Endosomal sorting complexes required for transport III (ESCRT-III) proteins have been implicated in sealing the nuclear envelope in mammals, spindle pole body dynamics in fission yeast, and surveillance of defective nuclear pore complexes in budding yeast. Here, we report that Lem2p (LEM2), a member of the LEM (Lap2-Emerin-Man1) family of inner nuclear membrane proteins, and the ESCRT-II/ESCRT-III hybrid protein Cmp7p (CHMP7), work together to recruit additional ESCRT-III proteins to holes in the nuclear membrane. In Schizosaccharomyces pombe, deletion of the ATPase vps4Δ leads to severe defects in nuclear morphology and integrity. These phenotypes are suppressed by loss-of-function mutations that arise spontaneously in lem2 or cmp7, implying that these proteins may function upstream in the same pathway. Building on these genetic interactions, we explored the role of LEM2 during nuclear envelope reformation in human cells. We found that CHMP7 and LEM2 enrich at the same region of the chromatin disk periphery during this window of cell division and that CHMP7 can bind directly to the C-terminal domain of LEM2 in vitro. We further found that, during nuclear envelope formation, recruitment of the ESCRT factors CHMP7, CHMP2A, and IST1/CHMP8 all depend on LEM2 in human cells. We conclude that Lem2p/LEM2 is a conserved nuclear site-specific adaptor that recruits Cmp7p/CHMP7 and downstream ESCRT factors to the nuclear envelope.

Eukaryotic genomes are sequestered within the nucleus, an organelle with a boundary that comprises the double-membranated nuclear envelope (NE) (1). The inner and outer bilayers of the NE are perforated by annular channels that contain nuclear pore complexes (NPCs), each a massive assembly that regulates the trafficking of macromolecules like mRNA and proteins between the cytoplasm and nucleoplasm. The evolution of the envelope, among other roles, helped safeguard genome duplication and mRNA transcription from parasitic nucleic acids (2). The isolation of nucleoplasm from cytoplasm, however, presents a challenge during cell division when duplicated chromosomes must be separated for daughter-cell inheritance. Chromosome inheritance depends on assembly of a mitotic spindle, which pulls chromosomes toward opposite sides of the duplicating cell. Spindle assembly begins when two microtubule-organizing centers (MTOCs) nucleate polymerization of antiparallel arrays of microtubules to capture daughter chromosomes. Despite functional conservation throughout Eukarya, the mechanisms by which spindle microtubules breach the NE to gain access to metaphase chromosomes vary markedly (3–6). In vertebrates and other organisms that have an “open mitosis,” the NE disassembles completely, so that nucleoplasmic identity is lost. Certain protists and fungi, by contrast, maintain NE integrity throughout a “closed mitosis” (3, 5).

The fission yeast Schizosaccharomyces pombe and the budding yeast Saccharomyces cerevisiae integrate their MTOC, known as the spindle pole body (SPB), into the NE so that microtubule assembly can occur in the nucleoplasm (3, 4, 7, 8). In budding yeast, duplication of the SPB is coupled with NE remodeling so that mother and daughter SPBs reside within the envelope throughout the cell cycle (4, 9). Fission yeast, by contrast, restricts SPB access to the nucleoplasm during mitosis only (7, 8). Upon mitotic entry, a fenestration through the NE opens transiently, and the mother and daughter SPBs are incorporated (3, 5, 6, 8, 10). For every cell cycle, therefore, the fission yeast NE must open and reseal twice: once when the SPBs are inserted and again when the SPB is ejected from the envelope after a successful cell cycle.

Incorporating NPCs and SPBs into the NE requires certain factors and mechanisms in common, including membrane-remodeling activities (6, 11–15). We and others have previously reported strong genetic interactions between transmembrane nucleoporins, SPB components, and endosomal sorting complexes required for transport (ESCRT) genes—portraying a role for certain ESCRT proteins in nuclear membrane remodeling (16, 17). In general, ESCRT components are recruited to different target membranes by site-specific adaptors that ultimately recruit the membrane-remodeling ESCRT-III subunits and their binding partners, including VPS4-family adenosine triphosphatases (ATPases) (18–20). We previously showed that certain ESCRT-III mutants and vps4Δ cells displayed an apparent overamplification of SPBs (or defective fragments) in fission yeast and that the severity of this SPB phenotype in fission yeast
waned over time, suggesting possible genetic suppression (16). In budding yeast, Webster et al. reported that, without ESCRT-III/ Vps4 activity, misassembled NPCs accumulate in a compartment they named the SINC (for storage of improperly assembled NPCs) (21). They also showed that LEM family (Lap2-Emerin-Man1) inner nuclear membrane (INM) proteins Heh1p and Heh2p in budding yeast associate with defective NPC assembly intermediates (but not with mature NPCs) and that Heh1/2 proteins may recruit ESCRT-III and Vps4 activities to malformed NPCs to clear them from the NE (21).

In mammals, VPS4 depletion induces nuclear morphology defects (22), and several recent reports have demonstrated that ESCRT pathway proteins are recruited transiently to seal gaps in reforming mammalian nuclear membranes during anaphase (23, 24) and to rupture sites in the nuclei of interphase mammalian cells (25, 26). Depletion of ESCRT factors delays sealing of the reforming NE and impairs mitotic spindle disassembly (23, 24). Moreover, depletion of SPASTIN, another meiotic clade VPS4-family member and ESCRT-III-binding enzyme (27), also delays spindle disassembly and envelope resealing (24, 28). Similar effects were seen upon depletion of several ESCRT-III proteins, including the poorly characterized ESCRT factor CHMP7, which has features of both ESCRT-II and -III proteins (29). These observations support a model in which ESCRT-II and -III factors (including SPASTIN) coordinate microtubule severing with the closure of annular gaps in the NE. This model is conceptually similar to the mechanism of cytokinetic abscission, where SPASTIN disassembles the residual microtubules that pass between daughter cells, while ESCRT-III and VPS4 proteins constrict the midbody membrane to the point of fission (19, 28, 30).

Here, we address the key question of what upstream factor(s) serves as the membrane-specific adaptor that facilitates CHMP7 recruitment to function in sealing NE breaches. To identify factors in this pathway, we returned to the genetically tractable fission yeast system. We report that deletion of vps4 in S. pombe leads to severe defects in nuclear membrane morphology and nuclear integrity, with secondary defects in NPCs and SPB dynamics. Remarkably, these phenotypes are suppressed spontaneously when cells acquire loss-of-function mutations in cmp7, an ortholog of human CHMP7, or in lem2, a LEM domain INM protein and ortholog of human LEM2. We also show that, in human cells, recruitment of CHMP7 and downstream ESCRT-III proteins to the reforming NE during anaphase depends on LEM2, most likely through a direct interaction between CHMP7 and the C-terminal nucleoplasmic domain of LEM2. Together, these observations implicate LEM2 as a nucleus-specific adaptor that recruits ESCRT pathway activities to remodel the NE during both open and closed mitoses across Eukaryotes.

Results

vps4Δ Fission Yeast Cells Grow Very Slowly, and Loss of Either cmp7 or lem2 Rescues Growth. The AAA ATPase VPS4 has a primary role in disassembling ESCRT-III polymeric structures in the different settings where the ESCRT pathway mediates membrane remodeling. To determine whether and how reported phenotypes that result from deletion of VPS4 were suppressed over time (16), we monitored the growth of individual colonies over time (16), we monitored the growth of individual colonies over time (16), we monitored the growth of individual colonies over time (16), we monitored the growth of individual colonies over time (16), we monitored the growth of individual colonies over time (16), we monitored the growth of individual colonies over time (16), we monitored the growth of individual colonies (Fig. 1A). This growth defect spontaneously reverted over time, so that when mutant spores were streaked on rich medium, some vps4Δ colonies exhibited growth rates comparable to WT colonies (Fig. 1B). To identify potential suppressor mutations, we sequenced complete genomes for 12 strains that spontaneously reverted to WT growth rates and compared them with genomes of both WT and apparently unsuppressed vps4Δ strains. The analysis revealed that 7 of the 12 suppressors had different loss-of-function mutations in the ESCRT-II/III hybrid gene, cmp7 (Table S1). The remaining five each had equivalent independent mutations in a LEM domain family member, lem2, within what appears to be a slippery poly-T tract (Table S1). These 12 mutant alleles were further confirmed by Sanger sequencing, and none of the suppressors were found to harbor mutations in both cmp7 and lem2 simultaneously.

To determine whether these potentially suppressive alleles rescued the growth of vps4Δ cells, we engineered cmp7Δ/+ and lem2Δ/+ genotypes within our vps4Δ/+ diploid background and isolated lem2Δ, cmp7Δ, and vps4Δ single mutants and their corresponding double mutants via sporulation and tetrad dissection. Quantitative growth rates in liquid culture for biological and technical triplicates demonstrated that cmp7Δ single mutants had WT growth rates and that the vps4Δcmp7Δ double-mutant cells grew much faster than vps4Δ single-mutant cells (albeit slightly more slowly than WT cells; Fig. 1C). Similarly, lem2Δ single mutants displayed a modest growth defect compared with WT, but vps4Δlem2Δ double-mutant cells again grew much faster than vps4Δ cells (Fig. 1C). Thus, our unbiased whole-genome
Cells Have NE Defects, Which Are Suppressed by Loss of cmp7 or lem2. Next, we sought to discover the cellular defects that correlated with the slow growth of vps4Δ cells and to test whether those defects were also rescued by cmp7Δ or lem2Δ. In light of our prior work on mitotic and SPB defects in vps4Δ cells, we first examined NE and SPB morphology and dynamics. More than 80% of vps4Δ cells from recently dissected haploid spores displayed severe NE morphology defects. These defects were rescued by loss of either cmp7 or lem2 (Fig. 1 D–F). Moreover, ~30% of vps4Δ cells displayed clearly asymmetric SPB segregation errors and anucleate daughter cells (Fig. S1A and B). In this case, loss of cmp7 rescued these phenotypes, but loss of lem2 did not. Indeed, even lem2Δ single-mutant cells displayed similar SPB segregation defects (Fig. S1C). Thus, defects in NE morphology and integrity were the features that correlated best with the slow-growth phenotype, suggesting that these defects were primarily responsible for the vps4Δ slow-growth phenotype.

vps4Δ Cells Display a Series of Mitotic Errors Associated with NE Defects. Live cell imaging using NE (Ish1p-mCherry) and SPB (Cut12p-YFP) markers enabled us to monitor the development and consequences of NE defects in mutant vs. WT cells. Abnormal NE morphologies or asymmetric and even failed karyokinesis were observed in the majority of cells (Fig. 2), and only ~30% of vps4Δ cells displayed normal, symmetric karyokinesis (Fig. 2A). An apparent proliferation or overgrowth of Ish1p-marked membranes was a particularly common defect in vps4Δ cells. Approximately 25% of mutant cells displayed these long-lived NE “outgrowths” that we later determined were karmellae (see below). In cells with karmellae, daughter SPBs often failed to separate normally or displayed extensive delays in separation (Fig. 2B). Indeed, separation of duplicated SPBs was significantly prolonged in vps4Δ cells, whether or not they exhibited abnormal NE malformations (Fig. S2). Together, these observations suggest that Vps4p plays a central role in regulating NE morphology in fission yeast, particularly during SPB extrusion or insertion through the NE, and perhaps during karyokinesis.

vps4Δ Nuclei Leak and Their Integrity Is Largely Restored by Loss of cmp7 or lem2. Our observations, together with recent reports that the ESCRT pathway closes holes in the mammalian NE (23, 24), prompted us to test the integrity of vps4Δ nuclei. Image analysis revealed that a large nuclear import cargo, NLS-GFP-LacZ, was enriched within nuclei by >10-fold in 98% of WT cells (Fig. 3A). By contrast, ~55% of vps4Δ cells displayed <10-fold nuclear enrichment (partial leaking; Fig. 3B, arrowheads), and ~10% of vps4Δ cells displayed <2-fold nuclear enrichment of NLS-GFP-LacZ (severe leaking; Fig. 3B, arrow; Fig. 3 C and D, quantification). Remarkably, loss of cmp7 or lem2 rescued this abnormal nuclear integrity phenotype to a large extent, although a small minority of single and double cmp7 or lem2 cells still displayed partial leaking (Fig. 3 C and D). Live cell imaging also revealed that the extent of nuclear integrity loss correlated with NE morphology defects (Fig. S3). Cells that initially displayed normal GFP reporter localization and normal NE morphology gradually accumulated cytoplasmic signal over the course of tens of minutes (Fig. S3A). Cells with abnormal NE morphology at the beginning of the experiment, by contrast, lost nuclear GFP completely over the time course (Fig. S3B). Thus, cytoplasmic GFP resulted from loss of nuclear integrity rather than from defects in nuclear import.

vps4Δ NEs Are Persistently Fenestrated and Have Karmellae and Disorganized Tubular Extensions. We used electron tomography of high-pressure frozen and freeze-substituted cells to examine whether we could detect morphological defects in the NE that could account for the loss of nuclear integrity. Serial 400-nm sections were imaged and reconstructed to generate 3D volumes of >1-μm thickness. WT nuclei had evenly spaced inner and outer lipid bilayers with embedded NPCs evenly distributed around the periphery (Fig. 4A and Movie S1). vps4Δ nuclei, by contrast, displayed at least four structural abnormalities. First, large fenestrations through both the inner and outer nuclear membranes were observed (Fig. 4B, bracket). Second, karmellae or concentric layers of membrane were present around certain regions of the mutant nuclei (Fig. 4B, arrowheads, and Fig. 5B). Third, extensive whorls of disorganized tubulo-vesicular membranes that were topologically continuous with the adjacent karmellae were apparent (Fig. 4B, asterisk), and the NPCs that were present were localized to regions that were largely free of karmellae and tubulo-vesicular structures (Fig. 4D). The 3D reconstruction of these features confirmed the presence of very large gaps (>400 nm) in the NE and topologically continuous karmellae and whorls of tubular extensions (Fig. 4C and Movies S2–S4). Persistent fenestraations explain the loss of nuclear integrity in vps4Δ cells, whereas
Defects Are Suppressed by Loss of lem2 (1.1%, (Fig. 5). Remarkably, n=8.9, E2169 Movie S5 Table S4 lem2 vps4 n=82,56,67, 0.9%; partial leaking (2- to A 0.01; *** n=80,59,61, 5.9%; severe leaking (>2 fold) WT: n=82,59,61, lem2/Δ: n=68,61,69, 93.4% (Fig. 6A)). The mean Δ lem2±: 93.2 ± 1.6%. lemm2: 86.6 ± 2.3%. vps4Δlemm2/Δ: 93.4 ± 0.4%; partial leaking (2- to 10-fold GFP nuclear enrichment) WT: 1.6 ± 0.9%, vps4Δ: 54.1 ± 8.1%. comp7/Δ: 5.9 ± 1.1%, vps4Δcomp7/Δ: 6.8 ± 1.6%, lem2/Δ: 11.5 ± 2.3%. vps4Δlemm2/Δ: 5.9 ± 1.4%; severe leaking (>2 fold) GFP nuclear enrichment) WT: 0%, vps4Δ: 9.4 ± 2.4%. cmp7/Δ: 0%, vps4Δcmp7/Δ: 0%, lem2/Δ: 2.0 ± 1.3%. vps4Δlemm2/Δ: 0.7 ± 0.7%; WT: n=82,56,67, vps4Δ: n=60,59,61, cmp7/Δ: n=62,63,50, vps4Δcmp7/Δ: n=82,59,61, lem2/Δ: n=68,61,69, vps4Δlemm2/Δ: n=76,50,63. *P < 0.05; **P < 0.01; ***P < 0.001.

karmellae and other disorganized membrane extensions may underlie the kinetic delays, asymmetries, and outright failures of SPB separation and karyokinesis.

vps4Δ NE Defects Are Suppressed by Loss of cmp7 or lem2. Thin-section electron microscopy of single- and double-mutant cells demonstrated that karmellae formation in vps4Δ cells was completely suppressed by loss of cmp7 or lem2 (Fig. 5). Remarkably, double-mutant cells displayed WT-like nuclei, although a few examples of probable nuclear fenestrations were observed in lemΔ2 single-mutant cells (Fig. 5G). These observations indicate that, in the absence of vps4Δ, karmellae formation depends on both Lmp2p and Cmp7p. Similarly, overexpressing Lmp2p in S. pombe, or compromising nuclear import of Heh2p, an ortholog of Lmp2p in S. cerevisiae, induces the formation of similar abnormalities (31, 32). Together, these results suggest that, in the absence
Fig. 4. vps4Δ NEs have persistent fenestrations, karmellae, and disorganized tubular extensions. (A, Left) Single tomographic slice showing the WT NE. NPCs are marked by asterisks. Segmented NE (green) with NPCs (red) reconstructed from 150 tomographic slices is shown in a merged view (A, Left Center), a front view (A, Right Center), and a side view (A, Right). (B, Left) Single tomographic slice showing the vps4Δ NE. The following features and defects are highlighted: a fenestration (bracket), an NPC (asterisk), karmellae layers (white arrowheads), and a disorganized whorl of tubules (arrows). (B, Right) Segmented model of NE reconstructed from 100 tomographic slices is shown in a merged view with karmellae in gold and a whorl of tubules in purple. (C) A segmented model of vps4Δ NE reconstructed from 200 tomographic slices is shown from the front (Left), back (Left Center), top (Right Center), and bottom (Right). (D, Left) Single vps4Δ tomographic slice showing NPCs are absent from karmellae region. The following features and defects are highlighted: a fenestration (bracket), an NPC (asterisk), and karmellae layers (white arrowheads). Segmented NE (green) with NPCs (red) and SPB (yellow) reconstructed from 150 tomographic slices shown in a merged view (D, Left Center), a front view (D, Right Center), and a side view (D, Right). (Scale bars: 200 nm.)
Discussion

Pioneering work on the ESCRT pathway in budding yeast led to our understanding of its roles in multivesicular body biogenesis (18), while work in human cells is leading to a broader view of ESCRT roles at a variety of different target membranes (20). Our results indicate that the role of the ESCRT pathway in closing holes in the NE is evolutionarily ancient (ref. 16 and this work). The different nuclear ESCRT functions (NPC surveillance, NE reformation, and SPB insertion/removal) can now be unified by the hypothesis that all of these processes generate fenestrations in the NE that are closed by the action of the ESCRT machinery (refs. 21, 23–26, and 36 and this study). Conserved activities include roles for nucleus-specific adaptors of the LEM domain family and the ESCRT-II/-III hybrid protein CHMP7/Cmp7p (refs. 21 and 29 and this study). This conserved pathway may likewise underlie the requirement for Srp1, a LEM-domain protein in Aspergillus nidulans, in the formation of stable nuclei (37). This work suggests that LEM2 plays a specific, initiating role in coordinating membrane remodeling events, particularly during nuclear assembly, in addition to the other roles it plays as a NE resident during anaphase (31, 35, 38–43).

Two LEM family members, Heh1p and Heh2p, are involved in ESCRT recruitment in S. cerevisiae, so multiple LEM family proteins may also be involved in recruiting ESCRT-III activities in mammalian cells. In this regard, we found that knockdown of LEM2 in HeLa cells led to a less severe IST1/CHMP8 recruitment phenotype than knockdown of CHMP7 (Tables S2 and S3). It will therefore be of great interest to determine whether additional LEM-domain family members, which are present at the nascent NE (44, 45), also serve as ESCRT recruitment factors in human cells. Like other ESCRT-III proteins, CHMP7 can also interact with membranes, and this activity contributes to its targeting (46), so further biochemical studies will be required to elucidate how the dynamic interplay between LEM2, CHMP7, and lipids regulates the recruitment and activity of the ESCRT pathway at the nascent NE. It will additionally be informative to determine the mechanistic basis of vps4Δ phenotypes in fission yeast and the underlying toxicity of unrestricted Lem2p Cmp7p activity. We speculate that in the absence of Vps4p, Lem2p Cmp7p complexes stabilize nuclear membrane fenestrations aberrantly and/or promote NE remodeling events that—when unregulated by Vps4p—result in karmellae, large gaps, and other malformations that ultimately compromise cell division (Fig. S5). Similarly, overexpression of Lem2p in fission yeast induces NE malformations (31), and impairment of Heh1p/2 nuclear import in budding yeast induces karmellae formation (32). We predict that both of these phenotypes depend on CHMP7/Cmp7p proteins and other downstream ESCRT pathway activities. Studies of these and related activities in the future will benefit from the facile S. pombe genetic system for investigating how the ESCRT pathway senses and seals breaches of the envelope—a nuclear membrane integrity pathway that is conserved from yeast to human.

Materials and Methods

Yeast Strains and Growth Medium. S. pombe, diploid strain SP286 (h×i+, leu1-32/leu1-32, ura4-D18/ura4-D18, ade6-M210/ade6-M216) was used for all haploid constructions. All other strains used in this study are listed in Tables S1 and S5. Cells were routinely grown in YE5 rich medium [yeast extract 0.5% (wt/vol), glucose 3.0% (wt/vol) supplemented with 225 mg/L histidine, leucine, uracil, and lysine hydrochloride, and 250 mg/L adenine] or Edinburgh Minimal Media.
and a 500-bp genomic DNA fragment corresponding to 5' of the stop codon. The fragment was transformed into heterozygous diploids and selected on YE5 supplemented with G418 disulfate. This robust category was graphed and statistical analysis was performed, comparing the siControl dataset to each depletion condition dataset (siControl: 63 ± 6%, n = 18, 58, 24; siLEM2-1: 0 ± 0%, n = 40, 44, 12; siLEM2-2: 6 ± 6%, n = 34, 22, 6; siCHMP7-1: 0 ± 0%, n = 42, 20, 12; siCHMP7-2: 0 ± 0%, n = 22, 20, 2).

(c) Widefield images illustrating CHMP2A localization in anaphase B cells after siControl, siLEM2-1, or siLEM2-2 treatment. (D) CHMP2A recruitment to chromatin disks at anaphase B, assessed as in B (siControl: 70 ± 9%, n = 48, 48, 52; siLEM2-1: 9 ± 5%, n = 108, 86, 38; siLEM2-2: 11 ± 5%, n = 98, 52, 42; siCHMP7-1: 4 ± 1%, n = 78, 102, 47; siCHMP7-2: 21 ± 8%, n = 112, 58, 36). Analysis of parallel samples confirmed that LEM2 and CHMP7 depletion profoundly disrupted IST1/CHMP8 recruitment as before and is shown in Fig. S7G. (E) Widefield images of cells costained for IST1/CHMP8 and LEM2 illustrates the differential sensitivity of their localization at anaphase chromatin disks after siCHMP7-1 or siCHMP7-2 treatment. Signal detected at the midzone with LEM2 antibody is likely nonspecific (Fig. 7A). (F) Quantification of LEM2 recruitment to chromatin disks at anaphase B. Images were used for blind scoring the presence of chromatin-associated LEM2 (siControl: 95 ± 4%, n = 28, 35, 39; siCHMP7-1: 99 ± 6%, n = 18, 72, 45; siCHMP7-2: 99 ± 1%, n = 35, 56, 7). Analysis of parallel samples confirmed that CHMP7 depletion profoundly disrupted IST1/CHMP8 recruitment as before and is shown in Fig. S7D. *P < 0.05; **P < 0.01; ***P < 0.001. N.S., not significant. (Scale bars: 10 μm.) All graphs plot mean ± SEM.

** Knockout Cassettes and Plasmids.**

**vsps deletion cassette.** The vspsΔ::natMX6 template (16) was amplified to create an amplicon covering 756 bp upstream of the ATG and 499 bp downstream of the stop codon. This 2,439-bp amplicon was transformed into SP286 and plated on YES + ClonNAT to select for heterogeneous vspsΔ::natMX6/+ diploids.

**cmp7 deletion cassette.** A fragment including 420 bp upstream of the ATG and 377 bp downstream of the stop codon was amplified and cloned into BamHI/ BgIII and EcoRV/SpeI of pAG32 (a pFA6-derived plasmid containing hphMX4, which confers resistance to hygromycin B, was a gift from David Stillman, Department of Pathology, University of Utah). The cmp7Δ::hphMX4 fragment was amplified and transformed into heterogeneous vspsΔ::natMX6/+ diploid intermediate strain and selected on YES supplemented with both ClonNAT and hygromycin B.

**lem2 deletion cassette.** A 3.1-kb lem2 genomic fragment spanning 600 bp upstream of the ATG to 500 bp downstream of the stop codon was subcloned into pGEM vector. The ORF region was replaced with a hphMX4 hygromycin resistance cassette to make the final knockout construct (pMGF130). The lem2Δ::hphMX4 cassette was amplified and transformed into heterozygous vspsΔ::natMX6/+ diploids and selected on YES supplemented with both ClonNAT and hygromycin B.

**ish1-mCherry cassettes.** A 1-kb ish1 genomic DNA fragment corresponding to 500 bp upstream and 500 bp downstream of the ish1 stop codon was subcloned into the pGEM vector. A flexible linker (GGTGGSGGT) and mCherry fusion cassettes were assembled, followed by the yADH1 terminator and MX46 drug resistance or auxotrophic markers. These cassettes were integrated at the native ish1 locus to make the final fusion constructs: ish1-mCherry::natMX6 (pMGF170), ish1-mCherry::kanMX6 (pMGF169), ish1-mCherry::hphMX4 (pMGF157), and ish1-mCherry::ura4(+)(pMGF172).

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**Fig. 7.** Recruitment of CHMP2A and IST1/CHMP8 during mammalian nuclear reformation depends on LEM2, whose targeting is independent of CHMP7. (A) Confocal images illustrating IST1/CHMP8 localization in anaphase B cells after siControl, siLEM2-1, or siLEM2-2 treatment. (B) Quantification of IST1/CHMP8 recruitment to chromatin disks during anaphase B. IST1/CHMP8 recruitment was scored as robust, weak, or no chromatin-associated foci. The robust category was graphed and statistical analysis was performed, comparing the siControl dataset to each depletion condition dataset (siControl: 63 ± 6%, n = 18, 58, 24; siLEM2-1: 0 ± 0%, n = 40, 44, 12; siLEM2-2: 6 ± 6%, n = 34, 22, 6; siCHMP7-1: 0 ± 0%, n = 42, 20, 12; siCHMP7-2: 0 ± 0%, n = 22, 20, 2).

(c) Widefield images illustrating CHMP2A localization in anaphase B cells after siControl, siLEM2-1, or siLEM2-2 treatment. (D) CHMP2A recruitment to chromatin disks at anaphase B, assessed as in B (siControl: 70 ± 9%, n = 48, 48, 52; siLEM2-1: 9 ± 5%, n = 108, 86, 38; siLEM2-2: 11 ± 5%, n = 98, 52, 42; siCHMP7-1: 4 ± 1%, n = 78, 102, 47; siCHMP7-2: 21 ± 8%, n = 112, 58, 36). Analysis of parallel samples confirmed that LEM2 and CHMP7 depletion profoundly disrupted IST1/CHMP8 recruitment as before and is shown in Fig. S7G. (E) Widefield images of cells costained for IST1/CHMP8 and LEM2 illustrates the differential sensitivity of their localization at anaphase chromatin disks after siCHMP7-1 or siCHMP7-2 treatment. Signal detected at the midzone with LEM2 antibody is likely nonspecific (Fig. 7A). (F) Quantification of LEM2 recruitment to chromatin disks at anaphase B. Images were used for blind scoring the presence of chromatin-associated LEM2 (siControl: 95 ± 4%, n = 28, 35, 39; siCHMP7-1: 99 ± 6%, n = 18, 72, 45; siCHMP7-2: 99 ± 1%, n = 35, 56, 7). Analysis of parallel samples confirmed that CHMP7 depletion profoundly disrupted IST1/CHMP8 recruitment as before and is shown in Fig. S7D. *P < 0.05; **P < 0.01; ***P < 0.001. N.S., not significant. (Scale bars: 10 μm.) All graphs plot mean ± SEM.
**Isolation of vps4Δ and Suppressors.** Individual petite colonies from random spore analysis plates of YES+ClonNAT were selected, resuspended in 200 μL of YES medium, plated onto two YES+ClonNAT plates, and incubated at 32 °C for 3 d. Isolates that grew across one plate without apparent suppression were frozen with glycerol without further culturing by scraping and resuspension in YES with 15% (wt/vol) glycerol. Cells from the other plate were picked, resuspended in 200 μL of YES medium, and plated onto two YES plates. After 2 d, cells were harvested as described above for glycerol stocks and genomic DNA extraction.

**Yeast Genomic DNA Extraction, Illumina Sequencing, and Analysis.** Genomic DNA extraction. Frozen pellets of WT, vps4Δ, or suppressor cells (200 μL) were thawed on ice. A total of 250 μL of resuspension buffer (20 mM Tris HCl, pH 8.0, 100 mM EDTA, and 0.5 M β-mercaptoethanol) and 50 μL of lyticase (50 units) were then added to remove the cell wall. Genomic DNA was extracted by using phenol/chloroform/isomyl alcohol, precipitated with ethanol, and rehydrated with RNase, followed by DNeasy Blood Tissue purification according to the manufacturer’s protocol (Qiagen catalog no. 69504).

Illumina sequencing. Libraries were constructed by using the Illumina TruSeq DNA Sample Preparation Kit (catalog nos. FC-121-2001 and FC-121-2002). Briefly, genomic DNA was sheared in a volume of 52.5 μL by using a Covaris S2 Focused-ultra-sonicator with the following settings: intensity, 5.0; duty cycle, 10%; cycles per burst, 200; treatment time, 120 s. Sheared DNA was converted to blunt-ended fragments and size-selected to an average length of 275 bp by using AMPure XP (Beckman Coulter catalog no. A63882). After denaturation of the DNA, adapters containing a T-base overhang were ligated to the A-tailed fragments. Adapter-ligated fragments were enriched by PCR (eight cycles) and purified with AMPure XP. The amplified libraries were qualified on an Agilent Technologies 2200 TapeStation by using a D1K ScreenTape assay (catalog no. 5067-5363), and quantitative PCR was performed by using the Kapa Biosystems Kapa Library Quant Kit (catalog no. KK4824) to define the molarity of adapter-modified molecules. Molarities of all libraries were subsequently adjusted to 10 nM, and equal volumes were pooled in preparation for Illumina sequencing.

Sequencing data analysis. Raw reads were aligned to the S. pombe genome, obtained from Ensembl Fungi, by using NovoClast Novoalign, allowing for no more than 1 mismatch or 1 indel per alignment. Alignments were converted to BAM-formatted files by using samtools (samtools.sourceforge.net). Sequence pileups were generated with samtools pileup, and variants were called by using the bcftools utility (options `-g -v`). Variants were filtered by using the included varFilter Perl script included with samtools and written out as a vcf file. To distinguish unique variants in each strain from common variants, sample vcf files were intersected with one another by using the Perl script intersect_SNPs (https://github.com/tjparnell). Variants were annotated with the Perl script locate_SNPs (https://github.com/tjparnell) by using a GF3 gene annotation file obtained from Ensembl. From the resulting table, variants were further filtered by the fraction of reads supporting the alternate allele, the presence of codon changes, and visual inspection in a genome browser. The summary statistics are reported in Table S1.

**Fluorescence Microscopy.** All yeast strains were cultured in either YES or EMMS, if the desired protein was induced by the nmt1 promoter. Cells were imaged after reaching log phase. Hoechst staining was conducted at a concentration of 1 μg/mL in water for 15 min. Images were collected on a Zeiss Axio Observer Z1 microscope by using a 100x oil-immersion objective. HeLa cells were fixed in −20 °C methanol for 10 min. The primary antibodies used for immunodetection were rabbit α-IST1/CHMP8 (46), α-LEMD2 (HPA017340; Sigma), rat α-tubulin (YL12; Accurate Chemical & Scientific), rabbit α-CHMP2A (UT 634; Covance), and mouse α-IST1/CHMP8 (UT 697; Covance). Full-length CHMP2A and IST1/CHMP8 protein (49) were used as antigens to produce custom antibodies by Covance Immunochemistry Services. The anti-IST1/CHMP8 antibody (UT 697) was affinity-purified (50) before use. After incubation with fluorescein labeled secondary antibodies (Thermo Fisher), coverslips were mounted by using DAPI ProLong Gold (Thermo Fisher) and imaged. For the purpose of illustration, images of anaphase B cells were acquired by spinning-disk confocal microscopy and adjusted so that cytoplasmic IST1/CHMP8 intensity was comparable between samples. Images acquired by widefield microscopy at 100x were used to score the IST1/CHMP8 and CHMP2A phenotypes after minimal adjustment that was applied uniformly. For each graph, IST1/CHMP8 and CHMP2A localization to anaphase B chromosomes were assessed in three independent experiments. Each chromatin disk (two per cell) was scored as having robust, weak, or no recruitment for IST1/CHMP8 or CHMP2A. Robust recruitment was characterized by distinctive foci organized at chromatin masses, whereas weak recruitment was characterized by less intense, often fewer, and less organized foci at the chromatin surface. Images of anaphase B cells from all treatments and experiments were randomized and quantified blindly by three scorers. The majority score was used in cases where the three scores differed. In an analogous set of experiments, anaphase B cells from all treatments were scored blindly for the absence or presence of chromatin-associated LEM2. As a positive control, anaphase B cells were scored, in parallel, as having robust, weak, or no IST1/CHMP8 recruitment.

**Time-Lapse Light Microscopy Analysis.** WT and vps4Δ cells were grown in YES at 32 °C for 8 h and loaded into the CellASIC ONIX Microfluidic system (catalog no. EVE262, EMD Millipore), which immobilizes the cells in a single focal plane, maintains a constant temperature (32 °C), and pumps fresh medium over the cells. Images from multiple positions per chamber were captured every 10 min for 16 h. A lens heater was used to maintain constant temperature inside the chamber. Images were collected with an Andor Clara CCD camera attached to a Nikon Ti-E microscope using a 60× Plan Apo Lambda 5 NA 1.4 lens. The samples were illuminated with a Lumencor Sola LED at 20% intensity, which was further reduced by the insertion of an ND8 filter. Exposure times ranged between 1 and 3 s for both YFP and mCherry channels. Five Z plane images separated by 1 μm were collected. Maximum intensity projection images were created to follow the Cut12p-YFP signals within a given cell. For Ish1p-mCherry signals, only the Z plane that bisected the nucleus was chosen for analysis.

For time-lapse colocalization experiments in HeLa cells, cells were plated on fibronectin-coated Mat-Tek dishes and incubated overnight. Cells were then transiently cotransfected with pCMV(Δ5)-GFP-CHMP7 and pCMV(Δ5)-LEMD2-mCherry using Lipofectamine 3000 (Thermo Fisher) to coexpress GFP-CHMP7 and LEM2-mCherry under attenuated CMV promoters (47). For siRNA depletion and GFP-CHMP7 expression experiments, HeLa cells, either parental or stably expressing H2B-mCherry, were plated on fibronectin-coated Mat-Tek dishes in the presence of siRNA (siControl, siLEM2-1, or siLEM2-2, as described below). After 24 h, pCMV(Δ5)-GFP-CHMP7 was delivered by transient transfection with Lipofectamine 3000. In all live imaging experiments, cells were transiently transfected for 24 h before being arrested at G1/S and released, as described below. Twelve hours after release, cells were live-imaged by spinning disk confocal microscopy.

**Quantification of Nuclear Enrichment of NLS-GFP-LacZ.** Fluorescence microscopy of fixation yeast was performed as described above by using 0.2-s exposures for five Z-sections separated by 0.29-μm steps. Integrated pixel intensities for GFP were measured within a 100 × 100-pixel square box at both the center of nucleus and cytoplasm near the pole of the cell. The average background pixel intensity was also measured from cell-free regions of the image and, this value was subtracted from both the nuclear sum and the cytoplasmic sum. The fold nuclear GFP enrichment was calculated as the ratio of nuclear/cytoplasmic integrated intensity. A minimum of 150 cells were quantified for each genotype.

**Quantification of CHMP7 Recruitment During Anaphase.** Live-cell fluorescence microscopy was performed as described above. Images of cells from 1 min before cleavage furrow ingression were selected for scoring. The “Find Maxima” function with variable noise tolerance values, as implemented in ImageJ, was used to identify CHMP7 foci around the contour of chromatin disks in each cell. The absolute number of those foci were recorded and scored blindly between siControl and siLEM2.

**Electron Microscopy.** Yeast strains were grown to log phase and harvested by using gentle vacuum filtration on a filter paper. The cell pellet was scraped from the filter, mixed with cryoprotectant (20% [wt/vol] BSA in PBS), transferred to the well of a 100-μm specimen carrier (type A), and covered with the flat side of a type B specimen carrier (51). The loaded specimens...
were immediately frozen with a high-pressure freezer (EM-HPM100; Leica Microsystems). Frozen cell were transferred for freeze substitution (FS) to a preheated 55 °C (Leica Microsystems) and processed in 75% ethanol, followed by 100% ethanol for 8 h, and polymerized at 60 °C for 48 h. Ultrathin sections (70 nm) were cut using an ultramicrotome (Leica Microsystems) and transferred to formvar- and carbon-coated copper grids (Electron Microscopy Sciences; FC-200-Cu) and poststained with 3% (wt/vol) uranyl acetate. The grids were carbon-coated to enhance stability in the electron beam.

Electron Tomography. Blocks of embedded WT and vps4Δ S. pombe cells were trimmed to 100 × 100 × 100 μm. Serial tomographic sections were cut using a UC6 ultramicrotome (Leica Microsystems) by using a diamond knife (Diatome Ltd.) and ribbons of 10–20 sections were placed on formvar-coated copper grids (Electron Microscopy Sciences) for 24 h. Four sections were recorded at intervals of 3° and 6°, and images were recorded at 1° intervals. The number of serial semithick (400-nm) sections were placed on surfaces of the sections to serve as fiducial markers for subsequent tomographic image alignment, and the grids were carbon-coated to enhance stability in the electron beam. The number of serial sections were trimmed to 20 sections placed on Formvar-coated, carbon-coated grids (Electron Microscopy Sciences) and recorded with 3% (wt/vol) uranyl acetate and Lead citrate. The sections were viewed with a JEOL-1400 Plus transmission electron microscope (JEOL, Ltd.) at 120 kV and images collected on a Gatan Ultramat CCD (Gatan, Inc.).

siRNA-Mediated Depletion and Cell-Cycle Synchronization. HeLa cells were plated in six-well dishes and subjected to similar conditions as above. Cell pellets were resuspended in lysis buffer supplemented with lysozyme and protease inhibitors and eluted in four steps (1 CV per step) with wash buffer supplemented with increasing imidazole concentrations (50, 150, 250, and 400 mM). The eluted protein was dialyzed against gel filtration buffer (50 mM Tris, pH 8.0, 150 mM KCl, 1 mM DTT, 5% (wt/vol) glycerol). Ulp1 protease (0.75 mg per 30 mL) was added to the dialysis bag to remove the affinity tag, and the dialysis reaction was performed overnight. The cleaved protein was incubated with 2 mL of Ni-NTA agarose beads (Qiagen) to remove His6-Sumo and Ni-NTA agarose binding contaminants. The eluate was concentrated to 5 mL with a Vivaspin 20 (30,000 nominal molecular weight cutoff (MWCO), polyethersulfone (PES) membrane). Monomeric Chmp7 was isolated by gel filtration chromatography. Monomeric Chmp7 was concentrated to 24–42 μM by using a Vivaspin 20 concentrator (30,000 nominal MWCO, PES membrane), aliquoted, and snap-frozen in liquid N2. For binding experiments, the protein was thawed on ice and cleared from aggregations by centrifugation at 20 min at 98,600 × g, using a TLA-55 rotor (Beckman Coulter). Yields were 300–500 μg of protein per 1L of bacterial culture.

Protein Expression and Purification. We individually expressed full-length human Chmp7 (Uniprot ID Q8VUX9) and the terminal domains of LEM2, encoded by LEM2 (NTD 1–208, CTD 395–530; Uniprot ID Q8BCN6) with a N-terminal His6-Sumo affinity tag by using a pC5A28 vector (WISP08–174; DNASU Plasmid Repository) in BL21(DDE)-RPL Escherichia coli. The cells were grown in autoinduction medium ZYP-5052 (1.5 L cultures each). The cells were grown at 37 °C to OD 0.8 with vigorous shaking in baffled flasks, moved to 19 °C and grown for an additional 19 h. Cells were then harvested by centrifugation, and bacterial pellets were snap-frozen in liquid nitrogen. Subsequent purification steps were performed at 4 °C. Cells were thawed and lysed for 30 min with lysosome in 2.5 times the pellet volume of lysis buffer followed by sonication. The supernatant was clarified by centrifugation (40,000 × g, 45 min).

**CHMP7 purification.** A total of 20–30 g of bacterial pellet were lysed (50 mM Tris, pH 8.0, 1 M NaCl, 20 mM imidazole, 10% (wt/vol) glycerol, 5 mM beta-mercaptoethanol (BME), 0.1% Triton X-100, 2 μg/mL DNase1, and protease inhibitors (84 μM leupeptin, 0.3 μM aprotinin, 1 μM pepstatin, 100 μM phenylmethylsulfonyl fluoride (PMSF)), and incubated with Ni-NTA agarose beads (Qiagen) for 2 h. The bound protein was washed with 20 column volumes (CVs) of lysis buffer without lysosome and protease inhibitors, 20 CVs of wash buffer (50 mM Tris (pH 8.0), 1 M NaCl, 20 mM imidazole, 5% (wt/vol) glycerol, 5 mM BME, 0.009% Triton X-100) and eluted in four steps (1 CV per step) with wash buffer supplemented with increasing imidazole concentrations (50, 150, 250, and 400 mM). The eluted protein was dialyzed against gel filtration buffer (50 mM Tris, pH 8.0, 150 mM KCl, 1 mM DTT, 5% (wt/vol) glycerol). Ulp1 protease (0.75 mg per 30 mL) was added to the dialysis bag to remove the affinity tag, and the dialysis reaction was performed overnight. The cleaved protein was incubated with 2 mL of Ni-NTA agarose beads (Qiagen) to remove His6-Sumo and Ni-NTA agarose binding contaminants. The eluate was concentrated to 5 mL with a Vivaspin 20 (30,000 nominal molecular weight cutoff (MWCO), polyethersulfone (PES) membrane). Monomeric Chmp7 was isolated by gel filtration chromatography. Monomeric Chmp7 was concentrated to 24–42 μM by using a Vivaspin 20 concentrator (30,000 nominal MWCO, PES membrane), aliquoted, and snap-frozen in liquid N2. For binding experiments, the protein was thawed on ice and cleared from aggregations by centrifugation at 20 min at 98,600 × g, using a TLA-55 rotor (Beckman Coulter). Yields were 300–500 μg of protein per 1 L of bacterial culture.

**LE2174 purification.** LEM2 (HPA017340; Sigma-Aldrich), Hsp90-SUMO, and vps4Δ S. pombe cells were snap-frozen in liquid N2. Yields were 2.5 mg of protein per 1 L of bacterial culture. Protein Binding Experiments. Binding experiments were performed at room temperature by using 40 μL of Ni-NTA agarose beads (Qiagen) in 1 mL centrifuge columns (Pierce). Proteins were mixed and incubated for 1 h. For control reactions, proteins were incubated with corresponding buffers to mimic binding conditions. The protein reactions were added to the beads, which were equilibrated with binding buffer (25 mM Tris, pH 7.0, 150 mM NaCl, 20 mM imidazole, 10% glycerol). Protein binding experiments were performed at 4 °C. Cells were then harvested by centrifugation, and bacterial pellets were snap-frozen in liquid nitrogen. Cell pellets were resuspended in lysis buffer supplemented with increasing imidazole concentrations (50, 150, and 400 mM). The eluted protein was dialyzed against gel filtration buffer (50 mM Tris, pH 8.0, 150 mM KCl, 1 mM DTT, 5% (wt/vol) glycerol) and incubated with Ni-NTA agarose beads (Qiagen) for 2 h. The bound protein was washed with 20 CVs of lysis buffer without lysosome and protease inhibitors, 20 CVs of wash buffer (50 mM Tris (pH 8.0), 1 M NaCl, 20 mM imidazole, 5% (wt/vol) glycerol, 5 mM BME, and 0.009% Triton X-100) and eluted in four steps (1 CV per step) with wash buffer supplemented with increasing imidazole concentrations (50, 150, 250, and 400 mM). The eluted protein was dialyzed against gel filtration buffer (50 mM Tris, pH 8.0, 150 mM KCl, 1 mM DTT, 5% (wt/vol) glycerol). Ulp1 protease (0.75 mg per 30 mL) was added to the dialysis bag to remove the affinity tag, and the dialysis reaction was performed overnight. The cleaved protein was incubated with 2 mL of Ni-NTA agarose beads (Qiagen) to remove His6-Sumo and Ni-NTA agarose binding contaminants. The eluate was concentrated to 5 mL with a Vivaspin 20 (10,000 KDa MWCO, PES), and monomeric His-Sumo-LE2174 (NTD) was isolated by gel filtration chromatography. The eluate was concentrated to 5 mL with a Vivaspin 20 (10,000 KDa MWCO, PES), and monomeric His-Sumo-LE2174 (NTD) was isolated by gel filtration chromatography. Monomeric CHMP7 was concentrated to 24–42 μM by using a Vivaspin 20 concentrator (30,000 nominal MWCO, PES membrane), aliquoted, and snap-frozen in liquid N2. For binding experiments, the protein was thawed on ice and cleared from aggregations by centrifugation at 20 min at 98,600 × g, using a TLA-55 rotor (Beckman Coulter). Yields were 300–500 μg of protein per 1 L of bacterial culture.

**LE2174(CTD) purification.** Cells were suspended in buffer with 50 mM Tris (pH 8.0), 150 mM KCl, and 1 mM DTT plus protease Inhibitor mixture and were lysed by freeze–thaw cycles, and the supernatant was harvested after 16,350 × g for 45 min. The lysate was incubated with Ni-beads (Qiagen) for 45 min, washed with 20 mM imidazole, and eluted with 750 mM imidazole. The eluted protein was dialyzed against gel–filtration buffer (50 mM Tris, pH 8.0, 150 mM KCl, and 1 mM DTT) and applied to 120 mL of HiloPure Super75 PGH (GE Healthcare). Fractions containing pure LEM2-CTD were collected and snap-frozen in liquid nitrogen. Yields were ~2.5 mg of protein per 1 L of bacterial culture.
KCl, and 20 mM imidazole) and incubated for an additional 45 min. The resin was washed with 20 CVs of binding buffer. Excess His-Sumo-tagged-LEM2 (CTD or NTD) was eluted with 3 CVs of binding buffer supplemented with 150 mM imidazole. Note that the majority of LEM2 and bound CHMP7 did not elute at this step. Protein remaining on beads was eluted with two CVs of 2× SDS sample buffer and the eluate was analyzed with SDS/PAGE.

Note. While our study was under review, corroborative evidence for these roles for CHMP7 in human cells (46) and LEM domain family proteins in budding yeast were also published by others (59).

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