SUPPLEMENTAL INFORMATION

Title: Removal of reactive oxygen species induced 3'-blocked ends by XPF-ERCC1

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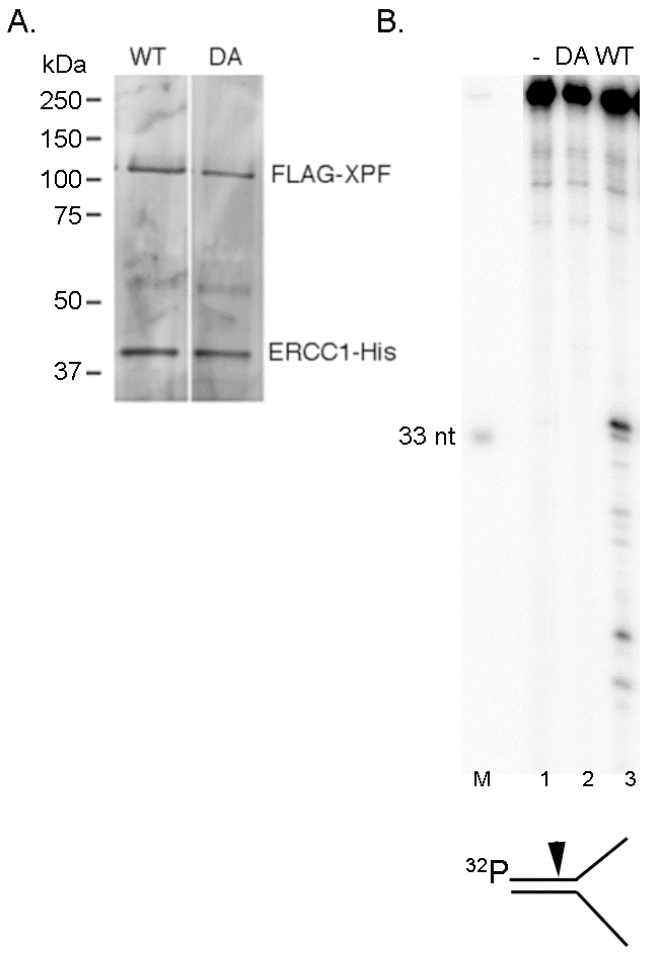

Fig. S-1. **Purification of the XPF-ERCC1 complex from insect cells.** Wild type and the endonuclease deficient XPF(DA)-ERCC1 complex were purified as described in "Experimental Procedures." The XPF(DA) mutant was generated as described in Fisher at al. (A) Approximately 500 ng of the complex were analyzed on an 8% SDS-PAGE with sliver staining. XPF was tagged with FLAG peptide in the N-terminus and ERCC1 was tagged with 6 x His at the C-terminus. (B) In vitro endonuclease assay (Fisher et al.). XPF-ERCC1 (35 nM) was incubated with a 5'-end labeled splay substrate (2 nM) in the reaction buffer (10 mM HEPES, 25 mM KCl, 0.05 mM EDTA, 0.5 mM DTT, 10% glycerol, and 5 mM $MgCl_2$) at 30°C for 30 min. The reaction products were analyzed on a 10% denaturing PAGE. M, a 33 nt size marker; lane 1, no XPF-ERCC1; lane 2, the endonuclease defective XPF(DA)-ERCC1; lane 4, wild type XPF-ERCC1.

Fisher, L. A.; Bessho, M.; Bessho, T., Processing of a psoralen DNA interstrand cross-link by XPF-ERCC1 complex in vitro. *J Biol Chem* **2008,** 283, (3), 1275-81.

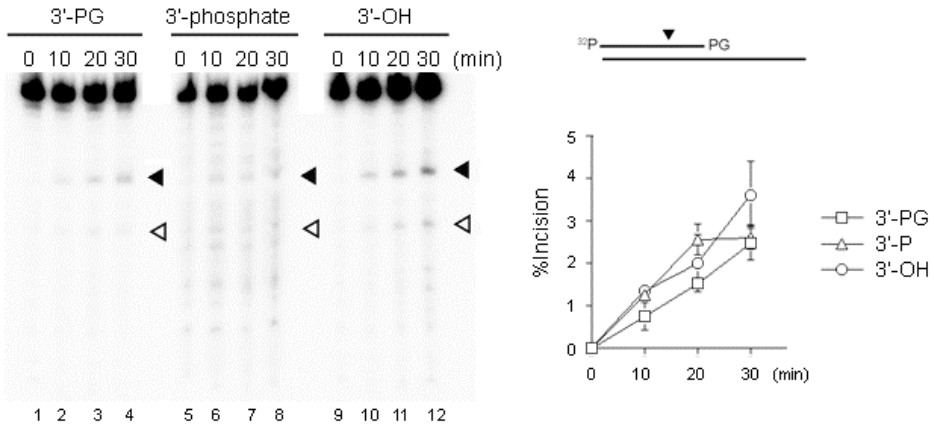

Fig. S-2. XPF-ERCC1 (35 nM) was incubated with recessed substrate (DS/SS) at 30°C for the indicated time points. The reaction products were analyzed on 10% sequencing gels. Closed triangles and open triangles represent 16 nt and 14 nt fragments, respectively. The average of the percent of the incision products to the total amount of substrate at each time point was determined from three independent experiments and plotted in a graph. The error bars represent standard deviations. No preferential incision by XPF-ERCC1 on the damaged 3'-end was detected.

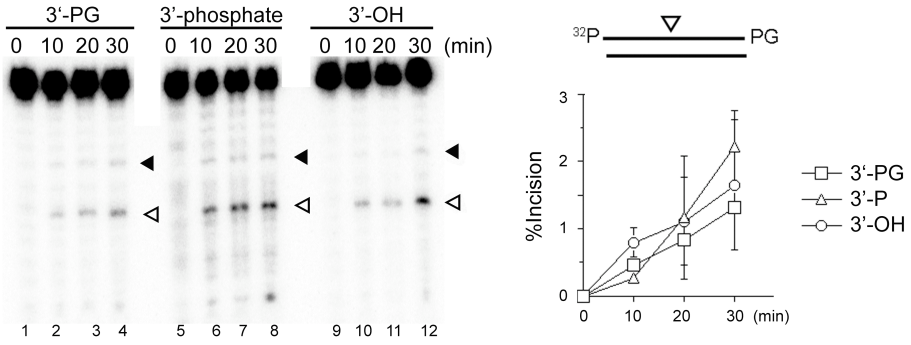

Fig. S-3. XPF-ERCC1 (35 nM) was incubated with blunt end substrate (DS) at 30°C for the indicated time points. The reaction products were analyzed on 10% sequencing gels. Closed triangles and open triangles represent 16 nt and 14 nt fragments, respectively. The average of the percent of the incision products to the total amount of substrate at each time point was determined from three independent experiments and plotted in a graph. The error bars represent standard deviations. No preferential incision by the XPF-ERCC1 on damaged 3'-end was detected.

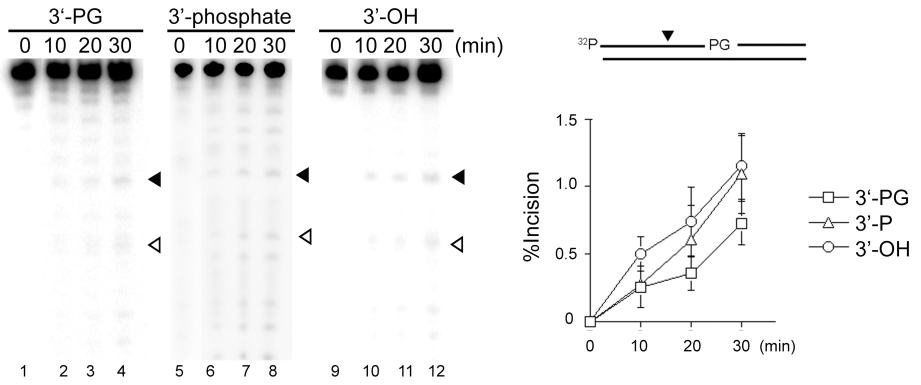

Fig. S-4. XPF-ERCC1 (35 nM) was incubated with nicked substrate (Nick) at 30°C for the indicated time points. The reaction products were analyzed on 10% sequencing gels. Closed triangles and open triangles represent 16 nt and 14 nt fragments, respectively. The average of the percent of the incision products to the total amount of substrate at each time point was determined from three independent experiments and plotted in a graph. The error bars represent standard deviations. No preferential incision by XPF-ERCC1 on the damaged 3'-end was detected.

Table S-1. Survival fractions after treatment with ROS-generating agents. The values are the average of three independent experiments with ± standard deviations.

	No treatment	0.2 mM H_2O_2	2 mM H_2O_2
AA8	1	0.39 ± 0.04	0.16 ± 0.01
UV41	1	0.14 ± 0.03	0.004 ± 0.001
UV41 + XPF	1	0.67 ± 0.04	0.17 ± 0.03
UV20	1	0.14 ± 0.03	0.008 ± 0.003

	No treatment	50 μM bleomycin	100 μM bleomycin
AA8	1	0.76 ± 0.05	0.55 ± 0.04
UV41	1	0.49 ± 0.03	0.44 ± 0.17

	No treatment	0.05 μM paraquat	0.1 μM paraquat
AA8	1	0.18 ± 0.008	0.058 ± 0.03
UV41	1	0.11 ± 0.02	0.025 ± 0.001