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Supplemental Methods

Patient samples and cell culture

Peripheral blood samples from patients with newly diagnosed AML and healthy donors were obtained from the First Affiliated Hospital of Anhui Medical University. Peripheral blood mononuclear cells were isolated using a standard Ficoll lymphocyte separation solution (GE Healthcare Life Sciences, Stockholm, Sweden).

The MOLM-13 cell line was purchased from Beyotime Biotechnology (Shanghai, China). MV4-11 and THP-1 cell lines were obtained from Procell Life Science & Technology (Wuhan, China) and were cultured in RPMI 1640 medium (Hyclone, Logan, UT, USA) containing 10% (FBS; foetal bovine serum, FBS; Gibco, Waltham, MA, USA) and 1% penicillin-streptomycin (Beyotime Biotechnology). MV4-11 cells were maintained in Iscove's modified Dulbecco's medium (Biosharp, Hefei, China) supplemented with 10% FBS and 1% penicillin-streptomycin. Cells were grown at 37 °C in a humidified atmosphere containing 5% CO2.

Culture and differentiation of CD34+ HSPCs

Human HSPCs were isolated from the umbilical cord blood of healthy donors at the First Affiliated Hospital of Anhui Medical University using Ficoll-Paque PLUS solution (GE Healthcare Life Sciences). CD34+ cells were isolated using anti-CD34 antibodies and magnetic isolation via the CD34 MicroBead Kit UltraPure magnetic separator (cat. # 130-100-453, Miltenyi Biotech, Bergisch Gladbach, Germany), according to the manufacturer's instructions.

Sorted CD34+ cells were cultured in StemSpan SFEM medium (StemCell Technologies,

Vancouver, Canada) supplemented with 2 mmol/l L-glutamine (Sigma-Aldrich, St. Louis, MO, USA), 1% lipid mixture 1 (L0288, Sigma-Aldrich), 100 ng/ml stem cell factor, 2 ng/ml IL-3 (both human origin, PeproTech, Waltham, MA, USA), and 1% penicillin-streptomycin. To induce granulocyte differentiation, a cytokine cocktail of 50 μg/ml G-CSF (human origin, PeproTech) and 25 μg/ml IL-3 was freshly added to the culture media.

Cell transfection

The pHBLV-CMV-MCS-3flag-EF1-BSD overexpression plasmid vector and pHBLV-U6-MCS-EF1-Luc-T2A-Puro knockdown lentiviral vectors were obtained from Hanbio Biotechnology (Shanghai, China). The Ubi-MCS-SV40-puromycin overexpression plasmid vector was constructed using Genomeditech (Shanghai, China). For lentivirus transfection, cells were cultured with viral supernatants supplemented with 5 μg/ml polybrene for three days at 37 °C. The cells were then washed and cultured overnight in a fresh medium. Stably transfected cells expressing luciferase were obtained by selection with blasticidin or puromycin (2 μg/ml).

Immunofluorescence assays

Cells were spun onto glass coverslips, fixed using 4% paraformaldehyde, permeabilised using 0.5% Triton X-100 for 10 min, blocked using 3% bovine serum albumin for 1 h, and incubated with primary antibody (1:200) overnight at 4 °C. The cells were then washed thrice using phosphate-buffered saline (PBS) at room temperature and incubated with fluorochrome-conjugated secondary antibodies in the dark for 1 h at room temperature. Nuclei were counterstained using 4'-6-diamidino-2-phenylindole for

5 min, and imaged using a fluorescence inversion microscope (Olympus, Tokyo, Japan).

Flow cytometry

A cell cycle analysis propidium iodide kit (BestBio, Nanjing, China) was used to detect intracellular DNA content according to the manufacturer's instructions. The cell cycle data were acquired using a CytoFLEX flow cytometer (Beckman Coulter, Brea, CA, USA), and the ratio of cells in the G0/G1, S, and G2/M phases was determined using the Modfit software (Verity Software House, Topsham, ME, USA). To evaluate AML cell differentiation, the cells were incubated with an anti-CD11b-PE human monoclonal antibody (BioLegend, San Diego, CA, USA) for 30 min in the dark at room temperature. The expression of CD11b was measured using a CytoFLEX cytometer (Beckman Coulter), and the data were analysed using the CytExpert software. A minimum of 10 000 events was recorded.

Protein extraction and western blotting

The cells were collected, and total proteins were extracted using RIPA lysis buffer (Beyotime Biotechnology) containing phenylmethylsulfonyl fluoride (Beyotime Biotechnology) and then centrifuged for 30 min at 12 000 rpm at 4 °C. Equal amounts of protein were separated using sodium dodecyl sulphate-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride membrane (Millipore, Burlington, MA, USA). The membranes were then blocked using 5% defatted milk for 2 h at room temperature and incubated overnight at 4 °C with primary antibodies diluted in Tris-buffered saline with Tween (TBST). The cells were incubated with a horse radish peroxidase-conjugated secondary antibody (ZSGB-Bio, Beijing, China) for 1 h

at room temperature. After washing thrice with TBST, the protein blots were visualised using an enhanced chemiluminescence kit (EpiZyme, China). Primary antibodies against FLT3, p-FLT3, CDK2, PCNA, cyclin D1 (Abcam, Cambridge, UK), and E2F1 (Cell Signalling Technology) were used at a dilution of 1:1000, while the primary antibody against β-actin (Proteintech, Rosemont, IL, USA) was used at 1:500 dilution.

RNA extraction and qPCR

Total RNA was isolated from cells using TRIzol reagent (Invitrogen, Waltham, MA, USA) and reverse transcribed into complementary DNA (cDNA) using a High Capacity cDNA Reverse Transcription Kit (Accurate Biology, China) according to the manufacturer's instructions. Relative gene mRNA expression levels were evaluated by qPCR using a SYBR-Green PCR kit. The primers used for qPCR are listed in Table 1 and were synthesised by Sangon Biotech Company (Shanghai, China). qPCR results were analysed using the 2-ΔΔCT method. All data were normalised to β-actin levels as an internal control.

Cell viability and colony growth analysis

For the CCK-8 assay, 6 x 103 cells were seeded in 96-well plates and viability was observed for five days. CCK-8 solution (10 μl; TargetMol, Shanghai, China) was added to each well. Each cell group was treated according to the experimental design, and the plates were incubated at 37 °C in a humidified, 5% CO2 atmosphere. After incubation for 5 h, optical density (OD) values were measured at 450 nm using a standard enzyme instrument (Spectra Max iD3, Molecular Devices, San Jose, CA, USA), and cell viability was expressed as the OD value. Cells transfected with lentivirus were seeded

on MethoCult (StemCell Technologies) and incubated at 37 °C in a humidified atmosphere of 5% CO2 for 14 days.

Morphological analysis

Cell morphology was analysed using Wright-Giemsa staining; the cytoplasm stained slightly blue, and the nucleus stained purple. Briefly, cells were collected, washed twice using PBS, fixed on glass slides, dried at room temperature, and stained with Wright-Giemsa stain solution. Finally, the morphology of the cell nucleus was observed using a fluorescence inversion microscope (Olympus).

ChIP-seq

ChIP assays were performed according to the manufacturer's instructions (cat. # 9003, Cell Signalling Technology, Danvers, MA, USA). Briefly, the cells were fixed with 1% formaldehyde for 10 min, quenched, and enzymatically digested for 20 min at room temperature. Fragmented chromatin was prepared using nucleases and sonication. The fragmented chromatin was approximately 300–1000 bp in size, as analysed on agarose gels. Approximately 1 × 106 cells (~10 μg of DNA) were used for all IPs. Sheared chromatin-protein complexes were incubated with antibodies against IgG and E2F1 overnight at 4 °C. The complexes were precipitated using ChIP-grade protein G magnetic beads. After IP, DNA fragments were released from the immunoprecipitated complexes by reversing the cross-linkages at 65 °C for 3 h, and then purified using a spin column. Immunoprecipitated DNA was quantified and stored at -80 °C. cDNA library construction and sequencing were performed on the Illumina HiSeq-PE150 platform by Bioacme Biotechnology Co., Ltd. (Wuhan, China).

Metabolomic analysis

Each group comprised four independent biological replicates. For each sample, 1 x 107 cells were collected, wash 2–3 times using pre-cooled PBS (4 °C), centrifuged at 1000 g for 10 min at 4 °C, and the supernatants were discarded. Metabolomics was performed by Chi-Biotech (Shenzhen, China). For LC-MS analysis, the samples were re-dissolved in 100 μl acetonitrile/water, vortexed, and centrifuged (14 000 g, 4 °C, 15 min). Supernatants were collected for LC-MS analysis using an ultra-performance LC-MS system (1290 Infinity LC, Agilent Technologies, Santa Clara, CA, USA) coupled with a quadrupole time-of-flight (5500, SCIEX, Framingham, MA, USA).

PPI docking

The PPI between E2F1 and FLT3 was predicted and refined using the Dock Protein (ZDOCK) and Refined Protein (RDOCK) protocols available in the Discovery Studio software (2017 R2 client). The best position was selected based on ZDOCK and RDOCK energy values.

AML xenograft model

NOD/SCID mice (animal certificate number: 20170010015280) were purchased from the Shanghai Model Organisms Centre (Shanghai, China). All animals were raised in a specific pathogen-free animal breeding facility at the Laboratory Animal Centre of Anhui Medical University. The animal experiments were approved by the Animal Ethics Committee at the Anhui Medical University and performed in accordance with ethical guidelines and approved protocols (ethics approval number: LISC20190751). To assess the anti-leukaemic activity of E2F1 in vivo, we transplanted 5 × 106 MOLM-

13 cells expressing luciferase in 100 µl PBS into NSG mice, which enabled us to quantify leukaemia growth using bioluminescence imaging. The mice were sacrificed two weeks after inoculation. Engraftment was validated by assessing the percentage of human CD45+ cells in mouse peripheral blood using flow cytometry.

Bioluminescence imaging

Mice were depilated and intraperitoneally injected with D-luciferin potassium salt (150 mg/kg, Abcam) and then anaesthetised using 2% isoflurane. After 10–15 min, the fluorescence signal was acquired using bioluminescence imaging on a spectral Ami HTX (USA).

Statistical Analysis

Data are presented as the mean ± standard error of the mean and were analysed using a paired or unpaired Student's t-test. Multiple groups were analysed using one-way ANOVA with Tukey's multiple comparison test. Survival was estimated using the Kaplan-Meier method and compared using the log-rank test. Statistical significance was set as $p < 0.05$ for all experiments. Data were analysed and plotted using the GraphPad Prism 8 software.

Supplemental Table 1

Forward and reverse primers of target genes used for the qRT-PCR assay

Gene	Forward	Reverse
ACTIN	CGAGCGCGGCTACAGCTT	CCTTAATGTCACGCACGATT
E2F1	CCCAACTCCCTCTACCCT	CTCCCATCTCATATCCATCCTG
ADCY1	AGGCACGACAATGTGAGCATC	TTCATCGAACTTGCCGAAGAG
ADCY5	TCTCCTGCACCAACATCGTG	CATGGCAACATGACGGGGA
GART	GGAATCCCAACCGCACAATG	AGCAGGGAAGTCTGCACTCA
PDE6D	TCCTGAGGGGCTTCAAACTAA	TCTGTTCCTTGCCAGAGTATCTT
ENPP3	GCTTGGACTCAGGAAACTGGA	TCACCTCGGTCTTTACATGCC
ENTPD4	TTATGGTCATTAGTGTCCTGGCT	TTCAATGTCGGTAACTCGTGC
PGM2	GAGGCAGTGAAACGACTAATAGC	CTGTCCCAAACTCCATTCGGG
PRPS1L1	TGGCGAAATCAACGACAGTCT	GGGACCGGCTCTTATCCTTC
NT5C3A	TCACATGGTTTGCTTGTTCAGC	AGCATAACGTCAGATTCTGCC
DCTD	TGCAAGAAACGGGACGACTAT	ATCACTGCACCCATTTGGCAT

Supplemental Figure Legends

Figure S1. E2F1 inhibition blocks the survival and growth of AML cells

A-D The protein and mRNA expression of E2F1 in Mv4-11 cells (**A**, **B**) and Molm-13 cells (**C**, **D**) was assessed by Western blotting and qPCR after treatment with shE2F1 for 3 days. **E**, **F** The overexpression efficiencies of E2F1 in THP-1**(E)** and Molm-13 **(F)** cells were confirmed by q-PCR. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Figure S2. Decreased E2F1 expression in cells increases sensitivity to retinoic acid treatment

A,B Western blot and q-PCR analysis of E2F1 expression in THP-1 cells treated with ATRA (100nM), ATPR (100nM) for 72h, and quizartinib (10nM) for 24 hours. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Figure S3. E2F1 knockdown abrogates the enhanced proliferation induced by FLT3-ITD overexpression

A,B THP-1 cells were treated with quizartinib (10nM) at different time points (0, 4, 8, 12, 24 and 48h). Then, the protein and mRNA expression of E2F1 was assessed by western blot **(A)** and q-PCR **(B)**. **C** Schematic diagram of co-transfection process (Created with BioRender.com). **D,E** qPCR analysis showing E2F1 knockdown. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Figure S4. FLT3-ITD/E2F1 axis initiates transcriptional programs associated with purine metabolism

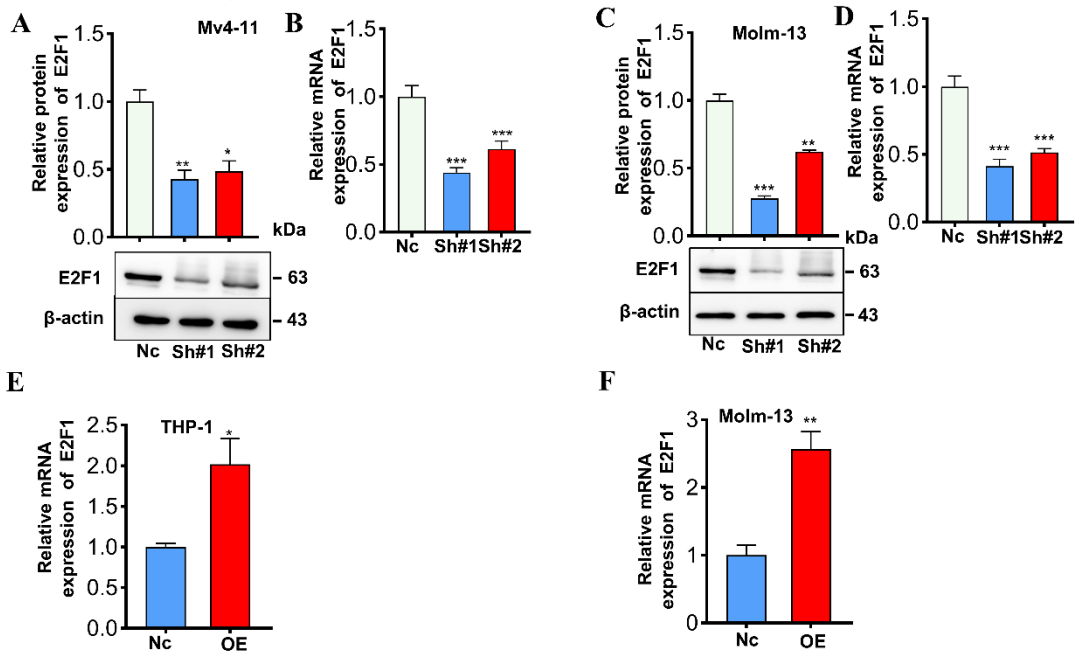
A The expression of purine synthesis pathway genes in AML dataset GSE114868 (n=214). **B** qPCR was used to compare the expression of purine biosynthesis pathway

genes in AML cells transduced with Sh-Nc or Sh-E2F1. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

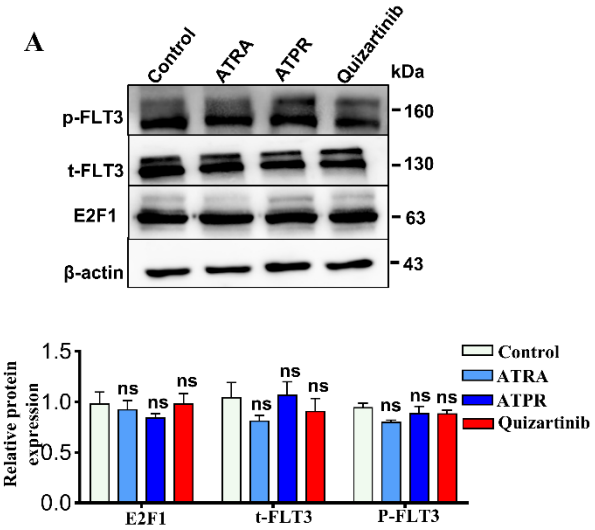
Figure S5. The FLT3-ITD/E2F1 axis maintains pools of purine metabolites

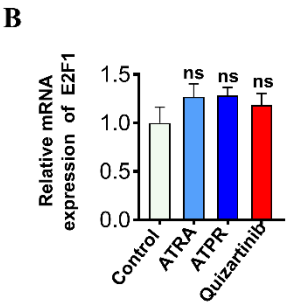
A Pyruvate metabolites levels in each treatment group. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Supplemental Figure1

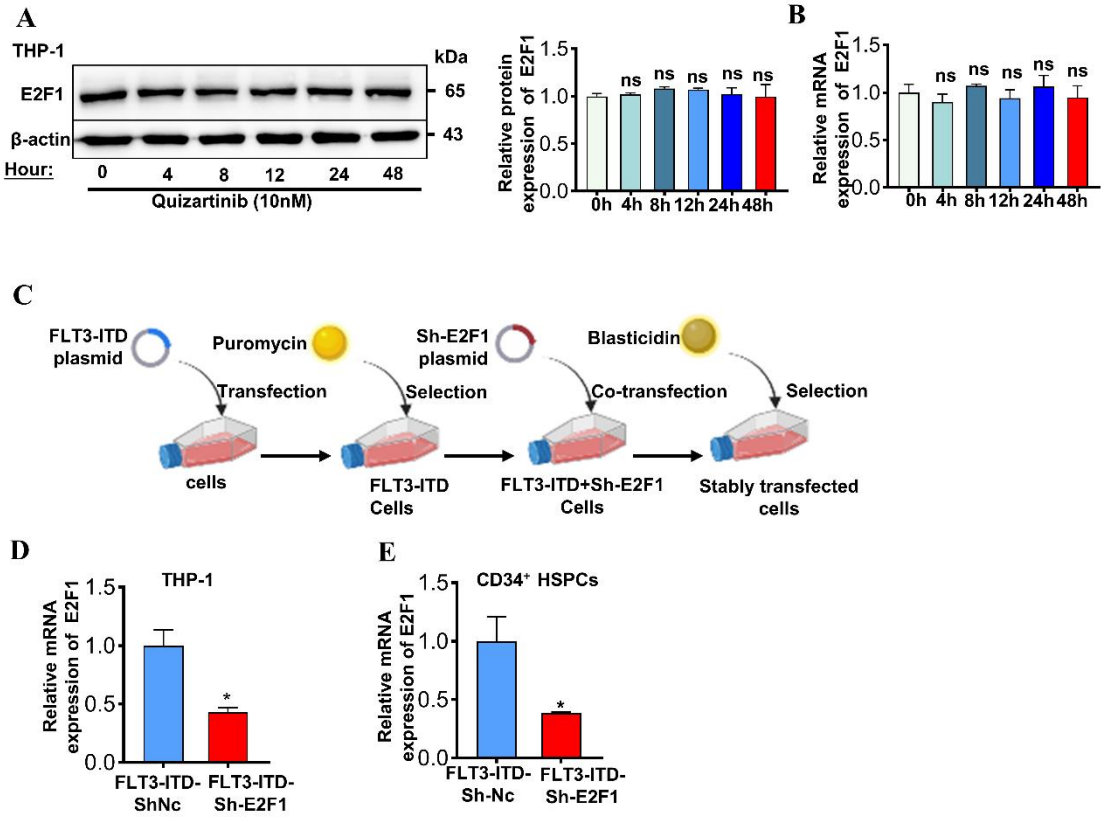


Supplemental Figure2

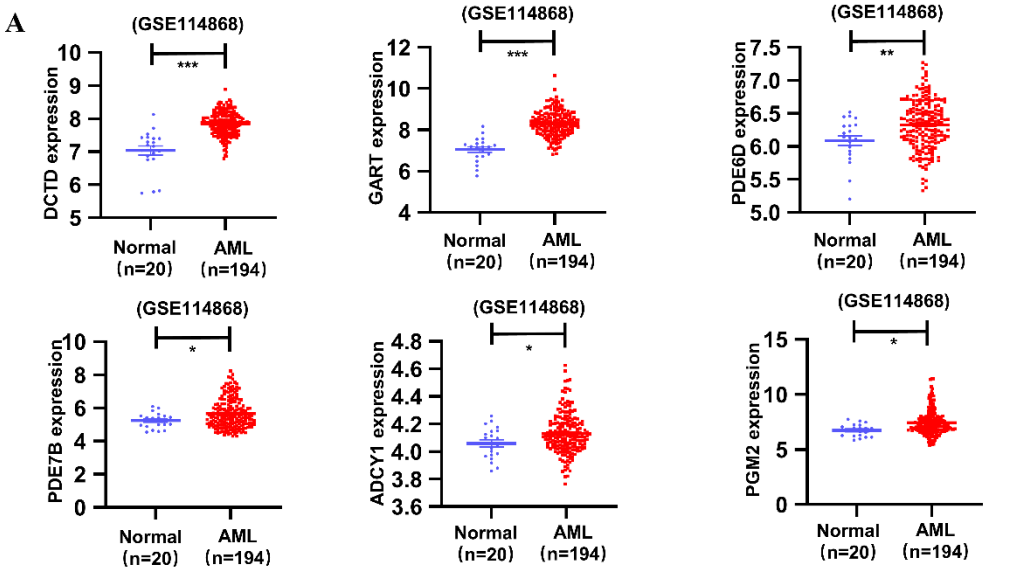


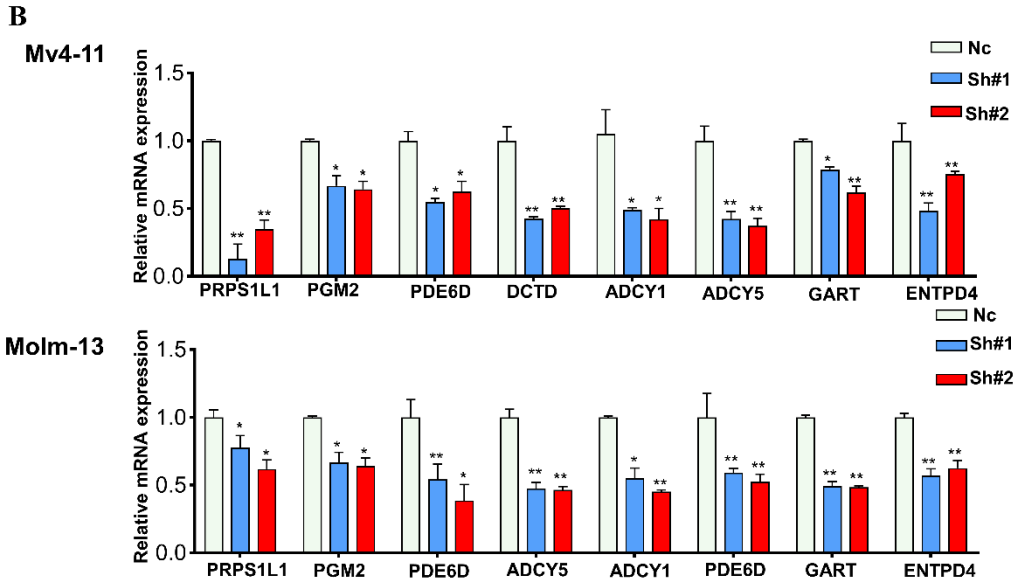


Supplemental Figure3



Supplemental Figure4





Supplemental Figure5

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