Online Supplementary Information

Online Methods

Cell Culture

HeLa and 293T cells were kind gifts from Prof Juan Martin-Serrano (KCL) and were cultured in DMEM containing 10% FBS, Penicillin (100U/ml) and Streptomycin (0.1 mg/ml). 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 (100U/ml) and Streptomycin (0.1 mg/ml). Stable cells lines were generated by transduction using MLV-based retroviruses as described previously[9], and selected using Puromycin (200 ng/ml), G418 (500 μg/ml) or hygromycin (200 μg/ml) as necessary. Cells were sorted to monoclonality by limiting dilution or FACS. HeLa cells stably expressing mCh-Tubulin[29] or GFP-CHMP4B[12] have been described previously and were kind gifts from Prof Juan Martin-Serrano (KCL). MycoSensor (Agilent) was used to screen for contamination.

Plasmids

Plasmids encoding TSG101, EAP20, EAP30, EAP45, CHMP1A, CHMP1B, CHMP2A, CHMP2B, CHMP3, CHMP4A, CHMP4B, CHMP4C, CHMP5, VPS4A, LIP5, UBPY, CEP55, TAL, LAP2β and ALG2 were kind gifts from Prof. Juan Martin-Serrano (King's College London) and have been described previously[8,9,13,30]. Coding sequences for p97, p47, NPL4, UFD1, CHMP7, VPS4B and SPARTIN were amplified from IMAGE clones (6502535, 3635947, 5017718, 3507963, 5551762, 6042862, 5313378) respectively and were cloned into mammalian expression (pCR3.1-YFP) and yeast 2-hybrid plasmids (pHB18 and pGBKT7). A plasmid encoding HD-PTP was a kind gift from Prof Phillip Woodman (University of Manchester) and was cloned similarly. siRNA resistant CHMP2A constructs were created by introducing Q132Q, A133A, E134E, I135I, and D137D silent mutations in the CHMP2A coding sequence by PCR. 5' EcoRI and 3' NotI sites were added to facilitate cloning. Additional CHMP2A truncations, mutations and deletions were created by standard PCR procedures. UFD1 and its deletions were cloned with 5' and 3' NotI sites into relevant expression vectors. CHMP2A constructs were subcloned into pGBKT7 and UFD1 constructs were cloned into pHB18 for yeast 2-hybrid analysis. For recombinant protein expression, CHMP2A was cloned into pGEX-EcoRI-NotIXhoI and UFD1 constructs were cloned into pET28a (kind gifts from Prof Juan Martin-Serrano). A lentiviral expression vector (pLVXP) was a kind gift from Dr Mark Dodding (KCL) and modified to express a GFP-EcoRI-NotI-XhoI polylinker by replacing its SnaBI/XbaI fragment with a GFP-EcoRI-XhoI-NotI-STOP-XbaI fragment, obtained by PCR from pCR3.1-GFP-EcoRI-XhoI-NotI (a kind gift from Prof Juan Martin-Serrano, KCL). SnaBI/XhoI fragments of pHM840 (encoding GFP-NLS-βGAL and obtained from Thomas Stamminger (Universitätsklinikum Erlangen) via Addgene)

were cloned into the SnaBI/XhoI sites of pLVXP-GFP-ENX to produce pLVXP-GFP-NLS-βGAL. pLVXP-GFP-NLS was obtained by amplifying GFP using primers to incorporate a C-terminal SV40 NLS.

A SnaBI/NotI fragment from pH2B-mCherry-IRES-Neo3 (a kind gift from Dr Ulrike Eggert (King's College London)) was subcloned into SnaBI/Not sites of pLHCX-MCS (a modified version of pLHCX containing a HindIII/MluI/SalI/XhoI/NotI/HpaI/BamHI/NsiI/ClaI MCS, a kind gift from Prof T. Ng, King's College London) to create pLHCX-H2B-mCh. LAP2β residues 244-454 were amplified with in-frame NotI sites and cloned into the NotI site of pMSCVneo-YFP-EXN to create pMSCVneo-YFP-LAP2β. siRNA-resistant CHMP2A constructs were cloned with C-terminal FLAG extensions into EcoRI and NotI sites of pCR3.1-EXN and subcloned into pNG72-ENX (kind gifts from Prof Juan Martin-Serrano).

For retroviral transduction, above constructs within pMSCVneoYFP-EXN, pNG72 pLHCX-MCS retroviral packaging vectors were transfected with pVSVG into 293GP2 cells (all from Clontech). Supernatants were harvested, clarified by centrifugation (200 x g, 5 minutes), filtered (0.45 μm) and used to infect target cells in the presence of 8μg/ml polybrene (Millipore) at MOI < 1. For lentiviral transduction, 293T cells were transfected with pCMV8.91, pVSVG and either pLVXP-GFP-NLS-βGAL, pLVXP-GFP-NLS-PK or pLVXP-GFP-NLS. Supernatants were harvested, clarified by centrifugation (200 x g, 5 minutes), filtered (0.45 μm) and used to infect target cells in the presence of 8μg/ml polybrene (Millipore) at MOI < 1. In both cases, antibiotic selection was applied after 48 hours.

Antibodies

Antibodies against HSP90 (H114) were from Santa Cruz Biotechnology, TSG101 (T5701) 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, UFD1 (106151-AP) was from Proteintech, anti-p24 Gag (183-H12-5C) was from the NIH AIDS Research and Reference Reagent Program, EGFR (2232) was from Cell Signaling Technology, GFP (7.1/13.1) was from Roche, LBR (SAB10400151) 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 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 antisera in 5% milk. HRP-conjugated secondary antibodies were incubated with ECL Prime enhanced 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 manufacturers instructions. 293GP2 and 293T cells were transfected using linear 25-kDa polyethylenimine (PEI, Polysciences, Inc.)

siRNA transfections

Cells were seeded at a density of 1E5 cells/ml (HeLa, BJ) or 2.6E5 cells/ml (293T) and were transfected with siRNA at 100nM, 2 hours after plating using Dharmafect-1 (Dharmacon). To minimize toxicity associated with CHMP2A and UFD1 depletion, single transfections were performed for 72 hours. The following targeting sequences that have already been demonstrated to achieve potent and specific suppression of the targeted CHMP were employed: Control – Dharmacon Non-targeting control D-001810-01. CHMP2A-1 : aggcagagaucauggauaudTdT[16], CHMP2A-2 : AAGAUGAAGAGGAGAGUGAdTdT[17], CHMP2B : UCGAGCAGCUUUAGAGAAAdTdT[17], CHMP3 ggaagaagcagaaauggaadTdT[17], CHMP4A Q-SI04268845[13], CHMP4B Q-SI00325199[13], CHMP4C Q-SI04279674[13], CHMP1A CCAAGAAGGCGGAGAAGGAdTdT[17], CHMP1B UGGACAAAUUCGAGCACCAdTdT[17], UFD1-1 GAGGCAGAUUCGUCGCUUUdTdT, UFD1-2 MQ-017918-03-0002, UFD1-3 GUGGCCACCUACUCCAAAUdTdT[31], LEM4 GAGAAGACGCUGAGAAAUUdTdT[18]. UFD1-2 was excluded from much of the analysis due to toxicity and morphological changes specific to this oligo.

Yeast Two-Hybrid assays

Yeast Y190 cells were co-transformed with plasmids encoding the indicated proteins fused to the VP16 activation domain (pHB18) or the Gal4 DNA-binding domain (pGBKT7). Co-transformants were selected on SD-Leu-Trp agar for 3 days at 30 ^{o}C, harvested, and *LacZ* activity was measured using a liquid β-galactosidase assay employing chlorophenolred-ß-D-galactopyranoside (Roche) as a substrate. Average β-galactosidase activities presented.

Lentiviral release

293T cells were transfected with siRNA as described above, except that the second transfection contained additionally either 300 ng of HIV-1 pCMVd8.91 (a kind gift from Prof T. Ng, King's College London), 100ng of pLenti-SEW (a packaging vector encoding GFP, a kind gift from Prof A. Ridley, King's College London) and 100 ng pVSVG. After 48 hours, virions were harvested from 293T supernatants by filtration (0.45 μm) and centrifugation through 20% sucrose (14000 rpm, 120 minutes), lysed, resolved by SDS-PAGE and examined by western blotting. Additionally, HeLa cells were infected with 50 μl of viral supernatant and GFP-expression in these cells was measured by western blotting. Virion Release was calculated by quantifying $Gag^{Virion}/Gag^{Cellular}$ as determined densitometry using ImageJ.

Co-Precipitation Assays

293T cells were co-transfected equal quantities of the indicated pCAGGS-GST construct and the relevant pCR3.1-YFP construct for 48 hours. Cells were harvested and lysed in 1 ml of 50 mM Tris·HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 5% glycerol, 1% Triton X-100, a protease inhibitor mixture (complete mini-EDTA-free, Roche). Clarified lysates were incubated with glutathione-Sepharose beads (Amersham Biosciences) for 3 h at 4 °C and washed three times with wash buffer (50 mM Tris·HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 5% glycerol, 0.1% Triton X-100). Bead-bound proteins were recovered in Laemmli sample buffer, resolved by SDS-PAGE and examined by western blotting.

Production of recombinant proteins

BL21* DE3 *E. coli* expressing plasmids encoding GST-tagged or HIS-tagged proteins were collected in bacterial lysis buffer (20mM Hepes (pH 7.4), 500 mM NaCl, supplemented with complete mini, EDTA-free protease inhibitor (Roche) and 1 mM PMSF; buffers for HIS-tagged protein purification were supplemented with 20mM Imidazole. Cells were lysed by addition of lysosyme (1 mg/ml, 15 minutes), Triton X100 (0.25%, 15 minutes) and were snap frozen in liquid nitrogen. Cells were thawed on ice, clarified through addition of DNAse1 (20 μg/ml) and soluble proteins were collected by centrifugation at 28000 x g for 30 minutes. Proteins were immobilised on Glutathione Sepharose 4β or Ni-NTA-sepharose resin, washed in wash buffer (20mM Hepes, pH7.4, 150mM NaCl), containing 20mM Imidazole if required. Proteins were eluted from Glutathione Sepharose 4β resin in wash-buffer supplemented with 10mM reduced Glutathione (pH 8), or were eluted from Ni-NTA sepharose with a step gradient of imidazole. CHMP2A was cleaved from the GST using AcTEV protease (Life Technologies). Protein concentrations were measured using the Qubit assay system (Life Technlogies).

Microscale thermophoresis (MST)

Measurements were preformed using a Monolith NT.115 instrument (Nanotemper). Briefly, recombinant CHMP2A was labeled with Alexa-647 using an NHS Amine-reactive labeling kit (Nanotemper), labeling was verified by infrared imaging (Licor). 360nM Alexa-647 CHMP2A was combined with serial dilutions of GST, HIS-UFD1 or HIS-UFD1 1-257 (maximally 52.8 μM). Interactions were performed in 150 mM NaCl, 20 mM Hepes, 0.04% Tween-20 pH 7.4. Temperature jump and thermophoresis experiments were conducted using 100% LED illumination and 40% IR laser power and were analysed using Nanotemper'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 MeOH (for CHMP2A-staining) or 4% PFA and subject to processing for immunofluorescence as described previously[9]. For multinucleation and midbody arrest assays, at least 300 cells per experiment were quantified. For telophase NE localisation, at 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 hours after siRNA transfection (10.5 hours 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 hours using a 20x dry objective and a 1.5 x magnification lens. For mitotic rim formation, 3 co-ordinates 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-ßGal, frames were acquired every 1 min. The ratio of background-corrected, area-normalised, GFP-positive pixel intensities within the cytoplasm and mCh-H2B demarcated nuclei at the indicated intervals were obtained using NIS-elements. We excluded 2/98 cells from CHMP2A-1 analysis, 2/60 cells from UFD1-1 analysis and 2/60 cells from UFD1-3 analysis as these gave anomalous N/C ratios > 10 x S.D. from the average. For imaging of GFP-CHMP4B recruitment to the telophase NE, cells were imaged using a 100x oil-immersion objective and confocal slices were acquired every 30 seconds 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 hours post 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 minutes post-bleach.

Correlative Light Electron Microscopy

500,000 HeLa cells were seeded in a 3.5cm Mattek gridded dish (P35G-2-14-C-GRID). The following morning, cells were fixed in phosphate buffer containing 1% PFA for 3 minutes. Cells were permeabilised with 0.1% saponin in PHEM (60mM PIPES, 25mM HEPES, 10mM EGTA, 2mM $MgCl_2$, pH6.9) and processed for immunofluorescence using anti-CHMP2A primary and goat anti-rabbit Alexa^{594}-conjugated fluoronanogold (Nanoprobes) and DAPI. Cells were subjected to a subsequent 10-minute 3% PFA fixation and quenching prior to 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 OsO_4 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[32]. For retracing of the cells of interest sections were imaged on a FEI Tecnai12 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 tomograms.

Statistical analysis

Variance wan analysed using an F-test and type-relevant 2-tailed Student's T-tests were used to assess significance between test samples and controls.

ImageStream Analysis

siRNA-treated HeLa cells in 6-well dishes were detached, fixed in 4% PFA, permeabilised with 0.1% Tx100 and stained in suspension with mAb414, Alexa^{594} conjugated secondary antibodies and DAPI (at 0.1 μg/ml). In-focus, single-cellular populations were acquired and a mask was applied to the DAPI channel and duplicated then dilated by 3 pixels to encompass the mAb414 signal surrounding the nuclei. The difference in 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.

Supplementary Video 1 : CHMP2A forms a reticular network around telophase nuclei

Deconvolved 3D reconstruction of HeLa cells stained with anti-CHMP2A and DAPI and analysed by widefield microscopy, from Extended Data Figure 1C.

Supplementary Video 2 : GFP-CHMP4B transiently localises to telophase nuclei

Movie of GFP-CHMP4B localisation during the anaphase to telophase transition, from Extended Data Figure 1D.

Supplementary Video 3 : CHMP2A decorates nucleo-cytoplasmic channels

3D reconstruction ofHeLa cells stained with anti-CHMP2A and DAPI and analysed by correlative light and electron tomography, From Figure 1D.

Supplementary Video 4 : Tomographic reconstruction of nascent nuclear envelope

3D reconstruction ofHeLa cells stained with anti-CHMP2A and DAPI and analysed by correlative light and electron tomography, as depicted in Figure 1D and Extended Data Figure 2.

Supplemental References

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