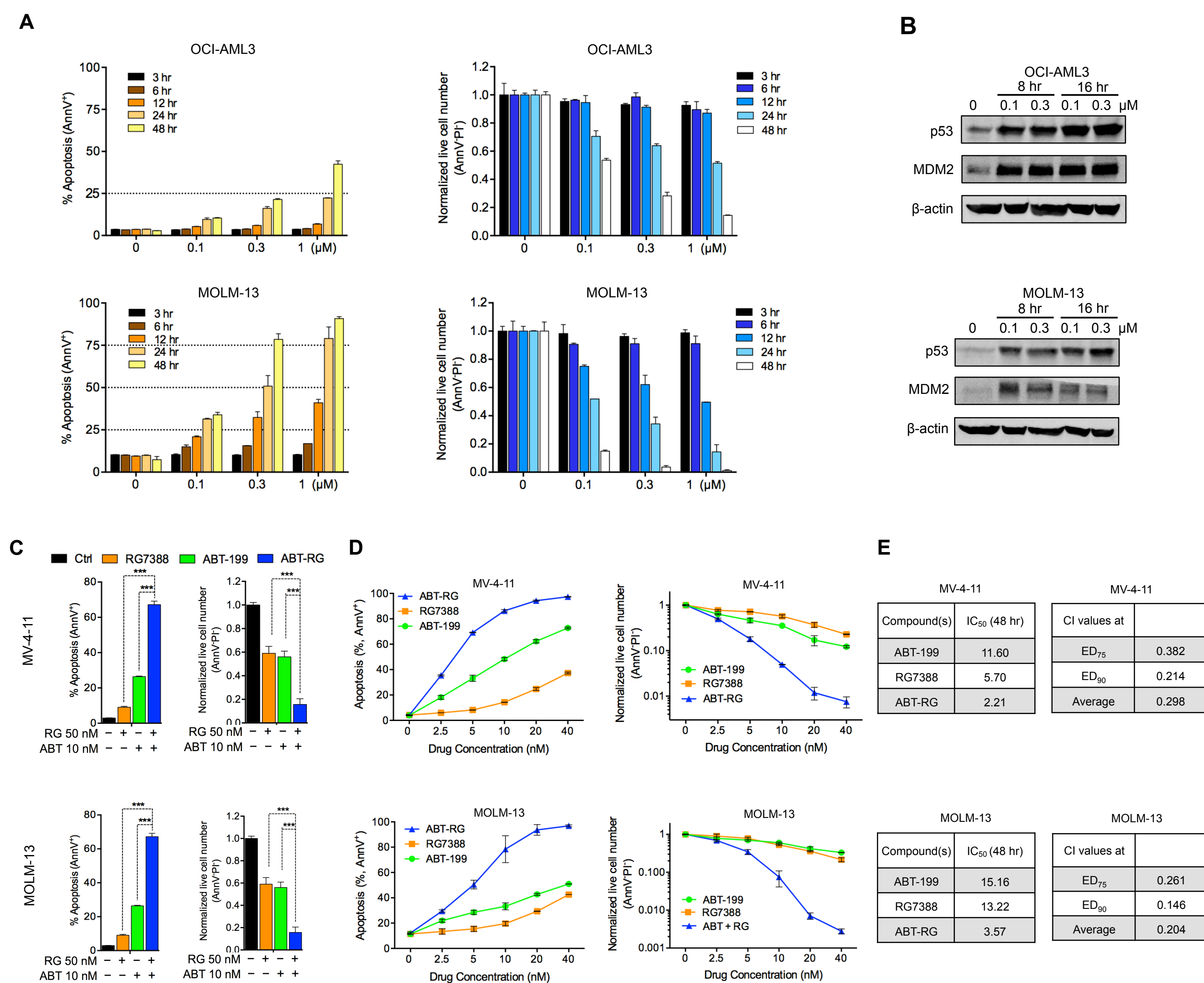

MV-4-11

Compound(s)	IC_{50} (48 hr)
ABT-199	11.60
RG7388	5.70
ABT-RG	2.21

MV-4-11

CI values at	
ED_{75}	0.382
ED_{90}	0.214
Average	0.298

MOLM-13

Compound(s)	IC_{50} (48 hr)
ABT-199	15.16
RG7388	13.22
ABT-RG	3.57

MOLM-13

CI values at	
ED_{75}	0.261
ED_{90}	0.146
Average	0.204

Figure S1, related to Figure1.
(A) Apoptosis induction and decrease of live cell numbers in OCI-AML3 and MOLM-13 cells after the indicated RG treatment.
(B) Immunoblots of p53 and MDM2 after the indicated RG treatment. β-actin served as loading control.
(C-E) ABT-sensitive MV-4-11 and MOLM-13 cells were treated with ABT/RG combination at a 1:5 (C) or 1:1 ratio (D) for 48 hr. The combination index (CI) and IC_{50} values were calculated using Calcusyn software based on live cell numbers (E). ED_{75}, 75% effective dose; ED_{90}, 90% effective dose.
Data in bar/line graphs represent the means of triplicate experiments (A, C, D). Error bars, mean ± SD. *** $p < 0.001$ as determined by two-tailed unpaired Student's *t*-test.

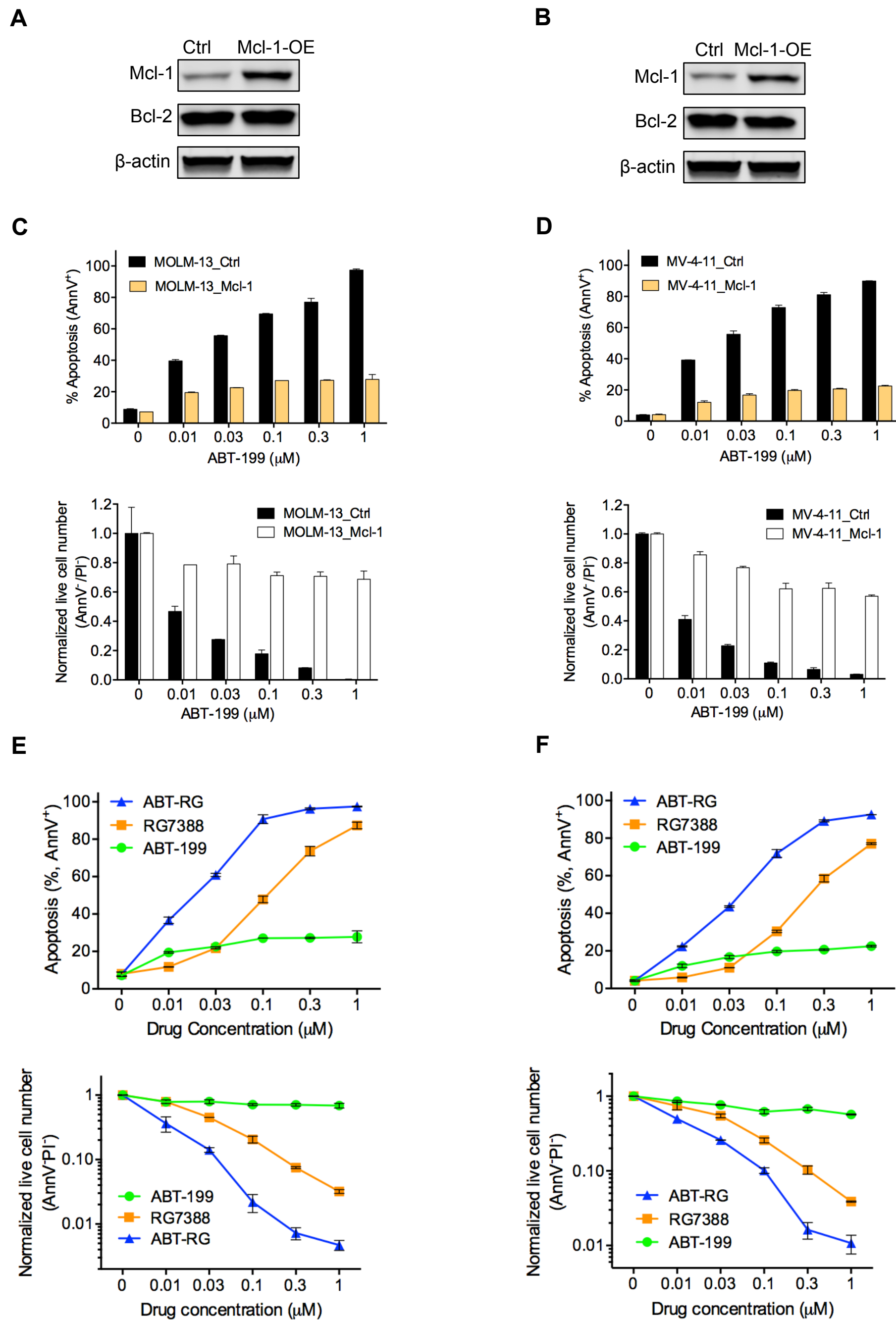

Figure S2, related to Figure 2. Mcl-1 overexpression confers resistance to ABT, which can be abrogated by p53 activator RG.

(A, B) Immunoblot showing ectopic overexpression of Mcl-1 in MOLM-13_Mcl-1 (A) and MV-4-11_Mcl-1 (B) cells. β-actin served as loading control.

(C, D) Apoptosis percentage and live cell numbers of the control cells and Mcl-1 overexpressing MOLM-13 (C) or MV-4-11 (D) cells after 48 hr ABT treatment.

(E, F) Apoptosis percentage and live cell numbers of MOLM13_Mcl-1 (E) and MV-4-11_Mcl-1 (F) cells after treatment with RG and ABT at a 1:1 ratio for 48 hr.

Data in bar/line graphs represent the means of triplicate experiments. Error bars, mean ± SD.

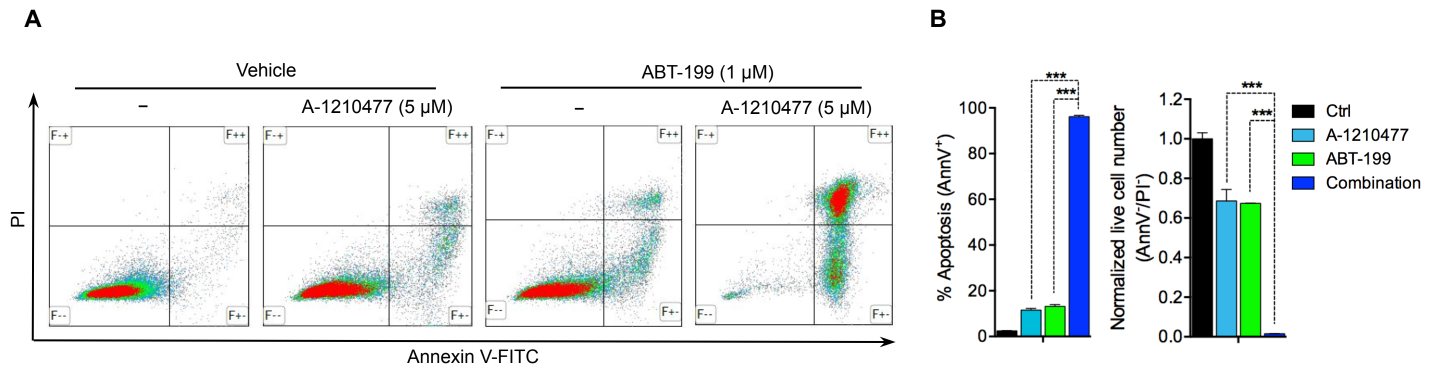

Figure S3, related to Figure 3. Mcl-1 inhibition overcomes inherent ABT resistance of OCI-AML3 cells.
(A) Representative flow cytometry plots of OCI-AML3 cells after 24 hr treatment with 5 μM Mcl-1 antagonist A-1210477 in the presence or absence of 1 μM ABT.
(B) The cell numbers in panel A were enumerated by flow analysis using CountBright counting beads and then normalized to untreated control. Data represent the means of triplicate experiments. Two-tailed unpaired Student's *t*-test was used to calculate p values. *** $p < 0.001$. Error bars, mean ± SD.

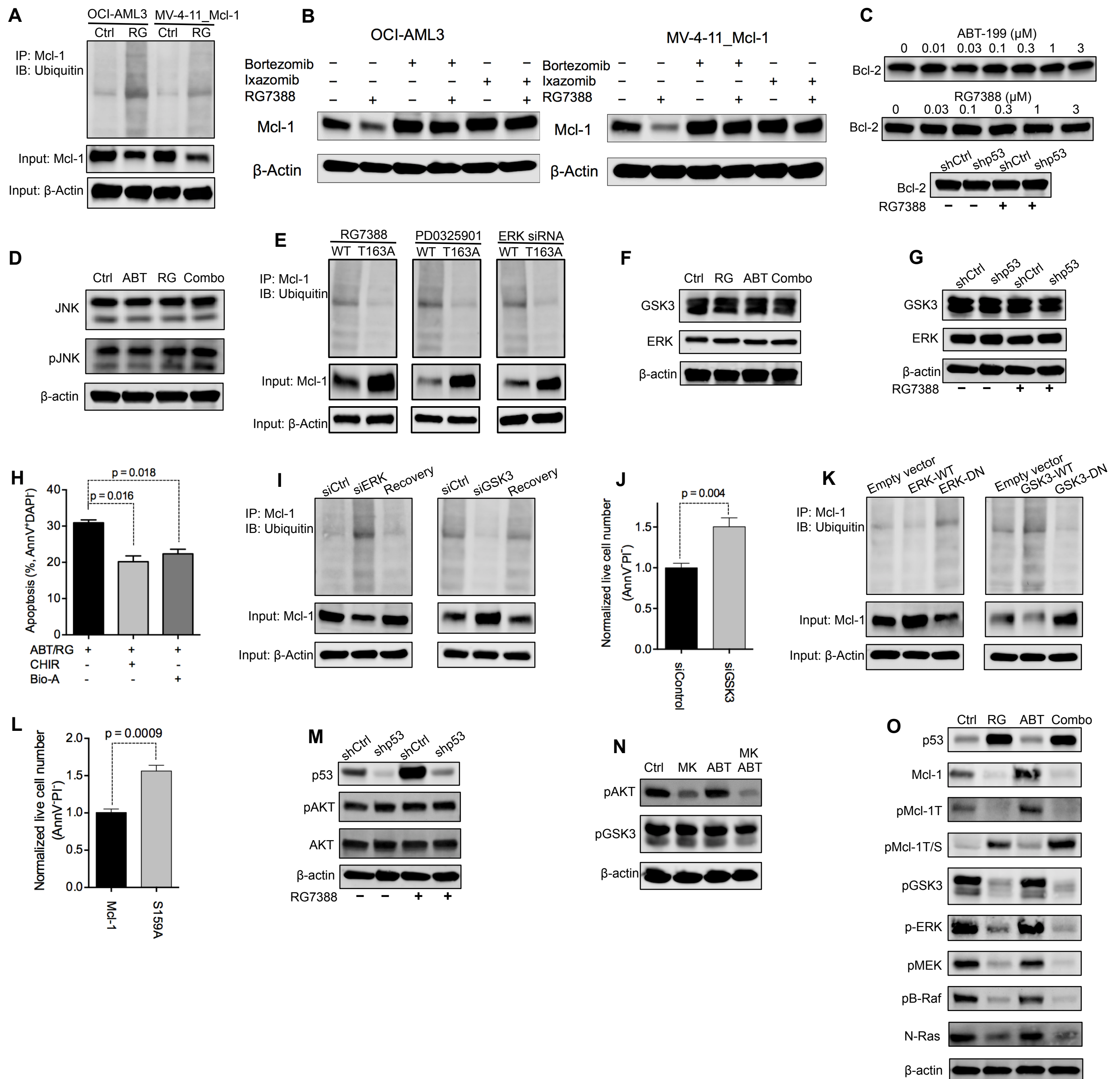

Figure S4, related to Figure 5.

(A) OCI-AML3 cells were treated with 1 μM RG or vehicle for 12 hr. Mcl-1 was immunoprecipitated and then analyzed by immunoblotting as described in STAR METHORDS.

(B) OCI-AML3 and MV-4-11_Mcl-1 cells were pre-treated with 1 μM RG for 6 hr. Proteasome inhibitors bortezomib or ixazomib were then added to a final concentration of 0.1 μM. Cells was harvested and then analyzed by immunoblotting after another 6 hr co-treatment.

(C) Effects of ABT, RG, or p53 knockdown on Bcl-2 protein levels. The cell lysates and immunoblots of loading control (not shown here) are the same as in related Figures 4C, 4D or 5B, respectively.

(D) Immunoblots of pJNK or total JNK after the indicated treatment. Cell lysates are the same as in related Figure 5A.

(legend continued on next page)

(Figure S4 legend, continued)

(E) OCI-AML3 cells overexpressing WT Mcl-1 or T163A mutant were treated with 1 μM RG for 12 hr (left), 100 nM PD0325901 for 12 hr (middle) or ERK siRNA as described in STAR METHORDS (right). Mcl-1 was immunoprecipitated and analyzed by immunoblotting.
(F) Immunoblots of total GSK3 or ERK in OCI-AML3 cells after treatment with 1 μM RG, 1 μM ABT, or the combination for 24 hr. The cell lysates and the blot of loading control are the same as in related Figure 5A.
(G) Immunoblots of total GSK3 or ERK in control or p53 knockdown OCI-AML3 cells. The cell lysates and the blot for loading control are the same as in related Figure 5B.
(H) Apoptosis of OCI-AML3 cells after treatment with different combinations of 1 μM ABT, 1 μM RG, and GSK3 inhibitors CHIR-99021 (CHIR, 1 μM) or BIO-Acetoxime (BIO-A, 1 μM) for 24 hr.
(I) The effects of ERK or GSK3 knockdown on Mcl-1 ubiquitination in OCI-AML3 cells. For the right panel, OCI-AML3 cells were also treated with 1 μM ABT/RG for 24 hr. The treatment and the blots of Mcl-1 and β-Actin are the same as in related Figures 5E and 5I.
(J) Normalized live cell numbers of control and GSK3 knockdown OCI-AML3 cells after treatment with 1 μM ABT/RG for 24 hr.
(K) OCI-AML3 cells were transduced to overexpress wild-type or dominant-negative ERK or GSK3. Cells overexpressing GSK3 (right) were also treated with 1 μM ABT/RG before Mcl-1 immunoprecipitation and immunoblotting. Mcl-1 and β-Actin blots are the same as in related Figures 5G and 5J.
(L) Normalized live cell number of MV-4-11 cells overexpressing wild-type or S159A Mcl-1 after treatment with 1 μM ABT/RG for 24 hr.
(M) Effects of p53 knockdown or p53 activation on pAKT levels. The cell lysates and the blots of p53 and β-actin are the same as in related Figure 5B.
(N) Immunoblots of pGSK3 and pAKT in response to AKT inhibition. OCI-AML3 cells were treated with vehicle, 1 μM MK-2206 (MK, AKT inhibitor), 1 μM ABT, or the combination for 12 hr.
(O) Immunoblot of indicated proteins in MV-4-11_Mcl-1 cells after treatment with vehicle (Ctrl), 1 μM RG, 1 μM ABT, or the combination for 24 hr.
Note: For ubiquitin blots (panels A, E, I, and K), Mcl-1 protein was immunoprecipitated from the same number of viable cells. Immunoprecipitated Mcl-1 from 1 million cells was then analyzed using a ubiquitin-specific antibody. Data in panels H, J, and L represent the means of triplicate experiments. Error bars, mean ± SD.

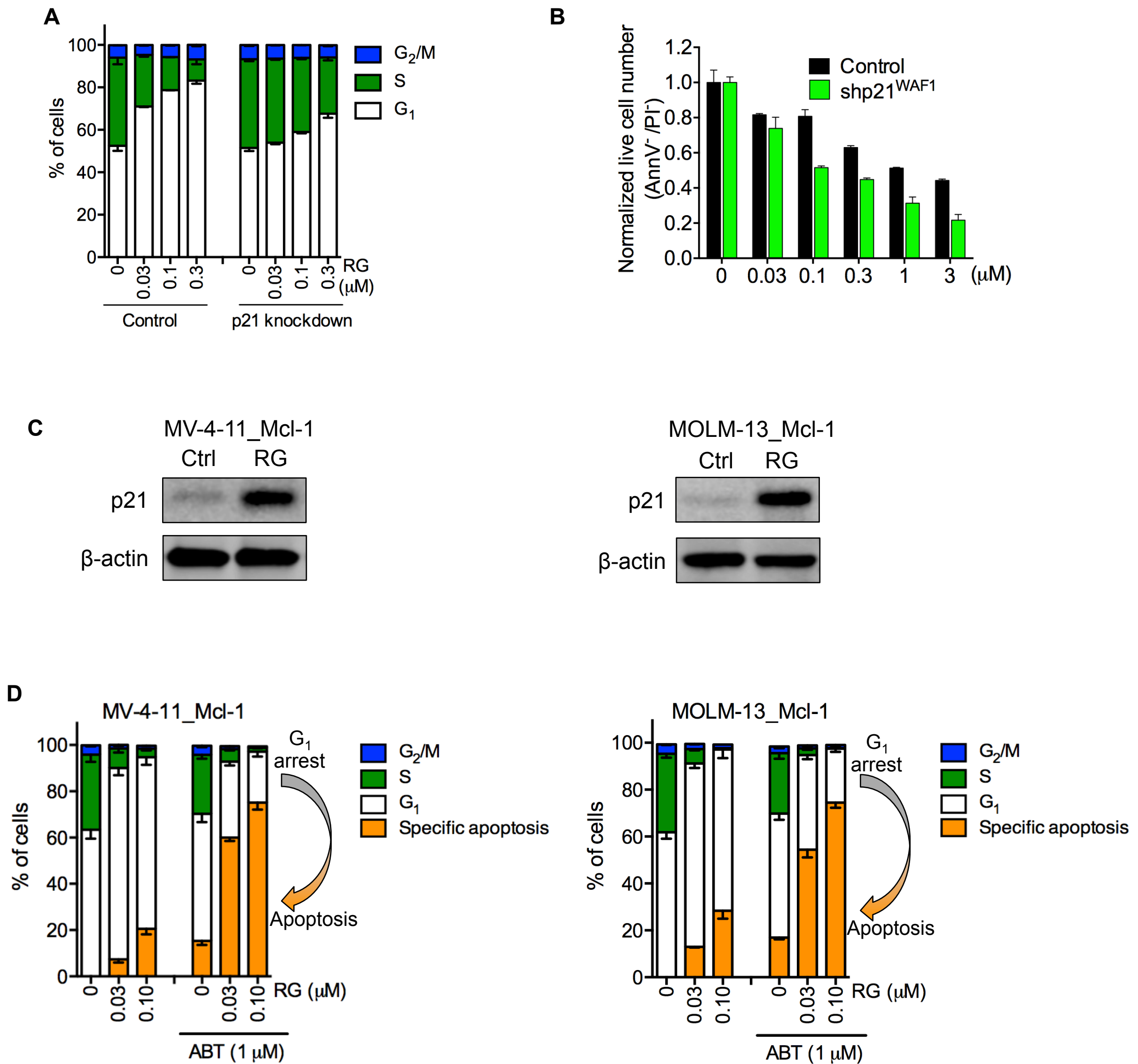

Figure S5, related to Figure 6.

(A) The effects of p21 knockdown on cell cycle distribution. OCI-AML3 cells were treated with RG for 24 hr, and then pulsed with 10 µM Edu for 1 hr. The cells was next fixed, permeabilized, stained with Click-iT Plus reaction cocktail (Life Technologies), and analyzed by flow cytometry.

(B) Cell numbers of OCI-AML3 cells (control and p21 knockdown) after treatment for 48 hr with indicated concentrations of RG.

(C) Immunoblots of p21 after RG treatment. Cells were treated with vehicle DMSO (Ctrl) or 1 µM RG for 24 hr before immunoblotting analysis.

(D) Apoptosis and cell cycle analysis of ABT-resistant MV-4-11_Mcl-1 and MOLM-13_Mcl-1 cells after 24 hr treatment with RG in the absence or presence of 1 µM ABT. The percentage of specific apoptosis was calculated as follows: 100 × (experimental apoptosis % – spontaneous apoptosis %)/(100 % – spontaneous apoptosis %).

Data in panels A, B, and D represent the means of triplicate experiments. Error bars, mean ± SD.