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**General information\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

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**Related article:**

**Introductory information\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**1. Extended data 1:** Excel file containing raw data for the quantification of spontaneously stalled forks (Figure 4b), replication track length before and after exposure to camptothecin (CPT) (Figure 4c) and CPT-induced RAD51 foci (Figure 4d) in WT fibroblasts (WT1) and fibroblasts from the affected proband (patient).

**2. Extended data 2:** Pdf file containing uncropped images of the Western blots used in figure 4a and 4d.

**Methodological information**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**1. Extended data 1:** Immunofluorescence and DNA fibre spreading were carried out as previously described in Burrage et al. (2019). Am. J. Hum. Genet. 104:422-438. Images of DNA fibres or cells with RAD51 foci were captured on a Nikon Eclipse Ni microscope using the Nikon NIS Elements software and were analysed using ImageJ v1.48. Replication fork stalling was quantified by eye from multiple images across at least independent experiments. The percentage of cells with RAD51 foci were quantified by eye from multiple images across at least independent experiments. RAD51 foci were quantified in a minimum of 300 cells were quantified per experiment. Statistical analysis was carried out using GraphPad Prism v10.4.1. An unpaired Student’s T-test (two-sided, equal variance) was used to calculate statistical significance in Figure 4a and 4d. A Mann-Whitney rank sum test was used to calculate statistical significance in Figure 4c. (\* *p*<0.05; \*\* *p*<0.01; \*\*\**p*<0.001).

**2. Extended data 2:** Images of uncropped Western blots. SDS-PAGE and Western blotting were carried as previously described in Burrage et al. (2019). Am. J. Hum. Genet. 104:422-438. Western blots were scanned using an Epson Perfection V350 Photo scanner and processed using Adobe Photoshop.

**Data-specific information**\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

**Definitions/acronyms/data labels:** WT1: WT fibroblast; Exp: Experiment; IdU: 5-Iodo-2’-deoxyuridine; CldU: 5-chloro-2’-deoxyuridine; CPT: camptothecin;