LETTER

Autophagy mediates degradation of nuclear lamina

Zhixun Dou1, Caiyue Xu1, Greg Donahue1, Takeshi Shimii2, Ji-An Pan1, Jiajun Zhu1, Andrejs Ivanov4,5, Brian C. Capelli1, Adam M. Drake1, Parisha P. Shah1, Joseph M. Catanzaro3, M. Daniel Ricketts6, Stephen A. Adam2, Ronen Marmorstein5,7,8, Wei-Xing Zong3, Terje Johansen6, Robert D. Goldman2, Peter D. Adams4 & Shelley L. Berger1

Macroautophagy (hereafter referred to as autophagy) is a catabolic membrane trafficking process that degrades a variety of cellular constituents and is associated with human diseases1–3. Although extensive studies have focused on autophagic turnover of cytoplasmic materials, little is known about the role of autophagy in degrading nuclear components. Here we report that the autophagy machinery mediates degradation of nuclear lamina components in mammals. The autophagy protein LC3/Atg8, which is involved in autophagy membrane trafficking and substrate delivery4–6, is present in the nucleus and directly interacts with the nuclear lamina protein lamin B1, and binds to lamin-associated domains on chromatin. This LC3–lamin B1 interaction does not downregulate lamin B1 during starvation, but mediates its degradation upon oncogenic insults, such as by activated RAS. Lamin B1 degradation is achieved by nucleus-to-cytoplasm transport that delivers lamin B1 to the lysosome. Inhibiting the LC3–lamin B1 interaction prevents activated RAS-induced lamin B1 loss and attenuates oncogene-induced senescence in primary human cells. Our study suggests that this new function of autophagy acts as a guarding mechanism protecting cells from tumorigenesis.

Several mammalian autophagy proteins are present in the nucleus, including LC3 (refs 7, 8), Atg5 (ref. 9), and Atg7 (ref. 10). However, whether nuclear LC3 is involved in degrading nuclear components is not understood. We investigated LC3 distribution by subcellular fractionation of primary human IMR90 cells and found a substantial amount of endogenous LC3 and a small amount of lipidated LC3–II in the nucleus (Fig. 1a). We used bacterially purified glutathione S-transferase (GST)–LC3B (hereafter ‘LC3’, unless specified otherwise) to pull down the nuclear fraction (Fig. 1b). One protein that we found to interact with LC3 is the nuclear lamina protein lamin B1 (Fig. 1b). The nuclear lamina is a fibrillar network located beneath the nuclear envelope whose major components are the four nuclear lamina isoforms: lamin B1, B2, and A/C, and their associated proteins11. Nuclear lamina provides the nucleus with mechanical strength and regulates higher-order chromatin organization, modulating gene expression and silencing11. In contrast to lamin B1, laminas A/C and lamin B2 bind poorly, if at all, to LC3 (Fig. 1b). We detected a direct interaction of purified lamin B1 (Extended Data Fig. 1a) with LC3B (Fig. 1c) and other members of the Atg8 protein family, including LC3A, LC3C, and GABARAP (Extended Data Fig. 1b, c). Co-immunoprecipitation (co-IP) revealed that LC3–lamin B1 interaction occurs at the endogenous level in the nucleus (Fig. 1d, e and Extended Data Fig. 1d). Lipidated LC3–II is involved in mediating lamin B1 interaction (Fig. 1d and Extended Data Fig. 1e–g), and the LC3 G120A lipidation-deficient mutant showed impaired binding to lamin B1 (Fig. 1f). A bimolecular fluorescence complementation (BiFC) assay2 showed that LC3–lamin B1 interaction happens at the nuclear lamina and is dependent on LC3 lipification (Extended Data Fig. 1h–j). Together, these data suggest that LC3 directly interacts with lamin B1, and that LC3 lipidation facilitates this interaction, possibly by tethering LC3 to the inner nuclear membrane where the interaction with nuclear lamina occurs.

**Figure 1** | LC3 interacts with nuclear lamina protein lamin B1.

**a.** Proliferating young IMR90 cells were subjected to subcellular fractionation and immunoblotting. SE, short exposure; LE, long exposure.

**b.** The nuclear fraction of IMR90 cells was pulled down with bacterially purified GST or GST–LC3.

**c.** HEK293T cells were transfected with plasmids expressing GFP, GFP–LC3, GFP–Atg5, or GFP–GABARAP, and subjected to GFP immunoprecipitation and immunoblotting. Bars, mean ± s.e.m.; n = 3; *P < 0.001. **P < 0.0001; one-way analysis of variance (ANOVA) coupled with Tukey’s post hoc test (b); unpaired two-tailed Student’s t-test (f). Uncropped blots are in Supplementary Figure 1.

1Epigenetics Program, Department of Cell and Developmental Biology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA. 2Department of Cell and Molecular Biology, Feinberg School of Medicine, Northwestern University, Chicago, Illinois 60611, USA. 3Department of Molecular Genetics and Microbiology, Stony Brook University, Stony Brook, New York 11794, USA. 4Institute of Cancer Sciences, University of Glasgow and Beatson Institute for Cancer Research, Glasgow G61 1BD, UK. 5Molecular Cancer Research Group, Institute of Medical Biology, University of Tromsø – The Arctic University of Norway, 9037 Tromsø, Norway. 6Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA. 7Abramson Family Cancer Research Institute, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA. 8Present address: Centre for Haematology-Oncology, Barts Cancer Institute, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK.

© 2015 Macmillan Publishers Limited. All rights reserved
Lamin B1 associates with transcriptionally inactive heterochromatin domains called LADs (lamin-associated domains)\(^{11,13}\). We used chromatin immunoprecipitation (ChIP) to investigate the association of LC3 with LADs. ChIP of LC3 showed that in its lipiddated form, LC3 associates with LADs but poorly with euchromatin regions, such as β-actin and PCNA promoters, similarly to that of lamin B1 (Fig. 2a, b and Extended Data Fig. 2a–c). We then performed endogenous lamin B1 and LC3 ChIP followed by genome-wide sequencing (ChIP-seq), done in two independent biological replicates, R1 and R2 (Fig. 2c for whole chromosome 3 and a zoom-in window in Extended Data Fig. 2d). We used enriched domain detector (EDD), an algorithm that detects wide enrichment domains\(^{14}\) to define LADs and LC3-associated domains (LC3ADs) (Fig. 2c and Extended Data Fig. 2d, black rectangles beneath the tracks). Analyses of lamin B1 and LC3 ChIP-seq revealed high reproducibility between R1 and R2 over LADs and LC3ADs (Fig. 2d, top two panels, and Extended Data Fig. 2e, f); LADs defined here correlate well with previously identified LADs from lamin B1 ChIP-seq\(^{15,16}\) and DamID\(^{13}\) (Extended Data Fig. 2g). We further found that LADs and LC3ADs significantly overlap (Fig. 2d, bottom panel; permutation test \(P < 0.001, 1,000\) iterations). Comparing LADs with an equal number of size-matched and randomly selected non-LAD control regions, we observed that both lamin B1 and LC3 are strongly enriched in LADs, for both replicates (Fig. 2e; permutation test for LC3: \(P < 0.01, 100\) iterations, for both replicates). A similar enrichment is also detected over LC3ADs (Extended Data Fig. 2h). As expected, Lys9 trimethylation on histone H3 (H3K4me3) is highly enriched in LADs (Fig. 2e, permutation test \(P < 0.01, 100\) iterations), whereas H3K4me5 is relatively depleted (Fig. 2e, permutation test \(P = 1, 100\) iterations). We also found that both lamin B1 and LC3 from our ChIP-seq are strongly enriched in LADs mapped by other published studies\(^{13,15}\) relative to non-LAD control regions (Extended Data Fig. 2i), in line with our findings from Fig. 2e. Collectively, these results indicate that LC3 associates with LADs on chromatin at the genome-wide scale.

Next, we examined the biological functions of this interaction, and found that neither starvation nor rapamycin treatment downregulates lamin B1 protein (Fig. 3a), suggesting that autophagy does not degrade lamin B1 during starvation. One scenario that involves lamin B1 loss is oncogenic insult, such as induced by oncogenic RAS\(^{17-19}\). In fact, most primary cells and tissues cope with oncogenic RAS activity by inducing cellular senescence, a stable cell-cycle arrest that serves as a potent tumour suppressive mechanism\(^{20,21}\). We and others have shown that lamin B1, but not lamin A/C or B2, is dramatically downregulated during oncogene-induced senescence\(^{17-19}\). Importantly, autophagy is
upregulated during oncogene-induced senescence, and is required for the mitosis-to-senesence transition\(^2,23\). We thus hypothesized that activated oncopgenes trigger autophagic degradation of lamin B1 in primary human cells.

Consistent with previous findings\(^17,18\), primary, but not immortalized, human cells show downregulation of lamin B1 but not other lamin isoforms (Fig. 3b and Extended Data Fig. 3a). Although starvation does not alter lamin B1 nuclear lamina localization, HRasV12 expression induces nuclear membrane blebbing and cytoplasmic lamin B1 signals (Extended Data Fig. 3b). Transmission electron microscopy (TEM) analysis of HRasV12-expressing cells confirmed the induction of autophagosomes, reduction of perinuclear heterochromatin, and induction of nuclear membrane blebs (Extended Data Fig. 3c–e). Unlike yeast piecemeal microautophagy, in which nuclear blebs directly contact cytoplasmic autophagic vacuoles\(^24\), the nuclear blebs in human senescent cells are morphologically distinct and do not directly contact these vacuoles (Extended Data Fig. 3c–e).

We further used an mCherry–GFP–lamin B1 construct to investigate the hypothesis that lamin B1 is degraded by the autophagy–lysosome pathway. Here, a yellow signal (due to merged mCherry and GFP) indicates that the fusion protein is in a neutral pH environment, whereas a red signal (due to quenching of GFP) indicates that the protein has entered acidic lysosomes\(^25,26\). mCherry–GFP–lamin B1 showed a merged yellow nuclear peripheral pattern in control cells, but displayed cytoplasmic red-only bodies in HRasV12-expressing cells (Extended Data Fig. 3f). Inhibiting lysosomal acidification by bafilomycin A1 prevents GFP quenching and results in merged yellow signals in the cytoplasm (Extended Data Fig. 3g). Furthermore, we co-stained with antibodies against LC3 and LAMP1, and found that the cytoplasmic mCherry-only lamin B1 bodies stain positively for endogenous LC3 and LAMP1 (Fig. 3c). Super-resolution microscopy analysis revealed that the cytoplasmic lamin B1 and LC3 co-localizes within the LAMP1-decorated vesicle (Fig. 3c and Extended Data Fig. 4a). Cytoplasmic lamin B1 and nuclear membrane blebs are specifically induced by HRasV12, but not by starvation or rapamycin treatment (Fig. 3d). In addition, we performed live-cell imaging on mCherry–GFP–lamin B1–expressing HRasV12 IMR90 cells, and confirmed a nucleus-to-lysosome transport process, through nuclear membrane blebbing, which then leads to lamin B1 degradation in the cytoplasm (Extended Data Fig. 4b).

Cytoplasmic lamin B1 in HRasV12 cells is reminiscent of the cytoplasmic chromatin fragments (CCF) that we previously described in senescent cells, which are fragments of heterochromatin budded off from the nucleus\(^19\). Consistent with the behaviour of lamin B1, we found cytoplasmic DAPI (4',6-diamidino-2-phenylindole) specifically appearing in response to HRasV12 (Fig. 3d). The cytoplasmic DAPI staining bodies are positive for H3K27me3 and H3K9me3, and co-localize with LC3 and lamin B1 (Extended Data Fig. 5a–c). Immuno-TEM analysis revealed that lamin B1 specifically localizes at the nuclear lamina in control cells (Extended Data Fig. 5d, left), whereas HRasV12-expressing cells showed decreased presence of lamin B1 at the nuclear lamina, and the appearance inside autophagosomes and autolysosomes (Fig. 3e and Extended Data Fig. 5d, right). Taken together, these data indicate that lamin B1 is an autophagy substrate upon oncogenic insult, which, through a nucleus-to-lysosome transport process, leads to its autophagic degradation in the cytoplasm.

We subsequently investigated the consequence of autophagy inhibition. Knockdown of Atg7 impairs the downregulation of lamin B1 protein in HRasV12 cells (Fig. 4a and Extended Data Fig. 6a). Lamin B1 messenger RNA (mRNA) has been shown to decrease upon HRasV12 expression\(^17,18\). Here the mRNA of lamin B1 is reduced both in control and in Atg7 knockdown cells (Extended Data Fig. 6b), whereas the protein level of lamin B1 is maintained in Atg7-deficient cells (Fig. 4a). These data suggest that lamin B1 is downregulated both at mRNA and at protein levels, and are consistent with the observation that nuclear lamins are among the most long-lived proteins in cells\(^27\). Besides RAS-induced senescence, we found that Atg7 inhibition also attenuates lamin B1 loss triggered by oxidative stress and DNA damage-induced senescence (Extended Data Fig. 6c–e). Further, mCherry–GFP–lamin B1 expressed in Atg7 knockdown HRasV12 cells displayed normal induction of nuclear membrane blebs but deficient cytoplasmic mCherry signals (Extended Data Fig. 6f, g). These data suggest that inhibition of autophagy leads to a profound defect in the nucleus-to-lysosome transport of lamin B1.

Lamin B1 plays an important role in cell proliferation and senescence\(^27\). Forced knockdown of lamin B1 causes premature senescence\(^16,17\), whereas overexpression of lamin B1 delays senescence\(^17\). Restoration of lamin B1 in already-established senescent cells is not sufficient to revert senescence in vitro (Extended Data Fig. 6h, i). Consistent with the compromised lamin B1 degradation, we found that Atg7 knockdown cells showed delayed HRasV12-induced senescence, as judged by reduced levels of p16 (Fig. 4a and Extended Data Fig. 6j) and delayed induction of senescence-associated β-galactosidase (β-gal) (Extended Data Fig. 6k).

We mapped the LC3–lamin B1 interaction and discovered that LC3 R10 and R11 are essential for lamin B1 binding, from in vitro pull-down, in vivo co-IP, BiFC, and ChIP experiments (Fig. 4b and Extended Data Fig. 7a–f). Moreover, while LC3-wild type (WT) showed co-localization with CCF, the LC3 mutant failed to do so (Extended Data Fig. 7g). On the lamin B1 end, the region between Coil 2 and the immunoglobulin (Ig)-fold of lamin B1 is necessary for LC3 binding (Fig. 4c and Extended Data Fig. 8a–c). Notably, this region...
(390–438) is the most evolutionarily conserved domain among all vertebrate lamin B1 (Extended Data Fig. 8d, e). The region, along with 20 amino-acid flanking sequence at the amino and carboxy (N and C) termini (resulting in the fragment 370–458), is sufficient to bind LC3 (Fig. 4c, d and Extended Data Fig. 8f), while the homologous regions on other lamins fail to bind LC3 (Fig. 4d). Examination of the amino-acid sequences revealed that lamins A/C harbour several distinct residues compared with lamin B1, and that lamin B2 has two insertions in the region (Extended Data Fig. 8d), which possibly alters the proper peptide folding for LC3 interaction.

The 370–458 region of lamin B1 contains its nuclear localization signal (NLS) (Fig. 4c), hence the fragment localizes to the nucleus (Extended Data Fig. 8g) and is able to interact with endogenous LC3 (Fig. 4e). Overexpression of this fragment decreases endogenous LC3–lamin B1 interaction, but does not affect LC3 lipidation, LC3 binding to p62 (Fig. 4e), or p62 degradation upon starvation (Extended Data Fig. 8h). When expressed in HRasV12 cells, the fragment impairs lamin B1 downregulation, accompanied by an attenuated senescence (Fig. 4f and Extended Data Fig. 8i–k).

We further identified the essential residues within lamin B1 for binding to LC3, and found that simultaneously substituting the residues S393, S395, S396, R397, and V398 to alanine abrogates the interaction with LC3 (Fig. 5a and Extended Data Fig. 9a–g). In control cells, this mutant has a profound deficiency in nucleus-to-cytoplasm transport. Consequently, the lamin B1 mutant-expressing cells delayed LC3–lamin B1 interaction occurred in the basal cellular state, and, upon restriction of oncogenic and tumorigenic insults. Our study suggests that LC3–lamin B1 interaction is required for lamin B1 degradation and cellular senescence. a, In vitro translated proteins were subjected to GST–LC3B pull-down. Uncropped blots are in Supplementary Figure 1.

**Figure 5** | LC3–lamin B1 interaction is required for lamin B1 degradation and cellular senescence. a, In vitro translated proteins were subjected to GST–LC3B pull-down. b, Lamin B1 mutant-expressing cells were analysed by immunoblotting. Uncropped blots are in Supplementary Figure 1. c, d, Colony formation analysis of BJ ERHrasV12 cells. A390 nm absorbance at 590 nm. e, Mid-life BJ fibroblasts stably expressing mCherry–GFP-tagged HRasV12-induced senescence with a higher efficiency than WT lamin B1 (Fig. 5b and Extended Data Fig. 9i), and significantly promoted the growth of colonies in colony-formation analysis (Fig. 5c). Furthermore, we used our lamin B1 370–458 peptide that blocks the LC3–lamin B1 interaction and inhibits senescence (Fig. 4e, f). Introducing point mutations as mapped above (Fig. 5a) abrogates the peptide association with LC3 (Extended Data Fig. 10a). While the 370–458 peptide delayed cellular senescence induced by HRasV12, the 370–458 mutant failed to do so (Fig. 5d and Extended Data Fig. 10b). Besides oncogene-induced senescence, the peptide also significantly delayed replicative senescence and the appearance of CCF (Fig. 5e–g and Extended Data Fig. 10c–e).

Taken together, these data indicate that the LC3–lamin B1 interaction plays an essential role in reinforcing cellular senescence, which both suppresses oncogene activity and limits cellular lifespan.

In this study, we discovered lamin B1 as a selective mammalian autophagy substrate upon oncogenic and genotoxic insults (illustrated in Fig. 5h). Recently, starvation-induced nuclear autophagy was discovered in yeast28, which is devoid of nuclear lamina and malignancies. In contrast, we show that mammalian lamin B1 degradation does not occur during starvation. Recent studies reveal that downregulation of lamin B1 impairs cell proliferation and DNA repair16,17,29,30, and leads to large-scale alterations in chromatin16. These dramatic changes are unlikely to happen during starvation, but are probably beneficial in restraining oncogenic and tumorigenic insults. Our study suggests that LC3–lamin B1 interaction occurs in the basal cellular state, and, upon aberrant cellular activities, initiates lamin B1 degradation (Fig. 5h) thus driving senescence to restrain cell proliferation. Hence, selective nuclear lamina degradation by autophagy may play a role in restricting tumorigenesis and maintaining cell and tissue integrity.

Although our current work focuses on lamin B1, we anticipate that other nuclear substrates of autophagy have roles in tumour suppression.
and other physiological/pathological scenarios. This study establishes a new perspective in understanding mammalian autophagy—from the nucleus.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

**Received 16 July; accepted 4 September 2015.**

**Published online 28 October 2015.**

1. Levine, B. & Kroemer, G. *Autophagy in the pathogenesis of disease*. Cell **132**, 27–42 (2008).

2. Mizushima, N., Levine, B., Cuervo, A. M. & Klionsky, D. J. *Autophagy fights disease through cellular self-digestion*. Nature **451**, 1069–1075 (2008).

3. Choi, A. M., Ryter, S. W. & Levine, B. *Autophagy in human health and disease*. *N. Engl. J. Med.* **368**, 651–662 (2013).

4. Kabeya, Y. et al. L34, a mammalian homologue of yeast *Apg8p*, is localized in autophagosome membranes after processing. *EMBO J.* **19**, 5720–5728 (2000).

5. Mizushima, N., Yoshimori, T. & Ohsumi, Y. *The role of Atg proteins in mammalian lysosome formation*. *Annu. Rev. Cell Dev. Biol.* **27**, 107–132 (2011).

6. Rogov, V., Dotsch, V., Johansen, T. & Kirkin, V. Interactions between autophagy receptors and ubiquitin-like proteins form the molecular basis for selective autophagy. *Mol. Cell* **53**, 167–178 (2014).

7. Drake, K. R., Kang, M. & Kenworthy, A. K. Nucleocytoplasmic distribution and dynamics of the autophagosome marker *EGFP-LC3*. *PLoS One* **5**, e9806 (2010).

8. Huang, R. et al. Deacetylation of nuclear LC3 drives autophagy initiation under starvation. *Mol. Cell* **57**, 456–466 (2015).

9. Simon, H. U., Yousefi, S., Schmid, I. & Friis, R. *ATG5 can regulate* p53 *expression and activation*. *Cell Death Dis.* **5**, e1339 (2014).

10. Lee, I. H. et al. *ATG7* modulates p53 activity to regulate cell cycle and survival during metabolic stress. *Science* **336**, 225–228 (2012).

11. Shimi, T. et al. The A- and B-type nuclear lamin networks: microdomains involved in chromatin organization and transcription. *Genes Dev.* **22**, 3409–3421 (2008).

12. Kerppola, T. K. *Bimolecular fluorescence complementation (BiFC) analysis* as a probe of protein interactions in living cells. *Annu. Rev. Biophys.* **37**, 465–487 (2008).

13. Guelen, L. et al. Domain organization of human chromosomes revealed by mapping of nuclear lamina interactions. *Nature* **453**, 948–951 (2008).

14. Lund, E., Oldenburg, A. R. & Collas, P. Enriched domain detector: a program for detection of wide genomic enrichment domains robust against local variations. *Nucleic Acids Res.* **42**, e92 (2014).

15. Sadaie, M. et al. Redistribution of the Lamin B1 genomic binding profile affects rearrangement of heterochromatic domains and SAHF formation during senescence. *Genes Dev.* **27**, 1800–1808 (2013).

16. Shah, P. P. et al. Lamin B1 depletion in senescent cells triggers large-scale changes in gene expression and the chromatin landscape. *Genes Dev.* **27**, 1787–1799 (2013).

17. Shimi, T. et al. The role of nuclear lamin B1 in cell proliferation and senescence. *Genes Dev.* **25**, 2579–2593 (2011).

18. Freund, A., Laberge, R. M., Demaria, M. & Campisi, J. *Lamin B1 loss is a senescence-associated biomarker*. *Mol. Biol. Cell* **23**, 2066–2075 (2012).

19. Ivanov, A. et al. *Lysozyme-mediated processing of chromatin in senescence*. *J. Cell Biol.* **202**, 129–143 (2013).

20. Serrano, M., Lin, A. W., McFerrach, M. E., Beach, D. & Lowe, S. W. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* **88**, 593–602 (1997).

21. Collado, M., Blasso, M. A. & Serrano, M. Cellular senescence in cancer and aging. *Cell* **130**, 223–233 (2007).

22. Young, A. R. et al. *Autophagy mediates the mitotic senescence transition*. *Genes Dev.* **23**, 798–803 (2009).

23. Liu, H. et al. Down-regulation of autophagy-related protein 5 (ATG5) contributes to the pathogenesis of early-stage cutaneous melanoma. *Sci. Transl. Med.* **5**, 202ra123 (2013).

24. Roberts, P. et al. *Piecemeal microautophagy* of nucleus in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* **14**, 129–141 (2003).

25. Mizushima, N., Yoshimori, T. & Levine, B. Methods in mammalian autophagy research. *Cell* **140**, 313–326 (2010).

26. Pankv, S. et al. *p62/SQSTM1* binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. *J. Biol. Chem.* **282**, 24131–24145 (2007).

27. Toyama, B. et al. Identification of long-lived proteins reveals exceptional stability of essential cellular structures. *Cell* **154**, 971–982 (2013).

28. Mochida, K. et al. Receptor-mediated selective autophagy degrades the endoplasmic reticulum and the nucleus. *Nature* **522**, 395–396 (2015).

29. Butin-Israeli, V. et al. Role of lamin B1 in chromatin instability. *Mol. Biol. Cell* **35**, 884–898 (2015).

30. Dreesen, O. et al. Lamin B1 fluctuations have differential effects on cellular proliferation and senescence. *J. Cell Biol.* **200**, 605–617 (2013).

**Supplementary Information** is available in the online version of the paper.

**Acknowledgements** We thank members of the Berger, Adams, and Goldman laboratories for technical assistance and discussions. We acknowledge A. L. Stout for help with confocal microscopy, and the electron microscopy resource laboratory for assistance on TEM. We thank Z. Yue for sharing the GFP antibody and reading the manuscript, and M. Narita and R. Salama for help with LAOs definition. Z.D. is supported by a fellow award from the Leukemia & Lymphoma Society. B.C.C. is supported by career development awards from the Dermatology Foundation, Melanoma Research Foundation, and American Skin Association. S.L.B., P.D.A. and R.M. are supported by NIA P01 grant (P01AG031862). S.L.B. is also supported by NIH R01 CA078831. R.D.G. is supported by R01 GM106223 and the Progeria Research Foundation.

**Author Contributions** Z.D., A.L., P.D.A. and S.L.B. conceived the project. Z.D. performed most of the experiments. C.X., G.D., B.C.C., A.M.D., and P.P.S. performed and analysed ChlP-seq. T.S. performed three-dimensional structural illumination microscopy imaging. T.S., S.A.A., and R.D.G. contributed novel lamin reagents and experimental design. J.-A.P., J.M.C., and W.-X.Z. contributed novel lamin reagents and experimental design. J.Z. performed Atg7 knockdown. T.S. performed illumintaion microscopy imaging. T.S. performed and analysed ChIP-seq. T.S. performed dimensional structural illumination microscopy imaging. T.S., S.A.A., and R.D.G. contributed novel lamin reagents and experimental design. J.-P., J.M.C., and W. contributed novel lamin B1 and senescence reagents. J.Z. performed Atg7 knockdown. M.D.R. and R.M. contributed to the biochemistry characterization of L34-lamin B1 interaction. T.L. and J.T. contributed novel autophagy constructs and experimental design. Z.D., P.D.A., and S.L.B. composed the manuscript. All authors reviewed the manuscript and discussed the work.

**Author Information** L34 and lamin B1 ChIP-seq data have been deposited in the NCBI Gene Expression Omnibus (GEO) database under accession number GSE65440. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to S.L.B. (bergers@upenn.edu).
METHODS

Data reporting. No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Cell lines and culture. IMR90, mouse embryonic fibroblasts, and HEK293T were described previously16,17. Primary BJ fibroblasts were purchased from ATCC. Cell line identities were not further authenticated. The cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), 100 U ml−1 penicillin, and 100 μg ml−1 streptomycin (Invitrogen), and were intermittently passed for necrosis and serum deprivation. Cells were cultured in Hank’s buffer (with calcium and glucose) supplemented with 10% dialysed FBS and 1% HEPES (Invitrogen). For amino-acid starvation, cells were incubated in Hank’s buffer plus 0.5% BSA in PBS supplemented with 10% dialysed FBS and 1% HEPES (Invitrogen). For amino-acid and serum deprivation, cells were cultured in Hank’s buffer plus 1% HEPES.

Retrovirus and lentivirus infection. Stable cell lines were made by retrovirus or lentivirus infection, as previously described18, with slight modifications. Retroviral constructs were transduced to Phoenix packaging cell line. Lentiviral pLKO constructs were transduced with packaging plasmids to HEK293T cells. Viral supernatant was filtered through a 0.45-μm filter, supplemented with 8 μg ml−1 polybrene, and mixed with transduced recipient cells. pLNCX-ER-HRasV12, W2L-hygro, and W2L-HRasV12-hygro viral constructs were described elsewhere19,20, sh-Atg7 hairpin sequence GGAGTCACAGCTCTTTTCATC was from ref. 22, and cloned into Tet-pLKO-puro and Tet-pLKO-sh-Atg7-inducible vector21. Doycyclin 100 ng ml−1 was added to IMR90 to induce knockdown of Atg7. Another pLKO-shAtg7 construct (TRCN000007587) was purchased from Sigma-Aldrich and used in BJ fibroblasts. The infected cells were selected with puromycin, neomycin, or hygromycin for about 1 week.

Reagents and antibodies. Rapamycin was purchased from Millipore. H2O2 was from Fisher Scientific. 4-Hydroxytamoxifen and etoposide was from Sigma-Aldrich. The following antibodies were used: LC3 (MBL PM036 for WB of mouse embryonic fibroblasts; Cell Signaling Technology 3868 for immunoprecipitation, ChIP IE, WB; Cell Signaling Technology 2775 for WB), β-tubulin (Sigma-Aldrich T4026), calreticulin (Cell Signaling Technology 12238), COX IV (Cell Signaling Technology 4850), Atg5 (Cell Signaling Technology 8540), Atg7 (Cell Signaling Technology 8558), lamin B1 (Abcam ab16048), lamin B2 (Abcam ab8983), laminas A/C (Millipore AB3211), GFP (Roche 11 814 460 001 and Abcam ab2890), p62 (Abnova H0000878-001), GAPDH (Fitzgerald Industries 10R-G109A), p16 (Abcam ab16123), Ras (Millipore 05-016), HA (Sigma-Aldrich H3663), H3K27me3 (Active Motif 39538), H3K9me3 (Abcam ab8898), LAMAP (Iowa Hybridoma Bank HAa-3), and Flag (Sigma-Aldrich F1804).

Plasmids. GST, GST–LC3A, B, and C, and GST–LC3B mutants/truncations were described elsewhere22,23. GFP, HA/Flag/GFP–LC3 WT and mutants, GFP–Beclin 1, GFP–ULK1, GFP–lamin B1, and split Venus constructs were described previously17,18,19,35. pBabe–mCherry–GFP–lamin B1 was detected with a GFP antibody35 diluted 1:50 in 5% BSA, 0.1% fish gelatin, and LC3 was truncated to make pBabe–mCherry–GFP, and then lamin B1 sequences were cloned. Lamin B1 truncations/mutations were made from pPEGF–lamin B1 for direct transfection, pBabe–mCherry–GFP–lamin B1 for retrovirus, or pP7–NHA–lamin B1 for in vitro translation. Tet-inducible lentiviral GFP–lamin B1 was made by cloning the GFP–lamin B1 fragment into pTRIPZ. All new constructions in this study were verified by DNA sequencing.

Western blotting. Cells were lysed in buffer containing 50 mM Tris pH 7.5, 0.5 mM EDTA, 1% SDS, 50 μg ml−1 polybrene, and with 1:100 Halt protease and phosphatase inhibitor cocktail (Thermo Scientific). The lysates were briefly sonicated, and supernatants were subjected to electrophoresis using NuPAGE Bis-Tris precast gels (Life Technologies). After transferring to nitrocellulose membrane, 5% milk in TBS supplemented with 0.1% Tween 20 (TBST) was used to block the membrane at room temperature (−25°C) for 1 h. Primary antibodies were diluted in 5% BSA in TBST, and incubated at 4°C overnight. The membrane was washed three times with TBST, each for 10 min, followed by incubation of HRP-conjugated secondary antibodies at room temperature for 1 h, in 5% milk/2% TBST. The membrane was washed again three times, and imaged by a Fujifilm LAS-4000 imager.

Immunoprecipitation. Cells were lysed in immunoprecipitation buffer containing 20 mM Tris, pH 7.5, 137 mM NaCl, 1 mM MgCl2, 1 mM CaCl2, 1% NP-40, 10% glycerol, supplemented with 1:100 Halt protease and phosphatase inhibitor cocktail (Thermo Scientific) and benzonase (Novagen) at 12.5 U ml−1. Benzonase is essential to release chromatin-bound proteins to supernatant, and MgCl2 is critical for its activity. The lysates were rotated at 4°C for 30–60 min. The supernatant was incubated with antibody-conjugated Dynabeads (Life Technologies), and rotated at 4°C overnight. The immunoprecipitation was washed and collected by magnet, for five times with immunoprecipitation buffer, and boiled with NuPAGE loading dye. Samples were analysed by western blotting.

In vitro translation. Cell-free in vitro translation was performed using the 1-Step In Vitro Translation Kit (Thermo Scientific), following the manufacturer’s guidance. Target proteins were cloned into pT7CEF1-NHA vector (with N-terminal HA tag) and translated in vitro at 30°C.

GST pull-down. GST-tagged constructs were transformed into BL21-CodonPlus Esherichia coli and purified with glutathione beads (Life Technologies). Lamin B1 370–458 and 390–438 fragments were cloned into GST construct with a TEV protease recognition site between GST and the cloned sequences. The expressed proteins were loaded and purified with glutathione agarose beads, and digested with His-tagged TEV protease. The resulting supernatant was further purified with Ni-NTA beads (Qiagen) to remove His-tagged TEV protease.

For GST pull-down, bacterial lysates were incubated with glutathione beads at 4°C for 2 h and washed four times with buffer containing 30 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM DTT, supplemented with 100 μM PMSE. The purified proteins or in vitro translated proteins were diluted in binding buffer (20 mM Tris, pH 7.5, 137 mM NaCl, 1 mM MgCl2, 1 mM CaCl2, 1% NP-40, 10% glycerol, supplemented with 1:1000 Halt Protease inhibitor cocktail) and then pre-cleared with GST at 4°C for 1 h. The resulting supernatant was then subjected to GST pull-down with GST or GST fusion proteins. The product was washed four times with binding buffer and boiled with NuPAGE loading dye for immunoblot analysis. Purified lamin B1 protein was purchased from Origene.

Immunofluorescence and live-cell imaging. For immunofluorescence, cells were fixed in 4% paraformaldehyde in PBS for 30 min at room temperature. Cells were washed twice with PBS, and permeabilized with 0.5% Triton X-100 in PBS for 10 min. After washing two times, cells were blocked in 10% BSA in PBS at 1 h at room temperature. Cells were incubated with primary antibodies in 5% BSA in PBS supplemented with 0.1% Tween 20 (PBST) overnight at 4°C. The next day, cells were washed four times with PBST, each for 10 min, followed by incubation with Alexa Fluor-conjugated secondary antibody (Life Technologies) in 5% BSA/PBST for 1 h at room temperature. Cells were then washed four times in PBST, incubated with 1 μg ml−1 DAPI in PBS for 5 min, and washed twice with PBS. The slides were then mounted with ProLong Gold (Life Technologies) and imaged with a Leica TCS SP8 fluorescent confocal microscope. The slides were mounted with ProLong Diamond (Life Technologies) for 5 days at room temperature for super-resolution microscopy.

Three-dimensional structural illumination microscopy was performed using N-SIM Super-resolution Microscope System (Nikon) with an oil immersion objective lens CFI SR (Apoachromat TIRF ×100, 1.49 numerical aperture; Nikon). Twenty to forty-one optical sections were collected with a 200 nm interval between neighbouring sections.

For live-cell imaging, mCherry–GFP–lamin B1 HRasV12 cells were plated onto a 35 mm glass bottom dish (MatTek P35G-0-14-C) pre-coated with poly-L-lysine (Sigma-Aldrich). The dish was imaged with a spinning disk fluorescent confocal microscope (Olympus IX71 and IX81 Inverted System, coupled with an Andor iXon3 EMCCD camera, with motorized x–y stage, Okolab stagetop incubation chamber, and MetaMorph acquisition software). Cells were imaged overnight every 15 min. Twelve z-sections were acquired covering the entire individual cell. Images were viewed and presented as the maximum projection from all z-sections.

TEM. For immunoo-gold TEM, GFP–lamin B1 expressing IMR90 cells were subjected to high-pressure freezing. The samples were then dehydrated by freeze substitution methods for 72 h at −90°C in 0.1% uranyl acetate/acetone followed by embedding in Lowicryl HM20 at −50°C with 360 nm light polymerization of the resin. Thin sections (80–120 nm) were cut with a UC 6 ultramicrotome (Leica) and stained with 1% uranyl acetate and lead citrate. The sections were observed using a Jeol JEM-100 CX II electron microscope.

ChIP-seq and ChIP-chip. These assays were performed as described previously16,18 with slight modification. In brief, cells were crosslinked with 1% formaldehyde diluted in PBS, without the addition of other co-crosslinkers, for 5 min at room temperature. After glycine quenching, the cell pellets were lysed in buffer containing 50 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.1% Na-deoxycholate, 0.1% SDS, supplemented with complete protease inhibitor

© 2015 Macmillan Publishers Limited. All rights reserved
cocktail (Thermo Scientific), and sonicated with a Covaris sonicator, resulting in chromatin fragments with an average size of 250 base pairs. The supernatant was diluted ten times with the above buffer without SDS, and subjected to immunoprecipitations with 2 μg of antibody or control IgG conjugated with Dynabeads Protein A or G (Invitrogen) at 4°C overnight. The beads were then washed five times with buffer containing 50 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, and once with final wash buffer (50 mM Tris, pH 8.0, 10 mM EDTA, 50 mM NaCl), followed by elution with incubation of elution buffer (final wash buffer plus 1% SDS) at 65°C for 30 min with agitation in a thermomixer. The ChIP and input were then purified and used for qPCR analysis or for constructing sequencing libraries with a NEBNext Ultra kit (New England Biolabs). For ChIP-sequencing, the libraries were quantified (Kapa Biosystems) and were single-end sequenced on an Illumina NextSeq 2000.

The following primers were used for qPCR analyses of LADs. LAD1: forward, AGAGACCTGGCCTTGTCTG; reverse, GGAGCTAATCTGCTTGAGG; forward, ATTTGCACTGAAGCCCTTGT; reverse, CTGGGCAATTCCCTTGGTAGT (chromosome 7: 35434121–35434171). LAD3: forward, GCATCCATTTCACATCCTTGG; reverse, CCCATTGGCTCTGAAGTTTTGT (chromosome 8: 130184820–130184870).

Subcellular fractionation. This was performed with the subcellular fractionation kit for cultured cells (Thermo Scientific 78840) according to the manufacturer instructions, with slight modification. Benzonase (Novagen) was used to digest chromatin-bound proteins in the nuclear fraction, in the buffer supplemented with 5 mM MgCl2.

Senescence-associated β-gal assay. β-Galactosidase assays were performed using a cellular senescence assay kit (Chemicon KA0002), according to the manufacturer's protocol. Cells were incubated with β-gal detection solution at 37°C overnight, and quantified under final regular light microscopy. At least 200 cells were scored for β-gal positivity with over four different fields.

Statistical analysis. Student’s t-test was used for comparison between two groups. One-way ANOVA coupled with Tukey’s post hoc test was used for comparisons over two groups. Significance was considered when the P-value was less than 0.05.
**Extended Data Figure 1** | Characterization of LC3 and lamin B1 association. **a**, Protein gel staining of purified lamin B1 protein. **b, c**, Purified lamin B1 protein was subjected to GST pull-down. **d**, Endogenous LC3 immunoprecipitation in HEK293T cells. **e**, IMR90 stably expressing GFP–LC3 constructs were starved and imaged. **f**, Endogenous co-IP in wild-type and Atg5 knockout mouse embryonic fibroblasts. **g**, Nuclear fractions of control and Atg7 knockdown IMR90 cells were analysed by LC3 immunoprecipitation. **h–j**, BiFC analysis of LC3–lamin B1 interaction. HeLa cells were transfected with the indicated combination of split Venus constructs and analysed as follows. **h**, Cells were fixed and imaged. **i**, Lysates were analysed by immunoblotting. **j**, Cells were scored for Venus positivity. Bars, mean ± s.d.; *n* = 4, with over 500 cells; *P* < 0.001; unpaired two-tailed Student’s *t*-test.
Extended Data Figure 2 | LC3 interacts with LADs on chromatin.

a, b, ChIP–qPCR of proliferating IMR90. c, ChIP–qPCR of LC3 knockdown IMR90. Bars, mean ± s.e.m. (a, b), s.d. (c); n = 3; *P < 0.05, **P < 0.005, ***P < 0.001; NS, non-significant; unpaired two-tailed Student’s t-test.

d–i, ChIP-sequencing analyses. d, Related to Fig. 2c, a zoom-in window of chromosome 3. e, f, Analyses of two replicates at LADs and LC3ADs.

g, Per-nucleotide overlap of published data sets with the LADs called from this study. Number unit: megabases. h, Enrichment over LC3ADs. *P < 2.2 × 10^{-16}; one-sided Wilcoxon test. i, Analysis of our lamin B1 and LC3 ChIP-seq at LADs defined by other studies, and randomly sampled non-LAD loci (Ctrl). *P < 2.2 × 10^{-16}; one-sided Wilcoxon test.
Extended Data Figure 3 | Lamin B1 degradation upon HRasV12-induced senescence. a, Related to Fig. 3b. Immunoblotting of immortalized IMR90. b, GFP–lamin B1 stably expressing IMR90 cells were treated as indicated and imaged. Cytoplasmic signals are indicated by arrows. c–e, TEM analyses of IMR90. Nu, nucleus. f, IMR90 cells stably expressing mCherry–GFP–lamin B1 were imaged and quantified. g, Cells as in f were treated with bafilomycin A1 and imaged under confocal microscopy.
Extended Data Figure 4 | Imaging analyses of mCherry–GFP–lamin B1 HRasV12 cells. a, Related to Fig. 3c. mCherry–GFP–lamin B1 HRasV12 cells stably expressing IMR90 were imaged by three-dimensional super-resolution microscopy. Sections shown span the top, middle, and bottom layers of the cell. The mCherry channel was deliberately under-exposed to prevent over-saturation of the cytoplasmic signals. Scale bar, 5 μm. The insets are presented in Fig. 3c. b, Live-cell imaging of mCherry–GFP–lamin B1 HRasV12 IMR90. Images shown are the maximum-projection combining all z-sections. Nucleus-to-cytoplasm transport events are labelled sequentially as indicated. Note the initial yellow signal, followed by disappearance of GFP then mCherry, in events 1 and 3; event 2 was not yet degraded by the end of the imaging.
Extended Data Figure 5 | CCF and lamin B1 are targeted by autophagy. a, b, IMR90 cells stably expressing GFP–LC3 and HRasV12 were stained with indicated antibodies and imaged under confocal microscopy. Cytoplasmic events are labelled by arrows. c, HRasV12 IMR90 cells were stained with LC3 antibody. d, Related to Fig. 3e, immuno-TEM analysis of GFP–lamin B1 IMR90 cells. Cells were stained with a GFP antibody and conjugated with 10 nm gold particles. Gold particles are indicated by arrows.
Extended Data Figure 6 | Knockdown of Atg7 attenuates lamin B1 downregulation. a, Related to Fig. 4a, quantification of lamin B1 immunoblots. Bars, mean ± s.e.m.; n = 3; *P < 0.05, **P < 0.005, ***P < 0.0001, compared with sh-NTC day 0; NS, non-significant.
b, Reverse transcribed qPCR of cells as in Fig. 4a. Data are the mean normalized to GAPDH ± s.e.m.; n = 3. c, d, IMR90 cells were treated as indicated and analysed by immunoblotting. e, f, Atg7 knockdown inhibits mCherry–GFP–lamin B1 nucleus-to-cytoplasm transport. Bars are mean ± s.d.; n = 4, over 100 cells; *P < 0.0001.
h, i, ER:HRasV12 BJ cells stably expressing Dox-inducible GFP or GFP–lamin B1 were either left uninduced (bars 1 and 2), or induced with 4-OHT for 3 weeks (3–6). Cells were then induced with Dox (in the presence of 4-OHT) for an additional 2 weeks (5 and 6). i, Quantification of β-gal positivity. Bars, mean ± s.d.; n = 4, over 200 cells. j, k, Related to Fig. 4a, quantification of p16 immunoblots. Bars, mean ± s.d.; n = 4; *P < 0.05, **P < 0.0005, ***P < 0.0001. One-way ANOVA coupled with Tukey’s post hoc test for a and i; all other tests were unpaired two-tailed Student’s t-tests.
Extended Data Figure 7 | LC3 R10 and R11 are essential for lamin B1 binding.  
a, b, HEK293T cells were transfected as indicated and analysed by co-IP.  
c–e, BiFC analyses in HeLa cells transfected with the indicated combination of split Venus constructs. Bars, mean ± s.d.;  
n = 4, over 500 cells; *P < 0.0001.  
f, IMR90 cells stably expressing the indicated constructs were analysed by Flag ChIP. Bars, mean ± s.e.m.; *P < 0.05, **P < 0.005; unpaired two-tailed Student’s t-test for e and f.  
g, LC3 R10 and R11 are necessary for co-localization with CCF in HRasV12 IMR90. CCFs are indicated with arrows.
Extended Data Figure 8 | Mapping of LC3–lamin B1 interaction.

a, HEK293T cells transfected with indicated constructs were analysed by GST–LC3B pull-down. b, c, In vitro translated constructs were subjected to GST–LC3B pull-down. d, e, Evolutionary analyses of vertebrate lamin B1 and the corresponding regions of other lamin isoforms. e, Number of conserved residues normalized to total residues. f, Bacterially purified fragments were analysed by GST–LC3B pull-down. g, mCherry–GFP–lamin B1 370–458 localizes to the nucleus. h, Cells were starved and analysed by immunoblotting. i, j, Related to Fig. 4f, quantification of lamin B1 and p16 immunoblots; n = 3. k, ER-HRasV12 IMR90 cells were scored for β-gal positivity; n = 4, over 200 cells. Bars, mean ± s.e.m. (i, j), s.d. (k); NS, non-significant; *P < 0.05; **P < 0.0005; ***P < 0.0001; unpaired two-tailed Student’s t-test.
Extended Data Figure 9 | Additional characterization of lamin B1 substitution mutant. a–f. Related to Fig. 5a, in vitro translated proteins were analysed by GST–LC3B pull-down. g, LC3 immunoprecipitation in HEK293T cells transfected as indicated. The remaining interaction with the mutant is probably due to the endogenous lamin B1 that interacts with LC3 and the mutant, as shown in j. h, i, IMR90 cells were imaged under confocal microscopy and quantified. Bars, mean ± s.d.; n = 4, over 200 cells; *P < 0.05, **P < 0.005, ***P < 0.0001; unpaired two-tailed Student's t-test. j, HEK293T transfected cells were analysed by immunoprecipitation. k, ER:HRasV12 IMR90 cells were induced with OHT and harvested for immunoblotting. l, IMR90 cells were quantified for β-gal positivity. Bars, mean ± s.d.; n = 4, over 200 cells; *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, NS, non-significant; one-way ANOVA coupled with Tukey’s post hoc test.
Extended Data Figure 10 | Lamin B1 370–458 fragment extends cellular lifespan. a, In vitro translated proteins were analysed by GST–LC3B pull-down. b, ER:HRasV12 IMR90 cells were quantified for β-gal positivity. Bars, mean ± s.d.; n = 4, over 200 cells; *P < 0.05; NS, non-significant; one-way ANOVA coupled with Tukey’s post hoc test. c, d, Related to Fig. 5f, representative images of β-gal. e, Related to Fig. 5g, cells were fixed and stained with DAPI. CCFs are indicated by arrows.