Supplemental Figures and Tables

Azacitidine is synergistically lethal with XPO1 inhibitor Selinexor in acute myeloid leukemia by Targeting XPO1/eIF4E/c-MYC Signaling

Huideng Long[1#], Yue Hou[1#], Jun Li, Chunhua Song[2,3] and Zheng Ge[1*]

[1]Department of Hematology, Zhongda Hospital, School of Medicine, Southeast University, Institute of Hematology Southeast University, Nanjing 210009, China

[2]Hershey Medical Center, Pennsylvania State University Medical College, Hershey, PA17033, USA

[3]Division of Hematology, The Ohio State University Wexner Medical Center, the James Cancer Hospital, Columbus, OH 43210, USA

These authors contributed equally to the work.

Correspondence to:
Zheng Ge, M.D., Ph.D.
Department of Hematology, Zhongda Hospital, School of Medicine, Southeast University
Institute of Hematology Southeast University
87 Dingjiaqiao Street, Nanjing 210009, China,
Telephone: +86 25-83262468; Fax: +86 25-83262471
E-mail: zhengge@seu.edu.cn

ORCID: https://orcid.org/0000-0001-8028-1612

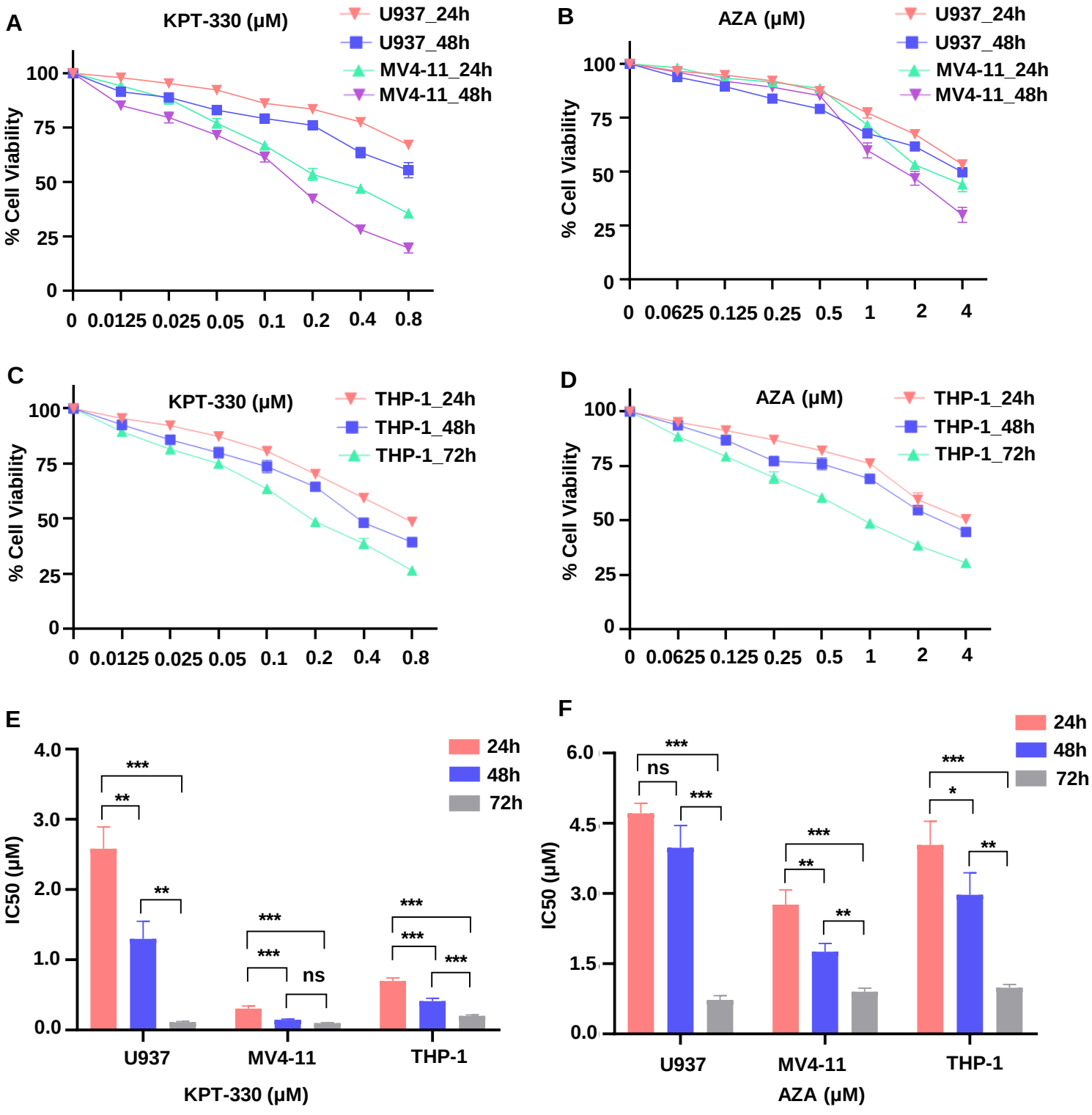

Figure S1 Growth inhibition of KPT-330 or AZA on AML cell lines. (A) Cell proliferation assay result (dose-dependent curve) for KPT-330 in U937 and MV4-11 for 24h and 48h. (B) Cell proliferation assay result (dose-dependent curve) for AZA in U937 and MV4-11 for 24h and 48h. (C) Cell proliferation assay result (dose-dependent curve) for KPT-330 in THP-1 for 24h, 48h and 72h. (D) Cell proliferation assay result (dose-dependent curve) for AZA in THP-1 for 24h, 48h and 72h. (E-F) IC50 values of three cell lines were calculated and an IC50 curve was observed based on the activity of KPT-330 or AZA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns:$p > 0.05$

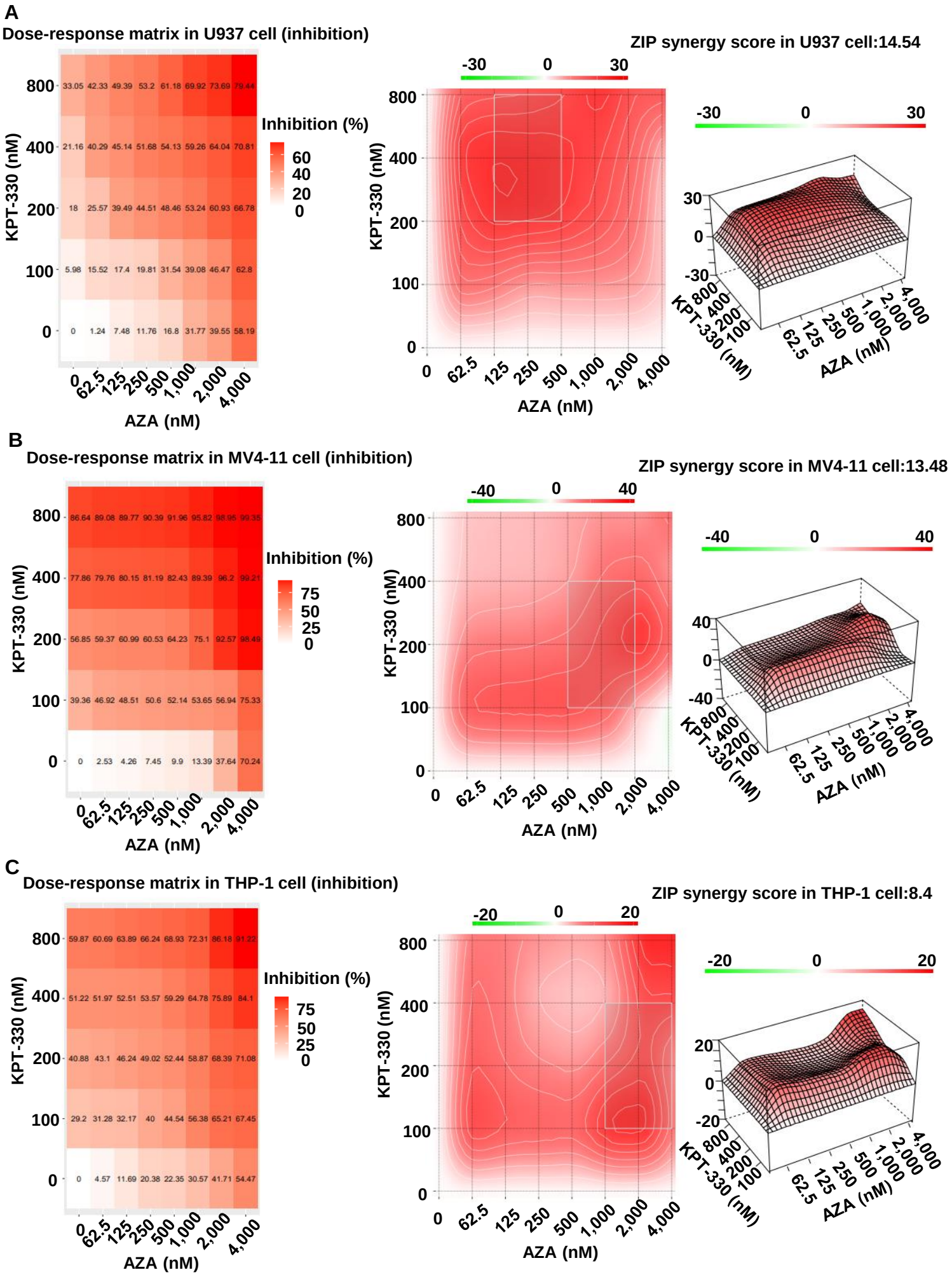

Figure S2 Bliss analysis of Synergy of the combination on cell proliferation arrest. (A-C) Cell viability was calculated for every dose combination of KPT-330 and AZA in U937 (A), MV4-11 (B) and THP-1 (C) cells using the SynergyFinder Web application. Synergy score: < -10, antagonistic; from -10 to10, additive; >10, synergistic.

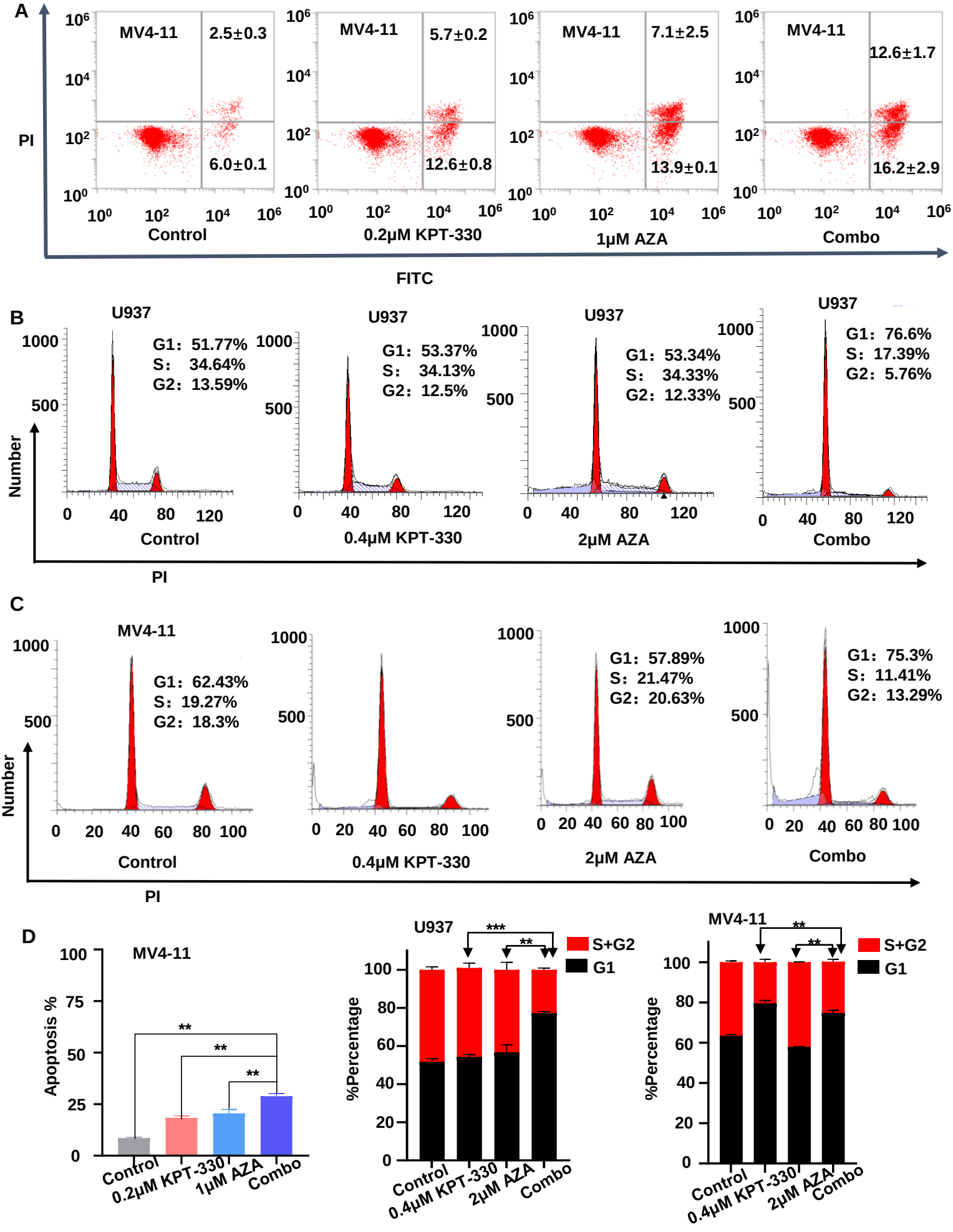

Figure S3 Effect of KPT-330 with AZA on apoptosis and cell cycle of AML cells by flow cytometry. (A) Effect of KPT-330 with AZA on apoptosis of MV4-11 cells. (B) Effect of KPT-330 with AZA on cell cycle of U937 cells. (C) Effect of KPT-330 with AZA on apoptosis of MV4-11 cells. (D) Quantitative bar figures in the cell lines (U937 and MV4-11). $^{**}p < 0.01$, $^{***}p < 0.001$

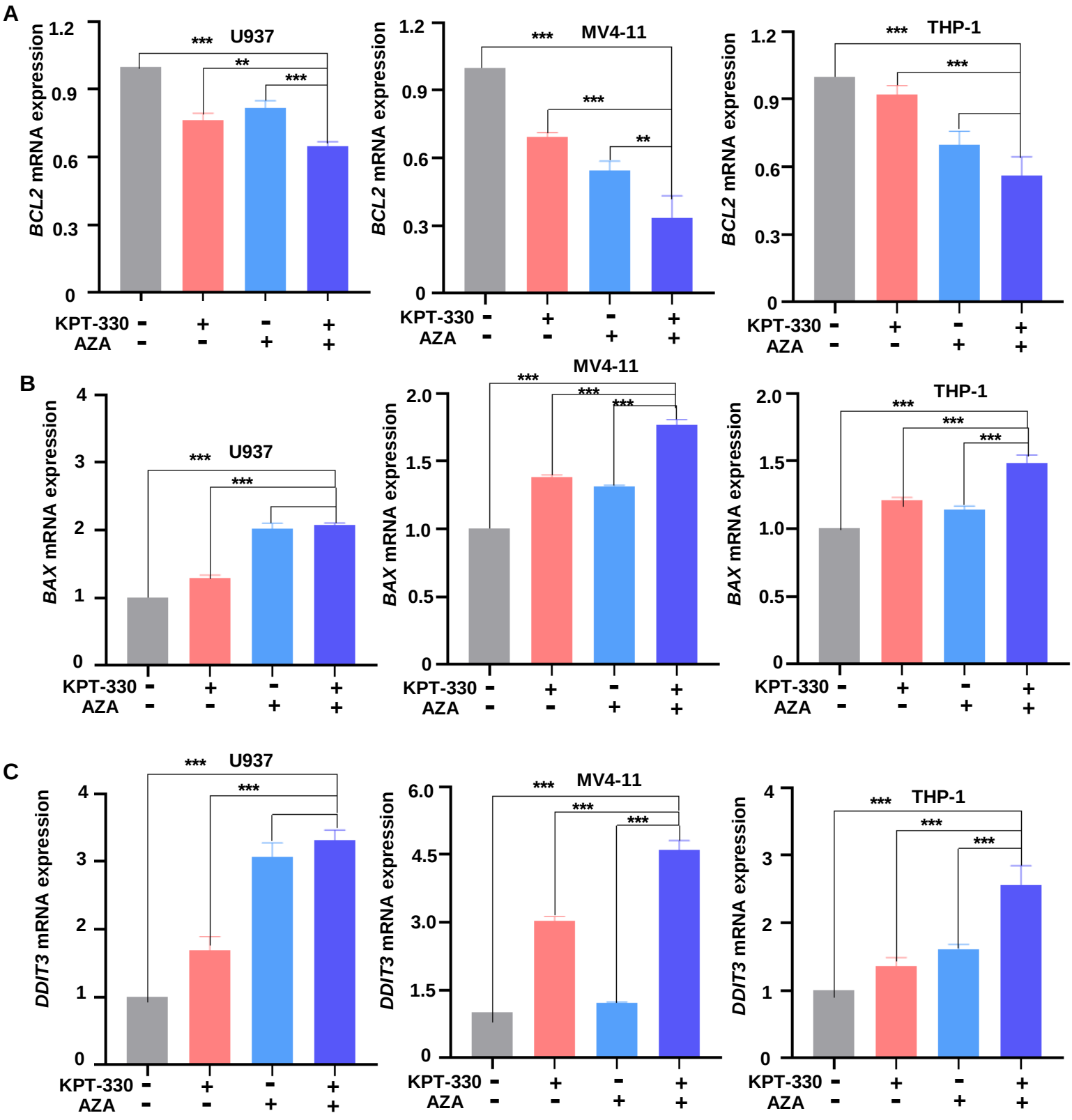

Figure S4 Change of mRNA level in the apoptosis-related genes (*BCL2, BAX and DDIT3*) upon the drug treatment in U937 (A), MV4-11 (B) and THP-1 (C) cells. $^{**}p < 0.01$, $^{***}p < 0.001$

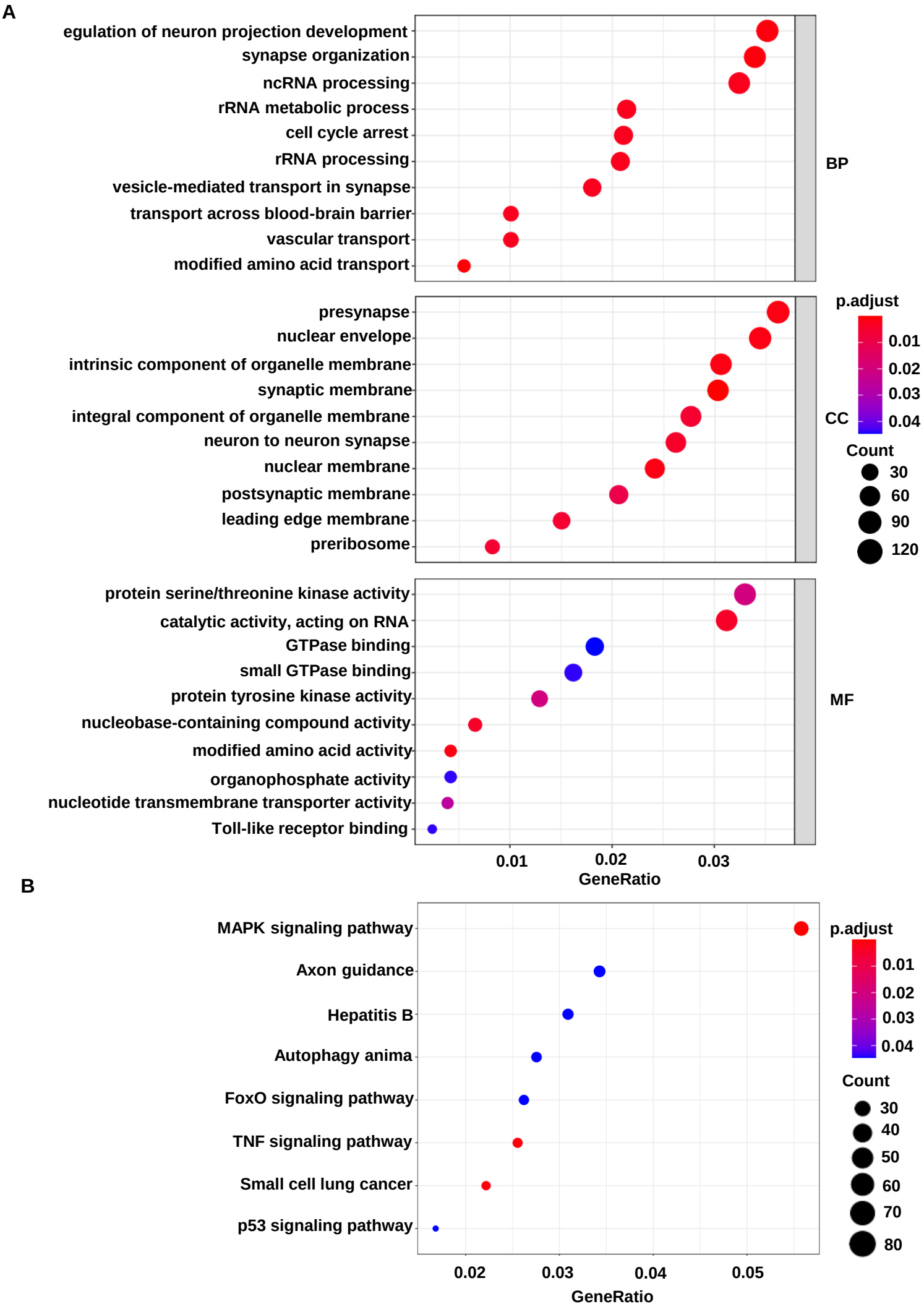

Figure S5 Transcriptome differential gene analysis to identify key signaling pathways by bioinformatics. (A) GO analysis figure. (B) KEGG analysis figure.

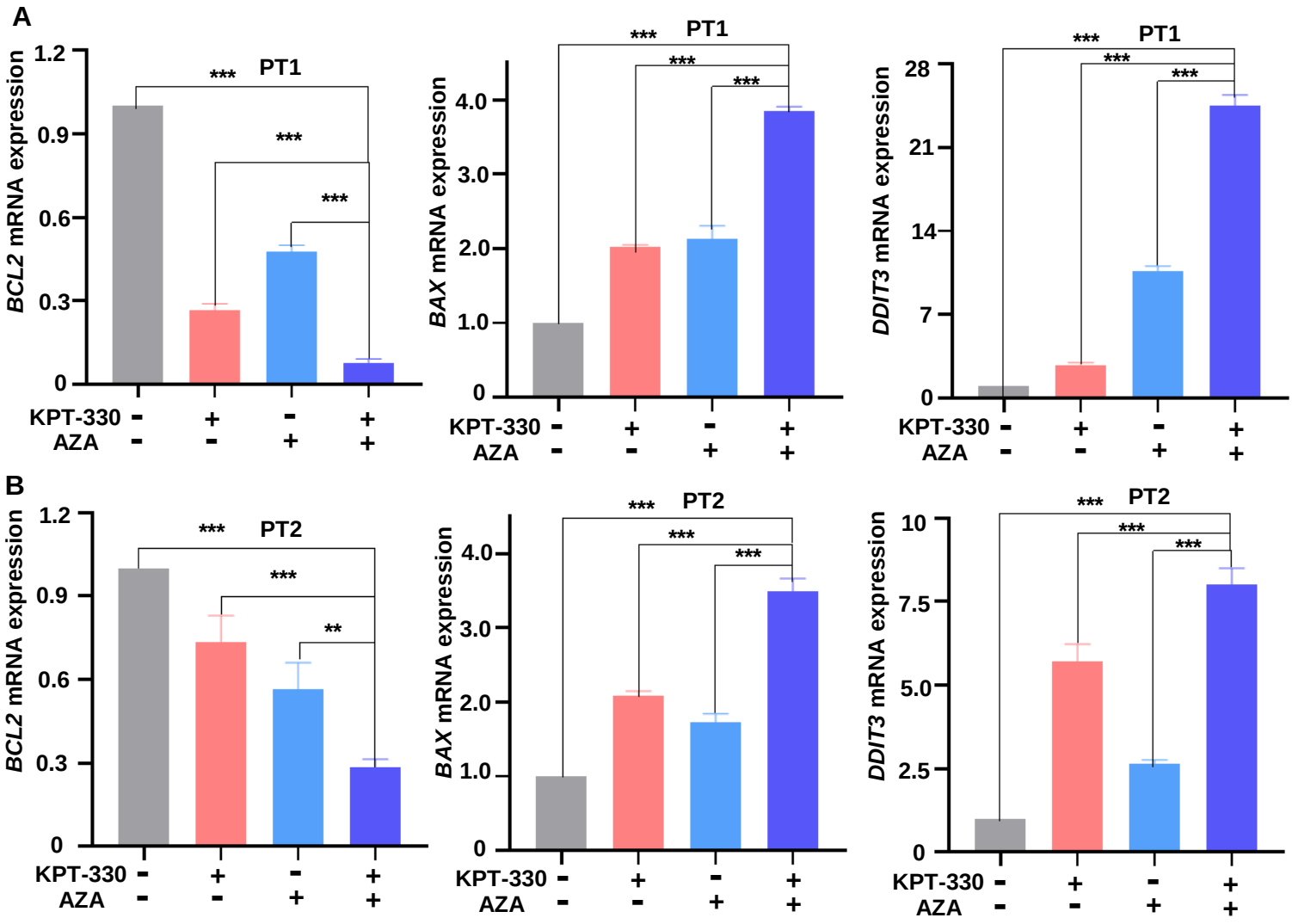

Figure S6 Change of mRNA level in the apoptosis-related genes (*BCL2, BAX and DDIT3*) were determined after drug exposure in PT1 (A) and PT2 (B). $^{**}p < 0.01$, $^{***}p < 0.001$

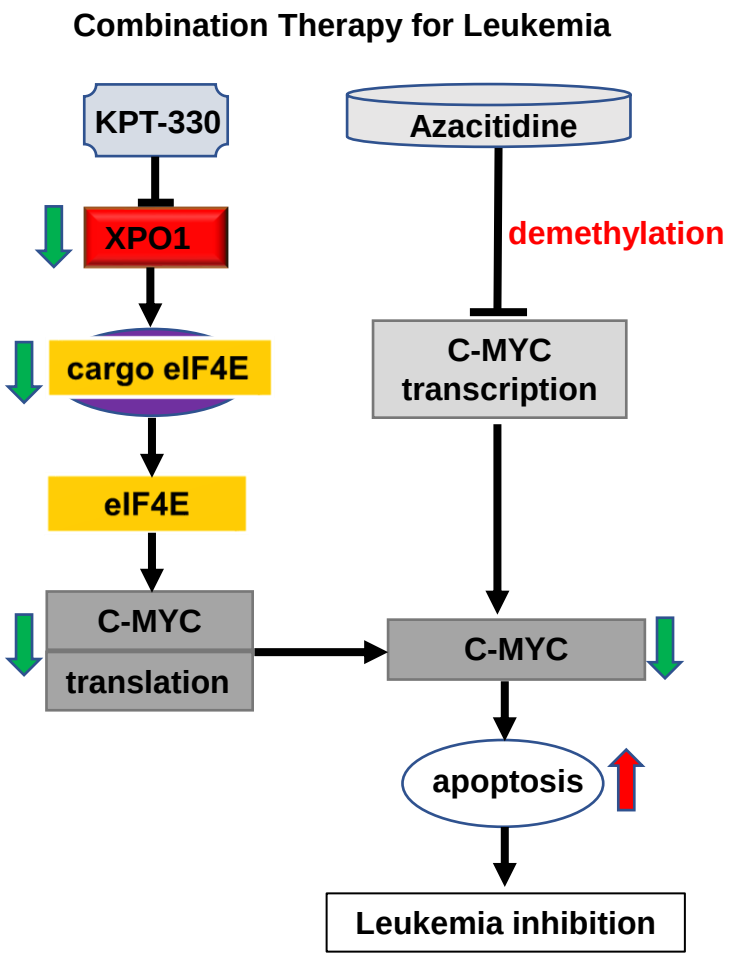

Figure S7 Anti-tumor mechanism model of the combination in AML.

Table S1. Correlation of characteristics of AML patients with *XPO1* and *eIF4E* expression in our center.

Characteristics	XPO1 expression (n=53)		P	eIF4E expression (n=53)		P
	low (n=40)	high (n=13)		low (n=40)	high (n=13)	
Sex, n (%)			0.534			0.338
Male	17(42.5)	7(53.8)		20(50)	4(30.8)	
Female	23(57.5)	6(46.2)		20(50)	9(69.2)	
FAB, n			0.597			0.256
M1	3	1		2	2	
M2	20	4		20	4	
M4	4	2		3	3	
M5	11	6		13	4	
NA	2	0		2	0	
Age, n			0.757			0.757
<60	19(47.5)	7(53.8)		19(47.5)	7(53.8)	
≥60	21(52.5)	6(46.2)		21(52.5)	6(46.2)	
BM blasts, %			0.385			0.620
Median(range)	60.6(23.5-96)	54.4(19-89)		64.25(19-96)	63.2(33.2-94.9)	
WBC(×109/L)			0.301			0.385
Median(range)	13.02(1.91-242.88)	4.8(0.4-73.97)		18.29(0.69-94.47)	33.34(0.4-242.88)	
Hb(g/L)			0.602			0.888
Median(range)	79(36-112)	70.5(36-152)		70.5(36-152)	71.5(42-112)	
ELN risk, n (%)			0.898			0.107
Favorable	8(20)	3(23.1)		10(25)	1(7.7)	
Intermediate/Poor	30(75)	9(69.2)		29(72.5)	10(76.9)	
NA	2(5)	1(7.7)		1(2.5)	2(15.4)	
Karyotype, n (%)			0.625			0.174
normal	22(55)	9(69.2)		22(55)	9(69.2)	
abnormal	15(37.5)	3(23.1)		16(40)	2(15.4)	
NA	3(7.5)	1(7.7)		2(5)	2(15.4)	
FLT3, n (%)			0.805			0.586
Wt	36(90)	12(92.3)		37(92.5)	11(84.6)	
Mut	4(10)	1(7.7)		3(7.5)	2(15.4)	
TP53, n (%)			0.982			0.249
Wt	37(92.5)	12(92.3)		38(95)	11(84.6)	
Mut	3(7.5)	1(7.7)		2(5)	2(15.4)	

There was no difference in clinical characteristics between the high and low-expression groups of *XPO1*. The *eIF4E* high group tended to have more *FLT3* (2/11 vs. 3/37) and *TP53* (2/11 vs. 2/38) mutations than the *eIF4E* low expression group.