SAMHD1 is a biomarker for cytarabine response and a therapeutic target in acute myeloid leukemia

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The nucleoside analog cytarabine (Ara-C) is an essential component of primary and salvage chemotherapy regimens in acute myeloid leukemia (AML). After cellular uptake, Ara-C is converted into its therapeutically active triphosphate metabolite, Ara-CTP, which exerts anti-leukemic effects primarily by inhibiting DNA synthesis in proliferating cells[1]. Currently, a substantial fraction of AML patients fails to effectively respond to Ara-C therapy and reliable biomarkers are lacking[2,3]. SAMHD1 is a deoxynucleoside triphosphate (dNTP) triphosphohydrolase that cleaves physiological dNTPs into deoxyribonucleosides and inorganic triphosphate[4,5]. Although it has been postulated that SAMHD1 sensitizes cancer cells to nucleoside analog derivatives through depletion of competing dNTPs[6], we show here that SAMHD1 reduces Ara-C cytotoxicity in AML cells. Mechanistically, dGTP-activated SAMHD1 hydrolyzes Ara-CTP, resulting in a drastic reduction of Ara-CTP in leukemic cells. Loss of SAMHD1 activity - through genetic depletion, mutational inactivation of its triphosphohydrolase activity, or proteasomal degradation using specialized virus-like particles[7,8] - potentiates the cytotoxicity of Ara-C in AML cells. In mouse retroviral AML transplantation models as well as in retrospective analyses of adult AML patients, the response to Ara-C-containing therapy was inversely correlated with SAMHD1 expression. These results identify SAMHD1 as a potential biomarker for the stratification of AML patients to Ara-C-based therapy and as a target for treating Ara-C-refractory AML.

The current backbone of AML therapy is treatment with the cytidine analog Ara-C and the anthracycline daunorubicin. Despite a high rate of initial remissions, a substantial fraction of AML patients relapses and acquires resistance to Ara-C[1,9]. The prognosis of AML patients, especially elderly ones, remains dismal[2,9]. Mutations in the *SAMHD1* gene, encoding Sterile alpha motif and histidine-aspartic domain-containing protein 1 (SAMHD1), have been associated with the Aicardi-Goutières autoimmune syndrome[10] and the development of malignancies including cutaneous T-cell lymphoma, chronic lymphatic leukemia, and colon cancer[11-13]. SAMHD1, a dNTP triphosphohydrolase that cleaves dNTPs into deoxyribonucleosides and inorganic triphosphate, enhances the efficacy of certain nucleoside analog drugs for the treatment of human immunodeficiency virus type-1 (HIV-1) by decreasing the levels of intracellular dNTPs[14,15], which apparently compete with the thymidine analog triphosphates for incorporation into HIV-1 cDNA during reverse transcription[16]. We postulated that SAMHD1 could have a similar effect on nucleoside analog-based therapy in leukemia[6].

To investigate whether SAMHD1 expression enhances Ara-C cytotoxicity in AML cells, we tested whether Ara-C sensitivity in 13 AML cell lines, determined by the half maximal inhibitory concentration (IC_{50}), is correlated with SAMHD1 protein and mRNA levels. Both SAMHD1 expression (**Fig. 1a** and **Supplementary Fig. 1**) and Ara-C sensitivity (**Supplementary Table 1**) varied considerably among these cell lines. Unexpectedly, SAMHD1 levels inversely correlated with Ara-C cytotoxicity (p=0.0037, **Fig. 1b** and **Supplementary Fig. 2a,b**), as well as with the levels of early (Caspase 3 and 7 activity, p=0.02, **Supplementary Fig. 3a,b**) and late (sub-G1 cells, apoptotic DNA fragmentation, p=0.029, **Supplementary Fig. 3c,d**) markers of apoptosis. In contrast, no significant correlation could be established between Ara-C IC_{50} values and the expression of cellular

proteins previously implicated in Ara-C uptake or its conversion to Ara-CTP[1], including equilibrative nucleoside transporter (ENT1/SLC29A1), deoxycytidine kinase (DCK), cytidine deaminase (CDA), deoxycytidilate deaminase (DCTD), or 5'-nucleotidase (NT5C2) (**Fig. 1a,c-g**).

To further investigate its role in Ara-C resistance, we tested the effects of SAMHD1 deficiency by a number of approaches: (i) depletion of SAMHD1 in AML cell lines expressing high endogenous SAMHD1 levels using either lentiviral vectors encoding *SAMHD1*-specific shRNA or transfection with *SAMHD1*-specific siRNA; (ii) CRISPR/Cas9-mediated disruption of the *SAMHD1* gene; and (iii) targeted degradation of SAMHD1 using virus-like particles (VLPs) which shuttle the SAMHD1-interacting lentiviral Vpx protein (Vpx-VLPs) into cells[7,8,17] (**Fig. 2a** and **Supplementary Fig. 4**). Vpx recruits SAMHD1 to a cullin4A-RING E3 ubiquitin ligase ($CRL4^{DCAF1}$), which targets the enzyme for proteasomal degradation[7,8].

SAMHD1 depletion in AML cell lines by RNA interference (OCI-AML3, THP-1), *SAMHD1* knockout ($THP\text{-}1^{-/-}$), or transduction with Vpx-VLPs (MonoMac6 cells, THP-1) markedly sensitized AML cell lines to Ara-C toxicity relative to the respective controls (**Fig. 2a,b** and **Supplementary Fig. 4**). In contrast, *SAMHD1* siRNA had only a marginal effect on Ara-C toxicity in low SAMHD1-expressing HEL cells (**Fig. 2a,b**). Interestingly, we observed SAMHD1 dependency, although less pronounced, for the purine analog fludarabine (**Supplementary Fig. 5a**); however, the IC_{50} values for the topoisomerase II inhibitors etoposide and daunorubicin, as well as for dFdC (2',2'-difluorodeoxycytidine; gemcitabine), were not consistently affected by SAMHD1 down-modulation (**Supplementary Fig. 5b-d**), indicating a certain degree of drug specificity.

In HEL cells, an AML cell line that expresses very low endogenous levels of SAMHD1 (**Fig. 1a** and **Fig. 2a,c**), constitutive overexpression of wild-type SAMHD1 (SAMHD1-WT), but not of the dNTPase-deficient SAMHD1-D311A mutant, increased the IC_{50} values for Ara-C and fludarabine (**Fig. 2c** and **Supplementary Fig. 5a**). In contrast, the toxicity of daunorubicin, etoposide, or dFdC was largely unaltered (**Supplementary Fig. 5b-d**). These results indicate that SAMHD1's enzymatic activity is critically involved in mediating resistance of AML cells to Ara-C and, to a lesser extent, fludarabine. Notably, Ara-C at concentrations mimicking the steady state levels of Ara-C in plasma during standard Ara-C regimens (100-200 mg/m^2)[1] or the minimum blood plasma concentration (C_{min}; achieved 12h after completion of i.v. infusion) during high-dose Ara-C therapy (3000 mg/m^2)[18] only partially affected the viability of SAMHD1-expressing AML cell lines, whereas SAMHD1-depleted cells were effectively killed (**Supplementary Fig. 6**).

Although previous *in vitro* studies provided no evidence that triphosphorylated anti-viral nucleoside analogs can be hydrolyzed by SAMHD1[19,20], we hypothesized that SAMHD1 might be able to hydrolyze Ara-CTP, which differs from the physiological dCTP substrate by only a hydroxyl substituent in the up-position at carbon atom-2 of the pentose moiety (**Supplementary Fig. 7**). To directly test whether Ara-CTP is hydrolyzed by SAMHD1, we quantified Ara-CTP levels relative to SAMHD1 expression following short-term exposure of AML cells to $^{13}C_3$-Ara-C, using liquid chromatography tandem mass spectrometry (LC-MS/MS). We detected 47-fold higher Ara-CTP levels in SAMHD1-deficient THP-1$^{-/-}$ cells compared to THP-1$^{+/+}$ control cells (**Supplementary Fig. 8a**), whereas SAMHD1 deficiency did not affect dFd-CTP levels after drug treatment (**Supplementary Fig. 8b**). Moreover, HEL SAMHD1-WT cells harbored 97- and 69-fold lower levels of Ara-CTP compared to parental HEL cells and HEL cells expressing the SAMHD1-D311A mutant, respectively (**Fig. 2d**).

Notably, lack of enzymatically active SAMHD1 in THP-1$^{-/-}$ cells or in parental and SAMHD1-D311A-expressing HEL cells also resulted in elevated dNTP levels (**Supplementary Fig. 9a,b**), which, however, did not counteract the cytotoxicity of the concomitantly increased Ara-CTP levels (**Fig. 2b,c**).

dGTP or GTP binding to the primary allosteric site of SAMHD1 leads to formation of a catalytically active tetramer, whereas the second allosteric site of the enzyme accommodates dNTPs[21-23]. To elucidate whether Ara-CTP can serve as a substrate or as both an activator and substrate for the triphosphohydrolase, we performed an enzymatic *in vitro* assay using bacterially expressed full-length SAMHD1. Analysis of reaction products by ion-pair reverse-phase HPLC revealed that SAMHD1 hydrolyzes Ara-CTP to Ara-C, requiring the presence of the activator dGTP, which itself is also cleaved to dG (**Fig. 2e**). In specificity control experiments, the physiological substrate TTP, but not dFd-CTP, was hydrolyzed by SAMHD1 (**Supplementary Fig. 10a,b**). Thus, Ara-CTP is a direct substrate, but not an activator, of SAMHD1's triphosphohydrolase activity in AML cells.

To explore whether SAMHD1 might contribute to the development of Ara-C resistance, we gradually adapted three AML cell lines (HEL, HL-60, and Molm13), characterized by low endogenous SAMHD1 expression levels and high Ara-C sensitivity, to growth in the presence of the nucleoside analog. The resulting drug-resistant sublines were cultivated in medium containing Ara-C at a concentration of 2 µg/ml (designated ^{r}Ara-C$^{2\mu g}$) and displayed increases in Ara-C IC_{50} values ranging from 1643- to 4250-fold relative to the parental cell lines (**Supplementary Table 2**). In addition to changes in the expression of some cellular factors previously implicated in acquired Ara-C resistance (i.e., DCK, CDA, and NT5C2), the levels of SAMHD1 were markedly increased in the Ara-C-resistant sublines relative to their parental counterparts (**Fig. 3a** and **Supplementary Fig. 11**). Ara-C resistance

was accompanied by drastically decreased Ara-CTP levels (**Fig. 3b**) and exposure of Ara-C-resistant AML cells to Vpx-VLPs depleted SAMHD1, increased Ara-CTP levels, and re-sensitized the cells to Ara-C by up to 11.5-fold relative to Vpr-VLP-treated control cells (**Fig. 3c,d**). Whereas cultivation of the parental AML cell lines in the presence of the drug for 24 h did not acutely induce SAMHD1 levels (**Supplementary Fig. 12a**), selection for 20 d in Molm13 cells resulted in an upregulation of SAMHD1 levels (**Supplementary Fig. 12b**).

To study the role of SAMHD1 in AML drug sensitivity *in vivo*, we transformed mouse myeloid progenitor cells using the oncogenes Hoxa9/Meis1 or MN1 and subsequently deleted the *SAMHD1* gene using CRISPR/Cas9 genome editing with two independent guide RNAs (gRNA). $SAMHD1^{+/+}$ control AML blasts and knockout ($SAMHD1^{-/-}$) AML blasts were transplanted into irradiated recipient mice, and were treated with either Ara-C or phosphate-buffered saline (PBS) on day 18 and 19 after transplantation (**Supplementary Fig. 13a-d**). Overall survival of the two independent mouse cohorts transplanted with $SAMHD1^{-/-}$ AML was dramatically prolonged compared to $SAMHD1^{+/+}$ AML by administration of Ara-C (**Fig. 4a,b** ($p=2.97x10^{-6}$ for both cohorts), **Supplementary Fig. 13e** ($p=3.05x10^{-6}$) and **Supplementary Fig. 13f** ($p=4.32x10^{-6}$)), but not by PBS treatment (**Fig. 4a,b** and **Supplementary Fig. 13e,f**). Similar results were obtained for two mouse cohorts for which a second SAMHD1 gRNA was used (**Supplementary Fig. 14**).

In human blasts isolated from bone marrow of therapy-naïve AML patients, basal SAMHD1 expression correlated with Ara-C IC_{50} values ($p=0.04$, **Supplementary Fig. 15**). Moreover, we tested the effects of transient depletion of SAMHD1 in blasts of six patients (**Supplementary Table 3**, patients A to F). Two days after transfection with control or *SAMHD1*-targeting siRNA, the blasts were cultured in the presence of Ara-C or daunorubicin. SAMHD1 depletion diminished Ara-C IC_{50} values by 3- to 15-fold (**Fig. 4c**), whereas the

sensitivity of the blasts to daunorubicin was unaltered (**Supplementary Table 4**). Similarly, treatment with SAMHD1-degrading Vpx-VLPs sensitized primary AML blasts to Ara-C, but not to daunorubicin, compared to Vpr-containing control VLPs (**Supplementary Fig. 16** and **Supplementary Table 5**). Concomitantly with this sensitization, intracellular Ara-CTP levels were elevated (**Supplementary Fig. 8c,d**). Finally, we examined the suitability of SAMHD1 expression as a biomarker for predicting the response of AML to standard Ara-C-containing therapy. We analyzed an AML cohort of 150 adult patients who had received one to two courses of Ara-C-containing induction therapy in two University Hospital study centers in Germany. Patients received either two cycles of "7+3" or "7+3" plus high-dose Ara-C in combination with mitoxantrone (HAM) according to standard German AML protocols (**Supplementary Table 6**). The "7+3" treatment regime refers to 7 days of standard-dose Ara-C with the addition of daunorubicin for 3 days during the 7 day-chemotherapy induction cycle (see Online Methods). Retrospective analysis of SAMHD1 protein levels by immunohistochemical (IHC) staining of blasts in sections of paraffinized bone marrow isolated at primary diagnosis revealed that expression of SAMHD1 was highly variable between patients and, importantly, markedly increased in the group of AML patients that did not reach a complete remission ("No CR", n=38) at the end of induction therapy, as compared to the group of patients with documented complete remission ("CR", n=112) ($p=6.3x10^{-8}$, Wilcoxon Rank Sum test) (**Fig. 4d,e** and **Supplementary Table 6**). Of the 150 patients, 112 achieved CR; of these 90 were scored as "SAMHD1-low" and 22 were scored as "SAMHD1-high". The CR rate in the "SAMHD1-high" cohort was 22/50 (44%), whereas the CR rate in the "SAMHD1-low" cohort was 90/100 (90%). This difference between the two SAMHD1-stratified cohorts was highly statistically significant ($p=3.477x10^{-9}$, Chi-Squared Test with 1 degree of freedom). As a validation of the IHC staining and scoring system, levels of

SAMHD1 protein expression in blasts as determined by IHC scoring and in parallel by flow cytometry showed a positive correlation ($p<0.0001$, **Supplementary Fig. 17b**). All CR patients received post-remission therapy: a high-dose Ara-C-containing chemo-consolidation regimen and/or allogeneic stem cell transplantation. In our AML cohort, the level of SAMHD1 expression in blasts at initial diagnosis was highly predictive for event-free survival (EFS) ($p=1.86 \times 10^{-11}$, **Fig. 4f**), where an event was defined as failure to achieve CR, relapse or death. Also, relapse-free survival (RFS) of CR patients was significantly worse in "SAMHD1-high" compared to "SAMHD1-low" patients ($p=2.02 \times 10^{-4}$, **Fig. 4g**), indicating that the depth of remission in "SAMHD1-high" patients was less pronounced than in "SAMHD1-low" patients. Although the median observation time was relatively short, "SAMHD1-high" patients experienced a significantly worse overall survival (OS) ($p=4.85 \times 10^{-3}$, **Fig. 4h**). When patients were censored from the analyses at the time of allogeneic transplantation, all differences (EFS, RFS, OS) remained statistically significant (**Supplementary Fig. 18a-c**). Notably, no correlation could be established between cytogenetic risk groups and SAMHD1 expression (**Supplementary Fig. 19a**). In a multivariate analysis including age, gender, initial leukocyte count and cytogenetic risk group, and type of second induction cycle (HAM versus "7+3"), high SAMHD1 expression proved to be an independent risk factor for EFS (hazard ratio 1.72, $p=5.23 \times 10^{-8}$) and OS (1.33, $p=0.0228$) (**Supplementary Fig. 19b,c**).

Furthermore, mining of the publicly available AML data set of the TCGA cohort[24] confirmed a correlation between low SAMHD1 mRNA expression with CR for patients who had received an Ara-C-containing therapy (**Supplementary Fig. 20**). Thus, SAMHD1 levels at diagnosis inversely correlate with the clinical response to Ara-C-based therapy in two

different adult AML patient cohorts, and this prominent role of SAMHD1 in Ara-C response is corroborated by results from murine AML transplantation models.

Taken together, this study identifies SAMHD1 as a cellular biomarker for stratification of patients to Ara-C-based therapy and uncovers a patient- and drug-specific interference mechanism (**Supplementary Fig. 21**) that, in addition to established molecular and cytogenetic risk factors[25], determines the outcome of AML disease. Ara-C's mimicry of the physiological nucleoside cytidine endows it with anti-leukemic activity, but this mimicry is detrimental in AML blasts that express the Ara-CTP-inactivating SAMHD1 protein. This concept applies to a lesser extent also to the purine analog fludarabine, but not to dFdC, indicating that SAMHD1 should be considered a critical cellular factor in the evaluation of all nucleoside analog-based drugs or drug candidates in AML. Future studies will determine whether protein- or mRNA-based quantification of SAMHD1 in AML blasts at diagnosis is a better predictor of the therapeutic response. *In vivo* strategies aiming at transient downregulation of SAMHD1 by either RNA interference or application of SAMHD1-degrading Vpx-VLPs, or, conceivably, through administration of an inhibitor of SAMHD1's enzymatic activity, may improve the Ara-C-based treatment of AML and other malignancies.

METHODS

Methods and any associated reference are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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Author Contributions

J.C. and O.T.K. conceived the study and together with C.S., T.O., H.-M.B., and S.-M.S. designed and analyzed the majority of experiments. D.T., R.F., H.B., L.S., M.Ma, and F.C. conducted experiments, analyzed data, and provided discussion. A.C., J.L., M. Mi., and V.H. provided critical reagents and discussion. G.G., M.-L.H., L.K., A.F.Y., P.S., N.F., C.S., W.E.B., W.H., E.W., and H.S. analyzed data and provided discussion. J.C. and O.T.K. wrote and all authors edited the manuscript.

Competing Financial Interests Statement

The Johann Wolfgang Goethe-University has filed a patent application, on which several of the coauthors are listed as inventors.

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Legends to Figures

Figure 1| SAMHD1 expression levels inversely correlate with Ara-C cytotoxicity in AML cell lines. (**a**) Representative immunoblots of SAMHD1 and other proteins previously reported to be involved in Ara-C uptake and its conversion to the active Ara-C metabolite, Ara-CTP, in the indicated AML cell lines. β-actin served as a loading control. Three independent experiments were performed. Uncropped images are shown in **Supplementary Figure 22**. (**b-g**) Correlation analyses between Ara-C IC_{50} values for these AML cell lines in **a** and the relative expression levels of SAMHD1 (**b**), ENT1 (**c**), DCK (**d**), CDA (**e**), DCTD (**f**), or NT5C2 (**g**). Expression levels were normalized to β-actin and are shown as arbitrary units (a.u.); the relative expression of each protein in THP-1 cells was set to 1. For **b-g**, closed circles and error bars represent mean ± s.e.m. of three independent experiments each performed with technical replicates (n=3). Data in **b-g** were analysed using a linear regression model. R^2 values indicate goodness of fit of the regression model to the data, and represent variance explained by the independent variable divided by total variance of the IC_{50} Ara-C values.

Figure 2 |SAMHD1 counteracts Ara-C toxicity in AML cell lines via hydrolysis of the active metabolite, Ara-CTP. (**a**) Representative immunoblots for SAMHD1 after CRISPR/Cas9-mediated *SAMHD1* knockout in THP-1 (THP-$1^{-/-}$) cells or after shRNA or siRNA-mediated silencing of *SAMHD1* in OCI-AML3 and HEL cells. Alternatively, OCI-AML3 and MonoMac6 cells were transduced with VLPs carrying either lentiviral Vpr (Vpr-VLPs, control) or Vpx (Vpx-VLPs) proteins. β-actin served as a loading control. Uncropped images are shown in **Supplementary Figures 22** and **23**. (**b**) Ara-C IC_{50} values of the

experimental groups shown in **a**. Each circle represents a technical replicate (n=3) of three independent experiments performed. Horizontal lines and error bars represent means ± s.e.m. Numbers at the top indicate the factor of decrease of Ara-C IC_{50} values in SAMHD1-depleted relative to control cells. (**c**) Ara-C IC_{50} values (top) and immunoblot for SAMHD1 (bottom) in parental HEL cells or HEL cells stably transduced with either wildtype SAMHD1 (SAMHD1-WT) or the dNTPase-inactive D311A SAMHD1 mutant (SAMHD1-D311A). For the IC_{50} data, the numbers above the bars indicate the factor by which the IC_{50} value differed relative to HEL SAMHD1-WT cells. The IC_{50} values are shown as means ± s.e.m. of three independent experiments each performed with technical replicates (n=3) represented by individual circles. β-actin served as a loading control for the SAMHD1 immunoblot. Uncropped images are shown in **Supplementary Figure 23**. (**d**) Representative liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of Ara-CTP in parental HEL cells (blue chromatogram), HEL SAMHD1-WT cells (black chromatogram), or HEL SAMHD1-D311A cells (red chromatogram). (**e**) HPLC analysis of products from an enzymatic *in vitro* assay using bacterially expressed full-length SAMHD1. Ara-CTP was incubated by itself (top), in the presence of SAMHD1 (middle), or in the presence of SAMHD1 and the allosteric activator/substrate dGTP (bottom). Chromatogram peaks corresponding to Ara-C, dG, and Ara-CTP are indicated. Statistical analyses were performed using unpaired two-tailed Students' *t*-test. For (**b**) the degree of freedom was 16 and $p< 0.0001$ for all except for siRNA-treated HEL cells (df =4, p=0.0004). df was 16 and $p< 0.0001$ for (**c**). *$p\leq0.05$; **$p<0.01$, ***$p<0.001$.

Figure 3 | SAMHD1 contributes to the acquired resistance of AML cells to Ara-C. (**a**) Representative immunoblots of proteins involved in Ara-C uptake and metabolism in parental AML cell lines (HEL, HL-60, and Molm13) and in their respective Ara-C-resistant sublines (HEL^{r}Ara-C$^{2\mu g}$, HL-60^{r}Ara-C$^{2\mu g}$, and Molm13^{r}Ara-C$^{2\mu g}$). β-actin served as a loading control. Three independent experiments were performed. Uncropped images are shown in **Supplementary Figure 24**. (**b**) Quantitative analysis by LC-MS/MS of Ara-CTP levels in parental and Ara-C-resistant AML cell lines. The means ± s.d. of triplicates of one representative experiment are shown; three independent experiments were performed. Numbers above the bars represent the factor of decrease in Ara-CTP levels in Ara-C-resistant cell lines relative to their parental counterparts. (**c**) Ara-C-resistant cell lines were treated with the indicated VLPs and subsequently analyzed for Ara-C cytotoxicity (top) and SAMHD1 expression (bottom). Ara-C IC_{50} values of three independent experiments each performed with technical replicates (n=3) are presented with center lines showing the medians. The box limits are quartiles 1 and 3, and whiskers show maximum and minimum values. Numbers above the bars indicate the factor of decrease in IC_{50} values in Vpx-VLP-treated cells relative to Vpr-VLP-treated controls. (**d**) Representative LC-MS/MS chromatograms of Ara-CTP in Molm13^{r}Ara-C$^{2\mu g}$ cells treated with either Vpr-VLPs (control, black), Vpx-VLPs (red), or left untreated (blue). Statistical analyses were performed using unpaired two-tailed Students' *t*-test. For (**b**) the degree of freedom for all was 4 and exact p-values 0.0073 for HEL cells, 0.0037 for HL-60 cells and 0.0004 for Molm13 cells. df was 16 and $p< 0.0001$ for (**c**).*$p\leq0.05$; **$p<0.01$, ***$p <0.001$.

Figure 4 | SAMHD1 expression in leukemic blasts predicts response to Ara-C-containing therapy in mouse transplantation models and AML patients. (**a,b**) Kaplan-

Meier survival analyses of Hoxa9/Meis1- (**a**) or MN1-driven (**b**) AML transplantation models using myeloid progenitors with endogenous SAMHD1 expression ($SAMHD1^{+/+}$) or SAMHD1-deleted ($SAMHD1^{-/-}$) myeloid progenitors. Ara-C: i.p. administration of 75 mg/kg Ara-C on days 18 and 19 after transplantation; Control: PBS. The difference in overall survival between Ara-C-treated $SAMHD1^{+/+}$ and $SAMHD1^{-/-}$ mice was statistically significant (logrank test, Hoxa9/Meis1: $p=2.97x10^{-6}$, n=10 both groups; MN1: $p=2.97x10^{-6}$, n=10 both groups). The differences between the two control groups and between $SAMHD1^{+/+}$Ara-C and both control groups were not significant (Hoxa9/Meis1: p=0.42, $SAMHD1^{-/-}$ (n=10) vs $SAMHD1^{+/+}$ (n=9); MN1: p=0.196, n=10 both groups). (**c**) Blasts isolated from bone marrow from six adult AML patients (A–F) were transfected with *SAMHD1*-specific or control siRNAs and two days later analyzed for Ara-C cytotoxicity (top) and SAMHD1 expression (bottom). Ara-C IC_{50} values are presented as the means ± s.d. of the triplicates shown. The numbers above the data points indicate the factor of difference between the siCTRL and si*SAMHD1* groups. Uncropped images are shown in **Supplementary Figure 25**. (**d**) Representative IHC micrographs showing SAMHD1 and CD34 expression in bone marrow (BM) from one No CR patient (#39), one CR patient (#28), and one healthy donor. Scale bar: 50 µm. (**e**) Comparison of SAMHD1 expression levels (IHC scores) in CR and No CR patients. See Online Methods for an explanation of the IHC scores. Shown are relative frequencies (in percent) of patients with IHC scores of 0, 1, 2 or 3 among CR (n=112) and No CR (n=38) patients. **f-h,** Kaplan-Meier analyses for event-free survival (**f**), relapse-free survival (**g**), and overall survival (**h**), for which AML patients were grouped into "No / low SAMHD1 expressors" (IHC scores 0 or 1, red curves) versus "High SAMHD1 expressors" (IHC scores 2 or 3, black curves). Numbers above the plots indicate the absolute number of patients in each of the two groups at the respective time points. Significance of difference

between survival curves in **f**,**g**,**h** was assessed using the logrank test (p-values indicated in figure).

ONLINE METHODS

Ethics statement. Whole blood and bone marrow biopsies of AML patients were obtained and collected pre- and post-treatment. All patients gave informed consent according to the Declaration of Helsinki to participate in the collection of samples. The use of whole blood and bone marrow aspirates was approved by the Ethics Committee of Frankfurt University Hospital (approval no. SPO-01-2015) and University Hospital Münster (approval no. 2007-390-f-S).

Plasmids. The SIVmac251-based *gag-pol* expression constructs pSIV3+R- (Vpr-deficient) and pSIV3+X- (Vpx-deficient) were previously reported[17]. pLKO.1-puro-control-shRNA and pLKO.1-puro-SAMHD1-shRNA#1-3 for shRNA-mediated silencing of SAMHD1 were previously described[15]. pHR-based transfer vectors expressing SAMHD1-WT or the D311A mutant were generated by site-directed mutagenesis in a codon-optimized SAMHD1 expression construct (kindly provided by Dr. Thomas Gramberg, Institute of Clinical and Molecular Virology, FAU Erlangen-Nürnberg, Erlangen, Germany) and subcloned into pHR-luc transfer vectors. pPAX2 was purchased from Addgene and pVSV-G has been previously described[26].

Cells and Reagents. Human AML cell lines including THP-1 (DSMZ no. ACC16; FAB M6), OCI-AML2 (DSMZ No. ACC 99; FAB M4), OCI-AML3 (DSMZ No. ACC 582; FAB M4), Molm13 (DSMZ No. ACC 554; FAB M5a), PL-21 (DSMZ No. ACC 536; FAB M3), HL-60

435 (DSMZ No. ACC 3; FAB M2), MV4-11 (DSMZ No. ACC 102; FAB M5), SIG-M5 (DSMZ
436 No. ACC 468; FAB M5a), ML2 (DSMZ No. ACC 15; FAB M4), NB4 (DSMZ No. ACC
437 207; FAB M3), KG1 (DSMZ No. ACC 14; FAB not indicated), MonoMac6 (DSMZ No.
438 ACC 124; FAB M5), and HEL (DSMZ No. ACC 11; FAB M6) were obtained from DSMZ
439 (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH). All cell lines were
440 cultured in IMDM (Biochrom) supplemented with 10% FBS (SIG-M5 20% FBS), 4 mM L-
441 Glutamine, 100 IU/ml penicillin, and 100 mg/ml streptomycin at 37°C in a humidified 5 %
442 CO_2 incubator. Cells were routinely tested for mycoplasma contamination (LT07-710, Lonza)
443 and authenticated by short tandem repeat profiling, as reported[27]. THP-1 cells deficient for
444 SAMHD1 (THP-$1^{-/-}$) and control cells (THP-$1^{+/+}$) were generated as previously described[28]
445 and cultivated in RPMI supplemented with 10% FCS, 100 IU/ml penicillin, and 100 mg/ml
446 streptomycin.
447 Mononuclear cells from blood or bone marrow AML samples were purified by Ficoll-
448 Hypaque gradient centrifugation[26]. Leukemic cells were enriched by negative selection with a
449 combination of CD3- (130-150-101), CD19- (130-150-301) and CD235a-microbeads (130-
450 150-501, all from Miltenyi Biotec) according to the manufacturer's instructions and separated
451 by the autoMACS™ Pro Separator. All preparations were evaluated for purity resulting in
452 >90% leukemic blasts. The AML-393 sample carrying a MLL-AF10 translocation was
453 derived from a 47 year old female with AML at relapse after bone marrow transplantation.
454 Primary cells were amplified in NSG mice and re-isolated from enlarged spleens as
455 described[29]. Frozen cells were kindly provided by Dr. Irmela Jeremias (Department of Gene
456 Vectors, Helmholtz Zentrum München, German Research Center for Environmental Health,
457 Munich, Germany) and thawed for experiments.

458 AML blasts (2 x 10^6) were cultivated in X-vivo 10 medium (Lonza) supplemented with 10%
459 HyClone FCS (Perbio), 4 mM L-glutamine, 25 ng/ml hTPO (130-094-013), 50 ng/ml hSCF
460 (130-096-695), 50 ng/ml hFlt3-Ligand (130-096-479) and 20 ng/ml hIL3 (130-095-069, all
461 from Miltenyi Biotec) in 96-well plates in the presence or absence of drugs.
462 The ecotropic GP+E86 packaging cell line was cultured in DMEM (Life Technologies) with
463 10% heat-inactivated FCS, 2 mM L-Glutamine, 100 U/ml Penicillin and 100 µg/ml
464 Streptomycin. Murine bone marrow cells were cultured in DMEM with 10% heat-inactivated
465 FCS, 2 mM L-Glutamine, 100 U/ml Penicillin and 100 µg/ml Streptomycin supplemented
466 with 10ng/ml murine recombinant IL3 (213-13), 10 ng/ml human recombinant IL6 (200-06),
467 and 100ng/ml murine recombinant SCF (250-03) (all from Peprotech).
468 Ara-C was purchased from Tocris (147-94-4), daunorubicin from Selleckchem (S3035),
469 etoposide from TEVA (45891.00), fludarabine from Tocris (21679-14-1) and dFdC
470 (gemcitabine) from Accord Healthcare GmbH (82092.00.00). Deoxynucleosides (dNs)
471 (Sigma-Aldrich), which are dNTP precursors, were used as previously reported[15]. All
472 nucleotide standards and internal standards for the LC-MS/MS analysis were obtained from
473 Sigma-Aldrich, Silantes or Alsachim[30]. Labeled cytarabine, $^{13}C_3$-Ara-C (SC-217994, Santa
474 Cruz), was used for LC-MS/MS analysis.
475
476 **Generation of Ara-C-resistant cell lines.** Ara-C-resistant cell lines were established by
477 continuous exposure of Ara-C sensitive cell lines HL-60, HEL, Molm13, THP-1, MV4-11,
478 OCI-AML3 to increasing drug concentrations as previously described[31,32] and are part of the
479 Resistant Cancer Cell Line (RCCL) collection
480 (http://www.kent.ac.uk/stms/cmp/RCCL/RCCLabout). Briefly, cells were cultured at
481 increasing Ara-C concentrations starting with concentrations that inhibited viability of the

parental cell lines by 50 % (IC_{50}). Ara-C concentrations were doubled every 2 to 6 weeks until cells readily grew in the presence of 2 µg Ara-C. Resistant cell lines were designated as HL-60^{r}Ara-C^{2µg}, HEL^{r}Ara-C^{2µg}, Molm13^{r}Ara-C^{2µg}, THP-1^{r}Ara-C^{2µg}, MV4-11^{r}Ara-C^{2µg} and OCI-AML3^{r}Ara-C^{2µg} and were continuously cultured in the presence of 2 µg Ara-C. Cells were routinely tested for mycoplasma contamination (LT07-710, Lonza) and authenticated by short tandem repeat profiling[27].

Cell viability assay. The viability of AML cell lines treated with various drug concentrations was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction assay after 96 hours of incubation as described previously[33]. IC_{50} values were determined using CalcuSyn (Biosoft).

Cytotoxic assay for AML blasts *ex vivo*. The cell viability of AML blasts was determined after 96 hours of incubation by the quantification of ATP levels in cell culture supernatants using the CellTiter-Glo® Luminescent Cell Viability Assay (G7573, Promega) according to the manufacturer's instructions. Luminescence was measured on a Tecan infinite M200 (TECAN) instrument at a wavelength of 560 nm (reference wavelength 620 nm). IC_{50} values were determined using CalcuSyn (Biosoft).

Apoptosis assays. Sub-G1 cells as a marker for DNA fragmentation in late apoptotic cells were measured according to Nicoletti by flow cytometry[34]. Briefly, Ara-C-treated and untreated cells were washed once in 1x PBS, incubated for at least 2h at 4°C with Nicoletti buffer (0.1 % trisodiumcitrate-dihydrate pH 7.4, 0.1% Triton X-100, 50 µg/ml propidium iodide), and diluted prior to measurement in 1x PBS. Samples were analyzed using a

506 FACSVerse (BD Biosciences) and FlowJo software (TreeStar). Alternatively, Caspase 3/7
507 activity as a surrogate for early apoptosis was quantified by luminescence. Briefly, Ara-C-
508 treated and untreated cells were measured using the Caspase-Glo® 3/7 assay (G8091,
509 Promega) according to the manufacturer's instructions. Luminescence was measured on a
510 Tecan infinite M200 (TECAN) instrument at a wavelength of 560 nm (reference wavelength
511 620 nm).
512
513 **Flow cytometry.** Intracellular SAMHD1 staining was performed as previously described[15].
514 Staining for surface markers (CD33, CD34, CD45) was applied prior to fixation. The
515 following fluorochrome-conjugated antibodies were used: CD33-PE (130-091-732), CD34-
516 FITC (130-081-001, both from Miltenyi Biotech), and CD45-V450 (560373, BD
517 Pharmingen), all diluted 1:11 per 10^7 cells, and Alexa-Fluor-660 (A-21074, Invitrogen, Life
518 technologies, 1:200). Samples were analyzed using a FACSVerse or FACSCanto II flow
519 cytometer (BD Biosciences) and FlowJo software (TreeStar).
520 For *in vitro* characterization of Hoxa9/Meis1- and MN1-transformed myeloid progenitor cells,
521 surface-marker expression was characterized by flow cytometry using a LSRFortessa flow
522 cytometer (BD Biosciences). Briefly, cells ($2x10^5$) were washed twice with 2% FCS in PBS
523 and stained with the following antibodies: PerCP-Cy5.5-conjugated anti-mouse Gr1 (45-
524 5931), V450-conjugated anti-mouse Mac1 (48-0112), APC-conjugated anti-mouse c-kit (17-
525 1171), PE-Cy7-conjugated anti-mouse Sca1 (25-5981), PE-conjugated anti-mouse FcεRI (12-
526 5898, all from eBioscience), and APC-H7-conjugated anti-mouse CD19 (560245, BD
527 Bioscience). 7-AAD (BD-Bioscience) was used for exclusion of dead cells.
528

Immunoblotting. Cells were lysed in Triton X-100 sample buffer and proteins separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Proteins were blotted onto a nitrocellulose membrane (Thermo Scientific). The following primary antibodies were used at the indicated dilutions, SAMHD1 (12586-1-AP, Proteintech, 1:1000), β-actin (3598R-100, BioVision via BioCat, 1:2000), DCK (sc-393099, Santa Cruz, 1:500), CDA (sc-365292, Santa Cruz, 1:100), ENT1 (ab135756, Abcam, 1:500), and NT5C2 (H00022978-M02, Abnova, 1:200), DCTD (NBP1-75825, Novus, 1:200). Visualization and quantification was performed using fluorescently labeled secondary antibodies (926-32210 IRDye® 800CW Goat anti-Mouse and 926-32211 IRDye® 800CW Goat anti-Rabbit, LI-COR, 1:20000) and an Odyssey CLx Imaging system (LI-COR Biosciences).

Patients. Patients were admitted to the University Hospital Frankfurt between 2010 and 2014 and treated for newly diagnosed AML with regimens containing standard dose Ara-C and daunorubicin ("7+3") (83 patients) or Ara-C alone (1 patient) (**Supplementary Table 6**); an additional 66 patients admitted to the University Hospital Münster were included in the IHC analyses. In addition, viable AML cells were purified from bone marrow of patients that were admitted to the University Hospital Frankfurt in 2015 and 2016 (**Supplementary Table 3**). Patients at the University Hospitals of Frankfurt and Münster are routinely advised to undergo a bone marrow biopsy at diagnosis. All patients consented to the scientific analyses of their data and to scientific analyses of biomaterial that was obtained for diagnostic purposes. All patients received at least one course of Ara-C at a dose of 100 mg/m^2 over 7 days and daunorubicin at a dose of 60 mg/m^2 over 3 days ("7+3") (if not otherwise stated). All patients below the age of 60 received a second cycle of induction therapy. Patients over the age of 60 received a second induction cycle only if their day 15 bone marrow aspirate showed more

than 5% blasts. For the analyses, patient records were reviewed by physicians who were unaware of the SAMHD1 expression results in the diagnostic biopsies. Remission criteria and cytogenetic risk groups were assessed according to the ELN guidelines.

The initial response to induction therapy was analyzed in bone marrow biopsies/aspirates and defined as complete (CR) if the blast count was <5%, and as "No CR" if the blast count was >5%. For the calculation of event-free survival (EFS), events were defined as failure to achieve complete remission (CR, CRi, CRp) within 40 days after the last induction cycle, relapse, or death at any time after start of therapy. Relapse-free survival (RFS) and remission duration were calculated only in patients that achieved CR.

Immunostaining of bone marrow samples. Bone marrow samples from 154 patients, including 150 AML patients and four healthy donors, were provided by the University Hospital Frankfurt and University Hospital Münster, Germany. Tissues were fixed in 4% buffered formalin, descaled by EDTA and embedded in paraffin. Immunohistochemical staining was performed as previously described[35]. Briefly, 2-μm bone marrow tissue sections were incubated with EnVision Flex Target Retrieval Solution, pH low (K8005, DAKO) and stained with primary antibodies directed against SAMHD1 (12586-1-AP, Proteintech, 1:3000) and against CD34 (IR632, DAKO) for 40 min at room temperature. Polymeric secondary antibodies coupled to HRPO peroxidase and DAB were used for visualization (REAL EnVision Peroxidase/DAB+, K5007, DAKO). Tissue samples were analyzed by light microscopy after counterstaining with Meyer's haematoxylin (K8008, DAKO).

Two pathologists, who were blinded to clinical history and therapeutic response, independently scored the SAMHD1 IHCs. They evaluated all tissue sections for nuclear SAMHD1 staining using a four-stage staining score: 0 = negative, 1 = weak intensity of

staining, 2 = strong intensity of staining in less than 25% of blasts, 3 = strong intensity of staining in more than 25% of blasts. IHC staining scores of 0 and 1 were defined as "No / low expression" and IHC staining scores of 2 and 3 were defined as "High SAMHD1 expression". Membranous CD34 staining for the quantification of the number of AML blasts was evaluated using a two-stage staining score: 0 = negative, 1 = positive.

Retrospective analysis of the TCGA AML cohort. Clinical data, mutational profiles and normalized gene expression data for a cohort of 200 AML patients from The Cancer Genome Atlas (TCGA, run date 20150821)[24] were retrieved using the RTCGA-Toolbox R/BioConductor package, version 2.2.2[36]. All analyses were performed using R 3.3.0 and custom scripts. To test the association between SAMHD1 expression and first complete remission, all Ara-C-treated patients with available gene expression data were considered. To test the association between SAMHD1 expression and cytogenetic risk, all patients with available gene expression data were considered. For analysis of mutation rates, all patients with available mutational profiles were considered.

Mice and retroviral infection of lineage-depleted bone-marrow cells. C57BL/6J female mice (age: 8-12 weeks) were obtained from Janvier-Labs (Le Genest-Saint-Isle, France). All animal experiments were performed according to the regulations of the United Kingdom Home Office and German authorities. All animal experiments were performed according to national and international standards. Bone marrow cells were harvested from mice, and lineage-negative cells were obtained by negative selection using the Lineage Cell Depletion Kit (130-090-858, Miltenyi Biotec) as recommended by the manufacturer. Lineage-negative cells were co-cultured with GP+E86 cells packaging MSCV-Hoxa9-PGK-neo in the presence

of polybrene (10 µg/ml, Sigma-Aldrich) for 3 days followed by co-incubation with GP+E86 MSCV-Meis1-IRES-YFP for 1 day. Hoxa9-expressing cells were selected with 0.6 mg/ml G418 (Sigma-Aldrich) for at least 5 days. MN1-overexpressing myeloid progenitor cells were generated as described previously[37]. After selection, cells were sorted with a FACS BD Aria III cell sorter. Lentiviral transductions of cultured cells with pLentiCRISPRv2 vectors encoding *SAMHD1*-specific CRISPR or control vectors were performed as described previously[35] using BU033 (5' CACCGgacgatcctcatccaaaaa 3') and BU034 (5' AAACtttttggatgaggatcgtcC 3') (**Fig. 4a,b**), or BU035 (5' CACCGgatgattctgataaggaga 3') and BU036 (5' AAACtctccttatcagaatcatcC 3') (**Supplementary Fig. 14**).

Transplantation and monitoring analyses of transplanted mice. $7.5x10^4$ cells were transplanted together with $2x10^5$ "support cells" by injection into the tail vein of lethally irradiated (9.5 Gy) recipient mice (C57BL/6J). Wild-type mononuclear bone marrow cells isolated from C57BL/6J mice and purified on a Ficoll gradient (Sigma-Aldrich) were used as support cells to reconstitute hematopoiesis in irradiated recipient mice. 18 days after transplantation, mice were treated with 75 mg/kg cytarabine or PBS (i.p.) for 2 subsequent days.

Blood and bone marrow cells were isolated from mice for further analyses. Blood counts were analyzed with ScilVet abc animal blood cell counter (Scil Animal Care Company). Cells from spleen and bone marrow were incubated for 10 min with erythrocyte lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA) and then washed twice with 2% FCS in PBS. Staining was performed as described above.

Blood smears and purified cells from bone marrow and spleen were centrifuged on cover slips ($2x10^5$ cells/slip). Subsequently, cells were fixed for 10 min with methanol and stained with a

May-Grünwald solution for 8 min followed by Giemsa (both Merck Millipore) staining for 20 min.

mRNA analyses. RNA extraction and TaqMan-based mRNA quantification of SAMHD1 (Applied Biosystems: assay no. Hs00210019-m1) and RNaseP (Applied Biosystems: TaqMan® RNase P Control Reagents Kit (4316844), endogenous reference control) were performed essentially as reported[38].

LC-MS/MS analysis. Cells (5 x 10^5) were treated with 10 µg/ml $^{13}C_3$-Ara-C (SC-217994, Santa Cruz) and incubated at 37°C in a humidified 5 % CO_2 incubator for 6 h. Subsequently, cells were washed twice in 1 ml PBS, pelleted and stored at -20°C until measurement. The concentrations of dNTPs, $^{13}C_3$-Ara-CTP, and dFdC-TP in the samples were analyzed by liquid chromatography-electrospray ionization-tandem mass spectrometry essentially as previously described[30]. Briefly, the analytes were extracted by protein precipitation with methanol. An anion exchange HPLC column (BioBasic AX, 150 x 2.1 mm, Thermo) was used for the chromatographic separation and a 5500 QTrap (Sciex) instrument was used to analyze the samples, operating as triple quadrupole in positive multiple reaction monitoring (MRM) mode. Analysis of dNTPs was performed as previously described[30]. Additionally, $^{13}C_3$-Ara-CTP and dFdC-TP were quantified using Cytidine-$^{13}C_9$,$^{15}N_3$-5'-triphosphate as an internal standard (IS). The precursor-to-product ion transitions used as quantifiers were *m/z* 487.0 → 115.1 for $^{13}C_3$-Ara-CTP and m/z 504 → 326 for dFd-CTP. Due to the lack of commercially available standards of $^{13}C_3$-Ara-CTP and dFd-CTP, relative quantification was performed by comparing the peak area ratios (analyte/IS) of the differently treated samples.

Production of lentiviral expression vectors and VLPs. Lentiviral vectors expressing SAMHD1-WT or SAMHD1-D311A were generated by co-transfection of packaging vector pPAX2, either pHR-SAMHD1-WT or pHR-SAMHD1-D311A and a plasmid encoding VSV-G. VLPs, carrying either Vpx or Vpr from SIVmac251, were produced by co-transfection of 293T cells with pSIV3+ *gag pol* expression plasmids and a plasmid encoding VSV-G. The SAMHD1 degradation capacity of Vpx-VLPs was determined in THP-1 cells 24 h post transduction by intracellular SAMHD1 staining.

Manipulation of intracellular SAMHD1 levels. For shRNA-mediated silencing of *SAMHD1*, OCI-AML3 cells were transduced by spinoculation with VSV-G pseudotyped lentiviral vectors carrying either pLKO.1-puro-control-shRNA or pLKO.1-puro-SAMHD1-shRNA#1-3. On day 10 after transduction, successfully transduced cells were selected with puromycin (P8833, Sigma-Aldrich) (7.5 µg/ml). SAMHD1 levels were monitored by intracellular SAMHD1 staining and Western blotting. For siRNA-mediated silencing, AML cells (1.2×10^6) were transfected with 2.5 µM ON-TARGET plus human *SAMHD1* siRNA SMART-pool (L-013950-01-0050, Dharmacon) in resuspension electroporation buffer R (Invitrogen) using the Neon transfection system (Invitrogen). Additionally, ON-TARGET plus Non-targeting Pool (D-001810-10-50, Dharmacon) was transfected in parallel. Electroporation was performed using one 20 msec pulse of 1700 V and analyzed 48 h post-transfection via Western blotting and cell viability assay. The following siRNA duplexes were used: non-targeting (UGGUUUACAUGUCGACUAA;UGGUUUACAUGUUGUGUGA; UGGUUUACAUGUUUUCUGA; UGGUUUACAUGUUUUCCUA), *SAMHD1* (GACAAUGAGUUGCGUAUUU; CAUGUUUGAUGGACGAUUU; AAGUAUUGCUAGACGUGAA; UUAGUUAUAUCCAGCGAUU).

673 HEL cells were transduced by spinoculation with VSV-G pseudotyped lentiviral vectors
674 carrying either SAMHD1-WT or the D311A mutant. Expression of SAMHD1 was monitored
675 by intracellular SAMHD1 staining and Western blotting. AML cell lines and primary AML
676 blasts were spinoculated with VSV-G pseudotyped VLPs carrying either Vpx or Vpr.
677
678 **HPLC assays for hydrolysis**. To investigate whether or not Ara-CTP or dFd-CTP are
679 hydrolyzed by SAMHD1, HPLC assays were performed as described previously[39]. The
680 reaction mixtures (50 µl) contained 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 100 mM NaCl,
681 1 mM DTT, 1 mM Ara-CTP or dFdCTP, 0.2 mM activator (dGTP), and SAMHD1 (4 µM). In
682 some experiments, the SAMHD1 substrate TTP was used as positive control. Reaction
683 mixtures were incubated at 37°C and passed through 10K VWR Centrifugal Filters to quench
684 the reactions and remove the protein. Reaction products were analyzed by ion-pair reverse-
685 phase HPLC using a Varian Pursuit C18 column (150 × 4.6 mm) in a Varian ProStar HPLC
686 system with a photodiode array detector set at 260 nm. The mobile phase for separation of
687 nucleotides consisted of two eluants: 0.1 mM KH_2PO_4 (pH 6.0) with 8
688 mM tetrabutylammonium hydroxide and 0.1 mM KH_2PO_4 (pH 6.0) with 8
689 mM tetrabutylammonium hydroxide and 30 % methanol.
690
691 **Statistical analysis.** Statistical data analysis was performed using GraphPad Prism. Linear
692 regression and nonlinear fitting using one phase decay was used to assess correlation of
693 protein expression, mRNA levels, or IC_{50} values (**Fig. 1, Supplementary Figs. 2,3,15,17**). R^2
694 were used to assess the quality of the fit. Unpaired group comparisons were performed using
695 Student's *t*-test. In **Supplementary Fig. 11**, paired group comparisons were performed using
696 Student's *t*-test. F-test was used to assess significance of regression models (**Supplementary**

Fig. 3). Comparisons of multiple groups were performed using 1-way ANOVA. Statistical data analysis of AML patient data was performed in R version 3.2.5 (R Core Team, 2016, https://www.R-project.org/), using Kaplan-Meier analysis and logrank test for survival analysis.

Data availability

The Cancer Genome Atlas data (TCGA) can be retrieved using the RTCGA-Toolbox R/BioConductor package, version 2.2.2. Plasmids are available upon request.

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744 **Editorial summary**

745 The therapeutic response of acute myeloid leukemia to the nucleoside analog Ara-C is
746 regulated by SAMHD1, an enzyme which is differentially expressed in this cancer and which
747 hydrolyzes the active metabolite Ara-CTP.

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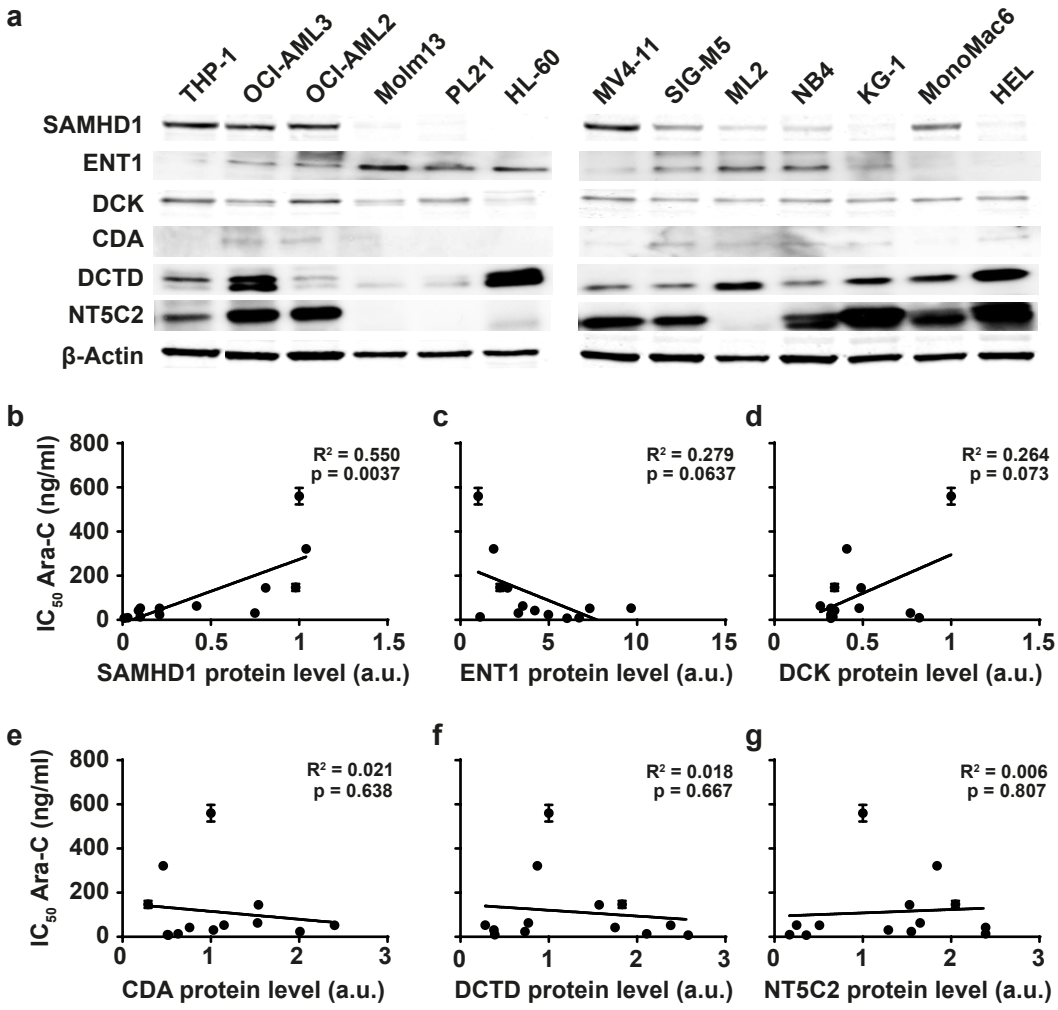
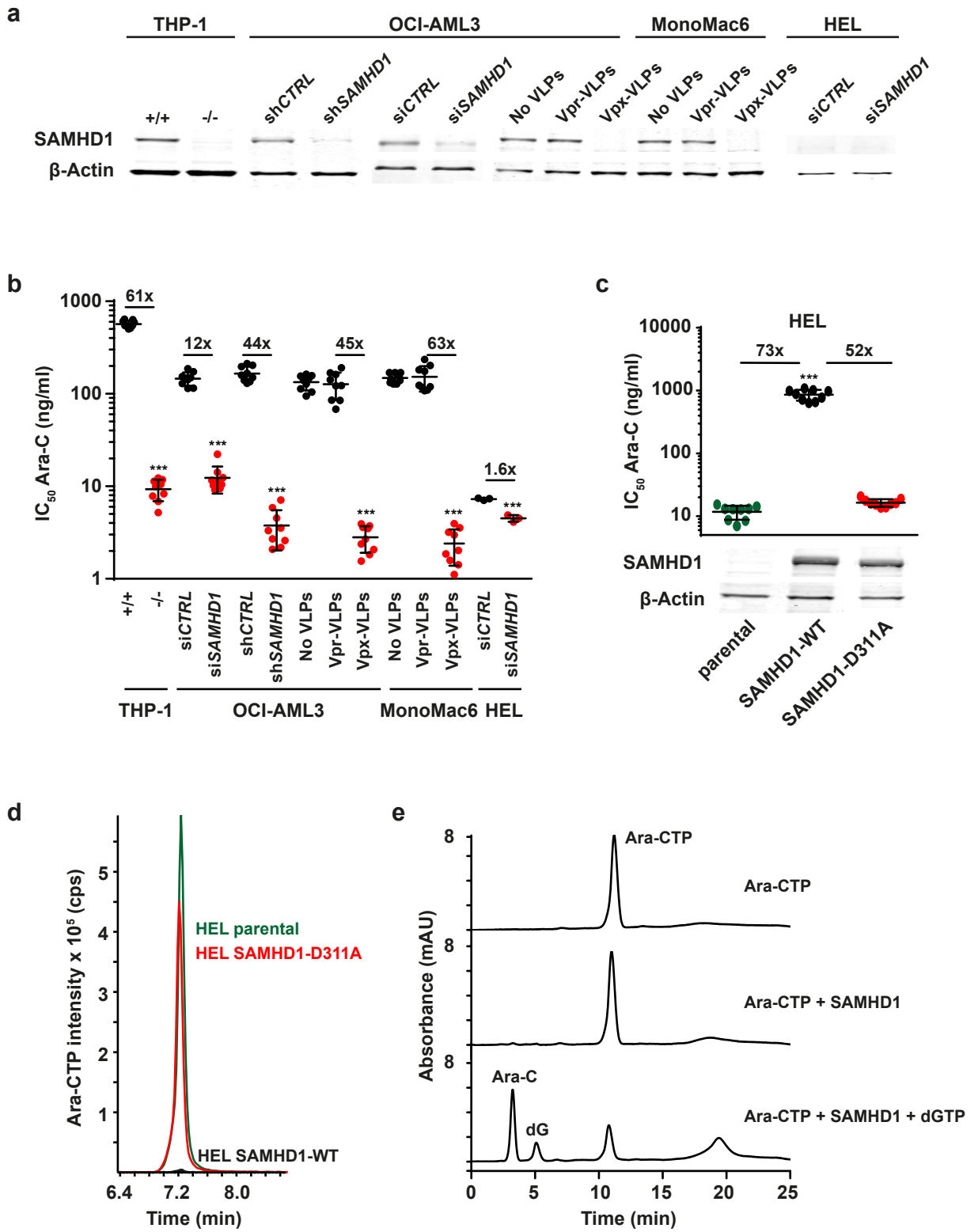
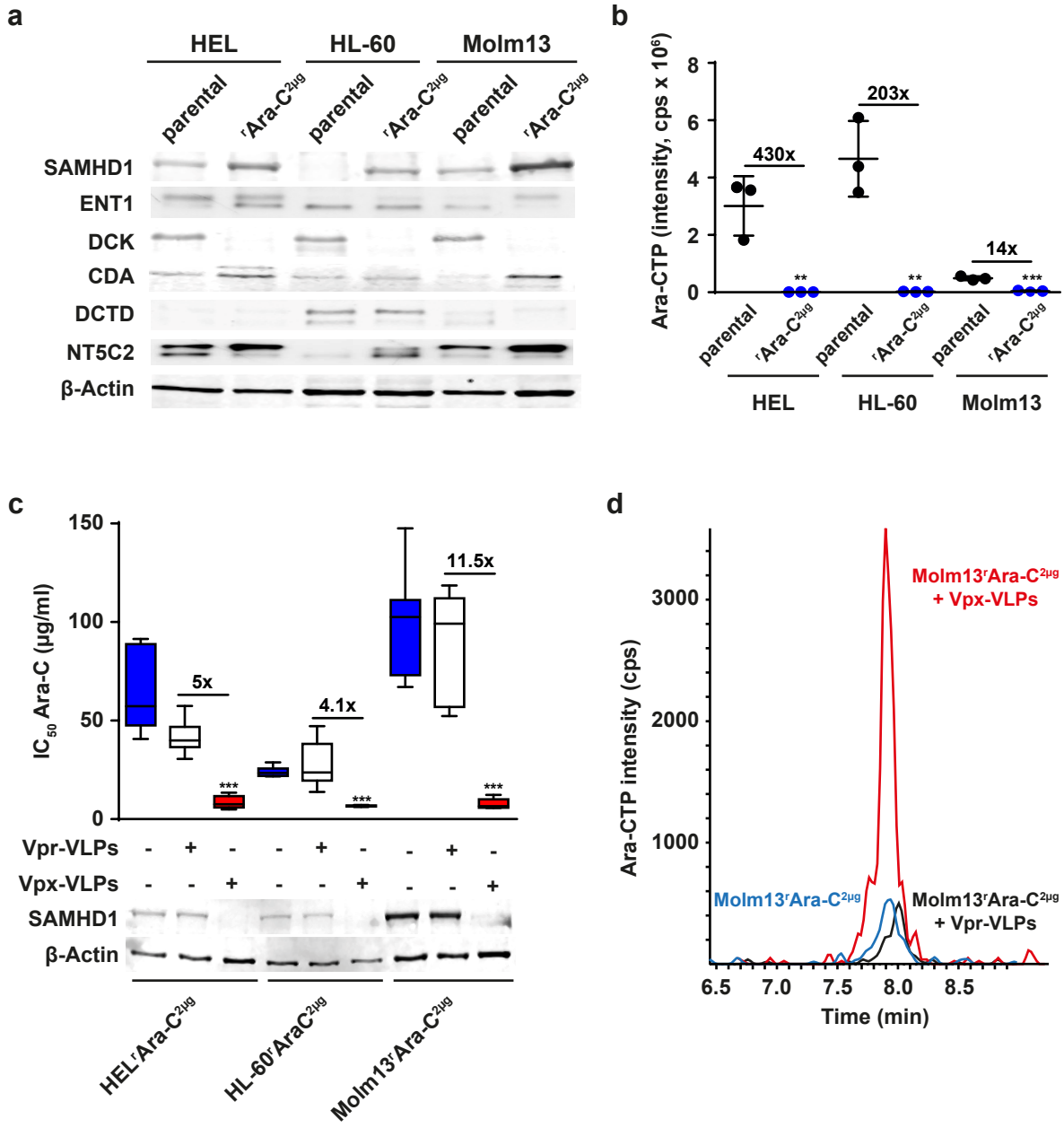
Schneider *et al.*, Figure 1



Schneider *et al.*, Figure 2



Schneider *et al.*, Figure 3



Schneider *et al.*, Figure 4