**Supplementary materials and methods**

**Generation of cell line conditionally expressing tagged CLASPIN**

To generate a cell line in which tagged CLASPIN could be inducibly expressed, the full-length CLASPIN coding sequence was PCR amplified from an image clone (IMAGE:9021657) using forward (5’-GCCACAGCCGCCACCATGGGAACAGGCGAGGTGGGTTCTG-3’) and reverse primers (5’-CCGGCAGCGCCTCCGCTCTCCAAATATTTGAAGATG-3’) and cloned in-frame with a dual C-terminal Flag/OneSTrEP tag contained within a modified version of the pcDNA5/FRT/TO plasmid (pAB1) using ligation independent cloning. Stable cell lines conditional expressing tagged-CLASPIN were generated by co-transfection of pAB1-CLASPIN plasmid and pOG44 plasmid into the Flp-In T-REx 293 Cell Line (Life technologies) using FuGENE HD (Promega). Cells that underwent FRT-directed recombination of the pAB1-Clapsin plasmid were selected using Hygromycin (100 g/ml) and individual clones were isolated and are referred to as Strep-CLASPIN cells. Conditional expression of Strep-CLASPIN was routinely induced by addition of doxycycline (1 g/ml) to cell culture media to alleviate TET-repression. Control cells were generated as above using an empty pAB1 plasmid and are referred to as empty-vector cells.

**Mass spectrometry and data analysis.**

Peptide mixtures were desalted and concentrated on home-made C18 desalting tips before being injected onto a UHPLC (Easy-nLC 1000 Proxeon, Denmark). Peptide separation occurred on a home-made 25 cm long reverse-phase spraying fused silica capillary column (75 μm i.d.) packed with 1.7 μm ReproSil AQ C18 (Dr. Maisch GmbH, Germany). A gradient of eluents A (pure water with 2% v/v ACN, 0.1% v/v formic acid) and B (ACN with 20% v/v pure water with 0.1% v/v formic acid) was used to achieve separation, from 12% to 50% of B over 20 minutes, at a constant flow rate of 250 nl/min. The LC system was connected to a QExactive mass spectrometer (ThermoScientific, Bremen, Germany) equipped with a nanoelectrospray ion source (Proxeon Biosystems, Odense, Denmark). Full scan mass spectra were acquired in the mass spectrometer with the resolution set to 70,000 (at 200 m/z) to a target value of 1e6 for a maximum injection time of 120 msec. The acquisition mass range for each sample was from m/z 300 to 1750 Da and samples were run in duplicates. The ten most intense doubly and triply charged ions were automatically selected and HCD fragmented after accumulation to a ‘target value’ of 1e5 for a maximum injection time of 120 msec, and recorded with resolution of 35000 (at 200 m/z). Normalized Collision energy was set to 25% and isolation width to 3.0 m/z. Target ions already selected for the MS/MS were dynamically excluded for 5 sec. Identification and quantification of peptides and proteins were performed with MaxQuant 1.5.1.2 against the human Uniprot complete proteome set using the Andromeda search engine in which trypsin specificity was used with up to two missed cleavages allowed. Criteria for high-confidence protein identification were at least 2 peptides (1 unique), 6 amino acids of minimal length for high-confidence protein identification and quantified with at least 2 ratio counts. Cysteine carbamidomethylation was used as fixed modification, methionine oxidation and protein N-terminal acetylation as variable modifications. Mass deviation for MS/MS peaks was set at 4.5 ppm. The peptides and protein false discovery rates (FDR) were set to 0.01. The lists of identified proteins were filtered to eliminate reverse hits and known contaminants. For quantitative analysis the options Re-quantify and second peptide were selected. Significant outliers scores were calculated using Perseus 1.5.1.6 (1) and those with a p-value <0.05 have been selected for further analysis. Proteins were selected to be interactors and further investigated if they display a fold change higher than 3, based on the ratio distribution of all the quantified proteins

**Cell culture.** hTERT immortalized RPE1 cells were obtained from ATCC. Cells were cultured at 37°C, 5% CO2 in DMEM F-12 (Lonza) supplemented with 1% penicillin-strep and heat inactivated 10% Fetal bovine serum (Sigma-Aldrich).

**Immunofluorescence microscopy.** For USP9X localisation studies, U2OS cells grown on coverslips were treated with either 40 nM leptomicin B (Sigma) or 2 mM HU (Sigma) for 2, 4, 6 and 8 hr. Cells were fixed with 4% paraformaldehyde for 10 min at room temperature. After three washes with PBS, cells were permeabilised with PBS-TX (PBS 0.1% Triton X-100) for 10 min at room temperature. Cells were stained with rabbit anti-USP9X antibody (Bethyl Laboratories cat n. A301 351A) and secondary Alexa Fluor 488 goat anti-rabbit antibody (Invitogen). Nuclei were counterstained stained with DAPI (Sigma) and coverslips were mounted using Slow Fade Gold reagent (Invitrogen). Cells were examined using an IX71 Olympus microscope with a 60× oil immersion objective.

**Confocal laser scanning microscopy.** Confocal image stacks were recorded on a LSM-510 confocal microscope (Zeiss) using a 100x 1.40 NA Plan-Apochromat oil immersion objective. Fluorochromes were excited with a 405nm diode laser and the 488 line of an Argon laser. Setting used were 1024x1024 pixels frame size, 44 nm pixel size, 129 nm Z-distance between sections; 1 Airy unit pinhole diameter; 4-times averaging.

**Image processing and analysis.** Huygens software (Scientific Volume Imaging B.V., Netherlands) was used for all processing and analysis. Deconvolution of confocal datasets was performed using estimated point spread functions and CMLE algorithm (settings: maximum iterations: 40; signal-to-noise: 20; quality criterion: 0.05). Maximum intensity projections, axial, frontal and transversal views were prepared using the Ortho slicer module. 3D Segmentation of nuclei and USP9X signals was done using the Object Analyzer module. The Colocalization Analyzer module was used to filter non-intersecting USP9X objects, and to quantify volumes of intersection of USP9X objects that intersected with the nucleus. Graphs were prepared in Prism 6 (GraphPad). Data shown are volume fractions (% voxels intersecting) for each individual USP9X object, with the median.

**Reference**

1. Cox J, Mann M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. Nat Biotechnol. 2008;26:1367–72.