Supplemental Experimental Procedures

Generation of BLM protein segments and mutants.

For immunoprecipitation experiments with topoisomerase IIα, BLM N-terminal segments were generated by PCR amplification from the *pEGFP-BLM* vector using the following forward primers: BLM-N1 (5'AAGCTGGATCCTAATACGACTCACTATAG GGAGCCACCATGGCTGCTGTTCCTC-3'), BLM-N101 (5'AAGCTGGATCCTAATA CGACTCACTATAGGGAGCCACCATGGGTGGATCAAAATC-3'), BLM N203 (5'-AAGCTGGATCCTAATACGACTCACTATAGGGAGCCACCATGGTAAAGACTG ATTTGC-3'), BLM-N301 (5'-AAGCTGGATCCTAATACGACTCACTATAGGGAGCC ACCATGCCACCTTCTCCAGAAG-3'), and BLM-N400 (5'AAGCTGGATCCTAATA CGACTCACTATAGGGAGCCACCATGCTTCAGCAGCGGAAC-3') with BLM-NR587 (5'GATCTTTACTTGATGGGTTGATAGGC-3') as the reverse primer. BLM 1-488 was generated using forward primer BLM-N1 and reverse primer BLM-NR488 (5'-GATCTTTACCTTTCAAAAAGATTCTTCC-3').

pEGFP-BLM$^{\Delta 489\text{-}587}$ was generated by sequential mutations of bases encoding amino acids 488-489 and 587-588 within the *pEGFP-BLM* vector to *BspE*1 restriction sites using QuikChange PCR (Stratagene) with the following primers: BLM-489BspE1F (5'-CCAGGAAGAATCTTTTTGTCCGGACTTTATTCAATACCCATTTACAG-3') and BLM-489BspE1R (5'-CTGTAAATGGGTATTCAATAAAGTCCGGACAAAAGAT CTTCCTGG-3') for mutation of amino acid 489, and BLM-587BspE1F (5'-CCACAGCCTGCCTATCAACCCATCCGGAAAGGTCGGCCAATTAAATCAG-3') and BLM-587BspE1R (5'-CTGATTTAATTGGCCGACCTTTCCGGATGGGTTGATA GGCAGCTGTGG-3') for mutation of amino acid 587. Following mutagenesis, digestion by *BspE*I, gel purification and re-ligation of *pEGFP-BLM*, correction of bases coding for amino acids 488 and 588 was carried out with the following primers: BLM-488-588F (5'-CCAGGAAGAATCTTTTTGAAAGGGAAGGTCGGCCAATTAAATCAG-3') and BLM-488-588R (5'-CTGATTTAATTGGCCGACCTTCCCTTTCAAAAAGATTCTTCC TGG-3').

Protein purification.

The *pYES* expression vectors encoding 6xHis-tagged-BLM, BLM^{D795A} (helicase-dead mutant) or β-galactosidase were over-expressed in *S. cerevisiae* strain *JEL1*. Transformed yeast were grown in 1L of –Ura DO + minimal base and 2% glucose at 30°C. Protein expression was induced at an OD_{600} of 1.2-1.4 by switching cells to growth in media containing 2% galactose. Cells were grown for an additional 16 hours, pelleted and resuspended in 30 ml of lysis buffer (50 mM

KPO_4 pH 7.0, 500 mM KCl, 10% glycerol, 1 mM PMSF and protease inhibitor cocktail (Sigma)). Cells were lysed using a french press at 20K psi, cleared by ultracentrifugation at 40,000 rpm for 40 minutes. Lysates were loaded on nickel resin, Ni-NTA (Qiagen). An initial 35 mM imidazole wash was followed by a 50 ml gradient from 35 mM to 250 mM imidazole. All 6xHis-tagged proteins eluted from the nickel resin at 121-185 mM imidazole. Fractions were pooled, dialyzed (50 mM Tris pH 7. $5_{R/T}$, 10 % glycerol, 100 mM NaCl, 1 mM PMSF and protease inhibitor cocktail), and loaded on a Q-sepharose column (Sigma) and washed at 100 mM NaCl followed by a 10 ml gradient from 150 mM to 1 M NaCl. Pure 6xHis-tagged proteins eluted at 221-351 mM NaCl. Fractions were dialyzed in final storage buffer (25 mM Tris pH $7.5_{R/T}$, 20% glycerol, 1 mM DTT, 1 mM EDTA, 200 mM NaCl, 1 mM PMSF and protease inhibitor cocktail) and snap-frozen at -80°C.

Helicase assays.

3' overhang substrate was generated by radio-labeling NT38 (5'-ATGAGAAGCAGCCGTATCAGGAAGAGGGAAAGGAAGAA-3') at the 5' terminus with [γ-^{32}P]-ATP and T4 polynucleotide kinase prior to annealing to NT68 (5'-TTCTTCCTTTCCCTCTTCCTGATACGGCTGCTTCTCATCTACAACGTGATCC GTCATGGTTCGGAGTG-3'). Bubble substrate was generated by radio-labeling BT (5'-GACGCTGCCGAATTCTGGCTTGCTCGGACATCTTTGCCCACGTTGACCCG-3') at the 5' terminus with [γ-^{32}P]-ATP and T4 polynucleotide kinase prior to annealing to BB (5'-CGGGTCAACGTGGGCAAAGCCAATGCGATCGGCCAGAATTCGGCAGC GTC-3'). X-junction substrate was generated by radio-labeling X12-1 (5'-GACGCTGCCGAATTCTGGCTTGCTAGGACATCTTTGCCCACGTTGACCCG-3') at the 5' terminus with [γ-^{32}P]-ATP and T4 polynucleotide kinase prior to annealing to X12-2 (5'-CGGGTCAACGTGGGCAAAGATGTCCTAGCAATGTAATCGTCTATGA CGTC-3') and annealing X12-3 (5'-GACGTCATAGACGATTACATTGCTAGGACAT GCTGTCTAGAGACTATCGC-3') to X12-4 (5'GCGATAGTCTCTAGACAGCATGTC CTAGCAAGCCAGAATTCGGCAGCGTC-3'). X12-1/2 was then annealed to X12-3/4 at 4°C overnight.

Decatenation assays.

Decatenation assays were carried out with the Topoisomerase II Assay Kit as per manufacturer's directions (TopoGEN). Catenated DNA was used at 100 ng per reaction with indicated amounts of topoisomerase IIα and BLM, BLM^{D795A}, or β-galactosidase for 30 minutes at 37°C. Assays were terminated with supplied stop-dye and treated with proteinase K (50 μg/ml) for 30 minutes at 37°C before separation using 1% agarose gel electrophoresis and visualization by ethidium bromide-staining.

siRNA transfections and comet assays.
HeLa cells were transfected with 30 nM *BLM* and *TOP2 siRNA* (*BLM* siRNA ID: 146899, *TOP2A* siRNA ID: 110840, Ambion) separately or in combination. Alkali comet assays used embedding in 1% low melting agarose, lysis in alkaline lysis solution and electrophoresis at 0.6 V/cm for 25 minutes. DNA was stained with propidium iodide (25 μg/ml) and comets visualized with a Zeiss Axiovert 200M microscope using Axio Vision 4.5 software. Percent DNA in comet tails were analyzed using CometScore (http://autocomet.com/products_cometscore.php).

Figure S1

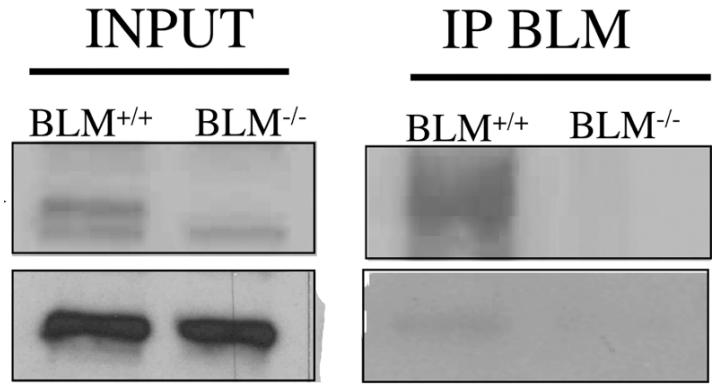


Figure S1. The BLM and topoisomerase IIα (TOPO IIα) association is absent in BS cells. Whole cell lysates of GM01806 (wild-type human cells) and GM03403 (BS cells) were used to precipitate BL with 2 mg of nuclear extract and 2μg of BLM antibody (Ab-1, Calbiochem) per mg of extract or 2μg of rabbit IgG (Jackson ImmunoResearch) per mg extract. Western blotting was carried out with BLM (ab476, Abcam) and topoisomerase IIα (Ab-1, Calbiochem) antibodies.

Figure S2

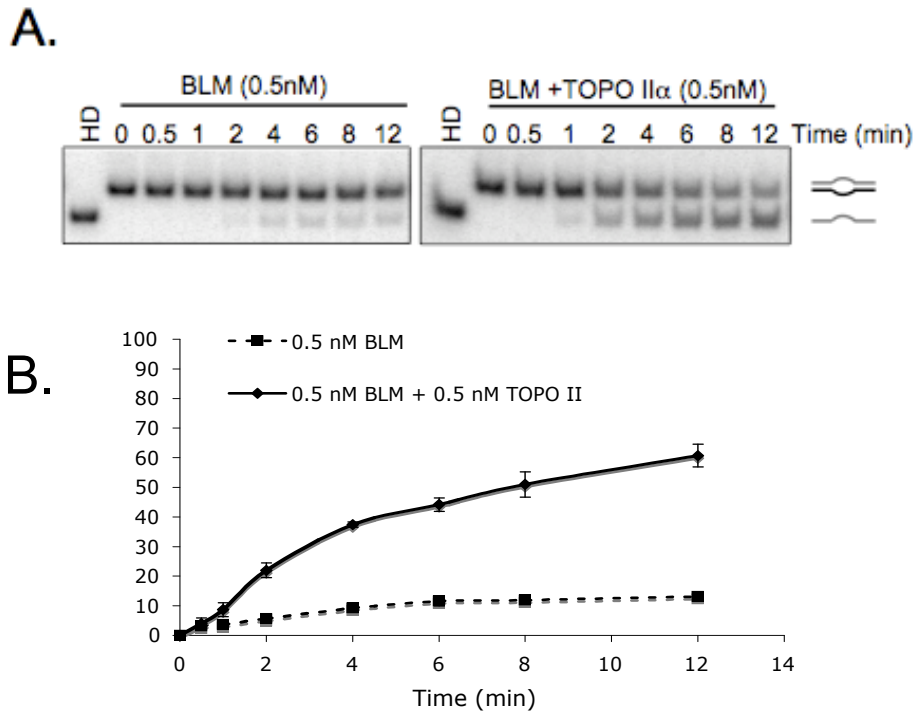


Figure S2. Topoisomerase IIα enhances BLM helicase activity with bubble substrate. (A) Autoradiograph of a time-course helicase assay with indicated amounts of BLM and topoisomerase IIα with 2 fmoles of bubble substrate. DNA products were resolved using 12% non-denaturing PAGE. (B) Quantitation of percent unwinding of BLM by itself or with equimolar topoisomerase IIα.

Figure S3

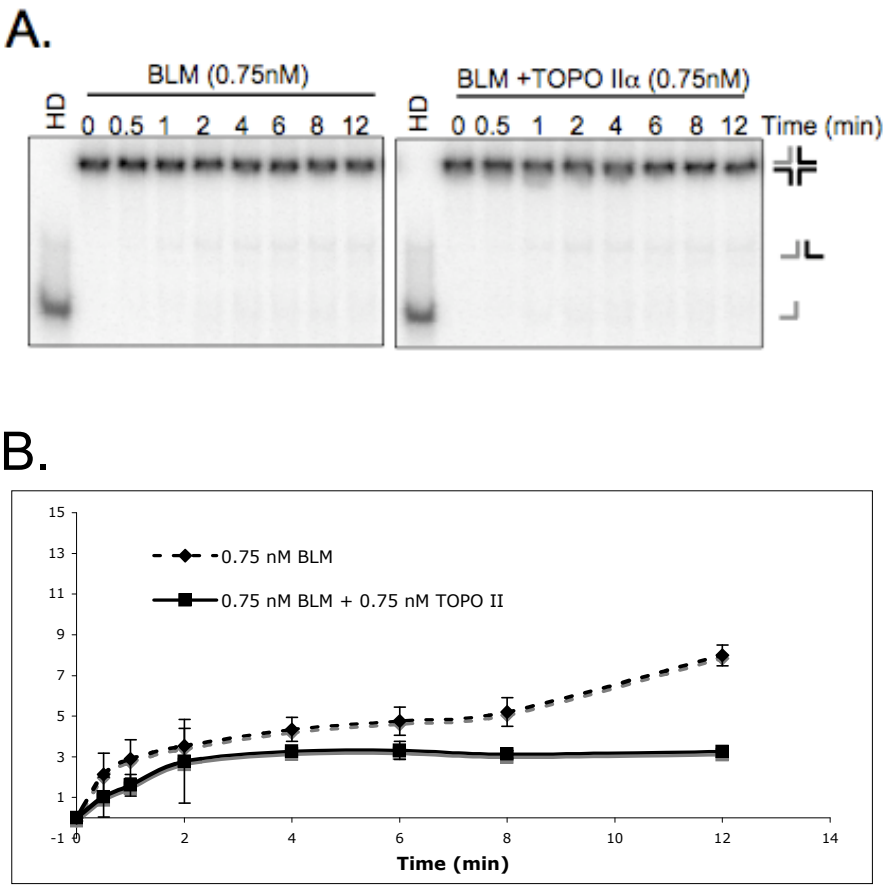
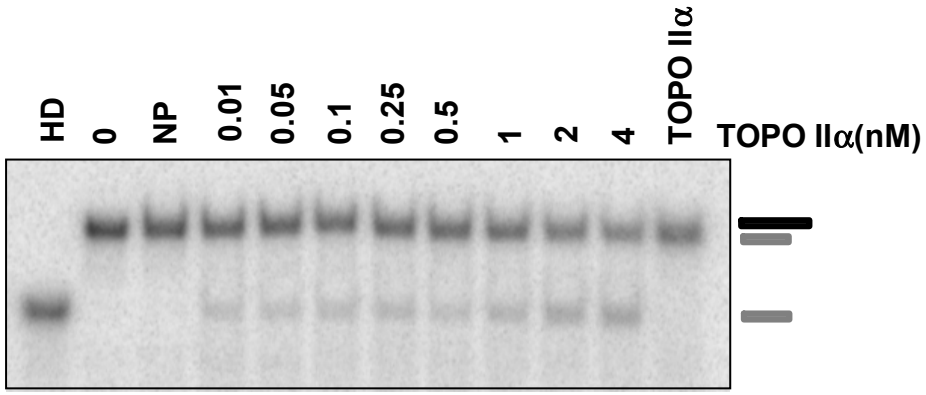
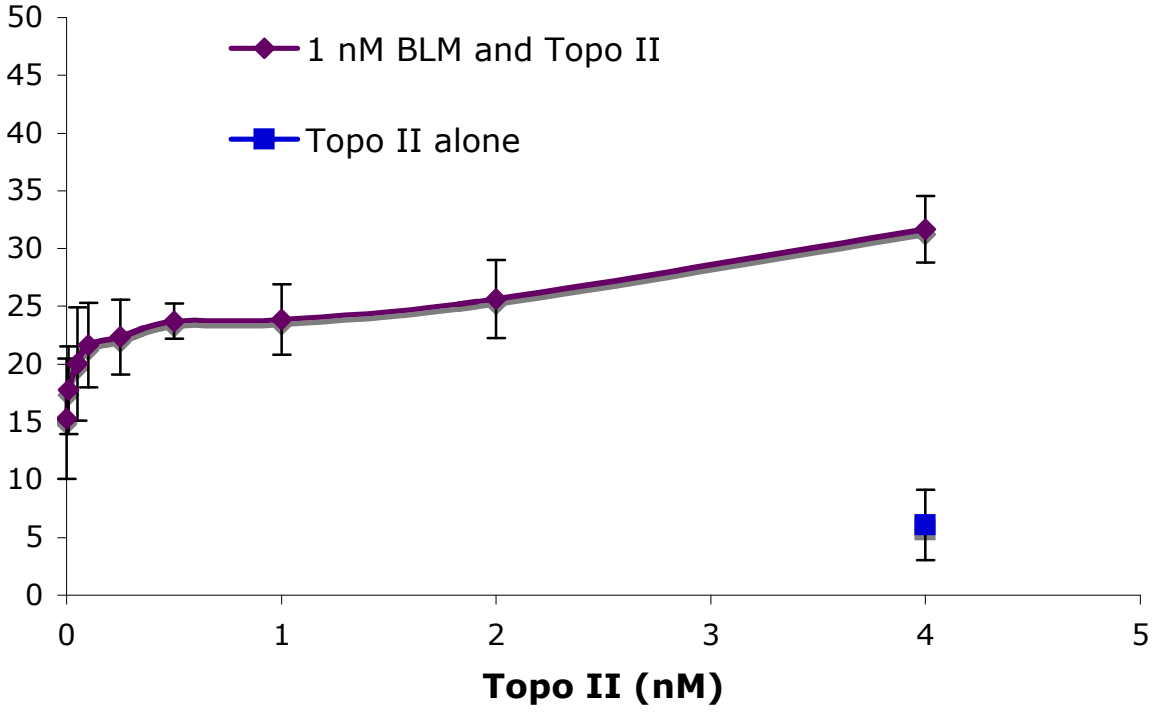


Figure S3. Topoisomerase IIα inhibits BLM helicase activity with X-junction substrate. (A) Autoradiograph of a time-course helicase assay with indicated amounts of BLM and topoisomerase IIα with 2 fmoles of X-junction substrate. DNA products were resolved using 10% non-denaturing PAGE. (B) Quantitation of percent unwinding by BLM only or with equimolar topoisomerase IIα.

Figure S4

A.


HD
0
NP
0.01
0.05
0.1
0.25
0.5
1
2
4
TOPO IIα
TOPO IIα(nM)


1 nM BLM and Topo II
Topo II alone
50
45
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
Topo II (nM)

B.

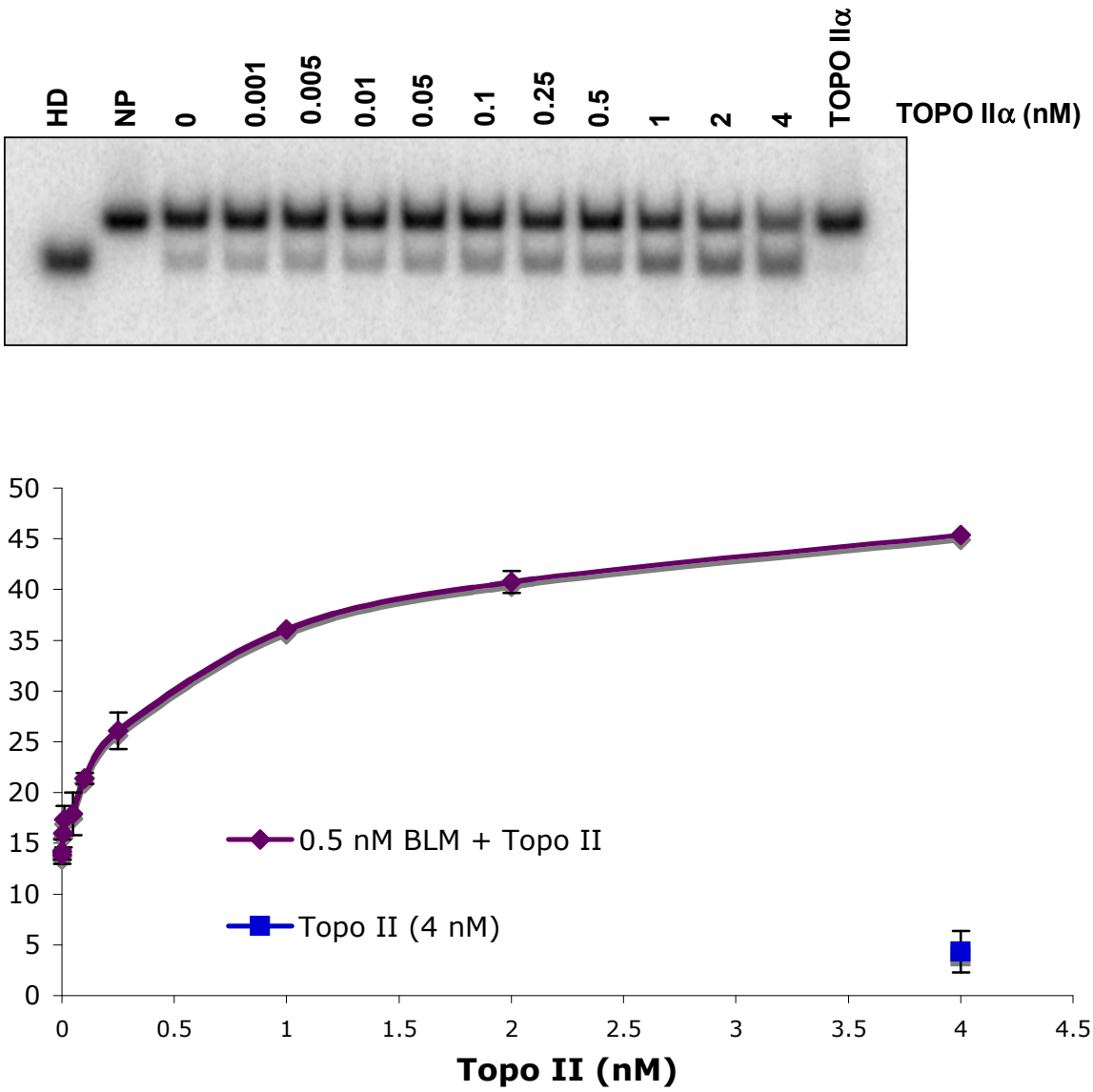
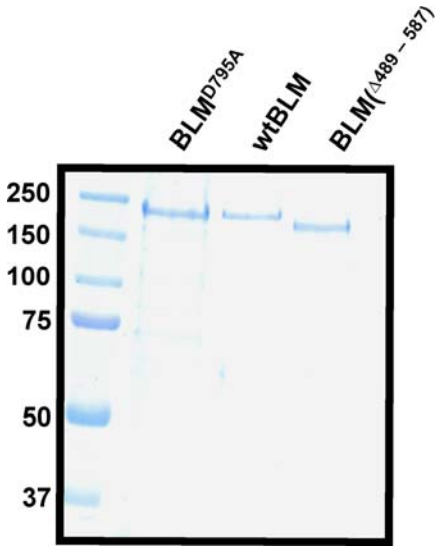


Figure S4. Topoisomerase IIα (TOPO IIα) stimulates BLM helicase on 3'-overhang and bubble substrates. (A) Different concentrations of topoisomerase IIα (0.01 – 4 nM) were added to 2 fmoles of 3'-overhang substrate and incubated for 5 minutes prior to adding 1 nM BLM. The reactions were continued for 12 minutes and the products resolved by 12% PAGE. Percent unwinding of the substrate was quantified and plotted. (B) Different concentrations of topoisomerase IIα (0.0001 – 4 nM) were added to 2 fmoles of bubble substrate and incubated for 5 minutes prior to adding 0.5 nM BLM. The reactions were continued for 12 minutes and the products resolved by 12% PAGE. Percent unwinding of the substrate was quantified and plotted.

Figure S5

A.



B.

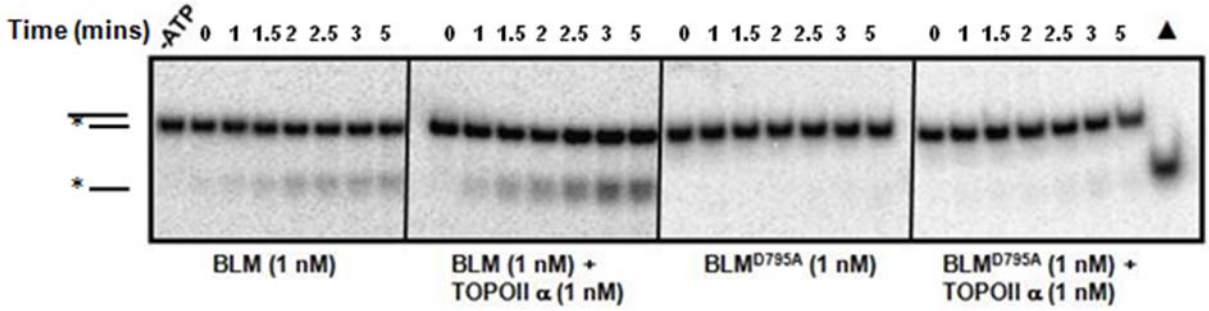


Figure S5. Topoisomerase IIα does not stimulate BLM helicase-dead mutant BLM^{D795A}. (A) Helicase assays were conducted as described in 'Experimental Procedures' in the presence and absence of 1 nM topoisomerase IIα with wild-type or mutant BLM. Unwinding of the 3'-overhang substrate was monitored over time. Minus ATP control was included to indicate ATP-dependent unwinding of the substrate and ▲ represents heat-denatured substrate. (B) Biosafe Coomassie-stained gel depicting purified recombinant helicase-dead BLM^{D795A}, wild-type and topoisomerase IIα interaction domain deletion mutant BLM used in the *in vitro* assays. Molecular weight markers are indicated on the left.

Figure S6

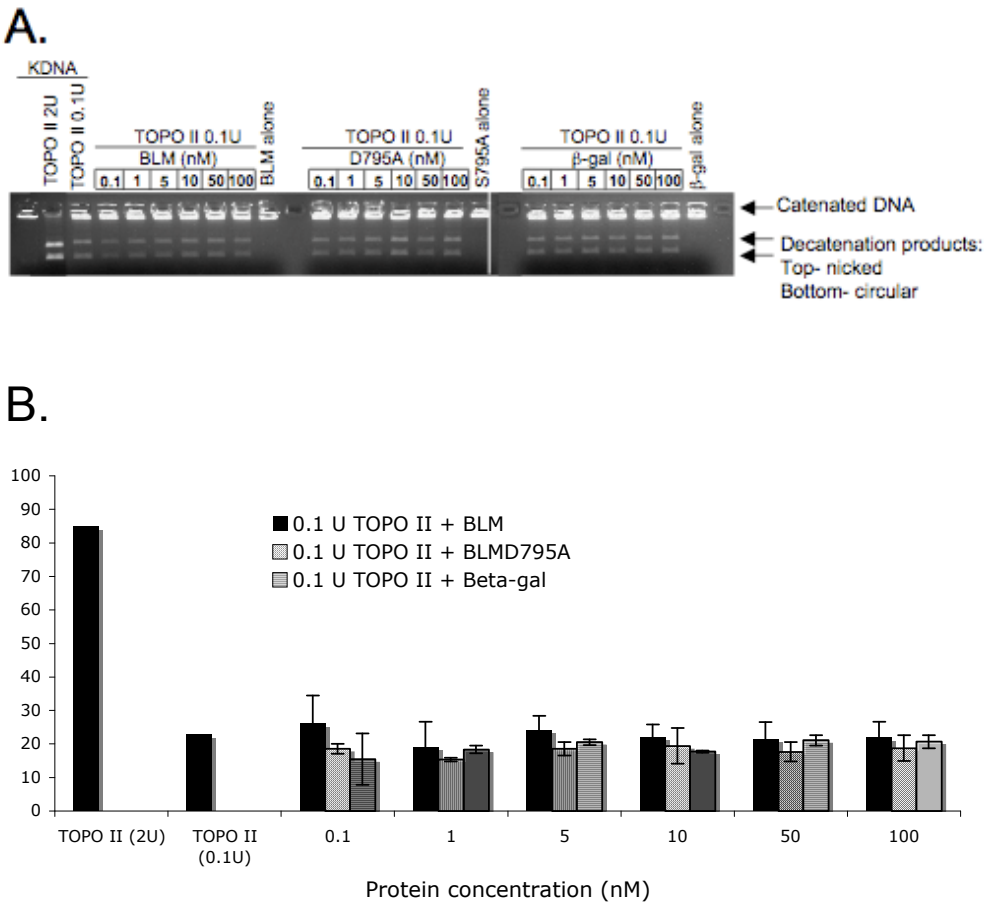
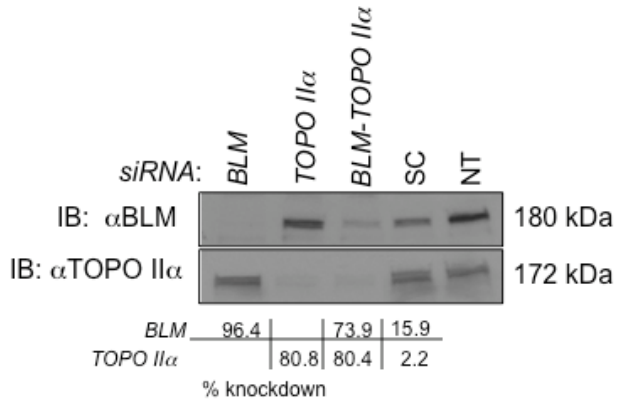


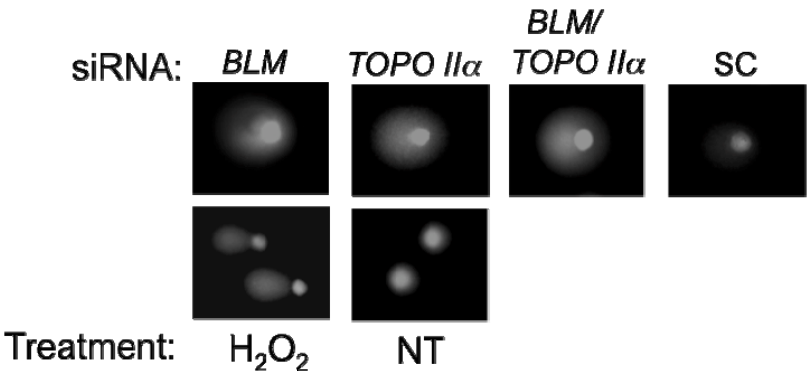
Figure S6. BLM does not affect topoisomerase IIα decatenation activity. (A) Decatenation assays were carried out with 100 ng of catenated DNA, indicated units of topoisomerase IIα (USB) and increasing amounts of purified 6xHis-tagged BLM, the helicase-dead mutant BLM^{D795A}, or β-galactosidase (β-gal). Decatenation products were separated by gel electrophoresis using 1% agarose. A catenated DNA control (KDNA) demonstrates the mobility of fully catenated DNA. A decatenated control (KDNA + 2U TOPO IIα) demonstrates the migration of both decatenation products. A decatenation reaction containing 0.1U of topoisomerase IIα (KDNA + 0.1U TOPO IIα) demonstrates approximately 20% decatenation and is the starting topoisomerase IIα concentration for all other reactions containing either wild-type BLM, the helicase-dead mutant, BLM^{D792A} or β-gal. Control reactions with BLM, BLM^{D795A} and β-gal only are included. (B) Graphical representation of the mean decatenation activities and standard deviations from at least three separate experiments.

Figure S7

A.


siRNA:
BLM
TOPO IIα
BLM-TOPO IIα
SC
NT
IB: αBLM
IB: αTOPO IIα
180 kDa
172 kDa
BLM 96.4 73.9 15.9
TOPO IIα 80.8 80.4 2.2
% knockdown

B.


siRNA:
BLM
TOPO IIα
BLM/ TOPO IIα
SC
Treatment:
H2O2
NT

C.

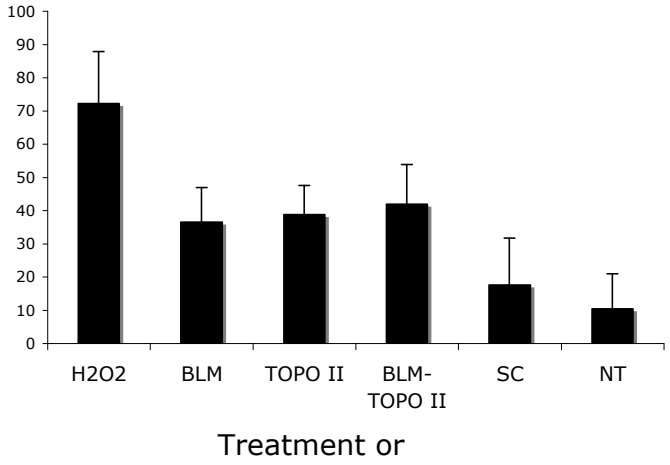


Figure S7. BLM and topoisomerase IIα function in the same pathway to prevent DNA damage. (A). Western blots show BLM and topoisomerase IIα in HeLa nuclear extracts prepared from non-transfected (NT) cells or those transfected with *siRNA*s targeting *BLM*, *TOP2*A, both, or a scrambled control. Percent reduction of each protein is shown. Topoisomerase III, a known protein partner of BLM that affects DNA damage (33), remained unchanged following topoisomerase IIα knock-down (data not shown). No change in BLM or topoisomerase IIα protein levels was observed in cells transfected with a scrambled control *siRNA* (B) Representative comet assays of transfected cells. Hydrogen peroxide-treated (H_2O_2) and untreated cells are positive and negative controls. (C) This shows the percent of DNA in comet tails and represent the average length of 100 comet tails per sample from three experiments analyzed 24 hours post-transfection of *siRNA* or 1 hour after H_2O_2 treatment (positive control). Hydrogen peroxide treatment resulted in 72.2% of DNA in the tail; untreated controls and scrambled control-transfected cells resulted in 10.5 and 14.0% of DNA in the tail, respectively. BLM, topoisomerase IIα and BLM/topoisomerase IIα knock-downs increased the percentage of DNA in the comet tail, (36.5, 38.8 and 41.9%, respectively) compared to negative controls. There was no difference between the DNA strand breaks caused by BLM, topoisomerase IIα or BLM/topoisomerase IIα knock-downs. Treatments that showed no significant difference in average comet tail length from one another are bracketed above (p=<.05 using one-way ANOVA with Tukey-Kramer multiplier to control family-wise error rate).