Supplemental Data

Supplemental Methods:

Flow Cytometry for Mitochondrial Mass and Glucose Uptake

Monocytes were stimulated for 24 hours with LPS (200ng/mL), IFNγ (1000U/mL), or the combination of LPS and IFNγ. Cells were then washed and stained with MitoTracker Green (Invitrogen, M7514) or incubated with 2-NBDG (BioVision, #K682-50) for 30 minutes before analysis. Data were collected with the use of FACS Diva (BD Biosciences) and analyzed with the use of FlowJo (Treestar). Geometric mean fluorescence intensities are reported.

Agilent Seahorse Mito Fuel Flex Test

Healthy control monocytes were left untreated or treated with etomoxir (4uM), UK5099 (2uM), or BPTES (3uM), throughout the 24 hour IFNγ stimulation period. Oxygen consumption was assessed by Seahorse assay according to a modified Mito Fuel Flex Test protocol (Agilent, 103260-100). Basal OCR measurements were captured after the 24 hour stimulation. PMA (100ng/mL) was injected during the assay and PMA-stimulated OCR measurement were collected. Rotenone and antimycin A were then injected and OCR measurements were collected.

LDH Cytotoxicity Assay

Cellular cytotoxicity was assessed by LDH release (QuantiChrom LDH Cytotoxicity, BioAssay Systems, C2LD-100) per manufacturer’s protocol and optical density was assessed at 500nm.

cDNA synthesis and qRT-PCR:

RNA was extracted from cells using RLT buffer and the RNeasy kit (Qiagen Catalog# 74106) per the manufacturer’s protocol. Purified RNA was used for cDNA synthesis using the SuperScript III Frist Strand Synthesis System kit (Invitrogen catalog# 18080-051). Quantitative
PCR was performed using TaqMan detection with the QuantStudio3 Real Time PCR System. All qPCR assays were normalized to β-actin transcript levels and the relative gene expression was determined using the ΔΔCT method. TaqMan primers/probes for NAMPT, CD38, P2RX7 and β-actin were pre-designed by Applied Biosystems.

Statistical analyses

Statistical analyses were performed in GraphPad Prism 9 software. Data are expressed as mean ± s.e.m., and P values were calculated using one-way analysis of variance (ANOVA) with Tukey’s multiple comparisons test or two-way ANOVA with Sidak’s multiple comparisons test. A confidence interval of 95% was used for all statistical tests. Sample sizes were determined based on the experiment type and standard practice in the field.
Supplemental Figures:

Supplemental Figure 1:

Supplemental Figure 1: IFNγ-induced increases in OCR are time-dependent, but not dose-dependent.

(A-B) Real time changes in OCR measured by Seahorse following treatment for 24 hours prior to the start of the assay with media alone or IFNγ at (A) various doses as indicated in legend or (B) for 1h, 6h, 18h, or 24h as indicated in legend. Data in (A-B) were analyzed by two-way ANOVA with Sidak’s multiple comparisons test. Error bars are mean ± s.e.m *p<0.05, **p<0.01 ****p<0.0001.
Supplemental Figure 2: FK866 reversibly inhibits both OCR and ECAR in IFNγ stimulated monocytes, but does not affect monocyte viability.

(A-B) Real time changes in OCR measured by Seahorse following treatment for 24 hours prior to the start of the assay with media alone or IFNγ with (A) exogenous NAM or (B) FK866 at indicated doses. (C) Real time changes in OCR and ECAR measured by Seahorse following treatment for 24 hours prior to the start of the assay with medium alone or IFNγ with or without FK866 (C), Rotenone or DPI (D). (E) Cytotoxicity measured by LDH release in supernatants of M(0) or M(IFNγ) monocytes with or without various doses of inhibitors (FK866 or DPI as labeled on X-axis). Data in (E) were analyzed by one-way ANOVA with Dunnett’s multiple comparisons test. Error bars are mean ± s.e.m **p<0.01 ****p<0.0001. (F-G) Real time changes in OCR and ECAR demonstrating that high dose (10mM) nicotinamide mononucleotide (NMN) can rescue both OCR and ECAR in M(IFNγ) monocytes treated with FK866.
Supplemental Figure 3: IFN\(\gamma\) induced increases in OCR are pSTAT1, NAMPT and Complex I dependent

(A-C) Gene expression measured by qPCR in primary monocytes from 3 healthy controls, and a STAT1 GOF patient before and after initiating treatment with Ruxolitinib. (D-F) Primary monocytes from a homoplasmic Leigh syndrome patient (proband), a heteroplasmic Leigh syndrome patient (sibling) and their unaffected father were stimulated with media alone or IFN\(\gamma\) for 24 hours and OCR was measured by Seahorse. (E-F) Basal and PMA-stimulated OCR values from (D) are summarized in bar graphs and data were analyzed by two-way ANOVA with Sidak’s multiple comparisons test. Error bars are mean ± s.e.m *p<0.05, ***p<0.001 and ****p<0.0001.
Supplemental Figure 4: IFNγ regulates expression of genes involved in NAD(P)H oxidation and biosynthesis

Heat map generated from RNA-seq analysis of human monocyte-derived macrophages stimulated with IFNγ for 24 hours displaying genes involved in (A) NADPH oxidase complex, (B) Mitochondrial complex I (nuclear encoded genes), and (C) De novo and Preiss-Handler pathways of NAD⁺ biosynthesis. ND, normal donor.
Supplemental Figure 5: IFNγ-induced increases in OCR depend on electron transport, not ATP production

(A) Diagram highlighting the metabolic pathways connecting NAD⁺ metabolism to oxygen consumption, including the inhibition of PDH by PDK4 and chemical inhibitors of mitochondrial electron transport chain components. (B-D) OCR measured by Seahorse in primary human monocytes treated with media alone or IFNγ with or without (B) mitochondrial complex I inhibitor, rotenone, and mitochondrial complex III inhibitor, antimycin A (0.5 µM), (C) complex V inhibitor, oligomycin (1µM), or (D) mitochondrial inner membrane protonophore, FCCP (0.5 µM), for 24 hours prior to the start of the assay. Basal OCR was measured then PMA (100ng/mL) was injected during the assay and OCR was monitored (n = 5 technical replicates). Data in (B-D) are representative tracings of three independent experiments.