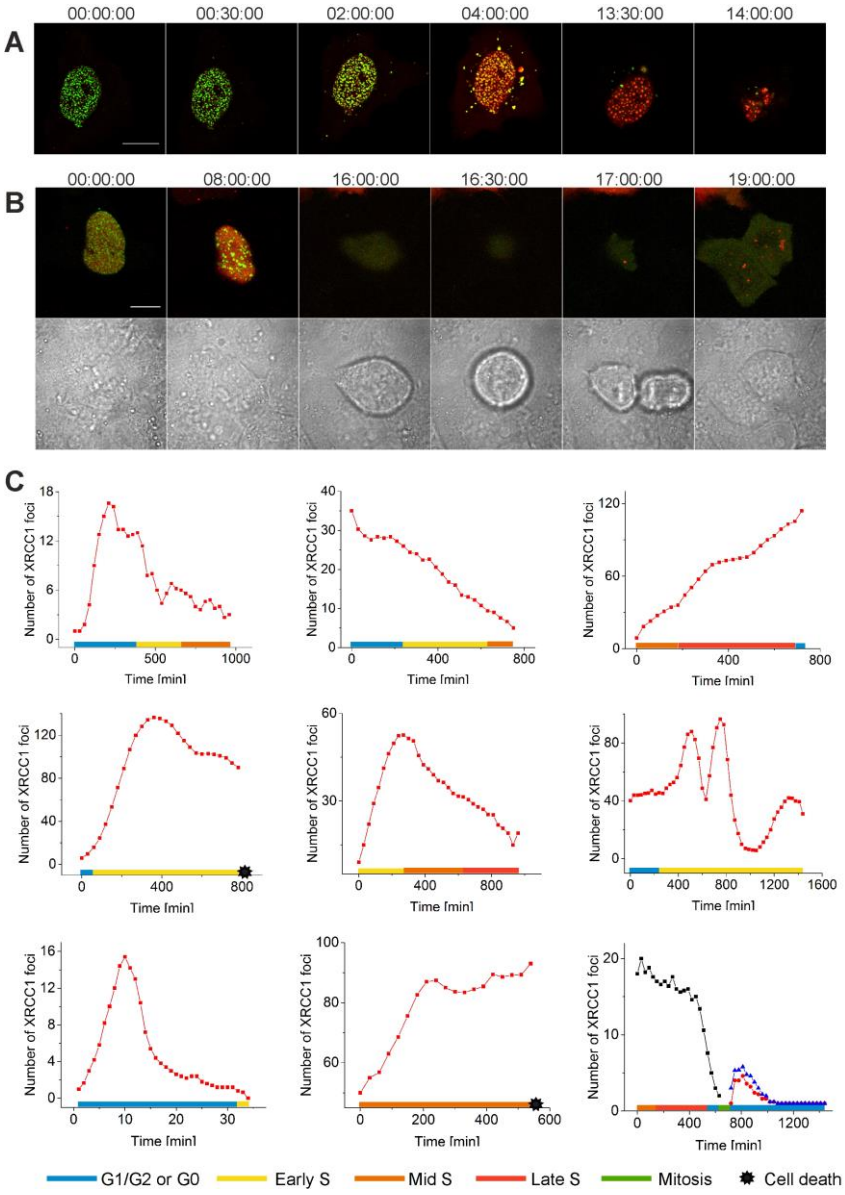
Suppl. Data Set 1.
Various fates of cells containing large numbers of nuclear XRCC1 foci - damage and recovery



Supplementary Figure 1. *Examples of two different fates of cells that develop high numbers of XRCC1 foci in response to extensive endogenous DNA damage (A - cell death, B - recovery followed by mitosis). XRCC1 foci and replication sites are shown (mRFP-XRCC1 (red), mEGFP-PCNA (green)); the bottom row - transmitted light images.*
The plots (C) illustrate changes of the number of XRCC1 foci in time per a single cell nucleus, in nine cells. Scale bars: 10 µm.

Most HeLa and HSF cells contain only a few large XRCC1 foci, however, a small subpopulation contain a high number (over 10, even over a hundred) of these structures. A large majority of these foci are formed in positions adjacent to replicating DNA. We have not detected any foci formed aside and subsequently migrating towards regions of replication. Moreover, the foci dynamically roaming from one replication region to another were detected only rarely. Thus, in most of time-lapse sequences, in which cells that suffered heavy DNA damage and contained numerous XRCC1 foci were followed for several hours, these structures were found near replicating regions and remained associated with them during this time (Suppl. Fig. 1). Sequential live cell imaging showed that the morphology and succession of events in each such cell seemed almost unique. Some frequently occurring scenarios could, however, be identified. Filming of cells with numerous XRCC1 foci for several hours demonstrated that approximately 40% of these cells eventually perished (Suppl. Fig. 1A, Mov. 1 available at http://helios.wbbib.uj.edu.pl/publikacje), while 60% remained viable. This observation supports the notion that XRCC1 foci are indeed formed in response to DNA damage. Interestingly, in some cells the number of XRCC1 foci increased continuously, suggesting a progressing induction of massive endogenous damage within the sites of replication, subsequently, however, the number of these foci dwindled. Eventually these cells went into a successful division (Suppl. Fig. 1B, Mov. 2, available at http://helios.wbbib.uj.edu.pl/publikacje). Apparently, in these cases, constituting approximately 12% among all recorded cells, the extensive endogenous DNA damage in replicating regions was eventually repaired. The number of repair foci was often seen to change in time dynamically, and quantitative analysis shows how diverse cell response can be throughout the entire cell population (Suppl. Fig. 1C).

Surprisingly, in a few cells large numbers of XRCC1 foci were formed immediately after the division, or later in G1 phase (data not shown). These observations indicate that although formation of XRCC1 foci in response to endogenous DNA damage was generally linked to replication, a sudden large scale induction of endogenous DNA damage was occasionally also possible even without active replication.