 and GDF15 were purchased from R&D Systems and were carried out according to the manufacturer's instructions with appropriately diluted cell supernatants added to each plate in duplicate or triplicate. IFN β was determined using a DuoSet ELISA kit from R&D Systems or Abcam (ab252363). Quantikine ELISA kit for IFN β (R&D Systems) was used for determination of IFN β concentrations in serum samples and from human cells, and these were also carried out according to the manufacturer's instructions. Absorbance at 450 nm was quantified using a FLUOstar Optima plate reader. Corrected absorbance values were calculated by subtracting the background absorbance, and cytokine concentrations were subsequently obtained by extrapolation from a standard curve plotted using GraphPad Prism 9.2.0.

Quantification and statistical analyses

Details of all statistical analyses performed are provided in the figure legends. Data are expressed as the mean \pm s.e.m. unless stated otherwise. Representative western blots are shown. For metabolomics data, MetaboAnalyst (v.5.0)⁶⁶ was used to analyse, perform statistics and visualize the results. Autoscaling of features (metabolites) was used for heatmap generation. One-way analysis of variance (ANOVA) corrected for multiple comparisons using the Tukey statistical test was used, and a *P*-adjusted < 0.05 was set as the cut-off. For proteomics data, protein signal intensity was converted to a log₂ scale, and biological replicates were grouped by experimental condition. Protein-wise linear models combined with empirical Bayes statistics were used for the differential expression analyses. The Bioconductor package limma was used to carry out the analysis using a R-based online tool⁶⁸. Data were visualized using a heatmap with autoscaled features (genes) and a volcano plot, which shows the log₂(fold change) on the *x* axis and the -log₁₀(adjusted *P* value) on the *y* axis. The proteomics cut-off values for analysis were a log₂(fold change) of 0.5 and a FDR < 0.05, which were determined using *t*-statistics. RNA-seq cut-off values were set to log₂(fold change) of 1 and FDR < 0.05. Overrepresentation analysis of significant changes were assessed using Enrichr and the Bioconductor package clusterProfiler 4.0 in R (v.3.6.1). Further information on this visualization method is available⁶⁹. Emapplots were generated using the enrichplot package in R (v.3.6.1). GSEA analysis of RNA-seq was performed using the Broad Institutes GSEA (v.4.1.0)⁷⁰. Graphpad Prism v.9.2.0 was used to calculate statistics in bar plots using appropriate statistical tests depending on the data, including one-way ANOVA, two-tailed unpaired *t*-test and multiple *t*-tests. Adjusted *P* values were assessed using appropriate correction methods, such as Tukey, Kruskal–Wallis and Holm–Sidak tests. Sample sizes were determined on the basis of previous experiments using similar methodologies. All depicted data points are biological replicates taken from distinct samples unless stated otherwise. Each figure consists of a minimum of three independent experiments from multiple biological replicates unless

stated otherwise. For in vivo studies, mice were randomly assigned to treatment groups. For metabolomics, proteomics and RNA-seq analyses, samples were processed in random order and experimenters were blinded to experimental conditions.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Proteomics data from Fig. 1d were previously deposited¹¹ to the ProteomeXchange Consortium through the PRIDE partner repository with the dataset identifier PXD029155. All other proteomics, RNA-seq data and metabolomics data have been deposited to Dryad (<https://doi.org/10.5061/dryad.6wwpzn28>). All other data are available from the corresponding authors upon request. Source data are provided with this paper.

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Author contributions A.H., C.G.P., D.G.R. and L.A.J.O. conceptualized the project. A.H., C.G.P. and D.G.R. were lead experimentalists, provided intellectual input, designed all experiments, analysed and visualized the data and co-wrote the paper with input from all authors. E.A.D. performed in vivo experiments. E.N.M., L.H., G.D.L.S., M.I., D.J.W., S.V. and C. Jefferies generated data from patients with SLE. J.E.T.-K. assisted with immunofluorescence experiments. C.F., M. Yang, A.S.H.C. and E.N. assisted with metabolomics. A.B.-C. and A.V.K. assisted with proteomics. A.F.M., M. Yin, T.A.J.R., A.M.C. and H.A.P. performed in vitro experiments. C.F. and V.Z. provided inducible *Fh1tm* and *Fh1^{fl/fl}* mouse tissue. N.F. verified protein succination with 2SC antibody on provided macrophage lysates. C. Johansson provided *Mavs^{-/-}* mouse tissue. M.P.M. and C.F. provided intellectual input and oversaw a portion of the research programme. L.A.J.O. obtained funding and oversaw the research programme.

Competing interests The authors declare no competing interests.

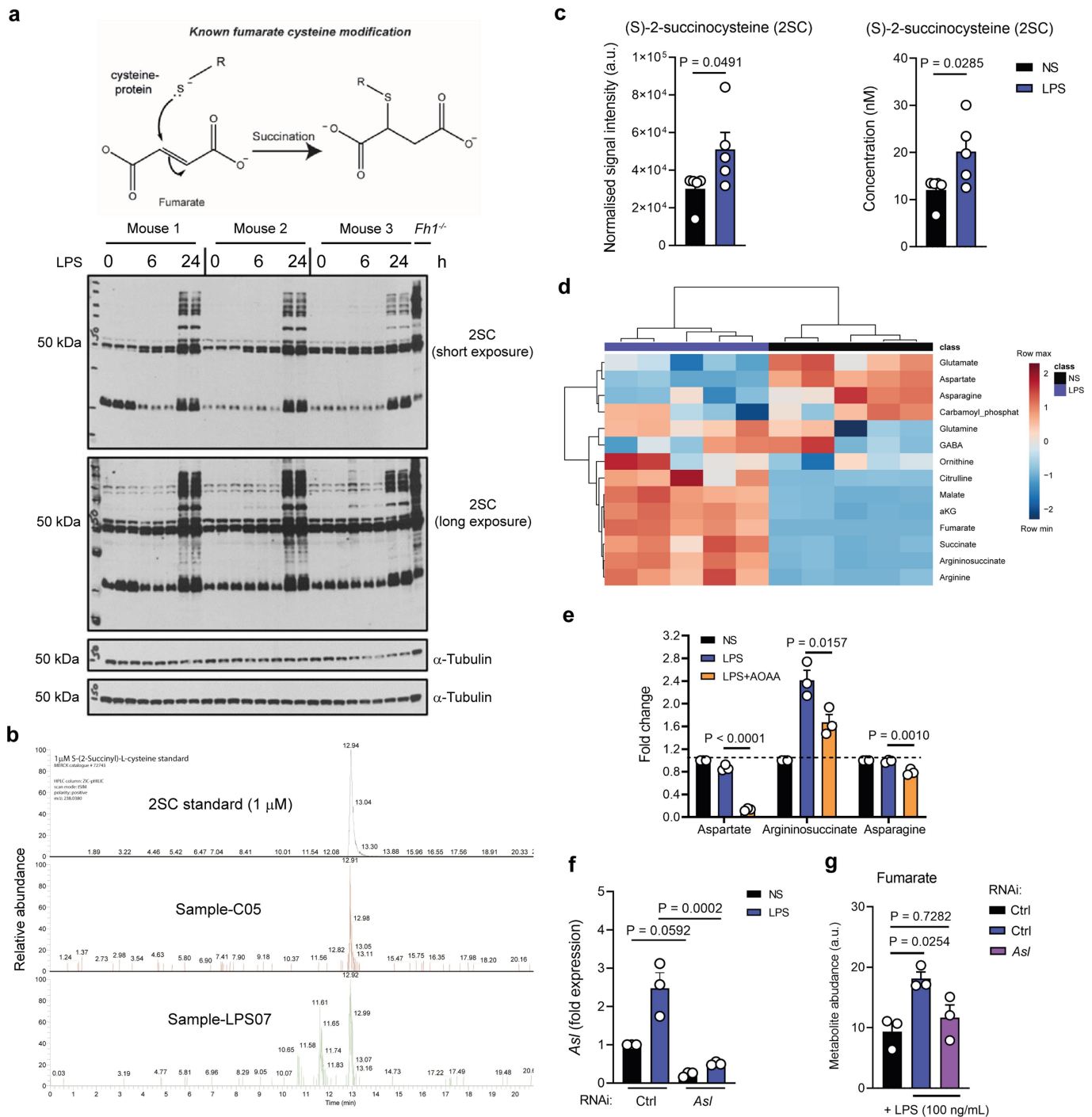
Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-023-05720-6>.

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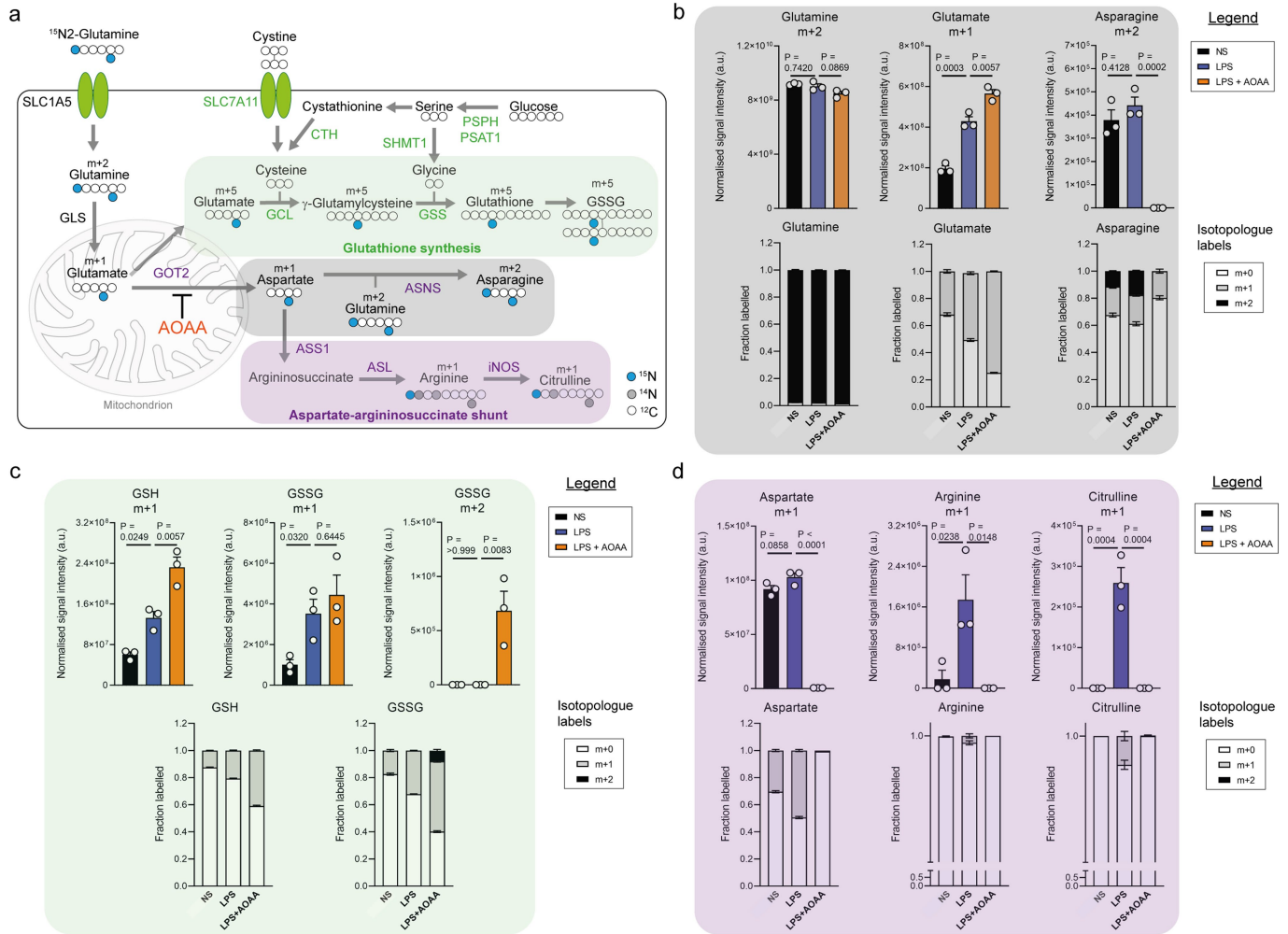
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Extended Data Fig. 1 | LPS stimulation drives fumarate accumulation and protein succination. **a-c**, Fumarate-mediated protein succination with LPS ($n = 3$) and 2SC abundance in NS and LPS-stimulated BMDMs ($n = 5$; LPS 4 h). **d**, Heatmap of metabolites linked to aspartate-argininosuccinate shunt in NS and LPS-stimulated BMDMs ($n = 5$; LPS 24 h). **e**, Metabolite abundance of aspartate-argininosuccinate shunt metabolites in LPS-stimulated BMDMs

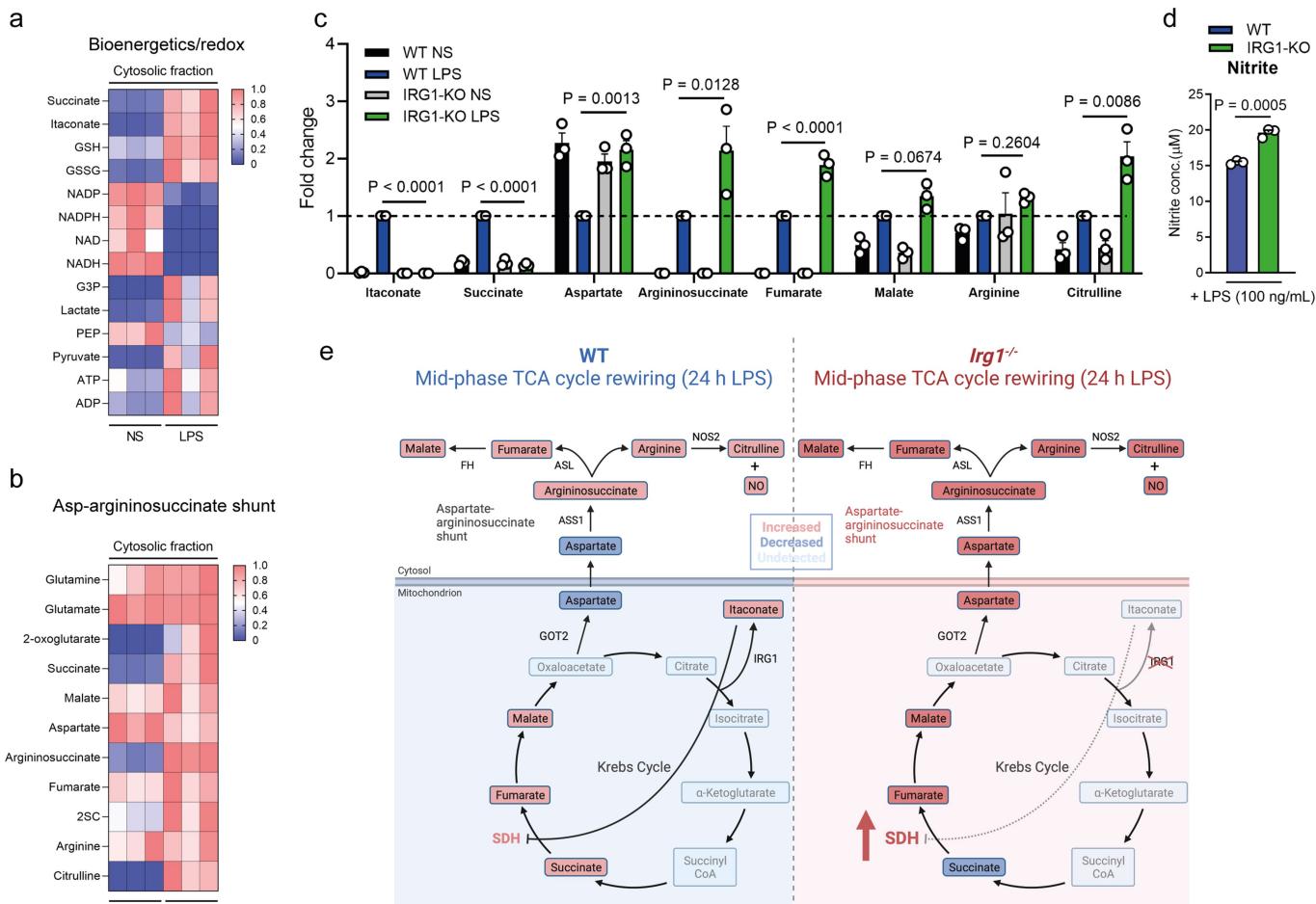
pre-treated with DMSO or AOAA ($n = 3$; LPS 4 h; aspartate ($P = 0.000005$)). **f**, *Asl* expression with silencing of *Asl* following LPS stimulation ($n = 3$; LPS 24 h). **g**, Fumarate levels with silencing of *Asl* following LPS stimulation ($n = 3$; LPS 24 h). **c-e-g**, Data are mean \pm s.e.m. **a**, 1 representative blot of 3 shown. $n =$ biological replicates. P values calculated using two-tailed Student's t -test for paired comparisons or one-way ANOVA for multiple comparisons.



Extended Data Fig. 3 | LPS stimulation drives fumarate accumulation via glutamine anaplerosis and an aspartate-argininosuccinate shunt.

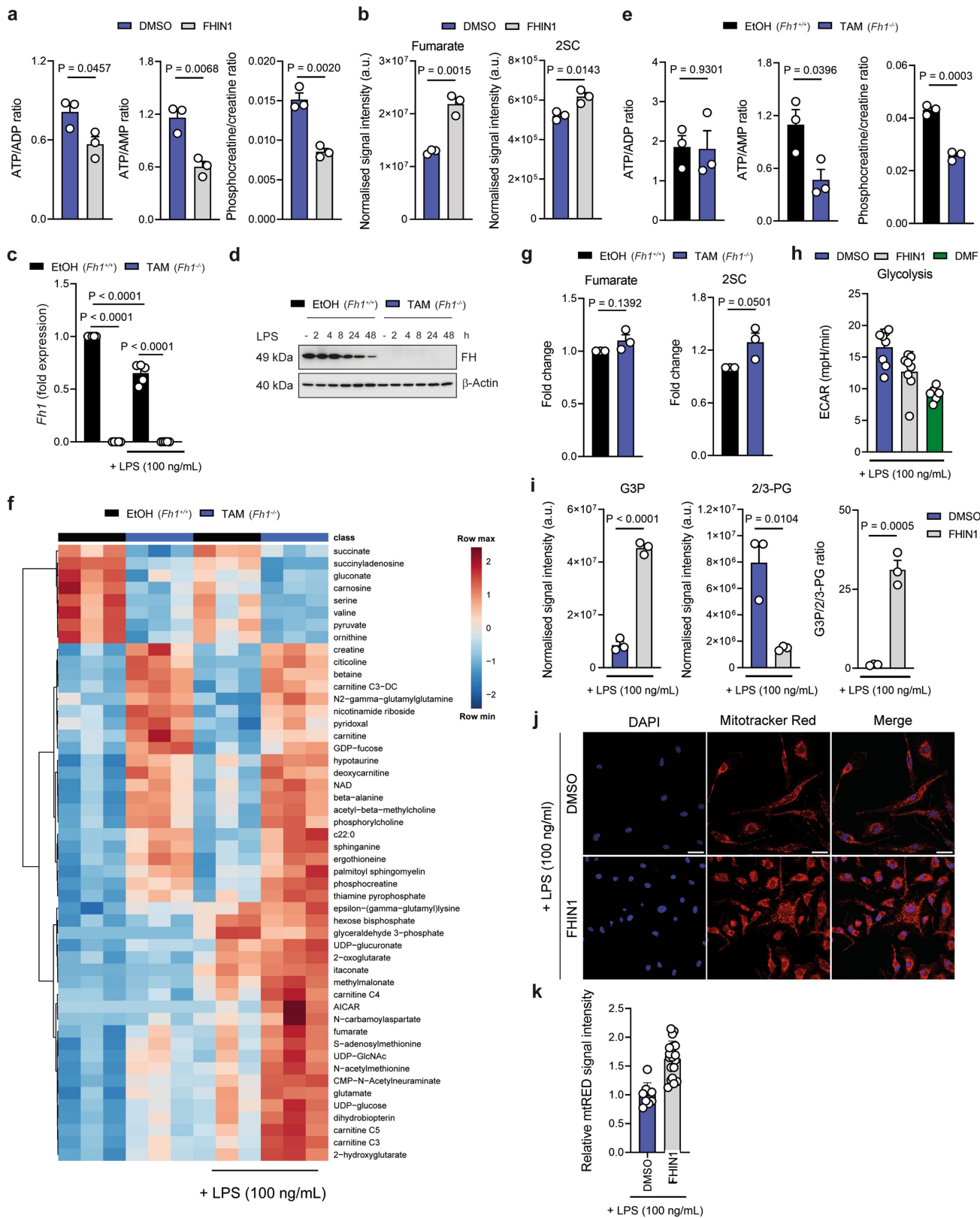
a, Schematic diagram indicating $^{15}\text{N}_2$ -glutamine tracing into distinct metabolic modules. **b**, $^{15}\text{N}_2$ -glutamine tracing into glutamate and asparagine in LPS-treated BMDMs (m+1 and m+2 labelling intensity and total isotopologue fraction distribution) ($n = 3$; LPS 4 h). **c**, $^{15}\text{N}_2$ -glutamine tracing into GSH and GSSG in

LPS-treated BMDMs (m+1 and m+2 labelling intensity and total isotopologue fraction distribution) ($n = 3$; LPS 4 h). **d**, $^{15}\text{N}_2$ -glutamine tracing into aspartate, arginine and citrulline in LPS-treated BMDMs (m+1 labelling intensity and total isotopologue fraction distribution) ($n = 3$; LPS 4 h; aspartate ($P = 0.000001$)). Data are mean \pm s.e.m. $n =$ biological replicates. P values calculated using one-way ANOVA for multiple comparisons.



Extended Data Fig. 4 | Increase in aspartate-argininosuccinate shunt metabolites in cytosol and *Irg1*^{-/-} macrophages. Heatmap (min-max) of metabolites linked to mitochondrial bioenergetics and redox signalling (**a**) and the aspartate-argininosuccinate shunt (**b**) in NS and BMDMs ($n = 3$; LPS 24 h). **c**, Metabolite abundance of TCA cycle and aspartate-argininosuccinate shunt metabolites in WT and *Irg1*^{-/-} BMDMs ($n = 3$; LPS 24 h); itaconate ($P = 0.000000000000002$, succinate ($P = 0.00000003$), fumarate

($P = 0.000018$)). **d**, Nitrite levels in WT and *Irg1*^{-/-} BMDMs ($n = 3$; LPS 24 h). **e**, Schematic of metabolic changes occurring during mid-phase TCA cycle rewiring in WT and *Irg1*^{-/-} BMDMs. Data are mean \pm s.e.m. n = biological replicates. P values calculated using two-tailed Student's t-test for paired comparisons or one-way ANOVA for multiple comparisons. Schematic in panel **e** was created using BioRender (<https://biorender.com>).

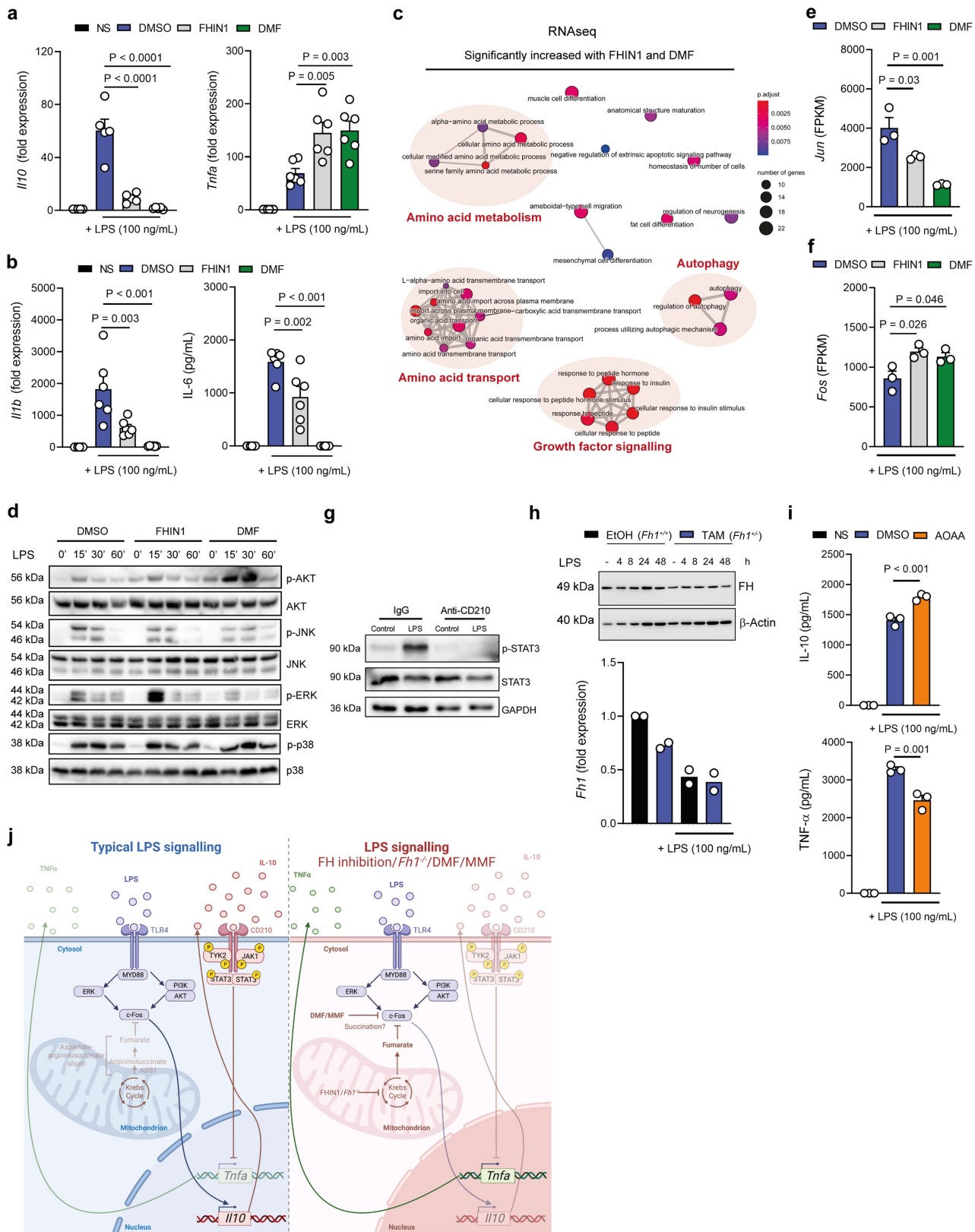


Extended Data Fig. 5 | See next page for caption.

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Extended Data Fig. 5 | FH deletion increases bioenergetic stress, fumarate, and mitochondrial membrane potential. a, Bioenergetic ratios in BMDMs treated with DMSO or FHINI ($n = 3$). **b**, Fumarate and 2SC levels in BMDMs treated with DMSO or FHINI ($n = 3$). qPCR ($n = 5$) (**c**) and western blot ($n = 2$) (**d**) analysis of *Fh1* expression in *Fh1*^{+/+} and *Fh1*^{-/-} BMDMs (EtOH/TAM 72 h; LPS 4 h; *Fh1*^{+/+} NS vs *Fh1*^{+/+} LPS ($P = 0.00000002$), *Fh1*^{+/+} NS vs *Fh1*^{-/-} NS ($P = 0.00000000000002$), *Fh1*^{-/-} NS vs *Fh1*^{-/-} LPS ($P = 0.0000000000014$)). **e**, Bioenergetic ratios in *Fh1*^{+/+} and *Fh1*^{-/-} BMDMs ($n = 3$; EtOH/TAM 48 h). **f**, Heatmap of top 50 significantly abundant metabolites in *Fh1*^{+/+} and *Fh1*^{-/-} BMDMs ($n = 3$; LPS 4 h). **g**, Fumarate and 2SC levels in *Fh1*^{+/+} and *Fh1*^{-/-} BMDMs ($n = 3$; EtOH/TAM 72 h). **h**, Glycolysis as measured by ECAR in BMDMs pre-treated with DMSO, FHINI or DMF ($n = 8$ (DMSO/FHINI); $n = 6$ (DMF); LPS 4 h).

$n =$ technical replicates from 1 experiment performed with 3 pooled biological replicates. Data are mean \pm s.d. **i**, Glyceraldehyde 3-phosphate (G3P) and 2,3-phosphoglycerate (2/3-PG) levels and ratio in BMDMs pre-treated with DMSO or FHINI ($n = 3$; LPS 4 h; G3P ($P = 0.00004$)). Immunofluorescence (**j**) and quantification (**k**) of Mitotracker red staining in BMDMs pre-treated with DMSO or FHINI ($n = 8$ (DMSO); $n = 19$ (FHINI); LPS 4 h). $n =$ technical replicates from representative experiment. Scale bar = 20 μ m. Data are mean \pm s.d. **a-c, e, g, i** Data are mean \pm s.e.m. Representative blots or images of 2 (**d**) or 1 experiment(s) (**j**) shown. $n =$ biological replicates unless stated otherwise. P values calculated using two-tailed Student's t-test for paired comparisons or one-way ANOVA for multiple comparisons.

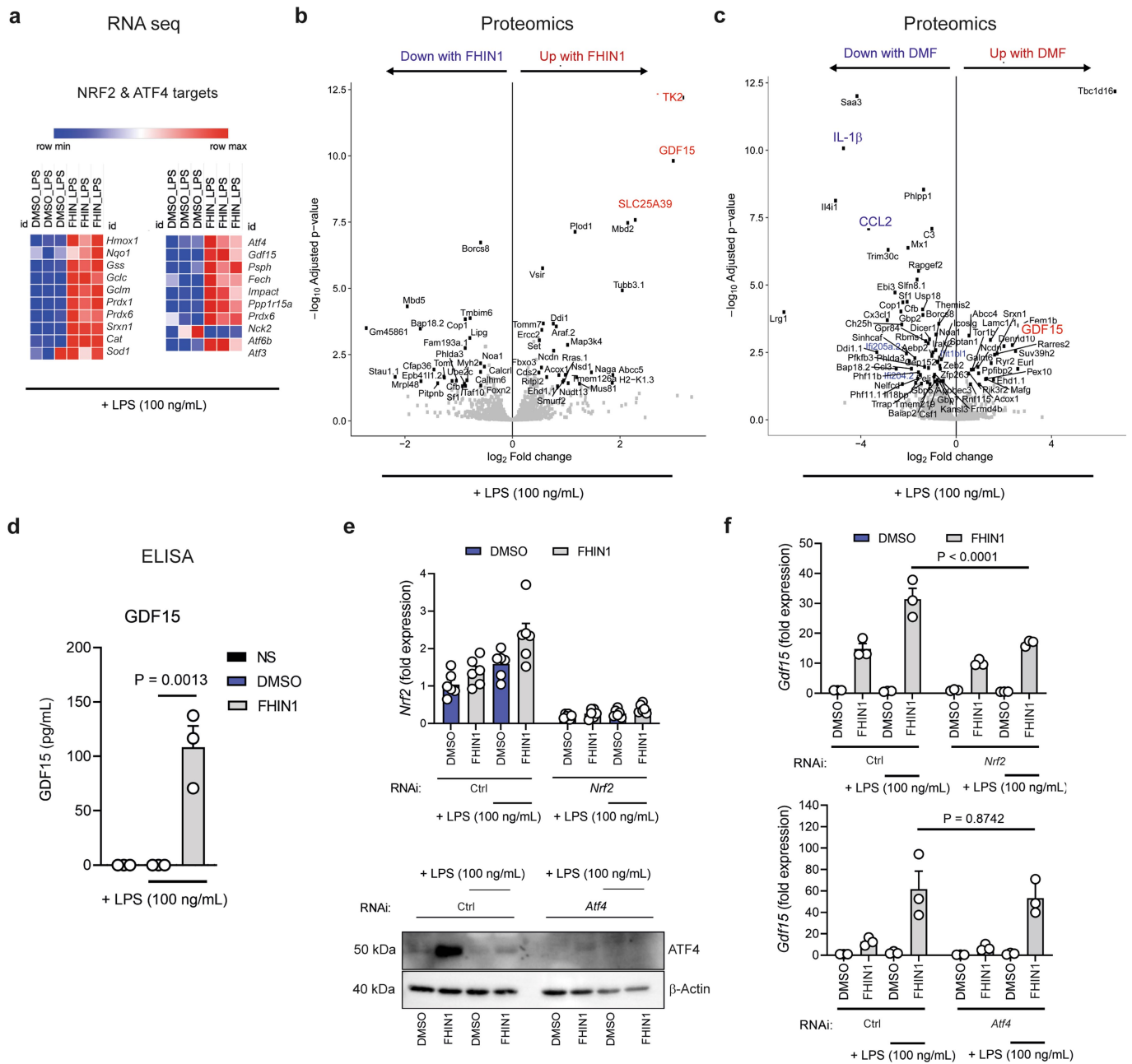


Extended Data Fig. 6 | See next page for caption.

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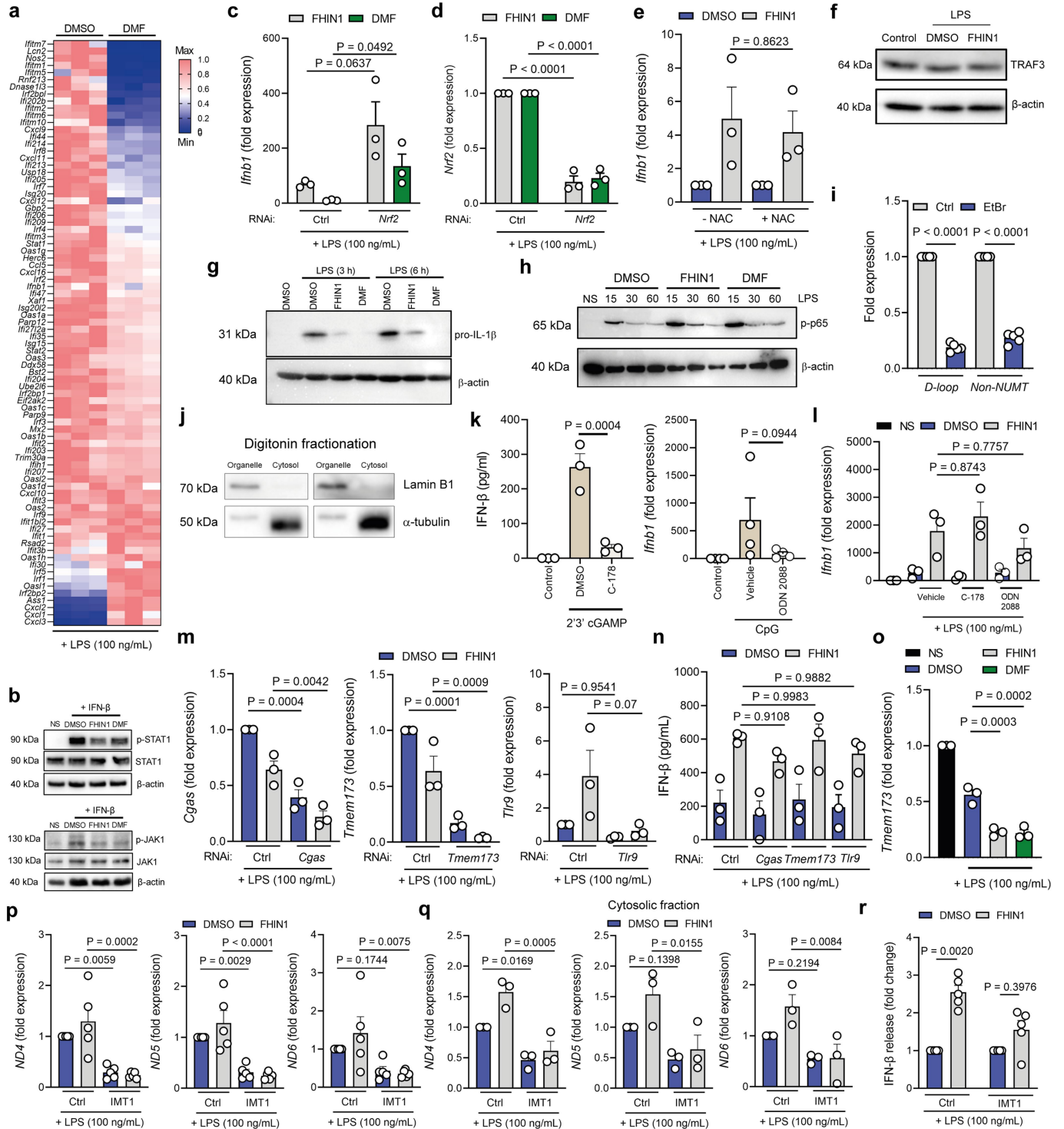
Extended Data Fig. 6 | FH inhibition remodels inflammatory gene expression. **a**, *Il10* and *Tnfa* expression in BMDMs pre-treated with DMSO, FHIN1 or DMF ($n = 5$ (*Il10*); $n = 6$ (*Tnfa*); LPS 4 h; FHIN1/*Il10* $P = 0.000002$, DMF/*Il10* $P = 0.0000004$). **b**, *Il1b* expression and IL-6 release in BMDMs pre-treated with DMSO, FHIN1 or DMF ($n = 6$; 4 h LPS; DMF/*Il1b* ($P = 0.000046$), DMF/IL-6 ($P = 0.0000002$)). **c**, Enrichment map plot of shared significantly increased genes in BMDMs pre-treated with DMF or FHIN1 compared to DMSO control ($n = 3$; LPS 4 h). **d**, Western blot of total and phospho-AKT, JNK, ERK and p38 levels in BMDMs pre-treated with DMSO, FHIN1 or DMF ($n = 2$). **e**, *Jun* expression in RNA seq from BMDMs pre-treated with DMF or FHIN1 compared to DMSO control ($n = 3$; LPS 4 h). **f**, *Fos* expression in RNA seq from BMDMs pre-treated with DMF or FHIN1 compared to DMSO control ($n = 3$; LPS 4 h).

g, Western blot of total and phospho-STAT3 levels in BMDMs pre-treated with anti-CD210 antibody (1 h) ($n = 4$; LPS 4 h). **h**, FH protein and gene expression levels in *Fh1^{+/+}* and *Fh1^{-/-}* BMDMs ($n = 2$; EtOH/TAM 72 h). Data are mean. **i**, ELISA of IL-10 and TNF- α release in BMDMs pre-treated with DMSO or AOAA ($n = 3$; LPS 4 h; IL-10 ($P = 0.000483$)). **j**, Schematic depicting mild suppression of IL-10 expression during typical LPS signalling (left), and increased suppression of IL-10 following FH inhibition, leading to dysregulated TNF- α release (right). **a, b, e, f, i** Data are mean \pm s.e.m. 1 representative blot of 2 (**d, h**) or 4 (**g**) shown. $n =$ biological replicates. P values calculated using two-tailed Student's t -test for paired comparisons or one-way ANOVA for multiple comparisons. Schematic in panel **j** was created using BioRender (<https://biorender.com>).



Extended Data Fig. 7 | FH inhibition triggers the NRF2 and ATF4 stress response and promotes GDF15 release. **a**, Heatmap of significantly differentially expressed RNA seq data in BMDMs pre-treated with FHIN1 compared to DMSO control ($n = 3$; LPS 4 h). **b**, Volcano plots of proteomics in BMDMs pre-treated with DMSO, FHIN1 (**b**) or DMF (**c**) ($n = 5$; LPS 4 h). **d**, ELISA of GDF15 in BMDMs pre-treated with DMSO or FHIN1 ($n = 3$; LPS 4 h). **e**, *Nrf2*

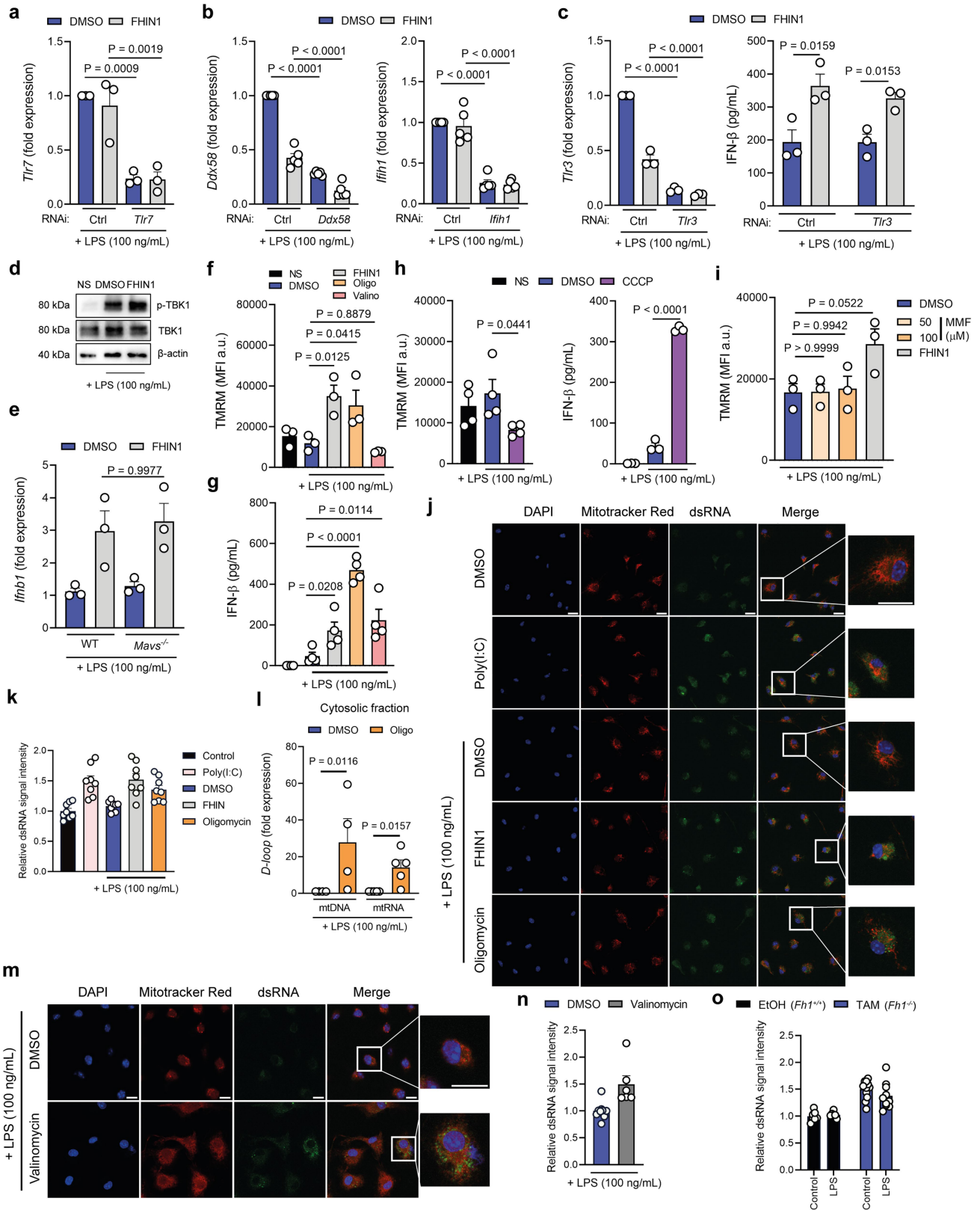
expression or ATF4 protein levels after silencing of *Nrf2* or *Atf4*, respectively, in BMDMs pre-treated with DMSO or FHIN1 ($n = 6$; LPS 4 h). **f**, *Gdf15* expression after silencing of *Nrf2* or *Atf4* respectively in BMDMs pre-treated with DMSO or FHIN1 ($n = 3$, LPS 4 h; FHIN1/*Nrf2* RNAi ($P = 0.000048$)). **d-f**, Data are mean \pm s.e.m. **e**, 1 representative blot of 6 shown. $n =$ biological replicates unless stated otherwise. P values calculated using one-way ANOVA for multiple comparisons.



Extended Data Fig. 8 | See next page for caption.

Extended Data Fig. 8 | IFN- β release following FH inhibition is independent of cGAS-STING. **a**, Heatmap (min-max) of significantly differentially expressed RNA seq data in BMDMs pre-treated with DMSO or DMF ($n = 3$; LPS 4 h). **b**, Phospho-STAT1, STAT1, phospho-JAK1 and JAK1 levels in BMDMs pre-treated with DMSO, FHIN1 or DMF ($n = 3$; LPS 4 h). **c**, *Ifnb1* expression after silencing of *Nrf2* in BMDMs pre-treated with DMSO, FHIN1 or DMF ($n = 3$, LPS 4 h). **d**, *Nrf2* expression after silencing of *Nrf2* in BMDMs pre-treated with DMSO, FHIN1 or DMF ($n = 3$, LPS 4 h; FHIN1 ($P = 0.0000008$), DMF ($P = 0.0000012$)). **e**, *Ifnb1* expression in BMDMs pre-treated with DMSO or FHIN1 in the presence of NAC ($n = 3$; LPS 4 h). **f**, TRAF3 levels in BMDMs pre-treated with DMSO or FHIN1 ($n = 3$; LPS 4 h). **g**, IL-1 β levels in BMDMs pre-treated with DMSO, FHIN1 or DMF ($n = 3$). **h**, p-p65 levels in BMDMs pre-treated with DMSO, FHIN1 or DMF ($n = 3$). **i**, *D-loop* and *Non-NUMT* DNA fold expression in ethidium bromide (EtBr)-treated BMDMs ($n = 5$; *D-loop* ($P = 0.00000000031$), *Non-NUMT* ($P = 0.0000000012$)). **j**, Lamin B1 and α -tubulin in cytosolic and membrane-bound organelle fractions following digitonin fractionation ($n = 3$). **k**, IFN- β release from 2',3' cGAMP- or

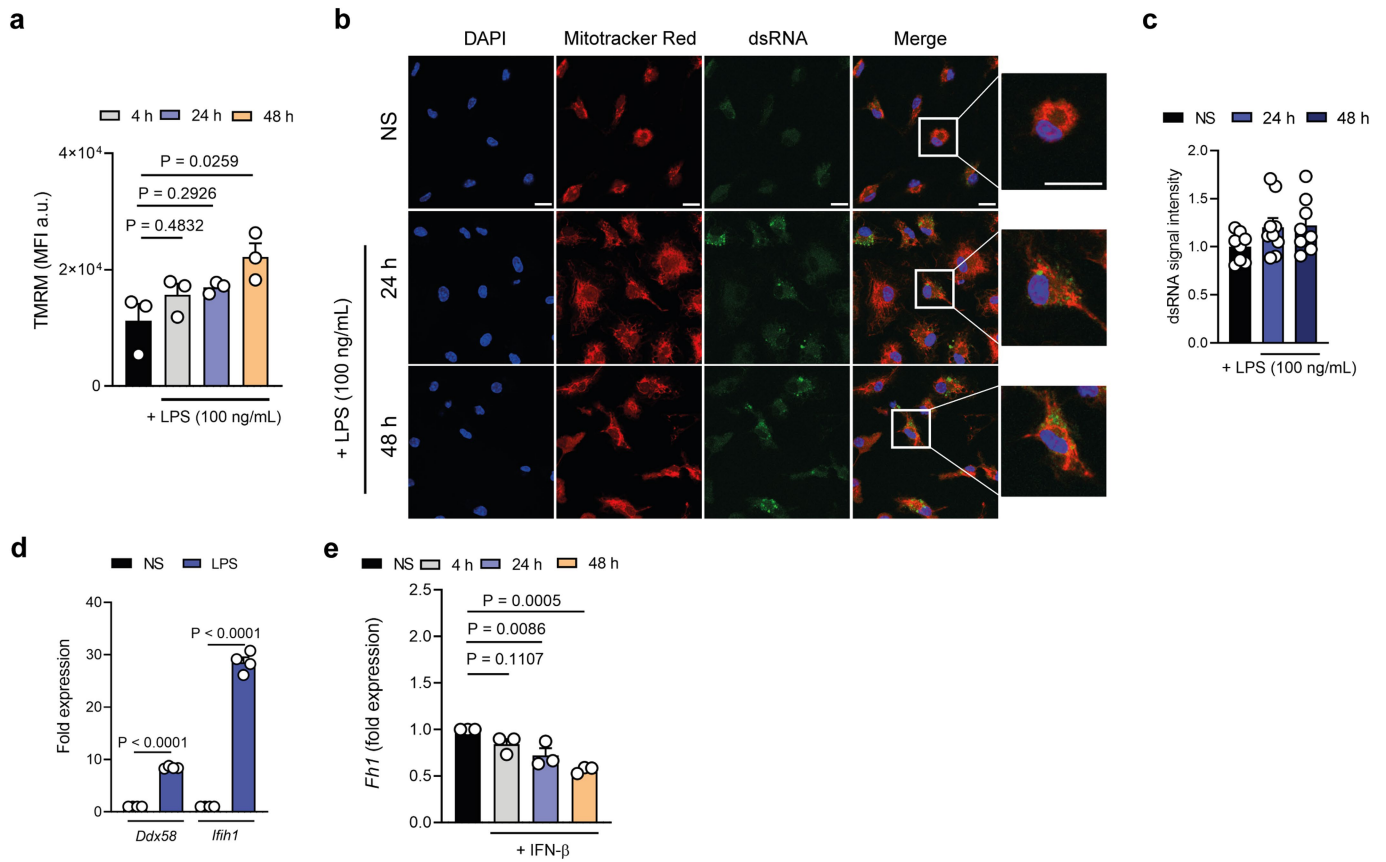
CpG-transfected BMDMs pre-treated (1 h) with C-178 or ODN2088 ($n = 3$ (cGAMP); $n = 4$ (CpG); 3 h). **l**, *Ifnb1* expression in BMDMs pre-treated with DMSO or FHIN1 in conjunction with C-178 or ODN2088 (1 h) respectively ($n = 3$; LPS 4 h). **m**, *Cgas*, *Tmem173* and *Tlr9* expression with silencing of *Cgas*, *Tmem173* and *Tlr9* respectively in BMDMs pre-treated with DMSO or FHIN1 ($n = 3$; LPS 4 h). **n**, IFN- β release with silencing of *Cgas*, *Tmem173* and *Tlr9* respectively from BMDMs pre-treated with DMSO or FHIN1 ($n = 3$; LPS 4 h). **o**, *Tmem173* expression in BMDMs pre-treated with DMSO, FHIN1 or DMF ($n = 3$, LPS 4 h). **p**, *ND4*, *ND5* and *ND6* RNA levels in whole cell extracts of BMDMs pre-treated with DMSO or FHIN1 in the presence of IMT1 ($n = 5$; LPS 4 h; *ND5* ($P = 0.000052$)). **q**, *ND4*, *ND5* and *ND6* RNA levels in cytosolic extracts of BMDMs pre-treated with DMSO or FHIN1 in the presence or absence of IMT1 ($n = 5$; LPS 4 h). **r**, IFN- β release in BMDMs pre-treated with DMSO or FHIN1 in the presence of IMT1 ($n = 3$; LPS 4 h). **c-e, i, k-r**, Data are mean \pm s.e.m. **b, f, h, j**, 1 representative blot of 3 shown. $n =$ biological replicates. P values calculated using two-tailed Student's t -test for paired comparisons or one-way ANOVA for multiple comparisons.



Extended Data Fig. 9 | See next page for caption.

Extended Data Fig. 9 | Mitochondrial membrane potential modifiers increase mtRNA and trigger IFN- β release. **a**, *Tlr7* expression with silencing of *Tlr7* in BMDMs pre-treated with DMSO or FHINI ($n = 3$; LPS 4 h). **b**, *Ddx58* and *Irfih1* expression with silencing of *Ddx58* and *Irfih1* respectively in BMDMs pre-treated with DMSO or FHINI ($n = 5$; LPS 4 h; DMSO/*Ddx58* ($P = 0.000000000002$), FHINI/*Ddx58* ($P = 0.000000813792$), DMSO/*Irfih1* ($P = 0.00000009$), FHINI/*Irfih1* ($P = 0.00000014$)). **c**, *Tlr3* expression and IFN- β release with silencing of *Tlr3* in BMDMs pre-treated with DMSO or FHINI ($n = 3$; LPS 4 h; DMSO/*Tlr3* ($P = 0.000000007$), FHINI/*Tlr3* ($P = 0.000013487$)). **d**, TBK1 and p-TBK1 in BMDMs pre-treated with DMSO or FHINI ($n = 3$; LPS 4 h). **e**, *Irfnb1* expression in WT and *Mavs*^{-/-} BMDMs pre-treated with DMSO or FHINI ($n = 3$; LPS 4 h). **f**, MFI of TMRM staining in BMDMs pre-treated with DMSO, FHINI, oligomycin or valinomycin ($n = 3$; LPS 4 h). **g**, IFN- β release from BMDMs pre-treated with DMSO, FHINI, oligomycin or valinomycin ($n = 4$; LPS 4 h; oligomycin ($P = 0.0000003$)). **h**, MFI of TMRM staining and IFN- β release from BMDMs pre-treated with DMSO or CCCP ($n = 4$ (TMRM), $n = 3$ (IFN- β); LPS 4 h; CCCP/IFN- β ($P = 0.00000008$)). **i**, MFI of TMRM staining in BMDMs pre-treated

with DMSO or MMF ($n = 3$; LPS 4 h). Immunofluorescence (**j**) and quantification (**k**) of dsRNA in BMDMs pre-treated with DMSO, FHINI or oligomycin or transfected with poly (I:C) ($n = 8$; LPS 4 h). $n =$ technical replicates from representative experiment. Data are mean \pm s.d. Scale bar = 20 μ m. **l**, *D-loop* fold expression in DNA and RNA isolated from cytosolic fractions of digitonin-fractionated BMDMs pre-treated with DMSO or oligomycin ($n = 4$ for mtDNA, $n = 5$ for mtRNA). Immunofluorescence (**m**) and quantification (**n**) of dsRNA in BMDMs pre-treated with DMSO or valinomycin ($n = 9$ (DMSO); $n = 6$ (Valinomycin); LPS 4 h). $n =$ technical replicates from representative experiment. Data are mean \pm s.d. Scale bar = 20 μ m. **o**, Quantification of dsRNA immunofluorescence in *Fhl1*^{+/+} and *Fhl1*^{-/-} BMDMs ($n = 7$ (*Fhl1*^{+/+} Control); $n = 6$ (*Fhl1*^{+/+} LPS); $n = 12$ (*Fhl1*^{-/-} Control); $n = 10$ (*Fhl1*^{-/-} LPS); EtOH/TAM 72 h; LPS 4 h). $n =$ technical replicates from representative experiment. Data are mean \pm s.d. **a-c, e-i, l** Data are mean \pm s.e.m. **d, j, m, n** representative blot or image of 3 experiments shown. $n =$ biological replicates unless stated otherwise. P values calculated using two-tailed Student's t -test for paired comparisons, one-way ANOVA for multiple comparisons.



Extended Data Fig. 10 | Prolonged LPS stimulation increases mitochondrial membrane potential and dsRNA. **a**, MFI of TMRM staining in BMDMs ($n = 3$). Immunofluorescence (**b**) and quantification (**c**) of dsRNA in BMDMs ($n = 8$ (0/48 h); $n = 9$ (24 h)). $n =$ technical replicates from representative experiment. Data are mean \pm s.d. Scale bar = 20 μ m. **d**, *Ddx58* and *Ifih1* expression in BMDMs

($n = 4$; LPS 4 h; *Ddx58* ($P = 0.0000000010$), *Ifih1* ($P = 0.00000012$)). **e**, *Fh1* expression in IFN- β -stimulated BMDMs ($n = 3$). **a, d, e**, Data are mean \pm s.e.m. **b**, 1 representative image of 3 experiments shown. $n =$ biological replicates unless stated otherwise. P values calculated using two-tailed Student's t -test for paired comparisons, one-way ANOVA for multiple comparisons.

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a Confirmed

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection XCalibur Qual Browser, XCalibur Quan Browser, Tracefinder 5.0, Leica LAS X Life Science Microscope Software

Data analysis Metaboanalyst 5.0, DESeq2 R package, Bioconductor Limma (3.16), DIA-NN 1.8, Agilent Seahorse Wave (2.4.3), Enrichr, Bioconductor clusterProfiler 4.0, enrichplot package in R (version 3.6.1), Broad Institutes GSEA 4.1.0, GraphPad Prism 9.2.0, Leica LAS X Life Science Microscope Software Platform, FlowJo v10, Image Lab 6.0.1, ImageJ (1.53t)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

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Proteomics data from Fig. 1d were previously deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD0291556. All other proteomics, RNA sequencing data and metabolomics data have been deposited to Dryad (doi:10.5061/dryad.6wwpzgn28). All original gel images are provided in the source data file. All other source data are available from the corresponding author(s) upon request.

Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	We have used at least 1 biological replicate for each individual experiment, which was performed on 3 independent occasions unless otherwise stated. Although we did not use statistical methods to calculate sample size, we decided to use a minimum of 3 biological replicates per experiment to account for biological variability, taking into account the 3 Rs principle and the fact that the majority of experiments were performed in primary murine macrophages from inbred mice. See statistical analyses section of methods for full details.
Data exclusions	No data were excluded from in vitro experiments. Data points were excluded from the in vivo experiment (Fig. 4l) on the basis that blood was harvested from the liver of these samples as it could not be harvested retroorbitally (as was performed with all other samples) due to technical issues.
Replication	The in vitro experiments were highly reproducible. Each experiment was repeated on at least 3 independent occasions unless otherwise stated. The in vivo experiment was performed on two separate occasions, both of which were successful and included in the analysis, in order to ensure reproducibility.
Randomization	For in vivo studies, mice were randomly assigned to treatment groups. For metabolomics, RNA sequencing and proteomics experiments, samples were processed in random order and experimenters were blinded to experimental conditions. All in vitro treatment groups were randomly assigned.
Blinding	In vitro and in vivo experiments were not blinded due to lack of available experimenters with required expertise. For metabolomics, RNA sequencing and proteomics experiments, samples were processed in random order and experimenters were blinded to experimental conditions.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
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<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

Methods

n/a	Involved in the study
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<input type="checkbox"/>	<input checked="" type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	Anti-mouse Lamin B1 (12586), STAT1 (9172), p-STAT1 (9167), JAK1 (3344), p-JAK1 (3331), TBK1 (3504), p-TBK1 (5483), STAT3 (30835), p-STAT3 (9145), FH (4567), ASS1 (70720), α -tubulin (2144), MAVS (4983), ATF4 (11815), p-AKT (13038), AKT (2920), p-JNK (9255), JNK (9252), p-ERK1/2 (9101), ERK1/2 (4695), p-p38 (4511), p-38 (9212), TRAF3 (4729), p-p65 (3033) and GAPDH (2118) antibodies were purchased from Cell Signaling. Anti-2SC antibody was kindly provided by Dr. Norma Frizzell (University of South Carolina, US). Anti-mouse β -actin antibody (A5316) was purchased from Sigma Aldrich. Horseradish peroxidase (HRP)-conjugated anti-mouse (115-035-003), anti-goat (705-035-003) and anti-rabbit (111-035-003) immunoglobulin G (IgG) antibodies were purchased from Jackson ImmunoResearch. Anti-mouse CD210 (112710) and anti-mouse IgG (406601) antibodies were purchased from Biolegend. Anti-dsRNA antibody (clone rJ2) was purchased from Merck (MABE-1134). Alexa Fluor 488 goat anti-mouse IgG1 antibody (A21121) was purchased from Invitrogen.
Validation	Antibodies were used according to validation listed in manufacturer's instructions. All details of antibody validation are given in Table S1.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	In vitro experiments were performed with BMDMs isolated from 6-18-week-old female and male mice. In vivo models were performed with 6-week-old male mice and littermates were randomly assigned to experimental groups. All mice were on a C57BL/6J0laHsd background unless stated below. Wild-type (WT) mice were bred in-house. Mice were kept in 12 hour light/dark cycles, and the facility was maintained at 20-24°C and 45-65% humidity. The inducible Fh1+/fl and Fh1fl/fl mice were generated on the C57BL/6 genetic background and their hind legs were generously donated by Dr. Christian Frezza (University of Cambridge, UK). Vehicle (ethanol) treated Fh1+/+ and Fh1-/l were used as controls. Upon treatment with 4-hydroxy tamoxifen, Cre-mediated chromatin excision results in the loss of either one (Fh1+/-) or both (Fh1-/-) copies of Fh1, thus generating either heterozygous or null animals. Hind legs from WT and Mavs-/- mice were generously donated by Dr Cecilia Johansson (Imperial College London, UK). These strains, originally obtained from S. Akira (World Premier International Immunology Frontier Research Center, Osaka University, Osaka, Japan), were Ifna6gfp/+ but since Ifna6 expression was not a primary readout the mice are designated as WT and Mavs-/-.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve field-collected animals.
Ethics oversight	Animals were maintained under specific pathogen-free conditions in line with Irish and European Union regulations. All animal procedures were ethically approved by the Trinity College Dublin Animal Research Ethics Committee prior to experimentation and conformed with the Directive 2010/63/EU of the European Parliament.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	For experiments performed in TCD which involved isolation of human PBMCs and macrophages for in vitro experiments: donors were defined as healthy and no further information was recorded. For experiments performed in Cedars-Sinai: All SLE patients (as per ACR diagnostic criteria) were recruited from Cedars-Sinai Medical Center, CA, USA. Age- and sex-matched healthy donors who had no history of autoimmune diseases or treatment with immunosuppressive agents were included. There were no significant covariates between the two groups.
Recruitment	For experiments performed in TCD: Blood samples were obtained anonymously and written informed consent for the use of blood for research purposes was obtained from the donors. For experiments performed in Cedars-Sinai: All participants provided informed written consent and the study received prior approval from the institutional ethics review board (IRB protocol. 19627). No selection bias was noted.
Ethics oversight	School of Biochemistry and Immunology Research Ethics Committee (TCD), Cedars-Sinai Medical Center Institutional Ethics Review Board (IRB protocol 19627)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Murine BMDMs were plated in 12-well plates and treated as desired. CellROX Green (5 µM, Thermo Fisher) or TMRM (20 nM, Thermo Fisher) was added to cells 30 mins prior to end of cell treatments. Cells were washed once in PBS and scraped into 200 µl FACS buffer (2 mM EDTA, 0.5% FCS in PBS). Acquisition of samples was performed on a BD Accuri C6 flow cytometer. Debris was excluded by FSC vs SSC analysis and 10,000 cells was acquired per condition.
Instrument	BD Accuri C6
Software	FlowJo V10
Cell population abundance	Purity of samples was not determined. Debris was excluded by FSC vs SSC analysis.

Gating strategy

The gating strategy used for all flow cytometry experiments consisted of debris exclusion by FSC-A vs SSC-A analysis and subsequent doublet exclusion by FSC-A vs FSC-H analysis. A sample gating strategy is provide in Supplementary Fig. 2.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.