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Corresponding author(s): Patel KJ

☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

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For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

For the analysis of Mendelian segregation of alleles, samples size was determined by power analysis using the following site http://biomath.info/power/chsq1gp.htm. Sufficient animals were used in order to detect a 50% reduction in expected frequency, using power of 0.8 and alpha 0.05. For all other animal experiments, no statistical methods were used to predetermine sample size.

2. Data exclusions

Describe any data exclusions.

No data were excluded from analyses.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All experiments were replicated as stated in the text. All experiments were reproducible on all occasions reproduced.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

No randomisation was employed

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

The investigators were blinded to the genotypes of mice throughout the study and data was acquired relying purely on identification numbers.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Data was analysed using Graphpad prism. NGS data which was analysed with CaVEman, pindel and Brass. Flow cytometry data was analyzed with FlowJo.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	All materials are available

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	SCE ASSAY: mouse FITC-conjugated anti-BrdU antibody (Clone B44, BD Biosciences) and goat anti-mouse Alexa fluor-488 secondary antibody (Life Technologies, A-11001). FLOW CYTOMETRY: Micronucleus assay, FITC-CD71 antibody (GenTex, clone R17217.1.4). HSCs FITC-conjugated lineage cocktail with antibodies anti-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 anti-CD41 (FITC, clone MWReg30, BD Pharmigen); anti-c-Kit (PerCP-Cy5.5, clone 2B8, eBioscience), anti-Sca-1 (PE-Cy7, clone D7, eBioscience), anti-CD150 (PE, clone TC15-12F12.2, BioLegend) and anti-CD48 (biotin, clone HM48-1, BioLegend). ENGRAFMENT anti-CD4 (FITC, clone H129.19, BD Pharmingen), anti-CD8a (FITC, clone 53-6.7, BD Pharmingen), anti-CD45R/B220 (PerCP-Cy5.5, clone RA3-6B2, BioLegend), anti-CD11b/Mac-1 (PE, clone M1/70, BD Pharmingen), anti-Ly-6G/Gr-1 (PE, clone 1A8, BD Pharmingen), anti-TER-119 (PE-Cy7, clone TER-119, BioLegend), anti-CD45.1 (BV421, clone A20, BioLegend) and anti-CD45.2 (APC, clone 104, BioLegend). MATURE LINEAGES: anti-CD45R/B220 (PE, clone RA3-6B2, BD Pharmingen) and anti-IgM (FITC, clone II/41, BD Pharmingen) for B cell progenitors; anti-TER-119 (FITC, clone TER-119, BD Pharmingen) and anti-CD71 (PE, clone C2, BD Pharmingen) for erythroid maturation; anti-CD11b/Mac-1 (PE, clone M1/70, BD Pharmingen) and anti-Ly-6G/Gr-1 (FITC, clone 1A8, eBioscience) for monocyte/granulocyte progenitors. INTRACELLULAR ANTIGENS: anti-p53 (AlexaFluor647, clone 1C12, Cell Signalling), anti-cleaved caspase-3 (AlexaFluor647, clone D3E9, Cell Signalling). WESTERN BLOT: The FANCA antibody (Cell Signalling, D1L2Z) was used at 1:1000 in 5% w/v BSA, 1X TBS, 0.1% Tween20 at 4°C with gentle shaking, overnight. The anti-VINCULIN (abcam, 129002) was used at 1:4000 in the same conditions. Dako swine anti-rabbit immunoglobulins HRP was used as secondary antibody, at 1:2000 for one hour at room temperature. The antibody was validated with FANCA KO samples.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

DT40 cell lines used in this study were described previously. They were generated in our lab (FANCC, FANCC/XRCC2, Nidezwiedz et al., Mol Cell. 2004) or the lab of Shunichi Takeda (HR mutants: Takata, Mol Cell Biol., 2001; Al Abo, Caner Research, 2014).

b. Describe the method of cell line authentication used.

DT40 genetic mutants were validated by southern blot and sensitivity to DNA damaging agents, see above reports for detailed information.

c. Report whether the cell lines were tested for mycoplasma contamination.

All cells lines tested mycoplasma negative

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

N/A

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Aldh2-/-Fancd2-/- mice on a C57BL/6 x 129S4S6/Sv F1 background were generated for this study. To this end, the previously reported Fancd2 allele (Fancd2tm1Hou, MGI ID: 2673422, a gift from M. Grompe) was backrossed onto the C57BL/6Jo1a background for ten 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 (Aldh2tm1a(EUCOMM)Wtsi; MGI ID: 4431566, EUCOMM8) was backrossed 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 F1 hybrids from crosses between Aldh2+/-Fancd2+/- females (129S4S6/Sv, Harwell, UK) and Aldh2+/-Fancd2+/- males (C57BL/6, Harwell UK).

To generate Fanca-/-Ku70-/- mice in a pure C57BL/6 background, Fanca+/-mice (Fancatm1a(EUCOMM)Wtsi; MGI ID: 4434431, C57BL/6N, EUCOMM the generation of which was described in Garaycoechea JI, Nautre 2012) were crossed with Ku70+/-mice (Xrcc6tm1Fwa, MGI ID: 2179954 the generation of which was described in Gu, Y Immunitry 1997), and the Fanca+/-Ku70+/- progeny were then intercrossed to generate all possible genotypes. Pups from these crosses were genotyped at between 2-3 weeks old. For the generation of FancaF/-Ku70-/- Vav1-iCre+ tissue-specific double mutants (also pure C57BL/6), Fanca+/-mice were first crossed with FLP deletor mice (Farley, FW Genesis 2000) to produce the Fanca floxed allele (FancaF or Fancatm1c(EUCOMM)Wtsi). Recombination of the Frt sites was verified by PCR (FL033: GCCTTTGCTGCTCTAATTCCATGT, FL040: TCAGCTCACTGAGACGCAACCTTTT ACACT; and En2A: GCTTCACTGAGTCTCTGGCATCTC) and reconstitution of FANCA expression was verified by western blotting FancaF/F spleen extracts (Figure 3). Fanca+/F mice were then crossed with Ku70+/- mice to eventually produce FancaF/F Ku70+/- mice. Finally, these mice were crossed with Fanca+/-Ku70+/-Vav1-iCre to generate FancaF/-Ku70-/- Vav1-iCre and control mice. The Vav1-iCre allele directs the expression of the iCre recombinase to HSCs and hematopoietic tissues 41, and in this case yields the Fanca null allele (FancaΔ or Fancatm1d(EUCOMM)Wtsi). FancaΔ/Δ mice phenocopy the Fanca-/- mice reported previously, as judged by FANCA expression, sterility and sensitivity to mitomycin C (Figure 3, Extended Data Figure 5).
Similarly to Aldh2-/-Fancd2-/- F1 mice, Aldh2-/-Fancd2-/-p53-/- mice were also generated in a C57BL/6 x 129S4S6/Sv F1 background. Briefly, the p53 allele reported previously 42 was backcrossed onto 129S6/Sv or C57BL/6J for six generations. p53+/- mice were then intercrossed with Aldh2-/-Fancd2+/- mice to establish parental (F0) strains on both genetic backgrounds, which were finally crossed to obtain Aldh2-/-Fancd2-/-p53-/- and control F1 mice.
For single HSC transplantation experiments, we employed CD45.1 homozygous mice on a C57BL/6J x 129S6/Sv F1 background as recipients. CD45.1 (or Ptprca) 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 x 129S4S6/Sv hybrid background. The resulting mice were inter-crossed to obtain Aldh2-/-Fancd2-/- BigBlue λLIZ and control mice.

All animals were maintained in specific pathogen-free conditions. In individual experiments all mice were matched for gender and age (8-12 weeks).

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

N/A

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Corresponding author(s): KJ Patel

☐ Initial submission ☐ Revised version ☒ Final submission

Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.

Treated or untreated mice (8-12 weeks of age) were bled and 62 µl of blood were mixed with 338 µl of PBS supplemented with 1000 U/mL of heparin (Calbiochem). 360 µl of blood suspension were then added to 3.6 ml of -80°C methanol and placed at -80°C for at least 12 hours. 1 ml of fixed blood cells were then washed with 6 ml of bicarbonate buffer (0.9% NaCl and 5.3 mM NaHCO3). The cells were resuspended in 150 µl of bicarbonate buffer and 20 µl of this suspension was used for subsequent staining. 72 µl of bicarbonbate buffer, 1 µl of FITC-CD71 antibody (GenTex, clone R17217.1.4) and 7 µl of RNaseA (Sigma) were premixed and added to 20 µl of each cell suspension. The cells were stained at 4°C for 45 minutes, followed by addition of 1 ml of bicarbonate buffer and centrifugation. Finally, cells pellets were resuspended in 500 µl of bicarbonate buffer supplemented with 5 µg/ml propidium iodide (Sigma). The samples were analysed immediately on an LSRII analyser (BD) and data analysed with FlowJo 10.0.7

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 of red cell lysis buffer (MACS Miltenyi Biotec) for 10 minutes 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). 10x106 bone marrow cells were resuspended in 200 µl of staining buffer and stained as described in the methods. The samples were incubated for 15 minutes at 4°C and washed with 2 ml of buffer. The cell pellets were resuspended in 200 µl of staining buffer containing streptavidin-BV421 and incubated for another 15 minutes at 4°C. Finally, cells were washed, resuspended in 500 µl of staining buffer, data acquired on a Fortessa analyser (BD)

To assess the engraftment of single HSCs into irradiated recipients, 50 µl of blood were obtained from the tail vein of recipient mice every two weeks. Red blood cells (RBC) were lysed by the addition of 1 ml of ammonium chloride lysis buffer (155 mM NH4Cl, 10 mM KHCO3, 0.1 mM Na2EDTA, pH 7.2) and incubated for 10 minutes at room temperature. After centrifugation, the cell pellets were resuspended in 100 µl of staining

	buffer and stained with antibodies described in the methods. After the 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.
6. Identify the instrument used for data collection.	LSRII analyser (BD) Fortessa Analyser (BD) iCyt Synergy Sorter (Sony)
7. Describe the software used to collect and analyze the flow cytometry data.	Data was collected using FACSDiva (BD) and processed using FlowJo 10.0.7
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Post sort analysis was not conducted on single cells for HSC transplant. These cells were assessed for their functional ability to engraft and provide long term multi-lineage reconsitution.
9. Describe the gating strategy used.	The gating strategies are outline in the Suppl Figure.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒