Spontaneous DNA breakage in single living cells of *Escherichia coli*
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**Supplementary Figure 4** Low frequencies of inviable, propidium-iodide stained cells among spontaneously SOS-induced green cells for ≥8 hours after becoming green. (a) Representative flow cytometry analysis of growing wild-type cells for propidium-iodide-positive (PI⁺) dead cells (upper two quadrants), GFP⁺ cells (rightmost two quadrants), and cells both GFP⁺ and PI⁺ (dead green cells, upper right quadrant). (b) Percent of spontaneously green and non-green cells that are also PI⁺ during growth. Mean ± SEM of 3 experiments. (c) Once formed, green cells do not die substantially over the next 8 hours. We tested whether green cells already formed die substantially, and become PI⁺, over the next 8 hours by halting cell divisions during this time, by the withdrawal of their carbon source. Proliferation of log-phase cultures (grown as described above) was stopped by resuspending in the same medium but without the carbon source and cells withdrawn, stained with PI, and analyzed by flow cytometry either immediately upon resuspension or after 8h further incubation (37°). Without cell proliferation, the proliferation-capable green cells already formed should not lose greenness by cell division and dilution of the GFP. Also, if DNA replication is the major source of spontaneous SOS induction (Fig. 4 main text) then new green cells should not be added in the absence of cell proliferation. However, if those green cells formed during the prior log-phase growth begin to die, the fraction of green cells that are PI⁺ should increase. As expected, the number of colony-forming viable cells is not significantly different at 0 and 8h (3.1±0.4 x 10⁸ and 2.8±0.2 x 10⁸ cfu/mL, respectively), nor is the fraction of all cells that are green (0.9±0.3%, and 2.1±1.1%, respectively). Informatically, even after 8 h, the majority of green cells are alive; only 1.7±0.2% of green cells were PI⁺ at 8h, compared with 1.1±0.3% PI⁺ at 0 h. This death rate—(1.7/1.1 =) 1.5 times more PI⁺ green cells at 8 than 0 h—is negligible and statistically not significant (single-factor ANOVA and Tukey test). It is also the same or lower than the increase in PI⁺ cells in the non-green cells, a useful comparison to control for any general affects of resuspension and holding for 8h without carbon source. We found that the number of PI⁺ non-green cells at 8 than 0h were 0.62(±0.3)% and 0.15(±0.06)%, respectively: 4.1 times more dead cells at 8 than 0h. Therefore, the 65% green cells that do not form colonies (main text and above) are not mostly dead 8 hours after they become green—they remain viable in the long term of at least 8 hours. Data for (c) are mean ± SEM of 3 experiments.