Supplemental Data

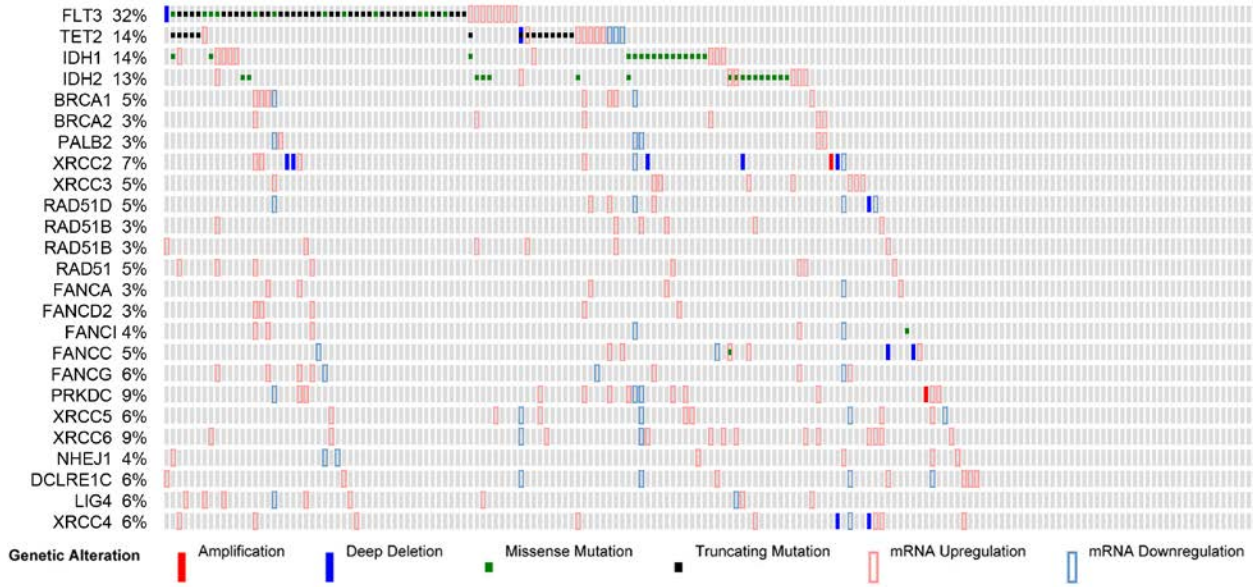
Supplementary Table 1. Clinically relevant mutations of AML samples.

UPN	Mutations
125	FLT3(ITD)
188	FLT3(ITD); KIT(D816V)
194	FLT3(ITD); MLL-AF9
499	FLT3(ITD); NPM1(type A mutation)
10KM1950	FLT3(ITD); DNMT3A(R882H); NPM1(type A mutation)
9KM3949	FLT3(ITD); DNMT3A(R882H); NPM1(type A mutation)

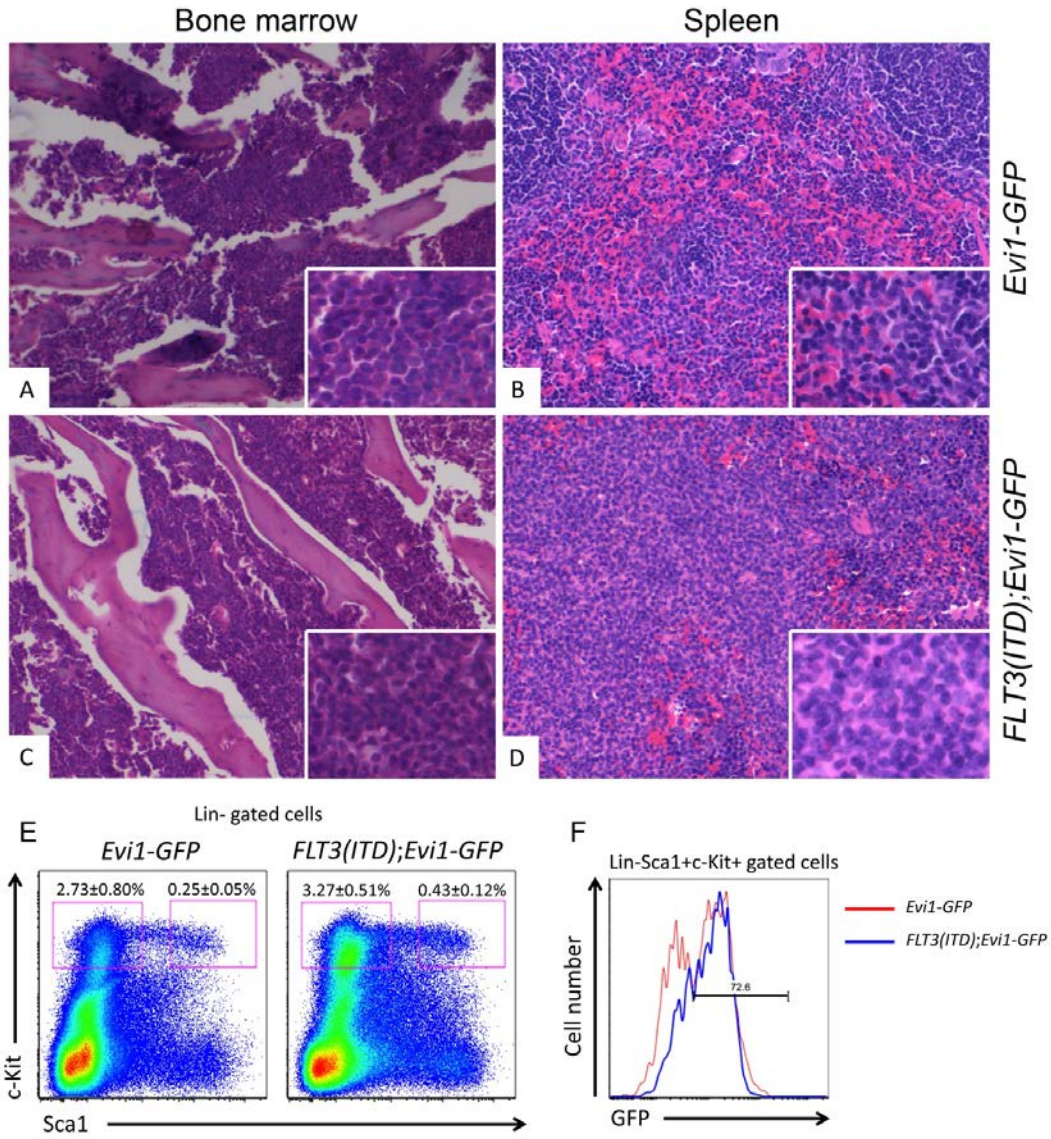
Supplementary Table 2. Toxicity of AC220+BMN673 in hematopoietic organs of C57Bl/6 mice.

Parameter		Control (+1 day)	Treated (+1 day)	Treated (+14 days)
Peripheral blood	WBC $x10^3/\mu L$	5.684±0.735	5.06±1.37	6.26±0.619
	NE $x10^3/\mu L$	0.932±0.079	0.936±0.384	1.275±0.249
	LY $x10^3/\mu L$	4.31±0.729	3.8±0.878	4.08±0.684
	MO $x10^3/\mu L$	0.212±0.045	0.170±0.077	0.196±0.06
	EO $x10^3/\mu L$	0.193±0.013	0.086±0.063	0.08±0.163
	BA $x10^3/\mu L$	0.055±0.021	0.02±0.012	0.05±0.06
	RBC $x10^6/\mu L$	8.11±0.908	7.56±1.391	9.402±0.527 *
	HB g/dL	11.14±1.172	11.56±2.371	13.32±0.589 **
	HCT %	38.54±4.517	41.425±3.611	44.96±1.826 *
	MCV fL	47.5±0.579	47.96±0.498	47.84±1.372
	MCH pg	13.74±0.279	14.225±0.222 *	14.2±0.4
	PLT $x10^3/\mu L$	933.75±103.106	951.0±119.0	887.8±146.633
Marrow	White cells/femur ($x10^6$)	15.27±1.95	8.723±1.084 *	13.4±2.241
	Lin^-cKit^+ ($x10^6$)	0.378±0.057	0.798±0.196 *	0.376±0.074
	Lin^-Sca1^+ ($x10^6$)	0.184±0.019	0.209±0.045	0.145±0.033
	$Lin^-cKit^+Sca1^+$ ($x10^6$)	0.02±0.002	0.049±0.01 **	0.028±0.005
Spleen weight (g)		0.067±0.013	0.065±0.011	0.083±0.007
Body weight (g)		20.3±1.639	19.28±1.366	21.34±0.994

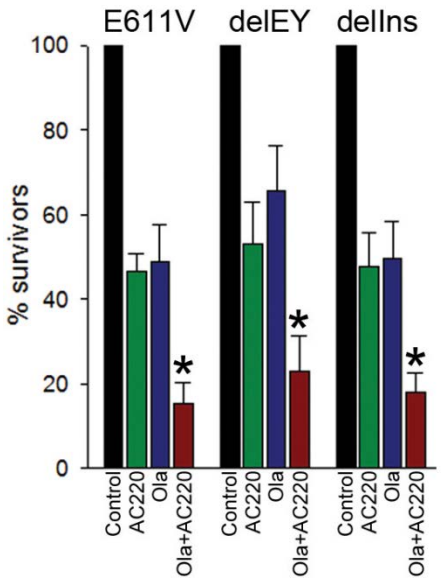
Hematological parameters of mice (n=5 per group) examined one day after the end of treatment with vehicle [Control (+1 day)] and the combination of AC220+BMN673 [Treated (+1 day)], or 2 weeks after the end of treatment [Treated (+14 days)]; treatment regimen is described in Figure 4. Blood was collected into heparinized syringes by cardiac puncture. Peripheral blood parameters (WBC = white blood cells, NE = neutrophils, LY = lymphocytes, MO = monocytes, EO = eosinophils, BA = basophils, RBC = red blood cells, HB = hemoglobin, HCT = hematocrit, MCV = mean red blood cell volume, MCH = mean corpuscular hemoglobin, PLT = platelets) were tested using Hemavet 950FS (Drew). Lin-cKit+, Lin-Sca1+, and Lin-cKit+Sca1+ cells in bone marrow were counted by flow cytometry as described in Materials and Methods. *$p<0.05$ and **$p<0.01$ in comparison to Control.



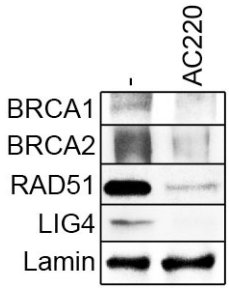
Supplemental Figure S1. cBioportal OncoPrint of the DSB repair genes showing mutations and gene expression changes in AML patients in the TCGA data set in the context of mutations associated with AML.



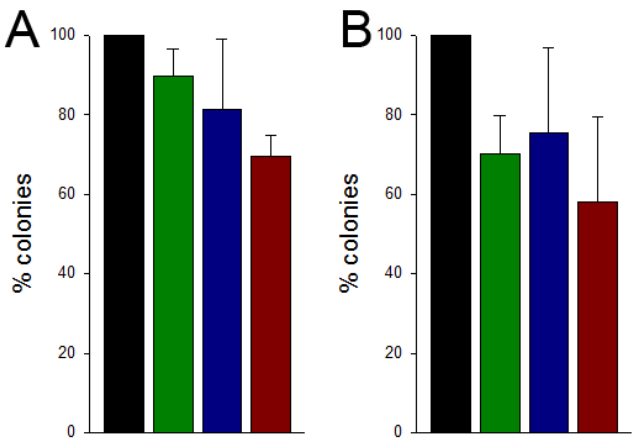
Supplemental Figure S2. FLT3(ITD);Evi1-GFP mice develop myeloid leukemia. (**A-D**) Bone marrows from femurs and spleens were analyzed by microscopy (n=3). The harvested tissues were fixed in formalin, and embedded in paraffin as described before [1]. Sections of the tissue blocks were deposited on glass slides, stained with H&E and evaluated microscopically. The representative images (20x magnification, insets: 40x magnification) were captured with a camera attached to the microscope. (**E, F**) Expansion of $Lin^{-}Sca1^{+}cKit^{+}$ (LSK) compartment (containing long-term and short-term HSC, as well as multipotent progenitors cells) and $Lin^{-}Sca1^{-}cKit^{+}$ (LK) myeloid progenitor ($Lin^{-}Sca1^{-}ckit^{+}$) populations in bone marrow of *FLT3(ITD);Evi1-GFP* mice over *Evi1-GFP* mice. (**E**) Representative flow cytometry data showing HSC-containing LSK fraction (right purple gates) and LK fraction (left purple gates) for control (*Evi1-GFP*) and *FLT3-ITD;Evi1-GFP* mouse. Numbers indicate percent cells within total bone marrow cells (mean± SEM). Shown is 1 representative of 3 independent experiments. (**F**) Representative flow cytometry data showing GFP+ population for control (*Evi1-GFP*) and *FLT3-ITD;Evi1-GFP* mouse. One representative of 3 independent experiments is shown.



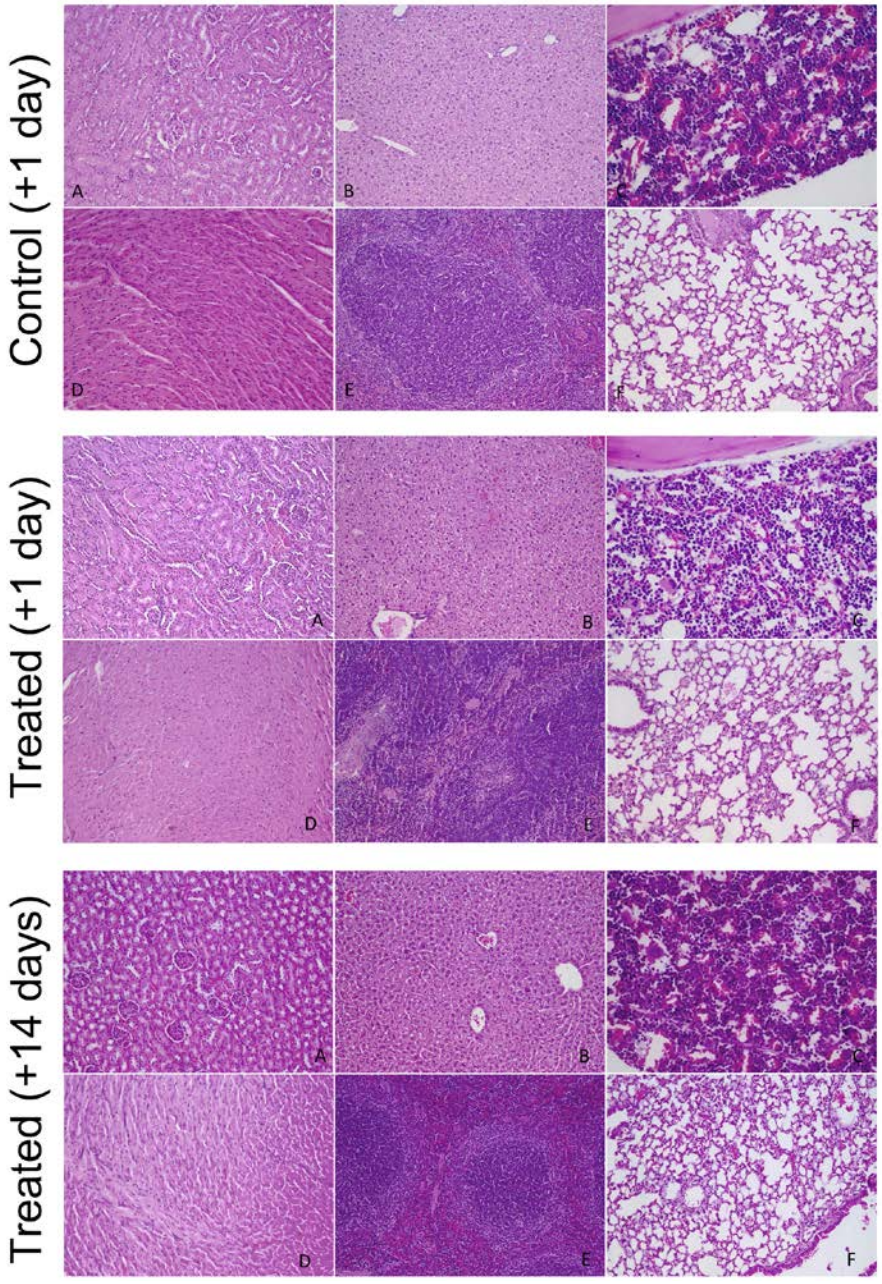
Supplemental Figure S3. FLT3i AC220 enhanced anti-leukemia effect of PARP1i in FLT3(mut)-positive cell lines. BaF3 cells expressing indicated mutants were left untreated (Control) or were incubated with PARP1i olaparib (Ola, 1.25 μM), FLT3i AC220 (10 nM) and AC220+Ola for 96 hrs in the presence of IL-3. Results from 3 experiments are represented by the mean % ± SD of living cells detected by trypan blue exclusion in comparison to untreated counterparts; *p<0.001 in comparison to individual treatment using two-tailed Student t test.



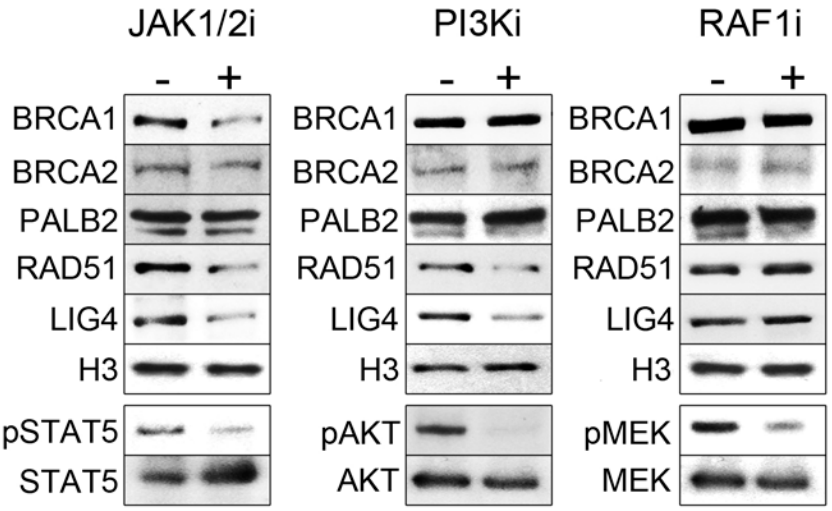
Supplemental Figure S4. FLT3i AC220 downregulated HR and D-NHEJ proteins in primary AML cells. Western analysis of the indicated nuclear proteins in $Lin^{-}CD34^{+}$ AML (patient 10KM1950) cells after 24 hrs incubation with 10 nM AC220 or vehicle (-) in the presence of growth factors.



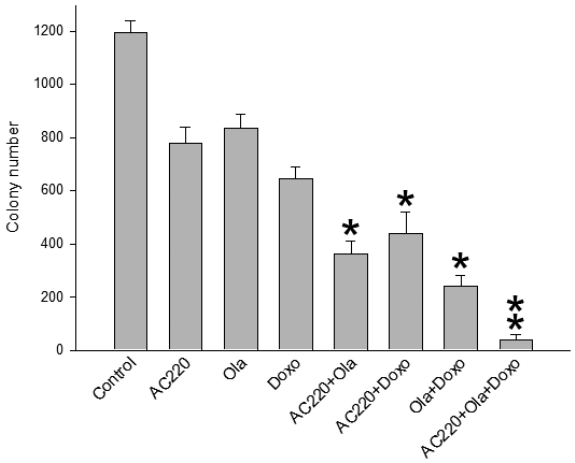
Supplemental Figure S5. AC220 did not enhance the effect of olaparib against primary hematopoietic cells from healthy donors. (**A**) Lin^-CD34^+ cells from 3 healthy donors were left untreated (black bar) or treated with FLT3 inhibitor AC220 (10 nM, green bar), PARP1i olaparib (Ola, 2.5 μM, blue bar) and the combination of AC220 and olaparib (red bar) in IMDM supplemented with 10% FBS and growth factors for 3 days followed by plating in methylcellulose. (**B**) Lin^-CD34^+ cells from 3 healthy donors were left untreated (black bar) or treated with FLT3 inhibitor AC220 (535 nM, green bar), PARP1i olaparib (Ola, 6 μM, blue bar) and the combination of AC220 and olaparib (red bar) in 100% FBS supplemented with growth factors for 7 days followed by plating in methylcellulose. Results represent mean % ± SD of colonies in comparison to untreated samples.



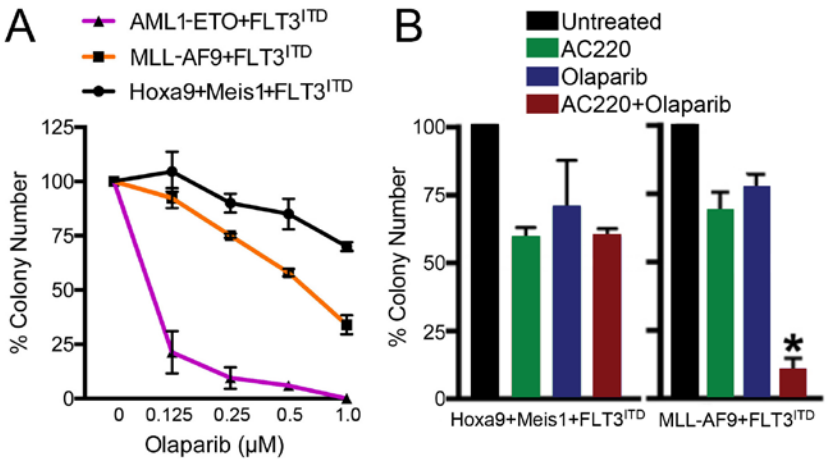
Supplemental Figure S6. AC220 + BMN673 treatment does not cause any major cellular and organ toxicity. (**A**) Kidney, (**B**) liver, (**C**) tibia bone marrow, (**D**) heart, (**E**) spleen and (**F**) lung were examined one day after the end of treatment with vehicle [Control (+1 day)] and the combination of AC220+BMN673 [Treated (+1 day)], or 2 weeks after the end of treatment [Treated (+14 days)]; treatment regimen is described in Figure 4. The harvested tissues were fixed in formalin, and embedded in paraffin as described before [1]. Sections of the tissue blocks were deposited on glass slides, stained with H&E and evaluated microscopically. The representative images (20x magnification) were captured with a camera attached to the microscope.



Supplemental Figure S7. The role of JAK2, PI3K and RAF1 kinases in regulation of expression of DSB repair proteins in FLT3(ITD)-positive cells. Western analysis of DSB repair proteins BRCA1, BRCA2, PALB2 RAD51 and LIG4 in nuclear cell lysates from FLT3(ITD)-positive BaF3 cells after 24 hrs incubation in the presence of IL-3 with 400 nM JAK1/2 inhibitor ruxolitinib (Selleckchem), 5 μM PI3K inhibitor buparlisib (Selleckchem), 1 μM RAF1 inhibitor LY3009120 (Selleckchem), or vehicle. Histone 3 (H3) served as loading control. Phosphorylated proteins (pSTAT5, pAKT and pMEK) and corresponding total proteins (STAT5, AKT, MEK) were detected to confirm the inhibition of specific pathways by the inhibitors. The following primary antibodies were used: anti-BRCA1 (R&D #MAB22101), anti-BRCA2 (Abcam #ab75335), anti-RAD51 (Abcam #ab88572), anti-PALB2 (Thermo Fisher PA5-20796), anti-DNA Ligase 4 (Thermo Fisher PA5-40826), anti-Histone H3 (Thermo Fisher #AHO 1432), anti-pSTAT5 (Tyr694) (Cell Signaling #9359), anti-STAT5 (Santa Cruz #sc-28685), anti-pAKT (Ser473) (Cell Signaling #4060), anti-AKT (Cell Signaling #4691), anti-pMEK1/2 (Ser217/221) (Cell Signaling #9121) and anti-MEK1/2 (Cell Signaling #9122). The secondary were: goat anti-mouse IgG-HRP (Millipore #AP181P) and goat anti-rabbit IgG-HRP (Millipore #12-348).



Supplemental Figure S8. AC220 enhanced the sensitivity of FLT3(ITD)-positive AML primary cells to olaparib combined with doxorubicin. Lin-CD34+ AML primary cells from FLT3(ITD)-positive AML patient #125 were incubated with vehicle (Control), AC220 (A, 10 nM), olaparib (O, 2.5 μM)), doxorubicin (D, 2.5 nM) and the combinations for 72 hrs in the presence of growth factors following the plating in methylcellulose. Colonies were counted after 7 days. Results represent mean number of colonies from triplicate experiment ± SD; *p<0.02 in comparison to individual drug treatment; **p≤0.001 in comparison to combinations of two drugs using Student t test.



Supplemental Figure S9. Additional mutations regulate the sensitivity of FLT3(ITD)-positive murine bone marrow cells to AC220 +/- olaparib. mBMCs expressing the indicated genes were described before [2]. (**A**) Clonogenic activity of mBMCs expressing the indicated genes and treated with olaparib for 96 hrs in the presence of growth factors [100 ng/ml SCF; 10 ng/ml Flt3 ligand; 20 ng/ml IL-3, IL-6, G-CSF and GM-CSF] followed by plating in methylcellulose [2]. Colonies were counted after 7-10 days. Results represent mean percentage ± SD of colonies compared to untreated counterparts from 3 experiments. (**B**) Cells expressing the indicated genes were untreated or treated with 0.125μM olaparib, 20 nM AC220 and olaparib + AC220 for 96 hrs in the presence of growth factors [100 ng/ml SCF; 20 ng/ml IL-3 and IL-6] followed by plating in methylcellulose. Colonies were counted after 7 days. Results represent mean percentage ± SD of surviving colonies compared to untreated counterparts from 3 experiments. *p<0.001 in comparison to individually treated MLL-AF9+FLT3(ITD) cells and *p≤0.002 when compared to HOXA9+MEIS1+FLT3(ITD) cells treated with olaparib + AC220.

References

1. Nieborowska-Skorska M, Maifrede S, Dasgupta Y, et al. Ruxolitinib-induced defects in DNA repair cause sensitivity to PARP inhibitors in myeloproliferative neoplasms. Blood. 2017;130:2848-2859.

2. Maifrede S, Martinez E, Nieborowska-Skorska M, et al. MLL-AF9 leukemias are sensitive to PARP1 inhibitors combined with cytotoxic drugs. Blood Adv. 2017;1:1467-1472.