SUPPLEMENTARY INFORMATION

SUMOylation of xeroderma pigmentosum group C protein regulates DNA damage recognition during nucleotide excision repair

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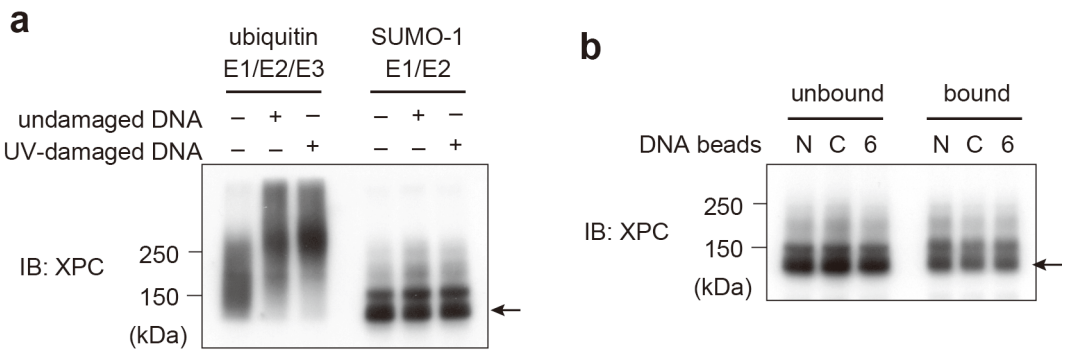
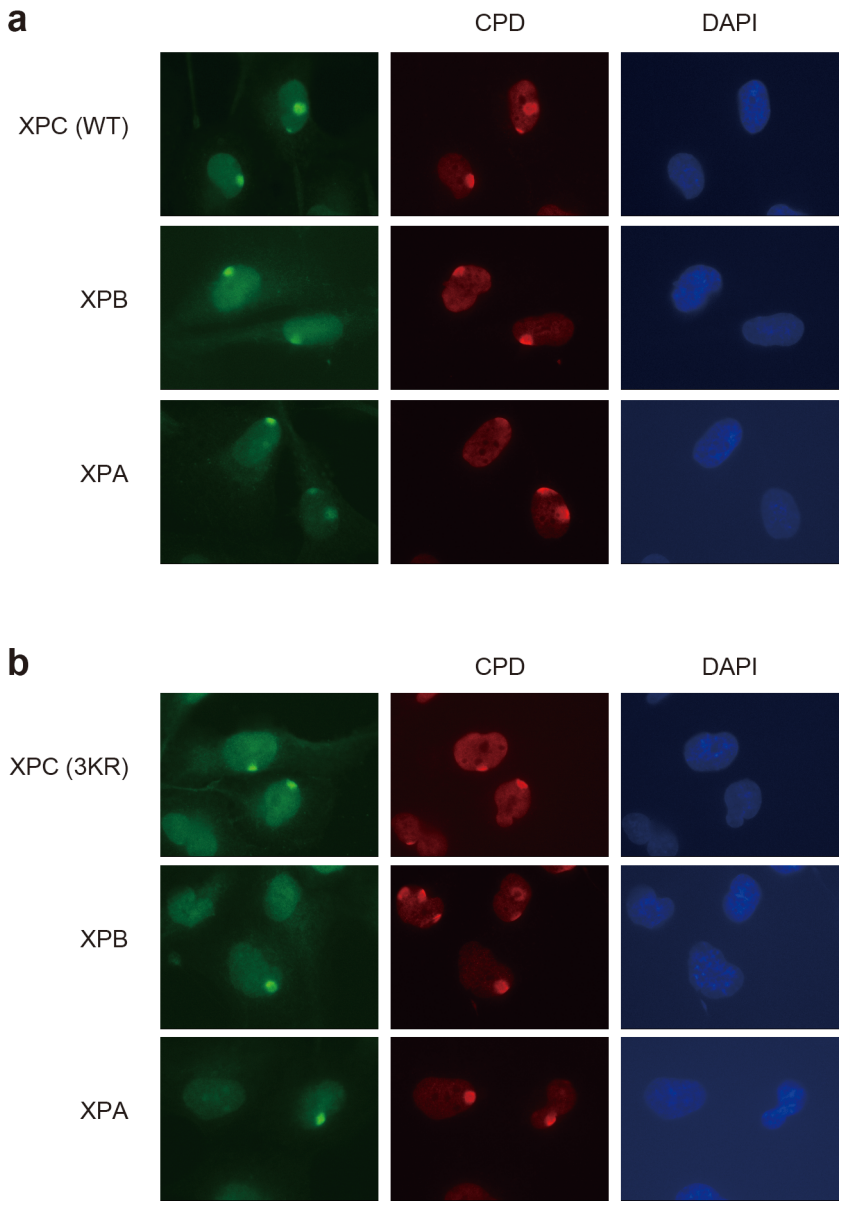
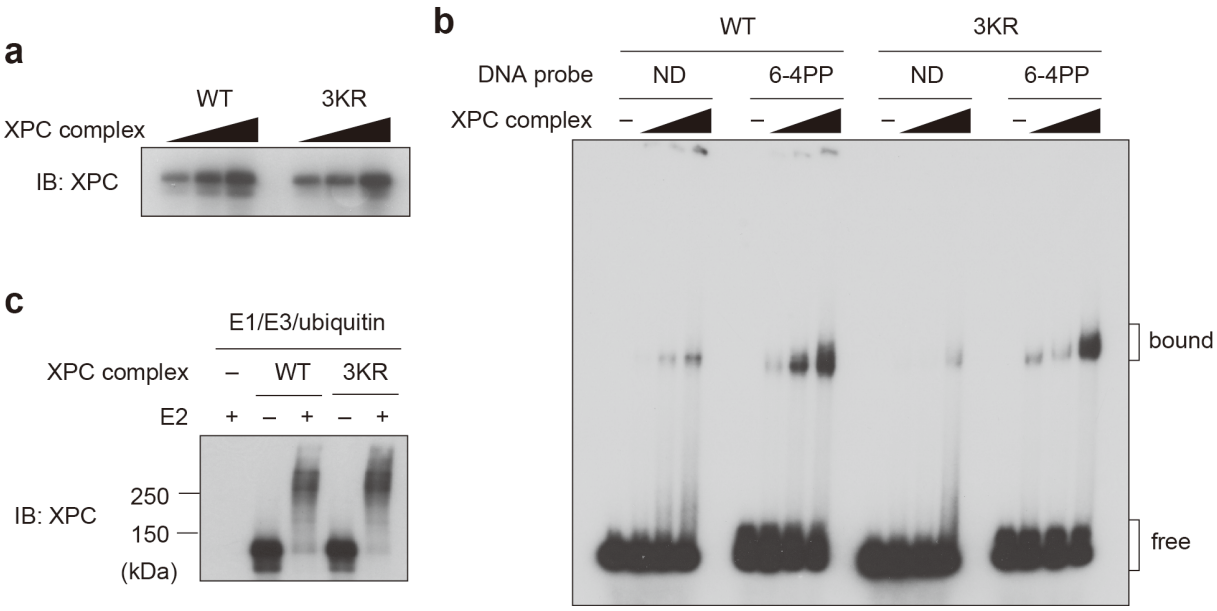


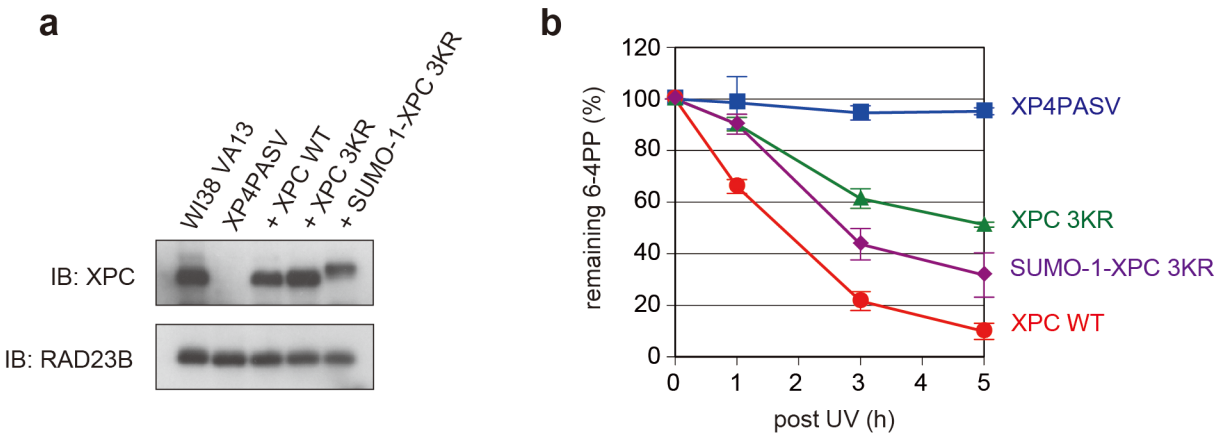
Supplementary Figure S1 | XPC SUMOylation is not stimulated by DNA and does not affect its DNA-binding activity. (a) Immunoblot analyses of XPC from *in vitro* ubiquitination and SUMOylation reactions performed in the presence or absence of undamaged or UV-damaged DNA. (b) Immunoblot analyses of XPC from *in vitro* SUMOylation reactions performed in the presence of paramagnetic beads on which DNA containing no damage (N), cyclobutane pyrimidine dimers (C), or 6-4PP (6) was immobilized. After incubation, the proteins that were bound and unbound to the DNA beads were separated and subjected to immunoblot analyses. The arrows indicate unmodified XPC.



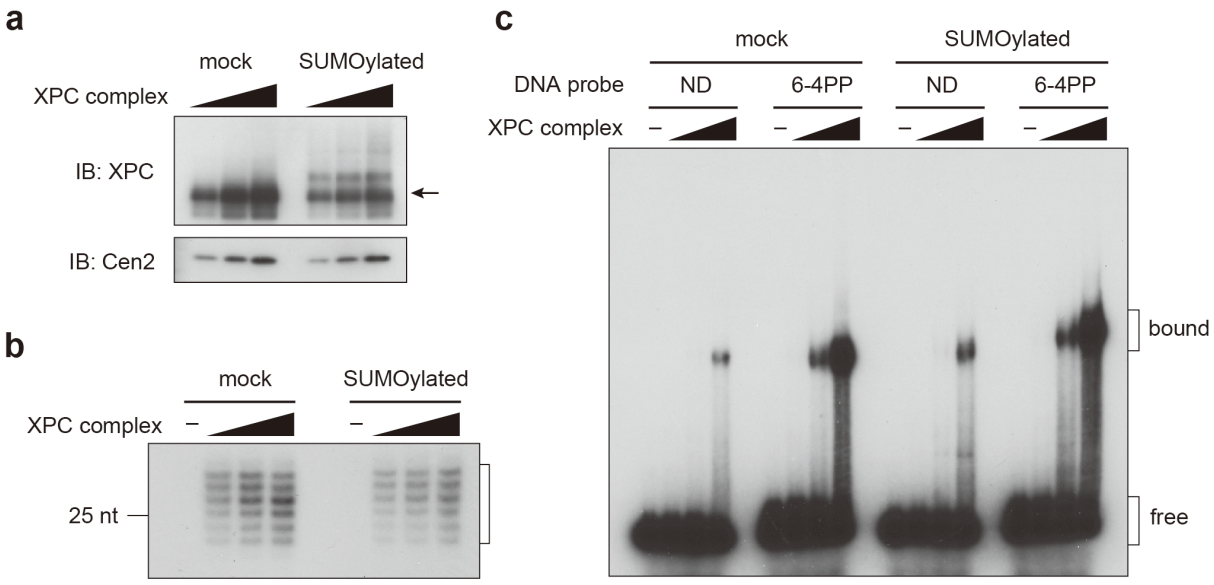
Supplementary Figure S2 | The XPC 3KR mutant is normally recruited to the sites with UV-induced photolesions but compromises recruitment of the downstream NER factors. The transformed XP4PASV cell lines stably expressing the XPC WT (a) or 3KR (b) protein were irradiated with UVC (at 100 J/m^2) through isopore membrane filters. At 30 min post irradiation, the cells were subjected to immunofluorescence staining with antibodies against the indicated NER proteins (green). The presence of UV-induced CPD was also visualized (red), and nuclei were counterstained with DAPI (blue).



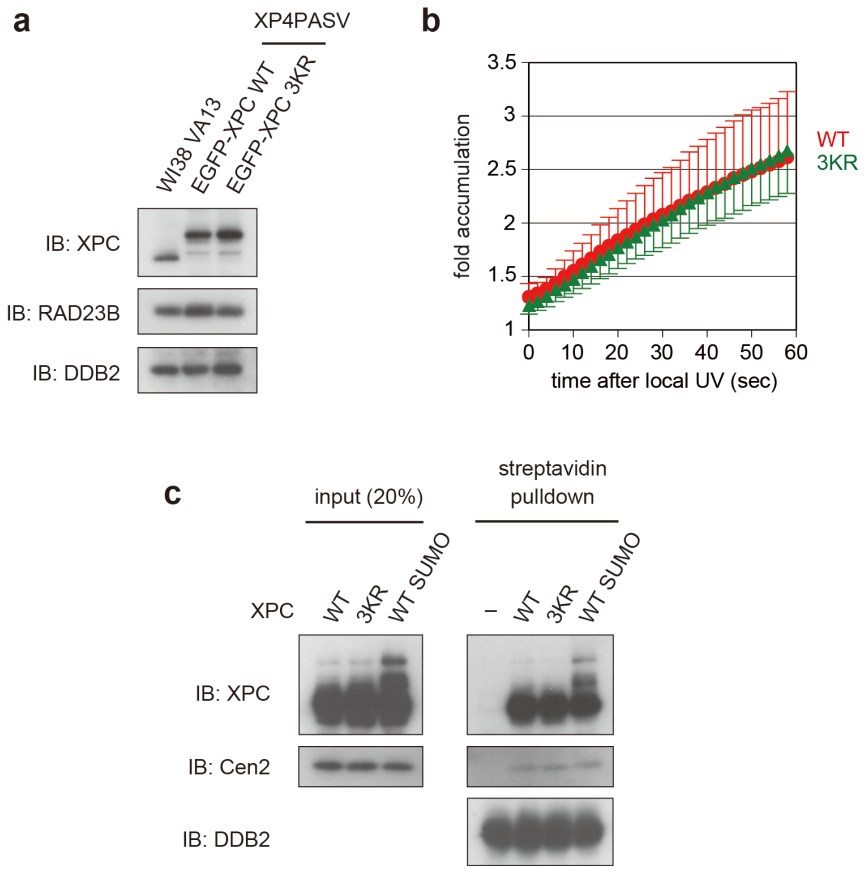
Supplementary Figure S3 |The XPC 3KR mutant protein exhibits normal damage recognition activity and CRL4^{DDB2}-mediated ubiquitination *in vitro*. (a) Immunoblot analyses of the XPC WT and 3KR protein complexes confirming that comparable amounts of proteins were included in the electrophoretic mobility shift assays. (b) Electrophoretic mobility shift assays using the XPC WT or 3KR complex and DNA probes (approximately 180 bp) containing no damage (ND) or a single 6-4PP at a specific site. The positions of the free DNA and XPC-DNA complex (bound) are indicated. (c) Immunoblot analyses of XPC WT and 3KR in cell-free ubiquitination reactions performed in the presence (+) or absence (-) of E2.



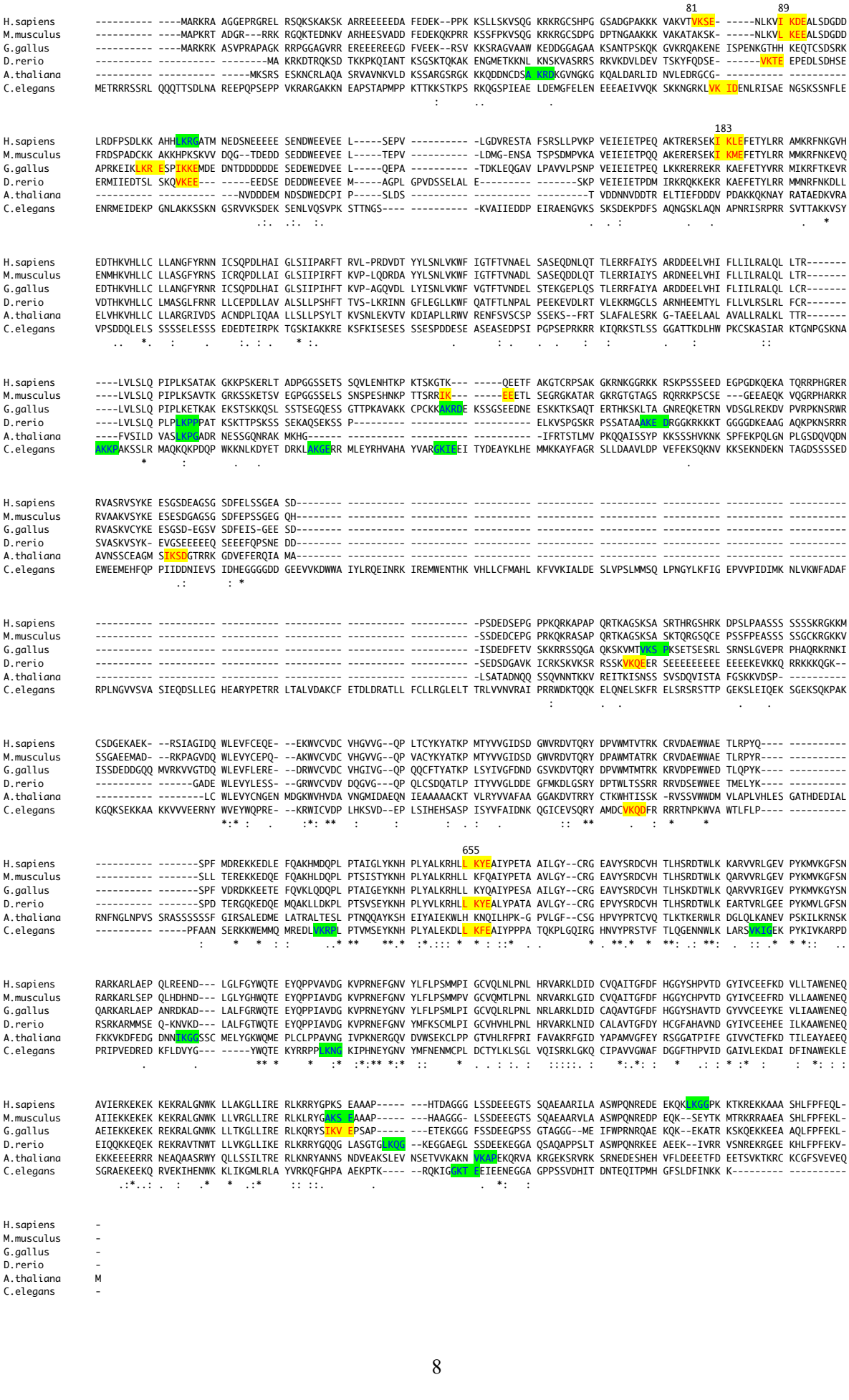
Supplementary Figure S4 | SUMO-1 fusion partially alleviates the NER defect caused by the XPC 3KR mutations. (a) Immunoblot analyses comparing the levels of the ectopically expressed XPC proteins. RAD23B was detected on the same blot as a loading control. (b) DNA repair assays of the indicated cell lines following exposure to UVC (10 J/m^2). The percentages of 6-4PP remaining in the genomic DNA were quantified and plotted as a function of time. The mean values and standard errors were calculated from two independent experiments.



Supplementary Figure S5 | The effects of SUMOylation on NER and the DNA damage recognition activity of the XPC protein *in vitro*. (a) Immunoblot analyses of the recombinant FLAG-XPC WT protein bound to RAD23B-His and centrin-2 (Cen2) and then immobilized on anti-FLAG M2 affinity beads and subjected to *in vitro* SUMOylation reactions. For the mock treatment, a similar reaction was carried out in parallel without E1. After an extensive wash, the proteins were recovered by incubating with the FLAG peptide, and varying amounts were subjected to immunoblot analyses, which confirmed that comparable amounts of proteins were included in the following assays. (b) Cell-free NER dual incision assays using increasing amounts of the protein complexes shown in (A). The excised oligonucleotides containing a 6-4PP are indicated. (c) Electrophoretic mobility shift assays using SUMOylated and unmodified XPC WT proteins and DNA probes containing no damage (ND) or a single 6-4PP at a specific site. The positions of the free DNA and XPC-DNA complex (bound) are indicated.



Supplementary Figure S6 | XPC SUMOylation does not affect its recruitment to local UV damage or physical interaction with UV-DDB. (a) Immunoblot analyses of the transformed XP4PASV cells stably expressing EGFP-tagged XPC WT or 3KR. The expression levels of RAD23B and DDB2 in the same samples were measured as controls. (b) Time-lapse images of the cells stably expressing EGFP-tagged XPC WT or 3KR were obtained after a pulse of focused deep UV light was applied to a certain area within a cell nucleus. For each image, the relative fluorescence intensity of the irradiated area relative to that of a non-irradiated area within the same nucleus was plotted as a function of time. The mean values and standard errors were calculated from analyses of 20 cells per condition. (c) The UV-DDB complex containing biotinylated DDB2 was immobilized on streptavidin-coated paramagnetic beads and used to pull down the purified XPC/RAD23B/centrin-2 heterotrimeric complex containing XPC WT, SUMOylated XPC WT, or XPC 3KR protein. The bound proteins and input samples were subjected to immunoblot analyses using the indicated antibodies.



Supplementary Figure S7 | Evolutionary conservation of the XPC SUMOylation motifs. Amino acid sequences of the XPC orthologs from indicated species are aligned by ClustalW, in which putative SUMOylation motifs predicted by the SUMOplot analyses are highlighted. The motifs with high probability (score 0.9 or higher) are shown in red, whereas those with moderate probability (higher than 0.65 and lower than 0.9) are in blue. The four SUMOylation motifs in human XPC are numbered. Accession numbers of the reference sequences used are as follows: *H. sapiens*, NP_004619.3; *M. musculus*, NP_033557.2; *G. gallus*, XP_414379.3; *D. rerio*; NP_001038675.1; *A. thaliana*, NP_197166.2; *C. elegans*, NP_500156.2.