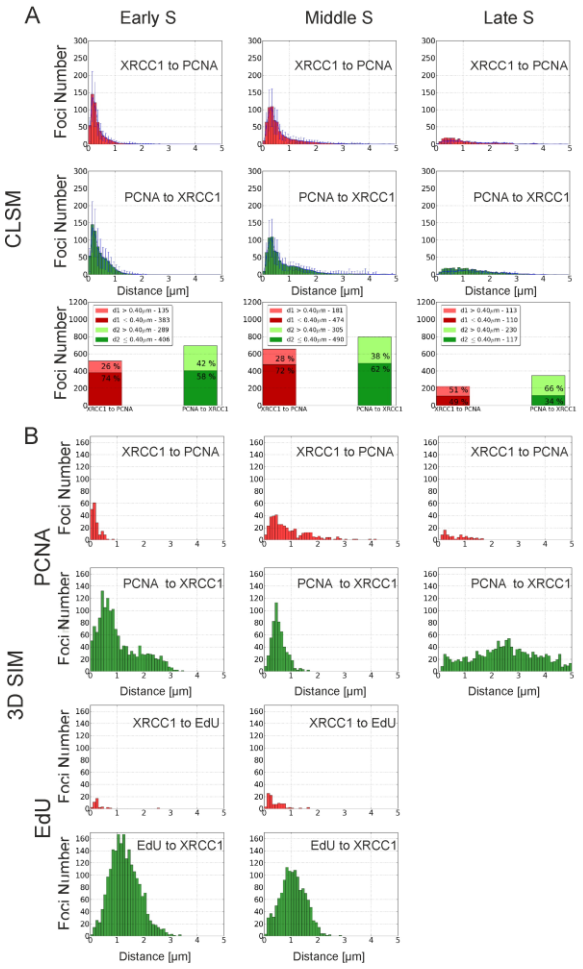
Suppl. Data Set 3.
Distances between XRCC1 foci and replication measured in confocal and SIM super-resolution images



Supplementary Figure 3. *Histograms of the distances measured from XRCC1 foci to the nearest, neighboring replication site (red) and from replication sites to the nearest XRCC1 focus, for the three substages of S-phase (early, middle and late)(A), and representative examples of histograms of such distances measured in 3D SIM super-resolution images of XRCC1 and replication (detected via PCNA or an incorporated precursor EdU) (B). The presented analysis was performed for the cells expressing fluorescently labeled proteins.* ***A****. The shapes of the histograms and proportions of foci located in close proximity (bar charts) in subphases of S indicate that the proximity of XRCC1 foci and replication sites is preserved (red histograms), and that a large proportion of replication sites have a neighboring XRCC1 focus throughout the whole S-phase, that is during replication of eu- as well as heterochromatin,* ***B****. Each histogram illustrates the distances from XRCC1 to replication (red) and replication to XRCC1 (green) measured in five, individual representative 3D super-resolution (3D SIM) image stacks. The shapes of these histograms demonstrate the general nature of the spatial patterns and correlations between the positions of replication sites and XRCC1 foci. Two maxima in these histograms indicate that there are two subpopulations of XRCC1 foci and two subpopulations of replication foci. XRCC1 foci of a large subpopulation are located very close to the sites of active replication (presumably groups of replication forks). Foci of the other smaller group have no adjacent replication regions; these foci likely represent stress bodies formed afar of the sites of DNA damage, as described in* (1).

The images and time-lapse recordings of cells fluorescently labeled for XRCC1 and DNA replication revealed a close proximity rather than a full overlap of the foci of the two types. Only 10% of XRCC1 foci were found within a distance shorter than 100 nm from the nearest replication site (Fig. 2A), yet most of the barycenters of replication and repair foci were separated by a distance of 200 - 400 nm (Fig. 2AB). Almost no cases of a full overlap between XRCC1 and replication were detected, as demonstrated by the left end (short distances) of the histograms shown in Fig. 2. Preliminary estimates revealed that the number of mRFP-XRCC1 molecules in individual repair foci reached at least a few hundred (estimated as in (2)).

Quantitative analysis of the distances measured from XRCC1 foci to the nearest replication sites, as presented in Fig. 2, represents an average measured for the whole S-phase. We also performed such analyses, based on 3D confocal images of replicating cells, for the three substages of S-phase separately. These analyses confirmed that the spatial association between XRCC1 foci and replication regions occurs throughout the whole S-phase (Suppl. Fig. 3A).

The notion of proximity without overlap between XRCC1 foci and replication regions was confirmed by analyzing 3D SIM super-resolution images. The shapes of histograms of distances measured from barycenters of XRCC1 foci to barycenters of the nearest replication sites (detected via PCNA or EdU) (Suppl. Fig. 3B, red histograms) confirm that XRCC1 foci are rarely formed afar of replication regions. The shapes of histograms of distances measured from barycenters of replication regions to barycenters of the nearest XRCC1 foci (green) show the presence of a subpopulation of damaged replication sites accompanied by an XRCC1 focus, and a population of replication sites with no accompanying XRCC1 focus, suggesting that there was no DNA damage at these sites (Suppl. Fig. 3B, green histograms).

References

1. Solarczyk, K. J., Kordon, M., Berniak, K., and Dobrucki, J. W. (2016) Two stages of XRCC1 recruitment and two classes of XRCC1 foci formed in response to low level DNA damage induced by visible light, or stress triggered by heat shock. *DNA Repair (Amst).* **37**, 12–21
2. Verdaasdonk, J. S., Lawrimore, J., and Bloom, K. (2014) Determining absolute protein numbers by quantitative fluorescence microscopy. In *Methods in Cell Biology* vol. 123, pp. 347–365, Elsevier Inc.