**SUPPLEMENTARY MATERIAL AND METHODS**

**Plasmids and shRNA Constructs**

shRNA constructs directed against human and murine target genes – sequences listed below – were cloned into a doxycycline-inducible GEPIR vector ([1](#_ENREF_1)), containing a miRE expression cassette, as XhoI–EcoRI fragments, which were generated by amplifying 97-mer oligonucleotides (Invitrogen) using 5′miRE-XhoI (TGAACTCGAGAAGGTATATTGCTGTTGACAGTGAGCG) and 3′miRE-EcoRI (TCTCGAATTCTAGCCCCTTGAAGTCCGAGGCAGTAGGC) primers and the Vent polymerase kit (Invitrogen) with the following conditions: 50 μl reaction containing 0.05 ng oligonucleotide template, 1× Vent buffer, 0.3 mM of each dNTP, 0.8 μM of each primer, and 1.25 U Vent polymerase; cycling: 94 °C for 3 min; 35 cycles of 94 °C for 30 s, 54 °C for 30 s, and 75 °C for 20 s; 75 °C for 5 min. SPI1/Spi1-directed shRNA constructs cloned into a pRRL-PGK-GFP vector were kindly provided by Dr. Ileana Antony-Debre and Dr. Ulrich G. Steidl (Albert Einstein College of Medicine, New-york city, NY, USA).

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| **Designation** | **Species** | **Target Sequence** |
|   |   |   |
| sh*BRD4*\_1 | Homo Sapiens | TTCTTTTTTCTTTTCCTTCTTG |
| sh*BRD4*\_2 | Homo Sapiens | TTTTTTATTCTCTTCCACTTCC |
| sh*DHFR*\_1 | Homo Sapiens | TTAGAAATAATTATAGATCTAA |
| sh*DHFR*\_2 | Homo Sapiens | TTCATATTAATCATTCTTCTCA |
| sh*DHFR2*\_1 | Homo Sapiens | TTAAATCACAATTTCTTACCAC |
| sh*DHFR2*\_2 | Homo Sapiens | TTCACTTAAATTTCAGTCTTGG |
| sh*EED*\_1 | Homo Sapiens | TTAGAAAGTATCAAATCGCCTA |
| sh*EED*\_2 | Homo Sapiens | TTTAATTTTATCTATATCATCT |
| sh*EHMT1*\_2 | Homo Sapiens | TTATTTAAGATGTATCCGTTGC |
| sh*EHTM1*\_1 | Homo Sapiens | TTAAACATCTCAATCACCGTCC |
| sh*MTHFR*\_1 | Homo Sapiens | TTCAAAGACACTTTCTTCACTG |
| sh*MTHFR*\_2 | Homo Sapiens | TCAGAATAGACAACTGTGCCAA |
| sh*Mthfr*\_1 | Mus Musculus | TTTTCTATCAACTCTCTCTGTA |
| sh*Mthfr*\_2 | Mus Musculus | TTAATTGGAAAAATGCAATGTG |
| sh*MTR*\_1 | Homo Sapiens | TAGATTTTCATCTAACAGCTGG |
| sh*MTR*\_2 | Homo Sapiens | TTTATAATCTTGAATATTCCTA |
| sh*SETDB1*\_1 | Homo Sapiens | TTTGTTGTCAAATTTCACCTTG |
| sh*SETDB1*\_2 | Homo Sapiens | TCAATTATTTCTGTAGGCCGGG |
| sh*SHMT1*\_1 | Homo Sapiens | TTTATTTTCCTAGAATTATGTC |
| sh*SHMT1*\_2 | Homo Sapiens | TTAAATTCCAGAGTCATAGCTT |
| sh*SPI1*\_1/sh*Spi1*\_1 | Homo Sapiens / Mus Musculus | CAAGAAGATGACCTACCAGAA |
| sh*SPI1*\_2/sh*Spi1*\_2 | Homo Sapiens / Mus Musculus | GAAGCTCACCTACCAGTTCAG |
| sh*MTAP\_1* | Homo Sapiens | TAAAATTTCTGGATCATCCAGG |
| sh*MTAP\_2* | Homo Sapiens | TATATATAATTCTTTGTATGCAT |
| shControl |   | CCTAAGGTTAAGTCGCCCTCG |
|  |  |  |

AgeI-XhoI *MTHFR* fragments were synthesized by PCR-amplification of the *MTHFR* cDNA which was generated from RNA extracted from U937 cells using an RNeasy Kit (Qiagen). These fragments were then digested using AgeI and XhoI restriction enzymes prior to their T4 PNK phosphorylation (NEB, M0201) and their ligation into a DD-tagged pLVX-pTUNER vector (Clontech, # 632173). Positive clones were validated using Sanger Sequencing (Eurofins).

**Cell Culture**

U937 and KG1a cell lines were purchased from the American Type Culture Collection and OCI-AML2 cell line was purchased from DSMZ. MOLM-13 was provided by Dr. Benjamin Ebert; and MOLM-14 cell lines by Dr. Scott Armstrong (Dana-Farber Cancer Institute, Boston, MA, USA). Identity of these two cell lines was confirmed by STR loci profiling. All cell lines tested negative for mycoplasma. All cell lines, except MOLM-13 and Kasumi-3, were maintained in RPMI 1640 (Invitrogen) supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum (FBS, Sigma-Aldrich) at 37 °C with 5% CO2. MOLM-13 cells were maintained in RPMI 1640 supplemented with 1% penicillin-streptomycin and 20% FBS. Kasumi-3 cells were maintained in RPMI 1640 supplemented with 1% penicillin/streptomycin and 10% FBS with 20 ng per ml GM-CSF (Peprotech). The 293T cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% FBS (Sigma-Aldrich) and 100 units per ml penicillin/streptomycin (Invitrogen).

Mononuclear cells isolated using Ficoll-Paque Plus (Amersham Biosciences) were thawed before drug treatment. These cells were maintained in RPMI 1640 medium supplemented with 20% FBS, 20 ng/ml IL-3 (#200-03, Peprotech), 20 ng/ml IL-6, 20 ng/ml GM-CSF (#300-03, Peprotech), 10 ng/nl G-CSF (#300-23, Peprotech), 10 ng/ml EPO (#100-64, Peprotech), 50 ng/ml TPO (#300-18, Peprotech), 100 ng/ml FLT3-Ligand (#300-19, Peprotech) and 100 ng/ml SCF (#300-07, Peprotech).

For folate starvation experiments, cell lines were washed twice in PBS, and maintained for 18 hours in no folic acid RPMI 1640 medium (Invitrogen, #27016-021) supplemented with 10% dialyzed FBS (Sigma-Aldrich, #F0392) and 100 units per ml penicillin/streptomycin (Invitrogen) prior to treatment with drugs or infection with shRNAs. Primary patient cells were kept in the same medium as cell lines supplemented with the same concentration of cytokines as previously stated. The corresponding + folic acid RPMI 1640 medium was generated using no folic acid RPMI 1640 medium supplemented with 1mg/L folic acid (Sigma-Aldrich-Aldrich, #F8758).

For functional characterization of the effect of single amino acid starvation on cell response to BET inhibitors, cell lines were washed twice in PBS, and maintained for 18 hours in no folate and no amino acid RPMI 1640 medium (Genaxxon Bioscience) supplemented with all amino acids and folic acid as indicated in the table below at the exception of the amino acid to test, 10% dialyzed FBS, and 100 units per ml penicillin/streptomycin prior to treatment with BET inhibitors.

|  |  |  |  |
| --- | --- | --- | --- |
| **Product** | **Concentration (mg/L)** | **Reference** | **Provider** |
|   |   |   |   |
| **Amino Acids** |   |   |   |
| L-Arginine x HCl | 240 | A6969 | Sigma Aldrich |
| L-Asparagine x H2O | 50 | A4284 | Sigma Aldrich |
| L-Aspartic acid | 20 | A7219 | Sigma Aldrich |
| L-Cystine | 50 | C6727 | Sigma Aldrich |
| L-Glutamic acid | 20 | G8415 | Sigma Aldrich |
| Glycine | 10 | G8790 | Sigma Aldrich |
| L-Histidine base | 15 | H5659 | Sigma Aldrich |
| L-Hydroxyproline | 20 | H5534 | Sigma Aldrich |
| L-Isoleucine | 50 | I5281 | Sigma Aldrich |
| L-Leucine | 50 | L6914 | Sigma Aldrich |
| L-Lysine x HCl | 40 | L7039 | Sigma Aldrich |
| L-Methionine | 15 | M5308 | Sigma Aldrich |
| L-Phenylalanine | 15 | P5482 | Sigma Aldrich |
| L-Proline | 20 | P8865 | Sigma Aldrich |
| L-Serine | 20 | S4311 | Sigma Aldrich |
| L-Threonine | 20 | T8441 | Sigma Aldrich |
| L-Tryptophan | 5 | T8941 | Sigma Aldrich |
| L-Tyrosine | 20 | T4321 | Sigma Aldrich |
| L-Valine | 20 | V0513 | Sigma Aldrich |
|   |   |   |   |
|   |   |   |   |
| **Vitamins** |   |   |   |
| Folic acid | 1 | F8758 | Sigma Aldrich |
|   |   |   |   |

**Chemicals**

OTX015 (# S7360), THZ-1 (# S7549), EPZ-6438 (# S7128) and JQ1 (# S7110) were purchased from Selleckchem. Venetoclax (# HY-15531) was purchased from MedChemExpress. Bulk quantity of JQ1 for *in vivo* experiments was provided by Jun Qi’s laboratory (Dana-Farber Cancer Institute). SAH (# A9384) was purchased from Sigma-Aldrich and 5-CH3-THF (sc-214334) from Santa-Cruz.

**Methylcellulose Assay**

Colony forming assays with AML cell lines were performed in methylcellulose-based medium ClonaCellTCS respectively (Stem Cell Technologies). Cells were seeded 0.5×103 cells per plate in triplicate and scored for colony formation 10 days later. The number of colonies was counted after MTT staining. For folate rescue experiments, 2% methylcellulose base solution was prepared using methylcellulose (Sigma-Aldrich, #M0512) dissolved into boiling water and then pre-chilled prior to adding no folic acid RPMI 1640 media supplemented with 10% dialyzed FBS, and 100 units per ml penicillin/streptomycin to have a final concentration of 0.8% no folic acid methylcellulose.

**Growth Assessment**

To assess growth, cells were plated in a minimum of four replicates in 384-well plates and growth was measured using CellTiter Glo (Promega) per the manufacturer’s instructions. For assessment of cell number, cells were plated in triplicate and manually counted with Trypan blue exclusion on the indicated days.

**Apoptosis Assay**

Cell death was assessed using flow cytometric analysis of Annexin V and Propidium Iodide staining according to manufacturer’s instructions (eBioscience). For each condition, 20,000 cells were analyzed on a BD FACSCanto II HTS. Data analysis was completed using FlowJo software.

**Methyltransferase Activity Assay**

Nuclear extracts from AML cell lines were prepared according to the nuclear extraction kit’s protocol from Epigentek (# OP-0002-1). H3K9 and H3K27 methyltransferase activities (Epigentek, # P-3003 and # P-3005) were evaluated in nuclear lysates (in biological triplicate, 15 µg per replicate) according to the manufacturer’s instructions.

**Metabolomic Analyses**

To determine the relative levels of intracellular metabolites, extracts were prepared and analyzed by LC/MS/MS. 16 hours prior to metabolite extraction, 15×106 U937 cells were plated in quadruplicate in no folic acid RPMI 1640 medium supplemented with 10% dialyzed FBS and 100 units per ml penicillin/streptomycin with or without 1mg/L folic acid. Metabolites were extracted on dry ice with 4 mL of 80% methanol (−80°C), as described previously ([2](#_ENREF_2)). Insoluble material was pelleted by centrifugation at 3000*g* for 5 min, followed by two subsequent extractions of the insoluble pellet with 0.5 ml of 80% methanol, with centrifugation at 16000*g* for 5 min. The 5 ml metabolite extract from the pooled supernatants was dried down under nitrogen gas using an N-EVAP (Organomation Associates, Inc).

Dried pellets were re-suspended using 20 μL HPLC grade water for mass spectrometry. 10 μl were injected and analyzed using a 5500 QTRAP triple quadrupole mass spectrometer (AB/SCIEX) coupled to a Prominence UFLC HPLC system (Shimadzu) via selected reaction monitoring (SRM) of a total of 547 endogenous water soluble metabolites for steady-state analyses of samples. Some metabolites were targeted in both positive and negative ion mode for a total of 391 SRM transitions using pos/neg polarity switching. ESI voltage was +4900V in positive ion mode and –4500V in negative ion mode. The dwell time was 3 ms per SRM transition and the total cycle time was 1.55 seconds. Approximately 10–14 data points were acquired per detected metabolite. Samples were delivered to the MS via normal phase chromatography using a 4.6 mm i.d × 10 cm Amide Xbridge HILIC column (Waters Corp.) at 350 μL/min. Gradients were run starting from 85% buffer B (HPLC grade acetonitrile) to 42% B from 0–5 minutes; 42% B to 0% B from 5–16 minutes; 0% B was held from 16–24 minutes; 0% B to 85% B from 24–25 minutes; 85% B was held for 7 minutes to re-equilibrate the column. Buffer A was comprised of 20 mM ammonium hydroxide/20 mM ammonium acetate (pH=9.0) in 95:5 water:acetonitrile. Peak areas from the total ion current for each metabolite SRM transition were integrated using MultiQuant v2.0 software (AB/SCIEX). A Student’s t-test was performed to assess significance of pairwise comparisons. All data are log2-transformed and normalized against the average control condition. No or small variations in metabolite production compared to average control condition appears closest to the white color on the heatmap. Decreased or increased metabolite production compared to the average control condition is represented by a gradient of color from blue to red respectively. The MetaboAnalyst software ([www.metaboanalyst.ca](http://www.metaboanalyst.ca/)) was used to perform pathway enrichment analysis on the set of top metabolites hits identified by steady-state profiling. No specific manual settings were needed to analyze the list of metabolites.

**Pooled CRISPR/Cas9 Epigenetic Screen**

This CRISPR screen is a tool adapted from published and publically available resources.  The constructs were cloned into a single-vector system which drives transcription of both sgRNA and Cas9 ([3](#_ENREF_3)).  The sgRNAs were pulled out from a published library to form our custom library featuring 5 sgRNAs per gene (([4](#_ENREF_4)), and **Table S6**). OCI-AML2 cells were seeded into six-well plates at a density of 3x106 cells per well and transduced at an MOI of 0.2. A total of 15x106 cells were transduced in 5 wells. 24 hours after viral transduction, cells were replated into puromycin-containing media. A sample was collected at 48 hours of puromycin exposure to confirm library coverage in the transduced population. Transduced cells were expanded in puromycin for a total of 10 days prior to drug introduction, at which point the transduced cell population was split into vehicle (DMSO) and JQ1 conditions and maintained for two weeks. All conditions were performed in replicate. JQ-1 was used at 50nM, a dose sufficient to achieve 20-30% loss of viability. Cells were counted, replated, and drug replenished every 3-4 days. At any given point during the screen, each replicate was represented by a minimum of 1.2x106 cells, sufficient to provide 1000x coverage of the library (~1000 cells per unique sgRNA). Samples of 2.5x106 cells were collected upon screen initiation, termination, and at weekly intervals. Following completion of the screens, DNA was extracted (DNeasy Blood & Tissue Kit, QIAGEN) and prepared for sequencing as previously described ([5](#_ENREF_5)). Deep sequencing was performed on an Illumina Nextseq platform (75 bp, single-ended) to identify differences in library composition. All sequencing was performed by Hudson Alpha Institute for Biotechnology. Barcoded reads were matched and binned into guide-level counts. Determinations of genetic essentiality and drug sensitization/resistance were made by evaluating differential guide compositions between the initial population and subsequent drug-treated and vehicle-treated cells populations. Briefly, the fractional representation (FR) for a guide within a sample was normalized to the sum of all guides attributed to that sample. A direct comparison between two samples entailed the quotient of the respective FRs, which we term the depletion metric (DM). The five guide-level DMs for each gene were then collapsed to gene-level scores by taking the average. Guides which totaled fewer than 200 counts for a given sample were excluded from analysis. Genetic essentiality was calculated by considering the depletion/enrichment of the vehicle-treated population over time (DMSOfinal / initial). Drug sensitization/resistance was calculated by considering the depletion/enrichment of the drug-treated population relative to the vehicle-treated population (Drugfinal / DMSOfinal). All depletion/enrichment effects are reported as log2 ratios. All described manipulations were performed in R.

**Western-Blot**

Western immunoblotting was performed as described previously ([2](#_ENREF_2)) using cell lysates normalized for total protein content. Lysates were boiled in Laemmli sample buffer and run on SDS–polyacrylamide gel electrophoresis (PAGE) prior to transfer to nitrocellulose membranes, and immunoblotting. A list of antibodies used for western immunoblotting is listed below.

|  |  |  |
| --- | --- | --- |
| **Target** | **Reference** | **Provider** |
|   |   |   |
| ACTIN | MA5-11869 | Thermo Fisher Scientific |
| DHFR | H00001719-D01P | Novus Biologicals |
| DHFR2 | PA5-48563 | Thermo Fisher Scientific |
| EED | AF5827 | Novus Biologicals |
| EHMT1 | NBP1-77400 | Novus Biologicals |
| H3 | # 12648 | Cell Signaling Technology |
| H3K27me3 | C15410069 | Diagenode |
| H3K9me2 | C15200154 | Diagenode |
| MTAP | 4158S | Cell Signaling |
| MTHFR | ab203786 | Abcam |
| MTR | H00004548-A01 | Abnova |
| MYC | # 9402 | Cell Signaling Technology |
| SETDB1 | sc-166621 | Santa-Cruz Biotechnology |
| SHMT1 | # 80715 | Cell Signaling Technology |
| VINCULIN | ab18058 | Abcam |
| SPI1 | sc-390405 | Santa Cruz   |

**CRISPR/Cas9-Mediated Introduction of Single Nucleotide Polymorphisms On MTHFR**

Top and bottom sgRNAs targeting the C677 and A1298 *MTHFR* sites (Top\_C677: 5’-CACCGAAGCTGCGTGATGATGAAAT-3’ and Bottom\_C677: 5’-AAACATTTCATCATCACGCAGCTTC-3’ ; Top\_A1298: 5’-CACCGTTCAAAGACACTTTCTTCAC-3’ and Bottom\_A1298: 5’-AAACGTGAAGAAAGTGTCTTTGAAC-3’) were annealed and phosphorylated according to the protocol previously described (24157548), and ligated into the BbSSI-digested pSpCas9(BB)-2A-GFP (PX458) vector (Addgene, # 48138) to generate PX458\_sgC677 and PX458\_sgA1298 constructs. 1x106 KG1a cells were washed in PBS prior to be resuspended into 100 μl Opti-MEM medium (ThermoFisher Scientific, # 31985-047). 10 μg PX458\_sgC677 or PX458\_sgA1298 vector and 0.3 μM SSODN\_C677 (5’- TGGCAGGTTACCCCAAAGGCCACCCCGAAGCAGGGAGCTTTGAGGCTGACCTGAAGCACTTGAAGGAGAAGGTGTCTGCGGGAGTCGATTTCATCATCACGCAGCTTTTCTTTGAGGCTGACACA-3’) or SSODN\_A1298 (5’-TTGGGGAGCTGAAGGACTACTACCTCTTCTACCTGAAGAGCAAGTCCCCCAAGGAGGAGCTGCTGAAGATGTGGGGGGAGGAGCTGACAAGTGAAGCAAGTGTCTTTGAAGTCTTCGTTCTTTAC-3’) were then added into the mix of Opti-MEM and cells to generate isogenic KG1a clones expressing either the *MTHFR* 677 C>T, A1298 A>C, or wild-type genetic variant, respectively.

Cells were then electroporated using a NEPA21 eletroporator (Nepagene) following a poring pulse of 150V, length: 5 ms, interval: 50 ms, No: 2, D. rate: 10% and polarity: +, and a transfer pulse of 20V, length: 50 ms, interval: 50 ms, No: 5, D. rate: 40% and polarity: +/-. Electroporated cells were then incubated for 48 hours in presence of 10 μM SCR7 (Selleckchem, # S7742) prior to single cell sorting into 96-well plates. Clones were then grown and *MTHFR* genetic status was screened by allelic discrimination assay.

**ChiP-Sequencing Analyses**

U937 and IMS-M2 cells were grown for 72 hours in regular versus folate-deprived medium prior to a 24-hour treatment with 300nM and 1μM OTX015, respectively. Cells were fixed with 1% formaldehyde for 10 min at room temperature, lysed in nuclei incubation buffer (15 mM Tris, pH 7.5, 60 mM KCl, 150 mM NaCl, 15 mM MgCl2, 1 mM CaCl2, 250 mM sucrose, 1 mM DTT, 0,3% NP-40) for 10 min at 4°C. Nuclei were isolated by centrifugation and digested in digest buffer (10 mM NaCl, 10 mM Tris, pH 7.5, 3 mM MgCl2,1 mM CaCl2) using 0,8 units of micrococcal nuclease (USB) per 50 ug of chromatin for 1 hour at 37°C to generate mononucleosomal particles. Digestion reaction was stopped by addition of EDTA (20mM). Nuclei were lysed in nuclei lysis buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, 1\* SDS) followed by sonication using a Bioruptor Pico (Diagenode). The equivalent of 1.10^6 cells of chromatin was used per ChIP reaction. Chromatin was immunoprecipitated overnight using the anti-H3K27me3 or anti-H3K9Me2 antibody using Auto-iDeal Histone ChIP-seq kit (Diagenode) in an IP-Star Compact (Diagenode). Enriched DNA from ChIP and input DNA fragments were end-repaired, extended with an 'A' base on the 3′ end, ligated with indexed paired-end adaptors (NEXTflex, Bioo Scientific), size-selected with AMPure XP beads (Beckman Coulter) and amplified by PCR for 14 cycles. Libraries were single-end sequenced (50bp) using Illumina HiSeq 4000 (Illumina, San Diego, CA). Sequencing reads were aligned to the human hg19 version of the genome using Bowtie2. Normalized bigwig files for profile representations were generated using Deeptools2 with the --normalizeUsing RPGC --exactScaling --effectiveGenomeSize 2685511504 --extendReads 100 and --ignoreDuplicates option.

Differential profiling of H3K27me3 and H3K9me2 between cells cultured with or without FA was performed using SICER v1.1 ([6](#_ENREF_6)) using the following parameters: WINDOW\_SIZE=1000, GAP\_SIZE=3000, EFFECTIVEGENOME=0.86, FRAGMENT\_SIZE=150, FDR=0.001, FDR\_WT\_KO=0.001.

**RNA-Sequencing Analyses**

For RNA-sequencing analysis, total RNA was extracted and profiled by RNA sequencing (100bp paired-end HiSeq, Illumina) at Macrogen Europe (Amsterdam, The Netherlands) using TruSeq stranded mRNA library preparation (Illumina). The total number of reads for individual samples ranged from 60 to 130 million with at least 94% of reads >Q30. Quality-control tests for the unmapped reads were performed using the FASTQC software (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). The reads were aligned to the GRCh37 (hg19) GENCODE v.17 annotated human reference genome by using TopHat2 ([7](#_ENREF_7)) splice-junction mapper for RNA-Seq reads (version 2.1.1) with default parameters and the Cuffdiff program (version 2.2.1) for all analyses of differential expression. CuffDiff was run using the following parameters: --library-type=fr-firststrand --compatible-hits-norm --library-norm-method geometric --min-reps-for-js-test 3 --dispersion-method per-condition -u -b. In all such analyses, the difference in expression of a gene was considered significant if the p-value was less than 0.05 and the fold change was superior to 1.2 (corresponding to more than one standard deviation of all fold changes).

**Gene set enrichment analysis (GSEA) and volcano plot.**

The GSEA v3.0 software ([8](#_ENREF_8), [9](#_ENREF_9)) was used to identify functional associations of the molecular phenotypes induced by any indicated cross-compared conditions with a compendia of gene signatures including the MSigDB v6.2 ([9](#_ENREF_9), [10](#_ENREF_10)) c2 collection of 4,762 curated gene sets, the c3 collection of 836 motif gene sets, the c6 collection of 189 oncogenic signatures, and the ENCODE and CHEA datasets available from Enrichr ([11](#_ENREF_11), [12](#_ENREF_12)). Gene sets with less than 15 genes or with more than 500 genes were excluded from the analysis. GSEA was performed using weighted enrichment statistic on a pre-ranked list of genes after filtering out non expressed genes (genes with median FPKM expression below 1 in both compared conditions). Genes were ranked using their log2 fold change of expression between compared conditions. Gene sets with an FDR ≤ 0.25 and a nominal P ≤ 0.05 were considered highly significant hits. Volcano plots were generated under R environment (v3.5.2) using the ggplot2 package.

**Integrated ChIP- and RNA-sequencing analysis.**

SICER-identified significantly decreased peaks of H3K9me2 (14651 regions in IMS-M2; 4842 regions in U937) and H3K27me3 (4048 regions in IMS-M2; 4855 regions in U937) in OTX-FA versus OTX+FA conditions were annotated for the 2 nearest genes using GREAT ([13](#_ENREF_13)) (http://great.stanford.edu/public/html/) within a 50kb window upstream and downstream. The union of lists of annotated genes per cell line was kept defining a list of all genes in the vicinity of lost H3K9me2 or H3K27me3 (3961 genes for IMS-M2; 4416 genes for U937).

Significantly upregulated genes (fold change > 1.2; p-value < 0.05) identified by RNA-seq experiments comparing OTX-FA versus OTX+FA conditions were selected (623 genes in IMS-M2; 1342 genes in U937) compared to the lists of genes proximal to decreased H3K9me2 and H3K27me3 regions identified previously leading to an overlap of 93 genes in IMS-M2 and 202 genes in U937.

***In silico*tests for Pearson correlation calculation between MYC-related and metabolic gene sets**

Single-sample GSEA (ssGSEA) was used to calculate separate enrichment scores for each pairing of a sample whose transcriptomic data was available from TCGA-LAML (n=198 AML samples) or GSE14468 (n = 526 AML samples) ([14](#_ENREF_14), [15](#_ENREF_15)) and a given gene set (queried from MSigDB database for MYC and KEGG-related gene signatures or manually curated from BIOCYC for other metabolic gene sets). Data were downloaded from the InSilico DB Genomic Data Sets Hub ([http://www.genomespace.org](http://www.genomespace.org/)). A Pearson correlation matrix was then computed between each ssGSEA score for the core MYC signature and all gene sets of interest obtained across all patients from a given cohort. Connected metabolic pathways were clustered based on the median of Pearson correlation scores obtained from each individual pathway.

***Mthfr* Genotyping**

Mouse tails were dissolved in a mix of DNA release and dilution buffer (Phire Tissue Direct PCR Master Mix Kit, F170L, ThermoFisher Scientific) at room temperature for 5 min followed by 2 min at 99°C prior to proceed with PCR using the KAPPA 2X mix (Kappa biosystem, KM5101), and the forward 1 mmREX3s (GAAGCAGAGGGAAGGAGGCTTCAG), forward 2 miN3 (AGCCTGAAGAACGAGATCAGCAGC) and the reverse NeoS5 (GACTAGCTGGCTATCCTCTCATCC) primers. PCR products were run onto a 2% agarose gel at 100V for one hour in TAE buffer. The observed bands for *Mthfr*-/- mice is 216bp and Mthfr+/+ is 145bp.

**H3 Methylation Profiling**

Histones from AML cell lines were extracted according the histone extraction kit’s protocol from Epigentek (# OP-0006). Histone H3 modification profile was evaluated on histone extracts (in biological triplicate, 75ng and 50ng per replicate of IMS-M2 and U937 cells respectively) according to the manufacturer’s instructions (Epigentek, # P-3100). Each histone H3 modified at specific sites were captured by an antibody that is coated on the strip wells and specifically targets the appropriate [histone modification pattern](https://www.epigentek.com/catalog/images/products/p3000/p3100plate.gif). The captured histone modified at specific sites were detected with a detection antibody, followed by a color development reagent. The ratio of modified histone was proportional to the intensity of absorbance measured. Total histone H3 sets were measured using coated anti-H3 antibodies which were used for normalizing total histone H3 levels for relative comparison of histone H3 content between the different samples and treatment conditions.

**Allelic Discrimination Assay**

gDNA was extracted from each KG1a clone using a DNeasy Blood & Tissue Kit (Qiagen, # 69506). A total of 20 ng of gDNA was PCR-amplified in a 96-well plate using a mix of TaqMan Universal PCR Master Mix (ThermoFisher Scientific, # 4304437) and either the TaqMan SNP Genotyping Assay Human rs1801131 or rs1801133 probe used for the detection of the 1298A>C or the 677C>T MTHFR variant, respectively. Data were acquired on a SteOnePlus instrument (Applied Biosystems).

**Intracellular SAH and SAM Content Measurement**

AML cell lines were sonicated on ice in PBS (6 cycles of 30 seconds on and 60 seconds off) prior to evaluate the intracellular SAH and SAM concentrations using an SAM/SAH Elisa kit (antibodies-online.com, ABIN5564170) in biological triplicate according to the manufacturer’s instructions.

**RNA Extraction and qRT-PCR Analysis**

RNA was extracted from cells with an RNeasy Kit (Qiagen). Primers and probes for each target gene were obtained from Applied Biosystems. The list of probes and their references is available below. Data were collected in technical quadruplicate, analyzed using the ΔΔCT method, and plotted as percentage of transcript compared to the negative control condition.

|  |  |  |
| --- | --- | --- |
| **Target** | **Reference** | **Provider** |
|   |   |   |
| BCL2A | Hs06637394\_s1 | Thermo Fisher Scientific |
| DNASE2 | [Hs00923078\_g1](https://www.thermofisher.com/taqman-gene-expression/product/Hs00923078_g1?CID=&ICID=&subtype=) | Thermo Fisher Scientific |
| ICAM1 | [Hs00959180\_g1](https://www.thermofisher.com/taqman-gene-expression/product/Hs00959180_g1?CID=&ICID=&subtype=) | Thermo Fisher Scientific |
| S100A4 | [Hs00243202\_m1](https://www.thermofisher.com/taqman-gene-expression/product/Hs00243202_m1?CID=&ICID=&subtype=) | Thermo Fisher Scientific |
| SLC6A6 | [Hs00161778\_m1](https://www.thermofisher.com/taqman-gene-expression/product/Hs00161778_m1?CID=&ICID=&subtype=) | Thermo Fisher Scientific |
| LPXN | [Hs00183105\_m1](https://www.thermofisher.com/taqman-gene-expression/product/Hs00183105_m1?CID=&ICID=&subtype=) | Thermo Fisher Scientific |
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**In Vivo Transplantation**

The French National Committee on Animal Care reviewed and approved all mouse experiments described in this study. Sample size was chosen in light of the fact that the MLL-AF9-driven *in vivo* models were historically highly penetrant and consistent. Animals were excluded from any studies if any signs of distress were observed without clinical signs of leukemia: absence of leukemic blasts in bone marrow, spleen, and blood. None of our animals were excluded based on these criteria. Blinded observers visually inspected mice for obvious signs of distress, such as loss of appetite, hunched posture, and lethargy.

For the generation of the *Mthfr* knockout mouse model of AML, BALB/cJ mice were purchased from Charles River Laboratories. Each recipient mouse was transplanted with transduced Sca1- /c-Kit+ myeloid progenitors sorted from the total bone marrow of three donor mice. To do so, 4-week-old male *Mthfr*+/+, *Mthfr*+/-, and *Mthfr*-/- donor mice (obtained through a collaboration with Dr. Hava Golan’s laboratory from Gurion University, Beer-Sheva, Israel and Dr. Rima Rozen’s laboratory from McGill University, Montreal, QC, Canada) were primed with intraperitoneal injection of 5′-fluorouracil (150 mg/kg) and subsequently sacrificed after 6 days by CO2 asphyxiation. Bone marrow was harvested from femur, tibia, and humerus, and red blood cells were lysed (Red Blood Cell Lysis, RBCL buffer, Sigma). Cells were maintained in transplant medium made with 20 ng/ml Il-3 (#213-13, Peprotech), 20 ng/ml Il-6 (#216-16, Peprotech), 100 ng/ml Flt3-Ligand (#250-31L, Peprotech) and 100 ng/ml Scf (#250-03, Peprotech) in StemSpan SFEM (#09650, StemCell Technologies Inc.). The Sca-1- /c-Kit+ myeloid progenitor fraction was sorted from the total bone marrow following exclusion of Cd5+, Cd127+, Cd45R+, and Ter-119+ cell populations using FACS Aria (BD Biosciences) and were transduced with a pMIG-MLL-AF9 retroviral vector (gift from Dr. Scott Armstrong’s laboratory) by two rounds of spin-infection, at a three-hour interval, and let grown for 6 hours in CO2 incubator. All remaining bone marrow cells were maintained in culture during these steps of spin-infection and were then mixed with the infected myeloid fraction before tail vein injection into recipient mice. For each spin-infection, plates were coated with 50 μg/mL retronectin (Takara) per the manufacturer’s instructions and fresh viral supernatant was added onto each retronectin-coated well before spinning for two hours at 2000g at 32 °C to allow virus binding to the retronectin. 10×106 cells in 3 mL transplant media containing 6 μg/mL polybrene (Sigma) and 7.5 mM HEPES buffer (Sigma) were then seeded into each of these wells and centrifuged for 2 hours at 1800g to promote cell transduction. Transduced cells were washed in PBS, resuspended in Hanks balanced salt solution (Life Technologies) and then mixed with the remaining non-infected bone marrow cells before injection into the lateral tail vein of lethally irradiated (2×450cGy) BALB/cJ male recipient mice. Mice were housed in microisolator cages with autoclaved chow and acidified water. Approximatively two months after transplantation, sick mice were euthanized and their bone marrow was harvested prior to flow cytometry sorting of the MLL-AF9-positive granulo-monocytic bone marrow progenitor population (GFP+/Sca-1−/c-Kit+/Cd16/32+/Cd34+) and confirmation by PCR genotyping of the *Mthfr* knockout status. 0.2x106 MLL-AF9-positive Mthfr+/+, Mthfr+/-, and Mthfr-/- cells were then reinjected into sublethally-irradiated secondary recipient mice. Twelve days after injection, mice were randomized and treated daily by intraperitoneal injection with 50mg/kg JQ1 (10% DMSO + 90% G5W) for 8 days. Bone marrow was harvested from euthanized animals at day 21 post-injection and the proportion of GFP-positive cells was quantified by flow cytometry. The list of antibodies used for this study is referenced below.

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| **Target** | **Reference** | **Provider** |
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| Cd127 | 17-1271 | Thermo Fisher Scientific |
| Cd16/32 | 45-0161-82 | Thermo Fisher Scientific |
| Cd34 | 48-0341-82 | Thermo Fisher Scientific |
| Cd45R | 17-0452 | Thermo Fisher Scientific |
| Cd5 | 17-0051 | Thermo Fisher Scientific |
| c-Kit | 12-1172-82 | Thermo Fisher Scientific |
| Lineage | BDB558074 | BD Biosciences |
| Sca-1 | 25-5981-82 | Thermo Fisher Scientific |
| Ter-119 | 17-5921 | Thermo Fisher Scientific |
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For all the other mouse studies, primary granulo-monocytic mouse progenitor cells (Linlow, Sca-1-, c-Kit+, CD16/32+, and CD34+) were purified from C57BL/6 Actin-DsRed mice using flow cytometry, transduced with a pMSCV-MLL-AF9-Neo vector, and transplanted into lethally irradiated C57BL/6J recipients (Envigo) as previously described ([16](#_ENREF_16)). After disease onset, cells were harvested from the spleens and transplanted into sublethally-irradiated secondary recipients. Transplantation of bulk splenocytes from leukemic secondary mice was subsequently repeated twice to generate leukemia cells from quaternary transplant leukemic mouse spleens. These serially transplantable Actin-DsRed MLL-AF9-positive L-GMP cells were then used for all the mouse studies. For folate starvation experiments, 5-week old male C57BL/6J mice were fed with regular or folate-deficient casein-reconstituted diet (U8958 Version 0262, Safe-Diets) for 4 weeks prior to sublethal irradiation (350cGy) and injection with 0.2 x 106 MLL-AF9-positive L-GMP cells. Mouse cages were changed twice a week. Once disease burden reached at least 0.5% of leukemic blasts in bone marrow, mice were randomized and treated daily by intraperitoneal injection with 35mg/kg or 50mg/kg JQ1 (10% DMSO + 90% G5W) for 7 days. Three days after JQ1 treatment was stopped, proportion of DsRed-positive leukemic blasts in bone marrow was assessed by flow cytometry. Homocysteine and methionine plasma concentrations were measured as previously described ([17](#_ENREF_17)). Briefly, plasmas were mixed up with deuterated internal standards of homocysteine and methionine used for quantification. Oxidized forms of homocysteine were released by the action of dithiothreitol to obtain total plasma homocysteine. After deproteinization by methanol, homocysteine and methionine were butylated and analyzed by positive ion mode electrospray tandem mass spectrometry.

 For *Mthfr* knockdown experiments, MLL-AF9-positive L-GMP cells or Cbfb-MYH11-driven leukemic blasts (kindly provided by Lucio H. Castilla’s laboratory, ([18](#_ENREF_18))) were infected with a control or two *Mthfr*-directed shRNAs cloned into an MSCV-miRE-SV40-eBFP vector modified from the MSCV-miRE\_shBRD9\_561-SV40-GFP vector (Addgene, # 75139) by substitution of the GFP cassette with an eBFP fluorescent marker. 0.2 x 106 and 0.4 x 106 infected MLL-AF9 and Cbfb-MYH11 infected cells, respectively, were injected into sublethally-irradiated 5-week old C57BL/6J recipient mice. Once disease burden reached at least 1% of leukemic blasts in blood, mice were randomized and treated daily by intraperitoneal injection with 35mg/kg JQ1 (10% DMSO + 90% G5W) for 7 days. Three days after JQ1 treatment was stopped, proportion of DsRed-positive leukemic blasts in bone marrow was assessed by flow cytometry.

**Quantification of Extra- and Intra-Cellular Folate Levels**

For *in vivo* folate measurement, 5-week old male C57BL/6J mice were fed with folate-deficient casein-reconstituted diet (U8958 Version 0262, Safe-Diets) for 2, 4, or 8 weeks. Mouse cages were changed twice a week. Blood was harvested every two weeks at 10:00am by cardiac puncture and transferred into clot activator and gel for serum separation added tube (BD, #356968) and K2EDTA coated collection tube (BD, #356975). For sera, tubes were centrifugated at 1000g at 25°C for 15 min. Red blood cells were hemolyzed with folate RBC hemolyzing reagent for 90 minutes in the dark. 100mg of liver was smashed in folate RBC hemolyzing reagent (Roche, #05944317 190) and sonicated on ice (6 cycles of 30 seconds on and 60 seconds off). Blood and liver samples were spanned at the highest speed for 10 minutes at 25°C and 4°C, respectively.

For quantification of intracellular folic acid levels in the IMS-M2 cell line, cells were washed twice in PBS, and maintained for 48 hours in 10% dialyzed FBS and 100 units per ml penicillin/streptomycin no folic acid RPMI 1640 medium supplemented with 4000, 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.8125 or 0ng/mL folic acid (Sigma-Aldrich, #8758). Cells were washed once with PBS, lysed with folate RBC hemolyzing reagent and sonicated on ice (6 cycles of 30 seconds on and 60 seconds off) followed by incubation in the dark for 90 minutes.

For quantification of intracellular folic acid levels in the MLL-AF9 blasts, C57BL/6J mice were fed with folate-deficient casein-reconstituted diet (U8958 Version 0262, Safe-Diets) for 2 weeks prior to transplantation with MLL-AF9 cells. After two weeks, bone marrow was harvested and 5 million MLLAF9-dsRED+ were sorted, washed once with PBS and pellet. Folic acid extraction was carried out as described for IMS-M2 cells.

All samples were diluted with diluent universal (Roche, #07299001190) prior to measurement. Samples were measured with Elecsys Folate III Assay on a Cobas e801 analyzer (Roche Diagnostics).

**Quantification of Folic acid, Tetrahydrofolic (THF) and 5-Methyltetrahydrofolic acid (5-CH3-THF)**

40 x 106 IMS-M2 cells infected with either doxycycline-inducible control, *DHFR*, *DHFR2*, or *MTHFR*-directed shRNAs were washed twice with 1X PBS and seeded in no folic acid RPMI 1640 and induced with 1μg/mL doxycycline for 24 hours. Cells were then replenished with 1000ng/mL folic acid for 16 hours prior to sorting.

For each condition four replicates of 7.5x106 sorted cells were frozen in 1 mL of methanol/water (8/2) containing dithiothreitol, ascorbic acid and citric acid all three at 100µg/mL pending subsequent analysis. Then samples were thawed, and the cells were disrupted by vortexing and scraping the tube. Cellular debris were removed by spinning at 20 000 g for 15 min at +4 °C and transferring the supernatant to a fresh tube. The LC-MS/MS system consisted of an UPLC Acquity I-CLASS and a Xevo-TQ-XS triple quadruple mass spectrometer (Waters SAS, Saint-Quentin-en-Yvelines, France) with an ESI interface. An Acquity UPLC HSS PFP column (1.8 µm, 2,1 mm x 150 mm) maintained at 50°C was used for chromatographic separation. The mobile phases consisted of water (phase A) and acetonitrile (phase B), both containing formic acid 0.1%. The flow rate of the mobile phase was 0.4 mL/min. The gradient conditions ramped from 0% to 65% B between 0.5 and 3 min, then ramped to 100% and maintained up to 3.5 min, ramped to initial conditions up to 5.5 min for re-equilibration. The MS analysis operated in positive ion electrospray MRM mode. Monitored transitions were m/z 442.1>295.1, 446.07>299.04, 460.1>313.1, for folic acid, tetrahydrofolic acid, and 5-methyltetrahydrofolic acid, respectively. Under these conditions, these compounds displayed a mean retention time of 2.6, 2.4 and 2.8 min, respectively.

**BH3 Profiling**

Cytochrome C loss assay was performed as described previously ([19](#_ENREF_19)). 3.0 x 106 cells were washed in PBS, then stained with Zombie Aqua Fixable Viability dye (BioLegend). Cells were then resuspended in DTEB buffer and permeabilized with digitonin. They were then incubated with different concentrations (100, 10, 1, 0.1, 0.01µM) of Venetoclax for 1 hour. Cells were then fixed with 8% formaldehyde. After fixation termination with N2 buffer, cells were labeled with anti-human cytochrome c Alexa Fluor 647 (BD Biosciences, clone 6H2.B4). All experiments included two DMSO 2% samples (without BH3 mimetics peptide), one labeled with anti-cytochrome c and one unlabeled, as positive and negative controls for mitochondrial cytochrome c content, respectively. Flow cytometry analysis was then performed on a BD Fortessa analyzer (BD Biosciences).

**Drug interaction analysis**

The expected dose-inhibitory fraction relationships for the combination therapy of OTX015 and methotrexate were assessed with Chou–Talalay combination index (CI) for Loewe additivity ([20](#_ENREF_20), [21](#_ENREF_21)), which employs a dose–effect strategy while the combination of drugs for OTX015 and EZP-6438 were accessed with the Bliss independence model ([22](#_ENREF_22), [23](#_ENREF_23)), which uses an effect-based strategy. Loewe additivity is a basic dose–effect approach that estimates the effect of combining two drugs based on the dose of each individual drug that produces the same quantitative effect. Chou and Talalay showed that Loewe equations are valid for enzyme inhibitors with similar mechanisms of action, either competitive or noncompetitive toward the substrate. They introduced the CI scores to estimate the interaction between the two drugs. If CI < 1, the drugs have a synergistic effect, and if CI > 1, the drugs have an antagonistic effect. CI = 1 means the drugs have additive effect.

The Bliss independence model is based on the principle that drug effects are outcomes of probabilistic processes and compares the effect resulting from the combination of two drugs directly with the effects of its individual components. Bliss independence assumes that the drugs have independent mechanisms of action and can bind simultaneously and mutually nonexclusively. The model computes a quantitative measure called excess over Bliss (eob). Positive eob values are indicative of synergistic interaction, whereas negative eob values are indicative of antagonistic behavior. Null eob values indicate additive effect.

**Statistical Analysis**

Statistical analysis was done using Microsoft Excel, Prism 5.03 (GraphPad) or indicated software for more dedicated analysis. Statistical significance was determined by unpaired Student’s t-test after testing for normal distribution. For samples with significantly different variances, Welch’s correction was applied. Samples with non-normal distribution (with the assumption of no Gaussian distribution of the group) were analyzed using a nonparametric Mann-Whitney test, and the level of significance (alpha) was always set at 0.05. For comparison of three or more groups, nonparametric Kruskall-Wallis and Dunn’s multiple comparisons tests were used, and the level of significance was always set at 0.05.

**Data Availability**

Transcriptomic data from primary patient samples with AML are available from TCGA-LAML (GDC Data Portal, National Cancer Institute) and from GEO database under accession numbers: GSE14468. U937 and IMS-M2 ChIP-seq and RNA-seq data are available at GEO database under accession code GSE152442.

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