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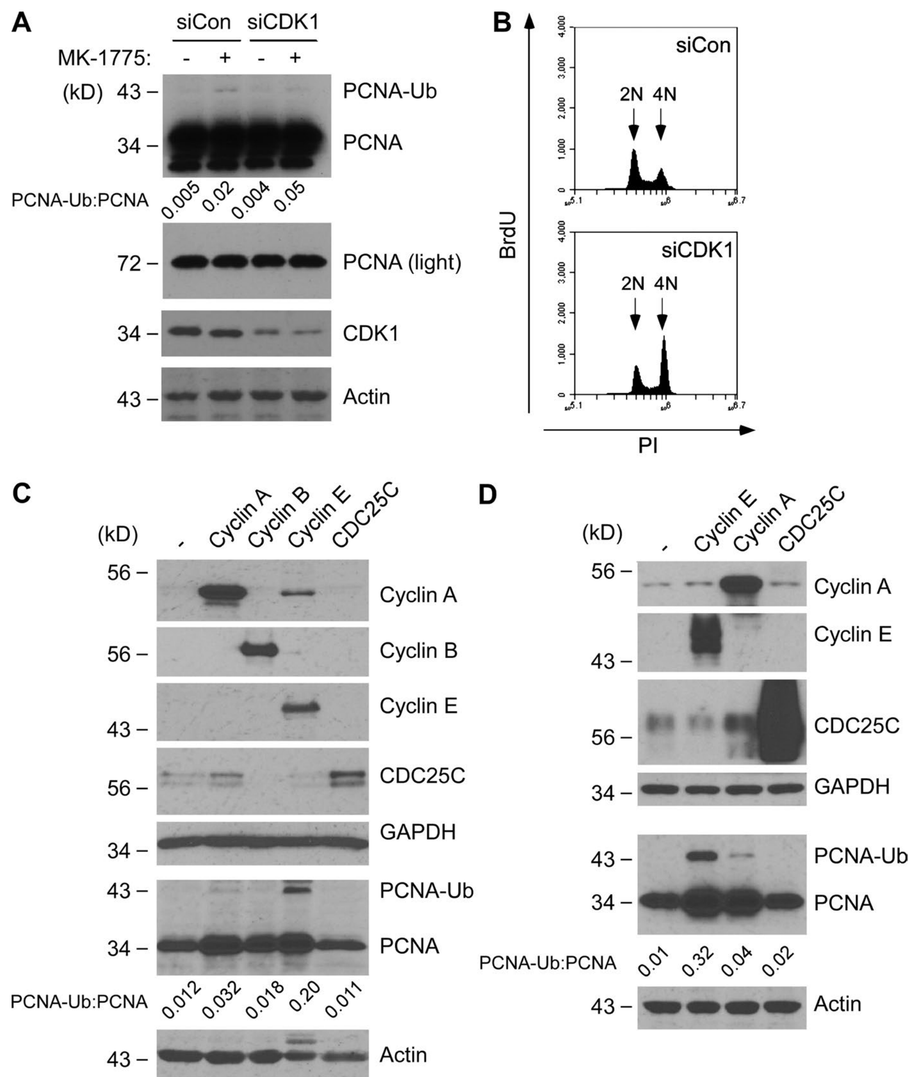

Figure S1. **Differential effects of CDK2 and CDK1 activators on PCNA monoubiquitination.** (A and B) Effect of CDK1 depletion on MK-1775–induced PCNA monoubiquitination (A) and cell cycle distribution (B) of U2OS cells. 48 h after transfection with siCDK1 or siCon, U2OS cells were treated with 10 µM MK-1775 for 4 h. Lysates from the resulting cells were separated by SDS-PAGE and analyzed by immunoblotting with the indicated antibodies. PI, propidium iodide. (C and D) Effect of adenovirally expressed Cyclin E, Cyclin A, Cyclin B (C), and CDC25C (D) on PCNA monoubiquitination in NHFs. Exponentially growing U2OS cells (C) or NHFs (D) were infected with the indicated adenoviral vectors. 48 h later, cell extracts were prepared for immunoblot analysis of PCNA and overexpressed CDK activators.

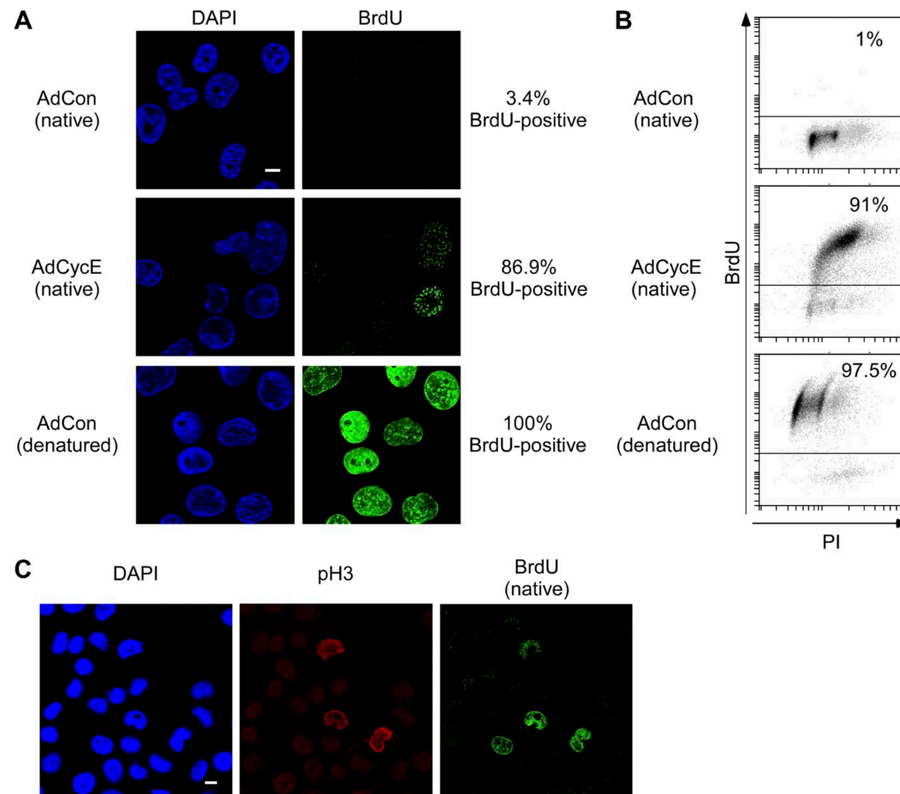

Figure S2. **Comparison of immunofluorescence microscopy and flow cytometry–based ssDNA assays.** (A and B) Replicate cultures of AdCyclin E– or AdCon-infected U2OS cells were incubated with BrdU for 24 h to label replicating DNA. Cells were then fixed with or without acid denaturation, stained with antibodies against BrdU, and analyzed by immunofluorescence microscopy as described by Raderschall et al. (1999) (A) or by flow cytometry (B). The numbers of ssDNA-containing nuclei (detected by anti-BrdU staining without prior acid denaturation) are indicated for each experimental condition. Both microscopy and flow cytometry–based methods detect negligible incorporated BrdU in AdCon-infected nuclei maintained under native conditions. However, all acid-denatured nuclei from AdCon-infected cultures are BrdU positive. As expected from previous work (Neelson et al., 2013a), nearly all native nuclei from Cyclin E–overexpressing cultures stain positively with anti-BrdU, indicative of ssDNA accumulation. (C) The result of an experiment showing that the condensed state of native chromatin in post–S phase nuclei does not preclude detection by anti-BrdU antibody. RAD18-depleted H1299 cells were BrdU labeled for 24 h before a 6-h treatment with 10 µM MK-1775. The resulting nuclei were fixed under native conditions and then costained using antibodies against phospho–histone H3 and BrdU. The immunofluorescence images show that within the same field, many nuclei were stained with both pH3 and BrdU (ssDNA) antibodies. We conclude that condensed chromatin of phospho-H3–positive nuclei does not prevent detection of ssDNA.

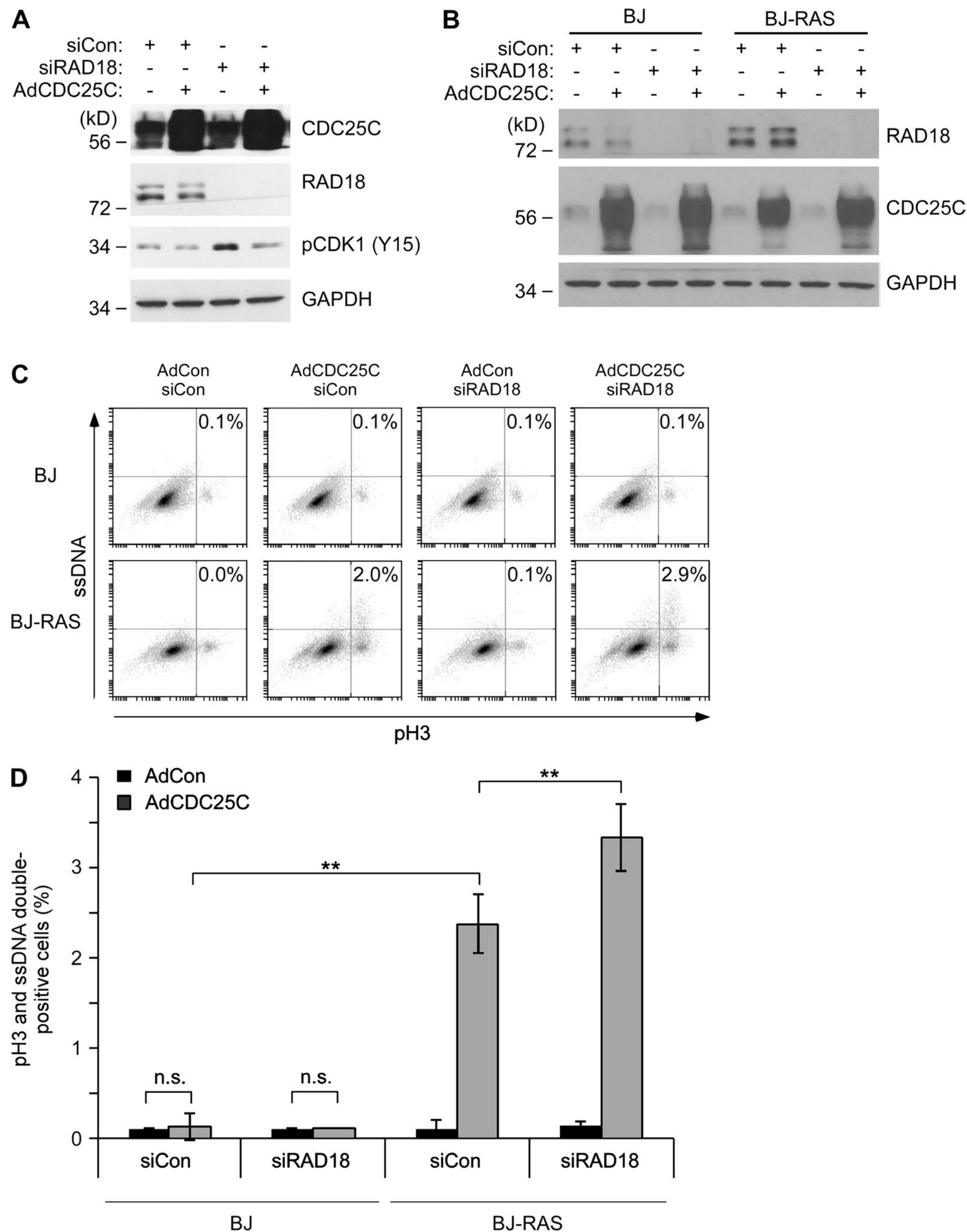

Figure S3. **CDC25C overexpression dephosphorylates CDK1 and promotes the accumulation of mitotic cells harboring persistent ssDNA.** (A) H1299 cells were transfected with siRAD18 or nontargeting (siCon) RNA. 24 h after transfection, cultures were infected with AdCon or AdCDC25C. 48 h later, cells were collected for immunoblot analysis with the indicated antibodies. (B–D) Effect of CDC25C expression on persistent mitotic ssDNA in untransformed BJ fibroblasts and the isogenic transformed BJ-RAS cell line. Replicate cultures of cells were electroporated with siRAD18 or nontargeting control siRNA (siCon). The resulting cultures were infected with AdCDC25C or with AdCon for 24 h. One plate of each replicate pair was analyzed by SDS-PAGE and immunoblotting with the indicated antibodies (C). The remaining plates were harvested and analyzed for phospho–histone H3 and ssDNA content using flow cytometry. The FACS profiles show the results obtained with a single representative experiment. The histogram in D compiles the results of three independent experiments in which we measured ssDNA levels under the same experimental conditions. Columns represent the mean values for ssDNA, and error bars represent the range. We performed ANOVA between groups followed by Tukey's multiple comparison of means test to determine the significance of differences in levels of ssDNA between experimental conditions. Results of the Tukey test are as follows: BJ AdCon versus BJ AdCDC25C, $P > 0.05$ (indicating that CDC25C overexpression does not induce ssDNA in parental nontransformed cells), BJ-RAS siCon AdCon versus BJ-RAS siCon AdCDC25C, $P < 0.01$ (indicating that CDC25C overexpression induces ssDNA in RAS-transformed cells); BJ-RAS siCon AdCDC25C versus BJ-RAS siRAD18 AdCDC25C, $P < 0.01$ (indicating that RAD18 depletion exacerbates ssDNA accumulation in RAS-transformed and CDC25C-overexpressing cells but not in untransformed parental cells). **, $P < 0.01$.

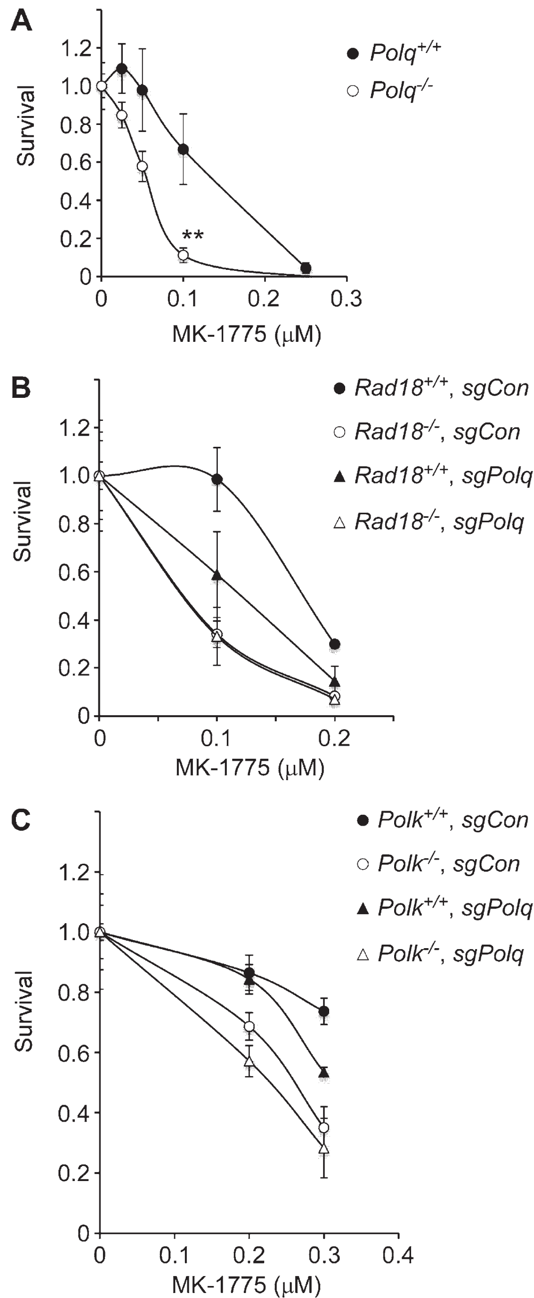

Figure S4. **MK-1775 sensitivity of *Polq*-deficient cells.** (A) Cultures of isogenic MEFs derived from $Polq^{+/+}$ and $Polq^{-/-}$ mice were treated with different concentrations of MK-1775, and sensitivity to Wee1 inhibition was evaluated by clonogenic survival assay. The number of surviving colonies from MK-1775–treated cultures was expressed as a percentage of colony number from cells that received vehicle (DMSO) for control. On the survival curves, each data point represents the mean of triplicate determinations, and error bars represent the range. We performed a Student's *t* test with data obtained using the 0.1-μM MK-1775 concentration. When comparing $Polq^{+/+}$ and $Polq^{-/-}$ cells, $P = 0.0072$, indicating that *Polq*-deficient cells are MK-1775 sensitive. The data shown are from a representative experiment that was repeated three times and yielded similar results on each occasion. (B and C) $Rad18^{+/+}$ and $Rad18^{-/-}$ MEFs (B) or $Polk^{+/+}$ and $Polk^{-/-}$ MEFs (C) were infected with lentiviruses encoding nontargeting control guide RNA or sgRNA targeting the murine *Polq* gene. After lentiviral transduction, pools of puromycin-resistant cells were treated with different concentrations of MK-1775 and evaluated in clonogenic survival assays. The number of surviving colonies from MK-1775–treated cultures was expressed as a percentage of colony number from cells that received vehicle (DMSO) for control. On the survival curves, each data point represents the mean of triplicate determinations, and error bars represent the range. We performed ANOVA between groups followed by Tukey's multiple comparison of means test to determine the significance of differences in MK-1775 sensitivity between different genotypes. For data obtained using 0.1 μM MK-1775 in the $Rad18^{+/+}$ and $Rad18^{-/-}$ cells, results of the Tukey test were as follows: $Rad18^{+/+}$, *sgCon* versus $Rad18^{+/+}$, *sgPolq*, $P < 0.05$ (indicating MK-1775 sensitivity of *Polq*-targeted cultures, consistent with MK-1775 sensitivity observed for $Polq^{-/-}$ MEFs in A); $Rad18^{+/+}$, *sgCon* versus $Rad18^{-/-}$, *sgCon*, $P = 0.001$ (indicating MK-1775 sensitivity of $Rad18^{-/-}$ MEFs, as expected); $Rad18^{-/-}$, *sgCon* versus $Rad18^{-/-}$, *sgPolq*, $P = 0.15$ (indicating no additive MK-1775 sensitivity when cells lack both *Rad18* and *Polq*). For data obtained using 0.3 μM MK-1775 in the $Polk^{+/+}$ and $Polk^{-/-}$ cells, results of the Tukey test were as follows: $Polk^{+/+}$, *sgCon* versus $Polk^{+/+}$, *sgPolq*, $P = 0.02$ (indicating MK-1775 sensitivity of *Polq*-targeted cultures, consistent with MK-1775 sensitivity of *Polq*-deficient cells shown in A and B); $Polk^{+/+}$, *sgCon* versus $Polk^{-/-}$, *sgCon*, $P = 0.001$ (demonstrating the expected MK-1775 sensitivity of *Polk*-deficient cells); $Polk^{-/-}$, *sgCon* versus $Polk^{-/-}$, *sgPolq*, $P = 0.6$ (indicating no additive MK-1775 sensitivity when cells lack both *Polk* and *Polq*). All survival curves shown are representative of experiments that were repeated three times and yielded similar results on each occasion. **, $P < 0.01$.

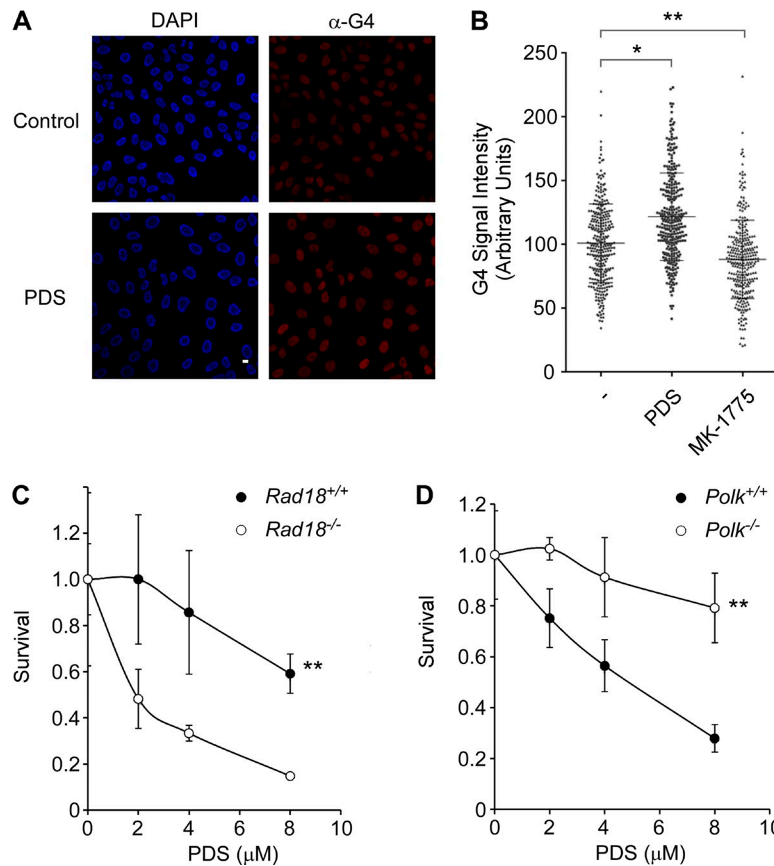

Figure S5. **Separable roles of *Rad18* and *Polk* in PDS tolerance.** (A) U2OS cells were treated with PDS (2 µM) or MK-1775 (0.25 µM) for 24 h and then fixed and stained using anti-G4 antibody. Specific anti–G4-reactive immunofluorescence was analyzed by measuring the mean signal intensity within each nucleus (as marked by DAPI staining) with ImageJ. 300 cells were quantified for each experimental condition. Bar, 10 µm. (B) To determine the statistical significance of differences in G4 staining, we performed ANOVA between groups followed by a Tukey multiple comparison of means test. Results of the Tukey test indicated significant PDS-induced increases in staining intensity when compared with control (-) samples ($P < 0.001$). The Tukey test also indicated a significant decrease in staining intensity of MK-1775–treated samples relative to control cells ($P < 0.001$). (C and D) *Rad18*$^{+/+}$ and *Rad18*$^{-/-}$ MEFs (C) or *Polk*$^{+/+}$ and *Polk*$^{-/-}$ MEFs (D) were treated with different concentrations of PDS, and PDS tolerance was evaluated using clonogenic survival assays. For each experiment, the number of surviving colonies from PDS-treated cultures was expressed as a percentage of the colony number from cells that received vehicle (DMSO) for control. On the survival curves, each data point represents the mean of triplicate determinations, and error bars represent the range. For each experiment, we performed a Student's *t* test with the data obtained using 8 µM PDS. When comparing *Rad18*$^{+/+}$ versus *Rad18*$^{-/-}$ cells, the p-value was <0.01, indicating that *Rad18*$^{-/-}$ MEFs are PDS sensitive relative to isogenic *Rad18*$^{+/+}$ control cells. When comparing *Polk*$^{+/+}$ versus *Polk*$^{-/-}$ cells, P was <0.01, indicating that *Polk*$^{-/-}$ MEFs are PDS-resistant relative to isogenic *Polk*$^{+/+}$ cells. All data shown are from representative experiments that were repeated at least three times and yielded similar results on each occasion.

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