Metabolic reprogramming by PRDM16 drives cytarabine resistance in acute myeloid leukemia

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Abstract

Acute myeloid leukemia (AML) patients with high *PRDM16* expression frequently experience induction failure and have a poor prognosis. However, the molecular mechanisms underlying these clinical features remain elusive. We found that murine AML cells transformed by *MLL::AF9* fusion and oncogenic short-isoform Prdm16 overexpression (hereafter, *MF9/sPrdm16*) exhibited resistance to cytarabine (AraC), but not to anthracycline, both *in vitro* and *in vivo*. Intriguingly, *MF9/sPrdm16* cells displayed a gene expression signature of high oxidative phosphorylation (OxPHOS) and increased mitochondrial respiration. The inhibition of mitochondrial respiration with metformin or tigecycline abrogated AraC resistance in *MF9/sPrdm16* cells via an energetic shift toward low OxPHOS status. Furthermore, *sPrdm16* up-regulated *Myc* and the glutamine transporter Slc1a5, activating the TCA cycle and glutaminolysis. Of note, both OxPHOS and MYC-target gene signatures were significantly enriched in AML patient samples with high *PRDM16* expression. Together, we showed that *PRDM16* overexpression activates mitochondrial respiration through metabolic reprogramming via the MYC-SLC1A5-Glutaminolysis axis, thereby conferring AraC resistance on AML cells. These results suggest that targeting mitochondrial respiration might be a novel treatment strategy to overcome chemoresistance in AML patients with high *PRDM16* expression.

Introduction

PRDM16 is one of the 16 members of PR/SET domain (PRDM) family,[1] which is critical for the function of hematopoietic stem cells (HSC).[2] In pediatric acute myeloid leukemia (AML), patients with high *PRDM16* expression showed significantly worse overall survival (OS) and event-free survival (EFS) than those with low *PRDM16* expression.[3] Further analyses of clinical parameters have revealed that the percentage of patients who did not achieve complete remission (CR) after induction chemotherapy was significantly higher in those with high *PRDM16* expression than in those with low *PRDM16* expression.[3] High *PRDM16* expression is also observed in 31% of adult AML cases.[4] Remarkably, high *PRDM16* expression was frequent among adult AML patients who did not achieve CR after induction chemotherapy.[4] Moreover, OS was significantly worse in those with high *PRDM16* expression than in those with low *PRDM16* expression.[4] Several other studies have also demonstrated that high PRDM16 expression in adult AML predicts poor OS, relapse-free survival (RFS), and low CR rate after induction therapy.[5-7] These findings suggest that high *PRDM16* expression is a pivotal clinical determinant of poor prognosis and induction failure in both pediatric and adult AML.

PRDM16 was originally isolated as a transcriptionally activated translocation partner gene in t(1;3)(p36;q21) AML.[8] Analysis using *Prdm16*-deficient mice revealed that *Prdm16* is a critical regulator of hematopoietic and neural stem cell maintenance, partly by regulating oxidative stress.[9] *Prdm16* is selectively expressed in the earliest HSC compartment

and is crucial for the establishment and maintenance of the HSC pool.[2] Full-length PRDM16 (fPRDM16) is a zinc finger transcription factor which also possesses an N-terminal PR domain, homologous with a SET chromatin remodeling domain. Another isoform, short-isoform PRDM16 (sPRDM16), lacks the PR domain and this sPRDM16 was mainly detected in the t(1;3)(p36;q21)-positive AML and myelodysplastic syndromes (MDS).[10,11]

Of note, elevated *sPRDM16* expression due to promoter hypomethylation was frequently observed in cytogenetically normal (CN) AML.[12] The roles of these two distinct PRDM16 isoforms on myeloid leukemogenesis seem to be slightly different. Although both *fPrdm16* and *sPrdm16*-transduced cells cause myeloid diseases *in vivo*, *fPrdm16* requires longer disease latency than *sPrdm16*.[13] In addition, histone H3 lysine 4 methyltransferase activity of the PR domain within fPrdm16 was shown to be required for specific suppression of MLL-rearranged leukemogenesis both *in vitro* and *in vivo*, indicating that *fPRDM16* functions as a tumor suppressor in this context.[14] On the other hand, a number of studies have demonstrated the apparent leukemogenic effect of *sPRDM16*. Overexpression of *sPRDM16* blocked granulocyte colony-stimulating factor (G-CSF)-induced granulocytic differentiation in myeloid L-G3 cells, whereas fPRDM16 could not block the differentiation.[10] Moreover, overexpression of sPrdm16 can co-operate with p53 loss or Hoxb4 overexpression to induce myeloid leukemia *in vivo*.[12,15] More recently, sPrdm16 was shown to transform megakaryocyte-erythroid progenitors into myeloid leukemia-initiating cells by activating myeloid gene regulatory networks.[13] These studies indicate that *sPRDM16* is a bona fide oncogene in myeloid leukemia. However, how this oncogene drives high-risk myeloid leukemia with chemotherapy resistance remains elusive.

Here, we performed cell viability, transcriptome, metabolome and *in vivo* analysis using murine leukemic cells and patient samples and explored response to chemotherapeutic agents used in standard induction therapy for AML in leukemic cells with high expression of *sPRDM16*. Our study uncovered unique *sPRDM16*-driven metabolic reprogramming, leading to chemoresistance of AML cells with high *sPRDM16* expression.

Methods

Animals

C57BL/6JJmsSlc (CD45.2) or C57BL/6-Ly5.1 (CD45.1) mice were purchased from Japan SLC (Shizuoka, Japan) and Sankyo Laboratory Service Corporation (Tokyo, Japan), respectively. *Flt3*^{ITD} mice have been previously described.[16] Mice were housed in a special pathogen-free environment at our institutional animal facility. All animal procedures were approved by the Institutional Animal Care Committees in Yokohama City University.

Patient samples and RNA-sequencing

We analyzed RNA-sequencing data obtained from 139 of all the pediatric AML patients enrolled in the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) AML-05 trial.[17] All patients provided informed consent. These 139 patients were divided into two groups based on a *PRDM16/ABL1* expression ratio of 0.010 as the cutoff using RT-qPCR: 39 patients with high *PRDM16* expression and 100 patients with low *PRDM16* expression, as previously described.[3] This research was conducted in accordance with the Declaration of Helsinki and approved by the institutional review boards of Yokohama City University, the participating institutes, and the ethical review board of the JPLSG AML-05 trial.

Results

High *sPRDM16* expression drives cytarabine resistance in acute myeloid leukemia

To establish murine bone marrow (BM) and leukemic cells with high *sPRDM16* expression, we transduced murine normal BM or *MLL::AF9*-transformed cells with either mock or sPrdm16-expressing retroviral vector to establish models for *sPrdm16*-low or high murine BM and *MLL::AF9* cells (Figure 1A, B). We confirmed transforming capacity of *sPrdm16* in normal BM cells *in vitro* as previously described (*Online Supplementary Figure S1A-C*).[13] Of note, murine *MLL::AF9* leukemia cells did not substantially change immunophenotype (*Online Supplementary Figure S1D, E*), cellular growth (*Online Supplementary Figure S1F*), and *in vivo* leukemogenicity (*Online Supplementary Figure S1G, H*) by ectopic *sPrdm16* expression, suggesting non-tumor suppressive role of *sPrdm16* in *MLL::AF9* leukemia cells, which is in sharp contrast to *fPRDM16*.[14] Using these cells as cellular models of low or high *sPrdm16* expression, we first examined the sensitivity of these cells to either cytarabine (AraC) or daunorubicin by cell viability assays. Strikingly, *MLL::AF9* leukemia cells with high *sPrdm16* expression (*MA9/sPrdm16* cells) showed significantly higher 50% inhibitory concentration (IC_{50}) against AraC compared to *MLL::AF9* leukemia cells transduced with mock vector (MA9/control cells) (Figure 1C). In contrast, IC_{50} against daunorubicin were comparable in these leukemia cells (Figure 1C). Importantly, murine normal BM cells with high *sPrdm16* expression (*WT/sPrdm16* cells) also showed higher IC_{50} against AraC compared to murine normal BM cells transduced with mock vector (*WT/control* cells) (*Online Supplementary Figure S2A*). Consistent with cell viability assays, *WT/sPrdm16* cells showed decreased sensitivity to AraC but not to daunorubicin compared to WT/control cells in colony forming unit (CFU) assays (*Online Supplementary Figure S2B, C*). To validate these findings in human leukemia cells, we transduced human AML cell lines, MOLM13 and THP-1, with either mock or sPRDM16-expressing retroviral vector. As expected, both MOLM13 and THP-1 cells with high *sPRDM16* expression showed higher IC_{50} against

AraC compared to cells transduced with mock vectors (Figure 1D, E). Similar to murine leukemia cells, IC_{50} against daunorubicin were comparable in these human leukemia cells (Figure 1D, E). AraC is an antimetabolic agent which

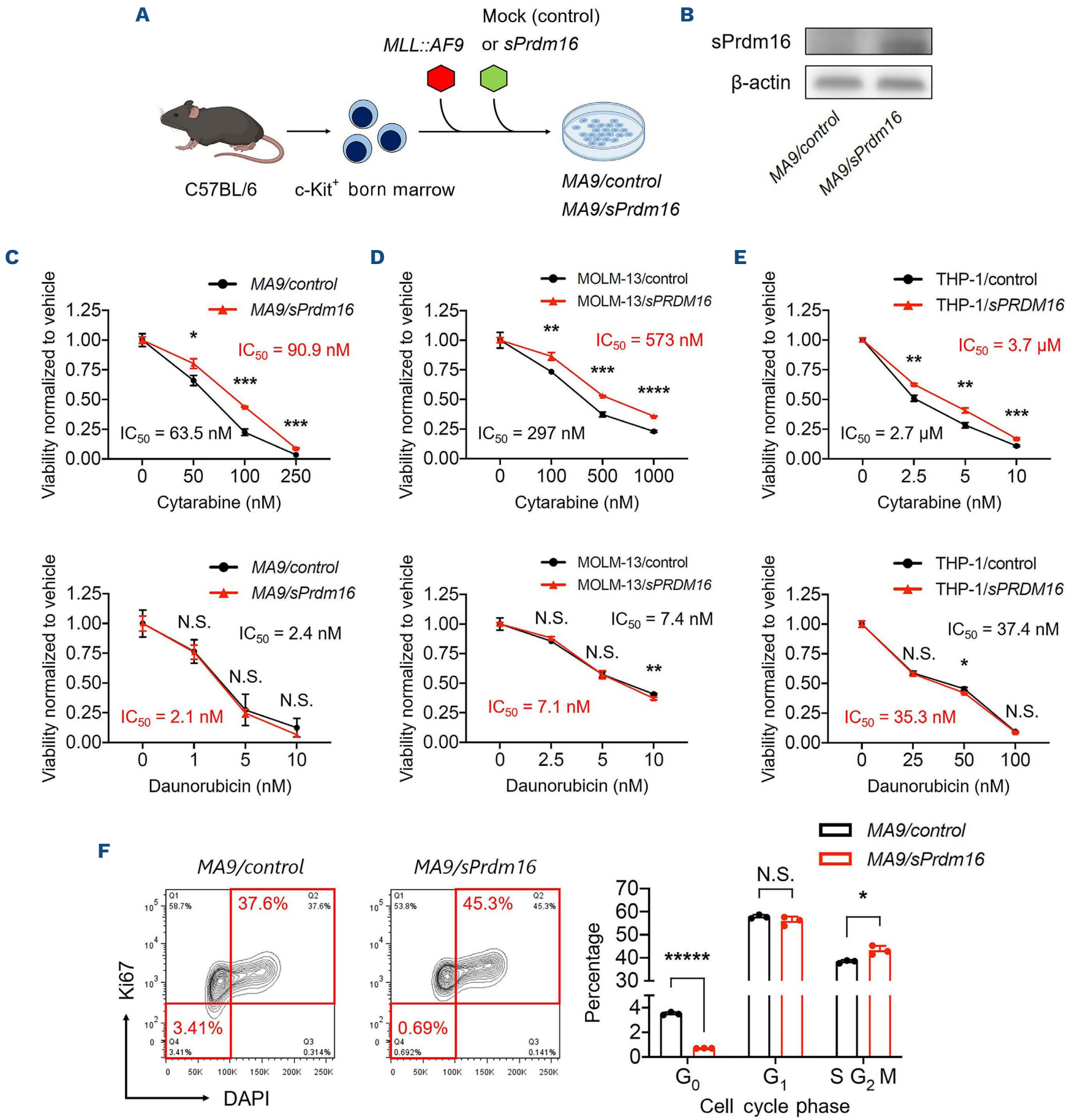

Figure 1. sPRDM16 induces cytarabine resistance in acute myeloid leukemia cells in a cell cycle-independent manner. (A) Experimental schema for the establishment of MA9/control or *MA9/sPrdm16* cells. (B) Protein expression levels of sPrdm16, detected by western blotting analysis, were up-regulated in *MA9/sPrdm16* cells. (C) *In vitro* drug sensitivity assay for cytarabine (upper panel) or daunorubicin (lower panel) using *MA9/control* and *MA9/sPrdm16* (N=3 for each group). (D, E) Drug sensitivity assay using human acute myeloid leukemia (AML) cell lines, MOLM-13 (D) and THP-1 (E), transduced with either mock or sPRDM16-expressing vector (N=3 for each group). (F) Cell cycle analysis of *MA9/control* and *MA9/sPrdm16* cells (N=3 for each group). Representative flow cytometry images (left panels) and percentages of each phase of the cell cycle (right panel) are shown. *MA9/sPrdm16* cells exhibited an increased S/G2/M phase, which typically exhibits high sensitivity to cytarabine. All data are represented as mean ± standard deviation. An unpaired Student *t* test was used to calculate *P* values. N.S.: not significant; **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001; ******P*<0.00001.

is converted to cytarabine-5'-triphosphate after cellular uptake and incorporated into DNA during DNA synthesis, thereby inhibiting DNA polymerase and inducing cell cycle arrest in an S phase-specific manner.[18] We, therefore, checked the percentage of S phase in *MA9/sPrdm16* and MA9/control cells, hypothesizing that lower percentage of S phase might have conferred AraC resistance in *MA9/sPrdm16* cells. Intriguingly, however, *MA9/sPrdm16* cells showed a significantly higher percentage of S/G2/M fraction compared to *MA9/control* cells (Figure 1F). Likewise, WT/sPrdm16 cells also showed a higher percentage of $S/G_2/M$ fraction compared to *WT/control* cells (*Online Supplementary Figure S2D*). Of note, the percentage of $S/G_2/M$ fraction was higher in AraC-treated *WT/sPrdm16* cells compared to vehicle-treated *WT/sPrdm16* cells (*Online Supplementary Figure S2E*). These data suggest that sPrdm16 overexpression drives AraC resistance of *MA9/sPrdm16* and *WT/sPrdm16* cells independently of S phase. We further examined the effect of high *sPrdm16* expression on drug sensitivity using a murine *Flt3*^{ITD} model.[16] In line with the murine *MLL::AF9* model, murine *Flt3*^{ITD} BM cells with high sPrdm16 expression (*Flt3*^{ITD}*/sPrdm16*) also showed relative resistance to AraC, but not to daunorubicin, compared to control *Flt3*^{ITD} cells (*Flt3*^{ITD}*/control*) in both cell viability and CFU assays (*Online Supplementary Figure S2F-H*). In addition, *Flt3*^{ITD}*/sPrdm16* cells and AraC-treated *Flt3*^{ITD}*/sPrdm16* cells showed a higher percentage of S/G2/M fraction compared to *Flt3*^{ITD}*/control* cells or vehicle-treated *Flt3*^{ITD}*/control* cells, respectively, again indicating that high *sPrdm16* expression drives AraC resistance independently of S phase (*Online Supplementary Figure S2 I-J*).

High *sPrdm16* expression activates oxidative phosphorylation

To explore the molecular basis of AraC resistance driven by *sPrdm16* expression, we next performed RNA-sequence (RNA-seq) using *MA9/control* and *MA9/sPrdm16* cells and compared their transcriptome. Notably, 7,658 genes (96.5% of differentially expressed genes) were up-regulated in *MA9/sPrdm16* cells compared to *MA9/control* cells (Figure 2A). Gene Ontology (GO) analysis revealed that "cell cycle"-, "cell division"- or "DNA replication"-related genes were enriched in the up-regulated genes (*Online Supplementary Figure S3A*). Gene set enrichment analysis (GSEA) revealed that the gene signature related to "oxidative phosphorylation (OXPHOS)" was strongly enriched in *MA9/sPrdm16* cells compared to *MA9/control* cells (Figure 2B, C). We also performed RNA-seq using *WT/control* and *WT/sPrdm16* cells. Similar to RNA-seq data derived from an *MLL::AF9* leukemia model, the majority of differentially expressed genes (1,015 genes, 74.0%) were up-regulated in *WT/sPrdm16* cells compared to *WT/control* cells (*Online Supplementary Figure S3B*). GO analysis confirmed enrichment of "cell cycle"-, "cell division"- or "DNA replication"-related genes in relatively up-regulated genes in *WT/sPrdm16* cells (*Online Supplementary Figure S3C*). Importantly, GSEA showed that the gene signature related to "OXPHOS" was also significantly enriched in *WT/sPrdm16* cells compared to *WT/control* cells (*Online Supplementary Figure S3D, E*), suggesting that high sPrdm16 expression drives OXPHOS-related transcriptional output. To confirm if mitochondrial respiration is elevated in cells with high *sPrdm16* expression, we conducted the Mito Stress Test and the Glycolysis Stress Test using *MA9/control* and *MA9/sPrdm16* cells. Consistent with the GSEA results, *MA9/sPrdm16* cells showed significantly higher basal and maximum mitochondrial respiration, and higher glycolytic function compared to *MA9/control* cells in the Mito Stress and the Glycolysis Stress Test, respectively (Figure 2D). To further evaluate mitochondrial reactive oxygen species (ROS) and membrane potential of these leukemic cells, we labeled cellular mitochondria with either MitoSOX, Mitotracker or tetramethylrhodamine methyl ester (TMRM) dye and quantified their fluorescence with flow cytometry. In line with the results of the GSEA and the Mito Stress Test, *MA9/sPrdm16* cells exhibited significantly higher median fluorescence intensity (MFI) of MitoSOX and relative TMRM compared to *MA9/control* cells (Figure 2E). Likewise, the Mito Stress Test of *WT/sPrdm16* cells showed significantly higher basal and maximum mitochondrial respiration and higher glycolytic function compared to *WT/control* cells (*Online Supplementary Figure S3F*). Taken together, these data suggest that high *sPrdm16* expression in both murine normal and leukemic BM cells drive mitochondrial respiration to activate OXPHOS-related transcriptional output.

Pharmacological inhibition of mitochondrial respiration sensitizes *MA9/sPrdm16* cells to cytarabine

A previous study has shown that AraC-resistant human AML cells critically require oxidative metabolism.[19] To determine if activated mitochondrial respiration driven by high *sPrdm16* expression is responsible for AraC resistance in AML cells with high sPrdm16, we examined the effect of metformin and tigecycline, electron transport chain complex I inhibitor and mitochondrial protein synthesis inhibitor, respectively, on AraC resistance induced by sPrdm16 overexpression. Cell viability assays using *MA9/control* and *MA9/sPrdm16* cells revealed that addition of 2 mM metformin or 10 μM tigecycline decreased the IC_{50} against AraC in *MA9/sPrdm16* cells to the same level as those in *MA9/control* (Figure 3A, B). IACS-010759, a selective mitochondrial complex I inhibitor, also sensitized *MA9/sPrdm16* cells to AraC (Figure 3C). We next validated if metformin, tigecycline or IACS-010759 treatment reduces mitochondrial respiration in *MA9/sPrdm16* leukemia cells. Treatment with either 1 mM metformin alone or a combination of AraC and metformin significantly reduced mitochondrial respiration (*Online Supplementary Figure S4A*). Similar results were also observed using tigecycline

and IACS-010759 (*Online Supplementary Figure S4B, C*). Collectively, these data indicate that activated mitochondrial respiration driven by high *sPrdm16* expression is directly responsible for AraC resistance in AML cells with high *sPrdm16* and that pharmacological inhibition of this metabolic pathway can reverse their AraC resistance.

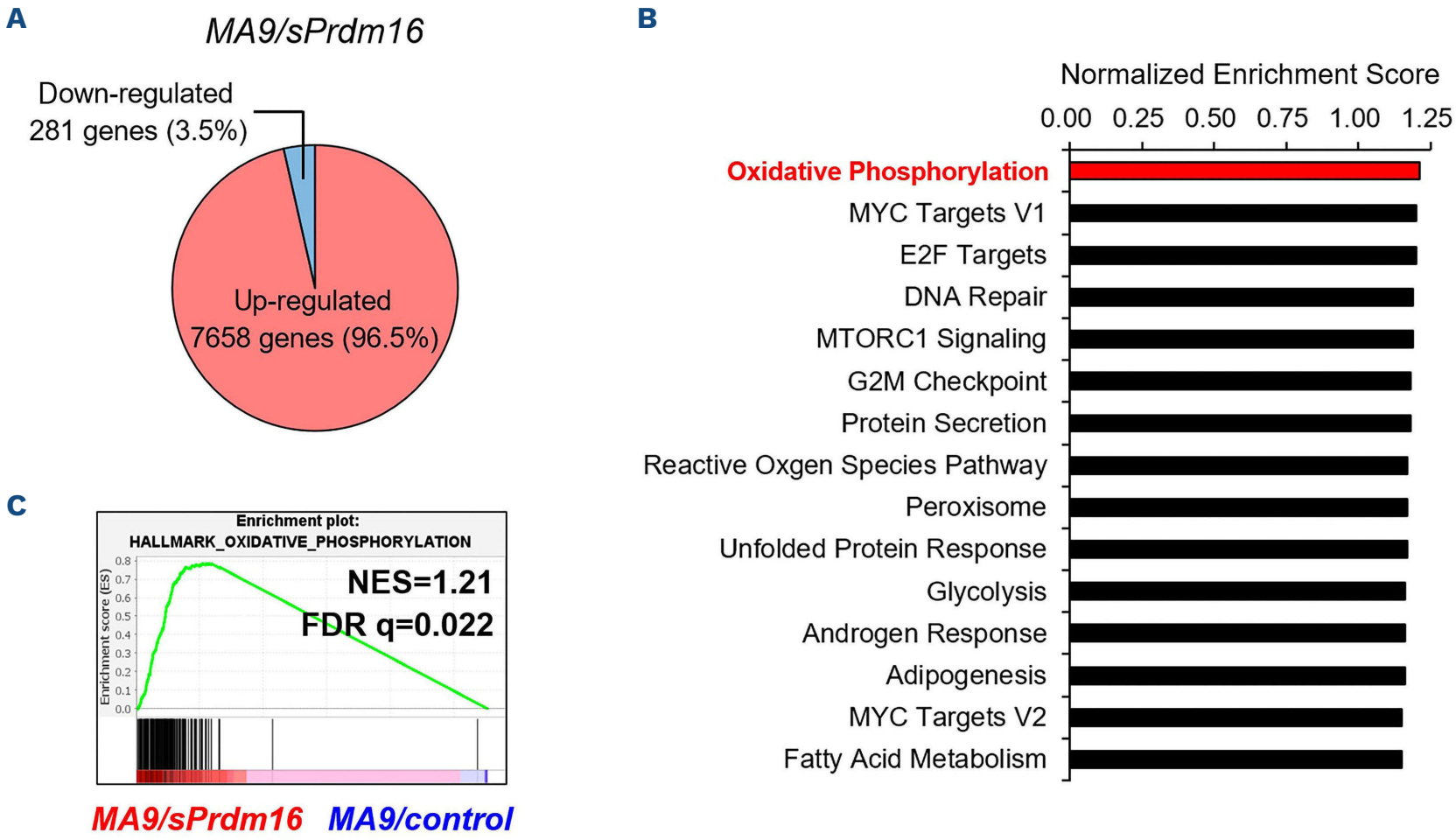

Figure 2. sPrdm16 activates oxidative phosphorylation. (A) Differentially expressed genes in *MA9/sPrdm16* cells compared to *MA9/control* cells. There were 7,658 up-regulated genes and 281 down-regulated genes. (B, C) Top 15 gene signatures significantly changed in gene set enrichment analysis (GSEA) using RNA-seq data from *MA9/sPrdm16 versus MA9/control* cells (B). The gene signature related to "OXIDATIVE PHOSPHORYLATION" was the most significantly enriched in *MA9/sPrdm16* cells (B, C). (D) Mito Stress Test (left panel) and Glycolysis Stress Test (right panel) in *MA9/control* and *MA9/sPrdm16* cells (N=3 for each group). (E) Mitochondrial reactive oxygen species (ROS) and relative membrane potential in *MA9/sPrdm16* cells were measured using MitoSOX and the ratio of tetramethylrhodamine methyl ester (TMRM) and mitotracker, respectively (N=3 for each group). All data are represented as mean ± standard deviation. An unpaired Student *t* test was used to calculate *P* values. $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$. MFI: mean fluorescence intensity.

Myc activation and Slc1a5-mediated glutaminolysis drive metabolic reprogramming in acute myeloid leukemia with high *sPrdm16* expression

To explore the rationale for activated OXPHOS in AML cells with high *sPrdm16* expression, we performed capillary electrophoresis time-of-flight mass spectrometry-based metabolome profiling of *MA9/control* and *MA9/sPrdm16* leukemia cells. We found significantly higher amounts of glutamine and trends toward higher amounts of succinate and fumarate in *MA9/sPrdm16* cells (Figure 4A). To further seek metabolic profiles of AraC-exposed leukemia cells, we next profiled the metabolic status of AraC-treated *MA9/control* and *MA9/sPrdm16* leukemia cells. Notably, AraC-exposed *MA9/sPrdm16* cells showed significantly higher amounts of glutamine, α-ketoglutarate, and malate, and trends toward higher succinate and fumarate compared to AraC-exposed *MA9/control* cells (*Online Supplementary Figure S5A*). These data underscore activated glutamine

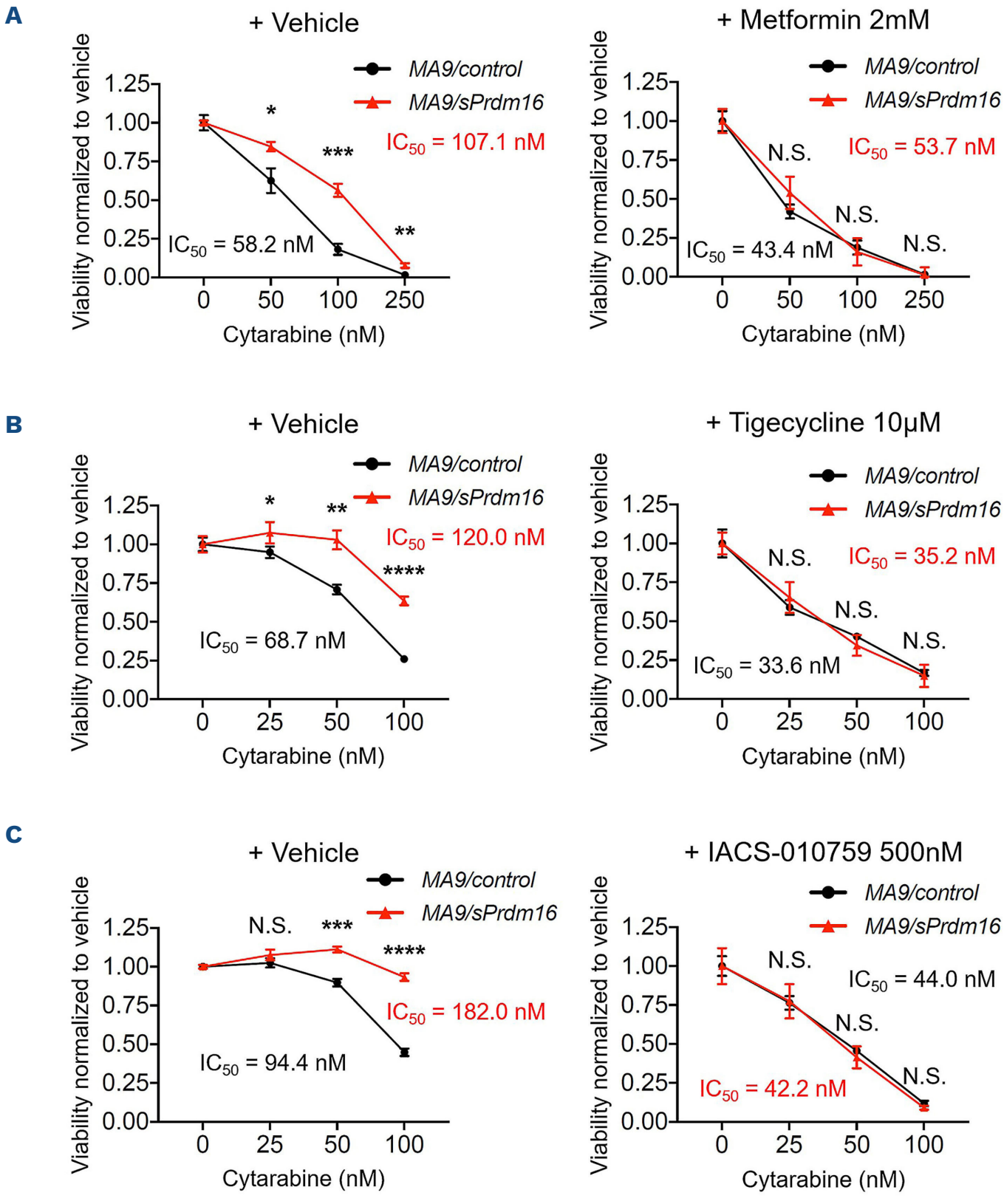

Figure 3. Pharmacological inhibition of mitochondrial respiration abrogated cytarabine resistance in *MA9/sPrdm16* cells. (A, B) *In vitro* drug sensitivity assay for cytarabine with vehicle (left panel) or metformin (right panel), an electron transport chain complex I inhibitor (A), and with vehicle (left panel) or tigecycline (right panel), a mitochondrial protein synthesis inhibitor (B). Metformin and tigecycline abrogated cytarabine resistance in *MA9/sPrdm16* cells (N=3 for each group). (C) *In vitro* drug sensitivity assay for cytarabine with vehicle (left panel) or IACS-010759 (right panel), a selective mitochondrial complex I inhibitor. IACS-010759 also abrogated cytarabine resistance in *MA9/sPrdm16* cells (N=3 for each group). All data are represented as mean ± standard deviation. An unpaired Student *t* test was used to calculate *P* values. N.S.: not significant; $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$.

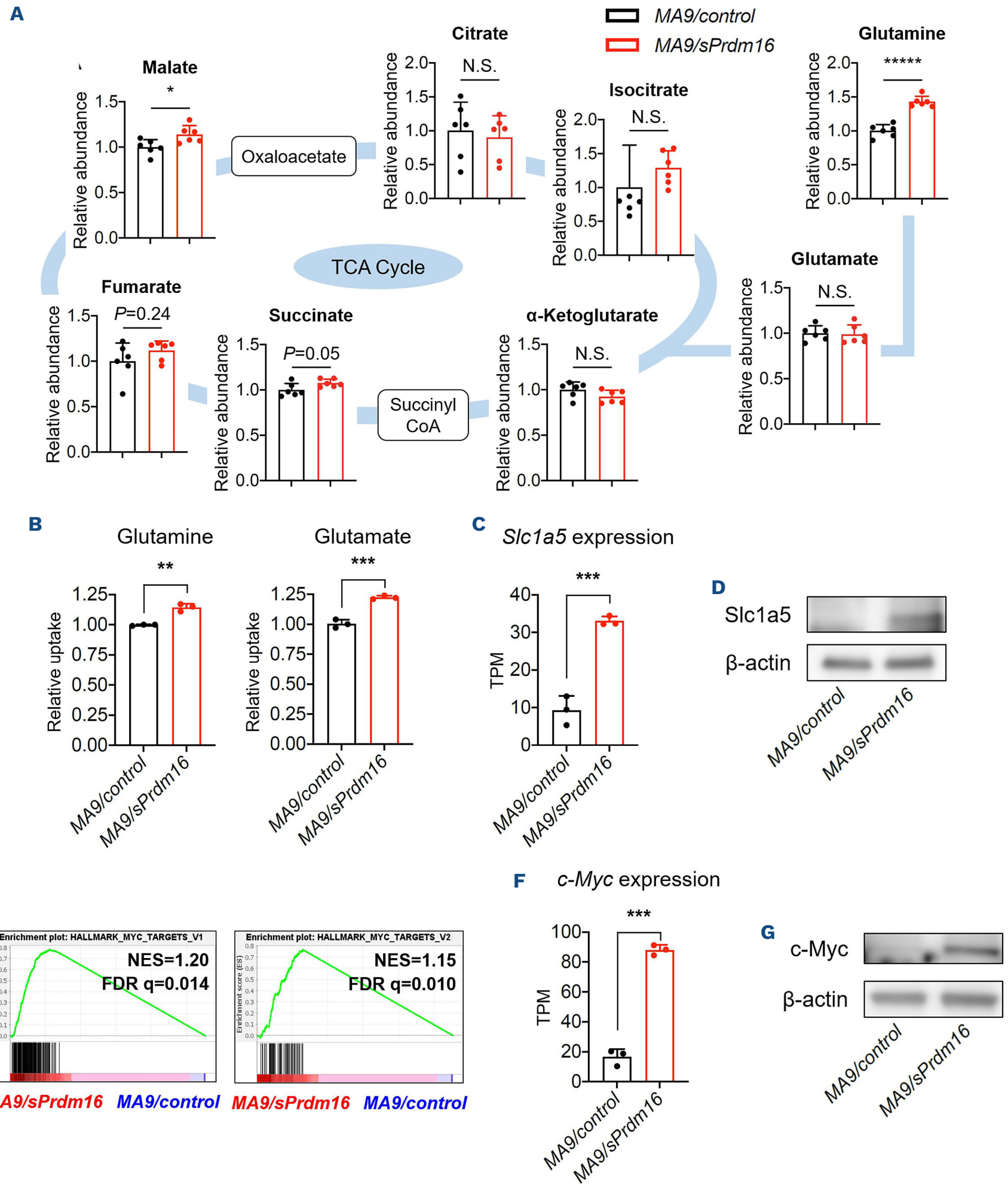

Figure 4. sPrdm16 induces metabolic reprogramming through the activation of glutaminolysis via the Myc-Slc1a5 axis. (A) Metabolite concentrations in the TCA cycle of *MA9/control* or *MA9/sPrdm16* cells were measured using capillary electrophoresis time-of-flight mass spectrometry-based metabolome profiling (N=6 for each group). (B) Intracellular glutamine (left panel) and glutamate uptake (right panel) levels, measured by Glutamine/Glutamate-Glo Assay, were higher in *MA9/sPrdm16* cells compared to control (N=3 for each group). (C) Transcripts per million (TPM) values of Slc1a5 gene were up-regulated in MA9/sPrdm16 cells (N=3 for each group). (D) Protein expression levels of *Slc1a5*, detected by western blotting analysis, were also up-regulated in MA9/sPrdm16 cells. (E) The gene signatures related to "MYC-TARGET" were significantly enriched in *MA9/sPrdm16* cells. (F) TPM values of Myc gene were up-regulated in *MA9/sPrdm16* cells (N=3 for each group). (G) Protein expression levels of Myc, detected by western blotting analysis, were also up-regulated in *MA9/sPrdm16* cells. All data are represented as mean ± standard deviation. An unpaired Student *t* test was used to calculate *P* values. NS: not significant; $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{*****}P<0.00001$.

uptake, glutaminolysis and subsequent reprogramming of the tricarboxylic acid (TCA) cycle in non-treated and AraC-exposed *MA9/sPrdm16* leukemia cells.

Glutaminase is a mitochondrial enzyme crucial for glutaminolysis which deaminates glutamine to form glutamate.[20] To explore whether activated glutaminolysis is a functional hub driving AraC resistance in *MA9/sPrdm16* leukemia cells, we treated *MA9/control* or *MA9/sPrdm16* leukemia cells with various concentrations of AraC with BPTES, a glutaminase inhibitor, and performed cell viability assays. Addition of BPTES sensitized *MA9/sPrdm16* cells to AraC to the same level as *MA9/control* cells, indicating that glutaminase-mediated glutaminolysis contributes to AraC resistance in *MA9/sPrdm16* cells (*Online Supplementary Figure S5B*). To accurately quantify intracellular glutamine and glutamate levels, we applied a luminescence-based Glutamine/Glutamate-Glo Assay to *MA9/control* and *MA9/sPrdm16* leukemia cells. In agreement with the metabolome data, we found higher amounts of glutamine and glutamate in *MA9/sPrdm16* cells compared to *MA9/control* cells, suggesting that glutamine uptake and subsequent glutamate synthesis via glutaminolysis are relatively active in *MA9/sPrdm16* cells (Figure 4B).

A recent study has shown that MYC-induced transcription of the neutral amino acid transporter SLC1A5 promotes intracellular uptake of glutamine and subsequent TCA cycle/OXPHOS activity in leukemia stem cells (LSC) of human AML for energy metabolism and survival.[21] Therefore, we hypothesized that *MA9/sPrdm16* leukemia cells also drive the Myc-Slc1a5 axis to promote glutamine uptake, fuel TCA cycle metabolites and subsequently activate OXPHOS. In line with this notion, we observed significant upregulation of Slc1a5 in *MA9/sPrdm16* cells compared to *MA9/control* cells, at both transcriptional and protein levels (Figure 4C, D). Importantly, GSEA based on RNA-seq data of *MA9/control* and *MA9/sPrdm16* cells revealed significant enrichment of "MYC-TARGET" gene signatures in *MA9/sPrdm16* cells (Figure 4E). Moreover, Myc was significantly up-regulated in *MA9/sPrdm16* cells compared to *MA9/control* cells, at both transcriptional and protein levels (Figure 4F, G). Of note, GSEA based on RNA-seq of *WT/control* and *WT/sPrdm16* cells also showed significant enrichment of "MYC-TARGET" gene signatures in *WT/sPrdm16* cells (*Online Supplementary Figure S5C*). The transcription of Slc1a5 and Myc were significantly higher in *WT/sPrdm16* cells compared to *WT/control* cells (*Online Supplementary Figure S5D*). Next, to explore if MYC-SLC1A5 axis-mediated metabolic reprogramming is also relevant in human AML cells with high *sPRDM16* expression, we checked MYC and SLC1A5 expression in MOLM13 and THP-1 cells transduced with either mock or *sPRDM16*-expressing retroviral vector. Consistent with murine data, immunoblot analysis showed higher MYC and SLC1A5 expression in MOLM13/*sPRDM16* and THP-1/*sPRDM16* cells compared to control cells (*Online Supplementary Figure S5E*). Glutamine/Glutamate-Glo Assay revealed higher amounts of glutamine and glutamate in MOLM13/*sPRDM16* and THP-1/*sPRDM16* cells compared to control cells (*Online Supplementary Figure S5F, G*). Above all, the Mito Stress Test confirmed significantly higher basal and maximal respiration in MOLM13/*sPRDM16* and THP-1/*sPRDM16* cells compared to control cells (*Online Supplementary Figure S5H, I*). Together, these data suggest that high sPrdm16 expression in murine and human AML cells drives Myc-Slc1a5 axis to promote glutamine uptake, fuel TCA cycle metabolites, and subsequently activate OXPHOS, which is a critical determinant of *sPrdm16*-mediated AraC resistance.

Myc is a functional hub of *sPrdm16*-mediated metabolic reprogramming and cytarabine resistance

Next, we sought to examine if Myc is functional in *sPrdm16*-mediated metabolic reprogramming and AraC resistance. To this end, we knocked down Myc using retroviral shRNA vectors in *MA9/sPrdm16* cells and tested for a cell viability assay (Figure 5A). Consistent with our hypothesis, knockdown of Myc significantly sensitized *MA9/sPrdm16* cells to AraC (Figure 5B). In addition, the Glutamine/Glutamate Glo assay detected significantly lower amounts of glutamine and glutamate in Myc knocked-down *MA9/sPrdm16* cells compared to Scramble *MA9/sPrdm16* cells (*Online Supplementary Figure S6A*). The Mito Stress Test revealed reduced basal and maximum mitochondrial respiration upon Myc knockdown in *MA9/sPrdm16* cells (*Online Supplementary Figure S6B*). To further confirm the role of Myc in *sPrdm16*-mediated AraC resistance, we performed a cell viability assay using an Myc inhibitor, 10058-F4. In *MA9/control* cells, addition of 10058-F4 did not sensitize leukemia cells to AraC (Figure 5C). In sharp contrast, addition of 10058-F4 significantly sensitized *MA9/sPrdm16* cells to AraC (Figure 5C). Furthermore, Synergy Finder detected a significantly high synergy score between AraC and 10058-F4 in *MA9/sPrdm16* cells but not in *MA9/control* cells, indicating that Myc inhibition synergizes with AraC to kill leukemia cell specifically in *MA9/sPrdm16* cells (*Online Supplementary Figure S6C*). We also conducted a reciprocal experiment to determine if *MLL::AF9* leukemia cells with Myc overexpression mimic the phenotype of *MF9/sPrdm16* cells such as resistance to AraC and high OXPHOS status (Figure 5D, E). As expected, *MLL::AF9* leukemia cells with Myc overexpression (*MA9/Myc* cells) showed significantly higher IC_{50} against AraC compared to *MA9/control* cells, confirming that Myc overexpression drives AraC resistance in *MLL::AF9* leukemia cells (Figure 5F). Myc-induced AraC resistance was also associated with high amounts of glutamine and glutamate (*Online Supplementary Figure S6D*), high basal and maximum mitochondrial respiration (*Online Supplementary Figure S6E*), and high mitochondrial ROS and relative membrane potential (*Online Supplementary Figure S6F*). Overall, these data demonstrate that Myc does, indeed,

function as a cornerstone of *sPrdm16*-mediated metabolic reprogramming and AraC resistance.

sPrdm16 overexpression in acute myeloid leukemia leads to cytarabine resistance *in vivo*

To validate if AML cells with high *sPrdm16* expression exert AraC resistance *in vivo*, we transplanted *MA9/control* or *MA9/sPrdm16* leukemia cells into sub-lethally irradiated recipient mice, treated them with either vehicle or AraC via intraperitoneal injection, and compared survival. AraC treatment prolonged survival of *MA9/control* mice (Figure 6). On the other hand, AraC treatment failed to improve survival of *MA9/sPrdm16* mice, suggesting that sPrdm16 overexpression directly drives AraC resistance in AML *in vivo* (Figure 6).

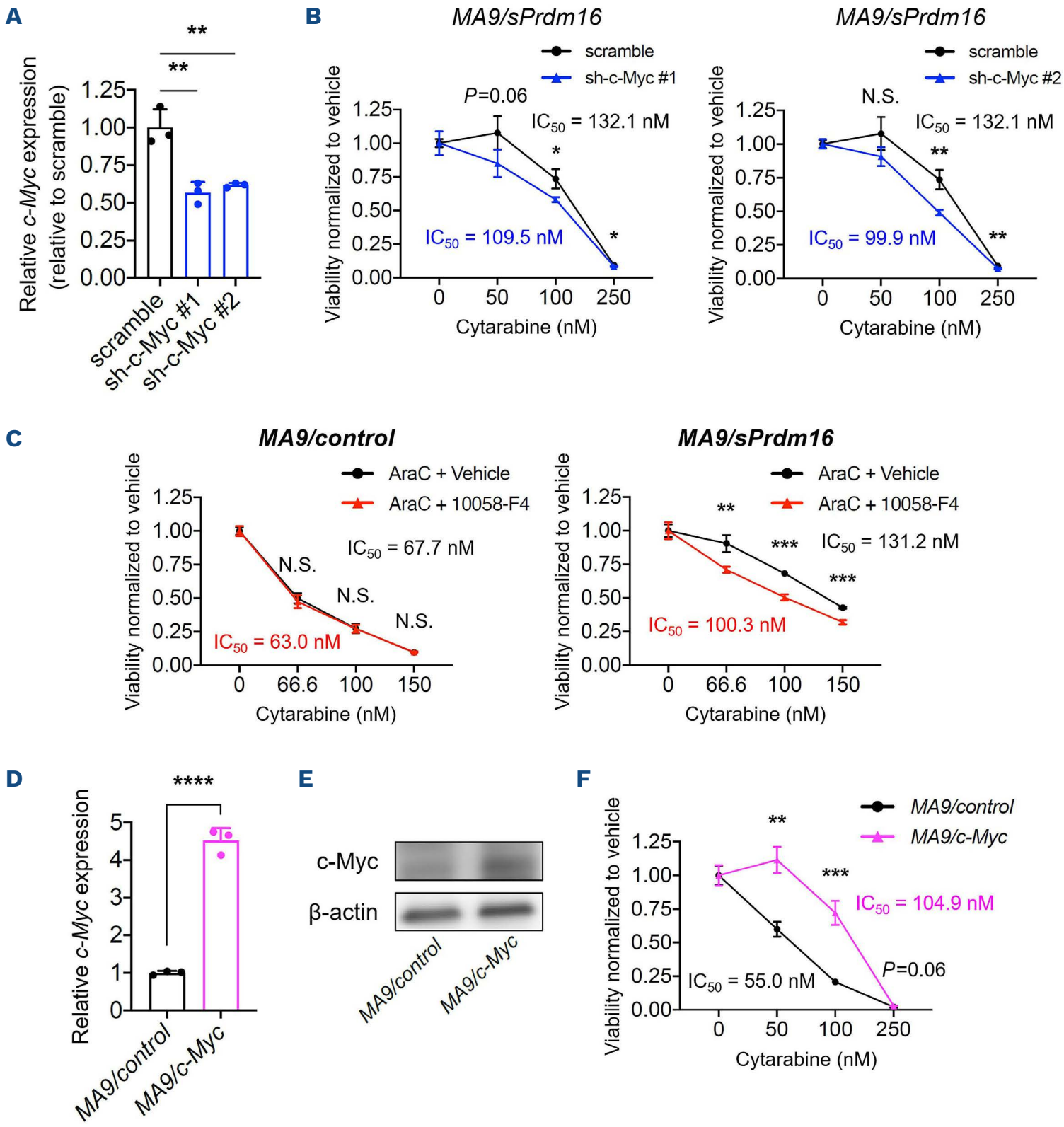

Figure 5. Myc is a key regulator of sPrdm16-mediated resistance to cytarabine. (A) Relative Myc expression in *MA9/sPrdm16* cells with Myc knockdown (N=3 for each group). (B) *In vitro* drug sensitivity assay for cytarabine in *MA9/sPrdm16* cells with Myc knockdown (N=3 for each group). (C) *In vitro* drug sensitivity assay for cytarabine with vehicle or 10058-F4, a Myc inhibitor, in *MA9/control* or *MA9/sPrdm16* cells (N=3 for each group). In contrast to MA9/control cells, the combination of cytarabine and 10058-F4 reduced the IC_{50} value of cytarabine in *MA9/sPrdm16* cells. (D) Relative Myc expression in *MLL::AF9* leukemic cells transduced with Myc-expressing vector (MA9/Myc) (N=3 for each group). (E) Protein expression levels of Myc, detected by western blotting analysis, were also up-regulated in MA9/Myc cells. (F) *In vitro* drug sensitivity assay for cytarabine in *MA9/Myc* cells (N=3 for each group). MA9/Myc cells mimicked the phenotype of MA9/sPrdm16 cells as evidenced by the resistance to cytarabine. All data are represented as mean ± standard deviation. An unpaired Student *t* test was used to calculate *P* values. NS: not significant; $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$.

Acute myeloid leukemia patient samples with high *PRDM16* expression display activated "OXPHOS" and "MYC Targets" gene expression signatures

Finally, we aimed to clarify if AML patients with high *PRDM16* expression also present evidence of MYC-mediated metabolic reprogramming. We analyzed RNA-seq data derived from 139 pediatric AML patient samples registered in the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) AML-05 clinical trial and classified into *PRDM16* low and high groups (Figure 7A). Strikingly, GSEA analysis revealed that the gene signatures related to "OXPHOS" and "MYC Targets" were significantly enriched in *PRDM16*-high samples compared to *PRDM16*-low samples (Figure 7B, C). Taken together, these data indicate that MYC-mediated metabolic reprogramming is a common transcriptional hallmark in both murine and human AML cells with high *PRDM16* expression, which is a functional determinant of *PRDM16*-mediated AraC resistance.

Discussion

PRDM16 is one of the 16 members of PR/SET domain (PRDM) family.[1] *sPRDM16*, which lacks the PR domain, is mainly detected in t(1;3)(p36;q21)-positive AML and MDS.[10-12] In addition, high *sPRDM16* expression is also observed in cytogenetically normal (CN) AML.[12] Likewise, a gene encoding a short isoform of PRDM3 lacking the PR domain, known as *Ecotropic Viral Integration site 1 (EVI1)*, is also highly expressed in high-risk MDS/AML with inv(3)/t(3;3).[1] Altogether, PRDM16 is one of the two PRDM family members highly expressed in a subset of MDS/AML patients. Notably, there is a growing body of evidence to suggest that high *PRDM16* expression is a transcriptional determinant of poor OS, RFS, and low CR rate after induction therapy both in pediatric and adult AML.[3-7] Importantly, a number of studies have shown leukemogenic effects of sPRDM16.[10,12,13,15] Based on these observations, we hypothesized that *PRDM16* functions as an oncogene, which drives chemoresistance in AML.

In this study, we have shown that overexpression of *sPRDM16* drives resistance to AraC but not to daunorubicin, the two major chemotherapeutic agents used in standard induction therapy for AML, in murine and human leukemic cells *in vitro* and *in vivo*. AraC is an antimetabolic agent which is incorporated into DNA during DNA synthesis, inhibits DNA polymerase, and induces cell cycle arrest in an S phase-specific manner.[18] Paradoxically, murine BM cells and *MLL::AF9* leukemia cells with high *sPrdm16* expression showed a higher percentage of S/G_2/M fraction compared to control cells, suggesting that sPrdm16 drives AraC resistance in an S phase-independent manner. GSEA based on RNA-seq data revealed that both *MA9/sPrdm16* and *WT/sPrdm16* cells showed enrichment of OXPHOS-related gene signature. The Mito Stress Test exhibited higher basal and maximum mitochondrial respiration in both *MA9/sPrdm16* and *WT/sPrdm16* cells compared to control cells. Moreover, metabolome profiling demonstrated activated glutamine uptake, glutaminolysis and reprogramming of the TCA cycle in both untreated and AraC-exposed *MA9/sPrdm16* leukemia cells. Together, these data strongly denote activated mitochondrial oxidative metabolism in these cells with high *sPrdm16* expression. Previous studies have shown a direct link between mitochondrial oxidative metabolism and AraC-resistance in AML. The ectonucleotidase CD39 (ENTPD1) is reported to be up-regulated in AraC-resistant leukemic cells from both AML cell lines and patient samples *in vivo* and *in vitro*.[22] High CD39 activity was shown to promote AraC resistance by enhancing mitochondrial activity and biogenesis through activation of cAMP-mediated adaptive mitochondrial stress response.[22] More recently, expression profiling of circular RNA, together with functional assays, revealed that downregulation of circFAM193B enhances OXPHOS, inhibits the lipid peroxidation of LSC, and promotes AraC resistance in AML.[23] In fact, low circFAM193B expression was significantly associated with poor survival in AML patients.[23] Of note, attenuation of mitochondrial OXPHOS by either metformin or tigecycline dramatically reversed AraC resistance of leukemic cells with

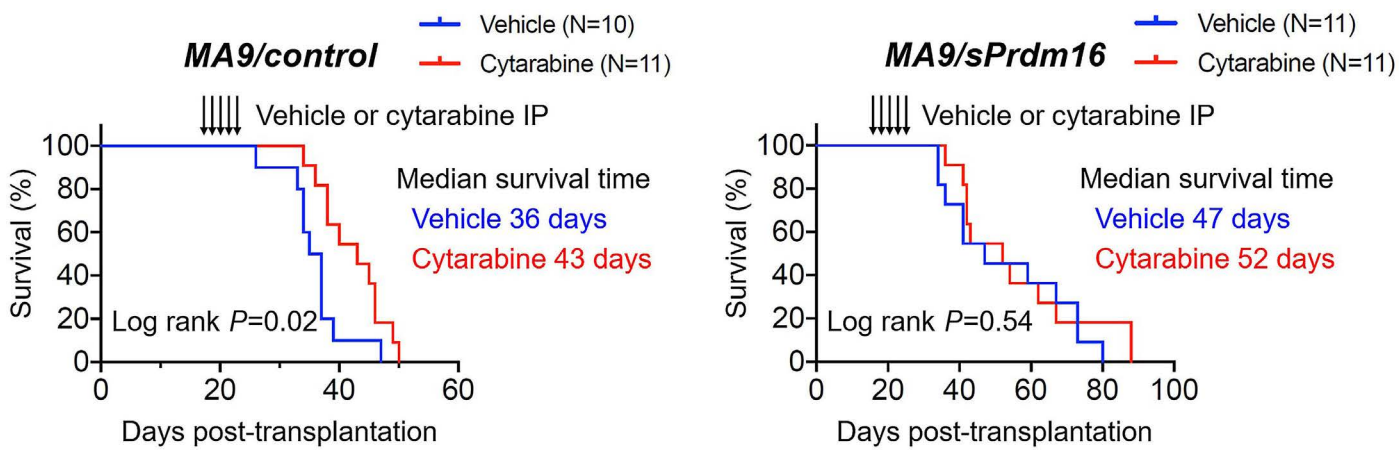

Figure 6. *MA9/sPrdm16* mice are resistant to cytarabine. Kaplan-Meier survival curve of *MA9/control* (left panel) and *MA9/sPrdm16* (right panel) mice treated with either vehicle (N=10-11) or cytarabine (N=11). 1.0x10^4 leukemia cells were transplanted to each sublethally irradiated (6.0 Gy) recipient mouse. *MA9/sPrdm16* mice exhibited resistance to cytarabine. The log rank test was used in survival analysis. IP: intraperitoneal injection.

high sPrdm16 expression. Collectively, these data clearly indicate that *PRDM16* drives AraC resistance via activated mitochondrial respiration.

Molecular mechanisms by which cancer cells activate mitochondrial respiration have been explored. Mutations driving constitutive activation of phosphatidylinositol-3 kinase (PI3K)/AKT signaling are among the most common mutations in human cancer. Growth factor signaling through PI3K/AKT drives membrane localization of transporters required for the uptake of critical nutrients such as glucose, amino acids and iron.[24-26] Additionally, oncogenic transcription factors such as MYC and hypoxia inducible factor (HIF) can also activate nutrient acquisition by increasing the expression of genes involved in the cellular uptake of crit-

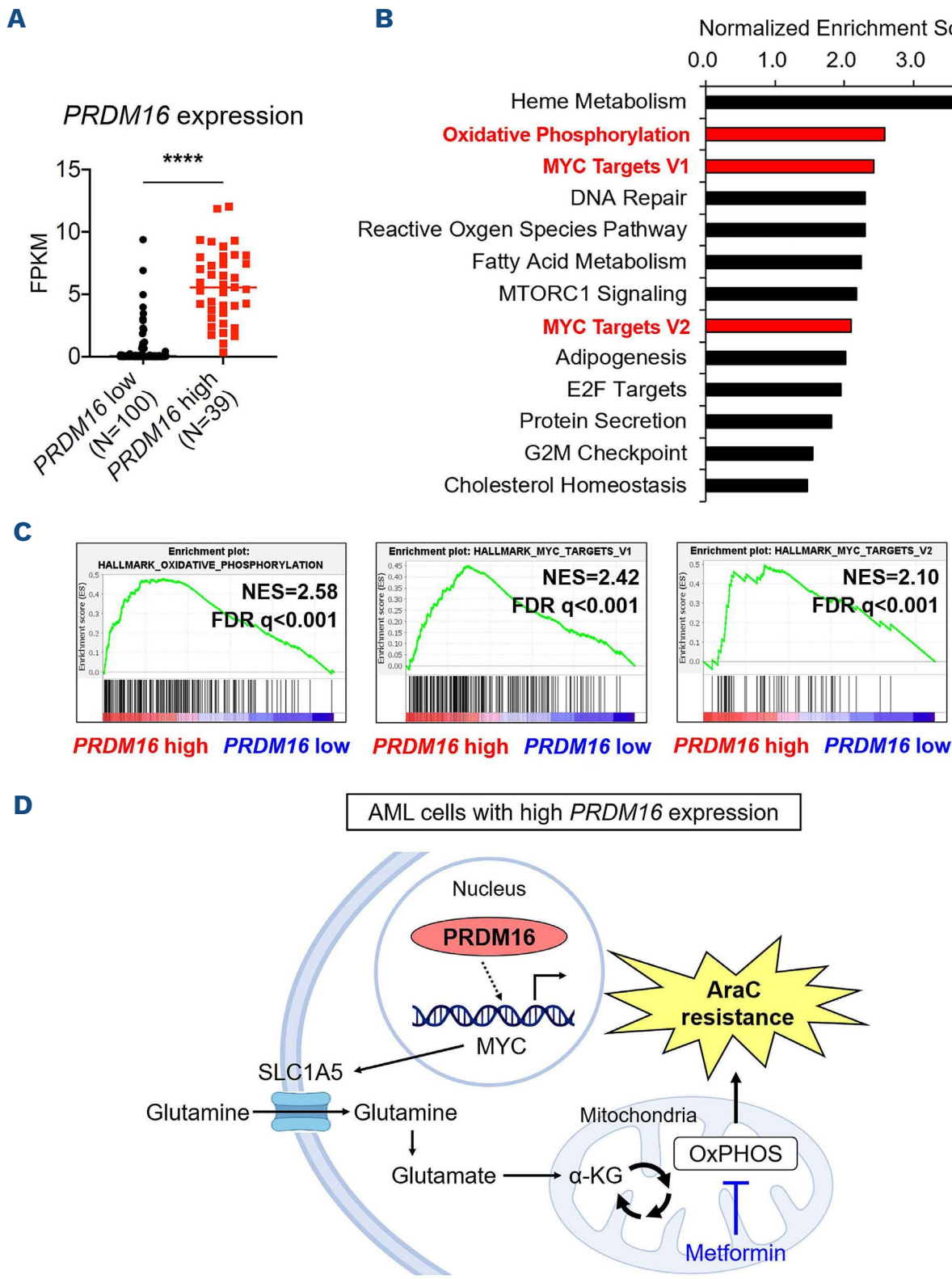

Figure 7. Primary acute myeloid leukemia patient samples with high *PRDM16* expression exhibit gene expression signatures associated with "OXPHOS" and "MYC-Targets". (A) Relative total *PRDM16* expression (including both full-length and short isoform) in acute myeloid leukemia (AML) patients with low (left, N=100) or high (right, N=39) *PRDM16* expression. (B) Gene signatures significantly changed in gene set enrichment assay (GSEA) using RNA-seq data from 139 pediatric AML patient samples registered in the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) AML-05 clinical trial. (C) The gene signatures related to "OXIDATIVE PHOSPHORYLATION" (left panel) and "MYC-TARGET" (middle and right panels) were significantly enriched in AML samples with high *PRDM16* expression. (D) Proposed model of cytarabine resistance mechanisms in AML with high *Prdm16* expression. PRDM16 drives cytarabine resistance through upregulation of mitochondrial respiration via upregulation of MYC and glutamine transporter SLC1A5, activation of glutamine uptake, and glutaminolysis. Therefore, targeting mitochondrial respiration might be a novel treatment strategy to overcome cytarabine resistance in AML patients with high PRDM16 expression. Statistical analyses were performed using the Mann-Whitney U test. ****P<0.0001.

ical nutrients such as glucose and glutamine.[27,28] Activated cellular uptake of these nutrients can ultimately fuel the TCA cycle, leading to metabolic reprogramming resulting in upregulation of mitochondrial respiration in cancer cells. In agreement with this, human AML LSC utilize pro-survival mechanisms where oncogenic transcription factor MYC induces transcription of amino acid transporter SLC1A5 to promote cellular uptake of glutamine, fuel the TCA cycle, and activate OXPHOS activity.[21] Here, we demonstrate that both murine BM cells and *MLL::AF9* leukemia cells with high *sPrdm16* expression showed enrichment of "MYC-TARGET" gene signatures, upregulation of Slc1a5 transcription and glutamine uptake. Addition of glutaminase inhibitor BPTES neutralized *sPrdm16*-mediated AraC resistance in *MLL::AF9* leukemia cells. MYC-SLC1A5 axis-mediated metabolic reprogramming was also relevant in human AML cells with high *sPRDM16* expression. Knockdown or pharmacological inhibition of Myc also reversed *sPrdm16*-mediated AraC resistance in murine *MLL::AF9* leukemia cells. Most importantly, transcriptomic analysis of pediatric AML patient samples with high *PRDM16* expression identified significant enrichment for OXPHOS and *MYC* target signatures, which is adherent to the murine data.

Although multiple functional studies have shown an oncogenic role of *sPrdm16* in myeloid leukemia, the precise biological effect of *sPrdm16* on hematopoietic cells is pleiotropic.[10,12,13,15] Nishikata *et al*. have shown that *sPrdm16* blocks G-CSF-induced myeloid differentiation in murine myeloid L-G3 cells.[10] *sPrdm16*-overexpressing murine p53$^{-/-}$ BM cells, Hoxb4-co-expressing murine BM cells and murine megakaryocyte-erythroid progenitors (MEP) can develop AML in recipient mice *in vivo*.[12,13,15] *sPrdm16*-overexpressing murine p53$^{-/-}$ BM cells also showed increased clonogenicity in CFU assays.[12] In this study, we confirmed MYC-SLC1A5 axis-mediated metabolic reprogramming as a molecular basis for *sPRDM16*-induced Ara-C resistance in multiple cell types, including WT murine BM cells, *MA9* murine leukemia cells, human AML cell lines, and primary AML patient samples. On the other hand, growth curve analysis showed comparable cell growth in *MA9/sPrdm16* cells compared to *MA9/control* cells (*Online Supplementary Figure 1F*). In addition, the untreated *MA9/sPrdm16* cell-bearing mice lived longer than the untreated *MA9/control* cell-bearing mice when we injected 1.0x10^4 leukemia cells to sublethally irradiated (6.0 Gy) recipients (Figure 6). The difference in OS between these two groups diminished when we injected a greater number of leukemia cells (5.0x10^4 cells) to sublethally irradiated (6.0 Gy) recipients (*Online Supplementary Figure 1G*). These data suggest that sPrdm16-induced Ara-C resistance driven by metabolic reprogramming may be a common biological effect shared by a wide spectrum of normal hematopoietic and myeloid leukemia cells, whereas effect of sPrdm16 on cell growth and leukemogenicity may be context dependent, such as cell type or genetic background of leukemia cells. Taken together, these data suggest that PRDM16 drives AraC resistance through MYC-induced upregulation of the glutamine transporter SLC1A5, activation of glutamine uptake, glutaminolysis and subsequent mitochondrial oxidative metabolism (Figure 7D). This is further supported by the recent study showing that EVI1, another PRDM family member, also triggers metabolic reprogramming toward activated glutaminolysis and OXPHOS status in AML for leukemia cell survival, sensitizing *EVI1*-high AML cells to L-asparaginase which depletes intracellular asparagine and glutamine.[29] Our study revealed MYC-glutaminolysis-mitochondrial OXPHOS pathway to be a fundamental basis for *PRDM16*-driven AraC resistance, providing a rationale for mitochondrial respiration-targeted therapies against *PRDM16*-mediated chemoresistance which should be further clarified in future clinical studies.

Disclosures

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Contributions

JI, HK, YS, KM, IK, MFT and HN are responsible for study concept and methodology. JI, YS, AM, TK, KM, IK and NS are responsible for validation, formal analysis, investigation and data curation. YS, ST, MT, MTF, AY and TT are responsible for resources. JI and HK wrote the original draft. JI, HK and HN wrote, reviewed and edited the paper. JI, HK and YS are responsible for visualization. HK and HN are responsible for funding acquisition, and project supervision and administration.

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Data-sharing statement

For original data, please contact kunimoto@yokohama-cu.ac.jp RNA-seq data are available at GEO under accession number GSE280735.

References

1. Morishita K. Leukemogenesis of the EVI1/MEL1 gene family. Int J Hematol. 2007;85(4):279-286.
2. Aguilo F, Avagyan S, Labar A, et al. Prdm16 is a physiologic regulator of hematopoietic stem cells. Blood. 2011;117(19):5057-5066.
3. Shiba N, Ohki K, Kobayashi T, et al. High PRDM16 expression identifies a prognostic subgroup of pediatric acute myeloid leukemia correlated to FLT3-ITD, KMT2A-PTD, and NUP98-NSD1: the results of the Japanese Paediatric Leukaemia/Lymphoma Study Group AML-05 trial. Br J Haematol. 2016;172(4):581-591.
4. Yamato G, Yamaguchi H, Handa H, et al. Clinical features and prognostic impact of PRDM16 expression in adult acute myeloid leukemia. Genes Chromosomes Cancer. 2017;56(11):800-809.
5. Dao F, Chen W, Long L, et al. High PRDM16 expression predicts poor outcomes in adult acute myeloid leukemia patients with intermediate cytogenetic risk: a comprehensive cohort study from a single Chinese center. Leuk Lymphoma. 2021;62(1):185-193.
6. Xiang X, Lu Q, Xu X, et al. Prognostic impact of PRDM16 expression in acute myeloid leukemia with normal cytogenetics. Hematology. 2022;27(1):499-505.
7. El-Meligui YM, Hassan NM, Kassem AB, et al. Impact of HOXB4 and PRDM16 gene expressions on prognosis and treatment response in acute myeloid leukemia patients. Pharmgenomics Pers Med. 2022;15:663-674.
8. Mochizuki N, Shimizu S, Nagasawa T, et al. A novel gene, MEL1, mapped to 1p36.3 is highly homologous to the MDS1/EVI1 gene and is transcriptionally activated in t(1;3)(p36;q21)-positive leukemia cells. Blood. 2000;96(9):3209-3214.
9. Chuikov S, Levi BP, Smith ML, Morrison SJ. Prdm16 promotes stem cell maintenance in multiple tissues, partly by regulating oxidative stress. Nat Cell Biol. 2010;12(10):999-1006.
10. Nishikata I, Sasaki H, Iga M, et al. A novel EVI1 gene family, MEL1, lacking a PR domain (MEL1S) is expressed mainly in t(1;3)8p36;q21)-positive AML and blocks G-CSF-induced myeloid differentiation. Blood. 2003;102(9):3323-3332.
11. Xiao Z, Zhang M, Liu X, Zhang Y, Yang L, Hao Y. MEL1S, not MEL1, is overexpressed in myelodysplastic syndromes patients with t(1;3)(p36;q21). Leuk Res. 2006;30(3):332-334.
12. Shing DC, Trubia M, Marchesi F, et al. Overexpression of sPRDM16 coupled with loss of p53 induces myeloid leukemias in mice. J Clin Invest. 2007;117(12):3696-3707.
13. Hu T, Morita K, Hill MC, et al. PRDM16s transforms megakaryocyte-erythroid progenitors into myeloid leukemia-initiating cells. Blood. 2019;134(7):614-625.
14. Zhou B, Wang J, Lee SY, et al. PRDM16 suppresses MLL1r leukemia via intrinsic histone methyltransferase activity. Mol Cell. 2016;62(2):222-236.
15. Yu H, Neale G, Zhang H, et al. Downregulation of Prdm16 mRNA is a specific antileukemic mechanism during HOXB4-mediated HSC expansion in vivo. Blood. 2014;124(11):1737-1747.
16. Lee BH, Williams IR, Anastasiadou E, et al. FLT3 internal tandem duplication mutations induce myeloproliferative or lymphoid disease in a transgenic mouse model. Oncogene. 2005;24(53):7882-7892.
17. Shiba N, Yoshida K, Hara Y, et al. Transcriptome analysis offers a comprehensive illustration of the genetic background of pediatric acute myeloid leukemia. Blood Adv. 2019;3(20):3157-3169.
18. Braess J, Fiegl M, Lorenz I, Waxenberger K, Hiddemann W. Modeling the pharmacodynamics of highly schedule-dependent agents: exemplified by cytarabine-based regimens in acute myeloid leukemia. Clin Cancer Res. 2005;11(20):7415-7425.
19. Farge T, Saland E, de Toni F, et al. Chemotherapy-resistant human acute myeloid leukemia cells are not enriched for leukemic stem cells but require oxidative metabolism. Cancer Discov. 2017;7(7):716-735.
20. Adamoski D, Dias MM, Quesnay JEN, et al. Molecular mechanism of glutaminase activation through filamentation and the role of filaments in mitophagy protection. Nat Struct Mol Biol. 2023;30(12):1902-1912.
21. Amaya ML, Inguva A, Pei S, et al. The STAT3-MYC axis promotes survival of leukemia stem cells by regulating SLC1A5 and oxidative phosphorylation. Blood. 2022;139(4):584-596.
22. Aroua N, Boet E, Ghisi M, et al. Extracellular ATP and CD39 activate cAMP-mediated mitochondrial stress response to promote cytarabine resistance in acute myeloid leukemia. Cancer Discov. 2020;10(10):1544-1565.
23. Yang X, Liu J, Liu W, et al. circFAM193B interaction with PRMT6 regulates AML leukemia stem cells chemoresistance through altering the oxidative metabolism and lipid peroxidation. Leukemia. 2024;38(5):1057-1071.
24. Edinger AL, Thompson CB. Akt maintains cell size and survival by increasing mTOR-dependent nutrient uptake. Mol Biol Cell. 2002;13(7):2276-2288.
25. Plas DR, Talapatra S, Edinger AL, Rathmell JC, Thompson CB.

Akt and Bcl-xL promote growth factor-independent survival through distinct effects on mitochondrial physiology. J Biol Chem. 2001;276(15):12041-12048.
26. Barata JT, Silva A, Brandao JG, Nadler LM, Cardoso AA, Boussiotis VA. Activation of PI3K is indispensable for interleukin 7-mediated viability, proliferation, glucose use, and growth of T cell acute lymphoblastic leukemia cells. J Exp Med. 2004;200(5):659-669.
27. DeBerardinis RJ, Lum JJ, Hatzivassiliou G, Thompson CB. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation. Cell Metab. 2008;7(1):11-20.
28. Wise DR, DeBerardinis RJ, Mancuso A, et al. Myc regulates a transcriptional program that stimulates mitochondrial glutaminolysis and leads to glutamine addiction. Proc Natl Acad Sci USA. 2008;105(48):18782-18787.
29. Saito Y, Sawa D, Kinoshita M, et al. EVI1 triggers metabolic reprogramming associated with leukemogenesis and increases sensitivity to L-asparaginase. Haematologica. 2020;105(8):2118-2129.