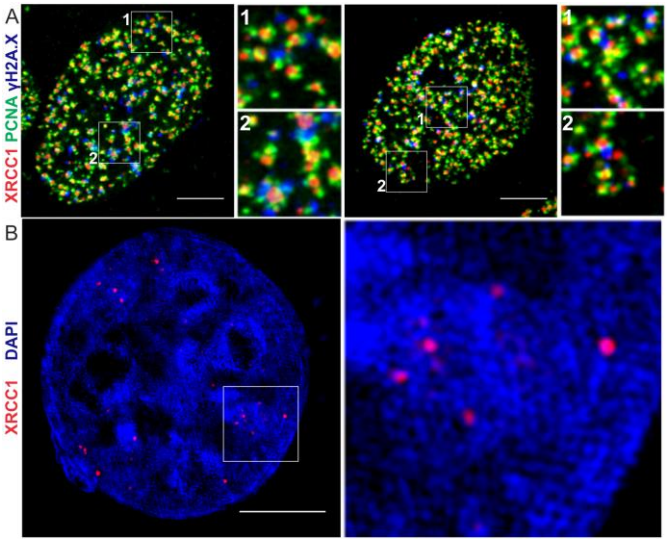
Suppl. Data Set 5.
Conversion of SSBs into DSBs and localization of XRCC1 foci in inter-chromatin compartment



***Supplementary Figure 5**. Localization of XRCC1-containing nuclear bodies in relation to DSBs and heterochromatin.*
***A**. DNA double strand breaks in nuclei of cells containing XRCC1 foci (mRFP-XRCC1 (red), mEGFP-PCNA (green), γH2A.X (blue, IF)). γH2A.X foci are located close to the regions of replication and, at the same time, XRCC1 bodies.*
***B**. Localization of XRCC1 foci (mRFP-XRCC1) in perichromatin regions of the cell nucleus. XRCC1 bodies are always located in the regions of low DNA density (perichromatin regions). It is detectable even though the presented 3D SIM images of DNA show remnants of artifactual periodic pattern often encountered in SIM images. Exclusion from regions of high DNA density may be a consequence of the size of XRCC1 bodies (300 - 400 nm in diameter). Scale bars: 5 µm, ROIs: 4.3 x 4.3 µm.*

Frequent cell death, which we observed in cells containing large numbers of XRCC1 foci adjacent to replication, could result from an overwhelming quantity of SSBs, or from conversion of many SSBs into DSBs. In order to establish if the replication-related SSBs, described here, were subsequently converted to DSBs, we recorded images of replicating cells with mRFP-XRCC1 foci and immunofluorescently labeled histone γH2A.X. The images presented in Suppl. Fig. 5A show that generally less γH2A.X foci, likely marking DNA double-strand breaks (1), than XRCC1 foci, that mark the single-strand DNA breaks, could be detected. A great majority of γH2A.X foci were located close to replicating DNA. This observation suggests that some (altough not all) replication-related SSBs, were transformed into DSBs. Notably, only very few γH2A.X foci were detected at a distance from XRCC1 foci and replication regions, suggesting that the existing DSBs were created as a result of a conversion from replication-related SSBs (Suppl. Fig. 5A).

A thorough examination of the localization of XRCC1 foci in relation to the regions of high and low DNA density demonstrated that these foci were located exclusively in the so-called inter-chromatin compartment or perichromatin region (2–5). High resolution 3D SIM images showed XRCC1 foci mostly localized at the edges or within the compartment of chromatin characterized by low chromatin density and compaction (Suppl. Fig. 5B). This observation agrees with the detected formation of repair foci outside of active replication sites marking heterochromatin regions in mid S, after inducing local DNA damage (Fig. 5A). It is also in agreement with the hypothesis, which states that chromatin is remodeled in response to DNA damage, and DNA, which is undergoing repair, is pulled out of the regions of high DNA compaction (6–9).

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