2 min rinse in 1× PBS, both at room temperature. Finally, slides were mounted with SlowFade Gold mounting solution containing DAPI (Thermo Fisher Scientific). M-FISH images were visualized on a Zeiss AxioImager D1 fluorescent microscope equipped with narrow band-pass filters for DAPI, DEAC, FITC, Cy3, Texas Red and Cy5 fluorescence and an ORCA-EA CCD camera (Hamamatsu). M-FISH digital images were captured using the SmartCapture software (Digital Scientific UK), and processed using the SmartType Karyotyper software (Digital Scientific UK). Thirty metaphases for each sample were karyotyped by M-FISH.

DT40 clonogenic survival. DT40 cells were grown in RPMI Medium 1640 (Life Technologies, 61870), supplemented with 7% fetal bovine serum (FBS, Life Techonologies, 10270), 3% chicken serum (Life Technologies, 16110), 50 μM β-mercaptoethanol and penicillin/streptomycin, at 37 °C in a 5% CO_2 incubator. Sensitivity assays were performed as previously described[6]. In brief, 10^5 cells were incubated with drug-containing medium in a sealed FACS tube at 37 °C for 2 h (acetaldehyde) or 1 h (mitomycin C or cisplatin). Dilutions were plated in 6-well plates containing semi-solid medium (4000 cP methyl cellulose (M0512 Sigma), DMEM/F-12 powder (Life Technologies, 32500-043), 7% FBS, 3% chicken serum, 50 μM β-mercaptoethanol and penicillin/streptomycin). Plates were incubated for 7–10 days, after which time colonies were counted manually. Survival is plotted as a percentage relative to untreated cells. Each data point represents the mean of three independent experiments each carried out in quadruplicate.

Sensitivity assays of primary mouse B cells. These assays were performed as described previously[5]. Lymphocytes purified from the spleen using Lympholyte M (Cederlane) were stimulated with lipopolysaccharide (L4391, Sigma) at a final concentration of 40 μg ml^{-1}. Cells (4×10^5) were then plated with acetaldehyde in one well of a 24-well plate. After seven days, viable cells were counted using trypan blue exclusion from 100 images on a Vi-Cell XR cell viability counter (Beckman Coulter). Each data point represents the mean of three independent experiments each carried out in quadruplicate.

Survival assays of colony-forming units (CFU). Bone marrow cells were isolated using IMDM medium, and single cell suspensions were obtained by passing the bone marrow through a 70-μm cell strainer (Falcon). Nucleated cells were counted by diluting cells tenfold in a 3% solution of acetic acid with methylene blue (Stem Cell Technologies) using a Vi-Cell XR cell viability counter (Beckman Coulter). Cells were resuspended to make up 1.5 ml of IMDM containing 30×10^6 cells and 250 μl of each suspension was mixed with 250 μl of IMDM containing 2× acetaldehyde to give final concentrations of 0, 1, 2, 4 and 8 mM acetaldehyde. The cells were incubated at 37 °C for 4 h in sealed tubes, after which two tenfold serial dilutions were made. 400 μl of cells were then added to 4 ml of MethoCult M3534 (StemCell Technologies), and the total volume of each dilution was plated in two wells of a six-well plate each containing 10^6, 10^5 and 10^4 cells, respectively. After seven days of culture at 37 °C with 5% CO_2, the colonies were counted and the relative survival was plotted. Each data point represents the average of experimental duplicates carried out on three mice of each genotype.

Flow cytometry. The micronucleus assay was performed essentially as described previously[38]. Treated or untreated mice (8–12 weeks of age) were bled and 62 μl blood was mixed with 338 μl PBS supplemented with 1,000 U ml^{-1} of heparin (Calbiochem). 360 μl of blood suspension was then added to 3.6 ml of methanol at −80 °C and stored at −80°C for at least 12 h. 1 ml of fixed blood cells was then washed with 6 ml of bicarbonate buffer (0.9% NaCl, 5.3 mM $NaHCO_3$). The cells were resuspended in 150 μl of bicarbonate buffer and 20 μl of this suspension was used for subsequent staining. 72 μl of bicarbonate buffer, 1 μl of FITC-conjugated CD71 antibody (GenTex, clone R17217.1.4) and 7 μl RNase A (Sigma) were premixed and added to 20 μl of each cell suspension. The cells were stained at 4 °C for 45 min, followed by addition of 1 ml bicarbonate buffer and centrifugation. Finally, cell pellets were resuspended in 500 μl bicarbonate buffer supplemented with 5 μg ml^{-1} propidium iodide (Sigma). The samples were analysed immediately on an LSRII FACS analyser (BD) and the data analysed with FlowJo v10.0.7.

For HSC quantification, bone marrow cells were isolated from tibiae and femurs with staining buffer (PBS supplemented with 2.5% FCS) and strained through 70-μm meshes. Red cells were lysed by resuspending the cells in 10 ml red cell lysis buffer (MACS Miltenyi Biotec) for 10 min at room temperature. After centrifugation, the cell pellet was resuspended in staining buffer and nucleated cells were counted with 3% acetic acid (StemCell Technologies) on a Vi-Cell XR cell viability counter (Beckman Coulter). Bone marrow cells (10×10^6 cells) were resuspended in 200 μl of staining buffer containing the following antibody solution: FITC-conjugated lineage cocktail with antibodies against CD4 (clone H129.19, BD Pharmingen), CD3e (clone 145-2C11, eBioscience), Ly-6G/Gr-1 (clone RB6-8C5, eBioscience), CD11b/Mac-1 (clone M1/70, BD Pharmingen), CD45R/B220 (clone RA3-6B2, BD Pharmingen), Fcε R1α (clone MAR-1, eBioscience), CD8a (clone 53-6.7, BD Pharmingen), CD11c (clone N418, eBioscience), TER-119 (clone Ter119, BD Pharmingen) and CD41 (FITC, clone MWReg30, BD Pharmigen); c-Kit (PerCP-Cy5.5, clone 2B8, eBioscience), Sca-1 (PE-Cy7, clone D7, eBioscience), CD150 (PE, clone TC15-12F12.2, BioLegend) and CD48 (biotin, clone HM48-1, BioLegend). The samples were incubated for 15 min at 4 °C and washed with 2 ml buffer. The cell pellets were resuspended in 200 μl staining buffer containing streptavidin-BV421 and incubated for another 15 min at 4 °C. Finally, cells were washed, resuspended in 500 μl staining buffer, data were acquired on a Fortessa FACS analyser (Becton Dickinson) and analysed with FlowJo v10.0.7. LKS cells were defined as lineage$^-$ CD41$^-$Sca-1$^+$ Kit$^+$ and HSCs were defined as LKS CD48$^-$ CD150$^+$.

To assess engraftment of single HSCs into irradiated recipients, 50 μl of blood was obtained from the tail vein of recipient mice every two weeks. RBCs were lysed by the addition of 1 ml of ammonium chloride lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH 7.2) and incubated for 10 min at room temperature. After centrifugation, the cell pellets were resuspended in 100 μl of staining buffer containing antibodies against: CD4 (FITC, clone H129.19, BD Pharmingen), CD8a (FITC, clone 53-6.7, BD Pharmingen), CD45R/B220 (PerCP-Cy5.5, clone RA3-6B2, BioLegend), CD11b/Mac-1 (PE, clone M1/70, BD Pharmingen), Ly-6G/Gr-1 (PE, clone 1A8, BD Pharmingen), TER-119 (PE-Cy7, clone TER-119, BioLegend), CD45.1 (BV421, clone A20, BioLegend) and CD45.2 (APC, clone 104, BioLegend). After incubation, cells were washed with 3 ml of staining buffer before being resuspended in 250 μl of the same buffer. Samples were run on the Fortessa analyser (BD) with the HTS module and the multilineage chimaerism was calculated using FlowJo v10.0.7. TER-119 was used to exclude RBC debris and chimaerism was calculated for each of the WBC lineages (CD45$^+$ total WBCs, B220$^+$ B cells, CD4$^+$CD8$^+$ T cells and Gr-1$^+$ Mac-1$^+$ myeloid cells) as the proportion of cells derived from the single HSC (CD45.2$^+$) over the total number of CD45$^+$ cells, which includes cells derived from the recipient or carrier cells (CD45.1$^+$).

For intracellular staining of p53 and cleaved caspase-3, total bone marrow cells were stained with the lineage-depletion kit (130-090-858, MACS Miltenyi Biotec) following the manufacturer's instructions and passed through LS magnetic columns. Lineage-depleted cells were spun down for 5 min at 1,200 r.p.m. and the pellets were resuspended in 200 μl MACS buffer with the antibodies described above for HSC quantification. In parallel, 3×10^6 total bone marrow cells were stained with antibodies against committed lineages: CD45R/B220 (PE, clone RA3-6B2, BD Pharmingen) and IgM (FITC, clone II/41, BD Pharmingen) for B cell progenitors; TER-119 (FITC, clone TER-119, BD Pharmingen) and CD71 (PE, clone C2, BD Pharmingen) for erythroid maturation; and CD11b/Mac-1 (PE, clone M1/70, BD Pharmingen) and Ly-6G/Gr-1 (FITC, clone 1A8, eBioscience) for monocyte/granulocyte progenitors. After antibody staining, cells were washed, then fixed and permeabilized with BD Cytofix/Cytoperm solution (554722, BD Pharmingen) following the manufacturer's instructions. Finally, cells were stained with either anti-p53 (AlexaFluor647, clone 1C12, Cell Signalling) or anti-cleaved-caspase-3 (AlexaFluor647, clone D3E9, Cell Signalling) antibodies.

Gating strategies are described in Supplementary Fig. 2.

Blood counts. Total blood was collected in K3EDTA MiniCollect tubes (Greiner bio-one) and analysed on a VetABC analyser (Horiba).

Western blot. The FANCA antibody (Cell Signalling, D1L2Z) was used at 1:1000 in 5% w/v BSA, 1× TBS, 0.1% Tween-20 at 4 °C with gentle shaking, overnight. The β-actin antibody (Abcam, 8227) was used at 1:3,000 in the same conditions. Swine anti-rabbit immunoglobulins HRP (Dako) was used as secondary antibody at 1:2,000 for 1 h at room temperature.

Histological analysis. Tissue samples were fixed in 10% neutral-buffered formalin for at least 24 h. The femur samples were then decalcified and embedded in paraffin, and 4 μm sections were cut before staining with haematoxylin and eosin using standard methods.

***In vivo* point mutation assay.** The λ select-cII (BigBlue) mutagenesis assay was performed following the manufacturer's instructions. This assay allows the detection of point mutations *in vivo* and is based on the ability of coliphage λ to multiply either through the lytic or lysogeneic cycles in *Escherichia coli*.

In brief, the RecoverEase DNA-isolation kit (Stratagene) was used to extract genomic DNA from the bone marrow of 8-to-12-week old *Aldh2*$^{-/-}$*Fancd2*$^{-/-}$ and control mice that carry Big Blue λLIZ repeats. The shuttle vector that was recovered from mouse genomic DNA was packaged into phage with the Transpack packaging extract (Stratagene), which was then used to infect *E. coli* G1250 (Stratagene).

To assess the frequency of mutations within the *cII* gene, infected *E. coli* were diluted in TB1 top agar, spread on ten 100-mm TB1-agar plates and incubated at 24 °C for 48 h. The plaques were enumerated, picked and replated to confirm that they were lytic. This provided the number of mutated phage within the sample. To determine the total number of phage undergoing the lysogenic cycle and the mutation frequency, 10-and-50-fold dilutions of the stock of infected *E. coli* in TB1 top agar were spread on two 100-mm TB1-agar plates and incubated at 37 °C for 24 h. Incubation at 37 °C switches all phage to the lytic cycle, and therefore allows the total number of replication-competent phage to be assessed. The mutation