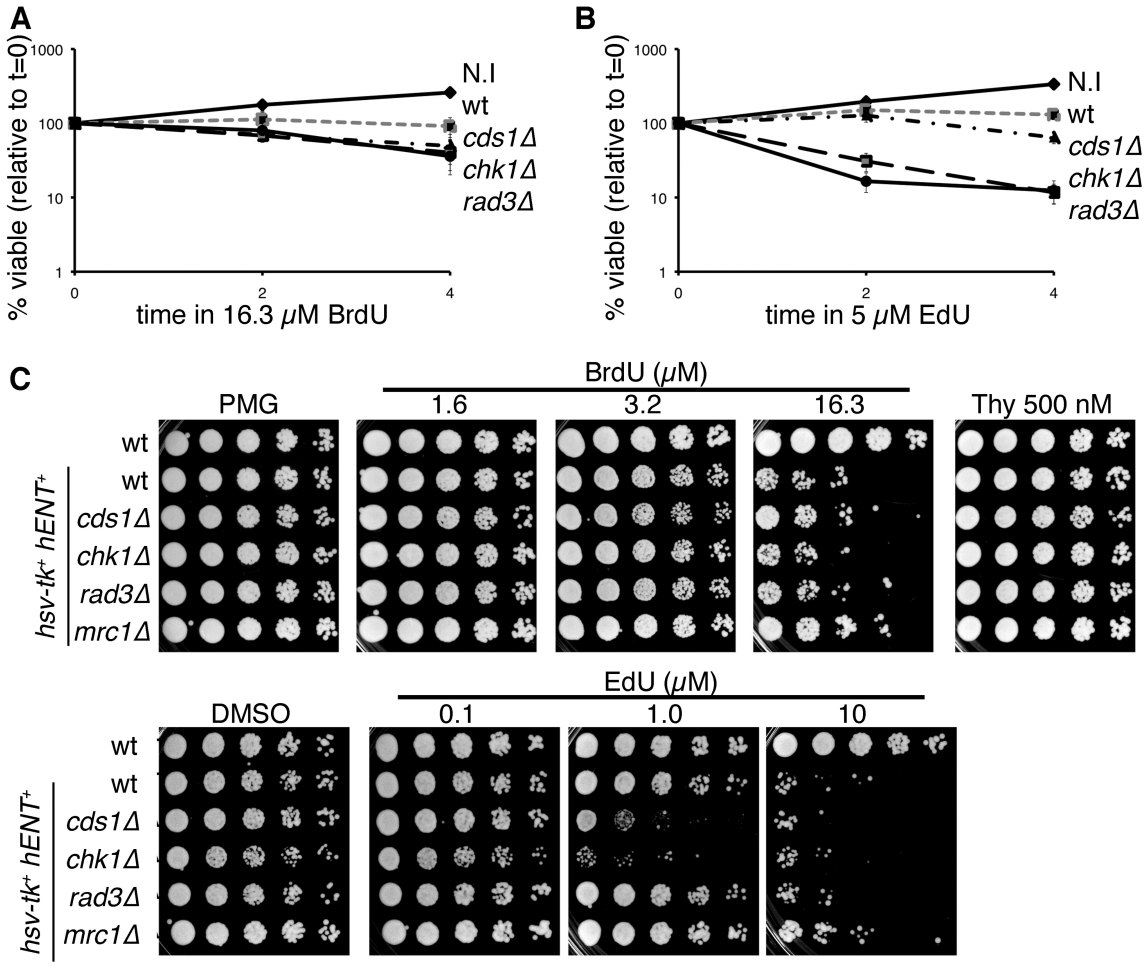

Figure S1 BrdU and EdU dose affects cell viability (refer to Figures 1 and 2). **A.** Relative viability of *hsv-tk*$^{+}$ *hENT*$^{+}$ wild-type, *cds1Δ, chk1Δ,* and *rad3*Δ cells at 16.3 μM BrdU (compare with Figure 1B) with non-incorporating (N.I.) control. Shown are the means of two independent experiments ±SEM. **B.** As in A, viability in lower dose EdU (5 μM) (compare with Figure 1D). Shown are the means of two independent experiments ±SEM. **C.** Spot tests on poor-nitrogen source medium PMG for non-incorporating wild-type (wt) and *hsv-tk*$^{+}$ *hENT*$^{+}$ cells of indicated genotype (compare with Figure 2).

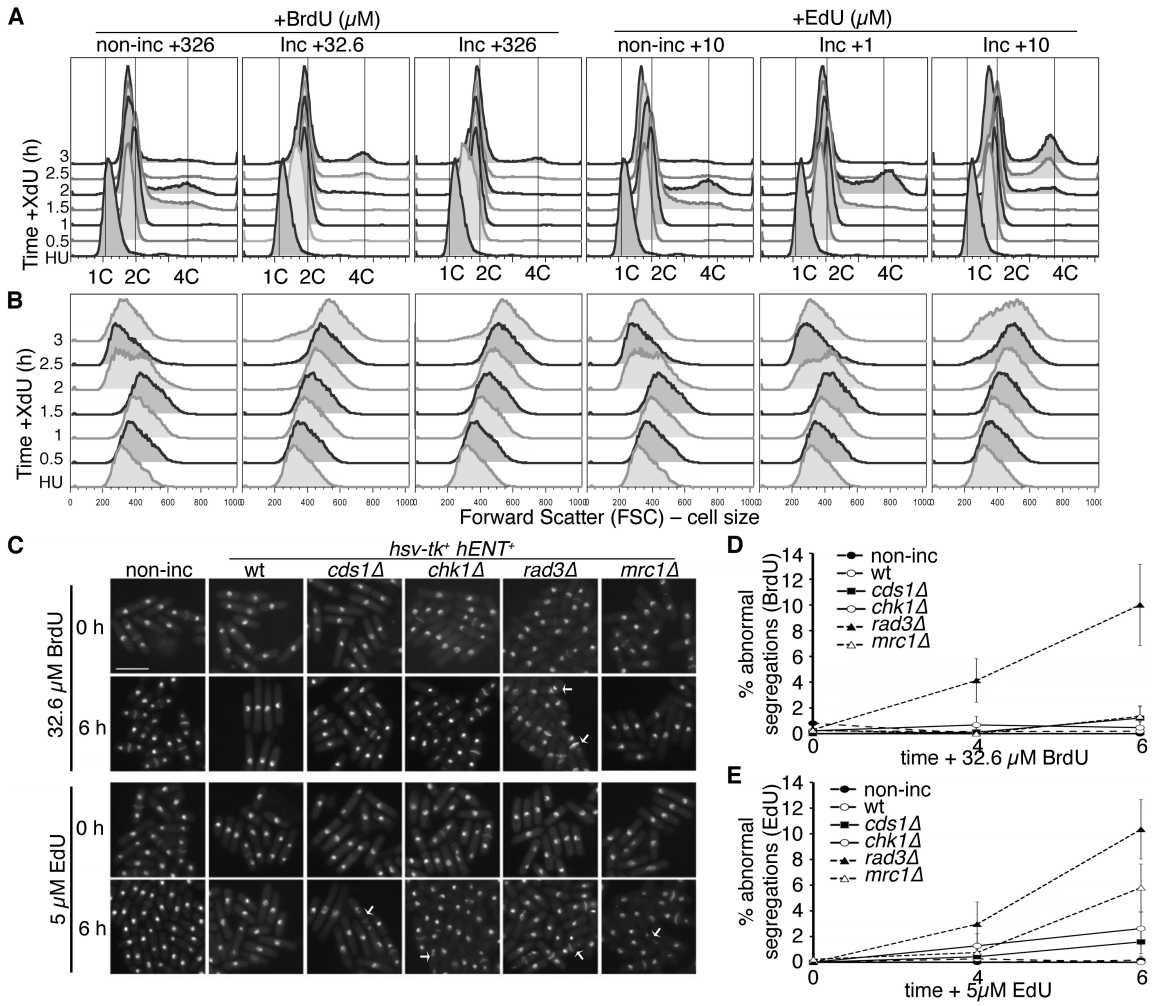

Figure S2 BrdU and EdU cause prolonged DNA synthesis, cell cycle slowing and DNA damage (related to Figure 3). **A.** Sytox Green stained cells (from Figures 3A, 3B) were analyzed by flow cytometry to highlight progression to 4C DNA (second S-phase; using modified cytometer settings). Non-incorporating (non-inc) or *hsv-tk*$^{+}$ *hENT*$^{+}$ cells (Inc) at indicated doses of BrdU or EdU. Note that 4C peak accumulation is consistent with septation index peaks (Figure 3A, 3B), indicating the second S-phase after release. **B.** Forward scatter (FSC) dynamics of cells in A, indicating cell size during experiment. Left-shift toward smaller cell size (M-phase) occurs slightly later than septation (S-phase; Figure 3A, 3B). **C.** Cells were stained with DAPI and aniline blue to detect nuclei and septa, respectively, before or after 6h of BrdU or EdU treatment. Wild-type (wt) incorporating cells elongate during prolonged exposure. Both *chk1*Δ and rad3Δ *hsv-tk*$^{+}$ *hENT*$^{+}$ cells continue to septate and divide, and many cells mis-segregate DNA (indicated by arrows). *mrc1*Δ and *cds1*Δ *hsv-tk*$^{+}$ *hENT*$^{+}$ cells show an intermediate phenotype in EdU. Scale bar 10 μm. **D.** Abnormal DNA segregation events were scored as the percentage of cut or anucleate cells in the total population during BrdU treatment. Shown are combined data from 2 independent experiments, displayed as proportion of abnormal segregants ±95% CI. **E.** Abnormally segregated nuclei during EdU exposure. Shown are combined data from 2 independent experiments, displayed as proportion of abnormal segregants ±95% CI.

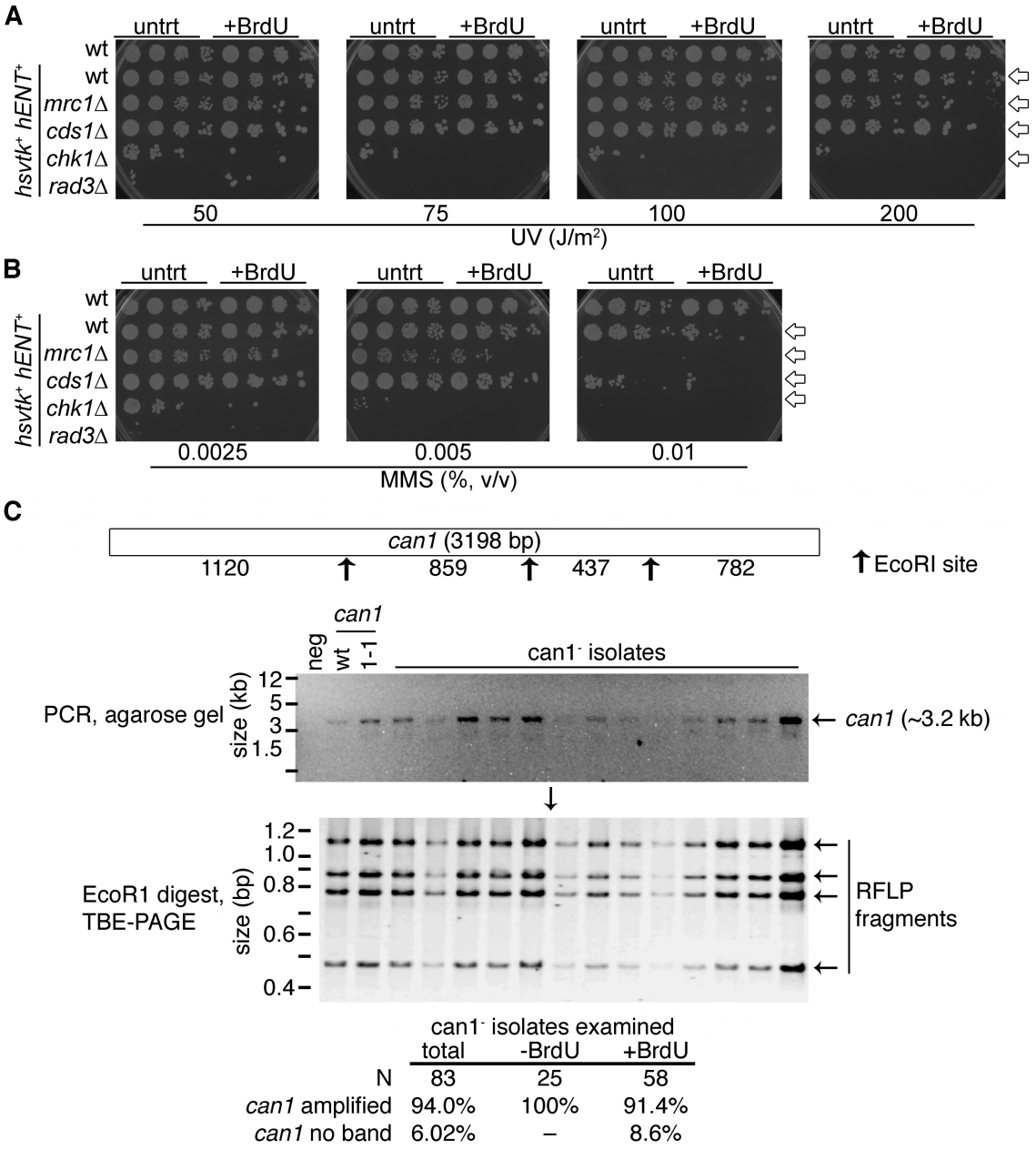

Figure S3 BrdU pre-treatment changes sensitivity to mutagens (refer to Figure 6). **A, B.** Cells were untreated (untrt) or pre-treated (+BrdU) with 32.6 µM BrdU (2h at 32°C), and then spotted onto drug plates (YES), 1/5 dilutions. Arrows indicate greater sensitivity to drug +BrdU. Refer to Figure 6A for control. **A**. Sensitivity to UV, irradiated after plating yeast. **B.** Sensitivity to MMS after BrdU treatment. **C.** Analysis of can1$^-$ isolates from forward mutation study. Strains were pooled to assess *can1* amplification and RFLP ±BrdU; no differences were seen between genotypes. The *can1* locus was amplified by PCR, and produces a 3.2 kb band by agarose gel electrophoresis. PCR product was digested with EcoRI, producing 4 restriction fragments which were screened on 8% TBE-PAGE gels. Lane 1 (top) is a negative (water) control for PCR. Lanes 2 and 3 are non-incorporating strains that were known *can1*$^+$ or *can1-1* genotypes. Restriction fragment length differences were not detected in any of the can1- isolates. Instead, a minority of BrdU-treated isolates failed to amplify a detectable *can1* band (8.6% of all BrdU treated isolates).

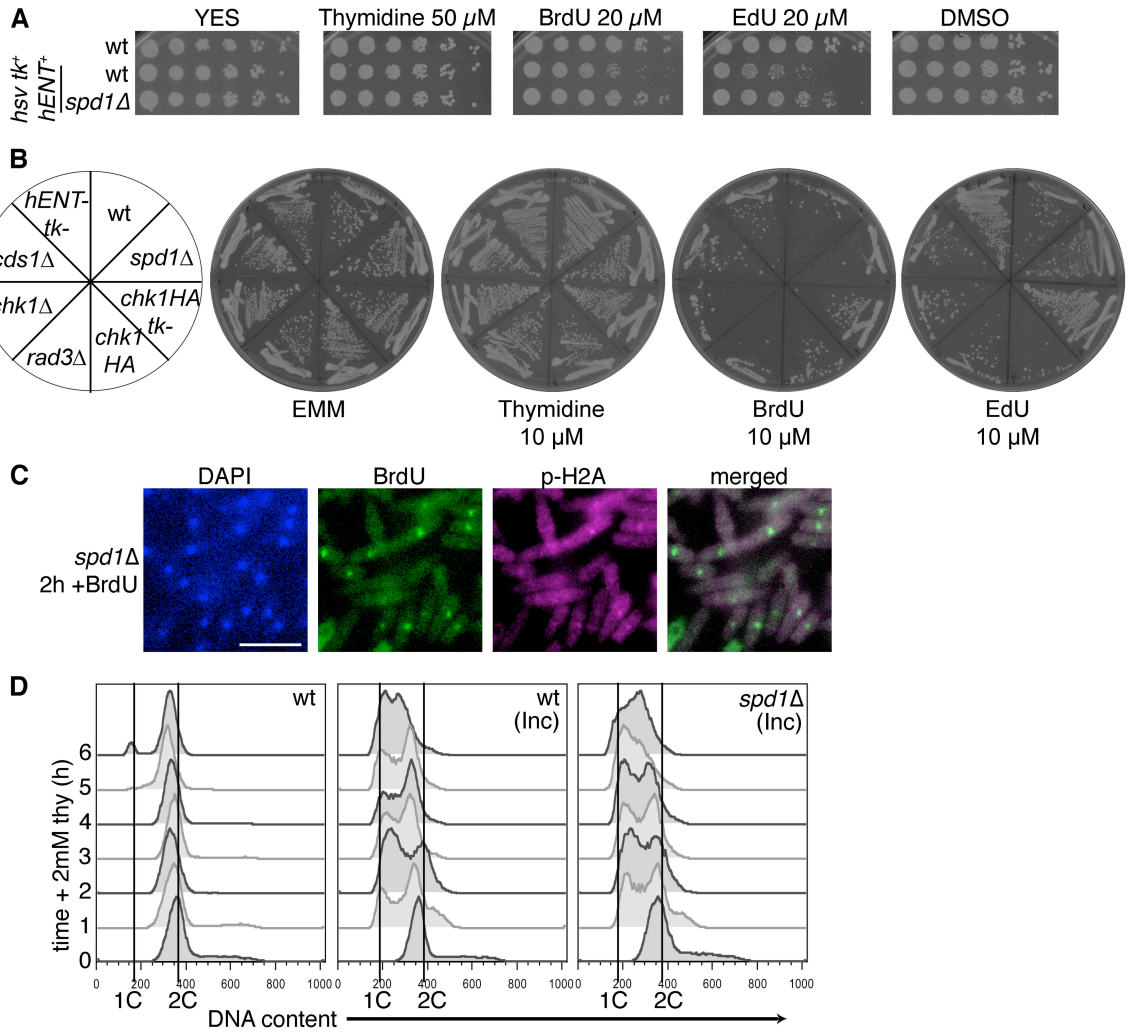

Figure S4 Spd1 protects cells from division and mutation during dNTP imbalance (refer to Figure 7). **A.** On YES medium, *spd1*Δ cells withstand high doses of EdU and BrdU. DMSO is a vehicle control for EdU. Note that wild-type (wt) *hsv-tk*$^{+}$ *hENT*$^{+}$ cells were also resistant to 50 µM thymidine on rich media. **B.** Strains (FY 2317, 3454, 6427, 5030, 5031, 5150, 5149, 5148) were streaked onto supplemented EMM with thymidine, BrdU or EdU to assess growth on plates. *spd1*Δ *hsv-tk*$^{+}$ *hENT*$^{+}$ cells formed some large colonies, but also a background of small colonies, also seen for *cds1*Δ *hsv-tk*$^{+}$ *hENT*$^{+}$. **C.** Immunofluorescence of spd1Δ *hsv-tk*$^{+}$ *hENT*$^{+}$ cells, after 2h BrdU treatment for nuclei (DAPI), BrdU incorporation, phospho-histone H2A (p-H2A), and merged BrdU/p-H2A. Scale 10 µm. **D.** Addition of 2 mM thymidine over prolonged periods in non-incorporating wild-type (wt), wt and *spd1*Δ *hsv-tk*$^{+}$ *hENT*$^{+}$ cells (Inc). The 1C and 2C DNA content peaks are indicated; G1 arrest causes a shift toward the 1C peak.

Supporting Files

Available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.112.145730/-/DC1.

File S1 Wild-type (Movie #1) *hsv-tk*$^{+}$ *hENT*$^{+}$ cells in a microfluidics chamber were treated with 32.6 µM BrdU for 3h (pink border) and switched to BrdU-free medium for 3h afterward, to monitor Rad52-YFP foci (yellow).

File S2 *cds1*Δ (Movie #2) *hsv-tk*$^{+}$ *hENT*$^{+}$ cells were treated with 32.6 µM BrdU for 3h (pink border) in a microfluidics chamber, and then switched to BrdU-free medium for 3h afterward, to monitor Rad52-YFP foci (yellow).

File S3 Wild-type (Movie #3) *hsv-tk*$^{+}$ *hENT*$^{+}$ cells were treated with 10 µM EdU for 3h (pink border) then media was switched to EdU-free medium to monitor Rad52-YFP foci (yellow).

File S4 *cds1*Δ (Movie #4) *hsv-tk*$^{+}$ *hENT*$^{+}$ cells were treated with 10 µM EdU for 3h in a microfluidics chamber (pink border) before media switch (EdU-free) for 3h, to monitor Rad52-YFP foci (yellow).