

Supplementary Figure 1: AK2 and AK1 transcriptional expression in T-ALL cell lines

(A) AK2 transcriptional expression of (A) 14 T-ALL cell lines and (C) 80 Primary T-ALLs classified according to the maturation stage arrest. (B) AK1 transcriptional expression of (B) 12 T-ALL cell lines and (D) 80 Primary T-ALLs classified according to the maturation stage arrest.

Supplementary Figure 2: AK2 protein expression in thymic subpopulations

(A) Western blots showing protein expression of AK2 in thymic subpopulations compared to a whole thymus and a total bone marrow. AK2 protein level relative to Actin is represented under each band. The depicted blot is representative of three independent experiments. (B) AK2 protein expression as determined by Western blot in early or immature blasts (3 types) *versus* late or cortical ones (2 types). Means + SEM are depicted and data shown here is an example of three independent experiments.

Supplementary Figure 3: AK2 is expressed irrespective of T-ALL molecular subgroups

(A) AK2 expression in T-ALL primary samples stratified by their ETP status determined by immunophotyping. (B) AK2 expression in T-ALL primary samples sorted by their molecular subtype. (C) AK2 expression in NOTCH1 pathway-altered, or PTEN-altered, or Ras-mutated T-ALL samples.

Supplementary Figure 4: AK2 protein expression post-shAK2 or shScramble transduction

(A-B) Western blot showing protein expression of AK2 in (A) 5 T-ALL cell lines and (B) 2 B-ALL cell lines at day 3 post-sh Scramble or shAK2 transduction.

Supplementary Figure 5: Efficiency of inducible IPTG-inducible shAK2

Western blot showing AK2 expression at day 3 (D3) and day 12 (D12) post-sh Scramble or shAK2 induction by IPTG treatment. AK2 protein level relative to Actin is represented under each band.

Supplementary Figure 6: AK1 overexpression does not rescue AK2-depleted cells from apoptosis in JURKAT cell line

(A) Western blot showing protein expression of AK2 and AK1 with and without overexpression of AK1 (AK1+) downregulation of AK2 (shAK2). (B) Percentage of Annexin V and Propidium iodide (PI) positive cells overexpressing either AK1 alone (AK1+) or shAK2 alone, or both in JURKAT cell line every two days from day 2 (D2) post shAK2 or sh Scramble transduction to day 8 (D8). (C) Absolute number of cells overexpressing either AK1 (AK1+) or shAK2 or both in JURKAT cell line over time post shAK2 or sh Scramble transduction. (D) Analysis of the different phases of the cell cycle (G0/G1, S or G2/M) at day 2 in the shAK2 or sh Scramble condition after transduction in JURKAT cells overexpressing or not AK1 (AK1+).

Supplementary Figure 7: Antioxidant treatment does not rescue AK2-depleted cells from apoptosis in T-ALL cell lines

Percentage of Annexin V and Propidium iodide negative cells in (A) JURKAT cells and (B) RPMI cells and absolute cell number in (C) JURKAT cells and (D) RPMI cells every two days from day 1 onwards (D1) post shAK2 or shScramble transduction to day 8 (D8) with or without 5 mM N-acetylcystein (NAC) treatment.

Supplementary Figure 8: AK2 expression does not correlate with venetoclax sensitivity

Correlation between AK2 mRNA expression determined by qRT-PCR and venetoclax sensitivity determined by cell mortality upon 1 μ M venetoclax treatment. Each dot represents a primary sample (n=18). Spearman' r and p-value are indicated.

Supplementary Table 1: Immunophenotype of the T-ALL cell lines used in this study

T-ALL cell lines were immunophenotyped using a validated flow cytometry panel (see refs 17-20).

Supplementary Table 2: Biological and clinical features of the cohort

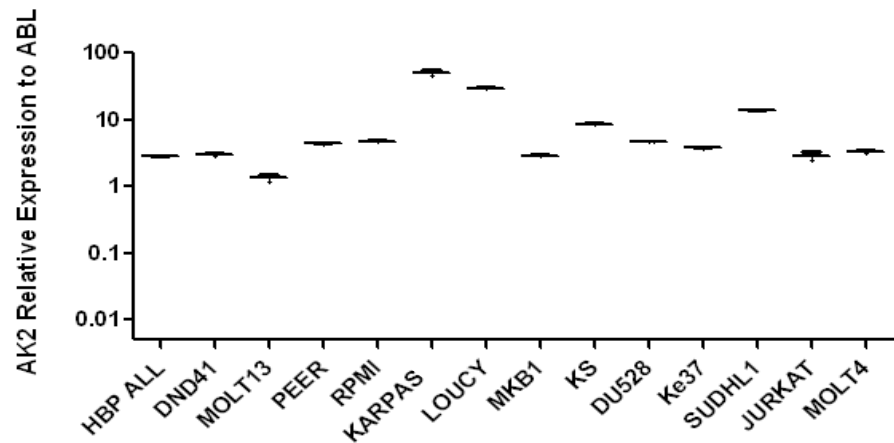
Patients were stratified in quartiles of AK2 expression. TCR status and oncogenetics were determined as previously described (see refs 17-20). WBC: white blood cells, CNS: central nervous system involvement, MRD: minimal residual disease.

Supplementary Figure 1

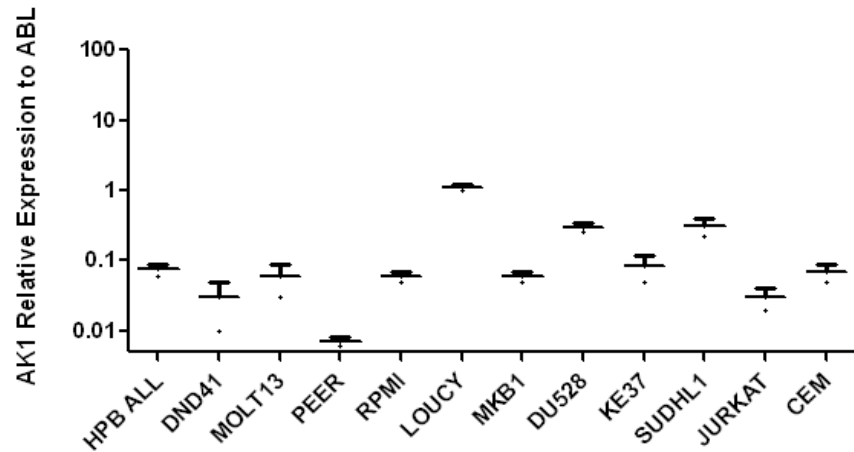
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AK1

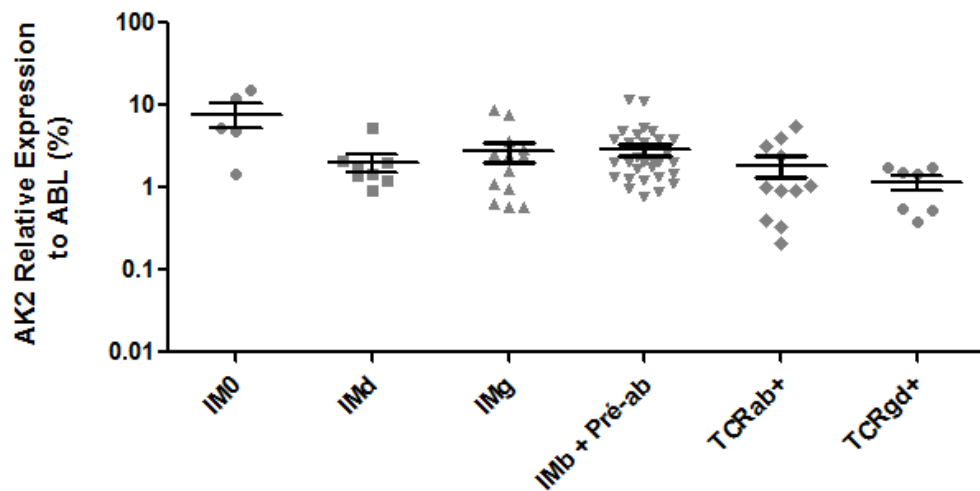
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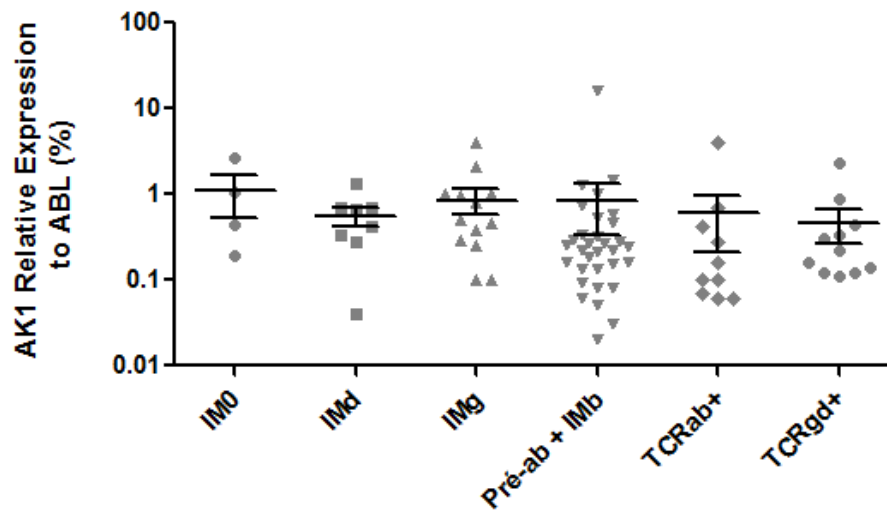
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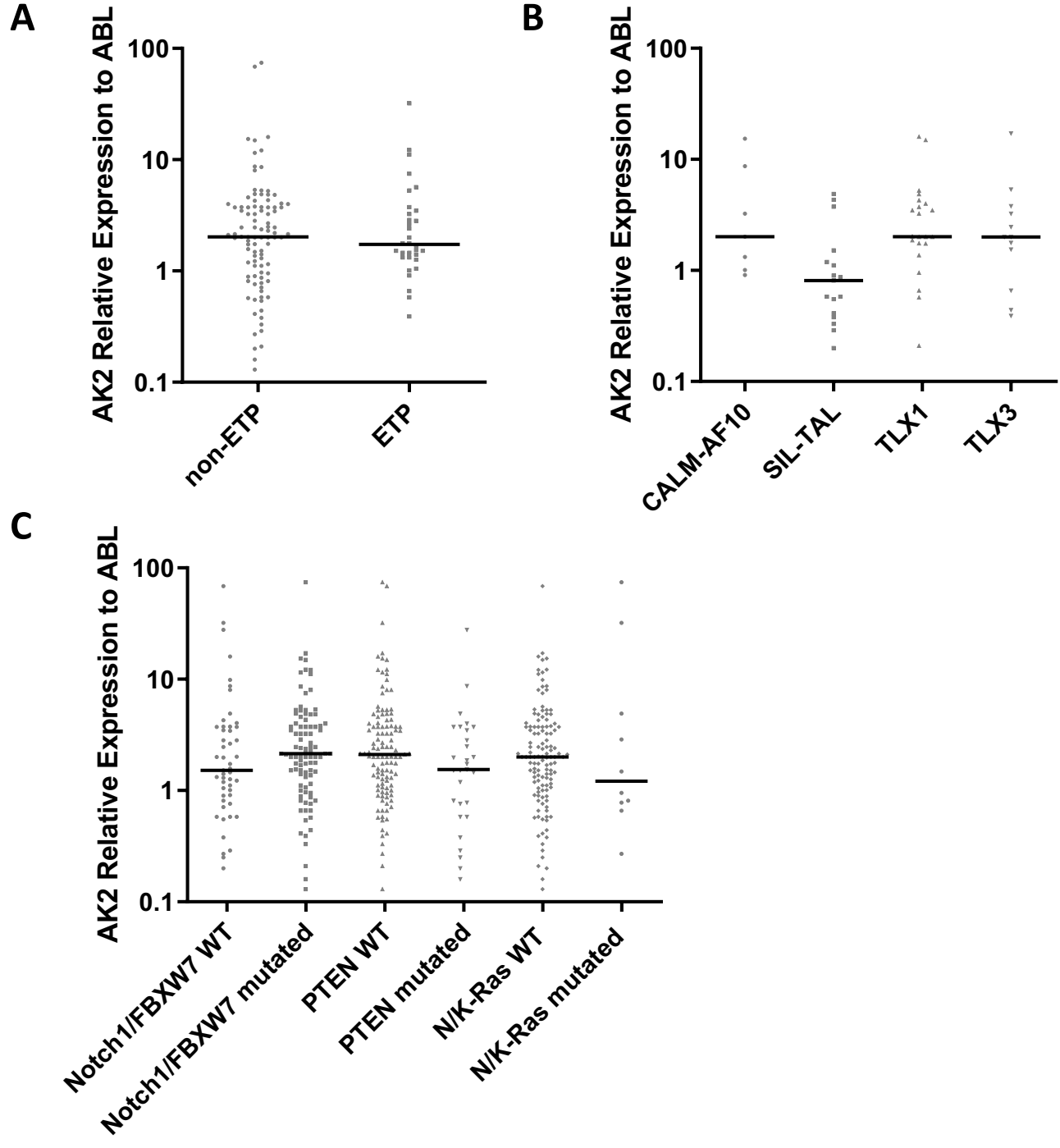
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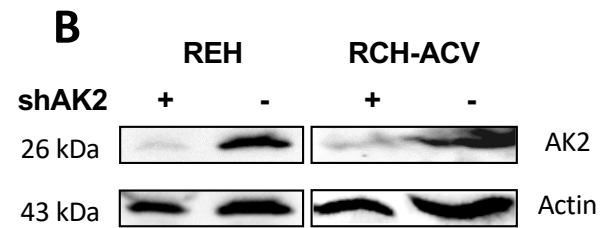
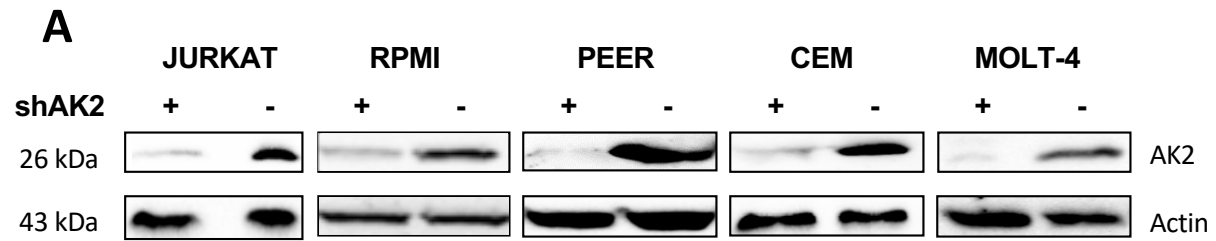
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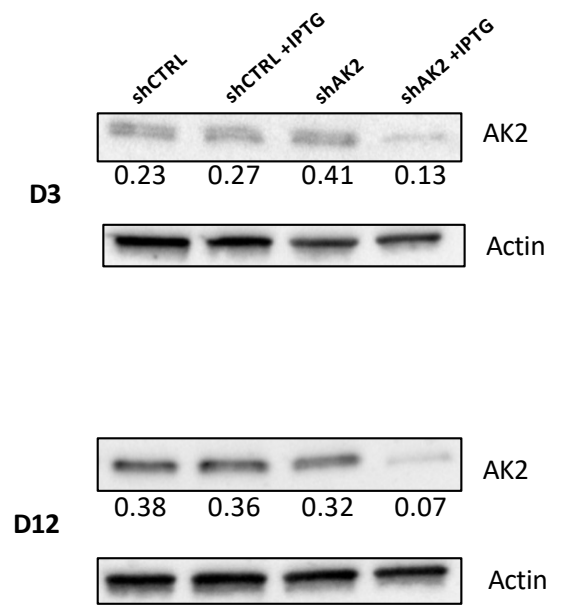
Supplementary Figure 3



Supplementary Figure 4

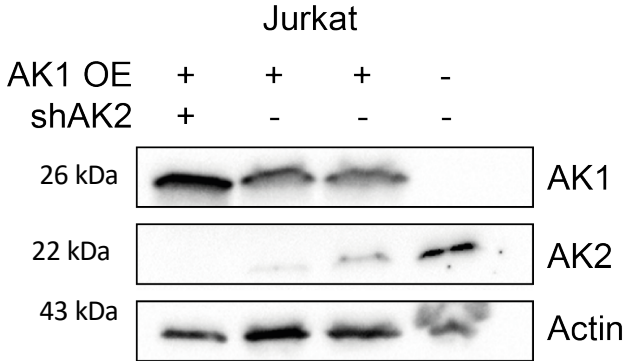


Supplementary Figure 5

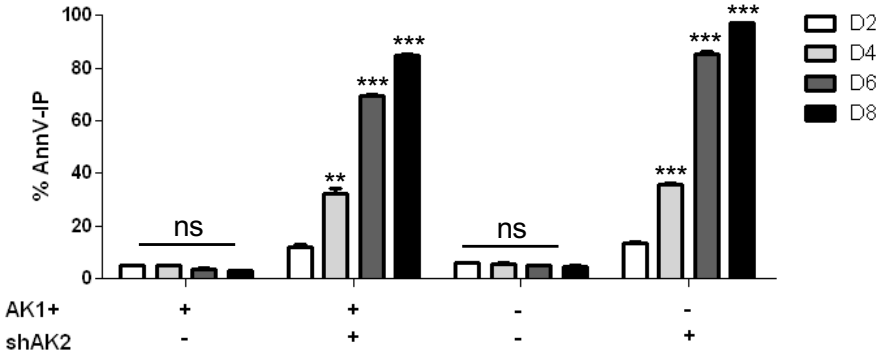


Supplementary Figure 6

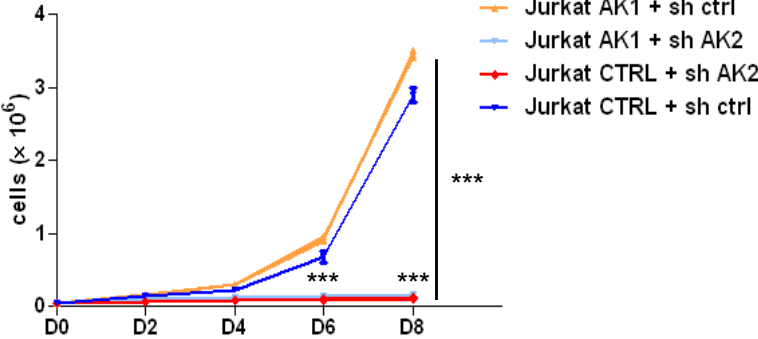
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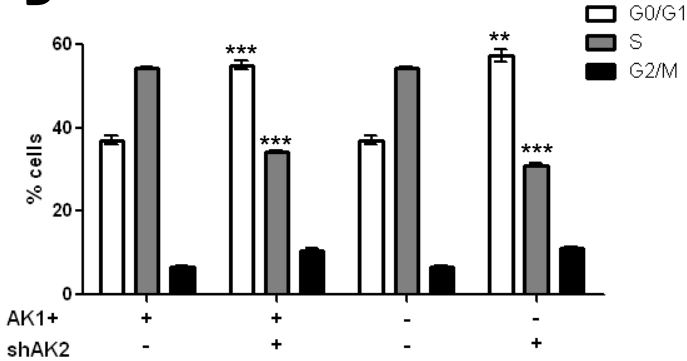
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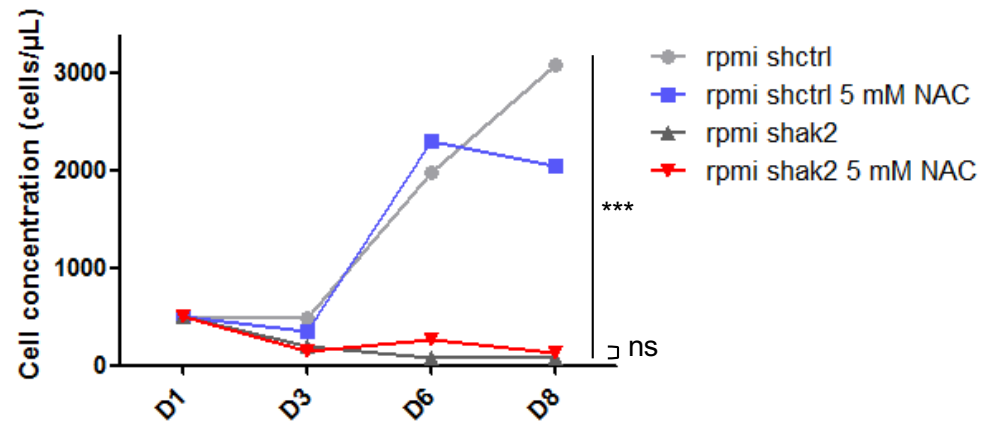
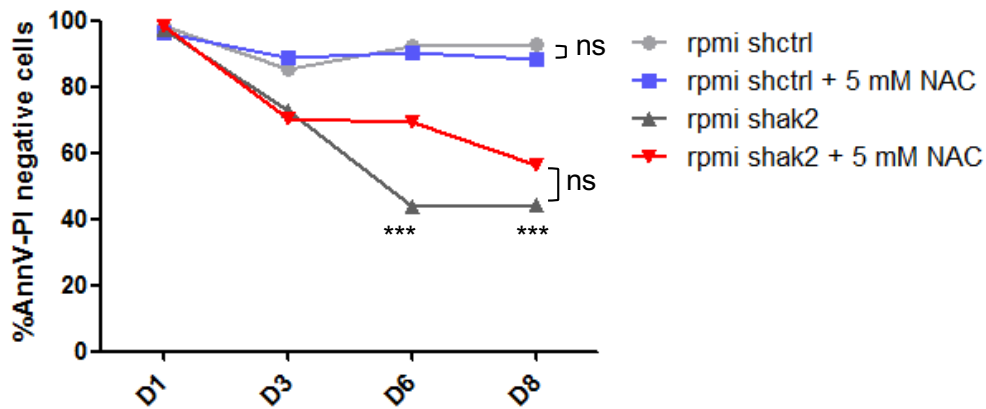
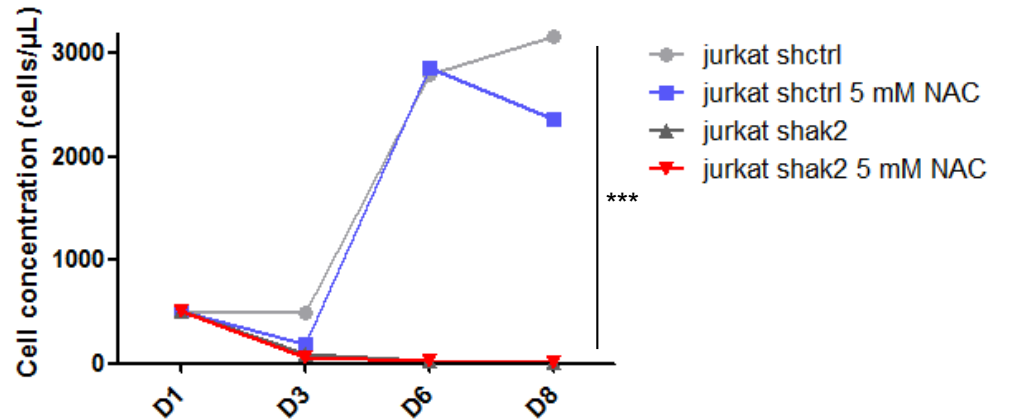
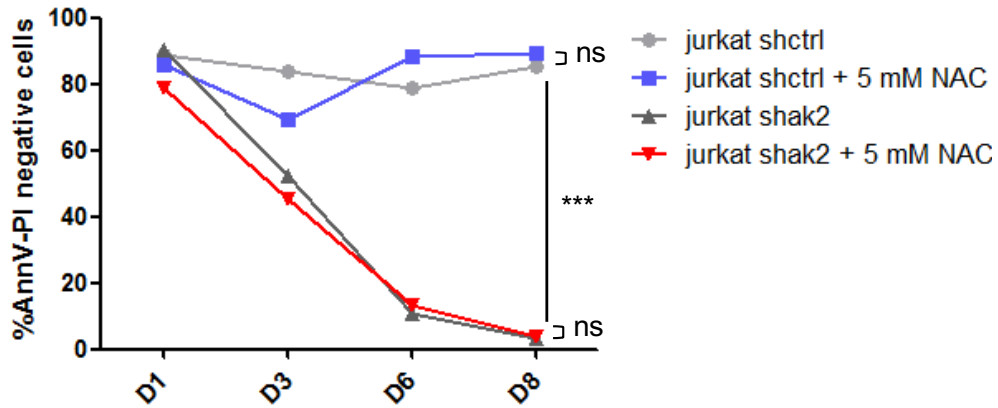
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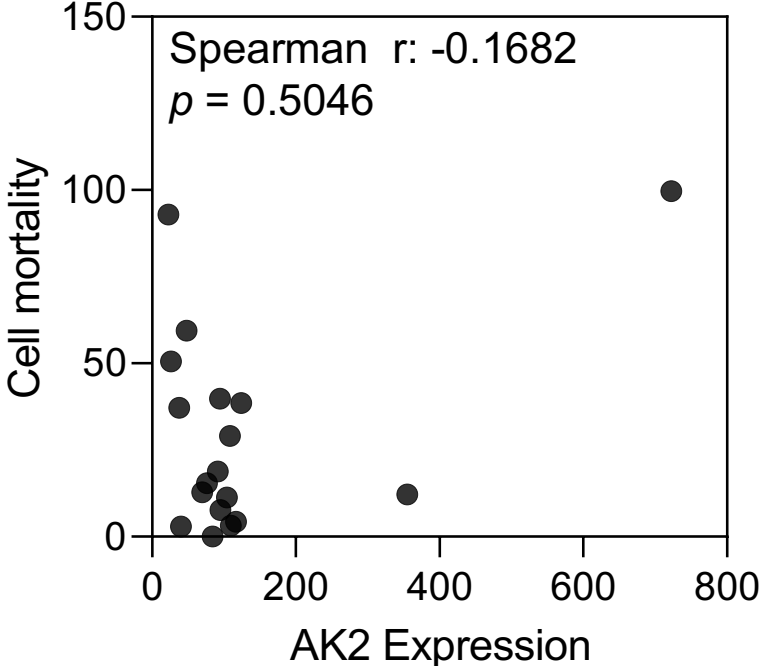


Supplementary Figure 7



Supplementary Figure 8

Correlation between AK2 expression and venetoclax sensitivity



Supplementary Table 1

Cell line	CD3	CD4	CD8	TCRab	TCRgd	CD19	cylgM
JURKAT	+	low	-	+	-	-	-
RPMI-8402	-	-	-	-	-	-	-
PEER	+	+	-	-	+	-	-
MOLT4	-	+/-	+/-	-	-	-	-
CEM	+	+	-	+	-	-	-
RCH-ACV	-	-	-	-	-	+	+
REH	-	-	-	-	-	+	-

Supplementary Table 2

Characteristic	Overall, N = 145	1, N = 37 (26%) ¹	2, N = 32 (22%) ¹	3, N = 40 (28%) ¹	4, N = 36 (25%) ¹	p-value ²
Median Age (y)	31.93 ± 10.72 (16.79-59.15)	30.50 ± 11.16 (17.40-58.97)	34.48 ± 11.35 (16.79-59.15)	31.79 ± 9.49 (18.64-53.76)	33.16 ± 11.04 (17.56-56.45)	0.49
Sex						0.97
Male	100 / 145 (69%)	25 / 37 (68%)	22 / 32 (69%)	27 / 40 (68%)	26 / 36 (72%)	
Female	45 / 145 (31%)	12 / 37 (32%)	10 / 32 (31%)	13 / 40 (32%)	10 / 36 (28%)	
Median WBC (G/l)	36.80 ± 115.04 (1.50-645.00)	37.55 ± 114.08 (4.40-604.40)	52.84 ± 103.67 (1.50-328.00)	25.58 ± 114.09 (1.54-645.00)	48.08 ± 126.62 (2.81-456.00)	0.45
CNS	15 / 145 (10%)	5 / 37 (14%)	3 / 32 (9%)	3 / 40 (8%)	4 / 36 (11%)	0.85
Immunophenotypic classification						0.044
ETP	32 / 134 (24%)	5 / 35 (14%)	13 / 30 (43%)	8 / 36 (22%)	6 / 33 (18%)	
Alpha Beta	73 / 134 (54%)	24 / 35 (69%)	13 / 30 (43%)	17 / 36 (47%)	19 / 33 (58%)	
Gamma Delta	13 / 134 (10%)	4 / 35 (11%)	3 / 30 (10%)	5 / 36 (14%)	1 / 33 (3%)	
Immature	16 / 134 (12%)	2 / 35 (6%)	1 / 30 (3%)	6 / 36 (17%)	7 / 33 (21%)	
Oncogenomic classification						0.11
AF10 Fusion	10 / 67 (15%)	2 / 21 (10%)	4 / 14 (29%)	2 / 16 (12%)	2 / 16 (12%)	
MLL Fusion	2 / 67 (3%)	0 / 21 (0%)	0 / 14 (0%)	1 / 16 (6%)	1 / 16 (6%)	
SET-NUP	3 / 67 (4%)	0 / 21 (0%)	0 / 14 (0%)	2 / 16 (12%)	1 / 16 (6%)	
SIL-TAL	17 / 67 (25%)	11 / 21 (52%)	3 / 14 (21%)	0 / 16 (0%)	3 / 16 (19%)	
TLX1	22 / 67 (33%)	4 / 21 (19%)	4 / 14 (29%)	8 / 16 (50%)	6 / 16 (38%)	
TLX3	13 / 67 (19%)	4 / 21 (19%)	3 / 14 (21%)	3 / 16 (19%)	3 / 16 (19%)	
Corticosensitivity	73 / 145 (50%)	17 / 37 (46%)	16 / 32 (50%)	22 / 40 (55%)	18 / 36 (50%)	0.89
Chemosensitivity	78 / 144 (54%)	19 / 37 (51%)	17 / 31 (55%)	19 / 40 (48%)	23 / 36 (64%)	0.53
MRD1 > 10e-3	19 / 77 (25%)	4 / 14 (29%)	6 / 21 (29%)	6 / 23 (26%)	3 / 19 (16%)	0.77
Allograft	50 / 145 (34%)	12 / 37 (32%)	11 / 32 (34%)	15 / 40 (38%)	12 / 36 (33%)	0.97

¹Statistics presented: median ± SD (minimum-maximum); n / N (%)

²Statistical tests performed: One-way ANOVA; chi-square test of independence