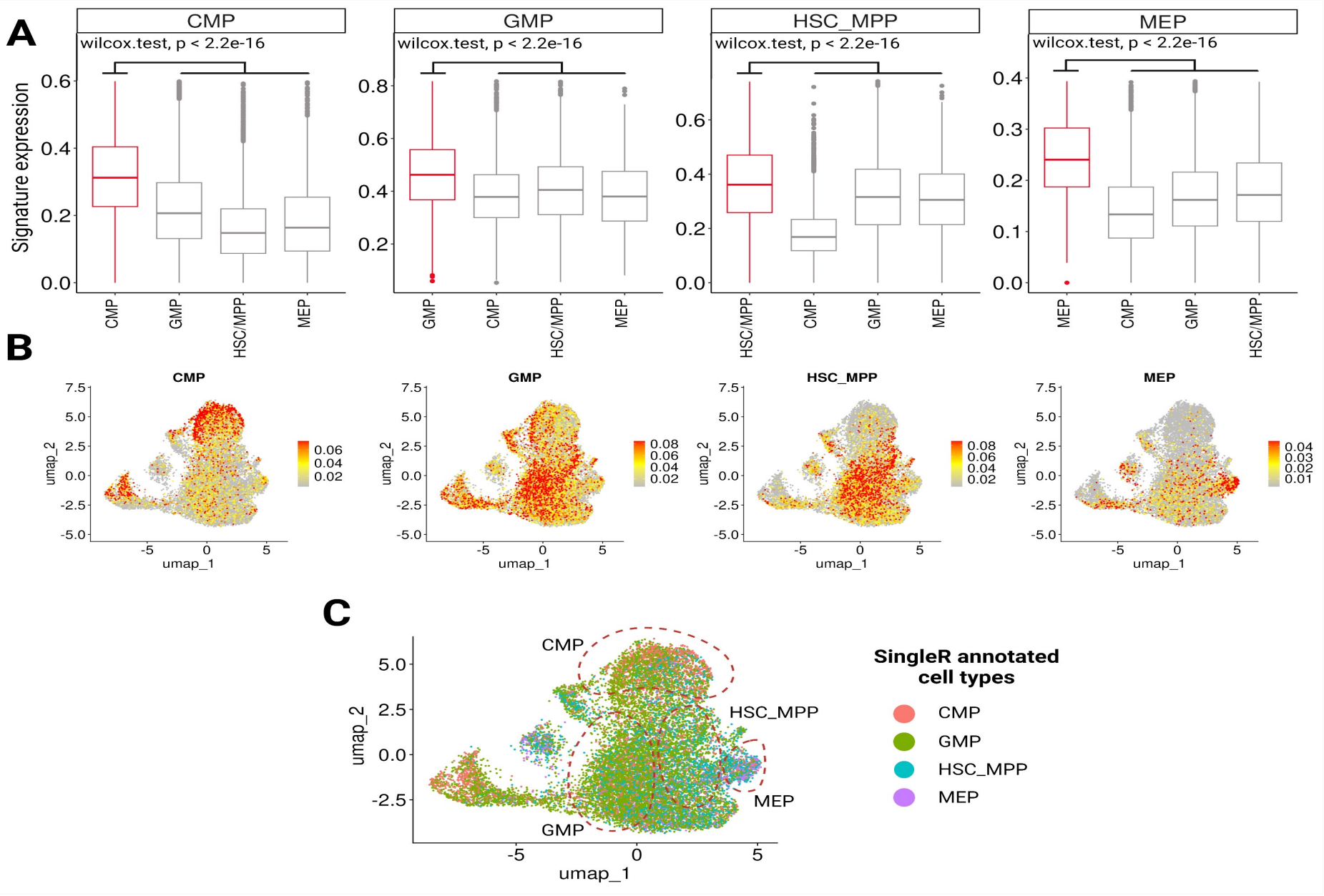
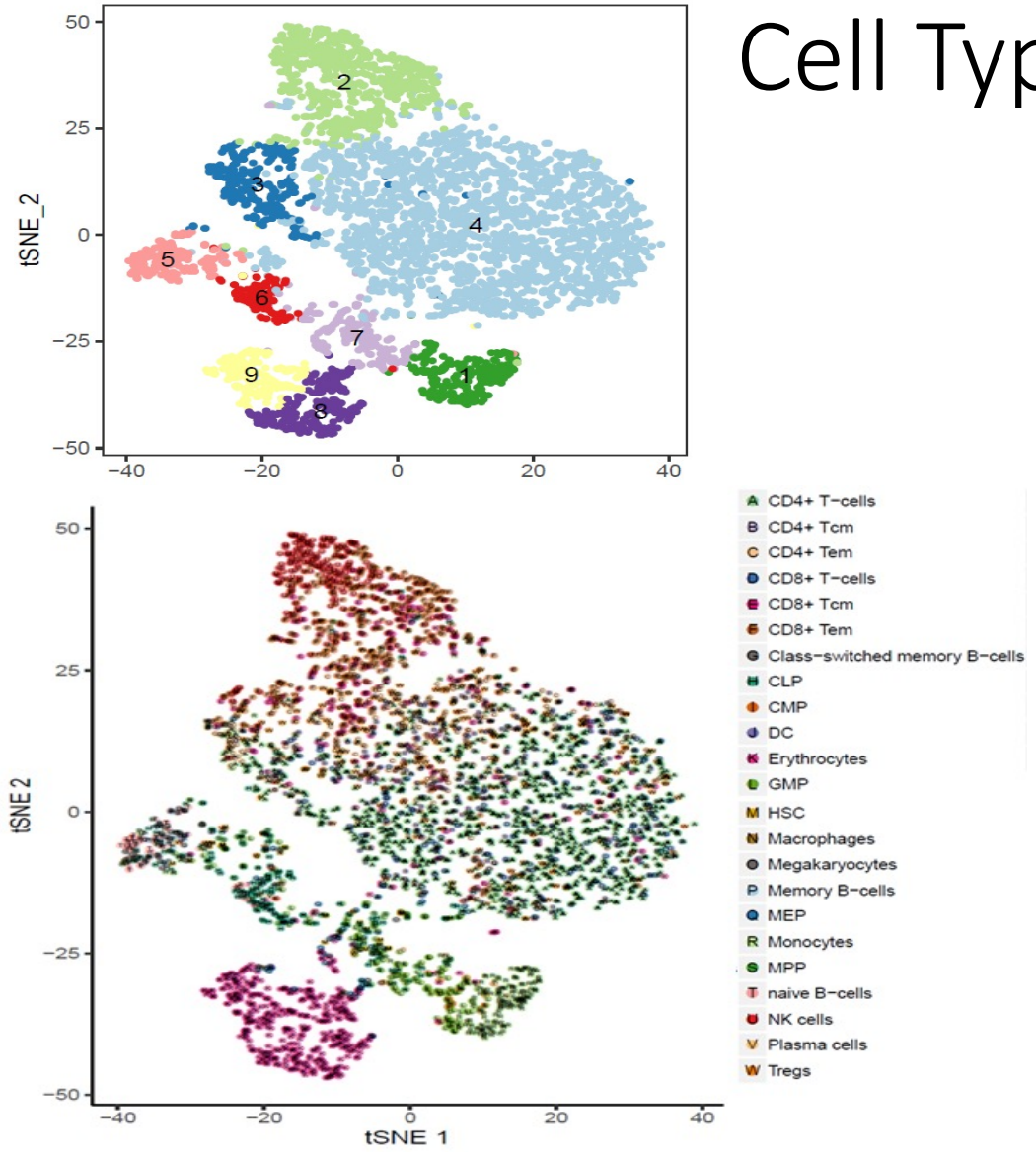
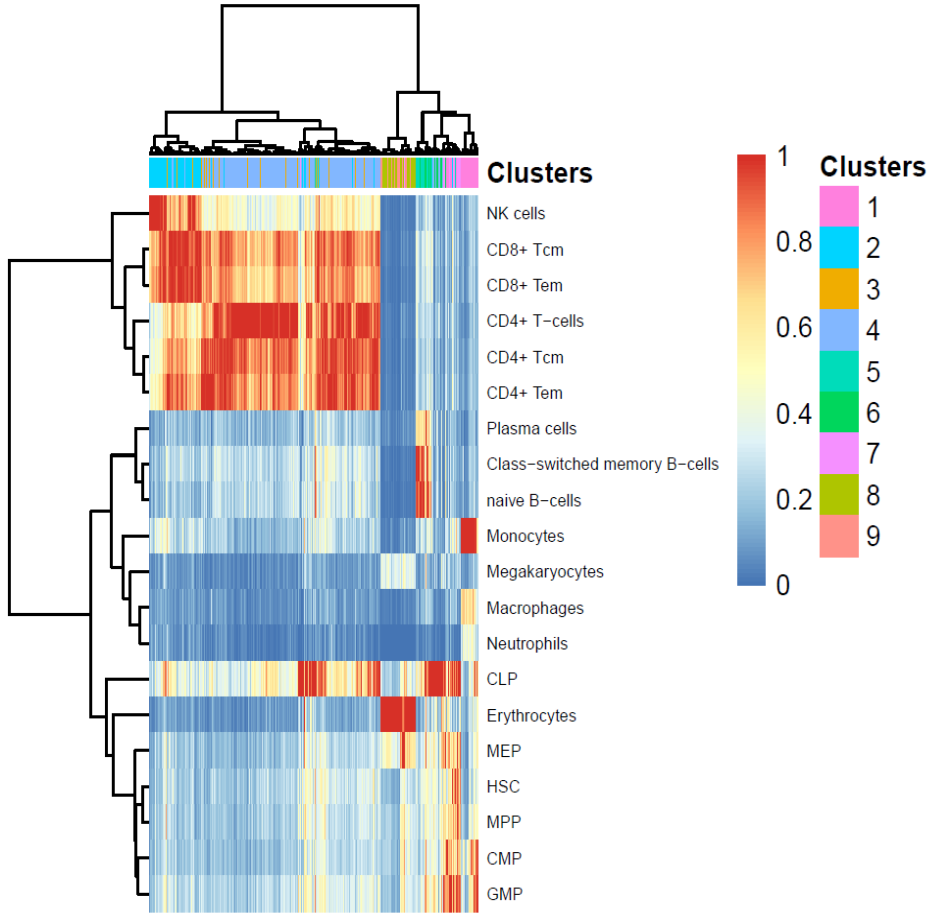


Supplementary Figure S1: SingleR annotation validation in myeloid progenitor cells.
(A) Each facet shows the expression of a cell-type signature (Signature expression) from sorted CMP, GMP, HSC_MPP, and MEP cells (Y-axis, facet labels), across SingleR annotated single-cells (individual boxes). (B) Same data as in A, visualized at the single-cell level depicting the similarity of cells with high respective signature expression levels.
(C) Integrated UMAP with cells colored by SingleR-annotated cell types, highlighting regions enriched in cells annotated with a specific type.

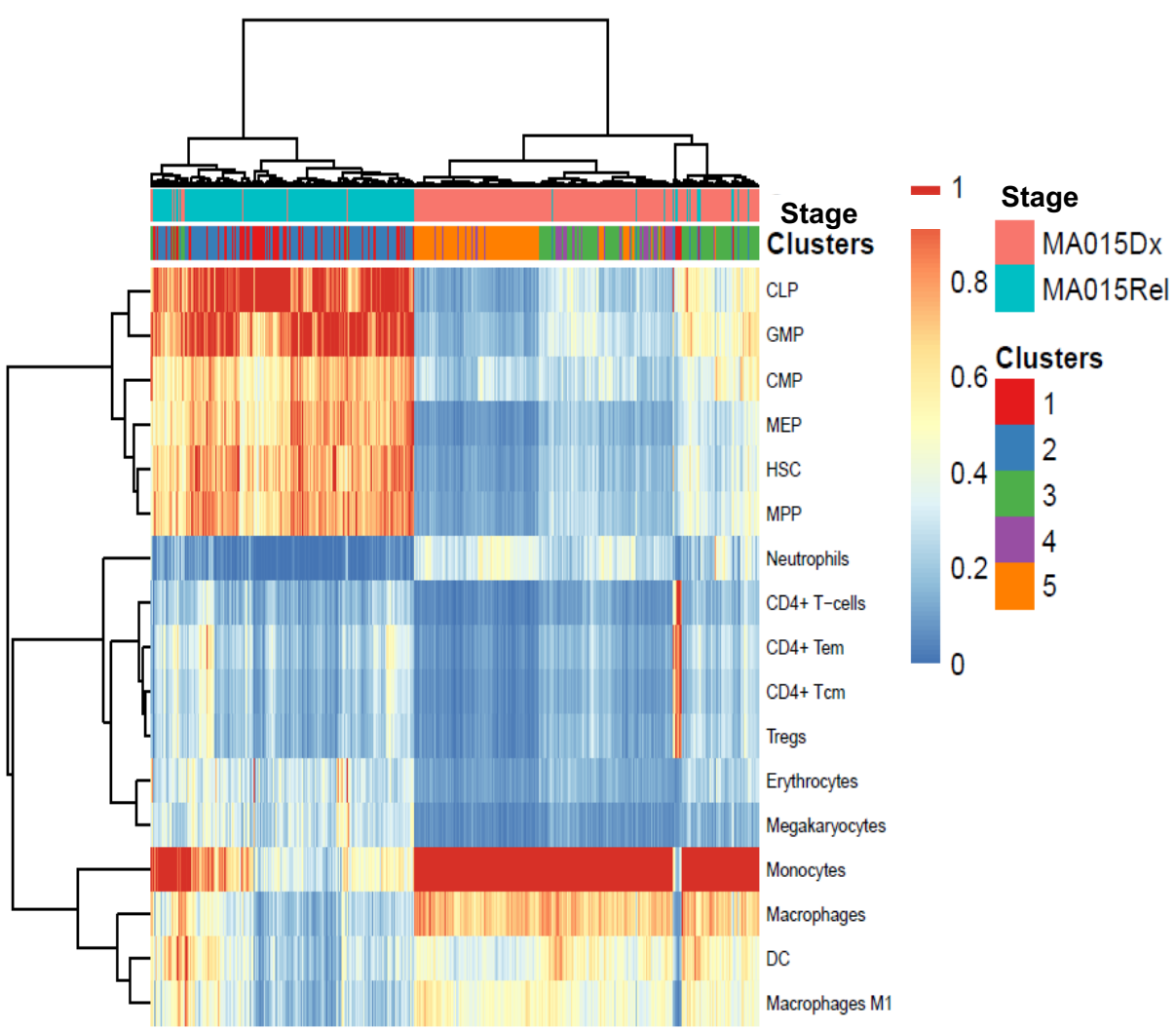
Cell Type Assignment for NBM pool

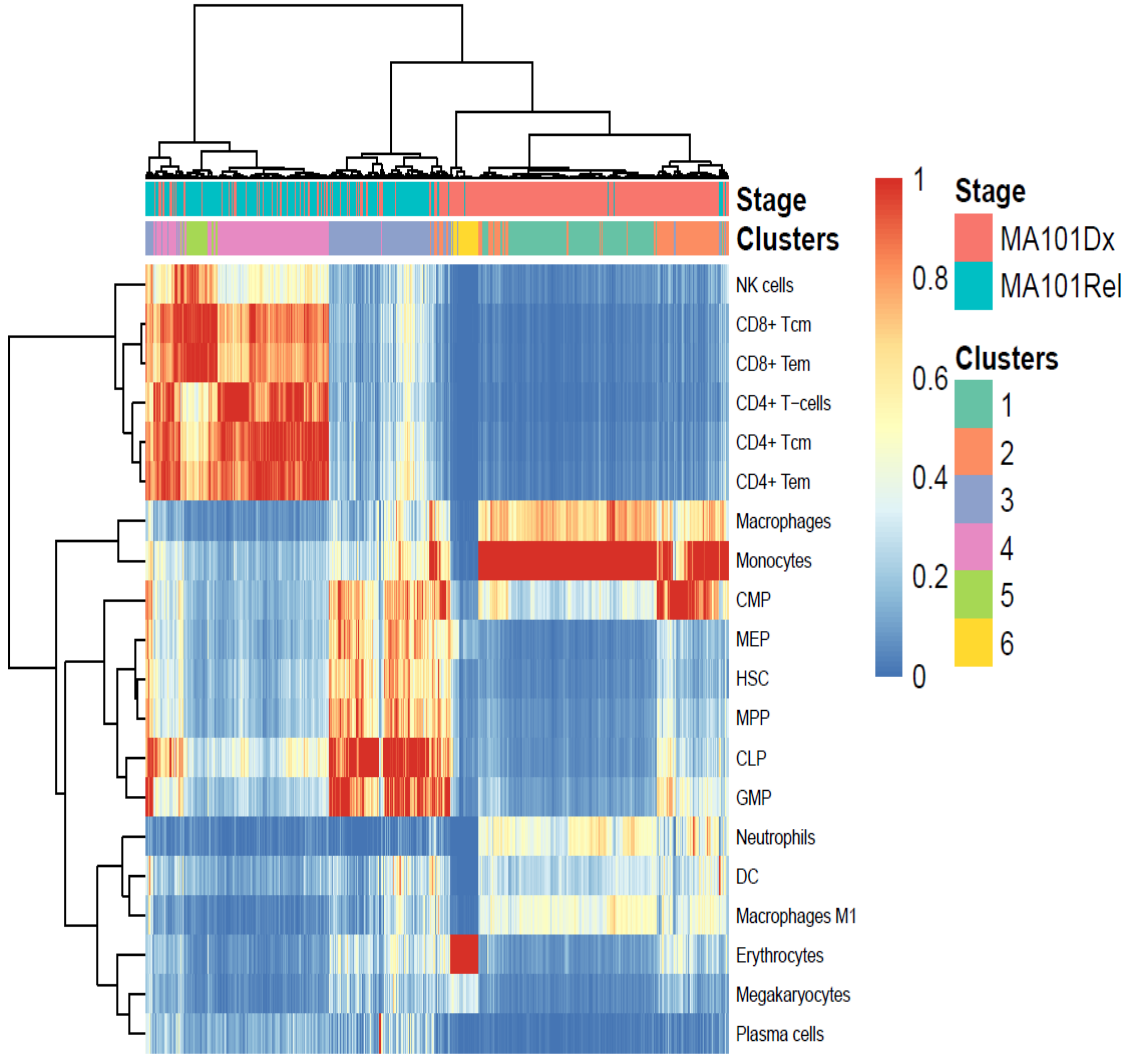




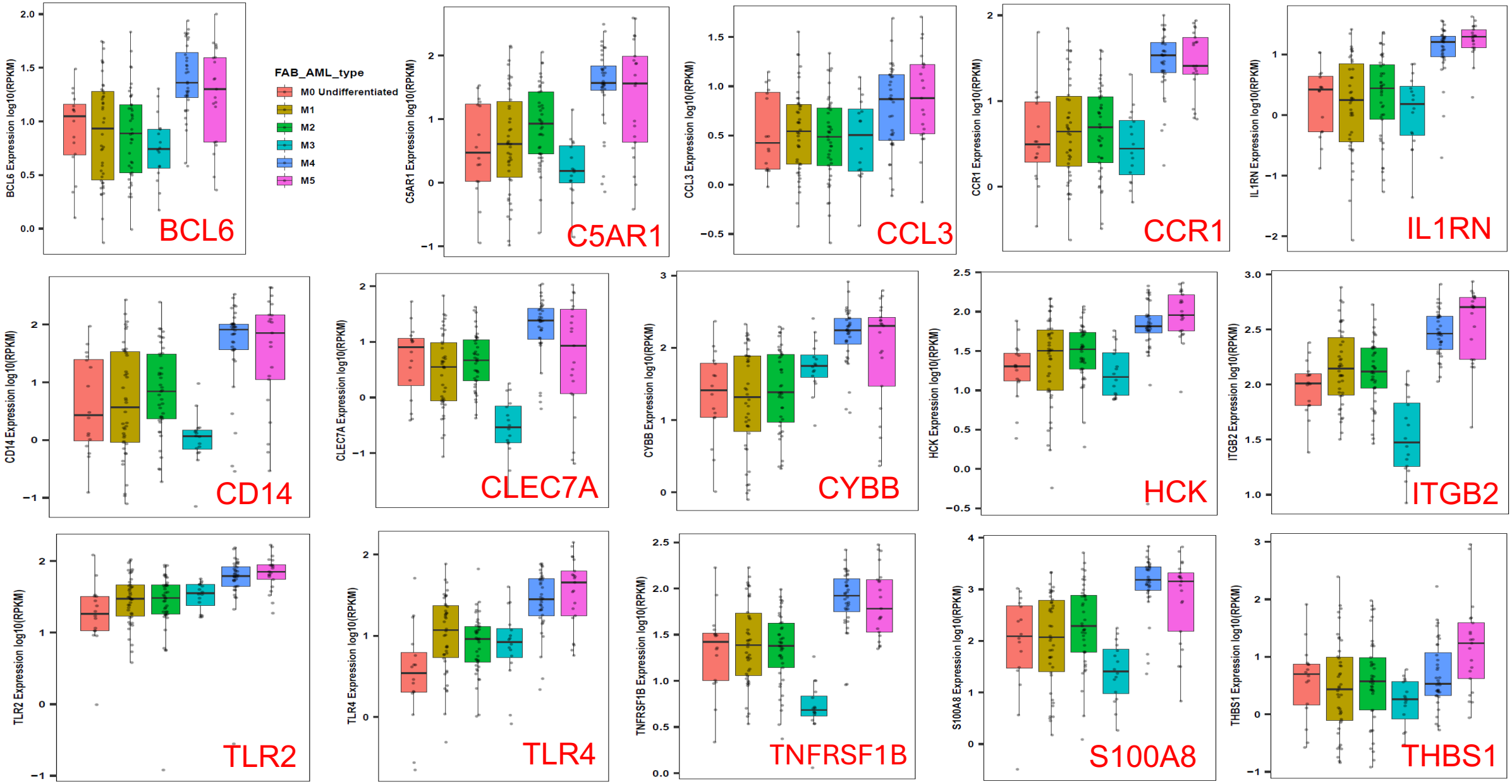
Supplementary Figure S2: Cell type assignment in pooled normal human bone marrow based on single cell gene expression correlation analyses.
Left: t-SNE displays of cell clusters of pooled scRNA-seq data from five normal human bone marrows and cell type assignment. Cell type assignment as described in the *methods* using SingleR and sorted progenitor fractions as in Blueprint and ENCODE data. Right: Heatmap of statistical gene expression correlation analyses and cell type assignment. Red: high identity; Blue: low identity. The t-SNE clusters assignment for individual cells is at the top and the color code is on the right.

Assigning Cell Types for Dx-Rel Pairs in AML monoblast/ monocyte-rich AML at Diagnosis

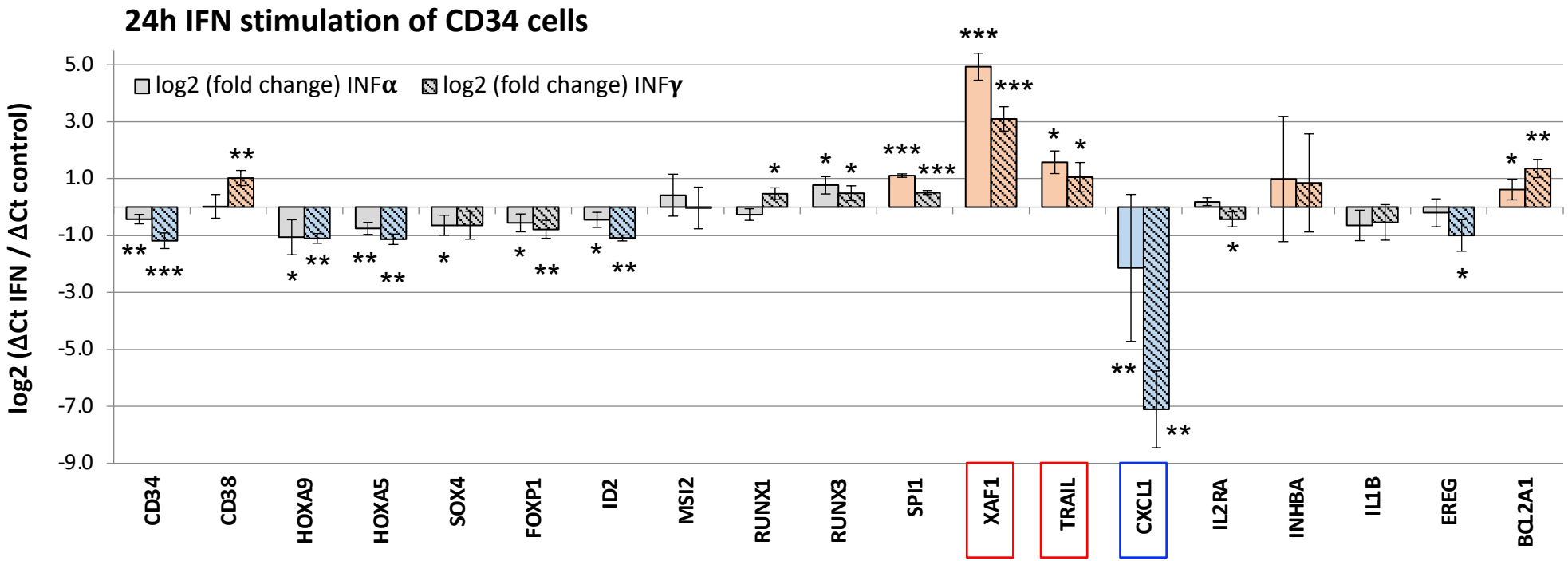




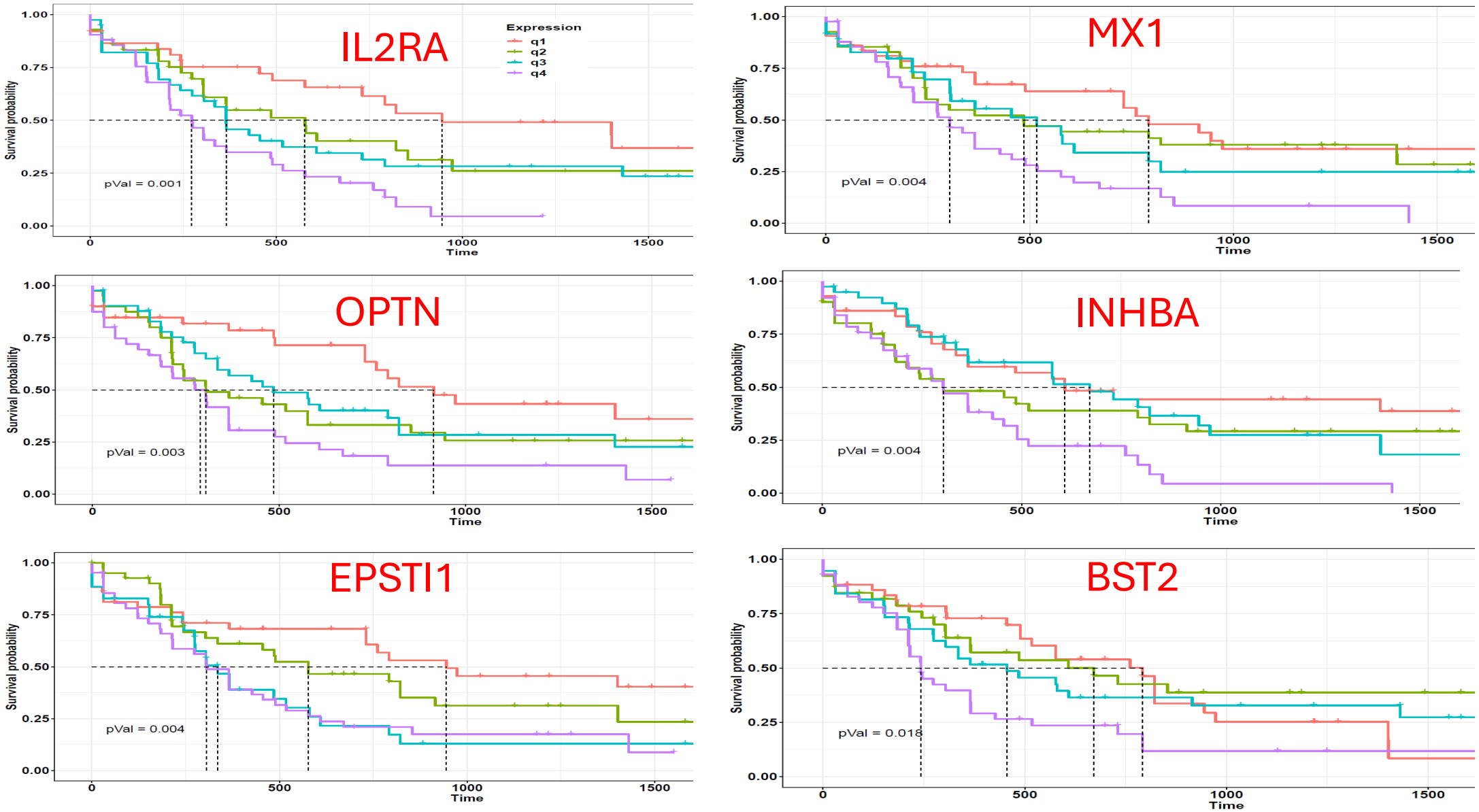
Supplementary Figure S3: Examples of cell type assignment of two mature monoblast/ monocyte-rich AML cases using statistical gene expression correlation analyses and resulting heatmaps. The stage of the disease is color-coded at the top. A color-coded cluster assignment is provided. Red: high identity; Blue: low identity.
The t-SNE cluster assignment for individual cells is at the top and the color code is on the right.



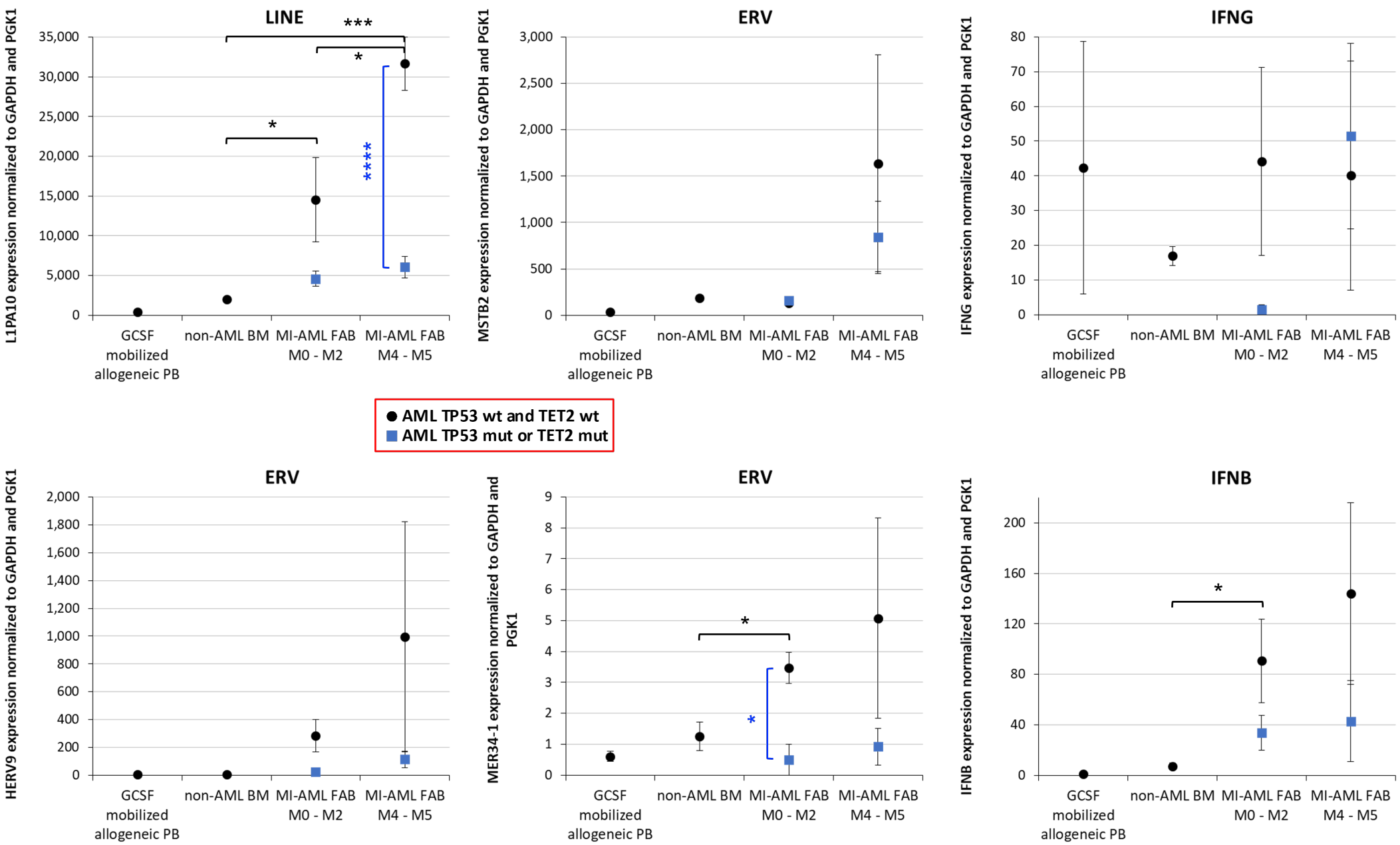
Supplementary Figure S4: Expression of inflammation associated genes in AML TCGA RNA sequencing data grouped by FAB types M0-M5.



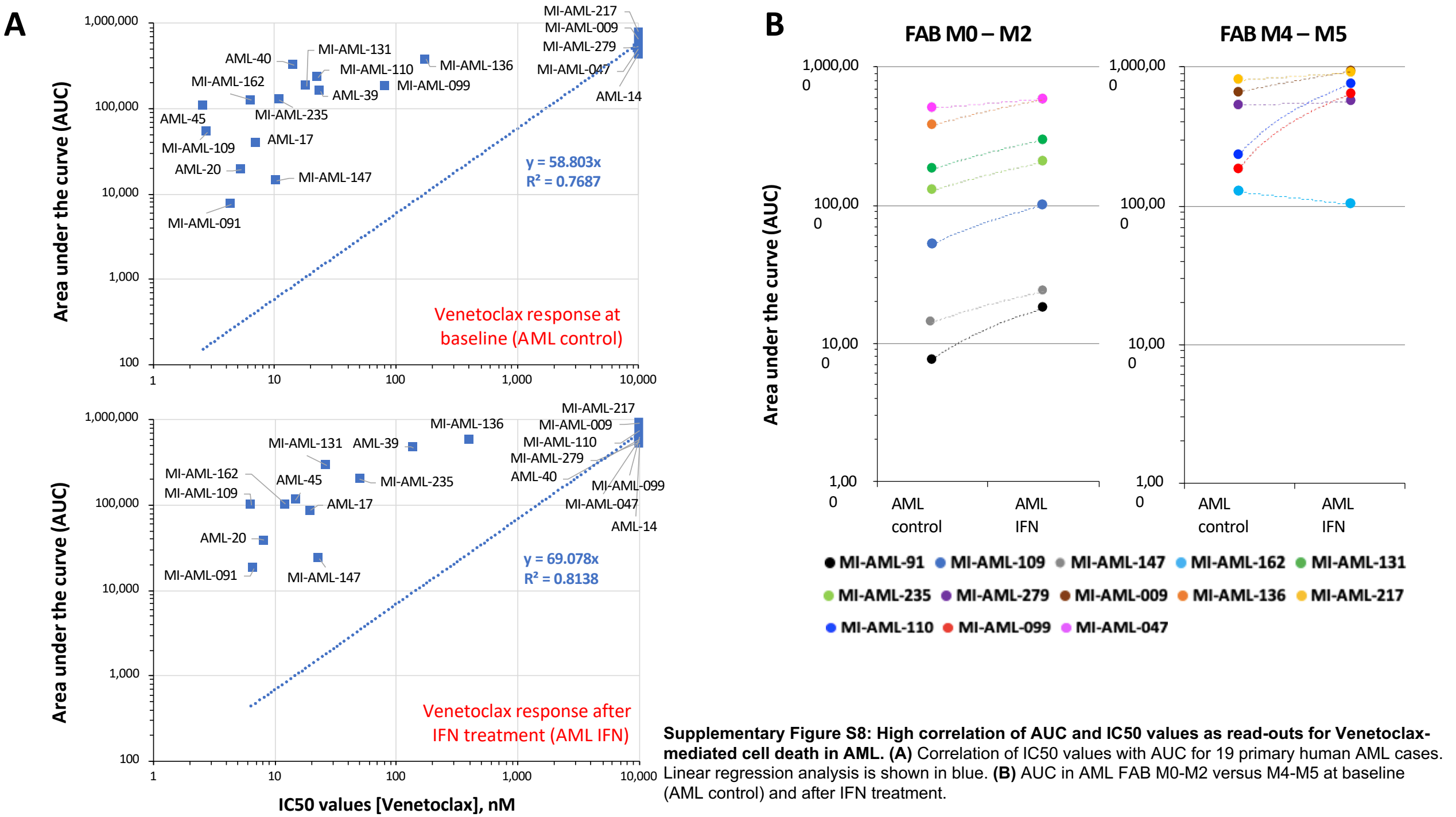
Supplementary Figure S5: Selected target gene expression in primary human CD34+ cells after 24h IFN stimulation (qPCR).
Purified human CD34+ cells were treated for 24h with 1,000 I.U. IFNα or IFNγ in parallel to untreated control samples, respectively. Log_2 fold change of target gene expression of IFN stimulation normalized to internal control genes (GAPDH and PGK1) are displayed as mean values ± SEM. Stars indicate the results of a paired two-tailed t-test (*p<0.05, **p<0.01, ***p<0.001).



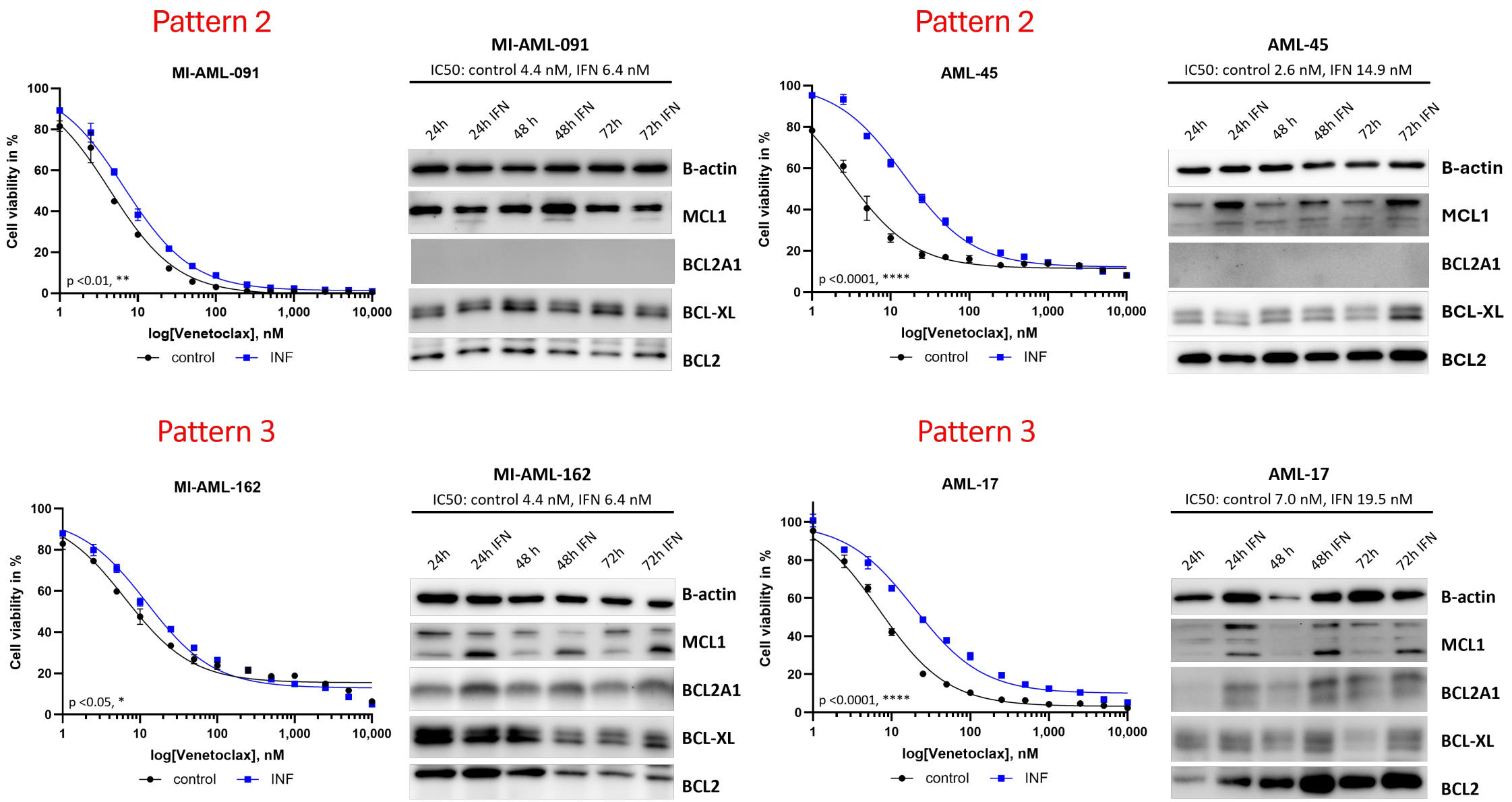
Supplementary Figure S6: Poor survival of AML patients expressing IFN-induced genes (KM-plots). RNA-seq data for individual genes from the AML TCGA dataset were partitioned into quartiles (q1 lowest, q4 highest) and the survival of AML patients in the quartiles was computed using the KM method. Very adverse day 1,000 survival for patients with the highest gene expression (q4, purple) is evident. log-rank test FDR corrected.



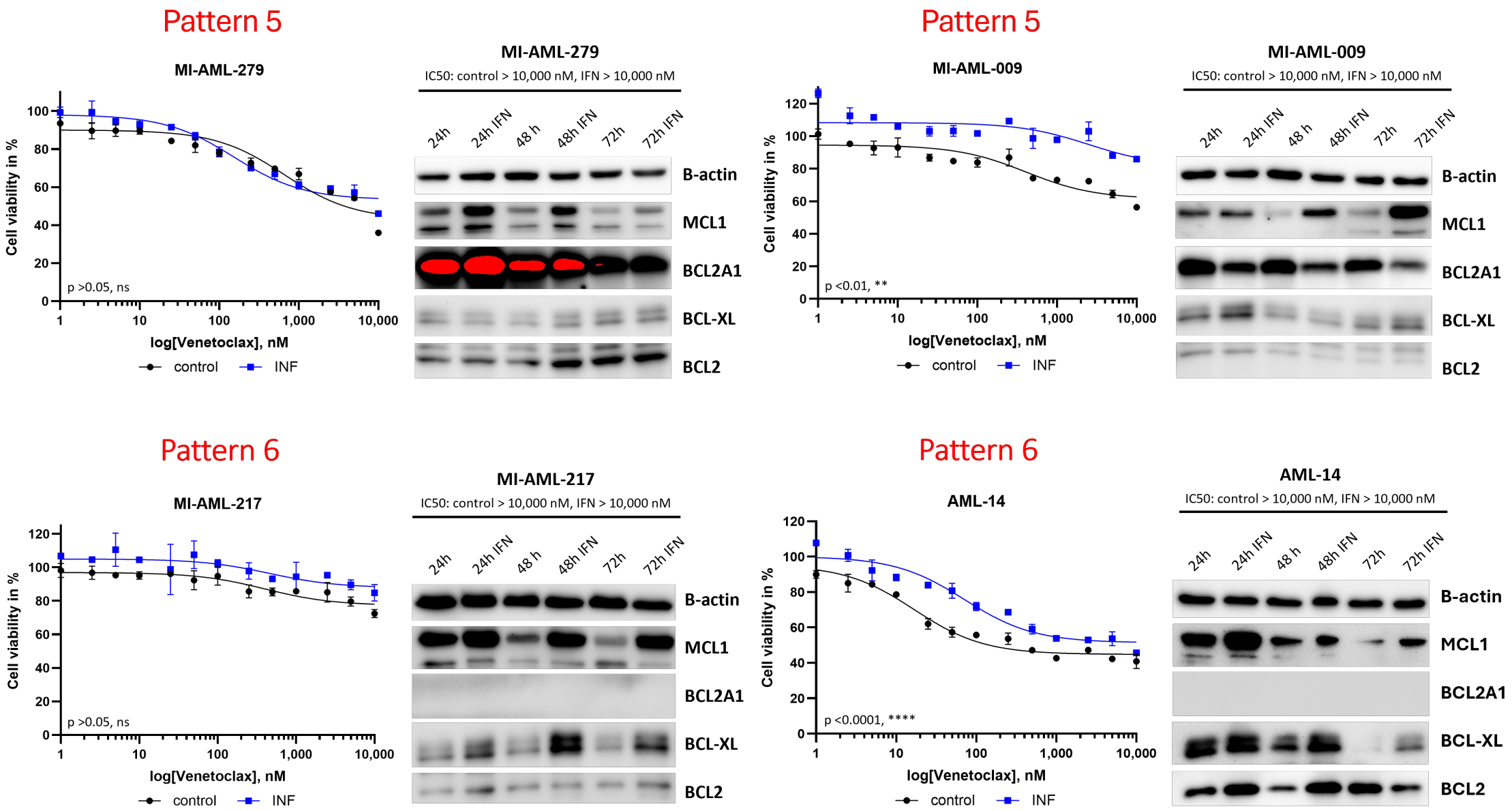
Supplementary Figure S7: Analysis of LINE/ERV/IFN expression in fourteen primary human AML. qPCR data for L1PA10, MSTB2, HERV9 and MER34-1 are shown as examples for LINE and ERV expression in AML, respectively. Displayed are mean values ± SEM of target gene expression normalized to internal control genes (GAPDH and PGK1) in different AML cases with WT or MUT *TP53* and/or *TET2*. IFNB and IFNG mRNA expression was measured for the same AML cases. Stars indicate the results of an unpaired two-tailed t-test (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).



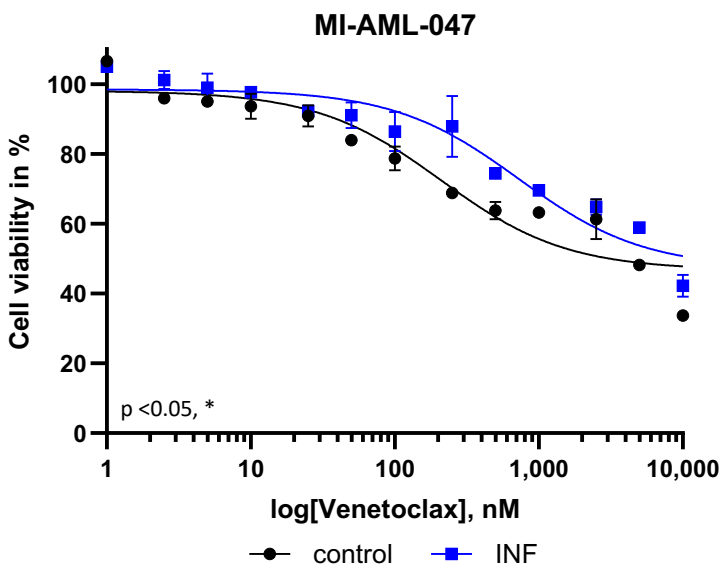
Supplementary Figure S8: High correlation of AUC and IC50 values as read-outs for Venetoclax-mediated cell death in AML. (A) Correlation of IC50 values with AUC for 19 primary human AML cases. Linear regression analysis is shown in blue. **(B)** AUC in AML FAB M0-M2 versus M4-M5 at baseline (AML control) and after IFN treatment.

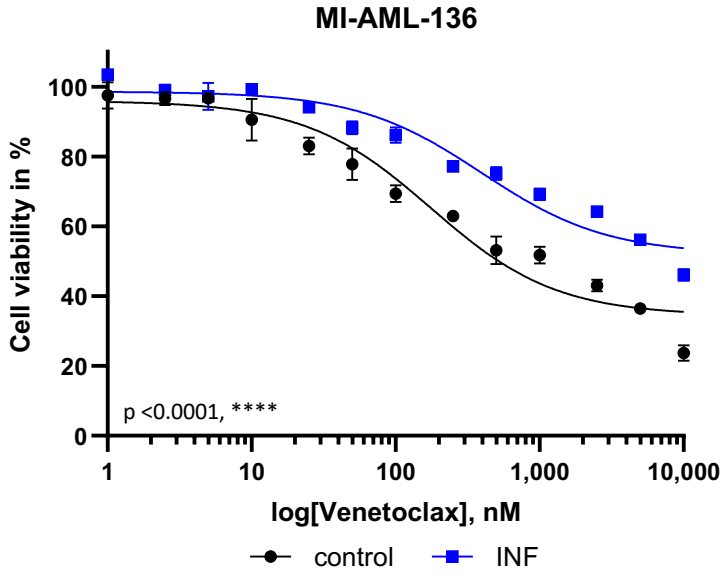


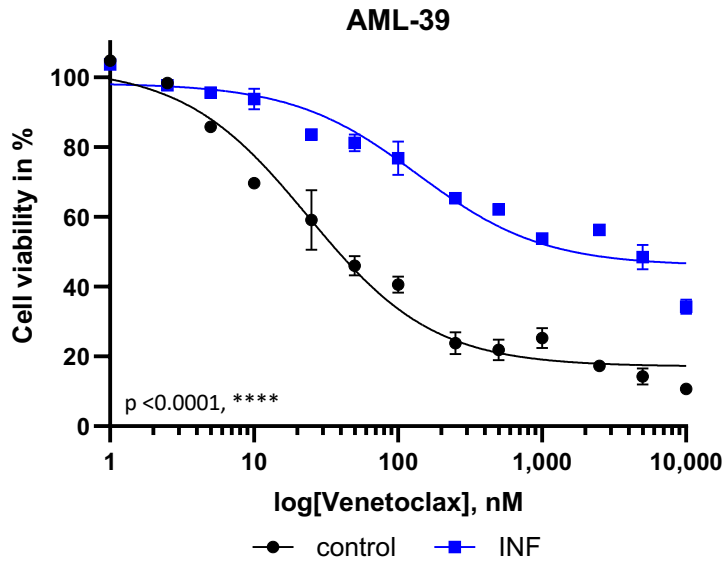
Supplementary Figure S9: MCL1, BCL2A1, BCL-XL and BCL2 protein expression in primary human AML following IFN stimulation identify patterns in relation to Venetoclax dose-responses. Dose-response curves: AML cases were pre-stimulated for 4h with 1,000 I.U. IFNα and IFNγ each in parallel to untreated control AML samples. Samples were then incubated with varying concentrations of Venetoclax for 24h while IFN was still present. Immunoblots: Primary AMLs were treated for 24h, 48h and 72h with 1,000 I.U. IFNα and IFNγ in parallel to untreated control AML samples and data for various BCL2 family members are shown. Stars indicate the results of a two-way ANOVA analysis (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). **Pattern 2:** Venetoclax-sensitive AML with absent BCL2A1 and low MCL1 expression. **Pattern 3:** Venetoclax sensitive AML with low BCL2A1 and MCL1 expression.



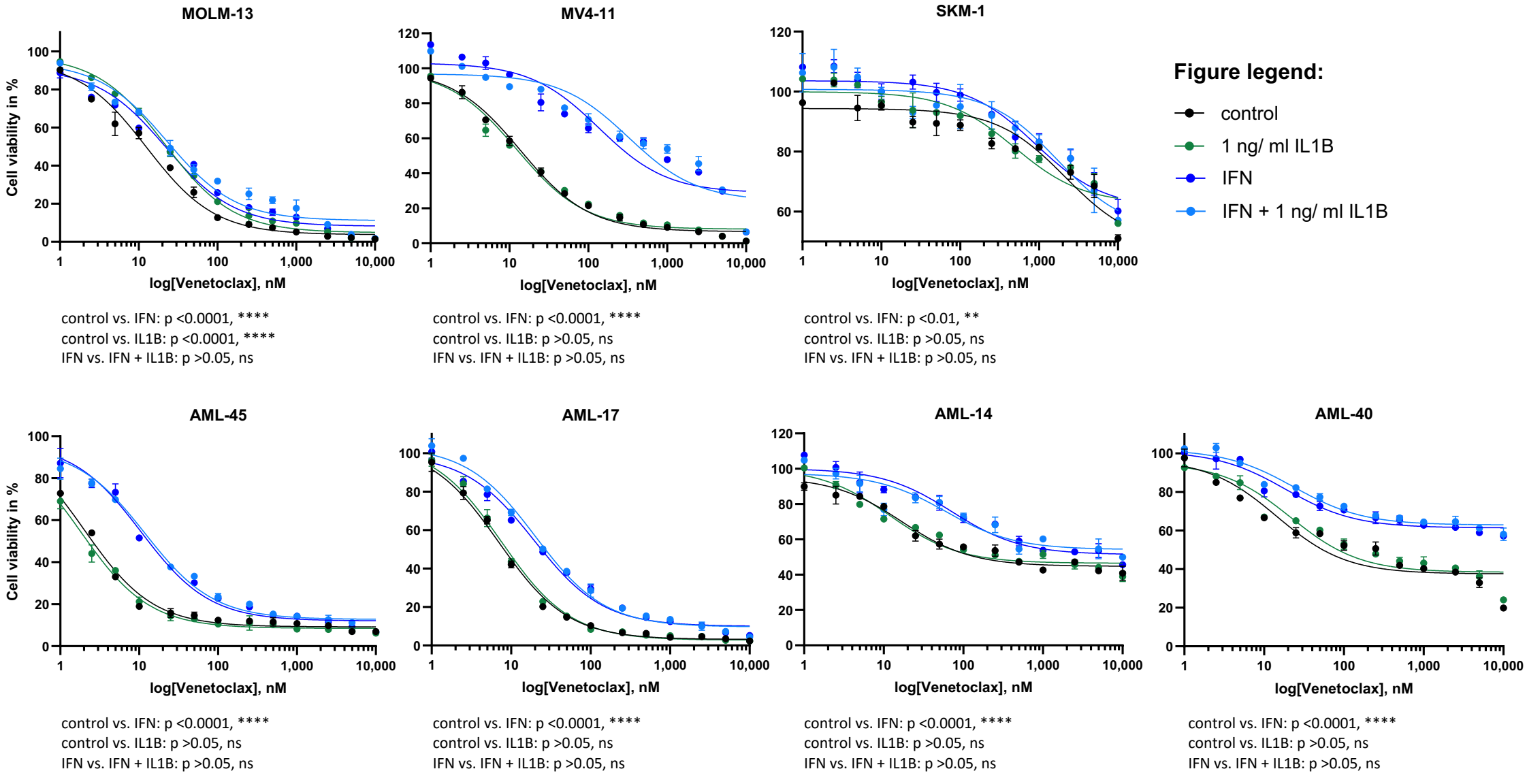
Supplementary Figure S10: MCL1, BCL2A1, BCL-XL and BCL2 protein expression in primary human AML following IFN stimulation identify patterns in relation to Venetoclax dose-responses. Dose-response curves: AML cases were pre-stimulated for 4h with 1,000 I.U. IFNα and IFNγ each in parallel to untreated control AML samples. Samples were then incubated with varying concentrations of Venetoclax for 24h while IFN was still present. Immunoblots: Primary AMLs were treated for 24h, 48h and 72h with 1,000 I.U. IFNα and IFNγ in parallel to untreated control AML samples and data for various BCL2 family members are shown. Stars indicate the results of a two-way ANOVA analysis (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001). **Pattern 5:** Venetoclax-resistant AML with high BCL2A1 and moderate MCL1 expression. **Pattern 6:** Venetoclax-resistant AML with high MCL1 and IFN-inducible BCL-XL expression.



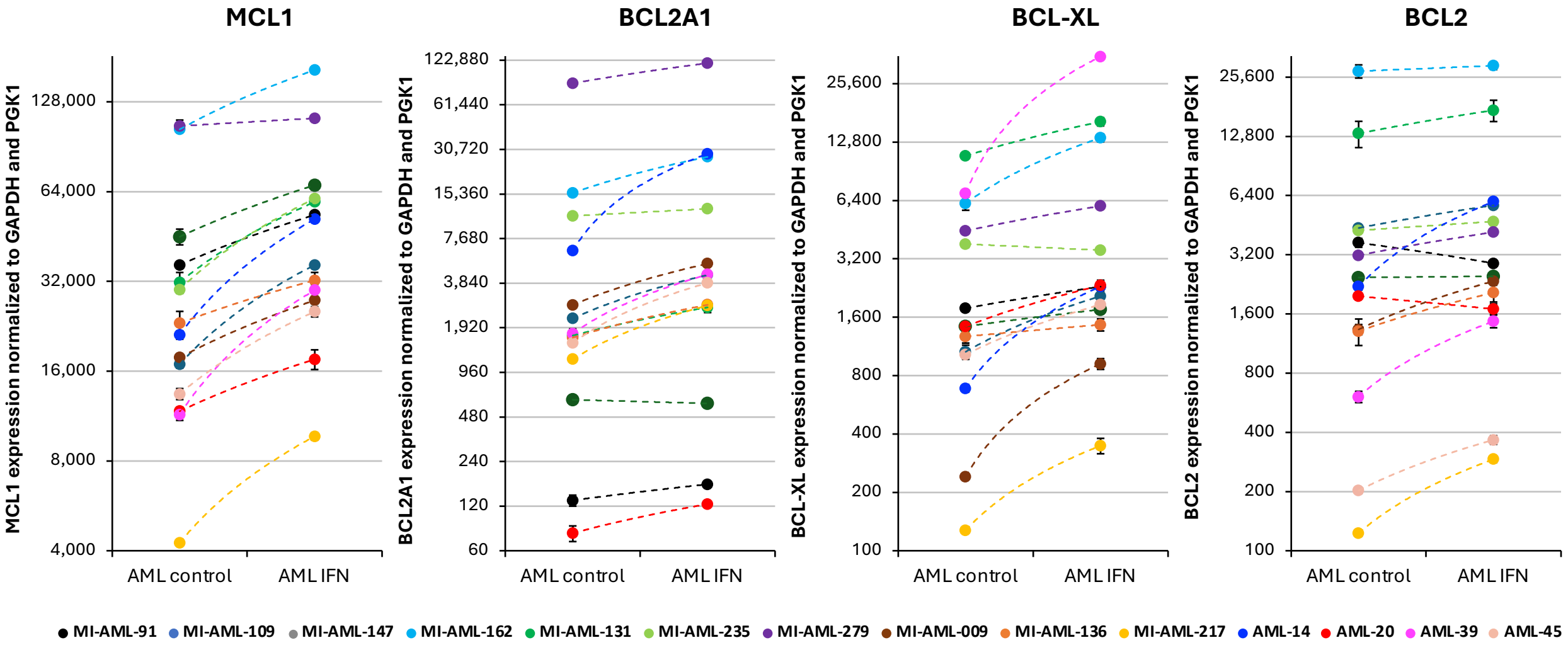




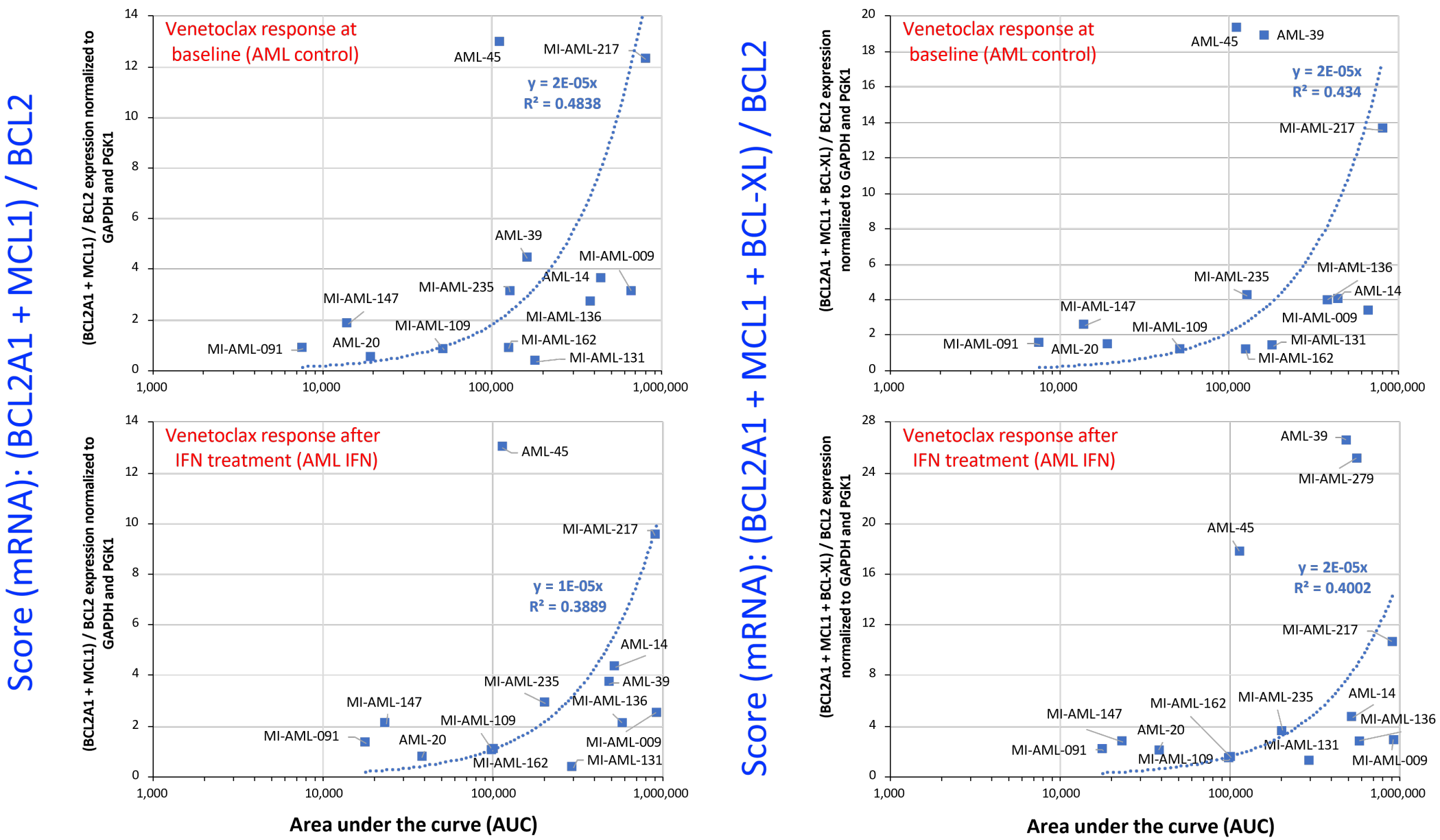
Supplementary Figure S11: Venetoclax dose-response curves for three additional primary human AML without immunoblot data.
AML cases were pre-stimulated for 4h with 1,000 I.U. IFNα and IFNγ each in parallel to untreated control AML samples. Samples were then incubated with varying concentrations of Venetoclax for 24h while IFN was still present. Three examples for the shift in cell viability of IFN treated samples (blue) versus untreated AML (black) are displayed.
Due to low cell amounts these AML cases are shown without immunoblot data. Stars indicate the results of a two-way ANOVA analysis (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).



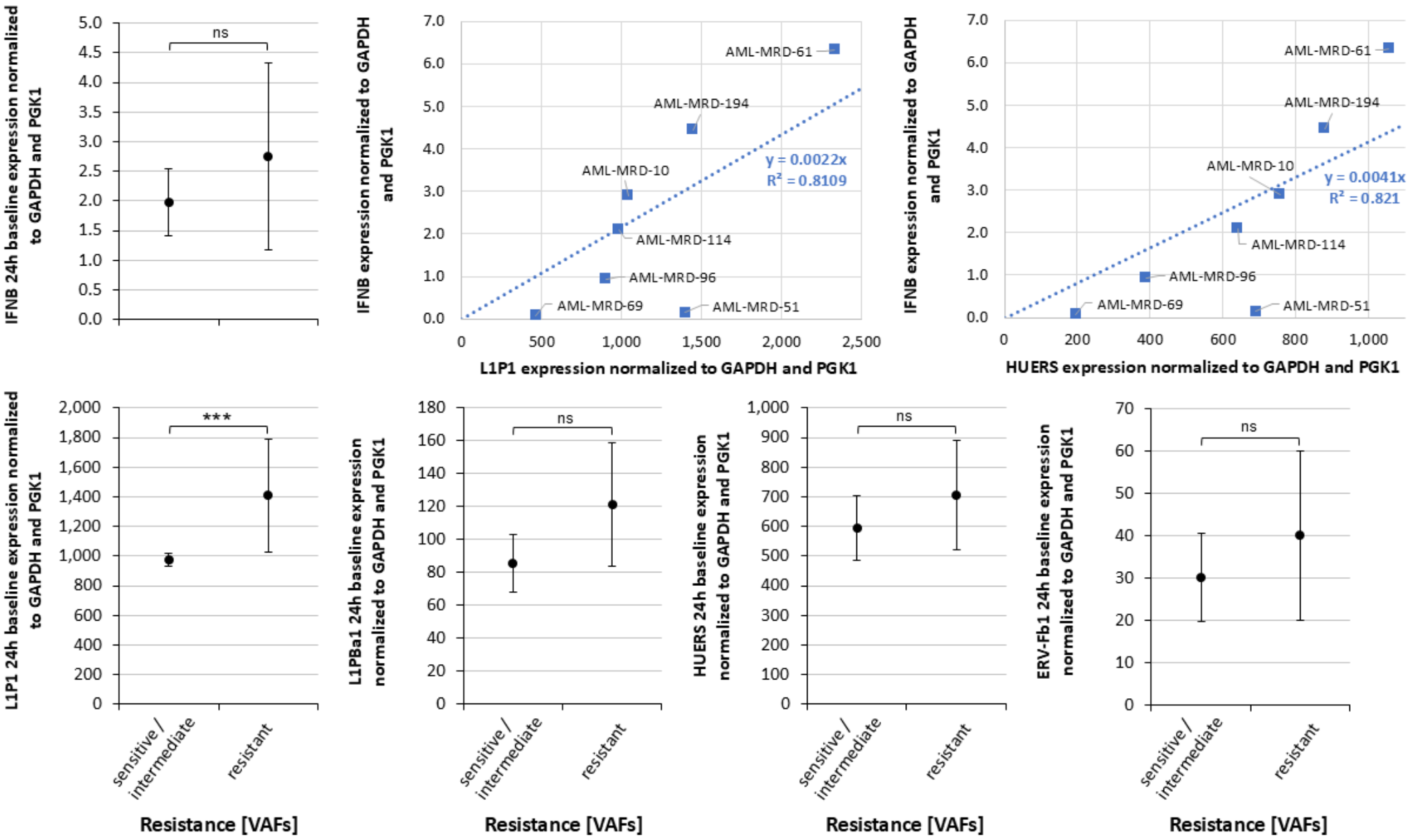
Supplementary Figure S12: Venetoclax dose-response curves for AML cell lines and primary human AML treated with IFNα and IFNγ, or IL1β or combined.
AML cell lines and primary AML cases were pre-stimulated for 4h with 1,000 I.U. IFNα and IFNγ, or IL1β or combined each in parallel to untreated control samples. Samples were then incubated with varying concentrations of Venetoclax for 24h while the stimulants were still present. The shift in cell viability of IFN treated samples (dark blue), or IFN + IL1β treated samples (light blue), or IL1β treated samples (green) versus untreated AML (black) are displayed.



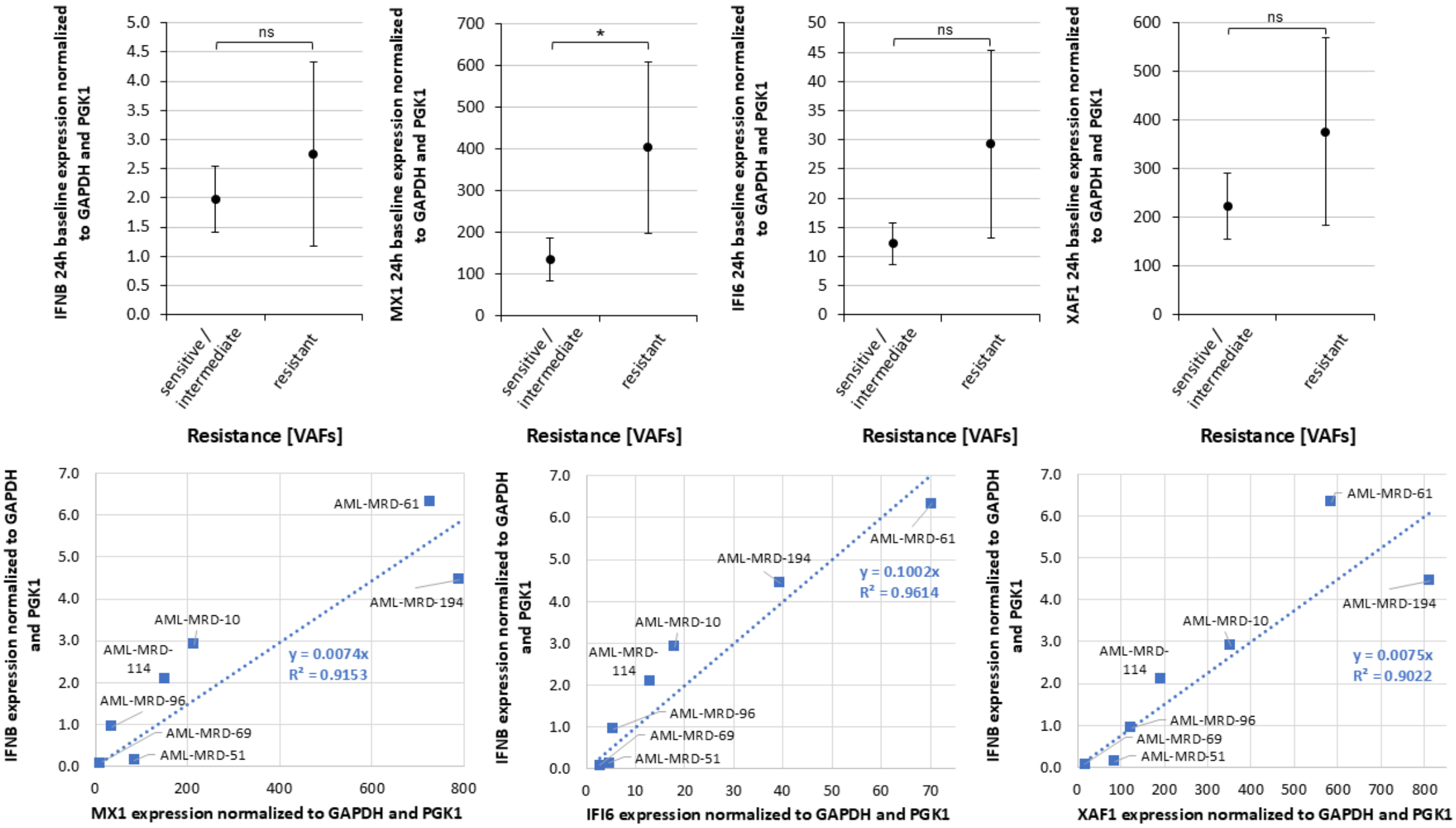
Supplementary Figure S13: MCL1, BCL2A1, BCL-XL and BCL2 mRNA expression in 14 primary human AML after IFN stimulation (qPCR). Primary AML were treated for 4h with 1,000 I.U. IFNα and IFNγ each in parallel to untreated control AML samples. qPCR data of target gene expression normalized to internal control genes (GAPDH and PGK1) are displayed as mean values ± SD of quadruplicates on a $\log_2$ y-axis scale.



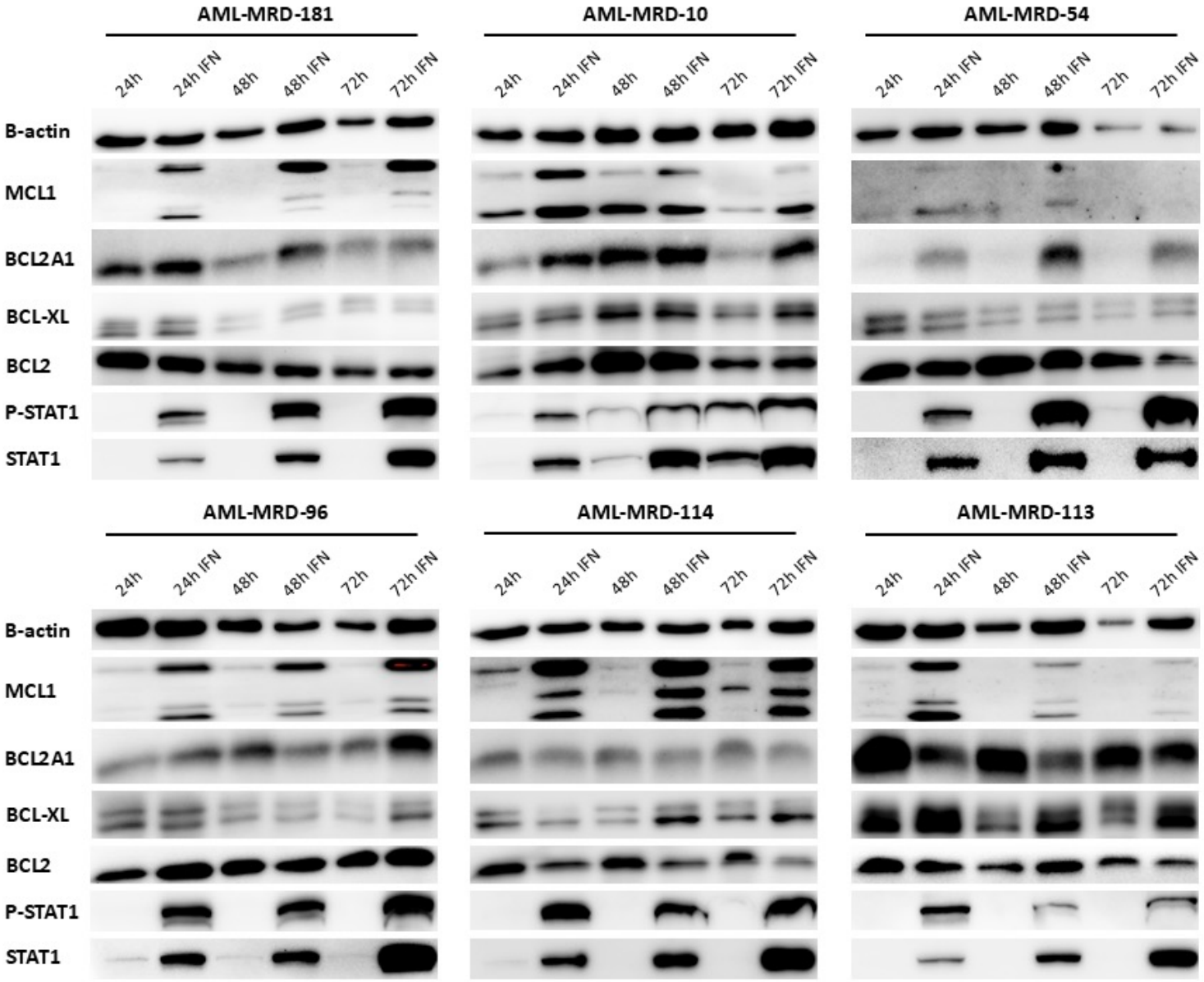
Supplementary Figure S14: Correlation of normalized ratios of (BCL2A1+MCL1):BCL2 or (BCL2A1+MCL1+BCL-XL):BCL2 mRNA expression with Venetoclax-induced responses in primary human AML (Area Under the Curves; AUCs). BCL2 family mRNA expression was normalized to internal control genes (GAPDH and PGK1). mRNA scores of different ratios have been calculated by dividing the normalized expression value for a target gene of a sample by the mean expression value of all samples for the same gene followed by the calculation of the score of interest. Correlation of mRNA ratios with Venetoclax responses at baseline (top) and after IFN-treatment (bottom) are displayed and linear regression analysis is shown in blue. Please note the $\log_{10}$ x-axis scale used for Venetoclax AUC values.



Supplementary Figure S15: Analysis of LINE/ERV/IFN expression in seven primary human AML. qPCR data for L1P1, L1PBa1, HUERS and ERV-Fb1 are displayed as mean values ± SEM of target gene expression normalized to internal control genes (GAPDH and PGK1) in sensitive/ intermediate and resistant AML cases. IFNB mRNA expression was measured for the same AML cases and correlated with L1P1 and HUERS expression as one example for retroelements and ERVs, respectively. Linear regression analysis is shown in blue. Stars indicate the results of an unpaired two-tailed t-test (ns = p>0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).



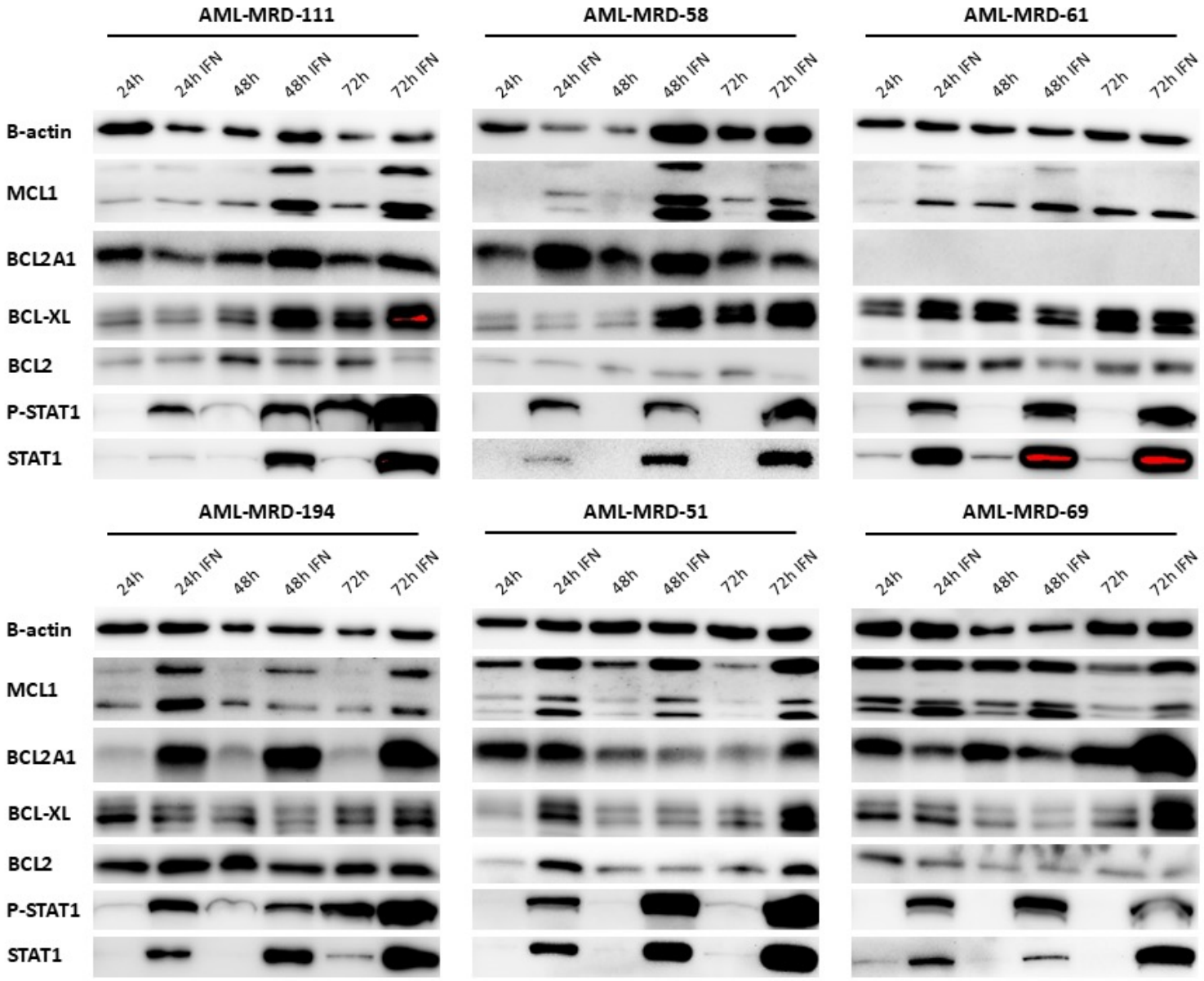
Supplementary Figure S16: Analysis of IFN response gene expression in seven primary human AML. qPCR data for MX1, IFI6 and XAF1 are displayed as mean values ± SEM of target gene expression normalized to internal control genes (GAPDH and PGK1) in sensitive/ intermediate and resistant AML cases. IFNB mRNA expression was measured for the same AML cases and correlated with the expression of IFN response genes. Linear regression analysis is shown in blue. Stars indicate the results of an unpaired two-tailed t-test (ns = p>0.05, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).



Overview of sensitive / intermediate AML cases with individual ddPCR MRD VAF measurements	
	ddPCR VAFs
AML-MRD-181	NRAS 0.009% (BM); FLT3-835 0% (BM)
AML-MRD-96	NPM1 0.003% (PB); IDH2 0.15% (PB)
AML-MRD-10	WNT9A 0.153% (PB), 0.394% (BM)
AML-MRD-114	NPM1 0.496% (BM)
AML-MRD-54	IDH1 0.11% (PB)
AML-MRD-113	NPM1 0.45% BM; 0.01% PB

Overview of sensitive / intermediate AML cases with gDNA mutation data	
	mutation
AML-MRD-181	KMT2A-MLLT3, FLT3-ITD, NRAS
AML-MRD-96	ASXL1, SRSF2, IDH2, NPM1
AML-MRD-10	TET2, KIT, WNT9A
AML-MRD-114	FLT3, TET2, SMC1A, NPM1, DNMT3A, CEBPA
AML-MRD-54	SRSF2, TET2, IDH1, JAK2
AML-MRD-113	ZRSR2, TET2, NPM1

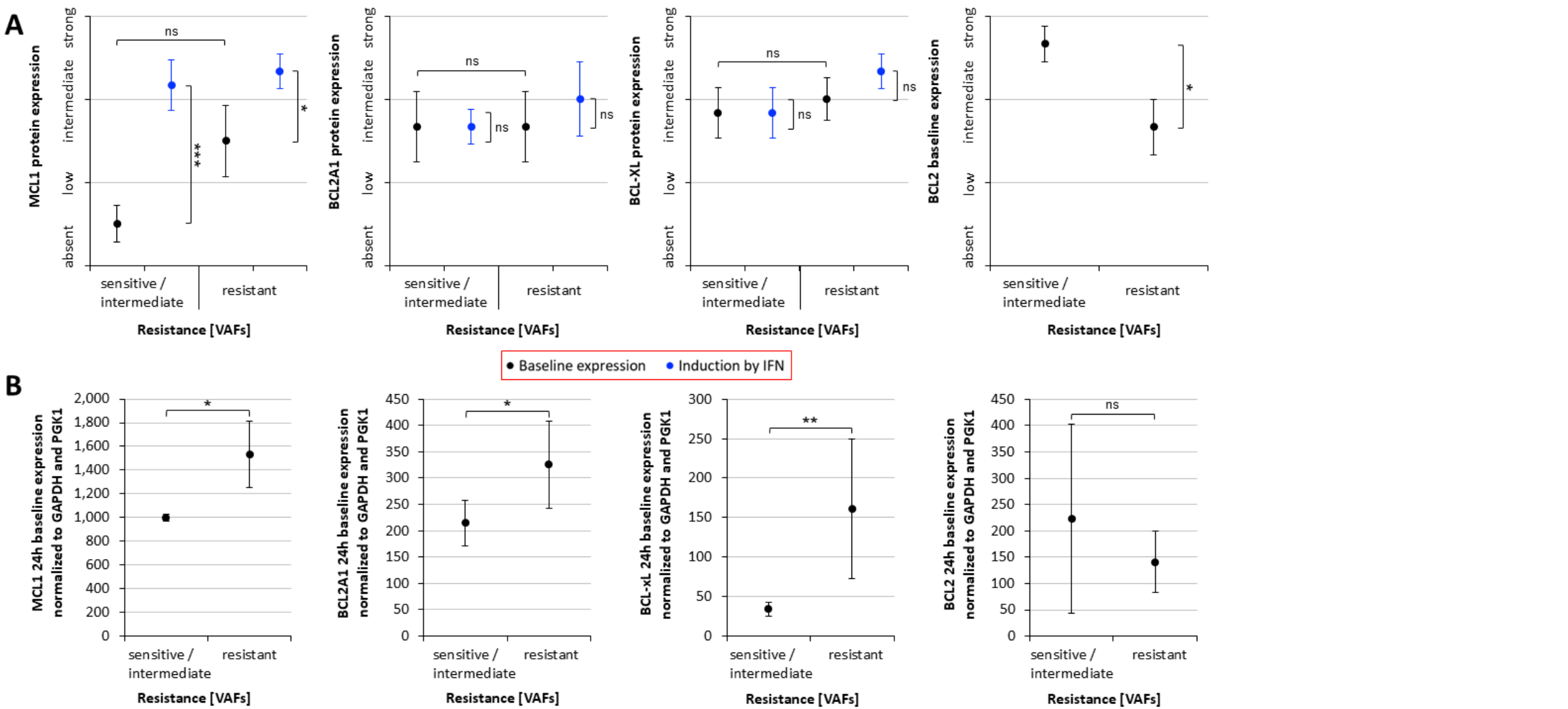
Supplementary Figure S17: MCL1, BCL2A1, BCL-XL, BCL2 and P-/STAT1 protein expression in primary human AML samples at baseline and following IFN stimulation. Immunoblot data for human AML that were sensitive/ intermediate clinically to Decitabine/Venetoclax therapy are displayed. The corresponding post HMA/Ven cycle 1-2 ddPCR MRD VAF measurements (upper table right) and their genomic DNA mutations (lower table right) are displayed. Primary AMLs were treated for 24h, 48h and 72h with 1,000 I.U. IFNα and IFNγ in parallel to untreated control AML samples and immunoblot data for various BCL2 family members and p-Y701-STAT1/STAT1 are shown. BM – bone marrow, PB – peripheral blood, MRD – minimal residual disease



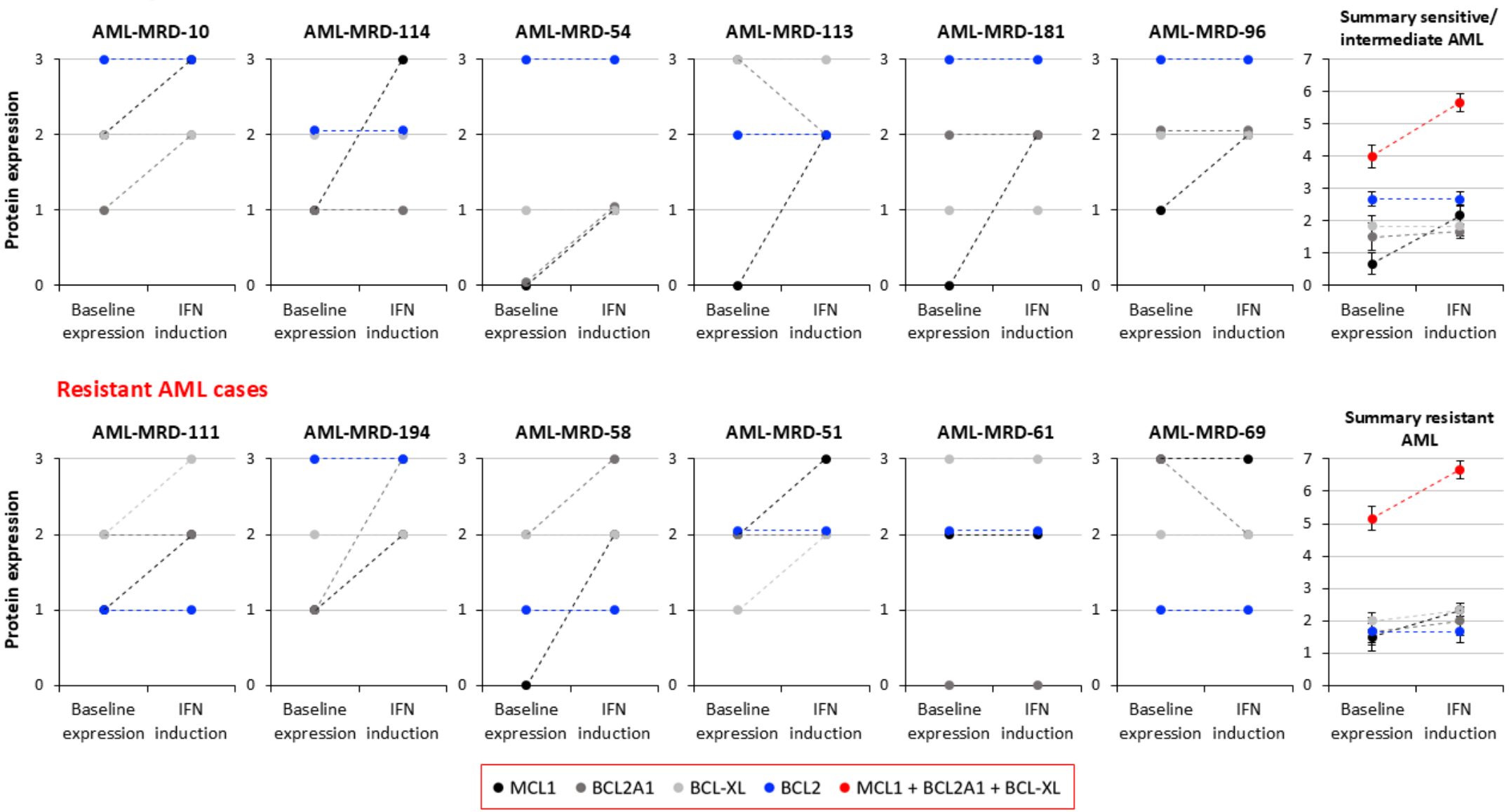
Overview of resistant AML cases with individual ddPCR MRD VAF measurements	
	ddPCR MRD VAFs
AML-MRD-111	NPM1 36.9% BM; 32.3% PB, IDH2 33.39%
AML-MRD-194	IDH2 8.21% (BM)
AML-MRD-58	NPM1 3.09% (BM); 1.43% (PB); GALK2 6.213 % (BM); 4.76% (PB)
AML-MRD-51	NRAS 25.36% (BM), NPM1 0.003% (BM)
AML-MRD-61	TP53 14.545% (BM), 9.95% (PB); DDAH1 1.4% (BM); 0.86% (PB)
AML-MRD-69	IDH2 2.31% (BM), 2.697% (PB), NRAS 0.993% (PB)

Overview of resistant AML cases with gDNA mutation data	
	mutation
AML-MRD-111	IDH2, NPM1
AML-MRD-194	FLT3-ITD, IDH2, RUNX1, DNMT3A, SRSF2
AML-MRD-58	TET2, NPM1, DNMT3A, FLT3, NF1, GALK2
AML-MRD-51	TET2, ASXL1, NRAS, SETD1B, NPM1
AML-MRD-61	TP53, PPM1D
AML-MRD-69	ASXL1, SRSF2, NRAS, IDH2, XPO1

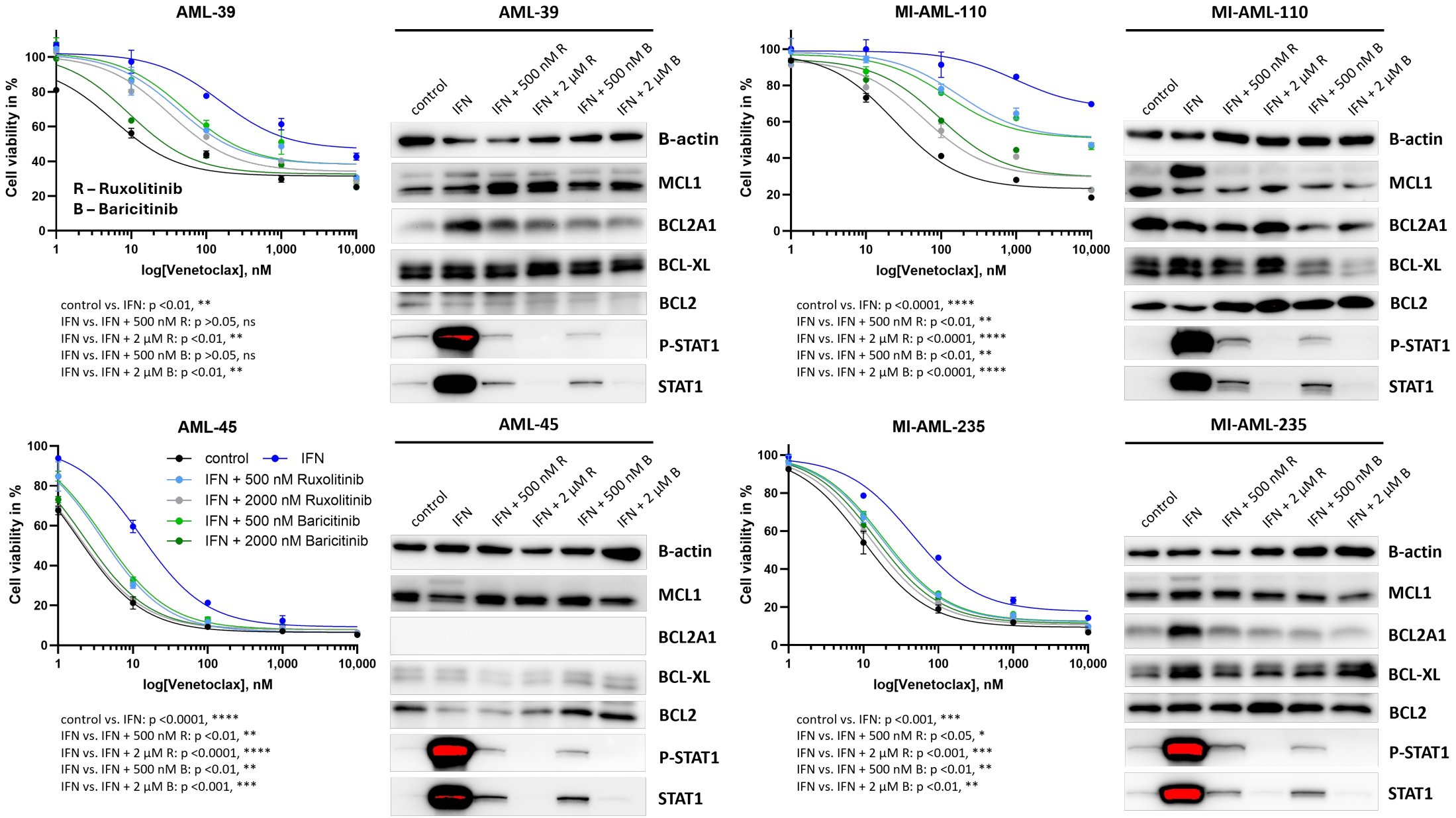
Supplementary Figure S18: MCL1, BCL2A1, BCL-XL, BCL2 and P-/STAT1 protein expression in primary human AML samples at baseline and following IFN stimulation. Immunoblot data for human AML that were resistant clinically to Decitabine/Venetoclax therapy are displayed. The corresponding post HMA/Ven cycle 1-2 ddPCR MRD VAF measurements (upper table right) and their genomic DNA mutations (lower table right) are displayed. Primary AMLs were treated for 24h, 48h and 72h with 1,000 I.U. IFNα and IFNγ in parallel to untreated control AML samples and immunoblot data for various BCL2 family members and p-Y701-STAT1/STAT1 are shown. BM – bone marrow, PB – peripheral blood, MRD – minimal residual disease



Supplementary Figure S19: Analysis of BCL2 family member expression in primary human AML. Primary AML cases were grouped into sensitive/ intermediate and resistant to Decitabine/Venetoclax therapy based on post HMA/Ven cycle 1-2 ddPCR MRD VAF measurements. (A) Individual plots of various BCL2 family members based on scores of protein expression at baseline and after IFN treatment for 24h, 48h and 72h with 1,000 I.U. IFNα and IFNγ (Supplementary Table 8). Mean values ± SEM of AML cases within one group were calculated for all BCL2 family members and analyzed using an unpaired two-tailed t-test. Based on individual protein semiquantitative assessment coded as 0 (absent expression), 1 (low), 2 (intermediate) and 3 (high). (B) qPCR data for BCL2 family members are displayed as mean values ± SEM of target gene expression normalized to internal control genes (GAPDH and PGK1) in sensitive/ intermediate and resistant AML cases. Stars indicate the results of an unpaired two-tailed t-test (ns = $p>0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$).



Supplementary Figure S20: Plots of MCL1, BCL2A1, BCL-XL and BCL2 protein expression in primary human AML at baseline and following IFN stimulation. Individual plots of various BCL2 family members based on scores of protein expression at baseline and after IFN treatment for 24h, 48h and 72h with 1,000 I.U. IFNα and IFNγ (Supplementary Table 8). Primary AML cases were grouped into sensitive/ intermediate and resistant to Decitabine/Venetoclax therapy based on post HMA/Ven cycle 1-2 ddPCR MRD VAF measurements. Mean values ± SEM of AML cases within one group were calculated for all BCL2 family members and for the sum of MCL1+BCL2A1+BCL-XL and are displayed as summary plots (last plots right). Based on individual protein semiquantitative assessment coded as 0 (absent expression), 1 (low), 2 (intermediate) and 3 (high).



Supplementary Figure S21: JAK inhibition reverses resistance induction to Venetoclax in IFN-treated AML cells. Four primary human AML were pre-treated with 500 nM or 2 µM of Ruxolitinib or Baricitinib, respectively, followed by 4h treatment with 1,000 I.U. IFNα and IFNγ each in parallel to untreated controls. Samples were incubated with varying concentrations of Venetoclax for 24h while IFN and JAK-inhibitors were still present. Dose-response curves are shown and color coded. Immunoblotting results for BCL2 family members and p-Y701-STAT1/STAT1 with and without IFN treatment +/- JAK-inhibitors. Stars indicate the results of a two-way ANOVA analysis (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001).