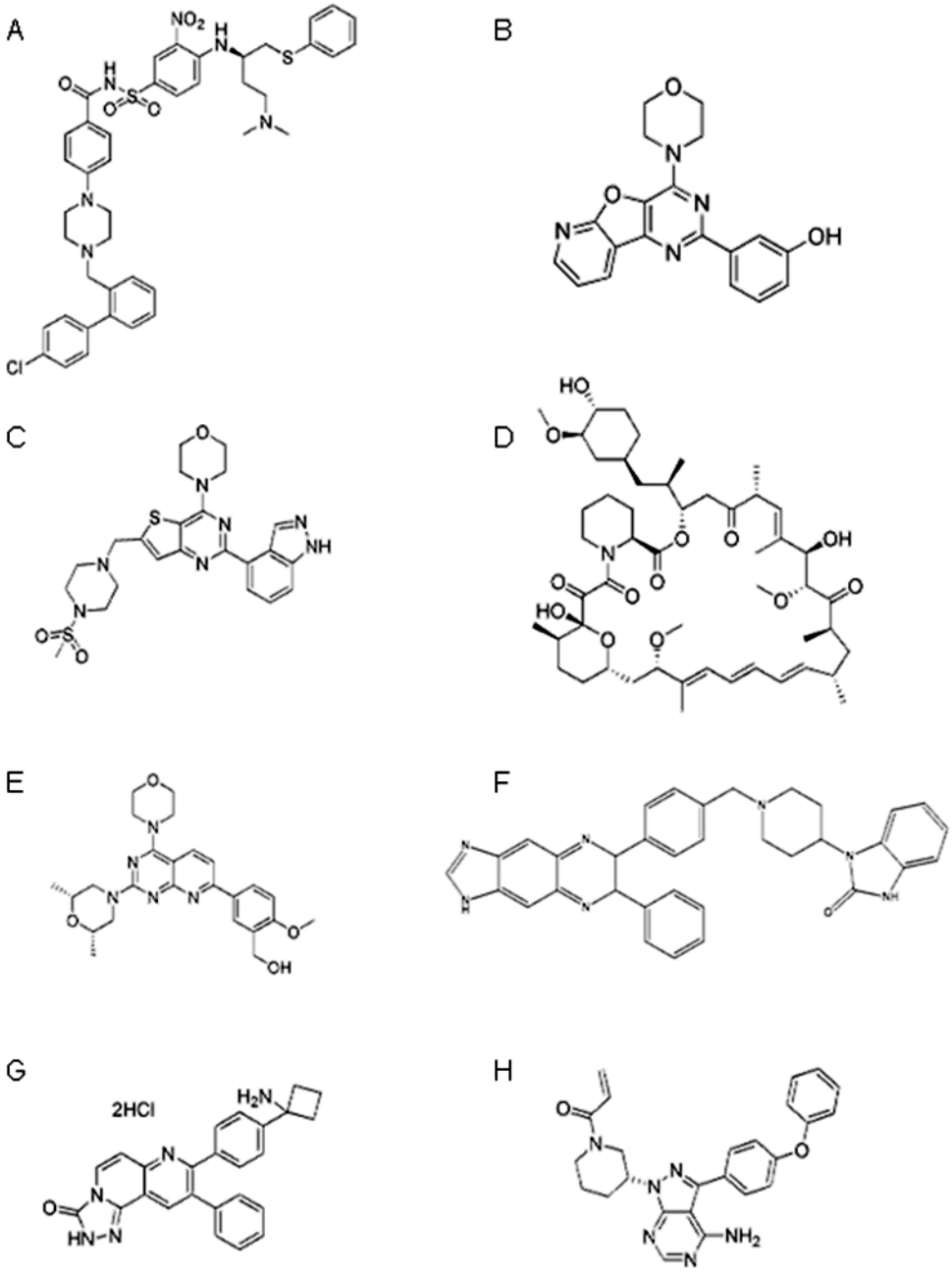

Figure W1. Structures of inhibitors used in study. (A) ABT-737, (B) PI-103, (C) GDC-0941, (D) rapamycin, (E) KU-0063794, (F) AKTi1/2, (G) MK-2206, and (H) PCI-32765.

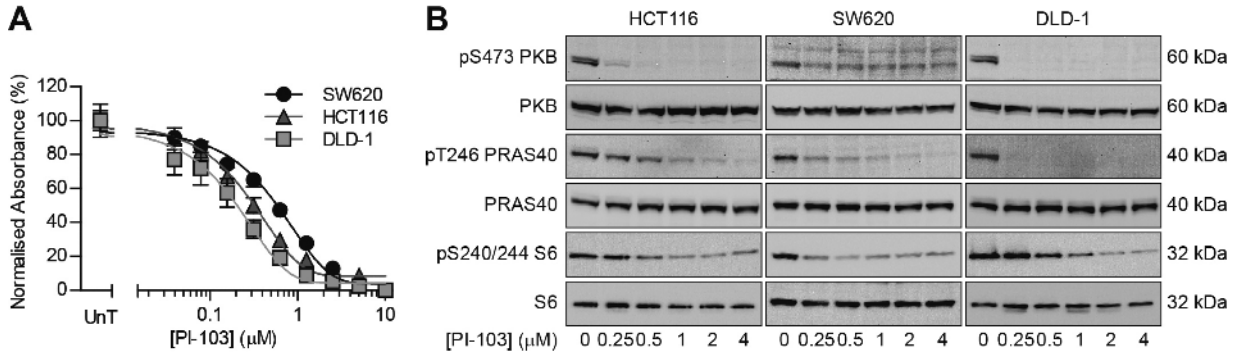

Figure W2. PI-103 inhibited cell proliferation and PI3K and mTOR signaling. (A) Cells were exposed to the indicated concentration of PI-103 for 3 days. Cells were fixed and stained with SRB, and the absorbance relative to untreated (UnT) cells was determined. Data represent the means of three independent experiments carried out in triplicate ± SEM. (B) Cells were exposed to the indicated concentration of PI-103 for 4 hours, and the effect on level of pS473 PKB, total PKB, pT246 PRAS40, total PRAS40, pS240/244 S6, and total S6 was determined by Western blot analysis. Results are representative of three independent experiments.

Table W1. Effect of MCL-1 RNAi on ABT-737 GI_{50}.

Cell Line	Treatment*	ABT-737 GI_{50} (μM ± 95% CI)	Significance†	
			vs NT DMSO	*vs* MCL-1 DMSO
HCT116	NT DMSO	5.83 (5.39-6.31)		
	NT PI-103	3.64 (3.20-4.15)	0.0037	
	MCL-1 DMSO	3.07 (2.59-3.65)	0.0027	
	MCL-1 PI-103	1.90 (1.75-2.06)	<0.0001	0.0079
SW620	NT DMSO	18.0 (17.1-19.0)		
	NT PI-103	3.54 (2.75-4.55)	0.0002	
	MCL-1 DMSO	2.09 (1.76-2.48)	<0.0001	
	MCL-1 PI-103	0.71 (0.56-0.92)	<0.0001	0.0022

The table relates to Figures 4*B*, *C*, and W6.

*Cells were transfected with either nontargeting siRNA (NT) or MCL-1 targeting siRNA (MCL-1). Cells were also treated with 2 μM PI-103 or a DMSO equivalent.

†Two-tailed unpaired *t* test *versus* indicated treatment for the same cell line.

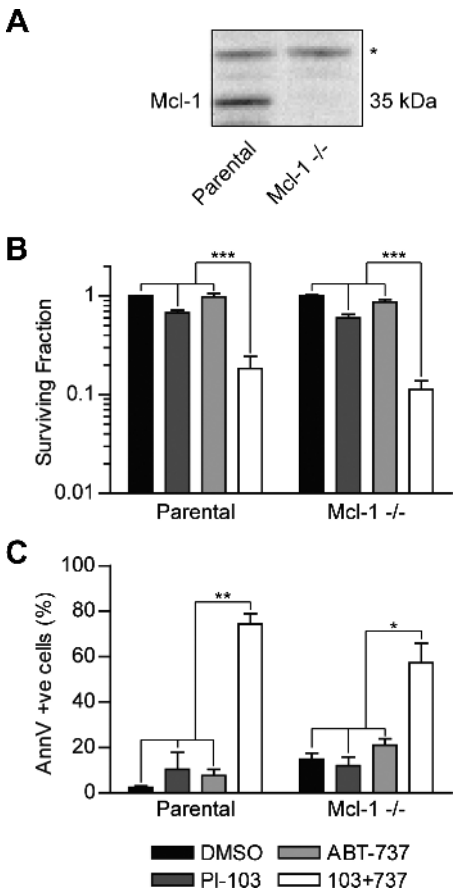

Figure W3. MCL-$1^{-/-}$ MEFs were sensitized to ABT-737–induced apoptosis by PI-103. (A) The level of MCL-1 in parental and MCL-$1^{-/-}$ MEFs was determined by Western blot analysis. *, nonspecific background band that acts as a loading control. (B) Parental and MCL-$1^{-/-}$ MEFs seeded at a low density were exposed to 2 μM PI-103 and/or 10 μM ABT-737 (parental) or 0.15 μM ABT-737 (MCL-$1^{-/-}$) for 3 days. Drugs were removed, and cells were left for 1 week for colonies to form. The number of colonies were counted and expressed as a surviving fraction relative to DMSO control. (C) Parental and MCL-$1^{-/-}$ MEFs were exposed to the same concentrations of PI-103 and ABT-737 as in B for 24 hours and stained with APC-conjugated annexin V. All graphs represent the means of three independent experiments carried out in duplicate ± SEM.

Table W2. Effect of PI3K Activity on ABT-737 GI_{50}.

Cell Line	PIK3CA Status	ABT-737 GI_{50} (μM ± 95% CI)	Significance*
HCT116	Parental	4.89 (4.55-5.24)	
	Mutant	8.16 (7.18-9.26)	0.0023
	Wild-type	3.40 (3.13-3.71)	0.0031
DLD-1	Parental	7.92 (7.29-8.61)	
	Mutant	6.97 (5.37-9.04)	0.4085
	Wild-type	3.76 (2.96-4.76)	0.0043

The table relates to Figure 3*B*.
*Two-tailed unpaired *t* test *versus* GI_{50} of parental cells for same cell line.

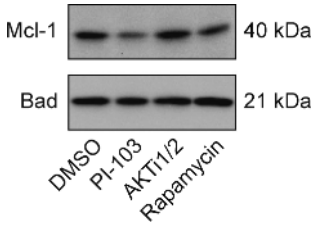

Figure W4. AKT and mTORC1 inhibition did not effect MCL-1 expression. HCT116 cells were treated with DMSO equivalent, 2 μM PI-103, 1 μM AKTi1/2, or 10 nM rapamycin for 24 hours, and the level of MCL-1 and Bad was determined by Western blot analysis.

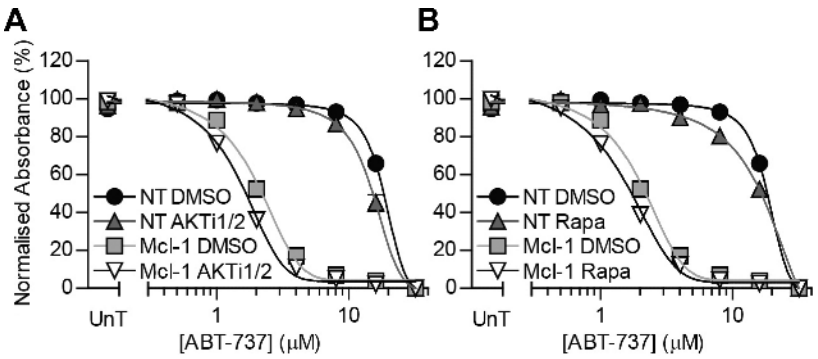

Figure W5. Neither AKTi1/2 nor rapamycin further sensitized MCL-1 knockdown SW620 cells to ABT-737. SW620 cells were transfected with NT RNAi or MCL-1 RNAi and plated for experiments 24 hours later. Cells were treated with 1 μM AKTi1/2 (A), 10 nM rapamycin (Rapa; B), or DMSO equivalent and the indicated concentration of ABT-737 for 3 days and processed as in Figure 1*A*. All graphs represent the means of three independent experiments carried out in triplicate ± SEM.

Table W3. Effect of MCL-1 RNAi on ABT-737 GI_{50}.

Cell Line	Treatment*	ABT-737 GI_{50} (μM ± 95% CI)	Significance†	
			vs NT DMSO	*vs* MCL-1 DMSO
SW620	NT DMSO	17.7 (17.3-18.2)		
	NT AKTi1/2	15.1 (13.0-17.7)	0.12	
	NT rapamycin	16.2 (14.5-18.1)	0.20	
	MCL-1 DMSO	2.1 (1.8-2.5)	<0.0001	
	MCL-1 AKTi1/2	1.6 (1.3-2.0)	<0.0001	0.11
	MCL-1 rapamycin	1.6 (1.3-2.1)	<0.0001	0.17

The table relates to Figure W5.
*Cells were transfected with either nontargeting siRNA (NT) or MCL-1 targeting siRNA (MCL-1). Cells were also treated with 1 μM AKTi1/2 or 10 nM rapamycin or a DMSO equivalent.
†Two-tailed unpaired *t* test *versus* indicated treatment for the same cell line.

Table W4. Results of siRNA Screen.

siRNA SMARTpool	Robust *Z* Score	*P* Value
MCL1	–4.669	4.66E-15
SOS1	–1.348	.018
PLEKHB2	–1.213	.030
BMX	–1.080	.002
SGK1	–0.944	.006
PIK3R2	–0.915	.074
AKT1	–0.746	.114
PHLDB3	–0.718	.013
PLEKHA2	–0.706	.089
SGK3	–0.649	.032
PIK3CD	–0.619	.067
AKT2	–0.535	.147
MCF2	–0.488	.261
DAPP1	–0.484	.156
FGD6	–0.456	.067
PREX2	–0.444	.054
ARAP3	–0.393	.149
VAV2	–0.382	.127
VAV1	–0.359	.140
ARHGEF4	–0.338	.183
DOCK1	–0.311	.336
GSK3B	–0.290	.163
PLEKHA1	–0.250	.216
TIAM1	–0.224	.206
PHLDB1	–0.197	.249
ADAP2	–0.186	.305
MTOR	–0.169	.320
GAB3	–0.141	.355
PIK3CA	–0.127	.419
ARAP1	–0.119	.434
SH3BP2	–0.079	.651
NT	–0.077	.539
SGK2	–0.056	.497
ARHGAP1	–0.055	.541
ADAP1	–0.045	.632
RASA2	–0.043	.552
VAV3	–0.025	.559
GSK3A	0.015	.726
SBF1	0.023	.733
PLCXD2	0.029	.732
CYTH4	0.031	.716
PLEK2	0.032	.758
ITK	0.046	.851
TEC	0.049	.796
PTPN9	0.067	.833
GAB1	0.078	.848
ARHGEF6	0.080	.865
SWAP70	0.083	.924
GAB2	0.084	.879
RASA3	0.131	.995
PREX1	0.167	.890
RICTOR	0.223	.830
PDPK1	0.224	.773
DOCK2	0.230	.712
CYTH2	0.301	.519
AKT3	0.303	.580
CYTH1	0.318	.498
ARAP2	0.345	.482
MYO10	0.482	.426
RASA1	0.572	.133
CYTH3	0.612	.201
BTK	0.751	.104
RPTOR	0.825	.075
AKAP13	0.861	.013
PLCL2	1.015	.008
PIK3R1	1.404	.001
PIK3CB	1.571	.002

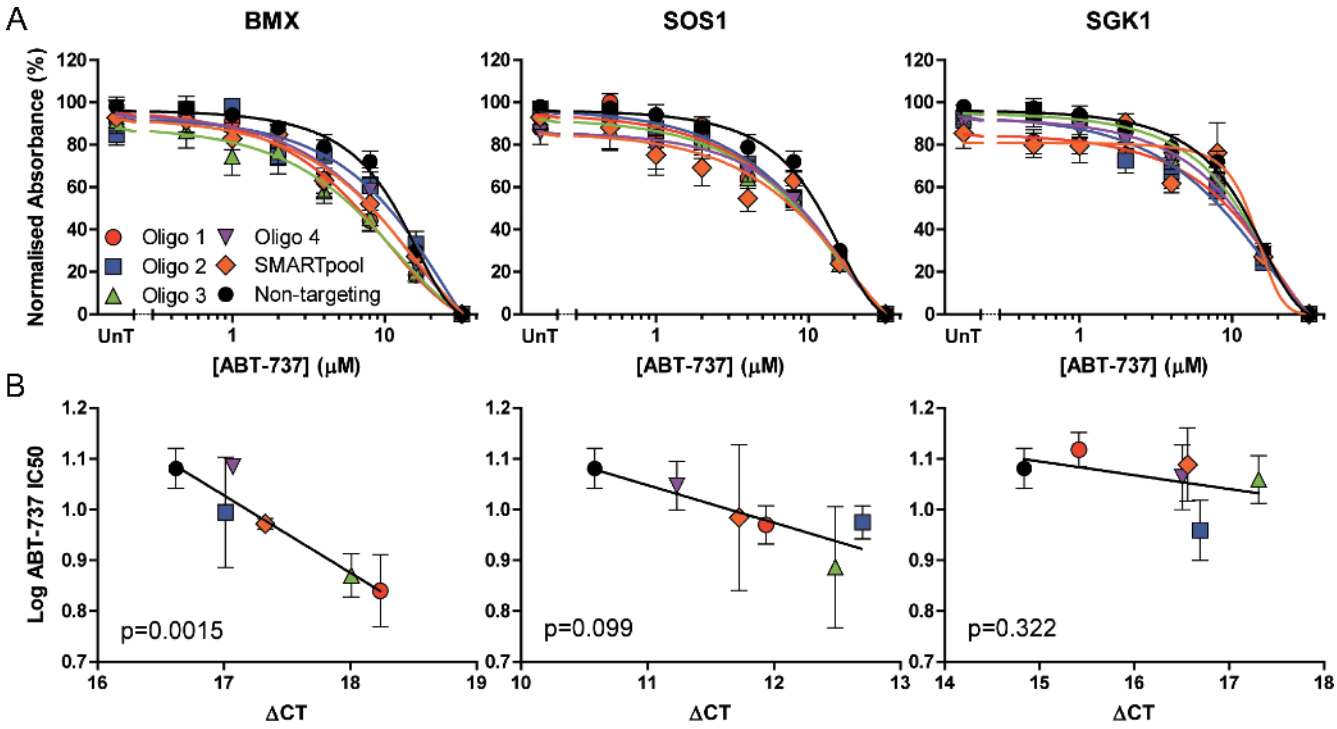

Figure W6. Deconvolution of BMX, SOS1, and SGK1 SMARTpool. SW620 cells were transfected with nontargeting siRNA, SMARTpool siRNA, or individual oligos targeting BMX, SOS1, or SGK1, and an ABT-737 concentration response was carried out, or the appropriate mRNA level was determined by qPCR. (A and B) A shows the concentration response curves, and B shows the correlation between knockdown efficiency and ABT-737 efficacy. All graphs represent the means of three independent experiments carried out in triplicate ± SEM.

Table W5. ABT-737 GI_{50} from Deconvolved siRNA Transfection.

RNAi Target	Oligo	ABT-737 GI_{50} (μM ± 95% CI)	Significance*
Nontargeting	SMARTpool	12.04 (10.07-14.10)	
BMX	1	6.92 (5.02-9.52)	0.041
	2	9.87 (6.06-16.08)	0.496
	3	7.42 (6.13-8.99)	0.022
	4	8.80 (4.68-16.56)	0.402
	SMARTpool	9.38 (8.95-9.83)	0.057
SOS1	1	9.33 (7.87-11.06)	0.113
	2	9.42 (8.16-10.91)	0.107
	3	7.70 (4.49-13.20)	0.198
	4	11.13 (8.98-13.81)	0.613
	SMARTpool	9.64 (5.04-18.44)	0.552
SGK1	1	13.11 (11.26-15.26)	0.516
	2	9.10 (6.96-11.89)	0.163
	3	11.46 (9.26-14.17)	0.744
	4	11.58 (8.69-15.44)	0.832
	SMARTpool	12.25 (8.85-16.96)	0.931

The table relates to Figures 4, *B* and *C*, and W4.
*Two-tailed unpaired *t* test *versus* nontargeting siRNA ABT-737 GI_{50}.

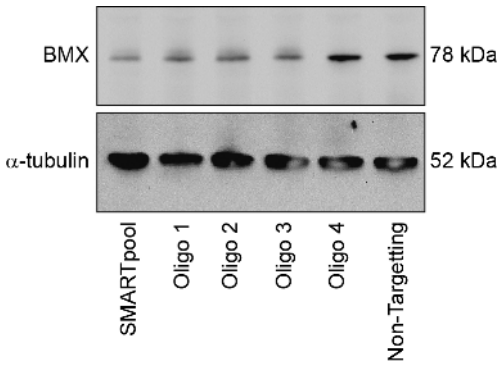

Figure W7. BMX RNAi reduced levels of BMX protein. SW620 cells were transfected with BMX siRNA SMARTpool, individual BMX siRNA oligos, or nontargeting control siRNA SMARTpool. After 48 hours, cells were harvested, and the level of BMX was assayed by Western blot analysis. Tubulin was used as a loading control. Blots are representative of three independent experiments.

Table W6. ABT-737 GI_{50} from HCT116 BMX RNAi.

RNAi Target	ABT-737 GI_{50} (μM ± 95% CI)	Significance*
Nontargeting	5.32 (4.40-6.43)	
BMX	2.94 (2.83-3.05)	0.027
MCL-1	1.70 (1.28-2.24)	0.022

The table relates to Figure 4*D*.
*Two-tailed unpaired *t* test *versus* nontargeting siRNA ABT-737 GI_{50}.

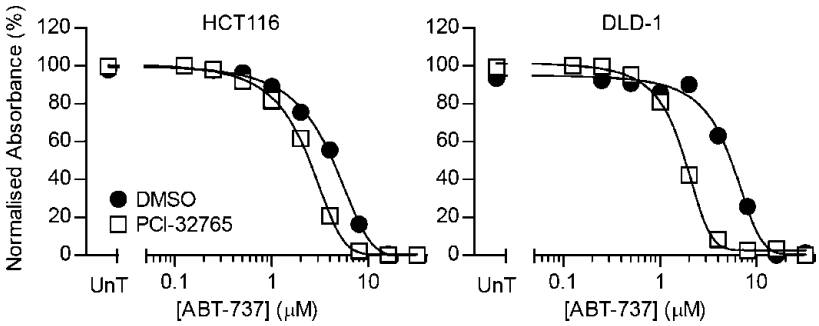

Figure W8. PCI-32765 increased the sensitivity of HCT116 and DLD-1 cells to ABT-737. HCT116 and DLD-1 cells were treated with 4 μM PCI-32765 or DMSO equivalent and the indicated concentration of ABT-737 for 3 days and processed as in Figure 1*A*. All graphs represent the means of three independent experiments carried out in triplicate. SEM error bars are not visible due to being smaller than the symbols.

Table W7. ABT-737 GI_{50} from SW620, HCT116 and DLD-1 PCI-32765 Treatment.

Cell Line	Treatment	ABT-737 GI_{50} (μM ± 95% CI)	Significance*
SW620	DMSO	10.8 (10.3-11.2)	
	4 μM PCI-32765	6.4 (5.9-6.9)	0.0005
HCT116	DMSO	4.2 (3.8-4.7)	
	4 μM PCI-32765	2.3 (2.0-2.7)	0.005
DLD-1	DMSO	5.4 (5.1-5.8)	
	4 μM PCI-32765	1.8 (1.7-1.8)	>0.0001

The table relates to Figures 4*E* and W8.
*Two-tailed unpaired *t* test *versus* nontargeting DMSO-treated ABT-737 GI_{50}.

Table W8. ABT-737 GI_{50} from SW620 BMX RNAi +/– PI-103.

Treatment*	ABT-737 GI_{50} (μM ± 95% CI)	Significance†	
		vs NT DMSO	*vs* NT PI-103
NT DMSO	15.4 (14.3-16.6)		
NT PI-103	3.79 (3.02-4.76)	0.0003	
BMX oligo 1 DMSO	8.0 (7.2-8.87)	0.0006	
BMX oligo 1 PI-103	2.93 (2.4-3.57)	0.0001	0.171

The table relates to Figure 4*F*.
*Cells were transfected with either nontargeting siRNA (NT) or BMX oligo 1. Cells were also treated with 2 μM PI-103 or a DMSO equivalent.
†Two-tailed unpaired *t* test *versus* indicated treatment for the same cell line.

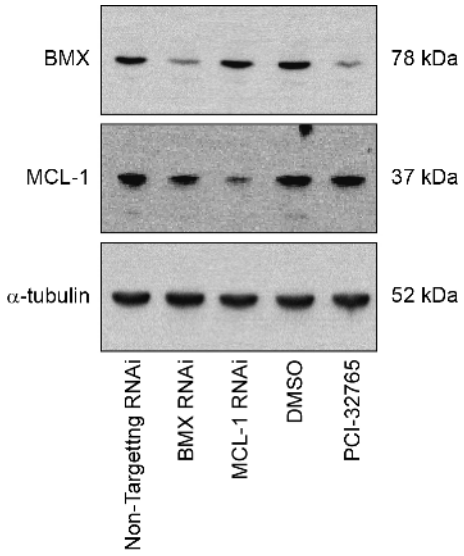

Figure W9. Neither BMX RNAi nor inhibition affected levels of MCL-1. SW620 cells were transfected with nontargeting siRNA, BMX siRNA, or MCL-1 siRNA SMARTpool and harvested 48 hours later to be treated with 4 μM PCI-32765 or DMSO equivalent and harvested 24 hours later. The level of BMX and MCL-1 was assayed by Western blot analysis. Tubulin was used as a loading control. Blots are representative of three independent experiments.