**SUPPLEMENTAL FIGURE LEGENDS**

**Supplemental Figure 1. AML marrow progression is associated with increased hypoxia.**
Expression of the hypoxia marker pimonidazole (Hypoxyprobe Store, Burlington, MA, USA) was examined in the bone marrow of irradiated SCID mice prior to and following systemic engraftment with luciferase-transfected human AML (HEL-luc) cells. (A) Measurements of total flux (photons/s) and whole animal bioluminescent imaging (BLI) demonstrate increased leukemia growth over time in mice. (B) Bone marrows of mice sacrificed prior to and at intermittent intervals following leukemia inoculation show increased pimonidazole expression over time correlating with increased leukemia disease burden (representative images, 10x light microscopy). (C) Shown is the mean ± SEM number of pimonidazole-positive cells per 20x field at each time point. P values comparing each time point with the preceding one using t-test analysis are denoted * for p<0.05 and ** for p<0.001.

**Supplemental Figure 2. The number of hypoxic marrow cells correlate with AML burden.**
(Top row) Shown from left to right are marrow sections stained with isotype control or pimonidazole with minimal (0.86%) versus moderate (35%) leukemic marrow disease as confirmed by flow cytometry for human CD33 antigen expressing cells. (Bottom row) Hypoxic (pimonidazole-expressing brown staining) cells remained in the marrows of leukemia-engrafted mice three days after treatment with the chemotherapy drugs cytarabine or doxorubicin or the vascular endothelial growth factor (VEGF) inhibitor aflibercept (VEGF Trap, Regeneron/Sanofi Aventis, NJ) (10x magnification, Olympus Bx40 light microscope).

**Supplemental Figure 3. Cytarabine induces apoptosis of human AML (HL60/PAR) cells in a dose and time-dependent manner.** (A) Mean numbers of viable HL60/PAR cells as measured by trypan blue exclusion following continuous treatment with cytarabine (5-500 nM)
for 24-72 hours are shown. Each bar represents the mean ± SD of triplicate wells from a representative experiment. (B) Cleaved PARP levels by western blot in cell lysates continuously treated with cytarabine (5-100 nM) for 24-72 hours are also shown. All of the above experiments were repeated three times with cells plated in multiple cells.

Supplemental Figure 4. Hypoxia attenuates cytarabine-induced apoptosis of human AML cells. Hypoxia attenuates chemotherapy-induced apoptosis of human AML cells. Bulk human AML cells (HL60, ML-2, HEL) were cultured in the presence or absence of cytarabine 100 nM under normoxic (21% O₂) or hypoxic (1% O₂) conditions for 24-72 hours. (A-B) (Top panel) Viability of HL60 and ML-2 human AML cells as determined by trypan blue exclusion under light microscopy was performed. Shown are mean ± SD of viable normoxic cells (solid black line with diamonds -♦-), hypoxic cells (dashed line with open box - -○- -), normoxic cells treated with cytarabine (solid line with filled circles -●-), and hypoxic cells treated with cytarabine (100 nM) as a dotted line with open triangles ...Δ...). (Bottom panel) Corresponding western blot analysis demonstrating relative decreased cytarabine-induced apoptosis of AML (HL60, ML-2) cells following 24-72 hours of hypoxia versus normoxia. Each of these experiments was independently performed three times with similar results noted in all cell lines. One representative experiment is shown. (C-E) Representative dot blots demonstrating the number of apoptotic AML cells (as determined by flow cytometry for 7-AAD/annexin-V expression) treated under differing conditions. Data with HL60, ML-2 and HEL cells are shown. (D) Mean number of apoptotic ML-2 cells ± SEM from triplicate samples under varying conditions. Student’s t test was performed with significant p values denoted as * for p<0.05, ** for p<0.001, and *** for p≤0.0001.
Supplemental Figure 5. Effects of hypoxia and drug treatment on ROS generation by AML cells. (A) Hypoxia (1% O₂) induces time-dependent increases in overall reactive oxygen species generated by AML (HEL) cells as compared to ROS levels under normoxia (21% O₂). One representative experiment out of three shown. White bars indicate normoxia; grey bars indicate hypoxia. (B) Hypoxia-induced ROS generation in AML cells was significantly attenuated in the presence of the NADPH oxidase inhibitor, diphenyleneiodonium (DPI) 5 μM followed by incubation for 48 hours. White bars indicate normoxia. Grey bars indicate hypoxia. (C) Human AML cells (HEL) were cultured in the presence or absence of cytarabine 100 nM under normoxic (21% O₂) or hypoxic (1% O₂) conditions for 72 hours followed by CM-H2DCFDA staining and flow cytometric analysis for mean fluorescent intensity. Vehicle (PBS) is indicated in white bars; cytarabine treatment is in diagonal shaded bars. All of the above experiments were repeated at least twice with cells plated in multiple cells. Student’s t test analysis compared conditions versus controls. Significant p values are denoted as * for p<0.05, ** for p<0.001, and *** for p≤0.0001.