The American Journal of Human Genetics, Volume *92*

Supplemental Data

Mutations in *ERCC4*, Encoding the DNA-Repair Endonuclease XPF, Cause Fanconi Anemia

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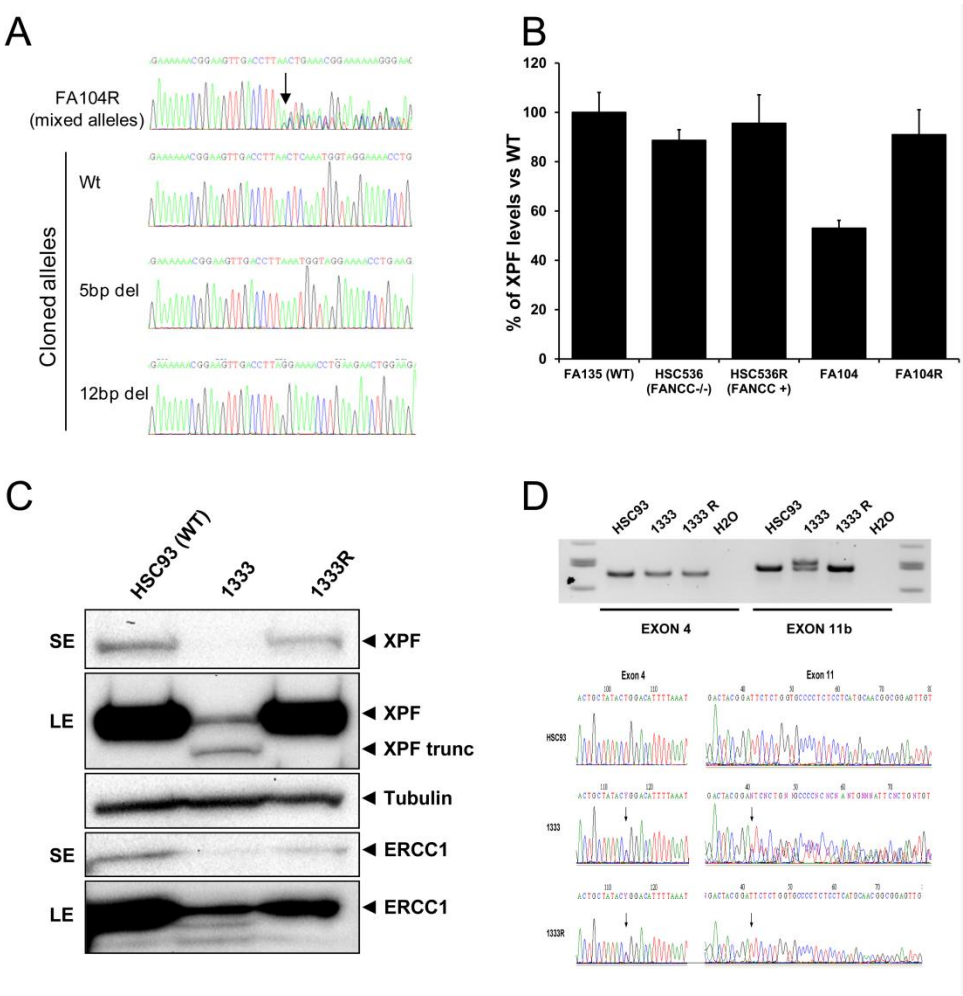

Figure S1. **Genetic Analysis of Back Mutations in *ERCC4* in Reverted MMC-Resistant FA Cell Lines**

(A) Sequence analysis of individual exon 8 alleles cloned from the FA104R cell line. Exons 8 was amplified from FA104R DNA and the PCR products were cloned with the Topo TA Cloning kit (Invitrogen) and transfected into Library Efficiency DH5alpha Competent Cells (Invitrogen). The plasmids from single bacterial colonies were prepared with the NucleoSpin® Plasmid QuickPure Kit (Macherey-Nagel). Sequencing of single bacterial clones revealed the presence of a 12 bp deletion in exon 8 encompassing the pathogenic 5 bp deletion and restoring the reading frame of the *ERCC4* gene.

(B) Quantification of XPF expression by immunoblot in lymphoblasts from FA104, FA104R, HSC536 (FA-C), HSC536R (HSC536 reverted to wt) and FA139 (wt). XPF levels are expressed as a ratio of the loading control (vinculin). Further details on antibodies used can be obtained upon request. The histogram represents XPF levels in the different cell lines normalized to the levels of the loading control. Means and SEM of at least three independent experiments are shown.

(C) Immunoblot analysis showing low levels of two XPF proteins in 1333 and a normal size XPF protein in the reverted cell line 1333R.

(D) Absence of the 28 bp duplication in *ERCC4* exon 11 in 1333R eliminating the longer *ERCC4* mutant allele with the 28 bp duplication (upper panel) and restoring the wt sequence in exon 11 (lower panel).

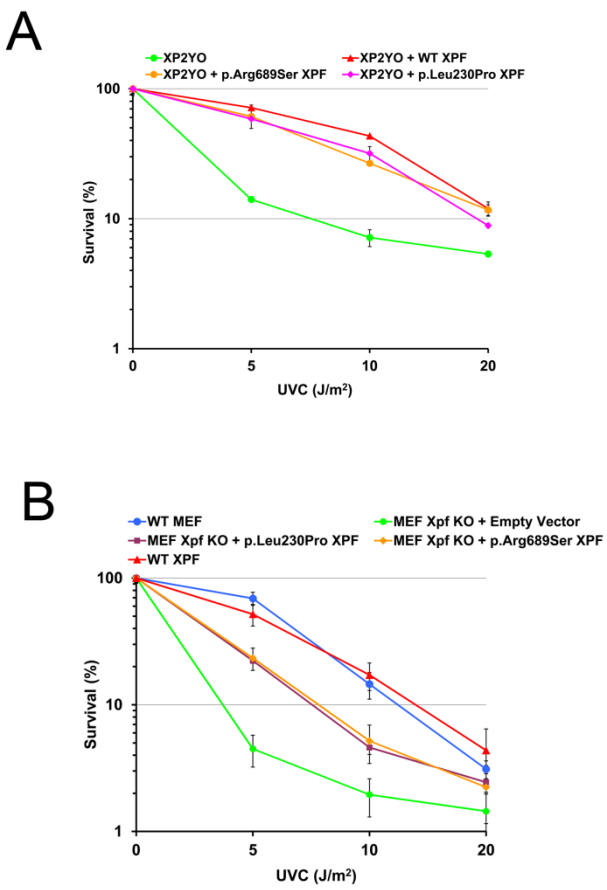

Figure S2. XPF Mutants Leading to FA Restore UVC Resistance of NER-Deficient Human and Mouse Fibroblasts

(A) UVC-induced growth inhibition of human XPF-deficient immortal cell lines from XP and FA individuals (XP2YO and 1333, respectively) transduced with lentiviral particles carrying cDNAs coding for XPF-WT, XPF-p.Arg689Ser and XPF-p.Leu230Pro. Data represent means and SD of two independent experiments.

(B) UV-induced growth inhibition of *Ercc4* KO MEFs transduced with lentiviral particles expressing GFP (negative control vector), wild type XPF, XPF-p.Arg689Ser and XPF-p.Leu230Pro. Data represent means and SD of at least four independent experiments. Lentivirus mediated cDNA transduction and survival analysis were performed as shown in Fig. 1 and 2 (main text).

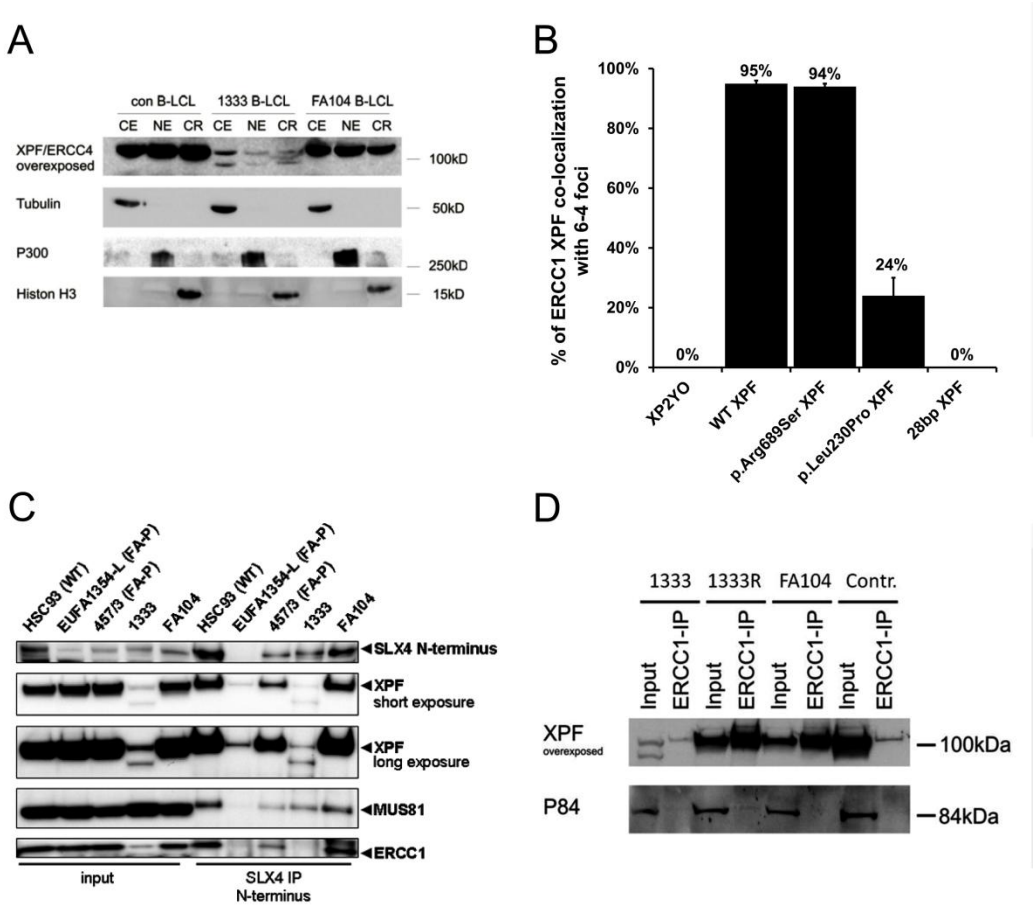

Figure S3. XPF Relocation to DNA Damage and Protein-Protein Interactions

(A) Immunoblot analysis of XPF protein in cytoplasmic (CE), nucleoplasmic (NE) and chromatin (CR) fractions from wild type, 1333 and FA104 lymphoblast cell lines. FA104 and FA104 show an abundance and a distribution of XPF between the cytoplasmic, nuclear and chromatin compartments comparable to a normal control, whereas 1333 reveals reduced abundance and two species of that protein with sizes predicted by its mutations but, of note, XPF is still detected in the nucleus and on chromatin with grossly unaffected ratios to the cytoplasmic fraction. Specificity of the separation of extracts from lymphoblasts is confirmed by the compartment-specific marker proteins tubulin, p300 and histone H3. Subcellular Protein Fractionation Kit from Pierce (Thermo Scientific) following the manufacturer's instructions. Further details on antibodies used and subfractioning conditions can be optained upon request.

(B) Graphical representation of the percent co-localization of XPF with (6-4)PP in XP2YO cells expressing various forms of XPF. XP2YO cells were transduced with HA-tagged wild type XPF, XPF-p.Arg689Ser, XPF-p.Leu230Pro, or XPF-28bp dup, irradiated with UVC (120 J m^{-2}) through a polycarbonate filter with 5 μm pores, incubated for 0.5 h, fixed and stained with antibodies to (6-4)PP and antibodies to HA. Data represent the average of at least 3 independent experiments ± the SD. For each experiment 100 cells were counted.

(C) Normal SLX4 interactions in XPF-deficient FA individuals. SLX4 was immunoprecipitated with a polyclonal antibody raised against the first 300 amino acids of SLX4 (SLX4 N-terminus; gift from J. Rouse, Dundee). Precipitated proteins were visualized by immunoblotting with antibodies to SLX4 N-terminus, XPF, ERCC1 and MUS81. Reduced XPF and ERCC1 protein expression was found in lymphoblasts of individual 1333. In these cells, full-length and truncated XPF and MUS81 were coprecipitated with SLX4, whereas ERCC1 is barely detectable. In lymphoblasts of individual FA104, the interaction between SLX4 and its binding partners XPF-ERCC1 and MUS81 is normal. Wild type lymphoblasts (HSC93) and lymphoblasts of FA-P individuals (EUFA1354-L and 457/3) were used as controls.

(D) Normal ERCC1-XPF interactions in FA104 and 1333 lymphoblast cell lines. ERCC1 was immunoprecipitated with a polyclonal antibody against ERCC1 and the precipitated proteins were visualized by immunoblotting with antibodies against XPF and P83 as internal control. Further technical details on co-immunoprecipitation conditions and antibodies used can be obtained upon request.

Table S1. List of Candidate Genes with Biallelic Mutations after Whole-Exome Sequencing

Chrom	Pos	Ref	Alt	Ensembl pred	AA change	GN	NRR	SNV Q	GT Q
1	169489751	A	W	SS	-	*F5*	42	171	171
1	169525877	T	Y	SS	-	*F5*	52	36	36
2	73675227	-	CTC	NFC	S/SP	*ALMS1*	16	N/A	N/A
2	73678183	G	R	NSC	G1509D	*ALMS1*	156	120	120
3	49094490	G	S	NSC	N381K	*QRICH1*	122	228	228
3	49095011	C	S	NSC	G208R	*QRICH1*	109	43	43
4	126238305	C	M	NSC	P247T	*FAT4*	52	178	178
4	126355484	C	M	NSC	A2368E	*FAT4*	56	190	190
5	156479444	TTG	-	NFC	TS/S	*HAVCR1*	61	N/A	N/A
5	156479568	-	GTT	NFC	T/TT	*HAVCR1*	106	N/A	N/A
6	31238942	G	W	NSC	A176V	*HLA-C*	23	61	39
6	31239577	A	C	NSC	S48A	*HLA-C*	21	90	90
6	32709309	A	R	SS	-	*HLA-DQA2*	29	84	84
6	32713044	C	Y	NSC	T64M	*HLA-DQA2*	192	228	228
6	32713188	C	Y	SS	-	*HLA-DQA2*	126	228	228
6	38840915	A	R	NSC	I2479V	*DNAH8*	72	216	216
6	38879340	A	T	NSC	E3267D	*DNAH8*	12	34	34
7	100686777	C	Y	NSC	T4027M	*MUC17*	323	228	228
7	100687107	G	R	SS	-	*MUC17*	66	79	79
8	30700598	T	Y	NSC	N1979S	*TEX15*	33	97	97
8	30701995	A	M	NSC	D1513E	*TEX15*	141	228	228
10	69682773	T	Y	NSC	D920G	*HERC4*	64	69	69
10	69785435	-	A	SS	-	*HERC4*	9	N/A	N/A
16	14029271	AACTC	-	FC	-	*ERCC4*	22	N/A	N/A
16	14041518	C	M	NSC	R689S	*ERCC4*	121	228	228
16	72137553	C	S	NSC	Q564E	*DHX38*	56	85	85
16	72142141	A	R	NSC	S994G	*DHX38*	52	106	106
17	74272839	C	Y	NSC	V1593M	*QRICH2*	54	33	33
17	74277009	T	Y	NSC	Q1264R	*QRICH2*	23	81	81
18	14105016	C	M	NSC	R508I	*ZNF519*	136	228	228
18	14105853	C	M	NSC	R229I	*ZNF519*	23	51	51
19	51918360	A	R	NSC	S445P	*SIGLEC12*	43	39	39
19	52004795	G	CT	FC	-	*SIGLEC12*	19	N/A	N/A
X	53561632	A	W	NSC	F4226I	*HUWE1*	42	53	53
X	53642759	C	M	NSC	E665D	*HUWE1*	16	33	33

Chrom: chromosome number; Pos: genomic position (GRCh37/hg19);Ref: reference allele; Alt: sample allele; Ensembl pred: consequence prediction of variants on transcript according to Ensembl v59. This column contains one of the following values: SS=splice site, NSC=non-synonymous coding, FC=frameshift coding, NFC=non-frameshift coding; AA change: amino acid change in the affected protein; GN: Gene name; NRR: Number of non-redundant reads; SNV Q: the phred-scaled likelihood that the genotype is identical to the reference; GT Q: Phred-scaled likelihood that the genotype is wrong.

Table S2. Comparative Summary of Clinical and Cellular/Molecular Features of XP, XFE, and FA Individuals with Mutations in *ERCC4*

Clinical/Cellular Features	XPF	XFE	FA
Skin phosensitivity	mild	severe	no
Atrophic epidermis	variable	yes	no
Neurologic features	rare	yes	no
Hematology	normal	anemia[a]	anemia, BMF
Growth retardation[b]	no	yes	yes
Premature death	no	16yo	4yo (FA104). 1333 alive at age 10
UV sensitivity	mild	severe[c]	none (FA104)[d], mild (1333)
UDS defect	mild	severe[c]	mild (1333), ND in (FA104)[d]
MMC sensitivity	mild	severe	severe
DEB-test	negative	positive	positive
MMC induced G2/M arrest	negative	positive	positive
Nuclease activity on stem loop susbtrates	yes[e]	yes[e]	no

[a]It is not known whether anemia evolved to BMF in the XFE individual (Laura Niedernhofer, personal communication).
[b]Include microsomy in 1333, FA104 and XFE and microcephaly in XFE and 1333.
[c]Reported in Niedernhofer et al., 2005.
[d]UDS assay was not done in FA104 due to the lack of skin fibroblasts but FA104 lymphoblasts were resistant to UV.
[e]Reported in Ahmad et al., 2010 for XP mutation p.Arg799Trp and XFE mutation p.Arg153Pro. Typically XP and FA features are marked in yellow and green, respectively.