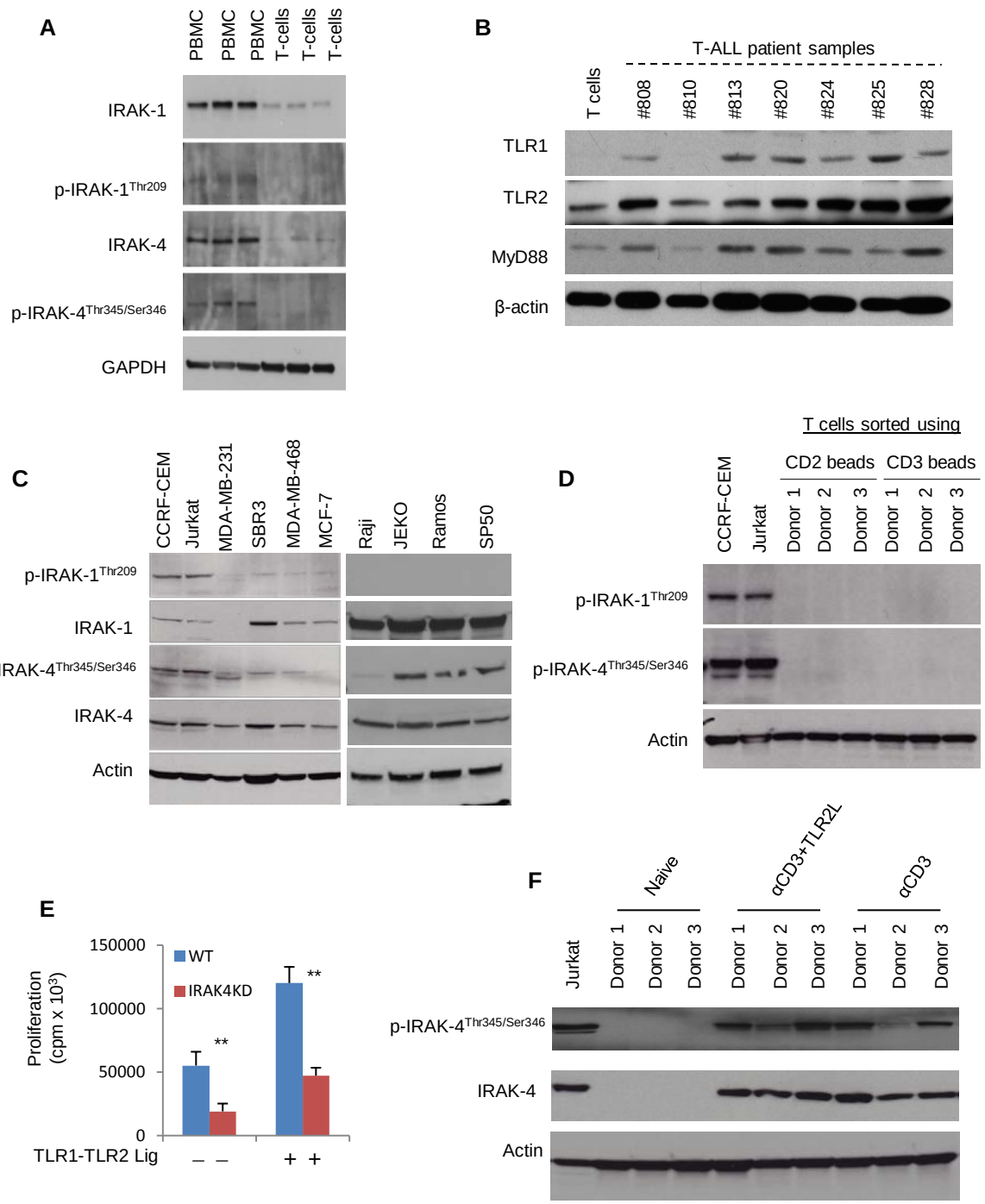
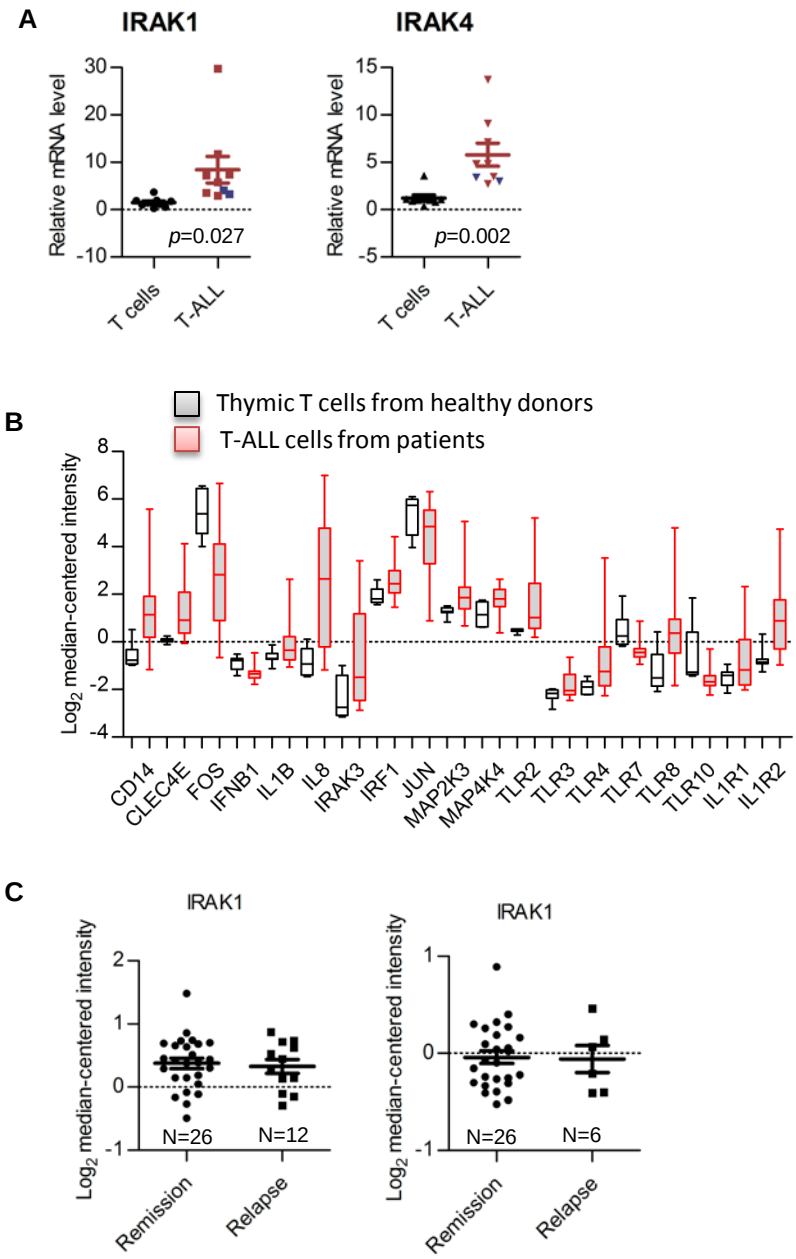
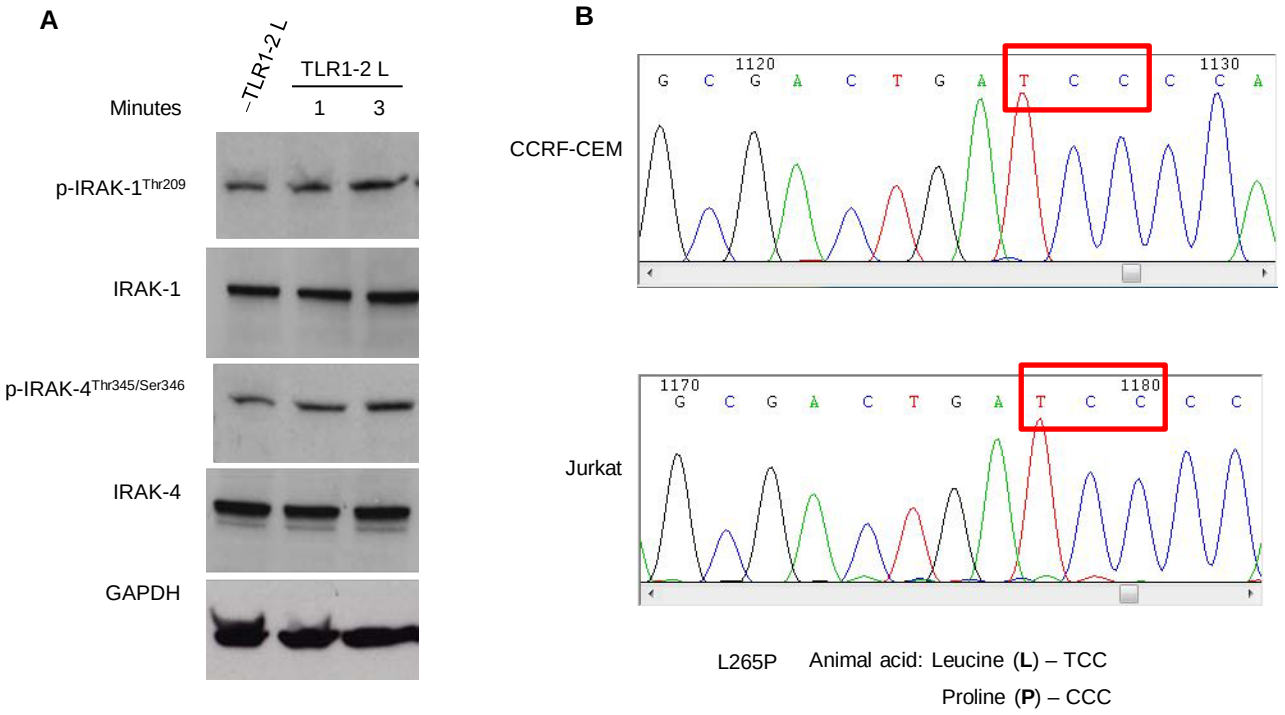


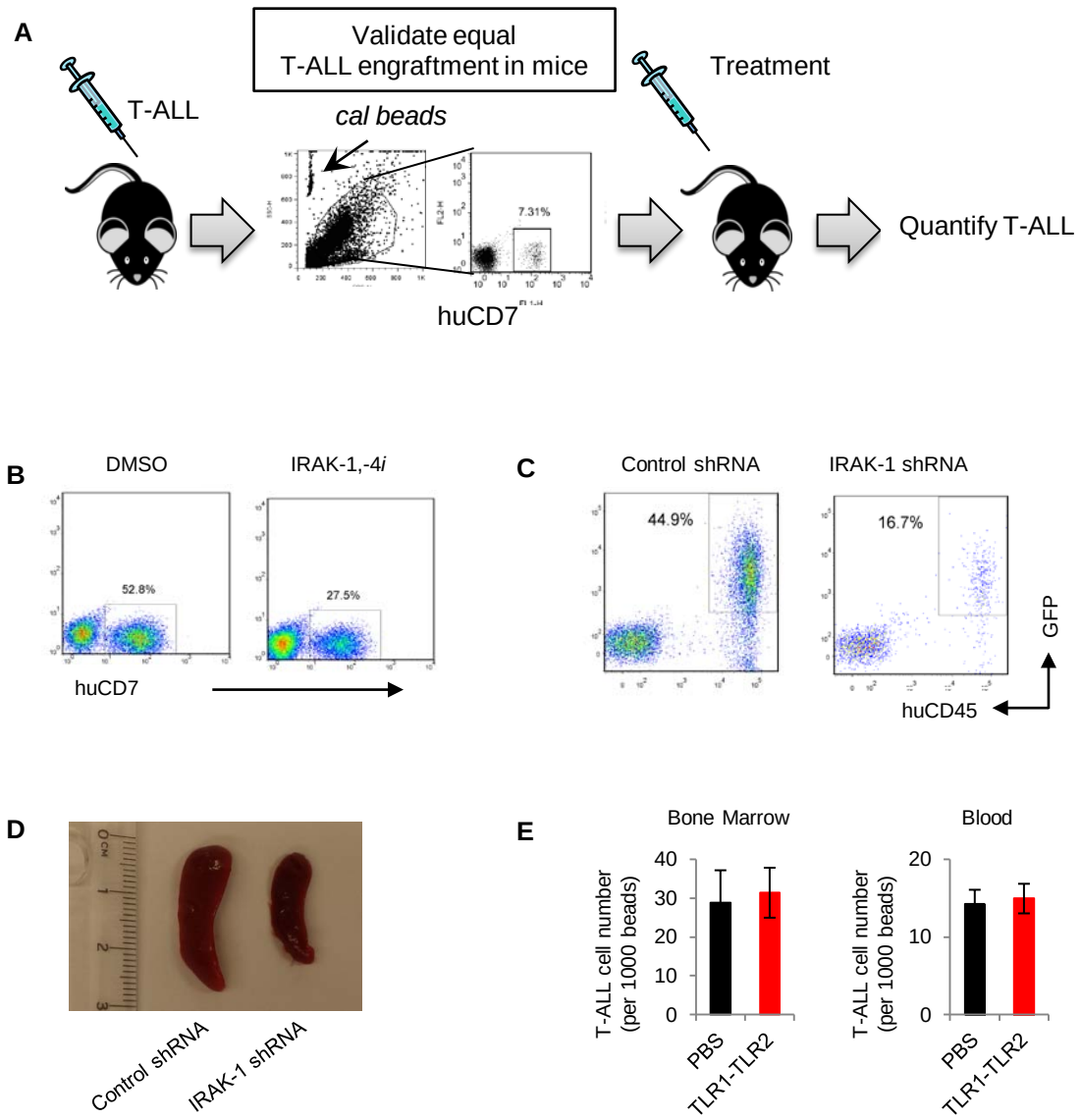
Supplemental Figure 1. (**A**) Western blot analysis of human PBMC and purified peripheral T-cells and (**B**) T-ALL patient samples examining the expression levels of the indicated proteins. (**C**) Western blot analysis of indicated protein levels in breast tumor cell lines (MB-231, SBR3, MB-468, MCF-7) Raji and Ramos (Burkitt's Lymphoma) JEKO (Mantle cell lymphoma), SP-50 (B cell lymphoma). (**D**) T cells were sorted using CD2 or CD3 beads and the levels of the indicated proteins were determined by western blot. CCRF-CEM and Jurkat cells served as controls both panels C and D. (**E**) Total T cells from IRAK-4 kinase dead (IRAK4KD) or wild type mice were purified by negative and activated using plate-bound CD3 antibody (0.5ug/ml) with or without TLR1-TLR2 ligand (1ug/ml). Proliferation was determined by ^{3}[H] thymidine incorporation measuring the average of triplicate readings. Analysis of data shown are representative of two independent experiments (mean±SD). **p<0.01; T-test. (**F**) Western blot of total and phosphorylated IRAK-4 in naïve T cells and T cells activated with anti-CD3 with or without TLR1-TLR2 ligand (1ug/ml).



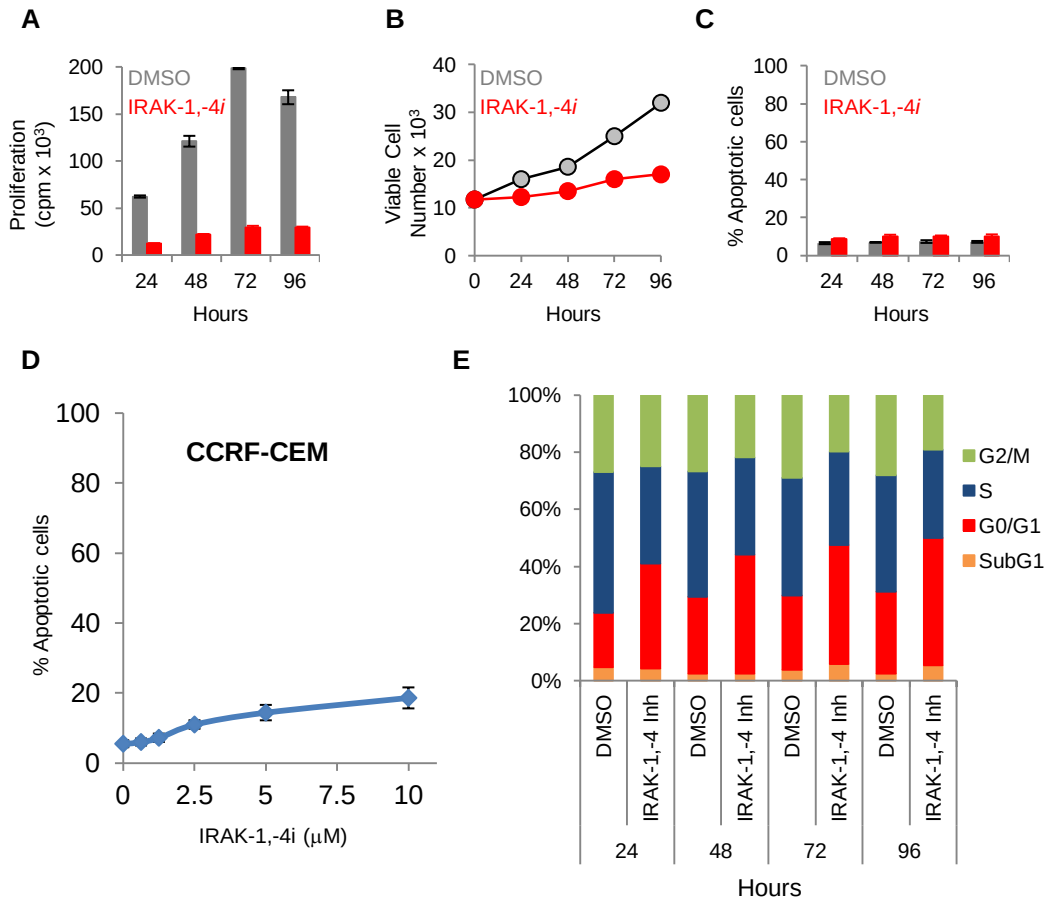
Supplemental Figure 2. (**A**) RT-PCR analysis of IRAK1 and IRAK4 levels in sorted CD3+ T cells from healthy donor blood (n=7) and T-ALL samples from patient peripheral blood (red; n=6) or patient bone marrow (blue; n=2). Each symbol represents one donor. (**B**) Expression levels of IRAK signaling-related genes in T-ALL cells and thymic T cell controls. Analyses were conducted using the online available microarray data set (GSE46170). T-test, $P<0.05$. Line, bar, and whiskers represent median, quartiles, and min-max values, respectively. (**C**) *IRAK1* transcript levels were not associated with chemoresistance. Microarray analysis of IRAK1 mRNA levels between patients with remission and relapse from two independent data sets (left, Yeoh, et al. 2002 (http://www.stjuderesearch.org/site/data/ALL1)); right, Chiaretti, et al. 2004 (http://master.bioconductor.org/packages/2.13/data/experiment/html/ALL.html).



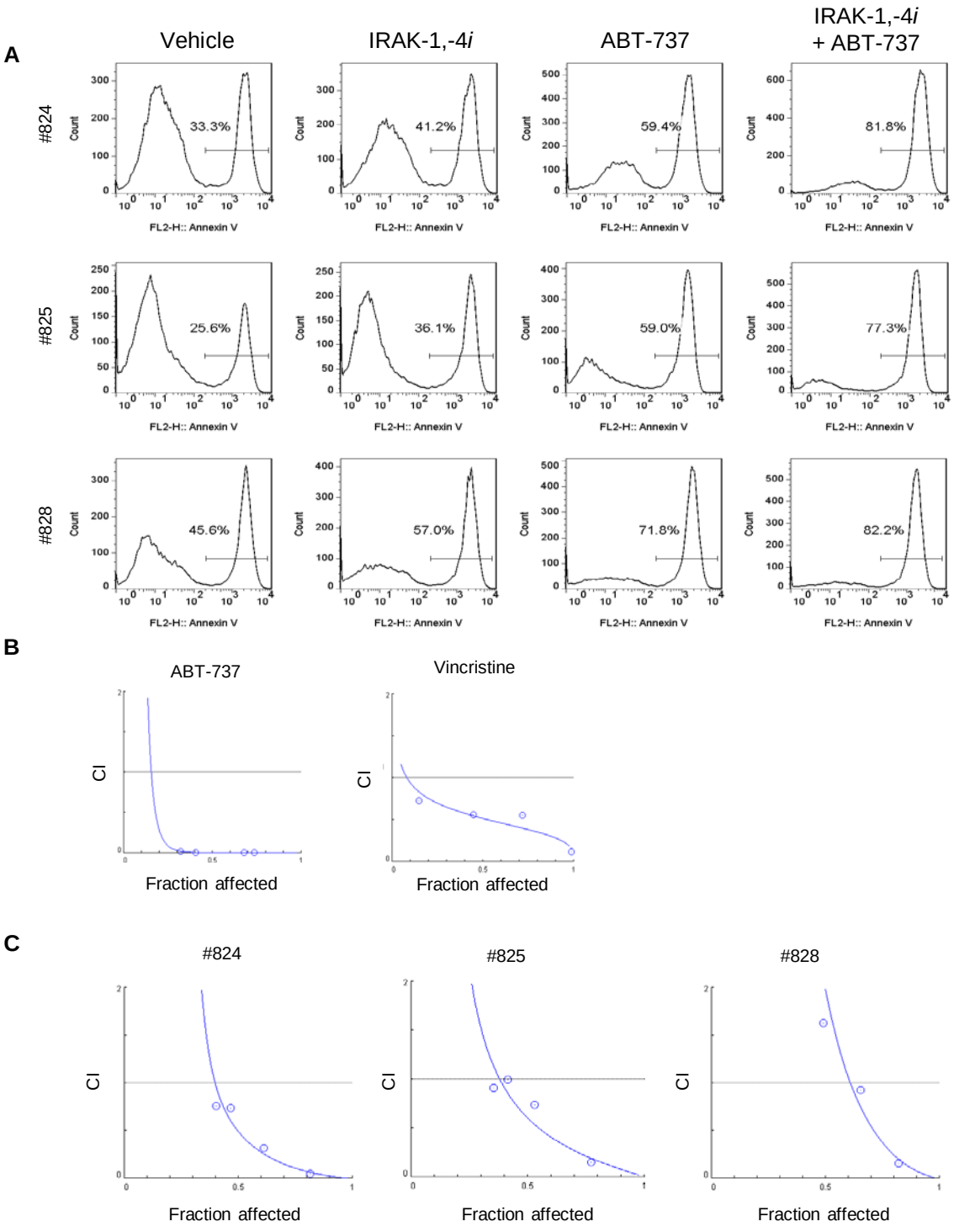
Supplemental Figure 3. (**A**) Western blot analysis of the indicated proteins from untreated CCRF-CEM cells or cells treated with TLR1-2 ligand (1.5 μg/ml) for the indicated time points. (**B**) Gene sequencing of MyD88 in CCRF-CEM and Jurkat cells. Three independent sequences were conducted in reverse and forward orientation. Partial electropherograms focusing on the region of interest in the MyD88 gene are shown.



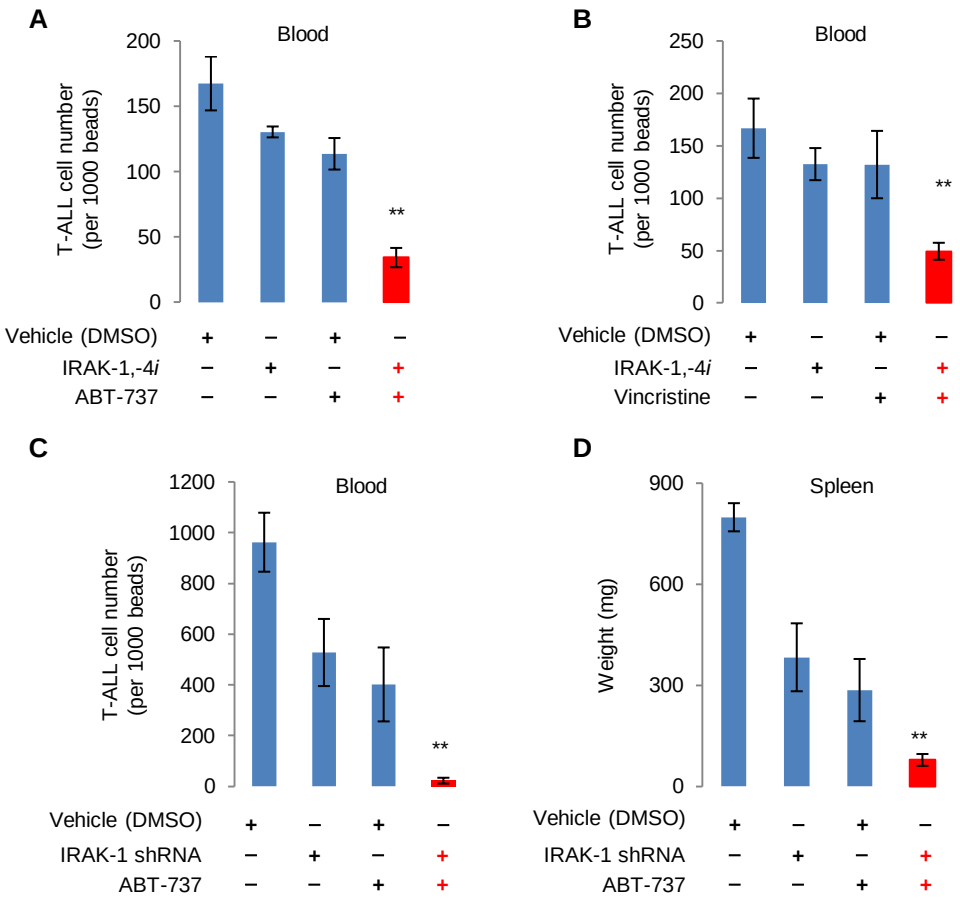
Supplemental Figure 4. (**A**) A schematic representation of the protocol used to normalize the T-ALL cell count between mice. T-ALL cell count between mice were normalized by adding 10μl of calibration beads to 50μl of blood (or bone marrow) and setting the instrument gates to count a constant number of beads. (**B**) CCRF-CEM cells (2 x 10^6 cells) were injected intravenously into NSG mice (n=5), followed by intraperitoneal injection with IRAK-1,-4 inhibitor (10 mg/kg) or control vehicle (DMSO) on days 3, 6 and 9. Numbers of T-ALL cells ($CD7^+$) in blood were determined on day 21 by flow cytometry. Representative dot plots were shown. (**C**) 2 x 10^6 Jurkat IRAK-1 knockdown cells or control cells were injected i.v. into NSG mice. On day 21, numbers of T-ALL cells (GFP^+CD45^+) in blood were measured by flow cytometry. Representative dot plots were shown. (**D**) Representative spleens from NSG mice with i.v. injected control or IRAK-1 knockdown Jurkat cells. (**E**) Patient T-ALL cells (#824; 5 x 10^6 cells) were injected intravenously into NSG mice, 7 days later, the mice were injected with TLR1-TLR2 ligand (Pam_3CysK_4, 2mg/kg) or control (PBS). On day 14, the number of T-ALL cells in bone marrow or blood were measured by staining with anti-human CD7 and CD5 antibodies and analyzed by flow cytometry.



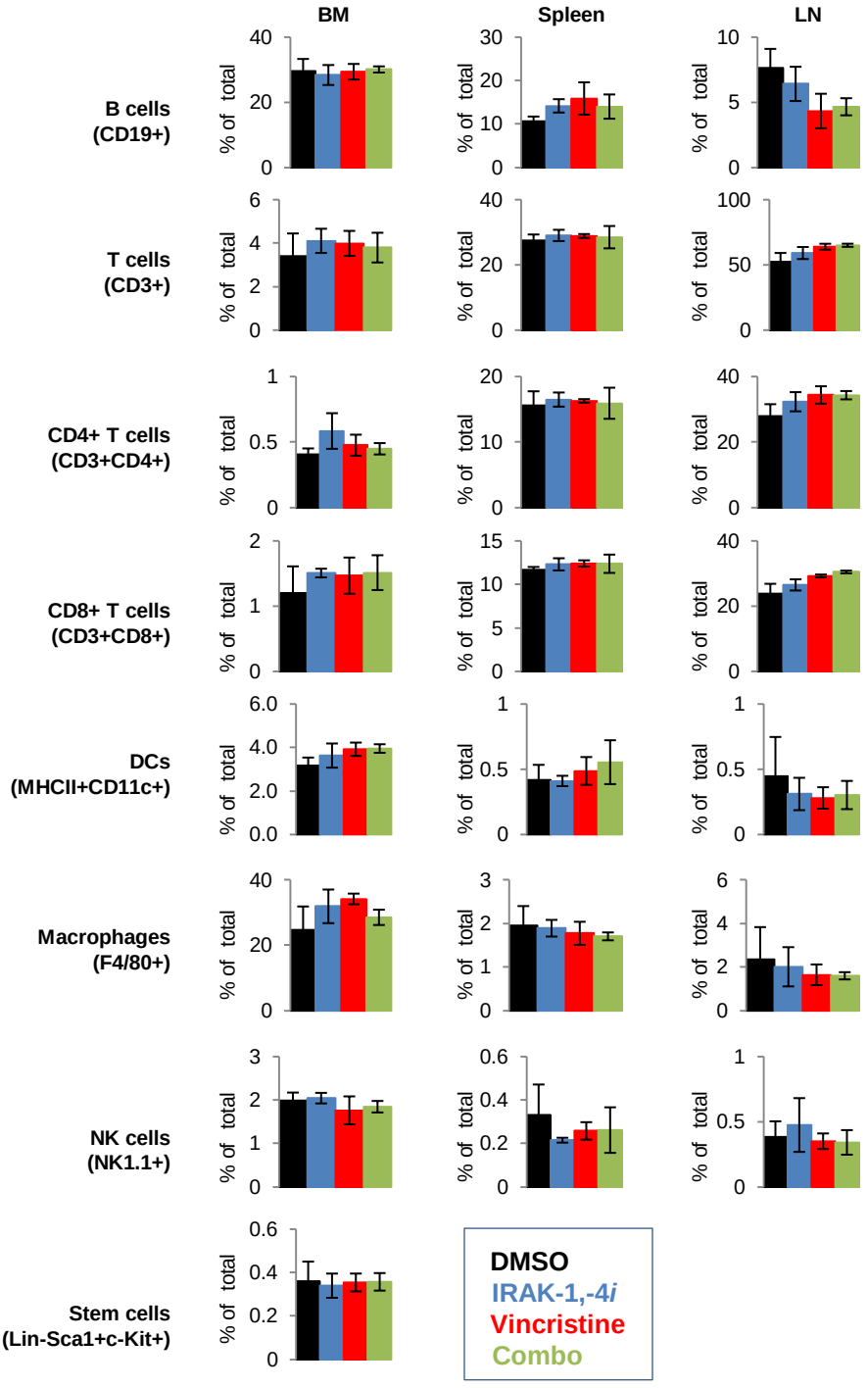
Supplemental Figure 5. CCRF-CEM cells were treated with IRAK-1,-4 inhibitor (2.5 μM) or DMSO for 24, 48, 72, and 96 hours and then, proliferation (**A**), cell number (**B**), apoptosis (**C**), and (**E**) cell cycle were determined by ^{3}H-thymidine incorporation, counting viable cells using trypan blue exclusion, by PI/Annexin-V staining, respectively. (**D**) CCRF-CEM cells were treated with the indicated concentrations of IRAK-1,-4 inhibitor for 48 hours followed by quantification of apoptosis assay by staining cells with Annexin-V and7-AAD.



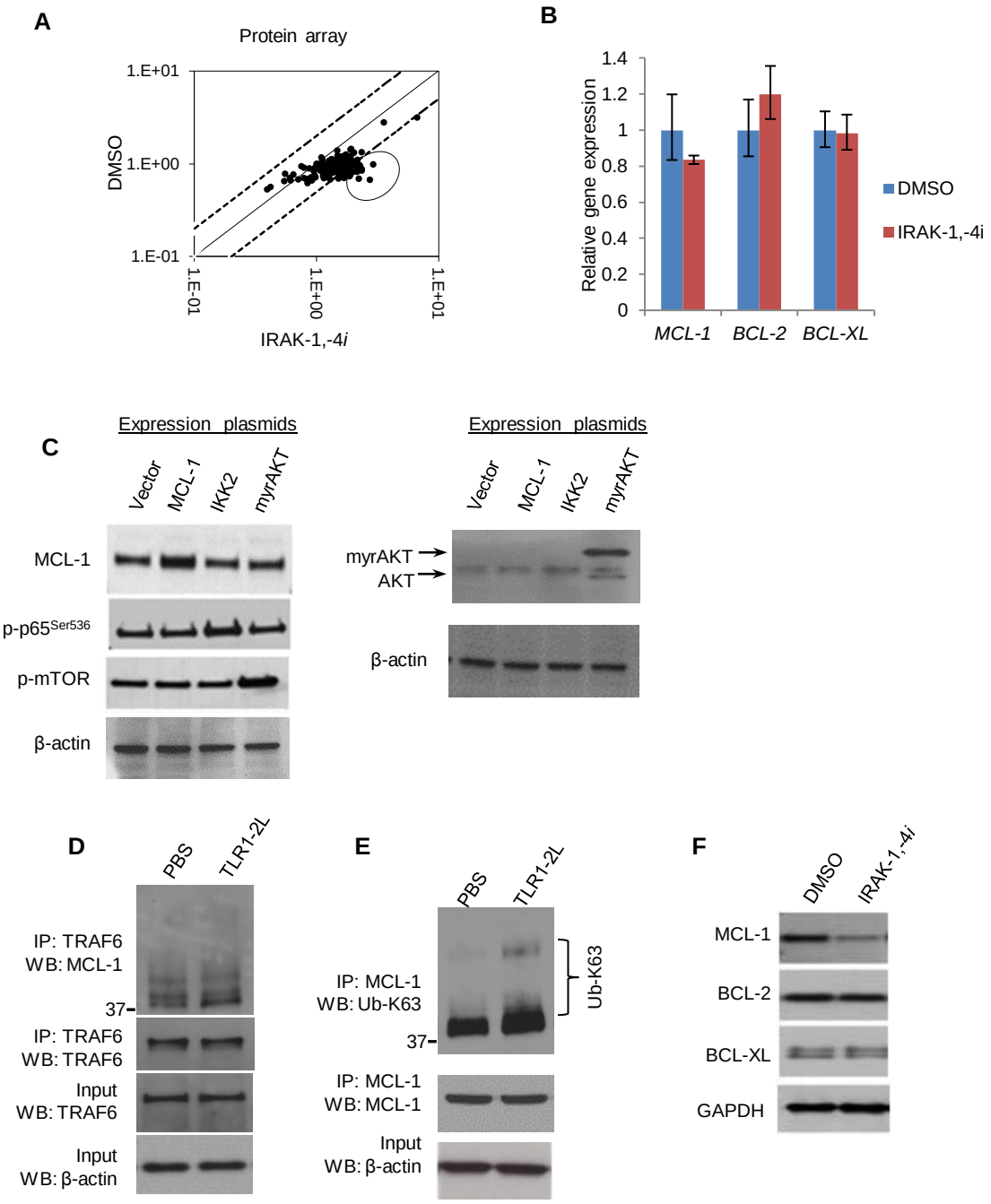
Supplemental Figure 6. **(A)** Representative histograms of patient T-ALL cells treated with IRAK-1,-4 inhibitor and ABT-737 alone or together and analyzed by flow cytometry. **(B and C)** CI, combination index, for combined ABT-737 or vinscristine and IRAK-1,-4 inhibitor is shown in Figure B for Jurkat cells and for patient T-ALL in panel C and was determined using Compusyn software.



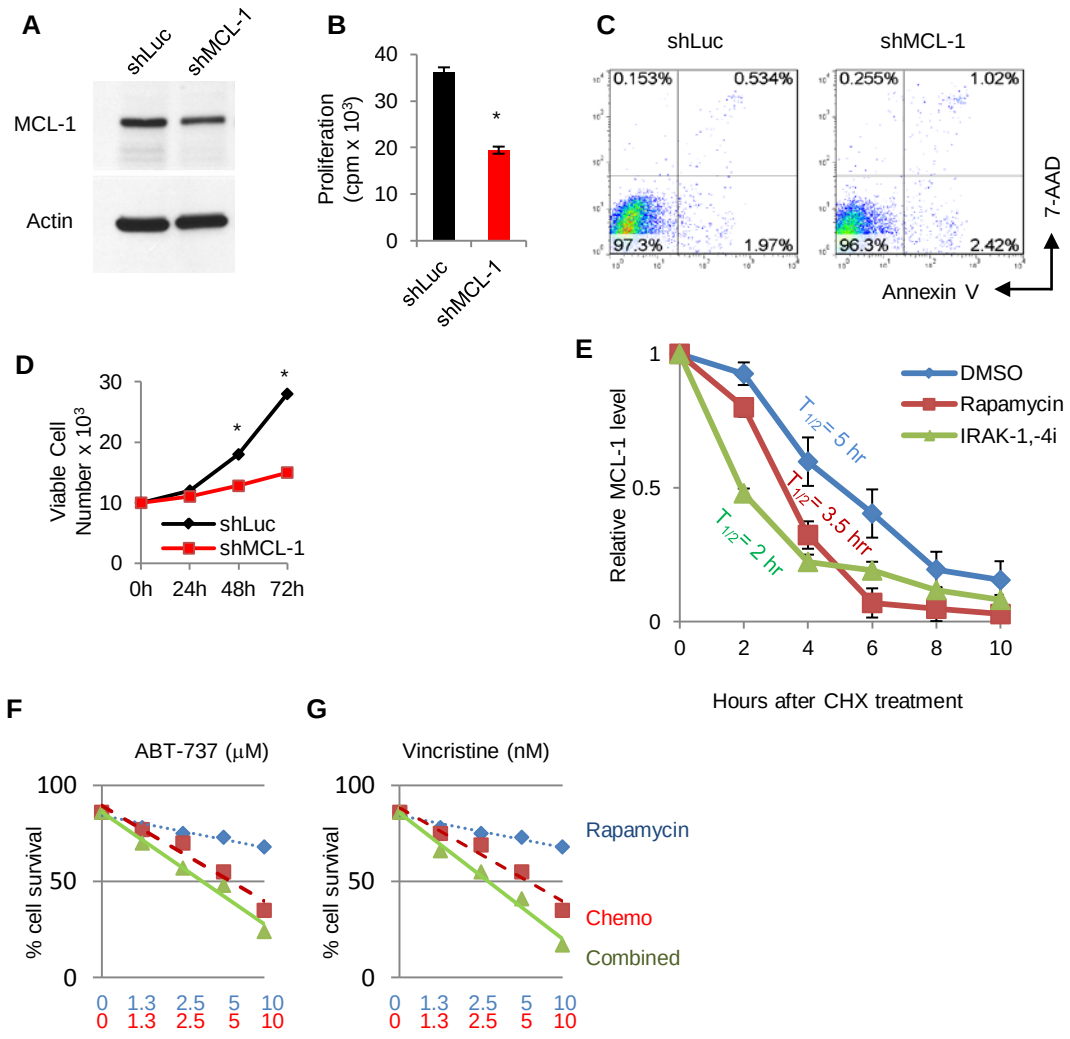
Supplemental Figure 7. **(A, B)** NSG mice (n=5/group) were injected i.v. with $3x10^6$ CCRF-CEM cells. On day 3, 6, 9, IRAK-1,-4 inhibitor (5mg/kg), ABT-737 (40mg/kg), or vincristine (0.5mg/kg)) were injected i.p. into the mice. T-ALL cell numbers were measured by flow cytometry. ** *P<0.01, T-test.* **(C, D)** NSG mice (n=5/group) were injected i.v. with 3×10^6 Jurkat engineered to downregulate IRAK-1 using cells using shRNA lentiviral vectors (or control vectors). On day 3, 6, 9, ABT-737 was injected i.p. into the mice. T-ALL cell numbers were measured by flow cytometry on day 21. T-ALL cell numbers in blood are shown. **(D)** Average weight of spleens collected from the different treatment groups is shown. ** *P<0.01, T-test.*



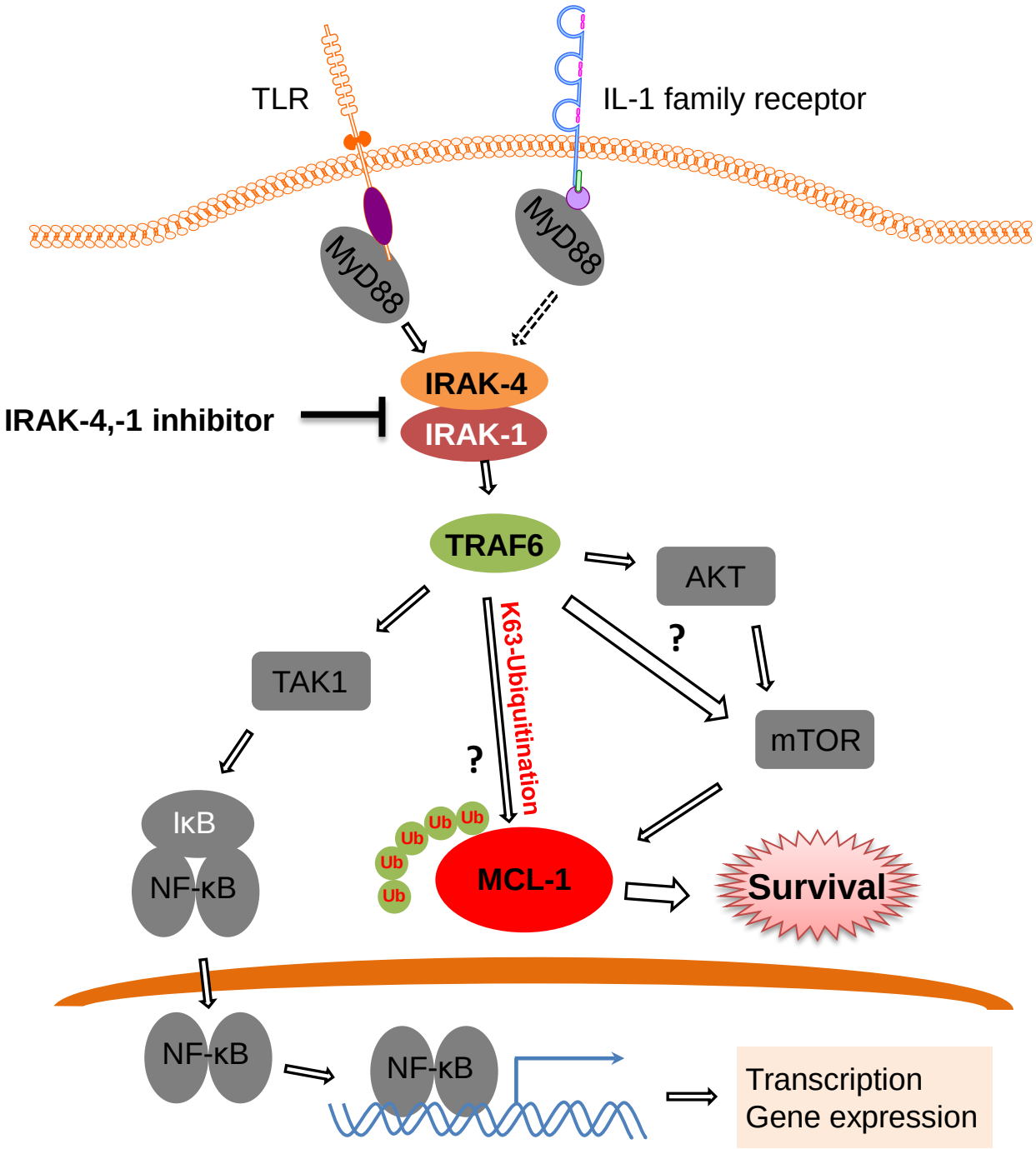
Supplemental Figure 8. C57BL/6 mice were injected i.p. with IRAK-1,-4 inhibitor, vincristine or both using the same concentrations as used in NSG mice (IRAK-1,-4 inhibitor (5mg/kg); Vincristine (0.5mg/kg)) on day 3, 6, 9. On day 21, the indicated immune cell populations in bone marrow (BM), spleen, and lymph node (LN) were analyzed by flow cytometry.



Supplemental Figure 9. (**A**) CCRF-CEM cells were treated with IRAK-1,-4 inhibitor (5 μM) or DMSO for 48 hours. Cell lysates were analyzed using Fullmoon Apoptosis antibody array. (**B**) RT-PCR analysis of *MCL-1*, *BCL-2*, and *BCL-XL* expression levels from CCRF-CEM cells treated with IRAK-1,-4 inhibitor (5μM) or DMSO. (**C**) CCRF-CEM cells transfected with control plasmid (p-Vector) and plasmids for MCL-1 (p-MCL-1), mutated IKK2 (p-IKK2), and AKT (p-myrAKT) and the indicated protein levels were examined by western blot analyses. (**D**) and (**E**) Co-immunoprecipitation assay and western blot analysis. CCRF-CEM cells were treated with TLR1-TLR2 ligand (2.5 μg/ml) or PBS, and lysates were subjected to a IP using anti-TRAF6 antibody (**D**) or anti-MCL-1 antibody (**E**) and immunoblotted (WB) with anti-MCL-1 and anti-TRAF6 (**D**) or anti-Ub-K63 and anti-MCL-1 (**E**) antibodies. Direct WB analysis was used to monitor protein expression levels.



Supplemental Figure 10. CCRF-CEM cells were transiently transfected with plasmids containing the MCL-1 shRNA (shMCL-1-GFP) or control shRNA (shLuc-GFP). GFP positive cells were sorted by flow cytometry. (**A**) Western blot analysis of MCL-1 levels in CCRF-CEM cells transfected with MCL-1 shRNA (shMCL-1) or control shRNA (shLuc). Proliferation (**B**), apoptosis (**C**) and cell number (**D**) were determined by ^{3}H-thymidine incorporation, 7AAD/Annexin-V staining, and cell enumeration using trypan blue exclusion, respectively. (**E**) CCRF-CEM cells were treated with Rapamycin (5nM), IRAK-1,-4 inhibitor (5μM) or DMSO for 6 hours. The cells were then treated with cycloheximide (CHX) for 0, 2, 4, 6, 8, 10 hours. The total cell lysates were analyzed by western blot for MCL-1 expression. Densitometric values (±SD) for MCL-1 protein relative to 0 hour MCL-1 of each treatment group are shown taken from the average of three independent experiments. (**F** and **G**) CCRF-CEM cells were cultured with Rapamycin or chemotherapy alone or together at the indicated drug concentrations. After 48 hours, apoptosis was examined by flow cytometry (AnnexinV/7AAD) to determine cell death. Data is represented of two experiments.



Supplemental Figure 11. Model describing how IRAK signaling in T cell acute lymphoblastic leukemia contributes to cell survival and chemotherapy sensitization. In T-ALL IRAK-4,-1 is activated in the absence of exogenous TLR ligands. However, further activation of IRAK-4 and IRAK-1 signaling can occur via the engagement of TLRs or IL-1R. It possible that IRAK-4,-1 activation might also arise activating mutations in MyD88. IRAK-4 activates IRAK-1 which activates TRAF6 and leads to the activation of NF-κB via TAK1. IRAK signaling also impact the levels of p-AKT and p-mTOR. Whether reduced levels of p-AKT that occur in response to IRAK-4,-1 inhibition are directly responsible for the reduced p-mTOR levels after IRAK inhibition have yet to be determined. TRAF6 associates with MCL-1 and increases K63-linked ubiquitination, resulting in increased protein stability. TLR, Toll like receptor; MyD88, myeloid differentiation primary-response 88; IRAK, interlukin-1 (IL-1)-receptor-associated-kinase; TRAF6, tumor-necrosis-factor-associated factor 6; TAK1, transforming-growth-factor-β-activated kinase 1; IκB, inhibitor of nuclear factor-κB; NF-KB, nuclear factor-κB; MCL-1, myeloid cell leukemia sequence 1; AKT, also known as protein kinase B; mTOR, mammalian target of rapamycin;.

Supplemental Table 1. Proliferative effects of TLR agonists on T-cell neoplasms.

Proliferative effects of TLR ligands on T-cell subsets																
	Acute Lymphoblastic Leukemia									Cutaneous T-cell Lymphoma / T-cell Leukemia					Primary T-cells	
	CCRF-CEM	CEM-C1	CEM-C2	CEM-C7	Jurkat	Loucy	Molt-4	HSB-2	SupT1	Hut-78	HH	Hut 102	MO	H9	Non-Act	Activated
TLR1/2 (Pam3Cysk4)	+ (1.2)	+ (2.5)	+ (2.5)	+ (2.5)	+ (10)	+ (2.5)	∘	– (5)	∘	∘	∘	∘	∘	∘	∘	+ (10)
TLR3 (pI:C)	∘	∘	∘	– (10)	∘	∘	∘	– (0.6)	– (2.5)	∘	∘	– (10)	∘	– (1.2)	∘	∘
TLR4 (LPS)	∘	∘	– (5.0)		∘	∘	∘	– (0.3)	∘	∘	∘	– (10)	– (5)	– (5)	∘	∘
TLR5 (Flagellin)	∘	∘	∘	– (2.5)	∘	∘	∘	– (10)	∘	∘	∘	∘	∘	– (10)	∘	+ (10)
TLR2/6 (HKML)	∘	∘	∘	∘	∘	∘	– (5.0)	– (5)	– (10)	∘	∘	∘	∘	– (2.5)	∘	∘
TLR7 (Imiquimod)	∘	∘	∘	∘	– (10)	∘	∘	– (1.2)	– (0.6)	∘	∘	∘	∘	∘	∘	– (5)
TLR8 (ssRNA)	∘	∘	∘	∘	∘	+ (5)	∘	– (0.1)	– (5)	∘	∘	– (5)	– (10)	– (1.2)	∘	– (5)
TLR9 (CpG-ODN)	∘	∘	∘	∘	∘	∘	∘	– (0.6)	– (1.2)	∘	∘	– (5)	– (10)	– (0.6)	∘	∘

The various malignant cell lines or healthy T cells were cultured in the presence of varying concentrations (10–0.1 μg/ml) of the different TLR ligands shown for 72 hrs. Proliferation was determined by measuring ^{3}[H]-thymidine update. Changes in proliferation greater than 20% are represented by symbol "+" (increased proliferation), "–" (reduced proliferation), or "∘" for no change. The values in parentheses indicate concentrations at which the noted differences were observed.

Supplemental Table 2. Association between mutations, translocations and response to IRAK inhibitor

Cell Line	Tumor Type	Cytogenetics	Mutation	Note	Response to IRAK-1,-4*i*
CCRF-CEM	T-ALL	t(5;14)(q35.1;q32)	Notch1, p53	Gamma-secretase-resistant	Yes
CEM/C1	T-ALL	t(5;14)(q35.1;q32)	TLX3	Camptothecin (CPT) resistant derivative	Yes
CEM/C2	T-ALL	t(5;14)(q35.1;q32)	TLX3	Camptothecin (CPT) resistant derivative	Yes
CEM/C7	T-ALL	t(5;14)(q35.1;q32)	TLX3	Glucocorticoid-sensitive derivative	Yes
Jurkat	T-ALL	46,XY,-2,-18,del	Notch1, p53	Gamma-secretase-resistant	Yes
MOLT4	T-ALL	hypertetraploid	Notch1	Gamma-secretase-resistant	No
Mo	T leukemia	no information			Yes
HuT78	T lymphoma	t(2;8)(q34;q24)	MYC, TCL4, p53		Yes
H9	T lymphoma	near triploid			Yes
HH	T leukemia	no information			Yes
Patient #					
#808	T-ALL	normal cytogenetics			Not tested
#810	T-ALL	t(11;14)(p13;q11.2)	LMO1		Not tested
#813	T-ALL	normal cytogenetics			Yes
#820	T-ALL	t(11;14)(p13;q11.2)	LMO1		Yes
#824	T-ALL	t(11;19)(q23;p13.3)	LMO2		No
#825	T-ALL	normal cytogenetics			Yes
#828	T-ALL	t(7;9)(q34;q34.3)	Notch1		Yes

Supplemental Table 3. Real-Time PCR primer sequences

Primer Name	Sequences (5' - 3')
MCL-1 forward	CCAAGAAAGCTGCATCGAACCAT
MCL-1 reverse	CAGCACATTCCTGATGCCACCT
BCL-2 forward	ATCGCCCTGTGGATGACTGAGT
BCL-2 reverse	GCCAGGAGAAATCAAACAGAGGC
BCL-XL forward	GCCACTTACCTGAATGACCACC
BCL-XL reverse	AACCAGCGGTTGAAGCGTTCCT
β-actin forward	CACCATTGGCAATGAGCGGTTC
β-actin reverse	AGGTCTTTGCGGATGTCCACGT
IRAK1 forward	TCAGAACGGCTTCTACTGCCTG
IRAK1 reverse	TACCCAGAAGGATGTCCAGTCG
IRAK4 forward	ATGCCACCTGACTCCTCAAGTC
IRAK4 reverse	CCACCAACAGAAATGGGTCGTTC
GAPDH forward	GTCTCCTCTGACTTCAACAGCG
GAPDH reverse	ACCACCCTGTTGCTGTAGCCAA