Supplementary Information for

Nuclear lamin isoforms differentially contribute to LINC complex-dependent nucleocytoskeletal coupling and whole cell mechanics

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Materials and Methods

Immunofluorescence and microscopy

WT, KO and KD MEFs were seeded on glass coverslips (#1.5). For all studies, MEFs were fixed in 4% paraformaldehyde for 10 min at RT followed by permeabilization in PBS-containing 0.05% Triton X-100 for 10 min at RT. Following fixation and permeabilization, MEFs were incubated in PBS with 10% normal goat or donkey serum containing primary antibodies for 30 min at RT. Subsequently the coverslips were washed 3X (3 min each) in PBS containing 0.01% Tween followed by a final wash in PBS. Cells were next incubated with the appropriate secondary antibodies for 30 min at RT and then washed in PBS as above. For morphometric studies, the glass cover slips were mounted using a glycerol mounting medium with p-phenylenediamine anti-fading agent. For all other studies, coverslips and membranes were mounted on glass slides using Prolong Diamond (Thermo Fisher Scientific, MA).

The following primary antibodies were used: rabbit anti-LA (1:500; #323; (1)), mouse monoclonal anti-LC (1:50; EM-11, Novus Biologicals), goat polyclonal anti-LB1 (1:500; SC-6217, Santa Cruz Biotechnology), rabbit monoclonal anti-LB2 (1:100; EPR9701, Abcam), mouse anti-γH2AX (1:500; EMD Millipore), rabbit anti-H3K9me2 (1:1000; #39239, Active Motif), rabbit anti-H3K9me3 (1:1000; ab8898, Abcam), rabbit anti-H3K27me3 (1:1,000; 07-449, Millipore Sigma), rabbit anti-nesprin-2G (2), rabbit anti-nesprin-3 (kind gift from Dan Starr (3)), chicken anti-vimentin (1:500; 919101, BioLegend) and Hoechst 33342 (1:10,000). The secondary antibodies used in our study were AlexaFluor 488- or 647-labeled donkey anti-rabbit (1:400; Thermo Fisher), AlexaFluor 488-, 568-, or 647-labeled donkey anti-mouse (1:400; Thermo Fisher), donkey anti-goat Alexa Fluor 488, 568 (1:400; Thermo Fisher), Alexa Fluor PLUS 488- or 568-labeled goat anti-rabbit (1:400, Thermo Fisher), and Alexa Fluor 488-labeled goat anti-chicken (1:400 Thermo Fischer). The actin cytoskeleton was labeled with Alexa Fluor 488- or 568-labeled phalloidin (1:400; Thermo Fisher).
3D morphometrics, nuclear sphericity, and nuclear surface roughness analyses

Confocal z-stacks (200nm optical sections) of the cell/nucleus from WT, lamin KO, lamin KD, and rescued MEFs were imported into Imaris to generate 3D renderings for cell and nucleus area/volume measurements as described previously (4).

The measured nuclear volumes and surface areas from 3D renderings were then used to calculate nuclear sphericity as:

\[
\text{Sphericity} = \frac{\pi^{1/3}(6V^{2/3})}{A}
\]

where \(V\) is the nuclear volume and \(A\) is the surface area. A value of 1 corresponds to a perfect sphere with the value reducing to less than 1 as the object becomes more oblate.

For nuclear surface roughness measurements, the 3D nuclear profile was used to create a 200nm thick rim (equal to the size of 1 pixel) on the nuclear surface. Using the “particle” feature, the center of the nucleus was defined as the center of the smallest sphere that can fully surround the nucleus. The sum of squares for the distance between voxels on the nuclear surface and nuclear center was then calculated as:

\[
\text{Sum of squares} = \sum_{i=1}^{n} (r_i - \bar{r})^2
\]

Where \(r_i\) is the distance from a voxel on the nuclear surface to the nuclear center and \(\bar{r}\) is the average distance between the nuclear surface and the center. Higher values of the sum of squares indicate increased levels of nuclear surface roughness.

Analyses of perinuclear F-actin caps and VIF distribution

Confocal z-stacks (200nm optical sections) of the WT and lamin KO MEFs stained for DNA (DAPI), F-actin (phalloidin), and vimentin were first used to identify the apical and basal surface of the nucleus. The corresponding z-stacks in the F-actin and vimentin channels were then used to determine the presence or absence of F-actin caps at the nuclear apical surface as previously described by Kim et al. (5, 6) and the perinuclear VIFs cage as we described previously (7). The confocal stacks were further used to create an orthogonal section of WT and lamin KO MEFs to examine the overall localization of VIFs and F-actin at the cell cortex level.

To examine the uniformity of the perinuclear VIF distribution, the Julia programming language (8) was used to load raw images of the WT, lamin KO, and rescued MEFs stained for the nucleus and VIFs using the Julia package, BioFormatsLoader.jl, a wrapper for the Bioformats library in Java (9), and maximum-intensity projections were
then calculated. Using the Julia package Images.jl, the nucleus was segmented by applying smoothing with a Gaussian kernel with an SD of 4 pixels (0.8 \( \mu m \)). The method of Otsu (10) was then used to automatically calculate a threshold based on the smoothed image. The threshold was subsequently used to binarize the smooth image to provide segmentations of the nuclei from the pixels above the threshold. The nuclei were then subjected to connected component analysis, and the centroids and areas were calculated. Segmentations of the nuclei were accepted if the calculated area was \( \geq 120 \mu m^2 \). A Euclidean distance map relative to the segmented nuclei was calculated, and a watershed transformation was done to partition the image into regions closest to each nucleus. Within each region, pixels were discretized into 2-\( \mu m \) increments (10 pixels) away from the boundaries of the nucleus. A total of 60 equidistant angular samples were measured. The function “circ r” was calculated as in the CircStats Toolbox for Matlab (11) to calculate the resultant vector lengths (R) based on the 60 angular samples. The circular variance (S) was then calculated as:

\[
\text{Circular Variance} = 1 - R.
\]

The circular variance for a perfectly uniform circular distribution is 1; thus, lower values of “R” indicate higher levels of non-uniformity in the distribution.

**Chromatin immunofluorescence intensity measurement**

Fluorescence intensity measurements were made using confocal z-stacks (200 nm optical sections) of nuclei using a 60x (oil, 1.4 NA) objective. Maximum projections of the images of nuclei were obtained in ImageJ using automatic threshold detection. The background fluorescence was determined by selecting a 50 x 50 pixel cell free area. The average fluorescence intensity of the nucleus was determined, and the average background intensity present within the image was subtracted (12). All imaging and threshold settings were kept constant when generating and processing the data for comparison of the different MEF lines.

**Generation of the LA KD and LC KD MEFs**

Selective KD of LA or LC was carried out by transduction of WT MEFs with lentiviruses prepared from pLKO.1 constructs containing a neomycin/G418 resistance cassette as well as the shRNAs targeting the non-conserved regions of LA or the 3’ UTR of the LC mRNA (13). Viral particles were produced in 293FT cells co-transfected with the LA- or LC-
targeting pLKO.1 constructs as well as psPAX2 and pVSV-G. Culture medium containing viral particles was harvested 48 hrs following transfection and incubated with WT MEFs overnight with the inclusion of 8µg/ml polybrene. The transduced MEFs were then selected with 400µg/ml G418 for 7 days and subsequently screened for LA or LC KD by immunofluorescence microscopy and immunoblotting with antibodies specific for either LA (rabbit #321) or LC (EM-11 Novus Biologicals).

**Generation of rescued lamin KO MEFs**

The re-expression of lamin isoforms in the KO MEFs was achieved by transiently transfecting these cells with pPyCAGIP constructs that encode mouse LA, LB1, or LB2 using Lipofectamine 3000 (Thermo Fisher Scientific) (14). Forty-eight hours following transfection, the cells were selected for 2 days with 2 µg/ml puromycin and immediately used for AFM. The expression of lamins was confirmed by immunofluorescence with antibodies specific for each lamin isoform as described in the section on Immunofluorescence and microscopy. The average lamin concentration in the rescued lamin KO MEFs was determined by quantitative immunoblotting with the following antibodies: LA (rabbit 266, 1:500 (15)); LB1 (Proteintech AG3631, 1:2000); LB2 (mouse 2B2 1:1000;(15)). The average amount of specific lamin expression in each of the rescued MEF lines compared to WT MEFs was 63% for LA, 80% for LB1, and 290% for LB2 determined as described in the section on Immunoblotting.

**RNA interference.**

The siRNA pool (3 gene specific siRNAs, SYNE2 Mouse siRNA Oligo Duplex (Locus ID 319565), Cat# SR423729) and the scrambled negative control (Trilencer-27 Universal Scrambled Negative Control siRNA Duplex, Cat# SR30004) for Nesprin-2 were purchased form ORIGENE. Nesprin-3 siRNA SMARTpool (4 individual siRNAs, Mouse Syne3 siRNA, Gene ID: 212073) and its ON-TARGETplus Non-targeting control pool (Cat# D-001810-10-05) was from Horizon Discovery. siRNA transfection on day one at 20nM for Nesprin-2 and 50nM for Nesprin-3 followed by a second transfection at similar siRNA concentration after 24hrs gave the highest knockdown efficiency in the examined MEFs 48hrs after initial transfection as evidenced by immunofluorescence examination for Nesprin-2 and Nesprin-3.
**Cell cycle analysis**

Cells were harvested and washed twice with PBS at 335xg for 5 min followed by fixation in 70% ethanol for 30 min at -20°C. After fixation, the cells were diluted with PBS and centrifuged at 1126xg for 10 min followed by a PBS wash. The fixed cells were then stained by resuspending in 0.5 ml of DAPI solution (1 µg/ml of DAPI in 0.1% Triton X-100) for 30 min at 4°C. The stained cells were analyzed on a BD FACS Melody Cell Sorter (BD Biosciences, CA).

**Immunoblotting**

Whole cell lysates of MEFs were prepared by trypsinizing cells, quenching in DMEM with 10% FBS, washing twice in PBS by centrifugation at 200xg and finally solubilizing the pellet in 1x SDS sample buffer containing equal numbers of the WT MEFs, the lamin KO MEFs, and the lamin KD MEFs. Lysates were run in triplicate on an SDS-PAGE gel and electrophoretically transferred to nitrocellulose. The membranes were stained for total protein with the Revert Protein Staining kit (LI-COR) and imaged at 700 nm using a LI-COR Odyssey Fc. The protein stain was then removed using the destaining solution included in the kit. After blocking the membranes in 5% non-fat dry milk in PBS with 0.1% Tween 20, the blots were probed with specific antibodies in blocking buffer overnight at 4°C. Antibodies used: LA/C rabbit 266 (1:500) (15); LB1 rabbit Proteintech AG3631 (1:2000); LB2 mouse 2B2 (1:1000) (15); H3K9me mouse Cell Signaling 6F12 (1:2000); H3K27me rabbit Cell Signaling C36B11 (1:1000). After washing 3X for 5 mins each in PBS with 0.1% Tween 20, the blots were probed with 1:15,000 dilution of IRDye 800CW Donkey anti-mouse IgG or 800CW donkey anti-rabbit secondaries (LI-COR) in 5% non-fat dry milk in PBS with 0.2% Tween 20 for 45 mins at RT. After washing twice for 5 mins each in PBS containing 0.2% Tween 20, the blot was allowed to air-dry in the dark. Imaging of the membranes was performed on a LI-COR Odyssey Fc and the resulting images were analyzed using LI-COR Empiria software. The amount of protein present in each lane was quantified from the Revert protein stain image and used to correct for sample loading across the different lanes of the gel.

**Determination of the fluorescence intensity and time of half-recovery (t_{1/2}) for the FRAP experiments**
Nikon Elements (NIS-Elements) was used to quantify the average fluorescence intensity in the region of interest and total cellular intensity. It was then normalized to the changes in total fluorescence intensity as 

\[ I_{\text{rel}} = \frac{T_0 I_t}{T_t I_0}, \]

where \( T_0 \) is the total cellular intensity during the prebleach, \( T_t \) is the total cellular intensity at time point \( t \), \( I_0 \) is the average intensity in the bleached area during prebleach, and \( I_t \) is the average intensity in the region of interest at time point \( t \) (16). The normalized fluorescence was then plotted against the time after bleaching. To account for the differences of the immobile fraction (the difference between the fluorescence intensity in the bleached area prebleach and the intensity at infinity after bleach) between different cell types and EGFP-tagged constructs, we used a modified time of half-recovery value (\( t_{1/2} \)) where \( t_{1/2} \) is the time after bleach required for the fluorescence levels to reach the median between levels immediately after bleach and prebleach, rather than using the median between prebleach levels and steady-state levels (17). To determine \( t_{1/2} \), we used a modification of the method described by Harrington et al. (18). We plotted \( \ln(1 - i_t) \) versus time after bleach, where it is the mean normalized fluorescence intensity in the bleach region at time \( t \) and 1 is the mean normalized fluorescence intensity in the bleach region prebleach. The curves were fitted using Excel, and \( t_{1/2} \) was calculated as \( t_{1/2} = \ln 2 \times (-1/\text{slope}) \) (17). Data from the first 31 s after bleach were used in all experiments.

**Analysis of AFM data and calculations of Young’s modulus**

AFM measurements were performed at 200-400nm indentations (less than 10-20% of cell height) to avoid substrate effects (19, 20) and with a ramp speed of 800 nm/sec, which is slow enough to make measurements rate independent (21). Data from AFM measurements were obtained in the form of tip deflection “d” as a function of tip travel distance “z” (Fig. S11A) (22). The indentation, “\( \delta \)”, which is the net distance the tip indents into the sample, is then defined as \( \delta = (z - z_0) - (d - d_0) \), where \( z_0 \) and \( d_0 \) are respectively the probe position in the z direction and the deflection of the tip at the initial contact point between the probe and the sample (Fig. S11A). We use an inhouse written MATLAB code based on an objective automatic method proposed by Crick and Yin (23) to determine the initial contact point \((z_0, d_0)\). Next, the force vs indentation plot was derived (Fig. S11B); The indentation force, “F”, was calculated using Hooke’s law, \( F = k \times d \), where \( k \) is the spring constant of the cantilever.
To determine the aggregate cell modulus (E) for a rounded AFM tip of radius R, we used the following Hertz relationship (24):

$$E = \frac{3F(1-v^2)}{4R^{1/2}\delta^{3/2}}$$  \hspace{1cm} (S1)

where F is the applied force, δ is the indentation, and v=0.5 is the Poisson ratio (25-27) (Fig. S11C). For sharp tips, we used a corrected model for pyramidal indenters that accounts for the spherical cap at the apex of the AFM tip (28, 29):

$$F = \frac{2E}{(1-v^2)} \left\{ a\delta - \frac{\sqrt{2}a^2}{\pi \tan \theta} \left[ \frac{\pi}{2} - \sin^{-1} \left(\frac{b}{a}\right) \right] - \frac{a^2 - b^2}{3R} + \frac{b}{\pi \tan \theta} \left[ \frac{\sqrt{2}b}{3R} + \frac{(a^2-b^2)}{3R} \right] \right\}$$

(S2a)

$$\delta + \frac{a}{R} \left[ a - (a^2 - b^2)^{1/2} \right] + \frac{2\sqrt{2} a}{\pi \tan \theta} \left[ \frac{\pi}{2} - \sin^{-1} \left(\frac{b}{a}\right) \right] = 0$$

(S2b)

where b=R cosθ (θ=20°), and R is the radius of the spherical cap (20 nm). Equation (S2b) is used to find the effective radius of contact “a” for any indentation δ, and then used in equation (S2a) to find E for a given force F (Fig. S11D).

We (21) and others (19, 20) have shown that these models eventually asymptote to values of E that are relatively independent of δ (In our case, for values of δ greater than approximately 100 nm, Figs. S11 C-D). These δ-independent moduli were then averaged and reported as estimated apparent Young’s Modulus.

**Fourier Transform Traction Microscopy**

Acrylamide-based hydrogels were made on glass bottom 6-well plates as previously described (30) and the Young’s modulus of the gels was 8 kPa, as determined by AFM. After coating the hydrogels with fluorescent markers to visualize displacement and bovine collagen (40 μg/mL) to promote cell attachment, we sparsely seeded cells on the hydrogels and incubated overnight. We used an epifluorescence microscope equipped with an environment control chamber (Leica DMi8, Germany) to capture images of the cells and the fluorescent markers in the hydrogels. Based upon these images, displacements made by cells on the hydrogels were calculated using particle image velocimetry (31) and traction was retrieved from the resulting displacement fields using FTTM (32). As a measure of cellular contractile force, we used both strain energy and net contractile moment, of which the former is less sensitive to the variation in cell spreading area than the latter (32).
Optical tweezers

A laser beam (10 W, 1,064 nm) was tightly focused through a series of Keplerian beam expanders and a 100x Nikon objective (oil, 1.45 NA). A high-resolution quadrant detector was used for position detection. To measure the mechanical properties of living cells, latex beads with a diameter of 0.5 μm (L3280, Sigma) were added into the culture medium and were endocytosed by the cells overnight at 37°C with 5% CO₂. To measure the stiffness of the cytoplasm, we selected particles away from both the thin lamellar region at the cell periphery and the nucleus to avoid any interactions with the mechanically distinct cell cortex and nucleus. The selected particle was then dragged away from the nucleus. To measure the stiffness of the nucleus, we selected particles adjacent to the nucleus, and dragged them towards the nucleus. Particles were dragged at a constant velocity of 1 μm/s by the optical trap and the force-displacement curve was recorded. The slope in the linear range of the force-displacement curve was taken as the local stiffness (33).

Wound healing assay

Wound healing assays were performed in 35mm μ-dishes with 4 well silicon inserts (Ibidi GmbH). Cells were seeded at 50,000 cells per well and incubated overnight at 37°C with 5% CO₂. The next day, the inserts were removed, and the migrating cells were imaged for 8 hrs of wound closure time using phase contrast microscopy performed with a 20x (air, 0.3 NA) objective at 37°C with 5% CO₂. Image analysis was performed using ImageJ to calculate the remaining wound area and the wound closure rate after 8 hrs.

Transwell migration assays

Polycarbonate transwell membranes with pore diameters of either 3 μm or 5 μm (Corning, NY) were used for migration assays. Membranes were pre-coated with rat-tail collagen 1 (50 μg/mL, Thermo Fisher, MA) for 30 min, rinsed with PBS, incubated with pre-warmed growth medium for 60 min, and then seeded with 10,000 cells/well followed by incubation at 37°C for 18 hours. The membranes were fixed and stained for immunofluorescence with LA, LB1, and γH2AX antibodies (see above) and then mounted on glass coverslips using Prolong Diamond (Thermo Fisher Scientific). The membranes were imaged with a 20x (air, 0.3 NA) objective in 600 x 600 μm² fields of view (10 consistent locations on the membrane per condition) and nuclear count and area were determined by lamin staining and through thresholding and automated particle analysis in ImageJ (34). Bi-nucleated cells
and nuclei with areas less than 75 μm², which were likely to be micro-nuclei, were excluded. The migration percentage across the membrane was then calculated as the ratio of the number of cells on the bottom of the membrane to the sum of cells on top and bottom of the filter. For analysis of double-stranded DNA damage induced by transwell migration, confocal images of the membranes were acquired with a 40x (oil, 1.4 NA) objective in 200 x 200 μm² fields of view (10 locations per condition). The background fluorescence and average fluorescence intensity of the nuclei were determined as described in the section for “Chromatin immunofluorescence intensity measurement”. For comparison between the cell types, the results were then normalized to the average fluorescent intensity in the WT MEFs. All experiments were conducted a minimum of two times with two replicates for each group per experiment.
Supplementary Figures

A

|   | LA | LC | LB1 |
|---|----|----|-----|
| WT| ![Image](WT_LA.png) | ![Image](WT_LC.png) | ![Image](WT_LB1.png) |
| LA KD| ![Image](LA_KD_LA.png) | ![Image](LA_KD_LC.png) | ![Image](LA_KD_LB1.png) |
| LC KD| ![Image](LC_KD_LA.png) | ![Image](LC_KD_LC.png) | ![Image](LC_KD_LB1.png) |
Figure S1: Validation of the lamin knockdown and rescued MEF lines used in this work. (A) Representative wide-field immunofluorescence images of the nuclei from the indicated MEF lines stained for anti-LA, anti-LC, and anti-LB1. (B) Quantitative immunoblot for the expression level of the knockdown protein in LA KD and LC KD MEFs. (C) Representative wide-field immunofluorescence images of the nuclei in LA/C-, LB1-, and LB2- MEFs respectively expressing LA, LB1, or LB2. (D) Quantitative immunoblot for the expression level of the rescued protein in LA/C-, LB1-, and LB2-MEFs expressing LA, LB1, and LB2 respectively. Scale bar is 20 μm.
Figure S2. Loss of nuclear lamins alters nuclear and cell morphology. Violin plots of (A) Sum of squares for the distance between the nuclear surface and center in WT (n=3, 25 cells), LA/C- (n=4, 28 cells), LB1- (n=4, 52 cells), and LB2- (n=4, 62 cells) MEFs, (B) nuclear sphericity, (C) nuclear volume, (D) cellular volume, and (E) cellular spreading area in the WT (n = 3, 55 cells), LA KD (n = 3, 66 cells), and LC KD (n = 3, 82 cells) MEFs. (F) Scatter plot of cellular vs. nuclear volume in the WT (slope = 6.72, P < 0.0001), LA/C- (slope = 6.14, P < 0.0001), LB1- (slope = 4.69, P < 0.0001), and LB2- (slope = 2.04, P < 0.01) MEFs. The regression slopes in LB1- and LB2- MEFs are significantly different from WT and LA/C- MEFs (P < 0.0001). (G) Scatter plot of cellular vs. nuclear volume showing the correlation between the two variables in WT (slope = 6.72, P < 0.0001), LA KD (slope = 6.47, P < 0.0001), LC KD (slope = 4.37, P < 0.0001) MEFs. The regression slope for LC KD MEFs is significantly different than those in WT and LA KD MEFs (P < 0.0001). The solid bars in the violin plots represent the median and the dashed lines mark the 25th and the 75th percentiles. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure S3. Flow cytometry analysis of the MEF lines used in this work. Histograms of the cell cycle distributions of (A) WT, (B) LA/C-, (C) LA KD, (D) LC KD, (E) LB1-, and (F) LB2- MEFs. The data are quantified as the percentage of the cells in G1, S, and G2 stages of the cell cycle (n=3). The x and y axes denote the DNA content and cell number, respectively.
Figure S4. Knockdown of LA or LC compromises cellular mechanics. (A) Violin plots of the AFM round probe measurements for the cytoplasmic stiffness in the WT (n = 4, 75 cells), LA KD (n = 2, 55 cells), and LC KD (n = 2, 53 cells) MEFs. (B) Box plots of OT measurements of the cytoplasmic stiffness in the WT (n = 1, 9 cells), LA KD (n = 1, 8 cells), and LC KD (n = 1, 13 cells) MEFs. (C) Violin plots of the AFM sharp probe measurements for the apical cortex stiffness in the WT (n = 4, 147 cells), LA KD (n = 2, 99 cells), LC KD (n = 2, 100 cells) MEFs. (D) Violin plots of the logarithmically transformed SE in the WT (n = 2, 105 cells), LA KD (n = 1, 36 cell), and LC KD (n = 1, 39 cells) MEFs. (E) Violin plots of the logarithmically transformed net contractile moment in the WT (n = 2, 105 cells), LA KD (n = 1, 36 cells), and LC KD (n = 1, 39 cells) MEFs. The solid bars in the violin plots represent the median and the dashed lines mark the 25th and 75th percentiles. The bars and the whiskers in the box plots represent the median and the minimum/maximum, respectively. *P < 0.05, **P < 0.01, ***P < 0.001.
Figure S5. The nuclear lamin isoforms distinctively regulate the dynamics of LINC complex components at the NE of MEFs. (A) Representative fluorescent images of the WT, LA/C-, LB1-, and LB2- MEFs lines expressing the indicated EGFP-tagged LINC complex component constructs and subjected to FRAP. The white boxes indicate the photobleached regions of interest. (B) Bar plots of the average $t_{1/2}$ of recovery of the indicated EGFP-tagged LINC complex constructs expressed in the WT, LA/C-, LB1-, and LB2- MEF lines (n ≥ 2; 10-15 cells per experimental condition). The data are shown as mean ± SE. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Scale bar is 10 μm.
Figure S6. Immunofluorescence analyses of perinuclear F-actin and VIFs. (A) Representative images of the nucleus (blue), F-actin (magenta) and vimentin (green) in WT and lamin KO MEFs shown as maximum projections and their corresponding confocal stacks at the bottom and top surface of the nucleus (marked via dashed square). (B) Bar plots for quantification of F-actin cap presence on the apical nuclear surface in WT (n=4, 48 cells), LA/C- (n = 3, 32 cells), LB1- (n = 3, 53 cells), and LB2- (n = 4, 59 cells) MEFs. (C) Orthogonal sections of WT and lamin KO MEFs stained for F-actin and vimentin. The data in bar plots are shown as mean ± SE. Scale bar is 20 μm.
Figure S7. Microscopic verification of the dominant negative inhibition or selective siRNA disruption of LINC complexes. (A) Representative wide-field immunofluorescence images of nesprin-2G staining in the WT MEFs expressing the indicated SS-EGFP-tagged constructs. (B) Representative wide-field immunofluorescence images of nesprin-2G (top) or nesprin-3α (bottom) staining in a MEF line (shown here is LB2-) treated with the nesprin-2G (top) or the nesprin-3α (bottom) targeting siRNA or the non-coding control siRNA followed by staining for nesprin-2 (top) and nesprin-3 (bottom). Scale bar is 20 μm.
**F**

WT  LA  LA KD  LC KD  LB1  LB2

**G**

WT  LA  LA KD  LC KD  LB1  LB2

**H**

WT  LA  LA KD  LC KD  LB1  LB2

**I**

H3K9me

WT  LA  LA KD  LC KD  LB1  LB2

H3K27me

WT  LA  LA KD  LC KD  LB1  LB2

25 kDa
Figure S8. Loss or knockdown of lamin isoforms affects nuclear stiffness and alters heterochromatin levels in MEFs. Violin plots of AFM round probe measurements of the nuclear stiffness in the WT (n = 4, 188 cells), lamin KO (n = 4, 69-73 cells), and rescued (n = 2, 33-39 cells) MEFs (A) and LA KD (n = 2, 54 cell), and LC KD (n = 2, 55 cells) MEFs (B). Box plots of OT measurements for the nuclear stiffness in the WT (n = 1, 9 cells), lamin KO (n = 1, 9-12 cells), and rescued (n = 1, 9-12 cells) MEFs (C) and LA KD (n = 1, 14 cells), and LC KD (n = 1, 9 cells) MEFs (D). (E) Representative maximum projections of confocal z-stacks of nuclei in the WT, LA/C-, LA KD, LC KD, LB1-, and LB2- MEFs stained for H3K9me2, H3K9me3, or H3K27me3. Violin plots of the mean fluorescent intensity of (F) H3K9me2, (G) H3K9me3, and (H) H3K27me3 in the WT (n = 2, 54-93 cells), LA/C- (n = 2, 44-59 cells), LA KD (n = 2, 30-77 cells), LC KD (n = 2, 71-85 cells), LB1- (n = 2, 61-132 cells), and LB2- (n = 2, 91-117 cells) MEFs. (I) Immunoblot for the expression levels of the H3K9me (top) and H3K27me (bottom) in the WT, lamin KO and lamin KD MEFs. The solid bars in the violin plots represent the median and the dashed lines mark the 25th and 75th percentiles. The bars and the whiskers in the box plots represent the median and the minimum/maximum, respectively. *P < 0.05, **P < 0.01, ***P < 0.001. Scale bar is 20 μm.
A  

\( t = 0 \)  
\( t = 8 \text{ hours} \)

B  

Average 2D Migration Rate (\( \mu \text{m}^2/s \))

C  

Average 2D Migration Rate (\( \mu \text{m}^2/s \))

D  

Average Migration (%)

E  

Average Migration (%)

F  

Average Migration (%)
Figure S9. Loss or knockdown of lamin isoforms changes migratory behavior in MEFs. (A) Representative phase contrast images of the indicated MEF lines migrating in a 2D wound healing assay for 8 hours. The t = 0 is when the silicon insert is removed from the dish. The yellow lines demarcate the leading edge of the wound. Scale bar is 100 μm. Bar plots of the average 2D migration rate during the 2D wound healing assay (n = 8) for the WT, lamin KO (B), and KD (C) MEFs. Bar plots of the average migration percentage for the WT, lamin KO (D), and KD (E) MEFs challenged with transwell membranes with 3 μm or 5 μm pore diameters (n ≥ 3 for each set of experiments). Comparison of the nuclear to pore diameter ratio and migration through 3 μm and 5 μm pores in lamin KO (F) and KD (G) MEFs to the WT MEFs (n ≥ 50 cells per condition). (H) Bar plots of the average migration percentage through transwell membranes with 3 μm pores for the indicated MEF lines (n ≥ 3). (I) Scatter plots of the average nuclear area for the indicated MEF lines present at the top and bottom of the transwell membranes. (J) Same as (I) for the the WT, LA KD, and LC KD MEFs. (n ≥ 3, at least 50 cells per experiment condition). Data are shown as mean ± SE. *P < 0.05, **P < 0.01, ***P < 0.001.
### A

|               | Before migration | After migration |
|---------------|------------------|-----------------|
| **WT**        |                  |                 |
| **LA/C-**     |                  |                 |
| **LB1-**      |                  |                 |
| **LB2-**      |                  |                 |
| **LA KD**     |                  |                 |
| **LC KD**     |                  |                 |

- **DAPI**
- **γ-2AX**
Figure S10. Loss or knockdown of lamin isoforms increases constricted migration-induced DNA damage in MEFs. (A) Representative maximum projections of confocal z-stacks of DAPI- and γ-H2AX-stained nuclei in the indicated MEF lines before and after their migration through transwells with 3 μm pores. Bar plots of the normalized post/pre migration γ-H2AX foci counts in the WT, lamin KO (B), and KD (C) MEFs that migrated through transwell membranes with 3 μm pores. (n = 3, at least 150 cells per experiment condition). Data are shown as mean ± SE. *P < 0.05, **P < 0.01, ***P < 0.001. Scale bar is 20 μm.
Figure S11. Procedure for AFM data analyses and Young’s Modulus calculations. (A) Representative plot for the tip deflection (d) as a function of tip traveling distance (z). The black arrow indicates the identified contact point \((z_0, d_0)\) between the tip and cell surface. (B) Calculated force vs indentation curve for an AFM measurement with sharp tip (purple) and 10 µm round tip (orange). Representative plot for calculated Young’s modulus for a 10 µm round tip (C) and a sharp tip (D) measurement.
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