SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. NOL12 regulates fibrillarin and nucleolin protein levels and nucleolar area. **(A)** NOL12 (green) and tubulin (grey) immunostaining of mock and NOL12-depleted (siNOL12) fibroblasts. DNA was stained with DAPI (blue). Scale bars, 10µm. **(B)** Western blot analysis of NOL12 protein levels in cell extracts from mock and siNOL12 fibroblasts. Tubulin levels were used as loading control. Values are mean ± SD from three independent experiments and normalized to mock controls. $**p \leq 0.01$ by Mann-Whitney statistical test. **(C)** qPCR analysis of *NOL12* transcript levels in mock and siNOL12 fibroblasts. *G6PD* was used as housekeeping gene. Values are mean ± SD from four independent experiments and normalized to mock controls. $*p \leq 0.05$ by Mann-Whitney statistical analysis. **(D)** Western blot analysis of fibrillarin protein levels. Tubulin levels were used as loading control. Values are mean ± SD from four independent experiments and normalized to mock controls. $*p \leq 0.05$ by Mann-Whitney statistical test. **(E)** Western blot analysis of nucleolin protein levels. Tubulin levels were used as loading control. Values are mean ± SD from four independent experiments and normalized to mock controls. $*p \leq 0.05$ by Mann-Whitney statistical test. **(F)** Nucleolar area (μm^2) in mock, siNOL12 and siXRN2 cells. Values are mean ± SD of n=total number of cells. $****p \leq 0.0001$ by Kruskal-Wallis statistical test. **(G)** Nuclear area (μm^2) in mock, siNOL12 and siXRN2 cells. Values are mean ± SD of n= total number of cells. $****p \leq 0.0001$ by Kruskal-Wallis statistical test.

Supplementary Figure 2. RPL11 is required for Actinomycin D-induced p53 stabilization in human dermal fibroblasts. **(A)** p53 and p21 protein levels in cell extracts from neonatal fibroblasts incubated with 0nM, 8nM and 16nM Actinomycin

D (ActD) for 4 hours. Tubulin was used as loading control in the immunoblotting. In the graph, bars are the protein levels normalized to the untreated control from a single experiment. **(B)** Western blot analysis of p53 and RPL11 protein levels in cell extracts from control and siRpL11 cells treated with 8nM Actinomycin D (ActD) for 4 hours. Tubulin was used as the loading control.

Supplementary Figure 3. NOL12 repression inhibits cell proliferation in an apoptosis-independent manner. **(A)** Immunostaining of Ki67 cell proliferation marker (red) in mock, siNOL12, siP53 and siNOL12+siP53 human dermal fibroblasts. DAPI staining was used for nuclei masking (white solid lines). Scale bars, 10µm. **(B)** Cell cycle duration was measured as the interval between mother cell mitosis and daughter cell mitosis over three generations (1^{st} – 3^{rd} Gen), in mock, siNOL12, siP53 and siNOL12+siP53 fibroblast cultures. Each dot represents a single cell. n= total number of cells analyzed. n.s., not significant. **(C)** Representative flow cytometry cell cycle profiles from control mock, siNOL12, siP53 and siNOL12+siP53 cell cultures. **(D)** Western blot analysis of the apoptotic marker cleaved-caspase 3 in cell extracts from mock, siNOL12 and siNOL12+siP53 fibroblasts. Extracts from cells treated with DMSO or 5µM staurosporine (STS) for 4 hours were used as negative and positive controls, respectively. Tubulin was used as the loading control.

Supplementary Figure 4. Quantification of senescence markers in naturally aged and replicative senescent fibroblasts. **(A)** Percentage of Ki67 negative cells in neonatal (Neo2) vs. elderly (Old; 77y, 84y, 85y and 87y average), as well as in neonatal low vs. high passage (Neo1 P<9 and Neo1 P>25) fibroblast cultures. Values are mean ± SD

from at least two independent experiments. ****$p \leq 0.0001$ by $\chi 2$-square statistical test. **(B)** Percentage of cells double positive for p21/53BP1 staining. Values are mean ± SD from two independent experiments. ****$p \leq 0.0001$ by $\chi 2$-square statistical test. **(C)** Percentage of SA-β-galactosidase (SA-β-Gal) positive cells. Values are mean ± SD from two independent experiments. ****$p \leq 0.0001$ by $\chi 2$-square statistical test. **(D)** Nucleolar area (μm^2). Values are mean ± SD. ****$p \leq 0.0001$ by Kruskal-Wallis statistical test. **(E)** Nuclear area (μm^2). Values are mean ± SD. ****$p \leq 0.0001$ by Kruskal-Wallis statistical test. In all graphs (A-E), values were normalized to Neo (red bars) or Neo1 (dark grey bar) and n= total number of cells analyzed.

Supplementary Figure 5. Overexpression of NOL12 in elderly donor cells negligibly impacts nucleolar stress and senescence phenotypes. **(A)** NOL12 immunoblotting in cell extracts from neonatal (Neo1), 84-year-old (84y) and 84-year-old fibroblasts overexpressing NOL12 (84y+NOL12 OE). Tubulin was used as the loading control. **(B)** Percentage of Ki67 negative cells in Neo1, 84y and 84y+NOL12 OE cell cultures. Values are mean ± SD from two independent experiments. ****$p \leq 0.0001$ by $\chi 2$-square statistical test. **(C)** Scatter plot of the mean pixel intensity of fibrillarin nuclear levels in Neo1, 84y and 84y+NOL12 OE nuclei. Each dot represents a single cell. Horizontal lines represent the mean. **$p \leq 0.01$ and ***$p \leq 0.001$ by Kruskal-Wallis statistical test. **(D)** Ratio between nucleolar and nuclear areas in neonatal Neo1, 84y and 84y+NOL12 OE cells. Values are mean ± SD and normalized to 84y mean value. ***$p \leq 0.001$ by Kruskal-Wallis statistical test. **(E)** Distribution curves of the percentage of Neo1, 84y and 84y+NOL12 OE cells exhibiting a total number of nucleoli as indicated. **(F)** Percentage of SA-β-galactosidase (SA-β-Gal) positive cells in Neo1, 84y and 84y+NOL12 OE cell cultures. Values are mean ± SD from two

independent experiments. ****$p \leq 0.0001$ by $\chi 2$-square statistical test. n=total number of cells analyzed in each experiment.

Supplementary Movies 1-4. Related to Figure 3. Long-term phase-contrast live-cell imaging (Movies 1-4) of mock, siNOL12, siP53 and siNOL12+siP53 cell cultures, respectively. Movie records started 28 hours and ended 70 hours after post-transfection. Images were acquired every 2.5 min. Scale bars, 100µm.

Supplementary Table 1. Human dermal fibroblasts (HDFs) used in this study.

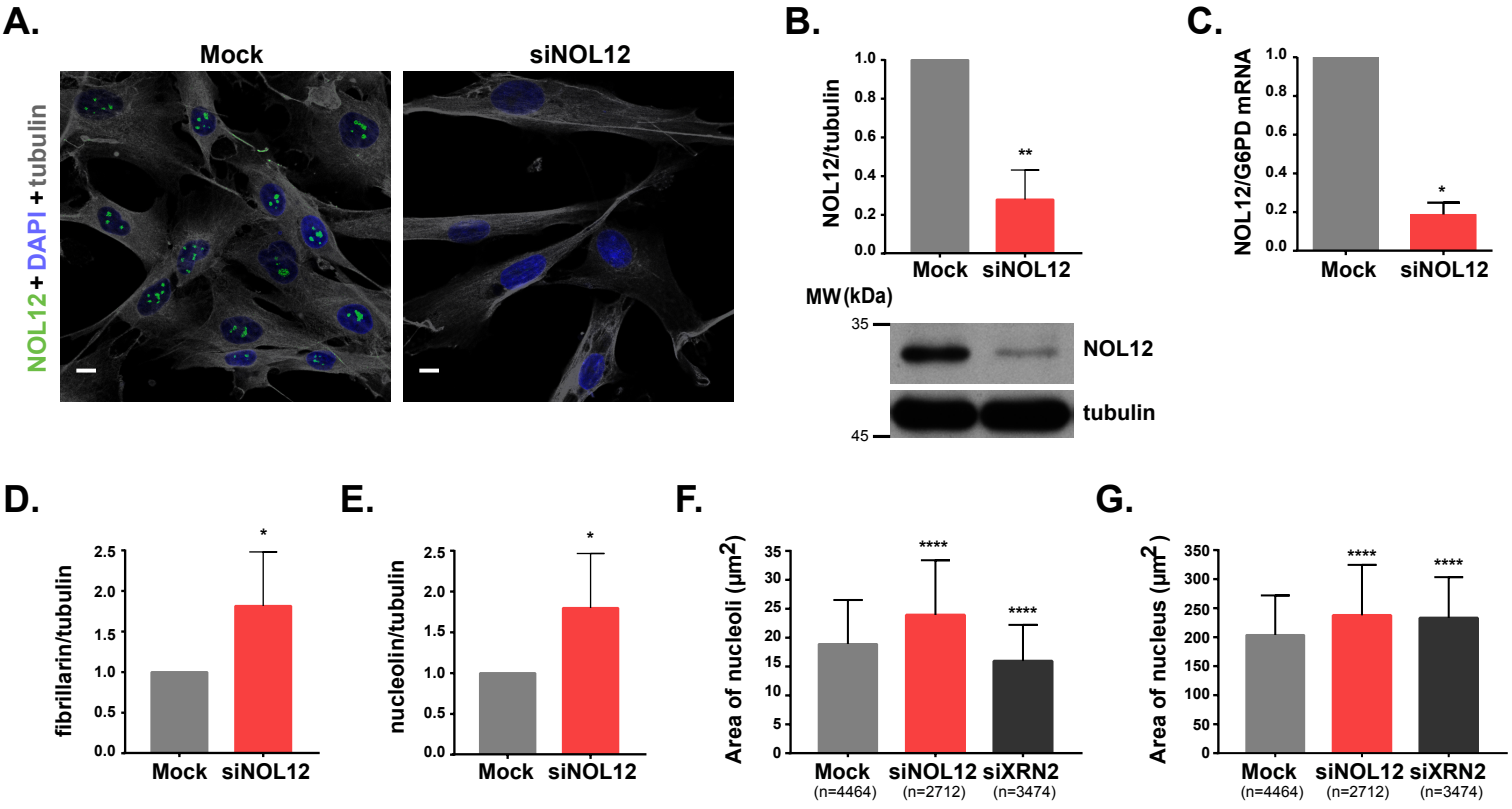
HDFs (age)	***Reference, Repository***	***Referred as***
Neonatal	DFM021711A, Zen Bio	Neo1
1 day	GM21811, Coriell Cell Repository	Neo2
77 years	AG07135, Coriell Cell Repository	77y
84 years	AG11488, Coriell Cell Repository	84y
85 years	AG09271, Coriell Cell Repository	85y
87 years	AG10884, Coriell Cell Repository	87y

Supplementary Table 2. Sequences of siRNAs used in this study.

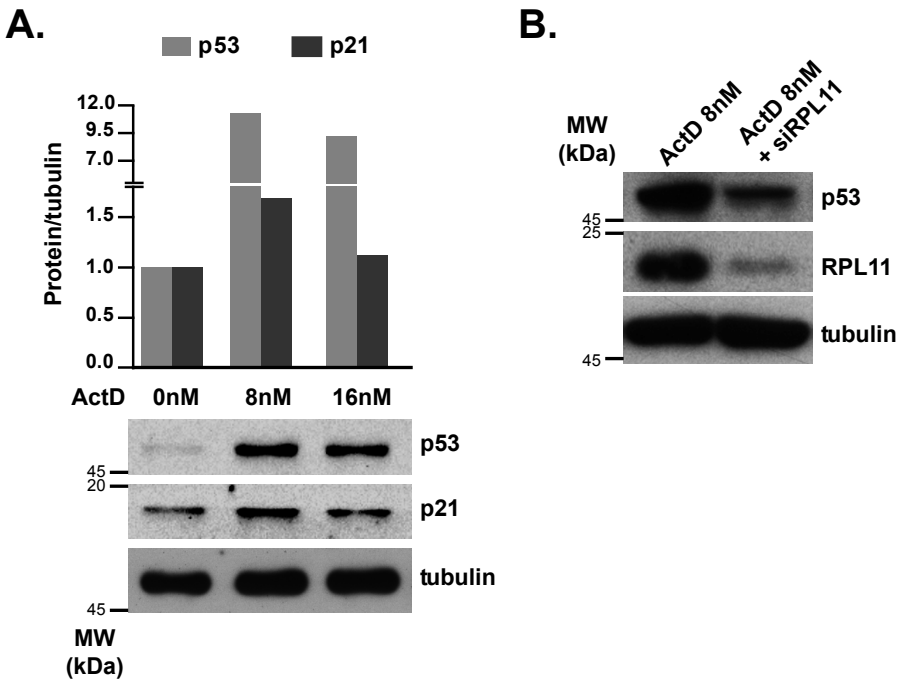
mRNA targets	***Sequence 5'-3'***
NOL12	(SASI_Hs01_00047859) CAGAUGAGCUGGACCGGUU[dT][dT] (sense) AACCGGUCCAGCUCAUCUG[dT][dT] (antisense)
P53	(SASI_Hs02_00302766) GAGGUUGGCUCUGACUGUA[dT][dT] (sense) UACAGUCAGAGCCAACCUC[dT][dT] (antisense)
RPL11	CGCGAGCAGCCAAGGUGUUGGAGCA[dT][dT] (sense) UGCUCCAACACCUUGGCUGCUCGCG[dT][dT] (antisense)
XRN2	siRNA 1: AAGAGUACAGAUGAUCAUG[dT][dT] (sense) CAUGAUCAUCUGUACUCUU[dT][dT] (antisense) siRNA 2: GGGAAGAAAUAUUGGCAAA[dT][dT (sense) UUUGCCAAUAUUUCUUCCC[dT][dT (antisense)

Supplementary Table 3. Sequences of primers used in qPCR experiments.

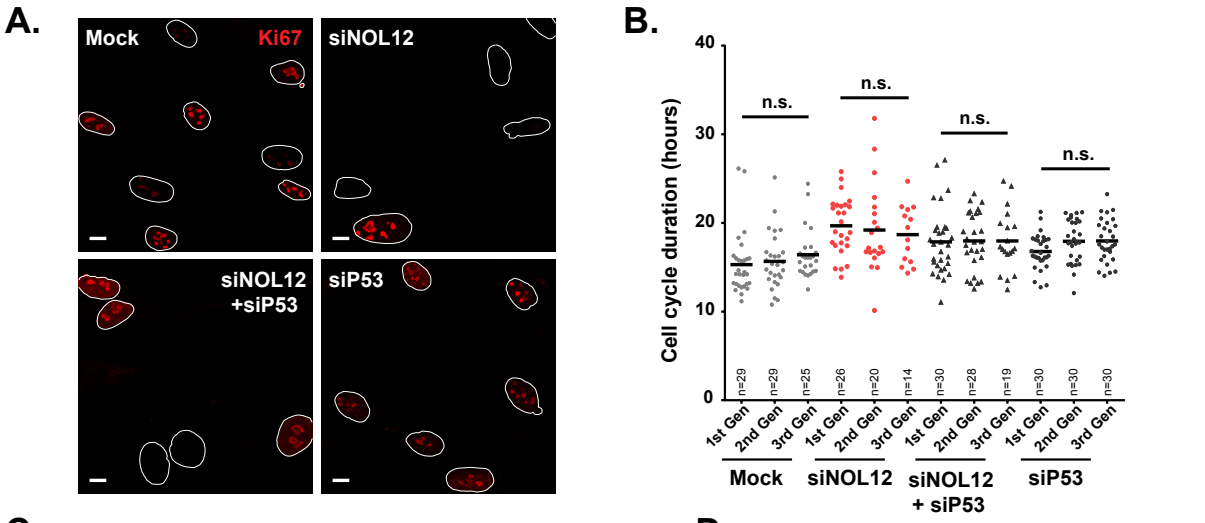
Gene	Sequence (5'-3')	
	Forward	Reverse
G6PD	AACATCGCCTGCGTTATCCTC	ACGTCCCGGATGATCCCAA
NOL12	GGCCGAGGCTCGTTCTTAG	TGCCTTCTTTCGCTCGACC
CDKN1A	TGGACCTGGAGACTCTCAGG	CGGATTAGGGCTTCCTCTTGG
MMP1	AGCGTGTGACAGTAAGCTAACC	AACTTCCGGGTAGAAGGGATTTG
CXCL8	GCCTTCCTGATTTCTGCAGCT	GCACTGACATCTAAGTTCTTTAGCA
TSPAN13	CGCCATGTGCTCCAATCATAG	GTAGGTCAGCCAAACACCCA
TBP	GAGCCAAGAGTGAAGAACAGTC	GCTCCCCACCATATTCTGAATCT



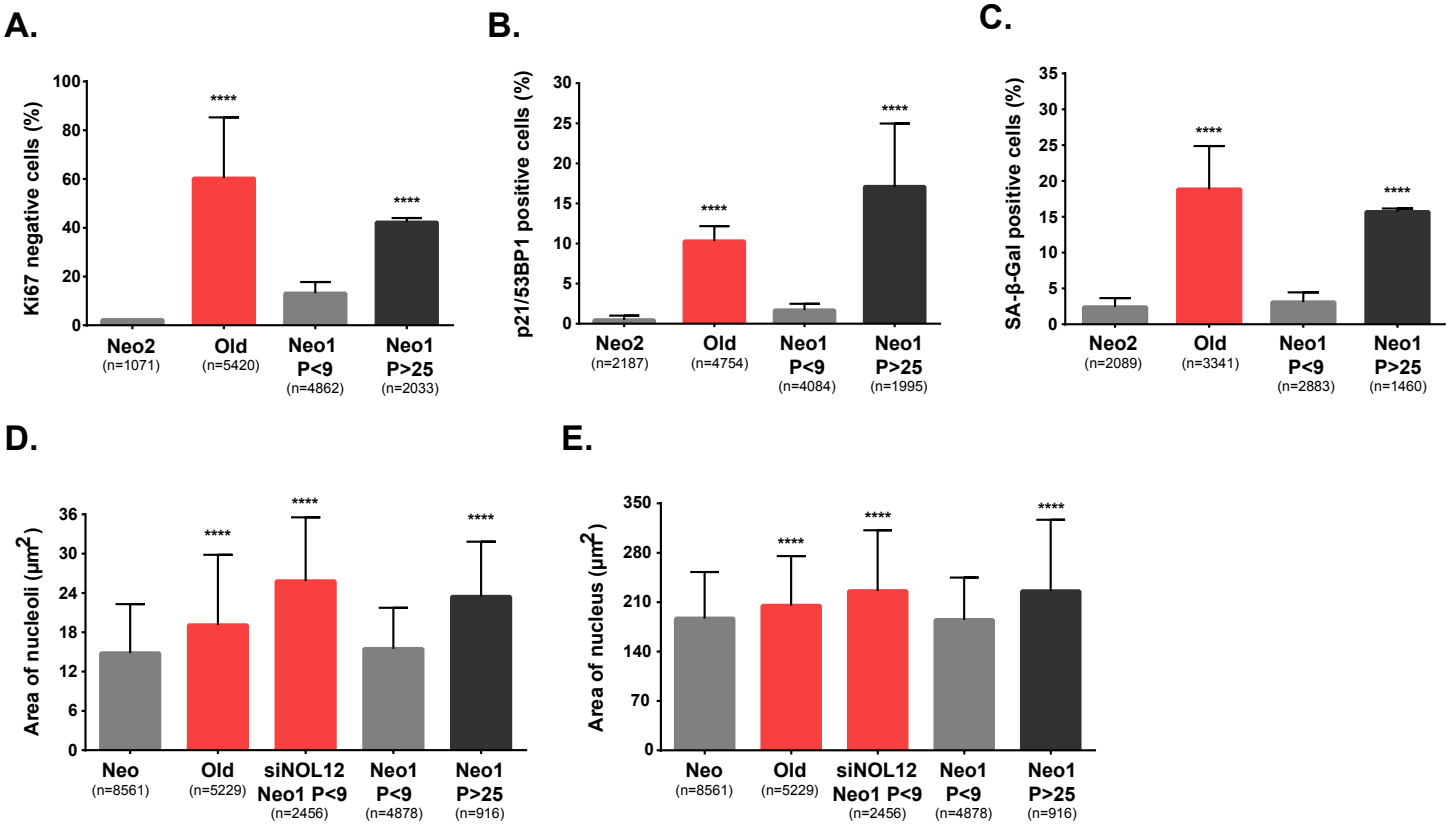
Supplementary Figure 1. Pinho M et al, 2018



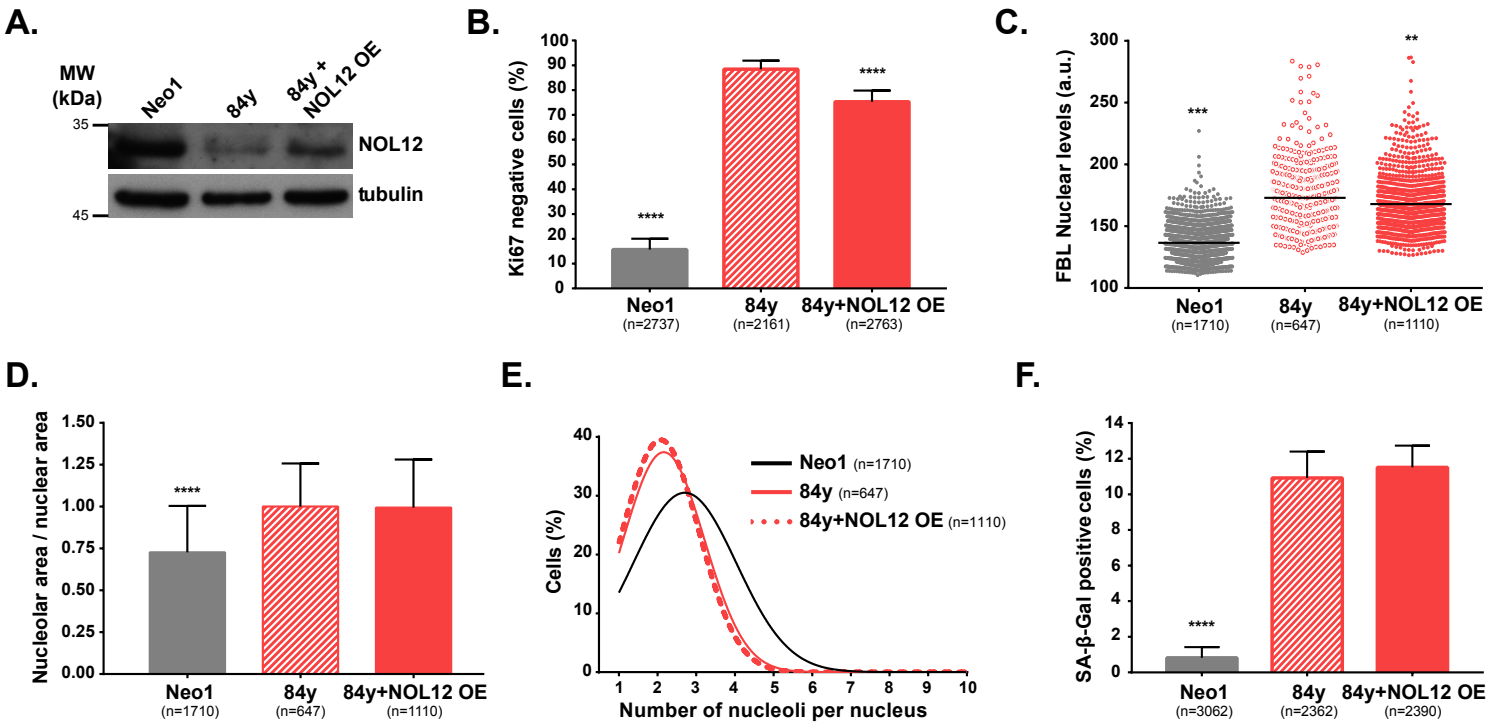
Supplementary Figure 2. Pinho M et al, 2018



Supplementary Figure 3. Pinho et al, 2018



Supplementary Figure 4. Pinho et al, 2018



Supplementary Figure 5. Pinho et al, 2018