

Supplemental Materials For:

Lamin C conserves DNA replication factors via phase separation during oxidative stress for DNA replication recovery

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Materials and Methods

Plasmid construction

The GFP-lamin A and mCherry-BAF plasmids have been described in our previous publication (Lin et al., 2020). The GFP-lamin C plasmid was generated by cloning human lamin C cDNA into pEGFP-C2. The GFP-lamin A and GFP-lamin C truncations and mutants were derived from GFP-lamin A and GFP-lamin C, respectively. The mCherry-PRDX1, mCherry-PRDX2, mCherry-PRDX6 and LIG1-mKate plasmids were generated by cloning their human cDNAs from a cDNA library, prepared by the Core Facilities at the College of Life Sciences, Peking University (Beijing, China), into the pmCherry-C2 (a derivative of pEGFP-C2) or pmKate2-N vectors, respectively. pCDH-EGFP-lamin A and pCDH-EGFP-lamin C were generated by inserting EGFP-lamin A and EGFP-lamin C into the lentiviral vector pCDH-CMV-MCS-EF1-Puro, provided by the Jing Yi laboratory, Shanghai Jiao Tong University (Shanghai, China). pCDH-E-mCherry-lamin A, pCDH-E-mCherry-lamin C-WT and pCDH-E-mCherry-lamin C-5A were generated by inserting mCherry-lamin A, mCherry-lamin C-WT and mCherry-lamin

C-5A into the lentiviral vector pCDH-E-CMV-MCS-EF1-Puro (pCDH-CMV-MCS-EF1-Puro inserted with an enhancer in front of CMV), respectively. pX458-mCherry was created by replacing the GFP segment of pX458 with mCherry. The FLAG-TagBFP plasmid was generated by replacing the EGFP segment of pEGFP-C2 with 3× FLAG-TagBFP. FLAG-TagBFP-lamin C-WT, FLAG-TagBFP-lamin C-5A and FLAG-TagBFP-lamin C-5A-FUSN were produced by inserting lamin C-WT, lamin C-5A and lamin C-5A-FUSN into the FLAG-TagBFP plasmid, respectively.

Cell culture, transfection, lentiviral transduction and cell line generation

HeLa and HEK293T cells were obtained from the American Type Culture Collection (ATCC, USA) and were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS). The cells were maintained at 37 °C in an atmosphere of 5% CO₂. Lipofectamine 2000 (Thermo Fisher Scientific, USA) was used to transfect the plasmids into the HeLa cells, and PEI was used to transfect the plasmids into the HEK293T cells according to the manufacturer's instructions. For transient overexpression, the plasmids were expressed for 24–48 h.

For lentiviral transduction, HEK293T cells were transfected with pCDH-EGFP-lamin A, pCDH-EGFP-lamin C, pCDH-E-mCherry-lamin A, pCDH-E-mCherry-lamin C-WT or pCDH-E-mCherry-lamin C-5A, along with pMD2.G and psPAX2, using PEI in serum-free DMEM. The medium was replaced at 6 h after transfection with fresh DMEM supplemented with 30% FBS. After 72 h, the virus-containing supernatant was collected by centrifugation at 3000 × *g* for 15 min. Next, 20% PEG-it virus precipitation solution (SBI, USA) was added, and the mixture was incubated at 4 °C overnight. Then, the virus particles were collected by centrifugation at 1500 × *g* for 30 min at 4 °C, followed by resuspension in PBS or serum-free DMEM. The viruses and 8 µg/mL polybrene (Sigma, USA) were subsequently added to dishes containing HeLa cells. After 6 h, the medium was replaced with fresh DMEM supplemented with 10% FBS. After 72 h, the cells were sorted by flow cytometry.

The RFP-H2B stable cell line was described in our previous publication (Lin et al., 2020). Single GFP-positive colonies were isolated by flow cytometry after the transduction of HeLa cells with the lentivirus to establish stable GFP-lamin A and GFP-lamin C cell lines, and

one colony expressing low levels of GFP-lamin A or GFP-lamin C was used for subsequent experiments. For the establishment of stable mC-LA, mC-LC-WT and mC-LC-5A cell lines, after the transduction of *LMNA*-KO HeLa cells with a lentivirus, pools of cells with a moderate fluorescence intensity were screened by flow cytometry, and the screens were performed three times. These three cell lines presented the same fluorescence intensity pattern. *LMNA*-KO HeLa cells and LAO-LCmC cells were generated using CRISPR-Cas9 technology. All the cell lines were free of mycoplasma contamination.

CRISPR-mediated gene editing and *LMNA*-KO and LAO-LCmC cell line generation

CRISPR-Cas9 gene editing was performed according to published protocols (Ran et al., 2013; Koch et al., 2018). Briefly, the *LMNA* knockout (KO) HeLa cell line was generated by transfecting the pX458 plasmid containing the guide sequence (5'-CATCGACCGTGTGCGCTCGC^{3'}) into HeLa cells. After 48 h, the cells were dissociated into single cells, and the GFP-positive cells were seeded on 96-well plates using a flow cytometer. After two weeks, single colonies were picked, and the genomic fragment of the human *LMNA* gene was amplified by PCR using the primer pair 5'-GCACCTACACCAGCCAACCCAGAT^{3'}/5'-CGCGCTCCTTGGCTACTGAGTCAAG^{3'}. The PCR products were sequenced, and the corresponding cells were examined by western blotting (WB) and immunofluorescence (IF) staining. One colony was used for subsequent experiments. Lamin C-mClover knock-in (LAO-LCmC) cells were generated by cotransfecting the pX458-mCherry plasmid containing the guide sequence (5'-AGCGGCGGCTACCACTCACG^{3'}) and the donor plasmid into HeLa cells. Forty-eight hours later, the mCherry-positive cells were collected using a flow cytometer and cultured for 4–6 days. The cells were subsequently dissociated into single cells, and the mClover-positive cells were seeded in 96-well plates using a flow cytometer. After two weeks, single colonies were picked, and the genomic fragment of the human *LMNA* gene was amplified by PCR using the primer pair 5'-CCCTTGCTGACTTACCGGTTCCCACCAAAGTTCA^{3'}/5'-GTGACACTGGAGGCAGAAGAGCCAGAGGAGAT^{3'}. The expected PCR products were sequenced, and the corresponding cells were examined by WB and IF staining. One colony was used for subsequent experiments.

Cell treatments

For H₂O₂ treatment, cells cultured on dishes or plates were washed twice with PBS or HBSS (Solarbio, China). HBSS containing the indicated concentration of H₂O₂ (Aladdin, China) was subsequently added to the dishes or plates, and the dishes or plates were incubated at 37 °C in an atmosphere of 5% CO₂ until use. The process must be performed away from light. Afterward, the cells were washed three times with preheated PBS. The cells were then collected, or preheated fresh medium was added to allow for cell recovery. For 1,6-hexanediol treatment, the cells were cultured on 35 mm glass-bottom dishes supplemented with 2 ml of medium containing the indicated concentration of 1,6-hexanediol (Sigma, USA), and images were acquired every 10 s. For other related treatments, the cells were seeded on plates or dishes with glass coverslips, fresh medium containing the drugs was added, and the cells were cultured at 37 °C in an atmosphere of 5% CO₂ until use. The drug treatment concentrations and times were as follows: VP-16 (MCE, USA) at 100 µM for 4 h, cisplatin (MCE, USA) at 20 µM for 24 h, menadione (Selleck, USA) at 50 µM for 1 h, hydroxyurea (HU) (Sigma, USA) at 5 mM for 2 h, actinomycin D (MCE, USA) at 2 µM for 4 h, sucrose at 0.2 M for 2 h, D- sorbitol (Aladdin, China) at 0.4 M for 2 h, 5,6-dichlorobenzimidazole 1-β-D-ribofuranoside (DRB; Sigma, USA) at 75 µM for 4 h, MG132 (Selleck, USA) at 20 µM for 2 h, and olaparib (MCE, USA) at 10 µM for 18 h. For serum starvation, the cells were cultured in serum-free DMEM at 37 °C with 5% CO₂ for 18 h, and for heat shock, the cells were incubated at 42 °C for 2 h, followed by an incubation at 37 °C for 0.5 h in an atmosphere of 5% CO₂.

Immunofluorescence staining

The cells were seeded on glass coverslips, fixed with 4% PFA at room temperature for 15 min, permeabilized with 0.2% Triton X-100 for 5 min, and incubated with primary antibodies (diluted in PBS containing 3% BSA) overnight at 4 °C. For the detection of PCNA, RPA2, RPA1, POLD1, PRDX1, PRDX2, PRDX6 and LAP2, the cells were fixed with cold methanol on ice for 5 min. After washing, the cells were incubated with primary antibodies overnight at 4 °C and secondary antibodies (diluted in PBS containing 3% BSA) for 1 h at room temperature. The coverslips were mounted on glass slides in Mowiol with

or without 1 µg/ml DAPI. Immunofluorescence was detected using a Delta Vision Elite microscope (Applied Precision, USA) or a high-speed spinning disk confocal microscope (Dragonfly; Andor, England). The images were processed and analyzed using Volocity software. All the images of immunofluorescence staining were processed for maximum intensity projection.

The antibodies used in this study were anti-lamin A/C (sc-7292, Santa Cruz, USA), anti-phospho-lamin A/C (Ser22) (PA5-17113, Invitrogen, USA), anti-lamin B1 (sc-6216, Santa Cruz, USA), anti-lamin C (LS-B2972, LSBio, USA), anti-BrdU/CldU (Ab6326, Abcam, England), anti-BrdU/IdU (347580, BD Biosciences, USA), anti-GAPDH (60004-1-Ig, Proteintech, China), anti-GFP/mClover (sc-9996, Santa Cruz, USA), anti-GFP/mClover (A11122, Invitrogen, USA), anti- α -tubulin (T9026, Sigma, USA), anti-SC35 (ab11826, Abcam, England), anti-PSPC1 (sc-374181, Santa Cruz, USA), anti-Bmi1 (6964, CST, USA), anti-PML (sc-966, Santa Cruz, USA), anti-HSF1 (ab52757, Abcam, England), anti-coilin (sc-55594, Santa Cruz, USA), anti-RPA194 (sc-28714, Santa Cruz, USA), anti-B23 (B0556, Sigma, USA), anti-PCNA (sc-56, Santa Cruz, USA; 10205-2-AP, Proteintech, China), anti-RPA2 (2208, CST, USA; 10412-1-AP, Proteintech, China), anti-RPA1 (ab79398, Abcam, England), anti-POLD1 (sc-17776, Santa Cruz, USA), anti-LAP2 (611000, BD Biosciences, USA), anti-PRDX1 (15816-1-AP, Proteintech, China), anti-PRDX2 (10545-2-AP, Proteintech, China), anti-PRDX6 (13585-1-AP, Proteintech, China), anti-LIG1 (18051-1-AP, Proteintech, China), anti-FLAG (MA1-142, Invitrogen, USA), anti-mouse/rabbit/rat/goat IgG and Alexa Fluor 405/488/594/647 (secondary antibodies for IF, Thermo Fisher Scientific, USA).

EdU incorporation assay and flow cytometry

The EdU (5-ethynyl-2'-deoxyuridine) incorporation assay was performed using kits (C0081 or C0071, Beyotime Biotechnology, China) according to the manufacturer's instructions. Briefly, HeLa cells were treated with 10 µM EdU for 30 min and fixed with 75% ethanol or 2% PFA (followed by permeabilization with 0.2% Triton X-100 for 10 min). The cells were subsequently incubated in the Click reaction cocktail for 30 min at room temperature, followed by staining with FxCycle™ PI/RNase Staining Solution (F10797, Thermo Fisher Scientific,

USA) for 15 to 30 min or with 10 $\mu\text{g/mL}$ Hoechst 33342 for 10 min at room temperature. A total of 10,000 cells per sample were processed and analyzed using a flow cytometer (LSRFortessa or FACSVerser, Becton Dickinson, USA), and the data were analyzed with FlowJo.

DNA fiber assay

The cells were sequentially pulse labeled with 60 μM 5-chloro-2-deoxyuridine (CldU) and 120 μM 5-iodo-2-deoxyuridine (IdU) for 30 min each. Following labeling, the cells were trypsinized and resuspended at a concentration of 1×10^6 cells/ml in PBS. A 2- μl aliquot of the cell suspension was placed onto a glass slide and lysed in 10 μl of lysis buffer (200 mM Tris-HCl, pH 7.5, 50 mM EDTA, 0.5% w/v SDS). After 3 min, the slides were tilted at an angle of 30–40 degrees, allowing the drops to run down the slides slowly. The DNA spreads were air-dried overnight and subsequently fixed with methanol/acetic acid (3:1) at 4 $^{\circ}\text{C}$ for 1 h. The DNA fibers were denatured with 2.5 M HCl for 1 h at room temperature, neutralized with sodium borate buffer (pH 8.5) for 7 min, washed three times with PBST (PBS supplemented with 0.1% Tween-20), and blocked with 5% BSA in PBST for 20 min. After blocking, the slides were incubated for 1.5 h in the dark at 37 $^{\circ}\text{C}$ with a rat anti-CIdU antibody (1:200), washed three times, and incubated for 45 min in the dark at 37 $^{\circ}\text{C}$ with anti-rat IgG Alexa Fluor 488 (1:200) to detect CldU. Thereafter, the slides were washed three times, blocked again, incubated for 1.5 h in the dark at 37 $^{\circ}\text{C}$ with a mouse anti-IdU antibody (1:100), and washed once with PBST containing 500 mM NaCl and four times with PBST. The slides were subsequently incubated for 45 min in the dark at 37 $^{\circ}\text{C}$ with anti-mouse IgG Alexa Fluor 594 (1:200) to detect IdU. The slides were then washed three times and mounted in Mowiol. Images were acquired using a Dragonfly microscope. The IdU tract lengths were measured using the segmented line tool of Imaris. Data from two independent experiments were combined.

Live-cell imaging

For live-cell imaging, cells were seeded on glass-bottom dishes. After transfection for approximately 30 h, the dishes were placed in a heated chamber (37 $^{\circ}\text{C}$) supplemented with 5% CO_2 . Images were captured at 3-minute intervals or at the indicated time intervals using

a Dragonfly microscope (63×/1.40 NA) and processed with Volocity software. All the images of immunofluorescence staining were processed for maximum intensity projection.

FRAP

The cells were seeded on glass-bottom dishes. WT HeLa cells were transfected with GFP-lamin A and C for 24 h, or LAO-LCmC HeLa cells were treated with H₂O₂ for 40 min. FRAP experiments were performed on a spinning disk microscope (60×/1.42 NA; UltraView VoX, PerkinElmer, USA). The condensates were bleached using a laser at 488 nm. Recovery was recorded at the indicated time intervals. The fluorescence intensity was normalized to the reference intensity before bleaching. The data were analyzed using Volocity and GraphPad Prism 8.

Protein expression and purification

Genes encoding His-GFP-lamin C-Δ1BΔ2B59 or His-GFP-lamin C-Δ1BΔ2B59-5A were cloned and inserted into the pET-28a vector to obtain soluble recombinant proteins. *Escherichia coli* T7 express strains (BC206, Biomed, China) were transformed with these plasmids, inoculated into LB media supplemented with 30 μg/ml kanamycin, and cultured in shaker flasks at 37 °C. When the cultures reached an OD₆₀₀ of 0.8, IPTG (0.3 mM) was added to induce expression, and the cultures were incubated for 15 h with shaking at 18 °C. The cell pellets were subsequently collected and resuspended in lysis buffer (25 mM Tris-HCl, pH 8.0, 500 mM KCl, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride (PMSF) and protease mixture inhibitor cocktail). The cells were sonicated and clarified by centrifugation for 20 min at 14,600 × *g*. The clarified lysates were incubated with Ni NTA beads (Smart Lifesciences, China) for 2 h. The beads were washed with wash buffer (25 mM Tris-HCl, pH 8.0, 500 mM KCl, 10% glycerol, 20 mM imidazole), and the proteins were eluted with elution buffer (25 mM Tris-HCl, pH 8.0, 500 mM KCl, 10% glycerol, 400 mM imidazole). Thereafter, imidazole was removed, and the proteins were concentrated using an Amicon® Ultra 30K centrifugal filter device (Millipore, USA). The protein concentration was measured using an *Easy Protein Quantitative Kit* (TransGen, China). The proteins were suspended in storage buffer (25 mM Tris-HCl, pH 8.0, 500 mM KCl, and 10% glycerol), aliquoted into PCR tubes, flash-frozen in liquid nitrogen, and stored at –80 °C.

In vitro phase separation assay

His-GFP-lamin C- $\Delta 1B\Delta 2B59$ or His-GFP-lamin C- $\Delta 1B\Delta 2B59$ -5A proteins in storage buffer were diluted to various concentrations in a buffer composed of 25 mM Tris-HCl (pH 7.5), 3% glycerol, and 150 mM KCl supplemented with or without 10% polyethylene glycol 3350 (PEG3350). After 30 min, the mixtures were added to a slide chamber (81506, ibidi, Germany). The images were captured using an UltraView VoX microscope (60 $\times$ /1.42 NA).

Immunoprecipitation

HEK293T cells transfected with the indicated plasmids or LAO-LCmC cells subjected to the indicated conditions were collected and lysed in lysis buffer (20 mM Tris-HCl, pH 7.5, 130 mM NaCl, 1% Triton X-100, 10 mM NaF, 2 mM Na₃VO₄, 0.1 mM CaCl₂, 2 mM MgCl₂, 10% glycerol) supplemented with 500 U/ml benzonase, 1 mM PMSF, and protease inhibitor cocktail at 4 °C for 10 min and subsequently at 37 °C for 30 to 60 min. The lysates were centrifuged at 15,000 $\times g$ for 10 min twice. The supernatants were incubated with GFP-Trap beads (ChromoTek, Germany) for 2 h at 4 °C. Following five washes with lysis buffer, the beads were suspended in 1 $\times$ SDS sample buffer and boiled at 95 °C for 5 min. Then, Western blotting or mass spectrometry was performed on the samples.

Mass spectrometry analysis

An immunoprecipitation assay was performed using LAO-LCmC cells treated with DMEM (control) or HBSS containing 500 μ M H₂O₂ for 1 h to identify the phosphorylation sites of lamin C.

LMNA-KO cells transiently expressing mClover and LAO-LCmC cells incubated with HBSS or 250 μ M H₂O₂ for 1 h were treated with 1% formaldehyde (Sigma, USA) for 10 min to crosslink proteins within the lamin C bodies and to identify the protein components of lamin C bodies. The assay was performed via rapid immunoprecipitation mass spectrometry (RIME) (Mohammed et al., 2016). Briefly, cells crosslinked with formaldehyde were treated with glycine to quench the reaction and lysed in cold LB1 medium (50 mM HEPES-KOH, pH 7.5, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100) supplemented with 1 mM PMSF and a protease inhibitor cocktail at 4 °C for 10 min. The

lysates were then clarified by centrifugation at $2,000 \times g$ for 5 min at 4 °C. The pellets were suspended in cold LB2 medium (10 mM Tris-HCl, pH 8.0, 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA) supplemented with 1 mM PMSF and a protease inhibitor cocktail and rotated at 4 °C for 5 min. The lysates were again clarified by centrifugation, and the pellets were suspended in cold LB3 medium (10 mM Tris-HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% (wt/vol) sodium deoxycholate and 0.5% (vol/vol) N-lauroylsarcosine) supplemented with 1 mM PMSF and a protease inhibitor cocktail. The mixtures were subsequently sonicated on ice. Afterward, 1% Triton X-100 was added before the lysates were clarified by centrifugation at $20,000 \times g$ for 10 min at 4 °C. The supernatants were incubated with GFP-Trap beads and rotated at 4 °C overnight. The next day, the beads were washed with RIPA buffer (50 mM HEPES-KOH, pH 7.6, 1 mM EDTA, 0.1% (wt/vol) sodium deoxycholate, 1% NP-40, 0.5 M LiCl) supplemented with 1 mM PMSF and a protease inhibitor cocktail at 4 °C. Finally, 2× SDS sample buffer supplemented with 200 mM dithiothreitol (DTT) was added to the beads, which were boiled at 95 °C for 35 min.

The resulting samples were processed and analyzed on a Thermo Orbitrap Fusion Lumos (Thermo Fisher Scientific, USA) or a Thermo Orbitrap Exploris 480 (Thermo Fisher Scientific, USA). The MS data were searched against the Human Reviewed Swiss-Prot protein database with Proteome Discoverer 2.2 software.

In Situ Proximity Ligation Assay

The cells were grown on coverslips, treated with 10 μ M EdU for 10 min, and fixed with -20 °C methanol. The cells were then incubated in Click reaction cocktail (one-twentieth azide 488/647 for HBSS and one-fifth azide 488/647 for H₂O₂) for 30 min at room temperature. Afterward, the cells were washed three times with PBS, and a proximity ligation assay (PLA) was performed using Duolink PLA technology (Sigma, USA) according to the manufacturer's instructions. The primary antibodies used in this assay were rabbit anti-PCNA (1:400) and mouse anti-POLD1 (1:100). For WT and *LMNA*-KO HeLa cells, red fluorescence detection reagents were used, whereas for mC-LA cells transfected with FLAG-TagBFP, FLAG-TagBFP-lamin C-WT, 5A or 5A-FUSN, farRed fluorescence detection reagents were used. The mC-LA cells transfected with the indicated plasmids were then

incubated with a rat anti-FLAG antibody (1:200) overnight at 4 °C and anti-rat IgG Alexa Fluor 405 antibody (1:200) for 1 h at room temperature after PLA procedures. The coverslips were mounted on glass slides in Mowiol with or without DAPI. Immunofluorescence was detected using a Dragonfly microscope. The images were processed and analyzed using Volocity software. The images were processed for maximum intensity projection.

Statistical analysis

All the statistical analyses were performed with the unpaired two-tailed *t* test using GraphPad Prism 8 software. Not significant (ns), $P > 0.05$ * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

Supplemental Figures and Legends

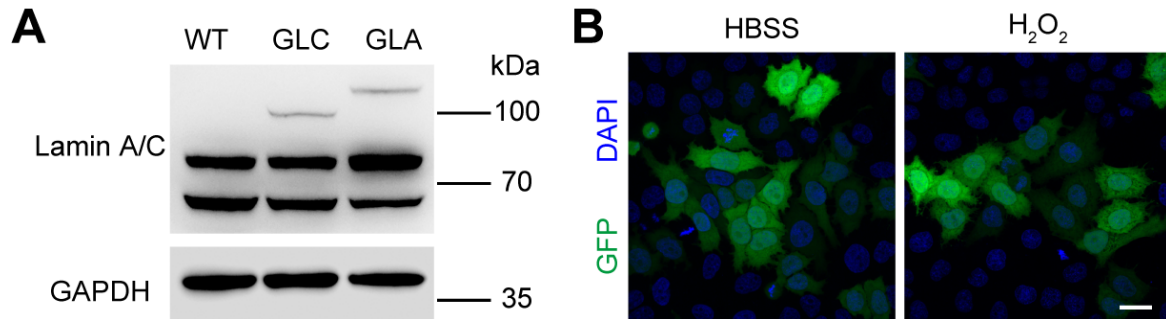


Figure S1. Stable GFP-lamin A- and GFP-lamin C-expressing cells. (A) Western blot of stable GFP-lamin A (GLA)- and GFP-lamin C (GLC)-expressing cells. Endogenous lamin A/C, exogenous GFP-lamin A and GFP-lamin C were detected by anti-lamin A/C antibodies. **(B)** Representative images of HeLa cells transiently expressing GFP treated with HBSS or 250 μ M H₂O₂ for 1 h. DAPI (blue). Scale bar, 20 μ m.

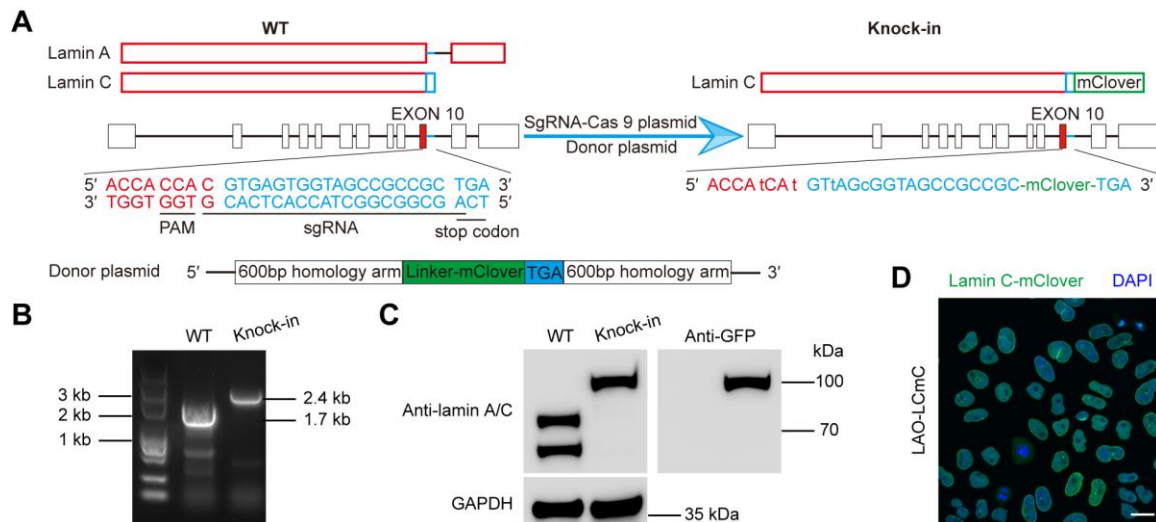


Figure S2. Generation and validation of the endogenously tagged lamin C-mClover (LAO-LCmC) HeLa cell line. (A) Schematic of the knock-in strategy using CRISPR/Cas9. Red indicates the shared sequence of lamin A and lamin C. Cyan indicates the specific sequence of lamin C. The small letters indicate synonymous mutations introduced by CRISPR/Cas9. **(B)** Agarose gel of genotyping PCR for clonal lines of LAO-LCmC HeLa cells. **(C)** Immunoblot validation of the endogenous lamin C expression level in the LAO-LCmC HeLa cell line. Lysates from WT and LAO-LCmC HeLa cells were subjected to immunoblotting with the indicated antibodies. The shift in molecular weight is of the size expected for the mClover fusion protein. **(D)** Representative images of lamin C-mClover (green) in the LAO-LCmC HeLa cell line. The cells were fixed with PFA and stained with DAPI (blue). Scale bar, 20 μ m.

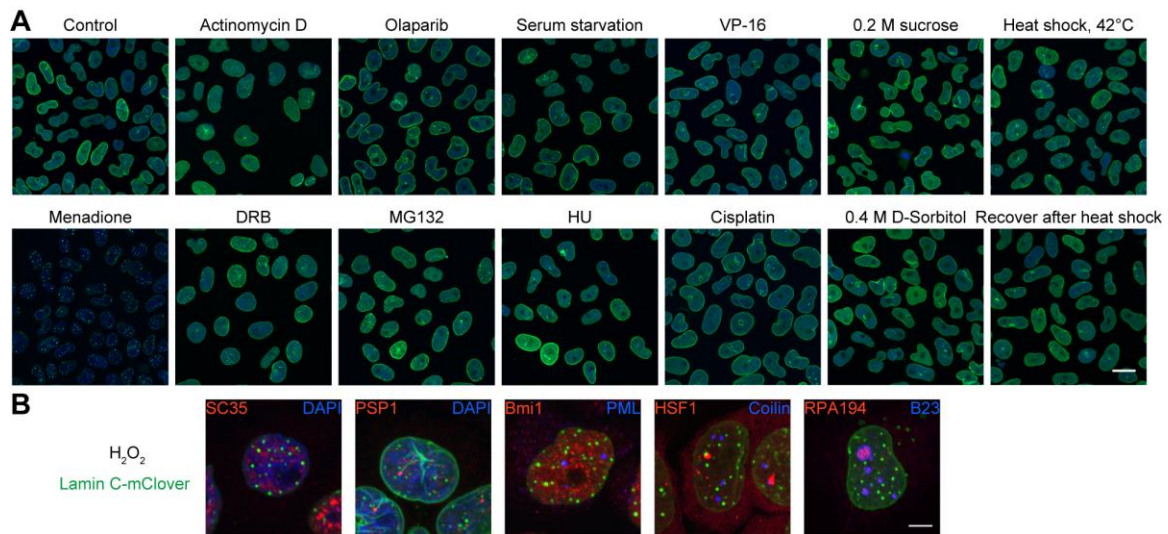


Figure S3. Lamin C condensates are oxidative stress-specific nuclear bodies. (A) In addition to oxidative stress (menadione), other stresses we tested, such as DNA damage (VP-16 and cisplatin), heat shock (42 °C), DNA replication stress (HU), RNA transcription stress (actinomycin D and DRB), hyperosmotic stress (sucrose and D-sorbitol), proteasome inhibition (MG132), PARP inhibition (olaparib) and serum starvation, failed to induce the formation of lamin C condensates. Representative images of lamin C-mClover (green) in LAO-LCmC cells under the indicated conditions. After the treatments, the cells were fixed with PFA and stained with DAPI (blue). Scale bar, 20 μ m. **(B)** The nuclear lamin C condensates host no known markers of other nuclear bodies that we tested, including SC35 for nuclear speckles, PSP1 for paraspeckles, Bmi1 for polycomb bodies, PML for PML bodies, HSF1 for nuclear stress bodies, Coilin for Cajal bodies, and RPA194 and B23 for nucleoli. Representative images of lamin C-mClover condensates and other nuclear bodies in LAO-LCmC HeLa cells. The LAO-LCmC HeLa cells were treated with 250 μ M H_2O_2 for 40 min, fixed with methanol and stained with antibodies against SC35 (red)/DAPI (blue), PSP1 (red)/DAPI (blue), Bmi1 (red)/PML (blue), HSF1 (red)/Coilin (blue) or RPA194 (red)/B23 (blue). Scale bar, 5 μ m.

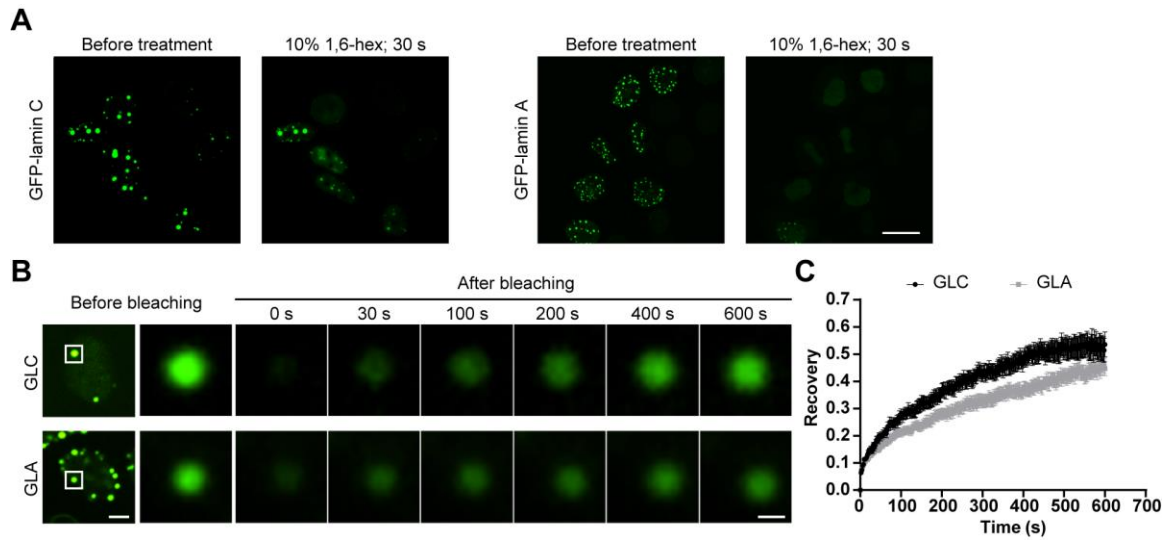


Figure S4. Lamin condensates formed upon transient overexpression possess liquid-like properties. **(A)** Representative images of HeLa cells transiently overexpressing GFP-lamin A and GFP-lamin C before and after treatment with 10% 1,6-hexanediol (1,6-hex) for 30 s. Scale bar, 10 μ m. **(B)** Representative images of the fluorescence intensity recovery of GFP-lamin A and GFP-lamin C condensates formed upon transient overexpression after photobleaching. Scale bars, 5 μ m and 1 μ m (zoom). **(C)** Quantitative FRAP data from **(B)**. Fifteen GLC condensates and 21 GLA condensates were counted. GFP-lamin A (GLA), GFP-lamin C (GLC).

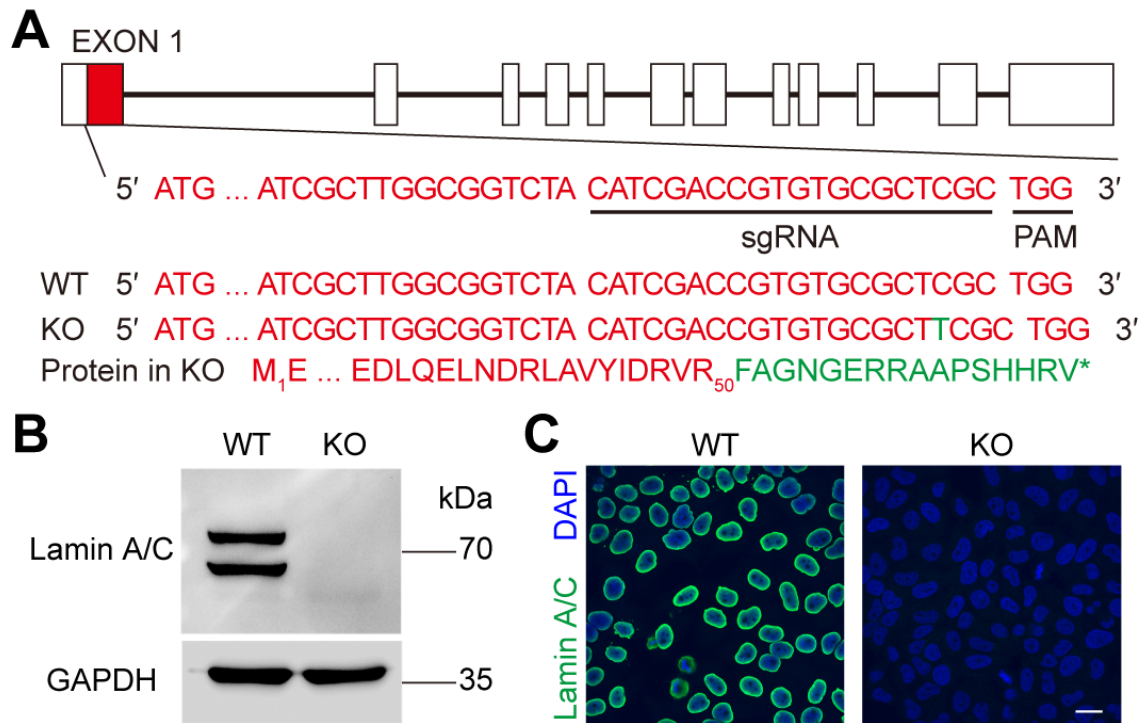


Figure S5. Generation of the *LMNA*-KO HeLa cell line. (A) Schematic of the *LMNA* knockout strategy. The *LMNA* gene sequences of WT and *LMNA*-KO HeLa cells and the protein sequences of *LMNA*-KO HeLa cells are shown. The lower numbers indicate amino acid residue positions, and the asterisk (*) indicates the translation termination site. The green letters indicate mutations in *LMNA*-KO compared with WT HeLa cells. (B) Western blot of *LMNA*-KO HeLa cells with anti-lamin A/C antibodies. (C) Immunofluorescence staining of *LMNA*-KO HeLa cells with anti-lamin A/C antibodies. The cells were fixed with PFA and stained with anti-lamin A/C antibodies (green) and DAPI (blue). Scale bar, 20 μ m.

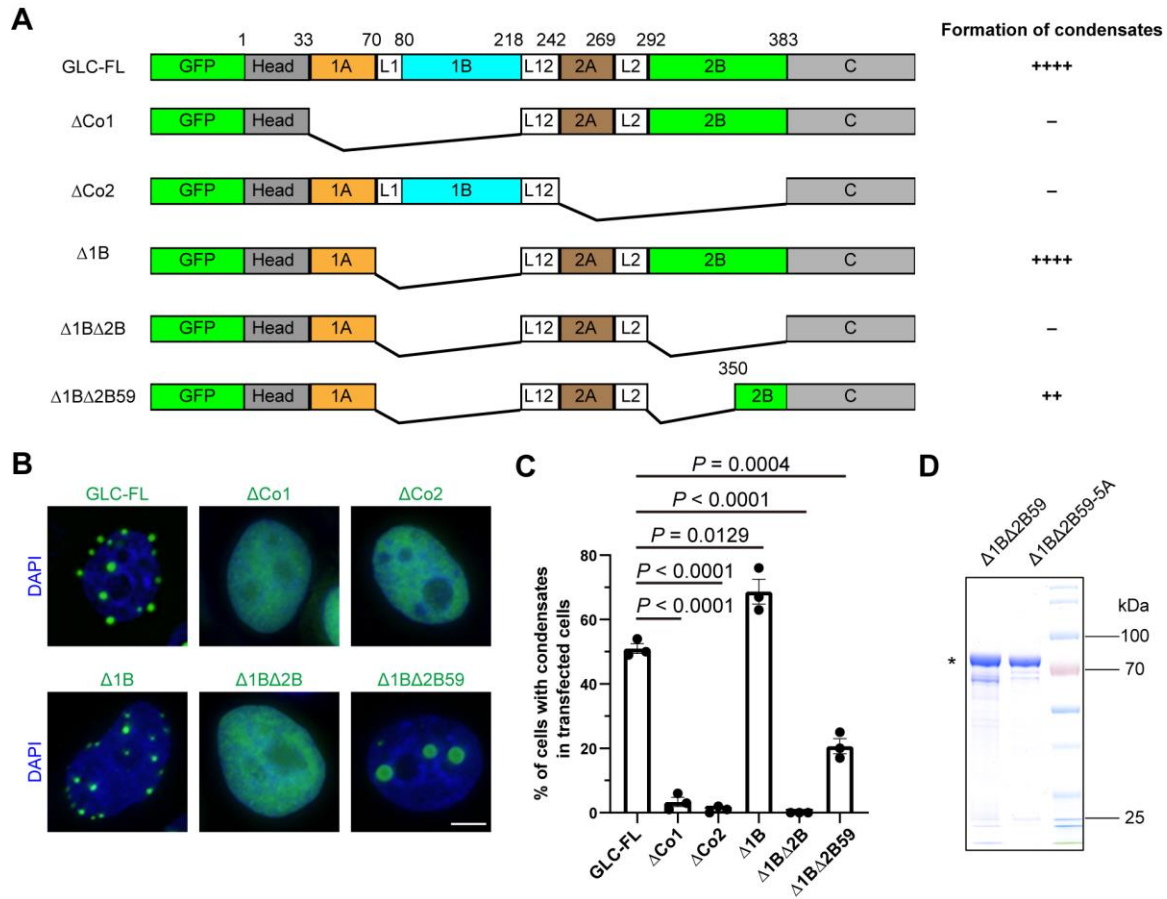


Figure S6. Some lamin C truncations lacking a partial coiled-coil domain still have the ability to form condensates. (A) Schematic diagram illustrating the truncated mutants of GFP-lamin C. The numbers denote amino acid positions in the lamin C sequence, indicating the extent of different domains. “+/-” represents the statistical range of the indicated proteins in **(C)**. “-”, < 5%; “+”, 5–15%; “++”, 15–30%; “+++”, 30–40%; “++++”, > 40%. **(B)** Representative images of *LMNA*-KO HeLa cells transiently overexpressing truncated GFP-lamin C (green), fixed with PFA and stained with DAPI (blue). Scale bar, 5 μ m. **(C)** Quantification of the frequency of cells with condensates in the transfected cells in **(B)**. The data are presented as the means $\pm$ SEMs from three independent experiments. One hundred cells were counted per sample in each experiment. **(D)** Coomassie-stained SDS-PAGE gel containing the purified GLC- Δ 1B Δ 2B59 and GLC- Δ 1B Δ 2B59-5A (a mutant of the five arginines within GLC- Δ 1B Δ 2B59) to alanines) proteins, revealing molecular weights of approximately 70 kDa for both proteins. The asterisk indicates the target band. GFP-lamin C (GLC).

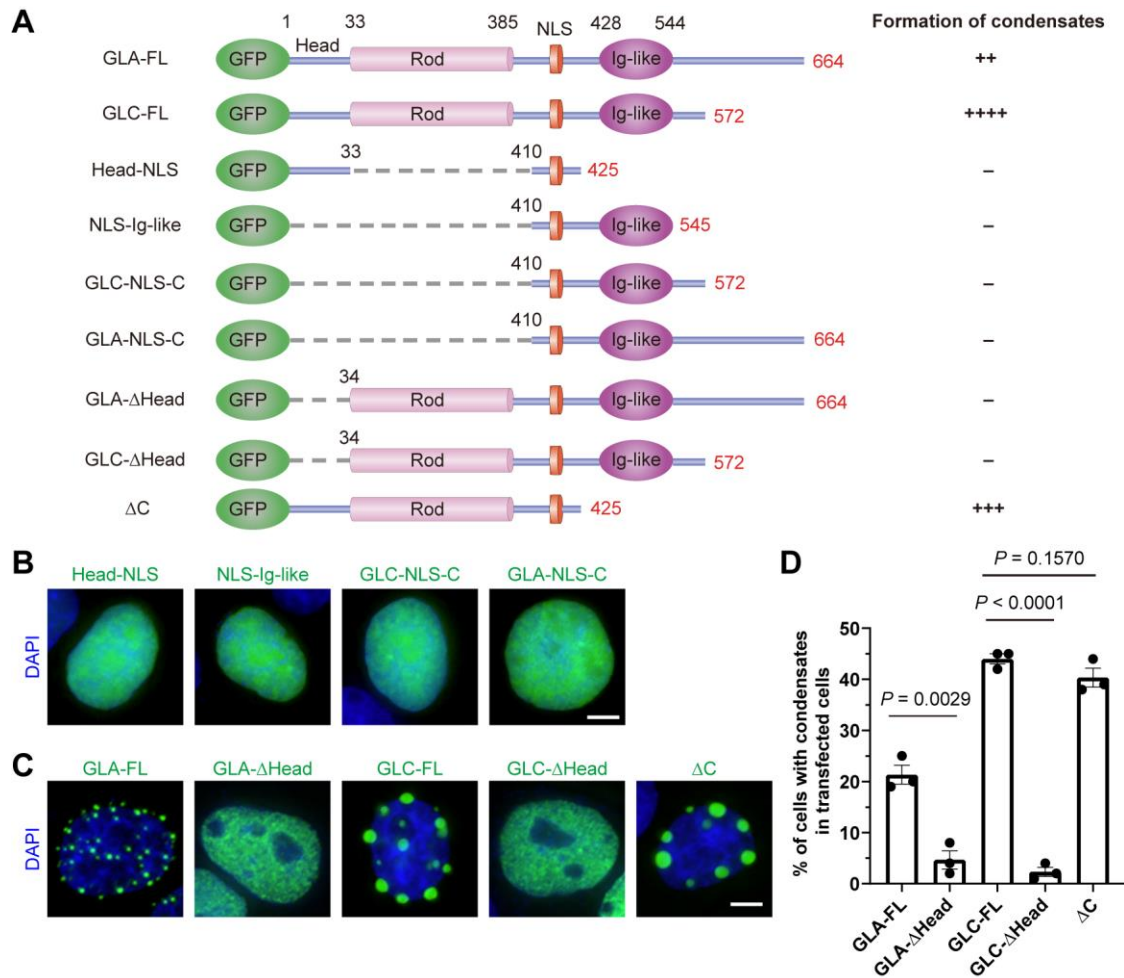


Figure S7. The head and rod domains of lamins A and C collectively contribute to phase separation. (A) Schematic of truncated mutants of GFP-lamin A/C. The numbers denote amino acid residue positions within the lamin A/C sequences, indicating the extent of different domains. The red numbers denote the termini of various truncated lamin A/C constructs. “+/-” represents the statistical range of the indicated proteins in **(D)**. “-”, < 5%; “+”, 5–15%; “++”, 15–30%; “+++”, 30–40%; “++++”, > 40%. **(B and C)** Representative images of LMNA-KO cells transiently overexpressing truncated mutants of GFP-lamin A/C (green). The cells were fixed with PFA and stained with DAPI (blue). Scale bars, 5 μ m **(B and C)**. **(D)** Quantification of the frequency of cells with condensates in the transfected cells in **(C)**. The data are presented as the means $\pm$ SEMs from three independent experiments. One hundred cells were counted per sample in each experiment. GFP-lamin A (GLA), GFP-lamin C (GLC).

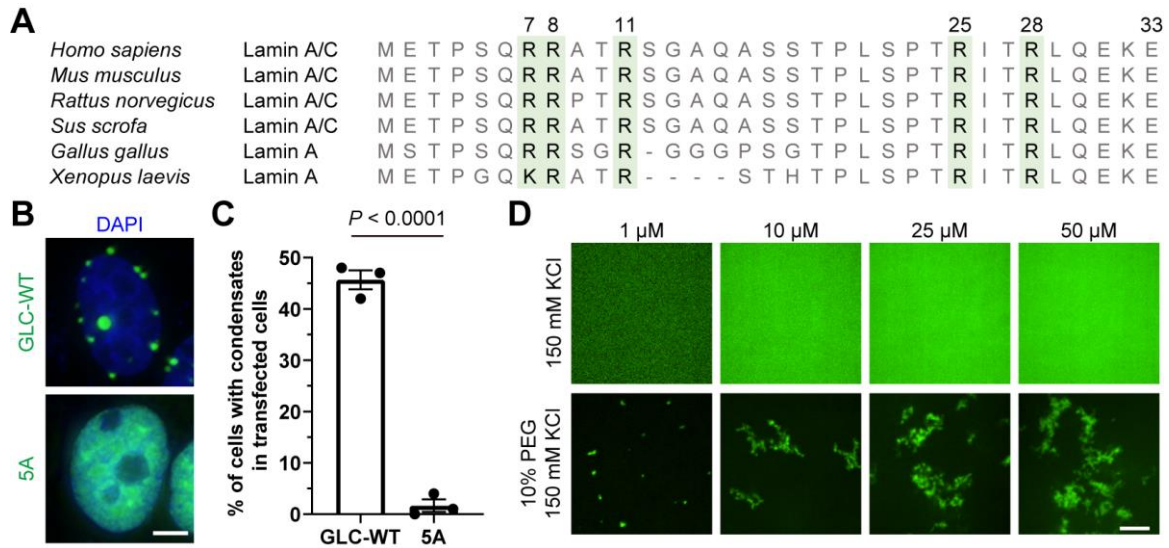


Figure S8. Positive charges of the head domain promote lamin C phase separation. (A) Multiple protein sequence alignment of the head domains of lamin A/C across different species. The numbers represent amino acid residue positions in the protein sequence. **(B)** Representative images of *LMNA*-KO HeLa cells transiently overexpressing GFP-lamin C-WT and GFP-lamin C-5A (green). The cells were fixed with PFA and stained with DAPI (blue). Scale bars, 5 μ m. **(C)** Quantification of the frequency of cells with condensates in the transfected cells in **(B)**. The data are presented as the means $\pm$ SEMs from three independent experiments. One hundred cells were counted per sample in each experiment. **(D)** In vitro phase separation assay for GFP-lamin C- Δ 1B Δ 2B59-5A, which contains five arginine-to-alanine mutations in the head domain. Representative images of the protein at the indicated concentrations in solution (25 mM Tris-HCl, pH 7.5, 3% glycerol, 150 mM KCl) with or without 10% PEG. Scale bar, 10 μ m. GFP-lamin C (GLC).

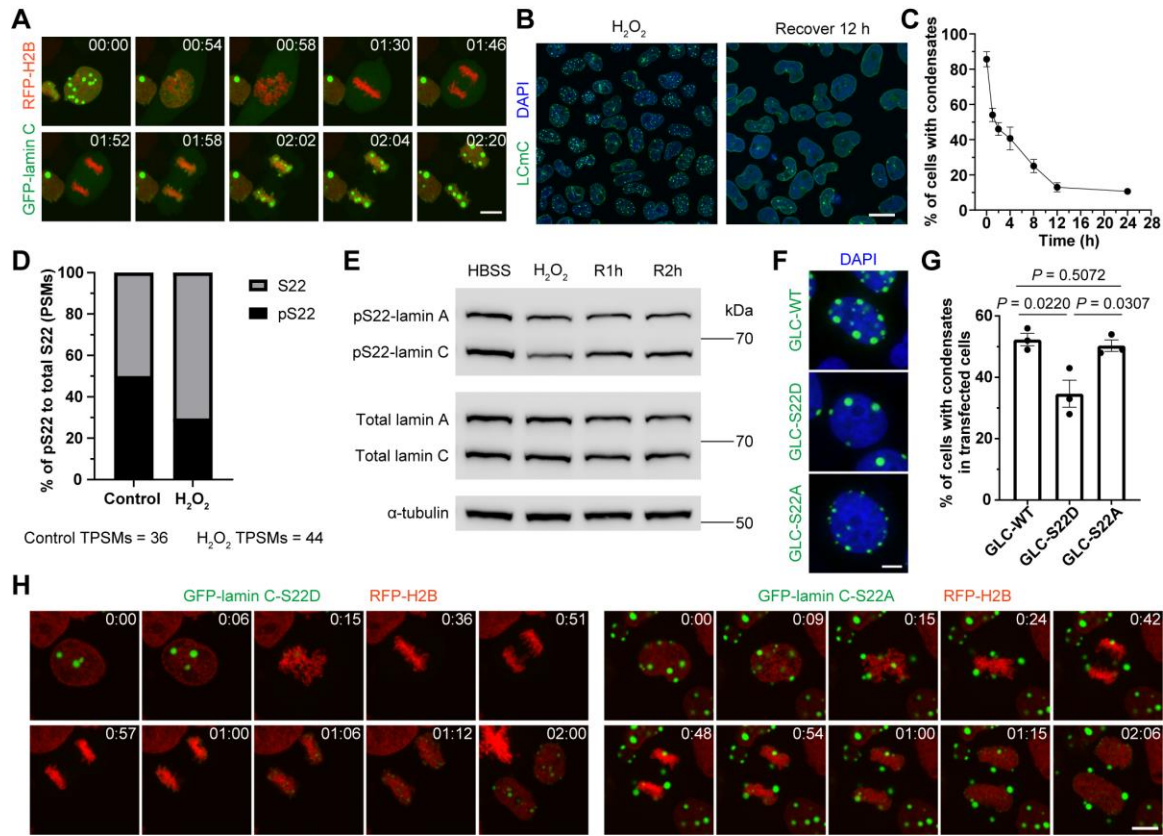


Figure S9. Lamin C phase separation is regulated by its phosphorylation at S22. (A) GFP-lamin C bodies disassemble during mitosis. GFP-lamin C (green) was overexpressed in stable RFP-H2B-expressing (red) cells. Then, live-cell imaging was performed. Scale bar, 10 μ m. (B and C) Lamin C bodies disappeared after the removal of oxidative stress. (B) Representative images of LAO-LCmC HeLa cells treated with 250 μ M H_2O_2 for 40 min and after the removal of H_2O_2 for 12 h. The LAO-LCmC HeLa cells were treated with 250 μ M H_2O_2 for 40 min, washed with PBS and incubated with fresh medium to allow cell recovery. The cells were fixed with PFA and stained with DAPI (blue) at the indicated times. LCmC, lamin C-mClover. Scale bar, 20 μ m. (C) Quantification of the frequency of cells with condensates at the indicated times after H_2O_2 removal. The data are presented as the means $\pm$ SEMs from three independent experiments. One hundred cells were counted per sample in each experiment. (D) Percentages of pS22-containing peptides to total S22-containing peptides under the indicated conditions. LAO-LCmC HeLa cells were treated with DMEM (control) or HBSS containing 500 μ M H_2O_2 for 1 h and analyzed for phosphorylation sites on lamin C by mass spectrometry. Peptide-spectrum matching (PSM) of total S22-containing peptides (TPSMs) and phospho-S22 (pS22). (E) The levels of pS22-lamin A and C in HeLa cells under the indicated conditions. The cells were treated with HBSS or H_2O_2 (250 μ M in HBSS) for 40 min, followed by recovery by washing with PBS and an incubation in medium for 1 h (R1h) or 2 h (R2h). The cell

lysates from these cells were subjected to immunoblot analysis using the indicated antibodies. pS22-lamin A/C, lamin A/C phosphorylated at S22. **(F)** Representative images of *LMNA*-KO HeLa cells expressing GFP-lamin C (GLC) mutants with serine-to-aspartic acid (GLC-S22D, green) or serine-to-alanine (GLC-S22A) substitutions. The cells were fixed with PFA and stained with DAPI (blue). Scale bar, 5 μ m. **(G)** Quantification of the frequency of cells with condensates in the transfected cells in **(F)**. One hundred cells were counted per sample in each experiment. **(H)** The disruption of S22 phosphorylation inhibits the disassembly of lamin C bodies during mitosis. GFP-lamin C-S22D/A (green) was expressed in stable RFP-H2B-expressing (red) cells. Live-cell imaging was performed using a Dragonfly microscope (63 $\times$ /1.40 NA). Scale bar, 10 μ m.

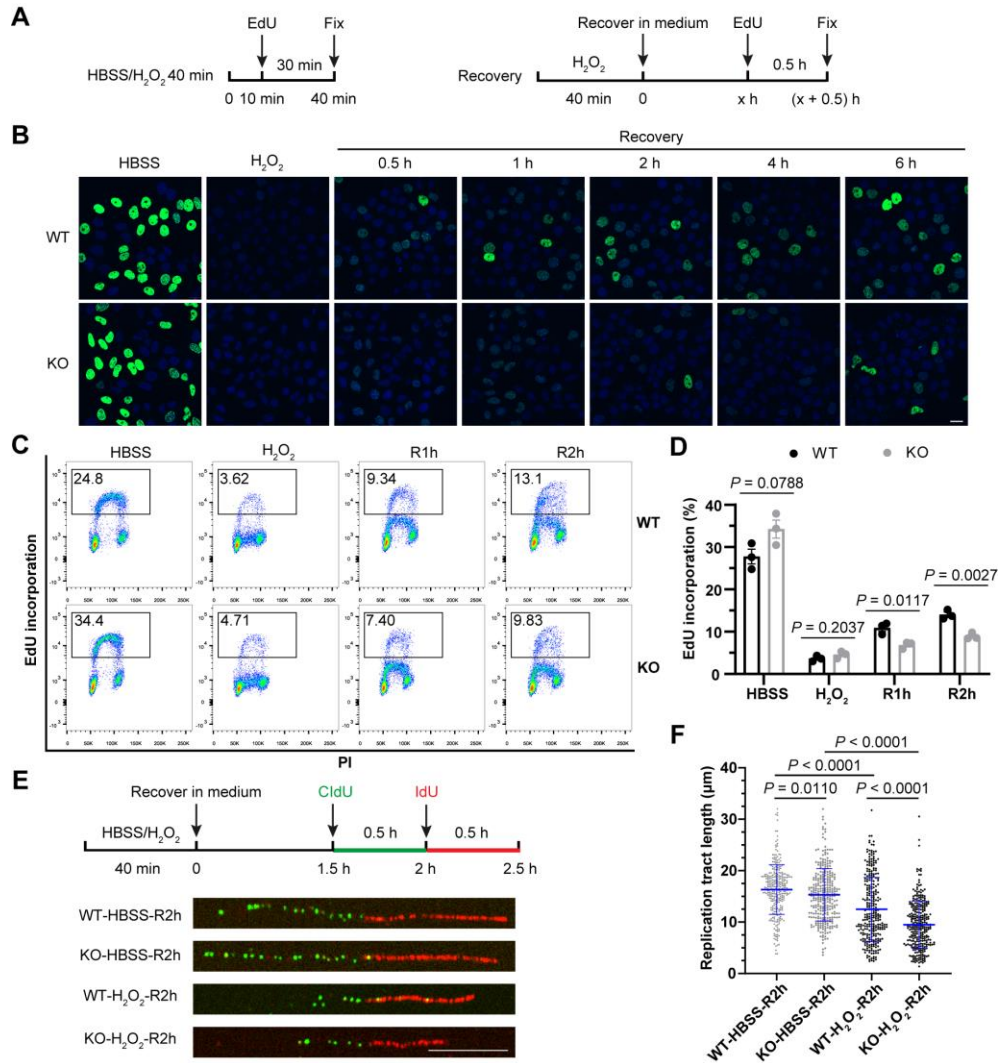


Figure S10. Lamin A/C enhances DNA replication recovery after oxidative stress. (A) Experimental scheme for (B) and (C). For the HBSS and H₂O₂ groups, 10 μ M EdU (5-ethynyl-2'-deoxyuridine) was added after treatment with HBSS/H₂O₂ (250 μ M) for 10 min, and then the cells were fixed after labeling for 30 min. For the recovery group, after treating the cells with H₂O₂ (250 μ M), the cells were washed with PBS and incubated with medium to allow cell recovery. Subsequently, 10 μ M EdU was added at the indicated times, and the cells were fixed after labeling for 30 min. (B) Representative images of EdU incorporation in WT and LMNA-KO HeLa cells under the indicated conditions. EdU (green), DAPI (blue). Scale bar, 20 μ m. (C) Flow cytometry analysis of EdU incorporation in WT and LMNA-KO HeLa cells under the indicated conditions. The x-axis represents the DNA content indicated by propidium iodide (PI) staining, and the y-axis represents the EdU intensity. The numbers represent the percentage of cells (high EdU intensity). (D) Quantification of the percentage of cells with a high EdU intensity in (C). The data are presented as the means $\pm$ SEMs from three independent experiments. (E) Scheme and representative images of the DNA fiber assay. Green indicates the CldU tract, and red indicates the IdU tract. The replication tract (IdU tract) lengths were measured. Scale bar, 10 μ m. (F) Quantification of the replication tract lengths in (E). Data points (n > 290 tracts per condition from two independent experiments) and means $\pm$ SDs are plotted. The cells were allowed to recover for 1 h (R1h) or 2 h (R2h).

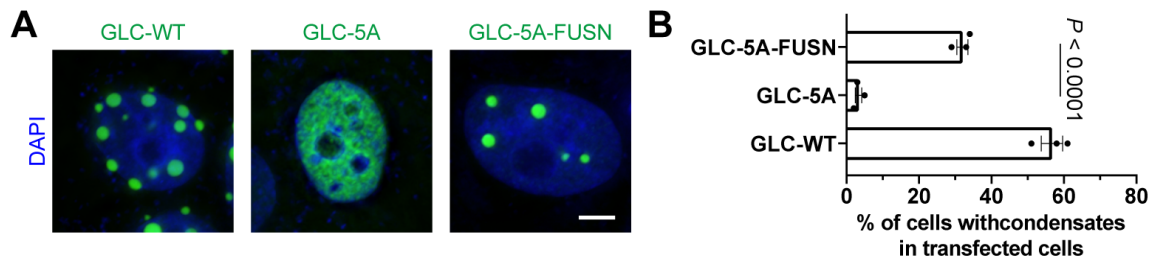


Figure S11. FUSN restores the ability of GFP-lamin C-5A to undergo phase separation. (A) Representative images of *LMNA*-KO HeLa cells transiently overexpressing GLC-WT, GLC-5A or GLC-5A-FUSN (green). The cells were fixed with PFA and stained with DAPI (blue). Scale bar, 5 μ m. **(B)** Quantification of the frequency of cells with lamin bodies in the transfected cells in **(A)**. The data are presented as the means $\pm$ SEMs from three independent experiments. One hundred cells were counted per sample in each experiment. GFP-lamin C (GLC).

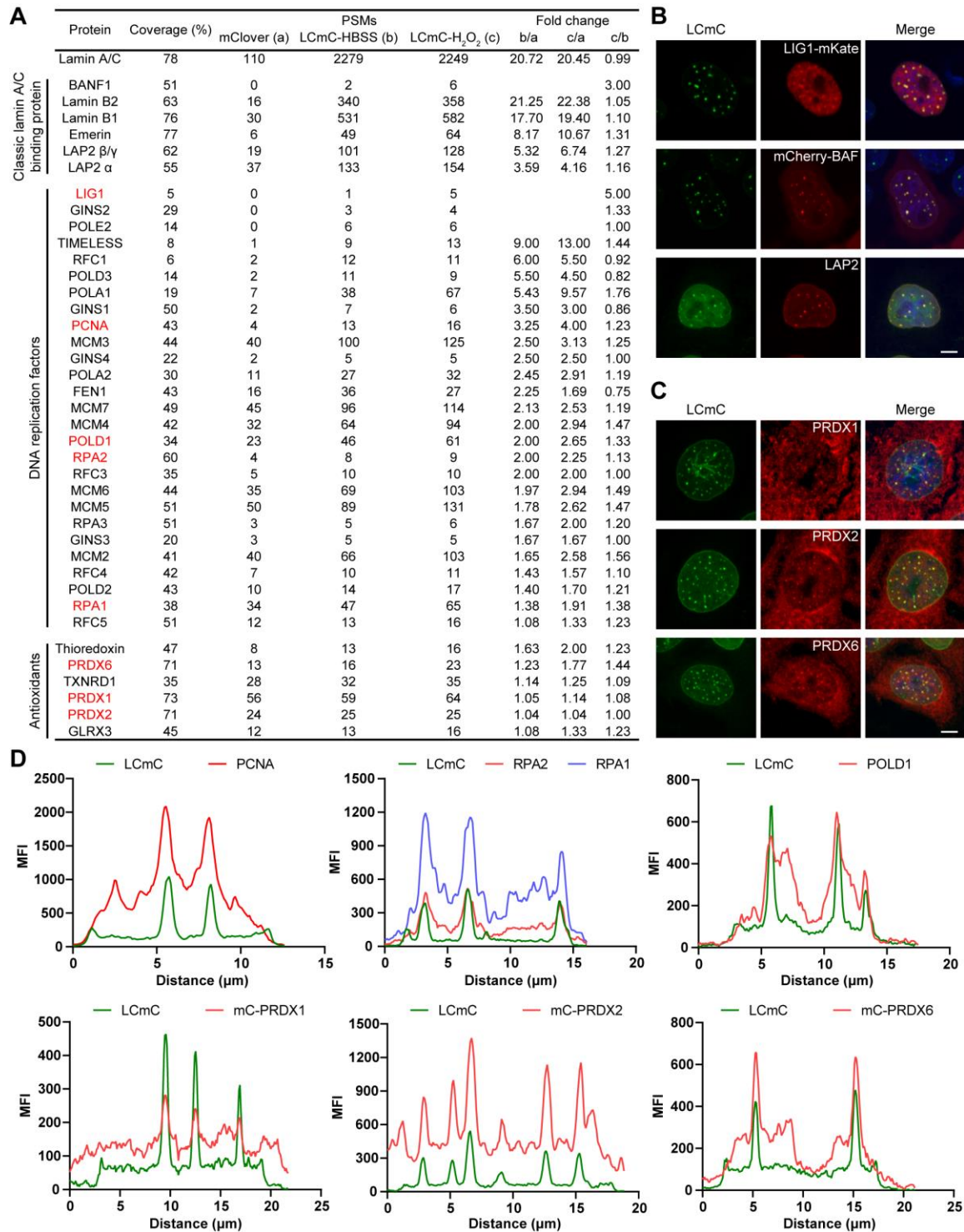


Figure S12. Lamin C bodies concentrate DNA replication factors and antioxidants under oxidative stress. (A) Proteins present in lamin C bodies identified by RIME. *LMNA*-KO HeLa cells transiently expressing mClover were treated with HBSS (a), and LAO-LCmC HeLa cells were treated

with HBSS (b) or 250 μ M H₂O₂ (c) for 1 h and then incubated with 1% formaldehyde for 10 min to crosslink proteins within the lamin C bodies. The mass spectrometry analysis was subsequently conducted according to the protocol outlined in the Methods section. "b/a" indicates the fold change in PSMs of the proteins in the LCmC-HBSS group relative to the mClover group, and if b/a > 1, it suggests that this protein may bind to lamin C under normal conditions. "c/a" indicates the fold change in PSMs of the proteins in the LCmC-H₂O₂ group relative to the mClover group, and if c/a > 1, it suggests that this protein may exist in lamin C bodies. "c/b" indicates the fold change in PSMs of the proteins in the LCmC-H₂O₂ group relative to the LCmC-HBSS group, and if c/b > 1, it suggests that the binding of this protein to lamin C is enhanced under oxidative stress. The red words indicate the proteins of interest. **(B and C)** DNA replication factors, lamin A/C-binding proteins and antioxidants are concentrated in lamin C bodies, related to **Fig. 2E and 2F**. LAO-LCmC HeLa cells transiently expressing LIG1-mKate (red), mCherry-BAF (red) or LAO-LCmC HeLa cells were treated with 250 μ M H₂O₂ for 40 min and fixed with methanol or PFA. The cells expressing LIG1-mKate and mCherry BAF were stained with DAPI (blue), and the other cells were stained with anti-LAP2 (red)/DAPI (blue), anti-PRDX1 (red)/DAPI (blue), anti-PRDX2 (red)/DAPI (blue) or anti-PRDX6 (red)/DAPI (blue). Lamin C-mClover (LCmC, green). Scale bars, 5 μ m **(B and C)**. **(D)** Quantification of the mean fluorescence intensity in **Fig. 2E and 2F**. The mean fluorescence intensity of the indicated proteins in the area crossed by the line shown in **Fig. 2E and 2F** was analyzed. The "0" in the images in **Fig. 2E and 2F** indicates the starting points of the measurements. The x-axis represents the distance from the starting point. MFI, mean fluorescence intensity.

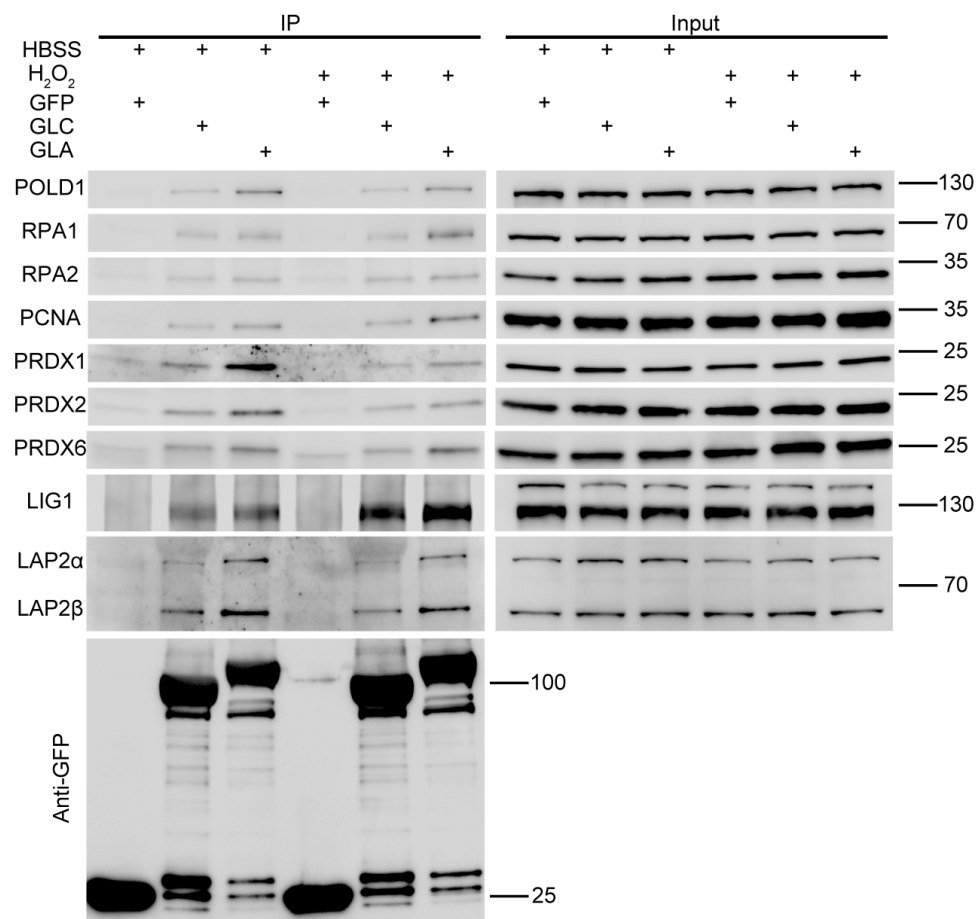


Figure S13. Lamin A and lamin C interact with DNA replication factors and PRDXs. HEK293T cells transfected with GFP, GFP-lamin A (GLA), or GFP-lamin C (GLC) were treated with HBSS/H₂O₂ (250 μM) for 1 h and collected for immunoprecipitation. The binding proteins were detected using the indicated antibodies.

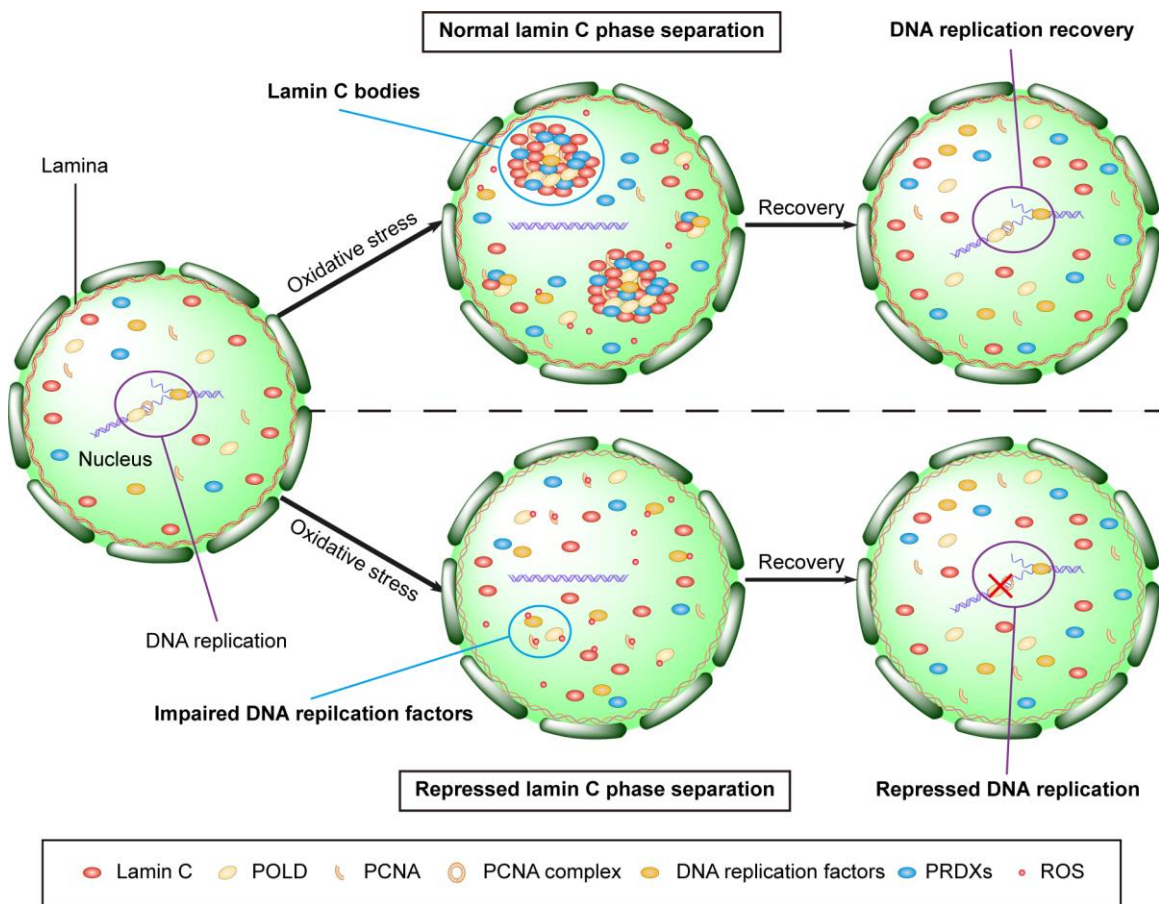


Figure S14. A working model of the role of lamin C in preserving DNA replication factors during oxidative stress. Oxidative stress induces a deceleration or arrest of DNA replication. Under such circumstances, lamin C undergoes phase separation to assemble the lamin C bodies. Lamin C binds DNA replication factors (PCNA, POLD and others) and antioxidants (PRDXs) and gathers them into these lamin C bodies. Lamin C bodies protect DNA replication factors during oxidative stress, which may be achieved by the more effective removal of ROS through the antioxidants in lamin C bodies. Upon the removal of oxidative stress, lamin C bodies disassemble, liberating DNA replication factors to enable them to participate in DNA replication recovery. The loss of lamin C or repression of lamin C phase separation leads to impaired functions of DNA replication factors and hence the repression of DNA replication recovery after oxidative stress.

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