Supplementary Information

Targeting polyamine metabolism and eIF5A hypusination in pediatric acute myeloid leukemia

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Supplementary Figure Legends:

Table S1 Table containing patient specimen information including age, sex, diagnosis and cytogenetics/mutation status.

Fig. S1. (A) Putrescine, spermidine, spermine, and DENSpm abundance in two pediatric AML PDX specimens (AML1 and AML2) following 24 h treatment with 10 μM DENSpm or vehicle, as determined by mass spectrometry. Statistical significance was determined using an unpaired t-test. **(B)** Colony-forming ability of eight pediatric AML PDX specimens following 24 h treatment with 10 μM DENSpm or vehicle. Colony numbers were quantified 14–21 days post-plating. Statistical significance was determined using multiple unpaired t-tests. **(C)** Putrescine, spermidine, and spermine abundance in two pediatric AML PDX specimens (AML1 and AML2) following 24 h treatment with 2.5 mM DFMO, 400 nM AMXT-1501, DFMO + AMXT-1501 (2.5 mM and 400 nM, respectively), or vehicle, as determined by mass spectrometry. Statistical significance was determined using one-way ANOVA. **(D)** Colony-forming ability of eight pediatric AML PDX specimens following 24 h treatment with 2.5 mM DFMO, 400 nM AMXT-1501, DFMO + AMXT-1501 (2.5 mM and 400 nM, respectively), or vehicle. Colony numbers were quantified 14–21 days post-plating. Statistical significance was determined using two-way ANOVA. All error bars represent standard deviation. ns, not significant; *P < 0.05, **P < 0.01, ***P < 0.001, ***P < 0.0001.

Fig. S2. (A) Expression of hypusinated eIF5A in three pediatric AML PDX specimens following 24 h treatment with 10 μM DENSpm, 2.5 mM DFMO + 400 nM AMXT-1501 (D+A), or vehicle, as determined by Western blot. **(B)** Expression of hypusinated eIF5A in three pediatric AML PDX specimens following 24 h treatment with 50 μM GC7 or vehicle, as determined by Western blot. **(C)** Colony-forming ability of eight pediatric AML PDX specimens following treatment with vehicle, with 50 μM GC7 added directly to the MethoCult medium. Colony numbers were quantified 14–

21 days post-plating. Statistical significance was determined using multiple unpaired t-tests. All error bars represent standard deviation. *P < 0.05, **P < 0.01, ***P < 0.001, ***P < 0.0001.

Fig. S3. PCA plot of the protein expression in three pediatric AML PDX samples following vehicle, DENSpm, or GC7 treatment.

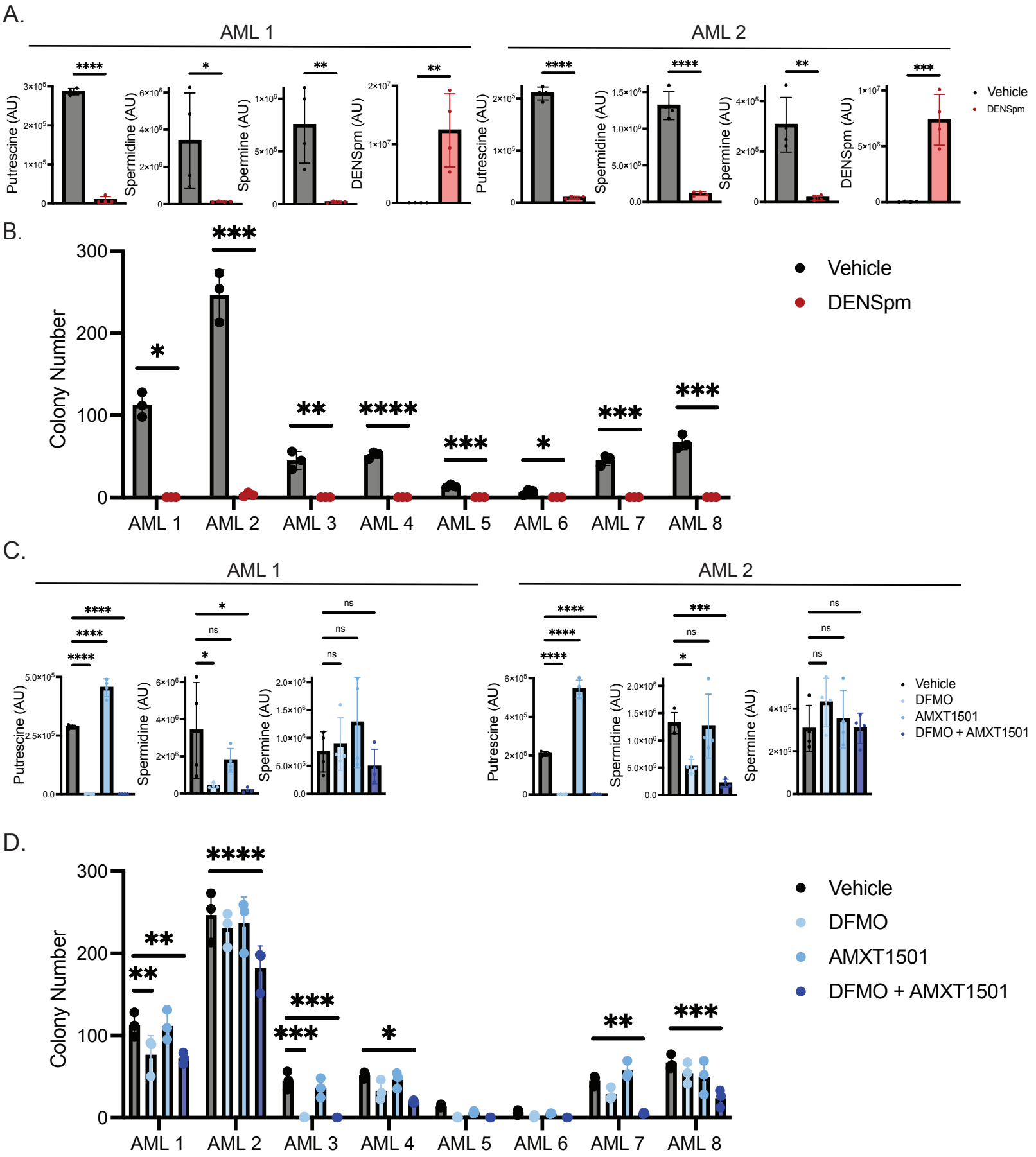
Fig. S4. (A) Venn diagram showing the overlap of mRNAs with differential ribosome occupancy following DENSpm and GC7 treatment compared to vehicle control. **(B)** KEGG pathway analysis of mRNAs with differential ribosome occupancy in both DENSpm and GC7 treatment groups. **(C)** Marker analysis of mRNAs with differential ribosome occupancy in both DENSpm and GC7 treatment groups.

Fig S5. (A) Ribosome occupancy and **(B)** mRNA expression of cell cycle related proteins identified in the proteomic analysis.

Fig. S6. Median fluorescence intensity of CD11b, CD14, and CD15 in AML1 and AML2 treated with low dose DENSpm or GC7 post colony formation.

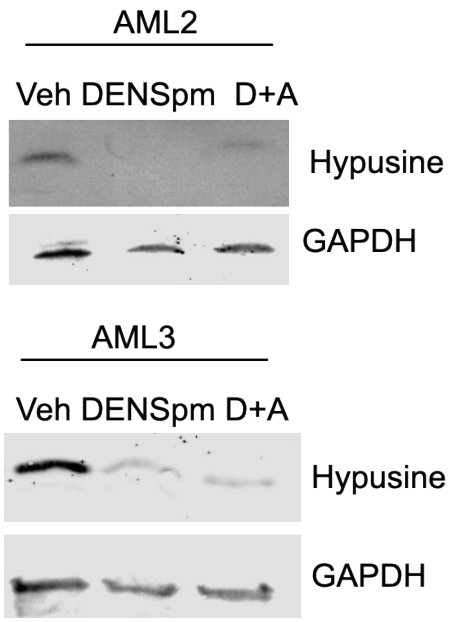
Fig. S7. (A) Synergy analysis of three pediatric AML PDX specimens (AML1, AML2, and AML3) following 48 h treatment with DENSpm (10, 5, 2.5, and 1.25 μM), cytarabine (10, 5, 2.5, and 1.25 μM), or all pairwise combinations of DENSpm and cytarabine at the indicated concentrations. Synergy scores were calculated using SynergyFinder.

Supplemental Figure 1

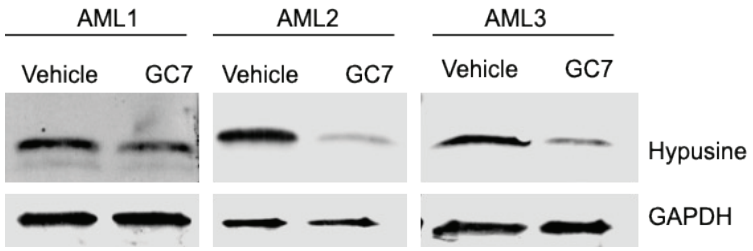


Supplemental Figure 2

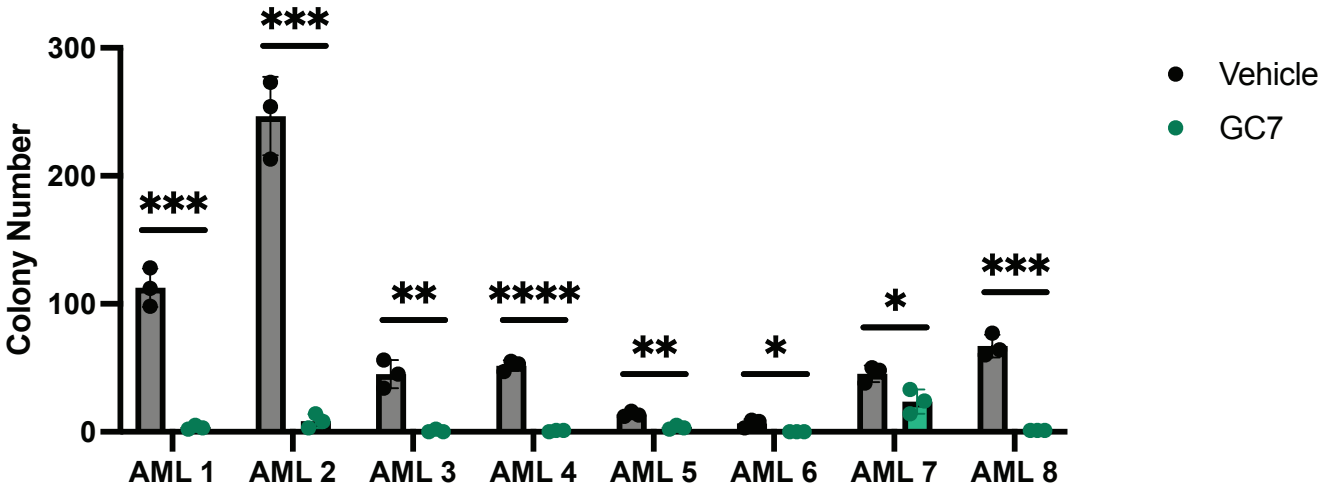
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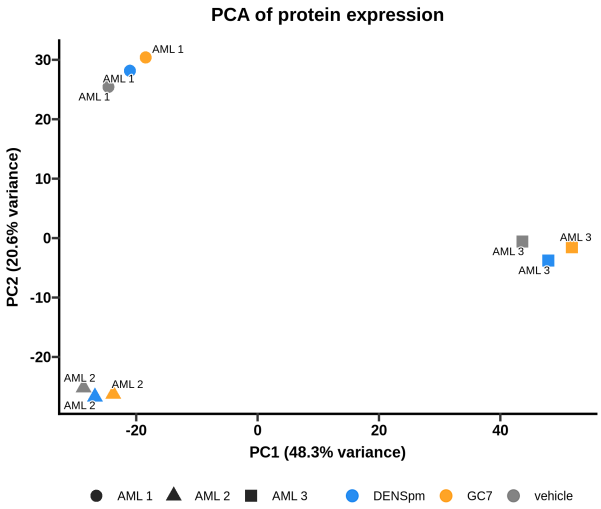


C.



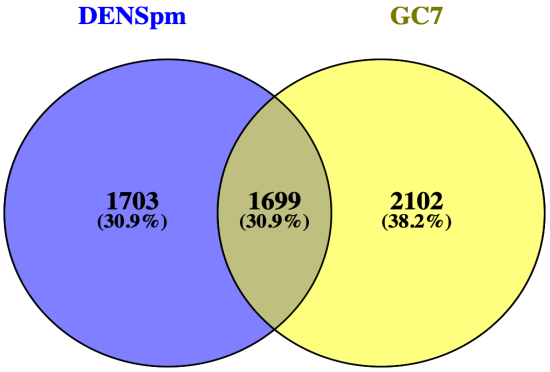
Supplemental Figure 3

A.

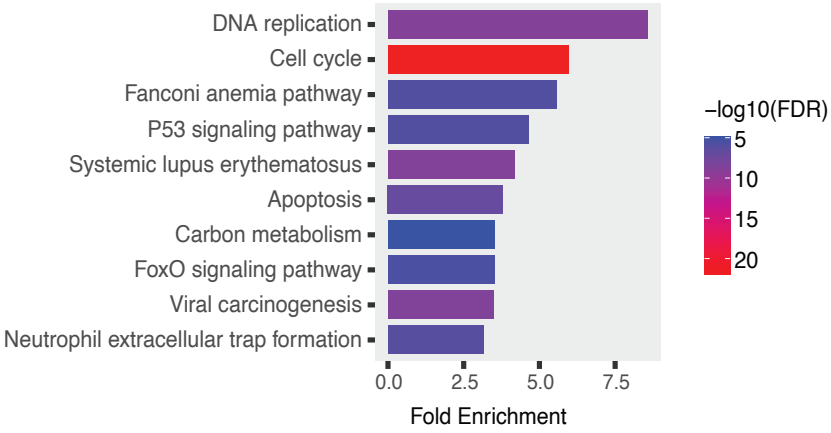


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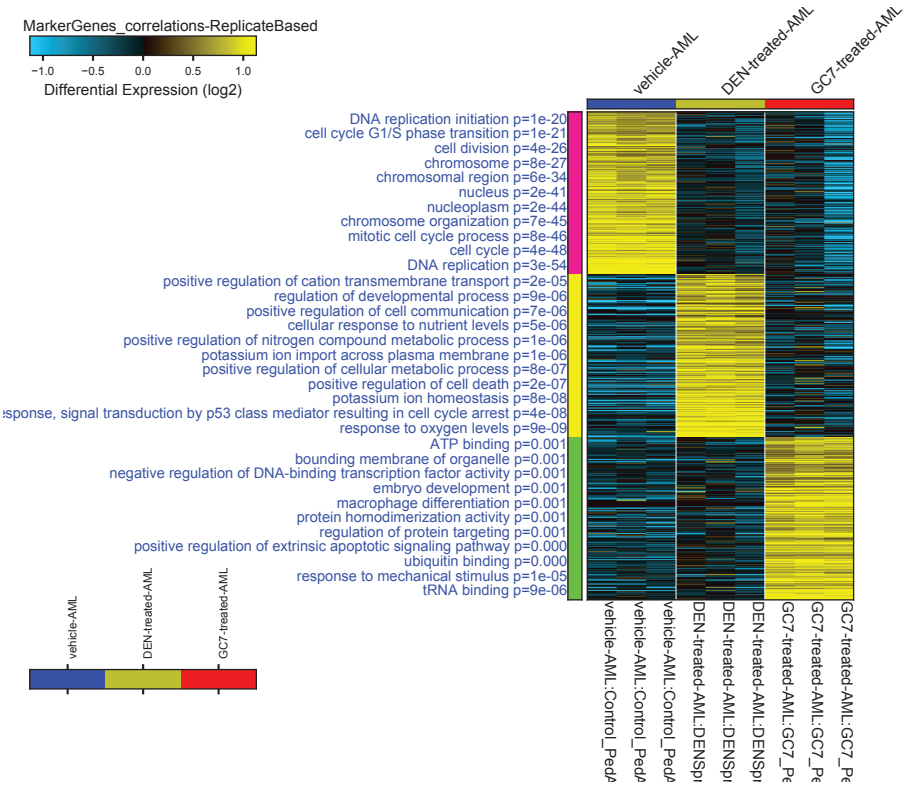
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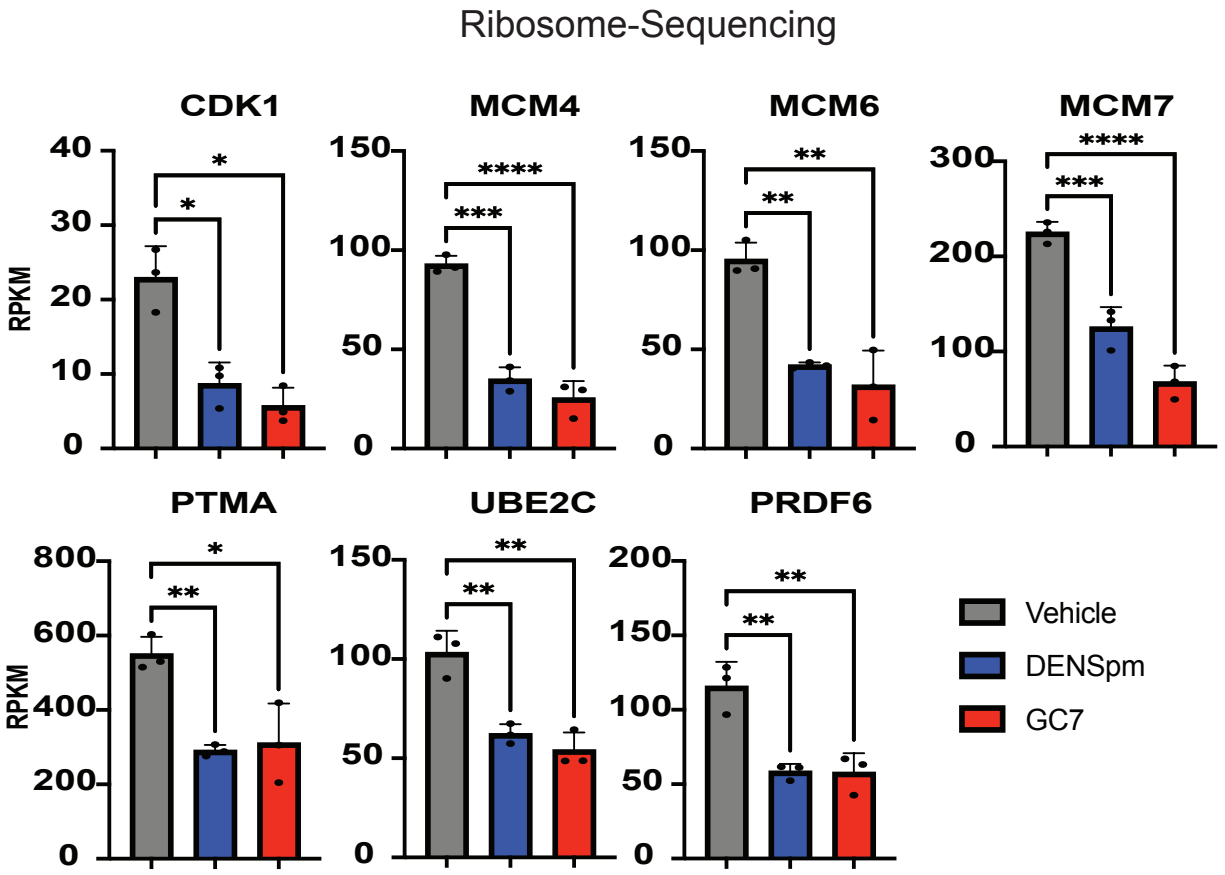


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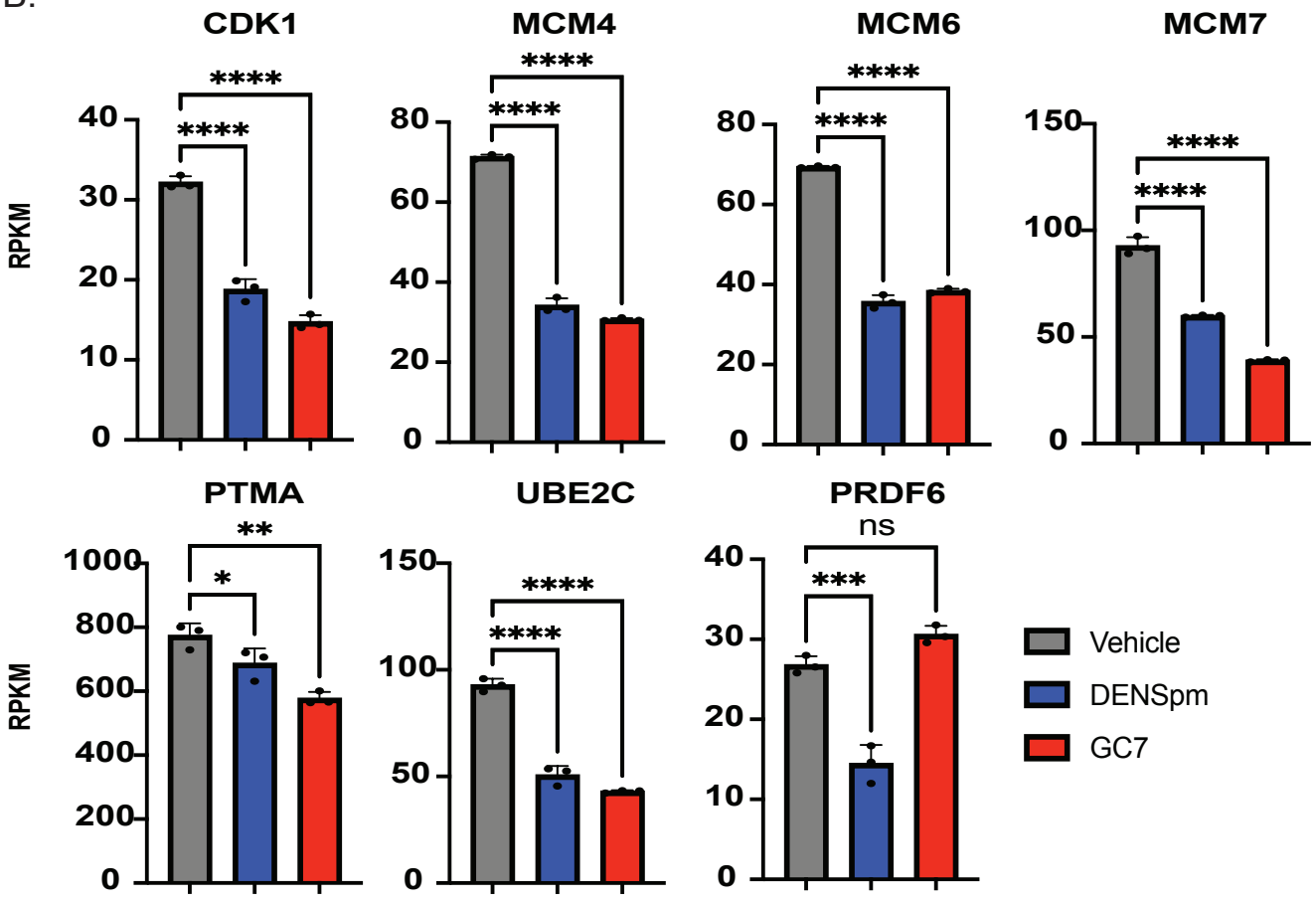


Supplemental Figure 5

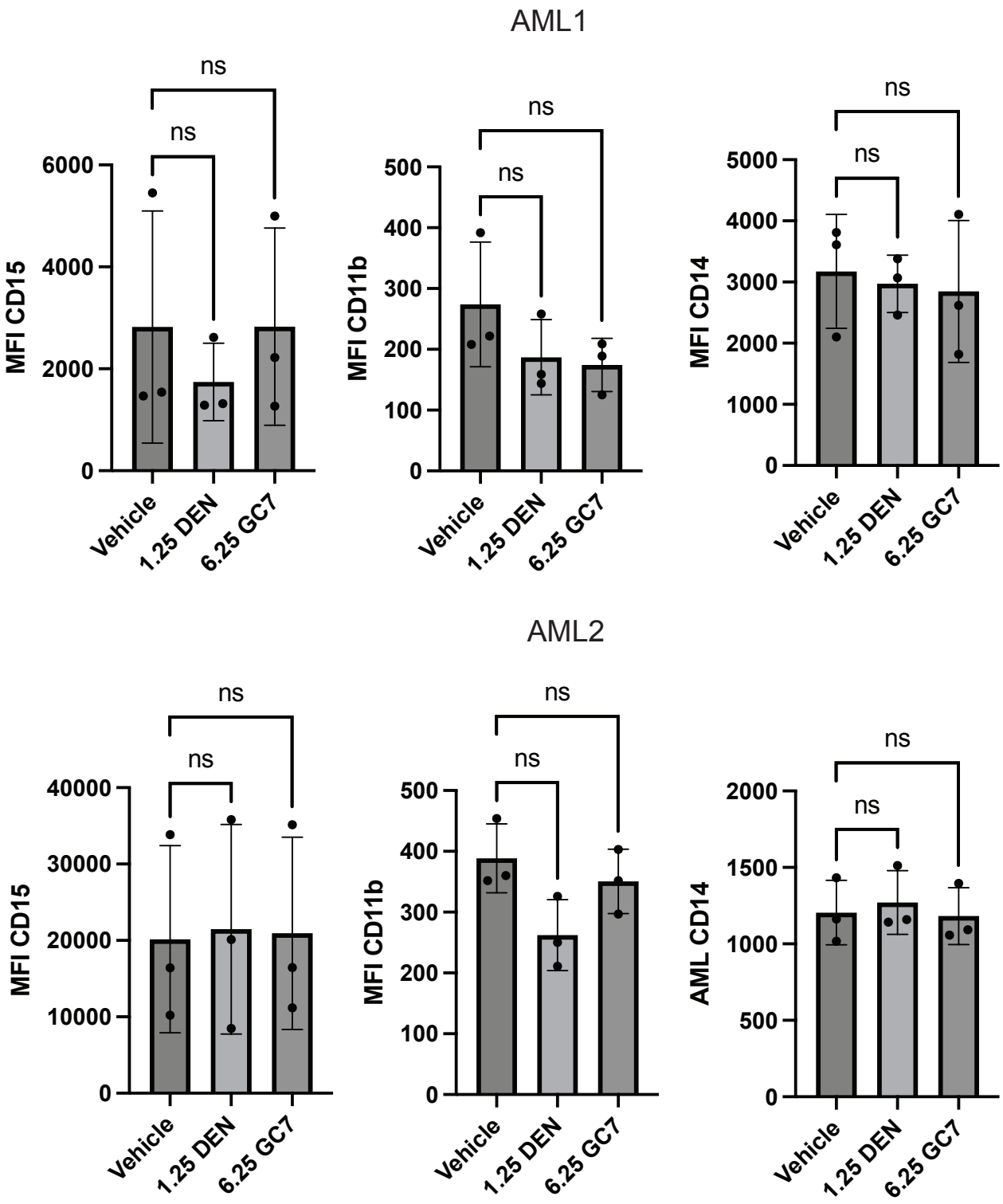
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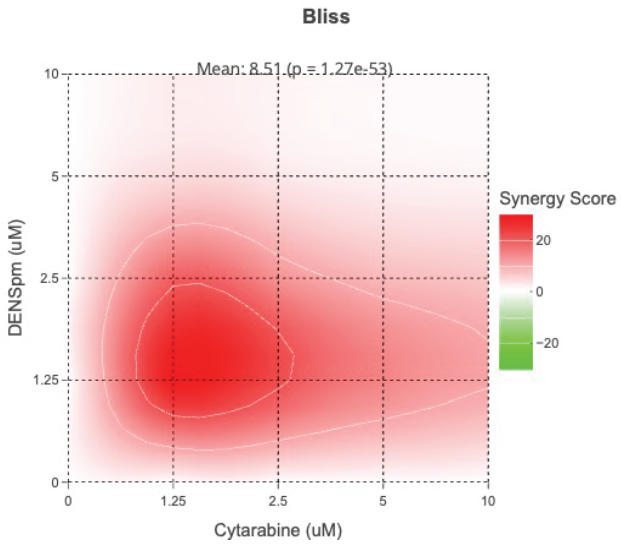


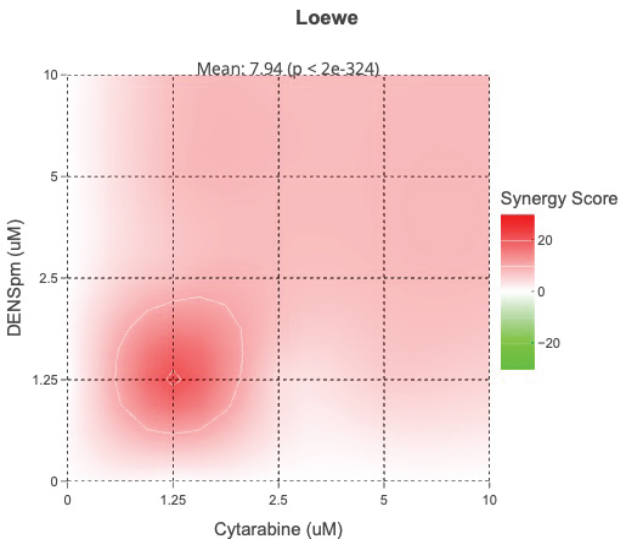
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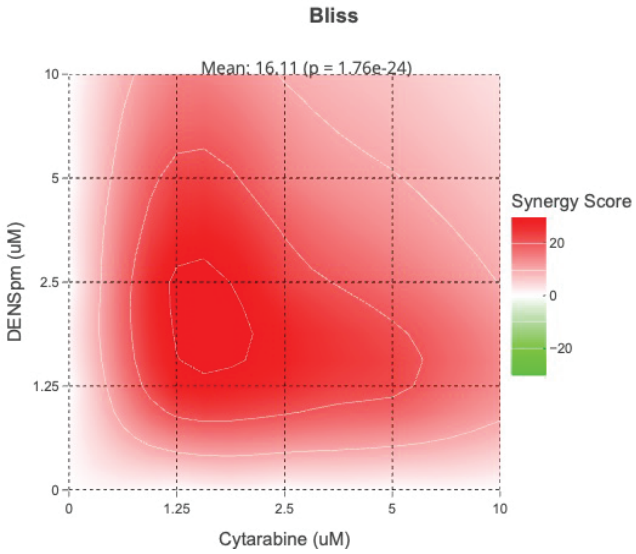
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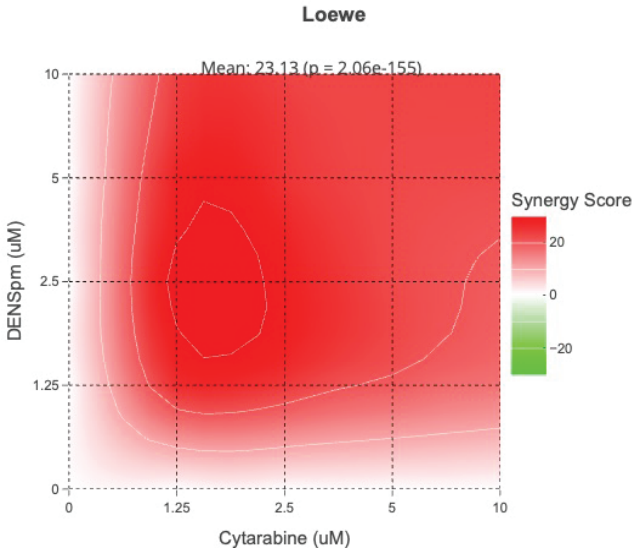
AML1





AML2





AML3

