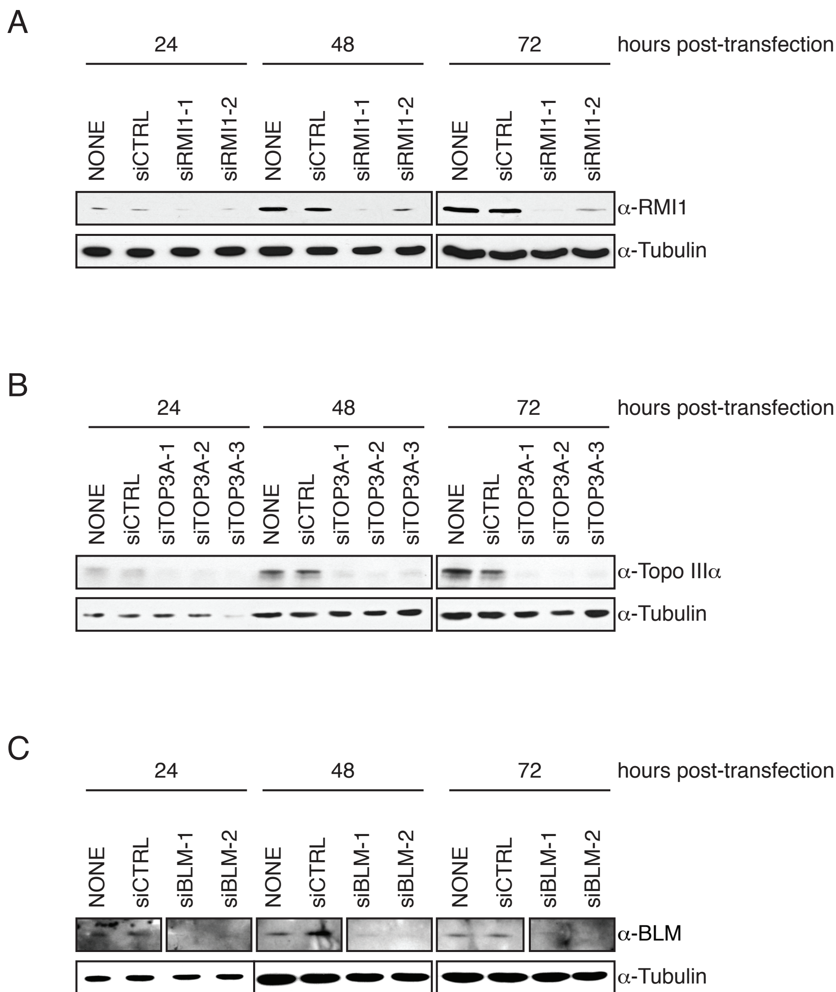
Supplementary information

Supplementary Table 1: List of siRNA sequences with 3' dTdT overhangs.

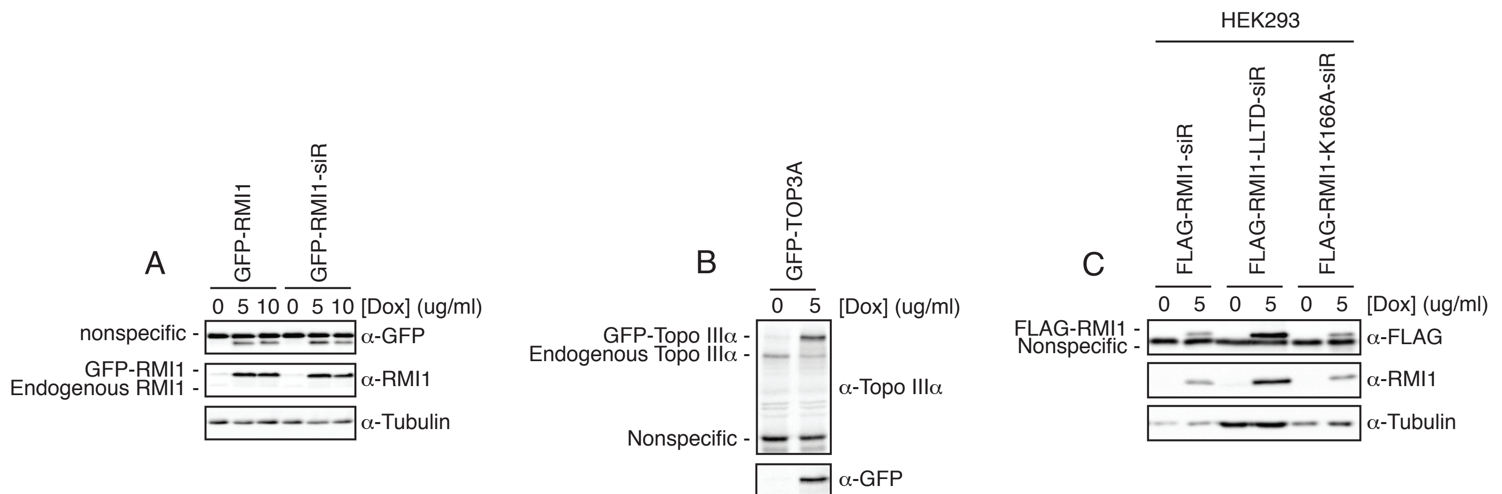
Name	Sequence
siCTRL	5' AUUGUAUGCGAUCGCAGAC 3'
siRMI1-1	5' AGCCUUCACGAAUGUUGAU 3'
siRMI1-2	5' UCUAGUUACAGCUGAAGCA 3'
siTOP3A-1	5' ACAUCGGGUUUGAGAUUAU 3'
siTOP3A-2	5' CCAGAAAUCUUCCACAGAA 3'
siTOP3A-3	5' GGACAAAUUUGUGGUUCUA 3'
siBLM-1	5' AGCAGCGAUGUGAUUUGCA 3'
siBLM-2	5' AUCAGCUAGAGGCGAUCAA 3'

Supplementary Table 2: List of PCR primer sequences

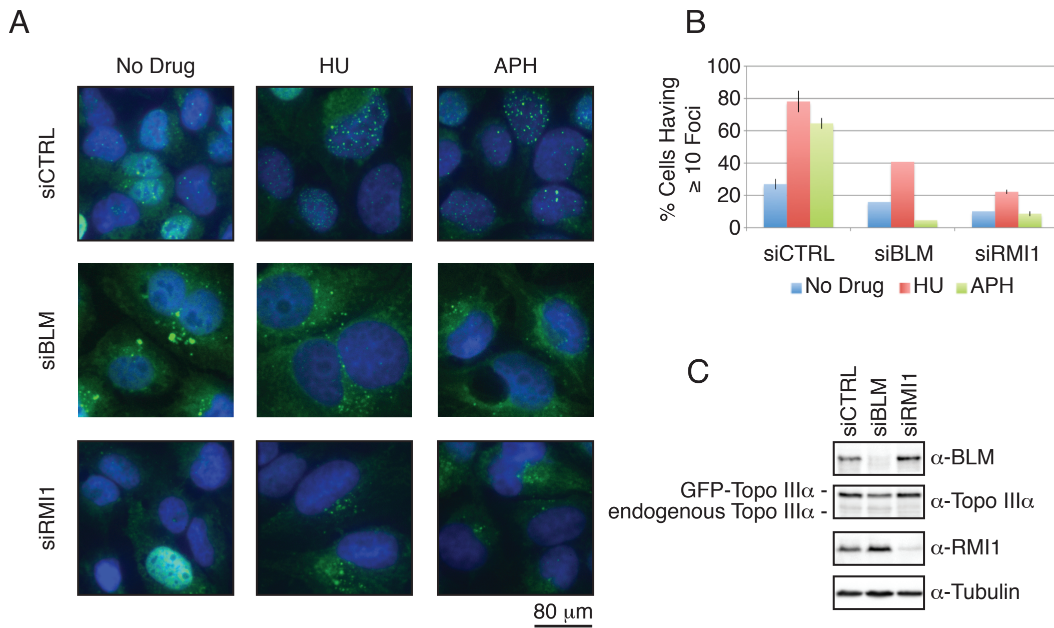
Primer		Sequence
Amplify RMI1 gene to be cloned into pcDNA5/FRT/TO	Fwd	5' TTGGCGCGCCAATGAATGTGACTAGTATTGC 3'
	Rev	5' GATCAGCTCGAGTTATTTATTTAACCGCTTCTTTAG 3'
Amplify TOP3A gene to be cloned into pcDNA5/FRT/TO	Fwd	5' TTGGCGCGCCAATGATCTTTCCTGTCGCCCG 3'
	Rev	5' GATCAGCTCGAGTCATCTGTTCTGAGGACAAAAG 3'
Introduce silent mutations to RMI1 gene	Fwd	5' AACCTTGGGAAGCAAAACCAAGCC- -GCATGCTCATGCTGCAGCTAACTG 3'
	Rev	5' CAGTTAGCTGCAGCATGAGCATGC- -GGCTTGGTTTTGCTTCCCAAGGTT 3'
Amplify TOP3A gene for Gateway cloning	Fwd	5' GGGGACAAGTTTGTACAAAAAAGCA- -GGCTTCATGATCTTTCCTGTCGCCC 3'
	Rev	5' GGGGACAAGTTTGTACAAAAAAGCA- -GGCTTCATGATCTTTCCTGTCGCCC 3'
Amplify RMI2 gene for Gateway cloning	Fwd	5' GGGGACAAGTTTGTACAAAAAAGCA- -GGCTTCATGGCGGCGGCTGCGGACTC 3'
	Rev	5' GGGGACCACTTTGTACAAGAAAGCT- -GGGTCAGGAATATTCCTGTGTAAATC 3'



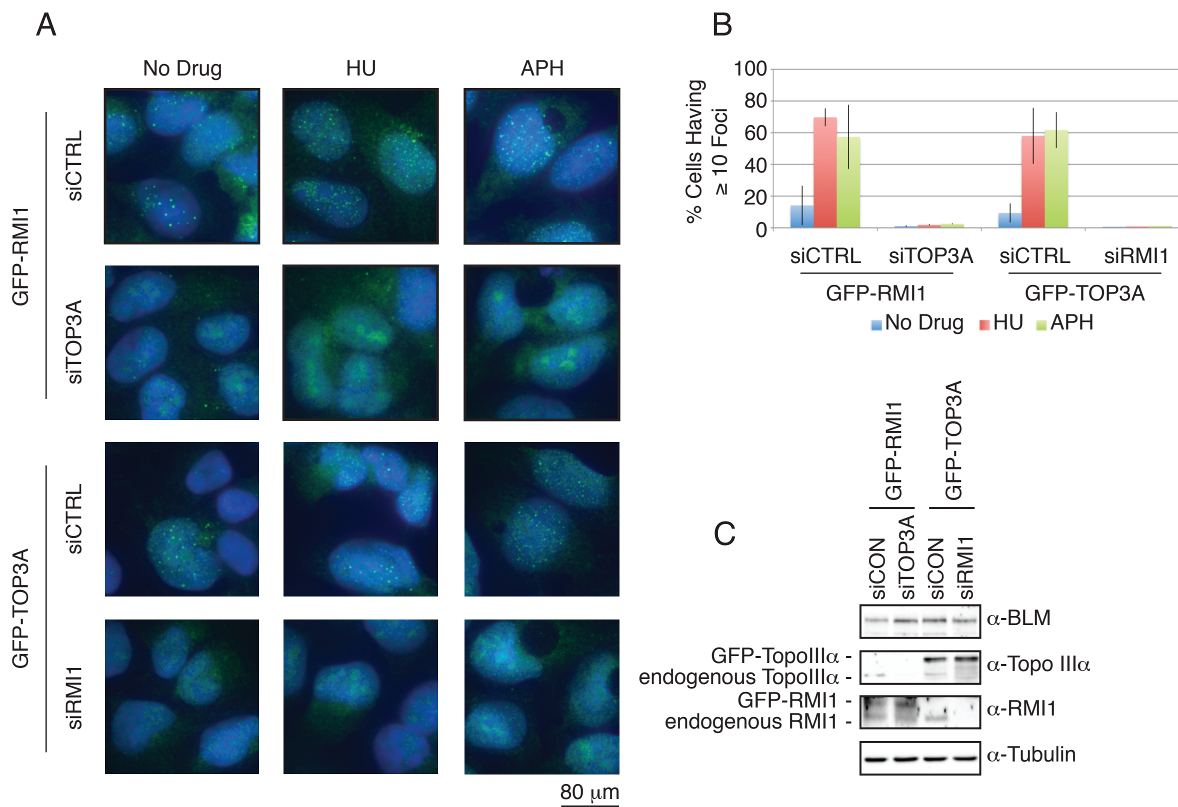
Supplementary Figure 1 siRNA knockdown experiments in U2OS cells. (A) U2OS cells were transfected with RMI1 siRNA oligos (siRMI1-1 and siRMI1-2) for 24, 48, or 72 hours. U2OS cells that were not transfected with any siRNA oligos (NONE) or were transfected with scrambled RNA sequence (siCTRL) were included as negative controls. Extracts were subjected to immunoblotting analysis, probing for RMI1 protein levels. Anti-Tubulin protein level was included as loading control. (B and C) The same experiments were repeated as described in (A), except that U2OS cells were transfected either with Topo IIIα siRNA oligos (siTOP3A-1, siTOP3A-2, and siTOP3A-3) (B), or with BLM siRNA oligos (siBLM-1 and siBLM-2) (C).



Supplementary Figure 2 Generation of stable cell lines expressing GFP-RMI1 that are resistant to siRMI1 treatment. (A) U2OS stable cell lines expressing N-terminally GFP-tagged wild type (GFP-RMI1) or siRMI1 resistant (GFP-RMI1-siR) RMI1 were induced with 0, 5, or 10 μg/ml doxycycline for 24 hours before being subjected to immunoblotting analysis, probing for RMI1 protein levels. Anti-Tubulin is included as loading control. (B) U2OS stable cell line expressing N-terminally GFP-tagged Topo IIIα (GFP-TOP3A) was induced with 5 μg/ml doxycycline for 24 hours before being subjected to immunoblotting analysis, probing for Topo IIIα protein level. Anti-Tubulin is included as loading control. (C) HEK293 stable cell lines expressing N-terminally FLAG-tagged RMI1 variants were induced with 5 μg/ml doxycycline for 24 hours before being subjected to immunoblotting analysis, probing for RMI1 protein levels. Anti-Tubulin is included as loading control.



Supplementary Figure 3 Topo IIIα nuclear foci formation is dependent on the presence of BLM and RMI1. (A) Representative confocal microscopy images of Topo IIIα (green) foci in the nucleus (blue; DAPI) following treatment with siCTRL, siBLM, or siRMI1 in cells grown in no drug, 4 mM HU, or 30 µM aphidicolin for 24 hours. (B) Images from the experiment in (A) were analyzed to determine the percentage of cells having more than 10 Topo IIIα nuclear foci. (C) U2OS stable cell line expressing GFP-Topo IIIα was treated with siCTRL siBLM, or siRMI1 oligos for 48 hours. Extracts were subjected to immunoblotting analysis, probing for BLM, Topo IIIα, and RMI1 protein levels. Anti-Tubulin is included as loading control.



Supplementary Figure 4 BLM nuclear foci formation is dependent on the presence of Topo IIIα and RMI1. (A) Representative confocal microscopy images of BLM (green) foci in the nucleus (blue; DAPI) following treatment with siCTRL, siTOP3A, or siRMI1 in cells grown in no drug, 4 mM HU, or 30 µM aphidicolin for 24 hours. (B) Images from the experiment in (A) were analyzed to determine the percentage of cells having more than 10 BLM nuclear foci. (C) U2OS stable cell line expressing GFP-RMI1 or GFP-Topo IIIα was treated with siCTRL siTOP3A, or siRMI1 oligos for 48 hours. Extracts were subjected to immunoblotting analysis, probing for BLM, Topo IIIα, and RMI1 protein levels. Anti-Tubulin is included as loading control.

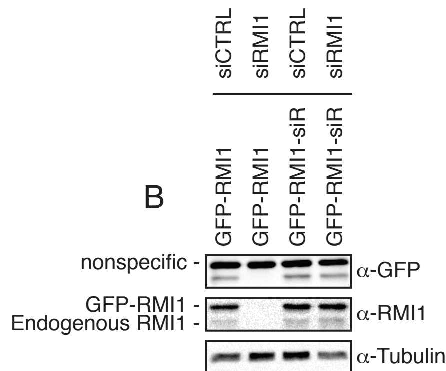
A

silent mutations: A AAGC C C C
DNA: GCACAAGTAACCCCAAAACCTTGGGAAGCAAAGCCTTCACGAATGTTGATG 408
Ptn: A Q V T P K P W E A K P S R M L M 136

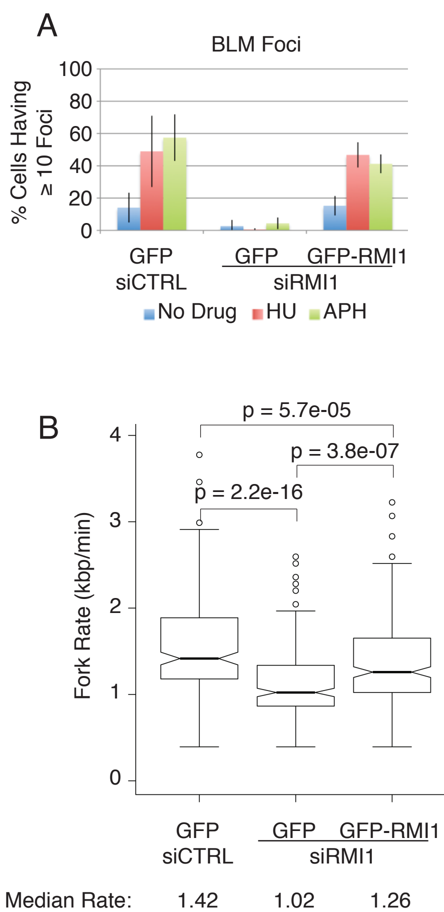
GFP-RMI1: ---TAACCCCAAAACCTTGGGAAGCAAAGCCTTCACGAATGTTGATGCTGCAGCTAACTGATGGA---

GFP-RMI1-siR: 5' AACCTTGGGAAGCAAAACCAAGCCGCATGCTCATGCTGCAGCTAACTG 3'
SphI

B



Supplementary Figure 5 Eight silent mutations in GFP-RMI1-siR that is resistant to siRMI1 treatment. (A) Nucleotide sequence of RMI1 that is resistant to siRMI1 treatment (GFP-RMI1-siR) is shown containing eight silent mutations (red). siRMI1 target site is underscored. (B) U2OS stable cell lines expressing N-terminally GFP-tagged wild type (GFP-RMI1) or siRMI1 resistant (GFP-RMI1-siR) RMI1 were transfected with siRMI1-1 for 24 hours before being induced with 5 μg/ml doxycycline for additional 24 hours. Extracts were subjected to immunoblotting analysis, probing for RMI1 protein levels. Anti-Tubulin is included as loading control.



Supplementary Figure 6 GFP-RMI1 restores BLM foci formation and suppresses the fork progression defect when endogenous RMI1 is depleted. (A) Images from the experiment in Figure 5D were analyzed to determine the percentage of cells having more than 10 BLM nuclear foci. Data from cells depleted of endogenous RMI1 while expressing GFP or GFP-RMI1 were identical in this figure and in Figure 5E. (B) Distributions of the rates of replication fork progression in U2OS stable cell lines expressing GFP, or GFP-RMI1 are represented in a boxplot. The median fork rate for each experiment is shown. P values were determined by a two-tailed Mann-Whitney U test to compare the distributions of fork rates between two samples. Data from cells depleted of endogenous RMI1 while expressing GFP or GFP-RMI1 were identical in this figure and in Figure 5F.