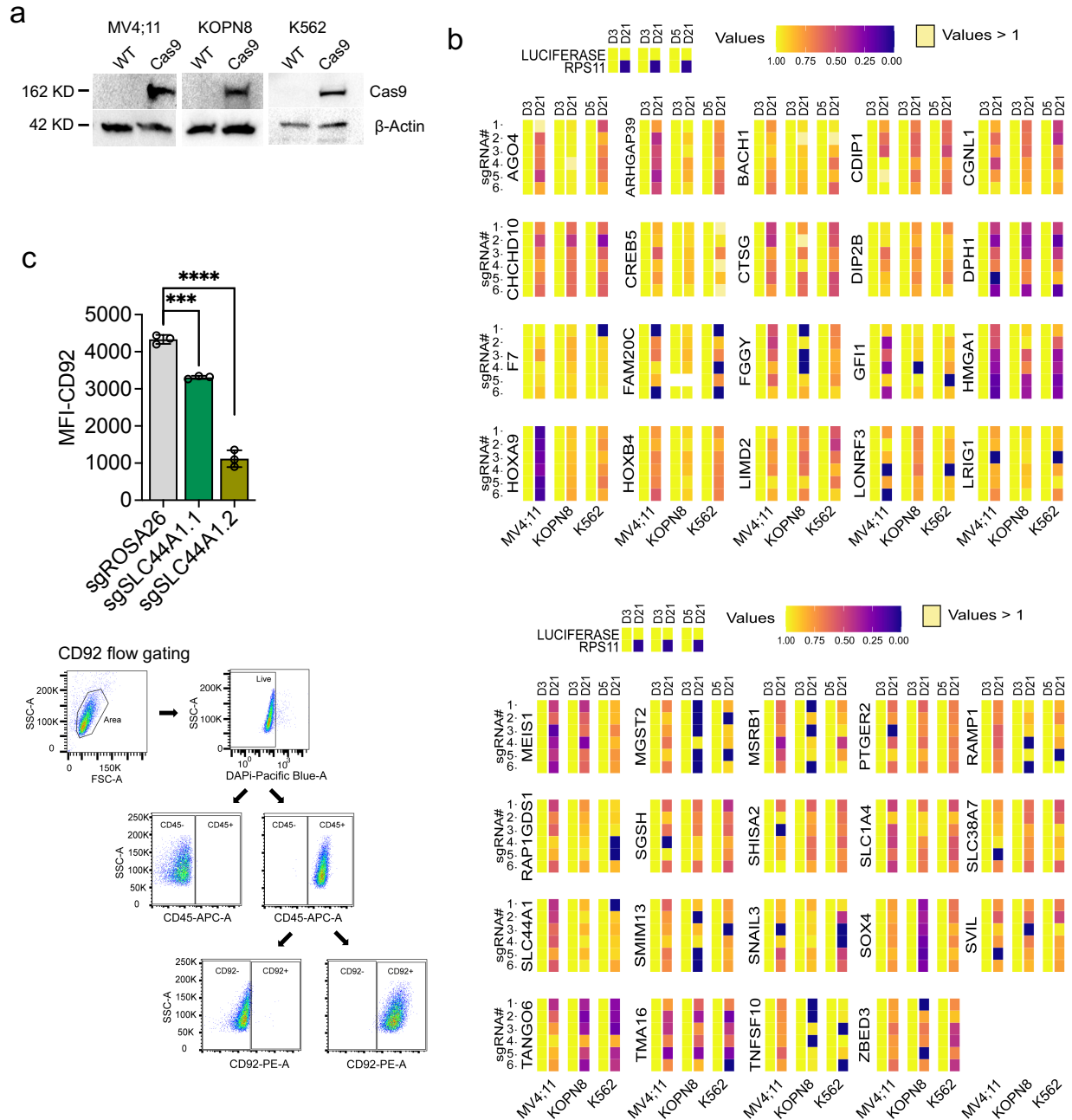


Choline-dependent methionine metabolism supports leukemia progression

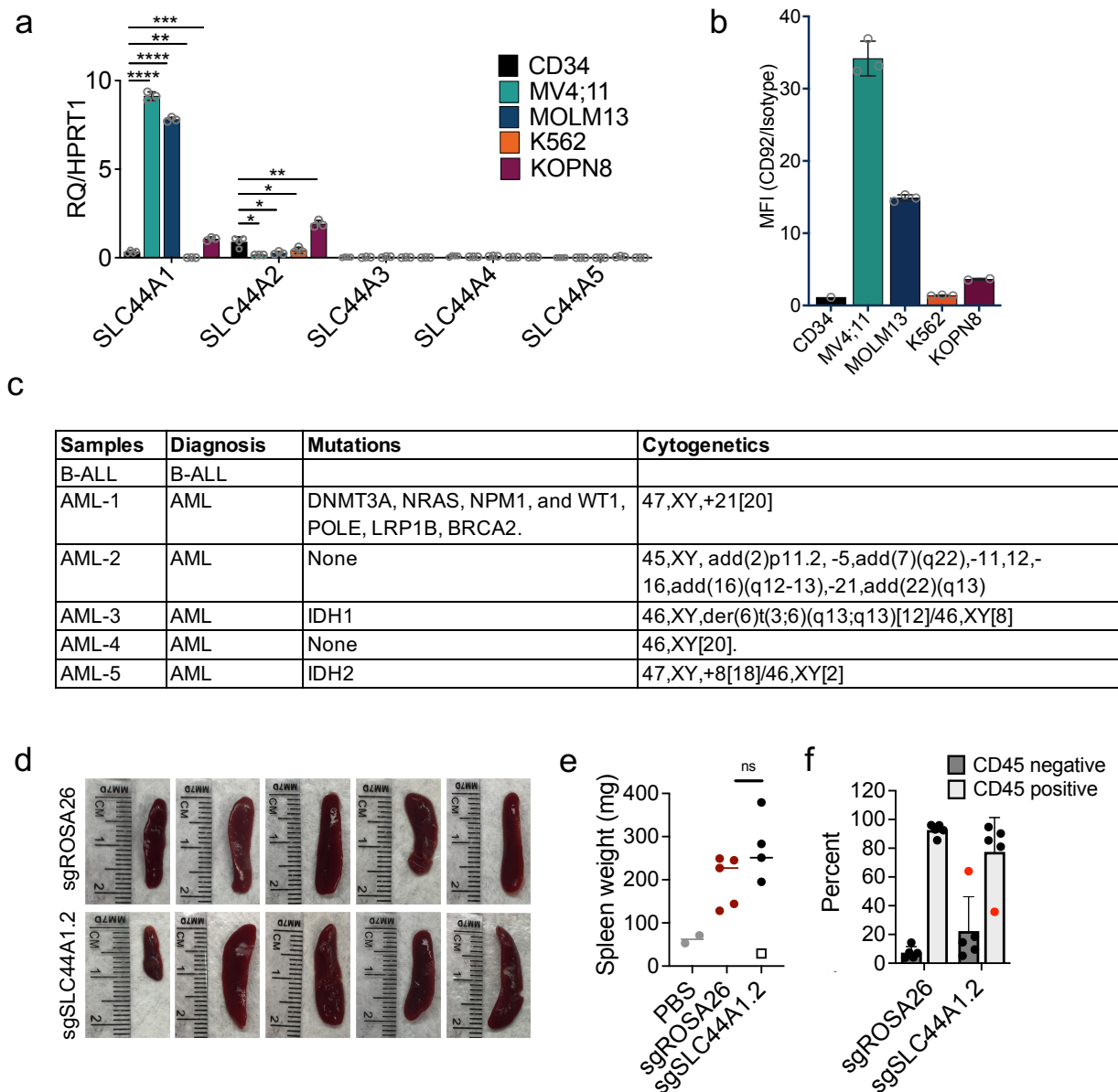
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Supplemental Information



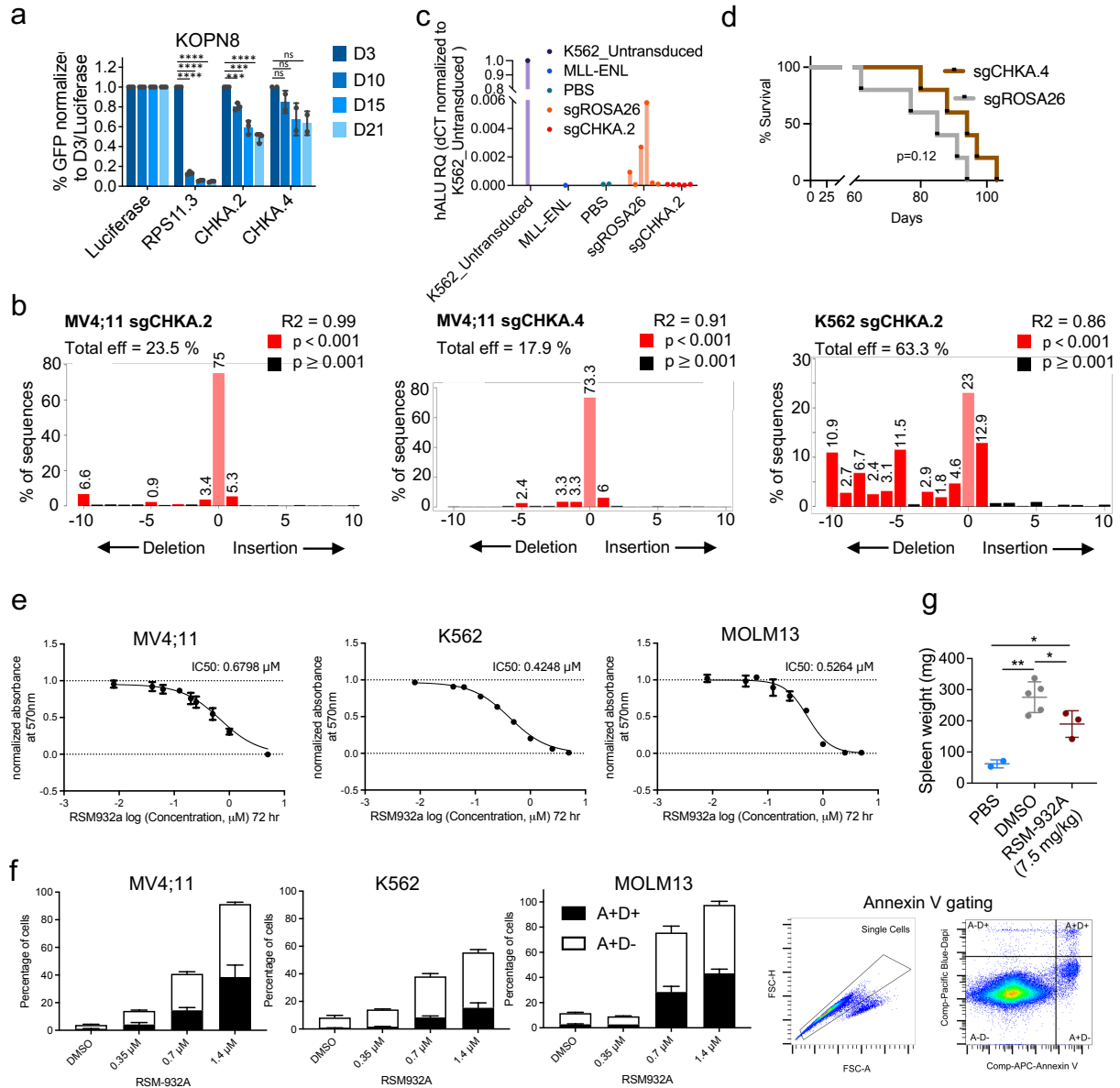
Supplemental Figure 1

(a) Western blot for spCas9 in human leukemic cells. (b) Arrayed CRISPR-Cas9 screen of 39 genes. The data is represented as heatmap of Day 21 values normalized to Day 3 or Day 5 and normalized to non-targeting control Luciferase. (c) Verification of SLC44A1 targeting by SLC44A1.1 and SLC44A1.2 sgRNA in MV4;11-Cas9 cells. The data is collected on day 10 following transduction using CD92 antibody for flow cytometry. Unpaired two-tailed T-test was applied. *** p -value < 0.001, **** p -value < 0.0001



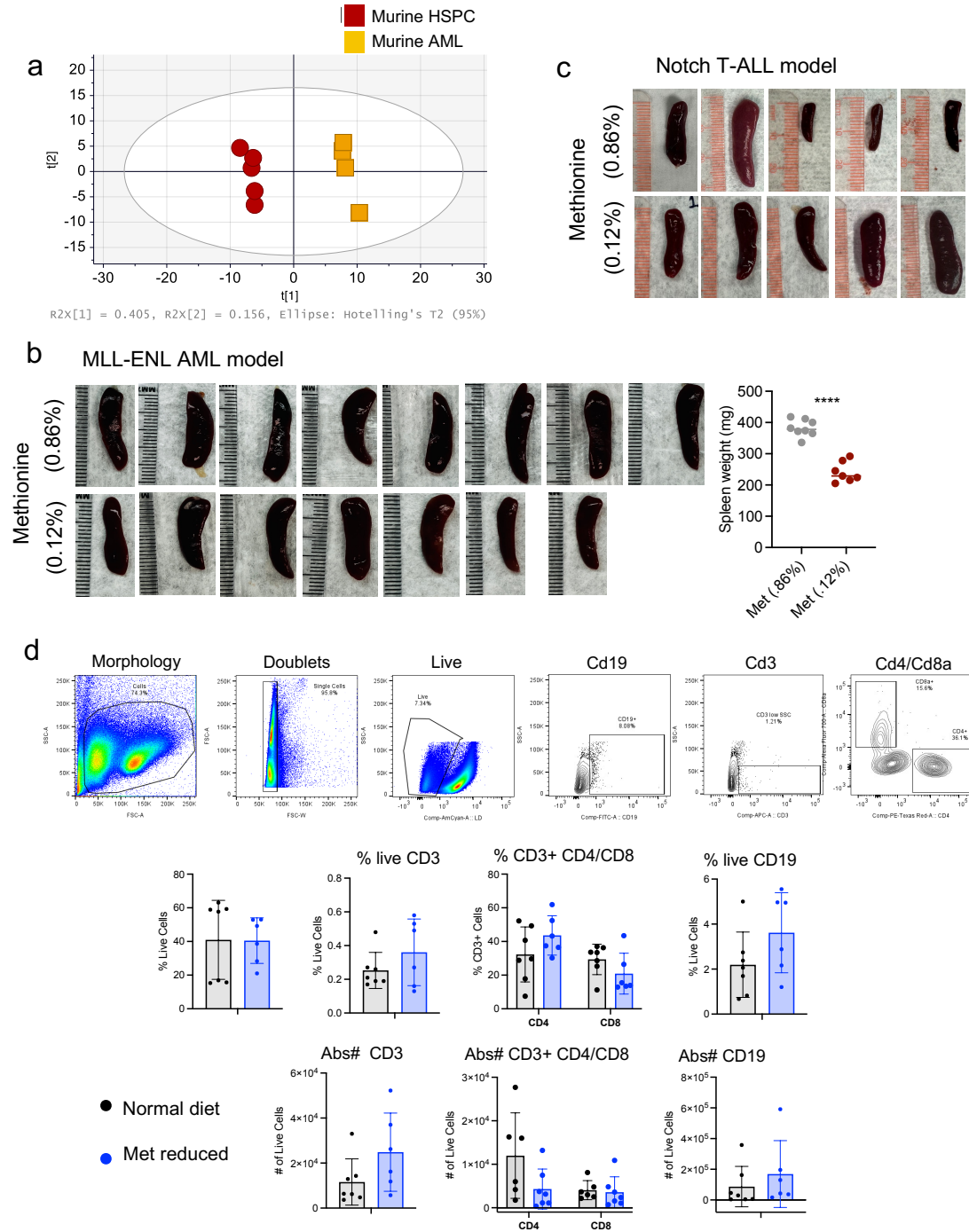
Supplemental Figure 2

(a) qRT-PCR analysis showing expression of SLC44A family of genes in various human leukemic cell lines compared to normal cord blood CD34⁺ cells; $n=3$. Unpaired two-tailed T-test was applied. (b) Cell surface expression of SLC44A1 in human leukemic cells compared to normal CD34⁺ cells using anti-CD92 antibody. Normal CD34⁺ cells were isolated from peripheral blood mononuclear cells. (c) Table showing the clinical and genetic data for the primary patient samples used in this study. (d) Spleens collected at the moribund stage from the *in vivo* leukemogenesis assay. Ruler scale = cm. (e) Plot showing the distribution of spleen weights from the *in vivo* leukemogenesis assay. Square denotes censored mouse. Unpaired two-tailed T test applied. (f) Plot showing bone marrow aspirates from the *in vivo* leukemogenesis assay stained with antibody against human CD45. Red points denote censored mouse. * p -value < 0.05, ** p -value < 0.01, *** p -value < 0.001, **** p -value < 0.0001



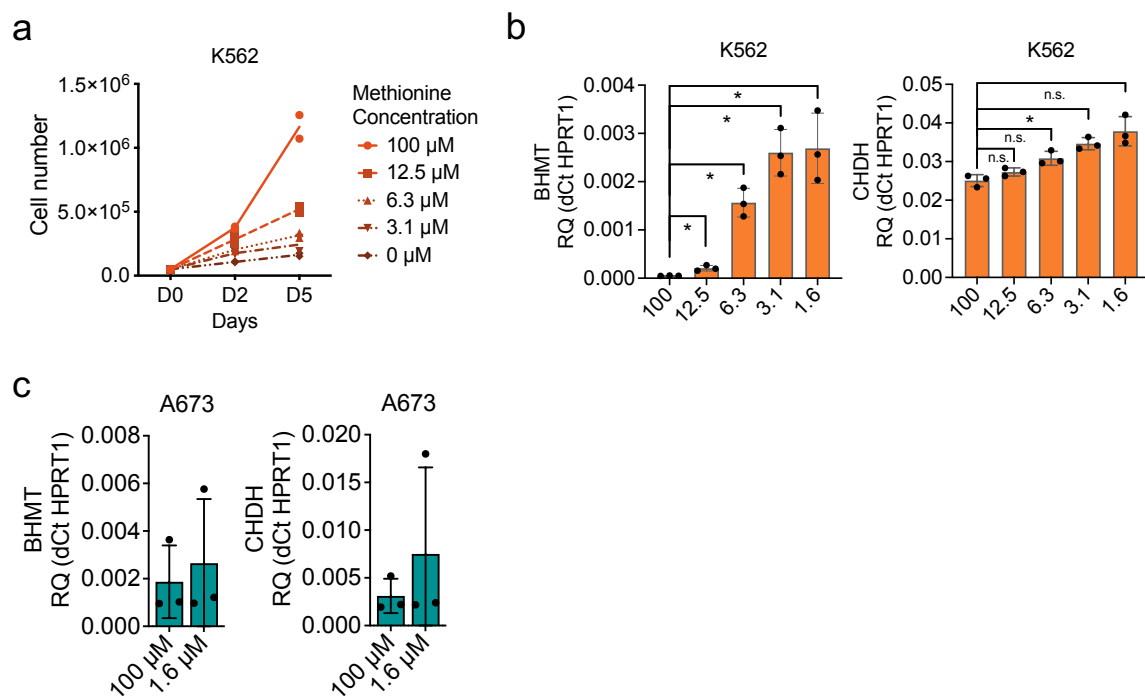
Supplemental Figure 3

(a) CRISPR-Cas9 based competition drop-out assays in KOPN8 cells testing sgCHKA.2 and sgCHKA.4. Paired two-tailed T-test applied. (b) TIDE analysis testing targeting efficiency for shCHKA.2 in MV4;11-Cas9 cells and K562-Cas9 cells and shCHKA.4 in MV4;11-Cas9 cells. (c) qPCR analysis to detect human ALU elements in the bone marrow of the *in vivo* CRISPR-Cas9 targeted CHKA leukemogenesis assay. (d) MV4;11-Cas9 xenograft leukemogenesis assay following transduction with sgCHKA.4 or control sgROSA26. Log-Rank (Mantel-Cox) applied. (e) MTT assays showing response to RSM-932A treatment. (f) Apoptosis analysis was performed using Annexin V staining on human leukemic cells following treatment with RSM-932A. A; Annexin V, D; Dapi. (g) Spleen weights from leukemogenesis assay testing the efficacy of RSM-932A on leukemia compared to PBS injected controls. Unpaired two-tailed T test applied. * p-value < 0.05, ** p-value < 0.005 *** p-value < 0.001, **** p-value < 0.0001.



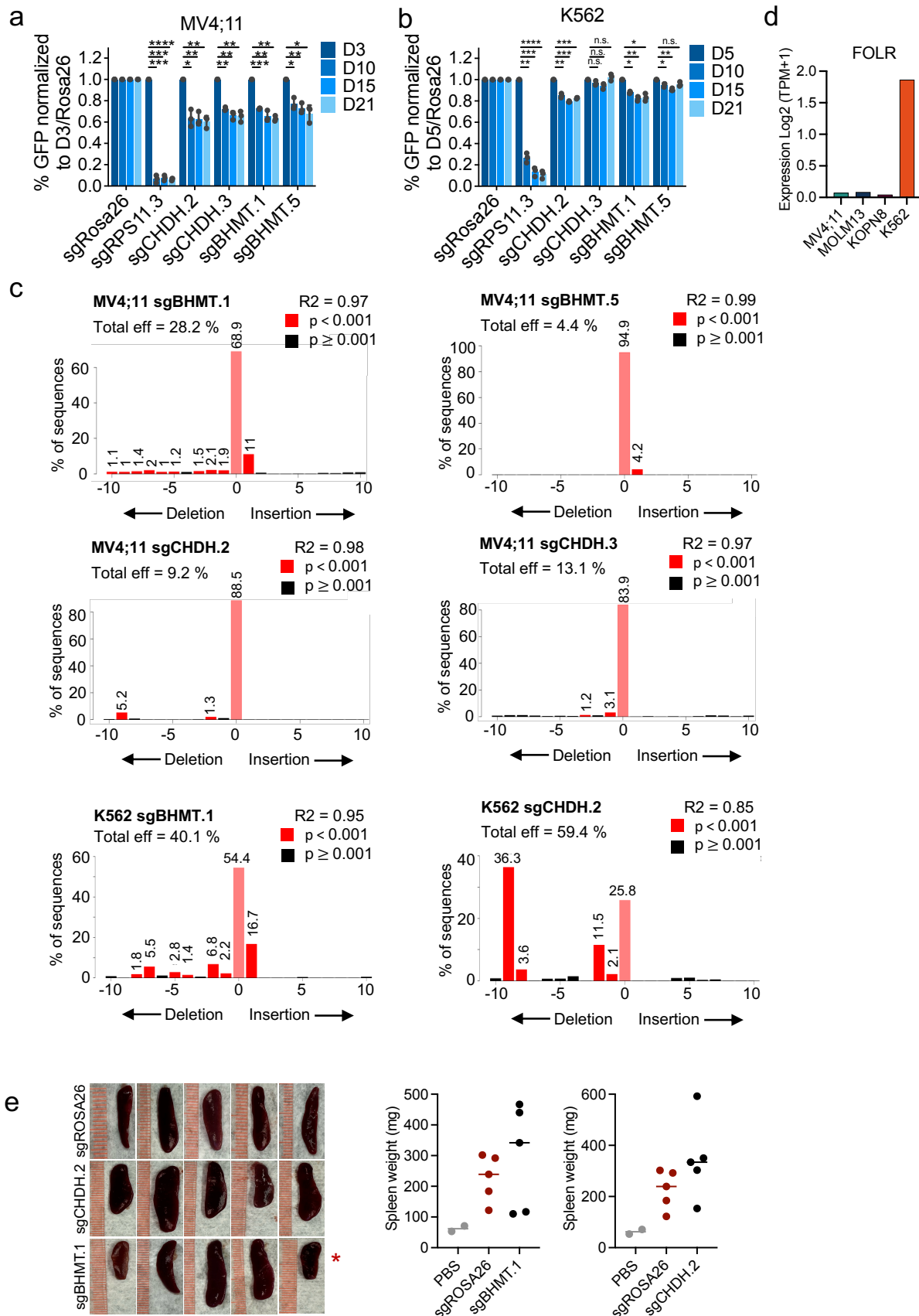
Supplemental Figure 4

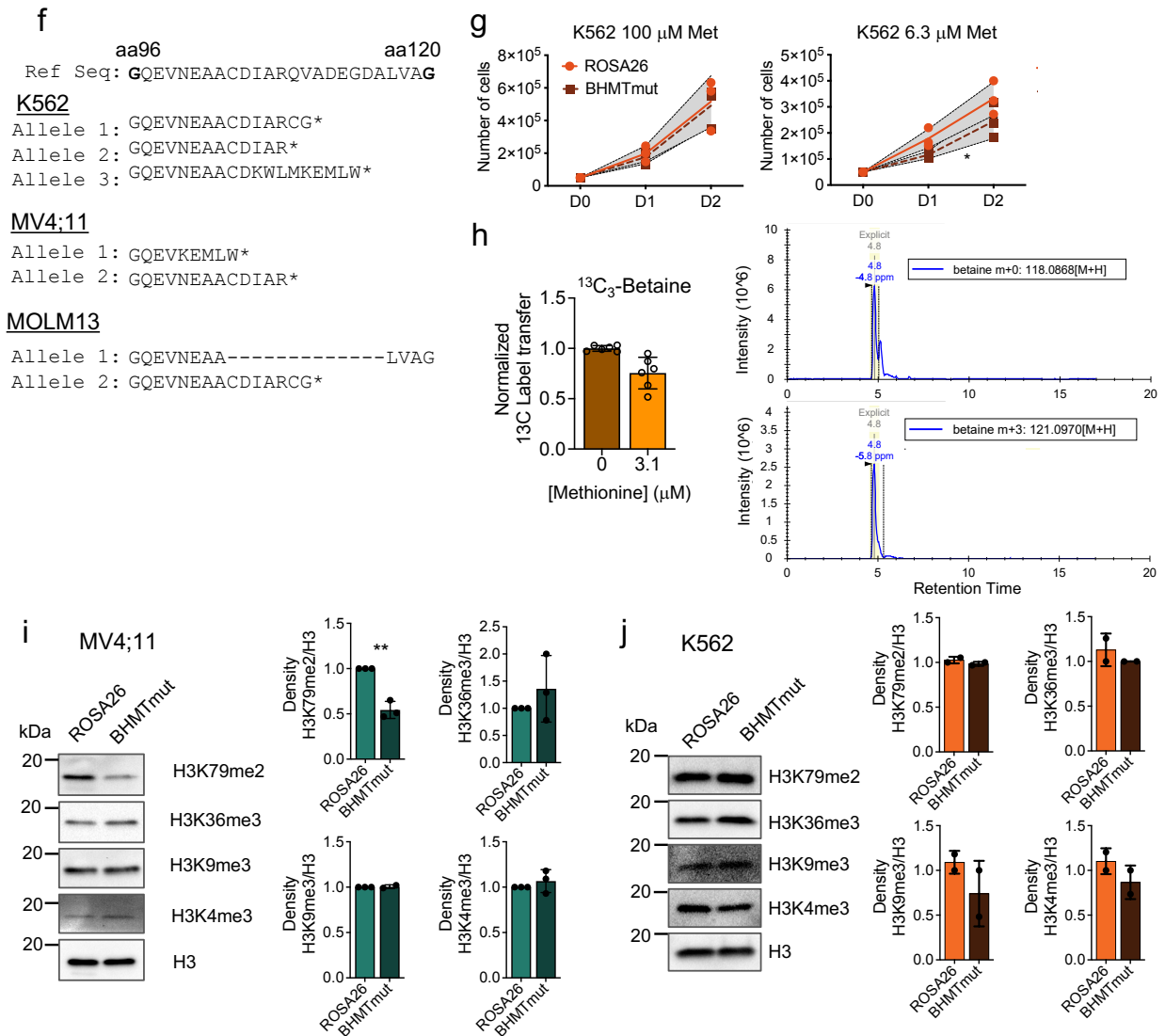
(a) Multivariate analysis of metabolic data from HSPCs and murine AML samples. (b, c) Spleens at the moribund stage from *in vivo* assays testing methionine restriction on leukemogenesis. Spleens from MLL-ENL driven AML (b) and Notch induced T-ALL (c). Ruler scale = mm. Unpaired two-tailed T test applied. (d) Flow cytometry analysis of B and T cells from bone marrow collected from MLL-ENL induced AML diseased mice at time of sacrifice. Data shows percentage and absolute numbers of CD3, CD4/CD8 and CD19 cells from mice fed a normal diet or methionine reduced diet. Error bars denote standard deviation. **** p-value < 0.001.



Supplemental Figure 5

(a) Cell proliferation assay of K562 cells with decreasing concentration of methionine media. (b) qRT-PCR analysis showing expression of BHMT or CHDH with decreasing concentration of methionine in K562; $n=3$. Paired two-tailed T-test was applied. (c) qRT-PCR analysis showing expression of BHMT or CHDH in human Ewings Sarcoma cancer cell line A673, under standard or restricted methionine conditions; $n=3$. Paired two-tailed T-test was applied. * p -value < 0.05 .

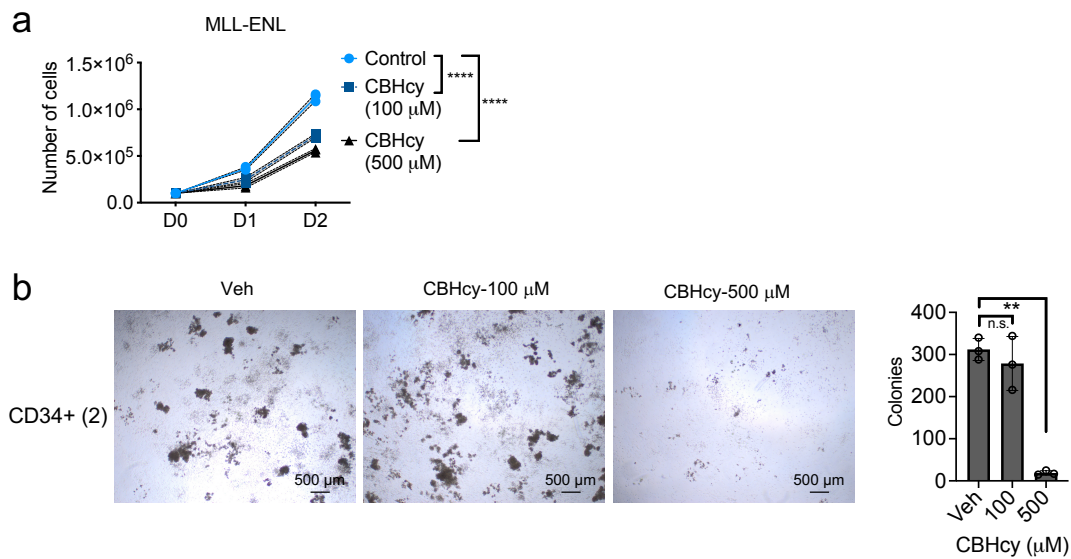




Supplemental Figure 6

(a, b) CRISPR-Cas9 based competition drop-out assays in MV4;11 (a) and K562 cells (b) targeting ROSA26 (negative control) sgRPS11 (positive control) and BHMT or CHDH. Paired two tailed T-test applied. (c) TIDE analysis showing efficiency of CRISPR-mediated targeting of BHMT and CHDH in MV4;11 and K562 cells. (d) Analysis of DEPMap data showing expression of the folate transporter, FOLR, in MV4;11, MOLM13, KOPN8 and K562 cells. (e) Spleens at the moribund stage from BHMT and CHDH in vivo leukemogenesis assays. Ruler scale = mm. Red asterisk represents spleen of mouse sacrificed at experimental endpoint without signs of disease. (f) BHMT protein sequence in CRISPR-targeted clonal human leukemic cell lines compared to wild type BHMT protein sequence. * Represents stop codon. (g) Cell proliferation assay of BHMTmut-K562 cells compared to ROSA26-K562 cells. Upper panel; cells grown in standard media methionine, lower panel; cells grown in restricted media methionine; n=3. 2-Way Anova test. Grey filled areas represents standard deviation. (h) (left) $^{13}\text{C}_3$ carbon tracing experiment. MV4;11 cells were cultured in $^{13}\text{C}_3$ choline for 24 hours before processing for MS detection of betaine. Graph shows normalized transfer of $^{13}\text{C}_3$ into betaine in cells cultured in either 0 or 3.1 μ M methionine. (Right) Representative LCMS chromatograms from experimental

samples for betaine m+0 and betaine m+3 quantitation. The peak quantified is encased within the dashed black lines. The yellow bar indicates the retention time range permitted for a positive identification, with the explicit number indicating the expected retention time for each compound. The blue number indicates the actual retention time, with the mass error indicated as ppm below. Maximum mass error cutoffs of ± 10 ppm for betaine m+0, m+3 were used. (i) Western blot analysis of histone modifications in control and MV4;11-BHMTmut cells following culture in 3.1 μ M methionine. Quantification of blots using densitometric analysis is represented as bar plots. Unpaired two-tailed T test applied. (j) Western blot analysis of histone marks in K562 and K562 BHMTmut cells. Cells were grown in media containing 6.3 μ M methionine and protein extract was prepared after 5 days. Quantification of blots using densitometric analysis is represented as bar plots; n=3. Unpaired T-test was applied. * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001, **** p-value < 0.001.



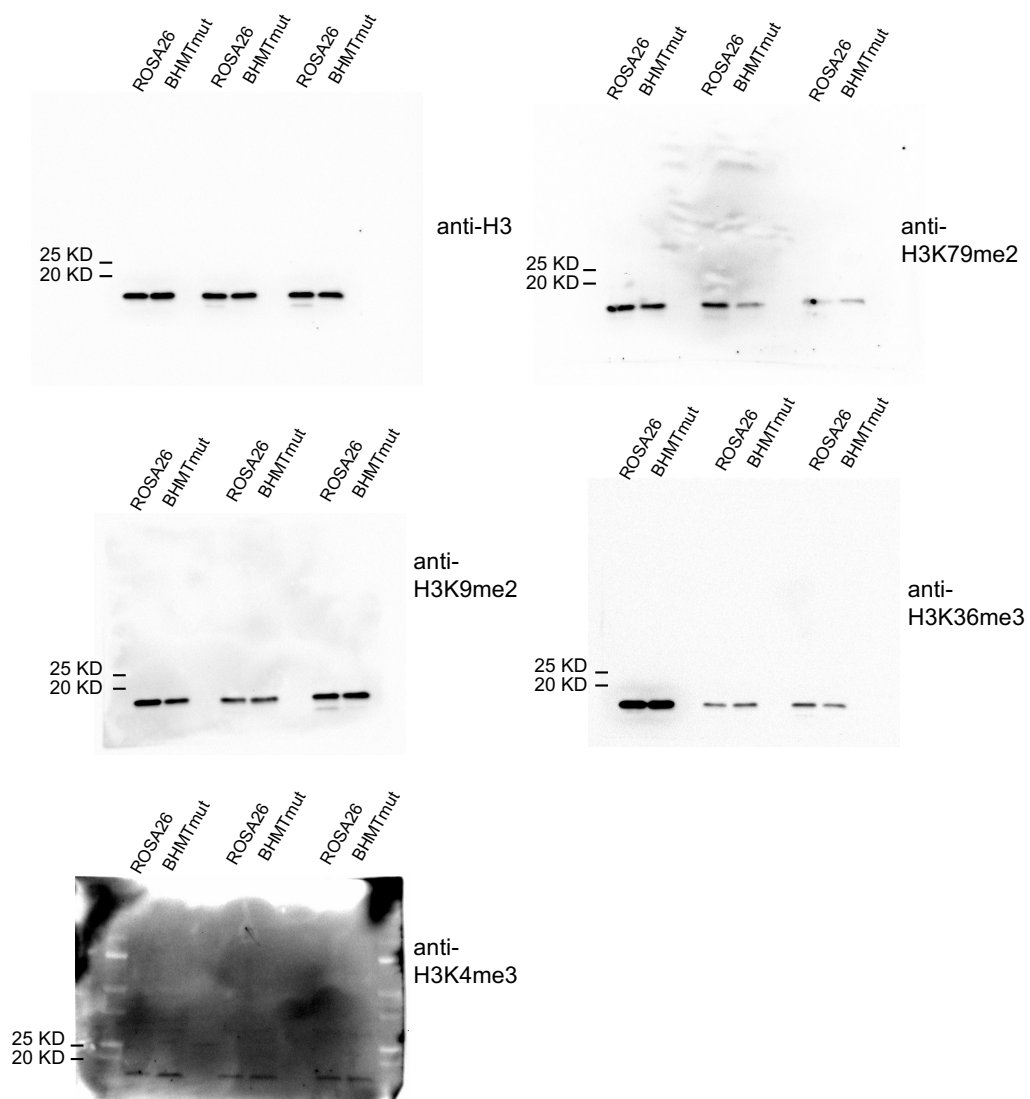
Supplemental Figure 7

(a) Cell proliferation of murine MLL-ENL leukemic cells treated with vehicle control or CBHcy at indicated concentrations. Cells were grown in restricted methionine (3.1 μ M). n=3. 2-Way Anova test applied. Grey filled areas represents standard deviation. (b) 2X images of second CD34+ colony formation assay following treatment of cells with CBHcy at indicated concentrations. Images were taken after 11 days culture in semi-solid media. Quantitation is shown on the right. n=3, paired two-tailed T-test applied. ** p-value < 0.01. **** p-value < 0.0001.

Uncropped Gels

Supplemental Figure 6i

MV411



Uncropped Gels

Supplemental Figure 6j

K562

