Supplemental Data: Gutierrez et al.

Supplemental Figure 1

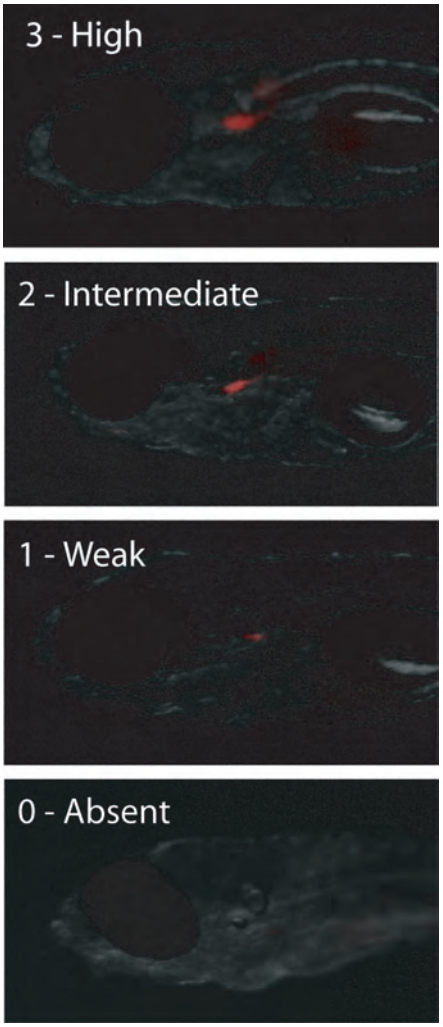

Figure S1. Representative images from the zebrafish primary screen depicting the fluorescence scoring system utilized: 3 (high or normal fluorescence), 2 (intermediate), 1 (weak) and 0 (absent) thymic fluorescence in 7 dpf zebrafish larvae expressing rag2:dsRed2.

Supplemental Figure 2

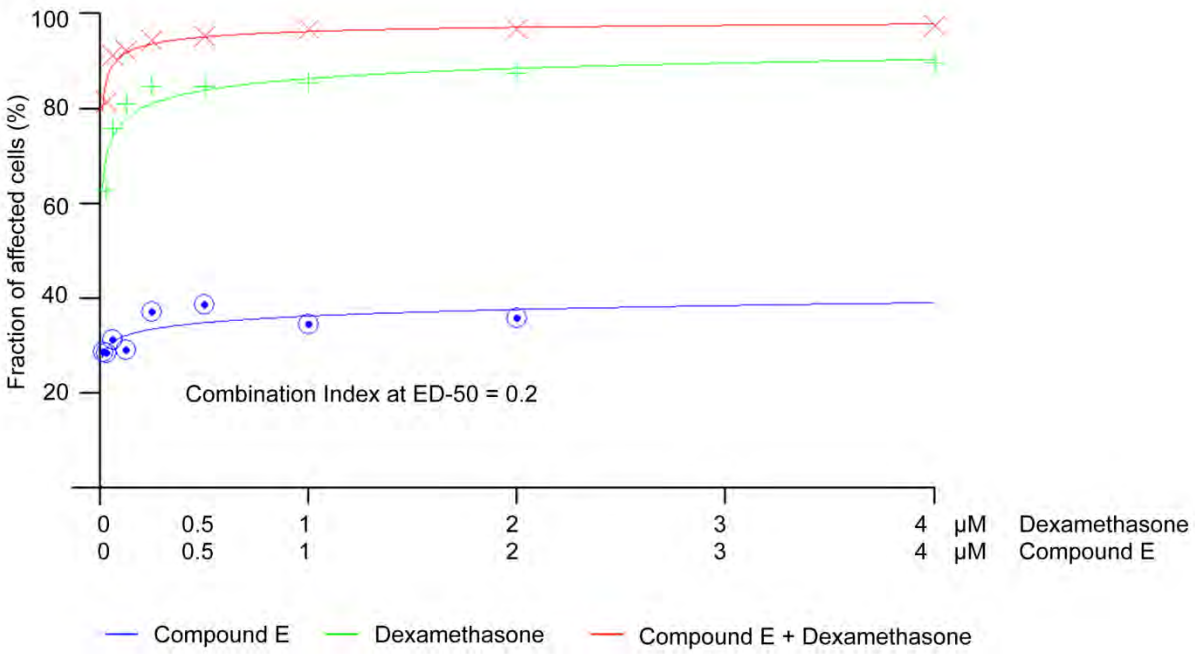

Figure S2. Synergistic suppression of KOPT-K1 cell growth by compound E and dexamethasone. KOPT-K1 cells were incubated with various concentrations of dexamethasone and compound E for 48hr, and then incubated with Cell-Titer Blue for an addition 4hr. Cell numbers were determined by measuring fluorescence at 595 nm in a plate reader. Data were processed and subjected to isobologram analysis using CalcuSyn software to determine the combination index (CI) at effective dose 50 (ED_{50}) (see methods). A combination index (CI) <0.7 at ED_{50} is taken to be indicative of synergism.

Supplemental Figure 3

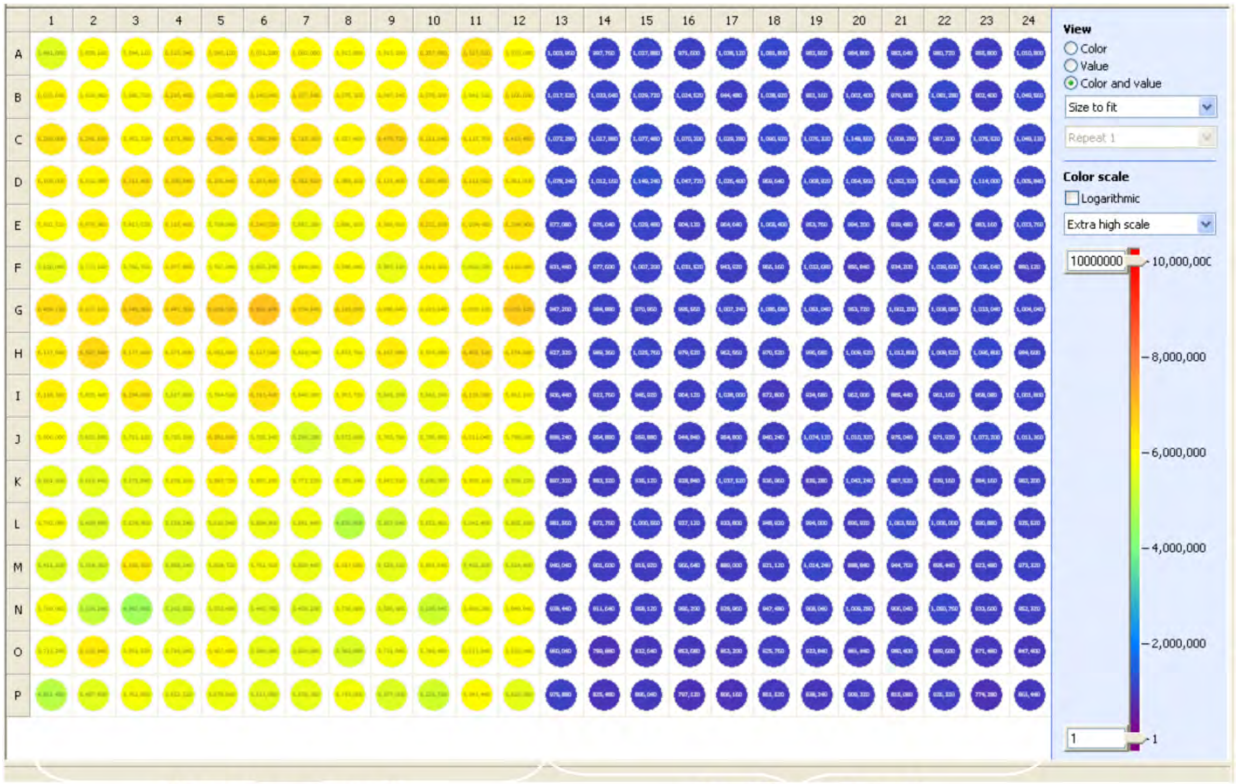

Figure S3. **Control 384-well plate showing differential effects of GSI alone and GSI plus dexamethasone on KOPT-K1 cell line growth.** The drug concentrations used produce synergistic effects on KOPT-K1 growth, as judged by prior isobologram analysis (see Supplemental Figure 2). The color of each well is proportional to cell numbers assessed by Cell-Titer Blue, with red/orange being high and blue/violet being low.

Supplemental Figure 4.

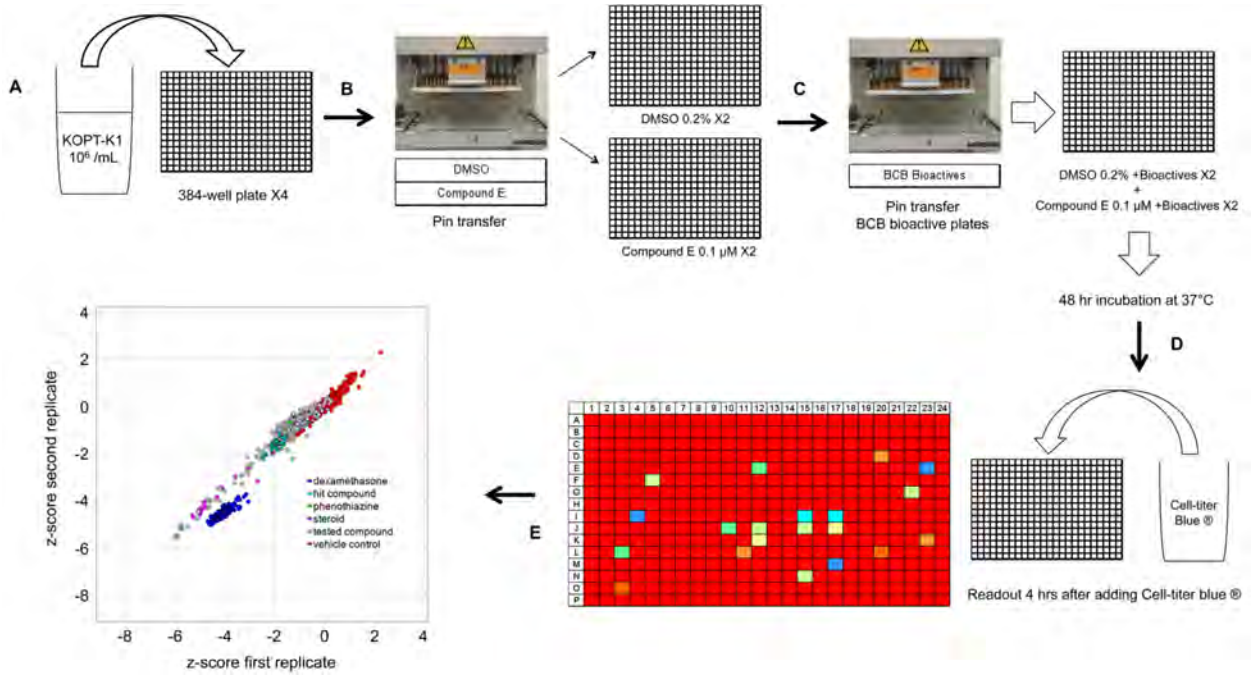

Figure S4. Small molecule screening strategy in KOPT-K1 cells. (A) Plating in 384-well format. (**B**) Pin transfer of DMSO or GSI (compound E). (**C**) Pin transfer of Broad bioactive compounds. (**D**) Incubation at for 48hr, followed by 4hr with Cell-Titer Blue (**E**) Data collection and analysis.

Supplemental Figure 5

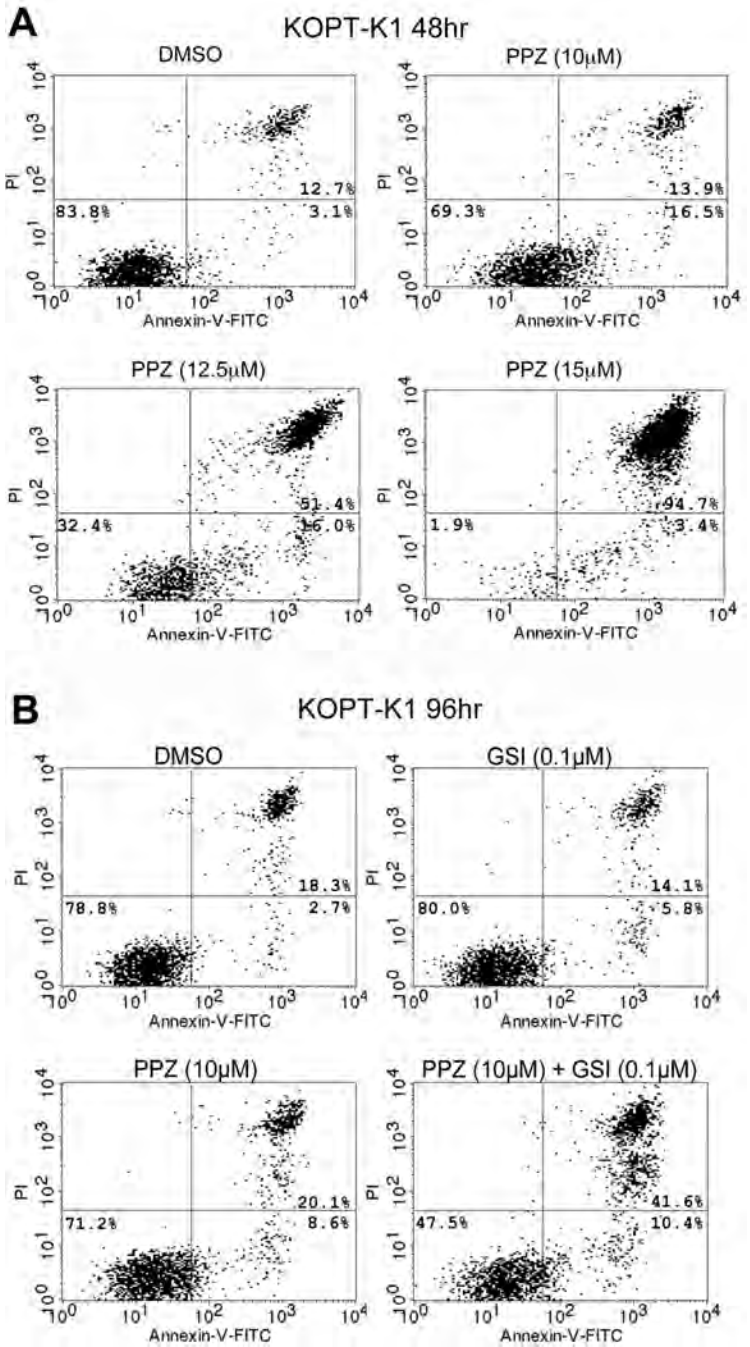

Figure S5. Induction of apoptosis in KOPT-K1 cells treated with perphenazine alone (**A**) or perphenazine and GSI (**B**). Apoptosis was assessed by staining for annexin V and propidium iodide as described under Methods.

Supplemental Figure 6.

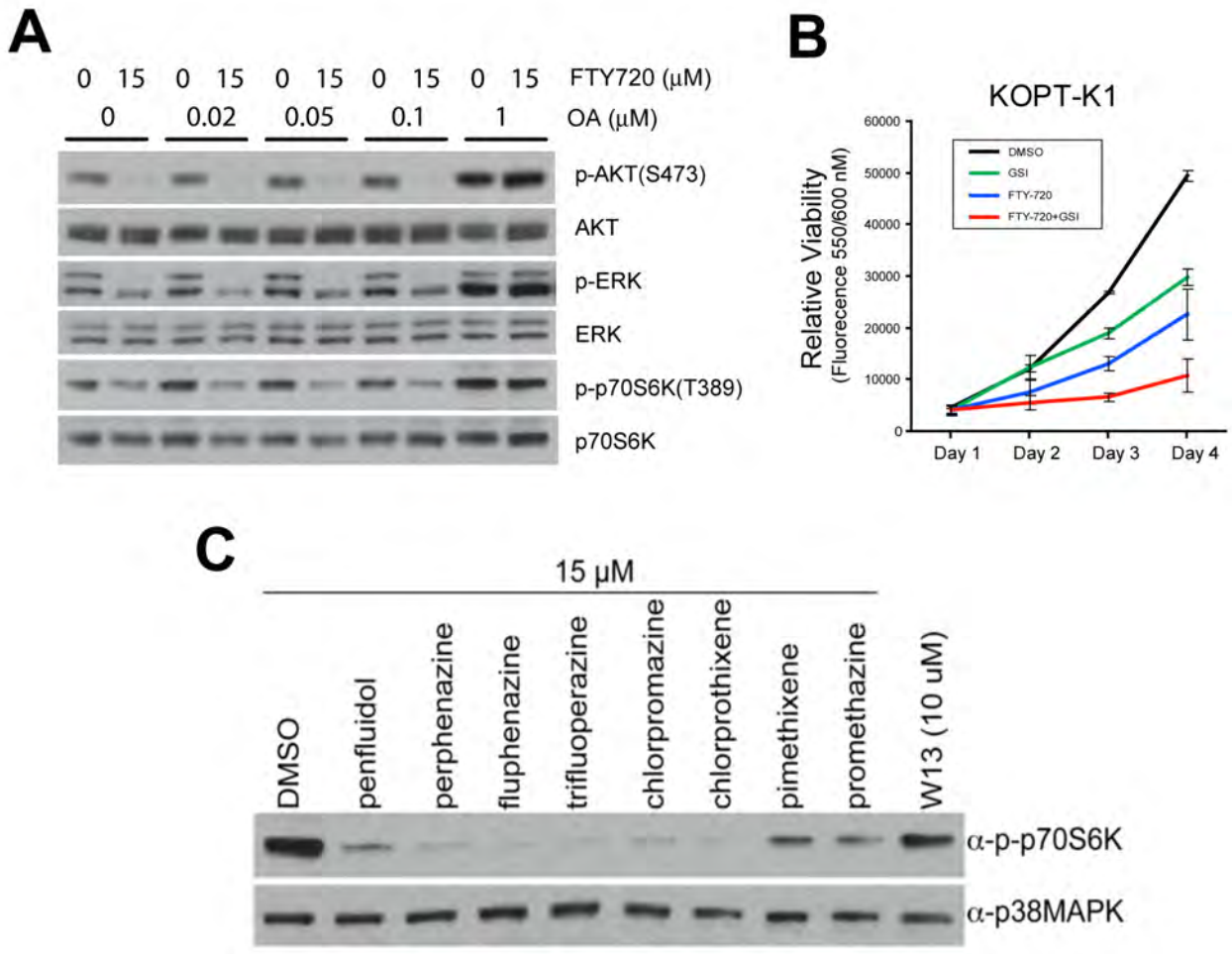

Figure S6. Effects of FTY-720 and W13 on PP2A targets in T-ALL cells. (A) Effect of FTY-720 treatment for 15 min on phosphorylation of PP2A targets in KOPT-K1 cells. (B) Effect of FTY-720 (15μM) alone and in combination with GSI (compound E, 1μM) on KOPT-K1 cell growth. (C) Comparison of effects of various phenothiazines and the calmodulin inhibitor W13 on phosphorylation of the PP2A target p70S6K. In (C), cells were incubated for 30min with the indicated drug concentrations and p38 was used as a loading control.

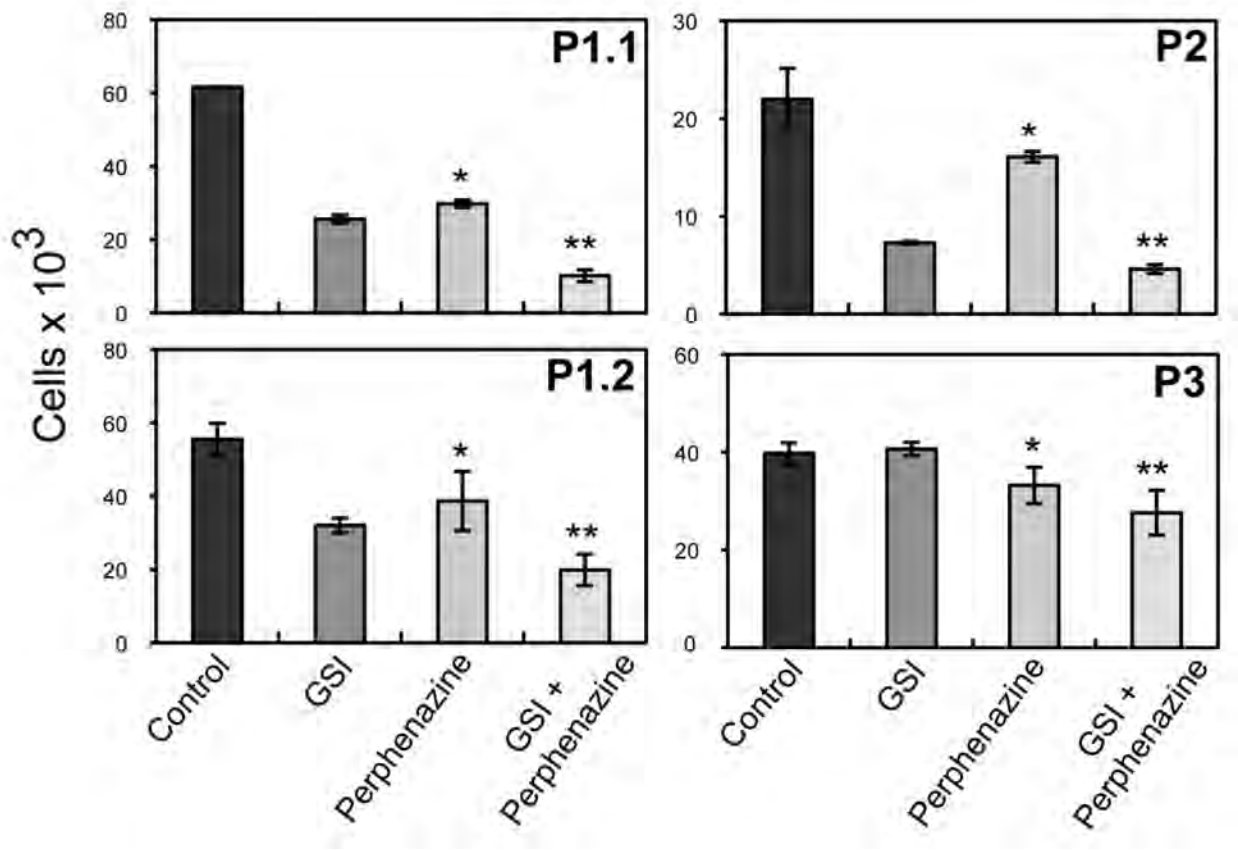

Figure S7. Effects of perphenazine and GSI on primary human T-ALLs. Primary T-ALLs were co-cultured for 7 days with MS5 bone marrow stromal cells expressing the Notch ligand DLL1. P1.1 is a primary sample with wild type *NOTCH1* alleles, and P1.2 is the same tumor following expansion in immunodeficient mice. P2 is a primary T-ALL with a NOTCH1 PEST domain mutation, whereas P3 is a primary T-ALL with a NOTCH1 HD domain mutation. Cells were treated with DMSO (control), GSI (compound E, 0.1μM), and/or perphenazine (8μM) in triplicate, and growth was monitored by counting viable cells. *, $p < 0.05$, Mann-Whitney test, in comparison to the vehicle treated control cells; **, $p < 0.05$, Mann-Whitney test, in comparison to the GSI-treated cells.

Table S1. Hits identified in zebrafish screen that are toxic to developing thymocytes.

Novel hits	**Steroids and antineoplastics**
Perphenazine	Dexamethasone
(±)-Butaclamol hydrochloride	Halcinonide
Indirubin	Isofluprednone acetate
Lidoflazine	Beclomethasone dipropionate
Nalidixic acid	Betamethasone
Huperzine A	Budesonide
Kasugamycin hydrochloride	Desonide
AG-1296	Desoxymetasone
Apigenin	Diflorasone Diacetate
5-Fluoroindole-2-carboxylic acid	Fludrocortisone acetate
L(-)-vesamicol hydrochloride	Fluocinolide
Ethacridine lactate	Flurandrenolide
Ginkgolide A	Hydrocortisone butyrate
Gedunol	Isoflupredone acetate
Melbiose	Mometasone furoate
SP600125	Prednicarbate
Cimaterol	Triamcinoline
	Pregnenolone
	Melengestrol acetate
	Sobuzoxane
	ICRF-193
	10-Hydroxycamptothecin
	Mycophenolic acid
	Idarubicin
	Thioguanosine
	Carmustine
	Thiotepa
	Trichlormethine
	Ellipticine
	Amsacrine

Table S2. Perphenazine/GSI Synergism in T-ALL cell lines

Cell Line	Notch Status	GSI Sensitive	CI (ED_{50})*
KOPT-K1 (human)	Mutated	Yes	0.299
DND41 (human)	Mutated	Yes	0.448
PF382 (human)	Mutated	No	0.660
SUP-T13 (human)	Wild type	No	1.08
142 (murine)	Mutated	Yes	0.338
144 (murine)	Mutated	Yes	0.489

*CI(ED_{50}), combination index (effective dose at which 50% of cells are affected). CI(ED_{50}) <0.7 is taken as evidence of a synergistic drug interaction.

Table S3. Characteristics of primary human T-ALL samples

Cell Line	NSG Passage #	*NOTCH1* Mutation Status
P1.1	0	Wild Type
P1.2	1	Wild Type
P2	0	PEST mutation
P3	0	HD mutation
hTALL1	2	PEST mutation
hTALL2	2	PEST mutation
hTALL6	2	PEST mutation
hTALL8	1	PEST mutation

PEST, NOTCH1 C-terminal PEST degron domain; HD, NOTCH1 extracellular heterodimerization domain