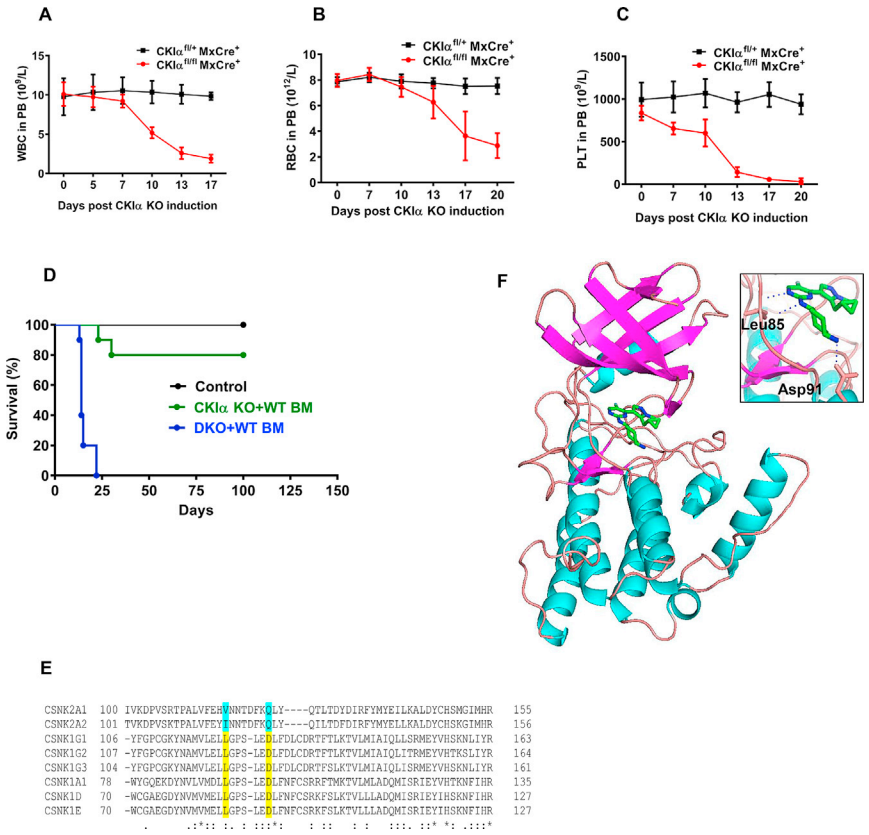

Figure S1. CKIα Ablation Compromise Hematopoietic System and pan-CKI Inhibitors Specificity, Related to Figure 1

(A-C) White blood cells (WBC), red blood cells (RBC) and platelets (PLT) counts in peripheral blood (PB) over time of CKIα$^{fl/+}$ Mx1-Cre (black line, Het- control; N = 5) and CKIα$^{fl/fl}$ Mx1-Cre (red line, CKIα KO; N = 5) mice treated with 3 consecutive pIpC injections. Graphs show mean ± SD values.

(D) Kaplan-Meier survival curves showing that whereas BMT from GFP transgenic WT mice (WT BM) rescues CKIα ablated mice (CKIα KO, green; N = 10), but does not rescue CKIα/p53 DKO mice (blue; N = 10). CKIα$^{fl/+}$ Mx1-Cre (Control, black, N = 10) transplanted mice showed no signs of successful donor engraftment.

(E) Multiple sequence alignment of all human isoforms of CKI and CK2 family shows highly conserved interacting amino acids (highlighted in Yellow) within the ATP binding domains of CKI family, but not in the CK2 family (highlighted in gray).

(F) Structure of A86 in complex with CKIδ: CKIδ is shown as a cartoon, A86 as a stick representation (C and H atoms in green, N in blue, F in gray). Inset: Leu85 and Asp91 of the ATP binding pocket forming hydrogen bonds with A86. Interacting residues shown in stick, hydrogen bonds in blue dotted line.

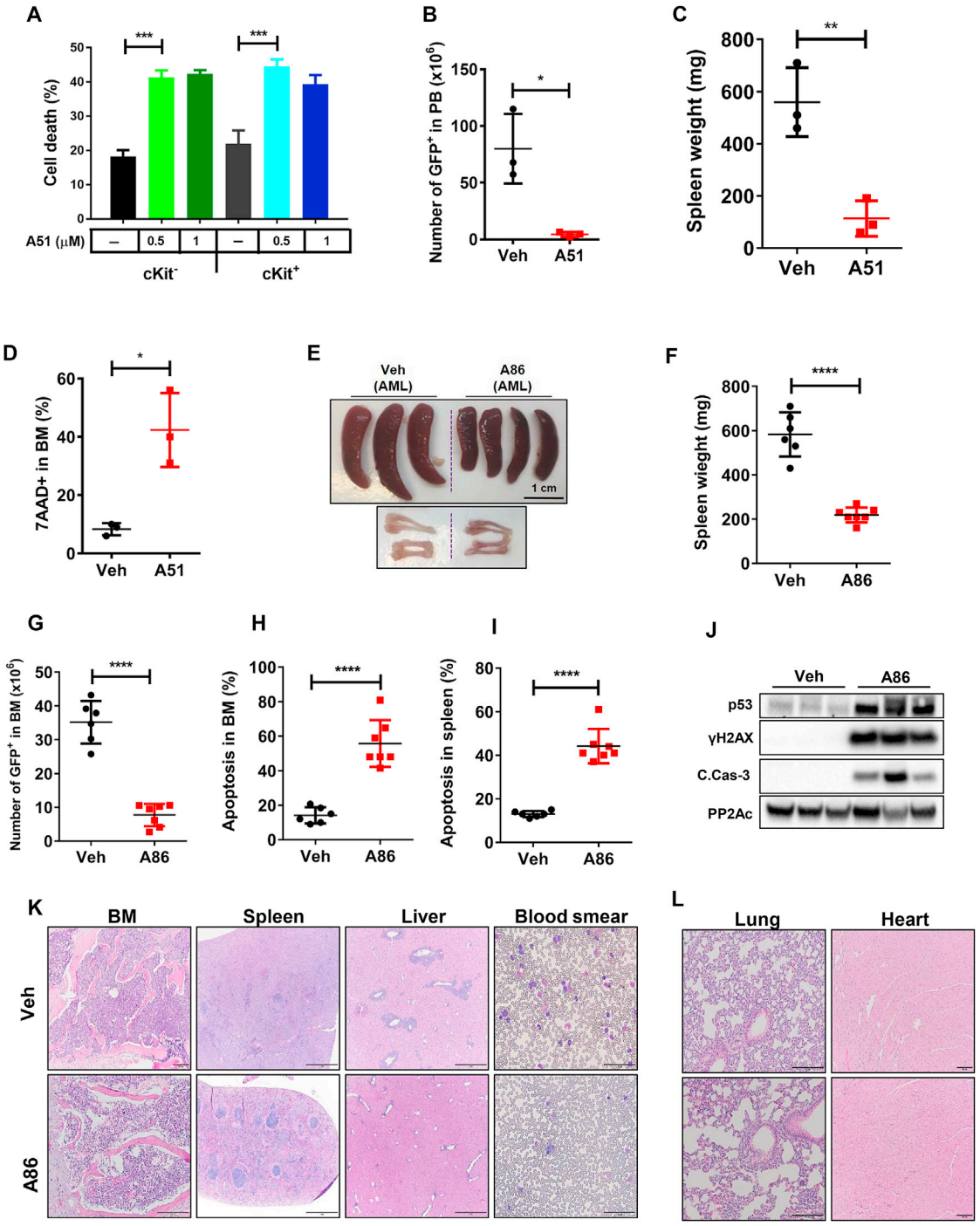

Figure S2. Short-Term Anti-leukemia Activity of the CKI Inhibitors A51 and A86 in AML Mouse Model, Related to Figure 2

(A) FACS analysis of the percentage of 7AAD$^+$ cells of cKit$^+$ versus cKit$^-$ BM cells isolated from AML mice, cultured for 4hrs with A51 at the indicated concentrations or with DMSO. Graphs show Mean ± SD values; N = 3. Statistical analysis by Student's t test.

(B-D) Short-term treatment of AML mice treated for 16hrs with a single dose of A51 (20mg/kg) or vehicle. Spleen weight (B), absolute numbers of GFP$^+$ leukemia blasts in peripheral blood (PB) (C) and percentage of 7AAD$^+$ cells in BM (D). Data are presented as mean ± SD; N = 3 for both groups. Statistical analysis is done by Student's t test.

(E-L) Short-term treatment of AML mice with a single dose of A86 (10mg/kg) or vehicle. Mice were sacrificed 5 hr post treatment. N = 7 for A86 group, N = 6 for vehicle. Graphs show Mean ± SD values. Statistical analysis by Student's t test.

(E) Representative spleen image (upper panel), tibia and femur (bottom panel). (F) Spleen weight.

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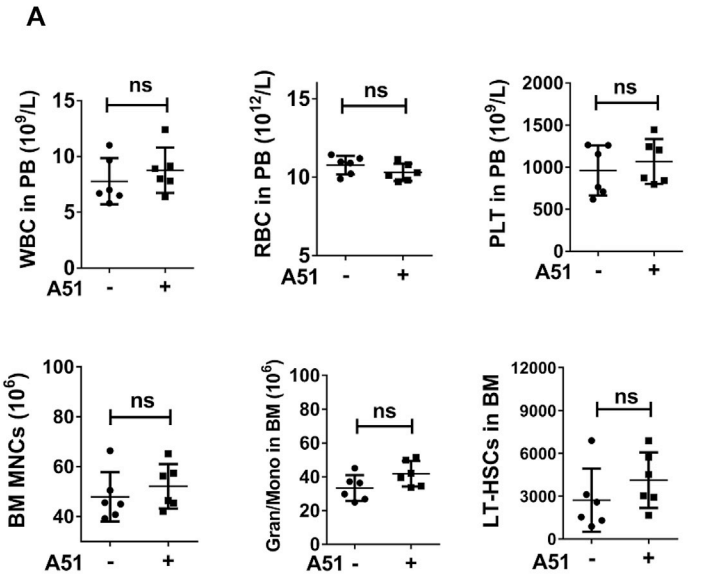
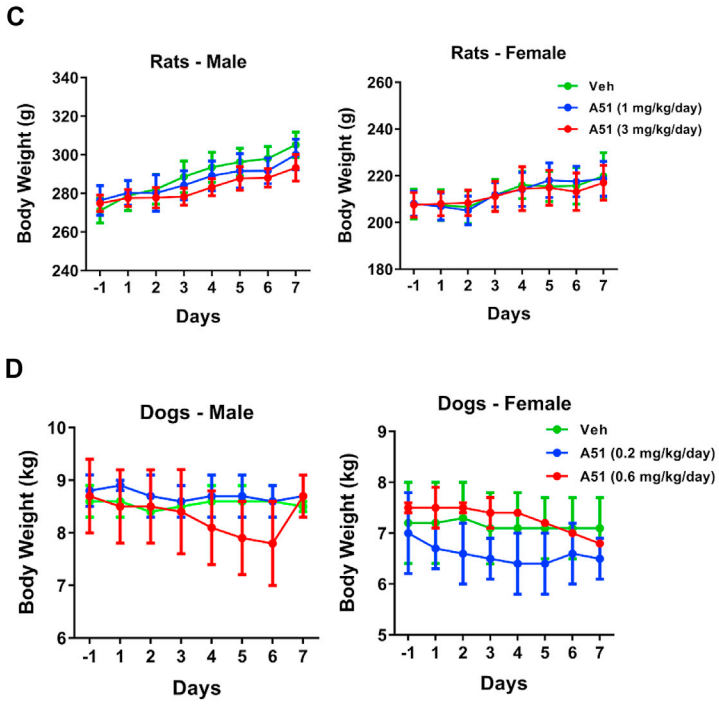
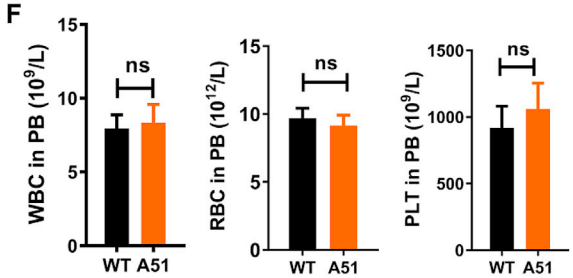
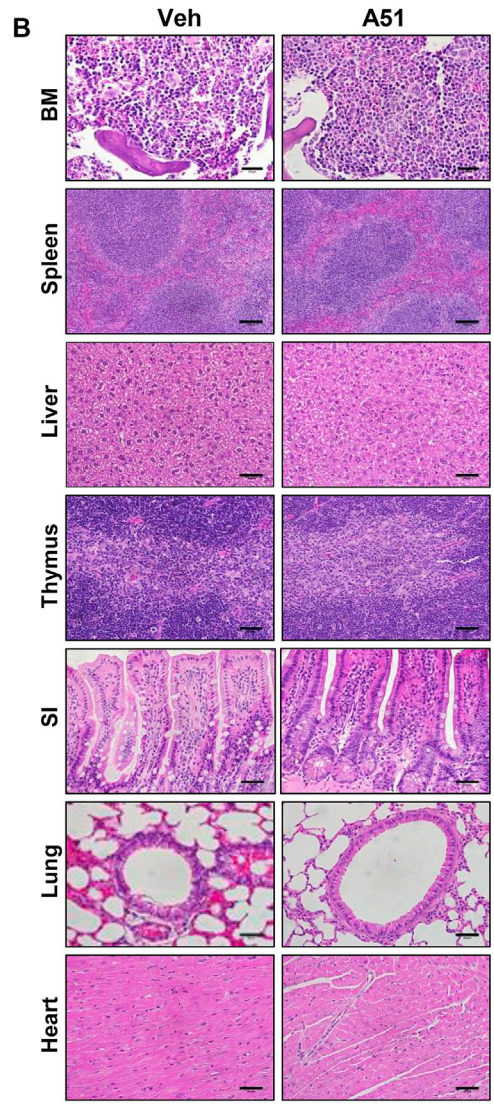
(G) Absolute numbers of GFP^+ leukemic cells in BM. (H) Percentage of Annexin V^+ apoptotic cells in BM and (I) spleen.
(J) WB analysis of leukemic BM cells. PP2Ac is a loading control.
(K and L) Representative H&E-stained sections of the BM, spleen, liver and blood smear (scale bar, 200 μm, 1mm, 400 μm and 30μm, respectively), Lung and heart (200 μm and 400 μm, respectively).









E

Assay with A51	Parameter	Result
hERG Inhibition	%Inhibition at 10 μM	57
	IC_{50} (μM)	7.1
AMES mutagenesis test	*S. typhimurium*: TA98, TA100, TA1535, TA1537 and E.Coli combo	None

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Figure S3. Toxicity Study of A51-Treated Healthy C57BL/6 Mice, Rats, Dogs, and Long-Term Therapeutic Effects of the CKI Inhibitor A51 in AML Mice, Related to Figure 3

(A) Cell counts in BM and PB of healthy C57BL/6 mice at day 9 following treatment with A51 (5mg/kg/day, for 7 days) (N = 6). MNCs, mononuclear cells. Gran/ Mono, Granulocytes/ Monocytes. LT-HSCs were defined as described in the methods section. Graphs show mean ± SD values.

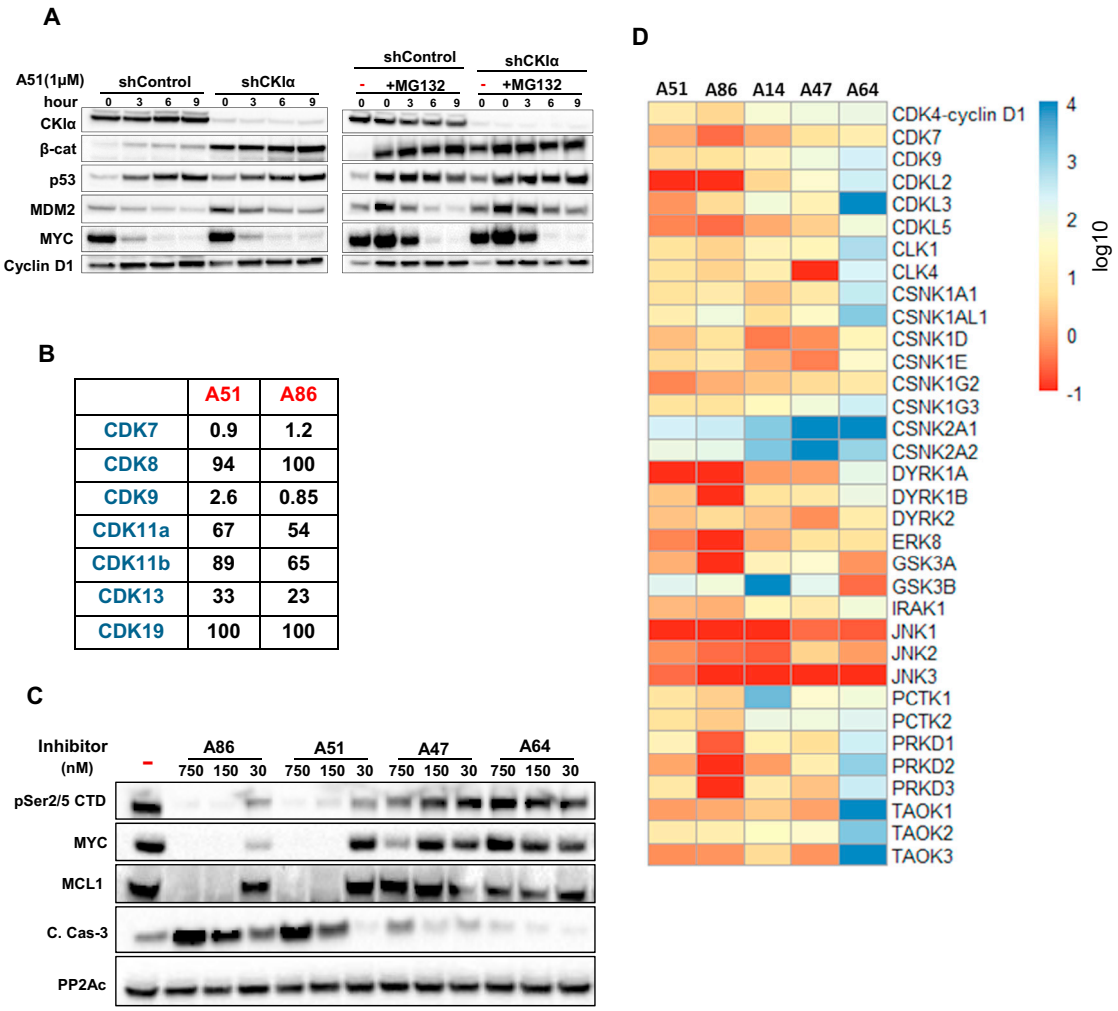
(B) Representative H&E stained sections from vehicle and A51 (5mg/Kg/day) treated mice (as detailed in A). Shown are sections of BM, spleen, liver, thymus, small intestine (SI), lung and heart (N = 6). Scale bar for BM, 30μm; for liver, thymus, small intestine (SI), lung and heart, 50μm; for spleen, 100 μm.

(C) Rats were orally treated once-daily with 1 or 3 mg/kg/day A51 (Di-PTSA salt) for 7 consecutive days and 1 mg/kg/day treated rats had no meaningful test article-related findings in either sex. Noteworthy findings at 3 mg/kg/day included mild clinical signs of toxicity, with no body weight loss, or decreased body weight gain, and mild changes in hematology and clinical chemistry parameters in females only; males were not affected. N = 4 and graphs show mean ± SD values.

(D) Dogs were orally treated once-daily with 0.2 or 0.6 or 2 mg/kg/day A51 (Di-PTSA salt); where the 2 mg/kg/day A51 was not tolerated and resulted in mortality/ morbidity on Days 1 and 2. Once-daily oral administration of 0.2 and 0.6 mg/kg/day A51 for 7 consecutive days was tolerated in both sexes with test article-related ante- and post-mortem findings suggestive of dose-limiting toxicity in both sexes. N = 2 and graphs show mean ± SD values.

(E) hERG inhibition and AMES mutagenesis assays of A51.

(F) WBC, RBC and PLT counts in the PB of surviving mice post A51 inhibitor treatment in comparison to WT mice. Blood counts were measured 135d post leukemia inoculation. N = 10 and graphs show mean ± SD values.



	A51	A86
CDK7	0.9	1.2
CDK8	94	100
CDK9	2.6	0.85
CDK11a	67	54
CDK11b	89	65
CDK13	33	23
CDK19	100	100

Figure S4. CKIα Inhibitor Treatment Co-targets CDK7 and CDK9, Related to Figure 4

(A) WB analysis of CKIα-depleted (shCKIα) in comparison to shControl A51-treated RKO cells. Cells were treated with 1 μM A51 for the indicated time intervals with or without the proteasome inhibitor MG132 (20μM). Shown is the A51 inhibitor effect on the expression of β-catenin, p53, MDM2 and MYC. Cyclin D1 protein levels did not change and serves as a loading control.

(B) Percent control binding (KINOMEScan) values of selected A-Series at 100nM (A51 and A86) inhibitors to 7 known transcriptional kinases.

(C) WB analysis of MV4-11 human AML cells treated with different A-series inhibitors at the indicated concentration for 18hrs. Shown is a dose-dependent inhibition of CDK7&9-mediated Pol II C-terminal domain phosphorylation (pSer2/5 CTD), reduced expression of MYC and MCL1, and caspase 3 activation, preferentially by A86 and A51 treatment. PP2Ac is a loading control.

(D) Kinome targeting analysis (measured Kd, or estimated Kd from %Control binding) of A-series inhibitors, A51, A86, and A14 (apoptotically active compounds) and A47 and A64 (inactive compounds); 100nM kinome scan (DiscoverX). Shown are target kinases hit by at least one active inhibitor, at %Control $\leq$ 10; CSNK2 (CK2) family serves as a kinase binding reference.

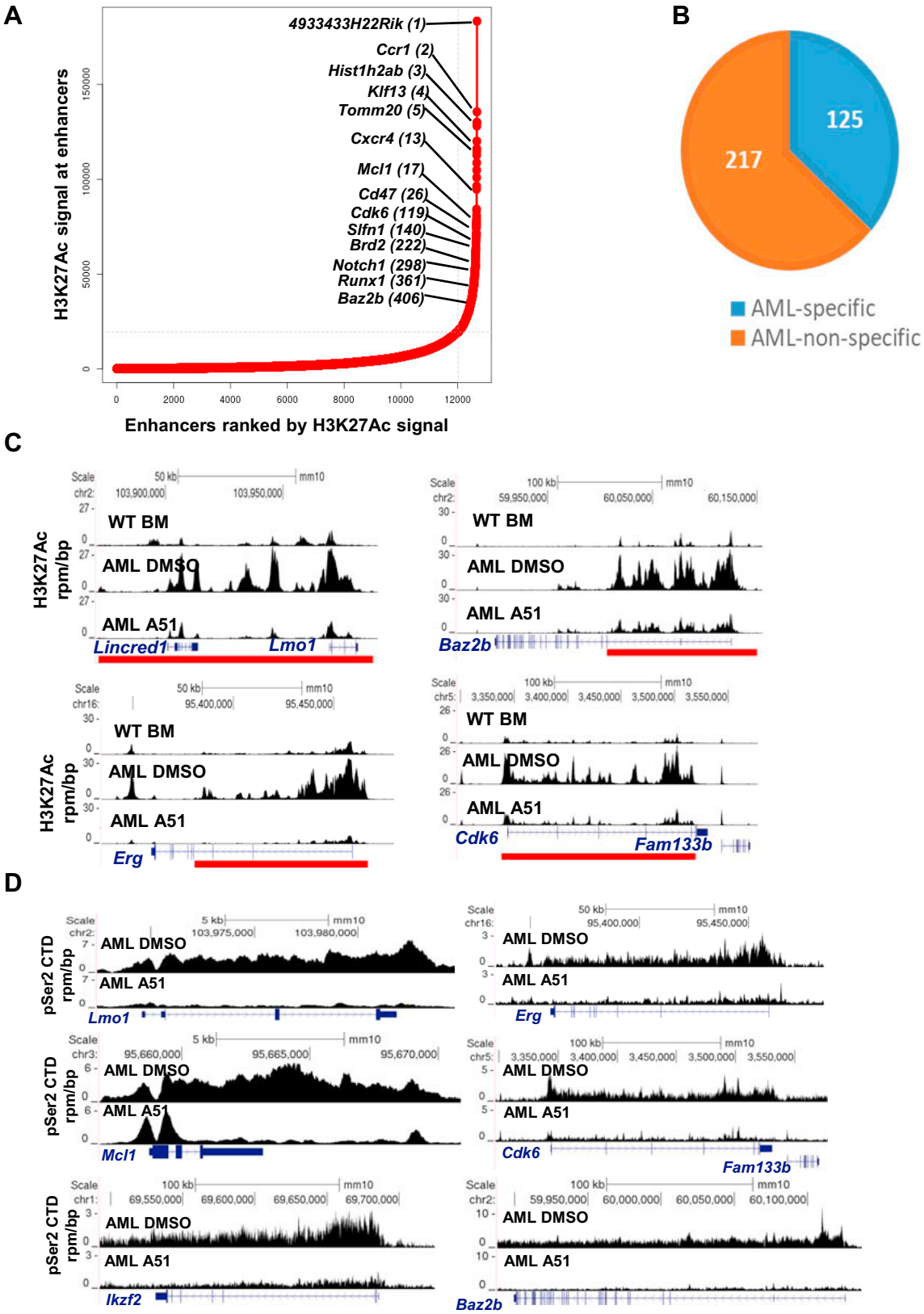


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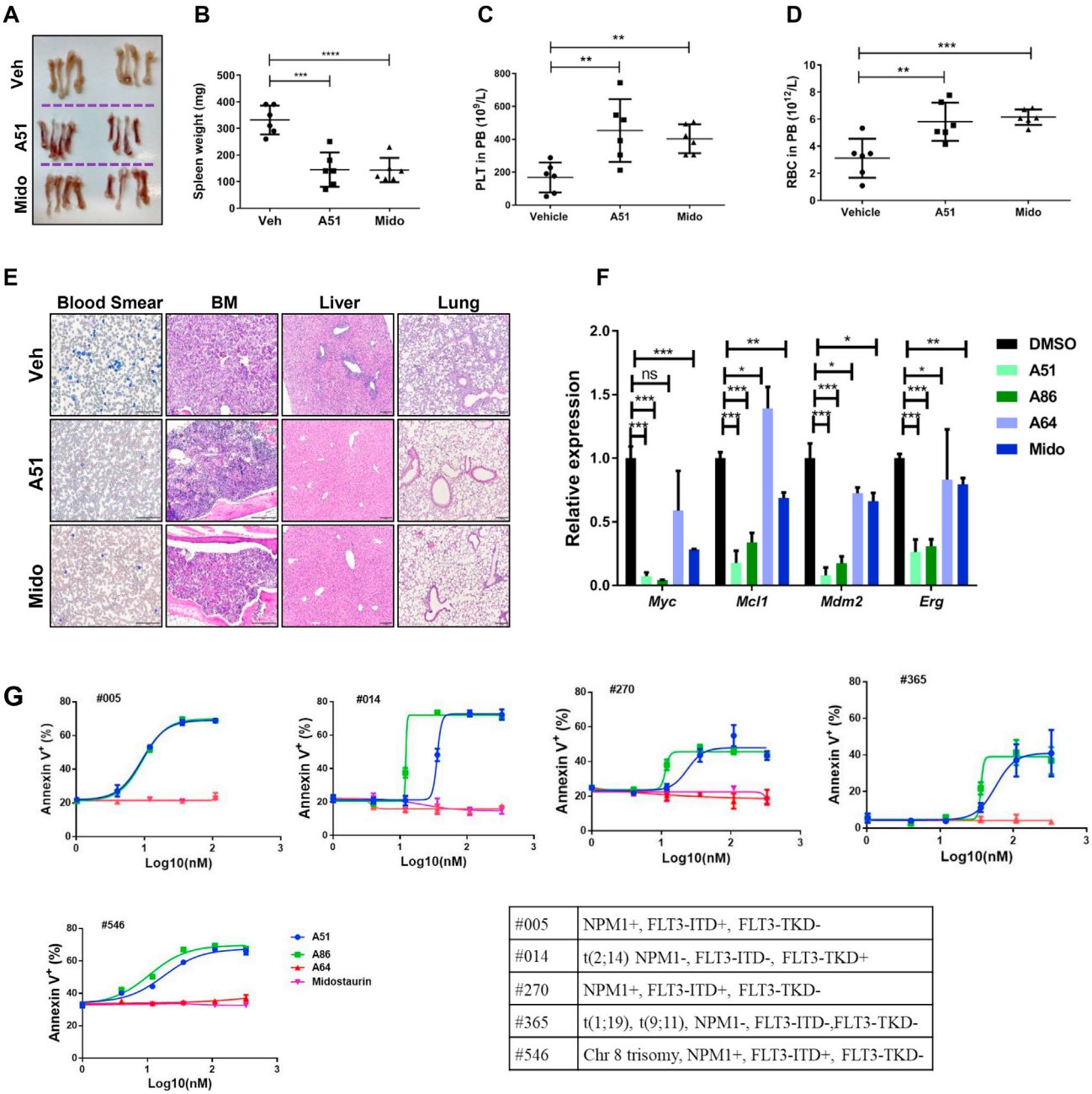
Figure S5. Blocking CDK7 and CDK9 Disrupts Leukemia SEs and Halts the Transcription Elongation of SE-Driven Leukemia Oncogenes, Related to Figure 5

(A) Ranking plot of enhancers identified in WT BM cells, ranked by increasing H3K27Ac signal (units: rpm). SEs defined as enhancer clusters, excluding signals at the promoter region, ranked above the inflection point of the curve. Selected genes are indicated with ranks.

(B) SE analysis summary of 342 SE-associated genes that were identified in AML BM; 125 are AML specific (non-detected in normal BM).

(C) Individual gene tracks of ChIP-seq signal for H3K27Ac at multiple loci (*Lmo1*, *Baz2b*; *Erg* and *Cdk6*) in WT BM or primary AML cells treated with A51 (1μM) or DMSO for 4hrs. Super enhancers identified in DMSO treated AML cells are indicated in red bars. Y axis shows ChIP-seq signal (rpm/bp). The X axis depicts genomic position.

(D) Individual gene tracks of pSer2 CTD ChIP-seq signals at multiple loci (*Lmo1, Erg, Mcl1, Cdk6, Ikzf2* and *Baz2b*) after 4hr treatment of primary mouse AML BM cells with A51 (1 μM) or DMSO. Y axis shows ChIP-seq signal (rpm/bp). X axis depicts genomic position.



#005	NPM1+, FLT3-ITD+, FLT3-TKD-
#014	t(2;14) NPM1-, FLT3-ITD-, FLT3-TKD+
#270	NPM1+, FLT3-ITD+, FLT3-TKD-
#365	t(1;19), t(9;11), NPM1-, FLT3-ITD-,FLT3-TKD-
#546	Chr 8 trisomy, NPM1+, FLT3-ITD+, FLT3-TKD-

Figure S6. Effects of A-Series Inhibitors in *Tet2*$^{-/-}$;*Flt3*^{ITD}-Derived AML Mouse Model and Primary AML Patient Samples, Related to Figure 7

(A-F) *Tet2*$^{-/-}$;*Flt3*^{ITD} mouse data: (A) Representative image of femur and tibia of mice treated with A51, midostaurin (Mido) or vehicle (Veh) as in 7A at time of sacrifice.

(B) Spleen weight quantification of treated mice. N = 10 and graphs show mean ± SD values.

(C) PLT and RBC (D) counts of A51 or Mido versus Veh treated mice at the day of sacrifice.

(E) Representative Blood smear (scale bar 100μm) and H&E images of BM, Liver and Lung (scale bar: 200μm, 200μm and 200 μm, respectively) from A51, Mido or Veh treated mice at time of sacrifice. N = 6 for all groups.

(F) Quantitative real-time PCR (qPCR) mRNA expression of *Myc*, *Mcl1*, *Mdm2* and *Erg* in primary AML cells isolated from the spleen of Tet2$^{-/-}$;Flt3^{ITD} mice treated *ex vivo* with 1μM of A51, A86, A64 or Mido, in comparison to DMSO control for 4hr (N = 3, Student's t test); Graph show Mean ± SD values.

(G) Primary human BM AML cells data: cells were isolated from 5 different patients and treated *ex vivo* with A51, A86, A64 and Mido at the indicated concentrations for 20 hr. FACS analysis was done on CD45^{+}SSC^{low} population representing the blast population. Chromosomal and molecular profiles of the AML samples are specified in the table. Experiment done in triplicates; Y axis intersection corresponds to DMSO control and graphs show mean ± SD values.

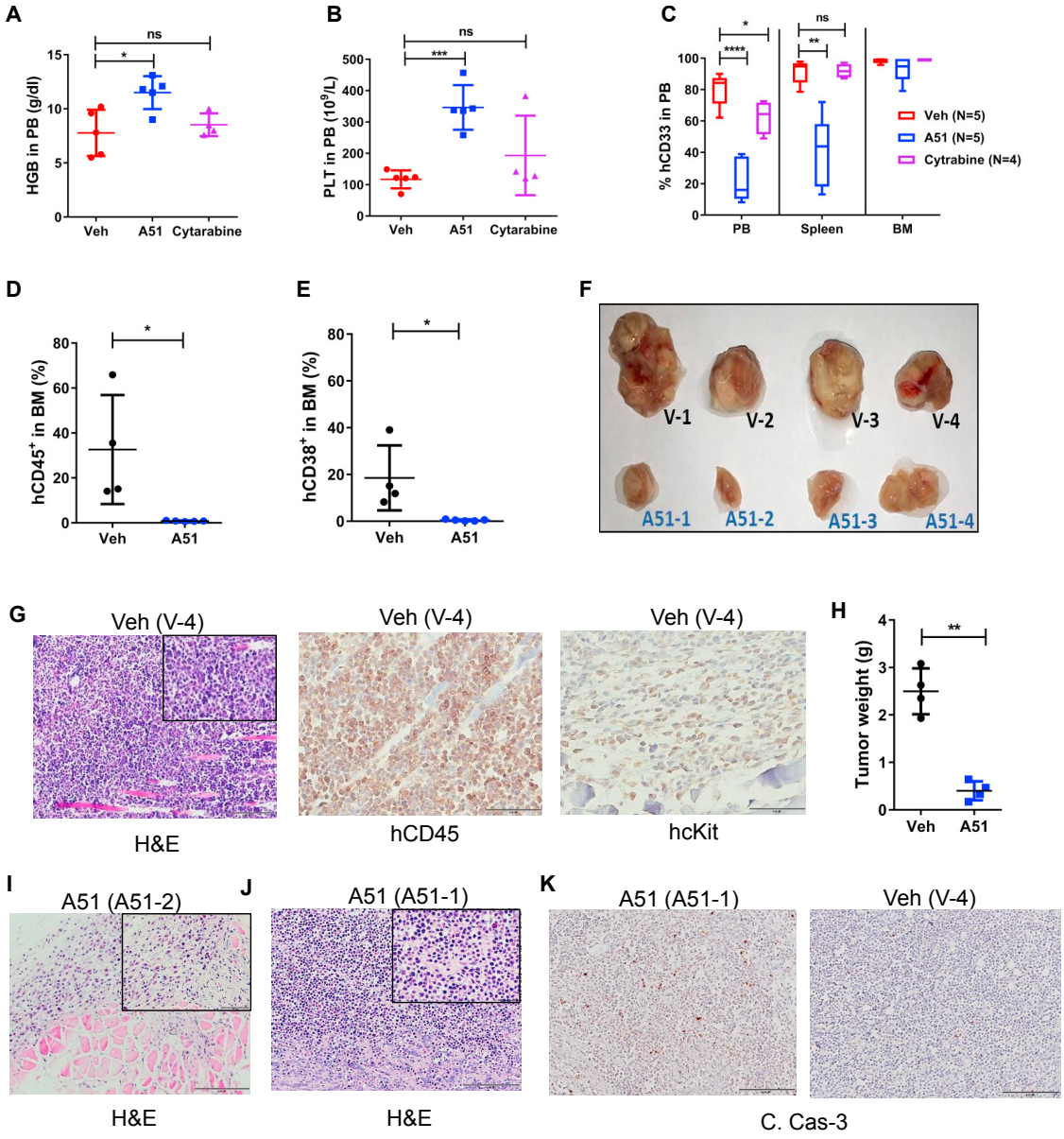


Figure S7. Long-Term Therapeutic Effects of CKI Inhibitor in Multiple Patient-Derived Xenograft Models, Related to Figure 7

(A-C) PDX-1 mouse data: HGB (A) and PLT (B) in PB; and ratios of hCD33$^+$ (C) in indicated tissues of PDX mice treated with A51 (7.5mg/Kg), Cytarabine (30mg/Kg), or vehicle. Samples were collected at the time of sacrifice. N = 6 and graphs show mean ± SD values.

(D & E) PDX-2 mouse data: Ratios of hCD45$^+$ and hCD38$^+$ in PB of A51 or vehicle-treated PDX mice at the time of sacrifice. N = 4 for Veh, and 5 for A51 and graphs show mean ± SD values.

(F-K) PDX-3 mouse data (of normal karyotype, *NPM1+, FLT3-ITD+, FLT3-TKD-*): (F) Tumor image of A51 and vehicle-treated mice. (G) Representative images of H&E (scale - 200μm, inset scale – 50μm) and IHC of hCD45 and hcKit from vehicle treated mice (scale – 100μm). (H) Weight values of tumors dissected from A51 and vehicle treated mice. N = 4 and graph shows mean ± SD values. (I) Representative H&E image (scale - 200μm, inset scale – 50μm) of A51-treated mouse with minimal residual disease. (J) Representative H&E image (scale - 200μm, inset scale – 50μm) of A51 treated mouse with reduced tumor mass. (K) Cleaved Caspase 3 (C. Cas-3) IHC of A51, and a vehicle treated mouse as control (scale - 200μm).