Figure 6. Co-treatment with decitabine and panobinostat, or 17-DMAG inhibits clonogenic survival greater than treatment with the single agents alone. **A**. K562 cells were treated with the indicated doses of DAC and/or panobinostat for 48 hours. Following treatment the cells were washed and plated in methocult media for 7 days. (*) represents values significantly less than those following treatment with either agent alone at the indicated concentrations (p=0.04 and p=0.007, respectively). **B-C**. K562 cells were treated with the indicated doses of DAC and/or 17-DMAG for 48 hours. The cells were washed and plated as before. (*) represents values significantly less than those following treatment with either agent alone at the indicated concentrations (p=0.04). **D**. K562 cells were treated with the indicated concentrations of panobinostat and/or 17-DMAG for 48 hours. Following treatment the cells were washed and plated in methocult media for 7 days.

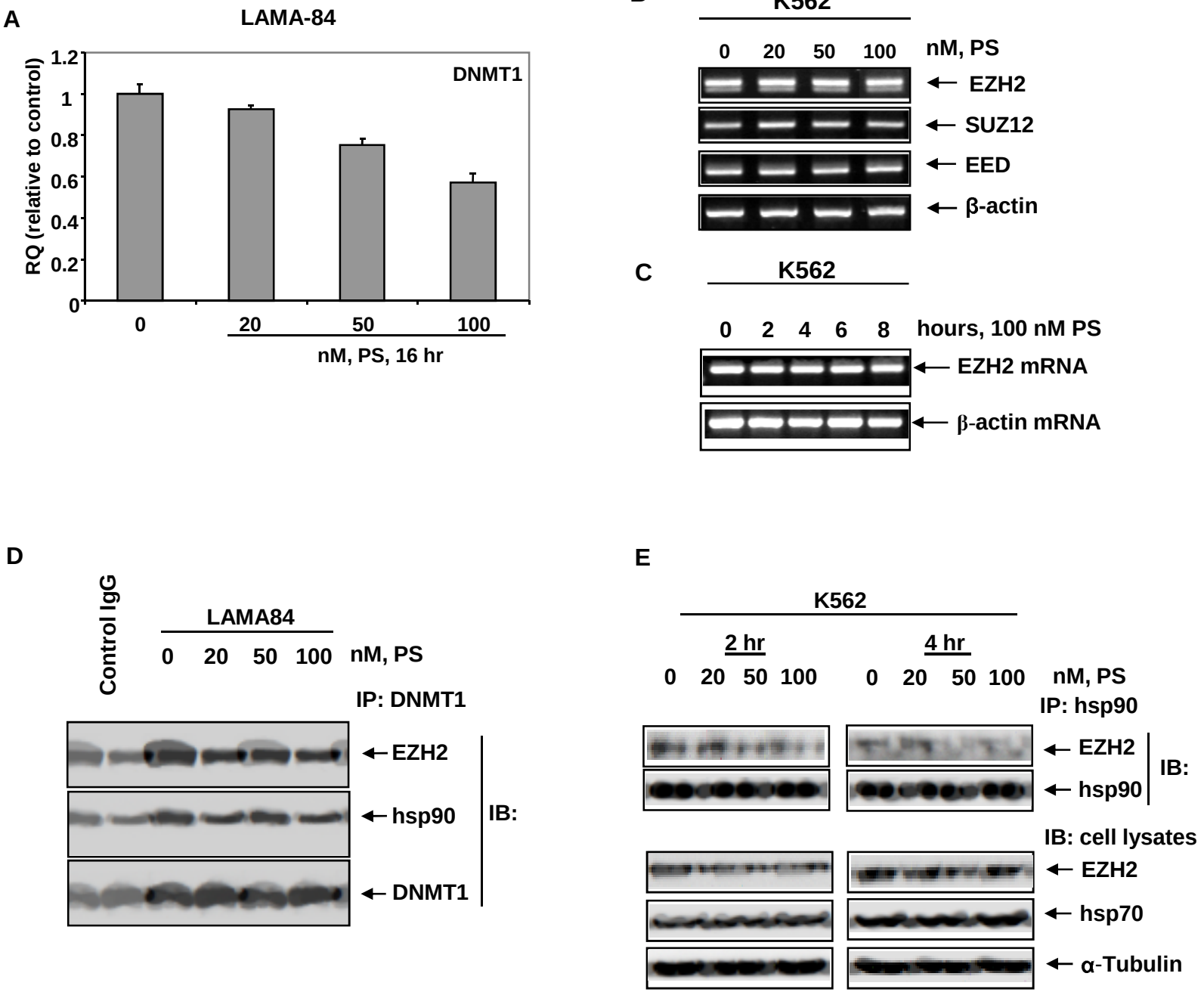
Figure 7: Co-treatment with panobinostat and decitabine exerts superior anti-leukemic activity against primary AML and CML cells than treatment with either agent alone. Peripheral blood or bone marrow from 8 AML, 4 CML-BC and 2 normal CD34+ patients were treated with the indicated doses of 17-DMAG or panobinostat and DAC for 48 hours. Then, the percentages of non-viable cells for each drug alone or drug combination were determined by trypan blue uptake in a hemocytometer. Values represent the percentage of non-viable cells from each condition as compared to the untreated cells.

Supplementa1 Figure 1. Panobinostat depletes the levels of DNMT1 and EZH2 by disrupting its chaperone association with hsp90. **A**. LAMA84 cells were treated with the indicated concentrations of panobinostat (PS) for 16 hours. Total RNA was isolated, reverse transcribed, and qPCR was performed for DNMT1 mRNA. The relative quantity (RQ) of DNMT1 mRNA expression was normalized against expression of GAPDH. **B**. K562 cells were treated with the indicated doses of panobinostat for 16 hours. Total RNA were harvested and RT-PCR was performed for EZH2, SUZ12 and EED. **C**. K562 cells

were treated with 100 nmol/L of panobinostat for the indicated times. Total RNA were harvested and RT-PCR was performed for EZH2 mRNA expression. A β-actin specific reaction served as the internal loading control for the PCR reactions. **D**. LAMA84 cells were treated with the indicated concentrations of panobinostat for 8 hours. Cell lysates were harvested and DNMT1 was immunoprecipitated. Immunoblot analysis was performed for EZH2, hsp90 and DNMT1 on the immunoprecipitates. **E**. K562 cells were treated with the indicated concentrations of panobinostat for 2 and 4 hours. Following this, cell lysates were harvested and hsp90 was immunoprecipitated. Immunoblot analysis was performed for EZH2 and hsp90 in the immunoprecipitates. Western blot analysis was also performed for EZH2 and hsp70 on the total cell lysates. The levels of α-tubulin in the lysates served as the loading control.

Supplemental Figure 2 Depletion of EZH2 by panobinostat is proteasome, but not caspase dependent. **A**. LAMA84 cells were treated with the indicated concentrations of bortezomib (BZ) and/or panobinostat (PS) for 8 hours. Total cell lysates were prepared and immunoblot analysis was performed for DNMT1, EZH2 and c-Raf on the cell lysates. The expression of β-actin in the lysates served as the loading control. **B**. K562 cells were treated with ZVAD and/or panobinostat for 24 hours. Following treatment, immunoblot analysis was performed for EZH2, Caspase 9, Caspase 3 and p21. The levels of α-tubulin in the lysates served as the loading control.

Supplemental Figure 1



Supplemental Figure 2