Comprehensive Transcriptome Profiling of Cryptic *CBFA2T3-GLIS2* Fusion-positive AML Defines Novel Therapeutic Options – A COG and TARGET Pediatric AML Study

Supplemental Figures

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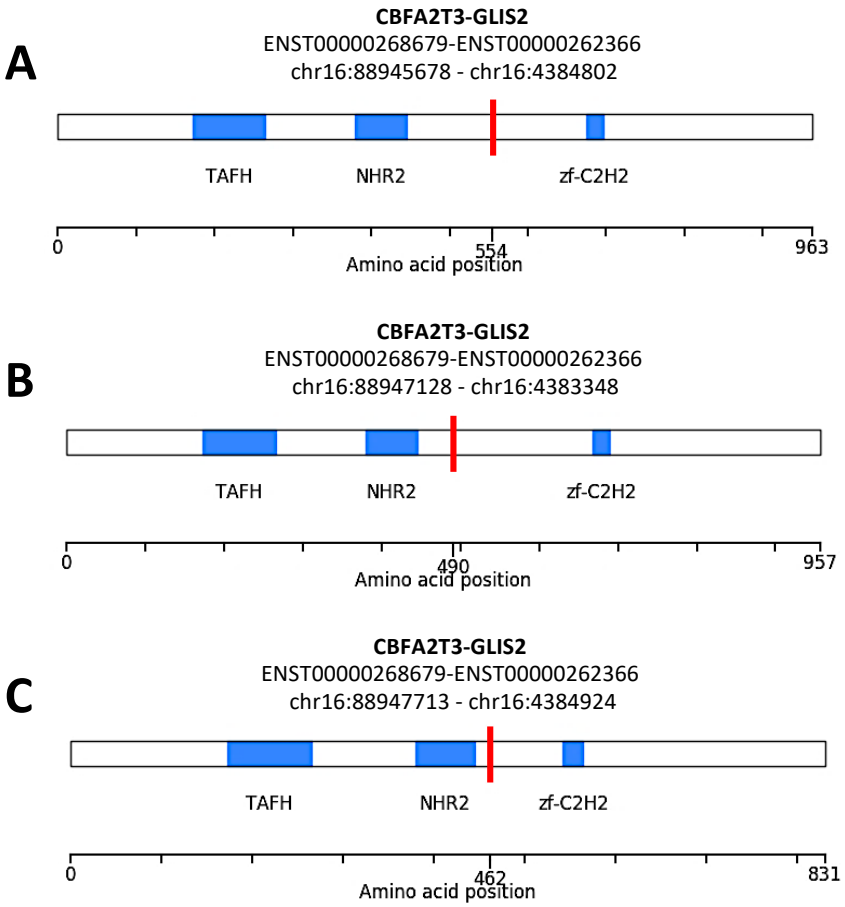
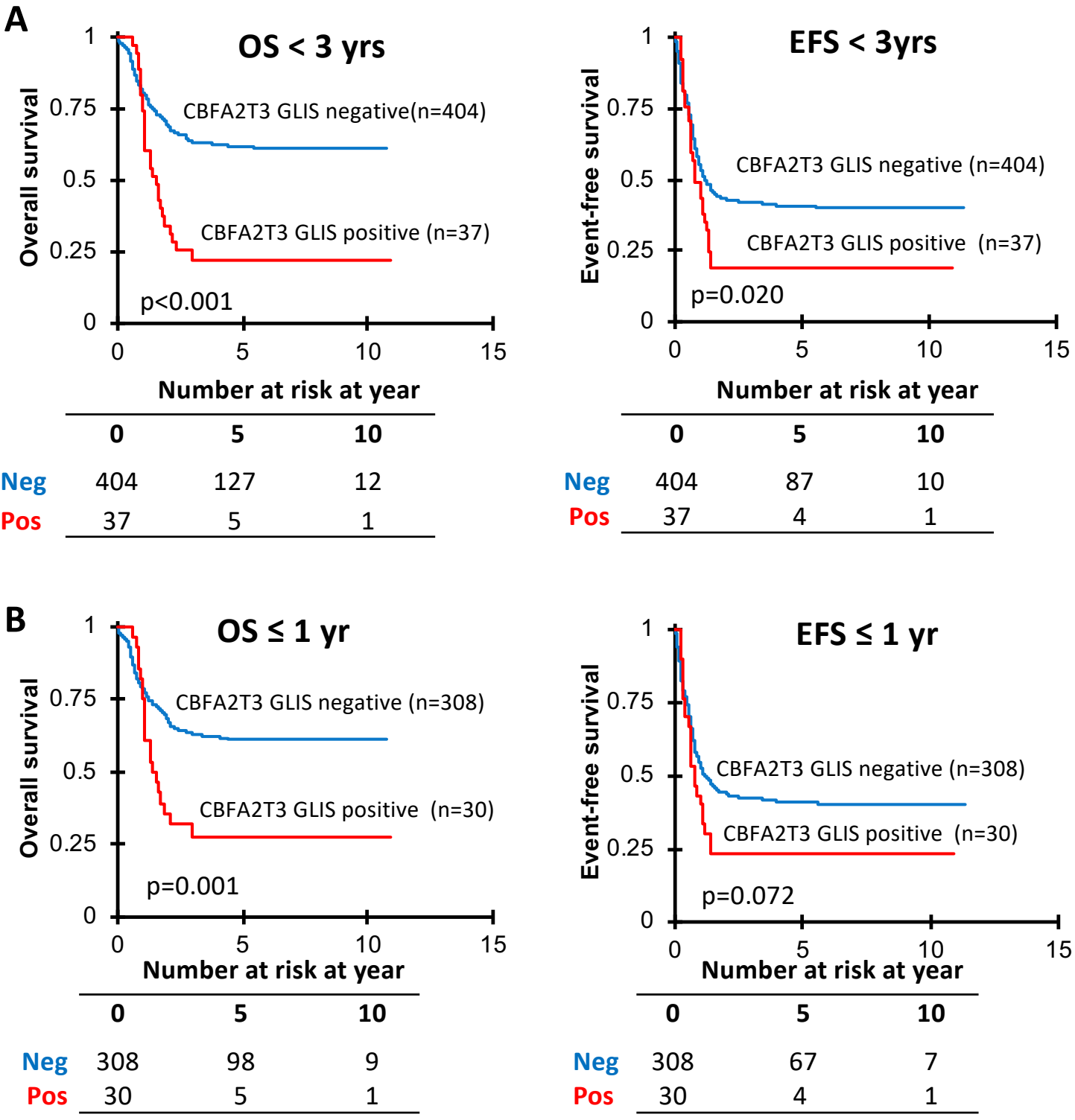
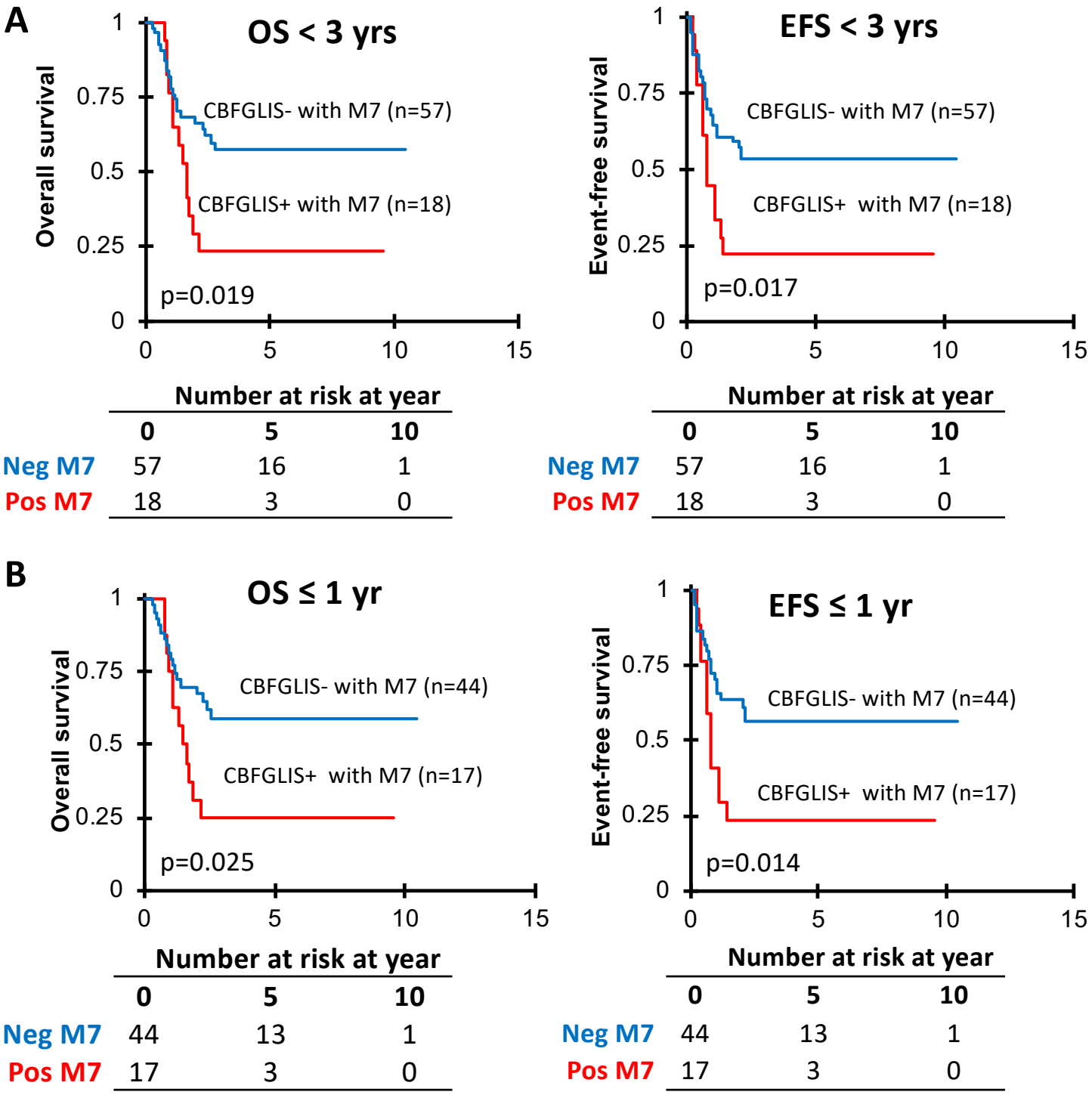


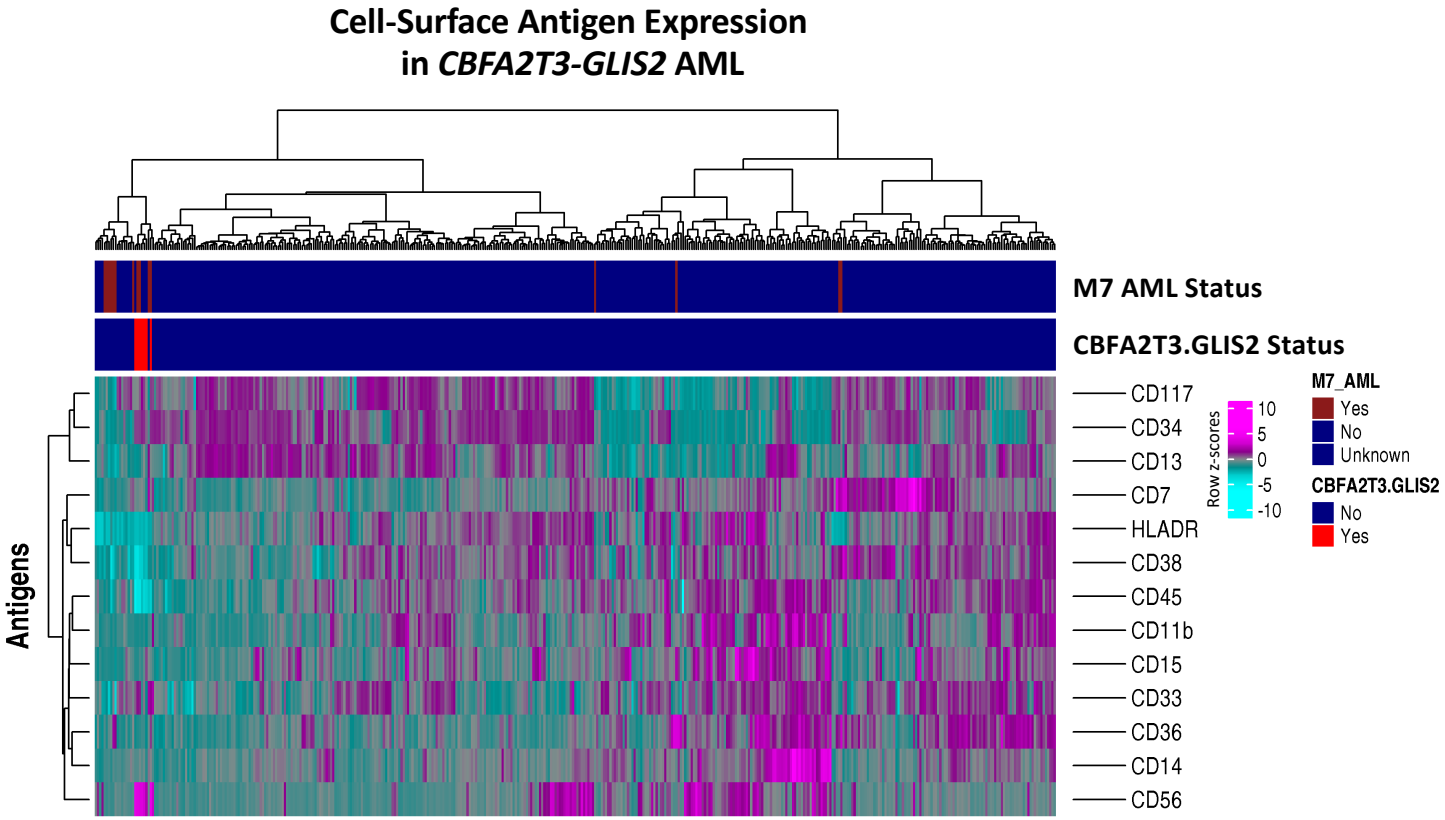
Supplemental Figure 1. Schematic of fusion proteins for each unique *CBFA2T3-GLIS2* breakpoint detected by RNA-sequencing. A) The most common breakpoint and **B-C)** the two non-typical breakpoints detected are represented. The breakpoint position is denoted by the red vertical line.



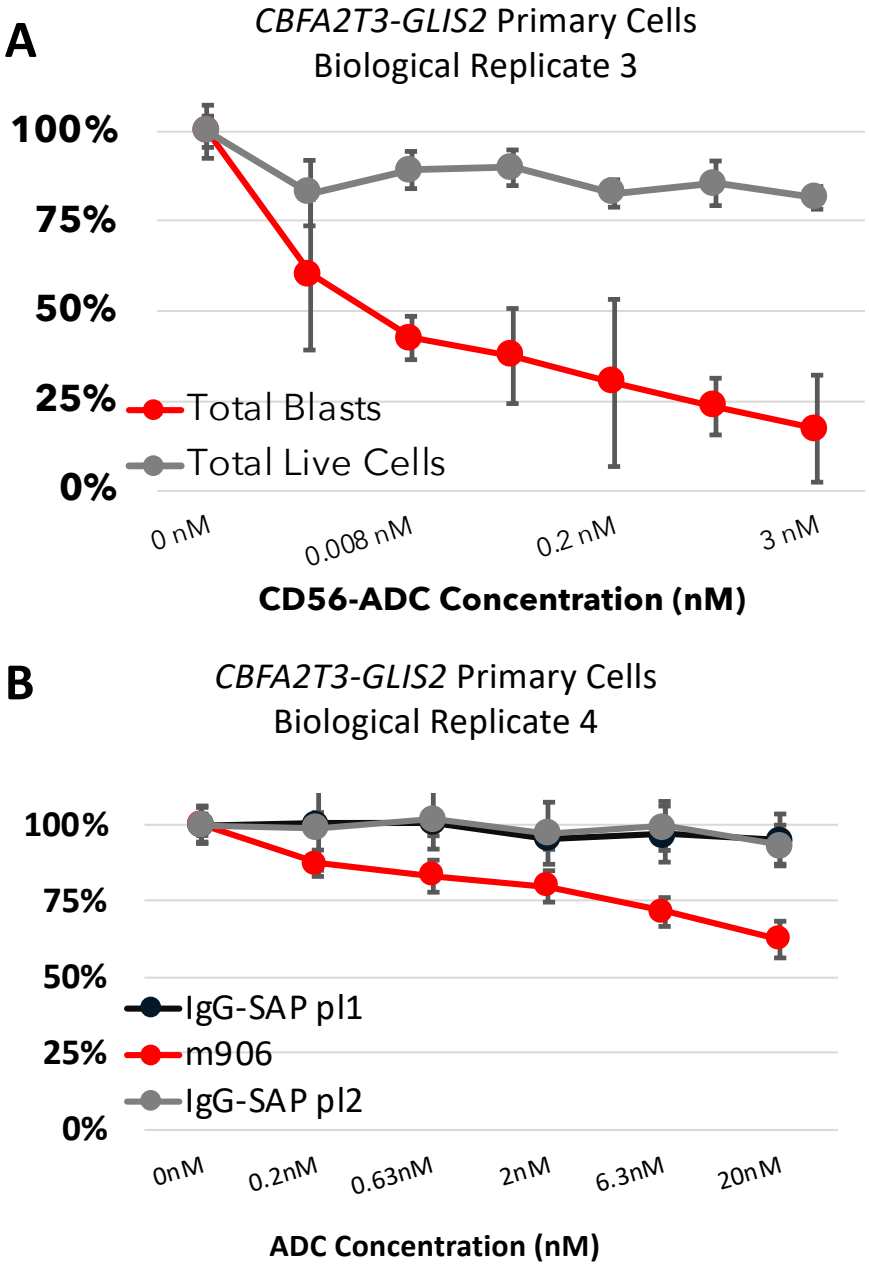
Supplemental Figure 2. Overall Survival and Event-free survival in infant AML with and without the *CBFA2T3-GLIS2* Fusion. A) Kaplan-Meier curves demonstrating *CBFA2T3-GLIS2* AML patients (< 3 years old) have an adverse outcome compared to fusion-negative cohorts who are < 3 years old and **B)** similarly, *CBFA2T3-GLIS2* who are ≤ 1 year old compared to age matched heterogenous fusion negative population.



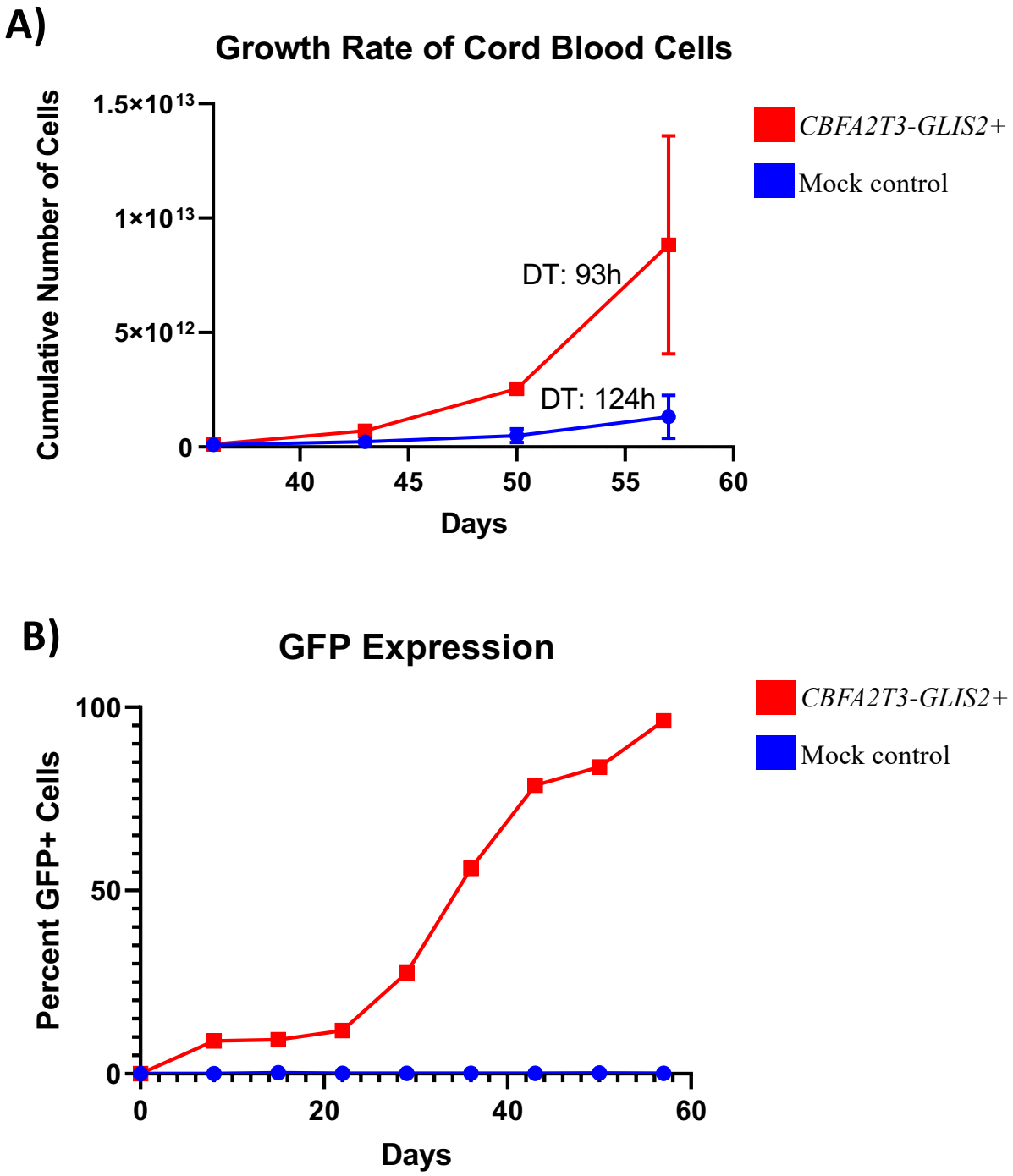
Supplemental Figure 3. Overall Survival and Event-free survival in infant M7 (megakaryoblastic) AML with and without the *CBFA2T3-GLIS2* Fusion. A) Kaplan-Meier curves demonstrating M7 *CBFA2T3-GLIS2* AML patients (< 3 years old) have an adverse outcome compared to fusion-negative M7 cohorts of the same age. **B)** *CBFA2T3-GLIS2* cases ≤ 1 year old who have M7 morphology have poor outcomes for OS and EFS compared to heterogenous M7 fusion-negative patients.



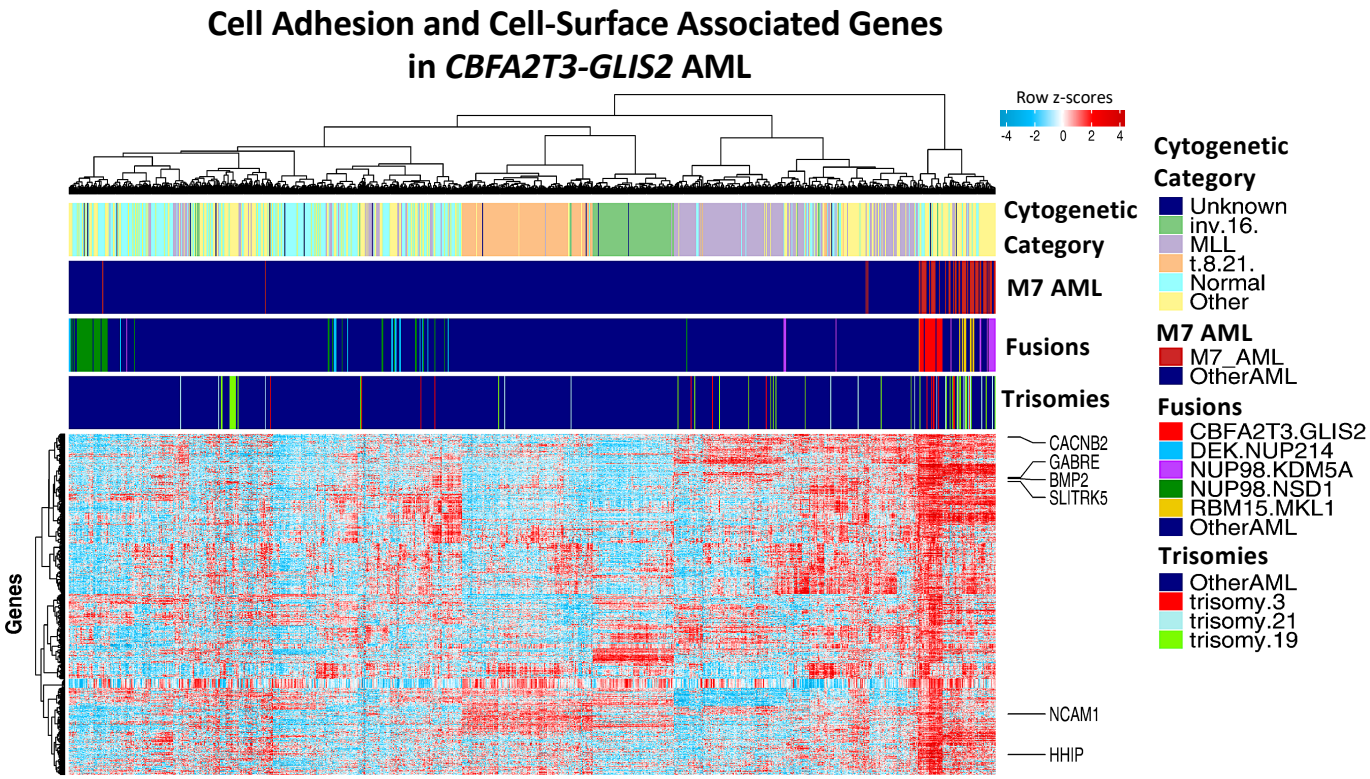
Supplemental Figure 4. Fusion-positive cases display a unique immunophenotype. Unsupervised hierarchal clustering of mean fluorescent intensity (MFI) values from 13 cell surface antigens in the pediatric AML cohort AAML0531 (N = 437). *CBFA2T3-GLIS2* AML (N=7) cluster closely together and exhibit high expression of CD56, and dim to absent expression of HLADR, CD38, and CD45, a pattern representative of RAM phenotype.



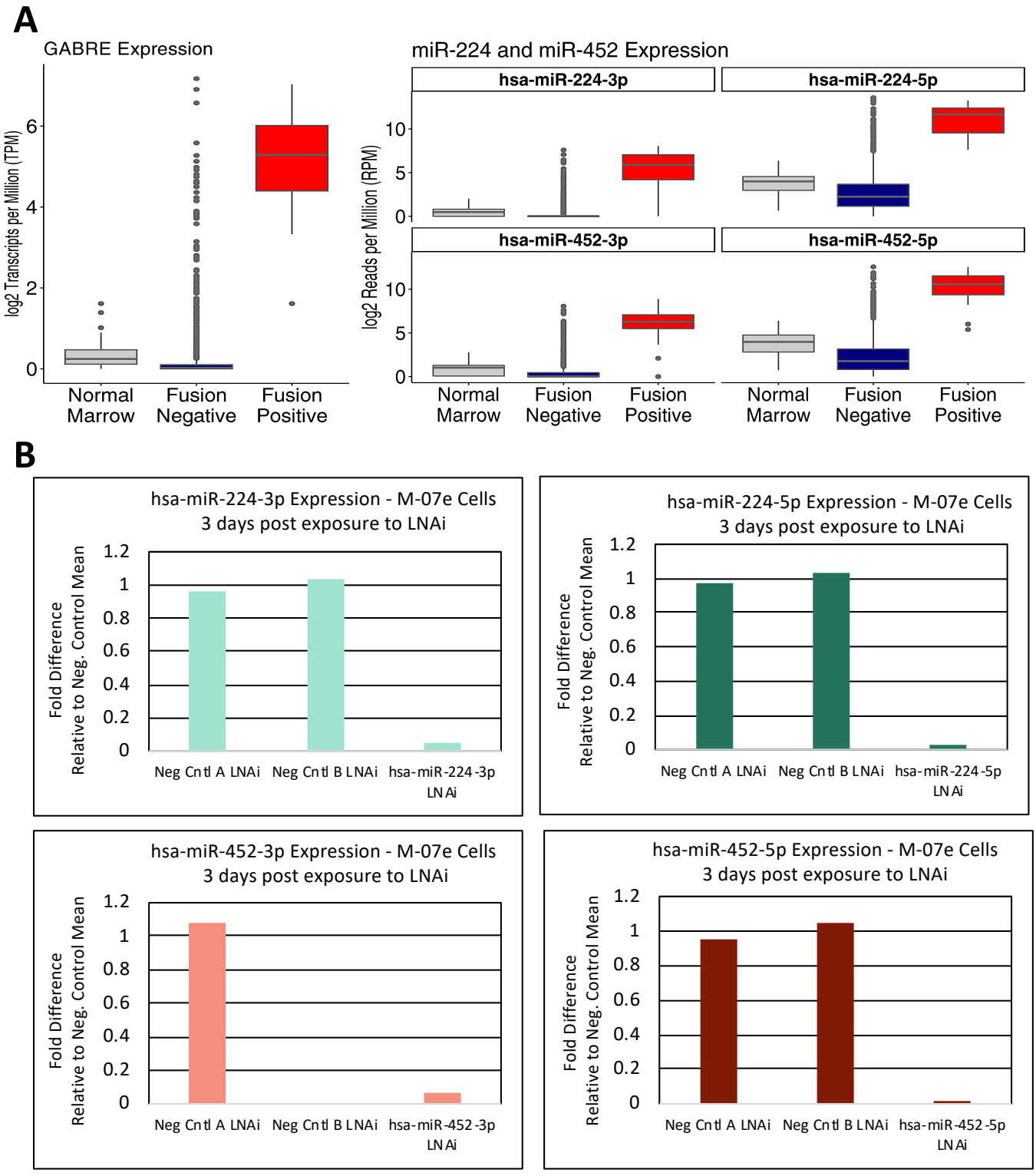
Supplemental Figure 5. The CD56 anti-body drug conjugate (ADC) exhibits potent cytotoxicity. A) An additional *CBFA2T3-GLIS2* patient primary sample exposed to varying concentrations of the CD56-ADC. **B)** Cytotoxicity assay with *CBFA2T3-GLIS2* primary cells exposed CD56-ADC or the non-targeting conjugate control, IgG-SAP ADC.



Supplemental Figure 6. *CBFA2T3-GLIS2* confers enhanced proliferative potential in long-term culture of cord blood cells. **A)** Transduced human CD34+ cord blood stem cells (CBSC) with a lentivirus encoding the *CBFA2T3-GLIS2* fusion transcript and GFP (red) showed increased cumulative cell number compared to mock control (blue). **B)** Proportions of GFP+ and CD34+ cells are shown for *CBFA2T3-GLIS2*+ cultures and mock control. Error bars denote standard deviation of duplicates.

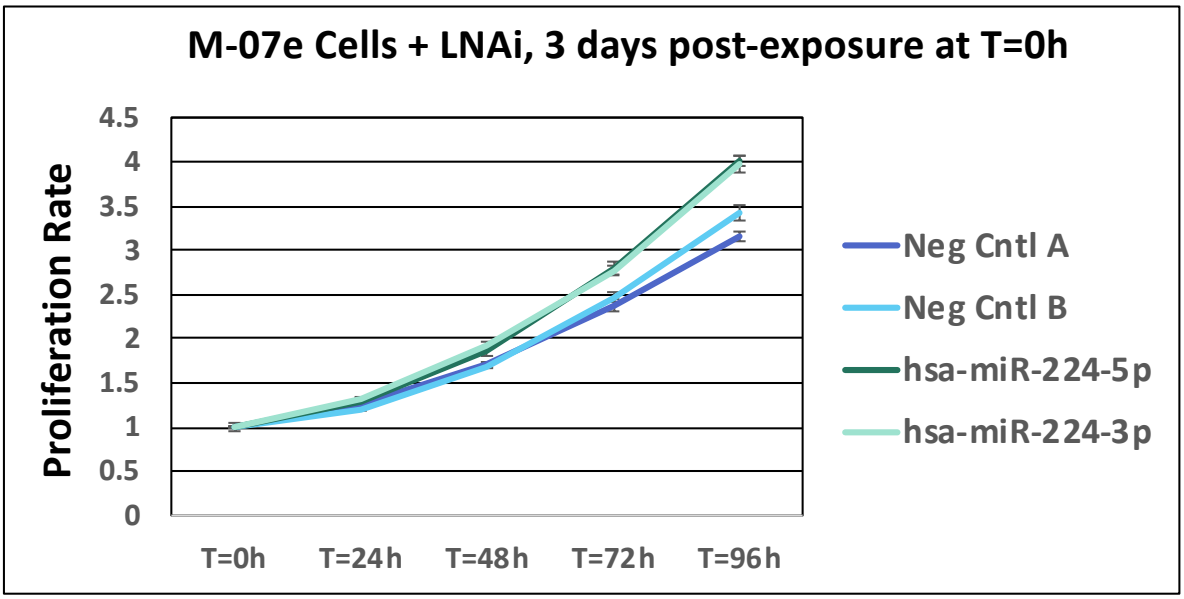


Supplemental Figure 7. *CFBA2T3-GLIS2* patients highly over-express cell surface, cell adhesion and extracellular matrix associated genes. Unsupervised hierarchal clustering of 1,049 AML patients RNA-sequencing data. Gene included are significantly up-regulated cell surface, cell-adhesion, and extracelluar matrix gene markers in *CBFA2T3-GLIS2* AML (789 genes with log2 old-change > 1.0 and FDR < 0.001).). Color bars indicate primary cytogenetic code, M7 AML classification, fusion status (*CBFA2T3-GLIS2* in red), and trisomies associated with CBFA2T3-GLIS2. Labeled genes are highly over-expressed members of TGFB/BMP, Hedgehog, and NCAM1 interaction pathways (*BMP2, HHIP*, *NCAM1* and *CACNB2*), as well as two gene targets (*SLITRK5* and *GABRE*) with miRNA associations.



Supplemental Figure 8. Efficiency of LNA Knockdown of miR-224 and miR-452 microRNAs in MO7E cell-lines. A) *CBFA2T3-GLIS2* fusion-positive primary patient samples have increased transcript expression of *GABRE*, and the mature miRNAs *miR-224* and *miR-452,* which are transcribed from *GABRE* intronic regions. **B)** Knockdown of *miR-224-5p/3p* and *miR-542-5p/3p* expression by LNA miRNA inhibitors resulted in > 90% knockdown efficiency at 72 hours post-exposure measured by quantitative PCR.

A



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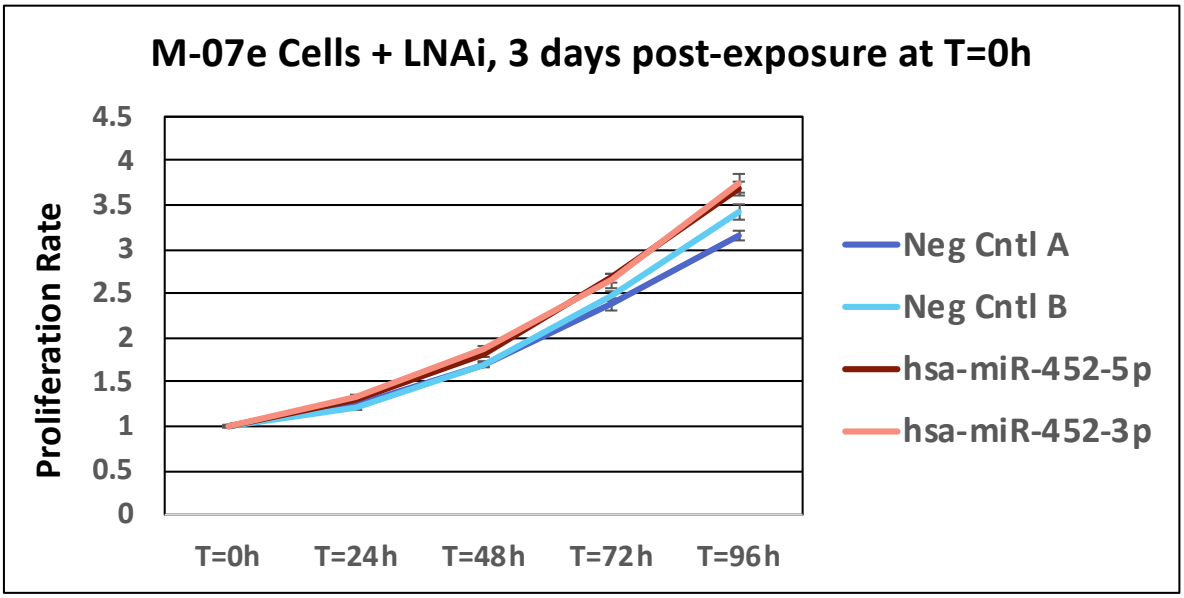
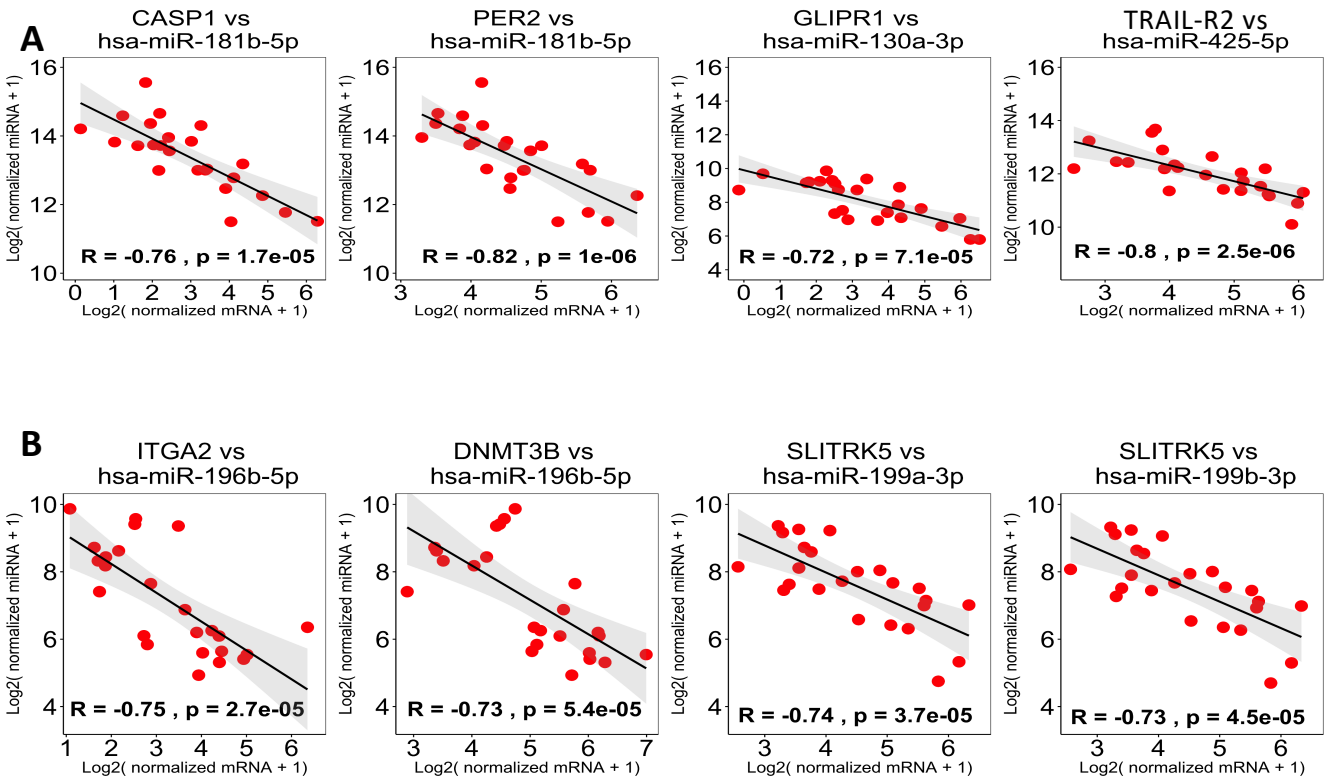


Supplemental Figure 9. LNA Knockdown of miR-224 and miR-452 shows a subtle increase in proliferation in MO7E cells. A) Proliferation rates of *CBFA2T3-GLIS2* positive MO7E cultures were assessed for 4 days following exposure to LNA miRNA inhibitors directed against *hsa-miR-224-5p* or *hsa-miR-224-3p*, as well as **B**) *hsa-miR-452-5p* or *hsa-miR-452-3p*. Proliferation rates in knockdown conditions were compared to negative control A (p=0.001) and control B (p=0.009) after 96-hours.



Supplemental Figure 10. MicroRNA-mRNA interactions in *CBFA2T3-GLIS2* AML involved with tumor suppressor and apoptotic functional roles. Scatter plots of mRNA-miRNA interacting partners demonstrating anti-correlation of expression for **A)** apoptotic and tumor suppressor genes identified as significantly down-regulated in *CBFA2T3-GLIS2* AML with the over-expressed miRNAs, or **B)** significantly down-regulated tumor suppressor miRNAs whose gene partners were significantly up-regulated in the fusion-positive cohort.