

## Appendix

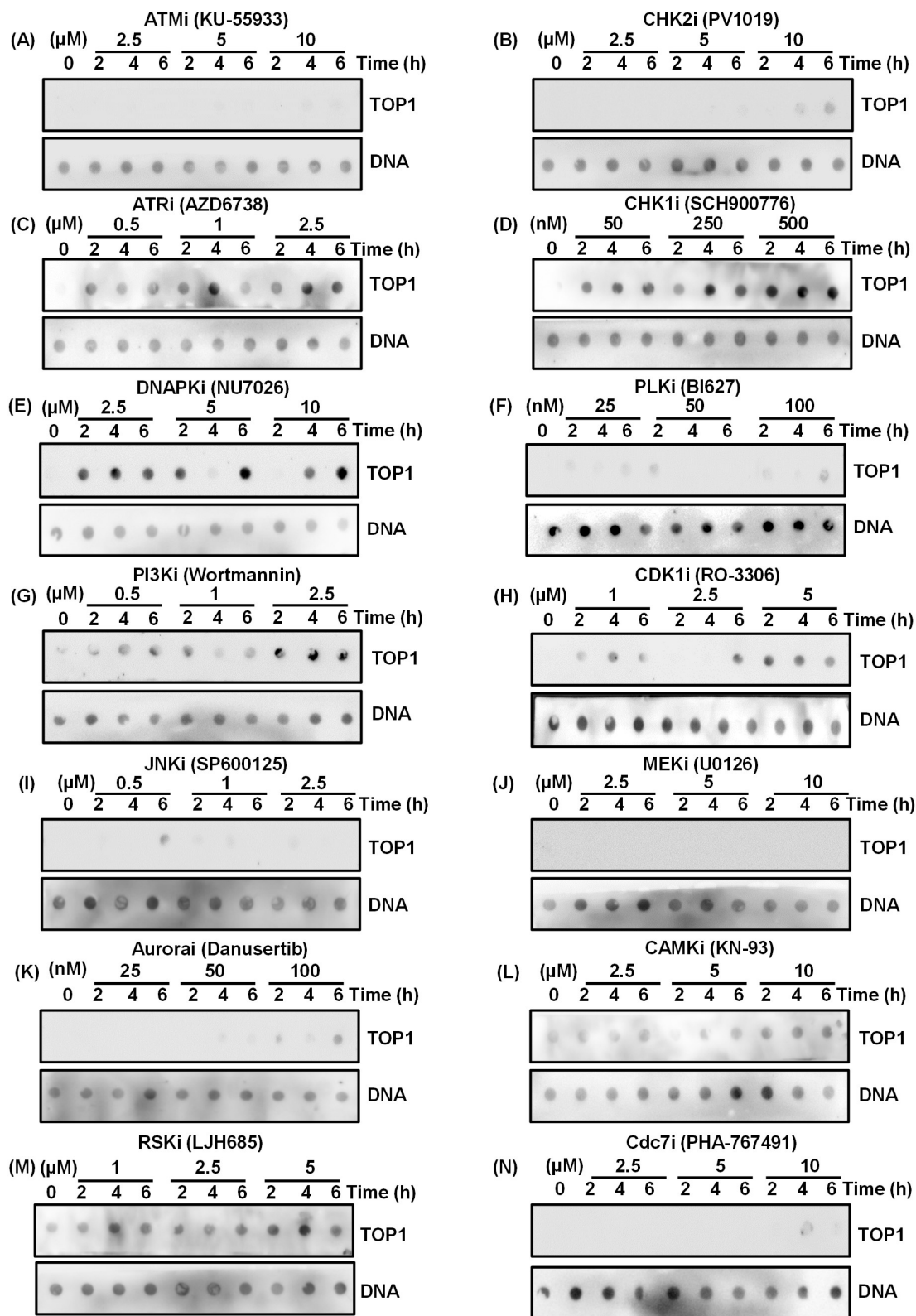
### A CHK1-mediated phosphorylation switch suppresses human Topoisomerase 1-associated genomic instability

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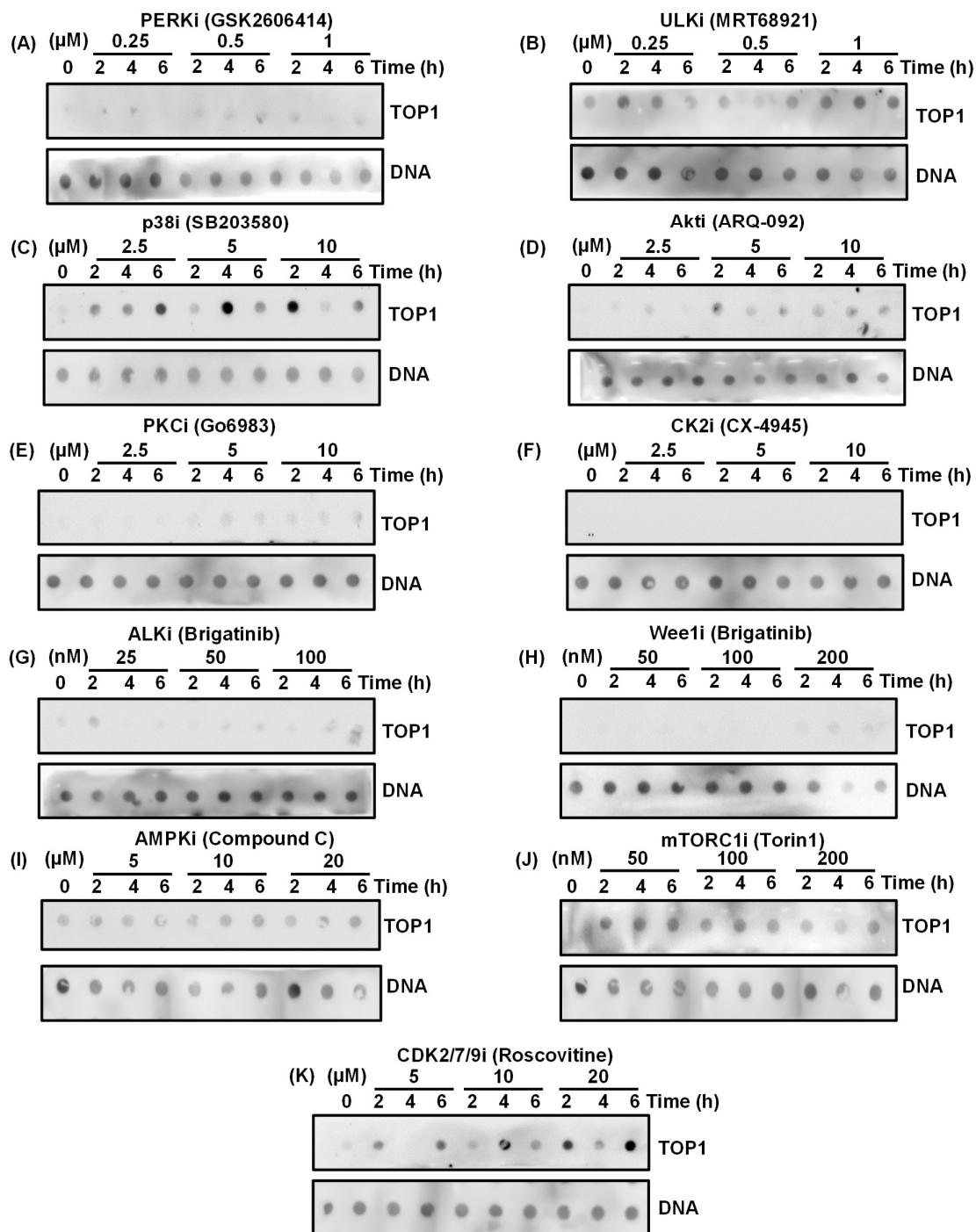
#### Contents

|                                                                                                                                                                                                                                                 | Page No. |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|
| Appendix Figure S1. RADAR screening of kinase inhibitors. ....                                                                                                                                                                                  | 3        |
| Appendix Figure S2: RADAR screening of kinase inhibitors. ....                                                                                                                                                                                  | 5        |
| Appendix Figure S3. Regulation of TOP1 dynamics by CHK1. ....                                                                                                                                                                                   | 7        |
| Appendix Figure S4. Analysis of DNA damage status of cells exposed to CHK1 inhibition. ....                                                                                                                                                     | 9        |
| Appendix Figure S5. Single stranded DNA accumulation in cells exposed to CHK1 inhibition. ....                                                                                                                                                  | 11       |
| Appendix Figure S6. Characterization of CHK1i-induced stabilization of TOP1ccs (contribution of replication and transcription, and targeting by cellular machinery). ....                                                                       | 13       |
| Appendix Figure S7. <i>In silico</i> analysis of CHK1-TOP1 interaction. ....                                                                                                                                                                    | 15       |
| Appendix Figure S8. Characterization of TOP1-CHK1 interaction. ....                                                                                                                                                                             | 16       |
| Appendix Figure S9. Characterization of expression levels and immunoprecipitation efficiencies of EGFP-TOP1 <sup>WT</sup> and EGFP-TOP1 <sup>S320A</sup> . ....                                                                                 | 18       |
| Appendix Figure S10. Replication-dependent DNA damage and replication perturbations associated with EGFP-TOP1 <sup>S320A</sup> expression. ....                                                                                                 | 19       |
| Appendix Figure S11. Transfection efficiency of EGFP-TOP1 <sup>WT</sup> and EGFP-TOP1 <sup>S320A</sup> . ....                                                                                                                                   | 21       |
| Appendix Figure S12. Characterization of transcription-associated DNA damage in cells expressing EGFP-TOP1 <sup>S320A</sup> . ....                                                                                                              | 22       |
| Appendix Figure S13. Transcription-associated genomic instability in cells expressing EGFP-TOP1S320A. ....                                                                                                                                      | 23       |
| Appendix Table S1: Kinase inhibitors, their targets, and concentrations employed in the RADAR screen. ....                                                                                                                                      | 24       |
| Appendix Table S2: Prediction of CHK1 target motifs on TOP1. While the entire motif is highlighted in green, the target residue is depicted in red. ....                                                                                        | 25       |
| Appendix Table S3: Details of sites found to be phosphorylated on catalytically active TOP1. Whether they have previously been reported elsewhere is also indicated. References pertaining to these studies are provided in the main text. .... | 26       |
| Appendix Table S4: Details of primers used in qPCR. ....                                                                                                                                                                                        | 27       |



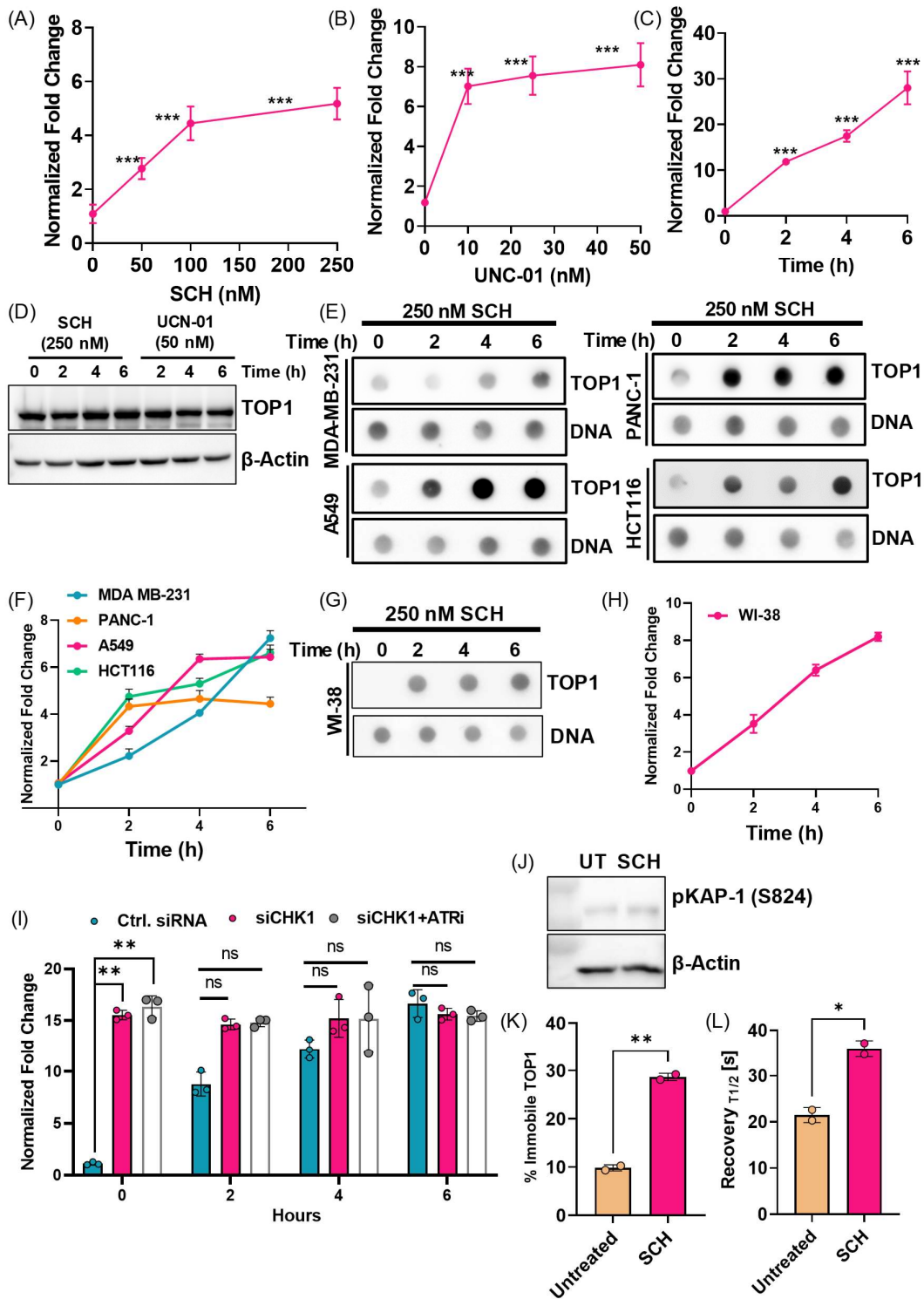
### **Appendix Figure S1. RADAR screening of kinase inhibitors.**

(A-N) RADAR assay screen for TOP1cc trapping by small molecule kinase inhibitors. U2-OS cells were treated with indicated concentrations of inhibitors for 2, 4 or 6 h, followed by evaluation of TOP1cc stabilization through RADAR assay.



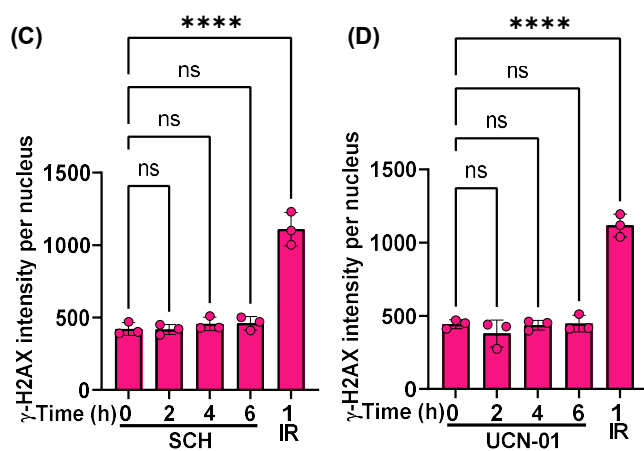
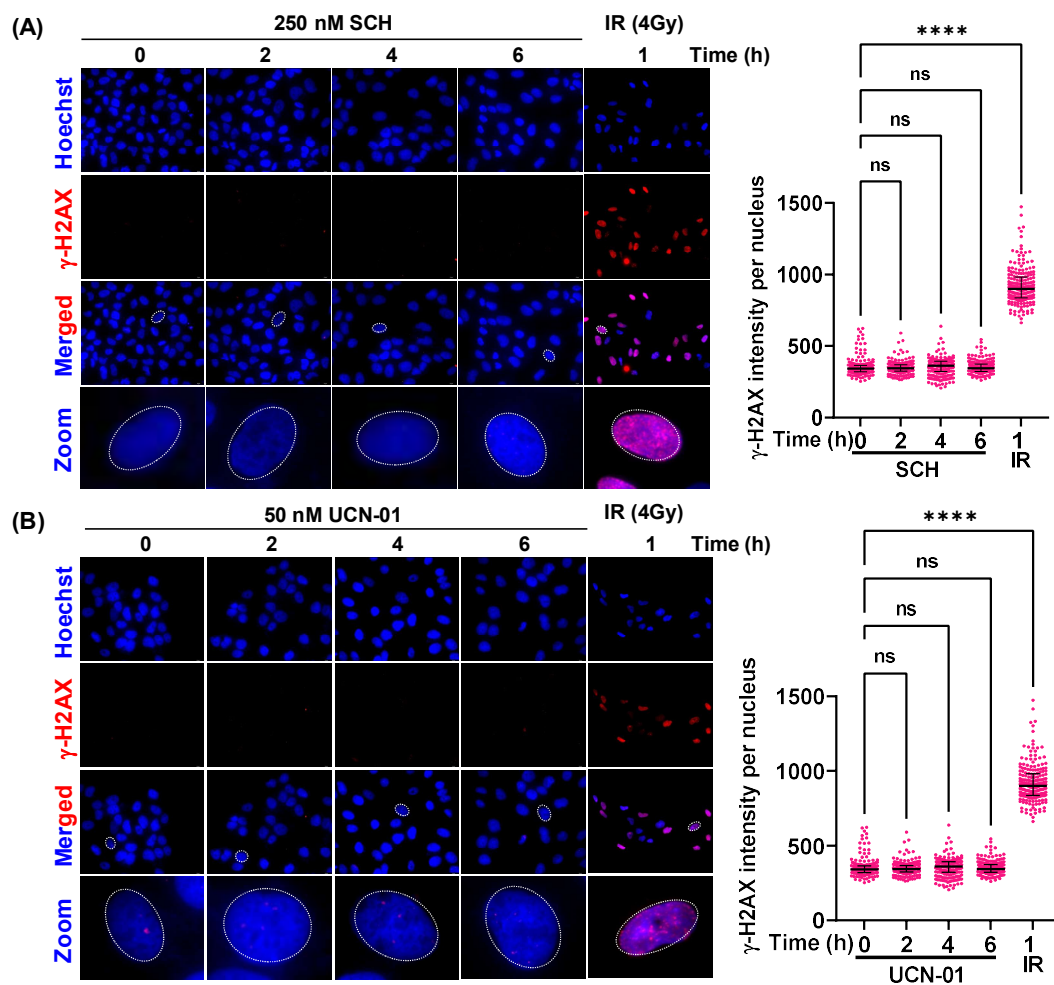
## **Appendix Figure S2: RADAR screening of kinase inhibitors.**

(A-K) RADAR assay screen for TOP1cc trapping by small molecule kinase inhibitors. U2-OS cells were treated with indicated concentrations of inhibitors for 2, 4 or 6 h, followed by evaluation of TOP1cc stabilization through RADAR assay.



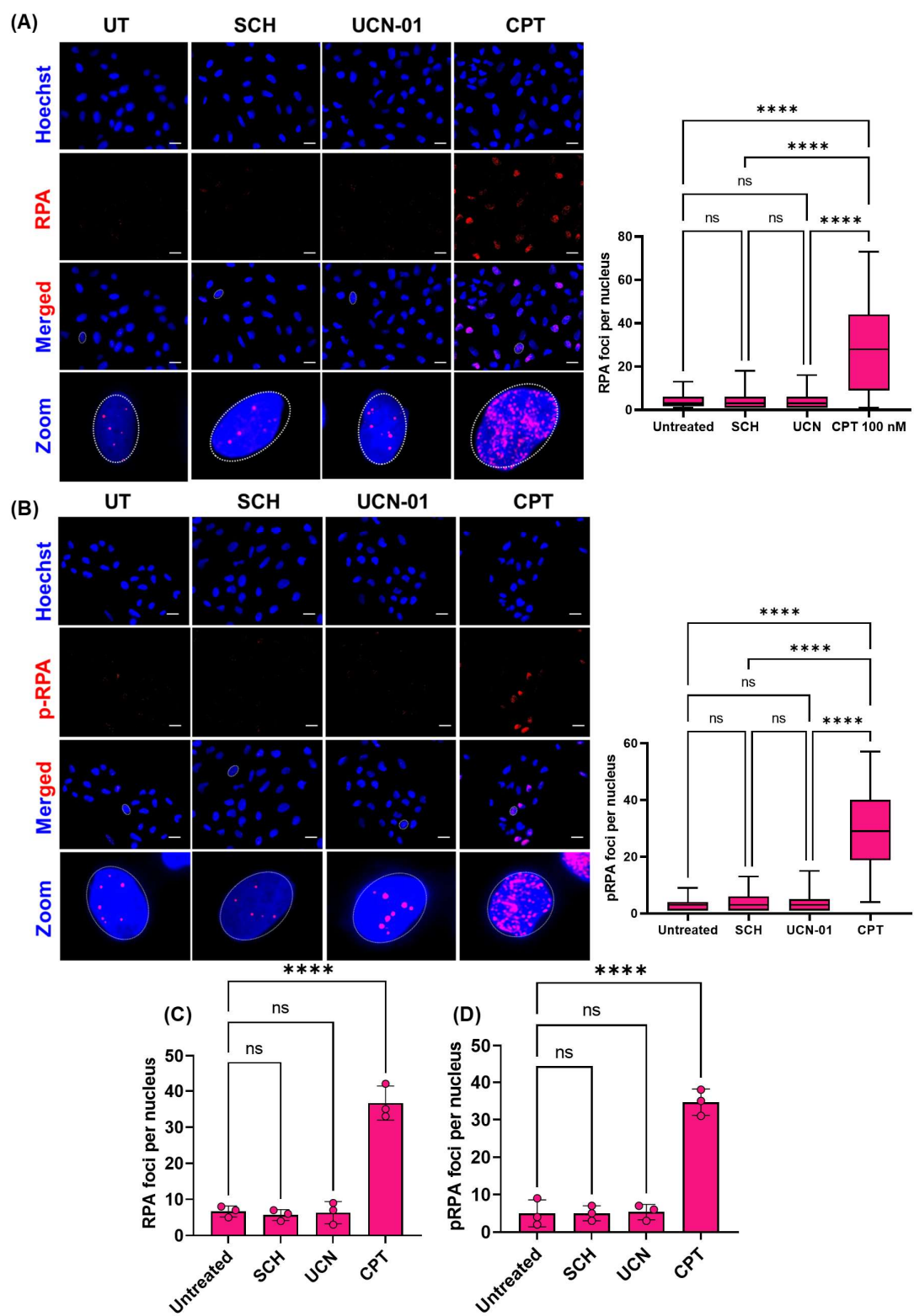
### **Appendix Figure S3. Regulation of TOP1 dynamics by CHK1.**

(A-C) Quantitative densitometric analyses of dot blots shown in Fig. 1C-E. (D) Global TOP1 levels in U2-OS cells treated with SCH (250 nM) or UCN-01(50 nM) for 2-,4- or 6 h using TOP1 downregulation assay (alkaline lysis method). (E, F) SCH-mediated TOP1cc stabilization in different cancer cells. MDA-MB-231, PANC-1, A549 and HCT116 cells were treated with SCH (250 nM) for 2, 4 or 6 h, followed by RADAR assay. (G, H) SCH-mediated TOP1cc stabilization in non-transformed WI-38 (human fetal lung) cells. (I) Densitometric quantification of Fig. 1E (mean $\pm$ SEM) (J) Immunoblot for phospho-KAP-1 (S824) in U2-OS cells treated with SCH (250 nM) for 6 h. (K, L) Quantification across three replicates of data presented in Fig. 1K, L. Error bars represent SEM. ns: not significant. \*\*  $P<0.01$ ; \*\*\*  $P<0.001$  (Data from three independent experiments; significance was determined using one-way ANOVA). Error bars represent SD.



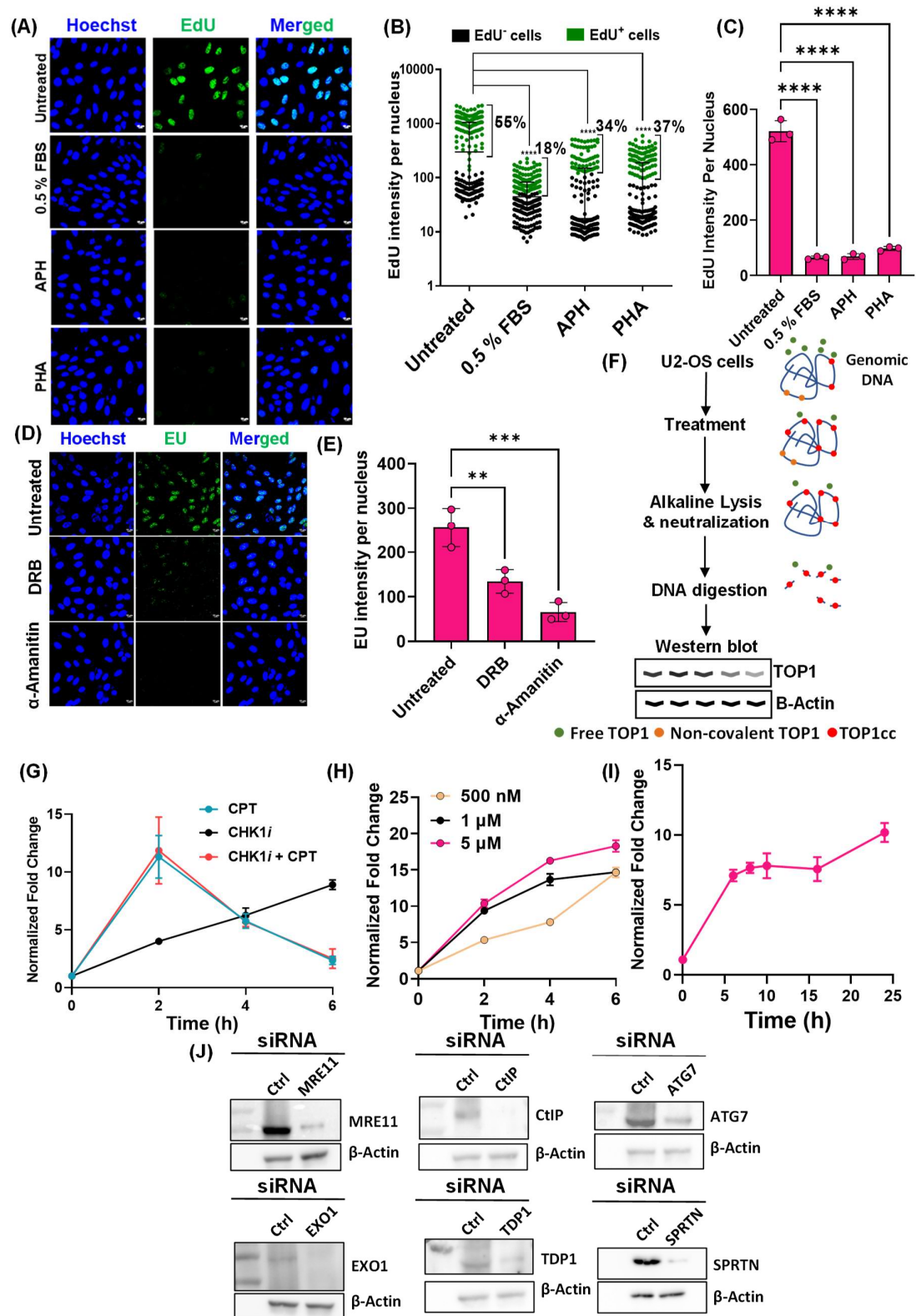
**Appendix Figure S4. Analysis of DNA damage status of cells exposed to CHK1 inhibition.**

(A) Representative microscopic images (and quantification of the shown replicate) showing  $\gamma$ -H2AX levels in U2-OS cells treated with SCH (250 nM) for 2-, 4- or 6 h. (B) Representative microscopic images (and quantification of the shown replicate) showing  $\gamma$ -H2AX levels in U2-OS cells treated with UCN-01 (50 nM) for 2-, 4- or 6 h. In both cases, IR (4 Gy) was used as positive control. Cells were subjected to 4 Gy IR and samples were processed 1 h post irradiation. (C,D) Quantification of all replicates of (A) and (B) respectively. Data from three independent experiments with 200 cells per condition. Error bars represent interquartile range (A, B) or SEM (C, D). ns: not significant, \*\*\*\*  $P < 0.0001$  (Kruskal Wallis test with Dunn's post-hoc analysis). Scale bars = 10  $\mu$ m.



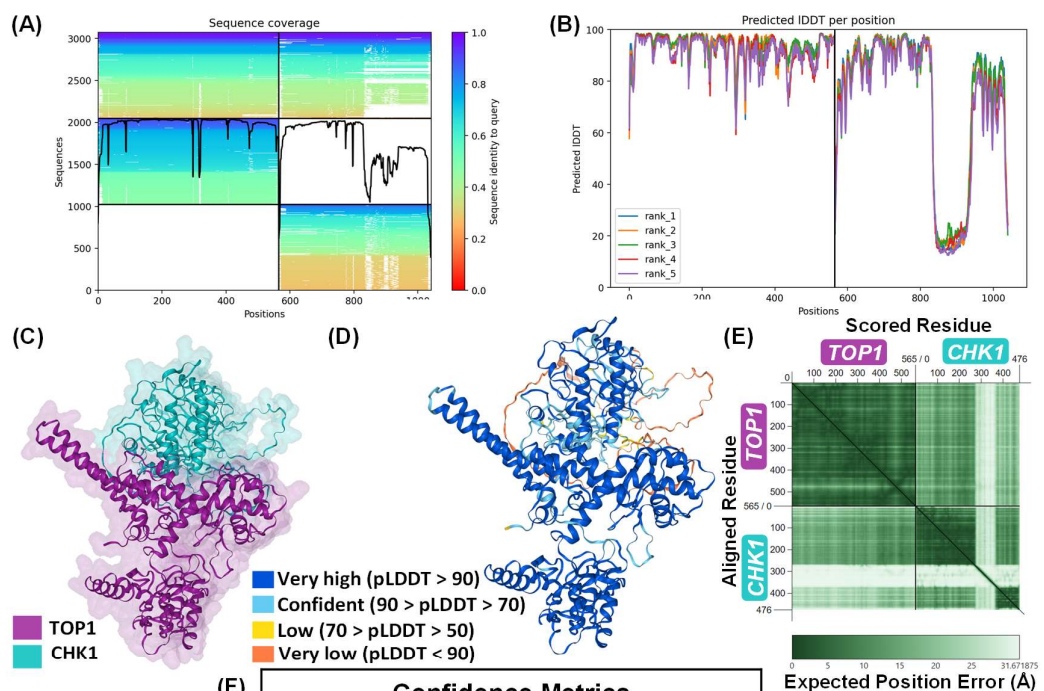
**Appendix Figure S5. Single stranded DNA accumulation in cells exposed to CHK1 inhibition.**

(A) Representative microscopic images (and quantification of the same experiment) showing RPA-32 foci in U2-OS cells treated with SCH (250 nM) or UCN-01 (50 nM) for 2 h or CPT (100 nM) for 1 h (B) Representative microscopic images (and quantification of the same experiment) showing RPA-32 foci in U2-OS cells treated with SCH (250 nM) or UCN-01 (50 nM) for 2 h or CPT (100 nM) for 1 hour. (C, D) Quantification of all replicates of (A) and (B), respectively. Data from three independent experiments with 200 cells per condition. ns: not significant. Error bars represent maximum and minimum values (A, B) or SEM (C, D). \*\*\*\*  $P < 0.0001$  (Kruskal Wallis test with Dunn's post-hoc analysis for DNA). Scale bars = 10  $\mu\text{m}$ .



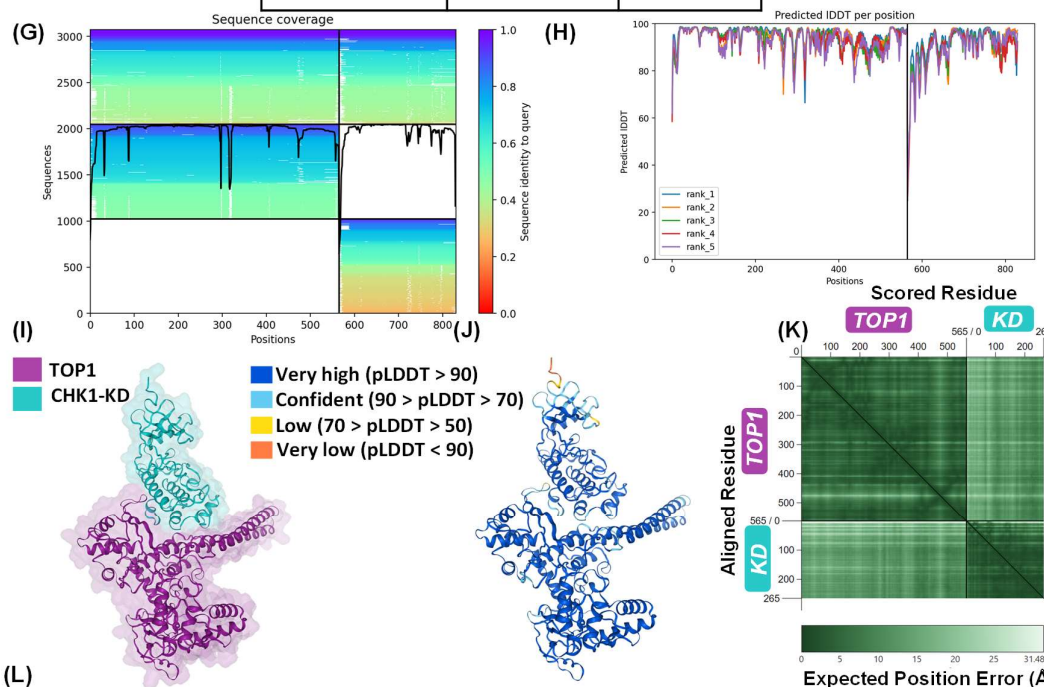
**Appendix Figure S6. Characterization of CHK1i-induced stabilization of TOP1ccs (contribution of replication and transcription, and targeting by cellular machinery).**

(A, B) Representative microscopic images (and quantification of the shown replicate) of EdU incorporation in U2-OS cells under untreated, serum starvation (0.5 % serum *i.e.*, FBS for 48 h), aphidicolin (APH; 250 nM, 16 h) or CDC7 inhibitor (PHA; 5  $\mu$ M, 4 h) conditions. U2-OS cells were subjected to requisite treatments, followed by incubation with EdU (10  $\mu$ M) for 20 min prior to termination of experiment. EdU incorporation was measured using Click chemistry. Percentage of EdU positive cells is indicated against each sample. (C) Quantification of data from three replicates of the experiment shown in (A). (D, E) Representative microscopic images (and quantification across three replicates) of EU incorporation in cells treated with the transcription inhibitors DRB and  $\alpha$ -Amanitin. U2-OS cells were treated with DRB (100  $\mu$ M) for 4 h or  $\alpha$ -Amanitin (40  $\mu$ g/mL) for 16 h, prior to incubation with EU (1 mM) for 1 h, followed by termination of experiment. EU incorporation was visualized employing click chemistry. (F) Schematic representation of TOP1 downregulation assay (alkaline lysis method) of assessing global TOP1 downregulation. (G) Densitometric quantification of Fig 3B. (H, I) Densitometric quantification of RADAR assay blots shown in Fig 3E, F. (J) Immunoblots showing siRNA mediated depletion of the indicated proteins. All experiments were performed in three independent replicates. For microscopy, 200 cells were scored per condition. Scale bars: 10  $\mu$ m. Error bars represent SEM. \*\*\*\*  $P < 0.0001$  (Kruskal Wallis test with Dunn's post-hoc analysis).



(F) Confidence Metrics

| Mean pLDDT | pTM    | ipTM   |
|------------|--------|--------|
| 84.0622    | 0.7200 | 0.6100 |

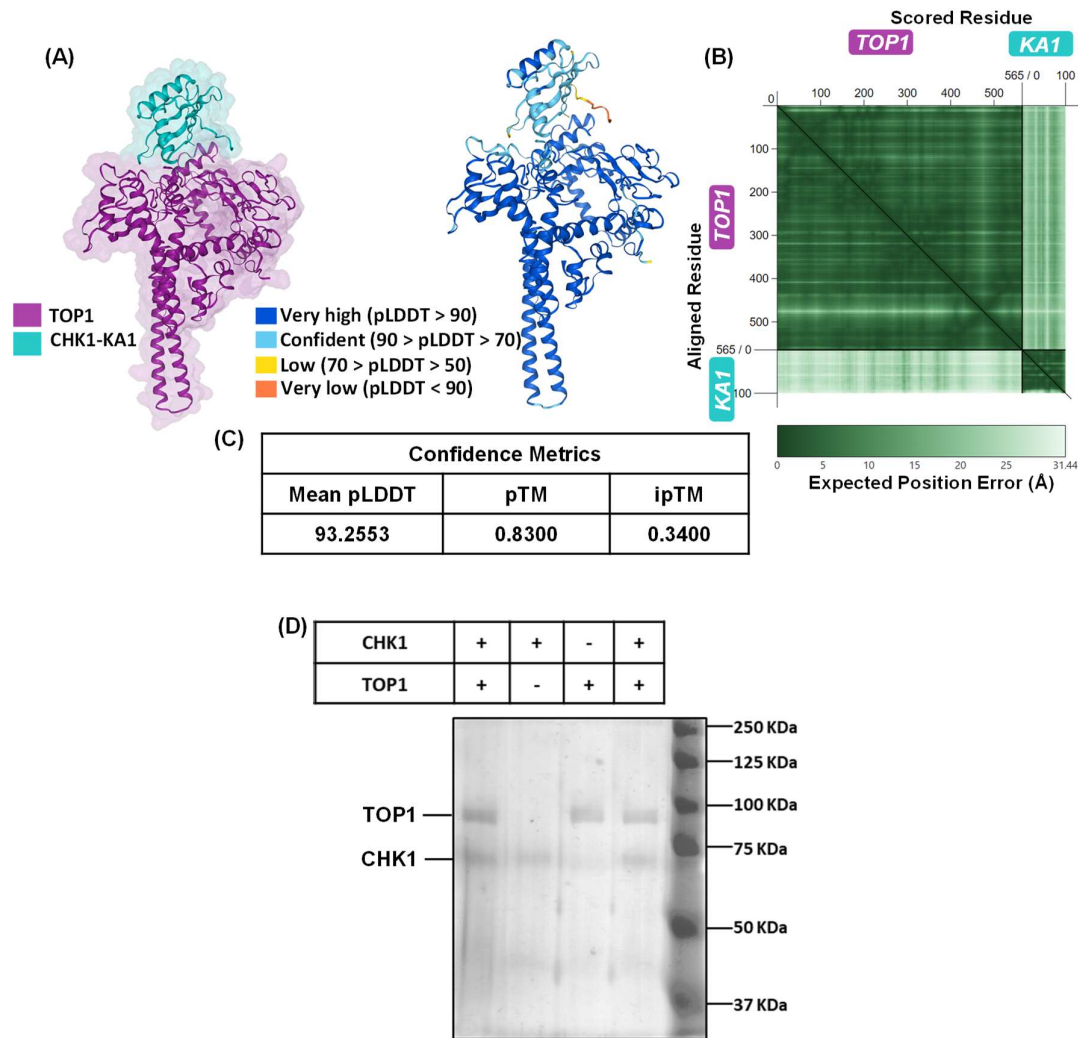


(L) Confidence Metrics

| Mean pLDDT | pTM    | ipTM   | SPOC Score | pDOCKQ | Mean PAE |
|------------|--------|--------|------------|--------|----------|
| 94.2633    | 0.8200 | 0.6400 | 0.414      | 0.349  | 7.5      |

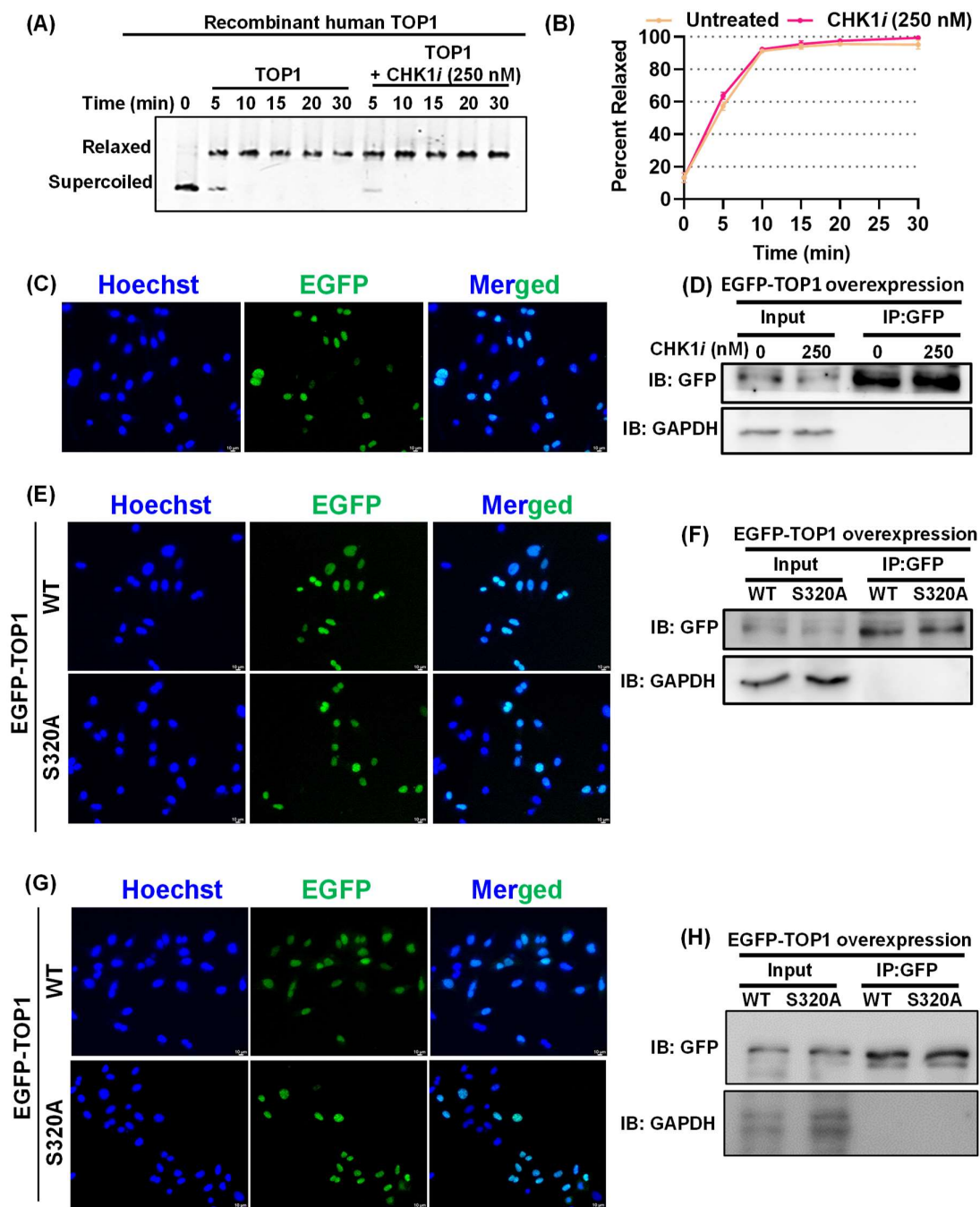
**Appendix Figure S7. *In silico* analysis of CHK1-TOP1 interaction.**

Sequence coverage (A) and pLDDT (B) plots of 5 models for TOP1-CHK1 interaction produced by Alphafold Multimer V3. The top ranked model has been reproduced in Figure 2. The second ranked model has been shown in (C) and (D). (E, F) PAE plot and confidence metrics of the second ranked model. Sequence coverage (G) and pLDDT (H) plots of 5 models for interaction between TOP1 and CHK1 kinase domain (KD) produced by Alphafold Multimer V3. (I, J) Visualization of the top-ranked model. (K, L) PAE plot and confidence metrics of the top ranked model.



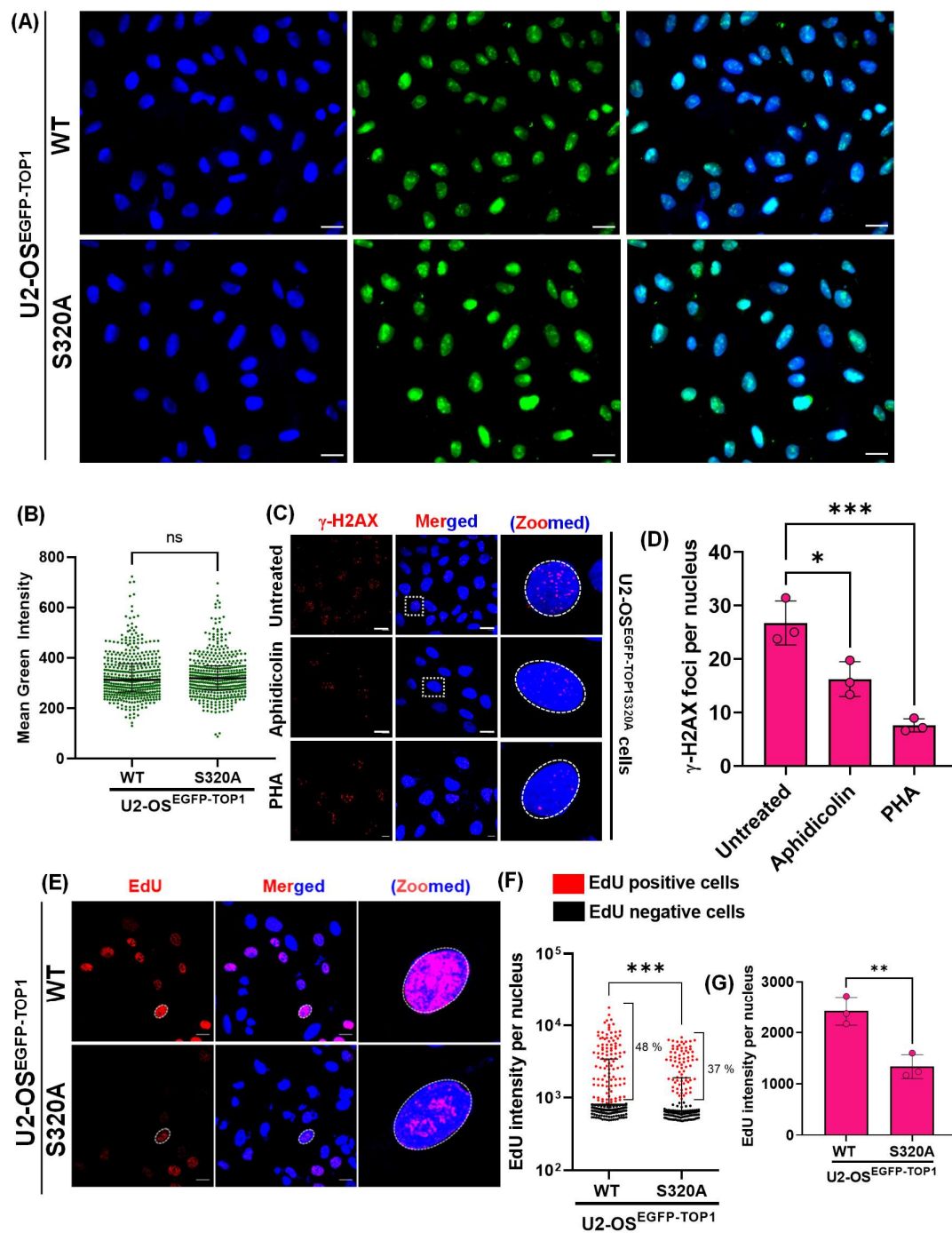
### Appendix Figure S8. Characterization of TOP1-CHK1 interaction.

Visualization (A) and PAE plot (B) of the best ranked model for interaction between TOP1 and CHK1 KA1 domain produced by AlphaFold Multimer V3. (C) Confidence metrics of the prediction. (D) Silver-stained gel corresponding to the *in vitro* kinase assay reaction. Parallel gels were run for autoradiography and silver stain.



**Appendix Figure S9. Characterization of expression levels and immunoprecipitation efficiencies of EGFP-TOP1<sup>WT</sup> and EGFP-TOP1<sup>S320A</sup>.**

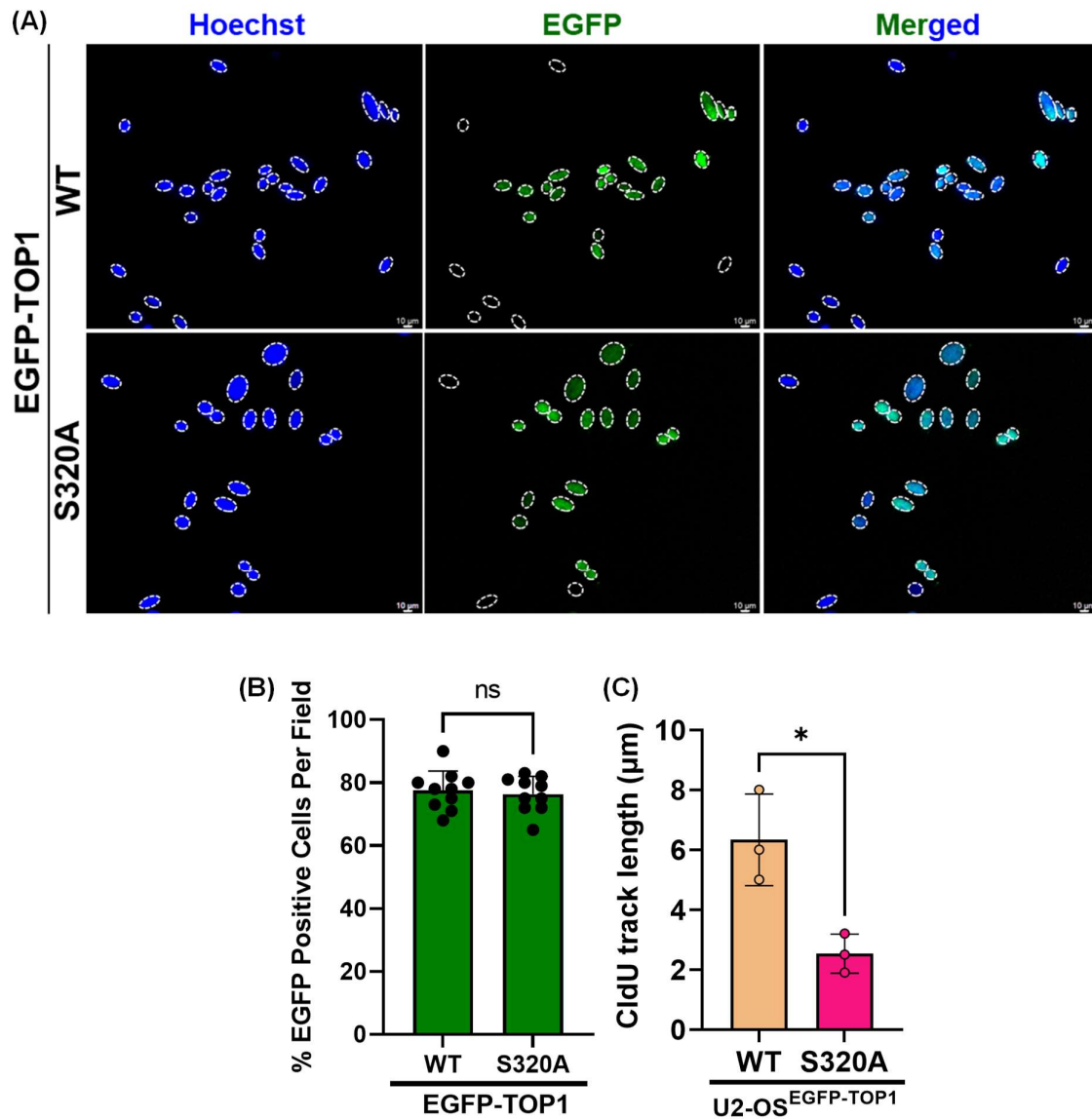
(A, B) Plasmid relaxation assay with recombinant human TOP1 in the absence or presence of CHK1i (250 nM). Data from two independent replicates. (C) Representative live-cell microscopy images showing transfection efficiency pertaining to the experiment shown in Fig. 6C. (D) Immunoprecipitation efficiency of EGFP-TOP1<sup>WT</sup> from U2-OS cells under untreated or CHK1i-treated conditions. (E) Representative live-cell microscopy images showing transfection efficiency pertaining to the experiment shown in Fig. 6E. (F) Immunoprecipitation efficiency of EGFP-TOP1<sup>WT</sup> and EGFP-TOP1<sup>S320A</sup> from U2-OS cells. (G) Representative live-cell microscopy images showing transfection efficiency pertaining to the experiment shown in Fig. 6H, J. (H) Immunoprecipitation efficiency of EGFP-TOP1<sup>WT</sup> and EGFP-TOP1<sup>S320A</sup> from U2-OS cells. These immunocomplexes were employed for TOP1 cleavage and religation assays. N=3. Error bars represent SEM.



**Appendix Figure S10. Replication-dependent DNA damage and replication perturbations associated with EGFP-TOP1<sup>S320A</sup> expression.**

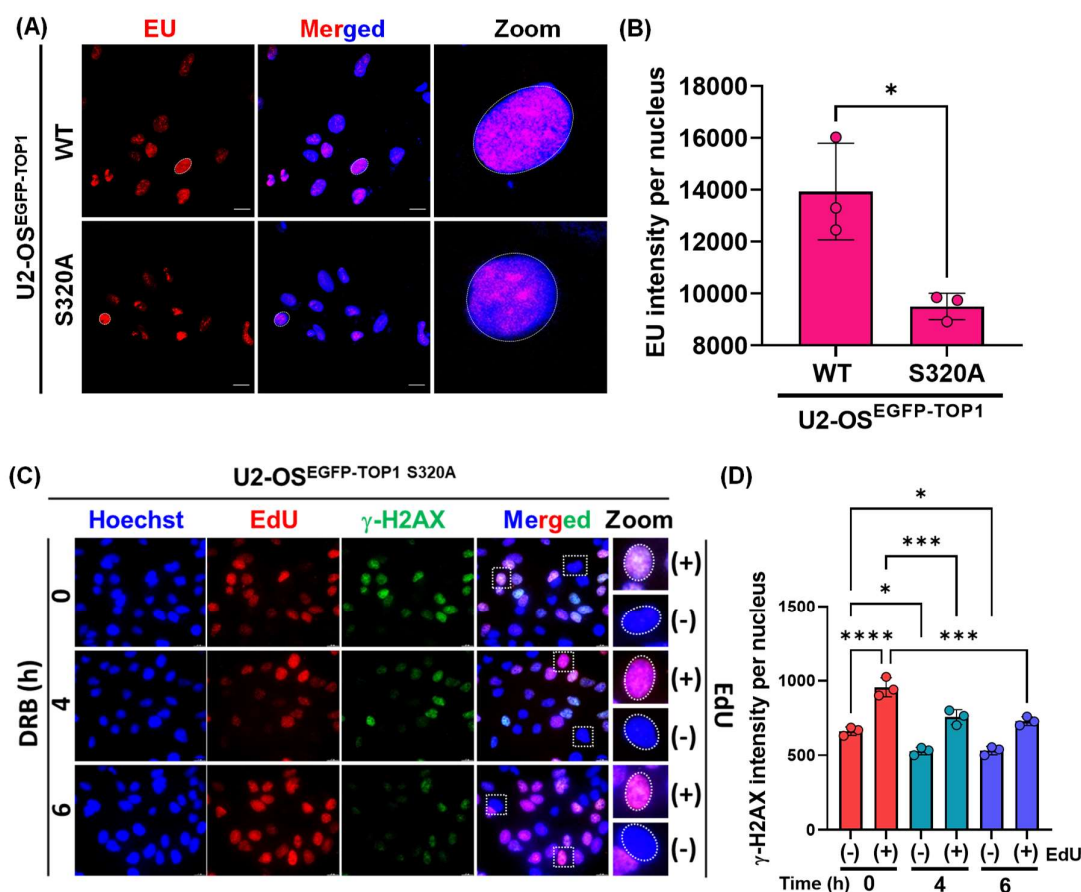
(A, B) Representative live cell fluorescence microscopy images (and quantification) showing expression of EGFP-TOP1<sup>WT</sup> and EGFP-TOP1<sup>S320A</sup> in U2-OS<sup>EGFP-TOP1<sup>WT</sup></sup> and U2-OS<sup>EGFP-TOP1<sup>S320A</sup></sup>

TOP1S320A cells, respectively. Data from the shown replicate with 500 cells per condition. (C, D) Representative microscopic images (and quantification across three replicates) of  $\gamma$ -H2AX levels in U2-OS<sup>EGFP-TOP1S320A</sup> cells treated with replication inhibitors Aphidicolin or CDC7i (PHA). Cells were treated with aphidicolin (200 nM) for 16 h or PHA (5  $\mu$ M) for 6 h before immunofluorescent detection of  $\gamma$ -H2AX foci. Data from three independent experiments with 200 cells per condition. (E-G) Representative microscopic images (and quantification of the shown replicate, as well as data from three replicates) of EdU incorporation assay in U2-OS<sup>EGFP-TOP1WT</sup> and U2-OS<sup>EGFP-TOP1S320A</sup> cells. Cells were labelled with EdU (10  $\mu$ M) for 30 min, followed by detection through click chemistry. Scale bars 10  $\mu$ m. Data from three independent experiments with 200 cells per condition. Error bars represent interquartile range (B, F) or SEM (D, G). \*  $P < 0.05$ , \*\*  $P < 0.01$ ; \*\*\*\*  $P < 0.0001$  (Kruskal Wallis test with Dunn's post-hoc analysis).



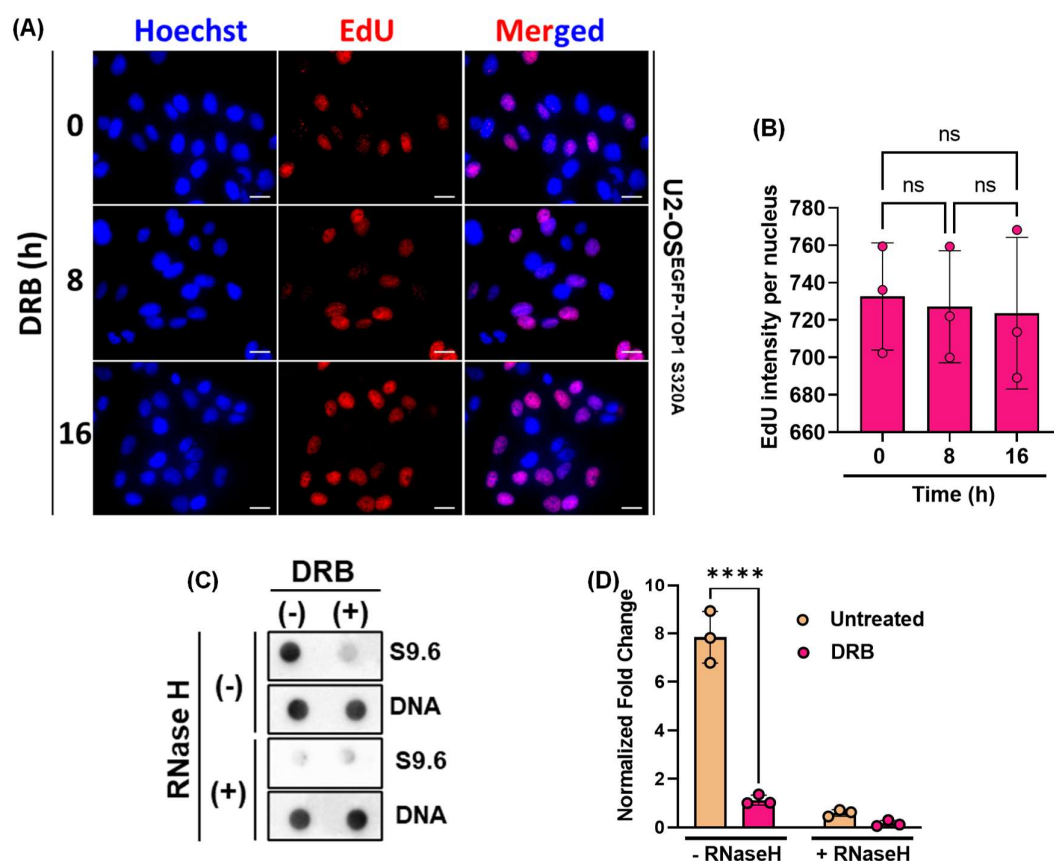
**Appendix Figure S11. Transfection efficiency of EGFP-TOP1<sup>WT</sup> and EGFP-TOP1<sup>S320A</sup>.**

(A) Representative live-cell microscopy images showing transfection efficiency pertaining to the experiment shown in Figure 7G. (B) Quantification of experiment shown in (A) across 10 fields. (C) Quantification across all replicates pertaining to experiment shown in Fig. 7G. Error bars represent SEM. ns: not significant,  $P < 0.05$  (Kruskal Wallis test with Dunn's post-hoc analysis).



**Appendix Figure S12. Characterization of transcription-associated DNA damage in cells expressing EGFP-TOP1<sup>S320A</sup>.**

(A, B) Representative microscopic images (and quantification across three replicates) of EU incorporation assay in U2-OS<sup>EGFP-TOP1</sup> WT and U2-OS<sup>EGFP-TOP1S320A</sup> cells. Cells were labelled with EU (1 mM) for 1 h, followed by detection through click chemistry. Data from three independent experiments with 200 cells per condition. (C, D) Representative microscopic images (and quantification across three replicates) showing EdU/ γ-H2AX dual staining in U2-OS<sup>EGFP-TOP1S320A</sup> cells treated with DRB (100 μM) for 4 or 6 h. Data from three independent experiments with 200 cells per condition. Scale bars 10 μm. Error bars represent SEM. \* $P < 0.05$ ; \*\*\*  $P < 0.0001$ ; \*\*\*\*  $P < 0.0001$  (Kruskal Wallis test with Dunn's post-hoc analysis).



**Appendix Figure S13. Transcription-associated genomic instability in cells expressing EGFP-TOP1S320A.**

(A, B) Representative microscopic images (and quantification across three replicates) of EdU incorporation levels in U2-OS<sup>EGFP-TOP1S320A</sup> cells upon treatment with DRB (20 μM) with 8 or 16 h. U2-OS<sup>EGFP-TOP1S320A</sup> cells were treated with DRB (20 μM) for 8 or 16 h, followed by labelling with EdU (10 μM) for 20 min prior to termination of experiment. Data from three independent experiments with 200 cells per condition. (C, D) Dot blot showing R-loop levels in U2-OS<sup>EGFP-TOP1S320A</sup> cells treated with DRB (20 μM) for 16 h. Data from three independent experiments with 200 cells per condition. Scale bars 10 μm. Error bars represent SEM. ns: not significant,  $P < 0.05$ ; \*\*\*\*  $P < 0.0001$  (Kruskal Wallis test with Dunn's post-hoc analysis).

| INHIBITOR            | TARGET KINASE (S)                                                                  | C1<br>( $\mu$ M) | C2<br>( $\mu$ M) | C3<br>( $\mu$ M) |
|----------------------|------------------------------------------------------------------------------------|------------------|------------------|------------------|
| KU-55933             | ATM                                                                                | 2.5              | 5                | 10               |
| PV1019               | CHK2                                                                               | 2.5              | 5                | 10               |
| AZD6738              | ATR                                                                                | 0.5              | 1                | 2.5              |
| SCH900776            | CHK1                                                                               | 0.1              | 0.25             | 0.5              |
| NU7026               | DNAPK                                                                              | 2.5              | 5                | 10               |
| Volasertib (BI627)   | PLK1, PLK2, PLK3                                                                   | 0.025            | 0.05             | 0.1              |
| Wortmannin           | PI3K                                                                               | 0.5              | 1                | 2.5              |
| RO-3306              | CDK1                                                                               | 1 1              | 2.5              | 5                |
| SP600125             | JNK                                                                                | 5                | 10               | 20               |
| U0126                | MEK1, MEK2                                                                         | 2.5              | 5                | 10               |
| Danuserib            | Aurora A, Aurora B, Aurora C                                                       | 0.025            | 0.05             | 0.1              |
| KN-93                | CAMK                                                                               | 5                | 10               | 20               |
| LJH685               | RSK1, RSK2, RSK3                                                                   | 1                | 2.5              | 5                |
| PHA-767491           | Cdc7                                                                               | 2.5              | 5                | 10               |
| GSK2606414           | PERK                                                                               | 0.25             | 0.5              | 1                |
| MRT68921             | ULK1, ULK2                                                                         | 0.25             | 0.5              | 1                |
| SB203580             | p38                                                                                | 2.5              | 5                | 10               |
| ARQ-092              | Akt1, Akt2, Akt3                                                                   | 2.5              | 5                | 10               |
| Go6983               | PKC $\alpha$ , PKC $\beta$ , PKC $\gamma$ , PKC $\delta$ , PKC $\zeta$ , PKC $\mu$ | 2.5              | 5                | 10               |
| CX-4945              | CK2                                                                                | 2.5              | 5                | 10               |
| Brigatinib (AP26113) | ALK                                                                                | 0.025            | 0.05             | 0.1              |
| MK1775               | Wee1                                                                               | 0.05             | 0.1              | 0.2              |
| Compound C           | AMPK                                                                               | 5                | 10               | 20               |
| Torin1               | mTORC1                                                                             | 0.1              | 0.25             | 0.5              |
| Roscovitine          | CDK2, CDK7, CDK9                                                                   | 5                | 10               | 20               |

**Appendix Table S1: Kinase inhibitors, their targets, and concentrations employed in the RADAR screen.**

|                  | Query Motif                     | Hits      |            |
|------------------|---------------------------------|-----------|------------|
|                  |                                 | Position  | Sequence   |
| Minimal Sequence | [RK]-x-x-[ST]                   | 44 – 47   | KDREKSKH   |
|                  |                                 | 64 - 67   | KEKEKTKH   |
|                  |                                 | 70 – 73   | KHKDGSSE   |
|                  |                                 | 151 – 154 | PKKIKTED   |
|                  |                                 | 284 – 287 | WRKEMTNE   |
|                  |                                 | 310 – 313 | YFKAQTEA   |
|                  |                                 | 317 – 320 | ARKQMSKE   |
|                  |                                 | 391 – 394 | DAKVPSP    |
|                  |                                 | 443 – 446 | WQKYETAR   |
|                  |                                 | 567 – 570 | FDRLNTGI   |
|                  |                                 | 603 – 606 | QLKELTAP   |
|                  |                                 | 615 – 618 | PAKILSYN   |
| Full Sequence    | [KRHV]-[RK]-x-x-[ST]            | 69 – 73   | TKHKDGSSE  |
|                  |                                 | 150 – 154 | KPKKIKTED  |
|                  |                                 | 283 – 287 | DWRKEMTNE  |
|                  |                                 | 316 – 320 | EARKQMSKE  |
| Full Sequence    | [RFPGAILV]-[KRHV]-[RK]-x-x-[ST] | 149 – 154 | YKPKKIKTED |
|                  |                                 | 315 – 320 | TEARKQMSKE |

**Appendix Table S2: Prediction of CHK1 target motifs on TOP1.** While the entire motif is highlighted in green, the target residue is depicted in red.

| Site | Untreated | 250 nM SCH 2h | 250 nM SCH 6h | 200 nM CPT 2h | 1 $\mu$ M CPT 2h | Previously Detected? |
|------|-----------|---------------|---------------|---------------|------------------|----------------------|
| S320 | +         | +             | +             | +             | +                | No                   |
| S394 | +         | +             | -             | -             | +                | Yes                  |
| T570 | +         | -             | -             | +             | -                | Yes                  |
| Y231 | +         | -             | -             | -             | -                | No                   |
| S250 | +         | -             | -             | +             | -                | No                   |
| Y480 | +         | -             | -             | +             | -                | Yes                  |
| T446 | -         | +             | -             | -             | -                | No                   |
| Y461 | -         | -             | +             | +             | -                | No                   |
| S534 | -         | -             | -             | +             | -                | No                   |
| Y538 | -         | -             | -             | +             | -                | No                   |
| T706 | -         | -             | -             | +             | -                | Yes                  |

**Appendix Table S3: Details of sites found to be phosphorylated on catalytically active**

**TOP1.** Whether they have previously been reported elsewhere is also indicated. References pertaining to these studies are provided in the main text.

|                                           | <b>Forward Primer</b> | <b>Reverse Primer</b>    |
|-------------------------------------------|-----------------------|--------------------------|
| <b>MYO3A TSS</b>                          | GTCAGATCCGGAGGACC     | GTTTCATCCCTCTCCTCCC      |
| <b>MYO3A Gene Body</b>                    | CTACAGCAGCCCACTCAAG   | CATTATCCAGTTTCTTGATTCATG |
| <b><math>\beta</math>-Actin TSS</b>       | CGGGGTCTTTGTCTGAGC    | CAGTTAGCGCCCAAAGGAC      |
| <b><math>\beta</math>-Actin Gene Body</b> | GGAGCTGTCACATCCAGGGTC | TGCTGATCCACATCTGCTGG     |
| <b>g-Actin TSS</b>                        | CCGCAGTGCAGACTTCCGAG  | CGGGCGCGTCTGTAACACGG     |
| <b>g-Actin Gene Body</b>                  | GTGACACAGCATCACTAAGG  | ACAGCACCGTGTTGGCGT       |
| <b>PTB Gene Body</b>                      | GCCGTTGGTACAAAGGTAGG  | GCCCCTTAGGAATGAAAAAG     |
| <b>Geminin 7 Gene Body</b>                | TCTTCTTCCACCTGGACCAC  | GGGACAGAGAGAGTGCCTTG     |
| <b>IL4 Gene Body</b>                      | TTCAGGTGACAAGTGCCACAG | CTGGTTGGCTTCCTTCACAG     |
| <b>Intergenic</b>                         | ACCCAGCACCCCCTAATACC  | AGCCGGACATGCTTCCAGAG     |

**Appendix Table S4: Details of primers used in qPCR.**