Supplementary Information

Supplementary Methods

Co-immunoprecipitation.

The co-immunoprecipitation experiments for MEFs and U2OS cells were carried out as follows. 150 mm plates were used per condition. In the case of U2OS cells, a pretreatment with either 100 μM or 500 μM H_2O_2 for 30 min was included. Cells were washed twice in PBS and lysed in lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, and 1x mini EDTA-free protease inhibitor cocktail (Sigma)) at 4°C for 30 min. The suspension was centrifuged at 14,000 × g for 15 min. The extracts were then pre-cleared for 1 h with protein A-agarose beads (pre-washed with PBS) (30 μl; Sigma). Immunoprecipitation was carried out on 500 μl (1 mg of protein) of the pre-cleared lysate with anti-lamin A/C antibody (2 μg; sc-20681; Santa Cruz; rabbit polyclonal) and protein A-agarose beads (30 μl) overnight, rotating, at 4°C. In the case of U2OS cells, a negative control was incorporated in which normal rabbit IgG antibody (2 μg; sc-2027; Santa Cruz) was used in place of anti-lamin A/C antibody. Bound proteins were eluted by boiling in SDS sample buffer and were run on SDS-PAGE. Blots were analyzed using anti-lamin A/C (Santa Cruz), anti-βactin (AMB-7229), anti-PARP1 (614302; Nordic Biosite), anti-OGG1 (NB100-106, Novus), anti-APE1(ab194, Abcam); anti-LAP2α (GTX803750, GeneTex) was used as a positive control in the case of MEFs, and anti-emerin (NCL-EMERIN, Leica Biosystems) as a positive control in the case of U2OS cells. Protein bands were detected by chemiluminescence (ECL, GE Healthcare).

Supplementary Tables

Supplemental Table S1. PCR primers

Primer name	Primer sequence
Lmna left	AGCAAAGTGCGTGAGGAGTT
Lmna right	AACAAGTCCCCCTCCTCCTT
p53 left	CTAGCATTCAGGCCCTCATC
p53 right	CCTCCATGGCAGTCATCC
Parp1 left	AGGCCGCCTACTCTATCCTC
Parp1 right	GATTCACTGCTGCCTTGAGA
Lig3 left	GCGCAAGTCGGTTACTGC
Lig3 right	TGTCTCTTTAAATACGGGTACTCCA
Polβ left	GACTCGAGTTACTGGCATTGG
Polβ right	TCTTAATTCCTTCATCTACAAACTTCC
Ape1 left	AAAGAAAGGTTTGGATTGGGTAA
Ape1 right	CTGACCAGTACTGATGGGTGAG
Ogg1 left	TTATCATGGCTTCCCAAACC
Ogg1 right	GTACCCCAGGCCCAACTT
Nrf2 left	CATGATGGACTTGGAGTTGC
Nrf2 right	CCTCCAAAGGATGTCAATCAA

Supplemental Table S2A. Oligonucleotides used as a substrate in the APE1 DNA incision assay

Specificity	Name	Sequence
AP site (=F)	THF28	5'-GAACGACTGT**(F)**ACTTGACTGCTACTGAT
Complementary strand	OG 28-comp	3'- CTTGCTGACACTGAACTGACGATGACTA

Supplemental Table S2B. Oligonucleotides used as a substrate in the POLβ nucleotide incorporation assay

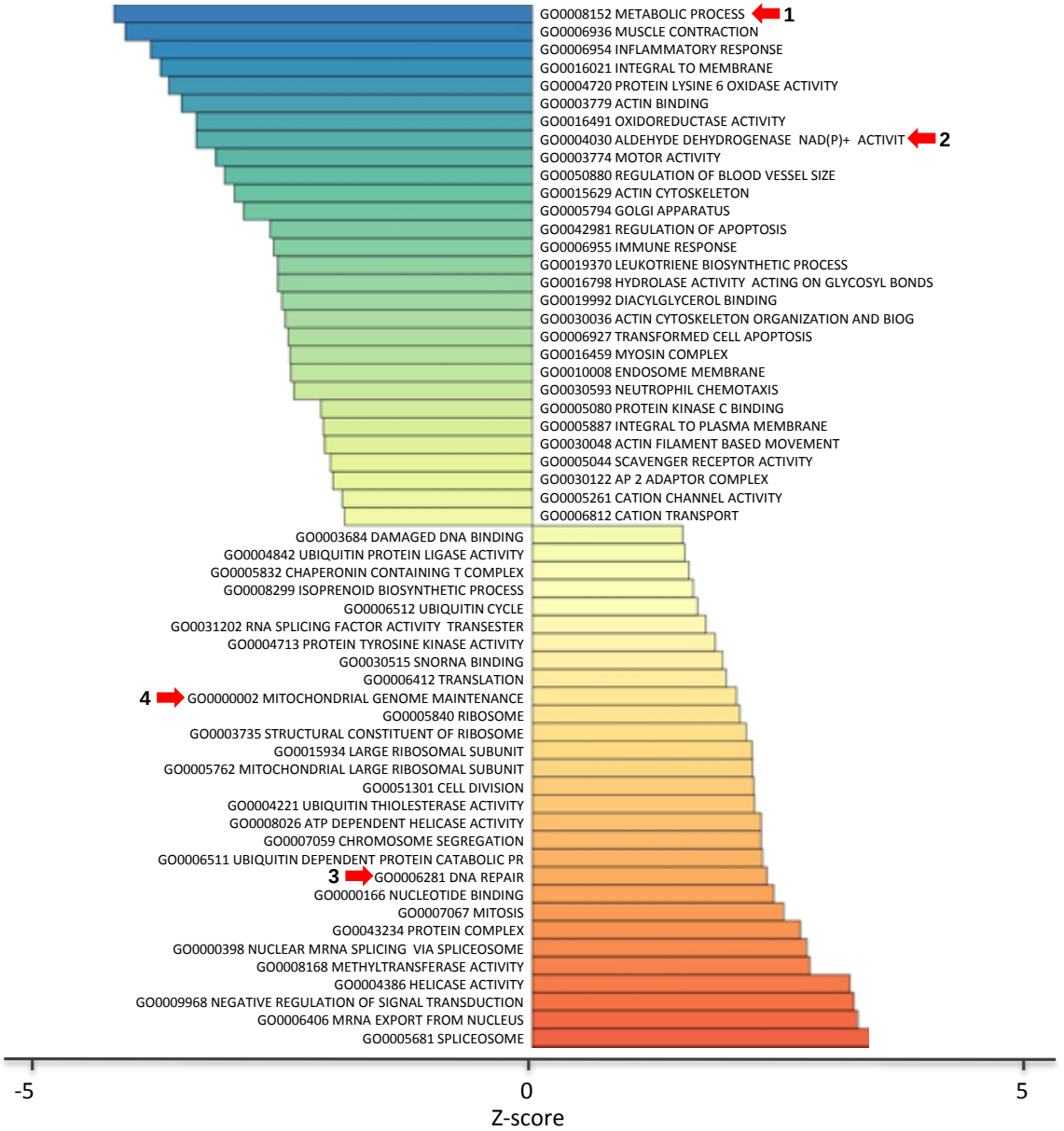
Specificity	Sequence
18 nucleotide upstream	5´-TAMRA-CATATCCGTGTCGCCCTC
17 nucleotide downstream	5´-TTCCGATAGTGACTACA
36 nucleotide template	GTATAGGCACAGCGGGAGTAAGGCTATCACTGATGT-5´

Supplementary Table S3. Top Canonical Pathways including "Molecules"

Name	*P*-value	Ratio	Molecules
Pancreatic Adenocarcinoma Signaling	1.01E-08	23/106 (= 0.217)	IGFBP4, MYH10, CTGF, COL4A5, IL1RL1, COL4A2, PDGFC, FAS, TGFBR2, TIMP1, PDGFRA, TGFB2, MYL4, STAT1, COL18A1, IL1RAP, TNFRSF11B, EGFR, TIMP2, COL4A1, COL2A1, VEGFC, MMP2, IGFBP5, MET, COL1A1, IGF2, ACTA2, TGFB3, CD14, COL7A1
Hepatic Fibrosis / Hepatic Stellate Cell Activation	2.79E-08	32/197 (= 0.162)	CDKN2A, SUV39H1, TYK2, VEGFC, MDM2, STAT3, E2F3, CDKN2B, PDGFC, RAD51, TGFBR2, RB1, HMOX1, GAB1, CDKN1A, E2F5, TGFB3, TGFB2, PIK3CB, STAT1, FRS2, CDK2, EGFR, ATM
Aryl Hydrocarbon Receptor Signaling	1.40E-07	25/140 (= 0.179)	CDKN2A, SRC, GSTM1, MGST1, CCNE2, NQO1, APAF1, MDM2, FAS, CYP1B1, TGM2, RB1, FOS, MGST2, ALDH1L2, CDKN1A, TGFB2, TGFB3, RXRB, GSTO2, CDK2, ALDH6A1, HSPB1, GSTK1, ATM
Molecular Mechanisms of Cancer	1.68E-06	43/365 (= 0.118)	RAP1B, CDKN2A, BMP4, SUV39H1, GNA11, CRK, E2F3, CDKN2B, FAS, TGFBR2, RB1, ZBTB17, ARHGEF19, PAK1, ITGA3, IKBKG, HHAT, RHOB, TGFB2, E2F5, ARHGEF3, CASP8, FZD2, FRS2, ATM, SRC, CCNE2, TYK2, SMAD6, APAF1, MDM2, FADD, FOS, GAB1, CDKN1A, TGFB3, PRKAG2, FZD6, ARHGEF18,CYCS,PIK3CB,CDK2,CASP7,WNT5A,FZD7
NRF2-mediated Oxidative Stress Response	5.26E-06	26/180 (= 0.144)	DNAJA4, HMOX1, PMF1/PMF1-BGLAP, ATF4, FOSL1, GCLM, ACTG2, FKBP5, FRS2, ACTA1, ATM, GSTK1, GSTM1, MGST1, ACTB, NQO1, HERPUD1, DNAJB9, MAFF, FOS, GAB1, ACTA2, MGST2, CAT, PIK3CB, SQSTM1, GSTO2, EPHX1

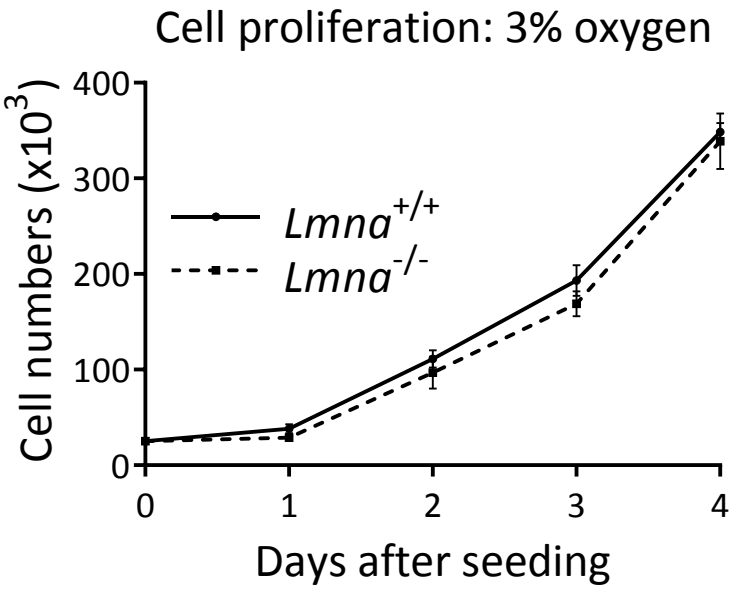
Supplementary Table S4. Fold Change for genes in the NRF2 Top Canonical Pathway.

Symbol	Entrez Gene Name	Expr Fold Change	Location	Type(s)
ACTA1	actin, alpha 1, skeletal muscle	-6.08	Cytoplasm	other
ACTA2	actin, alpha 2, smooth muscle, aorta	-2.41	Cytoplasm	other
ACTB	actin beta	5.54	Cytoplasm	other
ACTG2	actin, gamma 2, smooth muscle, enteric	-10.63	Cytoplasm	other
ATF4	activating transcription factor 4	5.16	Nucleus	transcription regulator
ATM	ATM serine/threonine kinase	3.22	Nucleus	kinase
CAT	catalase	-2.65	Cytoplasm	enzyme
DNAJA4	DnaJ heat shock protein family (Hsp40) member A4	-2.17	Nucleus	other
DNAJB9	DnaJ heat shock protein family (Hsp40) member B9	-1.67	Nucleus	other
EPHX1	epoxide hydrolase 1	-18.37	Cytoplasm	peptidase
FKBP5	FK506 binding protein 5	1.8	Nucleus	enzyme
FOS	Fos proto-oncogene, AP-1 transcription factor subunit	-4.11	Nucleus	transcription regulator
FOSL1	FOS like 1, AP-1 transcription factor subunit	2.1	Nucleus	transcription regulator
FRS2	fibroblast growth factor receptor substrate 2	-2.03	Plasma Membrane	kinase
GAB1	GRB2 associated binding protein 1	-4.07	Cytoplasm	kinase
GCLM	glutamate-cysteine ligase modifier subunit	2.06	Cytoplasm	enzyme
GSTK1	glutathione S-transferase kappa 1	3.25	Cytoplasm	enzyme
GSTM1	glutathione S-transferase mu 1	-3.43	Cytoplasm	enzyme
GSTO2	glutathione S-transferase omega 2	2.11	Cytoplasm	enzyme
HERPUD1	homocysteine inducible ER protein with ubiquitin like domain 1	-2.67	Cytoplasm	other
HMOX1	heme oxygenase 1	-2.35	Cytoplasm	enzyme
MAFF	MAF bZIP transcription factor F	-2.11	Nucleus	transcription regulator
MGST1	microsomal glutathione S-transferase 1	2.29	Cytoplasm	enzyme
MGST2	microsomal glutathione S-transferase 2	-2.02	Cytoplasm	enzyme
NQO1	NAD(P)H quinone dehydrogenase 1	2.21	Cytoplasm	enzyme
PIK3CB	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit beta	-2.04	Cytoplasm	kinase
PMF1/PMF1-BGLAP	polyamine modulated factor 1	1.75	Nucleus	transcription regulator
SQSTM1	sequestosome 1	-2.07	Cytoplasm	transcription regulator

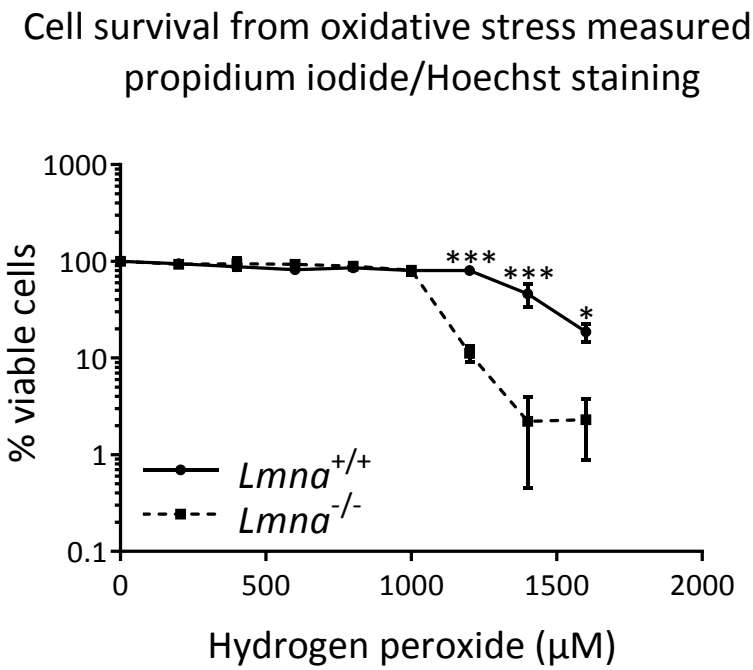


Supplementary Fig. S1. Microarray data. List of significantly changed GO terms in *Lmna*$^{-/-}$ MEFs compared to WT (*Lmna*$^{-/-}$) MEFs. Z score indicates the extent of elevated or inhibited (negative value) gene expression as a result of the *Lmna* knockout. Four relevant GO terms, that are shown in Table 1, are numbered and indicated by red arrows: 1, Chemical reactions including DNA repair, replication, and protein synthesis/degradation; 2, Catalysis of the reaction: an aldehyde + NAD(P)$^+$ + H2O = an acid + NAD(P)H + H$^+$; 3, DNA repair pathways that include BER; 4, The maintenance of the structure and integrity of the mitochondrial genome; suggestive of BER since this is a very robust DNA repair pathway in mitochondria (due to constant oxidative stress on mitochondrial DNA).

Fig. S1.

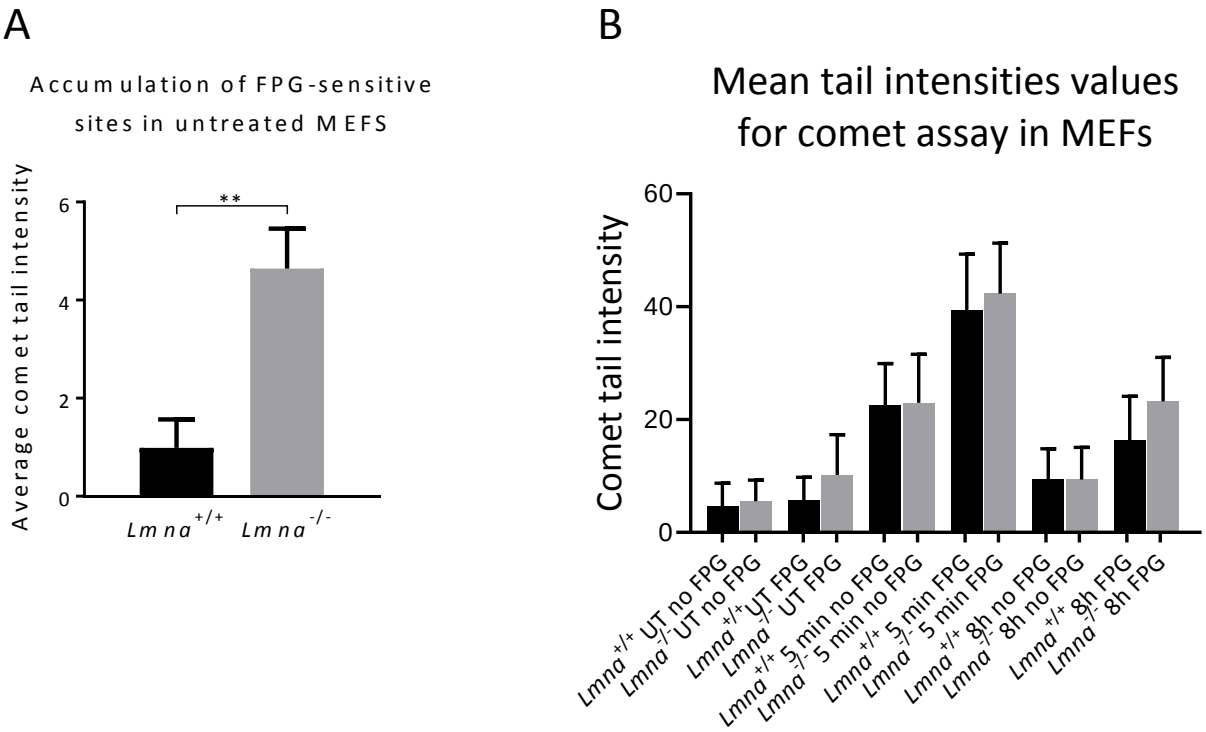


Supplementary Fig. S2. *Lmna*$^{-/-}$ MEFs no longer have defective cell proliferation (compared to *Lmna*$^{+/+}$ MEFs) when incubated at 3% oxygen instead of 20% oxygen. There was no significant difference (by Student's t-test) at any of the time points (n = 3; mean ± SD). The cells were conditioned by incubating at 3% oxygen over a period of one week prior to performance of the growth curve.



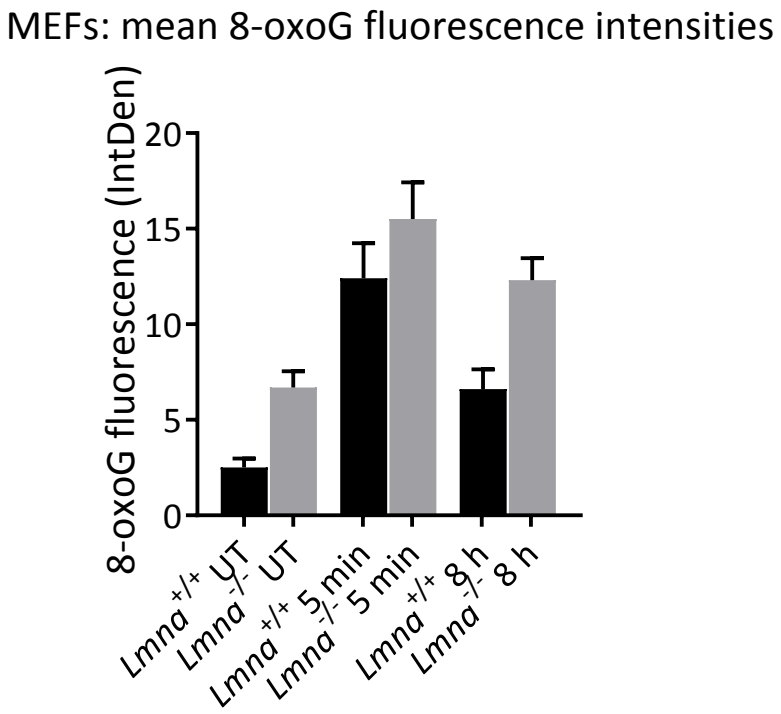
Supplementary Fig. S3. *Lmna*$^{-/-}$ MEFs have reduced cell viability, after oxidative stress, compared to *Lmna*$^{+/+}$ MEFs. Cells in a 96 well plate were stained with medium containing propidium iodide (5 mg/ml) and Hoechst (2µM). Viability of the cells (total cells minus dead cells) after treatment for 4 h with the indicated concentration of hydrogen peroxide, was determined using a Celigo imager cytometer (Nexcelom). All data points are the mean live cell count (as percent of the mean live cell counts from untreated cells) from three wells (in 96 well dishes) ± SD. Cells were seeded at 20,000 cells per well, 24 h prior to treatment. The *P* values were determined by 2-way ANOVA Sidak's multiple comparisons test; ****P* < 0.0001; **P* < 0.05.

Fig. S3.



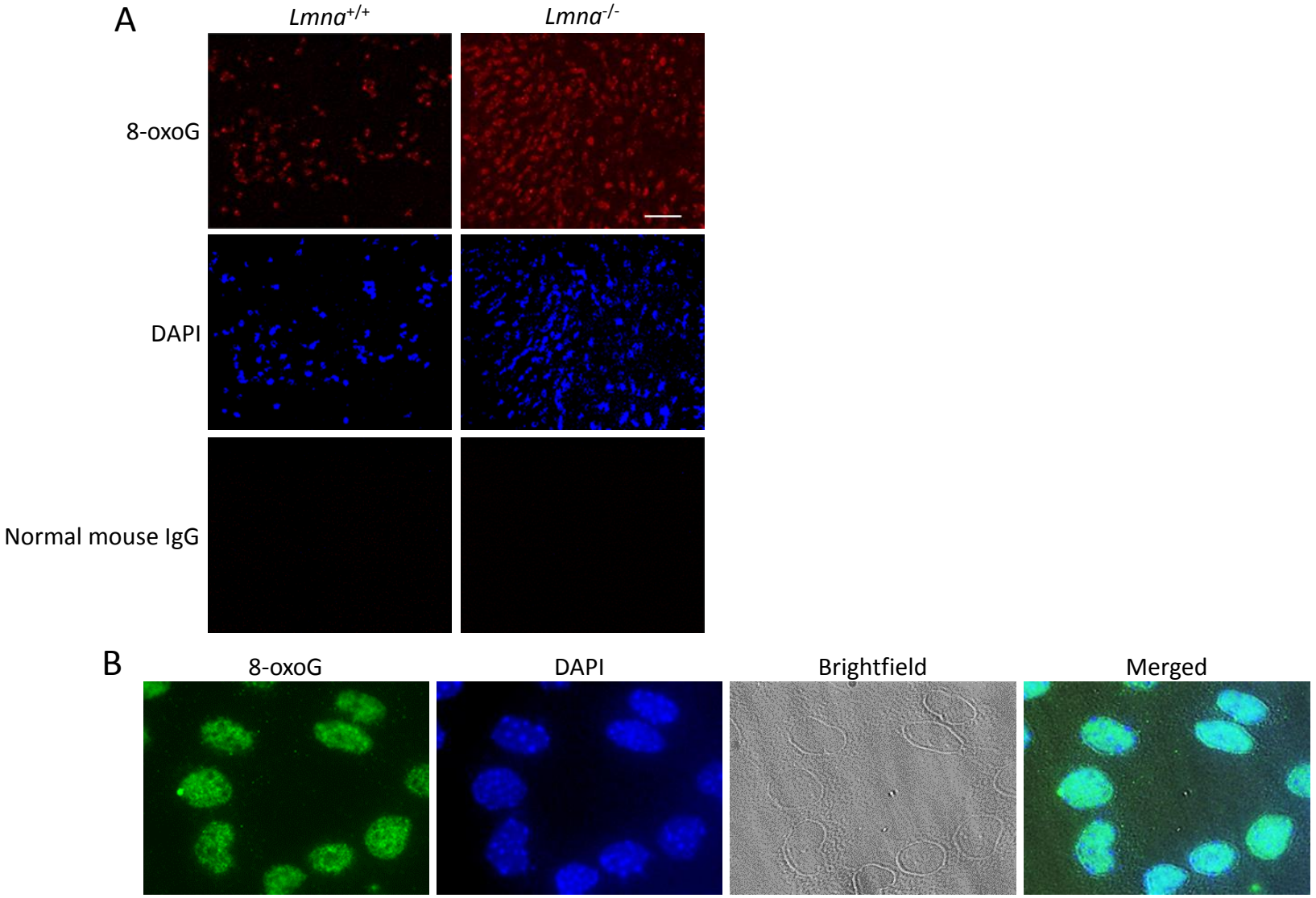
Supplementary Fig. S4. (**A**) FPG-sensitive sites in untreated MEFs (prior to addition of H_2O_2) from the comet assay shown in Figure 1A. For estimation of oxidative damage (FPG-sensitive sites), the tail intensity for buffer treated cells ("no FPG") is subtracted from the tail intensity for FPG-treated cells. *P* value was determined by Student's t-test; ***P* < 0.005. (**B**) Raw mean tail intensity (i.e. % DNA in tail) from the comet assay shown in Figure 1A. n = 100 comet tails, mean ± SD. UT, untreated.

Fig. S4.



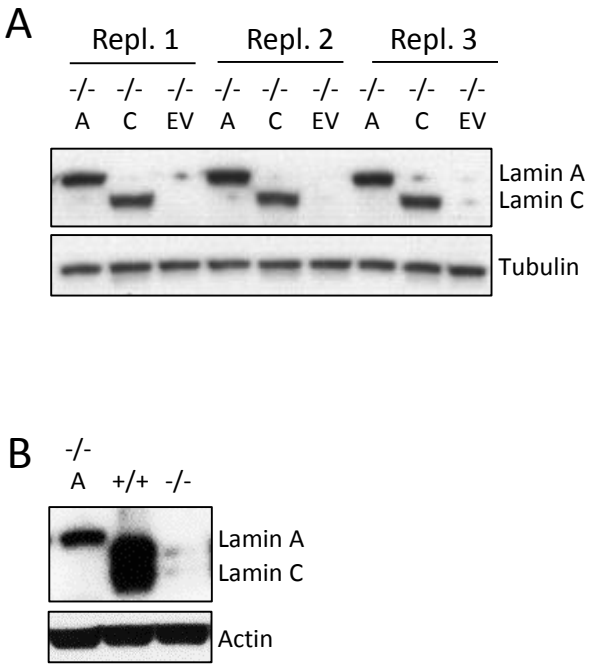
Supplementary Fig. S5. Immunofluorescence intensities of 8-oxoG DNA lesions at all time points before and after subjecting the MEFs to oxidative insult (100 µM H_2O_2 for 30 min): untreated (UT), 5 min and 8 h after treatment. Quantitation was performed using Integrated Density (IntDen) in Image J software.

Fig. S5.



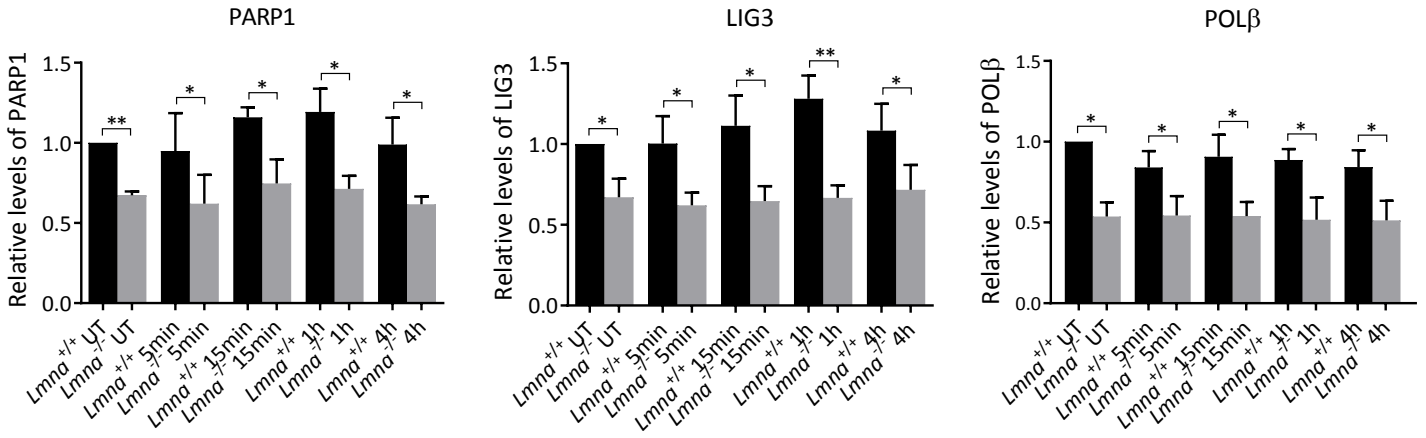
Supplementary Fig. S6. (**A**) Immunofluorescence images of untreated cells from Figure 1B, showing the DAPI staining and normal mouse IgG control staining (as negative control). This staining verifies that the 8-oxoG staining is nuclear. Bar represents 10 µm. The brightness of the images were enhanced to enable strong DAPI visualization; all frames were enhanced to the same extent. (**B**) Immunofluorescence of 8-oxoG DNA lesions in *Lmna*$^{-/-}$ MEFs shown at higher magnification (60X); includes a brightfield channel to reveal the cytoplasm (which is damaged as expected from the protocol). The cells were stained with 8-oxoG and DAPI after treatment with 100 µM H_2O_2 for 30 min: these images further verify that 8-oxoG staining is nuclear.

Fig. S6A,B



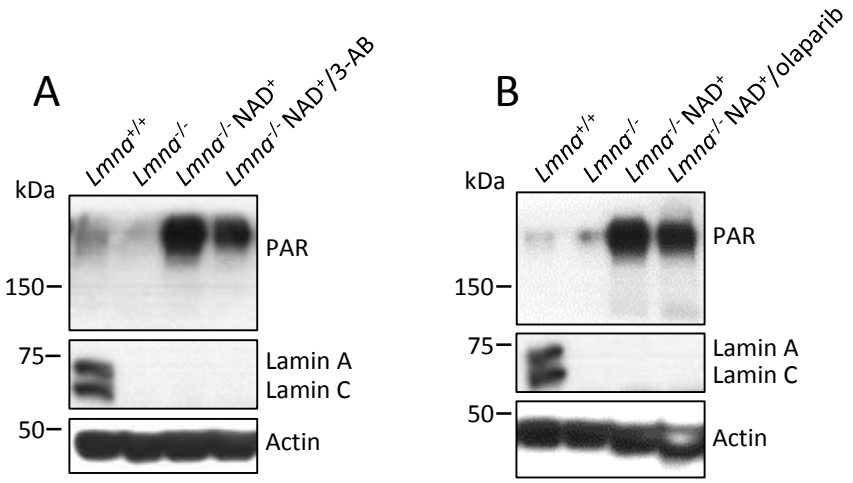
Supplementary Fig. S7. Western blots showing expression of Lamin A and Lamin C in *Lmna*$^{-/-}$ MEFs, produced by retroviral transduction. (**A**) Lamin A and lamin C are expressed at near equal levels in the *Lmna*$^{-/-}$ MEFs; designated as -/- A (*Lmna*$^{-/-}$ MEFs overexpressing lamin A), -/- C (*Lmna*$^{-/-}$ MEFs overexpressing lamin C) or -/- EV (*Lmna*$^{-/-}$ MEFs transfected with empty vector). (**B**) *Lmna*$^{-/-}$ MEFs retrovirally express lamin A at a level that are lower than the endogenous lamin A or C in *Lmna*$^{+/+}$ MEFs (designated as +/+). -/-, *Lmna*$^{-/-}$ MEFs; Repl., replicate. The tubulin antibody was purchased from abcam (ab52866).

Fig. S7A,B.



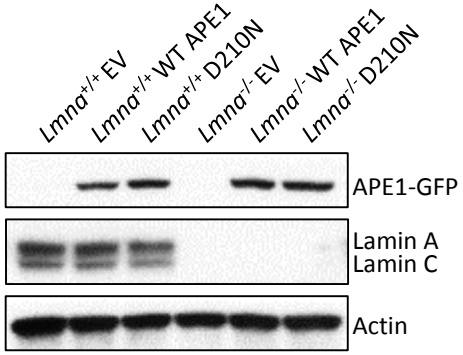
Supplementary Fig. S8. Average protein levels of PARP1, LIG3, and POLβ in *Lmna*$^{+/+}$ and *Lmna*$^{-/-}$ MEFs, at the indicated DNA repair time points, from three separate experiments ± SD, as determined from western blotting (depicted as protein levels relative to untreated *Lmna*$^{+/+}$ cells). This verifies the alterations seen in these BER-associated proteins in Figure 3D. *P* value was determined by Student's t-test; ***P* <0.005; *P < 0.05.

Fig. S8.

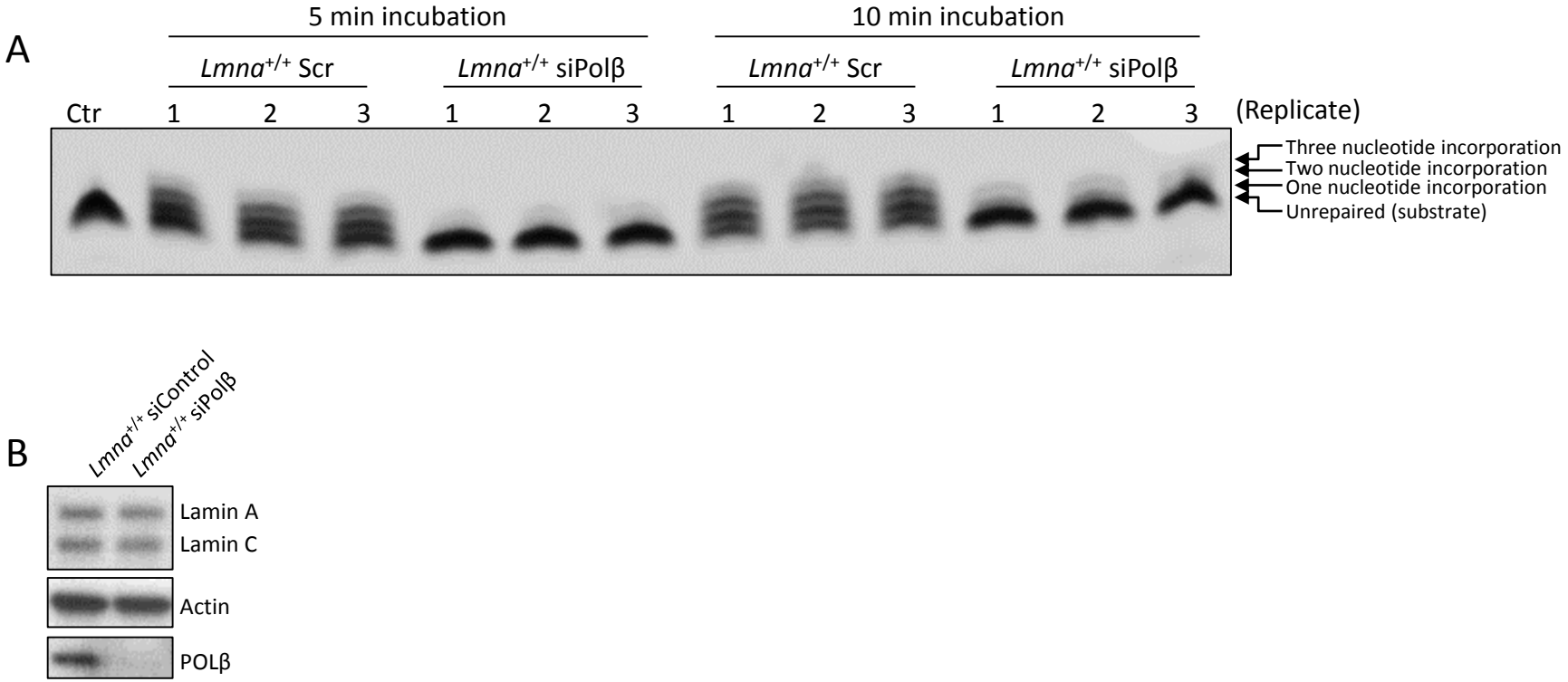


Supplementary Fig. S9. Western blotting to verify that NAD^+ supplementation enhances PAR formation in *Lmna*$^{-/-}$ MEFs. PAR formation was induced by addition of NAD^+ (4 mM; 2 h) into the medium (since NAD^+ is a substrate for PARylation), and this induction was attenuated by inclusion of either of two different PARP inhibitors, namely 3-aminobenzamide (3-AB; 10 mM) (**A**) or olaparib (2 μM) (**B**), together with the NAD^+, for 2 h. We reduced the concentration of NAD to 2mM (2h) for the relevant experiments in the manuscript (so that the PARP inhibitor would have more effect at reducing PAR when NAD^+ is present in the medium).

Fig. S9A,B.

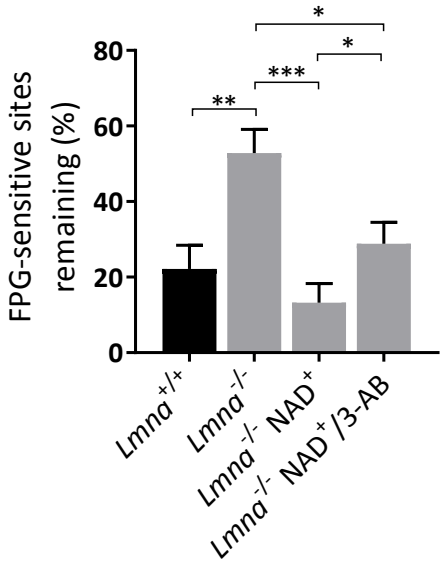


Supplementary Fig. S10. Western blots showing expression of GFP tagged wild-type (WT) APE1 and DNA incision mutant APE1 (D2010) in *Lmna*$^{+/+}$ and *Lmna*$^{-/-}$ MEFs. EV, empty vector. The GFP antibody was purchased from Santa Cruz (sc-9996).



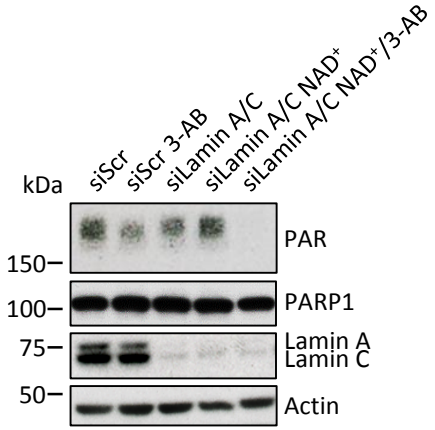
Supplementary Fig. S11. (**A**) Gel image showing POLβ nucleotide incorporation assay on extracts of MEFs with siRNA of Polβ. *Lmna*$^{+/+}$ MEFs were transfected with scrambled (Scr) siRNA or with siRNA against Polβ (siPolβ). Ctr, Extracts included but with zero incubation time. (**B**) Western blotting showing that effectiveness of the siPolβ to knock down the Polβ protein. The target sequences for mouse siPolβ (Dharmacon, SMARTpool, L-042850-01-0005) are 5′-AAUAAGAACAUGAGAGCGC-3′, 5′-CCACACAGGAGAAUCGAUA-3′, 5′-GAACCAUCAUCAACGAAUU-3′, and, 5′-CAACUGGAAAAUUGCGUAA-3′.

Fig. S11A,B.

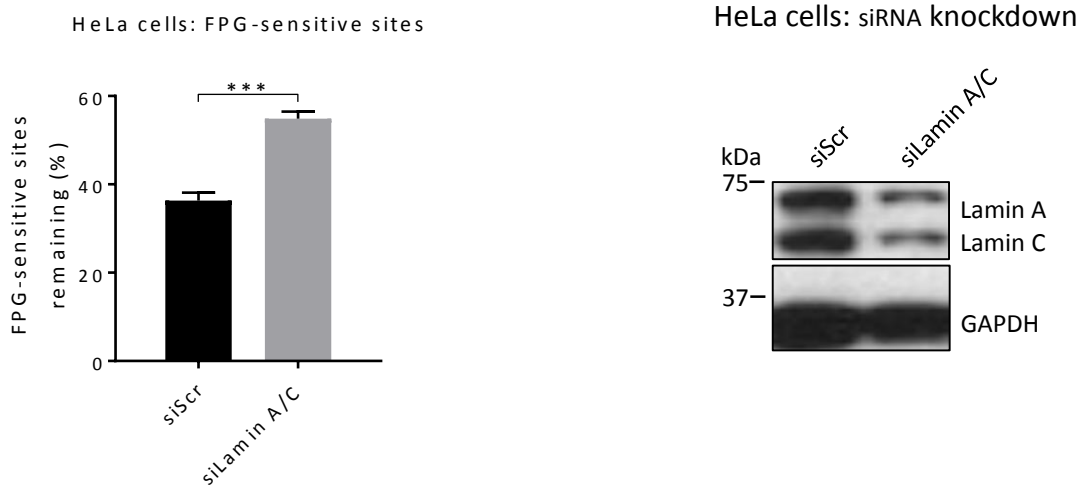


Supplementary Fig. S12. FPG comet assay showing that NAD^+ supplementation in the cell medium (2mM, 2 h) corrects the BER defect in *Lmna*$^{-/-}$ MEFs, which is partially reversed by including PARP inhibitor 3-AB (10mM, 2h) with the NAD^+. n = 100 comet tails, mean ± SD. This data supports the comet assay of Figure 5C which was done in same manner but using a different PARP inhibitor (olaparib). *P* value was determined by Student's t-test; ****P* < 0.0001; **P < 0.005; *P < 0.05.

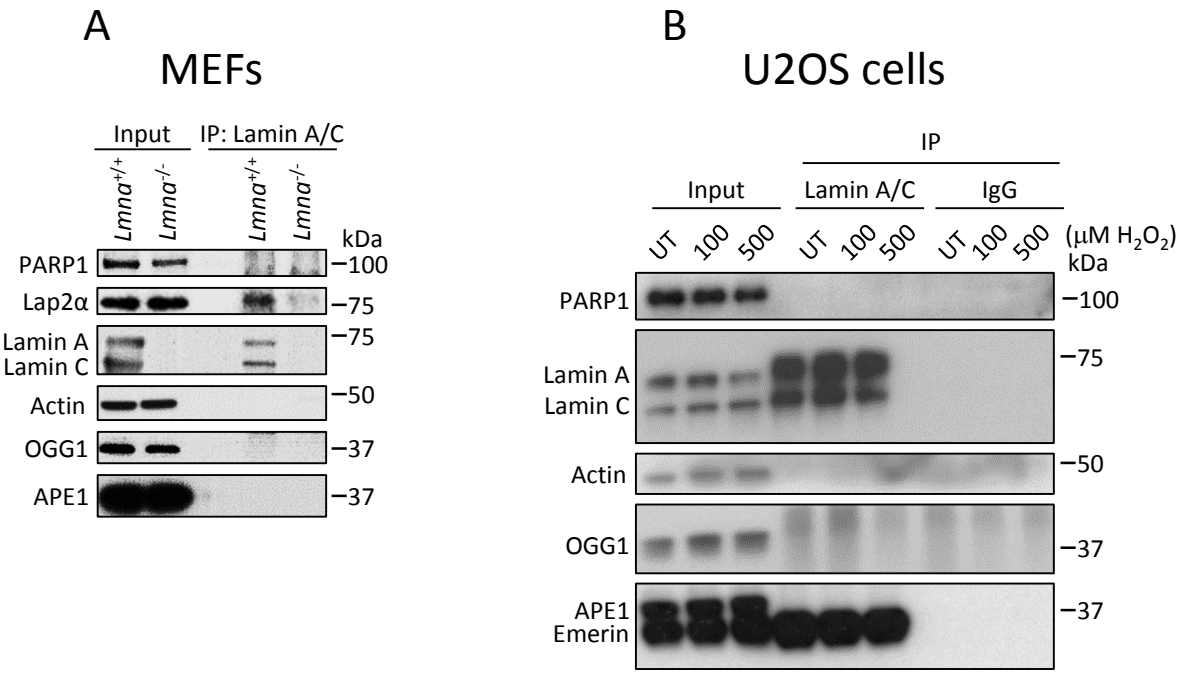
Fig. S12.



Supplementary Fig. S13. Western blotting on lysates prepared 1 h into the repair period of the comet assay shown in Figure 7C. This verifies that the NAD^+ supplementation (2 mM) enhances PAR formation, and the 3-aminobenzamide (3-AB; 10 mM) suppresses PAR formation, in the U2OS cells, as indicated.



Supplementary Fig. S14. HeLa cells, with siRNA knockdown of lamin A/C, display impaired BER, mirroring the data seen in MEFs and U2OS cells. FPG-comet assay on HeLa cells transfected with scrambled siRNA (siScr) or siRNA against lamin A/C (siLamin A/C). The HeLa cells were treated with oxidative stress (100 µM H_2O_2 for 30 min) and then allowed to repair in normal medium (DMEM with 10% FBS) for 8 h. DNA repair efficiency was expressed as percent of FPG-sensitive sites (estimate of 8-oxoG DNA lesions; see Methods) remaining, after 8 h of repair, relative to 5 min repair, after correction for the FPG-sensitive sites in untreated cells. n = 100 comet tails, mean ± SEM. The western blot shows the effective siRNA knockdown of lamin A/C in HeLa cells at 72 h post-transfection. The siRNA knockdown was performed as described for U2OS cells in the Methods section.



Supplementary Fig. S15 Co-immunoprecipitation using lamin A/C antibody (rabbit, sc-20681; Santa Cruz). PARP1, OGG1, and APE1 are not co-immunprecipitated with lamin A/C in either MEFs (**A**) or U2OS cells (**B**). (**A**) The LAP2α was used as a positive control, and *Lmna*$^{-/-}$ MEFs as a negative control. (**B**) Emerin was used as a positive control, and normal rabbit IgG as a negative control. The U2OS cells were either untreated (UT) or treated with either 100 μM or 500 μM H_2O_2 for 30 min.

Fig. S15.