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TopBP1 condensates orchestrate nuclear actin assembly to ensure genome stability

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Abstract

Replication stress triggers extensive remodelling of nuclear architecture to safeguard genome stability, but how this reorganization is coordinated remains unclear. Here we identify TopBP1 condensates as central regulators of nuclear actin remodelling during this response. Sustained replication stress promotes TopBP1 condensate formation in S phase at both the nuclear interior and the nuclear periphery. We show that these condensates drive nuclear F-actin assembly through ATR-mTOR-N-WASP-dependent nucleation, while separately stabilizing actin filaments through an mTOR-dependent, ATR-independent mechanism involving cofilin inhibition. Nuclear F-actin, in turn, is required for proper TopBP1 condensate positioning, revealing a reciprocal relationship between actin network organization and condensate localization. At the nuclear periphery, TopBP1 condensates further require Lamin B1 and stabilize Emerin, coupling nuclear envelope integrity to the spatial organization of DNA repair. Notably, this TopBP1-dependent nuclear actin programme is enhanced in oxaliplatin-resistant colorectal cancer cells, where it promotes survival under chemotherapy. Our findings establish TopBP1 condensates as key architectural hubs that integrate replication stress signaling with nuclear actin dynamics and envelope organization, and identify TopBP1 condensation as a potential therapeutic target in chemo-resistant cancer.

Introduction

The spatial organization of the genome and the regulation of nuclear processes are fundamental to cellular function and identity. Among the key players in these processes, nuclear F-actin has emerged as a critical structural and functional component, challenging the traditional view of actin as a purely cytoplasmic element (1,2). Growing evidence demonstrates that actin shuttles into the nucleus through active transport mediated by Importin α (3), where it participates in essential nuclear functions, including cell cycle regulation, DNA replication and repair (4–7), chromatin organization (8), transcription regulation and initiation (9) or functional control of chromatin remodeling complexes (10). These activities require a finely tuned balance between nuclear F-actin filament assembly and turnover, regulated by actin nucleators such as the ARP2/3 complex and its activator WASP (Wiskott–Aldrich syndrome protein), as well as by actin-severing factors like cofilin 1, whose activity is suppressed by LIMK-mediated phosphorylation (11,12).

Beyond its structural role, nuclear F-actin has emerged as a dynamic and essential player in the cellular response to genotoxic stress. The DNA damage response (DDR) is a complex network that detects, signals, and repairs damaged DNA (13,14), activating cell cycle checkpoints to prevent the progression of cells with unresolved double-strand breaks (DSBs). While this response is crucial for avoiding chromosomal rearrangements and cellular transformation, cancer cells often proliferate despite persistent DNA damage, relying on DDR pathways, particularly the master checkpoint kinase ATR to activate cell cycle checkpoints (15), promote DNA repair (16), and regulate dNTP pools and replication origin firing (17).

Recent studies have revealed that DNA damage leads to rapid assembly of F-actin filaments in the nucleus (18), with the ATR-mTOR signaling axis playing a pivotal role in their polymerization and elongation (19). These filaments form a functional scaffold that facilitates the recruitment and redistribution of DNA repair factors (4), enhancing the mobility of stressed replication foci and chromatin domains surrounding DSBs (5). Additionally, nuclear F-actin filaments counteract replication stress-induced nuclear envelope deformations (19), direct the movement of heterochromatic breaks toward repair-competent regions (20), and control access of replication and repair machinery during replication stress (7). Through these mechanisms ATR-mTOR dependent nuclear F-actin filaments integrate mechanical and signaling functions to preserve genome integrity (21–23).

The main activator of ATR kinase is TopBP1 (24,25) (Topoisomerase II β -binding protein 1), a multifunctional scaffold protein that interacts with numerous factors to coordinate transcriptional regulation (26,27), DNA replication initiation (28), DNA replication stress signaling (24,25) and DNA repair (29,30). Furthermore, we demonstrated that TopBP1 self-assembles into biomolecular condensates that activate ATR under replication stress underscoring its central role in maintaining genome stability (31). However, the molecular consequences of these condensates are still poorly understood. Here, we showed that

prolonged replication stress in S phase induces TopBP1 condensates at both the interior and periphery of the nucleus. Acting as organizational hubs, these condensates orchestrate ATR-mTOR-WASP-dependent nuclear actin assembly. Furthermore, TopBP1 self-assembly limits F-actin turnover through mTOR-dependent yet ATR-independent mechanism. By forming condensates, TopBP1 activates signaling factors to safeguard replication fork integrity. At the nuclear periphery, TopBP1 engages Lamin B1 to position condensates where they stabilize Emerin, functionally linking nuclear envelope integrity to actin dynamics and DNA repair. Conversely, disrupting nuclear actin increases TopBP1 condensate formation and coincides with heightened DNA damage signaling, consistent with impaired repair under sustained replication stress. Notably, TopBP1-driven nuclear actin assembly promotes survival of oxaliplatin-resistant cancer cells, highlighting this signaling axis as a candidate target for overcoming chemoresistance. Altogether, our results demonstrate a positive feedback loop between TopBP1 condensation and nuclear F-actin polymerization, that facilitates the resolution of DNA-damage events induced by replicative stress.

Results

Spatial organization and ultrastructure of CPT-induced TopBP1 foci

TopBP1 has the capacity to form condensates but importantly, its subnuclear localization and composition are highly context-dependent, varying with the nature of the lesion and the phase of the cell cycle. Overexpression of recombinant TopBP1 in cells or treating with the transcription inhibitor actinomycin D promotes its accumulation within nucleoli, resulting in ATR-dependent repression of rRNA transcription and nucleolar segregation (32). Mild replication stress induced by hydroxyurea or aphidicolin leads to the formation of large TopBP1 foci in nucleoli, as well as smaller foci distributed throughout the nucleoplasm (33). Optogenetic, light-induced activation of TopBP1 results in the formation of large condensates distributed throughout the nucleus that amplify ATR activity (31). Together, these observations indicate that TopBP1 localization and functions are dictated by the nature of the cellular stress and the DNA intermediates that are generated. In this study, we challenged cells with high concentrations of the DNA replication stress-inducing agent camptothecin (CPT) to promote replication fork stalling and reversal, as well as fork collapse-associated DNA double-strand breaks (DSBs). We investigated the spatial organization and functional properties of TopBP1 condensates under these conditions using structured illumination microscopy (SIM). To this end, we used a HEK293T cell line engineered by CRISPR-Cas9 to express endogenous TopBP1 tagged at its carboxyl terminus with HaloTag on both alleles (TopBP1^{Halo/Halo} HEK293T). Exposure of cells to high doses of CPT for 3 hours induced the formation of TopBP1 condensates specifically during the S phase of the cell cycle and mitosis (Fig. 1a). Halo-tagged TopBP1 condensates triggered activation of the ATR pathway under CPT treatment. Indeed, TopBP1 depletion using a HaloPROTAC3 ligand for 3 hours significantly reduced TopBP1 levels and ATR signaling, as evidenced by decreased phosphorylation of Chk1 on Ser345, one of the main ATR substrates (Supplementary Fig. 1a,b). CPT-induced condensates were primarily

localized within the central part of the nucleus; however, during mid and late-S phase, TopBP1 condensates displayed a striking peripheral nuclear distribution that closely followed the pattern of EdU incorporation as described in (34) (Fig. 1a). Colocalization analysis further revealed that peripheral TopBP1 foci associated with EdU-labeled replication sites following CPT treatment (Supplementary Fig. 1c). In addition, lattice-based SIM (LSIM) provided high-resolution visualization of the increased peripheral localization of TopBP1 condensates in response to CPT (Fig. 1b,c). To quantify this redistribution, TopBP1 foci distribution was analyzed across six concentric nuclear bins spanning from the nuclear center area (Bins 1–5) to the periphery (Bin 6) (Fig. 1d). Under control conditions, small TopBP1 foci arising from constitutive replication stress displayed a gradual enrichment toward the nuclear periphery. However, following camptothecin treatment, this distribution shifted further towards the nuclear periphery (Fig. 1d,e and Supplementary Fig. 1d). Indeed, while no significant differences were observed in the central bins (Bins 1–5), CPT induced a significant increase in TopBP1 foci in the outer nuclear regions, particularly in Bin6 (Fig. 1e). Collectively, these results indicate that sustained DNA replication stress promotes the redistribution of TopBP1 foci toward the nuclear periphery.

Furthermore, LSIM revealed a high degree of colocalization between TopBP1 condensates and DNA following CPT treatment (Supplementary Fig. 1e,f), indicating that the majority of TopBP1 foci form at sites of DNA damage. To explore the ultrastructure of these foci, we employed high resolution LSIM, named SIM², achieving a resolution of ~60 nm, and observed that CPT-induced large foci are composed of clusters of smaller condensates (Fig. 1f). Quantitative analysis using CellProfiler software showed that foci sizes were heterogeneous, with diameter ranging from 80 to 300 nm (Fig. 1g). Next, we explored the dynamics of TopBP1 molecules using single-particle tracking (SPT). Halo-tagged TopBP1 expressed in HEK293 cells were labelled with a high concentration of the Fluorescent probe JF549 HaloTag[®] ligand (Hhmi Janelia) to visualize TopBP1 condensates and a low concentration of the Fluorescent probe JF646 HaloTag[®] ligand to visualize TopBP1 molecules with or without CPT treatment and observed with homemade objective-type total internal reflection fluorescence (TIRF) setup allowing multicolor single-molecule imaging (see Materials and Methods section) (35). The data were analyzed using the custom-built software Patrack which includes a neural network-based classification of trajectories (36). We observed that most TopBP1 molecules exhibited confined behaviour, with a median apparent diffusion coefficient of ~ 0.094 $\mu\text{m}^2/\text{s}$ (data not shown) and a confined diameter median of 220 nm (range 80 to 230 nm, Fig. 1h). These confinement areas match the size of TopBP1 condensates observed in Fig. 1g, strongly suggesting that these molecules are physically restricted within the foci. Interestingly, we also identified a population of very mobile molecules, which were very difficult to track. Proteins very rapidly diffused out of the evanescent field, most likely corresponding to molecules freely diffusing in 3D in the nucleoplasm (data not shown). Taken together, these observations demonstrate that, in response to sustained DNA damage, TopBP1 forms heterogeneous foci that localize both centrally and at peripheral mid-S phase nuclear DNA damage sites.

Fig. 1 | CPT-induced TopBP1 condensates concentrate at the nuclear periphery during mid S phase.

a, Structured illumination microscopy (SIM) of a single z-plane from HEK293 cells stained with the Janelia Fluor 549 HaloTag[®] ligand and click-it EdU Cell Proliferation Kit after treatment with 2 μ M CPT for 3 h. **b**, Lattice structured illumination microscopy (LSIM) of a single z-plane of HEK293 cells stained with the Janelia Fluor 549 HaloTag[®] ligand after 2 μ M CPT treatment for 3 h. CTR - control cells. **c**, Peri-center TopBP1 foci density ratio (area-normalized) from the experiments shown in **(b)**. The density of peripheral and central TopBP1 foci were calculated by dividing foci counts by their respective compartment areas. The peripheral-to-central (“Peri-center”) density ratio was then calculated for each nucleus. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m. of 2 biological replicates, scoring ≥ 23 nuclei per condition and replicate. **d**, CellProfiler radial distribution pipeline applied to HEK293 cells stained with Janelia Fluor 549 and click-it EdU Cell Proliferation Kit after treatment with 2 μ M CPT for 3 h. EdU-positive nuclei were selected and segmented into 6 concentric bins scaled to the nuclear area, from the centre (bin 1) to the periphery (bin 6); TopBP1 foci centroids were assigned to each bin. **e**, Relative radial distribution of TopBP1 foci from the experiments shown in **(d)**. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m. of 8 biological replicates, scoring ≥ 707 nuclei per condition and replicate. Statistical analysis was performed using two-tailed paired Student’s t-tests on biological replicate means, followed by Holm-Bonferroni correction for multiple comparisons: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (FWER-adjusted P-values). **f**, LSIM (120 nm lateral resolution) of a single z-plane from HEK293 cells stained with Janelia Fluor 549 after 2 μ M CPT treatment for 3 h, compared with SIM² (60 nm lateral resolution). **g**, TopBP1 focus area (μm^2) from the experiments shown in **(f)**. **h**, Confinement diameter (μm) of TopBP1 foci from HEK293 cells using single molecule tracking experiments. TopBP1 is stained with low-dose Janelia Fluor 549 and high-dose Janelia Fluor 646 after treatment with 2 μ M CPT for 1 h; confined trajectories were identified using a neural network classifier. Differences between control and CPT-treated cells were not statistically significant. For **(a)**, **(b)** and **(f)**, scale bars, 10 μm .

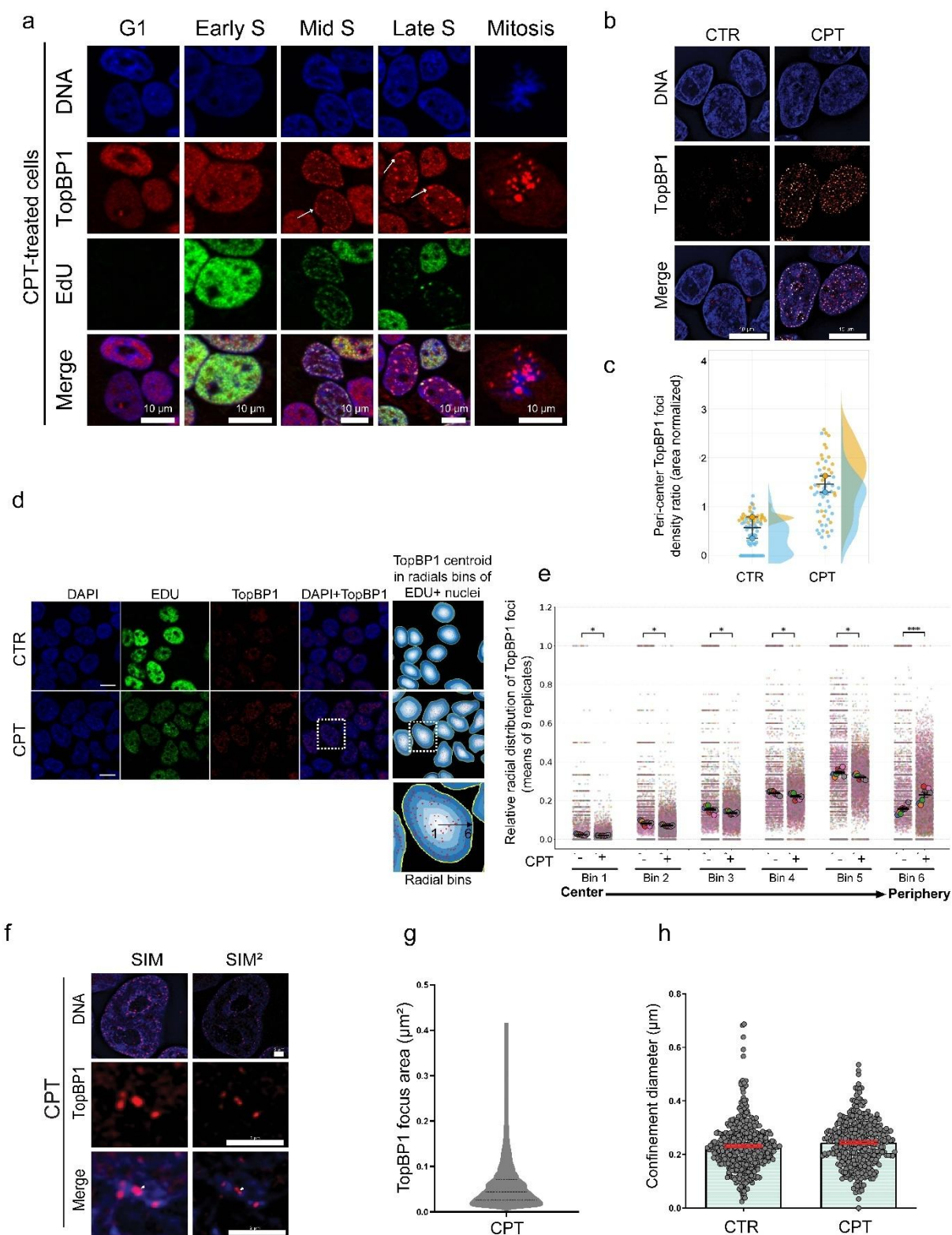


Figure 1

TopBP1 condensates regulate nuclear F-actin polymerization through ATR-mTOR-WASP signaling

Genotoxic stress triggers the polymerization of nuclear actin into filamentous networks (4,19). Using LSIM, we observed that camptothecin (CPT) treatment induces the formation of distinct nuclear F-actin architectures in Halo-TopBP1 HEK293 cells transiently expressing the NLS-GFP-actin chromobody. These are consistent with sequential stages of filament maturation, including nucleation, elongation, and the establishment of steady-state filaments (Supplementary Fig. 2a). These nuclear actin filaments predominantly assemble during S phase (19) and coincide temporally with the emergence of TopBP1 foci (Fig. 1a). To further elucidate their spatial relationship, we harnessed the subdiffraction resolution of SIM, revealing that CPT-induced TopBP1 condensates frequently localize in proximity to, and occasionally colocalize with, nuclear F-actin filaments (Fig. 2a). Furthermore, CPT treatment enhances the association of TopBP1 condensates with the actin nucleation factor WASP (Fig. 2b,c). Given the established involvement of the ATR–mTOR signaling axis in nuclear F-actin polymerization (19), and our prior work linking TopBP1 self-assembly to ATR activation (31), we next investigated whether TopBP1 directly regulates this process. While CPT treatment stabilized WASP expression, this effect was abolished by ATR inhibition with berzosertib (VE-822) or mTOR inhibition with sapanisertib (INK128) (Fig. 2d). Moreover, acute depletion of TopBP1 via HaloPROTAC3 reduced WASP stabilization and impaired ATR signaling, as evidenced by decreased Chk1 Ser345 phosphorylation (Fig. 2d). Consistent with attenuated mTOR activity, phosphorylation of the downstream effector p70S6 kinase at Ser371/Thr389 was also reduced following TopBP1 depletion or ATR inhibition (Supplementary Fig. 2b), supporting a role for TopBP1 upstream of the ATR–mTOR signaling axis. Moreover, nuclear actin polymerization contributes to DNA damage-induced nuclear expansion (19). Accordingly, TopBP1 depletion or pharmacological inhibition of ATR, mTOR, or LIM kinase significantly reduced nuclear area (μm^2), suggesting that TopBP1-mediated actin polymerization underpins this morphological response (Supplementary Fig. 2c). Next, we performed an *in vitro* actin polymerization assay using pyrene-labeled actin on nuclear extracts from CPT-treated Halo-TopBP1 293HEK cells. This further confirmed that CPT enhances actin polymerization. Crucially, this effect was abolished upon TopBP1 depletion (Fig. 2e and Supplementary Fig. 6a). Finally, to test whether TopBP1 condensation is required for nuclear F-actin assembly, we employed an optogenetic clustering system in HEK293 Flp-In cells expressing TopBP1-Cry2-mCherry construct (31). Light-induced TopBP1 condensation produced a slight rise in WASP (Supplementary Fig. 2d) and, within 15 minutes, triggered nuclear actin rods (Supplementary Fig. 2e), marking the onset of actin nucleation. This response was lost in cells expressing the condensate-deficient TopBP1 W1145R mutant reported by Frattini et al. (31) (Supplementary Fig. 2f). Together, these findings support a model in which TopBP1 self-assembly into condensates regulates nuclear F-actin polymerization through an ATR-TopBP1-WASP signaling axis under genotoxic stress.

Fig. 2 | TopBP1 self-assembly drives nuclear F-actin formation through an ATR-mTOR-WASP axis.

a, Lattice structured illumination microscopy of a representative z-stack image of HEK293 cells transfected with the fluorescent nuclear actin chromobody and stained with the Janelia Fluor 549 HaloTag[®] ligand and anti-lamin B1 after treatment with 2 μ M CPT for 3 h. Six representative cells are shown. **b**, Structured illumination microscopy of a single z-plane of HEK293 cells stained with the Janelia Fluor 549 HaloTag[®] ligand and anti-N-WASP after treatment with 2 μ M CPT for 3 h. **c**, TopBP1/N-WASP foci per nucleus from the experiments shown in **b**. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m. of 2 biological replicates, scoring ≥ 584 nuclei per condition and replicate. **d**, Western blot analysis of whole-cell extracts from HEK293 cells treated with 2 μ M CPT for 3 h in the presence or absence of 1 μ M HaloPROTAC3, 10 μ M VE-822 or 200 nM INK128. **e**, Normalized time course of pyrene-labelled actin assembly in the presence or absence of HEK293 nuclear extracts. One of three independent biological replicates is shown. For **(a)** and **(b)**, scale bars, 2 and 10 μ m, respectively.

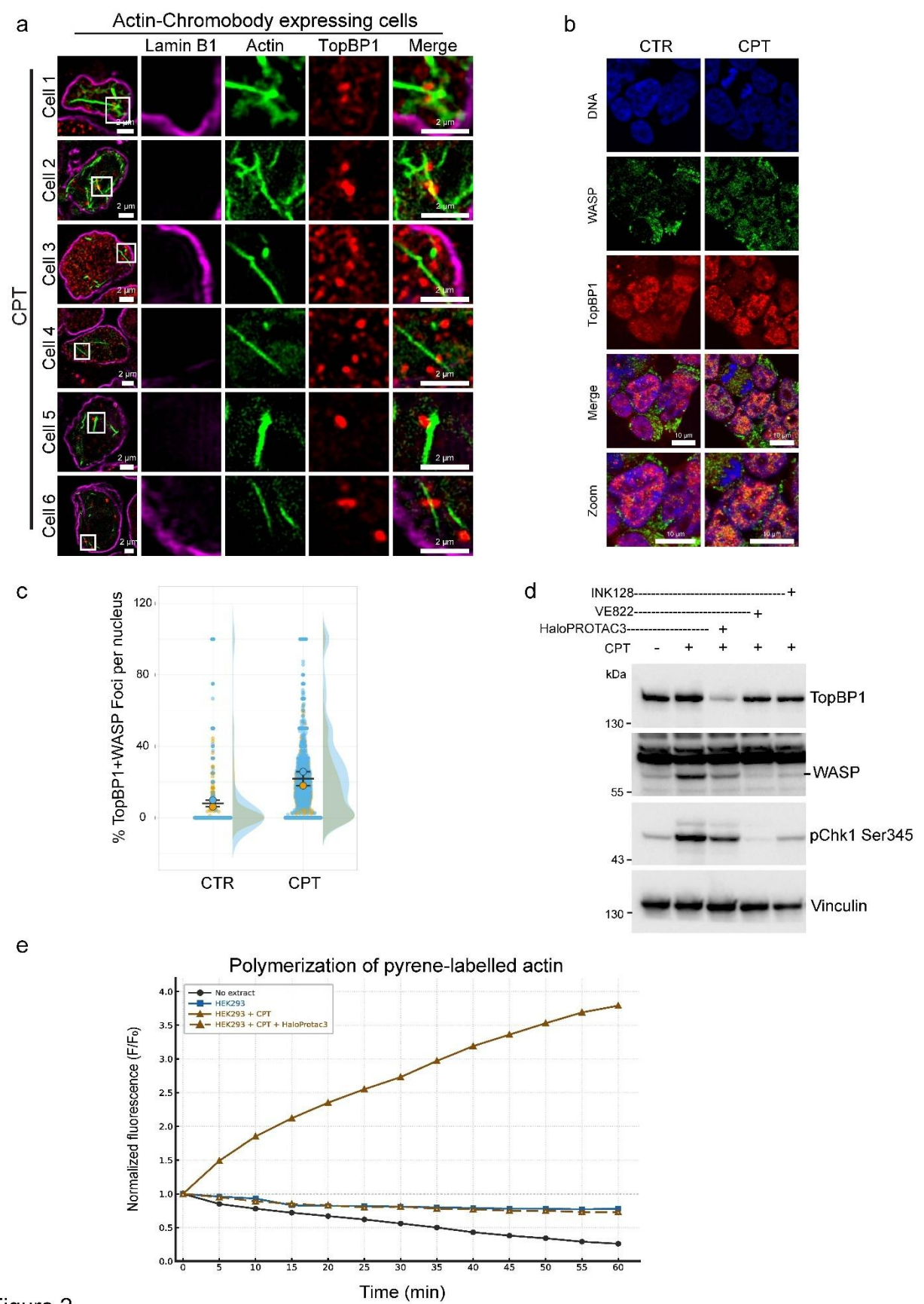


Figure 2

TopBP1 maintains actin polymerization through an mTOR-dependent but ATR-independent pathway

Given the observed link between TopBP1 and actin nucleation, we investigated its role in nuclear actin filament stabilization by examining cofilin, an actin-binding protein critical for filament turnover. Cofilin severs actin filaments, and its phosphorylation by LIM kinase at Ser3, inhibits F-actin binding, thereby promoting actin depolymerization (11,12). Following CPT treatment, we detected an increase in LIM kinase 1/2 activation, as evidenced by phosphorylation at Thr508 and Thr505, alongside elevated cofilin phosphorylation at Ser3, suggesting enhanced stabilization of actin filaments (Fig. 3a). However, depletion of TopBP1 via HALOPROTAC3 significantly reduced the phosphorylation of both LIM kinase and cofilin, indicating that TopBP1 contributes to protecting nuclear actin filaments from degradation (Fig. 3a). To place this regulation within the ATR–mTOR signaling axis, cells were treated with the ATR inhibitor VE-822 or the mTOR inhibitor INK128 (Fig. 3b). mTOR inhibition substantially impaired cofilin phosphorylation in response to CPT, whereas ATR inhibition had no discernible effect on cofilin phosphorylation status (Fig. 3b).

We next considered SSH1, a phosphatase that dephosphorylates LIMKinase and cofilin to promote filament severing (37). We hypothesized that, in response to DNA replication stress, SSH1 phosphatase activity would be downregulated to prevent cofilin activation and protect filamentous actin integrity. Immunofluorescence revealed a marked increase in nuclear SSH1 foci after CPT treatment, with a partial colocalization with TopBP1 foci (Fig. 3c,d). Immunoblotting revealed that nuclear SSH1 protein levels are stabilized after CPT treatment, and this stabilization was reduced upon TopBP1 depletion (Fig. 3e). These results suggest that a subset of TopBP1 condensates may sequester SSH1, thereby restraining its activity toward cofilin and protecting F-actin from severing. Collectively, these data demonstrate that TopBP1 safeguards nuclear actin filaments through an mTOR-dependent, ATR-independent mechanism, linking the DNA replication stress response to the regulation of actin dynamics.

Fig. 3 | TopBP1 protects nuclear F-actin disassembly through an mTOR-dependent, ATR-independent pathway.

a, Western blot analysis of whole-cell extracts from HEK293 cells treated or not with 2 μ M CPT for 3 h in the presence or absence of 1 μ M HaloPROTAC3. **b**, Western blot analysis of whole-cell extracts from HEK293 cells treated with 2 μ M CPT for 3 h in the presence or absence of 1 μ M HaloPROTAC3, 10 μ M VE-822 or 200 nM INK128. **c**, Structured illumination microscopy of a single z-plane from HEK293 cells stained with the Janelia Fluor 549 HaloTag[®] ligand and anti-SSH1 after treatment with 2 μ M CPT for 3 h, quantified by the number of SSH1/TopBP1 foci per nucleus (**d**). Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m. of 3-4 biological replicates, scoring $\geq$ 484 nuclei per condition and replicate. Statistical analysis was performed using two-tailed paired Student's t-tests on biological replicate means, followed by Holm-Bonferroni correction for multiple comparisons. ****** $p < 0.01$ (FWER-adjusted p-value). **e**, Western blot analysis of SSH1 and Cdt1 in nuclear extracts from HEK293 cells treated with 2 μ M CPT for 3 h in the presence or absence of 1 μ M HaloPROTAC3. Scale bars in (**c**), 10 μ m.

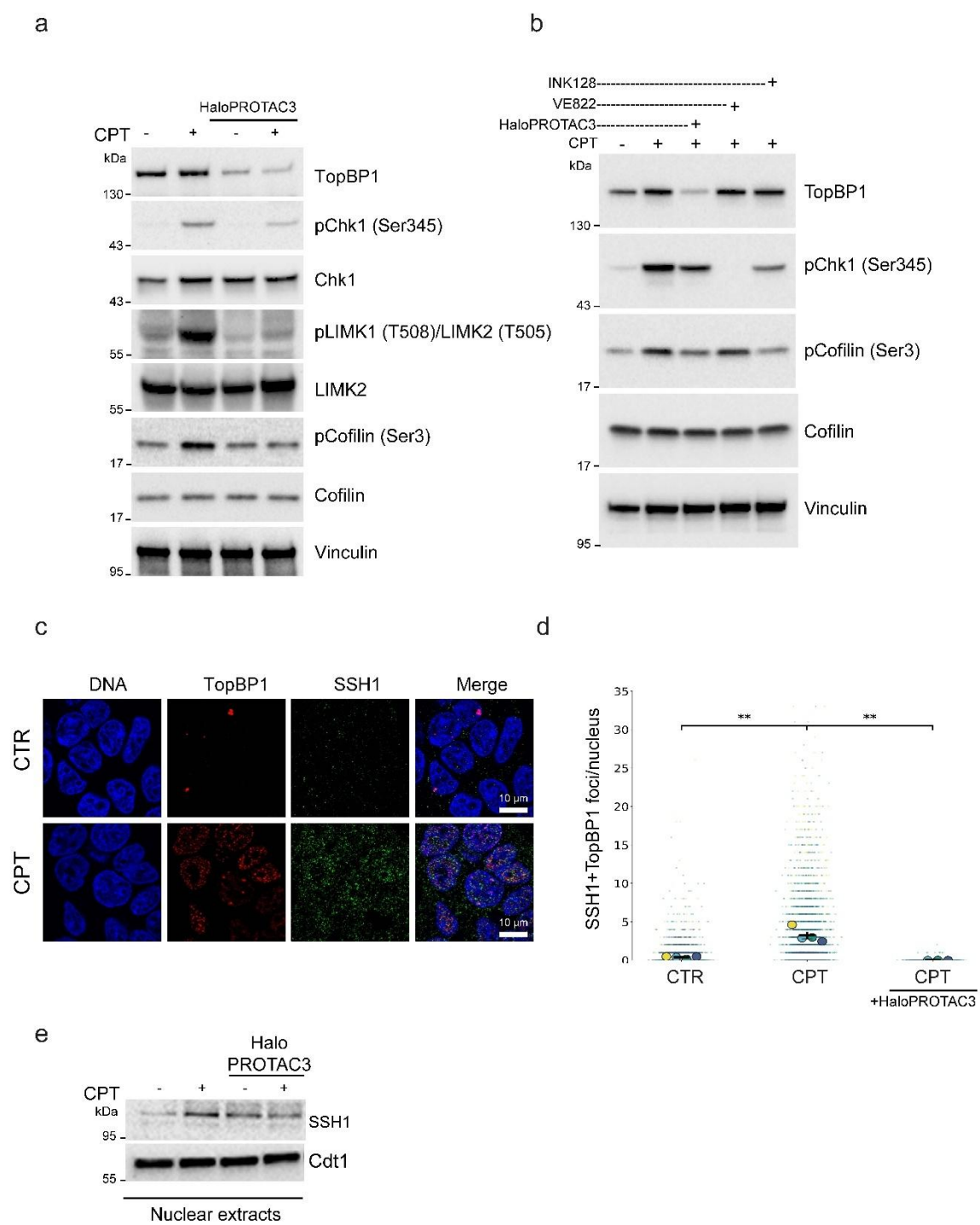


Figure 3

TopBP1 condensates interact with nuclear lamina components and regulate Emerin stability during replication stress

To investigate the functional role of peripheral nuclear TopBP1 condensates, we analyzed their molecular composition at these specific nuclear sites. Using HEK293 cells expressing endogenous Halo-tagged TopBP1, we performed Halo-Trap pull-down assays on nuclear peripheral fractions (see Materials and Methods section). Upon CPT treatment, we co-purified Lamin A/C and Emerin with TopBP1 (Fig. 4a). Notably, we also detected a significant enrichment of γ H2AX, a marker of DNA double strand, in the TopBP1-associated fraction, suggesting that these DNA structures are involved in mediating the DNA damage response at the nuclear periphery (Fig. 4a). In addition, LSIM (Fig. 4b) and apotome fluorescence microscopy (Fig. 4c) revealed substantial colocalization of TopBP1 with Lamin A/C and Lamin B1, respectively. Together, these findings support an association of TopBP1 condensates with nuclear lamina components under conditions of elevated DNA replication stress. Interestingly, we observed that depletion of TopBP1 resulted in a marked reduction in Emerin protein levels within the nuclear fraction. Emerin is an actin-binding factor that supports nucleo-cytoskeletal transport (Fig. 4a). This finding was further validated using siRNA-mediated knockdown of TopBP1: while Emerin intensity increased following camptothecin treatment in control cells, it was significantly diminished in TopBP1-deficient cells under both basal and CPT-treated conditions (Fig. 4d,e and Supplementary Fig. 3a). These data suggest that TopBP1 is required for the stabilization of nuclear Emerin, particularly during high DNA replication stress.

Given that lamin mutations mislocalize Emerin and ATR and impair ATR signaling pathway and DNA repair foci formation (38), we tested whether Lamin B1 sustains TopBP1 condensation. We found that siRNA-mediated depletion of Lamin B1 significantly reduced the overall number of TopBP1 foci in CPT-treated cells (Fig. 4f,g). Importantly, this depletion did not affect TopBP1 protein intensity (Supplementary Fig. 3b) nor alter the proportion of S-phase cells, as EdU incorporation remained unchanged in Lamin B1-depleted cells (Supplementary Fig. 3c). These results indicate that the integrity of TopBP1 condensates at the nuclear periphery depends on interactions with nuclear lamina components, and that TopBP1, in turn, contributes to the stability of Emerin during the cellular response to replication stress.

Fig. 4 | TopBP1 condensates couple nuclear lamina interactions to Emerin stability during replication stress.

a, Western blot analysis of total and peripheral nuclear fractions after HaloTrap pulldown of HEK293 cells treated or not with 2 μ M CPT for 3 h in the presence or absence of 1 μ M HaloPROTAC3. **b**, representative z-stacks of cells stained with the Janelia Fluor 549 HaloTag[®] ligand and anti-lamin A/C antibodies after treatment with 2 μ M CPT for 3 h, observed using structured illumination microscopy (SIM). **c**, Left, a representative CellProfiler segmentation image is shown with TopBP1 foci in red and TopBP1 foci close to lamin B1 in green (overlapped red and green colors). Quantification of the percentage of lamin B1-proximal TopBP1 foci during S-phase. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m. of 3 biological replicates, scoring ≥ 1522 nuclei per condition and replicate. Statistical analysis was performed using two-tailed paired Student's t-tests on biological replicate means; $*p < 0.05$. **d**, Structured illumination microscopy (SIM) of a single z-plane of cells treated with control siRNA (siCTR) or siRNA targeting TOPBP1 (siTopBP1) and stained with the Janelia Fluor 549 HaloTag[®] ligand and anti-Emerin antibodies after treatment with or without 2 μ M CPT for 3 h. **e**, Quantitative analysis of Emerin expression at the nuclear periphery from the experiments shown in **d**, expressed as mean fluorescence intensity (MFI) (see the Materials & Methods section for the determination of the nuclear periphery areas). Data are mean $\pm$ s.e.m. $n = 2$ biological replicates, scoring ≥ 432 nuclei per replicate. For siTopBP1-treated cells, the subsequent analysis was strictly restricted to successfully depleted cells via pre-gating on TopBP1 nuclear MFI ≤ 0.012 A.U. **f**, Structured illumination microscopy (SIM) of a single z-plane of cells treated with control siRNA (siCTR) or siRNA targeting Lamin B1 (siLaminB1) and stained with the Janelia Fluor 549 HaloTag[®] ligand and with anti-Lamin B1 antibodies after treatment with or without 2 μ M CPT for 3 h. **g**, Quantitative analysis of TopBP1 foci per nucleus from the experiments shown in **f**. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m. of 7 biological replicates, scoring ≥ 283 nuclei per condition and replicate. For siLamin B1-treated cells, the subsequent analysis was strictly restricted to successfully depleted cells via pre-gating on Lamin B1 MFI at nuclear periphery ≤ 0.03 A.U. Statistical analysis was performed using two-tailed paired Student's t-tests on biological replicate means, followed by Holm-Bonferroni correction for multiple comparisons: $*p < 0.05$, $***p < 0.001$ (FWER-adjusted p-values). Scale bars, **b** 2 μ m, **(d)** and **(f)**, 10 μ m.

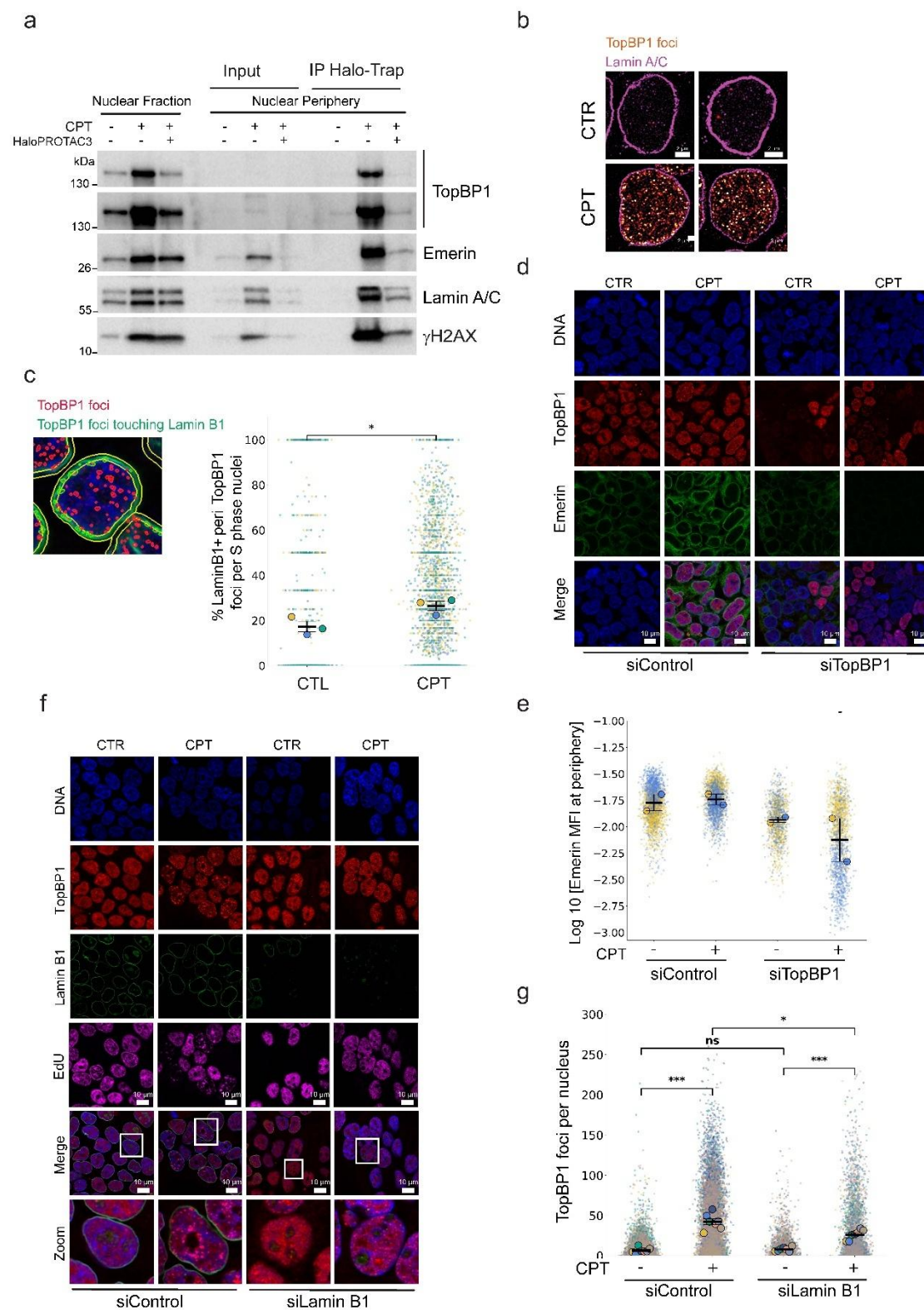


Figure 4

TopBP1 condensates regulate the spatial organization and stability of DNA damage response factors during replication stress

Next, to assess the spatial organization of TopBP1 condensates during high DNA replication stress, we analyzed the radial distribution of the DNA damage markers, γ H2AX and phospho-53BP1 (Ser1778), across the nucleus following camptothecin treatment (Fig. 5a). Quantitative analysis revealed a redistribution of p53BP1 foci toward the nuclear periphery in response to CPT treatment at 1 and 3 hours (Supplementary Fig. 4a), mirroring the behavior of TopBP1 condensates described in Figure 1e. Supporting this, double-positive TopBP1- γ H2AX and TopBP1-p53BP1 foci, as well as triple-positive TopBP1- γ H2AX-p53BP1 foci, displayed a similar peripheral redistribution over time, with pronounced colocalization in Bin 6 (Fig. 5b and Supplementary Fig. 4b,c). In addition, we observed a marked increase in chromatin-bound RPA32 foci after 3 h of CPT treatment, both in the nuclear interior and at the nuclear periphery (Fig. 5c). In fission yeast, severe replication stress has been reported to drive large RPA foci to the nuclear periphery, where they colocalize with Crb2, the functional homolog of human 53BP1 and with Man1, a LEM domain nuclear inner membrane protein (39). Similarly, in HEK293 Halo-TopBP1 cells treated with high doses of camptothecin, chromatin bound RPA32 foci colocalized with TopBP1 not only in the nuclear interior but also in bin 4, corresponding to the nuclear periphery (Fig. 5d). Together, these findings indicate that DNA damage-associated condensates progressively relocate to the nuclear periphery during prolonged replication stress.

To further assess the contribution of TopBP1 to the organization of DNA damage foci, we depleted TopBP1 using siRNAs molecule. Loss of TopBP1 resulted in a reduction in the intensity of γ H2AX foci following CPT treatment (Fig. 5e), an effect corroborated by Western blot analysis of TopBP1 depleted-nuclear fractions using an acute TopBP1 depletion by HaloPROTAC3 strategy (Fig. 4a). In addition, given that FANCD2 foci formation is regulated by ATR kinase activity and is described to be a marker of DNA replication stress (40), we sought to determine whether TopBP1 condensates contribute to FANCD2 foci regulation through ATR activation. To address this, we performed immunofluorescence assays in TopBP1-deficient cells. We observed a significant attenuation of FANCD2 foci formation in this context (Fig. 5f and Supplementary Fig. 4d). Our results indicate that TopBP1 condensates are required for efficient ATR-dependent regulation of γ H2AX and FANCD2 foci during the DNA damage response. However, this signaling defect observed in cells depleted from TopBP1 was accompanied by an increased accumulation of single-stranded DNA under sustained replication stress, as reflected by elevated chromatin-bound RPA32 foci in cells exposed to HaloPROTAC3 ligand (Fig. 5c and Supplementary Fig. 4e,f). Together, these findings establish TopBP1 condensates as key organizers of the spatial assembly and stability of DNA damage response factors in the context of high level of DNA replication stress.

Fig. 5 | TopBP1 condensates govern DNA damage response factor organization in replication stress conditions

a, Structured illumination microscopy (SIM) of a single z-plane of HEK293 cells stained with the Janelia Fluor 549 HaloTag[®] ligand and antibodies directed against γ H2AX or the Ser1778 mutant p53BP1, after treatment with or without 2 μ M CPT for 3 h. Scale bars, 10 μ m. **b**, Relative radial distribution of TopBP1/ γ H2AX/p53BP1 Ser1778 triple-positive foci from bin 1 (centre) to bin 6 (periphery) in HEK293 Halo-TopBP1 cells after treatment with 2 μ M CPT for 0, 1 and 3 h. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m. of 3 biological replicates, scoring ≥ 576 nuclei per condition and replicate. Statistical analysis was performed on bin 6 using two-tailed paired Student's t-tests using paired Student's t-tests on biological replicate means, followed by Holm-Bonferroni correction for multiple comparisons: $*p < 0.05$ (FWER-adjusted P-values). **c**, Absolute radial distribution of RPA32 foci from bin 1 (centre) to bin 4 (periphery) in HEK293 Halo-TopBP1 cells after treatment with or without 2 μ M CPT for 3 h in the presence or absence of 1 μ M HaloPROTAC3 and after CSK-extraction. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m. of 3 biological replicates, scoring ≥ 410 nuclei per condition and replicate. Statistical analysis was restricted to bin 4. The expected CPT-induced increase in RPA32 foci (CPT > CTR) was assessed as a one-tailed paired Student's t-tests on biological replicate means as a technical positive control. The sole biological comparison (HaloPROTAC3 + CPT vs CPT) was then assessed in a two-tailed paired Student's t-test on biological replicate means: $*p < 0.05$. **d**, Absolute radial distribution of TopBP1-RPA32 foci from bin 1 (centre) to bin 4 (periphery) in HEK293 Halo-TopBP1 cells after treatment with or without 2 μ M CPT for 3 h in the presence or absence of 1 μ M HaloPROTAC3. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m. of 3 biological replicates, scoring ≥ 410 nuclei per condition and replicate. Statistical analysis was performed on bin 4. Expected control differences in TopBP1-RPA32 colocalized foci (CPT > CTR and CPT > HaloPROTAC3 + CPT) were assessed using one-tailed paired Student's t-tests on biological replicate means. As these comparisons served strictly as independent, predefined controls, no multiplicity correction was applied $*p < 0.05$. **e**, γ H2AX foci per nucleus in HEK293 Halo-TopBP1 cells treated with 2 μ M CPT for 3 h in siCTR and siTopBP1 conditions. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m. of biological replicates, scoring ≥ 432 nuclei per replicate. **f**, FANCD2 foci per nucleus from HEK293 Halo-TopBP1 cells treated with or without 2 μ M CPT for 3 h in the presence or absence of 1 μ M HaloPROTAC3. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m. of 2 biological replicates, scoring ≥ 546 nuclei per condition and replicate.

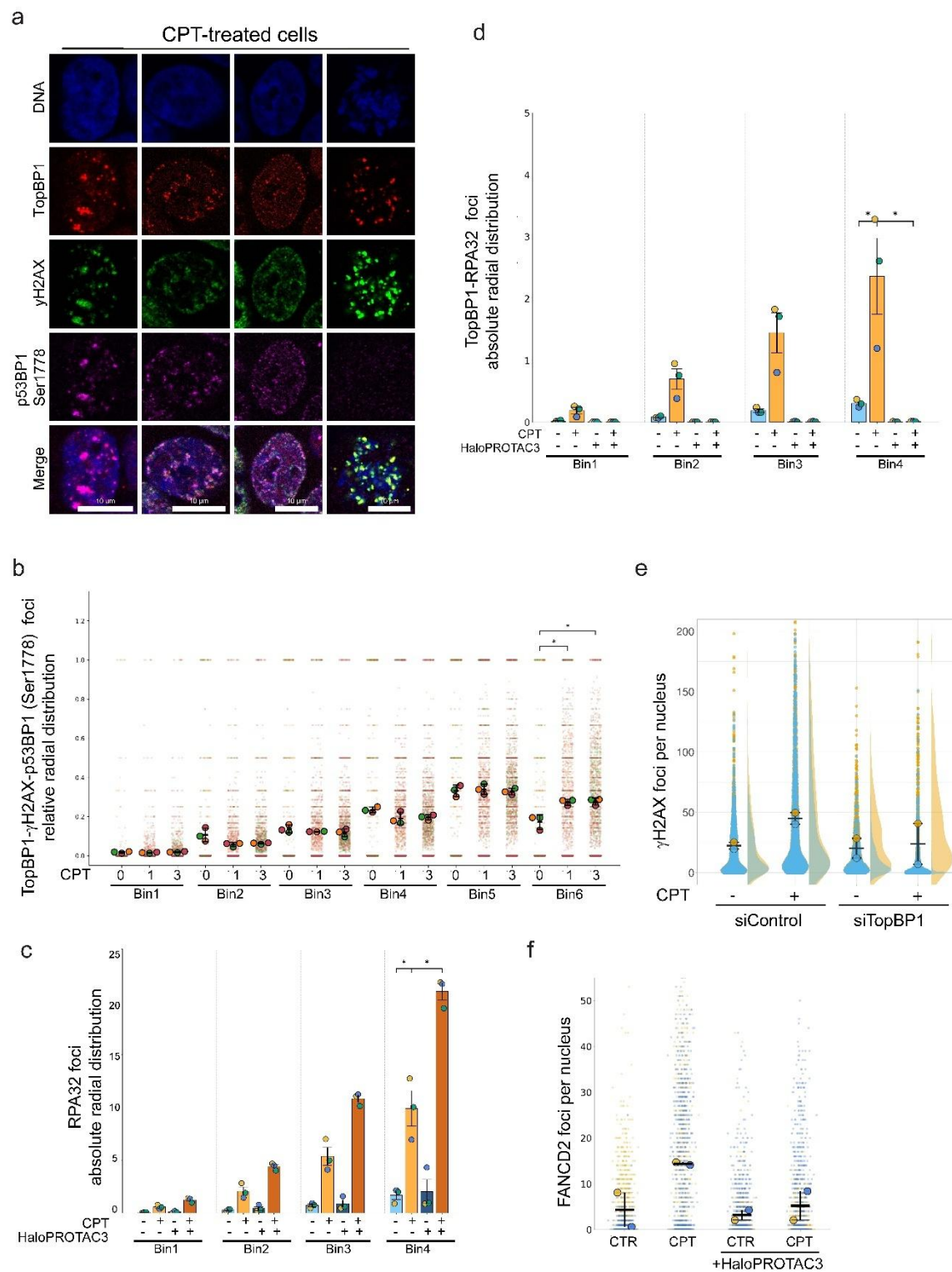


Figure 5

Loss of actin nucleation enhances DNA damage-induced TopBP1 condensates formation

Nucleation and polymerisation of nuclear actin filaments are essential for efficient DNA repair in response to DNA damage (4). To assess how TopBP1 condensates behave when actin integrity is compromised, we disrupted the nuclear actin pool by depleting Importin-9 (IPO9), the principal nuclear import receptor for actin (Fig. 6a), or by knocking down IQGAP1, a scaffold essential for actin filament assembly and organization (Fig. 6a). Both perturbations significantly increased the number of TopBP1 condensates (Fig. 6b). These foci were more intense (Supplementary Fig. 5a), and, under actin-disrupting conditions, a subset of CPT-induced condensates clustered within discrete intranuclear domains (Supplementary Fig. 5b–d). In addition, we observed concomitant increases in γ H2AX and Chk1 Ser315 phosphorylation in IPO9- and IQGAP1-deficient cells (Fig. 6c,d). Next, we treated CPT-challenged cells with Latrunculin A (LatA), a potent inhibitor of actin polymerization. Under these conditions, we did not observe a substantial increase in the number of TopBP1 condensates (Fig. 6e,f). However, under conditions affecting actin filaments by LatA, a subset of the CPT-induced TopBP1 foci colocalized with FANCD2 foci (Fig. 6g,h) and displayed a clustering pattern similar to that observed in IPO9- and IQGAP1- deficient cells (Supplementary Fig. 5f). Collectively, these findings indicate that disruption of nuclear actin filaments increases TopBP1 condensate formation and DNA damage signalling, consistent with the accumulation of unresolved DNA damage intermediates.

Fig. 6 | Nuclear actin breakdown causes an increase in TopBP1 condensates and amplifies DNA damage signaling

a, Structured illumination microscopy (SIM) of a single z-plane from HEK293 cells treated with control siRNA (siControl) or siRNAs targeted IPO9 or IQGAP1. Cells were then stained with the Janelia Fluor 549 HaloTag[®] ligand and antibodies directed against IPO9 or IQGAP1, after treatment with or without 2 μ M CPT for 3 h. **b**, Quantitative analysis of TopBP1 foci per nucleus from the experiments shown in **a**. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m of 3 biological replicates, scoring ≥ 642 nuclei per replicate. Statistical significance of predefined directional hypotheses (siIPO9 CPT > siCTR CPT and siIQGAP1 CPT > siCTR CPT) was determined using one-tailed paired Student's t-tests on biological replicate means, followed by the Holm-Bonferroni correction for multiple comparisons, * $p < 0.05$, ** $p < 0.01$ (FWER-adjusted P-values). Assay validations for CPT-induced foci formation were evaluated as independent quality controls using uncorrected one-tailed paired Student's t-tests on biological replicate means: * $p < 0.05$, *** $p < 0.001$. **c**, **d**, Western blot analysis of whole-cell extracts from **a** with the indicated antibodies. **e**, Structured illumination microscopy (SIM) microscopy of a single z-plane from HEK293 cells stained with the Janelia Fluor 549 HaloTag[®] ligand after treatment with or without 2 μ M CPT for 3 h in the presence or absence of 200 nM latrunculin A. **f**, Number of TopBP1 foci per nucleus from the experiment shown in **(e)**. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m of 2 biological replicates, scoring ≥ 546 nuclei per condition and replicate. **g**, Structured illumination microscopy (SIM) of a single z-plane from HEK293 cells stained with the Janelia Fluor 549 HaloTag[®] ligand and anti-FANCD2 after treatment with or without 2 μ M CPT for 3 h in the presence or absence of 200 nM latrunculin A. **h**, TopBP1-FANCD2 nuclear Pearson Correlation Coefficient (PCC) from the experiment shown in **(g)**. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m of 2 biological replicates, scoring ≥ 546 nuclei per condition and replicate. For **(a)**, **(e)** and **(g)**, scale bars, 10 μ m.

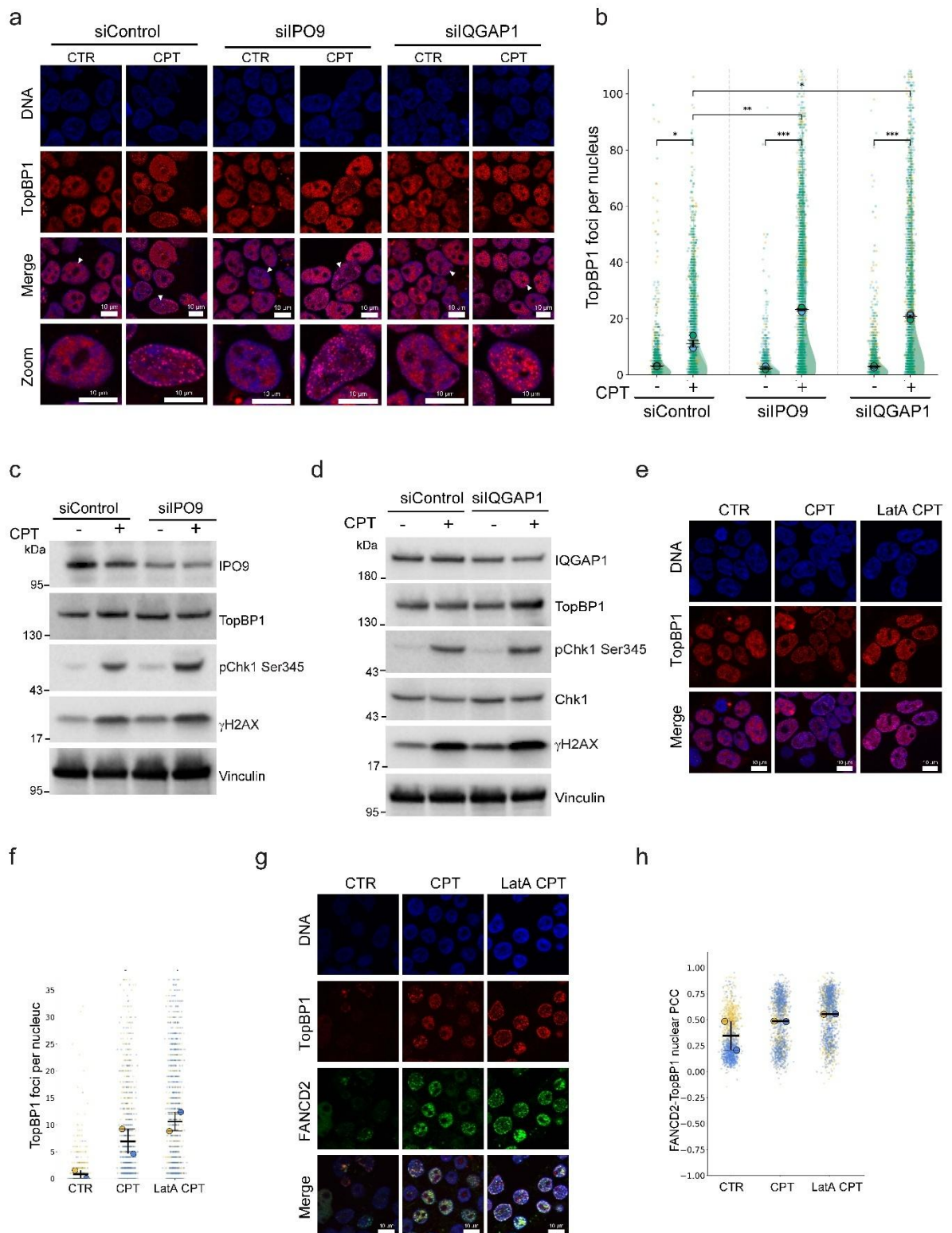


Figure 6

TopBP1-mediated actin polymerisation supports the survival of oxaliplatin-resistant cancer cells

TopBP1 is overexpressed across multiple solid tumour types, where elevated expression correlates with adverse clinical outcomes. To define the mechanistic contribution of TopBP1 to tumour progression, with a particular focus on its role in regulating actin cytoskeleton dynamics, we characterized its expression alongside key modulators of actin remodelling in colorectal cancer (CRC) cell lines exhibiting acquired resistance to oxaliplatin (44). This experimental framework is motivated by converging lines of evidence: TopBP1 has emerged as a functionally relevant factor in CRC pathobiology and therapeutic response (42), while ATR signaling, a canonical downstream target of TopBP1, has been identified as a central driver of oxaliplatin resistance, with pharmacological ATR inhibition being sufficient to restore oxaliplatin sensitivity in preclinical CRC models (44).

In oxaliplatin-resistant colorectal (CRC OxR) cancer cells, we observed an increase in TopBP1 expression and a strong activation of the mTOR and ATR signaling pathways, demonstrated by increased phosphorylation of mTOR at Ser2481, ATR at Ser1989, and Chk1 at Ser345 compared with non-resistant cells (CRC parental) (Fig. 7a). In parallel, phosphorylation of the actin-severing factor cofilin was also elevated, indicating a protection mechanism of actin dynamics in the cancer context (Fig. 7a). Notably, depletion of TopBP1 in oxaliplatin-resistant cells led to a marked increase in γ H2AX. This effect is specific to the cancer context in which multiple induced signaling pathways may converge to elevate H2AX phosphorylation, an indicator of DNA damage accumulation (Fig. 7b). Consistent with a functional impact on the actin cytoskeleton, oxaliplatin-resistant cells displayed enhanced actin polymerization capacity as shown by *in vitro* polymerization assay based on pyrene-labeled actin, an effect that was sharply abolished upon TopBP1 depletion (Fig. 7c and Supplementary Fig. 6a,b). Together, these findings implicate TopBP1 as a critical regulator of ATR–mTOR signaling and actin dynamics in oxaliplatin-resistant CRC cells, linking its overexpression to both genome instability and cytoskeletal remodeling in the chemo-resistant state.

Fig. 7 | TopBP1-mediated actin polymerization promotes survival of oxaliplatin-resistant cancer cells.

a, Western blot analysis of whole-cell extracts from parental and oxaliplatin-resistant colorectal cancer (CRC) cells. **b**, Western blot analysis of whole-cell extracts treated with control siRNA or siRNA targeted TopBP1 in parental and oxaliplatin-resistant CRC cells. **c**, Normalized timecourse of pyrene-labelled actin assembly in the presence or absence of nuclear extracts from parental CRC, oxaliplatin-resistant CRC and TopBP1-depleted oxaliplatin-resistant CRC cells. One of two independent biological replicates is shown. **d**, Schematic summary of the proposed model. (1) Actin nucleation is driven through ATR–mTOR–N-WASP signaling in response to DNA damage. (2) Actin filament protection from degradation is mediated through the mTOR–cofilin pathway. (3) Actin stabilization at the nuclear periphery is achieved through Emerin stabilization. Conditions are shown for: (A) normal response to DNA damage, (B) response in the absence of TopBP1 and (C) response in the absence of nuclear actin.

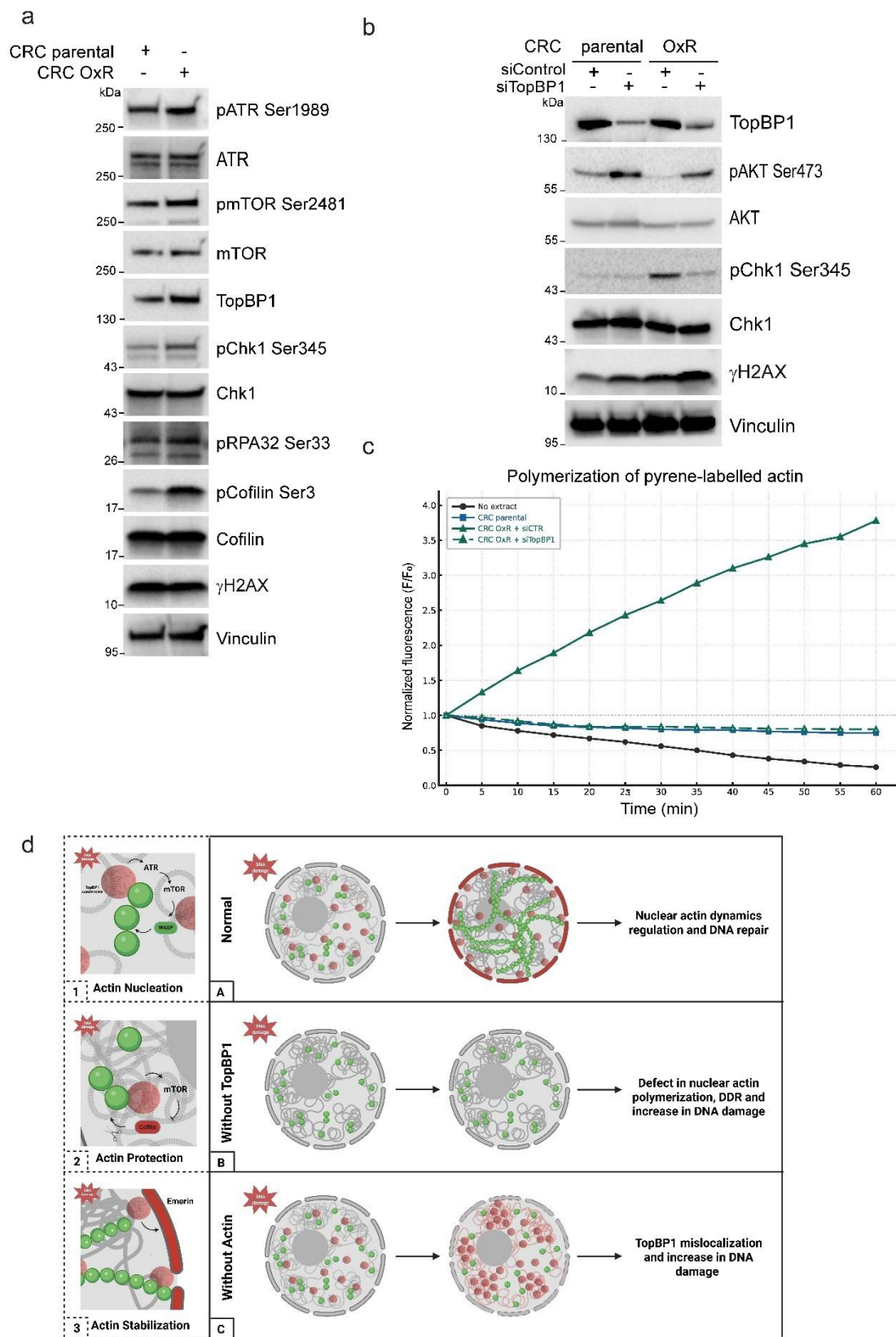


Figure 7

Discussion

Biomolecular condensates are increasingly recognized as key organizers of cellular signaling, yet how they coordinate genome surveillance with nuclear architecture remains incompletely understood. Here, we identify TopBP1 condensates as central regulators of nuclear actin dynamics during sustained replication stress. Our findings support a model in which TopBP1 condensates function not merely as signaling assemblies, but as adaptive nuclear compartments that integrate replication-stress responses with cytoskeletal control (Fig. 7d).

A central insight from this study is that TopBP1 condensates appear to coordinate both the assembly and the stabilization of nuclear actin filaments. Under severe replication stress, TopBP1 condensates selectively accumulated actin regulatory factors, including WASP and ARP2/3 (data not shown), and their stabilization required TopBP1, ATR and mTOR signaling. Moreover, optogenetic clustering of TopBP1 was sufficient to partially stabilize WASP and initiate nuclear actin nucleation even in the absence of exogenous DNA damage, indicating that TopBP1 condensation can actively instruct this process. Beyond nucleation, TopBP1 was also required for DNA-damage-induced phosphorylation of cofilin at Ser3, thereby limiting filament severing. Notably, this branch was largely ATR-independent but sensitive to mTOR inhibition, suggesting that TopBP1 condensates may locally tune kinase-phosphatase balance to preserve actin filament integrity. Together, these findings place TopBP1 condensates upstream of multiple regulatory steps that shape nuclear actin assembly during replication stress.

These observations have important implications for how cells spatially organize replication-stress responses. Nuclear actin has emerged as a critical determinant of DNA repair, replication fork remodeling and the trafficking of damaged chromatin within the nucleus (19). Our data support the idea that TopBP1 condensates help couple these processes by creating permissive microenvironments in which actin-dependent nuclear remodelling can be coordinated with checkpoint signaling. Consistent with this view, perturbation of nuclear actin led to the accumulation of TopBP1 condensates in discrete intranuclear regions associated with heightened DNA damage signaling. This suggests that intact nuclear actin networks may facilitate the positioning or mobility of stalled forks and promote productive engagement of repair machinery, whereas aberrant TopBP1 condensate accumulation may reflect a compensatory but inefficient response to unresolved replication stress.

Our findings also extend the function of TopBP1 condensates beyond canonical replication-stress signaling by linking them to the nuclear periphery and lamina-dependent organization. The enrichment of TopBP1 at the nuclear periphery, together with the requirement for Lamin B1 in TopBP1 foci formation and for TopBP1 in Emerin stability, points to a functional connection between TopBP1 condensates, nuclear lamina components and actin-dependent nuclear architecture. In this context, it is notable that ATR-dependent phosphorylation of lamin A/C has been implicated in nuclear envelope fragility in homologous recombination-

deficient tumours (45). Our data broaden this framework and raise the possibility that TopBP1 condensates contribute to the maintenance of nuclear envelope integrity under genotoxic stress. Whether these condensates directly organize repair events at the nuclear periphery or instead reinforce mechanical resilience of the nucleus will require further investigation.

The peripheral accumulation of RPA32 observed upon TopBP1 depletion is also intriguing. To our knowledge, such localization has not been described previously in mammalian cells and may indicate that a subset of stalled or collapsed replication forks is redirected towards the nuclear periphery for processing (39). More broadly, our findings support a model in which TopBP1 condensates, together with nuclear actin filaments and associated motors, may help spatially reorganize persistent replication intermediates into specialized nuclear compartments. In the setting of prolonged replication stress, such compartmentalization may not only promote repair but could also contribute to downstream quality-control pathways, potentially including nucleophagy (46). In the same line, Case et al. reported that phase separation at lipid membranes increases the specific activity of actin regulatory proteins toward Arp2/3-dependent actin assembly by prolonging their membrane dwell time (47). By analogy, phase separation of TopBP1 condensates might likewise modulate the residence of actin-associated factors at the nuclear periphery, although this remains to be directly tested.

Finally, the TopBP1-actin axis may have direct relevance to cancer therapy. TopBP1 overexpression is observed across multiple tumour types, and oxaliplatin-resistant cells displayed increased TopBP1 stability together with enhanced ATR-Chk1 and mTOR signaling and elevated cofilin phosphorylation. These features suggest that resistant cells may become particularly dependent on TopBP1-driven nuclear actin regulation to tolerate persistent replication stress. Interfering with TopBP1 condensate formation, or with the actin-regulatory pathways that they control, may therefore represent a strategy to simultaneously compromise nuclear mechanics and DNA damage signaling in therapy-resistant tumours.

In summary, our work identifies TopBP1 condensates as multifunctional organizers that connect replication-stress signaling, nuclear actin dynamics and nuclear architecture. By coordinating both actin nucleation and filament stabilization, these condensates provide a mechanistic framework for understanding how biomolecular compartmentalization supports genome maintenance. More broadly, these findings expand the role of biomolecular condensates in nuclear stress responses and highlight TopBP1-dependent actin regulation as a potential vulnerability in genome instability-associated disease.

Methods

Cell culture

Flp-In™ 293HEK T-REx cells were obtained from Thermo Fisher and cultured in Dulbecco's Modified Eagle Medium (DMEM)-GlutaMAX medium (Merck-Sigma-Aldrich, #D5796) supplemented with 10% heat-inactivated fetal bovine serum (BioWest FBS, Eurobio Scientific, #S1810). For the generation of stable cell lines that express TopBP1 fused to the photoreceptor cryptochrome 2 (Cry2) and mCherry (optoTopBP1), please refer to (31). The HCT116 human colorectal cancer (CRC) cell line was obtained from Horizon (catalog #HD-PAR-082). The control human colon epithelial cell line CCD 841 CoN was purchased from ATCC (#CRL-1790). An oxaliplatin-resistant derivative of HCT116 was generously provided by Dr. C. Gongora's laboratory, originally described by Gourdier et al. (48) where drug-specific resistance to oxaliplatin was linked to defects in apoptosis. These cell lines were cultured in RPMI 1640 medium (Merck-Sigma-Aldrich, #R8758) supplemented with 10% heat-inactivated fetal bovine serum (FBS). Cells were maintained at 37 °C in a humidified incubator with 5% CO₂. Authentication of cell lines was confirmed by short tandem repeat (STR) profiling (Eurofins Genomics), and all cultures were routinely tested for mycoplasma contamination.

293T KnockIN Halo tagged TopBP1 cell lines we received from Biocytogen. To generate endogenous Halo-Tagged fusion TopBP1 protein, Biocytogen Extreme Genome Editing System (EGE) was used, CRISPR/cas9 based technology was improved to attach the Halo tag to the C-terminus of endogenous TopBP1 gene.

If necessary, the cells were eventually treated with DMSO (Sigma-Aldrich, #276855), CPT (Sigma-Aldrich, #C9911), LatA (Cayman Chemical, #10010631), INK128 (Cayman Chemical, #11811), VE822 (Selleckchem, #S7102), Wiskostatin (Sigma-Aldrich, #W2270), LIMKi 3 (Calbiochem, #435930).

Antibodies

The following primary antibodies were used: anti-BrdU (mouse monoclonal for IdU detection, BD Biosciences #347580, 1:5); anti-BrdU (rat monoclonal for CldU detection, Abcam, #ab6326, 1:25); anti-FANCD2 (Abcam, #ab2187, 1:250); anti-lamin A/C (Sigma-Aldrich, #L1293, 1:1,000); anti-vinculin (Sigma-Aldrich, #V9131, 1:5,000); anti-mTOR (Ozyme, #2972S), anti-mTOR (phosphorylated Ser 2448), anti-P70S6 (phosphorylated Ser 371), anti-4E-BP1 (phosphorylated Thr 37/46) (mTOR substrates kit, Ozyme, #9862T, 1:1,000); anti-4E-BP1 (Cell Signaling Technology, 9644, 1:1,000); anti-P70S6 (Ozyme, #9234T, 1:1,000); anti-IQGAP1 (Ozyme, #29016S, 1:2,500 for western blot and 1:200 for immunofluorescence); anti-cofilin 1 (Cell Signaling Technology, #5175, 1:2,000); anti-cofilin1 (phosphorylated Ser 3) (Cell Signaling Technology, #3313, 1:1,000); anti-importin 9 (Euromedex, #AL-A20901-50UL, 1:1,000); anti-Arp2 (Euromedex, #GTX103311-S, 1:100); anti-Arp3 (Euromedex, #GTX115345-S, 1:100); anti-CHK1 (Cell Signaling Technology, #2360, 1:1,000); anti-CHK1 (phosphorylated Ser 345) (Ozyme, #2348S, 1:1,000); Anti-Phospho-LIMK1 ((Thr508)/LIMK2 (Thr50) (Ozyme, #3841S,

1:1,000); anti-LIMK2 (8C11) Rabbit mAb (Ozyme, #3845S, 1:1,000); anti-WASP (Euromedex, #GTX133749, 1:1,000); Emerin Antibody (CL0203) (Biotech, #NBP2-52877, 1:1,000)

The following secondary antibodies were used: goat anti-mouse, Alexa Fluor 488 (Thermo Fisher Scientific, #A28175, 1:1,000 for fixed nuclei staining); goat anti-rabbit, Alexa Fluor 488 (Thermo Fisher Scientific, #A11034, 1:1,000); goat anti-rabbit Alexa, Fluor 568 (Thermo Fisher Scientific, #A-11011, 1:750); goat anti-rabbit Alexa, Fluor 647 (Thermo Fisher Scientific, #A21245, 1:750); goat anti-rabbit, HRP (Dako, #P0448, 1:1,000 for anti-phosphorylated primary antibodies, otherwise 1:3,000), goat anti-mouse, HRP (Dako, #P0447, 1:1,000 for anti-phosphorylated primary antibodies, otherwise 1:3,000)

Western blotting

Whole cell extracts were obtained by lysing cells with 1X Laemmli Sample buffer (Biorad, #C161-0737) and heated 5min at 95°C. 40 µg of protein samples were resolved on precast SDS-PAGE gels (4-15%, 7.5% and 10%, BioRad) and transferred to nitrocellulose membranes using the Bio-Rad Trans-Blot Turbo transfer device. Membranes were saturated in 10% non-fat milk diluted in TBS (20 mM Tris, 150 mM NaCl, pH 7.4)-0.2% Tween 20, and incubated with primary antibodies at 4°C overnight. Membranes were then incubated with anti-mouse (Cell Signaling Technology, #7076S) or anti-rabbit HRP (Cell Signaling Technology, #7074S) secondary antibodies for 1 h. Revelation was carried out with ECL Clarity (BIO-RAD, #170-5061) or ECL select (BIO-RAD, #1705062) according to the manufacturer instructions.

Perinuclear fractionation

Cells depleted or not of TopBP1 upon addition of HaloProtac3 ligand (#GA3110, Promega) were treated with 2 µM of CPT for 2 hours. The nuclear envelope was extracted using the Minute™ Nuclear Envelope Protein Extraction kit (#NE-013, Invent Biotechnologies, Inc.), according to the manufacturer's protocol. Briefly 60-70 million cells were collected, washed with cold PBS, then resuspended in 1.5 ml of buffer A and incubated on ice for 10 minutes. The cell suspension was then vortexed for 10 seconds, transferred to filter cartridges, and centrifuged at 14,000 rpm for 30 seconds. The nuclei pellet was washed with cold PBS, vortexed for 10 seconds, then centrifuged at 3000 rpm at 4°C for 2 minutes. 1 ml of Buffer B was then added to the pellet, incubated on ice for 5 minutes, then vortexed for 10 seconds. Incubation and vortexing steps were repeated a second time, followed by centrifugation at 8000 rpm for 5 minutes at 4°C. The supernatant was recovered in a fresh tube, mixed with 2.4 ml of cold PBS, inverted 10 times to precipitate the extracted nuclear envelope, then centrifuged at 14,000 rpm at 4°C for 15 minutes. The pellet obtained (perinuclear fraction) was dissolved in ice cold RIPA buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.05% SDS, 1% Triton X-100, 1% deoxycholate, supplemented with 1mM PMSF and Roche protease inhibitor cocktail, #4693159001).

Halo-Trap pulldown assay

Cells depleted or not of TopBP1 (upon addition of HaloProtac3 ligand), were treated with 2 μ M CPT for 2 hours. 10 million cells were harvested per condition in ice cold PBS and centrifuged at 2500 rpm at 4°C for 5 minutes. The pellets were resuspended in ice cold RIPA buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.1% SDS, 1% Triton X-100, 1% deoxycholate, supplemented with 1mM PMSF and Roche protease inhibitor cocktail), and incubated on a rotating wheel at 4°C for 30 minutes for cell lysis. Immunoprecipitation of Halo-TopBP1 bound proteins, either from whole cell lysates or from perinuclear fractions, was performed using Halo-Trap Agarose beads from Chromotek (#PR-OTA-100) according to the instructions of the manufacturer. Briefly, Halo-Trap agarose beads were equilibrated by resuspending and washing in cold dilution buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5 mM EDTA), then sedimented by centrifugation at 2500 g at 4°C for 5 minutes. The lysates were added to the equilibrated beads and incubated on a rotating wheel at 4°C for 1 hour. Samples were then centrifuged at 2500 g at 4°C for 5 minutes. Beads were then washed 3 times with cold wash buffer (10 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.05% NP-40, supplemented with 1mM PMSF and Roche protease inhibitor cocktail), sedimenting the beads after each wash at 2500 g at 4°C for 5 minutes. The remaining supernatant was completely removed after the last wash and the beads were resuspended in Laemmli buffer.

Immunofluorescence analysis

Cells were grown on coverslips and after incubation with the corresponding drugs (see above). Cells were then fixed with 4% PFA/PBS at RT for 20 min. Non-specific epitopes were saturated with a blocking solution (5% BSA/PBS) at RT for 30 min. For immunostaining, primary antibodies and the anti-mouse coupled to fluorochrome (Invitrogen, #A-11011, diluted at 1/500) secondary antibody were diluted in blocking solution and incubated for 1 h or 45 min, respectively. Three 5-min washes between incubation steps were performed in PBS/0.1% Tween 20 at RT. Hoechst 33342 was used at 1 μ g/mL for DNA staining and coverslips were mounted on slides with the Prolong Gold antifade reagent (Invitrogen, #P36930). Images were captured using a 63 X objective and a Zeiss AxioImager with ApoTome (structured illumination) at the Montpellier Ressources Imaging facility (NA 1.46 oil).

Lattice Structured illumination microscopy (LSIM)

SIM imaging was performed using a ZEISS Elyra7 Lattice SIM inverted microscope equipped with a 63 $\times$ Plan-Apochromat oil immersion objective (NA 1.4) and a monochrome sCMOS PCO.edge camera (pixel size: 6.5 μ m, 16-bit depth). Fluorophores were excited using laser diodes at 405 nm (DAPI), 488 nm (GFP), 561 nm (DsRed), and 642 nm (far-red dyes). Emission was collected using a LBF 405/488/561/642 BGOR filter set for blue, green, and far-red channels, and a BP 495–550 / BP 570–620 GO filter set for DsRed emission.

All acquisitions were performed using a structured illumination lattice grid (pattern: G4, 32 μ m, 13 phases). Three-dimensional SIM (3D-SIM) images were acquired with a z-step of 0.091 μ m. Image reconstruction and SIM processing were carried out using ZEN Black software (ZEISS, version 16.0). Chromatic aberrations and channel alignment were corrected using

multispectral calibration beads. Sharpness parameters for SIM reconstruction were set to 6.5 for the DAPI and GFP channels, 6.7 for DsRed, and 7.0 for the far-red channel. SIM² deconvolution was performed with 20 iterations across all channels. Regulation weights were set at 0.1 for the blue, green, and far-red channels, and 0.01 for the red channel.

Opto-TopBP1 activation

Cells were plated at around 70% confluency in DMEM. Expression of opto-TopBP1 was induced for 16 h with 2 µg/ml doxycycline. For light activation, plates were transferred into a custom-made illumination box containing an array of 24 LEDs (488nm) delivering 10mW/cm² (light intensity measured using a ThorLabs-PM16-121-power meter). Cry2 oligomerisation was induced using 3 min of light-dark cycles (4 s light followed by 10 s dark).

Cell cycle assays

Cell cycle profiling using 5-bromo-2'-deoxyuridine (BrdU)/propidium iodide (PI) was performed as previously described (Egger et al., 2022). Briefly, cells were pulsed with 30 µM BrdU for 15 min and fixed in ice-cold PBS - 70% ethanol. Cells were digested in 30 mM HCl, 0.5 mg/mL pepsin (37°C, 20 min). DNA was denatured in 2 N HCl (RT, 20 min) and BrdU was immunodetected using the anti-BrdU clone BU1/75 in PBS/2% goat serum/0.5% HEPES/0.5% Tween 20 and revealed with a goat anti-rat Alexa Fluor 488 secondary antibody. DNA was stained with 25 µg/mL PI in PBS and 20,000 cells/sample were analyzed on a Gallios flow cytometer (Beckman Coulter) at the IRCM. Data were analyzed using the Kaluza software.

Software tools

Immunofluorescence data were acquired with a Zeiss AXIOIMAGER microscope (Zeiss) and the Zen toolkit connect software. For foci quantification, the pipelines generated on CellProfiler 4.2.1 and described in Egger et. al, 2024 were used. For western blot images acquisition, ChemiDoc Imaging System (Biorad) and ImageLab 6.1 were used. GraphPad version 10 was used for graphical representation and statistical analyses.

Preparation of nuclear and cytoplasmic extracts

When appropriate, cells were treated with corresponding treatments for 3 h. Cultures were washed once with cold 1× PBS, scraped carefully and centrifuged at 800 g for 10 min. The pellet was kept at -80°C for 45 min, and then resuspended in buffer P1 (5 × 10⁶ cells in 250 µl of a buffer composed of 10 mM HEPES, 0.1 mM EGTA, 1 mM dithiothreitol (DTT), complete protease inhibitors (Roche)). 0.5% riton X100 was added to the samples which were vortexed for 10 s. The nuclei were sedimented at 10,000 g for 10 min and the supernatant was retained as cytoplasmic extract. The nuclear pellet was then washed with buffer P1 before lysis in buffer P2 (5 × 10⁶ cells lyzed in 100 µl of a buffer composed of 20 mM HEPES, 25% glycerol, 400 mM NaCl, 1 mM EGTA, 1 mM DTT, protease inhibitors) for 90 min on a rotary shaker at 4°C. Insoluble material was sedimented at 16,000 g for 30 min and the remaining supernatant was retained as nuclear extract. Extract purity was determined by immunoblotting for α tubulin

and histone H2. For western blots, a 5× greater volume of nuclear extract was loaded per lane as compared with cytoplasmic extracts.

Pyrenyl–actin assembly assays

The assay was adapted from previously described protocols (60). Nuclear extracts were dialysed using Slide-A-Lyzer Dialysis Cassettes (3,500-D protein molecular weight cutoff; Thermo Fisher Scientific) against 10 mM HEPES, pH 7.7, 100 mM KCl, 2 mM MgCl₂, 0.1 mM CaCl₂, 5 mM EGTA, 1 mM DTT buffer for at least 3 h. Nuclear extracts were then incubated with pyrene labelled actin (Cytoskeleton, BK003) and fluorescence was measured at 407 nm with excitation at 365 nm using a Plate Reader Clario BMG labtech.

Chromobody transfection

The NLS–GFP–actin chromobody plasmid (Chromotek, acgn, nuclear actinCB) was purchased through a material transfer agreement from Chromotek. Plasmid was transfected with Jet prime (Polyplus, #101000046). Cells were seeded on sterile cover slips and transfected 24h later. Chemical inhibitors were added 24h after transfection for 3h. Samples were fixed in 3.7% paraformaldehyde/1× PBS for 10 min, permeabilized with 0.5% Triton X100/1× PBS for 10 min at room temperature and then mounted using Prolong Gold.

siRNA transfection

Nontargeting (control siRNA, D-001206-13-20), TopBP1 (M-012358-01-0010), IQGAP1 (M-004694-02-0010), IPO9 (M-016863-01-0010), Emerin (EMD, M-011025-01-001) and LaminB1 (LMNB1, M-005270-01-0010) were transfected using INTERFERin (Polyplus, #409-01).

Volume and sphericity measurements

To analyze nuclear morphology under conditions of replication stress, a 2D morphometric study was performed using FIJI (ImageJ) software on Structured Illumination Microscopy (SIM2) images of DAPI-stained nuclei. Six conditions were analyzed, with 20 nuclei per condition. For each nucleus, Maximum Intensity Projections (MIP) were generated from .czi files after channel splitting. Individual nuclei were isolated using polygon selection and cropped with the "Clear Outside" function, followed by MIP projection of the DAPI channel. Each isolated nucleus was saved as a separate .tiff image. A fine polygonal selection was then applied around each nucleus to define the region of interest (ROI), which was added to the ROI Manager. Morphometric parameters were extracted using the "Measure" function after enabling Area, Perimeter, Circularity (Shape Descriptors), and Feret's Diameter in the "Set Measurements" menu. Graphical representation of the extracted data was performed using Microsoft Excel and descriptive statistical analysis conducted using Jamovi software.

Single-molecule tracking

SMT experiments were carried out as previously described (49). Briefly, cells plated on coverslips were incubated in red phenol-free DMEM at 37 °C for 10 min with CPT for 2 hours

A homemade objective-type TIRF setup allowing multicolor single-molecule imaging and equipped with a Plan Fluor 100×/1.45 NA objective (Zeiss, Le Peck, France Brattleboro, VT) was used. A 1.5x telescope was used to obtain a final imaging 150-fold magnification corresponding to a pixel size of 107 nm. 3 lasers were used for excitation: 488 nm (OBIS, LX 488-50, Coherent Inc.), 532 nm (Ventus, Novantaphotonics), and 640 nm (OBIS, LX 640-100, Coherent Inc.). Laser lines were expanded, and coupled into a single beam using dichroic mirrors and an acousto-optic tunable filter (AOTFnc-400.650-TN, AA opto-electronics) was used to modulate laser intensity. Two achromatic lenses were used to expand the excitation laser and an additional dichroic mirror (488/532/638rpc, Chroma) to direct it towards the back focal plane of the objective. Fluorescence light was spectrally filtered with emission filters (ET525/50m, ET600/50m and ET700/75m, Chroma Technology) and imaged on an EMCCD camera (iXon Ultra897, Andor Technologies). All the experiments were performed with a 100 ms integration time.

Movies were analyzed using homemade software (named 'PaTrack') implemented in visual C++ (36). Briefly, trajectories were constructed using the individual diffraction limited signal of each molecule. The center of each fluorescence peak was determined with subpixel resolution by fitting a two-dimensional elliptical Gaussian function. The two-dimensional trajectories of single molecules were constructed frame per frame. Only trajectories containing at least 41 points were retained. Diffusion coefficient values were determined from a linear fit to the MSD (mean square displacement)- τ plots between the first and the fourth points (D1-4) according to the equation $MSD(t) = 4Dt$. The determination of the motional modes was performed using a homemade algorithm based on a neural network. The MSD curve was linearly fitted (Brownian) or adjusted with a quadratic curve ($4Dt + v^2t^2$) (directed diffusion) or exponential curve $L^2/3(1 - \exp(-12Dt/L^2))$ (confined diffusion), where L is the side of a square domain, the confinement diameter being related to L by $d_{conf} = (2/\sqrt{L})$ [50]. The apparent diffusion coefficient values were determined from a linear fit between the first and fourth points (D1-4). The algorithm has been tested with simulated trajectories displaying pure Brownian, confined or directed behavior or a combination of these 3 modes.

Click-iT EdU Cell Proliferation Assay

To assess the cell cycle phases, cells were treated with CPT for 2 hours then with EdU and Janelia fluor dye. We then used the Click-iT™ EdU Cell Proliferation Kit for Imaging (Thermo Fisher Scientific, #C10337). Cells were incubated with the thymidine analog EdU, which is efficiently incorporated into newly synthesized DNA during replication. Following fixation, EdU was fluorescently labeled using a copper-catalyzed azide-alkyne cycloaddition ("click" chemistry) with Alexa Fluor™ dye JF647. Normal immunofluorescence protocol then was performed.

Image quantifications

QuantImagin performed the microscopy image quantifications. CellProfiler 4.2.8 was used for image quantification. Briefly, metadata were extracted from ApoTome .czi files converted

from RAW (Zeiss, Zen Blue 2.3). Channels were identified in the NamesAndTypes module. Nuclear segmentation was performed via the RunCellpose plugin (GitHub, Cellpose (50)) using the DAPI channel. Nuclei touching image borders and objects < 3000 pixels² (debris) were filtered out. Fluorescent signals (foci) were then quantified after enhancement (EnhanceOrSuppressFeatures module, “speckles” mode), using the IdentifyPrimaryObjects module and related to their parent nuclei and sub-nuclear regions masks (RelateObjects module). Colocalizing objects were identified as the intersection of different channels foci masks. The percentage of colocalizing foci per nucleus was calculated as the ratio of colocalized foci to total foci per individual nucleus. Pearson correlations were calculated following polynomial illumination corrections. Segmentation and foci identification were systematically validated using the GrayToColor and OverlayOutlines modules, producing images outlining each identified object as quality controls.

For the radial distribution analyses, foci were reduced to their centroids using ShrinkToObjectCenters module, and converted into binary masks. Radial distributions were then quantified using the MeasureObjectIntensityDistribution module in 6 radial bins scaled to the nuclear area (bin 1 = center; bin 6 = periphery). For the relative radial distribution, the fraction of centroids within each bin was calculated per cell. For the absolute foci count per bin, cells with zero foci were strictly assigned a value of 0 for each bin to ensure unbiased averaging.

All source data underlying the quantitative figures, including object-level measurements, replicate-level tables and detailed statistical reports, are publicly available.

Statistical analysis

Data were organized using Microsoft Excel, statistical analyses were performed using GraphPad Prism 11 and Python 3.11 with Pandas, NumPy, SciPy, statsmodels, Matplotlib and Seaborn. To maximize scientific transparency, replicate-aggregated excel files (tidy format) were used to produce SuperPlots as described (51). These graphs display the data distribution using replicate-specific colors, overlaying biological replicate means. Bars represent the grand mean (mean of the biological replicate means), and inter-replicate standard error of the mean (s.e.m). To account for the matched experimental design and inter-replicate variability, and as visually confirmed by inter-replicate covariance patterns in SuperPlots, matched conditions originating from the same independent experiment were processed as paired blocks. Therefore, unless otherwise stated, two-tailed paired Student’s t-tests performed on biological replicate means were used to determine the significance of the specific treatment effects. For pre-defined directional hypotheses, one-tailed paired Student’s t-tests were applied to biological replicate means. Holm-Bonferroni corrections for multiple comparisons were applied when indicated to control the Family-Wise Error Rate (FWER). Significance thresholds of the adjusted p-values are: ns: non-significant; *: $p < 0.05$; **: $p < 0.01$; ***: $p <$

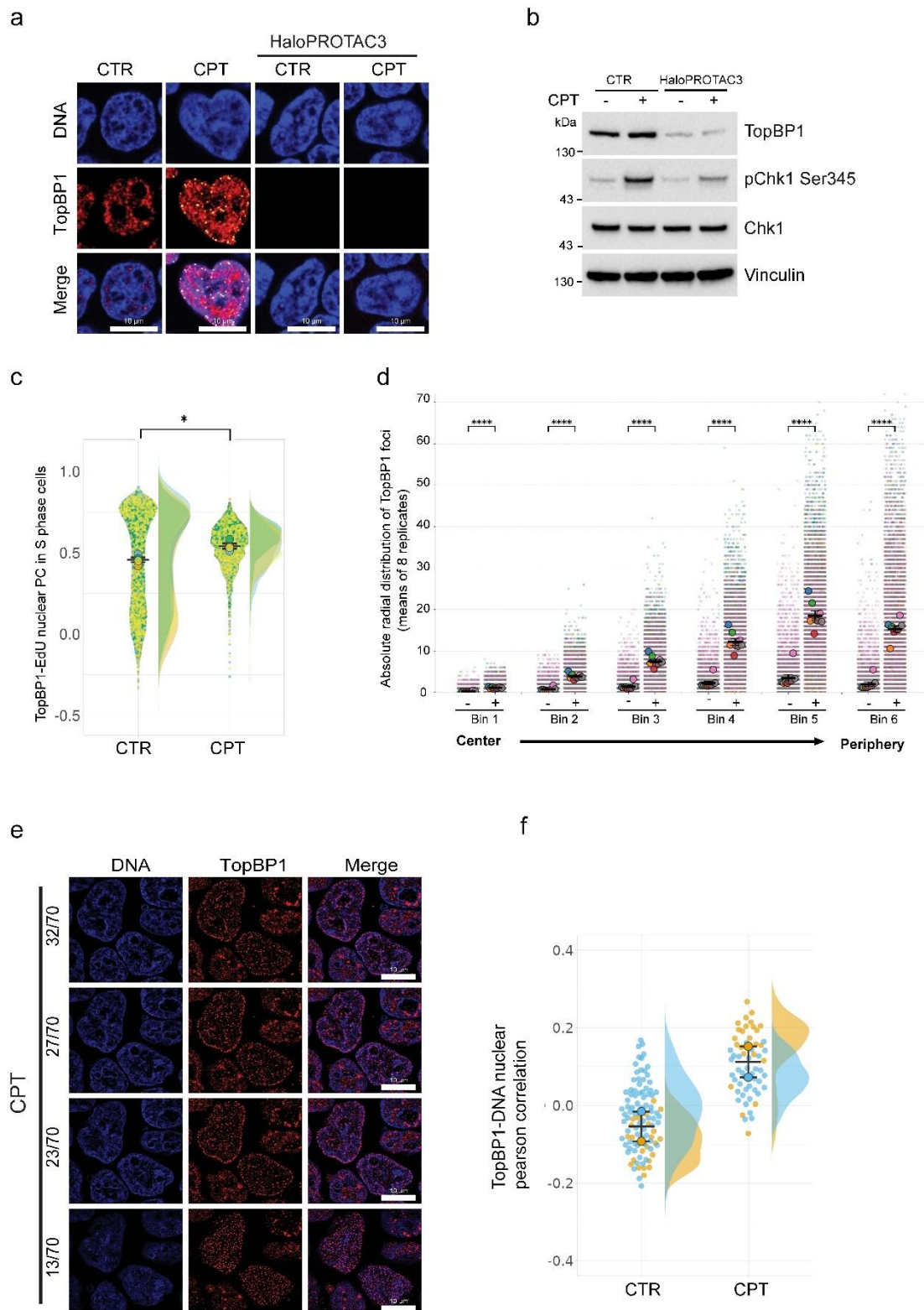
0.001; ****: $p < 0.0001$. Nuclear and foci fluorescence intensities measurements were log10-transformed before calculation of biological replicate means and statistics, when indicated.

Detailed statistical reports are included for each figure panel in the raw data archive. These include biological replicate means, scored object counts per condition and replicate, test types FWER adjustments, as well as raw and adjusted P-values.

Supplementary Figures

Supplementary Fig. 1 | Peripheral localisation of nuclear TopBP1 condensates under sustained DNA replication stress

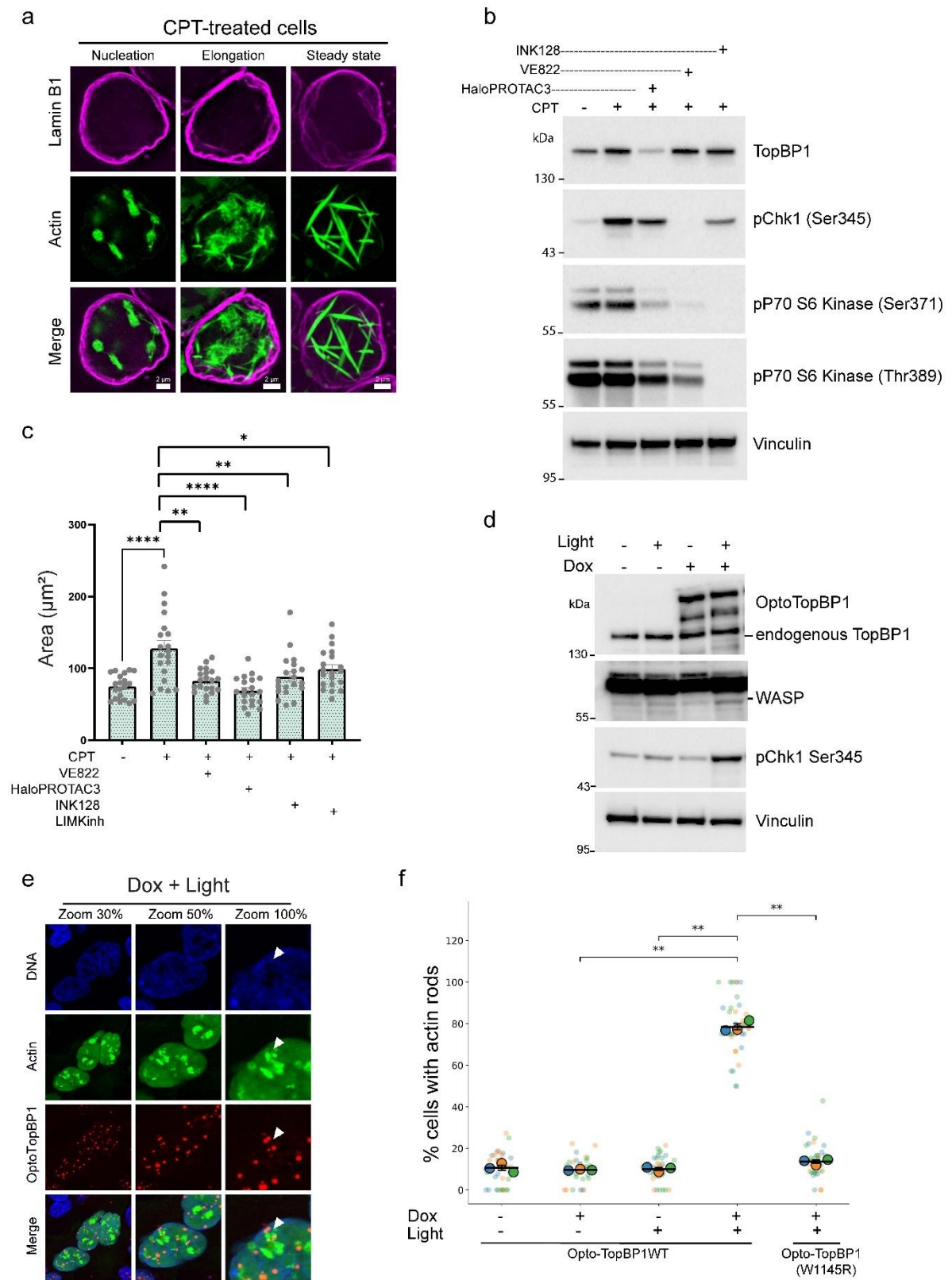
a Structured illumination microscopy (SIM) of a single z-plane from HEK293 Halo-TopBP1 cells stained with Janelia Fluor 549 after treatment with 2 μ M CPT for 3 h in the presence or absence of 1 μ M HaloPROTAC3. **b**, Western blot analysis of whole-cell extracts from HEK293 Halo-TopBP1 cells treated with 2 μ M CPT for 3 h in the presence or absence of 1 μ M HaloPROTAC3. **c**, TopBP1–EdU nuclear Pearson correlation coefficient in S-phase HEK293 cells after treatment with 2 μ M CPT for 3 h. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m of 4 biological replicates, scoring ≥ 707 nuclei per replicate. Statistical analysis was performed using two-tailed paired Student's t-tests on biological replicate means: * $p < 0.05$. **d**, Absolute radial distribution of TopBP1 foci from bin 1 (centre) to bin 6 (periphery) in HEK293 Halo-TopBP1 cells after treatment with 2 μ M CPT for 3 h. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m of 8 biological replicates, scoring ≥ 707 nuclei per condition and replicate. Statistical analysis was performed using two-tailed paired Student's t-tests on biological replicate means, followed by Holm-Bonferroni correction for multiple comparisons: **** $p < 0.0001$ (FWER-adjusted P-values). **e**, Structured illumination microscopy (SIM) of a representative z-plane from HEK293 Halo-TopBP1 cells stained with Janelia Fluor 549 after treatment with 2 μ M CPT for 3 h. **f**, TopBP1–DNA nuclear Pearson correlation coefficient in HEK293 Halo-TopBP1 cells after treatment with 2 μ M CPT for 3 h. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m of 2 biological replicates, scoring ≥ 23 nuclei per condition and replicate. For **(a)** and **(e)**, scale bars, 10 μ m.



Supplementary Figure 1

Supplementary Fig. 2 | Self-assembled TopBP1 promotes nuclear F-actin formation via ATR-mTOR-WASP

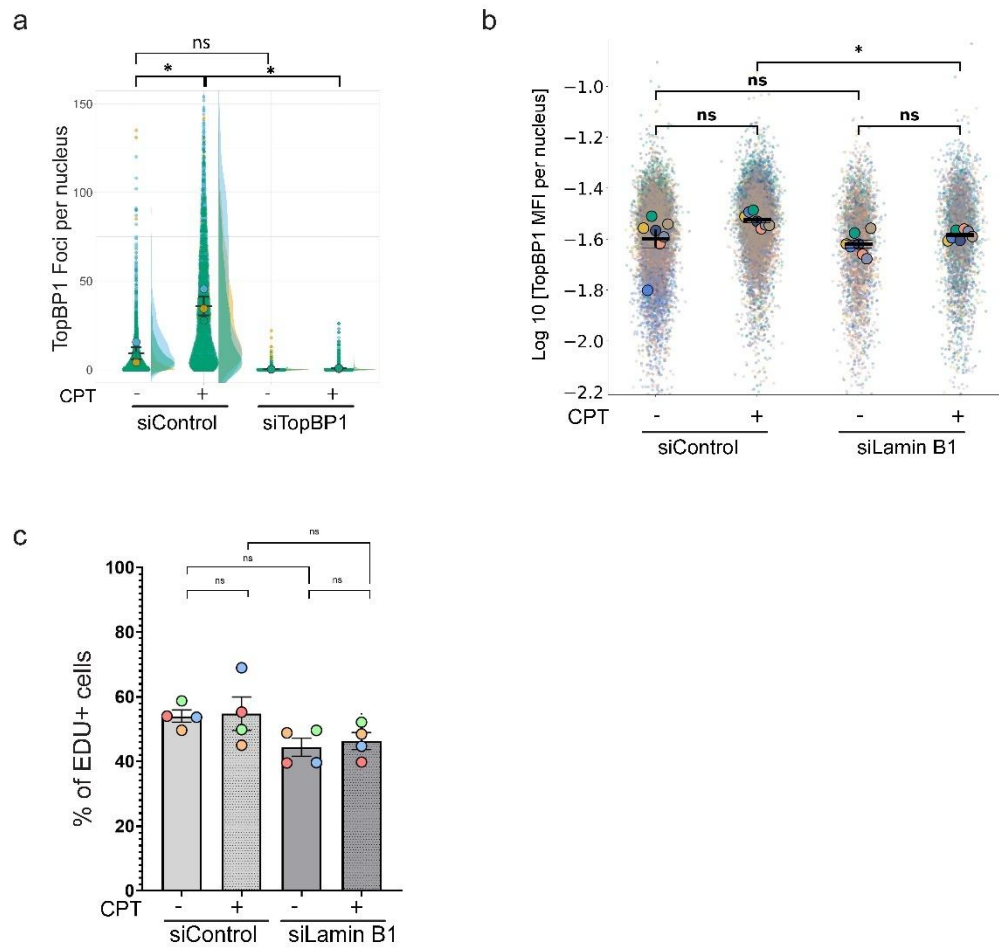
a, Structured illumination microscopy (SIM) of a representative z-plane from a z-stack of HEK293 Halo-TopBP1 cells transfected with the nuclear actin chromobody and treated with 2 μ M CPT for 3 h. **b**, Western blot analysis of whole-cell extracts from HEK293 cells treated with 2 μ M CPT for 3 h in the presence or absence of 1 μ M HaloPROTAC3, 10 μ M VE-822 or 200 nM INK128. **c**, Nuclear area (μm^2) of HEK293 Halo-TopBP1 cells treated with 2 μ M CPT for 3 h in the presence or absence of 1 μ M HaloPROTAC3, 10 μ M VE-822, 200 nM INK128 or 3 μ M LIMKi3. Data are mean $\pm$ s.e.m. $n = X$ biological replicates, scoring $\geq X$ nuclei per replicate. **d**, Western blot analysis of whole-cell extracts from Opto-TopBP1 HEK293 cells treated with or without doxycycline (2 $\mu\text{g}/\text{ml}$) and exposed or not to 5 min of blue light. **e**, Structured illumination microscopy (SIM) of a single z-plane from Opto-TopBP1 HEK293 cells treated with doxycycline and transfected with the nuclear actin chromobody, exposed to 5 min of blue light. **f**, Percentage of cells with actin rods per microscopy field in Opto-TopBP1 HEK293 cells treated with or without doxycycline, transfected with the nuclear actin chromobody and exposed or not to 5 min of blue light. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m of 3 biological replicates, scoring ≥ 99 nuclei per condition and replicate. Statistical analysis was performed using two-tailed paired Student's t-tests on biological replicate means, followed by Holm-Bonferroni correction for multiple comparisons: *** $p < 0.001$ (FWER-adjusted P-values). For **(a)** and **(e)**, scale bars, 2 μm .



Supplementary Figure 2

Supplementary Fig. 3 | Lamin B1 modulates CPT-induced TopBP1 condensates

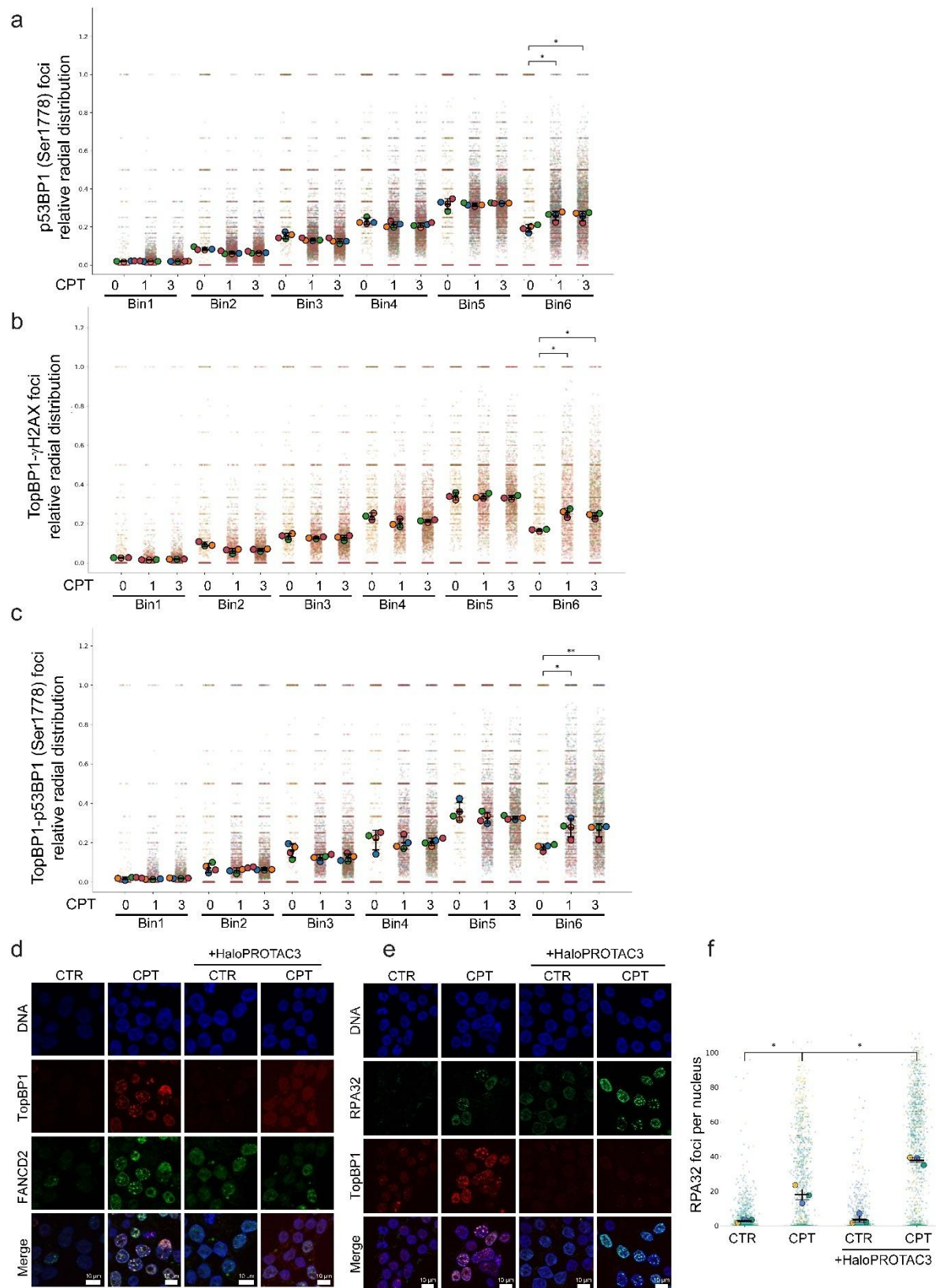
a, TopBP1 foci per nucleus in HEK293 Halo-TopBP1 cells treated with 2 μ M CPT for 3 h in siCTR and siTopBP1 conditions. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m of 3 biological replicates, scoring ≥ 650 nuclei per condition and replicate. For siTopBP1-treated cells, the subsequent analysis was strictly restricted to successfully depleted cells via pre-gating on TopBP1 nuclear MFI ≤ 0.012 A.U – see Figure 4E. Statistical analysis was performed using two-tailed paired Student's t-tests on biological replicate means, followed by Holm-Bonferroni correction for multiple comparisons: ns: non-significant, * $p < 0.05$ (FWER-adjusted P-values). **b**, Log10-transformed TopBP1 mean fluorescence intensity (MFI) per nucleus in HEK293 Halo-TopBP1 cells treated with 2 μ M CPT for 3 h in siCTR and siLamin B1 conditions. For siLamin B1-treated cells, the subsequent analysis was strictly restricted to successfully depleted cells via pre-gating on Lamin B1 MFI at nuclear periphery ≤ 0.03 A.U – see figure 4G. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m of 6 biological replicates, scoring ≥ 283 nuclei per condition and replicate. Statistical analysis was performed using two-tailed paired Student's t-tests on biological replicate means, followed by Holm-Bonferroni correction for multiple comparisons: ns: non-significant, * $p < 0.05$ (FWER-adjusted P-values). **c**, Percentage of EdU-positive cells in HEK293 Halo-TopBP1 cells treated with 2 μ M CPT for 3 h in siCTR and siLamin B1 conditions. Data are mean $\pm$ s.e.m. $n = 4$ biological replicates, scoring ≥ 898 nuclei per condition and replicate. Statistical analysis was performed using two-tailed paired Student's t-tests on biological replicate means, followed by Holm-Bonferroni correction for multiple comparisons: ns: non-significant (FWER-adjusted P-values).



Supplementary Figure 3

Supplementary Fig. 4 | TopBP1 condensates colocalize with distinct DNA damage markers at the nuclear periphery

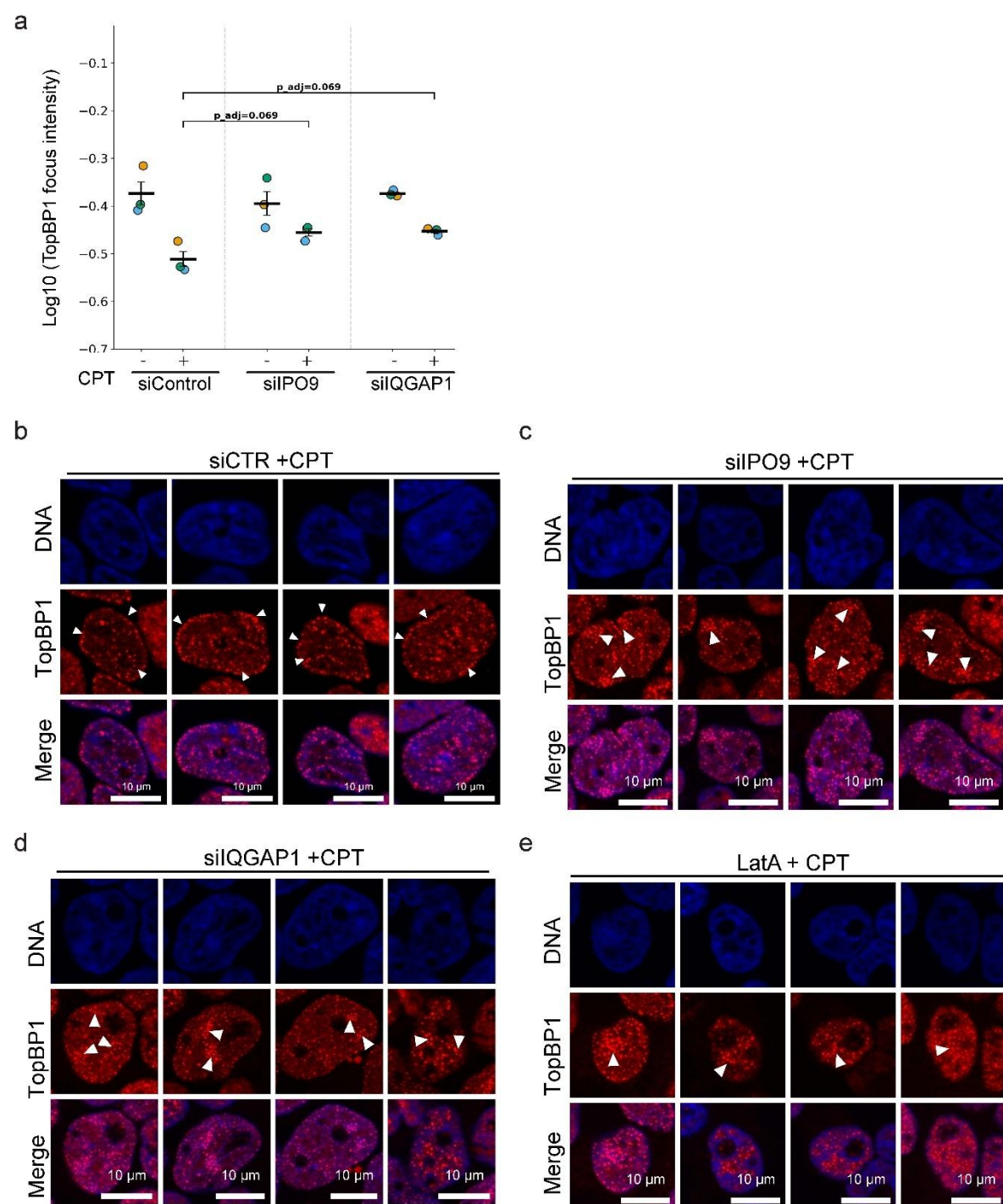
a, Relative radial distribution of p53BP1 Ser1778 foci from bin 1 (centre) to bin 6 (periphery) in HEK293 Halo-TopBP1 cells after treatment with 2 μ M CPT for 0, 1 and 3 h. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m of 4 biological replicates, scoring ≥ 576 nuclei per condition and replicate. Statistical analysis was performed on bin 6 using two-tailed paired Student's t-tests on biological replicate means, followed by Holm-Bonferroni correction for multiple comparisons: * $p < 0.05$ (FWER-adjusted P-values). **b**, Relative radial distribution of TopBP1/ γ H2AX foci from bin 1 (centre) to bin 6 (periphery) in HEK293 Halo-TopBP1 cells after treatment with 2 μ M CPT for 0, 1 and 3 h. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m of 3 biological replicates, scoring ≥ 576 nuclei per condition and replicate. Statistical analysis was performed on bin 6 using two-tailed paired Student's t-tests on biological replicate means, followed by Holm-Bonferroni correction for multiple comparisons; * $p < 0.05$ (FWER-adjusted P-values). **c**, Relative radial distribution of TopBP1/p53BP1 Ser1778 foci from bin 1 (centre) to bin 6 (periphery) in HEK293 Halo-TopBP1 cells after treatment with 2 μ M CPT for 0, 1 and 3 h. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m of 4 biological replicates, scoring ≥ 576 nuclei per condition and replicate. Statistical analysis was performed on bin 6 using two-tailed paired Student's t-tests on biological replicate means, followed by Holm-Bonferroni correction for multiple comparisons; * $p < 0.05$, ** $p < 0.01$ (FWER-adjusted P-values). **d**, Structured illumination microscopy (SIM) of a single z-plane from HEK293 Halo-TopBP1 cells stained with Janelia Fluor 549 and anti-FANCD2 after treatment with or without 2 μ M CPT for 3 h in the presence or absence of 1 μ M HaloPROTAC3. STAT. **e**, Structured illumination microscopy (SIM) of a single z-plane from HEK293 Halo-TopBP1 cells stained with Janelia Fluor 549 and anti-RPA32 after treatment with or without 2 μ M CPT for 3 h in the presence or absence of 1 μ M HaloPROTAC3. STAT. **f**, RPA32 foci per nucleus from the experiments shown in **e**. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m of 3 biological replicates, scoring ≥ 410 nuclei per condition and replicate. The expected CPT-induced increase in RPA32 foci (CPT > CTR) was assessed as a technical positive control by a one-tailed paired Student's t-tests on biological replicate means. The sole biological comparison (HaloPROTAC3 + CPT vs CPT) was then assessed in a two-tailed paired Student's t-test on biological replicate means : * $p < 0.05$. X. For **(d) and (e)**, scale bars, 10 μ m.



Supplementary Figure 4

Supplementary Fig. 5 | Defective nuclear actin filament formation increases TopBP1 condensates and alters their localization

a, Log10-transformed TopBP1 focus intensity in siCTR, siIPO9 and siIQGAP1 HEK293 Halo-TopBP1 cells after treatment with or without 2 μ M CPT for 3 h. Data are presented as SuperPlots, displaying means of biological replicates as colored circles, bars represent the grand-mean $\pm$ s.e.m of 3 biological replicates, scoring ≥ 1273 foci per condition and replicate. One-tailed paired t-tests on biological replicate means were applied to assess the significance of the pre-specified hypotheses (TopBP1 focus intensity increases after IPO9 and IQGAP1 depletion upon CPT treatment). The Holm-Bonferroni correction for multiple comparisons was applied. Adjusted P-values are indicated on the graph. **b**, Structured illumination microscopy (SIM) of a single z-plane from siCTR HEK293 Halo-TopBP1 cells stained with the Janelia Fluor 549 HaloTag[®] ligand after treatment with 2 μ M CPT for 3 h. Arrowheads indicate TopBP1 foci. **c**, Structured illumination microscopy (SIM) of a single z-plane from siIPO9 HEK293 Halo-TopBP1 cells stained with the Janelia Fluor 549 HaloTag[®] ligand after treatment with 2 μ M CPT for 3 h. Arrowheads indicate TopBP1 foci. **d**, Structured illumination microscopy (SIM) of a single z-plane from siIQGAP1 HEK293 Halo-TopBP1 cells stained with Janelia Fluor 549 after treatment with 2 μ M CPT for 3 h. Arrowheads indicate TopBP1 foci. **e**, Structured illumination microscopy (SIM) of a single z-plane from HEK293 Halo-TopBP1 cells stained with the Janelia Fluor 549 HaloTag[®] ligand after treatment with 2 μ M CPT for 3 h in the presence of 200 nM latrunculin A. Arrowheads indicate TopBP1 foci. For **b–e**, scale bars, 10 μ m.

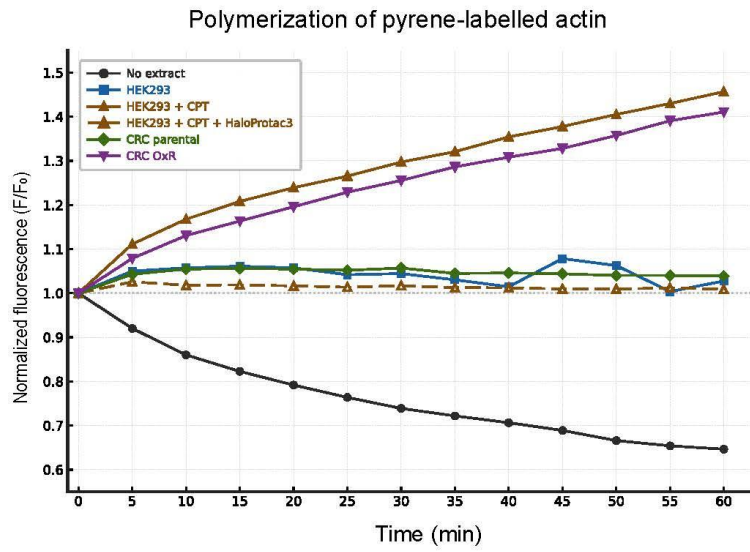


Supplementary Figure 5

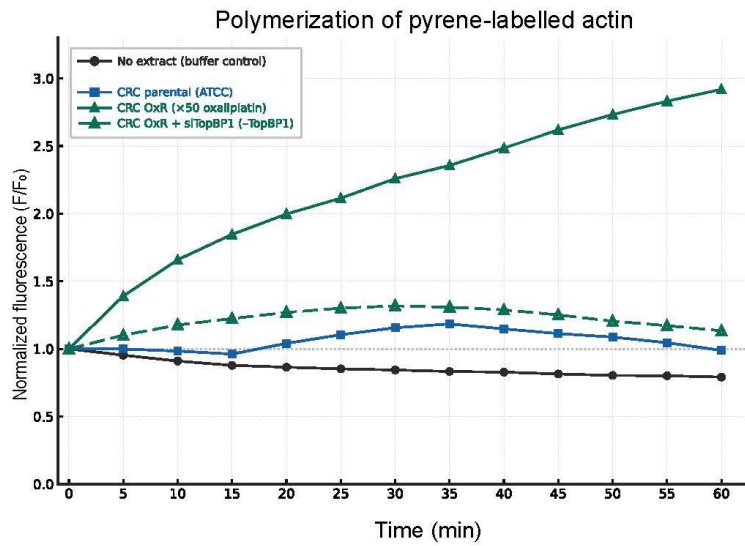
Supplementary Fig. 6 | Actin polymerization in oxaliplatin-resistant CRC depends on TopBP1

a, Normalized timecourse of pyrene-labelled actin assembly in the presence or absence of nuclear extracts from HEK293 Halo-TopBP1 cells untreated or treated with 2 μ M CPT for 3 h, HEK293 Halo-TopBP1 cells treated with 2 μ M CPT in the presence of 1 μ M HaloPROTAC3, and parental and oxaliplatin-resistant colorectal cancer (CRC) cells. One of two independent biological replicates is shown. **b**, Normalized time course of pyrene-labelled actin assembly in the presence or absence of nuclear extracts from parental CRC, oxaliplatin-resistant CRC and TopBP1-depleted oxaliplatin-resistant CRC cells. One of two independent biological replicates is shown.

a



b



Supplementary Figure 6

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Author Contributions

Conceptualization: EM, JB, AC, PEM and CD conceptualized experiments

Methodology: EM, JB, DH, AD, YF, CD, PEM

Formal analyses: EM, JB, DH, HZ, SZJ, VN, YF, , CC, CD, PEM

Investigation: EM, JB, DH, HZ, YF, PC, CC, CD, PEM

Writing original draft: EM, JB, PEM

Writing, review and editing: EM, JB, PC, , PEM, CD

Visualization: AC, JB, PEM

Supervision: AC, JB, PEM

Project Administration: AC, JB, PEM

Funding acquisition: AC, PEM

Declaration of Interests

The authors declare no competing interests.

Inclusion and diversity

We support inclusive, diverse, and equitable conduct of research.

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