Supplemental Information

Pre-clinical activity of combined LSD1 and mTORC1 inhibition in *MLL*-translocated acute myeloid leukaemia

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Table S5. Karyotypes of primary AML samples.

| Biobank number | BM or PB | Karyotype |
|----------------|----------|-----------|
| 104            | BM       | 46,XX,t(6;9;11)(p2?1;p22;q23)[6]/ 45,idem,der(15)t(15;17)(p11.2;q11.2),-17[4] |
| 108            | BM       | 46,XX,t(6;11)(q27;q23)[10] |
| 148            | PB       | 46,XY,t(6;11)[10] |
| 419            | PB       | 46,XX,t(1;22)(p21;p11.2),ins(10;11)(p12;q23q1?4)[10] (MLL gene rearrangement confirmed by FISH) |
| 514            | BM       | 46,XX,t(9;11)(p22;q23)[1]/ 46,XX[7] |
| 518            | BM       | 45,XY,der(10)t(10;11)(p1?2;q23),der(10;12)(q10;q10),der(11)t(10;11)inv(11)(q21 q23)[7]/ 45,XY,add(7)(q?22),add(10)(p?11.2),der(10;12)(q10;q10),der(11)t(10;11)inv(1 1)(q21q23),?i(17)(q10)[3] |
| 582            | BM       | 46,XX,der(1)t(1;1)(p36;q25),t(5;9;11)[5]/ 46,XX,t(5;9;11),add(17)(p11.2)[3]/46,XX,t(5;9;11),add(12)(q24)[2] |
Figure S1. CRISPR screen quality control.

(A) Histogram shows distribution of sgRNA sequencing reads in the amplified GeCKOv2 library; 99% of sgRNAs were represented by at least one sequencing read. (B) Read statistics. Scatter plots show sgRNA representation between technical replicates of (C) early time point (Day 0) samples or (D) Day 0 versus plasmid pool samples (nr = normalized reads). (E) Venn diagram shows the overlap between genes depleted in both DMSO and OG-86 Day 18 samples by comparison with Day 0 and a reference ‘core essential’ gene set.\(^\text{15}\)
Figure S2. RRAGA and MLST8 validation experiments in THP1 AML cells.

(A) Western blots show RRAGA and MLST8 knockdown 96 hr following lentiviral infection. (B) Mean±SEM fold expansion over 96 hr in liquid culture in the indicated conditions (n=4). P<0.05 or P<0.001 (t-test) indicated by * or ** respectively for OG-86 versus control condition. * indicates P<0.05 for the indicated comparisons (one way ANOVA with Fisher’s least significant difference post hoc test). (C) Exemplar flow cytometry plot shows expression of the indicated markers after 48 hr of drug treatment. (D) Mean±SEM (n=6) expression of CD11b and CD86 in the indicated conditions after 48 hrs drug treatment with (E) exemplar flow cytometry plots. * indicates P<0.05 for comparison of the indicated vehicle or OG-86-treated condition versus their respective controls (one way ANOVA with Fisher’s least significant difference post hoc test) (F) Exemplar cytospin preparations after seven days in liquid culture. Scale bar applies to all images. (G) Cell cycle and (H) apoptosis analysis of cells five days after initiation of knockdown and culture in the indicated conditions (mean±SEM; n=4); * indicates P<0.05 (paired t-test) for comparison of OG-86 condition with DMSO condition. * indicates P<0.05 (one way ANOVA with Fisher’s least significant difference post hoc test) for comparison of RRAGA KD with NTC conditions. NS – not significant. NTC – non-targeting control. MFI – mean fluorescence intensity.
Figure S3. Combined pharmacologic inhibition of LSD1 and mTORC1 impairs THP1 AML cell growth.

CD11b expression (upper panels) and cellular viability (annexin V/7-AAD negative cells) (lower panels) in THP1 cells after 48hrs or 120hrs treatment respectively with OG-86 250nM (red bars) or DMSO vehicle (blue bars) and (A) MK2206 or (B) PP242. (C) & (D) Western blots for the indicated proteins under the indicated conditions.
Figure S4. Amino acid depletion.

THP1 cells were subjected to amino acid starvation for five days in liquid culture. (A) Mean±SEM relative cell growth. (B) Mean±SEM cell viability (annexin V/7-AAD negative cells). (C) Mean±SEM relative mean fluorescence intensity (MFI) of CD86 as determined by flow cytometry. Conditions highlighted in bright red are those where MFI in vehicle treated cells is more than 2-fold higher than control cells. (D) Exemplar flow cytometry plots showing CD86 expression following methionine starvation for 48hr in the presence or absence of OG-86 250nM.
Figure S5. Gene set enrichment analyses.

Enrichment of gene sets positively and negatively correlated with leukemia stem cell potential in murine *MLL* leukemias (Table S4).
Figure S6. Structure and properties of tranylcypromine-derivative inhibitors of LSD1.

(A) Structure of OG-98 (2-((trans-2-(4-benzyloxy)phenyl)cyclopropyl)amino)-1-(4-methylpiperazin-1-yl)ethanone) and OG-86 (trans-N-((2-methoxypyridin-3-yl)methyl)-2-phenylcyclopropan-1-amine). (B) IC50 and \( K_{inact}/K_i \) values for OG-98 versus LSD1, MAOA and MAOB. (C) Pharmacokinetic data for OG-98 following a single oral 3 mg/kg dose delivered to CD1 mice (\( T_{\text{max}} \) - time to maximum plasma concentration; \( C_{\text{max}} \) - maximum concentration; \( t_{1/2} \) - plasma half-life; AUC\(_{\text{inf}} \) - area under the curve extrapolated to infinity).

| Enzyme | \( K_i \) | \( k_{\text{inact}}/K_i \) |
|--------|----------|------------------|
| LSD1   | 30nM     | 18750 M\(^{-1}\)s\(^{-1}\) |
| MAOA   | 11\( \mu \)M | 244 M\(^{-1}\)s\(^{-1}\) |
| MAOB   | 9\( \mu \)M  | 4933 M\(^{-1}\)s\(^{-1}\) |

| Value          | Value          |
|----------------|----------------|
| Plasma \( T_{\text{max}} \) | 60 min |
| Plasma \( C_{\text{max}} \) | 14mg/mL |
| Plasma \( t_{1/2} \) | 3.27 hr |
| AUC\(_{\text{inf}} \) | 56.5 hr*ng/mL |
| Bioavailability | 19% |

Figure S7. Minimal residual disease xenografts.

(A) Example images of primary human MLL leukaemia colonies. At the end of the colony assay, methylcellulose was washed out and cells counted and analysed for cell surface marker expression. (B) Example flow cytometry plots (C) Mean±SEM murine cells in semi-solid culture. (D) Scatter plot shows number of cells recovered from clonogenic assays correlates strongly with the number of colonies formed (n=20).
Figure S8. Stromal co-culture experiments with primary AML cells.

Primary patient AML cells were thawed, recovered in stromal co-culture for 10 days and then treated with DMSO vehicle or the indicated compounds in stromal co-culture for seven days. (A) Relative cell numbers (upper panel) and relative expression of CD11b (lower panels) after a further seven days of culture are shown. (B) Table shows genotypes of primary AML samples evaluated. * indicates P<0.05 by one-way ANOVA with Fisher’s least significant difference post hoc test for comparison of OG-86/RAD001 condition with all others.
Supplementary Materials and Methods

Human tissue, cell lines & cell culture

Human THP1 cells were from DMSZ (Braunschweig, Germany) and were cultured under standard conditions (5% CO₂, 37°C) in RPMI1640 supplemented with 10% FBS, 10U/mL penicillin, 10µg/mL streptomycin and 2mM L-glutamine (all from Life Technologies, Carlsbad, CA), or methylcellulose (H4320, Stem Cell Technologies, Vancouver, Canada). Cells were verified for authenticity by STR analysis and confirmed to be free from mycoplasma contamination. Culture densities were 5x10⁴ - 5x10⁵ for cells in liquid culture and the starting density was 10³/ml in semi-solid culture. Colonies were enumerated after 5-7 days.

Cryopreserved leukaemic blast cells collected from the bone marrow or blood of patients at presentation were thawed and co-cultured on MS5 stromal cells, as described, in α-MEM medium supplemented with 12.5% heat-inactivated FBS, 12.5% heat-inactivated horse serum, 2mM L-glutamine, 57.2µM β-mercaptoethanol, 1µM hydrocortisone (Sigma Aldrich, St. Louis, MO) and IL-3, G-CSF and TPO (all at 20ng/ml; Peprotech, Rocky Hill, NJ) for seven days. This was to allow for recovery from cryopreservation. Cells were then transferred to fresh stromal layers and cultured for a further seven days in OG-86 250nM, RAD001 2µM or DMSO vehicle. Leukaemic cells (single cells) were readily separated from stromal cells (adhesive clumps) through disruption of the stromal layer by pipetting and then filtering the whole through a 75µm filter basket (Partec, Gorlitz, Germany).

Reagents

Inhibitors used were: MK2206 (#A10003), PP242 (#A10746), CCI779 (#A10906), BEZ235 (#HY-50673), AZD2014 (#A11303) (all from Generon, Slough, UK) and RAD001 (#07741, Sigma Aldrich). OG-86 was synthesised in house, as described. The water-soluble compound OG-98 was obtained from Oryzon Genomics S.A. Resorufin (alamarBlue) was from Invitrogen (Carlsbad, CA).

Antibodies, protein extraction and western blotting

For western blotting cells were lysed in a high salt lysis buffer (45mM HEPES (pH7.5), 400mM NaCl, 1mM EDTA, 10% glycerol, 0.5% Nonidet P-40, 6.25mM NaF, 20mM β-glycerophosphate, 1mM DTT, 20mM sodium orthovanadate and 1x Protease Inhibitor Cocktail (Roche)) and equal amounts of protein were loaded and separated by SDS-PAGE. Horseradish peroxidase-linked secondary antibodies (GE Healthcare, Little Chalfont, UK) and ECL (enhanced chemiluminescence; GE Healthcare) or SuperSignal (Pierce, Rockford, IL, USA) were used to detect immune complexes. Antibodies for western blotting were: ACTB (MAB1501; Millipore), RRAGA (#4357), MLST8 (#3274), phospho-p70 S6 Kinase (#9234), p70 S6 Kinase (#2708), phospho-p44/42 MAPK (#4370), p44/42 MAPK (#4695), phospho-4E-BP1 (#2855), 4E-BP1 (#9644) (all from Cell Signaling Technology; Danvers, MA, USA). All antibodies were used at a dilution of 1:1000 except ACTB (1:10,000).
Flow cytometry

Flow cytometry analyses were performed using either an LSR Model II BD FACSArray (BD Biosciences, Oxford, UK) or a Novocyte (Acea Biosciences, San Diego, CA, USA) flow cytometer. Cell-sorting experiments were performed using either Influx or FACSARia fluorescence-activated cell sorters (both from BD Biosciences). Antibodies used were anti-human CD11b-PE, anti-human CD14-FITC (fluorescein isothiocyanate), anti-human CD86-PerCP-eFluor710, and anti-Human CD117-PE (all eBioscience, Hatfield, UK). Apoptosis was assessed using a BD Pharmingen APC Annexin V kit (Oxford, UK), according to the manufacturer’s instructions. Propidium iodide cell cycle analyses were performed as described.4

LSD1 and MAO biochemical assays

To determine the inhibitory activity of OG-98 versus LSD1, human recombinant LSD1 enzyme (#50100, BPS Bioscience, Abingdon, UK) was incubated for 15 minutes with OG-98 on ice in the assay buffer (50 mM sodium phosphate pH 7.4). The enzymatic reaction was initiated by the addition of KM dimethyl-H3K4 peptide substrate (#63677, Anaspec, Fremont, CA). After 30 min at 37°C, Amplex Red reagent and horseradish peroxidase solution were added according to manufacturer’s instructions (Invitrogen) and left to incubate at room temperature in the dark. Conversion of the Amplex Red reagent to the fluorescent resorufin (λex = 540 nm, λem = 590 nm) was measured after 5 minutes using an Infinite F200 Tecan microplate reader (Tecan, Mannedorf, Switzerland). Kinetic parameters of LSD1 inhibition were assessed similarly, monitoring the formation of resorufin over time. To determine the inhibitory activity of OG-98 versus MAOA and MAOB, recombinant enzymes (#M7316 and # M7441 respectively, Sigma Aldrich) were incubated for 15 minutes with OG-98 in reaction buffer (100 mM Hepes pH 7.5). The enzymatic reaction was initiated by the addition of KM kynuramine and incubated for 1 hour at 37°C and stopped by adding NaOH 2N (v/v). The conversion of kynuramine to 4-hydroxyquinoline was measured by fluorescence (λex = 320 nm, λem = 360 nm) using an Infinite F200 Tecan microplate reader. Kinetic parameters of MAOA and MAOB inhibition were assessed similarly monitoring the formation of 4-hydroxyquinoline over time. Fluorescence signals were background corrected and the IC50 value was calculated with GraphPad Prism Software. Ki and K inactive were calculated using the equations previously described for tranylcypromine.54

Murine experiments

Cryopreserved leukaemic blast cells from a patient with a t(10;11) MLL gene rearrangement (BB518) were thawed and subjected to human CD3+ immunomagnetic bead depletion (#130-050-101, Miltenyi Biotec, Bergisch Gladbach, Germany) to remove residual T-cells using the POSSELD program of an AutoMACS Pro device (Miltenyi Biotec), according to the manufacturer’s instructions. Cells were placed overnight in StemSpan™ (Stem Cell Technologies) at a density of 2.5x10⁶/ml supplemented with 100ng/ml each of FL, IL-6, TPO and SCF (Peprotech) to allow for cell recovery from cryopreservation. 10x10⁶ million cells were then injected into sub-lethally irradiated (150cGy) NOD-SCID IL2Rγ− (NSG) mice (female, aged 6–12 weeks; Envigo, Shardlow, UK) via the tail vein. Ten
weeks later mice were euthanized and engrafted human AML cells in BM and spleen cryopreserved. To establish four cohorts of primary cell xenografted mice for drug treatment experiments (n=7 per cohort), sub-lethally irradiated (100cGy) mice were injected via the tail vein with 1x10⁶ CD45⁺ human AML BB518 cells cryopreserved after the first round of xenotransplantation. Fourteen weeks later, when circulating human AML cells could be detected in a majority of animals, mice were allocated to four groups balanced for human CD45⁺ blood chimerism and treated via oral gavage with either vehicle (H₂O), RAD001 (5mg/kg), OG-98 (3mg/kg), or a combination of RAD001 (5mg/kg) and OG-98 (3mg/kg) for five days. At termination of the experiment, bone marrow cells were harvested from the long bones by crushing with a mortar and pestle. An oral dose of 5mg/kg RAD001 is expected to deliver a maximum concentration of ~4,500ng/mL one hour following administration, with a t½ of 4.3hrs.⁵⁵

To model minimal residual human AML, we transplanted leukaemic blast cells from patient BB518 into NSG mice (female, aged 6–12 weeks; Envigo, Shardlow, UK) via the tail vein (1x10⁶ per mouse). Prior to injection, cells were expanded in vitro in stromal co-culture (as described above) for 14 days. Three weeks after injection, mice were randomized into four groups (five mice per cohort) and treated via oral gavage with either vehicle (H₂O), RAD001 (5mg/kg), OG-98 (3mg/kg), or a combination of RAD001 (5mg/kg) and OG-98 (3mg/kg) for four weeks (five days treatment, two days off).

CRISPR screening

Human GeCKOv2 half libraries A and B in lentiCRISPRv2 (Addgene, Cambridge, MA) were amplified and maxi-prepped as described.¹⁰ Details of verification of library representation, lentivirus manufacture and CRISPR screening procedures are in the Supplemental Information. Next, 2x10⁸ THP1 AML cells were infected with lentiviral particles (8µg/mL Polybrene; Millipore, Billerica, MA) at a multiplicity of infection of 0.3 (see Supplemental Information for details) and selected with 3ug/ml puromycin for seven days. 6x10⁷ cells (representing 500-fold coverage of the library) were then washed with PBS and frozen for subsequent genomic DNA (gDNA) isolation. This was the early time point (Day 0). The remaining cells were treated with either DMSO or 250nM OG-86 for 18 days (sufficient for ten population doublings). Cells were divided between two groups of ten T225 flasks; each flask was seeded at 2x10⁵ cells/mL in a total volume of 120mL. Cells were split every three days and medium and compound replaced. At the end of the experiment cells were frozen at -80°C for subsequent genomic DNA isolation using a QIAamp Blood Maxi kit (Qiagen, Hilden, Germany) as per manufacturer's instructions.

The sgRNA cassettes were amplified from genomic DNA and sequenced as detailed in the Supplemental Information. Sequencing reads were de-multiplexed using forward primer barcodes. Reads were trimmed using cutadapt (v1.7.1). The 20bp sgRNA sequences were then aligned to the GeCKO library using Bowtie (v1.0.1) allowing for up to one mismatched base. Mapped sgRNAs were filtered according to the following criteria before quantification: (i) any sgRNAs with other targets in the genome that match exactly or differ by only one base were discarded and (ii) for each biological
sample, any sgRNA with only a single read was removed. Normalised abundance for each sgRNA in
a given sample was calculated as follows:

\[ \text{Normalised reads per sgRNA} = \left( \frac{\text{reads per sgRNA}}{\text{total reads for all sgRNAs in the sample}} \right) \times 10^6 \]

To identify significantly depleted sgRNAs and genes in the screen we used the Model-based Analysis
of Genome-wide CRISPR/Cas9 Knockout (MAGeCK) method.\(^1^1\)

**Verification of uniform representation of CRISPR library**

To verify uniform representation of sgRNAs a two-step PCR was performed in which PCR1 amplifies
the lentiviral sequence containing the 20bp sgRNA cassette and PCR2 attaches Illumina sequencing
adapters and barcodes. In PCR#1, 50ng of each half library was amplified using V2 adaptor primers
(http://genome-engineering.org/gecko/) using Phusion High Fidelity master mix (Thermo). The
thermocycling conditions were: 98C for 3min, 15 cycles of (98C for 20s, 60C for 20s, 72C for 30s)
and 72C for 7min. For each half library there were two PCR#1 replicates. 5uL of each #PCR1 product
was run in 1.5% TAE-agarose ethidium bromide gel to verify amplicon length (~300bp). For the
PCR#2 reaction, which is carried out to add appropriate Illumina sequencing adapters to the PCR#1
product, 5uL of each PCR#1 product was used as template. Unique barcoded forward and reverse
primer combination were used for each PCR#2 reaction as described.\(^1^2\) Thermocycling conditions and
cycle numbers were the same as for PCR#1. 5uL of PCR#2 product was run on a 1.5% TAE-agarose
ethidium bromide gel to verify amplicon length (~350bp). All PCR#2 products were then pooled and
gel purified from 2% TAE-agarose ethidium bromide gel using a QiaQuick kit, as per manufacturer’s
instructions (Qiagen, Hilden, Germany). The pooled library was then quantified by Qubit fluorometric
quantitation (Thermo Fisher, Waltham, MA) and sequenced with 10% PhiX using a MiSeq System
(150 cycles, v3 chemistry) (Illumina, San Diego, CA).

The sgRNA cassettes recovered from lentivirally-infected cells were amplified using PCR#1
and PCR#2 reactions as described above, but with some modifications. To preserve full library
complexity and representation during sequencing, 244µg genomic DNA (~300-fold coverage) was
used in each PCR#1 reaction per sample. For each PCR#1, we used 5ug gDNA using V2 adaptor
primers and Phusion High Fidelity master mix (Thermo Fisher). The thermocycling conditions were:
98C for 30s, 19 cycles of (98C for 1s, 62C for 5s, 72C for 35s), 72C for 1min. 50 PCR#1 reactions
were carried out per sample to capture the full representation of the screen. All the PCR#1 reactions
for each biological sample were pooled together and 10uL of pooled #PCR1 product was used as
template for PCR#2 reactions using barcoded primers for Illumina sequencing. The thermocycling
conditions were: 98C for 30s, 19 cycles of (98C for 1s, 70C for 5s, and 72C for 35s), 72C for 1 min.
For each sample, we performed at least 15 PCR#2 reactions (1 per 10K constructs). We included
PCR#2 technical replicates for every sample (barcoded differently) to avoid duplicate sequences due
to PCR over-amplification of constructs during sequencing. PCR#2 products of each technical
replicate were pooled together and gel purified. The gel purified products were quantified by Qubit
fluorometric quantitation (Thermo Fisher). Pooled PCR#2 products for each biological sample were
then normalized before combining uniquely barcoded separate PCR replicates for all biological samples. The amount of amplifiable DNA was assessed by real time PCR using P5 and P7 Illumina primers. High throughput sequencing was performed with 15% PhiX and a HiSeq 2500 system.

**Generation of lentivirus for CRISPR screening & determination of MOI**

For lentivirus production, both hGeCKO v2 half libraries were combined in an equimolar ratio. HEK293FT cells at 80-90% confluence in 20 T225 flasks in D10 were transfected with the combined library using polyethylenimine (PEI). For each flask 16µg hGeCKO v2 library (A+B), 8µg pCMVΔ8.91 (containing HIV gag, pol, rev and tat sequences) and 4µg pMD2.G (containing sequence for vesicular stomatitis virus envelope glycoprotein) were mixed with 84µL PEI. The solution was pulse vortexed and incubated at room temperature for 30min prior to dropwise addition in 40mL D10 to each flask. After overnight incubation the medium was replaced and 24hr later viral particles were harvested and stored at 4C; this step was repeated to generate a second viral harvest which was mixed with the first. Supernatant was centrifuged at 3,000 rpm at 4°C for 10 minutes and filtered through a 0.45μm low protein-binding membrane (Millipore). Lentiviral particles were concentrated as follows: 30% (w/v) polyethylene glycol (Bioultra, 6000) (PEG) (Sigma Aldrich) was prepared in 0.5M NaCl and the solution autoclaved and stored at 4°C. 20mL ice-cold 30% PEG was added to 20mL viral supernatant and incubated overnight at 4°C on a roller shaker. The virus-PEG mixture was then centrifuged at 3000g for 15min at 4°C. The supernatant was discarded and the pellet re-suspended in 1mL R10 per 50mL centrifuge tube. Concentrated virus was stored for later use at -80°C.

To determine the viral titre of concentrated lentivirus stocks, 3×10^6 THP1 AML cells per well of a 12-well plate were spinoculated (2000rpm for 30min at 37°C) with varying dilutions of virus in R10 with 8µg/mL Polybrene (Sigma Aldrich). Next day, each well was divided between two wells of a 6-well plate and 3ug/ml puromycin was added to one of the replicate wells. After 72hr cells were counted using Trypan Blue dye exclusion and a haemocytometer. The viral dilution that resulted in 20-30% cell survival following puromycin selection in library transduced cells was used for transduction during the screen, and corresponds to a multiplicity of infection (MOI) of 0.2-0.3, assuming all infection events occur independently. At 20% cell survival following puromycin treatment, the expected percentage of cells infected by a single viral particle is estimated to be ~89%.

**Lentiviral vectors for knockdown experiments**

Lentiviral constructs (pLKO.1) used in knockdown experiments were from Sigma Aldrich: *RRAGA* #1 (TRCN0000291690), *RRAGA* #2 (TRCN0000291754), *MLST8* #1 (TRCN0000039761), *MLST8* #2 (TRCN0000039760), non-targeting control (NTC) (SHC002).

**RNA sequencing and data analysis**

Total RNA was extracted from AML cells using QIAshredder spin columns and an RNaseasy Plus Micro Kit (Qiagen). RNA quality was confirmed using an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA). Indexed PolyA libraries were prepared using either an Agilent SureSelect Strand Specific RNA Library Prep Kit (Agilent, Santa Clara, CA) (THP1 cells, 200ng total RNA) or a QuantSeq 3'
mRNA-Seq Library Prep Kit for Illumina Sequencing (Lexogen, Greenland, NH) (primary patient cells, 10ng total RNA). Libraries were quantified by quantitative PCR using a Kapa Library Quantification Kit for Illumina sequencing platforms (Roche, Basel, Switzerland). Paired-end (THP1 cells) or single end (primary cells) 75bp sequencing was carried out by clustering 1.8pM of the pooled libraries on a NextSeq500 sequencer (Illumina, San Diego, CA). Reads were mapped to the human genome (GRCm38 Gencode release 25) with STAR version 2.4.2a with the settings --outFilterMultimapNmax 20, --outFilterType BySJout, --alignSJoverhangMin 8 and --quantMode GeneCounts using the matching feature annotation GTF file for GRCh38 Gencode release 25. The number of uniquely mapped reads was 30-40 million. Differential gene expression analysis was performed using DESeq2 version 1.20.0. RPKM or FPKM (Reads or Fragments Per Kilobase of transcript per Million mapped reads) normalized values were also calculated using the DESeq2 package. Pre-ranked gene set enrichment analysis was performed with GSEA v2.0.14 software from www.broadinstitute.org/gsea. Genes were rank ordered according to log2 fold change in expression.

**Cytospin analyses**

2-5x10^4 cells were suspended in 150μl PBS and, through centrifugation at 600xg for 5 minutes, were spun onto a microscope glass slide and left to air dry. Cells were fixed by incubation in methanol for 10 minutes and then stained by May-Grünwald (Sigma Aldrich; diluted 1:1 with Sorenson’s Buffer (33.3mM KH₂PO₄, 64.75mM Na₂HPO₄, pH 6.8)) staining for 20 minutes and then Giemsa (Sigma Aldrich; 10x diluted with Sorenson’s Buffer) staining for 30 minutes. Stained slides were washed under running tap water and left in Sorenson’s buffer for five minutes prior to one final brief wash with tap water. Slides were left to air dry before cells were permanently mounted with a coverslip and DPX neutral mounting media (VWR, Radnor, PA). Images were obtained using a Leica SCN400 histology scanner (Leica, Solms, Germany).

**alomarBlue assay**

2500 cells were plated into each well of a 96-well plate and incubated with inhibitors or DMSO vehicle for 5 days. 20μl alamarBlue was added to the cells and incubated for 4h at 37°C. Resorufin fluorescence was measured using a fluorescence-based plate reader (POLARstar Omega, BMG Labtech, Aylesbury, UK).

**Amino acid depletion assay**

Amino acid-free RPMI medium powder (R8999-04A, US Biological, Salem, MA) was complemented with 2g/L sodium bicarbonate and 0.8g/L sodium phosphate, dissolved in water, adjusted to pH7.4 and sterile filtered. Complete RPMI containing a 1x concentrated solution of amino acids was obtained by complementing amino acid-free RPMI medium with RPMI 1640 amino acids solution (R7131, Sigma Aldrich), adjusted to pH7.4 and filtered. 10U/mL penicillin, 10µg/mL streptomycin, 2mM L-glutamine were added shortly before use. Amino acid depletion media were obtained by supplementing the amino acid free RPMI with individual amino acids (all from Sigma Aldrich) as follows: L-Arginine (0.2mg/ml), L-Asparagine (0.05mg/ml), L-Aspartic acid (0.02mg/ml), L-
Cysteine.2HCl (0.0652mg/ml), L-Glutamic acid (0.02mg/ml), L-glutamine (0.3mg/ml), Glycine (0.01mg/ml), L-Histidine (0.015mg/ml), Hydroxy-L-Proline (0.02mg/ml), L-Isoleucine (0.05mg/ml), L-Leucine (0.05mg/ml), L-Lysine.HCl (0.04mg/ml), L-Methionine (0.015mg/ml), L-Phenylalanine (0.015mg/ml), L-Proline (0.02mg/ml), L-Serine (0.03mg/ml), L-Threonine (0.02mg/ml), L-Tryptophan (0.005mg/ml), L-Tyrosine.2Na2H2O (0.03mg/ml), L-Valine (0.02mg/ml). 2500 cells per well of a 96 well plate were incubated with OG-86 250nM in the indicated media. Up regulation of cell surface markers such as CD86 was assessed after two days by flow cytometry. Viability was assessed after 5 days using an alamarBlue assay.

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