Supporting Information

Fluorescent labeling of the nuclear envelope by localizing GFP on the inner nuclear membrane

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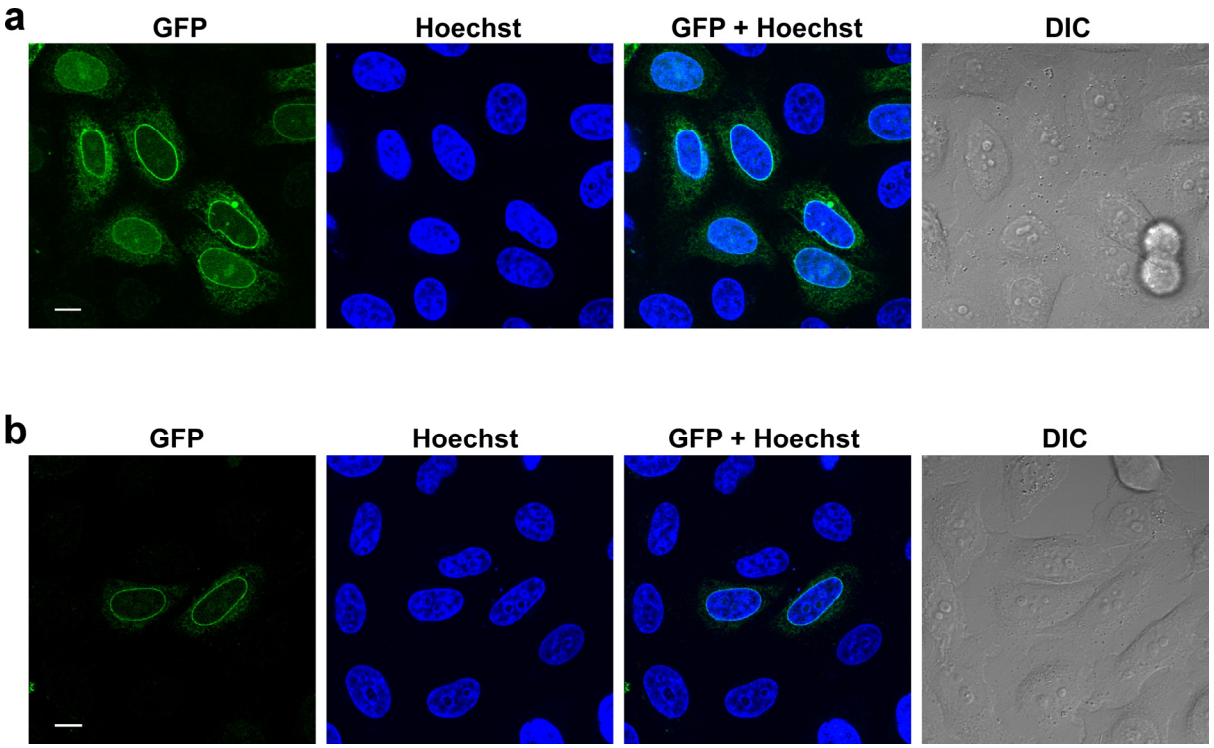


Figure S1. Confocal images of the cells expressing TM-BPL and BCCP-GFP-NLS. (a) HeLa cells were transfected with 2 μg of each expression plasmid for TM-BPL and BCCP-GFP-NLS using 4 μL of Lipofectamine 2000. Twenty-four hours after transfection, the cells were stained with Hoechst 34580 and observed by confocal microscopy on green (*left)* and blue (*second from the left*) channels for GFP and Hoechst, respectively. The *third panel from the left* represents an overlay of signals from both channels. The *right panel* is a differential interference contrast (DIC) image of the cells. (b) HeLa cells were transfected with 2 and 0.5 μg of the expression plasmids for TM-BPL and BCCP-GFP-NLS, respectively, using 4 μL of Lipofectamine 2000, and 24 hr after the transfection, the cells were imaged in the same procedures as those in panel (a). Bars, 10 μm.

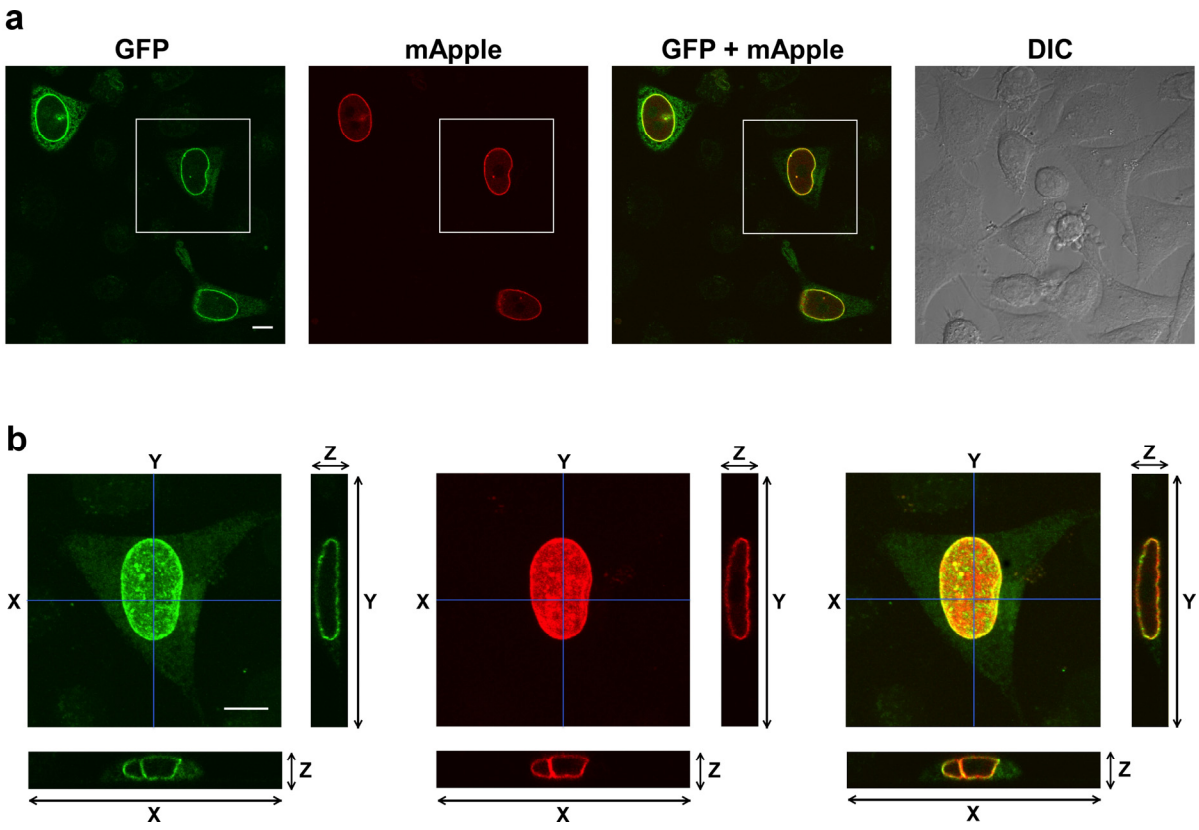
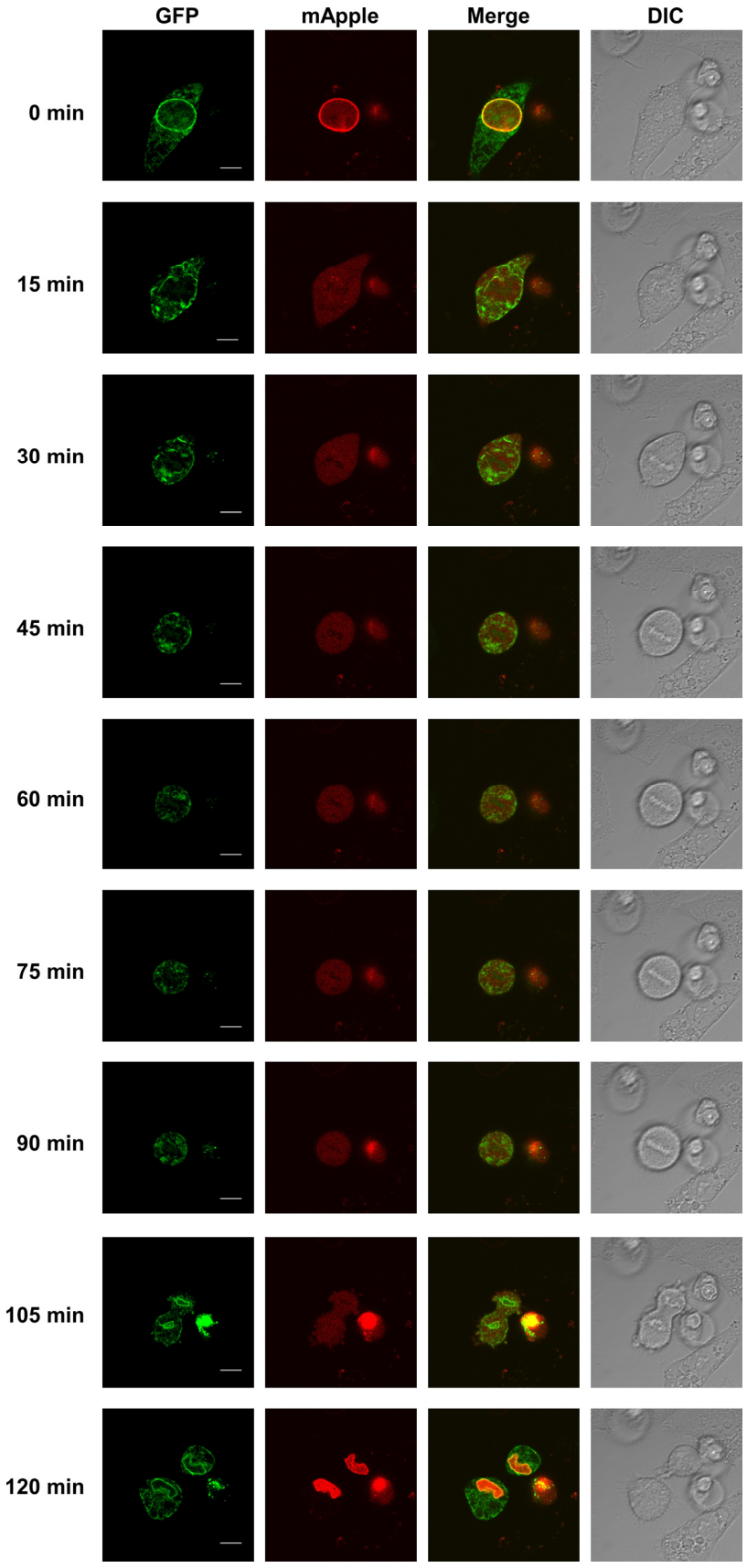


Figure S2. Comparison of the distribution of the NE and the nuclear lamina. HeLa cells were transfected with 2, 1, and 2 μg of the expression plasmids of TM-BPL, BCCPGFP-NLS, and mApple-Lamin A, respectively, using 8 μL of X-tremeGene 9. Twenty-four hours after the transfection, the cells were observed by confocal microscopy on green and red channels for GFP and mApple, respectively. (a) Cross-sectional images of the cells obtained by monitoring fluorescence from GFP (*left*) and mApple (*second from the left*). *Third panel from the left:* An overlay of signals from both channels. *Right panel:* A differential interference contrast (DIC) image of the cells. (b) Three-dimensional reconstruction from a series of the sectional images. A series of the sectional images were taken from the bottom to the top of the cell at 0.25-μm intervals. Three-dimensional images of a labeled cell in the boxed region of panel (a) were reconstructed by stacking the 40 sectional images on both channels. *Left and middle panels:* The stacked images from the fluorescence of GFP and mApple, respectively, along with the sectional images in XZ- and YZ- planes. *Right panel:* An overlay of the left and middle panels. Bars: 10 μm.

Figure S3 (continued)



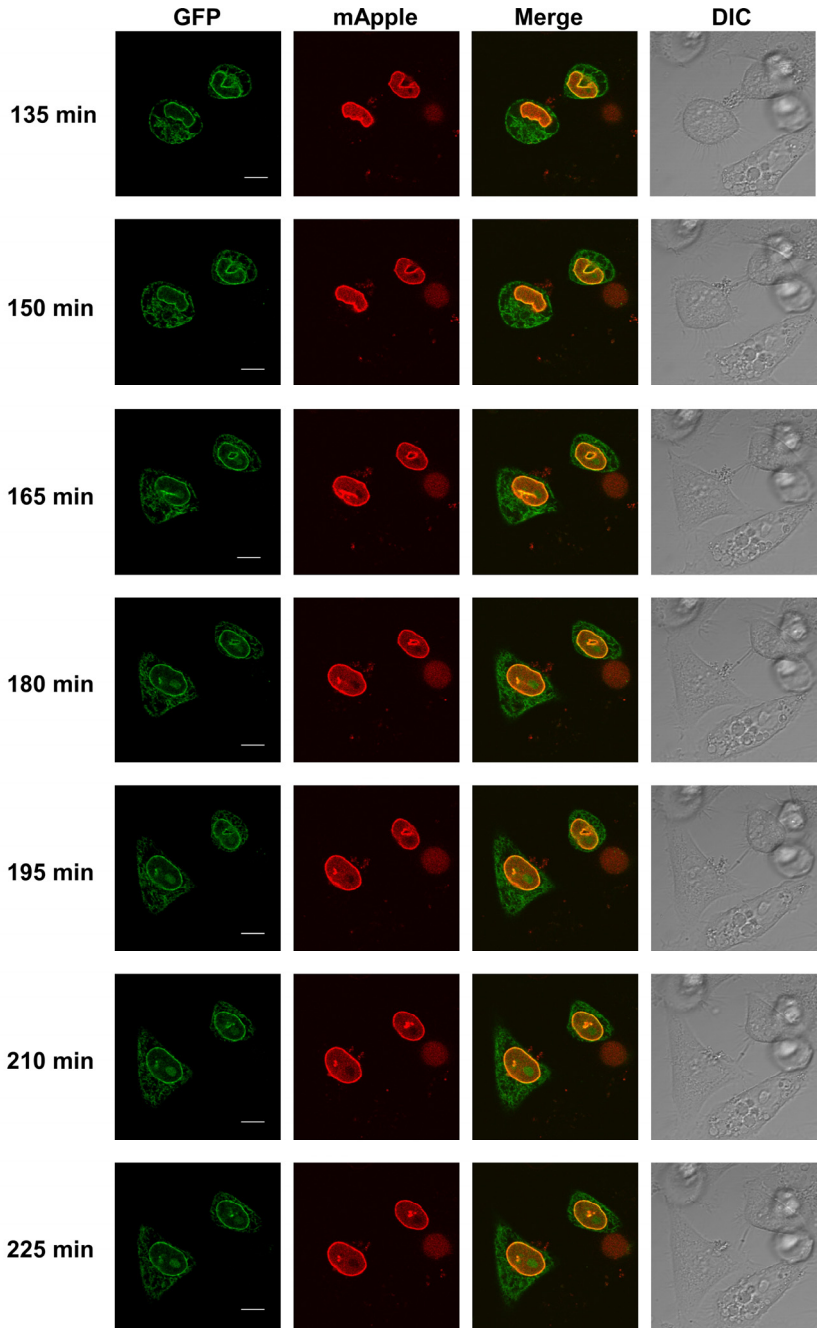


Figure S3. Time-lapse sequences of the dividing cell recorded every 15 min by confocal microscopy. HeLa cells were transfected with the expression plasmids for fusion proteins and the time-lapse sequences of the labeled cell were recorded under the same conditions as those in the legend of Figure 3 of the main text. *First and second columns from the left:* Time-lapse sequences of the cell recorded on green and red channels, respectively. *Third column from the left:* An overlay of signals from both channels. *Right column:* DIC image of the cell. Bars, 10 μm.

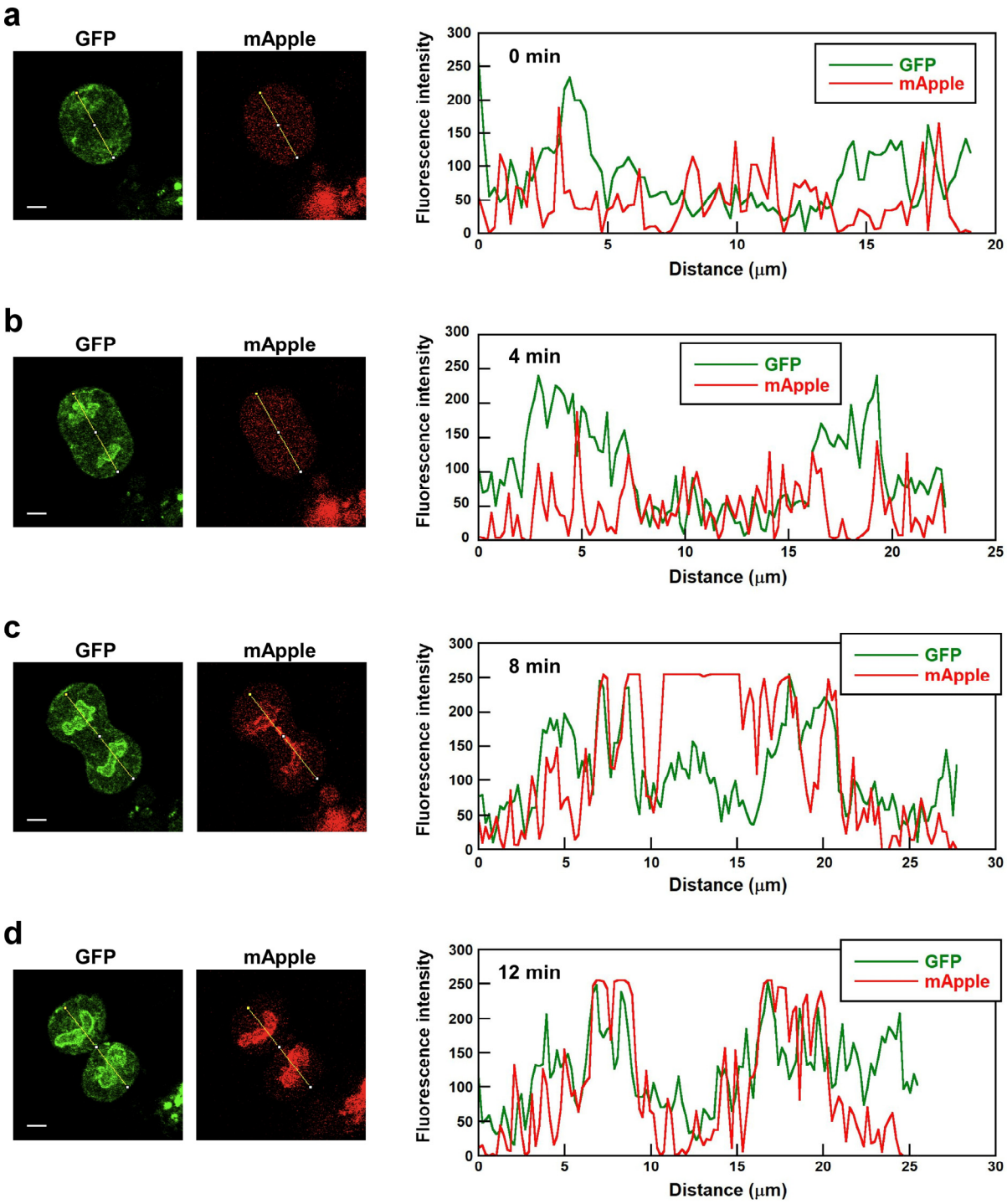


Figure S4. Quantitative analysis of the fluorescence intensities from GFP and mApple during mitosis. Some of the imaging data shown in Figure 4 of the main text were analyzed with ImageJ software; the sectional images at 0, 4, 8, and 12 min were used for calculation, and the analyzed data are shown in panels a, b, c, and d, respectively. The fluorescence intensities from GFP and mApple on the line in each sectional image were calculated and plotted against distance; the plotted data based on the fluorescence intensity from GFP (*green lines*) and mApple (*red lines*) are shown. Bars: 5 μm.

Legends of Movies

Movie S1

A time-lapse movie of the dividing cell based on the sectional images recorded every 15 min. HeLa cells were transfected with 2, 1, and 2 μg of the expression plasmids of TM-BPL, BCCP-GFP-NLS, and mApple-Lamin A, respectively, using 8 μL of X-tremeGene 9. Twenty-four hours after the transfection, the time-lapse sequences of the cells were recorded every 15 min by confocal microscopy on green and red channels for GFP and mApple, respectively. The time-lapse movie was constructed by assembling each imaging frame sequentially on ImageJ. *Upper left and right panels:* Movies based on the fluorescence from GFP and mApple, respectively. *Lower left panel*: A movie based on the overlaid images of the fluorescence from GFP and mApple. *Lower right panel:* A movie based on a differential contrast image of the cells. Bar, 10 μm.

Movie S2

A time-lapse movie of the dividing cell based on the sectional images recorded every 2 min. The time-lapse sequences of HeLa cells expressing TM-BPL, BCCP-GFP-NLS, and Lamin A-mApple were recorded every 2 min and the movie was constructed in the same procedure as those in the legend of Movie S1. Bar, 10 μm.

Construction of expression plasmids for fusion proteins

The expression plasmid for TM-BPL was constructed from a plasmid, pBS-α7-B2R-BCCPΔ100, which was used in the previous work[S1]. In pBS-α7-B2R-BCCPΔ100, the DNA sequences coding for human bradykinin B2 receptor (B2R) and *S. tokodaii* BCCP lacking N-terminal 100 amino acids were tandemly inserted into BamHI/EcoRI and EcoRI/NotI sites in pBluescript II SK(+) (Stratagene), respectively. This plasmid also contains the DNA sequences for the N-terminal signal peptide of α7 nicotinic acetylcholine receptor[S2] and for the FLAG tag in the XhoI/HindIII and HindIII/BamHI sites at the upstream region of B2R, respectively. The DNA sequence coding for the transmembrane domain of the human platelet-derived growth factor receptor (TM) was amplified by PCR with an expression vector, pDisplay (ThermoFisher Scientific), as a template using the following primers: 5'-GGATCCA**GGAGGCGCT**GTGGGCCAGGACACG-3' and 5'-GAATTC**ACCGCCTCC**ACGTGGCTTCTTCTGCCAAAGC-3' (restriction enzyme sites are underlined and the extra codons for glycine residues are shown in bold).

The resulting PCR product was TA cloned into pTAKN-2 vector (BioDynamic Laboratory), and following sequence analysis, the DNA fragment for TM was cut out from the vector by digestion with BamHI/EcoRI. The obtained fragment was inserted into the pBS-α7-B2R-BCCPΔ100 digested with BamHI/EcoRI to give a plasmid, pBS-α7-TM-BCCPΔ100. Also, the DNA sequence coding for *S. tokodaii* BPL was PCR-amplified with a plasmid, pBS-BPL (opt), constructed previously[S1] as a template using the following primers: 5'-GAATTCATGGGCATCAGCATGCTGATCTTTAAG-3' and 5'-GCGGCCGC**TTA**TTCTTCGATGACCCGGAGAATG-3' (restriction enzyme sites are underlined and the stop codon is shown in bold). The PCR product obtained was TA cloned into pTAKN-2 vector, and following sequence analysis, the DNA fragment for BPL was cut out from the vector by digestion with EcoRI/NotI. The obtained fragment was inserted into the pBS-α7-TM-BCCPΔ100 digested with EcoRI/NotI to give a plasmid, pBS-α7-TM-BPL. This plasmid was digested with XhoI/NotI and the resulting fragment was recloned into an expression vector, pCI-neo (Promega), to give an expression plasmid for TM-BPL, pTM-BPL.

The expression plasmid for TM-GFP was constructed by replacing the gene of BPL with that of GFP in pTM-BPL. Thus, the DNA sequence coding for GFP was PCR-amplified with an expression vector, pEGFP-N1 (Clontech), as a template using the following primers: 5'-GAATTCATGGTGAGCAAGGGCGAGG-3' and 5'-GCGGCCGC**TTA**CTTGTACAGCTCGTCCATGCC-3' (restriction enzyme sites are underlined and the stop codon is shown in bold). The PCR product obtained was TA cloned into pTAKN-2 vector, and following sequence

analysis, the DNA fragment for GFP was cut out from the vector by digestion with EcoRI/NotI. The obtained fragment was inserted into the pTM-BPL digested with EcoRI/NotI to give an expression plasmid for TM-GFP, pTM-GFP.

The expression plasmid for BCCP-GFP was constructed by inserting the DNA sequence of *S. tokodaii* BCCP into an expression vector, pQBI25-fA1 (Wako Chemicals USA). Thus, the DNA sequence coding for *S. tokodaii* BCCP lacking N-terminal 100 amino acids was PCR-amplified with a plasmid, pBS-BCCPΔ100(opt), constructed previously[S1] as a template using the following primers: 5'-GGATCCAGGTGAAATCCTGTCTCCCATGC-3' and 5'-GAATTC CCTTGATGATGAGGAGCAGATC-3' (restriction enzyme sites are underlined). The PCR product obtained was TA cloned into pTAKN-2 vector, and following sequence analysis, the DNA fragment for BCCP was cut out from the vector by digestion with BamHI/EcoRI. The obtained fragment was inserted into the pQBI25-fA1 digested with BamHI/EcoRI to give an expression plasmid for BCCP-GFP, pBCCP-GFP.

The expression plasmid for BCCP-GFP-NLS was constructed using pBCCP-GFP and pAcGFP1-Nuc (Clontech). The pAcGFP1-Nuc carries a DNA sequence coding for GFP with three copies of the nuclear localization signal (NLS) of the simian virus 40 large T-antigen on its C-terminus (GFP-NLS). The DNA sequence of GFP-NLS was PCR-amplified with pAcGFP1-Nuc as a template using the following primers: 5'-GCTAGCAAGGGCGCCGAGC TGTTC-3' and 5'-ATCGAT**TTA**TCTAGATCCGGTGGATCC-3' (restriction enzyme sites are underlined and the stop codon is shown in bold). The PCR product obtained was TA cloned into pTAKN-2 vector, and following sequence analysis, the DNA fragment for GFP-NLS was cut out from the vector by digestion with NheI/ClaI. The obtained fragment was inserted into the pBCCP-GFP digested with NheI/ClaI to give an expression plasmid for BCCP-GFP-NLS, pBCCP-GFP-NLS.

The expression plasmid for mApple-Lamin A was constructed using mApple-C1 and pBABE-puro-GFP-wt-lamin A; mApple-C1 was a gift from Michael Davidson (Addgene plasmid # 54631)[S3] and pBABE-puro-GFP-wt-lamin A was a gift from Tom Misteli (Addgene plasmid # 17662)[S4]. The DNA sequence coding for Lamin A was PCR-amplified with pBABE-puro-GFP-wt-lamin A as a template using the following primers: 5'-AGATCTATGGAGACCC CGTCCCAGC-3' and 5'-GAATTCCCAGA**TTA**CATGATGCTGCAGTTCTG-3' (restriction enzyme sites are underlined and the stop codon is shown in bold). The PCR product obtained was TA cloned into pTAKN-2 vector, and following sequence analysis, the DNA fragment for Lamin A was cut out from the vector by digestion with BglII/EcoRI. The obtained fragment

was inserted into mApple-C1 digested with BglII/EcoRI to give an expression plasmid for mApple-Lamin A, pmApple-Lamin A.

References

S1. Sueda, S., Yoneda, S., and Hayashi, H. (2011) Site-specific labeling of proteins by using biotin protein ligase conjugated with fluorophores. *Chembiochem. 12*, 1367-1375.

S2. Weill, C., Ilien, B., Goeldner, M., and Galzi, J.L. (1999) Fluorescent muscarinic EGFP-hM1 chimeric receptors: design, ligand binding and functional properties. *J. Recept. Signal Transduct. Res. 19*, 423-436.

S3. Kremers, G.J., Hazelwood, K.L., Murphy, C.S., Davidson, M.W., and Piston, D.W. (2009) Photoconversion in orange and red fluorescent proteins. *Nat. Methods. 6*, 355-358.

S4. Scaffidi, P., and Misteli, T. (2008) Lamin A-dependent misregulation of adult stem cells associated with accelerated ageing. *Nat. Cell Biol. 10*, 452-459.