Supplementary Figure S1.

Effects of UBL5 knockdown on cell cycle distribution and sister chromatid cohesion

A. Representative examples of flow cytometry profiles of HeLa cells transfected with indicated siRNAs for 72 h and stained with Propidium Iodide (FL2A). Cells with sub-G1 DNA content are marked by the boxed area (blue).

B. Representative examples of flow cytometry profiles of HeLa cells transfected with indicated siRNAs for 72 h and stained with Propidium Iodide (FL2-A) and Histone H3-pSer10 antibody (FL1-H). Cells in mitosis are marked by the boxed area (magenta).

C. Sister chromatid cohesion status was scored in metaphase spreads of U2OS cells transfected with non-targeting control (CTRL), UBL5, or SCC1 siRNAs for 48 h. At least 50 metaphases were analyzed per condition.

Supplementary Figure S2.

Interactions between UBL5 and pre-mRNA splicing factors

A. A UBL5 D22A mutation does not impair interaction with SART1. Whole cell extracts (WCE) of U2OS cells transfected with indicated combinations of SART1-GFP and Strep-HA-UBL5 constructs were subjected to Strep-Tactin pull-down and analyzed by immunoblotting with GFP and HA antibodies.

B. UBL5 associates with EFTUD2 independently of SART1. U2OS cells were transfected or not with SART1 siRNA. Twenty-four h later, cells were transfected with Strep-HA-UBL5 or empty vector (−) plasmids for an additional 24 h. The cells were then collected and subjected to Strep-Tactin pull-down and analyzed by immunoblotting with EFTUD2, HA, and MCM6 antibodies.

C. UBL5 D22A rescues the cohesion defect induced by knockdown of endogenous UBL5. HeLa cells carrying inducible Strep-HA tagged siRNA-resistant form (si^R) of UBL5 D22A were transfected with control or UBL5 siRNA in the absence or presence of doxycycline (DOX). Two days later, cells were treated with Nocodazole for 3 h and harvested for chromosome analysis.

Supplementary Figure S3.

RNA-Seq analysis of cells lacking UBL5 or SART1

A. Scatter plot showing correlation (Pearson coefficent (r)) between expression levels of all detected transcripts in two replicates (a and b) of RNA-Seq analysis of cells treated with UBL5 siRNA.

B. Scatter plot showing correlation (Pearson coefficent (r)) between expression levels of all detected transcripts in cells treated with independent UBL5 siRNAs (UBL5#58 and UBL5#82).

C. Median IF values from the distributions shown in Fig. 3D.

Supplementary Figure S4.

Correlation between phenotypes resulting from knockdown of factors involved in pre-mRNA splicing and cohesion

A. Immunoblot analysis of the experiment in Fig 4A. HeLa cells were transfected with siRNAs and processed for immunoblotting of the indicated proteins.

B. Correlation between mitotic defects induced by knockdown of pre-mRNA splicing and sister chromatid cohesion factors. Phenotypes arising from siRNA-mediated depletion of the indicated human proteins were obtained from the MitoCheck database (http://mitocheck.org/).

Supplementary Figure S5.

Impact of UBL5 knockdown on expression levels and chromatin loading of cohesion factors

A. HeLa cells transfected with non-targeting control (CTRL) or UBL5 siRNA were synchronized by thymidine plus nocodazole block and released for the indicated times. Chromatin-enriched and soluble fractions were analyzed using indicated antibodies.

B. HeLa cells transfected with indicated siRNAs were fixed and immunostained with SGO1 antibody. Scale bar, 10 μm.

C. HeLa cells transfected with indicated siRNAs were processed for immunoblotting.

D. HeLa cells transfected with indicated siRNAs were lysed, and total RNA was extracted. After reverse transcription, cDNA was subjected to PCR using primers targeting exons 1 and 2 or exons 5 and 6 as indicated by arrows.

E. Immunoblot analysis of the experiment in Fig 4F. HeLa cells were transfected with siRNAs and processed for immunoblotting of the indicated proteins.

F. Sister chromatid cohesion status in metaphase spreads from HeLa cells transfected with indicated siRNAs and HA-Sororin cDNA [mean ± SD (error bars); N=3]. At least 100 metaphase spreads were counted. The relative efficiency of HA-Sororin expression to reverse the sister chromatid cohesion defect in UBL5-depleted cells was determined by comparing the degree of rescue to that of expressing HA-Sororin in siSororin-treated cells (Fig 4G).

Supplementary Table S1.

Mass spectrometry-based analysis of cellular UBL5-interacting proteins

HeLa cells expressing Strep-HA-UBL5 or not were cultured in heavy (H) or light (L) SILAC medium, respectively. Strep-HA-UBL5 and associated proteins enriched on Strep-Tactin Sepharose were analyzed by MS. The Excel workbook shows all proteins quantified in the experiment.

Supplementary Table S2.

RNA-Seq analysis of transcriptome changes resulting from knockdown of UBL5 or SART1

List of fold change in expression levels between samples treated with control siRNA (Control) and SART1 (Sart1) or either of three independent UBL5 siRNAs (UBL5#57, UBL5#58, and UBL5#82). Expression levels are expressed in RPKM (reads per kilobase per million reads). Two replicates of each experiment (a and b) were performed. In the "Control vs UBL5 pooled" list, expression values from all three UBL5 siRNA-treated samples are averaged.

Supplementary Table S3.

Isoform switching in UBL5- and SART1-depleted cells

List of genes showing most severe isoform switching upon knockdown of UBL5 or SART1. The tabulated output from the spliceR analysis was merged with full-length gene names and descriptions, obtained from NCBI's FTP gene database.

Supplementary Movie S1.

Mitotic progression in the absence of UBL5

Chromosome dynamics during mitosis in live HeLa/H2B-mCherry cells transfected with UBL5 siRNA was monitored by time-lapse microscopy. A representative example of mitotic progression in these cells is shown.