Supplemental Material to:

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Regulation of HIF-1alpha signaling and chemoresistance in Acute Lymphocytic Leukemia under hypoxic conditions of the bone marrow microenvironment

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Figure S1

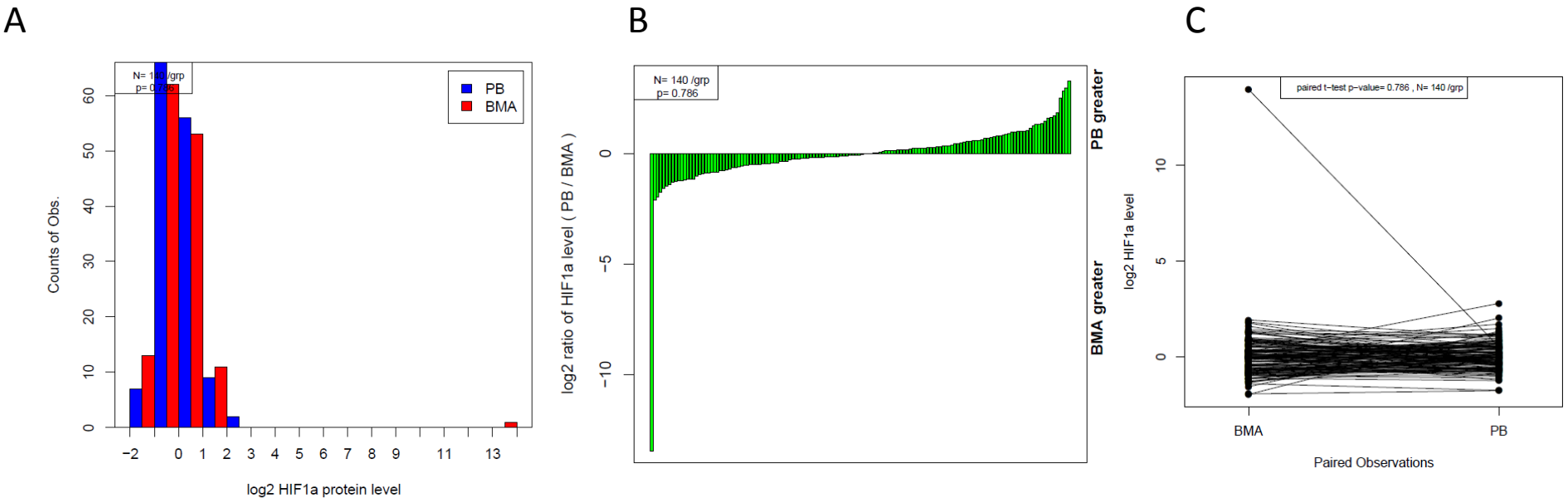

Figure S1. Comparison of HIF-1α levels in paired samples from different sources (BM vs PB). One hundred and forty (140) pairs of BM and PB samples from the same patient were compared to assess the effect of sample source on protein level (paired t test). A. Distribution of protein levels for pairs of samples from BM and PB. B. Distribution of protein level difference for pairs of samples from BM and PB. C. Comparison of protein levels for each pair of samples from BM and PB.

Figure S2

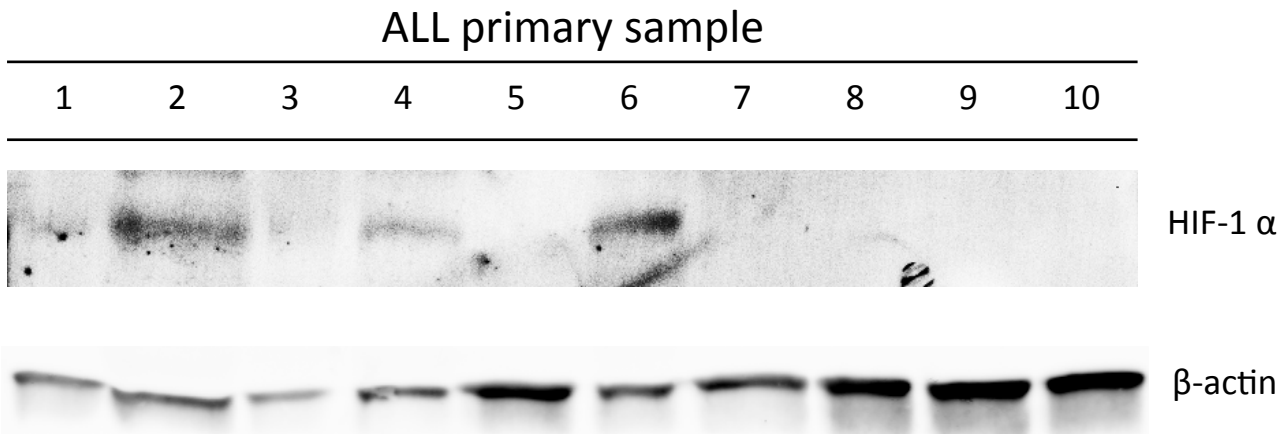

Figure S2. A subset of primary ALL samples from RPPA study (10 samples) was subjected to conventional Western blotting for HIF-1α expression. The blot was probed for β-Actin as loading control.

Figure S3

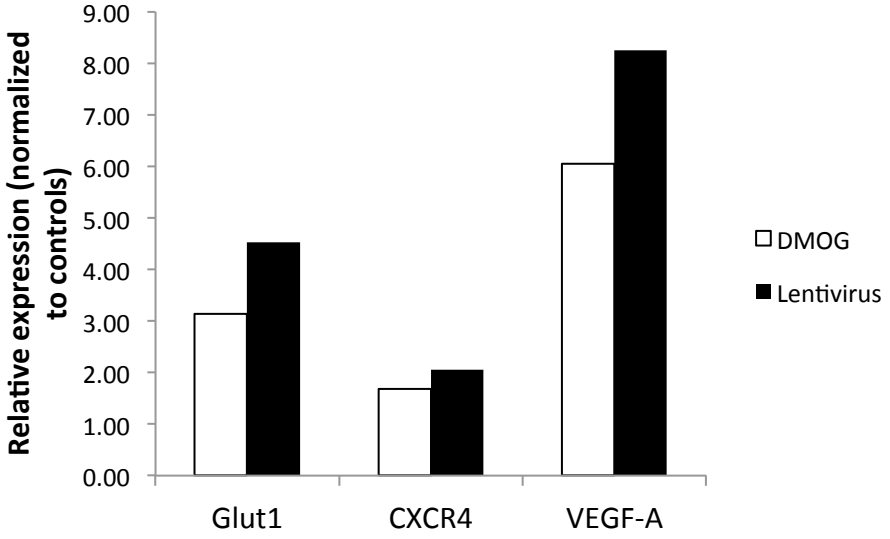

Figure S3. REH cells were treated with 100uM DMOG (24hr) or infected with empty vector or HIF-1α lentivirus and induced 48hr with Doxocycline (1ug/ml). After inducing HIF-1α expression at 21%O_2, RNA lysates were prepared and HIF-1α downstream targets (Glut-1, CXCR-4 and VEGF-A) were determined by quantitative real time PCR. Results are expressed as fold change realative to controls (DMSO for DMOG treated cells and empty vector for lentivirus infected cells).

Figure S4

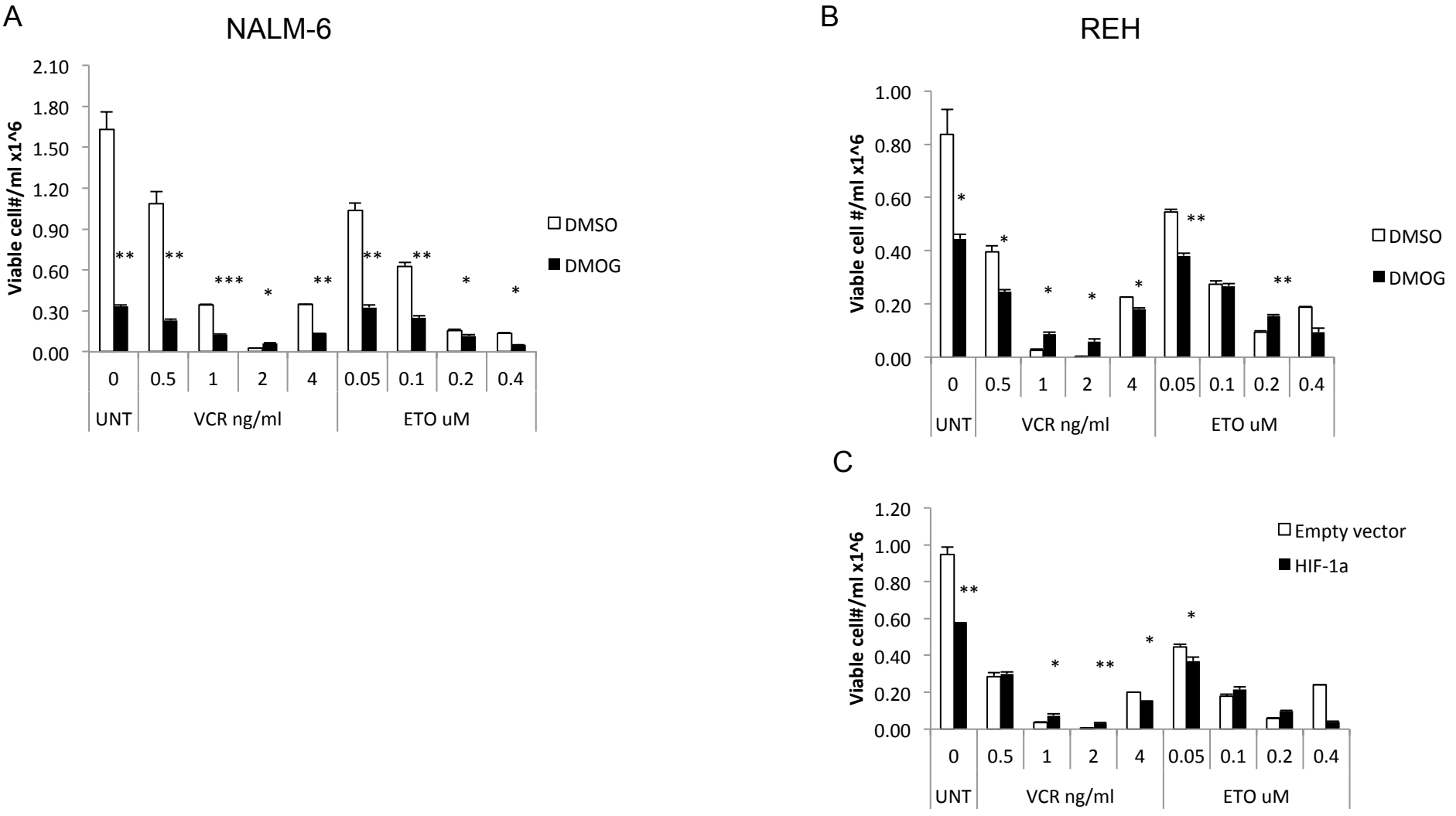

Figure S4. HIF-1α and cytotoxic effects of chemotherapy at 1% O_2. NALM-6 or REH cells **(A,B, C)** were treated with 100μM DMOG (A,B) or infected with empty vector or HIF-1α lentivirus and induced with 1μg/ml Doxocycline (REH, C). After inducing HIF-1α expression at 21%O_2, cells were treated with chemotherapy (VCR or ETO). After 72 hrs, effects on viable cell number were determined by FACS. *P<0.05; ** P<0.01.

Figure S5

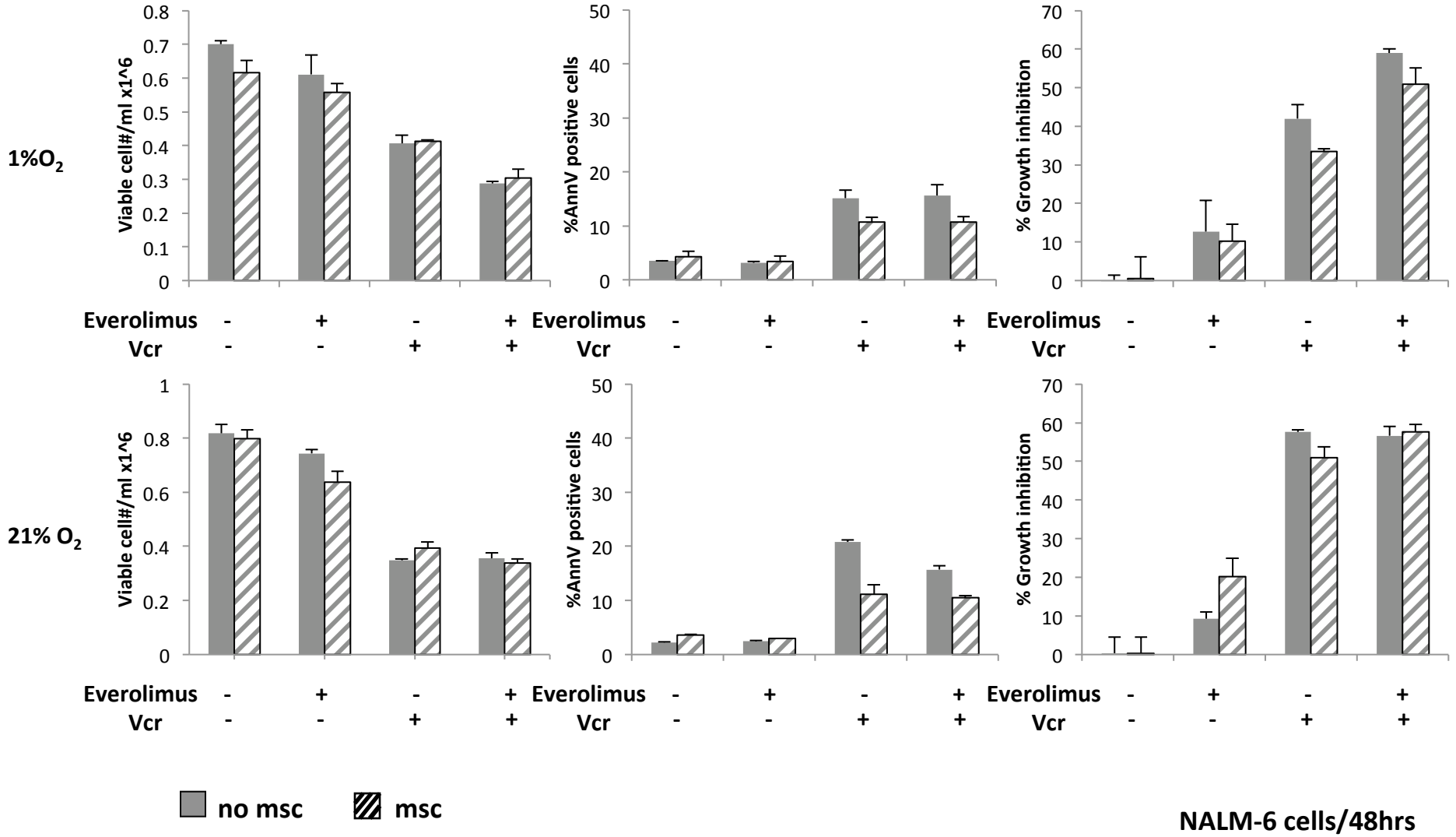

Figure S5. Inhibition of mTOR signaling sensitized leukemic cells to chemotherapy under hypoxic conditions mimicking BM microenvironment. Exponentially growing NALM-6 cells were cultured with or without MSC for 48 hrs under normoxia (21% O_2) and then were grown under normoxic (21% O_2) or hypoxic (1% O_2) conditions in the presence or absence of 20nM everolimus and/or 2ng/ml Vcr. After 48 hrs, effects on cell growth and apoptosis induction were determined by viable cell count and annexin V flow cytometry).