

B-type lamins maintain transcriptional homeostasis by spatially controlling chromatin–speckle proximity

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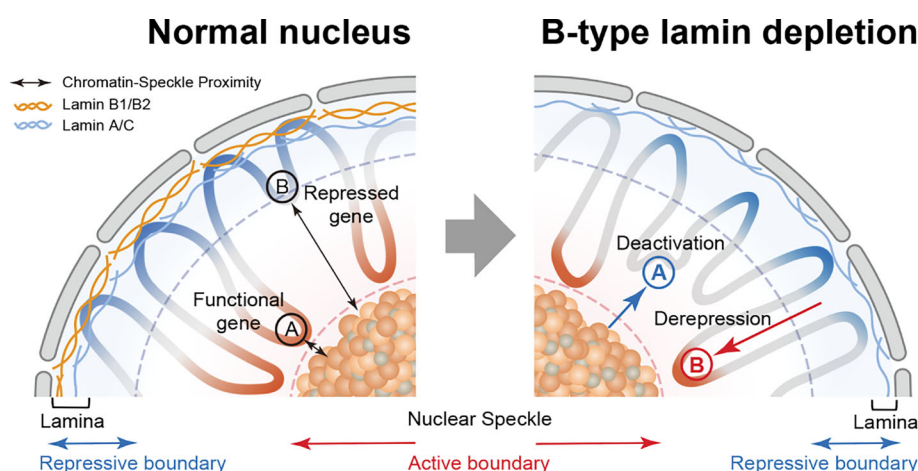
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Abstract

B-type lamins are essential nuclear scaffold proteins known to maintain peripheral heterochromatin through lamina-associated domains (LADs). Here, we identify an unexpected role for B-type lamins in preserving transcriptional output by regulating chromatin proximity to nuclear speckles, key hubs of transcription and RNA processing. Using TSA-Seq mapping and RNA-Seq in lamin-depleted human cells, we show that most differentially expressed genes are located in non-LAD, speckle-associated chromatin domains. Lamin depletion causes euchromatin to relocate away from nuclear speckles, resulting in global transcriptional repression accompanied by alterations in RNA processing and reduced cell viability. In contrast, heterochromatin gains speckle proximity and exhibits aberrant transcriptional activation. Integrative epigenomic analyses reveal that these spatial chromatin rearrangements precede disruption of speckle-associated domains (SPADs), including genes involved in cell viability and RNA metabolism. Together, our findings uncover a speckle-centered regulatory axis governed by B-type lamins and highlight chromatin–speckle proximity as a key spatial parameter in nuclear gene regulation.

Graphical abstract



Introduction

The eukaryotic nucleus is characterized by a highly organized genome and interconnected nuclear compartments that are essential for normal cellular functions and architecture. Among these compartments, the nuclear lamina—a meshwork of A-type and B-type lamins, which are type V intermediate filament (IF) proteins—plays a crucial role in nuclear organization and gene regulation [1]. B-type lamins, an-

chored to the nuclear envelope by farnesyl and carboxymethyl groups at their tail domains, differ from A-type lamins, which lack these modifications and interact with various transcriptional and epigenetic regulators [2]. Conventional electron microscopy has shown that the nuclear lamina is closely associated with densely stained chromatin at the nuclear periphery [3]. Genome-wide mapping has identified lamina-associated domains (LADs), which are primarily transcriptionally

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repressive and include both facultative (fHet) and constitutive heterochromatin (cHet) [4–6]. This finding suggests that the nuclear lamina is not only a structural scaffold but also an active regulator of genome organization and gene expression [1, 7]. The concept of stochastic radial positioning posits that repressive genes are typically located at the nuclear periphery, while active genes are situated toward the nuclear center, resulting in a gradient of transcriptional activity based on radial positioning [8, 9]. However, recent research has expanded this view, showing that transcriptional activity also varies along an axis between the nuclear lamina and intranuclear organelles, such as nuclear speckles, rather than in a strictly radial position-dependent manner [10] (Fig. 1A, left).

Initial studies suggested a straightforward correlation between reduced lamina–chromatin interactions and increased gene activation in lamin-deficient cells or during cell differentiation [11–13, 14] (Fig. 1A, Concept I). However, this perspective has been challenged by subsequent research. For instance, the deletion of lamina components does not always alter the expression of genes within LAD regions, and relocating a gene away from the lamina does not necessarily lead to its activation [15–18]. Moreover, genome-wide gene expression profiling of lamin-deficient conditions has revealed extensive dysregulation of genes independent of LAD positioning, suggesting additional layers of regulation [16, 19, 20] (Fig. 1A, Concept II). Although advances in chromatin mobility and compartment switching have been observed through high-throughput sequencing [21–23], the underlying mechanisms of widespread gene dysregulation in lamin-depleted cells remain poorly understood.

Nuclear speckles, also known as interchromatin granule clusters (IGCs), are membraneless nuclear bodies enriched with RNA-binding proteins (RBPs) such as SON and SC35 (SRSF2), which facilitate pre-messenger RNA (mRNA) processing and recycling speckle components [24–26]. A growing evidence suggests that nuclear speckles act as hubs for coordinating gene expression regulation, including chromatin modification, transcription, mRNA processing, and export, all closely linked with RNA polymerase II activity [27–29]. These speckles are spatially distinct from the nucleoplasm, and assemble into discrete and membraneless entities through liquid–liquid phase separation (LLPS) [30]. These dynamic structures exhibit diffusible behavior, enabling them to function in a distance-dependent manner without direct contact with DNA [31–33].

Chromatin is nonrandomly organized into hierarchical structural units in a nucleus and its spatial structure and position are closely related to the temporal regulation of genome functions [34]. Conventional high-throughput sequencing methods such as chromatin immunoprecipitation (ChIP), DNA adenine methyltransferase identification (DamID), and cleavage under targets and release using nuclease (CUT&RUN) have been widely used to map 3D genome architecture, but they are limited in revealing chromatin positioning with respect to phase-separated compartments due to their reliance on molecular interactions [28, 35–37]. To overcome these limitations, recent adaptations of the tyramide signal amplification (TSA) method, combined with high-throughput sequencing (TSA-Seq), have provided a novel approach for mapping genome-wide chromatin interactions with membraneless nuclear compartments. This technique leverages the gradient exponential decay of diffusible tyramide labeling, enabling the identification of chromatin regions in-

fluenced by nuclear speckles and predicting chromatin trajectories in the three-dimensional (3D) nuclear space [10, 38].

In this study, we investigate how B-type lamins regulate 3D chromatin organization and transcriptional regulation by modulating chromatin proximity to nuclear speckles. By integrating SC35 TSA-Seq with transcriptomic, genomic, and epigenomic data, we demonstrate that loss of B-type lamins leads to large-scale repositioning of chromatin domains, particularly affecting euchromatin–speckle proximity. This spatial reorganization disrupts the functional segregation of chromatin states, with euchromatin regions moving away from speckles and heterochromatin becoming aberrantly enriched near them. These alterations coincide with widespread gene expression changes, indicating that B-type lamins are critical for maintaining nuclear topological balance and ensuring transcriptional stability. In turn, these changes are associated with downstream effects on cellular function, including impaired RNA processing and reduced cell viability.

Materials and methods

Cell cultures

All the cell culture reagents used in the present study were purchased from Welgene (Seoul, Republic of Korea). H1299 (human nonsmall cell lung carcinoma) cells were purchased from the Korean Cell Line Bank (Seoul, Republic of Korea). H1299 cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin at 37°C in an incubator with 5% CO₂ in a humidified atmosphere. For synchronization at G1/S, cells were cultured in the presence of 2 mM thymidine (Sigma) for 18 h, washed three times with phosphate-buffered saline (PBS), and released in fresh medium without thymidine for 9 h. After another 16 h in thymidine, cells were provided for experiments.

Generation of knockout (KO) stable cell lines

Each sgRNA target sequence for *LMNB1* and *LMNB2* was designed by using the single-guide RNA (sgRNA) prediction program (<http://crispr.mit.edu>). 20 bp guide sequences indicated in [Supplementary Table S10](#). Each oligo was phosphorylated and annealed using T4 polynucleotide kinase (NEB; MA, USA). The annealed oligo was ligated into the BsmBI-digested lentiCRISPRv2 vector purchased from Addgene. The CRISPR vector-transfected cells were selected with 1 µg/ml of puromycin for 4–7 days. Selected cells were recovered approximately for 5 days. The sequence of the single clone was verified by DNA sequencing.

Immunocytochemistry

After growing on sterile glass coverslips in 60 mm dishes for 24 h, cells were fixed with 4% paraformaldehyde (#15710, Electron Microscopy Sciences) for 10 min at 37°C, followed by 5 min quenching with 0.125 M glycine. Cells were permeabilized for 30 min in PBS containing 0.5% Triton X-100, and then incubated for 1 h in a blocking solution (5% normal goat serum and 0.2% Triton X-100 in PBS). Primary antibodies were diluted in blocking solution and incubated for overnight at 4°C. Secondary antibodies incubated for 1 h at 23–25°C in the dark condition after washed with PBS. The list of antibodies was described in [Supplementary Table S9](#). For 3 min in the dark, the nucleus was counterstained with 5 µg/ml of 4',6-diamidino-2-phenylindole (DAPI). The confocal images

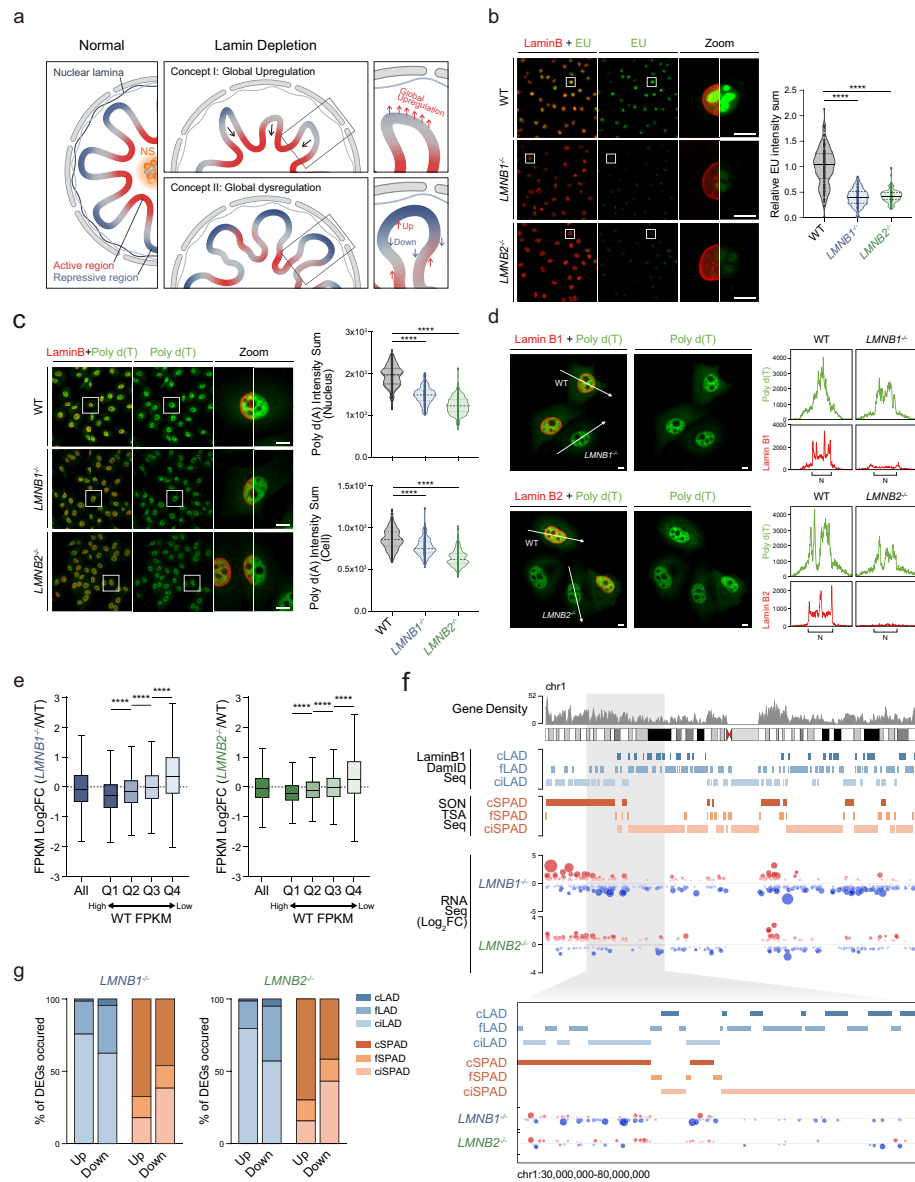


Figure 1. The absence of B-type lamins changes the global expression pattern of genes especially adjacent to the nuclear speckles. **(a)** Schematic diagram contrasting chromatin activity in a normal state (left) and possible transcription regulation defect in lamin-depleted cells (right). **(b)** Staining of EU incorporation (green) and immunostaining for Lamin B (red; WT and LMNB2^{-/-}: Lamin B1 Ab, LMNB1^{-/-}: Lamin B2 Ab). Cells were incubated for 4 h after treatment with 0.2 mM EU. Confocal images display EU-positive signals in the nuclei of three different cell types. *P*-values are calculated using a two-tailed, unpaired *t*-test (*****P* < 0.0001); scale bar: 10 μ m. **(c)** RNA FISH detecting poly(A) mRNA expression (green; poly(dT) probe), with DNA stained using DAPI (blue), and quantification of poly(dT) signals in both the nucleus and the whole cell area, with significance determined by a two-tailed, unpaired *t*-test (*****P* < 0.0001); scale bar: 10 μ m. **(d)** WT and LMNB1^{-/-} or WT and LMNB2^{-/-} cells were respectively co-cultured. RNA FISH detecting poly(A) mRNA expression (green; poly(dT) probe) and immunostaining for Lamin B (red), along with quantification of poly(dT) and Lamin B intensities in the whole cell area; scale bar: 10 μ m. **(e)** Box plot of expression changes (log₂-fold changes in FPKM ratios) categorized into quartiles based on expression levels in WT cells (Q1 = upper quartile; most active genes), with the box representing the interquartile range (IQR) and whiskers plotted using the Tukey method, ignoring outliers; significance was assessed using a two-tailed Mann–Whitney test (*****P* < 0.0001). **(f)** Genome tracks of LADs and SPADs detected by the EDD with a 20 kb bin size in four different cell types (H1, K562, HCT116, and HFFc6), showing constitutive, facultative, and constitutive-inter LADs and SPADs. Up- and downregulated genes in LMNB1^{-/-} and LMNB2^{-/-} cells are separately visualized in red and blue, respectively, and plotted onto their genomic loci, with point size representing the *P*-value and height denoting the level of expression change (log₂-fold changes in FPKM ratios). **(g)** Bar plot showing the proportion of up- and downregulated genes in each type of LAD and SPAD. See also Supplementary Figs S1 and 2, and Supplementary Tables S1, 2, 3, 8, and 10.

were created using a Nikon Ti2-E confocal microscope and the SIM images were created using Super Resolution Microscope N-SIM S with the NIS-elements BR program (v.5.21.00) (Nikon, Japan).

5-Ethynyl uridine staining

To visualize nascent RNA synthesis, cells were pulsed with growth media containing 0.2 mM 5-ethynyl uridine (EU) (E10345, Invitrogen) for 4 h, according to the manufacturer's instructions. Subsequent "click" reaction was performed utilizing the components included in Click it EdU-Alexa-Fluor488 Imaging Kit (ab219801, Abcam). After fixation, permeabilization, and blocking of the cells, EU incorporation was visualized through being crosslinked with a fluorescent azide (iFluor-488). Cells were then washed and proceeded to immunocytochemistry (ICC) process described above.

DNA and RNA fluorescence *in situ* hybridization

Cells were fixed with 4% paraformaldehyde (#15710; Electron Microscopy Sciences) in 1× PBS for 10 min at 37°C and quenched with 0.125 M glycine for 5 min. For DNA FISH, probes were made from human BACs (ordered from BACPAC; RP11-94L15, RP11-268H17, RP11-948G15 for *LMNB1*^{-/-}, and RP11-59J16, RP11-221E20 for *LMNB2*^{-/-}) using the FISH Tag™ DNA Kits (F32947, F32948, Invitrogen), followed by EtOH precipitation with 0.2 mg/ml Salmon sperm DNA (D7656, SIGMA) and 0.1 µg/µl Cot-1 (18440016, Invitrogen). Fixed cells were equilibrated in 20% glycerol/PBS for at least 1 h at 4°C, subjected to three freeze-thaw cycles in LN2 and permeabilized in 0.5% saponin/0.5% Triton X-100/PBS for 30 min at RT. Cells were then equilibrated in 50% formamide/2× SSC for at least 30 min at RT before hybridization. Hybridization was performed in DNA hybridization buffer (50% deionized formamide, 15% dextran sulfate in 2× SSC) containing fluorescence-labeled probes. Samples were incubated at 45°C for 1 h, at 80°C for 5 min for DNA denaturation, and hybridized at 37°C for 3 days in a humidified chamber. For RNA FISH, fixed cells were permeabilized for 1 h at 4°C in PBS containing 0.5% saponin and 1.5 mg/ml BSA, and then incubated for 2 h at RT in RNA hybridization solution [1.5 mg/ml BSA, 5% dextran sulfate, 35% deionized formamide, 2 µg/µl tRNA, and 1 U/µl RNasin® Plus (N2611, Promega) in 4× SSC]. After rinsing with 4× SSC containing 0.1% saponin, cells were hybridized for 36 h at RT in the presence of 75 ng/µl of Cy5-conjugated oligo-d(T)30 in hybridization solution. After hybridization, RNA FISH samples were washed in 4× SSC, whereas DNA FISH samples were washed sequentially in 50% formamide/2× SSC at 45°C, 0.2× SSC at 65°C, and 2× SSC at 45°C. All samples were then washed in 2× SSC and processed for ICC as described above.

Image analysis

Intensities of EU and poly d(T) signals were measured within each cell's nucleus or whole cell body by using "automated measurement" plugin NIS software.

Statistical analysis

GraphPad PRISM statistic software was used to examine the significance of differences in result data. The results were described using the mean ± standard error of the mean (SEM)

and were obtained from two or three separate experiments. Statistically significant *P*-values were <0.05.

Immunoprecipitation and immunoblot

The cells were lysed in a solution including 0.1% sodium dodecyl sulfate (SDS), 1% Nonidet P-40, and 1 mM PMSF, 1% Triton X-100, 150 mM NaCl, 50 mM Tris-HCl (pH 7.5). After homogenizing on ice, the cell suspensions were centrifuged at 15 000 × *g* for 10 min at 4°C. The protein samples were electrophoresed on a 10% SDS-PAGE and transferred to a nitrocellulose membrane (ProtranTM; Whatman, Maidstone, UK). After blocking the membrane with 5% skim milk in TBS-T buffer (137 mM NaCl, 20 mM Tris-HCl, pH 7.6, and 0.1% Tween-20), the primary antibody was diluted and interacted with the membrane for an overnight at 4°C. [Supplementary Table S9](#) contains a list of antibodies. Membranes were washed three times with TBST for 10 min before being incubated for 1 h with a 1:5000 dilution of horseradish peroxidase-conjugated anti-rabbit antibodies. Following product directions, blots were washed three times using TBST before being developed using the western blotting luminol reagent (sc-2048, Santa Cruz).

Total RNA isolation and RT-qPCR

Following the manufacturer's instructions, total RNA was extracted using the TRIzol solution (15596018, Invitrogen, CA, USA). By mixing 20 units of Rnase-free Dnase I (New England Biolabs) and 4 units of Rnase inhibitor (New England Biolabs) with DEPC-treated water, contaminated genomic DNA was eliminated from 10 µg of total RNA. The reaction mixture was incubated for 10 min at 50°C after incubation for 1 h at 37°C. All RNA extracts had an OD260:OD280 ratio between 1.8 and 2.0, indicating that RNA was clearly extracted. Oligo-dT (6110A, Takara) was applied as the primer in the first step of cDNA synthesis. Total RNA (500 ng) was mixed with 1 µl of oligo dT, and distilled water and then preheated at 65°C for 5 min to denature the secondary structures of RNA. The mixture was quickly cooled to 4°C, before adding 10 mM DTT, 4 µl of 5X PrimeScript buffer, and 200 units of PrimeScript reverse transcriptase (#RR036A, PrimeScript™ RT Master Mix, Takara) to make a total volume of 20 µl. The reverse-transcriptase mixture was incubated at 25°C for 5 min then incubated at 42°C for 60 min before being stopped by heating at 70°C for 15 min. The stock of cDNA was kept at -20°C. According to the manufacturer's instructions, the CFX96 Real-time PCR detection equipment (Bio-Rad) and the iQ SYBR Green PCR Supermix (#1708880, Bio-Rad) were used to detect amplified complementary DNA (cDNA) samples for real-time quantitative PCR. Melting curve analysis was used to demonstrate the uniqueness of each amplified product. The β -actin gene was used as for normalization. The relative mRNA expression was calculated by the $2^{-(\Delta\Delta Ct)}$ method.

RNA sequencing and bioinformatical analysis

According to the manufacturer's instructions, the TruSeq mRNA Library Prep Kit (Illumina) generated the RNA-Seq library. To be brief, 100 ng of total cells were extracted, and then reverse transcription was carried out using an oligo-dT primer that had an Illumina-compatible sequence at its 5' end. After degradation of the RNA template, a random primer with an Illumina-compatible linker sequence at its 5' end started

the second strand synthesis. The AMPure magnetic beads (A63881, Beckman Coulter) were used to remove all reaction components from the double-stranded library during purification. The library was amplified to include all of the adapter sequences required for cluster generation. The finished library is purified from PCR components. High-throughput sequencing was carried out as paired-end 101 sequencing reads using NextSeq 500 (Illumina). STAR (v.2.7.1a) was used to align mRNA-Seq reads. Indices were generated from either the genome assembly sequence or representative transcript sequences for alignment to the genome and transcriptome. The alignment file was used to assemble transcripts, estimate their abundances, and detect gene expression differences using RSEM (v.1.3.3). Differentially expressed transcripts were identified using EdgeR (v.3.36.0) within R version 4.1.0 (R development Core Team, 2011) using the R package. Differentially expressed genes (DEGs) were visualized using karyoploteR R package [39]. The information for key algorithms are listed in [Supplementary Table S11](#).

Chromatin immunoprecipitation sequencing

Cells were crosslinked with 1% paraformaldehyde in PBS at RT for 10 min and quenched with 0.125 M glycine for 5 min. Pelleted cells were lysed in SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris–HCl, pH 8.1), and chromatin was fragmented using a Bioruptor sonicator (Diagenode) for 40 cycles (30 s on/30 s off, repeated twice) at high power setting. The lysates were cleared by centrifugation, and the supernatant was subsequently diluted with ChIP dilution buffer (0.01% SDS, 1.2 mM EDTA, 1.1% Triton X-100, 167 mM NaCl, 16.7 mM Tris–HCl, pH 8.1). Chromatin was incubated overnight at 4°C with 5 µg of the indicated antibodies: anti-H3K9me3 (ab8898, Abcam) or anti-H3K4me3 (ab8580, Abcam). Protein A/G magnetic beads (#26162, Thermo Fisher) were added and incubated for 4 h, followed by sequential washes with low-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 150 mM NaCl, and 20 mM Tris–HCl, pH 8.1), high-salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 500 mM NaCl, and 20 mM Tris–HCl, pH 8.1), LiCl wash buffer (0.25 M LiCl, 1% NP-40, 1% sodium deoxycholate, 1 mM EDTA, and 10 mM Tris–HCl, pH 8.1), and TE buffer (1 mM EDTA and 10 mM Tris–HCl, pH 8.0). Chromatin was eluted twice with IP elution buffer (1% SDS and 0.1 M NaHCO₃) and reverse-crosslinked overnight at 65°C. DNA was purified by EtOH precipitation, air-dried, and resuspended in nuclease-free water for subsequent analyses. ChIP-Seq libraries were prepared using the TruSeq ChIP Library Preparation Kit (IP-202-1012, IP-202-1024, Illumina) according to the manufacturer's instructions and high-throughput sequencing used 101-bp paired-end reads generated on Illumina NextSeq 1000 and NovaSeq X instruments (Illumina).

Tyramide signal amplification assay

TSA staining and TSA-Seq procedures were mostly based on previous methods. Brief experimental procedures are below. Cells were treated with trypsin for 5 min and immediately crosslinked by adding sample volume of 8% paraformaldehyde freshly prepared in calcium and magnesium free (CMF)-PBS for 20 min at 37°C. After quenching unreacted aldehyde with glycine at a final concentration of 0.125 M, cells were transferred to tubes for subsequent reaction. Cells were permeabilized for 1 h in CMF-PBS containing 0.7% saponin

and rinsed with CMF-PBS. To quench endogenous peroxidases, cells were incubated in 1.5% H₂O₂ (#216763, Sigma) in CMF-PBS for 1 h at RT and washed using washing buffer (0.1% saponin in CMF-PBS). Samples are incubated for 1 h in a blocking solution (5% normal goat serum and 0.2% saponin in CMF-PBS). Primary antibodies were diluted in blocking solution and samples were incubated for 24 h at 4°C. After washing with washing buffer, cells were resuspended in blocking solution containing secondary antibody and incubated for 24 h at 4°C. To label tyramide under different conditions, cells were resuspended in Buffer A indicated in [Supplementary Fig. S3A](#) and same volume of Buffer B was added. For TSA v1.0, the labeling was done at RT for 10 min, but for TSA v2.0, total two rounds of reactions were performed at RT for 30 min each. Among cells, small portion was used to confirm TSA labeling with Streptavidin-PE and remaining cells were subjected to modified genomic DNA (gDNA) isolation method.

Because tyramide preferentially labeled proteins over DNA, avoiding protein contamination is necessary to efficiently purify biotinylated DNA. Thus, we performed two different DNA purification methods. First, tyramide labeled cells were lysed by using PureLink™ Genomic Digestion Buffer (K182301, Invitrogen), followed by 1 h of RNase (350 ng/µl) reaction at 37°C and 6 h of Proteinase K (1 µg/µl) reaction at 55°C. After adding NaCl solution (200 mM), samples were incubated at 65°C for an overnight period to reverse protein–DNA crosslinks. Then, gDNA was extracted by using UltraPure™ Phenol: Chloroform: Isoamyl alcohol (PCI; 15 593 049, Invitrogen), followed by EtOH precipitation. gDNA was dissolved in distilled water (W4502, Sigma) and sheared using a bioruptor sonicator (Diagenode) at high power for 30 cycles (30 s on/30 s off). For the second round of purification, the AMPure magnetic beads (A63881, Beckman Coulter, CA, USA) were used to remove all protein contaminants from the biotinylated DNA. After bead purification, the samples were sheared again until the size of DNA fragments were ranged from 100 to 300 bp. Biotinylated DNA fragments were isolated by using Dynabeads M-270 streptavidin (65305, Invitrogen) according to the manufacturer's instructions. Immobilized DNA fragments were eluted in TSA elution buffer (10 mM Tris, 10 mM EDTA, 0.5% SDS, 1 µg/µl proteinase K, and 0.6 mM biotin) and final round of DNA extraction with PCI and subsequent EtOH precipitation was performed before proceeding to library preparation.

TSA-Seq library preparation and bioinformatical analysis

The Kapa Hyper Prep Kit (KK8502, Kapa Biosystems/07962347001, Roche) was used to create the TSA-Seq library. To summarize, 15 ng of input and TSA-enriched DNA were end-repaired, 3'-end A-tailed, and ligated with KAPA Unique Dual-Indexed Adapter Kit (KK8727, Kapa Biosystems/08861919702, Roche) before being amplified. The Illumina HiSeq X Ten platform was used for paired-end sequencing of all TSA libraries. All TSA reads in FASTQ format were aligned to the GRCh38.p13.103 human genome using Bowtie2 (v.2.4.4) and removed redundant reads using SAMtools (v.1.14). After confirmed the consistency between each replicate, we pooled extended reads and generated the bigwig track for visualization with IGV (v.2.8.7). All bigwig files were generated from sorted bam files using deepTools (v 3.5.1).

The TSA-Seq normalization process was carried out following a similar scheme as previously described [10, 38]. First, the read count within 100 bp genomic bins were obtained using the “bamCoverage” command in deepTools. To compute the TSA-Seq enrichment score, the read counts were normalized based on the input read number, N'_{TSA} , according to equation (1):

$$N'_{TSA} = \frac{N_{TSA} \times Ave(N_{input})}{N_{input}} \quad (1)$$

where, N_{TSA} and N_{input} represent the number of mapped reads within each 100 bp bins of TSA sample and input control. This calculation was skipped for bins with a read count of zero. Next, TSA-Seq enrichment score (ES) was defined as log2 ratio of N'_{TSA} and the average N'_{TSA} , as shown in equation (2):

$$ES = \log_2 \frac{N'_{TSA}}{Ave(N'_{TSA})} \quad (2)$$

To compare chromatin positioning on a large scale among different genotype of TSA-Seq, Hanning window methods applied to ES with 20 kb bins, according to equation (3):

$$w(n) = 0.5 - 0.5 \cos\left(\frac{2\pi n}{M-1}\right), \quad 0 \leq n \leq M-1 \quad (3)$$

where, M is the number of points in the window size. We set the $M = 21$ for 20kb bins. From Hanning window, discrete convolution operation was applied to ES of 100bp bin for smoothed ES (sES), according to equation (4):

$$sES_i = \sum_{j=i-10}^{i+10} w(n)_j ES_{i-j} \quad (4)$$

where i is the index of given window. The sES from equations (3) and (4) implemented hanning function and convolve function in numpy (v.1.21.5) and Python (v.3.8.12).

To detect SPAD regions, we identified peak regions in two steps: first, we detected high-confidence regions (defined as those above the 70th percentile of sES) then we detected low-confidence regions (defined as those above the 50th percentile of sES). SPAD regions were considered separated by gaps, which were characterized by deviations in sES between adjacent SPADs. Finally, we defined SPAD regions by merging high-confidence regions that fell within the corresponding low-confidence regions.

Enhancer region identification

To characterize enhancer regions for each gene (Fig. 2 and Supplementary Figs 5–7), we applied previously predicted enhancers in human lung fibroblasts based on the Activity-by-Contact (ABC) model [40]. The genomic positions, originally in the hg19 assembly, were converted to hg38 using “CrossMap.” Promoter regions were excluded from the predicted regions, and the remaining regions were then intersected with H3K27ac ChIP-Seq data (GSE172506) from H1299 cells. Regions with the highest ABC score for each gene were defined as the enhancers.

Gene ontology and GSEA analysis

Significant DEGs and gene sets residing in cSPAD regions were clustered into functional gene ontologies using DAVID (<https://davidbioinformatics.nih.gov/>). Metascape was used to identify enriched gene ontology terms, and REVIGO was used to

create scatter plots of ontology terms. GSEA analysis was carried out using (GSEA 4.1.0; <http://www.broadinstitute.org/gsea/index.jsp>) and the MsigDB v7.1 human database.

Enrichment domain detector

To identify LAD from Lamin B1 and SPADs from SON TSA-Seq profiles across the different cell types (H1, K562, HFFc6, and HCT116), we utilized SON TSA-Seq and Lamin B1 DamID-Seq data [38]. FASTQ reads were aligned to GRCh38.p13.103 reference genome using Bowtie2 (v.2.4.4), and duplicated reads were removed. Enriched domains were identified using EDD (v.1.1.19) with the options “–bin-size 20 –g 2.”

Chromatin state modeling using ChromHMM

H3K4me1, H3K4me3, H3K9ac, H2AK9ac, H2BK5ac, H3K36me3, H3K27me3, H3K9me3 (GSE81322) H3K27ac (GSE172506), PolII (GSE161321), and ATAC-Seq (GSE145663) were used for chromatin state modeling. All FASTQs also aligned to the GRCh38.p13.103 human genome using Bowtie2 (v.2.4.4). Non-duplicated aligned used as input for “BinarizedBAM” command in ChromHMM (v.1.24) with default parameter. To choose the optimal number of state, we modeled from 6 to 15 states and selected the most distinct state based on the transition probabilities.

Gene annotation and clustering

To classify the chromatin state of genes, we choose eight state defined by previously obtained ChromHMM results: “Active Promoter,” “Weak Promoter,” “Open Chromatin,” “Active Transcription,” “Weak Transcription,” “Constitutive Heterochromatin,” “Facultative Heterochromatin,” and “Weak Facultative Heterochromatin.” For each gene, the proportion of each chromatin state was calculated by dividing the number of occurrences of that state by the total number of states in the gene. These proportions were then used as feature for applying the Louvain algorithm, resulting in 36 clusters, which were manually annotated into six meta-cluster groups. All analyses were conducted using the default parameters of the Scanpy package (v.1.9.1).

Detection of alternative splicing defects and retained intron

To identify significantly differentially expressed transcript variants for each gene, we applied the Chi-square test to the expression levels of transcripts in each gene using scipy package (v.1.8). The Chi-square test was performed on transcripts with a biotype of protein-coding and an expression level of 1 in at least one sample. Genes with only one or no transcript meeting these criteria were excluded from the analysis. To detect and quantify retained intron (RI) events in all known introns, we used IRFinder (v.1.3.0). IRFinder was run on trimmed FASTQ with default parameters. Differentially expressed RI events were calculated AC-test using “analysisWithLowReplicates.pl” script in IRFinder.

Motif discovery

The promoter sequences of splicing factor genes were analyzed using the *de novo* motif finder MEME-ChIP (<http://meme.nbcr.net/meme/tools/meme-chip>, date last accessed,

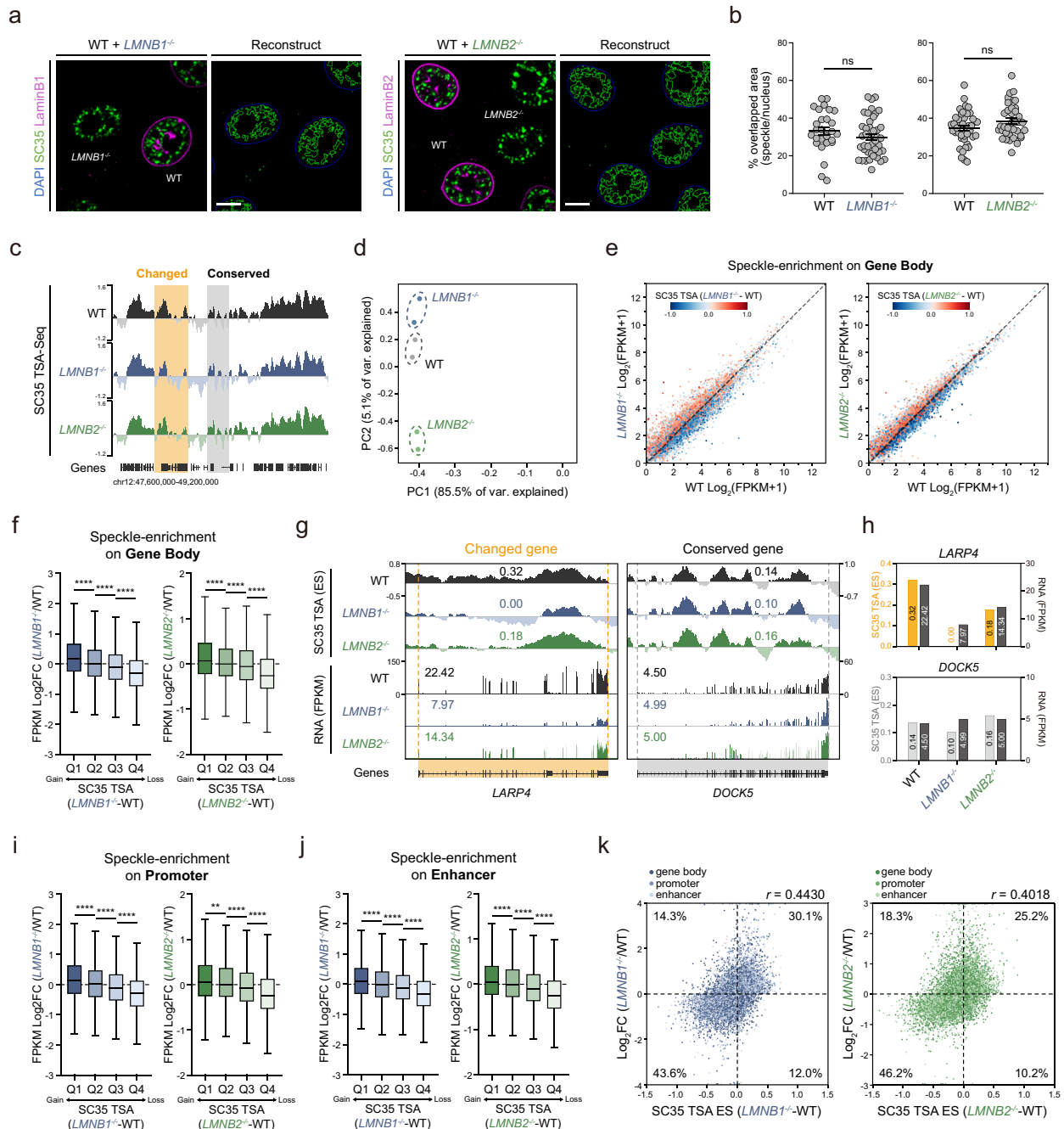


Figure 2. The loss of B-type lamins mediates the changes of chromatin–speckle association which are tightly related to the global gene expression changes. **(a and b)** Co-cultures of WT and *LMNB1*^{-/-} (left) or WT and *LMNB2*^{-/-} cells (right) were subjected to immunofluorescent confocal microscopy. Nuclear staining was performed using DAPI (blue), SC35 (green), and Lamin B (purple). DAPI and SC35 signals are reconstructed (A), and the % area occupied by speckles within each nucleus is quantified using Imaris software (B); scale bar: 10 μ m. **(c)** Region representing changed or conserved speckle associations, with normalized SC35 TSA-Seq enrichment scores (ES) analyzed at a 100 bp bin size for WT, *LMNB1*^{-/-}, and *LMNB2*^{-/-} cells. **(d)** PCA of SC35 TSA-Seq data from *LMNB1*^{-/-} and *LMNB2*^{-/-} cells, where the numbers indicate the amount of variation attributed to each principal component, and small dots indicate individual samples with a larger dashed line showing each group. **(e)** Comparison of RNA expressions ($\log_2$ -FPKM + 1) between *LMNB1*^{-/-} and WT (left) and *LMNB2*^{-/-} and WT (right) for all protein-coding genes (FPKM $\geq$ 1), with each spot representing a single gene and color indicating the mean changes in SC35 TSA-Seq ES on the gene body region. **(f)** Box plot of expression changes of genes ($\log_2$ -fold changes in FPKM ratios) partitioned into quartiles based on the changes in SC35 TSA-Seq ES on the gene body region (Q1 = upper quartile; most increase in SC35 association compared to WT), with the box representing the IQR and whiskers plotted using the Tukey method. Outliers are ignored. *P*-values are calculated using a two-tailed Mann–Whitney test (*****P* < 0.0001). **(g)** Zoomed view of SC35 TSA-Seq (top) and RNA-Seq (bottom) profiles are represented for the two genes (*LARP4* and *DOCK5*). **(h)** Calculated mean ES of TSA-Seq and FPKM of RNA-Seq values. **(i and j)** Box plot of expression changes of genes ($\log_2$ -fold changes in FPKM ratios) partitioned into quartiles based on the changes in TSA-Seq ES on gene promoter (i) and enhancer region (j) (Q1 = upper quartile; most increase in SC35 association compared to WT). Box represents IQR and whiskers are plotted with the Tukey method. Outliers are ignored. *P*-values are calculated using a two-tailed Mann–Whitney test (*****P* < 0.0001, ***P* < 0.01). **(k)** Scatter plots showing the correlation between speckle association changes and RNA expression changes, with each spot indicating the most positively correlated genomic region, dashed lines representing zero value, and the proportions of spots in each quadrant with Pearson correlation coefficients (*r*) calculated. See also [Supplementary Figs S3–8](#) and [Table S4](#).

July 2015). Also, the motif binding probabilities on promoter regions were obtained via MEME-Suite.

Image analysis

Intensities of EU and poly d(T) signals were measured within each cell's nucleus or whole cell body by using "automated measurement" plugin NIS software. Intensities of H3K9me3 signal were measured by using 'histogram' plugin ImageJ software.

Statistical analysis

A statistic software, GraphPad PRISM was used to examine the significance of differences between result data with independent two-sample *t*-test. The results were described by mean $\pm$ standard error of the mean (SEM) and gained from two or three separated experiments. *P*-values <0.05 were considered statically significant.

Results

Absence of B-type lamins changes the global expression pattern of genes, particularly in regions adjacent to nuclear speckles

Cells lacking Lamin B1 (*LMNB1*^{-/-}) or Lamin B2 (*LMNB2*^{-/-}) were generated using CRISPR-Cas9, and complete loss of each protein was confirmed (Supplementary Fig. S1A–C). When comparing total RNA levels among wild-type (WT), *LMNB1*^{-/-}, and *LMNB2*^{-/-} cells, we observed a significant reduction in total RNA abundance following the loss of B-type lamins (Supplementary Fig. S1D–F). Moreover, EU staining combined with ICC for Lamin B (Lamin B1 in WT and *LMNB2*^{-/-} cells, and Lamin B2 in *LMNB1*^{-/-} cells) revealed that nascent RNA synthesis in the nucleus was reduced by more than half in both *LMNB1*^{-/-} and *LMNB2*^{-/-} cells compared to WT (Fig. 1B). RNA fluorescence *in situ* hybridization (FISH) using poly-d(T) probes further confirmed that total mRNA expression, both in the nucleus and across the entire cell, was diminished in *LMNB1*^{-/-} and *LMNB2*^{-/-} cells compared to WT (Fig. 1C and D). Together, these results indicate that loss of B-type lamins leads to a net decrease in global transcriptional activity, extending their functional impact beyond heterochromatin tethering.

To assess the broad impact of B-type lamin depletion on transcription at the individual gene level, we performed RNA-Seq to profile gene expression in WT, *LMNB1*^{-/-}, and *LMNB2*^{-/-} cells. The principal component analysis (PCA) plot revealed strong consistency among biological replicates, with distinct alterations in gene expression patterns following the loss of B-type lamins (Supplementary Fig. S1G and H). In line with the findings from EU staining and RNA FISH, overall gene expression (measured as log₂-fold changes in FPKM ratios) for all protein-coding genes (FPKM ≥ 1) exhibited a general decrease (Fig. 1E). Notably, when we categorized these genes into quartiles based on their expression levels in WT cells, we found that genes actively expressed in WT cells were more likely to be downregulated, whereas genes with repressive profiles tended to be up-regulated in the absence of Lamin B.

Given the pivotal roles of nuclear lamina in 3D gene positioning and expression, we integrated RNA-Seq data with

genome-wide maps illustrating genomic associations with nuclear compartments, specifically the nuclear lamina, and speckles. We reanalyzed Lamin B1 DamID-Seq and SON TSA-Seq profiles across four different WT cell lines (H1, K562, HCT116, and HFFc6), identifying LADs and speckle-associated domains (SPADs) using the enriched domain detector (EDD) tool [38, 41] (Fig. 1F and Supplementary Fig. S2). Statistically significant DEGs were identified (FDR < 0.01 , absolute log₂-fold changes > 0.5), revealing 4444 DEGs in *LMNB1*^{-/-} cells and 3020 DEGs in *LMNB2*^{-/-} cells. The log₂-fold changes and *P*-values for each DEG were plotted according to their chromosomal positions. Strikingly, most gene expression changes were concentrated in chromatin regions associated with constitutive SPADs (cSPADs) and facultative SPADs (fSPADs), rather than in constitutive LADs (cLADs) or facultative LADs (fLADs) (Fig. 1F, bottom). Over 60% of DEGs were located within cSPAD or fSPAD regions, while only $<5\%$ were found in cLADs (Fig. 1G). These findings suggest that a significant portion of dysregulated genes cluster in chromatin regions strongly associated with nuclear speckles, rather than nuclear lamina, even in the absence of B-type lamins.

Loss of B-type lamins alters chromatin–speckle associations closely linked to global gene expression changes

Since most DEGs were located near transcriptionally active nuclear speckles, we hypothesized that the B-type lamin depletion might disrupt speckle-chromatin associations. To explore this, we generated speckle enrichment genomic maps for WT, *LMNB1*^{-/-}, and *LMNB2*^{-/-} cells using SC35 TSA-Seq 2.0 [38] (Supplementary Fig. S3A–D). Although SC35 antibodies also recognize SRRM2, these major speckle components are collectively referred to here as SC35. SC35 TSA-Seq data from WT cells showed strong concordance with previously published SON TSA-Seq datasets across multiple WT cell types, indicating conserved speckle association patterns (Supplementary Fig. S4A and B).

Even though the overall structure or distribution of speckles remained unaffected (Fig. 2A and B, and Supplementary Fig. S4C and D), comparing SC35 TSA-Seq scores between WT, *LMNB1*^{-/-}, and *LMNB2*^{-/-} cells showed that the loss of B-type lamins altered speckle association in specific genomic regions (Fig. 2C). This variation in SC35 TSA-Seq signals was also evident in the PCA plot (Fig. 2D). To assess the functional relevance of these spatial changes, we integrated gene body-averaged SC35 TSA-Seq scores with RNA-Seq data. Across all protein-coding genes, upregulated genes tended to exhibit increased speckle association at their gene bodies, whereas downregulated genes showed reduced association (Fig. 2E).

Consistently, changes in SC35 TSA-Seq signals were positively correlated with changes in mRNA expression, with genes moving closer to speckles displaying increased expression and those moving away showing decreased expression (Supplementary Fig. S5A and B). When genes were categorized into quartiles based on the extent of speckle association changes, those with the highest increase in SC35 TSA-Seq scores (Q1) showed the greatest rise in expression, while those in the lowest quartile (Q4) showed the most significant drop in gene expression (Fig. 2F and Supplementary Fig. S5C). For example, the *LARP4* gene showed a decrease in speckle

association following Lamin B loss, corresponding with a similar decrease in mRNA expression. In contrast, the *DOCK5* gene, which maintained consistent speckle association across all cell types, did not exhibit significant changes in mRNA expression (Fig. 2G and H).

Gene expression is controlled by complex molecular regulatory networks, with promoters and enhancers serving as crucial regulatory elements of the genome. Therefore, the 3D positioning of gene promoters or enhancers within nuclear speckles may be closely linked to gene activation. Changes in SC35 TSA-Seq scores at promoter and enhancer regions positively correlated with gene expression changes in both *LMNB1*^{−/−} and *LMNB2*^{−/−} cells (Fig. 2I and J, and [Supplementary Fig. S6A and B](#)). In some cases, such as *ABTB2*, increased promoter–speckle association coincided with elevated expression despite stable gene body association ([Supplementary Fig. S6C and D](#)). Similarly, for *NCAPG2*, altered enhancer association was linked to expression changes ([Supplementary Fig. S6E and F](#)). Overall, approximately 75% of genes showed a strong positive correlation between expression changes and speckle association at least one regulatory region (Fig. 2K), accounting for 78.9% and 75.6% of DEGs in *LMNB1*^{−/−} and *LMNB2*^{−/−} cells, respectively ([Supplementary Fig. S7](#)).

We also examined whether the genes exhibiting altered speckle association at any regulatory region (gene body, promoter, or enhancer) overlapped between the two knockout conditions ([Supplementary Fig. S8](#)). Approximately 20% of genes moving closer to speckles and about 30% of genes moving farther from speckles were shared between *LMNB1*^{−/−} and *LMNB2*^{−/−} cells, while each lamin isoform also regulated a unique set of genes. Together, these results indicate that each B-type lamin contributes to isoform-specific regulation, while both lamins commonly maintain spatial speckle–chromatin interactions closely linked to gene expression.

B-type lamin deficiency induces large-scale chromatin mispositioning relative to nuclear speckles

To assess large-scale transcriptional changes following B-type lamin depletion, we mapped DEGs along chromosomal coordinates. Both *LMNB1*^{−/−} and *LMNB2*^{−/−} cells exhibited extensive and continuous domains of gene upregulation or downregulation spanning megabase-scale regions (Fig. 3A). For example, *LMNB1*^{−/−} cells showed broad upregulation across the 130–180 Mb region of chromosome 5, whereas *LMNB2*^{−/−} cells displayed continuous downregulation across the 60–130 Mb region of the same chromosome.

Because coordinated expression changes across large genomic regions are consistent with spatial chromatin mispositioning, we examined whether these domains coincided with altered speckle proximity. Using smoothed SC35 TSA-Seq profiles generated with 20 kb bins, we observed that chromatin regions showing coordinated transcriptional upregulation or downregulation exhibited corresponding increases or decreases in speckle association, respectively (Fig. 3B and [Supplementary Fig. S9A](#)). These changes extended over large genomic segments, with an average span of approximately 11 Mb, and reached maximal extents of ~90 Mb in *LMNB1*^{−/−} cells and ~88 Mb in *LMNB2*^{−/−} cells ([Supplementary Fig. S9B](#)).

To further characterize these spatial changes, we converted SC35 TSA-Seq enrichment scores into predicted distances from nuclear speckles using established TSA-Seq distance modeling (Condition E; $R_0 = -3.79$) [10]. Comparison of linear chromosome trajectories toward nuclear speckles revealed substantial shifts in speckle–chromatin distance following loss of B-type lamins (Fig. 3C and D). Notably, although absolute speckle proximity changed, the overall linear chromatin profiles—reflected by the relative arrangement of peaks and valleys—remained largely intact, indicating that large-scale shifting occurs without disruption of fine-scale chromatin structure. Quantitative analysis of local TSA-Seq slopes ($\Delta ES/\Delta bin$) confirmed strong conservation of linear chromatin architecture despite marked changes in speckle association (Fig. 3E and F, and [Supplementary Fig. S9C](#)).

Then, we explored whether the conservation of linear chromatin architecture is unique in the absence of B-type lamins or a general phenomenon observed across different cell types. To investigate this, we reanalyzed published SON TSA-Seq profiles and compared chromatin organization changes relative to nuclear speckles during cell differentiation and under heat shock conditions ([Supplementary Fig. S9D and F](#)). When we compared the linear chromatin structure relative to nuclear speckles in H1 cells with those in three differentiated cell types (K562, HCT116, and HFFc6), both the SON TSA-Seq scores (ES) and the linear slopes ($\Delta ES/\Delta bin$) differ significantly between differentiated cells and H1 embryonic stem cells, indicating that linear chromatin structures relative to nuclear speckles change substantially during cell differentiation ([Supplementary Fig. S9D and E](#)). In contrast, under heat shock conditions, only minor changes were observed in the SON TSA-Seq scores, while the $\Delta ES/\Delta bin$ values were altered, indicating that heat shock modifies fine chromatin structure without significantly impacting large-scale chromatin association with nuclear speckles ([Supplementary Fig. S9F and G](#)).

Additionally, we used Spearman's rank correlation to evaluate the conservation of speckle association (ES) and chromatin linear slope ($\Delta ES/\Delta bin$) across various conditions, including the loss of B-type lamins, cell differentiation, and heat shock (Fig. 3G, and [Supplementary Fig. S9H and I](#)). Although the ES values showed a lower correlation between WT and B-type lamin-deficient cells compared to other conditions, the correlation coefficients for $\Delta ES/\Delta bin$ scores were notably high ($\rho \geq 0.84$). This indicates that while the loss of B-type lamins significantly alters chromatin–speckle association, it causes only minor changes in linear chromatin architecture.

Finally, large-scale spatial alterations of chromatin were further validated by immuno-FISH analysis, in which consecutive chromatin regions with reduced SC35 TSA-Seq enrichment in B-type lamin depleted cells were used as probes (P1–P3 for *LMNB1*^{−/−} and P4–P5 for *LMNB2*^{−/−}) (Fig. 3H). When we directly measured the physical distances between these loci and nuclear speckles, all regions with decreased TSA-Seq enrichment showed significantly increased distances from speckles (Fig. 3I and [Supplementary Fig. S10](#)). Furthermore, comparison of TSA-Seq scores with FISH-measured distances revealed a clear inverse correlation, supporting that the TSA-Seq signal corresponds to actual spatial mispositioning of chromatin (Fig. 3J). These findings suggest that B-type lamins are essential for maintaining the 3D nuclear positioning of chromatin relative to nuclear speckles but are not crucial for conserving the linear structure of chromatin.

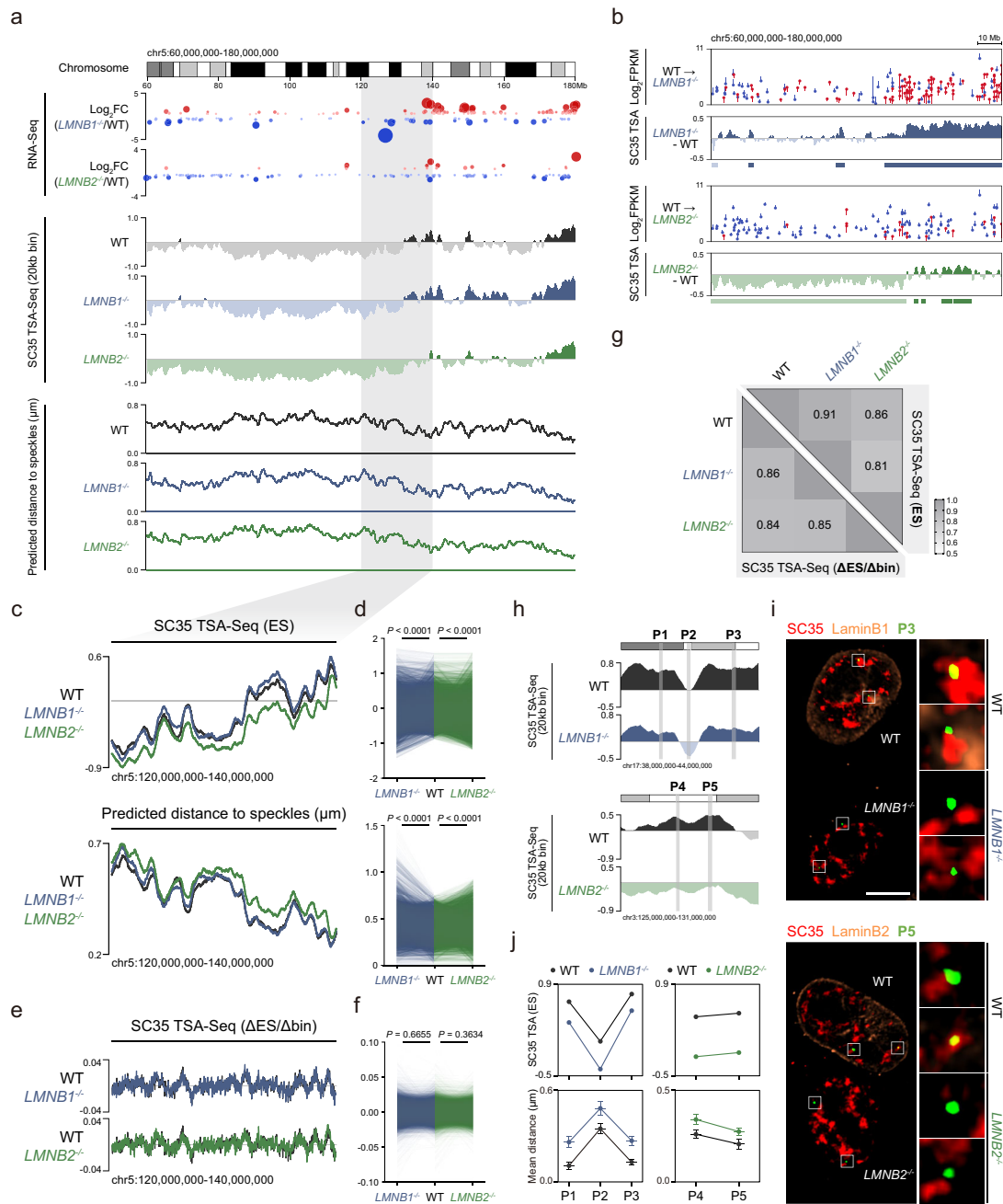


Figure 3. The B-type lamin deficiency induces large-scale chromatin shift toward or away from the nuclear speckle. **(a)** Up- and downregulated genes in *LMNB1*^{-/-} and *LMNB2*^{-/-} cells are visualized in red and blue, respectively, and plotted on their genomic loci (top). The size of the points represents the *P*-value, while the height of the points indicates the level of expression changes (log₂-fold changes in FPKM ratios). Genome tracks for chromosome 5 (60–180 Mb) regions are generated using smoothed and normalized SC35 TSA-Seq with a 20 kb bin size (middle) and predicted speckle distance (bottom) in WT, *LMNB1*^{-/-}, and *LMNB2*^{-/-} cells. **(b)** The RNA expression tracks for each DEG are represented by a line and dot, where the line depicts the level of expression changes (log₂(FPKM + 1)) in *LMNB1*^{-/-} (top) and *LMNB2*^{-/-} (bottom), and their expression in each cell is dotted on the plot. The up- or downregulation pattern of each DEG is further shown in red or blue, respectively. Smoothed SC35 TSA-Seq ES for *LMNB1*^{-/-} and *LMNB2*^{-/-} cells are compared to WT by subtraction and the changed regions are determined by two criteria (|ES of boundary| ≥ 0.01, |Mean ES of changed region| ≥ 0.1). Dark blue or green bars represent positively changed regions, while light blue or green bars denote negatively changed regions in *LMNB1*^{-/-} and *LMNB2*^{-/-} cells, respectively. **(c)** Overlapped signal intensity profiles of smoothed SC35 TSA-Seq ES (top) and predicted speckle distance (bottom) for WT, *LMNB1*^{-/-}, and *LMNB2*^{-/-} cells. **(d)** Smoothed SC35 TSA-Seq ES (top) and predicted speckle distance (bottom) are compared for each bin (20 kb). *P*-values are calculated using a two-tailed Wilcoxon matched-pairs signed rank test. **(e)** Overlapped signal intensity profiles of SC35 TSA-Seq ΔES/Δbin for WT, *LMNB1*^{-/-}, and *LMNB2*^{-/-} cells. **(f)** SC35 TSA-Seq ΔES/Δbin values are compared for each bin (20 kb). *P*-values are calculated using a two-tailed Wilcoxon matched-pairs signed rank test. **(g)** Correlation coefficients (ρ) of SC35 TSA-Seq ΔES/Δbin (left) and ES (right) among WT, *LMNB1*^{-/-}, and *LMNB2*^{-/-} cells are calculated based on Spearman's rank correlation. Correlation coefficients (ρ) between the two cells are represented. **(h)** Genome tracks of consecutive chromatin regions showing reduced SC35 TSA-Seq enrichment in B-type lamin depleted cells. Probes P1–P3 were used for *LMNB1*^{-/-} cells, and P4–P5 for *LMNB2*^{-/-} cells. **(i)** WT and *LMNB1*^{-/-} or WT and *LMNB2*^{-/-} cells were co-cultured and subjected to immuno-FISH. Representative images for probes P3 (*LMNB1*^{-/-}) and P5 (*LMNB2*^{-/-}) are shown. SC35 (red), probes (green), and Lamin B (orange) are visualized; scale bar: 10 μm. **(j)** The comparison between SC35 TSA-Seq ES and mean speckle-chromatin distances at each 5 probed locus is shown. See also [Supplementary Figs S9 and 10](#).

Absence of B-type lamins reduces euchromatin–speckle associations while increasing heterochromatin proximity to nuclear speckles

To characterize the functional nature of chromatin regions exhibiting altered speckle association, we applied ChromHMM [42] to integrate 11 epigenomic datasets, including ATAC-Seq and ChIP-Seq profiles for RNA polymerase II and nine histone modifications [43–46], and annotated the genome into 13 chromatin states based on previous models [47, 48] (Fig. 4A and Supplementary Fig. S11).

Active states, such as promoters and transcriptionally active regions, were strongly enriched near nuclear speckles, whereas constitutive heterochromatin (cHet) states were preferentially located farther away (Fig. 4A and Supplementary Fig. S12). Notably, the mean SC35 TSA-Seq score for facultative heterochromatin (fHet) states was relatively high compared to that of cHet, indicating the presence of heterochromatin near nuclear speckles, consistent with previous findings [10].

We next examined whether specific chromatin states were preferentially affected by B-type lamin depletion. By applying established methods [13, 38], we identified regions with significantly increased or decreased speckle associations in *LMNB1*^{−/−} and *LMNB2*^{−/−} cells (Fig. 4B). Regions showing significantly gained speckle association were predominantly enriched for repressive heterochromatic states, whereas regions that lost speckle association were enriched for active euchromatic states (Fig. 4C and D, and Supplementary Figs S13 and S14A). Correspondingly, genes within heterochromatic regions that gained speckle proximity tended to be up-regulated, while genes located in euchromatic regions that lost speckle association were preferentially downregulated (Fig. 4E).

Immunostaining further supported these findings. Loss of B-type lamins led to dispersion of H3K9me3-positive heterochromatin from both perinuclear and intranuclear regions, consistent with previous observations [49], resulting in increased spatial proximity between heterochromatin and nuclear speckles (Fig. 4F and G, and Supplementary Fig. S14B and C). In contrast, changes in euchromatin–speckle distance assessed by H3K9ac immunostaining were less readily resolved at the available spatial resolution (Supplementary Fig. S14D).

To validate these spatial changes at the genome-wide level, we analyzed ChIP-Seq profiles of representative active (H3K4me3) and repressive (H3K9me3) histone marks. Regions enriched for H3K4me3 in WT cells showed reduced speckle association and decreased H3K4me3 signal upon B-type lamin depletion, whereas H3K9me3-marked regions exhibited increased speckle association accompanied by reduced H3K9me3 enrichment (Fig. 4H and I). Together, these data indicate that loss of B-type lamins redistributes euchromatin away from nuclear speckles while allowing heterochromatic regions to aberrantly gain speckle proximity, coinciding with transcriptional deactivation and derepression, respectively.

Single-gene resolution mapping of spatial shifts and transcriptional changes following B-type lamin loss

To examine the relationship between chromatin positioning and transcriptional changes at single-gene resolution following B-type lamin loss, we analyzed the spatial organization of individual genes relative to nuclear speckles. We focused

on eight functional chromatin states associated with transcriptional activity and calculated state enrichment for each protein-coding gene. Based on their epigenetic features in WT cells, genes were grouped into six clusters using integrated SC35 TSA-Seq and RNA-Seq data: actively transcribed genes, weakly transcribed genes, inactive genes in open chromatin, active but non-speckle-associated genes, facultative heterochromatin (fHet) genes, and constitutive heterochromatin (cHet) genes (Fig. 5A and B). In WT cells, actively and weakly transcribed genes were preferentially located adjacent to nuclear speckles and exhibited high transcriptional output. In contrast, inactive genes in open chromatin regions showed low expression levels despite their proximity to speckles. Active but non-speckle-associated genes displayed features of an active promoter but were positioned farther from nuclear speckles, with reduced accessibility to RNA polymerase II. fHet genes were generally closer to nuclear speckles than cHet genes, although both gene sets showed low transcriptional activity.

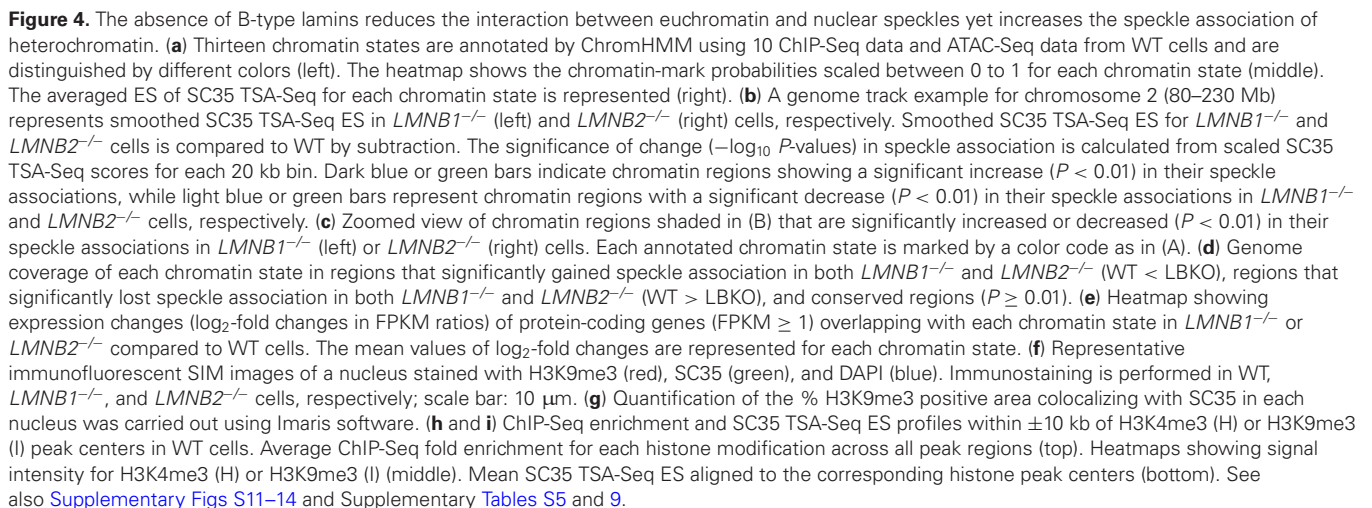
To visualize the spatial distribution of these gene clusters, we generated a speckle-centered genome-wide circos map. In WT cells, this representation revealed active and repressive boundaries that spatially separate transcriptionally active and repressive gene clusters relative to nuclear speckles (Fig. 5C). Loss of Lamin B1 or Lamin B2 markedly disrupted this organization, resulting in large-scale shifts in gene positioning and boundary crossing between clusters.

We next examined the spatial changes of statistically significant DEGs in *LMNB1*^{−/−} and *LMNB2*^{−/−} cells. Consistent with the global patterns observed above, these DEGs frequently exhibited shifts across spatial boundaries, which correlated with corresponding changes in gene expression (Supplementary Fig. S15). For example, in a downstream region of chromosome 5 in *LMNB1*^{−/−} cells, several genes that were previously inactive or heterochromatic shifted closer to nuclear speckles and showed increased expression (Fig. 5D). In contrast, in the same chromosomal region of *LMNB2*^{−/−} cells, genes with active or weak transcription, as well as those not associated with speckles, were predominantly downregulated due to a large-scale shift of chromatin away from the nuclear speckles (Fig. 5E). Together, these results demonstrate that loss of B-type lamins is associated with large-scale repositioning of genes relative to nuclear speckles, accompanied by corresponding changes in transcriptional output at single-gene resolution.

B-type lamins depletion disrupts constitutive speckle-associated domains enriched for genes essential for cell viability

Previous studies defined SPADs as genomic regions within the top 5% of TSA-Seq enrichment scores [10, 38]. To evaluate the broader impact of B-type lamin depletion on speckle-associated chromatin, we applied a relaxed definition and identified SPADs as regions within the top 30% of SC35 TSA-Seq scores, allowing for gaps exceeding 50% (Supplementary Fig. S16A). cSPADs were defined as regions showing conserved speckle association across all five WT cell types.

Analysis of SC35 TSA-Seq profiles revealed that loss of B-type lamins markedly altered speckle association within cSPAD regions (Supplementary Fig. S16B). Although more than half of the cSPADs were retained in B-type lamin-deficient cells (Supplementary Fig. S16C and E),



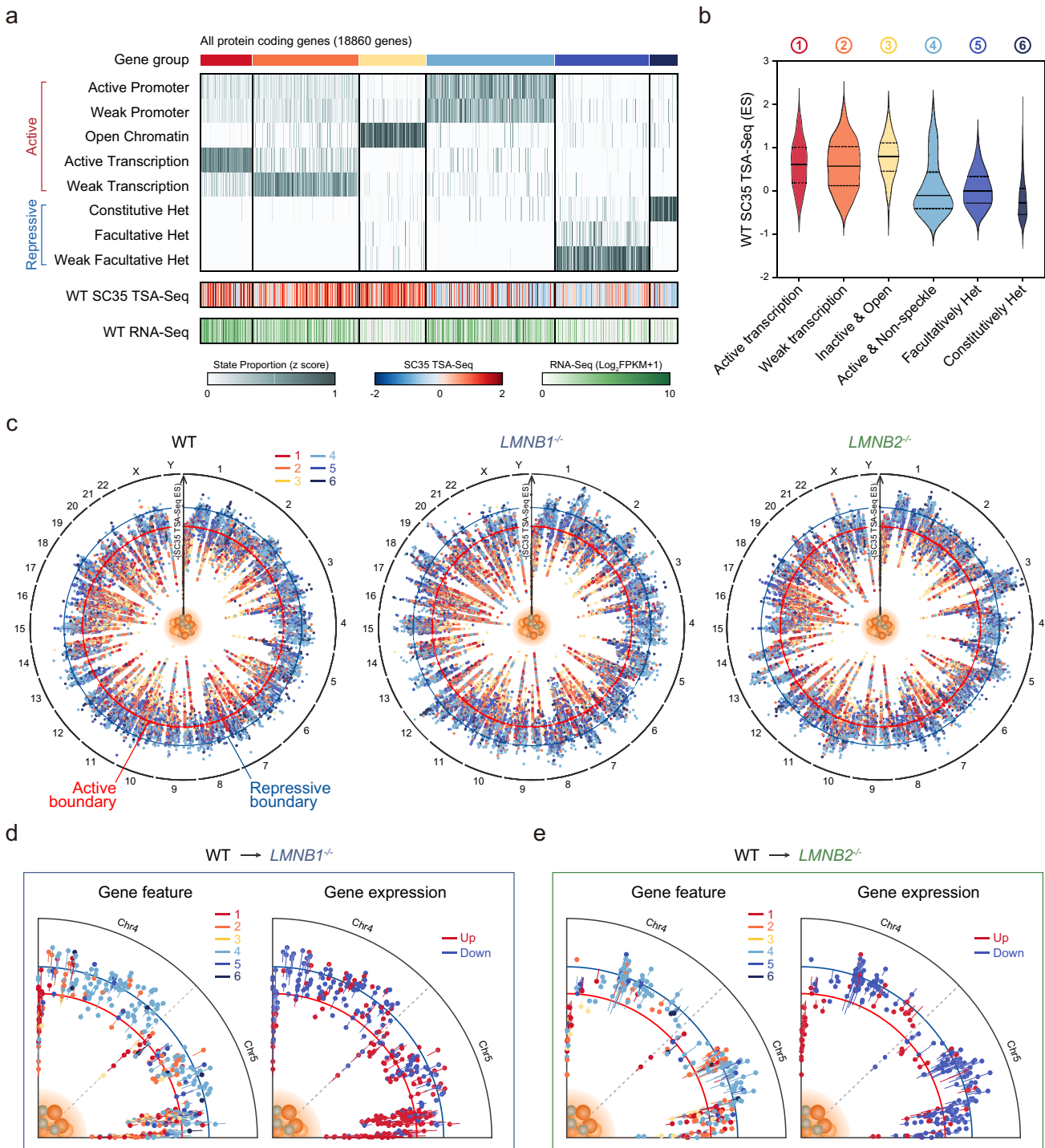


Figure 5. Single-gene level integration of chromatin features, speckle proximity, and transcriptional changes upon B-type lamin loss. **(a)** Six gene groups are clustered using eight chromatin states defined by ChromHMM in WT cells. The heatmap shows the state enrichment for each gene group (top). The averaged ES of SC35 TSA-Seq (middle) and FPKM of RNA-Seq data (bottom) are integrated for each gene and indicated by the heatmap. **(b)** Six gene groups are reannotated based on their chromatin features, and the distribution of SC35 TSA-Seq ES for WT cells is represented in a violin plot for each gene group. **(c)** The Circos plot illustrates the genome-wide gene positioning relative to nuclear speckles for WT (left), *LMNB1*^{-/-} (middle), and *LMNB2*^{-/-} cells (right). The distance from the center of each gene was determined by its averaged $-(SC35\ TSA-Seq\ ES)$ value and genes are colored according to their gene groups. Active and repressive boundaries include 90% of active transcribing genes and constitutively heterochromatic genes, respectively. **(d)** and **(e)** Relative speckle association changes of each DEG in *LMNB1*^{-/-} (D) and *LMNB2*^{-/-} cells (E) are visualized on chromosomes 4 and 5. The line depicts the level of changes for the gene positioning relative to nuclear speckles, and their final position is dotted on the plot. DEGs are colored based on their gene groups (left), and the up- or downregulation pattern of each DEG is further represented in red or blue, respectively (right). See also [Supplementary Figs S15](#) and [Table S6](#).

approximately one-quarter (24.13%) of cSPADs lost their speckle associations upon lamin depletion (Supplementary Fig. S16C and F). In addition, new SPAD regions emerged in lamin-deficient cells that were not associated with nuclear speckles in any WT condition (Supplementary Fig. S16D and G).

Genes located within disrupted cSPAD regions showed a global decrease in expression in both *LMNB1*^{-/-} and *LMNB2*^{-/-} cells and were enriched for functional categories related to essential cellular processes, including signal transduction, protein processing, and chromatin remodeling (Supplementary Fig. S17). When disrupted cSPADs were aligned with chromatin state annotations, these regions were strongly enriched for euchromatic states associated with active transcription relative to genome-wide averages (Supplementary Fig. S18A and B). In contrast, newly formed SPADs in lamin-deficient cells were preferentially enriched for repressive heterochromatin states (Supplementary Fig. S18C), and genes within these regions exhibited increased expression.

The absence of B-type lamins leads to widespread dysregulation of genes involved in multiple cellular processes

To assess the global transcriptional consequences of B-type lamin depletion, we compared the transcriptomes of *LMNB1*^{-/-} and *LMNB2*^{-/-} cells. This analysis identified 746 genes that were commonly upregulated and 781 genes that were commonly downregulated in both knockout conditions (Fig. 6A, and Supplementary Fig. S19A and C). Gene ontology (GO) analysis revealed that the co-upregulated genes were enriched for biological processes related to apoptosis, cellular senescence, lineage-associated differentiation and developmental programs (e.g. astrocyte differentiation and ossification), which are inconsistent with the identity of the studied epithelial cancer cell line, reflecting a loss of transcriptional fidelity (Fig. 6B and Supplementary Fig. S19B). In contrast, the co-downregulated genes were predominantly associated with RNA processing, transcription, protein folding, and cell cycle regulation (Fig. 6C and Supplementary Fig. S19D). Gene set enrichment analysis (GSEA) further supported these findings, showing coordinated changes in apoptotic signaling pathways and components of the spliceosomal machinery following B-type lamin loss (Supplementary Fig. S19E and F).

In addition to these shared effects, analysis of uniquely regulated genes in each knockout condition revealed both isoform-specific and overlapping patterns (Supplementary Fig. S20). Apoptosis- and differentiation-related pathways were enriched among uniquely upregulated genes in *LMNB1*^{-/-} and *LMNB2*^{-/-} cells, respectively, while also appearing in the co-upregulated gene set. Similarly, RNA processing pathways were consistently enriched among uniquely downregulated genes and overlapped with those identified in the co-downregulated group. Isoform-specific effects were also observed, including reduced DNA damage response pathways in *LMNB1*^{-/-} cells and selective downregulation of translation-related genes in *LMNB2*^{-/-} cells.

To determine whether these transcriptional changes were associated with functional cellular outcomes, we examined cell viability and proliferation. Live/dead assays revealed a significant increase in cell death in both *LMNB1*^{-/-} and *LMNB2*^{-/-} cells compared with WT controls (Fig. 6D and

Supplementary Fig. S21A), accompanied by a time-dependent decline in overall cell viability (Fig. 6E). Consistent with impaired proliferative capacity, *LMNB2*^{-/-} cells also exhibited a reduced fraction of Ki67-positive cells (Supplementary Fig. S21B).

We next assessed apoptotic phenotypes in lamin-deficient cells. Annexin V and propidium iodide (PI) staining demonstrated a significant increase in apoptotic cell populations in both *LMNB1*^{-/-} and *LMNB2*^{-/-} cells (Fig. 6F and Supplementary Fig. S21C). In parallel, cleaved Caspase-3 staining revealed a marked increase in apoptotic signaling, with at least a threefold elevation in cleaved Caspase-3-positive cells relative to WT (Fig. 6G and Supplementary Fig. S21D). Similar apoptotic phenotypes were observed following short-hairpin RNA (shRNA)-mediated acute depletion of each B-type lamin, confirming that these effects were not restricted to the knockout system (Supplementary Fig. S22).

Given the consistent downregulation of genes associated with RNA processing, we examined the expression of spliceosome-related factors. Spliceosomal components [50, 51] that were highly expressed in WT cells (FPKM > 20) showed a global reduction in expression upon B-type lamin depletion (Supplementary Fig. S23A and B), which was validated by RT-qPCR for a subset of representative genes (Supplementary Fig. S23C and D). Consistent with these changes, transcript-level analysis revealed alterations in transcript variant proportions and increased retained intron events in lamin-deficient cells (Fig. 6H–J), indicating widespread perturbation of RNA processing downstream of B-type lamin loss.

Discussion

Our study identifies a previously underappreciated role for B-type lamins in maintaining transcriptional homeostasis through spatial regulation of chromatin relative to nuclear speckles, in addition to their established functions at the nuclear lamina. This regulatory effect operates at gene body, promoter, and enhancer regions and is associated with large-scale chromatin reorganization. In the absence of B-type lamins, euchromatic genes are preferentially downregulated, whereas subsets of heterochromatic genes become derepressed (Fig. 7).

Previous studies have highlighted the importance of three-dimensional genome organization in coordinating gene activation and repression in a cell-type-specific manner, with the nuclear lamina serving as a major architectural determinant [52–54]. Given that B-type lamins anchor heterochromatin to the nuclear periphery and create a transcriptionally repressive environment, one might expect extensive activation of lamina-associated genes upon lamin loss. However, multiple nuclear envelope components, including LBR and LEM-domain proteins (LAP2, Emerin, MAN1), cooperate to tether chromatin to the nuclear membrane, thereby limiting large-scale detachment [55, 56]. Indeed, even combined deletion of multiple lamin proteins results in only modest LAD displacement, despite widespread transcriptional dysregulation occurring predominantly outside LAD regions [16, 19–22]. Advanced approaches examining chromatin compartment switching, chromatin decompaction, and chromatin mobility have provided only limited insight into relationship between B-type lamins and gene expression [21–23], suggesting that B-type lamins regulate transcription through mechanisms distinct from direct lamina-chromatin tethering.

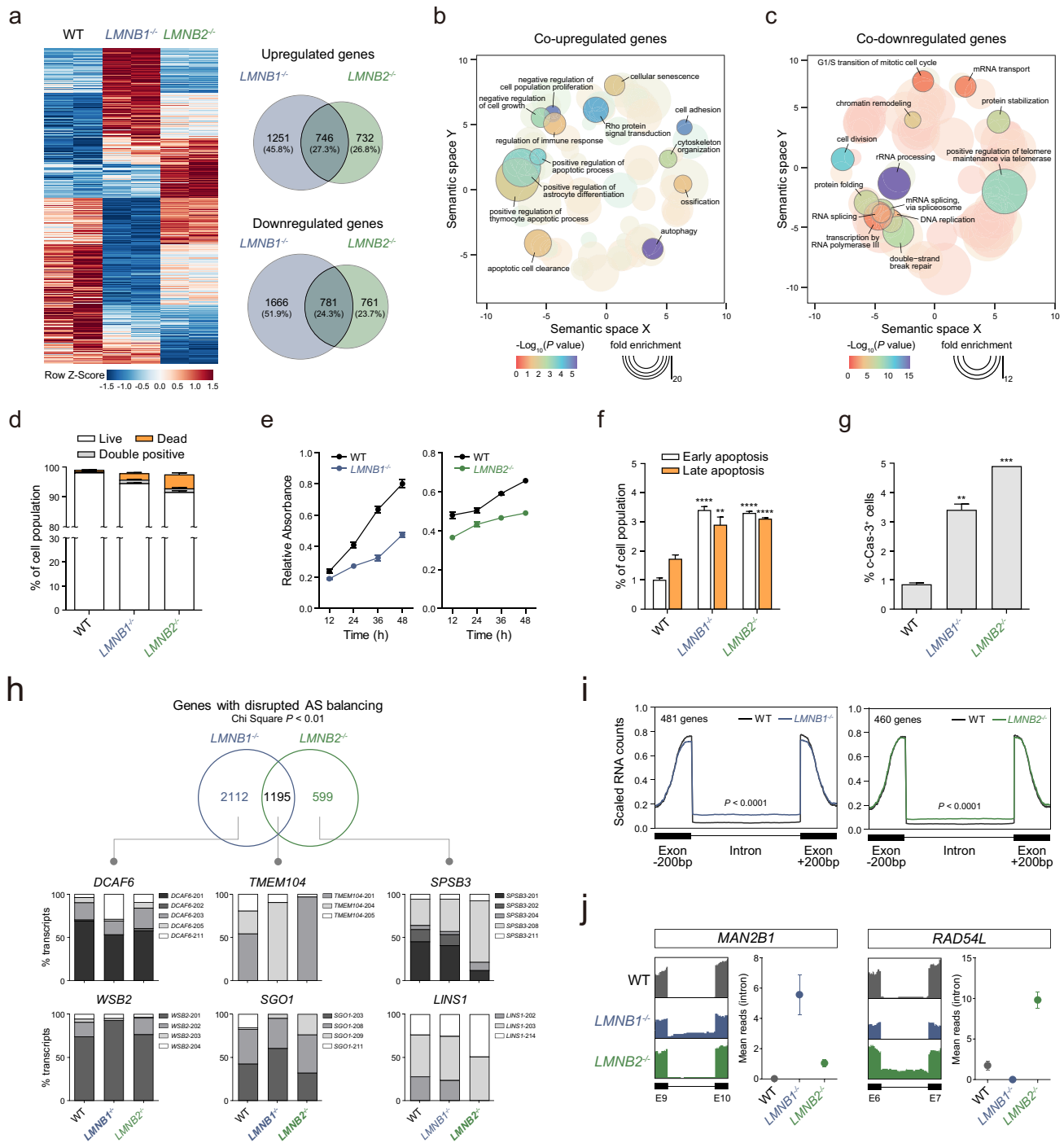


Figure 6. The absence of B-type lamins causes global dysregulation of gene sets crucial for cell survival. **(a)** Hierarchical clustering of genes with the following filtering criteria: $abs(\log_2FC) > 0.5$, $FDR < 0.01$ in WT, $LMNB1^{-/-}$, and $LMNB2^{-/-}$ cells (left). Overlapping proportions of up- (top right) and downregulated (bottom right) genes between $LMNB1^{-/-}$ and $LMNB2^{-/-}$ compared to WT cells. **(b)** and **(c)** Gene ontologies of biological processes for co-upregulated (B) and co-downregulated (C) genes in $LMNB1^{-/-}$ and $LMNB2^{-/-}$ cells are identified. A scatter plot of confidence scores for enriched gene ontologies associated with DEGs, with ontologies clustered by functional similarity in the semantic space. Circle sizes correspond to fold enrichment, with representative values of 5, 10, 15, and 20 in panel (B), and 4, 8, and 12 in panel (C). **(d)** Cell viability was determined using a live/dead assay. The proportion of each cell population (Live, Dead, and double-positive signal) is quantified. Error bars show mean $\pm$ SEM. **(e)** Time-dependent viability changes are measured using an MTT assay in $LMNB1^{-/-}$ and $LMNB2^{-/-}$ cells compared to WT cells, respectively. Error bars show mean $\pm$ SEM. **(f)** Apoptosis of WT and B-type lamin-deficient cells is tested by flow cytometry. Cells are stained with Annexin V-FITC and PI. The proportion of each cell population (early and late apoptosis) is quantified. Error bars show mean $\pm$ SEM. P -values are calculated using a two-tailed and unpaired t -test (**** $P < 0.0001$, ** $P < 0.01$). **(g)** Cleaved caspase-3 is immunostained with an anti-c-Cas-3 antibody, and the cell population with active Caspase-3 is quantified. Error bars show mean $\pm$ SEM. P -values are calculated using a two-tailed and unpaired t -test (*** $P < 0.001$, ** $P < 0.01$). **(h)** Genes with significantly disrupted transcript balance in $LMNB1^{-/-}$ and $LMNB2^{-/-}$ cells are detected (Chi-Square $P < 0.01$), and the overlapping numbers are shown in a Venn diagram. **(i)** Profile plots of scaled RNA count in the intron region retained in $LMNB1^{-/-}$ and $LMNB2^{-/-}$ cells. $P < 0.0001$. **(j)** Representative view of RNA-Seq profiles is plotted, and mean reads in intron regions are counted. Genes with retained introns in $LMNB1^{-/-}$ (left; *MAN2B1*) and $LMNB2^{-/-}$ (right; *RAD54L*) are represented. Error bars show mean $\pm$ SEM. P -values are calculated using a two-tailed and paired t -test. See also [Supplementary Figs S19–23](#) and [Supplementary Tables S7 and 10](#).

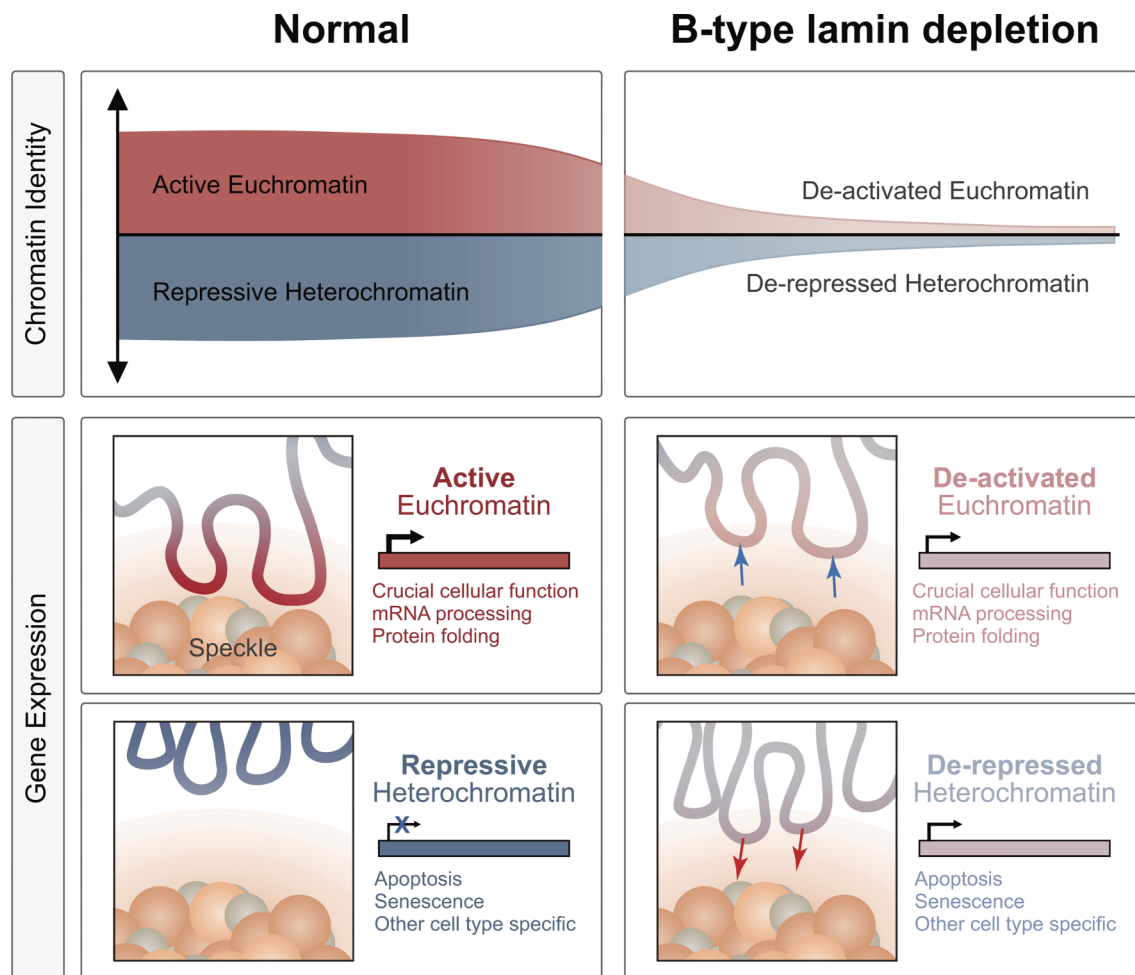


Figure 7. B-type lamins organize spatial chromatin architecture to maintain their transcriptional identity by regulating proximity to the nuclear speckles.

Nuclear speckles are dynamic, membraneless compartments enriched in transcriptional and RNA-processing factors that enhance gene expression in a proximity-dependent manner [57–59]. Although nuclear speckles do not physically bind DNA [60, 61], their spatial relationship with chromatin has emerged as a key determinant of transcriptional output. In this context, our data support a model in which B-type lamins contribute to stabilizing chromatin positioning along a speckle-lamina axis, thereby maintaining proper spatial access to speckle-associated regulatory environments.

Consistent with this model, loss of B-type lamins caused megabase-scale chromatin repositioning and disrupted conserved SPAD, leading to coordinated transcriptional dysregulation at the domain level (Fig. 3C and Supplementary Fig. S16). Comparative analysis of published TSA-Seq datasets revealed that neither cell differentiation nor acute stress conditions induce chromatin shifts of comparable magnitude (Supplementary Fig. S9D–I), underscoring a unique role for B-type lamins in global chromatin positioning. At single-gene resolution, both active and repressive gene classes exhibited extensive spatial disorganization relative to nuclear speckles, closely mirroring changes in transcriptional output (Fig. 5D and E). These findings underscore a central role for B-type lamins in tethering specific chromatin regions to the nuclear lamina and facilitating regular associations between chromatin and speckles to maintain transcriptional homeostasis.

However, because B-type lamins do not directly associate with nuclear speckles, the specific chromatin-based factors and molecular mechanisms that govern speckle-chromatin proximity downstream of lamins remain to be elucidated.

Although altered speckle association accounts for approximately 75% of the DEGs following B-type lamin depletion (Fig. 2K and Supplementary Fig. S7), a subset of genes shows expression changes that do not directly correlate with speckle repositioning. These effects likely reflect indirect regulatory cascades involving transcription factors, epigenetic modifiers, and chromatin structural proteins [62]. For example, splicing-related genes were globally downregulated in lamin-deficient cells (Supplementary Fig. S23), consistent with reduced expression of potential upstream regulators, including the ETS family that preferentially bind promoters of RNA-processing genes [63–65]. Similarly, modest reductions in epigenetic regulators such as KDM3B, whose expression and speckle association were both decreased in *LMNB2*^{−/−} cells (FPKM log₂FC (*LMNB2*^{−/−}/WT) = −0.3, SC35 TSA ES (*LMNB2*^{−/−}/WT) = −0.3), may amplify apoptotic signaling pathways [66]. In addition, reduced expression of chromatin structural proteins such as HP1α (*CBX5*) in both B-type lamin depleted cells (FPKM log₂FC (*LMNB1*^{−/−}/WT) = −1.3, FPKM log₂FC (*LMNB2*^{−/−}/WT) = −0.6) may compromise heterochromatin integrity, thereby contributing to the derepression of normally silenced regions [67, 68].

Moreover, loss of B-type lamins may perturb the complementary and isoform-specific functional interplay among lamin isoforms, thereby introducing additional indirect routes to transcriptional change. In *LMNB1*^{−/−} cells, we observed a modest increase in both nucleoplasmic and laminal pools of A-type lamins. Since nucleoplasmic Lamin A/C regulate transcription through LAP2α-mediated manner and enhancer-associated chromatin interactions, such changes may affect the expression of multiple gene sets beyond the direct impact of speckle distance. Furthermore, the broad downregulation of RNA processing and splicing factors in lamin-deficient cells raises the possibility that secondary perturbations in *LMNA* splicing, potentially followed by imbalanced production of Lamin A and Lamin C, which reported in diverse disease contexts. These considerations further support the interpretation that B-type lamins regulate the transcriptional regulatory network primarily by maintaining proper speckle–chromatin proximity, while also indirectly modulating gene expression through cascading effects on transcription factors, epigenetic modifiers, chromatin-associated proteins, and lamin isoform balance.

Chromatin organization is inherently hierarchical, encompassing loops, topologically associating domains, compartments, and chromosome territories, all of which contribute to genome function. While diverse genomic methods capture complementary aspects of nuclear organization, including chromatin accessibility [69–71], contact frequencies with nuclear features [10, 35, 37, 72], proximity-based DNA interactions [73], and radial genome positioning [74], functional interpretation based on any single dimension remains challenging. By integrating multidimensional epigenomic annotation (ChromHMM), spatial chromatin profiling, and transcriptomic analysis, our study demonstrates that loss of B-type lamins induces a coordinated redistribution of euchromatin away from nuclear speckles and aberrant relocation of heterochromatin toward them (Fig. 4H and I). These spatial shifts are accompanied by inversion of canonical transcriptional states, with deactivation of active chromatin and derepression of repressive chromatin domains (Fig. 4E).

At the single gene level, classification into six epigenetically defined clusters revealed that genes corresponding to previously defined Type I hot zones (actively and weakly transcribed, speckle–proximal) and Type II hot zones (active but non-speckle associated) are particularly sensitive to chromatin repositioning upon B-type lamin loss [10] (Fig. 5). Although genes classified as active but non-speckle-associated or inactive within open chromatin were considered to be regulated independently of speckles, both clusters showed speckle proximity-mediated expression changes following B-type lamin depletion (Fig. 5D and E). These gene clusters therefore appear to be spatially organized to enable appropriate transcriptional states, either by maintaining moderated expression at intermediate speckle distances or by remaining poised for rapid activation in close proximity to speckles. Notably, both clusters include stress-responsive genes, such as heat shock- and hypoxia-inducible genes, which can be rapidly activated either by epigenetic modifications or by relocation toward nuclear speckles [38, 75–77]. Thus, extensive chromatin disorganization resulting from the loss of B-type lamins not only disrupts basal gene expression programs but may also impair rapid transcriptional responses to sudden environmental stimuli. Such defective transcriptional

adaptability to environmental stimuli may contribute to diverse cellular phenotypes associated with aging and lamin-associated disorders (e.g. ADLD and Microcephaly), potentially through impaired coordination of speckle-associated transcription, even though many B-type lamin-linked diseases are primarily driven by lamin overexpression or specific mutations.

In summary, our findings support a role for B-type lamins in transcriptional regulation through spatial positioning of chromatin relative to nuclear speckles. B-type lamins contribute to maintaining appropriate proximity of gene bodies, promoters, and enhancer regions to nuclear speckles, thereby supporting balanced gene activation and repression. Loss of B-type lamins is associated with widespread gene expression changes, altered RNA processing, reduced cell viability, and increased apoptosis. Together, these results highlight the importance of spatial genome organization in coordinating transcriptional and post-transcriptional regulation within the nucleus.

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Supplementary data

Supplementary data is available at NAR online.

Conflict of interest

The all authors declare no competing interests.

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Data availability

RNA-Seq and TSA-Seq data that support the findings of this study have been deposited in NCBI GenBank with the primary accession code GSE275659 and GSE275661, respectively. All other data are present in the article and its Supplementary files or are available from the corresponding author upon reasonable request. Source data are provided with this paper.

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