Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Information

A comprehensive method to study the DNA's association with lamin and chromatin compaction in intact cell nuclei at super resolution

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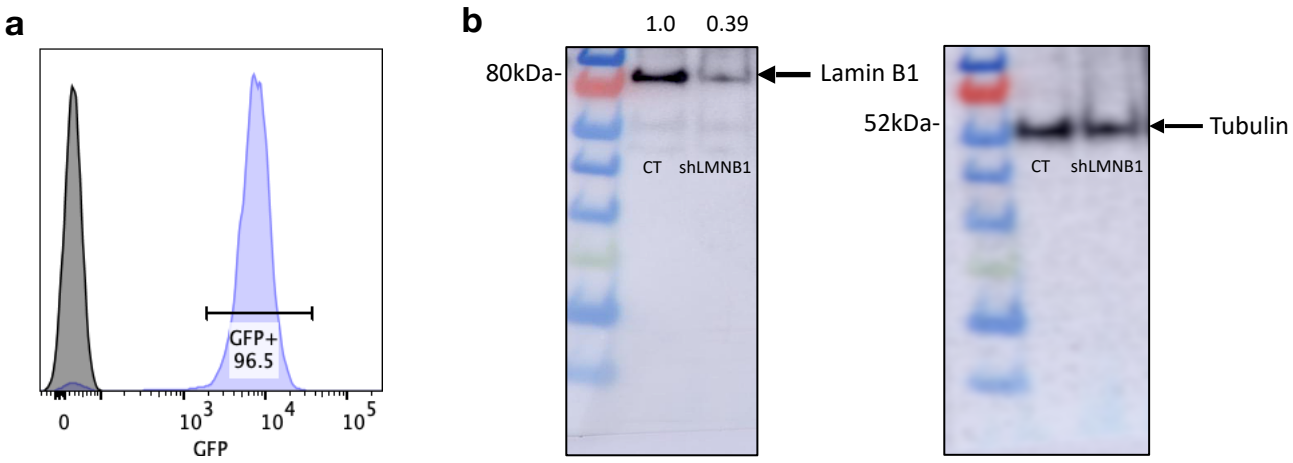

Figure S1: shRNA-mediated knockdown of LaminB1 in BL-2 lymphoma cells. (a) Flow cytometry chart showing the percentage of GFP+ BL2 cells upon the induction of shRNA with doxycycline. Control BL-2 cells are shown in grey and shLMNB1 in blue. (b) Immunoblot of total LaminB1 levels in BL-2 cells upon inducing shRNA. Tubulin was used as a loading control. Image was acquired using Amersham Image 600 and analyzed in ImageJ. Normalized ratio of LaminB1 to Tubulin in control and shLMNB1 sample is indicated.

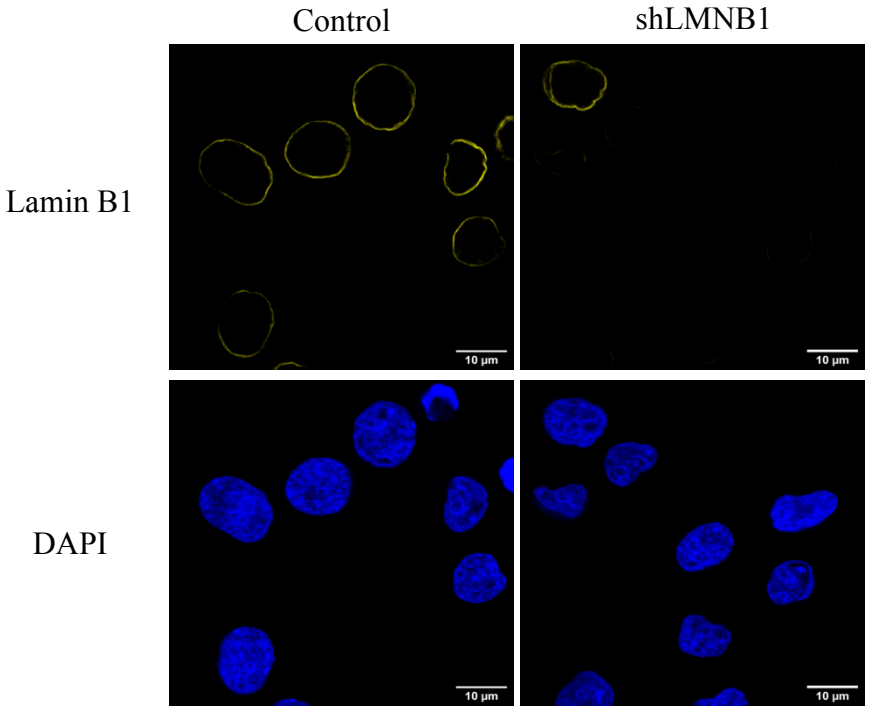

Figure S2: Confocal microscopy of LaminB1 after performing shRNA-mediated knockdown. Nuclear LaminB1 was assessed 72 hours after inducing shLMNB1 by Zeiss LSM 710 confocal microscope. DAPI was used as a nuclear stain. Scale bar represents 10 µM.

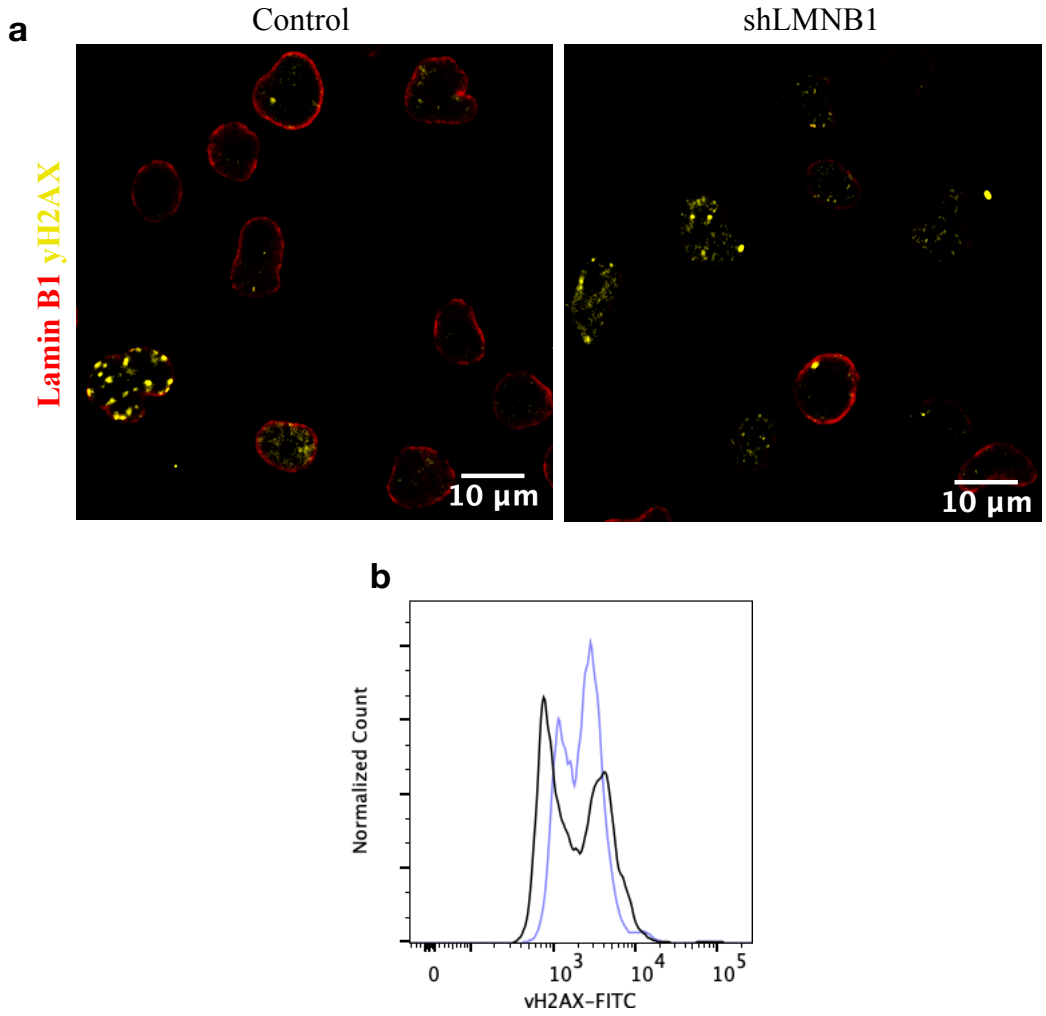

Figure S3: Assessment of LaminB1 and γH2AX after performing shRNA-mediated knockdown of LaminB1 prior to SMLM imaging. (a) Nuclear LaminB1 was assessed 72 hours after inducing shLMNB1 by Zeiss LSM 710 confocal microscope. Scale bar represents 10 µM (b) Flow cytometry chart of γH2AX intensity in control (black) and shLMNB1 (blue) BL-2 cells. At least 10,000 cells were acquired for the analysis.

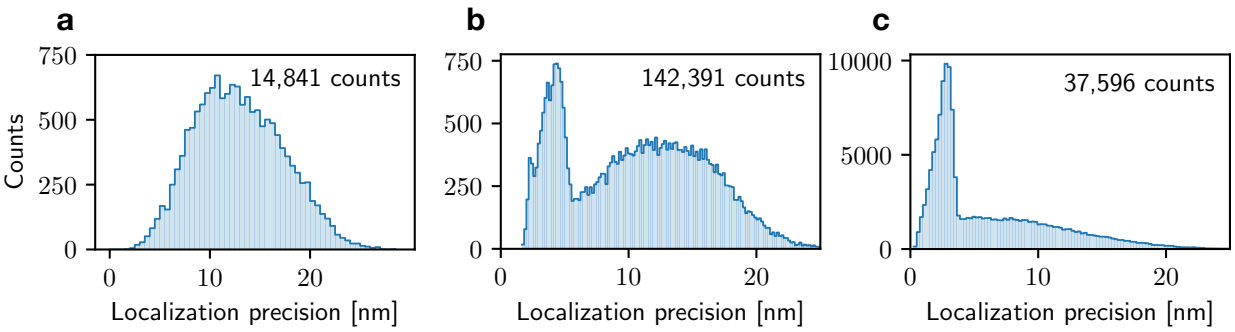

Figure S4: Representative localization precision distributions for samples imaged with (a) the original SMLM method in ProLong Gold (here shown for LaminB1) and (b, c) dual-colored samples imaged with fBALM buffer and ProLong Gold (b shows Sytox Orange localizations, c shows LaminB1). Each distribution shows the data collected from 2,000 image frames of a single cell and the number of localizations detected in that particular measurement. Measurements using the original SMLM method (a) were analyzed with SMLM.py and fBALM measurements (b, c) were analyzed with ThunderSTORM.

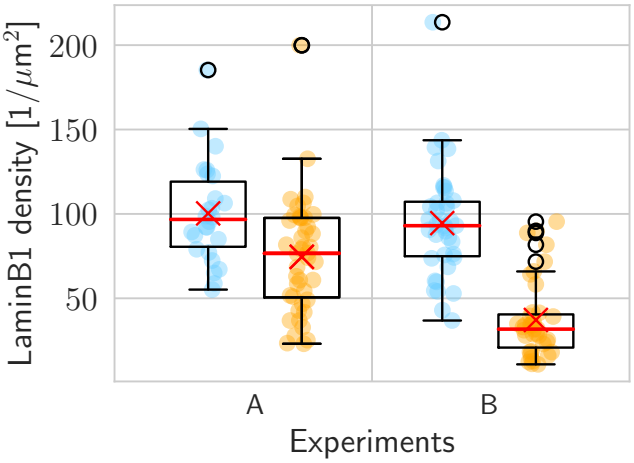

Figure S5: Effect of the LaminB1 knockdown on LaminB1 localizations per area. The LaminB1 signal density was compared between two identically prepared batches, experiment A and B. In experiment A, the LaminB1 knockdown led to distinct holes in the lamina, while in experiment B a globally disintegrated structure was observed. This led to a reduced incorporation of LaminB1 in the nucleus in experiment B as compared to A. 26 control and 44 shLMNB1 nuclei were measured in experiment A, 39 control and shLMNB1 nuclei in B. Each colored point shows the data of one nucleus. Blue represents control and orange shLMNB1 nuclei. The mean is represented with a red x, and the median is indicated by a red line. The box shows the interquartile range (IQR). The IQR has values ranging from the first quartile (Q1) to the third quartile (Q3). The end of the lower whisker is defined by Q1 - 1.5 · IQR, and the upper end is defined by Q3 + 1.5 · IQR. The circles are outliers.

Antibody	Dilution	Time	Host Species	Manufacturer and catalogue number
Lamin B1	1:800	1 hour (RT)	Rabbit	Abcam ab16048
Lamin B1	1:400	Overnight (4°C)	Mouse	Abcam ab8982
γH2AX	1:200	1 hour (RT)	Mouse	Abcam ab26350
H3K9me3	1:300	45 minutes (37°C)	Rabbit	ActiveMotif 39161
Goat Anti- Mouse IgG H&L (Alexa Fluor594)	1:600 (for ab8982)	1 hour (RT)	Goat	ThermoFisher A11005
Goat Anti- Rabbit IgG H&L (Alexa Fluor594)	1:600 (for ab16048) 1:300 (for H3K9me3)	1 hour (RT)	Goat	ThermoFisher A11037
Goat Anti-Mouse IgG H&L (Alexa Fluor647)	1:500 (for yH2AX)	1 hour	Goat	Abcam ab150115

Table S1: Summary of antibodies and corresponding dilutions for immunofluorescent staining of BL-2 cells.