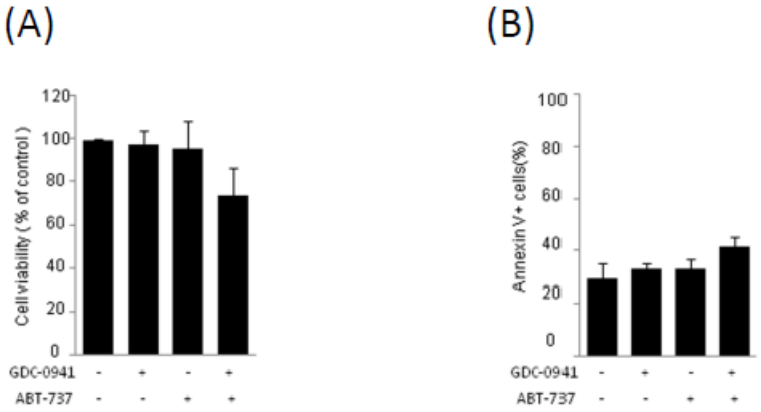
J Mol Med 2013
PI3K inhibitor GDC-0941 enhances apoptotic effects of BH-3 mimetic ABT-737 in AML cells in the hypoxic bone marrow microenvironment.
Linhua Jin, Yoko Tabe, Kensuke Kojima, Masato Shikami, Juliana Benito, Vivian Ruvolo, Rui-Yu Wang, Teresa McQueen, Stefan O. Ciurea, Takashi Miida, Michael Andreeff, Marina Konopleva

Supplementary Table 1. Clinical characteristics of AML patients

Patient number	source	age/sex	FAB	WBC ($x10^9$/L)	% BM Blast (PB Blast)	Disease status	Cytogenetics
1	PB	80/F	M4	48.9	84.4(49)	Primary refractory	46,XX (20/20)
2	BM	22/M	M5	11.4	95.4(71)	Relapse / refractory	46,XY,t(6;11)(q27;q23) (19/20) 47,sl,+mar (1/20)
3	BM	63/M	M5	265.0	79.2(73)	Primary refractory	46,XY(20/20)
4	BM	30/M	M1	175	91.4(95.8)	Newly diagnosed	46,XY(20/20)

FAB, French-American-British (classification) of AML.

Supplementary Figure 1



Supplementary Figure 1. Normal $CD34^+$ cells (n=3) co-cultured with normal BM-derived MSCs were incubated for 24 hours with 0.5μM GDC-0941, 0.05μM ABT-737, or both, under normoxic conditions. The viable cells number was measured by trypan blue exclusion assay (A). The annexin V−positive fractions were measured by flow cytometry (B). Graphs show the means ± SEM of results of three independent experiments.

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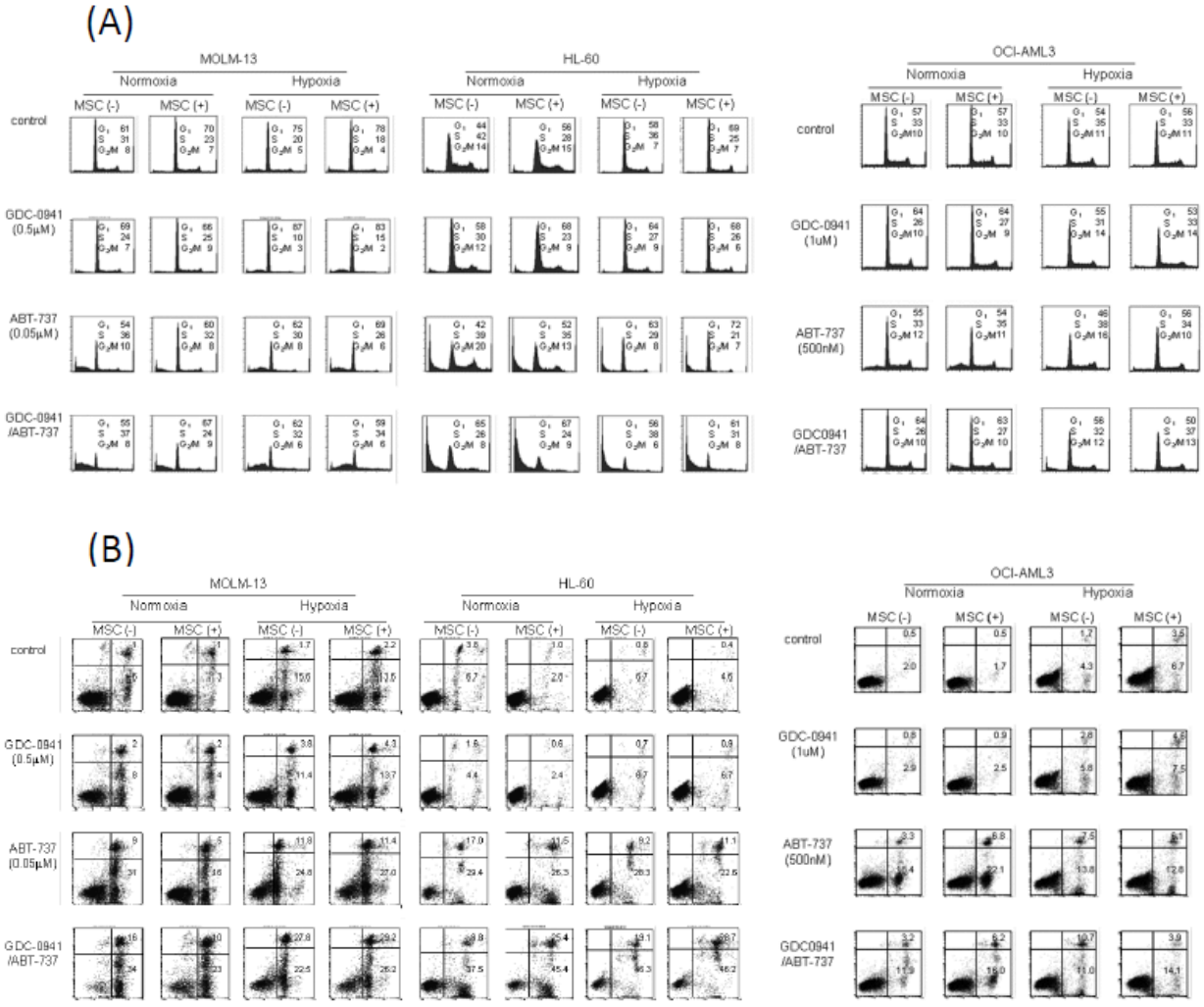
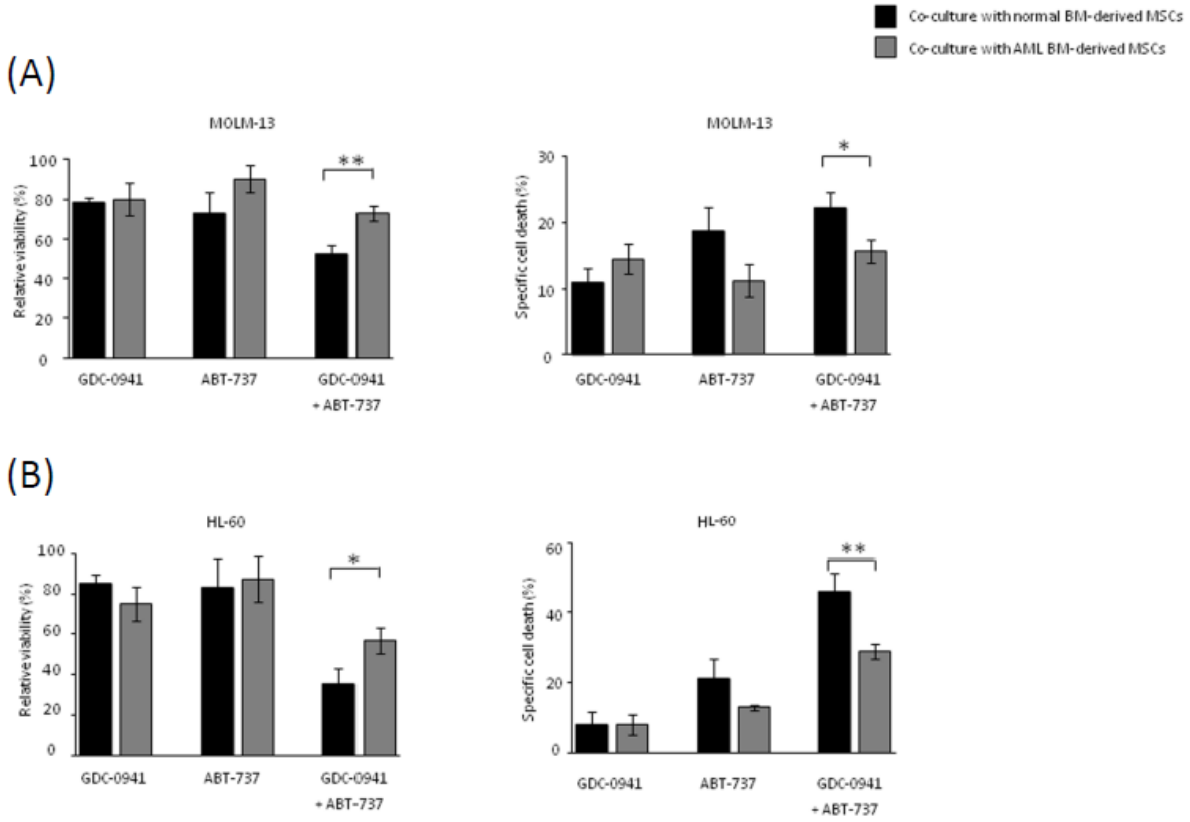
Supplementary Figure 2



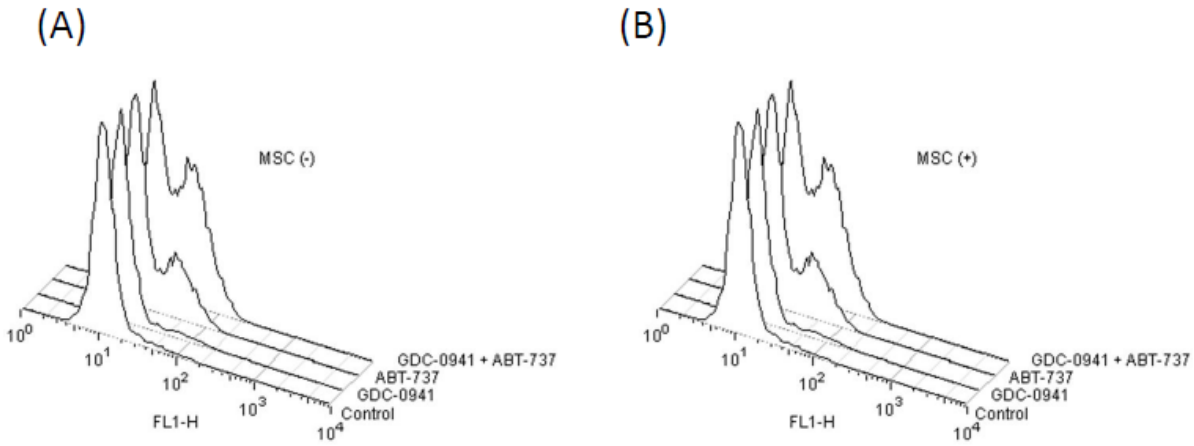
Supplementary Figure 2. MOLM-13, HL-60 and OCI-AML3 cells were incubated for 48 hours with 0.5μM GDC-0941, 0.05μM ABT-737, or both, with or without MSC co-culture under normoxic or hypoxic conditions. Representative results of three independent experiments for DNA content (A) and annexin V–positive fractions (B) measured by flow cytometry are shown.

Supplementary Figure 3



Supplementary Figure 3. MOLM-13 (A) and HL-60 (B) cells were incubated for 48 hours with 0.5μM GDC-0941, 0.05μM ABT-737, or both, in the presence of absence of MSCs derived from 3 normal BM or 3 AML patients BM under normoxic condition. Relative viability and specific cell death induction were calculated (trypan blue exclusion assay). Relative viability (%) = (viable cell numbers in treated group/viable cell numbers in control group) x 100. Specific cell death (%)= (%viable trypan blue positive cell numbers in control group - % trypan blue positive cell numbers in treated group) / (100 - % trypan blue positive cell numbers in treated group) x100. *p<0.05, ** p<0.01.

Supplementary Figure 4



Supplementary Figure 4. Combination of GDC-0941 and ABT-737induced mitochondrial apoptosis in MOLM-13 cells. MOLM-13 cells were treated with 0.5μM GDC-0941, 0.05μM ABT-737, or both, with or without MSC co-culture conditions; representative flow cytometry results of Bax conformational change after 16 hours of treatment.

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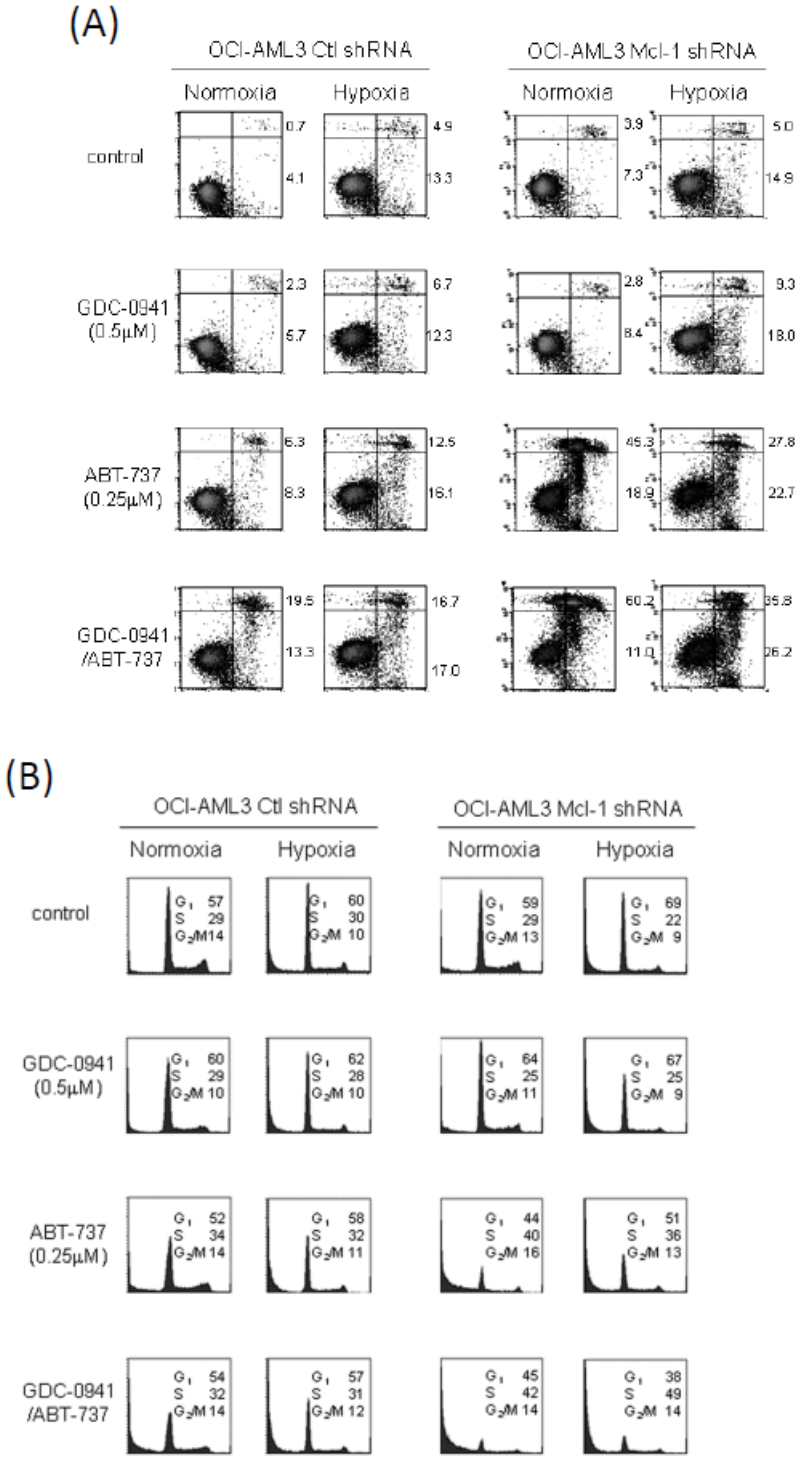
Supplementary Figure 5



Supplementary Figure 5. Mcl-1 shRNA or scrambled control shRNA transfected OCI-AML3 cells were incubated for 48 hours with 0.5μM GDC-0941, 0.25μM ABT-737, or both, with or without MSC co-culture under normoxic or hypoxic conditions. Representative results of three independent experiments for annexin V–positive fractions (A) and DNA content (B) measured by flow cytometry are shown.