SUPPLEMENTARY METHODS

Samples and cell lines
Genomic DNA was obtained from 82 individuals with Fanconi anemia and / or their parents with informed consent. These individuals came from several different clinicians and repositories. All had a clinical diagnosis of Fanconi anemia. This diagnosis was confirmed by study of baseline and DEB- or mitomycin C-induced chromosomal breakage or cell cycle arrest in PHA-stimulated cultured peripheral blood lymphocytes as previously described\textsuperscript{1,2}, except in cases that died before suitable samples could be obtained. In some cases, diagnosis of Fanconi anemia was based on fibroblast analysis. None of the cases included in this study were known to be due to an existing Fanconi anemia gene. However, the extent to which known genes were formally excluded varied, depending on the available samples. Methods used for exclusion were sequencing, retroviral complementation group analysis\textsuperscript{3}, and western blot analysis for FANCD1 (ref. 4), FANCD2 (ref. 5) and FANCJ\textsuperscript{6}. BRCA2 mutations had been excluded in all cases by sequencing of all exons and intron/exon boundaries in genomic DNA from either the case and/or parents. The 176 samples from normal individuals were from the 1958 Birth Cohort Collection (see http://www.els.ioe.ac.uk/Cohort/Ncgs/mainncgs.htm). This research was approved by the London Multicentre Research Ethics Committee (05/MRE02/17).

PALB2 sequencing
Primers were designed to amplify the 13 exons and intron-exon boundaries of PALB2 (Supplementary Table 1). We used a touchdown 68-50°C protocol to amplify all products which were sequenced using the BigDye Terminator Cycle sequencing kit and a 3730 automated sequencer (ABI Perkin Elmer). Sequencing traces were analysed using Mutation Surveyor software (www.softgenetics.com) and by visual inspection.

Analysis of splice site mutations
To isolate RNA we used the PureLink Micro-to-Midi Total RNA Purification System (Invitrogen) from which cDNA was prepared using oligo(dT)20 primers and
SuperScriptII (Invitrogen). cDNA was amplified with primers
ggggttaggatcaggaataattagagagtattctgagctCAGTCTGTCACAAAGCCTATTCTG
GAAACGAAATCAGAGAGATCAGGAATTATAGGGTTAATCACAATGAGCTGAAACreverse to show exon skipping of 9 and 10 in LOV (father of LOAO) and CAGTCTGTCACAAAGCCTATTCTGforward/GAGTCATCCCTGTGCCAAAGreverse to show the insertion of 4bps in LNEY. Amplified DNA was sequenced as detailed above.

**Immunoblotting**
PALB2 immunoblots were performed with samples containing 50 µg total protein each on 7% NuPage Tris-Acetate polyacrylamide gels (Invitrogen). Membranes were probed with polyclonal rabbit anti-PALB2 antiserum raised against the first 120 amino acids of PALB2 at a concentration of 1:1000 (gift of B Xia). Secondary antibody was ECL donkey anti-rabbit IgG horseradish peroxidase-linked whole antibody (GE Healthcare) at 1:2000. Detection was by the chemiluminescence technique using the ECL system (Amersham). BRCA2 immunoblots were performed with samples containing 80 µg total protein each on 3-8% NuPage Tris-Acetate polyacrylamide gels (Invitrogen). Membranes were probed with polyclonal rabbit anti-BRCA2 antiserum (Ab-2, Calbiochem), raised against amino acids 3245-3418 of BRCA2, at a concentration of 1:200. Secondary antibody (1:5000) and detection were the same as for the PALB2 immunoblots. Immunoblotting for detection of monoubiquitinated FANCD2 was performed using previously described methods.

**Transduction and complementation**
For complementation studies, the γ-retroviral vectors pOZC-PALB2 (ref 7) and pS11EG (expressing GFP) were packaged in PG 13 cells and used for transduction of patient and control cell lines. Gene transfer was monitored by CD25 (pOZC-PALB2) or GFP (pS11EG) expression. Transduced cells were grown for 48 h in the presence of MMC at concentrations of 12 ng/ml (fibroblasts) or 15 ng/ml (LCL). The cells were vitally stained with Hoechst 33342 fluorescent dye (Molecular Probes) at 16 µg/ml. DNA histograms were recorded by flow cytometry.

**Lymphocyte survival assay**
Lymphocyte survival was determined using CD3/CD28/IL2-stimulated lymphocytes (Supplementary Fig 1b). Transduction was with FANC cDNAs and GFP cDNA as a
control, separately cloned into a S11-type γ-retroviral vector as described\textsuperscript{3}. The cells were exposed to various concentrations of MMC for 5 days. Live/dead cell ratios were determined by propidium iodide exclusion/uptake on flow cytometry.

**Immunofluorescence**

To examine RAD51 foci formation, nuclear foci were induced by ionizing irradiation with 8 Gy of fibroblast cultures grown on glass slides. Cells were fixed 8 h later using 4% paraformaldehyde in PBS (pH 6.8) and permeabilized with 0.1% Triton X-100. Primary antibody was mouse monoclonal anti-RAD51 (GeneTex) at a dilution of 1:100. Secondary antibody was Alexa594-conjugated goat anti-mouse IgG (Molecular Probes) at 1:200. DAPI was used as DNA counterstain. Nuclear foci were counted on a fluorescence microscope. All slides were evaluated independently by several investigators.

**References**


