Tuning mTORC1 activity dictates the response of acute myeloid leukemia to LSD1 inhibition

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Supplemental Methods

Reagents

DDP38003 (referred to as Compound 15) was synthesized as described before. MC2805 (Compound 14e in [1]) was kindly provided by Prof. Antonello Mai. Rapamycin (Sirolimus) (Catalog.No.R-5000) was purchased from LC laboratories. AZD8055 (Catalog.No.S1555) and U0126 (Catalog.No.S1102) were purchased from Selleckchem. 2-Deoxy-D-glucose (Catalog.No.D8375), all-trans-retinoic acid (Catalog.No.R2625), May-Grunwald, Giemsa and ROCHE Complete™ EDTA-free Protease Inhibitor Cocktail (Catalog.No.11836170001) were purchased from Sigma Aldrich. NT-157 (Catalog.No.23442) was purchased from Cayman. Bio-Rad DC™ Protein Assay (Catalog.No.500-0114) was purchased from Biorad.

Antibodies

Primary antibodies against p-mTOR (S2448) (5536), total mTOR (2972), p-p70 S6K (T389) (9234), total p70 S6K, pS6 (S235/236) (2211), total S6 (54D2), p-4E-BP1 (T37/46) (2855), total 4E-BP1 (9644), p-AKT (S473) (9271), total AKT (9272), p-AMPK, total AMPK, p-acetyl CoA carboxylase, total acetyl CoA carboxylase (3676), p-ERK1/2 (T202/Y204) (4370), total ERK1/2 (4695), raptor (24C12), IRS1 (2382S) and LSD1 (2139) were purchased from Cell Signaling Technology. H3K4me2 (ab32356), H3K4me3 (ab8580), H3K27Ac (ab4729) and LSD1 (ab17721) antibodies were purchased from Abcam. H3K9Ac antibody was purchased from Diagenode. Antibodies against vinculin and β-actin were purchased from Sigma Aldrich. Rabbit IgG was purchased from Jackson Immunoresearch Labs Inc. Anti-human CD45 PerCP (345809) was purchased from BD Biosciences. Anti-human nuclei (4383) was purchased from Millipore.

Cell proliferation and ATP Cell Titer Glo™ assays

AML cells were cultured in T175 flasks and treated as indicated. Cell counts were performed in triplicates using trypan blue (0.2%) to identify viable AML cells using Biorad TC20™ automated cell counter. Unless otherwise specified, AML cells were cultured and treated in 96-well plate as indicated and their ATP levels were then determined using a Cell Titer-Glo™ assay (Promega Corporation, USA). Luminescence was measured using Glomax™ Multi-Detection System.
Retroviral constructs and retroviruses production

shRNA constructs were prepared in the MSCV-based pLMP retroviral vector as previously described\(^1\). The hairpins used in the study are outlined in Table S6. Supernatants from transfected Phoenix packaging cells were collected 48 and 72 h post-transfection and immediately used for infection cycles.

Retroviral transduction of AML cells

For transducing THP-1, OCI-AML3 and KASUMI-1 cells, cells were diluted in retroviral supernatants added with 8μg/mL polybrene and seeded in 24-well plate. Spin infection was performed for 2 consecutive days (2 cycles of infection/day) at 1800 rpm for 45 min. For THP-1 and OCI-AML3 cells, puromycin selection was initiated 24 h after the last cycle of infection. Early passages of puromycin-resistant pool of cells were used for subsequent experiments. For KASUMI-1 cells, GFP positive cells were sorted and used for subsequent experiments.

Establishment of LSD1i-resistant AML cells

To generate LSD1i-resistant cells, parental LSD1i-naïve/responsive KASUMI-1 cells (designated KASUMI-1/P) were continuously exposed to increasing concentrations of DDP38003 as previously described\(^3\). After twelve months, descendent KASUMI-1 cells started to grow and proliferate in the presence of DDP38003. The derived resistant cells were named KASUMI-1/R cells. Resistance index (RI) was determined as the ratio of the median inhibitory concentration (IC\(_{50}\)) of KASUMI-1/R subline divided by the IC\(_{50}\) of KASUMI-1/P. Cross resistance of KASUMI-1/R cells to varying concentrations of MC2580 was confirmed using Cell Titer-Glo™ Luminescent assay.

Western blot analysis

Cells were washed and then lysed in HEPES lysis buffer supplemented with protease/phosphatase inhibitor cocktail as previously described\(^4\). After 20 min incubation on ice, cell lysates were centrifuged at 14,000 rpm for 10 min at 4°C. Cell lysates were resolved onto 12% SDS-PAGE and proteins were transferred to nitrocellulose membranes (Protran BA85, GE Healthcare life sciences) and then blocked for 1 h at using 5% skimmed milk in TBST. Incubation with the specific primary antibodies was performed in blocking buffer at 4°C. Horseradish peroxidase-conjugated anti-IgG was used as secondary antibody.
Immunoreactive bands were detected by ECL chemiluminescent substrate (Amersham ECL Prime Western Blotting Detection Reagent, GE Healthcare life sciences).

_Apoptosis detection using Annexin V/PI staining_

Extent of apoptosis in untreated and treated AML cells was assessed using Annexin V/Propidium iodide (PI) assay as previously described. Data were analyzed using FlowJo software.

_Cell cycle analysis_

Briefly, following the treatment of AML cells as indicated, cells were fixed in 70% ethanol and treated with RNase and stained with PI overnight before FACS analysis was carried out as previously indicated. Data were analyzed using FlowJo software.

_Real time-reverse transcriptase quantitative PCR (RT-qPCR)_

RNA was extracted from the cells using RNA extraction kit (Direct-zol™ RNA kit, Zymo research Corp). RNA quantity was evaluated spectrophotometrically using NanoDrop (ND-1000 Technologies Inc.). cDNA was used to carry out quantitative PCR using SYBR Green Reaction Mix (Perkin Elmer, Boston, MA). mRNA levels were normalized against the house keeping gene (GAPDH) mRNA. The sequences of the primers used in the present study are listed in Table.S7.

_RNA-Sequencing and bioinformatic analysis_

Cells were pelleted and RNA was isolated using an RNA extraction kit (Direct-zol™ RNA kit, Zymo research Corp). RNA-seq libraries were constructed using the Illumina TruSeq RNA sample preparation protocol. Single-end reads were aligned to the _hg18_ reference genome using TopHat v2.0.13 with default parameters, except for using the option _--no-coverage-search_. The normalized coverage tracks files (bigWig) for the UCSC genome browser were generated considering just reads with a unique mapping position on the genome, those reads were fished out from the bam files using the option _-q 1_ of SAM tools. Profile were obtained using _genomeCoverageBed_ from Bed Tools v2.17.0 and then linearly re-scaled according to sequencing depth (RPM, Reads Per Million sequenced reads). Reads quantification was calculated using the _featureCount_ function of the Subread package. _edger_ was used to assess differential expression. Libraries were normalized according to TMM normalization. Differentially expressed genes (DEGs) were defined as those showing a
FDR ≤0.05 and a linear fold-change ≥1.5. R was used to run edger and to generate the heatmaps and plots. Functional annotation and enrichment of differentially expressed genes (DEGs) were analyzed through the use of Ingenuity Pathway Analysis (IPA; QIAGEN Inc.) at (https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis). The datasets generated in this study have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE125719 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE125719).

Chromatin immunoprecipitation quantitative PCR (ChIP-qPCR)

Chromatin immunoprecipitation (ChIP) assays for LSD1, H3K4me2, H3K4me3, H3K9Ac, H3K27ac and IgG were performed after treating the cells as indicated. For LSD1 and histone marks immunoprecipitation, 200×10⁶ and 5×10⁶ human AML cells per immunoprecipitation were used respectively. Briefly, after crosslinking with 1% paraformaldehyde for 10 min and quenching with 0.125M glycine for 5 min, cells were washed twice with ice-cold PBS, lysed and resuspended in IP buffer and sonicated. Sonicated lysates were then precleared with BSA-blocked Protein A Sepharose beads. An aliquot was used as an input control, whereas the rest of the sonicated samples were incubated overnight at 4°C with the indicated antibodies and precipitated with 30 μl of Protein G Dynabeads. Following washing cycles, the precipitated magnetic beads were treated with elution buffer. Cross-links of the eluted samples were reversed by incubating them overnight at 65°C. After protein digestion with Proteinase K, DNA was eventually purified using a PCR Purification Kit (Qiagen) and analyzed by real-time qPCR.

Clonogenic assay

AML cells were treated as indicated and cultured in MethoCult H4435 Enriched (StemCell Technologies, Vancouver, BC) according to the manufacturer instructions for the indicated time point of treatment. Colonies were then counted and cytospin preparations of the obtained cells were then stained using May Grunwald Giemsa staining.

FACS analysis of infiltration of human AML cells

Peripheral blood samples were collected via tail bleeding. Spleen and femur bone marrow samples were also harvested two weeks after treatment. RBCs were lysed using RBC lysis buffer and the remaining mononucleated cells were pelleted by centrifugation. The pellet was washed once using ice-cold PBS. Cells were then stained with anti-human CD45 PerCP
diluted in 5% BSA in PBS at 4°C for 30 min, washed in 1% BSA in PBS and fixed in formaldehyde (final concentration 1%) for 20 min on ice. Fixed cells were then washed in 1% BSA in PBS and resuspended in PBS. FACS acquisition was carried out using FACS Calibur and data were analyzed using FlowJo software.

**Histopathological examination**

Paraformaldehyde-fixed (4%) samples of murine spleen, bone marrow and muscle tissues were paraffin embedded using Diapath automatic processor. Haematoxylin and eosin (Diapath) staining was performed according to the standard protocol and samples were mounted in EuKitt (Bio-Optica). The degree of leukemia infiltration was scored by a pathologist blinded to the experimental groups.

**Immunohistochemistry**

For IHC analysis, paraffin was removed using xylene and sections were rehydrated in graded alcohol. Antigen retrieval was carried out using a preheated target retrieval solution for 45 min. Tissue sections were blocked using FBS in PBS for 60 min and incubated overnight with one of the following primary antibodies; anti-human nuclei antibody (1:200) to clearly identify/distinguish infiltrating primary human leukemic cells and anti-phospho-S6 ribosomal protein (1:300) as readout of mTOR activity. Antibody binding was detected using polymer detection kit (GAR-HRP and GAM-HRP, Microtech) followed by diaminobenzidine chromogen reaction (Peroxidase substrate kit, DAB, SK-4100; Vector Lab). All sections were counterstained with Mayer's hematoxylin and visualized using bright-field microscope.

**Statistical analysis**

Analysis of data was performed using GraphPad Instat (Version 2) as follows; data are presented as mean ± standard deviation (SD). Unless otherwise indicated, Student’s t-test was used to compare two different treatment groups. Multiple comparisons for more than two treatment groups were carried out using either one way analysis of variance (ANOVA) followed by Dunett test for post-hoc analysis or two-way ANOVA followed by Bonferroni post-hoc test. Mantel-Cox test was used to analyze Kaplan-Meier survival curve results. Statistical significance was acceptable at P value less than 0.05. Graphs were presented using Graphpad Prism software program (Version 5).
Table S1. Predicted upstream regulators obtained from IPA upstream analysis of KASUMI-1 cells 6h following their treatment with DDP38003 (0.5μM).

| Upstream Regulator | Predicted Activation State | Activation z-score | P-value of overlap | Target molecules in dataset |
|--------------------|-----------------------------|--------------------|--------------------|-----------------------------|
| DOCK8              | Activated                   | 2,000              | 1.60E-02           | EDN1,LTA,RILPL1,TLR3        |
| Ifnar              | Activated                   | 2,224              | 7.55E-03           | CCL2,CD86,GBP2,OAS1,TLR3    |
| IL4                | Activated                   | 2,975              | 1.93E-02           | CD163,CD2,CNR2,CXCR3,GBP2,GZMA,IL1RN,LRP1,LTA,PMP22,STAB1 |
| CD44               | Activated                   | 2,414              | 1.38E-03           | ACKR3,ADGRE1,CXCR3,IL1RN,SELL,TIAM1,TLR8 |
| SAMSN1             | Activated                   | 2,000              | 3.52E-02           | EDN1,LTA,RILPL1,TLR3        |
| MET                | Activated                   | 2,000              | 6.44E-03           | EDN1,LTA,RILPL1,TLR3        |
| HNF1A              | Activated                   | 2,236              | 2.61E-02           | DCT,EBF1,FFAR2,PRLR,RNASE4,TMPRS4 |
| SASH1              | Activated                   | 2,000              | 1.76E-02           | EDN1,LTA,RILPL1,TLR3        |
| IL6                | Activated                   | 2,200              | 2.68E-02           | A2M,ADGRE1,CXCR3,HFE,IL1RN,ITGAM |

*Appendix Supplementary Tables*
Table S2. Predicted upstream regulators obtained from IPA upstream analysis of KASUMI-1 cells 72h following their treatment with DDP38003 (0.5μM).

| Upstream Regulator | Predicted Activation State | Activation z-score | P-value of overlap | Target molecules in dataset |
|--------------------|-----------------------------|--------------------|--------------------|----------------------------|
| IL4                | Activated                   | 2,813              | 1.93E-02           | CD163, CD2, CNR2, CXCR3, GBP2, GZMA, IL1RN, LRP1, LTA, PMP22, STAB1 |
| CHUK               | Activated                   | 2,200              | 6.38E-03           | ACKR3, GBP2, IL1A, IL1RN, TLR3, TMEM176B |
| CD44               | Activated                   | 2,414              | 1.38E-03           | ACKR3, ADGRE1, CXCR3, IL1RN, SELL, TIAM1, TLR8 |
| HNF1A              | Activated                   | 2,236              | 2.61E-02           | DCT, EBF1, FFAR2, PRLR, RNASE4, TMPRSS4 |
| IL6                | Activated                   | 2,200              | 2.68E-02           | A2M, ADGRE1, CXCR3, HFE, IL1RN, ITGAM |
Table S3. Predicted upstream regulators obtained from IPA upstream analysis of THP-1 cells 24h following their treatment with DDP38003 (0.5μM).

| Upstream Regulator | Predicted Activation State | Activation z-score | P-value of overlap | Target molecules in dataset |
|--------------------|----------------------------|--------------------|--------------------|----------------------------|
| Alpha catenin      | Inhibited                  | -2.380             | 1.52E-04           | FFAR2,GPR34, MPEG1, PLBD1  |
| TRAF2              | Inhibited                  | -2.000             | 7.34E-03           | FFAR2,GPR34, MPEG1, PLBD1  |
| TRAF3              | Inhibited                  | -2.000             | 1.31E-02           | CYBB,CYP19A1, IL1A, VCAN   |
| ERK1/2             | Activated                  | 2.000              | 3.03E-02           | IL15,LTA,RILPL1, TLR3      |
| DOCK8              | Activated                  | 2.000              | 3.33E-02           | CCL2,CD86,GBP2, IL15,OAS1,TLR3 |
| Ifnar              | Activated                  | 2.434              | 4.34E-03           | ITGAM,MYOG, S100A8,S100A9,TRIM63 |
| TNFSF12            | Activated                  | 2.219              | 1.15E-02           | CCL2,CD14,CD163, CD86,FCGR2B,GBP2 ,GPR34,IL15,IL1A, ITGAM,LTA,RILPL1 ,SLC6A12,TLR3, TRIM63 |
| TLR4               | Activated                  | 2.438              | 1.19E-04           | ADAM19,ANXA2, CD163,CD2,CLEC10 A,CNR2,CXCR3, GBP2,GZMA,IL18R1 ,IL1RN,LRP1,LTA,P |
| Gene | Status   | Value 1  | Value 2  |
|------|----------|---------|---------|
| MP22,STAB1,VIM | | | |
| IL4  | Activated | 2,554   | 1.27E-03 |
| CRHBP,GPR65,IL1A,IL1RN,LY96,S100A9 | | | |
| mir-223 | Activated | 2,449   | 1.06E-02 |
| ACKR3,ADGRE1,CXCR3,IL1RN,MOGAT1,SELL,TIAM1,TLR8 | | | |
| CD44 | Activated | 2,611   | 1.15E-03 |
| IL15,LTA,RILPL1,TLR3 | | | |
| CRKL | Activated | 2,000   | 1.10E-04 |
| CCL2,CXCL14,EBF1,IL15,IL1A,LTA | | | |
| CDKN2A | Activated | 2,000   | 1.02E-02 |
| IL15,LTA,RILPL1,TLR3 | | | |
| MET | Activated | 2,000   | 1.40E-02 |
| CCL2,CD86,FPR1,IL15,IL1A,TLR3 | | | |
| TICAM1 | Activated | 2,401   | 2.02E-02 |
| BLNK,CD86,ID3,PGR | | | |
| SATB1 | Activated | 2,000   | 3.18E-02 |
| CD86,IL1A,S100A8,S100A9 | | | |
| IL12B | Activated | 2,000   | 4.76E-03 |
| IL15,LTA,RILPL1,TLR3 | | | |
| SASH1 | Activated | 2,000   | 3.64E-02 |
| CYP19A1,MYOG,TMEM176A,TMEM176B,VWF | | | |
| LIF | Activated | 2,236   | 1.21E-02 |
| IL15,LTA,RILPL1,TLR3 | | | |
| ARHGAP21 | Activated | 2,000   | 6.77E-03 |
| BLNK,CD86,CLEC10A,CLEC4M,GEM | | | |
| **SPIB** | Activated | 2,213 | 1,28E-02 | FFAR2,GPR34, MPEG1,PLBD1 |
**Table S4.** Predicted upstream regulators obtained from IPA upstream analysis of THP-1 cells 72h following their treatment with DDP38003 (0.5μM).

| Upstream Regulator | Predicted Activation State | Activation z-score | P-value of overlap | Target molecules in dataset |
|--------------------|-----------------------------|---------------------|--------------------|-----------------------------|
| Alpha catenin      | Inhibited                   | -2.380              | 1.52E-04           | ADAMTS12, ADAMTS5, CXCL12, FMNL2, ITGAM, LYZ, S100A8, S100A9, VIM |
| ERK1/2             | Activated                   | 2.000               | 3.03E-02           | CYBB, CYP19A1, IL1A, VCAN    |
| MGEA5              | Activated                   | 2.121               | 4.68E-03           | ADAMTS5, CALCRL, CCL2, CD14, CD53, CD86, GP9, GPR34, IL21R |
| DOCK8              | Activated                   | 2.000               | 3.33E-02           | IL15, LTA, RILPL1, TLR3     |
| Ifnar              | Activated                   | 2.434               | 4.34E-03           | CCL2, CD86, GBP2, IL15, OAS1, TLR3 |
| TNFSF12            | Activated                   | 2.219               | 1.15E-02           | ITGAM, MYOG, S100A8, S100A9, TRIM63 |
| TLR4               | Activated                   | 2.948               | 1.19E-04           | CCL2, CD14, CD163, CD86, FCGR2B, GBP2, GPR34, IL15, IL1A, ITGAM, LTA, RILPL1, SLC6A12, TLR3, TRIM63 |
| IL4                | Activated                   | 3.065               | 1.27E-03           | ADAM19, ANXA2, CD163, CD2, CLEC10A, CNR2, CX3, GBP2, GZMA, IL18R1, IL1RN, LRP1, LTA, PMP22, STAB1, VIM |
| mir-223            | Activated                   | 2.236               | 1.06E-02           | CRHBP, GPR65, IL1A,         |
| Gene    | Status    | Value  | P-value | Additional Genes |
|---------|-----------|--------|---------|------------------|
| IL1RN,LY96,S100A9 |            |        |         |                  |
| CD44    | Activated | 2,611  | 1,15E-03| ACKR3,ADGRE1,CXCR3, IL1RN,MOGAT1,SELL,TI AM1,TLR8 |
| CRKL    | Activated | 2,000  | 1,10E-04| IL15,LTA,RILPL1,TLR3 |
| CDKN2A  | Activated | 2,000  | 1,02E-02| CCL2,CXCL14,EBF1,IL15, IL1A,LTA |
| MET     | Activated | 2,000  | 1,40E-02| IL15,LTA,RILPL1,TLR3 |
| TICAM1  | Activated | 2,401  | 2,02E-02| CCL2,CD86,FPR1,IL15, IL1A,TLR3 |
| SATB1   | Activated | 2,000  | 3,18E-02| BLNK,CD86,ID3,PGR |
| IL12B   | Activated | 2,000  | 4,76E-03| CD86,IL1A,S100A8, S100A9 |
| SASH1   | Activated | 2,000  | 3,64E-02| IL15,LTA,RILPL1,TLR3 |
| LIF     | Activated | 2,236  | 1,21E-02| CYP19A1,MYOG,TMEM1 76A,TMEM176B,VWF |
| ARHGAP21| Activated | 2,000  | 6,77E-03| IL15,LTA,RILPL1,TLR3 |
| SPIB    | Activated | 2,213  | 1,28E-02| BLNK,CD86,CLEC10A, CLEC4M,GEM |
Table S5. List of differentially expressed genes (DEGs) in THP-1 and KASUMI-1 cells following the indicated time points of treatment with DDP38003 (0.5μM).

| KASUMI-1_6h N=175 | KASUMI-1_24h N=192 | THP-1_24h N=205 | THP-1_72h N=196 |
|-------------------|-------------------|----------------|----------------|
| A2M               | A2M               | ABCG2          | ABCA6          |
| ABI3              | ABCA6             | ACADL          | ACOX2          |
| ACADL             | ABCG2             | ACOX2          | ACVR1C         |
| ACVR1C            | ABI3              | ADAM19         | ADAM19         |
| ADAM28            | ACVR1C            | ADAM28         | ADAM28         |
| ADAP2             | ADAM28            | ADAMTS12       | ADAMTS12       |
| AMICA1            | ADAMTS12          | ADAMTS5        | ADAMTS5        |
| ASPN              | AF064861.93       | ADAP2          | ADAP2          |
| B3GALT2           | AIM1              | ADORA1         | AIM1           |
| BAI3              | AKR1C4            | AF064861.93    | AKR1C4         |
| BCL2L14           | ALOX5             | AIM1           | ALDH7A1        |
| BHLHE41           | APOL3             | AKR1C4         | ALOX5          |
| BX296545.5        | ASB15             | ALDH7A1        | AMDHD1         |
| C10orf67          | ASPN              | ALOX15         | AMICA1         |
| C11orf41          | B3GALT2           | ALOX5          | ANXA2          |
| C1orf62           | BAI2              | AMDHD1         | APOA2          |
| C1QTNF1           | BAI3              | AMICA1         | APOL3          |
| C20orf26          | BHLHE41           | ANXA2          | ARL4C          |
| C4orf18           | BLNK              | APOL3          | ASB15          |
| C4orf44           | BX296545.5        | BAI2           | BAI2           |
| CABP4             | C10orf141         | BIRC7          | BIRC7          |
| CALCB             | C10orf67          | BLNK           | BLNK           |
| CALCRL            | C11orf41          | BMX            | BMX            |
| CALHM3            | C15orf53          | BTBD11         | C10orf67       |
| CAMSAP1L1         | C16orf45          | C11orf41       | C16orf45       |
| CASP5             | C17orf99          | C16orf45       | C1orf62        |
| CCDC46            | C1orf62           | C1QL4          | C1QL4          |
| CCL2              | C1QL4             | C21orf93       | C20orf26       |
| CD163 | C20orf26 | C4orf44 | C21orf93 |
|-------|----------|---------|----------|
| CD1C  | C4orf18  | C7orf23 | C4orf44  |
| CD5   | C7orf51  | C7orf51 | C7orf23  |
| CD86  | CABP4    | C7orf58 | C7orf51  |
| CEACAM6 | CACNA1G | CACNA2D1 | C7orf58 |
| CFH   | CACNA2D1 | CADM1   | CACNA1G  |
| CLUL1 | CALCB    | CALCB   | CADM1    |
| CMKLR1 | CALCRL  | CAMK2A  | CALCRL   |
| CNR2  | CALHM3   | CASP5   | CALHM3   |
| CRHBP | CAMK2A   | CBLN1   | CASP5    |
| CSAG2 | CAMSAP1L1 | CCDC109B | CCDC109B |
| CXCL11 | CCDC46  | CCDC129 | CCDC46   |
| CXCL14 | CD14    | CCDC46  | CCL13    |
| CXCL5 | CD163    | CCL2    | CCL2     |
| CXCR3 | CD2      | CD14    | CD14     |
| CXCR7 | CD3E     | CD163   | CD163    |
| CYP2C18 | CD5     | CD1A    | CD1C     |
| DCT   | CD86     | CD1B    | CD2      |
| DDX43 | CFH      | CD1C    | CD5      |
| ECM2  | CHN2     | CD1E    | CD53     |
| EDN1  | CHRNA6   | CD2     | CD86     |
| EPB41L3 | CLEC12A | CD5     | CEACAM6  |
| EPN3  | CLEC4A   | CD53    | CFH      |
| ETV1  | CNR2     | CD86    | CHN2     |
| F9    | COL28A1  | CEACAM6 | CHRNA6   |
| FAIM3 | COLEC12  | CFH     | CLEC12A  |
| FAM108A6 | CRHBP  | CHN2    | CLEC4A   |
| FAM162B | CSAG2  | CHRNA6  | CLUL1    |
| FCRLA | CTNBP2   | CLEC4A  | CNR2     |
| FFAR2 | CXCL14   | CLUL1   | COL28A1  |
| FGD6  | CXCL5    | CNR2    | CPT1C    |
| FRG2B | CXCR7    | CPT1C   | CSPG2    |
| GABRA4 | CYP26B1 | CRHBP | CXCL11 |
|--------|---------|-------|--------|
| GCNT1P | CYP2C18 | CSAG2 | CXCL14 |
| GEM    | DCT     | CSPG2 | CXCR7  |
| GIMAP4 | DDIT4L  | CXCL11| CYBB   |
| GLIPR1 | DDX43   | CXCR3 | CYP19A1|
| GNGT2  | DES     | CXCR7 | DDIT4L |
| GPR143 | DMD     | CYBB  | DMD    |
| GPR15  | DMGDH   | DDIT4L| DSG2   |
| GPR174 | DSG2    | DDX43 | DTX4   |
| GPR177 | EBF1    | DMD   | EBF1   |
| GPR183 | ECM2    | DOCK4 | ECM2   |
| GPR65  | EDN1    | DTX4  | EMR1   |
| GPR84  | EGFLAM  | EBF1  | EPB41L3|
| GRIA3  | EMR1    | ENPP1 | EPHB3  |
| GZMA   | ENPP1   | EPB41L3| EPN3  |
| HFE    | EPHB3   | EPHB3 | ETV1   |
| HORMAD1| EPN3    | ETV1  | EVPL   |
| HPGD   | ETV1    | EVPL  | FAIM3  |
| ID3    | F9      | FAIM3 | FAM162B|
| IGKC   | FAIM3   | FAM162B| FAM65B|
| IGSF3  | FCGBP   | FAM65B| FAP    |
| IL1RN  | FCGR2B  | FAP   | FCAMR  |
| IL24   | FCRLA   | FCAMR | FCGBP  |
| IL28B  | FFAR2   | FCGR2B| FCGR2B |
| IL31RA | FGD6    | FCRLA | FCRLA  |
| KCTD12 | FPR1    | FFAR2 | FFAR2  |
| KIAA1644| FRG2B  | FGD2  | FGD2   |
| KISS1R | GABBR2  | FMNL2 | FGD6   |
| KLF4   | GABRA4  | FPR1  | FMNL2  |
| KLHL34 | GATA1   | GABRA4| FPR1   |
| LAMB4  | GBP2    | GABRD | GABRA4 |
| LGALS2 | GEM     | GALR2 | GABRD  |
| LRFN5 | GIMAP4 | GBP2 | GALR2 |
|-------|--------|------|-------|
| LRRC66 | GLIPR1 | GCNT1P | GBP2 |
| LTA | GLYATL2 | GCNT2 | GCNT2 |
| LY96 | GNGT2 | GEM | GIMAP4 |
| LYZ | GP9 | GIMAP4 | GLIPR1 |
| MAOA | GPR174 | GLIPR1 | GNGT2 |
| MICF | GPR177 | GPR143 | GP9 |
| MPEG1 | GPR183 | GPR177 | GPR143 |
| MS4A4A | GPR34 | GPR34 | GPR177 |
| MS4A4E | GPR65 | GPR65 | GPR183 |
| MTUS1 | GPR84 | GPR84 | GPR65 |
| NLRP1 | GRAMD1C | GRAP2 | GPR84 |
| OMD | GRAP2 | GRIA3 | GRAMD1C |
| OR1L8 | GZMA | GZMA | GRAP2 |
| OR2L1P | HFE | HERC5 | GZMA |
| OSBPL11 | HLA-DMB | HFE | HERC5 |
| P2RX6 | IGKC | HLA-DMB | HFE |
| P2RX7 | IGSF3 | HORMAD1 | HLA-DMB |
| P2RY5 | IL1RN | HSPA6 | HSPA6 |
| PGLYRP3 | IL31RA | ID3 | ID3 |
| PGLYRP4 | ITGAM | IGSF6 | IGKC |
| PHOSPHO1 | JHDM1D | IL15 | IGSF6 |
| PI16 | JSRP1 | IL18R1 | IL15 |
| PITPNM3 | KCNK13 | IL1RN | IL18R1 |
| PLN | KCTD12 | IL24 | IL1RN |
| PLVAP | KIF13A | IL28B | IL21R |
| PMP22 | KISS1R | IL31RA | IL24 |
| PRG4 | KLF4 | IRS1 | IRS1 |
| PRL | KLHL10 | ITGAM | ITGAM |
| PRLR | LGSN | JHDM1D | JHDM1D |
| PRSS35 | LPAR3 | KCNK13 | KCNK13 |
| PSD | LPXN | KIAA1244 | KCTD12 |
| Gene 1 | Gene 2 | Gene 3 | Gene 4 |
|-------|-------|-------|-------|
| RAB17 | LRFN5 | KIF13A | KIAA1244 |
| RASGEF1B | LRP1 | KISS1R | KIAA1644 |
| RGMB | LRRRC66 | KLF4 | KIF13A |
| RIN2 | LY96 | KLHL10 | KLHL10 |
| RNASE1 | LYZ | LILRB1 | KLHL34 |
| RNASE4 | MAB21L2 | LPAR3 | LGSN |
| RNASE6 | MAOA | LPAR3 | LILRB1 |
| RNF126P1 | MBNL2 | LPXN | LPXN |
| RPL8P2 | MICF | LRFN5 | LRFN5 |
| RPS27AP7 | MS4A4A | LRP1 | LRP1 |
| S100A12 | MS4A4E | LRRRC66 | LRRRC66 |
| S100A5 | MTUS1 | LY96 | LTA |
| S1PR1 | MYO1A | LYZ | LY96 |
| SEL1L2 | NAGS | MBNL2 | LYZ |
| SGMS2 | NLRP1 | METTL7A | MBNL2 |
| SGSH | OAS1 | MPZL1 | METTL7A |
| SHH | OR1L8 | MS4A4A | MPZL1 |
| SHROOM3 | OR2L1P | MYO1A | MS4A4A |
| SIRPB2 | OSBPL11 | NAGS | MTUS1 |
| SLC1A3 | P2RX7 | NLRC4 | MYO1A |
| SLC26A11 | P2RY5 | NLRP1 | NAGS |
| SLC35D3 | PCDHA1 | OLFML3 | NLRC4 |
| SLC7A7 | PGLYRP3 | OMD | NLRP1 |
| SLC7A8 | PGLYRP4 | OSBPL11 | OAS1 |
| SMARCA1 | PI16 | P2RX7 | OLFML3 |
| SNCAIP | PLBD1 | PCDH7 | OMD |
| SNX7 | PLVAP | PDPN | OSBPL11 |
| SP140 | PMP22 | PGLYRP4 | P2RX7 |
| ST6GALNAC5 | PPP1R1C | PHOSPHO1 | PCDH7 |
| ST8SIA1 | PRG4 | PIGR | PCDHA1 |
| SULF2 | PRLR | PITPNM3 | PDPN |
| TAS2R42 | PRSS35 | PLBD1 | PGLYRP4 |
| Gene 1 | Gene 2 | Gene 3     | Gene 4       |
|--------|--------|------------|--------------|
| TEAD3  | PSD    | PLEKHC1    | PHOSPHO1     |
| TGM7   | RAB17  | PLVAP      | PIGR         |
| TLR1   | RASGEF1B | PMP22    | PLBD1        |
| TLR3   | RGMB   | PPP1R1C    | PLEKHC1      |
| TLR8   | RILPL1 | PRG4       | PRKAR2B      |
| TMEM176A | RNASE4 | PRKAR2B    | PRL          |
| TMEM176B | RPS27AP7 | PRL     | PRLR         |
| TMEM178 | S1PR1  | PRLR       | RASGEF1B     |
| TMPRSS4 | SDS    | RAB17      | RBM47        |
| TNFAIP8L3 | SEL1L2 | RBM47      | RGMB         |
| TRAT1  | SEL    | RGMB       | RIN2         |
| TRIM2  | SLC1A3 | RILPL1     | RNASE6       |
| U66059.1-2 | SLC7A7 | RIN2       | SDS          |
| VIT    | SMARCA1 | RNASE6     | SEL1L2       |
| VWF    | SMTNL1 | RNF126P1   | SELL         |
| Z82248.1 | SNAI2  | RPS27AP7   | SLC26A11     |
| Z85996.1-2 | SNCAIP | SEL1L2     | SLC37A2      |
| ZDHHC15 | SNX7   | SELL       | SLC7A7       |
| ZNF366 | SP140  | SEPT10     | SLC7A8       |
| ST8SIA1 | SEPT10 | SLC15A3    | SNAI2        |
| STAB1  | SLC1A3 | SNX7       | SLC26A11     |
| SULF2  | SLC1A3 | SYN3       | SP140        |
| SYN3   | SLC26A11 | SLC37A2 | ST8SIA1     |
| TLE2   | SLC7A7 | STAB1      | SLYN3        |
| TLR3   | SLC7A8 | SYN3       | TLR8         |
| TLR5   | SLC7A8 | TSLFN5     | TBC1D4       |
| TMEM176A | SMARCA1 | TDRD9     |              |
| TMPRSS4 | SNAI2  | TIMP3      |              |
| TRAT1  | SNX7   | TLE2       |              |
| TRIM2  | SP140  | TLR3       |              |
| TRIM22 | SULF2  | TLR5       |              |
| U66059.1-2 | SYN3 | TLR8 |
|------------|------|------|
| YPEL4      | TBC1D4 | TMEM176A |
| ZCCHC24    | TDRD9 | TRIM22 |
| ZDHHC15    | TLE2 | TRPM1 |
|            | TLR3 | VIM |
|            | TLR8 | VIT |
|            | TMEM176A | YPEL4 |
|            | TRIM2 | ZNF366 |
|            | TRIM22 | |
|            | TRPM1 | |
|            | VIM | |
|            | VIT | |
|            | VWF | |
|            | ZDHHC15 | |
|            | ZFPM2 | |
|            | ZFPM2 | |
|            | ZNF366 | |
**Table S6.** Oligonucleotide sequences of the small hairpin RNAs (shRNAs) used to knock down LSD1.

| Target of shRNAs | Oligos sequence          |
|------------------|--------------------------|
| Scramble shRNAs  | AGTACGCGAAGAATACTATCGA  |
| shRNAs against LSD1 #1 | AAGTGATACTGTGCTTGTCCAC |
| shRNAs against LSD1 #2 | ATTCAGAAGATGAGTATTATT |

**Table S7.** Sequences of primers used for RT-qPCR.

| Primer | Primers’ sequence          |
|--------|-----------------------------|
| LSD1   | F: AGACGACAGTTCTGGAGGGTA     |
|        | R: TCTTGAGAAGTCATCCGGTCA    |
| CD11b  | F: AACCCTGGTGTCCTTCCTC      |
|        | R: CATGACATAAGGTCAAGGCTGT   |
| GAPDH  | F: TTCGCTTCCTGCTCTCCTCTG    |
|        | R: CCTAGCCTCCGGTTCCTC       |

**Table S8.** Sequences of IRS1 primers used for ChIP-qPCR.

| Primer | Primers’ sequence          |
|--------|-----------------------------|
| A      | F: AGGCCAAAACCTACTGTGCA     |
|        | R: AGCTGGCACCACCTTGTTT      |
| B      | F: GGTAAAGACTGACCCACGGGA    |
|        | R: GATCCCCCTACCATGGCCTA     |
| C      | F: GAGGCTCCGAAAAACAACCG     |
|        | R: CGTGGATTTCAGAGTCGGGG     |
| D      | F: ATCAGTGAACCGGAAGGAAA     |
|        | R: CTGGAAGGACAGCTCGAAA      |
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Fig. S1

A

SKNO-1  
KASUMI-1  
UF1  

NB4  
OCI-AML3  
THP-1  

B

Vehicle  
DDP38003  

C

KASUMI-1  
THP-1  

D

SKNO-1  

E

NB4  

F

MC2580  

G

MC2580  

Cellular ATP level (%)  

DDP38003 concentration (µM)  

KASUMI-1  

OCI-AML3  

Vehicle  
DDP38003  

Cell number (Fold Change)  

Days from treatment  

Cell number (Fold Change)  

Days from treatment  

- p-p70S6K T389  

Total p70S6K  

Vinculin  

SKNO-1  

- p-p70S6K T389  

Total p70S6K  

Vinculin  

NB4  

- p-p70S6K T389  

Total p70S6K  

Vinculin  

NB4
Fig. S2

A

B

THP-1

DDP38003

2DG

p-S6 S235/236

37 KDa

-    +    +    -

-     -     +    +

Total S6

37 KDa

Vinculin

100 KDa

C

Cell Number (Fold Change)

Days from treatment

+    +    +    -

% Cellular ATP level

Cell number (Fold Change)

Days from treatment

-    +    +    -

-     -     +    +

2-DG concentration (mM)

% Cellular ATP level

2-DG concentration (mM)
Fig. S3

(A) CFU (%)

Vehicle, DDP38003, AZD8055, DDP38003+AZD8055

(B) Microscopy images of cells treated with different compounds.
A. Stepwise prolonged exposure to increasing concentrations of DDP38003 for 12 months.

Parental LSD1i-naive KASUMI-1 cells (KASUMI-1/P)

LSD1i-resistant KASUMI-1 subline (KASUMI-1/R)

B. Cellular ATP level (%)

DDP38003 concentration (µM)

C. Cellular ATP level (%)

MC2580 concentration (µM)

D. 

DDP38003

p-S6 S235/236

Total S6

β-actin

37 KDa

E. Cellular ATP level (%)

RAPAMYCN concentration (nM)

F. Cellular ATP level (%)

AZD8055 concentration (nM)

G. Apoptotic cells (%)

KASUMI-1/P + Veh

KASUMI-1/P + DDP

KASUMI-1/R + DDP + AZD

Annexin V+/−

Annexin V+/−
Fig. S5

A

THP-1

DDP38003 (µM) 0 0.5 0.1

p-AMPK T172

Total AMPK

p-ACC S79

Total ACC

Vinculin

B

KASUMI-1

DDP38003 (µM) 0 0.5 0.1

p-AMPK T172

Total AMPK

p-ACC S79

Total ACC

Vinculin

C

NB4

DDP38003 (µM) 0 0.5 0.1

p-AMPK T172

Total AMPK

p-ACC S79

Total ACC

Vinculin

D

OCI-AML3

DDP38003 (µM) 0 0.5 0.1

p-AMPK T172

Total AMPK

p-ACC S79

Total ACC

Vinculin

E

THP-1

DDP38003 - +

Raptor

β-actin

F

SKNO-1

DDP38003 - +

Raptor

β-actin

G

OCI-AML3

DDP38003 - +

Raptor

β-actin
**Fig. S6**

**A**
- KASUMI-1
- DDP38003
- p-ERK1/2 T202/Y204
- Total ERK1/2
- p-p70S6K T389
- Total p70S6K
- Vinculin

**B**
- THP-1
- DDP38003
- p-ERK1/2 T202/Y204
- Total ERK1/2
- p-p70S6K T389
- Total p70S6K
- Vinculin

**C**
- NB4
- DDP38003 (µM) 0 0.5 0.1
- p-ERK1/2 T202/Y204
- Total ERK1/2
- Vinculin

**D**
- THP-1
- DDP380003
- U0126
- p-S6 S235/236
- Total S6
- β-actin

**E**
- Cell number (Fold Change)
- Days from treatment

**F**
- Cellular ATP levels (%)
- Pimsertib concentration (µM)

**G**
- Cellular ATP levels (%)
- Trametinib concentration (nM)

**H**
- Cellular ATP levels (%)
- Trametinib concentration (nM)
Fig. S7

A PHYSIOLOGICAL SYSTEM DEVELOPMENT AND FUNCTION

KASUMI-1

THP-1

B MOLECULAR AND CELLULAR FUNCTION

KASUMI-1

THP-1
**Fig. S8**

**A**

KASUMI-1 Control shLSD1

IRS1

β-actin ~37 KDa

**B**

B

NB4 LSD1 [0-40]

KASUMI LSD1 [0-20]

SKNO-1 LSD1 [0-20]

**C**

C

NB4

LSD1 enrichment (% input)

**D**

D

OCI-AML3

LSD1 enrichment (% input)

**E**

E

H3K4me2 enrichment (% input)

A

B

C

D

**F**

F

H3K4me2 ChIP-Seq

DMSO

GSK860

RN-1
Fig. S9

A  B  C  D  E

Relative IRS1 mRNA level
Veh DDP ATRA DDP+

ATRA

Relative IRS1 mRNA level
Veh ATRA

C

NB4
ATRA

IRS1

β-actin

150 KDa

37 KDa

F  G  H  I

H3K4me2 enrichment (% input)

H3K27Ac enrichment (% input)

Vehicle
ATRA

A

B

C

D

E

H3K4me2 enrichment (% input)

H3K27Ac enrichment (% input)

IgG

H3K4me2

IgG

H3K27Ac

IgG

H3K27Ac

IgG

H3K27Ac
Fig. S10

A

![Graph showing cell number (Fold Change) over days from treatment with Vehicle and DDP3803.]

B

![Bar graph showing cellular ATP levels (%). Veh vs DDP.]

C

![Western blot images of DDP3803, p-p70S6K T389, Total p70S6K, p-AKT S473, Total AKT, and Vinculin with molecular weights indicated.]

| Protein       | Molecular Weight |
|---------------|------------------|
| p-p70S6K T389 | 75 KDa           |
| Total p70S6K  | 75 KDa           |
| p-AKT S473    | 50 KDa           |
| Total AKT     | 50 KDa           |
| Vinculin      | 100 KDa          |

Cell number (Fold Change)

Days from treatment

Cellular ATP levels (%)

Veh vs DDP

DDP3803

- Veh  
+ DDP

DDP3803
Fig. S11

A. Graph showing cell count (Fold Change) over time with Vehicle and DDP38003.

B. Bar graph comparing relative CD11b mRNA levels between Veh and DDP.

C. Bar graph comparing relative CD86 mRNA levels between Veh and DDP.

D. Western blot images of p-mTOR S2448, Total mTOR, p-AKT S473, Total AKT, and β-actin at 250 KDa, 50 KDa, and 37 KDa.

E. Bar graph showing relative number of coarse colonies between Veh, MC, and DDP.

F. Bar graph showing relative number of disperse colonies between Veh, MC, and DDP.

G. Images showing cell count (Fold Change): Vehicle, MC2805, DDP38003.
Fig. S12

C

|                | Vehicle | DDP38003 | Rapamycin | DDP38003 + Rapamycin |
|----------------|---------|----------|-----------|----------------------|
| Blood smear    |         |          |           |                      |
| 20x            |         |          |           |                      |
| 40x            |         |          |           |                      |
| Spleen         |         |          |           |                      |
| 20x            |         |          |           |                      |
| 40x            |         |          |           |                      |
| Bone marrow    |         |          |           |                      |
| 20x            |         |          |           |                      |
| 40x            |         |          |           |                      |
|      | Liver | Spleen | BM    | Muscle |
|------|-------|--------|-------|--------|
| NT   | ![Liver NT](image) | ![Spleen NT](image) | ![BM NT](image) | ![Muscle NT](image) |
| A2   | ![Liver A2](image) | ![Spleen A2](image) | ![BM A2](image) | ![Muscle A2](image) |
| A3   | ![Liver A3](image) | ![Spleen A3](image) | ![BM A3](image) | ![Muscle A3](image) |
| B11  | ![Liver B11](image) | ![Spleen B11](image) | ![BM B11](image) | ![Muscle B11](image) |
| B12  | ![Liver B12](image) | ![Spleen B12](image) | ![BM B12](image) | ![Muscle B12](image) |
| C21  | ![Liver C21](image) | ![Spleen C21](image) | ![BM C21](image) | ![Muscle C21](image) |
| C25  | ![Liver C25](image) | ![Spleen C25](image) | ![BM C25](image) | ![Muscle C25](image) |
| D33  | ![Liver D33](image) | ![Spleen D33](image) | ![BM D33](image) | ![Muscle D33](image) |
| D36  | ![Liver D36](image) | ![Spleen D36](image) | ![BM D36](image) | ![Muscle D36](image) |

(20X)
| Anti human nuclei (20x) | Spleen | BM | Muscle | Liver |
|------------------------|--------|----|--------|-------|
| NT                     | ![Spleen NT] | ![BM NT] | ![Muscle NT] | ![Liver NT] |
| A2                     | ![Spleen A2] | ![BM A2] | ![Muscle A2] | ![Liver A2] |
| A3                     | ![Spleen A3] | ![BM A3] | ![Muscle A3] | ![Liver A3] |
| B11                    | ![Spleen B11] | ![BM B11] | ![Muscle B11] | ![Liver B11] |
| B12                    | ![Spleen B12] | ![BM B12] | ![Muscle B12] | ![Liver B12] |
| D33                    | ![Spleen D33] | ![BM D33] | ![Muscle D33] | ![Liver D33] |
| D36                    | ![Spleen D36] | ![BM D36] | ![Muscle D36] | ![Liver D36] |
| C21                    | ![Spleen C21] | ![BM C21] | ![Muscle C21] | ![Liver C21] |
| C25                    | ![Spleen C25] | ![BM C25] | ![Muscle C25] | ![Liver C25] |
|     | BM       | Muscle   | Liver   |
|-----|----------|----------|---------|
| NT  | ![Image](NT.bmp) | ![Image](NT.bmp) | ![Image](NT.bmp) |
| A2  | ![Image](A2.bmp) | ![Image](A2.bmp) | ![Image](A2.bmp) |
| A3  | ![Image](A3.bmp) | ![Image](A3.bmp) | ![Image](A3.bmp) |
| B11 | ![Image](B11.bmp) | ![Image](B11.bmp) | ![Image](B11.bmp) |
| B12 | ![Image](B12.bmp) | ![Image](B12.bmp) | ![Image](B12.bmp) |
| D33 | ![Image](D33.bmp) | ![Image](D33.bmp) | ![Image](D33.bmp) |
| D36 | ![Image](D36.bmp) | ![Image](D36.bmp) | ![Image](D36.bmp) |
| C21 | ![Image](C21.bmp) | ![Image](C21.bmp) | ![Image](C21.bmp) |
| C25 | ![Image](C25.bmp) | ![Image](C25.bmp) | ![Image](C25.bmp) |

**Fig. S12**

Liver BM Muscle Liver

Phospho-S6 (20X)
Legends of Supplemental Figures

Figure.S1. Heterogeneous responses of AML cells to LSD1 inhibitor-based therapy correlates with mTORC1 activity.

A) Metabolic activity or viability (cellular ATP %) of AML cell lines (SKNO-1, KASUMI-1, UF1, NB4, OCI-AML3 and THP-1 cells) following 6 days of treatment using varying concentrations of DDP38003 (0.0625 – 1 μM) normalized to vehicle-treated cells was assessed using ATP Cell Titer-Glo™ assay (n=3).

B) Flowcytometry dot plots showing the effect of 6 days of vehicle or DDP38003 treatment on the viability/apoptosis of the indicated AML cells assessed by Annexin V/PI staining.

C) Heatmap representation of the transcript changes in a subset of LSD1 target genes analyzed using RNA-Seq (LY96: Lymphocyte antigen 96; CD86: T-lymphocyte activation antigen CD86; ID3: Inhibitor of DNA binding 3; ITGAM: Integrin Subunit Alpha M (CD11b); ACVR2A: Activin A Receptor Type 2A; PI16: peptidase inhibitor 16; KCTD12: Potassium Channel Tetramerization Domain Containing 12; MS4A4A: Membrane Spanning 4-Domains A4A; C14orf109: Chromosome 14 Open Reading Frame 109; FGD6: FYVE, RhoGEF And PH Domain Containing 6) in THP-1 and KASUMI-1 cells following their treatment with vehicle or DDP38003. Values are row-scaled.

D-E) Growth curves of SKNO-1 (D) and NB4 (E) cells treated with either vehicle (DMSO) or MC2580 (2 μM) for the indicated time points of treatment assessed using trypan blue cell counting. Data were statistically analyzed using two way ANOVA followed by Bonferroni post-hoc test, a: P < 0.05 compared to vehicle-treated cells (n=3).

F-G) Western blot analysis of lysates obtained from SKNO-1 (F) and NB4 (G) cells 6 days after their treatment with either vehicle (DMSO) or MC2580 (2 μM). Vinculin served as the loading control.

Figure.S2. Mimicking energetic or nutritional stress using the non-metabolizable glycolytic inhibitor - 2-deoxyglucose - inhibits mTOR and reverses the resistance of AML cells to LSD1 inhibition.

A) Cell growth curve of THP-1 cells treated with vehicle or DDP38003 (0.5 μM) or 2DG (5 mM) or their combination for the indicated time points. Data were statistically analyzed using
two way ANOVA followed by Bonferroni *post-hoc* test, \(^{a,b,c}\): \(P < 0.05\) compared to vehicle, DDP38003 or 2DG treated cells respectively (n=3).

**B)** Western blot analysis of lysates obtained from (A) after 72h of treatment. Vinculin served as the loading control.

**C)** Viability (cellular ATP%) of THP-1 cells following their treatment with varying concentrations of 2-deoxyglucose (2-DG) together with either vehicle (PEG) or DDP38003 (0.1 or 0.5 μM) assessed using Cell Titer-Glo™ assay. Data were statistically analyzed using two way ANOVA followed by Bonferroni *post-hoc* test, \(^{a,b}\): \(P < 0.05\) compared to vehicle or DDP38003 (0.1 or 0.5 μM) treated cells respectively (n=3).

**Figure.S3.** mTOR inhibition augments the anti-clonogenic activity of LSD1 inhibition further promoting myeloid differentiation of THP-1 cells in methylcellulose semisolid culture assays.

**A)** Percent of the number of THP-1 colonies treated with either vehicle, DDP38003 (DDP: 0.5 μM), AZD8055 (AZD: 10 nM) or their combination. Data were statistically analyzed using one way ANOVA followed by Bonferroni post-test, \(^{\ast}\): \(P < 0.05\) compared to vehicle-treated cells.

**B)** Upper panel: Representative images of THP-1 colonies in semisolid culture with the indicated inhibitors. Lower panel: Representative images of cytospin preparations of THP-1 cells recovered at the end of the semisolid culture assay and stained with May Grunwald Giemsa stain.

**Figure.S4.** Abrogating mTOR signalling reverses the acquired resistance of primarily sensitive AML cells to LSD1 inhibition.

**A)** Schematic outline illustrating the method of generating LSD1i-resistant KASUMI-1 (designated as KASUMI-1/R) subline from the parental LSD1i-sensitive KASUMI-1 (KASUMI-1/P) cells.

**B-C)** Cell viability (Cellular ATP levels %) of LSD1i-naive KASUMI-1/P (P) and resistant KASUMI-1/R (R) cells following their treatment using varying concentrations of (B) DDP38003 (0.015–1μM) or (C) MC2580 (0.078–5μM) for 72h was assessed using Cell Titer-Glo™ assay (n=3). Data were statistically analyzed using two way ANOVA followed by Bonferonni post-test, \(^{\ast}\): \(P < 0.05\) compared to parental KASUMI-1 (KASUMI-1/P) cells.
D) Immunoblot analysis of lysates obtained from LSD1i-naive KASUMI-1/P and resistant KASUMI-1/R cells 72h following their treatment using either vehicle or DDP38003 (0.5 μM). β-actin served as the loading control.

E-F) Cell viability (Cellular ATP levels %) of LSD1i-naive KASUMI-1/P and resistant KASUMI-1/R cells was determined 72h after their treatment with varying concentrations of either AZD8055 (E) or rapamycin (F) using Cell Titer-Glo™ assay (n=3). Data were statistically analyzed using two way ANOVA followed by Bonferroni post-test, *: P < 0.05 compared to parental KASUMI-1 (KASUMI-1/P) cells.

G) Percent of early (Annexin V+/PI−) and late (Annexin V+/PI+) apoptotic cells of KASUMI-1/P and KASUMI-1/R treated as indicated with either vehicle (Veh), DDP38003 (DDP) and/or AZD8055 (AZD) and analyzed using Annexin V/PI staining by flowcytometer. *: P < 0.05.

**Figure S5. Effect of LSD1 inhibition on AMP-activated protein kinase (AMPK) signalling and Raptor level in AML cells.**

A-D) Western blot analysis of lysates obtained from THP-1 (A), KASUMI-1 (B), NB4 (C) and OCI-AML3 (D) cells 144h following their treatment with vehicle or DDP38003 (0.1 and 0.5 μM). Vinculin served as the loading control. The presented blots are derived from replicate samples run on parallel gels and controlled for even loading.

E-G) Western blot analysis of lysates obtained from THP-1 (E), SKNO-1 (F) and OCI-AML3 (G) cells 144h following their treatment with vehicle or DDP38003 (0.5 μM). β-actin served as the loading control.

**Figure S6. Activation of extracellular-signal regulated kinases 1 and 2 (ERK1/2) contributes to mTOR activation in resistant AMLs following LSD1 inhibition.**

A-B) Western blot analysis of lysates obtained from sensitive KASUMI-1 (A) and resistant THP-1 (B) cells after 6 and 24h respectively of their treatment with either vehicle or DDP38003 (0.5 μM). Vinculin was used as the loading control. The presented blots are derived from replicate samples run on parallel gels and controlled for even loading.

C) Western blot analysis of lysates obtained from NB4 cells treated with vehicle or DDP38003 (0.1 and 0.5 μM) for 72h. Vinculin served as the loading control.
D) Western blot analysis of lysates obtained from THP-1 cells treated as indicated for 72h. β-actin served as a loading control.

E) Cell growth curves of THP-1 cells treated with vehicle, DDP38003, the selective MEK1/2 inhibitor U0126 (2.5 μM), and combination of DDP38003 and U0126. Data were statistically analyzed using two way ANOVA followed by Bonferroni post-hoc test, $a,b,c$: $P < 0.05$ compared to vehicle, DDP38003 or U0126 alone treated cells respectively (n=3).

F-H) Cell viability (Cellular ATP levels %) of NB4 (F and G) and OCI-AML3 (H) cells following their treatment with the indicated concentrations of pimasertib or trametinib (C) was assessed using Cell Titer-Glo™ assay.

Figure.S7. Genome-wide transcriptome analysis of KASUMI-1 and THP-1 cells following LSD1 inhibition.

Top enriched biological pathways categorized in physiological system, development and function (A) and molecular and cellular function (B) analysed by Ingenuity Pathway Analysis of differentially expressed genes (DEG) of KASUMI-1 and THP-1 cells 72h following LSD1 inhibition using DDP38003 (0.5 μM). P values were determined using Fisher exact test.

Figure.S8. LSD1 binding to insulin receptor substrate 1 (IRS1) promoter regions in AML cells.

A) Western blot analysis of lysates obtained from transduced KASUMI-1 cells expressing the indicated shRNAs. β-actin served as a loading control.

B) Tracks of LSD1 chromatin immunoprecipitation sequencing (LSD1 ChIP-Seq) at IRS1 promoter in NB4, KASUMI-1 and SKNO-1 AML cells (KASUMI-1 and SKNO-1 tracks were obtained from the cistrome database: Mcgrath et al., 2016).

C-D) LSD1 ChIP-qPCR analyses were performed in NB4 (C) and OCI-AML3 (D) cells. Enrichment values at the indicated sites (A–D) were normalized against input DNAs. Values are means ± SD. *: $P < 0.05$.

E) H3K4me2 ChIP-qPCR analyses were performed in THP-1 cells 72h following their treatment with either vehicle or DDP38003 (0.5 μM). Enrichment values at the indicated sites (A–D) were normalized to input DNAs. Values are means ± SD. *: $P < 0.05$. 

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F) Tracks of H3K4me2 chromatin immunoprecipitation sequencing (H3K4me2 ChIP-Seq) on IRS1 gene in KASUMI-1 cells 72h following their treatment with vehicle or irreversible LSD1 inhibitors (GSK690 or RN-1) (From cistrome database deposited by Mcgrath et al., 2016).

Figure S9. ATRA reverses LSD1i-induced IRS1 upregulation sensitizing resistant AML cells to LSD1 inhibition.

A-B) Normalized IRS1 mRNA levels assessed in THP-1 cells following their treatment with either vehicle (Veh) or ATRA (1 μM) for 72h and using two different primers (A and B) analyzed using RT-qPCR. Data were statistically analyzed using either Student’s t test *: P < 0.05.

C) Western blot analysis of lysates obtained from NB4 cells following their treatment with either vehicle (Veh) or ATRA (1 μM) for 72h. β-actin served as a loading control.

D-E) Normalized IRS1 mRNA levels assessed in NB4 cells following their treatment with either vehicle (Veh) or ATRA (1 μM) for 72h and using two different primers (D and E) analyzed using RT-qPCR. Data were statistically analyzed using either Student’s t test *: P < 0.05.

F-G) H3K4me2 (F) and H3K27Ac (G) ChIP-qPCR analyses were performed in NB4 cells 72h following their treatment with either vehicle or ATRA (1 μM). Enrichment values at the indicated sites (A–D) were normalized to input DNAs. Values are means ± SD. *: P < 0.05.

H) Relative cell number of OCI-AML3 cells treated with vehicle or DDP38003 (0.5 μM) or all-trans retinoic acid (ATRA) or their combination. Data were statistically analyzed using one way ANOVA followed by Bonferonni post-hoc test, *: P < 0.05 (n=3).

I) Normalized IRS1 mRNA levels of OCI-AML3 cells treated as indicated in (H) assessed using RT-qPCR. Data were statistically analyzed using one way ANOVA followed by Bonferonni post-hoc test, *: P < 0.05.

Figure S10. Effect of LSD1 inhibition using DDP38003 on primary human CD34+ cord blood cells.

A) Effect of DDP38003 treatment (0.3 μM) on the proliferation of primary human CD34+ cord blood cells assessed using trypan blue cell counting (n=3).
B) Metabolic activity or viability (ATP %) of primary human CD34⁺ cord blood cells treated with vehicle or DDP38003 (0.3 μM) for 72h assessed using Cell Titer-Glo™ assay (n=3).

C) Western blot analysis of primary human CD34⁺ cord blood cells treated with vehicle or DDP38003 (0.3 μM) for 72h. Vinculin served as the loading control.

Figure S11. Effect of LSD1 inhibition on primary human CD34⁺ cord blood cells transduced with lentiviral vector expressing human MLL-AF9 (hCD34⁺ MLL-AF9).

A) Effect of DDP38003 treatment (0.5 μM) on the proliferation of hCD34⁺ MLL-AF9 cells assessed using trypan blue cell counting (n=3). Data were statistically analyzed using two way ANOVA followed by Bonferonni post-test.*: P < 0.05.

B-C) Normalized CD11b (B) and CD86 (C) mRNA levels assessed in hCD34⁺ MLL-AF9 cells 6 days following their treatment with either vehicle or DDP38003 (0.5 μM). Data were statistically analyzed using Student’s t test.*: P < 0.05 compared to vehicle-treated cells.

D) Western blot analysis of lysates obtained from hCD34⁺ MLL-AF9 cells 6 days following their treatment with either vehicle or DDP38003 (0.5 μM). β-actin served as the protein loading control.

E-F) Relative number of coarse (E) and disperse (F) colonies of hCD34⁺ MLL-AF9 cells treated as with either vehicle, MC2580 (2 μM) or DDP38003 (0.5 μM) and cultured in semisolid medium (n=3). Data were statistically analyzed using either one way ANOVA followed by Bonferroni post-hoc test*: P<0.05 compared to vehicle-treated cells.

G) Representative phase contrast images of hCD34⁺ MLL-AF9 colonies treated as indicated (first column) and their cytospin preparations recovered at the end of the semisolid culture assay and after being stained with May Grunwald Giemsa stain (2nd and 3rd columns). Red arrows refer to macrophages.

Figure S12. Inhibiting mTOR sensitizes primary human MLL-AF9 expressing (AML-IEO20) leukemia cells to LSD1 inhibition.

A-B) Cell cycle analysis (A) and percent of apoptotic cells (B) of patient-derived AML-IEO20 leukemic cells following their treatment with either vehicle (designated as V),
DDP38003 (D), rapamycin (referred to as R) or AZD8055 (referred to as A) analyzed using FACS analysis.

C) May Grunwald-Giemsa stained blood smear as well as cytospin preparations of spleen and bone marrow obtained from AML-IEO20 transplanted NSG mice 15 days after initiating treatment with vehicle or DDP38003 or rapamycin or a combination of DDP38003 and rapamycin (20 and 40x).

D) Histopathological examination of the spleen, bone marrow as well as muscular tissues harvested from non-transplanted NSG mice (NT) as well as mice xenotransplanted with AML-IEO20 leukemia 15 days after initiating treatment with vehicle (#A2 and #A3), DDP38003(#B11 and #B12), rapamycin (#D33 and #D36) or a combination of DDP38003 and rapamycin (#C21 and #C25).

E) Immunohistochemical assessment of human nuclei in the spleen, bone marrow as well as muscular tissues harvested from non-transplanted NSG mice (NT) as well as mice xenotransplanted with AML-IEO20 leukemia 15 days after initiating treatment with vehicle (#A2 and #A3), DDP38003(#B11 and #B12), rapamycin (#D33 and #D36) or a combination of DDP38003 and rapamycin (#C21 and #C25).

F) Immunohistochemical assessment of ribosomal S6 phosphorylation as a readout of mTORC1 activity in the bone marrow as well as muscular tissues harvested from non-transplanted NSG mice (NT) as well as mice xenotransplanted with AML-IEO20 leukemia 15 days after initiating treatment with vehicle (#A2 and #A3), DDP38003 (#B11 and #B12), rapamycin (#D33 and #D36) or a combination of DDP38003 and rapamycin (#C21 and #C25).