Supplemental Material to:
Michaela Clever, Tomoko Funakoshi, Yasuhiro Mimura, Masatoshi Takagi and Naoko Imamoto. The nucleoporin ELYS/Mel28 regulates nuclear envelope subdomain formation in HeLa cells. Nucleus 2012; 3(2):
http://dx.doi.org/10.4161/nucl.3.2.19595
http://www.landesbioscience.com/journals/nucleus/article/19595

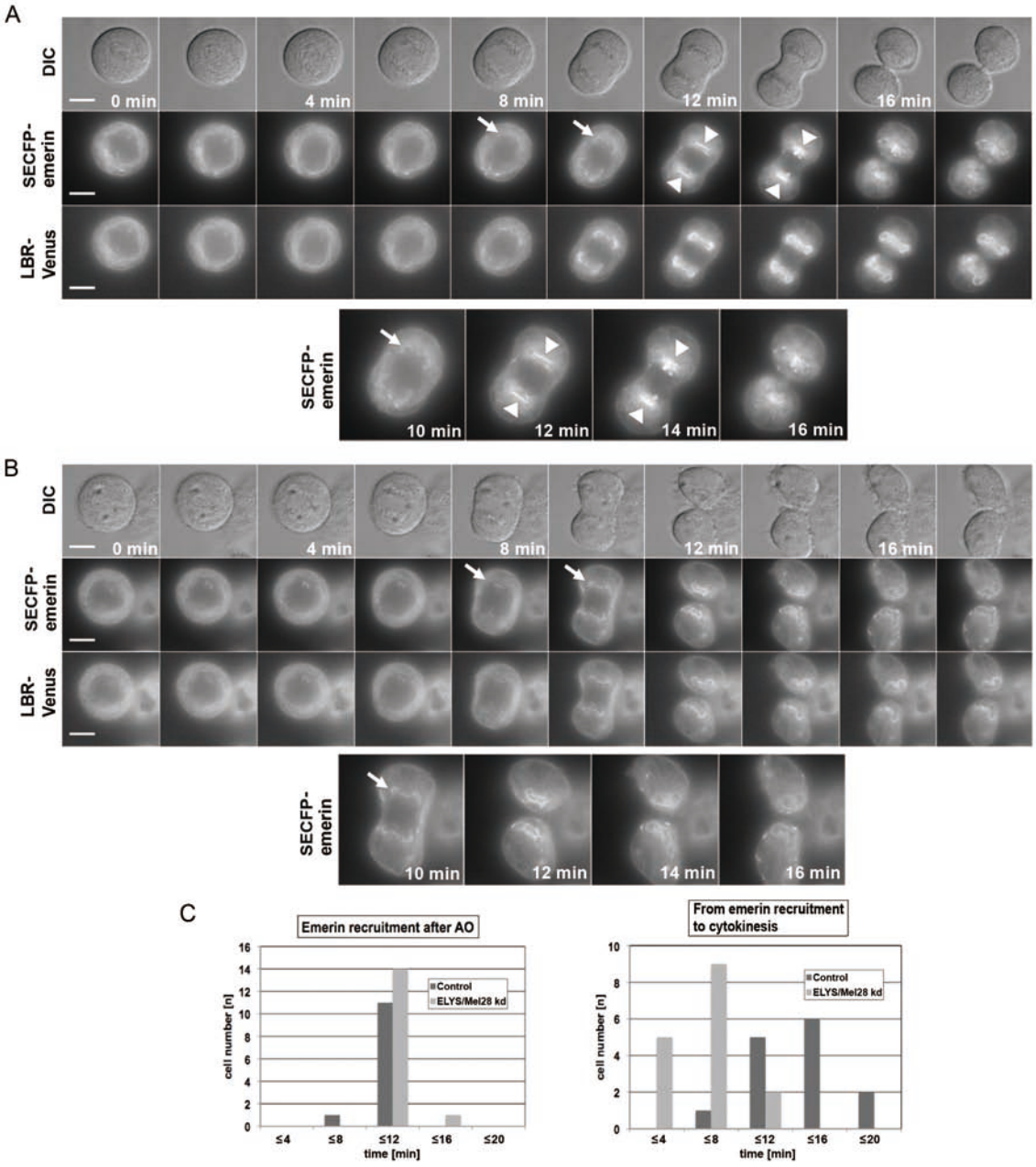

A
DIC
SECFP-emerin
LBR-Venus
0 min
4 min
8 min
12 min
16 min
10 min
14 min
B
C
Emerin recruitment after AO
From emerin recruitment to cytokinesis
Control
ELYS/Mel28 kd
cell number [n]
time [min]
≤4
≤8
≤12
≤16
≤20

Figure S1. The depletion of ELYS/Mel28 hinders accumulation of SECFP-emerin at the core region but does not change the time of its recruitment and accelerates entry into cytokinesis. Live imaging with HeLa cells stably expressing LBR–Venus and SECFP-emerin was performed as described in **Figure 1**. **(A)** Cells treated with RNAi oligos either against firefly luciferase (control). **(B)** Cells treated with RNAi oligos against ELYS/Mel28 (ELYS/Mel28 kd) for 48 h. Arrowheads show SECFP-emerin accumulating at the core region, and arrows show initial targeting of SECFP-emerin to the chromosomes. Scale bars = 10 μm. **(C)** SECFP-emerin recruitment after anaphase onset (AO) and the time between recruitment of SECFP-emerin and cytokinesis was determined in live imaging experiments, described in **Figure 1**. Data from three independent experiments were analyzed (n = 15 for the ELYS/Mel28 kd, n = 12 for the control). Note, that the data used for this analysis derived from live imaging experiments in **Figure 1**.

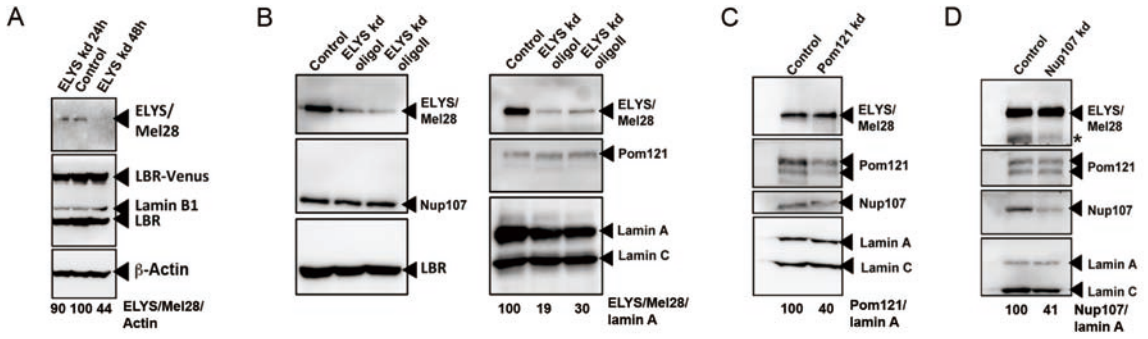

A
ELYS kd 24h
Control
ELYS kd 48h
ELYS/
Mel28
LBR-Venus
Lamin B1
LBR
β-Actin
90 100 44
ELYS/Mel28/
Actin
B
Control
ELYS kd
oligo I
ELYS kd
oligo II
ELYS/
Mel28
Nup107
LBR
Pom121
Lamin A
Lamin C
100 19 30
ELYS/Mel28/
lamin A
C
Pom121 kd
Pom121
Nup107
100 40
Pom121/
lamin A
D
Nup107 kd
*
100 41
Nup107/
lamin A

Figure S2. RNAi depletion of different nucleoporins is shown by Western blotting. (A) The Western blot analysis demonstrated the depletion of ELYS/Mel28 with an RNAi oligo I for the indicated times in HeLa cells stably expressing LBR-Venus. Extracts of 2×10^5 cells/lane were analyzed using SDS-PAGE (7–20% gradient gel), followed by immunoblotting and detection of indicated proteins. Detection of β-actin served as the loading control. **(B)** Unsynchronized HeLa cells were treated with two RNAi oligos recognizing different sequences against ELYS/Mel28 for 50 h. Extracts of 1×10^5 cells were loaded in duplicates for SDS-PAGE (7% polyacrylamide gel), immunoblotting and detection of indicated proteins. The Western blot analysis showed that RNAi reduced Pom121 **(C)** and Nup107 **(D)** in mitotic extracts under the conditions described in **Figure 2**. Extracts of 2×10^5 cells/lane were analyzed by SDS-PAGE, immunoblotted used for detection of indicated proteins. The anti-Pom121 antibody recognizes a double band when used to detect Pom121 from mitotic extracts (see Funakoshi et al.[57]). Detection of lamin A/C served as the loading control in (B-D). Asterisks mark the nonspecific signals. Depletion efficiencies using signal intensities were evaluated with ImageJ software.

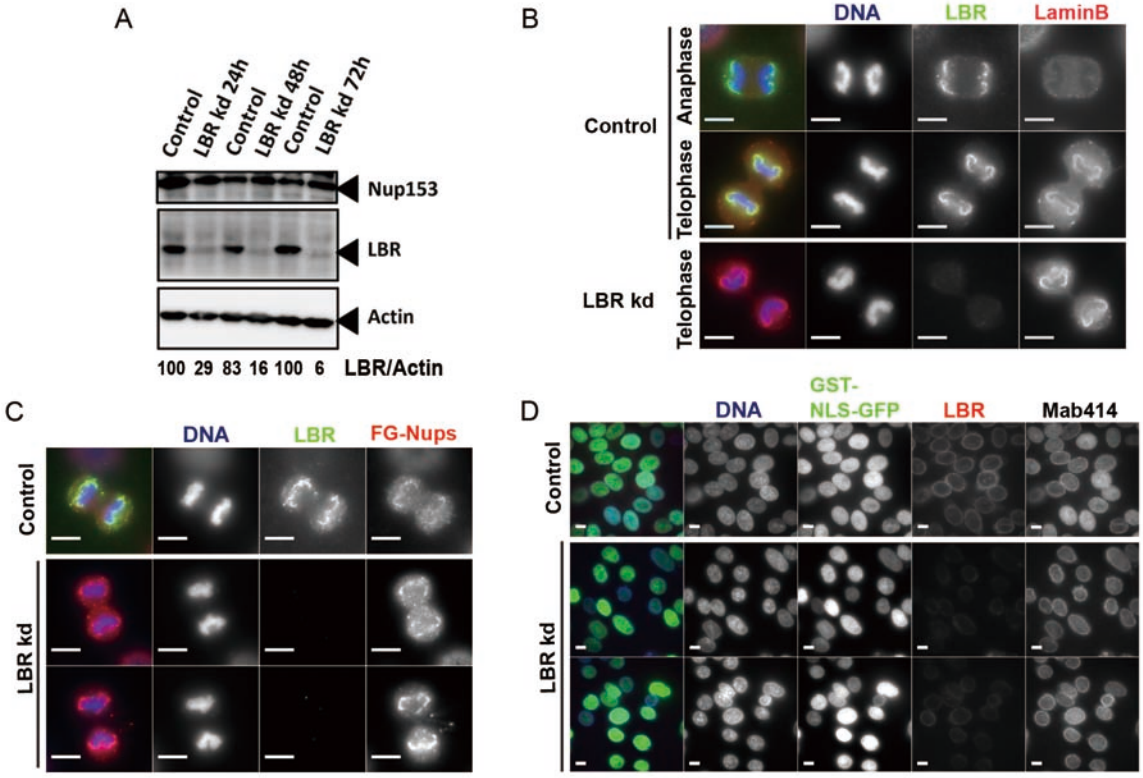

A
Control
LBR kd 24h
Control
LBR kd 48h
Control
LBR kd 72h
Nup153
LBR
Actin
100 29 83 16 100 6 LBR/Actin
B
DNA
LBR
LaminB
Control
Anaphase
Telophase
LBR kd
Telophase
C
DNA
LBR
FG-Nups
Control
LBR kd
D
DNA
GST-NLS-GFP
LBR
Mab414
Control
LBR kd

Figure S3. The depletion of the LBR does not affect postmitotic NPC assembly, the nuclear transport in interphase or the recruitment of lamin B to mitotic chromosomes. (A) The Western blot analysis shows the reduction of the LBR after siRNA treatment for the indicated time. 2×10^5 cells/lane were applied to SDS-PAGE, immunoblotting and detection of indicated proteins. The depletion efficiency using signal intensities was evaluated with ImageJ software. Cells depleted of LBR by RNAi for 65 h were immunostained for lamin B and LBR **(B)** or for nucleoporins (FG-Nups) with an antibody against Nup153, which recognizes Nup62, Nup153 and Nup 214 **(C)**. All pictures shown in are projections of image stacks (distance = 0.2 µm; three images). **(D)** Hela cells reduced of LBR by RNAi for 50 h were tested in an *in vitro* transport assay[S1] and immunostained for LBR and nucleoporins (mAB414). GST–NLS–GFP served as the cargo in the transport assay. Scale bars = 10 µm.

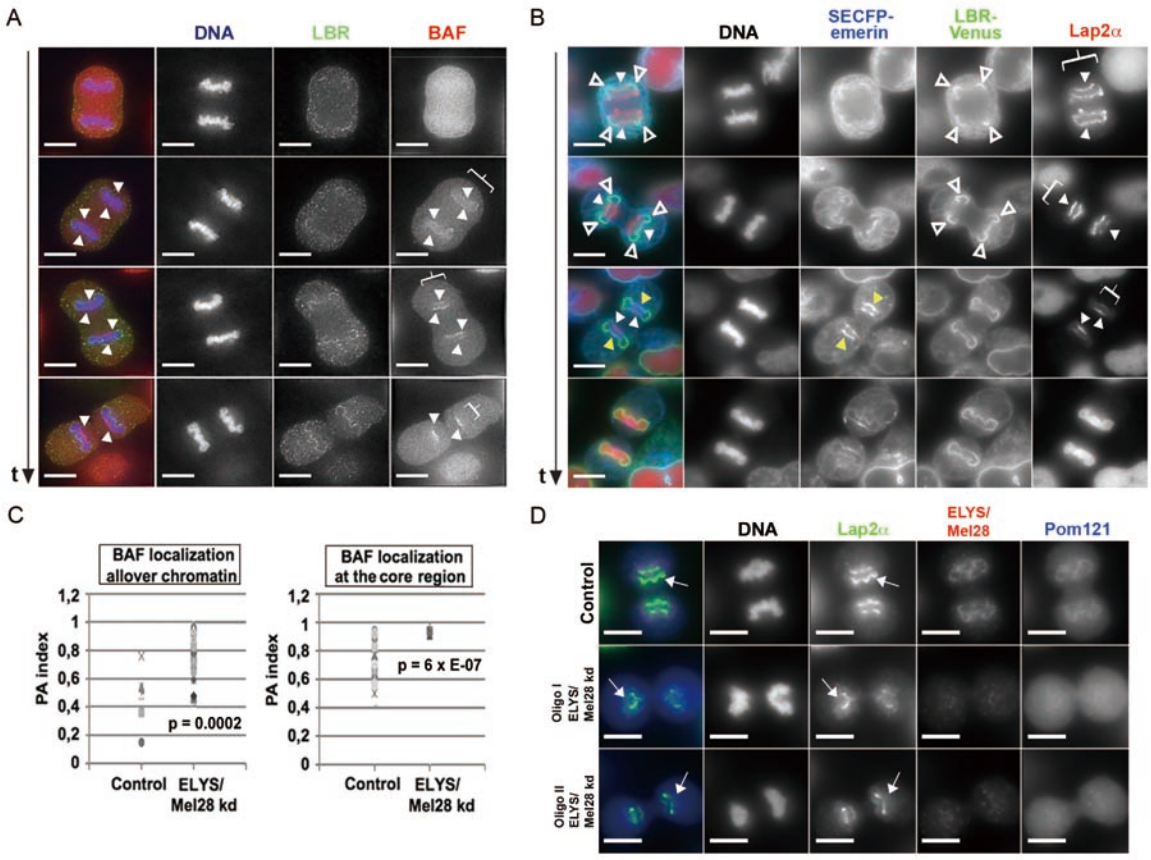
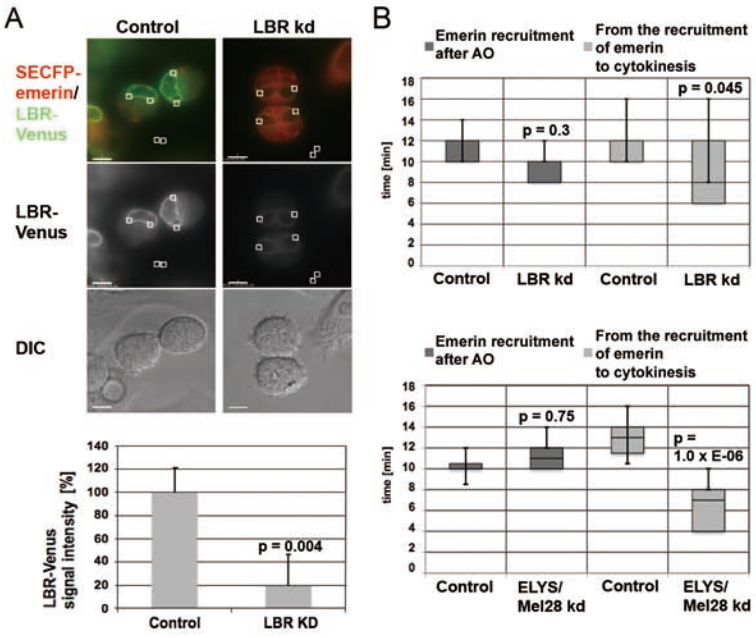
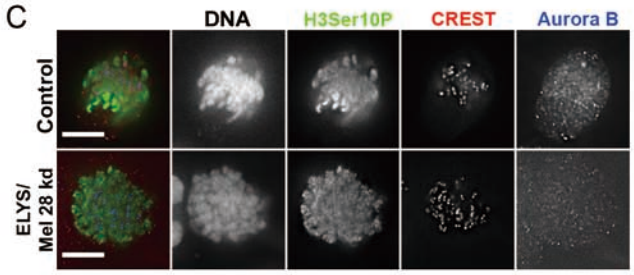
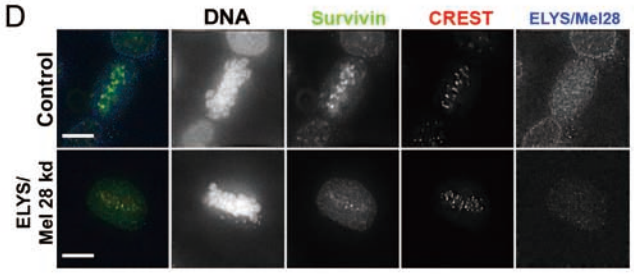

A
DNA
LBR
BAF
t
B
DNA
SECFP-emerin
LBR-Venus
Lap2α
t
C
BAF localization allover chromatin
PA index
1,2
1
0,8
0,6
0,4
0,2
0
p = 0.0002
Control
ELYS/ Mel28 kd
BAF localization at the core region
PA index
p = 6 x E-07
Control
ELYS/ Mel28 kd
D
DNA
Lap2α
ELYS/ Mel28
Pom121
Control
Oligo I ELYS/ Mel28 kd
Oligo II ELYS/ Mel28 kd

Figure S4. The dynamic localization of BAF and Lap2α at the core region is disturbed by the depletion of ELYS/Mel28. (A) BAF and the LBR were co-stained in HeLa cells by IF and aligned according their progression through mitosis. Pictures were deconvoluted using Softworx software. Arrowheads indicate BAF at the core region. **(B)** SECFP–emerin (yellow arrowheads)- and LBR–Venus (open arrowheads)-expressing cells were stained by IF for Lap2α (white arrowheads) to examine the localization of Lap2α relative to SECFP-emerin during mitosis. The white brackets correspond to the length of the core region, which becomes more focused during mitosis. **(C)** The progression from anaphase to telophase is expressed by the post-anaphase progression (PA) index, which considers the length and width of the mitotic cell, which changes with the furrowing of the cell membrane (PA index = 1 – width/length; Uehara and Goshima[67]). As observed in **Figure 4D**, BAF localization with regard to the PA index was examined (n = 43 for the control, n = 45 for the ELYS/Mel28 kd cells). **(D)** Cells were treated with two RNAi oligos against ELYS/Mel28 for 50 h and stained for Lap2α. Scale bars = 10 μm. The pictures are projections of image stacks (distance = 0.2 μm; three images).


A
Control
LBR kd
SECFP-emerin/ LBR-Venus
LBR-Venus
DIC
LBR-Venus signal intensity [%]
p = 0.004
Control
LBR KD
B
Emerin recruitment after AO
From the recruitment of emerin to cytokinesis
time [min]
p = 0.3
p = 0.045
Control
LBR kd
Control
LBR kd
Emerin recruitment after AO
From the recruitment of emerin to cytokinesis
time [min]
p = 0.75
p = 1.0 x E-06
Control
ELYS/ Mel28 kd
Control
ELYS/ Mel28 kd


C
DNA
H3Ser10P
CREST
Aurora B
Control
ELYS/ Mel 28 kd


D
DNA
Survivin
CREST
ELYS/Mel28
Control
ELYS/ Mel 28 kd

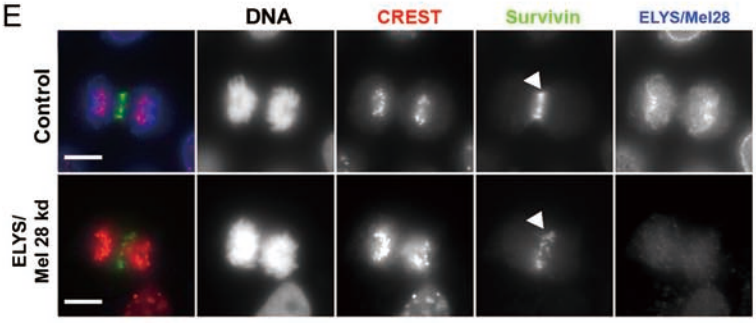

E
DNA
CREST
Survivin
ELYS/Mel28
Control
ELYS/ Mel 28 kd

Figure S5. Different from the depletion of the LBR, the ELYS/Mel28-depletion accelerates entry into cytokinesis, which might be connected to the mislocalization of the chromosomal passenger complex (CPC). (A) The depletion efficiency of LBR in live imaging of **Figure 5** was estimated by quantification of the LBR-Venus signal according to the same method used to quantify ELYS/Mel28 as in **Figure 1**. The LBR-Venus signal was compared in control cells and in cells depleted of the LBR (n = 12 for control cells, n = 10 for LBR kd cells). Scale bar = 5 µm. **(B)** The recruitment of SECFP-emerin after AO and the time between SECFP-emerin recruitment to cytokinesis was determined in live experiments in **Figures 1 and 5**. The mislocalization of the CPC components upon ELYS/Mel28-depletion was detected by IF staining of Aurora B kinase and its substrate histone 3, which is phosphorylated on serine 10 **(C)** or of survivin and CREST **(D)** in prophase cells. The pictures were deconvoluted using Softworx. Scale bars = 10 µm. **(E)** The localization of survivin after AO to the central spindle was disrupted in ELYS/Mel28-depleted cells, as shown by the IF staining of survivin and CREST. The white arrows indicate the midzone. The pictures are projections of image stacks (distance = 0.2 µm; three images). Scale bars = 10 µm.

Supplementary movies

Movie S1: Live imaging of control cells associated with Figure 1A.
Live imaging was conducted with HeLa cells stably expressing LBR-Venus/SECFP-emerin. Images were captured every 2 min as described in Materials and Methods. Red: emerin, Green: LBR.

Movie S2: Live imaging of control cells associated with Figure 1A.
Live imaging was conducted with HeLa cells stably expressing LBR-Venus/SECFP-emerin. Images were captured every 2 min as described in Materials and Methods. Emerin alone is shown.

Movie S3: Live imaging of ELYSkd cells associated with Figure 1B.
Live imaging was conducted with HeLa cells stably expressing LBR-Venus/SECFP-emerin depleted of ELYS/Mel28 by siRNA transfection for 48h. Images were captured every 2 min as described in Materials and Methods. Red: emerin, Green: LBR.

Movie S4: Live imaging of ELYSkd cells associated with Figure 1B.
Live imaging was conducted with HeLa cells stably expressing LBR-Venus/SECFP-emerin depleted of ELYS/Mel28 by siRNA transfection for 48h. Images were captured every 2 min as described in Materials and Methods. Emerin alone is shown.

Movie S5: Live imaging of LBR kd cells associated with Figure 5B.
Live imaging was conducted with HeLa cells stably expressing LBR-Venus/SECFP-emerin depleted of LBR by siRNA transfection for 48h. Images were captured every 2 min as described in Materials and Methods. Red: emerin, Green: LBR.

Movie S6: Live imaging of LBR kd cells associated with Figure 5B.
Live imaging was conducted with HeLa cells stably expressing LBR-Venus/SECFP-emerin depleted of LBR by siRNA transfection for 48h. Images were captured every 2 min as described in Materials and Methods. Emerin alone is shown.

Supplementary material and methods:

Antibodies. The following antibodies were used in this study: mouse anti-β-Actin (cat. no. F3022, SIGMA), mouse anti-Aurora B (cat. no. A78720, Transduction Laboratories), human anti-CREST (cat. no. CS1058, human nuclear ANA-centromere autoantibody, CORTEX Biochem), rabbit anti-phospho histone 3 serine 10 (cat. no. 9701, cell signaling technologies), rabbit anti-Survivin (cat. no. ab469, Abcam) and mouse anti-Nup153 (cat. no. ab24700, Abcam). The following secondary antibodies were used (Molecular Probes, Invitrogen): goat anti human Alexa 594 (cat. no. A11014).

***In vitro* transport assay.** The *in vitro* transport assay was performed as described previously.[S1] For siRNA transfection, HeLa cells were plated onto 16-chamber slides (cat. no. 178599, LabTek™, Nunc), synchronized, and transfected with siRNA oligos for the indicated times, then subjected to the assay.

References:

S1. Kose S, Imamoto N, Tachibana T, Shimamoto T, Yoneda Y. Ran-unassisted nuclear migration of a 97-kD component of nuclear pore-targeting complex. J Cell Biol 1997; 139:841-9.

The other refs. 57 and 67 can be found in the reference list of the main manuscript.