Neelsen et al., http://www.jcb.org/cgi/content/full/jcb.201212058/DC1

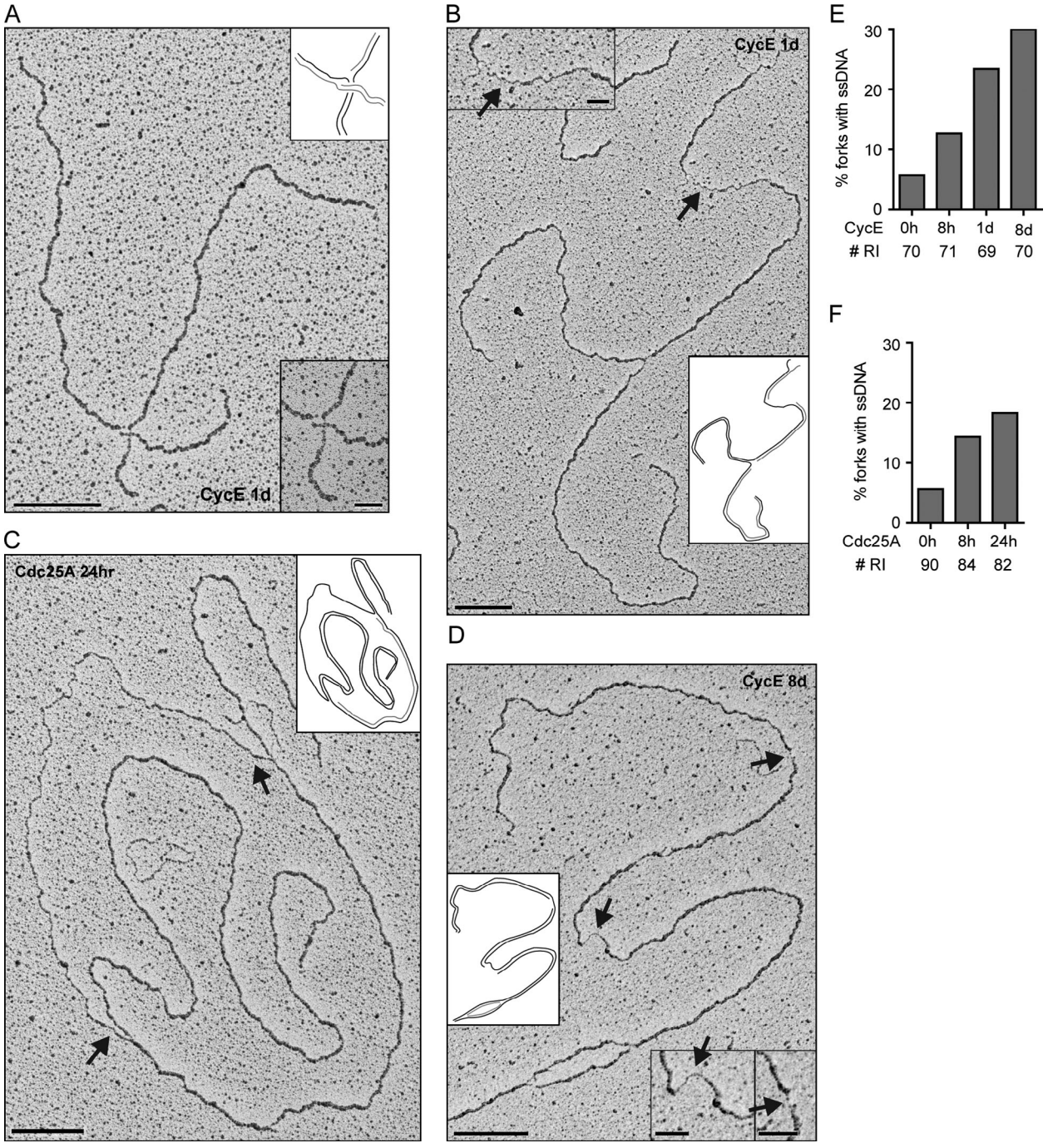

Figure S1. **CycE and Cdc25A promote replication fork reversal and accumulation of single-stranded DNA.** (A) Electron micrograph showing a reversed fork from cells overexpressing *CycE*. The regressed arm is connected to one of the daughter strands. (B) Replication fork from *CycE*-overexpressing cells displaying a gap on one of the daughter strands. (C) A hemireplicated bubble isolated from cells overexpressing *Cdc25A* for 24 h. (D) A replication bubble with gaps on the parental DNA strand from cells overexpressing *CycE* for 8 d. Insets in A–D show the magnified forks or regions containing single-stranded DNA (ssDNA), and schemes of the fork structure. Black and gray lines describe parental and newly synthesized DNA strands, respectively; black arrows indicate regions of ssDNA. (E and F) Frequency of replication intermediates with exposed ssDNA at the indicated time points after induction of *CycE* and *Cdc25A*, respectively. The majority of exposed ssDNA was found as gaps on newly replicated duplexes as shown in B. The threshold for ssDNA at the fork was 500 nucleotides. "# RI" is the number of analyzed replication intermediates. Data in E and F were reproduced in at least one independent experiment. Bar: (main panels) 200 nm (500 bp); (insets) 50 nm. For overexpression levels, see Fig. 1.

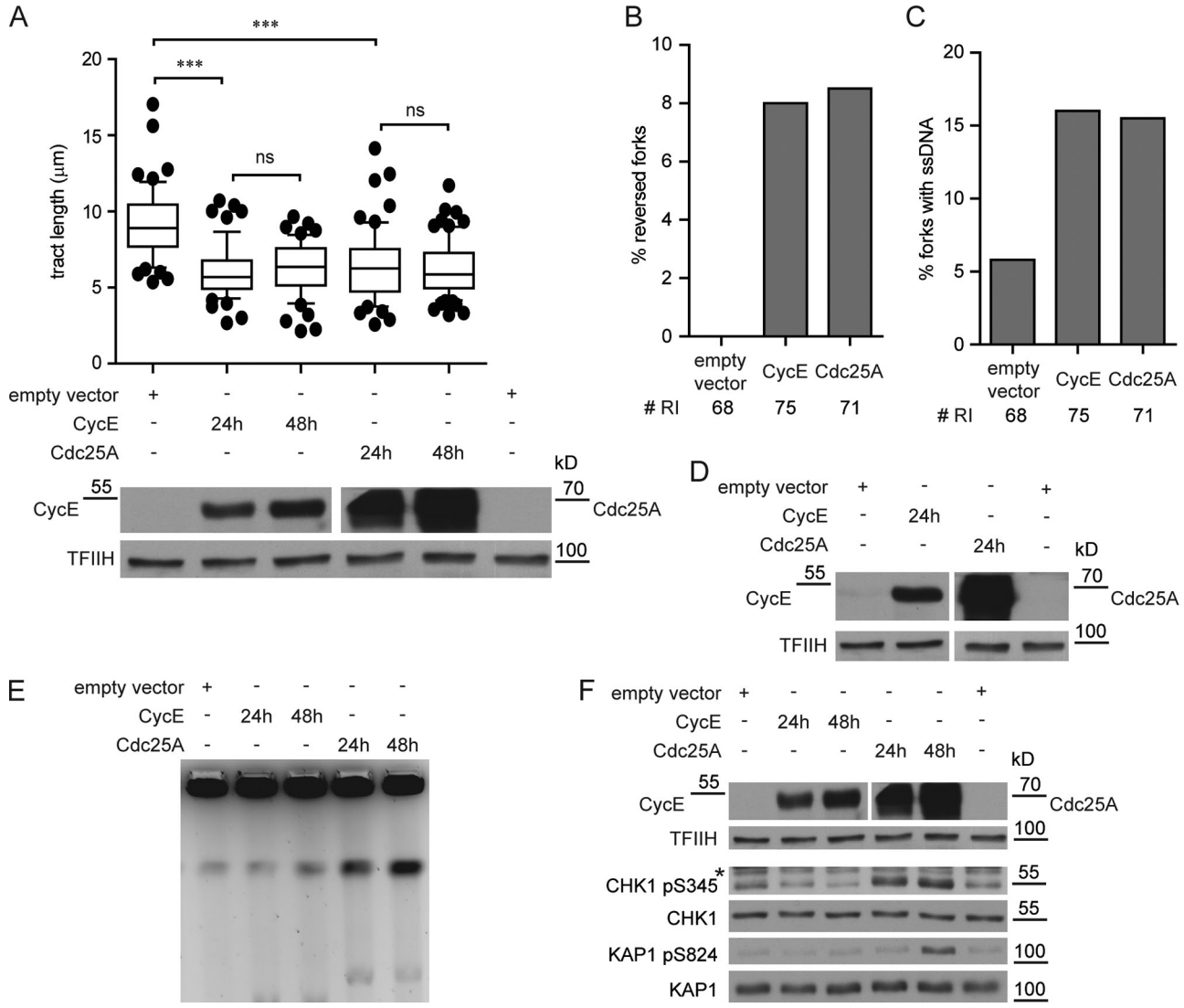

Figure S2. **Overexpression of *CycE* and *Cdc25A* induces replication stress in MRC5 cells.** (A) Replication fork progression in MRC5 cells transfected with empty vector, or plasmid encoding *CycE* and *Cdc25A*, respectively, at the indicated time points. The bottom panels show overexpression of the oncogenes. The samples shown were run on a single gel and transferred to a membrane that was cut and the relevant portions probed individually for CycE, Cdc25A, and TFIIH (the loading control). The complete Western blotting data in A are shown again in F, and the empty vector lanes in A also are shown again in D. (B) Frequency of reversed replication forks in MRC5 cells 24 h after transfection with empty vector or *CycE*- and *Cdc25A*-encoding plasmids. (C) Frequency of replication intermediates with exposed ssDNA in samples from B. (D) Immunoblot showing overexpression of *CycE* and *Cdc25A* in the samples in B and C. (E) Oncogene-induced DNA breakage upon expression of the indicated oncogenes monitored by pulse-field gel electrophoresis. (F) Activation of the DNA damage response (pCHK1, pKAP1) and total damage response proteins (CHK1, KAP1) upon expression of *CycE* and *Cdc25A*, respectively, in the same samples as were analyzed in E. Total CHK1 and KAP1 levels control for loading of the relevant phosphorylated proteins on the same gel in F. The data in A, D, E, and F are derived from one independent experiment. The experiment in B and C was completed once. For A, at least 100 tracts were scored per sample. Whiskers indicate 10–90th percentile (***, $P < 0.0001$; ns, not significant, Mann-Whitney test). Molecular weight in kD of nearest protein size marker is indicated for all Western blots. Asterisk indicates an unspecific band.

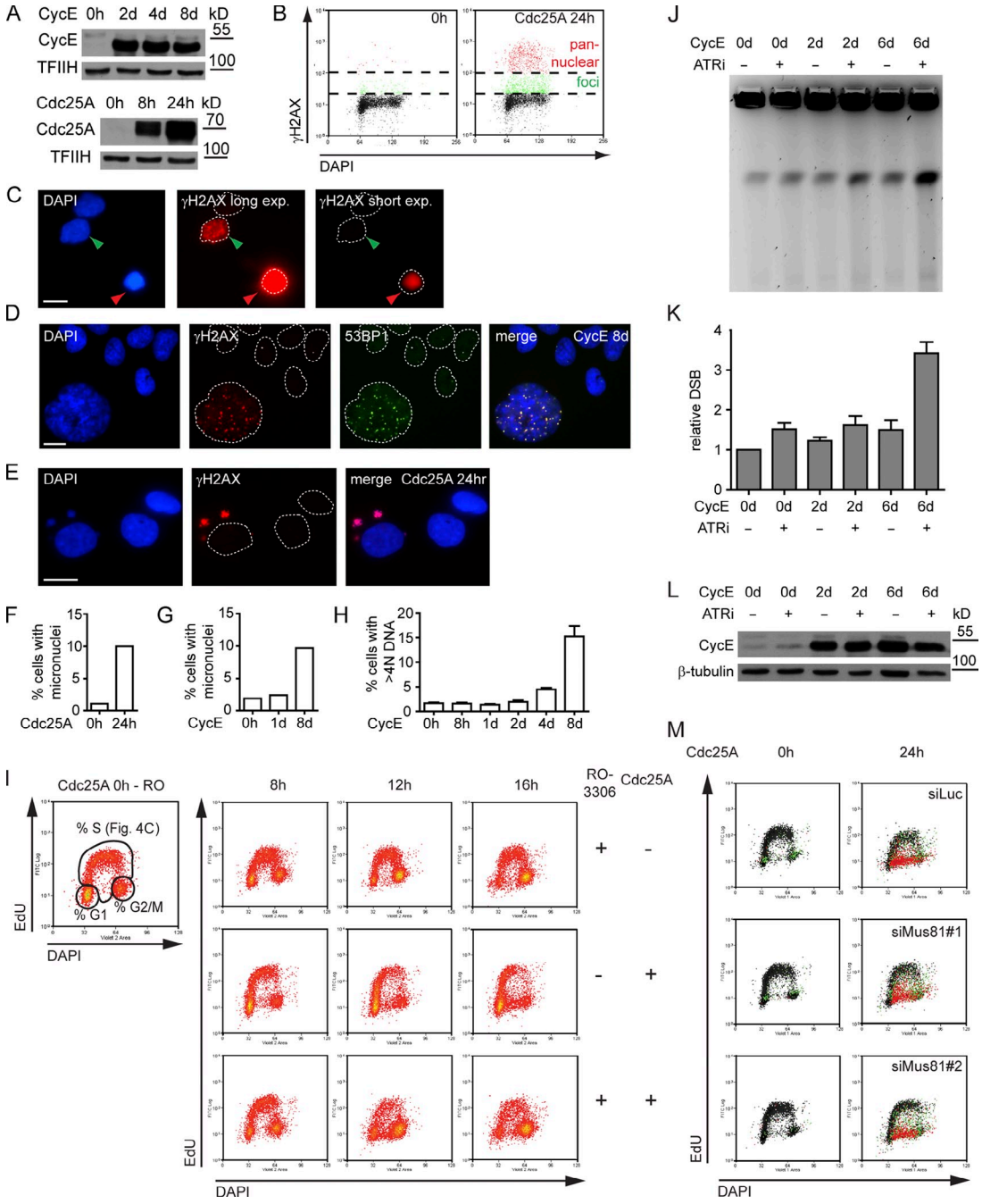

Figure S3. **CycE and Cdc25A induce different patterns of DDR markers, cell cycle analysis of Cdc25A-overexpressing cells upon CDK1 inhibition or MUS81-depletion, and the role of ATR in *CycE*-induced DNA breakage.** (A) Immunoblot showing overexpression of *CycE* and *Cdc25A* in samples in Fig. 3 and this figure. (B) Scheme illustrating the immunodetection of γ-H2AX coupled to detection of DNA content (DAPI) and DNA synthesis (EdU) on control cells (0 h) and cells overexpressing Cdc25A for 24 h. The intensity thresholds for the identification of cells displaying foci and pan-nuclear γ-H2AX are set based on comparison of patterns of γ-H2AX in immunofluorescence microscopy and γ-H2AX signal intensity in the FACS assay. The threshold between γ-H2AX–negative cells and cells with foci was set based on microscopic quantification of γ-H2AX–positive cells in an untreated sample (0 h); the threshold between cells with foci and pan-nuclear γ-H2AX pattern was determined by quantifying the number of cells with pan-nuclear γ-H2AX observed after 24 h of Cdc25A overexpression (24 h). Data are from a single representative experiment out of four repeats. (C) γ-H2AX patterns observed after 24 h of Cdc25A expression as described in Fig. 3 and in panel A of this figure. Green arrowhead, cell with γ-H2AX foci. Red arrowhead, cell with pan-nuclear γ-H2AX and high intensity DAPI signal indicating condensed DNA. (D) Immunostaining for γ-H2AX and 53BP1 of cells overexpressing CycE for 8 d. At late time points after CycE induction, cells with giant nuclei with γ-H2AX/53BP1-positive foci accumulate. (E) γ-H2AX–positive micronuclei observed after overexpression of Cdc25A for 24 h. (F and G) Quantification of cells with micronuclei before (0 h) and at the indicated time points after induction of CycE and Cdc25A, respectively. Data in F and G were reproduced in at least one independent experiment. (H) Quantification of cells with >4n DNA upon *CycE* overexpression. Mean + SEM, $n = 4$. (I) EdU/DAPI-FACS analysis of samples shown in Fig. 4 C. The panel on the left illustrates the quantification of the cell cycle phases based on EdU/DAPI-FACS analysis. This experiment was completed once. (J) DNA breakage after *CycE* overexpression in the absence or presence of the ATR inhibitor (ETP-46464) monitored by pulse-field gel electrophoresis at the indicated time points. (K) Quantification of chromosomal breakage by PFGE in cells treated as in J. Mean + SEM, $n = 3$. (L) Immunoblot showing overexpression of *CycE* in samples in J. β-Tubulin serves as loading control. Molecular weight in kD of nearest protein size marker is indicated. (M) Flow cytometric analysis of cells overexpressing *Cdc25* after mock (siLUC) and MUS81 depletion (siMus81#1, #2). Cells with γ-H2AX foci and pan-nuclear γ-H2AX are shown in green and red, respectively. Data were reproduced in one independent experiment.