Supplementary Materials

Progerin and telomere dysfunction collaborate to trigger cellular senescence

Kan Cao[1,2], Cecilia D.Blair[1], Dina A. Faddah[1], Julia E. Kieckhaefer[2], Michelle Olive[1], Michael R. Erdos[1], Elizabeth G. Nabel[1,3], and Francis S. Collins[1]*

[1]Genome Technology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892-8004, USA

[2] Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, USA

[3]Current address: Brigham and Women's Hospital, Boston, MA 02116, USA

* Corresponding author:

Francis S. Collins, M.D., Ph.D.

National Institutes of Health

One Center Drive, Room 126

Bethesda, Maryland 20892-0148

Phone: 301-496-2433

e-mail: Francis.Collins@nih.gov

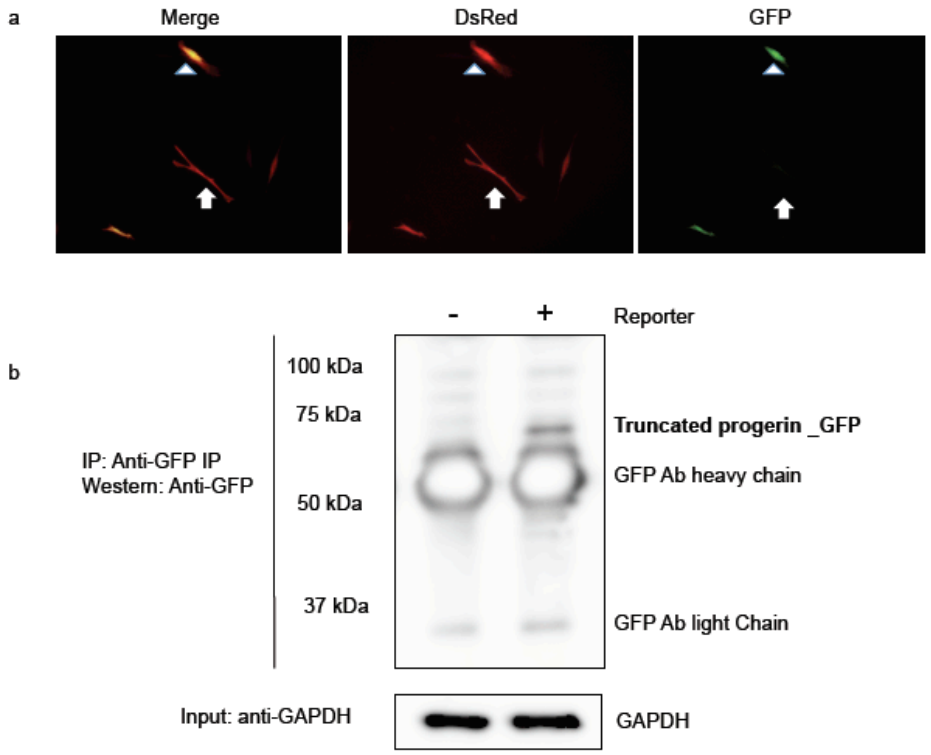

Figure S1: **a.** Live cell imaging of normal primary fibroblasts (AG06299) transfected with the progerin-splicing reporter. Arrow: an example of a DsRed-only cell; Arrowhead: an example of a double-positive cell. **b.** Immunoprecipitation followed by western blotting analysis with an anti-GFP antibody in whole cell lysates from an un-transfected fibroblast line (-), and a reporter-transfected cell lines (+). GAPDH was used as a loading control.

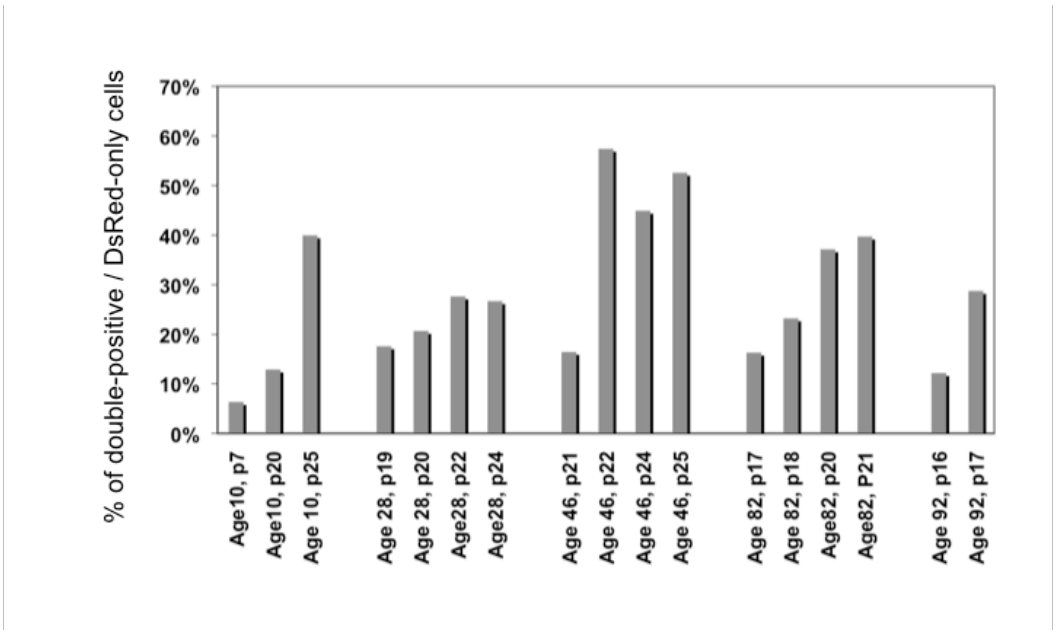

Figure S2: The percentage of fibroblast cells that utilize the cryptic *LMNA* splice site increases with cell passage number, but not with age of the skin biopsy donor.

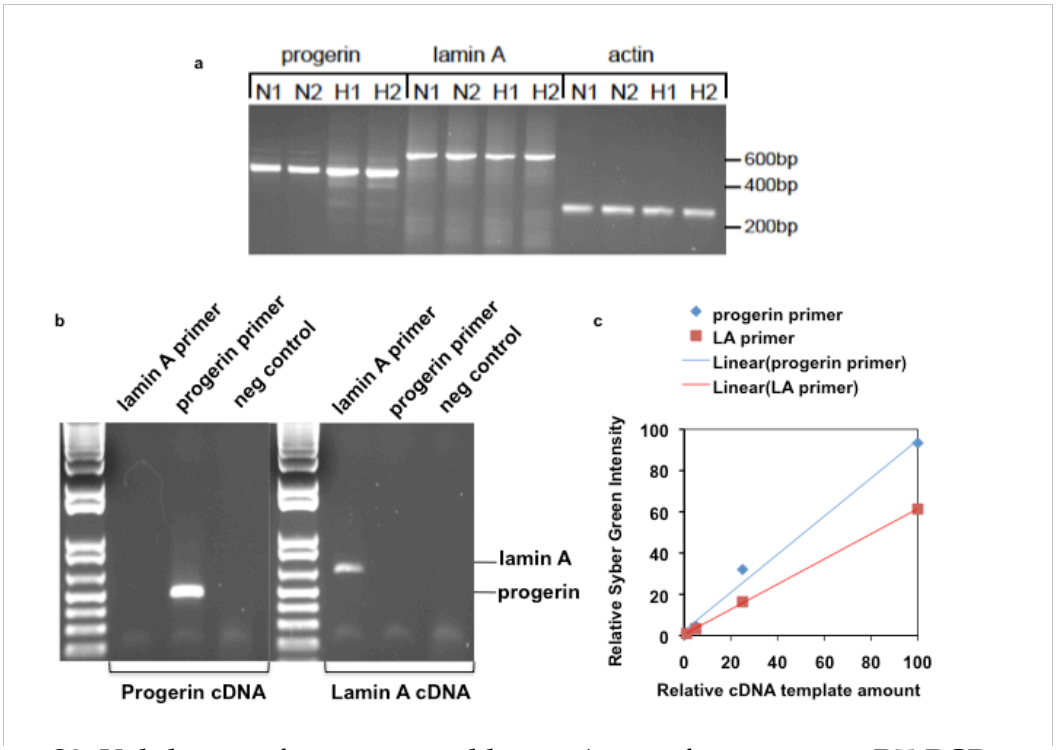

Figure S3: Validation of progerin and lamin A specific primers. **a** RT-PCR analysis of normal and HGPS fibroblasts using primers detecting either lamin A or progerin. N1: HGADFN168 (normal); N2: HGADFN090 (normal); H1: HGADFN003 (HGPS); H2: HGADFN167 (HGPS). Final PCR products after 40 amplification cycles were shown, so this is not a quantitative analysis. **b** PCR analysis using purified progerin or lamin A cDNA as template. **c.** Amplification efficiency of progerin and lamin A primers on purified cDNA. The efficiencies are 93.3% and 61.3% for progerin and lamin A primers, respectively.

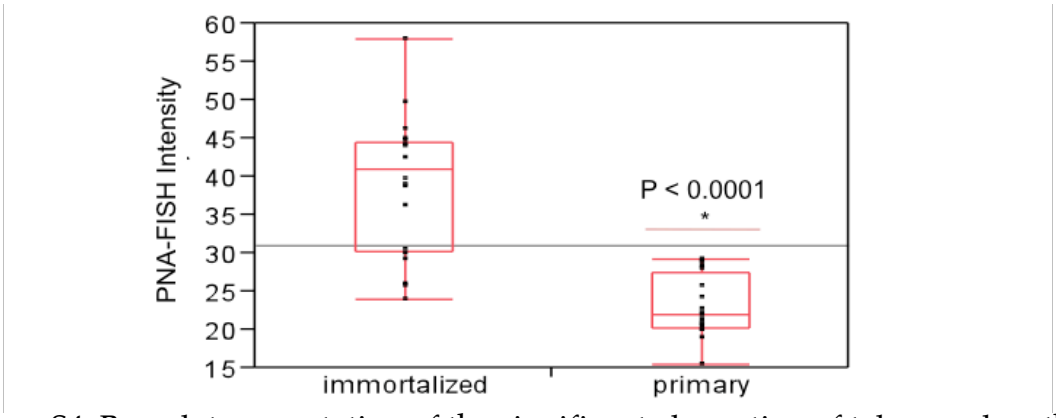

Figure S4: Box plot presentation of the significant elongation of telomere length in hTERT-immortalized fibroblast cells AG09838 in comparison to untreated primary parent cells ($p < 0.0001$).

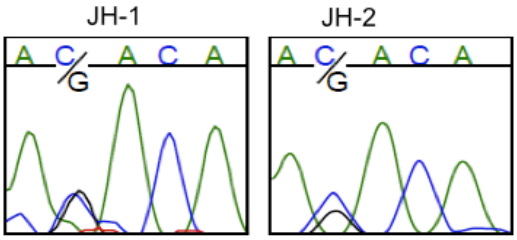

Figure S5: DNA sequencing confirms the heterozygous point mutation (K902N, C=>G) in the *hTERT* gene in cell lines JH-1 and JH-2.

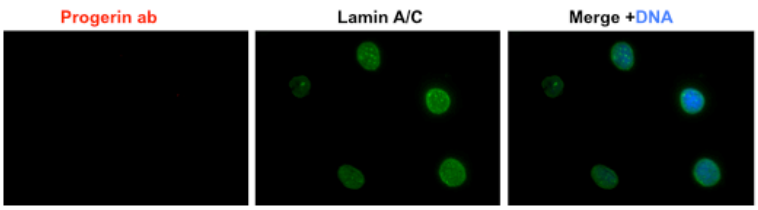

Figure S6: Immunostaining of a normal fibroblast cell line AG08470 with anti-lamin A/C and anti-progerin antibody.

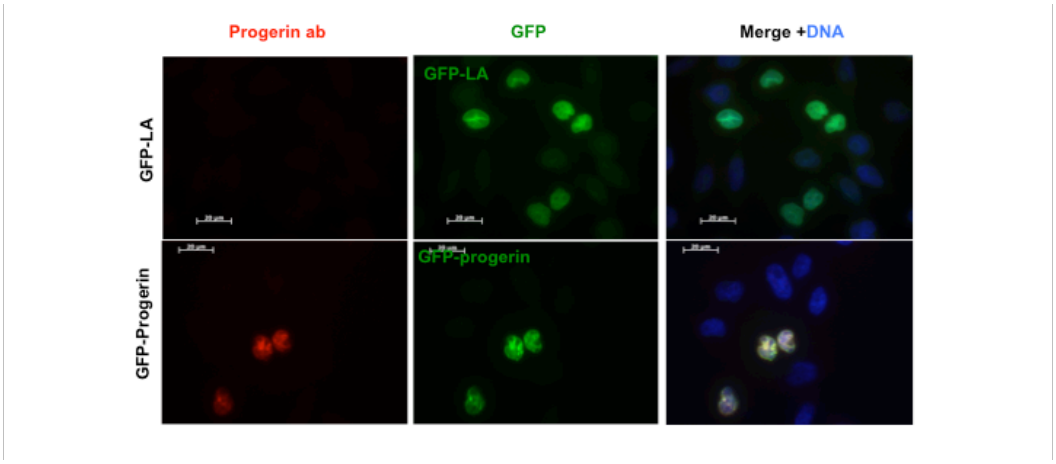

Figure S7: Immunofluorescence staining with an anti-progerin antibody on HeLa cells transfected with GFP-lamin A or GFP-progerin. The pictures were taken with the sample exposure time. The progerin antibody only stains the cells with GFP-progerin signals, and does not label the GFP-lamin A expressing cells.

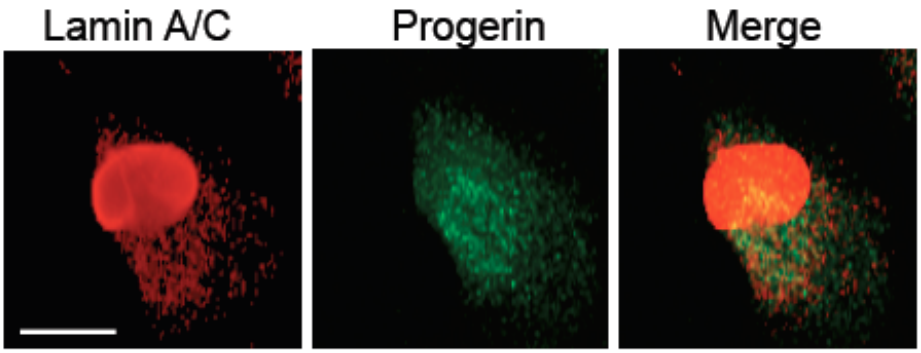

Figure S8: Images of lamin A/C cytoplamsmic stainings under over-exposure condition. We observed some co-localization of signals from progerin and lamin A/C antibodies. Scale bar: 20μm.

Table S1: Gene lists as described in Figure 7A. Quantitative RT-PCR assays were carried out to validate these changes: out of the 8 randomly-selected genes, 5 were validated (62.5%).

Table S2: The 82 overlapping genes from Figure 7B that exhibit significant changes in alternative splicing as cells senesce.

Table S3: The cell lines used in this study.