



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 *chk1Δ* and *rad3Δ hsv-tk⁺ hENT⁺* cells continue to septate and divide, and many cells mis-segregate DNA (indicated by arrows). *mrc1Δ* and *cds1Δ hsv-tk⁺ hENT⁺* cells show an intermediate phenotype in EdU. Scale bar 10 μ m. **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 \pm 95% CI. **E.** Abnormally segregated nuclei during EdU exposure. Shown are combined data from 2 independent experiments, displayed as proportion of abnormal segregants \pm 95% CI.