Supplemental Experimental Procedures

Plasmid construction

shRNA directed against human CAND1 or NRF2 were, respectively, directed against 5'-CGTGCAACATGTACAACTA-3', 5'-GCAACAGGACATTGAGCAA-3'recognition sequences and cloned into the pSIHH1puro plasmid (System Biosciences). pSIHH1puro-shLuc (System Biosciences) was used as a non-targeting control in experiments. NRF2 knockdown experiments as described in figure 6 were performed with shRNA directed against the 5'-GTAAGAAGCCAGATGTTAA–'3 recognition sequence and cloned into the pLVTHM/GFP plasmid (Addgene, #12247), further using pLVTHM-shGL2 (Zhang et al., 2015) as a non-targeting control. The pRetrosuperblast plasmid expressing progerin shRNA was kindly provided by P. Fernandez (NCI, NIH, Bethesda). Constitutively active human NRF2 (caNRF2) was generated by stitch PCR using 5'-TCACCGGTTACTCGAGCAATGTTTCTCCCAATTCAGCCAGCCCAGCACATCC-3' and 5'-GAAGAATGTACTGGTGTGAAAGCTTTGCAAAGTG-3' as well as 5'-CACTTTGCAAAGCTTTCACACCAGTACATTCTTC-3' and 5'-GCGGCCGCGATATCCTAGTTTTTCTTAACATCTGG-3' primer combinations, generating NRF2 in which both Neh2 and Neh6 destabilizing domains were removed (McMahon et al., 2004), and subcloned into the pCDHblastMCSNard lentiviral plasmid (Kubben et al., 2012) containing an HA-tag and a nuclear localization signal (NLS) using 5'AgeI/3'NotI digestion, generating the HA-tagged caNRF2 expressing pCDHblastMCSNard HA-NLS-NRF2ΔNeh2ΔNeh6 lentiviral plasmid. BamHI/NotI digestion was used to transfer this construct via the pENTR1A-no-ccDB plasmid into the doxycycline-inducible pLentiCMVTRE3G-Neo plasmid, generating the inducible HA-caNRF2 lentiviral plasmid. Overexpression of caNRF2 in HGPS patient or wild-type fibroblasts was performed by multiple rounds of lentiviral infection with pLentiCMVTRE3G-NeoHA-NLS-NRF2ΔNeh2ΔNeh6 and pLentiCMVrtTA3-Hygro, and induction of expression for 96 hours by addition of 1µg/ml doxycycline. Overexpression constructs for caNRF2 used in iPSC-based cell models (Figure 6 and S6) were generated by cloning NRF2 cDNA with an E82G (A245G) mutation (amplified from the pCDNA3-Myc3-Nrf2^{A245G} plasmid , a gift from Dr. Yue Xiong) into a PLE4 plasmid (a gift from Dr. Tomoaki Hishida), further using PLE4-Luciferase (Duan et al., 2015) as a control in these experiments.

For immunoprecipitation experiments NRF2, lamin A and progerin constructs were cloned into pCDNA3.1 (Life Technologies). pcDNA3.1 OST-NLS-mCherry was created by triple ligation of a 5'BamHI/3'AsuII digested OneSTrep (OST)-tag from pCDHblastMCSNard OST-LMNA (Addgene, #22661) with a 3'XhoI/ 5'AsuII NLS-mCherry PCR product from the pQXCIN-Tetr-mCherry plasmid (Addgene, #59417) into BamHI/NotI digested pcDNA3.1 plasmid. pcDNA3.1 OST-NLS-mCherry-NRF2 was generated by introducing a 5'XhoI/3'NotI full length human NRF2 PCR fragment into this plasmid. pCDNA3.1 expressing NLS-mCherry-NRF2 with 9 additional N-terminal FLAG tag sequences was generated by replacing the OST-tag of pcDNA3.1 OST-NLS-mCherry-NRF2 with 9 FLAG tags. pcDNA3.1OST-LaminA and OST-Progerin were generated by BamH1/EcoRI mediated cloning from previously published plasmids (Kubben et al., 2010)(Addgene, #22661& #22662) into the pcDNA3.1 backbone. For pcDNA3.1 HA-LaminA and HA-Progerin, the OST tag was replaced by BamHI/SalI compatible annealed oligos containing the hemagglutinin (HA) tag sequence.

Antibodies

The following antibodies were used for western blotting: α-lamin A/C goat polyclonal (SantaCruz, Sc-6215), α-NRF2 mouse monoclonal (R&D systems, MAB3925), α-OneSTrEP mouse monoclonal (IBA Tagnology, 2-1507-001), α-FLAG mouse monoclonal (Sigma, F3165), α-HSC70 rat monoclonal (Abcam, Ab19136), α-βactin mouse monoclonal (Sigma, A2228), α-GAPDH mouse monoclonal (SantaCruz, Sc-32233), α-αtubulin rat monoclonal (Gentaur, #YSRTMCA77S) and α-Hemagglutinin mouse monoclonal (SantaCruz, sc-7392). For FACS the following antibodies were used: PE α-CD73 mouse monoclonal (BD, 5550257), FITC α-CD90 mouse monoclonal (BD, 555595) and APC α-CD105 mouse monoclonal (eBioscience, 17-1057-42). For immunofluorescence the following antibodies were used: α-progerin mouse monoclonal (Alexis Biochemicals, ALX-804-662), α-lamin B1 goat polyclonal (SantaCruz, Sc-6217), α-LAP2 rabbit polyclonal (SantaCruz, Sc-28541), α-HP1γ mouse monoclonal (Chemicon, MAB3450), α-trimethylated histone 3 lysine 27 rabbit polyclonal (Upstate, #07-449), α-Serine 139 phosphorylated H2AX mouse monoclonal (Millipore, #05-636), α-53BP1 rabbit polyclonal (Novus, NB100-304), α-NRF2 rabbit monoclonal (Abcam, 62352; Figure 2 and S2), α-NRF2 rabbit polyclonal (SantaCruz, Sc-722; Figure 3A-B), α-Serine 40 phosphorylated NRF2 rabbit polyclonal (Abcam, Ab76026), α-KEAP1 goat polyclonal

(SantaCruz, Sc-15246), α-MAF-F/G/K mouse monoclonal (SantaCruz, Sc-166548), α-CBP rabbit monoclonal (Cell Signaling, #7389). Secondary antibodies used for immunofluorescence detection were Alexa Fluor Donkey-anti-Mouse 568 and 647 (Invitrogen, #A10037, #A31571), Alexa Fluor Donkey-anti-Rabbit 568 and 647 (Invitrogen, #A10042, #A31573) and Alexa Fluor Donkey-anti-Goat 568 and 647 (Invitrogen, #A11057, #A21447). All IF stainings were performed after fixation in 4% paraformaldehyde (see below), with the exception of the α-NRF2 rabbit polyclonal antibody (SantaCruz, Sc-722; Figure 3A-B), which was used after standard methanol fixation (5 minutes at -20˚C) (Voncken et al., 2005). All antibodies were used at dilutions recommended by the manufacturers.

Immunofluorescence staining

All steps for IF staining were performed at ambient temperature. 96 hours post-transfection cells were fixed by addition of 25µl formaldehyde per well (10 minutes), permeabilized for 10 minutes (PBS/0.5% triton-X 100), and washed once with PBS/0.05% Tween-20. Next, cells were incubated for 1 hour with primary antibodies (γH2AX, 1:1000, Millipore, #05-636; lamin B1, 1:500, SantaCruz, Sc-6217) diluted in blocking buffer (PBS, 0.05% tween-20, 5% bovine serum albumin). After two consecutive washes in wash buffer (PBS, 0.5% Tween-20), and one wash in PBS/0.05% Tween-20, cells were incubated for 1 hour with secondary antibodies (Donkey-anti-Mouse 568, 1:500 Invitrogen, #A10037; Alexa Fluor Donkey-anti-Goat 647, Invitrogen, #A21447) together with 5µg/ml DAPI. After washing, cells could be stored for extended periods of time in PBS/0.05% Tween-20.

One-STrEP pull-down

293FT cells grown to 80% confluency in 15 cm tissue culture dishes were transfected with 1ml of Optimem reduced serum medium (Invitrogen) containing 40 µl X-tremeGENE HP DNA transfection reagent (Roche, #06366236001), 7 µg of pcDNA3.1 containing OST-tagged NRF2 orlamin A/ Progerin bait, and 3 µg of the pcDNA 3.1 plasmid for either HA-tagged lamin A/progerin or FLAG-tagged NRF2 prey (see above). 18 hours post-transfection a pull-down of OST-tagged proteins was performed with streptactin matrix as described previously after 0.1% formaldehyde fixation (Figure 3D-E) (Kubben et al., 2010). For interaction experiments in figure 3F dermal fibroblasts expressing OST-lamin A or OST-progerin were used for purification of OST-lamin A and OST-progerin by OST-pull-down as previously described (Kubben et al., 2010), without the use of any fixation. Purified immobilized lamin proteins were next incubated with recombinant His6-tagged full length NRF2 (Origene, TP760529) in ChiP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl pH=8.1, 167 mM NaCl), and after 6 hours of incubation at 4°C, washed 6 times with ChiP dilution buffer.

Western blotting

Western blots were performed as described (Kubben et al., 2010; Zhang et al., 2015). Nuclear extracts were prepared as previously published (Andersen et al., 2002), with the addition of 0.033% Triton X-100 to buffer A (10 mM HEPES-KOH, 1.5 mM MgCl2, 10 mMKCl, 0.5 mM DTT). For OST detection PVDF membranes were blocked in 5% BSA/TBS-Tween, and subjected to an additional pre-blocking step with biotin blocking buffer (IBA TAGnology, #2-0501-002)) according to manufacturer's instructions.

Microarrays and quantitative analysis of gene expression

RNA samples, prepared as previously described (Pegoraro et al., 2009), of HGPS patients dermal fibroblast (HGADFN001, HGADFN003, HGADFN127 from Progeria Research Foundation; AG01972, AG06297, AG11513 from Coriell Cell Repository), and control dermal fibroblast cell lines (GM00038, GM05565 from Coriell Cell Repository; CRL-1474 from ATCC), were analyzed on Genechip human genome 133 (HG-U133_plus_2) microarrays (Affymetrix) at the Frederick Laboratory of Molecular Technology Microarray Facility. All microarray data are publically available under GEO accession number GSE69391. After normalization of intensity values to the median intensity of the arrays, ratios of the average HGPS versus control mRNA expression levels were calculated

for each gene and plotted for non-NRF2 targets, NRF2 transcriptional targets, previously defined as genes that interact with NRF2 in ChIP-on-Chip experiments and show decreased mRNA expression upon genetic disruption of NRF2 expression (Malhotra et al., 2010). Ratio expression profiles were statistically compared by the non-parametric two-sample Kolmogorov-Smirnov test. Analysis of mRNA expression levels of NRF2 target genes in HGPS patient and control cells, or upon induction of GFP-progerin in P1 cells, was carried out as previously described (Pegoraro et al., 2009) using primer combinations as indicated in Supplementary Table 1, and normalized to cyclophilin A. Geneset enrichment analysis (GSEA) was performed on mRNA expression datasets of wild-type and HGPS patient fibroblasts using the C3 transcription factor targets mySig database v3.0 (GSEA, Broad Institute) with the addition of an NRF2 signature set as described above (Malhotra et al., 2010), using standard settings as previously used (Fernandez et al., 2014). Gene signatures were considered enriched for false discovery rate (FDR) q-values < 0.01 and Family-Wise Error Rate (FWER) p-values < 0.05.

Luciferase reporter assay

Fibroblast were transfected in absence or presence of 1 µg/ml doxycycline according to the manufacturer's instructions with X-tremeGENE HP DNA transfection reagent (Roche, #06366236001) and 9µg of the pGL-8xARE firefly luciferase reporter, which contains 8 copies of a minimal functional NRF2 responsive antioxidant responsive element (ARE) sequence as previously described (Wang et al., 2006), or 9µg of the pGL-0xARE plasmid, which does not contain ARE recognition sequences and serves as a negative control. Co-transfection with 1 µg pRL-TK (Promega) was used for normalization of transfection efficiencies. After 36 hours cells were harvested and both firefly and renilla luciferase actvity were measured using the Dual-Luciferase Reporter Assay System (Promega).

Reactive oxygen species detection

To measure oxidative stress levels skin fibroblasts were grown in 384-wells clear bottom tissue culture plates (Perkin Elmer, #6007558), washed once with Optimem reduced serum medium (Invitrogen), and incubated with 20 µM of the cell permeate reagent 2',7'-dichlorofluorescein diacetate (DCFDA) (Abcam, ab113851) at 37˚C, which becomes fluorescent upon oxidation by ROS. After 45 minutes the medium was replaced with regular tissue culture medium supplemented with the DNA stain DRAQ5 (1µM;Biostatus Limited). Fluorescent DCFDA and DRAQ5 co-staining were immediately imaged under 37˚C, 5%CO_2 and 60% humidity life imaging conditions according to the manufacturer's instructions using Perkin Elmer's Opera system. The total cellular intensity of DCFDA was quantified by standard Acapella Image Analysis scripts as described above. Oxidative stress levels were quantified in iPSC based cell models through H2-DCFDA (Invitrogen, C6827 or Abcam, ab186028) based flow cytometry as per manufacturer's instructions.

Apoptosis and senescence associated β-galactosidase detection

The FITC annexin V Apoptosis detection kit (BD, 556547) was used to determine cell apoptotic level according to the manufacturer's instructions. Cell survival was measured by counting MSC cell number 72 hours after plating after trypan blue exclusion staining. Senescence associated β-galactosidase **(**SA-β-gal) staining was performed as described previously (Endisha et al., 2015). Briefly, cultured cells were washed in PBS and fixed at room temperature for 3 min in 2% formaldehyde and 0.2% glutaraldehyde. Fixed cells were stained with fresh staining solution for SA-β-gal activity at 37°C overnight, and counted for positivity (N>300).

In vivo luminescence measurements

MSC implantation experiments were performed as previously described (Pan et al., 2016; Zhang et al., 2015) on GC- and HGPS-iPSC-MSCs that express control or NRF2 targeting shRNA, control or caNRF2, or were vehicle or Oltipraz (20µM; 3 weeks) pretreated. In brief, 100µl of $1X10^6$ MSCs transduced with lentivirus expressing luciferase were injected into the midportion of the tibialis anterior (TA) muscle of immunodeficient mice (Pan et al., 2016; Zhang et al., 2015). 5 days after implantation, mice were anaesthetized and treated with D-luciferin. Then, photon emission was measured by the IVIS lumina system (PerkinElmer). Bioluminescence images were acquired at auto-set model. Photons were counted according to the digital false-color photon emission image of the mouse, and

the values were normalized by average cellular luciferase intensity before implantation. All animal experiments were conducted with the approval of the Institute of Biophysics, Chinese Academy of Science.

Gene	NRF2 Target?	Forward primer (5'->3')	Reverse primer (5'->3')	Corresponding Figure
GCLC	Yes	CAAGAGAAGGGGGAAAGGAC	GACCTCGGGCAGTGTGAAC	Fig 4, S4, S6
GCLM	Yes	TCAGGGAGTTTCCAGATGTC	CAATAGGAGGTGAAGCAATG	Fig 4, S4, S6
TALDO	Yes	GGGCCGAGTATCCACAGAAG	GGCGAAGGAGAAGAGTAACG	Fig 4, S4, S6
GSR	Yes	CCCAAGCCCACAATAGAGG	ACCTGCACCAACAATGACG	Fig 4, S4, S6
TKT	Yes	GGCTGTCCTCTTTTTCCAC	CCCAGCAAGCAATAGACTC	Fig 4, S4, S6
GSTM1	Yes	GCATGATCTGCTACAATCC	CTTGGGCTCAAATATACGG	Fig 4, S4, S6
GSTM4	Yes	CCTTGCTCCCTGAACACTC	GTCGTCACTTCCAACCAAC	Fig 4, S4, S6
GPX7	Yes	ACTTCAAGGCGGTCAACATC	GGCAAAGCTCTCAATCTCC	Fig 4, S4, S6
HO1	Yes	AAGACTGCGTTCCTGCTCAAC	AAAGCCCTACAGCAACTGTCG	Fig 6, S6
NQO1	Yes	GGCATTCTGCATTTCTGTG	GGCGTTTCTTCCATCCTTC	Fig 4, S4, S6
TXNDR1	Yes	CTGCGGGAGAAAAAAGTCG	CAGGGATGCCCAAGTAACG	Fig 4, S4, S6
TBP	No	CGCCAGCTTCGGAGAGTTC	ACAACCAAGATTCACTGTGGATACA	Fig 4, S4, S6
CYPA	No	CTGTTGCTGTAGCCAAATTCGT	ACCCACTCCTCCACCTTTGA	Fig 4, S4
18S	No	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG	Fig 6, S6
CAND1	No	AGCGCCTCGTACCACATTTC	TGCAGTTCCGTCATCAAATCA	Fig S1, S6
progerin	No	GCGTCAGGAGCCCTGAGC	GACGCAGGAAGCCTCCAC	Fig S6

Table S1. Primers used for quantitative real-time PCR analysis, related to main figure 4, main figure 6 and supplemental figure S1, S4 and S6. Primer sequences for real-time PCR quantitative analysis of indicated human mRNAs in a 5' to 3' direction. CypA and 18S were used as housekeeping controls.

Primary Screening Results Candidates										
Target	Gene ID	Normalized HGPS aging parameters and Z-scores								
		Progerin		Lamin B1		γH2AX		LaminB1 + γH2AX combined		Cell number
		Value	Z	Value	Z	Value	Z	Value	Z	Value
CAND1	55832	**0.43**	**2.99***	1.40	-0.62	**1.76**	**2.06***	**1.58**	**-2.15***	1.41
WSB1	26118	**0.48**	**2.72***	1.20	-0.02	**1.97**	**2.48***	**1.59**	**-2.15***	1.31
FLJ25076	134111	**0.52**	**2.55***	1.61	-1.22	**1.96**	**2.44***	**1.78**	**-2.85***	1.24
FBXO38	81545	**0.56**	**2.31***	1.70	-1.50	**1.84**	**2.18***	**1.77**	**-2.81***	0.73
TRIM2	23321	**0.52**	**2.51***	**1.86**	**-1.89***	0.81	0.01	1.33	-1.21	0.63
RNF39	80352	**0.57**	**2.25***	**1.88**	**-2.03***	1.00	0.38	1.44	-1.58	0.71
HERC3	8916	**0.57**	**2.29***	**1.90**	**-2.18***	0.83	0.06	1.37	-1.33	0.81
Neg Control		1.00	-	1.00	-	1.00	-	1.00	-	1.00
Pos Control		0.02	-	2.00	-	2.00	-	2.00	-	1.13
Secondary Verification Results										
CAND1	55832	**0.53**	**2.56***	**1.70**	**-2.08***	**1.89**	**2.20***	**1.80**	**-2.56***	1.25
WSB1	26118	1.05	0.41	1.10	-0.78	0.85	0.04	0.98	-0.03	1.17
FBXO38	81545	0.81	1.02	1.04	-0.56	**1.56**	**2.02***	1.30	-1.21	1.21
FLJ25076	134111	1.12	0.35	1.03	-0.48	0.95	0.07	0.99	-0.02	0.83
Neg Control		1.00	-	1.00	-	1.00	-	1.00	-	0.89
Pos Control		0.03	-	2.00	-	2.00	-	2.00	-	1.00

Table S2. RNAi screening results of candidates, related to main figure 1. Primary screening and verification results of candidates that lower induced GFP-progerin levels, as well as either prevent formation of defects in laminB1 or γH2AX. Progerin, laminB1, γH2AX, and combined laminB1+γH2AX parameters were quantified as described in the Experimental Procedures, and compared to negative control (non-targeting siRNA) and positive control (GFP-targeting siRNA) transfected GFP-progerin induced wild-type fibroblasts (96 hours), which values were arbitrarily set as indicated in the table at 1.00 and 2.00. Significant Z-scores, as defined by cut-off values as listed in Experimental Procedures, are indicated in bold and with asterisk. Underlined targets were selected for secondary verification with structurally distinct on-target plus siRNA pools.

Gene Signatures Upregulated in WT vs. HGPS						
Gene Signature	Number of genes	ES	NES	Nominal P-value	FDR Q-Value	FWER P-value
NRF2	179	0.483258	2.13967	0	4.01E-04	0
GCCATNTTG_V$YY1_Q6	360	0.4135507	1.9734445	0	7.61E-04	0.01
V$E2F1_Q6	206	0.4288878	1.9346342	0	8.99E-04	0.016
V$E2F_Q4	205	0.42010823	1.894692	0	0.001323	0.030
V$E2F_Q6	204	0.41962507	1.8883432	0	0.001285	0.034
Gene Signatures Upregulated in HGPS vs. WT						
V$NRSF_01	91	-0.42931843	-1.9478011	0	0.005654	0.013
KRCTCNNNNMANAGC_UNKNOWN	58	-0.4771294	-1.9479508	0	0.011308	0.013

Table S4. Geneset Enrichment Analysis, related to main figure 2. Geneset Enrichment Analysis of mRNA expression datasets for wild-type and HGPS human dermal fibroblasts using the C3 transcription factor targets mySig database v3.0 (GSEA, Broad Institute) with the addition of an NRF2 signature set (See Experimental Procedures). ES = Enrichment Score; NES = Normalized Enrichment Score; FDR = False discovery rate; FWER = Family-Wise Error Rate. All significantly enriched gene signatures with FDR Q-values < 0.01 and FWER P-value < 0.05 are listed.