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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

We have used at least 3 biological replicates for each experiment. This is designed to account for biological variability taking into account that the majority of experiments were performed in murine macrophages from inbred mice. See statistical analyses section of methods for full details.

2. Data exclusions

Describe any data exclusions.

Any exclusions from in vitro data were deemed outliers in GraphPad Prism. From the LPS in vivo cytokine data one LPS-treated sample has been removed as it appears that I did stimulate in response to LPS, most like an injection error.

3. Replication

Describe whether the experimental findings were reliably reproduced.

The in vivo trial required some optimization with various doses of compound as this was the first time it had been used in vivo. In vitro experiments were highly reproducible. All experimental findings were reproduced as biological replicates at the value stated in figure legends, unless otherwise indicated.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

See statistical analyses section of methods. For in vivo studies, mice were randomly assigned to treatment groups. For MS analyses, samples were processed in random order and experimenters were blinded to experimental conditions.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The in vivo trials were blinded. For MS analyses, samples were processed in random order and experimenters were blinded to experimental conditions.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Flowjo was used to flow cytometry analysis. Metabolite spectra were analysed using XCalibur Qual Browser and XCalibur Quan Browser software (Thermo Scientific). Labsolutions software (Shimadzu) was used to assess itaconate uptake. SoftMax Pro software was used for ELISAs. MS data was analysed with PEAKS Studio 8 (Bioinformatics Solutions). GraphPad Prism was used for all graphing and statistical tests.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	4-octyl itaconate is available from the authors.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Antibodies used were goat anti-mouse IL-1β (R&D Systems, AF401-NA), mouse anti-β-actin (Sigma-Aldrich, AC-74), rabbit anti-HIF-1α (Novus, NB100-449), rabbit anti-phospho-pIRF3 (Cell Signalling Technology, 4947) rabbit anti-total IRF3 (Cell Signalling Technology, 4302), rabbit anti-phospho-pTBK1 (Cell Signalling Technology, 5483) rabbit anti-total TBK1 (Cell Signalling Technology, 3013), rabbit anti-IKKε (Cell Signalling Technology, 3416), Nrf2 (Cell Signalling Technology, 12721), Hmox1 (Enzo Life Sciences, ADI-SPA-896-D), Ass1 (Abcam, SAB5300141). Secondary horseradish peroxidase-conjugated anti-mouse IgG, anti-rabbit IgG and anti-goat IgG were from Jackson ImmunoResearch Inc.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

C2C12, Hepa1c1c7 cells and COS1 cells were from American Type Culture Collection (ATCC). The L929 cells are from Sigma, cat no is 85011425.HEK293T cells were obtained from the Centre for Applied Microbiology and Research, Wiltshire, UK.

b. Describe the method of cell line authentication used.

No authentication was used

c. Report whether the cell lines were tested for mycoplasma contamination.

They were not tested.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

8 week old, C57BL/6JOlaHsd female mice were purchased from Envigo UK. The rats were 10-12 week old female Wistar Rats from Charles River Laboratories, Strain Code:003. Nrf2-deficient mice and their wild type counterparts, both on the C57Bl/6 genetic background (used for isolation of BMDM cells) were bred and maintained in the Medical School Resource Unit of the University of Dundee.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

The blood was from anonymous donors so I do not have this information.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. 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).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Murine bone marrow derived macrophages
6. Identify the instrument used for data collection.	Dako CyAn flow cytometer
7. Describe the software used to collect and analyze the flow cytometry data.	FlowJo
8. Describe the abundance of the relevant cell populations within post-sort fractions.	This was pure in vitro prepared bone marrow derived macrophages
9. Describe the gating strategy used.	Cells were gated first on side-scatter (area) versus forward scatter (area) (Population; P1; to determine the macrophage population) Then on forward-scatter (height) versus forward scatter (area) (P2; to eliminate doublets) Then on side-scater (area) versus pacific blue (to eliminate dead cells) Finally on counts versus APC (as a meaure of CellROX staining).

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