Maerki et al., http://www.jcb.org/cgi/content/full/jcb.200906117/DC1

A

Protein name	ID (UNIPROT)	Identified peptides KLHL9 IP	Identified peptides KLHL13 IP
Kelch-like protein 9	KLHL9_HUMAN	APMTTVR, QQLVVSK, AMFTGGMK, TAVDTVFR, GHLYAVGGR, SLAPMDAPR, SLAPMDAPR + Oxidation (M), TFFHLSALK, IIDFIYTAK, MNEWSYVAK, LYVIGGNHFR, LAFVLSSNSLK, WMQVASLNEK, KIIDFIYTAK, RTFFHLSALK, AMMASASDYFK, WMQVASLNEK + Oxidation (M), AMMASASDYFK + Oxidation (M), AMMASASDYFK + 2 Oxidation (M), VFDLPESLGGIR, YDPEKDEWHK, GQSDVGVAVFENK, IANTYNLIEVDK, IYVVGGYSWNNR, NFPALLSTGEFLK, SDSTHLVTLGGVLR, FFTSNTHSSVVLQGFDQLR	APMTTVR, QQLVVSK, TAVDTVFR, GHLYAVGGR, SLAPMDAPR, SLAPMDAPR + Oxidation (M), YVNNFILK, TFFHLSALK, IIDFIYTAK, MNEWSYVAK, MNEWSYVAK + Oxidation (M), LYVIGGNHFR, LAFVLSSNSLK, KIIDFIYTAK, RTFFHLSALK, AMMASASDYFK, AMMASASDYFK + Oxidation (M), AMMASASDYFK + 2 Oxidation (M), VFDLPESLGGIR, YDPEKDEWHK, GQSDVGVAVFENK, IANTYNLIEV DK, IYVVGGYSWNNR, NFPALLSTGEFLK, SDSTHLVTLGGVLR, FFTSNTHSSVVLQGFDQLR, YQHGIAVIGNFLYVVGGQSNYDTK
Kelch-like protein 13 (BTB and kelch domain containing protein 2)	KLH13_HUMAN	APMTTVR, QQLVVSK, AMFTGGMK, TAVDTVFR, GYLYAVGGR, SLAPMDAPR, YVNSFVLK, SLAPMDAPR + Oxidation (M), GLSSHLQSSK, TFFHLSALK, IIDFIYTAK, TNEWTYVAK, LYVIGGNHFR, LAFVLSSNSLK, WMQVASLNEK, KIIDFIYTAK, RTFFHLSALK, WMQVASLNEK + Oxidation (M), VMMASASDYFK, VMMASASDYFK + Oxidation (M), VFDLPESLGGIR, GQSDVGVAVFENK, IANTYNLTEVDK, IYVVGGYSWNNR, NFPALLSTGEFLK, SDTTHLVTLGGVLR, IFTSNTHSSVVLQGFDQLR	APMTTVR, QQLVVSK, TAVDTVFR, GYLYAVGGR, SLAPMDAPR, YVNSFVLK, SLAPMDAPR + Oxidation (M), GLSSHLQSSK, TFFHLSALK, IIDFIYTAK, TNEWTYVAK, LYVIGGNHFR, LAFVLSSNSLK, KIIDFIYTAK, RTFFHLSALK, VMMASASDYFK, VMMASASDYFK + Oxidation (M), VMMASASDYFK + 2 Oxidation (M), VFDLPESLGGIR, YDPDKDEWHK, GQSDVGVAVFENK, ANTYNLTEVDK, IYVVGGYSWNNR, NFPALLSTGEFLK, SDTTHLVTLGGVLR, IFTSNTHSSVVLQGFDQLR, YQHGIAVIGNFLYVVGGQSNYDTK
Kelch-like variant (Fragment) Kelch-like protein 2	Q53GT1_HUMAN	LLETVR, IYVLGGR, LDPSPLR, RGEDYLK, YIYAVAGR, HFTASLAPR, LAELFDLSR, SAQHSQALLR, AWHGMATLLNK, DTVASALMYHR, FPLMEAEVLQR, LTEQLDTYILK, DYHNDLNAVER, LYVIGGSNNDAGYR, NESLQPSLQSPQTELR, YDPATNSWAYVAPLKR	QLPLEK, LLETVR, IYVLGGR, GMFAGGLK, NFVAFSR, YIYAVAGR, SLLLEPPR, VYSLLSSNR, LAELFDLSR, SAQHSQALLR, YLNPLLGEWK, AWHGMATLLNK, DTVASALMYHR, LHDKLDPSPLR, FPLMEAEVLQR, LTEQLDTYILK, DYHNDLNAVER, LYVIGGSNNDAGYR, NESLQPSLQSPQTELR, YDPATNSWAYVAPLKR
Kelch-like protein 21	KLH21_HUMAN	EEAAYQLALR, AVLAAASPYFR	AVLAAASPYFR, VAVSGDNAEPLLR
Kelch-like protein 26	Q8TAP0_HUMAN		IYALGGR, ESVDAFTFR, LVFFLQSNR, EASQDVIELK

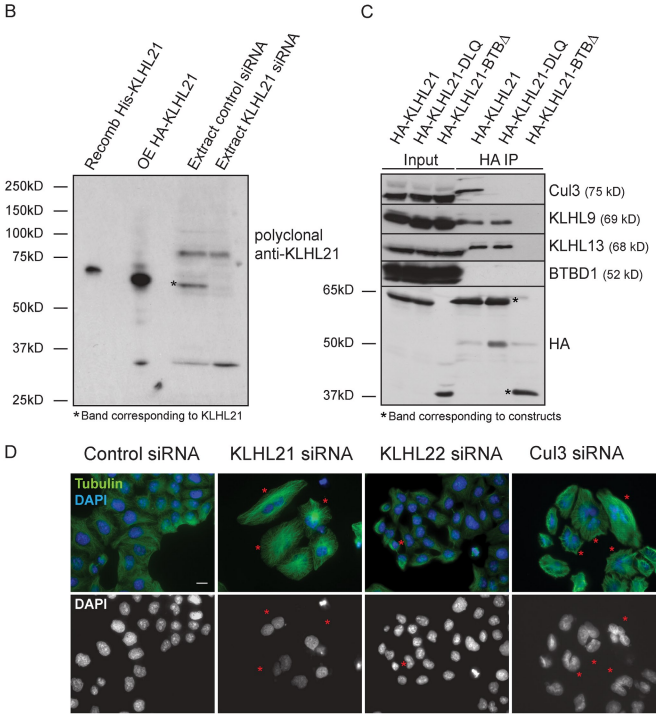


Figure S1. **KLHL21 is a novel interactor of Cul3, KLHL9, and KLHL13 and is required for cytokinesis.** (A) Table showing the BTB proteins identified by mass spectrometry analysis in immunoprecipitations performed with anti-KLHL9 or -KLHL13 antibodies in HeLa extracts. Protein names, UniProt IDs, and identified peptides are listed. (B) Recombinant GST-Cul3–Rbx1–His-KLHL21, extracts from cells overexpressing HA-KLHL21 and extracts from cells treated with control or KLHL21 siRNA were analyzed by immunoblotting with polyclonal KLHL21 antibodies. The asterisk marks the band detected in HeLa extracts that corresponds to the size of KLHL21 and disappears specifically in KLHL21 siRNA. (C) HeLa cells were transfected with a vector containing HA-KLHL21, HA-KLHL21 with a triple mutation in the BTB domain (D114A/L115A/Q117A; HA–KLHL21-DLQ), or with HA-KLHL21 lacking the entire BTB domain (HA–KLHL21-BTBΔ). Extracts were prepared and immunoprecipitated with anti-HA antibodies. Inputs and eluates (HA IP) were analyzed by immunoblotting. The bands corresponding to the different constructs detected with the anti-HA antibody are marked with an asterisk (bottom). (D) HeLa cells treated with the indicated siRNAs for 72 h were stained for α-tubulin to visualize microtubules. DNA was stained with DAPI. Asterisks mark multinucleated cells. Quantification is shown in Fig. 1 E. Bar, 10 µm.

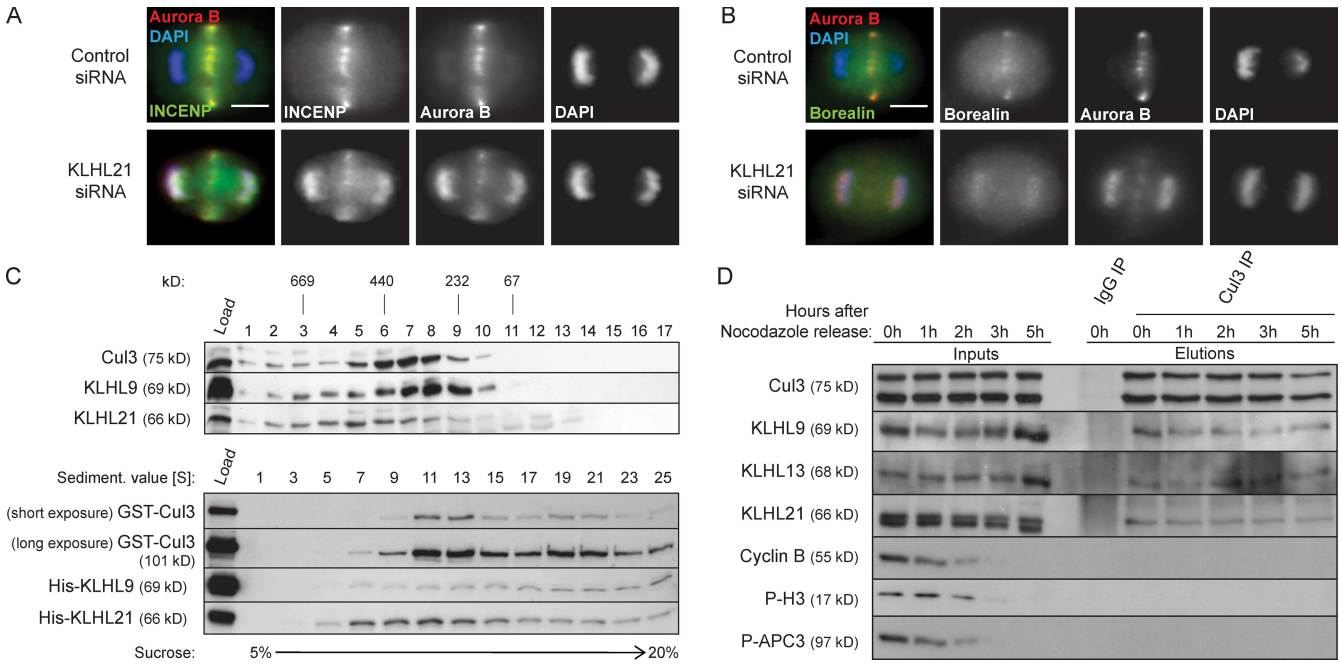


Figure S2. **KLHL21 regulates midzone accumulation of the CPC and forms a high molecular mass complex with Cul3 throughout mitosis.** (A and B) HeLa cells treated with control or KLHL21 siRNA were analyzed by IF for INCENP (A) or Borealin (B) and Aurora B. DNA was stained with DAPI. (C) Extracts prepared from HeLa cells or Sf9 insect cells simultaneously expressing GST-Cul3–Rbx1 and His-KLHL9, -KLHL13, and -KLHL21 were separated by gel filtration chromatography (top) or sucrose gradient centrifugation (bottom), respectively. Fractions were analyzed by immunoblotting. The positions of marker proteins with known molecular mass (kilodaltons) or sedimentation value (S) are indicated. An aliquot of the input was loaded in the first lane (load). (D) HeLa cells were synchronized by double thymidine block and release. 6 h after the second release, cells were arrested with 100 ng/ml nocodazole for 14 h and then released for the indicated time (hours). Extracts were prepared and immunoprecipitated with anti-Cul3 or control (anti-IgG) antibodies (only time point 0 h). Inputs and eluates were analyzed by immunoblotting. Bars, 5 µm.

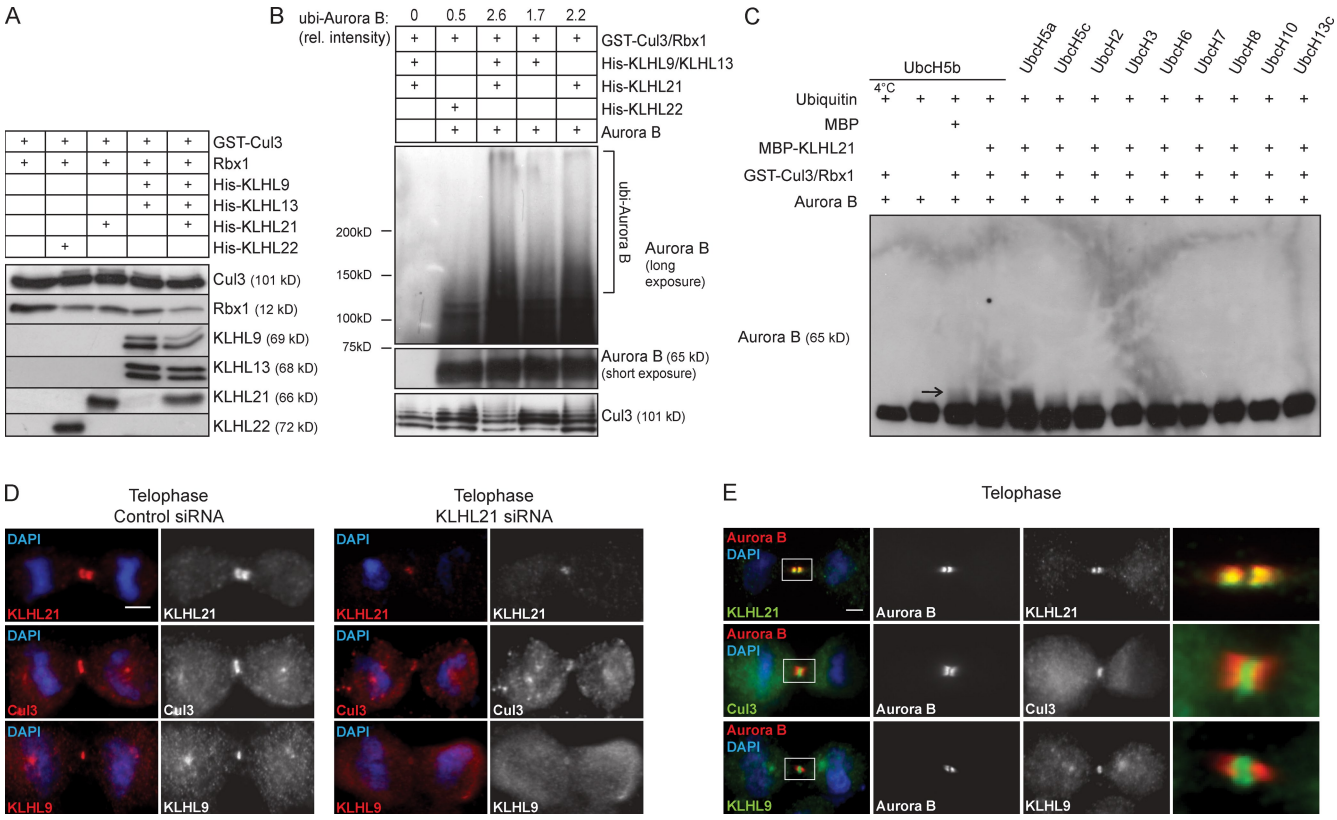
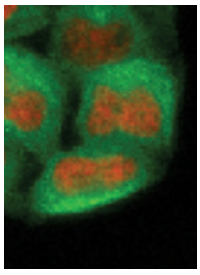
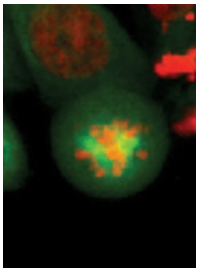


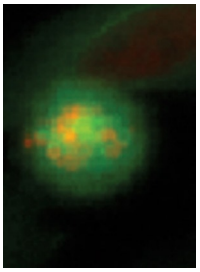
Figure S3. **The Cul3–KLHL21 E3 ligase shows ubiquitination activity toward Aurora B in vitro and Cul3, KLHL9, and KLHL21 localize to distinct midbody structures.** (A) GST-Cul3–Rbx1, GST-Cul3–Rbx1–His-KLHL22, GST-Cul3–Rbx1–His-KLHL21, GST-Cul3–Rbx1–His-KLHL9–His-KLHL13, and GST-Cul3–Rbx1–His-KLHL9–His-KLHL13–His-KLHL21 complexes were purified from Sf9 cells and analyzed by immunoblotting. (B) Recombinant Aurora B was added as indicated to in vitro ubiquitination reactions containing the different Sf9-purified Cul3 complexes described in A and the E2 enzyme UbcH5c. Reactions were incubated for 1 h and analyzed by immunoblotting. Higher molecular mass conjugates of ubiquitin–Aurora B (ubi–Aurora B) are shown in brackets. The intensities of the ubiquitin–Aurora B signal from the different gel lanes were quantified, and relative intensities are depicted, which correspond to intensities with subtracted background (background: first gel lane, reaction without the addition of Aurora B). (C) Recombinant Aurora B was added as indicated to in vitro ubiquitination reactions with GST-Cul3–Rbx1 complexes purified from Sf9 cells and MBP or MBP-KLHL21 purified from *E. coli*. Reactions were incubated for 1 h with the indicated E2 enzymes, and Aurora B ubiquitination was analyzed by immunoblotting. The arrow indicates the position of monoubiquitinated Aurora B. (D) HeLa cells treated with control or KLHL21 siRNA were pre-extracted before fixation and analyzed by IF for KLHL21, Cul3, or KLHL9. DNA was stained with DAPI. (E) HeLa cells pre-extracted before fixation were analyzed by IF for KLHL21, Cul3, and KLHL9 and Aurora B. DNA was stained with DAPI. Boxes in the first column outline areas magnified in the last column. Bars, 5 µm.



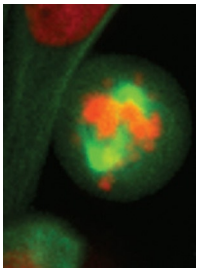
Video 1. **Control siRNA–treated HeLa cells, stably expressing H2B-mRFP and mEGFP–α-tubulin, progress through mitosis.** Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 510). Frames were taken every 15 min for 24 h, out of which 2 h are shown. This video corresponds to still images presented in Fig. 1 C and is shown at 5 frames/s.



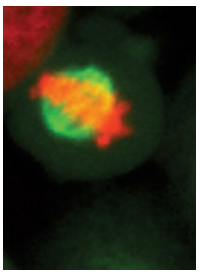
Video 2. **KLHL21 siRNA–treated HeLa cells, stably expressing H2B-mRFP and mEGFP–α-tubulin, show a delay from prophase to anaphase.** Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 510). Frames were taken every 15 min for 24 h, out of which 3 h are shown. This video corresponds to still images presented in Fig. 1 C and is shown at 5 frames/s.



Video 3. **KLHL22 siRNA–treated HeLa cells, stably expressing H2B-mRFP and mEGFP–α-tubulin, show a delay from prophase to anaphase.** Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 510). Frames were taken every 15 min for 24 h, out of which 5 h are shown. This video corresponds to still images presented in Fig. 1 C and is shown at 5 frames/s.



Video 4. **Cul3 siRNA–treated HeLa cells, stably expressing H2B-mRFP and mEGFP–α-tubulin, show a delay from prophase to anaphase.** Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 510). Frames were taken every 15 min for 24 h, out of which 3 h are shown. This video corresponds to still images presented in Fig. 1 C and is shown at 5 frames/s.



Video 5. **KLHL21 siRNA–treated HeLa cells, stably expressing H2B-mRFP and mEGFP–α-tubulin, fail to complete cytokinesis.** Images were analyzed by time-lapse confocal microscopy using a laser-scanning confocal microscope (LSM 510). Frames were taken every 15 min for 24 h, out of which 4 h are shown. This video corresponds to still images presented in Fig. 1 C and is shown at 5 frames/s.