Supplemental material

Selective drug combination vulnerabilities in *STAT3* and *TP53* mutant malignant NK-cells

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Table of Contens

Supplemental material

Supplemental Tables

Table S1. Cell numbers in 3-day drug screens and 9-day extended assays.

Table S2. Antibodies, buffers and concentrations used in flow cytometry assay.

Table S3. ZIP synergy scores for NK-cell lines and PBMCs.

Supplemental Figures

Figure S1. BCL2 homology domain 3 (BH3) profiling.

Figure S2. Extended drug response assays: 9-day treatment and 3-day treatment, 6-day recovery assay.

Figure S3. Time-to-progression assay graphs for ruxolitinib combined with dexamethasone or S63845.

Figure S4. Comparison of top drug combination responses on NK-cell lines and PBMCs.

Figure S5. Effect of dexamethasone or S63845 combined with ruxolitinib on healthy PBMC populations.

Supplemental Materials and Methods

Supplemental References

Supplemental Tables

Table S1. Cell numbers in 3-day drug screens and 9-day extended assays.

Cell line and reference / PBMC	STAT3/ TP53 mutation [1]	Exonic function [1]	Original disease	Cell line established	EBV status	3- day assays, 384-well plate (cells/ ml)	3-day assays, 384-well plate (cells/ well)	9-day assays, 96-well plate (cells/ ml)	9-day assays, 96-well plate (cells/ well)	IL-2 concentration in growth media (ng/ml)
NKL [2,3]	Intronic splicing *TP53* mutation	NA	NK-LGL	1996	Negative	150000	3000	25000	2000	10
NK-92 [3,4]	*TP53*(E326X)	stopgain	ANKL	1992	Positive	150000	3000	50000	4000	10
IMC-1 [5]	*TP53*(L344P) *TP53*(V157F)	nonsynonymous SNV	ANKL	2004	Negative	200000	4000	18750 or 25000	1500 or 2000	20
KHYG-1 [3,6]	*TP53*(R248W)	nonsynonymous SNV	ANKL	1997	Negative	200000	4000	NA	NA	10
NK-YS [3,7]	*STAT3*(Y640F)	nonsynonymous SNV	NKTCL	1996	Positive	100000	2000	18750 or 25000	1500 or 2000	10
YT [3,8,9]	*STAT3*(Y640F)	nonsynonymous SNV	Acute lymphoblastic lymphoma with thymoma	1983	Positive	200000	4000	50000	4000	10
SNK-6 [10]	*STAT3*(D661Y)	nonsynonymous SNV	NKTCL	2001	Positive	200000	4000	50000	4000	10
Healthy PBMC	NA	NA	NA	NA	NA	500000	10000	NA	NA	10

Abbreviations used in table:

NK-LGL = NK-cell large granular lymphocytic leukemia

ANKL = Aggressive NK-cell leukemia

NKTCL = Natural-killer/T cell lymphoma

EBV = Epstein-Barr virus

NA = not applicable

Table S2. Antibodies, buffers and concentrations used in flow cytometry assay.

Antibody	Clone	Dilution	Vendor	Buffer	Mix
CD14 (FITC)	MφP9	1:100	BD Biosciences	staining buffer (10% FBS and 0.02% NaN3 in RPMI-1640 medium)	Antibody mix
CD56 (PE-Vio770)	ICRF44	1:100	BD Biosciences	staining buffer (10% FBS and 0.02% NaN3 in RPMI-1640 medium)	Antibody mix
CD3 (APC)	8G12	1:100	BD Biosciences	staining buffer (10% FBS and 0.02% NaN3 in RPMI-1640 medium)	Antibody mix
CD33 (BV421)	HIT2	1:500	BD Biosciences	staining buffer (10% FBS and 0.02% NaN3 in RPMI-1640 medium)	Antibody mix
CD19 (BV510)	104D2	1:100	BD Biosciences	staining buffer (10% FBS and 0.02% NaN3 in RPMI-1640 medium)	Antibody mix
CD45 (BV786)	HI30	1:100	BD Biosciences	staining buffer (10% FBS and 0.02% NaN3 in RPMI-1640 medium)	Antibody mix
7-Aminoactinomycin D (7-AAD)		1:50	BD Biosciences	Annexin V binding buffer	Dead and apoptotic cell discrimination mix
PE-Annexin V		1:50	BD Biosciences	Annexin V binding buffer	Dead and apoptotic cell discrimination mix

Table S3. ZIP synergy scores for NK-cell lines and PBMCs.

Target		Glucocorticoids		MCL-1	BCL-2/-xL	BCL-2	MDM-2		Farnesyltransferase	
Cell line/ Sample	Readout	Dexamethasone + ruxolitinib	Methylprednisolone + ruxolitinib	S63845 + ruxolitinib	Navitoclax + ruxolitinib	Venetoclax + ruxolitinib	Idasanutlin + ruxolitinib	Siremadlin + ruxolitinib	Tipifarnib + ruxolitinib	Lonafarnib + ruxolitinib
YT	Toxicity	2,388	-1,232	2,902	-4,583	3,123	-0,645	0,056	3,993	1,314
	Viability	16,62	7,411	11,742	1,085	0,665	7,754	5,521	8,053	2,649
NK-YS	Toxicity	14,715	9,558	3,628	0,155	11,64	9,172	4,931	8,282	7,122
	Viability	9,956	4,481	2,492	2,165	5,114	6,388	3,116	2,306	10,168
SNK-6	Toxicity	2,057	-2,492	5,446	-8,474	3,235	-0,333	3,404	3,925	1,965
	Viability	7,128	1,211	4,135	0,391	2,188	8,009	4,944	6,756	1,432
NK-92	Toxicity	-1,13	-1,263	1,232	0,079	6,854	0,984	2,438	2,188	5,361
	Viability	11,888	2,879	3,982	-0,701	1,304	3,221	3,839	6,541	0,531
IMC-1	Toxicity	2,726	2,988	4,214	0,274	7,671	2,289	2,563	2,67	-4,652
	Viability	9,665	4,775	4,926	0,029	-0,51	-0,797	0,548	9,74	-1,429
KHYG-1	Toxicity	8,382	6,028	5,706	-3,632	4,848	0,19	0,391	-7,531	1,937
	Viability	6,709	3,027	3,124	-0,368	4,017	6,189	1,133	8,661	8,591
NKL	Toxicity	0,27	1,865	1,575	-4,212	3,934	-0,79	1,459	-1,483	3,966
	Viability	6,33	2,58	9,543	2,706	7,395	-0,992	1,547	1,047	-3,038
PBMC_1	Toxicity	-0,918	ND	-1,055	ND	ND	-0,165	ND	1,043	ND
	Viability	0,704	ND	0,777	ND	ND	4,876	ND	0,763	ND
PBMC_2	Toxicity	1,234	ND	-0,078	ND	ND	1,624	ND	2,368	ND
	Viability	2,707	ND	1,266	ND	ND	1,428	ND	-3,491	ND

>5
3
1
0
-1
-3
<5

ND = not determined

Supplemental Figures

Figure S1. BCL2 homology domain 3 (BH3) profiling.

Short-term drug treatment (16 h) coupled with the BH3 profiling assay could predict the cytotoxic effect of ruxolitinib. 1000 nM ruxolitinib induced BIM-, BID- and HRK- dependent apoptosis in NK-YS cells more efficiently than in NKL cells.

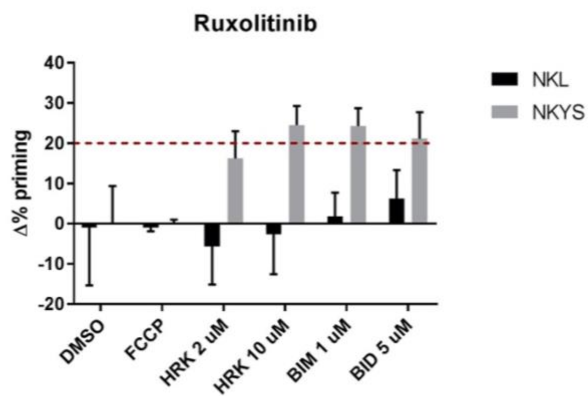


Figure S2. Extended drug response assays: 9-day treatment and 3-day treatment, 6-day recovery assay.

Box indicates standard deviation, line median, plus (+) sign mean and bar minimum and maximum of *STAT3* and *TP53* mutant cell lines. Cell viabilities are normalized to positive and negative controls.

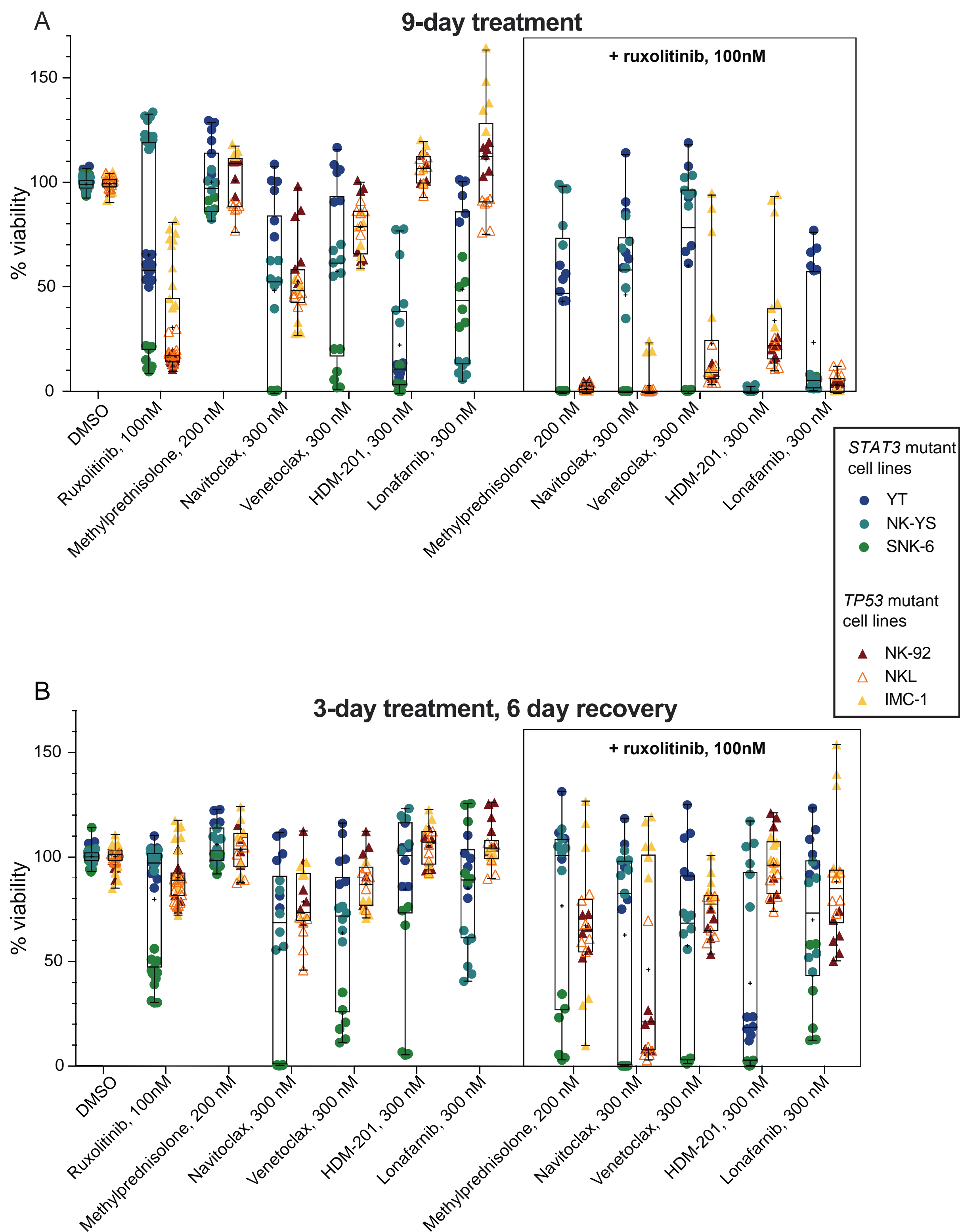


Figure S3. Time-to-progression assay graphs for ruxolitinib combined with dexamethasone or S63845.

Treatments were maintained for 30 days (gray area) with single drugs and their combinations, and followed for another 30 to 32 days after drug removal (white area). Dexamethasone treated **a-c** *STAT3* mutant and **d-f** *TP53* mutant cells. MCL-1 inhibitor S63845 treated **g-l** *STAT3* mutant cells and **j-i** *TP53* mutant cells. Cells were counted and drug and media were replenished every third to fourth day and set to 3 to 3.5×10^5 cells/ml cell density. Cultures were stopped when reaching 20 million (2 duplications from start).

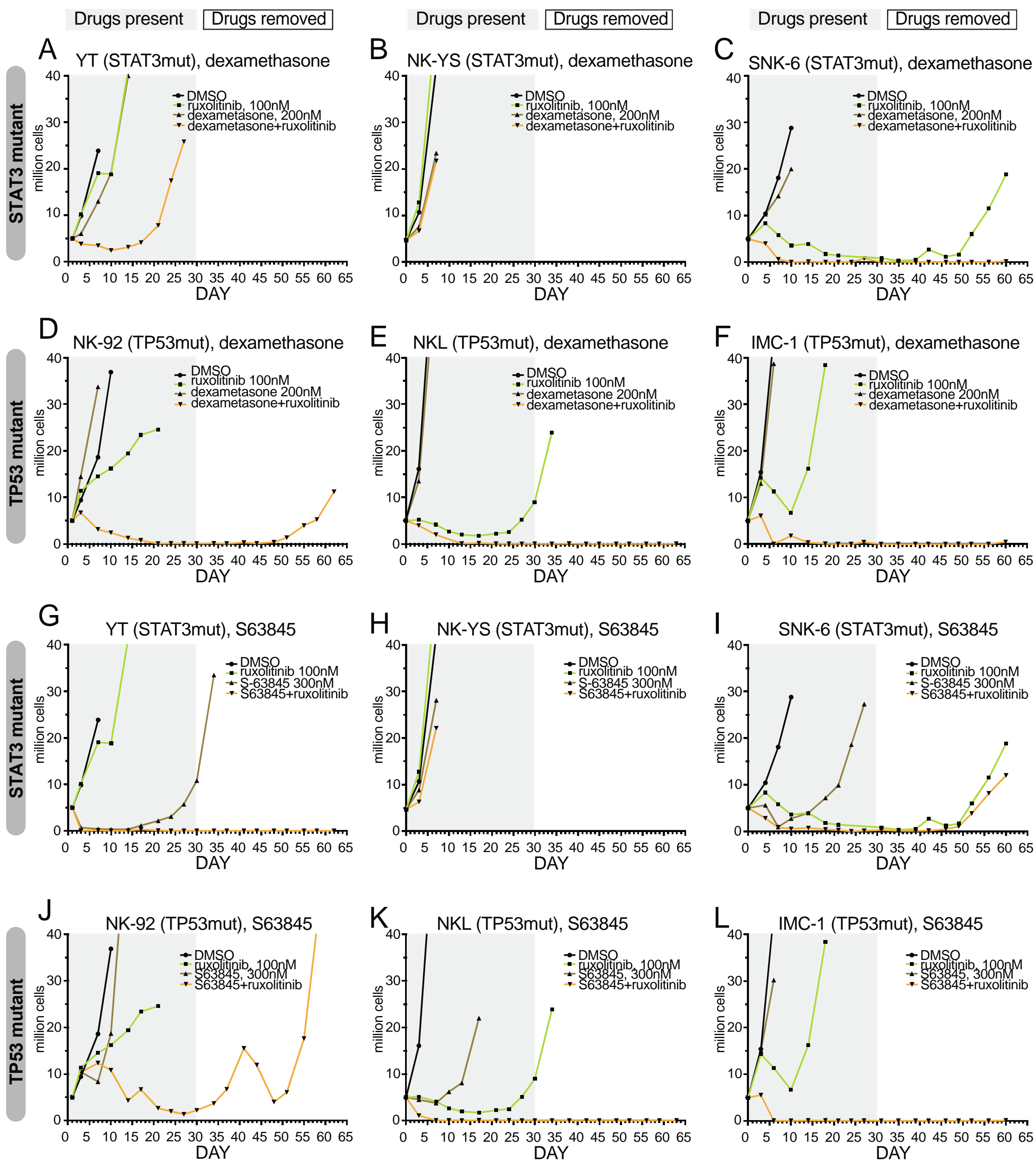


Figure S4. Comparison of top drug combination responses on NK-cell lines and PBMCs. Ruxo. = ruxolitinib; dexa/dexameth. = dexamethasone. Supporting data for Fig. 6.

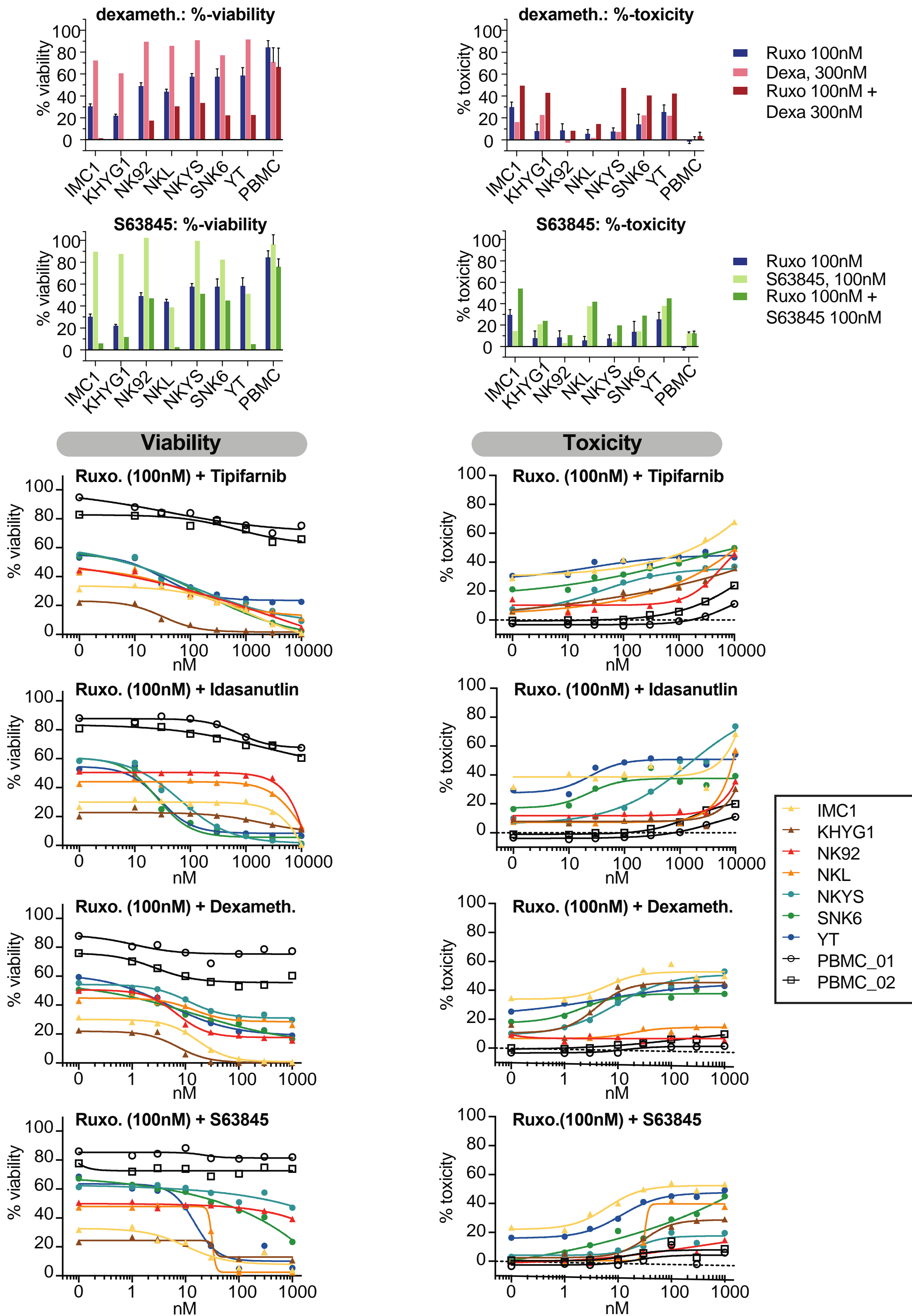
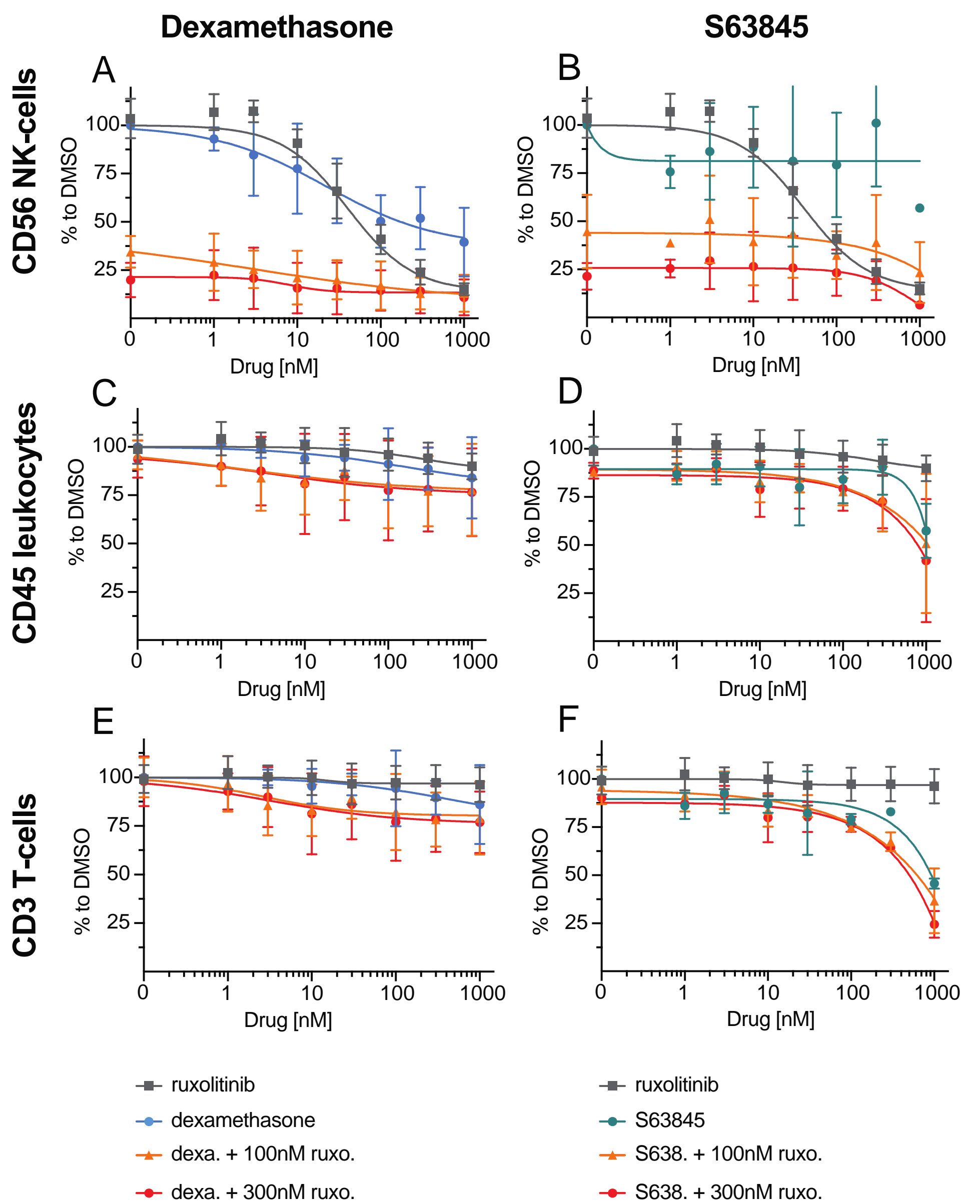


Figure S5. Effect of dexamethasone or S63845 combined with ruxolitinib on healthy PBMC populations.

a-b CD56+ NK-cells, **c-d** CD45+ leukocytes and **e-f** CD3+ T-cells.



Supplemental Materials and Methods

NK-YS and NKL cells were treated with 1000 nM ruxolitinib for 16 h prior to BH3 profiling. The BH3 profiling was performed according to the plate reader-based protocol by Ryan and Letai [11], with minor modifications: 25 nl of 1000x BH3 peptide stocks or alamethicin control in DMSO were dispensed using an acoustic liquid dispenser (Echo 550, Labcyte Inc.) to 384-well plates (Corning) and 25 µl of cell suspension in 1x digitonin/JC1 solution was added to treatment and control wells. Kinetic changes in the total well fluorescence (590 nm) was measured using Cytation5 imaging multi-mode reader (BioTek) at 30°C for 3 h with 15 min intervals. Mitochondrial depolarization was quantified using the kinetic area under the curve (AUC) values according to formula: % depolarization = 1-[(sample-alamethicin)⁄(DMSO-alamethicin)]. Drug-induced apoptotic priming, or delta (% depolarization), was quantified as a difference between % depolarization values for BH3 only treated cells and ruxolitinib + BH3 treated cells.

Supplemental References

1. Dufva O, Kankainen M, Kelkka T, et al. Aggressive natural killer-cell leukemia mutational landscape and drug profiling highlight JAK-STAT signaling as therapeutic target. *Nat Commun.* 2018;9(1):1567.
2. Robertson MJ, Cochran KJ, Cameron C, Le JM, Tantravahi R, Ritz J. Characterization of a cell line, NKL, derived from an aggressive human natural killer cell leukemia. *Experimental hematology.* 1996;24(3):406-415.
3. Drexler HG, Matsuo Y. Malignant hematopoietic cell lines: in vitro models for the study of natural killer cell leukemia-lymphoma. *Leukemia.* 2000;14(5):777-782.
4. Gong JH, Maki G, Klingemann HG. Characterization of a human cell line (NK-92) with phenotypical and functional characteristics of activated natural killer cells. *Leukemia.* 1994;8(4):652-658.
5. Chen IM, Whalen M, Bankhurst A, et al. A new human natural killer leukemia cell line, IMC-1. A complex chromosomal rearrangement defined by spectral karyotyping: functional and cytogenetic characterization. *Leuk Res.* 2004;28(3):275-284.
6. Yagita M, Huang CL, Umehara H, et al. A novel natural killer cell line (KHYG-1) from a patient with aggressive natural killer cell leukemia carrying a p53 point mutation. *Leukemia.* 2000;14(5):922-930.
7. Tsuchiyama J, Yoshino T, Mori M, et al. Characterization of a novel human natural killer-cell line (NK-YS) established from natural killer cell lymphoma/leukemia associated with Epstein-Barr virus infection. *Blood.* 1998;92(4):1374-1383.
8. Yodoi J, Teshigawara K, Nikaido T, et al. TCGF (IL 2)-receptor inducing factor(s). I. Regulation of IL 2 receptor on a natural killer-like cell line (YT cells). *J Immunol.* 1985;134(3):1623-1630.
9. Yoneda N, Tatsumi E, Kawano S, et al. Detection of Epstein-Barr virus genome in natural-killer-like cell line, YT. *Leukemia.* 1992;6(2):136-141.
10. Nagata H, Konno A, Kimura N, et al. Characterization of novel natural killer (NK)-cell and gammadelta T-cell lines established from primary lesions of nasal T/NK-cell lymphomas associated with the Epstein-Barr virus. *Blood.* 2001;97(3):708-713.
11. Ryan J, Letai A. BH3 profiling in whole cells by fluorimeter or FACS. *Methods.* 2013;61(2):156-164.