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Corresponding author(s): Jose M.P. Freije
Carlos López-Otín

Life Sciences Reporting Summary

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For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Experimental design

1. Sample size

   Describe how sample size was determined.

   Sample size was determined based on previous experiments of our laboratory, carried out with the same animal model under identical environmental conditions.

2. Data exclusions

   Describe any data exclusions.

   Two AAV9-treated mice (one from the control cohort and another one from the sgRNA-LCS2 group) were excluded from the survival plot due to perinatal death. This event could be explained by an early manipulation and a higher frailty of progeroid mice. The analysis of indel mutations in the liver of a LCS2-transduced mouse was also excluded due to a low number of reads in NGS (only 24 reads compared to more than 100,000 in the rest of analyzed samples). These exclusion criteria had not been pre-established.

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   Three independent infections were done for in vitro experiments. All attempts at replication were successful.

4. Randomization

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

   Allocation of both mice and cell cultures to each group was random.

5. Blinding

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

   Nuclear abnormalities and progerin analysis in cultures were quantified by investigators who were blinded to the identity of the analyzed cells. In the same way, histological analysis (Gomori staining, TUNEL assay and progerin immunohistochemistry) were also performed by investigators blinded to group identity.

Note: all in vivo studies must report how sample size was determined and 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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes 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 in all relevant figure captions (with explicit mention of central tendency and variation)

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.

-Microsoft Excel v. 15.21.1 and GraphPad Prism v. 6.0.2 were used for the statistical analysis.
-BWA v. 0.7.5a-r405 and Samtools v. 1.3.1 were used for MiSeq analysis.
-ImageJ v. 1.48v was used for Western blot and TUNEL analysis.
-FIJI v. 1.52i was used for Gomori quantification.

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 third party.

Unique materials used are available from the authors upon Material Transfer Agreement signature. AAVs were obtained from The Viral Vector Production Unit (UPV) of the Universitat Autònoma de Barcelona (Barcelona, Spain).

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

The primary antibodies used were:
-Mouse monoclonal anti-lamin A/C (MANLAC1) was provided by Prof. Glenn Morris (Wolfson Centre for Inherited Neuromuscular Disease, UK) and used at a dilution 1:500. For validation, cultured mouse fibroblast cell extracts were used.
-Rabbit polyclonal anti-lamin A/C (H-110; cat: sc-20681) from Santa Cruz Biotechnology was used at a dilution 1:1,000. According to the websites of the manufacturer, this antibody reacts against Lamin A/C from mouse, rat and human origin.
-Mouse monoclonal anti-beta-actin (AC-15; cat: A5441; lot: 014M4759) was pursached from Sigma and used at a dilution 1:10,000. According to the websites of the manufacturer, reacts against guinea pig, canine, Hirudo medicinalis, feline, pig, carp, mouse, chicken, rabbit, sheep, rat, human and bovine orthologs.
-The anti-progerin polyclonal antibody was generated using peptide immunogens and standard immunization procedures (S. Nourshargh et al., manuscript in preparation). 1:200-1:300 dilutions were used and its specificity was confirmed by nuclear staining of LmnaG609G/G609G mice-derived fibroblasts, which was negative in the case of wild-type cells.

The secondary antibodies used were:
-Goat anti-mouse IgG HRP-linked antibody from Jackson ImmunoResearch (cat: 115-035-062; lot:121006) diluted 1:10,000.
-Goat anti-rabbit IgG HRP-linked antibody from Cell Signaling (cat: 7074S; lot: 27) diluted 1:3,000.
-Goat anti-rabbit IgG - H&L 488 from Alexa Fluor (cat: A11034; lot: 1670152) diluted 1:500.

10. Eukaryotic cell lines

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

HEK-293T cells are from ATCC. Controls and progeroid mouse fibroblasts cultures were established in our laboratory from control and mutant mice. Human control and HGPS fibroblasts are from Coriell.

b. Describe the method of cell line authentication used.

The identity of control and progeroid fibroblasts was confirmed by Western blot of lamin A/C. PCR-based microsatellite characterization of HEK-293T cells was performed at the University of Oviedo.

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

The cell lines were not tested for mycoplasma contamination.

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.

HEK-293T cells are widely used for infection experiments. The identity of HEK-293T was assessed by PCR-based microsatellite characterization.

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

Progeria LmnaG609G/G609G mouse model (Lmna tm1.1Otin) was used in a C57BL/6N background. Both males and females were used for the study. Samples from wild-type and LmnaG609G/G609G sgRNA-transduced mice were collected at the age of 3.5 months.

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.

The study did not involve human research participants.