Figure S1. Sequence alignment among human, mouse, and chicken Xpf. Purple, green, and orange lines indicate disrupted SF2-family helicase domains, Ercc4 domain, and Ercc1 binding region, respectively. Grey and black blocks represent identical amino residues in two and three species, respectively. Red (D674) and blue (D702) blocks are replaced by alanine for inactivation of nuclease activity. The yellow boxes indicate amino-acid sequences determined by mass spectrometric analysis of proteins precipitated together with tagged Eme1.

Figure S2. Identification of the interaction partners for Xpf in DT40 cells. (A) Ercc1 and Slx4 are immunoprecipitants of tagged Xpf. Lysates derived from *wild-type* and *XPF*$^{-/-}$*/GdXPF-Flag* DT40 cells were subjected to Immunoprecipitation with anti-FLAG antibody. Immunoprecipitants were separated on an SDS-PAGE gel and visualized by silver staining. Bands indicated by arrows were excised and subjected to mass spectrometric analysis. (B) The sequence alignment among human, mouse, and chicken Ercc1. Grey and black blocks represent identical amino residues in two and three species, respectively. The yellow boxes indicate amino acid sequences determined by mass spectrometric analysis of proteins precipitated together with tagged Xpf.

Figure S3. Ortholog gene of *MUS81* is not registered in the chicken database. (A) Human *MUS81* was compared with each of chimpanzee, dog, mouse, rat, chicken, fugu, and zebrafish. (B) Sequence alignment among human, mouse, and chicken Eme1. Chicken Eme1 was cloned by using synthesized cDNAs derived from DT40 cells. Grey and black blocks show identical amino residues in two and three species, respectively.

Figure S4. Co-immunoprecipitation of Xpf with Eme1 or Slx4. (A) Xpf is an immunoprecipitant of tagged Eme1. Lysates derived from *wild-type* and GdEme1-TAP-expressing DT40 cells were subjected to tandem affinity purification (TAP). TAP-purified proteins were separated on a 10% SDS-PAGE gel and visualized by silver staining. Bands indicated by arrows were excised and subjected to mass spectrometric analysis. (B) Lysates from HEK293 cells co-expressing TAP-GdXpf or TAP-GdEme1with Flag-GdEme1 or Flag-HA-GdXpf were immunoprecipitated with IgG beads, respectively, and the immunoprecipitates were probed with anti-CBP and anti-Flag antibodies. (C) Upper panels, gel filtration profiles of mono S fractions. Lower

panels, the gel filtration fractions were analyzed by Western blotting against Flag-Xpf and Eme1. Flag-Xpf and Eme1 were co-purified from fraction 6 to 9. (D) Lysates from HEK293 cells co-expressing Flag-HA-GdXpf with GFP-GdSlx4 were immunoprecipitated with anti-Flag or anti-GFP antibody, and the immunoprecipitates were probed with anti-GFP and anti-Flag antibodies.

Figure S5. Schematic representation of *EME1* disruption in DT40 cells. (A) Disruption of the chicken *EME1* gene. Left: Schematic representation of partial restriction map of the Gd*EME1* locus, the two gene disruption construct and the configuration of the targeted loci. Black boxes indicate the positions of exons. Relevant *Eco*RI restriction sites are shown here. Right: Southern blot analysis of *wild type* (+/+), heterozygous mutant (+/-) and homozygous mutant (-/-) clones. *Eco*RI digested genomic DNA was hybridized with the probe shown in the left. (B) Growth curves of cells with the indicated genotype. The experiment was repeated at least three times. (C) Sensitivity to the indicated genotoxic agents in *wild-type* and *EME1*$^{-/-}$ DT40 cells. Cells with the indicated genotype were exposed to each genotoxic agent. The experiment was repeated at least three times and three independent *EME1*$^{-/-}$ clones was used.

Figure S6. Schematic representation of *XPF* disruption in DT40 cells. (A) Southern blot analysis for disruption of the chicken *XPF* gene. *Eco*RV-digested genomic DNA of *wild-type* (+/+), heterozygous mutant (+/-), heterozygous mutant with marker gene being excised (+/-*), and homozygous mutant (-/-) cells was hybridized with the probe shown in Fig. 1A. (B) Strategy for generating *XPF*$^{-/-}$*GdXPF-loxP* cells. (C) Transgene excision by the chimeric Cre recombinase carrying the TAM-binding domain. (D) Transgene excision was confirmed by monitoring the loss of GFP expression (x-axis) before (left) and 1, 2, 3, and 4 days after addition of TAM at time zero. Propium-iodine (PI) staining of dead cells is indicated on the y-axis. (E) Xpf mRNA in *XPF*$^{-/-}$*GdXPF-loxP* cells was confirmed by RT-PCR. Cells were extracted before, and 2 and 3 days after addition of TAM, and same amounts of total RNA were used for RT-PCR. (F) Growth curve without the TAM treatment in the indicated cells. (G) Top panels, all plasmids made in this study (TAP-Xpf *wild-type*, TAP-Xpf D674A, and TAP-Xpf D702A) can work well under conditions of transient expression, although we could not obtain any stable DT40 clones expressing the TAP-tagged mutant Xpf in contrast to a *wild-type* Xpf transgene. Bottom

panels, the number of clones analyzed by Western blotting to obtain the stable DT40 clones.

Figure S7. Xpf and Mus81 are compensatory to each other in the completion of HR in human and mouse cell lines. (A) HeLa cells were treated with either control siRNA or siRNA against *MUS81* and/or *XPF*. Depletion efficiency was analyzed in whole-cell extracts by western blotting against Xpf and Mus81. β-Actin provided a loading control. (B) Mouse *wild-type* or *MUS81*$^{-/-}$ ES cells were treated with either control siRNA or siRNA against *XPF*. Depletion efficiency was analyzed in whole-cell extracts by western blotting against Xpf. β-Actin provided a loading control.

1. Kikuchi K, Taniguchi, Y., Hatanaka, A., Sonoda, E., Hochegger, H., Adachi, N., Matsuzaki, Y., Koyama, H., van Gent, D.C., Jasin, M., and Takeda, S. **.** Fen-1 facilitates homologous recombination by removing divergent sequences at DNA break ends. Mol. Cell. Biol. 2005;25:6948-55.
2. Okada M, Cheeseman, I.M., Hori, T., Okawa, K., McLeod, I.X., Yates, J.R. 3rd, Desai, A., and Fukagawa, T. The CENP-H-I complex is required for the efficient incorporation of newly synthesized CENP-A into centromeres. Nat. Cell Biol. 2006;8:446-57.
3. Kikuchi K, Niikura, Y., Kitagawa, K., and Kikuchi, A. Dishevelled, a Wnt signalling component, is involved in mitotic progression in cooperation with Plk1. EMBO J. 2010;29(20):3470-83.
4. Zhang Y, Wienands, J., Zurn, C., and Reth, M. Induction of the antigen receptor expression on B lymphocytes results in rapid competence for signaling of SLP-65 and Syk. EMBO J. 1998;17:7304-10.
5. Takata M, Sasaki, M.S., Tachiiri, S., Fukushima, T., Sonoda, E., Schild, D., Thompson, L.H., and Takeda, S. Chromosome instability and defective recombinational repair in knockout mutants of the five Rad51 paralog. Mol. Cell. Biol. 2001;21:2858-66.
6. Hanada K, Budzowska, M., Davies, S.L., van Drunen, E., Onizawa, H., Beverloo, H.B., Maas, A,, Essers, J., Hickson, I.D., and Kanaar, R. The structure-specific endonuclease Mus81 contributes to replication restart by generating

double-strand DNA breaks. . Nat. Struct. Mol. Biol. 2007;14:1096-104.