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Topoisomerase II α controls the decatenation checkpoint

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Abstract

Topoisomerase II (Topo II) is required to separate intertwined sister chromatids before chromosome segregation can occur in mitosis¹. However, it remains to be resolved whether Topo II has any role in checkpoint control. Here we report that when phosphorylated, Ser 1524 of Topo II α acts as a binding site for the BRCT domain of MDC1 (mediator of DNA damage checkpoint protein-1), thereby recruiting MDC1 to chromatin. Although Topo II α –MDC1 interaction is not required for checkpoint activation induced by DNA damage, it is required for activation of the decatenation checkpoint. Mutation of Ser 1524 results in a defective decatenation checkpoint. These results reveal an important role of Topo II in checkpoint activation and in the maintenance of genomic stability.

Sister chromatids become entangled after DNA replication². Normally, the chromosome catenation status is monitored by the decatenation checkpoint, which arrests cells at the G2 phase, delaying the onset of mitosis until sister chromatids are fully separated^{3,4}. The checkpoint is activated when cells are treated with catalytic Topo II inhibitors, such as bis (2, 6-dioxopiperazine) derivatives ICRF-193 and ICRF-187 (refs 3, 4). These inhibit the ATPase activity of Topo II and stabilize the enzyme in a closed-clamp form, rather than stabilizing the Topo II DNA-cleavable complex, which is the mechanism of action of Topo II inhibitor etoposide⁵. Therefore, in contrast to etoposide^{5,6}, ICRF-193 and ICRF-187 do not induce extensive DNA breaks. Indeed, many studies suggest that the decatenation checkpoint is distinct from the DNA damage checkpoint. For example, stem cells, progenitor cells and certain lung and bladder cancer cells have an intact DNA damage checkpoint, whereas they lack a decatenation checkpoint^{7–9}. Furthermore, ICRF-193 induces G2-phase arrest that is independent of the DNA checkpoint kinases ATM, Chk1 and Chk2 (ref. 3). Despite these differences, these two checkpoints do require some common checkpoint proteins, notably, ATR, BRCA1 and WRN^{3,7,10}. Here we report an unexpected role for Topo II α in the activation of the decatenation checkpoint.

MDC1 is an important multi-functional checkpoint protein¹¹. To identify proteins associated with its BRCT domain we performed affinity purification using glutathione *S*-transferase (GST)–BRCT. As BRCT domains bind phospho-Ser or phospho-Thr motifs^{12,13}, we also

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Author contributions

K.L., J.Y. and Z.L. performed the experiments and analysed the data; Z.L. and J.C. wrote the paper. All authors discussed the results and commented on the manuscript.

Competing financial interests

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treated HeLa cell lysates with λ -phosphatase. In pulldown assays, three bands showed specific binding to the MDC1 BRCT domain (Fig. 1a). These interactions were abolished by λ -phosphatase treatment. We excised these bands and sequencing by mass spectrometry revealed that they were Topo II α , DNA ligase III and XRCC1. Western blotting using anti-Topo II α antibodies confirmed that the BRCT domain of MDC1 binds Topo II α in a phosphorylation-dependent manner (Fig. 1b). We further verified the interaction between MDC1 and Topo II α *in vivo*, by co-immunoprecipitation (Fig. 1c, d). Similarly, we observed the interaction between MDC1 and DNA ligase III-XRCC1 *in vivo* (data not shown).

As Topo II α was isolated by the BRCT domain of MDC1 *in vitro*, we generated MDC1 with the BRCT domain deleted, and examined its interaction with Topo II α *in vivo*. Deletion of the BRCT domain markedly decreased the MDC1–Topo II α interaction (Fig. 1e), confirming that the MDC1 BRCT domain is required for the MDC1–Topo II α interaction *in vivo*.

To map the region of Topo II α that binds MDC1, we generated a series of Topo II α deletion mutations. We found that deletion of the 71 residues in the carboxy-terminal region significantly reduced the MDC1–Topo II α interaction (Fig. 2a). The MDC1 BRCT domain is predicted to preferentially bind a phospho-pSer(Thr)xxPhe motif¹⁴. Within the 71 amino acids of the Topo II α C-terminal region (residues 1461–1531), Ser 1496 is followed by Phe at the +3 position, but Ser 1496 is not known to be phosphorylated *in vivo*. On the other hand, Ser 1524, which is known to be phosphorylated *in vivo*¹⁵, has Asp not Phe at the +3 position. We mutated these two sites individually and tested whether they are required for MDC1 binding. *In vitro* pulldown assays (Fig. 2b) and co-immunoprecipitation (Fig. 2c) showed that mutation of Ser 1496 did not affect the interaction between the MDC1 BRCT domain and Topo II α (data not shown), whereas mutation of the Ser 1524 site abolished this interaction. These results indicate that Ser 1524 is required for MDC1 binding.

To verify these results, we performed *in vitro* pulldown assays using peptides containing non-phosphorylated Ser 1524 or phosphorylated Ser 1524 (pSer 1524). Peptides containing pSer 1524 preferentially bound the MDC1 BRCT domain (Fig. 2d), confirming that the MDC1–Topo II α interaction is mediated by the BRCT domain of MDC1 and by phosphorylation at the Ser 1524 site of Topo II α .

Although phospho-peptide mapping and sequencing¹⁵ has shown that phosphorylation at Ser 1524 of Topo II α occurs *in vivo*, no antibody is available to specifically detect this event. To verify that the Ser 1524 site of Topo II α is phosphorylated *in vivo*, we generated anti-pSer 1524 antibodies and confirmed their specificity (Supplementary Information, Fig. S1a–c). Using these antibodies, we found that phosphorylation of Ser 1524 occurs preferentially when cells are at the G2–M transition (Fig. 2e), suggesting a potential functional role of pSer 1524 at this stage of the cell cycle.

Although phosphorylation of Topo II α at Ser 1524 was identified more than 10 years ago, the functional significance of this event is unclear. It has been suggested that casein kinase II (CK2) phosphorylates Ser 1524 (ref. 15). However, when cells were treated with either of the CK2 inhibitors 5,6-dichlorobenzimidazole riboside (DRB) or 4,5,6,7-tetrabromobenzotriazole (TBB), we did not observe any decrease in pSer 1524 (data not shown), suggesting that kinases other than CK2 may be responsible for Topo II α Ser 1524 phosphorylation *in vivo*. There are also conflicting results regarding the relationship between Topo II α activity and its phosphorylation. Although some reports suggest that CK2-mediated phosphorylation of Topo II α enhances its decatenation activity¹⁶, other reports show no such effect^{17,18}. To further investigate this phosphorylation event we expressed FLAG-tagged wild-type Topo II α (Topo II α ^{WT}) or mutant Topo II α (Topo II α ^{S1524A}), in 293T cells, and purified the recombinant Topo II α by affinity purification. Decatenation assays were performed using purified Topo II α ^{WT}

and Topo II α ^{S1524A}, with kinetoplast DNA as the substrate. We did not observe any marked difference in activity between Topo II α ^{WT} and Topo II α ^{S1524A} (Fig. 3a), which suggests that Topo II α phosphorylation at Ser 1524 does not have a major regulatory role in Topo II α decatenation activity.

Thus, the functional significance of Topo II α Ser 1524 phosphorylation and its interaction with MDC1 *in vivo* remains unclear. As MDC1 is known to be important for checkpoint regulation, we speculated that MDC1 may also have a role in the decatenation checkpoint. Indeed, downregulation of MDC1 in HT1080 (human fibrosarcoma) cells resulted in defective decatenation and DNA damage checkpoints: cells depleted of MDC1 failed to block mitotic entry after ICRF-193, ionizing radiation (IR), mitomycin C (MMC) or UV treatment (Fig. 3b, c; Supplementary Information, Fig. S1d, e). These results implicate MDC1 in the regulation of the decatenation checkpoint, in addition to its role in checkpoint activation induced by DNA damage.

As MDC1 interacts with Topo II α , we further proposed that the MDC1–Topo II α interaction, and phosphorylation of Topo II α Ser 1524, may be involved in the decatenation checkpoint. We generated HT1080 cells stably expressing short interfering RNA (siRNA)-resistant Topo II α ^{WT} or Topo II α ^{S1524A}, both of which contain silent mutations in the region targeted by *Topo II α* siRNA. These cells were transfected with *Topo II α* siRNA to deplete endogenous Topo II α . When siRNA-transfected cells were treated with ICRF-193, those expressing Topo II α ^{WT} had an intact decatenation checkpoint (Fig. 3d; Supplementary Information, Fig. S1e). Interestingly, cells expressing Topo II α ^{S1524A} had a defective decatenation checkpoint (Fig. 3d; Supplementary Information, Fig. S1e), indicating that Ser 1524 of Topo II α is required for the decatenation checkpoint. On the other hand, UV-, MMC- or IR-treated Topo II α ^{S1524A} cells showed an intact G2 arrest induced by DNA damage (Fig. 3e; Supplementary Information, Fig. S1d, e). These results suggest that Topo II α Ser 1524 phosphorylation specifically regulates the decatenation checkpoint. However, the higher mitotic index in cells with MDC1 downregulation, or in cells expressing Topo II α ^{S1524A}, could also be due to enhanced mitotic arrest rather than defective G2 arrest. To distinguish between these possibilities, we treated these cells with both ICRF-193 and nocodazole, and examined the mitotic population at several time-points. Control cells remained blocked at mitotic entry, whereas cells with MDC1 downregulation or expressing Topo II α ^{S1524A} continued to accumulate in mitosis (Fig. 3f), suggesting a failure to arrest at G2 in these cells. Finally, to confirm that the ICRF-193-induced checkpoint is due to its action on Topo II α , we used Topo II α ^{R162Q}, a mutant shown to be resistant to ICRF-type agents¹⁹. We found that ICRF-193 failed to induce G2 arrest in cells expressing Topo II α ^{R162Q} (Fig. 3g), suggesting that ICRF-193 acts through Topo II to induce the decatenation checkpoint.

In contrast to previous studies suggesting that ICRF-type Topo II inhibitors induce DNA damage^{20,21}, we did not observe induction of foci or changes in levels of the well-recognized DNA damage marker, γ H2AX, under our treatment conditions (ICRF, 2 μ M for 1 h; (Supplementary Information, Fig. S2a, e). We were also unable to detect induction of NBS1 or BRCA1 foci formation or the stabilization of p53 (Supplementary Information, Fig. S2a, b). Interestingly, we observed a slight increase in ATM phosphorylation after ICRF-193 treatment (Supplementary Information, Fig. S2c), even though no induction of phospho-ATM foci was found (Supplementary Information, Fig. S2a). This is consistent with a previous report⁷ suggesting that ICRF-193 could induce a conformational change in chromosomes in the absence of DNA damage²². However, ATM is not required for the decatenation checkpoint activation (Supplementary Information, Fig. S2f, g). We did not detect Chk1 phosphorylation (Supplementary Information, Fig. S2d). There was no detectable DNA damage and results suggest that the classical DNA damage checkpoint was not induced under the conditions used for the decatenation assay. However, conditions used in previous studies to detect DNA

damage^{20,21}, involving prolonged treatment with ICRF-193 (>3 h), did result in the induction of γ H2AX, phospho-ATM, BRCA1 and NBS1 foci (Supplementary Information, Fig. S2a). It is likely that some cells escaped G2 arrest, entering mitosis in the presence of catenated chromosomes, causing DNA damage.

Defects in the decatenation checkpoint lead to genomic instability because cells enter mitosis in the presence of chromatid entanglement (pseudomitosis)⁹. Indeed, we found that ICRF-193 treatment resulted in an increased incidence of pseudomitosis in cells expressing Topo II α ^{S1524A}, compared with Topo II α ^{WT} cells (Fig. 3h). Additionally, cells expressing Topo II α ^{S1524A} showed an increased incidence of spontaneous pseudomitosis in the absence of ICRF-193 (Fig. 3i). These results provide evidence that the MDC1–Topo II α interaction is important in maintaining genomic stability following chromosome entanglement.

Although the MDC1–Topo II α interaction is important for activation of the decatenation checkpoint, it is not clear how cells sense chromosome catenation and activate the checkpoint. Interestingly, we found that ICRF-193 treatment induced a significant increase in the MDC1–Topo II α interaction in both HT1080 cells and mouse embryonic fibroblasts (Fig. 4a; Supplementary Information, Fig. S2h). However, although the MDC1–Topo II α interaction requires Ser 1524 phosphorylation, we did not observe an increase in Ser 1524 phosphorylation after Topo II α inhibition (Fig. 4b). This raises the question of how ICRF-193 treatment increases MDC1–Topo II α interaction. It has been shown previously that methylation at histone H3 and H4 can recruit DNA damage factors 53BP1 to sites of DNA damage^{23,24}, whereas the overall methylation level of histone H3 and H4 does not change. A possible interpretation is that DNA breaks induce conformational changes, exposing previously buried methylation sites. We speculate that inhibition of Topo II α by ICRF-193 can also induce conformational changes²⁵ and expose pSer 1524 sites that were previously buried, without affecting overall Ser 1524 phosphorylation. The exposed pSer 1524 would then recruit MDC1 to chromatin and activate the decatenation checkpoint. To test this possibility, we performed nuclear fractionation experiments. We found that ICRF-193 treatment increased chromatin-associated MDC1 (Fig. 4c) but the amount of Ser 1524-phosphorylated Topo II α or total Topo II α remained unchanged. This increase in chromatin-bound MDC1 was abolished in the Topo II α ^{S1524A} mutants (Fig. 4d). These results support our hypothesis that after Topo II α inhibition, Topo II α pSer 1524 sites are exposed, recruiting MDC1 to chromatin and thus activating the decatenation checkpoint.

ATR-dependent inhibition of the mitosis regulator polo-like kinase 1 (PLK1) has been proposed to be one mechanism for G2 arrest after Topo II α inhibition²⁶. To further explore how the MDC1–Topo II α interaction may regulate the decatenation checkpoint, we assessed PLK1 kinase activity in MDC1-expressing or MDC1-deficient cells treated with ICRF-193. PLK1 activity was inhibited in cells expressing MDC1 but not in MDC1-deficient cells (Supplementary Information, Fig. S2i). To test whether disruption of the MDC1–Topo II α interaction specifically affects PLK1 inhibition, we used HT1080 cells stably expressing Topo II α ^{WT} or Topo II α ^{S1524A}. These cells were transfected with *Topo II α* siRNA, and then treated with ICRF-193. PLK1 activity was then evaluated using casein as the substrate. PLK1 activity was inhibited in cells expressing Topo II α ^{WT} but not in Topo II α ^{S1524A} (Fig. 4e). These results indicate that the MDC1–Topo II α interaction regulates the blockade of PLK1 activity in response to Topo II α inhibition.

Although PLK1 activity is not essential for mitotic entry, inhibition or downregulation of PLK1 delays mitotic entry²⁷. We also found that inhibition of PLK1 using the specific inhibitor ON01910 blocked mitotic entry in the initial hours of treatment (Supplementary Information, Fig. S2j). To further confirm that PLK1 inhibition is required for ICRF-193-induced G2 arrest, we expressed wild-type PLK1 (PLK1^{WT}) or constitutively active PLK1 (PLK1^{TD}) in cells and

treated these cells with ICRF-193. Expression of PLK1^{TD}, but not PLK1^{WT}, overcame ICRF-193-induced G2 arrest (Fig. 4f). These results are consistent with a previous study²⁶ and suggest that inhibition of PLK1 is important for activation of the decatenation checkpoint.

In conclusion, our data suggest that Topo II α directly controls the decatenation checkpoint. Although it has been speculated that Topo II α has a role in checkpoint signalling^{28,29}, experimental evidence supporting this hypothesis has been lacking as it is difficult to separate this role from its role in DNA decatenation. However, we were able to achieve this by using the Topo II α ^{S1524A} mutant. Our results indicate that the S1524A mutation affects only ICRF-193-induced checkpoint activation, but not IR-, UV- or MMC-induced checkpoint activation, suggesting that the checkpoint regulated by Topo II α Ser 1524 phosphorylation is different from the classical DNA damage checkpoint. We hypothesize that the Topo II α pSer 1524 site becomes exposed after ICRF-193 treatment, and can then bind and recruit MDC1 to chromatin, thereby activating the decatenation checkpoint in response to chromosome entanglement (Fig. 4g). These results reveal a mechanism by which cells maintain genomic stability.

Methods

Protein purification

GST or GST–MDC1 BRCT domain was conjugated to glutathione-Sepharose beads and then incubated with nuclear extracts (untreated or treated with λ -phosphatase) prepared from 5 l of HeLa cells (obtained from NCCC) at 4 °C for 2 h. The Sepharose beads were then washed extensively and proteins retained on the beads were eluted with reduced glutathione (2 mM), separated by SDS–PAGE, and stained with Coomassie blue. Bands specifically identified in GST–MDC1 BRCT pulldown samples were excised and analysed using mass spectrometry.

Immunoprecipitation and western blotting

Cells were lysed with modified NETN lysis buffer (0.5% NP40, 250 mM NaCl, 50 mM Tris, 1 mM EDTA). Cell lysates were then centrifuged and supernatants were subjected to either immunoprecipitation or pulldown with the indicated antibodies or GST–MDC1 BRCT domain. Anti-FLAG and anti- β -actin antibodies were from Sigma. Anti-PLK1 antibodies (monoclonal) were from Zymed (Invitrogen). Anti-MDC1 antibodies were generated in rabbit using GST-1-250 of MDC1 (CoCalico). Anti-Topo II α antibodies were generated in rabbit using the peptide IKYLEESDEDDL, or purchased from NeoMakers. Anti-phospho-Ser 1524 Topo II α antibodies were generated in rabbit using phosphorylated peptide IKYLEEpSDEDDL. Prebleeds from rabbits were used as controls in immunoprecipitation experiments. For western blotting, antibodies were used at a concentration of 0.5 μ g ml⁻¹.

siRNA transfection

All siRNAs were synthesized by Dharmacon. The sequence for *Topo II α* siRNA corresponded to *Topo II α* cDNA 78–97 (with the sense strand sequence 5'-AAGACTGTCTGTGAGA-3'). The sequence for *MDC1* siRNA and the siRNA transfection method were as described previously³⁰.

Decatenation checkpoint assay

Cells were treated with ICRF-193 (2 μ M) for 1 h, fixed and stained with anti-phospho-H3 antibodies or anti-cyclinB1 antibodies (Santa Cruz Biotechnology). Mitotic populations were then determined by FACS and microscopic analysis.

Nuclear fractionation

Cells were treated with dimethylsulphoxide (DMSO) or ICRF-193 (2 μ M) for 1 h, then collected. After washing with PBS, cells were resuspended in 80 μ l high salt buffer with NP40 (50 mM Hepes, 150 mM NaCl, 1 mM EDTA, 0.05% NP40, 25 mM NaF, 10 mM β -glycerolphosphate, 5 μ g ml⁻¹ aprotinin, 5 μ g ml⁻¹ pepstatin, 5 μ g ml⁻¹ leupeptin, 1 mM Na₃VO₄), and incubated for 5 min on ice. Cell lysates were spun at 3,000g. for 5 min at 4 °C, the supernatants were collected as fraction I. Pellets were washed once with 80 μ l high-salt buffer with NP40 and the supernatants were collected as fraction II. Pellets were then resuspended in 80 μ l RIPA buffer (with protease inhibitor) and incubated for 20 min on ice. After centrifugation, the supernatants were collected as fraction III. The pellets as the chromatin fractions were then resuspended in SDS sample buffer. Each fraction (20 μ l) was loaded and separated by SDS–PAGE.

PLK1 kinase assay

Cells were treated with DMSO or ICRF-193 (2 μ M) for 1 h and lysed in NETN buffer. Cell lysates were then subjected to immunoprecipitation using anti-PLK-1 antibody (Zymed). The PLK1 immunoprecipitates and substrate (casein) were incubated in kinase buffer (25 mM Tris-HCl (7.5 pH), 5 mM β -glycerophosphate, 2 mM dithiothreitol (DTT), 0.5 mM Na₃VO₄, 10 mM MgCl₂, 0.2 mg ml⁻¹ BSA, 10 μ M ATP, 5 μ Ci[γ -³²P]) for 30 min at 30 °C. Reactions were stopped by addition of SDS sample buffer. Samples were separated by SDS–PAGE and incorporation of ³²P was examined by autoradiography. The gel was stained with Coomassie blue to demonstrate that equal amounts of proteins were included in these reactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. MDC1 interacts with Topo II α through its BRCT domain

(a) Sepharose beads coupled with GST or GST-MDC1 BRCT domain were incubated with HeLa cell lysates (untreated or treated with λ -phosphatase). Proteins pulled down by GST-BRCT were stained with Coomassie blue. (b) GST-MDC1 BRCT domain was used to pull down Topo II α from cell lysates (untreated or treated with λ -phosphatase), and immunoblotted with anti-Topo II α antibodies. WCL: whole cell lysates. (c, d) HeLa cell lysates were subjected to immunoprecipitation with anti-MDC1 (c) or anti-Topo II α (d) antibodies and the immunoprecipitates were blotted with the indicated antibodies. Rabbit prebleeds were used as a control. (e) 293T cells were transfected with FLAG-tagged full-length (FL) MDC1 or MDC1 deleted of the BRCT domain (dBRCT). Cell lysates were then subjected to

immunoprecipitation with anti-FLAG antibodies and the immunoprecipitates were then blotted with the indicated antibodies. Uncropped images of the scans in **b**, **d** and **e** are shown in Supplementary Information, Fig. S3.

Figure 2. The pSer 1524 site of Topo II α is the binding site of the MDC1 BRCT domain (a–c) FLAG-tagged full-length (FL) Topo II α , deletion mutations or point mutations (a, upper panel) were expressed in 293T cells, and cell lysates were incubated with Sepharose beads coupled with GST–MDC1 BRCT domain protein (a, lower panel, b) or subjected to immunoprecipitation with anti-FLAG antibodies (c). Proteins pulled down by GST–BRCT or co-immunoprecipitated with Topo II α were blotted with the indicated antibodies. (d) Sepharose beads conjugated with peptides containing unphosphorylated Ser 1524 or pSer 1524 were incubated with GST–MDC1 BRCT protein. Proteins retained on the Sepharose beads were then immunoblotted with anti-GST antibodies. (e) HT0180 cells were synchronized by double-thymidine block, then released into the cell cycle. Cells were collected at the indicated time-points and phosphorylation at Topo II α Ser 1524 was examined by immunoblotting. Cell-cycle distributions at different time-points were determined by FACS analysis and summarized below. Uncropped images of the scans in b–e are shown in Supplementary Information, Fig. S3.

Figure 3. The MDC1–Topo IIα interaction is important for activation of the decatenation checkpoint

(a) Ser 1524 phosphorylation of Topo IIα is not important for Topo IIα decatenation activity. S- and FLAG-tagged Topo IIα^{WT} (WT) or Topo IIα^{S1524A} (S1524A) was expressed in 293T cells and purified by sequential purification. Purified Topo IIα was then used to perform DNA decatenation assay. Equal amounts of WT and S1524A used in these assays were verified by anti-Topo IIα immunoblotting. (b–e) HT1080 cells were transfected with *MDC1* siRNA (b, c). HT1080 cells stably expressing siRNA-resistant (siRNA R) WT or S1524A were transfected with *Topo IIα* siRNA (d, e). After 72 h, transfected cells were treated with ICRF-193 (2 μM, b, d) or UV (40 J per m², c, e). After a further 1 h, cells were collected, fixed and stained

with anti-phospho-H3 antibodies to determine the mitotic population (mitotic index). Expression of MDC1 or Topo II α were examined using the indicated antibodies (**d**, **e**, lower panels). Uncropped images of the scans in **a** and **e** are shown in Supplementary Information, Fig. S3 (**f**) Cells were transfected as in **b** and **d**, then treated with ICRF-193 and nocodazole. Mitotic populations were determined at the indicated time-points. (**g**) HT1080 cells were transfected with *Topo II α* siRNA together with constructs encoding siRNA-resistant Topo II α ^{WT} (WT) or Topo II α ^{R162Q} (R162Q) Topo II. Cells were then treated with ICRF-193 and the mitotic index was determined. (**h**) HT1080 stably expressing siRNA-resistant WT or S1524A were transfected with *Topo II α* siRNA. After 72 h, transfected cells were treated with ICRF-193 (2 μ M, 1 h) and then treated with colcemid (0.1 μ g ml⁻¹) for 4 h and mitotic spreads were prepared using standard protocol. Representative pictures of mitotic spreads from mock-treated or ICRF-193-treated cells are shown (only S1524A is shown). Quantification of pseudomitosis in cells expressing WT or S1524A are shown in the lower panel. (**i**) HT1080 cells stably expressing siRNA-resistant WT or S1524A were transfected with *Topo II α* siRNA every other day. Mitotic spreads were then prepared at the indicated times and examined for pseudomitosis. Data shown are mean $\pm$ s.d. of three independent experiments (**b–i**).

Figure 4. Phospho-Topo IIα recruits MDC1 to chromatin and activates the decatenation checkpoint (a) HT1080 cells stably expressing FLAG-tagged Topo IIα^{WT} (WT) or Topo IIα^{S1524A} (S1524A) were treated with ICRF-193 (2 μM) and lysed 1 h later. Cell lysates were subjected to immunoprecipitation and immunoblotting with the indicated antibodies. (b) HT1080 cells were treated with ICRF-193 (2 μM) and lysed 1 h later. Cell lysates were subjected to immunoprecipitation and immunoblotting with the indicated antibodies. (c) HT1080 cells were treated with ICRF-193 (2 μM) and 1 h later, nuclear fractionations were performed. Nuclear fractions were blotted with the indicated antibodies. (d) HT1080 cells stably expressing siRNA-resistant WT or S1524A were transfected with *Topo IIα* siRNA. After 72 h, cells were collected and experiments similar to those described in c were carried out. (e) HT1080 cells stably expressing siRNA-resistant WT or S1524A were transfected with *Topo IIα* siRNA. After 72 h, cells were treated with ICRF-193 (2 μM) and collected 1 h later, and *in vitro* kinase assay of PLK1 was performed. (f) HT1080 cells were transfected with PLK1^{WT} or constitutively active PLK1 (PLK1^{TD}). Transfected cells were treated with ICRF-193 and mitotic populations were examined. Uncropped images of the scans in a–f are shown in Supplementary Information, Fig. S3. (g) Working hypothesis of decatenation checkpoint activation: ICRF-193 stabilizes the Topo IIα dimer and induces a conformational change²⁵, resulting in the

accumulation of MDC1 on chromatin through pSer 1524, which contributes to activation of the decatenation checkpoint.