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Supplemental material

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Provided online is one table in Excel. Table S1 provides individual z-scores for CHK1 levels upon siRNA-mediated knockdowns related to the screen results depicted in Fig. 3 A.

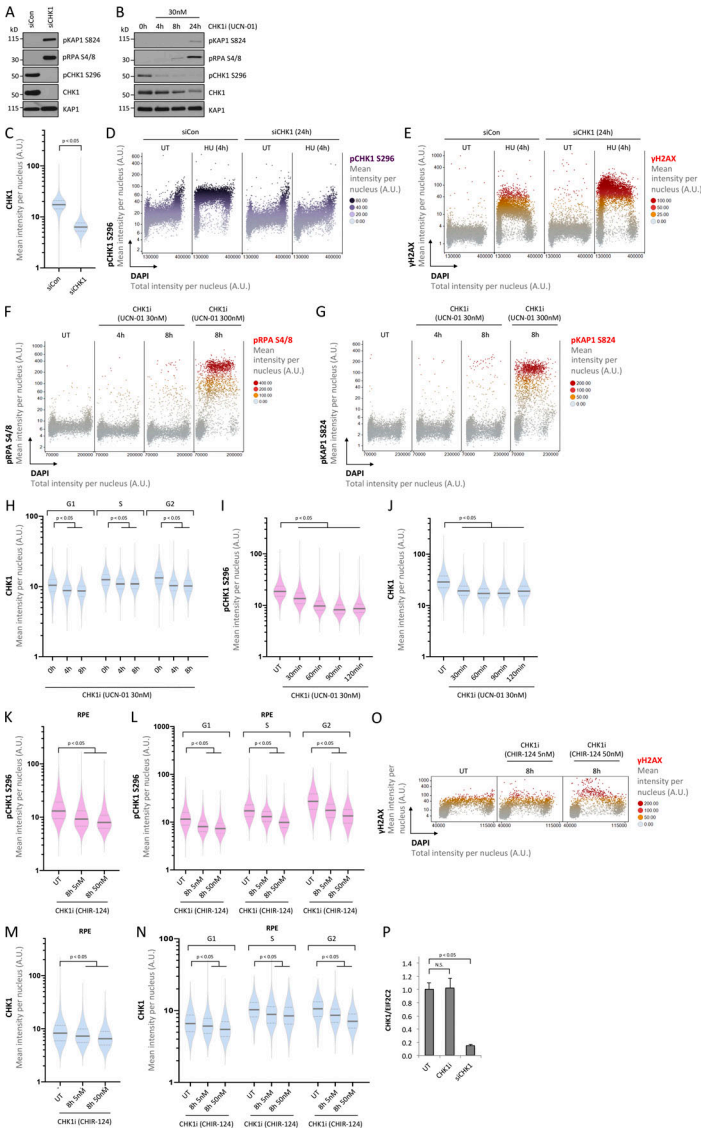

Figure S1. **Steady-state CHK1 activity maintains CHK1 levels in unchallenged conditions. (A)** U-2 OS cells were transfected with siRNA against CHK1 for 48 h as indicated, and whole cell extracts were analyzed for DNA damage markers and CHK1 levels by Western blot. **(B)** U-2 OS cells were treated with low-dose CHK1i for the indicated time periods, and whole cell extracts were analyzed for DNA damage markers and CHK1 levels by Western blot. **(C)** U-2 OS cells were transfected with siRNA for 24 h as indicated and stained for CHK1. QIBC-derived levels of CHK1 are shown with median (solid line) and quartiles (dashed lines) indicated (sample size: 9,799 and 9,622 cells). **(D)** U-2 OS cells were transfected with siRNA against CHK1 for 24 h, treated with 2 mM HU for 4 h, and stained for pCHK1 S296, γH2AX, and DNA content. QIBC-derived pCHK1 S296 levels are shown as a function of cell cycle progression (sample size: 10,325, 10,565, 10,049, and 9,895 cells). **(E)** Cell cycle–resolved γH2AX levels from D. **(F)** U-2 OS cells were treated with low- or high-dose CHK1i as indicated, stained for pRPA S4/8, and analyzed by QIBC (sample size: 4,915, 5,868, 5,204, and 4,798 cells). **(G)** U-2 OS cells treated as in F were stained for pKAP1 S824 and analyzed by QIBC (sample size: 4,781, 4,810, 5,203, and 5,183 cells). **(H)** U-2 OS cells were treated with CHK1i as indicated and stained for CHK1, EdU, and DNA content. CHK1 levels are depicted according to cell cycle position. Cell cycle staging was performed based on EdU and DAPI, with median (solid line) and quartiles (dashed lines) indicated (sample size: 1,419 G1 cells, 4,609 S-phase cells, and 1,983 G2 cells in untreated, 1,740 G1 cells, 5,003 S-phase cells, and 2,002 G2 cells after 4 h treatment, 2,006 G1 cells, 4,121 S-phase cells, and 1,993 G2 cells after 8 h treatment). **(I)** U-2 OS cells were treated as indicated and stained for pCHK1 S296. QIBC-derived levels of pCHK1 S296 are shown with median (solid line) and quartiles (dashed lines) indicated (sample size: 3,839, 3,720, 3,708, 3,622, and 3,689 cells). **(J)** U-2 OS cells were treated as in I and stained for CHK1. QIBC-derived levels of CHK1 are shown with median (solid line) and quartiles (dashed lines) indicated (sample size: 4,949, 4,657, 4,670, 4,612, and 4,636 cells). **(K)** RPE-1 cells were treated with CHK1i and stained for pCHK1 S296. QIBC-derived levels of pCHK1 S296 are shown with median (solid line) and quartiles (dashed lines) indicated (sample size: 6,623, 7,322, and 7,738 cells). **(L)** The data from K are depicted according to cell cycle position. Cell cycle staging was performed based on total DAPI intensity. Median (solid line) and quartiles (dashed lines) are indicated (sample size: 4,936 G1 cells, 1,007 S-phase cells, and 680 G2 cells in untreated, 5,348 G1 cells, 1,244 S-phase cells, and 730 G2 cells after 5 nM CHIR-124, 5,792 G1 cells, 993 S-phase cells, and 953 G2 cells after 50 nM CHIR-124). **(M)** RPE-1 cells were treated as above and stained for CHK1. QIBC-derived levels of CHK1 are shown with median (solid line) and quartiles (dashed lines) indicated (sample size: 5,398, 5,134, and 5,376 cells). **(N)** The data from M are depicted according to cell cycle position. Cell cycle staging was performed based on total DAPI intensity. Median (solid line) and quartiles (dashed lines) are indicated (sample size: 3,905 G1 cells, 915 S-phase cells, and 578 G2 cells in untreated, 3,606 G1 cells, 894 S-phase cells, and 634 G2 cells after 5 nM CHIR-124, 4,123 G1 cells, 691 S-phase cells, and 562 G2 cells after 50 nM CHIR-124). **(O)** γH2AX levels from M are shown as a function of cell cycle progression. **(P)** CHK1 mRNA levels in U-2 OS cells treated with 30 nM CHK1i UCN-01 for 30 min; siCHK1 was used as specificity control. Data are presented as mean ± SD of triplicates; P ≥ 0.05 was considered not significant (N.S.). Mann–Whitney *U* test in C and H–N; unpaired *t* test in P. A.U., arbitrary units.

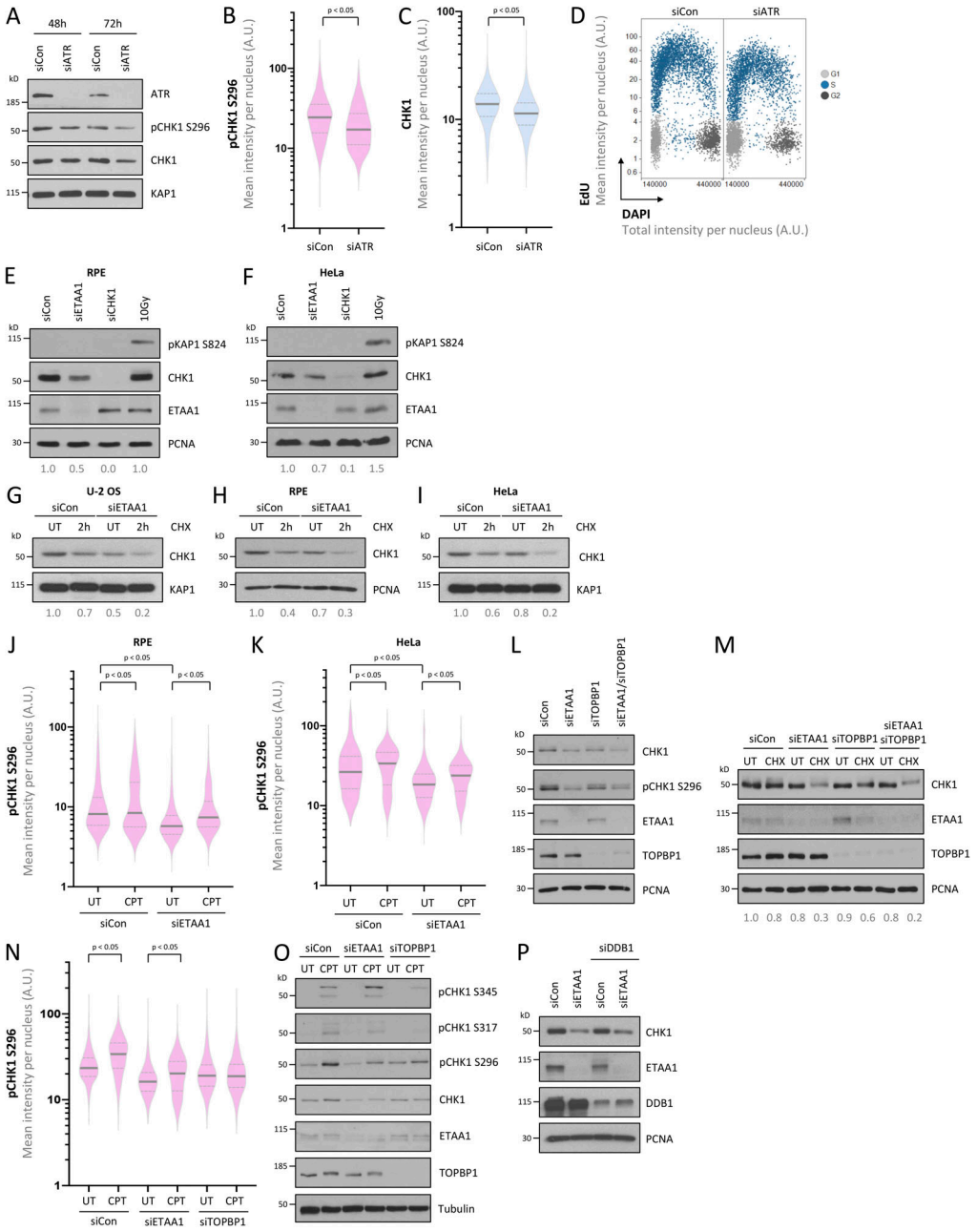

Figure S2. **ATR and its coactivator ETAA1 ensure steady-state CHK1 levels in the absence of genotoxic stress. (A)** U-2 OS cells were transfected with siRNA against ATR as indicated, and whole cell extracts were analyzed for CHK1 levels by Western blot. **(B)** U-2 OS cells were transfected with siRNA for 48 h as indicated and stained for pCHK1 S296, γH2AX, EdU, and DNA content. QIBC-derived levels of pCHK1 S296 are shown with median (solid line) and quartiles (dashed lines) indicated (sample size: 5,047 and 5,094 cells). **(C)** U-2 OS cells were treated as in B and stained for CHK1. QIBC-derived levels of CHK1 are shown with median (solid line) and quartiles (dashed lines) indicated (sample size: 5,910 and 7,524 cells). **(D)** QIBC-derived cell cycle–resolved EdU staining from B. **(E)** RPE-1 cells were transfected with siRNA as indicated for 72 h, and whole cell extracts were analyzed by Western blot for CHK1 levels and the DNA damage marker pKAP1 S824. Cells treated with 10 Gy and allowed to recover for 1 h were included as positive DNA damage control. **(F)** HeLa cells were treated as in E and analyzed by Western blot for CHK1 levels and the DNA damage marker pKAP1 S824. **(G)** U-2 OS cells were transfected with siRNA against ETAA1 for 48 h as indicated, exposed to CHX for 2 h, and immunoblotted for CHK1 and KAP1. **(H)** RPE-1 cells were treated and analyzed as in G. **(I)** HeLa cells were treated and analyzed as in G. **(J)** RPE-1 cells were transfected with siRNA for 48 h, treated with CPT (1 µM) for 1 h as indicated, and stained for pCHK1 S296. QIBC-derived levels of pCHK1 S296 are shown with median (solid line) and quartiles (dashed lines) indicated (sample size: 4,241, 3,501, 4,761, and 4,761 cells). **(K)** HeLa cells were treated and analyzed as in J. QIBC-derived levels of pCHK1 S296 are shown with median (solid line) and quartiles (dashed lines) indicated (sample size: 2,839, 2,807, 2,982, and 3,077 cells). **(L)** U-2 OS cells were transfected with siRNA against ETAA1 or TOPBP1 for 72 h as indicated, and CHK1 and pCHK1 S296 levels were analyzed by Western blot. **(M)** U-2 OS cells were transfected with siRNA against ETAA1 or TOPBP1, exposed to CHX for 1 h, and immunoblotted for CHK1. **(N)** U-2 OS cells were transfected with siRNA for 72 h, treated with CPT (1 µM) for 1 h as indicated, and stained for pCHK1 S296. QIBC-derived levels of pCHK1 S296 are shown with median (solid line) and quartiles (dashed lines) indicated (sample size: 1,698, 1,632, 1,614, 1,557, 1,586, and 1,564 cells). **(O)** Western blot analysis of U-2 OS cells transfected with siRNA for 72 h as indicated and treated with CPT (1 µM) for 1 h. **(P)** U-2 OS cells were transfected with siETAA1 (B) and siDDB1 for 48 h as indicated, and CHK1 levels were analyzed by Western blot. Mann–Whitney *U* test in B, C, J, K, and N. A.U., arbitrary units.

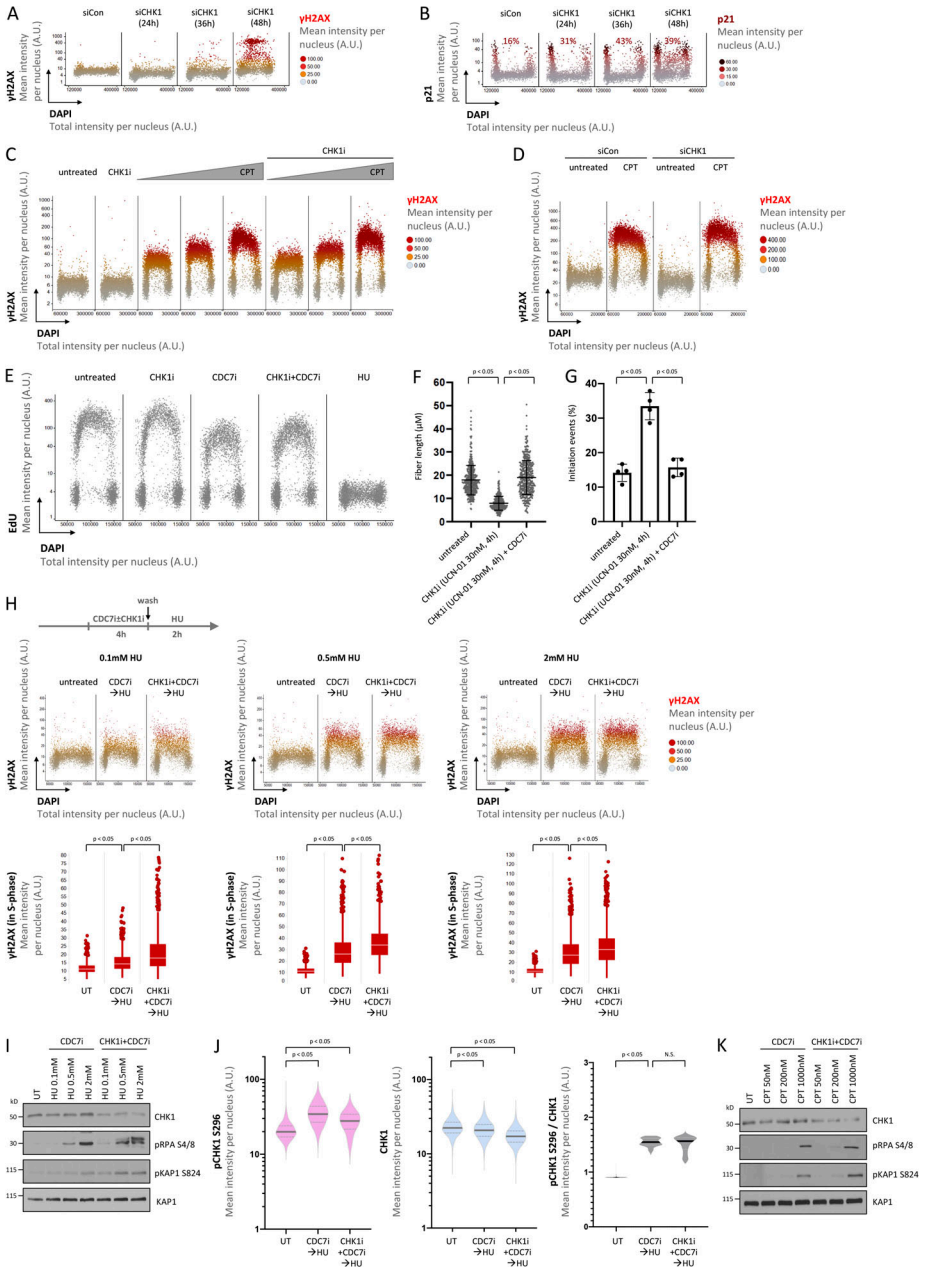

Figure S3. **CHK1 degradation upon impaired steady-state activity sensitizes to RS. (A)** U-2 OS cells were transfected with siRNA against CHK1 for the indicated time periods, stained for p21, γH2AX, and DNA content, and analyzed by QIBC. Cell cycle–resolved γH2AX levels are shown (sample size: 3,096, 3,076, 3,178, and 3,068 cells). **(B)** Cell cycle–resolved p21 levels for cells in A. The percentage of cells with high p21 levels is indicated. **(C)** U-2 OS cells treated or not with low-dose CHK1i (30 nM UCN-01) were exposed to increasing concentrations of CPT (50 nM, 200 nM, 1,000 nM) for 2 h, stained for γH2AX and DNA content, and analyzed by QIBC (sample size: 5,175, 5,592, 5,243, 4,743, 5,500, 5,263, 5,107, and 5,114 cells). **(D)** U-2 OS cells were transfected with siRNA against CHK1 for 24 h, treated with CPT (1 μM; 1 h), stained for γH2AX and DNA content, and analyzed by QIBC (sample size: 4,941, 7,102, 5,068, and 7,076 cells). **(E)** U-2 OS cells were treated with low-dose CHK1i (30 nM UCN-01), CDC7i, or HU (2 mM) for 4 h as indicated and stained for EdU and DNA content (sample size: 3,990, 4,405, 3,780, 4,582, and 4,074 cells). **(F)** DNA fiber assay to monitor replication fork speed. U-2 OS cells were treated with low-dose CHK1i in the presence or absence of CDC7i for 4 h and pulse-labeled with CIdU/IdU (30-min pulses each) and fiber track lengths were measured. Mean ± SD from two independent experiments, each performed in duplicate, is indicated. **(G)** Origin firing was assessed by scoring replication initiation events as percentage of the total number of DNA fibers analyzed in F. Results are depicted as means ± SD from two independent experiments, each performed in duplicate. **(H)** U-2 OS cells were treated with CDC7i and CHK1i (30 nM UCN-01) as indicated, the drugs were removed, and cells were then exposed to increasing concentrations of HU as indicated and stained for γH2AX and DNA content. γH2AX levels from cells in S-phase are additionally indicated as box plots, with white lines indicating the median (sample size: 4,918, 5,109, and 5,266 cells for 0.1 mM; 4,918, 6,938, and 5,586 cells for 0.5 mM; and 4,918, 5,333, and 7,089 cells for 2 mM). **(I)** Western blot analysis of DNA damage markers in cells treated as in H. **(J)** U-2 OS cells were treated with CDC7i and CHK1i (30 nM UCN-01) for 4 h as indicated, the drugs were removed, and cells were exposed to 2 mM HU for 2 h. pCHK1 S296 and CHK1 levels were monitored by QIBC. To compare the fold induction of pCHK1 S296 compared with total CHK1 levels, a corresponding normalization is shown on the right (sample size: 1,000 cells per condition). Median (solid lines) and quartiles (dashed lines) are indicated. **(K)** Western blot analysis of U-2 OS cells treated with CDC7i in the presence or absence of low-dose CHK1i for 4 h and treated with CPT for 2 h as indicated after washing out the inhibitors. Mann–Whitney *U* test in F, H, and J; unpaired *t* test in G. A.U., arbitrary units.