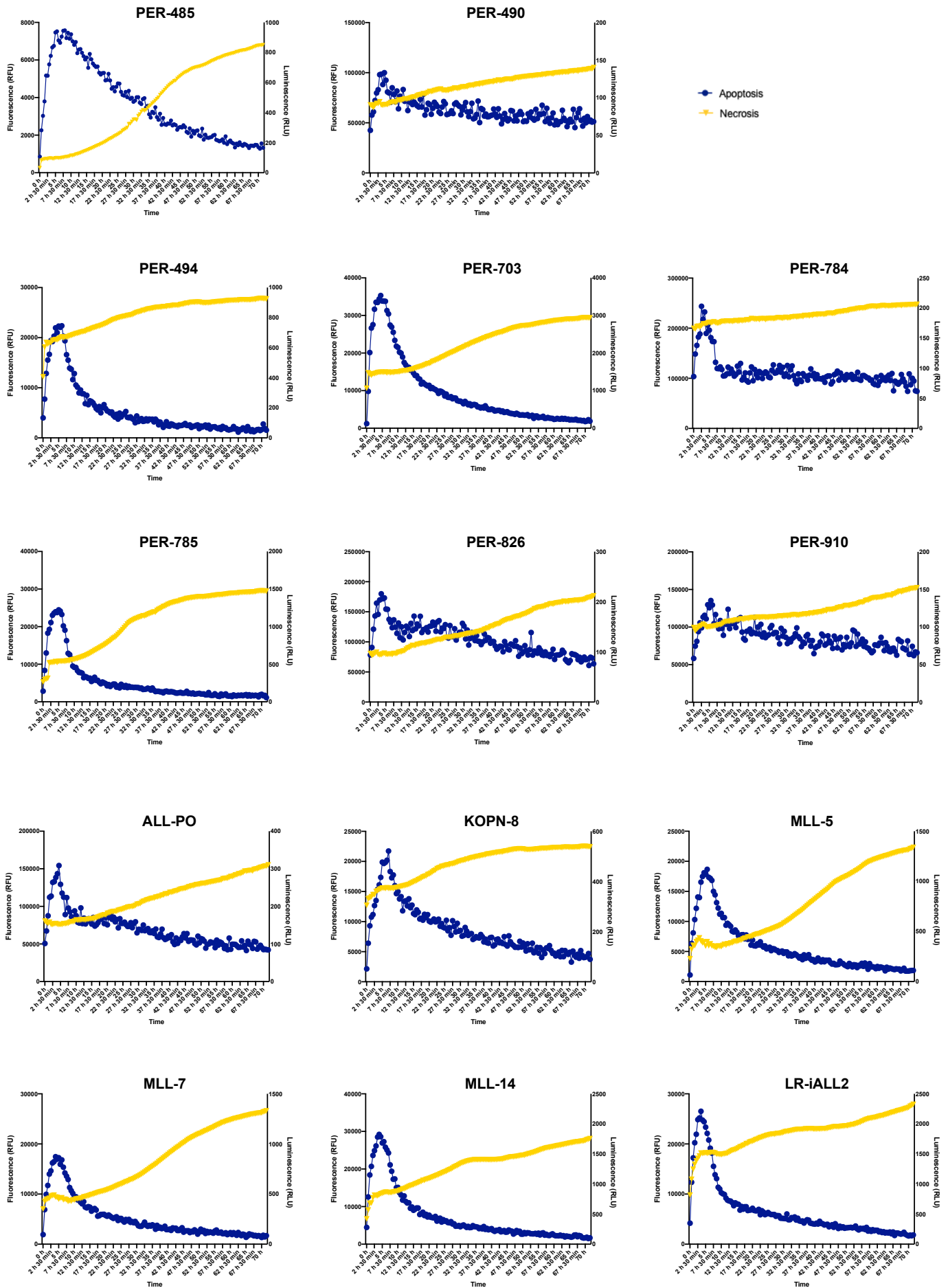
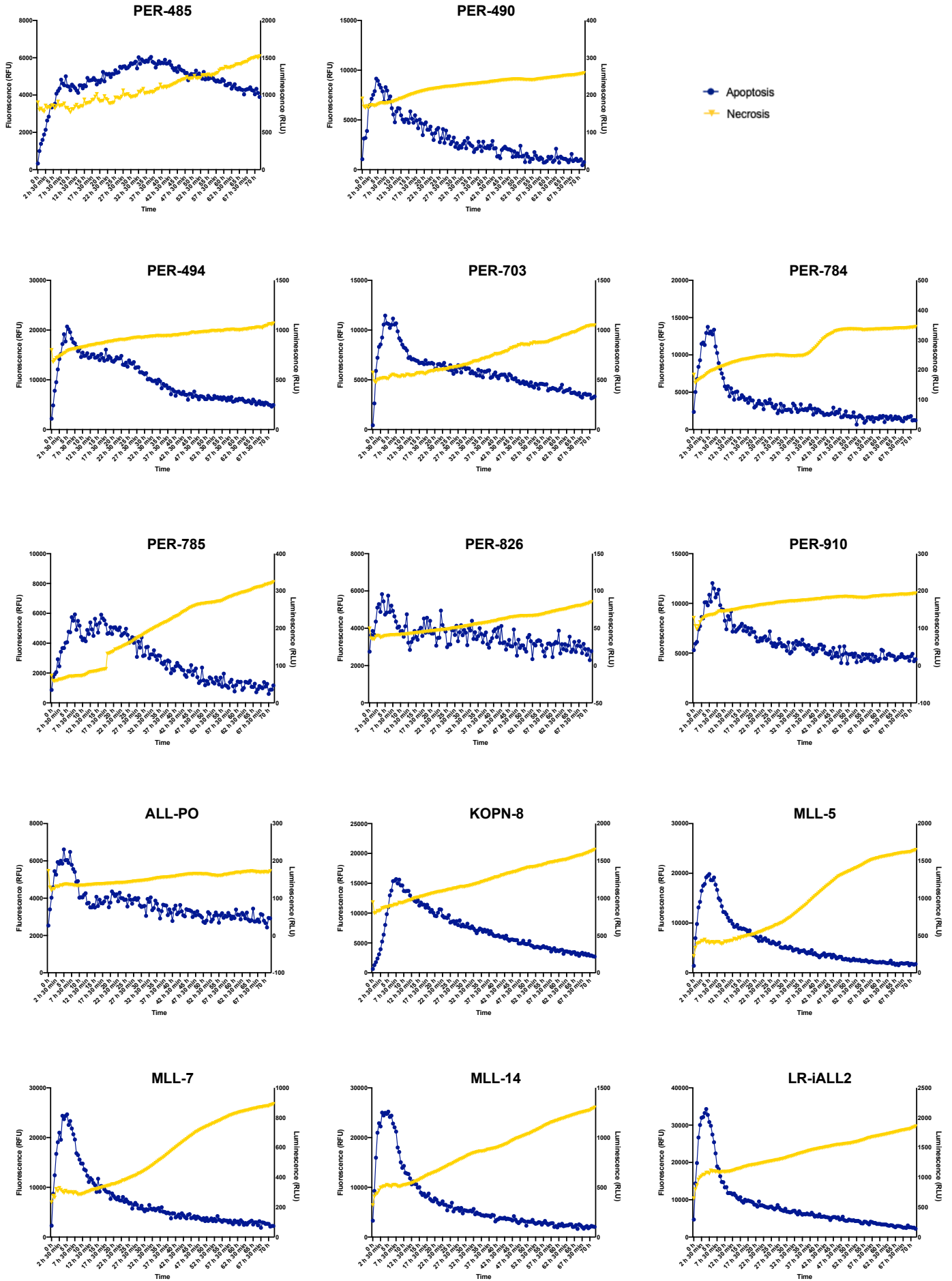


**Supplemental Figure S1. Azacitidine induces apoptosis and necrosis in *KMT2A*-rearranged infant acute lymphoblastic leukemia cell lines.** Cells were incubated with  $IC_{50}$  concentrations of azacitidine for 72 hours. Measurements of luminescence (apoptosis) and fluorescence (necrosis) were performed every 30 minutes.

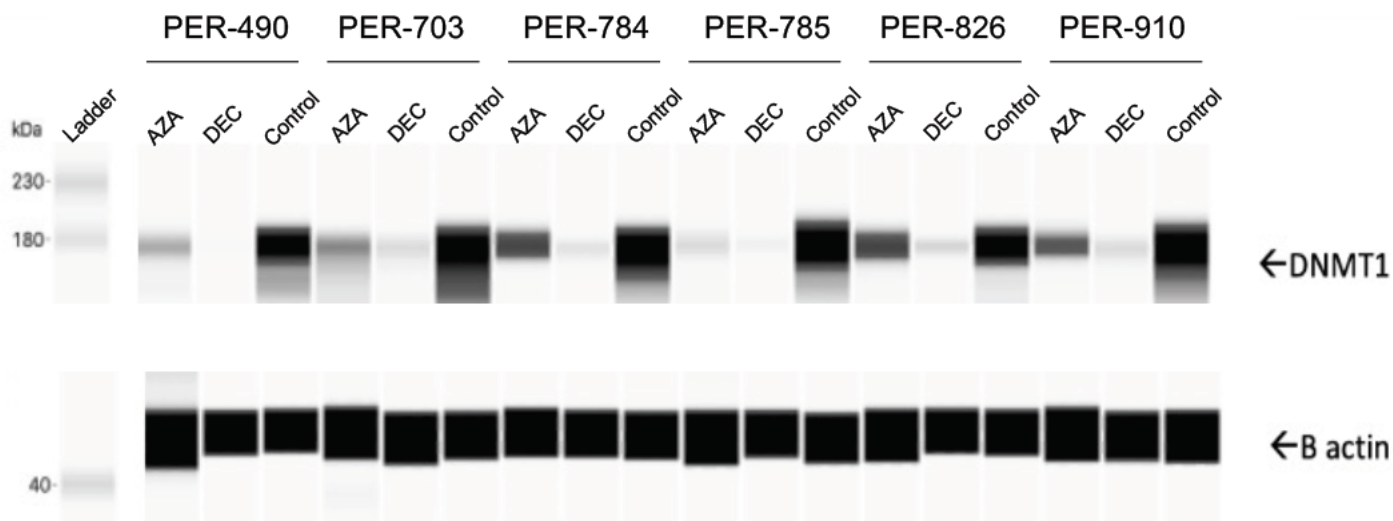


**Supplemental Figure S2. Decitabine induces apoptosis and necrosis in *KMT2A*-rearranged infant acute lymphoblastic leukemia cell lines.** Cells were incubated with IC<sub>50</sub> concentrations of decitabine for 72 hours. Measurements of luminescence (apoptosis) and fluorescence (necrosis) were performed every 30 minutes.

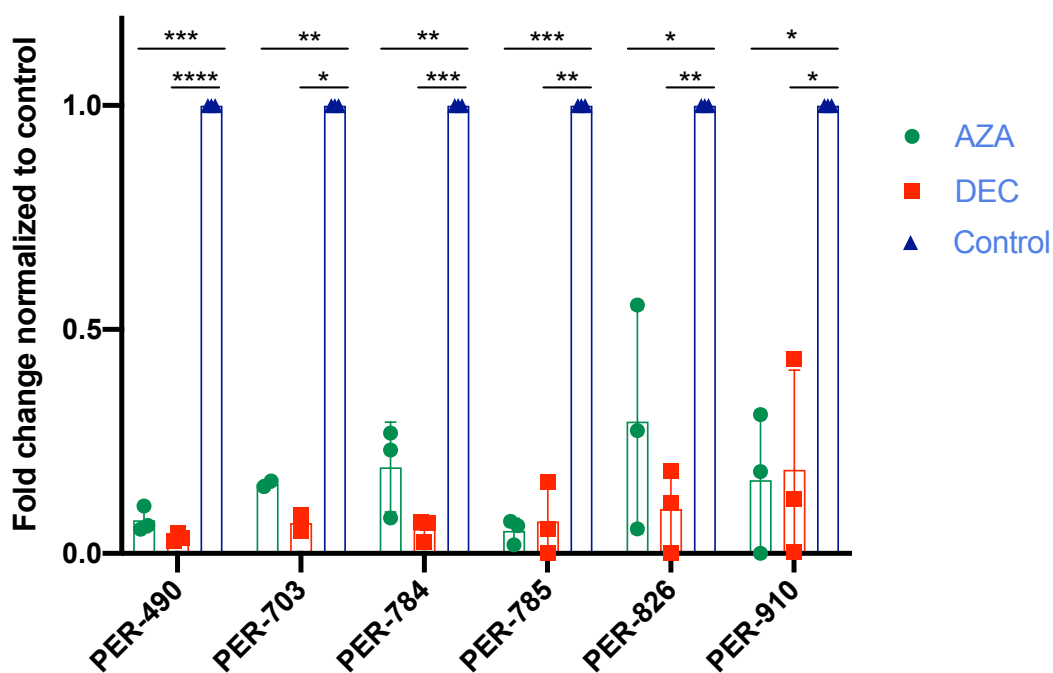


**Supplemental Figure S3. Azacitidine and decitabine reduce expression of DNMT1 in *KMT2A*-rearranged infant acute lymphoblastic leukemia cell lines.** Leukemia cell lines were treated with 1.5 $\mu$ M of azacitidine (AZA) or decitabine (DEC) for 72 hours before performing protein extraction. (A) Western blot against DNMT1 (~180kDa) and B actin (~47kDa). (B) Quantification of DNMT1 expression by densitometry from three independent experiments. The expression of DNMT1 is normalized to untreated control. \* $p$ <0.05, \*\* $p$ <0.01, \*\*\* $p$ <0.001, \*\*\*\* $p$ <0.0001.

A

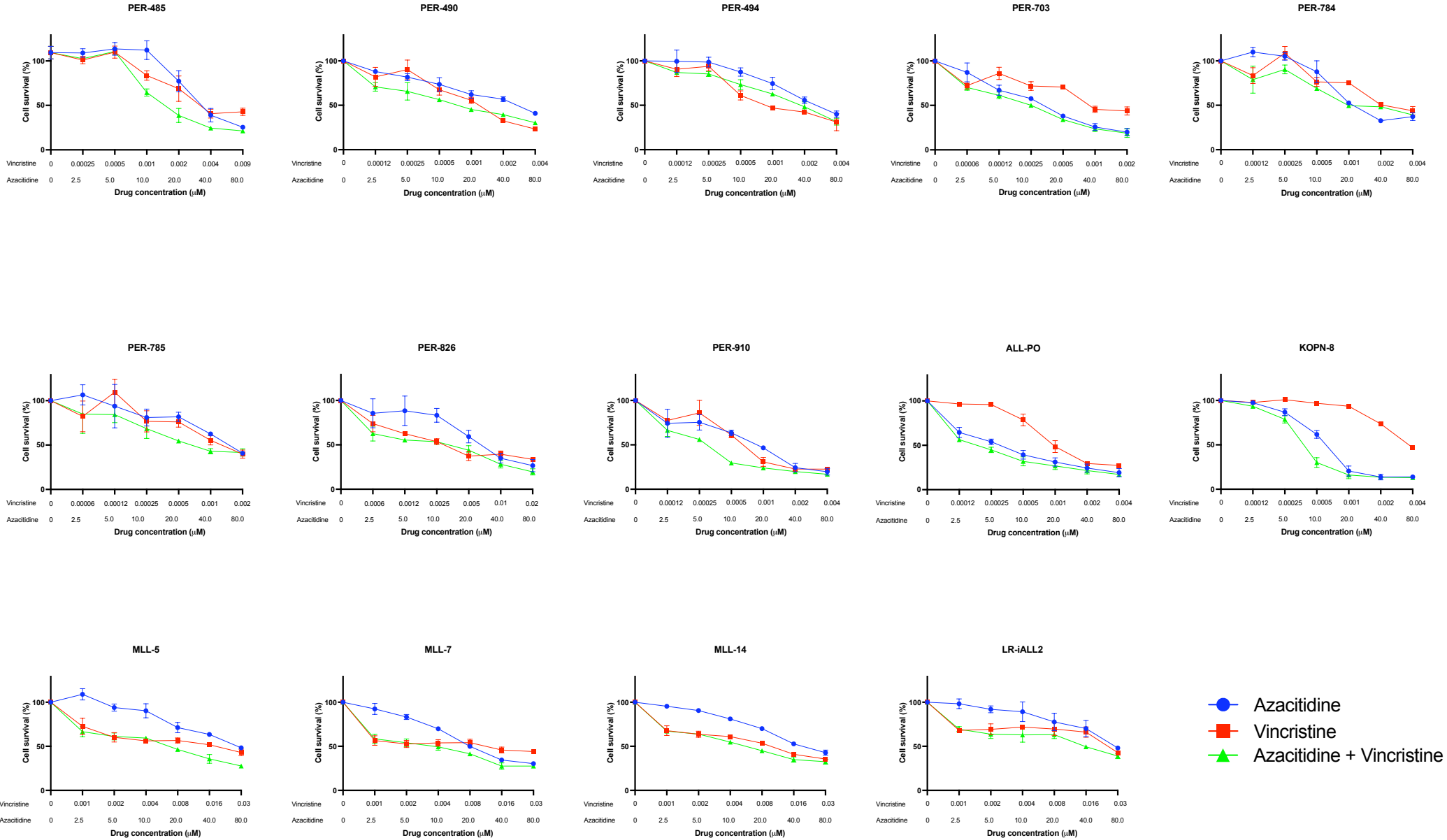


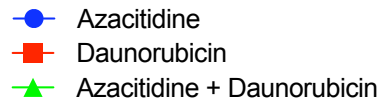
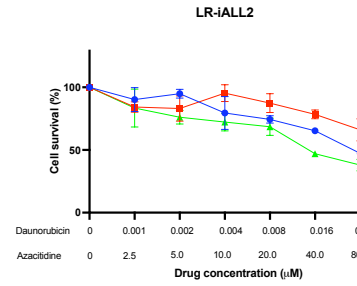
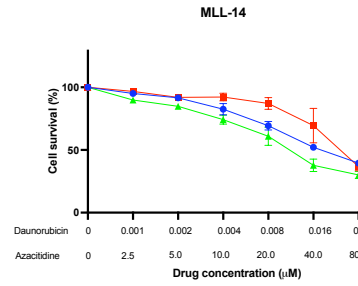
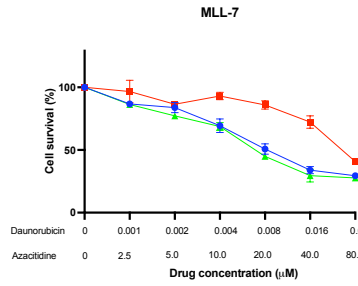
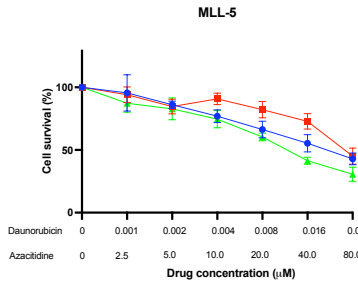
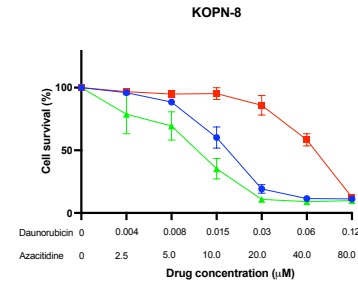
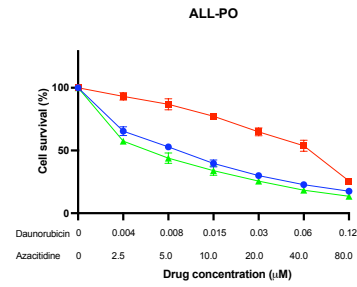
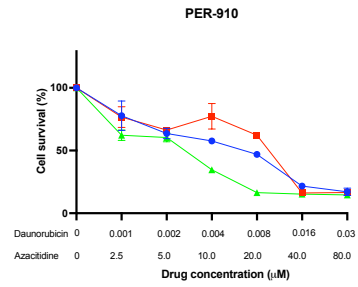
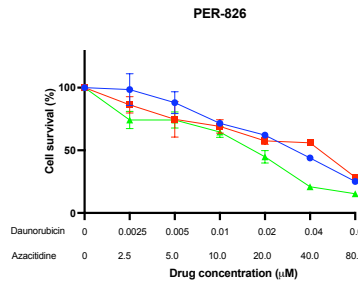
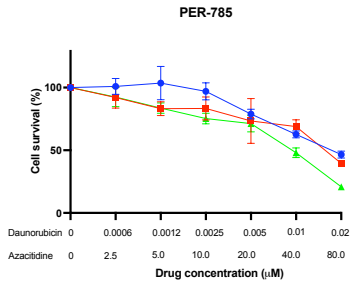
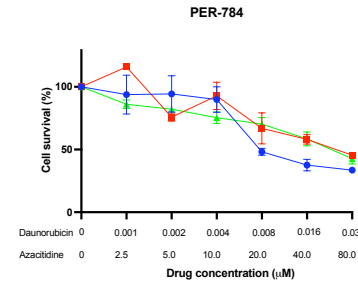
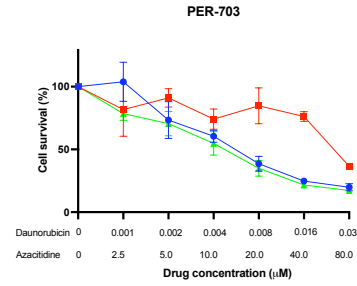
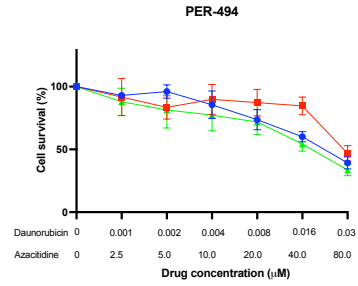
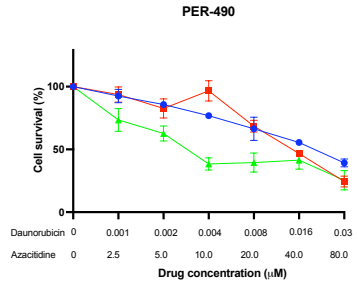
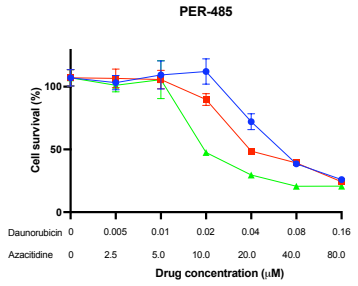
B



**Supplemental Figure S4. The combinatorial dose response of hypomethylating agents with conventional chemotherapeutic agents compared to single agent on cell survival.** Conventional chemotherapeutic agents include vincristine, daunorubicin, dexamethasone, cytarabine, methotrexate, 6-mercaptopurine, L-asparaginase, thioguanine and 4-hydroperoxycyclophosphamide (4-HPC). (A) Azacitidine with conventional chemotherapeutic agents. (B) Decitabine with conventional chemotherapeutic agents. (C) Zebularine with conventional chemotherapeutic agents.

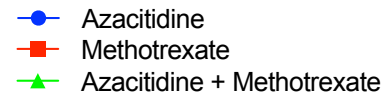
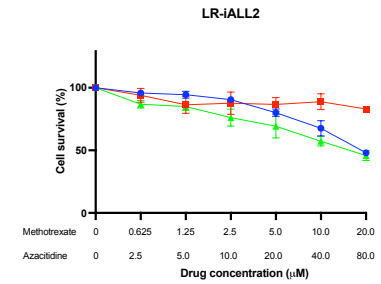
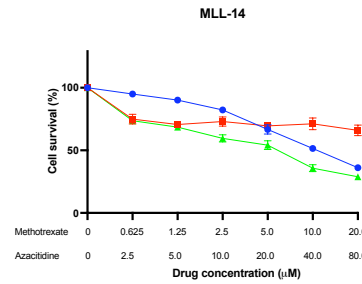
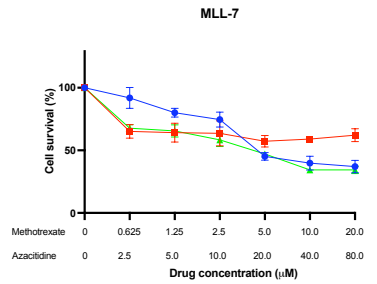
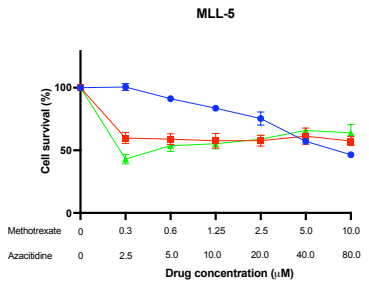
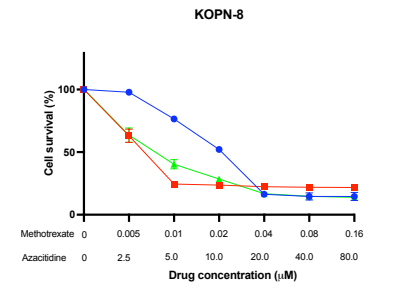
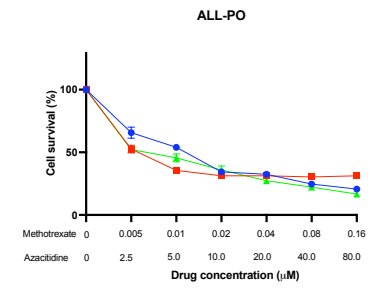
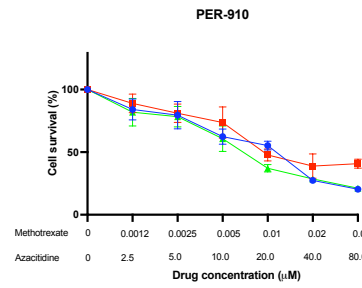
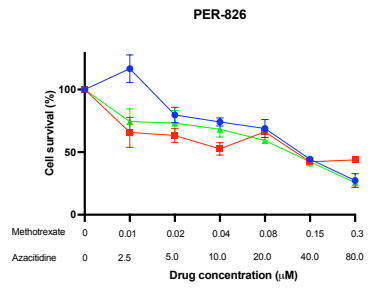
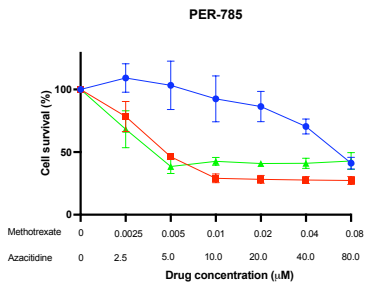
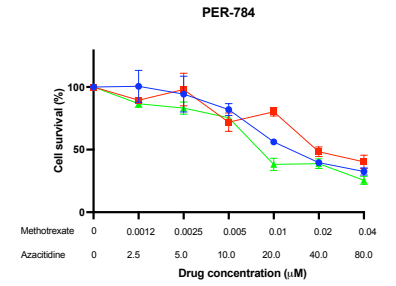
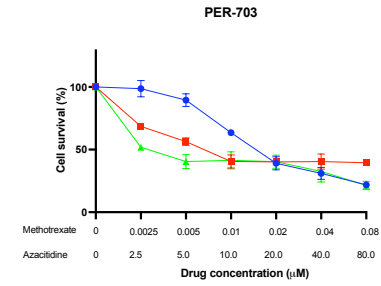
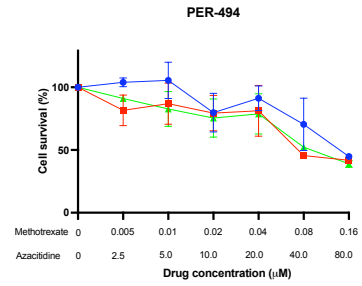
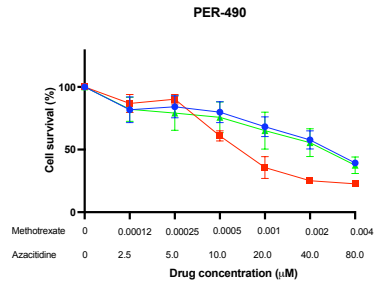
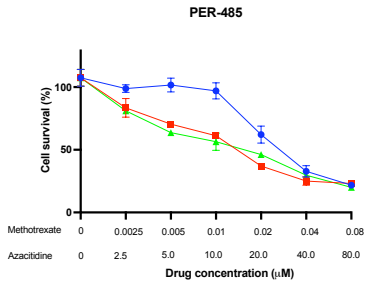
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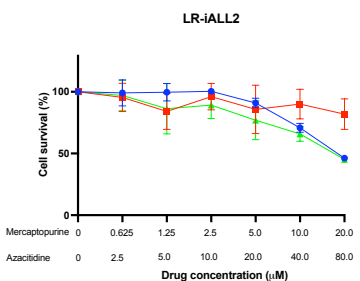
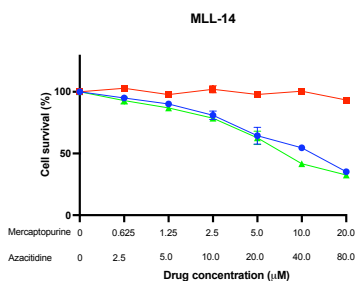
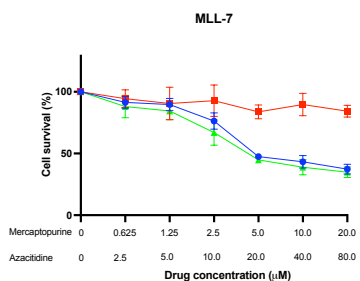
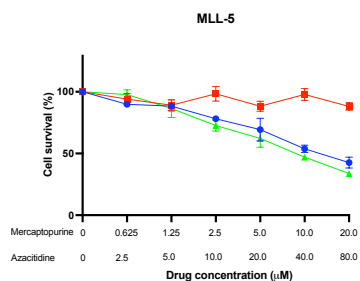
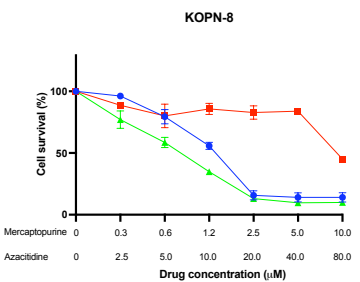
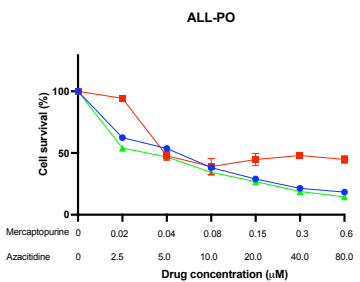
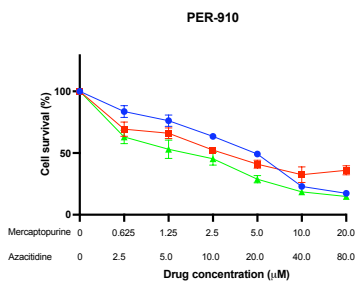
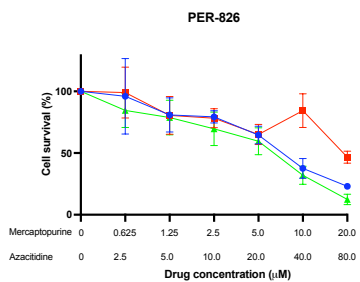
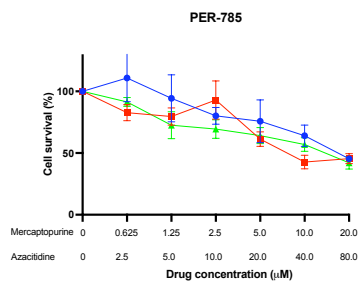
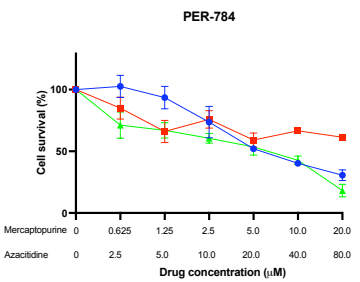
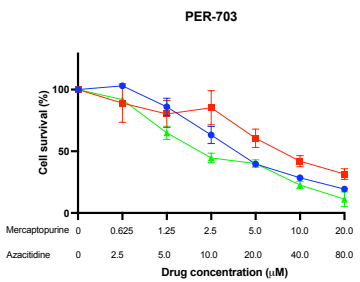
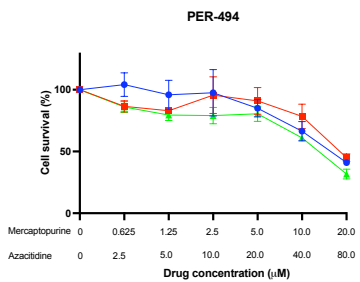
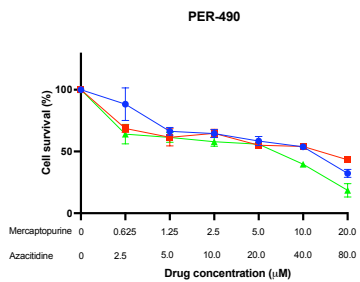
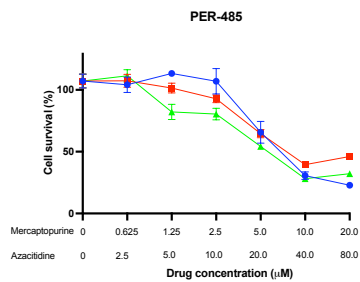






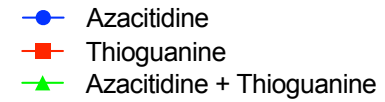
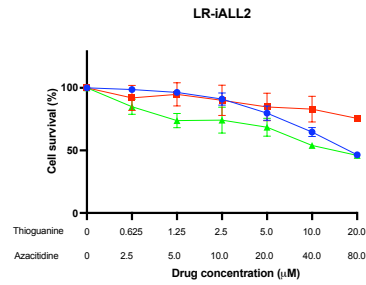
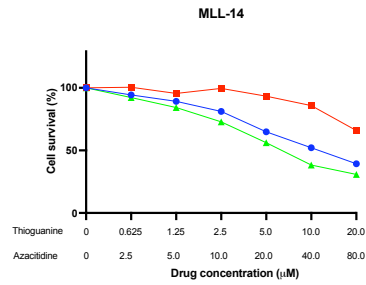
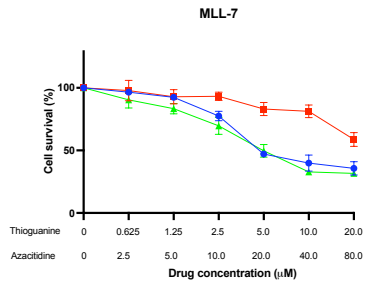
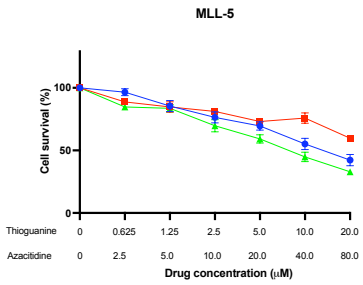
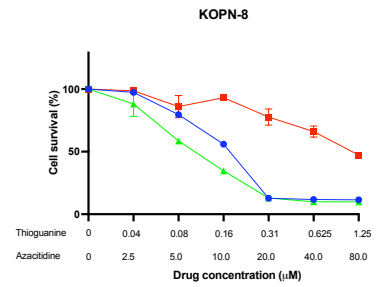
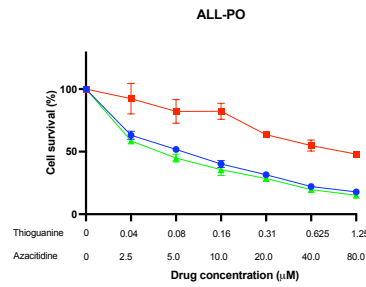
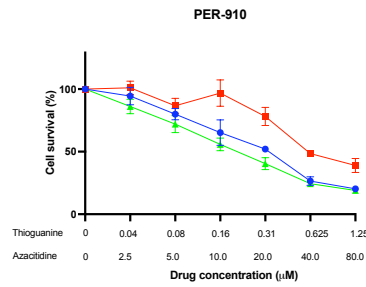
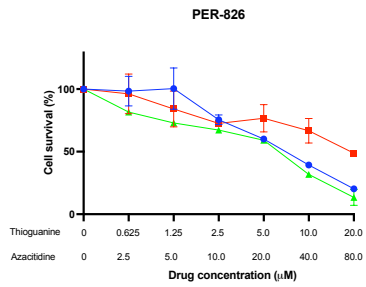
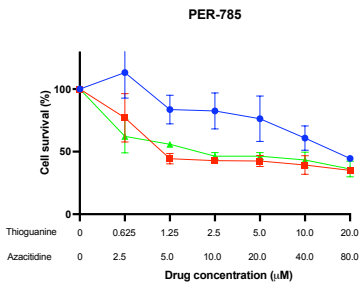
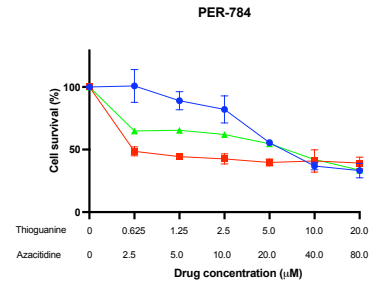
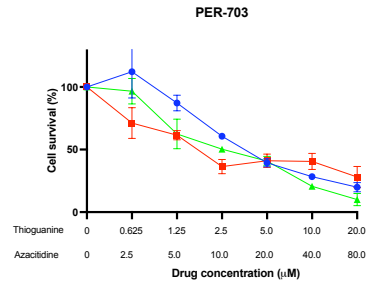
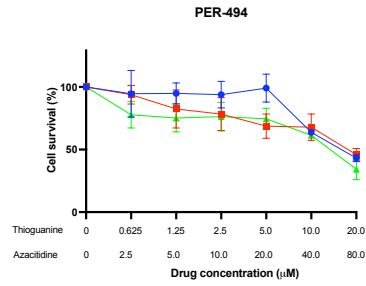
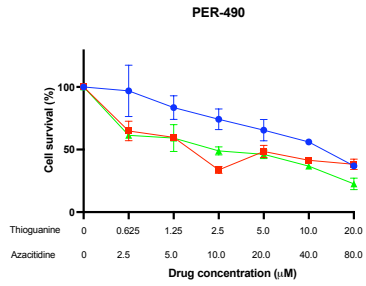
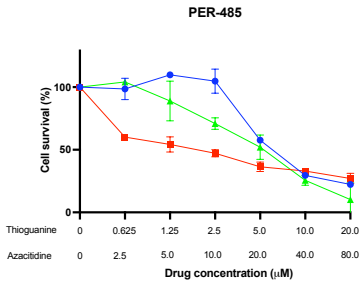




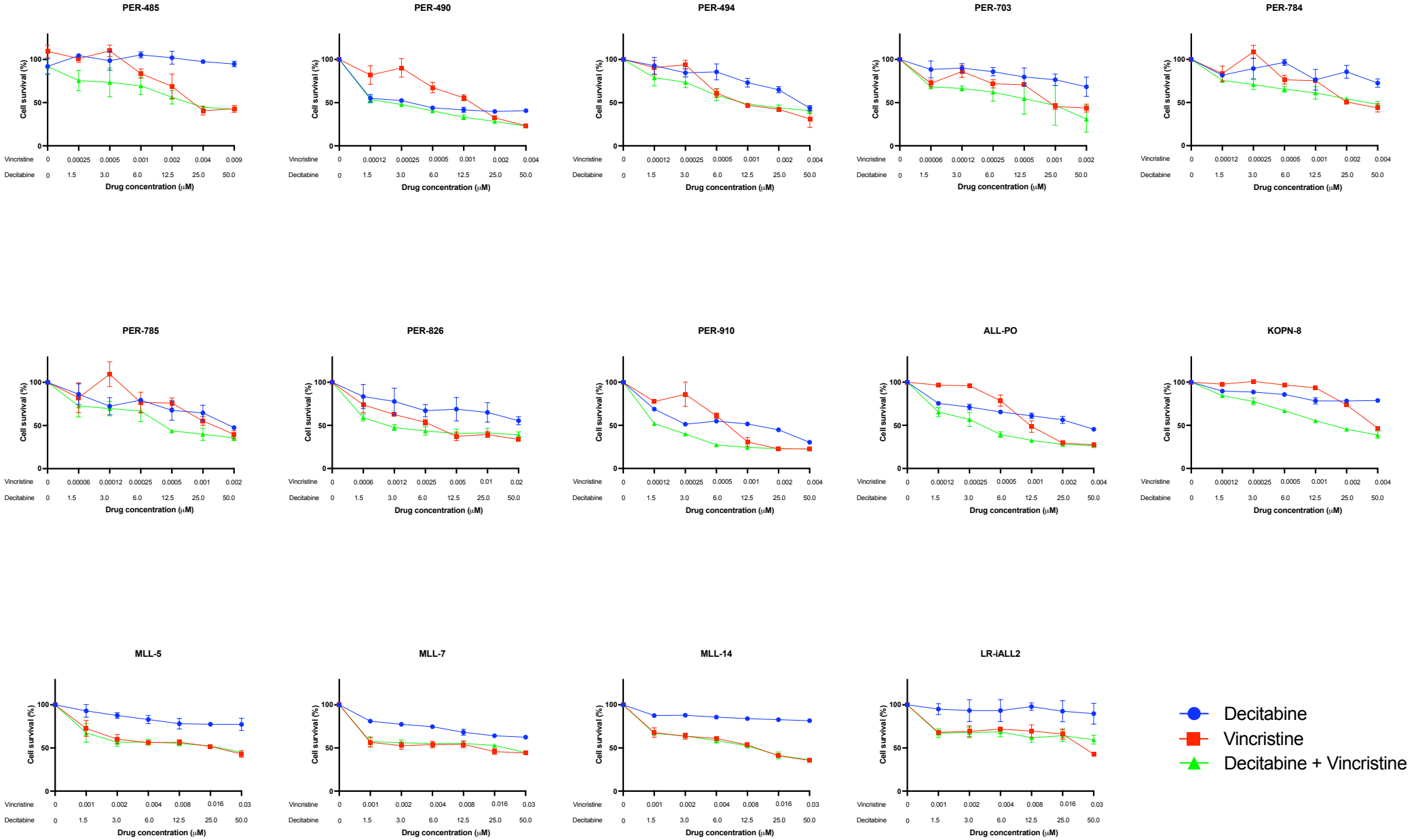


● Azacitidine  
 ■ 6-Mercaptopurine  
 ▲ Azacitidine + 6-Mercaptopurine

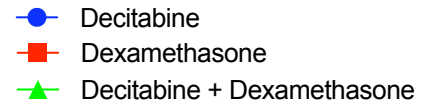
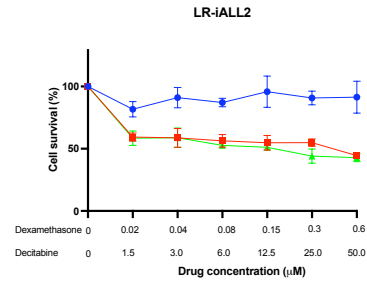
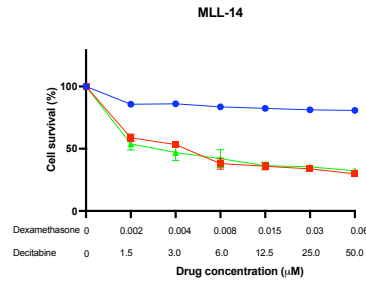
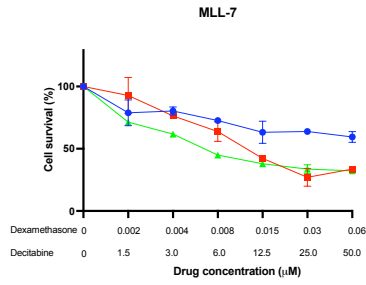
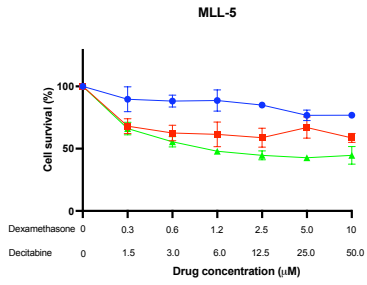
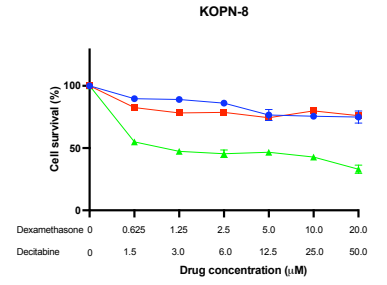
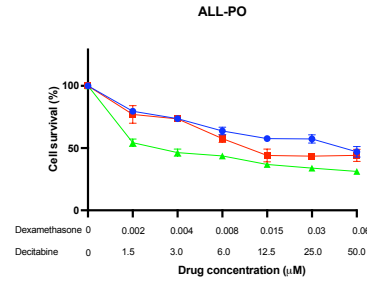
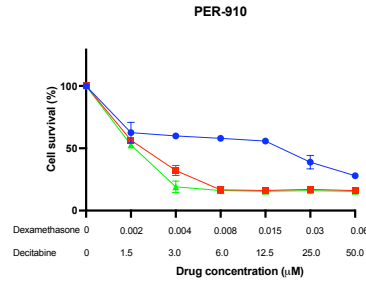
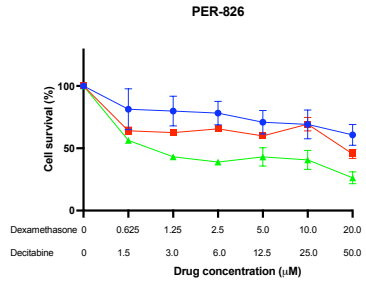
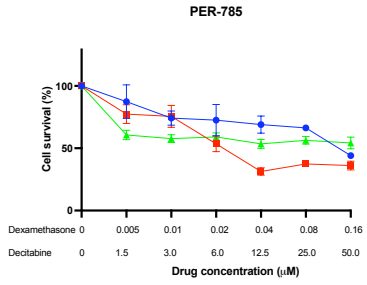
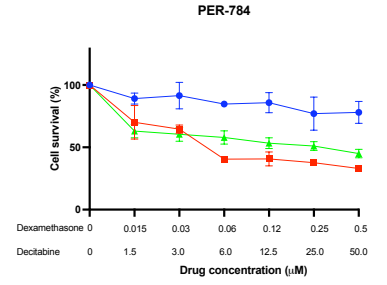
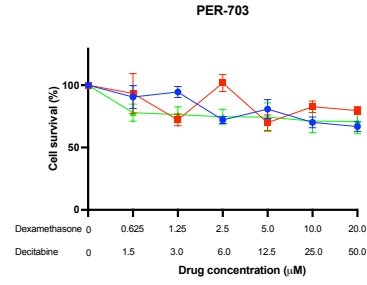
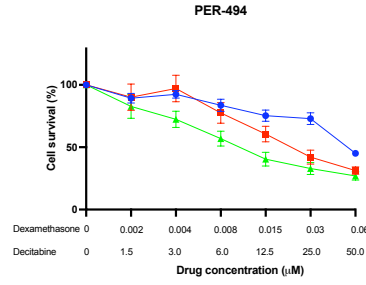
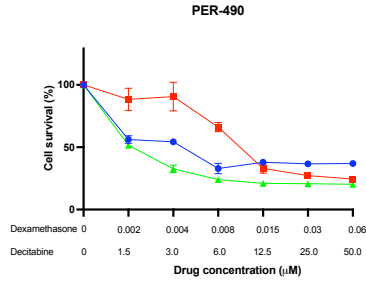
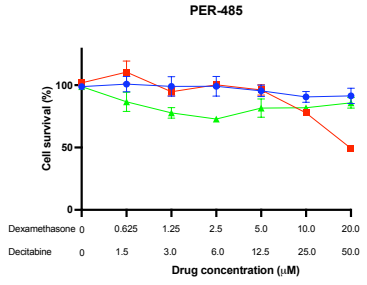


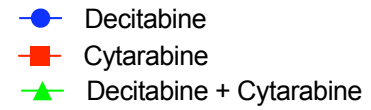
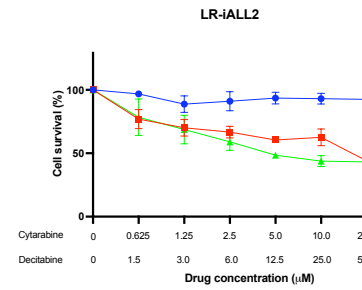
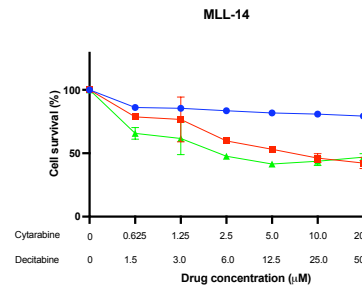
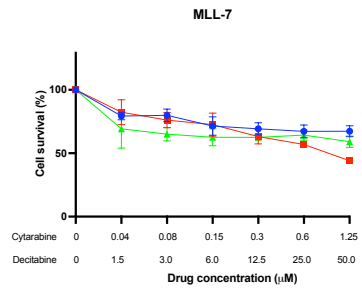
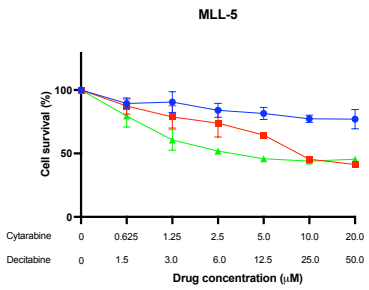
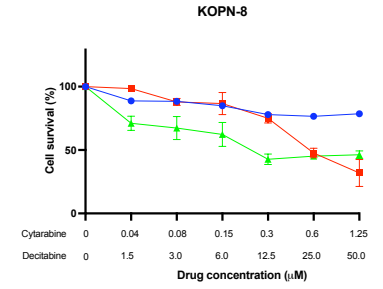
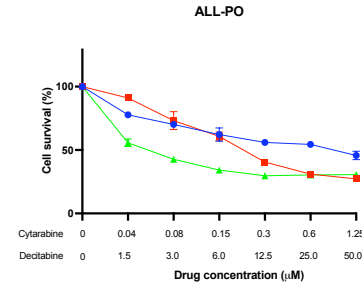
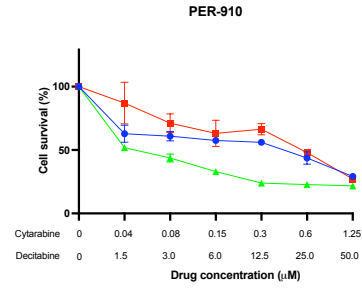
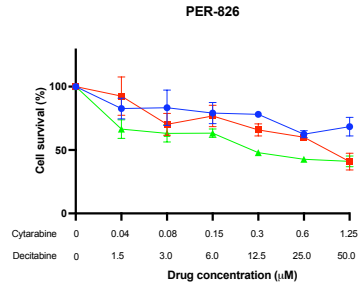
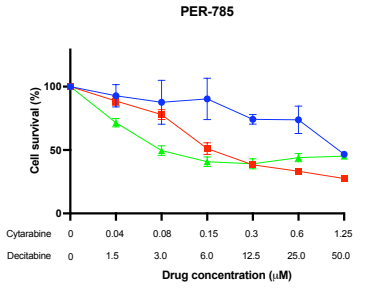
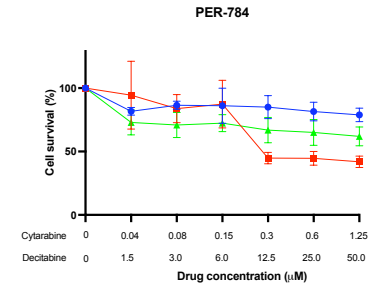
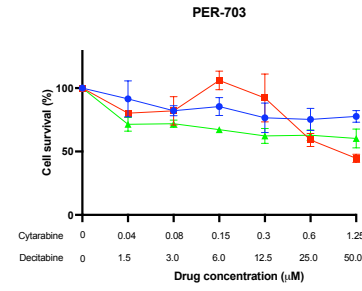
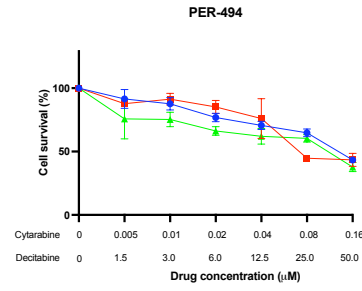
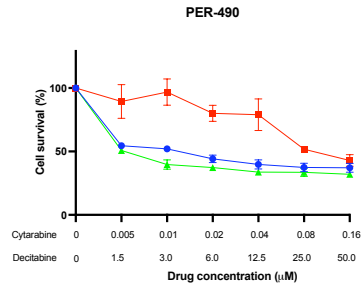
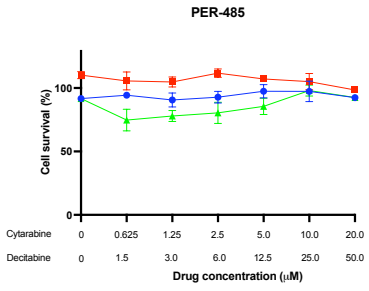




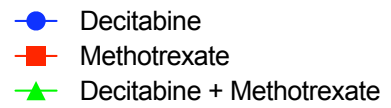
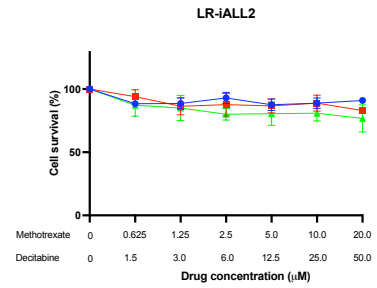
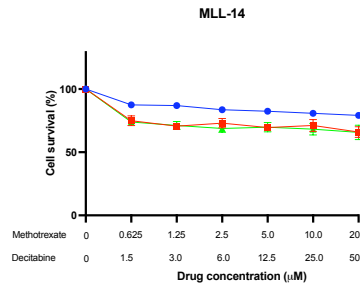
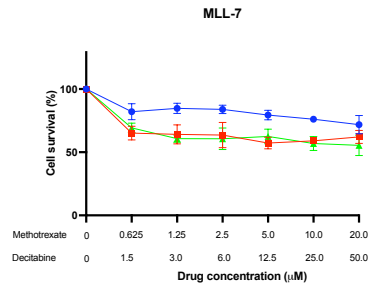
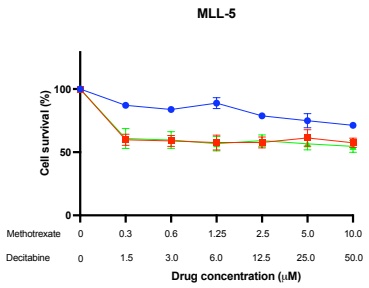
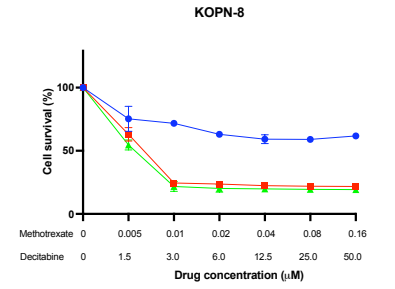
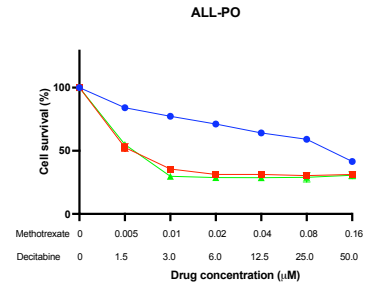
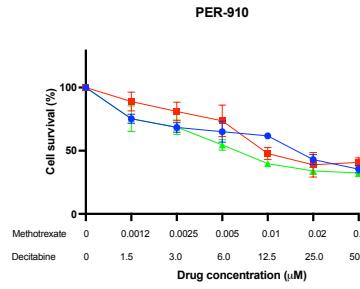
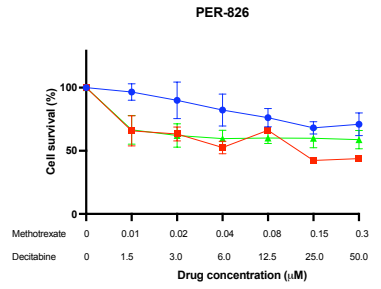
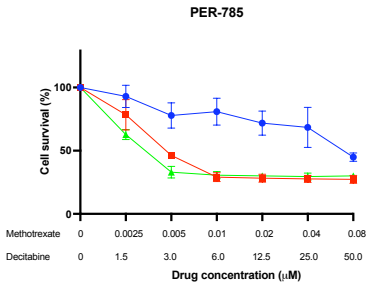
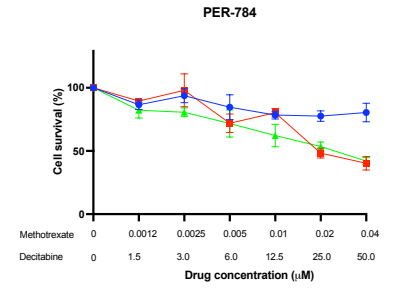
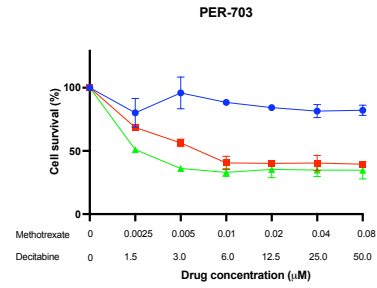
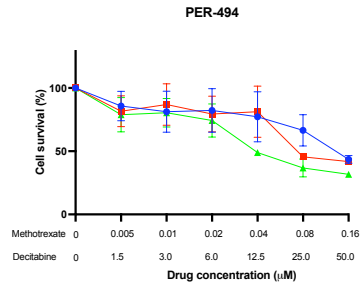
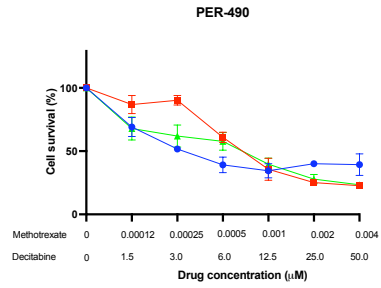
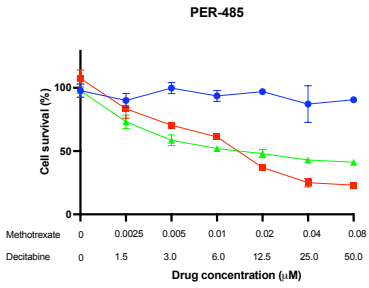
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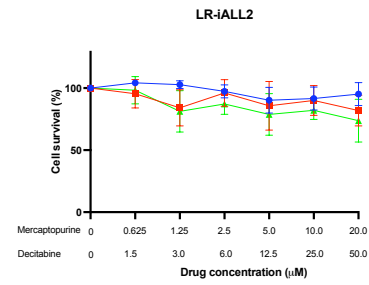
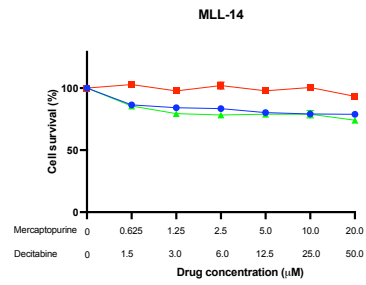
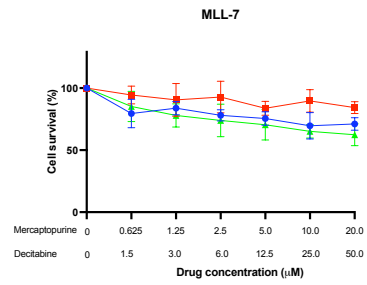
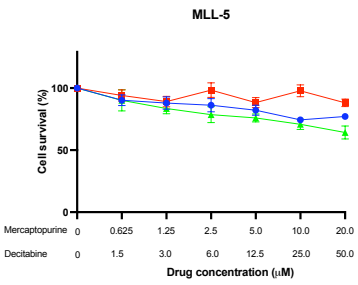
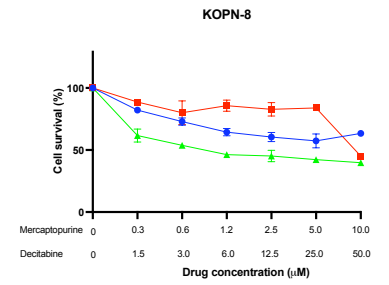
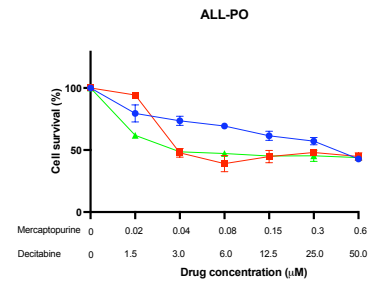
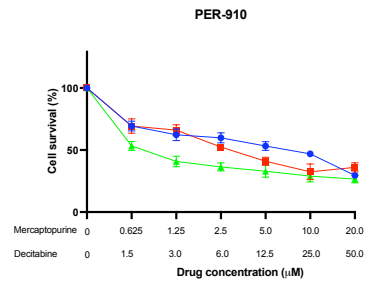
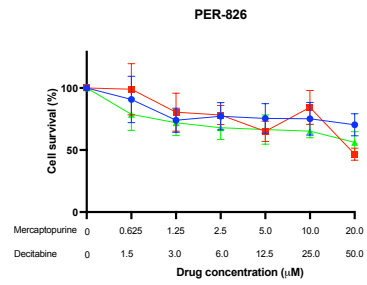
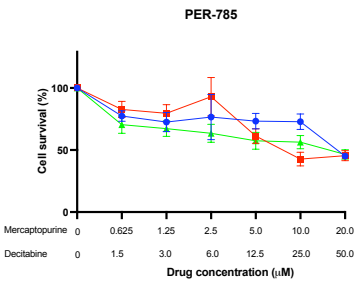
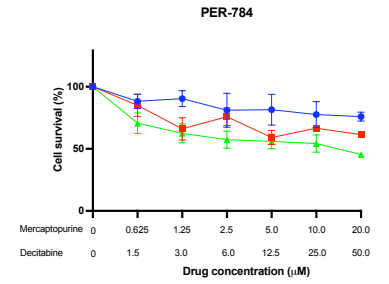
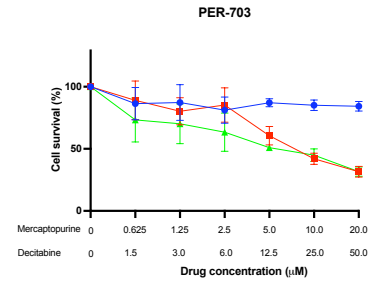
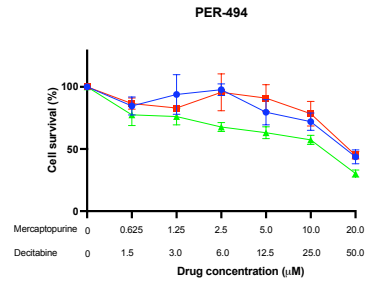
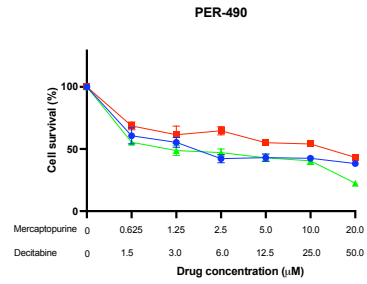
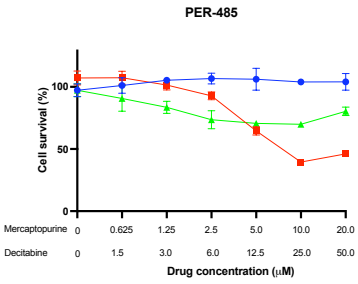




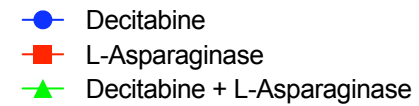
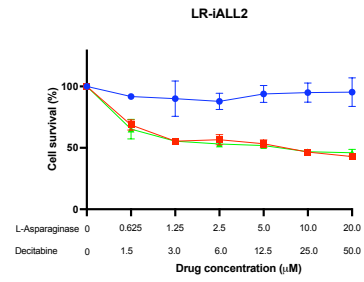
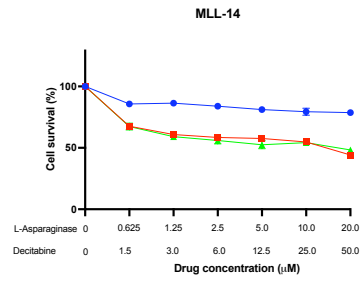
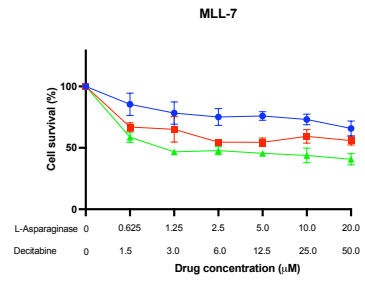
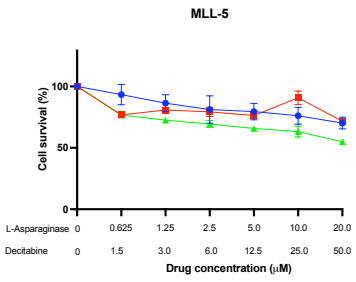
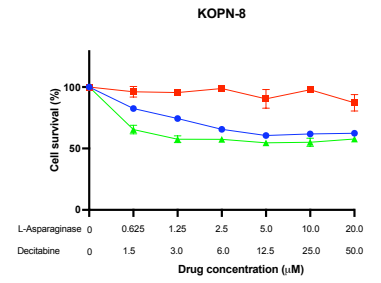
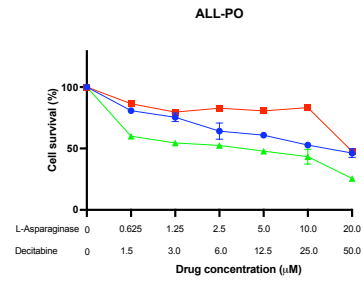
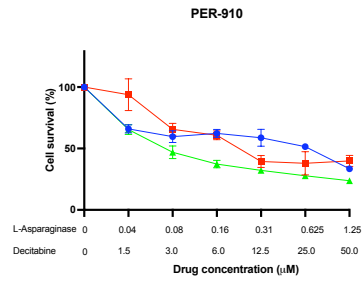
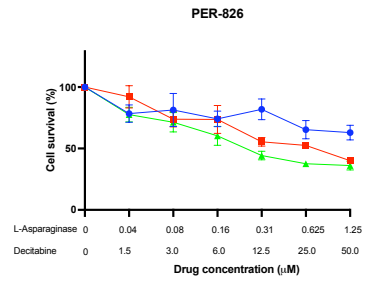
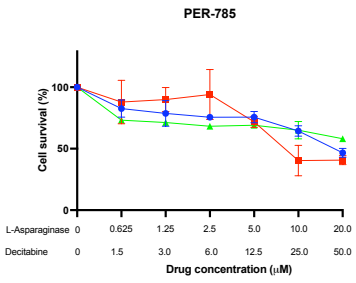
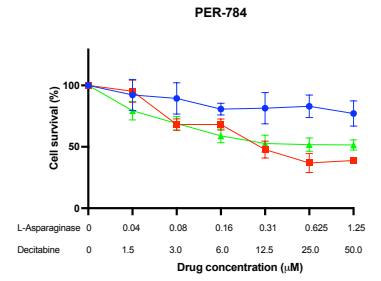
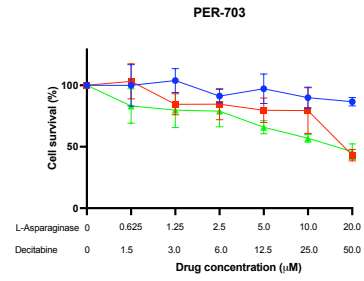
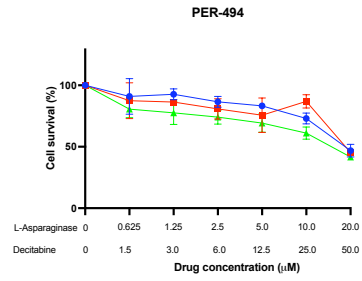
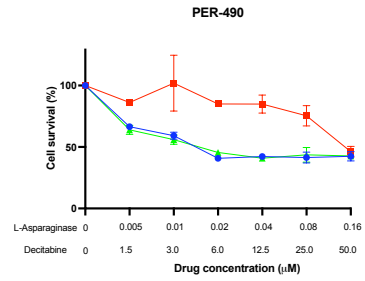
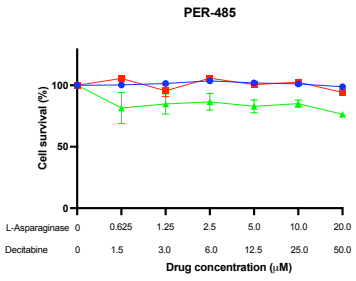


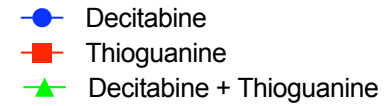
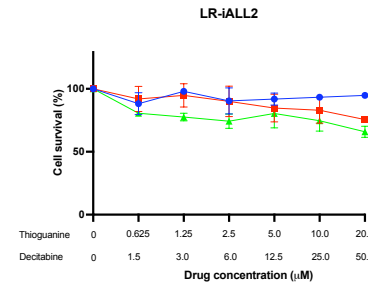
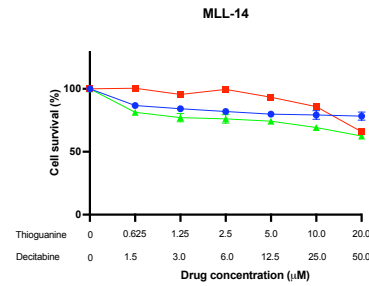
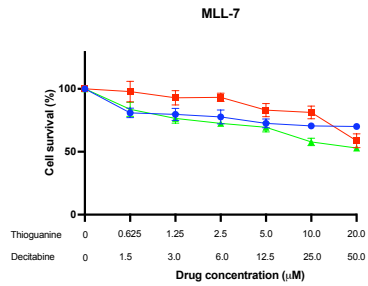
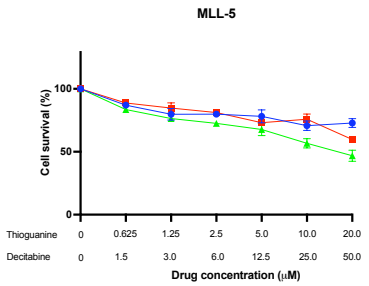
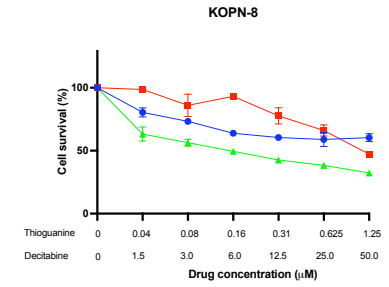
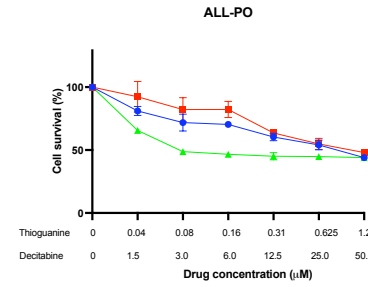
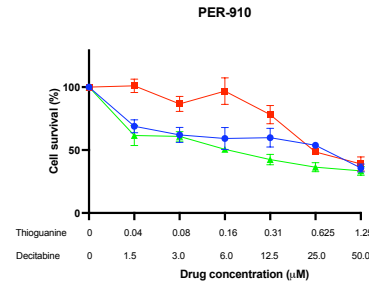
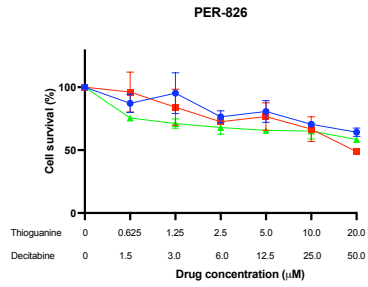
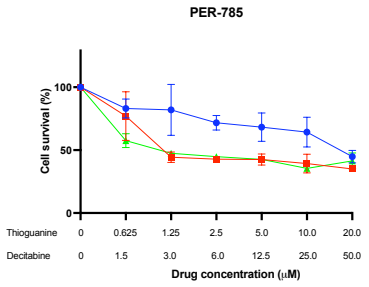
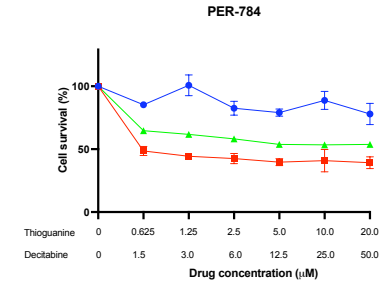
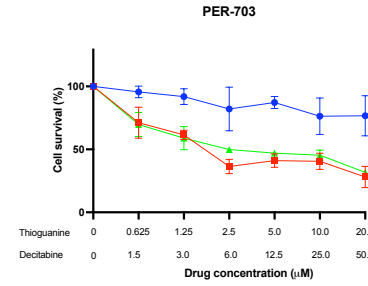
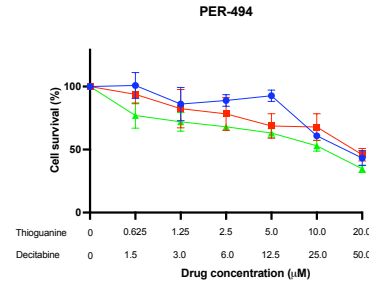
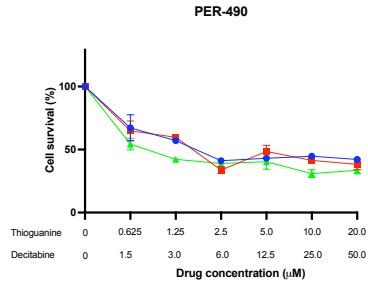
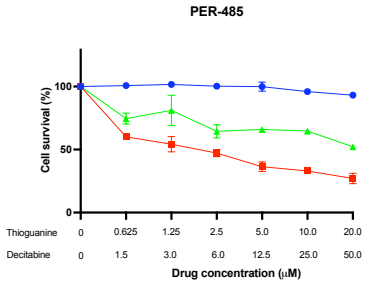






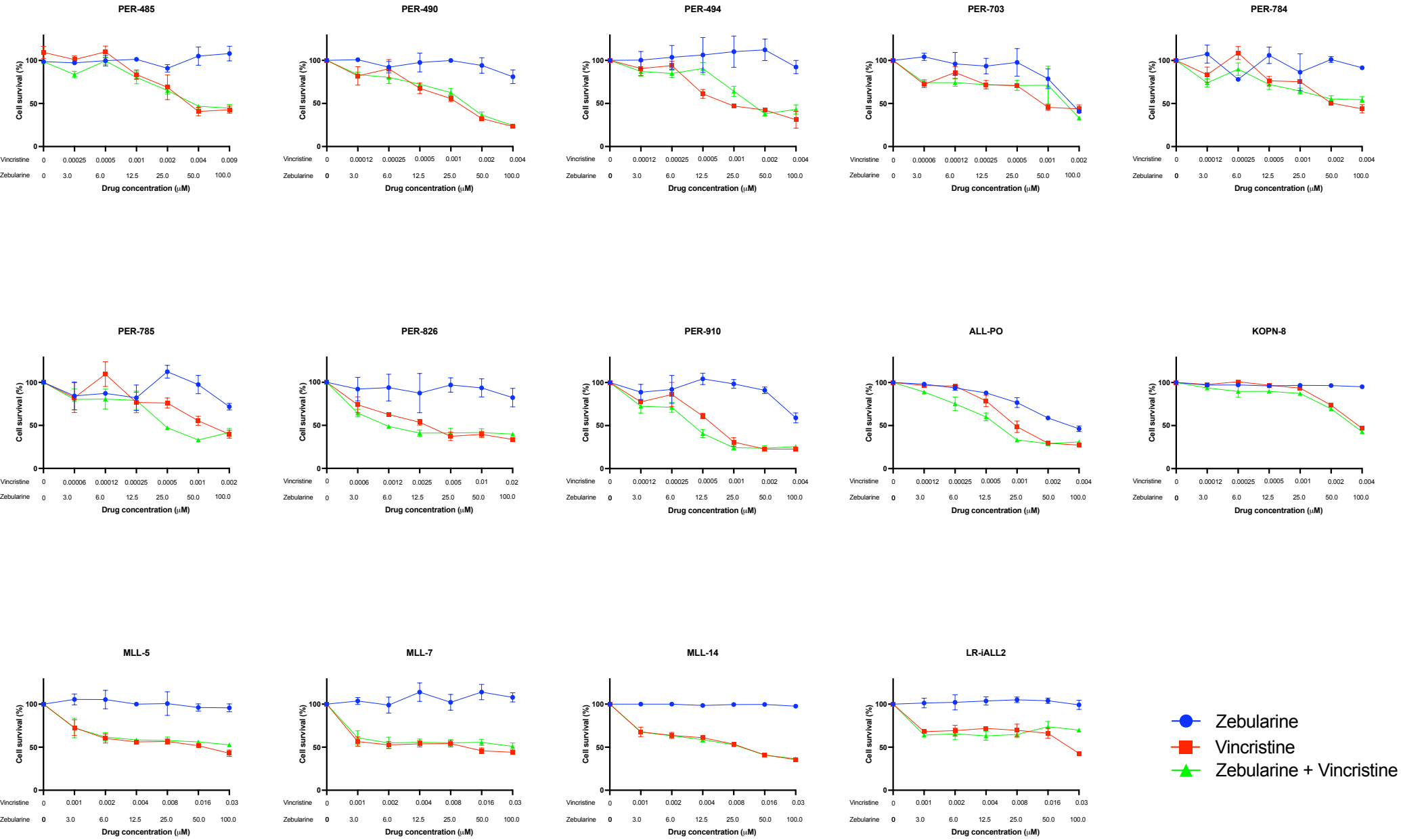
- Decitabine
- 6-Mercaptopurine
- ▲ Decitabine + 6-Mercaptopurine

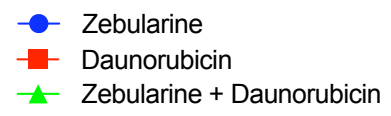
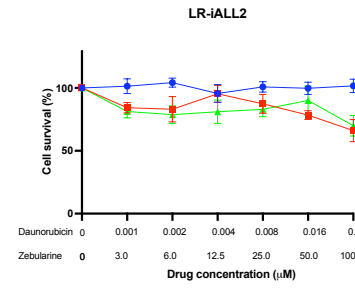
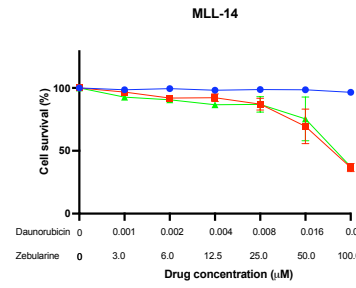
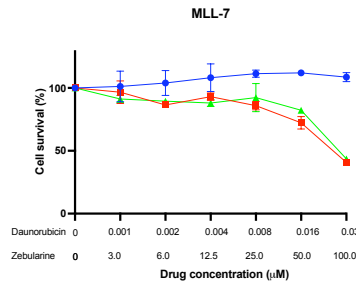
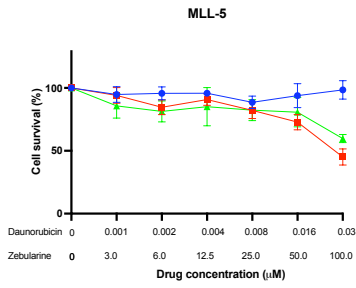
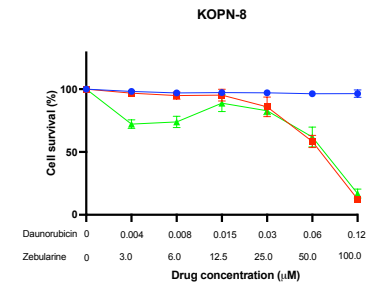
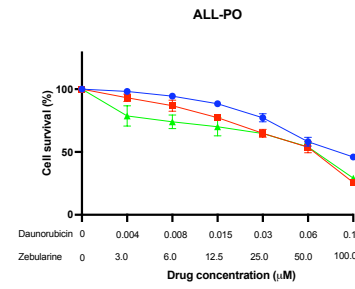
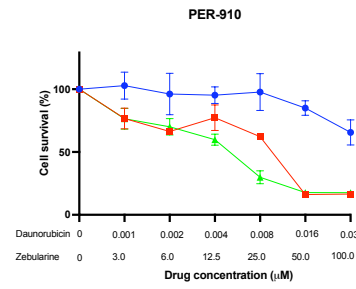
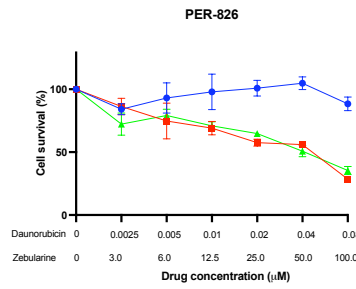
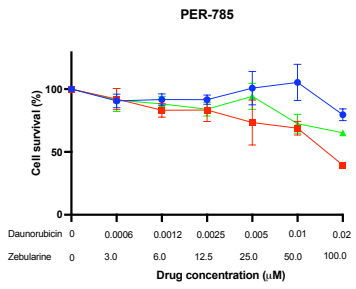
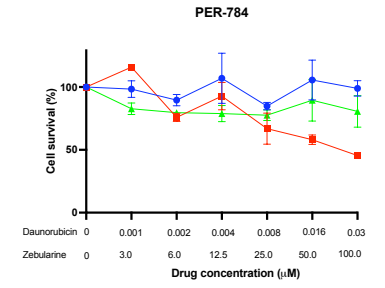
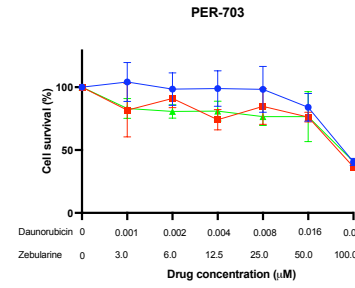
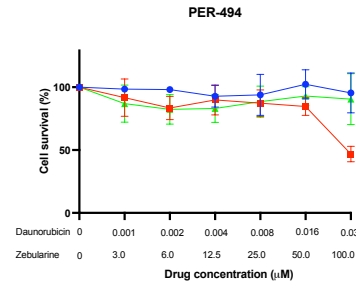
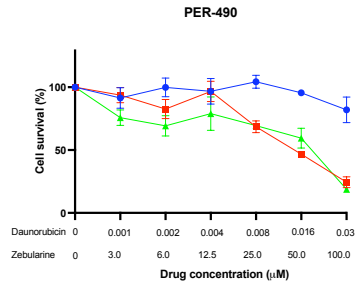
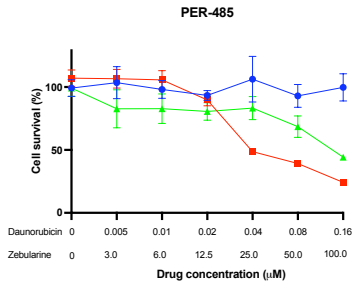


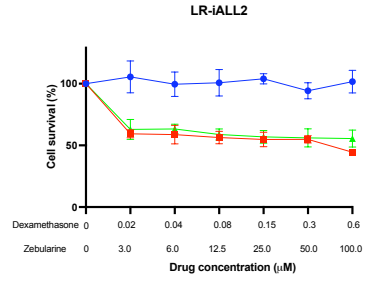
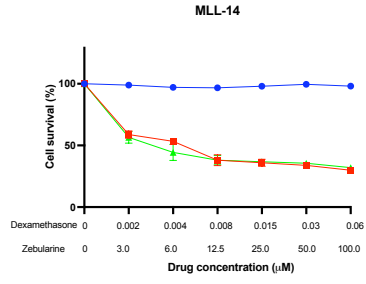
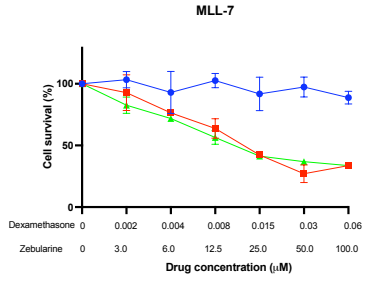
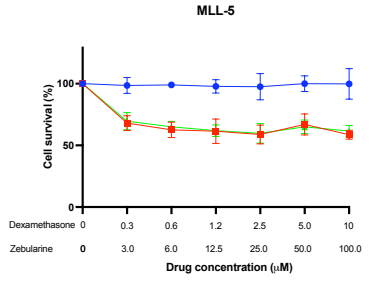
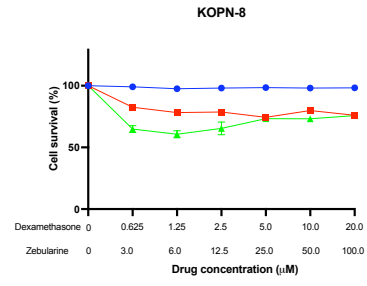
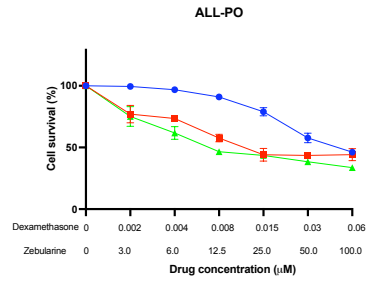
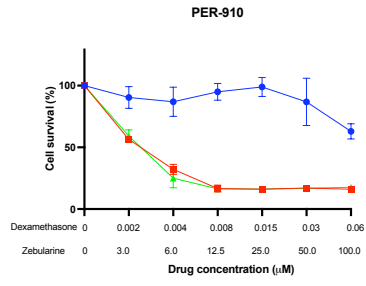
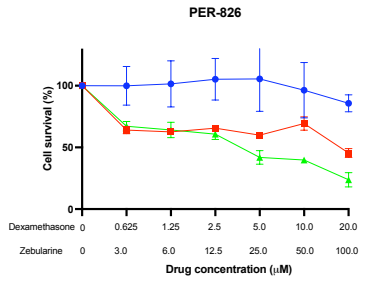
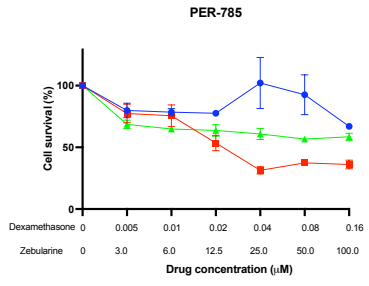
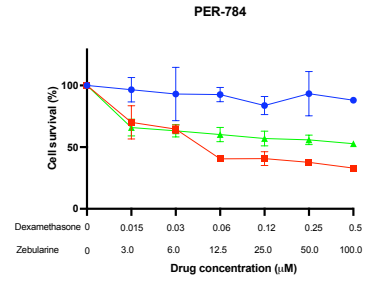
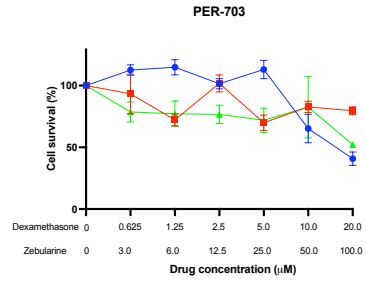
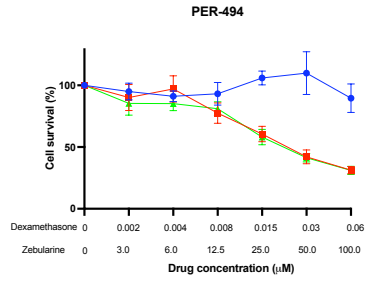
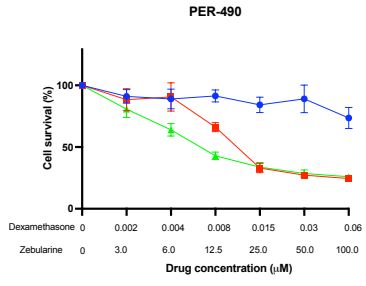
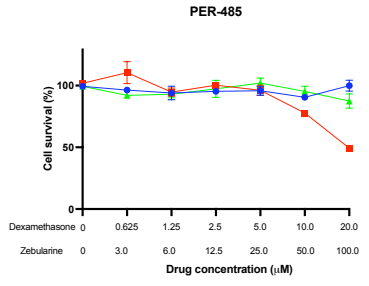




C





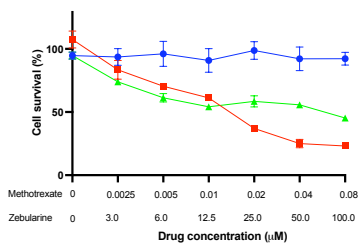


- Zebularine
- Dexamethasone
- ▲ Zebularine + Dexamethasone

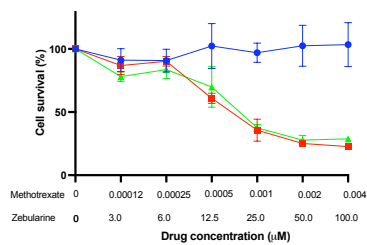




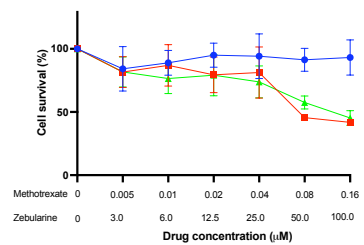
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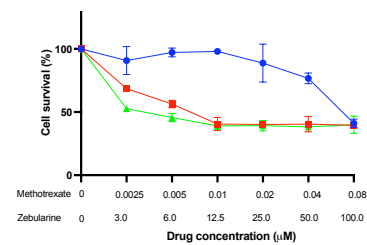
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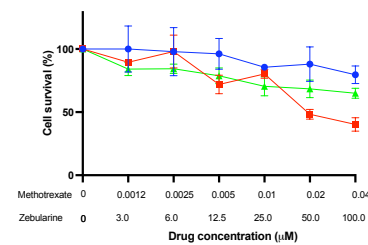
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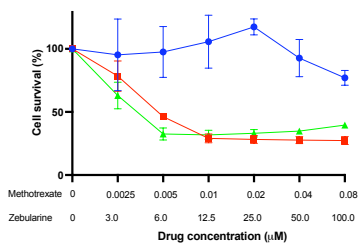
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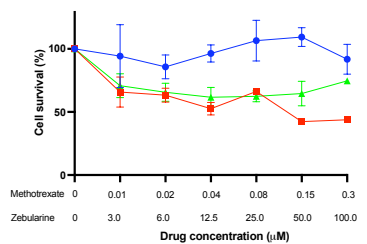
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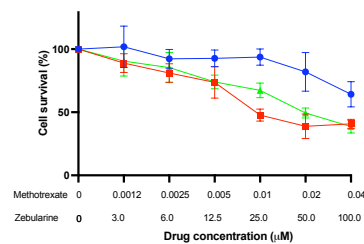
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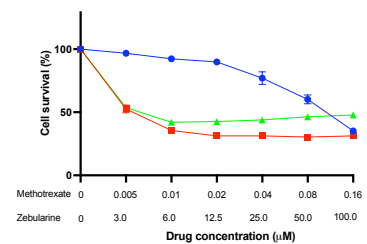
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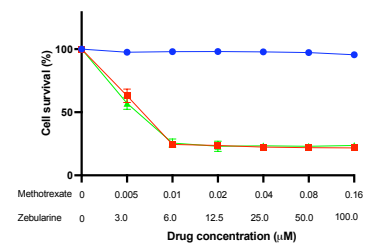
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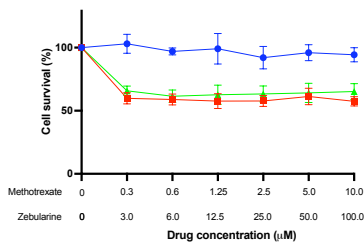
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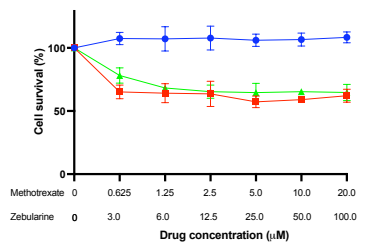
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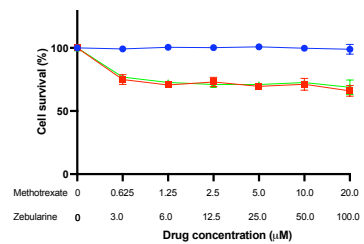
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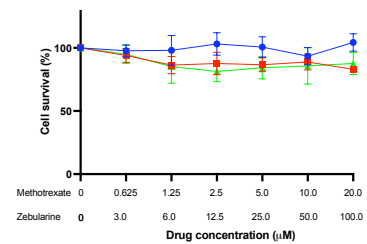
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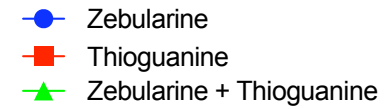
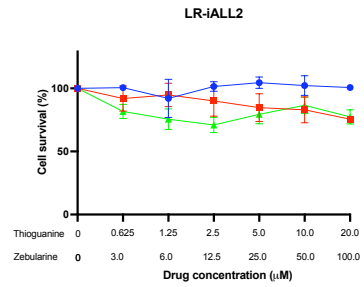
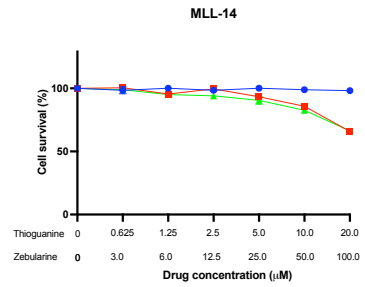
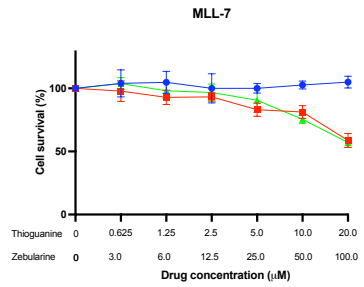
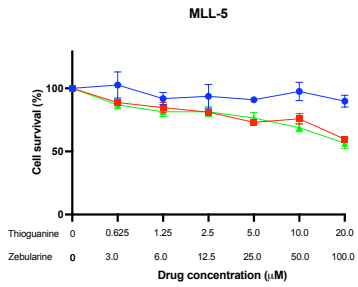
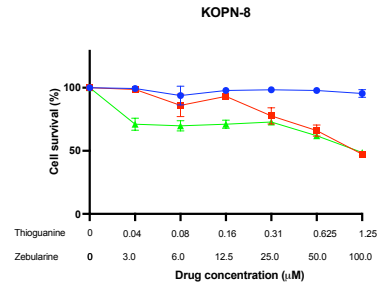
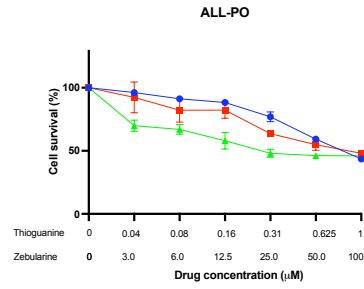
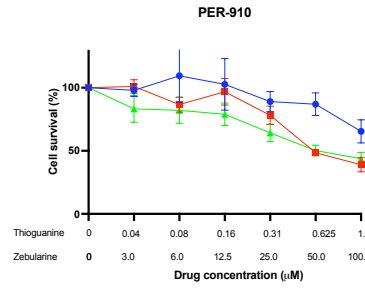
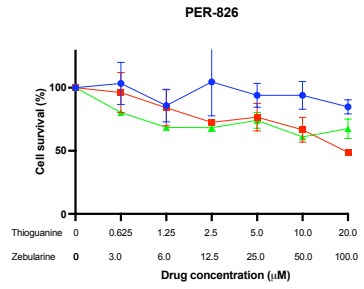
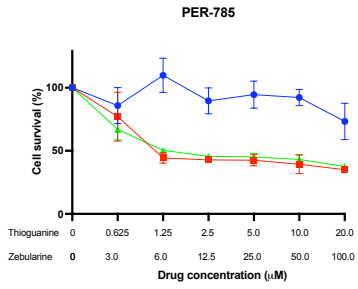
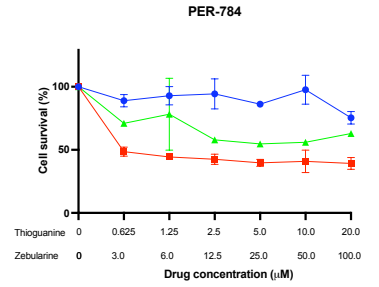
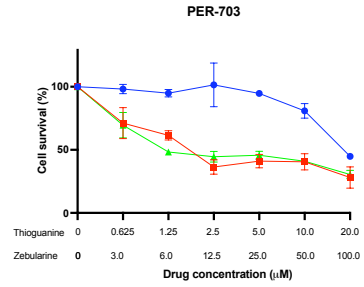
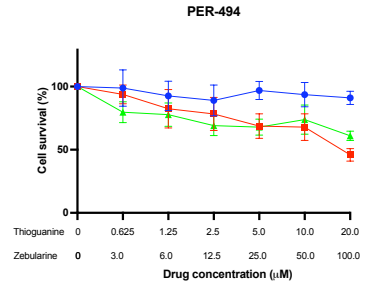
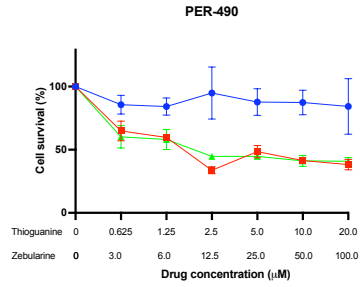
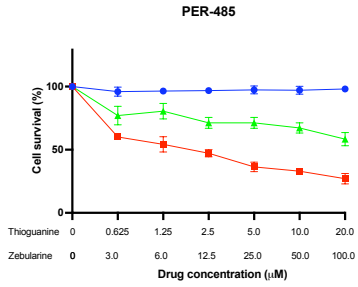
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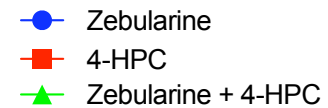
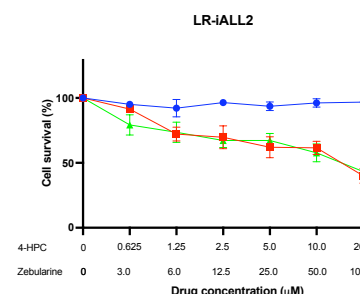
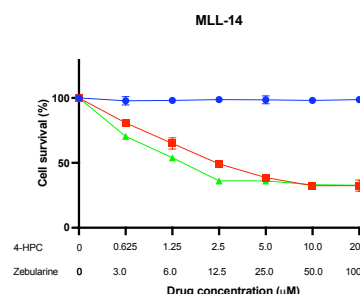
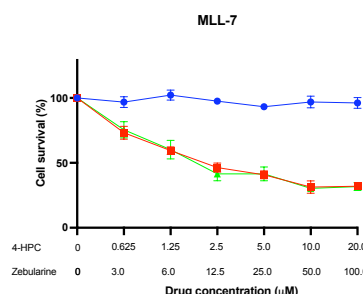
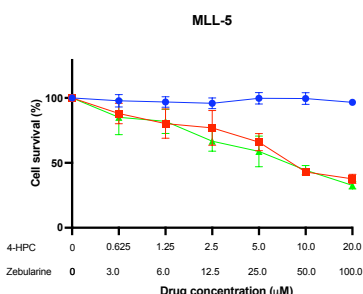
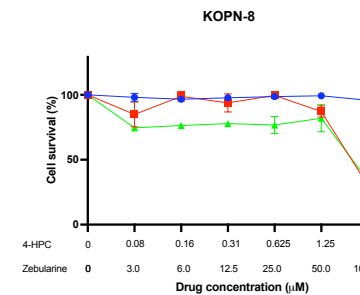
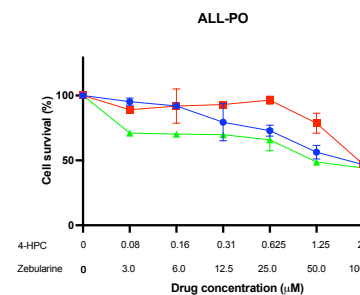
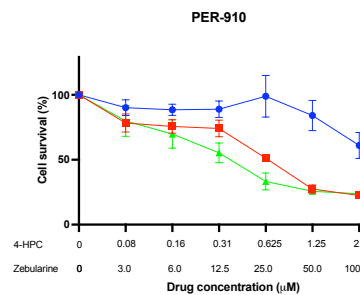
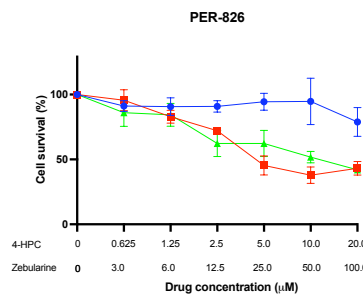
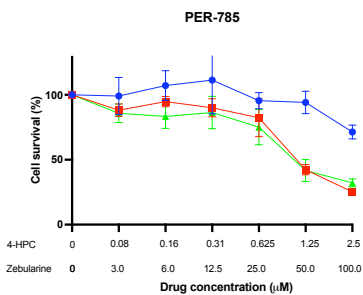
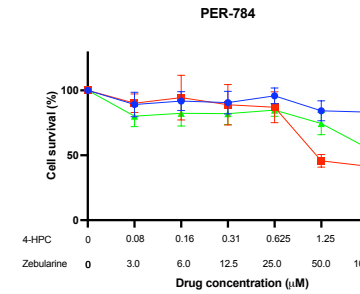
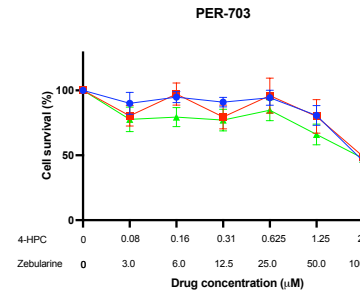
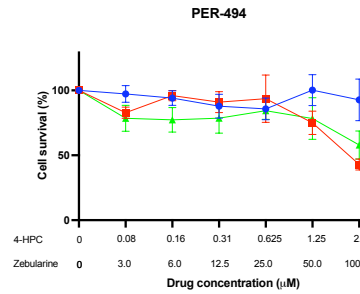
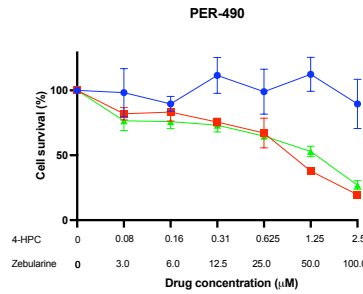
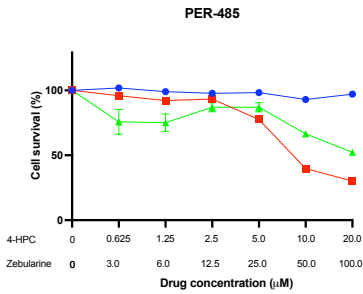


● Zebularine  
■ Methotrexate  
▲ Zebularine + Methotrexate



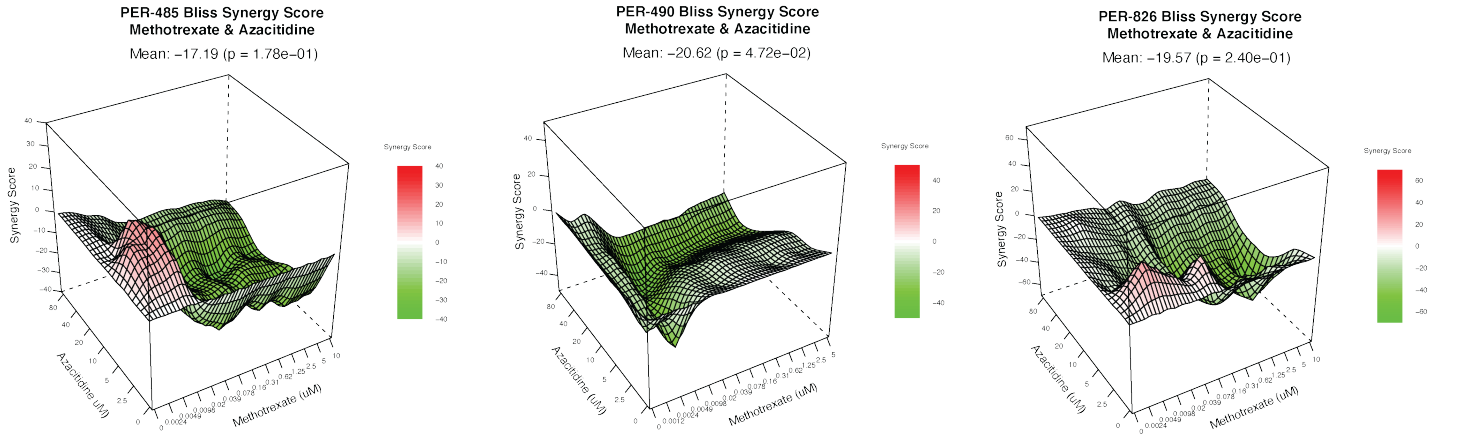




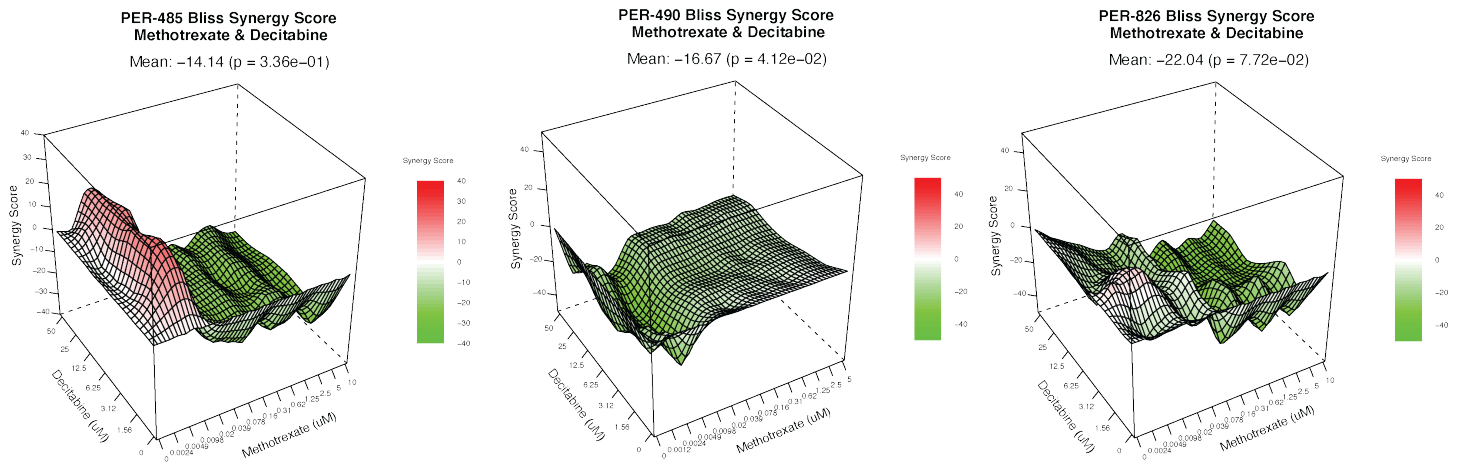


**Supplemental Figure S5. Antagonistic landscape of methotrexate in combination with (A) azacitidine or (B) decitabine in three *KMT2A*-rearranged infant acute lymphoblastic leukemia cell lines. Positive Bliss synergy scores indicate synergy and negative Bliss synergy scores indicate antagonism. Representative plots from three biological replicates shown.**

**A**

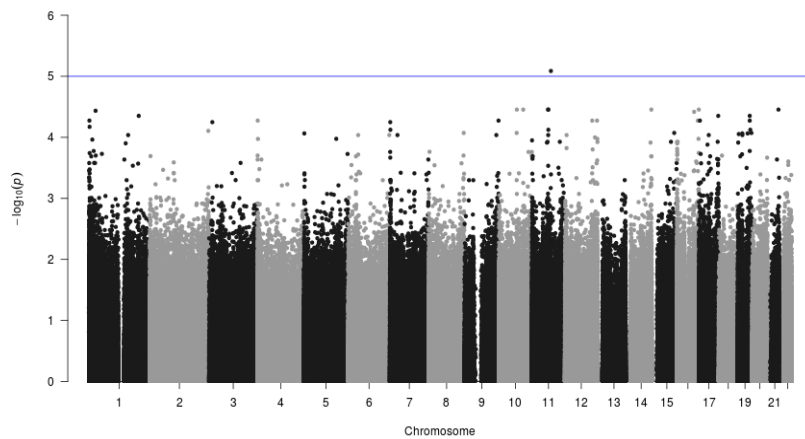


**B**



**Supplemental Figure S6.** (A) Manhattan plot showing differential methylation profile across genome-wide CpG sites of six *KMT2A*-rearranged infant acute lymphoblastic leukemia (ALL) cell lines treated with low dose (6.0 $\mu$ M) zebularine in comparison to untreated controls. There were no differentially methylated sites that reached the genome-wide significance threshold of  $5 \times 10^{-8}$ . The blue line indicates suggestive significance, valued at  $1 \times 10^{-5}$ . (B) Heatmaps featuring the expression levels (average log counts per million) of the most significant differentially expressed genes following treatment with azacitidine and decitabine across all six cell lines in contrast to untreated controls. (C) Volcano plots displaying the top differentially expressed genes from the transcriptome of the six cell lines following treatment with azacitidine and decitabine. Horizontal and vertical dashed lines indicate fold change and significance thresholds. (D) Heatmap displaying the normalized enrichment scores (NES) from gene set enrichment analyses performed on ranked differentially expressed genes following treatment with decitabine. NES are shown for each of the six cell lines and after merging the transcriptome of all six cell lines. Positive (red) and negative (blue) NES scores indicate enrichment of a specific gene set following treatment and in untreated controls, respectively. Gene sets with a false discovery rate  $< 0.1$  were considered significant. (E) Scatter plot comparing the mean beta fold change values, indicating hypomethylation (x axis), and  $\log_2$  fold change of the expression levels (y axis) for genes within the 617 hypomethylated regions following treatment with azacitidine and for genes within the 471 hypomethylated regions following treatment with decitabine. Fold changes for each gene were extracted from the merged comparison of all cell lines treated with azacitidine or decitabine against untreated controls. The mean beta fold change values indicate hypomethylation level ( $< -0.1$ ). Genes with  $\log_2$  fold change higher than 1 (dashed line) and false discovery rate  $< 0.05$  (red dots) are labelled. (F) Expression levels ( $\log_2$  fold change) of *MMP15*, *BAIAP3* and *CD82* measured by real-time quantitative PCR, in an extended cohort of 13 *KMT2A*-rearranged infant ALL *in vitro* models after treatment with azacitidine and decitabine. Mean expression values of three biological replicates with standard error of the mean are displayed for each model. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

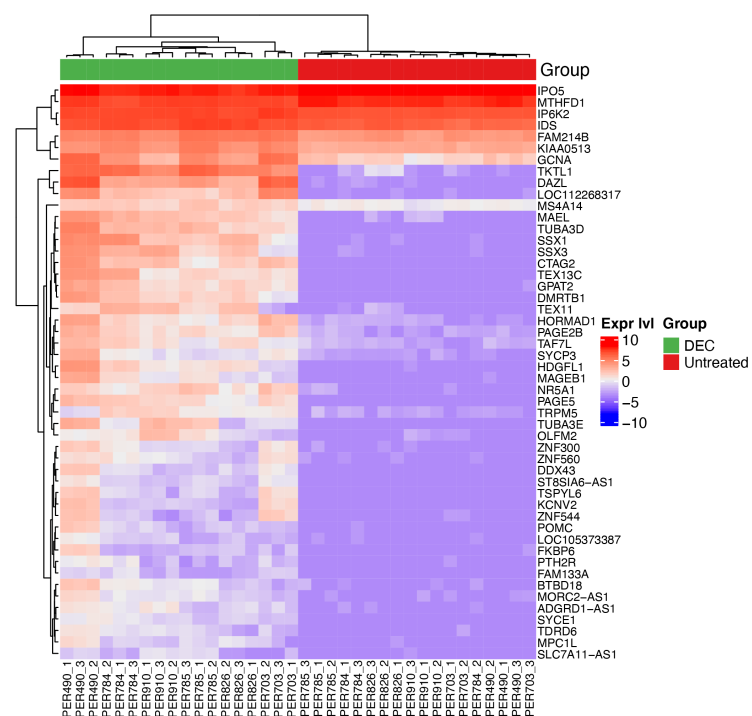
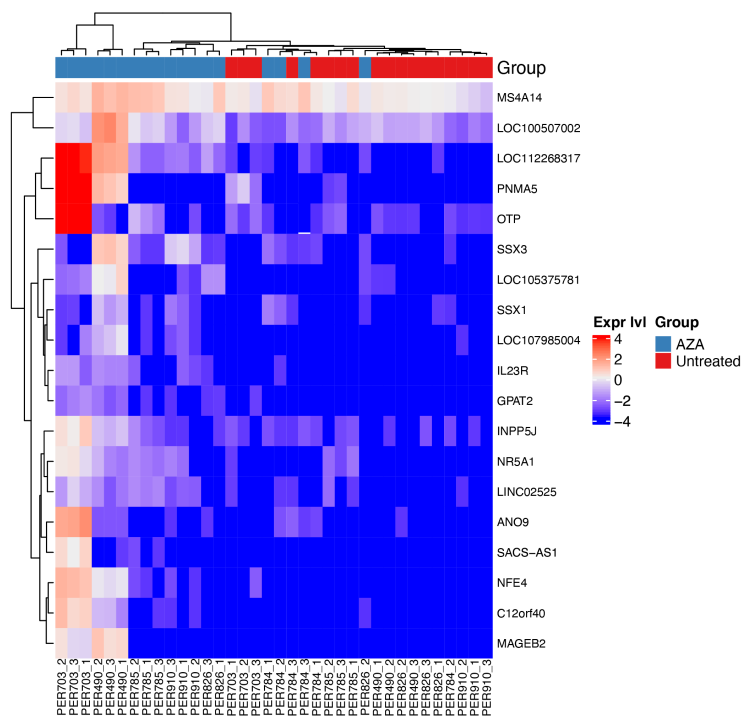
**A**



**B**

**Azacitidine**

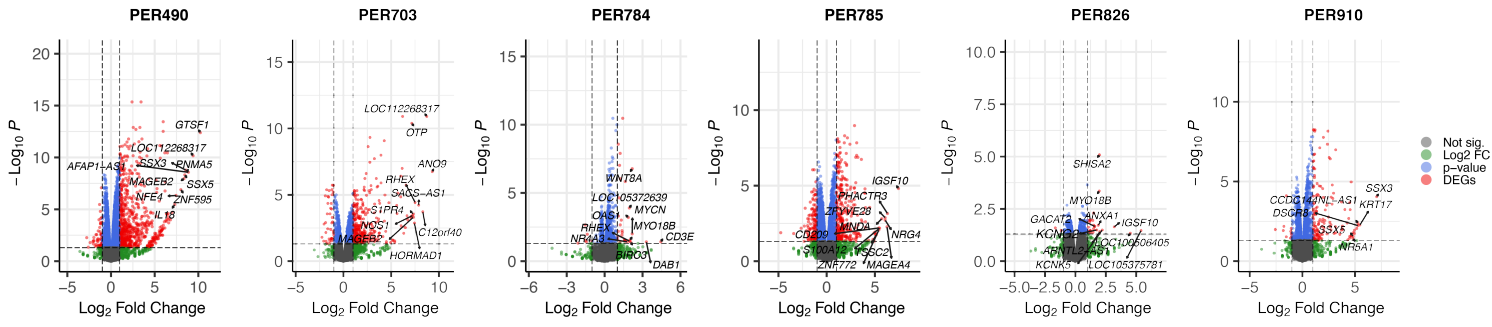
**Decitabine**



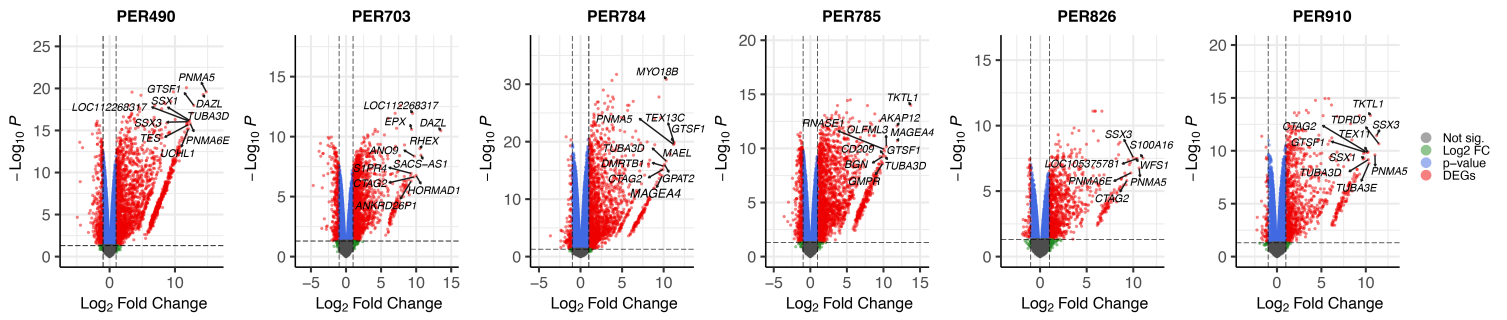


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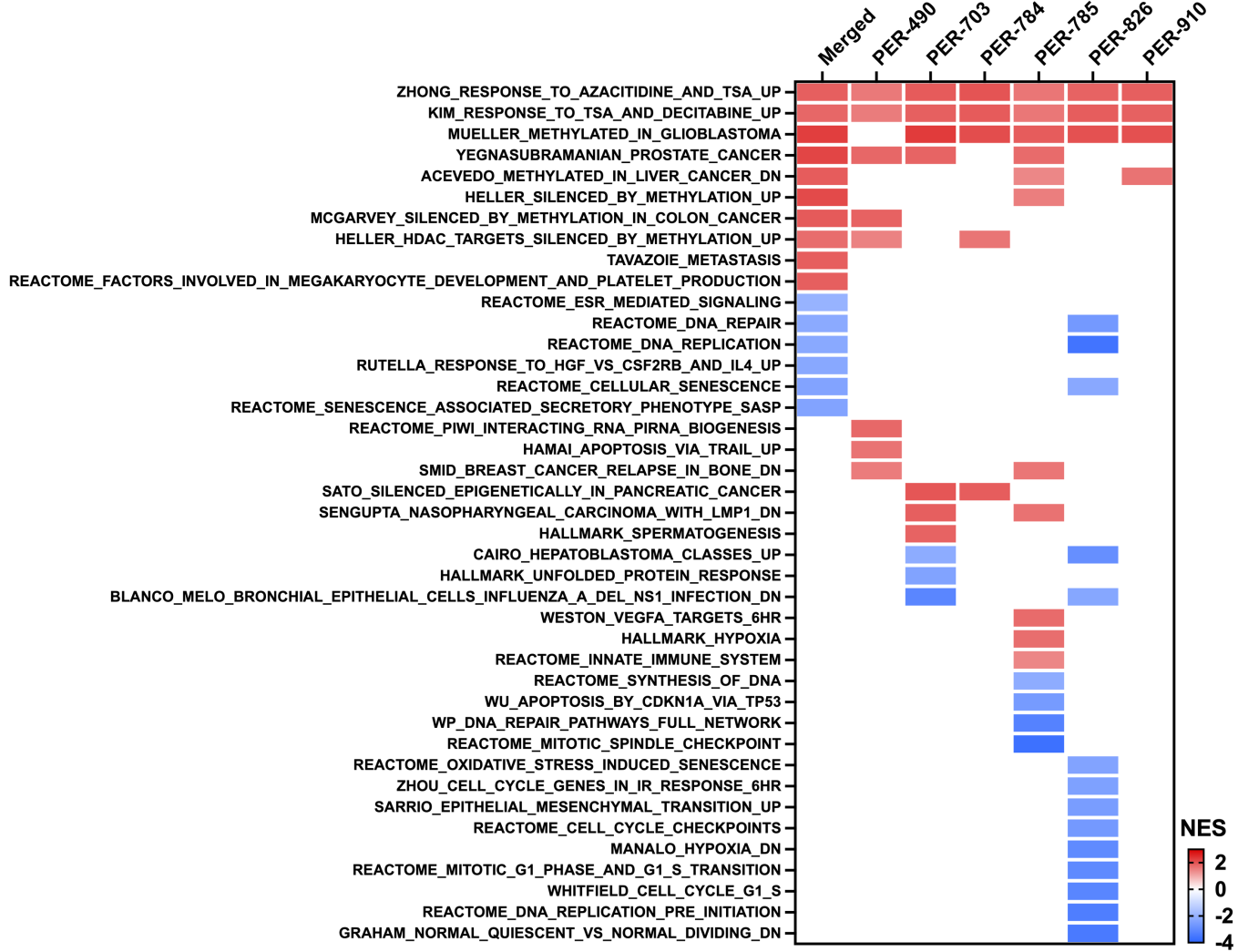
### Azacitidine



### Decitabine

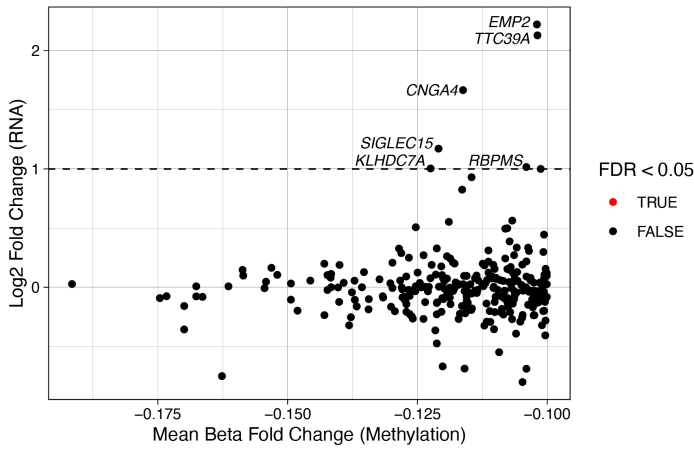


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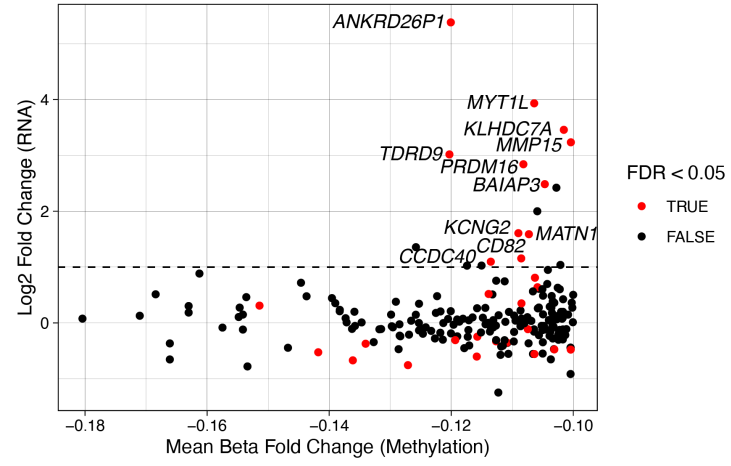


E

## Azacitidine

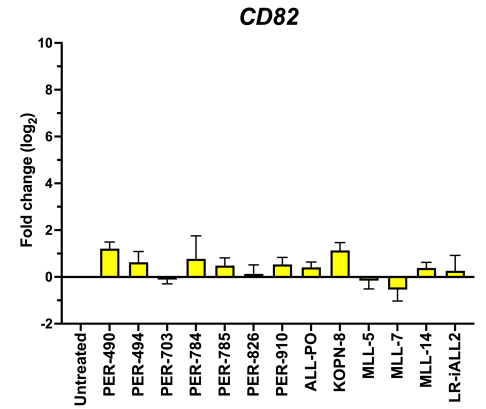
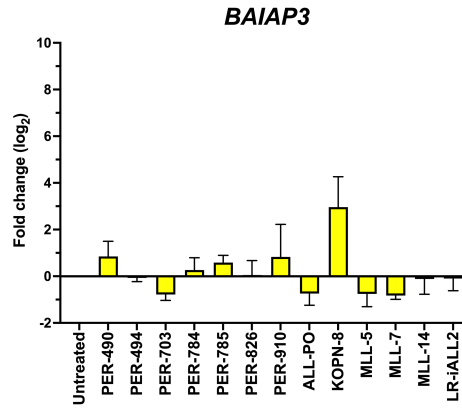
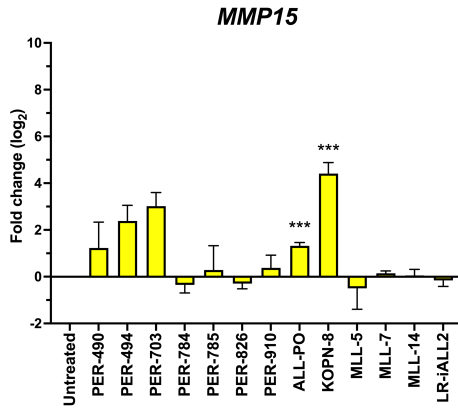


## Decitabine

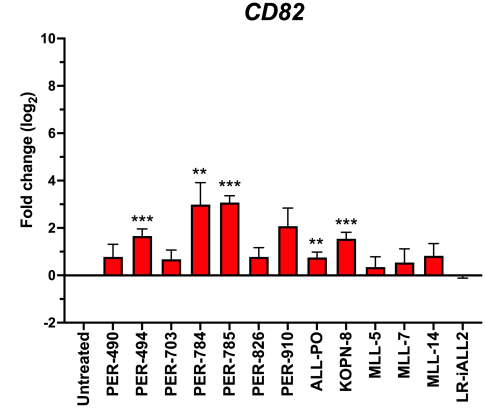
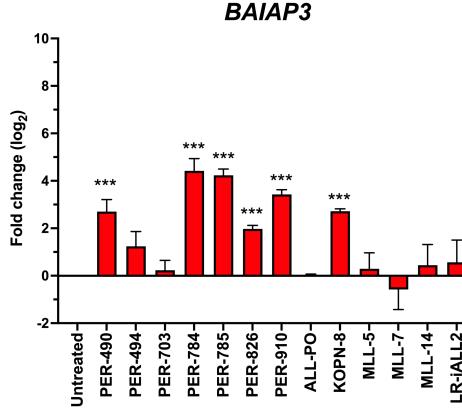
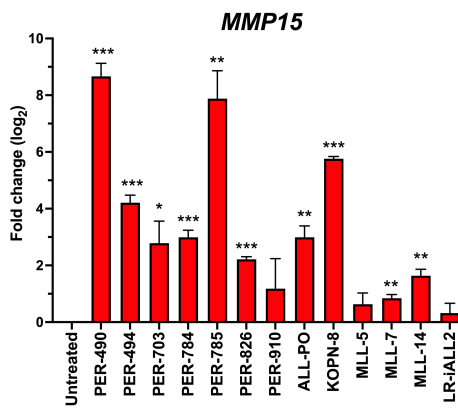


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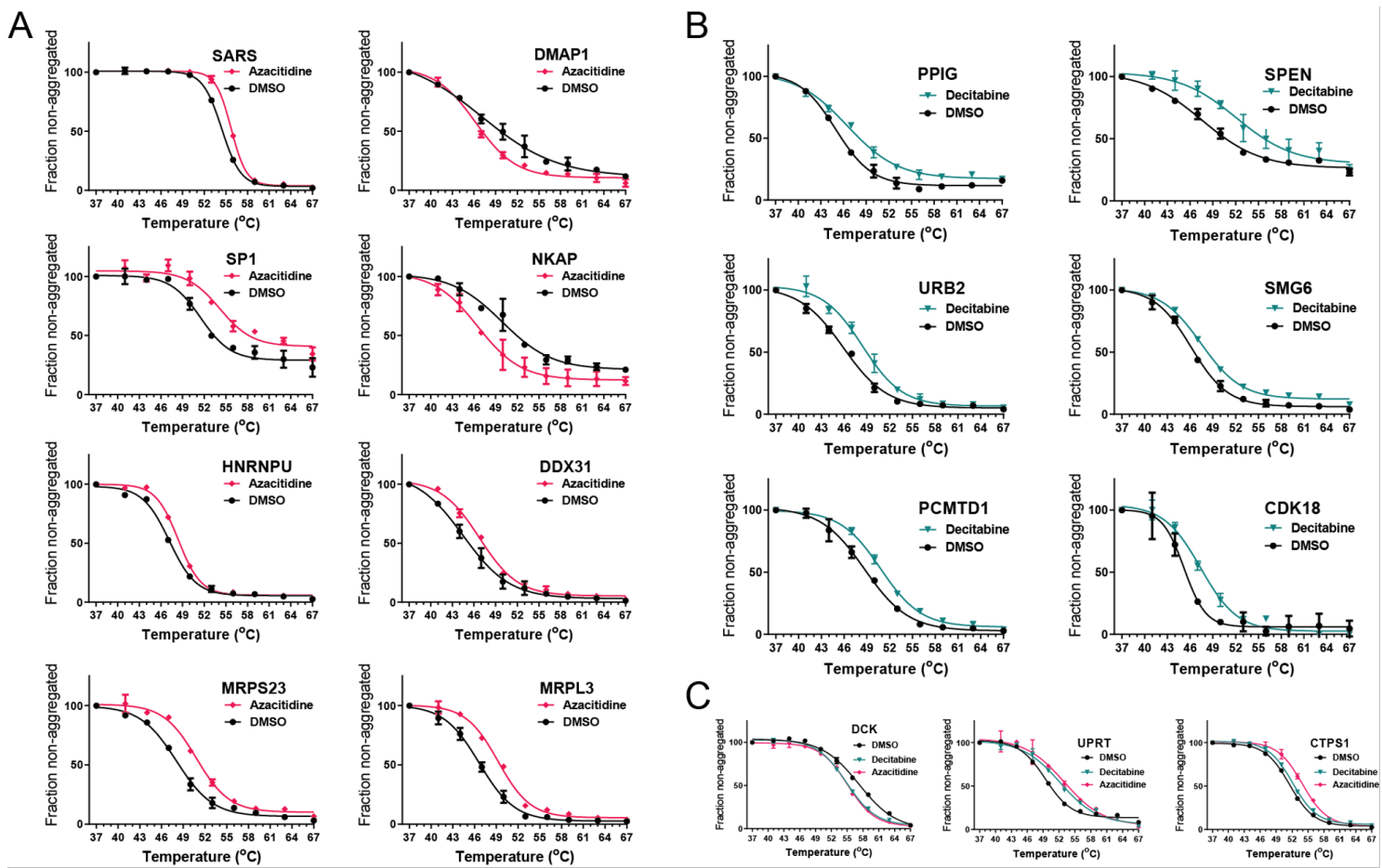
## Azacitidine



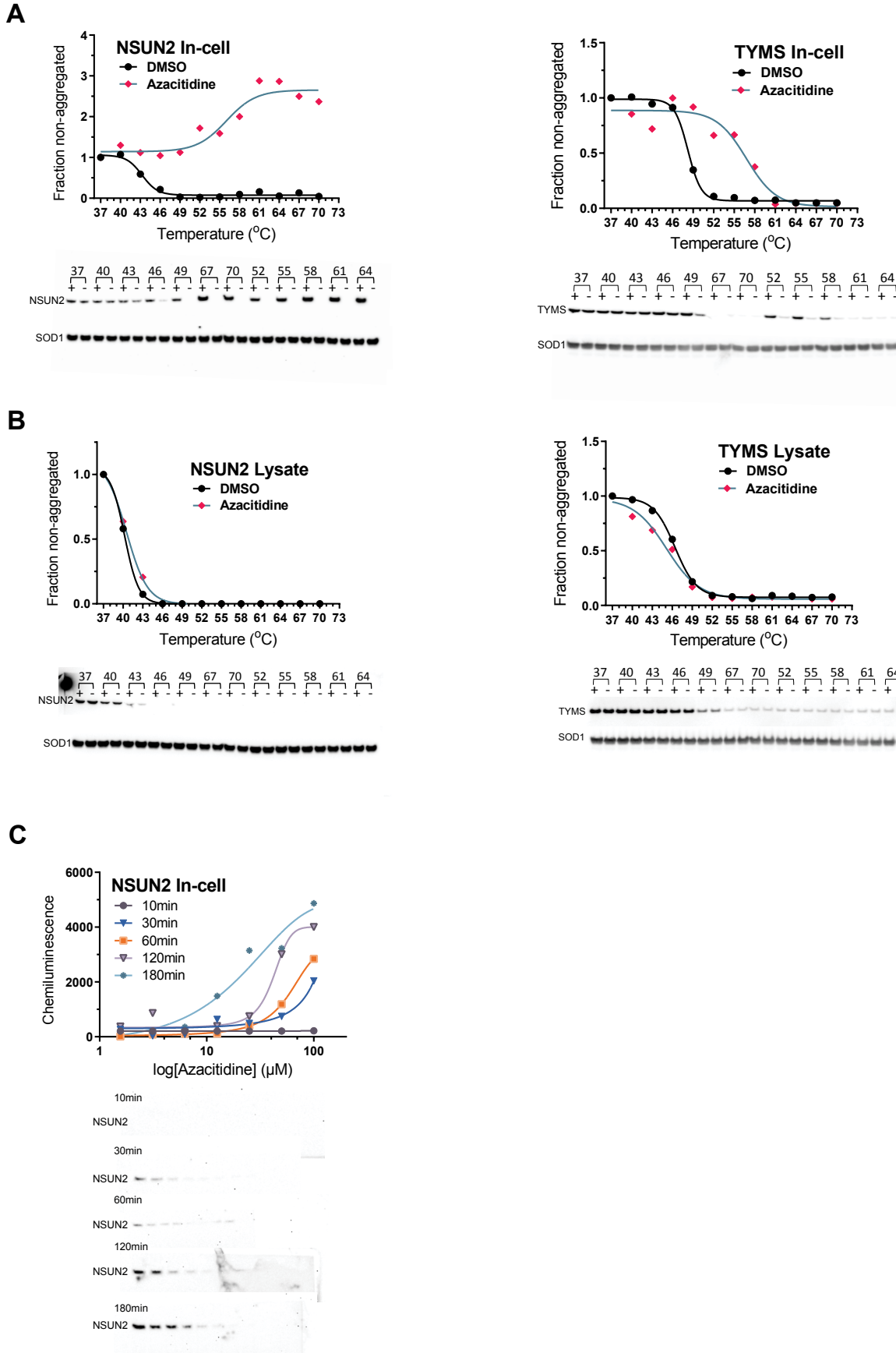
## Decitabine



**Supplemental Figure S7. Thermal proteome profiling of azacitidine and decitabine reveals target protein engagement.** Melt curves showing changes in aggregation temperature for selected proteins in (A) azacitidine and (B) decitabine treated ALL-PO cells. (C) Melt curves showing changes in aggregation temperature for selected overlapping proteins in both azacitidine and decitabine treated ALL-PO cells.



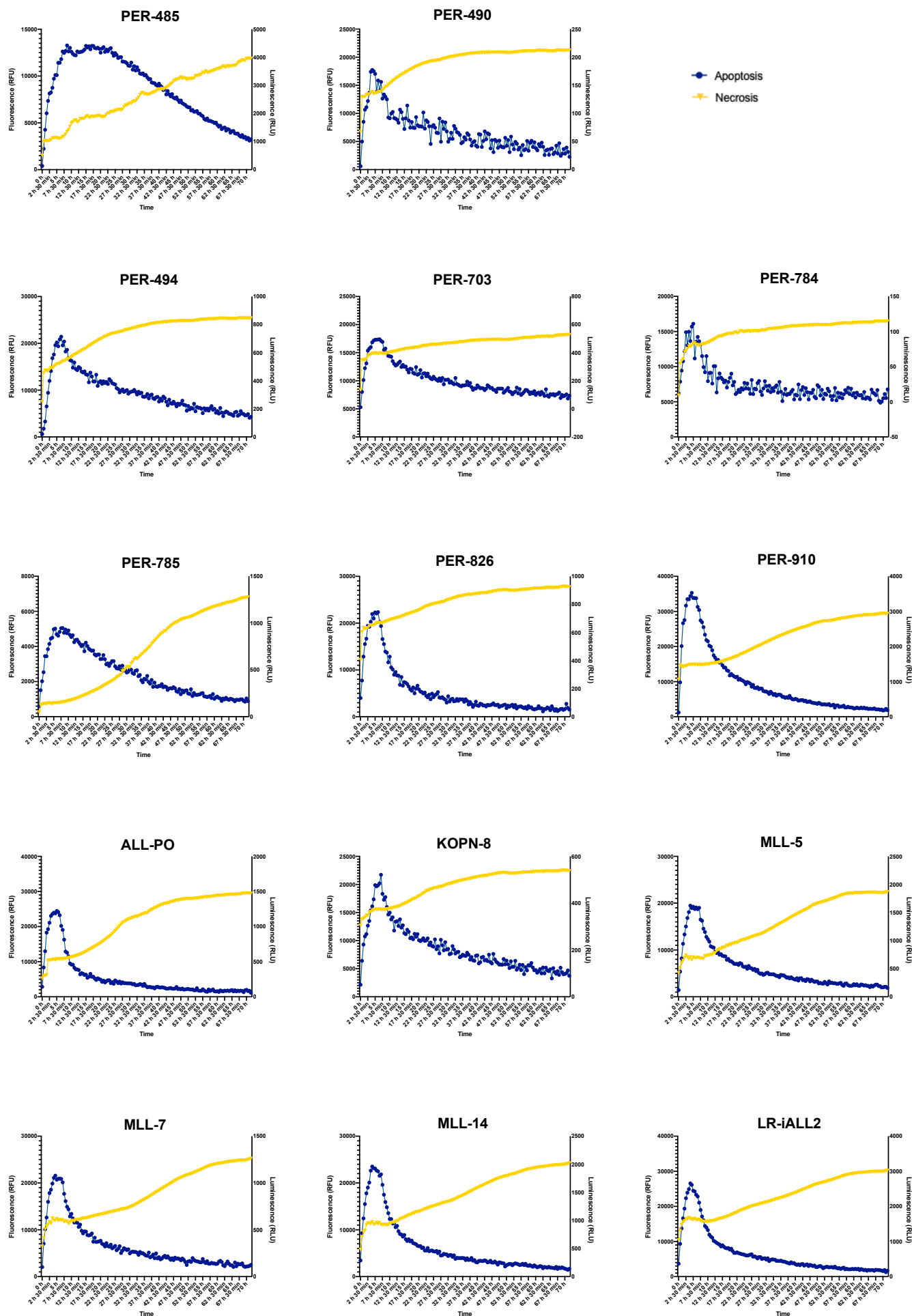
**Supplemental Figure S8. Azacitidine stabilizes NSUN2 and TYMS in the ALL-PO *KMT2A*-rearranged infant acute lymphoblastic leukemia cell line.** (A) Cellular thermal shift assay (CETSA) and western blot of NSUN2 and TYMS in ALL-PO cells with (+) and without (-) 100 $\mu$ M azacitidine treatment showing thermal stabilization of NSUN2 and TYMS in azacitidine treated samples. (B) ALL-PO cell lysates with (+) and without (-) 100 $\mu$ M azacitidine showing no thermal stabilization of NSUN2 or TYMS. SOD1 as loading control. (C) Western blot analysis and validation of thermal stabilization of NSUN2 in ALL-PO cells in a dose response time-course CETSA at 48 $^{\circ}$ C using a range of 100–1.56 $\mu$ M of azacitidine in eight steps during a time-course of 10–180-minute incubations showing increasing thermal stabilization over time at different concentrations.



**Supplemental Figure S9. Treatment of ALL-PO cells with venetoclax results in dose dependent thermal stabilization of BCL-2.** Western blot following dose response cellular thermal shift assay of two independent experiments at 60°C for BCL-2. Venetoclax dose ranged from 1 to 0.001μM.

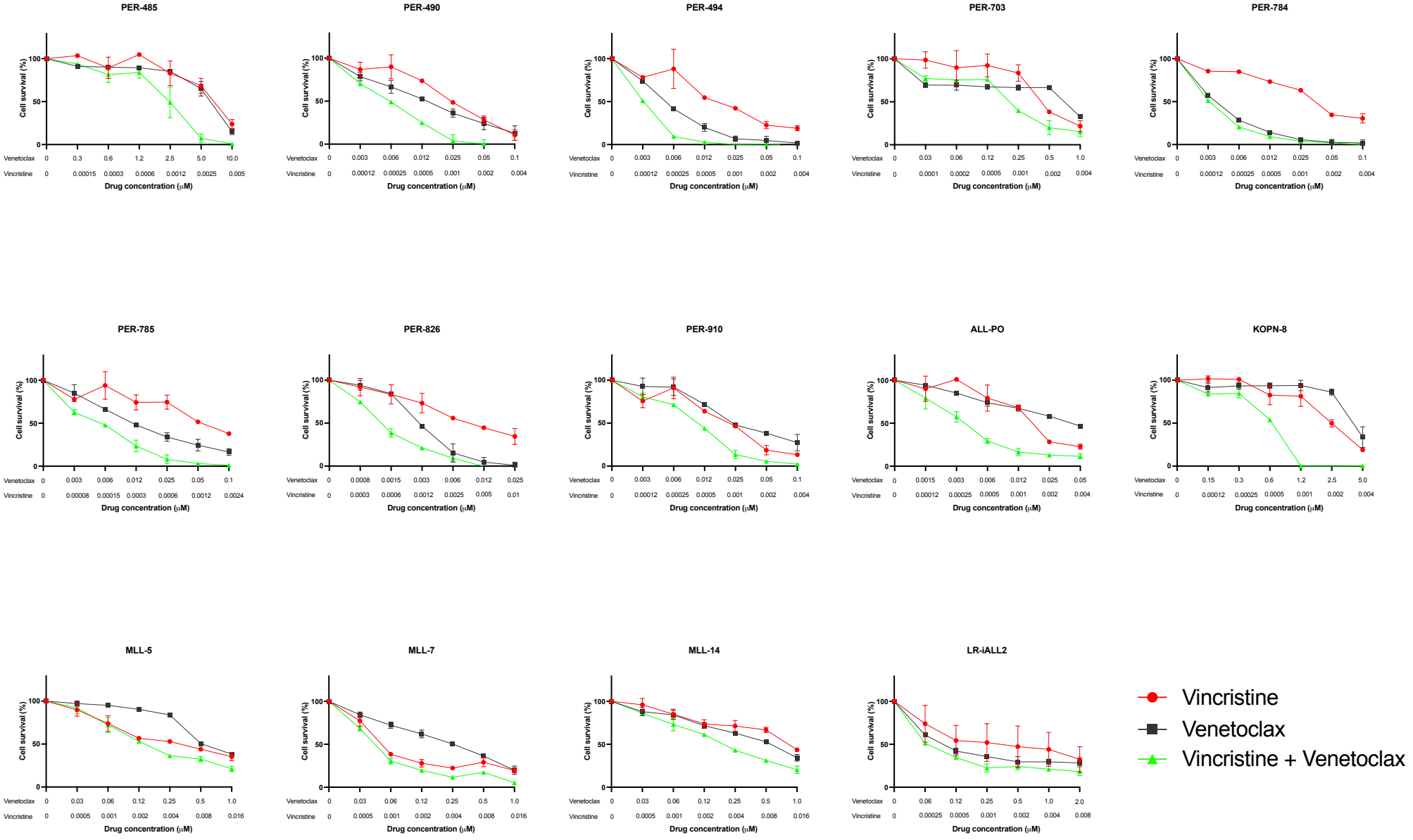


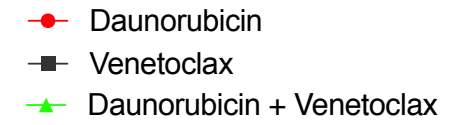
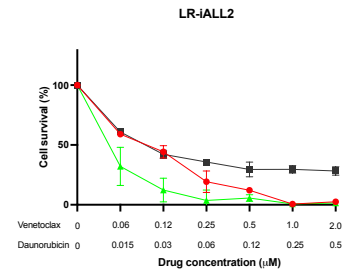
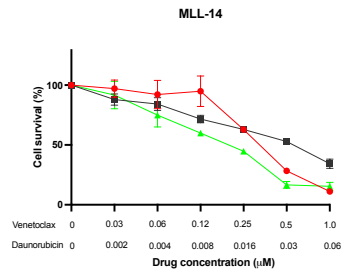
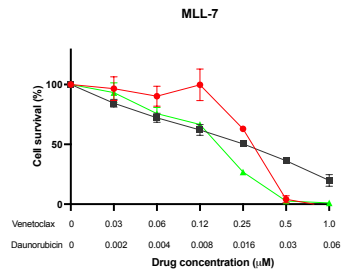
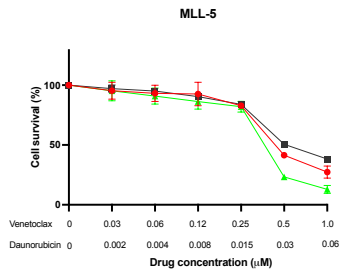
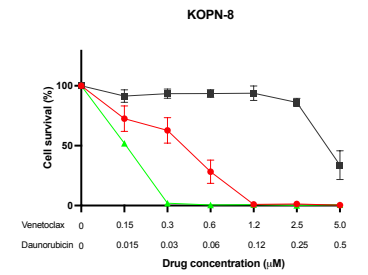
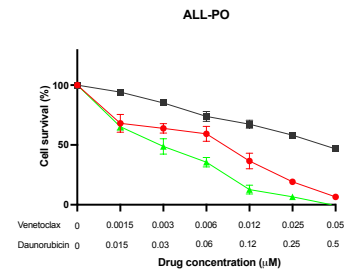
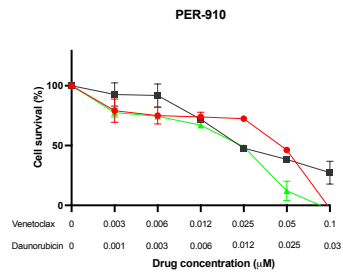
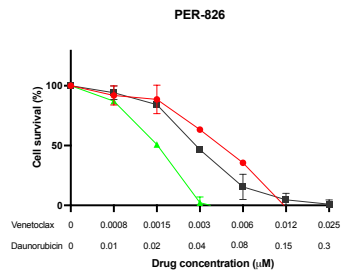
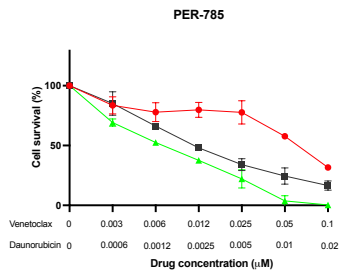
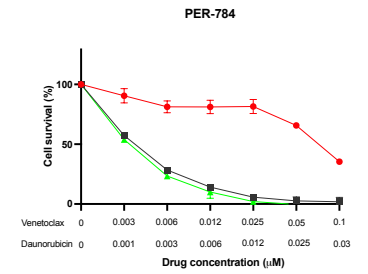
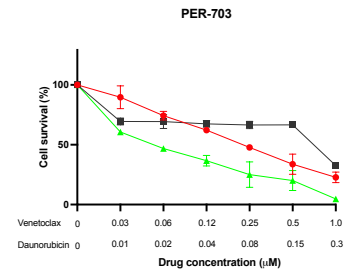
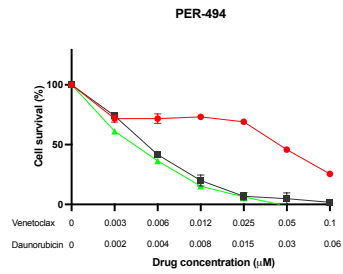
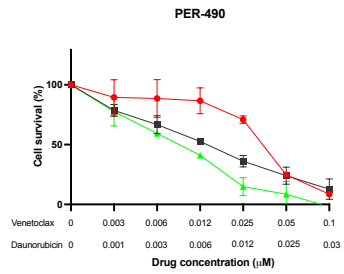
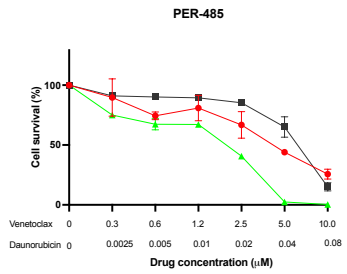
**Supplemental Figure S10. Venetoclax induces apoptosis and necrosis in *KMT2A*-rearranged infant acute lymphoblastic leukemia cell lines.** Cells were incubated with IC<sub>50</sub> concentrations of ventoclax for 72 hours. Measurements of luminescence (apoptosis) and fluorescence (necrosis) were performed every 30 minutes.



**Supplemental Figure S11. The combinatorial dose response of venetoclax with (A) conventional chemotherapeutic agents or (B) hypomethylating agents compared to single agent on cell survival.** Conventional chemotherapeutic agents include vincristine, daunorubicin, dexamethasone, cytarabine, methotrexate, 6-mercaptopurine, L-asparaginase, thioguanine and 4-hydroperoxycyclophosphamide (4-HPC). Hypomethylating agents include azacitidine, decitabine and zebularine.

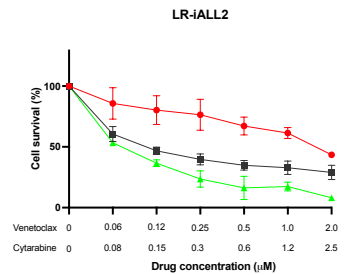
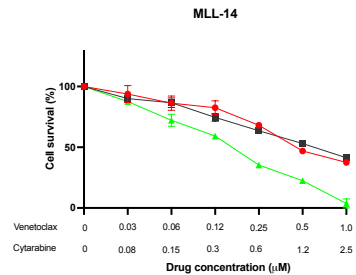
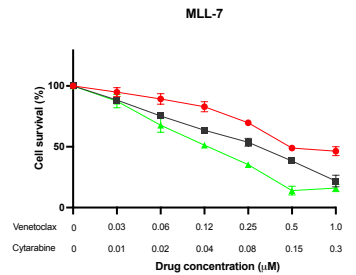
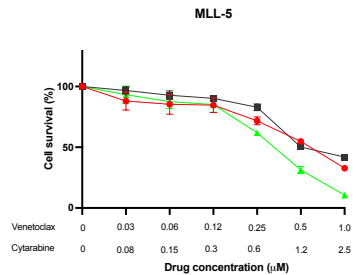
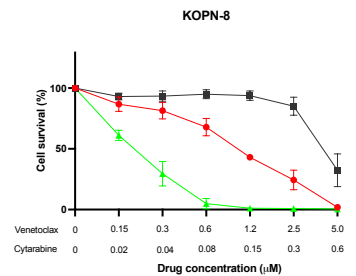
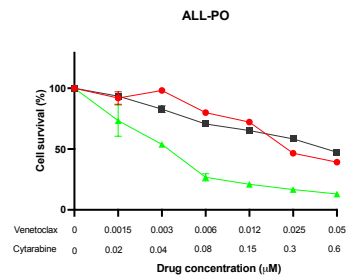
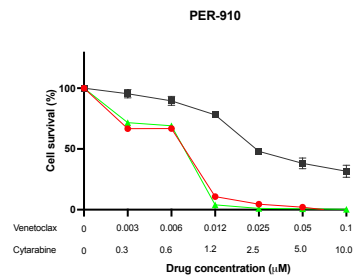
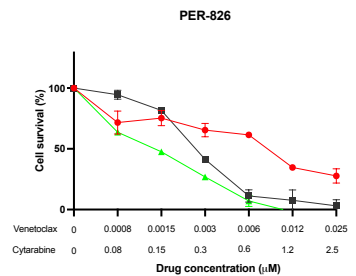
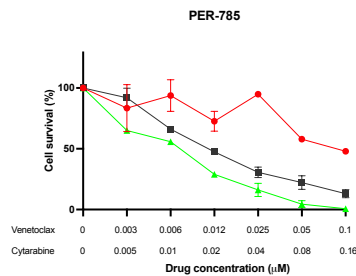
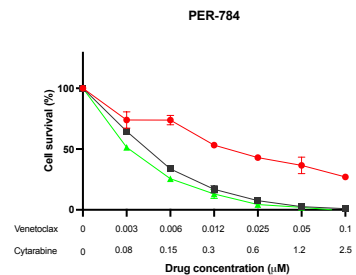
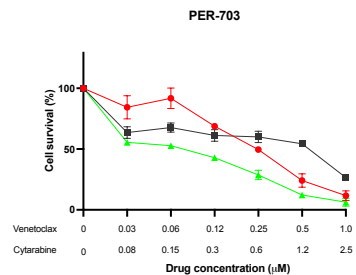
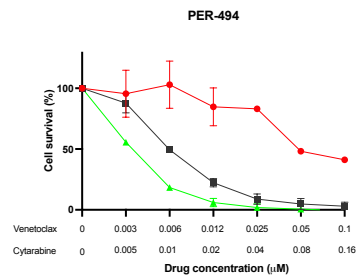
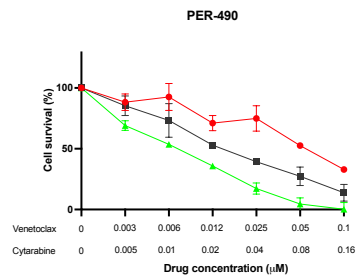
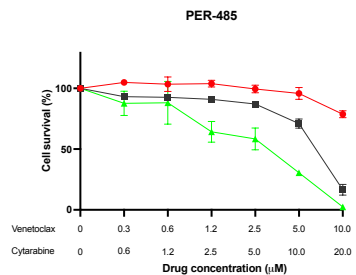
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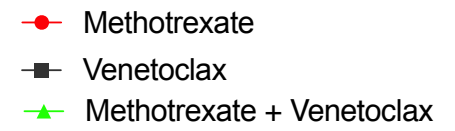
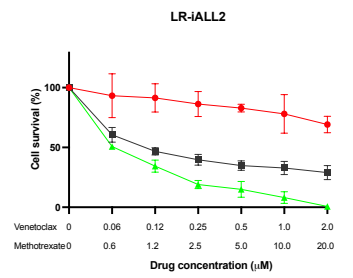
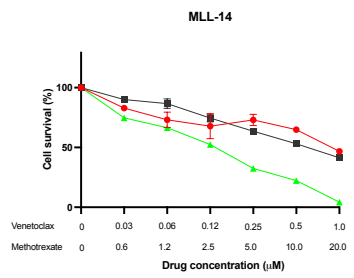
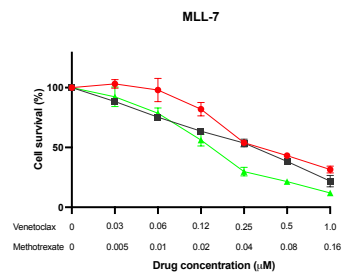
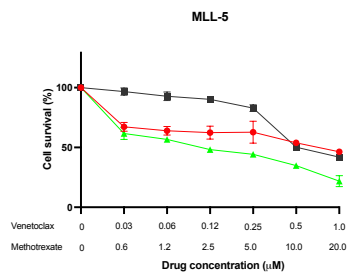
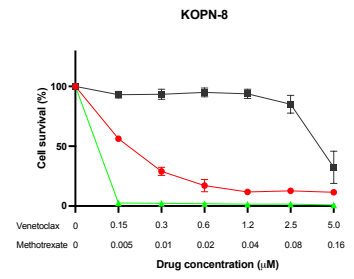
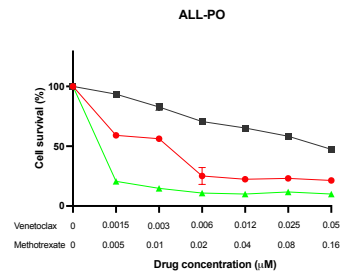
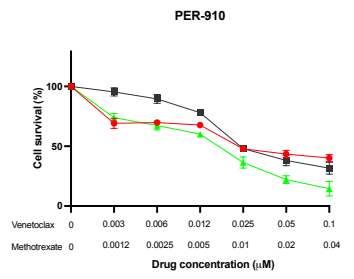
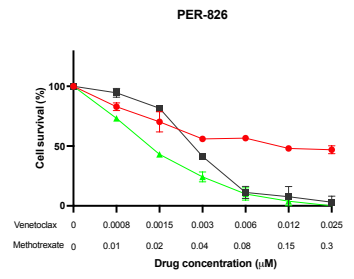
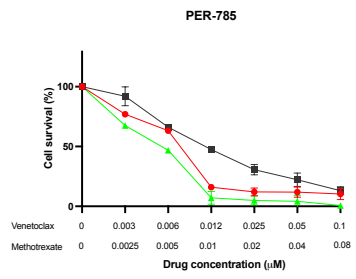
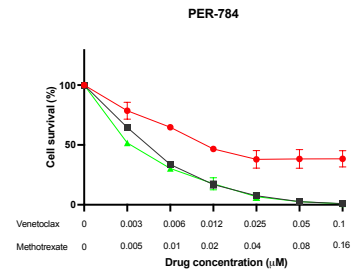
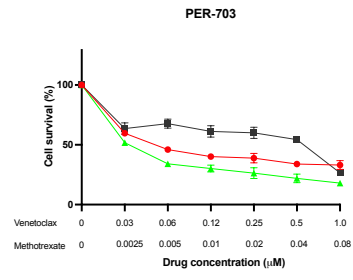
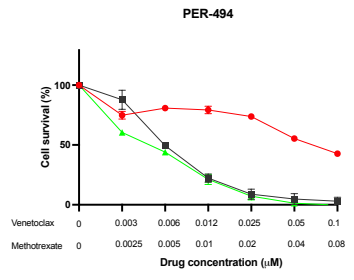
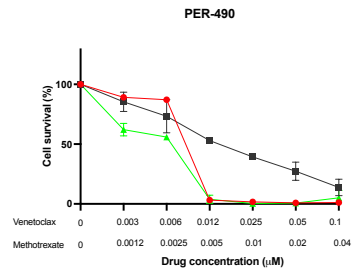
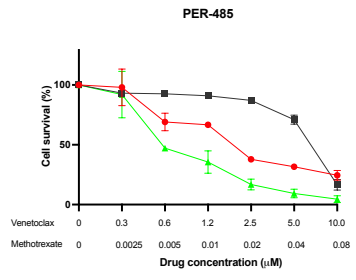




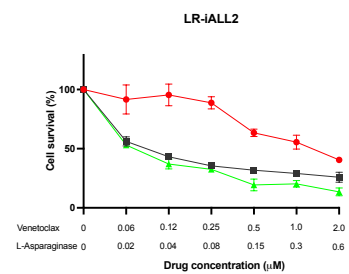
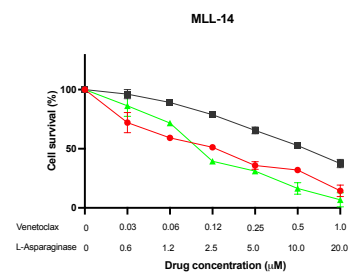
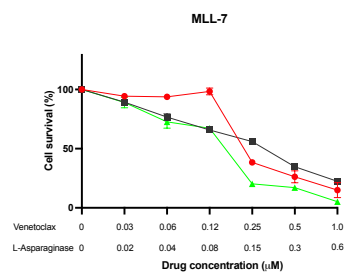
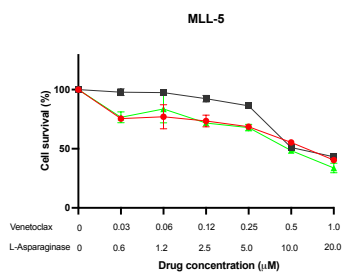
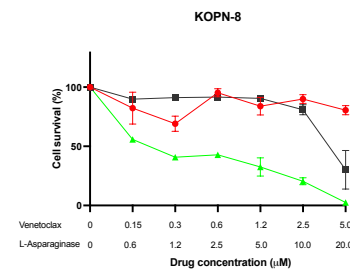
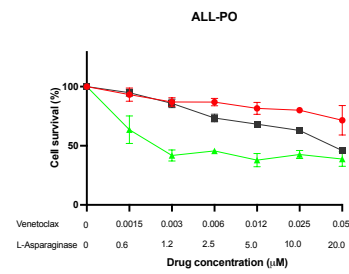
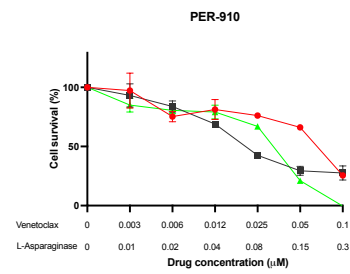
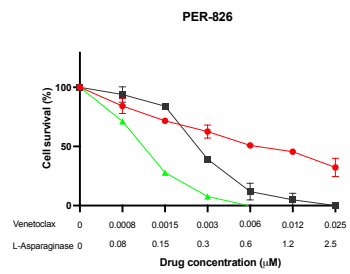
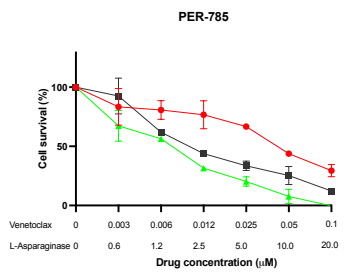
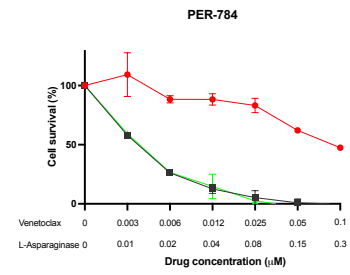
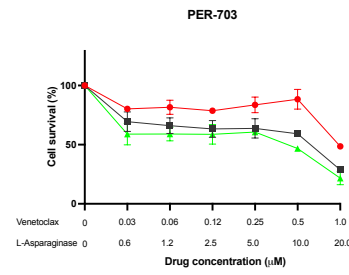
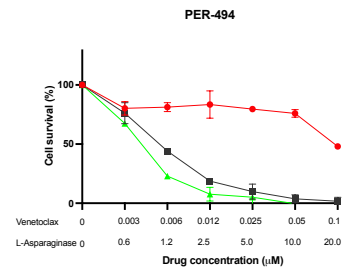
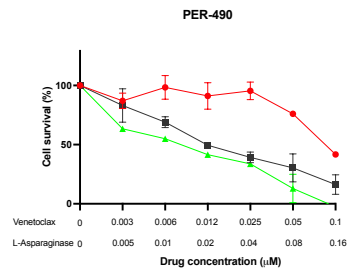
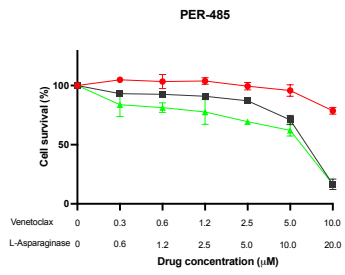




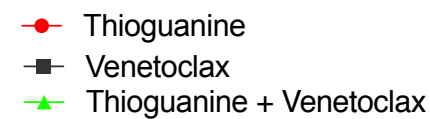
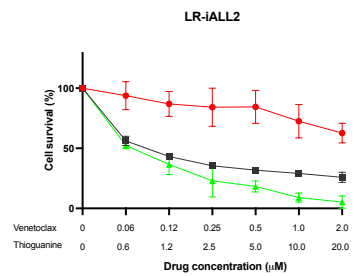
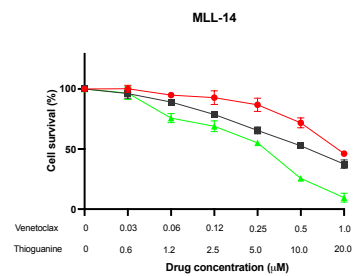
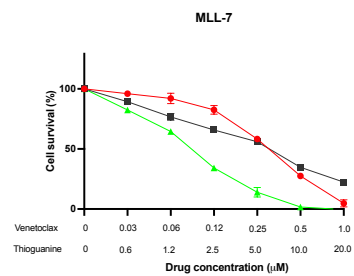
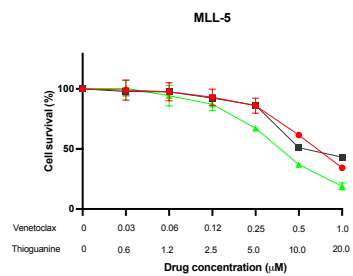
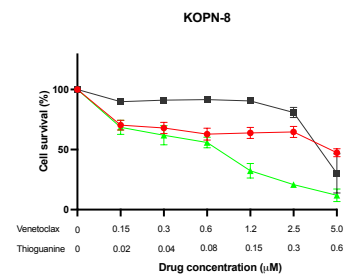
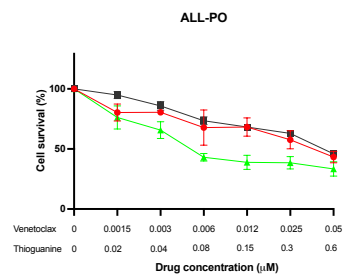
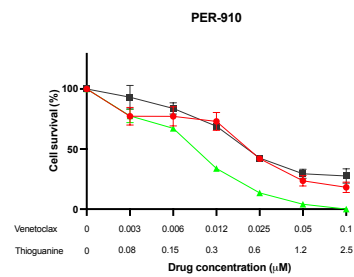
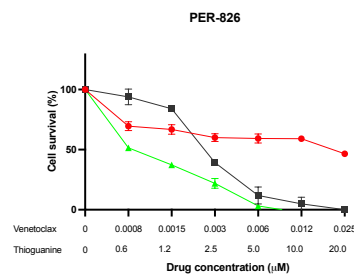
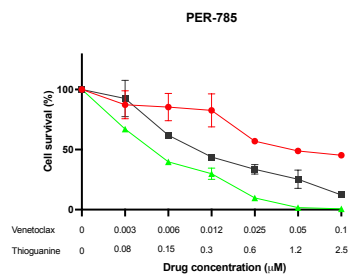
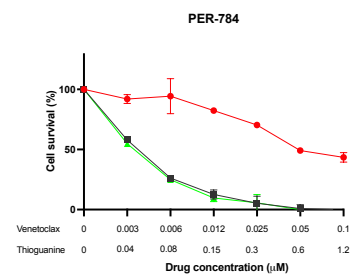
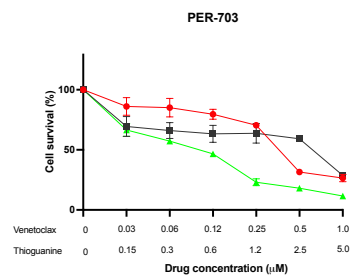
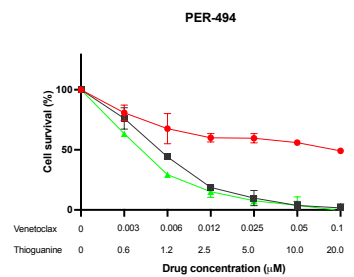
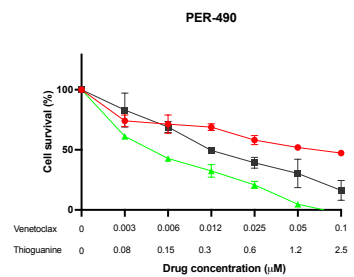
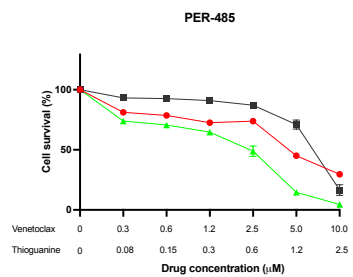
- Cytarabine
- Venetoclax
- ▲ Cytarabine + Venetoclax



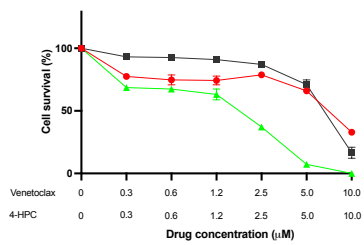




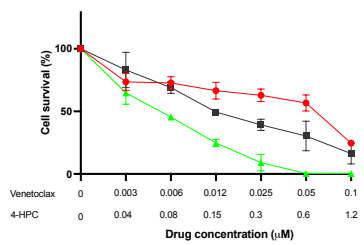
- L-Asparaginase
- Venetoclax
- ▲ L-Asparaginase + Venetoclax



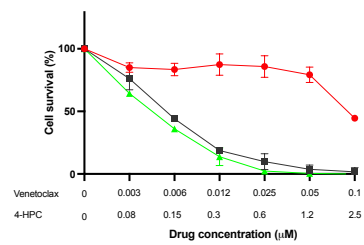
PER-485



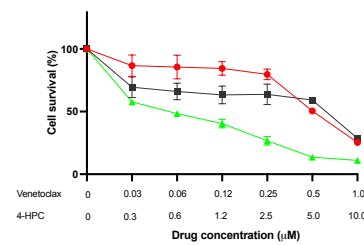
PER-490



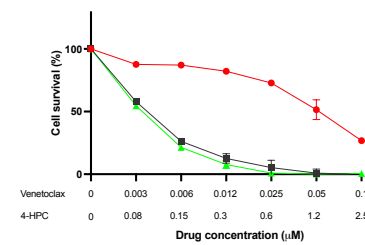
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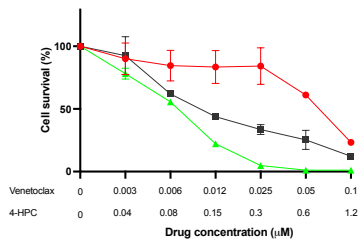
PER-703



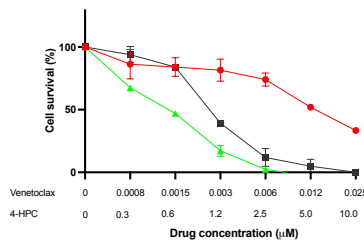
PER-784



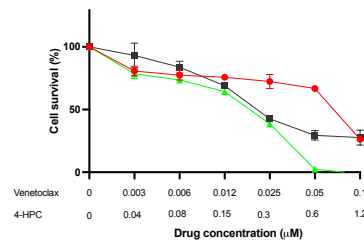
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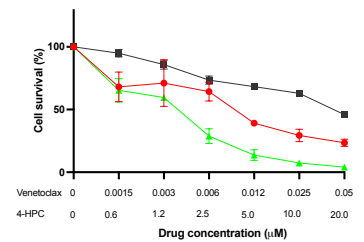
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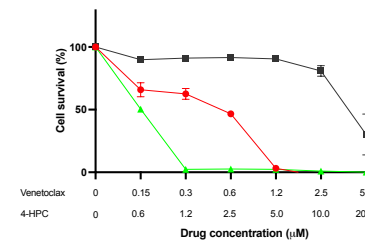
PER-910



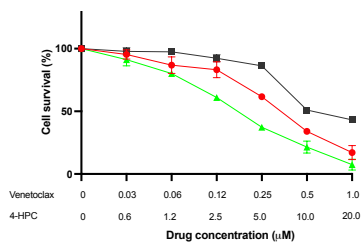
ALL-PO



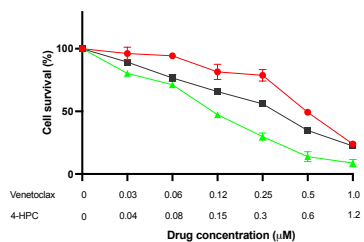
KOPN-8



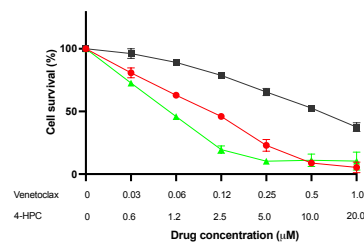
MLL-5



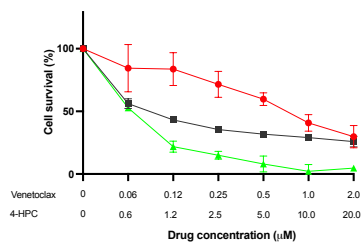
MLL-7



MLL-14



LR-iALL2



- 4-HPC
- Venetoclax
- ▲ 4-HPC + Venetoclax

**B**