**Supplementary table 1 legend**

**Protein groups identified by MaxQuant analysis.** For each protein group, the Uniprot accession number, gene and protein names are reported, as well as the number of peptides identified and used for the quantification, the log2 of the ratio and the log10 of the single and summed intensities in the medium and light channel. Proteins with a fold change higher than 3 are highlighted in green, while proteins identified only in the CLASPIN immunoprecipitated sample and not in the negative control are highlighted in red.

**Supplementary figure legends**

**Fig S1. Characterisation of inducible Strep-CLASPIN HEK-293 cellular system**. HEK-293 cells containing empty vector or Strep-CLASPIN were either induced with doxycycline or left untreated. (A) Growth curves were generated from total cell number as determined by trypan blue exclusion at time of induction (0 hr) and 24 hr, 48 hr, 72 hr and 96 hr post induction. (B) Cells were fixed at 0 hr, 24 hr, 48 hr and 72 hr post induction and DNA content was determined with PI/RNAase staining and FACS analysis. (C) Cells were fixed for and stained with primary anti-Strep antibody (Qiagen cat n. 34850) and the secondary Alexa Fluor 488 goat anti-mouse antibody (Invitrogen). DAPI (Sigma) was used to examine DNA content and subjected to FACS analysis.

**Fig S2. Extraction of Strep-CLASPIN from the chromatin.**

HEK-293 cells Strep-CLASPIN were induced with doxycycline and cells harvested 48 hr post induction, lysed in Buffer A 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1% Triton-X with varying concentrations of NaCl ranging from 100 mM to 400 mM. Proteins were separated into soluble and chromatin enriched fractions by centrifugation and analysed by immunoblotting. GAPDH and H2A (Abcam ab18255) were used as control for fractionation.

**Fig S3. USP9X depletion has no effect on cell cycle.**

HEK-293 cells or U2OS were transfected with either a siRNA targeting USP9X or siCTRL. After 48 hr cells were fixed and DNA stained with PI/RNAase (BD Bioscience). DNA content was determined by FACS analysis.

**Fig S4. WP1130 affects CLASPIN Stability in S-phase.**

The intensities of the Strep-CLASPIN bands shown in figure 3E were quantified on the Li-cor imaging system, normalized to the GAPDH loading control and graphed against the time in cell treatment with cycloheximide.

**Fig S5. Overexpression of WT but not CD USP9X can stabilise CLASPIN degradation.**

U2OS cells were transfected with constructs expressing either functional (WT) or catalytically dead C1566A (CD) USP9X. After 24 hr cells were either collected or treated with cycloheximide for a further 3, 6 or 9 hr, as indicated. Protein extracts were then prepared and analysed by immunoblotting (A). The intensities of the CLASPIN bands shown in figure S5 (a) were quantified on the Li-cor imaging system, normalized to the GAPDH loading control and graphed against the time in cell treatment with cycloheximide (B).

**Fig S6. USP9X promotes checkpoint signaling and USP9X depletion results in the accumulation of DNA damage.**

U2OS cells were transfected with control or 2 different siRNAs targeting USP9X. After 48 hr HU was added and samples taken 2 hours later. Phosphorylation of CHK1 at Ser317 as a marker of checkpoint activation was monitored by immunoblotting (A).

RPE1 cells were transfected with control or siRNA targeting USP9X. After 48 hr HU was added and samples taken 2 hours later. Phosphorylation of CHK1 at Ser317 as a marker of checkpoint activation was monitored by immunoblotting (B).

U2OS cells were transfected with control siRNA, CLASPIN siRNA or 2 different siRNAs targeting USP9X. Cells were stained with anti- -H2AX antibody detecting H2AX phosphorylated on serine 139 and anti-USP9X antibody (C).

**Fig S7. Treatment with Leptomycin B, a specific inhibitor of CRM1 mediated nuclear export, induces accumulation of USP9X in the nucleus in a time dependent manner.**

(A) U2OS cells were fixed before and at indicated time points after treatment with Leptomycin B (LMB) and stained with anti-USP9X antibodies (B) The intensity of USP9X nuclear signal was quantified with Operetta high content microscopy (Perkin Elemer). Each dot represents a single nucleus.

**Fig S8. Analysis of USP9X nuclear localization by confocal microscopy.**

(A) USP9X (grey) is seen over the whole cell in maximum intensity projections on the Z-axis. Orthogonal slices reveal little USP9X inside the nucleus (cyan) in untreated cells, but show a marked increase in nuclear USP9X after Leptomycin treatment. Bar: 10µm.

(B) USP9X (green) and DAPI (blue) signals of the cells shown in (A) were segmented in 3D, and USP9X objects that did not intersect with the DAPI region were filtered. Some USP9X is located at the surface of the nucleus (seen in the Opaque nucleus view), and some is located inside the nucleus (seen in the Transparent nucleus and No nucleus views).

 (C) The volume of each individual USP9X object that intersected with the nucleus was calculated for two untreated cells and two Leptomycin treated cells. The graph shows the proportion of that volume that is located inside the nucleus, the bar represents the median.

In untreated cells, half the USP9X objects (see median) have 57% (cell A) or 44% (cell B) of their volume inside the nucleus. After Leptomycin treatment, there is a marked increase in the amount of USP9X object located within the nucleus: half of the objects (see median) have 88% (cell C) or 99% (cell D) of their volume inside the nucleus.