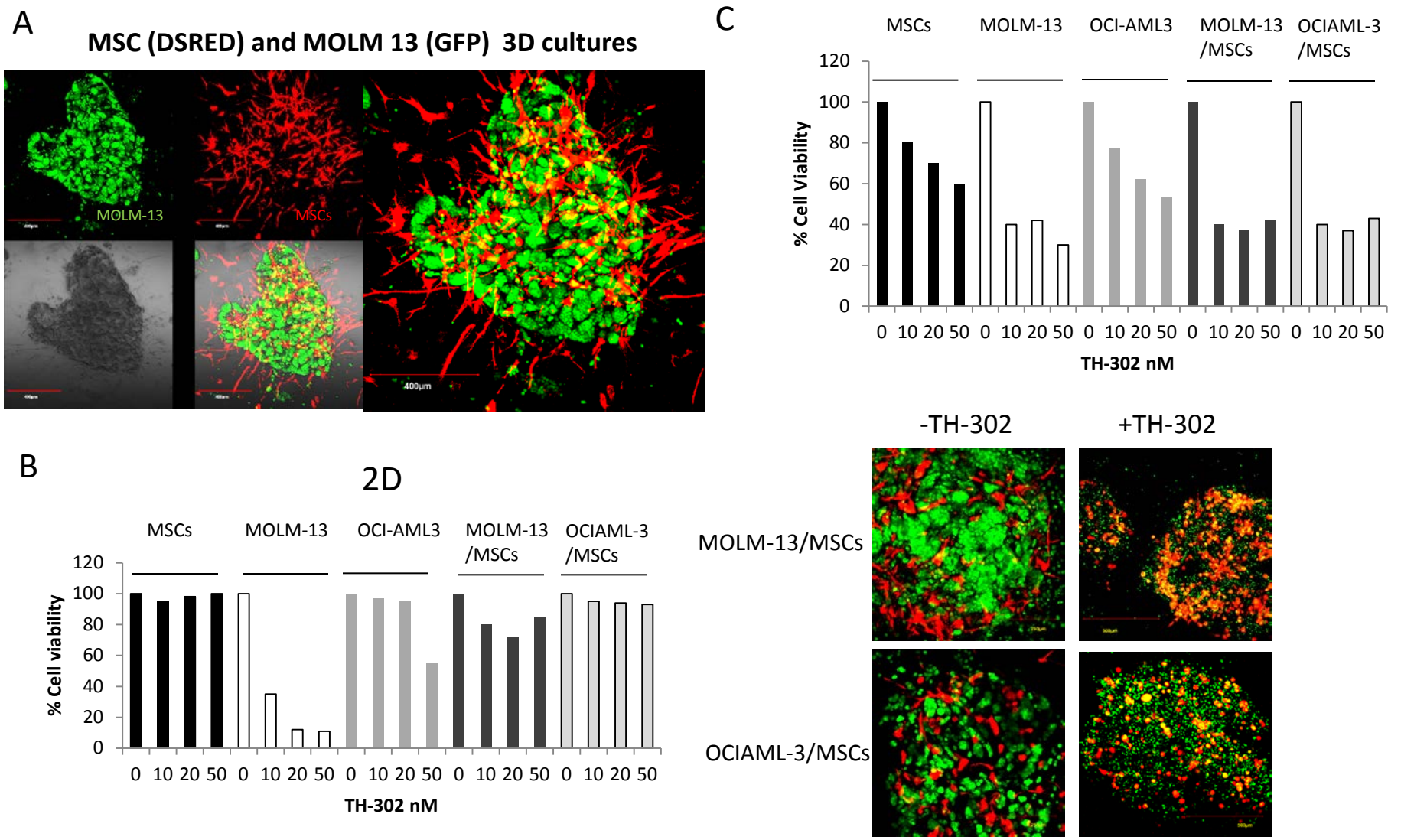
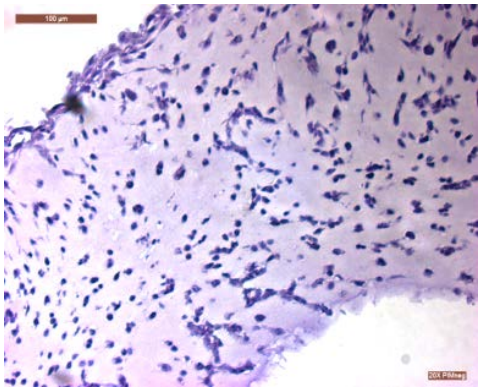
Supplementary Figure 1

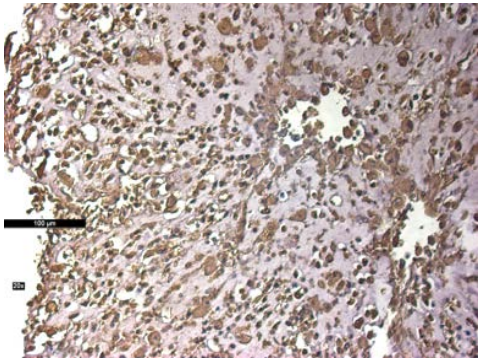


Supplementary Figure 1. TH-302 has potent antitumor activity in a 3D *in vitro* co-culture system of leukemia cells and bone marrow–derived mesenchymal stromal cells that recapitulates the multidimensional BM niche. A. Confocal images of a spheroid formed by mesenchymal stromal cells (MSCs; red) and MOLM-13 (green) cells. B and C. TH-302 cytotoxic activity, as determined by WST-1 assay, against 3D or 2D co-cultured MSC-leukemia cells, respectively, incubated with indicated concentrations of the drug for 72 h. Right panel shows confocal images of 3D MSC-leukemia cell spheroids incubated with or without TH-302 (50 nM) for 72 h.

Supplementary figure 2

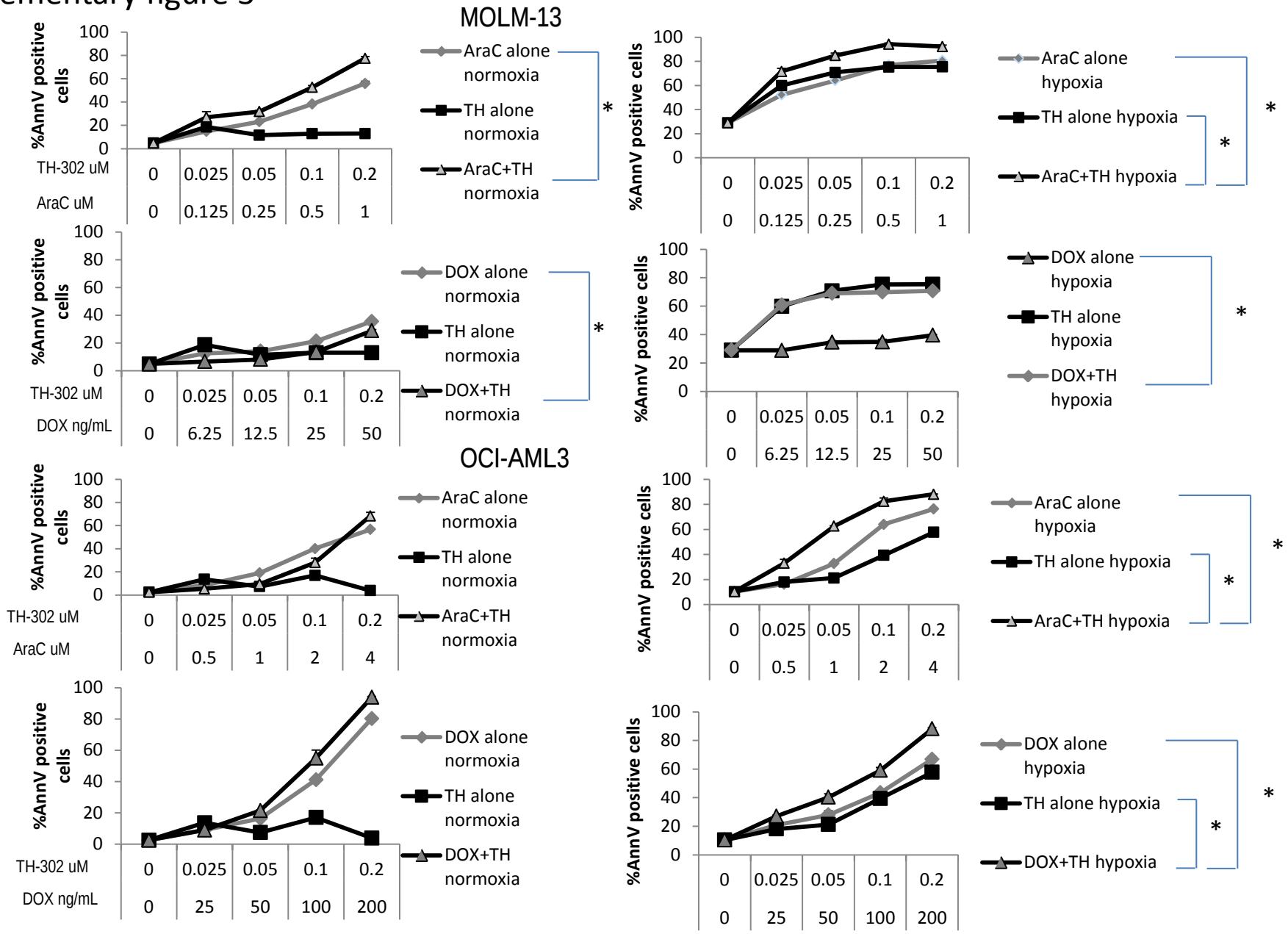


PIMO negative control



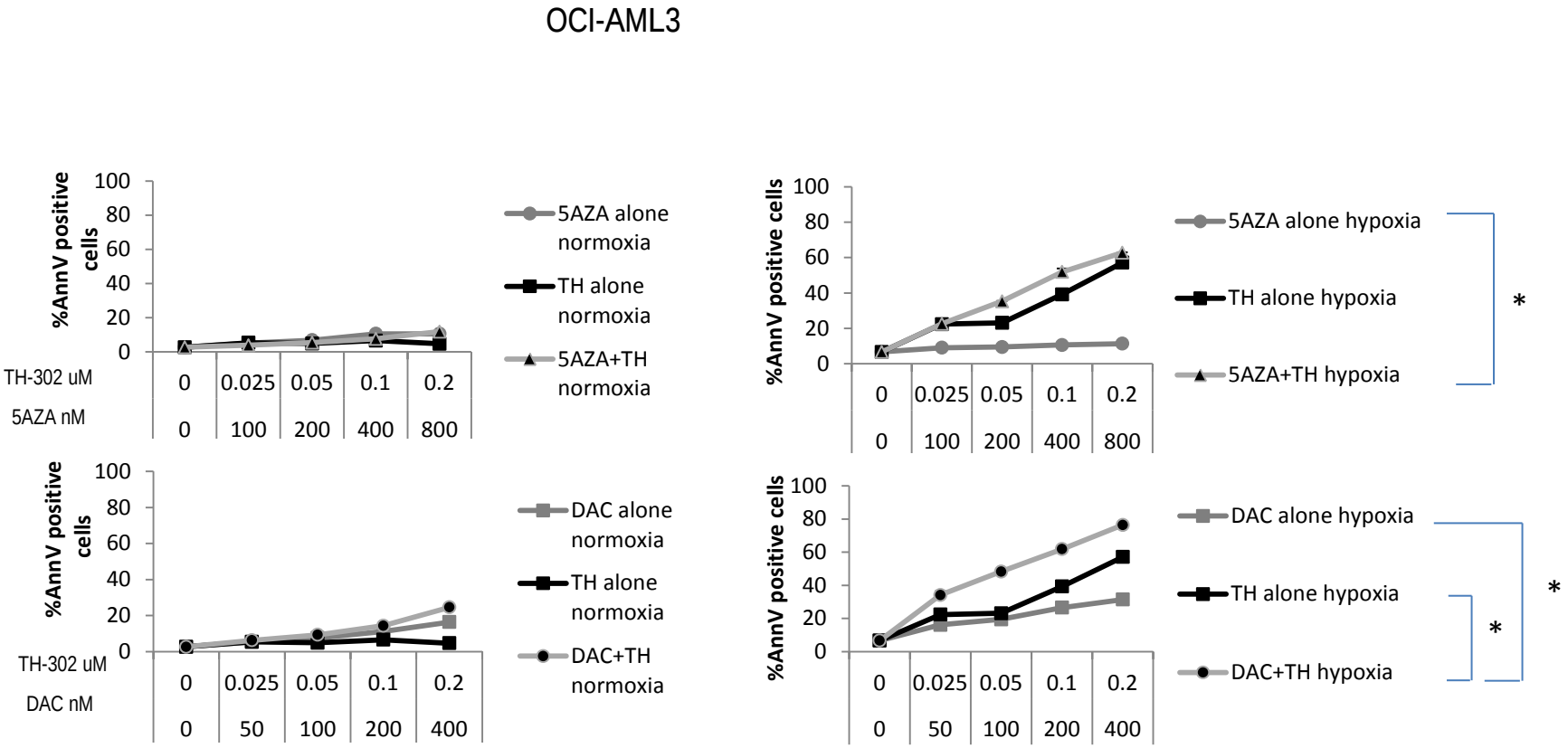
Supplementary Figure 2. Hypoxia is present in spheroids as indicated by PIMO positive staining in spheroids incubated with PIMO for 3hr before harvesting

Supplementary figure 3

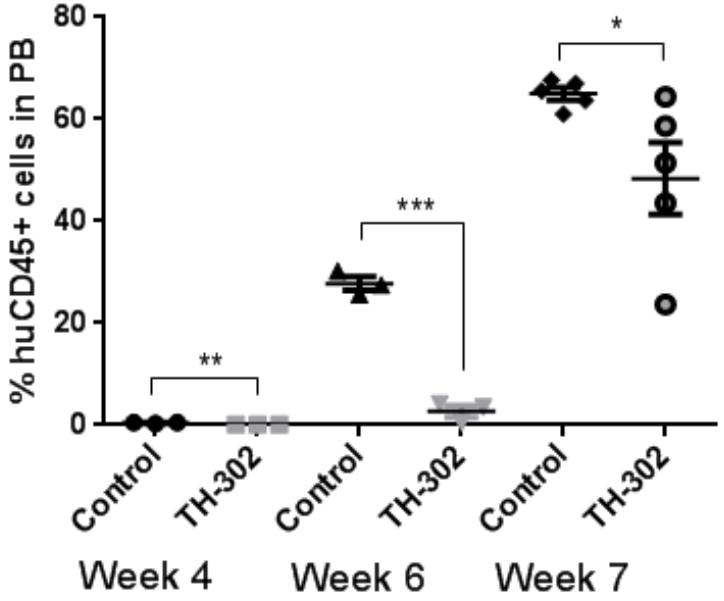


Supplementary figure 3. In vitro synergistic activity of TH-302 in combination with chemotherapy and demethylating agents. MOLM-13 and OCI-AML3 AML cell lines were exposed to TH-302 alone or in combination with AraC, 5AZA or DAC for 72hr under normoxic (21% O2; graphs on the left) or hypoxic (1%O2; graphs on the right) conditions. Effects on cell growth and apoptosis induction were determined by FACS. The asterisk next to the legend denotes a p value <0.05 (t-test).

Supplementary figure 3- continued



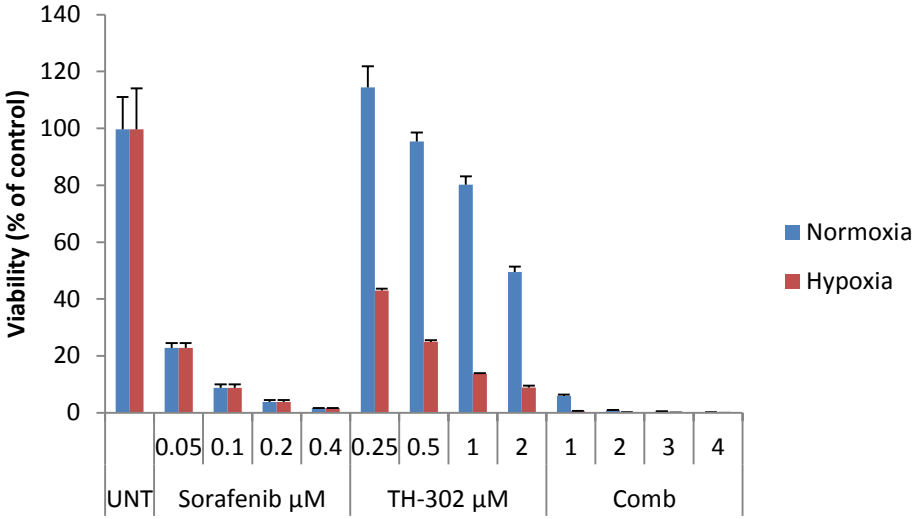
Supplementary figure 4

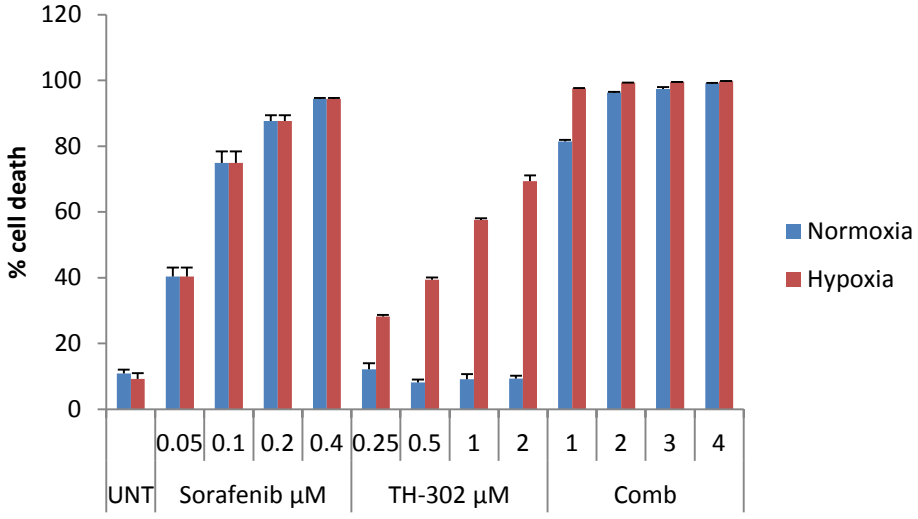


Supplementary Figure 4. TH-302 has anti-leukemia activity in an *in vivo* primary AML xenograft murine model. Secondary transplant mice (N=5/group). NSG mice were transplanted with 0.01 or 0.005×10^6 BM cells isolated from primary recipients treated with control or TH-302 (Figure 4). The percentage of circulating human CD45+ cells was determined by FACS in peripheral blood (PB) of mice transplanted with 0.01×10^6 cells at weeks 4, 6, and 7 after transplantation. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Supplementary figure 5

MOLM-13-48hr

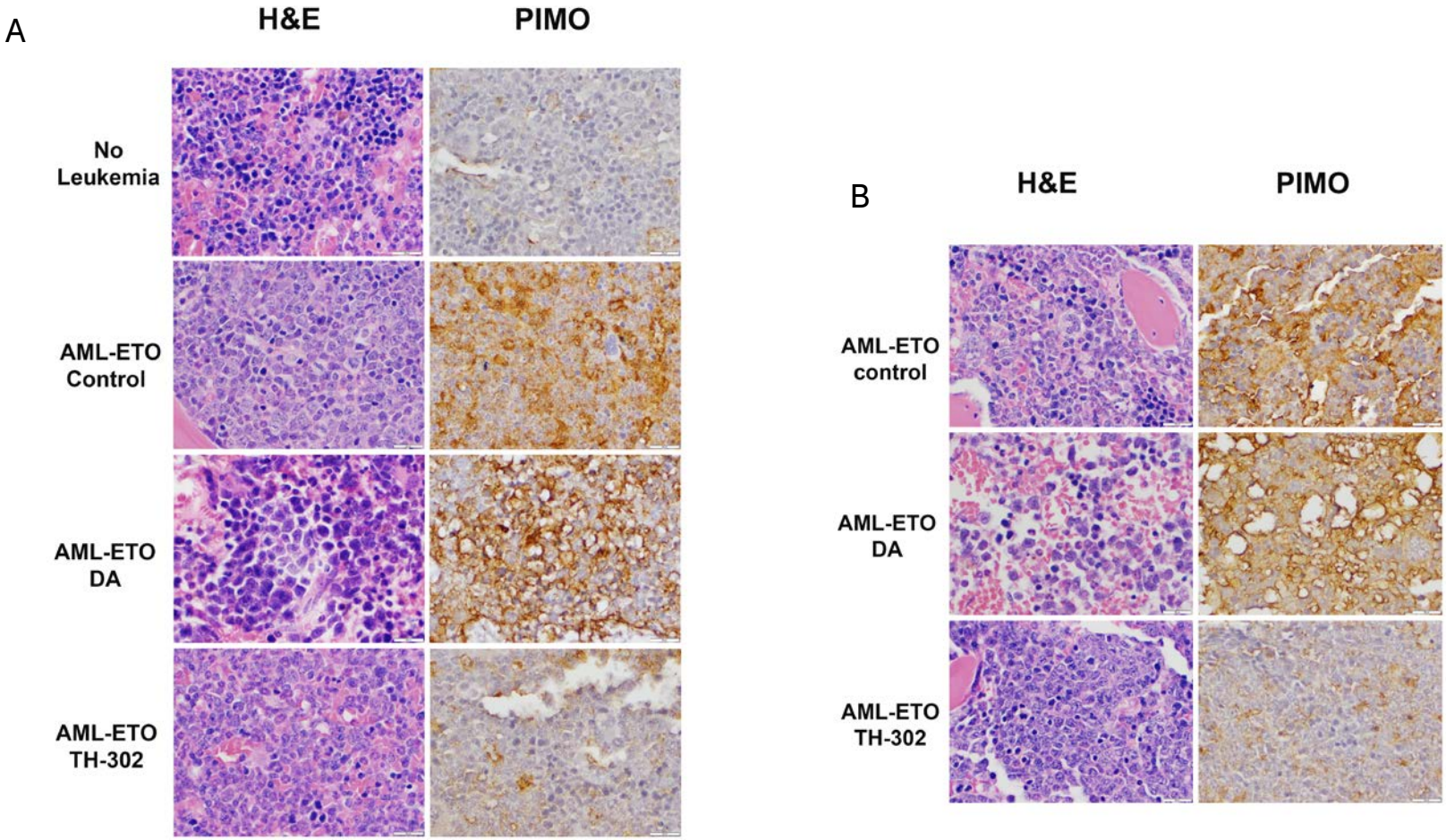




Drug	CI Values at		
	ED50	ED75	ED90
sorafenib+TH-302 normoxia	0.387	0.428	0.476
sorafenib+TH-302 hypoxia	0.053	0.055	0.057

Supplementary figure 5. In vitro synergistic activity of TH-302 in combination with sorafenib. MOLM-13 cells were exposed to TH-302 for 6 h under normoxic (21% O2) or hypoxic (1%O2). Cells were then washed and incubated with sorafenib for 48 h under normoxia. Effects on cell growth and apoptosis induction were determined by FACS.

Supplementary figure 6



Supplementary figure 6 A, B. Assessment of BM hypoxia in AML/ETO bearing mice treated with TH-302 or chemotherapy. C57Bl/6 mice were transplanted via tail vein injection with murine BM cells (1×10^6 viable cells per mouse) expressing the AML1/ETO oncoprotein. Three weeks after transplantation, mice were treated for 5 days with TH-302 (50mg/kg IP) or DA chemotherapy (doxorubicin: 1.5mg/kg IV Day 1-3 and cytarabine 50mg/kg, Day 1-5, first 3 injections IV and last 2 IP). Upon completion of the treatment, mice were injected with PIMO as described under Methods, sacrificed after 3 hrs and bones subjected to anti-PIMO immunohistochemistry. One healthy mouse was injected with PIMO and processed as above (6A, top panel). The extent of leukemia infiltration did not differ significantly between DA- or TH-302-treated mice (n=2/each, DA, 40% and 40%; TH-302, 30% and 50%). Images of H&E and PIMO staining are shown in respective animals identified by the legends. A, B: independent sets of mice.