Supplementary information

Materials and methods

γ-irradiation

^{137}Cs (0.02 Gy/s; Gammacell 40) was used for γ-irradiation.

Plasmid constructs

Two *XPF* disruption constructs, *XPF*-*hisD* and *XPF*-*bsr*, were generated from genomic PCR products combined with *hisD*- and *bsr*-selection marker cassettes (Fig. 1 A). Genomic DNA sequences of the *XPF* promoter region were amplified by inverse PCR using primers X-1(+),GGTGCTGGTGCTGAACACGAGC and X-2(-),CCTGATGGAACAGGTCCAGGAAGATCTGGC (for the left arm of the disruption construct) and cloned into the pCR2.1-TOPO vector (Invitrogen). This plasmid was digested with *Hind*III and self-ligated. Genomic DNA sequences of the *XPF* 3' region were amplified by X-3(+),GGTGCTGGTGCTGAACACGAGC and X-4(-),CAGTGGAAACAGAGGCAGGCTTAACATCTC (for the right arm of the disruption construct) and amplified PCR products were cloned into the pCR2.1-TOPO vector (Invitrogen). The 3.4 kb *Eco*RV-*Apa*I fragment from the right arm was cloned into the *Eco*RV and the *Apa*I site of the pCR2.1/XPF-5' arm. The *Eco*RV site between the two arms was used to clone blunt-ended marker-gene cassettes. For the probe used in the Southern blot analysis, the genomic DNA was amplified using primers X-5(+),GGTGCTGGTGCTGAACACGAGC and X-6(-),CAGTGGAAACAGAGGCAGGCTTAACATCTC and was digested with *Apa*I to obtain a 2.1 kb fragment. To generate the expression vector, pCR3-loxP-*GdXPF*/IRES-EGFP-loxP, we amplified *XPF* cDNA using the primers X-7(+),CGCGTCGACATGGCGGCGCTGCTGGAGCACGAGAGCCAG and X-8(-),CGCGGATCCTCATCTTTTGTTTTTTTCTTTAGATAGCGC, and inserted the *Sal*I-*Bam*HI fragment of the resulting cDNA between the *Sal*I and *Bam*HI sites of pCR3-*loxP*-MCS-*loxP* (1). Nuclease-deficient *XPF* cDNAs were amplified with primers X-9(+),CGCGGTACCATGGCGGCGCTGCTGGAGCACGAGAGCCAG, X-10(-),CGCGGGCCCTCATCTTTTGTTTTTTTCTTTAGATAGCGC, D674A(+),CTGCTCAACAGACTATAATAGTGGCTATGCGGGAATTTCGTAGTGAGCTT, D674A(-),AAGCTCACTACGAAATTCCCGCATAGCCACTATTATAGTCTGTTGAG

CAG,
D702A(+),GAGCCTGTTACTTTGGAAGTTGGAGCTTACATTTTAACTCCTGATAT
CTG, and
D702(-),CAGATATCAGGAGTTAAAATGTAAGCTCCAACTTCCAAAGTAACAGG
CTC. The primers
X-11(+),CGCGGATCCTATCCCTATGACGTCCCGGACTATGCATATCCCTATGAC
GTCCCGGACTATGCAGGAGGAGGAGGAGGAATGGCGGCGCTGCTGGAGCAC
GAGAGCCAG and
X-12(-),CGCGGGCCCTCATCTTTGTTTTTTCTTTAGATAGCGC were used to insert an HA-tagged *XPF* cDNA fragment into the pCMV-3Tag vector (Stratagene). The chicken *XPF*, each variant (1 to 5) of *EME1*, and *EME2* cDNA sequences have been submitted to the GenBank database under accession numbers AB085766, AB266761, AB266762, AB266763, AB266764, AB266765, and AB266766, respectively.

Identification of proteins that physically interact with Eme1 and Xpf

To identify proteins that are associated with Eme1 and Xpf, we ectopically expressed TAP-tagged GdEme1 and 3x Flag-2xHA-tagged GdXpf in DT40 cells. Cells (1×10^9) were suspended in lysis buffer (50 mM sodium phosphate at pH 8.0, 0.3 M NaCl, 0.1% NP-40, 5 mM β-mercaptoethanol) with phosphatase inhibitors (10 mM sodium pyrophosphate, 5 mM sodium azide, 10 mM NaF, 0.4 mM sodium orthovanadate, 20 mM β-glycerophosphate) and a complete protease inhibitor (Roche). After sonication (Tomy Seiko, Handy Sonic, UR-20P), the lysate was centrifuged at 15,000 rpm for 10 min at 4° C, after which a supernatant fraction was collected. Anti-Flag M2-beads (SIGMA) were incubated with the supernatant fraction for 2 h at 4° C, and the resin was washed with lysis buffer and then eluted with lysis buffer in the presence of 3x FLAG peptide (SIGMA). Mass-spectrometric identification of proteins was performed as previously described (2).

Western blotting and immunoprecipitation

Methods for western blotting and immunoprecipitation were described previously (3). To immunoprecipitate proteins, cells were washed twice with phosphate-buffered saline (PBS) and lyzed with lysis buffer (20 mM Tris-HCl at pH 8.0, 137 mM NaCl, 10% Glycerol, 1% N Nonidet P-40 (NP40) with protease inhibitors (1 mM

phenylmethylsulfonylfluoride (PMSF), 1 μg/ml leupeptin, and 1 μg/ml aprotinin) and protein phosphatase inhibitors (5 mM sodium fluoride, 1 mM sodium orthovanadate, and 10 mM β-glycerophosphate) for 20 min on ice. After centrifugation, the supernatant was collected and incubated with appropriate antibodies for 2 h at 4° C, and then 20 μl of protein G Sepharose (50% slurry) was added. The mixtures were placed on a rotary mixer for 1 h at 4°C. The beads were then washed once with lysis buffer, twice with 0.5 M LiCl/0.1 M Tris-HCl at pH7.5, and once with 10 mM Tris-HCl at pH 7.5, and finally resuspended in Laemmli's sample buffer.

Purification of Recombinant HsXpf-Eme1

Recombinant baculoviruse was constructed using a Bac-to-Bac system (Invitrogen). Flag-Xpf and Eme1 were co-expressed in HighFive insect cells. Cell monolayers cultured 150 mm dishes were washed twice with ice-cold PBS and lysed with 0.5 ml of ice-cold NP lysis buffer (25 mM Tris-HCl at pH 8.0, 1 mM EDTA, 10% Glycerol, 1% NP40, 1 mM dithiothreitol (DTT), 0.25 mM PMSF, 1× protease inhibitor cocktail [Complete; Roche Diagnostics]) containing 0.3 M NaCl. After 30 min of stirring on ice, the lysate was clarified by centrifugation (20,000*g* for 20 min), and dialyzed against buffer A (20 mM sodium phosphate at pH 7.8, 1 mM EDTA, 10% Glycerol, 1 mM DTT, and 0.25 mM PMSF). Insoluble materials were removed by centrifugation (20,000*g* for 30 min). The resulting extract was loaded onto HiPrep-heparin 10/16 column (Amersham Pharmacia Biotech) equilibrated with buffer B (20 mM sodium phosphate at pH 7.8, 0.1 M NaCl, 1 mM EDTA, 10% Glycerol, Triton X-100 0.01%, 1 mM DTT, and 0.25 mM PMSF) containing 0.1 M NaCl. After extensive washing with buffer B containing 0.1 M NaCl, the protein was eluted stepwise with buffer B containing 0.4, and 1 M NaCl, and the Xpf complex was recovered in the 0.4 M NaCl eluate. The haparin-Sepharose 0.4 M NaCl eluate was loaded onto anti-Flag M2 agarose column (Tricorn 10/50; GE Healthcare Bioscience), equilibrated with buffer B containing 0.1 M NaCl. After the column was washed with the same buffer, bound proteins were eluted with 0.1mg/ml Flag peptide. The fractions containing Flag-Xpf were loaded onto a Mono S PC 1.6/5 column that had been connected to a SMART system (GE Healthcare Bioscience) and equilibrated with buffer B containing 0.1 M NaCl. The bound proteins were eluated with a 3-ml gradient of 0.1 to 1 M NaCl in buffer B, where Flag-Xpf was collected from the fractions around 0.4 M NaCl. Flag-Xpf was detected by western blotting, and Eme1 was confirmed by MS

analysis. Finally, the sample was subjected to gel filtration chromatography using a Superdex *200 GL* column (GE Healthcare Bioscience) equilibrated with buffer B containing 0.5 M NaCl.

Generation of Xpf-deficient cells

Our experimental strategy is described in Supplementary Fig. S6B. Chicken DT40 cells were cultured at 39.5° C in RPMI-1640 medium supplemented with 10^{-5} M β-mercaptoethanol, 10% fetal bovine serum (FBS), and 1% chicken serum. The *XPF-bsr-loxP* targeting construct was transfected into *wild-type* cells harboring pANMerCreMer-*hyg* (4). To excise the *bsr-loxP* in $XPF^{+/-}$ cells, the cells were exposed to Tamoxifen (TAM) for 3 days and subcloned ($XPF^{+/-}$* clone). The $XPF^{+/-}$* clones were transfected with pCR3-*loxP*-*GdXPF*/IRES-EGFP-*loxP* expression vectors, followed by selection with G418 (2 mg/ml). Among stable transfectants, clones that expressed the greatest amounts of GFP were identified using FACScaliber (Becton Dickinson) and isolated, then exposed to TAM, as previously described. Deletion of the chicken *XPF* transgene was examined using FACScaliber. $XPF^{+/-}$**GdXPF-loxP* clones were transfected with the *XPF-bsr* targeting construct to obtain $XPF^{-/-}$*GdXPF-loxP* clones.

Generation of $XPF^{-/-}$*GdXPF-loxP/XRCC3*$^{-/-}$ cells

Both *XRCC3* alleles were deleted in the $XPF^{-/-}$*GdXPF-loxP* cells using the *XRCC3* targeting construct (5). Deletion of *XRCC3* was confirmed by Southern blot analysis. Deletion of the chicken *XPF* transgene was examined using FACScaliber.

Generation of $XPF^{-/-}$*GdXPF-loxP* cells expressing RusA, HsXpf-Ercc1, and HsMus81-Eme1

To generate $XPF^{-/-}$*GdXPF-loxP* cells expressing RusA, mammalian expression vector for RusA-fused nuclear localization signal and GFP (NLS-RusA-GFP) provided by M.C. Whitby was co-transfected with an Eco-gpt marker plasmid into $XPF^{-/-}$*GdXPF-loxP* cells, followed by selection with 30 μg/ml of mycophenolic acid. Expression of NLS-RusA-GFP was confirmed by western blotting and RT-PCR. To generate $XPF^{-/-}$*GdXPF-loxP* cells expressing HsXpf-Ercc1, pEYFP-N1-HsXpf provided by L.J. Niedernhofer was co-transfected with a puromycin-resistant-marker plasmid into $XPF^{-/-}$*GdXPF-loxP* cells, followed by selection with 0.5 μg/ml of puromycin. Expression

of EYFP-HsXpf was confirmed by RT-PCR. Next, pCMV6-XL4-ERCC1 purchased from OriGene (Rockville, MD, USA) was co-transfected with *hisD*^{R} marker plasmid into *XPF*$^{-/-}$*GdXPF-loxP* cells expressing EYFP-HsXpf. Expression of HsErcc1 was confirmed by western blotting. To generate *XPF*$^{-/-}$*GdXPF-loxP* cells expressing HsMus81-Eme1, hMus81 and hEme1 were introduced into *XPF*$^{-/-}$*GdXPF-loxP* cells by retroviral infection using pMSCV-hMus81 and pMSCV-hEme1. After subcloning, expression of HsMus81-Eme1 was confirmed by RT-PCR.

siRNA and antibodies for measurement of chromosome aberrations in HeLa cells.
HeLa cells were maintained in DMEM supplemented with 10% FBS and penicillin-streptomycin. The siRNAs and the antibodies used in this assay were obtained from commercial sources: *MUS81* siRNA; Thermo (L-016143-01-0005) pooled CAGCCCUGGUGGAUCGAUA, GGGUAUACCUGGUGGAAGA, CAGGAGCCAUCAAGAAUAA, GGGAGCACCUGAAUCCUAA, *XPF* siRNA; Thermo (L-019946-00-0005) pooled CCAAACAGCUUUAUGAUUU, GCACCUCGAUGUUUAUAAA, CGGAAGAAAUUAAGCAUGA, UGACAAGGGUACUACAUGA, Anti-Xpf antibody; Abcam (ab17798), Anti-Mus81 antibody; Abcam (ab14387), Anti-β actin antibody; SIGMA (A5441). Each primary antibody was used at a 1:1000 dilution. HRP-conjugated mouse IgG secondary antibody (Santa Cruz, sc2314) was used at a 1:5000 dilution.

siRNA and antibodies for measurement of chromosome aberrations in mouse ES cells.
Mouse *wild-type* (IB10) and *MUS81*$^{-/-}$ ES cells were cultured on gelatin-coated dishes in a 1:1 mixture of DMEM and buffalo rat liver conditioned medium, supplemented with 10% FBS, 0.1 mM nonessential amino acids, 50 μM β-mercaptoethanol and 500 U ml^{-1} leukemia inhibitory factor (6). The siRNAs used in this assay was obtained from commercial sources: *XPF* (*ERCC4*) siRNA; Thermo (L-045003-01-0005) pooled CCACGGAAGAGCAGCGCUA, GGAGCGUGCUUCCGCCAAA, GCUAUGAAGUCUACACGCA, GUAGAGAUUAAGCGUGAAU. Anti-Xpf antibody gifted by R. Kanaar was used at a 1:500 dilution. HRP-conjugated rabbit IgG secondary antibody (Jackson ImmunoResearch Laboratories) was used at a 1:5000 dilution.