SUPPLEMENTARY INFORMATION

A human XRCC4-XLF complex bridges DNA ends.

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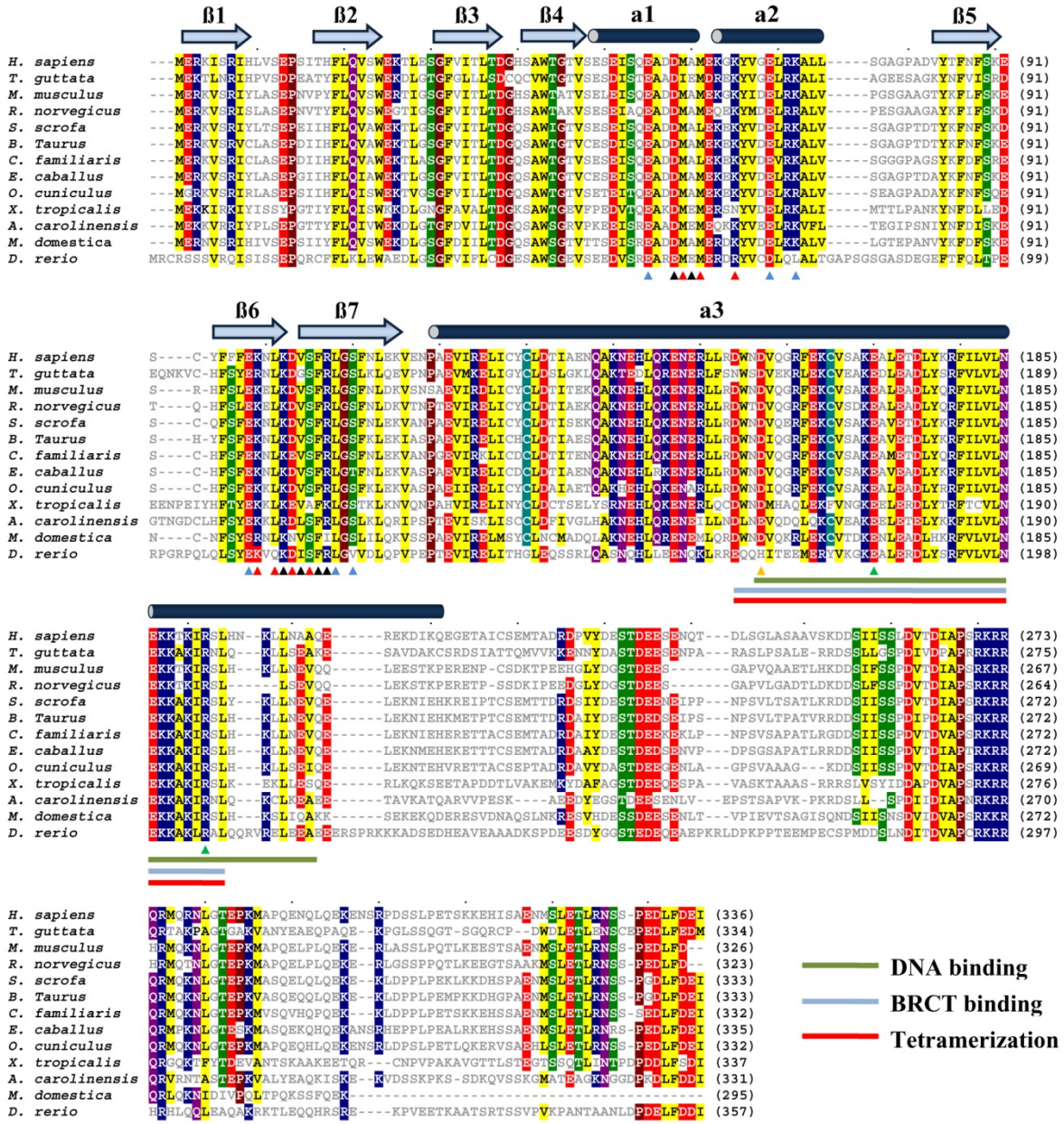
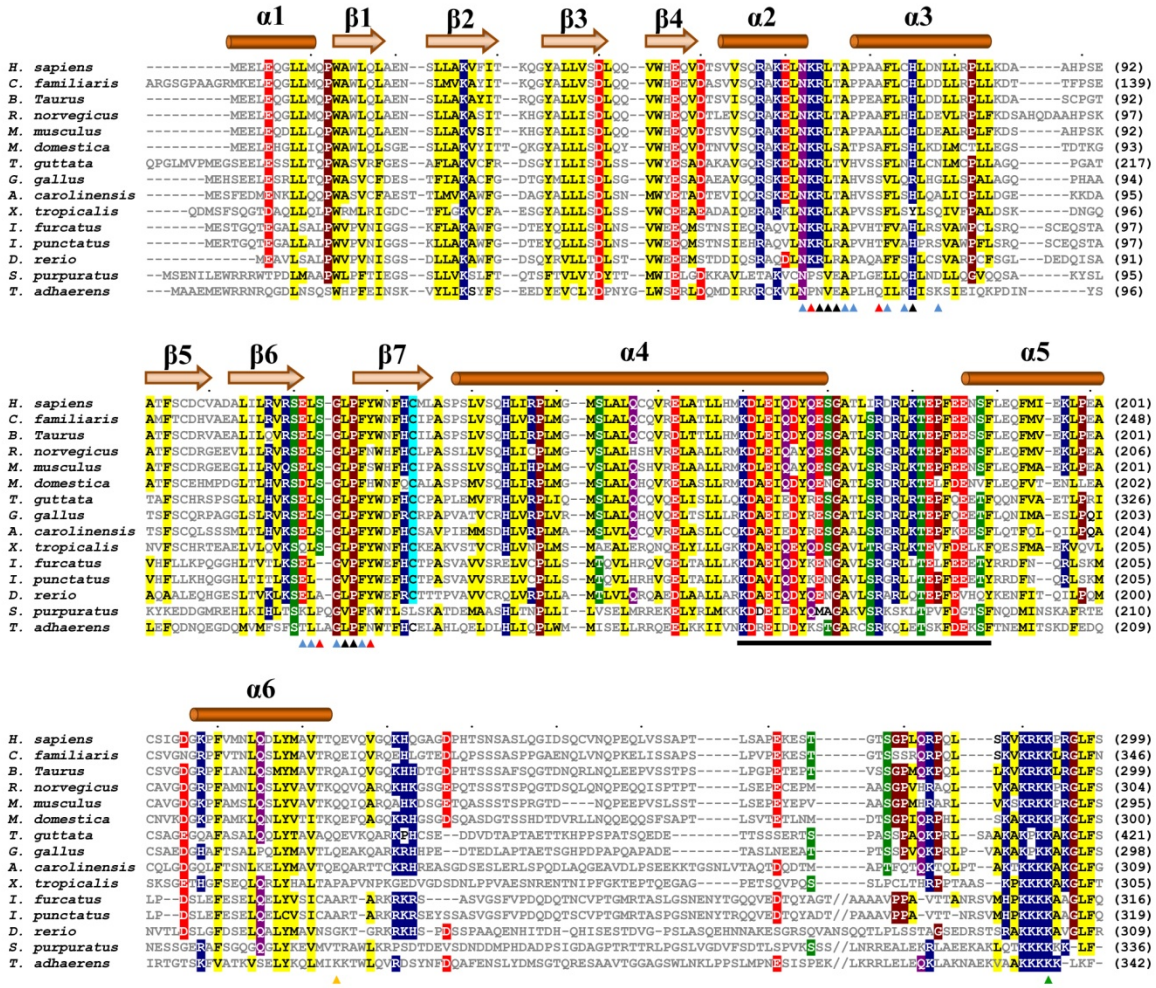
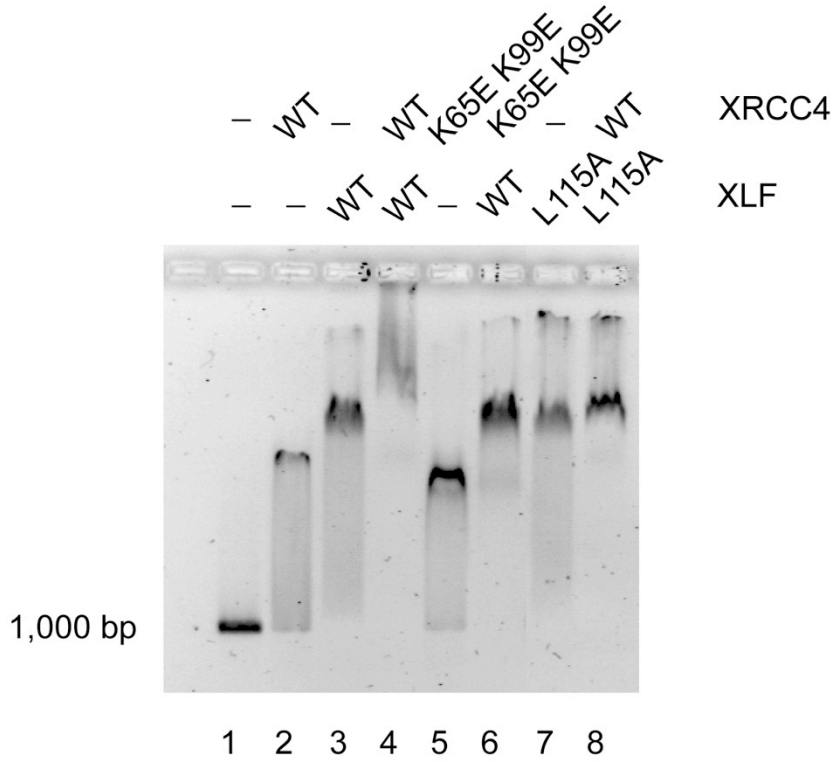
Supplementary Figures



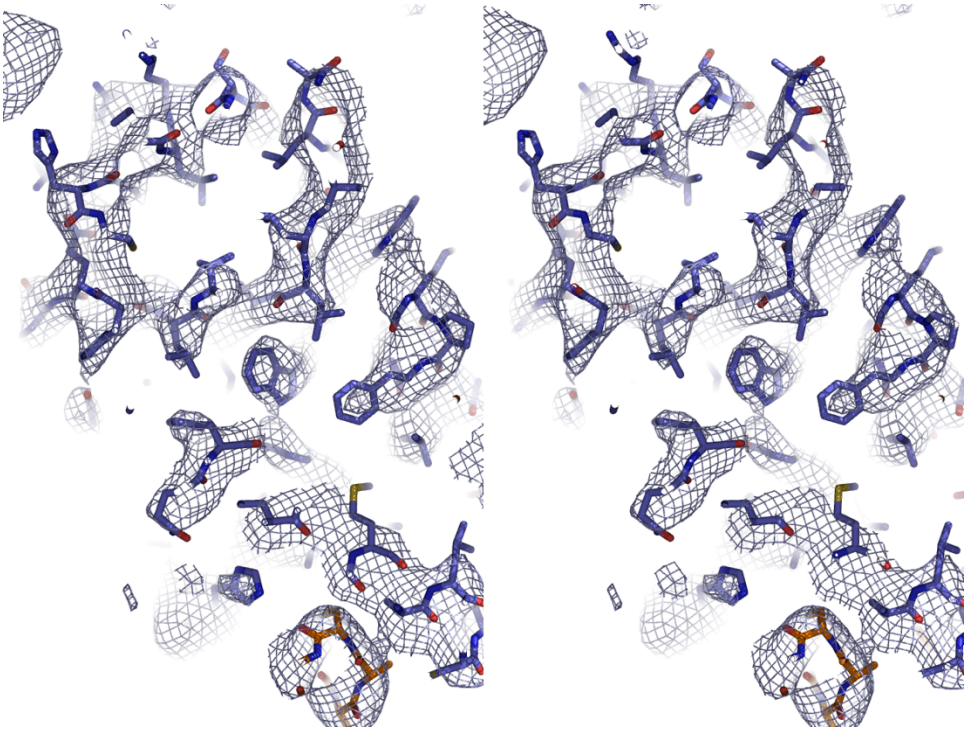
Supplementary Figure S1. XRCC4 sequence alignment. Highly conserved residues are colored as follows: hydrophobic (yellow), positive (blue), negative (red), small polar (green), large polar (purple), cysteine (cyan), glycine and proline (brown). Triangles indicate: end of protein in crystal structure D157 (yellow), DNA binding mutants E170A and R192A (green), residues at XRCC4-XLF interface burying 0-20, 20-50, 50-170 Å^2 (light blue, red and black respectively). Regions of XRCC4 mediating DNA binding, Ligase IV interaction and homo-tetramerization are indicated in green, light blue and red lines, respectively



Supplementary Figure S2. XLF sequence alignment. Highly conserved residues are colored as follows: hydrophobic (yellow), positive (blue), negative (red), small polar (green), large polar (purple), cysteine (cyan), glycine and proline (brown). Triangles indicate: end of protein in crystal structure Q224 (yellow), DNA binding mutant K293A (green), residues at XRCC4-XLF interface burying 0-20, 20-50, 50-170 Å^2 (light blue, red and black respectively). Hinge region, at which point the tails reverse direction, is underlined in black.



Supplementary Figure S3. DNA binding analysis of XLF and XRCC4 filament mutants. Previously reported XLF and XRCC4 mutations that prevent filament formation were analyzed for DNA binding activity (1).



Supplementary Figure S4. Refined 2fo-fc electron density map of XRCC4-XLF. The electron density of XRCC4^{157}-XLF^{224} after refinement is displayed.

Supplementary Tables

Supplementary Table S1. DNA binding activity of XRCC4 mutants with mutations between amino acids 157-200.

XRCC4 Mutant	Binds DNA
C93A/C165A	+
R161Q/K164Q[a]	+
C165F	+
C165A/K169V/L172E	-
K169A	+/-
K169E	-
E170A	-
F180D/I181D	+/-
L184Q/K187D/I191S[b]	-
K187D/K188D	-
I191D/L194E/L198E	-
R192A	-
H195D	-

[a] This XRCC4 mutant was previously identified as able to bind DNA (1).
[b] This XRCC4 mutant was previously identified as unable to bind BRCT domains (2).

Supplementary Table S2. XLF and XRCC4 interface residues with corresponding buried surface areas (BSA).

XLF	BSA(Å^2)	XRCC4	BSA(Å^2)
Asn – 62	3.0	Glu – 55	19.4
Lys – 63	29.5	Asp – 58	71.2
Arg – 64	167.5	Met – 59	19.8
Leu – 65	50.8	Ala – 60	56.4
Thr – 66	54.9	Met – 61	33.1
Ala – 67	17.2	Lys – 65	44.2
Pro – 68	22.4	Glu – 69	3.2
Ala – 71	37.7	Lys – 72	2.0
Phe – 72	1.7	Glu – 98	14.4
Cys – 74	21.09	Lys – 99	34.9
His – 75	70.6	Leu – 101	43.8
Asn – 78	2.3	Lys – 102	92.7
Glu – 111	16.3	Asp – 103	32.0
Leu – 112	14.9	Val – 104	63.3
Ser – 113	51.0	Ser – 105	31.1
Gly – 114	12.2	Phe – 106	86.4
Leu – 115	123.5	Arg – 107	56.1
Pro – 116	76.2	Leu – 108	0.8
Phe – 117	2.5	Ser – 110	1.34
Tyr – 118	24.9		

Supplementary Experimental Procedures

XRCC4$^{1-157}$ expression vector construction

XRCC4$^{1-157}$ construction was constructed as follows: Forward and reverse primers (5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGAAGGAGATAGAACCATGGAGAGAAAAATAAGCAG-3' and 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAGTGATGGTGATGGTGATGATCATTCCAATCTCTCAG-3') were used in conjunction with pWY-1088, an XRCC4 expression plasmid (3) to create XRCC4$^{1-157}$. This vector contained a C-terminal His_6-tag and was cloned into pDEST-14 using Gateway cloning technology (Invitrogen). Successful cloning was confirmed by DNA sequencing.

Electrophoretic Mobility Shift Assays for DNA Binding: DNA Substrate Preparation

The 1000 bp DNA substrate was prepared by PCR using Phusion DNA polymerase (NEB), primers 5'-GAGTTTTATCGCTTCCATGAC and 5'-AATTTATCCTCAAGTAAGGGGC and PhiX174 DNA as template. The PCR product was purified by gel electrophoresis and QIAquick Gel Extraction Kit (QIAGEN) and stored in 10 mM Tris pH 8, 1 mM EDTA.

DNA Bridging Assay

The one-end biotinylated 1000 bp DNA substrate was prepared by PCR using Phusion DNA polymerase, primers 5'-Biotin-GAGTTTTATCGCTTCCATGAC and 5'-AATTTATCCTCAAGTAAGGGGC and PhiX174 DNA as template. The 500 bp DNA substrate was similarly prepared using primers 5'-GAGTTTTATCGCTTCCATGAC and 5'-

CAGAAAATCGAAATCATCTTC. PCR products were purified and stored as described above. Magnetic streptavidin-coated beads (Dynabeads M-280 Streptavidin, Invitrogen) were first passivated by washing the bead suspension three times with one volume of binding buffer (20 mM HEPES pH 8, 75 mM KCl, 0.5 mM EDTA, 1 mM DTT, 5 % glycerol, 400 μg/ml acetylated BSA) and finally resuspended in the same volume of binding buffer. For each reaction, 200 ng of end biotinylated 1,000 bp DNA were added to 10 μL of passivated bead suspension and incubated for 5 min at room temperature (> 90% attachment). Next, 200 ng of 500 bp DNA fragment were added before final addition of the XRCC4, XLF or BRCTs proteins (each at 2 μM) in a total volume of 40 μL in binding buffer. Reaction mixtures were incubated for 30 min at room temperature after which beads were collected with the magnet without any centrifugation step. The 40 μL supernatant fractions were analyzed by electromobility shift assay and reducing polyacrylamide gel electrophoresis in the presence of SDS. The beads were washed 2 times with one volume of binding buffer and finally resuspended in 40 μL of binding buffer without BSA. A 10 uL fraction of the bead suspension was analyzed by reducing polyacrylamide gel electrophoresis in the presence of SDS. Proteinase K (40 μg) and Sarkosyl (0.5 % final) were added to the remainder 30 μL bead suspensions, incubated for 30 min at 50 °C and resolved by electrophoresis in 0.8 % or 1 % agarose gels in Tris-borate buffer as described above.

Scanning Force Microscopy: DNA and Protein Complex Preparation

The double-stranded DNA used in the SFM experiments was made by linearization of pDERI1 (Ristic et al., 2001). Digestion of this plasmid with PvuI produced 1821 bp linear double-stranded DNA. The resulting linear DNA was purified by GFX^{TM} column (Amersham) and checked for purity by gel electrophoresis.

XRCC4-XLF complexes were formed in 20 μl reactions containing 2μM XRCC4, 2μM

XLF, 20mM HEPES-KOH (pH8), 50mM KCl, 1mM DTT, 0.5mM EDTA, 5% glycerol. Complexes of XRCC4-XLF-DNA were produced by addition of 7.5μM DNA (concentration in bp) in reaction described above. Where present, DNA Ligase IV BRCT domains were added to binding reaction with final concentration of 2μM. Reactions were incubated at 19^0C for 10 min and then placed on ice. Reactions were diluted 40-fold in reaction buffer just before deposition. Mica was freshly cleaved and treated with 90mM spermidine. After 20 s spermidine was removed by washing with reaction buffer. The excess buffer was removed and diluted reaction mixture was transferred to mica. After 15 s the mica was washed with water and dried in a stream of filtered air.

Supplementary References

1. Andres, S.N., Modesti, M., Tsai, C.J., Chu, G., & Junop, M.S. (2007) Crystal structure of human XLF: a twist in nonhomologous DNA end-joining. *Mol. Cell* **28,** 1093-1101.

2. Modesti, M., Junop, M.S., Ghirlando, R., van de Rakt, M., Gellert, M., Yang, W., and Kanaar, R. (2003) Tetramerization and DNA ligase IV interaction of the DNA double-strand break repair protein XRCC4 are mutually exclusive. *J. Mol. Biol*. **334,** 215-228.

3. Junop, M.S., Modesti, M., Guarné, A., Ghirlando, R., Gellert, M., and Yang, W. (2000) Crystal structure of the XRCC44 DNA repair protein and implications for end joining. *EMBO J.* **22,** 5962-5970.