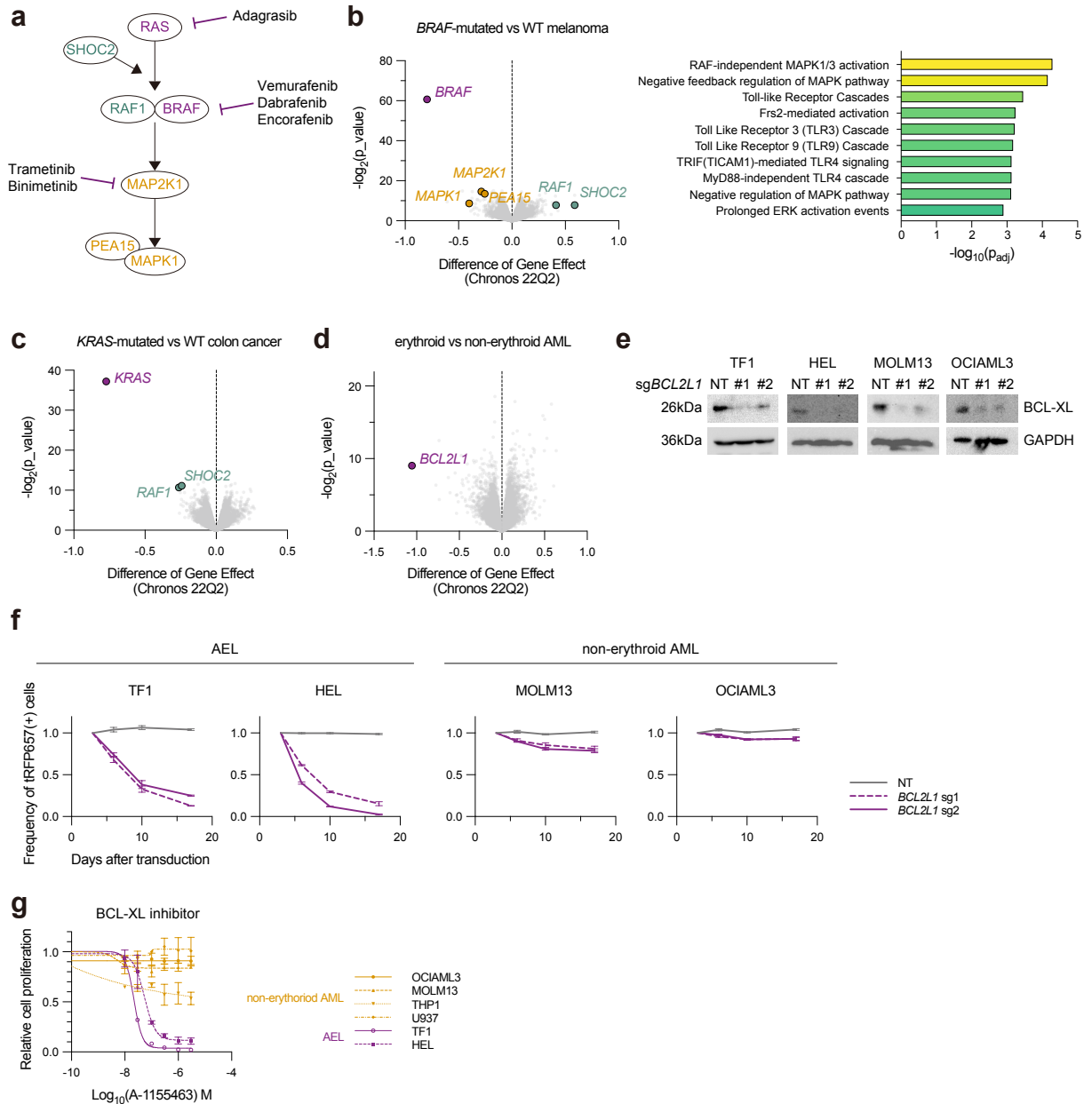


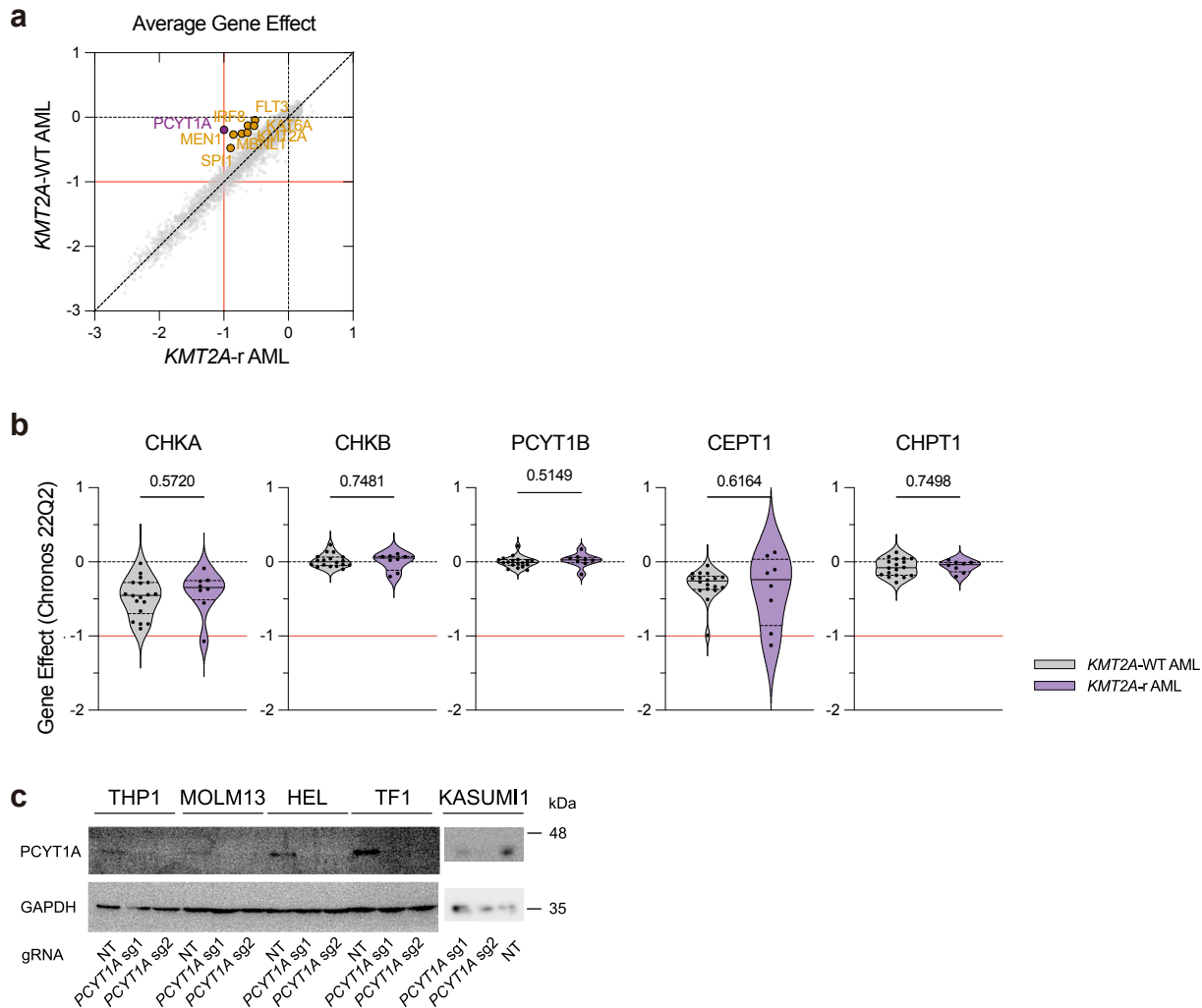
Supplemental Fig. 1



Supplemental Fig. 1 Validation of the DepMap-based two-group comparison system and validation of *BCL2L1* dependency.

(a) Schematic overview of the RAS-RAF-MAPK signaling pathway illustrating clinically approved inhibitors. (b) Volcano plot showing the difference in mean Gene Effect Scores between *BRAF*-mutated and *BRAF*-WT melanoma cell lines (left), and Reactome pathway enrichment analysis of the top 30 specific essential genes (right). (c) Volcano plot displaying the difference in mean Gene Effect Scores between *KRAS*-mutated and *KRAS*-WT colon cancer cell lines. (d) Volcano plot displaying the difference in mean Gene Effect Scores between erythroid and non-erythroid AML cell lines. (e) Western blot validation of BCL-XL expression across TF1, HEL, MOLM13, and OCIAML3 cells transduced with NT or *BCL2L1* sgRNAs. (f) Competitive growth assays monitoring the frequency of tRFP657+ cells following *BCL2L1* depletion in acute erythroid leukemia (AEL) and non-erythroid AML lines. (g) Dose-response proliferation curves of the BCL-XL inhibitor A-1155463 across non-erythroid (OCIAML3, MOLM13, THP1, TF1) and erythroid (TF1 and HEL) AML cell lines.

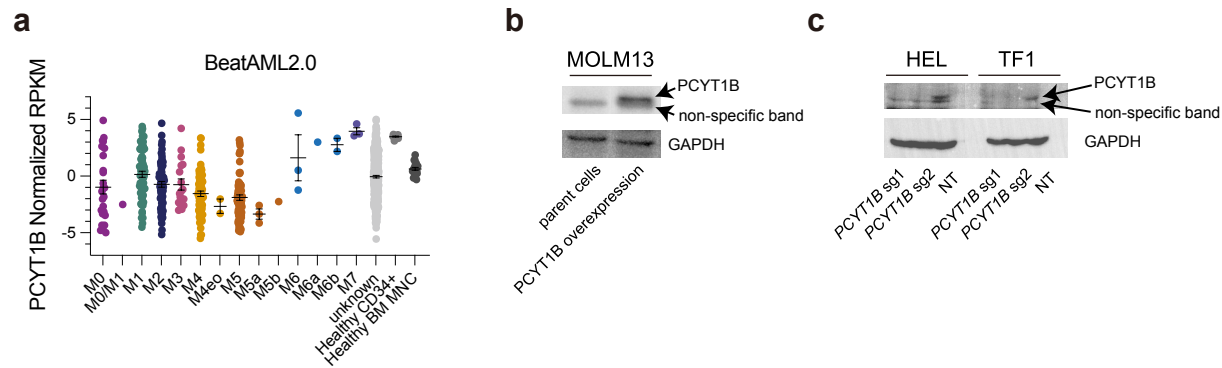
Supplemental Fig.2



Supplemental Fig. 2 DepMap two-group scatter plot and validation of Kennedy pathway components and PCYT1A depletion.

(a) Scatter plot comparing the average Gene Effect Scores between *KMT2A*-WT and *KMT2A*-r AML cell lines. **(b)** Violin plots showing the distribution of Gene Effect Scores for other Kennedy pathway components (*CHKA*, *CHKB*, *PCYT1B*, *CEPT1*, and *CHPT1*) between *KMT2A*-WT and *KMT2A*-r AML. **(c)** Western blot validation of CRISPR/Cas9-mediated *PCYT1A* protein depletion.

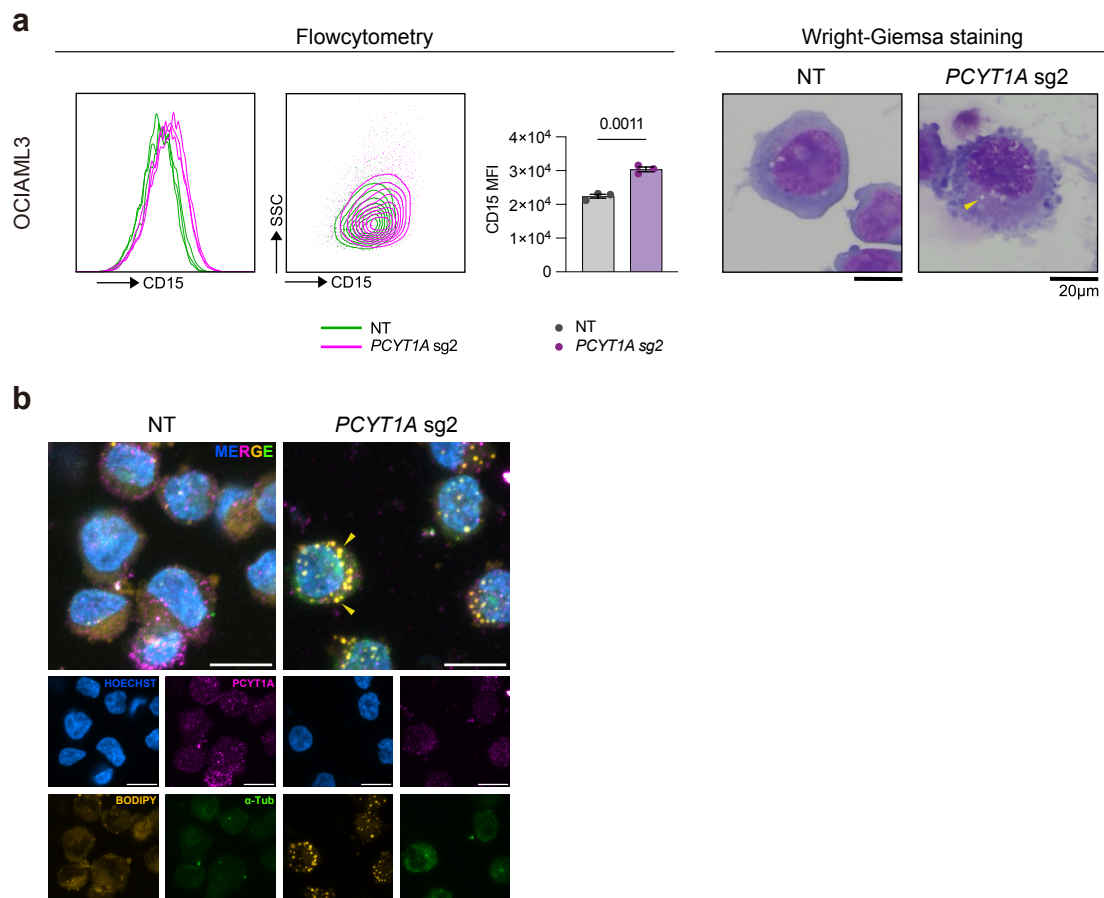
Supplemental Fig.3



Supplemental Fig. 3 *PCYT1B* expression across BeatAML cohort and validation of overexpression/knockout models.

(a) *PCYT1B* transcript levels (Normalized RPKM) across clinical AML FAB subtypes and healthy controls from the BeatAML 2.0 cohort. (b, c) Western blot confirmation of ectopic *PCYT1B* protein overexpression in MOLM13 cells (b), and CRISPR/Cas9-mediated *PCYT1B* protein depletion in HEL and TF1 cells (c).

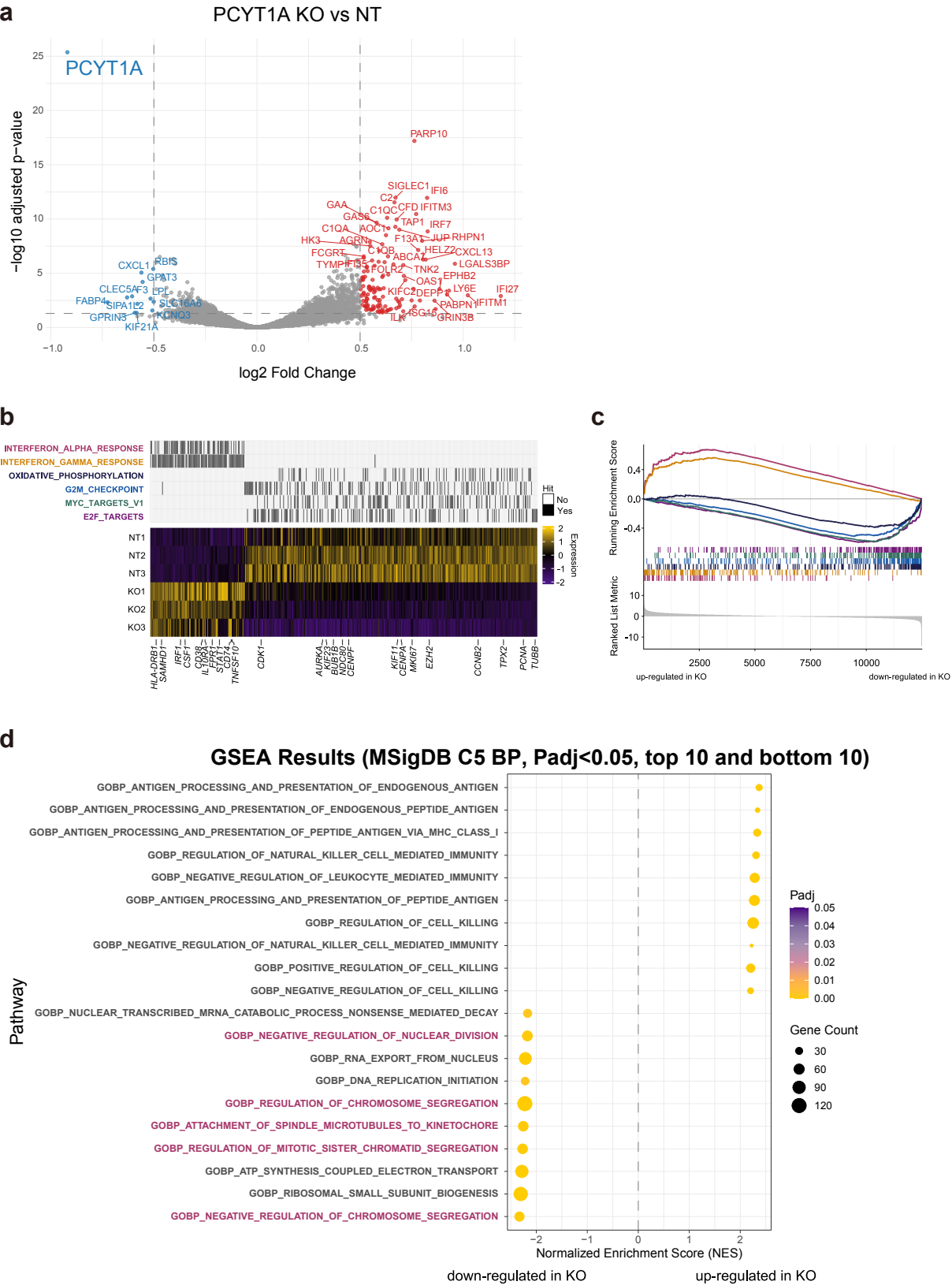
Supplemental Fig.4



Supplemental Fig. 4 Evaluation of myelomonocytic differentiation and lipid droplet morphology in AML cells.

(a) Flow cytometric analysis of CD15 expression and CD15 MFI quantification in OC1AML3 cells transduced with NT or *PCYT1A sg2* (left), alongside representative Wright-Giemsa staining (right). (b) Confocal imaging (maximum intensity projections of Z-stack images) of THP1 cells stained with Hoechst, BODIPY, PCYT1A and α -tubulin (scale bar: 10 μ m).

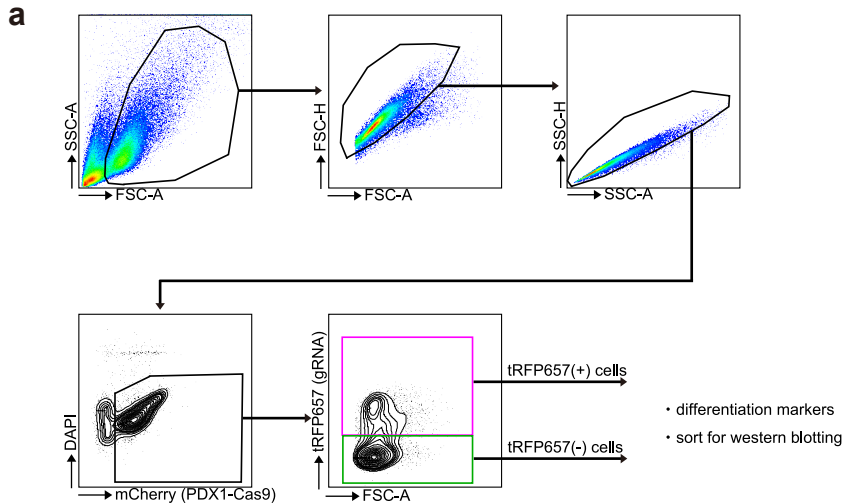
Supplemental Fig.5



Supplemental Fig. 5 Global transcriptomic changes and Gene Ontology analysis in PCYT1A-deficient THP1 cells.

(a) Volcano plot illustrating differentially expressed genes (DEGs) in *PCYT1A*-knockout versus NT THP1 cells. (b, c) Heatmap representation of leading-edge gene expression profiles (b) and running enrichment scores (c) derived from GSEA. Pathway labels in (b) are color-coded to match the corresponding enrichment score lines in (c). (d) GSEA bubble plots displaying the top 10 and bottom 10 enriched Gene Ontology Biological Process (GOBP) pathways.

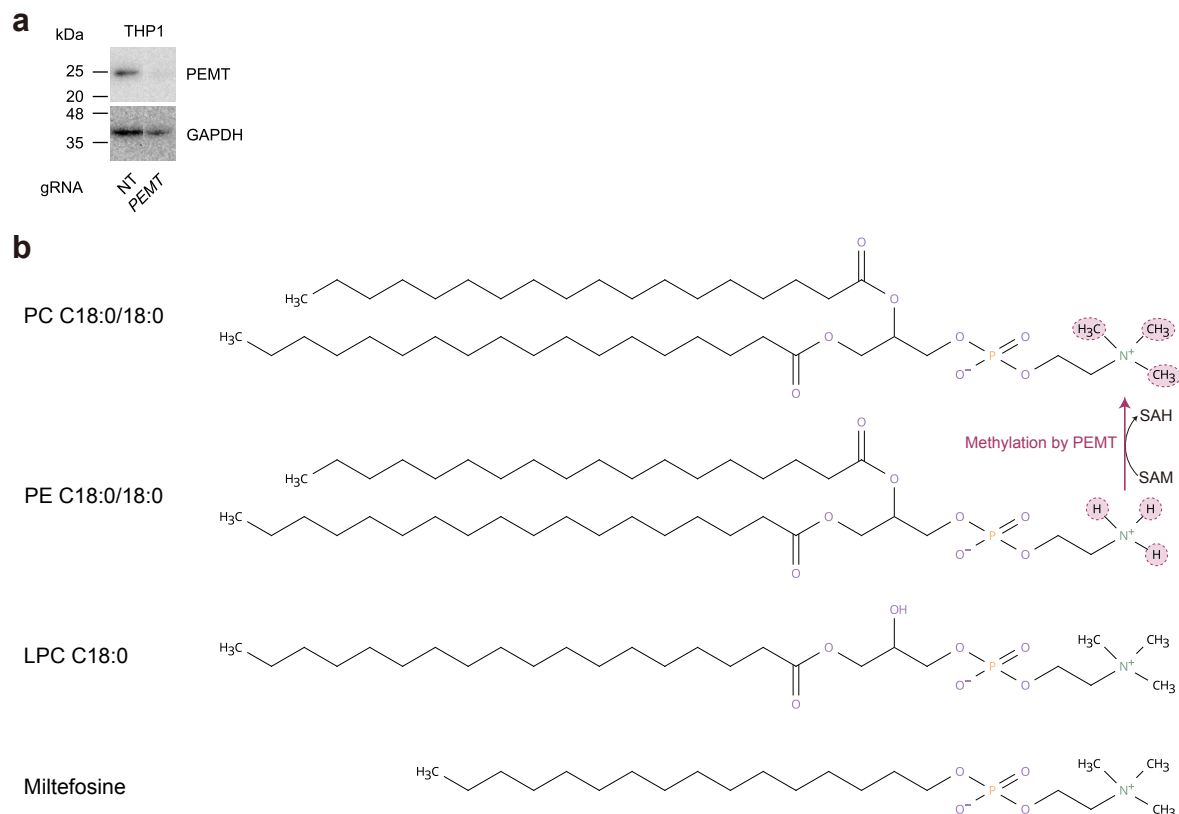
Supplemental Fig.6



Supplemental Fig. 6 Flow cytometric gating strategy for the analysis and sorting of PDX1 cells.

(a) Representative gating hierarchy for primary human AML cells harvested from the spleen of engrafted NSG mice, applied sequentially to track singlets, viability (DAPI-), Cas9 expression in AML cells (mCherry+), and sgRNA transduction (tRFP657+).

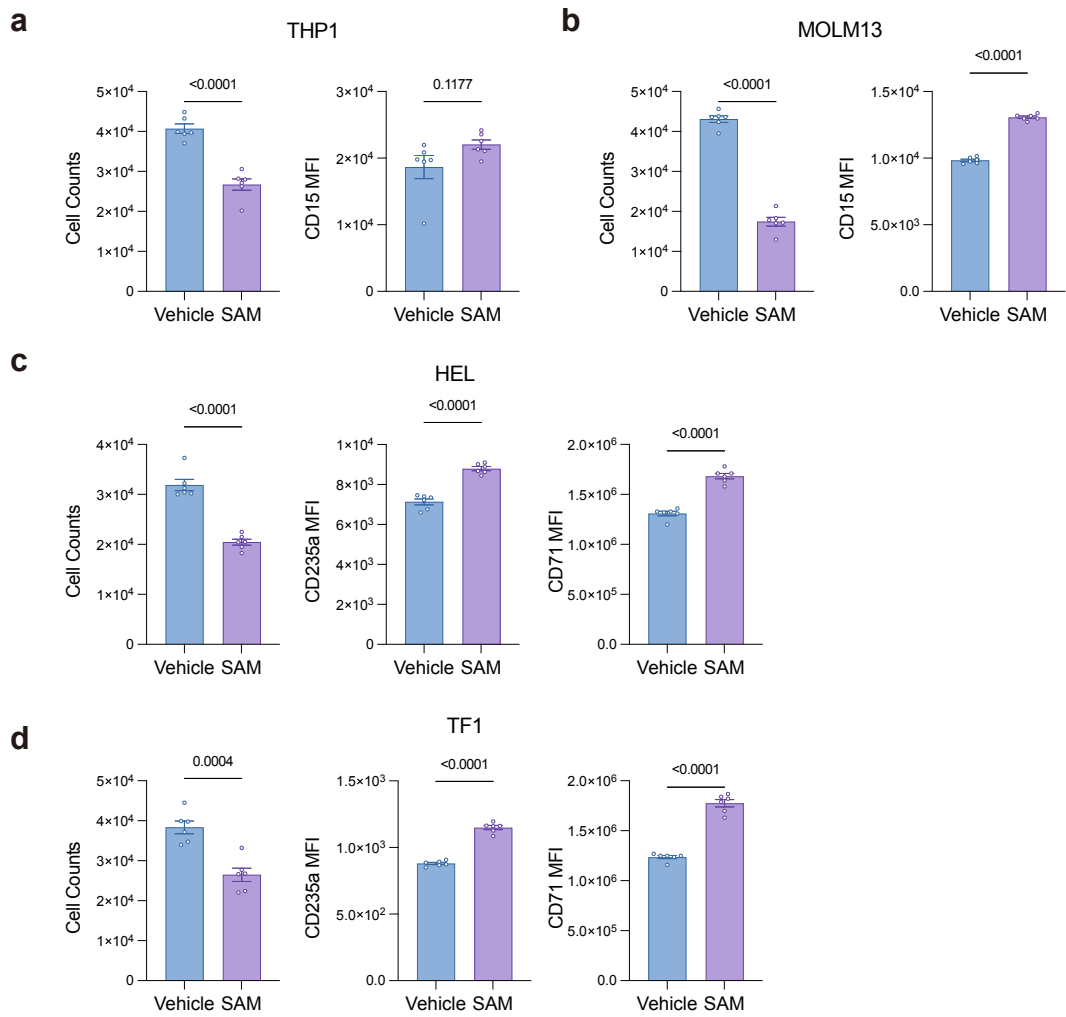
Supplemental Fig.7



Supplemental Fig. 7 Biochemical annotations of glycerophospholipids and target confirmation.

(a) Western blot validation of CRISPR/Cas9-mediated PEMT protein depletion in THP1 cells utilizing a puromycin-selectable sgRNA. (b) Chemical structures of phosphatidylcholine (PC 18:0/18:0), phosphatidylethanolamine (PE 18:0/18:0), lysophosphatidylcholine (LPC 18:0), and the PCYT1 inhibitor miltefosine, highlighting the PEMT-mediated methylation mechanism.

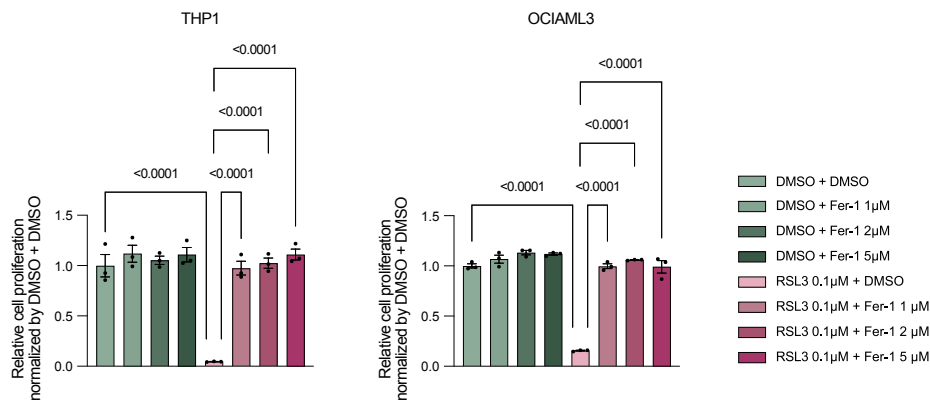
Supplemental Fig.8



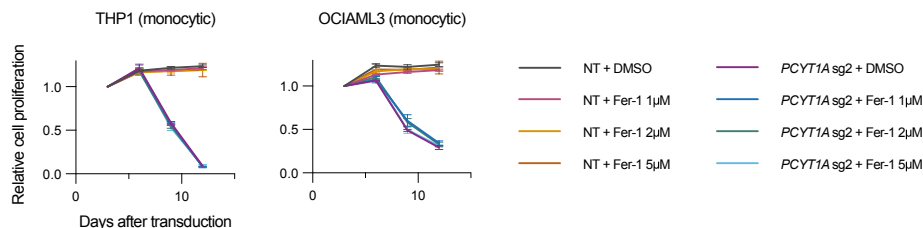
Supplemental Fig. 8 Exogenous S-adenosylmethionine (SAM) treatment induces lineage-specific differentiation in AML cell lines.
(a, b) Absolute cell counts and CD15 MFI quantification in monocytic AML lines THP1 (a) and MOLM13 (b) treated with SAM or vehicle. (c, d) Cell counts and erythroid differentiation markers (CD235a, CD71 MFI) in acute erythroid leukemia (AEL) lines HEL (c) and TF1 (d) following SAM treatment.

Supplemental Fig.9

a



b



Supplemental Fig. 9 Ferroptosis inhibition fails to rescue the growth defect induced by *PCYT1A* depletion.

(a) Validation of Ferrostatin-1 (Fer-1) efficacy against RSL3-induced ferroptosis in THP1 and OCIAML3 cells. Cells were pretreated with the indicated concentrations of Fer-1 (1, 2, and 5 μM) or DMSO vehicle for 1 hour before the addition of RSL3 (0.1 μM) or DMSO. Viable cell numbers were quantified by flow cytometry 72 hours post-RSL3 treatment and normalized to the DMSO + DMSO control condition (n = 3). Statistical significance was determined by one-way ANOVA. Data are presented as mean ± SEM (n=3). **(b)** Competitive growth assays monitoring the relative proliferation of *PCYT1A*-depleted (sg2) or non-targeting control (NT) THP1 and OCIAML3 cells over time in the continuous presence of indicated concentrations of Fer-1 (1, 2, and 5 μM) or DMSO vehicle. Cells were pretreated with Fer-1 for 1 hour prior to lentiviral transduction, and the indicated drug concentrations were maintained throughout the culture period. Data are presented as mean ± SEM (n=3).