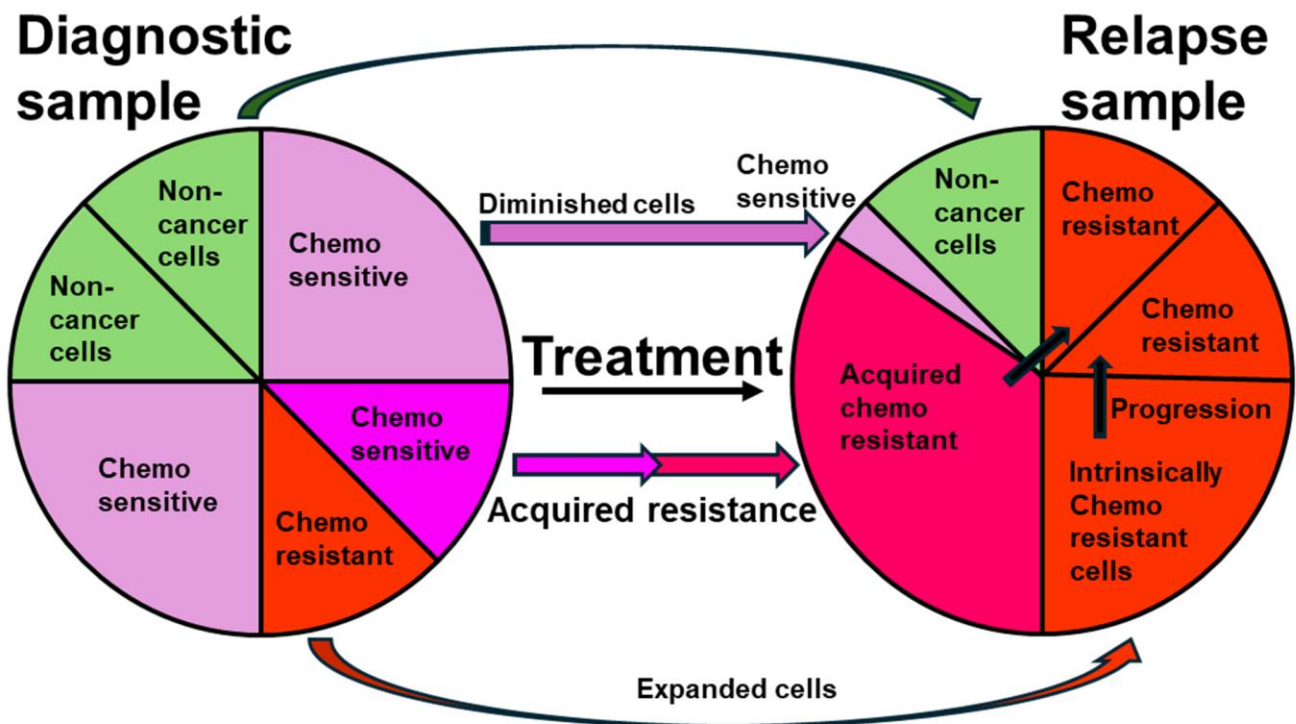


Model for the presentation of cells at diagnosis and relapse



Our model for cell populations before and after treatment

Figure S1. Our proposed model for the cell composition of pAML patient samples at diagnosis and relapse accounts for non-cancer cell types and both chemoresistant and chemosensitive pAML cells in the diagnosis sample. This model proposes the following: 1) the relative proportion of non-cancer cell types in the sample may increase or decrease between diagnosis and relapse, 2) the relative proportion of chemoresistant cells at diagnosis (expanded pAMLs) will increase during treatment, and 3) the relative proportion of some chemosensitive cells will diminish (diminished pAMLs), whereas others that display transformed transcriptomics may gain resistance to chemotherapy. We aim to characterize expanded and transforming cells that are predictive of pAML patient outcomes. Note that the transformation of pAML cell populations is strictly referred to as transcriptomic transformation and does not imply genetic clonality.

CyTOF profiles of representative pAML pairs

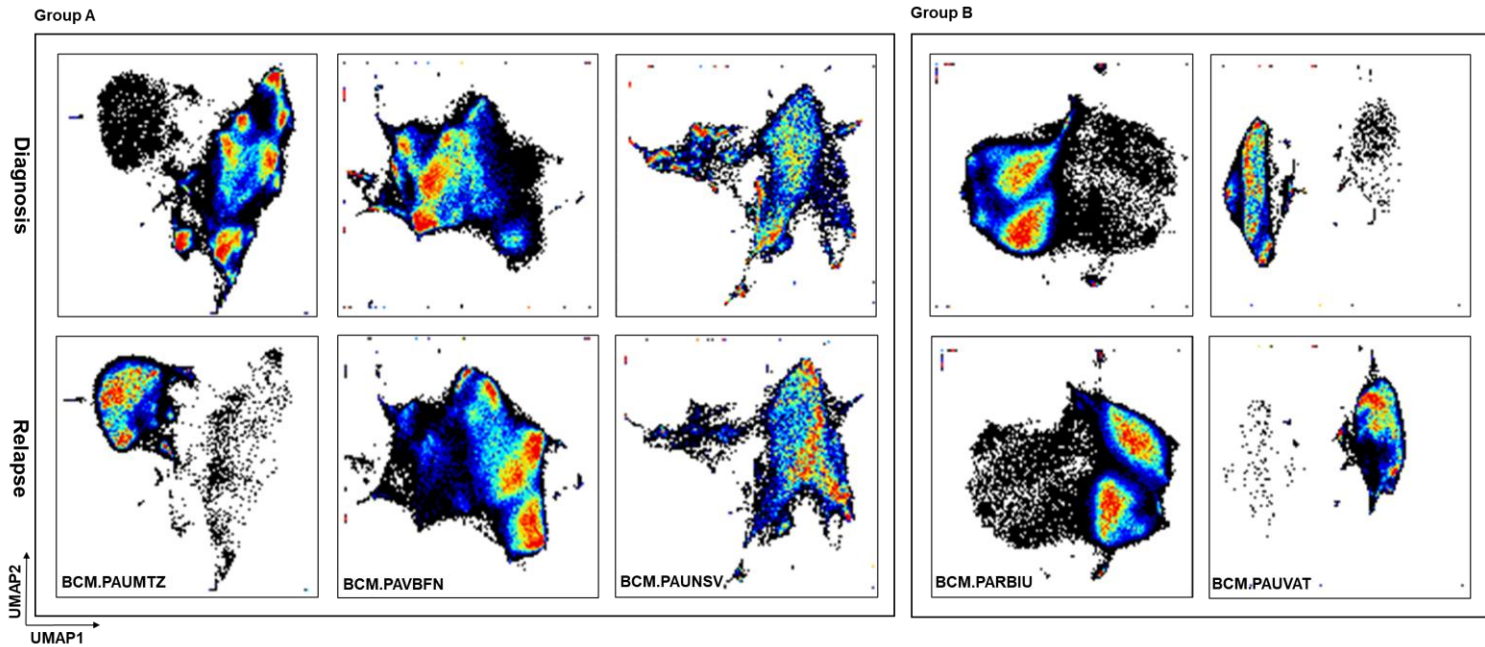


Figure S2. UMAP visualization of cytometry by time of flight (CyTOF) profiles of the five representative pAML pairs from Group A and B patients, shown in Figure 2D. UMAP color gradients represent cell density. Source data are provided as a Source Data file.

Single-cell trajectory plots

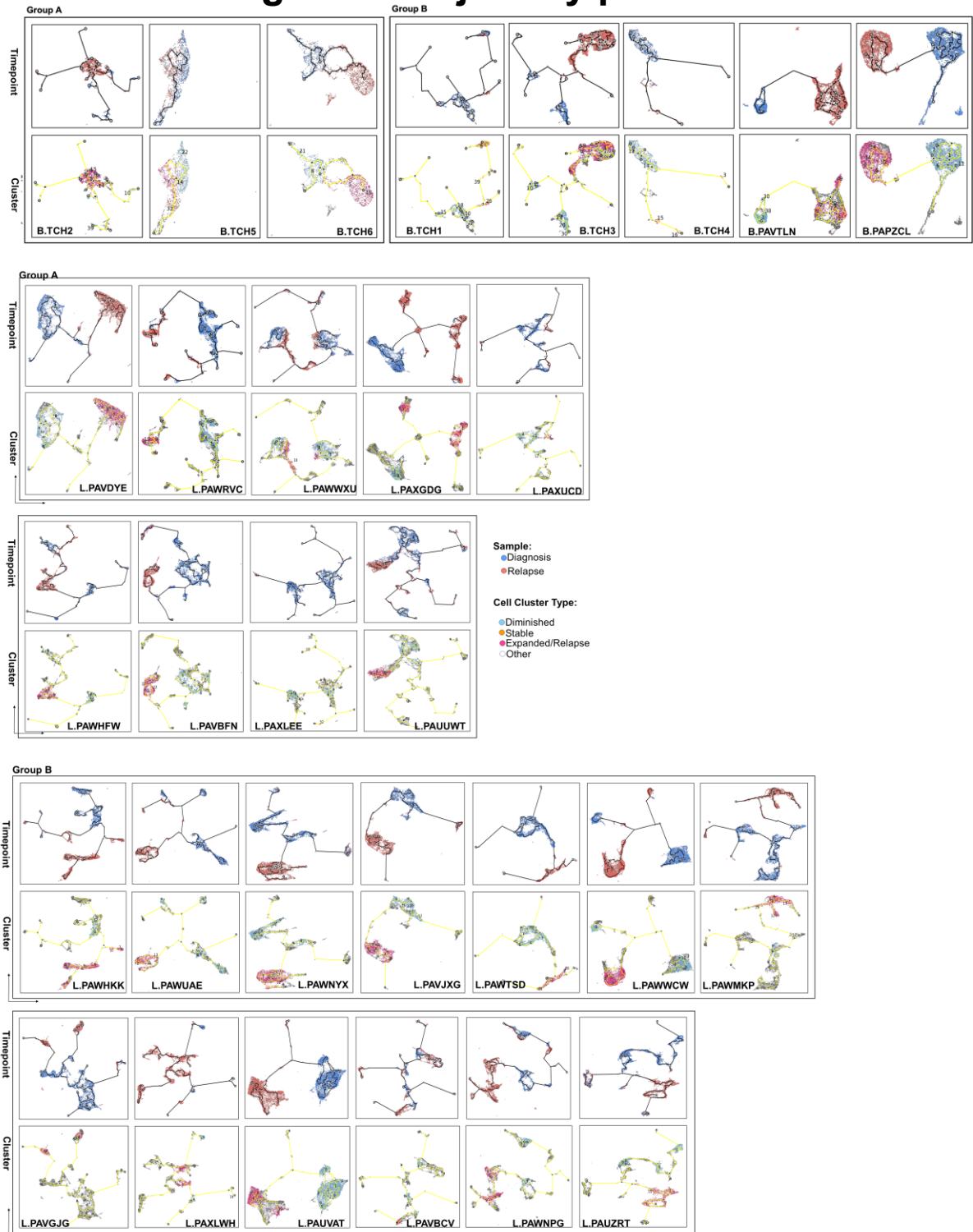


Figure S3. Single-cell trajectory plots, analogous to Figure 2D, depict profiles of samples from patients with or without expanded pAMLs in diagnostic samples (i.e., Group A and Group B for BCM and Lambo cohort patients). In the top panel, diagnosis and relapse time points are color-coded in red and blue, respectively, with the inferred trajectory path indicated by a solid black line. In the bottom panel, clusters are categorized as Expanded (in light red), Stable (orange), Diminished (in light blue), or Other (in white), with the inferred trajectory path represented by a solid yellow line. Trajectory paths predict cell transformations. Source data are provided as a Source Data file.

CNVs cells predicted to acquire resistance

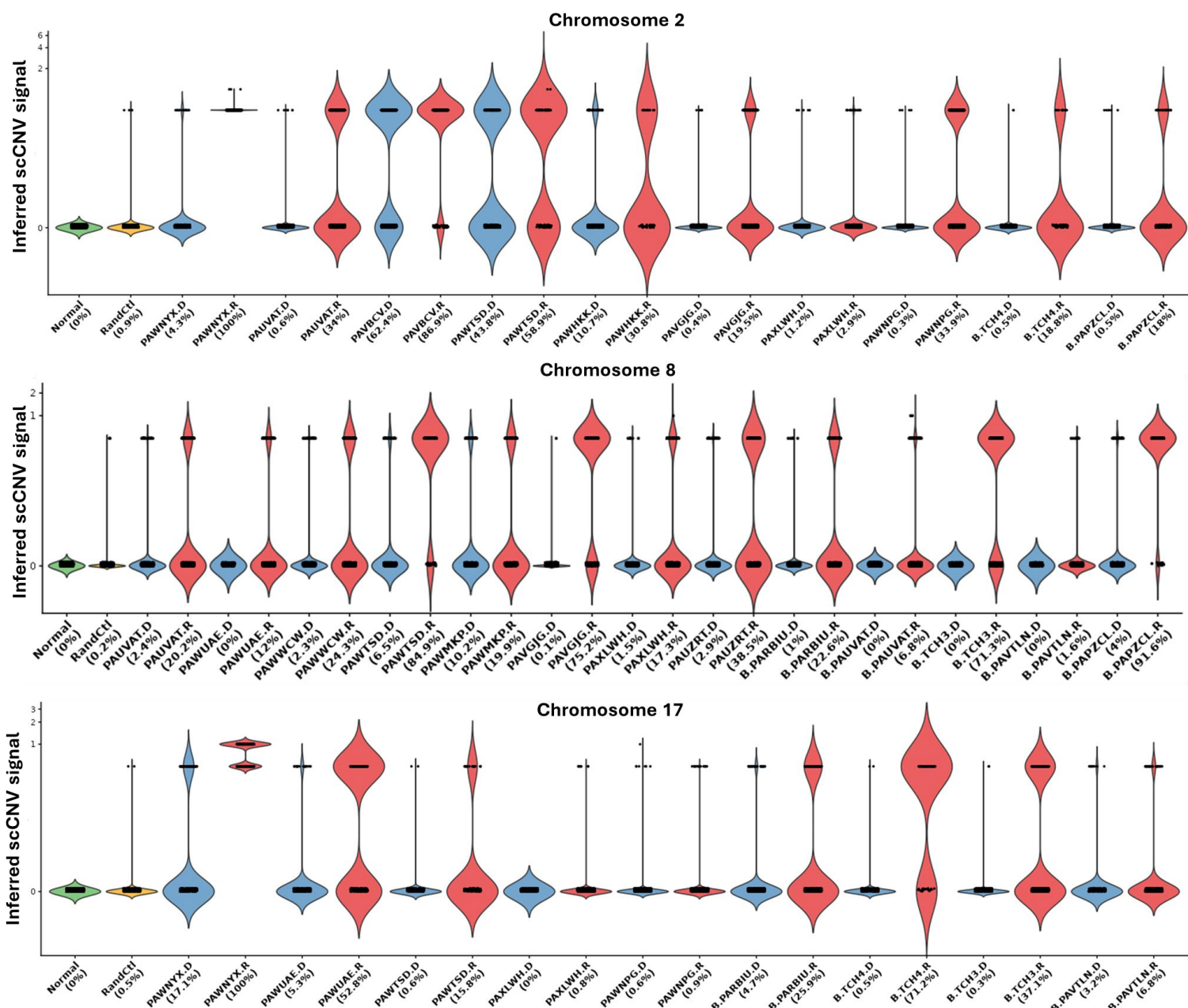


Figure S4. Inferred copy number variations (CNVs) in chromosomes 2, 8, and 17 in patients identified with cells predicted to acquire resistance to chemotherapy. Shown are the proportions of cells identified with chromosome gains according to inferCNV (gain rates) in these cell populations for diagnosis (.D) and relapse (.R) samples for each patient. Patients not identified with cells with specific gains at neither diagnosis nor relapse at a given chromosome were omitted. Normal cells were taken from non-cancer donors. Randomized controls (RandCntl) include randomly selected cells from the pretreatment samples included in the plot. The identification of diagnosis samples with gain rates above RandCntl that increased at relapse suggests a pre-treatment enrichment for these CNVs that expand after treatment. Source data are provided as a Source Data file.

Expanded–Enriched Gene Sets

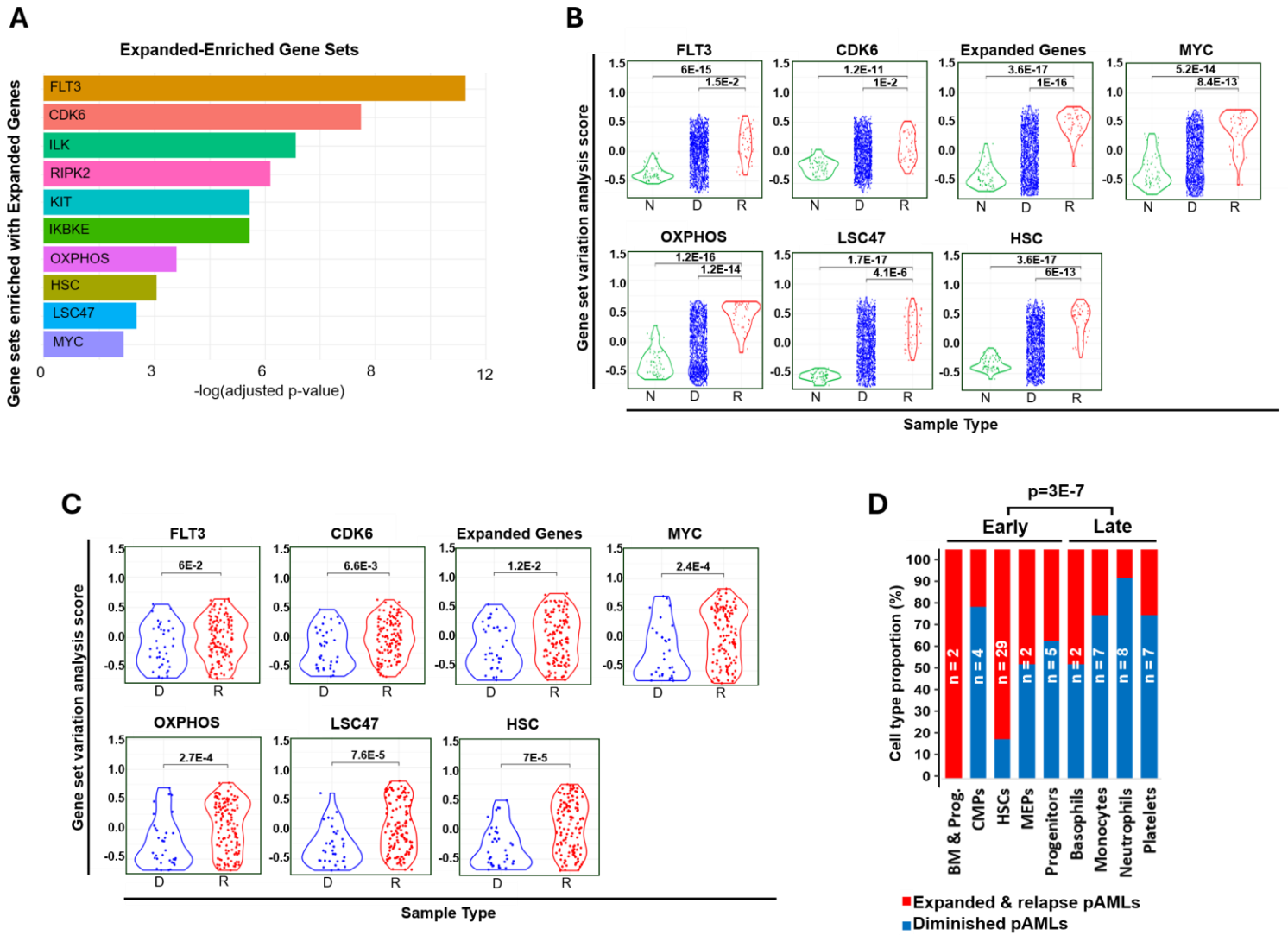


Figure S5. (A) Gene sets and pathways that were significantly enriched with Expanded Genes (Expanded–Enriched Gene Sets), including genes that are co-expressed with Fms-related receptor tyrosine kinase 3 (FLT3), cyclin-dependent kinase 6 (CDK6), integrin-linked kinase (ILK), receptor-interacting serine/threonine kinase 1 (RIPK), KIT proto-oncogene (KIT), and inhibitor of nuclear factor kappa-B subunit epsilon (IKBKE), as well as pathways including Hallmark oxidative phosphorylation (OXPHOS), hematopoietic stem cell (HSC), leukemia stem cell populations gene set (LSC47), and MYC targets. **(B, C)** Gene Set Variation Analysis (GSVA) revealed Expanded–Enriched Gene Sets that are upregulated in (B) AAML1031, n=1325, and (C) AML08 relapse samples, n=170; N, bone marrow aspirates from non-cancer donors; D, diagnosis samples; R, relapse samples. **(D)** The proportion of expanded and diminished pAML cell clusters in the BCM cohort as a function of pAML differentiation showed an enrichment of expanded and relapse pAML cell clusters in undifferentiated pAML aggregates; p values computed by t test. Source data are provided as a Source Data file.

OS prediction by GSVA of Expanded–Enriched Gene Sets

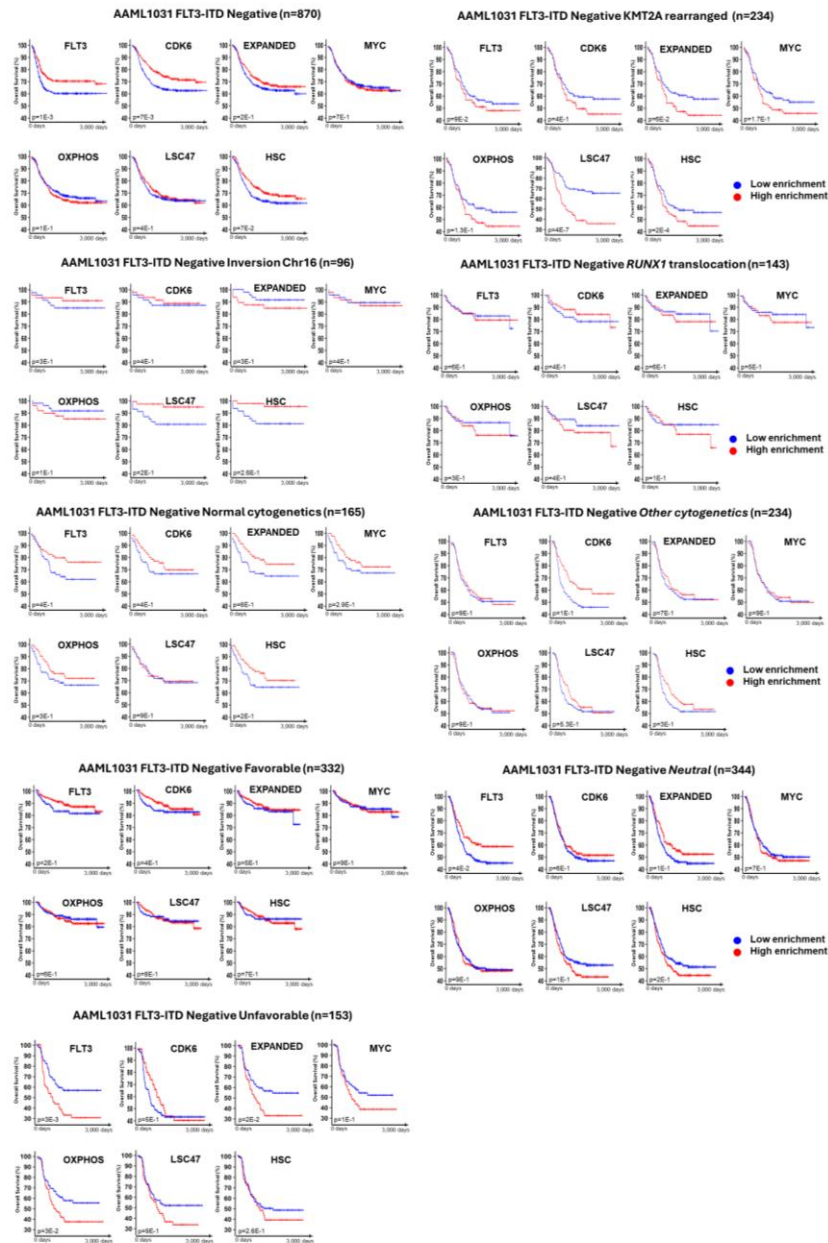


Figure S6. GSVA of Expanded–Enriched Gene Sets in cytogenetic classifications of AAML1031 without correction for multiple testing. We evaluated the outcomes-predictive power of previously proposed outcomes-predictive pathways and gene sets, including LSC47, HSC, and OXPHOS, as well as genes that were recurrently dysregulated in inferred chemoresistant pAML aggregates (*Expanded*). We also evaluated whether MSigDB-curated pathways were predictive of outcomes, and identified genes co-expressed with FLT3 and CDK6 and the MYC pathway as the most predictive. After multiple testing corrections, the following were identified as significantly predictive: LSC47 and HSC enrichment were significant in KMT2A rearranged pAML, but not in the Unfavorable Cytogenetics category or any other categories. Expanded Genes were enriched in the Unfavorable Cytogenetics category ($p=2E-2$); p values by cox regression. Source data are provided as a Source Data file.

KMT2A+ pAML aggregates are more abundant in samples with KMT2A rearrangements

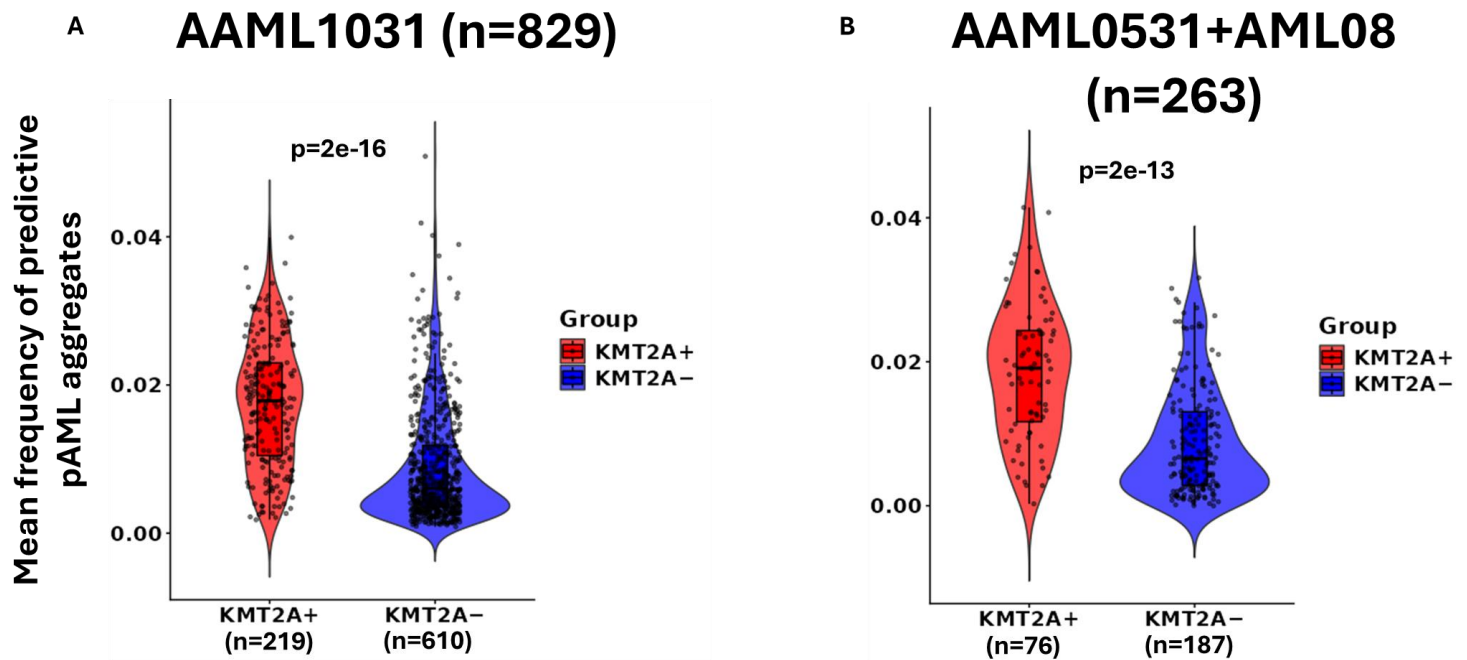


Figure S7. The estimated proportion of predictive pAML aggregates in samples from pAMLs with or without KMT2A rearrangements in the (A) AAML1031 and (B) AML08 and AAML0531 trials, according to SQUID. Shown are the means of the estimated frequency of each of the 23 predictive pAML aggregates studied. The data suggest a significantly (by t-test) higher proportion of predictive pAML aggregates in samples from pAMLs with KMT2A rearrangements; p values computed by t test. Source data are provided as a Source Data file.

EFS analysis of predictive aggregates

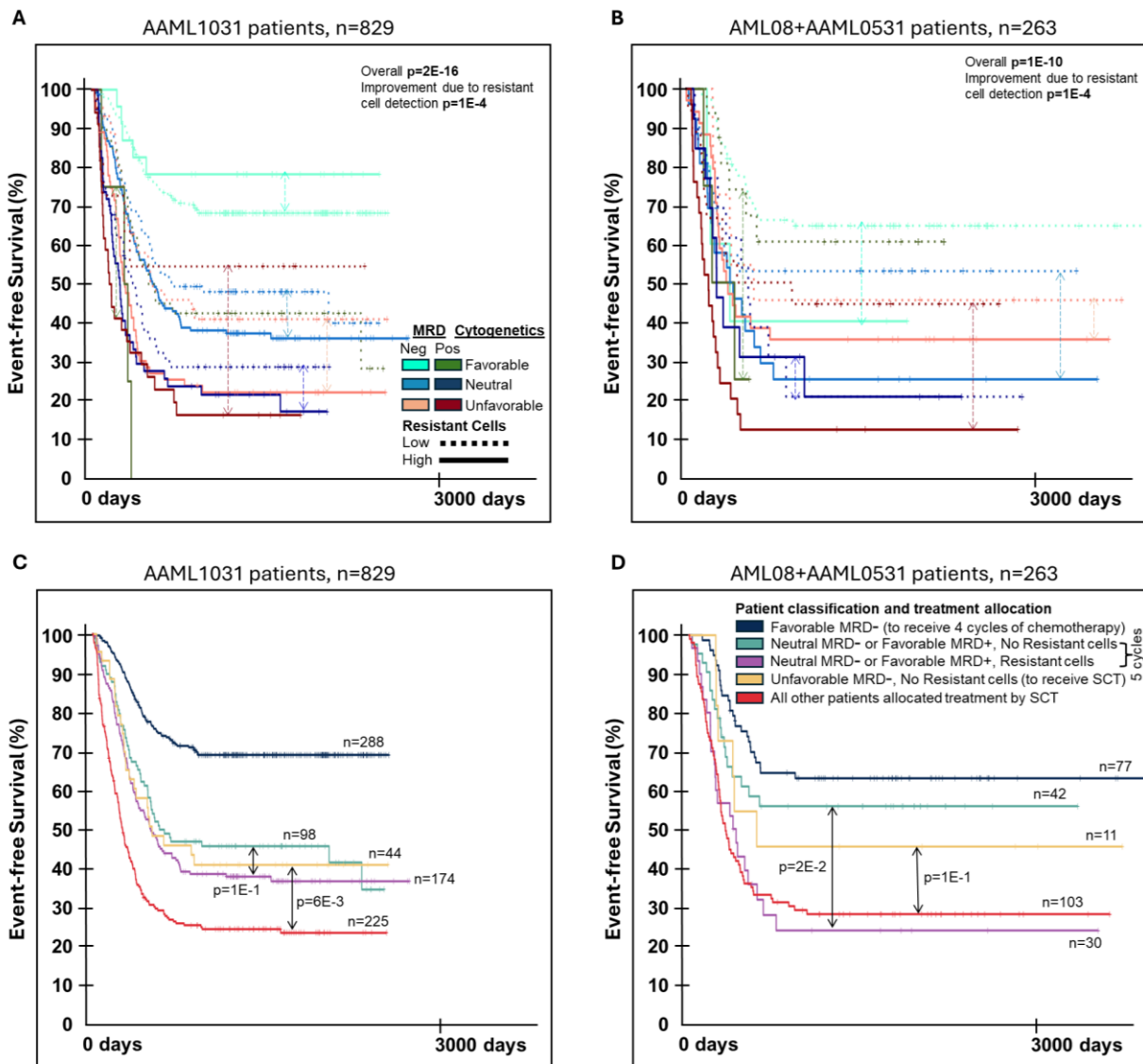


Figure S8. Analogous to Figure 3. **(A–B)** Estimated abundance of chemoresistant pAML aggregates (resistant cells) significantly predicted EFS for patients in (A) AAML1031 and (B) AML08 + AAML0531 cohorts, as determined by Kaplan–Meier survival analysis. These included patients classified by MRD after one chemotherapy cycle and stratified by cytogenetics (Favorable, Neutral, Unfavorable) according to the COG AAML1831 protocol. Across independent trials, resistant cell abundance improved EFS prediction ($p = 1E-4$). Solid and dotted lines represent high- and low-abundance patients, respectively. Arrows indicate patients within the same AAML1831 risk categories. **(C–D)** Inferred resistant cell abundance reclassified patients in (C) AAML1031 and (D) AML08 + AAML0531 who were allocated identical therapies under the current AAML1831 protocol. This analysis revealed higher-risk patients (purple) who would not be prescribed SCT under the COG AAML1831 protocol, with EFS < 0.4, and lower-risk patients (orange) allocated SCT under AAML1831 but achieving EFS > 0.4 without SCT across AAML1031 and AML08 + AAML0531. Source data are provided as a Source Data file.

Decision-curve analysis of predictive aggregates

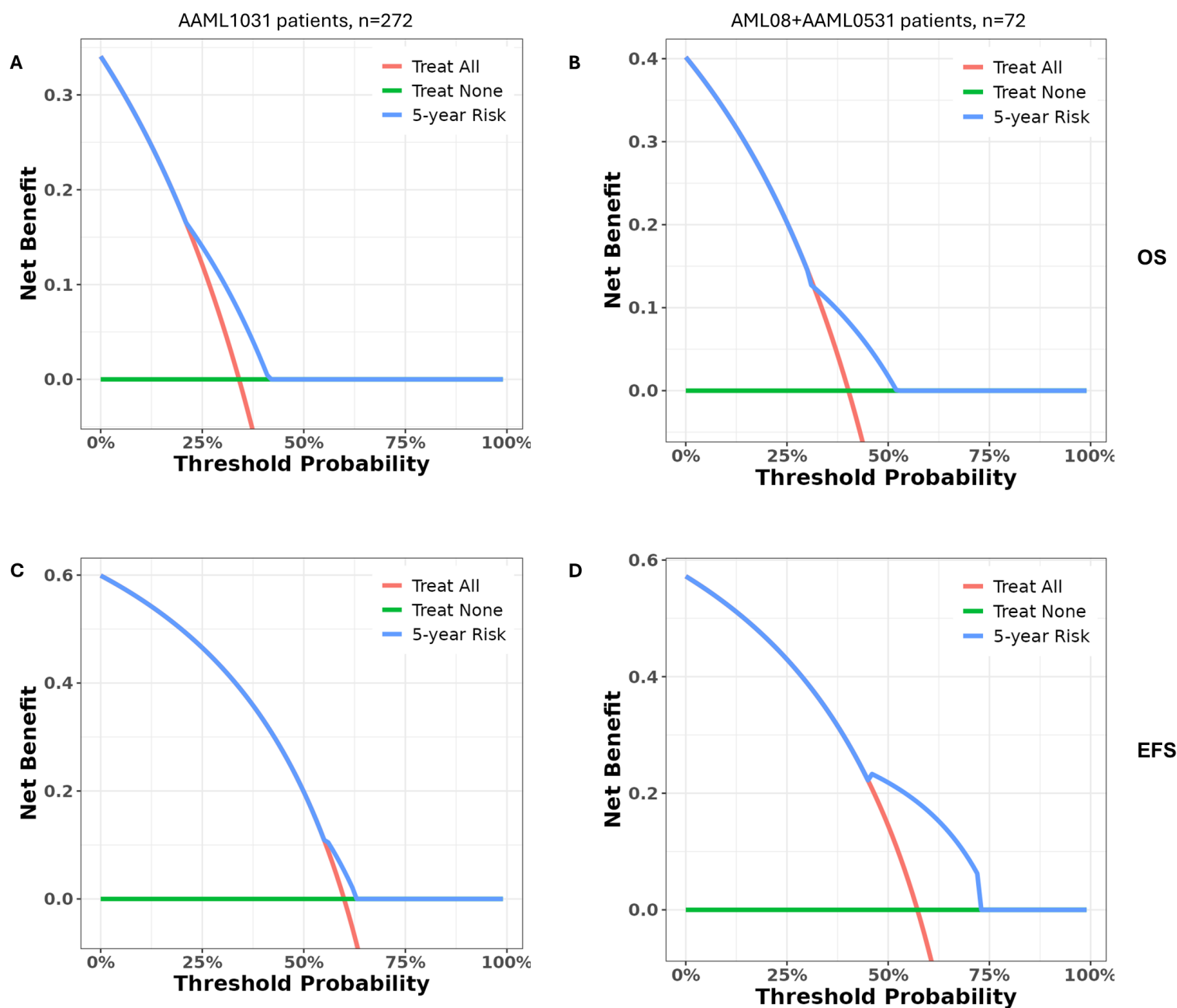


Figure S9. Decision-curve analysis for LR2 patients—patients with Favorable cytogenetics and positive MRD—was used to evaluate the net benefit of predictive pAML aggregate abundance (low vs. high) in classifying (A) AAML1031 OS, (B) AML08+AAML0531 OS, (C) AAML1031 EFS, and (D) AML08+AAML0531 EFS. The analysis revealed significant benefits for the classification within these patient cohorts. For example, for AML08+AAML0531 OS, the analysis identified significant benefits in inferring AML08+AAML0531 OS between 30% to 50% thresholds.

Multivariate analysis of predictive aggregates

AAML1031 patients, n= 829, 270 events

	HR	Std.Error	Statistic	P.value	95% CI Lower	95% CI Upper
Predictive pAML aggregates	6.437	0.485	3.836	1.25E-04	2.486	16.669
Cyto.Neutral	2.339	0.187	4.533	5.82E-06	1.620	3.377
Cyto.Unfavorable	2.848	0.208	5.039	4.67E-07	1.896	4.279
MRD.Yes	2.466	0.125	7.202	5.94E-13	1.929	3.153

AML08+AAML0531 patients, n=263, 103 events

	HR	Std.Error	Statistic	P.value	95% CI Lower	95% CI Upper
Predictive pAML aggregates	3.705	0.686	1.909	3.63E-02	0.965	14.218
Cyto.Neutral	3.324	0.333	3.612	3.04E-04	1.732	6.377
Cyto.Unfavorable	3.652	0.329	3.942	8.07E-05	1.918	6.954
MRD.Yes	1.659	0.201	2.520	1.17E-02	1.119	2.459

AAML1031 patients, n= 829, 442 events

	HR	Std.Error	Statistic	P.value	95% CI Lower	95% CI Upper
Predictive pAML aggregates	5.843	0.305	5.791	6.99E-09	3.215	10.619
Cyto.Neutral	2.335	0.187	4.526	6.02E-06	1.617	3.372
Cyto.Unfavorable	2.862	0.207	5.085	3.68E-07	1.908	4.292
MRD.Yes	2.464	0.125	7.198	6.12E-13	1.928	3.150

AML08+AAML0531 patients, n=263, 148 events

	HR	Std.Error	Statistic	P.value	95% CI Lower	95% CI Upper
Predictive pAML aggregates	8.918	0.651	3.361	7.77E-04	2.489	31.951
Cyto.Neutral	1.755	0.242	2.328	1.99E-02	1.093	2.819
Cyto.Unfavorable	1.848	0.245	2.507	1.22E-02	1.143	2.987
MRD.Yes	1.505	0.175	2.343	1.91E-02	1.069	2.119

Figure S10. Multivariate analysis revealed significant benefits for the inclusion of predictive pAML aggregates in predicting OS and EFS in AAML1031 and AML08 & AAML0531 patients; p values by ANOVA.

Predictive aggregates by cytogenetic classifications

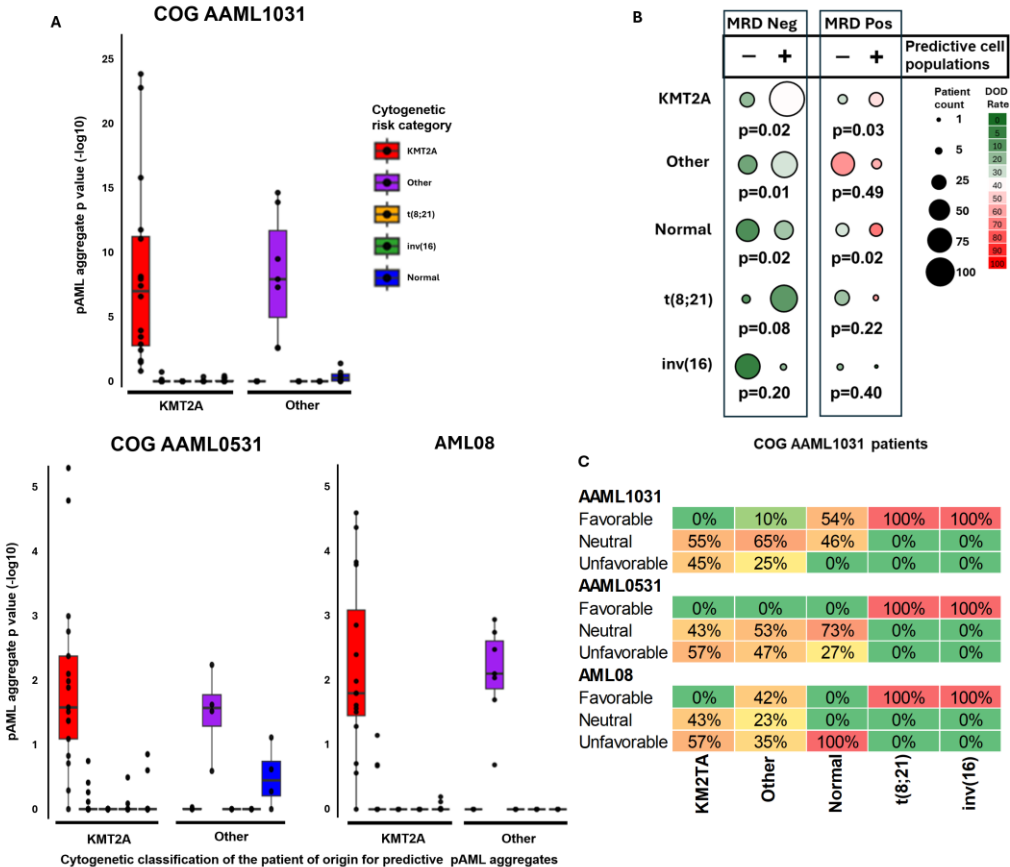
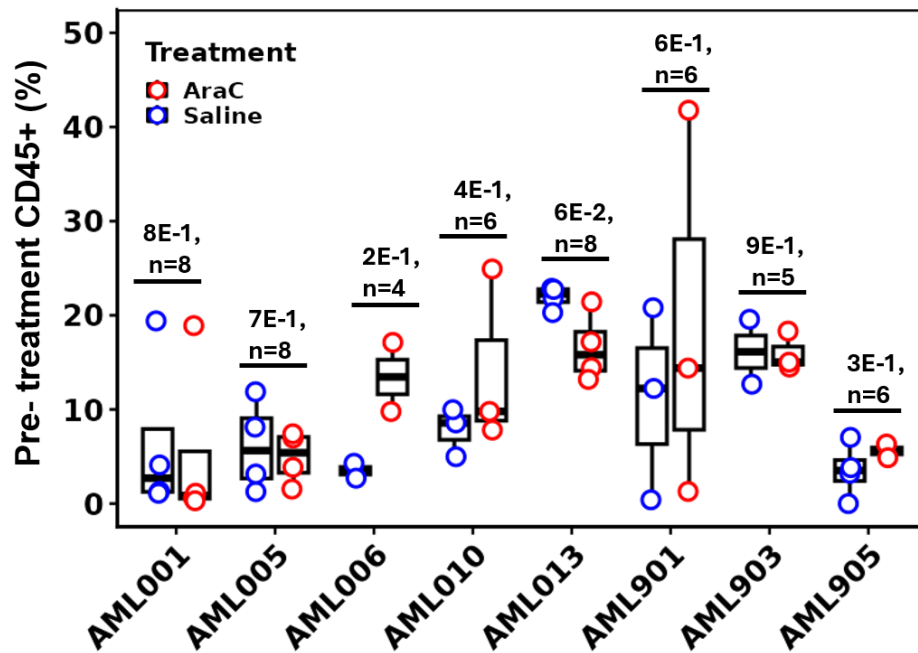


Figure S11. (A) We classified predictive pAML aggregates by the classic cytogenetic classifications of the patients that they were derived from, e.g., KMT2A predictive aggregates were derived from a pAML tumor with KMT2A rearrangements. Then, we classified AAML1031, AAML0531, and AML08 patients into the same cytogenetic classifications, producing patient classifications. Finally, for each pAML aggregate classification, we evaluated whether the estimated abundance of pAML aggregates from this classification is significantly greater in each patient classification relative to others. Our analysis revealed that the abundances of predictive aggregates from one classification is significantly greater in patients with the same classification, i.e., predictive aggregates with KMT2A alterations were significantly more abundant in patients with KMT2A alterations, etc. However, predictive aggregates were also identified and were predictive in patients who did not match their classification. **(B)** Focusing on AAML1031 patients, predictive aggregates were significantly predictive of outcomes in KMT2A, Other, and Normal cytogenetic classifications. Marker size denotes the number of patients from each cytogenetic classification with detected predictive aggregates, and the color denotes the rate of poor outcomes. **(C)** A mapping between the cytogenetic classification used here {KMT2A, Other, Normal, inv(16), and t(8;21)} and the risk categories proposed in the COG AAML1831 trial {Favorable, Neutral, and Unfavorable}. The detection of inv(16) or t(8;21) was consistently associated with low-risk, favorable prognosis; however, risk in other categories has been refined by COG AAML1831. DOD: died of disease; p values by t test. Source data are provided as a Source Data file.

A PDX treatment initiation



B

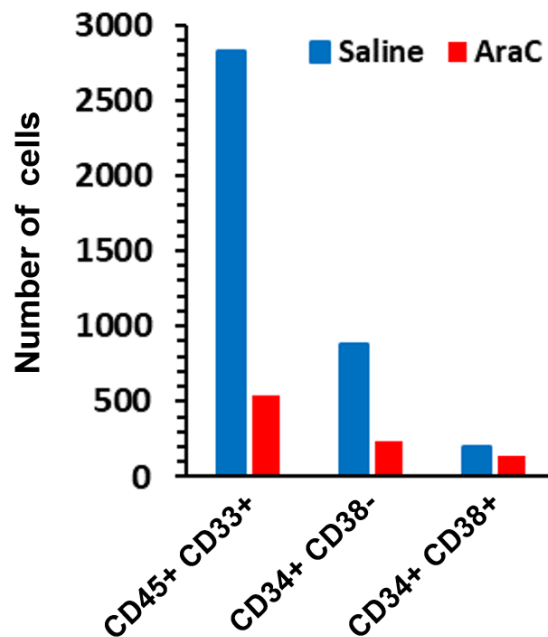


Figure S12. (A) Pre-treatment CD45⁺ cell abundance in PDX blood samples was used to determine the treatment initiation time point. Consequently, we expected no significant difference between the groups. The eight PDX models were represented by a total of 51 animals, as described in Supplementary Data 7. **(B)** Total CD45⁺CD33⁺, CD34⁺CD38⁻, and CD34⁺CD38⁺ pAML cells in PDX AML006 after treatment with AraC (red bars) or saline (blue bars). The total cell counts are shown. Source data are provided as a Source Data file.

Enriched network modules

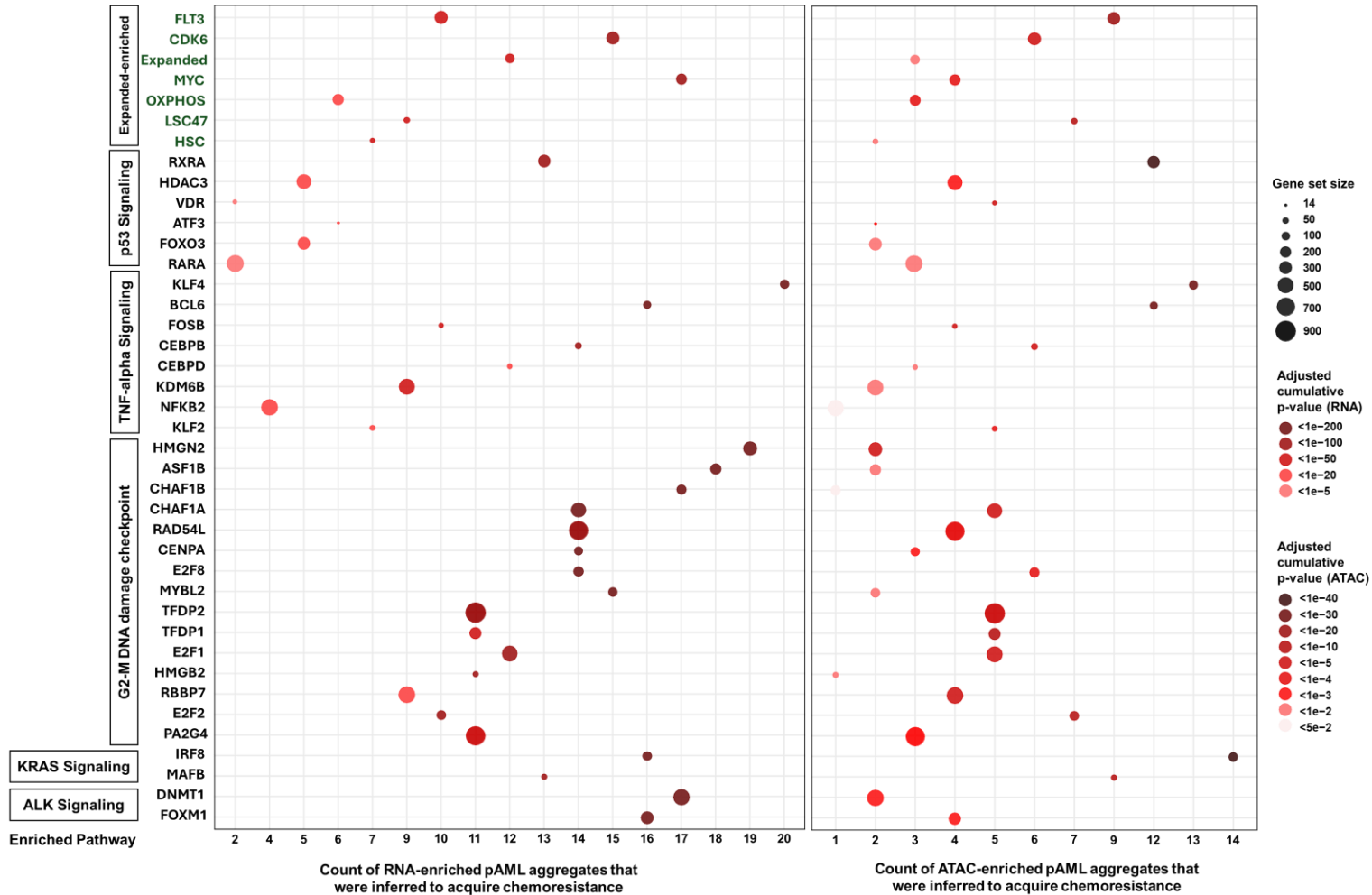


Figure S13. Gene sets and regulatory network modules that were enriched with differential gene expression (left) and chromatin accessibility (right) in pAML aggregates that were inferred to acquire chemoresistance, relative to pAML aggregates that were inferred to be chemosensitive in the same sample. The total number of pAML aggregates that were inferred to acquire chemoresistance is shown on the x-axis. Gene sets and regulatory network modules anchored at specific transcription factors are shown on the y-axis. The dot size and color denote the number of genes and the p-value for the enrichment (adjusted and cumulative over pAML aggregates); p values calculated by GSEA. Source data are provided as a Source Data file.

Response of enriched network modules to perturbations

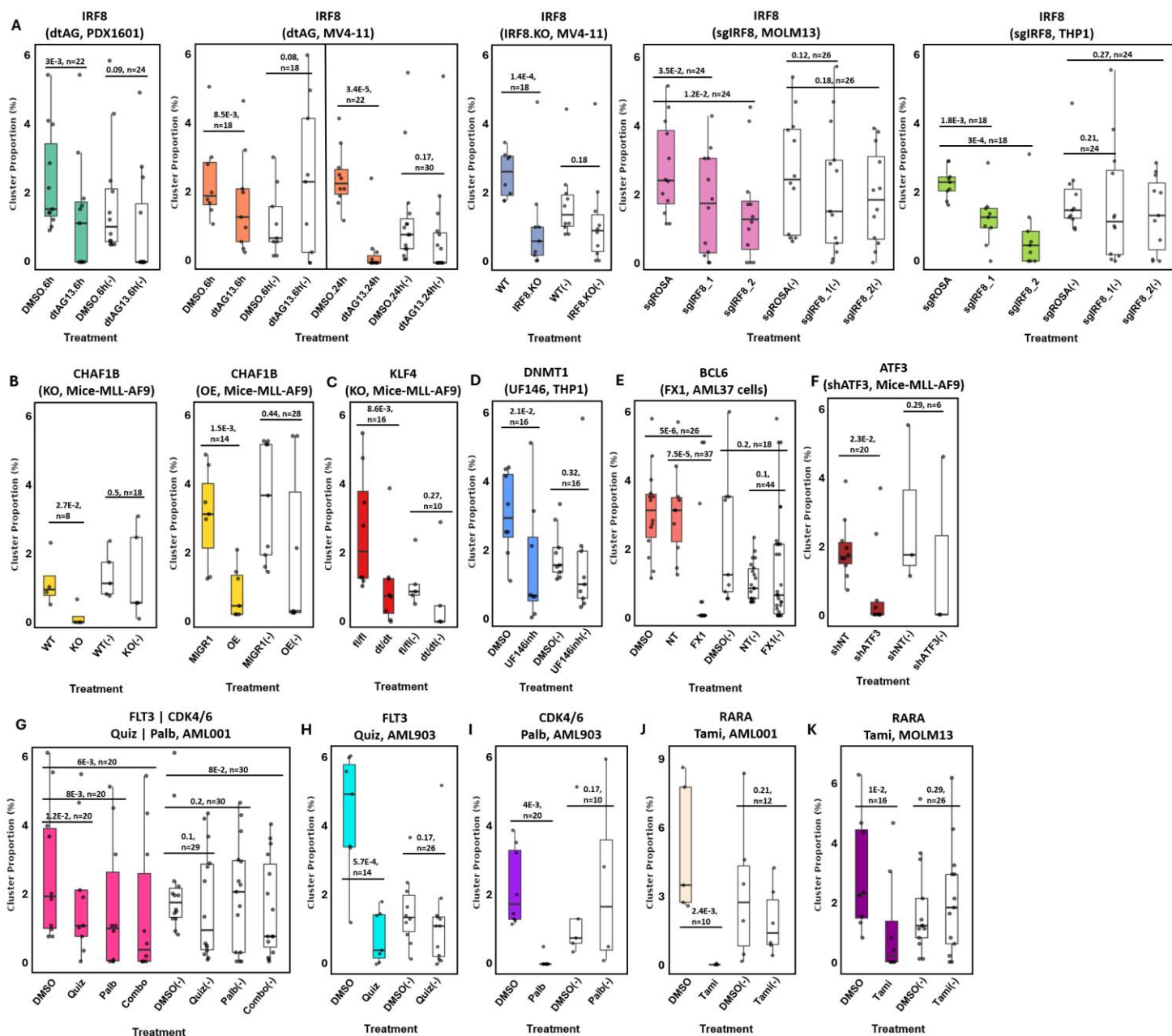


Figure S14. Specific evaluation of gene sets and regulatory network modules that were enriched with dysregulated genes and differential chromatin accessibility in diminished pAMLs that were inferred to acquire chemoresistance. We compared the inferred abundance of pAML aggregates (1) with predicted vulnerabilities in cells and tumors, and (2) those that were not predicted to be vulnerable to the effects of specific targeting. These results include a detailed comparison to the response of pAML aggregates not predicted to be targets corresponding to Figure 5L. The abundance of pAML aggregates that were not predicted to be vulnerable to each perturbation is shown in white for each gene set and regulatory network module. The figures present random selections of pAML aggregates to match the number of detected pAML aggregates that were predicted to respond to each perturbation; p values by FET. Source data are provided as a Source Data file.

Patient samples predicted to contain pAML aggregates

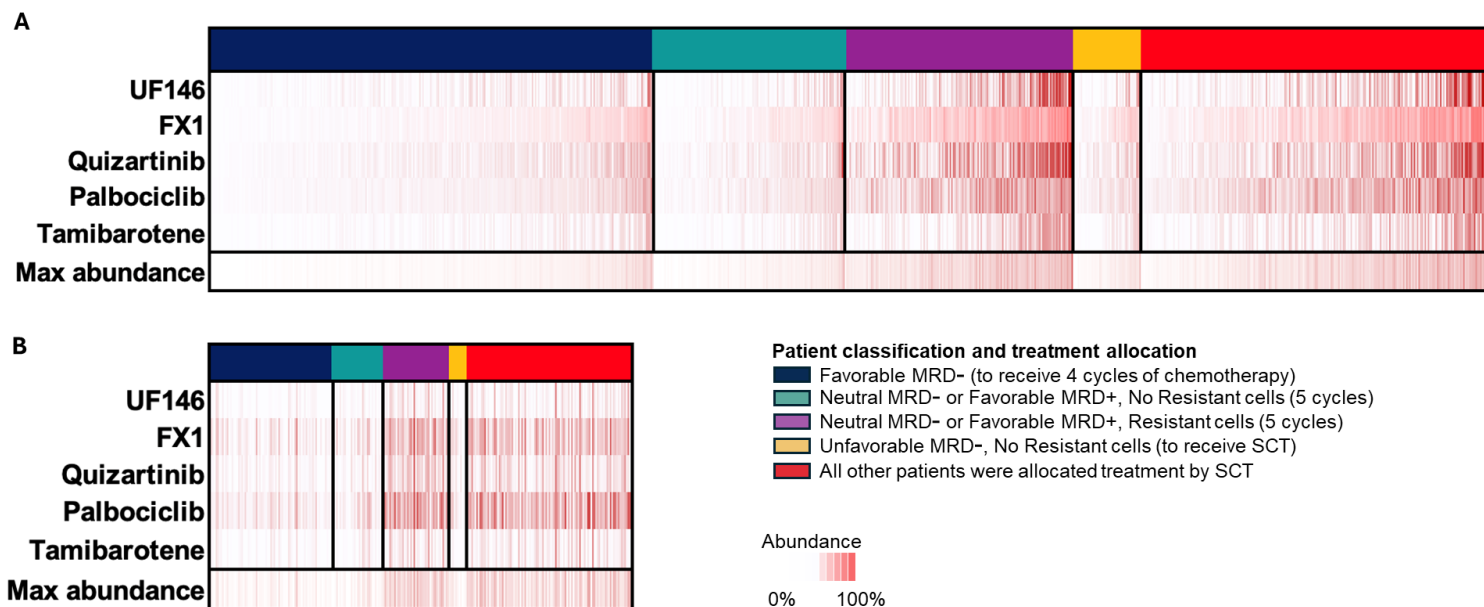


Figure S15. (A) AAML1031 and (B) AAML0531+AML08 patient samples predicted to contain pAML aggregates that respond to the five tested inhibitors in Figures 5 and S14. Namely, for each inhibitor, we collected the pAML aggregates whose abundance was significantly reduced, with significance estimated based on a null composed of non-target pAML aggregates (at least two standard deviations away). For each diagnostic patient sample, we evaluated the total inferred composition of these pAML aggregates. Color denotes the total proportion (from 0 to 1). Source data are provided as a Source Data file.

Fluorescence-activated cell sorting



Figure S16. Samples were enriched for mononuclear cells by density centrifugation and fluorescence-activated cell sorted to enrich for AML cells (side scatter [SSC] low, CD45 dim) prior to downstream analyses.