Mutations of the SLX4 gene in Fanconi anemia

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Fanconi anemia is a rare recessive disorder characterized by genome instability, congenital malformations, progressive bone marrow failure and predisposition to hematologic malignancies and solid tumors1. At the cellular level, hypersensitivity to DNA interstrand crosslinks is the defining feature in Fanconi anemia2. Mutations in thirteen distinct Fanconi anemia genes3 have been shown to interfere with the DNA-replication–dependent repair of lesions involving crosslinked DNA at stalled replication forks4. Depletion of SLX4, which interacts with multiple nucleases and has been recently identified as a Holliday junction resolvase5–7, results in increased sensitivity of the cells to DNA crosslinking agents. Here we report the identification of biallelic SLX4 mutations in two individuals with typical clinical features of Fanconi anemia and show that the cellular defects in these individuals’ cells are complemented by wildtype SLX4, demonstrating that biallelic mutations in SLX4 (renamed here as FANCP) cause a new subtype of Fanconi anemia, Fanconi anemia-P.

SLX4 is a multidomain scaffold protein that interacts with three distinct nucleases: SLX1, ERCC4/XPF-ERCC1 and MUS81-EME15–7. Although the SLX4-SLX1 interaction is largely responsible for the Holliday junction resolvase activity seen in the complex, SLX4 can also stimulate the activity of ERCC4/XPF and MUS81 nucleases, both of which have been previously implicated in the processing of interstrand crosslinks (ICLs)8. The finding that depletion of SLX4 leads to increased sensitivity to crosslinking agents and to camptothecin5–7 prompted us to investigate SLX4 as a candidate gene for Fanconi anemia1,2.

So far, mutations in thirteen genes have been shown to be responsible for Fanconi anemia3. Eight of the Fanconi anemia proteins (FANCA, FANCB, FANCC, FANCE, FANCF, FANCN, FANCL and FANCM) form a core complex, a nuclear E3 ubiquitin ligase which ubiquitinates FANCI and FANCD2 (refs. 9,10). These two activated proteins subsequently localize as an FANCI-FANCD2 complex to chromatin and direct repair4 partly through interaction with the newly identified nuclease FAN1 (refs. 11–14). Cells with mutations in the Fanconi anemia core complex (except for FANCM) lack monoubiquitination of FANCD2. The other Fanconi anemia proteins are FANCI (also known as BRIP1), a helicase, and the homologous recombination effectors FANCN (also known as PALB2) and FANCD1 (also known as BRCA2). Recently, RAD51C, also involved in homologous recombination repair, has been found to be mutated in three individuals with a Fanconi anemia–like disorder15. Cells mutated in FANCI (BRIP1), FANCN (PALB2), FANCD1 (BRCA2) and RAD51C have normal FANCD2 monoubiquitination, and their products are thought to work downstream of the FANCI-FANCD2 complex.

As depletion of SLX4 in a U2OS cell line does not affect FANCD2 ubiquitination (Fig. 1a,b), we sequenced SLX4 in the families from the International Fanconi Anemia Registry16 with unassigned Fanconi anemia complementation groups and normal FANCD2 modification (Fig. 1c) and identified two families carrying germline mutations, IFAR1084 and IFAR414 (Fig. 1d). Phenotypes of the two affected individuals are summarized in Table 1. The lymphoblastoid cell line (LCL) (RA3042) and fibroblasts (RA3083) from individual 1084/1 showed increased genomic instability (Fig. 1e and Table 2) and increased sensitivity to mitomycin C (MMC) (Supplementary Fig. 1a). The 414/1 individual’s LCL (RA 1376) was not sensitive to MMC, suggestive of reversion (Supplementary Fig. 1b); however, his skin fibroblasts (RA 3331) displayed a high degree of diepoxybutane (DEB)-induced chromosomal instability (Fig. 1e and Table 2) and sensitivity to MMC. We observed no ultraviolet sensitivity in fibroblasts from either of the affected individuals (Supplementary Fig. 1c,d). Fibroblasts from individual 414/1 (RA3331) but, interestingly, not individual 1084/1 (RA3083) were sensitive to camptothecin, a topoisomerase I inhibitor (Supplementary Fig. 1e,f).

Sequencing of the complementary DNA (cDNA) from the 1084/1 individual’s cells revealed skipping of exon 5 (Supplementary Fig. 2a) due to a homozygous point mutation in the canonical splice donor dinucleotide GT in intron 5 (c.1163+2T>A) in the genomic DNA (Supplementary Fig. 2b). We found both of this individual’s parents to be heterozygous and found an unaffected sibling to be negative for this mutation (Supplementary Fig. 2b). The predicted effect of this mutation is a 70-amino-acid deletion of amino acids 317–387 of SLX4 (p.Arg317_Phe387del) leading to an in-frame deletion of the conserved cysteine and leucine of the first UBZ domain and the whole second UBZ domain (Fig. 2a and Supplementary Fig. 2c).

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Immunoprecipitation of SLX4 from the cell line RA3083 confirmed the presence of a slightly shorter protein product (Fig. 2b lane 5 and Supplementary Fig. 2d).

In individual 414/1, we detected a heterozygous frameshift mutation in exon 2 (c.514delC) by sequencing of the full-length RT-PCR product (Supplementary Fig. 3a) and confirmed it in the genomic DNA of this individual and his father (Supplementary Fig. 3b). The predicted protein effect of this frameshift mutation is a truncated protein with N-terminal 171 amino acids of SLX4 followed by 22 non-SLX4 amino acids due to a frameshift (p.Leu672Val
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Fig. 4). The predicted effect of this mutation is a truncated protein with N-terminal 671 amino acids of SLX4 followed by 119 non-SLX4 amino acids due to a frameshift (p.Leu672ValX119) (Fig. 2a). Consequently, immunoprecipitation with the antibody against SLX4 failed to identify the full-length protein in the individual’s fibroblasts (RA3331) (Fig. 2b lane 6).

To prove that the mutations identified in SLX4 were causal for the Fanconi anemia phenotype of both affected individuals, we introduced the wild-type or the mutant SLX4 cDNAs into these individuals’ fibroblasts (RA3083 and RA3331) and performed functional complementation assays (Fig. 3 and Supplementary Fig. 4). Expression of wild-type SLX4 in both cell lines almost fully rescued the MMC sensitivity (Fig. 3a and Supplementary Fig. 4a,b), the late S/G2 arrest with MMC treatment (Fig. 3b and Supplementary Fig. 4c–e) and the chromosomal instability after treatment with DEB (Supplementary Fig. 4f). Some residual MMC sensitivity, cell cycle arrest and chromosomal breakage is most likely due to some cells losing expression of SLX4, as evident by immunofluorescence analysis (data not shown). Introduction of the mutant proteins did not rescue the Fanconi anemia phenotypes of these individuals’ cells, although we noted a slight improvement in the various assays, possibly due to over-expression of the mutant proteins, which might have residual function. These experiments demonstrate that biallelic SLX4 mutations cause a new subtype of Fanconi anemia, Fanconi anemia-P, and that FANCP becomes an alias for SLX4.

SLX4 interacts with multiple factors; two of which, ERCC4/XPF and MUS81, have been previously implicated in crosslink repair.

We therefore tested whether the mutant SLX4 proteins from both affected individuals still interacted with the ERCC4/XPF and MUS81 complexes. We found that ERCC4/XPF, MUS81 and ERCC1 coimmunoprecipitated with endogenous mutant SLX4 (p.Arg317_Phe387del) from RA3083 fibroblasts (Fig. 4a lane 5 and Supplementary Fig. 5a lane 4), although the levels of the mutant SLX4 protein were consistently lower in multiple experiments, leading to diminished immunoprecipitation of the interacting factors. The SLX4 p.Leu672ValX119 mutant protein, overexpressed in RA3331 fibroblasts, showed diminished but present interaction with ERCC4/XPF.

Table 1 Characteristics of individuals with Fanconi anemia and mutations in SLX4

| Individual | Maternal allele | Paternal allele | Ethnicity | Phenotypic and hematologic abnormalities |
|------------|----------------|----------------|-----------|----------------------------------------|
| 1084/1     | c.1163+2T>A, p.Arg317_Phe387del<sup>a</sup> | c.1163+2T>A, p.Arg317_Phe387del<sup>a</sup> | South Indian | Fifteen-year-old female, short stature (height –2.1 s.d., 1st percentile); vitiligo; presented at 9 years of age with isolated thrombocytopenia. |
| 414/1      | c.2013+225_3147del489insCC, p.Leu172PheX22<sup>c</sup> | c.514delC, p.Leu172PheX22<sup>c</sup> | American of European descent | Bilateral absent thumbs and right radial aplasia, undescended left testicle, pelvic kidney, malformed auricle and short stature; squamous cell carcinoma of the tongue at 21 years of age; platelets, 35,000 cells/µL; Hb, 10 g/dL; MCV, 105.5 fl; died at 22 years of age from complications of metastatic disease. |

<sup>a</sup>The predicted protein has an internal deletion from amino acids 317 to 387. <sup>b</sup>The predicted protein has 671 N-terminal amino acids of SLX4 followed by 119 non-SLX4 amino acids due to a frameshift. <sup>c</sup>The predicted protein has 172 N-terminal amino acids of SLX4 followed by 22 non-SLX4 amino acids due to a frameshift, s.d., standard deviation.
Table 2  Chromosome breakage analysis in the indicated cell lines with and without diepoxybutane treatment

|                | RA3042 (LCL) | RA3083 E6E7 | RA3331 E6E7 | BJ E6E7 |
|----------------|--------------|-------------|-------------|----------|
| DEB concentration (µg/ml) | 0            | 0.1         | 0           | 0.1      |
| Metaphases | 56           | 29          | 53          | 32       |
| Total breaks| 41 (1)       | 221         | 8 (1)       | 140 (1)  |
| Chromatid breaks | 29 (6)     | 123         | 6 (2)       | 92 (7)   |
| Triradials | 5            | 44          | 1 (1)       | 16 (1)   |
| Quadriradials | 1 (1)      | 5 (1)       | 8 (1)       | 0 (1)    |
| % of metaphases with breaks | 30 (10)    | 90 (13)     | 81 (14)     | 100 (11) |

Breaks per metaphase: 0.73 7.6 0.15 4.4 0.14 7.0 0.11 0.16

Fig. 4  Quantification of the data is shown in Supplementary Fig. 4a–c. Complementation of MMC sensitivity. We exposed fibroblasts from a healthy donor were used as a normal control. WT, wildtype.

(a) Schematic of SLX4 (based on ref. 7) showing the domain architecture, the interacting proteins and the predicted protein effect of SLX4 mutations in IFAR1084/1 and IFAR414/1 individuals. (b) Analysis of the mutant SLX4 protein in the cell lines. We subjected cell extracts of primary BJ, RA3083 and RA3331 fibroblasts to immunoprecipitation using a control rabbit antibody (control IgG) or the SLX4 antibody. Asterisks indicate the crossreacting bands. Note that the antibody does not identify SLX4 in straight protein blotting (lanes 7 to 9). WT, wildtype.

Fig. 3  Complementation of RA3083 and RA3331 cells with the SLX4 cDNA. (a) Complementation of MMC sensitivity. We exposed fibroblasts stably transduced with empty vector (control) or the vector expressing wildtype SLX4 or the mutant SLX4 cDNAs to different levels of MMC ranging from 0–100 nM. After 8 days, the cell number was determined using a counter. Total cell numbers at each dose were divided by the number of cells in the untreated sample to arrive at percent survival. Error bars indicate s.d. (b) Complementation of the cell cycle defect after MMC treatment. Indicated cells were treated with 100 nM MMC, and the cell cycle was analyzed 48 h later. Untreated samples were analyzed in parallel. Expression levels of the exogenous proteins are shown in Supplementary Figure 4a–c. Quantification of the data is shown in Supplementary Figure 4d.e. WT, wildtype.

and ERCC1 but not with MUS81 (Fig. 4b lane 3). This result is consistent with the previous findings that MUS81 interacts with the amino acid 684–1,834 fragment of the SLX4 protein, which is deleted in the p.Leu672ValfsX119 mutant protein. Immunoprecipitation with an antibody recognizing the N terminus of SLX4 from RA3331 cells showed greatly diminished interaction with ERCC4/XPF, ERCC1 and MUS81 (Supplementary Fig. 5b lane 6).

As UBZ domains are known to interact with ubiquitin, we hypothesized that the absence of the tandem UBZ domains in the mutant SLX4 from individual 1084/1 might disrupt the binding of the SLX4 complex to ubiquitin chains of repair proteins at the sites of DNA damage, as shown for the tandem UBZ domains of RAD80 (ref. 18). We therefore performed in vitro ubiquitin binding assays (Fig. 4c) that showed binding of the isolated UBZ domains of SLX4 to the K63 chains of ubiquitin (Fig. 4c lane 8). When the two conserved cysteines from each UBZ domain were mutated to alanines (Supplementary Fig. 2c), the binding was reduced to background levels seen with GST alone (Fig. 4c, compare lane 7 and 9), suggesting the possibility that SLX4 may localize to the sites of damage through binding to K63 ubiquitinated substrates. As SLX4 would localize other proteins, including ERCC4/XPF, MUS81 and SLX1, to sites of DNA damage, the SLX4-deficient cell lines described here are important tools to understand which interactions of SLX4 are essential for the repair of cross-linked DNA and ultimately to define the importance of the SLX4 (FANCP) function in the Fanconi anemia pathway. Phenotypes of the affected individuals also provide an important clue. Individuals with Fanconi anemia with mutations in PALB2 (FANCN) or BRCA2 (FANC1), which are essential for homologous recombination, have very early onset of childhood solid tumors and acute myelogenous leukemia.

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Individuals with Fanconi anemia having SLX4 (FANCP) mutations show a milder phenotype more akin to that seen in individuals with mutations in the Fanconi anemia core or the FANCI-FANC-D2 complex components. This suggests that the Holliday junction resolution, an integral step of homologous recombination, might not be the essential function of SLX4 in the somatic compartment during crosslink repair and that the repair depends on the other nucleases, ERCC4/XPF and MUS81, that interact with SLX4.

SLX4 (FANCP) represents a second protein (besides FANCM) that is conserved in lower eukaryotes, which do not have any other Fanconi anemia pathway components. Yeast Slx4, like human SLX4, interacts with orthologs of ERCC4/XPF and SLX1, and the work in this model organism will provide insight into the function of the Fanconi anemia pathway in human cells. Because germ-line mutations in three Fanconi anemia genes (BRCA1 (FANCD1), PALB2 (FANCN) and BRI1 (FANCI)) and RAD51C, mutated in an Fanconi anemia–like disorder, are associated with a high risk of developing familial breast and ovarian cancers,21–24 SLX4 should also be sequenced in individuals from pedigrees where no other predisposing mutations could be identified.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Accession codes. The SLX4 reference sequences are deposited in NCBI with the following reference sequences: NM_032444.2 and NP_115820.2.

Note: Supplementary information is available on the Nature Genetics website.

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AUTHOR CONTRIBUTIONS

The study was designed by A.S., Y. K. and F. P. L. Subject recruitment and sample collection was done by A. D. A., F. P. L. and A. S. Characterization with respect to Fanconi anemia subgroups was performed by A. S., F. P. L., H. H. and A. D. A. Mutation analysis and functional studies were performed by A. S., Y. K., F. P. L. and R. D. The manuscript was written by A. S. with help from other authors.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Mutagenesis. Mutagenesis was performed using a multisite mutagenesis kit (Agilent) using primers shown in Supplementary Table 2.

RNAi. Short interfering RNA (siRNA) transfections were performed using Lipofectamine RNAiMAX as suggested by the manufacturer with a final siRNA concentration of 50 nM. siRNAs (Invitrogen) are shown in Supplementary Table 3.

RT quantitative PCR. Superscript III reverse transcriptase followed by Platinum SybrGreen super mix (Invitrogen) was used according to the manufacturer’s instructions. Actin was used as the control.

Cell cycle studies. For cell cycle analysis, cells were left untreated or were treated with 100 nM MMC and were grown for 48 h. Collected cells were resuspended in 300 μl PBS. While vortexing, 700 μl of ice cold 100% (v/v) ethanol were added dropwise, and the suspension was stored at −20 °C at least overnight. Thirty minutes before fluorescence-activated cell sorting (FACS), cells were spun down, resuspended in propidium iodonium mix (1 ml PBS, 10 μl RNase (of stock solution of 20 mg/ml), 10 ml propidium iodonium (of stock solution of 1 mg/ml)) and analyzed using FACS Calibur (Becton Dickinson). Cell cycle analysis was performed using the FlowJo software (Tree Star, Inc).

Breakage analysis. Cells were treated with 0.1 μg DEB per ml of media for 72 h, arrested with colcemid (0.17 μg per ml of media) for 20 min (LCL) or 2 h for fibroblasts, harvested, incubated for 10 min at 37 °C in 0.075 M KCl and fixed in the freshly prepared methanol:glacial acid acid (3:1 vol/vol). Cells were stored at 4 °C and, when needed, dropped onto wet slides and air dried at 40 °C for 60 min before staining with KaryoMAX Giemsa (Invitrogen) Gurr Buffer for 3 min. After rinsing with fresh Gurr Buffer followed by distilled water, the slides were fully dried at 40 °C for 60 min and scanned using the Metasytems Metafer application.

Immunoprecipitations. For immunoprecipitations, cells were lysed in MCLB (50 mM Tris, 150 mM NaCl and 0.5% NP-40) supplemented with protease inhibitors (Roche) and phosphatase inhibitors (Calbiochem). One or two milligrams of protein extract was incubated with 5 μg of the indicated antibody and 10 μl of Protein A/G PLUS-Agarose (Santa Cruz). Following five washes in lysis buffer, the immunoprecipitates were eluted in tris-glycine SDS sample buffer and size fractionated on Novex 3%–8% Tris-Acetate gel (Invitrogen).

Mitomycin C sensitivity assay. Cells were plated in a six-well plate in triplicate at a density of 2.5 × 10^4 cells well. Twenty-four hours later, MMC was added at final concentrations from 0–100 nM. After 8 days in culture, cell numbers were determined using a Z2 Coulter Counter (Beckman Coulter). The cell number after MMC treatment was normalized to the cell number in the untreated sample to arrive at the percent survival.

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Figure S1. Damage sensitivity of patient cell lines.  

A. Mitomycin C (MMC) sensitivity of lymphoblasts from IFAR 1084 family.  

B. MMC sensitivity of lymphoblasts from IFAR 414 family.  

C. and D. UV sensitivity of the indicated cell lines.  

E and F. Camptothecin (CPT) sensitivity of the indicated cell lines.  

Cells were treated in triplicate with different levels of indicated damaging agents. After 8 days, cell number was determined using a coulter counter. Total cell numbers at each dose were divided by the number of cells in the untreated sample to arrive at percent survival.  

Error bars indicate standard deviation.
Figure S2. Sequencing of SLX4 in the IFAR1084. A. Chromatogram displaying skipping of the exon 5 as identified by direct sequencing of full length RT-PCR product from RA3042 cell line. B. Chromatogram displaying the splice donor site mutation and its segregation in the genomes of the IFAR1084 family. Genomic DNA from the fibroblast RA3083 cell line derived from the proband and the genomic DNA isolated from the peripheral blood (PB) of the other individuals were amplified and sequenced as described in Online Methods. C. Alignment of the UBZ domains of SLX4 from Mus musculus (Mus m.), Rattus norvegicus (Rattus n.), Homo sapiens (Homo s.), Pan troglodytes (Pan t.), Bos Taurus (Bos t.), Gallus gallus (Gallus g.), Danio Rerio (Danio r.). The residues that coordinate Zn are highlighted in red. Grey highlights the residues that are deleted in the patient from IFAR 1084 family. Magenta stars point to the Cys residues mutated for the experiment shown in Figure 4C (UBZ MUT). D. Cell extracts of primary BJ and RA3083 cells were subjected to immunoprecipitation using the SLX4 antibody and immunoprecipitates were bloted with another SLX4 antibody.
Figure S3. Sequencing of SLX4 in the IFAR414. A. Chromatogram displaying heterozygosity for the c.514delC mutation in direct sequencing of the cDNA from RA1376 cell line. B. Chromatogram displaying the first heterozygous mutation in the genomic DNA from the fibroblast RA3331 cell line derived from the proband and the genomic DNA isolated from the PB of the other individuals. C. Chromatogram displaying the second heterozygous mutation in IFAR414 family. Genomic DNA from the fibroblast RA3331 and RA1376 cell lines derived from the proband and the genomic DNA isolated from the mother's peripheral blood were amplified and sequenced as described in Online Methods. Note that there is apparent homozygosity in the patient and the carrier due to sequencing of a PCR product detecting only the allele carrying a genomic deletion. D. Chromatogram displaying heterozygosity for the deletion/insertion mutation in RA1376. The 3' junction is shown on the left and the 5' junction is shown on the right. E. PCR to identify the mutant deletion/insertion in the SLX4 allele in the indicated cell line and peripheral blood samples. Father's blood (sample RB09-1124.1), Mother's blood (sample RB09-1124.2).
**Figure S4.** Complementation of RA3083 and RA3331 cells with SLX4 cDNAs.  

A. Expression of SLX4 alleles in RA3083 hTERT cells used in studies of MMC sensitivity and cell cycle analysis after MMC treatment. WT, mutant SLX4 (MUT) and a degradation (DEG) product are indicated. 

B. Expression of SLX4 alleles in RA3331 E6E7 cells used in MMC sensitivity, breakage and cell cycle studies. 

C. Expression of SLX4 alleles in RA3083 E6E7 cells used in breakage studies. 

D. Quantification of cell cycle data shown in Figure 3B top panel. The experiment was done in triplicate and error bars indicate standard deviation. 

E. Quantification of cell cycle data shown in Figure 3B bottom panel. The experiment was done in triplicate and error bars indicate standard deviation. 

F. Complementation of the chromosome breakage phenotype. Indicated cells were treated with DEB at 0.1 μg/ml and chromosomal abnormalities were analyzed on metaphase spreads.

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| RA3083 E6E7 | RA3331 E6E7 |
|-------------|-------------|
| **CONTROL** | **WT SLX4** | **p.R317_F387del SLX4** | **CONTROL** | **WT SLX4** | **p.L672V_fsX119 SLX4** |
| **DEB concentration μg/ml** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| **# Metaphases** | 43 | 53 | 33 | 32 | 50 | 57 |
| **#Total Breaks** | 156 | 49 | 78 | 401 | 175 | 306 |
| **#Chromatid breaks** | 100 | 35 | 46 | 311 | 169 | 232 |
| **#Triradial** | 20 | 6* | 9 | 34 | 2 | 31 |
| **#Quadiradial** | 8 | 1* | 7 | 11 | 1 | 6 |
| **% Metaphases with breaks** | 51 | 13 | 48 | 97 | 22 | 74 |
| **# Breaks per metaphase** | 3.6 | 0.9 | 2.4 | 12.5 | 3.5 | 5.4 |

*1 cell had 3 triradial and one quadiradial chromosomes
Figure S5. Analysis of SLX4 interacting partners in SLX4 mutant cells. A. Cell extracts of primary BJ and RA3083 cells were subjected to immunoprecipitation using the SLX4 antibody. Interacting proteins were identified by immunoblotting with the indicated antibodies. Asterisk indicates SLX4 proteins. B. BJ E6E7 and RA3331 E6E7 cells were subjected to immunoprecipitation using antibody #392, which recognizes the N-terminus of SLX4. Interacting proteins were identified by immunoblotting with the indicated antibodies.
| Exon/Intron (i) | Primer Name | Forward Primer | Primer Name | Reverse Primer | PCR Product (bp) |
|----------------|-------------|----------------|-------------|----------------|-----------------|
| 2 FL043        | BTBD12-SLX4 ex2-1F | CCTCCCTGGAGAAGACTGCGCAATAA | FL044 BTBD12-SLX4 ex2-1R | TCTTGAGGAGGAGGAGGACTTCACT | 768 |
| 2 FL045        | BTBD12-SLX4 ex2-2F | AGGCTTCTGTGGTGTCACCTTTC | FL046 BTBD12-SLX4 ex2-2R | GCTGGCTGCTGGTTGTGTTAA | 711 |
| 3 FL019        | BTBD12-SLX4 ex3F | ACCACACCACACTCCTACCAAG | FL020 BTBD12-SLX4 ex3R | GACCTCTACCTTCTGCTGAGT | 675 |
| 4 FL005        | BTBD12-SLX4 ex4F | TTCGGAGCTGCTGCTGCTTCTTTC | FL006 BTBD12-SLX4 ex4R | TCACGGCCGCTCTACTCCAACTCC | 934 |
| 5 FL007        | BTBD12-SLX4 ex5F | GGGCCGACCTGGACATTTCCC | FL008 BTBD12-SLX4 ex5R | TGCTGACTGCAACCTTCTACCTTC | 889 |
| i5 FL009       | BTBD12-SLX5 ivs5-1F | TGCGAGCAGTAGTTCACACCTTAC | FL010 BTBD12-SLX5 ivs5-1R | AGCAGATGTCACCCAACCTTCTTT | 870 |
| i5 FL011       | BTBD12-SLX5 ivs5-2F | AACACTCTCTACTCTCCTGGT | FL012 BTBD12-SLX5 ivs5-2R | CACCCCTGCACTGCAATATCAG | 939 |
| 5 FL013        | BTBD12-SLX5 ivs5-3F | AGGCGGATAGTTCACACCTTC | FL014 BTBD12-SLX5 ivs5-3R | GGGTTGAGGTTCTCCTTACAG | 1105 |
| 6 FL015        | BTBD12-SLX5 ex6_7F | TGGGAGAATTTGATGGGTCAG | FL016 BTBD12-SLX5 ex6_7R | GCTGAGGCTGAGGCTGAGGAGG | 1099 |
| 7 FL081        | BTBD12-SLX4 ex8_9F | CTCGAGCAGTAGTTCACACCTTAC | FL082 BTBD12-SLX4 ex8_9R | AGCAGATGTCACCCAACCTTCTT | 1099 |
| 8_9 FL053      | BTBD12-SLX4 ex9-2F | CCGCCGCTCCTCTGAGT | FL054 BTBD12-SLX4 ex9-1R | GCTGAGGCTGAGGCTGAGGAGG | 736 |
| i9 FL055       | BTBD12-SLX4 ivs9-1F | AGGCGGATAGTTCACACCTTC | FL056 BTBD12-SLX4 ivs9-2R | GGGTTGAGGTTCTCCTTACAG | 722 |
| i9 FL057       | BTBD12-SLX4 ivs9-2F | AGGCGGATAGTTCACACCTTC | FL058 BTBD12-SLX4 ivs9-1R | GGGTTGAGGTTCTCCTTACAG | 998 |
| 10 FL023       | BTBD12-SLX4 ex10F | CATCTGCTGAGTTCACACCTTAC | FL024 BTBD12-SLX4 ex10R | GGGTTGAGGTTCTCCTTACAG | 428 |
| 10 FL057       | BTBD12-SLX4 ivs10-1F | CCAGTTCTAGAGCAGAGCAG | FL058 BTBD12-SLX4 ivs10-1R | CCAGTTCTAGAGCAGAGCAG | 939 |
| 10 FL059       | BTBD12-SLX4 ivs10-2F | GTTTGGCGAGGAGGACTTAC | FL060 BTBD12-SLX4 ivs10-2R | TCACCAGAGAGGACTTAC | 939 |
| 11 FL025       | BTBD12-SLX4 ex11F | GTAAAGCAGTACACACACTTAC | FL026 BTBD12-SLX4 ex11R | TGGGACACAACTCCTCAG | 413 |
| 11 FL061       | BTBD12-SLX4 ivs11-1F | CACATCTCATTACACACAGCACT | FL062 BTBD12-SLX4 ivs11-1R | AAAAGACATGGCATCACAGCAGAAT | 759 |
| 11 FL063       | BTBD12-SLX4 ivs11-2F | AGGGCGAGTACCTGCGCAATAATG | FL064 BTBD12-SLX4 ivs11-2R | GGGTTGAGGTTCTCCTTACAG | 944 |
| 12 FL047       | BTBD12-SLX4 ex12-1F | TGGGAGTGGTGTGGCCGGAG | FL048 BTBD12-SLX4 ex12-1R | GGGTTGAGGTTCTCCTTACAG | 915 |
| 12 FL049       | BTBD12-SLX4 ex12-2F | AGTAGATGCGGGAGGATTACGA | FL050 BTBD12-SLX4 ex12-2R | CGGCTCTGAGGCTGAGT | 913 |
| 12 FL051       | BTBD12-SLX4 ex12-3F | GGGCTTGTGCTGCTTGGT | FL052 BTBD12-SLX4 ex12-3R | CGGCTCTGAGGCTGAGT | 1111 |
| 12 FL065       | BTBD12-SLX4 ivs12-1F | GGGCGGCTGCTGCTTGGT | FL066 BTBD12-SLX4 ivs12-1R | GACCTCTGAGCTGCTTCTTACAT | 1344 |
| 12 FL067       | BTBD12-SLX4 ivs12-2F | TGGGAGGCTGACTAAGAAT | FL068 BTBD12-SLX4 ivs12-2R | TGGGAGGCTGACCTTACATAAT | 1261 |
| 12 FL083       | BTBD12-SLX4 ex12-3F | CAAGCTCTGACCTGCTTGGT | FL084 BTBD12-SLX4 ex12-3R | GTGAGAGCTGACCTTACATAAT | 1217 |
| 12 FL071       | BTBD12-SLX4 ex12-4F | GCCGCCGAGTGGCTGTCATTTT | FL072 BTBD12-SLX4 ex12-4R | TGGGAGGCTGACCTTACATAAT | 1068 |
| 13 FL073       | BTBD12-SLX4 ex13-1F | CACACAGGAGGAGGAGGAGGAC | FL032 BTBD12-SLX4 ex13R | CGGCTCTGAGGCTGAGT | 486 |
| 13 FL075       | BTBD12-SLX4 ivs13-1F | ACTGGGATAGTGGGCTGCTTCTT | FL074 BTBD12-SLX4 ivs13R | CGGCTCTGAGGCTGAGT | 893 |
| 15 FL033       | BTBD12-SLX4 ex14_15F | TAAGGACGAAGGCTGAGGTGTTG | FL034 BTBD12-SLX4 ex14_15R | ATGGGAGGCTGAGGTGTTG | 1399 |
| i5 FL086       | BTBD12-SLX4 ivs5-3R seq 2076_2096 | TTGGGCGCTGAGGAAAGAATA | FL087 BTBD12-SLX4 ivs9-1R seq 509_527 | ATGGGAGGCTGAGGTGTTG | 428 |
| i9 FL087       | BTBD12-SLX4 ex9-1R seq 509_527 | ATGGGAGGCTGAGGTGTTG | FL088 BTBD12-SLX4 ex9-1R seq 509_527 | ATGGGAGGCTGAGGTGTTG | 722 |
### Supplementary Table 2. Mutagenesis primers

| SLX4_C296A_C299A | GGAAAAGGTTGGTTCTCAGATCTCTAAAAG |
| SLX4_C336A_C339A | CCTCAGATCCCTGAGGCCATGCTGCTGAGAAAACCGTT |

### Supplementary Table 3. siRNA sequences

| SLX4_1 | TTTGGATGAAGATTTCTGAGATCTG, |
| SLX4_2 | TTCCGTGGCTCTGCTGCTGCTGTTG |
| SLX4_3 | AAGAGTTCTCAGTGAATTCGCCC |

**FANCI** (used as a combination of three at total concentration of 50 nM)

| TCTCCTCAGTTGTGTCAGATGTAT, |
| GGCAGCTGTGTTGACACCTTGTATA |
| GCTGGTGAAGCTGTGCTGATTCTCAT |

**ATR** (used as a combination of three at total concentration of 50 nM)

| GGGAAATAGTAGACACCTCATCTAAA |
| GGTCTGGAGTAAGAAGACCAATTTA |
| CCACCTGAGGGTAAGAACATGTATA |

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