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KDM4C inhibition reinforces NK cell cytotoxicity through the cGAS–STING pathway in *TP53*-mutated AML

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Wang**

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Supplementary Materials and Methods

Cell senescence assay

THP-1 and U937 cell lines were treated with QC6352 or DMSO for 48 hours, washed with $1 \times$ phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde fixative solution for 15 minutes at room temperature, and incubated with senescence β -galactosidase staining solution (provided in the CellEvent Senescence Green Flow Cytometry Assay Kit, Invitrogen, Cat. # C10840) at 37 °C for 4 hours in a dry incubator. The cells were washed and resuspended with $1 \times$ PBS.

Cell cycle analysis

After drug treatment, cells were collected, washed, and resuspended in ice-cold $1 \times$ PBS. Ice-cold 70% ethanol was added dropwise to the cell suspension with continuous gentle vortexing, and the cells were incubated at -20 °C overnight. The cells were then washed twice with $1 \times$ PBS and incubated with PI/RNase staining buffer (BD Pharmingen, Cat. # 550825) for 15 min. Cell-cycle distribution was measured by flow cytometry, and the data were analyzed using FlowJo software.

Cell lines, culture, and reagents

Human AML cell lines MOLM-13, OCI-AML3, Kasumi-1, KG-1a, THP-1, and U937 were obtained from Shanghai Cell Bank (Chinese Academy of Sciences, Shanghai, China). U937-Luc cell line was kindly provided by Dr. Mengqing Gao (The First Affiliated Hospital of USTC). The identities of all cell lines were verified by short tandem repeat DNA profiling, and all cell lines tested negative for mycoplasma contamination. MOLM-13, OCI-AML3, Kasumi-1, KG-1a, THP-1, U937, and U937-Luc cells were cultured in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin-streptomycin. The cells were maintained in a humidified incubator at 37 °C with 5% CO₂.

Immunohistochemistry

The bone marrow biopsy samples from AML patients were fixed overnight in 4% paraformaldehyde solution and paraffin-embedded after decalcification. Liver and spleen tissues collected from NCG mice were fixed overnight in 4% paraformaldehyde solution and subsequently embedded in paraffin. Six-micrometer-thick sections were obtained for immunohistochemical staining. Immunohistochemical stains were performed with KDM4C Polyclonal Antibody (Invitrogen, Cat. # PA523065) or Mouse Anti-human CD56 Antibody (Cell Signaling Technology, Cat. # 3576). Secondary antibody-only controls were included to exclude nonspecific staining. In addition, tissues from mice that did not receive human NK-cell infusion were used as negative tissue controls for hCD56 staining. Images were taken by the Mshot microscope (MF50) and analyzed by the MShot Image Analysis System (software).

Immunofluorescence

THP-1 and U937 cells were fixed in 4% paraformaldehyde and incubated with 5% goat serum in $1 \times$ PBS and 0.5% Triton X-100 (Sheng Gong) at room temperature for 1 h. The cells were then stained overnight at 4 °C with the primary antibody against dsDNA (Abcam; Cat. # ab27156), followed by incubation with Alexa Fluor 488 donkey anti-mouse IgG secondary antibody (Invitrogen; Cat. #

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A21202). The nuclei were counterstained with DAPI. Images were visualized and photographed under an LSM 800 confocal laser-scanning microscope (Carl Zeiss Microscopy GmbH, Jena, Germany).

Quantitative Real-Time PCR (qPCR) assays

Total RNA was extracted from isolated cells using TRIzol Reagent (Invitrogen, Cat. # 15596018CN). RNA was reverse-transcribed into complementary DNA using PrimeScript RT Master Mix (Takara, Cat. # RR036A). Then, qPCR was done using the TB Green Premix Ex Taq TM II (Takara, Cat. # RR820A) according to the manufacturer's instructions on the Applied Biosystems QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific, USA). Relative mRNA expression was calculated using the $2^{-\Delta\Delta C_t}$ method and normalized to *GAPDH* expression. The primer sequences are listed in **Supplementary Table 3**.

Intracellular cytokines and degranulation assays

For intracellular cytokine staining, NK cells were co-cultured with target cells at an effector-to-target (E: T) ratio of 5:1 for 6 h in the presence of monensin (10 μ g/mL; Sigma). Following incubation, the cells were stained for surface markers, fixed, permeabilized, and subsequently stained intracellularly for IFN- γ , TNF and Granzyme B. To evaluate NK cell degranulation, NK cells were co-cultured with target cells at an E: T ratio of 5:1 for 6 h with an anti-CD107a antibody and monensin added at the start of the assay.

Cell counting kit-8 (CCK-8) assay

THP-1 and U937 cells were seeded in 96-well plates following indicated treatments and incubated with culture medium containing 10% Cell Counting Kit-8 reagent (ApexBio, Cat. # K1018) for 4 h at 37 °C. Absorbance at 450 nm was measured using a microplate reader.

The enzyme-linked immunosorbent assay (ELISA)

The concentrations of 2'3'-cGAMP and IFN- α in cell culture supernatants were measured using ELISA kits (Cayman Chemical, Cat. # CAY-501700, for 2'3'-cGAMP; Dakewe, Cat. # 1110012, for IFN- α) according to the manufacturers' instructions.

Reactive oxygen species (ROS) assay

After treatment with QC6352 for 48 h, THP-1 and U937 cells were washed and resuspended in $1 \times$ PBS. The cells were incubated with MitoSOX Green (Invitrogen, Cat. # M36005) or H2DCFDA (MedChemExpress, Cat. # HY-D0940) at a final concentration of 5 μ M for 30 min. The cells were then washed twice, resuspended in $1 \times$ PBS, and analyzed by flow cytometry. The data were analyzed using FlowJo software.

For fluorescence microscopy, the cells were incubated with 5 μ M H2DCFDA for 30 min and subsequently stained with Hoechst 33342 (MedChemExpress, Cat. # HY-15559) at a final concentration of 10 μ g/mL for 10 min. The cell suspension was dropped onto glass slides and examined using an inverted fluorescence microscope.

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Public datasets

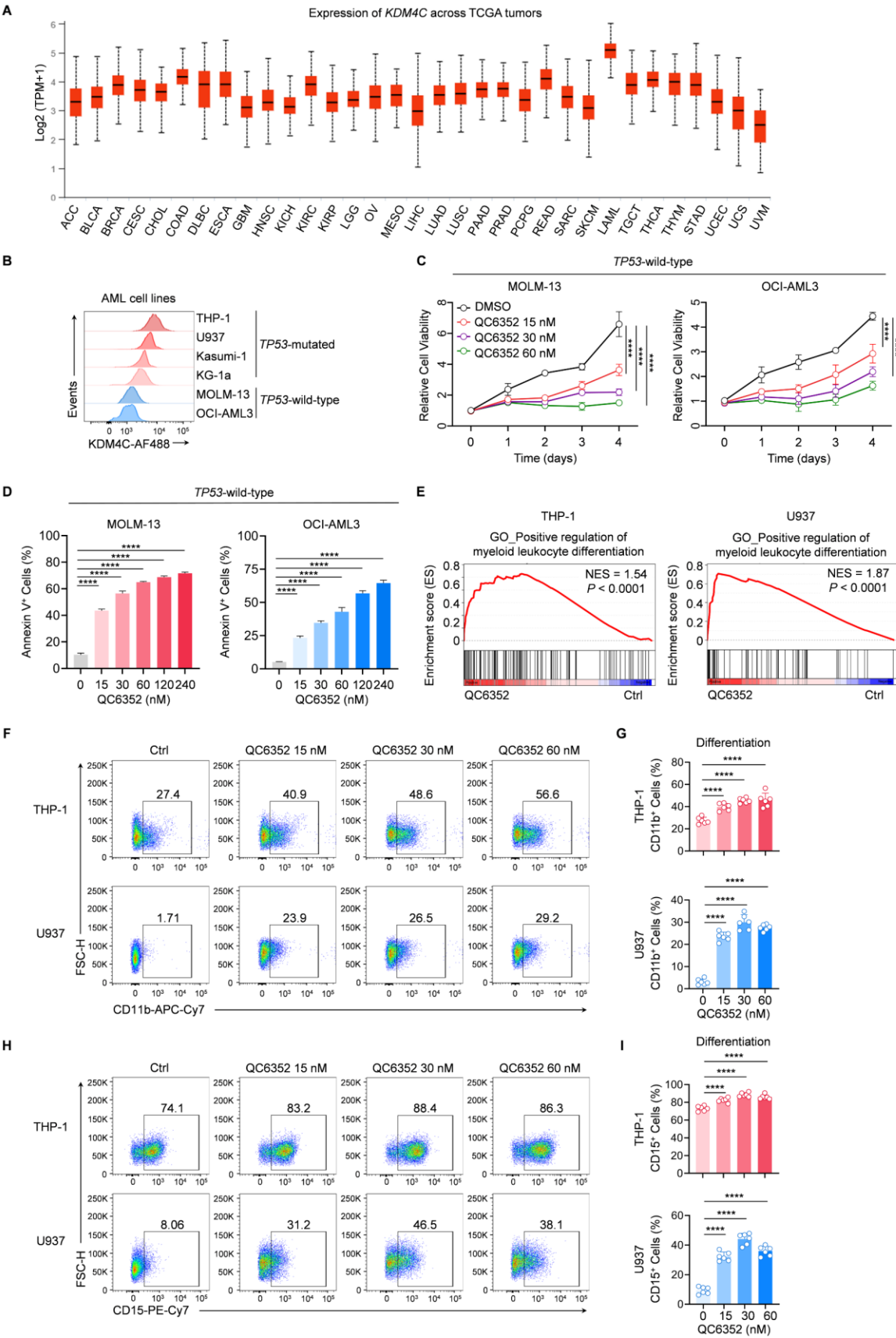
The Gene Expression Profiling Interactive Analysis (GEPIA) database (<http://gepia2.cancer-pku.cn/>), which is based on data from the UCSC Xena project, was used to analyze *KDM4C* mRNA expression in AML. Briefly, mRNA expression data from 173 AML samples and 70 normal bone marrow samples in The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) datasets were analyzed to compare *KDM4C* expression between the two groups. RNA-sequencing expression values were reported as transcripts per million (TPM). The following analysis parameters were used: the expression mode was set to “Expression DIY,” the dataset was set to “LAML,” the logarithmic scale was $\log_2(\text{TPM} + 1)$, and the *P*-value cutoff was set to 0.01. The results were presented as a box plot showing *KDM4C* expression and the number of samples in each group.

Kaplan–Meier survival analyses were performed in two AML cohorts (GSE6891 and GSE1159) after patients were stratified according to *KDM4C* expression levels. Patients were divided into high- and low-expression groups using the automatically selected optimal cutoff value in the Kaplan–Meier Plotter database. *P* values were calculated using the log-rank (Mantel–Cox) test.

The correlation between *KDM4C* expression and immune scores in tumor samples from TCGA datasets was analyzed using the “GENE” module of the Tumor Immune Estimation Resource (TIMER) database (<https://compbio.cn/timer3/>).

Supplementary Figures and Figure Legends

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Supplementary Figure 1. QC6352 induces differentiation in AML cells.

(A) *KDM4C* mRNA expression across 33 cancer types in The Cancer Genome Atlas (TCGA) cohort.

(B) Representative flow cytometry histogram showing KDM4C protein expression in AML cell lines, including OCI-AML3, MOLM-13, KG-1a, Kasumi-1, U937, and THP-1.

(C) MOLM-13 and OCI-AML3 cells were treated with the indicated concentrations of QC6352 for 1-4 days, and cell viability was assessed using the Cell Counting Kit-8 (CCK-8) assay (n = 6 biological replicates).

(D) MOLM-13 and OCI-AML3 cells were treated with the indicated concentrations of QC6352 for 48 hours, and apoptosis was assessed by Annexin V/7-AAD staining and flow cytometry (n = 6 biological replicates).

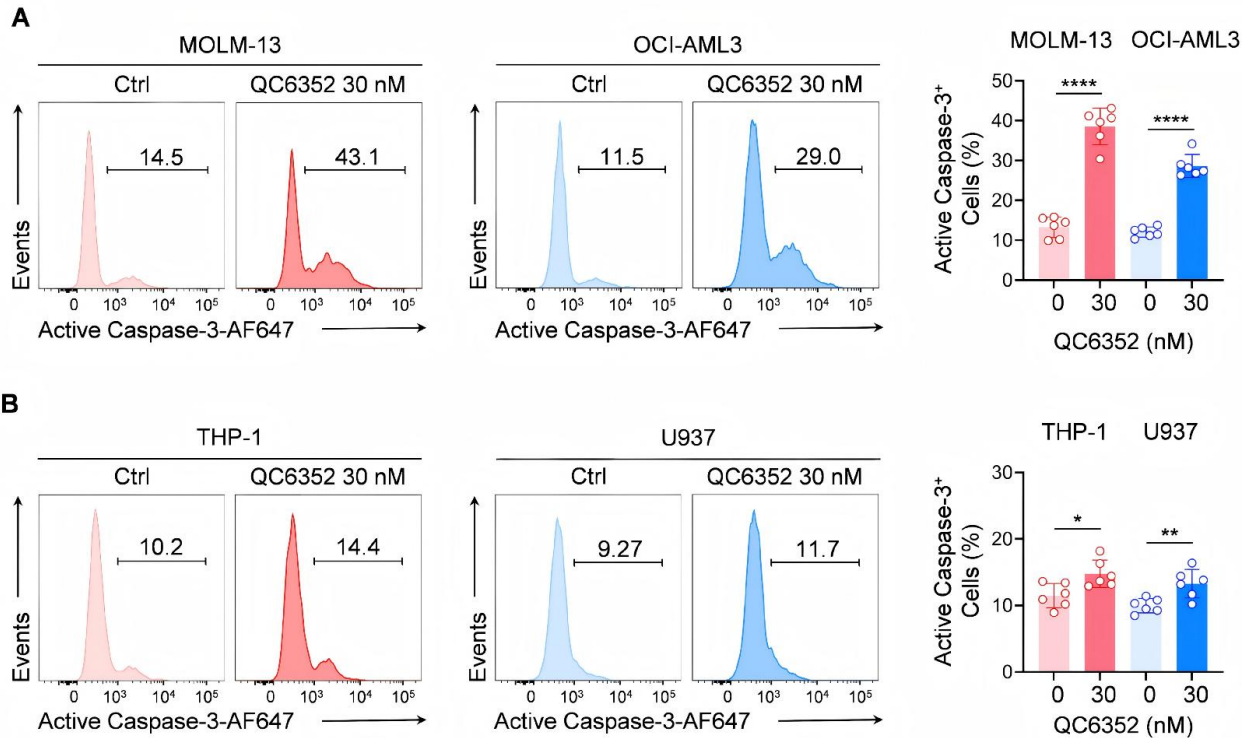
(E) GSEA plots showing enrichment of the gene set associated with the positive regulation of myeloid leukocyte differentiation in QC6352-treated THP-1 and U937 cells.

(F-G) Representative flow cytometry plots and bar diagrams showing the percentages of CD11b⁺ THP-1 and U937 cells after treatment with the indicated concentrations of QC6352 for 48 h (n = 6 biological replicates).

(H-I) Representative flow cytometry plots and bar diagrams showing the percentages of CD15⁺ THP-1 and U937 cells after treatment with the indicated concentrations of QC6352 for 48 h (n = 6 biological replicates).

Data in panels C, D, G and I were analyzed by one-way ANOVA with Tukey's multiple comparisons test. Adjusted *P* values are shown for all six pairwise comparisons in panels C, G and I, and for all 15 pairwise comparisons in panel D; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. The data are presented as mean ± standard deviation. ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LGG, brain lower grade glioma; OV, ovarian serous cystadenocarcinoma; MESO, mesothelioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; PAAD, pancreatic adenocarcinoma; PRAD, prostate adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; LAML, acute myeloid leukemia; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; STAD, stomach adenocarcinoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

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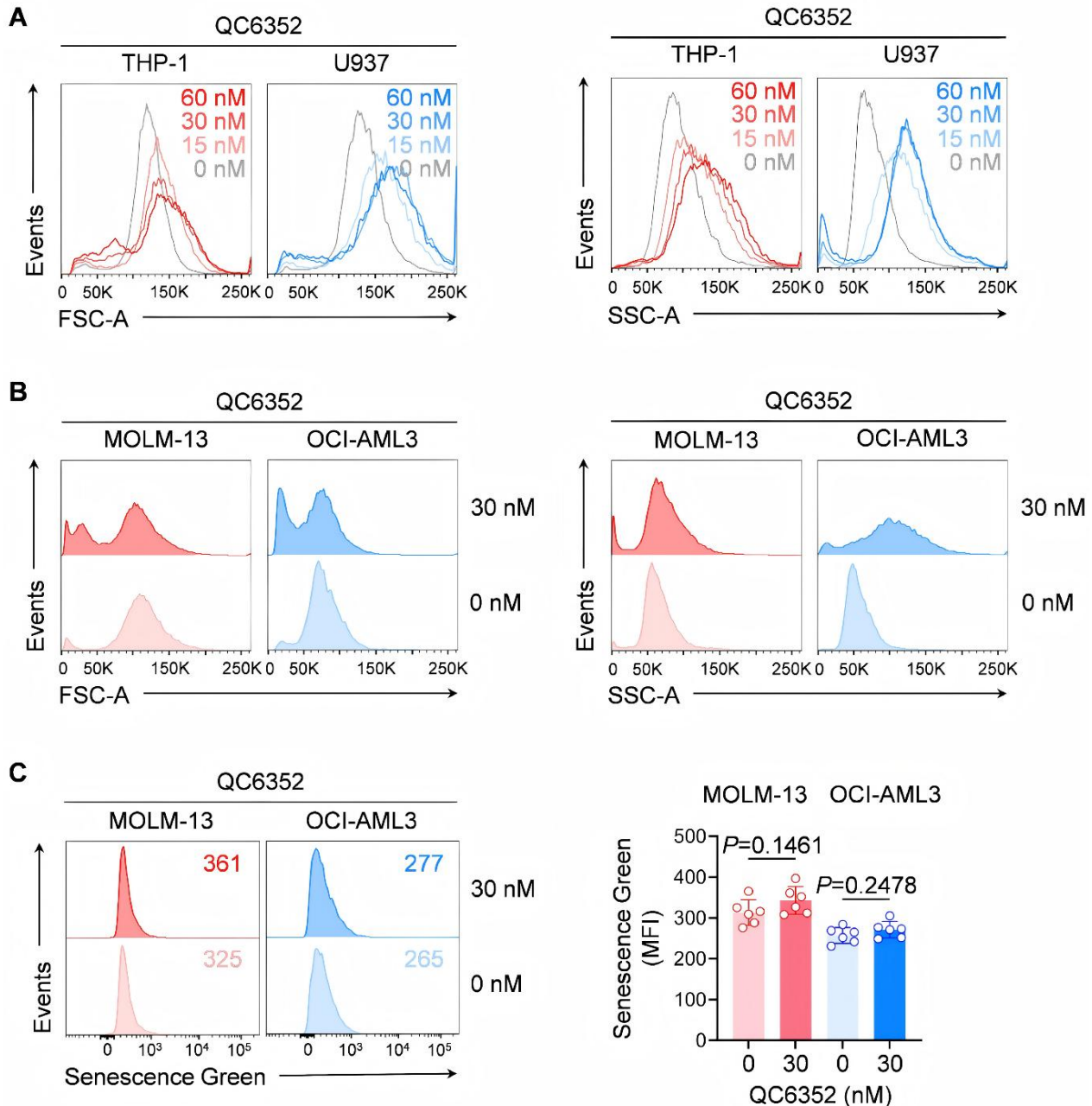
Supplementary Figure 2. QC6352 treatment increases active caspase-3 levels in *TP53*-wild-type AML cells.

(A) Representative flow cytometry histograms and the bar diagram showing the MFI of active caspase-3 in MOLM-13 and OCI-AML3 cells after treatment with or without QC6352 for 48 h (n = 6 biological replicates).

(B) Representative flow cytometry histograms and the bar diagrams showing the MFI of active caspase-3 in THP-1 and U937 cells after treatment with or without QC6352 for 48 h (n = 6 biological replicates).

Data were analyzed by two-tailed unpaired Student's *t* test; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. The data are presented as mean ± standard deviation.

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Supplementary Figure 3. QC6352 treatment induces apoptosis rather than senescence in *TP53*-wild-type AML cells.

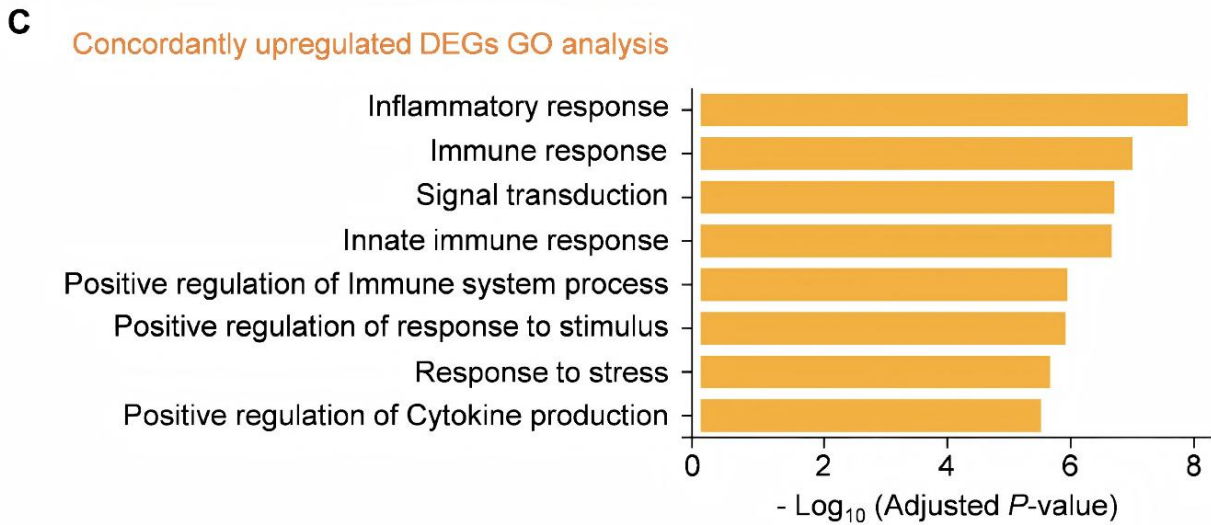
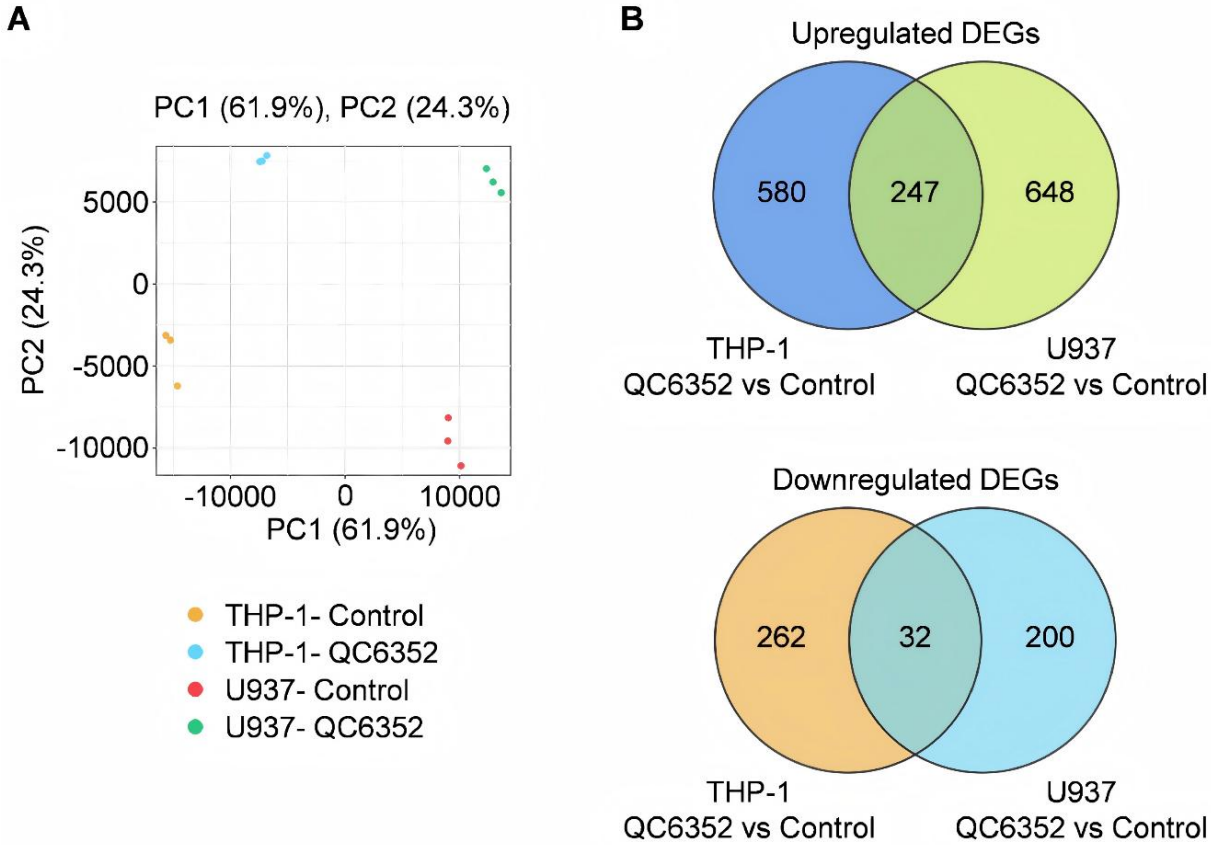
(A) Representative flow cytometry histograms for forward-scatter (FSC) and side-scatter (SSC) of THP-1 and U937 cells after treatment with the indicated concentrations of QC6352 for 48 h.

(B) Representative flow cytometry histograms for forward-scatter (FSC) and side-scatter (SSC) of MOLM-13 and OCI-AML3 cells after treatment with or without QC6352 for 48 h.

(C) Representative flow cytometry histograms and the bar diagram showing the MFI of SA- β -gal in MOLM-13 and OCI-AML3 cells after treatment with or without QC6352 for 48 h (n = 6 biological replicates). SA- β -gal was detected using Senescence Green fluorescent dye.

Data were analyzed by two-tailed unpaired Student's *t* test; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. The data are presented as mean $\pm$ standard deviation.

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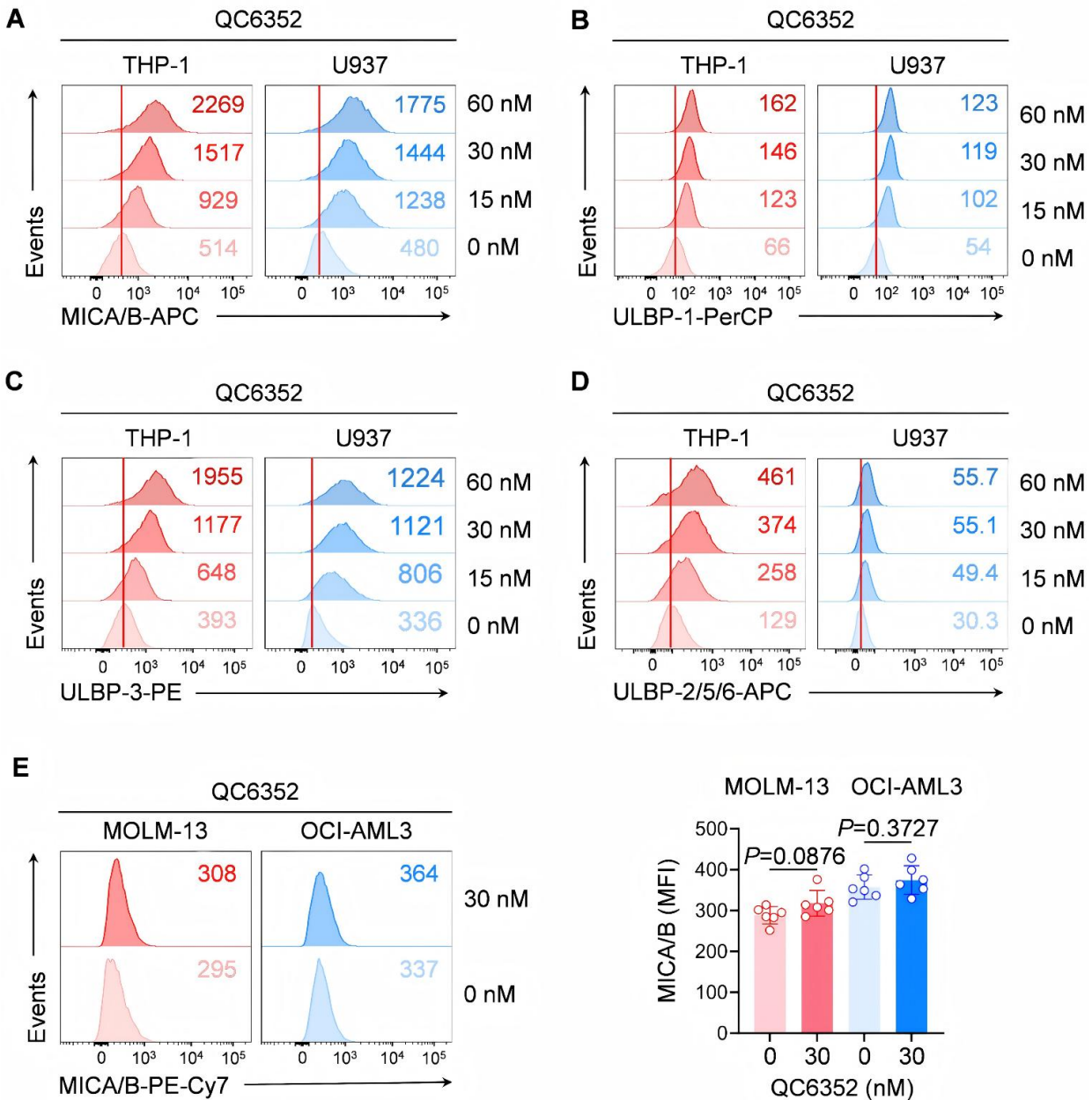
Supplementary Figure 4. QC6352 induces shared transcriptional responses in THP-1 and U937 cells.

(A) Principal component analysis (PCA) of RNA-sequencing samples from control- and QC6352-treated THP-1 and U937 cells.

(B) Venn diagrams showing the concordantly upregulated and downregulated DEGs shared between THP-1 and U937 cells.

(C) GO enrichment analysis of the concordantly upregulated DEGs shared between THP-1 and U937 cells.

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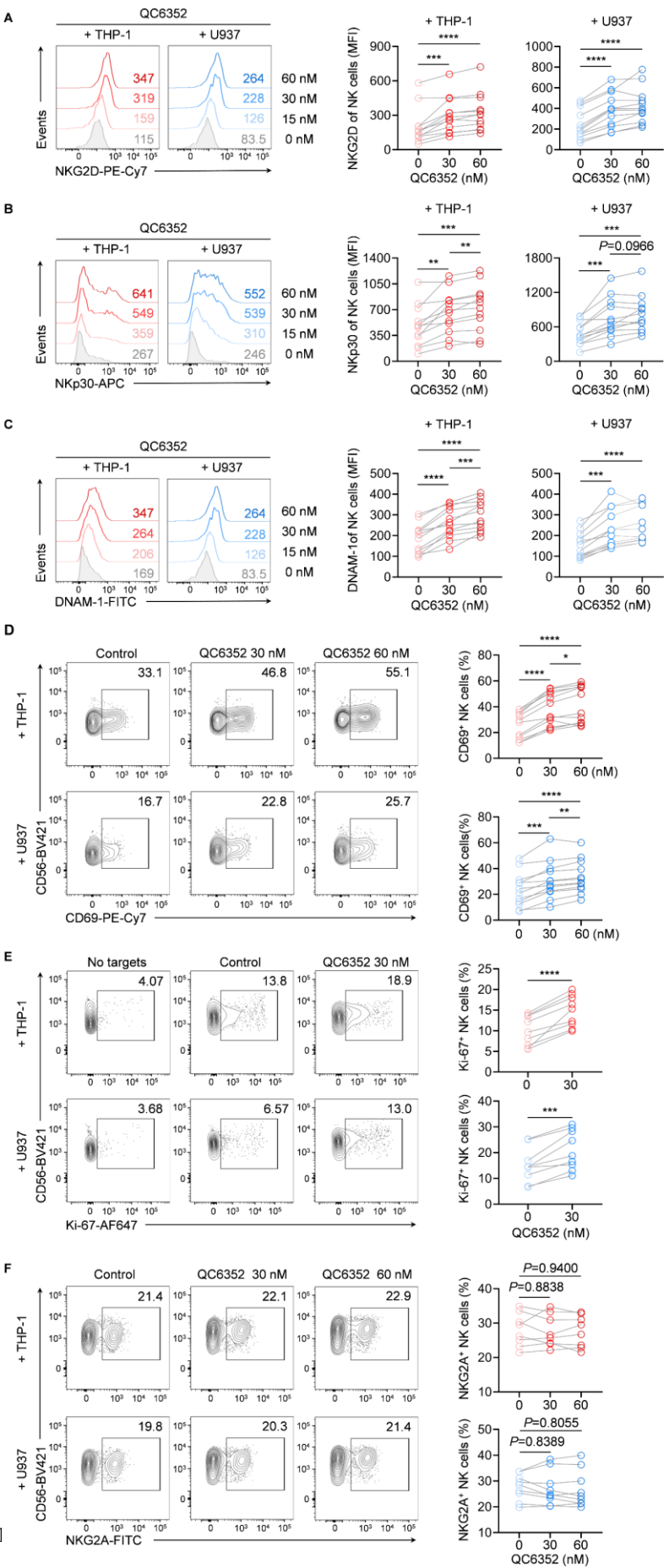
Supplementary Figure 5. QC6352 treatment upregulates NKG2D ligand expression on *TP53*-mutated AML cell lines.

(A-D) Representative flow cytometry histograms showing the MFI of (A) MICA/B, (B) ULBP-1, (C) ULBP-3, and (D) ULBP-2/5/6 on THP-1 and U937 cells after treatment with the indicated concentrations of QC6352 for 48 h.

(E) Representative flow cytometry histograms and bar diagrams showing the MFI of MICA/B on MOLM-13 and OCI-AML3 cells after treatment with or without QC6352 for 48 h (n = 6 biological replicates).

Data were analyzed by two-tailed unpaired Student's *t* test; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. The data are presented as mean ± standard deviation.

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Supplementary Figure 6. Pharmacological targeting of KDM4C with QC6352 in AML cells promotes NK-cell activation.

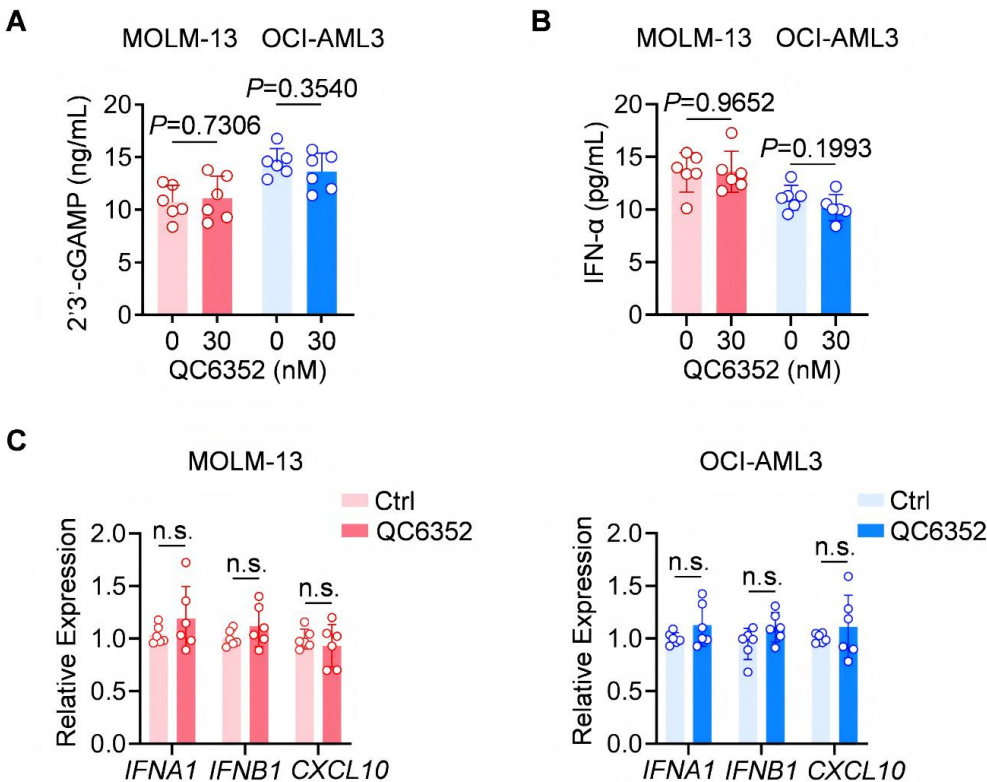
(A-C) Representative flow cytometry histograms and statistical charts showing the MFI of (A) NKG2D, (B) NKp30, and (C) DNAM-1 on NK cells after co-culture with either THP-1 or U937 cells for 48 h. THP-1 and U937 cells were pretreated with vehicle or QC6352 for 48 h before co-culture, and the E: T ratio was 5:1 (n = 12 NK-cell donors).

(D) Representative flow cytometry plots and statistical charts showing the percentages of CD69⁺ NK cells among total BMNK cells after co-culture with either THP-1 or U937 cells for 24 h. THP-1 and U937 cells were pretreated with vehicle or QC6352 for 48 h before co-culture, and the E: T ratio was 5:1 (n = 12 NK-cell donors).

(E) Representative flow cytometry plots and statistical charts showing the percentages of Ki-67⁺ NK cells among total BMNK cells after co-culture with either THP-1 or U937 cells for 48 h. THP-1 and U937 cells were pretreated with vehicle or QC6352 for 48 h before co-culture, and the E: T ratio was 5:1 (n = 10 NK-cell donors).

(F) Representative flow cytometry plots and statistical charts showing the percentages of NKG2A⁺ NK cells among total BMNK cells after co-culture with either THP-1 or U937 cells for 48 h. THP-1 and U937 cells were pretreated with vehicle or QC6352 for 48 h before co-culture, and the E: T ratio was 5:1 (n = 8-9 NK-cell donors).

Data in panel E were analyzed by two-tailed paired Student's *t* test; data in panels A-D and F were analyzed by one-way repeated-measures ANOVA with Tukey's multiple comparisons test, and adjusted *P* values are shown for all three pairwise comparisons; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. The data are presented as mean ± standard deviation.



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Supplementary Figure 7. QC6352 treatment does not activate the innate immune response in *TP53*-wild-type AML cells.

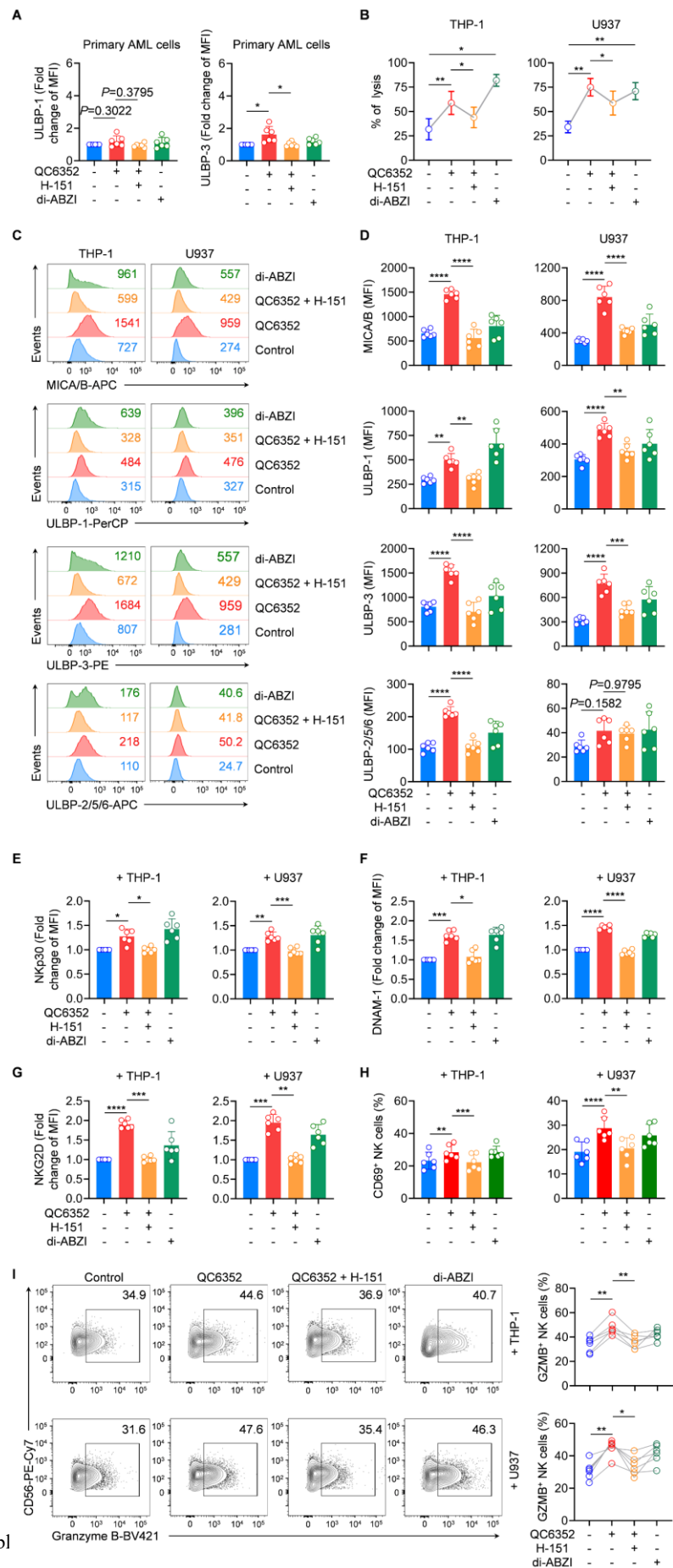
(A) ELISA results showing the concentrations of 2'3'-cGAMP in the culture supernatants of MOLM-13 and OCI-AML3 cells after treatment with or without QC6352 for 48 h (n = 6 biological replicates).

(B) ELISA results showing the concentrations of IFN- α in the culture supernatants of MOLM-13 and OCI-AML3 cells after treatment with or without QC6352 for 48 h (n = 6 biological replicates).

(C) RT-qPCR analysis of *IFNA1*, *IFNB1*, and *CXCL10* mRNA expression in MOLM-13 and OCI-AML3 cells after treatment with or without QC6352 for 48 h (n = 6 biological replicates).

Data were analyzed by two-tailed unpaired Student's *t* test; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. The data are presented as mean $\pm$ standard deviation.

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Supplementary Figure 8. Pharmacological inhibition of KDM4C with QC6352 promotes NK-cell activation via the cGAS–STING pathway.

(A) Bar diagrams showing the fold changes in the MFI of ULBP-1 and ULBP-3 on *TP53*-mutated primary AML cells after treatment with vehicle, QC6352, QC6352 plus H-151, or di-ABZI for 48 h ($n = 6$ patients).

(B) Statistical charts showing the percentage of specific lysis of THP-1 and U937 cells by BMNK cells. THP-1 and U937 cells were pretreated with vehicle, QC6352, QC6352 plus H-151, or di-ABZI for 48 h, and then co-cultured with BMNK cells for 4 h at an E: T ratio of 5:1 ($n = 6$ NK-cell donors).

(C and D) Representative flow cytometry histograms (C) and bar diagrams (D) showing the MFI of MICA/B, ULBP-1, ULBP-3, and ULBP-2/5/6 on THP-1 and U937 cells after treatment with vehicle, QC6352, QC6352 plus H-151, or di-ABZI for 48 h ($n = 6$ biological replicates).

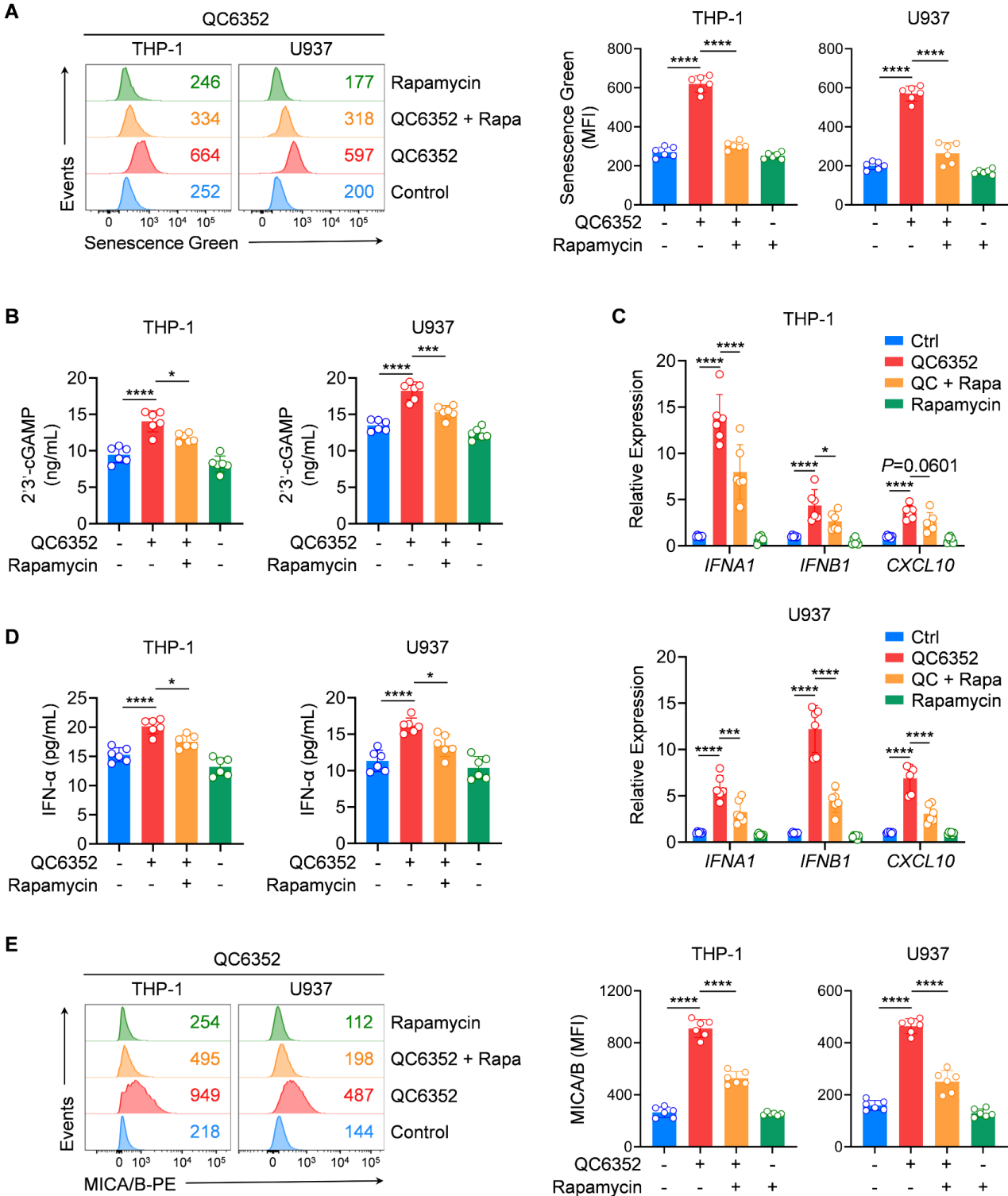
(E–G) Bar diagrams showing the fold changes in the MFI of (E) NKp30, (F) DNAM-1, and (G) NKG2D on BMNK cells after co-culture with either THP-1 or U937 cells for 48 h. THP-1 and U937 cells were pretreated with vehicle, QC6352, QC6352 plus H-151, or di-ABZI for 48 h before co-culture. The E: T ratio was 5:1 ($n = 6$ NK-cell donors).

(H) Bar diagrams showing the percentages of CD69⁺ NK cells among total BMNK cells after co-culture with either THP-1 or U937 cells for 24 h. THP-1 and U937 cells were pretreated with vehicle, QC6352, QC6352 plus H-151, or di-ABZI for 48 h before co-culture. The E: T ratio was 5:1 ($n = 6$ NK-cell donors).

(I) Representative flow cytometry plots and statistics showing the percentages of Granzyme B⁺ NK cells among total BMNK cells after co-culture with either THP-1 or U937 cells for 6 h. THP-1 and U937 cells were pretreated with vehicle, QC6352, QC6352 plus H-151, or di-ABZI for 48 h before co-culture. The E: T ratio was 5:1 ($n = 6$ NK-cell donors).

Data were analyzed by one-way repeated-measures ANOVA with Tukey's multiple comparisons test, and adjusted P values are shown for all six pairwise comparisons; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. The data are presented as mean $\pm$ standard deviation.

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Supplementary Figure 9. QC6352-induced senescence promotes the activation of the cGAS–STING pathway and the elevation of NKG2D ligands.

(A) Representative flow cytometry histograms and bar diagrams showing the MFI of SA- β -gal in THP-1 and U937 cells following treatment with vehicle, QC6352, QC6352 plus Rapamycin, or Rapamycin for 48 h (n = 6 biological replicates). SA- β -gal was detected using Senescence Green fluorescent dye.

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(B) ELISA results showing the concentrations of 2'3'-cGAMP in the culture supernatants of THP-1 and U937 cells after treatment with vehicle, QC6352, QC6352 plus Rapamycin, or Rapamycin for 48 h (n = 6 biological replicates).

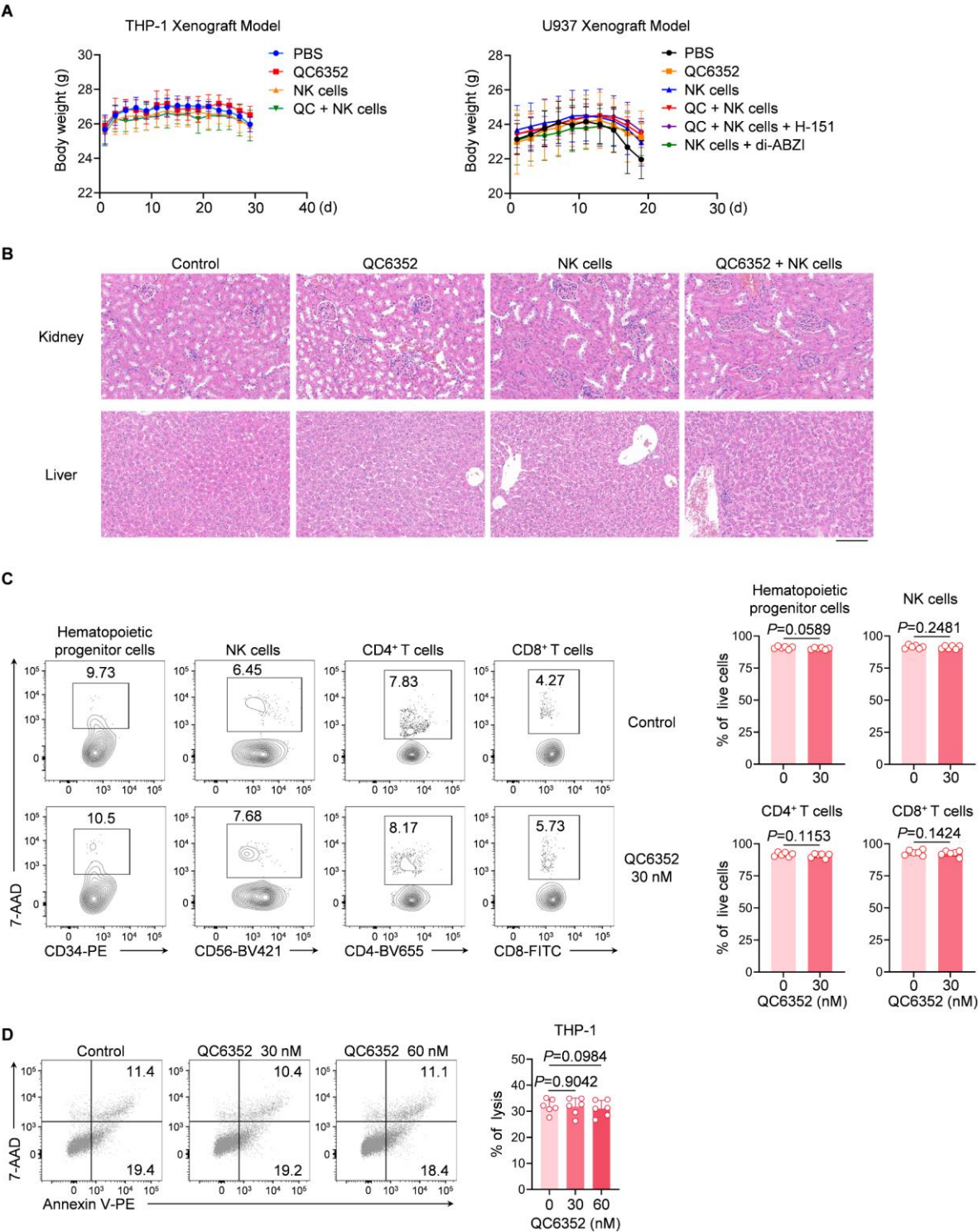
(C) RT-qPCR analysis of *IFNA1*, *IFNB1*, and *CXCL10* mRNA expression in THP-1 and U937 cells after treatment with vehicle, QC6352, QC6352 plus Rapamycin, or Rapamycin for 48 h (n = 6 biological replicates).

(D) ELISA results showing the concentrations of IFN- α in the culture supernatants of THP-1 and U937 cells after treatment with vehicle, QC6352, QC6352 plus Rapamycin, or Rapamycin for 48 h (n = 6 biological replicates).

(E) Representative flow cytometry histograms and bar diagrams showing the MFI of MICA/B on THP-1 and U937 cells following treatment with vehicle, QC6352, QC6352 plus Rapamycin, or Rapamycin for 48 h (n = 6 biological replicates).

Data were analyzed by one-way repeated-measures ANOVA with Tukey's multiple comparisons test, and adjusted *P* values are shown for all six pairwise comparisons; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. The data are presented as mean $\pm$ standard deviation.

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Supplementary Figure 10. QC6352 treatment is well tolerated and has limited effects on the viability of normal hematopoietic progenitor cells and immune cells.

(A) Body weight curves of NCG mice in the THP-1 and U937 xenograft models.

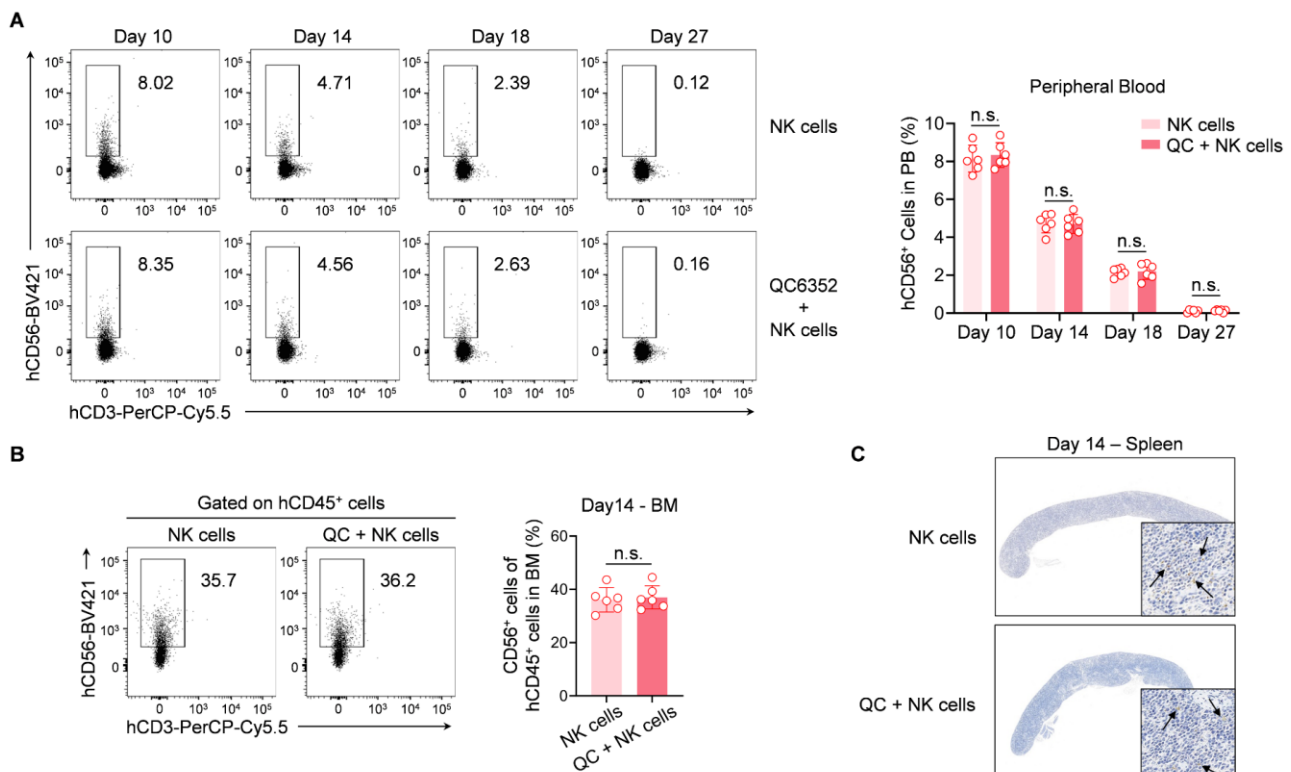
(B) Representative hematoxylin and eosin (H&E)-stained images of the kidneys and livers collected from NCG mice on Day 30.

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(C) Representative flow cytometry plots (left) showing the percentages of 7-AAD⁺ cells among CD34⁺ hematopoietic progenitor cells, NK cells, CD4⁺ T cells, and CD8⁺ T cells after treatment with or without 30 nM QC6352 for 48 h. Bar diagrams (right) show the percentages of viable cells in each cell population (n = 6 BMMC donors).

(D) Representative flow cytometry plots (left) and bar diagrams (right) showing the percentages of specific lysis of THP-1 cells by PBMCs. PBMCs were pretreated with vehicle, 30 nM QC6352 or 60 nM QC6352 for 48 h and then co-cultured with THP-1 cells at an E: T ratio of 5:1 (n = 6 PBMC donors).

Data in panel A were analyzed by two-way repeated-measures ANOVA with Tukey's multiple comparisons test, and adjusted *P* values are shown for all six pairwise comparisons in panel A (left), and for all 15 pairwise comparisons in panel A (right); data in panel D were analyzed by one-way repeated-measures ANOVA with Tukey's multiple comparisons test, and adjusted *P* values are shown for all three pairwise comparisons; data in panel C were analyzed by two-tailed paired Student's *t* test; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. The data are presented as mean ± standard deviation.



Supplementary Figure 11. Detection of adoptively transferred human NK cells in NCG mice.

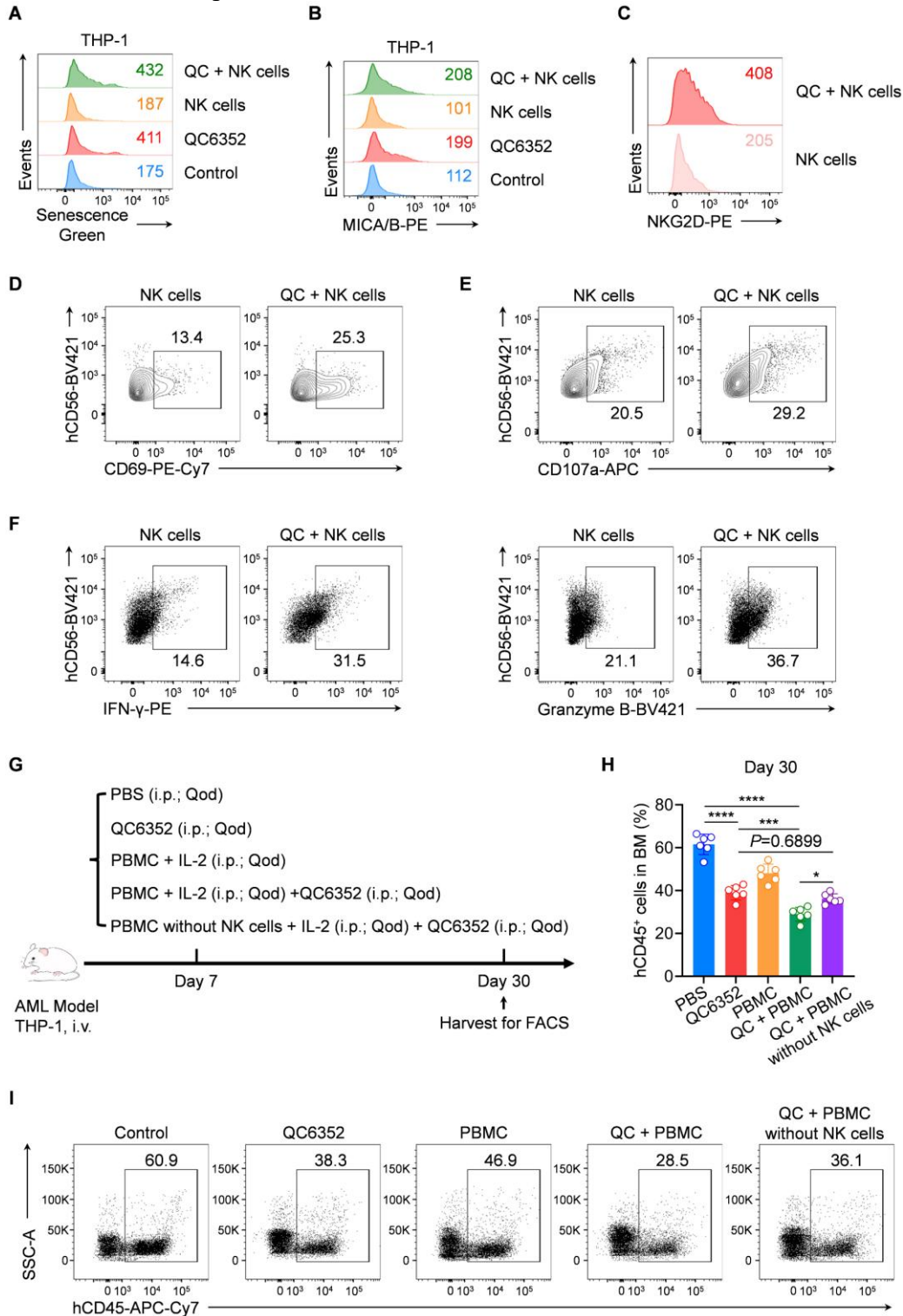
(A) Representative flow cytometry plots (left) and bar diagram (right) showing the percentages of hCD56⁺ cells in the peripheral blood of NCG mice receiving NK-cell infusion alone or in combination with QC6352 treatment on Days 10, 14, 18, and 27 (n = 6 mice per group).

(B) Representative flow cytometry plots (left) and bar diagram (right) showing the percentages of hCD56⁺ cells in the bone marrow of NCG mice receiving NK-cell infusion alone or in combination with QC6352 treatment on Day 14 (n = 6 mice per group).

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(C) Representative immunohistochemical images showing the infiltration of hCD56⁺ cells into the spleens of NCG mice receiving NK-cell infusion alone or in combination with QC6352 treatment on Day 14 (n = 6 mice per group).

Data were analyzed by two-tailed unpaired Student's *t* test; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. The data are presented as mean ± standard deviation.



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Supplementary Figure 12. QC6352 enhances NK-cell-mediated antileukemic activity *in vivo*.

(A) Representative flow cytometry histogram showing the MFI of SA- β -gal in THP-1 cells isolated from the bone marrow on Day 30 ($n = 6$ mice per group).

(B) Representative flow cytometry histogram showing the MFI of MICA/B on THP-1 cells isolated from the bone marrow on Day 30 ($n = 6$ mice per group).

(C) Representative flow cytometry histogram showing the MFI of NKG2D on NK cells in the bone marrow on Day 14 ($n = 6$ mice per group).

(D) Representative flow cytometry plots showing the percentages of CD69⁺ NK cells in the bone marrow on Day 14 ($n = 6$ mice per group).

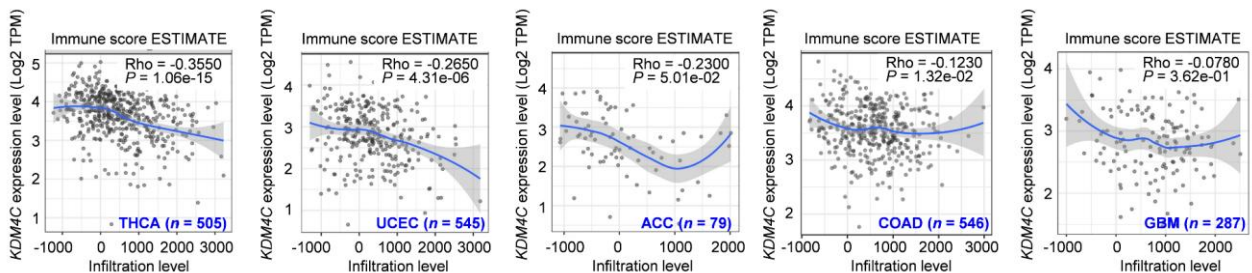
(E) Representative flow cytometry plots showing the percentages of CD107a⁺ NK cells in the bone marrow on Day 14 ($n = 6$ mice per group).

(F) Representative flow cytometry plots showing the percentages of IFN- γ ⁺ NK cells (left) and Granzyme B⁺ NK cells (right) in the bone marrow on Day 14 ($n = 6$ mice per group).

(G) Experimental design: NCG mice were injected with 1×10^6 THP-1 cells via the tail vein. The mice were randomly assigned to five groups and treated with PBS (control), QC6352 (10 mg/kg, Qod, i.p.), 1×10^7 PBMCs, 1×10^7 PBMCs plus QC6352, or 1×10^7 NK-cell-depleted PBMCs plus QC6352. On Day 30, the leukemic burden in the bone marrow was assessed by flow cytometry.

(H and I) Bar diagram (H) and representative flow cytometry plots (I) showing the percentages of hCD45⁺ cells in the bone marrow on Day 30 ($n = 6$ mice per group).

Data in panel H were analyzed by one-way ANOVA with Tukey's multiple comparisons test, and adjusted P values are shown for all ten pairwise comparisons; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. The data are presented as mean $\pm$ standard deviation.



Supplementary Figure 13. Correlation between *KDM4C* expression and immune scores in selected cancer types.

Spearman correlation analyses showing the relationships between *KDM4C* expression and immune scores in TCGA cohorts of thyroid carcinoma (THCA; $n = 505$), uterine corpus endometrial carcinoma (UCEC; $n = 545$), adrenocortical carcinoma (ACC; $n = 79$), colon adenocarcinoma (COAD; $n = 546$), and glioblastoma multiforme (GBM; $n = 287$). Spearman's rank correlation coefficients (Rho) and two-sided P values are shown.

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Supplementary Table 1. Clinical data of primary AML samples

Sample ID	Sample Type	Age	Sex	Blast (%)	Disease Status	Karyotype	Co-occurring gene mutations	TP53 - mutation	TP53 mutation frequency (%)	TP53 mutation site	Clinical treatment	Experiment
TP53-mutated AML samples												
Pat. 1-TP53-mut	AML	17	F	30	Relapse	46,XX,der(1)inv(1)(p34q21)t(1;11)(q21;q23.3)[24]/46,XX[1]	MLL-AF1Q, NRAS, TET2	Yes	61.2	NA	Ven + Aza +Selinexor	See Supplementary Table 2
Pat. 2-TP53-mut	AML	40	F	28	Relapse	47,XX,del(5)(q15q31),+add(8)(p21),del(9),(q12)(q12q23)[6]/46,XX[14]	RUNX1	Yes	32.6	NA	Ven + Aza	See Supplementary Table 2
Pat. 3-TP53-mut	AML	55	M	58	Relapse	44,XY,+add(4)(q34),del(5)(q21),-7,der(14)t(11;14)(q13;32),-18,+add(21)(p13)[20]	NRAS FLT3-ITD	Yes	9.13	c.1024del p.R342EfsTer3; c.724T>C p.C242R	IA	See Supplementary Table 2
Pat. 4-TP53-mut	AML	60	M	64	Newly diagnosed	46,XY,46,XY,t(8;12)(q22;q22)[10]/n[2]	FLT3-ITD, TET2	Yes	55.3	c.613T>G p.Y205D; c.423C>G p.C141W	Ven + Aza	See Supplementary Table 2
Pat. 5-TP53-mut	AML	33	M	31	Relapse	46,XY	NA	Yes	15.4	NA	HAA + Ven + Aza	See Supplementary Table 2
Pat. 6-TP53-mut	AML	21	F	72	Relapse	45,XX,-5,del(9)q(22),add(14)(p11.2),-16,-18,-21,+r,+mar1,+mar2[15]/46,XX[5]	RUNX1	Yes	66.2	c.375G>T p.T125=	IDAG	See Supplementary Table 2
Pat. 7-TP53-mut	AML	74	M	56	Relapse	46,XY	NPM1	Yes	54.5	c.524G>A p.R175H	Ven + Aza	See Supplementary Table 2
Pat. 8-TP53-mut	AML	66	F	68	Relapse	45,X,-X	FLT3-ITD, NPM1	Yes	NA	NA	CAVG	See Supplementary Table 2
Pat. 9-TP53-mut	AML	19	F	41	Relapse	NA	/	Yes	NA	NA	IDAG	See Supplementary Table 2
Pat. 10-TP53-mut	AML	76	M	89	Newly diagnosed	46,X,-Y,-4,+add(6)(q13),-7,del(7)(q22q32)/46,XY	NF1	Yes	47.3	NA	Ven + Aza	See Supplementary Table 2
Pat. 11-TP53-mut	AML	32	M	58	Newly diagnosed	46,XY,t(8;21)(q22,q22)/46,XY	ASXL1, TET2	Yes	30.9	c.817C>T p.R273C	IAE	See Supplementary Table 2
Pat. 12-TP53-mut	AML	40	F	90	Relapse	47,XX,del(5)(q15q31),+add(8)(p21),del(9)(q12q23)/46,XX	/	Yes	NA	NA	Ven + Aza	See Supplementary Table 2
Pat. 13-TP53-mut	AML	29	M	72	Relapse	46,XY	NPM1, FLT3-ITD	Yes	40.5	c.818A>G p.R273H	DAH	See Supplementary Table 2
TP53-wild-type AML samples												

SUPPLEMENTARY DATA

Pat. 1- TP53-wt	AML	21	M	44	Newly diagnos ed	46,XY, t(8;21)(q22;q22)	RUNX1	No	/	/	IA	See Supple mentary Table 2
Pat. 2- TP53-wt	AML	76	F	85	Newly diagnos ed	46, XX, t(6;9)(p22;q34)/ 46, XX	NA	No	/	/	CLA G+ Ven	See Supple mentary Table 2
Pat. 3- TP53-wt	AML	48	F	23	Relapse	46, XX	MLL- AF10, KRAS, CBL	No	/	/	IA	See Supple mentary Table 2
Pat. 4- TP53-wt	AML	54	M	47	Newly diagnos ed	46, XY	FLT3- ITD	No	/	/	IA, Aza + HAG	See Supple mentary Table 2
Pat. 5- TP53-wt	AML	55	M	39	Relapse	46, XY	SETD2, CEBPA, GATA2	No	/	/	Ven + Aza	See Supple mentary Table 2
Pat. 6- TP53-wt	AML	39	M	78	Newly diagnos ed	46,XY,inv(16)(p13 q22)	CBFB/M YH11	No	/	/	IA	See Supple mentary Table 2
Pat. 7- TP53-wt	AML	25	F	43	Newly diagnos ed	NA	NA	No	/	/	HAA + Ven + Aza	See Supple mentary Table 2
Pat. 8- TP53-wt	AML	48	F	71	Newly diagnos ed	46,XX	FLT3- ITD, NPM1, CEBPA	No	/	/	IA	See Supple mentary Table 2
Pat. 9- TP53-wt	AML	57	F	38	Newly diagnos ed	46,XX,t(1;11)(p32; q23)[18]/46,XX[2]	/	No	/	/	Ven +Aza	See Supple mentary Table 2
Non-AML control samples												
Ctrl. 1	MF											See Supple mentary Table 2
Ctrl. 2	MF											See Supple mentary Table 2
Ctrl. 3	AA											See Supple mentary Table 2
Ctrl. 4	Non- Hema tologi cal Disea se											See Supple mentary Table 2

SUPPLEMENTARY DATA

Supplementary Table 2. Patient sample-to-experiment and figure-panel mapping

Sample ID	Experimental assay	Figure panel
TP53-mutated AML samples		
Pat. 1-TP53-mut	KDM4C expression (FC); Ligands, differentiation and senescence (FC)	Fig. 1D, E and I; Fig. 2A; Fig. 3I; Fig. 5I; Fig. 8A
Pat. 2-TP53-mut	KDM4C expression (FC); Ligands, differentiation and senescence (FC)	Fig. 1D, E and I; Fig. 2A; Fig. 3I; Fig. 5I; Fig. 8A
Pat. 3-TP53-mut	KDM4C expression (FC); Co-culture Experiments (FC); Ligands, differentiation and senescence (FC); ELISA	Fig. 1D, E and I; Fig. 2A; Fig. 3I; Fig. 4A; Fig. 5G-O; Fig. 8A
Pat. 4-TP53-mut	KDM4C expression (FC); Co-culture Experiments (FC); Ligands, differentiation and senescence (FC)	Fig. 1D, E and I; Fig. 2A; Fig. 3I; Fig. 4A; Fig. 5I; Fig. 8A
Pat. 5-TP53-mut	Ligands, differentiation and senescence (FC)	Fig. 1I; Fig. 2A; Fig. 3I; Fig. 5I; Fig. 8A
Pat. 6-TP53-mut	Co-culture Experiments (FC); Ligands, differentiation and senescence (FC); ELISA	Fig. 1I; Fig. 2A; Fig. 3I; Fig. 4A; Fig. 5G-O; Fig. 8A
Pat. 7-TP53-mut	KDM4C expression (IHC)	Fig. 1C
Pat. 8-TP53-mut	KDM4C expression (IHC); Co-culture Experiments (FC)	Fig. 1C; Fig. 4A
Pat. 9-TP53-mut	KDM4C expression (IHC)	Fig. 1C
Pat. 10-TP53-mut	Co-culture Experiments (FC); ELISA	Fig. 4A; Fig. 5G, H and J-O
Pat. 11-TP53-mut	Co-culture Experiments (FC); ELISA	Fig. 4A; Fig. 5G, H and J-O
Pat. 12-TP53-mut	Co-culture Experiments (FC); ELISA	Fig. 4A; Fig. 5G, H and J-O
Pat. 13-TP53-mut	Co-culture Experiments (FC); ELISA	Fig. 4A; Fig. 5G, H and J-O
TP53-wild-type AML samples		
Pat. 1-TP53-wt	KDM4C expression (IHC)	Fig. 1C
Pat. 2-TP53-wt	KDM4C expression (IHC)	Fig. 1C
Pat. 3-TP53-wt	KDM4C expression (IHC)	Fig. 1C
Pat. 4-TP53-wt	KDM4C expression (IHC; FC)	Fig. 1C-E
Pat. 5-TP53-wt	KDM4C expression (IHC; FC)	Fig. 1C-E
Pat. 6-TP53-wt	KDM4C expression (FC)	Fig. 1D and E
Pat. 7-TP53-wt	KDM4C expression (FC)	Fig. 1D and E
Pat. 8-TP53-wt	KDM4C expression (FC)	Fig. 1D and E
Pat. 9-TP53-wt	KDM4C expression (FC)	Fig. 1D and E
Non-AML control samples		
Ctrl. 1	KDM4C expression (IHC)	Fig. 1C
Ctrl. 2	KDM4C expression (IHC)	Fig. 1C
Ctrl. 3	KDM4C expression (IHC)	Fig. 1C
Ctrl. 4	KDM4C expression (IHC)	Fig. 1C

SUPPLEMENTARY DATA

Supplementary Table 3. Primers Used for RT-qPCR

Gene Name	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
<i>CDKN1A</i>	ATGTCCGTCAGAACCCATGC	ATCTGTCATGCTGGTCTGCC
<i>CDKN2A</i>	AGGTCATGATGATGGGCAGC	AATCGGGGATGTCTGAGGGA
<i>CDKN1B</i>	AGTGTCTAACGGGAGCCCTA	TTGACGTCTTCTGAGGCCAG
<i>CXCL8</i>	TTTTGCCAAGGAGTGCTAAAGA	AACCCTCTGCACCCAGTTTTC
<i>IL6</i>	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTTCAGGTTG
<i>IL1B</i>	AGCTACGAATCTCCGACCAC	CGTTATCCCATGTGTCGAAGAA
<i>CXCL10</i>	GTGGCATTCAAGGAGTACCTC	TGATGGCCTTCGATTCTGGATT
<i>IFNA1</i>	CCTCGCCCTTTGCTTTACTG	CTGCTCTGACAACCTCCCAG
<i>IFNB1</i>	GACCAACAAGTGTCTCCTCCA	CTGACTATGGTCCAGGCACA