

Supplementary Figure S1. Lamin B1 levels are specifically increased in A-T primary fibroblast cells.

An increase of lamin B1 in two different A-T cell populations compared to two different wild-type primary fibroblasts strains was analyzed with two different lamin B1 antibodies. Neither lamin B2 nor lamin A/C was overexpressed in A-T cells compared to wild-type cells.

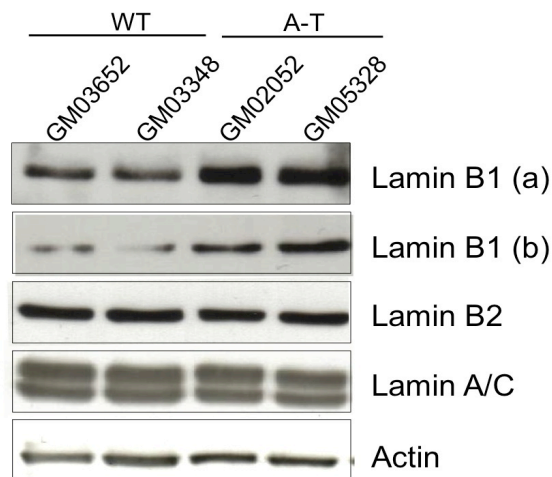


Figure S1: Western blot analysis of extracts from wild-type (GM03652 and GM03348) and A-T (GM02052 and GM05328) primary fibroblasts. An equal amount (30 μ g) of protein was loaded per sample. An increase of lamin B1 level was detected in A-T compared to the WT cells using two different antibodies. The first lane corresponds to monoclonal antibody ab20396 (a) staining, and the second lane was obtained using polyclonal antibody ab16048 (b) (Abcam Inc.). No change in lamin B2 and lamin A/C was detected (Abcam Inc., and Cell Signaling Technology, respectively).

Supplementary Figure S2.

A. Quantification of lamin B1 measured by immunofluorescence intensity

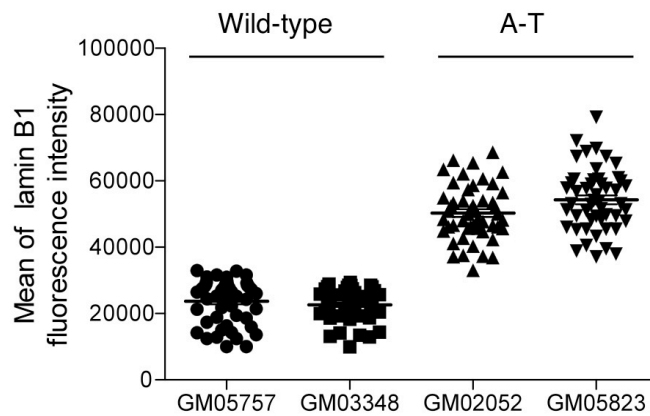


Figure S2A: The values on the graph correspond to the means of lamin B1 fluorescence intensity per nuclei quantified with the ImageJ software on at least 50 nuclei of wild-type (GM05757; GM03348) and A-T (GM02052; GM05823) human primary fibroblasts. We found a 3-fold increase of lamin B1 intensity in A-T cells compared to WT cells and confirm that lamin B1 levels increase using the quantified immunoblotting approach.

B. Negative effect of lamin B1 intensity on the nuclear shape

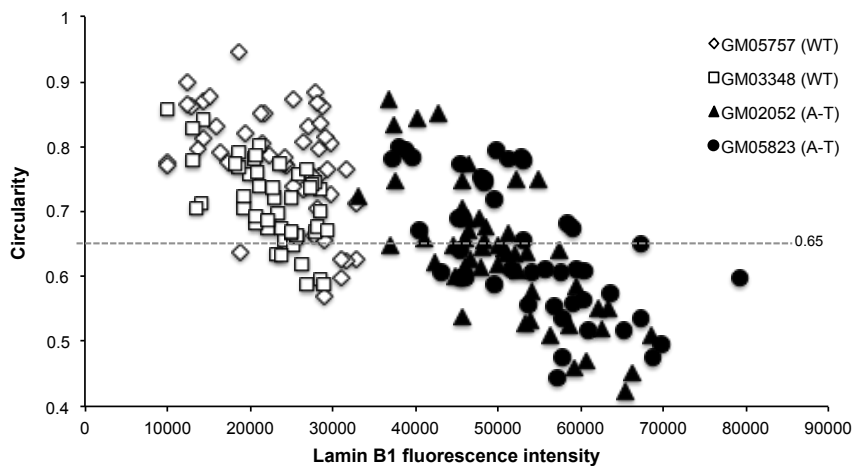


Figure S2B: The values on the graph correspond to the lamin B1 fluorescence intensity per nuclei on X axis and values of circularity of nuclei on Y axis, quantified by cell profiler software on at least 50 nuclei of wild-type (GM05757; GM03348) and A-T (GM02052; GM05823) human primary fibroblasts. We found that more the intensity of lamin B1 increases more the value of circularity decreases.

Supplementary Figure S3. Effect of the overexpression of lamin B1 on the DNA damage response.

Overexpression of lamin B1 did not lead to the phosphorylation of Chk2, Chk1 or H2AX or to γ -H2AX foci assembly in unchallenged cells. These data show that lamin B1 does not activate DDR.

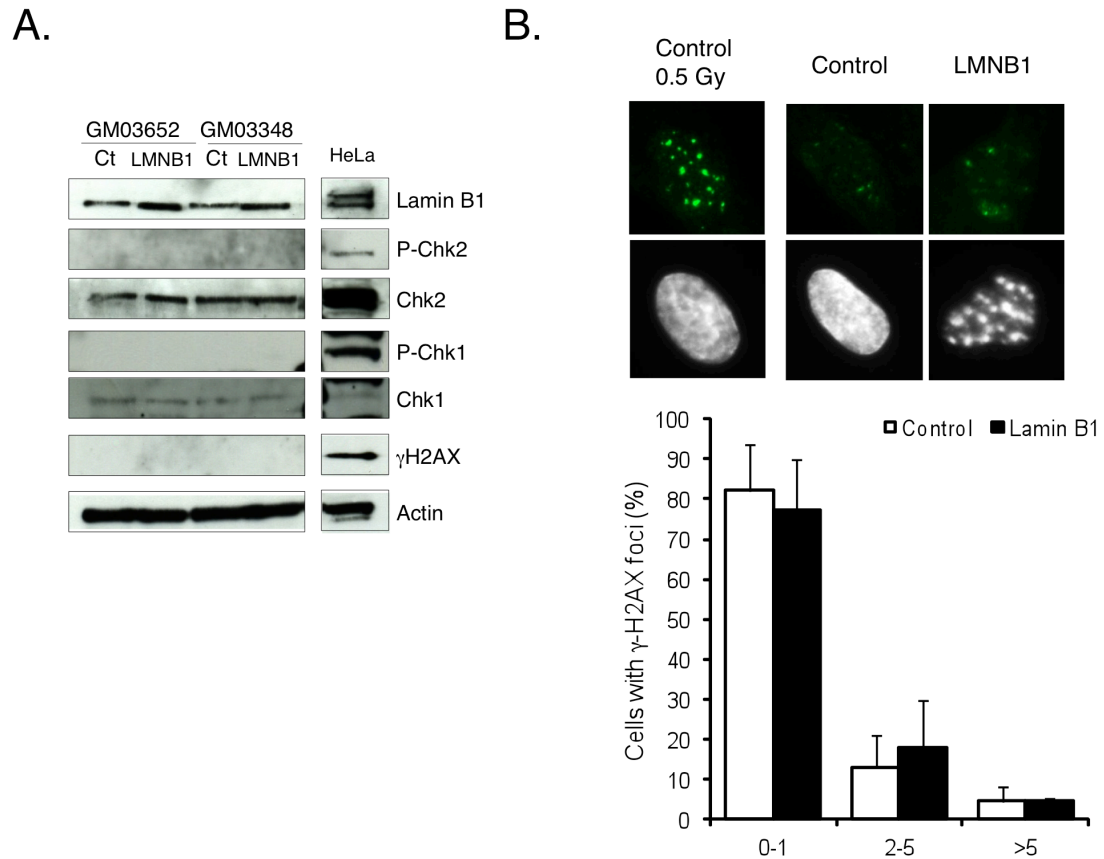


Figure S3 (A) Wild-type primary fibroblasts (GM03348 and GM03652) were transfected with an empty expression vector (Ct) or a vector containing lamin B1 cDNA (LMNB1). 48 h after transfection, protein levels were measured by immunoblotting with the respective antibodies. No activation of CHK2, CHK1 or γ -H2AX (measured using antibodies raised against their respective phosphorylated forms) was observed upon over-expression of lamin B1. **(B)** 48 h following transfection, quantification of γ -H2AX foci stained by immunofluorescence was performed as described in the Materials and Methods on at least 50 nuclei. Cells with 0-1, 2-5 and > 5 foci were analyzed. The values correspond to the means from three independent experiments. The error bars denote the SEM.

Supplementary Figure S4. Effect of antioxidant (NAC) treatment on lamin B1 and lamin A/C protein levels.

Treatment with NAC decreased lamin B1 levels in both wild-type (Figure S4A) and A-T cells (Figure S4B), showing that the level of lamin B1 protein depends on the level of endogenous ROS in wild-type and A-T cells. In contrast, the level of lamin A/C was not affected by NAC treatment, consistent with the fact that it is not increased in A-T cells (See Supplementary Figure S1), and shows specificity for lamin B1.

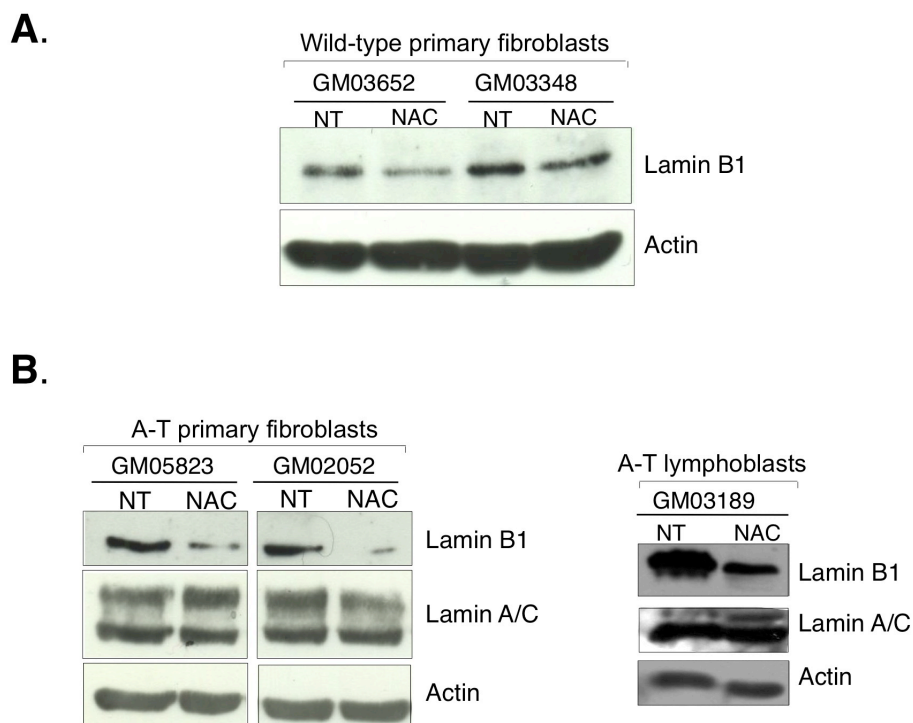
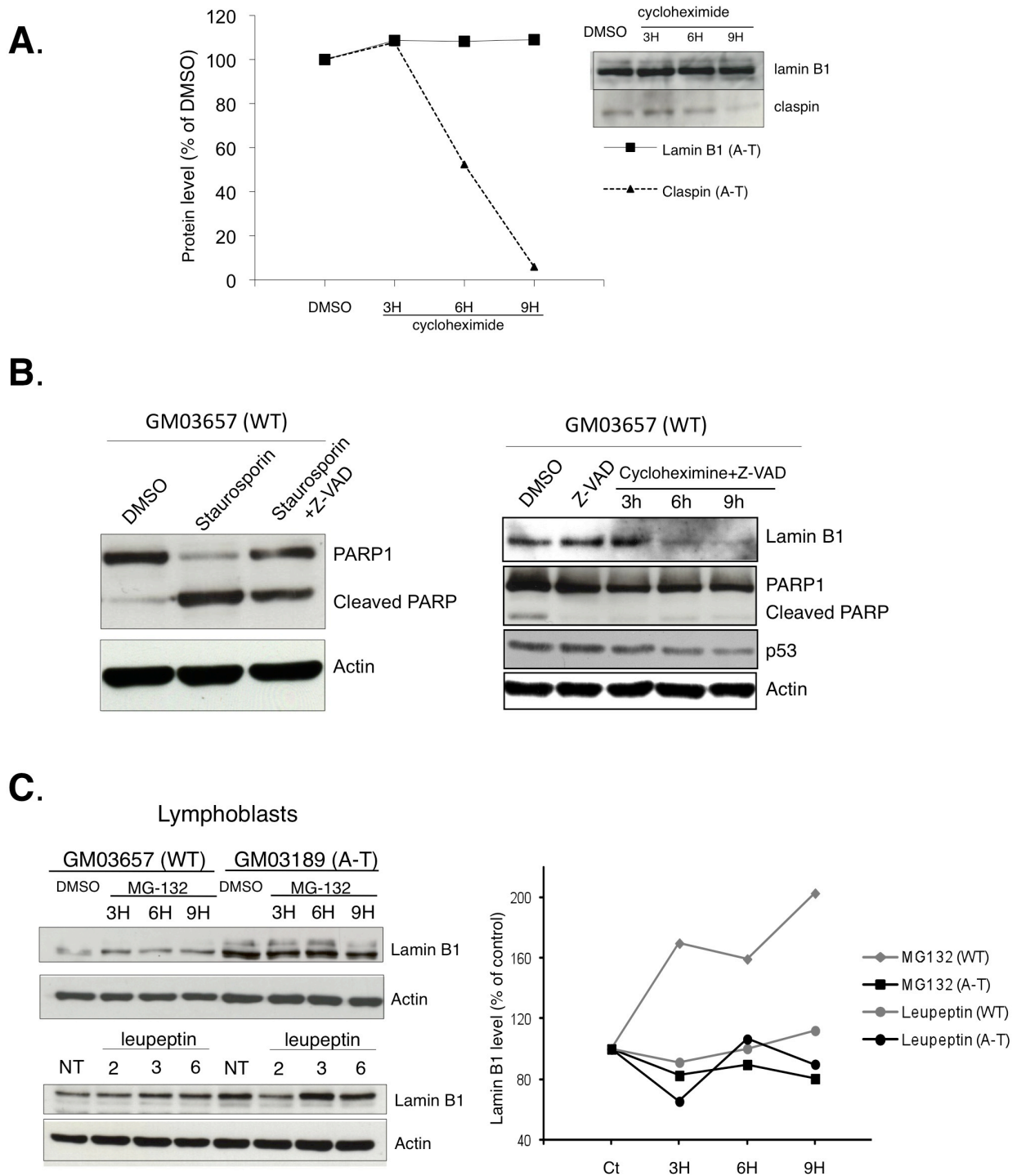


Figure S4. Wild-type (GM03348 and GM03652) and A-T (GM05823 and GM02052) primary fibroblasts and A-T lymphoblasts (GM03189) were treated for 72 h with 2 mM NAC. **(A)** Impact of NAC treatment on lamin B1 in WT primary fibroblasts. **(B)** Impact of NAC treatment on lamin B1 and lamin A/C protein levels in A-T primary fibroblasts (left panel) and A-T lymphoblasts (right panel).

Supplementary Figure S5. Stability of lamin B1 in A-T cells.



(A) Quantification of lamin B1 degradation in A-T versus wild-type cells (see Figure 6B). Degradation of claspain (see the insert) is a control showing that the protein degradation system was active in A-T cells treated with cycloheximide. The downregulation of claspain protein levels confirms the efficiency of cycloheximide treatment and the stabilization of lamin B1 in A-T cells.

(B) The degradation of lamin B1 upon cycloheximide treatment did not result from apoptosis.

Left panel: test of the efficiency of Z-VAD (an apoptosis inhibitor) treatment. Wild-type lymphoblasts (GM03657) were treated with 1 μ M staurosporine (a proapoptotic agent) for 1 hour alone or after a pretreatment with 20 μ M Z-VAD. The impact of apoptosis inhibition was verified on PARP protein cleavage by immunoblotting with a specific antibody. Under these conditions, Z-VAD treatment was efficient and downregulates the cleavage of PARP induced by staurosporine. Right panels: wild-type cells pretreated with Z-VAD 1 hour before the addition of 50 μ g/ml cycloheximide. Despite the Z-VAD treatment, the lamin B1 level still decreases in the presence of cycloheximide. **(C)** The proteasome is at least partly responsible for lamin B1 degradation in wild-type cells. Lamin B1 stability was assessed using MG132 or leupeptin treatment (Sigma-Aldrich, St. Louis, MO, USA). After 3, 6, or 9 h of treatment of wild-type cells with MG132 (10 μ M) or leupeptin (100 μ M), lamin B1 levels were then analyzed by western blot (left panels). The levels of lamin B1 (relative to actin) expressed as the percentage of control conditions in wild-type and A-T cells are represented by the curves on the right. Quantification of lamin B1 bands was performed using the ImageJ software.

Supplementary Figure S6. Activation of p38 MAPK

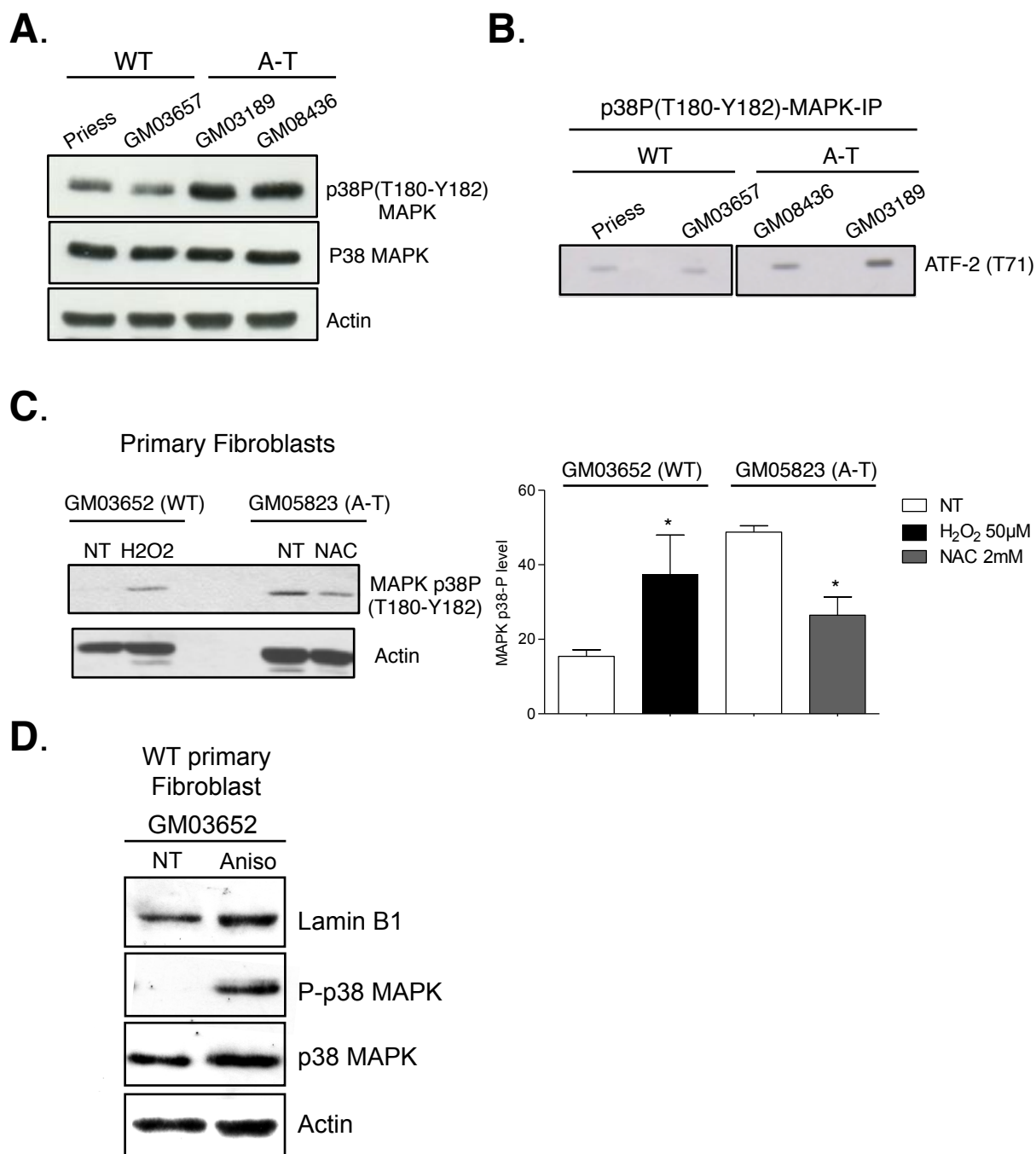


Figure S6. (A) Up-regulation of active p38 MAPK in A-T lymphoblasts. Western blot analysis of wild-type (WT: Priess and GM03657) and A-T (GM03189 and GM08436) protein extracts. Active p38 MAPK and total p38 MAPK were detected using a specific phospho(T180-Y182)-p38 MAPK (p38P MAPK) and p38 MAPK antibodies respectively. **(B)** Phosphorylation activity of p38 is increased in A-T cell extracts. P-p38 was immunoprecipitated with a specific anti-phospho(T180-Y182)p38 MAPK antibody in wild-type or A-T cell extracts. The phosphorylation activities immunoprecipitated from equal amounts of wild-type (WT: Priess and GM03657) and A-T (GM03189 and GM08436) extracts were assayed for ATF2, the specific substrate of p38 MAPK, and monitored using an anti-phospho(T71)-ATF2-specific antibody (see Materials and Methods, S6B). These two different approaches show that p38 MAPK is activated in A-T cells compared to WT cells. **(C)** Effect of oxidative stress on p38 MAPK activation. Left panels: 30 μg of protein extracts of wild-type (WT: GM03657) and A-T (GM05823) primary fibroblasts treated for 48 h with

50 μM H_2O_2 and 2 mM NAC, respectively, were loaded. Active p38 MAPK was detected using a specific phospho(T180-Y182)-p38 MAPK antibody. Right panel: quantification of phospho(T180-Y182)-p38 MAPK (relative to actin) after treatment of wild-type cells with 50 μM H_2O_2 and A-T cells with 2 mM NAC. All quantification values correspond to at least three independent experiments. The error bars denote the SEM. **(D)** Modifications of lamin B1 levels following p38 MAPK activation by anisomycin treatment in WT primary fibroblasts. Following 8 h of anisomycin treatment (10 $\mu\text{g}/\text{ml}$), immunoblotting of lamin B1 and phospho(T180-Y182)-p38 MAPK showed an increase in lamin B1 and P-p38 MAPK protein levels.

Material and methods S6B. Nonradioactive p38 MAPK activity assay

p38 MAPK activity was analyzed in lymphoblast extracts using a p38 MAPK assay kit (Cell Signaling Technology, Ozyme, France). First, to immunoprecipitate phospho(T180-Y182)-p38 MAPK, equal amounts of cell lysate in RIPA buffer were incubated with the immobilized phospho(T180-Y182)-p38 MAPK mAb primary antibody at 4°C overnight under rotary agitation. After centrifugation at 4°C, pellets were washed in kinase buffer and incubated for 30 minutes at 30°C in kinase buffer with 1 μl of ATF2 fusion protein and 200 μM ATP. The addition of SDS sample buffer terminated the reaction. After denaturation at 95°C for 3 minutes, an equal amount of each sample was separated on 10% SDS-PAGE. p38 MAPK activity was evaluated by the detection of phospho-ATF2 (T71) using a phospho-ATF2 (T71)-specific antibody.

Supplementary Figure S7. Inhibition of p38 MAPK prevents senescence and the increase in lamin B1 levels induced by H₂O₂ in WT primary fibroblasts.

Oxidative stress induced by H₂O₂ increases the percentage of senescent cells (β -Gal-positive cells) from 16.3 to 65.5 % (left panels), and the lamin B1 levels were 3 times as high compared to the DMSO condition (control condition) (Figure S7A). To confirm the efficiency of H₂O₂ and SB203580 treatments, the level of p-(S82)Hsp27, a p38 MAPK substrate, was detected. Inhibition of p38 MAPK with SB203580 rescues both senescence and lamin B1 levels induced by H₂O₂ in wild-type cells (Figure S7B).

Taken together, these data show that inhibition of p38 MAPK is sufficient to prevent both the senescence and increase in lamin B1 levels induced by H₂O₂.

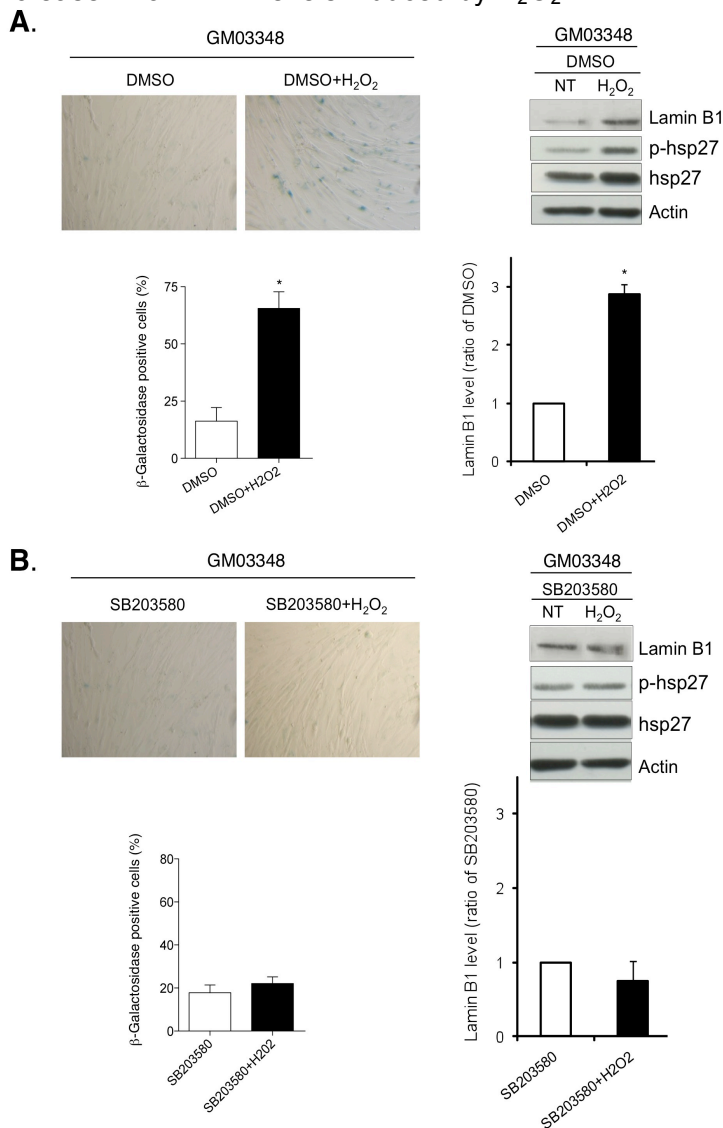


Figure S7. (A) Treatment of wild-type primary fibroblasts with H₂O₂-induced senescence (left panel) accompanied by high levels of lamin B1 protein (right panels). Senescence and lamin B1 protein levels were measured in WT primary fibroblasts (GM03348) following 3 days of treatment with 50 μ M H₂O₂. Histograms in lower panels correspond to the quantification. The values correspond to the means from three independent experiments. * represents a significant difference ($P < 0.006$, paired t-test). The error bars denote the SEM. **(B)** Inhibition of p38 MAPK rescues both senescence and lamin B1 protein levels. Cells were pretreated 24 h with 10 μ M SB203580 and then co-treated 72 h with 50 μ M H₂O₂ and 10 μ M SB203580. The values correspond to the means from three independent experiments. * represents a significant difference ($P < 0.006$, paired t-test). The error bars denote the SEM.

Supplementary Figure S8. Nuclear morphology alteration and stimulation of the lamin B1 protein level during stress-induced senescence (A) and oncogene-induced senescence (B).

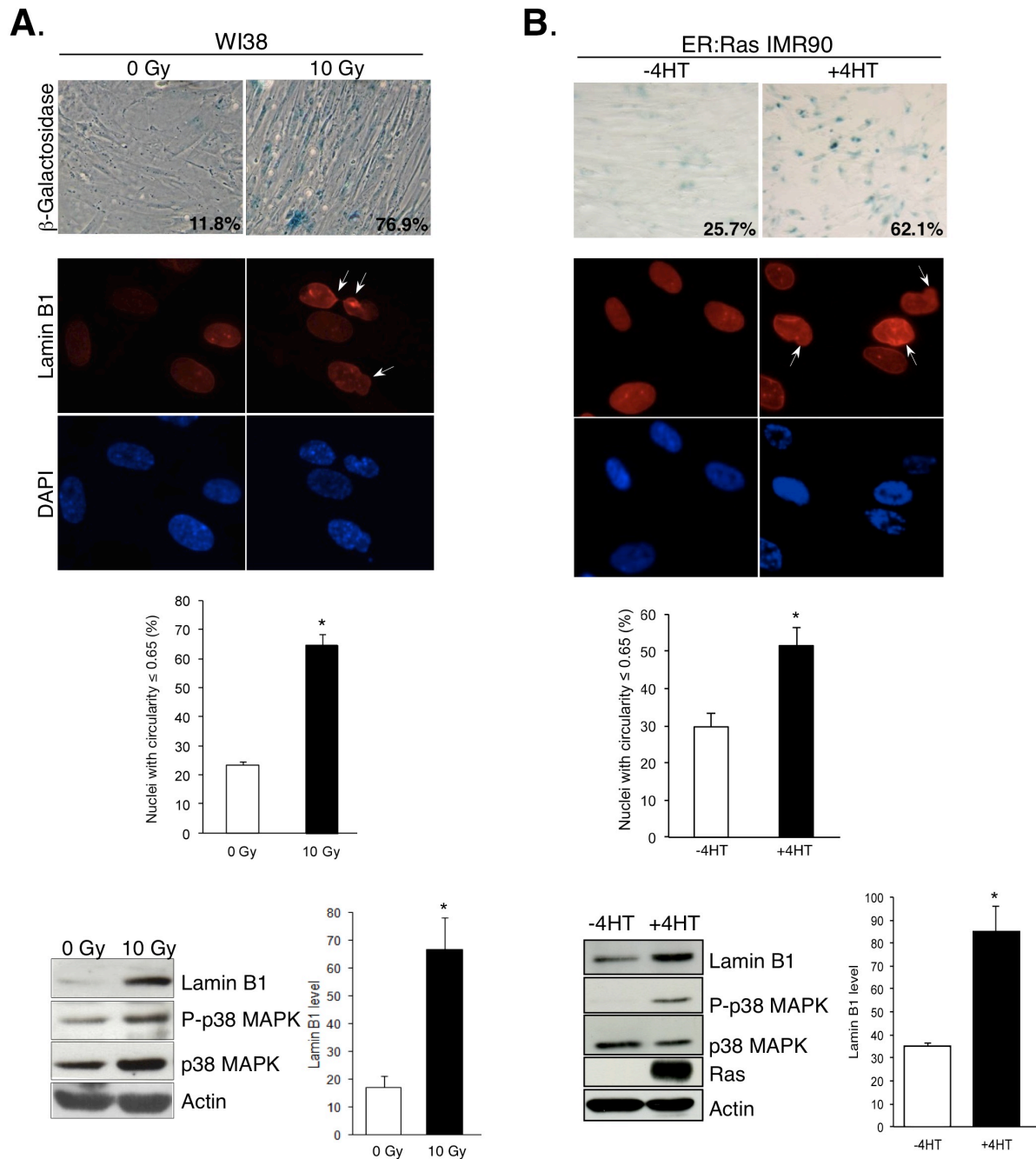


Figure S8. (A) Stress-induced senescence. Senescence, nuclear shape and protein expression were analyzed in primary fibroblasts WI38 10 days after irradiation with 10 Gy. Upper panel: SA- β -galactosidase staining. Middle panels: nuclear shapes of WI38 in the control condition and following 10 Gy were examined by immunofluorescence with the anti-lamin B1 antibody (red) and DAPI (blue). White arrows show examples of misshapen nuclei. The values on the histogram correspond to nuclei with circularity ≤ 0.65 from three independent experiments. Nuclear shape analysis was performed on at least 100 nuclei per condition. Lower panels: western blot analysis was performed on control and irradiated cells, and lamin B1 levels were quantified relative to actin. **(B) Oncogene-induced senescence.** Senescence, nuclear shape and protein expression were analyzed on ER:Ras IMR90 primary fibroblasts expressing the oncogene Ras following 7 days of treatment with 100 nM 4-hydroxytamoxifen (4HT). Upper panels: SA- β -galactosidase staining.

Middle panels: nuclear shapes of ER:Ras IMR90 cells treated with ethanol or 4HT were examined by immunofluorescence with the anti-lamin B1 antibody (red) and DAPI (blue). The values on the histograms correspond to nuclei with circularity ≤ 0.65 from three independent experiments. Nuclear shape analysis was performed on at least 100 cells per condition. Lower panels: western blot analysis on protein extracts was performed on ER:Ras IMR90 cells treated with ethanol or 4HT, and lamin B1 levels were quantified relative to actin. * represents a statistically significant difference ($P < 0.05$). The error bars denote the SEM.

(A) Stress-induced senescence. Ten days after irradiation (10 Gy), immunofluorescence was performed on extracts from WI38 cells, as already extensively characterized (Freund et al, 2011, EMBO J, 20:1536-48). SA- β -galactosidase staining (Figure S8A, upper panel) shows that irradiation (10 Gy) increases senescence (11.8 % in the control to 76.9 % in irradiated cells). Importantly, fluorescence analysis revealed an increase in misshapen nuclei (Figure S8A, middle panels) and in the level of lamin B1 protein (Figure S8A, lower panels) following irradiation.

(B) Oncogene-induced senescence. We used an extensively characterized experimental system kindly provided by Dr. Narita: inducible expression of the oncogene Ras (ER-expressing Ras V12) in IMR90 cells cultivated in physiological 3% O₂. Ras expression was induced by 100 nM 4-HT as described (Young et al., 2009, Genes Dev, 23, 798-803). Immunofluorescence of extracts was analyzed 7 days after Ras induction. 4HT treatment increases senescence (25.7 % in the control (-4HT or ethanol) and 62.1 % in cells treated with 4HT) (Figure S8B, upper panels). Importantly, fluorescence analysis revealed an increase in misshapen nuclei (Figure S8B, middle panels) and an increase in the level of lamin B1 protein (Figure S8B, lower panels) following Ras activation.

Supplementary Figure S9. Effect of lamin B1 overexpression on ROS levels in wild-type primary fibroblasts.

Wild-type (GM03652 and GM03348) primary fibroblasts were transfected with an empty expression vector (Control) or a vector containing the lamin B1 cDNA (LMNB1). Overexpression of lamin B1 decreases endogenous ROS levels in unchallenged WT primary fibroblasts (A) and p38 MAPK activation (B), consistent with the decrease in endogenous ROS. After treatment with H₂O₂, overexpression of lamin B1 decreased the level of ROS (C) and increased cell survival (D).

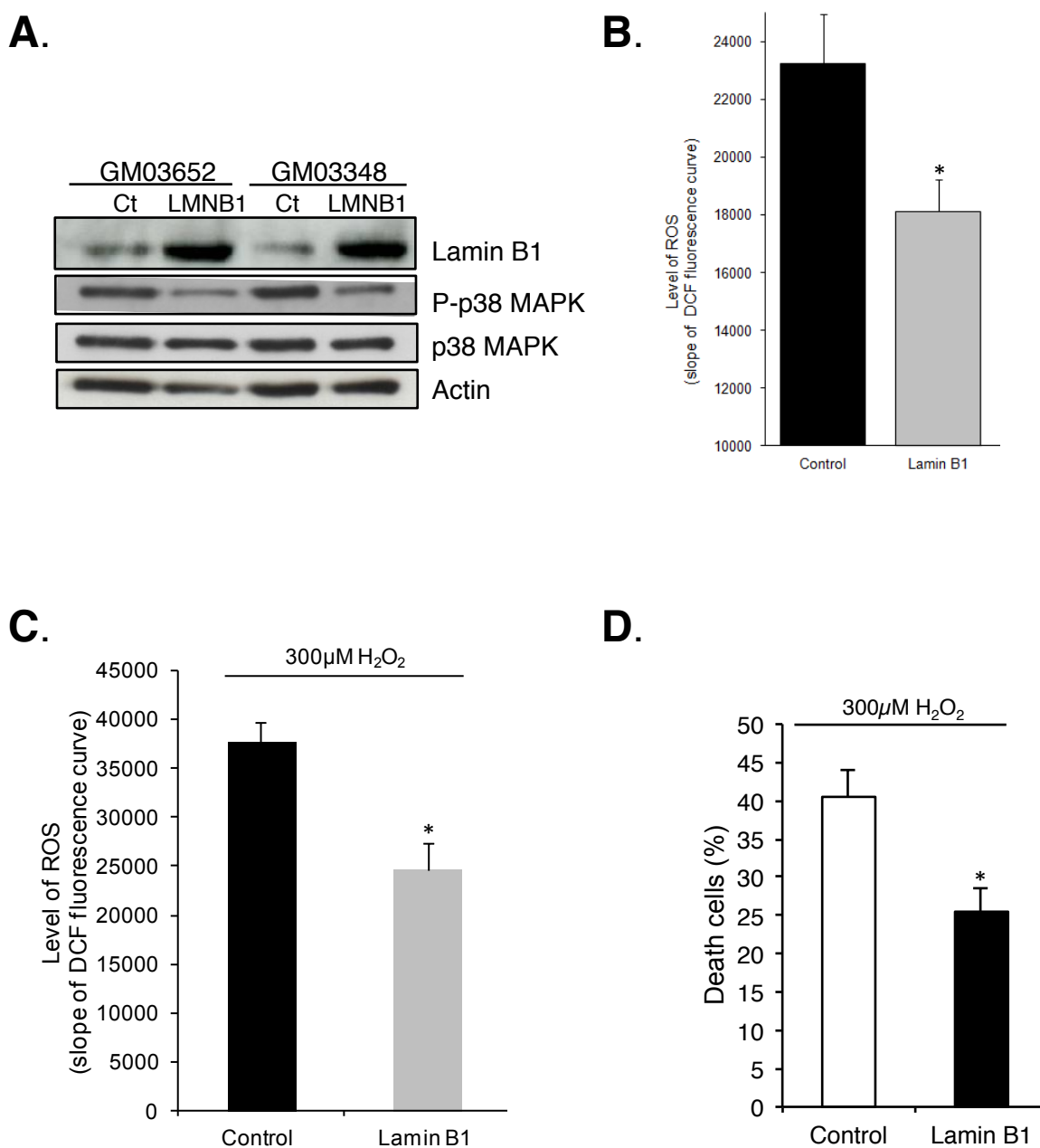


Figure S9. (A) Primary wild-type fibroblasts (GM03348) were transfected with control or lamin B1 plasmid and plated in 24-well plates. 24 h after transfection, cells were treated for 1 h at 37°C with H₂DCFDA. For 1 hour, ROS fluorescence levels were measured as the DCF fluorescence with a

multiwell fluorescence plate reader system, in primary fibroblasts overexpressing the control or lamin B1 plasmid. **(B)** 48 h after transfection, proteins were extracted to evaluate the impact of lamin B1 overexpression on p38 MAPK activation evaluated by immunoblotting using a P-p38 MAPK-specific antibody, **(C)** Treatment with H₂O₂: the same experimental conditions as in **(A)** were used except that preceding the measure of the fluorescence, the cells were treated with 300 μ M H₂O₂. For each condition in **(A)** and **(C)**, (left right panel), we determined the slope of the fluorescence curve corresponding to the velocity-time of the fluorescence signal. **(D)** Impact of 300 μ M H₂O₂ on the death of cells transfected with control or lamin B1 plasmids was evaluated using trypan blue staining. Histograms represent the means of three independent experiments. The error bars denote the SEM.

Material and Methods S9. Detection of intracellular reactive oxygen species (ROS)

To assess the generation of intracellular reactive oxygen species, we used the peroxide-sensitive fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (H2DCFDA; Molecular Probes). After diffusion into the cells, the nonfluorescent probe is converted to 2',7'-dichlorodihydrofluorescein (DCF), which is oxidized by intracellular ROS to the highly fluorescent DCF. Briefly, wild-type primary fibroblasts were first transfected with control or lamin B1 plasmid by the amaxa system (see the Materials and Methods) and plated at a density of 1×10^5 per well in 24-well plates in complete medium culture. 24 h later, cells were washed twice with PBS without CaCl₂ and MgCl₂ to remove residual serum of the medium and then incubated with 10 μ M CM-H2DCFDA for 1 hour at 37°C in serum-free medium without phenol red in the dark. Before the measure of relative fluorescence units (excitation/emission 485/535 nm) in a multiwell fluorescence plate reader (Wallac 1420 Victor2, Perkin-Elmer), the cells were treated with 300 μ M H₂O₂ (Figure S9C). The fluorescence was measured at 30 time points over 1 hour. For each condition, we determined the slope of the fluorescence curve corresponding to the velocity-time of the fluorescence