

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

|                                     |                                                                                                                                                                                                                                                                                                |
|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| n/a                                 | Confirmed                                                                                                                                                                                                                                                                                      |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                                          |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                                   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                           |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated                                                                                                                                                          |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-----------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data collection | ddPCR data was collected using QX200 Droplet Reader and QuantaSoft software v1.7.4.0917 (BioRad). Chromium Controller (Firmware v4.0) was used for scRNA-seq. Genomic DNA was quantified using NanoDrop 8000 Spectrophotometer or Qubit Fluorometers (APP 2.02 + MCU v0.26). PCR products were quantified using Qubit 4.0 Fluorometer (APP 2.02 + MCU v0.26) or Victor NIVO multimode plate reader with Control software v4.0.7 and characterized using Agilent Tapestation software v5.1. Flow cytometry data were collected using the Attune NxT Cytometer (with autosampler) and Attune NxT Software v3.2.1. For cell sorting experiments, the Sony SH800S device and Cell Sorter Software v2.1.6 were used.                                                                                                                                                         |
| Data analysis   | Microsoft® Excel for Mac (v16.84) and GrapPad Prism v10.0.3 (217) for Mac were used for data analysis and figures. Flow cytometry data were analyzed using FlowJo v10.8.2. Short-read sequencing were analyzed using CRISPResso2 (v2.0.20b) and ICE Synthego v3.0 for indel analysis. Long-read sequencing were analyzed using cutadapt (version 4.6), minimap 2 (version 2.24-r1122), deepTools (version 3.5.4), bioawk (version 1.0) and described at <a href="https://github.com/cornlab/summarizeOntDeletions">https://github.com/cornlab/summarizeOntDeletions</a> . R (4.2.1), infercnv(1.12.0), Seurat (5.1.0), dplyr (1.1.4), ggplot2 (3.5.1), ComplexHeatmap (2.20.0), were used for single-cell RNA sequencing analysis. CAST-seq were analyzed as described at <a href="https://github.com/AG-Boerries/CAST-Seq">https://github.com/AG-Boerries/CAST-Seq</a> |

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

## Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Sequencing data for short-read, long-read, and CAST-sequencing are available in the Sequence Read Archive under BioProject PRJNA1167903. GRCh38 was downloaded from GenBank. All the raw data supporting the findings of this study are available from the corresponding author upon reasonable request.

## Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

|                                                                    |                                                                                                                                                                                                  |
|--------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Reporting on sex and gender                                        | The primary cell samples used in this study were de-identified, so we do not have information on the donors' gender.                                                                             |
| Reporting on race, ethnicity, or other socially relevant groupings | The primary cell samples used in this study were de-identified, so we have no information on the race, ethnicity or other socially relevant groupings.                                           |
| Population characteristics                                         | The primary cell samples used in this study were de-identified, so we have no additional information on the population characteristics, other than the fact that they came from adult donors.    |
| Recruitment                                                        | The human G-CSF-mobilized CD34+ HSPC from adult healthy donors were purchased from the Fred Hutchinson Cancer Center. The human nasal epithelial samples were collected from healthy volunteers. |
| Ethics oversight                                                   | The human nasal epithelial samples were collected in accordance with ethical guidelines of ETH Zürich Ethics Commission (EK-2024-N-171-A) and Cantonal Ethics Committee Zürich (Req-2024-00558). |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

## Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://nature.com/documents/nr-reporting-summary-flat.pdf)

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

|                 |                                                                                                                                                                                                                             |
|-----------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sample size     | No formal sample size calculations were performed. The sample size was considered sufficient based on consistent measurable differences observed and in line with previously published studies conducting similar analyses. |
| Data exclusions | No data were excluded from the analyses                                                                                                                                                                                     |
| Replication     | Replicates were not performed, except for the gene editing assay using flow cytometry as the readout. In this case, the number of individual biological replicates is specified in the figure legends.                      |
| Randomization   | No randomization was performed. All independent biological experiments were treated equally                                                                                                                                 |
| Blinding        | Blinding was not performed as the data were not subjective.                                                                                                                                                                 |

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials &amp; experimental systems

|                                     |                                                           |
|-------------------------------------|-----------------------------------------------------------|
| n/a                                 | Involved in the study                                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies                       |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Plants                           |

## Methods

|                                     |                                                    |
|-------------------------------------|----------------------------------------------------|
| n/a                                 | Involved in the study                              |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging    |

## Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

The hTERT RPE-1 p53+/+ and hTERT RPE-1 p53-/- cells were a gift from Stephen Jackson (with hTERT RPE-1 originally purchased from ATCC).  
The human G-CSF-mobilized CD34+ HSPC from adult healthy donors were purchased from the Fred Hutchinson Cancer Center.  
The human nasal epithelial samples were collected from healthy volunteers.  
The K-562 cells were purchased from ATCC

Authentication

The hTERT RPE-1 p53+/+, hTERT RPE-1 p53-/-, K-562, and K-562 eGFP cell lines were STR-profiled.

Mycoplasma contamination

The hTERT RPE-1 p53+/+, hTERT RPE-1 p53-/-, K-562, and K-562 eGFP cell lines were routinely tested negative for mycoplasma (MycoAlert, Lonza).

Commonly misidentified lines  
(See [ICLAC](#) register)

No commonly misidentified cell lines were used.

## Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A

## Flow Cytometry

## Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ 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).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

## Methodology

Sample preparation

The FIRE K-562 and eGFP K-562 were directly transferred for analysis. Cell viability of GAPDH-edited HSPCs analyzed by single-cell RNA sequencing was also quantified using SYTOX™ Red Dead Cell Stain (1:1000 dilution).

Instrument

Attune NxT Cytometer (with autosampler) and SH800S Cell Sorter (Sony) for cell sorting.

Software

Attune NxT Software v3.2.1. and Cell Sorter Software v2.1.6

Cell population abundance

Purity of sorted fractions was not quantified.

## Gating strategy

Cells were first gated on morphology (FSC-A vs SSC-A), for single cells (FSC-A vs FSC-H) and then on mScarlet and eGFP expression. The gating strategy is shown in Extended Data Figure 2b.  
Edited K-562 cells expressing eGFP were first gated on morphology (FSC-A vs SSC-A), for single cells (FSC-A vs FSC-H) and then on eGFP expression. The gating strategy is shown in Extended Data Figure 4c.  
Edited HSPCs were first gated on morphology (FSC-A vs SSC-A), for single cells (FSC-A vs FSC-H) and then on SYTOX™ Red Dead Cell Stain level. The gating strategy is shown in Extended Data Figure 5a.

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