

SUPPLEMENTARY MATERIALS AND METHODS

Antibodies

The following antibodies were used: rabbit polyclonal antibodies against WDR43 (A302-478A, Bethyl Laboratories), NOL11 (HPA022010, Sigma, for immunoblotting and immunofluorescence), Cirhin (ab103357, Abcam; 11507-1-1AP, Proteintech; H00084916-A01, Abnova), Ki67 (sc-15402, Santa Cruz Biotechnology, Inc.), CENP-B (ab25734, Abcam), survivin (NB500-201, Novus Biologicals), Haspin (A302-241A, Bethyl Laboratories), hUTP15 (NBP2-58570, Novus Biologicals), and Rrn3 (TIF-1A: sc-133978, Santa Cruz Biotechnology); rabbit monoclonal antibodies against histone H3 (H3) phosphorylated at threonine 3 (H3T3ph; ab78351, Abcam) and NOL11 (ab181996, Abcam, for immunoprecipitation); mouse monoclonal antibodies against UBF (sc-13125, Santa Cruz Biotechnology), α -tubulin (DM1A, Millipore), β -actin (sc-47778, Santa Cruz Biotechnology), Aurora B/AIM-1 (6/AIM-1, BD Transduction Laboratories), heat shock protein 90 (HSP90; 610418, BD Transduction Laboratories), H3 phosphorylated at serine 10 (H3S10ph; MABI0312, MBL), FLAG (M2, Sigma), Sgo1 (H00151648-M01, Millipore), CENP-A (D115-3, MBL), Hec1 (ab3614, Abcam), CENP-E (ab5093, Abcam), hUTP10 (sc-390445, Santa Cruz Biotechnology), nucleolin (M019-3, MBL) and myc (MC045, Nacalai Tesque); rat monoclonal antibodies against hemagglutinin (HA; 3F10, Roche); and human polyclonal antibodies against centromere (ACA; NA-8184, MBL). Rabbit anti-H3 antibody was raised against human full-length H3 recombinant protein. Rabbit polyclonal anti-SMC2 antibodies were raised against synthetic peptide KSKAKPPKCAHVEV corresponding to the C-terminal sequence of human SMC2 (1). Rabbit polyclonal anti-PP1 antibodies were raised against synthetic peptide RPVTPPR corresponding to the C-terminal of common sequence of human PP1.

Subcellular fractionation

Mitotic HeLa cells were incubated with fractionation buffer [10 mM HEPES (pH 7.9), 10 mM KCl, 1.5 mM MgCl₂, 10% glycerol, and 0.34 M sucrose] containing 1 mM dithiothreitol (DTT), complete protease inhibitor cocktail (Nacalai Tesque), and phosphatase inhibitor (Nacalai Tesque) on ice for 5 min. The pellet as chromatin fraction was collected by centrifugation for 5 min at 1,500×g. Then, the chromatin fraction was washed by fractionation buffer and collected by centrifugation.

Tandem affinity purification and mass spectrometry (MS) analysis

Nocodazole-treated HeLa cells or those stably expressing FLAG/HA-NOL11 were collected and lysed in lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, and 0.5% NP-40] containing

complete protease inhibitor cocktail at 4°C for 30 min (2). The cleared lysate was mixed for 4 h with anti-FLAG M2-agarose beads. After washing three times with lysis buffer containing 300 mM NaCl and three times with lysis buffer, the bound proteins were eluted with 0.5 mg/ml lysis buffer containing FLAG peptide at 4°C for 1 h. The eluates were further purified by immunoprecipitation with anti-HA antibody-conjugated Sepharose. After washing three times with lysis buffer containing 300 mM NaCl and three times with lysis buffer, bound proteins were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), visualised by silver staining, and analysed by matrix-assisted laser desorption/ionisation time-of-flight MS (Bruker).

RNA purification and quantitative reverse transcription-PCR (RT-PCR)

RNA isolation was performed using Sepasol RNA I Super Reagent (Nacalai Tesque). Isolated RNA was reverse transcribed using random hexamers and PrimeScript reverse transcriptase (TaKaRa). cDNA was analysed by quantitative PCR with Thermal Cycler Dice TP800 (TaKaRa) using SYBR Premix Ex Taq (TaKaRa). The specific primer sets for PCR amplification were as follows: 5'-GGCGGTTTGAGTGAGACGAGA-3' and 5'-ACGTGCGCTCACCGAGAGCAG-3' for pre-rRNA (5'ETS), 5'-ATCGTCCACCGCAAATGCTTCTA-3' and 5'-AGCCATGCCAATCTCATCTTGTT-3' for β -actin mRNA, 5'-TGGACAAGACGAAAACCTCTG-3' and 5'-CAGCTGGACGTATTGGTATC-3' for NOL11 mRNA, 5'-CAGACCTCCTAATGAGAGCC-3' and 5'-GACAGGTTTCATCGGTAAGT-3' for WDR43 mRNA, and 5'-ATCAAGGAGCTCTGCATATTGTT-3' and 5'-ACACATGGCCTCCACTGTTC-3' for Cirhin mRNA.

RNA immunoprecipitation

RNA immunoprecipitation assay was performed using RiboCluster Profiler™ RIP-Assay Kit (MBL), following the manufacturer's instructions. Briefly, mitotic HeLa cells that stably expressed FLAG/HA-NOL11 were lysed in lysis buffer. The cleared lysate was incubated with control IgG or anti-FLAG antibody-immobilised protein G Sepharose. After washing with wash buffer, RNA was isolated from whole lysates or immunoprecipitated fractions. The subsequent procedure was conducted as described in RNA purification and quantitative RT-PCR.

GST pull-down assay

cDNAs encoding NOL11, WDR43, and Cirhin were cloned into pGEX-4T-1. GST fusion proteins were expressed in *Escherichia coli* BL21 (DE3) after induction with isopropyl β -D-1-thiogalactopyranoside and purified with glutathione Sepharose 4B beads (GE Healthcare). For the analysis of protein-RNA

interactions, GST pull-down assay was performed as described previously (3) with modification. The beads coupled with GST-tagged proteins were incubated with 1 µg purified RNA from HeLa cells, 5 µg yeast tRNA, and RNaseOUT (Invitrogen) in 100 µL PB-100 [20 mM HEPES-KOH (pH 7.6), 100 mM KCl, 0.05% NP-40, 0.5 mM PMSF, 0.5 mM EDTA, and, 1 mM DTT] for 90 min at 4°C. The beads were washed three times with PB-200 (PB-100 containing 200 mM KCl) and bound RNAs were extracted by Sepasol RNA I Super Reagent. The subsequent procedure was conducted as described in RNA purification and quantitative RT-PCR.

Flow cytometry

Flow cytometry analysis was done as described previously (4).

SUPPLEMENTARY REFERENCES

1. Takemoto, A., Kimura, K., Yokoyama, S. and Hanaoka, F. (2004) Cell cycle-dependent phosphorylation, nuclear localization, and activation of human condensin. *J. Biol. Chem.*, **279**, 4551-4559.
2. Hong, Z., Jiang, J., Lan, L., Nakajima, S., Kanno, S., Koseki, H. and Yasui, A. (2008) A polycomb group protein, PHF1, is involved in the response to DNA double-strand breaks in human cell. *Nucleic Acids Res.*, **36**, 2939-2947.
3. Schilders, G., Rajmakers, R., Raats, J.M. and Pruijn, G.J. (2005) MPP6 is an exosome-associated RNA-binding protein involved in 5.8S rRNA maturation. *Nucleic Acids Res.*, **33**, 6795-6804.
4. Hayashi, Y., Fujimura, A., Kato, K., Udagawa, R., Hirota, T. and Kimura, K. (2018) Nucleolar integrity during interphase supports faithful Cdk1 activation and mitotic entry. *Sci. Adv.*, **4**, eaap7777.

SUPPLEMENTARY FIGURE

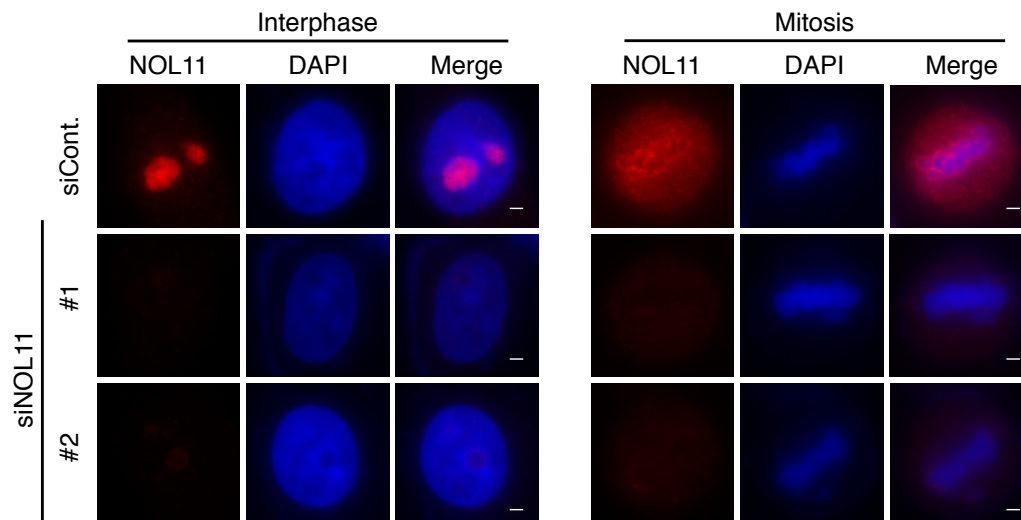


Fig. S1. Endogenous NOL11 localises at the chromosome periphery in mitotic cells.

HeLa cells transfected with siCont. or siNOL11 were fixed with ice-cold methanol and stained with anti-NOL11 antibodies (red) and DAPI (blue). Representative cells in interphase (left) and metaphase (right) are shown. Scale bar, 2 μ m.

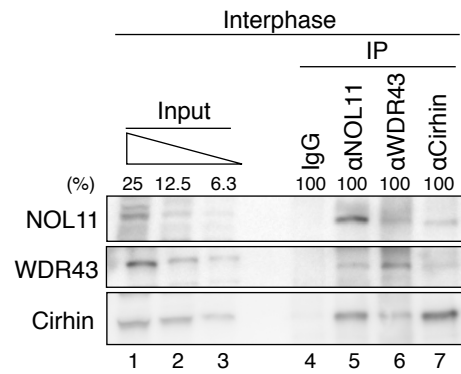


Fig. S2. NOL11 interacts with WDR43 and Cirhin in interphase cells.

Endogenous NOL11, WDR43, or Cirhin was immunoprecipitated from cell extracts prepared from HeLa cells synchronised in S phase and immunoblotted using the indicated antibodies.

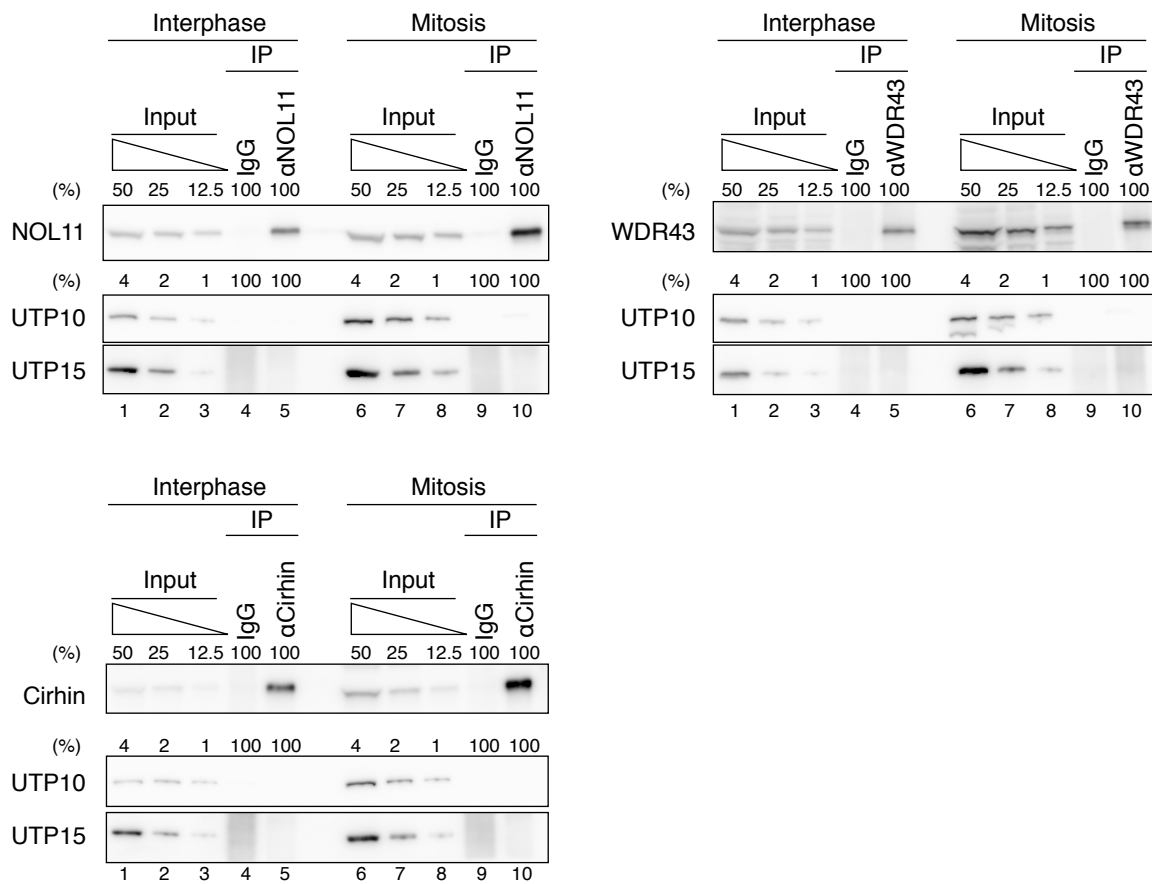


Fig. S3. The majority of the NWC complex do not comprise the t-UTP/UTPA complex. Endogenous NOL11, WDR43, or Cirhin was immunoprecipitated from logarithmically growing (Interphase) or mitotic (Mitosis) HeLa cell extracts and then immunoblotted with the indicated antibodies.

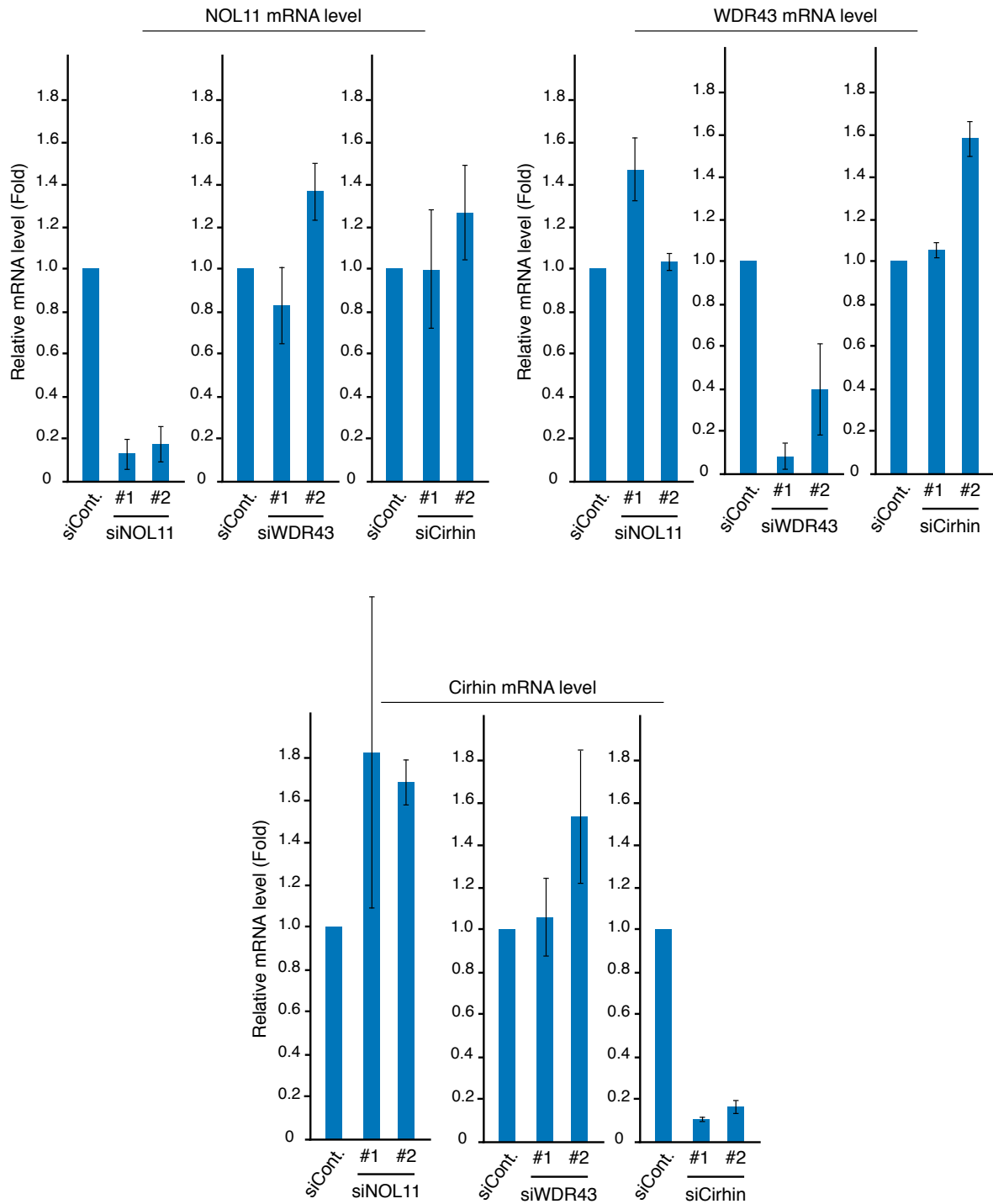


Fig. S4. NOL11, WDR43, or Cirhin depletion has little influence on mRNA levels of other genes.

HeLa cells were transfected with siCont., siNOL11, siWDR43, or siCirhin, and RNA was purified from cells after 48 h. Relative mRNA levels were measured by quantitative RT-PCR using primer sets specific for NOL11, WDR43, or Cirhin. Values are mean \pm SEM (n = 2).

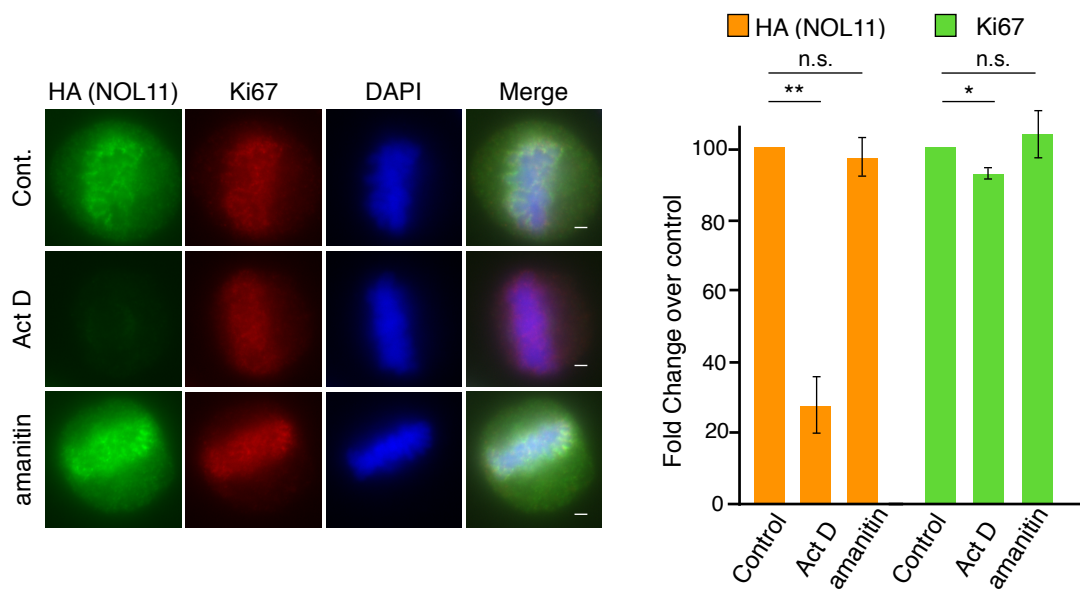


Fig. S5. RNA polymerase I inhibition causes the dissociation of NOL11 from the PR.

After treatment with 10 μ M MG132 for 2 h, HeLa cells treated with buffer alone (control), actinomycin D (Act D), or α -amanitin were co-stained with anti-HA (NOL11; green) or anti-Ki67 antibodies (red) and then DAPI (blue). Representative cells in metaphase are shown. Scale bar, 2 μ m. For each condition, the relative intensity of Aurora B or Ki67 signals at the PR was measured (right). Values are mean \pm SEM (n = 3). Chromosomes from more than 25 mitotic cells were measured. *p < 0.05, **p < 0.01, n.s. not statistically different (one-tailed Welch's t-test).

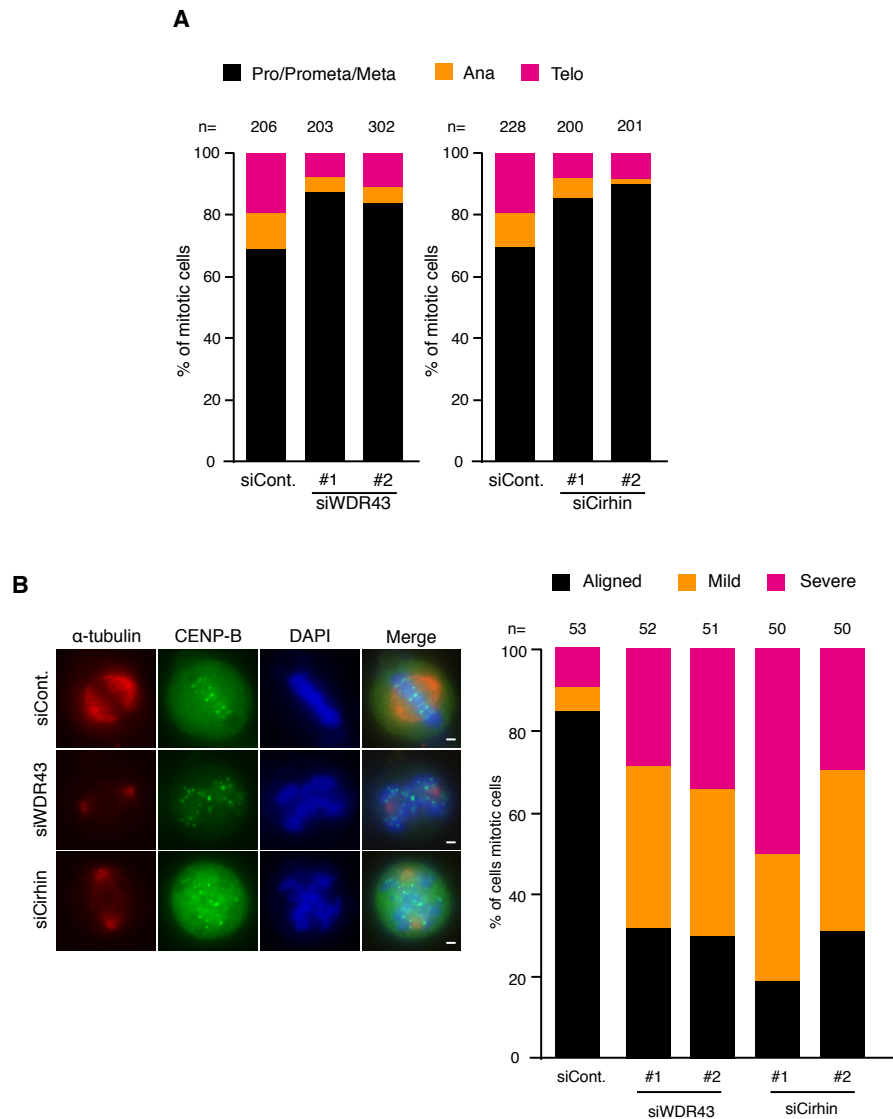


Fig. S6. WDR43 or Cirhin depletion delays progression through mitosis and causes chromosome misalignment.

(A) Metaphase arrest in cells depleted of WDR43 or Cirhin. HeLa cells transfected with siCont., siWDR43, or siCirhin for 48 h were fixed and stained. Mitotic cells were classified as Pro/Prmeta/Meta, Ana, and Telo. The distribution of mitotic cells in each phase is shown. **(B)** Defective chromosome alignment in cells depleted of WDR43 or Cirhin. After treatment with 10 μ M MG132 for 2 h, control, WDR43-depleted, and Cirhin-depleted cells were stained with anti- α -tubulin antibodies (red), anti-CENP-B antibodies (green), and DAPI (blue). Scale bar, 2 μ m. The percentage of mitotic cells with different degrees of chromosome alignment is presented (right). The classification of chromosome alignment was as follows: full alignment (Aligned), one to five misaligned chromosomes (Mild), and more than six misaligned chromosomes (Severe).

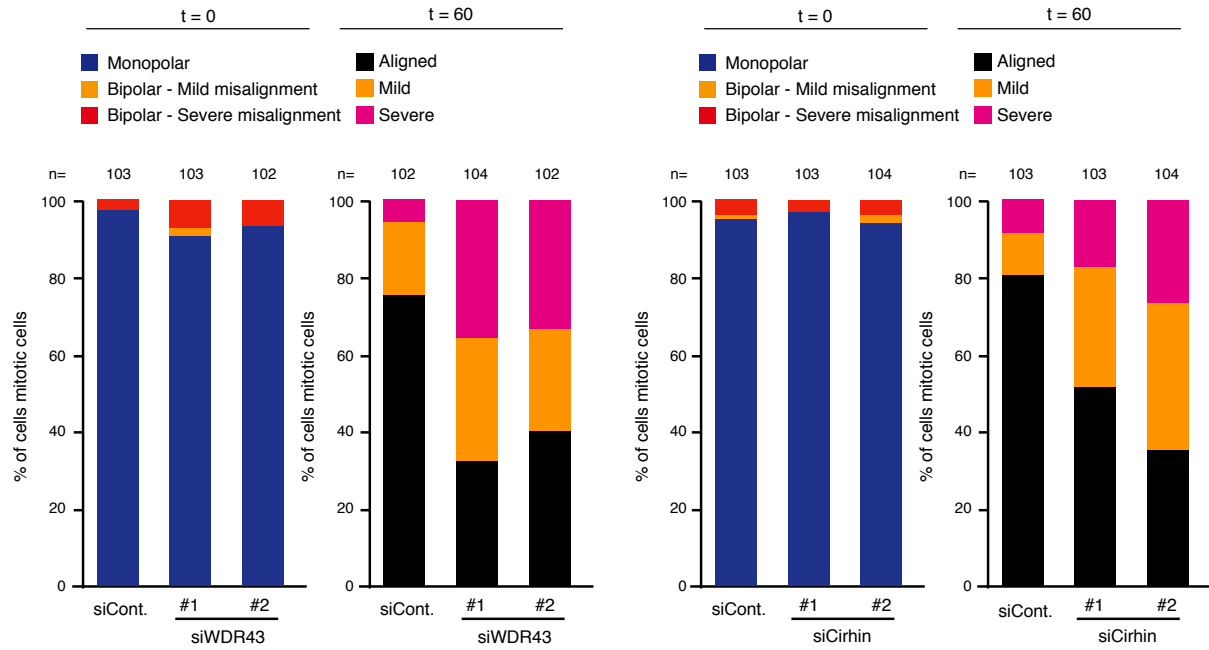


Fig. S7. WDR43 or Cirhin depletion causes defect in chromosome alignment after release from monastrol arrest.

HeLa cells were cultured for 48 h after transfection of siCont., siWDR43, or siCirhin, treated with 100 μ M monastrol for 4 h, and subsequently transferred in medium containing 10 μ M MG132.

Immunofluorescence and classification of chromosome alignment were performed as shown in Fig. 5A.

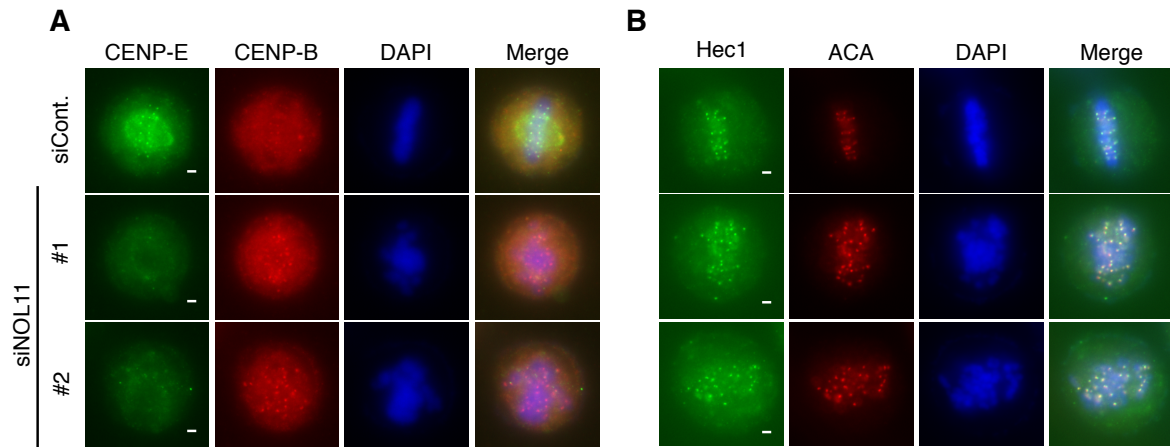


Fig. S8. NOL11 depletion causes CENP-E delocalisation.

HeLa cells transfected with siCont. or siNOL11 were fixed with ice-cold methanol (A) or 4% PFA (B) co-stained with anti-CENP-E (A, green), anti-CENP-B (A, red), anti-Hec1 (B, green), and ACA antibodies (B, red) and then DAPI (blue). Representative cells are shown. Scale bar, 2 μm.

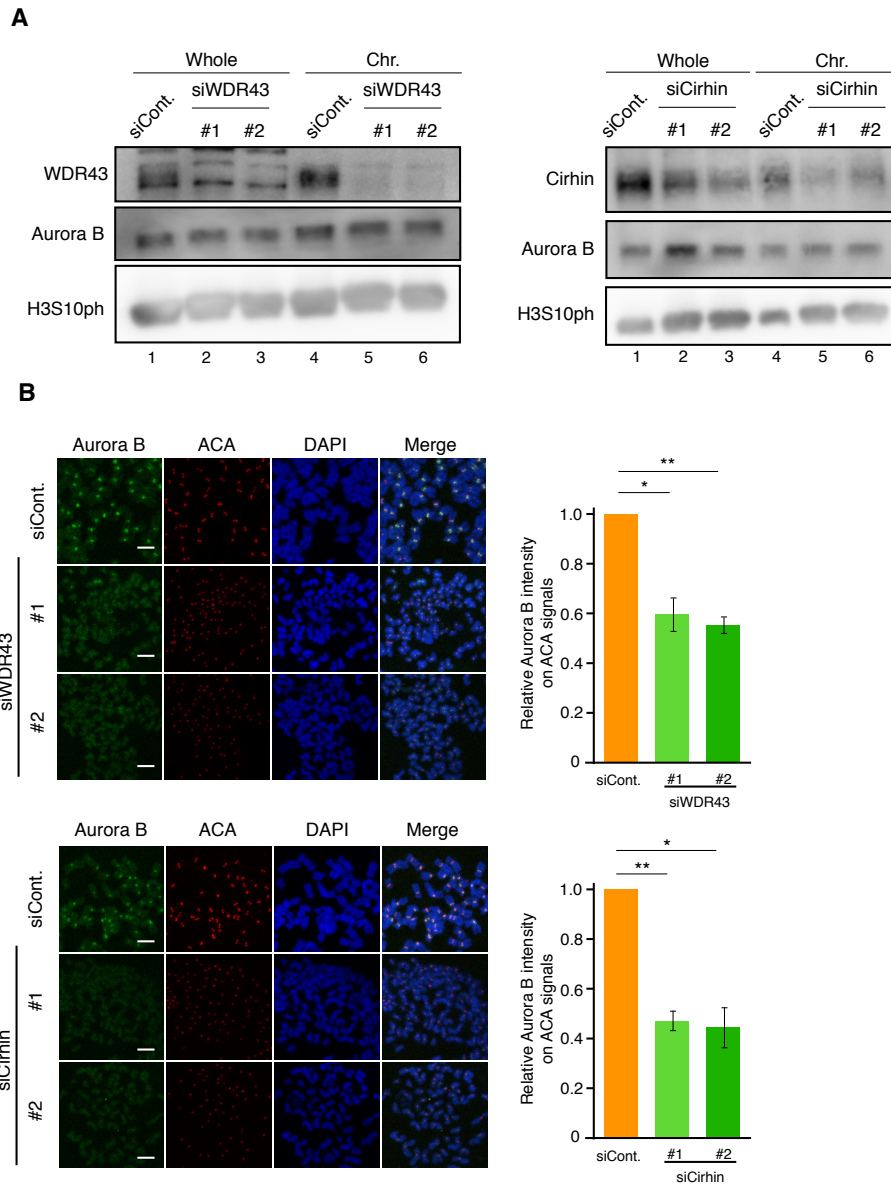


Fig. S9. WDR43 or Cirhin depletion causes enrichment failure of Aurora B at centromeres.

(A) Unvarying Aurora B levels on mitotic chromosomes from cells depleted of WDR43 or Cirhin. HeLa cells transfected with siCont., siWDR43, or siCirhin were synchronised in mitosis and fractionated. Whole-cell extracts and chromosomal fractions were immunoblotted with the indicated antibodies. **(B)** Reduced localisation of Aurora B at centromeres in WDR43- or Cirhin-depleted cells. Mitotic chromosome spreads were prepared 48 h after siRNA transfection and stained with anti-Aurora B antibodies (green), ACA (red), and DAPI (blue). Scale bar, 2 μ m. For each condition, the relative intensity of Aurora B signals at centromeres was measured (right). Values are mean \pm SEM (n = 3). Chromosomes from more than 15 mitotic cells were measured. *p < 0.05, **p < 0.01 (one-tailed Welch's t-test).

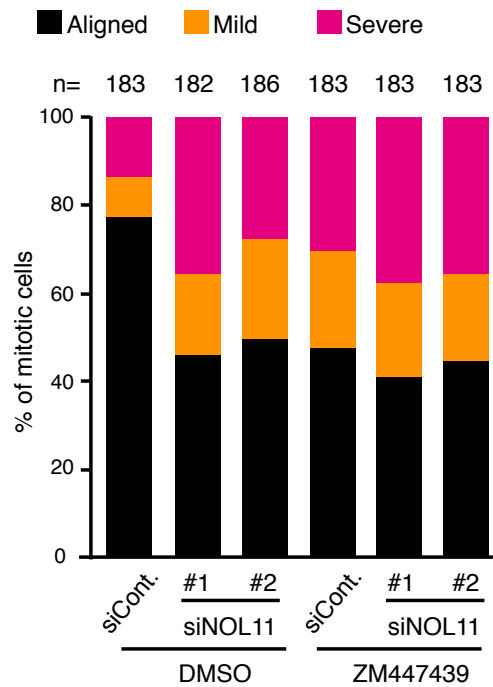


Fig. S10. Aurora B inhibitor eliminates differences in chromosome alignment status between control and NOL11-depleted cells.

HeLa cells were transfected with siCont. or siNOL11 for 46 h followed by the addition of 10 μ M MG132 for 2 h with or without 2 μ M ZM447439 in the cell culture medium, and their chromosome alignment status was examined. The percentage of mitotic cells with different degrees of chromosome alignment is presented (right). The classification of chromosome alignment was as follows: full alignment (Aligned), one to five misaligned chromosomes (Mild), and more than six misaligned chromosomes (Severe).

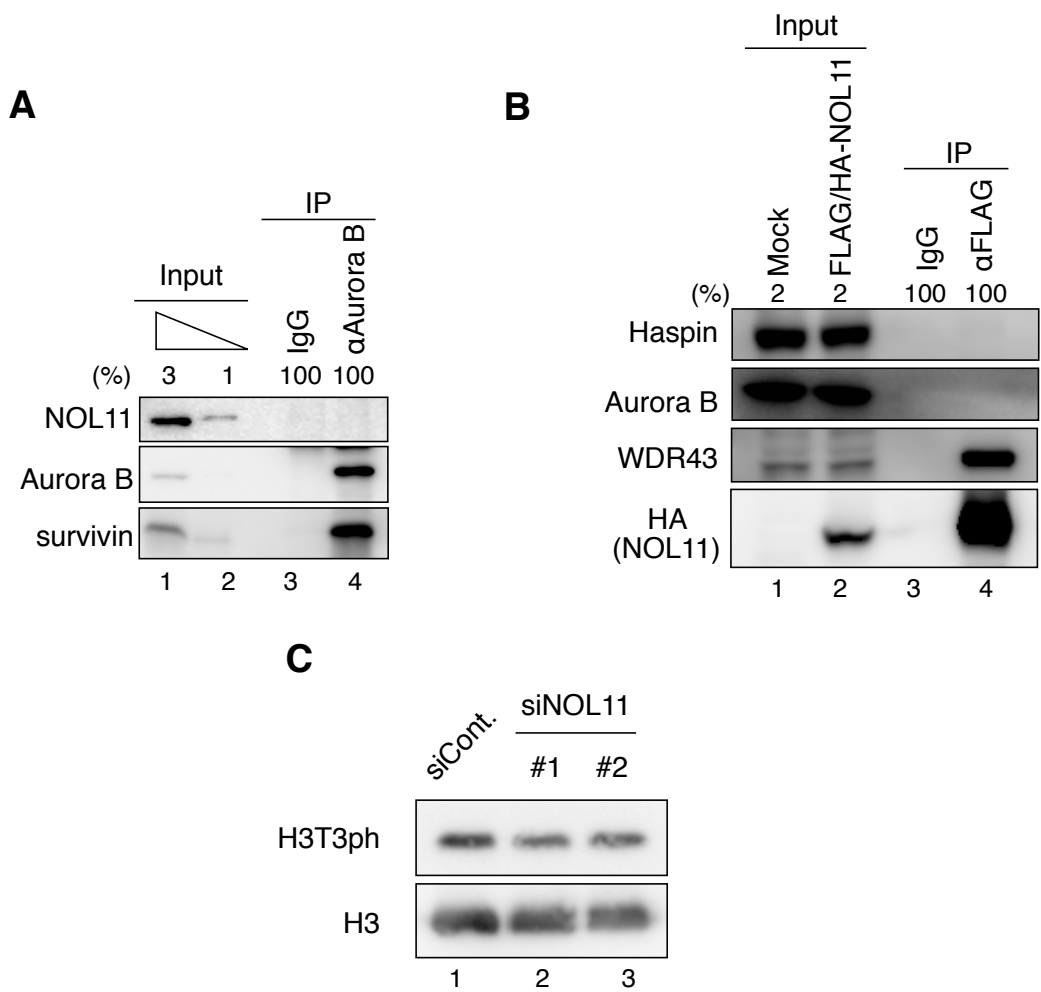


Fig. S11. NOL11 does not interact with Aurora B or Haspin.

(A) Mitotic HeLa cell extracts were subjected to immunoprecipitation with anti-Aurora B antibodies and immunoblotted with the indicated antibodies. **(B)** Cell extracts prepared from mitotic HeLa cells stably expressing FLAG/HA-NOL11 were subjected to immunoprecipitation with anti-FLAG antibodies and immunoblotted with the indicated antibodies. **(C)** Unvarying H3T3ph levels on mitotic chromosomes. HeLa cells transfected with siCont. or siNOL11 were synchronised in mitosis. Mitotic cell extracts were immunoblotted using the indicated antibodies.

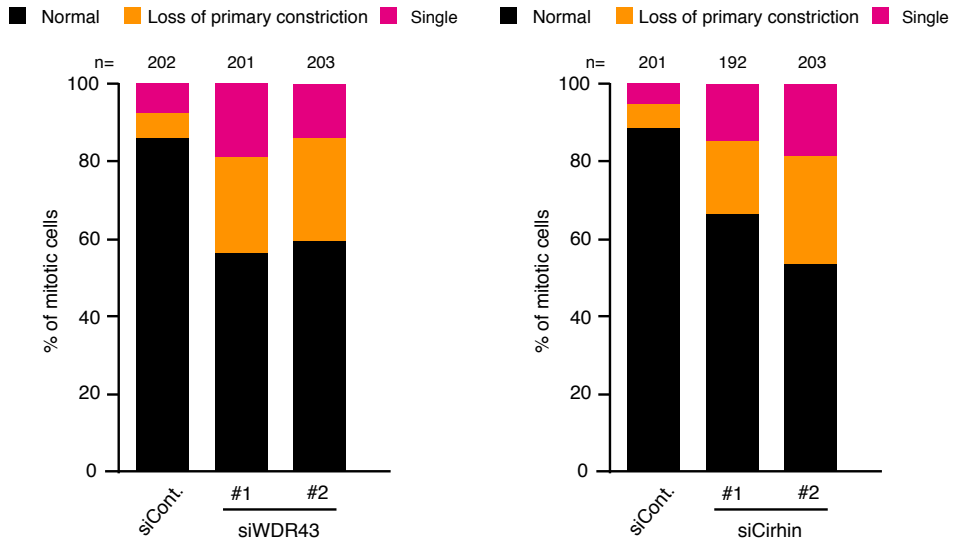


Fig. S12. WDR43 or Cirhin depletion disrupts sister chromatid cohesion.

Mitotic chromosome spreads were prepared from HeLa cells transfected with siCont., siWDR43, or siCirhin and stained with Giemsa. The percentages of normal and aberrant chromosomes are shown.

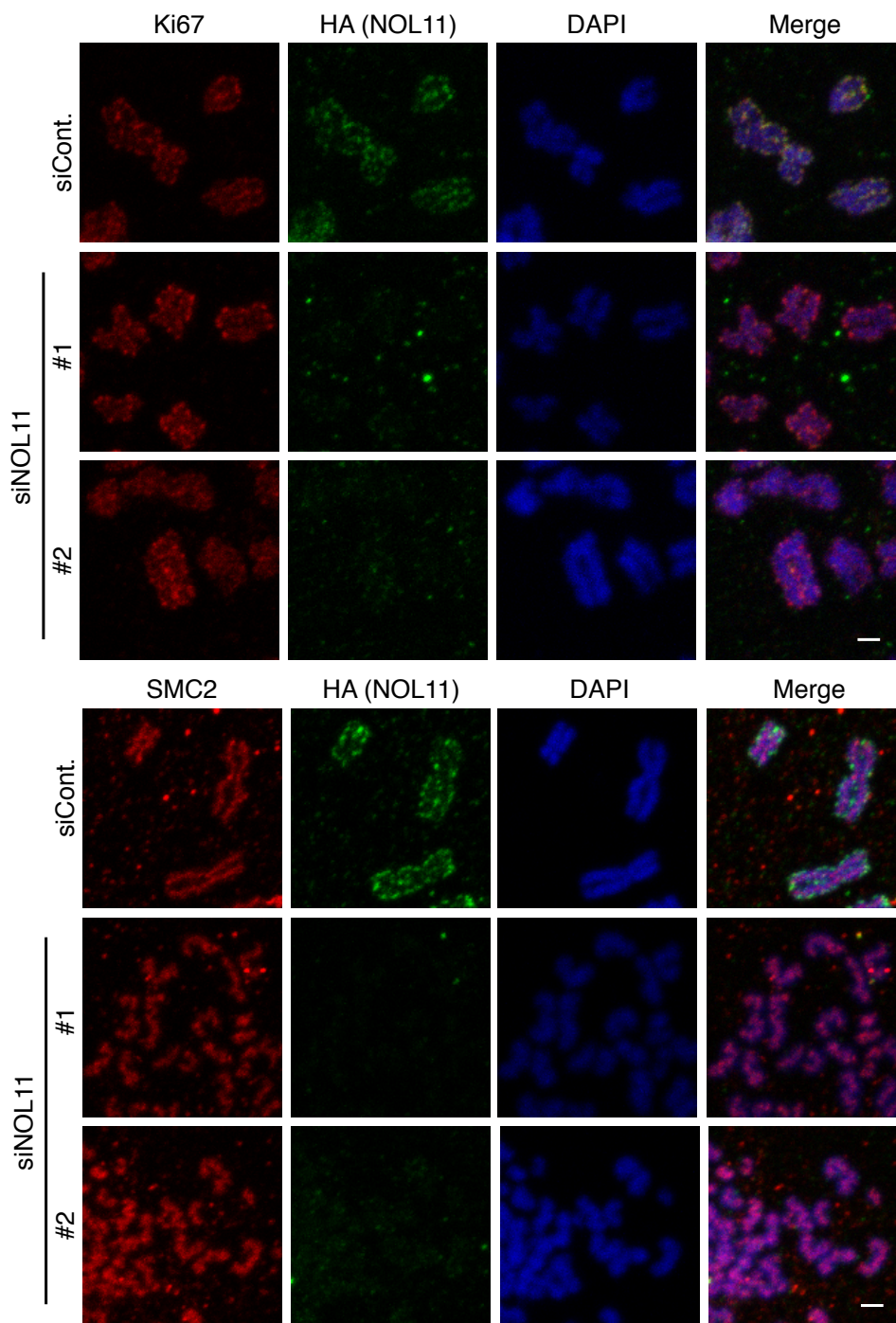


Fig. S13. NOL11 depletion does not affect the localisation of Ki67 or SMC2.

Mitotic chromosome spreads were prepared 48 h after transfection with siCont. or siNOL11 and stained with anti-Ki67 antibodies (red, top), human SMC2 antibodies (red, bottom), anti-HA antibodies (green), and DAPI (blue). Scale bar, 2 μ m.

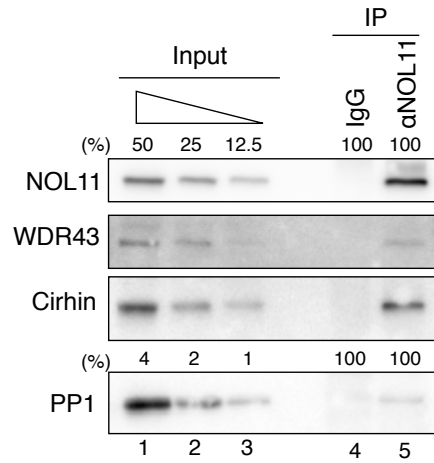


Fig. S14. The NWC complex associated with PP1.

Endogenous NOL11 was immunoprecipitated from mitotic HeLa cell extracts and the associated proteins were immunoblotted with the indicated antibodies.