Expanded View Figures

Figure EV1. Validation of the top-hit candidates.

A FANCD2 foci in HeLa cells depleted of the selected candidates. The mean of three independent experiments with at least 100 cells each was considered. *$P < 0.05$ (Mann–Whitney *U*-test). Red stars denote significant decreases. Representative images are shown on top.

B Relative mRNA levels of the indicated candidates as measured by RT–qPCR after siRNA depletion. Error bars represent relative target quantity (RQ) minimum and maximum from three technicals replicates.

C Relative S9.6 signal intensity per nucleus after nucleolus signal removal in HeLa cells transfected with the indicated siRNAs. More than 130 total cells from three independent experiments were considered. The median of each population is shown. Boxes and whiskers indicate 25–75 and 10–90 percentiles, respectively. ***$P < 0.001$ (Mann–Whitney *U*-test).

D Relative S9.6 signal intensity per nucleus after nucleolus signal removal in HeLa cells transfected with the indicated siRNAs and treated with caffeine (10 mM, 2 or 4 h) or the ATR inhibitor ETP-46464 (5 mM, 2 h). More than 600 total cells from five independent experiments were considered. The median of each population is shown. Boxes and whiskers indicate 25–75 and 10–90 percentiles, respectively. ***$P < 0.001$ (Mann–Whitney *U*-test).

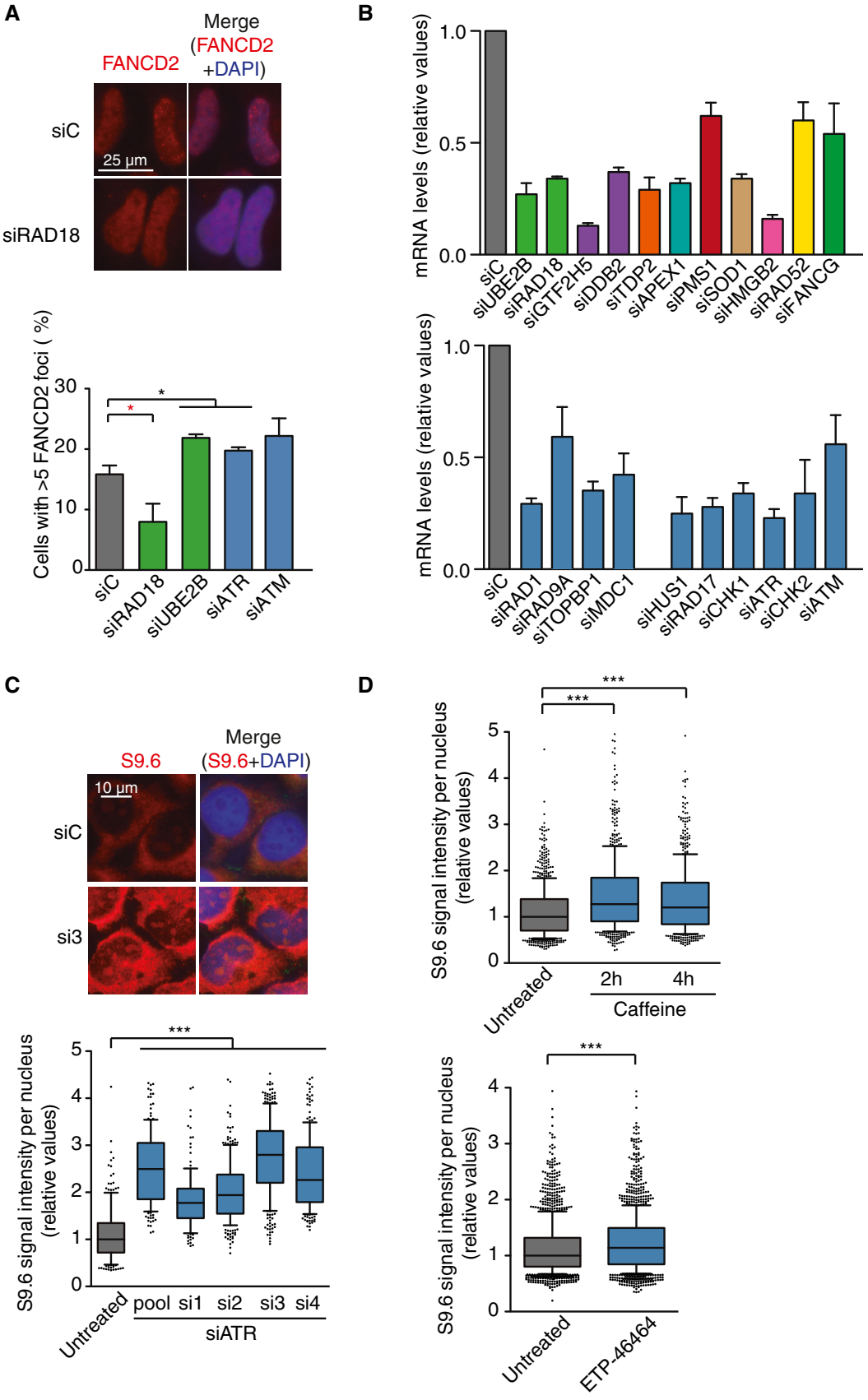
A
FANCD2
Merge (FANCD2 +DAPI)
siC
siRAD18
25 µm
Cells with >5 FANCD2 foci (%)
siC
siRAD18
siUBE2B
siATR
siATM
B
mRNA levels (relative values)
siC
siUBE2B
siRAD18
siGTF2H5
siDDB2
siTDP2
siAPEX1
siPMS1
siSOD1
siHMGB2
siRAD52
siFANCG
siC
siRAD1
siRAD9A
siTOPBP1
siMDC1
siHUS1
siRAD17
siCHK1
siATR
siCHK2
siATM
C
S9.6
Merge (S9.6+DAPI)
10 µm
siC
si3
S9.6 signal intensity per nucleus (relative values)
Untreated
pool
si1
si2
si3
si4
siATR
D
S9.6 signal intensity per nucleus (relative values)
Untreated
2h
4h
Caffeine
Untreated
ETP-46464

Figure EV1.

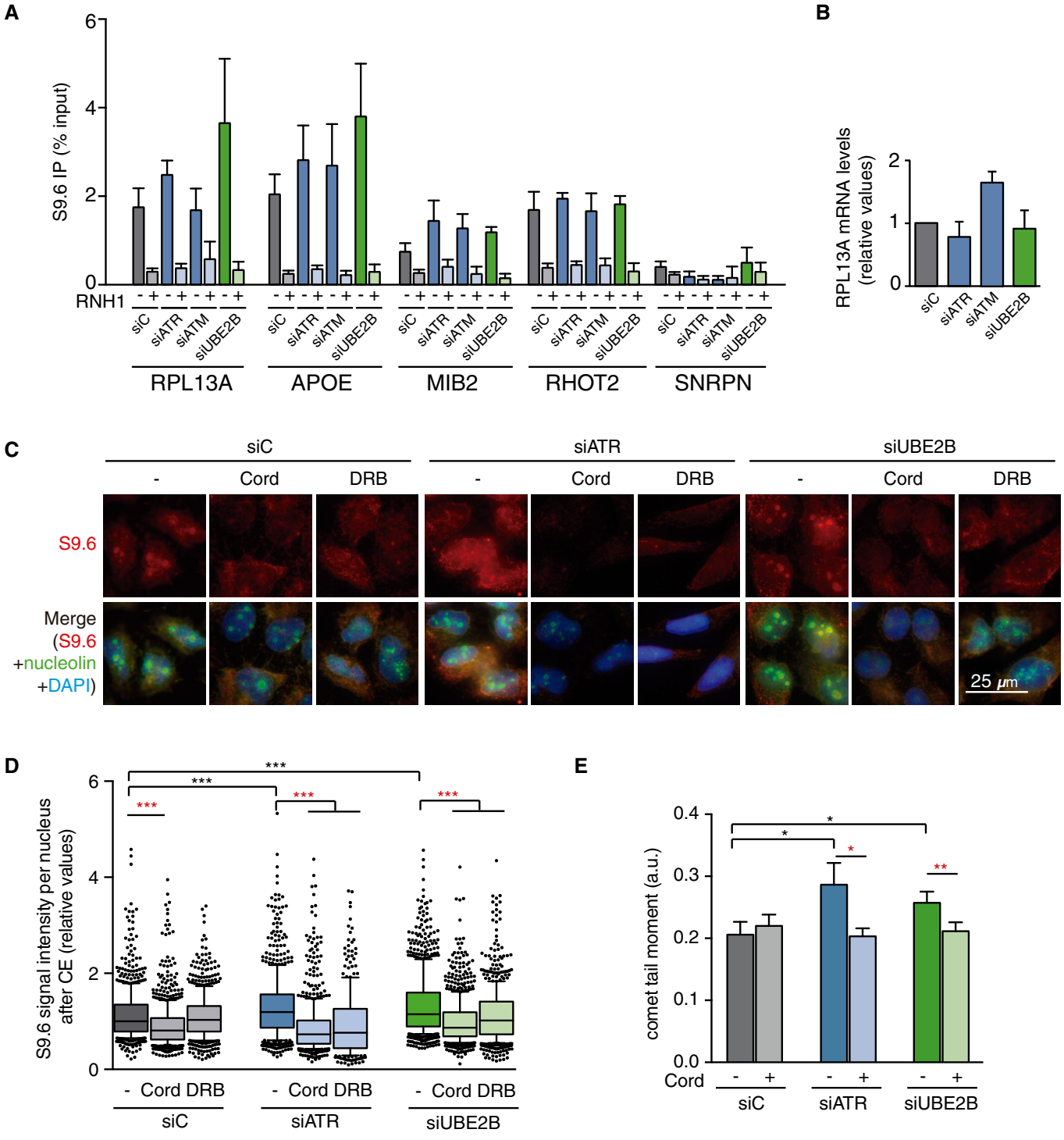

Figure EV2.

Figure EV2. Transcription dependency of the DNA–RNA hybrid accumulation and DNA breaks after DDR depletion.

A DRIP–qPCR signal values at *RPL13A*, *APOE*, *MIB2*, *RHOT2*, and *SNRPN* genes in HeLa cells transfected with the indicated siRNAs and treated *in vitro* with RNase H pre-immunoprecipitation where indicated. The mean ± SEM from at least three independent experiments is shown.

B Relative mRNA levels from the *RPL13A* gene in HeLa cells after transfection with the indicated siRNAs. The mean ± SEM from at least two independent experiments is shown.

C Representative images of HeLa cells immunostained with S9.6 and nucleolin antibodies after transfection with the indicated siRNAs and after cytoplasm pre-extraction (CE).

D Relative S9.6 signal intensity per nucleus in HeLa cells transfected with the indicated siRNAs and treated with the transcription inhibitors 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) or cordycepin (Cord). The median of the S9.6 signal intensity per nucleus relative to siC. Boxes and whiskers indicate 25–75 and 10–90 percentiles, respectively. More than 300 total cells from four independent experiments were considered. Values were normalized to the median of siC. ***$P < 0.001$ (Mann–Whitney *U*-test). Black stars denote significant increases, whereas red stars denote significant decreases.

E Tail moment from single-cell alkaline gel electrophoresis (comet assay) of HeLa cells transfected with the indicated siRNAs and treated with the transcription inhibitor cordycepin (Cord). More than 250 total cells were considered. The mean ± SEM of the median from five independent experiments is shown. *$P < 0.05$, **$P < 0.01$ (one-tailed unpaired *t*-test). Black stars denote significant increases, whereas red stars denote significant decreases.

Figure EV3. Representative cell cycle profiles of HeLa cells depleted of the selected DDC and PRR factors.

A Flow cytometry profiles showing EdU incorporation versus DNA content in the indicated HeLa cells. The percentage of cells in S (upper box), G0/1 (lower left box), and G2/M (lower right box) are indicated.

B Top row: flow cytometry histograms displaying the DNA content of the indicated HeLa cells after cytoplasm pre-extraction (CE). G1 (red), S (blue), and G2 (green) phases are calculated from the profile. Bottom row: flow cytometry histograms depicting intensity of S9.6 signal in each phase of the cell cycle. Quantification is shown in panel below representing the mean ± SD of four experiments. *$P < 0.05$, **$P < 0.01$ (ANOVA test with Bonferroni's post-test).

C Top left panel: flow cytometry histogram showing the DNA content of cells before (left) and after (right) sorting by DAPI signal to obtain samples enriched in G1 and S/G2 populations. A representative experiment of control cells is shown. Top right panel: representative images of S9.6 staining of G1 and S/G2 sorted fractions in control and UBE2B-depleted cells after cytoplasm pre-extraction (CE). Bottom left panel: relative S9.6 intensity in G1 and S/G2-sorted cells. Median values are indicated. Boxes and whiskers indicate 25–75 and 10–90 percentiles, respectively. At least three experiments, each with more than 150 cells per condition, were considered. Bottom right panel: fold change of S9.6 signal median values in S/G2 with respect to G1 sorted cells. **$P < 0.01$, ***$P < 0.001$ (Mann–Whitney *U*-test).

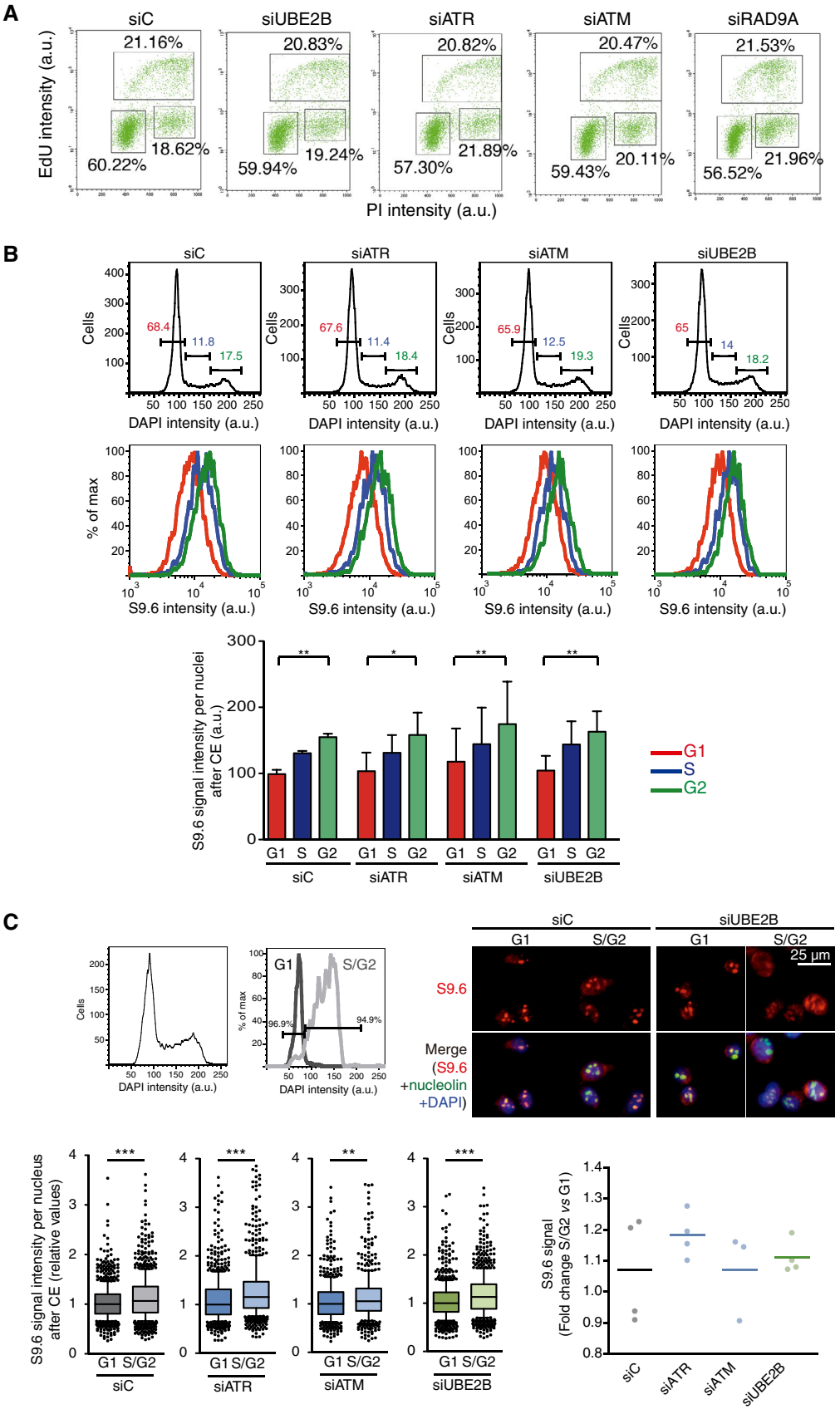

Figure EV3.

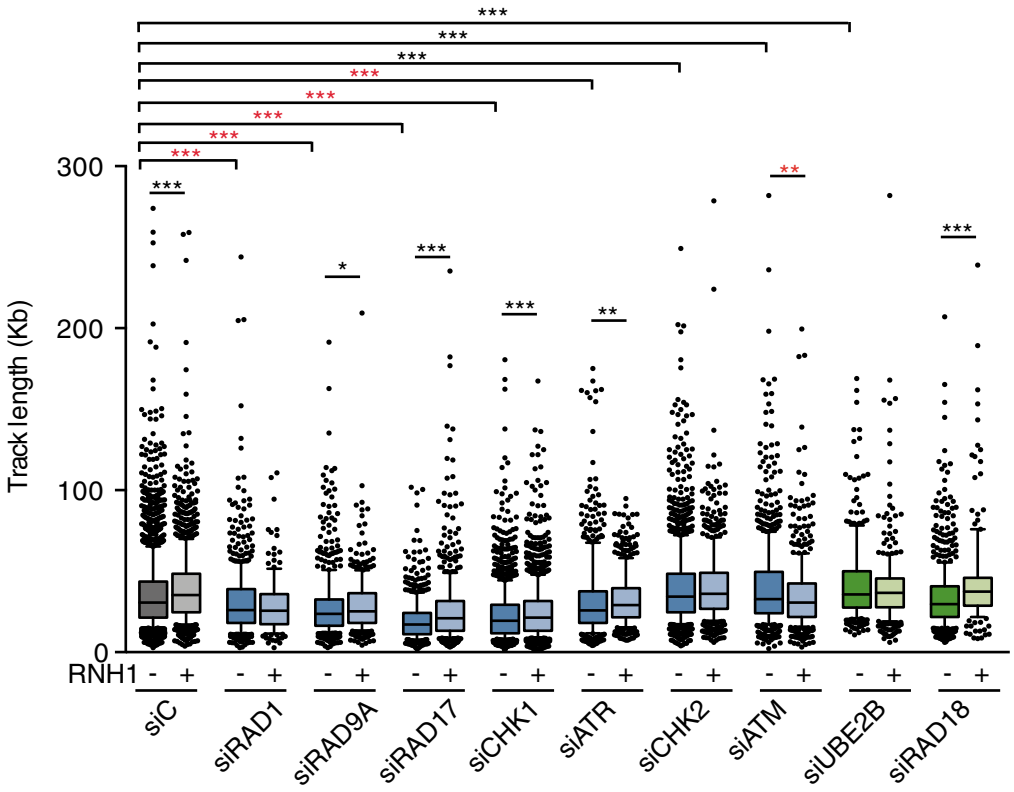

Figure EV4. Replication fork progression in HeLa cells depleted of DDC candidates.
Track length as measured by DNA combing assay in HeLa cells transfected with the indicated siRNAs and either pEGFP-C1 (RNH1^{-}) or pEGFP-M27 (RNH1^{+}). More than 300 tracks were considered except for siRAD1 + RNH1 (156) and RAD18 + RNH1 (179). Median values are indicated. Boxes and whiskers indicate 25–75 and 10–90 percentiles, respectively. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (Mann–Whitney *U*-test). Black stars denote significant increases, whereas red stars denote significant decreases.