Supplemental Methods

Analysis and sorting of mouse hematopoietic cells

Murine peripheral blood (PB), BM, and spleen cell counts were analyzed on a Drew Scientific Hemavet 950FS. Flow cytometry analysis was performed on a BD Fortessa X-20 Special Order Research Product Flow Cytometer. Fluorochrome-conjugated antibodies used to analyze mature hematopoietic populations were CD45.2-FITC, CD45.1-PE-Cy7, c-Kit-APC, CD3-PerCp-Cy5.5, CD19-AF700, B220-AF700, Gr-1-PE, CD11b-APC-Cy7. HSPC populations were analyzed using Lineage cocktail (CD8a-biotin, CD8b-biotin, B220-biotin, Ter119-biotin, CD3e-biotin, NK1.1-biotin, IgM-biotin, CD4-biotin, CD19-biotin, Gr1-biotin, CD11b-biotin and streptavidin-PE-Cy7), Sca-1-PE, cKit-APC-Cy7, Flt3-PE-Cy5, CD48-FITC, CD150-PerCp-Cy5.5, CD16/32-AF700 and CD34-APC. Purified ST-HSC (Lin-cKit+Sca1+CD48-CD150-), MPP (Lin-cKit+Sca1+CD48+CD150-) or GMP (Lin-Sca1-cKit+CD16/32+CD34+) populations were sorted using a BD FACS ARIA cell sorter.

Sorting of Human BM cells

For xenografts of purified HSPC, patient BM MNC were stained with antibodies to human antigens: CD45-FITC, CD3-biotin, CD11b-biotin, CD19-biotin, CD34-APC, CD38-PE, CD45RA-PE-Cy7, CD123-PE-Cy5, and CD90-PerCpCy5.5, then streptavidin-APC-Cy7. CMP (Lin-CD34+CD38+CD123+CD45RA-), GMP (Lin-CD34+CD38+CD123+CD45RA+) and MPP (Lin-CD34+CD38-CD90-) populations were sorted using a BD FACS ARIA cell sorter.

Blood Smears

Blood was collected in EDTA-coated tubes to prevent clotting. 1 uL of anti-coagulated blood was pipetted onto the center of the glass microscope slide lying flat on the benchtop. A second glass slide was then placed in front of the drop of blood at a 45 degree angle to the first slide, and slowly dragged toward the drop of blood until just making contact. After blood spread along the width of the slide, the second slide was quickly dragged away from the blood droplet to spread the blood across the first slide. Slides were air dried at room temperature for 30-90 min, then immersed in 100% methanol for 1 min and again air dried. Slides were immersed in Wright-Giemsa stain (MilliporeSigma) for 90 seconds, then diH_2O for 1 min, then rinsed with diH_2O and air dried before imaging under a 63x objective with immersion oil applied directly to the sample.

Drug Preparation

AC220 (Quizartinib, AdooQ) was suspended in DMSO to 33.33 mg/mL and stored at -20° C. For each treatment, a fresh aliquot was thawed and diluted to 0.625-1.25 mg/ml in 22% 2-hydroxypropyl-beta-cyclodextrin (MilliporeSigma). Cytarabine (Cayman Chemical) and Doxorubicin (Cayman Chemical) were suspended in sterile PBS and stored at -20° C. For each treatment, fresh aliquots were thawed.

Stromal coculture

To generate murine BM stromal layers, BM cells from CXCL12-KO mice and CXCL12-WT littermates were cultured in MesenCult Complete Expansion Medium (Stem Cell Technologies) for 10 days at 37°C in a hypoxic incubator (5% O_2), with half-medium change at day 7. After 10 days, expanded stromal cells were trypsinized and grown on 96-well plates (4,000 cells/well) in a hypoxic incubator. After 3 days, FACS-sorted LSK in StemSpan SFEM II (Stem Cell Technologies) were added (4,000 cells/well) and cultured for 3 hours before adding drugs suspended in DMSO and diluted in SFEM to obtain final concentrations. Cells were exposed to drugs for 3 days and analyzed by flow cytometry with CountBright Plus Absolute Counting Beads (ThermoFisher) and surface antibodies: CD45.2-PECy7, cKit-APCCy7, Sca-1-PE, CD48-FITC, and CD150-PerCpCy5.5.

Human AML BM stromal layers were generated by culturing MNC in alpha-MEM medium at 37°C with half-medium change every 3 days till confluent. Adherent cells were passaged and frozen in aliquots once confluent. For coculture stromal cells were thawed and plated in 96-well plates (5000 cells/well) in a hypoxic incubator. After 4 days, CD34+ cells from the same patient (5000cells/well) were added and exposed to drugs as described for murine cells, followed by flow cytometry analysis with CountBright Beads and surface antibodies: CD45-APCe780, CD34-APC, CD38-PE, CD45RA-PECy7.

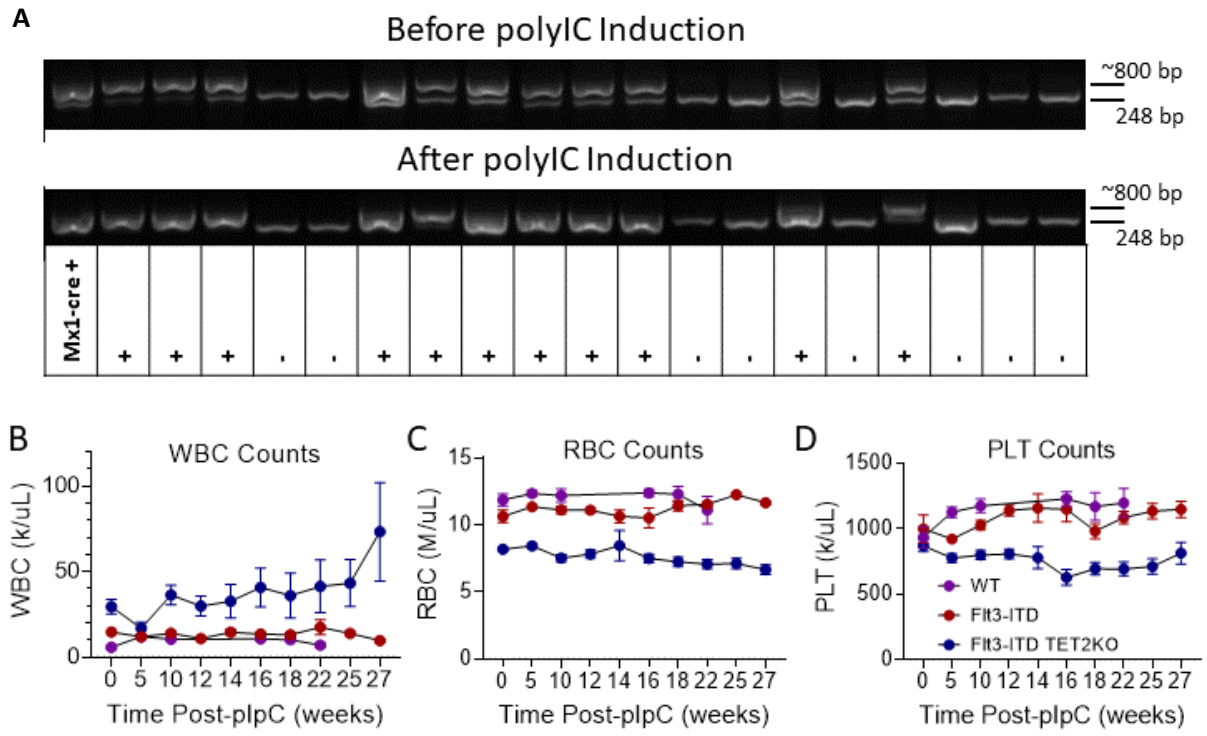
Supplementary Table 1 – Patient Characteristics

Patient	Sample type	Mutations	BM blast %	CD34+ (in MNC)	Fluorescence in situ hybridization
AML172	BM	FLT3-ITD, TET2,U2AF1,KDM6A	85	90%	Normal
AML395	BM	FLT3-ITD, DNMT3A,TET2,NPM1,NRAS	48	25%	Normal
AML521	BM	FLT3-ITD,NPM1,TET2,ZRSR2	73	3.4%	Normal

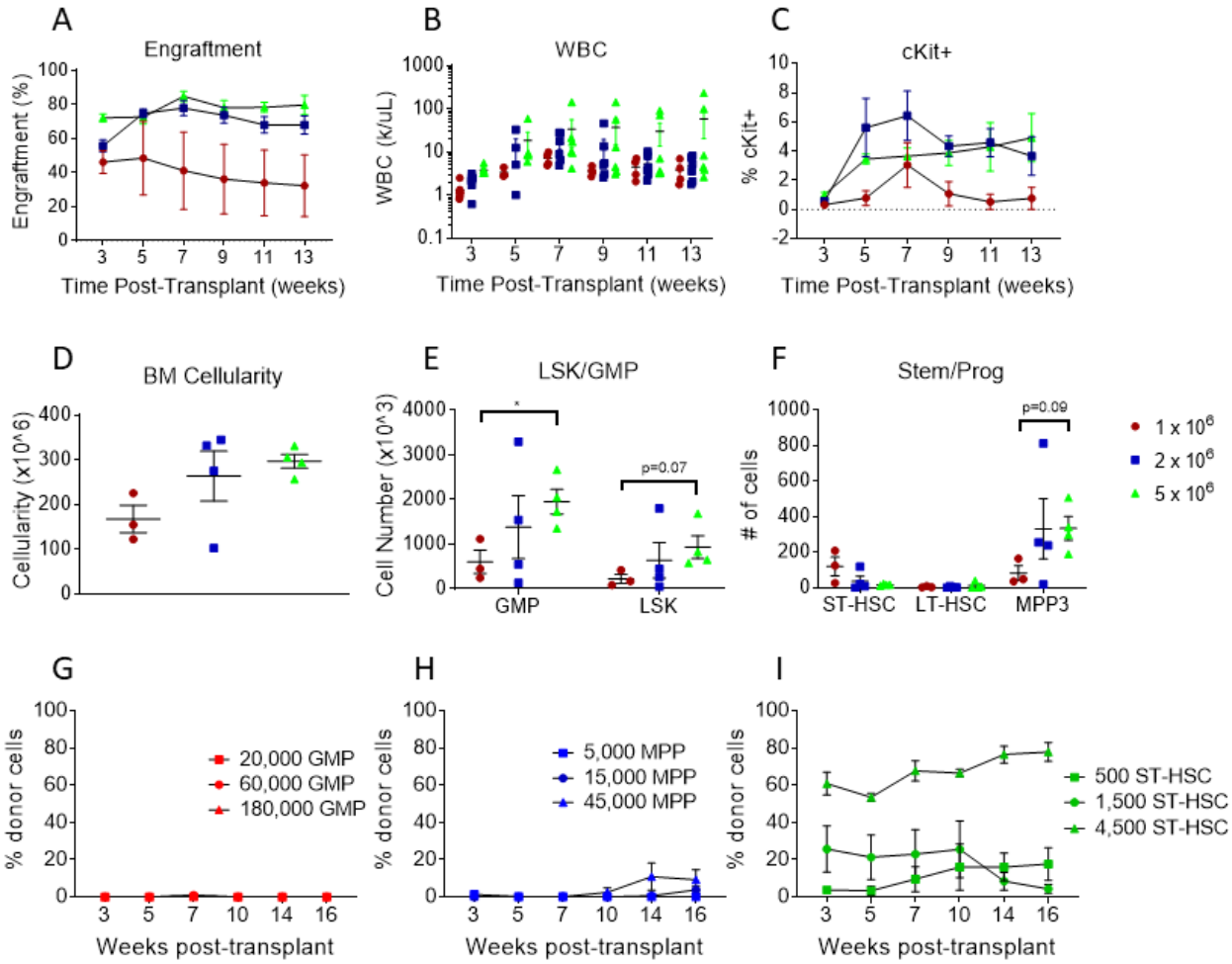
Supplementary Table 2: Details of antibodies uses

Company	Catalog number	Fluorochrome/ conjugate	Antibody	Clone
Mouse antibodies/reagents				
Biolegend	109806	FITC	CD45.2	104
Invitrogen	25-043-82	PE-Cy7	CD45.1	A20
Biolegend	105812	APC	c-Kit	2B8
Biolegend	100218	PerCp-Cy5.5	CD3	17A2
Invitrogen	56-0913-82	AF700	CD19	eBio103
Invitrogen	56-0452-82	AF700	B220	RA3-6B2
Invitrogen	12-5931-82	PE	Gr-1	RB6-BC5
Invitrogen	47-0112-82	APC-Cy7	CD11b	M1/70
Invitrogen	56-0451-82	AF700	CD45	30-F11
Bioelgend	116220	AF700	Ter119	Ter-119
Biolegend	124009	APC	Tie2	TEK4
E bioscience	46-1441-82	PerCp-eFluor710	VE-Cadherin	eBioBV13 (BV13)
E bioscience	48-0311-82	eFluor450	CD31	390
E bioscience	13-0512-85	biotin	CD51	RMV-7
BD	560654	APC-Cy7	Sca-1	D7
E bioscience	25-1401-82	PE-Cy7	CD140a	APA-5
Biolegend	405229	BV605	streptavidin	
E bioscience	13-0081-85	biotin	CD8a	53-6.7
E bioscience	13-0082-85	biotin	CD8b	54-6.7
E bioscience	13-0452-85	biotin	B220	RA3-6B2
Biolegend	116204	biotin	Ter119	TER-119
Invitrogen	13-0031-85	biotin	CD3e	145-2011
E bioscience	13-5941-85	biotin	NK1.1	PK136
Invitrogen	13-5790-85	biotin	IgM	1141
E bioscience	13-0041-85	biotin	CD4	GK1.5
E bioscience	13-0193-85	biotin	CD19	Ebio1D3
E bioscience	13-5931-85	biotin	Gr1	RB6-8C5
E bioscience	13-0112-85	biotin	CD11b	M1/70
BD	557598	PE-Cy7	streptavidin	
Invitrogen	12-5981-83	PE	Sca-1	D7
Biolegend	105826	APCCy7	cKit	2B8
Invitrogen	15-1351-82	PECy5	Flt3	A2F10
Invitrogen	48-0481-82	E450	CD48	HM48-1
Biolegend	115922	PerCpCy5.5	CD150	TC15-12F12.2
Biolegend	101337	AF700	CD16/32	93
BD	560518	APC	mCD34	RAM34
Human Antibodies/reagents				
Invitrogen	13-0199-82	biotin	CD19	HB19
Biolegend	306008	PECy5	CD123	6H6
Biolegend	405208	APC-Cy7	streptavidin	

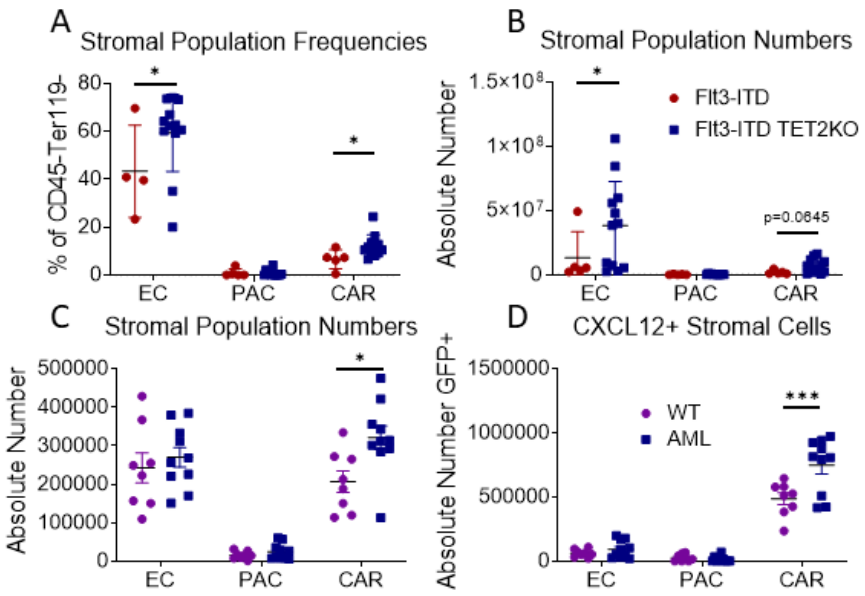
EBioscience	47-0459-42	APC-efluor780	CD45	H310
EBioscience	45-0909-42	PerCPCy5.5	CD90	eBio5E 10
Biolegend	25-0458-42	PECy7	CD45RA	H1264
Invitrogen	17-0349-42	APC	CD34	4H11
EBioscience	12-0388-42	PE	CD38	HB7



Supplementary Figure S1 – Characterization of the Flt3-ITD TET2 KO Mx-1 cre AML model
(A) TET2 excision before and after Mx1-cre induction by intraperitoneal injection of polyIC TET2_Flox_F primer (AAGAATTGCTACAGGCCTGC) and TET2_Flox_R primer (TTCTTTAGCCCTTGCTGAGC) amplify a 248 bp amplicon. TET2_Flox_F primer with Tet2_LoxP3R primer (TAGAGGGAGGGGGCATAAGT) span 4217 bp, including exon 3 of the TET2 gene (3205 bp), which is flanked by LoxP sites. When the floxed exon 3 is excised, the TET2_Flox_F and TET2_Flox_LoxP3R primer set amplify a ~800 bp amplicon. (B) WBC counts, (C) RBC counts, and (D) PLT counts in WT, Flt3-ITD, and Flt3-ITD TET2 KO mice over time after administration of pIpC.

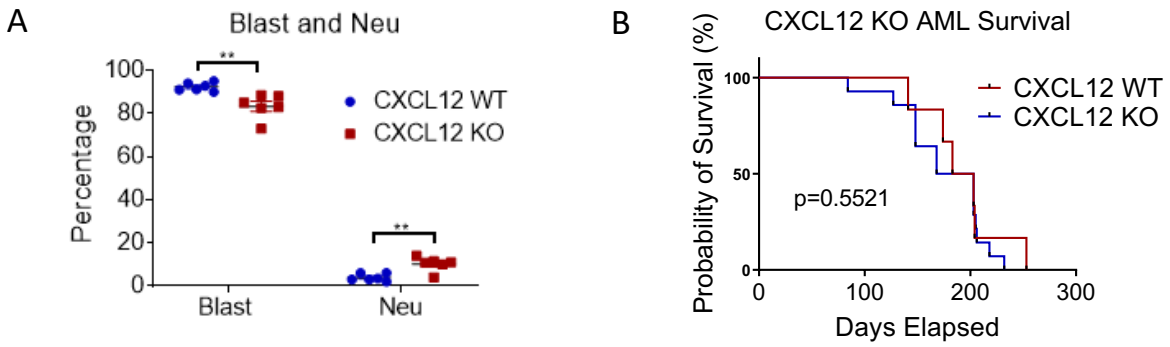


Supplementary Figure S2 – Transplantation from Flt3-ITD TET2 KO mice into WT recipients
(A) PB engraftment, (B) WBC counts, (C) %c-Kit+ cells in PB, (D) BM cellularity, (E) GMP and LSK numbers in BM, and (F) MPP and HSC numbers in BM of WT mice transplanted with 1 x10^6 (red), 2 x10^6 (blue) or 5x10^6 (green) BM MNC from Flt3-ITD TET2 KO mice. (G-I) Percentage engraftment of (G) GMP, (H) MPP, and (I) ST-HSC from Flt3-ITD TET2 AML mice transplanted into WT recipients. Significance values: *p<0.05. Results represent mean + SEM of multiple replicates.

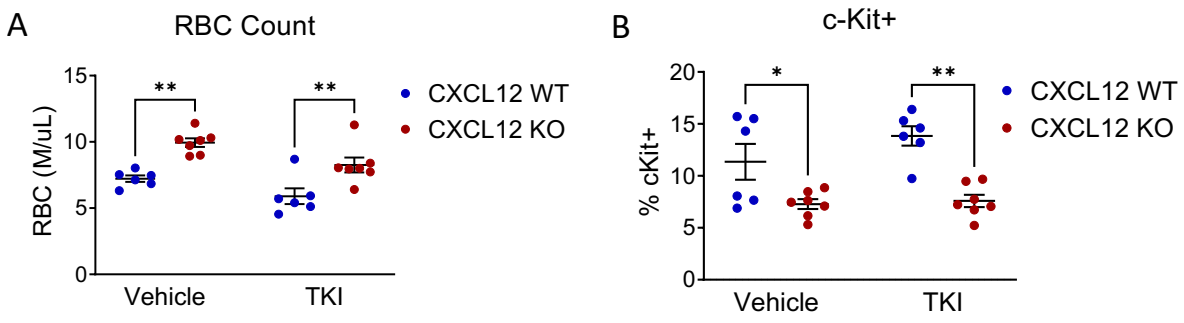


Supplementary Figure S3 – Characterization of Flt3-ITD TET2 KO AML effect on stromal populations and CXCL12 expression

(A) Frequencies and (B) absolute numbers of stromal populations by flow cytometry in Flt3-ITD and Flt3-ITD TET2 KO mouse BM. (C) Absolute numbers of cells and (D) absolute numbers of CXCL12+(GFP+) cells in various stromal populations in CXCL12-GFP mice transplanted with WT or Flt3-ITD TET2 KO AML BM MNC. Significance values: *p<0.05; ***p<0.001. Results represent mean + SEM of multiple replicates.

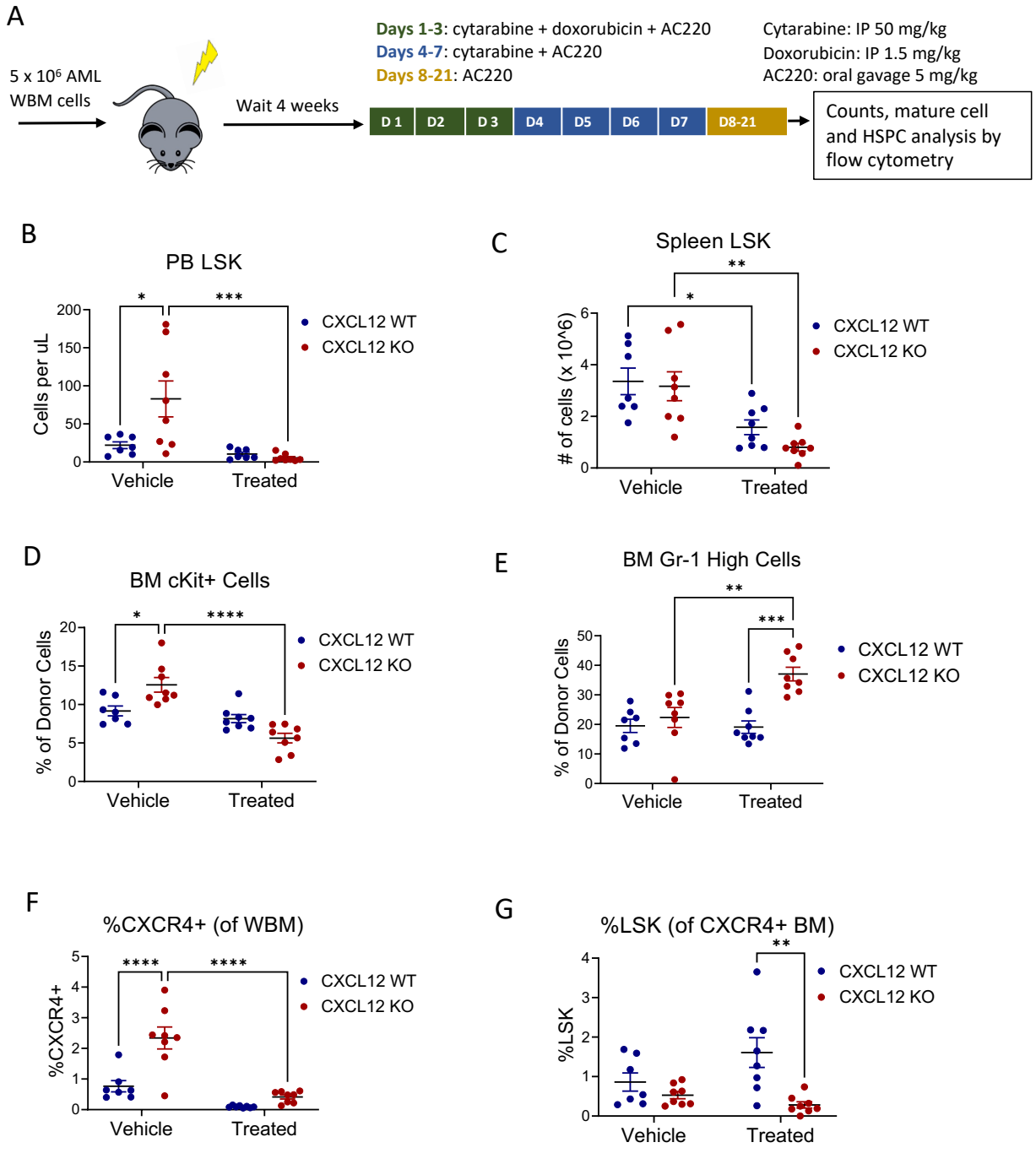


Supplementary Figure S4 – Effect of CXCL12 KO on myeloid maturation in AML
(A) Blast and neutrophil (neu) percentages by morphology on peripheral blood smears from CXCL12 WT vs. CXCL12 KO AML mice. (B) Survival of CXCL12-WT and CXCL12-KO mice transplanted with AML cells. Significance values: **p<0.01. Results represent mean + SEM of multiple replicates.



Supplementary Figure S5 – Effect of CXCL12 KO on TKI response

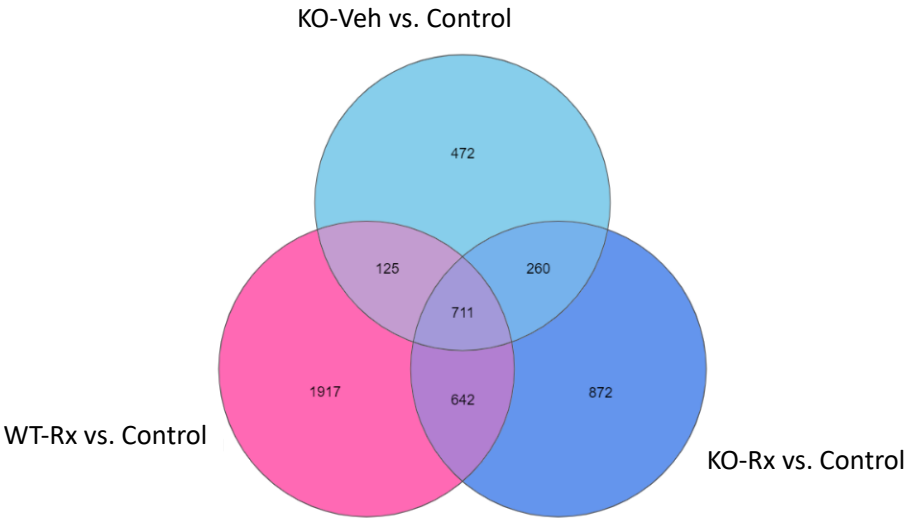
(A) RBC counts and (B) %cKit+ cells in peripheral blood of CXCL12 WT and CXCL12 KO mice transplanted with Flt3-ITD TET2 KO AML BM MNC and treated with vehicle control or TKI. Significance values: *p<0.05; **p<0.01; ***p<0.001. Results represent mean + SEM of multiple replicates.



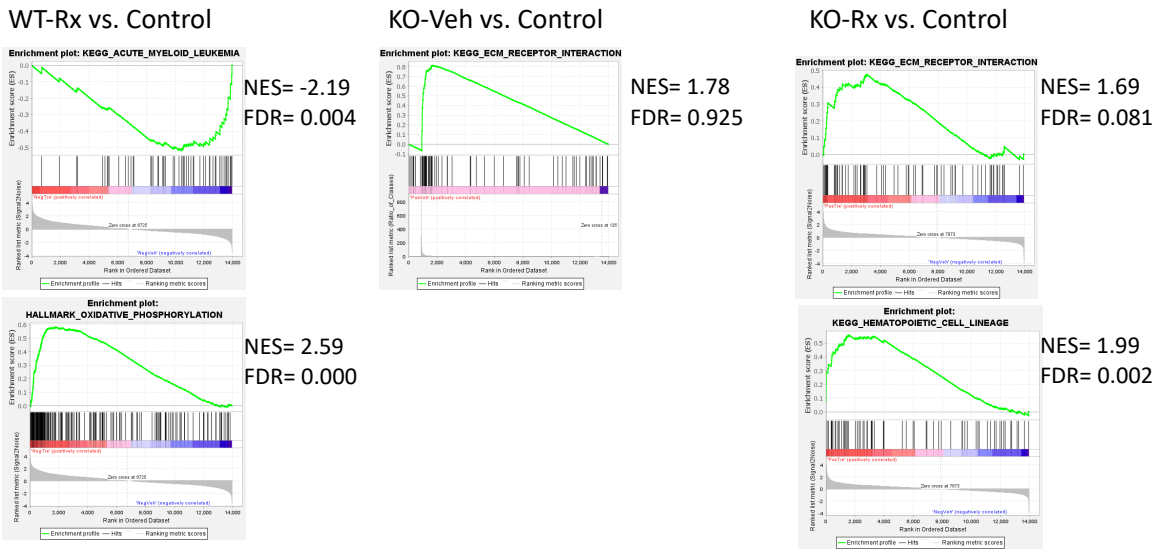
Supplementary Figure S6 – Effect of CXCL12 KO on Flt3-ITD TET2 KO AML response to chemo+TKI
(A) Experimental design for administration of combination chemotherapy plus quizartinib in CXCL12-WT and CXCL12-KO AML mice. (B) Number of LSK in PB, (C) number of LSK in spleens, (D) percentage of cKit+ cells in BM, and (E) percentage of Gr-1 high mature neutrophils in BM of CXCL12 WT or CXCL12 KO mice transplanted with Flt3-ITD TET2 KO AML BM and treated with chemo+TKI or vehicle. (F) Percentage CXCR4 positivity in BM MNC and (G) percentage of LSK within CXCR4+ BM MNC of CXCL12 WT or CXCL12 KO mice transplanted with Flt3-ITD TET2 KO AML BM and treated with either chemo+TKI or vehicle.

Significance values: *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. Results represent mean + SEM of multiple replicates.

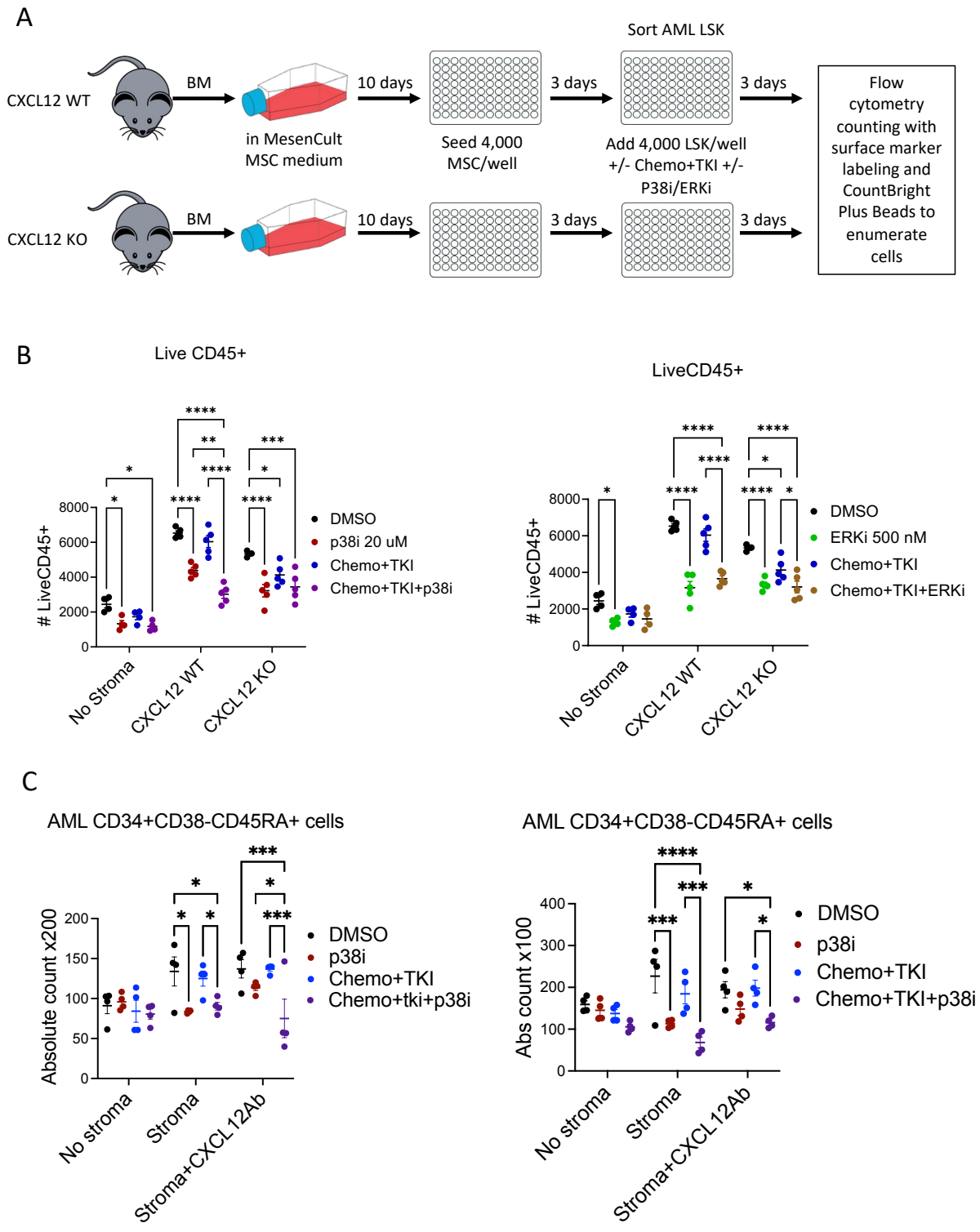
A



B



Supplementary Figure S7. Changes in gene expression in leukemia stem cells following CXCL12 KO and combination chemotherapy plus TKI. (A) Venn diagram of all differentially-expressed genes in ST-HSC from WT-Rx, KO-Veh, and/or KO-Rx vs control mice. (B) GSEA plots of gene sets enriched in CXCL12-WT AML mice treated with combination chemotherapy plus quizartinib (WT-Rx) vs. CXCL12-WT AML mice treated with vehicle (Control, left panel), CXCL12-KO AML mice treated with vehicle (KO-Veh) vs. Control (center), and CXCL12-KO AML mice treated with combination chemotherapy plus quizartinib (KO-Rx) vs. Control (right).



Supplementary Figure S8. p38 signaling contributes to AML LSK resistance to chemo + TKI
(A). Experimental design for co-culture experiments. MSC=mesenchymal stem cells; Chemo+TKI=combination chemotherapy plus quizartinib; p38i= losmapimod; ERKi=ulixertinib (B) Total

number of live (DAPI-) CD45+ cells after 3 days of culturing FACS-sorted LSK without stroma, on CXCL12 WT stroma, or on CXCL12 KO stroma and treated with vehicle, losmapimod, chemo + TKI, or chemo + TKI + losmapimod (left); or vehicle, ulixertinib, chemo + TKI, or chemo + TKI + ulixertinib (right). (C) Total number of human CD34+CD38-CD45RA+ cells following treatment of AML CD34+ cells with DMSO (vehicle), p38i, chemo+TKI, or chemo+TKI+p38i. with or without co-culture with stromal cells from the same patient, and with or without addition of CXCL12 blocking antibody. Results from two different AML samples AML395 (left) and AML172 (left) are shown. Significance values: *p<0.05; **p<0.01, ***p<0.001, ****p<0.0001. Results represent mean ± SEM of multiple replicates.