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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.

No sample size determination was performed. For metabolomics experiments, retrospective evaluation of data from pilot measurements indicated that 4 analytical replicates sufficed to yield statistical power that is acceptable for the value (<0.05) used in this manuscript to define experimental results as significant.

2. Data exclusions

Describe any data exclusions.

In some metabolomics experiments, up to one replicate was allowed to be excluded when technical failure of the mass spectrometry measurement was evident based on poor ion peak quality. Small debris was excluded from flow cytometry analysis.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All experimental findings presented in this study were reproducible based on independent replicate measurements as described in detail in the corresponding figure legends.

4. Randomization

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

For metabolomics analyses by GC-MS, samples were randomised using the relevant utility within the Agilent Chemstation software, and, for LC-MS analyses, randomisation was done using a random number generator in Microsoft Excel.

5. Blinding

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

No blinding was used as the types of measurements conducted were not susceptible to subjective bias.

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.

Graph plotting and statistical analysis: GraphPad Prism® 7.0b. In-house scripting: RStudio version 1.0.136, Python 3.5.5
GC-MS analysis: Agilent MassHunter Workstation software Quantitative Analysis Version B.06.00 for peak quantification, and custom R and Python scripts for downstream analysis.
LC-MS instrument control: Xcalibur 3.0.63
LC-MS analysis: Thermo Tracefinder 4.1 EFS
FACS analysis: BD FACSDiva 8.0.1 & FlowJo (TreeStar)
Plate-reader acquisition software: Tecan iControl 1.11

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.

DM-[13C5]-αKG and MOG were synthesised in-house and are available at limited quantities. All other materials are commercially available.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

mouse anti-α-tubulin (Clone DM1A, Sigma, T9026), 1:2000 in 5% BSA/TBS-T; rabbit anti-MCT1 (Millipore, AB3538P), 1:500 in 5% milk/TBS-T; rabbit anti-MCT2 (L-11, Santa Cruz, SC-22034-R), 1:500 in 5% milk/TBS-T (validated by overexpression and knock-down experiments (Suppl. Fig. 2e, Suppl. Fig. 5c); rabbit anti-MCT4 (H-90, Santa Cruz, SC-20329), 1:500 in 5% milk/TBS-T; mouse anti-β-actin antibody (Sigma, A2228), 1:1000 in 5% BSA/TBS-T. mouse anti-HIF1α (BD Biosciences 610958), 1:500 in 5% milk/TBS-T (validated by knock-out Suppl. Fig. 8b); rabbit anti-HKII (Cell Signaling Technology C64G5), 1:1000 in 5% BSA/TBS-T; rabbit anti-LDHA (Cell Signaling Technology 2012), 1:1000 in 5% BSA/ TBS-T (validated by knock-out in our lab); mouse-anti-puromycin clone 12D10 (Merck, MABE343), 1:25,000 in 5% BSA/ TBS-T (validated in our lab by loss of signal under conditions inhibiting translation). Secondary antibodies: Goat anti-rabbit IgG antibody conjugated to HRP (Millipore, AP132P), goat anti-mouse IgG antibody conjugated to HRP (Millipore, AP127P). Antibodies for which we do not specify in-lab validation above, specificity was based on evidence in the manufacturers' corresponding product pages.

10. Eukaryotic cell lines

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

All cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Human breast cancer cell lines (Fig. 1A) were obtained as NCI-ICBP45 kit.

b. Describe the method of cell line authentication used.

Cell identity was confirmed by short tandem repeat (STR) profiling by The Francis Crick Institute Cell Services Science Technology Platform.

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

All cell lines were tested mycoplasma-free by The Francis Crick Institute Cell Services Science Technology Platform.

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 mis-identified 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.

No animals were used.

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.

Study did not involve human participants.

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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.	MCF7 and HCC1569 cells were treated with vehicle, DMOG or cultured at 1% oxygen for 48h, before being trypsinised and resuspended in Phenol-red-free RPMI containing 2% foetal calf serum. Cells were passed through a filter and then stained with propidium iodide (final concentration 0.5 µg/ml).
6. Identify the instrument used for data collection.	BD LSRFortessa Analyser
7. Describe the software used to collect and analyze the flow cytometry data.	BD FACSDiva 8.0.1, FlowJo (TreeStar)
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Cell sorting was not applicable and was not used for this study.
9. Describe the gating strategy used.	Single cell nuclei were identified based on FSC-A and FSC-H; cell debris was excluded from the analysis. The gating strategy is described in detail in the Methods section.

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