Expanded View Figures

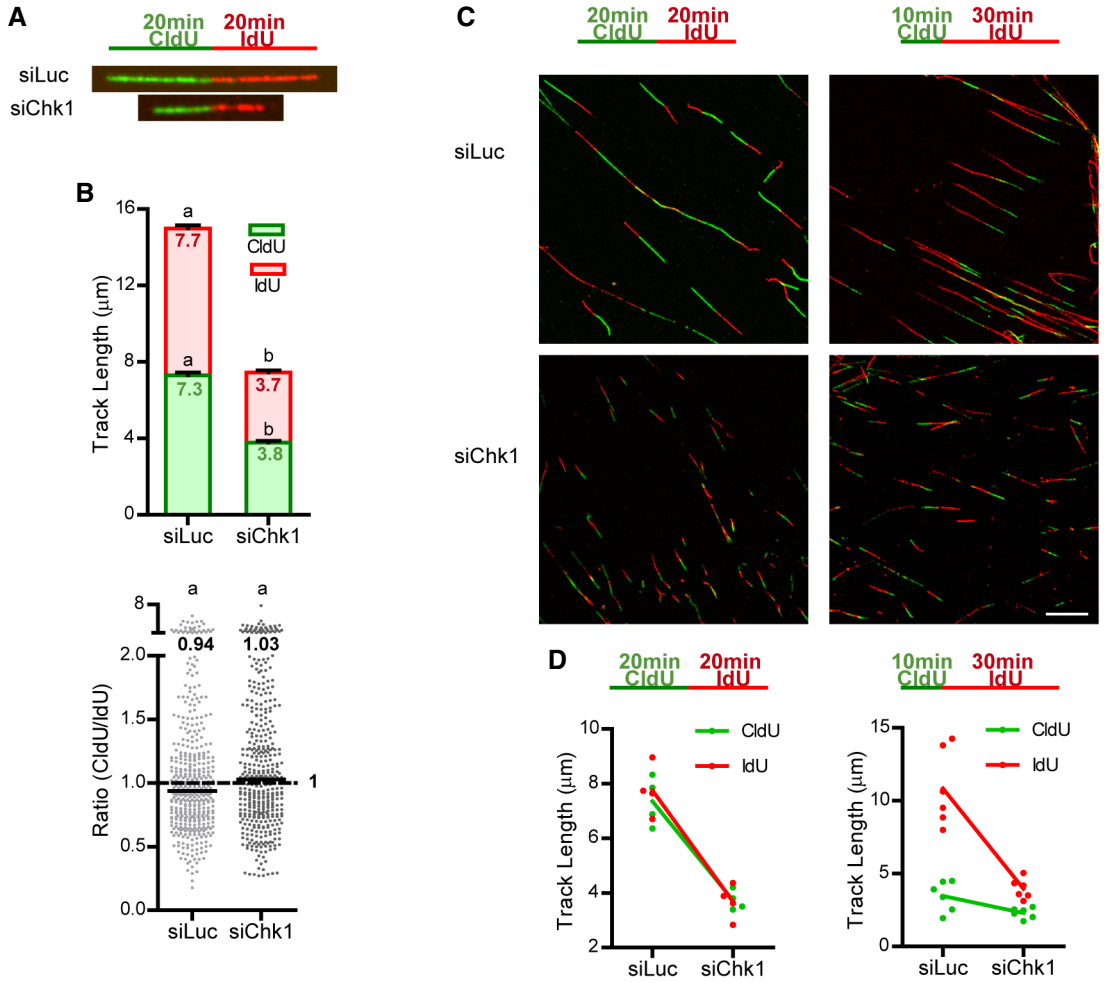

Figure EV1. Chk1 loss generates replication-associated obstacles that impair nascent DNA elongation.

A Labeling scheme and representative DNA fibers from control (siLuc) and Chk1-depleted (siChk1) U2OS cells.

B U2OS cells were labeled with CldU and IdU for 20 min each. Mean (± SEM) track lengths (top) and CldU/IdU ratios (bottom) are shown. Data come from four independent experiments, and a total of 436 (siLuc) and 422 (siChk1) fibers were scored.

C Representative fields of spread DNA from U2OS cells, labeled with CldU and IdU according to the schemes. Scale bar: 10 μm.

D Mean CldU and IdU track lengths from (B) and Fig 1C. The slope representing the drop in fork elongation in Chk1-depleted cells is equal for both analogues under a 20 + 20 min labeling protocol (left panel), but more pronounced for the second analogue (IdU) under a 10 + 30 min labeling scheme (right panel).

Data information: The bars/numbers on top of the distribution clouds indicate the median; different letters indicate significant differences, calculated by unpaired *t*-test.

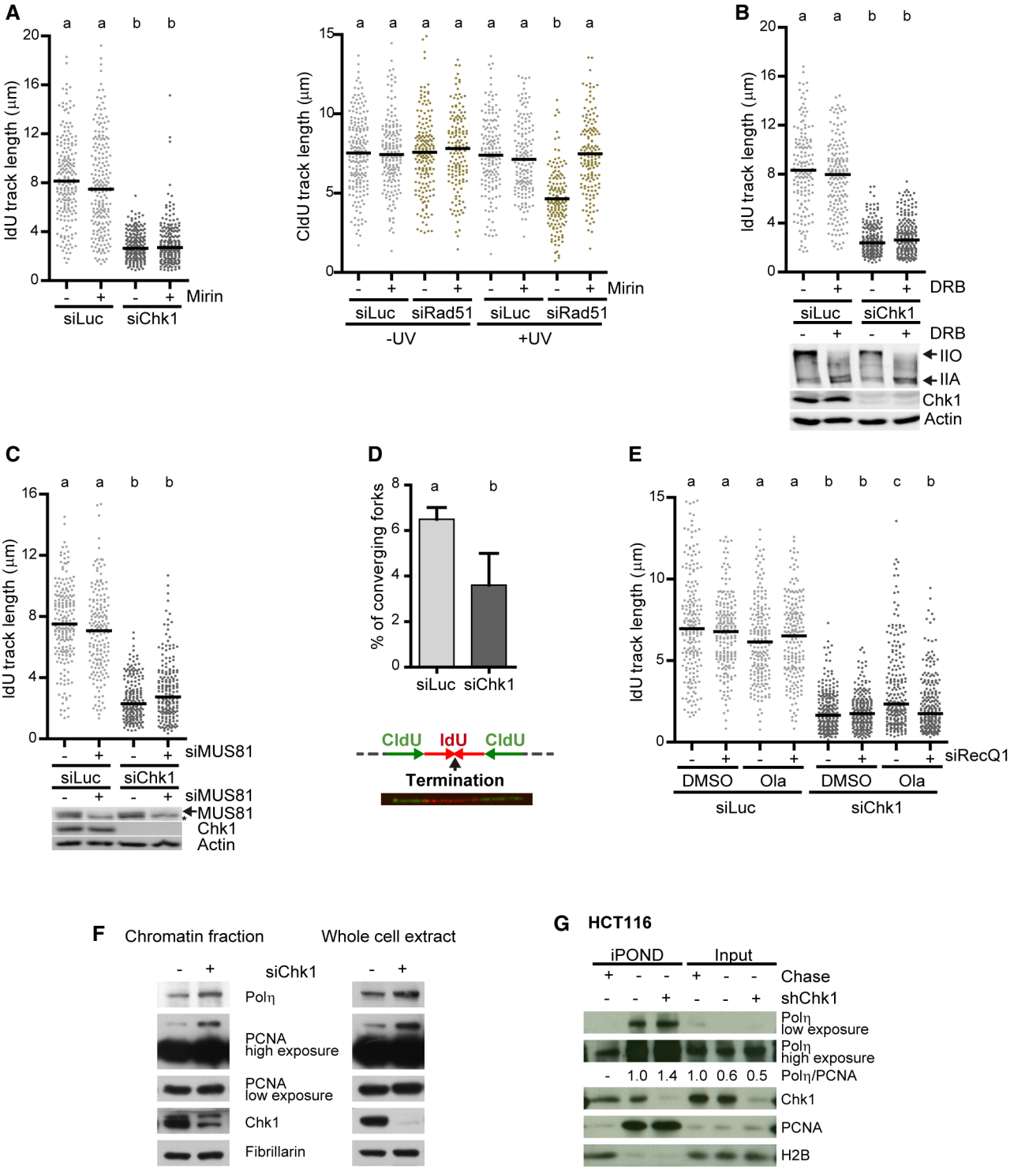

Figure EV2.

◀ **Figure EV2. Replication fork progression in Chk1-depleted cells is unaffected by Mre11-mediated degradation, conflicts with transcription, MUS81-dependent DSBs, or surplus converging forks, but it is associated with fork reversal and Polη enrichment at forks.**

A IdU track lengths from U2OS cells treated or not with mirin, an inhibitor of Mre11 exonuclease activity. > 200 DNA fibers obtained from two independent experiments were measured for each condition. The right panel serves as a control showing that mirin prevents degradation of nascent DNA in other conditions, specifically UV-irradiated, Rad51-depleted U2OS cells (Vallerga *et al*, 2015). > 150 DNA fibers obtained from 2 independent experiments were measured for each condition.

B IdU track lengths from U2OS cells treated or not with DRB, an inhibitor of RNA Pol II-dependent transcription elongation that limits R-loop formation (Kotsantis *et al*, 2016). > 150 DNA fibers obtained from two independent experiments were measured for each condition. The lower panel shows the corresponding Western blot of Rpb1 (the largest subunit of Pol II, whose hypo-phosphorylated and hyper-phosphorylation forms IIA and IIO indicate inactive and active transcription, respectively) and Chk1. Actin was used as a loading control.

C IdU track lengths from U2OS cells transfected or not with siMUS81. > 170 DNA fibers obtained from two independent experiments were measured for each condition. The lower panel shows the corresponding Western blot of MUS81 and Chk1. Actin was used as a loading control. The asterisk (*) indicates an unspecific band.

D Percentage of converging forks (mean ± SD) in U2OS cells. ~1,400 DNA fibers obtained from seven independent experiments were scored for each condition. The lower panel shows a representative image and scheme of two converging forks. Gray dotted lines represent unlabeled DNA.

E IdU track lengths from U2OS cells treated or not with the PARP inhibitor Olaparib (Ola), which prevents fork reversal, and siRNA against the RecQ1 helicase, which is required for the restart of regressed forks (Berti *et al*, 2013). > 200 DNA fibers obtained from two independent experiments were measured for each condition.

F Western blot of Polη, PCNA, and Chk1 in U2OS cells, after a 60-s extraction with CSK buffer to separate the insoluble and soluble fractions. Fibrillarin was used as a loading control.

G iPOND of HCT116 cells 96 h after transduction with shRNA targeting Chk1 (shChk1) or non-targeting shRNA (-, shScramble).

Data information: In (A–C and E), cells were labeled with CldU and IdU for 20 min each. The bars on top of the distribution clouds indicate the median; different letters indicate significant differences, calculated by one-way ANOVA with a Bonferroni post-test (A–C, E) or unpaired *t*-test (D).

Source data are available online for this figure.

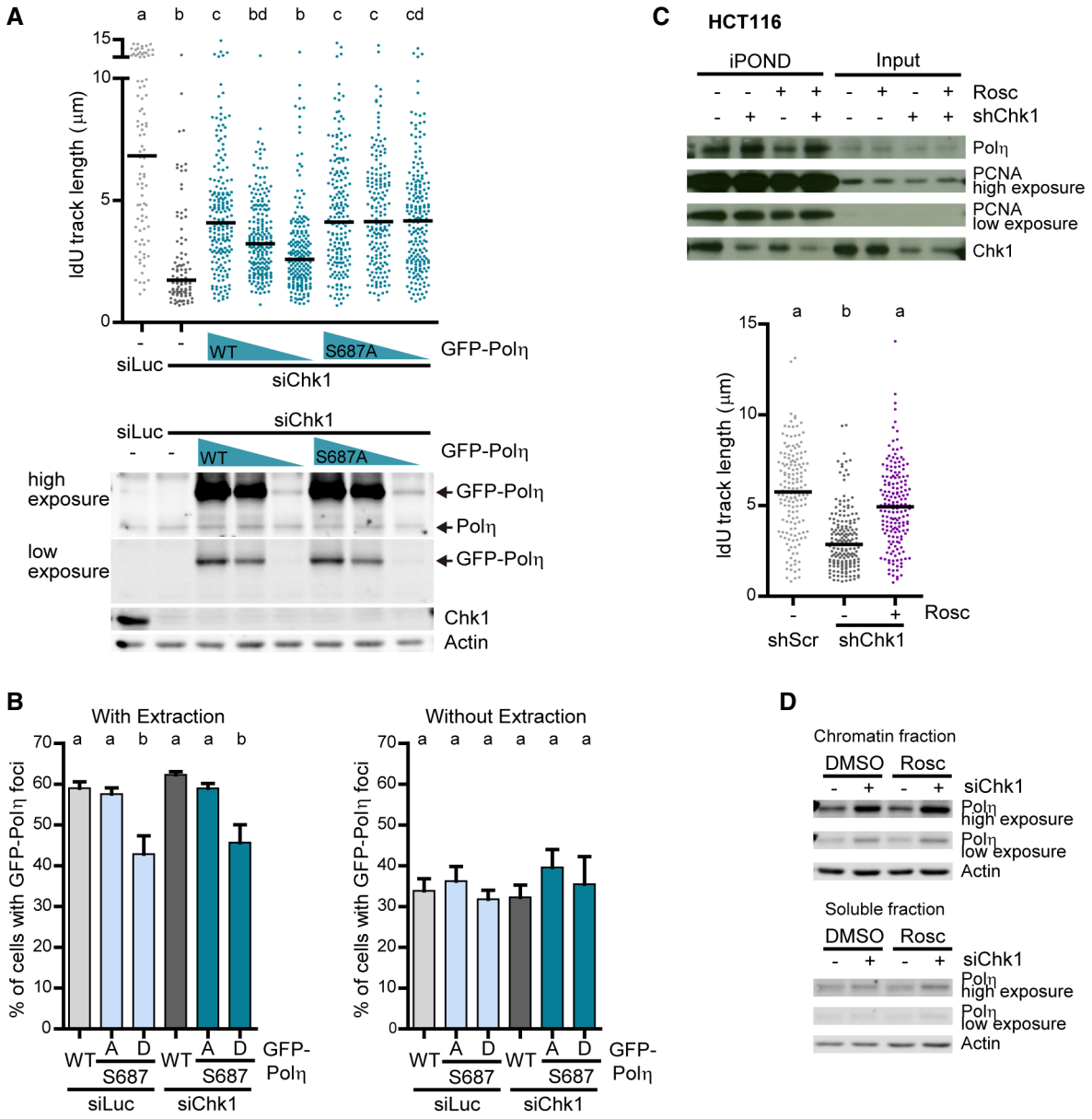

Figure EV3. Serine 687 phosphorylation status determines GFP-Polη function, not recruitment, at replication barriers created by Chk1 loss.

A IdU track lengths from U2OS cells transfected with GFP (–) or decreasing amounts of GFP-Polη-WT/S687A. > 175 fibers obtained from two independent experiments were measured for each condition. The lower panel shows the corresponding Western blot of Chk1, GFP-Polη, and endogenous Polη. Actin was used as a loading control.

B U2OS cells were subjected (left) or not (right) to a 15-s extraction with CSK buffer, and the percentage of cells with GFP-Polη (mean ± SD) was determined. > 600 cells/sample were analyzed in three independent experiments.

C iPOND of shChk1/shScramble-transduced HCT116 cells, treated for 1 h with 10 μM roscovitine. The lower panel shows a DNA fiber assay done under the same conditions as the iPOND. 157 (shScr) and 180 (shChk1 ± Rosc) fibers obtained from two independent experiments were measured for each condition.

D Western blot of Polη in siChk1/siLuc-transfected U2OS cells, treated for 1 h with 15 μM roscovitine or DMSO. A 30-s extraction with CSK buffer was used to separate the insoluble and soluble fractions. Actin was used as a loading control.

Data information: The bars on top of the distribution clouds indicate the median; different letters indicate significant differences, calculated by one-way ANOVA with a Bonferroni post-test (A–C) or repeated measures ANOVA with Newman–Keuls post-test (B).

Source data are available online for this figure.

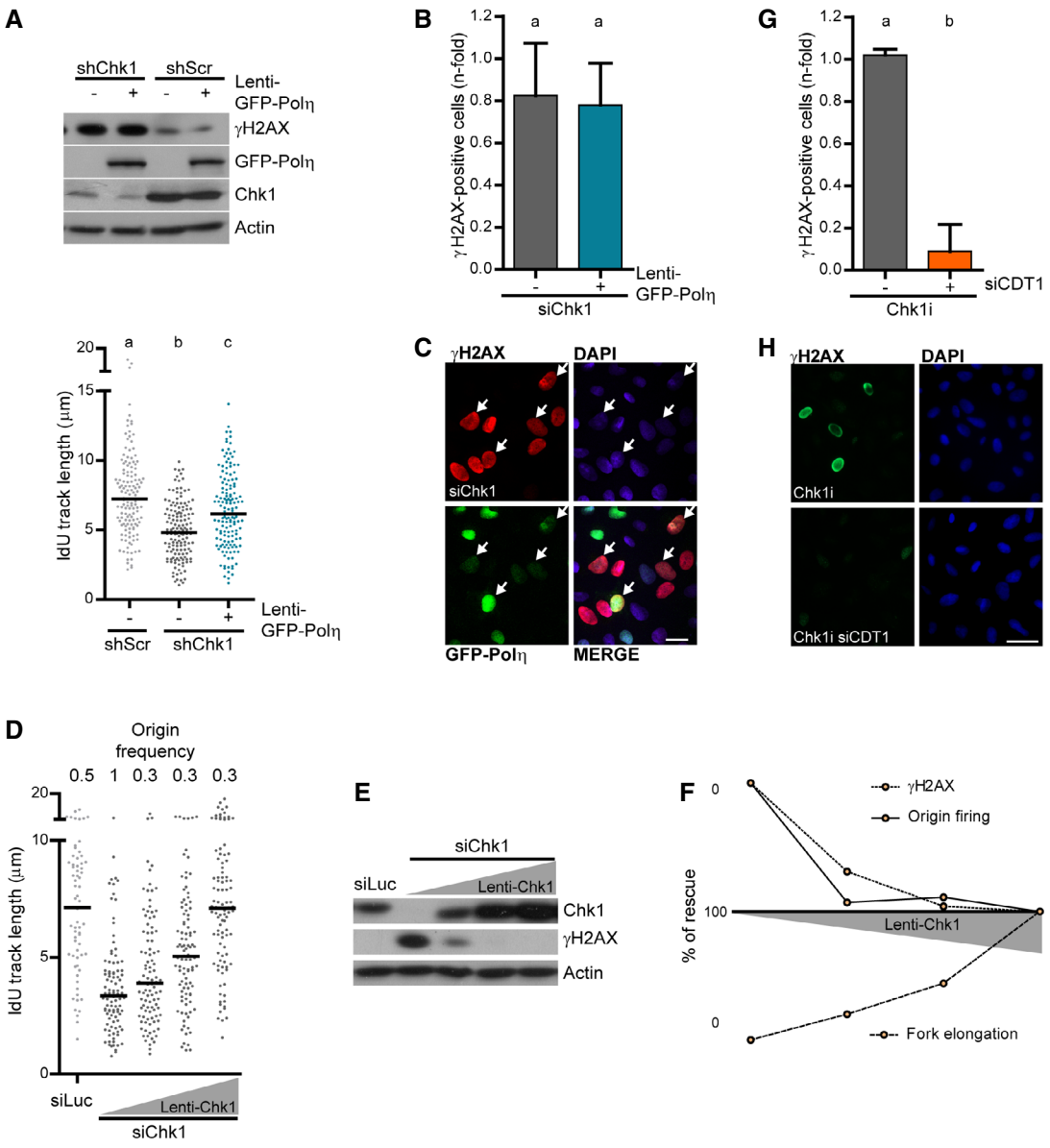

Figure EV4. Replication barrier bypass in Chk1-deficient cells does not alleviate DNA damage.

A Western blot of γH2AX, GFP-Polη, and Chk1 in U2OS cells, 48 h after transduction with shRNA and Lenti-GFP-Polη or Lenti-GFP (–). Actin was used as a loading control. The lower panel shows a DNA fiber assay performed under the same conditions as the Western blot. > 140 fibers obtained from two independent experiments were measured for each condition.

B Quantification of pannuclear γH2AX-positive U2OS cells (mean ± SD), transduced with Lenti-GFP-Polη and transfected with siChk1. > 900 cells/sample were analyzed in two independent experiments.

C Representative images of data shown in (B). White arrows indicate cells that accumulate γH2AX even if GFP-Polη is expressed. Scale bar: 25 μm.

D IdU track length and frequency of origin firing in Chk1-depleted U2OS cells transduced with increasing amounts of a lentivirus carrying the Chk1-WT (wild-type) sequence. A representative experiment is shown (n = 2). 100 (track length) and > 230 (origin frequency) DNA fibers were scored.

E Western blot of Chk1 and γH2AX of samples in (D). Actin was used as loading control.

F Percentage of rescue of fork elongation, origin firing, and γH2AX against the amount of exogenous Chk1 protein, calculated from data in (D–E).

G Quantification of pannuclear γH2AX-positive U2OS cells (mean ± SD), transfected with siLuc or siCDT1, and treated 5 h with Chk1i. > 450 cells/sample were analyzed in two independent experiments.

H Representative images of data shown in (G). Scale bar: 50 μm.

Data information: The bars on top of the distribution clouds indicate the median; different letters indicate significant differences, calculated by one-way ANOVA with a Bonferroni post-test (A) or unpaired t-test (B, G).

Source data are available online for this figure.

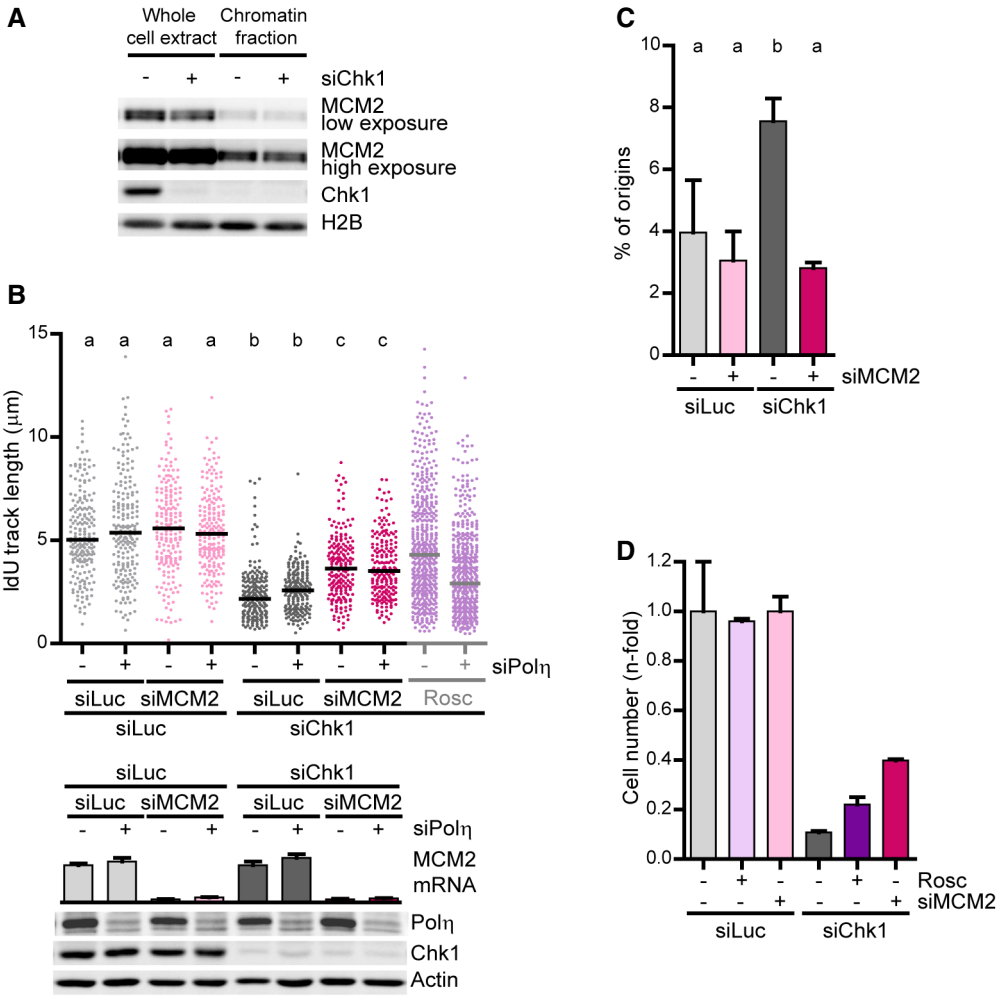

Figure EV5. MCM2-dependent generation of replication barriers compromise the fitness of Chk1-deficient cells.

A Western blot of MCM2 and Chk1 in U2OS cells after an extraction with CSK buffer to separate the insoluble and soluble fractions. H2B was used as a loading control.

B IdU track lengths from U2OS cells. 200 DNA fibers obtained from two independent experiments were measured for each condition. For comparison, the data showing that Polη is required for roscovitine-dependent rescue of fork elongation in Chk1-depleted cells (Fig 3B) are shown on the right. The lower panel shows the corresponding Western blot of Chk1 and Polη (actin was used as a loading control) and quantitative real-time PCR of MCM2 normalized to GAPDH (error bars represent the SD of 2 technical replicates).

C Percentage of origin firing (mean ± SD) from DNA fibers in (B) (n = 2). > 700 fibers per condition were scored.

D Sensitivity of U2OS cells to Chk1 and MCM2 depletion. Cell number was determined 7 days after transfection (5 days after maximal downregulation). A representative experiment is shown (n = 2). Error bars represent the SD of three technical replicates.

Data information: The bars on top of the distribution clouds indicate the median; different letters indicate significant differences, calculated by one-way ANOVA with a Bonferroni post-test (B) or repeated measures ANOVA with a Newman–Keuls post-test (C).

Source data are available online for this figure.