SUPPLEMENTARY TABLE 1

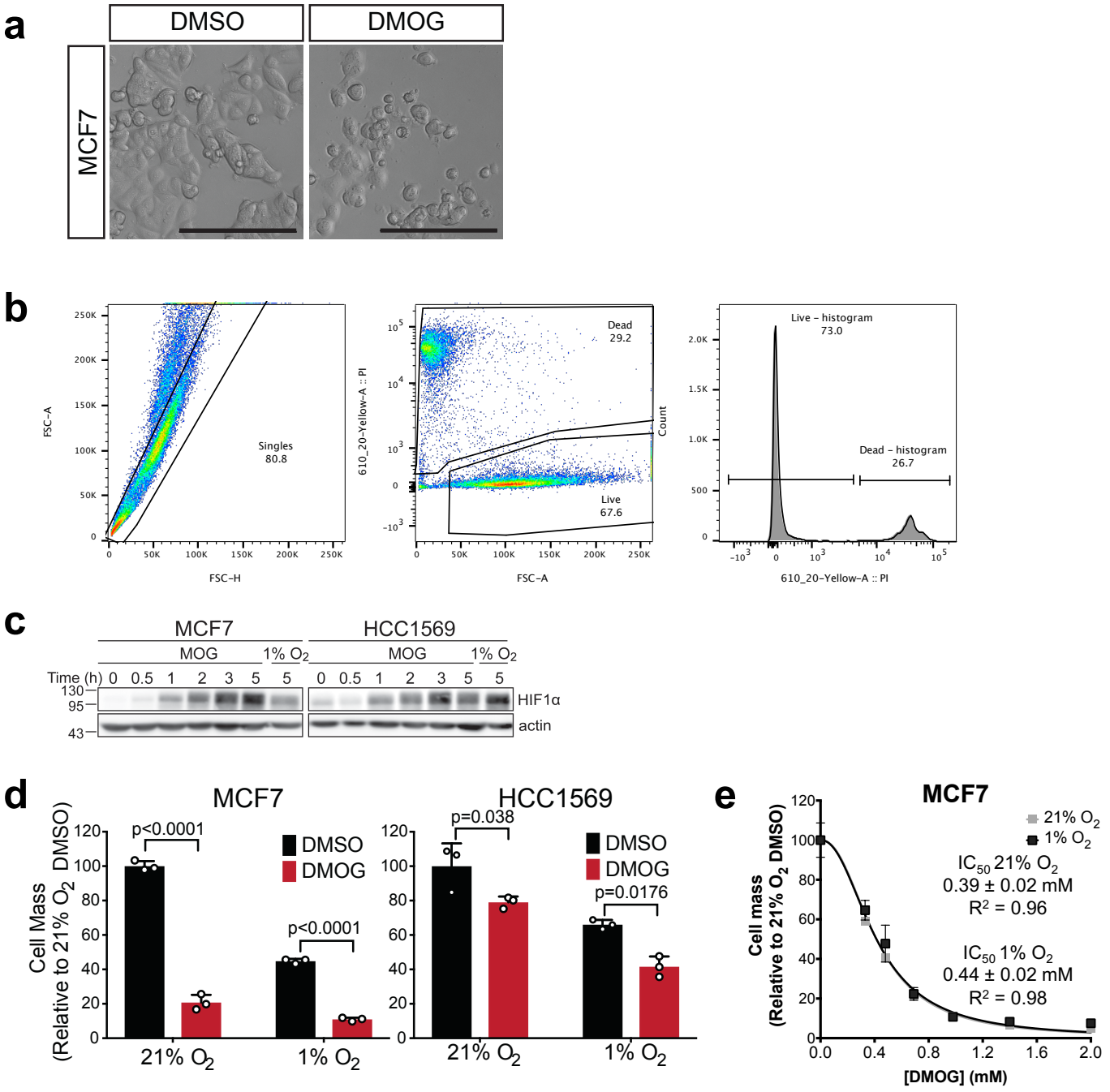
Primer name	cDNA	Sequence	Direction	Res. site
LF124	MCT1	cgc**ggatcc***acc*ATGCCACCAGCAGTTGGAGG	Forward	*Bam*HI
LF125	MCT1	cgc**tacgta**TCAGACTGGACTTTCCTCC	Reverse	*Sna*B1
oDA005	MCT2	cgc**ggatcc***acc*ATGCCACCAATGCCAAGTGCC	Forward	*Bam*HI
oDA006	MCT2	cgc**tacgta**TTAAATGTTAGTTTCTCTTTCTG	Reverse	*Sna*B1
LF127	MCT4	cgc**ggatcc***acc*ATGGGAGGGGCCGTGGTGG	Forward	*Bam*H1
LF128	MCT4	cgc**gaattc**TCAGACACTTGTTTCCGGGGTG	Reverse	*Eco*R1

Primers used to clone expression constructs (*lowercase italics*=Kozak sequence; **lowercase bold**=restriction enzyme cleavage site; UPPERCASE=insert; Res. site: restriction enzyme site)

SUPPLEMENTARY TABLE 2

Compound	Mode	Ion (m/*z*)	RT (min)
DMOG	Negative (M-H)	174.0406	4.4
MOG	Negative (M-H)	160.0252	8.4
NOG	Negative (M-H)	146.0095	13.8

Compound detection details using ZIC-pHILIC chromatography combined with the Q-Exactive Orbitrap (Thermo Scientific) as detailed in *Methods*.



SUPPLEMENTARY FIGURE 1
Fets *et al.*

SUPPLEMENTARY FIGURE 1

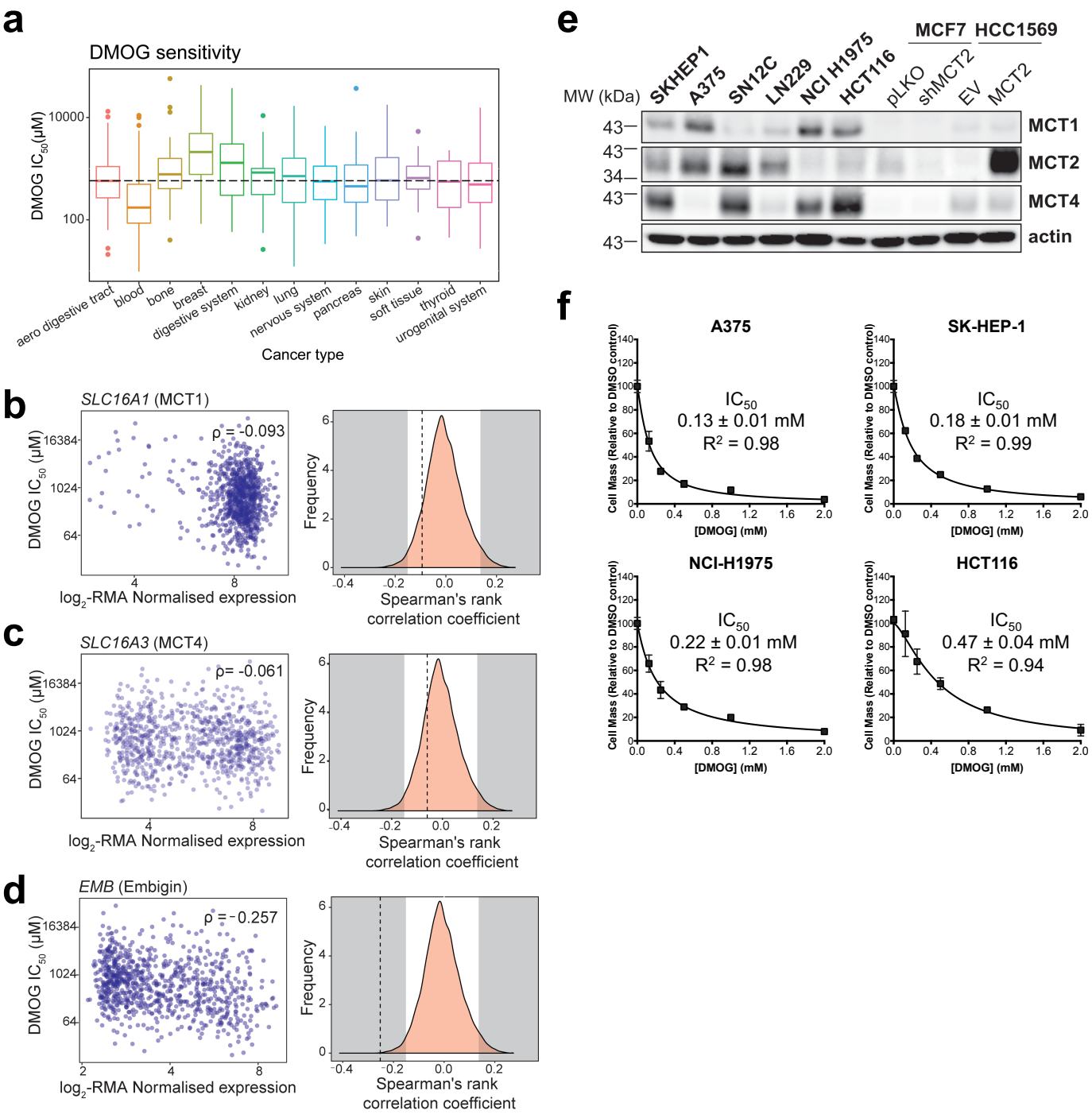
a) Phase-contrast images of the DMOG-sensitive MCF7 cells after 48 h treatment with either 0.1% DMSO (vehicle control) or 1 mM DMOG. Images representative of more than 5 experiments. Scale bars represent 200 μm.

b) Gating strategy for PI staining shown in Fig. 1c.

c) Western blot to assess HIF1α protein levels after treatment of the indicated cell lines with 1 mM DMOG, or incubation at 1% O_2. Different exposures were used for HIF1α in each cell line to aid comparison of stabilisation kinetics; uncropped blots available in **Supplementary Fig. 13b.** Experiment performed once.

d) Cell mass accumulation in MCF7 and HCC1569 cells after treatment with 1 mM DMOG at 21% O_2 or 1% O_2 for 48 h, relative to 21% O_2 vehicle-treated controls (0.1% DMSO). Data shown as mean ± SD (n = 3 cultures), significance tested by 2-way ANOVA with Tukey's multiple comparison correction.

e) Plot of MCF7 cell mass accumulation after treatment with different concentrations of DMOG for 48 h at either 21 or 1% O_2, relative to vehicle-only control (0.2% DMSO) at the respective O_2 concentration. Data shown as mean ± SD (n = 3 cultures). Curves were fitted using [inhibitor] vs normalised response (variable slope) algorithm in GraphPad Prism.



SUPPLEMENTARY FIGURE 2
Fets *et al.*

SUPPLEMENTARY FIGURE 2

a) Graph illustrating the distribution of DMOG IC_{50} values across 850 different cancer cell lines, separated by cancer type, shown as a Tukey-style boxplot (box shows median and inter-quartile range (IQR), whiskers show 1.5 IQR, or the maximum/minimums value, dependent on which is smallest). Dashed line shows the median IC_{50} of all cell lines. Data obtained from the Genomics of Drug Sensitivity in Cancer project (http://www.cancerrxgene.org).

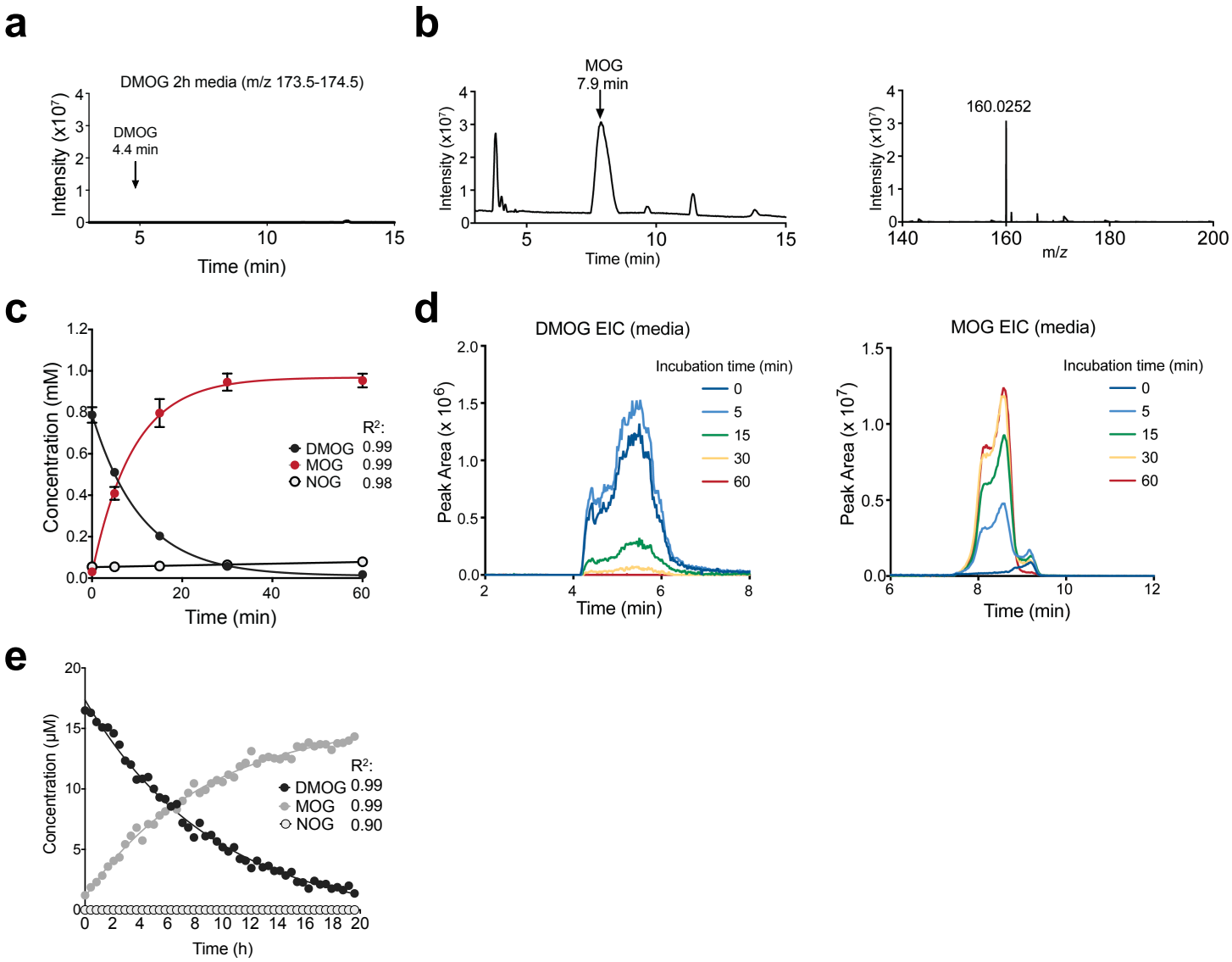
b) Left: Correlation of RMA-normalised *SLC16A1* (encoding MCT1) mRNA expression and IC_{50}^{DMOG} across 850 different cancer cell lines. Data source was as in (a). Spearman's rank correlation coefficient is shown in the top right corner. Right: Spearman's rank correlation coefficient of *SLC16A1* (black dashed line) with respect to those of all other genes. Grey shaded region either side shows ±2-standard deviations cut-off used to define sensitivity-associated genes.

c) As in (b), but showing correlation of IC_{50}^{DMOG} with *SLC16A3* (encoding MCT4).

d) As in (b), but showing correlation of IC_{50}^{DMOG} with *EMB* (encoding Embigin).

e) Western blot to assess MCT1, MCT2 and MCT4 expression in selected sensitive cell lines from a range of different cancer types. Specificity of MCT2 antibody was tested by knockdown and overexpression of MCT2, discussed in further detail in Fig. 3. This experiment was performed once. Uncropped blots available in **Supplementary Fig. 13c**.

f) IC_{50} curves of cell mass accumulation after treatment with different concentrations of DMOG for 48 h, in sensitive cell lines from different cancer types. All normalised to a vehicle-only control (0.2% DMSO). Data shown as mean ± SD (n = 3 cultures). Curves were fitted using [inhibitor] vs normalised response (variable slope) algorithm in GraphPad Prism.

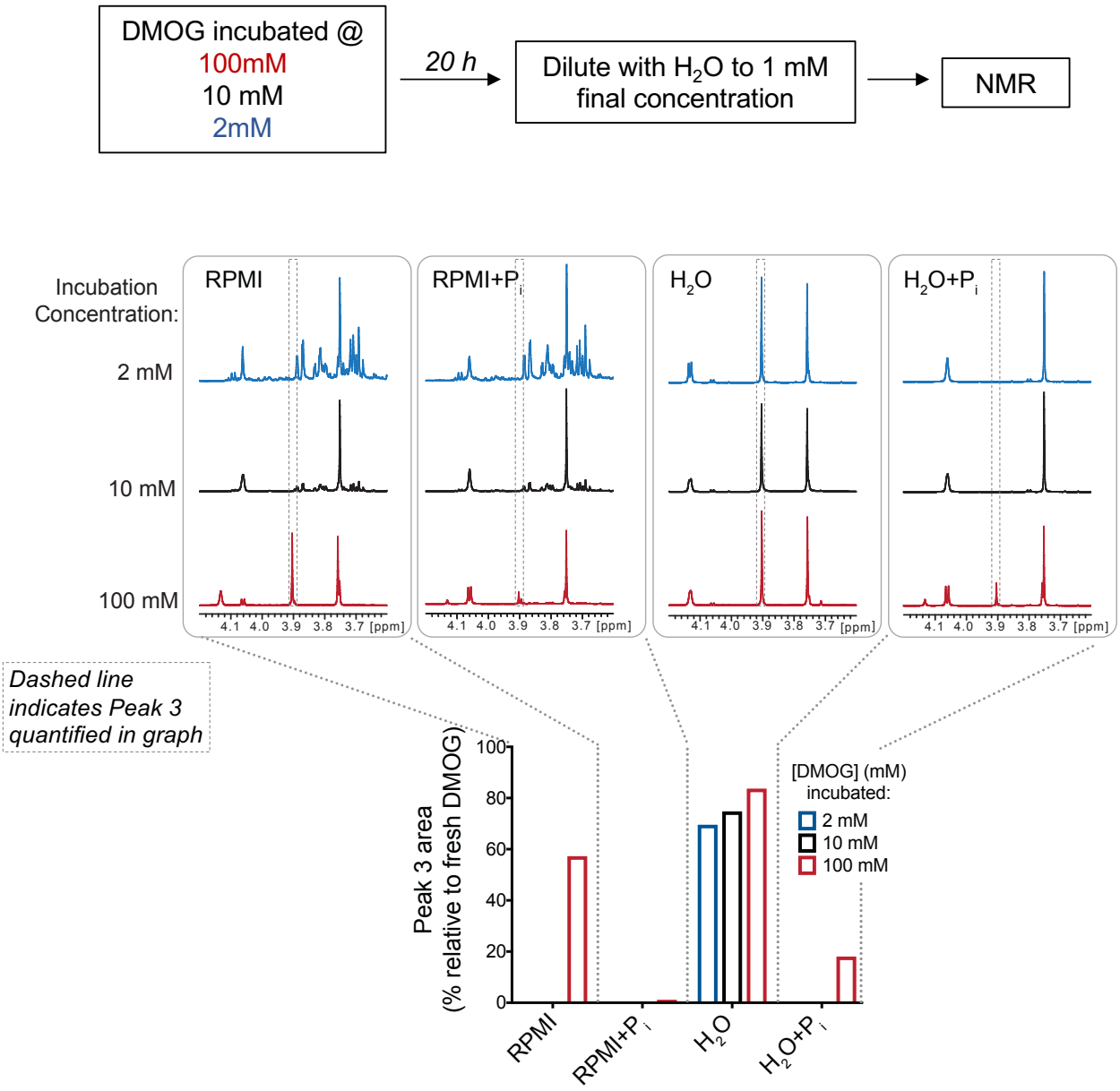


SUPPLEMENTARY FIGURE 3
Fets *et al.*

SUPPLEMENTARY FIGURE 3

a) Extracted LC-MS ion chromatogram of m/*z* 173.5-174.5 demonstrating loss of DMOG after 2h of incubation in RPMI medium (DMOG peak elutes at 4.4 mins with an m/*z* = 174.0406).
b) LC-MS base-peak chromatogram and mass spectrum of 10 µM MOG authentic standard in water, with peak and ion annotated.
c) Time course of 1 mM DMOG degradation to MOG in RPMI medium at 37 ^{o}C. Data shown as the mean ± SD of n = 3 technical replicates and are representative of two independent experiments. One-phase decay curves are fitted using GraphPad Prism.
d) Overlaid extracted ion chromatograms (EIC) of data from (c) showing the degradation of DMOG and the appearance of MOG in media over time.
e) Time course of 20 µM DMOG degradation to MOG in water, measured by LC-MS. One-phase decay curves are fitted using GraphPad Prism.

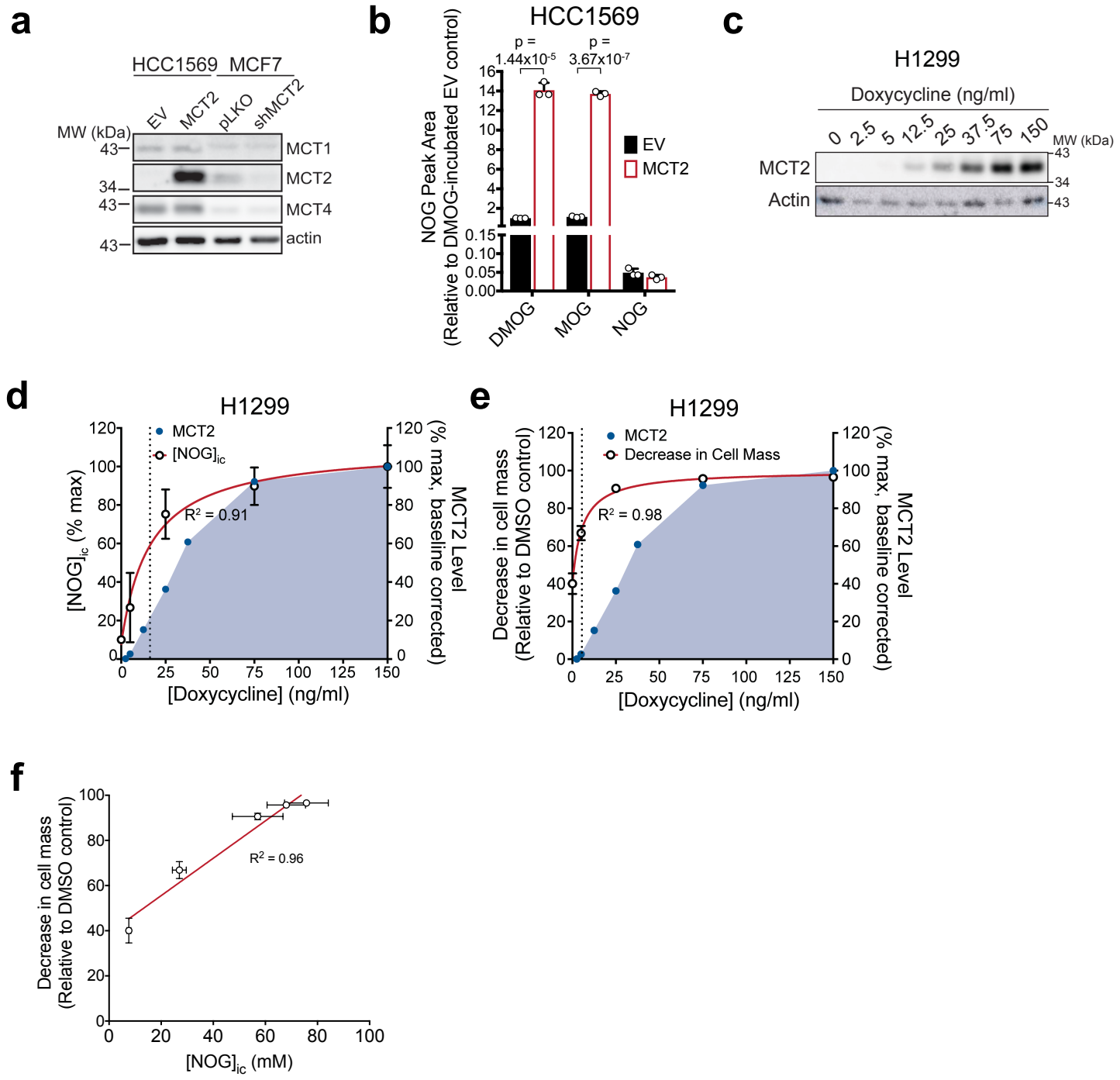
Data in a-e are representative of two independent experiments each with similar results.



SUPPLEMENTARY FIGURE 4
Fets *et al.*

SUPPLEMENTARY FIGURE 4

Incubation of varying concentrations of DMOG in RPMI media or water, with or without addition of 100 mM phosphate buffer, followed by NMR quantification of the oxoacetate methyl group-specific resonance (Peak 3, Fig. 2D), illustrating that the rate of DMOG-to-MOG conversion is pH-dependent. This experiment was performed once.



SUPPLEMENTARY FIGURE 5
Fets *et al.*

SUPPLEMENTARY FIGURE 5

a) Western blot to assess relative MCT1, MCT2 and MCT4 levels in HCC1569 cells over-expressing MCT2 (Fig. 3c, d) and in MCF7 cells in which MCT2 was knocked down (Fig. 3e, f), both shown compared to the respective control line. Experiment performed twice with similar results. Uncropped blots available in **Supplementary Fig. 13d**.

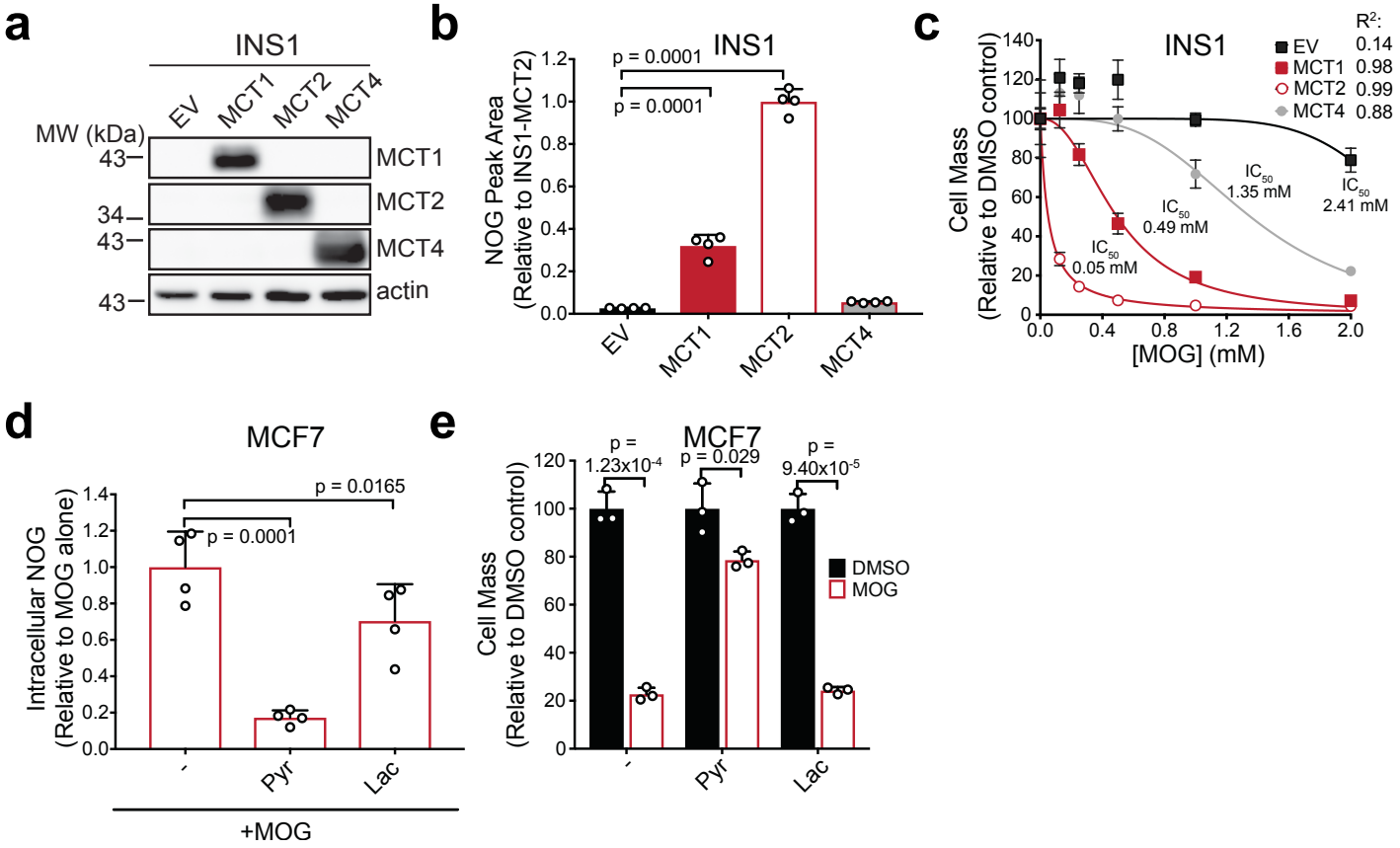
b) Relative NOG concentration in HCC1569 cells expressing either an empty vector (EV) or MCT2 as in (a), after 4 h incubation with either DMOG, MOG or NOG. Data shown as mean ± SD (n = 3 cultures) and significance was tested using 2-sided multiple t-tests with Holm-Sidak multiple comparison correction.

c) Western blot to assess doxycycline-inducible MCT2 expression in H1299-TetR cells stably expressing pLVX-Tight-MCT2 after 24 h incubation with the indicated doses of doxycycline. This experiment was repeated 3 times with similar results. Uncropped blots available in **Supplementary Fig. 13e**.

d) Relative $[NOG]_{ic}$ after 4 h incubation with MOG in cells as in (c) treated with increasing amounts of doxycycline (24 h). Data shown as mean ± SD (n = 4 parallel cultures). Relative MCT2 expression (shaded) determined from the western blot in (c) is plotted on the right y-axis. Dashed line represents the doxycycline concentration required for half-maximal $[NOG]_{ic}$ accumulation. Curve was fitted using the [agonist] vs response (3 parameters) algorithm in GraphPad Prism.

e) Decrease in cell mass (relative to DMSO) after treatment with 1 mM MOG (48 h) in H1299-pTightMCT2 cells pre-incubated with increasing amounts of doxycycline (24 h). Data shown as mean ± SD (n = 3 parallel cultures). Relative MCT2 expression as in (c) (shaded) plotted on the right y-axis. Dashed line represents the doxycycline concentration required for half-maximal decrease in cell mass as a result of MOG treatment. Curve was fitted as in (d).

f) Correlation (least-squares linear regression, red line) between $[NOG]_{ic}$ and decrease in cell mass from the experiments in d, e. Data shown as mean cell mass and $[NOG]_{ic}$ ± SD from n=3 and n=4 cultures, respectively.



SUPPLEMENTARY FIGURE 6
Fets *et al.*

SUPPLEMENTARY FIGURE 6

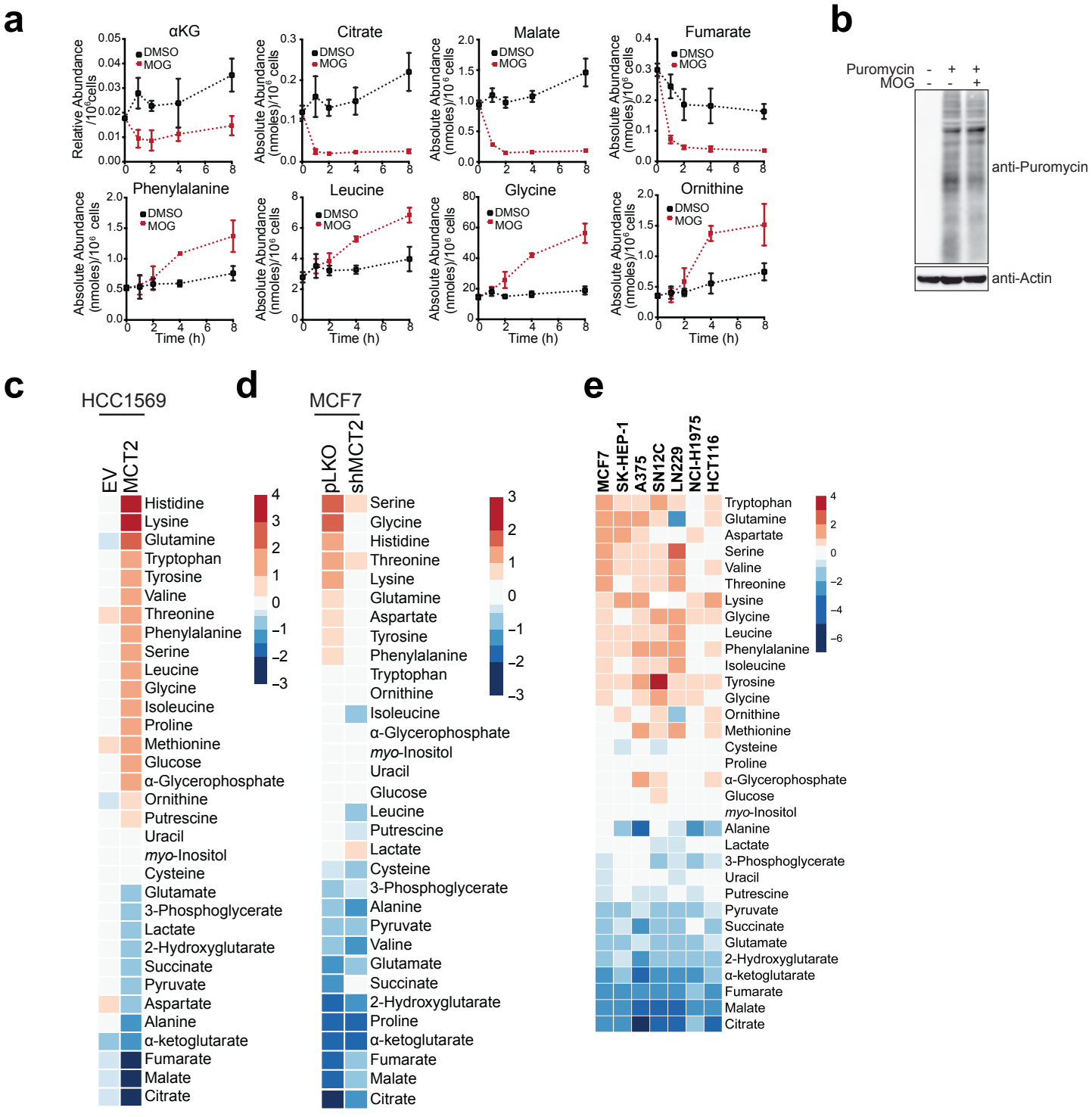
a) Western blot to assess MCT1, MCT2 and MCT4 protein expression in INS1 cells transduced with viruses encoding an empty vector (EV), MCT1, MCT2 or MCT4. Experiment performed twice with similar results. Uncropped blots available in **Supplementary Fig. 13f**.

b) $[NOG]_{ic}$ in INS1 cells described in (a), after incubation with MOG for 4 h. Data shown as mean ± SD (n = 4 cultures) and significance was tested using a one-way ANOVA with Dunnett's correction for multiple comparisons.

c) IC_{50} curves of cell mass accumulation for INS1 cells described in (a), after incubation with different concentrations of MOG for 48 h, relative to vehicle-only control (0.2% DMSO). Data shown as the mean ± SD of n = 3 parallel cultures and are representative of two independent experiments. Curves were fitted using the [inhibitor] vs normalised response (variable slope) algorithm in GraphPad Prism.

d) Relative $[NOG]_{ic}$ in MCF7 cells after 4 hours of incubation with 1 mM MOG alone or in the presence of 10 mM Pyruvate (Pyr) or 10 mM Lactate (Lac). Data shown as mean ± SD (n = 4 cultures) and significance was tested using a one-way ANOVA with Dunnett's correction for multiple comparisons.

e) Cell mass accumulation of MCF7 cells grown under the conditions described in (d) for 48 h. Data shown as mean ± SD (n = 3 cultures), and significance was tested using 2-sided multiple t-tests with Holm-Sidak multiple comparison correction.



SUPPLEMENTARY FIGURE 7

Fets *et al.*

SUPPLEMENTARY FIGURE 7

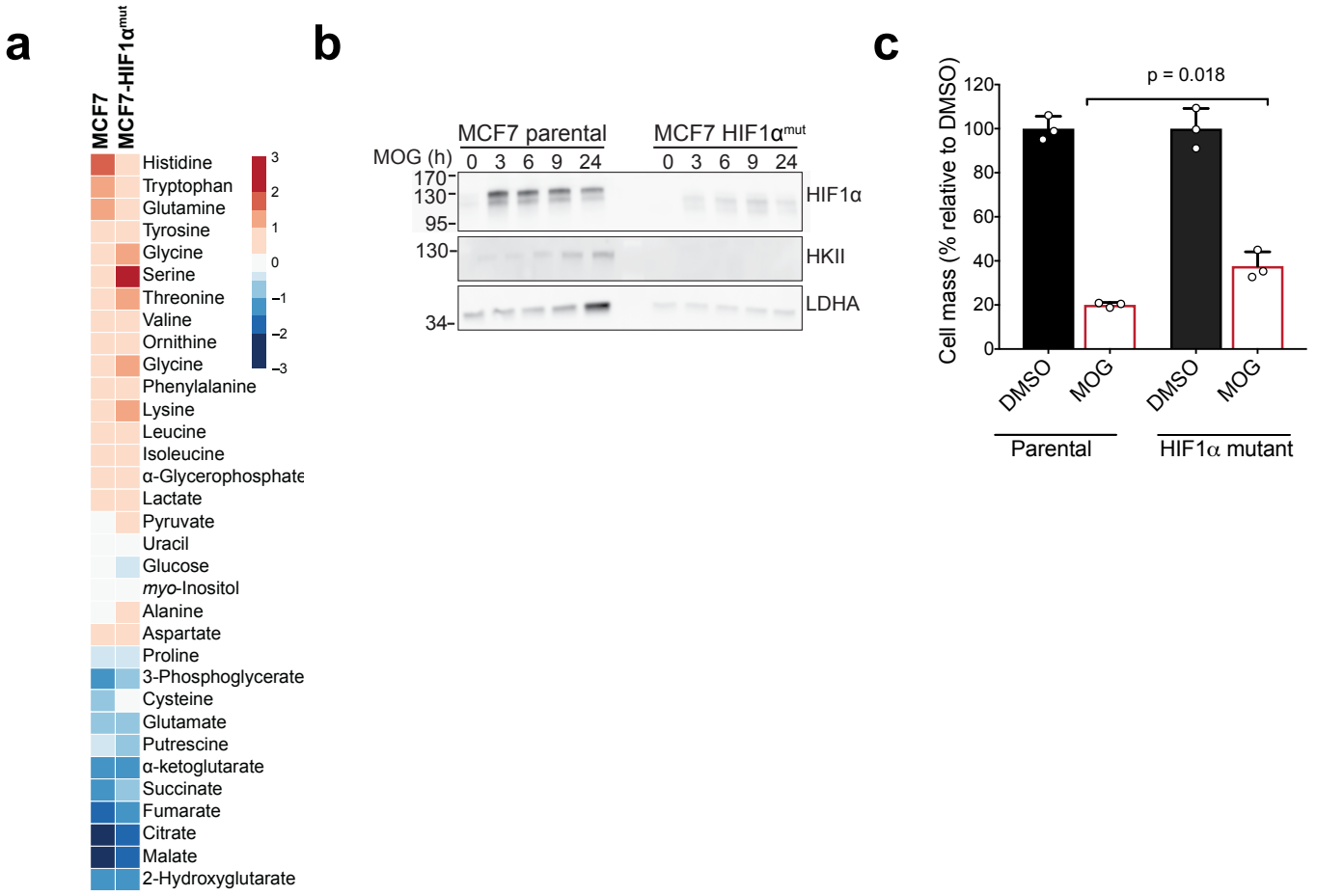
a) Abundance changes of selected metabolites from Fig 4a. Data shown as mean ± SD (n = 4 cultures).

b) Puromycin incorporation into newly-synthesised proteins (assessed via a puromycin-specific antibody) in MCF7 cells treated with 0.1% DMSO or 1 mM MOG for 4 hours with 90 µM puromycin added to the media 10 minutes prior to harvesting. Experiment carried out once. Uncropped blots available in **Supplementary Fig. 13g**.

c) Heatmap showing mean $\log_2$ fold-changes in the abundance of indicated metabolites after 4 h treatment with 1 mM MOG (relative to DMSO controls), in HCC1569 cells expressing either an empty vector (EV) or MCT2 (n = 4 cultures for each cell line). Metabolites are ordered from highest to lowest fold-change value in the MCT2-expressing HCC1569 cells.

d) Heatmap showing mean $\log_2$ fold-changes in the abundance of indicated metabolites after 4 h treatment with 1 mM MOG (relative to DMSO controls), in MCF7 cells expressing either an empty vector (pLKO), or an MCT2-targetting shRNA (n = 4 cultures for each cell line). Metabolites are ordered from highest to lowest fold-change value in the empty-vector-expressing MCF7 cells.

e) Heatmap showing mean $\log_2$ fold-changes in the abundance of indicated metabolites after 4 h treatment with 1 mM MOG (relative to DMSO controls), in DMOG-sensitive cancer cell lines from a range of different tissue types (n = 4 cultures for each cell line). Metabolites are ordered from highest to lowest fold-change value in MCF7 cells.



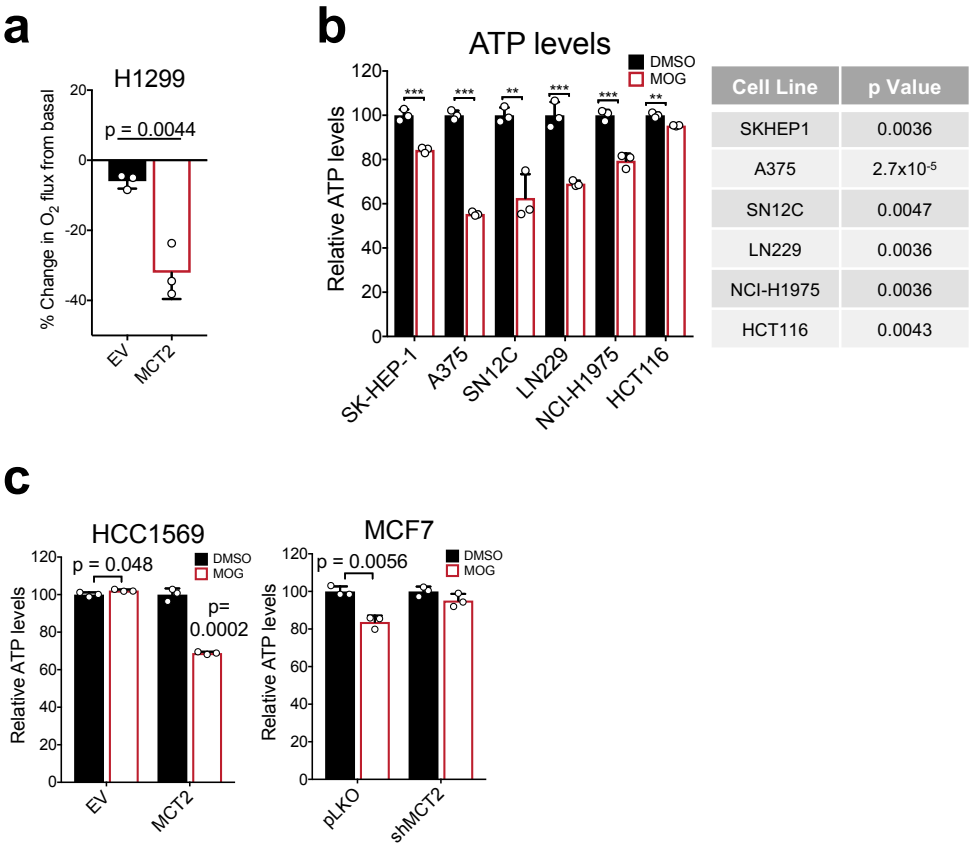
SUPPLEMENTARY FIGURE 8
Fets *et al.*

SUPPLEMENTARY FIGURE 8

a) Heatmap showing mean $\log_2$ fold-changes in the abundance of indicated metabolites after 4h treatment with 1 mM MOG (relative to DMSO controls), in parental MCF7 cells and MCF7-HIF1α mutant cells (n = 4 cultures for each cell line and treatment). Metabolites are ordered from highest to lowest fold-change value in the MCF7 parental cells.

b) Western blots to assess expression of HIF1α, and LDHA and HKII (encoded by HIF1α target genes), in parental and HIF1α-mutant MCF7 lines treated with 1 mM MOG. Experiment carried out once. Uncropped blots available in **Supplementary Fig. 13h**.

c) Cell mass accumulation of parental and HIF1α-mutant MCF7 lines treated with 1 mM MOG for 48 h relative to a 0.1% DMSO control. Data shown as mean ± SD (n = 3 cultures) and significance was tested by 2-way ANOVA with Sidak's multiple comparisons correction.



Cell Line	p Value
SKHEP1	0.0036
A375	$2.7x10^{-5}$
SN12C	0.0047
LN229	0.0036
NCI-H1975	0.0036
HCT116	0.0043

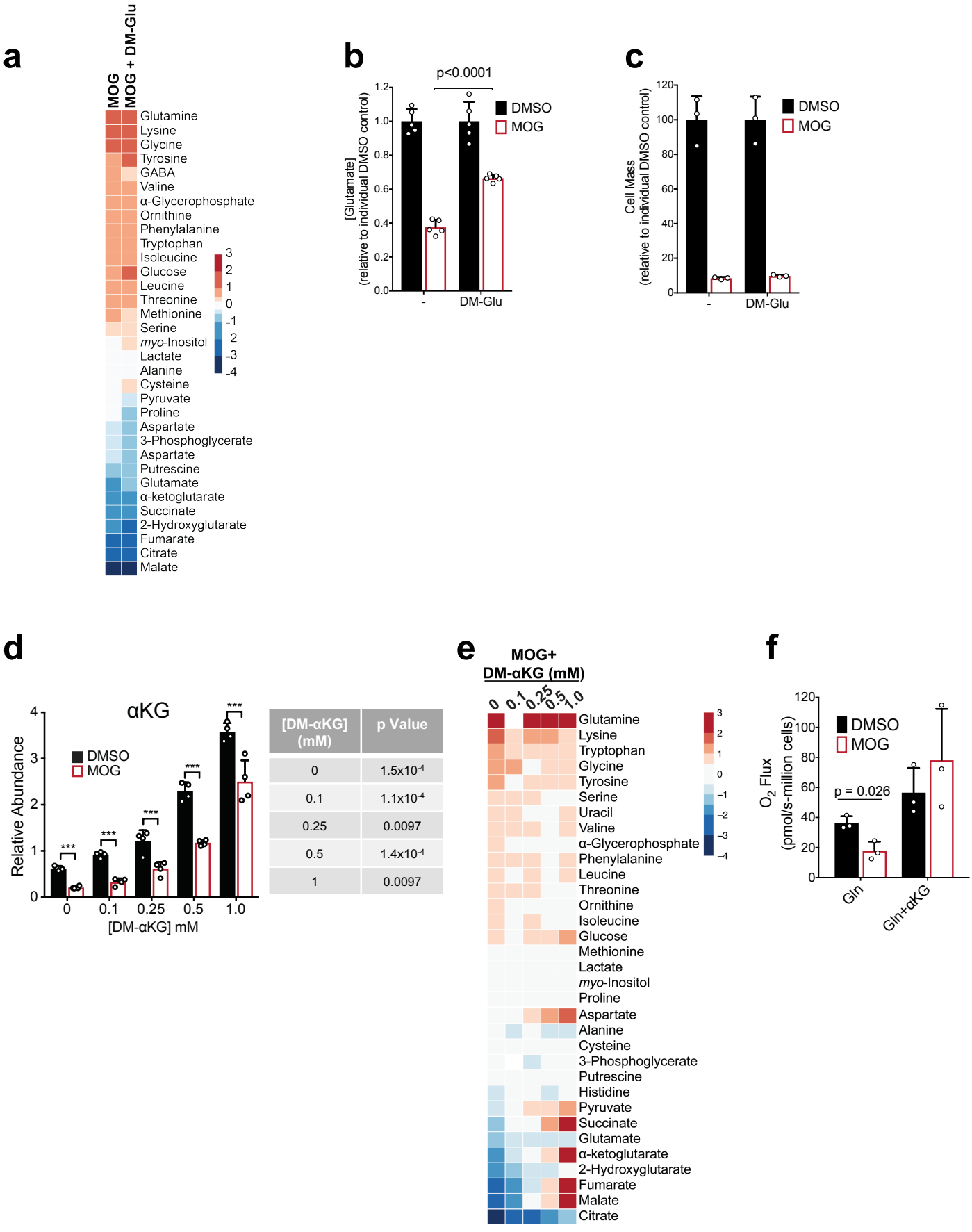
SUPPLEMENTARY FIGURE 9
Fets *et al.*

SUPPLEMENTARY FIGURE 9

a) Change in respiration from basal after treatment with 1 mM MOG in RPMI medium of H1299-TetR cells induced with doxycycline (75 ng/ml, 24 h) to express empty vector or MCT2. Data shown as mean ± SD (n = 3 cultures). Significance was tested using a 2-sided, unpaired t-test.

b) ATP levels in a panel of sensitive cell lines treated with 0.1% DMSO (vehicle) or 1 mM MOG for 4 h. Data shown as mean ± SD (n = 3 cultures), and significance was tested by 2-sided multiple t-tests with Holm-Sidak multiple comparisons correction.

c) ATP levels in HCC1569 cells expressing an empty vector (EV) or MCT2 and in MCF7 cells expressing an empty vector (pLKO) or an shRNA targeting MCT2, treated with 0.1% DMSO or 1 mM MOG for 4 h. Data shown as mean ± SD (n = 3 cultures), and significance was tested by 2-sided multiple t-tests with Holm-Sidak multiple comparisons correction.



SUPPLEMENTARY FIGURE 10
Fets *et al.*

SUPPLEMENTARY FIGURE 10

a) Heatmap showing mean $\log_2$ fold-changes in the abundance of indicated metabolites in MCF7 cells after 4 h treatment with 1 mM MOG alone or in the presence of 2 mM of DM-Glu, relative to the respective DMSO control (n = 5 cultures for each condition). Metabolites are ordered from highest to lowest fold-change value in MOG treated cells in the absence of DM-Glu.

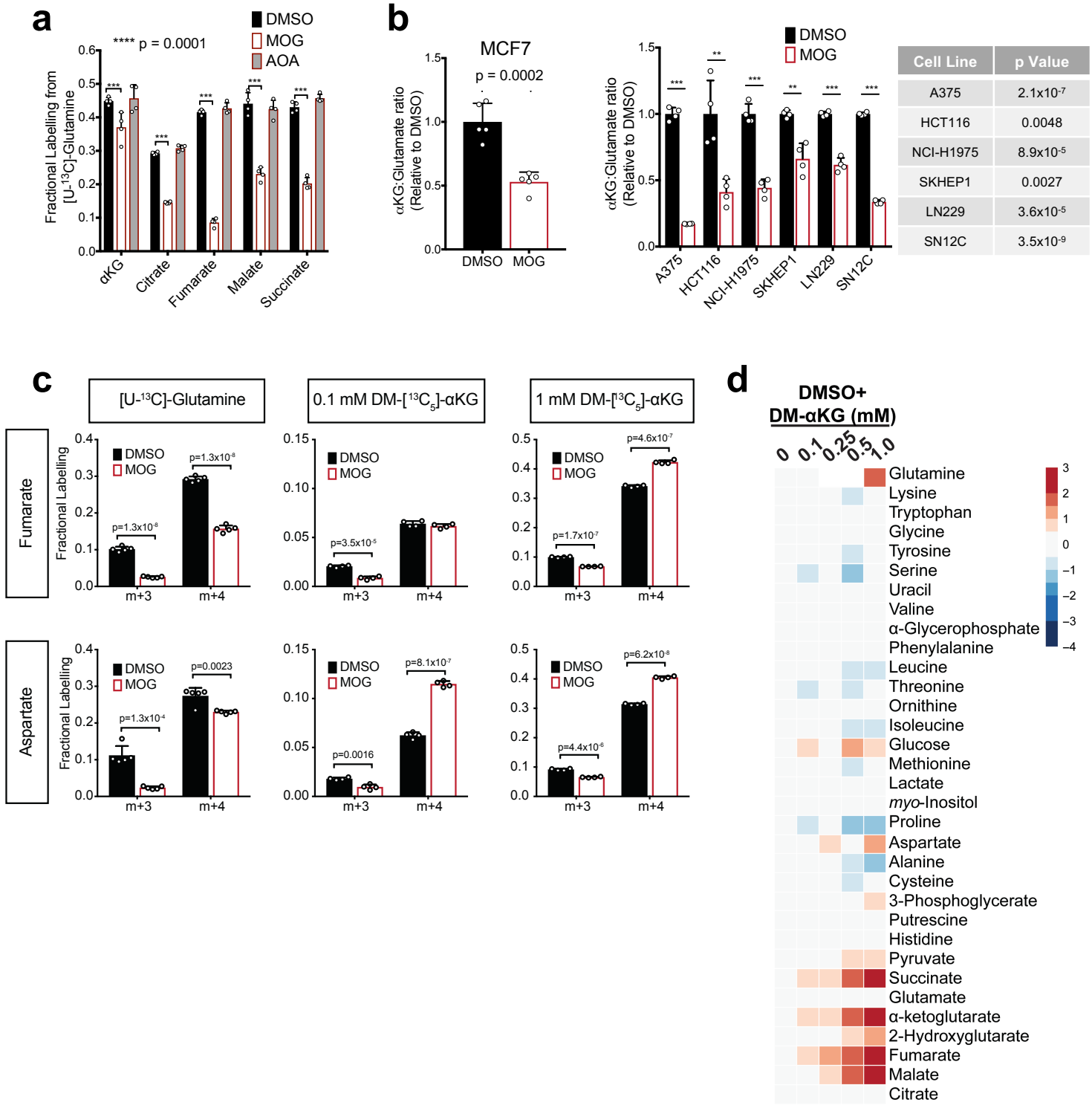
b) Intracellular concentrations of glutamate in MCF7 cells treated as in I. Data shown as mean ± SD (n = 5 cultures) and significance was tested by 2-way ANOVA with Sidak's multiple comparisons correction.

c) Cell mass accumulation in MCF7 cells treated with 1 mM MOG alone or in the presence of 2 mM DM-Glu, relative to the respective DMSO control. Data shown as mean ± SD (n = 3 cultures) and significance was tested by 2-way ANOVA with Sidak's multiple comparisons correction.

d) Relative abundance of αKG in cells treated for 4 h with increasing concentrations of DM-αKG in the absence or presence of 1 mM MOG. Data shown as the mean ± SD of n = 4 cultures for each condition. Significance was tested using 2-sided multiple t-tests with Holm-Sidak multiple comparisons correction.

e) Heatmap showing mean $\log_2$ fold-changes in the abundance of indicated metabolites in MCF7 cells after 4 h treatment with 1 mM MOG alone or in the presence of increasing concentrations of DM-αKG, relative to the respective DMSO controls (n = 4 cultures for each condition). Metabolites are ordered from highest to lowest fold-change value in MOG treated cells in the absence of DM-αKG.

f) State 3 (ADP-driven) respiration of xF-permeabilised MCF7 cells (after 30 min pre-incubation with 0.1% DMSO or 1 mM MOG) in the presence of 10 mM glutamine alone or 10 mM glutamine plus 10 mM αKG. Data shown as mean ± SD (n = 3 cultures). Significance was tested using 2-sided multiple t-tests with Holm-Sidak multiple comparison correction.



SUPPLEMENTARY FIGURE 11
Fets *et al.*

SUPPLEMENTARY FIGURE 11

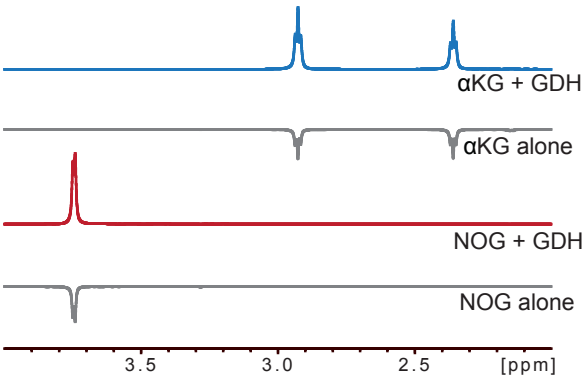
a) Fractional carbon labelling of TCA metabolites in MCF7 cells after 4 h of incubation with [U-^{13}C]-glutamine in the presence or absence of 1 mM MOG or 1 mM aminooxyacetate (AOA) compared to 0.1% DMSO control. Data shown as mean ± SD (n = 4 cultures). Significance was tested with 2-way ANOVA using Dunnett's correction for multiple comparisons.

b) Left – αKG:Glutamate ratio in MCF7 cells treated with 0.1% DMSO or 1 mM MOG for 4 hours. Data shown as mean ± SD (n = 5 cultures for each condition) and were tested for significance using a 2-sided unpaired t-test. Right – as for MCF7 cells but using a panel of MOG-sensitive cell lines (n = 4 cultures for each condition). Significance was tested with 2-sided multiple t-tests using with Holm-Sidak's correction for multiple comparisons.

c) Quantification of fumarate and aspartate m+4 isotopologues (generated by TCA in the oxidative direction) and m+3 isotopologues (generated by reductive carboxylation (RC) of αKG) in MCF7 cells incubated with [U-^{13}C]-glutamine (n = 5 cultures), tracer amounts (0.1 mM) of DM-[$^{13}C_5$]-αKG (n = 4 cultures) or rescue amounts (1 mM) of DM-[$^{13}C_5$]-αKG (n = 4 cultures) for 4 h in the presence of 0.1% DMSO or 1 mM MOG.
Data shown as mean ± SD. Significance was tested with 2-sided multiple t-tests using Holm-Sidak's correction for multiple comparisons

d) Heatmap of data from the experiment in Supplementary Fig. 7h showing mean $\log_2$ fold-changes in metabolite abundance with increasing concentrations of DM-αKG alone (without MOG) to assess the effect of different DM-αKG concentrations on metabolism (n = 4 parallel cultures for each condition). Metabolite order is the same as in **Supplementary Fig. 10e**.

a



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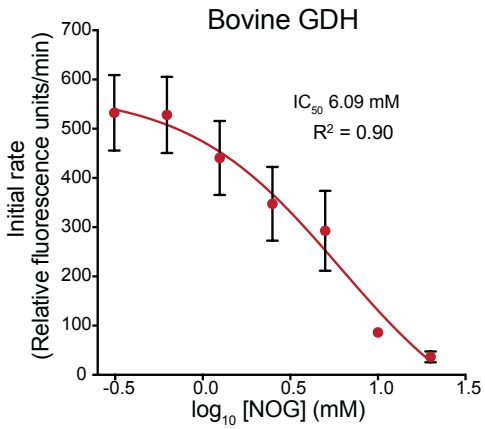

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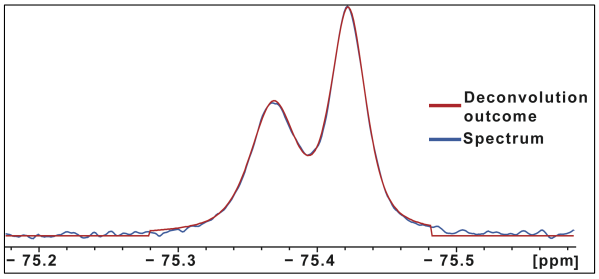
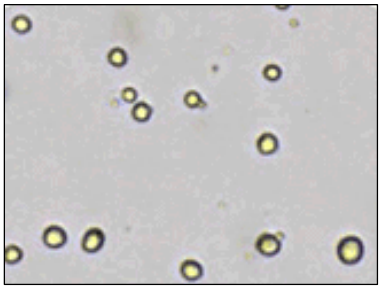




Trifluoroacetate NMR method

	Exp A	Exp B
Intracellular Peak Area	827	386
Extracellular Peak Area	1252	508
Total Volume (µL)	480	665
Total Cells	4.03E+07	7.18E+07
Total Cell Volume (uL)	191	287
Volume per cell (pL)	**4.74**	**4.00**

Imaging method (Cell diameter)

	Exp A	Exp B
Average cell diameter (µm)	18.03	20.28
Average cell radius (µm)	9.02	10.14
Volume per cell (pL)	**3.07**	**4.36**

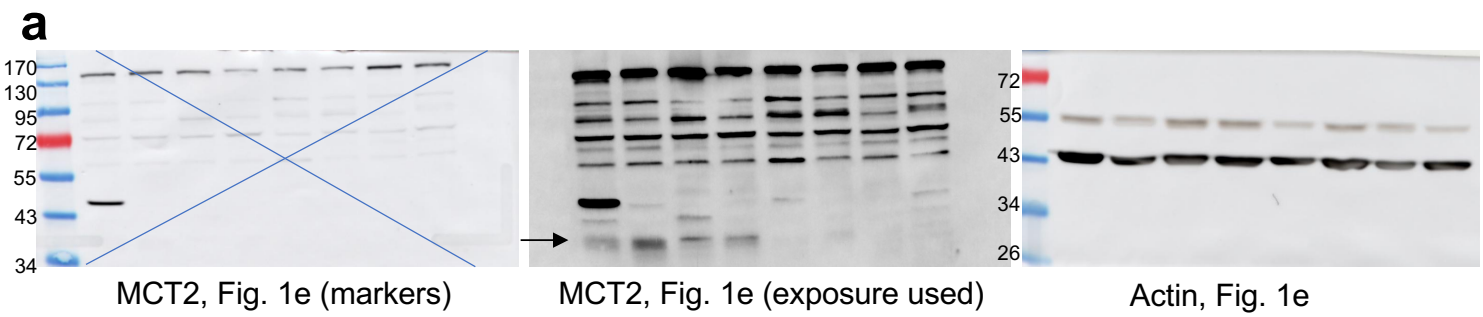
SUPPLEMENTARY FIGURE 12
Fets *et al.*

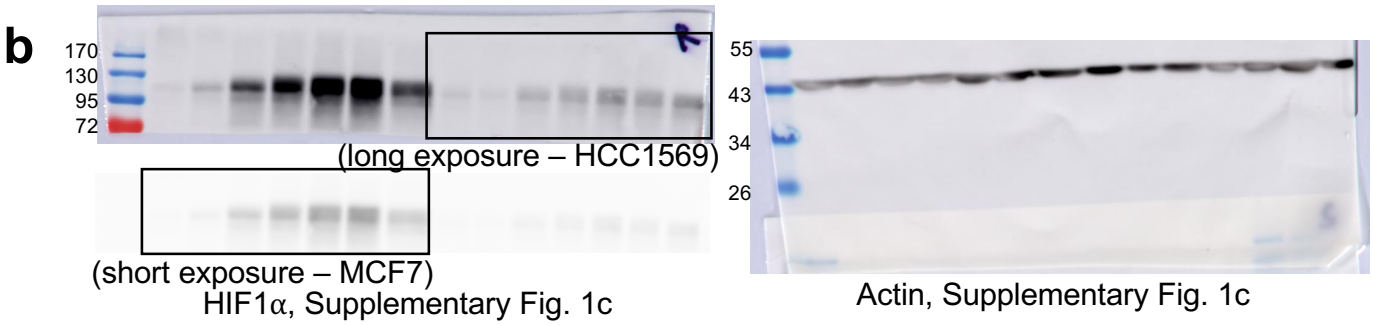
SUPPLEMENTARY FIGURE 12

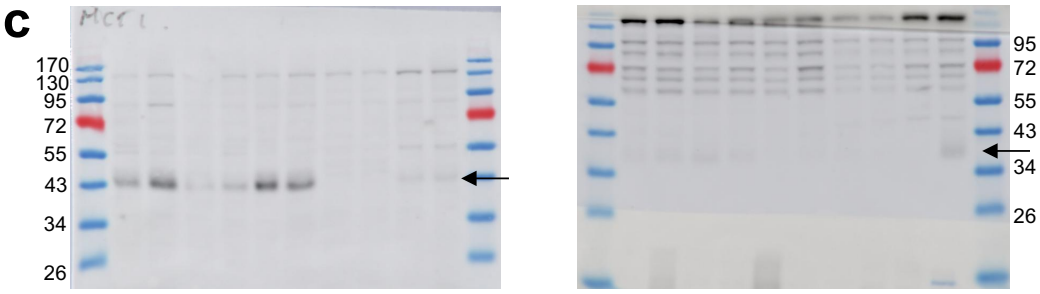
a) WaterLOGSY NMR spectra of 1 mM αKG and NOG in the presence or absence of 10 μM bovine GDH and 200 μM NAD^+. An inversion of the peak in the presence of GDH indicates ligand binding. Experiment performed three times with similar results.

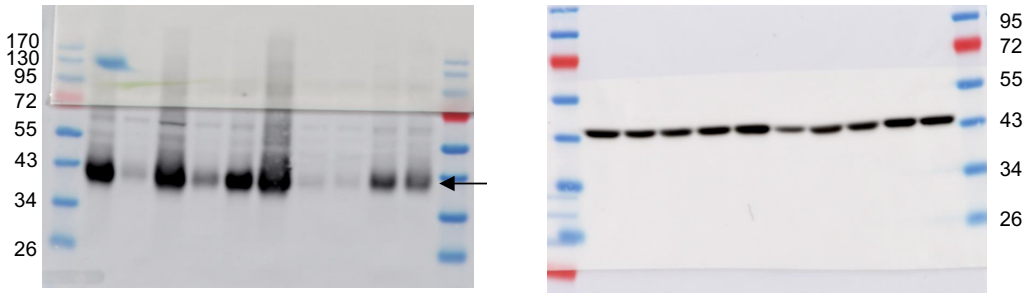
b) Activity of bovine GDH pre-incubated for 15 min in the presence of increasing concentrations of NOG. Data shown as mean ± SD (n = 3 technical repeats) and are representative of 2 independent experiments. IC_{50} determined by fitting a log[inhibitor] vs response (variable slope, 4 parameters) curve in GraphPad Prism.

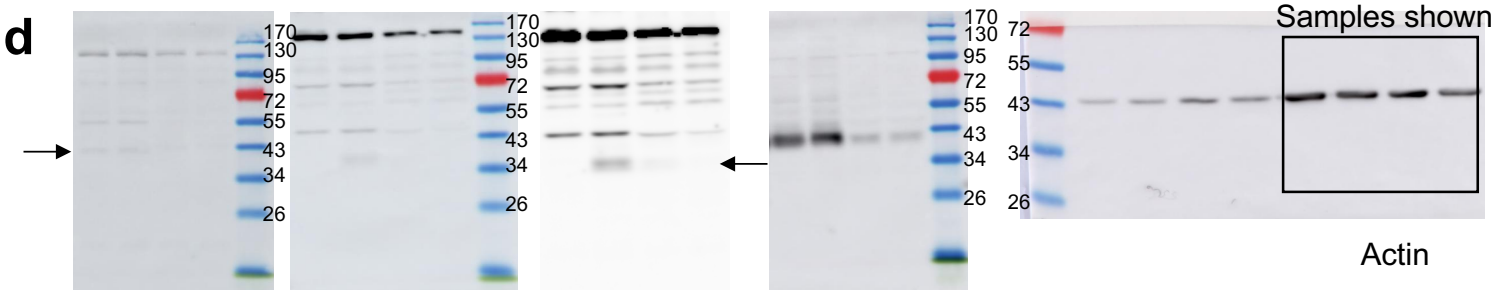
c) Outline of methods used to calculate cell volume for the determination of intracellular NOG concentrations. Volume was measured both using trifluoroacetate-uptake measured by NMR (data representative of two independent experiments) and also by measuring cell diameter (data representative of more than 10 independent experiments), as described in detail in Methods.



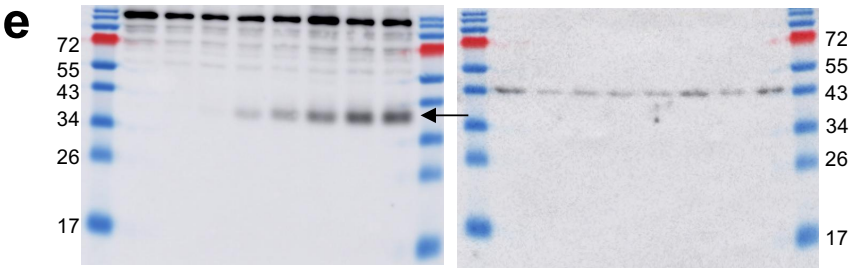






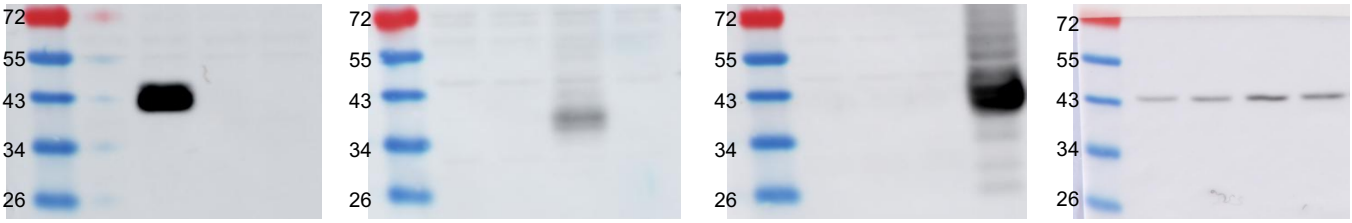


Supplementary Fig. 5a



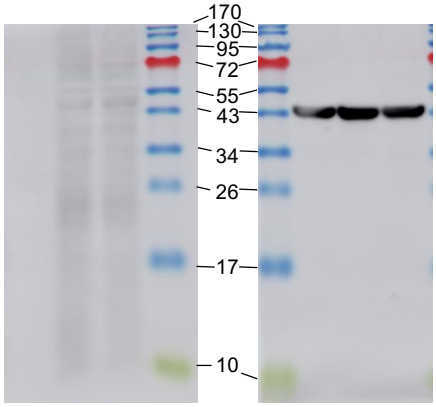
SUPPLEMENTARY FIGURE 13
Fets *et al.*

f



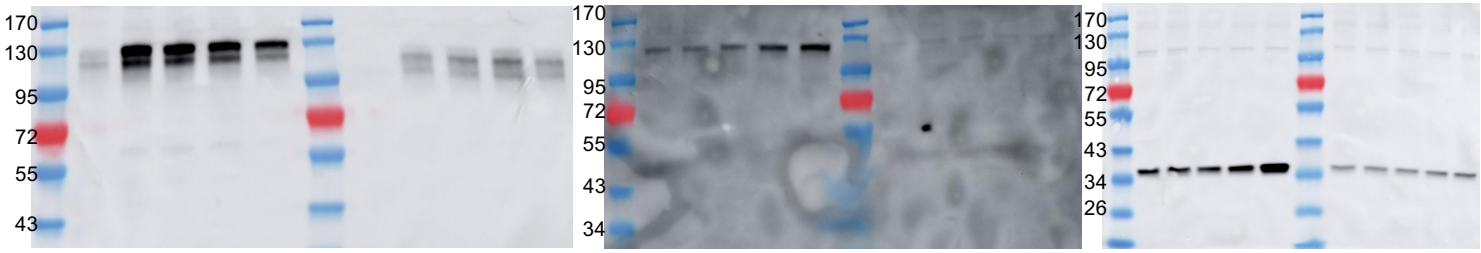
Supplementary Fig. 6a

g



Supplementary Fig. 7b

h



Supplementary Fig. 8b

SUPPLEMENTARY
FIGURE 13 (Cont'd)
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SUPPLEMENTARY FIGURE 13

a) Uncropped blots corresponding to Fig. 1e. Left MCT2 blot to show molecular weight markers, right blot shows actual exposure used.
b) Uncropped blots corresponding to Supplementary Fig. 1c. Different exposures were used for each of the two different cell lines (indicated by the boxes) in Supplementary Fig. 1c to aid comparison of the time course kinetics.
c) Uncropped blots corresponding to Supplementary Fig. 2e.
d) Uncropped blots corresponding to Supplementary Fig. 5a.
e) Uncropped blots corresponding to Supplementary Fig. 5c.
f) Uncropped blots corresponding to Supplementary Fig. 6a.
g) Uncropped blots corresponding to Supplementary Fig. 7b.
h) Uncropped blots corresponding to Supplementary Fig. 8b.

SUPPLEMENTARY DATASET 1
Spearman's rank correlation coefficients of gene transcripts with IC_{50}^{DMOG}, determined as described in Methods. Genes identified as positively or negatively correlating with sensitivity (as determined by a 5% FDR) are highlighted.

SUPPLEMENTARY DATASET 2
Spearman's rank correlation coefficients of gene transcripts with IC_{50}^{DMOG}, using only the top quartile of *SLC16A7*-expressing cell lines (213 lines) used in **Fig. 6e, f**. Genes identified as positively or negatively