**Article**

**LEM2 phase separation promotes ESCRT-mediated nuclear envelope reformation**

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During cell division, remodelling of the nuclear envelope enables chromosome segregation by the mitotic spindle. The reformation of sealed nuclei requires ESCRTs (endosomal sorting complexes required for transport) and LEM2, a transmembrane ESCRT adaptor. Here we show how the ability of LEM2 to condense on microtubules governs the activation of ESCRTs and coordinated spindle disassembly. The LEM motif of LEM2 binds BAF, conferring on LEM2 an affinity for chromatin, while an adjacent low-complexity domain (LCD) promotes LEM2 phase separation. A proline–arginine–rich sequence within the LCD binds to microtubules and targets condensation of LEM2 to spindle microtubules that traverse the nascent nuclear envelope. Furthermore, the winged-helix domain of LEM2 activates the ESCRT-II/ESCRT-III hybrid protein CHMP7 to form co-oligomeric rings. Disruption of these events in human cells prevented the recruitment of downstream ESCRTs, compromised spindle disassembly, and led to defects in nuclear integrity and DNA damage. We propose that during nuclear reassembly LEM2 condenses into a liquid-like phase and coassembles with CHMP7 to form a macromolecular O-ring seal at the confluence between membranes, chromatin and the spindle. The properties of LEM2 described here, and the homologous architectures of related inner nuclear membrane proteins, suggest that phase separation may contribute to other critical envelope functions, including interphase repair and chromatin organization.

In late anaphase, as the nuclear envelope surrounds the chromatin disc, LEM2 concentrates at the nuclear envelope core proximal to spindle microtubules. This is in contrast to the localization pattern of the non-core marker lamin B2 and to the even distribution of LEM2 around the nuclear envelope during interphase (Extended Data Fig. 1a). To determine how LEM2 concentrates within the core, we used live-cell imaging to monitor mutant LEM2–mCherry (mChr) constructs alongside GFP-tagged tubulin. Because, as expected, we found that the LEM domain of LEM2 bound with high affinity to BAF (Extended Data Fig. 1b), we tested whether a four-amino-acid substitution in the LEM domain of LEM2 (LEM2ΔNTD) would disable BAF binding. This mutation was sufficient to disrupt accumulation of LEM2 within the nuclear envelope and its subsequent core enrichment in anaphase cells, consistent with the role of BAF as an early nuclear envelope reformation factor and a constituent of the core region (Extended Data Fig. 1c). Unexpectedly, removing the adjacent LCD (LEM2ΔLCD; Δ AA 43–202) also compromised nuclear envelope enrichment, despite the presence of an intact LEM domain. This observation suggests that the LEM domain and the LCD are each necessary, but not sufficient alone, for proper targeting. By contrast, a deletion within the C-terminal winged helix (WH) domain of LEM2 (LEM2ΔWH; Δ amino acids AA 415–485) did not affect core localization of LEM2 (Fig. 1a).

Phase separation by LCDs, or the formation of biomolecular condensates, is a mechanism for organizing dynamic processes through the creation of membraneless, biochemically specialized compartments. To characterize the properties and roles of the LEM2 LCD in core localization, we purified the N-terminal domain (NTD) of LEM2, which contains the LEM and LCD domains (LEM2NTD; AA 1–208). The LCD is notable for its basic sequence (pI = 11.8), which results from overrepresentation of arginine, in addition to overrepresentation of proline and other residues (Extended Data Fig. 1e). After purification, LEM2NTD spontaneously formed spherical droplets with liquid-like properties under physiological conditions. These LEM2NTD droplets could undergo complete fusion and inter- and intra-droplet diffusion on a timescale of seconds (Fig. 1b, Supplementary Video 1).

We next sought to address whether and how cells exploit the ability of LEM2 to phase separate in mammalian open mitosis (in which the nuclear envelope breaks down). Phosphorylation regulates both the cell cycle and phase transitions, and LEM2 has several annotated phosphorylation sites within its LCD. To test whether LEM2 could be regulated by phosphorylation, we arrested HeLa cells in G1/S phase or mitosis and compared the phosphorylation status of LEM2 in each state. As expected, LEM2–mChr appeared to be more strongly phosphorylated in mitosis than in G1/S, and this was reversible with phosphatase treatment (Fig. 1e).

Constructs in which two subregions of the LCD had been deleted, each containing annotated phosphorylation sites (an SY-rich region and a PR-rich region, LEM2ΔSY–mChr ΔAA 75–123 and LEM2ΔPR–mChr ΔAA 26–75)
Fig. 1 | Targeting of LEM2 to the nuclear envelope core at anaphase chromatin discs depends on BAF binding and a LCD that can form liquid-like droplets. a, Top, cartoon of LEM2 sequence motifs and cellular localization. TM, transmembrane. Bottom, live-cell imaging of GFP–tubulin and LEM2–mCh constructs. Time 0 is time of complete cleavage furrow ingression (CFI). Representative of results from three biological replicates except for ∆LCD image, which is representative of two biological replicates. b, Top, partial and whole droplet fluorescence recovery after photobleaching (FRAP). Bottom, quantification shows mean ± s.d. of n = 3 independent samples. c, Real-time fluorescence imaging of LEM2NTD droplet fusion. Representative of two independent experiments. d, Concentration-dependent droplet formation of purified LEM2NTD. Representative of three independent experiments. e, Top, schematic indicating sites of LEM2NTD phosphomimetric mutants. Inset, LEM2NTD−Mim1–Mim2 phosphomimetic mutations in two constructs (Mim1 and Mim2). Bottom, LEM2 immunoblot assessing the migration pattern of full-length LEM2 (LEM2FL) or LEM2NTD following separation by Phos-tag SDS–PAGE; lysates treated with lambda phosphatase (λ-PP) as indicated. Representative of two biological replicates, with one and three technical replicates per biological replicate. For immunoblot source data, see Supplementary Fig. 1. Right, fluorescence imaging of purified LEM2NTD–phosphomimetic constructs. Representative of two technical replicates. Scale bar, 2 μm (a–e).

LEM2 forms a liquid-like coat on microtubules

Super-resolution stimulated emission depletion (STED) imaging of immunostained HeLa cells revealed that endogenous LEM2 enriched specifically in toroid-like structures around spindle microtubules at the chromatin surface (Fig. 2a, Extended Data Fig. 2a). To investigate whether LEM2 binds microtubules directly, we tested binding in vitro with fluorescently labelled proteins and observed that detergent-solubilized full-length LEM2 (LEM2FL) stabilized and bundled microtubules under physiological conditions (Fig. 2b). After reconstitution into proteoliposomes, moreover, membrane-embedded LEM2FL could still stabilize and bundle microtubules (Extended Data Fig. 2b). Finally, isolated LEM2NTD was sufficient to bundle microtubules (Fig. 2b–e, Extended Data Fig. 2c–f). We quantified microtubule bundling using light scattering and corroborated the assay using negative stain electron microscopy. LEM2NTD, which contains the LCD, bundled microtubules in a concentration-dependent and saturable manner, with a half-maximal scattering concentration (Kapparent value) of 1.3 μM (Extended Data Fig. 2c, d). Electron microscopy revealed that LEM2-bundled microtubules lost their characteristic tubulin lattice fine structure, which appeared to be occluded by an amorphous LEM2 coating (Fig. 2e). Furthermore, LEM2NTD droplets could form coacervates with unpolymerized tubulin and form microtubule bundles upon addition of GTP and MgCl2 (Extended Data Fig. 2e). These data demonstrate that the LEM2 LCD binds directly to microtubules to cause bundling in vitro.

Considering the liquid-like phase separation and complex coacervation properties of the LEM2 LCD, we investigated the state of microtubule-bound LEM2 in vitro. Using video fluorescence microscopy, we observed that both LEM2FL and LEM2NTD bound microtubule bundles dynamically, recovering fluorescence at similar rates after photobleaching, in contrast to tubulin in the microtubule lattice, which did not recover (Fig. 2c, Extended Data Fig. 2f). Notably, photobleached regions of LEM2NTD on microtubule bundles recovered fluorescence through coaxial flow, or a liquid-like gradient, rather than in a uniform recovery pattern across the bleached area (Fig. 2d,
A molecular O-ring seals the nuclear envelope

As predicted by direct binding between the WH domain of LEM2 (LEM2\textsubscript{WH}: AA 395–503) and CHMP7\textsuperscript{+}, LEM2\textsubscript{WH}–mChr was unable to recruit GFP–CHMP7 to the nuclear envelope (Fig. 3a). Furthermore, following depletion of endogenous LEM2, overexpression of small inhibitory RNA (siRNA)-resistant LEM2\textsubscript{WH}–mChr did not rescue the recruitment of IST1, an ESCRT-III protein downstream of LEM2 and CHMP7 that is required to recruit the microtubule-severing enzyme SPASTIN\textsuperscript{+} (Extended Data Fig. 4a). Overexpression of siRNA-resistant LEM2–mChr led to premature and enhanced recruitment of endogenous IST1 to the nascent nuclear envelope (Extended Data Fig. 4b), which indicates that LEM2 guides both the spatial and temporal patterns of CHMP7-dependent activation of ESCRT.

Incubation of monomeric full-length CHMP7 with LEM2\textsubscript{WH} but not with LEM2\textsubscript{NTD} triggered the assembly of looping protein polymers with an inner diameter of 50–100 nm (Fig. 3b, Extended Data Fig. 4c). To investigate the mechanism of CHMP7 autoinhibition and release by LEM2\textsubscript{WH}, we used quantitative cross-linking mass spectrometry (XL-MS) to isotopically distinguish between monomers and LEM2\textsubscript{WH}-induced polymers (Extended Data Fig. 5a). We identified 24 cross-links that were specific to monomeric CHMP7. Hybrid peptide mapping revealed that the N- and C-terminal regions of CHMP7 fold together, bringing the α1–α3 helices of the ESCRT-III domain of CHMP7 into proximity with its N-terminal WH domains (Fig. 3c, Extended Data Fig. 5, Supplementary Table 1). These interactions in the monodisperse CHMP7 sample were substantially reduced or absent in LEM2\textsubscript{WH}-induced CHMP7 polymers (Fig. 3c, Supplementary Tables 1, 2), consistent with a conformational change into the open and polymeric state as visualized by electron microscopy (Fig. 3b). In total, 19 cross-links were enriched in the LEM2\textsubscript{WH}-induced polymeric sample, mapping to interactions between LEM2\textsubscript{WH} and the α1–α3 helices of CHMP7. Polymer-specific cross-links between the N-terminal amines of neighbouring CHMP7 molecules, and between adjacent LEM2\textsubscript{WH} subunits, are consistent with copolymerization of both proteins (Fig. 3c, Extended Data Fig. 5). Consistent with the XL-MS findings, CHMP7 and LEM2\textsubscript{WH} copellet with a 1:1 stoichiometry, and mutations of conserved residues suggested by the hybrid peptides within the CHMP7 α1–α3 helices impaired co-polymerization of LEM2\textsubscript{WH} and CHMP7 (Extended Data Fig. 6a–c).

Model membranes were also sufficient to trigger polymerization of full-length CHMP7 (Extended Data Fig. 6f). Two-dimensional alignment and image averaging of membrane-induced CHMP7 polymers revealed a repeating unit that comprised a continuous, polymeric strand studded with repeating perpendicular spikes (Fig. 3d, Extended Data Fig. 6g). The periodic dimensions of this polymer matched those of homologous structures for ‘open’ human...
Finally, expression of LEM2∆WH–mChr also prominently impaired around spindle microtubules (Figs. 2f, 3f, Extended Data Fig. 7a, b). Consistent with this model, a truncated CHMP7 fragment comprised of only the ESCRT-III domain (CHMP7ESCRT-III; AA 229–453) spontaneously polymerized during purification into rings that lacked perpendicular spines, but had otherwise comparable dimensions (Extended Data Fig. 6h). The spontaneous polymerization of CHMP7ESCRT-III further supports an autoinhibitory function for the N-terminal WH domains of CHMP7. In summary, the LEM2 WH domain activates autoinhibited CHMP7 monomers through a domain-replacement mechanism, and this triggers their assembly.

To investigate the roles of the LEM2–CHMP7 partnership at the spindle–chromatin interface, we expressed LEM2 deletion mutants alongside an import cargo (NLS–3GFP) to assess nuclear compartmentalization during anaphase. Cells expressing sRNA-resistant LEM2mChr first had measurable enrichment of NLS–3GFP in reforming nuclei shortly after localization of LEM2 to the core, when spindle microtubules still present barriers to sealing the nuclear membrane (Figs. 1a, 3f, Extended Data Figs. 1a, 7a, b). Cells that expressed LEM2∆γ–mChr, which concentrated within the core normally, showed mild defects in compartmentalization (Fig. 2f, Extended Data Fig. 7a, b). By contrast, expression of LEM2∆ω–mChr, which did not concentrate within the core, considerably attenuated accumulation of NLS–3GFP, pointing to the importance of the ability of LEM2 to specifically condense around spindle microtubules (Figs. 2f, 3f, Extended Data Fig. 7a, b). Finally, expression of LEM2∆CHMP7–mChr also prominently impaired timely accumulation of NLS–3GFP. This shows that the WH domain has a key role in compartmentalization, presumably owing to its ability to recruit CHMP7 and subsequent ESCRT pathway proteins (Figs. 1a, 3f, Extended Data Fig. 7a, b). Accordingly, depletion of either LEM2 or CHMP7 impaired nuclear compartmentalization for an extended period, with defects persisting for at least 30 min after complete ingress of the cleavage furrow (Extended Data Fig. 7c).

Extended sRNA-mediated depletion of LEM2 causes nuclear malformations and is eventually lethal. To identify whether defects in nuclear morphology arise as a result of errors during mitotic exit, we synchronized cells following LEM2 depletion and imaged their progression from anaphase to late telophase. While LEM2-depleted cells progressed to anaphase without noticeable nuclear defects, a strong nuclear and tubulin morphology phenotype began to emerge in late anaphase and persisted through telophase (Extended Data Fig. 8a, Supplementary Video 3). Orthogonal views of LEM2-depleted cells revealed that aberrant microtubule bundles tunneled through the nucleus within a channel lined by nuclear envelope (Extended Data Fig. 8b). The appearance of DNA damage in telophase U2OS cells depleted of LEM2 underscores the important ramifications of these nuclear integrity phenotypes (Extended Data Fig. 8c).

Finally, to test the central hypothesis that the LCM and WH activities of LEM2 cooperate with CHMP7 to self-assemble around spindle microtubules, we engineered a simplified LEM2 construct bearing the extra-luminal domains of LEM2 connected by a flexible linker, LEM2STD-LINKER-WH. When incubated with microtubules, LEM2STD-LINKER-WH triggered copolymerization of CHMP7 and the resulting rings could be seen to loop upon and around microtubule bundles [Fig. 3g, Extended Data Fig. 8d]. Consistently, and underscoring the importance of their functional coordination, disruption of the microtubule-interacting or CHMP7-binding domains of LEM2 led to persistent interphase nuclear morphology phenotypes in cells (Extended Data Fig. 8e–g).

Our results suggest the following model: LEM2 concentrates within the nuclear envelope core through direct microtubule binding, and its LCM condenses into a liquid-liquid phase that wets the surface of spindle microtubules. The WH domain of LEM2 and CHMP7 then copolymerize to complete the formation of a macromolecular ‘O-ring’ seal that establishes a temporary barrier between the new nucleoplasm and the cytoplasm, before triggering complete spindle disassembly and membrane fusion (Fig. 3h, Supplementary Discussion).

Online content
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Methods

No statistical methods were used to predetermine sample size. The experiments were not randomized and, except where stated, investigators were not blinded to allocation during experiments and outcome assessment.

Cytoskeletal proteins

Porcine brain tubulin reagents were purchased from Cytoskeleton, including unpolymerized tubulin (T238P), unpolymerized, HiLyte-647-labelled Tubulin (TL670M), and pre-formed microtubules (MT002).

Purification of His₆-SUMO-tagged proteins

All purified proteins in this study, except full length LEM2, were expressed in a pCAGS vector (WISP08-174; DNASU Plasmid Repository) in BL21-(DE3)-RIPL *Escherichia coli* cells using an N-terminal His₆-SUMO affinity tag as described previously⁴. Full-length LEM2 was expressed in a pCAGS2 vector in C43(DE3). *E. coli* cells using an N-terminal His₆-SUMO affinity tag. All plasmids are listed in Supplementary Table 3. Expression cultures (3- to 4-L) were grown in ZY-3052 rich auto-induction medium containing kanamycin, shaking (220 rpm) for 3 h at 37 °C, then overnight at 19 °C. Cells were collected by centrifugation.

Purification of BAF

Purification of full length, human BAF protein (Uniprot ID O75531) was adapted from a published protocol⁵⁻²³. The purification was performed as described, except His₆-SUMO-BAF was cleaved with His₆-Ulp1 protease (30 min, room temperature) to suit the use of the His₆-SUMO affinity tag. Ultimately, Superdex 75 16/60 fractions containing BAF dimer were pooled, concentrated, and flash-frozen in liquid nitrogen and stored as single use aliquots at ~80 °C.

Purification of LEM2 proteins

Protein constructs originating from the human LEM2 protein (Uniprot ID Q8NC56), including LEM2₁⁻⁷², LEM2₁⁻¹⁰₈, LEM2₁⁻₂₀₈, LEM2₁⁻₅₀₃, LEM2₁⁻⁶⁵₀, and LEM2₁⁻⁷⁳₀, were purified as follows. Collected cells were resuspended in lysis buffer (5 ml per gram cell pellet) containing 5% glycerol, 10 mM imidazole, pH 8.0, 2 μg/ml DNaseI, lysozyme, and protease inhibitors), in lysis buffer (30 mM HEPES, pH 7.4, 500 mM KCl, 5% glycerol), pH 8.0. Droplet formation was induced to enrich for His₆-SUMO–LEM2std; the imidazole eluate was diluted with ice-cold buffer (40 mM HEPES, pH 7.4, 5% glycerol) to drop the concentration of KCl to 50 mM. After 20 min incubation on ice, protein droplets were pelleted by centrifugation (10,000g, 10 min, 0 °C), washed with two column volumes of ice-cold low-salt buffer (40 mM HEPES, pH 7.4, 50 mM KCl, 5% glycerol), and pelleted again. The pellet was resuspended in 5 ml high-salt buffer (40 mM HEPES, pH 7.4, 500 mM KCl, 5% glycerol), to dissolve protein droplets for the remaining purification steps, and incubated with His₆-Ulp1 protease (2 h). The mixture was incubated with Ni-NTA resin (2 ml, 1 h). Spin-concentrated, flow-through protein was further purified by size exclusion chromatography using the Superdex 75 16/60 column in high-salt buffer at 4 °C. Pooled, spin-concentrated, LEM2-containing fractions were dialysed overnight into storage buffer (40 mM HEPES, pH 7.4, 350 mM KCl, 5% glycerol), spin-concentrated, and aliquoted.

Protein construct aliquots were snap-frozen in liquid nitrogen and stored at ~80 °C for future experiments. Aliquots were thawed and used only once. Final protein concentrations were 260 μM LEM2₁⁻⁷², ~110 μM LEM2₁⁻¹⁰₈, 30 μM LEM2₁⁻₂₀₈, 190 μM His₆-SUMO–LEM2₁⁻⁶⁵₀, 100 μM LEM2₁⁻₅₀₃, and 110 μM LEM2₁⁻⁷₃₀. Note that concentrations of LEM2std without the tag were limited, and LEM2std was more stable with the His₆-SUMO tag.

Human, full-length LEM2 (LEM2std) was purified as follows. Collected cells (6-expression culture) were resuspended in lysis buffer (5 ml per gram cell pellet) containing 25 mM HEPES, pH 7.4, 500 mM KCl, 1 mM DTT, 5% glycerol, lysozyme, 2 μg/ml DNaseI, and protease inhibitors. Cells were lysed by sonication on ice and clarified (10,000g, 30 min, 4 °C). Membranes were pelleted and collected from clarified lysate by ultracentrifugation (100,000g, 1 h, 4 °C). LEM2std was extracted from membranes with 1% (w/v) n-dodecyl-B-d-maltopyranoside (DDM, Antrache D310) with stirring (1 h, 4 °C), and collected in the supernatant following centrifugation (35,000g, 30 min, 4 °C). DDM-extracted supernatant was supplemented with 10 mM imidazole and incubated with Ni-NTAagarose resin (Qiagen) (4 ml bed volume, 1 h, 4 °C), washed extensively with lysis buffer containing 0.1% DDM, and protein was eluted with five column volumes of lysis buffer containing 0.1% DDM and supplemented with 500 mM imidazole, pH 8.0. LEM2std-rich elution fractions were pooled and dialysed overnight at 4 °C to remove imidazole. The His₆-SUMO tag was cleaved by incubation with His₆-Ulp1 protease (30 min, room temp) and removed by reverse Ni-NTA chromatography. Cleaved, DDM-solubilized LEM2std was used unconcentrated, or spin-column concentrated (Vivaspin20, 100 kDa MWCO, PES) without freeze-thaw.

Purification of CHMP7 proteins

Purification procedures for full-length, human CHMP7 (Uniprot ID Q8WUX9) and point mutants CHMP7₁²₀–₁³₂₂, CHMP7₁⁻₂₀₈, and CHMP7₁⁻₃₃₀ were adapted from previously published purifications⁴. The truncated CHMP7₁⁻₁₁₂, CHMP7₁⁻₃₃₀ were purified as follows. Collected cells were resuspended in 5 ml ice-cold lysis buffer (40 mM HEPES, pH 8.0, 800 mM KCl, 5% glycerol, 10 mM imidazole, 2 μg/ml DNaseI, 5 mM BME, protease inhibitors, and lysozyme) per gram cell pellet. Cells were lysed by sonication on ice, and clarified (10,000g, 30 min, room temperature). Clarified CHMP7₁⁻₁₁₂ lysate was incubated with Ni-NTAagarose resin and spontaneously formed a gel composed of protein polymerized into rings, assayed by negative stain electron microscopy. His₆-SUMO–CHMP7₁⁻₁₁₂ rings were eluted with imidazole (350 μM), and the eluate was collected with low-speed spin (1,000g) as a gel phase on top of the resin. The gel was scooped off and washed three times with buffer (40 mM HEPES, pH 8.0, 800 mM KCl, 5% glycerol, 1 mM DTT), and polymers were collected by centrifugation (5,000g) each time.
The His-SUMO tag was cleaved by incubation with His$_{6}$-Ulp1 protease (2 h, 4 °C). Cleaved CHMP7, was washed three times with buffer and collected by centrifugation each time, and soluble His$_{6}$-SUMO and His$_{6}$-Ulp1 were discarded in the aqueous supernatant. Cleaved eluate was diluted with buffer to give a final protein concentration of about 60 μM.

Analytical size exclusion chromatography
Binding of LEM2$_{1–72}$ to BAF or BAF−dsDNA complex was assayed by gel filtration chromatography. DNA duplex was prepared as previously described32. Combinations of purified LEM2$_{1–72}$, BAF dimers, and DNA duplexes were combined in 1:2 molar ratio. Following incubation at room temperature for 30 min, 50 μl reaction mixture was applied to a Superose 6 3.2/300 column (GE Life Sciences) in buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 5 mM DTT, and 10% glycerol) equilibrated at 4 °C. The flow rate was 40 μl/min for all experiments. Retention volumes for major peaks absorbing at 280 nm (A280) were recorded. The peak contents of peak fractions were assayed by SDS–PAGE.

LEM2 LCD peptides
Chemically synthesized peptides bearing an N-terminal FITC-Ahx modification (Extended Data Fig. 3a) were purchased from GenScript, peptide stock solutions were prepared in milli-Q H$_{2}$O except for LEM2$_{75-122}$ stocks, which were prepared in DMSO. Phase separation of LEM2$_{1–72}$ was induced by dilution in milli-Q H$_{2}$O or buffer (25 mM HEPES, pH 7.4, 150 mM KCl) to 0.2 mg/ml stock.

Turbidity
Microtubule bundling by LEM2 was quantified by turbidity (absorbance at 340 nm) (Tecan Spark 10M spectrophotometer)33. Reactions (10 μl total volume) of purified LEM2$_{1–72}$, BAF dimers, and DNA duplexes were already turbid in the absence of microtubules. Microtubules were prepared following the Cytoskeleton protocol, to prevent droplet association to the silica surface34. Spinning disc confocal microscopy with FRAP To assay LEM2$_{1–72}$ droplet dynamics in FRAP and droplet fusion experiments, 384-well glass-bottom plates were PEGylated using PEG-Silane (LAYSAN BIO, MPEG-SIL-5000) followed by passivation with BSA, according to published protocols, to prevent droplet association to the silica surface35. Spinning disc confocal microscopy with FRAP was carried out using a W1-CSU with a Borealis upgrade (Andor) and ILE Laser Launch (laser lines 405 nm, 488 nm, 561 nm, 647 nm; Andor) on a Nikon Eclipse Ti microscope (Nikon Instruments) equipped with a Rapp Optoelectronic UGA-40 (Rapp Optoelectronic) photobleaching unit and operated with MicroManager 2.0beta (Open Imaging). Fluorescence images were collected with an Andor Zyla 4.2 cMOS (Laysanbio) or an AxioImager M2m (Carl Zeiss) (Laysanbio) equipped with a Rapp Optoelectronic UGA-series (Rapp Optoelectronic) photobleaching unit and operated with MicroManager 2.0beta (Open Imaging). Fluorescence images were collected with an Andor Zyla 4.2 cMOS Camera (Laysanbio). Samples were imaged using a C1 Probes Apochromat VC 100× Oil NA 1.4 objective (Laysanbio). Excitation wavelengths were: 488 nm for Alexa488 (Thermo Fisher Scientific) or FITC; 561 nm for Alexa647 (Thermo Fisher Scientific). Images were recorded with a frequency of 1 Hz, starting with at least one image recorded pre-bleach, including the bleach event, and up to 240 s post-bleaching. The ratio of the intensity within bleached regions of interest to background was calculated with ImageJ for each time point and normalized to the intensity at the time of bleaching. Replicates were aligned to the time of bleaching, averaged, and plotted ± s.d.

Negative stain electron microscopy
Continuous carbon grids (200–400 mesh copper, Quantifoil) were glow-discharged (PELCO EasiGlow, 15 mA, 0.39 mBar, 30 s). Samples (3–5 μl) were stained with 0.75% uranyl formate as described previously36. Images were collected with a Tecnai T12 microscope (FEI) with a LaB6 filament, operated at 120 kV, and data were captured with a Gatan Ultrascan CCD camera (Gatan). For microtubule-binding assays, reactions were prepared on grids and incubated for 2 min. Microtubules were used at a concentration of 1 μM αβ-tubulin in pre-formed microtubules. LEM2 constructs were imaged with microtubules (Fig. 2f) at the following concentrations: 6 μM LEM2$_{1–72}$, 4 μM LEM2$_{1–72}$ droplet formation was induced at room temperature by reducing the salt concentration to 150 mM KCl, keeping the concentrations of other buffer components the same; droplets were allowed to grow for 60 min before imaging. Binding of LEM2$_{1–72}$ droplets (8 to 24 μM) or detergent-solubilized LEM2$_{1–72}$ (8 μM) to microtubules was tested with the addition of fluorescently labelled microtubules (1 μM αβ-tubulin polymerized into microtubules). Microtubules were prepared following the Cytoskeleton protocol, in a molar ratio of 1.7 HiLyte647–tubulin:unlabelled tubulin, in G-PEM buffer (Cytoskeleton BST01). Unpolymerized tubulin, 1:7 labelled:unlabelled molar ratio, and Alexa555 labelled LEM2$_{1–72}$ were tested for association with LEM2$_{1–72}$ droplets at concentrations of 8 μM each. Note that 150 mM KCl promotes microtubule depolymerization in the absence of stabilizing proteins. To assay coacervate formation, LEM2$_{1–72}$ (8 μM) was fluorescently imaged directly after reducing the salt concentration for LEM2$_{1–72}$ alone or in the presence of 1 μM unpolymerized αβ-tubulin, 30 bases RNA (5′-GGGCCUCCGCCCCCAGUGAGGGGCCGGCC-3′), or 50 bases DNA (5′-AATGTATTGGTGGGGGCCTGCTCGGGATTGCGGATACGCCCTTGGATGCYC-3′).

Spinning disc confocal microscopy with FRAP
To assay LEM2$_{1–72}$ droplet dynamics in FRAP and droplet fusion experiments, 384-well glass-bottom plates were PEGylated using PEG-Silane (LAYSAN BIO, MPEG-SIL-5000) followed by passivation with BSA, according to published protocols, to prevent droplet association to the silica surface35. Spinning disc confocal microscopy with FRAP was carried out using a W1-CSU with a Borealis upgrade (Andor) and ILE Laser Launch (laser lines 405 nm, 488 nm, 561 nm, 647 nm; Andor) on a Nikon Eclipse Ti microscope (Nikon Instruments) equipped with a Rapp Optoelectronic UGA-40 (Rapp Optoelectronic) photobleaching unit and operated with MicroManager 2.0beta (Open Imaging). Fluorescence images were collected with an Andor Zyla 4.2 cMOS Camera (Laysanbio). Samples were imaged using a C1 Probes Apochromat VC 100× Oil NA 1.4 objective (Laysanbio). Excitation wavelengths were: 488 nm for Alexa488 (Thermo Fisher Scientific) or FITC; 561 nm for Alexa647 (Thermo Fisher Scientific). Images were recorded with a frequency of 1 Hz, starting with at least one image recorded pre-bleach, including the bleach event, and up to 240 s post-bleaching. The ratio of the intensity within bleached regions of interest to background was calculated with ImageJ for each time point and normalized to the intensity at the time of bleaching. Replicates were aligned to the time of bleaching, averaged, and plotted ± s.d.
LEM2-induced CHMP7 polymerization

LEM2 and CHMP7 proteins were mixed at concentrations of 4–8 μM in a total volume of 50 μl. LEM2<sub>WT</sub> or LEM2<sub>N396D-Linker-WT</sub> was present in twofold molar excess to CHMP7 proteins, unless otherwise stated. The buffer was adjusted to between 600 and 800 mM KCl. Reactions were dialysed for 9–12 h into low-salt buffer (30 mM HEPES, pH 8.0, 25 mM KCl, and 1 mM DTT) using Slide-A-Lyzer MINI Dialysis Device, 10K MWCO (Thermo Fisher Scientific). For experiments including microtubules, a 3× stock of pre-formed, lyophilized microtubules was prepared in buffer (30 mM HEPES, pH 8.0, 800 mM KCl, 1 mM DTT, 60 μM paclitaxel, and 3 mM MgCl<sub>2</sub>), and dialysis buffer was supplemented with 10 μM paclitaxel and 1 mM MgCl<sub>2</sub>. After dialysis, polymers were collected by low-speed centrifugation (5,000g, 5 min) and resuspended in 30 μl buffer for negative stain electron microscopy, SDS–PAGE, or cross-linking mass spectrometry analysis. To determine the stoichiometry of the CHMP7–LEM2<sub>WT</sub> polymer, CHMP7 (4 μM) was titrated with LEM2<sub>WT</sub> (0, 0.08, 1 and 4 μM), dialysed, and polymers were collected by low-speed centrifugation. The supernatant was collected such that 10 μl was left behind as the pellet fraction. Quantities of polymerized and unpolymerized CHMP7 at each LEM2 condition were determined by ImageJ. The data was normalized to samples containing 0 and equimolar amounts of LEM2<sub>WT</sub>.

Liposome preparation

Lipid solutions (Avanti Polar Lipids) were resuspended in chloroform, and liposomes were prepared as previously described<sup>28</sup>. In brief, lipids (2 mg total) were dried in a glass vial to give final ratio (mole %) of 30% egg phosphatidylserine (PS), 30% egg phosphatidylcholine (PC) and 40% phosphatidylethanolamine (PE). Lipid films were dispersed in buffer (40 mM HEPES, pH 8.0, 150 mM KCl) to produce liposomes at a final concentration of 1 mg/ml, and stored at −80 °C.

Microtubule bundling assay of full-length LEM2 in the presence of model membranes

A dried lipid film in a glass vial (1 mg total containing final ratio (mole %) 60% egg PC, 24.7% PE, 10% bovine liver phosphatidylinositol, 5% cholesterol, and 0.3% 18:1 Liss Rhod PE from Avanti Polar Lipids) was dispersed in buffer (40 mM HEPES, pH 8.0, 800 mM KCl, 30% glycerol, 0.1% DDM) and sonicated at 30 °C for 10 min to produce detergent-solubilized lipids at a concentration of 1 mg/ml. Equal volumes detergent-solubilized lipids, activated Bio-Beads SM-2 Resin (BioRad), and concentrated LEM2<sub>WT</sub> (1 mg/ml) or LEM2<sub>WT</sub> buffer were combined and incubated overnight at 4 °C. Detergent-depleted reactions were separated from resin and mixed with fluorescently labelled microtubule and G-PEM buffer (Cytoskeleton BSTD1) to a final KCl concentration of 150 mM and 1 μM tubulin. Reactions were incubated for 1 h at room temperature before imaging.

Membrane-induced CHMP7 polymerization

CHMP7 was dialysed or diluted to reduce the salt concentration from 800 mM to 150 mM KCl (supplemented with 5% glycerol, 40 mM HEPES, pH 8.0, 1 mM DTT) to give a final protein concentration of 0.1 mg/ml. Liposomes and CHMP7 were mixed 40:1 (w/w) and negative stain electron microscopy grids were prepared immediately.

Electron microscopy data acquisition and 2D classification

Membrane-induced CHMP7 polymers were prepared for negative stain electron microscopy and imaged with a Tecnai T20 microscope (FEI) with a LaB6 filament, operated at 200 kV. 227 micrographs were collected with a TemCam-F816 8k × 8k camera (TVIPS) using SerialEM software<sup>28</sup>, with a nominal pixel size of 1.57 Å. The defocus was 0.7–1.7 μm and the total dose was 20 e−/Å². Particles containing a repeating polymeric unit were picked manually along the polymeric protein chain, yielding 6,094 particles. Specifically, particles were picked from polymers detached from membrane, which were in a favourable orientation for subsequent classification. Particles were picked and 2D-classified using default parameters within RELION version 2.0 software.

Cross-linking mass spectrometry

Full length CHMP7 (60 μg) was polymerized with equimolar His<sub>6</sub>-SUMO–LEM2<sub>WT</sub>, following the described polymerization assay, and crosslinked with 2 mM light crosslinker (H<sub>2</sub>-BS3, Creative Molecules) for 30 min at 30 °C. His<sub>6</sub>-SUMO–LEM2<sub>WT</sub> was used to achieve a higher protein concentration. Full-length, monomeric CHMP7 (60 μg) was crosslinked with 2 mM heavy crosslinker (D<sub>2</sub>-BS3, Creative Molecules) under the same conditions. Reactions were quenched (10 mM ammonium bicarbonate, 10 min, room temperature), and light and heavy crosslinked reaction mixtures were combined and processed for mass spectrometry as described previously<sup>30,31</sup>. Crosslinked products were enriched by size-exclusion chromatography (Superdex Peptide, GE Healthcare Life Sciences) as described previously<sup>32</sup> and fractions eluting between 0.9 and 1.4 ml were dried and resuspended in 0.1% formic acid for MS analysis. The fractions starting at 0.9 ml and 1.3 ml were combined before evaporation to make four total SEC fractions.

LC–MS analysis was performed with a Q-Exactive Plus mass spectrometer (Thermo Scientific) coupled with a nanoelectrospray ion source (Easy-Spray, Thermo) and NanoAcquity UPLC system (Waters). Enriched fractions were separated on a 15 cm × 75 μm ID PepMap C18 column (Thermo) using a 60-min gradient from 5–28% solvent B (A: 0.1% formic acid in water, B: 0.1% formic acid in acetonitrile). Precursor MS scans were measured in the Orbitrap scanning from 350 to 1,500 m/z (mass resolution: 70,000). The ten most intense triply charged or higher precursors were isolated in the quadrupole (isolation window: 4 m/z) and dissociated by HCD (normalized collision energy: 24), and the product ion spectra were measured in the Orbitrap (mass resolution: 17,500). A dynamic exclusion window of 20 s was applied and the automatic gain control targets were set to 3 × 10<sup>5</sup> (precursor scan) and 5 × 10<sup>4</sup> (product scan).

Peaks were generated using Proteome Discoverer 2.2 (Thermo), and crosslinked peptides searched for with Protein Prospector 5.20.23<sup>33,34</sup>. 85 of the most intense peaks from each product ion spectrum were searched against a database containing His<sub>6</sub>-SUMO–LEM2<sub>WT</sub> along with the sequences of 10 other proteins comprising CHMP subunits and tubulin, concatenated with 10 randomized decoy versions of each of these sequences (121 sequences total). Search parameters were: mass tolerance of 20 ppm (precursor) and 30 ppm (product); fixed modifications of carboxamidomethylation on cysteine; variable modifications of peptide N-terminal glutamine conversion to pyroglutamate, oxidation of methionine, and ‘dead-end’ modification of lysine and tubulin, concatenated with 10 randomized decoy versions of each of these sequences (121 sequences total). Search parameters were: mass tolerance of 20 ppm (precursor) and 30 ppm (product); fixed modifications of carboxamidomethylation on cysteine; variable modifications of peptide N-terminal glutamine conversion to pyroglutamate, oxidation of methionine, and ‘dead-end’ modification of lysine and the protein N terminus by semi-hydrolysed heavy and light BS3; trypsin specificity was used with two missed cleavages and three lysine and the protein N terminus by semi-hydrolysed heavy and light BS3; trypsin specificity was used with two missed cleavages and three.
The peak areas were re-imported into R and summarized at the level of crosslinked residues for each light and heavy crosslink. Peak areas were summed for all peptides matching a given crosslink. Finally, log2 ratios of the heavy to light peak areas were determined. Filtered cross-links were mapped to the primary protein structure using x!NET41.

Homology modelling and cross-link mapping
Homology models of human CHMP7 and LEM2 domains were created with Phyre242 (Supplementary Table 2). Reference structures were selected based on confidence scores and homology to reference structure. Models were validated by mapping cross-linking data to the models using UCSF Chimera43 together with Xlink analyser44.

Immunostaining for fluorescence microscopy
Cells were fixed at room temperature in 2% paraformaldehyde for 25 min. The primary antibodies used for immunodetection were rabbit anti-IST145, rabbit anti-tubulin (ab18251; abcam), rat anti-tubulin (YL1/2; Accurate Chemical & Scientific), mouse anti-lamin B2 (AbCam; ab8983), mouse anti-SUN2 (gift from B. Burke), and mouse anti-53BP1 (MAB3802; Millipore). After incubation with fluorescently labelled secondary antibodies (anti-rabbit 488, anti-mouse 488, anti-rabbit 647, and anti-rabbit 568; Thermo Fisher), coverslips were mounted with DAPI ProLong Gold (Thermo Fisher) and imaged by widefield microscopy (Zeiss Axioskop 2; Axiovision software), spinning disc confocal microscopy (Nikon Eclipse Ti; Metamorph software), or stimulated emission depletion microscopy (see below). For quantifying IST1 localization: anti-rabbit 488 against rabbit anti-IST1; for assessing DNA damage in telophase cells: anti-mouse 488 against mouse anti-53BP1 and anti-rabbit 568 against rabbit anti-tubulin; for quantifying and imaging the tubulin and nuclear envelope phenotype in telophase cells: anti-mouse 488 against mouse anti-SUN2 (INM marker for quantitative experiments), and anti-rabbit 568 against rabbit anti-tubulin; for interphase phenotypes: anti-rabbit 488 against rabbit anti-tubulin; for STED: anti-mouse 568 against mouse anti-tubulin and anti-rabbit 647 against rabbit anti-LEMIEM2.

STED microscopy
STED microscopy was performed on a Leica TCS SP8 STED 3× confocal laser-scanning microscope equipped with a HC PL APO CS2 100×/1.40 oil objective. Confocal sections were imaged with Leica LAS X Core software and processed with Huygens Software Suite (SVI). Images were recorded using a 405-nm laser line at 1.4% laser power to image DAPI, and a 522-nm laser line at 5.6% laser power to image tubulin in confocal detector mode. LEM2 was imaged with a 653-nm laser line at 2.5% laser power in STED pulsed detector mode (gate start at 0.3 ns and gate end at 6.5 ns) with a Huygens saturation factor of 5.7. Deconvolved images were contrast enhanced in ImageJ (NIH); raw data are available upon request.

Quantification from fluorescence microscopy
For illustration, images of anaphase A and B cells were acquired by widefield microscopy at 100× and adjusted so that background fluorescence in the DNA, IST1, and LEM2–mCherry channels were comparable between samples. Raw images acquired by widefield microscopy at 100× were used to score the IST1 phenotype in anaphase A (early) and anaphase B (late). IST1 localization to anaphase chromatin masses was assessed in three independent experiments in which images were coded, randomized and scored blindly by three independent scorers. Images were decoded and blind scores were quantified. Each chromatin disc (two per cell) was scored as having extra robust, robust, weak, or no recruitment for IST1. Robust recruitment was characterized by distinctive foci organized at the core of chromatin masses, whereas weak recruitment was characterized by less intense, often fewer, and less organized foci at the chromatin surface, consistent with what has previously been shown4. Extra robust was characterized by strikingly intense IST1 fluorescence, often accompanied by recruitment over most of the chromatid disc. For clarity, the robust and extra robust categories were graphed together as a single category. The majority score was used in cases where the three scores differed.

For time-lapse colocalization experiments tracking GFP–CHMP7, images of anaphase cells were acquired by time-lapse light microscopy (described below) and were selected for scoring at the time of peak LEM2–mCherry or LEM2ΔWH–mCherry enrichment at the core region of anaphase chromatid discs. In FIJI, each cell was thresholded for either LEM2–mCherry or LEM2ΔWH–mCherry enrichment. The mean fluorescence (arbitrary units) was measured for each region of interest in the GFP–CHMP7 channel. The regions of interest were subtracted from the area of the whole cell to measure the mean fluorescence of cytoplasmic GFP–CHMP7 for each anaphase cell. The plotted values are the mean GFP–CHMP7 fluorescence of the region of interest, as determined by exogenous LEM2 enrichment, normalized to the mean fluorescence of cytoplasmic GFP–CHMP7.

Nuclear accumulation of NLS–3GFP in either late anaphase or telophase was determined by taking the ratio of nuclear to cytoplasmic NLS–3GFP at either 1-min intervals throughout anaphase (after imaging at 15 s intervals) or 30 min after complete cleavage furrow ingression, in telophase (time-lapse microscopy described below). In FIJI, regions of chromatin were defined as regions of interest in the NucBlue channel and used to measure mean fluorescence (arbitrary units) of nuclear NLS–3GFP in late anaphase or the integrated density (the product of area and mean grey value) in telophase cells. Cytoplasmic levels of NLS–3GFP were determined by selecting the whole cell, and subsequently deselecting the regions of chromatin. For anaphase cells, the mean fluorescence of nuclear NLS–3GFP for each disc was then divided by the mean fluorescence of cytoplasmic NLS–3GFP of the same cell. For telophase, the integrated density was used to account for changes in nuclear size due to decondensation.

For the purposes of illustration, interphase and telophase cells were acquired by widefield and time-lapse microscopy and were adjusted so that background fluorescence was comparable between samples. Spinning disc confocal microscopy at 60× was used to acquire z-stacks for orthographic projection. Raw images used to score the nuclear envelope and tubulin phenotype in telophase were acquired by widefield microscopy at 60×. To score DNA damage in telophase, images were acquired by widefield microscopy at 60× and thresholded uniformly in FIJI. Nuclear foci were detected using the find maxima function in FIJI and noise tolerance was held constant for all conditions. To score interphase nuclear circularity, images were acquired by widefield microscopy at 60×. The DNA channel was thresholded in FIJI and each nucleus was defined as a region of interest. The nuclei were then assessed for their circularity (circularity = 4π(area/perimeter2), as described by others46.

Time-lapse light microscopy analysis
All live imaging experiments were imaged at 60× by spinning disc confocal microscopy using glass-bottomed MatTek dishes. For experiments tracking chromatin, cells were treated with NucBlue (Thermo Fisher) 1 h before imaging, per the manufacturer’s instructions. Stable cell lines and plasmids for transient transfection used in this study are described in Supplementary Table 3. Cells stably expressing GFP–lamin B2 and LEM2–mCherry were cultured for 48 h and treated with 200 nM Sir-tubulin (CY-SC006; Cytoskelton) 1 h before imaging. Cells stably expressing GFP–BAF and LEM2–mCherry were also cultured asynchronously. All other experiments were subjected to cell-cycle synchronization, as described below. To track nuclear integrity, cells stably expressing NLS–3GFP (PL19) alone or in combination with the stable expression of siRNA-resistant LEM2–mCherry (PL13), LEM2ΔWH–mCherry (PL16), LEM2ΔPR–mCherry (PL17), or LEM2ΔWH–mCherry (PL18) were imaged at either 15 s intervals (to assess anaphase compartmentalization) or 3-min intervals for at least 30 min following complete cleavage furrow.
ingression (to assess telophase compartmentalization). In the latter experiments, 100 nM SirT-tubulin (CY-SC006; Cytoskeleton) was added 1 h before imaging. HeLa cells stably expressing H2B–mCherry and GFP–tubulin were treated with siRNA oligos, as described below. For transient expression experiments, cells were transfected with plasmids using Lipofectamine LTX Plus (Thermo Fisher) for 24 h, then re-plated and subjected to cell-cycle synchronization. Cells stably expressing GFP–tubulin19 were transiently transfected with siRNA-resistant pCMV(Δ5)-LEM2–mCherry (PL13), pCMV(Δ5)-LEM2Δm21–mCherry (PL14), pCMV(Δ5)-LEM2ΔLCD–mCherry (PL15), pCMV(Δ5)-LEM2ΔSY–mCherry (PL16), pCMV(Δ5)-LEM2Δp21–mCherry (PL17), or pCMV(Δ5)-LEM2Δh21–mCherry (PL18). For time-lapse colocalization experiments, HeLa cells stably expressing GFP–CHMP7 were transiently transfected with either siRNA-resistant pCMV(Δ5)-LEM2–mCherry (PL13) or siRNA-resistant pCMV(Δ5)-LEM2Δh21–mCherry (PL18).

siRNA-mediated depletion and cell-cycle synchronization

HeLa cells were a gift from M. Powers and U2OS cells were a gift from D. Ayer; both cell lines were authenticated by STR profiling. All HeLa cell lines were generated from the same parental HeLa cells, which tested negative for mycoplasma contamination. U2OS cells were not tested for mycoplasma contamination. HeLa and U2OS cells were plated on fibronectin-coated coverslips in the presence of 10 nM siRNA oligo, delivered by Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher). Specific sequences used were: siControl (siScr)17,18, siLEM2-1 (antisense sequence targeting nucleotides 78–98: UUCCGGUAAGACACCGGAAGdT dT18), siLEM2-2 (antisense sequence targeting nucleotides 1,297–1,317: UACAAUGCGCAAGCGGCUcdTdT18), and siCHMP7 (antisense sequence targeting nucleotides 650–669: CCCCCUUUCUUACUACUAdTdAAdTdT18). In experiments testing whether exogenous LEM2 could rescue function after endogenous LEM2 depletion, siLEM2-2 was used to deplete endogenous LEM2 as exogenous LEM2 constructs harbour silent mutations that confer resistance to the siLEM2-2 oligo. For cell-cycle synchronization experiments, 4–8 h after plating, 2 mM thymidine was added for 24 h to arrest cells at G1/S. Cells were then rinsed thoroughly with PBS, followed by the addition of culture medium. Twelve hours after release, cells were imaged live or fixed for microscopy. For experiments tracking interphase phenotypes, cells were fixed for immunostaining six hours after release. To assess the phosphorylation status of exogenous LEM2 at different stages of the cell cycle, cells were arrested at G1/S by treating with 2 mM thymidine for 24 h. G1/S–arrested cells were then either harvested or thoroughly rinsed with PBS before the addition of culture medium containing 100 ng/ml nocodazole. After 16 h of nocodazole treatment, mitotic cells were harvested by shake off. Cell pellets were rinsed in TBS before lysis and separation by Phos-Tag acrylamide SDS–PAGE19 and subsequent immunoblot (described below).

Immunoblots

To verify the efficacy of siRNA treatments and expression of siRNA-resistant constructs, cells were plated in six-well dishes and subjected to the same experimental conditions as those used to generate imaging data. Primary antibodies (anti-LEM2 (HPA017340; Sigma-Aldrich), anti-mCherry (Novus Biologicals, NB2-25157), anti-tubulin (ab18251; abcam)) were detected with HRP-coupled secondary antibodies (Thermo Fisher) and chemiluminescence. To assess phosphorylation of exogenous LEM2 by mobility shift, whole-cell lysates were prepared in lysis buffer lacking EDTA and phosphatase inhibitors. Aliquots of each lysate were then subjected to either lambda protein phosphatase treatment per the manufacturer’s instructions (P07535; NEB) or a control reaction treatment containing only PMP buffer and MnCl₂. Samples were run on 8% and 10% SDS–PAGE gels prepared with Phos-Tag Acrylamide reagent (AAL-107; Waco) per the manufacturer’s instructions. Detection of LEM2 by immunoblot was performed as described above.

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Raw data and peaklists from the quantitative crosslinking mass spectrometry analysis can be accessed with MassIVE: ftp://massive.ucsd.edu/MSV00084387. Crosslinked peptide spectral assignments are accessible using accession: rgefhrthsh at http://msviewer.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msviewer. The uncropped blots and gels are provided in Supplementary Fig. 1. Source Data for Figs. 1–3 and Extended Data Figs. 1–4, 5, 7, 8 are provided online.

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Extended Data Fig. 1 | The N terminus of LEM2 possesses a canonical BAF-binding LEM domain and an LCD. a, Live-cell imaging of GFP–lamin B2 and LEM2–mChr; DNA is stained with NucBlue and tubulin detected with SiR–tubulin. Time 0 refers to complete CFI. Representative of ten or more cells imaged across at least three biological replicates. Scale bar, 2 μm. b, Multiple sequence alignment of LEM domains across LEM family proteins, highlighting a conserved four-amino-acid sequence that, when mutated in emerin (EMD m24), disrupts BAF binding20. The position of an analogous mutation in LEM2 (LEM2 m21) is indicated. c, HeLa cells stably expressing LEM2–mCherry and EGFP–BAF live-imaged during anaphase. Representative of ten or more cells imaged across at least three biological replicates for both fixed and live-cell imaging. Scale bar, 10 μm. d, Top, a homology model for the LEM21–72–BAF–DNA complex51, based on Protein Data Bank code (PDB): 2BZF and PDB: 2ODG32,51. Middle, absorbance at 280 nm as a function of retention volume (ml) from analytical size exclusion chromatography. Retention volumes for major peaks (arrowheads) and predicted molecular weights for protein or protein–DNA complexes are listed. Bottom, SDS–PAGE of major peak for LEM21–72 + BAF + DNA sample. Representative of three technical replicates. For gel source data, see Supplementary Fig. 1. e, Percentage amino acid composition for the LEM2 LCD, and the compositions of two subregions, compared to an average amino acid composition. f, Schematic of LEM2NTD with amino acid substitutions (S, T, or Y to D) relative to SY-rich (yellow) and PR-rich (rust) regions, in LEM2 m20, Mim1 and Mim2 constructs. LEM2 immunoblot assessing the migration pattern of full-length (also shown in Fig. 1e) and mutant LEM2–mChr constructs following separation by Phos-tag SDS–PAGE. Cell lysates were prepared from G1/S- and prometaphase-arrested cells expressing the indicated exogenous LEM2; lysates treated with λ-PP are indicated. Representative data from two biological replicates, with one and three technical replicates per biological replicate. For immunoblot source data, see Supplementary Fig. 1. g, Top, amino acid sequences of the peptides corresponding to the SY-rich and PR-rich regions of LEM2. Middle, concentration-dependent droplet formation by the LEM2SY peptide, juxtaposed with similar data collected for full LEM2 NTD as in Fig. 1d. Bottom, SDS–PAGE of major peak for LEM21–72 + BAF + DNA sample. Representative of three technical replicates. For fluorescence microscopy of purified LEM2NTD, with indicated molecular anions. Image representative of two technical replicates. Scale bar, 2 μm.
Extended Data Fig. 2 | LEM2 wets the surface of microtubules with a liquid-like coat. 

a, Additional example of STED imaging of endogenous LEM2 localization during late anaphase. Scale bars, 150 nm. Representative of data from four cells. 
b, Fluorescence imaging of indicated combinations of LEM2 FL–Alexa488 (magenta), tubulin–Alexa647 (green), and lipids labelled with PE-rhodamine (cyan). Scale bar, 2 μm. Representative of two technical replicates. 
c, Light scattering at 340 nm (turbidity) of microtubule bundling by indicated LEM2 constructs. Half maximal concentration of LEM2 NTD is 1.303 ± 0.1 μM. Mean ± s.e.m. for n = 3 independent samples. 
d, Negative stain electron microscopy of microtubules alone or microtubules with indicated concentrations of LEM2 NTD, corresponding to the concentrations measured by light scattering. Images representative of at least six; scale bars, 25 nm. 
e, Fluorescence microscopy of LEM2 NTD–Alexa488 in combination with tubulin–Alexa647, LEM2 WH–Alexa555, and GTP/MgCl₂, as indicated. Scale bar, 10 μm. Images are representative of at least three. 
f, Example images for FRAP of LEM2 FL- and LEM2 NTD-coated microtubule bundles, representative of five independent samples (LEM2 FL) or 17 independent samples (LEM2 NTD). Scale bar, 2 μm. 
g, Top, kymograph across bleached region. Bottom, FRAP of LEM2 NTD-coated microtubule bundle. Images show fluorescent LEM2 NTD channel. Scale bar, 2 μm. Representative of eight technical replicates.
Extended Data Fig. 3 | LEM2–LCD bundles microtubules in vitro through a regulated microtubule-binding domain. a, Amino-acid sequences of six LEM2 peptides tiling the LCD. LEM2<sub>40–60</sub> is LEM2-PRA, and LEM2<sub>123–144</sub> is LEM2-PR<sub>b</sub>. b, Light scattering at 340 nm (turbidity) of microtubule bundling by indicated LEM2 peptides. Half maximal concentration of LEM2<sub>188–212</sub> (LEM2-PR<sub>b</sub>) is 85.11 μM. For LEM2<sub>40–60</sub>, LEM2<sub>61–81</sub>, LEM2<sub>123–144</sub>, and LEM2<sub>188–212</sub>, did not bundle. c, Negative stain electron microscopy of microtubules alone or microtubules with indicated concentrations of LEM2<sub>188–212</sub>. Corresponding to turbidity reactions. Scale bars, 25 nm. For each condition, images are representatives of seven. d, Live-cell imaging of indicated LEM2<sub>235–331</sub>-mChr and LEM2<sub>235–331</sub>-mChr deletion constructs (magenta) and GFP–tubulin (green). Time 0 refers to time of complete CFI. Scale bar, 2 μm. Images are representative of three independent experiments. e, Negative stain electron microscopy of microtubules co-incubated with the indicated phosphomimetic proteins. Scale bar, 25 nm. Images are representative of two technical replicates. f, Light scattering at 340 nm (turbidity) of microtubule bundling by phosphomimetic LEM2 constructs. Vertical lines are half maximum, shaded regions show s.e.; LEM2<sub>NTD</sub>, (green) half maximum 1.303 μM (1.144–1.493 μM), LEM2<sub>Mim1</sub> (red) half maximum 2.824 μM (2.283–3.397 μM), LEM2<sub>Mim2</sub> (blue) half maximum 8.442 μM (7.511–10.06 μM). Data plotted are mean ± s.e.m. from three technical replicates.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | The LEM2 WH domain is required for recruitment of IST1 to the nascent nuclear envelope and mediates polymer formation with CHMP7. a, Top, quantification of robust IST1 recruitment to chromatin discs in late anaphase, as assessed by blind scoring. Mean ± s.e.m. determined from three independent experiments (siControl parental: n = 80, 58, 106; siLEM2-2/parental: n = 78, 50, 58; siControl/LEM2–mChr: n = 46, 42, 62; siLEM2-2/LEM2–mChr: n = 78, 51, 62; siControl/LEM2∆WH–mChr: n = 138, 52, 42; siLEM2-2/LEM2∆WH–mChr: n = 112, 48, 51). Two-tailed unpaired t-test, no multiple comparisons. Bottom, representative images by widefield showing localization of endogenous IST1 in late anaphase cells depleted of endogenous LEM2 and expressing the indicated siRNA-resistant LEM2–mChr constructs. Scale bar, 2 μm. b, Top, quantification of the percent of early anaphase discs with robust IST1 recruitment, as assessed by blind scoring. Mean ± s.e.m. determined from three independent experiments (siControl/parental: n = 38, 16, 20; siLEM2-2/parental: n = 24, 10, 25; siControl/LEM2–mChr: n = 50, 20, 24; siLEM2-2/LEM2–mChr: n = 38, 14, 8; siControl/LEM2∆WH–mChr: n = 44, 16, 20; siLEM2-2/LEM2∆WH–mChr: n = 20, 4, 14). Two-tailed unpaired t-test, no multiple comparisons. Bottom, representative images by widefield showing localization of endogenous IST1 in early anaphase cells depleted of endogenous LEM2 and expressing the indicated siRNA-resistant LEM2–mChr constructs. Scale bar, 2 μm. c, Negative stain electron microscopy corresponding to the CHMP7 polymerization assay showing no polymerization for the control condition CHMP7 + LEM2NTD. Representative of two technical replicates.
Extended Data Fig. 5 | Homology modelling and XL-MS consistent with a WH domain-swap mechanism for activation of CHMP7 by LEM2.

**a,** Workflow of lysine–lysine hybrid peptide mapping using XL-MS. BS3 cross-links surface-accessible lysine residues with Cα–Cα distances below about 3 nm. **b,** Homology models for the WH domains of CHMP7 and LEM2 from reference structures described in Supplementary Table 2. WH1 of CHMP7 contains a membrane-binding region indicated as a loop.

**c,** Homology models of the CHMP7 ESCRT-III-fold in open and closed conformations. Green and orange, α-helices 1–2 and 3–4, respectively.

**d,** Distance restraints identified from XL-MS analysis of the CHMP7 monomer were mapped to open and closed homology models. Cα–Cα distances over 3 nm are considered violations. Blue, satisfied restraints; red, violated restraints. The open ESCRT-III conformation was rejected.

**e,** Top, a crystallographic interface between VPS25 and an ESCRT-III helix serves as a template for the XL-MS-informed homology model of the CHMP7 WH2 interaction with the CHMP7 ESCRT-III domain. For details on template structures see Supplementary Table 2. Middle, bottom, all cross-links are satisfied when mapped to the closed CHMP7-ESCRT-III model and agree with domain connectivity. A subset of cross-links was not satisfied when mapping WH1 instead to the same interface (data not shown).

**f,** Left, distance restraints identified from XL-MS analysis of CHMP7 monomer mapped to conformation of polymerized CHMP7 consistent with 2D class averages. Violated restraints suggest a hinge region between CHMP7 WH1 and WH2 that allows its WH1 to move closer to the ESCRT-III core (black arrow). Right, violated restraints to WH2.
Extended Data Fig. 6 | Activated CHMP7 forms polymeric rings via its ESCRT-III domain, exposing its tandem WH domains. a, CHMP7 point mutations are indicated in an open ESCRT-III fold, representing polymerized CHMP7. b, Negative stain electron microscopy of His₆-SUMO–LEM2WH co-incubated with CHMP7 mutants. Scale bars, 50 nm. Images representative of three technical replicates. c, SDS–PAGE of pellet (P) or supernatant (S) following centrifugation of LEM2WH incubated with wild-type or mutant CHMP7. For gel source data, see Supplementary Fig. 1. d, Quantification of pelleted protein after SDS–PAGE and Coomassie blue staining for mutant versus wild-type proteins. Mean ± s.d. quantified from n = 3 independent experiments. e, SDS–PAGE-based relative quantification of polymerized and pelleted CHMP7 with different ratios of LEM2WH present. Red dashed lines indicate expected fraction of CHMP7 in the pelleted polymer, assuming 1:1 stoichiometric polymer. Mean ± s.d. quantified from n = 3 independent experiments. For gel source data, see Supplementary Fig. 1. f, Negative stain electron microscopy of CHMP7 polymers on a liposome. Scale bar, 100 nm. g, Top, negative stain electron microscopy of membrane-induced CHMP7 polymers used for 2D averaging. Scale bar, 20 nm. Bottom, representative 2D class averages from manually picked particles from polymers shown at top. h, Negative stain electron microscopy of CHMP7 ESCRT-III (AA 229–453). Representative of three technical replicates. Scale bar, 20 nm.
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7 | LEM2 promotes early nuclear compartmentalization via cooperation between its LCD and WH domains. a, Example images of cells treated with siLEM2 and expressing NLS–3GFP in combination with siRNA-resistant LEM2 constructs corresponding to the quantification graphs shown in b and Fig. 3f. DNA labelled using NucBlue. Time 0 refers to the time of complete CFI. Scale bar, 2 μm. b, Mean + s.d. nuclear/cytoplasmic ratio of NLS–3GFP fluorescence over time in cells treated with the indicated siRNAs and expressing the indicated siRNA-resistant constructs. Cells imaged at 15-s intervals though 1-min increments are plotted. Data were collected across at least three biological replicates (siCon/LEM2–mChr: n = 26; siLEM2–2/LEM2–mChr: n = 44; siCon/LEM2ΔSY–mChr: n = 20; siLEM2–2/LEM2ΔSY–mChr: n = 24; siCon/LEM2ΔPR–mChr: n = 20; siLEM2–2/LEM2ΔPR–mChr: n = 20; siCon/LEM2ΔWH–mChr: n = 24; siLEM2–2/LEM2ΔWH–mChr: n = 16). Two-tailed unpaired t test was used to determine P values comparing deletion mutant lines to the full-length LEM2 line under endogenous LEM2 depletion conditions at each time point. No multiple comparisons. *P < 0.05, **P < 0.005; exact P values below. c, Quantification of nuclear/cytoplasmic ratio of NLS–3GFP approximately 30 min after complete CFI in parental HeLa cells treated with the indicated siRNAs. Data were collected across three biological replicates and plotted as mean ± s.d. (siControl: n = 11, 12, 6; siLEM2–2: n = 18, 18, 14; siCHMP7: n = 11, 6, 14). Two-tailed unpaired t test, no multiple comparisons.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Loss of LEM2 function leads to DNA damage and abnormal nuclear morphologies. 

**a**, Live-cell imaging of GFP–tubulin and H2B–mCh in siRNA-treated cells. Images representative of two biological replicates, quantified in **b**. Time 0 refers to the time of complete CFI. Scale bar, 2 μm. 

**b**, Left, orthogonal view of the tubulin phenotype following LEM2 depletion co-stained for the nuclear envelope protein SUN2. Images representative of three biological replicates. Scale bar, 5 μm. Right, mean ± s.e.m. percentage of telophase cells with nuclear tubulin defects, lined with inner nuclear membrane (as assessed by immunofluorescence of lamin B2). Three biological replicates (siControl: n = 75, 35, 37; siLEM2-1: n = 72, 35, 37; siLEM2-2: n = 45, 34, 34). Two-tailed unpaired t-test, no multiple comparisons.

**c**, Left, example images of 53BP1 localization by immunofluorescence in telophase U2OS cells following siRNA treatment. Scale bar, 5 μm. Right, mean ± s.e.m. percentage of telophase cells with five or more 53BP1 nuclear foci. Three biological replicates (siControl: n = 56, 52, 44; siLEM2-1: n = 64, 56, 26; siLEM2-2: n = 74, 50, 40). Two-tailed unpaired t-test, no multiple comparisons. Bottom, immunoblot confirming depletion of endogenous LEM2 in U2OS cells, using siRNA oligos previously validated in other human cell lines, including HeLa419 (immunoblot source data shown in Supplementary Fig. 1).

**d**, Negative stain electron microscopy of indicated combinations of microtubules, LEM2-NTD-linker-WH, and CHMP7β. Scale bars, 25 nm. Images representative of two technical replicates. e, Example images of cells expressing the indicated siRNA-resistant constructs and treated with the indicated siRNAs. Cells were arrested in S-phase and then allowed to progress through one round of division, resulting in an interphase population of cells that had just exited mitosis. We observed an increased number of highly irregular nuclei in cells expressing either LEM2∆PR–mChr or LEM2∆WH–mChr compared to cells expressing full-length LEM2 or even those depleted of LEM2. Notably, deformed nuclei were commonly associated with microtubule disorganization and aberrant accumulation of LEM2∆PR–mChr and LEM2∆WH–mChr. Representative nuclear, tubulin, and LEM2 phenotypes and the correspondence to nuclear circularity score are shown. Nuclear borders and circularity scores annotated in tubulin channel. Scale bar, 5 μm. These findings suggest that interfering with cooperation between the microtubule-interacting and ESCRT-binding domains of LEM2 alters nuclear morphology, indicating that both activities are necessary, but neither is sufficient for nuclear envelope reformation. Moreover, the presence of one activity without the other is detrimental to nuclear morphology.

**f**, Quantification of nuclear circularity in interphase parental HeLa cells and cells expressing the indicated siRNA-resistant LEM2 constructs, treated with the indicated siRNAs. Mean ± s.e.m. from three biological replicates (siControl/parental: n = 105, 46, 80; siLEM2-2/parental: n = 102, 116, 59; siControl/LEM2∆SY–mChr: n = 153, 53, 122; siLEM2-2/LEM2–mChr: n = 84, 81, 105; siControl/LEM2∆SY–mChr: n = 123, 68, 144; siLEM2-2/LEM2∆SY–mChr: n = 93, 95, 105; siControl/LEM2∆SY–mChr: n = 149, 123, 58; siLEM2-2/LEM2∆SY–mChr: n = 49, 31, 42; siControl/LEM2∆SY–mChr: n = 116, 96, 94; siLEM2-2/LEM2∆SY–mChr: n = 85, 32, 68). Two-tailed unpaired t-test comparing circularity scores less than 0.6 (indicated by blue) between the indicated treatments; no multiple comparisons. 

**g**, Immunoblot showing relative levels of the siRNA-resistant constructs fused with mCherry in parallel with endogenous LEM2. Representative of two technical replicates. For immunoblot source data, see Supplementary Fig. 1.
Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

- Software used for data collection: Proteome Discoverer 2.2 (Thermo), Protein Prospector 5.20.23, Skyline 4.1, SerialEM 3.5-9, ImageJ 1.52a, Leica Application Suite X 3.1.5, Metamorph Version 7.10.1.161, and Axiovision (AxioVision) version 4.8.2.0. All software packages used for data acquisition are described in the method section.

Data analysis

- ImageJ 1.52a, UCSF Chimera 1.14, Relion 2.0, Leica Application Suite X 3.7.1, Huygens Professional 17.04, For mammalian cell data: Fiji is just ImageJ 2.0.0-r-c-65/1.52b, GraphPad Prism Version 8.3.0

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Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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Raw data and peaklists from the quantitative crosslinking mass spectrometry analysis can be accessed with MassIVE: ftp://massive.ucsd.edu/MSV00084837/. Crosslinked peptide spectral assignments are accessible using accession: rgBlhrush at http://msviewer.ucsf.edu/prospector/cgi-bin/msform.cgi?form=msviewer. The data that supports the findings of this study are available within Supplementary Information files, and from the corresponding authors upon reasonable request.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | Sample sizes were not predetermined using statistical methods. Sample sizes are consistent with what is published in the field. |
| Replication | All attempts at replication were successful. For quantified mammalian cell data, no validated replicate experiments were excluded. For qualitative mammalian cell data, validated replicate experiments were not excluded when determining representative phenotypes. |
| Randomization | Only in the instance where a visual score of IST1 recruitment phenotypes by immunofluorescence was subjective in nature were data randomized. Specifically, all the images across all treatment conditions for each experiment (three replicate experiments) were coded and randomized as a single set of data to be scored visually by three blind scorers (blinding described below) using predetermined criteria. The results of blind scoring was recorded for all images, which were then decoded for quantification. Therefore, random allocation is not relevant — in other words, subjects/data were not assigned into groups for experimentation/study. For this reason, in combination with the fact the experimental samples were all treated in parallel (for each of the three independent experiments), covariates are not relevant. |
| Blinding | IST1 recruitment was scored blindly, consistent with previously published approaches. Experiments tracking cell phenotypes that were not blinded are consistent with what is published in the field, including using objective quantification methods where ever possible. |

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|---------------------------------|---------|
| n/a | Involved in the study |
| Antibodies | [x] ChiP-seq |
| Eukaryotic cell lines | [x] Flow cytometry |
| Palaeontology | [x] MRI-based neuroimaging |
| Animals and other organisms | |
| Human research participants | |
| Clinical data | |

Antibodies

| Antibodies used | |
|-----------------| |
| [anti-S3B1; Millipore, MA38802 [lot 2753986]; Mouse monoclonal BP13; reactivity: Human; application: Human cells, IF at 1:1,000;] | |
| [anti-Lamin B2; AbCam, ab8983 [lot GR141423]; Mouse monoclonal LN43; reactivity: Mouse, Hamster, Human, Xenopus laevis, Zebrafish; application: Human cells, IF at 1:2,000 and WB at 1:1,000;] | |
| [anti-LEMD2; Sigma-Aldrich; HPA017340 [lots B96759 and B106882]; Rabbit polyclonal; reactivity: rat, human, mouse; application: Human cells, IF at 1:100 and WB at 1:500;] | |
| [anti-mCherry; Novus Biologicals, NB27-25157 [lot 010519]; Rabbit polyclonal; reactivity: human, mouse, rat, non-species specific; application: detecting exogenous expression in Human cells, WB at 1:2,000;] | |
| [anti-Tubulin; AbCam, ab18251 [lots GR175278-1 and GR3198330-1]; Rabbit polyclonal; reactivity: Mouse, Rat, Chicken, Cow, Human, Drosophila melanogaster, Indian muntjac, African green monkey, Chinese hamster; application: Human cells, IF at 1:1,000 and WB at 1:2,000;] | |
| [anti-Tubulin; Accurate Chemical & Scientific, YSR17MC177G; Rat monoclonal Y11/2; reactivity: Yeast, Birds, Mammals; application: Human cells, IF at 1:1,000;] | |
| [anti-S1T1; Gift from Wes Sundquist [generated by the Sundquist lab, published in Bajorek, et al MBoC 2009; doi:10.1091/mbc.E08-05-0475]; Rabbit polyclonal; validated reactivity: Human; application: Human cells, IF at 1:500;] | |
| [anti-SUN2; unpurified hybridoma supernatant, gift from Brian Burke [unpublished]; Mouse monoclonal 3.1E; validated reactivity: Human; application: Human cells, IF at 1:100] | |
Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

- HeLa cells were a gift from Maureen Powers (Emory University School of Medicine).
- U2OS were a gift from Don Ayer (University of Utah School of Medicine).

Authentication

- Both cell lines were authenticated by STR profiling.

Mycoplasma contamination

- All HeLa cell lines were generated from the same parental HeLa cells, which tested negative for mycoplasma contamination.
- U2OS cells were not tested for mycoplasma contamination.

Commonly misidentified lines

- No commonly misidentified lines were used in this study.