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 rad3Δ 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<sup>−</sup> hENT<sup>−</sup> 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 chk1Δ and rad3Δ hsv-tk<sup>−</sup> hENT<sup>−</sup> cells continue to septate and divide, and many cells mis-segregate DNA (indicated by arrows). mrc1Δ and cdc11Δ hsv-tk<sup>−</sup> hENT<sup>−</sup> 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- 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, spd1Δ 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. spd1Δ hsv-tk+ hENT+ cells formed some large colonies, but also a background of small colonies, also seen for cds1Δ 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 spd1Δ 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


**File S1**  Wild-type (Movie #1) hsv-tk<sup>+</sup> hENT<sup>+</sup> 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**  cds1Δ (Movie #2) hsv-tk<sup>+</sup> hENT<sup>+</sup> 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<sup>+</sup> hENT<sup>+</sup> 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**  cds1Δ (Movie #4) hsv-tk<sup>+</sup> hENT<sup>+</sup> 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).