Figure S1. Addition of SLX4 to SLX4-depleted extract rescues ICL repair. Related to Figure 1.

(A) Schematic representation of replication-dependent ICL repair in Xenopus egg extract.

(B) Recombinant *xl*SLX4^{WT}, containing an N-terminal FLAG-tag and a C-terminal Strep-tag, was expressed in Sf9 insect cells and isolated via affinity purification using anti-FLAG M2 affinity gel (Sigma). Purity and concentration were determined using Coomassie blue staining of samples on SDS-PAGE.

(C) Recombinant FLAG-*xl*XPF-His-*hs*ERCC1 complex was isolated via affinity purification using NiNTA resin and anti-FLAG M2 affinity gel (Sigma). Purity and concentration were determined using Coomassie blue staining of samples on SDS-PAGE.

(D) Mock-depleted (Mock), SLX4-depleted (ΔSLX4), and SLX4-depleted NPE complemented with wild-type SLX4 (ΔSLX4+SLX4^{WT}) were analyzed by western blot using α-SLX4 antibody (right panel). These extracts, with SLX4-depleted HSS, were used to replicate pICL. Repair efficiency was calculated and plotted (left panel). Independent duplicate experiment related to Figure 1D.

(E) SLX4-depleted (ΔSLX4), and SLX4-depleted NPE complemented with wild-type SLX4 (ΔSLX4+SLX4^{WT}) or wild-type SLX4 and XPF-ERCC1 (ΔSLX4+SXE) were analyzed by western blot using α-SLX4 (upper right panel) or α-XPF antibodies (lower right panel). These extracts, with SLX4-depleted HSS, were used to replicate pICL. Repair efficiency was calculated and plotted (left panel). Line within blot indicates position where irrelevant lanes were removed. Independent duplicate experiment related to Figure 1E. #, SapI fragments from contaminating uncrosslinked plasmid present in varying degrees in different pICL preparations.

Figure S2. SLX1 is not required for ICL repair. Related to Figure 2.

(A) Schematic illustration of the *xl*SLX4^{SBD*} mutant protein. The C1753R mutation is indicated by a red line.

(B) Fragment of a sequence alignment between *Xenopus laevis* (*xl*SLX4), human (*hs*SLX4), and mouse (*mm*SLX4) by Clustal Omega. The red box in the alignment indicates the residue in the wild-type proteins that are mutated in SLX4^{SBD*}, the R above the red box indicates the substitution to an arginine residue. (*), conserved residue; (:), conservative residue, (.), semi-conservative residue.

(C) Recombinant *xl*SLX4^{SBD*}, containing an N-terminal FLAG-tag and a C-terminal Strep-tag, was expressed in Sf9 insect cells and isolated via affinity purification using anti-FLAG M2 affinity gel (Sigma). Purity and concentration were determined using Coomassie blue staining of samples on SDS-PAGE.

(D) Recombinant *xl*SLX4, containing an N-terminal FLAG-tag and a C-terminal Strep-tag, and recombinant *xl*SLX1, containing an N-terminal FLAG-tag, were co-expressed in Sf9 cells and isolated via affinity purification using anti-FLAG M2 affinity gel (Sigma). Purity and concentration were determined using Coomassie blue staining of samples on SDS-PAGE.

(E) SLX4-depleted (ΔSLX4), and SLX4-depleted NPE complemented with wild-type SLX4 (ΔSLX4+SLX4^{WT}) or mutant SLX4 (ΔSLX4+SLX4^{SBD*}) were analyzed by western blot using α-SLX4 antibody (right panel). These extracts, with SLX4-depleted HSS, were used to replicate pICL. Repair efficiency was calculated and plotted (left panel). Line within blot indicates position where irrelevant lanes were removed. Independent duplicate experiment related to Figure 2B.

(F) NPE was analyzed by western blot using α-SLX1 antibody alongside a dilution series of purified *xl*SLX4-*xl*SLX1 protein. Line within blot indicates position where irrelevant lanes were removed.

(G) Mock- and SLX1-depleted NPE were analyzed by western blot using α-XPF (upper panel) and α-ERCC1 antibodies (lower panel). A dilution series of undepleted extract was loaded on the same blots to determine the degree of depletion. A relative volume of 100 corresponds to 0.4 μL NPE.

(H) Mock-depleted (Mock), SLX1-depleted (ΔSLX1), and SLX1-depleted NPE complemented with wild-type SLX4 (ΔSLX1+SLX4^{WT}) or wild-type SLX4-SLX1 complex (ΔSLX4+SLX4/1) were analyzed by western blot using α-SLX1 (upper right panel) and α-SLX4 antibodies (lower right panel). These extracts, with SLX1-depleted HSS, were used to replicate pICL. Repair efficiency was calculated and plotted (left panel). Independent duplicate experiment related to Figure 2F. *, background band. #, SapI fragments from contaminating uncrosslinked plasmid present in varying degrees in different pICL preparations.

Figure S3. The C-terminal half of SLX4 is dispensable for ICL repair. Related to Figure 3.

(A) Schematic illustration of the *xl*SLX4$^{\Delta C}$ mutant protein. This protein is truncated at residue 840.

(B) Recombinant *xl*SLX4$^{\Delta C}$, containing an N-terminal FLAG-tag, was expressed in Sf9 insect cells and isolated via affinity purification using anti-FLAG M2 affinity gel (Sigma). Purity and concentration were determined using Coomassie blue staining of samples on SDS-PAGE.

(C) SLX4-depleted (ΔSLX4), and SLX4-depleted NPE complemented with wild-type SLX4 (ΔSLX4+SLX4^{WT}) or mutant SLX4 (ΔSLX4+SLX4$^{\Delta C}$), were used with SLX4-depleted HSS to replicate pICL. Repair efficiency was calculated and plotted. Independent duplicate experiment related to Figure 3C.

(D) Purified wild-type SLX4 (SLX4^{WT}), mutant SLX4 (SLX4$^{\Delta C}$) or buffer (-) were added to HSS. After incubation, recombinant SLX4 was immunoprecipitated using anti-FLAG M2 affinity gel (Sigma). The input (IN), flow-through (FT), and bound fractions (B) were analyzed by western blot using α-FLAG (upper panel) and α-XPF antibodies (lower panel). Line within blot indicates position where irrelevant lanes were removed. *, background band. #, SapI fragments from contaminating uncrosslinked plasmid present in varying degrees in different pICL preparations.

Figure S4. The MLR domain is essential for ICL repair by binding XPF and contributes to SLX4 recruitment to ICLs. Related to Figure 4.

(A) Schematic illustration of the *xl*SLX4$^{\Delta MLR}$ mutant protein. In this protein, residues 286-444 of xlSLX4, representing the MLR domain, are replaced by a short linker (TGSGTGST), indicated by the black line.

(B) Schematic illustration of the *xl*SLX4^{MLR*} mutant protein. In this protein, residues Thr418, Trp420, Tyr434, and Tyr435 are mutated to alanines, indicated by two red lines.

(C) Fragment of a sequence alignment between *Xenopus laevis* (*xl*SLX4), human (*hs*SLX4), and mouse (*mm*SLX4) by Clustal Omega. The red boxes in the alignment indicate the residues in the wild-type proteins that are mutated in SLX4^{MLR*}, the A's above the red boxes indicate the substitutions to alanine residues. (*), conserved residue; (:), conservative residue, (.), semi-conservative residue.

(D) Recombinant *xl*SLX4$^{\Delta MLR}$, containing an N-terminal FLAG-tag and a C-terminal Strep-tag, was expressed in Sf9 insect cells and isolated via affinity purification using anti-FLAG M2 affinity gel (Sigma). Purity and concentration were determined using Coomassie blue staining of samples on SDS-PAGE.

(E) As in (D), but for recombinant *xl*SLX4^{MLR*}.

(F) The structural integrity of *xl*SLX4$^{\Delta MLR}$ and *xl*SLX4^{BTB*} was assessed by circular dichroism (CD). The spectra of purified wild-type (SLX4^{WT}) or mutant SLX4 (SLX4^{MUT}) were obtained by subtraction of the spectrum for buffer without SLX4 protein. The measurements were repeated after addition of 3M guanidine hydrochloride (GuHCl) to denature the protein (only SLX4^{WT} shown here). The spectra are shown as mean residue ellipticity (MRE).

(G) SLX4-depleted (ΔSLX4), and SLX4-depleted NPE complemented with wild-type SLX4 (ΔSLX4+SLX4^{WT}) or mutant SLX4 (ΔSLX4+SLX4$^{\Delta MLR}$) were analyzed by western blot

using α-SLX4 antibody (right panel). These extracts, with SLX4-depleted HSS, were used to replicate pICL. Repair efficiency was calculated and plotted (left panel). Independent duplicate experiment related to Figure 4C.

(H) SLX4-depleted (ΔSLX4), and SLX4-depleted NPE complemented with wild-type SLX4 (ΔSLX4+SLX4^{WT}) or mutant SLX4 (ΔSLX4+SLX4$^{\Delta MLR}$) were analyzed by western blot using α-SLX4 antibody (right panel). These extracts, with SLX4-depleted HSS, were used to replicate pICL. Samples were taken at various times and immunoprecipitated with α-XPF (left panel) or α-SLX4 antibodies (middle panel). Co-precipitated DNA was isolated and analyzed by quantitative PCR using the pICL or pQuant primers. Recovery values of pQuant were subtracted from pICL recovery values. The resulting data were plotted as the percentage of peak value with the highest value within one experiment set to 100%. Independent duplicate experiment related to Figure 4D.

(I) As in (H) but using the *x*lSLX4^{MLR*} mutant protein. Independent duplicate experiment related to Figure 4F.

(J) As in (G) but using the *x*lSLX4^{MLR*} mutant protein. Line within blot indicates position where irrelevant lanes were removed. Independent duplicate experiment related to Figure 4G. *, background band. #, SapI fragments from contaminating uncrosslinked plasmid present in varying degrees in different pICL preparations.

Figure S5. The BTB domain contributes to SLX4-dimerization but is not crucial for ICL repair. Related to Figure 5.

(A) Schematic illustration of the *xl*SLX4^{BTB*} mutant protein. In this protein, residues Phe644 and Tyr646 are mutated to alanines, indicated by a red line.

(B) Schematic illustration of the *xl*SLX4$^{1-558}$ mutant protein. This protein is truncated at residue 558.

(C) Fragment of a sequence alignment between *Xenopus laevis* (*xl*SLX4), human (*hs*SLX4), and mouse (*mm*SLX4) by Clustal Omega. The red box in the alignment indicates the residues in the wild-type proteins that are mutated in SLX4^{BTB*}, the A's above the red boxes indicate the substitutions to alanine residues. (*), conserved residue; (:), conservative residue, (.), semi-conservative residue.

(D) Recombinant *xl*SLX4^{BTB*}, containing an N-terminal FLAG-tag and a C-terminal Strep-tag, was expressed in Sf9 insect cells and isolated via affinity purification using FLAG-beads. Purity and concentration were determined using Coomassie blue staining of samples on SDS-PAGE.

(E) As in (D) but for recombinant *xl*SLX4$^{1-558}$.

(F) SLX4-depleted (ΔSLX4), and SLX4-depleted NPE complemented with wild-type SLX4 (ΔSLX4+SLX4^{WT}) or mutant SLX4 (ΔSLX4+SLX4^{BTB*}) were analyzed by western blot using α-SLX4 antibody (right panel). These extracts, with SLX4-depleted HSS, were used to replicate pICL. Repair efficiency was calculated and plotted (left panel). Line within blot indicates position where irrelevant lanes were removed. Independent duplicate experiment related to Figure 5D.

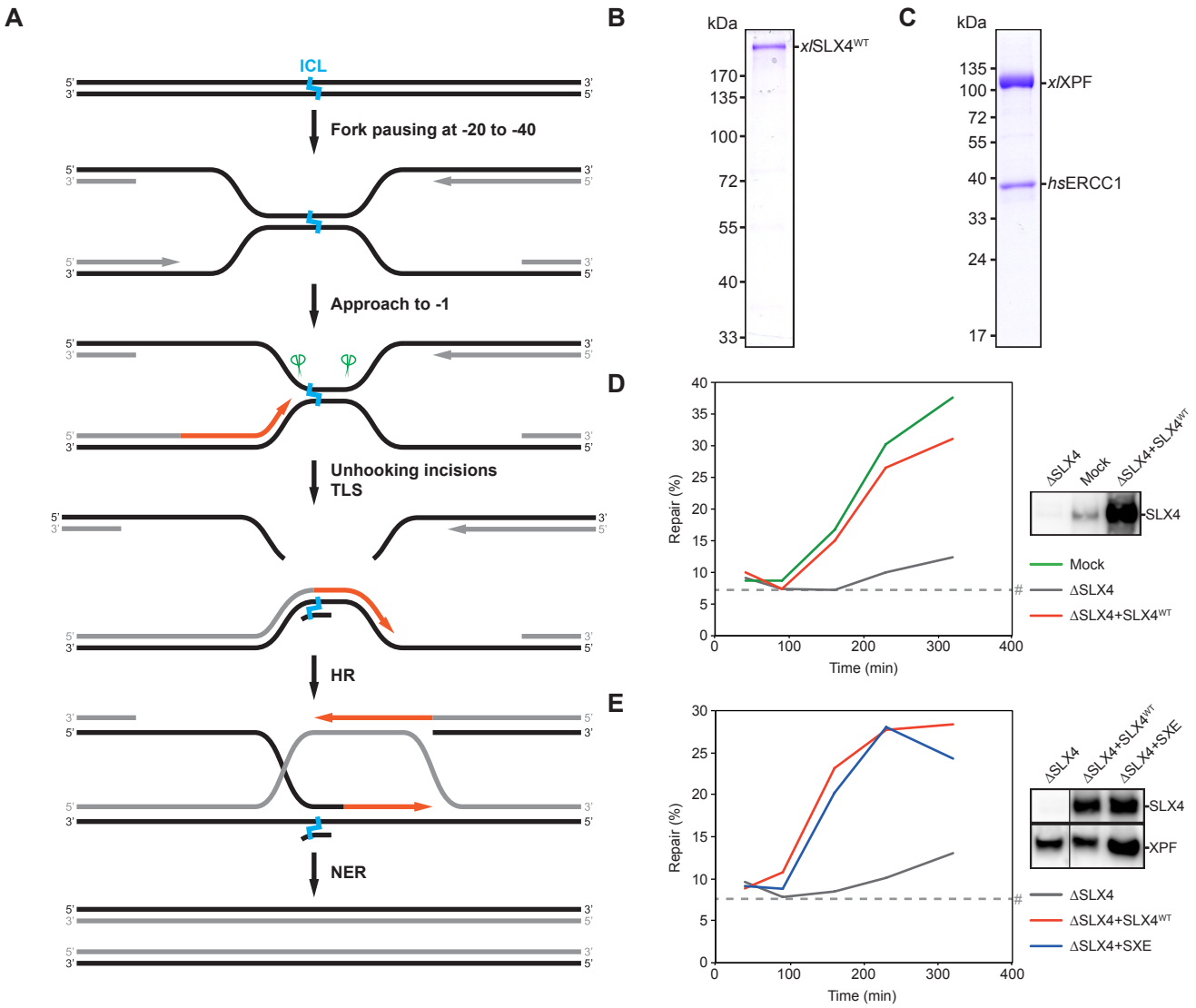
(G) Purified *xl*SLX4$^{\Delta C}$ and *xl*SLX4$^{1-558}$ mutant proteins or buffer (-) were added to HSS. After incubation, recombinant SLX4 was immunoprecipitated using anti-FLAG M2 affinity gel (Sigma). The input (IN), flow-through (FT), and bound fractions (B) were analyzed

by western blot using α-FLAG (upper panel) and α-XPF antibodies (lower panel). Line within blot indicates position where irrelevant lanes were removed.

(H) SLX4-depleted (ΔSLX4), and SLX4-depleted NPE complemented with wild-type SLX4 (ΔSLX4+SLX4^{WT}) or mutant SLX4 (ΔSLX4+SLX4$^{1-558}$), were used with SLX4-depleted HSS to replicate pICL. Repair efficiency was calculated and plotted (left panel). Protein dilutions of wild-type SLX4 (SLX4^{WT}) and mutant SLX4 (SLX4$^{1-558}$) were analyzed by western blot using α-FLAG antibody to ensure equivalent protein concentrations (right panel). Independent duplicate experiment related to Figure 5F.

(I) Wild-type (SLX4^{WT}) or mutant SLX4 (SLX4$^{1-558}$) containing an N-terminal FLAG-tag, were co-expressed with wild-type SLX4 containing an N-terminal His-tag and a C-terminal Strep-tag (His-SLX4) in Sf9 insect cells. Cells were lysed and FLAG-tagged SLX4 was immunoprecipitated using anti-FLAG M2 affinity gel (Sigma). To examine the contribution of DNA to SLX4-dimerization the cell lysates were split during the immunoprecipitation step and treated with benzonase (Sigma). The input (IN), flow-through (FT), and bound fractions (B) were analyzed by western blot using α-His antibodies. #, SapI fragments from contaminating uncrosslinked plasmid present in varying degrees in different pICL preparations.

Figure S1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D
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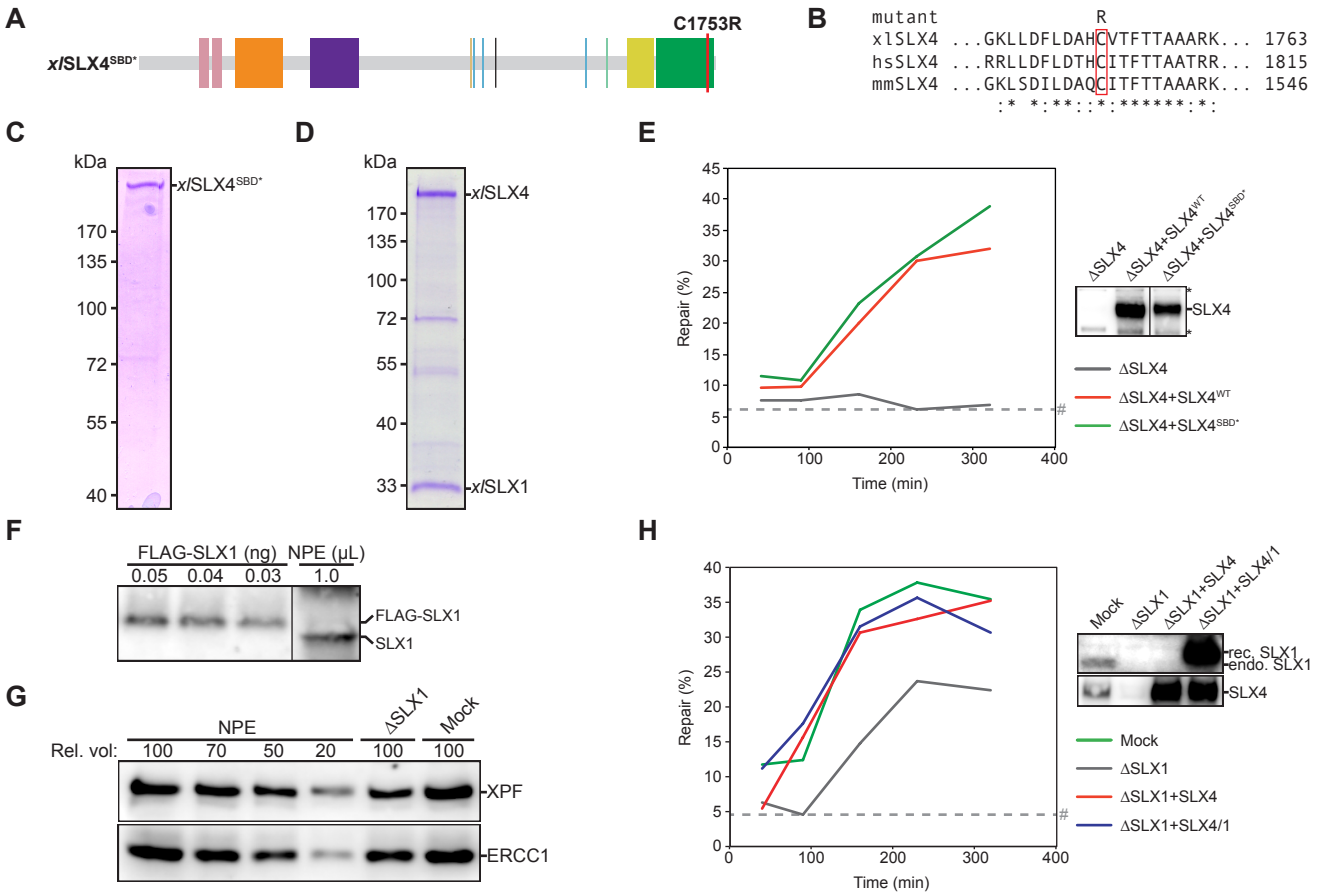
Figure S2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33
E
Repair (%)
Time (min)
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ΔSLX4+SLX4WT
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Figure S3

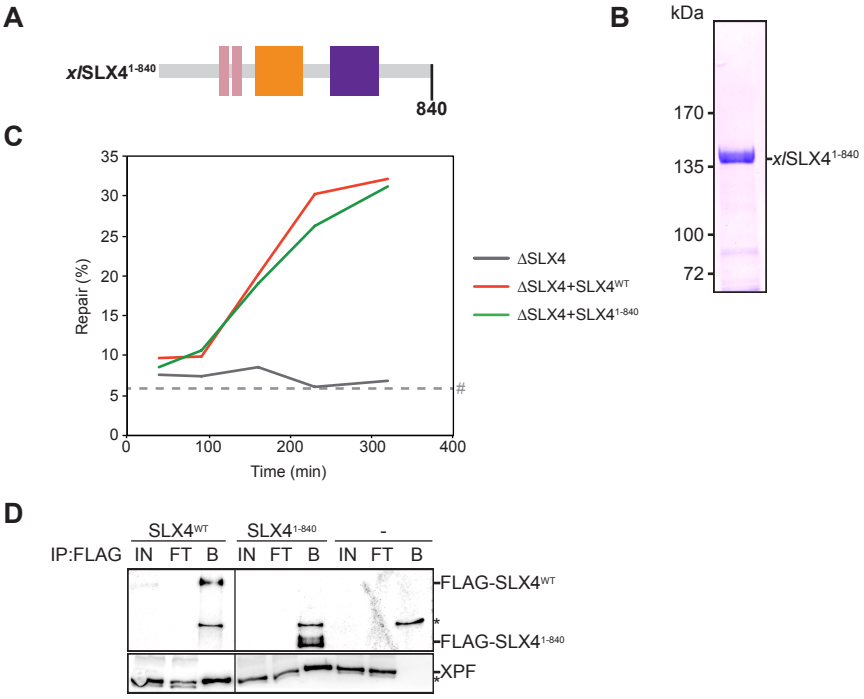
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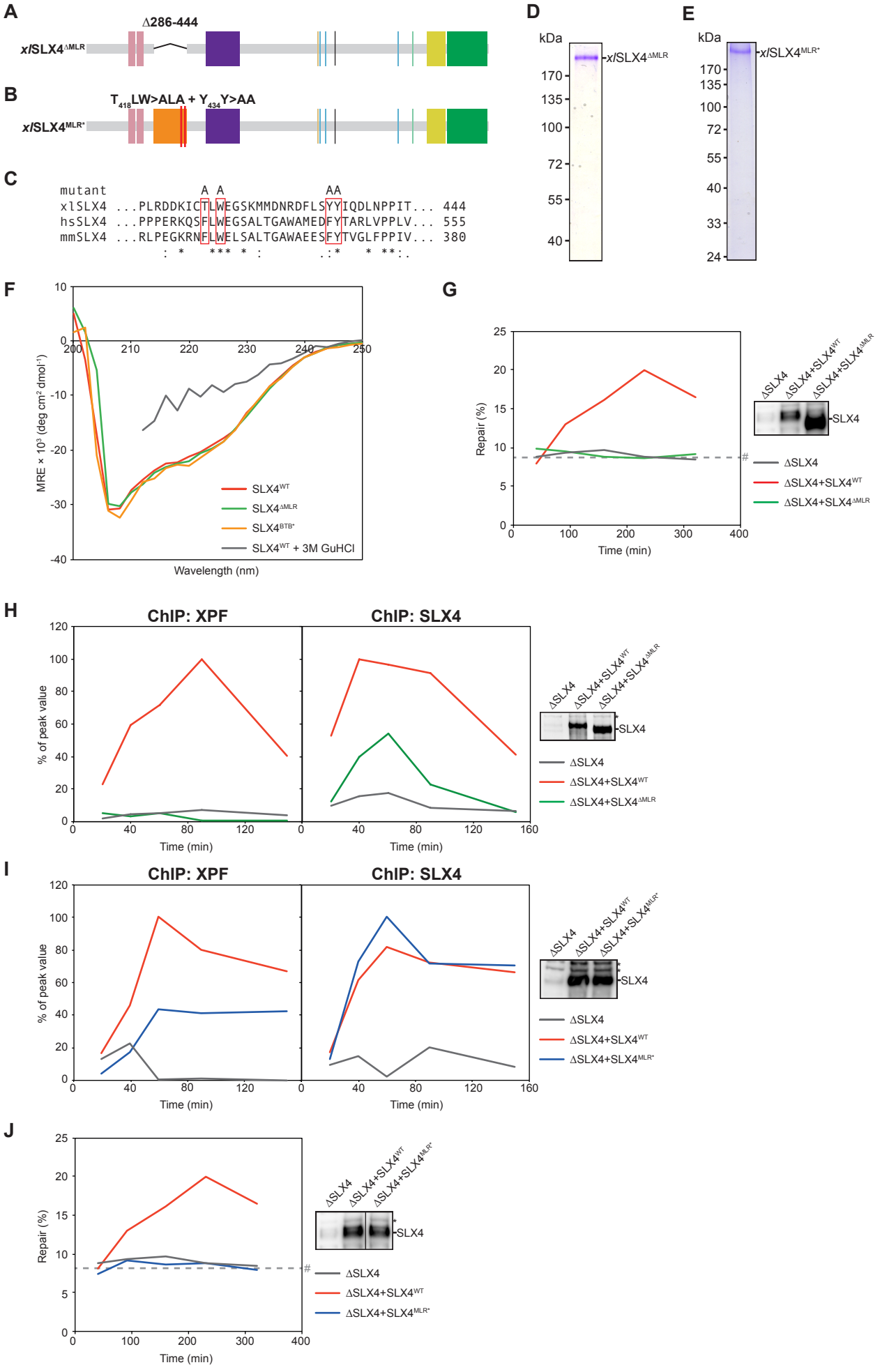
Figure S4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Wavelength (nm)
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SLX4BTB*
SLX4WT + 3M GuHCl
G
Repair (%)
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H
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ChIP: SLX4
% of peak value
Time (min)
ΔSLX4
ΔSLX4+SLX4WT
ΔSLX4+SLX4ΔMLR
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I
ChIP: XPF
ChIP: SLX4
% of peak value
Time (min)
ΔSLX4
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ΔSLX4+SLX4MLR*
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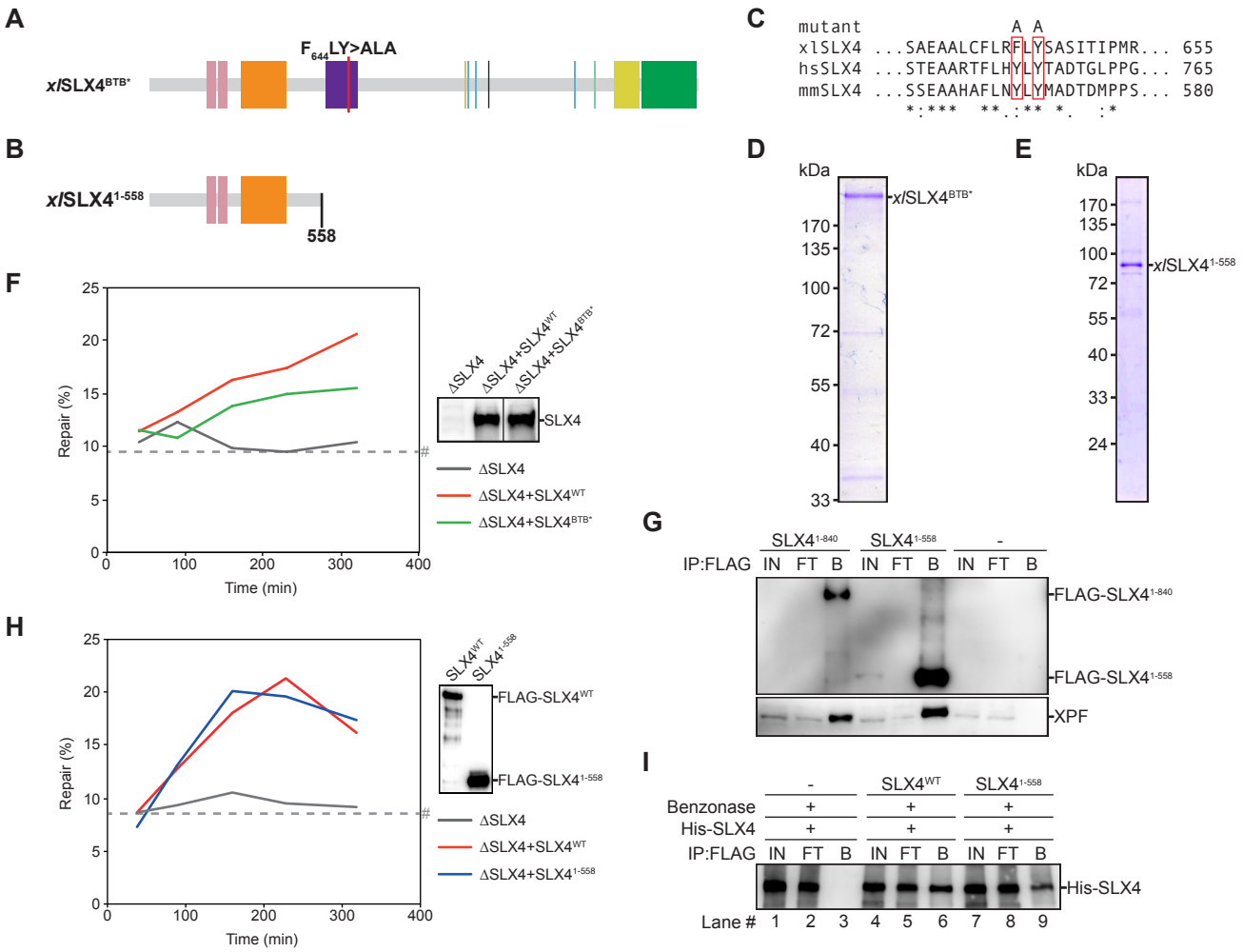
ΔSLX4+SLX4MLR*
SLX4

Figure S5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F
Repair (%)
Time (min)
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ΔSLX4+SLX4WT
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Time (min)
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