leukemia stem cells NMNAT1 prevents apoptosis of acute myeloid leukemia stem cells

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Metabolic dysregulation underlies malignant phenotypes attributed to cancer stem cells, such as unlimited proliferation and differentiation blockade. Here, we demonstrate that NAD+ metabolism enables acute myeloid leukemia (AML) to evade apoptosis, another hallmark of cancer stem cells. We integrated whole-genome CRISPR screening and pan-cancer genetic dependency mapping to identify NAMPT and NMNAT1 as AML dependencies governing NAD+ biosynthesis. While both NAMPT and NMNAT1 were required for AML, the presence of NAD+ precursors bypassed the dependence of AML on NAMPT but not NMNAT1, pointing to NMNAT1 as a gatekeeper of NAD+ biosynthesis. Deletion of NMNAT1 reduced nuclear NAD+, activated p53, and increased venetoclax sensitivity. Conversely, increased NAD+ biosynthesis promoted venetoclax resistance. Unlike leukemia stem cells (LSCs) in both murine and human AML xenograft models, NMNAT1 was dispensable for hematopoietic stem cells and hematopoiesis. Our findings identify NMNAT1 as a previously unidentified therapeutic target that maintains NAD+ for AML progression and chemoresistance.

INTRODUCTION

Acute myeloid leukemia (AML) represents heterogeneous myeloid malignancies with poor outcomes and few targeted therapies (1). The standard “7 + 3” induction regimen (7 days of cytarabine and 3 days of daunorubicin treatment) targeting bulk AML cells introduced in 1973 is still used to treat AML in many centers (2). Recent studies have revealed that AML, in particular the leukemia stem cells (LSCs) largely responsible for relapse and therapy resistance (3), has unique metabolic requirements, such as low reactive oxygen species and dependence on oxidative phosphorylation (OXPHOS) and adenosine 5′-monophosphate–activated protein kinase (AMPK), a master regulator of metabolism (4–7). These metabolic dependencies offer potential for therapeutic intervention, as exemplified by the finding that venetoclax, a B-cell lymphoma 2 (BCL-2) inhibitor, suppresses OXPHOS of LSCs to achieve remarkable responses against de novo AML (8–10) but not relapsed/refractory AML (11, 12). These findings underscore the need to identify novel metabolic vulnerabilities in AML, particularly in LSCs, for better therapeutic intervention.

Nicotinamide adenine dinucleotide (NAD+) is a foundational metabolite that is required for a variety of biological processes, including cell survival and genome integrity. In mammals, NAD+ is largely produced via the Preiss-Handler and salvage pathways due to the bioavailability of nicotinic acid (nicacin, NA), nicotinamide (NAM), and NAM riboside (NR) from food (13). A branch of the salvage pathway governed by NAM phosphoribosyltransferase (NAMPT), an enzyme that converts NAM to NAM mononucleotide (NMN), has gained attention as a potential cancer target. NAMPT inhibitors such as FK866 showed promising anticancer effects in vitro and in some animal models (14). However, NAMPT inhibitors had limited success in clinical trials (13), potentially due to the ability of NA and NR to bypass the requirement of NAMPT (15).

NAMPT is present in both intracellular and extracellular forms, the latter of which is predominantly produced from adipocytes and exhibits higher enzymatic activity (16). This raises the question of which source of NAMPT to target: NAMPT expressed in the tumors, in the tumor microenvironment, or in distal tissues such as adipocytes. NMN adenyltransferases (NMNATs) are downstream of NAMPT, which use NMN and NA mononucleotide (NaMN) from NAM, NR, and NA to produce NAD+ directly or indirectly. The three NMNATs have distinct cellular localizations (NMNAT1 in the nucleus, NMNAT2 in the cytoplasm, and NMNAT3 in the mitochondrion) to enable compartmentalization of NAD+ biosynthesis (17). However, some degree of NAD+ transportation exists between cellular compartments (18). Targeting NMNATs for cancer therapy may circumvent the redundant metabolites feeding into NAD+ production, but to develop new therapies, we must first determine which NMNAT enzyme is required for cancer cells.

In this study, we integrated whole-genome CRISPR screening and pan-cancer genetic dependency mapping and identified NAMPT and NMNAT1 as AML dependencies governing NAD+ biosynthesis. LSCs had increased expression of NAMPT, which contributed to the higher levels of NAD+ than non-LSCs and promoted AML progression. While the presence of NAD+ precursors circumvented the requirement of NAMPT, AML remained dependent on NMNAT1, pointing to NMNAT1 as a gatekeeper of NAD+ metabolism. Deletion of NMNAT1 reduced nuclear NAD+, activated p53 due to attenuated deacetylation by SIRT6/7, and sensitized AML to venetoclax. In contrast to LSCs, which depend on NMNAT1 for their maintenance, normal hematopoiesis and hematopoietic stem cells (HSCs) did not depend on NMNAT1. Our study highlights NMNAT1 as a previously unidentified genetic dependency in AML LSCs that prevents p53 activation by promoting NAD+ biosynthesis.

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RESULTS

The NAD⁺ salvage pathway is required for AML

To identify novel genetic dependencies in AML, we performed a CRISPR dropout screening in the MOLM13 human AML cell line. We transduced a single clone of MOLM13 cells expressing Cas9 with a human genome-wide CRISPR library consisting of 90,709 single guide RNAs (sgRNAs) targeting 18,010 genes. The cells were harvested at 10 and 25 days after transduction for sequencing, and the sgRNA depletion and enrichment was analyzed with the MAGeek package (Fig. 1A; fig. S1, A and B; and table S1) (19). By comparing the sgRNA abundance at day 10 and input, we identified 1889 genes essential for AML [false discovery rate (FDR) < 0.25] (fig. S1C). These genes were enriched for essential biological processes, including nucleic acid synthesis, translational initiation, and ribosomal RNA processing, similar to a report from previously performed essentiality screen (20). Our screen also identified 1665 genes that were further depleted from days 10 to 25 (FDR < 0.25), which were enriched for metabolic processes, such as amino acid, coenzyme, folic acid, vitamin, and carboxylic acid metabolism (fig. S1C). We identified known positive regulators of AML such as BCL2, BRD4, MYC, FLT3, and DHODH, as well as negative regulators such as TP53, CDKN1B, and CDKN2C in this analysis (Fig. 1B).

To find novel therapeutic targets for AML, we used the drug gene interaction database (http://dgidb.org/search_categories) and found 430 genes from the dropout gene list that encode druggable targets (table S2). We focused our attention to the NAD⁺ biosynthesis pathway, which has been reported as a dependency in many solid tumors (15). We found that two genes involved in the NAD⁺ salvage pathway, Nicotinamide Phosphoribosyltransferase (NAMPT) and Nicotinamide Nucleotide Adenylyltransferase 1 (NMNAT1), exhibited significant dropout in the screen (Fig. 1, C and D). NAMPT is an enzyme that converts NAM to NMN, while NMNAT1 converts NMN to NAD⁺ (Fig. 1C).

To systematically analyze the dependency of all genes involved in the NAD⁺ biosynthesis pathway across broad types of cancer, we used the DepMap dataset (https://depmap.org/portal/) that curates whole-genome CRISPR screening data in more than 500 cancer cell lines (21). We found that more than 20% of all cancers depend on NAMPT and NMNAT1 but not NMNAT2, NMNAT3, or genes involved in the Preiss-Handler or the de novo synthesis pathways (Fig. 1E). Further analysis of the dependency across cancers revealed that leukemia and other hematologic malignancies, such as lymphoma and multiple myeloma, are highly dependent on these two genes compared to other cancers (Fig. 1F and fig. S1, D and E). Moreover, NAMPT and NMNAT1 exhibited remarkable codependency among 563 cancer cell lines, consistent with the notion that NAMPT and NMNAT1 function in the same pathway (Fig. 1G and fig. S1F). To validate the unique dependency of AML on NAMPT and NMNAT1 within other genes involved in NAD⁺ biosynthesis, we designed two sgRNA per gene for a total of 10 genes involved in the de novo, Preiss-Handler, and salvage synthesis pathways and assessed their requirement in MOLM13 cells. Deletion of NAMPT or NMNAT1, but not other genes that regulate NAD⁺ biosynthesis, reduced the number of edited cells over time, confirming their requirement in AML (Fig. 1, H and I). Corroborating the importance of the NAD⁺ biosynthesis in AML, we found that NAD⁺ is more abundant in L-GMPs, the LSC population in the MLL-AF9–induced AML murine model (22), than normal in GMPs (Fig. 1J). Patient-derived AML cells with different genotypes also had more NAD⁺ compared to cord blood CD34⁺ normal hematopoietic stem and progenitor cells (HSPCs) (Fig. 1K). Thus, our genome-wide CRISPR screen in AML and cancer dependency analysis across hundreds of cancer cell lines unveiled the requirement of two components in the NAD⁺ salvage pathway, NAMPT and NMNAT1, in AML.

NMNAT1 acts as a gatekeeper in NAD⁺ homeostasis and AML survival

NAMPT is considered to be the rate-limiting enzyme in the NAD⁺ salvage pathway and has been demonstrated as a therapeutic target in multiple cancers with several inhibitors being developed. However, clinical trials with these inhibitors achieved limited success (13), potentially due to uptake of NAD⁺ precursors that bypass the requirement of NAMPT (15). For example, NR is phosphorylated by NR kinases 1 and 2 (NMRK1/2) to produce NMN (independently of NAMPT), which is used by NMNAT for NAD⁺ biosynthesis (Fig. 1C) (17). Since NMNAT1 catalyzes the last step in NAD⁺ synthesis, in which NMN produced by NAMPT and NMRK is converted to NAD⁺, we hypothesized that NMNAT1 may constitute an AML dependency that cannot be bypassed by NAD⁺ precursors.

To test this hypothesis, we treated MOLM13 and OCI-AML2 AML cell lines with FK866, a specific inhibitor of NMNAT1, with or without NAD⁺ precursors NMN, NA, NR, or NAM. FK866 potently reduced cell viability of these two AML cell lines (Fig. 2A). However, supplementation of NAD⁺ precursors significantly reduced the toxicity of FK866 (Fig. 2A and fig. S2, A and B). This effect was not due to an off-target effect of FK866, since NAD⁺ precursors also rescued the diminished cell expansion following deletion of NMNAT1 with CRISPR-Cas9 (fig. S2C). Deletion of NMNAT1 with CRISPR-Cas9 also significantly impaired expansion of four AML (THP1, NB4, OCI-AML2, and MV4-11) and a CML (K562) cell lines, but not pancreatic or breast cancer cells (fig. S2, D and E). NAD⁺ precursors failed to improve the defective expansion of NMNAT1-deleted cells (Fig. 2B). Thus, NAD⁺ precursors available in physiological settings cannot rescue the dependency of AML on NMNAT1.

The catalytic activity of NMNAT1 is required for AML

Previous studies have shown that NMNAT1 localizes to the nucleus and binds both NMN and adenosine 5′-triphosphate (ATP) via its acetyltransferase domain to produce NAD⁺ (23–25). Consistently, NMNAT1 protein was localized to the nucleus of MOLM13 cells and its deletion significantly reduced nuclear NAD⁺ (Fig. 2, C and D). Moreover, screening the NMNAT1 protein domain essentiality with eight sgRNA sequences showed that sgRNAs targeting the acetyltransferase domain, but not the C-terminal tail, significantly reduced survival of AML cells (Fig. 2E). To specifically determine whether the catalytic activity of NMNAT1 is required for its function, we expressed murine Nmnat1 (which is resistant to sgRNA against human NMNAT1) with mutations in tryptophan-170 (W170A) or histidine-24 (H24A), which impair binding to NMN or ATP, respectively (24), into NMNAT1-ablated MOLM13 cells. While wild-type (WT) murine Nmnat1 largely rescued the decline of NMNAT1-ablated cells in culture, Nmnat1 with W170A or H24A mutations failed to rescue the phenotype, indicating that the catalytic activity of NMNAT1 is essential for its function (Fig. 2F and fig. S2F).

To determine whether NMNAT1 and its catalytic function are essential for AML in vivo, we first transplanted MOLM13 cells with or without ablation of NMNAT1 into immunocompromised mice.
Fig. 1. Whole-genome CRISPR screen identifies NAMPT and NMNAT1 as genetic dependencies in AML. (A) A schematic outline of the genome-wide CRISPR-Cas9 screen. (B) A volcano plot showing both positively and negatively selected genes (FDR < 0.25). (C) A schematic showing the three NAD⁺ biosynthesis pathways, with metabolites and enzymes in normal and italic font, respectively. (D) The read numbers of four sgRNAs against NAMPT (left) and NMNAT1 (right) at day 25 compared to input. (E) A scatter plot showing the correlation between the dependency score of NAMPT and NMNAT1 (n = 563). (H) Immunoblots of NAMPT (left) and NMNAT1 (right) in MOLM13 cells at day 4 after deleting these genes. (I) De novo, Preiss-Handler, and Salvage pathway. (J) Measurement of intracellular NAD⁺ amounts (left) and NAD⁺/NADH (reduced form of NAD⁺) ratio (right) in GMP and MLL-AF9–driven L-GMPs (n = 4). (K) Intracellular NAD⁺ amounts (left) and NAD⁺/NADH ratio (right) in human cord blood CD34⁺ HSPCs and PDX-derived AML samples (n = 4). All data represent mean ± SD; *P < 0.05, **P < 0.01, and ***P < 0.001 by unpaired Student’s t test unless otherwise noted. See also fig. S1.
Fig. 2. NMNAT1 deletion decreases nuclear NAD⁺ and activates the p53 pathway. (A) Viability of MOLM13 and OCI-AML2 cells treated with FK866 (10 μM) with or without NAD⁺ precursors (NMN, NA, NR, and NAM; 1 mM). (B) Viability of MOLM13 and OCI-AML2 cells transduced with NMNAT1_sg (+) or negative control sgRNA [NC_sg (−)] with or without NAD⁺ precursors (NMN, NA, NR, and NAM; 1 mM). (C) Immunofluorescence images of NMNAT1 in MOLM13 cells. (D) Nuclear NAD⁺ levels in MOLM13 cells after deleting NMNAT1 or a negative control (NC) locus. (E) CRISPR domain screening using sgRNAs targeting different exons of NMNAT1 in Cas9-expressing MOLM13 cells. (F) Relative expansion of MOLM13 cells transduced with sgRNA against NMNAT1 or NC with or without the indicated mouse Nmnat1 constructs. (G) Survival curves of NSG-SGM3 mice transplanted with control or NMNAT1-deleted MOLM13 cells with the indicated murine Nmnat1 constructs. (H and I) Cell cycle analysis by BrdU incorporation (H) and annexin V staining (I) of MOLM13 cells after deleting NMNAT1 or NC. (J) Immunoblotting showing phospho-p53, γH2AX, and NMNAT1 in MOLM13 cells after lentiviral deleting NMNAT1. (K and L) Annexin V staining (K) or relative expansion (L) of MOLM13 cells after deleting NMNAT1 with or without consecutive targeting of TP53. (M) Relative expression of p53 target genes after deleting NMNAT1. (N) Immunoprecipitation assay showing increased acetylated lysine of p53 in NMNAT1-deleted MOLM13 cells. (O) Relative expansion of NMNAT1-deleted MOLM13 cells expressing WT or K320/373/381R-TP53 constructs. (P) Competitive growth assay after deleting the indicated genes. All data represent mean ± SD; *P < 0.05, **P < 0.01, and ***P < 0.001 by unpaired Student's t test unless otherwise noted. See also fig. S2.
In contrast to mice transplanted with control MOLM13, which all developed AML within 33 days after transplantation, mice infused with NMNAT1-ablated MOLM13 rarely developed AML (Fig. 2G). This defective leukemogenesis of NMNAT1-ablated MOLM13 cells was rescued by expressing murine WT Nmnat1 but not Nmnat1 with mutations (W170A or H24A) in the catalytic domain (Fig. 2G and fig. S2G). Together, these results establish that the catalytic function of NMNAT1 is essential for AML.

**NMNAT1 deletion activates p53 in AML**

We then determined whether NMNAT1 regulates cell cycle, apoptosis, or differentiation of AML cells. Bromodeoxyuridine (BrdU) incorporation assays showed that NMNAT1-deficient AML cells exhibited increased proportion of G0/G1 phase and reduced S phase, suggesting that AML undergoes cell cycle arrest (Fig. 2H). Moreover, deletion of NMNAT1 caused apoptosis (Fig. 2I). Gene expression and flow cytometry analyses shortly after NMNAT1 deletion did not detect significant increases in myeloid transcription factors or differentiation markers (fig. S2H). Immunoblotting showed that NMNAT1-deficient AML cells had increased levels of phospho-p53 and DNA damage response marked by γH2AX (Fig. 2J and fig. S2I). Expression of WT murine Nmnat1 diminished induction of γH2AX, demonstrating that off-target effects of the CRISPR-Cas9 system did not induce the DNA damage response in NMNAT1-deficient cells (fig. S2J).

To determine whether p53 mediates apoptosis upon NMNAT1 deletion, we simultaneously deleted NMNAT1 and TP53 with a dual guide CRISPR method. Codelletion of TP53 significantly reduced the frequency of apoptotic cells and improved the survival of NMNAT1-ablated AML cells, although NMNAT1/TP53 double-mutant cells were gradually outcompeted by unedited cells (Fig. 2, K and L). Consistent with the activation of p53 in NMNAT1-deficient AML, we detected increased expression of p53 target genes, such as CDKN1A (p21), NOXA, BAX, and PUMA (Fig. 2M and fig. S2K). We found that NMNAT1-deficient AML cells have increased acetylation of p53 (Fig. 2N and fig. S2L). Acetylation of p53 increases its DNA binding and transcriptional activity and is governed by acetyltransferases [p300/CREB-binding protein (CREB) and TIP60/MOF] and deacetylases, including NAD+−dependent sirtuins that are localized to the nucleus: SIRT1, SIRT6, and SIRT7 (26–30). We thus deleted SIRT1, SIRT6, or SIRT7 to examine whether they regulate apoptosis in AML. Deletion of SIRT6 or SIRT7, but not SIRT1, increased apoptosis and impaired the expansion of AML cells (Fig. 2O and fig. S2M). Consistent with prior findings, p53 interacted with SIRT6/7 in AML (fig. S2N). Mutating lysine-320, -373, and -381 of p53, the known SIRT6/7 deacetylation sites, to arginine (K320/373/381R) to prevent acetylation diminished induction of γH2AX, suggesting that AML undergoes cell cycle arrest (Fig. 2H). Moreover, NMNAT1-deficient AML cells were sensitive to venetoclax, exhibiting diminished cell viability even at doses that minimally affected unedited AML cells (Fig. 3G). Consistently, NAMPT and NMNAT1 were included in the dropout gene list in a recent CRISPR screen searching for genes that rendered AML cells sensitive to venetoclax when deleted (fig. S3B) (36). As shown before (32, 33, 36), TP53-deficient AML cells were resistant to venetoclax (fig. S3C). However, TP53 deletion was only able to partially rescue the venetoclax sensitivity of NMNAT1-deleted cells, and NMNAT1/TP53 double-knockout (KO) cells eventually became exhausted (Fig. 2L and fig. S3D). Together, these results establish the association between NAD+ levels and venetoclax resistance in AML.

**NMNAT1 deletion suppresses leukemogenesis in vivo**

To examine whether NMNAT1 regulates leukemogenesis, we deleted Nmnat1 or the control Rosa26 locus from murine MLL-AF9−induced AML cells by CRISPR and transplanted them into irradiated mice, as previously described (37, 38). Immunoblotting confirmed efficient deletion of NMNAT1 protein (fig. S4A). Ablation of Nmnat1 significantly delayed the onset of leukemogenesis, ameliorated leukocytosis, and reduced the frequency of AML cells marked by green fluorescent protein (GFP) expression, indicating that Nmnat1 is required for leukemogenesis in mice (fig. S4, B and C).

We then examined the Nmnat1 dependency of AML using a conditional KO model (39). We crossed Nmnat1fl+ mice with Mxl-Cre mouse to generate Mxl-Cre;Nmnat1fl−/fl, Mxl-Cre;Nmnat1fl+/fl, and control mice (Mxl-Cre;Nmnat1fl+/fl, Nmnat1fl+/fl, or Nmnat1fl−/fl), which were treated with polyinosinic:polycytidylic acid [poly(I:C)] to induce Cre (hereafter referred to as Nmnat1 KO, Het, and WT). We confirmed efficient deletion of the floxed exon in peripheral blood (PB) by polymerase chain reaction (PCR) (fig. S4D). We then isolated HSPCs and transduced the cells with MLL-AF9 retrovirus...
Fig. 3. NMNAT1 deletion sensitizes AML cells to venetoclax. (A) waterfall plot showing the sum of drug metabolite Pearson correlation in 53 hematopoietic malignancy cell lines. Pearson correlation r value was calculated between the concentration of metabolite and drug IC50 in the 53 cell lines. The x axis represents 225 metabolites, and the y axis represents the sum of all Pearson correlation r values for all the 395 drugs. The bar in red color indicates NAD+, which is ranked fourth among 225 metabolites. (B) waterfall plot showing the Pearson correlation r values (x axis) between the concentration of cellular NAD+ and IC50 of 395 drugs (y axis) in 53 hematopoietic malignancy cell lines. The bars in red color indicate venetoclax, which is ranked first among 395 drugs. (C) Plot showing the Pearson correlation between cellular NAD+ levels and venetoclax IC50 from 45 hematopoietic malignancy cell lines. Each dot represents one cell line. (D) Frequency of apoptotic MOLM13 cells treated with venetoclax (Ven, 1.5 to 3 mM) with or without NMN or NAM (1 mM) for 48 hours. (E) Effect of Ven (3 mM) on cell growth of MLL-AF9–driven L-GMPs with or without NMN or NAM (1 mM) supplementation for 72 hours. (F) Cell viability of MOLM13 cells treated with FK866 (0.625 to 2.5 mM) or Ven (0.75 to 3 mM) alone or in combination for 48 hours. (G) Cell viability of MOLM13 cells after deleting NMNAT1 or the control locus and treated with the indicated concentration of Ven for 48 hours. All data represent mean ± SD; *P < 0.05, **P < 0.01, and ***P < 0.001 by unpaired Student’s t test unless otherwise noted. See also fig.S3.

We found the AML model induced by the MOZ-TIF2 oncogene also required Nmnat1, indicating the requirement of Nmnat1 is not specific to the MLL-AF9–induced AML model. As shown before, transplantation of HSPCs transduced with MOZ-TIF2–expressing retrovirus caused lethal AML (6, 40). Similar to the MLL-AF9–induced AML model, we found that AML caused by MOZ-TIF2 exhibited increased NAD+ levels and NAD+/NADH (reduced form of NAD+) ratio compared to normal BM cells (fig. S4G), and deleting Nmnat1 significantly delayed the onset and attenuated the penetrance of AML (fig. S4H).

Next, we examined whether established AML depends on Nmnat1. We first established AML by transforming Mx1-Cre;Nmnat1fl/fl and Mx1-Cre;Nmnat1β/β cells [before poly(I:C)] with MLL-AF9 and transplanting cells into irradiated mice. We next transplanted AML from these primary recipient mice into secondary recipient mice, which we treated with poly(I:C) to delete Nmnat1. Deleting Nmnat1 in established AML significantly reduced the frequency of AML cells in the PB and extended survival (Fig. 4, I and J), demonstrating that NMNAT1 plays an important role in the maintenance of MLL-AF9–driven AML.

Our findings that NAD+ levels correlate with venetoclax resistance and that deleting NMNAT1 sensitized AML cells to venetoclax in vitro (Fig. 4) prompted us to test whether reducing the function
of NMNAT1 sensitizes AML to venetoclax in vivo. We thus generated cohorts of recipient mice transplanted with Nmnat1 WT or Het AML driven by MLL-AF9. These recipient mice were then treated with or without venetoclax (100 mg/kg per day) from days 5 to 21 after transplantation. Venetoclax treatment significantly reduced the frequencies of AML cells in the blood of both Nmnat1 WT and Het AML recipient mice (Fig. 4K) and extended median leukemia-free survival of Nmnat1 WT AML recipient mice from 25 to 32 days.
However, venetoclax was more potent in extending the median survival of mice with Nmnat1 Het AML (38.5 to 60 days; Fig. 4L). These effects correlated with reduced numbers of AML cells in the blood of Nmnat1 Het AML recipients treated with venetoclax compared to Nmnat1 WT recipients (Fig. 4K). Thus, reducing NMNAT1 function sensitizes AML to venetoclax.

We then examined whether NMNAT1 is required for human AML development in vivo. To this end, we generated two AML patient-derived xenograft (PDX) models, one with t(9;11) translocation resulting in a MLL-AF9 fusion gene and another with a FLT3-ITD mutation (41, 42). Transplanting these patient-derived cells into immunocompromised NSG-SGM3 mice led to lethal AML (7, 43). Electroporation of Cas9 protein together with sgRNA against NMNAT1 (or negative control) resulted in efficient deletion of NMNAT1 gene, leading to the depletion of approximately 90% of NMNAT1 protein and impaired expansion in vitro (fig. S4I). In both PDX models, NSG-SGM3 mice receiving NMNAT1-edited cells had reduced AML expansion in the BM and CD34+/CD38− LSCs, as well as extended survival compared to mice receiving AML edited for a control locus (Fig. 4, M to P, and fig. S4, J and K). Together, these AML PDX studies establish that NMNAT1 promotes human AML.

**NMNAT1 is dispensable for HSCs**

To better understand the role of NMNAT1 in normal hematopoiesis, we first measured the level of Nmnat1 mRNA in hematopoietic populations including HSCs, HSPCs, and mature hematopoietic cells. Compared to whole BM, the expression of Nmnat1 mRNA was higher in HSCs, immature HSPCs, lymphocytes, and erythroid cells (Fig. 5A). We next determined the impact of Nmnat1 deletion on hematopoiesis by analyzing Mx1-Cre;Nmnat1fl/fl, Mx1-Cre;Nmnat1+/fl, and Mx1-Cre;Nmnat1+/+ mice. We confirmed deletion of Nmnat1 after poly(I:C) induction by PCR analysis in HSC-derived colonies (fig. S5A). Four months after poly(I:C) injection, PB analyses of Nmnat1 Het and KO mice showed comparable numbers of white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), and slightly increased platelets (PLT) compared to Nmnat1 WT mice (Fig. 5B). Nmnat1 KO mice had fewer B cells and more T and myeloid cells in PB and a modest increase in BM myeloid cells compared to WT mice, although loss of Nmnat1 did not affect the total number of BM cells (Fig. 5, C and D, and fig. S5B). Consistent with increased myeloid cells in BM, Nmnat1-deficient mice showed increased frequency of myeloid progenitors (granulocyte-macrophage progenitors and common myeloid progenitors) but did not change the frequencies of immature HSPCs in the BM, including HSCs and multipotent progenitor cells (MPPs) (Fig. 5, E and F). Thus, HSCs and steady-state hematopoiesis are maintained in the absence of Nmnat1.

Last, to investigate the regenerative potential of Nmnat1-deficient HSCs, we performed long-term competitive transplantation assays by transplanting whole BM cells from Nmnat1 WT, Het, and KO mice into lethally irradiated recipient mice. Nmnat1 KO cells exhibited multilineage long-term reconstitution of all three lineages studied (B, T, and myeloid cells), with only a trend toward reduced B cell lineage contribution, consistent with the reduced B cells in the PB of Nmnat1 KO mice (Fig. 5G). Genotyping of the donor-derived cells showed that regenerating cells were deleted for Nmnat1 (fig. SSC). Together, these results establish that Nmnat1 is dispensable for normal hematopoiesis.

**DISCUSSION**

Understanding genetic dependency in AML and the mechanisms by which these dependencies drive disease progression is critical to developing novel therapeutics. Here, through a whole-genome CRISPR screening and pan-cancer dependency mapping analysis, we identify NMNAT1 as a previously unidentified therapeutic target for AML. Disruption of NMNAT1 reduced nuclear NAD+ levels and induced apoptosis partially through inactivation of sirtuin family proteins, particularly SIRT6/7, which deacetylates and keeps p53 under control (Fig. 5H). Deletion of NMNAT1 suppressed AML by reducing LSCs in both mouse and PDX models and sensitized AML to venetoclax treatment. The finding that NMNAT1 is dispensable for normal hematopoiesis further supports NMNAT1 as a therapeutic target for AML.

Past studies have highlighted the dependence of many cancers on NAD+ metabolism, with particular focus on NAMPT, an enzyme considered to be rate limiting in the NAD+ salvage pathway (15, 44). These studies led to the development of several small molecular inhibitors targeting NAMPT. However, NAMPT inhibitors had little success in clinical trials for several solid tumors (13), suggesting that targeting other components in the NAD+ biosynthesis pathway may achieve better responses. Our findings reveal that NAD+ precursors present in the serum and diet can substantially rescue the antineoplastic effects of NAMPT deletion or pharmacological inhibition. These results suggest that NAD+ precursors can maintain levels of NAD+ independently of NAMPT. However, NAD+ precursors failed to rescue the effects of NMNAT1 deletion, establishing NMNAT1 as an indispensable gatekeeper in NAD+ biosynthesis. Since the enzymatic activity of NMNAT1 is required for its function, NMNAT1 inhibitors may prove effective in suppressing NAD+ biosynthesis in cancer cells even in the presence of physiological NAD+ precursors.

Our study points to a role for NAD+-dependent deacetylases SIRT6/7 as potential key downstream targets of NMNAT1 in AML. In particular, we found that deletion of SIRT6 or SIRT7 suppressed AML cells in vitro. SIRT6/7 has been shown to interact with and deacetylate p53 (29, 30) to attenuate p53-dependent apoptosis (27, 28). Consistently, we showed that Nmnat1 deletion increased p53 acetylation and expression of p53 target genes involved in apoptosis. Moreover, deletion of TP53 in NMNAT1-deficient AML partially suppressed the increased apoptosis observed in NMNAT1-deficient cells. We note that the rescue by TP53 deletion was partial and do not exclude the possibility that other NAD+-dependent enzymes, such as the PARPs or NAD+-dependent dehydrogenases, are also affected by NMNAT1-mediated NAD+ biosynthesis. Our findings nonetheless demonstrate that a previously unidentified pathway consisting of NMNAT1-SIRT6/7-p53 mediates cell survival of AML.

A recent paper demonstrated that NAM mediates resistance to venetoclax in relapsed/refractory AML (45), which is consistent with our findings that NAD+ metabolism confers venetoclax resistance in AML. Jones et al. demonstrated that LSCs from relapsed/refractory AML have higher NAM and NAD+ levels than de novo AML LSCs, and that NAM promotes amino acid uptake and catalysis by the TCA cycle, a mitochondrial metabolic pathway suppressed by venetoclax (45, 46). Conversely, LSCs from relapsed/refractory AML were more sensitive to NAMPT inhibition than those from de novo AML. The mechanisms by which cells maintain mitochondrial NAD+ remains unclear, given that deletion of NMNAT3, which was considered to be exclusively localized to the mitochondria, did not affect mitochondrial NAD+ levels in mice (25, 47). Our study with mouse models does not distinguish the effects of targeting
Fig. 5. Nmnat1 is dispensable for normal hematopoiesis. (A) Quantitative PCR showing the relative expression levels of Nmnat1 in murine hematopoietic cell populations in the BM compared to whole BM (WBM) (n = 3). (B and C) Complete blood cell counts (B) and frequencies of B cell, T cell, and myeloid cell (C) in PB of Nmnat1 WT (Nmnat1+/+), Het (Mx1-Cre; Nmnat1 +/fl), and KO (Mx1-Cre; Nmnat1 fl/fl) mice at 4 months after poly(I:C) injection (n = 8 to 12). BM cellularity of poly(I:C)-treated Nmnat1 WT, Het, and KO mice (n = 5 to 6). (F) Frequencies of LSK, HSC, MPP, HPC1, and HPC2 in the BM of poly(I:C)-treated Nmnat1 WT, Het, and KO mice (n = 5 to 6). (G) Competitive BM transplantation with 500,000 Nmnat1 WT, Het, or KO BM cells (CD45.2+) along with 500,000 competitor BM cells (CD45.1+) into lethally irradiated recipient mice. Graphs show the overall percentage of CD45.2+ cells and those in B, T, and myeloid lineages (n = 5 to 6). (H) Model for the role of NMNAT1 in AML and venetoclax resistance.

NAD+ biosynthesis on de novo and relapsed/refractory AML. However, our finding that TP53-mutant AML, which are often refractory to treatments (48, 49), still requires NMNAT1 suggests that relapsed/refractory AML are dependent on NMNAT1-mediated NAD+ homeostasis, consistent with (45). Our data supports the model that impairing nuclear NAD+ homeostasis by targeting NMNAT1 activates TP53, which sensitizes cells to venetoclax (32, 33, 36). NAD+ in other cellular compartments such as the cytosol and mitochondria is also likely to promote cellular homeostasis in AML. Our findings that NMNAT1 is dispensable for HSCs provide a rationale to target nuclear NAD+ homeostasis as a therapeutic liability of AML.

MATERIALS AND METHODS

Mice
Mice were housed in The Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-accredited, specific pathogen–free animal care facilities at the Baylor College of Medicine, and the Institutional Animal Care and Use Committee (IACUC) at Baylor College of Medicine approved all animal protocols. Mice were genotyped by Southern blotting or PCR from tail genomic DNA (see Materials and Methods). All animal experiments were performed in specific pathogen–free animal care facilities at the Baylor College of Medicine.

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Cell lines

The 293T cells were purchased from American Type Culture Collection (catalog no. CRL-3216) and cultured in Dulbecco’s modified Eagle medium (DMEM). The leukemia cell lines MOLM13 and OCI-AML2 cells (from M. Goodell) were cultured in RPMI-1640 or minimum essential medium α, all supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Pen/Strep) (all reagents from Gibco). Cells were cultured in a humidified atmosphere containing 5% CO₂ at 37°C. All cell lines tested negative for mycoplasma using a PCR-based method.

Whole-genome CRISPR screening

Whole-genome CRISPR screening was performed as previously described, with minor modifications (51). First, lentiviral stocks of Cas9 (Addgene no. 68343, pKLV2-EFlαBsd2ACas9-W), Cas9 reporters (Addgene nos. 67979 for negative control and 67980 for targeting GFP expression), and a whole-genome sgRNA library (Addgene no. 67989) were generated by transfecting 293T cells with 5.4 μg of transfer plasmid, 5.4 μg of pSAPAX2 (Addgene no. 12260) packaging plasmid, and 1.2 μg of pMD2.G (Addgene no. 12259) envelope plasmid in a 10-cm culture plate using a polyethylenimine (PEI) transfection protocol. Sodium butyrate (5 mM) was added into the 293T cells 24 hours after transfection to increase lentivirus production. Culture medium was collected at 48 and 72 hours after transfection, pooled together, passed through a 0.45-μm filter, precipitated with polyethylene glycol, molecular weight 6000 (PEG-6000) solution [PEG-6000 (80 mg/ml), 100 mM NaCl, and 10 mM Heps-NaOH (pH 7.4)], and resuspended in culture media. We transduced leukemia cell lines including MOLM13 with the Cas9 lentivirus and performed blastocidin selection (10 μg/ml) 3 days after transduction to select for Cas9-expressing cells. After selection, the Cas9-expressing MOLM13 cells were single cell–sorted into 96-well plates and expanded. We functionally analyzed clonally derived Cas9-expressing cell lines by transduction with Cas9 reporter lentiviral vectors (Addgene nos. 67979 for negative control and 67980 for targeting GFP expression). We analyzed the ratio of blue fluorescent protein–positive (BFP⁺) single-positive and GFP⁺BFP⁺ double-positive cells on a BD LSRFortessa instrument (BD) at 3 to 4 days after transduction. Clones with the highest Cas9 activity (as assessed by the frequency of GFP⁺BFP⁺ double-positive cells) were used for the screen.

We performed the whole-genome CRISPR screen by transducing 3 × 10⁷ Cas9-expressing MOLM13 cells with the genome-wide sgRNA lentivirus at a transduction efficiency of 25 to 35% at 3 days after transduction, which was measured by BFP expression to achieve an average coverage of more than 100× per sgRNA. Three independent infections were conducted for both Cas9-expressing and Cas9-negative MOLM13 cells. Three days after transduction, the cells were treated with puromycin (1.5 μg/ml; Sigma-Aldrich) for 4 days. Approximately 10⁷ cells were harvested at day 10 after transduction for Cas9-negative MOLM13 cells to obtain input genomic DNA (gDNA). Cas9-expressing cells with the sgRNA library were cultured for up to 25 days with 5 × 10⁷ cells being seeded at every passage and 10⁶ cells harvested for gDNA extraction. We collected cells at days 10 and 25 for sequencing. gDNA was purified by phenol-chloroform extraction and ethanol precipitation, and 130 mg of gDNA was used to amplify sgRNA inserts using KAPA HiFi HotStart DNA Polymerase (Kapa Biosystems) with the primers listed in table S5. Illumina adapters (Illumina) were added during the second round of PCR. Resulting PCR products were purified and sequenced on a NextSeq500 sequencer (Illumina). We counted the numbers of reads for each guide with an in-house script. Enrichment and depletion of guides and genes were analyzed using MAGeCK (19).
Flow cytometry

All BM or PB cells were prepared and analyzed as described previously (6, 52). BM cells were isolated by crushing the long bones (tibias and femurs), pelvic bones, and spine with a mortar and pestle in Hank’s buffered salt solution (HBSS) without calcium and magnesium, supplemented with 2% heat-inactivated bovine serum (Gibco). Cells from BM or PB were filtered through a 100-μm nylon screen (Sefar America) to obtain single-cell suspension and then stained with antibodies.

To identify HSC (CD150° CD48° Lineage° Sca-1° c-kit°), MPP (CD150° CD48° Lineage° Sca-1° c-kit°), HPC1 (CD150° CD48° Lineage° Sca-1° c-kit°), and HPC2 (CD150° CD48° Lineage° Sca-1° c-kit°), BM cells were stained with antibodies against phycocerythrin (PE)–cyanine 5(Cy5)–conjugated anti-CD150, PE–Cy7–conjugated anti-CD48, allophycocyanin (APC)–conjugated anti–Sca-1, and biotin–conjugated anti–c-kit, together with PE–conjugated antibodies against the following lineage markers: CD2, CD3, CD8, CD127, and PE–conjugated lineage markers: Ter119, B220, Gr-1. We visualized biotin–conjugated antibody using streptavidin–conjugated APC–Cy7. For HSPC sorting, BM cells were positively selected for c-kit° HSPCs with anti-biotin microbeads and autoMACS (Miltenyi Biotec). To identify CMP (CD34 + CD16/32 − 4° CD45.2 antibodies; the remainder of the staining was described above. To analyze hematopoietic lineage composition in BM or PB, red blood cells were lysed with an ACK buffer and PE–conjugated anti-CD3 antibody for T cells. To distinguish stained with APC–conjugated anti-CD11b and PE–Cy7–conjugated position in BM or PB, red blood cells were lysed with an ACK buffer.

Xenotransplantation experiments

We generated the stable mouse Nmnat1 (Nmnat1) WT, H24A- or W170A-expressing MOLM13 cells through retrovirus transduction according to the protocol described above. MOLM13 cells were then transduced with NC_sg or NMNAT1_sg lentivirus (both expressing GFP) as negative control) or NMNAT1 (a gene involved in amelogenesis as negative control) or NMNAT1.

Colony-forming assays

Cells were cultured in MethoCult M3434 (catalog no. 03434, STEMCELL Technologies) supplemented with 1% Pen/Strep (Gibco). Single HSCs from Nmnat1 KO mice 4 months after poly(I:C) injection were sorted into 96-well plates and cultured for 7 days to form colonies, which were then used for genotyping.

Poly(I:C) and venetoclax treatments

Poly(I:C) (Amershams, Piscataway, NJ) was suspended in phosphate-buffered saline (PBS) at 50 μg/ml, and mice were injected intraperitoneally with 0.5 μg/g of body mass every other day for 6 days. AML recipient mice were treated with vehicle or venetoclax (100 mg/kg; LC Laboratories) daily by oral gavage from days 5 to 21.

Gene editing with Cas9-sgRNA ribonucleoprotein complex

sgRNA against murine Rosa26 (negative control) and Nmnat1 as well as human ENAM (negative control) and NMNAT1 were designed according to CRISPR DESIGN (http://crispr.mit.edu/). We cloned sgRNA with a T7 promoter sequence and transcribed using the HiScribe T7 In Vitro Transcription Kit (NEB, Ipswich, MA). We
introduced the Cas9-sgRNA complex to murine AML cells as previously described (38, 53). In brief, 1 μg of Cas9 protein (PNA Bio, Newbury Park, CA) was incubated with 1 μg of in vitro–transcribed sgRNA at room temperature for 5 min before being electroporated for murine AML cells (10⁵ cells per reaction) using the Neon Transfection System (MPK1096, Thermo Fisher Scientific) using the following parameters: 1700 V, 20 ms, and 1 pulse. Human AML cells were electroporated with the following parameters: 1350 V, 20 ms, and 1 pulse. Cells were then cultured for at least 12 hours before subsequent assays including transplantation, cell counting, and immunoblotting.

Cell cycle, cell apoptosis, and differentiation analyses

Cas9-expressing MOLM13 cells were transduced with sgRNA retroviruses targeting negative control or NMNAT1. We analyzed cell cycle and apoptosis on day 6 after transduction, while differentiation analyses were performed on days 6 and 11 after transduction. For cell cycle analysis, a BrdU incorporation assay was performed using a BrdU flow kit following the manufacturer’s specification (BD Pharmingen). Cells were pulsed with 10 μM BrdU for 1 hour in culture and then fixed with Cytofix/Cytoperm buffer for 30 min on ice. After permeabilization for 10 min, the cells were refixed with the Cytofix/Cytoperm buffer for 5 min on ice. Next, the cells were treated with deoxyribonuclease (300 μg/ml in PBS) for 1 hour at 37°C to expose incorporated BrdU, followed by staining with fluorescence isothiocyanate (FITC)–conjugated anti-BrdU antibody and PI for 1 hour at room temperature. We used FITC-conjugated annexin V (BD) to identify apoptotic cells. Cell differentiation analyses were performed with APC-conjugated antibodies against CD11b, CD14, CD15, or CD33.

Drug response experiments

The stock solutions of NAM (Sigma-Aldrich, catalog. no. N0636); β-NMN (Sigma-Aldrich, catalog no. N3501), NA (Selleck Chemicals, catalog no. S1744), and NR (Cayman Chemicals, catalog no. 23132) were prepared in water, and FK866 (Millipore Sigma, catalog no. 481908) and venetoclax (LC Laboratories, catalog no. V-3579) were dissolved in dimethyl sulfoxide (DCM). The drug response experiments were performed with a CellTiter 96 aqueous one solution cell proliferation assay (Promega) or by cell counting with a hemocytometer. Leukemia cells transduced with sgRNA lentiviruses were sorted on day 2 after transduction and treated with the indicated drugs at days 4 to 6 after transduction with seeding density of 10⁴ cells in a final volume of 100 μl per well in 96-well flat-bottom plates. After 48 hours of treatment, 20 μl of Aqueous One Solution Reagent (Promega) was added into each well of the 96-well assay plates, followed by incubation at 37°C for 4 hours. Cell viability was measured by recording the absorbance at 490 nm using Infinite M200 PRO (Tecan). The viability data were normalized to that of cells treated with vehicle alone.

Primary L-GMP cells were sorted from MLL-AF9 induced AML mice and cultured in X-Vivo 15 (Lonza), supplemented with mouse IL-3 (10 ng/ml; Peprotech), 10% FBS, and 1% Pen/Strep. Human AML cells were cultured in Iscove’s modified Dulbecco’s medium (Gibco), supplemented with human SCF (100 ng/ml), granulocyte colony-stimulating factor (20 ng/ml), IL-3 (20 ng/ml), FLT3 ligand (50 ng/ml; Peprotech), 15% FBS, and 1% Pen/Strep.

Immunofluorescence staining

MOLM13 cells were fixed with cold methanol at −20°C. Cells were cytospun to a slide and blocked in PBS containing 4% goat serum, BSA (4 mg/ml), and 0.1% NP-40, followed by staining overnight at 4°C with rabbit anti-NMNAT1 antibody (1:200, no. 98354, D7O4N, Cell Signaling Technology) diluted in blocking buffer. Primary antibody staining was developed with an anti-rabbit Alexa Fluor 488–conjugated secondary antibody (1:500) together with DAPI (2 μg/ml). Slides were analyzed on a Leica DMi6000 fluorescence microscope and a LAS software.

Immunoblotting

Cells were lysed with radioimmunoprecipitation assay (RIPA) buffer supplemented with a Halt Protease and Phosphatase inhibitor cocktail (Thermo Fisher Scientific) and phenylmethylsulfonyl fluoride (PMSF; Sigma-Aldrich) for 30 min on ice, followed by centrifugation at 15,000 rpm for 10 min at 4°C to remove cell debris. Protein concentration was measured using a Pierce BCA protein assay kit (Thermo Fisher Scientific), and the lysates were then boiled at 95°C in 1× lithium dodecyl sulfate (LDS) loading buffer (Life Technologies) for 5 min. Protein were separated on a bis-tris polyacrylamide gel (Life Technologies) and transferred to a polyvinylidene difluoride membrane (Millipore). Membranes were blocked in 5% (w/v) nonfat milk in 1× PBST (PBS with 0.1% Tween-20) for 1 hour at room temperature and then incubated overnight at 4°C with the following primary antibodies: rabbit anti-NMNAT1 (no. 98354, Cell Signaling Technology, 1:1000, for detection of human NMNAT1), rabbit anti-NMNAT1 (NB2-32107, Novus Biologicals, 1:1000, for detection of mouse NMNAT1), rabbit anti-NAMPT (no. 61122, Cell Signaling Technology, 1:1000), rabbit anti-p53 (no. 9282, Cell Signaling Technology, 1:1000), rabbit anti-p53 phospho-Ser 15 (no. 9284, Cell Signaling Technology, 1:1000), rabbit anti–acetylated lysine (no. 9441, Cell Signaling Technology, 1:1000), rabbit anti-FLAG M2 (no. 2368, Cell Signaling Technology, 1:1000), rabbit anti–γH2AX Ser 139 (no. 2577, Cell Signaling Technology, 1:1000), rabbit anti-SirT6 (no. 12486, Cell Signaling Technology, 1:1000), rabbit anti-SirT7 (no. 5360, Cell Signaling Technology, 1:1000), and mouse anti–β-actin (A1978, Sigma-Aldrich, 1:2000). After incubation with appropriate anti-mouse or anti-rabbit horseradish peroxidase–conjugated secondary antibodies (Cell Signaling Technology, 1:5000 to 1:10,000), we detected and acquired signals with a SuperSignal West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific, catalog no. 34580) and a KODAK X-OMAT 2000 Processor (Kodak).

Immunoprecipitation

We obtained protein lysates as described above. Primary antibodies or appropriate control immunoglobulin G was incubated with Protein G magnetic beads (Thermo Fisher Scientific) for 1 hour at 4°C, washed twice with RIPA buffer, and incubated with protein lysates overnight at 4°C. The beads were washed three times, resuspended in 1× LDS loading buffer, boiled for 5 min, and analyzed by immunoblotting.

NAD⁺/NADH measurement

We measured intracellular or nuclear NAD⁺/NADH levels using a NAD⁺/NADH Colorimetric Assay Kit Ver. 2 (CY-1253 V2, MBL) according to the manufacturer’s instructions. To measure nuclear NAD⁺, cells were incubated with Triton X-100 extraction buffer [PBS containing 0.5% Triton X-100 (v/v), 2 mM PMSF, 0.02% Na3 (w/v), 1 mM DDT, and 1× Halt Protease and Phosphatase inhibitor cocktail (at a cell density of 10⁴ cells/ml)] on ice for 10 min, followed by centrifugation for 10 min at 1000g at 4°C to isolate pelleted
nuclear fractions. Cells or the nuclear fractions were incubated with NAD\(^+\) extraction solution (0.5 M HClO\(_4\)) on ice or with NADH extraction solution (50 mM NaOH and 1 mM EDTA) at 60°C for 30 min, followed by neutralization with 0.55 M K\(_2\)CO\(_3\) (for NAD\(^+\) measurement) or 0.3 M potassium phosphate buffer (pH 7.4, for NADH measurement). The neutralized cell extracts were centrifuged at 15,000 rpm for 5 min at 4°C. The supernatants were incubated with NAD\(^+\)/NADH reaction mixture and the reading was taken at 450-nm absorbance. Data were normalized on the basis of protein concentration and expressed as picomole NAD\(^+\) per milligram of protein or as a ratio of NAD\(^+\)/NADH.

RNA-sequencing analysis
MOLM13 cells 4 days after NMNAT1 deletion were sorted into TRIzol, and RNA were extracted using a RNeasy MiniElute Cleanup kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. Complementary DNA (cDNA) libraries were generated using the Smart-seq2 protocol. Sequencing libraries were generated from 250 pg of amplified cDNA using a Nextera XT kit according to the manufacturer’s instructions. Barcoded libraries were purified and sequenced on a NextSeq platform. Raw sequencing data were converted to fastq files and aligned to the reference genome (hg38) using the Spliced Transcripts Alignment to a Reference (STAR) algorithm. DESeq2 was used for data processing, normalization, and differential expression analysis.

Quantitative real-time PCR
Cells were sorted into TRIzol (Thermo Fisher Scientific), and RNA was isolated according to the manufacturer’s instructions. cDNA was made with random primers and SuperScript IV reverse transcriptase (Thermo Fisher Scientific). We performed quantitative PCR using ViiA7 Real-Time PCR System (Thermo Fisher Scientific) with a SYBR Green master mix. Analysis was performed in triplicate by the \(2^{-\Delta\Delta C(T)}\) method, and \(\beta\)-actin or GAPDH was used as the housekeeping gene. Primers are listed in table S5.

Integrating metabolites and drug sensitivity in cancer cell lines
We downloaded the IC\(_{50}\) value for each drug of cancer cell lines (GDSC1 and GDSC2) from the GDSC (Genomics of Drug Sensitivity in Cancer) database (https://cancerrxgene.org/) (34). The intracellular metabolites level in cancer cell lines were from a previously published paper (35). We filtered out nonhematological malignancy cell lines. Calculation of Pearson correlations, \(P\) values, and visualization were performed using R. The correlation heatmap was row sorted by the row-wise sum of correlations.

Dependency scores for NAD\(^+\) biosynthesis genes
The dependency scores for NAD\(^+\) biosynthesis genes were downloaded from the DepMap dataset (https://depmap.org/) and further analyzed on the basis of subsets of hematopoietic malignancies, including leukemia, lymphoma, and multiple myeloma.

Quantification and statistical analysis
Data were analyzed with Stata, and the figures were generated with Prism software (GraphPad) and were presented as the mean ± standard deviation, unless otherwise stated. Paired or unpaired Student’s \(t\) test (two sided), Pearson’s \(\chi^2\) test, or Mantel-Cox log rank were performed to determine statistical significance. Statistical details are described in the

**SUPPLEMENTARY MATERIALS**
Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/30/eabf3895/DC1

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