SUPPLEMENTARY APPENDIX

Combinatorial targeting of XPO1 and FLT3 exerts synergistic anti-leukemia effects through induction of differentiation and apoptosis in *FLT3*-mutated acute myeloid leukemias: from concept to clinical trial

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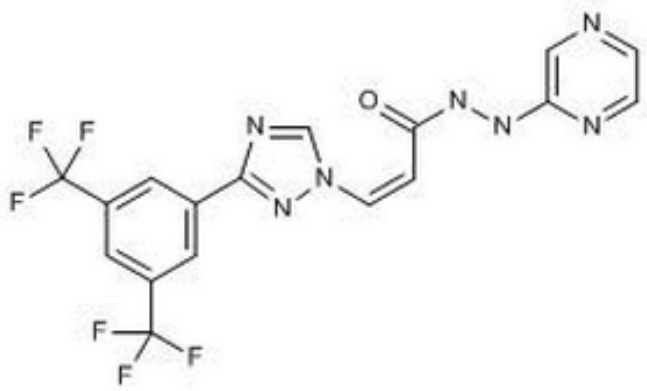
Supplementary Figures:

S1

A

Selinexor Chemical Structure

Molecular Weight: 443.31



B

Sorafenib Chemical Structure

Molecular Weight: 464.8

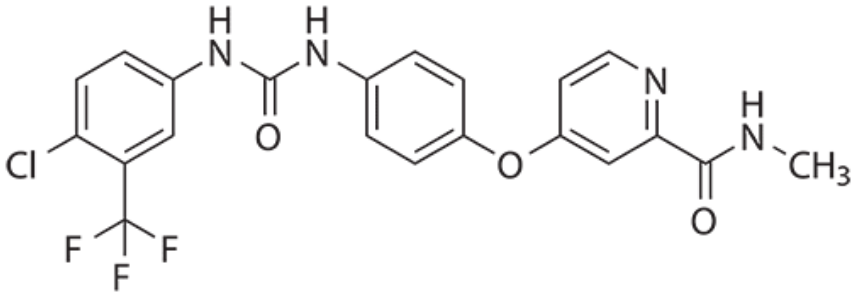
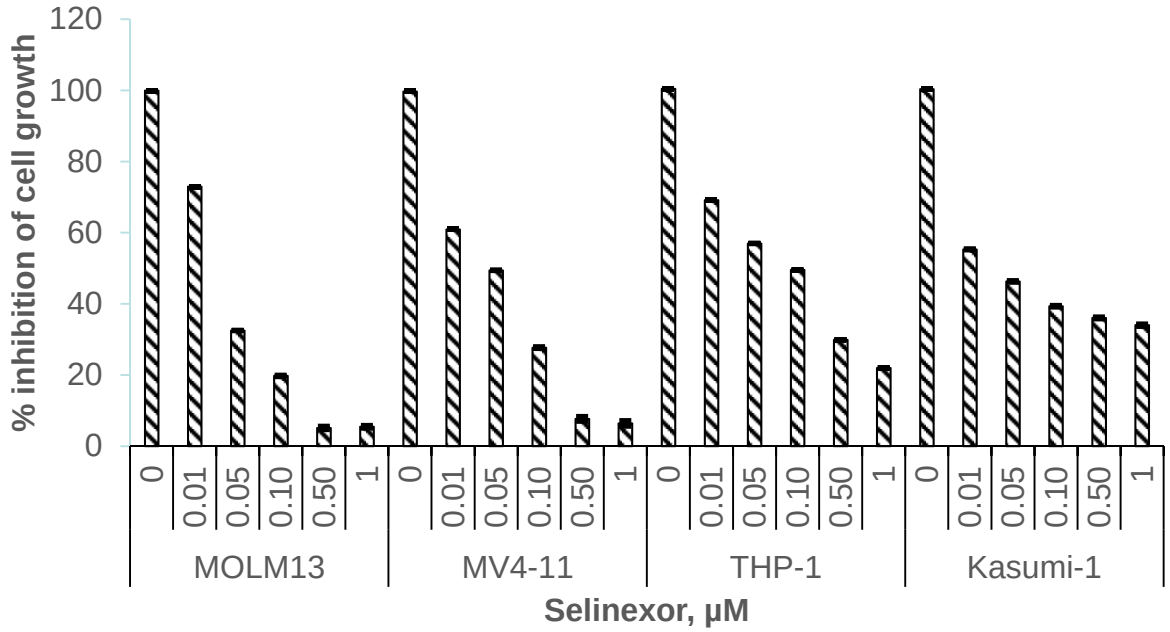


Fig. S1. Molecular structures of drugs used in this study.

S2

A



B

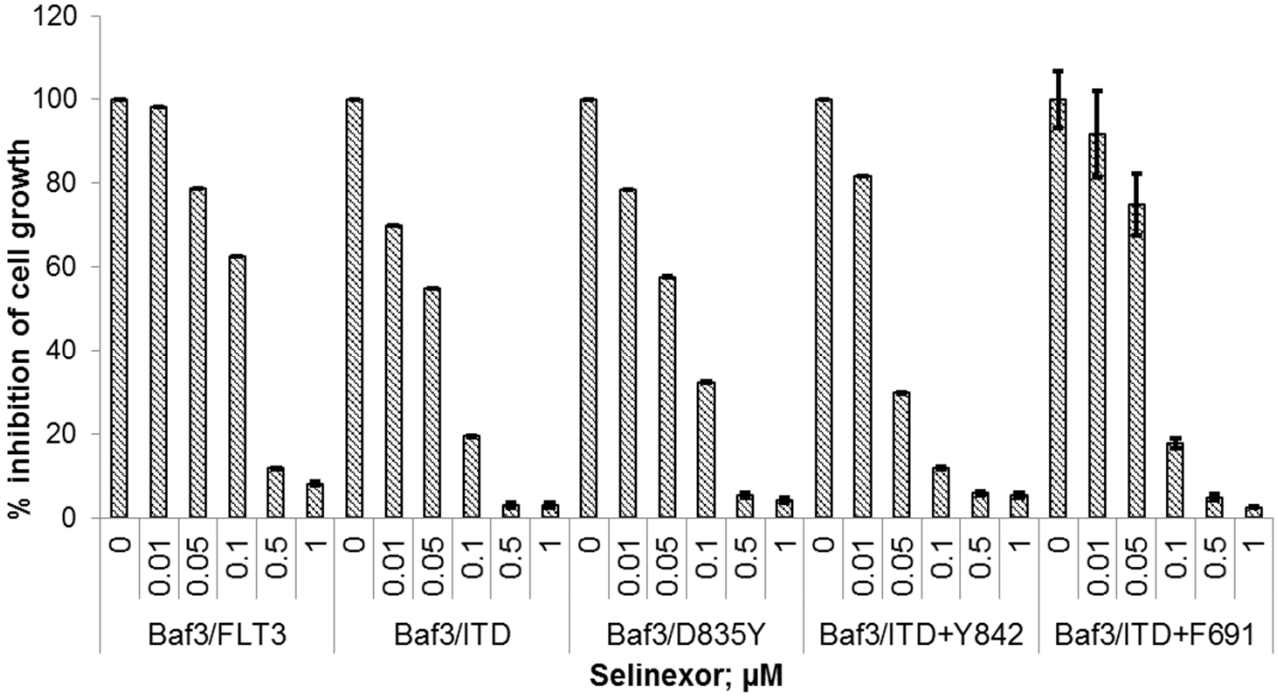


Fig. S2. Human (a) and murine (b) AML cells harboring wild type or ITD, TKD, or dual mutations of FLT3 were treated with indicated concentrations of selinexor for 72 h, Absolute viable cell number was counted using the Trypan blue dye exclusion method and relative percentage of anti-proliferative effect was assessed by compared with DMSO control group.

S3

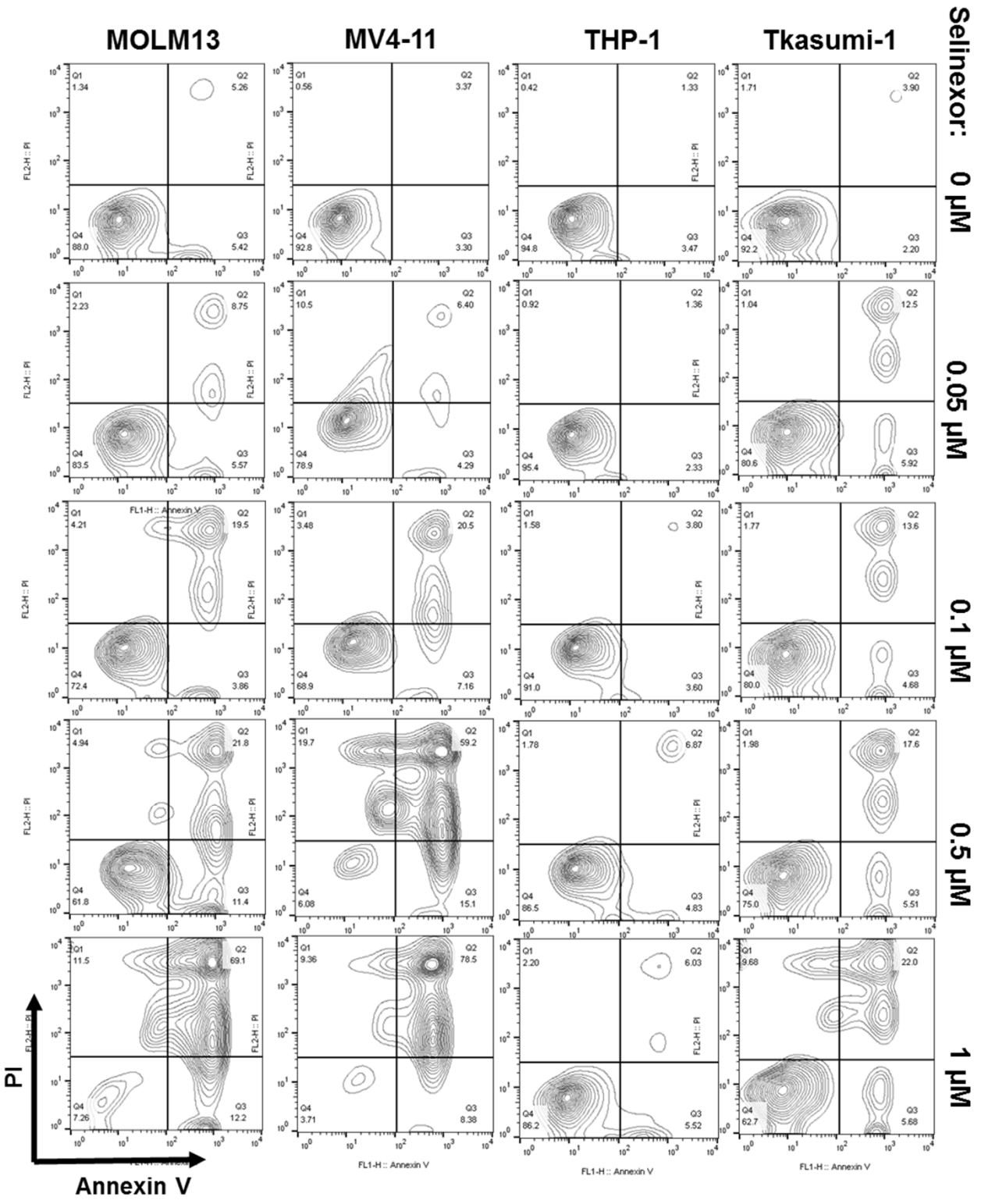


Fig. S3. Human AML cells MOLM13, MV4-11 (Harboring FLT3ITD mutations) and THP-1, Kasumi-1 (FLT3 wild type) cells were treated with indicated concentrations of selinexor for 72 h, Apoptosis induction was assessed by measuring annexin V positivity using flow cytometry.

S4

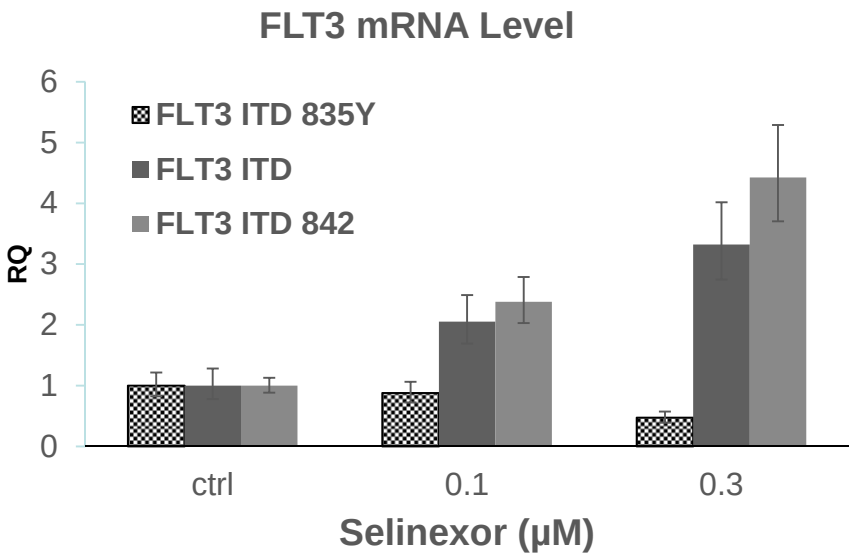


Fig. S4. Baf3-ITD, Baf3-ITD+842 and Baf3-ITD+D835Y cells were exposed in indicated concentrations of selinexor for 24 h. Total RNA was isolated with Trizol as directed by the manufacturer and reverse transcribed and qPCR were performed as described in our previous publication [Zhang, W, et al. Mol Cancer Ther. 2014;13:1848-59]. mRNA level of human FLT3 Hs00174690_m1. was assessed by qPCR. Results were analyzed using RQ Manager 1.2.1 software with 18S as housekeeping gene.

S5

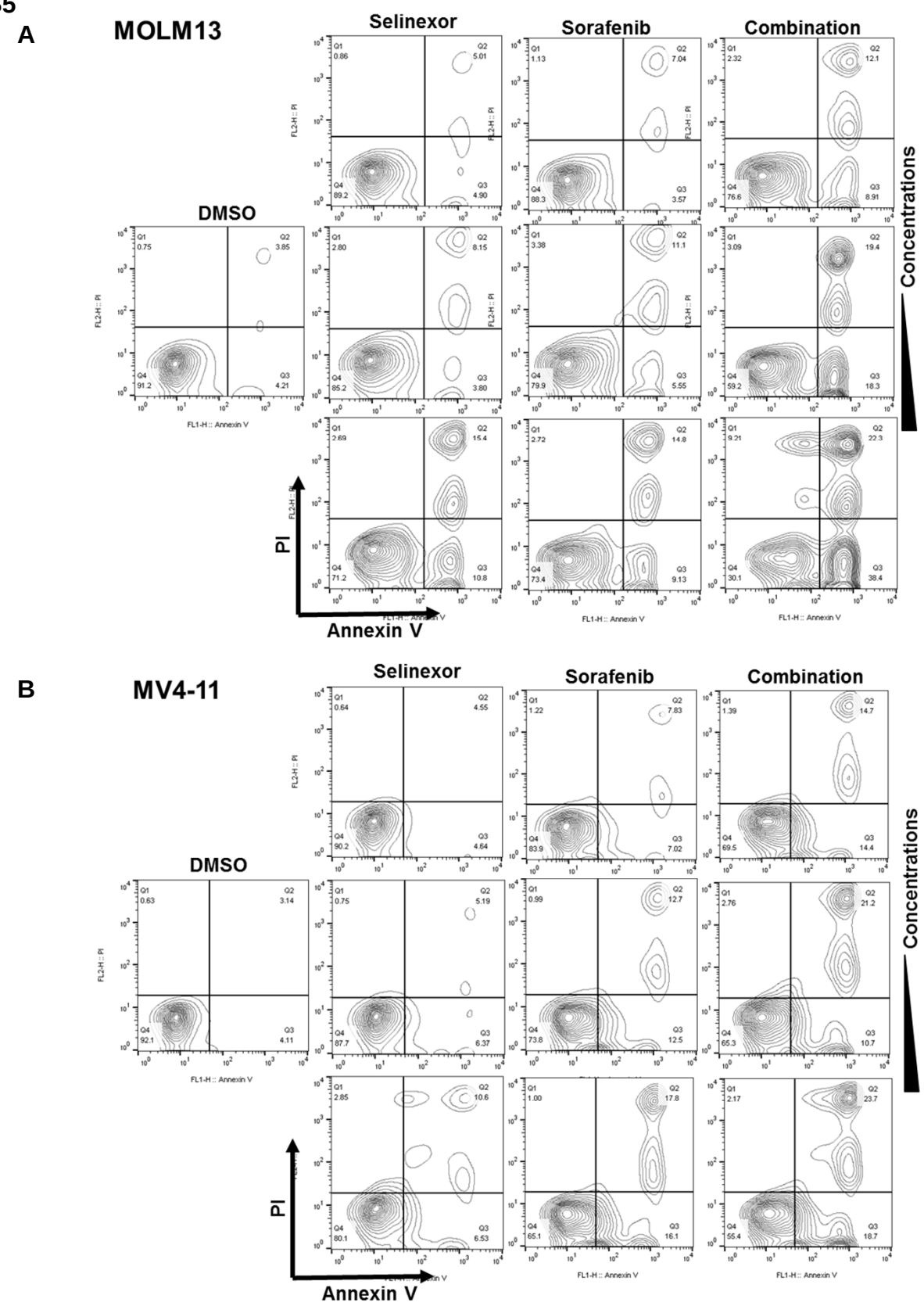


Fig. S5. Human AML cell lines MOLM13 (A) and MV4-11 (B) were treated with selinexor and/or sorafenib for 48 h, Cell apoptosis induction was assessed by measuring annexin V positivity using flow cytometry. DMSO was used as control group.

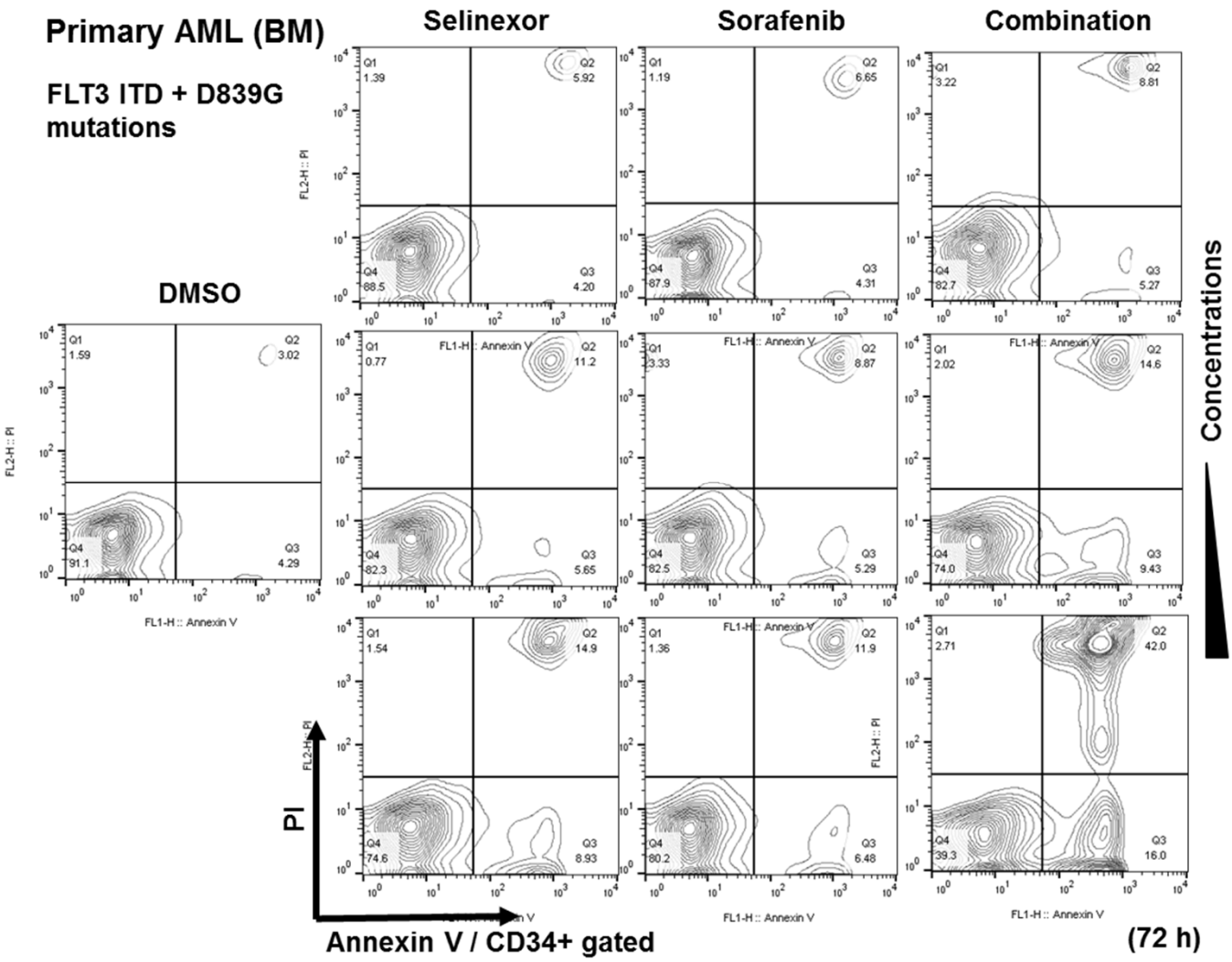


Fig. S6. Mononuclear cells form a primary AML bone marrow sample was isolated by Ficoll-Paque density gradient, and treated with increasing concentration of selinexor, sarafenib alone or the combination for 72 hours. Apoptosis induction was assessed by measuring annexin V positivity after gating with CD34 positive population using flow cytometry. DMSO was used as control. The patient harbored FLT3 ITD plus D839G mutations (ITD ratio = 0.527), and WT1, MLL mutations. Cytogenetics: 47, XX, inv(9)(p12q13), +13[19]/46, XX, inv(9)(p12q13)[1].

S7

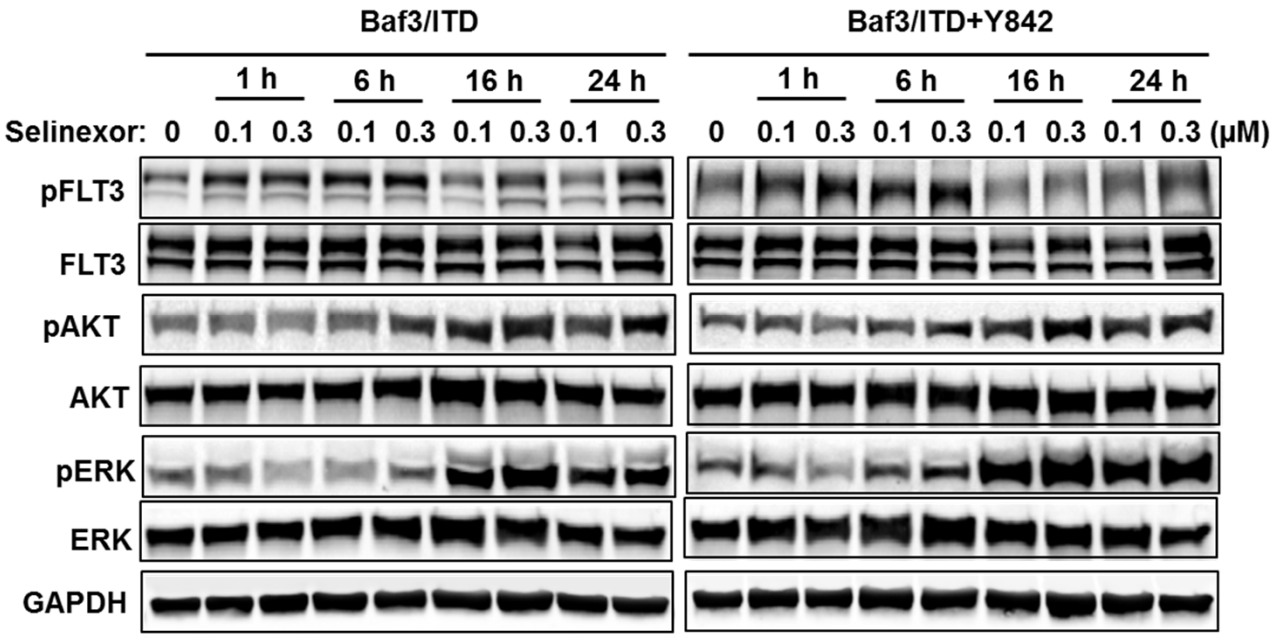


Fig. S7. Baf3-ITD and Baf3-ITD+842 cells were exposed in varying concentrations of selinexor for indicated time points and the cells were collected for lysis. Phosphorylated level of correlated proteins were assessed using immunoblotting. GAPDH was as loading control.

S8

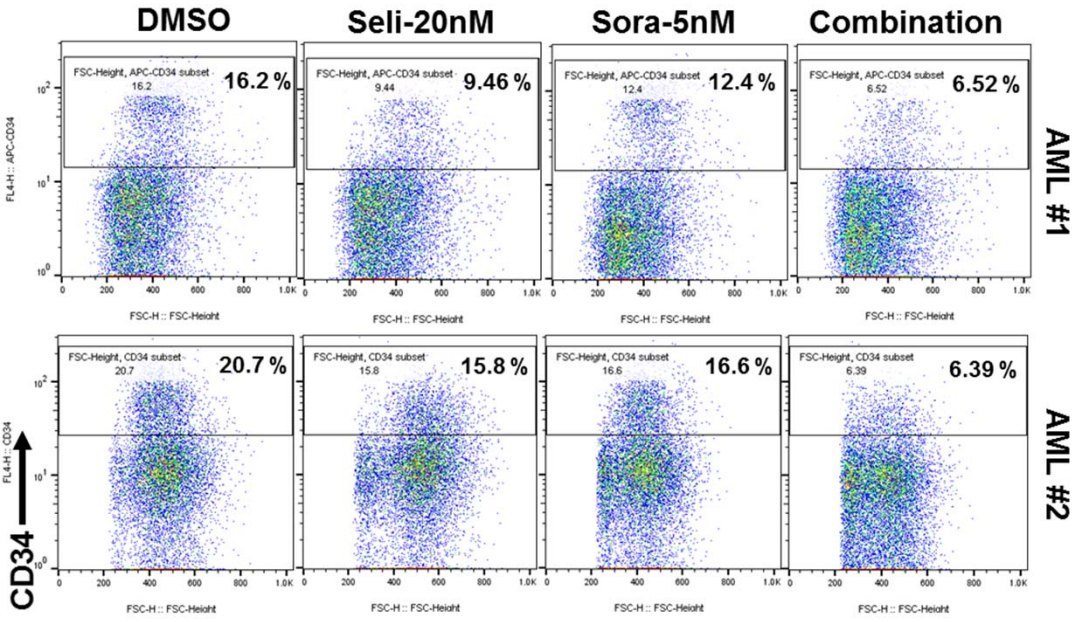


Fig. S8. Primary AML patient samples were treated with indicated concentrations of selinexor and/or sorafenib for 6 d in vitro. Percentage of CD34 positive population was measured by flow cytometry.

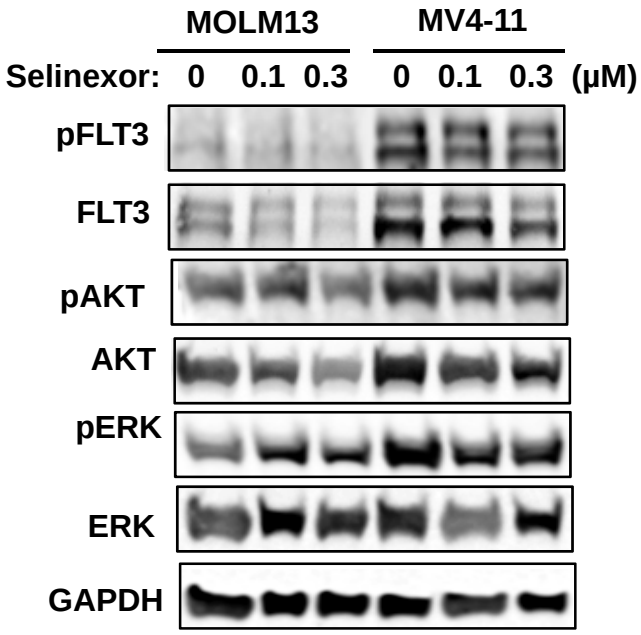


Fig. S9. Human leukemia cell lines MOLM13 and MV4-11 were exposed in indicated concentrations of selinexor for 24 h, and the cells were collected for lysis. Phosphorylated level of correlated proteins were assessed using immunoblotting. GAPDH was as loading control.

Table S1. Patient Characteristics for differentiation assays

Patients	Age	FLT3 Status (mut ratios)	Additional Mutations[a]	Karyotypes	Blasts (%)		WBC (k/μL)	Diagnosis	Prior Therapies
					PB	BM			
Case 1	73	D835 mut[b] (0.246)	EZH2, RUNX1, TET2	Diploid	7	21	46	Newly diagnosted AML	E6201
Case 2	73	ITD mut[b] (0.249)	NPM1, NMT3A, WT1	Diploid	96	94	21	Relapsed/ refractory AML	E6201

[a] Based on screening of BM samples with 28-gene mutation panel.

[b] mut = mutations