

Supplemental Data

Figure S1

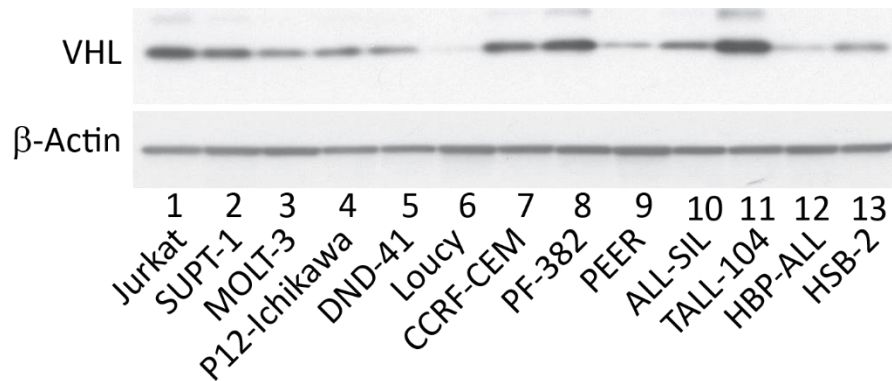
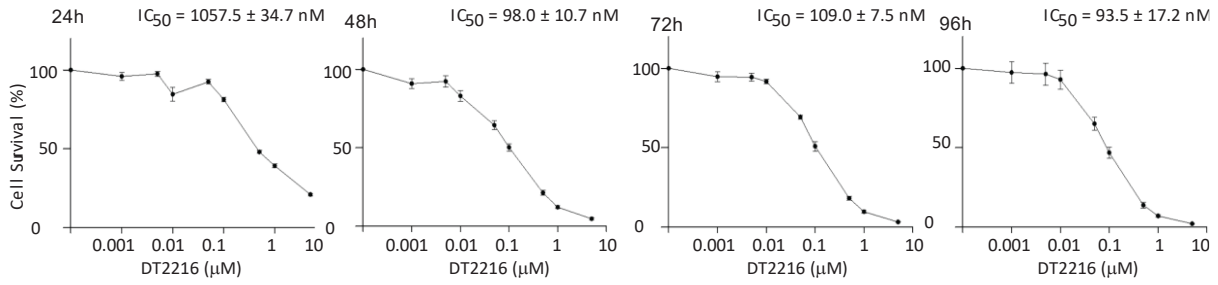


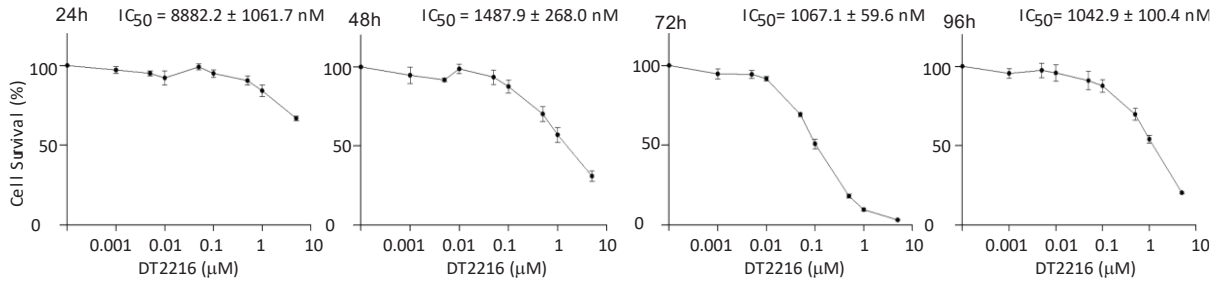
Figure S1- Steady state protein levels of VHL in 13 T-ALL cell lines. Western analysis of VHL in the 13 T-ALL lines tested for sensitivity to DT2216 indicates that there is no correlation of VHL levels with DT2216 response. For example, Loucy is the most sensitive cell lines and yet has the lowest level of VHL protein while SUPT1 is the most resistant cell line but has a moderate level of VHL.

Figure S2

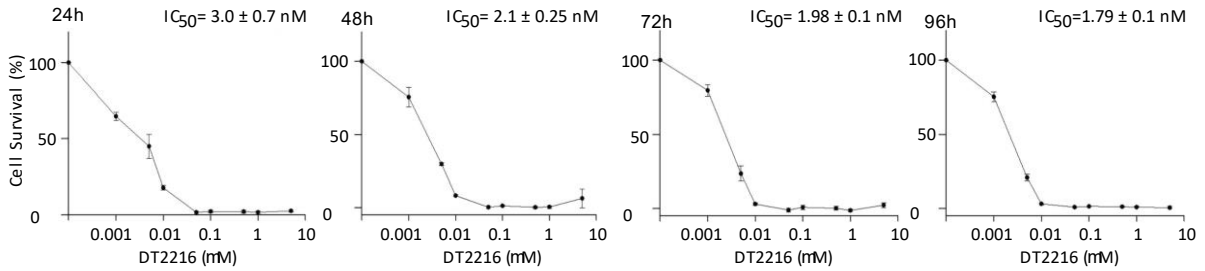
Jurkat



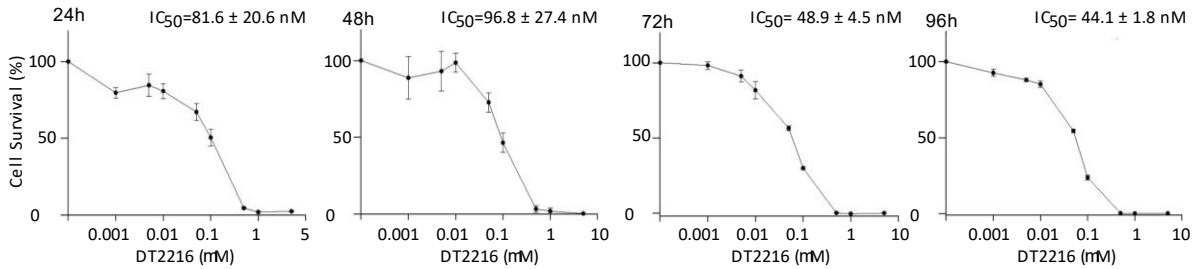
SupT1



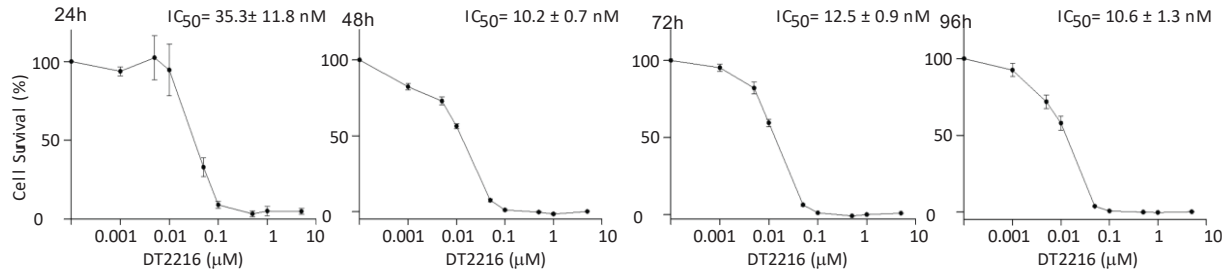
Loucy



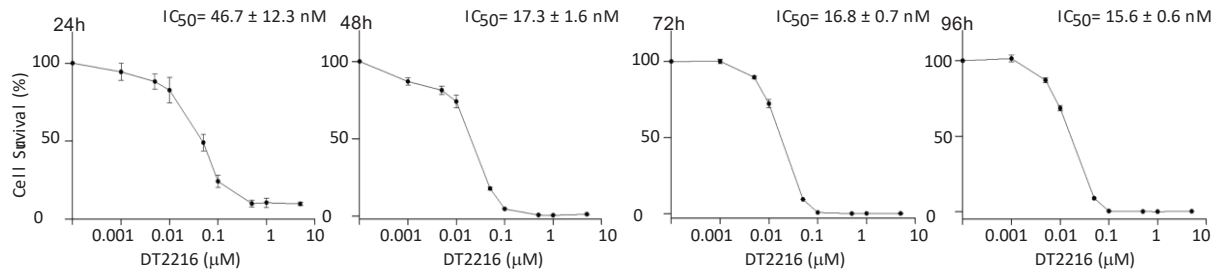
MOLT3



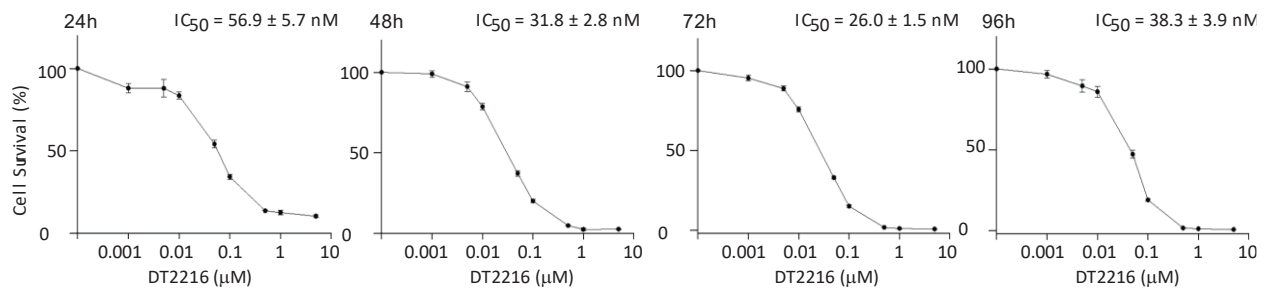
DND41



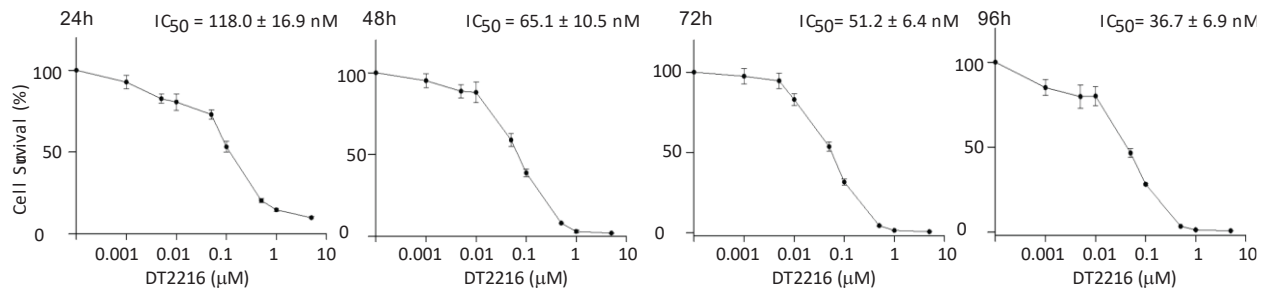
P12-Ichikawa



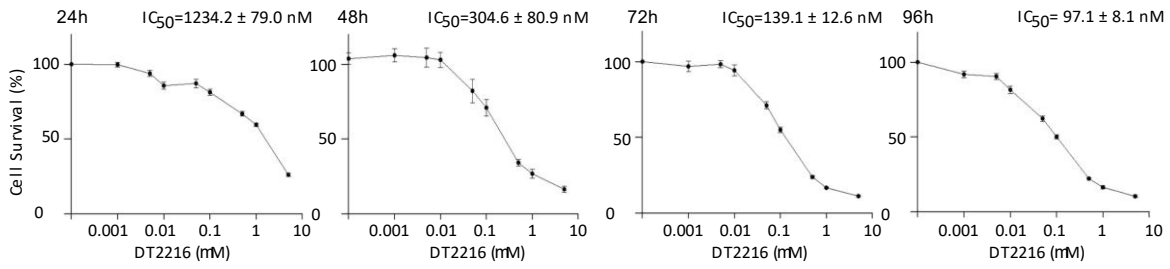
CCRF-CEM



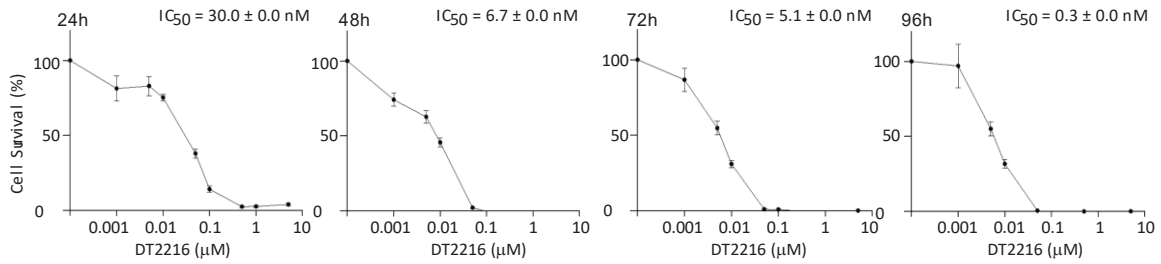
PF382



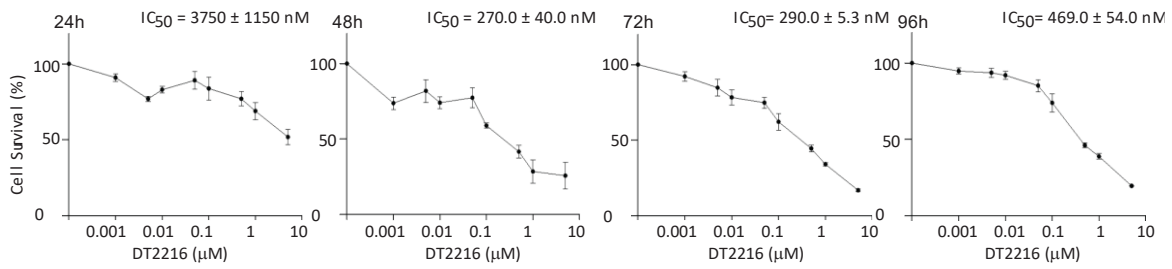
PEER



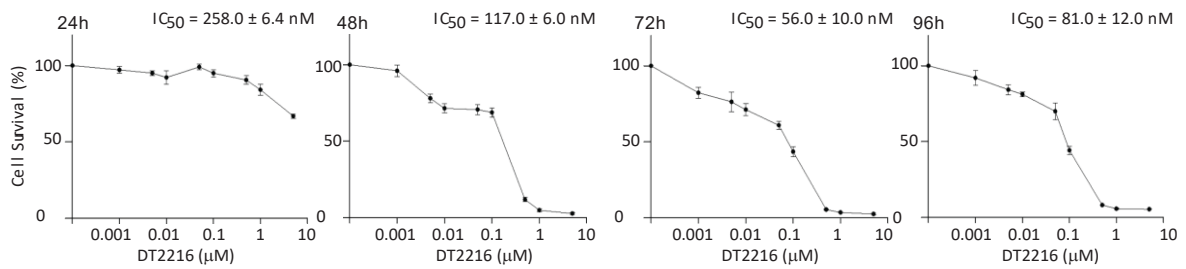
ALL-SIL



TALL-104



HBP-ALL



HSB-2

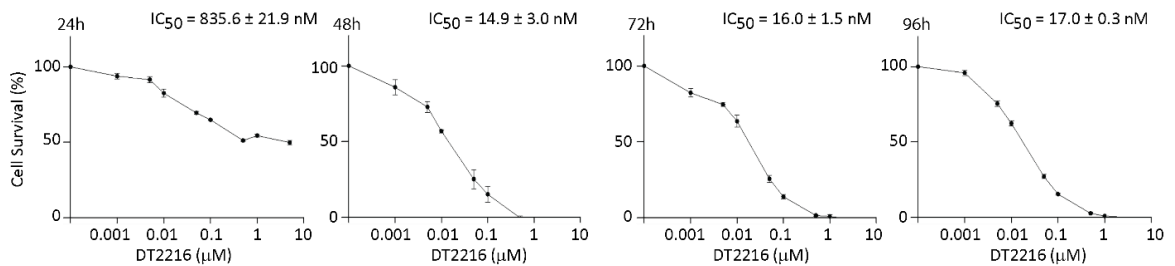


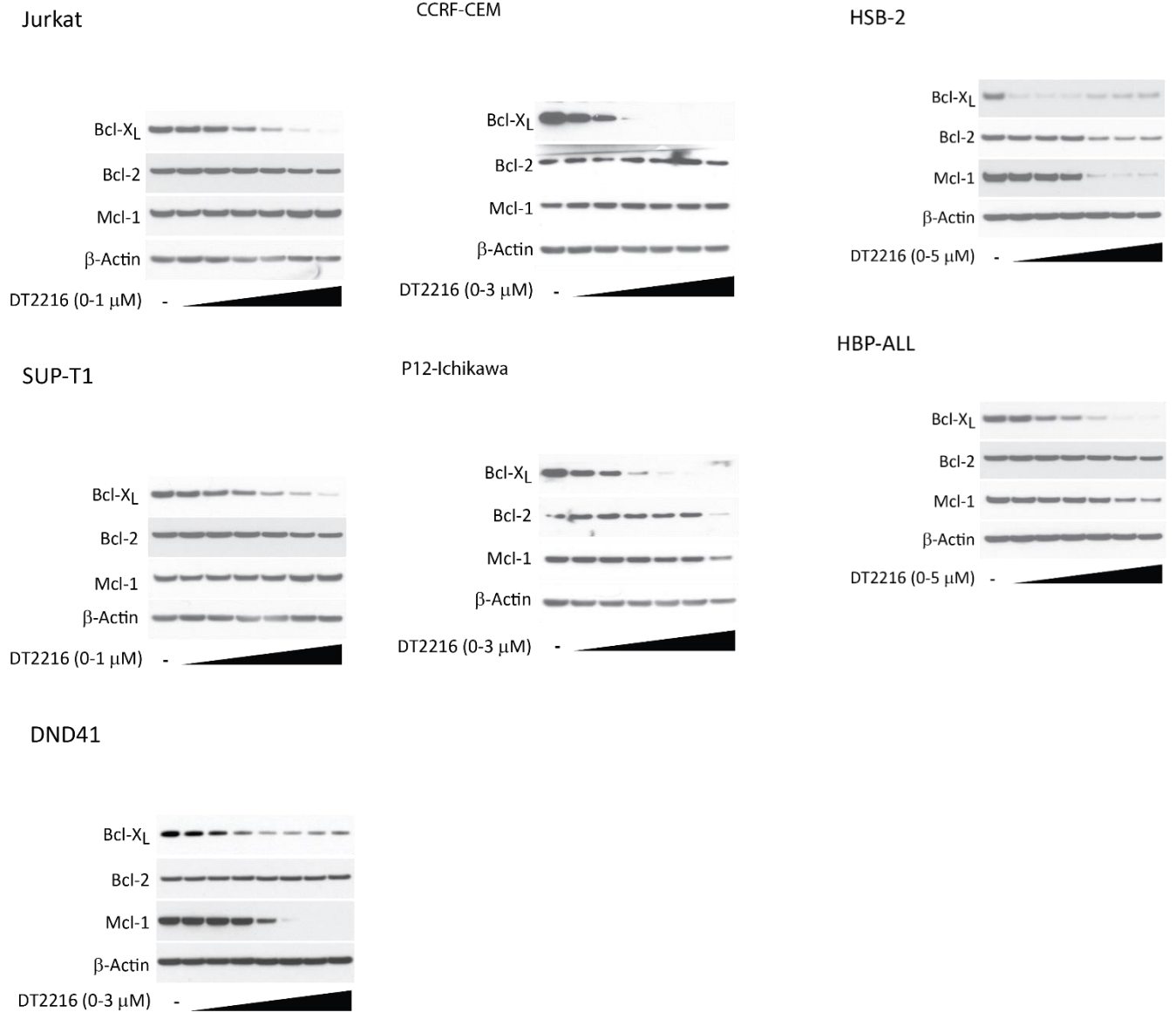
Figure S2. DT2216 survival curves for the 13 T-ALL lines. MTS assays in varying concentrations of DT2216 after 24, 48, 72, and 96 hours of exposure. All data points were performed in quadruplicate.

Figure S3

Cell Line	IC50 (nM)			
	24 h	48 h	72 h	96 h
Jurkat	1057.5 ± 34.7	98.0 ± 10.7	109.0 ± 7.5	93.5 ± 17.2
SUP-T1	8882.2 ± 1061.7	1487.9 ± 268.0	1067.1 ± 59.6	1042.9 ± 100.4
MOLT-3	81.60 ± 20.67	96.83 ± 27.42	48.89 ± 4.52	44.08 ± 1.84
P12-Ichikawa	46.74 ± 12.38	17.28 ± 1.66	16.82 ± 0.72	15.60 ± 0.58
DND-41	35.32 ± 11.87	10.19 ± 0.73	12.58 ± 0.96	10.57 ± 1.32
Loucy	3.00 ± 0.71	2.14 ± 0.25	1.98 ± 0.15	1.79 ± 0.17
CCRF-CEM	56.85 ± 5.74	31.82 ± 2.84	25.95 ± 1.45	38.26 ± 3.89
PF-382	118.00 ± 16.91	65.07 ± 10.47	51.18 ± 6.37	36.68 ± 6.85
PEER	1234.25 ± 79.00	304.63 ± 80.97	139.05 ± 12.61	97.05 ± 8.10
ALL-SIL	25.69 ± 3.72	6.67 ± 0.97	5.11 ± 0.47	0.35 ± 0.0
TALL-104	3749.75 ± 1152.7	274.8 ± 36.45	289.63 ± 53.19	468.72 ± 53.73
HBP-ALL	257.50 ± 64.17	117.18 ± 6.25	56.34 ± 9.60	80.52 ± 12.45
HSB-2	835.6 ± 21.9	14.9 ± 3.0	16.0 ± 1.5	17.0 ± 0.3

Figure S3- IC50 values over time of exposure to DT2216. MTS assays were performed on 13 T-ALL cell lines. Response to DT2216 appeared to plateau at 48 hrs so this was chosen as the main analytic timepoint.

Figure S4



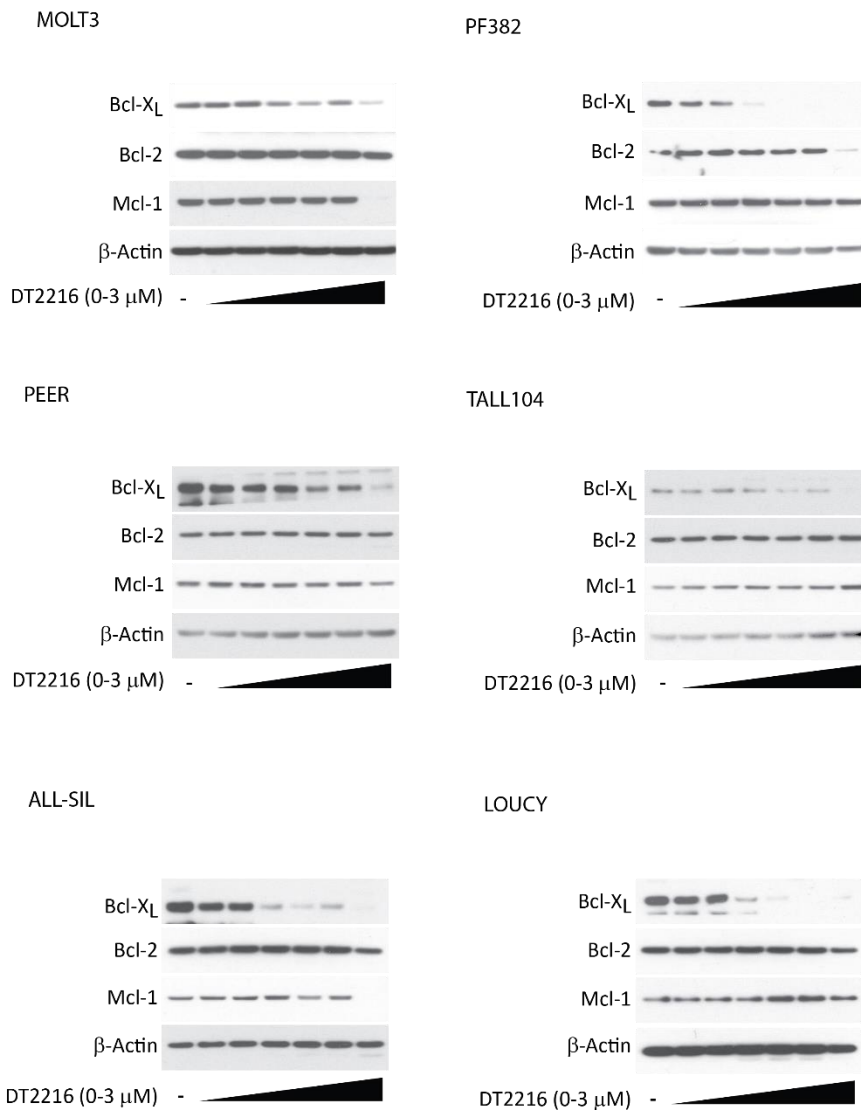


Figure S4. BCL-XL degradation from DT2216 after 24 hrs. Western analysis of BCL-XL, BCL-2 and MCL-1 protein levels after 24 hrs of exposure to varying concentrations of DT2216. Concentrations of DT2216 were 4, 12, 36, 110, 330, 1000, and 3000 nM. Even in the resistant SUP-T1 cell line, BCL-XL was degraded efficiently, albeit at significantly higher concentrations than other cell lines. BCL-2 and MCL-1 were not induced by DT2216. In some cell lines, MCL-1 was degraded due to activation of Caspases as reported [8].

Figure S5

SUP-T1

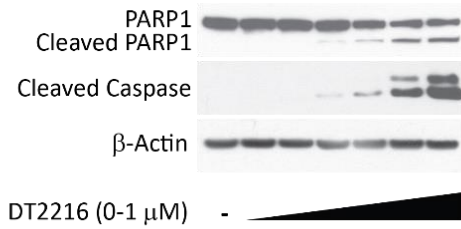


Figure S5- Western analysis of SUP-T1 cells of activation of apoptosis- Cleaved caspase 3 and cleaved PARP1 indicate that apoptosis is still activated in SUP-T1 cells even at concentrations of DT2216 where SUP-T1 cells survive (1 mM here and SUP-T1 IC₅₀ = 1.5 mM). Concentrations of DT2216 were 4, 12, 36, 110, 330, 1000, and 3000 nM.

Table S1- T-ALL cell lines with growth media and doubling time

Cell Line	Growth Medium	Doubling Time
CCRF-CEM	RPMI + 10% FBS	24-30 hr
Jurkat	RPMI + 10% FBS	25-35 hr
Loucy	RPMI + 10% FBS	50-60 hr
MOLT-3	RPMI + 10% FBS	40 hr
SUP-T1	RPMI + 10% FBS	30 hr
TALL-104	RPMI + 20% FBS + IL2	24 hr
ALL-SIL	RPMI + 20% FBS	48 hr
DND-41	RPMI + 10% FBS	35 hr
HBP-ALL	RPMI + 20% FBS	50 hr
HSB-2	Iscove + 10% FBS	40 hr
P12-ICHIKAWA	RPMI + 10% FBS	40-50 hr
PEER	RPMI + 20% FBS	50-60 hr
PF-382	RPMI + 10% FBS	70 hr