METHODS

Mice. *Aldh2*$^{-/-}$*Fancd2*$^{-/-}$ mice were generated on a C57BL/6 × 129S4S6/Sv F_1 background. To this end, the previously reported *Fancd2* allele (*Fancd2*^{tm1Hou}; MGI ID: 2673422, a gift from M. Grompe) was back-crossed onto the C57BL/6Jo1a background for 10 generations and crossed with *Aldh2*$^{+/-}$ (C57BL/6N) mice to generate *Aldh2*$^{+/-}$*Fancd2*$^{+/-}$ mice on a pure C57BL/6 background. Likewise, the previously reported *Aldh2* allele (*Aldh2*$^{tm1a(EUCOMM)Wtsi}$; MGI ID: 4431566, EUCOMM[6]) was backcrossed from C57BL/6N onto 129S6/Sv for five generations and crossed with *Fancd2*$^{+/-}$ mice to generate *Aldh2*$^{+/-}$*Fancd2*$^{+/-}$ mice on a 129S4S6/Sv background. Finally, *Aldh2*$^{-/-}$*Fancd2*$^{-/-}$ and control mice were generated as F_1 hybrids from crosses between *Aldh2*$^{+/-}$*Fancd2*$^{+/-}$ females (129S4S6/Sv) and *Aldh2*$^{+/-}$*Fancd2*$^{+/-}$ males (C57BL/6).

To generate *Fanca*$^{-/-}$*Ku70*$^{-/-}$ mice on a pure C57BL/6 background, *Fanca*$^{+/-}$ mice (*Fanca*$^{tm1a(EUCOMM)Wtsi}$; MGI ID: 4434431, C57BL/6N, EUCOMM[5]) were crossed with *Ku70*$^{+/-}$ mice (*Xrcc6*^{tm1Fwa}, MGI ID: 2179954[30]), and the *Fanca*$^{+/-}$*Ku70*$^{+/-}$ progeny were then intercrossed to generate all possible genotypes. Pups from these crosses were genotyped at between two and three weeks old. For the generation of *Fanca*$^{fl/-}$*Ku70*$^{-/-}$*Vav1*-iCre^{+} tissue-specific double mutants (also on a pure C57BL/6 background), *Fanca*$^{+/-}$ mice were first crossed with FLP deletor mice[31] to produce the *Fanca* floxed allele (*Fanca*^{fl} or *Fanca*$^{tm1c(EUCOMM)Wtsi}$). Recombination of the Frt sites was verified by PCR (using the primers FL033, GCCTTTGCTGCTCTAATTCCATGT; FL040, TCAGCTCACTGAGACGCAACCTTTT ACACT; and En2A, GCTTCACTGAGTCTCTGGCATCTC), and reconstitution of FANCA expression was verified by western blotting spleen extracts of *Fanca*$^{fl/fl}$ mice (Extended Data Fig. 3). *Fanca*$^{+/fl}$ mice were then crossed with *Ku70*$^{+/-}$ mice to eventually produce *Fanca*$^{fl/fl}$*Ku70*$^{+/-}$ mice. Finally, these mice were crossed with *Fanca*$^{+/-}$*Ku70*$^{+/-}$ *Vav1*-iCre to generate *Fanca*$^{fl/-}$*Ku70*$^{-/-}$*Vav1*-iCre and control mice. The *Vav1*-iCre allele directs the expression of the iCre recombinase to HSCs and haematopoietic tissues[32], and in this case yields the *Fanca*-null allele (*Fanca*$^{\Delta}$ or *Fanca*$^{tm1d(EUCOMM)Wtsi}$). *Fanca*$^{\Delta/\Delta}$ mice phenocopy the *Fanca*$^{-/-}$ mice reported previously, as judged by FANCA expression, sterility and sensitivity to mitomycin C (Fig. 3, Extended Data Fig. 3).

Similarly to *Aldh2*$^{-/-}$*Fancd2*$^{-/-}$ F_1 mice, *Aldh2*$^{-/-}$*Fancd2*$^{-/-}$*Trp53*$^{-/-}$ mice were also generated in a C57BL/6 × 129S4S6/Sv F_1 background. In brief, the *Trp53* allele reported previously[33] was backcrossed onto 129S6/Sv or C57BL/6J for six generations. *Trp53*$^{+/-}$ mice were then intercrossed with *Aldh2*$^{-/-}$*Fancd2*$^{+/-}$ mice to establish parental (F_0) strains on both genetic backgrounds, which were finally crossed to obtain *Aldh2*$^{-/-}$*Fancd2*$^{-/-}$*Trp53*$^{-/-}$ and control F_1 mice.

For single HSC transplantation experiments, we used CD45.1 homozygous mice on a C57BL/6J × 129S6/Sv F_1 background as recipients. CD45.1 (or *Ptprc*^{a}) had been serially backcrossed from B6.SJL onto 129S6/Sv for six generations, with selection at each generation by serotyping with anti-CD45.1 (A20, FITC, BioLegend) and anti-CD45.2 antibodies (104, PE-Cy7, BioLegend).

For the *in vivo* point-mutation assay, mice carrying BigBlue λLIZ shuttle vector repeats (Stratagene) were crossed with *Aldh2*$^{-/-}$*Fancd2*$^{+/-}$ mice on a C57BL/6 × 129S4S6/Sv hybrid background. The resulting mice were intercrossed to obtain *Aldh2*$^{-/-}$*Fancd2*$^{-/-}$ BigBlue λLIZ and control mice.

For the analysis of Mendelian segregation of alleles, sample size was determined by power analysis using http://biomath.info/power/chsq1gp.htm. Sufficient mice to detect a 50% reduction in expected frequency were used, using power of 0.8 and alpha 0.05. No statistical methods were used to predetermine sample size in the other animal experiments. No randomization was employed. The investigators were blinded to the genotypes of mice throughout the study and data were acquired by relying purely on identification numbers.

All animals were maintained in specific pathogen-free conditions. In individual experiments all mice were matched for gender and age (8–12 weeks). All animal experiments undertaken in this study were done so with the approval of the UK Home Office.

Ethanol treatment. For acute ethanol exposure, *Aldh2*$^{-/-}$*Fancd2*$^{-/-}$ mice and appropriate controls were injected intraperitoneally with ethanol. The total dose of 5.8 g kg^{-1} was split into two injections separated by 4 h. Ethanol (96%, Sigma) was diluted to 28% v/v in saline, and administered twice at 13 ml kg^{-1}. Mice were exsanguinated 48 h after the second injection and peripheral blood was analysed with the micronucleus assay. Alternatively, mice were injected with colchicine for the preparation of metaphase spreads for M-FISH. For SCE analysis, mice were injected with colchicine 12 h after the second ethanol injection.

For chronic ethanol treatment of *Aldh2*$^{-/-}$*Fancd2*$^{-/-}$*Trp53*$^{-/-}$ and control mice, ethanol was administered in drinking water for ten days as reported previously[6]. For the first five days, the drinking water supply was replaced by a solution of 10:15:75 blackcurrant Ribena:ethanol:water, followed by a 10:20:80 solution for the last five days. A 50 μl blood sample was taken from tail veins before alcohol exposure, and by cardiac puncture at the end of the experiment, to measure full blood counts. Femurs were dissected for histological analysis and to determine bone marrow cellularity.

Preparation of mouse bone marrow for metaphase spreads. Treated or untreated young mice (8–12 weeks of age) were injected intraperitoneally with 100 μl of colchicine (0.5% w/v in water, Sigma). After 30 min the mice were culled by cervical dislocation, femurs were harvested and placed in ice-cold PBS. Bone marrow cells were flushed with 10 ml of pre-warmed hypotonic solution (75 mM KCl, 37 °C) through a 70-μm cell strainer and incubated for 15 min in a water bath at 37 °C. After the incubation, 1 ml of fixative (3:1 methanol:acetic acid) was added dropwise to the hypotonic buffer, and mixed by gentle inversion of the tube. The tubes were spun down for 10 min at 250 *g* and the supernatant was aspirated, leaving 50 μl and the cell pellet in the tube. The cells were resuspended by flicking the base of the tube very gently, 3 ml of fixative were added dropwise and the volume was made up to 10 ml by pipetting fixative down the side of the tube. The cells were incubated at room temperature for 30 min and stored at −20 °C until further use.

SCE assay for mouse bone marrow. The staining of metaphase spreads for the quantification of SCEs was adapted from published protocols[34,35]. A 50 mg BrdU slow-release pellet (Innovative Research of America) was surgically implanted subcutaneously into 8-to-12-week-old mice. Unchallenged mice were injected with colchicine 24 h later and metaphases were prepared after 30 min. Mice challenged with ethanol were injected intraperitoneally with ethanol 8 h and 12 h after implantation of the BrdU pellet. A total ethanol dose of 5.8 g kg^{-1} was split between these two doses as described previously. Metaphases were prepared as outlined above. Cells were then dropped from a height of 30 cm onto chilled, humidified slides. The slides were then dried for 1 h at 62 °C in a hybridization oven. Cells were washed in 2× SSC for 5 min at room temperature. Cells were stained for 15 min at room temperature with 1 μg ml^{-1} Hoechst 33258 pentahydrate (H3569, Molecular Probes) in 2× SSC. The slides were then transferred to a Petri dish with 2× SSC and exposed to UV irradiation for 30 min in a Stratalinker Crosslinker (Stratagene). The slides were then dehydrated by passing them through an ethanol series (70%, 96% and 100%) and placed in PBS for 5 min at room temperature. The DNA was denatured by exposure to 0.07 N NaOH for 2 min at room temperature. The slides were then washed three times in PBS for 5 min. The slides were then blocked in PBS, 1% BSA, 0.5% Tween-20 for 1 h at room temperature and stained overnight with a FITC-conjugated mouse anti-BrdU antibody (Clone B44, BD Biosciences) diluted 1:1 in PBS, 3% BSA, 0.5% Tween-20 at room temperature. The slides were then washed three times with PBS, 1% BSA, 0.5% Tween-20 for 5 min at room temperature and stained with goat anti-mouse Alexa Fluor-488 secondary antibody (A-11001, Life Technologies) diluted 1:500 in PBS, 1% BSA, 0.5% Tween-20 for 6 h at room temperature. The slides were then washed three times in PBS, 1% BSA, 0.5% Tween-20 for 15 min and stained with Hoechst 33342 trihydrochloride (H3570, Molecular Probes) diluted 1:2000 in PBS for 15 min at room temperature. The slides were then washed three times in PBS for 10 min on each occasion, washed once in water for 5 min, mounted with ProLong Gold Antifade Mountant (P36930, Molecular Probes) and coverslips were lowered onto the slides. Thirty metaphases were captured per sample using a Zeiss LSM 780 confocal microscope (Zeiss). The number of sister-chromatid exchanges per metaphase was then counted blind.

M-FISH karyotyping. For M-FISH, chromosome-specific DNA libraries were generated from flow-sorted chromosomes provided by the Flow Cytometry Core Facility of the Wellcome Trust Sanger Institute, using the GenomePlex Complete whole-genome amplification kit (Sigma–Aldrich). A mouse 21-colour painting probe was prepared following the pooling strategy[36]. Five mouse-chromosome pools were each labelled with ATTO 425-, ATTO 488-, Cy3-, Cy5- and Texas Red-dUTPs (Jena Bioscience), respectively, using the GenomePlex WGA reamplification kit (Sigma–Aldrich) and a dNTP mixture as described previously[37]. The labelled products were pooled and sonicated to achieve a size range of 200–1,000 bp, optimal for use in chromosome painting. The sonicated DNA was ethanol-precipitated together with mouse Cot-1 DNA (Thermo Fisher Scientific), and resuspended in a hybridization buffer (50% formamide, 2× SSC, 10% dextran sulfate, 0.5 M phosphate buffer, 1× Denhardt's solution, pH 7.4). Bone marrow cells suspended in fixative as described above (3:1 methanol:acetic acid) were dropped onto precleaned microscope slides, followed by fixation in acetone (Sigma–Aldrich) for 10 min and dehydration through an ethanol series (70%, 90% and 100%). Metaphase spreads on slides were denatured by immersion in an alkaline denaturation solution (0.5 M NaOH, 1.0 M NaCl) for 2 min, followed by rinsing in 1 M Tris-HCl (pH 7.4) solution for 3 min, 1× PBS for 3 min and dehydration through a 70%, 90% and 100% ethanol series. The M-FISH probes were denatured at 65 °C for 10 min before being applied onto the denatured slides. The hybridization area was sealed with a 22 mm × 22 mm coverslip and rubber cement. Hybridization was carried out in a 37 °C incubator for approximately 44–48 h. The post-hybridization washes included a 5-min stringent wash in 0.5× SSC at 75 °C, followed by a 5 min rinse in 2× SSC containing 0.05% Tween-20 (VWR) and a