ESCRT-III controls nuclear envelope reformation

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During telophase, the nuclear envelope (NE) reforms around daughter nuclei to ensure proper segregation of nuclear and cytoplasmic contents1-4. NE reformation requires the coating of chromatin by membrane derived from the endoplasmic reticulum, and a subsequent annular fusion step to ensure that the formed envelope is sealed5-13. How annular fusion is accomplished is unknown, but it is thought to involve the p97 AAA-ATPase complex and bears a topological equivalence to the membrane fusion event that occurs during the abscission phase of cytokinesis14. Here we show that the endosomal sorting complex required for transport-III (ESCRT-III) machinery localizes to sites of annular fusion in the forming NE in human cells, and is necessary for proper post-mitotic nuclear-cytoplasmic compartmentalization. The ESCRT-III component charged multivesicular body protein 2A (CHMP2A) is directed to the forming NE through binding to CHMP4B, and provides an activity essential for NE reformation. Localization also requires the p97 complex member ubiquitin fusion and degradation 1 (UFD1).

Our results describe a novel role for the ESCRT machinery in cell division and demonstrate a conservation of the machineries involved in topologically equivalent mitotic membrane remodelling events. The ESCRT-III complex performs a topologically unique membrane fusion, allowing the release of enveloped retroviruses during viral budding, intraluminal vesicles during multivesicular body biogenesis, and daughter cells during the abscission phase of cytokinesis15,16. We found that as well as localizing to the midbody during late cytokinesis, endogenous ESCRT-III components CHMP2A and CHMP2B encircled the forming daughter nuclei during telophase (Fig. 1a, b and Extended Data Fig. 1a). CHMP2A localization was sensitive to CHMP2A-targeting short interfering RNA (siRNA; Extended Data Fig. 1b) and was not continuous; instead we found that CHMP2A adopted a transient punctate localization around the decondensing nuclei during telophase (Extended Data Fig. 1c and Supplementary Video 1). By scoring localization in HeLa cells stably expressing mCherry–tubulin (cell cycle of 21.5 ± 1.7 h (mean ± s.d.), n = 93), we estimate the duration of CHMP2A localization to be 96 ± 8.9 s. We found cells expressing green fluorescent protein (GFP)-tagged CHMP4B (ref. 12) also displayed a transient, punctate, juxta-nuclear localization during telophase with recruitment of GFP–CHMP4B lasting 225 ± 66 s (n = 8) and individual puncta lasting 75 ± 46 s (n = 92; Extended Data Fig. 1d and Supplementary Video 2). Telophase ESCRT-III localization was observed in other cell lines, including human diploid fibroblasts (Extended Data Fig. 1e). Using HeLa cells stably expressing a yellow fluorescent protein (YFP)-tagged nuclear envelope marker (lamin associated protein 2B, YFP–LAP2B)17, we determined that the juxta-nuclear localization corresponded to the forming nuclear envelope. Here, we observed colocalization with the lamin B receptor (LBR)18 (Fig. 1c) and demonstrated that CHMP2A localization occurred before appreciable formation of a nuclear lamina or nuclear pore complexes (Extended Data Fig. 1f, g). While mitotic chromatin association of ESCRT-III has been previously reported19, its function remains unknown. To investigate the role of ESCRT components at the NE, we used siRNA to deplete these proteins19. As described previously, depletion of ESCRT components produced aberrant nuclei, and these defects phenocopied those produced by depletion of proteins required for NE reformation18 (Extended Data Fig. 1h). NE reformation is thought to be a two-phase process, separable into membrane fusion events that create an expanding reticular network with subsequent annular fusion of holes within this network to create a sealed barrier20.

We next used correlative light-electron microscopy (Extended Data Fig. 2a–d) to examine telophase ESCRT-III NE localization. We found that at the stage of ESCRT-III recruitment, the NE had incompletely formed (Fig. 1d). Two populations of CHMP2A-positive membranes were found. First, isolated CHMP2A-decorated vesicles were observed in the cytoplasm, proximal to the forming NE (5.7 ± 4.2% of total cellular gold, Extended Data Fig. 2e, i). Second, CHMP2A-decorated double-membrane sheets were observed to coat the chromat (51 ± 1.7% of total cellular gold was within 100 nm of the NE). On these sheets, CHMP2A localized to discrete regions, with intact NE being devoid of label, but with CHMP2A preferentially (Extended Data Fig. 2h) decorating nucleo-cytoplasmic channels (mean diameter 38.4 ± 12.5 nm (± s.e.m.), n = 2 from 17 determinations) between the forming double membranes of the NE (Fig. 1d, Extended Data Figs 2d–g and 3a–d and Supplementary Videos 3 and 4). These channels must be resolved through annular fusion, and given the observed localization and topological equivalence with cytokinetic abscission (Fig. 1e), we speculated that ESCRT-III might be involved in this process.

Requirements for CHMP2A localization to the telophase NE were revealed through depletion of partner ESCRT proteins, with CHMP4B and CHMP3, as for other ESCRT-dependent membrane remodelling events, having a major role in this recruitment (Fig. 2a). We used siRNA-resistant Flag-tagged CHMP2A expressed at near-endogenous levels to report localization in the presence of CHMP2A siRNA (Fig. 2b, c). Through introduction of mutations targeting known binding partners, we found, as for midbody recruitment and cytokinetic abscission (Extended Data Fig. 4a, b), and consistent with the previously determined telophase localization of GFP–CHMP4B (Extended Data Fig. 1d), that while CHMP2AΔN–Flag localized to the forming NE, disrupting interaction with CHMP4 proteins by mutation of Arg24, Arg27 and Arg31 to Ala (CHMP2AΔN–Flag(RRR/AAA)) abolished this localization. Mutation of the amino-terminal CHMP2A α-helix19, or residues involved in the interaction with VPS4 (ref. 16) had no effect on NE localization (Fig. 2c and Extended Data Fig. 4a). These data indicate that CHMP2A is recruited to the forming NE through classical assembly of the ESCRT-III complex.

The p97 AAA-ATPase controls both phases of NE reformation; together with its adaptor protein p47, it regulates membrane delivery and NE expansion, whereas through its adaptors nuclear protein localization 4 (NPL4) and UFD1 it regulates annular fusion10. Through NPL4 and UFD1, the p97 complex extracts ubiquitinated aurora-B, a chromosomal passenger complex component, from chromatin to allow chromatin decondensation and membranation20,21. Given our observed ESCRT-III localization (Fig. 1) and known interactions of ESCRT-III components with the chromosomal passenger complex15, we screened the ESCRT machinery for interaction with the p97 AAA-ATPase complex.
complex by yeast two-hybrid assay (Extended Data Fig. 5a–d). We found that CHMP2A bound specifically to UFD1 and confirmed this interaction by direct binding and co-precipitation assays (Fig. 3a, b). To explore the integrity of the nascent NE in CHMP2A-depleted cells, we followed a protocol similar to that recently described and imaged synchronized cultures of cells stably expressing mCherry–tubulin and the indicated CHMP2AR–Flag with anti-Flag, to the midbody (Fig. 3d), recruitment of CHMP2A to the forming NE and the topological equivalence between annular fusion of the NE and ESCRT-III-dependent membrane fusion events. MVB, multivesicular body.

**Figure 1** | ESCRT-III localizes to the forming nuclear envelope. a, HeLa cells stained with anti-tubulin, either anti-CHMP2A or anti-CHMP2B, and 4', 6-diamidino-2-phenylindole (DAPI). Scale bars, 10 μm. Images representative of three acquired images in each case. b, Quantification of juxta-nuclear CHMP2A localization during mitosis from a, quantification from 20 cells in interphase, prophase, pro-metaphase and metaphase, 23 cells in anaphase, 24 cells in telophase, 36 cells in early cytokinesis and 20 cells in late cytokinesis. c, HeLa cells stained with DAPI, anti-CHMP2A or anti-CHMP2B, and either stably expressing YFP–LAP2β or stained with anti-LBR. Arrows indicate regions of colocalization. Scale bars, 10 μm. Images representative of two (anti-CHMP2B and YFP–LAP2β, anti-LBR and anti-CHMP2A) or four (anti-CHMP2A and YFP–LAP2β) acquired images. d, Tomographic slices of HeLa cells stained with fluoronanogold anti-CHMP2A. Correlation depicted in Extended Data Fig. 2a–c; arrow indicates nucleo-cytoplasmic channel. Scale bars, 200 nm. Images representative of 25 gold-decorated nucleo-cytoplasmic channels and quantified in Extended Data Fig. 2e. e, Schematic depicting topological equivalence between annular fusion of the NE and ESCRT-III-dependent membrane fusion events. MVB, multivesicular body.

was required for EGFR degradation\(^1\), we found cells depleted for UFD1 degraded EGFR normally (Extended Data Fig. 6b), allowed release of HIV-1-based lentivirus (Extended Data Fig. 6c), and completed cytokinesis normally as previously reported\(^2\) (Extended Data Fig. 6d). However, while cells depleted for UFD1 recruited CHMP2A to the midbody (Fig. 3d), recruitment of CHMP2A to the forming NE was impaired (Fig. 3c, d).

To examine mitotic roles for ESCRT-III in NE reformation, we imaged synchronized cultures of cells stably expressing both histone-2B–mCherry (H2B–mCh) and YFP–LAP2β, and quantified the time taken to enclose the chromatin with YFP–LAP2β-positive NE. We were surprised to find that cells lacking ESCRT-III, but not UFD1, enclosed their chromatin faster than control cells (Extended Data Fig. 7a–c). To explore the integrity of the nascent NE in CHMP2A-depleted cells, we followed a protocol similar to that recently described and imaged synchronized cultures of HeLa cells stably expressing both H2B–mCh and GFP-tagged β-galactosidase (βGal) fused to the nuclear localization signal (NLS) from Simian virus 40 (GFP–NLS–βGal)\(^2\). GFP–NLS–βGal is released from the nucleus after NE breakdown at mitotic onset, and returned after formation of transport-competent nuclear pores during NE reformation (Extended Data Fig. 8a, b). We found that the rate of GFP–NLS–βGal return to the nucleus was slower in ESCRT-III-depleted cells.
(Fig. 4a–c), despite the cells having enclosed their chromatin with NE membranes faster (Extended Data Fig. 7a). While nuclei were frequently malformed in ESCRT-III-depleted cells (Extended Data Fig. 1h), incorporation of nuclear pore complexes and import machineries were normal (Extended Data Fig. 8c–e). However, in CHMP2A−, CHMP3− or UFD1-depleted cells, the post-mitotic nucleo-cytoplasmic partitioning of GFP–NLS–βGal was reduced (Fig. 4b, c and Extended Data Fig. 9a, b), indicating that NE integrity

Figure 4 | ESCRT-III depletion disrupts nuclear envelope integrity. a, Timelapse analysis of NE sealing in siRNA-transfected HeLa cells stably expressing H2B–mCh and GFP–NLS–βGal. GFP signal presented according to pseudocolour scale at the indicated time points. Scale bars, 10 μm. A single image was pseudocoloured for demonstrative purposes. b, Quantification of NE sealing from siRNA-treated cells in a (cells were quantified at each time point; control, 140 cells from 7 independent experiments; CHMP2A−, 98 cells from 5 independent experiments, P = 0.047; CHMP2A÷, 80 cells from 4 independent experiments, P = 0.023; CHMP2A÷ + CHMP2B, 60 cells from 3 independent experiments, P = 0.006; CHMP3, 34 cells from 3 independent experiments, P = 0.002. All values are mean ± s.e.m.; two-tailed Student’s t-test used to assess significance after 85 min). c, Western blotting of cell lysates from b with anti-CHMP2A, anti-CHMP2B, anti-CHMP3 or anti-GAPDH antisera. d, Z-slices extracted from a correlative tomographic reconstruction of the NE at 60 min after anaphase onset from the indicated siRNA-transfected mCherry–tubulin HeLa cells. The numbered circles correspond to discontinuities labelled in the 3D reconstructions shown in Extended Data Fig. 10a. Scale bars, 200 nm. Images representative of 6 (control) and 12 (CHMP2A−/siRNA) tomographic reconstructions. e, The percentage of discontinuities smaller than 65 nm was scored. Discontinuities in this range that were not nuclear pore complexes (NPCs) as a percentage of total discontinuities (including NPCs) for n number of reconstructed tomograms: control, 9.4 ± 3.0, n = 6; CHMP2A−, 29.9 ± 4.7, P = 0.01, n = 12; CHMP2A÷, 28.3 ± 2.0, P = 0.021, n = 2. The increase in the percentage of non-NPC discontinuities was assessed by two-tailed Student’s t-test (average diameter of non-NPC discontinuities was 38 ± 22 nm (CHMP2A−) and 58 ± 19 nm (CHMP2A÷)). f, Western blotting of lysates from siRNA-treated HeLa cells stably expressing H2B–mCh, GFP–NLS–βGal and siRNA-resistant CHMP2A−/Flag with anti-CHMP2A, anti-Flag or anti-GAPDH antisera. g, Quantification of NE sealing from cells treated with siRNA as in f and imaged from 4 independent experiments (mean nuclear-cytoplasmic ratio given 85 min after anaphase onset ± s.d., two-tailed Student’s t-test was used to assess significance across 4 independent experiments (*); control, 8.9 ± 3.1, n = 174; CHMP2A siRNA, 5.4 ± 2.6, n = 171, P = 0.0006; CHMP2A siRNA + CHMP2A−/Flag, 8.4 ± 3.3, n = 132, not-significant; CHMP2A siRNA + CHMP2A−/RRR/AAA−/Flag, 5.4 ± 2.2, n = 196, P = 0.0001).
was compromised by treatments that prevent ESCRT-III assembly at the NE. Results were confirmed with a second reporter (GFP–NLS) (Extended Data Fig. 9c) and we demonstrated that nuclear retention of this probe was defective in post-mitotic ESCRT-III-depleted cells (Extended Data Fig. 9d, e). Using correlative live-cell electron tomography, we found that CHMP2A depletion resulted in the persistence of unsealed holes in the post-mitotic NE (Fig. 4d, e and Extended Data Fig. 10a, b). Paralleling CHMP2 requirements in lentiviral release and cytokinetic abscission (Extended Data Figs 6c and 9f), depletion of CHMP2B had minimal effect on NE integrity (Extended Data Fig. 9a, b), while co-depletion of CHMP2A and CHMP2B disrupted NE integrity to a greater extent than CHMP2A depletion alone (Fig. 4b). NE integrity could be rescued by stable expression of siRNA-resistant CHMP2A–Flag (CHMP2A–R–Flag), but, as with CHMP2A requirements in cytokinesis (Extended Data Fig. 4b) and HIV-1 release16, not by expression of CHMP2A–R–Flag(RRR/AAA) (Fig. 4f, g). We describe a novel localization and function of ESCRT-III in NE remodelling at sites of anuclear fusion, a process markedly similar to classical ESCRT-III-mediated membrane remodelling (Extended Data Fig. 10c). Localization is governed by classical ESCRT-III assembly mechanisms and also requires UF1D. An equivalent ESCRT-III-dependent membrane remodelling at the NE may allow viruses or megaRNP to traverse this membrane23–27, and in yeast, ESCRT-III has recently been shown to participate in surveillance and extraction of defective nucleoporins at the inner nuclear membrane28, indicating additional ESCRT-III activities on this membrane may exist throughout the cell cycle. ESCRT-III is thus involved in regulating the quality of the NE, and gene expansion within the ESCRT machinery may have resulted from an evolutionary drive to accommodate open mitoses.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper. References unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions J.G.C. conceived the study. P.V., L.H. and J.M. designed, performed and analysed electron microscopy experiments. J.G.C. and Y.O. conceived the study. P.V., L.H. and J.M. designed, performed and analysed electron microscopy experiments. J.G.C. and Y.O. designed, performed and analysed data from other experiments. J.G.C. wrote the manuscript with assistance from all other authors.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to J.G.C. (jeremy.carlton@kcl.ac.uk).
METHODS

Cell culture. HeLa and 293T cells were gifts from J. Martin-Serrano and were cultured in DMEM containing 10% FBS, penicillin (100 U ml\(^{-1}\)) and streptomycin (1 mg ml\(^{-1}\)). GP2-293 cells were obtained from Clontech and were cultured similarly. BJ fibroblasts were obtained from the ATCC and cultured in 4:1 DMEM:199 Media, supplemented with 15% FCS, penicillin (100 U ml\(^{-1}\)) and streptomycin (1 mg ml\(^{-1}\)). Stable cell lines were generated by transduction using MLV-based retroviruses as described previously\(^{29}\), and selected using Puromycin (200 \(\mu\)g ml\(^{-1}\)).

Plasmids. Plasmids encoding TSG101 (MQ-017918-03-0002), CHMP1A, CHMP2A, CHMP2B, CHMP3, CHMP4A, CHMP4B, CHMPC, CHMP5, VP4A, LIPS (VTA1), Ubpy, Cepps5, TAL, LAP2B (TMPO) and AL2G were gifts from J. Martin-Serrano and have been described previously\(^{20,32,23}\). Coding sequences for p73 (also known as VCP), p47 (NSFL1C), NPLA (NPLOCA), UFDI, CHMP7, VP4B and SPARTIN (SP2G) were amplified from IMAGE clones (6502535, 3635947, 5017718, 3507963, 5551762, 6042862 and 5313378), respectively, and were cloned into mammalian expression plasmids (pCR3.1-YFP) and yeast two-hybrid plasmids (pHB18 and pGBKT7). A plasmid encoding HD-PTP was a gift from P. Woodman and was cloned similarly. siRNA-encoding HD-PTP plasmids were obtained from T. Stamminger via Addgene and were subcloned into pLHCX containing a HindIII/MluI/SalI/XhoI/NotI/HpaI/BamHI/NsiI/ClaI cloning site. CHMP2A was cloned with 5' EcoRI and 3' NotI silent mutations into the pCAGGS-GST-EcoRI-NotI-XhoI (a gift from J. Martin-Serrano). A SnaBI/NotI fragment from pH2B-mCherry-IRES-Neo3 (a gift from U. Wiesmann) was inserted into pLHCX-MCS retroviral packaging vectors to create pLHCX-MCS-retroviral packaging vectors that were transfected with pNG72 or pLHCX-MCS retroviral packaging vectors were transfected with pCMV8.91, pVSVG and with pLVXP-GFP-NLS-luciferase (20) ng of viral supernatant were collected from 293T supernatants by filtration (0.45 \(\mu\)m) and centrifugation through a 20% sucrose (21,000 g, 120 min), lysed, resolved by SDS–PAGE and examined by western blotting. Additionally, HeLa cells were infected with 50 \(\mu\)l of viral supernatant and GFP-expression in these cells was measured by western blotting. Virion release was released by quantifying Gag\(^{\text{trunc}}\)/Gag\(^{\text{full}}\) as determined by densitometry using ImageJ.

Production of recombinant proteins. BL21(DE3) Escherichia coli-expressing plasmids encoding GST-tagged or His-tagged proteins were cultured in bacterial lysis buffer (20 mM Hepes, pH 7.4, 500 mM NaCl, 5 mM EDTA, 5% glycerol, 1% Triton X-100, a protease inhibitor mixture (complete mini-EDTA-free, Roche). Purified recombinant GST or His-tagged proteins were dialyzed against 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 5% glycerol, 0.1% Triton X-100. Bound proteins were recovered in laemmli sample buffer, resolved by SDS–PAGE and examined by western blotting.

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Antibodies. Antibodies against HSP90 (H114) were from Santa Cruz Biotechnology, TSG101 (T5071) was from Sigma, GAPDH (MAB374) was from Millipore, tubulin (DM1A) was from Sigma, CHMP2A (104771-AP) was from Proteintech, CHMP2B (ab33174) was from Abcam, CHMP4B (sc82556) was from Santa Cruz, CHMP3 (sc67228) was from Santa Cruz, UF1D (106151-AP) was from Proteintech, anti-p24 Gag (H18-132-S2) was from the NIH AIDS Research and Reference Reagent Program, EGF (2232) was from Cell Signaling Technology, GFA (7.1.3.13) was from Roche. LBR (NM_000578.3) was from Sigma, Lamin A/C (MAB3538) was from Millipore, mAb414 was from Covance, DYKDDDDK-Tag (Flag) was from Cell Signaling Technology. Alexa-conjugated secondary antibodies were from Invitrogen and horseradish peroxidase (HRP)-conjugated secondary antibodies were from Millipore.

SDS–PAGE and western blotting. Cell lysates were denatured in Laemmli buffer and resolved using SDS–PAGE gels. Resolved proteins were transferred onto nitrocellulose by western blotting and were probed with the indicated antiserum in 5% milk. HRP-conjugated secondary antibodies were incubated with ECL. Primeshine chemiluminescent substrate (GE Healthcare) and visualized by exposure to autoradiography film.

Transient transfection of cDNA. HeLa cells were transfected using Lipofectamine-2000 (Life Technologies) according to the manufacturer’s instructions. GP2-293 and HeLa cells were transfected using linear 25-kDa polyethyleneimine (P.E, Polysciences, Inc.)

siRNA transfections. Cells were seeded at a density of 1 \(\times\) 10\(^5\) cells per ml (HeLa, BJ) or 2.6 \(\times\) 10\(^5\) cells per ml (293T) and were transfected with siRNA at 100 nM, 2 h after plating using Dharmafect-1 (Dharmacon). To minimize toxicity associated with CHMP2A and UF1D depletion, single transfections were performed for 72 h.

The following target sequences have already been demonstrated to achieve potent and specific suppression of the targeted CHMP were used: control, Dharmacon non-targeting control D-001810-01. CHMP1A-1: ACGCGAGGAUCAUGGAUAUdTdT\(^{17}\); CHMP2A-2: AAGAUGAGAGGGAGAGAGAdTdT\(^{17}\); CHMP2B: UGGGACACUUAGAAGAAAdTdT\(^{17}\); CHMP3: GAAAGAAGCAGAAUAAAdTdT; CHMP4A: Q-SI04268845 (ref. 13); CHMP4B: Q-SI03225199 (ref. 13); CHMP6C: CAAAGGAGGCGGAGAAAdTdT\(^{17}\); UF1D-1: GAGGGACAUUGCGCGGCUAdTdT; UF1D-2: MQ-017918-03-0002; UF1D-3: GUGGCCACCUACUCCAAAdTdT; EM4: GAGAAGAAGCGUGAUAAdTdT. UF1D-2 was excluded from such analysis owing to toxicity and morphological changes specific to this oligonucleotide.

Yeast two-hybrid assays. Yeast Y190 cells were co-transformed with plasmids encoding the indicated proteins fused to the VP16 activation domain (pPH18) or the Gal4 DNA-binding domain (pGBK7). Co-transformants were selected on SD-Leu-Trp agar for 3 days at 30°C, collected, and LacZ activity was measured using a liquid β-galactosidase assay using chlorphenolred-β-galactopyranoside (Roche) as a substrate. Average β-galactosidase activities are presented.

Lentiviral release. 293T cells were transfected with siRNA as described above, except that the second transfection contained additionally 300 ng of HIV-1 pCMVd8.91 (a gift from T. Ng), 100 ng of pLenti-SEW (a packaging vector encoding GFP, a gift from A. Ridley) and 100 ng pVSVG. After 48 h, virions were collected from 293T supernatants by filtration (0.45 \(\mu\)m) and centrifugation through 20% sucrose (21,000 g, 120 min), lysed, resolved by SDS–PAGE and examined by western blotting. Additionally, HeLa cells were infected with 50 \(\mu\)l of viral supernatant and GFP-expression in these cells was measured by western blotting. Virion release was released by quantifying Gag\(^{\text{trunc}}\)/Gag\(^{\text{full}}\) as determined by densitometry using ImageJ.
0.04% Tween-20, pH 7.4. Temperature jump and thermophoresis experiments were conducted using 100% LED illumination and 40% infrared laser power and were analysed using NanoTemp's analysis suite. Binding curves could only be generated for the CHMP2A:UFD1 interaction, affinities were calculated by the software and averaged.

**Fixed cell imaging.** Cells were imaged using Nikon Eclipse microscopes teamed with widefield (Ti-E) and confocal (A1R or Spinning Disc) imaging systems. Widefield image stacks were iteratively deconvolved using Autoquant. Images were processed in NIS Elements and exported to Photoshop for assembly. HeLa cells were fixed in methanol (for CHMP2A staining) or 4% paraformaldehyde (PFA) and subject to processing for immunofluorescence as described previously. For multinucleation and midbody arrest assays, at least 300 cells per experiment were quantified. For telophase NE localization, between 10 and 20 telophase cells per experiment were scored. For fixed cell microscopical analysis, we scanned multiple coverslips and experiments before acquiring two to three representative images for presentation in figures.

**Live cell imaging.** HeLa cells stably expressing the indicated proteins were plated in Stickyslides (Ibidi) adhered to a glass number 1 coverslip and transfected with the indicated siRNA. Cells were synchronised using a double thymidine block, and 48 h after siRNA transfection (10.5 h after release from the second thymidine block), cells were transferred to a Nikon inverted spinning disc confocal microscope with attached environmental chamber and imaged live for 4 h using a 20× dry objective and a 1.5× magnification lens. For mitotic rim formation, three coordinates per condition were selected and frames were acquired every 1 min, rim formation was scored through manual analysis of individual frames. For nuclear accumulation of GFP–NLS and GFP–NLS–βGal, frames were acquired every 1 min. The ratio of background-corrected, area-normalized, GFP-positive pixel intensities within the cytoplasm and mCh–H2B demarcated nuclei at the indicated intervals were obtained using NIS-elements. We excluded 2 out of 98 cells from CHMP2A–1 analysis, 2 out of 60 cells from UFD1–1 analysis and 2 out of 60 cells from UFD1–3 analysis as these gave anomalous N/C (nuclear/cytoplasmic) ratios >10× s.d. from the mean. For imaging of GFP–CHMP4B recruitment to the telophase NE, cells were imaged using a 100× oil-immersion objective and confocal slices were acquired every 30 s using a spinning disc confocal microscope. For analysis of nuclear retention, siRNA-treated HeLa cells stably expressing GFP–NLS and mCh–H2B were imaged live using a Nikon A1R confocal microscope. Between 1 and 2 h after anaphase onset, cells were subject to photo-ablation of cytosolic GFP–NLS signal by point bleaching and the recovery of cytoplasmic fluorescence from the nuclear pool was quantified for 10 min after bleaching. For quantification of holes remaining in the NE after CHMP2A depletion, tomograms were acquired for CLEM as described above. Discontinuities in the NE were scored as being NPC or non-NPC on the basis of cross-sectional morphology. Internal diameters of these discontinuities were measured from reconstructed tomograms using Fiji. Discontinuities were segregated by size and whether they were identifiable as NPCs or not. A threshold was set at 65 nm (>2 s.d. smaller than the measured control NPC diameter) and the percentage of discontinuities smaller than this was displayed. At least 50 discontinuities were analysed per treatment across multiple cells from the indicated number of experiments.

**Statistical analysis.** Variance was analysed using an F-test, and type-relevant two-tailed Student's t-tests were used to assess significance between test samples and controls. No statistical methods were used to predetermine sample size. The experiments were not randomized, and the investigators were not blinded to allocation during experiments and outcome assessment.

**ImageStream analysis.** siRNA-treated HeLa cells in 6-well dishes were detached, fixed in 4% PFA, permeabilized with 0.1% Triton X-100 and stained in suspension with mAb414, Alexa-594-conjugated secondary antibodies and DAPI (at 0.1 µg ml⁻¹). In-focus, single-cellular populations were acquired and a mask was applied to the DAPI channel and duplicated then dilated by three pixels to encompass the mAb414 signal surrounding the nuclei. The difference in the mAb414 signal captured by these masks was given as the nuclear envelope mAb414 and presented as a histogram. Representative images of average mAb414 intensity were extracted for presentation.

**Correlative light electron microscopy.** Around 500,000 HeLa cells were seeded in a 3.5-cm Mattek gridded dish (P35G-2-14-C-GRID). The next morning, cells were fixed in phosphate buffer containing 1% PFA for 3 min. Cells were permeabilized with 0.1% saponin in PHEM (60 mM PIPES, 25 mM HEPES, 10 mM EGTA, 2 mM MgCl₂, pH 6.9) and processed for immunofluorescence using anti-CHMP2A primary and goat anti-rabbit Alexa-594-conjugated fluorophore (Nanoprobes) and DAPI. Cells were subjected to a subsequent 10-min 3% PFA fixation and quenching before imaging on a Leica SP5 or SP8 confocal microscope.

After fluorescent imaging, cells were postfixed in glutaraldehyde, subjected to silver enhancement (Aurion RGENT SE-EM), stained with OsO₄ and uranyl acetate, dehydrated through ethanol and embedded in Epon. Blocks were trimmed to the region identified by confocal imaging and 300-nm serial sections were cut using a diamond knife. For retraction of the cells of interest, sections were imaged on a FEI Tecnai2 and subsequently double tilt series of regions of interest were acquired on a FEI Tecnai20, Tilt series were reconstructed using IMOD and selected frames and movies were extracted using ImageJ.

For quantification of holes remaining in the NE after CHMP2A depletion, tomograms were acquired by CLEM as described above. Discontinuities in the NE were scored as being NPC or non-NPC on the basis of cross-sectional morphology. Internal diameters of these discontinuities were measured from reconstructed tomograms using Fiji. Discontinuities were segregated by size and whether they were identifiable as NPCs or not. A threshold was set at 65 nm (>2 s.d. smaller than the measured control NPC diameter) and the percentage of discontinuities smaller than this was displayed. At least 50 discontinuities were analysed per treatment across multiple cells from the indicated number of experiments.

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Extended Data Figure 1 | Localization of ESCRT components during the cell cycle. a, b, Immunofluorescence analysis of HeLa cells stained with anti-tubulin, anti-CHMP2A or anti-CHMP2B and DAPI (a). Images in a are representative of two acquired images per field of view. Cells in b were treated with control or CHMP2A-targeting siRNA; images representative of four (control) or two (CHMP2A siRNA) acquired images. c, Deconvolved projections of HeLa cells stained with anti-CHMP2A and DAPI, corresponding to stills from Supplementary Video 1. Images representative of two deconvolved image series. d, HeLa cells stably expressing GFP–CHMP4B were imaged live during the anaphase to telophase transition. Telophase frames at 30-s intervals are presented, corresponding to stills from Supplementary Video 2. Images representative of four acquisitions. e, Immunofluorescence analysis of human diploid fibroblasts stained with anti-CHMP2A, anti-tubulin and DAPI, images representative of three acquired cells per cell cycle phase. f, g, Immunofluorescence analysis of HeLa cells stained with anti-CHMP2A, DAPI and either anti-mAb414 (f) or anti-LaminA/C (g), images representative of five acquired cells. Arrowheads indicate regions of formed nuclear pores or lamina as indicated. h, Quantification of abnormal nuclei (the presence of multiple lobes, micronuclei, lamina ingression or invagination) in HeLa cells transfected with the indicated siRNA and stained with anti-LaminA/C (1,300 cells over 5 experiments quantified per treatment; data are mean ± s.d.). Images representative of three (control, CHMP2A siRNA) or two (LEM4 siRNA) acquired fields of view and resolved cell lysates were examined by western blotting with anti-CHMP2A, anti-CHMP2B or anti-GAPDH antisera as indicated. Scale bars, 10 μm.
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**Extended Data Figure 2** | Correlative light and electron microscopy (CLEM) of endogenous CHMP2A localization in telophase NE. a–c. Phase-contrast (a), correlative immunofluorescence (b) and transmission electron microscopy of HeLa cells stained with anti-CHMP2A, detected by Alexa-594-fluorophore and DAPI. Boxed region in a is shown in b; boxed region in b is shown in c. In all cases, images representative of three cells prepared for CLEM. d. 3D rendering of tomographic reconstruction of forming NE from boxed region in c and Fig. 1d; a single example of a nucleo-cytoplasmic channel was selected for 3D rendering. e–g. Z-slices extracted from tomographic reconstructions of forming NE depicting CHMP2A localization to isolated vesicles (e, i) and nucleo-cytoplasmic channels (arrows in e, ii, f, g) at the indicated Z-heights. Localization of CHMP2A to nucleo-cytoplasmic channels was observed in three independent cells; data from a second cell are presented in Extended Data Fig. 3. Note CHMP2A localization to nucleo-cytoplasmic channels is distinct from nuclear pores (asterisk in f). h. Quantification of CHMP2A labelling from two independently prepared cells. Channels were defined as discontinuities up to 80 nm, and gaps were defined as discontinuities over 80 nm. Distances of the gold-particles from channels or gaps were measured on the tomograms in three-dimensions and plotted as a histogram. Most (74.4%) of the gold label was found within 150 nm of nucleo-cytoplasmic channels, and most (70.6%) of the gold label was found more than 150 nm from the larger gaps in the NE. Scale bars, 10 μm (b) and 200 nm (f, g).
Extended Data Figure 3 | CLEM of endogenous CHMP2A localization in telophase NE. a–c, Phase-contrast (a), correlative immunofluorescence (b) and transmission electron microscopy (c) of a second HeLa cell stained with anti-CHMP2A, detected by Alexa-594-fluoronanogold and DAPI. Boxed region in a is shown in b; boxed region in b is shown in c. d, Z-slices extracted from tomographic reconstruction of forming NE from boxed region in c depicting CHMP2A-localization to nucleo-cytoplasmic channels at the indicated Z-heights. Arrow indicates nucleo-cytoplasmic channel. Images in all cases representative of 3 cells processed for CLEM, quantification of gold localization given in Extended Data Fig. 2H. Scale bars, 24 μm (b), 1 μm (c) and 200 nm (d).
Extended Data Figure 4 | Mitotic defects in cells reliant on mutated forms of CHMP2A. a, Quantification of CHMP2A recruitment to the telophase NE or the midbody from Fig. 2c (n = 3, 10 cells (midbody or telophase) scored per experiment). b, Quantification of cytokinetic failure from cells treated with the indicated siRNA (300 cells were quantified per experiment, from three independent experiments). Data are mean ± s.d.
Extended Data Figure 5 | Screening for ESCRT–p97 complex interactions.  

a–d, β-galactosidase activity of yeast co-transformed with the indicated Gal4 (ESCRT)- and VP16-fused proteins ($n = 2$).  
e, Resolved cell lysates and glutathione-bound fractions from 293T cells transfected with the indicated fusion proteins were examined by western blotting with anti-GFP ($n = 3$).  
f, β-galactosidase activity of yeast co-transformed with the indicated Gal4- and VP16-fused proteins ($n = 3$).  
g, Microscale thermophoresis experiments detailing binding of CHMP2A to GST ($n = 4$), His–UFD1 ($n = 5$) or His–UFD1(1–257) ($n = 4$). As no reduction in thermophoresis signal was observed for GST or His–UFD1(1–257) across the concentration range, we present here the average thermophoresis signal change at equivalent protein concentrations for these three proteins, normalized to zero at the concentration in capillary 1.  
h, Alexa-647-labelled CHMP2A, His–UFD1 and His–UFD1(1–257) were examined by infrared imaging or Coomassie staining. Data are mean ± s.d.
Extended Data Figure 6 | UFD1 depletion does not affect ESCRT-dependent receptor degradation, lentivirus release or cytokinetic abscission.  
a, Resolved cell lysates of HeLa cells transfected with the indicated siRNA were examined by western blotting with anti-UFD1 or anti-HSP90 antisera.  
b, Resolved lysates of human diploid fibroblasts transfected with the indicated siRNA and treated for the indicated times with epidermal growth factor (20 ng ml\(^{-1}\)) were examined by western blotting with anti-EGFR, anti-UFD1 and anti-GAPDH antisera. EGFR degradation was quantified by densitometry (\(n = 3\)).  
c, Resolved cell lysates from 293T cells transfected with the indicated HIV-1 based lentiviral plasmids, a virally packaged GFP-plasmid, and the indicated siRNA were examined by western blotting with anti-p24 capsid, -HSP90, -TSG101, -CHMP2A, -CHMP2B and -UFD1 antibodies. Viral supernatants were collected and used to infect target HeLa cells. Resolved virions present in the 293T supernatant were examined by western blotting with anti-p24 capsid. Resolved lysates of infected HeLa cells were examined by western blotting with anti-GFP. Virion release was the ratio of released to cellular p24 capsid, as quantified by densitometry (\(n = 2\)); infectivity was quantified as GFP signal in target cells, as quantified by densitometry (\(n = 2\)).  
d, siRNA-transfected HeLa cells were fixed and stained with anti-tubulin. Multinucleate cells (\(n = 5\)) or cells connected by midbodies (\(n = 5\)) were scored visually, 300 cells scored per experiment. Data are mean ± s.d.
Extended Data Figure 7 | ESCRT depletion impairs NE-rim formation.

a, b, Timelapse microscopy analysis and quantification of NE-rim formation in HeLa cells stably expressing YFP–LAP2β and mCh–H2B and treated with the indicated siRNA. Scale bars, 10 μm. Time for rim formation post anaphase onset given (mins) (control, 8.53 ± 0.09, 226 cells analysed over 8 independent experiments; CHMP2A-1, 7.60 ± 0.09, 205 cells analysed over 7 independent experiments; CHMP2A-2, 6.86 ± 0.12, 37 cells analysed over 2 independent experiments; CHMP2B, 6.92 ± 0.09, 79 cells analysed over 4 independent experiments; CHMP2A and CHMP2B, 6.84 ± 0.13, 50 cells analysed over 2 independent experiments; CHMP4B, 7.07 ± 0.14, 44 cells analysed over 2 independent experiments; UFD1, 9.2 ± 0.18, 39 cells analysed over 3 independent experiments). Data are mean ± s.e.m. (in minutes). Images representative of the indicated number of cell analysed. c, Resolved cell lysates from a were analysed by western blotting with the indicated antisera.
Extended Data Figure 8 | ESCRT depletion does not impair nuclear pore formation.  

**a**, Schematic of nuclear envelope integrity assay.  

**b**, Control-siRNA-treated HeLa cells reporting nucleo-cytoplasmic partitioning using the GFP–NLS–βGal assay, average NE compartmentalization from 20 cells presented. Nucleo-cytoplasmic partitioning stabilizes at 85 min (indicated by arrow).  

**c**, Immunofluorescence analysis of HeLa cells stably expressing YFP–LAP2β, transfected with the indicated siRNA then stained with anti-mAb414 and DAPI (n = 3). Scale bars, 10 μm.  

**d**, Mask used to quantify nuclear pore formation by image-based flowcytometry (Imagestream).  

**e**, Imagestream analysis of HeLa cells transfected with the indicated siRNA, then stained with anti-mAb414 and DAPI. Nuclear pore intensity quantified by mask described in **d**. Representative images from two independent experiments, histogram and population averages displayed, graphical quantification of NPC intensity from the indicated number of gated cells (control, 3,045; CHMP2A-1, 1,256; CHMP2A-2, 2,152; CHMP2B, 5,237; UFD1-1, 4,146; UFD1-3, 4,325). Data are mean ± s.d.
Extended Data Figure 9 | Requirements for nucleo-cytoplasmic compartmentalization. 

**a.** Quantification of NE sealing from siRNA-treated cells as in Fig. 4b (control, 140 cells from 7 independent experiments; UFD1-1, 60 cells from 3 independent experiments, \( P = 0.044 \); UFD1-3, 60 cells from 3 independent experiments, \( P = 0.021 \); CHMP2B 40 cells from 2 independent experiments; two-tailed Student’s \( t \)-test was used to assess significance at the 85-min time point).

**b.** Resolved cell lysates from **a** were analysed by western blotting with the indicated antisera.

**c.** NE integrity assay as performed with cells stably expressing mCh–H2B and GFP–NLS and transfected with the indicated siRNA. Differences in nucleo-cytoplasmic partitioning was assessed after plateau at the 65-min time point using a two-tailed Student’s \( t \)-test (control, 79 cells from 4 independent experiments, CHMP2A-1, 60 cells from 3 independent experiments, \( P = 0.048 \); CHMP2A-2, 52 cells from 3 independent experiments, \( P = 0.011 \); CHMP3, 28 cells from 3 independent experiments, \( P = 0.028 \)).

**d.** HeLa cells stably expressing mCh–H2B and GFP–NLS were transfected with the indicated siRNA and imaged live. 60 min after anaphase onset, cytoplasmic signal was photo-ablated (\( T = 0 \)) and recovery of cytoplasmic signal from the nuclear pool was calculated for the indicated conditions (cytoplasmic:nuclear ratio of GFP–NLS was normalized to \( T = 0 \); control, 21 cells from 4 independent experiments; CHMP2A-1, 24 cells from 4 independent experiments, \( P = 0.04 \); CHMP2A-2, 23 cells from 4 independent experiments, \( P = 0.05 \); CHMP3, 15 cells from 3 independent experiments, \( P = 0.004 \), two-tailed Student’s \( t \)-test was used to assess significance after 10 min). Scale bars, 10 \( \mu m \).

**f.** Scoring of multinucleate and midbody-connected HeLa cells transfected with the indicated siRNA and stained with anti-tubulin and DAPI (300 cells analysed per condition, \( n = 4 \)). Data are mean ± s.e.m. (a, c, d) and mean ± s.d. (f).
Extended Data Figure 10 | Effect of CHMP2A depletion on NE discontinuities. a, Presentation of reconstructed tomograms from Fig. 4d. b, CHMP2A-depleted cells exhibited more non-NPC discontinuities per unit area, while the number of NPC per unit area was constant. Tomograms as described in Fig. 4d, e were scored for discontinuities. The internal diameter of NPCs was slightly reduced in CHMP2A-depleted cells (control, 84 ± 7.6 nm, CHMP2A-1, 74 ± 8.8 nm; CHMP2A-2, 74 ± 5.7 nm). c, Schematic depicting topological equivalent of ESCRT-III-dependent membrane fusion events.