# **Supplemental Methods**

## Cell immunocytochemistry and imaging

Control and patient fibroblasts were immunostained for lamin A/C. HeLa cells were immunostained for Lamin B1. Cells were fixed with 3.7% formaldehyde in PBS, permeabilized with 0.1% Triton X-100 in PBS and blocked with 0.2% bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO). F-actin was labeled with Oregon green phallodin (2.5:100 dilution; Invitrogen, Carlsbad, CA). Lamin A/C was labeled by rabbit polyclonal antibody (1:100; Santa Cruz Biotechnology, Santa Cruz, CA) with Alexa fluor goat anti-rabbit 555 (1:200, Invitrogen, Carlsbad, CA). Lamin B was labeled by mouse polyclonal antibody (1:100; Santa Cruz Biotechnology, Carlsbad, CA) with Alexa fluor goat anti-mouse 488 (1:200, Invitrogen, Carlsbad, CA). DAPI (Invitrogen, Carlsbad, CA) was added to label DNA. Images, including those shown in Figure S1, were taken on a Leica DMI6000B inverted microscope with A, I3 and N2.1 filter cubes (Leica, Buffalo Grove, IL).



Figure S1: Imaging of nuclei with exogenous expression of progerin and HGPS patient cells. A) HeLa cells expressing either DsRed-lamin A or DsRed-progerin immunostained for lamin B1 and stained with Hoechst. The cells show colocalization of lamin B1 and DsRed-lamin A or DsRed-progerin. B) Control and patient fibroblast showing immunostain for lamin A/C, phallodin (actin), and Hoechst. In both the transfected HeLa cells and the patient fibroblasts the expression of progerin causes dysmorphic formations in the nucleus including creases, invaginations, and outward blebbing. Scale bars are 10 μm.

## Fluorescence localization and morphological analysis

A custom MATLAB (Natick, MA) script was used to analyze the immunostained lamin A/C channel in control and HGPS fibroblast or the transfected DsRed-lamin A or DsRed-progerin channel in HeLa, HUVEC, or Saos2 cells. The user first cropped the cells and a section of background. Otsu's method was used to segment the cell and create a mask from which circularity (Eqn. 1), solidity (Eqn. 2), and elongation (Eqn. 3) could be determined. An ellipsoid was fit to the nuclear mask and a line scan was taken across the major and minor axes of the ellipse (Fig S2).



**Figure S2: Characterization method for nuclear morphology.** A) Control fibroblasts immunostained with lamin A/C, Hoechst, and phallodin (actin). An example of the characterization of the localization of lamin A is shown. Briefly the lamin A channel is analyzed by fitting an ellipse to the cell and tracing intensity along the major and minor axis. B) HGPS fibroblast immunostained with lamin A/C, Hoechst, and phalloidin (actin). A similar analysis is employed for the HGPS fibroblast as the control fibroblast. Note the increased intensity a the cell periphery in the control and the interior peaks corresponding to nuclear creases in the HGPS patient fibroblast. Scale bars are 10 μm.

The user selected the local maxima for the major and minor axes corresponding to where the line scan crossed the nuclear periphery. The four points were then averaged and used to describe the intensity at the nuclear periphery for E (Equation 5). O describes the overall cell intensity (Equation 4). Quantification of these parameters can be found in table 1.

$$Circularity = \frac{4\pi (Area)}{(Perimeter)^2}$$
(1)  

$$Solidity = \frac{Area}{Convex Area}$$
(2)  

$$Elongation = \frac{Major Axis Length}{Perimeter}$$
(3)  

$$O = \frac{Average Cell Intensity}{Average Background Intensity}$$
(4)  

$$E = \frac{Average Periphery Intensity}{Average Cell Intensity}$$
(5)

#### Supplemental results

#### Characterization of lamin localization and nuclear morphology

To better characterize the cell types used in the study a series of morphological and intensity based quantifications were performed on cells immunostained for lamin A/C or expressing DsRed-lamin A or DsRed-progerin. The intensity based quantification included E (Equation 5), O (Equation 4), and the line scan across the major axis of the nucleus. For each cell type the magnitude of the values for O and E were similar between cells immunostained for lamins A/C or cells expressing DsRed-lamin A or DsRed-progerin of the same type (HeLa, HUVEC, Saos2, fibroblast) (Table S1). The line scans across the major axis (Figure S2) showed local maxima at the nuclear periphery in both progerin and lamin A cells (Figure S3). Another trend from the line scans was an increased interior intensity among the progerin expressing cells, possibly from out of plane folds. Similar to the values for O and E the line scan showed similar intensities across the nuclei of each cell type for both lamin A and progerin expression. This additional intensity is clearly seen in the line scans of the transfected HeLa cells and there is elevated intensity in the HUVEC and Saos2 cells at the centermost interior. The morphological parameters included circularity, solidity, and elongation (table S1). Similar to the intensity values, the values between cells of the same type expressing either lamin A or progerin showed similar values.



Figure S3: Characterization of nuclear morphology for the cell types used in this study. A) (Top row) Line scans normalized by background intensity along the major axis of the lamin A channel for the nuclei of multiple individual cells. Overlayed in black is the average of the line scans with arrows indicating the local maxima corresponding to the nuclear periphery. B) (Middle row) Same as A but with the HGPS patient cells or with cells expressing DsRed-progerin. C) (Bottom row) Comparison of the mean lamin A traces (black) and the mean progerin traces (red) along the major axis of the cells with SEM error bars.

Cell Line (n)	0	E	Circularity	Solidity	Elongation
	(mean ± SEM)	(mean ± SEM)	(mean ± SEM)	(mean ± SEM)	(mean ± SEM)
Control Fibroblasts (98)	$5.34 \pm 0.12*$	$1.05 \pm 0.01$	$0.92 \pm 0.01$	$0.98 \pm 0.001 *$	$0.37 \pm 0.002*$
HGPS Fibroblasts (127)	$6.62 \pm 0.14$	$1.07 \pm 0.01$	$0.93 \pm 0.01$	$0.97 \pm 0.001$	$0.36 \pm 0.002$
1101 0 110100 (127)	0.02 - 0.11	1.07 - 0.01	0.00 - 0.01	0.57 = 0.001	0.50 - 0.002
	0.01.0.00	1 (7 ) 0 0 1 4	0.55.0.014	0.04.0000	
DsRed-Lamin A	$9.01 \pm 0.32^*$	$1.65 \pm 0.01^*$	$0.75 \pm 0.01*$	$0.96 \pm 0.002$	$0.33 \pm 0.003*$
HeLa Cells (231)					
DsRed- Progerin	$9.96 \pm 0.32$	$1.42 \pm 0.01$	$0.81 \pm 0.01$	$0.96 \pm 0.002$	$0.36 \pm 0.002$
Hol a Colls (302)					
fiela Cens (502)					
DsRed- Lamin A	$12.36 \pm 1.92$	$1.39 \pm 0.03*$	$0.87 \pm 0.02$	$0.97 \pm 0.002$	$0.36 \pm 0.007$
HUVECs (34)					
DsRed-Progerin	$11.42 \pm 1.65$	$1.25 \pm 0.03$	$0.85 \pm 0.02$	$0.97 \pm 0.002$	$0.36 \pm 0.005$
$\mathbf{HIVEC}_{\mathbf{a}}(50)$	$11.12 \pm 1.05$	$1.25 \pm 0.05$	$0.05 \pm 0.02$	$0.97 \pm 0.002$	$0.50 \pm 0.005$
HUVECS (50)					
DsRed Lamin A	$10.73 \pm 1.75$	$1.32 \pm 0.07$	$0.81 \pm 0.04$	$0.95 \pm 0.01$	$0.36 \pm 0.01$
Saos2 (17)					
DeRed Progerin	$10.18 \pm 1.02$	$1.24 \pm 0.03$	$0.82 \pm 0.03$	$0.95 \pm 0.01$	$0.35 \pm 0.01$
	$10.10 \pm 1.02$	$1.27 \pm 0.03$	$0.02 \pm 0.03$	$0.75 \pm 0.01$	$0.55 \pm 0.01$
Saos2 (36)					

Table S1: Morphological characteristics of cell nuclei used in this study. \* p<0.05 progerin to control



**Figure S4: Movements and processing of intranuclear markers over time.** The left column shows raw data taken from a UBF1-GFP expressing HeLa cell under 0.1MPa compressive pressure for one hour. The right column shows corresponding images of the cell after cellular drift was removed and the cell was statistically segmented for particle track construction. The final panel shows the tracks of the particles from the initial image. Hoechst 33342 (blue) was used to stain the chromatin. UBF1-GFP is shown in green. Scale bar is 10 µm.



**Figure S5: Sample images of particle tracking.** (Top row) Sample images of nuclei for the cell lines used stained with Hoechst in blue and expressing the fiducial marker in green. (Bottom row) Tracks (red) representing the movement of each fiducial marker within the nucleus over a one hour time course. Scale bars are 10 µm.



**Figure S6: Chromatin dynamics of HGPS and control patient cells.** Chromatin dynamics in patient cells with and without HGPS measured by ensemble averaged MSD. Particle tracking measurements of punctate regions of GFP-tagged proteins in the nuclear interior demonstrate statistically different dynamics associated with HGPS patient cells relative to controls as evinced in the magnitude and trends of the MSD. See results for the statistical comparisons of the fits. Data was fit to the power-law equation (Equation 3) for calculation of the chromatin compliance (*Jeff*) and system forces (α) depicted in Figures 2 and 6.



**Figure S7: Chromatin dynamics of HeLa cells with and without exogenous progerin expressions.** Chromatin dynamics in HeLa cells for control and exogenous progerin expression measured by ensemble averaged MSD. Particle tracking measurements of punctate regions of GFP-tagged proteins in the nuclear interior demonstrate statistically different dynamics associated with progerin-expression relative to controls as evinced in the magnitude and trends of the MSD. Data was fit to the power-law equation (Equation 3) for calculation of the chromatin compliance (*Jeff*) and system forces (α) depicted in Figures 3 and 6.



Figure S8: Chromatin dynamics of HUVECs with and without exogenous progerin expressions under static and shear conditions. Chromatin dynamics in HUVECs for control and exogenous progerin expression measured by ensemble averaged MSD. Particle tracking measurements of punctate regions of GFP-tagged proteins in the nuclear interior demonstrate statistically different dynamics associated with progerin-expression relative to controls for both static conditions and with shear application as evinced in the magnitude and trends of the MSD. Data was fit to the power-law equation (Equation 3) for calculation of the chromatin compliance (*Jeff*) and system forces (α) depicted in Figures 4, 6 and 7.



