Table E1: Catalogue numbers and manufacturer's identification for siRNAs

Gene	siRNA a	siRNA b	siRNA c
ATIC	Hs_ATIC_4 Cat. No. SI00306110 *Referred to as siATICa in text*	Hs_ATIC_5 Cat. No. SI04211452 *Referred to as siATICb in text*	Hs_ATIC_6 Cat. No. SI04273682 *Referred to as siATICc in text*
ATM	Hs_ATM_3 Cat. No. SI00000840	Hs_ATM_4 Cat. No. SI00000847	Hs_ATM_8 Cat. No. SI00604730
NBS1	Hs_NBS1_1 Cat. No. SI00038479	Hs_NBS1_3 Cat. No. SI00038493	Hs_NBS1_6 Cat. No. SI02663570
MTPAP	Hs_PAPD1_4 Cat. No. SI00677516	Hs_PAPD1_5 Cat. No. SI03126242	Hs_PAPD1_6 Cat. No. SI04166645

Figure E1: Knockdown efficiency of siATICa in 3 different cancer cell lines: Cell lines were treated with the indicated concentration of siATICa for 48 hours and whole cell lysates were immunoblotted with anti-human ATIC.

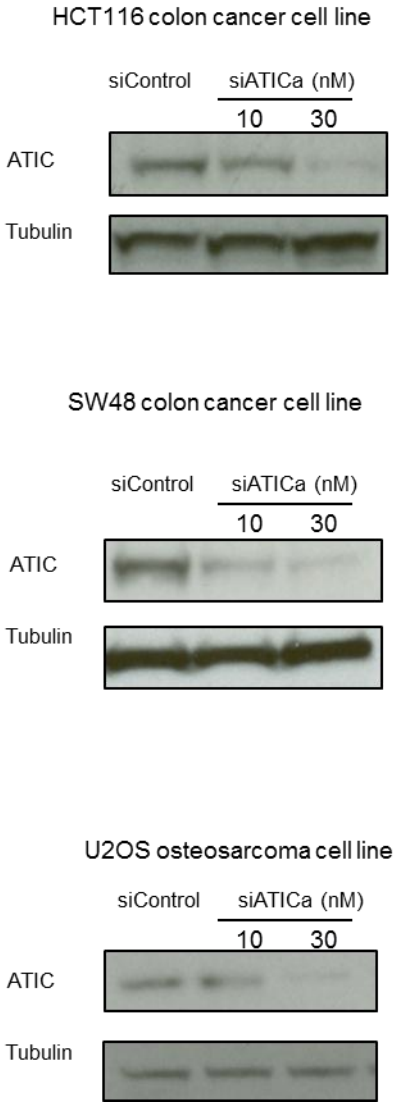

Figure E2. Effect of 3 different siRNAs directed against *ATIC* on the radiation survival of U2OS cells. (**A**) Cells were treated with the indicated concentrations of siATICa, siATICb, or siATICc for 48 hours prior to irradiation at 0, 1, 2, and 4 Gy. Surviving fraction relative to the 0 Gy treatment is plotted. (**B**) Representative pictures of colonies counted to generate (**A**).

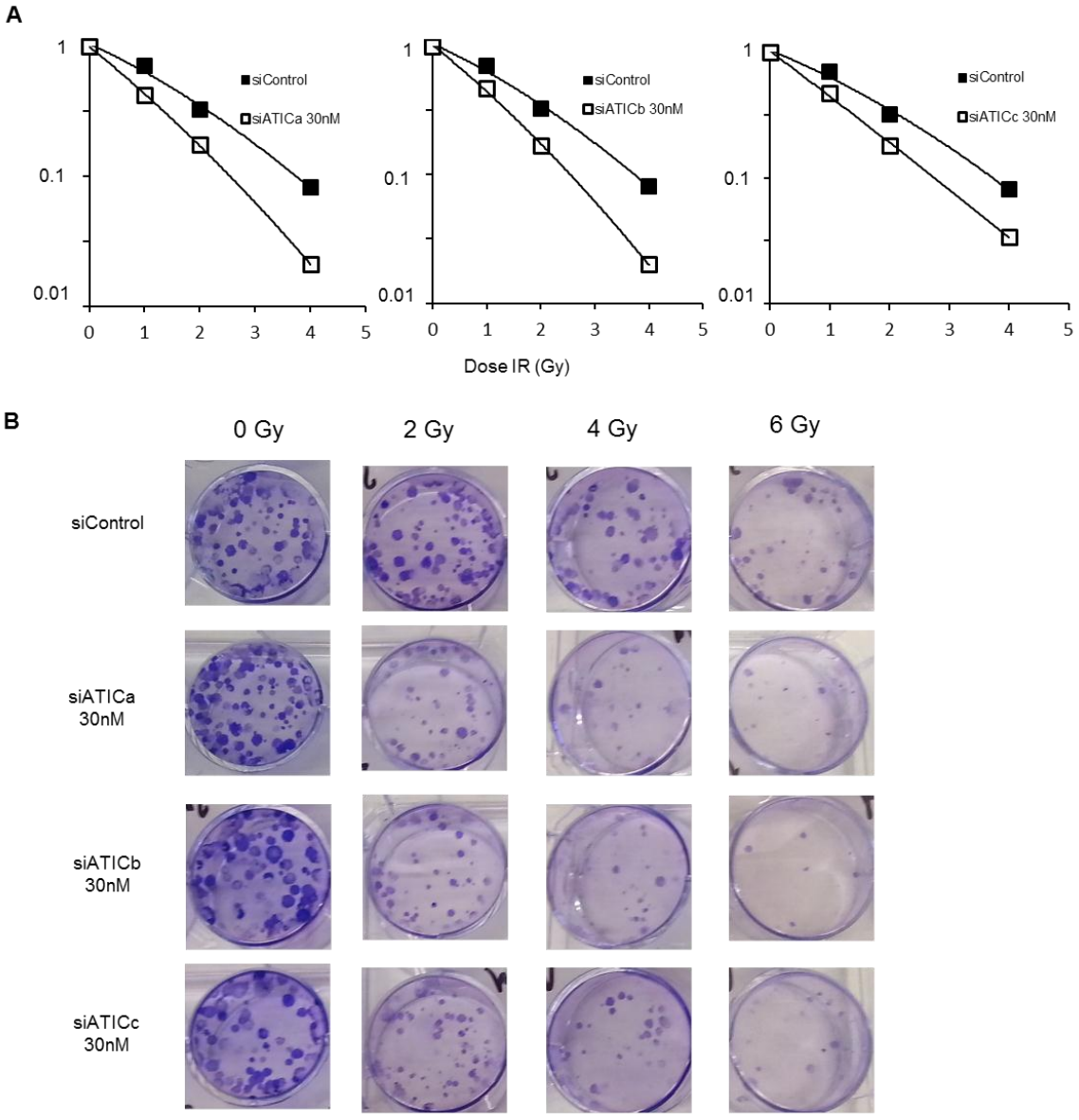

Figure E3. Small molecule inhibitors of ATIC transformylase activity radiosensitize. HCT116 cells were treated with the indicated concentrations of different ATIC inhibitors for 48 hours, treated with 0, 1, 2, or 4 Gy of ionizing irradiation, and plated. Colonies were counted after 3 weeks of growth. The surviving fraction was normalized to the colony number at 0 Gy.

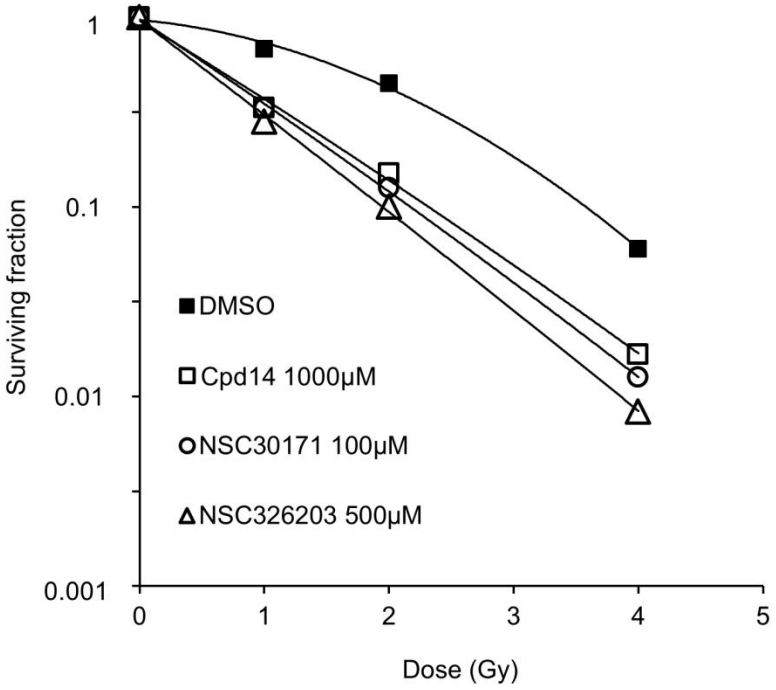

Figure E4. ATIC inhibition or depletion activates cell cycle checkpoints in SW48 cells. (A) DNA content was assessed by flow cytometry in untreated cells and cells treated with siATICa or Cpd14. Cell cycle distributions are indicated as: ☐ G1 and G2/M, ▨ S phase. SW48 cells treated with either siATICa (**B**) or Cpd14 (**C**) at indicated doses were immunoblotted for checkpoint proteins. β Actin serves as a loading control.

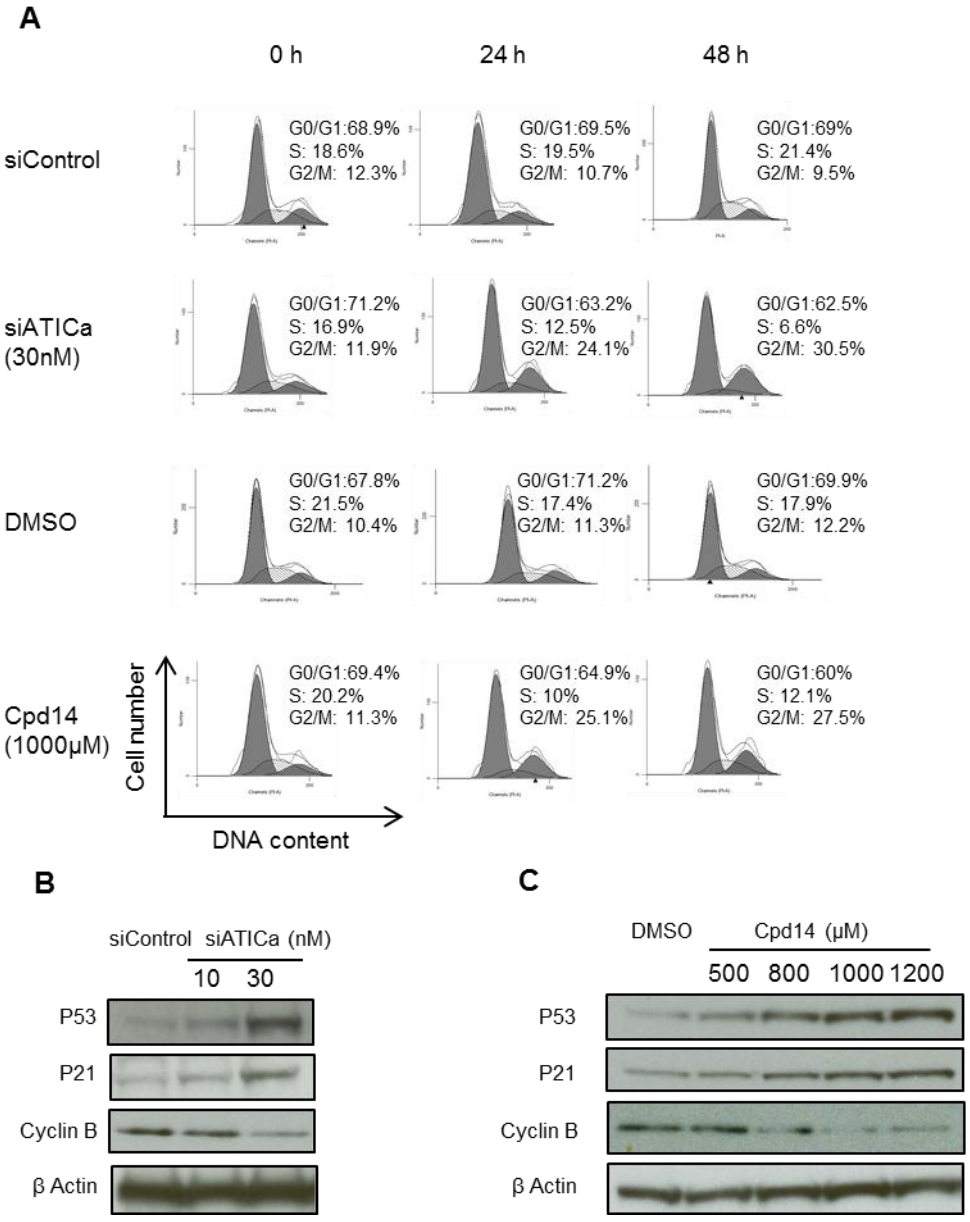

Figure E5. ATIC inhibition or depletion activates cell cycle checkpoints in U2OS cells. (A) DNA content was assessed by flow cytometry in untreated cells and cells treated with siATICa or Cpd14. Cell cycle distributions are indicated as: ☐ G1 and G2/M, ▨ S phase. U2OS cells treated with either siATICa (**B**) or Cpd14 (**C**) at the indicated doses were immunoblotted for checkpoint proteins. β Actin serves as a loading control.

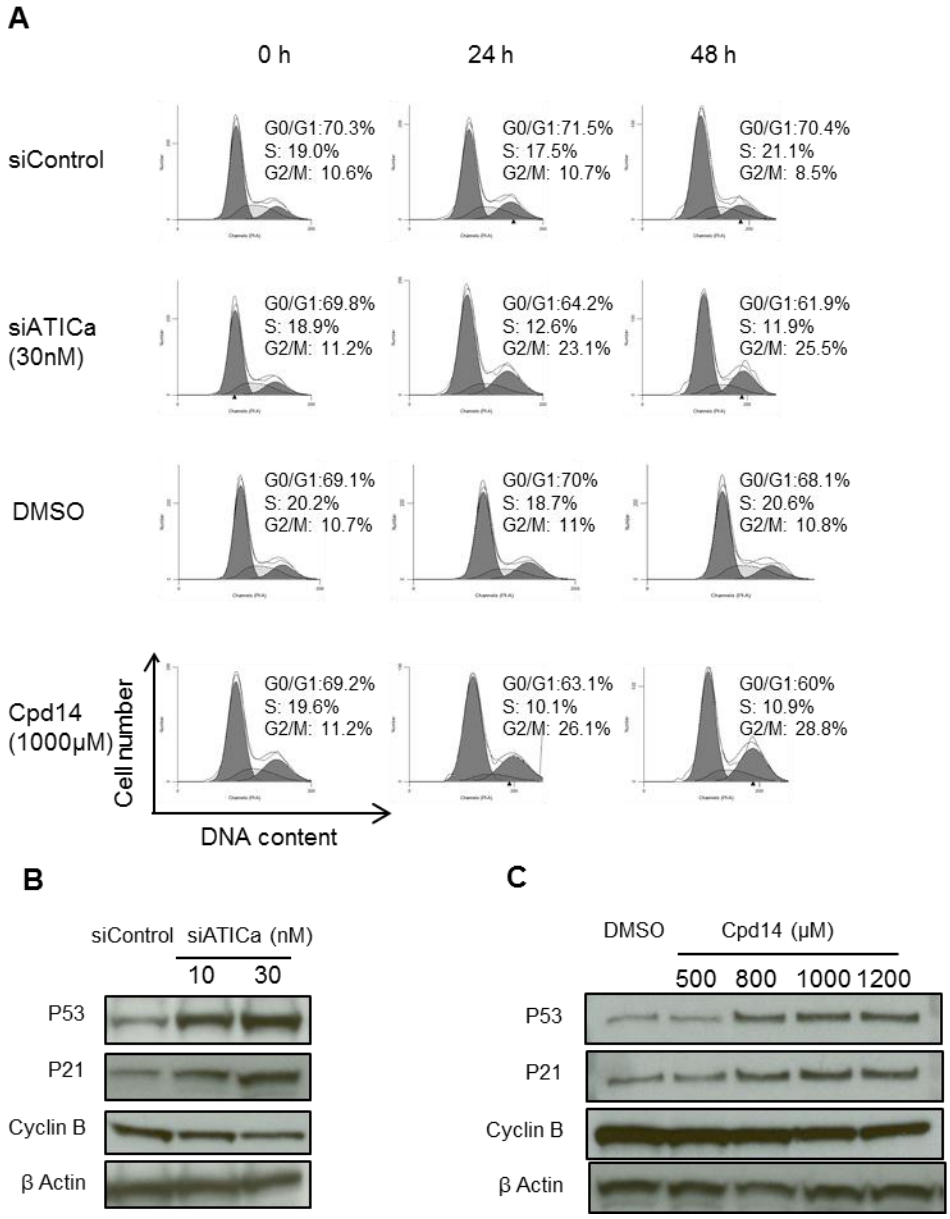

Figure E6. Effect of ATP supplementation on the reversion of siATICa induced effects. HCT116 cells were treated with a control siRNA (siControl) or 30nM siATICa for 48 hours and either not supplemented (open bars) or supplemented to the final indicated concentrations of ATP (filled bars) one hour prior to irradiation (2 Gy). DSB repair was measured via the neutral comet assay as described in the methods section. Percent repair (% DNA repair) was determined by monitoring the return of the tail moments (TM) of comets to baseline levels ((TM_{1hrs} -$TM_{12\ hrs}$)/TM_{1hrs}). * indicates $P<0.05$.

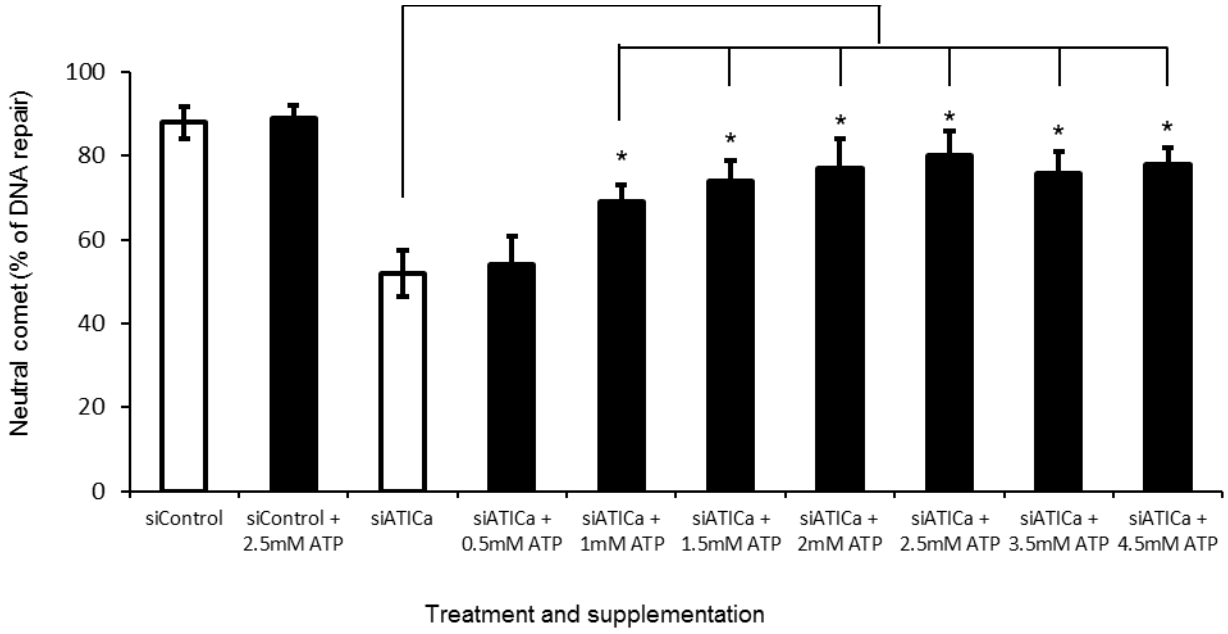

Figure E7. Effect of supplementation with different nucleotide triphosphates on the reversion of siATICa induced effects. HCT116 cells were treated with a control siRNA (siControl) or 30nM siATICa for 48 hours and either not supplemented (open bars) or supplemented with 2.5mM ATP, GTP, CTP, or TTP (filled bars) one hour prior to irradiation (2 Gy). DSB repair was measured via the neutral comet assay as described in method section. Percent repair (% DNA repair) was determined by monitoring the return of the tail moments (TM) of comets to baseline levels ((TM_{1hrs} -$TM_{12\ hrs}$)/TM_{1hrs}). * indicates $P<0.05$.