Supplemental Figure Legends

Supplemental Figure 1. Chemical structures of the molecules utilized in these studies. A. (S)-JQ1 (active enantiomer). **B**. (R)-JQ1 (inactive enantiomer). **C**. I-BET151. **D**. panobinostat.

Supplemental Figure 2. Treatment with R-JQ1 (inactive enantiomer) does not alter the cell cycle status or induce lethal effects against cultured AML cells. A. OCI-AML3 and MOLM13 cells were treated with the indicated concentrations of the inactive enantiomer of JQ1 (R-JQ1) for 48 hours. At the end of treatment, cells were stained with annexin V and TO-PRO-3 iodide and the percentages of apoptotic cells were determined by flow cytometry. Columns, mean of three experiments; Bars, standard error of the mean. **B-C.** OCI-AML3 and MOLM13 cells were treated with the indicated concentrations of (R)-JQ1 for 24 hours. Following this, the cells were fixed and stained with propidium iodide. Cell cycle status was determined by flow cytometry. Columns, mean of three independent experiments, Bars, standard error of the mean.

Supplemental Figure 3. Treatment with JQ1 reduces BRD4 and POL II occupancy on the promoters of c-MYC, BCL2, and CDK6 in cultured human AML cells. A-B. OCI-AML2 and MOLM13 cells were treated with the indicated concentration of JQ1 for 16 hours. Following this, chromatin immunoprecipitation was conducted with BRD4-specific antibody. The ChIP'ed DNA was subjected to real time PCR with primers for the promoter of c-MYC, BCL2, and CDK6. The fold enrichment was calculated using the Ct value of the ChIP DNA compared to the CT value of the input DNA. **C.** OCI-AML2 cells were treated with the indicated concentration of JQ1 for 16 hours. Following this, chromatin immunoprecipitation was conducted with RNA POL II antibody. The ChIP'ed DNA was subjected to quantitative real time PCR with primers against the promoter of c-MYC, BCL2 and CDK6. The fold enrichment was calculated using the Ct value of the ChIP DNA compared to the Ct value of the input DNA.

Supplemental Figure 4. Treatment with JQ1 depletes the mRNA expression of CDK6, with concomitant induction of p21 in AML cells. **A-B**. OCI-AML3, MOLM13 and primary AML cells were treated with the indicated concentrations of JQ1 for 16 hours. At the end of treatment, total RNA were isolated and reverse transcribed. The resulting cDNA was used for quantitative PCR analysis of CDK6. The relative mRNA expression was normalized to GAPDH. **C**. OCI-AML3 and MOLM13 cells were treated with the indicated concentrations of JQ1 for 16 hours. At the end of treatment, RNA was isolated and reverse transcribed. The resulting cDNA was used for real-time, quantitative PCR analysis of p21. The relative mRNA expression was normalized to GAPDH.

Supplemental Figure 5. Treatment with JQ1 depletes the mRNA expression of CDK6, c-MYC, and BCL2, with concomitant induction of p21 in FLT3-ITD expressing AML MV4-11 cells. A-B. MV4-11 cells were treated with the indicated concentrations of JQ1 for 16 hours. At the end of treatment, total RNA was isolated and reverse transcribed. The resulting cDNA was used for real-time, quantitative PCR analysis of CDK6, c-MYC, BCL2, and p21. The relative mRNA expression was normalized to GAPDH. **C**. MV4-11 cells were treated with the indicated concentrations of JQ1 for 24 hours. Following this, total cell lysates were prepared and immunoblot analyses were conducted for the expression levels of BRD4, c-MYC, NPM1, p21, p27, BIM, and β-actin in the lysates.

Supplemental Figure 6. Co-treatment with BET protein antagonists, JQ1 or IBET151, and pan-HDAC inhibitor panobinostat synergistically induces apoptosis of AML cells. A. OCI-AML3 and MOLM13 cells were treated with JQ1 and PS for 48 hours. Then, the percentage of annexin V-positive, apoptotic cells were determined by flow cytometry. Median dose effect and isobologram analyses were performed utilizing CalcuSyn, assuming mutual exclusivity. Combination index (CI) values less than 1.0 indicate a synergistic interaction of the two agents in the combination. **B-C.** HL-60 and MV4-11 cells were treated with JQ1 and PS for 48 hours. Then, the percentages of annexin V-positive, apoptotic cells were determined by flow cytometry. Median dose effect and isobologram analyses were conducted utilizing CalcuSyn assuming mutual exclusivity. Combination index (CI) values less than 1.0 indicate a synergistic interaction of the two agents in the combination. **D**. OCI-AML3 and MOLM13 cells were treated with I-BET151 and PS for 48 hours. Then, the percentage of annexin V-positive, apoptotic cells were determined by flow cytometry. Median dose effect and isobologram analyses were performed utilizing CalcuSyn, assuming mutual exclusivity. Combination index (CI) values less than 1.0 indicate a synergistic interaction of the two agents in the combination. **E**. HL-60 and OCI-AML2 cells were treated with JQ1 and/or PS for 48 hours. At the end of treatment, cells were washed free of the drug, plated in methylcellulose and cultured for 7-10 days. Colonies were counted and the % colony growth for each cell line is reported as a percentage of the untreated cells. Columns, mean of three experiments; Bars, standard error of the mean. **F**. MV4-11 cells were treated with the indicated concentrations of JQ1 and/or PS for 24 hours. Following this, cells were harvested and lysates were prepared. Immunoblot analyses were conducted for the expression levels of BRD4, pSer2 Pol II, c-MYC, p21, BIM and β-actin in the cell lysates.

Supplemental Table 1: Clinical presentation and mutation status of the primary AML samples

Note: The NPM1 status was determined by quantitative PCR utilizing primers for exon 12 of the NPM1 cDNA. The setup for this PCR utilizes a common forward primer and two separate

reverse primers, one that anneals and amplifies wild type NPM1 cDNA and the other which anneals to and amplifies the mutant NPM1 cDNA. The positive control for this qPCR is cDNA amplified from OCI-AML3, an AML cell line known to express mutant NPM1. The negative control is cDNA from HL-60 cells which only express wild type NPM1. For detection of FLT-ITD, total RNA was isolated and converted to cDNA. Exon 14 of FLT3, the location in which internal tandem duplications are known to occur, was amplified by PCR utilizing primers designed to specifically amplify exon 14 of FLT3. Amplified PCR products were resolved on a 2% agarose gel and documented with a UV transilluminator. Primary AML cells exhibiting amplicons that migrate at greater than 366 base pairs (the size of the wild type FLT3 exon 14) are considered to be positive for FLT3-ITD.

Supplemental Table 2: Fold expression change of the 40 most up (I.) and downregulated (II.) mRNAs in OCI-AML3 cells following treatment with 500 nM of JQ1 for 8 hours.

Supplemental Table 3: Ingenuity Pathway Analysis of the 5 most perturbed networks in OCI-AML3 following treatment with 500 nM of JQ1 for 8 hours.

Note: Ingenuity Pathway Analysis (IPA) was performed utilizing gene expression changes from 80 mRNA targets (40 most up regulated and 40 most down regulated mRNAs) identified by microarray expression analysis. A Score was assigned by IPA for each network. The score assigned by IPA for the associated network functions (i.e. a score of 36) indicates that there is a 1 in 10^{36} chance that the focus genes in the dataset are together in a network due to random chance alone.