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Supplementary Materials for

Mus81-Eme1–dependent aberrant processing of DNA replication intermediates in mitosis impairs genome integrity

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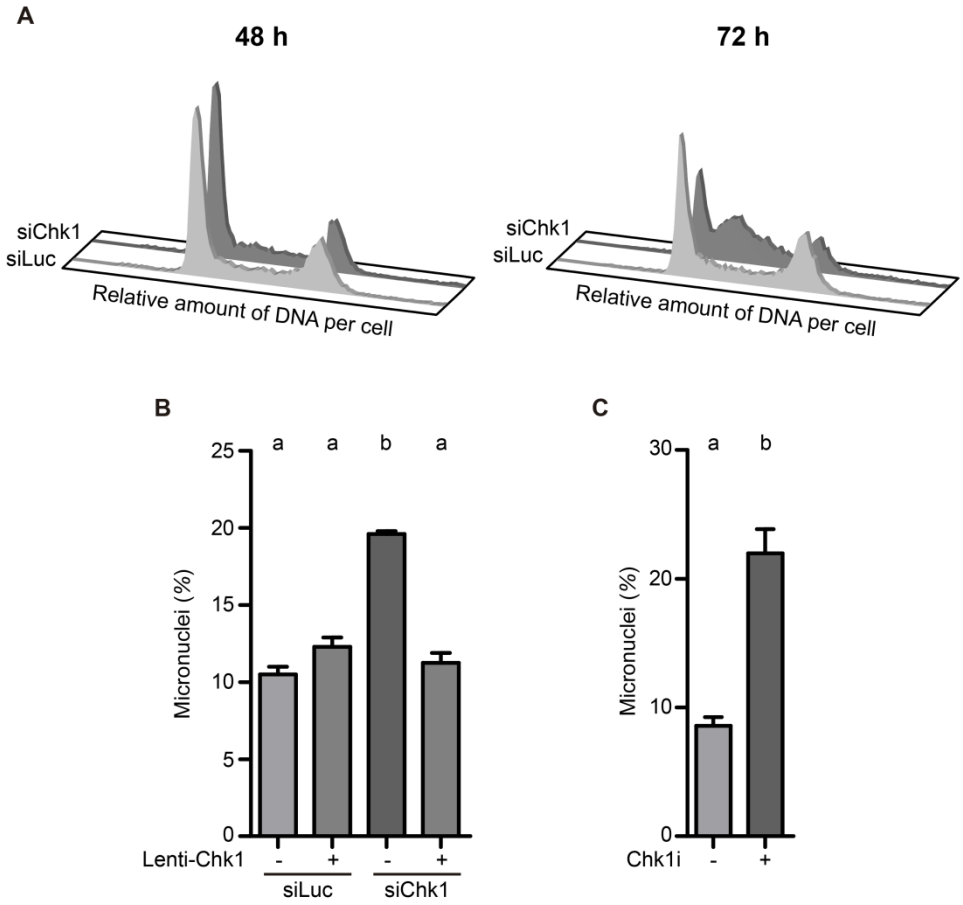
Figs. S1 to S9

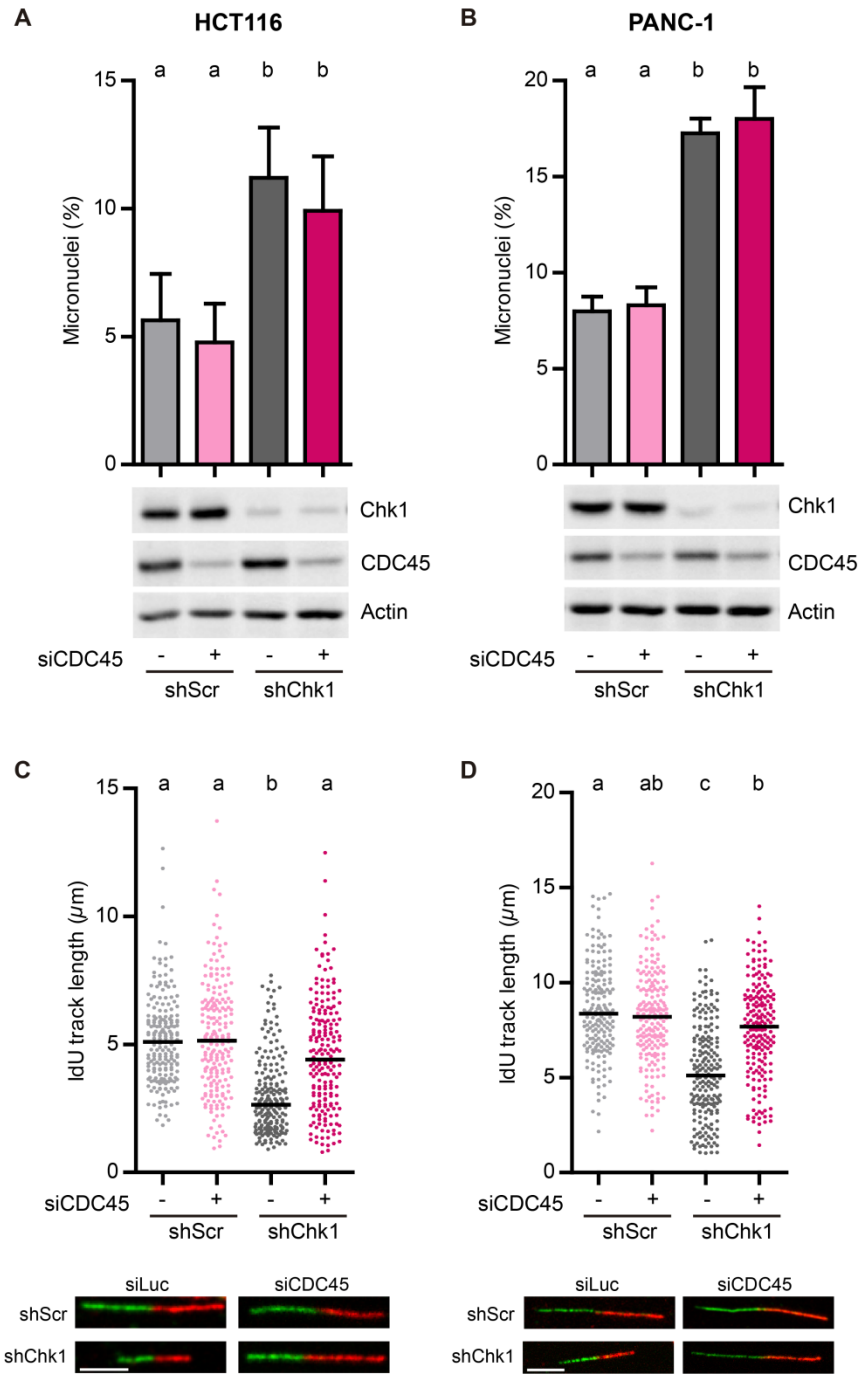
SUPPLEMENTARY FIGURES

Fig. S1



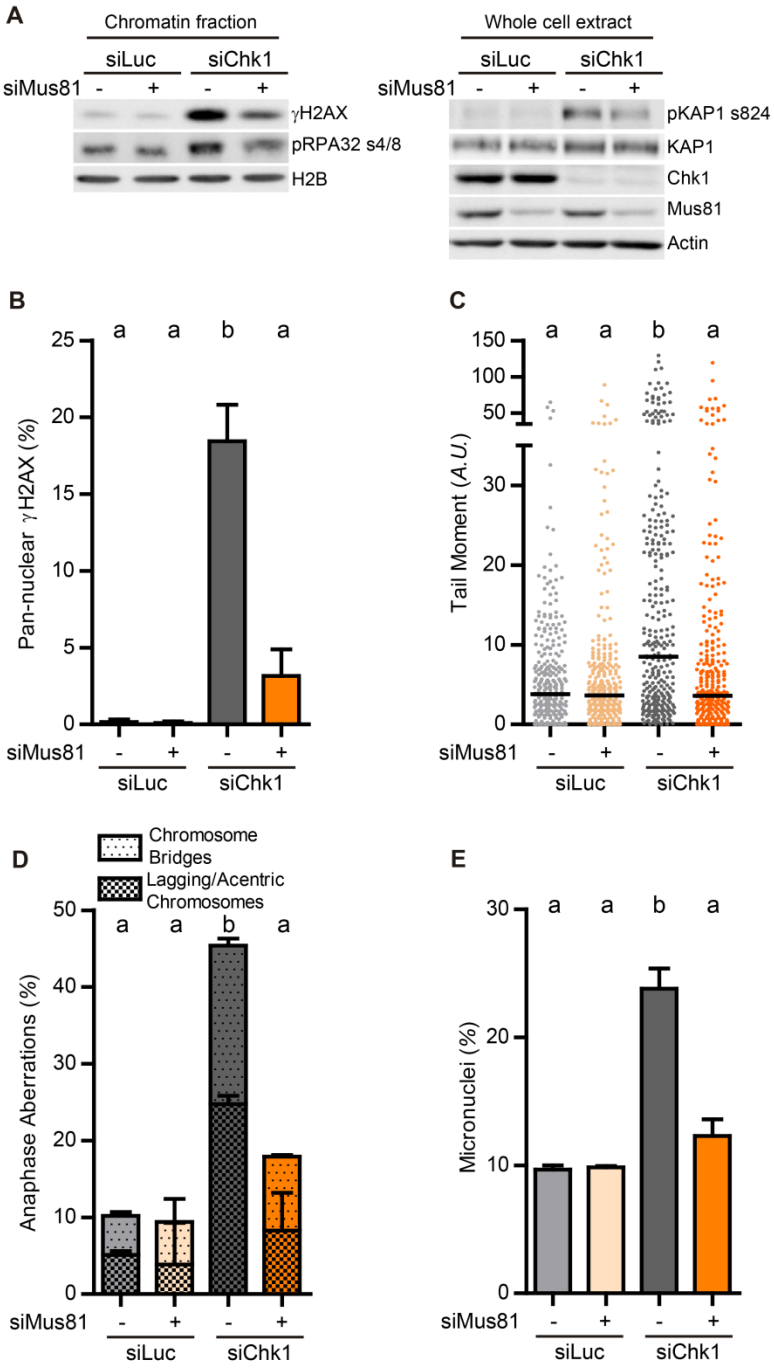
Chk1 expression and activity are required to prevent micronuclei accumulation in U2OS cells. (**A**) Cell cycle profiles of U2OS cells, 48 and 72 h after transfection with the indicated siRNAs. A representative experiment is shown (n = 2). (**B**) Percentage of binucleated cells with micronuclei (mean ± S.D.). U2OS cells were transfected with the indicated siRNAs and transduced 5 h later with a lentivirus carrying the Chk1 (Lenti-Chk1) or a control (-) sequence. ~400 binucleated cells/sample were analyzed in two independent experiments. (**C**) Percentage of binucleated U2OS cells with micronuclei (mean ± S.D.). The Chk1 inhibitor Gö6976 (Chk1i, 1 μM) was added 36 h before fixation. ~600 binucleated cells/sample were analyzed in three independent experiments.

Fig. S2



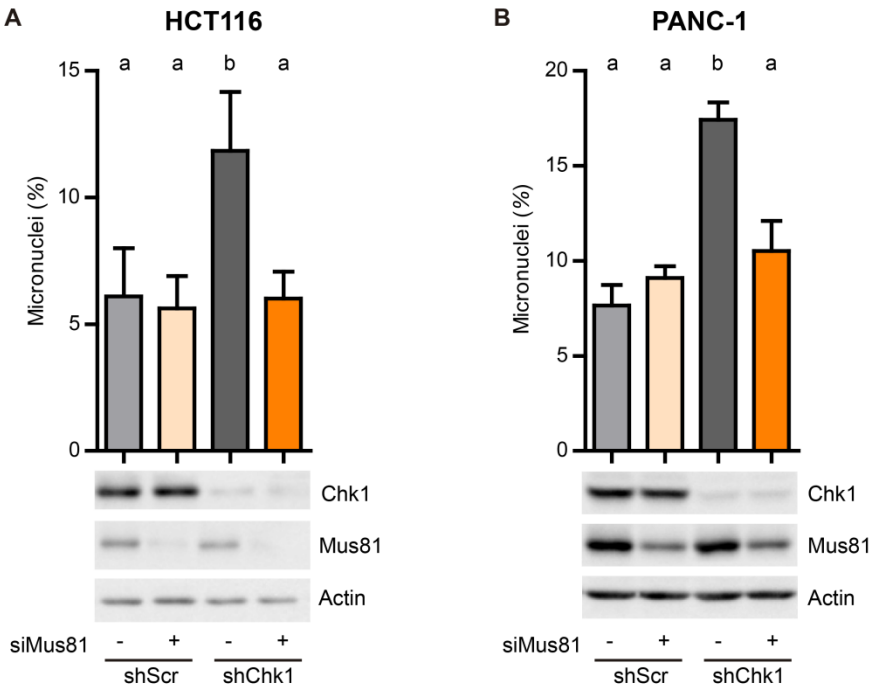
Micronuclei accumulation in Chk1-deficient HCT116 and PANC-1 cells is independent from CDC45. (**A** and **B**) Percentage of binucleated cells with micronuclei (mean ± S.D.). HCT116 (A) and PANC-1 (B) cells were transfected with the indicated siRNAs and transduced 5 h later with non-targeting shRNA (shScramble, shScr) or shRNA targeting Chk1 (shChk1). ~750 binucleated cells/sample were analyzed in three independent experiments. The lower panels show Western blots of Chk1 and CDC45 in HCT116 and PANC-1 cells, 48 h after transfection. Actin was used as a loading control. (**C** and **D**) IdU track lengths from HCT116 (C) and PANC-1 (D) cells. Cells were treated as in A-B. 200 DNA fibers obtained from two independent experiments were measured for each condition. The bars on top of the distribution clouds indicate the median. The lower panels show representative DNA fibers. Scale bar: 5 μm.

Fig. S3



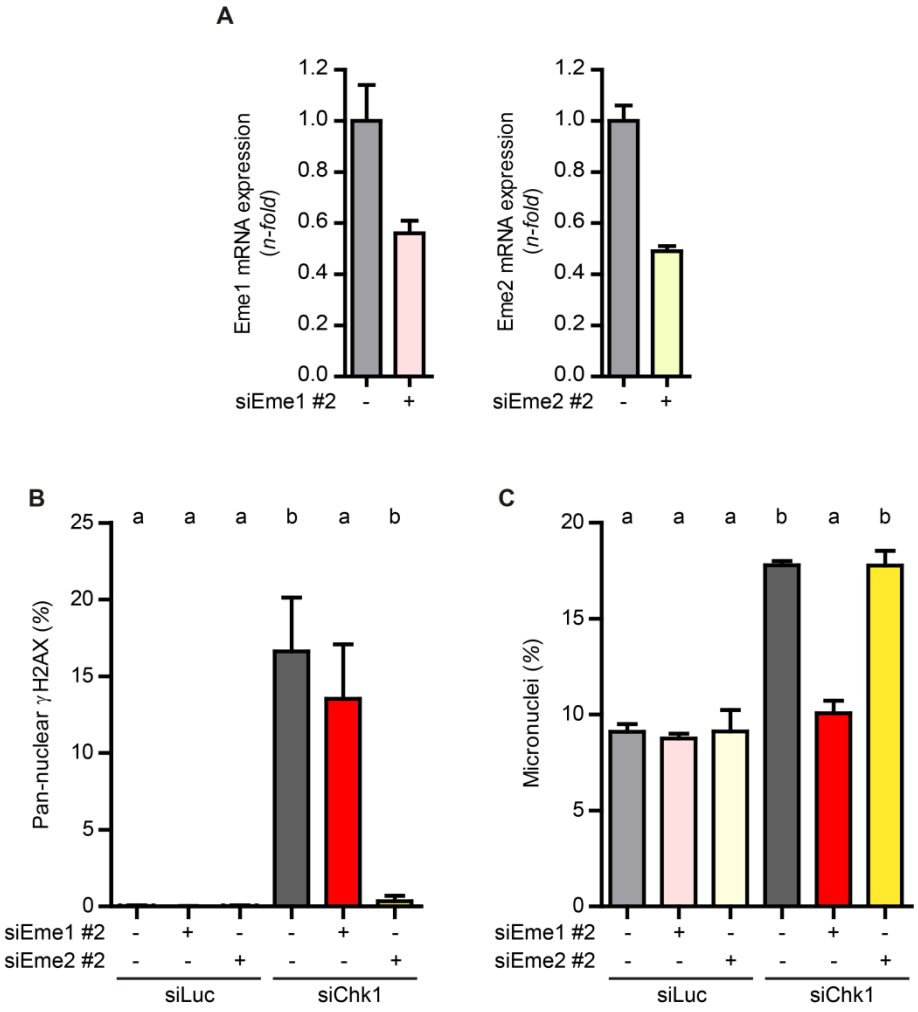
Mus81 triggers chromosome instability in Chk1-deficient cells. (**A**) Western blot of γH2AX, phospho-RPA32 ser4/8, phospho-KAP1 ser824, KAP1, Chk1 and Mus81 in U2OS cells, 48 h after transfection. The left panel shows the chromatin fraction, obtained after an extraction with CSK buffer; the right panel shows whole cell extracts. H2B and actin were used as loading controls. (**B**) Percentage of U2OS cells with pan-nuclear γH2AX staining (mean ± S.D.). >1800 cells/sample were analyzed in three independent experiments. (**C**) Quantification by neutral comet assay of DSB accumulation in U2OS cells (*A.U.*: arbitrary units). 300 cells/sample were analyzed in three independent experiments. The bars on top of the distribution clouds indicate the median. (**D**) Percentage of U2OS anaphase cells with aberrations (mean ± S.D.). ~100 anaphases/sample were analyzed in two independent experiments. The total percentage of aberrant anaphases (bridges plus lagging chromosomes) was used to calculate the statistics. (**E**) Percentage of binucleated U2OS cells with micronuclei (mean ± S.D.). ~600 binucleated cells/sample were analyzed in three independent experiments.

Fig. S4



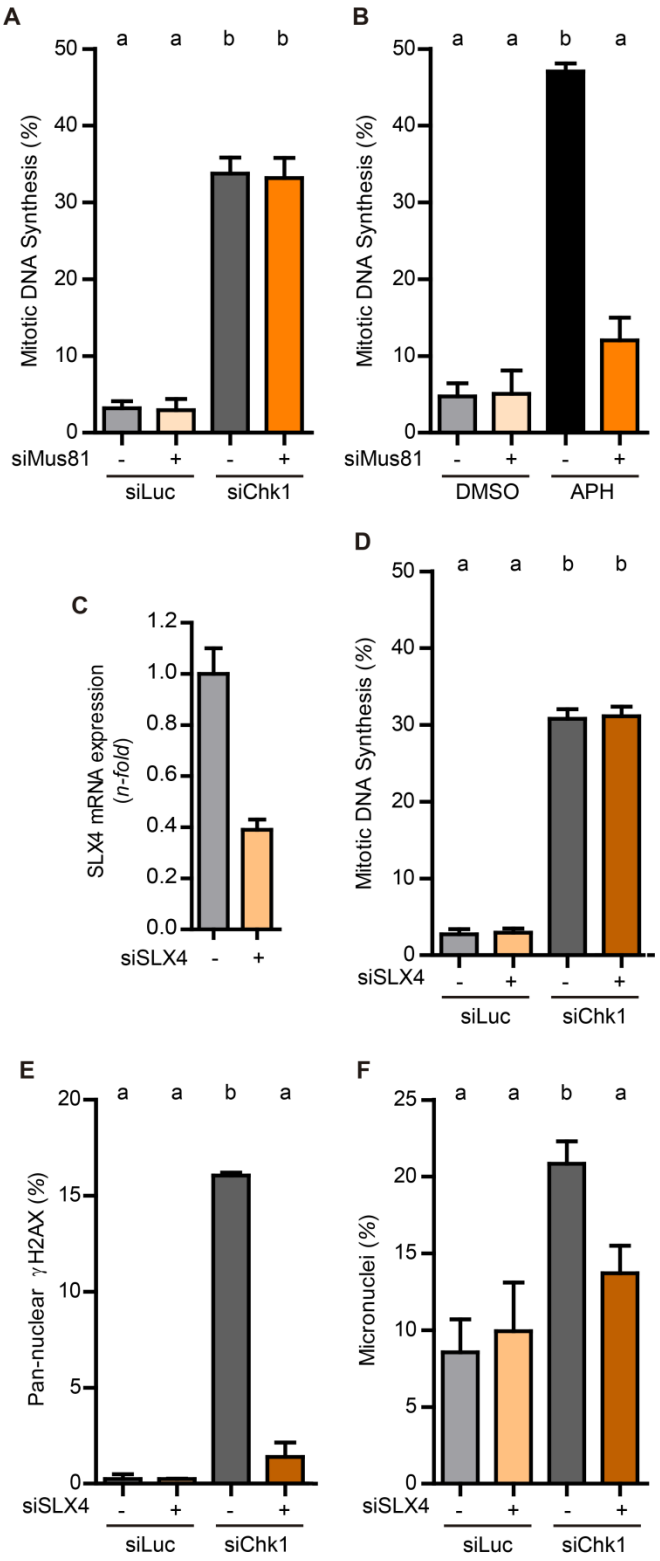
Micronuclei accumulation in Chk1-deficient HCT116 and PANC-1 cells depends on Mus81. (**A** and **B**) Percentage of binucleated cells with micronuclei (mean ± S.D.). HCT116 (A) and PANC-1 (B) cells were transfected with the indicated siRNAs and transduced 5 h later with the indicated shRNAs. ~750 binucleated cells/sample were analyzed in three independent experiments. The lower panels show Western blots of Chk1 and Mus81 in HCT116 and PANC-1 cells, 48 h after transfection. Actin was used as a loading control.

Fig. S5



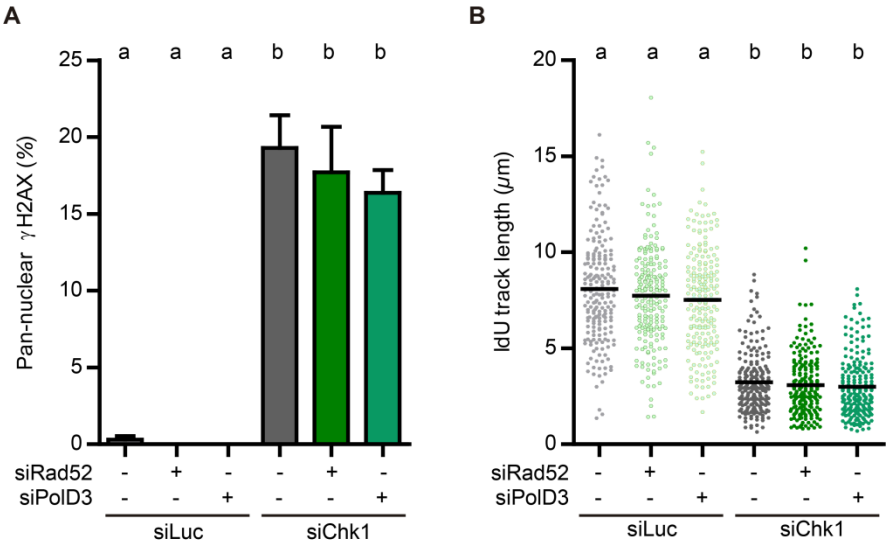
Eme1 depletion prevents micronuclei accumulation in Chk1-deficient cells, without avoiding replication catastrophe. (**A**) Quantitative real-time PCR of Eme1 and Eme2 normalized to GAPDH in U2OS cells; error bars represent the S.D. of 2 technical replicates. The siRNA sequences employed here are different from those in the main figures. (**B**) Percentage of U2OS cells with pan-nuclear γH2AX staining (mean ± S.D.). >1200 cells/sample were analyzed in two independent experiments. (**C**) Percentage of binucleated U2OS cells with micronuclei (mean ± S.D.). ~600 binucleated cells/sample were analyzed in three independent experiments.

Fig. S6



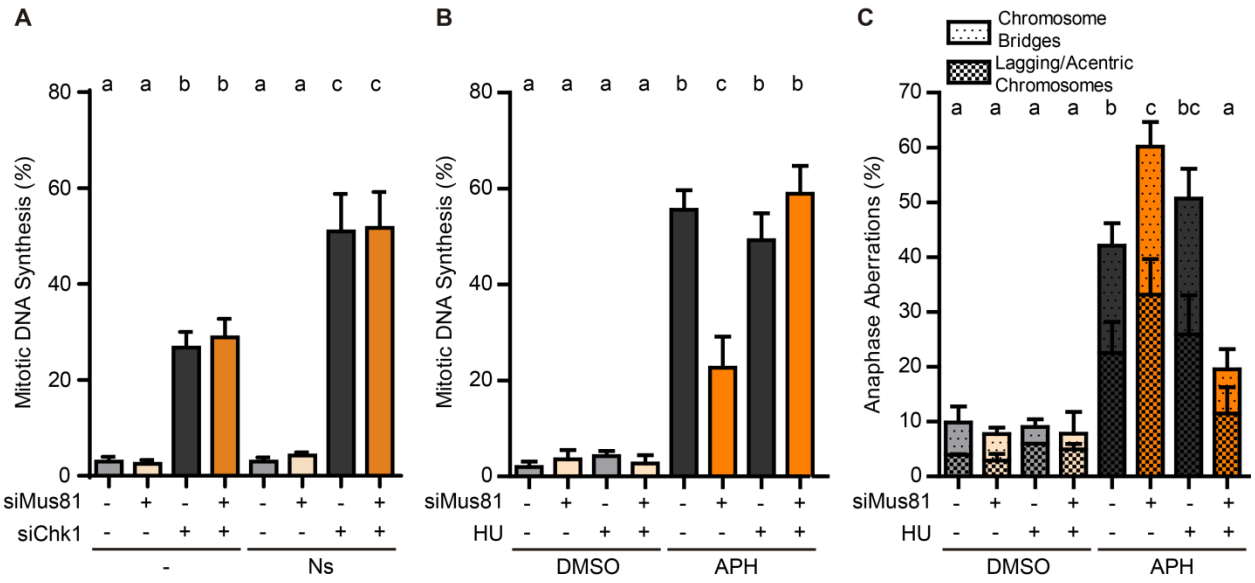
Mitotic DNA synthesis in Chk1-deficient cells is independent from Mus81 and SLX4. (**A**) Percentage of mitotic U2OS cells with EdU spots (mean ± S.D.). ~150 metaphases/sample were analyzed in three independent experiments. (**B**) Percentage of mitotic U2OS cells with EdU spots (mean ± S.D.). Aphidicolin (APH, 0.2 μM) was added 24 h before fixation. ~100 metaphases/sample were analyzed in two independent experiments. (**C**) Quantitative real-time PCR of SLX4 normalized to GAPDH in U2OS cells; error bars represent the S.D. of 2 technical replicates. (**D**) Percentage of mitotic U2OS cells with EdU spots (mean ± S.D.). ~150 metaphases/sample were analyzed in three independent experiments. (**E**) Percentage of U2OS cells with pan-nuclear γH2AX staining (mean ± S.D.). >1000 cells/sample were analyzed in two independent experiments. (**F**) Percentage of binucleated U2OS cells with micronuclei (mean ± S.D.). ~400 binucleated cells/sample were analyzed in two independent experiments.

Fig. S7



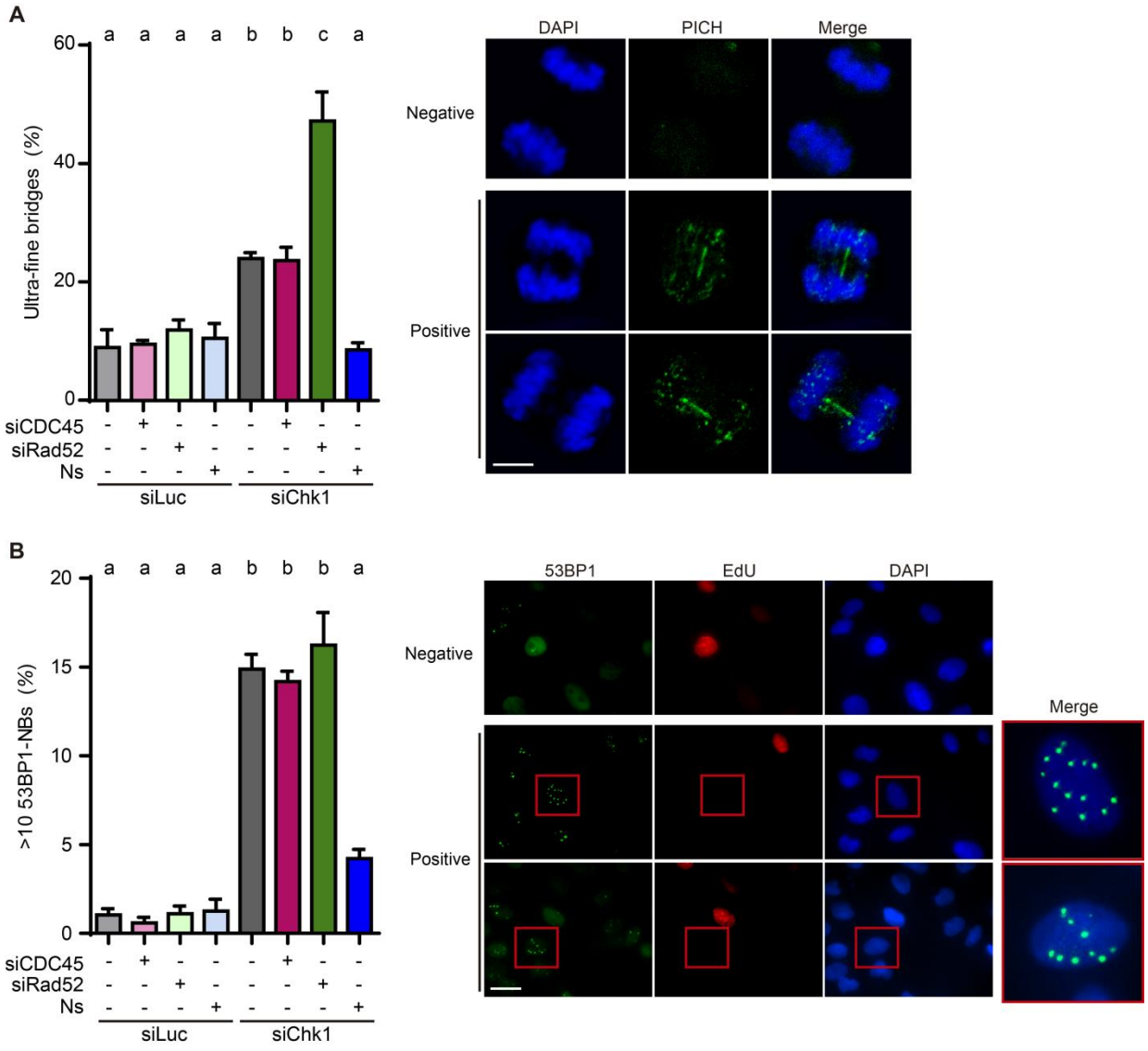
Neither PolD3 nor Rad52 triggers replication stress in Chk1-deficient cells. (**A**) Percentage of U2OS cells with pan-nuclear γH2AX staining (mean ± S.D.). >1500 cells/sample were analyzed in three independent experiments. (**B**) IdU track lengths from U2OS cells. 200 DNA fibers obtained from two independent experiments were measured for each condition. The bars on top of the distribution clouds indicate the median.

Fig. S8



Nucleotide deficiency during APH-induced mitotic DNA synthesis triggers Mus81-dependent chromosome segregation defects. (**A**) Percentage of mitotic U2OS cells with EdU spots (mean ± S.D.). Nucleosides (Ns) were added 24 h before fixation. ~100 metaphases/sample were analyzed in two independent experiments. (**B**) Percentage of mitotic U2OS cells with EdU spots (mean ± S.D.). Aphidicolin (APH, 0.2 μM) and Hydroxyurea (HU, 100 μM) were added 24 h before fixation. ~150 metaphases/sample were analyzed in three independent experiments. (**C**) Percentage of U2OS anaphase cells with aberrations (mean ± S.D.). Cells were treated as in B. ~100 anaphases/sample were analyzed in two independent experiments. The total percentage of aberrant anaphases (bridges plus lagging chromosomes) was used to calculate the statistics.

Fig. S9



Nucleotide deficiency during mitotic DNA synthesis leads to DNA under-replication in Chk1-deficient cells. (**A**) Percentage (mean ± S.D.) and representative images of U2OS anaphase cells with PICH-positive UFBs. Ns were added 24 h before fixation. >100 anaphases/sample were analyzed in two independent experiments. Scale bar: 5 μm. (**B**) Percentage (mean + S.D.) and representative images of EdU-negative U2OS cells with >10 53BP1 nuclear bodies (53BP1-NBs). Cells were treated as in A. ~450 nuclei/sample were analyzed in three independent experiments. Scale bar: 10 μm.