Supplementary information

Materials and methods

Cell lines and cell culture

The human multiple myeloma (MM) cell line NCI-H929 and the human plasmacytoma-derived cell line ARP-1 were cultured in RPMI-1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (Biological Industries, Israel) and 1% penicillin–streptomycin (Beyotime Biotechnology, China) at 37 °C in a humidified atmosphere containing 5% CO_2. The cytogenetic background of H929 is characterized by a t(4;14) translocation, whereas ARP-1 harbors a t(11;14) translocation. Cell lines were routinely authenticated by short tandem repeat profiling and tested negative for Mycoplasma infection.

Reagents

Carfilzomib, WYC-209, Ixazomib, Daratumumab, Pomalidomide, BMS961, and SR11237 were purchased from TargetMol (USA). ATRA, Bortezomib, Lenalidomide, Melphalan, Isotretinoin, Alitretinoin, AHPN, and Palovarotene were obtained from MedChemExpress (USA). DMSO, Dexamethasone, Cyclophosphamide, 5-Azacytidine, Acitretin, Bexarotene, Tamibarotene, Adapalene, Tazarotene, Trifarotene, and TTNPB were purchased from AmBeed (USA).

CRISPR-mediated gene knockout

The lentiCRISPR v2 vector (Addgene #52961) was used for human ZMYND8

knockout. Specific sgRNA sequences targeting ZMYND8 were cloned into the vector according to the manufacturer's instructions. Lentivirus was packaged in HEK293T cells and used to infect H929 and ARP-1 cell lines. Transduced cells were selected with puromycin (2 μg/mL). The guiding oligonucleotide sequences were sgZMYND8-1: TTTACGTGGAGAACATCCGC; sgZMYND8-2: CGTAGAGCACAGCGATTCGG.

Quantitative real-time PCR (qRT-PCR) analysis

Total RNA was extracted using SparkZol Reagent (Shandong Sparkjade Biotechnology Co., Ltd., China), and the RNA concentration was measured using a NanoDrop ONE spectrophotometer (Thermo Scientific, USA). Subsequently, cDNA was synthesized from the RNA samples using the HiScript II Q RT SuperMix for qPCR (+gDNA wiper) Kit (Vazyme Biotech, China). PCR experiments were carried out with AceQ qPCR SYBR Green Master Mix (Vazyme Biotech, China) on a StepOnePlus™ Real-Time PCR System (Thermo Fisher Scientific, USA). The expression levels of target genes were normalized to GAPDH and calculated using a $2^{-\Delta\Delta CT}$ method. All reactions were performed in three independent biological replicates. The primer sequences used in this study are listed below:

Gene symbol	Forward primer (5'-3')	Reverse primer (5'-3')
GAPDH	CGGAGTCAACGGATTTGGTC	TTCCCGTTCTCAGCCTTGAC
ZMYND8	TGCATCCACAGAGTCCCAAAT	ATGGAGGAAGGCTGCATTCAA
CEBPE	TTGCCTACCCTCCACATACCT	CTCCTCCTTCACCGCCACAGC

Chromatin immunoprecipitation (ChIP) assay

Cells were cross-linked with 1% formaldehyde for 20 min at room temperature, and the reaction was quenched with 0.125 M glycine for 10 min. Chromatin was then extracted and sonicated to generate DNA fragments of 200–500 bp. For immunoprecipitation, the chromatin solution was incubated overnight at 4°C through gentle rotation with 2 μg of specific primary antibody or IgG control, followed by a 2-hour incubation with protein A-Sepharose beads (GenScript, China) at 4°C. After washing and elution, immunoprecipitated complexes were treated with proteinase K (Invitrogen, USA) and cross-links were removed through heating at 65°C overnight. The DNA was purified by phenol-chloroform extraction and ethanol precipitation, resuspended in water, and analyzed by qPCR. The sequences of ChIP-qPCR primers are provided as follows:

Regions	Direction	Sequence information (5'-3')
P1	Forward	AGCAGCTCTGAACAACCAGTT
	Reverse	CGGCTTCTTTTCGTCCCCCAA
P2	Forward	CAGCTGGTATTTTTCCACAGG
	Reverse	AAACACAAATCTGACTTCCGT
P3	Forward	CCCCAGCTTCCCCTTCCCCTT
	Reverse	GGCTCACCCTCACCTGCCCCC

Dual-luciferase reporter gene assay

The ZMYND8 regulatory element sequence was synthesized by Tsingke Biotech (Beijing, China) and cloned into the pGL4.20 luciferase reporter vector. The

constructed plasmids were transfected into H929 cells. Subsequently, the cells were treated for 24 h with a panel of MM-relevant drugs or retinoid compounds, with concentrations predetermined by CCK-8 assays (> 80% viability) and dose-response reporter assays (maximal/near-maximal induction) for each agent. Luciferase activity was assayed using the Bio-Lumi™ Firefly Luciferase Reporter Gene Assay Kit (Beyotime Biotechnology, China) according to the manufacturer's instructions.

Western blot analysis

Protein lysates were obtained using the cell lysis buffer for Western blotting and IP (Beyotime, China). The protein concentration was determined using a bicinchoninic acid assay. After separation by SDS-PAGE, proteins were transferred to PVDF membranes (Roche, Switzerland). Following blocking with 5% non-fat milk in PBST for 1 h at room temperature, membranes were probed with primary antibodies (11633-1-AP, 14271-1-AP, 10494-1-AP, 50599-2-Ig, 12789-1-AP, and 25128-1-AP, Proteintech, China) overnight at 4°C and then with the corresponding HRP-conjugated secondary antibody. Protein bands were visualized using enhanced chemiluminescence reagents and imaged with a Tanon 5200 system. Immunoblot signals were quantified normalized to loading controls using ImageJ with three independent samples.

CCK-8 assay

To evaluate the cell viability after drug treatment, MM cells were inoculated into 96-well plates at equal densities (100 μL/well). After adding 10 μL of CCK-8 solution

(Vazyme Biotech, China) to each well, the cells were incubated at 37°C for 2 h in the dark. Absorbance at 450 nm was then measured using a Safire microplate reader (Tecan, Switzerland).

Apoptosis assay

The apoptosis rates of cells were assessed by flow cytometry using an Annexin V-FITC Apoptosis Detection Kit (Beyotime Biotechnology, China). Briefly, 1×10^5 cells were harvested, washed with cold PBS, and resuspended in 200 μL binding buffer. Each sample was stained with 5 μL Annexin V-FITC and 10 μL PI, followed by a 15 min incubation at room temperature in the dark. All stained samples were analyzed on an Attune NxT flow cytometer (Thermo Fisher, USA). Cells staining positive for Annexin V-FITC were classified as apoptotic. Data analysis was performed using FlowJo software (version 10).

Transmission electron microscopy (TEM)

Cell samples were fixed with 2.5% glutaraldehyde at room temperature for 30 min, rinsed three times with PBS, and post-fixed in 1% osmium acid fixative solution for 3 h. After dehydration through a graded ethanol series, the samples were embedded in EPON812 resin and solidified. Following double-staining with 2% uranyl acetate and lead citrate, ultrathin sections were examined under a TEM (Hitachi HT7800) operating at 80 kV.

Drug synergy assay

To assess the synergistic effects, MM cells were treated with varying concentrations of ATRA/WYC-209, CFZ, or their combination. After 6 days of treatment, cell growth inhibition was determined by CCK-8 assay. Potential synergy between ATRA/WYC-209 and CFZ was analyzed using the SynergyFinder tool (version 3.0) based on the zero interaction potency (ZIP) model. A ZIP score over 10 was interpreted as reflecting strong synergistic activity.

Mouse xenograft models and in vivo treatment

To establish xenograft MM models, female NSG mice aged 5 weeks were obtained from GemPharmatech Co., Ltd. (Nanjing, China). Animal experiments were approved by the Animal Ethics and Welfare Committee of Nanjing University (IACUC-2302005). All NSG mice were randomly assigned into groups and subsequently injected via the tail vein with 5×10^6 H929 cells transfected with sgNC or sgZMYND8 plasmids. Following injection, MM cells were allowed 14 days to establish residence into the bone marrow. Starting from day 15 after transplantation, mice received intraperitoneal injections of CFZ (2 mg/kg) and/or WYC-209 (10 mg/kg) every two days. The doses of CFZ and WYC-209 were adapted from previously established protocols (doi: 10.1182/blood.2020009856) and were well-tolerated in preliminary studies in non-tumor-bearing mice. In vivo drug safety was evaluated via body weight changes, complete blood counts, and serum biochemical parameters. During the treatment period, mice were monitored for survival status daily, with blood samples collected weekly.

Humane endpoints were strictly followed. Mice displaying any severe symptoms, including but not limited to loss of mobility, paralysis, or respiratory distress, were subjected to euthanasia. We assessed tumor burden via measuring human immunoglobulin light chains secreted by MM cells into mouse plasma using an enzyme-linked immunosorbent assay (ELISA). After euthanasia, bone marrow was harvested from femurs and processed for flow cytometric analysis to quantify the infiltration of MM cells.

Transcriptome sequencing and CUT&Tag assay

For RNA sequencing (RNA-seq), the quality of cDNA library was assessed using an Agilent 2100 Bioanalyzer. Sequencing was conducted on Illumina NovaSeq 6000 platform. Differentially expressed genes (DEGs) were identified using the DESeq package in R, with thresholds set at |fold change| > 2 and adjusted *P* value < 0.05. The ClusterProfiler package in R was utilized for the Gene Ontology (GO) analysis of DEGs.

For CUT&Tag assay, libraries were prepared with a pAG-Tn5 CUT&Tag Assay Kit (ABclonal, China) according to the manufacturer's instructions. After quantification and quality control, libraries were sequenced on a NovaSeq 6000 platform. Downstream sequencing data were analyzed using standard bioinformatic pipelines, and read distribution profiles were visualized with the Integrative Genomics Viewer.

The raw sequence data reported in this paper have been deposited in the Genome

Sequence Archive (Genomics, Proteomics & Bioinformatics 2025) in National Genomics Data Center (Nucleic Acids Res 2025), China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences (GSA-Human: HRA016924) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa-human. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Clinical samples

The collection of primary MM samples was approved by the Medical Ethics Committee of Nanjing Drum Tower Hospital, China (approval No. 2023-043-02). All patients provided written informed consent prior to collection. The clinical information of MM samples used in this study are summarized as follows:

Patient No.	Age	Gender	Cytogenetic abnormalities	R-ISS stage
#1	67	female	t(4;14)	III
#2	63	male	t(11;14), del(13q)	II
#3	49	female	1q21+	I
#4	57	female	t(11;14)	II
#5	54	male	1q21+, del(13q)	II
#6	56	male	del(13q)	I
#7	54	male	del(17p)	III
#8	82	male	t(4;14), 1q21+	III

Statistical analysis

All statistical analyses were conducted using GraphPad Prism (version 10.1.2) and R software (version 4.1.1). Data are presented as mean ± standard deviation (SD), and normality of data distribution was assessed using the Shapiro-Wilk test. Intergroup differences were analyzed using one-way or two-way analysis of variance (ANOVA) for parametric data, and the Kruskal-Wallis test for nonparametric comparisons. Survival data were analyzed with Kaplan-Meier curves, and differences were assessed using the Log-rank test. Statistical significance was defined as a two-tailed *P* value of less than 0.05.