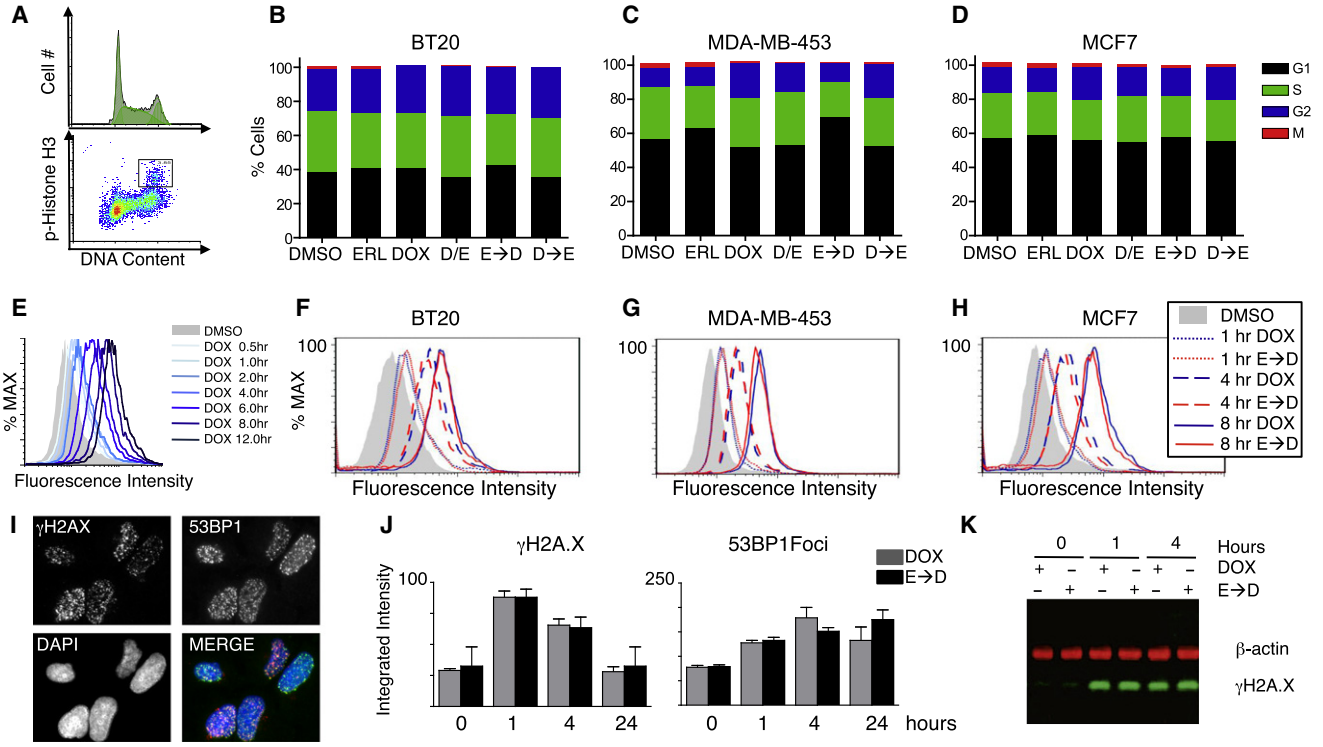

Figure 2. Prolonged Treatment with Erlotinib Does Not Change Cell-Cycle Profile, Doxorubicin Influx/Efflux, or the Level of DNA Damage

(A–D) Quantitative cell-cycle analysis. DNA content and the percentage of mitotic cells were measured by FACS. (A) Example FACS plots from untreated BT-20 cells. (B–D) Cell-cycle stage quantified from three experiments, each performed in duplicate. Cells were treated as in Figure 1, and data were collected at 6, 8, 12, 24, and 48 hr after DOX treatment. 8 hr data shown for each cell type.

(E–H) Doxorubicin retention measured by flow cytometry. (E) Sample time course of BT-20 cells treated with 10 μM DOX for the indicated times. (F–H) Cells treated with doxorubicin or pretreated with erlotinib for 24 hr prior to DOX (E → D). Cells were collected at 1, 4, or 8 hr after DOX exposure as indicated, and internal doxorubicin fluorescence was measured.

(I and J) Quantitative microscopy of the early DNA double-stranded break response. (I) Example image of cells treated with DOX for 1 hr and stained for γH2AX, 53BP1, or nuclear content (DAPI). (J) Integrated intensity per nucleus of γH2AX and 53BP1 foci was measured in BT-20 cells after the indicated treatments and times. Mean values ± SD from triplicate experiments shown.

(K) Western blot analysis of γH2AX in BT-20 cells. β-actin shown as a loading control.