**Supplemental Figure Legends**

**Supplemental Figure 1. Chemical structures of the agents utilized in these studies. A**. AC220. **B**. ponatinib. **C**. (S)-JQ1 (active enantiomer). **D**. I-BET151. **E**. panobinostat (PS).

**Supplemental Table 1: Clinical presentation and mutational 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 expresses 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 Figure 2. Treatment with BET protein antagonist or FLT3-TKI induces apoptosis of FLT-ITD expressing AML cells. A**. MOLM13 and MV4-11 cells were treated with the indicated concentrations of JQ1 for 48 hours. Following this, cells were washed with 1X PBS and stained with annexin V-FITC. The % of annexin V-positive apoptotic cells were determined by flow cytometry. Columns, mean of three experiments; Bars, standard error of the mean. **B**. MOLM13 and MV4-11 cells were treated with the indicated concentrations of AC220 for 48 hours. After this, cells were washed with 1X PBS, stained with annexin V-FITC and the % of annexin V-positive, apoptotic cells were determined by flow cytometry. Columns, mean of three experiments; Bars, standard error of the mean. **C-D**. MV4-11 cells were treated with the indicated concentrations of AC220 or ponatinib and/or JQ1 for 24 hours. Following this, cells were harvested and cell lysates were prepared. Immunoblot analyses were conducted for the expression levels of p-FLT3, FLT3 and β-actin in the cell lysates. **E-F**. MV4-11 cells were treated with the indicated concentrations of AC220 or ponatinib and/or JQ1 for 24 hours. At the end of treatment, cells were harvested and cell lysates were prepared. Immunoblot analyses were conducted for the expression levels of cleaved PARP and β-actin in the cell lysates. **G**. MOLM13 cells were treated with the indicated concentrations of AC220 and/or JQ1 for 24 hours. Following this, cells were harvested and cell lysates were prepared. Immunoblot analyses were conducted for the expression levels of c-MYC, p21, p27, BIM, and β-actin in the cell lysates.

**Supplemental Figure 3. Co-treatment with BET protein antagonist JQ1 and FLT3-TK inhibitors synergistically induces apoptosis of FLT3-ITD expressing AML cells. A-D.** MOLM13 and MV4-11 cells were treated with JQ1 and AC220 (**A, B**) or ponatinib (**C, D**) at a constant ratio for 48 hours. The % annexin V-positive, apoptotic cells were determined by flow cytometry. 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.

**Supplemental Figure 4. Co-treatment with BET protein antagonist I-BET151 and FLT3-TK inhibitor synergistically induces apoptosis of FLT3-ITD expressing AML cells. A-B**.MV4-11 and MOLM13cells were treated with JQ1 or I-BET151 and ponatinib at a constant ratio for 48 hours. The % of annexin V-positive, apoptotic cells was 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.

**Supplemental Figure 5. Co-treatment with JQ1 and ponatinib or AC220 exerts synergistic anti-leukemia activity against FLT3-ITD expressing primary AML cells. A.** Primary FLT3-ITD AML cells were treated with JQ1 and ponatinib at a constant ratio for 48 hours. Then, the % PI-positive, non-viable cells was determined by flow cytometry. Median dose effect and isobologram analyses were performed utilizing Calcusyn. Combination index (CI) values less than 1.0 indicate a synergistic interaction of the two agents in the combination. **B-C**. Primary FLT3-ITD AML cells were treated with JQ1 and AC220 at a constant ratio for 48 hours. Then, the % PI-positive, non-viable cells was determined by flow cytometry. Median dose effect and isobologram analyses were performed utilizing Calcusyn. Combination index (CI) values less than 1.0 indicate a synergistic interaction of the two agents in the combination.

**Supplemental Figure 6. Co-treatment with JQ1 and FLT3-TKI does not enhance depletion of pFLT3 but does increase cleaved PARP expression in primary AML cells. A-B**. Primary FLT3-ITD expressing AML cells were treated with the indicated concentrations of AC220 or ponatinib and/or JQ1 for 24 hours. Following this, cells were harvested and cell lysates were prepared. Immunoblot analyses were conducted for the expression levels of p-FLT3, FLT3 and β-actin in the cell lysates. **C**. Primary FLT3-ITD expressing AML cells were treated with the indicated concentrations of ponatinib and/or JQ1 for 24 hours. Then, cells were harvested and cell lysates were prepared. Immunoblot analyses were conducted for the expression levels of cleaved PARP and β-actin in the cell lysates.

**Supplemental Table 2. Comparison of MOLM13 and MOLM-TKIR response to anti-leukemia agents.** Note: MOLM13 and MOLM13-TKIR cells were treated with the indicated compounds for 48 hours. The % of annexin V-positive, apoptotic cells was determined by flow cytometry. IC50 values were calculated with GraphPad 5.0. The table shows the IC50 values for AC220, ponatinib, JQ1, panobinostat (PS) and vorinostat (VS) in MOLM13 and MOLM13-TKIR cells.

**Supplemental Figure 7. Co-treatment with JQ1 and FLT3-TKI is not more effective than treatment with JQ1 alone in MOLM13-TKIR cells**. MOLM13-TKIR cells were treated with the indicated concentrations of JQ1 and AC220 for 48 hours. At the end of treatment, cells were washed with 1X PBS and stained with annexin V and TO-PRO-3 iodide. The % of annexin V-positive, apoptotic cells was determined by flow cytometry. Columns, mean of three experiments; Bars, standard error of the mean.

**Supplemental Figure 8. Knockdown of BRD4 in MOLM13-TKIR cells increases sensitivity to treatment with JQ1. A**. MOLM13-TKIR cells were transduced with lentiviruses expressing non-targeting shRNA or BRD4 shRNA for 72 hours. Following this, cells lysates were prepared and immunoblot analyses were conducted for the expression levels of BRD4, HEXIM1 and β-actin in the cell lysates. **B**. MOLM13 and MOLM13-TKIR cells were transduced with sh-NT or sh-BRD4 for 72 hours. Then, cells were washed with complete media and treated with the indicated concentration of JQ1 for 48 hours. The % of annexin V-positive, apoptotic cells was determined by flow cytometry. Columns, mean of three experiments; Bars, standard error of the mean. \* indicates apoptosis values greater in MOLM13-TKIR cells transduced with shRNA to BRD4 compared to cells transduced with sh-NT (p< 0.05). **C**. MOLM13 and MOLM13-TKIR cells were treated with the indicated concentrations of ABT-199 for 48 hours. At the end of treatment, cells were washed with 1X PBS and stained with annexin V and TO-PRO-3 iodide. The % of annexin V-positive, apoptotic cells was determined by flow cytometry. Columns, mean of three experiments; Bars, standard error of the mean.

**Supplemental Figure 9. Co-treatment with JQ1 and PS synergistically induces apoptosis of MV4-11 cells with resistance to FLT3-TKI**.MV4-11-TKIR cells were treated with JQ1 and PS, as indicated for 48 hours. Following this, cells were washed with 1X PBS and stained with annexin V and TO-PRO-3 iodide. The % of annexin V-positive, apoptotic cells was determined by flow cytometry. Median dose effect and isobologram analysis was performed utilizing Calcusyn. CI values less than 1.0 indicate a synergistic interaction of the two agents in the combination.