

ADAM15 drives venetoclax resistance via HCK–AKT–FOXO3A signaling in AML

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Abstract

Venetoclax-based regimens have improved outcomes in acute myeloid leukemia (AML), but resistance frequently emerges and limits durable benefit. The upstream signaling mechanisms linking therapeutic stress to apoptotic evasion remain poorly defined. Here, we identify ADAM15 as a previously unrecognized regulator of venetoclax resistance in AML. Integrative multi-omics analyses across experimental models and clinical cohorts reveal that ADAM15 is consistently upregulated in venetoclax refractory AML and is associated with diminished drug sensitivity and adverse clinical outcomes. Ectopic expression of ADAM15 in parental sensitive cells confer venetoclax resistance, while genetic depletion of ADAM15 in resistant counterparts restores drug sensitivity and enhances mitochondrial apoptotic priming in vitro, and suppresses leukemia progression in resistant xenograft models. Mechanistically, ADAM15 engages HCK under venetoclax exposure to activate an AKT-FOXO3A survival program, thereby sustaining resistance. Collectively, these findings establish ADAM15 as a convergent and therapeutically actionable vulnerability in venetoclax-refractory AML and provide a translational rationale for targeting this axis to overcome resistance.

Introduction

Acute myeloid leukemia (AML) remains a leading cause of leukemia-related mortality in adults. The BCL-2 inhibitor venetoclax (VEN), combined with hypomethylating agents or low-dose chemotherapy, has substantially improved initial response rates in treatment-ineligible patients; however, responses are frequently transient and heterogeneous^{1,2}, and median overall survival remains only 9–14 months^{3–5}. The near-universal emergence of resistance is therefore the principal barrier to durable clinical benefit.

At the mechanistic level, VEN resistance converges on mitochondrial apoptotic rewiring: resistant AML cells reduce BCL-2 dependence and shift survival reliance toward MCL-1 or BCL-XL^{6,7}, reflected functionally by diminished mitochondrial priming and impaired apoptosis induction^{8,9}. While these downstream adaptations are well characterized, the upstream signaling mechanisms that dynamically orchestrate them under therapeutic stress remain poorly defined, limiting the development of rational combination strategies.

A Disintegrin And Metalloproteinase 15 (ADAM15) is a multifunctional transmembrane protein that acts predominantly as a signaling scaffold to promote tumor progression and therapeutic resistance. In solid tumors, ADAM15 drives therapeutic resistance by activating EGFR- and Src family kinase-dependent pro-survival pathways and recruiting kinase-active signaling complexes under stress^{10,11}. Despite these observations, its role in hematologic malignancies¹² — and specifically in AML therapy resistance — remains largely unexplored. Notably, AKT–FOXO3A signaling, a candidate effector axis downstream of scaffold-mediated kinase activation, is activated in adverse-risk AML and associated with inferior responses to VEN-based regimens^{13–16}, yet whether this axis is engaged in response to VEN treatment and how it interfaces with mitochondrial apoptotic rewiring remain unknown.

Integrating multi-omics data across experimental models and clinical cohorts, we identify ADAM15 as a convergent driver of VEN resistance that engages HCK to activate AKT–FOXO3A signaling and suppress mitochondrial apoptotic priming. Genetic and pharmacologic disruption of this axis restores VEN sensitivity in vitro and in vivo, defining the ADAM15–HCK–AKT–FOXO3A pathway as a therapeutically actionable vulnerability for overcoming BCL-2 inhibitor resistance in AML.

Results

Venetoclax resistance in AML is driven by phenotypic diversification and anti-apoptotic rewiring

Sensitivity profiling across a panel of human AML cell lines identified MOLM-13, OCI-AML-2, MV4-11, and KG1α as VEN-sensitive, and OCI-AML-3 and THP-1 as intrinsically resistant (Supplementary Fig. S1A). Stepwise dose escalation in three sensitive lines generated resistant derivatives (MOLM-13/R, OCI-AML-2/R, MV4-11/R) with > 10-fold increases in IC₅₀ (Fig. 1A, Table 1, Supplementary Fig. S1B).

Table 1
IC50 values and fold changes of Venetoclax in parental and resistant AML cell lines.

Cell Line	Parental IC50 (μM) [95% CI]	Resistant IC50 (μM) [95% CI]	Fold Change
MOLM-13	0.29 [0.26–0.34]	6.59 [5.44–7.95]	22.39
OCI-AML-2	0.04 [0.036–0.045]	14.02 [11.00–18.15]	345.24
MV4-11	0.03 [0.026–0.032]	2.28 [1.72–3.07]	79.19

VEN-resistant cells displayed markedly attenuated apoptotic responses to ABT-199 (Fig. 1B, Supplementary Fig. S1C). Cell-cycle analysis revealed a divergent response to drug exposure: parental cells underwent S-phase reduction and sub-G0 accumulation, whereas resistant cells evaded cell-cycle restraint and maintained S-phase progression (Fig. 1C). These findings suggest that VEN resistance is associated with a fundamentally altered proliferative response to drug exposure. In parallel, resistant cells exhibited enhanced migratory and invasive capacities compared with parental cells, indicating acquisition of a more aggressive cellular phenotype (Fig. 1D).

To determine whether these phenotypic changes reflected altered apoptotic regulation, we profiled BCL-2 family members at the transcript and protein levels. Transcriptomic analysis revealed consistent upregulation of MCL1, BAK1, and BID and downregulation of BAX and BMF across all resistant models (Fig. 1E, Supplementary Fig. S1D). These changes were corroborated at the protein level: MCL-1 was increased, while NOXA and PUMA were reduced, indicating a shift toward MCL-1-dominant survival signaling with diminished apoptotic priming (Fig. 1F, G).

Dynamic BH3 profiling confirmed these altered dependencies functionally. Resistant cells showed increased mitochondrial depolarization to NOXA and BID peptides but diminished responses to the BAD peptide, directly demonstrating reduced BCL-2 dependence and compensatory MCL-1 reliance (Fig. 1H, I). MOLM-13/R and MV4-11/R cells additionally displayed increased HRK sensitivity, implicating BCL-XL engagement, whereas OCI-AML-2/R cells exhibited a predominant MCL-1-dependent shift. Together, these data establish that acquired VEN resistance involves coordinated phenotypic diversification and context-specific rewiring of anti-apoptotic dependencies — from BCL-2 reliance toward MCL-1- and, in part, BCL-XL-mediated survival.

Integrative transcriptomic and metabolomic analysis identifies ADAM15 as a main regulator of venetoclax resistance

RNA sequencing of paired VEN-resistant and parental AML cell lines identified a conserved transcriptional signature comprising 27 upregulated and 222 downregulated genes shared across all three resistant models (Fig. 2A, E).

To assess clinical relevance, we interrogated the BeatAML functional genomics dataset. Differential expression analysis of ex vivo samples stratified by VEN sensitivity (AUC high vs. low) revealed extensive transcriptional reprogramming in VEN-refractory cases (Fig. 2B). Analysis of intrinsically resistant OCI-AML-3 cells relative to VEN-sensitive lines identified an additional set of differentially expressed genes associated with primary resistance (Fig. 2C). Cross-comparison of these datasets defined a restricted subset of concordantly regulated genes shared across experimental and clinical contexts (Fig. 2E, F; Supplementary Tables S1, S2). KEGG pathway enrichment analysis consistently identified cell adhesion-related programs, with the cell adhesion molecule (CAM) pathway emerging as a recurrent feature across both primary and acquired resistance (Fig. 2D, Supplementary Fig. S2A).

Given emerging evidence linking metabolic adaptation to VEN resistance, we performed untargeted metabolomic profiling of resistant and parental cells, which revealed coordinated alterations in metabolite abundance across all three models (Supplementary Fig. S2B, C). Correlation analysis between differentially expressed genes and resistance-associated metabolites identified ADAM15 as a highly connected node, showing strong correlations with a substantial fraction of altered metabolites across all cell line pairs ($|\text{Pearson's } r| > 0.8$; Fig. 2G). ADAM15 was consistently upregulated across all experimental and clinical resistance datasets, positioning it as a convergent molecular node at the interface of transcriptional and metabolic reprogramming in VEN-refractory AML.

ADAM15 expression correlates with venetoclax resistance and inferior clinical outcome in AML

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Having identified ADAM15 as a convergently upregulated candidate across multiple VEN-refractory contexts, we next sought to evaluate its clinical relevance in AML. To this end, we integrated ex vivo VEN sensitivity data, matched transcriptomic profiles, and clinical annotation from the BeatAML cohort (Fig. 3A). ADAM15 expression correlated positively with VEN AUC values (Fig. 3B) and was significantly elevated in VEN-resistant compared with VEN-sensitive samples (Fig. 3C). Kaplan–Meier analysis of 227 AML patients receiving VEN-based therapy demonstrated that high ADAM15 expression was associated with significantly inferior overall survival (Fig. 3D). These data indicate that elevated ADAM15 not only associates with reduced drug sensitivity but also identifies a subgroup of patients with adverse clinical outcomes in the context of VEN-based treatment.

To validate these findings independently, we analyzed an institutional cohort of AML patients stratified by treatment response. ADAM15 mRNA expression was significantly higher in refractory/relapsed cases compared with those achieving remission (Fig. 3E), a pattern corroborated at the protein level by immunoblotting (Fig. 3F, G). Together, these data establish ADAM15 upregulation as a consistent correlate of VEN refractoriness and adverse clinical outcome across independent datasets and patient cohorts.

Modulation of ADAM15 alters venetoclax sensitivity and mitochondrial apoptotic threshold in AML

ADAM15 was consistently elevated in VEN-resistant AML cells at both the mRNA and protein levels (Fig. 4A, B). To assess its functional contribution to resistance, we performed reciprocal gain- and loss-of-function studies. Ectopic ADAM15 overexpression in VEN-sensitive parental cells modestly but significantly reduced VEN sensitivity across all three lines (Fig. 4C, D). Conversely, stable ADAM15 depletion via two independent shRNAs in the corresponding resistant derivatives partially restored VEN sensitivity (Fig. 4E–G). These bidirectional findings identify ADAM15 as a functionally relevant modulator of VEN sensitivity in AML.

In addition to drug sensitivity, ADAM15 knockdown also significantly attenuated the migratory and invasive capacities of resistant cells (Fig. 4H, I) and reversed the aberrant cell-cycle response to VEN: whereas control resistant cells exhibited S-phase accumulation upon VEN treatment, ADAM15-depleted cells showed reduced S-phase fraction and increased sub-G₀ apoptotic population (Fig. 4J).

To investigate the molecular basis of restored sensitivity, we performed RNA sequencing in ADAM15-knockdown and control resistant cells. Although 479 shared differentially expressed genes were identified across the three models (Fig. 4K), BCL-2 family members did not show a consistent transcriptional pattern (Fig. 4L), suggesting that ADAM15-mediated resistance operates largely independently of uniform transcriptional reprogramming of core apoptotic machinery. We therefore assessed whether ADAM15 depletion alters mitochondrial apoptotic readiness at the functional level. Dynamic BH3 profiling revealed markedly increased mitochondrial priming in ADAM15-knockdown cells across all three resistant models, as reflected by enhanced BIM-induced depolarization (Fig. 4M). In OCI-

AML-2/R and MV4-11/R cells, increased priming extended to multiple BH3 peptides and BH3 mimetics, indicating a broad shift toward an apoptosis-permissive state. MOLM-13/R cells showed a similar pattern, with the exception of modestly reduced HRK-induced priming. These data demonstrate that ADAM15 depletion restores VEN sensitivity primarily by increasing mitochondrial apoptotic priming rather than by transcriptional reprogramming of apoptotic regulators.

ADAM15 depletion enhances in vivo venetoclax efficacy and prolongs survival in resistant AML models

To assess whether ADAM15 contributes to VEN resistance in vivo, we established xenograft models using parental OCI-AML-2, resistant OCI-AML-2/R scramble control, and ADAM15-knockdown OCI-AML-2/R cells (Fig. 5A, Supplementary Fig. S3A). VEN markedly suppressed leukemia progression in parental xenografts but was substantially attenuated in resistant scramble xenografts. ADAM15 depletion restored in vivo VEN responsiveness: mice bearing ADAM15-knockdown resistant cells showed significantly lower peripheral blood hCD45⁺ leukemic chimerism, reduced leukemic infiltration in bone marrow, liver, and spleen, and decreased spleen weight and tumor burden compared with VEN-treated resistant scramble controls (Fig. 5B, C). Histopathological analysis confirmed reduced systemic leukemic infiltration in the ADAM15-knockdown plus VEN group (Fig. 5D, Supplementary Fig. S3B).

Longitudinal monitoring of peripheral hCD45⁺ cells revealed that ADAM15 depletion significantly slowed leukemic expansion under VEN treatment, consistent with delayed disease progression (Fig. 5E). Notably, ADAM15 depletion alone exerted only a limited effect on leukemic burden, whereas its combination with VEN conferred substantially greater benefit, indicating that ADAM15 knockdown primarily sensitizes resistant cells to VEN rather than broadly suppressing leukemia growth. Mice engrafted with ADAM15-knockdown resistant cells and treated with VEN exhibited significantly prolonged survival compared with VEN-treated scramble controls (Fig. 5F).

Together, these data demonstrate that ADAM15 depletion restores VEN responsiveness in vivo, suppresses leukemia progression across multiple disease compartments, and prolongs survival in resistant AML xenografts.

ADAM15 drives adaptive survival signaling through HCK-dependent activation of AKT-FOXO3A axis under venetoclax stress

To identify effectors downstream of ADAM15, we performed protein–protein interaction network and hub gene analyses using shared differentially expressed genes across the three ADAM15-knockdown resistant models (Supplementary Table S3). HCK was consistently downregulated following ADAM15 depletion and predicted to interact with ADAM15, nominating it as a candidate proximal effector (Fig. 6A).

Immunofluorescence analysis revealed increased colocalization of ADAM15 and HCK in VEN-resistant cells following VEN exposure, whereas parental cells displayed substantially weaker overlap (Fig. 6B, Supplementary Fig. S4A, B). Quantitative colocalization metrics confirmed enhanced spatial coupling between ADAM15 and HCK specifically in resistant cells under therapeutic stress (Fig. 6C, D). Reciprocal co-immunoprecipitation demonstrated that ADAM15 and HCK formed a detectable complex in resistant OCI-AML-2/R cells following VEN treatment, but not in parental cells or under DMSO conditions (Fig. 6E–G). These data indicate that ADAM15–HCK engagement is preferentially induced in resistant cells in response to VEN exposure rather than constitutively maintained.

We next examined downstream signaling consequences of this interaction. Under VEN treatment, resistant cells displayed increased ADAM15 abundance together with enhanced phosphorylation of HCK, PDK1, AKT, GSK3 β , and FOXO3A compared with parental cells (Fig. 6H, I), consistent with activation of an HCK-PDK1-AKT-FOXO3A survival pathway downstream of ADAM15. VEN-treated resistant cells also showed increased p-BAD and reduced BIM expression alongside sustained MCL-1 elevation, indicating coupled anti-apoptotic remodeling (Fig. 6H, J, Supplementary Fig. S4E). Under basal DMSO conditions, these signaling changes were less consistent and key phosphorylation events were not robustly detected (Supplementary Fig. S4D), indicating that VEN exposure elicits a more coherent ADAM15-associated survival response than baseline rewiring alone. Nuclear–cytoplasmic fractionation confirmed enhanced cytoplasmic retention of FOXO3A in resistant cells with a corresponding reduction in the nuclear fraction (Fig. 6K, L), consistent with AKT-dependent suppression of FOXO3A pro-apoptotic transcriptional activity.

These results define a VEN stress-induced signaling mechanism in which ADAM15 engages HCK to activate the PDK1–AKT–FOXO3A axis, driving adaptive survival signaling and anti-apoptotic remodeling in resistant AML under therapeutic stress.

Pharmacologic and genetic disruption of the ADAM15-HCK axis re-sensitizes AML to venetoclax

GSEA revealed global suppression of FOXO-associated transcriptional programs in resistant cells (Fig. 7A), consistent with enhanced FOXO3A cytoplasmic sequestration in the resistant state. To determine whether this pro-survival signaling is functionally dependent on the ADAM15–HCK axis, we examined the effects of genetic ADAM15 depletion and pharmacologic HCK inhibition with PP2 in VEN-resistant OCI-AML-2/R cells. VEN treatment induced robust activation of the HCK–PDK1–AKT cascade in resistant cells, as evidenced by increased phosphorylation of HCK, PDK1, AKT, GSK3 β , and FOXO3A. Both ADAM15 knockdown and PP2 treatment markedly attenuated this signaling cascade under VEN exposure (Fig. 7B, Supplementary Fig. S4F), indicating that sustained AKT–FOXO3A activation requires ADAM15–HCK engagement. Disruption of this axis also reversed key anti-apoptotic features of the resistant state, reducing BAD phosphorylation and restoring BIM expression (Fig. 7B), consistent with reactivation of FOXO3A-dependent pro-apoptotic transcription.

Functionally, whereas resistant cells exhibited limited apoptotic response to VEN monotherapy, both ADAM15 depletion and HCK inhibition significantly enhanced VEN-induced apoptosis, with combined treatment producing a marked increase in apoptotic cell death across multiple resistant models (Fig. 7C). These findings establish that the ADAM15–HCK–AKT–FOXO3A axis is required for maintenance of the VEN-resistant state and is therapeutically actionable, with its disruption restoring apoptotic competence and re-sensitizing resistant AML to VEN.

Discussion

This study reveals that venetoclax resistance in AML is not solely a consequence of static anti-apoptotic rewiring but also depends on a dynamic, stress-responsive signaling program orchestrated by ADAM15. Under therapeutic pressure, ADAM15 engages HCK to activate AKT–FOXO3A signaling, suppressing mitochondrial apoptotic priming and sustaining the resistant state. This mechanism is therapeutically actionable: both genetic ADAM15 depletion and pharmacologic HCK inhibition restore VEN sensitivity in vitro and prolong survival in resistant xenograft models, defining the ADAM15–HCK axis as a candidate therapeutic target for VEN-refractory AML.

Resistance to VEN has been attributed to multiple non-mutually exclusive mechanisms, including compensatory reliance on alternative anti-apoptotic proteins such as MCL1 or BCL-XL^{7,17,18}, metabolic adaptation^{19,20}, transcriptional and epigenetic reprogramming²¹, and activation of pro-survival kinase pathways^{7,22}. Our findings extend current models of VEN resistance by identifying ADAM15 as a membrane-proximal signaling regulator that links therapeutic stress to adaptive kinase signaling and apoptotic remodeling. Although ADAM15 has been implicated in tumor cell adhesion, angiogenesis, and metastasis in various solid malignancies^{10–12,23}, its role in hematologic drug resistance has remained unexplored. In this context, our data establish that ADAM15 contributes not only to maintenance of the resistant state but also to associated phenotypic features, including altered cell-cycle dynamics, enhanced migratory and invasive behavior, and reduced mitochondrial apoptotic priming, broadening the biological scope of ADAM15 beyond solid tumor biology.

Mechanistically, activation of the ADAM15–HCK axis imposes a dual restraint on mitochondrial apoptotic competence through downstream AKT signaling. AKT-mediated BAD phosphorylation inactivates this pro-apoptotic BH3-only protein²⁴, while concurrent FOXO3A phosphorylation and cytoplasmic sequestration suppresses BIM transcription – consistent with studies in hematologic malignancies linking FOXO3A inactivation to reduced pro-apoptotic transcriptional output^{25–27}. Importantly, ADAM15–HCK engagement is not constitutive but preferentially induced under VEN exposure, distinguishing this mechanism from static anti-apoptotic rewiring and positioning it as a therapeutic stress-adaptive response.

These findings carry direct translational implications. High ADAM15 expression correlated with reduced ex vivo VEN sensitivity and inferior overall survival in public datasets, observations corroborated in our institutional cohort where ADAM15 was elevated in refractory/relapsed cases – supporting its potential

as a candidate biomarker of VEN response. Therapeutically, the pathway appears pharmacologically tractable. Although ADAM15 currently lacks a well-defined selective small-molecule inhibitor²⁸, its oncogenic functions appear to depend mainly on scaffold-mediated signaling rather than on its metalloproteinase activity^{10–12,23}. This scaffold-dependent mode of action suggests therapeutic strategies targeting protein degradation (e.g., PROTACs) or disruption of the ADAM15–HCK interface. More immediately, co-treatment with the SFK inhibitor PP2 and VEN markedly increased apoptosis in resistant cells, recapitulating genetic ADAM15 depletion. Given that SFK inhibitors (SFKi) such as dasatinib are already clinically deployed in hematologic malignancies, and that emerging evidence supports SFKi-containing regimens enhancing BCL-2-directed therapy^{29–31}, our findings provide a mechanistic rationale for SFK-based combination strategies in VEN-refractory AML.

Several limitations warrant acknowledgment. First, the downstream transcriptional programs through which FOXO3A executes this adaptive survival state remain incompletely defined and will require integrative chromatin profiling. Second, the functional relationship between this signaling axis and the metabolic reprogramming observed in resistant cells – whether downstream effector or parallel adaptation – also remains to be dissected. Finally, validation in patient-derived xenograft models will be necessary to assess translational feasibility more rigorously.

In conclusion, this study identifies ADAM15 as a convergent driver of VEN resistance in AML, operating through stress-induced HCK–PDK1–AKT–FOXO3A signaling to suppress mitochondrial apoptotic priming. These findings expand the molecular framework of BCL-2 inhibitor refractoriness and provide a rationale for therapeutic strategies targeting the ADAM15–HCK axis to restore VEN sensitivity.

Materials and methods

Detailed information on all shRNA sequences, primer sequences, BH3 peptides, and antibodies is provided in Supplementary Table S1. Detailed experimental protocols are provided in Supplementary Methods.

Patient samples

Peripheral blood or bone marrow mononuclear cells were isolated by Ficoll density gradient centrifugation from AML patients treated at Guangdong General Hospital. Samples were classified into a VEN-remission group (patients achieving CR/CRi following venetoclax-based therapy) and a VEN-refractory/relapsed group (patients failing to achieve CR/CRi or relapsing after initial response). Clinical characteristics are summarized in Supplementary Table S2. Written informed consent was obtained in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of Guangdong General Hospital (approval no. KY-Z-2020-551-02).

Cell culture and establishment of venetoclax-resistant cells

Human AML cell lines (MOLM-13, OCI-AML-2, OCI-AML-3, MV4-11, KG1 α , THP-1) and HEK293T cells were maintained under standard conditions (Supplementary Methods). VEN-resistant subclones of OCI-AML-2, MV4-11, and MOLM-13 were generated through continuous exposure to stepwise increasing concentrations of ABT-199, beginning at the IC₅₀ and escalating 1.5–2-fold per cycle until cells proliferated stably at concentrations exceeding 10-fold the initial IC₅₀. Resistant cells were thereafter maintained in ABT-199-containing medium. All cell lines were authenticated by STR profiling before and after resistance induction and routinely tested for Mycoplasma contamination.

Cell viability assays

Cell viability was assessed by CCK-8 assay following 48-h treatment with ABT-199 or vehicle control. IC₅₀ values were determined by four-parameter nonlinear regression. All experiments were performed with at least three biological replicates in technical triplicate.

Migration and invasion assays

Migration and invasion were assessed using Transwell inserts with or without Matrigel coating. Cells were seeded in serum-free medium in the upper chamber, allowed to migrate toward serum-containing medium for 24 h, and quantified by flow cytometry.

Flow cytometry

Apoptosis was assessed by Annexin V/PI staining following ABT-199 treatment. Cell cycle distribution was analyzed by PI/RNase staining and modeled using ModFit LT. Human cell engraftment in xenograft experiments was quantified as the percentage of hCD45⁺mCD45⁻ cells among total viable CD45⁺ leukocytes. All flow cytometric analyses were performed on a Cytex Aurora Evo flow cytometer and analyzed using FlowJo (v10.8.1). Detailed staining protocols, gating strategies, and quality control criteria are provided in Supplementary Methods.

Lentiviral-mediated gene knockdown and overexpression

ADAM15 overexpression and shRNA knockdown constructs were delivered by lentiviral transduction into parental and resistant AML cell lines, respectively. Transduced cells were selected with puromycin, and knockdown/overexpression efficiency was verified by RT-qPCR and immunoblotting. shRNA sequences are provided in Supplementary Table S1; vector and transduction details are provided in Supplementary Methods.

BH3 profiling

Dynamic BH3 profiling was performed on digitonin-permeabilized cells using a panel of BH3 peptides (BIM, BID, HRK, NOXA, BAD, PUMA) and BH3 mimetics (ABT-199, S63845, WHI-539). Mitochondrial depolarization was monitored kinetically by JC-1 fluorescence over 2 h, and priming was calculated relative to CCCP-induced complete depolarization. Peptide sequences, concentrations, and detailed protocols are provided in Supplementary Methods.

RNA extraction and real-time q-PCR

Total RNA was extracted using TRIzol, reverse-transcribed, and analyzed by SYBR Green-based qPCR on an ABI 7500 system. Relative expression was normalized to GAPDH using the $2^{-\Delta\Delta C_t}$ method. Each biological replicate was measured in technical triplicate where indicated.

RNA sequencing

Poly(A)-selected RNA libraries were constructed and sequenced on an Illumina NovaSeq 6000 platform in paired-end 150 bp mode. Reads were aligned to the reference genome using HISAT2, quantified using StringTie, and differential expression analysis was performed using DESeq2 ($|\log_2 FC| \geq 1$, adjusted $P < 0.05$). Raw sequencing data have been deposited in the NCBI Gene Expression Omnibus (GEO) under accession number GSEXXXXXX. (Raw sequencing data have been submitted to the GEO and are currently under processing; the accession number will be inserted prior to publication.).

Untargeted metabolomics

Parental and VEN-resistant cell line pairs (MOLM-13, OCI-AML-2, MV4-11) prepared in parallel with RNA-seq samples were subjected to untargeted metabolomic profiling. Metabolites were identified by accurate mass, retention time, and MS/MS fragmentation against reference databases, with differentially abundant metabolites defined by $VIP > 1.0$.

Co-immunoprecipitation

To assess the ADAM15–HCK interaction under therapeutic stress, cells were treated with either ABT-199 or DMSO for 12 h and subjected to reciprocal co-immunoprecipitation using crosslink-based IP with antibodies against ADAM15 and HCK, with corresponding isotype controls. Bound proteins were analyzed by immunoblotting.

Immunoblotting

Whole-cell lysates or subcellular fractions were resolved by SDS-PAGE, transferred to PVDF membranes, and probed with the indicated primary and HRP-conjugated secondary antibodies. For HCK inhibition studies, cells were pretreated with PP2 prior to ABT-199 exposure. Band intensities were quantified using ImageJ. Antibody details are provided in Supplementary Table S1; full protocols including treatment conditions, lysis buffers, and fractionation procedures are provided in Supplementary Methods.

Immunofluorescence staining and confocal microscopy

Cells were treated with ABT-199 or vehicle for 2 h, fixed, permeabilized with digitonin, and stained with primary antibodies followed by Alexa Fluor-conjugated secondary antibodies. Nuclei were counterstained with DAPI. Images were acquired by confocal microscopy, and colocalization was quantified using ImageJ.

In vivo xenograft assays

Female NOD/SCID mice (6 weeks) were intravenously injected with 4×10^6 cells from one of the following groups: parental OCI-AML-2, OCI-AML-2/R, OCI-AML-2/R-shADAM15, OCI-AML-2/R-scramble, or PBS alone (cell-free control). Engraftment was confirmed at day 7 by peripheral blood hCD45 flow cytometry and Wright-Giemsa morphology, with successful engraftment defined as $\geq 1\%$ hCD45⁺ cells. Engrafted mice were randomized ($n \geq 6$ per group) to receive venetoclax (50 mg/kg, oral gavage, QD) or vehicle beginning on day 7. Disease progression was monitored weekly by peripheral blood hCD45/mCD45 chimerism. Mice were euthanized upon reaching predefined humane endpoints, and tissues were collected for flow cytometry, histology, immunohistochemistry, and molecular analyses. All procedures were approved by the IACUC (approval no. G2025074) and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

BeatAML Cohort Analysis

Clinical, transcriptomic, and drug sensitivity data were obtained from the BeatAML database³². Patients were stratified into venetoclax-sensitive (Q1) and venetoclax-resistant (Q4) groups by venetoclax AUC quartiles. ADAM15 expression was compared between groups and correlated with venetoclax sensitivity. Patients were further dichotomized into ADAM15-high and ADAM15-low groups using the optimal cutoff, and overall survival was analyzed by Kaplan-Meier method with log-rank testing.

Statistical analysis

Experiments were performed with at least three independent biological replicates, and data are presented as mean $\pm$ SD unless otherwise stated. Comparisons between two groups were made using unpaired two-tailed Student's t-test; multiple-group comparisons used one-way ANOVA with Tukey's post hoc test. IC₅₀ values were determined by four-parameter nonlinear regression. Survival was analyzed by Kaplan-Meier method with log-rank test. $P < 0.05$ was considered statistically significant. All analyses were performed using GraphPad Prism (v9.5) or R (v4.3.1).

Declarations

Data availability

RNA sequencing data have been submitted to the NCBI Gene Expression Omnibus (GEO) and are currently under processing; the accession number will be inserted prior to publication. All other data supporting the findings of this study are available within the article and its supplementary files, or from the corresponding author upon reasonable request.

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Author contributions

KFL and PLL conceived and designed the study. KFL, LJZ and MZX performed the experiments and analyzed the data. KFL wrote the manuscript. MZX, SXG, YLW, MW, PLL and RHX reviewed and edited the manuscript. XH, MML, PW, XMC and YTL provided the clinical samples/datasets. XD, JYW and PLL supervised and acquired funding for this study. All authors have read and approved the final manuscript.

Ethics declarations

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Guangdong General Hospital (approval no. KY-Z-2020-551-02). All procedures involving animal subjects were approved by the Institutional Animal Care and Use Committee (IACUC; approval no. G2025074).

Conflict of interests

The authors declare no competing interests.

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Figures

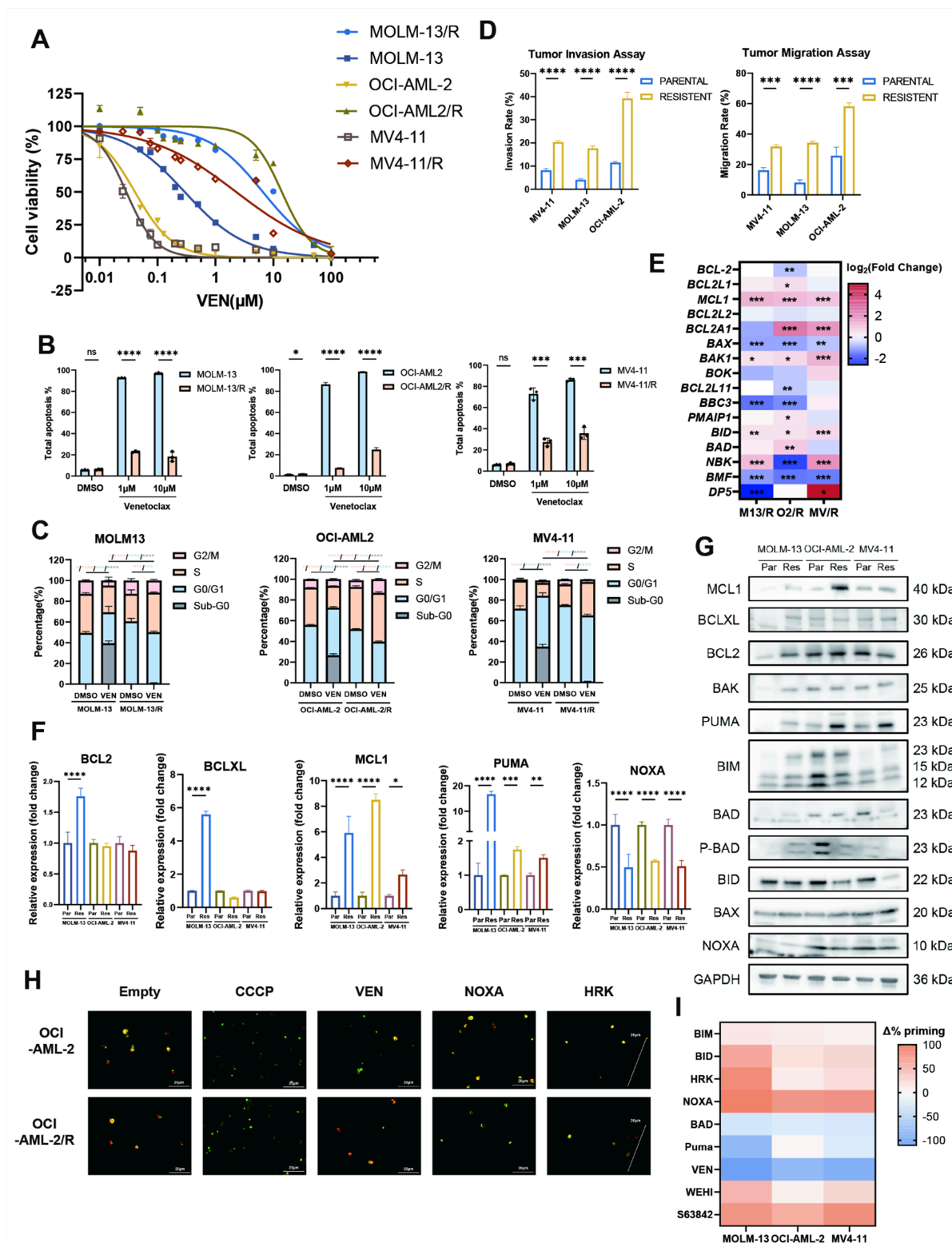


Figure 1

Venetoclax-resistant AML cells exhibit attenuated apoptotic responses, enhanced migratory and invasive capacities, and rewired anti-apoptotic dependencies.

(A) Dose–response curves of parental and venetoclax (VEN)-resistant AML cells treated with increasing concentrations of ABT-199 for 48 h. **(B)** Apoptosis in parental and VEN-resistant AML cells following

treatment with 1 μ M or 10 μ M ABT-199 for 24 h, assessed by Annexin V/propidium iodide (PI) staining. Total apoptosis represents the sum of early and late apoptotic cells. **(C)** Cell-cycle distribution of parental and VEN-resistant AML cells following treatment with ABT-199 or vehicle control. **(D)** Quantification of Transwell invasion (left) and migration (right) in parental and VEN-resistant AML cells. **(E)** Heatmap of RNA-seq analysis showing differential expression of apoptosis-related genes in VEN-resistant cells relative to parental controls, presented as $\log_2$ (fold change) based on TPM-normalized counts. **(F, G)** Representative immunoblots **(G)** and corresponding densitometric quantification **(F)** of anti- and pro-apoptotic BCL-2 family proteins. Protein levels were normalized to GAPDH and expressed relative to parental controls. **(H)** Representative JC-1 fluorescence images indicating mitochondrial membrane potential ($\Delta\Psi_m$). Green fluorescence indicates JC-1 monomers (depolarized mitochondria), whereas orange-red fluorescence indicates JC-1 aggregates (polarized mitochondria). Scale bar, 20 μ m. **(I)** Heatmap of dynamic BH3 profiling showing $\Delta\%$ priming in response to the indicated BH3 peptides and BH3 mimetics. $\Delta\%$ priming was calculated as the difference in priming between resistant and corresponding parental cells.

Data are presented as mean $\pm$ SD from three independent biological replicates. Statistical significance is indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$. For transcriptomic analyses, adjusted P values (P_{adj}) are indicated using the same thresholds.

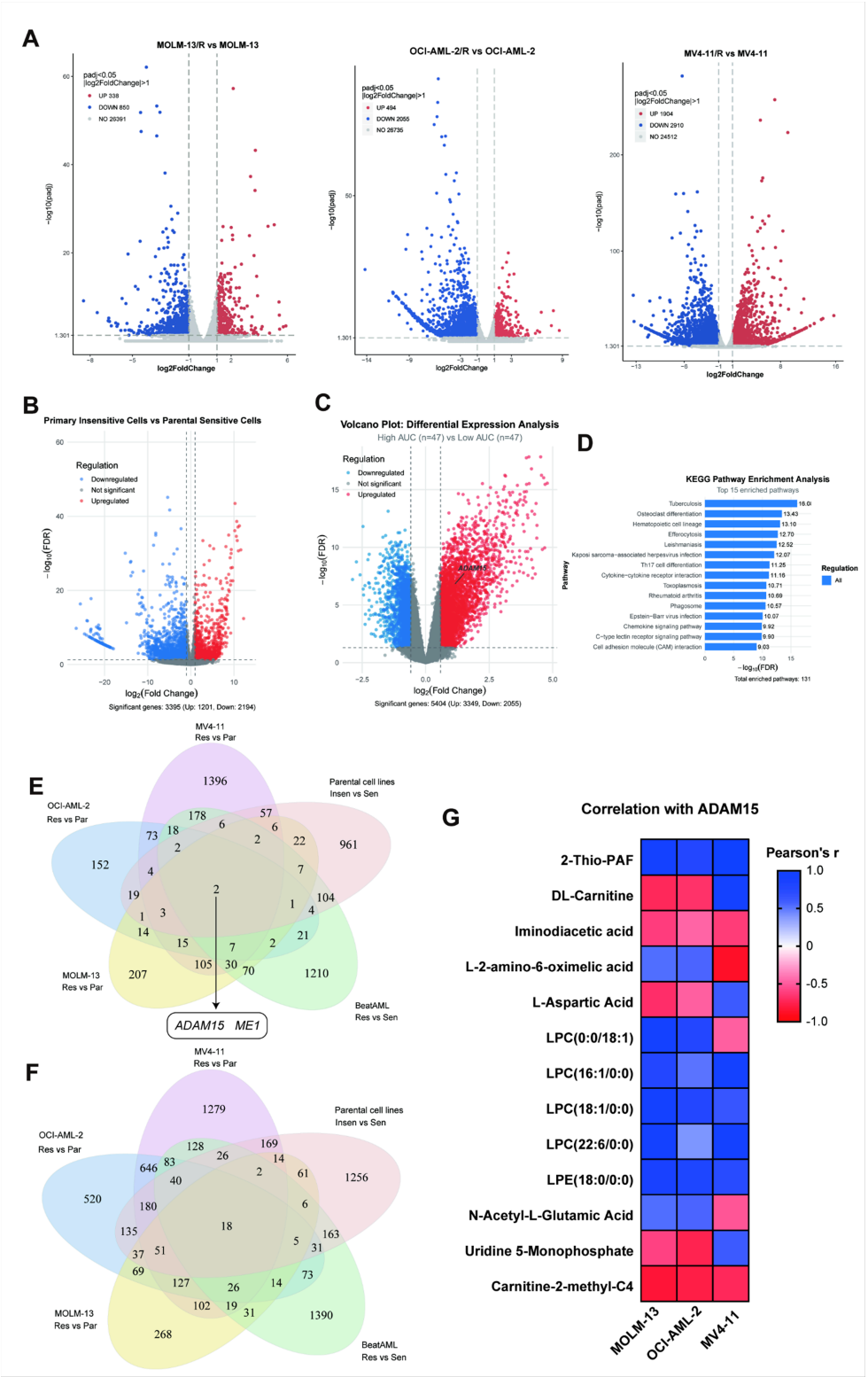


Figure 2

Integrative multi-omic analyses identify ADAM15 as a convergently upregulated feature across venetoclax-resistant AML states.

(A–C) Volcano plots depicting differentially expressed genes (DEGs) across distinct models of venetoclax (VEN) resistance: **(A)** paired VEN-resistant versus parental AML cell lines; **(B)** primary AML

samples stratified by VEN response in the BeatAML cohort; and **(C)** intrinsically VEN-insensitive versus VEN-sensitive AML cell lines. In **(B)**, samples were dichotomized based on the upper and lower quartiles of VEN area under the curve (AUC), corresponding to VEN-refractory (high AUC) and VEN-sensitive (low AUC) groups. Significantly upregulated and downregulated genes are highlighted in red and blue, respectively. **(D)** KEGG pathway enrichment analysis of DEGs derived from clinical and experimental resistance models. **(E, F)** Identification of a conserved resistance-associated transcriptional program. Venn diagrams illustrate the overlap of downregulated **(E)** and upregulated **(F)** DEGs across experimental systems and patient-derived datasets. **(G)** Integrative correlation analysis of resistance-associated DEGs and differentially abundant metabolites. ADAM15 emerges as a highly connected node, exhibiting strong correlations ($|\text{Pearson's } r| > 0.8$) with a substantial subset of resistance-associated metabolites across all three VEN-resistant models.

RNA-seq and untargeted metabolomics were performed with $n = 3$ independent biological replicates per group. For transcriptomic analyses, significance was defined as adjusted P value (P_{adj}) < 0.05 and $|\log_2 \text{fold change}| > 1$. Clinical data were obtained from the BeatAML functional genomics resource.

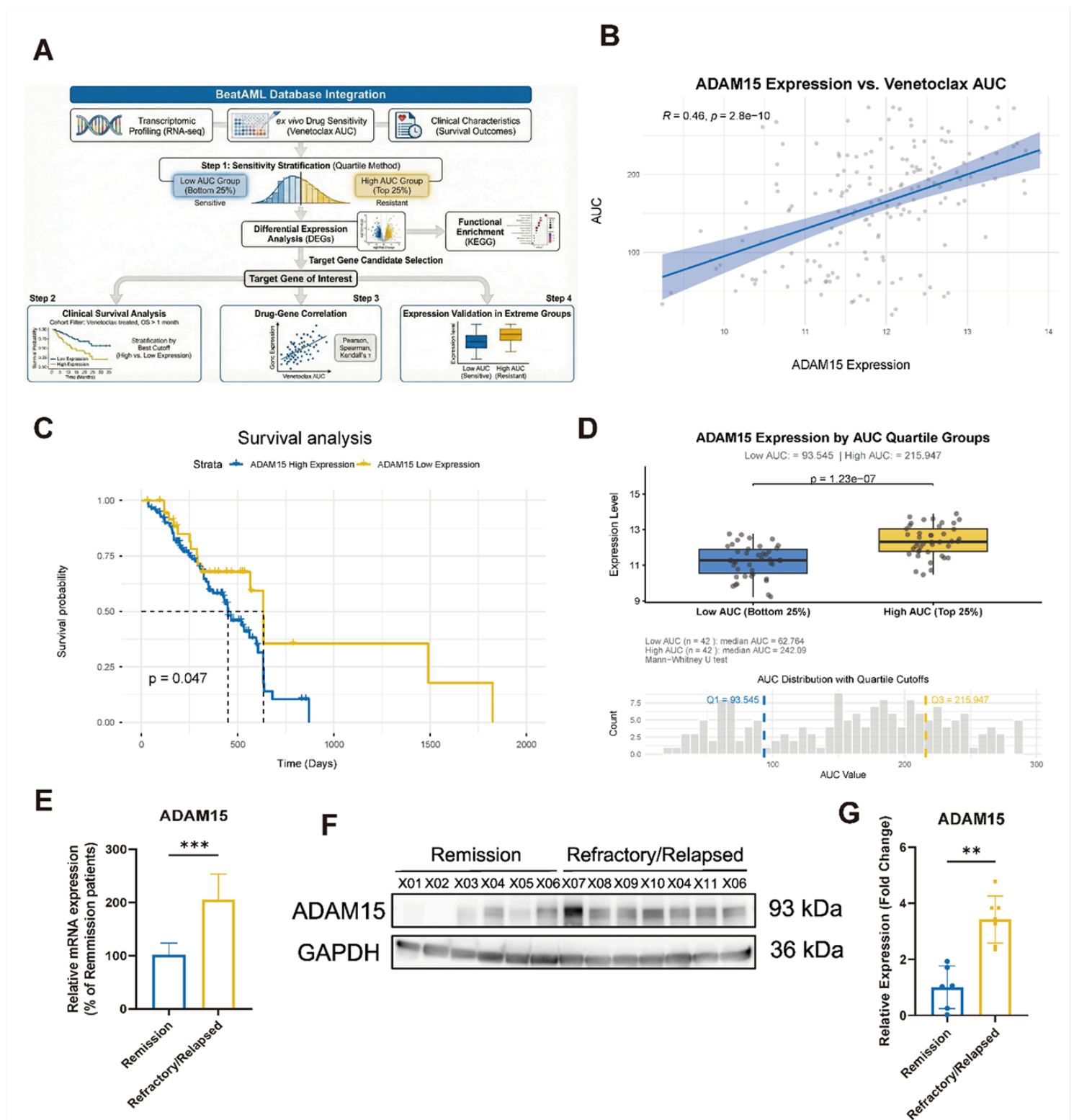


Figure 3

ADAM15 expression correlates with venetoclax resistance and inferior clinical outcomes in AML patients.

(A) Schematic workflow showing the integrative analysis of the BeatAML dataset, encompassing transcriptomic profiling, ex vivo drug sensitivity, and clinical survival outcomes. **(B)** Pearson correlation

analysis showing positive correlation between *ADAM15* mRNA expression and Venetoclax AUC values in the BeatAML cohort ($R = 0.46, p = 2.8 \times 10^{-10}$). **(C)** Comparison of *ADAM15* expression levels between the Venetoclax-sensitive (Bottom 25% AUC, $n=42$) and Venetoclax-resistant (Top 25% AUC, $n=42$) groups. Distribution of AUC values with quartile cutoffs is shown in the lower panel. $p = 1.23 \times 10^{-7}$ by Mann-Whitney U test. **(D)** Kaplan-Meier survival curves showing overall survival (OS) of AML patients stratified by *ADAM15* expression levels (High vs. Low). $p = 0.047$ by log-rank test. **(E)** Quantitative RT-PCR analysis of *ADAM15* mRNA levels in an independent institutional cohort of AML patients who achieved remission ($n=5$) versus those with refractory/relapsed disease ($n=6$) following VEN-based therapy. Data were normalized to GAPDH. **(F, G)** Representative immunoblots **(F)** and densitometric quantification **(G)** of *ADAM15* protein levels in the remission and refractory/relapsed patient groups. GAPDH served as the loading control.

Data are presented as mean $\pm$ SD. $**p < 0.01$, $***p < 0.001$ by Student's t-test (E, G). VEN, venetoclax; AUC, area under the curve; mRNA, messenger RNA.

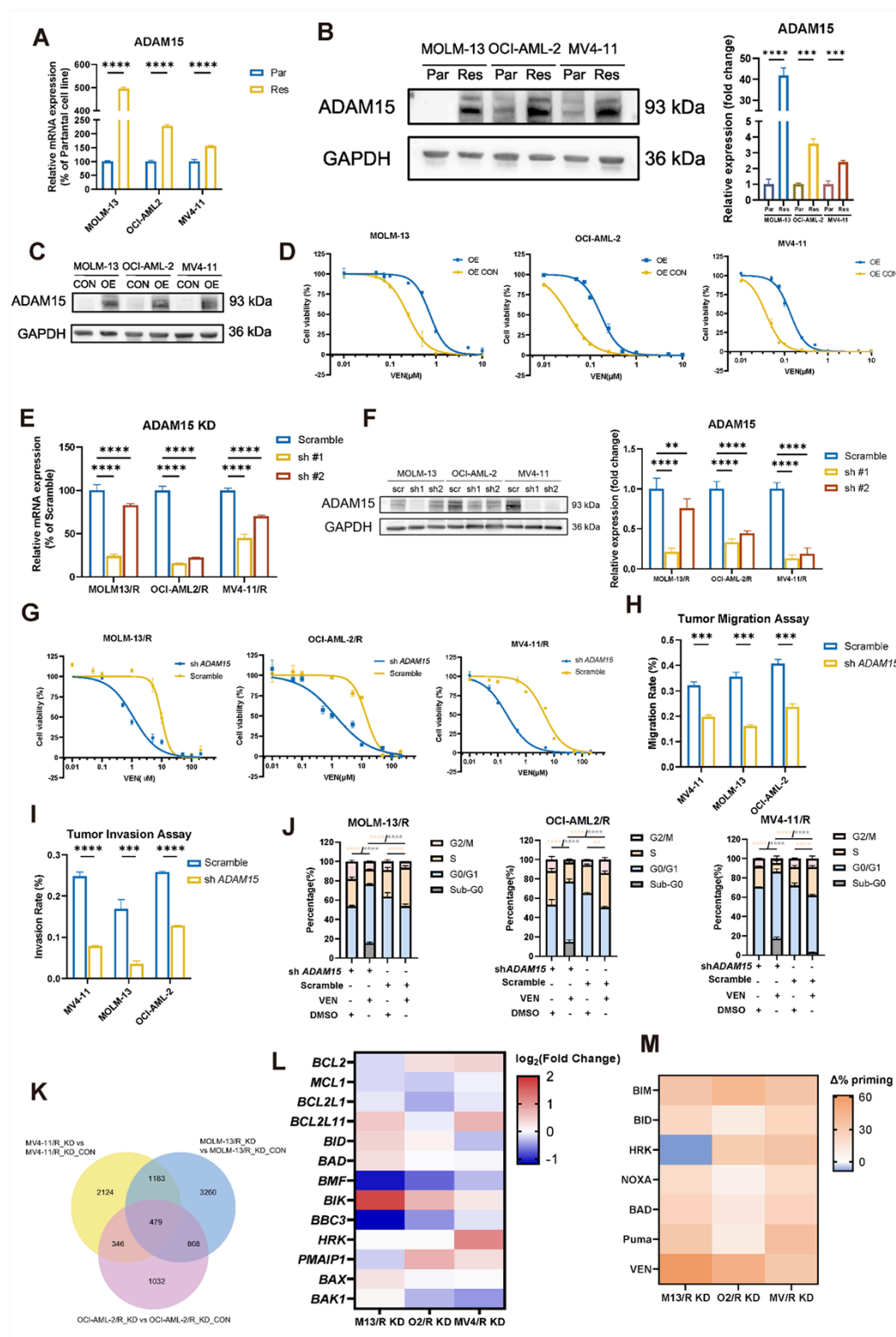


Figure 4

ADAM15 modulation alters venetoclax sensitivity through transcriptional reprogramming and mitochondrial priming dynamics in resistant AML cells

(A) Relative ADAM15 mRNA expression in parental (Par) and venetoclax-resistant (Res) MOLM-13, OCI-AML-2, and MV4-11 cells, quantified by RT-qPCR and normalized to the respective parental control (set to

1). **(B)** Representative immunoblots and densitometric quantification of ADAM15 protein abundance in parental and venetoclax-resistant derivatives across all three AML cell lines. GAPDH served as the loading control. **(C, D)** Ectopic ADAM15 overexpression in MOLM-13, OCI-AML-2, and MV4-11 cells was confirmed by immunoblotting (C). Cell viability of ADAM15-overexpressing and empty-vector control cells was assessed following 48-hour exposure to increasing concentrations of ABT-199 (D). **(E, F)** Stable ADAM15 knockdown efficiency in MOLM-13/R, OCI-AML-2/R, and MV4-11/R cells transduced with two independent shRNA constructs (sh#1 and sh#2) or a non-targeting scramble control, confirmed at the mRNA level by RT-qPCR (E) and at the protein level by immunoblotting with densitometric quantification (F). **(G)** Cell viability dose–response curves of shADAM15 and scramble-control MOLM-13/R, OCI-AML-2/R, and MV4-11/R cells treated with increasing concentrations of ABT-199 for 48 hours. **(H, I)** Transwell migration (H) and Matrigel invasion (I) assays in shADAM15 and scramble-control resistant AML cells. Migratory and invasive capacity is expressed as the percentage of input cells that traversed the membrane. **(J)** Cell-cycle distribution in shADAM15 and scramble-control cells following 24-hour treatment with venetoclax (10 μ M) or DMSO vehicle, assessed by propidium iodide staining and flow cytometry. Sub-G₀, G₀/G₁, S, and G₂/M fractions are presented as stacked bar graphs. **(K)** Three-way Venn diagram depicting the overlap of differentially expressed genes (DEGs) identified by RNA sequencing in each ADAM15-knockdown resistant model relative to its respective scramble control. The intersection contains 479 genes concordantly dysregulated across all three models (adjusted $P \leq 0.05$; $|\log_2FC| \geq 1.0$). **(L)** Heatmap of $\log_2$ (fold change) values for selected BCL-2 family members and BH3-only proteins derived from RNA sequencing of ADAM15-knockdown versus scramble-control resistant cells across the three models. **(M)** Dynamic BH3 profiling heatmap depicting the change in mitochondrial depolarization ($\Delta\%$ priming) in response to a panel of BH3 peptides and venetoclax (VEN) in ADAM15-knockdown resistant cells (MOLM-13/R KD, OCI-AML-2/R KD, and MV4-11/R KD) relative to scramble controls. Mitochondrial membrane potential was measured kinetically using JC-1 dye (1 μ M) at 37°C, with fluorescence acquisition every 5 minutes over a 2-hour period. Warmer colors indicate greater induction of mitochondrial priming.

All data represent mean $\pm$ SD from at least three independent biological replicates. Two-group comparisons were performed using an unpaired two-tailed Student's *t*-test; multi-group comparisons were assessed by one-way ANOVA with Tukey's post hoc correction. $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$.

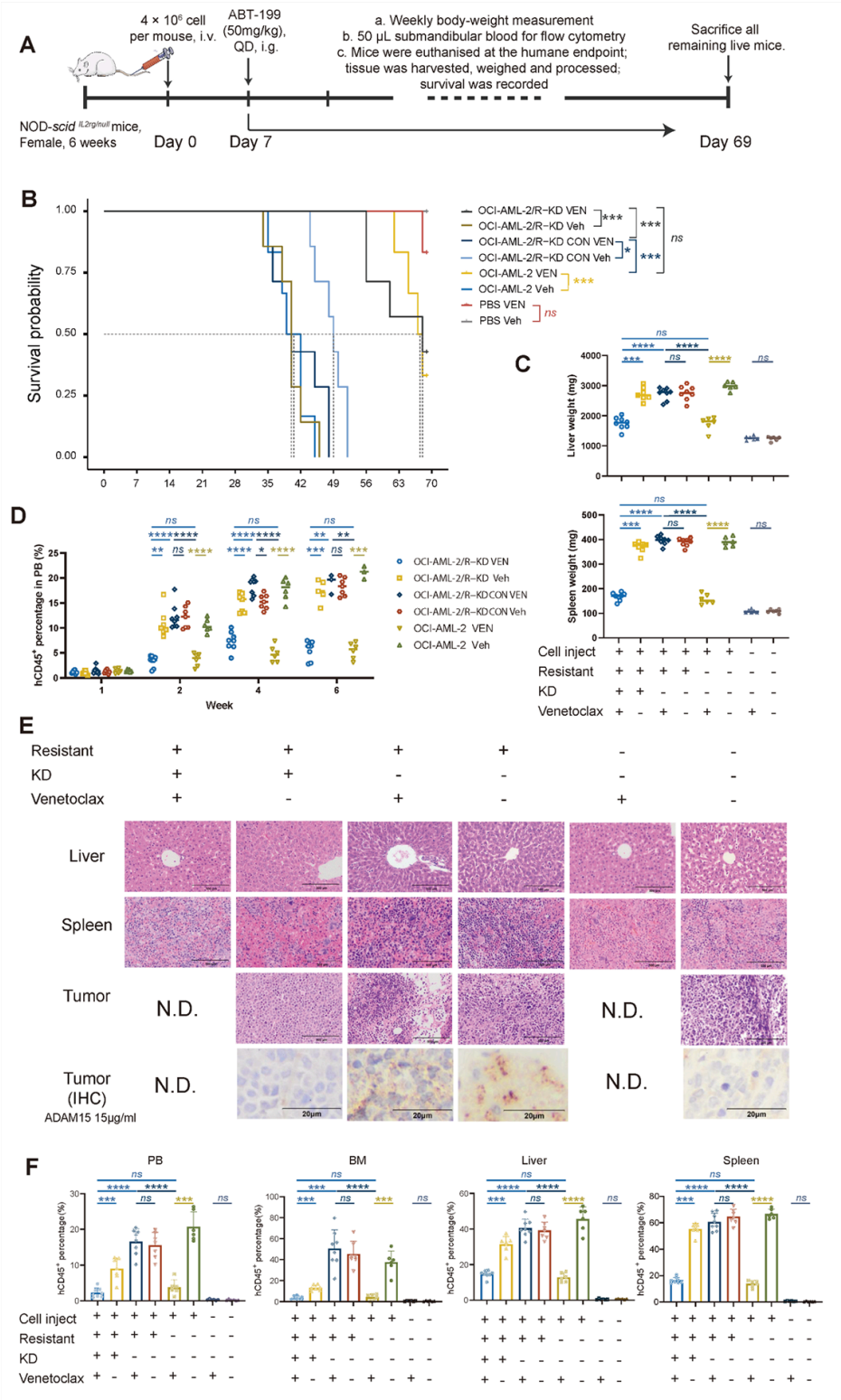


Figure 5

ADAM15 depletion enhances venetoclax efficacy and prolongs survival in venetoclax-resistant AML xenograft models.

(A) Schematic of the in vivo experimental design. Female NOD/SCID mice (6 weeks old) were engrafted via tail-vein injection with 4×10^6 cells or cell-free PBS on day 0. Starting on day 7, mice received ABT-

199 (50 mg/kg daily, administered orally, formulated in 10% DMSO/corn oil) or vehicle (10% DMSO/corn oil). Weekly body weight measurements, biweekly submandibular blood sampling (50 μ L), and continuous humane endpoint monitoring were performed throughout the study. Human CD45⁺ (hCD45⁺) chimerism was quantified by flow cytometry. All surviving animals were euthanized on day 69; tissues were then harvested, weighed, and processed for downstream analyses. **(B)** Liver weight (left) and spleen weight (right) at endpoint stratified by cell injection status, venetoclax resistance phenotype, ADAM15 knockdown, and treatment regimen. Each symbol represents an individual mouse ($n \geq 6$ per group); horizontal bars indicate mean $\pm$ SD. **(C)** Longitudinal peripheral blood hCD45⁺ chimerism monitored biweekly by flow cytometry across experimental groups. **(D)** Representative hematoxylin and eosin–stained sections of liver, spleen, and leukemic infiltrates (top three rows; scale bars, 500 μ m) and immunohistochemical staining for ADAM15 (15 μ g/mL; bottom row; scale bars, 20 μ m) in tumor sections from indicated experimental groups. N.D., not detected. **(E)** hCD45⁺ leukemic burden quantified by flow cytometry at endpoint in peripheral blood (PB), bone marrow (BM), liver, and spleen across experimental groups ($n \geq 6$ per group). **(F)** Kaplan–Meier survival curves for mice receiving different treatments. Cell-free PBS-injected animals served as non-engraftment controls. Survival differences were evaluated by log-rank test ($n \geq 6$ per group).

All data are presented as mean $\pm$ SD. Two-group comparisons were performed using unpaired Student's *t*-tests; multi-group comparisons used one-way ANOVA with Tukey's *post hoc* test. Survival analysis employed the log-rank test. Statistical significance was defined as follows: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; ns, not significant.

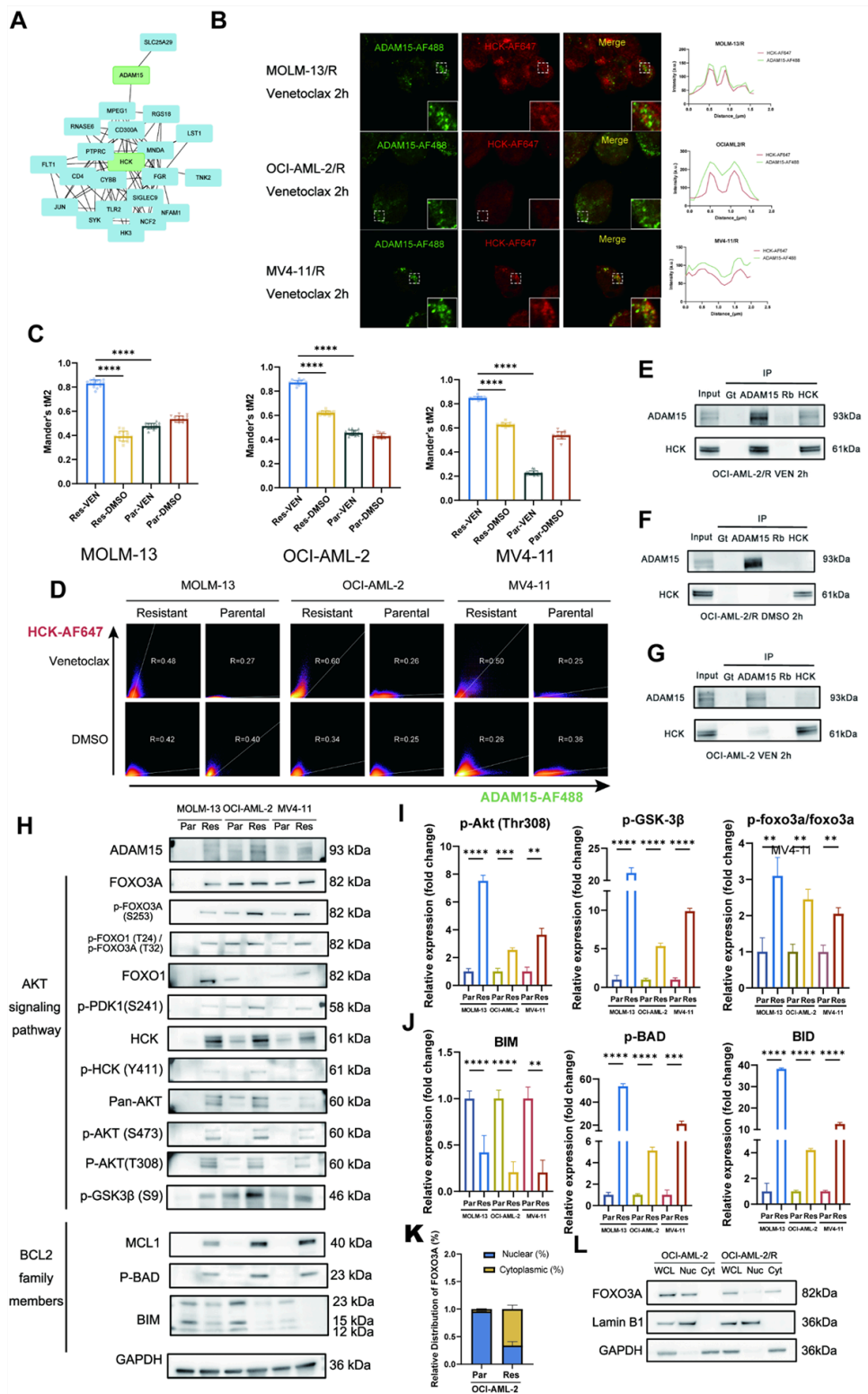


Figure 6

ADAM15 drives adaptive survival signaling through HCK-dependent activation of the AKT–FOXO3A axis under venetoclax stress.

(A) Protein–protein interaction (PPI) network derived from hub gene analysis of 479 differentially expressed genes shared across three ADAM15-knockdown venetoclax-resistant models. **(B)**

Representative confocal immunofluorescence images depicting colocalization of ADAM15 (AF488, green) and HCK (AF647, red) in MOLM-13/R, OCI-AML-2/R, and MV4-11/R cells following 2-hour exposure to venetoclax (10 μ M). Insets show magnified views of the boxed regions. Fluorescence intensity profiles along the indicated scan lines (right panels) illustrate the spatial overlap between ADAM15 and HCK signals. **(C)** Quantification of Manders' thresholded overlap coefficients (tM2), representing the fraction of ADAM15 signal overlapping with HCK, in venetoclax- versus DMSO-treated resistant (Res) and parental (Par) cells across MOLM-13 (left), OCI-AML-2 (middle), and MV4-11 (right) models. Each data point represents a single cell analyzed across at least three independent experiments (minimum five fields per experiment). **(D)** Pixel-by-pixel fluorescence intensity scatter plots depicting correlation between HCK-AF647 and ADAM15-AF488 signals in resistant and parental MOLM-13, OCI-AML-2, and MV4-11 cells treated with venetoclax (10 μ M) or DMSO for 2 hours. Pearson's correlation coefficients (R) are indicated within each panel. **(E–G)** Reciprocal co-immunoprecipitation (co-IP) analysis in OCI-AML-2/R cells treated for 2 hours with venetoclax (10 μ M; **E**) or DMSO (**F**), and in parental OCI-AML-2 cells treated with venetoclax (10 μ M; **G**). Immunoprecipitations were performed using anti-ADAM15, anti-HCK, goat IgG (Gt), or rabbit IgG (Rb) isotype control antibodies, as indicated. Precipitated complexes were probed by immunoblot for ADAM15 (93 kDa) and HCK (61 kDa). Whole-cell lysate (Input) is shown as reference. **(H)** Immunoblot analysis of AKT pathway components and BCL-2 family members in parental (Par) and venetoclax-resistant (Res) MOLM-13, OCI-AML-2, and MV4-11 cells following 12-hour venetoclax treatment. GAPDH served as the loading control. **(I)** Densitometric quantification of p-AKT (Thr308), p-GSK-3 β , and p-FOXO3A relative to total FOXO3A from immunoblots in panel H, expressed as fold change normalized to the respective parental control. **(J)** Densitometric quantification of BIM, p-BAD, and BID protein levels from immunoblots in panel H, expressed as fold change normalized to the respective parental control. **(K)** Relative nuclear and cytoplasmic distribution of FOXO3A in parental and resistant OCI-AML-2 cells, expressed as the fraction of total FOXO3A signal within each compartment. **(L)** Representative immunoblots of FOXO3A in whole-cell lysate (WCL), nuclear (Nuc), and cytoplasmic (Cyt) fractions from parental and OCI-AML-2/R cells. Lamin B1 and GAPDH served as nuclear and cytoplasmic compartment controls, respectively.

All quantitative data are presented as mean $\pm$ SD from at least three independent biological replicates. Two-group comparisons were performed using an unpaired two-tailed Student's *t*-test. ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001; ns, not significant.

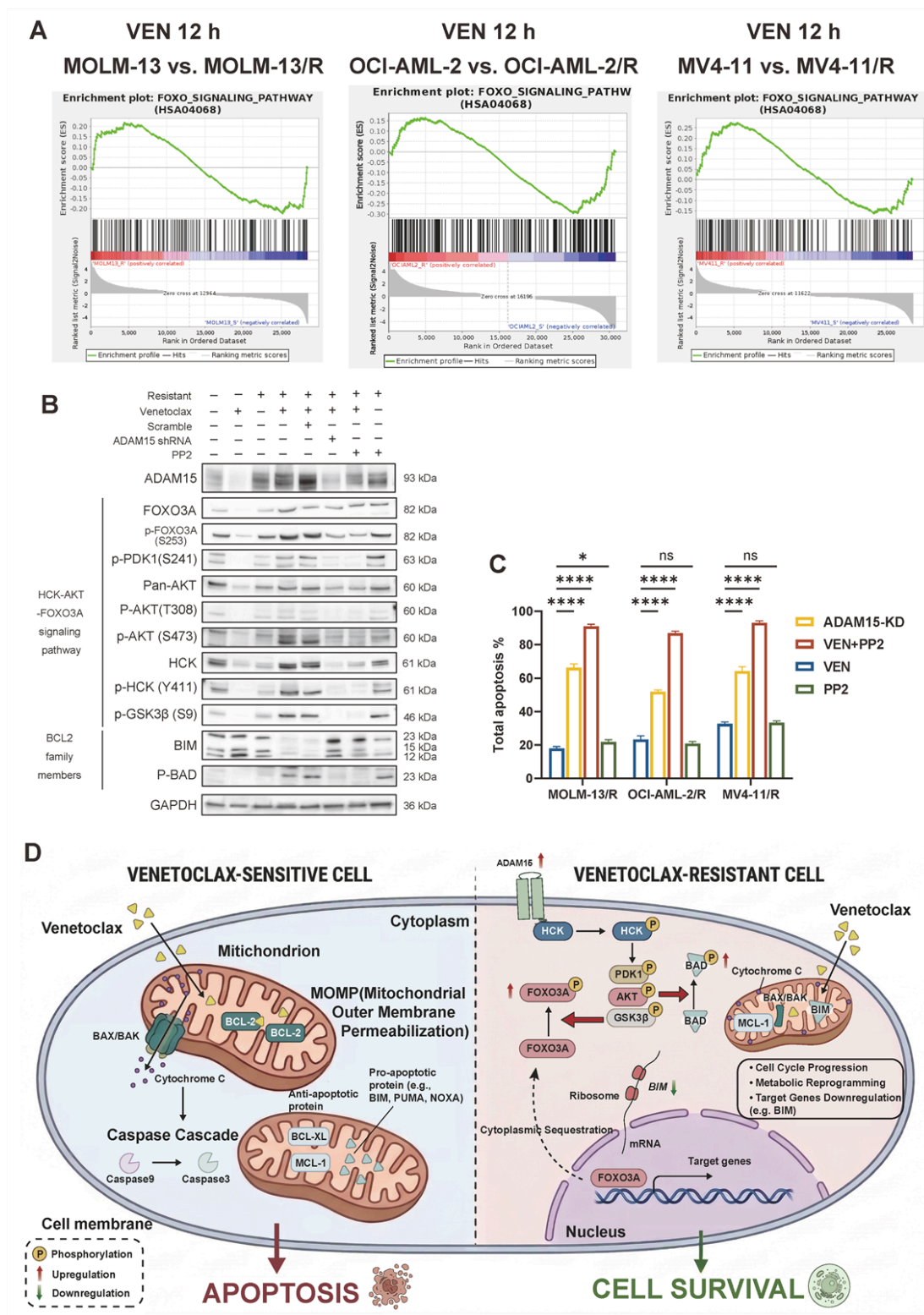


Figure 7

ADAM15 depletion abrogates HCK phosphorylation and AKT–FOXO3A activation, restoring venetoclax sensitivity.

(A) GSEA enrichment plot for the FOXO signaling pathway (KEGG: hsa04068) derived from transcriptomic profiling of three matched parental/resistant cell line pairs following 12 h venetoclax

treatment. The enrichment score profile (green curve), leading-edge gene positions (black vertical bars), and ranked list metric (grey bars) are shown. Genes positively and negatively correlated with the resistant signature are indicated in red and blue, respectively. The zero-crossing point is annotated. **(B)** Immunoblot analysis of HCK–AKT–FOXO3A pathway components and BCL-2 family members in OCI-AML-2/R cells under the following conditions: parental versus resistant background; venetoclax versus vehicle; scramble versus ADAM15 shRNA; and PP2 (10 μ M, HCK inhibitor) versus vehicle. GAPDH served as the loading control. Bracket annotations denote HCK–AKT–FOXO3A signaling components and BCL-2 family members, respectively. **(C)** Total apoptosis rates (%) in MOLM-13/R, OCI-AML-2/R, and MV4-11/R cells subjected to ADAM15 knockdown (ADAM15-KD), combined venetoclax and PP2 treatment (VEN+PP2; 10 μ M), venetoclax alone (VEN), or PP2 alone (10 μ M), as assessed by annexin V/propidium iodide staining and flow cytometry. **(D)** Schematic model illustrating the mechanistic basis of venetoclax sensitivity versus resistance in AML. In sensitive cells (*left*), venetoclax disrupts BCL-2–mediated sequestration of pro-apoptotic proteins, liberating BAX/BAK to induce mitochondrial outer membrane permeabilization (MOMP), cytochrome c release, and caspase-9/caspase-3–dependent apoptosis. In resistant cells (*right*), upregulated ADAM15 engages HCK and drives sequential phosphorylation of PDK1, AKT, and GSK-3 β . Activated AKT phosphorylates FOXO3A, promoting its cytoplasmic sequestration and consequent suppression of pro-apoptotic target gene transcription, including *BIM* downregulation. Concurrent BAD phosphorylation further attenuates pro-apoptotic signaling. Collectively, these adaptive events sustain cell survival, cell cycle progression, and metabolic reprogramming despite continuous venetoclax exposure.

All data are presented as mean $\pm$ SD from at least three independent biological replicates. Comparisons among multiple groups were assessed by one-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$; **** $p < 0.0001$; *ns*, not significant.

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