SUPPLEMENTARY INFORMATION

SUPPLEMENTAL TABLES

Supplemental Table 1. shRNA sequences cloned into the pLKO.1-puro lentiviral vector.

Supplemental Table 2. qRT-PCR primers.

Supplemental Table 3. Antibodies used for Western blot analysis and ChIP-PCR.

Supplemental Table 4. Summary of the small molecule screening results.

Supplemental Table 5. Summary of DepMap database CRISPR and RNAi screens.

Supplemental Table 6. Summary of RNA-seq results.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Activation of the *GIMAP* enhancer in T-ALL cells. (A) ChIP-seq gene tracks show that TAL1 and NOTCH1 bind to the *GIMAP* enhancer region (adapted from our previous study: Liau et al, Leukemia, 2017). Other proteins bound to the region include various transcription factors and regulators of transcription, as well as *ARID5B*, a TAL1 target. H3K27ac signals in Jurkat and normal thymus are also shown. Orange boxes indicate TAL1-bound regions. ChIP-seq datasets can be downloaded from the Gene Expression Omnibus under accession numbers GSE17954, GSE29181, GSE29600, GSE83777, GSE97512, GSM1013125, GSM1296384, GSM1442004. **(B)** Jurkat cells stably expressing the *GIMAP* reporter construct were treated with DMSO (negative control). Cell viability and *luciferase* activity were measured after 5 hours. Relative luminescence was determined by normalizing *luciferase* activity to cell viability and is presented as the fold change compared to untreated cells (0%). The values are shown as individual dots and the mean ± SD of technical triplicates and are normalized to untreated cells. **(C)** Jurkat cells were treated with THZ1. Cell viability was measured after 24 hours by a CellTiter-Glo assay.

The values are shown as individual dots and the mean ± SD of technical triplicates and are normalized to untreated cells. Representative results from multiple independent experiments were shown (B, C).

Supplemental Figure 2. Growth inhibitory effect of A66 on T-ALL cell lines. T-ALL cell lines were treated with A66. Cell viability was measured after 24 hours by CellTiter-Glo. The values shown are the mean ± SD of technical triplicates and are normalized to untreated cells.

Supplemental Figure 3. Apoptosis induction after PIK-75 treatment in T-ALL cell lines. (A) Jurkat and DND-41 cell lines were seeded at 10,000 cells per well, treated with DMSO or PIK-75 (120 nM) for 4 hours and stained with Annexin V-APC and PI. Cells were gated as shown. **(B)** Jurkat and DND-41 cell lines were treated with PIK-75 for 24 hours and stained with Annexin V-APC and PI. The values shown are the proportions of the total population. Early apoptosis, Annexin V-APC-positive and PI-negative; late apoptosis, Annexin V-APC-positive and PI-positive. **(C)** The values shown are the total Annexin V-APC-positive proportions of the total population ± SD of technical triplicates in DND-41 cells.

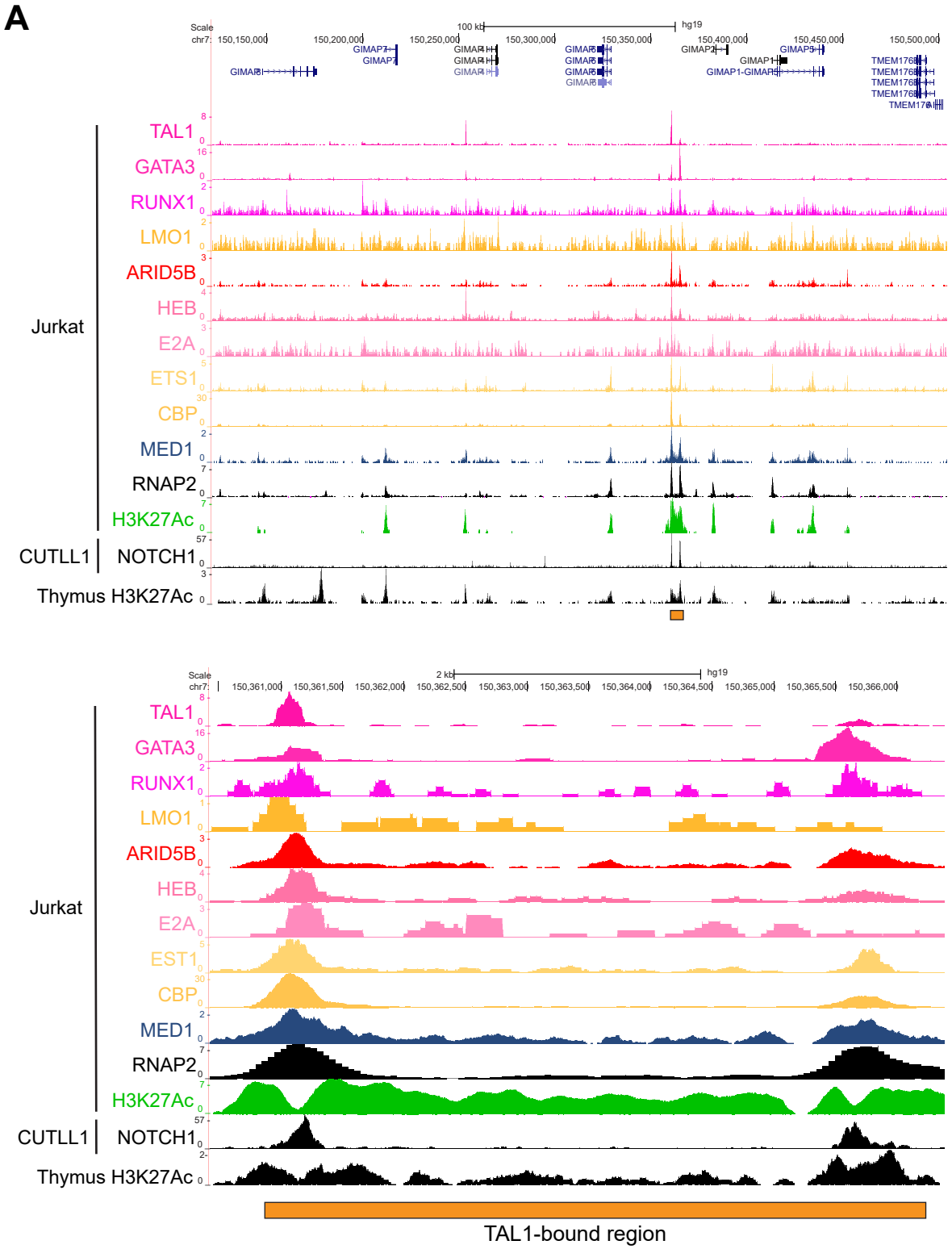
Supplemental Figure 4. Gene expression changes after PIK-75 treatment in T-ALL cells. (A) mRNA expression levels of *GIMAP* genes in a panel of T-ALL cell lines 4 hours after PIK-75 treatment (MOLT-16 IC_{80} = 80 nM; MOLT-4 IC_{80} = 210 nM; CCRF-CEM IC_{80} = 120 nM; RPMI-8402 IC_{80} = 160 nM). Expression values were normalized to spike-in RNA and shown as individual dots and the mean of technical duplicates. **(B)** mRNA expression levels of representative TAL1 target genes in Jurkat cells after treatment with PIK-75 at

different concentrations. Expression values were normalized to spike-in RNA and shown as individual dots and the mean of technical duplicates. Representative results from multiple independent experiments were shown (A, B).

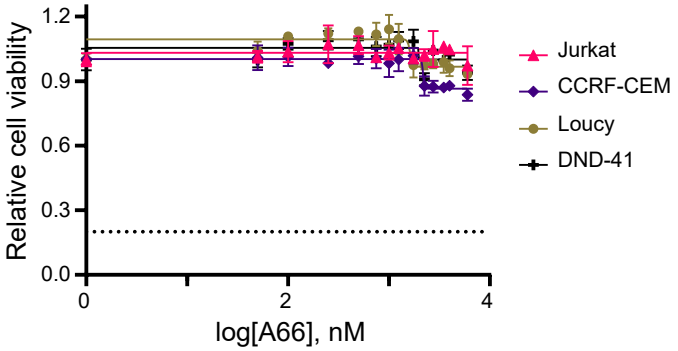
Supplemental Figure 5. The effect of various inhibitors on T-ALL cells. (A) T-ALL cell lines were treated with PIK-75, THZ1, JQ1 for 24 hours or DBZ for 7 days. Cell viability was measured by CellTiter-Glo. The values shown are the mean ± SD of technical triplicates and are normalized to untreated cells. **(B)** mRNA expression levels of representative TAL1 target genes (*GIMAP5, GIMAP7, ARID5B* and *MYB*) in Jurkat cells after treatment with each of inhibitors at different concentrations were measured by qRT-PCR. Expression values were normalized to spike-in RNA and shown as individual dots and the mean of technical duplicates. **(C)** mRNA expression levels of *HES1* and *MYC* in Jurkat cells after treatment with PIK-75 or DBZ at different concentrations were measured by qRT-PCR. Expression values were normalized to spike-in RNA and shown as individual dots and the mean of technical duplicates. Representative results from multiple independent experiments were shown (A-C).

Supplemental Figure 6. Potential involvement of JAK-STAT pathway in drug sensitivity to PIK-75. (A,B) SUP-T1 cells were induced with IL-7 (50 ng/mL) for 24 hours before treatment with PIK-75. Cell viability was measured after 24 hours by CellTiter-Glo (A). The values shown are the mean ± SD of technical triplicates and are normalized to untreated cells. Western blot was performed to measure the level of total and phosphorylated forms of JAK2, STAT5 and RNA polymerase II (B). **(C,D)** Jurkat cells expressing the *GIMAP* enhancer luciferase construct were transduced with BCL2 or empty

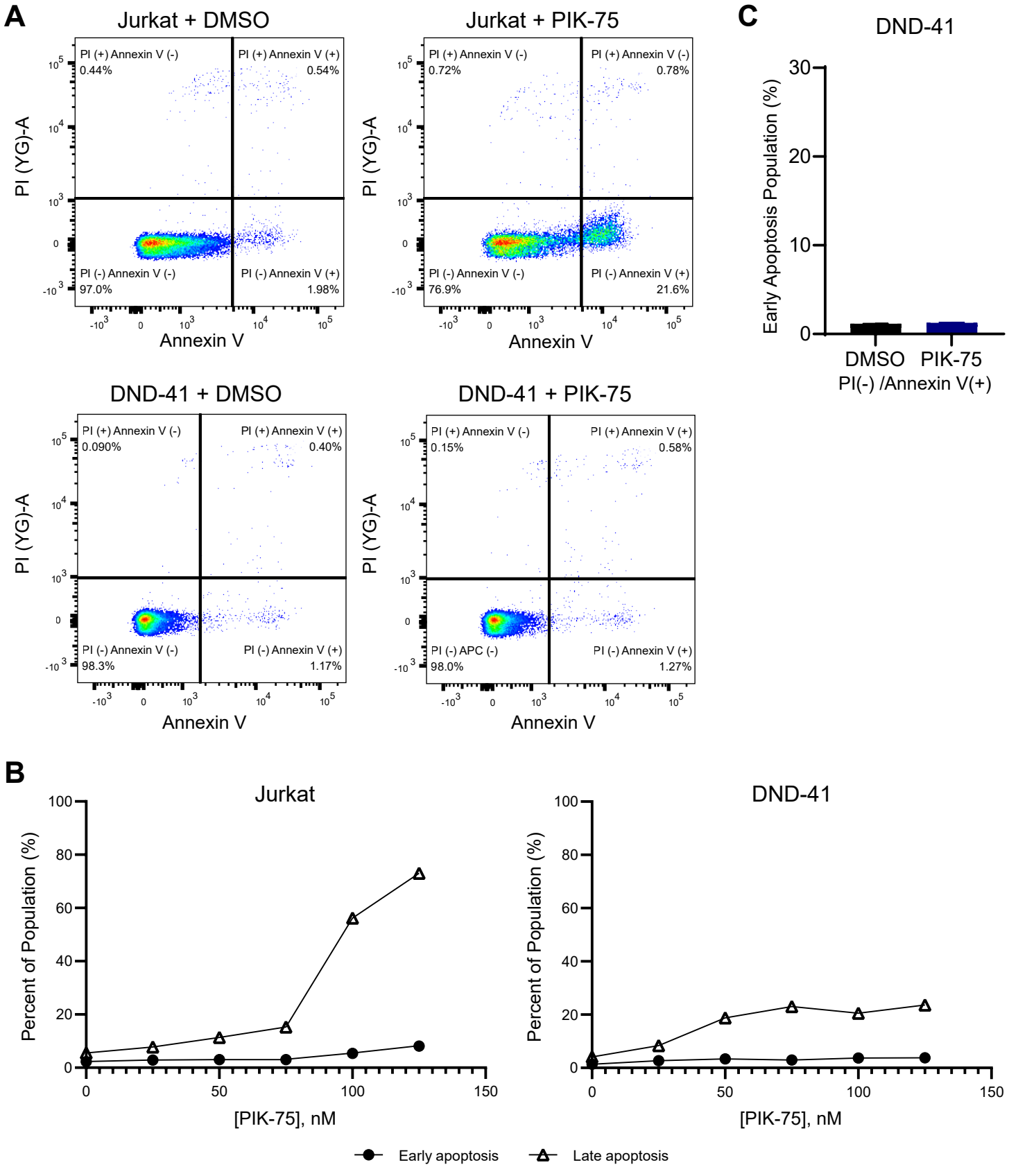
vector (EV) and then treated with PIK-75. Cell viability was measured after 24 hours by CellTiter-Glo (C). The *GIMAP* enhancer luciferase activity after treatment with PIK-75 at 5 hours was measured. Relative luminescence was determined by normalizing *luciferase* activity to cell viability and is presented as the fold change compared to untreated cells (0 nM). The values are shown as individual dots and the mean ± SD of technical triplicates (D). Representative results from multiple independent experiments were shown (D).



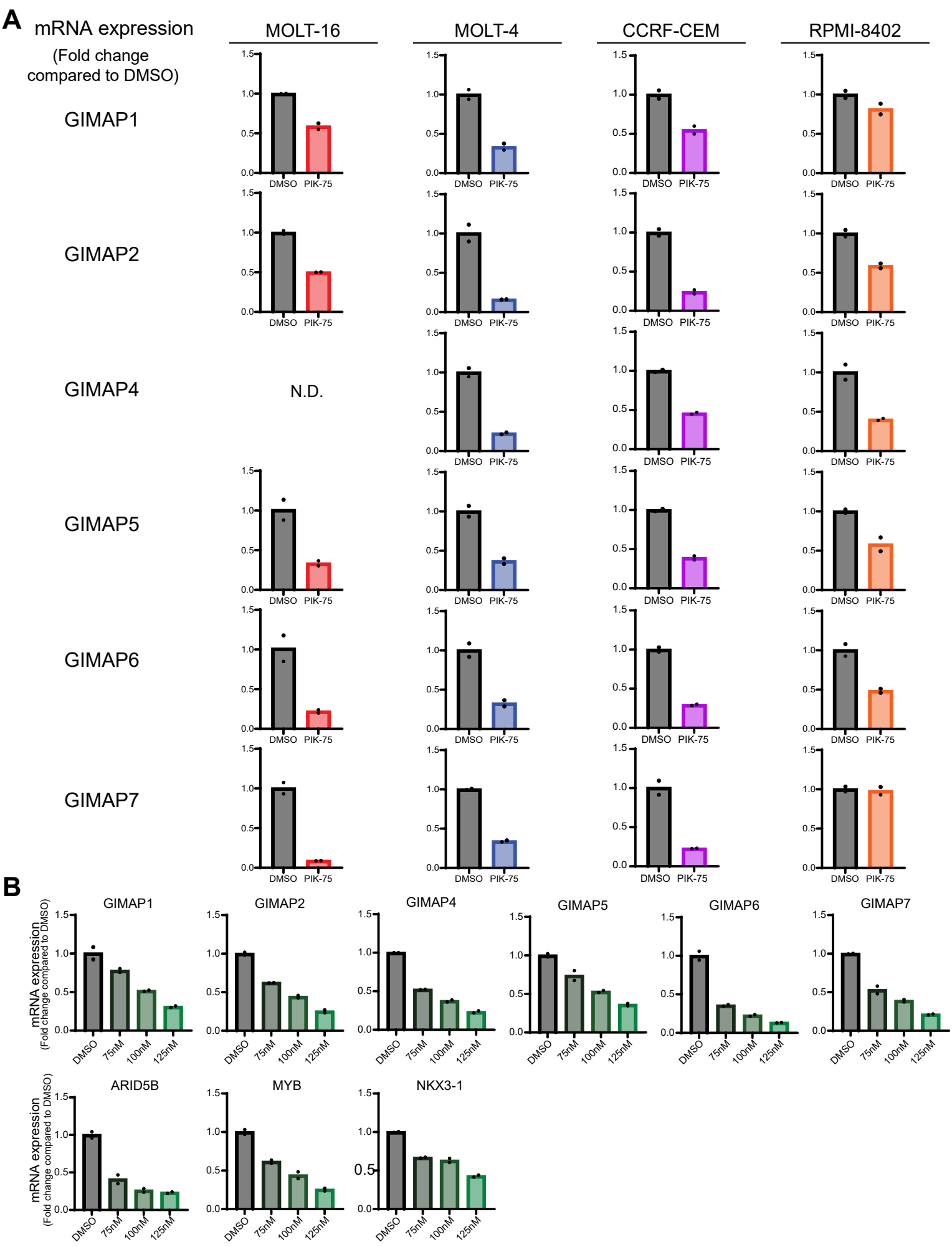
Supplemental Figure 1



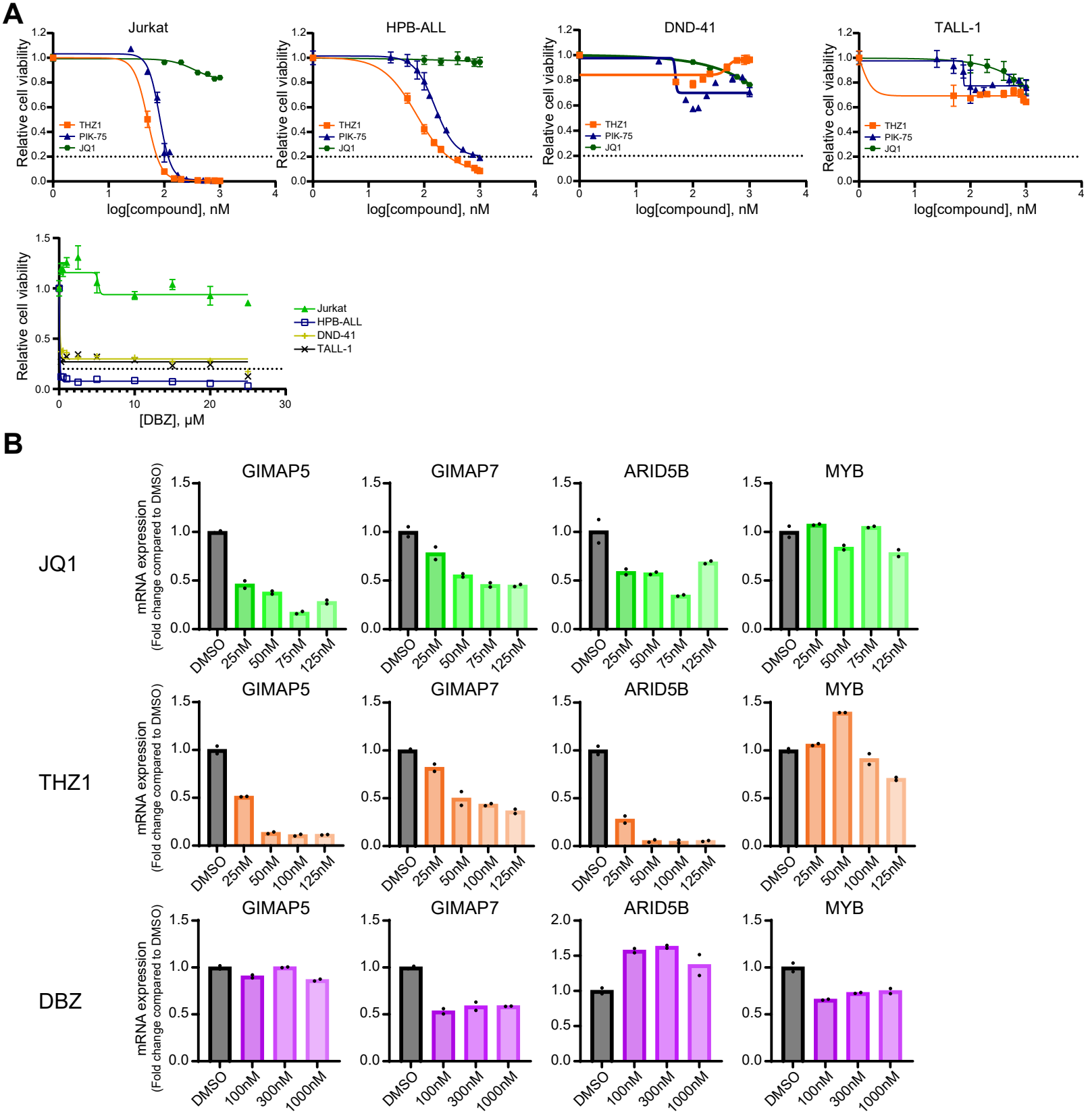
Supplemental Figure 2



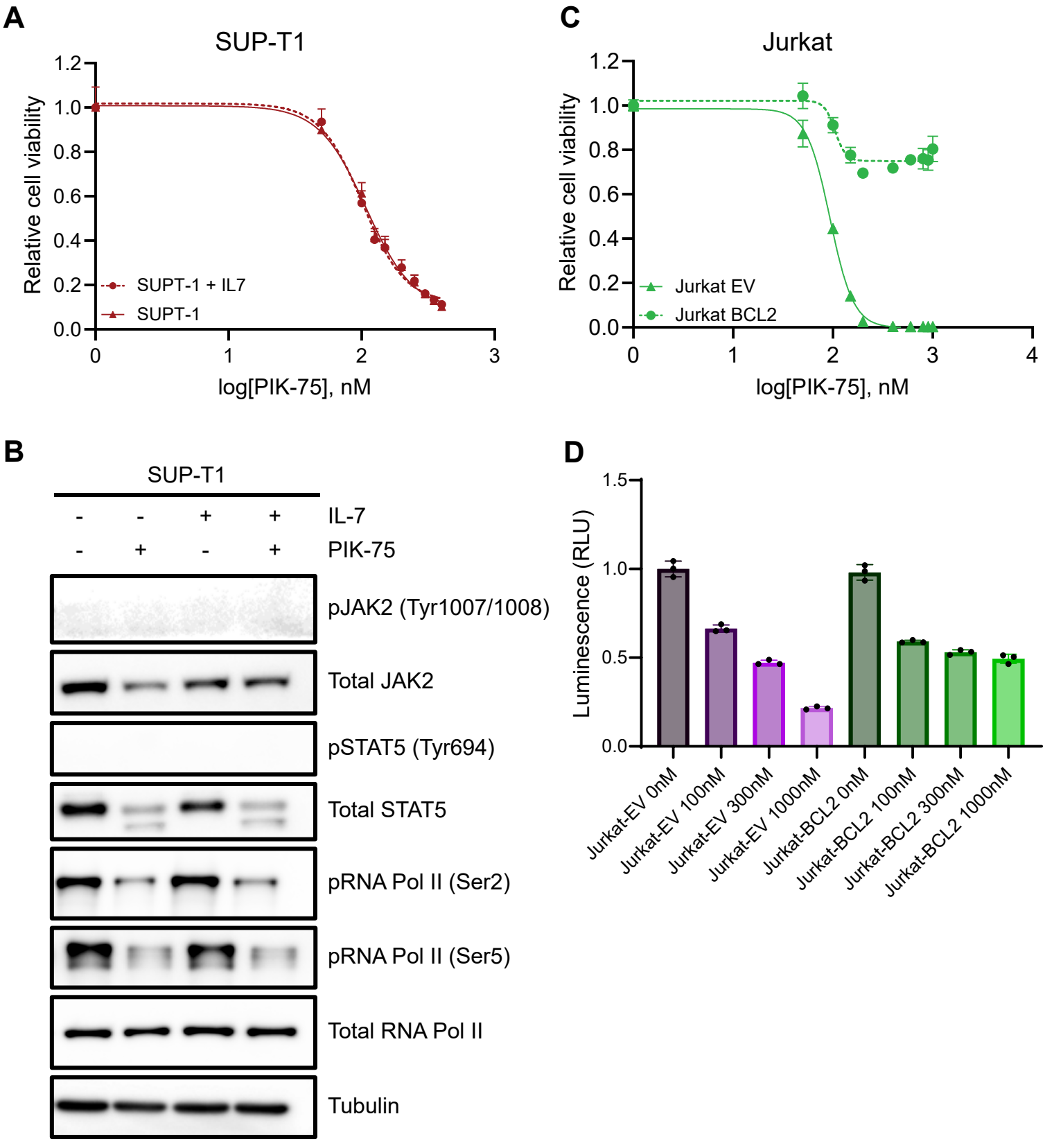
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6