

γ -Mangostin Induces Immunogenic Cell Death and Activates cGAS Signaling in Acute Myeloid Leukemia

Krzysztof Wiśniewski^{1*}, Małgorzata Nowak¹, Tomasz Adamczyk²

¹Department of Pharmacognosy and Immunogenic Cell Death, Faculty of Pharmacy, Warsaw University of Life Sciences, Warsaw, Poland.

²Department of Phytochemistry and Leukemia Research, Faculty of Pharmacy, Jagiellonian University, Krakow, Poland.

*E-mail ✉ krzysztof.wisniewski@sggw.edu.pl

Received: 21 October 2025; Revised: 18 November 2025; Accepted: 24 November 2025

ABSTRACT

Conventional treatments like chemotherapy and radiotherapy frequently provoke anti-tumor immunity by initiating immunogenic cell death (ICD), a process where expiring cancer cells extrude damage-associated molecular patterns to stimulate targeted immune reactions. Despite this, the capacity of natural compounds to precipitate ICD within leukemic contexts remains largely unexplored. The present investigation demonstrates that the dietary compound γ -mangostin successfully eliminates primary murine leukemic populations, thereby extending the lifespan of affected mice. Furthermore, this agent effectively suppresses primary malignant cells alongside $CD34^{+}$ leukemic progenitor cells derived from human patients. Notably, the anti-leukemic effects of γ -mangostin are mediated via ICD induction, confirmed by the marked expression of IL1B, ANXA1, and HSP90B1. Moreover, the compound expedites the formation of cytoplasmic chromatin fragments, which subsequently drives the DNA damage response and augments cGAS pathway activation, ultimately elevating chemokine levels. In parallel, γ -mangostin precipitates HDAC4 degradation while fostering the buildup of acetylated histone H3, mechanisms that jointly facilitate the transcription of chemokines. As a result, these elevated chemokines recruit and activate $CD8^{+}$ T cells within the local microenvironment. Ultimately, our findings reveal that γ -mangostin initiates ICD and engages cGAS networks via a combination of epigenetic alterations and the DNA damage response. Consequently, incorporating dietary γ -mangostin offers a promising pharmacological strategy for stimulating immune-mediated defenses in the clinical management and prophylaxis of leukemia.

Keywords: γ -mangostin, Leukemia, DNA damage, Immunogenic cell death, cGAS

How to Cite This Article: Wiśniewski K, Nowak M, Adamczyk T. γ -Mangostin Induces Immunogenic Cell Death and Activates cGAS Signaling in Acute Myeloid Leukemia. *Spec J Pharmacogn Phytochem Biotechnol.* 2025;5(2):119-38. <https://doi.org/10.51847/tsegLYiDly>

Introduction

Characterized by the unregulated expansion of leukemic stem cells (LSCs) and leukemic progenitor cells (LPCs) within the bone marrow, acute myeloid leukemia (AML) encompasses a diverse array of disease subtypes. While standard induction therapies utilizing cytarabine and anthracyclines yield complete remission in most individuals, extended survival outcomes remain distinctly unfavorable [1]. A growing body of literature underscores that successful therapeutic interventions are profoundly reliant upon a robustly active immune microenvironment and the inherent immunogenicity of the malignancy.

Certain modalities—including photodynamic therapy, physicochemical interventions, oncolytic viruses, chemotherapy, and radiotherapy—can precipitate immunogenic cell death (ICD). As malignant cells perish during ICD, they emit damage-associated molecular patterns (DAMPs) that incite a targeted immune reaction and foster overarching anti-tumor immunity. Nevertheless, the dynamic interplay connecting ICD to the molecular pathways governing tumor immunogenicity remains highly intricate [2].

By facilitating protein acetylation and subsequently modulating gene transcription, the suppression of histone deacetylases (HDACs) exerts formidable anti-neoplastic effects and corrects anomalous epigenetic profiles in cancer cells. At present, researchers are actively exploring HDAC-proteolysis-targeting chimeras (PROTACs) and traditional HDAC inhibitors for the management of diverse neoplasms, with a particular focus on hematological cancers [3, 4]. Given the profound interconnectedness of immune modulation, DNA damage pathways, and epigenetic regulation within malignant tissues, targeting HDACs holds the potential to dismantle immunosuppressive microenvironments, amplify anti-tumor immune reactions, and augment overall tumor immunogenicity [5-7]. Furthermore, prior studies have documented that epigenetic inhibitors can successfully restrict proliferation and activate innate immune pathways across multiple malignancies, including chondrosarcomas [8].

Derived primarily from the pericarp of the subtropical mangosteen fruit, xanthenes represent a family of natural polyphenolic secondary metabolites. Over the past several years, structurally varied xanthenes and their derivatives have demonstrated robust pharmacological properties, encompassing anti-malarial, anti-hypertensive, anti-inflammatory, and antioxidant functions [9]. Moreover, these compounds exhibit documented cytotoxicity against a spectrum of malignant lineages, notably including leukemia [10].

Historically utilized in traditional medicine to address infections, wounds, and diarrheal illnesses, γ -mangostin is a specific tetraoxygenated diprenylated xanthone. Importantly, this compound has surfaced as a compelling anti-cancer candidate capable of precipitating mitochondrial impairment, driving apoptosis, and halting the cell cycle at the G₀/G₁ transition. Mechanistically, γ -mangostin inhibits the β -catenin signaling cascade, thereby diminishing the expression levels of TCF4 alongside its downstream effectors [11]. It additionally mitigates malignant stemness and oncogenesis via the downregulation of the GSK3/ β -catenin/CDK6 axis [12]. Crucially, while γ -mangostin is highly lethal to neoplastic populations, it spares healthy cellular counterparts [11]. Consequently, probing how γ -mangostin might optimize therapeutic outcomes by altering tumor immunogenicity represents a highly valuable endeavor.

Within the current investigation, we illustrate that γ -mangostin successfully eradicates leukemic populations via the initiation of ICD. By prompting a DNA damage response and subsequently activating cGAS, γ -mangostin elevates chemokine production, a process integral to the activation of CD8⁺ T cells. Furthermore, we outline the precise mechanisms through which this agent drives histone acetylation to facilitate the transcription of these chemokines. Ultimately, our data underscore the viability of dietary γ -mangostin as a potent pharmacological tool for both treating and preventing leukemia.

Materials and Methods

Patients and samples

Clinical specimens, specifically peripheral blood mononuclear cells (PBMCs) and bone marrow mononuclear cells (BMMCs), were acquired from healthy donors and individuals diagnosed with AML at The Third Affiliated Hospital of Sun Yat-sen University's Department of Hematology. These cells were subsequently concentrated via Ficoll-Hypaque density gradient centrifugation. A CD34 MicroBead kit (Miltenyi Biotec, Bergisch Gladbach, Germany) was employed to separate LPCs. Ethical approval for this research was granted by the Research Ethics Committee of The Third Affiliated Hospital of Sun Yat-sen University ([2015]2–209), ensuring strict compliance with the principles outlined in the Declaration of Helsinki.

Reagents and cell culture

Extracted directly from mangosteen pericarp, the γ -mangostin utilized in this study demonstrated a purity exceeding 99.5%, verified via HPLC-MS analysis. This compound was formulated into a 100 mM stock utilizing dimethyl sulfoxide (DMSO) as the solvent, and subsequently preserved at -20°C . Reagents for evaluating lactate dehydrogenase (LDH) and Caspase 3 activity were sourced from Beyotime Biotechnology (Shanghai, China).

To accurately monitor apoptotic events and overall cellular viability, we utilized a CCK-8 assay (Dojindo Laboratories, Kumamoto, Japan), alongside an Annexin V-Phycoerythrin (PE)/7-Aminoactinomycin D (7-AAD) Kit (Beyotime Biotechnology, Shanghai, China) and an Annexin V-Fluorescein Isothiocyanate (FITC)/Propidium Iodide (PI) Kit (Vazyme, Nanjing, China). We procured 5(6)-Carboxyfluorescein diacetate N-succinimidyl ester (CFSE) from Sigma-Aldrich (St. Louis, MO, USA) and Z-VAD-FMK from APEX BIO (Houston, TX, USA).

The U937 and HL-60 cell lines were provided by the American Type Culture Collection (ATCC, Manassas, VA, USA), whereas MOLM13 cells were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany). Cultivation of all leukemic lines was performed using RPMI 1640 media (Gibco, Grand Island, NY, USA), which was enriched with standard antibiotic formulations and 10% fetal bovine serum (Gibco, Grand Island, NY, USA).

Homology modeling of HDACs

The amino acid sequences for human HDAC family members lacking determined crystallographic structures were retrieved from the UniProt database (HDAC5: Q9UQL6, HDAC9: Q9UKV0). Suitable structural templates for homology modeling were selected by performing sequence alignments of the HDACs with the NCBI Protein BLAST tool. Specifically, the core domain sequence of HDAC4 exhibited 98% and 99% similarity to the corresponding sequences of HDAC5 and HDAC9, respectively. Consequently, several crystallographic structures of HDAC4 (PDB code: 2VQJ, 4CBT, 2VQW, 4CBY, 5ZOO) available in the PDB database served as templates to construct homology models for HDAC5 and HDAC9. Similarly, the core domain of HDAC2 demonstrated 94% and 65% sequence similarity with HDAC1 and HDAC3, respectively. Thus, various HDAC2 crystallographic structures (PDB code: 3MAX, 4LXZ, 4LY1, 6G3O) from the PDB bank were employed for the homology modeling of HDAC1 and HDAC3. Following this, the Modeller 9.20 software [13] was utilized to perform the homology modeling, and the generated structures were subsequently validated using PROCHECK.

Computational docking and molecular dynamics refinement

The molecular docking of γ -mangostin into the various HDACs was executed using our proprietary FIPSDock software [14]. This docking simulation utilized the homology models of HDAC1, – 3, – 5, – 9 generated in the previous step alongside the established crystallographic structures for HDAC2, – 4, – 7, – 8 (PDB ID for HDAC2: 3MAX; PDB ID for HDAC4: 2VQJ; PDB ID for HDAC7: 3C10; PDB ID for HDAC8: 1T64). The grid center for each receptor structure was designated at its center of mass. The dimensions for each grid were set to $120 \times 120 \times 120$ points, featuring a spacing of $0.375 \text{ \AA}$. Both the starting conformation and spatial position of γ -mangostin were assigned randomly, followed by the execution of 70 independent docking runs. The receptor-ligand complexes demonstrating the optimal docking scores were selected for subsequent structural refinement via the Gromacs 2018.4 package [15]. Binding free energies for each complex were subsequently calculated in Discovery Studio using the CHARMM implicit solvation model.

Bioinformatics analysis

Following the treatment of HL-60, MOLM13, or U937 cells with γ -mangostin, RNA-Seq analysis was carried out by Novogene (Beijing, China). ChIP-Seq assays were performed by Shanghai Jiayin Biotechnology (Shanghai, China). The GSE116256 dataset was retrieved from the Gene Expression Omnibus (GEO, <https://www.ncbi.nlm.nih.gov/geo/>) [16]. Single-cell data integration was accomplished using the Seurat package within the R platform, version 4.1.3. Data from The Cancer Genome Atlas (TCGA) was accessed via the XENA browser (<https://xenabrowser.net/hub/>). Gene set enrichment analysis (GSEA) was executed utilizing the ClusterProfiler package [17]. Evaluations of immune cell infiltration were performed using the ssGSEA methodology and the xCell algorithm [18]. The identification and analysis of ICD-related genes were conducted following previously established protocols [19, 20].

HDAC activity assay

Intracellular HDAC activity was evaluated utilizing a colorimetric HDAC activity assay kit (BioVision, San Francisco, CA, USA) in strict accordance with the provided manufacturer protocols. In summary, cell nuclear extracts were combined with the corresponding substrate and assay buffer in a 96-well microplate, followed by a 1 h incubation period at 37°C . The reaction was subsequently terminated via the addition of a lysine developer buffer and incubated for an additional 30 min at 37°C . Finally, HDAC activity was quantified by recording the absorbance at 405 nm on a multi-well plate reader (Bio-Rad, Hercules, CA, USA).

Cellular thermal shift assay

To assess the intracellular target engagement between γ -mangostin and HDAC4, HL-60 and MOLM13 cells were exposed to either the vehicle control or γ -mangostin for the specified durations. Following treatment, the cells

were harvested, washed a single time in PBS, and resuspended in PBS containing either the vehicle or γ -mangostin. These cellular suspensions were aliquoted into PCR tubes and subjected to a temperature gradient for 3 min, followed by a subsequent 3 min equilibration phase at room temperature. Cellular lysis was achieved by subjecting the samples to two rapid freeze-thaw cycles using liquid nitrogen. The resulting cell lysates were collected and subsequently analyzed via western blotting.

Western blot analysis

Protein concentrations were quantified using the Bradford assay, and equivalent protein quantities from all samples were resolved via SDS-polyacrylamide gel electrophoresis. Following electrophoretic transfer, nitrocellulose membranes (Merck Millipore, Billerica, MA, USA) were blocked using 5% bovine serum albumin (BSA) for 1 h. The membranes were then probed overnight at 4 °C with primary antibodies targeting cGAS, STING, p-STING, TBK, p-TBK, c-abl, p21, Cleaved caspase 3, Cleaved PARP, Survivin, Bcl-2, histone H3 (acetyl Lys9), as well as a Histone Deacetylase Antibody Sampler Kit (Cell Signaling Technology, Danvers, MA, USA); Flt3, β -actin, and c-myc (Santa Cruz Biotechnology, Santa Cruz, CA, USA); and histone H3 (acetyl K27) (Abcam, Cambridge, MA, USA). This was followed by a 1 h incubation with the corresponding secondary antibodies. Antibody-antigen complexes were visualized using an enhanced chemiluminescence detection system (Vazyme, Nanjing, China).

Real-time PCR

Total RNA extraction was performed utilizing the TRIzol reagent system (Invitrogen Life Technologies, Carlsbad, CA, USA). Complementary DNA (cDNA) was reverse transcribed using the Thermo Scientific RevertAid RT Kit (Thermo Fisher Scientific, Waltham, MA, USA). This cDNA served as the template for evaluating mRNA expression levels via the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA).

T cell functional assay

Leukemic cell lines were exposed to varying concentrations of γ -mangostin for 24 h. Following a washing step, the cells were incubated in fresh culture medium for an additional 24 h, after which the supernatant was harvested as conditioned medium (CM). Peripheral blood mononuclear cells (PBMCs) were maintained in this CM for 48 h, subsequently transferred to 96-well plates, and stimulated with CD3/CD28 antibodies for 12 h. The expression levels of CD8 and CD69 were then quantified via flow cytometry. To assess T cell proliferation, PBMCs previously cultured in CM were labeled with CFSE, seeded into 96-well plates with CD3/CD28 antibody stimulation for a duration of 5 days, and subsequently analyzed by flow cytometry. To evaluate migratory capacity, CM was placed in the lower compartment of transwell chambers, while PBMCs were seeded into the upper inserts and allowed to migrate for 6 h. The extent of migration was determined by quantifying the viable cells present in the lower chamber.

Animal model

In the BALB/c nude mouse model, HL-60 cells were subcutaneously inoculated into the flanks. Tumor volumes were monitored on specified days and calculated using the formula $A \times B^2/2$, where A represents the longest diameter and B denotes the perpendicular diameter. In experiments involving NOD-SCID mice, subjects were engrafted with luciferase-expressing HL-60 cells prior to the intragastric administration of γ -mangostin. Longitudinal imaging of the mice was conducted at predetermined time points using an *in vivo* imaging system (PerkinElmer, Waltham, MA, USA). For the C57BL/6 mouse model, hematopoietic progenitor cells (HPCs) were isolated from donor mice pre-treated with 5-Fluorouracil (5-FU). These cells were subjected to overnight stimulation with mIL-3, mIL-6, and mSCF (Peprotech, Rocky Hill, NJ, USA) and subsequently transduced with a retrovirus harboring the MLL-AF9 fusion gene. Following X-ray irradiation, recipient mice were transplanted with the transduced HPCs through tail vein injections. Bone marrow (BM) samples from these leukemic mice were subsequently harvested for secondary transplantation assays or additional downstream analyses. All animal care protocols and experimental methodologies received approval from the Institutional Animal Care and Use Committee of Sun Yat-sen University (00181576), operating in strict compliance with the WMA statement regarding the ethical use of animals in biomedical research.

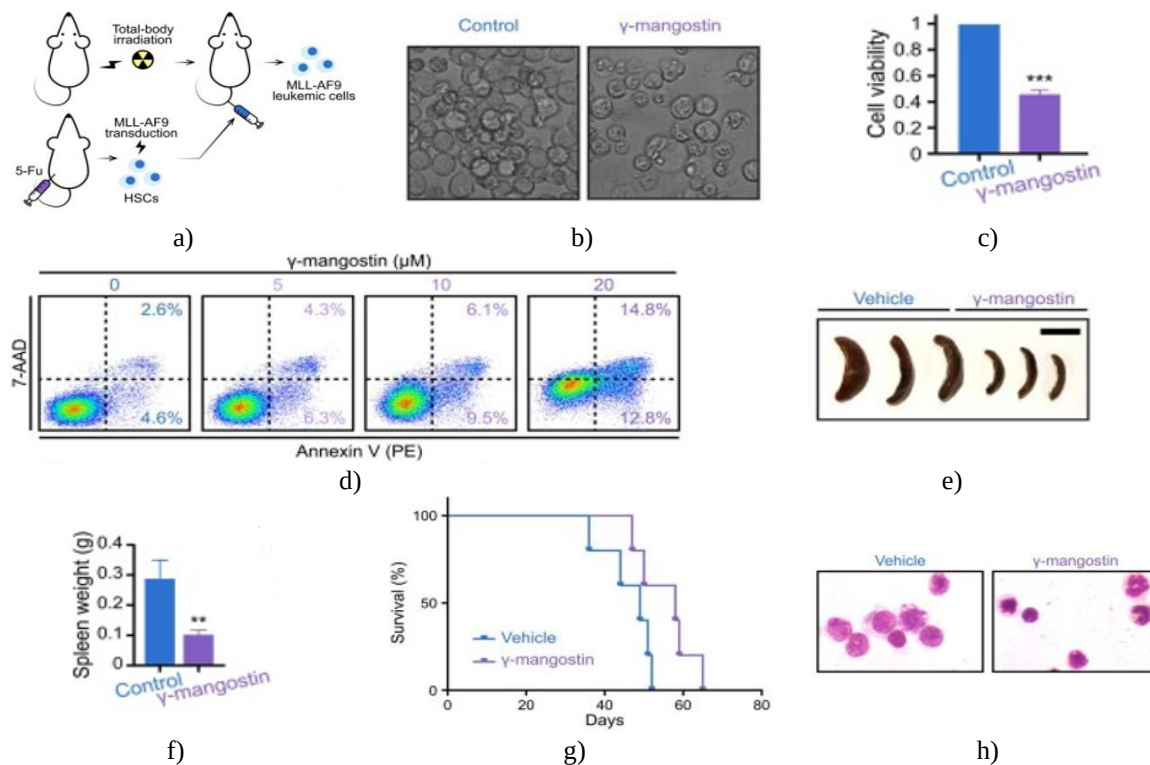
Statistics

All statistical evaluations were performed utilizing SPSS software, version 16.0. Statistical comparisons across experimental groups were executed using either the Student's t-test or ANOVA, with significance defined as $p < 0.05$.

Results and Discussion

γ -mangostin eliminates leukemic cells in both *in vitro* and *in vivo* settings

Following an initial screening of multiple naturally occurring compounds, we identified that the dietary compound γ -mangostin exhibited a robust capacity to clear malignantly transformed cells. To establish a leukemia model, murine bone marrow (BM) cells were transduced with the MLL-AF9 fusion gene and subsequently engrafted into recipient mice. Harvested BM cells were then subjected to CCK-8 assays and Annexin V-7AAD staining procedures (**Figures 1a and 1d**). Treating MLL-AF9 leukemic cells with γ -mangostin led to a marked decline in cellular viability alongside elevated levels of apoptotic cell death, relative to the untreated control group (**Figures 1b and 1d**). In a comparable manner, γ -mangostin suppressed the viability of cells undergoing malignant transformation driven by BCR-ABL or FLT3-ITD. Following a secondary engraftment of MLL-AF9 BM cells, the subjects received either a vehicle control or γ -mangostin via intragastric administration on alternate days. Treatment with γ -mangostin effectively mitigated splenomegaly in the MLL-AF9 murine model, which was demonstrated by significantly reduced splenic dimensions and masses (**Figures 1e and 1f**). Furthermore, subjects in the γ -mangostin treatment cohort exhibited a substantially extended overall survival (**Figure 1g**). Evaluations of cellular morphology indicated that γ -mangostin promoted an increased presence of leukemic blasts exhibiting segmented nuclei (**Figure 1h**). Flow cytometry further confirmed a reduction in the leukemic cell population, quantified by GFP⁺ expression, among the γ -mangostin-treated subjects (**Figure 1i**). Collectively, these findings demonstrated the capacity of γ -mangostin to effectively eliminate murine leukemic cells.



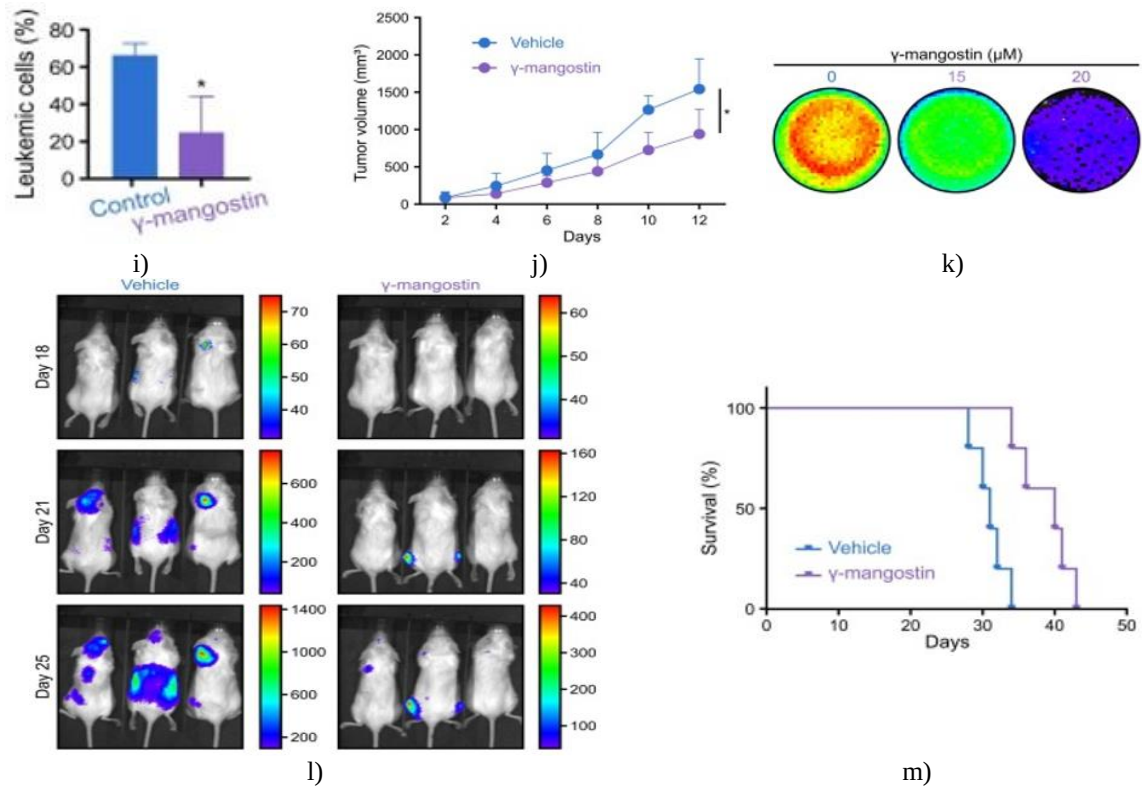


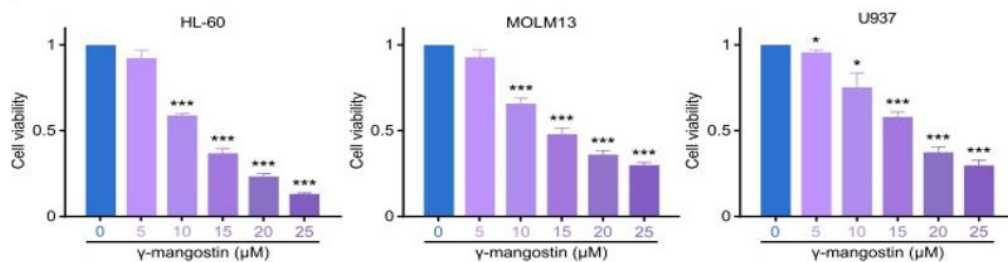
Figure 1. γ -mangostin suppresses LPC proliferation within a leukemia model. (a) Schematic outline detailing the experimental protocol for harvesting murine leukemic cells. Hematopoietic progenitor cells (HPCs) from mice were transduced with the MLL-AF9 retrovirus and engrafted into recipient subjects. Upon the onset of leukemia, bone marrow (BM) populations were harvested for subsequent evaluations. These isolated murine leukemic cells were cultured either in the presence or absence of γ -mangostin to assess phenotypic alterations (b), viability via CCK-8 analysis (c), and apoptosis (d). (e) Visual representation of spleens excised from MLL-AF9 leukemic subjects following treatment with or without γ -mangostin. (f) Quantification of spleen masses. (g) C57BL/6 subjects undergoing secondary MLL-AF9 transplantation received oral administrations of either γ -mangostin (50 mg/kg) or a control vehicle every alternate day over a 14-day period. Survival outcomes are depicted using Kaplan-Meier curves (n = 5). (h) Morphological assessment of BM populations via Wright-Giemsa staining comparing the γ -mangostin-treated cohort against the vehicle control. The proportion of MLL-AF9-driven (GFP-positive) leukemic cells within the BM was determined by flow cytometric evaluation of GFP expression (i). (j) HL-60 cells were subcutaneously implanted into the flank regions of 5- to 6-week-old male nu/nu BALB/c mice. The subjects were subsequently randomized into cohorts receiving either γ -mangostin (50 mg/kg, i.p.) or vehicle control every alternate day for a total of 12 days (n = 5). Tumor volumetric progression was graphically represented over time. *p < 0.05. (k) In vitro imaging of luciferase-expressing red fluorescent cells following a 24 h incubation with or without γ -mangostin. (l) NOD-SCID subjects bearing luciferase-labeled red fluorescent cells were intraperitoneally administered D-Luciferin and subjected to in vivo imaging at various intervals post- γ -mangostin treatment (n = 3). (m) Survival trajectories are illustrated through Kaplan-Meier analysis (n = 5).

Subsequently, the antineoplastic efficacy of γ -mangostin was evaluated in vivo using a nude mouse xenograft model engrafted with human leukemic cells. Specifically, nu/nu BALB/c mice subcutaneously injected with HL-60 cells were randomly assigned to either a γ -mangostin treatment group or an untreated control group. As depicted in **Figure 1j**, the administration of γ -mangostin robustly restricted tumor expansion. In parallel, in vitro exposure of luciferase-expressing HL-60 cells to γ -mangostin demonstrated a substantial decline in bioluminescent signaling (**Figure 1k**). These luciferase-tagged HL-60 cells were additionally engrafted into NOD-SCID subjects, which were then treated with either γ -mangostin or a vehicle control. A progressive, time-dependent reduction in bioluminescence intensity was recorded in the γ -mangostin-treated cohort relative to the control subjects (**Figure 1l**). Furthermore, the application of γ -mangostin significantly extended the overall

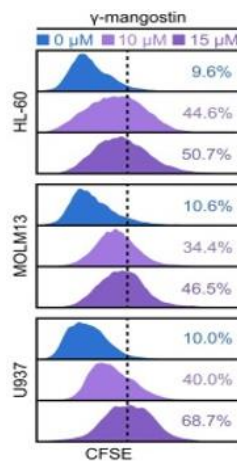
survival of the leukemic mice (**Figure 1m**). Taken together, these findings indicated the promising therapeutic potential of γ -mangostin for the treatment of acute myeloid leukemia (AML).

γ -mangostin induces immunogenic cell death and stimulates cGAS signaling in leukemic models

We subsequently explored the underlying mechanisms by which γ -mangostin restricts the viability of human leukemic cells. The findings demonstrated that γ -mangostin constrained the proliferative capacity of HL-60, MOLM13, and U937 cellular models in a dose-responsive fashion (**Figures 2a and 2b**). Importantly, this compound exhibited minimal cytotoxic effects on peripheral blood mononuclear cells (PBMCs) isolated from healthy individuals. Morphological examinations corroborated these findings, highlighting the appearance of nuclear fragmentation (**Figure 2c**). Flow cytometric evaluation using Annexin V-PI staining confirmed the induction of apoptosis across cell populations exposed to the specified concentrations of γ -mangostin (**Figures 2d and 2e**). Furthermore, the compound substantially elevated both the release of lactate dehydrogenase (LDH) and the enzymatic activity of Caspase 3 across the evaluated cell lines (**Figures 2f and 2g**). Treatment with γ -mangostin also upregulated the protein levels of cleaved Caspase 3 and PARP; notably, exposure to a 20 μ M concentration prompted a pronounced elevation in the expression of both apoptotic markers (**Figure 2h**).



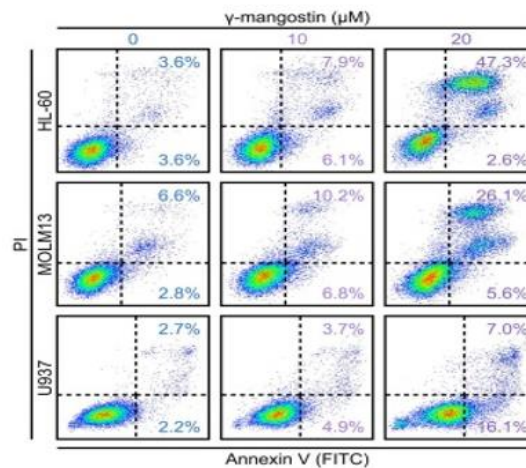
a)



b)



c)



d)

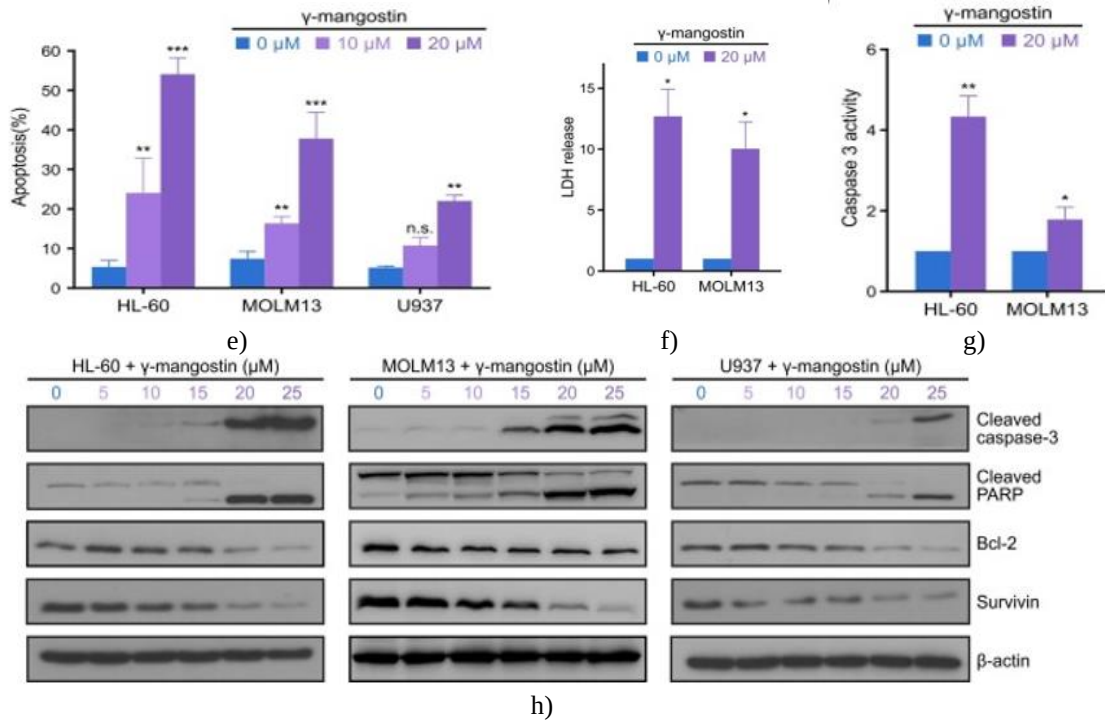
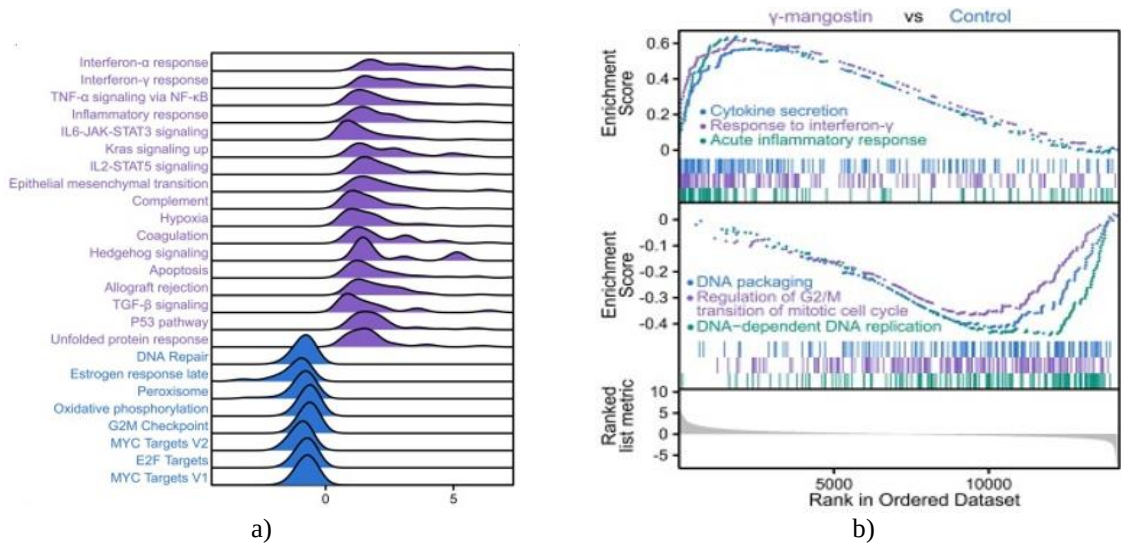


Figure 2. γ -mangostin promotes apoptotic cell death within human leukemic models. (a) Cellular proliferation was evaluated via a CCK-8 assay following a 24 h incubation with various concentrations of γ -mangostin. * $p < 0.05$, *** $p < 0.001$. Additional cellular evaluations included a CFSE tracking assay (b), Wright-Giemsa morphological staining (c), flow cytometric Annexin V-PI analysis (d, e), and quantification of LDH release (f). * $p < 0.05$, $p < 0.01$, *** $p < 0.001$. (g) Enzymatic activity of Caspase 3 was quantified in HL-60 and MOLM13 cell lines post- γ -mangostin treatment. * $p < 0.05$, $p < 0.01$. (h) Cellular lysates were generated to assess the expression levels of key apoptosis-associated proteins utilizing western blot methodologies.

Subsequent transcriptomic evaluation via RNA-Seq revealed that γ -mangostin substantially altered the global gene expression profile relative to untreated controls, which is illustrated in the corresponding heatmap. A ridge plot visualizing the enriched GSEA hallmark pathways indicated a marked upregulation of apoptotic cascades and interferon (IFN) signaling networks within the γ -mangostin-treated cohorts (**Figure 3a**). Furthermore, GSEA-GO assessments demonstrated a significant enhancement in gene sets associated with cytokine release and IFN responsiveness, alongside a concurrent downregulation in pathways governing DNA replication and chromosomal packaging (**Figure 3b**).



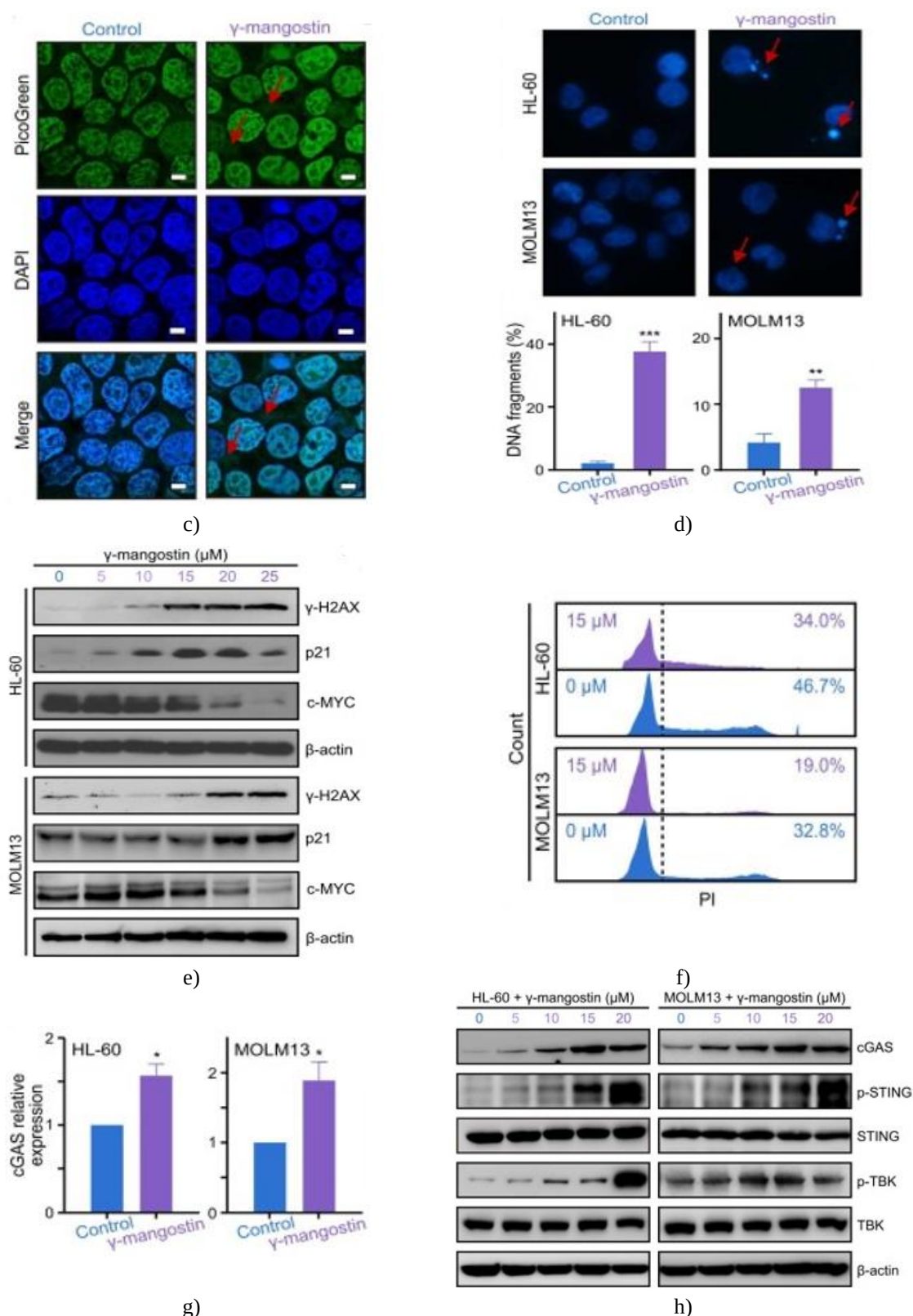


Figure 3. γ -mangostin provokes structural DNA damage and stimulates the cGAS/STING signaling cascade. Transcriptomic profiling was conducted via RNA-Seq on HL-60, MOLM13, and U937 cells following a 24 h exposure to γ -mangostin. Pathway enrichment was evaluated utilizing ridge plots for GSEA hallmarks (a) as well as GSEA-GO categorizations (b). (c) PicoGreen fluorescent labeling was utilized to visualize HL-60 cells post-treatment with 10 μ M γ -mangostin for 24 h. The presence of cytosolic DNA is denoted by arrows. Scale bar = 5 μ m. (d) The generation of DNA fragments in cells treated with 15 μ M γ -mangostin was assessed using Hoechst 33342 staining. $p < 0.01$, *** $p < 0.001$. Cells exposed to γ -mangostin were

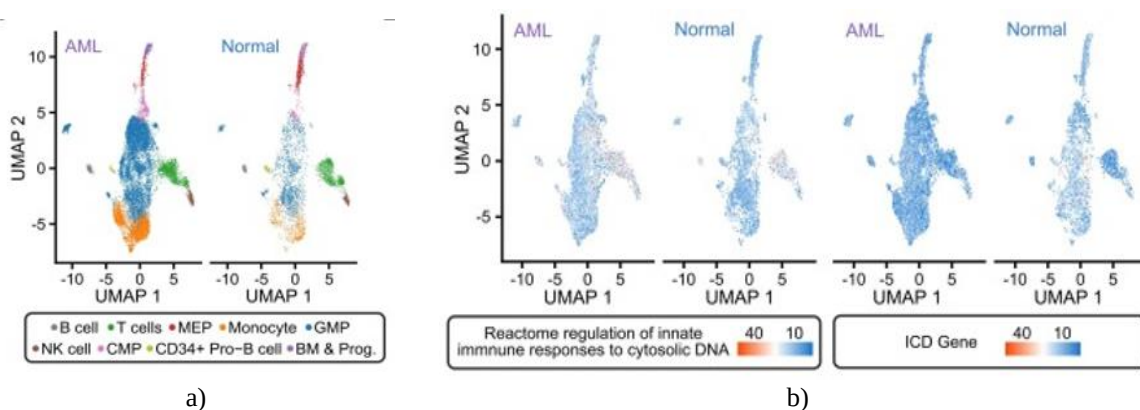
subsequently analyzed for the expression of γ -H2AX, p21, and c-MYC (e), alongside evaluations of cell cycle distribution (f). (g) Relative mRNA levels of cGAS were quantified in HL-60 and MOLM13 cells following treatment with 20 μ M γ -mangostin. * $p < 0.05$. (h) Western blot analyses of prepared cell lysates were performed to probe the components of the cGAS signaling pathway.

Notably, our observations indicated that even at reduced concentrations, γ -mangostin facilitated the accumulation of cytosolic DNA and cytoplasmic chromatin fragmentation (**Figures 3c and 3d**). Furthermore, western blot evaluations verified the upregulation of γ -H2AX following γ -mangostin treatment, serving as a primary indicator for the initiation of a DNA damage response (**Figure 3e**). Prior literature has established that the induction of DNA damage frequently leads to elevated p21 expression within neoplastic cells [21, 22]. Consistent with this, exposure to γ -mangostin amplified the protein levels of p21 while simultaneously suppressing its downstream target, c-MYC (**Figure 3e**). Additionally, we observed a pronounced cell cycle arrest at the G0/G1 phase among viable cell populations following γ -mangostin treatment (**Figure 3f**), an effect likely driven by the concurrent DNA damage signaling and subsequent p21 activation.

The cGAS protein, serving as a primary sensory molecule for cytosolic DNA, operates to impede DNA repair mechanisms, thereby hastening genomic instability and subsequent cellular demise [23]. Consequently, we proceeded to evaluate the activation status of the cGAS signaling axis following exposure to γ -mangostin. As anticipated, treatment resulted in an elevation of both the transcript and protein levels of cGAS, alongside the corresponding activation of its downstream effectors, STING and TBK (**Figures 3g and 3h**). Interestingly, in addition to stimulating these innate immune pathways, γ -mangostin provoked the upregulation of distinct markers of immunogenic cell death (ICD), notably HSP90B1, ANXA1, and IL1B. Collectively, these findings strongly suggest that γ -mangostin possesses the capacity to drive ICD within human leukemic models.

Chemokine production is induced by γ -mangostin via cGAS signaling

To evaluate the dynamic immunological alterations following γ -mangostin administration, the immune profile of individuals with acute myeloid leukemia (AML) was initially examined. Single-cell RNA sequencing data from the GSE116256 dataset, comprising 16 AML patients and 4 healthy controls, was analyzed. Following gene expression normalization and principal component analysis (PCA), cells were categorized into nine distinct lineages utilizing specific marker genes (**Figure 4a**). The distribution of these cell lineages differed substantially between healthy and leukemic cells. According to marker expression profiles, bone marrow (BM) cells from individuals with AML demonstrated significant clustering within the granulocyte-monocyte progenitor (GMP) population relative to healthy controls (**Figure 4a**). Furthermore, GMPs derived from both healthy donors and AML patients exhibited unique dominant gene expression signatures. Additionally, while a cytosolic-DNA immune response was detected in AML-derived GMPs, these cells presented diminished expression of immunogenic cell death (ICD)-related genes (**Figures 4a and 4b**), suggesting that the induction of ICD may represent a viable therapeutic approach for AML.



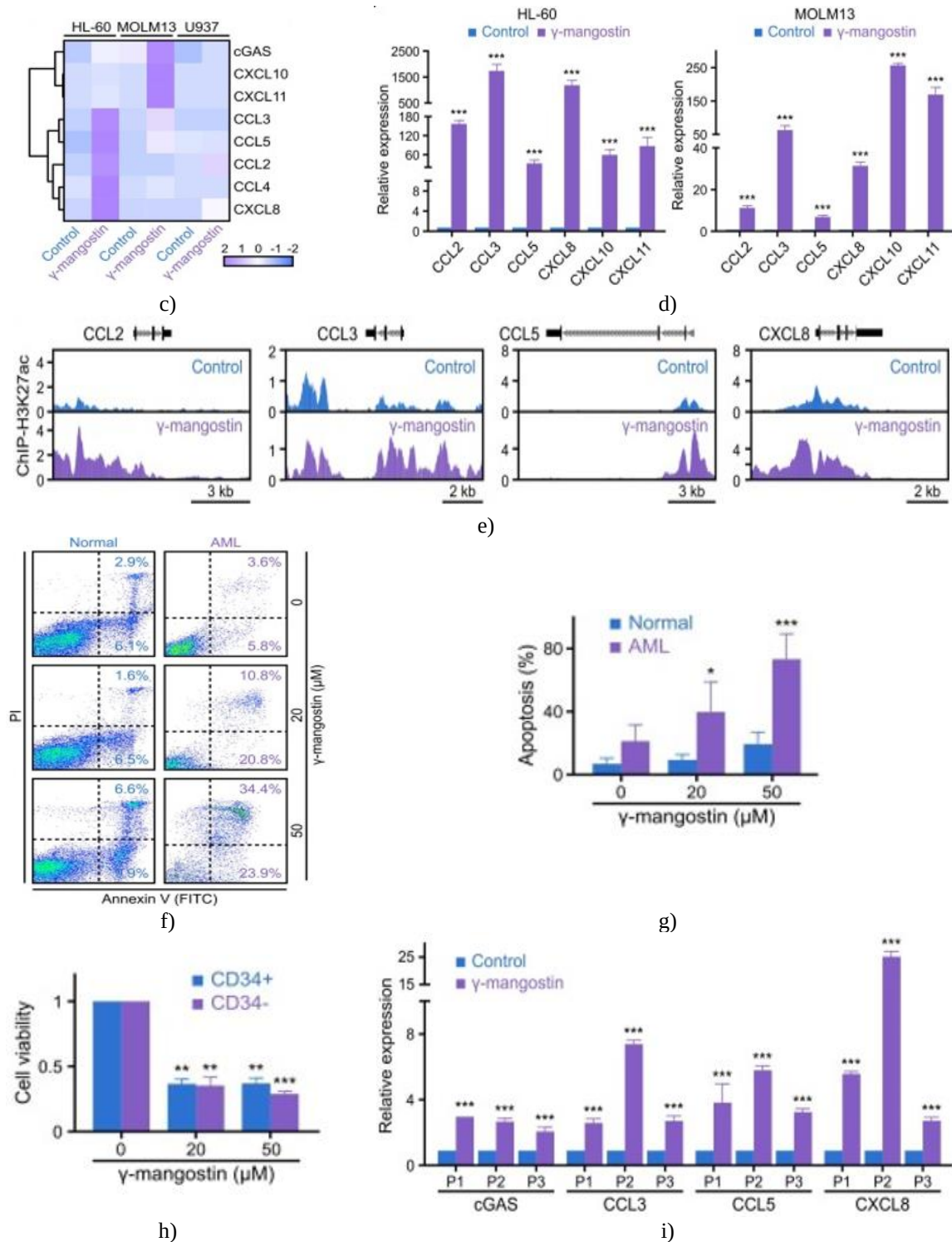


Figure 4. Chemokine expression is upregulated by γ -mangostin. (a) UMAP projection illustrating various cell populations derived from 16 AML patients and 4 healthy controls (GSE116256). (b) UMAP representations of cell populations linked to ICD gene expression and the cytosolic-DNA immune response (GSE116256). (c) RNA-Seq-derived heatmap displaying the expression of cGAS and chemokines across three evaluated cell lines. (d) Assessment of chemokine expression via real-time PCR. *** $p < 0.001$. (e) H3K27ac binding peaks at chemokine promoters identified through ChIP-Seq analysis in HL-60 cells. (f, g) Annexin V-PI apoptosis assays conducted on bone marrow cells sourced from 3 healthy controls and 10 AML patients following a 24-hour incubation with specified concentrations of γ -mangostin. * $p < 0.05$, *** $p < 0.001$. (h) Proliferation analysis of leukemic blasts (CD34+ and CD34- populations, $n = 3$) treated with

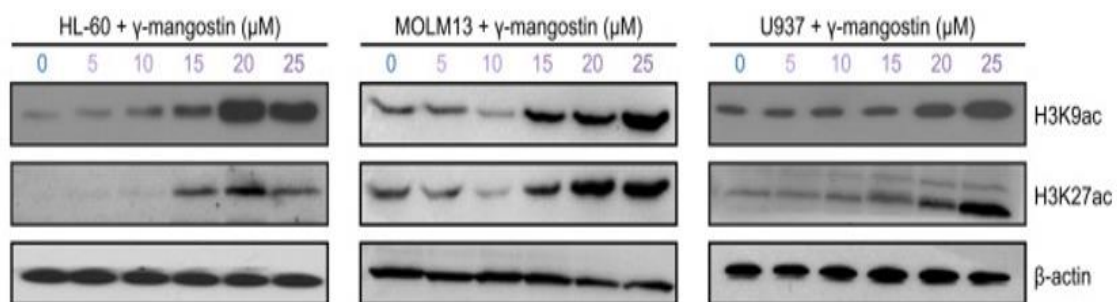
varying doses of γ -mangostin for 24 hours. $p < 0.01$, *** $p < 0.001$. (I) Real-time PCR evaluation of chemokine expression in patient-derived leukemia blasts. *** $p < 0.001$.

Subsequently, RNA-Seq validation of the heatmap corresponding to cGAS and ICD-associated chemokines demonstrated a marked upregulation following exposure to γ -mangostin (**Figure 4c**). Real-time PCR analysis corroborated the elevated expression levels of CCL2, CCL3, CCL5, CXCL8, CXCL10, and CXCL11 in HL-60 and MOLM13 cell lines (**Figure 4d**), mirroring the observations in bone marrow cells isolated from MLL-AF9 leukemic murine models. Prior studies have indicated that the upregulation of CCL chemokines necessitates an accumulation of histone acetylation within their promoter regions [7, 24]. Consequently, ChIP-Seq analysis utilizing an anti-H3K27ac antibody was conducted to evaluate histone acetylation status at these chemokine promoters. The findings confirmed that administration of γ -mangostin promoted the enrichment of histone H3 acetylation at the promoters of specific chemokines, including CCL2, CCL3, CCL5, and CXCL8 (**Figure 4e**). This provides further evidence that the γ -mangostin-induced enhancement of chemokine expression is driven by a combination of histone acetylation and cGAS pathway activation.

The capacity of γ -mangostin to eliminate primary leukemic blasts via ICD was subsequently investigated. Subjecting primary leukemic blasts to γ -mangostin for a 24-hour period triggered substantial apoptosis. Conversely, this natural compound caused negligible cytotoxicity in healthy bone marrow cells (**Figures 4f and 4g**), highlighting its favorable safety profile. Furthermore, γ -mangostin profoundly reduced the viability of both CD34⁺ and CD34⁻ fractions within primary leukemic cell populations (**Figure 4h**). Importantly, transcriptional levels of cGAS were upregulated in primary leukemic blasts following γ -mangostin treatment (**Figure 4i**). In alignment with the *in vitro* cell line data, the expression of CCL3, CCL5, and CXCL8 was significantly augmented in γ -mangostin-treated primary leukemic cells ($n = 7$); (**Figure 4i**). Therefore, these findings reinforce the hypothesis that γ -mangostin effectively eradicates primary leukemic cells and stimulates chemokine production through the initiation of ICD.

γ -mangostin inhibits the expression of HDAC4

The epigenetic activation of immune and inflammatory genes is mediated through histone modifications [7, 24, 25], and exposure to γ -mangostin resulted in H3K27ac enrichment at chemokine promoter sites (**Figure 4e**). Notably, previous research has shown that γ -mangostin elevates α -tubulin acetylation in MCF-7 and MDA-MB-231 breast carcinoma cell lines [26]. Consequently, the potential influence of γ -mangostin on broader epigenetic mechanisms was explored. As illustrated in **Figure 5a**, γ -mangostin significantly promoted the acetylation of histone H3. To determine if this alteration in histone H3 acetylation was linked to histone deacetylases (HDACs), homology modeling of the HDAC family was constructed. Subsequent molecular dynamics refinement and computational docking revealed that γ -mangostin acts as a broad inhibitor across the HDAC family. Among the various HDAC isoforms, γ -mangostin demonstrated a particularly strong binding affinity for HDAC4. Therefore, the specific modulatory interaction between γ -mangostin and HDAC4 warranted deeper investigation.



a)

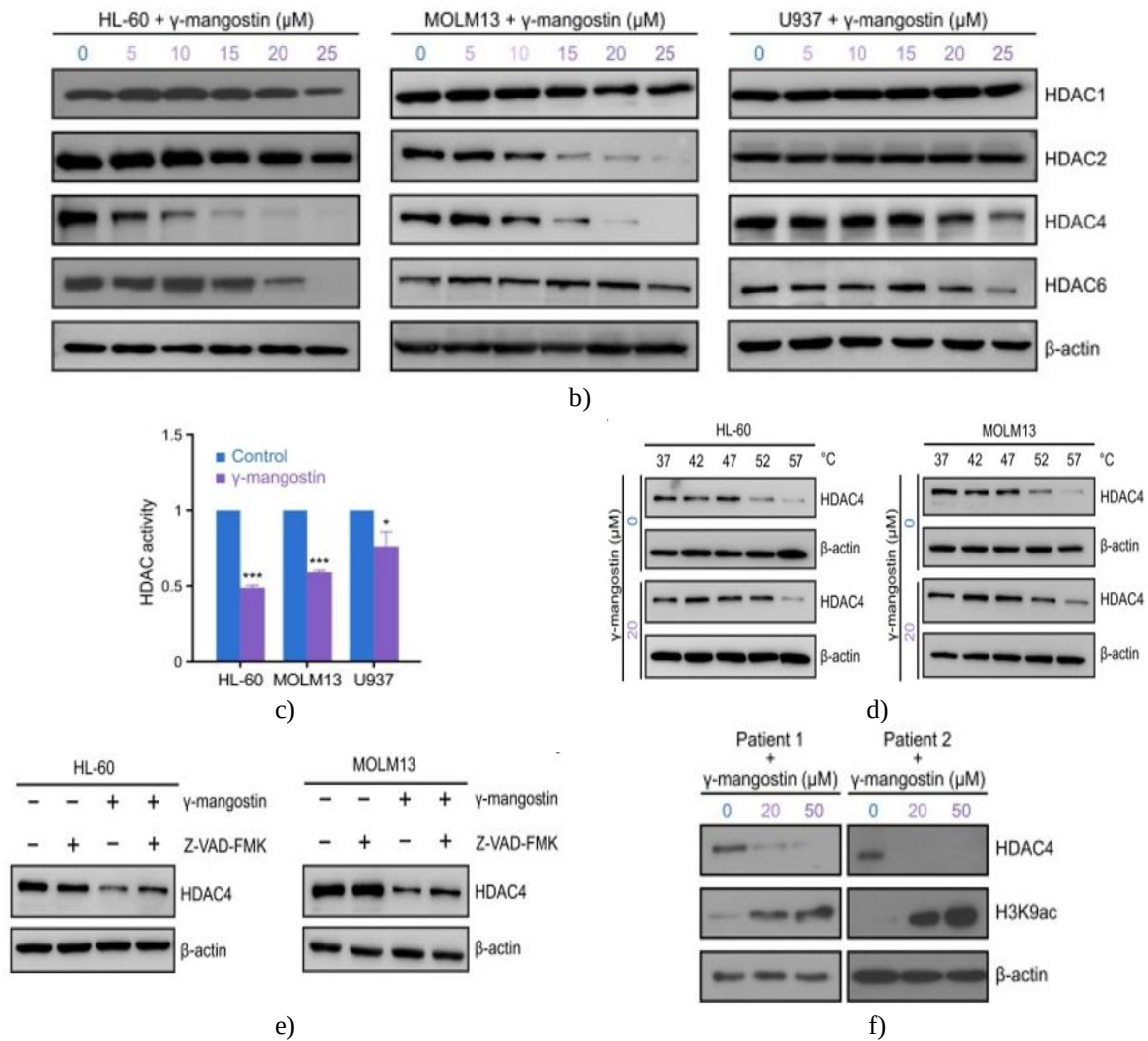
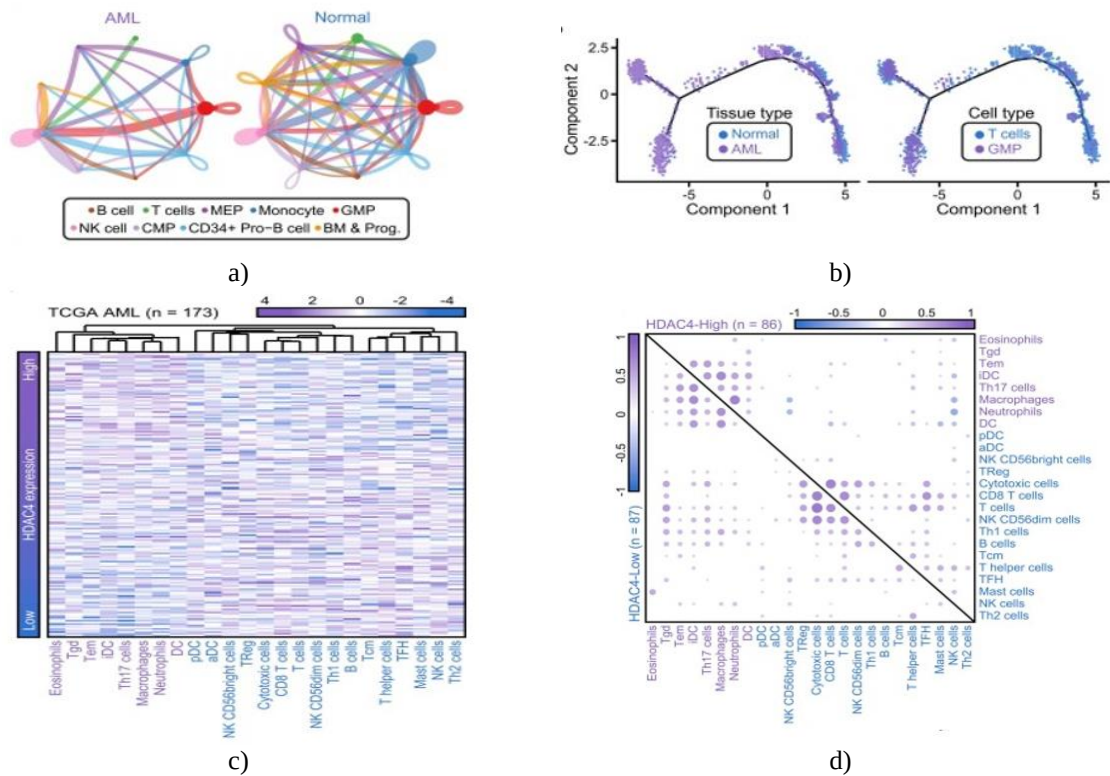


Figure 5. Downregulation of HDAC4 expression by γ -mangostin. (a) Western blot evaluation of histone H3 acetylation in cells exposed to specified concentrations of γ -mangostin for a 24-hour period. HL-60, MOLM13, and U937 cells were incubated with designated doses of γ -mangostin for 24 hours, followed by the assessment of HDAC expression via western blotting (b) and the measurement of overall HDAC enzymatic activity (γ -mangostin: 0 and 20 μ M) (c). * p <0.05, *** p <0.001. (d) Cellular models were administered either the vehicle control or 20 μ M γ -mangostin (HL-60: 1 hour and MOLM13: 2 hours). Following collection and washing steps, the cell suspensions containing the vehicle or γ -mangostin underwent temperature treatments for 3 minutes, stabilized at room temperature for another 3 minutes, and the resulting lysates were analyzed by western blot. (e) Western blot analysis of cells treated with 15 μ M γ -mangostin and/or 10 μ M Z-VAD-FMK for the specified durations (HL-60: 8 hours; MOLM13: 24 hours). (f) Western blot profiling of H3K9ac and HDAC4 levels in primary blasts derived from two leukemic patients.

The downregulation of HDACs mediated by γ -mangostin was further validated utilizing western blot techniques. As depicted in **Figure 5b**, γ -mangostin specifically diminished HDAC4 levels in leukemic cells, exerting minimal impact on alternative HDAC isoforms. Comparable outcomes were observed in K562 cells, as well as in cells transformed with FLT3-ITD or BCR-ABL. Furthermore, a colorimetric assay revealed that overall HDAC enzymatic activity was markedly suppressed by γ -mangostin in HL-60, MOLM13, and U937 cell lines (**Figure 5c**). Consistent with computational predictions, γ -mangostin demonstrated physical binding to HDAC4 within leukemic cellular models (**Figure 5d**), confirming a direct protein-compound interaction. The addition of 10 μ M of the caspase inhibitor Z-VAD-FMK successfully blocked the degradation of HDAC4 (**Figure 5e**), proving that γ -mangostin initiates HDAC4 breakdown through a caspase-dependent degradation pathway. In agreement with these findings, primary leukemic blasts exhibited a concurrent decrease in HDAC4 expression and an elevation in histone H3 acetylation upon γ -mangostin treatment (**Figure 5f**).

CD8⁺ T cell activation within the tumor microenvironment is triggered by γ -mangostin

Intercellular communication analysis derived from single-cell RNA-seq data indicated a profound functional disruption of immune cells in AML patients (**Figure 6a**). Trajectory analysis was subsequently utilized to evaluate the transcriptomic transitions of these immune populations. As expected, individuals with AML exhibited an overrepresentation of GMPs, whereas healthy controls presented higher frequencies of T cells (**Figure 6b**). This implies that the dysregulation of T cells may serve as a critical factor in the progression of AML. To investigate the association between T cell populations and HDAC4 in the context of AML, single-sample gene set enrichment analysis (ssGSEA) was executed utilizing The Cancer Genome Atlas (TCGA) dataset. The results established that patients with elevated HDAC4 expression demonstrated a diminished enrichment of CD8⁺ T cells (**Figure 6c**). While the general correlations among immune cell types were relatively similar regardless of HDAC4 status, both natural killer (NK) cells and CD8⁺ T cells showed a significant enrichment in patient cohorts characterized by low HDAC4 expression (**Figures 6d and 6e**). Subsequently, the relationship between HDAC4 levels and tumor microenvironment (TME) cellular infiltration was detailed. As illustrated in **Figure 6f**, cohorts with low HDAC4 expression exhibited a more robust infiltration of macrophages and NK56dim cells relative to the high-expression group. Importantly, within the elevated HDAC4 cohort, HDAC4 levels demonstrated a pronounced negative correlation with the infiltration of CD8⁺ T cells and NK cells, suggesting a potential immunosuppressive role for HDAC4 against T and NK cell populations in AML patients.



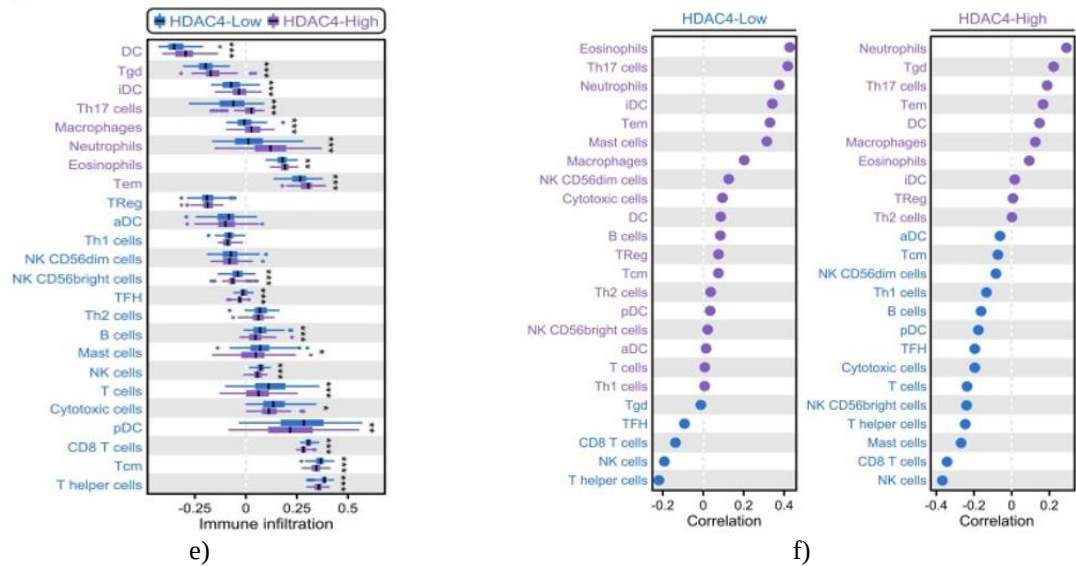
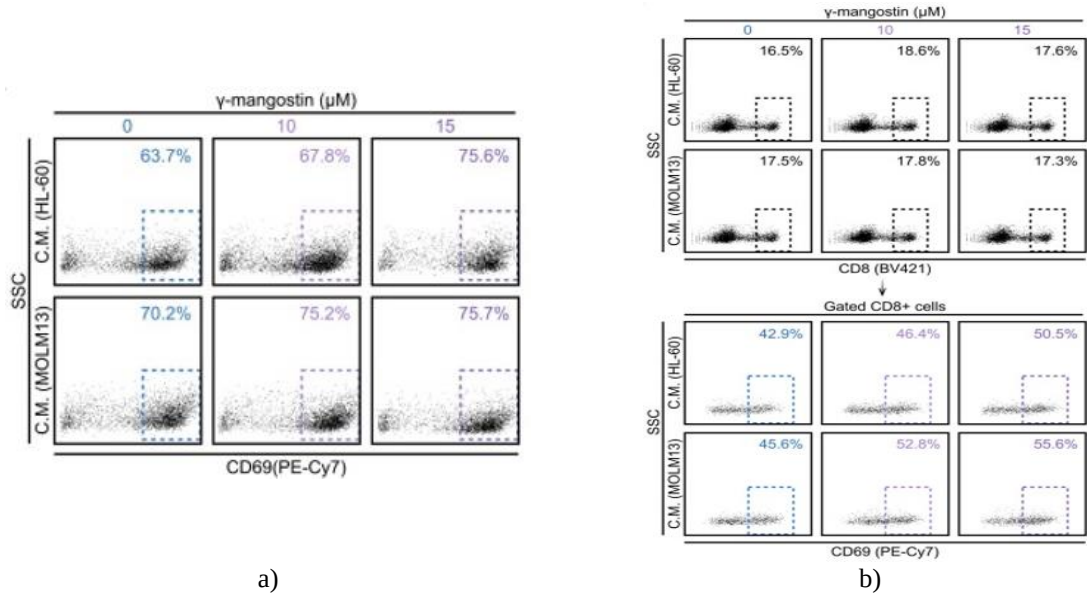


Figure 6. Analysis of the association between HDAC4 expression and T cell infiltration. (a) Intercellular communication networks among immune cells derived from healthy controls and AML patients (GSE116256). (b) Monocle 2-based trajectory mapping delineating sample origins (left) and cell types (right) across healthy donors and AML subjects (GSE116256). (c) Evaluation of immune cell infiltration profiles in cohorts with high versus low HDAC4 expression via ssGSEA. (d) Spearman analysis assessing the correlation between distinct infiltrating immune cell signatures. (e) Quantification of the relative abundance of specific infiltrating cell populations stratified by high and low HDAC4 expression. (f) ssGSEA-derived correlation analysis between individual infiltrating immune subsets and HDAC4 levels within the low and high expression groups.

Given the essential role of chemokines in orchestrating T cell development, preservation, and effector functions [27], it was hypothesized that the γ -mangostin-mediated pharmacological blockade of HDAC4 might counteract T cell suppression within the AML microenvironment. Consequently, exposure to conditioned medium (CM) harvested from γ -mangostin-treated leukemic cells successfully reactivated Jurkat cells and CD8⁺ T cells derived from peripheral blood mononuclear cells (PBMCs), as evidenced by the upregulation of the activation marker CD69 (**Figures 7a and 7c**). Crucially, this treatment also enhanced both the recruitment capacity and the proliferation of the PBMCs (**Figures 7d and 7e**). These findings suggest that the chemokine induction elicited by γ -mangostin likely facilitates the reactivation of CD8⁺ T cells, thereby amplifying the compound's anti-leukemic efficacy.



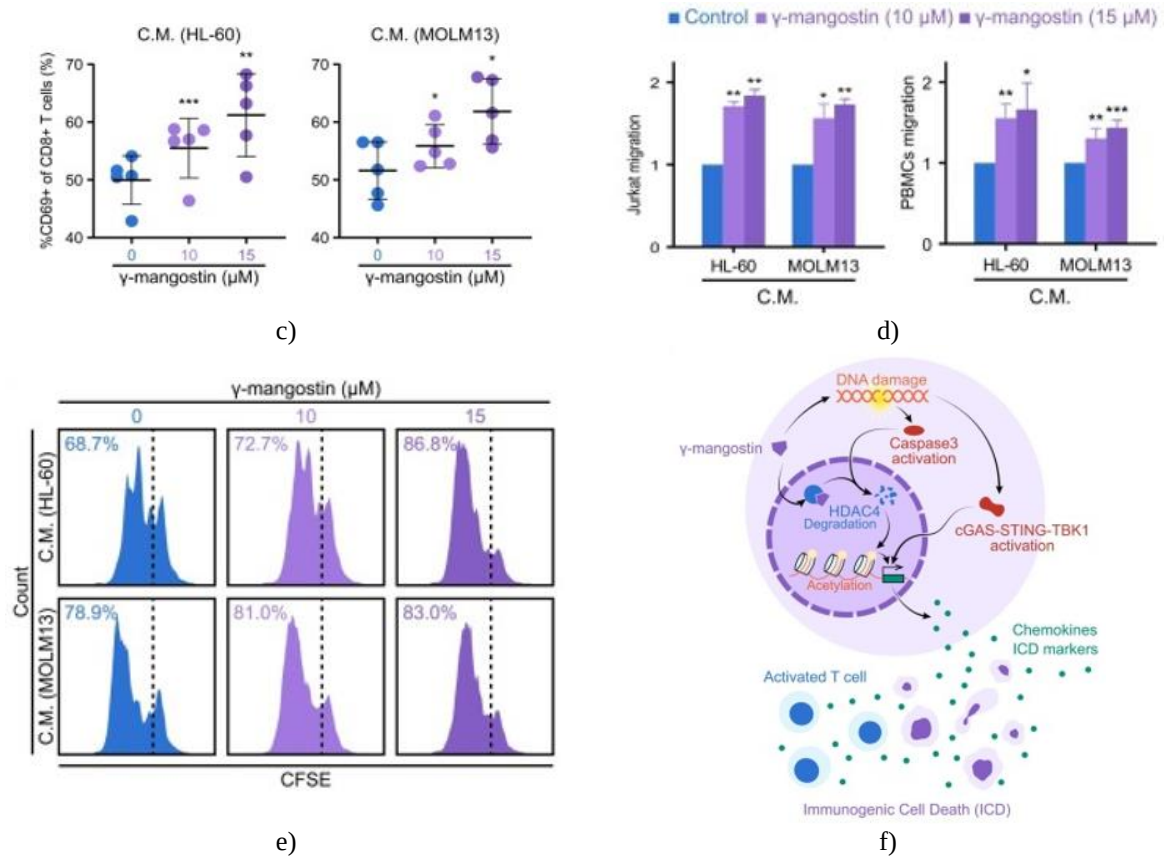


Figure 7. T cell activation within the microenvironment is stimulated by γ -mangostin. (a) Activation of Jurkat cells triggered by γ -mangostin-conditioned medium (CM), displaying representative data from a single experiment. (b) Representative data and (c) cumulative statistical analysis ($n = 5$) illustrating CD8+ T cell activation induced by γ -mangostin CM. * $p < 0.05$, $p < 0.01$, *** $p < 0.001$. (d) Enhanced chemotactic recruitment of Jurkat cells or PBMCs ($n = 5$) mediated by γ -mangostin, as evaluated via migration assays. * $p < 0.05$, $p < 0.01$, *** $p < 0.001$. (e) CFSE staining demonstrating the pro-proliferative effect of γ -mangostin CM on T cells ($n = 5$), featuring representative PBMC data from a single healthy volunteer. (f) Mechanistic schematic detailing the anti-leukemic action of γ -mangostin: The compound triggers ICD and activates the cGAS signaling cascade. Furthermore, γ -mangostin promotes the targeted degradation of HDAC4, leading to the accumulation of acetylated histone H3 proteins. This epigenetic modification synergizes with cGAS signaling to drive robust chemokine transcription, ultimately reactivating CD8+ T cells within the microenvironment.

Oncological immunotherapy modulates the immune system to generate an anti-tumoral immune reaction. Comprehending the underlying mechanisms or signaling cascades through which anti-cancer agents trigger DAMPs and stimulate immune activity holds promise for advancing effective ICD inducers and enhancing cancer immunotherapy. Importantly, discovering ICD inducers capable of leveraging innate immunity to amplify anti-tumor responses, while also stratifying patients based on their potential therapeutic benefit, would represent a significant clinical advancement [1].

DAMPs, such as nucleic acids, are recognized by the cGAS and stimulator of interferon genes (STING) signaling axis. The cGAS enzyme functions as an innate immune sensor responsible for monitoring cytosolic DNA, including pathogenic microbial DNA as well as endogenous DNA extruded from the nucleus during genomic stress [28-30]. Upon binding to double-stranded DNA, cGAS synthesizes the second messenger cyclic GMP-AMP. This event subsequently triggers downstream innate immunological cascades, ultimately driving the secretion of type I IFNs and various other cytokines [28, 31]. Aberrations within the cGAS pathway are associated with a multitude of pathological conditions, encompassing inflammatory pathologies, infectious diseases, and malignancies [31, 32]. Furthermore, cGAS exerts anti-neoplastic effects by sensing DNA released within malignant cells, thereby facilitating tumor immunogenicity following chemotherapeutic intervention [33, 34],

[35]. Within the current investigation, it was observed that γ -mangostin stimulated both ICD and cGAS pathway activation concurrent with DNA damage (**Figures 3e and 3h**). Primary downstream targets of cGAS signaling, namely CCL3, CCL5, and CXCL8, exhibited marked upregulation in cells subjected to γ -mangostin treatment (**Figures 4d and 4i**). Such chemokines serve as essential factors driving the chemotactic recruitment of CD8⁺ T lymphocytes, which is necessary to achieve optimal anti-tumor activity [36-39]. Additionally, this study demonstrated that the CM derived from γ -mangostin-exposed leukemic cells facilitated the activation, expansion, and recruitment of CD8⁺ T cells (**Figures 7b and 7e**). Corroborating these findings, analysis of TCGA datasets revealed that patient cohorts exhibiting diminished HDAC4 levels possessed greater infiltration of CD8⁺ T cells (**Figure 6e**). Consequently, the pharmacological suppression of HDAC4 by γ -mangostin promotes chemokine secretion, which beneficially modulates the leukemic microenvironment by alleviating CD8⁺ T cell suppression. Given that γ -mangostin induced immunogenic properties within leukemic cells, its underlying mechanistic action was further delineated. The findings indicated that γ -mangostin expedited the formation of cytoplasmic chromatin fragments, which subsequently activated cGAS and culminated in chemokine release. Alternatively, the transcriptional activation of chemokines is dependent upon histone acetylation modifications [7, 24, 25]. As a critical post-translational modification, acetylation governs both protein stability and gene transcription. The direct consequence of histone hyperacetylation, achieved via HDAC suppression, is the facilitated transcription of specific cytokines and chemokines [40]. In this research, γ -mangostin suppressed the enzymatic function of HDAC4 and promoted the accumulation of acetylated histone H3 protein (**Figure 5a**), thereby driving the transcriptional regulation of chemokines. The selectivity and potency of HDAC inhibitors are likely associated with the marked overexpression of HDACs frequently observed in malignant cells. Correspondingly, elevated expression of HDAC4 has been documented across various human solid tumors and leukemias [41-43]. The targeted inhibition of HDAC4 has been shown to impede leukemic cell proliferation, precipitate cell cycle arrest, and drive cells toward apoptosis [44, 45]. The current data suggested that γ -mangostin potentially establishes physical coordinates with the HDAC4 protein (**Figure 5d**). Prior investigations have indicated that HDAC inhibitors can stimulate the proteasome-dependent degradation of HDAC4 [46, 47]. Conversely, alternative reports have proposed that the degradation of HDAC4 does not rely on the proteasome but is instead caspase-dependent [48, 49]. Aligning with the latter, γ -mangostin was found to downregulate HDAC4 expression by initiating its caspase-dependent degradation (**Figure 5e**). Therefore, γ -mangostin likely contributes to HDAC inhibitory activity by structurally altering HDAC4 to expedite its degradation.

LPCs often endure rigorous chemotherapeutic regimens, ultimately driving recurrent disease. The well-documented oncogenes MLL-AF9 and BCR-ABL are recognized for imparting LPC characteristics and instigating leukemia in mice, rendering these *in vivo* models robust read-outs for studying leukemogenesis [50, 51]. In this investigation, γ -mangostin substantially attenuated the proliferative capacity of progenitor cells positive for MLL-AF9 or BCR-ABL (**Figures 1c and 1i**). Notably, γ -mangostin treatment proved highly efficacious against MLL-AF9-induced leukemia, significantly extending the survival of the leukemic mice (**Figure 1g**). Earlier research has reported that AML CD34⁺/CD38⁻ progenitor cells exhibit inherent chemoresistance coupled with reduced immunogenicity [52]. The present results demonstrated that γ -mangostin successfully suppressed not only CD34⁻ but also CD34⁺ leukemic cells (**Figure 4h**). More importantly, while γ -mangostin triggered apoptosis in leukemic blasts, its cytotoxic impact on normal cells was negligible (**Figure 4g**). Consequently, γ -mangostin exhibits a preferential lethality toward leukemic cells while demonstrating diminished toxicity against normal hematopoietic bone marrow cells, thereby mitigating drug-related myelosuppression.

Conclusion

In summary, the aggregated data reveal that γ -mangostin triggers ICD, which subsequently leads to the activation of cGAS signaling across both established leukemic cell lines and primary leukemic blasts. Evidence is also presented suggesting that γ -mangostin functions as a potential epigenetic modulator; this mechanism operates in tandem with cGAS activation to ultimately enhance the secretion of chemokines, facilitating the activation of CD8⁺ T cells within the microenvironment (**Figure 7f**). Such discoveries open novel therapeutic possibilities for leveraging γ -mangostin-mediated ICD in the treatment of leukemia. Furthermore, γ -mangostin holds potential utility as a dietary supplement, presenting a novel approach for cancer prevention.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Barrett AJ. Acute myeloid leukaemia and the immune system: implications for immunotherapy. *Br J Haematol.* 2020;188(2):147-58.
2. Ahmed A, Tait SWG. Targeting immunogenic cell death in cancer. *Mol Oncol.* 2020;14(12):2994-3006.
3. Cheng Y, He C, Wang M, Ma X, Mo F, Yang S, et al. Targeting epigenetic regulators for cancer therapy: mechanisms and advances in clinical trials. *Signal Transduct Target Ther.* 2019;4:62.
4. Fischer F, Alves Avelar LA, Murray L, Kurz T. Designing HDAC-PROTACs: lessons learned so far. *Future Med Chem.* 2022;14(2):143-66.
5. Wang X, Waschke BC, Woolaver RA, Chen SMY, Chen Z, Wang JH. HDAC inhibitors overcome immunotherapy resistance in B-cell lymphoma. *Protein Cell.* 2020;11(7):472-82.
6. Conte M, De Palma R, Altucci L. HDAC inhibitors as epigenetic regulators for cancer immunotherapy. *Int J Biochem Cell Biol.* 2018;98:65-74.
7. Liu X, Lu Y, Zhu J, Liu M, Xie M, Ye M, et al. A long noncoding RNA, antisense IL-7, promotes inflammatory gene transcription through facilitating histone acetylation and switch/sucrose nonfermentable chromatin remodeling. *J Immunol.* 2019;203(6):1548-59.
8. Sheikh TN, Chen X, Xu X, McGuire JT, Ingham M, Lu C, et al. Growth inhibition and induction of innate immune signaling of chondrosarcomas with epigenetic inhibitors. *Mol Cancer Ther.* 2021;20(12):2362-71.
9. Pinto MM, Sousa ME, Nascimento MS. Xanthone derivatives: new insights in biological activities. *Curr Med Chem.* 2005;12(21):2517-38.
10. Akao Y, Nakagawa Y, Iinuma M, Nozawa Y. Anti-cancer effects of xanthenes from pericarps of mangosteen. *Int J Mol Sci.* 2008;9(3):355-70.
11. Nauman MC, Johnson JJ. The purple mangosteen (*Garcinia mangostana*): defining the anticancer potential of selected xanthenes. *Pharmacol Res.* 2022;175:106032.
12. Wu AT, Yeh YC, Huang YJ, Mokgautsi N, Lawal B, Huang TH. Gamma-mangostin isolated from *Garcinia mangostana* suppresses colon carcinogenesis and stemness by downregulating the GSK3 β / β -catenin/CDK6 cancer stem pathway. *Phytomedicine.* 2022;95:153797.
13. Webb B, Sali A. Protein structure modeling with MODELLER. *Methods Mol Biol.* 2014;1137:1-15.
14. Liu Y, Zhao L, Li W, Zhao D, Song M, Yang Y. FIPSDock: a new molecular docking technique driven by fully informed swarm optimization algorithm. *J Comput Chem.* 2013;34(1):67-75.
15. Pronk S, Páll S, Schulz R, Larsson P, Bjelkmar P, Apostolov R, et al. GROMACS 4.5: a high-throughput and highly parallel open source molecular simulation toolkit. *Bioinformatics.* 2013;29(7):845-54.
16. van Galen P, Hovestadt V, Wadsworth MH II, Hughes TK, Griffin GK, Battaglia S, et al. Single-cell RNA-Seq reveals AML hierarchies relevant to disease progression and immunity. *Cell.* 2019;176(6):1265-81.e24.
17. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS.* 2012;16(5):284-7.
18. Aran D, Hu Z, Butte AJ. xCell: digitally portraying the tissue cellular heterogeneity landscape. *Genome Biol.* 2017;18:220.
19. Garg AD, De Ruyscher D, Agostinis P. Immunological metagene signatures derived from immunogenic cancer cell death associate with improved survival of patients with lung, breast or ovarian malignancies: a large-scale meta-analysis. *Oncoimmunology.* 2015;5(2):e1069938.
20. Pont F, Tosolini M, Fournié JJ. Single-cell signature explorer for comprehensive visualization of single cell signatures across scRNA-seq datasets. *Nucleic Acids Res.* 2019;47(21):e133.
21. Rong JJ, Hu R, Song XM, Ha J, Lu N, Qi Q, et al. Gambogic acid triggers DNA damage signaling that induces p53/p21(Waf1/CIP1) activation through the ATR-Chk1 pathway. *Cancer Lett.* 2010;296(1):55-64.
22. Nicolae CM, O'Connor MJ, Constantin D, Moldovan GL. NF κ B regulates p21 expression and controls DNA damage-induced leukemic differentiation. *Oncogene.* 2018;37(27):3647-56.

23. Jiang H, Xue X, Panda S, Kawale A, Hooy RM, Liang F, et al. Chromatin-bound cGAS is an inhibitor of DNA repair and hence accelerates genome destabilization and cell death. *EMBO J.* 2019;38(22):e102718.
24. Kiguchi N, Kobayashi Y, Saika F, Kishioka S. Epigenetic upregulation of CCL2 and CCL3 via histone modifications in infiltrating macrophages after peripheral nerve injury. *Cytokine.* 2013;64(3):666-72.
25. Saccani S, Pantano S, Natoli G. p38-Dependent marking of inflammatory genes for increased NF-kappa B recruitment. *Nat Immunol.* 2002;3(1):69-75.
26. Yeong KY, Khaw KY, Takahashi Y, Itoh Y, Murugaiyah V, Suzuki T. Discovery of gamma-mangostin from *Garcinia mangostana* as a potent and selective natural SIRT2 inhibitor. *Bioorg Chem.* 2020;94:103403.
27. Rahimi RA, Luster AD. Chemokines: critical regulators of memory T cell development, maintenance, and function. *Adv Immunol.* 2018;138:71-98.
28. Li T, Chen ZJ. The cGAS-cGAMP-STING pathway connects DNA damage to inflammation, senescence, and cancer. *J Exp Med.* 2018;215(5):1287-99.
29. Sun L, Wu J, Du F, Chen X, Chen ZJ. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science.* 2013;339(6121):786-91.
30. Härtlova A, Erttmann SF, Raffi FA, Schmalz AM, Resch U, Anugula S, et al. DNA damage primes the type I interferon system via the cytosolic DNA sensor STING to promote anti-microbial innate immunity. *Immunity.* 2015;42(2):332-43.
31. Yum S, Li M, Chen ZJ. Old dogs, new trick: classic cancer therapies activate cGAS. *Cell Res.* 2020;30(8):639-48.
32. Chen Q, Sun L, Chen ZJ. Regulation and function of the cGAS-STING pathway of cytosolic DNA sensing. *Nat Immunol.* 2016;17(10):1142-9.
33. Lu C, Guan J, Lu S, Jin Q, Rousseau B, Lu T, et al. DNA sensing in mismatch repair-deficient tumor cells is essential for anti-tumor immunity. *Cancer Cell.* 2021;39(1):96-108.e6.
34. Sen T, Rodriguez BL, Chen L, Corte CMD, Morikawa N, Fujimoto J, et al. Targeting DNA damage response promotes antitumor immunity through STING-mediated T-cell activation in small cell lung cancer. *Cancer Discov.* 2019;9(5):646-61.
35. Schadt L, Sparano C, Schweiger NA, Silina K, Cecconi V, Lucchiari G, et al. Cancer-cell-intrinsic cGAS expression mediates tumor immunogenicity. *Cell Rep.* 2019;29(5):1236-48.e7.
36. Schaller TH, Batich KA, Suryadevara CM, Desai R, Sampson JH. Chemokines as adjuvants for immunotherapy: implications for immune activation with CCL3. *Expert Rev Clin Immunol.* 2017;13(11):1049-60.
37. Gesser B, Lund M, Lohse N, Vestergaard C, Matsushima K, Sindet-Pedersen S, et al. IL-8 induces T cell chemotaxis, suppresses IL-4, and up-regulates IL-8 production by CD4⁺ T cells. *J Leukoc Biol.* 1996;59(3):407-11.
38. Jin L, Tao H, Karachi A, Long Y, Hou AY, Na M, et al. CXCR1- or CXCR2-modified CAR T cells co-opt IL-8 for maximal antitumor efficacy in solid tumors. *Nat Commun.* 2019;10:4016.
39. Wang Z, Chen J, Hu J, Zhang H, Xu F, He W, et al. cGAS/STING axis mediates a topoisomerase II inhibitor-induced tumor immunogenicity. *J Clin Invest.* 2019;129(11):4850-62.
40. Gatla HR, Muniraj N, Thevkar P, Yavvari S, Sukhvasi S, Makena MR. Regulation of chemokines and cytokines by histone deacetylases and an update on histone deacetylase inhibitors in human diseases. *Int J Mol Sci.* 2019;20(5):1110.
41. Wu H, Liu C, Yang Q, Xin C, Du J, Sun F, et al. MIR145-3p promotes autophagy and enhances bortezomib sensitivity in multiple myeloma by targeting HDAC4. *Autophagy.* 2020;16(4):683-97.
42. Gruhn B, Naumann T, Gruner D, Walther M, Wittig S, Becker S, et al. The expression of histone deacetylase 4 is associated with prednisone poor-response in childhood acute lymphoblastic leukemia. *Leuk Res.* 2013;37(10):1200-7.
43. Gaál Z, Oláh É, Rejtő L, Erdődi F, Csernoch L. Strong correlation between the expression levels of HDAC4 and SIRT6 in hematological malignancies of the adults. *Pathol Oncol Res.* 2017;23(3):493-504.
44. Li XH, Huang ML, Wang SM, Wang Q. Selective inhibition of bicyclic tetrapeptide histone deacetylase inhibitor on HDAC4 and K562 leukemia cell. *Asian Pac J Cancer Prev.* 2013;14(12):7095-100.
45. Kikuchi S, Suzuki R, Ohguchi H, Yoshida Y, Lu D, Cottini F, et al. Class IIa HDAC inhibition enhances ER stress-mediated cell death in multiple myeloma. *Leukemia.* 2015;29(9):1918-27.

46. Griffin EA Jr, Melas PA, Zhou R, Li Y, Mercado P, Kempadoo KA, et al. Prior alcohol use enhances vulnerability to compulsive cocaine self-administration by promoting degradation of HDAC4 and HDAC5. *Sci Adv.* 2017;3(11):e1701682.
47. Du J, Zhang L, Zhuang S, Qin GJ, Zhao TC. HDAC4 degradation mediates HDAC inhibition-induced protective effects against hypoxia/reoxygenation injury. *J Cell Physiol.* 2015;230(6):1321-31.
48. Liu F, Dowling M, Yang XJ, Kao GD. Caspase-mediated specific cleavage of human histone deacetylase 4. *J Biol Chem.* 2004;279(33):34537-46.
49. Zhou J, Li P, Chen Q, Wei X, Zhao T, Wang Z, et al. Mitogen-activated protein kinase p38 induces HDAC4 degradation in hypertrophic chondrocytes. *Biochim Biophys Acta.* 2015;1853(2):370-6.
50. Yuan H, Wang Z, Li L, Zhang H, Modi H, Horne D, et al. Activation of stress response gene SIRT1 by BCR-ABL promotes leukemogenesis. *Blood.* 2012;119(8):1904-14.
51. Stavropoulou V, Kaspar S, Brault L, Sanders MA, Juge S, Morettini S, et al. MLL-AF9 expression in hematopoietic stem cells drives a highly invasive AML expressing EMT-related genes linked to poor outcome. *Cancer Cell.* 2016;30(1):43-58.
52. Costello RT, Mallet F, Gaugler B, Sainty D, Arnoulet C, Gastaut JA, et al. Human acute myeloid leukemia CD34⁺/CD38⁻ progenitor cells have decreased sensitivity to chemotherapy and Fas-induced apoptosis, reduced immunogenicity, and impaired dendritic cell transformation capacities. *Cancer Res.* 2000;60(16):4403-11.