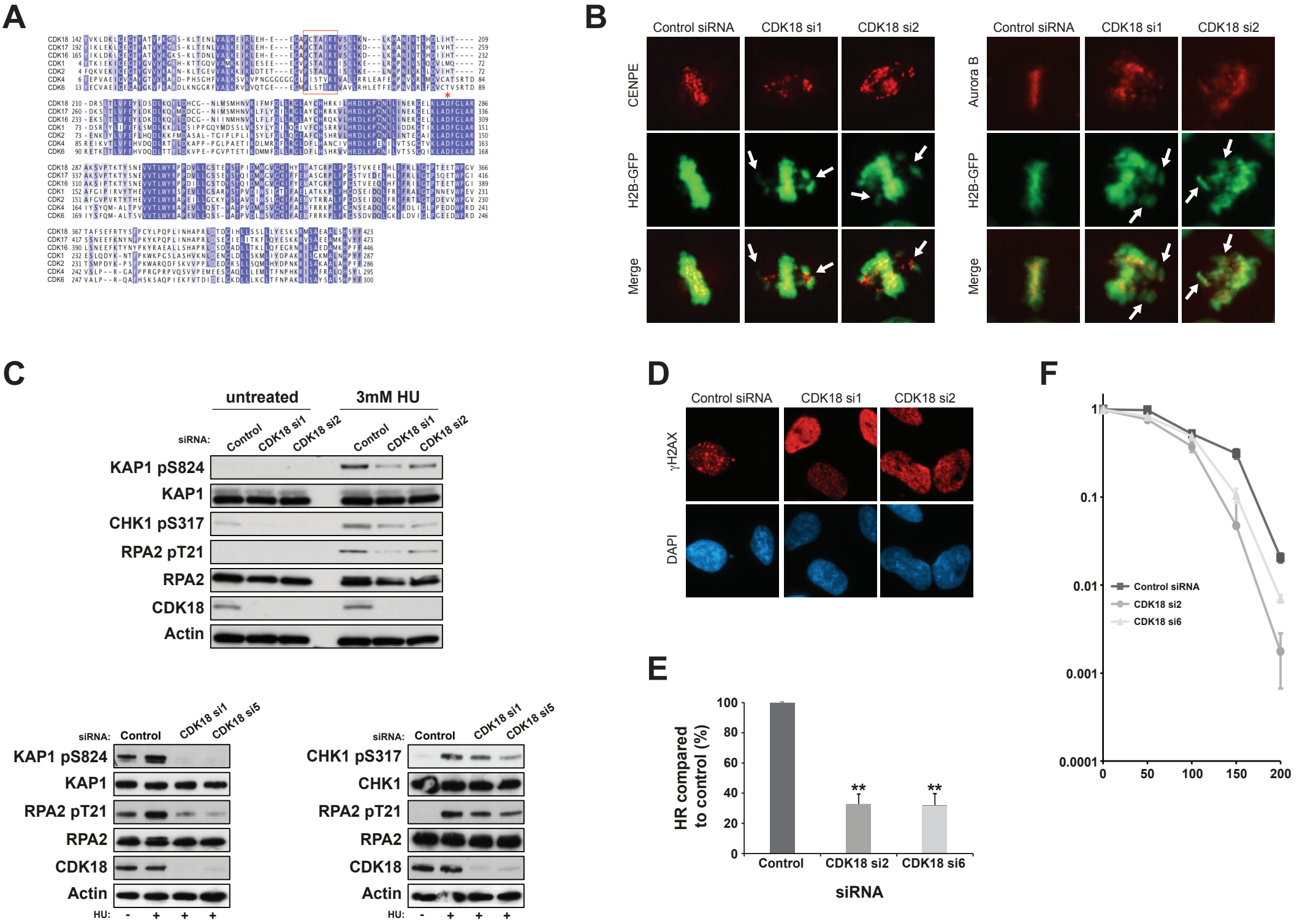
Supplementary Figure S1.

A: Schematic showing alignment of the human PCTAIRE family of CDK16, CDK17 and CDK18 alongside human CDK1, CDK2, CDK4 and CDK6 paralogues. Purple boxes highlight regions of homology, with shading increasing with homology. The red box represents the putative cyclin-binding motif and the asterisk identifies the conserved aspartic acid (D281) in the DFG magnesium-coordinating site, which was mutated to an alanine to create a kinase-inactive (D281A) CDK18 mutant for exogenous rescue experiments (see Figure 4). **B:** Representative images of chromosomal alignment defects (white arrows) in metaphase H2B-GFP HeLa cells transfected with either control or CDK18 siRNA for the quantified data shown in Figure 1E. CENPE (left panel) or Aurora B staining (right panel) was used to visualise centromeres, which align along the metaphase plate in control siRNA transfections. **C:** Upper panel; western blots showing phosphorylation status of KAP1 at Ser 824, CHK1 at Ser 317 and RPA2 at Thr 21 in HeLa cells transfected as described in Figure 3B, but using two additional CDK18 siRNA to those described in Figure 3B. Lower left and lower right panels show comparable data in RPE1 and U2OS cells respectively. **D:** Representative images of γH2AX pan-nuclear staining in control or CDK18 siRNA transfected HeLa cells treated with 3 mM HU for 6hrs for the data quantified in Figure 3F. **E:** Quantification of homologous recombination in control and CDK18 siRNA transfected HEK293 cells as assessed by reconstitution of an ISce-1-induced double-strand break in an EGFP-based transfected reporter plasmid. Data shown is the mean percentage recombination compared with control siRNA-transfected cells from at least two independent experiments with associated SEMs **p≤0.01 compared to control siRNA cells). **F:** HU clonogenic survival curves for HeLa cells treated with either control or CDK18 siRNA. Data shown represents the mean from at least two independent experiments with associated SEMs.

Supplementary Figure S2.

A: Kinase assays using purified WT or D281A kinase-inactive FLAG-CDK18 as indicated from tetracycline-inducible stable HeLa cell lines. Phosphorylated CDK18 (^{32}P-incorporation by autophosphorylation) was detected by autoradiography (top panels) and the total amount of CDK18 within each immunoprecipitate was compared by immunoblotting with anti-CDK18 antibodies (bottom panels) to

demonstrate equal loading. Duplicate lanes for each condition represent experimental replicates. **B:** Quantification of the phosphorylation of the indicated ATR substrates in both WT and KD expressing FLAG-CDK18 cells shown in Figure 4A. Each phospho signal was normalised to its respective total protein band and is represented as a fold change between -tet and +tet cells. **C:** Left panel; representative images of immunofluorescence detection of 53BP1 foci in stable HeLa cell lines as described in Figure 4B using anti-FLAG antibodies to confirm induced expression of WT FLAG-CDK18. Right panel; representative images of immunofluorescence detection of 53BP1 foci in cells as described in Figure 4C using anti-FLAG antibodies to confirm induced expression of D281 FLAG-CDK18. **D:** Quantification of pCDK signal in WT and S336A MYC-RAD9 IPs from HeLa cell transfected with either control or CDK18 siRNA shown in Figure 6G. Each pCDK signal was normalised to that present in control siRNA transfected cells expressing WT MYC-RAD9. **E:** Quantification of the chromatin fraction of the indicated protein in CDK18-depleted cells compared with control siRNA treated cells from the data presented in Figure 6A. All bands were normalised to ORC2. **F:** Quantification of the chromatin fraction of the indicated protein in untreated (top graph) and HU-treated (bottom graph) CDK18-depleted cells compared with control siRNA treated cells from the data presented in Figure 6B. All bands were normalised to ORC2. **G:** Quantification of the chromatin fraction of the indicated protein in parental, WT and KD expressing FLAG-CDK18 cells shown in Figure 6D. Each signal was normalised to its respective ORC2 loading control and is represented as a fold change compared to control siRNA treated parental cells (lane 1). **H:** Western blots evaluating levels of ATR, FANCD2 and Cyclin K in HeLa cells transfected with either non-targeting control siRNA, or individual siRNA targeting CDK18. Actin levels are used as a loading control and CDK18 blots conform efficient knockdown of endogenous CDK18 following transfection of targeted siRNA.



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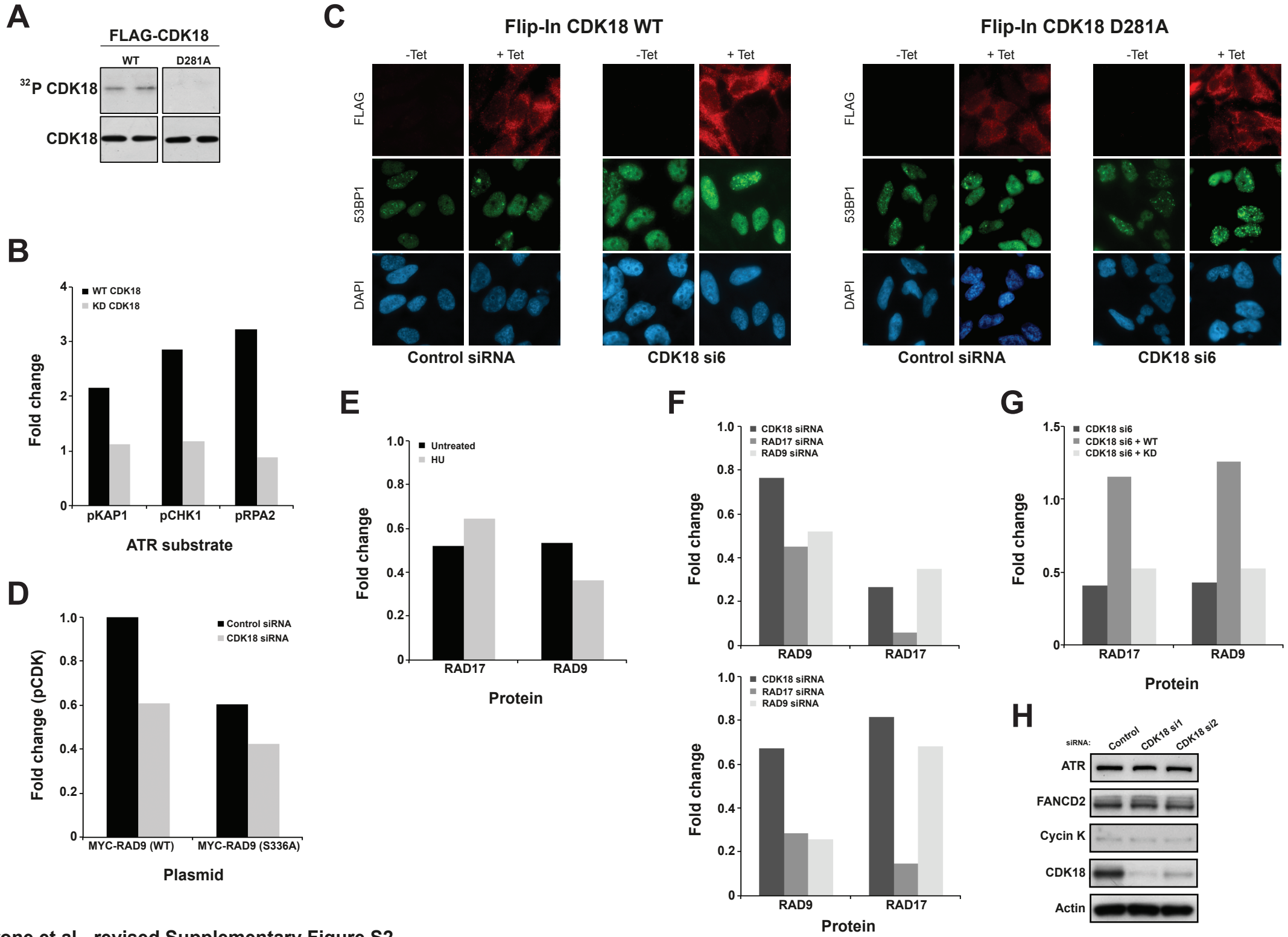

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