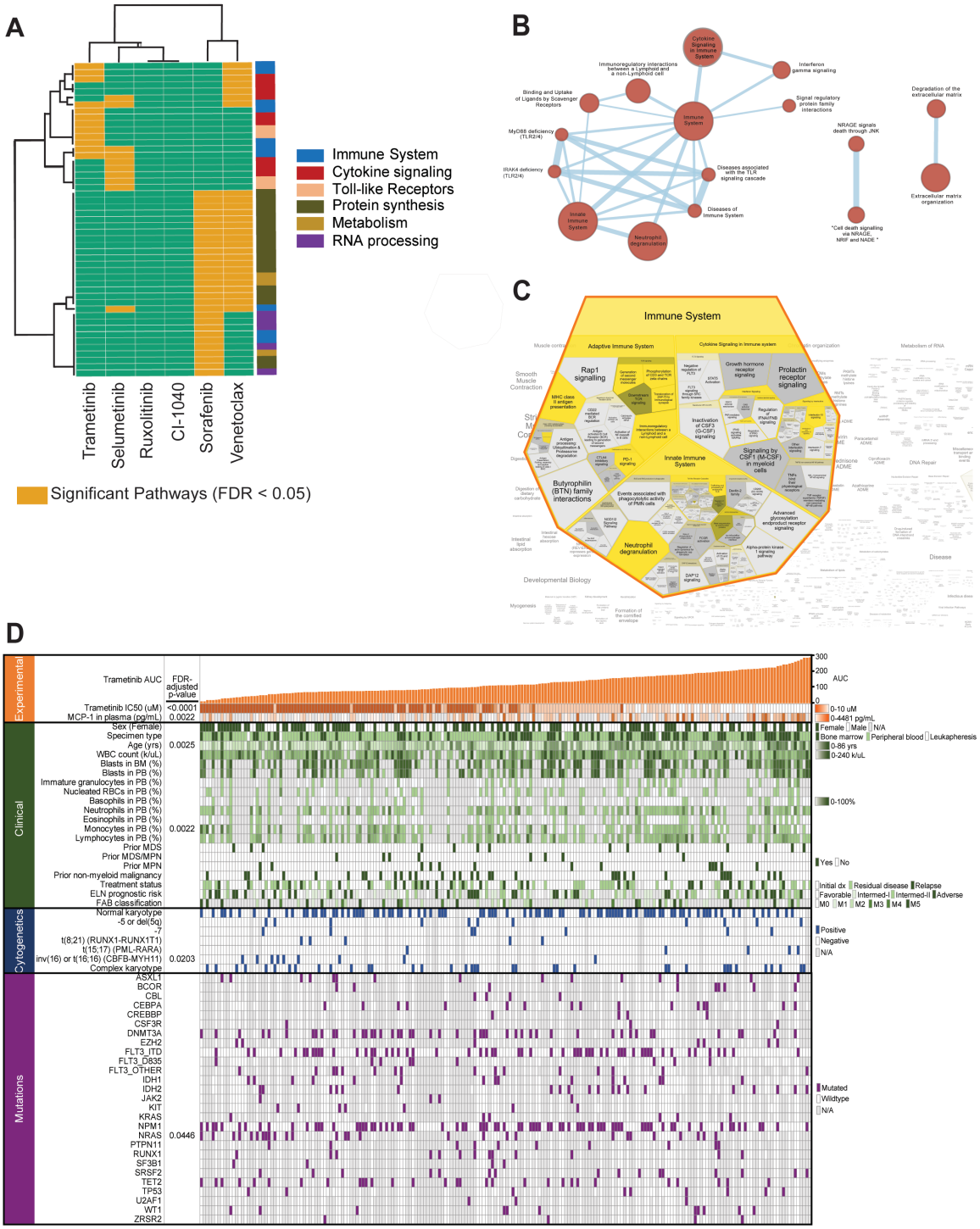
Targeting CCL2/CCR2 signaling overcomes MEK inhibitor resistance in Acute Myeloid Leukemia

INVENTORY FOR SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURES

SUPPLEMENTAL TABLES

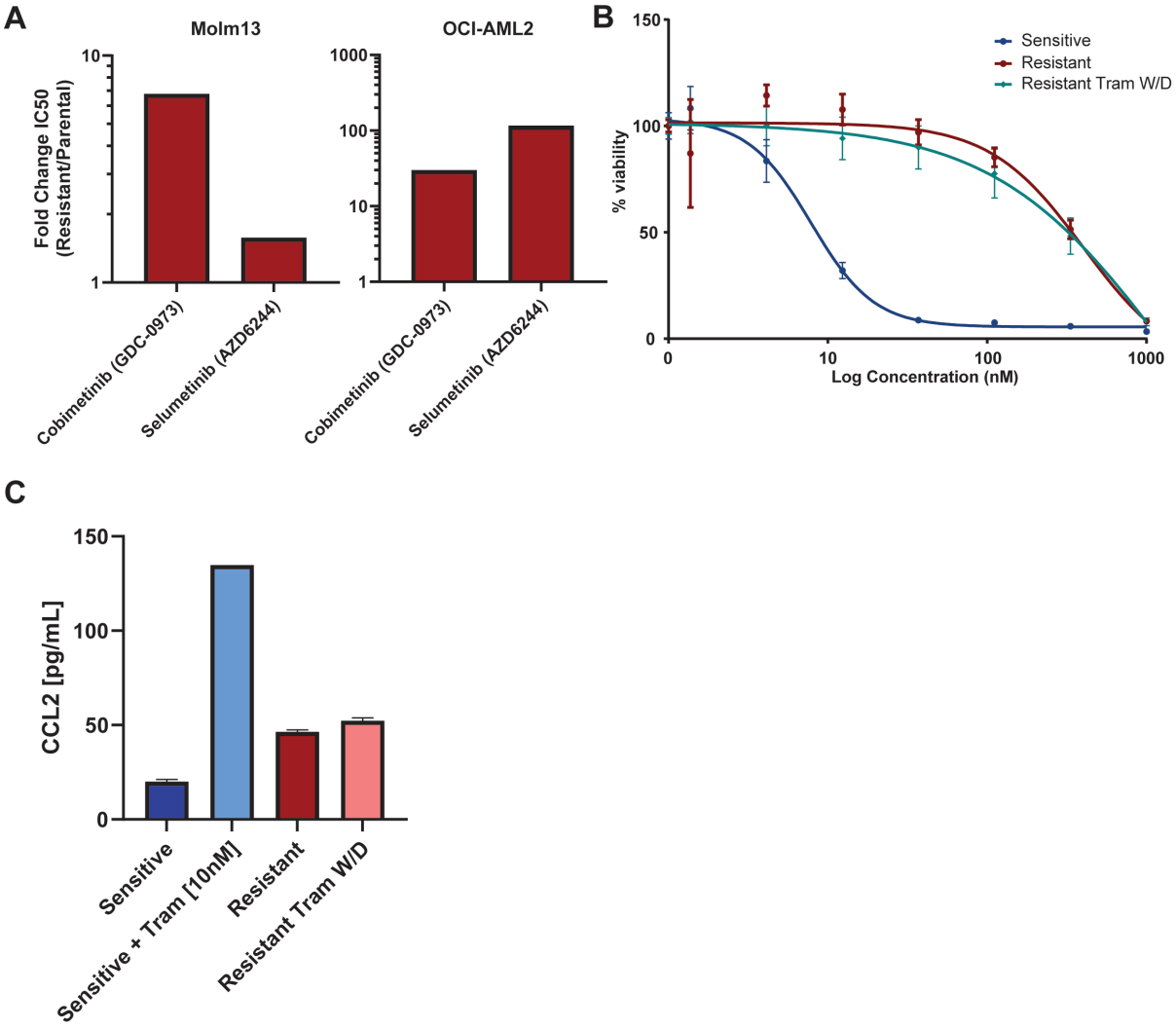
SUPPLEMENTAL FIGURE 1



SUPPLEMENTAL FIGURE S1. ***Trametinib resistance is associated with the dysregulation of immune signaling and high CCL2 levels in AML.*** **(A)** Hierarchical clustering of significantly altered pathways (FDR < 0.05) using the

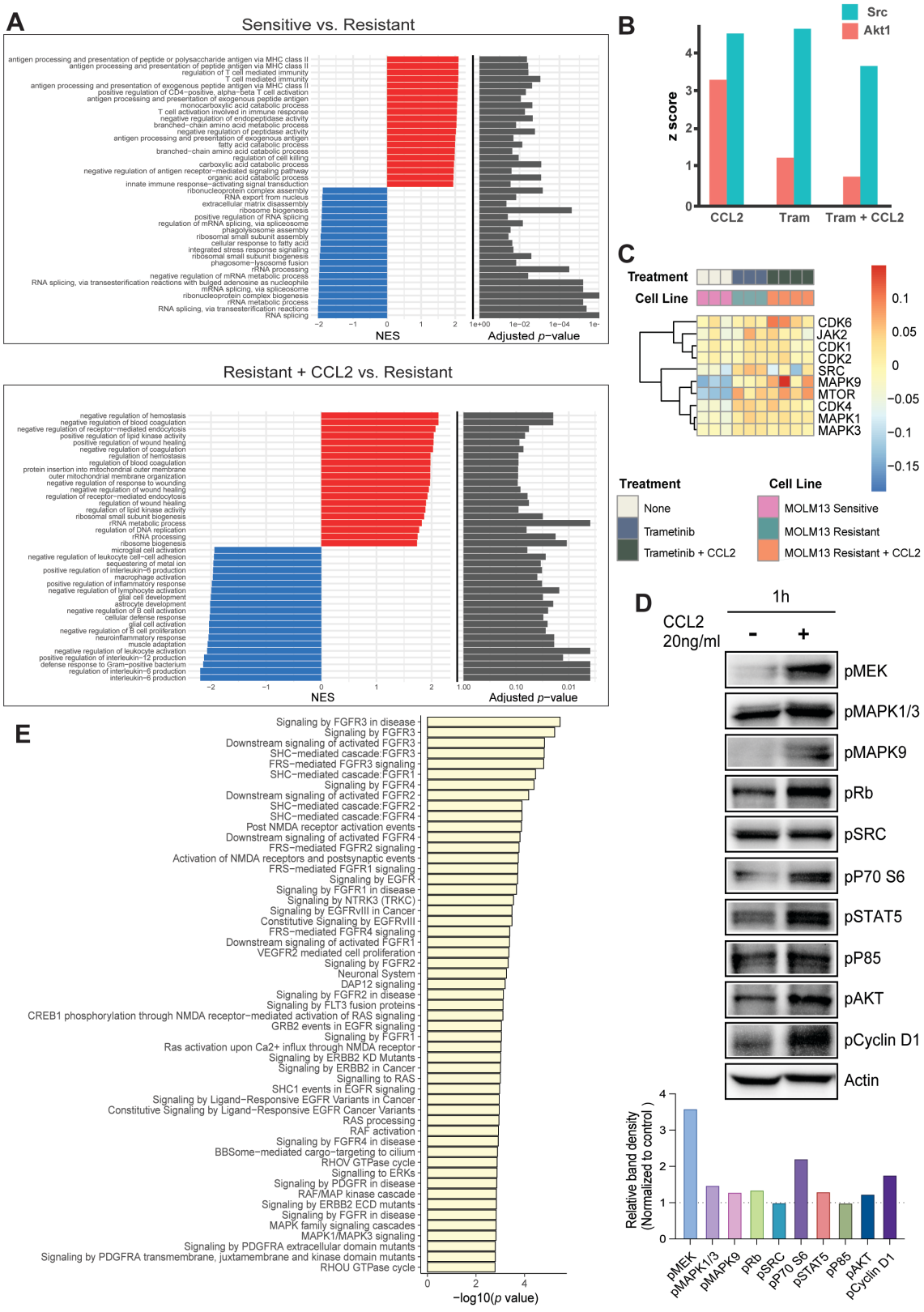
RNA-seq dataset correlated with the *ex vivo* sensitivity profile of the indicated inhibitors from the Beat AML cohort of 299 AML samples. Significance was calculated using a Binomial test. **(B)** Enrichment map of pathways for significantly enriched genes differentially expressed in trametinib-sensitive and resistant patient samples. Pathways with FDR < 0.05 are represented as nodes are linked together if two pathways have shared genes. The node sizes are proportional to the number of genes contained in pathways. **(C)** Highlighted pathways from 300 significantly altered genes using the Reactome Foam tree visualization data tool. **(D)** Clinical, cytogenetic, and mutational characteristics of AML patients are featured in Fig.1A from the Beat-AML database. Statistical significance calculations, features, and the number of samples are included in supplemental tables S4 and S5.

SUPPLEMENTAL FIGURE 2



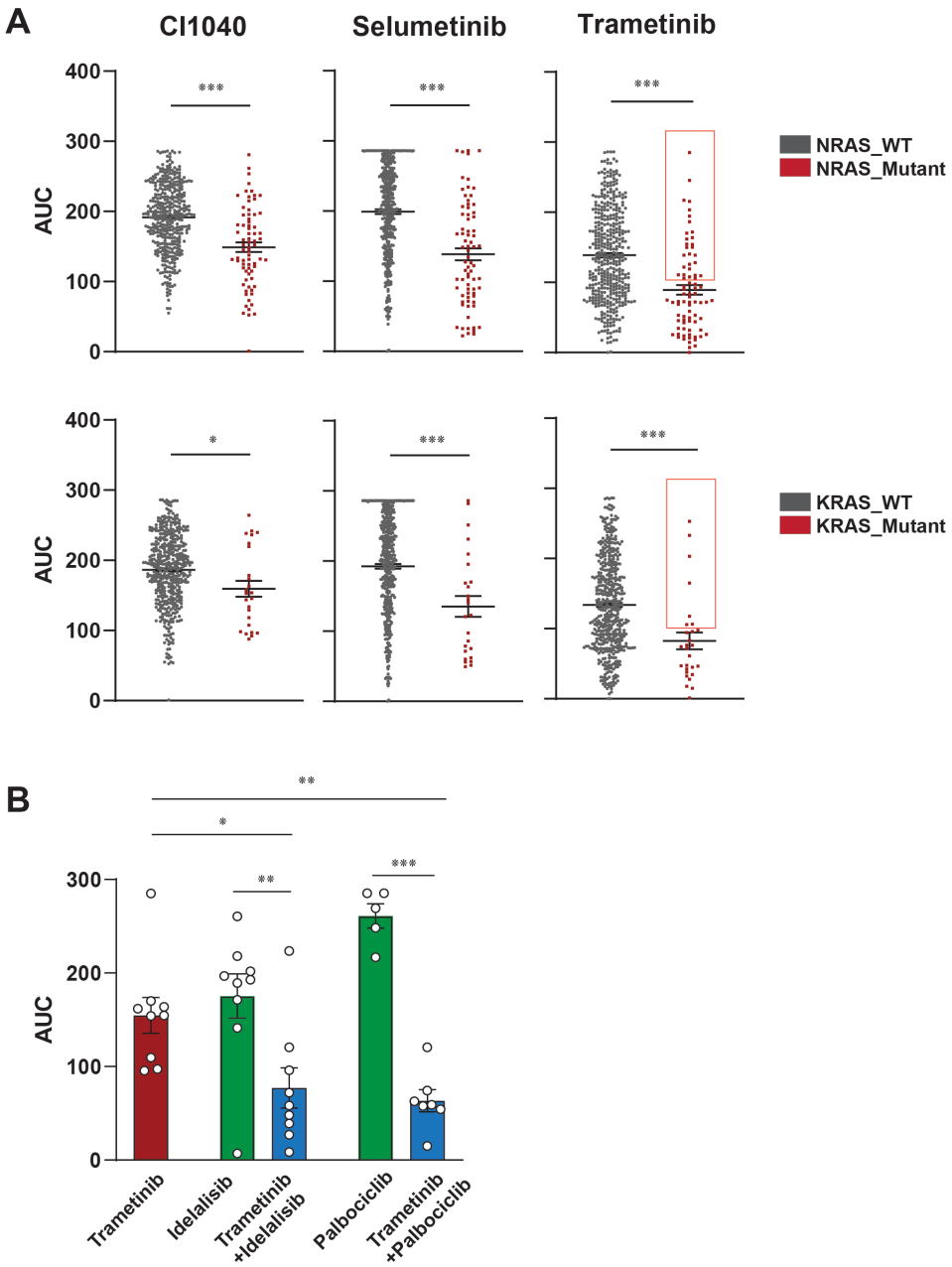
SUPPLEMENTAL FIGURE S2: Trametinib-resistant cell lines display resistance to MAPK inhibitors and stably express CCL2 in a trametinib independent manner. (A) MOLM13 and OCI-AML2 trametinib-resistant and sensitive cell lines were treated with cobimetinib and selumetinib using concentrations ranging from 0.014 to 10 μM and cell viability was determined using MTS cell viability assay after 72 hrs to calculate the $IC_{50.}$ The fold increase in IC_{50} of resistant cell lines over the IC_{50} of sensitive lines was represented as a bar graph. The data is represented as log scale **(B)** Resistant MOLM13 cells were cultured with trametinib and withdrawal (W/D) of trametinib for 2 weeks. Resistant, resistant withdrawal (W/D), and sensitive cell lines were treated with the indicated concentration of trametinib over concentrations ranging from 0.01 to 10 μM and viability was determined by MTS cell viability assay (data from 3 technical replicates). **(C)** Sensitive cells were cultured with or without trametinib (10nM) addition. Resistant MOLM13 cells were cultured with trametinib and withdrawal (W/D) of trametinib for 4 days. Levels of CCL2 were measured at day 4 by ELISA (data from 1-3 technical replicates).

SUPPLEMENTAL FIGURE 3



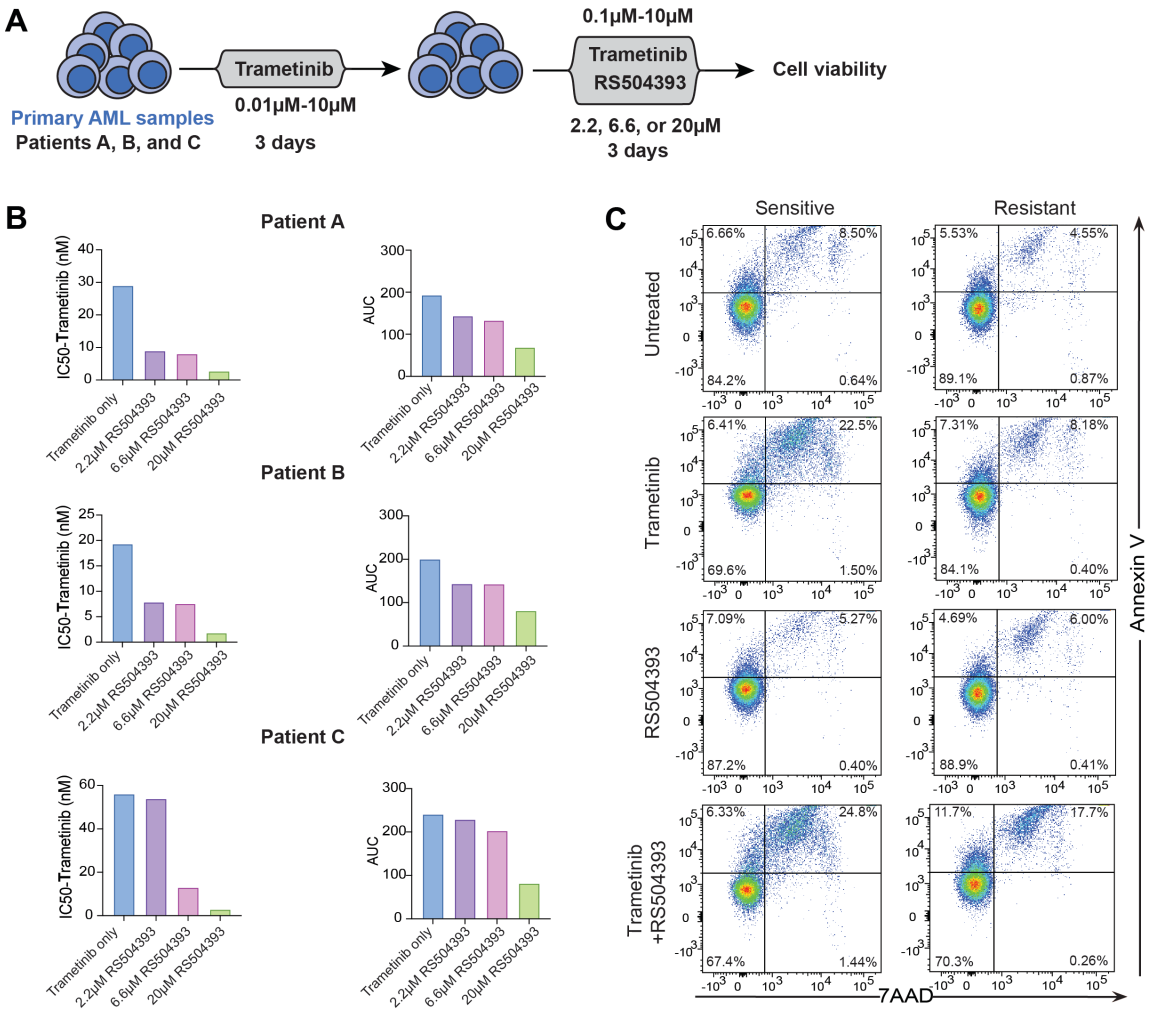
SUPPLEMENTAL FIGURE S3. ***Exposure to CCL2 or MEK inhibition activated many pro-survival pathways in AML cells.*** **(A)** Gene set enrichment analysis (GSEA) ($p \leq 0.05$) of global proteomics data from trametinib-sensitive and resistant MOLM13 cells treated with trametinib only (left panel) and trametinib + CCL2 (right panel) generated in Fig 2E. Red bars indicate an increase in pathway activity whereas blue bars indicate decreased activity. **(B)** KSEA analysis from proteomic analysis (Fig. 3A) of select kinases from AML cells upon 60 minutes of trametinib, CCL2, or combination treatments relative to vehicle treated. **(C)** Heatmap of protein levels between MOLM13 sensitive and trametinib-resistant lines generated in Fig 2E. **(D)** Immunoblot analysis of cell lysates from MOLM13 trametinib-resistant cells that were subjected to 18 hr starvation and stimulated for 1 hour with and without 20ng/ml CCL2 (top panel). Relative band intensity of the indicated phospho-proteins was normalized to the loading control b-ACTIN (bottom panel). **(E)** GSEA analysis for significantly enriched pathway in depleted sgRNAs in trametinib-treated versus DMSO control from Fig 3D.

SUPPLEMENTAL FIGURE 4



SUPPLEMENTAL FIGURE S4: RAS-mutated primary AML samples show sensitivity to MEK inhibition and combinations. (A) Using the Beat AML database, the sensitivity of primary samples to MEK inhibitors was examined. The data is shown for NRAS and KRAS mutated AML primary samples compared to RAS wild-type (WT) control samples. Area under the curve (AUC) values are represented. **(B)** Trametinib-resistant samples (red boxed in panel A) from the Beat AML database were used to see their sensitivity to combination inhibitors with idelalisib and palbociclib. P values are calculated using the student t-test. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

SUPPLEMENTAL FIGURE 5



SUPPLEMENTAL FIGURE S5. ***Targeting CCR2 in combination with trametinib reduced AML cell viability and induced apoptosis in primary AML samples.*** **(A)** Schematic for the ex vivo cell viability assays performed with primary AML patient samples. **(B)** Primary AML cells were pre-treated with trametinib (0.01μM-10μM) for 3 days followed by incubation with CCR2i RS540393 (0, 2.2, 6.6, and 20μM) for 3 additional days and IC_{50} values were determined using the MTS cell viability assay. **(C)** Representative flow cytometry plots for Annexin V and 7-AAD staining from trametinib-sensitive (S) and -resistant (R) MOLM13 cells treated with 0.1μM trametinib or 2.5 μM RS504393 or a combination of both for 72 hours.