Supplemental material

Single-strand DNA binding by the helix-hairpin-helix domain of XPF contributes to substrate specificity of ERCC1-XPF

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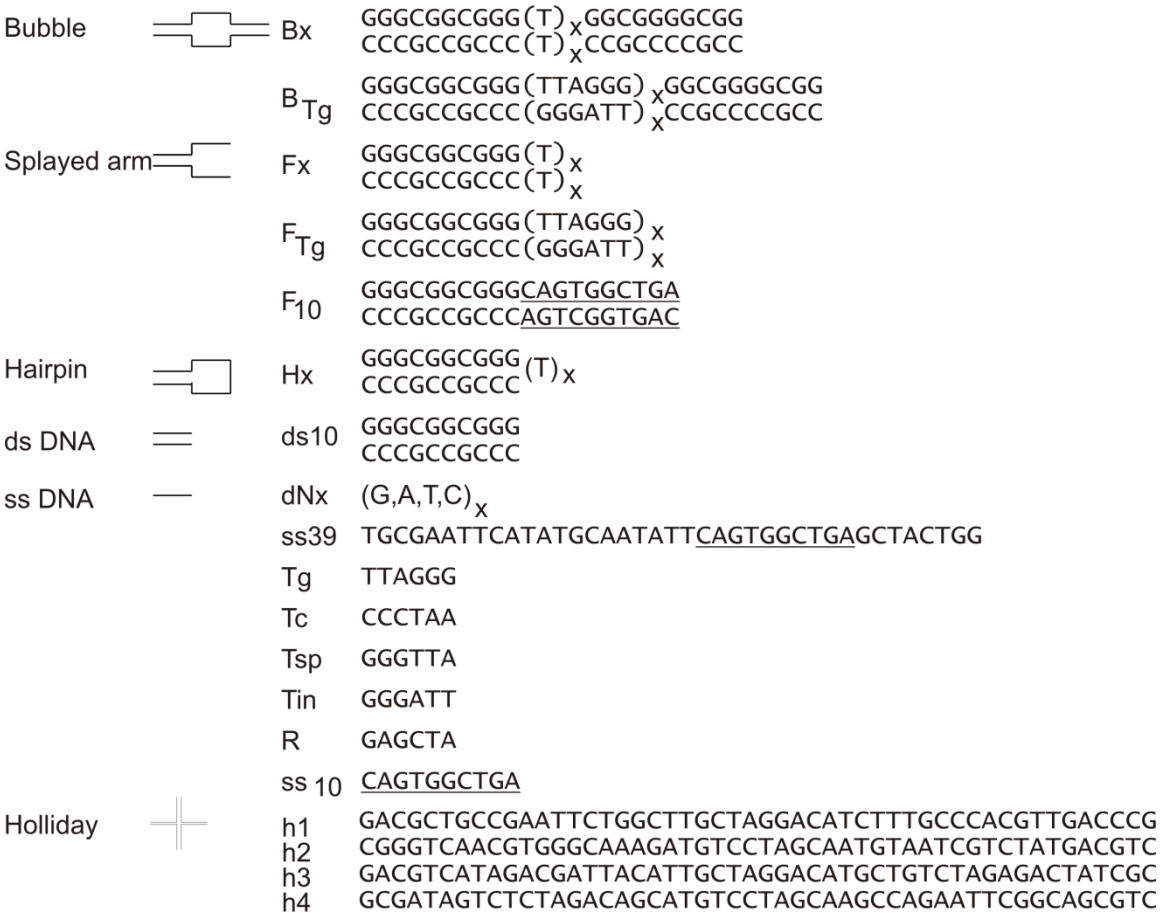
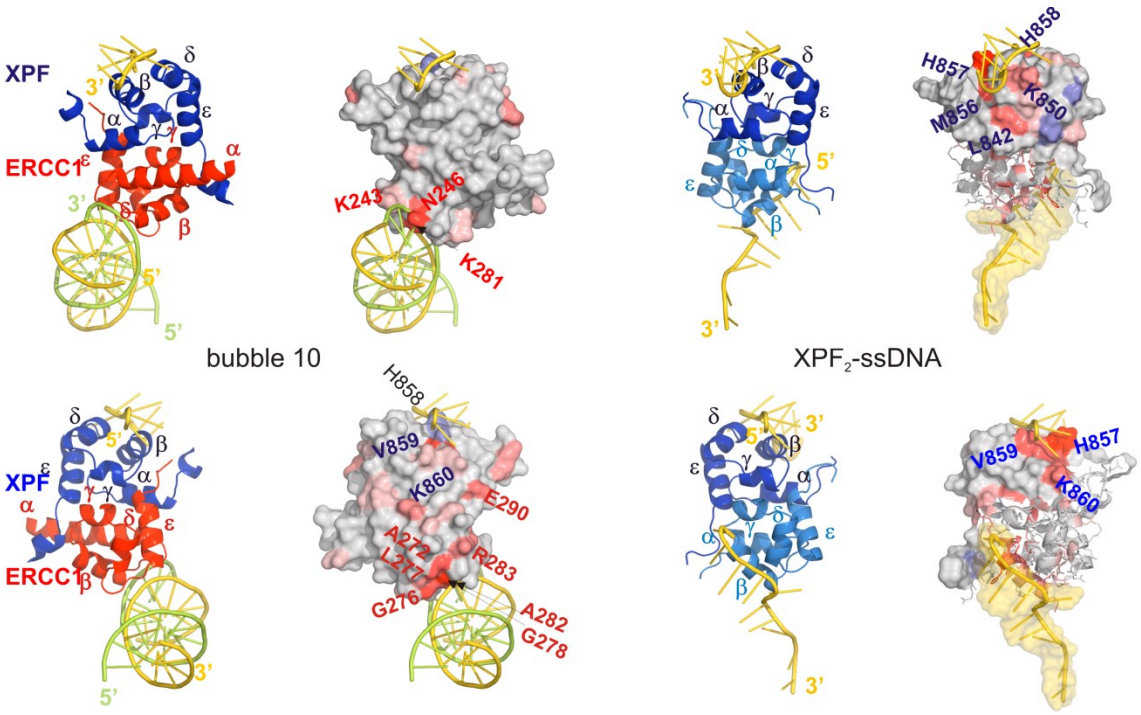


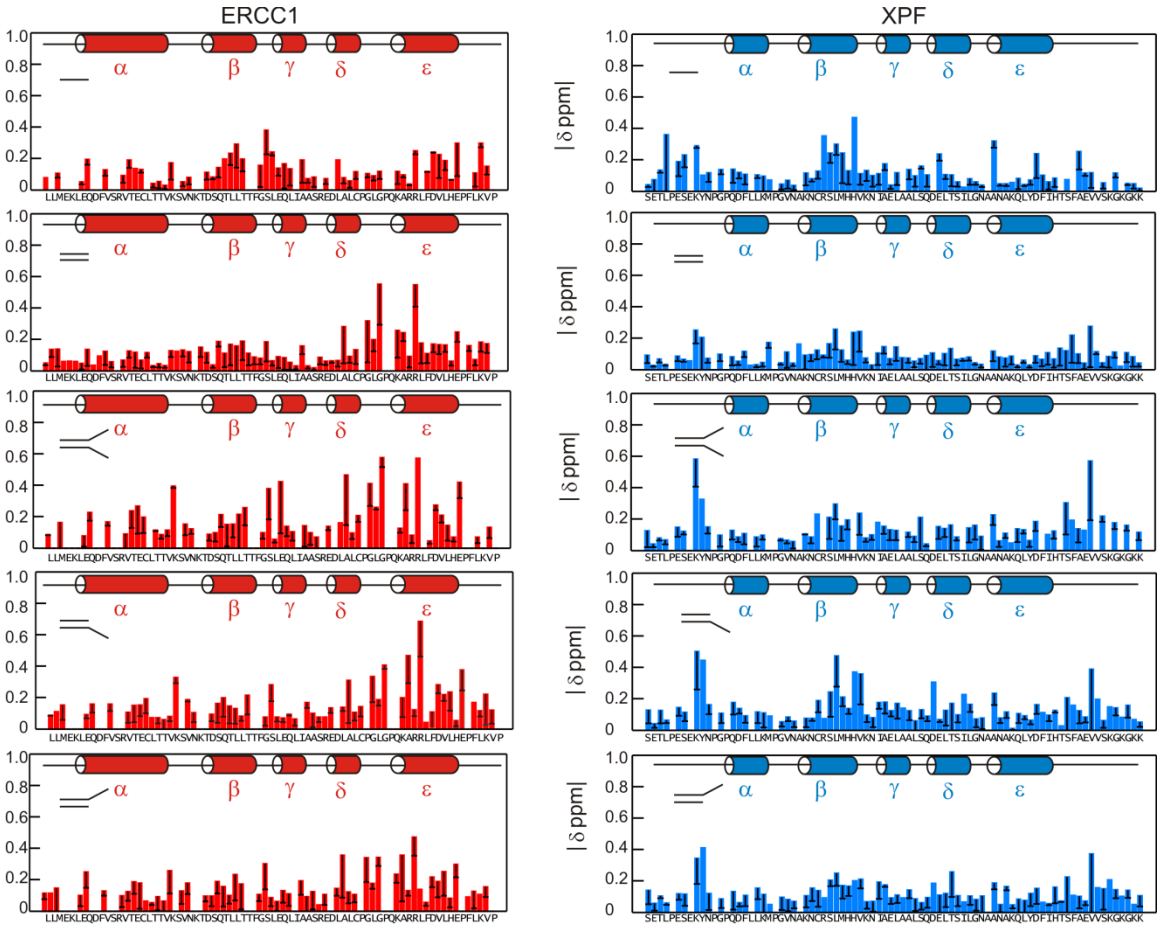
Supplemental Figure S1 *Probes used for DNA binding to the HhH domain of XPF.*

The figure shows the names, symbols and abbreviations and sequences of the various probes used in this study. The dsDNA probes are depicted as duplexes where the sequence of the upper strand is given 5' to 3' and the lower strand 3' to 5'. All ssDNA sequences and the 4 strands that together form the Holliday junction are all given 5' to 3'. ss_{10} (underlined) refers to the DNA sequence used for the structure determination of the homodimeric HhH domain XPF structure bound to ssDNA and in NMR titration experiments



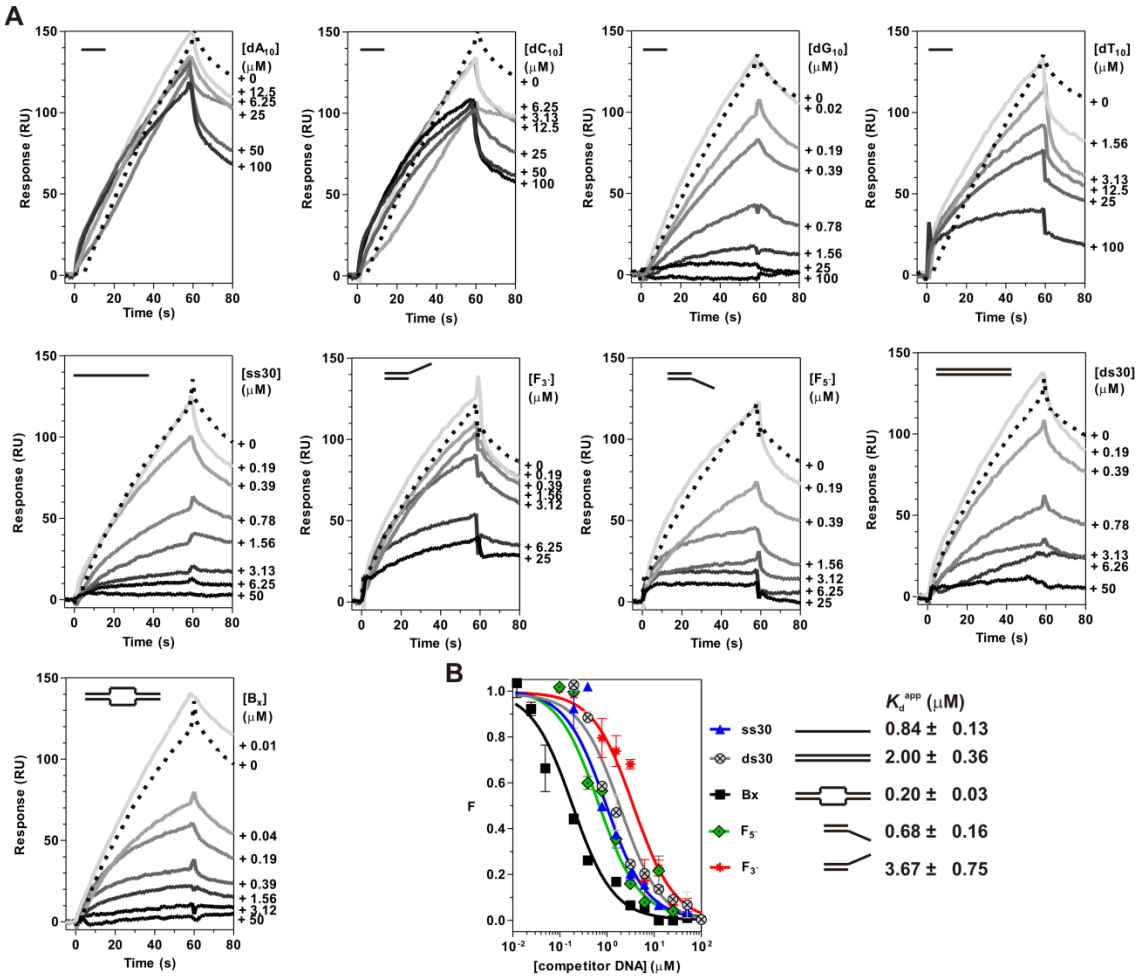
Supplemental Figure S2 Presentation of the p*reviously identified dsDNA and ssDNA interfaces in ERCC1 and XFP.*

For comparison of the results obtained before with the results presented in figure 4 of the main text we show the CSP data that were obtained for hairpin 20 binding to the ERCC1-XPF heterodimer (34) and ssDNA to the XPF homodimer (41). The CSP data are plotted on the surface in the same way as described in Figure 4



Supplemental Figure S3 *Binding of ERCC1-XPF to DNA substrates containing combinations of ssDNA and dsDNA sequences.*

The figure shows normalized average chemical shift perturbations (with the standard deviation indicated in black bars) for ERCC1 (red) and XPF (blue) induced by the addition of 3-4 fold excess of DNA (details on sequences used is presented in the Supplemental Table 1) to 25-100 μM ERCC1-XPF complex under various salt and buffer conditions. The previously determined secondary structure elements are depicted above. Missing bars are either proline residues or residues that could not be unambiguously assigned due to (exchange) line broadening or peak overlap.



Supplemental Figure S4 *ERCC1-XPF HhH domain binding to the various ssDNA or dsDNA probes followed by SPR competition experiment*s.

(A) The representative sensorgrams of 50 nM ERCC1-XPF HhH domain in an absence or a presence of various DNA probes were measured on the NTA SPR chip. (B) In order to calculate the apparent DNA binding affinity (K_D^{app}) the response values at the end of the loading (R_{60}) were divided by the R_{60} value of the ERCC1-XPF HhH domain in absence of DNA and plotted against the total concentration of the DNA and fitted considering a 1:1 binding model. This panel is identical to the results presented in Figure 3A of the main text

DNA	Sequence	Sequence	[ERCC 1-XPF] (μM)	Molar excess	[NaCl] (mM)	[NaHPO4] (mM)
ssDNA	CAGTGGCTGATT		100	4	10	5
	CAGTGGCTGATT		40	2.5	25	12.5
	CAGTGGCTGATT		100	4	50	25
dsDNA	gggcggcgggaatcagccactgcc	ggcagtggctgattcccgccgccc	150	3	100	50
	ggcggggcgggggcggcggg	cccgccgcccccgccccgcc	150	2	100	50
	gggcggcggg	cccgccgccc	40	2.5	25	12.5
	gggcggcggg	cccgccgccc	80	4	100	5
	gggcggcggg	cccgccgccc	100	4	50	25
Sa	gggcggcgggTTTTTTCAGTGGCTGA	CAGTGGCTGATTTTTTcccgccgccc	40	2.5	25	12.5
	gggcggcgggCAGTGGCTGA	CAGTGGCTGAcccgccgccc	80	4	100	5
	gggcggcgggCAGTGGCTGA	CAGTGGCTGAcccgccgccc	100	4	50	25
Sa 3’	ggcggggcgggggcggcgggCAGTGGCTGA	cccgccgcccccgccccgcc	150	2	100	50
	gggcggcgggTTTTTTCAGTGGCTGA	cccgccgccc	40	2.5	25	12.5
	gggcggcgggCAGTGGCTGA	cccgccgccc	80	4	100	5
	gggcggcgggCAGTGGCTGA	cccgccgccc	100	4	50	25
Sa 5’	CAGTGGCTGATcccgccgcccccgccccgcc	ggcggggcgggggcggcggg	150	2	100	50
	CAGTGGCTGATTTTTTcccgccgccc	gggcggcggg	40	2.5	25	12.5
	CAGTGGCTGAcccgccgccc	gggcggcggg	80	4	100	5
	CAGTGGCTGAcccgccgccc	gggcggcggg	100	4	50	25

Supplemental Table 1 *DNA sequences used for NMR titration experiments.*

The table shows the various DNA probes used in the NMR titration experiments and the corresponding sequences used to prepare these. The ERCC1-XPF HhH domain protein concentration is indicated. The molar excess DNA used for final evaluation of the chemical shift differences; the NaCl concentration and the phosphate buffer concentration for each probe is indicated. Sa: splayed arm probe containing of a dsDNA stem with either 1 or 2 ssDNA sequences