Caldas et al., http://www.jcb.org/cgi/content/full/jcb.201306054/DC1

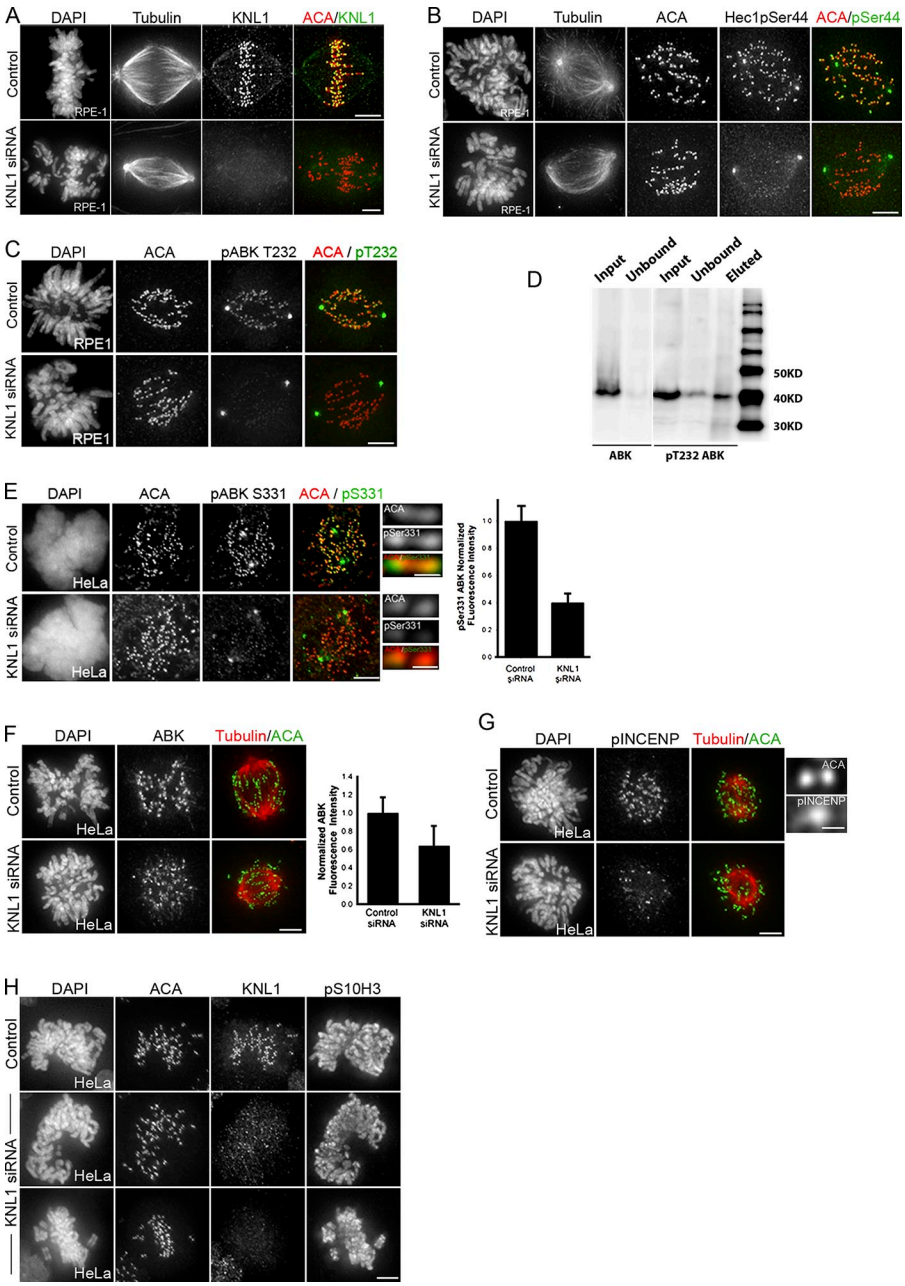


Figure S1. **KNL1 is required for full Aurora B activity at the kinetochore and at the inner centromere (related to Fig. 1).** (A) RPE-1 cells demonstrating the characteristic phenotype of KNL1 depletion. (B) Control and KNL1-depleted RPE-1 cells were immunostained with a Hec1 phosphospecific antibody, pSer44. (C) Control and KNL1-depleted RPE-1 cells were immunostained with an Aurora B phosphospecific antibody, pT232. As shown in HeLa cells, localization of pT232 is also reduced in RPE-1 cells upon KNL1 depletion. (D) Western blot of HeLa cell lysates probed with the Aurora B pT232 antibody. Input represents 5% of total protein used for immunoprecipitation assay. Input lane shows that the pT232 antibody primarily recognizes a band at 40 kD (right), the same size at which Aurora B is recognized by a pan-Aurora B antibody (left). The unbound lane represents cell lysate after Aurora B immunodepletion using a pan-Aurora B antibody (same amount of total protein as input lane, left). The intensity of the pT232 band is significantly decreased when lysates were immunodepleted of Aurora B (right). (E) Control and KNL1-depleted HeLa cells were fixed and immunostained with a phosphospecific antibody to Aurora B kinase, p-Ser331 (Petsalaki et al., 2011). This antibody recognizes phosphorylated Aurora B kinase at the kinetochore in control cells (top); however, this localization is reduced in cells depleted of KNL1 (bottom). Quantification is shown to the right; $n \geq 100$ kinetochores from at least 9 cells. (F–H) Control and KNL1-depleted HeLa cells were immunostained with a pan antibody to Aurora B kinase (AIM1, D), a phosphospecific antibody to INCENP (E, pSer893/pSer894; Wang et al., 2011), or a phosphospecific antibody to histone H3 (pSer10). Kinetochore fluorescence intensities are shown in F. Error bars represent SD from independent experiments ($n = 3$). For each experiment $n \geq 100$ kinetochores were measured from at least 10 cells. Bars: (cell panels) 5 µm; (kinetochore pair insets) 0.5 µm.

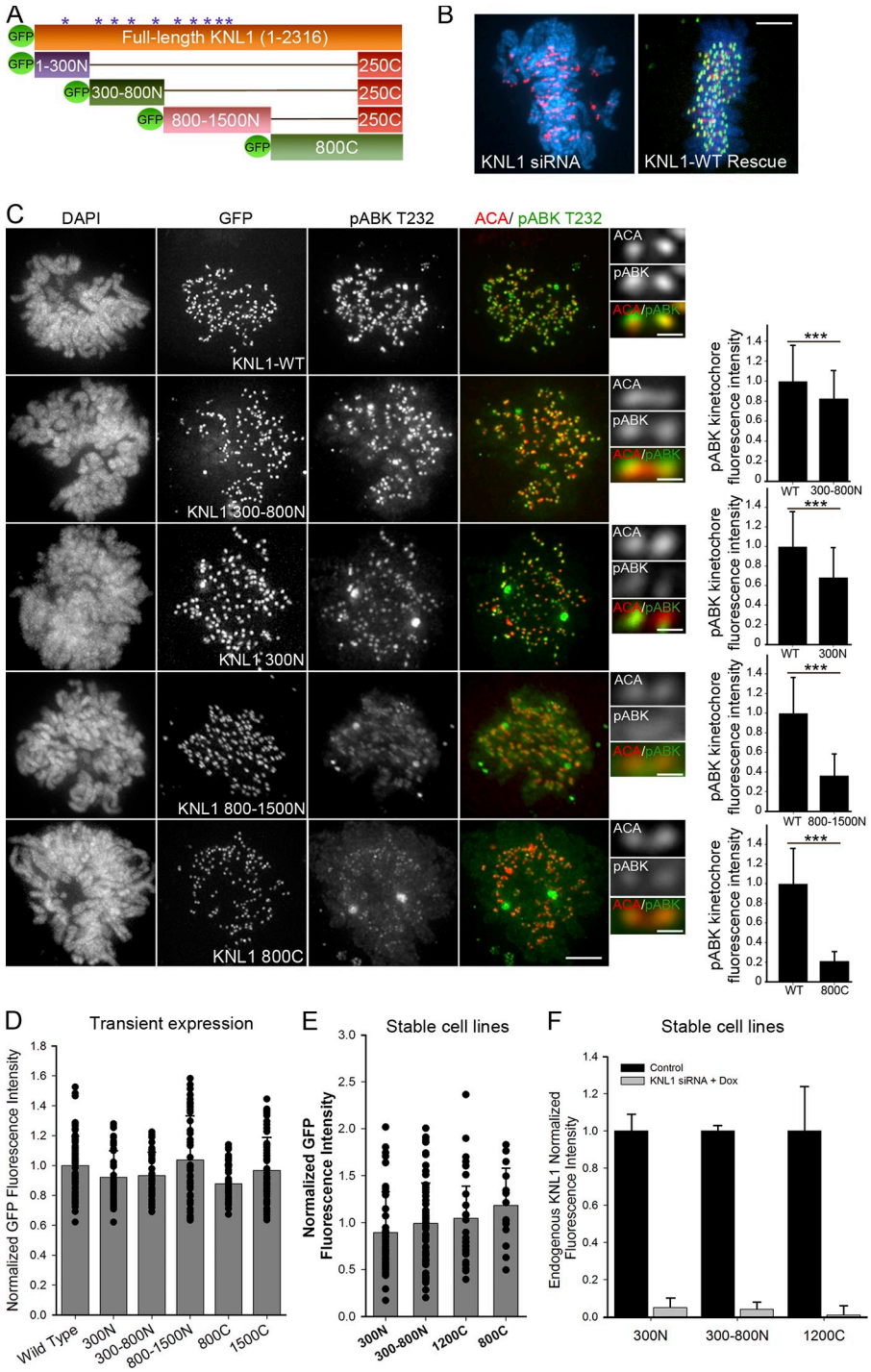


Figure S2. **The N terminus of KNL1 is sufficient to facilitate Aurora B activity (related to Fig. 3).** (A) Schematic of constructs used in silence/rescue transient expression experiments. "N" refers to amino acids from the N terminus of the protein. "C" refers to amino acids from the C terminus of the protein. Asterisks mark the MELT repeats present in the KNL1 constructs used in the study (London et al., 2012; Yamagishi et al., 2012). The full-length KNL1 sequence is similar to the KNL1 sequence published in Liu et al. (2010). The exact amino acids included in each construct are listed in Materials and methods. (B) Full-length KNL1 (KNL-WT) rescues the KNL1 depletion phenotype in HeLa cells. (C and D) HeLa cells were depleted of endogenous KNL1, rescued with the indicated GFP-KNL1 construct, and immunostained with antibodies to Aurora B pT232 (pABK). Kinetochore fluorescence intensities of pABK (C) and KNL1-GFP fusion proteins (D) in silence/rescue experiments were quantified. Error bars represent SD from independent experiments ($n = 2$). For each experiment $n \geq 60$ kinetochores were measured from at least 5 cells. ***, $P < 0.001$ (Mann-Whitney rank sum test). (E) Quantification of KNL1-GFP fusion protein levels at kinetochores in doxycycline-induced stable cell lines. Data represent at least 12 kinetochores from at least 3 cells, $n = 3$ independent experiments. (F) Quantification of endogenous KNL1 fluorescence intensity in the indicated HeLa inducible cell lines. Levels of endogenous KNL1 in cells rescued with the 300N fragment were examined with a KNL1 antibody targeted to aa 1413–1624 (Cheeseman et al., 2008). Levels of endogenous KNL1 in cells rescued with the 300–800N or the 1200C fragment were examined with a KNL1 antibody targeted to aa 1–22 (Materials and methods). For each experiment $n \geq 60$ kinetochores were measured from at least 5 cells. ***, $P < 0.001$ (Mann-Whitney rank sum test). Error bars represent SD from two independent experiments. For each experiment at least 12 kinetochores were measured from at least 5 cells. Bars: (cell panels) 5 µm; (kinetochore pair insets) 0.5 µm.

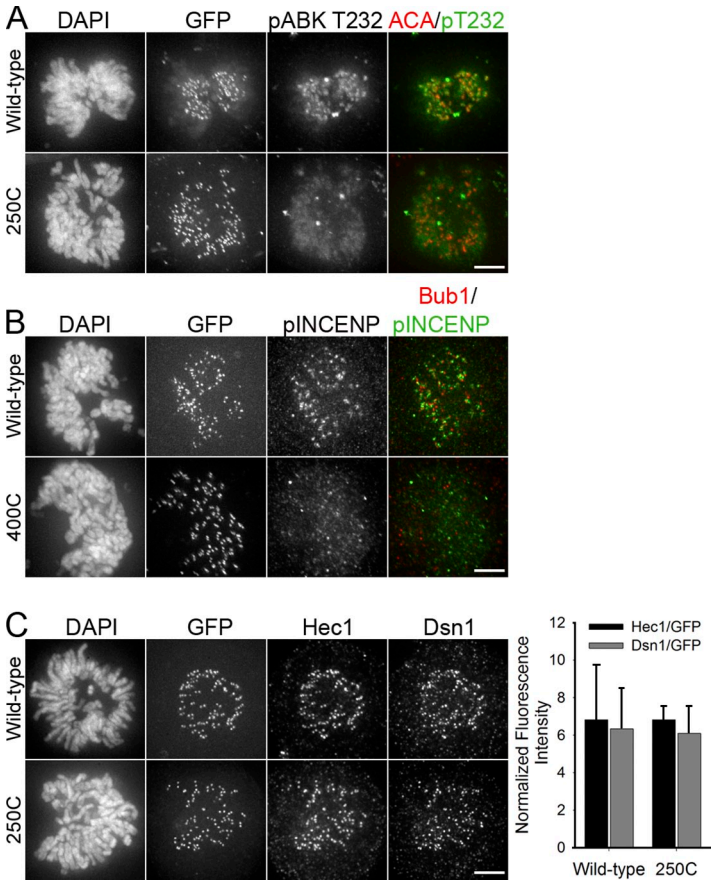


Figure S3. **KNL1 C terminus rescues wild-type levels of Hec1 and Dsn1 at kinetochores (related to Fig. 4).** HeLa cells were depleted of endogenous KNL1, rescued with the indicated GFP-KNL1 construct, and immunostained with antibodies to Aurora B pT232 (A), pINCENP (B), and Hec1 and Dsn1 (C). Kinetochore fluorescence intensities of Hec1, Dsn1, and KNL1-GFP fusion proteins (C) were quantified. Error bars represent SD between cells, $n \geq 100$ kinetochores were measured from at least 5 cells. Bars, 5 µm.

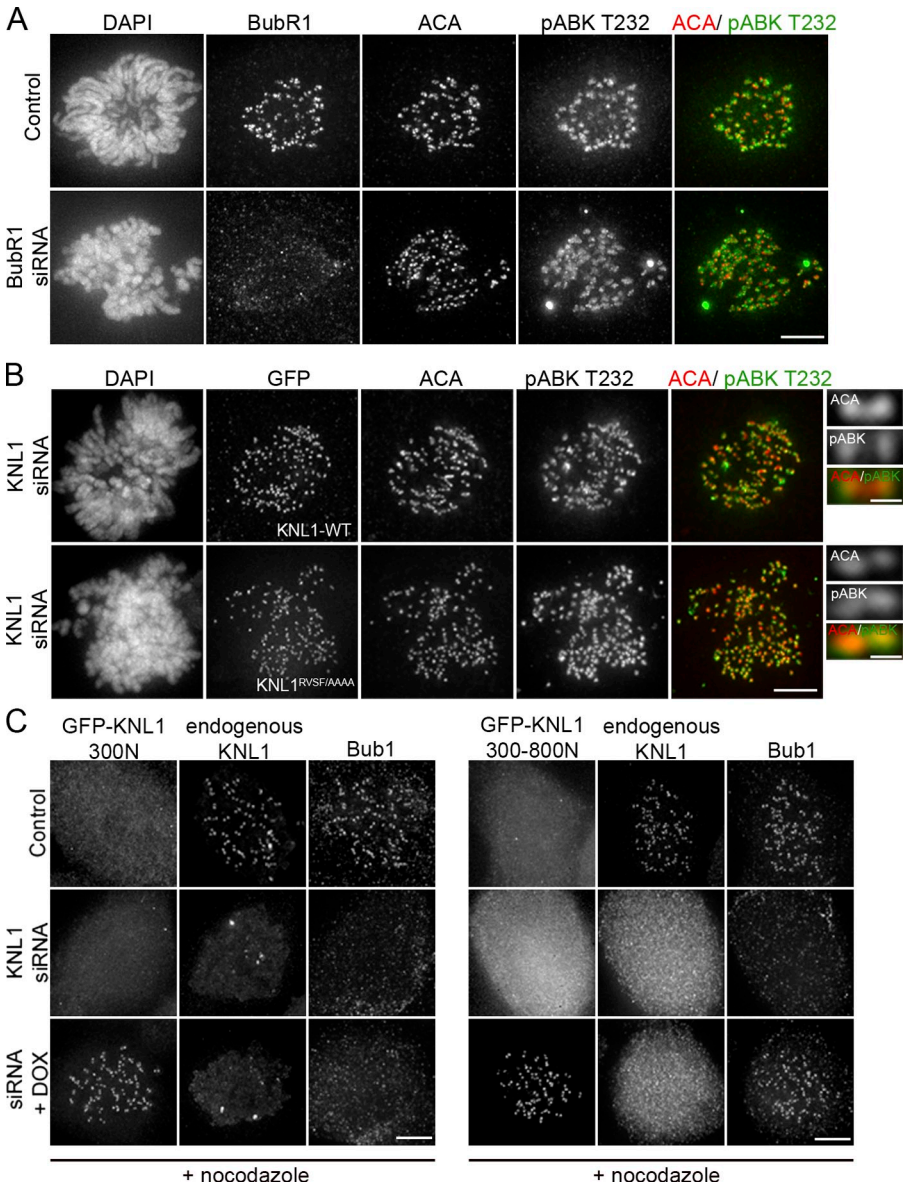


Figure S4. **KNL1 mediates Aurora B activity in a Bub1 accumulation–independent manner (related to Fig. 6).** (A) Control and BubR1-depleted cells were fixed and stained with pT232 phosphospecific Aurora B kinase and BubR1 antibodies. (B) HeLa cells were depleted of KNL1, rescued with the indicated GFP-KNL1 mutant (RVSF/AAAA), and stained with the pT232 antibody. (C) HeLa FlpIn KNL1 300N and 300–800N stable cell lines were depleted of KNL1 and rescued upon doxycycline addition. Cells were synchronized via a double-thymidine block and treated with 30 µM nocodazole for 3 h. Cells were subsequently fixed and immunostained with KNL1 and Bub1 antibodies. Bars: (cell panels) 5 µm; (kinetochore pair insets) 0.5 µm.

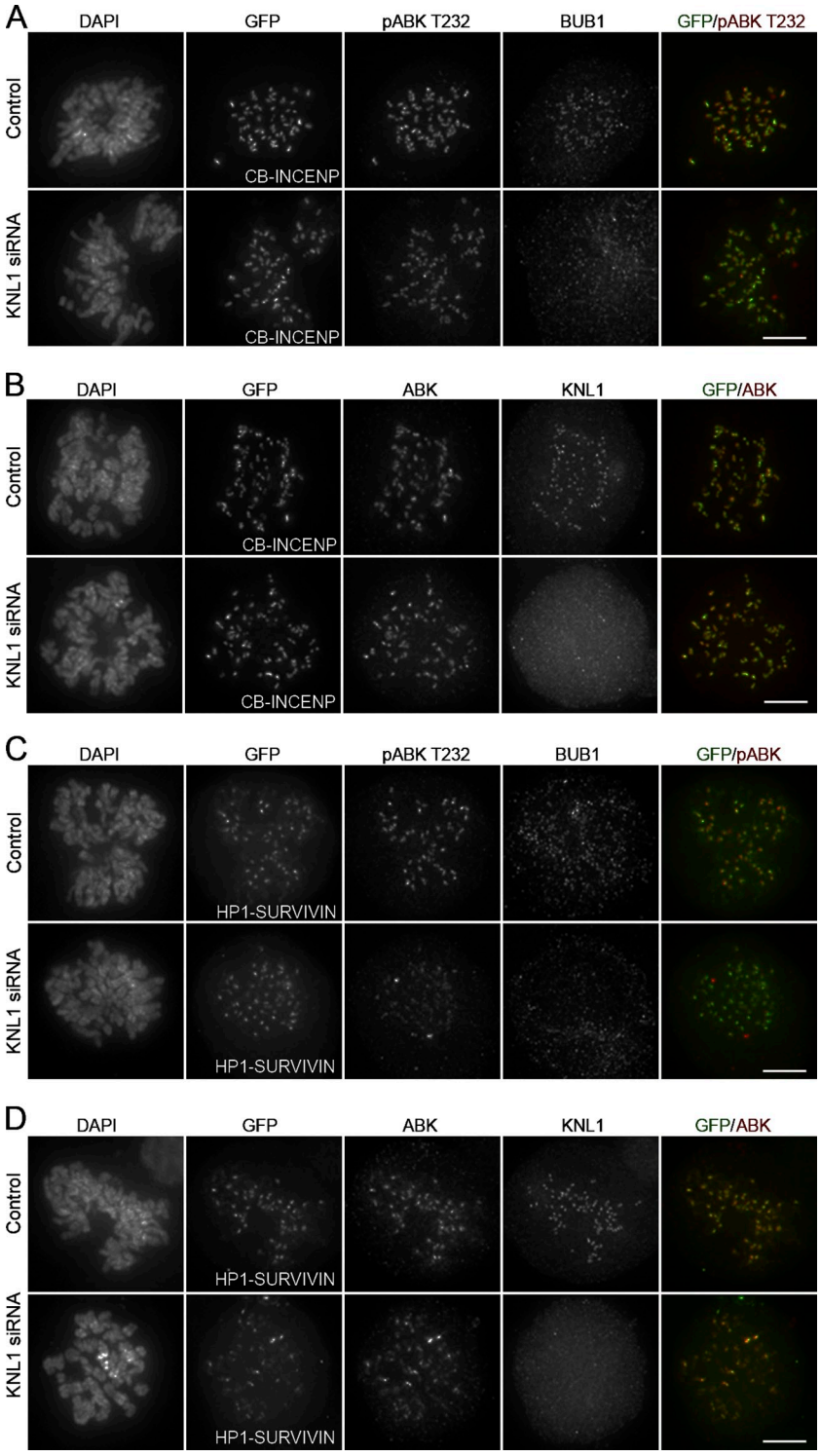
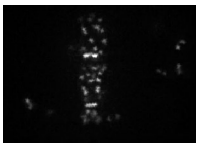


Figure S5. **Aurora B recruitment is not sufficient to restore full Aurora B activity in KNL1-depleted cells (related to Fig. 7).** (A–D) Control and KNL1-depleted HeLa cells expressing either CB-INCENP or HP1-Survivin and immunostained with antibodies to Aurora B pT232 (A and C) or pan Aurora B (AIM1, B and D). Bars, 5 µm.

Video 1. **Kinetochore oscillations in control HeLa cells.** Time-lapse sequence of a control HeLa cell treated with luciferase siRNA expressing EGFP-CENP-B and EGFP-centrin. Images were collected every 3 s for 10 min on an imaging system (DeltaVision Personal DV; Applied Precision). The movie is a projection of five optical sections played back at 20 frames per second (Related to Fig. 2).



Video 2. **Kinetochore oscillations in KNL1-depleted HeLa cells.** Time-lapse sequence of a HeLa cell depleted of endogenous KNL1 expressing EGFP-CENP-B and EGFP-centrin. Images were collected every 3 s for 10 min on an imaging system (DeltaVision Personal DV; Applied Precision). The movie is a projection of five optical sections played back at 20 frames per second (Related to Fig. 2).

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