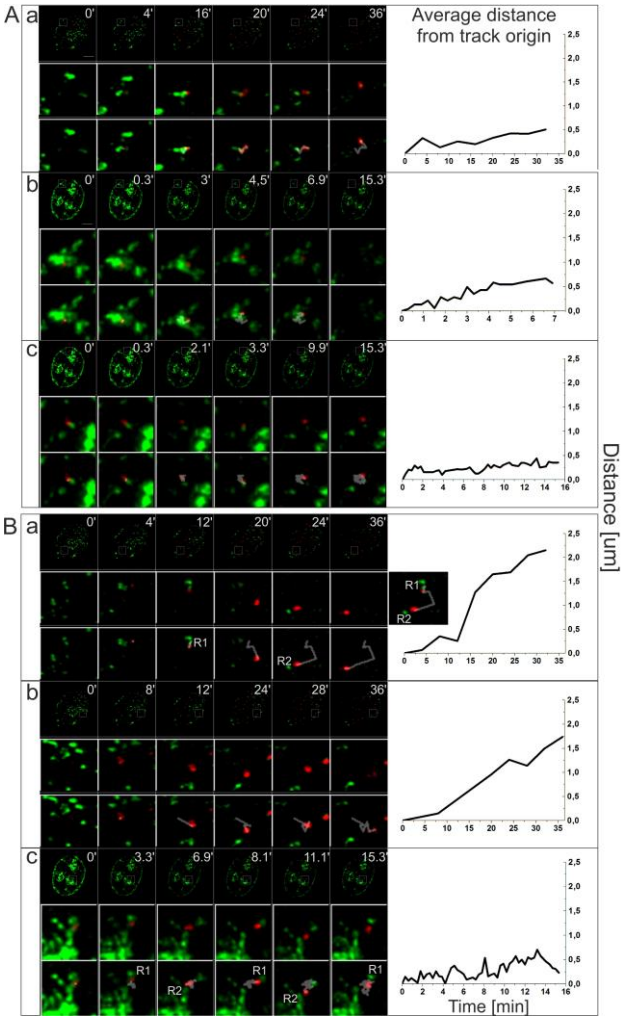
Suppl. Data Set 2.
Dynamics of XRCC1 foci



Supplementary Figure 2. *Dynamics of XRCC1 foci (mRFP-XRCC1) (red) in replicating cells expressing mEGFP-PCNA (green). Two subclasses of foci can be distinguished:*
A. *foci that do not travel over long distances and perform only oscillatory movements centered around relatively stable positions, very often near a replication site (examples Aa-c) (Mov. 3, 4, available at http://helios.wbbib.uj.edu.pl/publikacje);*
B. *foci that perform dynamic movements over long distances (examples Bab) or movements between replication sites (R1 and R2) (examples Bac); their movement can be often perceived as translocation from one to another distant replication site (example Ba) (Mov. 5, available at http://helios.wbbib.uj.edu.pl/publikacje), or shows signs of oscillatory movement between R1 and R2, as in the example Bc (Mov. 6, available at http://helios.wbbib.uj.edu.pl/publikacje). The plots present average distances from the track origin calculated for each case. Scale bars: 5 µm, ROIs: 3.5 x 3.5 µm.*

In order to provide clues as to the identity and function of XRCC1 foci associated with DNA lesions formed in the regions of replicating DNA, we analyzed 45 time-lapse 3D stack recordings, with 1 or 1.5 µm stacks registered at short (18 s) and long (4 min) intervals in order to observe the fast and long-range movements of XRCC1 foci in relation to replication sites. Tracking individual XRCC1 foci during S-phase confirmed the presence of two, previously described distinct subpopulations of these structures (1). The majority of XRCC1 foci made only oscillatory movements at short distances, centered around relatively stable positions (near replication) within the nucleus (Suppl. Fig. 2A, Mov. 3, 4; available at http://helios.wbbib.uj.edu.pl/publikacje). However, there was also a population of XRCC1 foci that were moving in one direction rather than in a Brownian fashion, at a velocity of approximately 1 nm/s, over long distances (approx. 2 µm over 35 min.) (Suppl. Fig. 2B, Mov. 5, 6; available at http://helios.wbbib.uj.edu.pl/publikacje). In some cases the direction of their movement was visibly correlated with the position of nearby replication sites. For instance, the XRCC1 focus shown in Suppl. Fig. 2Ba, or 2Bc, moved directly from one replication region to another (Mov. 5, 6). We hypothesize that the XRCC1 foci associated with DNA lesions in replicating regions occasionally transfer, as one stable entity, to another replication site. Such a transfer might occur upon completion of the repair process in the area, with which the XRCC1 focus was originally associated. The existence of these dynamic foci supports the view that the foci formed near the SSBs within a replication region, or at the site of experimentally induced DNA lesions, constitute reservoirs of numerous copies of XRCC1 and other proteins involved in DDR.

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