Supplementary Information

Williamson et al, Enhanced cytotoxicity of PARP inhibition in Mantle Cell Lymphoma harboring mutations in both ATM and p53

CONTENTS:

Primer Sequences used for quantitative PCR

- *CDKN1A*: CTCAAATCGTCCAGCGACCTT and CATTGTGGGAGGAGCTGTGAA
- NOXA: TAAAGCAAGAATGGAAGAC and GACCGAAGAAATCAACAC
- PUMA: GAAAGGCTGTTGTGCTGG and TCCCTCTTCCGAGATTTC
- *ACTB*: GGGCATGGGTCAGAAGGAT and GTGGCCATCTCTTGCTCGA
- *GAPDH:* AACAGCGACACCCACTTCTC and GGAGGGGAGATTCAGTGTGG

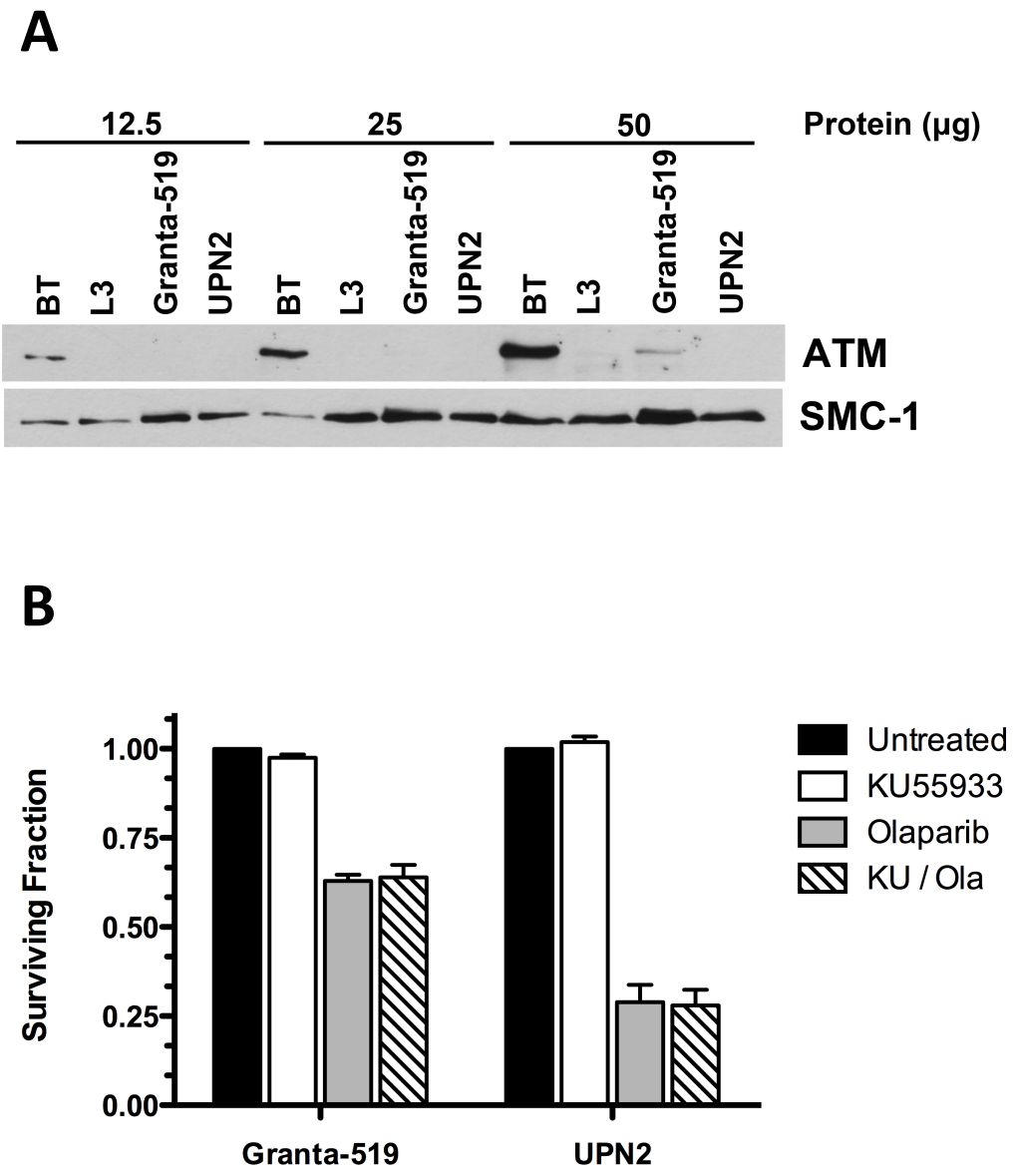
Supplemental Table 1: Genetic properties of MCL cell lines

Summary of the published genetic features of several Mantle Cell Lymphoma cell lines, with respect to *ATM*, ATM function (as determined in Williamson et al. MCT 2010), *TP53*, predicted p53 transcriptional activity (based on TP53 mutation / function data base search of the International Agency for Research on Cancer which predicts mutant p53 transcriptional function based on Kato *et al.* PNAS 2003) and presence of the t(11;14) translocation which defines MCL as a lymphoma subtype.

EBV, Epstein Barr Virus; ND, Not Determined; Del, Allele Deletion; Pm, polymorphism; Mut, Mutation; *, mutation in ATM kinase domain; NF, not functional; †, mutation in p53 DNA binding domain

Cell Line	*ATM* status (Allele 1 / Allele 2)	ATM Function	*TP53* status (Allele 1 / Allele 2)	Predicted p53 transcriptional activity	t(11;14)
Granta-519	Del / Mut (R2832C)*	-	WT / Del	WT	+
HBL-2	ND / ND	+	Del / Mut (D281G)†	NF	+
JVM-2	WT / WT	+	WT / WT	WT	+
UPN1	WT / Pm (S333F)	+	Del / Mut (E286K)†	NF	+
UPN2	Del / Mut (D2725V)*	-	Del / Mut (R175H)†	NF	+
Z138	ND / ND	+	WT / WT	WT	+

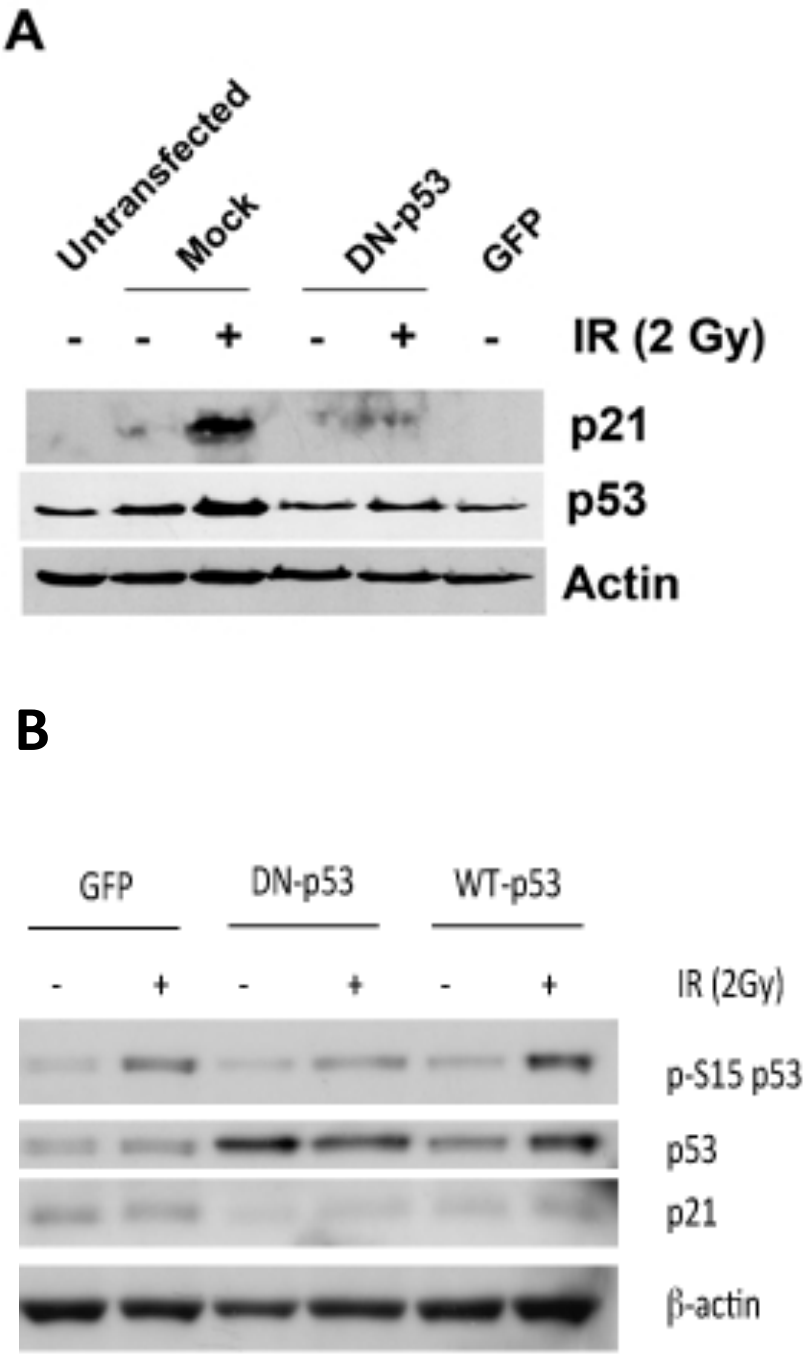
References: Kato S, Han S-Y, Liu W, et al. Proc Natl Acad Sci USA 2003;100:8424-9; Williamson CT, Muzik H, et al, Mol Cancer Ther 2010;9:347-57.



Supplementary Figure 1- Inhibition of ATM has no effect on the sensitivity of Granta-519 or UPN2 cells to olaparib

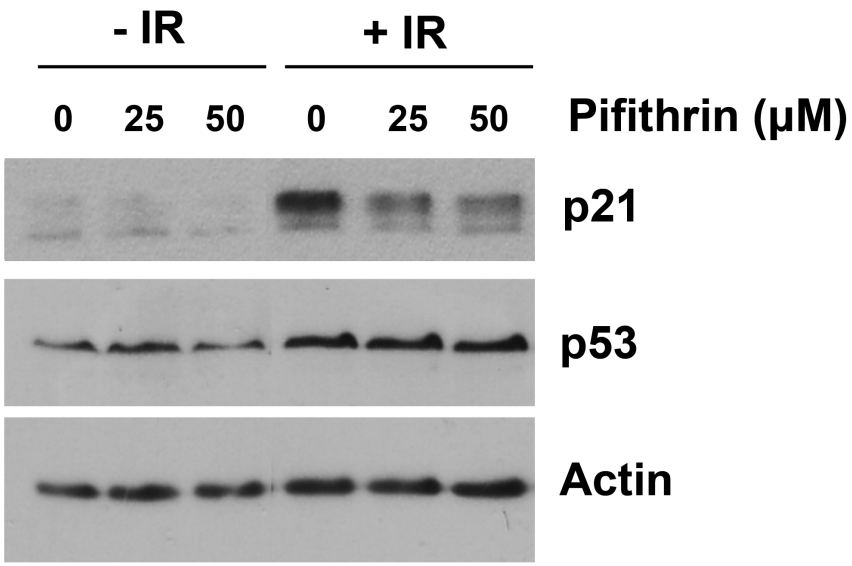
A) Whole cell lysates from BT, L3, Granta-519 and UPN2 cells, of 12.5, 25 and 50 µg of protein, was run on SDS-PAGE and western blot probed with the indicated antibody.

B) Granta-519 and UPN2 cells were exposed to KU55933 (5 µM), olaparib (5 µM) or both for 96 hours prior to determining cellular viability by trypan blue. N = 3 and error bars represent SEM.



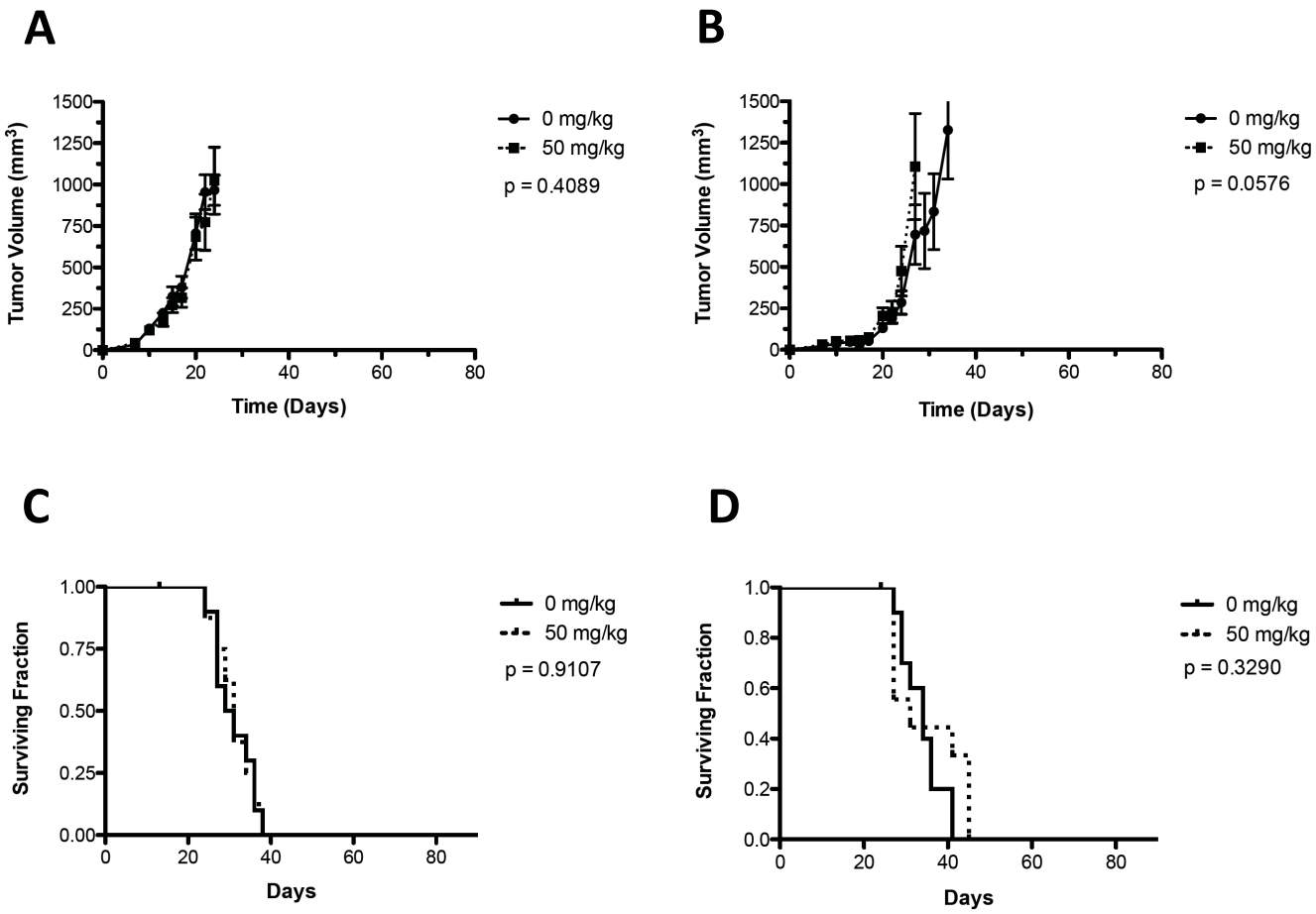
Supplementary Figure 2- Expression of dominant negative p53 in Granta-519 disrupts p53 transcriptional activity

Granta-519 (A) and UPN2 (B) cells were mock or transfected with dominant negative (DN) p53 or GFP expressing vectors. Following transfection cells were treated with 2 Gy IR, incubated 8 hrs and western blots examined for p21, p53 and actin.



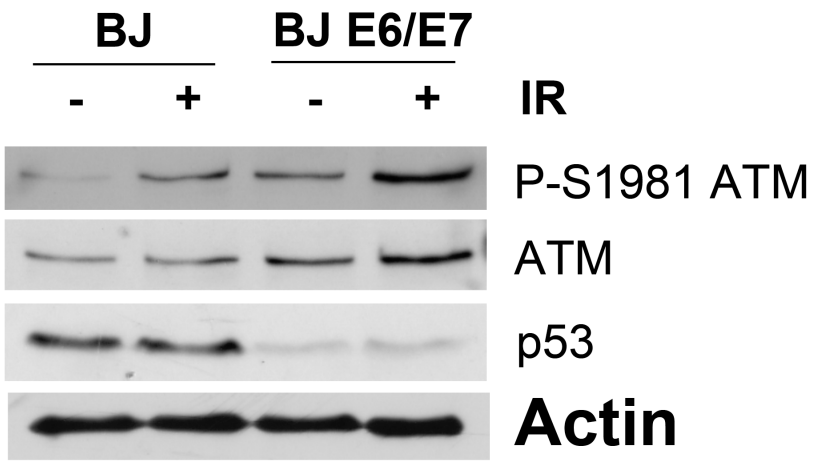
Supplementary Figure 3 – Pifithrin reduces p53 transcriptional activity in response to IR

BT cells were incubated with the indicated concentration of pifithrin for 1 hour prior to mock treatment or irradiation with 2 Gy. 8 hour following IR treatment cells were lysed by NET-N extraction and western blots of whole cell extracts were probed with the indicated primary antibodies



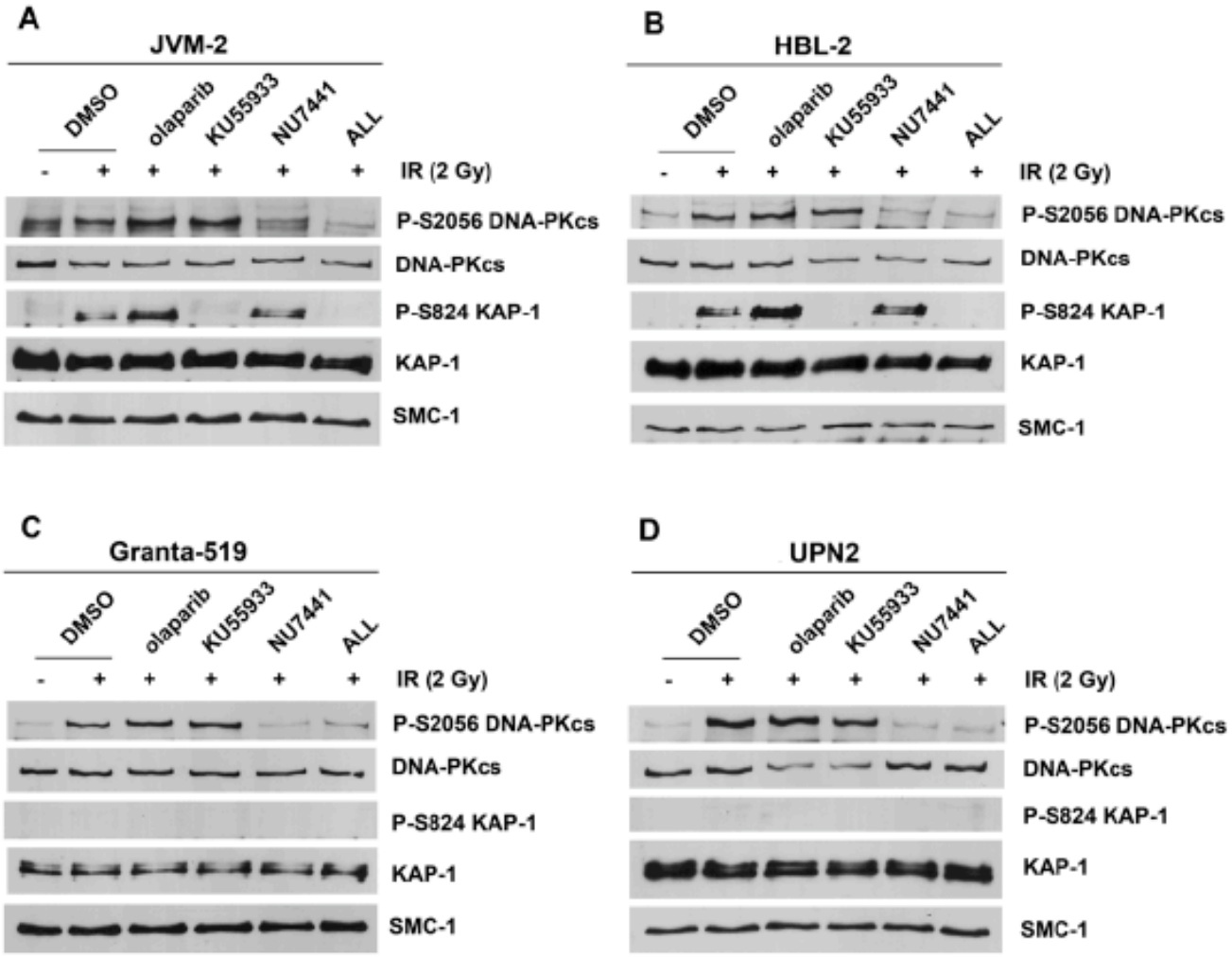
Supplementary Figure 4- Effect of olaparib on ATM-proficient MCL xenografts

Average tumor volume of RAG2-/- mice bearing xenografts with either JVM-2 (A) or HBL-2 (B) cell lines. Overall survival of mice bearing xenografts of JVM-2 (C) and HBL-2 (D) xenografts. Animals were treated with vehicle (solid line) or 50mg/kg olaparib (dotted line) daily. P-values on graph is from 2-way ANOVA comparing saline and drug treated TV, $p<0.05$ is considered statistically significant.

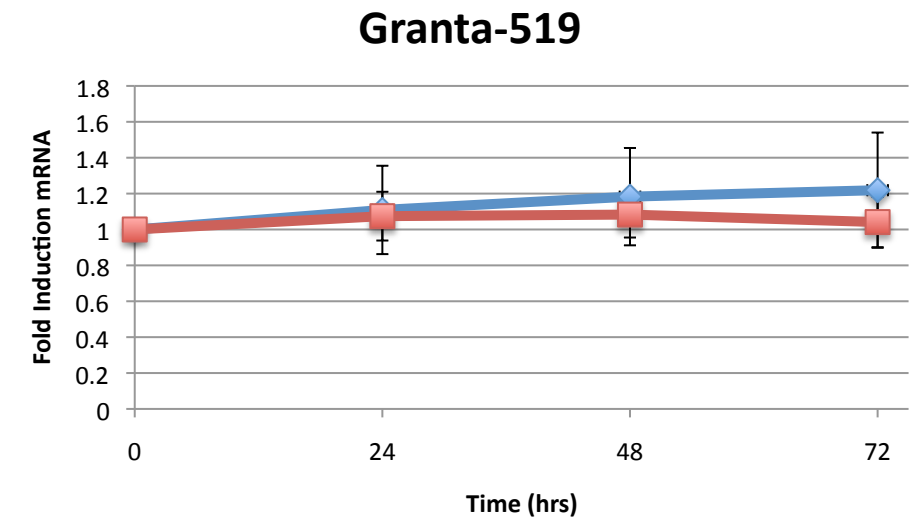


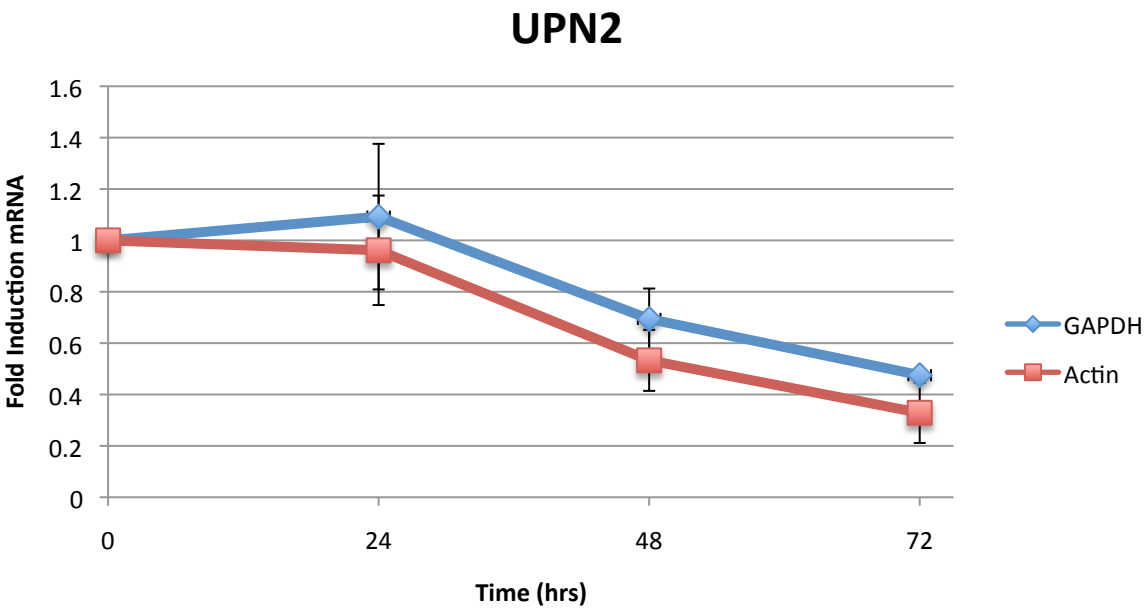
Supplementary Figure 5- Normal ATM activation in BJ and BJ-E6/7 cells

BJ cells and BJ E6/E7 cells were left untreated or exposed to 2 Gy of IR and incubated 1 hour prior to cell lysis. Extracts were probed with the indicated antibodies.

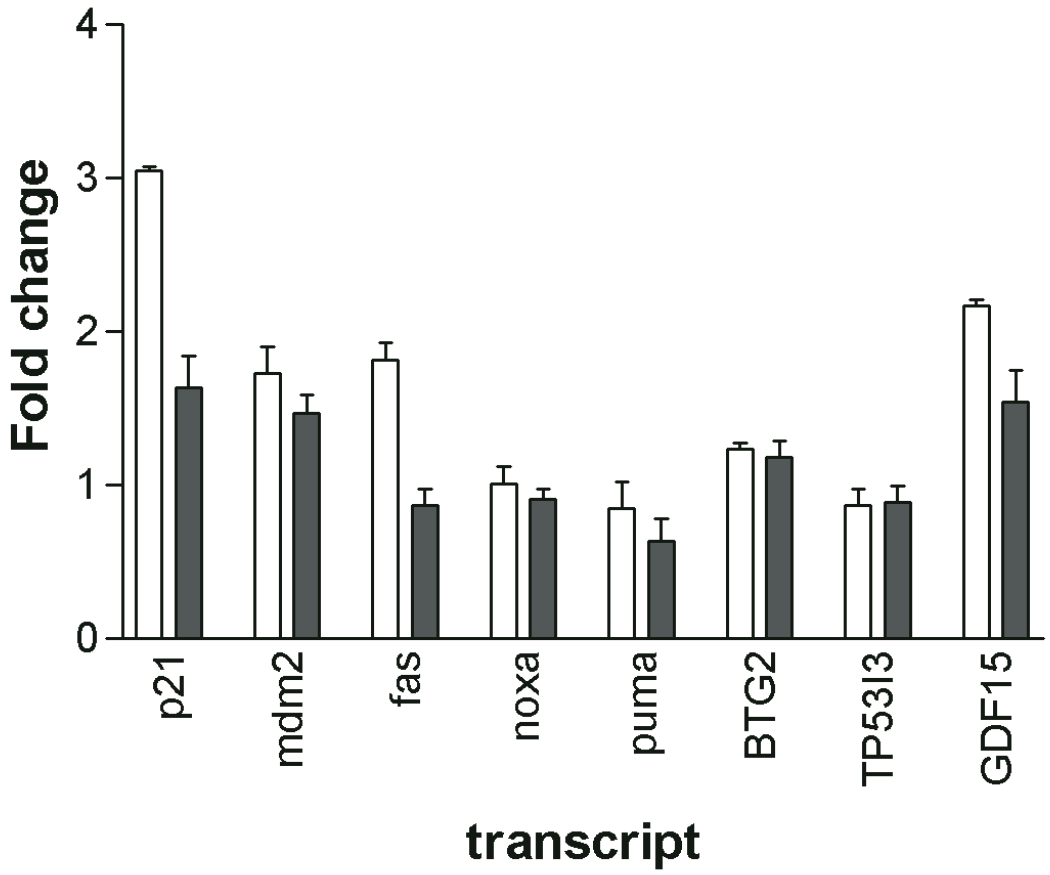


Supplementary Figure 6- Specificity of ATM and DNA-PK inhibitors in MCL cell lines
JVM-2 (A), HBL-2 (B), Granta-519 (C), and UPN2 (D) were incubated with the ATM inhibitor KU55933 (5 µM), DNA-PK inhibitor NU7441 (5 µM) or PARP inhibitor olaparib (5 µM) alone or in combination prior to IR (2 Gy) treatment. Cells were lysed following 1 hour in western blots probed with the indicated antibodies.

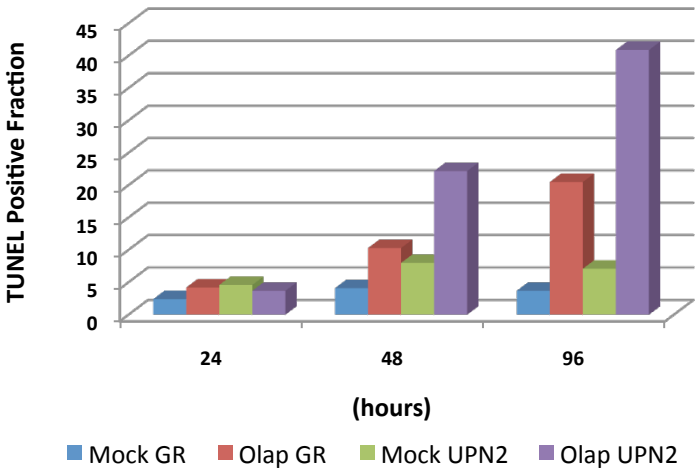




Supplementary Figure 7A-
Level of control mRNA genes (GAPDH and Actin) in Granta-519 and UPN2 cells exposed to Olaparib (2.5 µM) over time. Quantitative PCR was carried out as described in the main text. Equal amounts of RNA were used to generate cDNA for qRT-PCR, as described in the materials and methods. The amount of PCR product generated with GAPDH- and actin-specific primers was found to decrease in olaparib treated UPN2 cells.



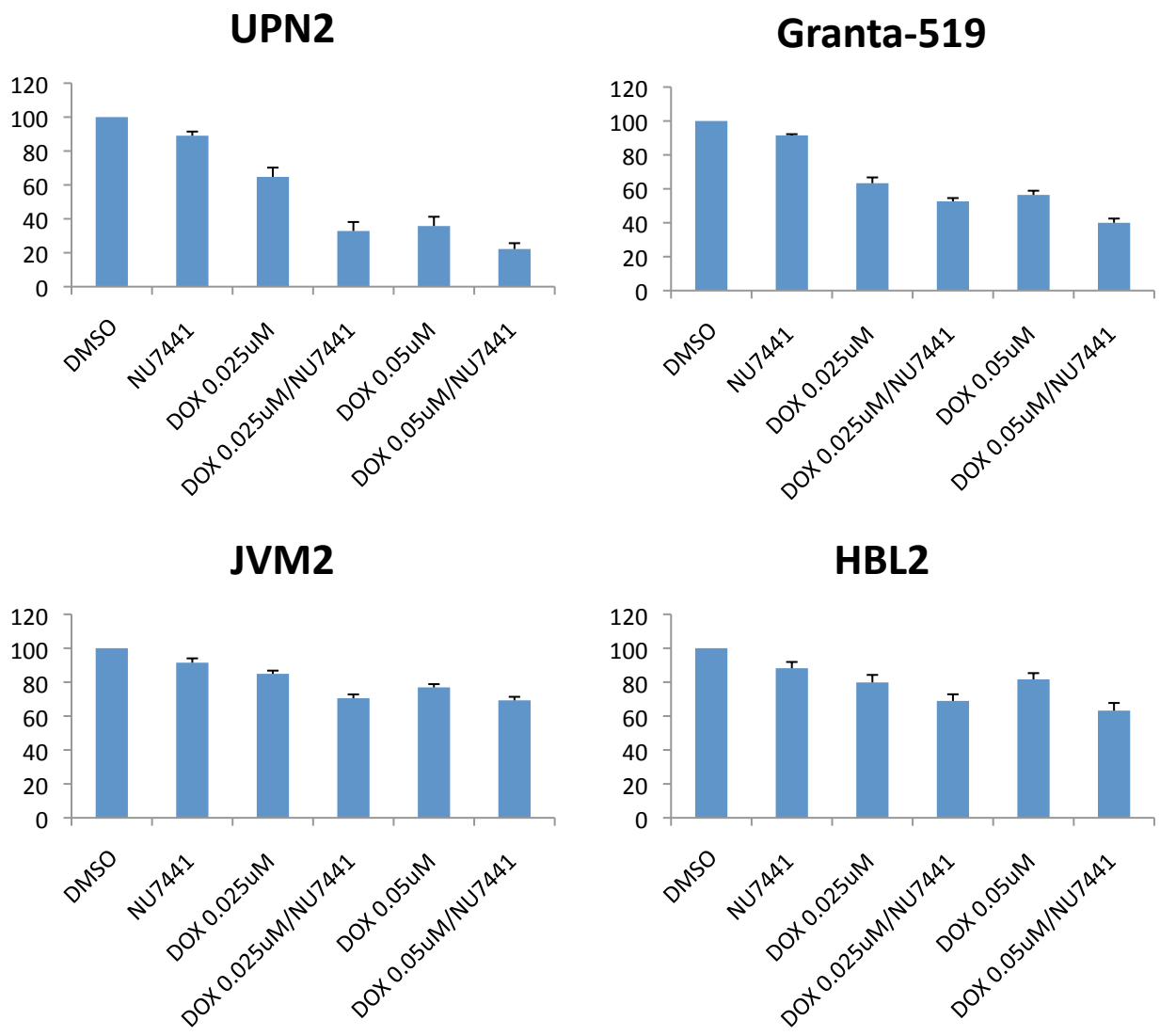
Supplementary Figure 7B-
Transcript level of mRNA of various p53-responsive genes in Granta-519 (white bars) and UPN2 (grey bars) 3 hours following 2 Gy of IR. Equal amounts of RNA were used to generate cDNA for qRT-PCR, as described in the materials and methods. The amount of PCR product generated with GAPDH- and actin-specific primers decreased in olaparib treated samples.



Granta-519		
Sample	Time (hour)	Apotosis (%)
Mock treated	24	2.4
Mock treated	48	4.1
Mock treated	96	3.7
2.5uM Olaparib	24	4.2
2.5uM Olaparib	48	10.3
2.5uM Olaparib	96	20.5
UPN2		
Mock treated	24	4.6
Mock treated	48	8
Mock treated	96	7.1
2.5uM Olaparib	24	3.7
2.5uM Olaparib	48	22.2
2.5uM Olaparib	96	40.9

Supplementary Figure 8- Olaparib induces apoptosis in UPN2 and Granta-519 cells

Granta and UPN2 cells were mock treated, or exposed to olaparib (2.5µM) for 24, 48 and 96 hours prior to TUNEL assay. TUNEL assays were carried out as described in Williamson et al, 2010.



Supplementary Figure 9- Inhibition of DNA-PK does not rescue the toxicity of doxorubicin in MCL cell lines. MCL cells were incubated with doxorubicin or NU7441 (5 µM) as indicated for 96 hours. Viability was determined by trypan blue staining. Results were analyzed by Student's T-test. N= 6. p values are indicated by * = $p<0.05$, ** = $p<0.01$. Actual P values are given below:

UPN2

DOX 0.025uM vs DOX 0.025uM/NU7441 (p = 0.0029),
DOX 0.05uM vs DOX 0.05uM /NU7441 (p = 0.0695)

Granta-519

DOX 0.025uM vs DOX 0.025uM/NU7441 (p = 0.0251),
DOX 0.05uM vs DOX 0.05uM /NU7441 (p = 0.0026)

JVM 2

DOX 0.025uM vs DOX 0.025uM/NU7441 (p = 0.0010),
DOX 0.05uM vs DOX 0.05uM /NU7441 (p = 0.0299)

HBL2

DOX 0.025uM vs DOX 0.025uM/NU7441 (p = 0.1168),
DOX 0.05uM vs DOX 0.05uM /NU7441 (p = 0.0082)