Supplementary figure legends

Supplementary Figure 1. Response of *Tet2*$^{-/-}$ *Flt3*^{ITD} leukemia model to 5-Aza therapy
Tet2$^{-/-}$*Flt3*^{ITD} leukemia model treated with vehicle or 5-Aza (n=5), **A,** gating for peripheral blood Mac1 and cKit compartments and **B,** response in bone marrow Myeloid progenitors (gated on lin^{-}Sca1^{-}cKit^{+}) response, numbers indicate mean ± sem MEP (CD34^{-}CD16/32^{-}) percentage. **C-E,** Tet2$^{-/-}$Flt3^{ITD} leukemia model treated with 5-Aza (n=4), followed by eRRBS analysis of LSK cells. Differentially methylated cytosines (DMCs) vs Vehicle are separated into (**C**) overall chromosomal hyper- and hypomethylation, (**D**) promoter, exon, intron, and intergenic regions and (**E**) CpG island, shores, shelves, and other regions. **p<.01 by t-test.

Supplementary Figure 2. *Idh2*^{R140Q}*Flt3*^{ITD} leukemia model phenotype
A, Genotyping strategy for *Idh2*^{R140Q} mice. Lox-stop-lox (LSL), excised, and wild-type alleles and associated primers. **B-D**, *Idh2*^{R140Q}*Flt3*^{ITD} leukemia model (**B**) serum 2-hydroxyglutarate (2HG) levels (n=3) and (**C**) hematocrit (HCT) and platelet (plts) counts (n=6-9). Graphs of mean ± sem, and (**D**) flow cytometry analysis of stem-progenitors. **E,** GSEA analysis of genes with differential methylation in the *Idh2*^{R140Q}*Flt3*^{ITD} leukemia model compared to a human *IDH2* mutant AML methylation signature. **F-G,** *Idh2*^{R140Q}*Flt3*^{ITD} BM cells (**F**) re-plating colony forming unity (CFU) assay with IDH2 inhibitor AG-187 and vehicle DMSO and (**G**) relative 2HG levels from first plating CFU cells. **H,** CFU assay with *Tet2*$^{-/-}$*Flt3*^{ITD} leukemic cells serial re-plating following treatment with IDH2 inhibitor. **p<.01 by t-test.

Supplementary Figure 3. *Idh2*^{R140Q}*Flt3*^{ITD} leukemia model treatment response to AG-221
Idh2^{R140Q}*Flt3*^{ITD} leukemia model treated with vehicle or AG-221 (n=6). Response of **A,** hematocrit and platelets, **B,** bone marrow (BM) CD71^{+}Ter119^{+} erythroid progenitors. Graphs of mean ± sem. **C,** Flow cytometry analysis of Mac1 and cKit in peripheral blood. **D,** Flow cytometry BM Myeloid progenitors and LSK (lin^{-}Sca1^{+}cKit^{+}) cells. **E-F,** Graph and gating of BM (**E**) Myeloid progenitors- Common myeloid progenitor (CMP, CD16/32$^{low/-}$CD34^{+}), Granulocyte-macrophage progenitor (GMP, CD16/32^{+}CD34^{+}), and Megakaryocyte-erythroid progenitors (MEP, CD16/32^{-}CD34^{-}) as fraction of lin^{-}Sca1^{-}cKit^{+} with statistical comparison of GMP fractions, and (**F**) Multipotent Progenitors (MPP, CD48^{+}, CD150^{-}) as percent of total bone marrow. Graphs of mean ± sem. ns- not significant, ** p<.01 by t-test.

Supplementary Figure 4. Genomic methylation analysis of *Idh2*^{R140Q} *Flt3*^{ITD} leukemia model response to AG-221 therapy
Idh2^{R140Q} *Flt3*^{ITD} leukemia model treated with vehicle or AG-221 (n=4) followed by ERRBS analysis of LSK (lin^{-}Sca1^{+}cKit^{+}) cells. **A-C,** Differentially methylated cytosines (DMCs) are separated into (**A**) overall chromosomal hyper- and hypomethylation, (**B**) promoter, exon, intron, and intergenic regions, and (**C**) CpG island, shores, shelves, and other regions.

Supplementary Figure 5. GSEA analysis of 5-Aza and AG-221 response
A, GSEA analysis of differentially expressed genes from LSK cells from *Tet2*$^{-/-}$*Flt3*^{ITD} and *Idh2*^{R140Q}*Flt3*^{ITD} leukemia models treated with 5-Aza and AG-221, respectively, compared to WT LSKs. GSEA comparison with gene sets at MSigdb:
JAATINEN_HEMATOPOIETIC_STEM_CELL_DN
HELLER_SILENCED_BY_METHYLATION_UP
TAKEDA_TARGETS_OF_NUP98_HOXA9_FUSION_10D_DN
B, Relative frequency of floxed *Tet2* exon in *Tet2*$^{-/-}$ *Flt3*^{ITD} Granulocyte-macrophage progenitors (GMPs) from mice treated with vehicle (n=3) or 5-Aza (n=4), and in *Tet2*$^{+/+}$, *Tet2*$^{+/-}$, *Tet2*$^{-/-}$ controls. **C,** Relative frequency of floxed LSL cassette in GMPs from *Idh2*^{R140Q}*Flt3*^{ITD} mice

treated with vehicle or AG-221 (n=3) and *Idh2*^{wt} and *Idh2*^{R140Q} controls. **D,** Frequency of multipotent progenitor (MPP) population in *Tet2*$^{-/-}$ *Flt3*^{ITD} mice treated with 5-Aza in total and CD45.1^{-} gated BM. And frequency of granulocyte-macrophage progenitor (GMP) population in *Idh2*^{R140Q} *Flt3*^{ITD} mice treated with AG-221 in total and CD45.1^{-} gated BM. Numbers of mean ± sem. **E,** Frequency of wild-type *Flt* expression compared to total *Flt3* expression in *Flt3*$^{+/+}$ (WT), *Flt3*$^{ITD/+}$, *Flt3*$^{ITD/ITD}$ controls and GMPs from *Tet2*$^{-/-}$*Flt3*^{ITD} (TF) and *Idh2*^{R140Q}*Flt3*^{ITD} (IF) mice treated with vehicle, 5-Aza, or AG-221 (n=3-4). ns, not significant by t-test.

Supplementary Figure 6. Response of *Tet2*$^{-/-}$*Flt3*^{ITD} leukemia model to 5-Aza and AC220 therapy

Tet2$^{-/-}$*Flt3*^{ITD} leukemia model treated with single and combination AC220 and 5-Aza (n=5). Response in bone marrow **A,** lineage^{-} (Lin) fraction, **B,** Mac1 Gr1 populations, and **C,** estimated blast population (CD45.2^{+}SSC^{low}). Graphs of mean ± sem. *p<.05, ** p<.01 by t-test.

Supplementary Figure 7. *Idh2*^{R140Q}*Flt3*^{ITD} and *Tet2*$^{-/-}$*Flt3*^{ITD} leukemia model response to combination therapy

A-H, *Idh2*^{R140Q}*Flt3*^{ITD} leukemia model treated with mono- or combination therapy (n=5-6). Response in (**A**) estimated bone marrow (BM) blast population (CD45.2^{+}SSC^{low}), (**B**) hematocrit (HCT) and platelets (plts), (**C**) peripheral blood (PB) Mac1 and cKit populations, (**D**) BM CD71^{+}Ter119^{+} erythroid progenitors, (**E**) BM long-term hematopoietic stem cell (LT-HSC, CD150^{+}CD48^{-} LSK), (**F**) BM CD45.1^{-} multipotent progenitor (MPP, CD150^{-}CD48^{+} LSK), (**G**) spleen Mac Gr1 immunophenotype, numbers indicate mean ± sem Mac1^{+}Gr1^{-}, and (**H**) BM Myeloid progenitors (gated on lin^{-}Sca1^{-}cKit^{+}) Common myeloid progenitor (CMP), Granulocyte-macrophage progenitor (GMP), and Megakaryocyte-erythroid progenitors (MEP). **I-J**, *Tet2*$^{-/-}$ *Flt3*^{ITD} leukemia model treated with mono- or combination therapy (n=5) and response in (**I**) long-term stem cell population (LT-HSC), and (**J**) CD45.1^{-} multipotent progenitor (MPP) population. Graphs of mean ± sem. *p<.05, ** p<.01 by t-test.

Supplementary Figure 8. Methylation and expression response of *Tet2*$^{-/-}$*Flt3*^{ITD} and *Idh2*^{R140Q}*Flt3*^{ITD} leukemia model to combination therapy

A-B, *Tet2*$^{-/-}$ *Flt3*^{ITD} leukemia model treated with single and combination AC220 and 5-Aza (n=3). (**A**) Heat map of coding region methylation for genes demonstrating combination 5-Aza and AC220 treatment effect and (**B**) *Gata2* locus CpG methylation following treatment. **C,** *Gata2* mRNA expression by RNA-seq in *Idh2*^{R140Q}*Flt3*^{ITD} leukemia following treatment (n=3-4) and **D,** *Gata2* mRNA expression by RNA-seq in *Tet2*$^{-/-}$*Flt3*^{ITD} leukemia following treatment (n=3-4). TPM, transcripts per million. **E,** Graph of log fold change in gene expression compared to wild-type in hypo- and hyper-methylated gene sets. Sets determined by annotated DMRs (differentially methylated regions) to nearest genes then divided into hypo- and hypermethylated groups. Sets statistically compared by Wilcoxon rank-sum test. **F,** GSEA of hypermethylated genes from combination treatment compared to signatures determined from genes decreased in RNA expression compared to wild-type.

Supplementary Figure 9. Response of *Tet2*$^{-/-}$ *Flt3*^{ITD} and *Idh2*^{R140Q}*Flt3*^{ITD} leukemia model to combination therapy

Tet2$^{-/-}$*Flt3*^{ITD} leukemia model treated with single and combination AC220 and 5-Aza (n=5). *Idh2*^{R140Q} *Flt3*^{ITD} leukemia model treated with single and combination AC220 and AG-221 (n=5-6). **A,** Gating for CD45.1 (wild type) and CD45.2 (leukemic) fractions in the bone marrow. **B-C,** Response and gating for spleen CD45.1^{+} (wild type) and CD45.2^{+} (leukemic) fractions in (**B**) *Tet2*$^{-/-}$ *Flt3*^{ITD} and (**C**) *Idh2*^{R140Q}*Flt3*^{ITD} leukemia model response to monotherapy and combination. **D,** Relative frequency of floxed *Tet2* exon in *Tet2*$^{-/-}$ *Flt3*^{ITD} mice treated with vehicle or combination therapy (n=3), and in *Tet2*$^{+/+}$, *Tet2*$^{+/-}$, *Tet2*$^{-/-}$ controls. **E,** Relative frequency of floxed

LSL cassette in *Idh2*^{R140Q}*Flt3*^{ITD} mice treated with vehicle, AG-221, AC220, and combined therapy (n=3) and in *Idh2*^{wt} and *Idh2*^{R140Q} controls. Graphs of mean ± sem. ns, not significant, * p<.05 by t-test.

Supplemental Methods

In vitro colony forming assays

Bone marrow cells (whole or sorted populations) were seeded into cytokine supplemented methylcellulose medium (M3434, Stem Cell Technologies). Colonies propagated in culture were scored at day 7 to 10. Cells were re-suspended and re-plated at 15,000 cells per well. Drug or vehicle (DMSO) was added at beginning of experiment.

Mice

To generate *Idh2* mutant mice, an 8.82 kb genomic DNA used to construct the targeting vector was first subcloned from a positively identified B6 BAC clone (RP23: 328H9). The mutation was introduced by PCR using the primer Idh2RQ. The floxed Neo stop cassette was introduced in intron 3-4 before the mutant codon. The targeting construct was electroporated into BAC-BA1 (C57BL/6 × 129/SvEv) hybrid embryonic stem cells. After selection, positive clones were injected into blastocycts. The mutation in was confirmed using Sanger sequencing.

Idh2RQ primer: AGCCCTAACGGAACGATCCAGAACATCCTTGGGGGAACCGTC

Idh2^{R140Q} mice were genotyped with the following primers:

IDH2-R	AACCATGCTGGGTTGTCAAT
IDH2-LSL	AAGCAATAGCATCACAAATTTCAC
IDH2-F	CACACTGGGGGATCAGTTTT

WT (F & R): ~260bp
LSL (LSL & R): ~340bp
LSL Excised (F & R): ~410bp

RT and Q-PCR analysis

RNA was isolated using the Qiagen RNeasy isolation kit. cDNA was synthesized using the Verso cDNA Synthesis kit. Genomic DNA was isolated using the QIAmp DNA mini kit. SYBR

Green Real-Time PCR master mixes were used according to manufacturer's instructions and run on an Applied Biosystems Real-Time PCR instrument. Allele levels were normalized to an internal *Fos* or *Tet2* control. Primers used -- *Tet2* exon excision: TCA GAA GCA CGC TGC CTT AA, ACG GCT TGG AGA GGA GAT CT; *Idh2* LSL excision: CCA CTC CCA CTG TCC TTT CC, CCG CCT CAG AAG CCA TAG AG; *Fos* internal control: GGC TGG CCC TGT ATT CCT GAT, TCT TCT GAC CCT TCC CTA CTG AGC; *Flt3* WT specific: ACT GGC CCC CTG GAT AAC, GCC ACC TGA ATT GAG ACT CC; *Flt3* common: CCC ACA GGG AAA GCT GTA AA, ATA ACT GGG GCA GTG TGC TC.

Antibodies for FACS and Flow cytometry

Antibodies used were as follows: (anti-mouse) Gr1 (Ly6G), B220 (RA3-682), CD34 (RAM34), FCγR (93), Sca-1 (D7), Mac-1 (CD11b) (M1/70), NK1.1 (PK136), Ter119 (Ter119,553673), CD3 (145-2C11), CD45.1 (A20), CD45.2 (104),CD150 (TC-15-12F2.2), CD48 (HM48-1), CD45.1 (A20), CD45.2 (104) from Biolegend and eBiosciences. The 'lineage cocktail' included CD3, Gr-1, Mac-1 (CD11b), NK1.1, B220, and Terr-119. For LSK isolation for RNA-seq and methylation studies, CD45.1 was added to the 'lineage cocktail' to eliminate wild-type derived cells in the isolation step.